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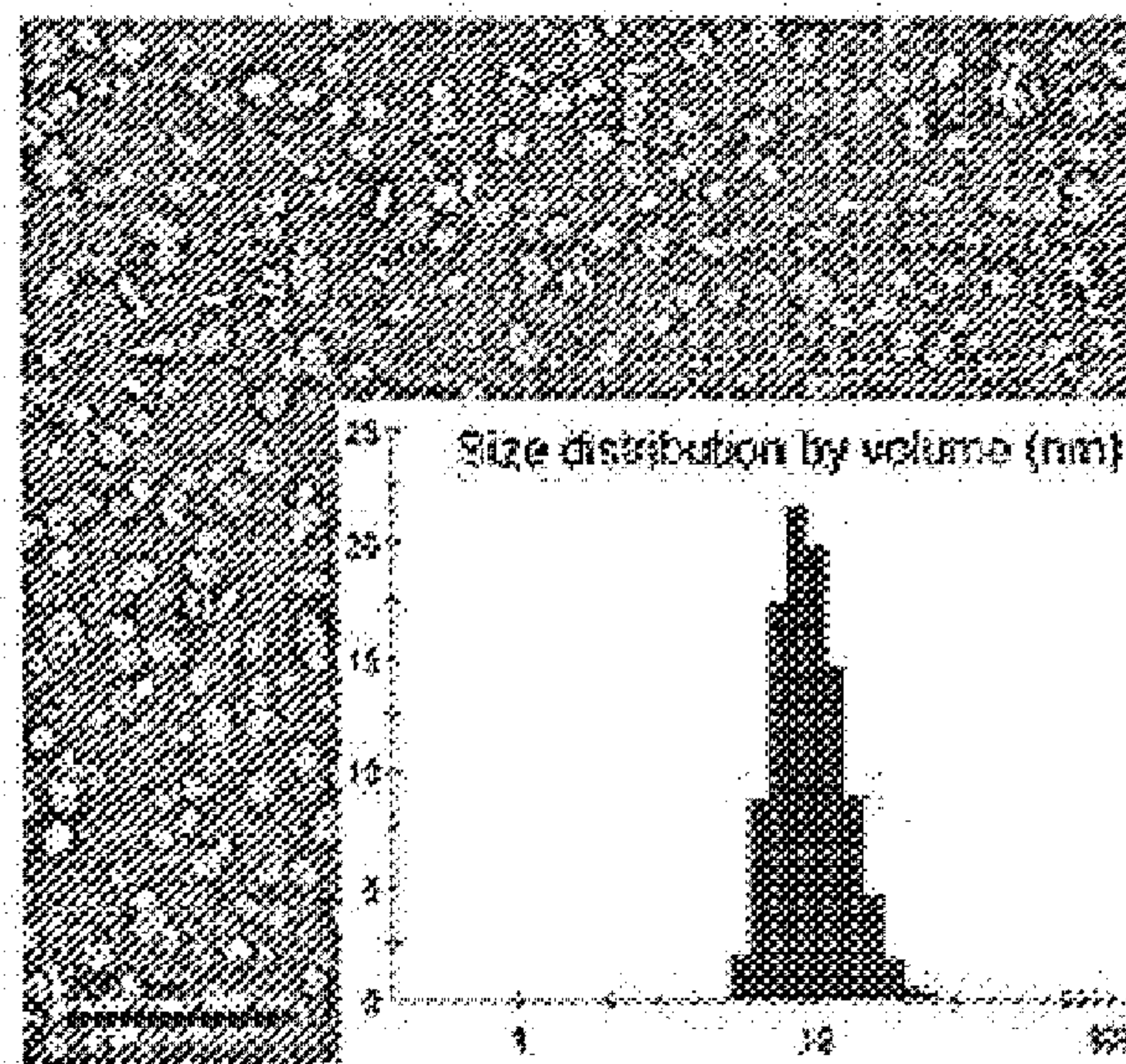
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(54) Titre : COMPOSITIONS ET METHODES POUR ADMINISTRER DES AGENTS BIOMACROMOLECULAIRES
 (54) Title: COMPOSITIONS AND METHODS FOR DELIVERY OF BIOMACROMOLECULE AGENTS

FIG. 1A



(57) **Abrégé/Abstract:**

The present invention relates to nanoparticles associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) biomacromolecule agents configured for treating, preventing or ameliorating various types of disorders, and methods of synthesizing the same. In particular, the present invention is directed to compositions comprising nanoparticles (e.g., synthetic high density lipoprotein (sHDL)) associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) biomacromolecule agents (e.g., nucleic acid, peptides, glycolipids, etc.), methods for synthesizing such nanoparticles, as well as systems and methods utilizing such nanoparticles (e.g., in diagnostic and/or therapeutic settings).

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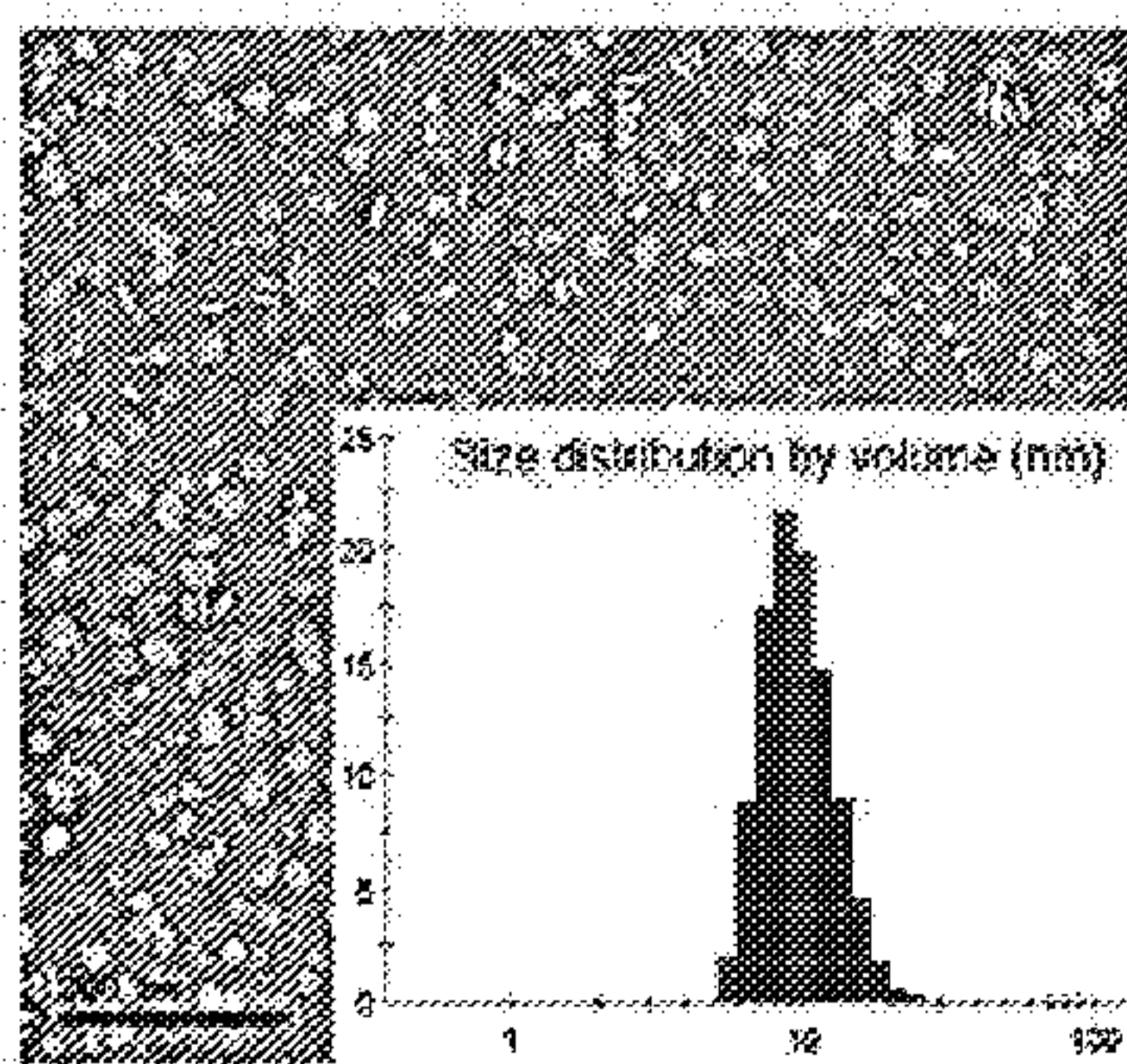
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(54) Title: COMPOSITIONS AND METHODS FOR DELIVERY OF BIOMACROMOLECULE AGENTS**FIG. 1A**

(57) Abstract: The present invention relates to nanoparticles associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) biomacromolecule agents configured for treating, preventing or ameliorating various types of disorders, and methods of synthesizing the same. In particular, the present invention is directed to compositions comprising nanoparticles (e.g., synthetic high density lipoprotein (sHDL)) associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) biomacromolecule agents (e.g., nucleic acid, peptides, glycolipids, etc.), methods for synthesizing such nanoparticles, as well as systems and methods utilizing such nanoparticles (e.g., in diagnostic and/or therapeutic settings).

COMPOSITIONS AND METHODS FOR DELIVERY OF BIOMACROMOLECULE AGENTS

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR 5 DEVELOPMENT

This invention was made with government support under AI097291 awarded by the National Institutes of Health. The Government has certain rights in the invention.

FIELD OF THE INVENTION

10 The present invention relates to nanoparticles associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) biomacromolecule agents configured for treating, preventing or ameliorating various types of disorders, and methods of synthesizing the same. In particular, the present invention is directed to compositions comprising nanoparticles (e.g., synthetic high density lipoprotein (sHDL)) associated with (e.g., complexed,
15 conjugated, encapsulated, absorbed, adsorbed, admixed) biomacromolecule agents (e.g., nucleic acid, peptides, glycolipids, etc.), methods for synthesizing such nanoparticles, as well as systems and methods utilizing such nanoparticles (e.g., in diagnostic and/or therapeutic settings).

BACKGROUND OF THE INVENTION

20 Peptide and nucleic acid based drugs have tremendous potential as the next generation therapeutics. Despite their huge potential, their clinical translation has been challenging, partially due to lack of drug delivery platforms that can efficiently deliver the drugs to the site of action while protecting the cargo materials against enzymatic degradation in vivo. One prime example is in the area of cancer vaccines; numerous clinical trials have been performed using
25 defined tumor-associated antigen peptides, but they have failed to demonstrate clinical efficacy because soluble peptides do not sufficiently reach the site of action (e.g., lymphoid tissues) and fail to generate strong immune responses.

Improved compositions and techniques for stable and targeted delivery (e.g., in vitro or in vivo) of biomacromolecules (e.g., peptides, nucleic acids, glycolipids) are needed.

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SUMMARY

Despite the tremendous potential of peptide-based cancer vaccines, their efficacy has been limited in humans. Recent innovations in tumor exome sequencing have signaled the new era of “personalized” immunotherapy with patient-specific neo-antigens (see, e.g., Yadav, M. et

al. *Nature* 515, 572-576 (2014); Kreiter, S. et al. *Nature* 520, 692-696 (2015); Schumacher, T.N. & Schreiber, R.D. *Science* 348, 69-74 (2015)), but a general methodology for stimulating strong CD8 α ⁺ cytotoxic T lymphocyte (CTL) responses remains lacking.

Experiments conducted during the course of developing embodiments for the present invention demonstrated that preformed high density lipoprotein-mimicking nanodiscs can be readily coupled with antigen (Ag) peptides and adjuvants, producing stable, ultrasmall nanoparticles that markedly improve Ag/adjuvant co-delivery to lymphoid organs and achieved sustained Ag presentation on dendritic cells. Strikingly, it was shown that these nanodiscs elicited up to 41-fold greater frequency of CTLs than soluble vaccines and even 9-fold greater than perhaps the strongest adjuvant in clinical trials (i.e. CpG in Montanide) (see, e.g., Speiser, D.E. et al. *J. Clin. Invest.* 115, 739-746 (2005); Fourcade, J. et al. *J. Immunother.* 31, 781-791 (2008)). Moreover, it was shown that the nanodisc platform can be easily adapted to neoantigens, generating potent anti-tumor immunity. Such results represent a new powerful approach for cancer immunotherapy and more broadly, suggest a general strategy for personalized nanomedicine.

Such results have significant clinical importance, as these nanodiscs, with an established manufacturing procedure and excellent safety profiles in humans, can drastically improve co-delivery of antigens and adjuvants to LNs, sustain antigen presentation on DCs, and drive T-cell responses with potent anti-tumor efficacy. As the majority of tumor mutations are unique to each patient, cancer vaccines would require a personalized approach (see, e.g., Yadav, M. et al. *Nature* 515, 572-576 (2014); Kreiter, S. et al. *Nature* 520, 692-696 (2015); Schumacher, T.N. & Schreiber, R.D. *Science* 348, 69-74 (2015)). Coupled with the recent technical innovations in neo-antigen screening, this approach provides powerful yet facile strategies for producing cancer vaccines designed for each patient. Furthermore, this platform technology is generally applicable for personalized therapeutics with a wide range of bioactive molecules and imaging agents.

Accordingly, in certain embodiments, the present invention provides methods for making a personalized neoplasia vaccine for a subject diagnosed as having a neoplasia. The present invention is not limited to particular methods for making a personalized neoplasia vaccine for a subject diagnosed as having a neoplasia. In some embodiments, such methods comprise obtaining a biological sample of the neoplasia from the subject; identifying a plurality of mutations in the neoplasia; analyzing the plurality of mutations to identify one or more neo-antigenic mutations predicted to encode neo-antigenic peptides, the neo-antigenic mutations selected from the group consisting of missense mutations, neoORF mutations, and any combination thereof; and producing a personalized neoplasia vaccine, wherein the personalized

neoplasia vaccine comprises a microparticle or nanoparticle associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) one or more neo-antigenic peptides specific for the analyzed and identified neo-antigenic mutations predicted to encode neo-antigenic peptides. In some embodiments, the nanoparticle is further associated with (e.g.,
 5 complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) with an adjuvant. In some embodiments, the identifying further comprises sequencing the genome, transcriptome, or proteome of the neoplasia.

In some embodiments, the size of the microparticle is between 0.5 microns to 100 microns.

10 In some embodiments, the one or more neo-antigenic peptides range from about 5 to about 50 amino acids in length. In some embodiments, the one or more neo-antigenic mutations peptides range from about 15 to about 35 amino acids in length. In some embodiments, the one or more neo-antigenic peptides range from about 18 to about 30 amino acids in length. In some
 15 embodiments, the one or more neo-antigenic peptides range from about 6 to about 15 amino acids in length.

In some embodiments, the adjuvant is selected from the group consisting of CPG, polyIC, poly-ICLC, 1018 ISS, aluminum salts (for example, aluminum hydroxide, aluminum phosphate), Amplivax, BCG, CP-870,893, CpG7909, CyaA, dSLIM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS,
 20 ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, OK-432, OM-174, OM-197-MP-EC, ONTAK, PepTel.RTM, vector system, PLGA microparticles, imiquimod, resiquimod, gardiquimod, 3M-052, SRL172, Virosomes and other Virus-like particles, YF-17D, VEGF trap, beta-glucan, Pam3Cys, Aquila's QS21 stimulon, vadimezan, AsA404 (DMXAA),
 25 3M MEDI9197, glucopyranosyl lipid adjuvant (GLA), GLA-SE, CD1d ligands (such as C20:2, OCH, AH04-2, α -galatosylceramide, α -C-galatosylceramide, α -mannosylceramide, α -fructosylceramide, β -galatosylceramide, β -mannosylceramide), STING agonists (e.g. cyclic dinucleotides, including Cyclic [G(3',5')pA(3',5')p], Cyclic [G(2',5')pA(3',5')p], Cyclic [G(2',5')pA(2',5')p], Cyclic diadenylate monophosphate, Cyclic diguanylate monophosphate),
 30 CL401, CL413, CL429, Flagellin, RC529, E6020, imidazoquinoline-based small molecule TLR-7/8a (including its lipidated analogues), virosomes, AS01, AS02, AS03, AS04, AS15, IC31, CAF01, ISCOM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), bacterial toxins (such as CT, and LT). In some embodiments, the adjuvant is any derivative of an adjuvant (e.g., cholesterol-modified CpG) or any combinations thereof.

The methods are not limited to a particular nanoparticle. In some embodiments, the average size of the nanoparticle is between 6 to 500 nm. In some embodiments, the nanoparticle is a sHDL nanoparticle. In some embodiments, the sHDL nanoparticle comprises a mixture of at least one phospholipid and at least one HDL apolipoprotein or apolipoprotein mimetic. In some
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In some embodiments, the phospholipid is selected from the group consisting of dipalmitoylphosphatidylcholine (DPPC), dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide],
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In some embodiments, the HDL apolipoprotein component is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A-II xxx (apo A-II-xxx), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), apolipoprotein E (apo E), apolipoprotein A-I milano (apo A-I-milano), apolipoprotein A-I paris (apo A-I-paris), apolipoprotein M (apo M), an HDL apolipoprotein mimetic, preproapolipoprotein, preproApoA-I, proApoA I, preproApoA-II, proApoA II, preproApoA-IV, proApoA-IV, ApoA-V, preproApoE, proApoE, preproApoA I_{Milano}, proApoA-I_{Milano}, preproApoA-I_{Paris}, proApoA-I_{Paris}, and mixtures thereof.
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In some embodiments, the ApoA-I mimetic is described by any of SEQ ID NOs: 1-336 and WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341), LKLLDNWDSVTSTFSKLREOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLR OEMS (SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO: 345), PYLDDDFQKKWQEEMEL YRQKVE (SEQ ID NO: 346), PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDALRTHLA (SEQ ID NO: 348), PYSDEL RQRLAARLEALKENGG (SEQ ID NO: 349), ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352),
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TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDYGKDLME (SEQ ID
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 NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358),
 5 LPVLVWLSIVLEGPAPAOGTPDVSS (SEQ ID NO:359),
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 (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362),
 HLRKLRKRLLRDADDLQKRLAVYOA (SEQ ID NO:363),
 AQAWGERLRARMEEMGSRTRDR (SEQ ID NO:364), LDEVKEQVAEVRACLEEQAQ
 10 (SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236),
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 PVLDFRELLNELLEALKQKL (SEQ ID NO:367), PVLDFRELLNELLEALKQKLA (SEQ
 ID NO:368), PVLDFRELLNELLEALKQKLK (SEQ ID NO:4),
 PVLDFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDFRELLNELLEALKKLLK
 15 (SEQ ID NO:370), PVLDFRELLNELLEALKKLLA (SEQ ID NO:371),
 PLLDFRELLNELLEALKKLLA (SEQ ID NO:372), and
 EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

In certain embodiments, the present invention provides methods for treating a subject
 diagnosed as having a neoplasia with a personalized neoplasia vaccine. The present invention is
 20 not limited to particular methods for treating a subject diagnosed as having a neoplasia with a
 personalized neoplasia vaccine. In some embodiments, such methods comprise obtaining a
 biological sample of the neoplasia from the subject; identifying one or more mutations in the
 neoplasia; analyzing the plurality of mutations to identify one or more neo-antigenic mutations
 predicted to encode expressed neo-antigenic peptides, the neo-antigenic mutations selected from
 25 the group consisting of missense mutations, neoORF mutations, and any combination thereof;
 producing a personalized neoplasia vaccine, wherein the personalized neoplasia vaccine
 comprises a microparticle or nanoparticle associated with (e.g., complexed, conjugated,
 encapsulated, absorbed, adsorbed, admixed) one or more neo-antigenic peptides specific for the
 analyzed and identified neo-antigenic mutations predicted to encode neo-antigenic peptides; and
 30 administering the personalized neoplasia vaccine to the subject, thereby treating the neoplasia.
 In some embodiments, the personalized neoplasia vaccine is coadministered with an adjuvant. In
 some embodiments, the nanoparticle is further associated with (e.g., complexed, conjugated,
 encapsulated, absorbed, adsorbed, admixed) an adjuvant. In some embodiments, the identifying
 further comprises sequencing the genome, transcriptome, or proteome of the neoplasia.

In some embodiments, the one or more neo-antigenic peptides range from about 5 to about 50 amino acids in length. In some embodiments, the one or more neo-antigenic mutations peptides range from about 15 to about 35 amino acids in length. In some embodiments, the one or more neo-antigenic peptides range from about 18 to about 30 amino acids in length. In some
5
embodiments, the one or more neo-antigenic peptides range from about 6 to about 15 amino acids in length.

In some embodiments, the adjuvant is selected from the group consisting of CPG, polyIC, poly-ICLC, 1018 ISS, aluminum salts (for example, aluminum hydroxide, aluminum phosphate), Amplivax, BCG, CP-870,893, CpG7909, CyaA, dSLIM, Cytokines (such as GM-
10 CSF, IL-2, IFN- α , Flt-3L), IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, OK-432, OM-174, OM-197-MP-EC, ONTAK, PepTel.RTM, vector system, PLGA microparticles, imiquimod, resiquimod, gardiquimod, 3M-052, SRL172, Virosomes and other Virus-like particles, YF-17D, VEGF trap,
15 beta-glucan, Pam3Cys, Aquila's QS21 stimulon, vadimezan, AsA404 (DMXAA), 3M MEDI9197, glucopyranosyl lipid adjuvant (GLA), GLA-SE, CD1d ligands (such as C20:2, OCH, AH04-2, α -galatosylceramide, α -C-galatosylceramide, α -mannosylceramide, α -fructosylceramide, β -galatosylceramide, β -mannosylceramide), STING agonists (e.g. cyclic dinucleotides, including Cyclic [G(3',5')pA(3',5')p], Cyclic [G(2',5')pA(3',5')p], Cyclic
20 [G(2',5')pA(2',5')p], Cyclic diadenylate monophosphate, Cyclic diguanylate monophosphate), CL401, CL413, CL429, Flagellin, RC529, E6020, imidazoquinoline-based small molecule TLR-7/8a (including its lipidated analogues), virosomes, AS01, AS02, AS03, AS04, AS15, IC31, CAF01, ISCOM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), bacterial toxins (such as CT, and LT). In some embodiments, the adjuvant is any derivative of an adjuvant (e.g.,
25 cholesterol-modified CpG) or any combinations thereof.

The methods are not limited to a particular nanoparticle. In some embodiments, the average size of the nanoparticle is between 6 to 500 nm. In some embodiments, the nanoparticle is a sHDL nanoparticle. In some embodiments, the sHDL nanoparticle comprises a mixture of at least one phospholipid and at least one HDL apolipoprotein or apolipoprotein mimetic. In some
30
embodiments, the average particle size of the sHDL nanoparticle is between 6-70 nm.

In some embodiments, the phospholipid is selected from the group consisting of dipalmitoylphosphatidylcholine (DPPC), dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-

maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide],
 5 phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and combinations thereof.

In some embodiments, the HDL apolipoprotein component is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A-II xxx (apo A-II-xxx), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), apolipoprotein E
 10 (apo E), apolipoprotein A-I milano (apo A-I-milano), apolipoprotein A-I paris (apo A-I-paris), apolipoprotein M (apo M), an HDL apolipoprotein mimetic, preproapolipoprotein, preproApoA-I, proApoA I, preproApoA-II, proApoA II, preproApoA-IV, proApoA-IV, ApoA-V, preproApoE, proApoE, preproApoA I_{Milano}, proApoA-I_{Milano}, preproApoA-I_{Paris}, proApoA-I_{Paris}, and mixtures thereof.

In some embodiments, the ApoA-I mimetic is described by any of SEQ ID NOs: 1-336 and WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341),
 LKLLDNWDSVTSTFSKLREOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLROEMS (SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO: 345), PYLDDFQKKWQEEMELYRQKVE (SEQ ID NO: 346),
 20 PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDALRTHLA (SEQ ID NO: 348), PYSDELQRRLAARLEALKENGG (SEQ ID NO: 349),
 ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352),
 25 TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDYGKDLME (SEQ ID NO:355), KVKSPELOAEAKSYFEKSKE (SEQ ID NO:356),
 VLTALVAVAGARAEVSADOVATV (SEQ ID NO:357), NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358), LPVLVWLSIVLEGPAPAOGTPDVSS (SEQ ID NO:359),
 30 LPVLVVVLSIVLEGPAPAQGTPDVSS (SEQ ID NO:360), ALDKLKEFGNTLEDKARELIS (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362),
 HLRKLRKRLLRDADDLQKRLAVYOA (SEQ ID NO:363), AQAWGERLRARMEEMGSRTRDR (SEQ ID NO:364), LDEVKEQVAEVRACLEEQAQ (SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236),

DWLKAFYDKVAEKLKEAFPDWAKAAYDKAAEKAKEAA (SEQ ID NO:366),
 PVLDLFRELLNELLEALKQKL (SEQ ID NO:367), PVLDLFRELLNELLEALKQKLA (SEQ
 ID NO:368), PVLDLFRELLNELLEALKQKLK (SEQ ID NO:4),
 PVLDLFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDLFRELLNELLEALKKLLK
 5 (SEQ ID NO:370), PVLDLFRELLNELLEALKKLLA (SEQ ID NO:371),
 PLLDLFRELLNELLEALKKLLA (SEQ ID NO:372), and
 EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

In some embodiments, the personalized neoplasia vaccine is coadministered with an an
 anti- immunosuppressive or immuno stimulatory agent. In some embodiments, the anti-
 10 immunosuppressive or immuno stimulatory agent is selected from the group consisting of anti-
 CTLA-4 antibody, anti-PD-1, anti-PD-L1, anti-TIM-3, anti-BTLA, anti-VISTA, anti-LAG3,
 anti-CD25, anti-CD27, anti-CD28, anti-CD137, anti-OX40, anti-GITR, anti-ICOS, anti-TIGIT,
 and inhibitors of IDO.

In certain embodiments, the present invention provides a composition comprising a
 15 microparticle or nanoparticle associated with (e.g., complexed, conjugated, encapsulated,
 absorbed, adsorbed, admixed) one or more neo-antigenic peptides, wherein each of the one or
 more neo-antigenic peptides is specific for a neo-antigenic mutation identified from a neoplasia
 biological sample obtained from a subject. In some embodiments, the subject is a human being.

In some embodiments, the size of the microparticle is between 0.5 microns to 100
 20 microns. In some embodiments, the average size of the nanoparticle is between 6 to 500 nm.

In some embodiments, the one or more neo-antigenic peptides range from about 5 to
 about 50 amino acids in length. In some embodiments, the one or more neo-antigenic peptides
 range from about 15 to about 35 amino acids in length. In some embodiments, the one or more
 neo-antigenic peptides range from about 18 to about 30 amino acids in length. In some
 25 embodiments, the one or more neo-antigenic peptides range from about 6 to about 15 amino
 acids in length.

In some embodiments, the nanoparticle is further associated with (e.g., complexed,
 conjugated, encapsulated, absorbed, adsorbed, admixed) with an adjuvant. In some
 embodiments, the adjuvant is selected from the group consisting of CPG, polyIC, poly-ICLC,
 30 1018 ISS, aluminum salts (for example, aluminum hydroxide, aluminum phosphate), Amplivax,
 BCG, CP-870,893, CpG7909, CyaA, dSLIM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L),
 IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac,
 MF59, monophosphoryl lipid A, Montanide IMS 1312, Montanide ISA 206, Montanide ISA
 50V, Montanide ISA-51, OK-432, OM-174, OM-197-MP-EC, ONTAK, PepTel.RTM, vector

system, PLGA microparticles, imiquimod, resiquimod, gardiquimod, 3M-052, SRL172, Virosomes and other Virus-like particles, YF-17D, VEGF trap, beta-glucan, Pam3Cys, Aquila's QS21 stimulon, vadimezan, AsA404 (DMXAA), 3M MEDI9197, glucopyranosyl lipid adjuvant (GLA), GLA-SE, CD1d ligands (such as C20:2, OCH, AH04-2, α -galatosylceramide, α -C-galatosylceramide, α -mannosylceramide, α -fructosylceramide, β -galatosylceramide, β -mannosylceramide), STING agonists (e.g. cyclic dinucleotides, including Cyclic [G(3',5')pA(3',5')p], Cyclic [G(2',5')pA(3',5')p], Cyclic [G(2',5')pA(2',5')p], Cyclic diadenylate monophosphate, Cyclic diguanylate monophosphate), CL401, CL413, CL429, Flagellin, RC529, E6020, imidazoquinoline-based small molecule TLR-7/8a (including its lipidated analogues), virosomes, AS01, AS02, AS03, AS04, AS15, IC31, CAF01, ISCOM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), and bacterial toxins (such as CT, and LT). In some embodiments, the adjuvant is any derivative of an adjuvant (e.g., cholesterol-modified CpG) or any combinations thereof.

In some embodiments, the nanoparticle is a sHDL nanoparticle. In some embodiments, the sHDL nanoparticle comprises a mixture of at least one phospholipid and at least one HDL apolipoprotein or apolipoprotein mimetic. In some embodiments, the HDL apolipoprotein is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), and apolipoprotein E (apo E). In some embodiments, the phospholipid is selected from the group consisting of dipalmitoylphosphatidylcholine (DPPC), dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and combinations thereof. In some embodiments, the HDL apolipoprotein mimetic is an ApoA-I mimetic.

In some embodiments, the ApoA-I mimetic is described by any of SEQ ID NOs: 1-336 and WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341), LKLLDNWDSVTSTFSLREOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLR OEMS (SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO: 345), PYLDDDFQKKWQEEMELYRQKVE (SEQ ID NO: 346),

PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDALRTHLA
 (SEQ ID NO: 348), PYSDELRQRLAARLEALKENGG (SEQ ID NO: 349),
 ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351),
 PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ
 5 ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352),
 TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDYGKDLME (SEQ ID
 NO:355), KVKSPELOAEAKSYFEKSKE (SEQ ID NO:356),
 VLTALVAVAGARAEVSADOVATV (SEQ ID NO:357),
 NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358),
 10 LPVLVWLSIVLEGPAPAOGTPDVSS (SEQ ID NO:359),
 LPVLVVVLSIVLEGPAPAQGTPDVSS (SEQ ID NO:360), ALDKLKEFGNTLEDKARELIS
 (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362),
 HLRKLRKRLLRDADDLQKRLAVYOA (SEQ ID NO:363),
 AQAWGERLRARMEEMGSRTRDR (SEQ ID NO:364), LDEVKEQVAEVRACLEEQAQ
 15 (SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236),
 DWLKAFYDKVAEKLKEAFPDWAKAAYDKAAEKAKEAA (SEQ ID NO:366),
 PVLDFRELLNELLEALKQKL (SEQ ID NO:367), PVLDFRELLNELLEALKQKLA (SEQ
 ID NO:368), PVLDFRELLNELLEALKQKLK (SEQ ID NO:4),
 PVLDFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDFRELLNELLEALKKLLK
 20 (SEQ ID NO:370), PVLDFRELLNELLEALKKLLA (SEQ ID NO:371),
 PLLDFRELLNELLEALKKLLA (SEQ ID NO:372), and
 EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

In some embodiments, the average particle size of the sHDL nanoparticle is between 6-70 nm.

25 Moreover, the present invention relates to nanoparticles associated with (e.g.,
 complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) biomacromolecule agents
 configured for treating, preventing or ameliorating various types of disorders, and methods of
 synthesizing the same. In particular, the present invention is directed to compositions
 comprising synthetic high density lipoprotein (sHDL) nanoparticles carrying biomacromolecule
 30 agents (e.g., nucleic acid, peptides, glycolipids, etc.), methods for synthesizing such sHDL
 nanoparticles, as well as systems and methods utilizing such sHDL nanoparticles (e.g., in
 diagnostic and/or therapeutic settings).

As such, in certain embodiments, the present invention provides methods for inhibiting a target gene in a cell, comprising introducing into the cell a composition comprising siRNA

encapsulated within a sHDL nanoparticle, wherein the siRNA is capable of inhibiting the target gene by RNA interference, wherein the siRNA comprises two RNA strands that are complementary to each other. In some embodiments, the siRNA is modified with cholesterol at the 3' sense strand. In some embodiments, the cell is in vivo, in vitro, or ex vivo. In some
5 embodiments, the cell is within a human being. In some embodiments, an imaging agent is encapsulated within the sHDL nanoparticle.

In certain embodiments, the present invention provides methods for reducing serum LDL-C levels in patient, comprising administering to the patient a therapeutically effective amount of a pharmaceutical composition comprising a PCSK9 siRNA encapsulated within a
10 nanoparticle, wherein the PCSK9 siRNA is capable of inhibiting the PCSK9 gene by RNA interference, wherein the PCSK9 siRNA comprises two RNA strands that are complementary to each other, wherein inhibiting of the PCSK9 gene results in reduction of serum LDL-C levels in the patient. In some embodiments, the patient is a human patient. In some embodiments, the PCSK9 siRNA is modified with cholesterol at the 3' sense strand. In some embodiments, an
15 imaging agent is encapsulated within the nanoparticle. In some embodiments, the nanoparticle is selected from the group consisting of sHDL nanoparticle, fullerenes, endohedral metallofullerenes buckyballs, trimetallic nitride templated endohedral metallofullerenes, single-walled and mutli-walled carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods, single-walled and multi-walled boron/nitrate nanotubes, carbon
20 nanotube peapods, carbon nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures, microstructures, or their derivatives formed using layer-by-layer processes, self-assembly processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles, nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable PLGA micro- and nano-spheres, gold nanoparticles, silver
25 nanoparticles, carbon nanoparticles, iron nanoparticles, a modified micelle. In some embodiments, the nanoparticle is a sHDL nanoparticle.

In certain embodiments, the present invention provides methods for treating coronary heart disease in a patient through reducing serum LDL-C levels in the patient, comprising administering to the patient a therapeutically effective amount of a pharmaceutical composition
30 comprising a PCSK9 siRNA encapsulated within a nanoparticle, wherein the PCSK9 siRNA is capable of inhibiting the PCSK9 gene by RNA interference, wherein the PCSK9 siRNA comprises two RNA strands that are complementary to each other, wherein inhibiting of the PCSK9 gene results in reduction of serum LDL-C levels. In some embodiments, the patient is a human patient. In some embodiments, the PCSK9 siRNA is modified with cholesterol at the 3'

sense strand. In some embodiments, an imaging agent is encapsulated within the nanoparticle. In some embodiments, the nanoparticle is selected from the group consisting of sHDL nanoparticle, fullerenes, endohedral metallofullerenes buckyballs, trimetallic nitride templated endohedral metallofullerenes, single-walled and multi-walled carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods, single-walled and multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures, microstructures, or their derivatives formed using layer-by-layer processes, self-assembly processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles, nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable PLGA micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles, iron nanoparticles, a modified micelle. In some embodiments, the nanoparticle is a sHDL nanoparticle. In some embodiments, the sHDL nanoparticle comprises a mixture of at least one phospholipid and at least one HDL apolipoprotein or apolipoprotein mimetic.

15 In certain embodiments, the present invention provides methods for inducing a natural killer T cell-mediated immune response in a cell comprising exposing the cell to a composition comprising an α GalCer glycolipid associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) within a nanoparticle, wherein such exposure results in the induction of a natural killer T cell-mediated immune response. In some embodiments, the cell is an in vivo cell, an ex vivo cell, or an in vitro cell. In some embodiments, the nanoparticle is selected from the group consisting of sHDL nanoparticle, fullerenes, endohedral metallofullerenes buckyballs, trimetallic nitride templated endohedral metallofullerenes, single-walled and multi-walled carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods, single-walled and multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures, microstructures, or their derivatives formed using layer-by-layer processes, self-assembly processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles, nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable PLGA micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles, iron nanoparticles, a modified micelle. In some 25 30 embodiments, the nanoparticle is a sHDL nanoparticle.

In certain embodiments, the present invention provides methods for inducing an immune response to an antigen comprising administering to a subject in need an effective amount of a composition comprising an nanoparticle, wherein the antigen is associated with (e.g.,

complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the nanoparticle, wherein an adjuvant is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) with the nanoparticle.

In some embodiments, the antigen is against PCSK9.

5 In some embodiments, the antigen is against gp100 melanoma.

In some embodiments, the antigen is selected from the group consisting of a peptide based antigen, a protein based antigen, a polysaccharide based antigen, a saccharide based antigen, a lipid based antigen, a glycolipid based antigen, a nucleic acid based antigen, an inactivated organism based antigen, an attenuated organism based antigen, a viral antigen, a
10 bacterial antigen, a parasite antigen, an antigen derived from an allergen, and a tumor antigen.

In some embodiments, the antigen is a tumor antigen selected from the group consisting of alpha-actinin-4, Bcr-Abl fusion protein, Casp-8, beta-catenin, cdc27, cdk4, cdkn2a, coa-1, dek-can fusion protein, EF2, ETV6-AML1 fusion protein, LDLR-fucosyltransferaseAS fusion protein, HLA-A2, HLA-A11, hsp70-2, KIAA0205, Mart2, Mum-1, 2, and 3, neo-PAP, myosin
15 class I, OS-9, pml-RAR α fusion protein, PTPRK, K-ras, N-ras, Triosephosphate isomeras, Bage-1, Gage 3,4,5,6,7, GnTV, Herv-K-mel, Lage-1, Mage-A1,2,3,4,6,10,12, Mage-C2, NA-88, NY-Eso-1/Lage-2, SP17, SSX-2, and TRP2-Int2, MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15(58), CEA, RAGE, NY-ESO (LAGS), SCP-1, Hom/Mel-40, PRAME, p53, H-Ras, HER-2/neu, BCR-ABL, E2A-PRL,
20 H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, β -Catenin, CDK4, Mum-1, p16, TAGE, PSMA, PSCA, CT7, telomerase, 43-9F, 5T4, 791Tgp72, α -fetoprotein, 13HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242,
25 CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), human EGFR protein or its fragments, such as human EGFR residues 306--325 (SCVRACGADSYEMEEDGVRK (SEQ ID NO:374)) and residues 897--915 (VWSYGVTVWELMTFGSKPY (SEQ ID NO:375)), HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB\70K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein\cyclophilin C-associated protein), TAAL6, TAG72, TLP, TPS, WT1
30 (and WT1-derivaed peptide sequences: WT1 126--134 (RMFP NAPYL (SEQ ID NO:376)), WT1 122--140 (SGQARMFPNAPYLPSCLLES (SEQ ID NO:377)), and WT1 122--144 (SGQARMFPNAPYLPSCLLESQPTI (SEQ ID NO:378))), MUC1 (and MUC1-derived peptides and glycopeptides such as RPAPGS (SEQ ID NO:379), PPAHGVT (SEQ ID NO:380), and PDTRP (SEQ ID NO:381)), LMP2, EGFRvIII, Idiotype, GD2, Ras mutant, p53 mutant,

Proteinase3 (PR1), Survivin, hTERT, Sarcoma translocation breakpoints, EphA2, EphA4, LMW-PTP, PAP, ML-IAP, AFP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, ALK, Androgen receptor, Cyclin B1, Polysialic acid, MYCN, RhoC, TRP-2, GD3, Fucosyl GM1, Mesothelin, sLe(animal), CYP1B1, PLAC1, GM3, BORIS, Tn, GloboH, NY-BR-1, RGS5, SART3, STn, Carbonic anhydrase IX, PAX5, OY-TES1, Sperm protein 17, LCK, HMWMAA, AKAP-4, XAGE 1, B7H3, Legumain, Tie 2, Page4, VEGFR2, MAD-CT-1, FAP, PDGFR-alpha, PDGFR- β , MAD-CT-2, Fos-related antigen 1, ERBB2, Folate receptor 1 (FOLR1 or FBP), IDH1, IDO, LY6K, fms-related tyrosine kinase 1 (FLT1, best known as VEGFR1), KDR, PADRE, TA-CIN (recombinant HPV16 L2E7E6), SOX2, aldehyde dehydrogenase, and any derivative thereof.

In some embodiments, the antigen is any type of viral, bacterial or self-antigen including, but not limited to, FimH against urinary tract infection; soluble F protein from respiratory syncytial virus (RSV); NEF, GAG, and ENV protein from HIV; Streptococcus pneumoniae proteins; HMGB1 protein; hemagglutinin and neuroamidase protein against influenza; Viral antigens derived from HPV type 16 and 18; gL2, ICP4, gD2 Δ TMR, gD2 Δ TMR, or ICP4.2 from HSV-2; antigens from *S. pneumoniae*, such as a pneumolysoid, Choline-binding protein A (CbpA), or Pneumococcal surface protein A (PspA), SP1912, SP1912, SP1912L, SP0148 with or without a signal sequence, SP2108 with or without a signal sequence; Antigens from *Chlamydia trachomatis*, such as a CT209 polypeptide antigen, a CT253 polypeptide antigen, a CT425 polypeptide antigen, a CT497 polypeptide antigen, and a CT843 polypeptide antigen; amyloid-beta peptide.

In some embodiments, the adjuvant is a dendritic cell targeting molecule. In some embodiments, the adjuvant is selected from the group consisting of CPG, polyIC, poly-ICLC, 1018 ISS, aluminum salts (for example, aluminum hydroxide, aluminum phosphate), Amplivax, BCG, CP-870,893, CpG7909, CyaA, dSLIM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, OK-432, OM-174, OM-197-MP-EC, ONTAK, PepTel.RTM, vector system, PLGA microparticles, imiquimod, resiquimod, gardiquimod, 3M-052, SRL172, Virosomes and other Virus-like particles, YF-17D, VEGF trap, beta-glucan, Pam3Cys, Aquila's QS21 stimulon, vadimezan, AsA404 (DMXAA), 3M MEDI9197, glucopyranosyl lipid adjuvant (GLA), GLA-SE, CD1d ligands (such as C20:2, OCH, AH04-2, α -galatosylceramide, α -C-galatosylceramide, α -mannosylceramide, α -fructosylceramide, β -galatosylceramide, β -mannosylceramide), STING agonists (e.g. cyclic dinucleotides, including Cyclic

[G(3',5')pA(3',5')p], Cyclic [G(2',5')pA(3',5')p], Cyclic [G(2',5')pA(2',5')p], Cyclic diadenylate monophosphate, Cyclic diguanylate monophosphate), CL401, CL413, CL429, Flagellin, RC529, E6020, imidazoquinoline-based small molecule TLR-7/8a (including its lipidated analogues), virosomes, AS01, AS02, AS03, AS04, AS15, IC31, CAF01, ISCOM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), and bacterial toxins (such as CT, and LT). In some embodiments, the adjuvant is any derivative of an adjuvant (e.g., cholesterol-modified CpG) or any combinations thereof.

In certain embodiments, the present invention provides methods for inducing an immune response to an antigen comprising administering to a subject in need an effective amount of a composition comprising a nanoparticle, wherein the antigen is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the nanoparticle. In some embodiments, the antigen is against PCSK9. In some embodiments, the nanoparticle is further associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) with an adjuvant. In some embodiments, the nanoparticle is co-administered with an adjuvant.

In some embodiments, the nanoparticle is selected from the group consisting of sHDL nanoparticle, fullerenes, endohedral metallofullerenes buckyballs, trimetallic nitride templated endohedral metallofullerenes, single-walled and multi-walled carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods, single-walled and multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures, microstructures, or their derivatives formed using layer-by-layer processes, self-assembly processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles, nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable PLGA micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles, iron nanoparticles, a modified micelle. In some embodiments, the nanoparticle is a sHDL nanoparticle.

In some embodiments, the adjuvant is a dendritic cell targeting molecule. In some embodiments, the adjuvant is selected from the group consisting of CPG, polyIC, poly-ICLC, 1018 ISS, aluminum salts (for example, aluminum hydroxide, aluminum phosphate), Amplivax, BCG, CP-870,893, CpG7909, CyaA, dSLIM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, OK-432, OM-174, OM-197-MP-EC, ONTAK, PepTel.RTM, vector system, PLGA microparticles, imiquimod, resiquimod, gardiquimod, 3M-052, SRL172, Virosomes and other Virus-like particles, YF-17D, VEGF trap, beta-glucan, Pam3Cys, Aquila's

QS21 stimulon, vadimezan, AsA404 (DMXAA), 3M MEDI9197, glucopyranosyl lipid adjuvant (GLA), GLA-SE, CD1d ligands (such as C20:2, OCH, AH04-2, α -galatosylceramide, α -C-galatosylceramide, α -mannosylceramide, α -fructosylceramide, β -galatosylceramide, β -mannosylceramide), STING agonists (e.g. cyclic dinucleotides, including Cyclic
 5 [G(3',5')pA(3',5')p], Cyclic [G(2',5')pA(3',5')p], Cyclic [G(2',5')pA(2',5')p], Cyclic diadenylate monophosphate, Cyclic diguanylate monophosphate), CL401, CL413, CL429, Flagellin, RC529, E6020, imidazoquinoline-based small molecule TLR-7/8a (including its lipidated analogues), virosomes, AS01, AS02, AS03, AS04, AS15, IC31, CAF01, ISCOM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), and bacterial toxins (such as CT, and LT). In
 10 some embodiments, the adjuvant is any derivative of an adjuvant (e.g., cholesterol-modified CpG) or any combinations thereof.

In some embodiments, the antigen is conjugated to the outer surface of the nanoparticle. In some embodiments, the adjuvant is conjugated to the outer surface of the nanoparticle. In some embodiments, the adjuvant is encapsulated within the nanoparticle.

15 In some embodiments, the composition is co-administered with a chemotherapeutic agent. In some embodiments, the chemotherapeutic agent is one or more of the following: aldesleukin, altretamine, amifostine, asparaginase, bleomycin, capecitabine, carboplatin, carmustine, cladribine, cisapride, cisplatin, cyclophosphamide, cytarabine, dacarbazine (DTIC), dactinomycin, docetaxel, doxorubicin, dronabinol, epoetin alpha, etoposide, filgrastim,
 20 fludarabine, fluorouracil, gemcitabine, granisetron, hydroxyurea, idarubicin, ifosfamide, interferon alpha, irinotecan, lansoprazole, levamisole, leucovorin, megestrol, mesna, methotrexate, metoclopramide, mitomycin, mitotane, mitoxantrone, omeprazole, ondansetron, paclitaxel (TAXOL), pilocarpine, prochloroperazine, rituximab, tamoxifen, taxol, topotecan hydrochloride, trastuzumab, vinblastine, vincristine and vinorelbine tartrate.

25 In certain embodiments, the present invention provides compositions comprising a nanoparticle, wherein an antigen is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the nanoparticle. In some embodiments, the nanoparticle is further associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) with an adjuvant. In some embodiments, the antigen is associated with (e.g., complexed, conjugated,
 30 encapsulated, absorbed, adsorbed, admixed) the adjuvant. In some embodiments, the antigen is not associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the adjuvant. In some embodiments, the antigen is conjugated with a hydrophobic molecule. In some embodiments, the adjuvant is conjugated with a hydrophobic molecule. In some embodiments, the average size of the nanoparticle is between 6 to 500 nm.

In some embodiments, the hydrophobic molecule is a lipid molecule. In some embodiments, the lipid molecule is a membrane-forming lipid molecule. In some embodiments, the lipid molecule molecule is a non-membrane-forming lipid molecule.

Examples of lipid molecules applicable with the embodiments of the present invention include, but are not limited to, phospholipids such as lecithin, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, egg sphingomyelin (ESM), cephalin, cardiolipin, phosphatidic acid, cerebrosides, dicetylphosphate, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoylphosphatidylethanolamine (DOPE), palmitoyloleoyl-phosphatidylcholine (POPC), palmitoyloleoyl-phosphatidylethanolamine (POPE), palmitoyloleoyl-phosphatidylglycerol (POPG), dioleoylphosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl-phosphatidylethanolamine (DPPE), dimyristoyl-phosphatidylethanolamine (DMPE), distearoyl-phosphatidylethanolamine (DSPE), monomethyl-phosphatidylethanolamine, dimethyl-phosphatidylethanolamine, dielaidoyl-phosphatidylethanolamine (DEPE), stearylloleoyl-phosphatidylethanolamine (SOPE), lysophosphatidylcholine, dilinoleoylphosphatidylcholine, and mixtures thereof. Other diacylphosphatidylcholine and diacylphosphatidylethanolamine phospholipids can also be used. The acyl groups in these lipids are preferably acyl groups derived from fatty acids having C₁₀-C₂₄carbon chains, e.g., lauroyl, myristoyl, palmitoyl, stearyl, or oleoyl.

Other non-limiting examples of lipid molecules include sterols such as cholesterol and derivatives thereof such as cholestanol, cholestanone, cholestenone, coprostanol, cholesteryl-2'-hydroxyethyl ether, cholesteryl-4'-hydroxybutyl ether, and mixtures thereof.

Other examples of lipid molecules suitable for use in the present invention include nonphosphorous containing lipids such as, e.g., stearylamine, dodecylamine, hexadecylamine, acetyl palmitate, glycerolricinoleate, hexadecyl stearate, isopropyl myristate, amphoteric acrylic polymers, triethanolamine-lauryl sulfate, alkyl-aryl sulfate polyethoxylated fatty acid amides, dioctadecyldimethyl ammonium bromide, ceramide, sphingomyelin, and the like.

Other examples of lipid molecules suitable for use in the present invention include fatty acids and derivatives or analogs thereof. They include oleic acid, lauric acid, capric acid (n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein (1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, C₁.

10 alkyl esters thereof (e.g., methyl, isopropyl and t-butyl), and mono- and di-glycerides thereof (i.e., oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, etc.) (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, p. 92; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1990, 7, 1-33; El Hariri et al., *J. Pharm. Pharmacol.*, 1992, 5 44, 651-654).

Other examples of lipid molecules suitable for use in the present invention include a lipid molecule modified with PEG (PEG-lipid). Examples of PEG-lipids include, but are not limited to, PEG coupled to dialkyloxypropyls (PEG-DAA) as described in, e.g., PCT Publication No. WO 05/026372, PEG coupled to diacylglycerol (PEG-DAG) as described in, e.g., U.S. Patent 10 Publication Nos. 20030077829 and 2005008689, PEG coupled to phospholipids such as phosphatidylethanolamine (PEG-PE), PEG conjugated to ceramides as described in, e.g., U.S. Pat. No. 5,885,613, PEG conjugated to cholesterol or a derivative thereof, and mixtures thereof. The disclosures of these patent documents are herein incorporated by reference in their entirety for all purposes. Additional PEG-lipids include, without limitation, PEG-C-DOMG, 2 KPEG- 15 DMG, and a mixture thereof.

PEG is a linear, water-soluble polymer of ethylene PEG repeating units with two terminal hydroxyl groups. PEGs are classified by their molecular weights; for example, PEG 2000 has an average molecular weight of about 2,000 daltons, and PEG 5000 has an average molecular weight of about 5,000 daltons. PEGs are commercially available from Sigma 20 Chemical Co. and other companies and include, for example, the following: monomethoxypolyethylene glycol (MePEG-OH), monomethoxypolyethylene glycol-succinate (MePEG-S), monomethoxypolyethylene glycol-succinimidyl succinate (MePEG-S-NHS), monomethoxypolyethylene glycol-amine (MePEG-NH₂), monomethoxypolyethylene glycol-tresylate (MePEG-TRES), and monomethoxypolyethylene glycol-imidazolyl-carbonyl (MePEG- 25 IM). Other PEGs such as those described in U.S. Pat. Nos. 6,774,180 and 7,053,150 (e.g., mPEG (20 KDa) amine) are also useful for preparing the PEG-lipid conjugates of the present invention. The disclosures of these patents are herein incorporated by reference in their entirety for all purposes. In addition, monomethoxypolyethyleneglycolacetic acid (MePEG-CH₂COOH) is particularly useful for preparing PEG-lipid conjugates including, e.g., PEG-DAA conjugates.

30 The PEG moiety of the PEG-lipid conjugates described herein may comprise an average molecular weight ranging from about 550 daltons to about 10,000 daltons. In certain instances, the PEG moiety has an average molecular weight of from about 750 daltons to about 5,000 daltons (e.g., from about 1,000 daltons to about 5,000 daltons, from about 1,500 daltons to about 3,000 daltons, from about 750 daltons to about 3,000 daltons, from about 750 daltons to about

2,000 daltons, etc.). In preferred embodiments, the PEG moiety has an average molecular weight of about 2,000 daltons or about 750 daltons.

In certain instances, the PEG can be optionally substituted by an alkyl, alkoxy, acyl, or aryl group. The PEG can be conjugated directly to the lipid or may be linked to the lipid via a linker moiety. Any linker moiety suitable for coupling the PEG to a lipid can be used including, e.g., non-ester containing linker moieties and ester-containing linker moieties. In a preferred embodiment, the linker moiety is a non-ester containing linker moiety. As used herein, the term “non-ester containing linker moiety” refers to a linker moiety that does not contain a carboxylic ester bond (—OC(O)—). Suitable non-ester containing linker moieties include, but are not limited to, amido (—C(O)NH—), amino (—NR—), carbonyl (—C(O)—), carbamate (—NHC(O)O—), urea (—NHC(O)NH—), disulphide (—S—S—), ether (—O—), succinyl ($\text{—(O)CCH}_2\text{CH}_2\text{C(O)—}$), succinamidyl ($\text{—NHC(O)CH}_2\text{CH}_2\text{C(O)NH—}$), ether, disulphide, as well as combinations thereof (such as a linker containing both a carbamate linker moiety and an amido linker moiety). In a preferred embodiment, a carbamate linker is used to couple the PEG to the lipid.

In other embodiments, an ester containing linker moiety is used to couple the PEG to the lipid. Suitable ester containing linker moieties include, e.g., carbonate (—OC(O)O—), succinoyl, phosphate esters (—O—(O)POH—O—), sulfonate esters, and combinations thereof.

Phosphatidylethanolamines having a variety of acyl chain groups of varying chain lengths and degrees of saturation can be conjugated to PEG to form the lipid conjugate. Such phosphatidylethanolamines are commercially available, or can be isolated or synthesized using conventional techniques known to those of skilled in the art. Phosphatidylethanolamines containing saturated or unsaturated fatty acids with carbon chain lengths in the range of C_{10} to C_{20} are preferred. Phosphatidylethanolamines with mono- or diunsaturated fatty acids and mixtures of saturated and unsaturated fatty acids can also be used. Suitable phosphatidylethanolamines include, but are not limited to, dimyristoyl-phosphatidylethanolamine (DMPE), dipalmitoyl-phosphatidylethanolamine (DPPE), dioleoylphosphatidylethanolamine (DOPE), and distearoyl-phosphatidylethanolamine (DSPE).

In some embodiments, the antigen is against PCSK9.

In some embodiments, the antigen is against gp100 melanoma.

In some embodiments, the antigen is selected from the group consisting of a peptide based antigen, a protein based antigen, a polysaccharide based antigen, a saccharide based antigen, a lipid based antigen, a glycolipid based antigen, a nucleic acid based antigen, an inactivated organism based antigen, an attenuated organism based antigen, a viral antigen, a

bacterial antigen, a parasite antigen, an antigen derived from an allergen, and a tumor antigen.

In some embodiments, the antigen is a tumor antigen selected from the group consisting of alpha-actinin-4, Bcr-Abl fusion protein, Casp-8, beta-catenin, cdc27, cdk4, cdkn2a, coa-1, dek-can fusion protein, EF2, ETV6-AML1 fusion protein, LDLR-fucosyltransferaseAS fusion protein, HLA-A2, HLA-A11, hsp70-2, KIAAO205, Mart2, Mum-1, 2, and 3, neo-PAP, myosin class I, OS-9, pml-RAR α fusion protein, PTPRK, K-ras, N-ras, Triosephosphate isomeras, Bage-1, Gage 3,4,5,6,7, GnTV, Herv-K-mel, Lage-1, Mage-A1,2,3,4,6,10,12, Mage-C2, NA-88, NY-Eso-1/Lage-2, SP17, SSX-2, and TRP2-Int2, MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15(58), CEA, RAGE, NY-ESO (LAGS), SCP-1, Hom/Mel-40, PRAME, p53, H-Ras, HER-2/neu, BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, β -Catenin, CDK4, Mum-1, p16, TAGE, PSMA, PSCA, CT7, telomerase, 43-9F, 5T4, 791Tgp72, α -fetoprotein, 13HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), human EGFR protein or its fragments, such as human EGFR residues 306–325 (SCVRACGADSYEMEEEDGVRK (SEQ ID NO:374)) and residues 897–915 (VWSYGVTVWELMTFGSKPY (SEQ ID NO:375)), HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB\70K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein\cyclophilin C-associated protein), TAAL6, TAG72, TLP, TPS, WT1 (and WT1-derivaed peptide sequences: WT1 126–134 (RMFP NAPYL (SEQ ID NO:376)), WT1 122–140 (SGQARMFPNAPYLPSICLES (SEQ ID NO:377)), and WT1 122–144 (SGQARMFPNAPYLPSICLESQPTI (SEQ ID NO:378))), MUC1 (and MUC1-derived peptides and glycopeptides such as RPAPGS (SEQ ID NO:379), PPAHGVV (SEQ ID NO:380), and PDTRP (SEQ ID NO:381))), LMP2, EGFRvIII, Idiotype, GD2, Ras mutant, p53 mutant, Proteinase3 (PR1), Survivin, hTERT, Sarcoma translocation breakpoints, EphA2, EphA4, LMW-PTP, PAP, ML-IAP, AFP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, ALK, Androgen receptor, Cyclin B1, Polysialic acid, MYCN, RhoC, TRP-2, GD3, Fucosyl GM1, Mesothelin, sLe(animal), CYP1B1, PLAC1, GM3, BORIS, Tn, GloboH, NY-BR-1, RGS5, SART3, STn, Carbonic anhydrase IX, PAX5, OY-TES1, Sperm protein 17, LCK, HMWMAA, AKAP-4, XAGE 1, B7H3, Legumain, Tie 2, Page4, VEGFR2, MAD-CT-1, FAP, PDGFR-alpha, PDGFR- β , MAD-CT-2, Fos-related antigen 1, ERBB2, Folate receptor 1 (FOLR1 or FBP), IDH1, IDO, LY6K, fms-related tyro- sine kinase 1 (FLT1, best known as VEGFR1), KDR, PADRE, TA-CIN (recombinant HPV16 L2E7E6), SOX2, and aldehyde dehydrogenase.

In some embodiments, the antigen is any type of viral, bacterial or self-antigen including, but not limited to, FimH against urinary tract infection; soluble F protein from respiratory syncytial virus (RSV); NEF, GAG, and ENV protein from HIV; Streptococcus pneumoniae proteins; HMGB1 protein; hemagglutinin and neuroamidase protein against influenza; Viral antigens derived from HPV type 16 and 18; gL2, ICP4, gD2ΔTMR, gD2ΔTMR, or ICP4.2 from HSV-2; antigens from *S. pneumoniae*, such as a pneumolysoid, Choline-binding protein A (CbpA), or Pneumococcal surface protein A (PspA), SP1912, SP1912, SP1912L, SP0148 with or without a signal sequence, SP2108 with or without a signal sequence; Antigens from *Chlamydia trachomatis*, such as a CT209 polypeptide antigen, a CT253 polypeptide antigen, a CT425 polypeptide antigen, a CT497 polypeptide antigen, and a CT843 polypeptide antigen; amyloid-beta peptide.

In some embodiments, the adjuvant is a dendritic cell targeting molecule. In some embodiments, the adjuvant is an immunostimulatory agent that activates dendritic cells. CPG, polyIC, poly-ICLC, 1018 ISS, aluminum salts (for example, aluminum hydroxide, aluminum phosphate), Amplivax, BCG, CP-870,893, CpG7909, CyaA, dSLIM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, OK-432, OM-174, OM-197-MP-EC, ONTAK, PepTel.RTM, vector system, PLGA microparticles, imiquimod, resiquimod, gardiquimod, 3M-052, SRL172, Virosomes and other Virus-like particles, YF-17D, VEGF trap, beta-glucan, Pam3Cys, Aquila's QS21 stimulon, vadimezan, AsA404 (DMXAA), 3M MEDI9197, glucopyranosyl lipid adjuvant (GLA), GLA-SE, CD1d ligands (such as C20:2, OCH, AH04-2, α -galatosylceramide, α -C-galatosylceramide, α -mannosylceramide, α -fructosylceramide, β -galatosylceramide, β -mannosylceramide), STING agonists (e.g. cyclic dinucleotides, including Cyclic [G(3',5')pA(3',5')p], Cyclic [G(2',5')pA(3',5')p], Cyclic [G(2',5')pA(2',5')p], Cyclic diadenylate monophosphate, Cyclic diguanylate monophosphate), CL401, CL413, CL429, Flagellin, RC529, E6020, imidazoquinoline-based small molecule TLR-7/8a (including its lipidated analogues), virosomes, AS01, AS02, AS03, AS04, AS15, IC31, CAF01, ISCOM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), and bacterial toxins (such as CT, and LT). In some embodiments, the adjuvant is any derivative of an adjuvant (e.g., cholesterol-modified CpG) or any combinations thereof.

In some embodiments, the antigen is conjugated to the outer surface of the nanoparticle. In some embodiments, the adjuvant is conjugated to the outer surface of the nanoparticle. In some embodiments, the adjuvant is encapsulated within the nanoparticle.

In some embodiments, the nanoparticle is selected from the group consisting of sHDL nanoparticle, fullerenes, endohedral metallofullerenes buckyballs, trimetallic nitride templated endohedral metallofullerenes, single-walled and mutli-walled carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods, single-walled and multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures, microstructures, or their derivatives formed using layer-by-layer processes, self-assembly processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles, nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable PLGA micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles, iron nanoparticles, a modified micelle. In some embodiments, the nanoparticle is a sHDL nanoparticle.

In certain embodiments, the present invention provides comprising siRNA encapsulated within a nanoparticle, wherein the siRNA is capable of inhibiting a target gene by RNA interference, wherein the siRNA comprises two RNA strands that are complementary to each other. In some embodiments, the siRNA is modified with cholesterol at the 3' sense strand. In some embodiments, an imaging agent is encapsulated within the nanoparticle.

In some embodiments, the nanoparticle is selected from the group consisting of sHDL nanoparticle, fullerenes, endohedral metallofullerenes buckyballs, trimetallic nitride templated endohedral metallofullerenes, single-walled and mutli-walled carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods, single-walled and multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures, microstructures, or their derivatives formed using layer-by-layer processes, self-assembly processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles, nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable PLGA micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles, iron nanoparticles, a modified micelle. In some embodiments, the nanoparticle is a sHDL nanoparticle.

In certain embodiments, the present invention provides comprising a PCSK9 siRNA encapsulated within a nanoparticle, wherein the PCSK9 siRNA is capable of inhibiting the PCSK9 gene by RNA interference, wherein the PCSK9 siRNA comprises two RNA strands that are complementary to each other. In some embodiments, the PCSK9 siRNA is modified with cholesterol at the 3' sense strand. In some embodiments, an imaging agent is encapsulated within the nanoparticle.

In some embodiments, the average size of the nanoparticle is between 6 to 500 nm.

In some embodiments, the nanoparticle is selected from the group consisting of sHDL nanoparticle, fullerenes, endohedral metallofullerenes buckyballs, trimetallic nitride templated endohedral metallofullerenes, single-walled and mutli-walled carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods, single-walled and multi-walled
 5 boron/nitrate nanotubes, carbon nanotube peapods, carbon nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures, microstructures, or their derivatives formed using layer-by-layer processes, self-assembly processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles, nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable PLGA micro- and
 10 nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles, iron nanoparticles, a modified micelle. In some embodiments, the nanoparticle is a sHDL nanoparticle.

In certain embodiments, the present invention provides comprising an α GalCer glycolipid associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) a nanoparticle.

15 Such methods and compositions are not limited to particular size, type or kind of nanoparticles. In some embodiments, the nanoparticle is selected from the group consisting of sHDL nanoparticle, fullerenes, endohedral metallofullerenes buckyballs, trimetallic nitride templated endohedral metallofullerenes, single-walled and mutli-walled carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods, single-walled and
 20 multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures, microstructures, or their derivatives formed using layer-by-layer processes, self-assembly processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles, nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable PLGA
 25 micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles, iron nanoparticles, a modified micelle.

In some embodiments, the nanoparticle is a sHDL nanoparticle. In some embodiments, the sHDL nanoparticle comprises a mixture of at least one phospholipid and at least one HDL apolipoprotein or apolipoprotein mimetic.

30 In some embodiments, the HDL apolipoprotein is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), and apolipoprotein E (apo E). In some embodiments, the HDL apolipoprotein is selected from preproapolipoprotein, preproApoA-I, proApoA-I, ApoA-I, preproApoA-II, proApoA-II, ApoA-II, preproApoA-IV, proApoA-IV, ApoA-IV, ApoA-V,

preproApoE, proApoE, ApoE, preproApoA-IMilano, proApoA-IMilano ApoA-IMilano preproApoA-IParis, proApoA-IParis, and ApoA-IParis and peptide mimetics of these proteins mixtures thereof.

In some embodiments, the phospholipid is selected from the group consisting of
 5 dipalmitoylphosphatidylcholine (DPPC), dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-
 10 maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and combinations thereof.

In some embodiments, the HDL apolipoprotein mimetic is an ApoA-I mimetic.

In some embodiments, the ApoA-I mimetic is described by any of SEQ ID NOs: 1-336
 15 and WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341), LKLLDNWDSVTSTFSKLREOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLRROEMS (SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO: 345), PYLDDFQKKWQEEMELYRQKVE (SEQ ID NO: 346),
 20 PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDALRTHLA (SEQ ID NO: 348), PYSDELQRRLAARLEALKENGG (SEQ ID NO: 349), ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352),
 25 TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDYGKDLME (SEQ ID NO:355), KVKSPLOAEAKSYFEKSKE (SEQ ID NO:356), VLTALVAVAGARAEVSADOVATV (SEQ ID NO:357), NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358), LPVLVWLSIVLEGPAPAOGTPDVSS (SEQ ID NO:359),
 30 LPVLVVVLSIVLEGPAPAQGTPDVSS (SEQ ID NO:360), ALDKLKEFGNTLEDKARELIS (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362), HLRKLRKRLLRDADDLQKRLAVYOA (SEQ ID NO:363), AQAWGERLRARMEEMGSRTRDR (SEQ ID NO:364), LDEVKEQVAEVRACLEEQAQ (SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236),

DWLKAFYDKVAEKLKEAFPDWAKAAYDKAAEKAKEAA (SEQ ID NO:366),
 PVLDLFRELLNELLEALKQKL (SEQ ID NO:367), PVLDLFRELLNELLEALKQKLA (SEQ
 ID NO:368), PVLDLFRELLNELLEALKQKLK (SEQ ID NO:4),
 PVLDLFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDLFRELLNELLEALKKLLK
 5 (SEQ ID NO:370), PVLDLFRELLNELLEALKKLLA (SEQ ID NO:371),
 PLLDLFRELLNELLEALKKLLA (SEQ ID NO:372), and
 EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

In some embodiments, the average particle size of the sHDL nanoparticle is between 6-
 70 nm.

10 In certain embodiments, the present invention provides methods for inducing an immune
 response to one or more antigens comprising administering to a subject in need an effective
 amount of a composition comprising a nanoparticle, wherein the one or more antigens is
 associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the
 nanoparticle, wherein an adjuvant is associated with (e.g., complexed, conjugated, encapsulated,
 15 absorbed, adsorbed, admixed) the nanoparticle. In some embodiments, the antigen is associated
 with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the adjuvant. In
 some embodiments, the antigen is not associated with (e.g., complexed, conjugated,
 encapsulated, absorbed, adsorbed, admixed) the adjuvant. In some embodiments, the antigen is
 conjugated with a hydrophobic molecule. In some embodiments, the adjuvant is conjugated with
 20 a hydrophobic molecule. In some embodiments, the average size of the nanoparticle is between
 6 to 500 nm.

In certain embodiments, the present invention provides compositions comprising a
 nanoparticle, wherein one or more antigens is associated with (e.g., complexed, conjugated,
 encapsulated, absorbed, adsorbed, admixed) the nanoparticle, wherein an adjuvant is associated
 25 with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the nanoparticle.
 In some embodiments, the antigen is associated with (e.g., complexed, conjugated, encapsulated,
 absorbed, adsorbed, admixed) the adjuvant. In some embodiments, the antigen is not associated
 with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the adjuvant. In
 some embodiments, the antigen is conjugated with a hydrophobic molecule. In some
 30 embodiments, the adjuvant is conjugated with a hydrophobic molecule. In some embodiments,
 the average size of the nanoparticle is between 6 to 500 nm.

In some embodiments, the hydrophobic molecule is a lipid molecule. In some
 embodiments, the lipid molecule is a membrane-forming lipid molecule. In some embodiments,
 the lipid molecule molecule is a non-membrane-forming lipid molecule.

Examples of lipid molecules applicable with the embodiments of the present invention include, but are not limited to, phospholipids such as lecithin, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, egg sphingomyelin (ESM), cephalin, cardiolipin, phosphatidic acid, cerebrosides, dicetylphosphate, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoylphosphatidylethanolamine (DOPE), palmitoyloleoyl-phosphatidylcholine (POPC), palmitoyloleoyl-phosphatidylethanolamine (POPE), palmitoyloleoyl-phosphatidylglycerol (POPG), dioleoylphosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl-phosphatidylethanolamine (DPPE), dimyristoyl-phosphatidylethanolamine (DMPE), distearoyl-phosphatidylethanolamine (DSPE), monomethyl-phosphatidylethanolamine, dimethyl-phosphatidylethanolamine, dielaidoyl-phosphatidylethanolamine (DEPE), stearylloleoyl-phosphatidylethanolamine (SOPE), lysophosphatidylcholine, dilinoleoylphosphatidylcholine, and mixtures thereof. Other diacylphosphatidylcholine and diacylphosphatidylethanolamine phospholipids can also be used. The acyl groups in these lipids are preferably acyl groups derived from fatty acids having C₁₀-C₂₄carbon chains, e.g., lauroyl, myristoyl, palmitoyl, stearyl, or oleoyl.

Other non-limiting examples of lipid molecules include sterols such as cholesterol and derivatives thereof such as cholestanol, cholestanone, cholestenone, coprostanol, cholesteryl-2'-hydroxyethyl ether, cholesteryl-4'-hydroxybutyl ether, and mixtures thereof.

Other examples of lipid molecules suitable for use in the present invention include nonphosphorous containing lipids such as, e.g., stearylamine, dodecylamine, hexadecylamine, acetyl palmitate, glycerolricinoleate, hexadecyl stearate, isopropyl myristate, amphoteric acrylic polymers, triethanolamine-lauryl sulfate, alkyl-aryl sulfate polyethoxylated fatty acid amides, dioctadecyldimethyl ammonium bromide, ceramide, sphingomyelin, and the like.

Other examples of lipid molecules suitable for use in the present invention include fatty acids and derivatives or analogs thereof. They include oleic acid, lauric acid, capric acid (n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein (1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, C₁₋₁₀ alkyl esters thereof (e.g., methyl, isopropyl and t-butyl), and mono- and di-glycerides thereof (i.e., oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, etc.) (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, p. 92; Muranishi, *Critical Reviews in*

Therapeutic Drug Carrier Systems, 1990, 7, 1-33; El Hariri et al., J. Pharm. Pharmacol., 1992, 44, 651-654).

Other examples of lipid molecules suitable for use in the present invention include a lipid molecule modified with PEG (PEG-lipid). Examples of PEG-lipids include, but are not limited to, PEG coupled to dialkyloxypropyls (PEG-DAA) as described in, e.g., PCT Publication No. WO 05/026372, PEG coupled to diacylglycerol (PEG-DAG) as described in, e.g., U.S. Patent Publication Nos. 20030077829 and 2005008689, PEG coupled to phospholipids such as phosphatidylethanolamine (PEG-PE), PEG conjugated to ceramides as described in, e.g., U.S. Pat. No. 5,885,613, PEG conjugated to cholesterol or a derivative thereof, and mixtures thereof. The disclosures of these patent documents are herein incorporated by reference in their entirety for all purposes. Additional PEG-lipids include, without limitation, PEG-C-DOMG, 2 KPEG-DMG, and a mixture thereof.

PEG is a linear, water-soluble polymer of ethylene PEG repeating units with two terminal hydroxyl groups. PEGs are classified by their molecular weights; for example, PEG 2000 has an average molecular weight of about 2,000 daltons, and PEG 5000 has an average molecular weight of about 5,000 daltons. PEGs are commercially available from Sigma Chemical Co. and other companies and include, for example, the following: monomethoxypolyethylene glycol (MePEG-OH), monomethoxypolyethylene glycol-succinate (MePEG-S), monomethoxypolyethylene glycol-succinimidyl succinate (MePEG-S-NHS), monomethoxypolyethylene glycol-amine (MePEG-NH₂), monomethoxypolyethylene glycol-tresylate (MePEG-TRES), and monomethoxypolyethylene glycol-imidazolyl-carbonyl (MePEG-IM). Other PEGs such as those described in U.S. Pat. Nos. 6,774,180 and 7,053,150 (e.g., mPEG (20 KDa) amine) are also useful for preparing the PEG-lipid conjugates of the present invention. The disclosures of these patents are herein incorporated by reference in their entirety for all purposes. In addition, monomethoxypolyethyleneglycolacetic acid (MePEG-CH₂COOH) is particularly useful for preparing PEG-lipid conjugates including, e.g., PEG-DAA conjugates.

The PEG moiety of the PEG-lipid conjugates described herein may comprise an average molecular weight ranging from about 550 daltons to about 10,000 daltons. In certain instances, the PEG moiety has an average molecular weight of from about 750 daltons to about 5,000 daltons (e.g., from about 1,000 daltons to about 5,000 daltons, from about 1,500 daltons to about 3,000 daltons, from about 750 daltons to about 3,000 daltons, from about 750 daltons to about 2,000 daltons, etc.). In preferred embodiments, the PEG moiety has an average molecular weight of about 2,000 daltons or about 750 daltons.

In certain instances, the PEG can be optionally substituted by an alkyl, alkoxy, acyl, or aryl group. The PEG can be conjugated directly to the lipid or may be linked to the lipid via a linker moiety. Any linker moiety suitable for coupling the PEG to a lipid can be used including, e.g., non-ester containing linker moieties and ester-containing linker moieties. In a preferred embodiment, the linker moiety is a non-ester containing linker moiety. As used herein, the term “non-ester containing linker moiety” refers to a linker moiety that does not contain a carboxylic ester bond (—OC(O)—). Suitable non-ester containing linker moieties include, but are not limited to, amido (—C(O)NH—), amino (—NR—), carbonyl (—C(O)—), carbamate (—NHC(O)O—), urea (—NHC(O)NH—), disulphide (—S—S—), ether (—O—), succinyl ($\text{—(O)CCH}_2\text{CH}_2\text{C(O)—}$), succinamidyl ($\text{—NHC(O)CH}_2\text{CH}_2\text{C(O)NH—}$), ether, disulphide, as well as combinations thereof (such as a linker containing both a carbamate linker moiety and an amido linker moiety). In a preferred embodiment, a carbamate linker is used to couple the PEG to the lipid.

In other embodiments, an ester containing linker moiety is used to couple the PEG to the lipid. Suitable ester containing linker moieties include, e.g., carbonate (—OC(O)O—), succinoyl, phosphate esters (—O—(O)POH—O—), sulfonate esters, and combinations thereof.

Phosphatidylethanolamines having a variety of acyl chain groups of varying chain lengths and degrees of saturation can be conjugated to PEG to form the lipid conjugate. Such phosphatidylethanolamines are commercially available, or can be isolated or synthesized using conventional techniques known to those of skilled in the art.

Phosphatidylethanolamines containing saturated or unsaturated fatty acids with carbon chain lengths in the range of C_{10} to C_{20} are preferred. Phosphatidylethanolamines with mono- or diunsaturated fatty acids and mixtures of saturated and unsaturated fatty acids can also be used. Suitable phosphatidylethanolamines include, but are not limited to, dimyristoyl-phosphatidylethanolamine (DMPE), dipalmitoyl-phosphatidylethanolamine (DPPE), dioleoylphosphatidylethanolamine (DOPE), and distearoyl-phosphatidylethanolamine (DSPE).

In some embodiments, the one or more antigens is against PCSK9, M30, M27, Adpgk, and ASMTNMELM (SEQ ID NO:383). In some embodiments, the one or more antigens are conjugated to the outer surface of the nanoparticle.

In some embodiments, the adjuvant is selected from the group consisting of CPG, polyIC, poly-ICLC, 1018 ISS, aluminum salts (for example, aluminum hydroxide, aluminum phosphate), Amplivax, BCG, CP-870,893, CpG7909, CyaA, dSLIM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312,

Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, OK-432, OM-174, OM-197-MP-EC, ONTAK, PepTel.RTM, vector system, PLGA microparticles, imiquimod, resiquimod, gardiquimod, 3M-052, SRL172, Virosomes and other Virus-like particles, YF-17D, VEGF trap, beta-glucan, Pam3Cys, Aquila's QS21 stimulon, vadimezan, AsA404 (DMXAA),
 5 3M MEDI9197, glucopyranosyl lipid adjuvant (GLA), GLA-SE, CD1d ligands (such as C20:2, OCH, AH04-2, α -galatosylceramide, α -C-galatosylceramide, α -mannosylceramide, α -fructosylceramide, β -galatosylceramide, β -mannosylceramide), STING agonists (e.g. cyclic dinucleotides, including Cyclic [G(3',5')pA(3',5')p], Cyclic [G(2',5')pA(3',5')p], Cyclic [G(2',5')pA(2',5')p], Cyclic diadenylate monophosphate, Cyclic diguanylate monophosphate),
 10 CL401, CL413, CL429, Flagellin, RC529, E6020, imidazoquinoline-based small molecule. TLR-7/8a (including its lipidated analogues), virosomes, AS01, AS02, AS03, AS04, AS15, IC31, CAF01, ISCOM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), and bacterial toxins (such as CT, and LT). In some embodiments, the adjuvant is any derivative of an adjuvant (e.g., cholesterol-modified CpG) or any combinations thereof. In some embodiments, the adjuvant is
 15 conjugated to the outer surface of the nanoparticle. In some embodiments, the adjuvant is encapsulated within the nanoparticle.

In some embodiments, the nanoparticle is selected from the group consisting of sHDL nanoparticle, fullerenes, endohedral metallofullerenes buckyballs, trimetallic nitride templated endohedral metallofullerenes, single-walled and multi-walled carbon nanotubes, branched and
 20 dendritic carbon nanotubes, gold nanorods, silver nanorods, single-walled and multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures, microstructures, or their derivatives formed using layer-by-layer processes, self-assembly processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles, nanorods, cellulose
 25 nanoparticles, glass and polymer micro- and nano-spheres, biodegradable PLGA micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles, iron nanoparticles, a modified micelle.

In some embodiments, the nanoparticle is a sHDL nanoparticle. In some embodiments, the nanoparticle is sHDL, wherein the sHDL nanoparticle comprises a mixture of at least one
 30 phospholipid and at least one HDL apolipoprotein or apolipoprotein mimetic.

In some embodiments, the HDL apolipoprotein is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), and apolipoprotein E (apo E), wherein the phospholipid is selected from the group consisting of dipalmitoylphosphatidylcholine (DPPC), dioleoyl-sn-glycero-3-

phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-
 5 [4-(p-maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide],
 phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and combinations thereof.

In some embodiments, the HDL apolipoprotein mimetic is an ApoA-I mimetic, wherein
 10 the thiol-reactive phospholipid is dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP).

In some embodiments, the ApoA-I mimetic is described by any of SEQ ID NOs: 1-336 and WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341),
 LKLLDNWDSVTSTFSKLREOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLR OEMS
 15 (SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO: 345), PYLDDFQKKWQEEMEL YRQKVE (SEQ ID NO: 346),
 PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDALRTHLA
 (SEQ ID NO: 348), PYSDEL RQRLAARLEALKENGG (SEQ ID NO: 349),
 ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351),
 20 PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352),
 TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDY GKDLME (SEQ ID NO:355), KVKSP ELOAEAKSYFEKSKE (SEQ ID NO:356),
 VLTALVAVAGARAEVSADOVATV (SEQ ID NO:357),
 25 NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358),
 LPVLVWLSIVLEG PAPAOGTPDVSS (SEQ ID NO:359),
 LPVLVVVLSIVLEG PAPAQGTPDVSS (SEQ ID NO:360), ALDKLKEFGNTLEDKARELIS
 (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362),
 HLRKLRKRLLRDADDLQKRLAVYOA (SEQ ID NO:363),
 30 AQAWGERLRARMEEMGSRTRDR (SEQ ID NO:364), LDEVKEQVAEVR AKLEEQAQ
 (SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236),
 DWLKAFYDKVAEKLKEAFPDWAKAAYDKAAEKAKEAA (SEQ ID NO:366),
 PVLDFRELLNELLEALKQKL (SEQ ID NO:367), PVLDFRELLNELLEALKQKLA (SEQ ID NO:368), PVLDFRELLNELLEALKQK LK (SEQ ID NO:4),

PVLDFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDFRELLNELLEALKKLLK (SEQ ID NO:370), PVLDFRELLNELLEALKKLLA (SEQ ID NO:371), PLLDFRELLNELLEALKKLLA (SEQ ID NO:372), and EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

5 Full-length apolipoprotein (typically over 20KDa) could be produced either by purification from out-dated plasma or by recombinant methodology. In contrast to full-length apolipoproteins, peptides as short as 18-25 amino acids are capable to form α -helix and could be produced by synthetic methodologies. Due to complexity of proteins, the cost associated with protein-based therapeutics (e.g. process development, manufacturing, and analysis) is typically
10 10 to 100 fold higher than peptide-based therapeutics. Additional experiments conducted during the course of developing embodiments for the present invention compared the use of apolipoprotein peptide with full length apolipoprotein. Such experiments demonstrate the advantages of utilizing apolipoprotein peptide in relation to manufacturing, ease of sHDL formation, and reduced manufacturing cost relative to utilization of full length apolipoprotein
15 (see, Examples VIII and IX).

To produce recombinant protein, an expression system and a purification method need to be developed in order to produce and purify protein from host cell-related impurities. Selection of expression system, development of fermentation, and purification methodology acceptable for scale-up under current Good Manufacturing Practices (cGMP) often take 1-2 years and
20 significant financial resources.

Typical cell-culture and purification process for biologics consists of several steps (see, Figs. 30 and 31). For example, certain methods involve the following steps: thawing of vial of working cell bank, preparation of inoculum (shake flask, small bioreactors), transfer to production bioreactor, harvest/filtration or cell destruction, purification with orthogonal
25 chromatography steps, including: ion-exchange chromatography, hydrophobic interaction chromatography, reverse phase chromatography, multiple concentration and buffer exchange steps, viral filtration, endotoxin/DNA removal filtration, final sterile filtration and freeze/storage of purified protein. All purification steps are required in order to remove impurities.

To produce and purify recombinant apolipoproteins, a large number of steps are required
30 to eliminate process- and product-related impurities. The process impurities include: host cell components, such as host cell proteins (HCP), DNA, membrane components, endotoxin, viral adventitious agents, mycoplasma, microbial adventitious agents, adenoviruses, and plasmid DNA. Their levels have to be measured prior to human use. Other impurities include anti-foam

agents, antibiotics, surfactants, leachables/extractables, organic solvents and other molecules added to control upstream or downstream processes.

The product-related impurities include: truncated apolipoprotein, apolipoprotein containing leader sequences like (pre and pro apolipoprotein A-I), aggregated, oxidized, de-
5 amidated and hydrolyzed protein.

All impurities have to be controlled at levels specified by FDA, WHO, and ICH guidelines and specification set for apolipoprotein product. To archive these specifications, multiple purification steps are required, leading to decreased process yields, longer manufacturing time and higher manufacturing costs.

10 To obtain and purify apolipoproteins from plasma, the following process needs to be established. Apolipoproteins can be separated and purified from human plasma. However, the supply of human plasma is mainly dependent on voluntary blood donation, and the availability of plasma may limit the manufacturing of apolipoproteins. In addition, the complexity of plasma itself also presents great challenges for the separation and purification of
15 apolipoproteins.

Cold ethanol fractionation of human plasma has also been developed for the production of apolipoproteins. Precipitates of cold ethanol fractionation are used as starting materials, followed by solubilization in guanidine hydrochloride and purification by gel filtration and anion-exchange chromatography.

20 However, for these methods, purification of apolipoproteins requires the use of solvent that has a high alkaline environment, which can cause partial degradation of apolipoproteins, triggering immunogenic responses. US Patent Application Serial No. 12/673,723 reported an improved method for apolipoprotein purification from plasma to reduce apolipoprotein degradation and improve the purity (Fig. 32). In this method, the fraction IV₁ paste from a cold
25 ethanol fractionation process-treated human plasma was used as starting materials for purification of apolipoprotein. The fraction IV₁ paste was suspended in a suspension buffer (100 mM Tris, pH 9.6), followed by pH/alcohol adjustment with ethanol and sodium acetate/acetic acid solution to precipitate apolipoprotein from other components. Separated apolipoprotein was further purified by passing through a cellulose filter coated with filter aid (Celite™ 574).
30 The purity of apolipoprotein was reported to be up to 89%, which corresponds to pharmaceutical grade purity. Other methods such as ion exchange chromatography and hydrophobic interaction chromatography have also been used for the purification of apolipoproteins.

The manufacturing process for chemical synthesis of apolipoprotein-mimetic peptides summarized in Fig. 33. Compared with endogenous apolipoproteins, apolipoprotein-mimetic peptides have several major advantages.

5 First, the starting materials for apolipoprotein-mimetic peptide synthesis are amino acids, and they assembled into peptides via solid-phase peptide synthesis on resin, followed by deprotection and cleavage from the resin and column purification to obtain the peptide (Fig. 34). Since amino acids are cheap and easy to obtain, the large-scale chemical synthesis of peptide can be easily achieved.

10 Second, compared with recombinant production of apolipoproteins and purification from human plasma, the chemical peptide synthesis is simpler and results in lower levels of product and process impurities. Therefore, it is easier to obtain highly pure form of peptide (>99% purity) – the level that is very challenging to achieve for apolipoproteins. The simplicity of peptides also makes it easier for quality control, compared with apolipoproteins, which may require complex assays to monitor the quality. More importantly, there is no risk of
15 contamination of chemically synthesized peptides by pathogens or other unknown chemicals, which may be present in human plasma. Third, the sequences of apolipoprotein-mimetic peptides can be optimized based on the binding affinity to lipids and homogeneity of formed HDL.

In certain embodiments, the present invention provides compositions comprising a
20 nanoparticle (as described herein), wherein any kind of biomacromolecule agent (e.g., nucleic acid, peptides, glycolipids, etc.) is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the nanoparticle.

In some embodiments, the biomacromolecule agent is a peptide.

For example, in some embodiments, the peptide is an antigen. In some embodiments
25 wherein the peptide is an antigen, the composition further comprises an adjuvant (as described herein).

As noted, the peptide is not limited to a particular type of peptide.

In some embodiments, the peptide is Adrenocorticotrophic Hormone (ACTH), a growth hormone peptide, a Melanocyte Stimulating Hormone (MSH), Oxytocin, Vasopressin,
30 Corticotropin Releasing Factor (CRF), a CRF-related peptide, a Gonadotropin Releasing Hormone Associated Peptide (GAP), Growth Hormone Releasing Factor (GRF), Lutenizing Hormone Release Hormone (LH-RH), an orexin, a Prolactin Releasing Peptide (PRP), a somatostatin, Thyrotropin Releasing Hormone (THR), a THR analog, Calcitonin (CT), a CT-precursor peptide, a Calcitonin Gene Related Peptide (CGRP), a Parathyroid Hormone (PTH), a

Parathyroid Hormone Related Protein (PTHrP), Amylin, Glucagon, Insulin, an Insulin-like peptide, NeuroPeptide Y (NPY), a Pancreatic Polypeptide (PP), Peptide YY (PYY), Cholecystokinin (CCK), a CCK-related peptide, Gastrin Releasing Peptide (GRP), Gastrin, a Gastrin-related peptide, a Gastrin inhibitory peptide, Motilin, Secretin, Vasoactive

5 Intestinal Peptide (VIP), a VIP-related peptide, an Atrial-Natriuretic Peptide (ANP), a Brain Natriuretic Peptide (BNP), a C-Type Natriuretic Peptide(CNP), a tachykinin, an angiotensin, a renin substrate, a renin inhibitor, an endothelin, an endothelin-related peptide, an opioid peptide, a thymic peptide, an adrenomedullin peptide, an allostatin peptide, an amyloid beta-protein fragment, an antimicrobial peptide, an antioxidant peptide, an apoptosis related peptide, a Bag

10 Cell Peptide (BCPs), Bombesin, a bone Gla protein peptide, a Cocaine and Amphetamine Related Transcript (CART) peptide, a cell adhesion peptide, a chemotactic peptide, a complement inhibitor, a cortistatin peptide, a fibronectin fragment, a fibrin related peptide, FMRF, a FMRF amide-related peptide (FaRP), Galanin, a Galanin-related peptide, a growth factor, a growth factor-related peptide, a G-Therapeutic Peptide-Binding Protein fragment,

15 Gualylin, Uroguanylin, an Inhibin peptide, Interleukin (IL), an Interleukin Receptor protein, a laminin fragment, a leptin fragment peptide, a leucokinin, Pituitary Adenylate Cyclase Activating Polypeptide (PAPCAP), Pancreastatin, a polypeptide repetitive chain, a signal transducing reagent, a thrombin inhibitor, a toxin, a trypsin inhibitor, a virus-related peptide, an adjuvant peptide analog, Alpha Mating Factor, Antiarrhythmic Peptide, Anorexigenic Peptide,

20 Alpha-1 Antitrypsin, Bovine Pineal Antireproductive Peptide, Bursin, C3 Peptide P16, Cadherin Peptide, Chromogranin A Fragment, Contraceptive Tetrapeptide, Conantokin G, Conantokin T, Crustacean Cardioactive Peptide, C-Telopeptide, Cytochrome b588 Peptide, Decorsin, Delicious Peptide, Delta-Sleep-Inducing Peptide, Diazepam-Binding Inhibitor Fragment, Nitric Oxide Synthase Blocking Peptide, OVA Peptide, Platelet Calpain Inhibitor

25 (P1), Plasminogen Activator Inhibitor 1, Rigin, Schizophrenia Related Peptide, Sodium Potassium Atherapeutic Peptidase Inhibitor-1, Speract, Sperm Activating Peptide, Systemin, a Thrombin receptor agonist, Tuftsin, Adipokinetic Hormone, Uremic Pentapeptide, Antifreeze Polypeptide, Tumor Necrosis Factor (TNF), Leech [Des Asp10]Decorsin, L-Ornithyltaurine Hydrochloride, P-Aminophenylacetyl Tuftsin, Ac-Glu-Glu-Val-Val-Ala-Cys-pNA, Ac-Ser-Asp-

30 Lys-Pro, Ac-rfwink-NH₂, Cys-Gly-Tyr-Gly-Pro-Lys-Lys-Lys-Arg-Lys-Val-Gly-Gly, D-Ala-Leu, D-D-D-D-D, D-D-D-D-D-D, N-P-N-A-N-P-N-A, V-A-I-T-V-L-V-K, V-G-V-R-V-R, V-I-H-S, V-P-D-P-R, Val-Thr-Cys-Gly, R-S-R, Sea Urchin Sperm Activating Peptide, a SHU-9119 antagonist, a MC3-R antagonist, a MC4-R antagonist, Glaspimod, HP-228, Alpha 2-Plasmin Inhibitor, APC Tumor Suppressor, Early Pregnancy Factor, Gamma Interferon, Glandular

Kallikrein N-1, Placental Ribonuclease Inhibitor, Sarcolecine Binding Protein, Surfactant Protein D, Wilms' Tumor Suppressor, GABAB 1b Receptor Peptide, Prion Related Peptide (iPRP13), Choline Binding Protein Fragment, Telomerase Inhibitor, Cardiostatin Peptide, Endostatin Derived Peptide, Prion Inhibiting Peptide, N-Methyl D-Aspartate Receptor Antagonist, and C-
5 Peptide Analog.

In some embodiments, the peptide is selected from 177Lu-DOTA0-Tyr3-Octreotate, Abarelix acetate, ADH-1, Afamelanotide, melanotan-1, CUV1647, Albiglutide, Aprotinin, Argipressin, Atosiban acetate, Bacitracin, Bentiromide, a BH3 domain, Bivalirudin, Bivalirudin trifluoroacetate hydrate, Blisibimod, Bortezomib, Buserelin, Buserelin acetate, Calcitonin,
10 Carbetocin, Carbetocin acetate, Cecropin A and B, Ceruletide, Ceruletide diethylamine, Cetrorelix, Cetrorelix acetate, Ciclosporine, Cilengitide, EMD121974, Corticorelin acetate injection, hCRF, Corticorelin ovine trifluoroacetate, corticorelin trifluoroacetate, Corticotropin, Cosyntropin, ACTH 1-24, tetracosactide hexaacetate, Dalbavancin, Daptomycin, Degarelix acetate, Depreotide trifluoroacetate (plus sodium pertechnetate), Desmopressin acetate,
15 Desmopressin DDAVP, Dulaglutide, Ecallantide, Edotreotide (plus yttrium-90), Elcatonin acetate, Enalapril maleate (or 2-butanedioate), Enfuvirtide, Eptifibatid, Exenatide, Ganirelix acetate, Glatiramer acetate, Glutathion, Gonadorelin, Gonadorelin acetate, GnRH, LHRH, Goserelin, Goserelin acetate, Gramicidin, Histrelin acetate, Human calcitonin, Icatibant, Icatibant acetate, IM862, oglufanide disodium, KLAKLAK, Lanreotide acetate, Lepirudin,
20 Leuprolide, Leuprolide acetate, leuprorelin, Liraglutide, Lisinopril, Lixisenatide, Lypressin, Magainin2, MALP-2Sc, macrophage-activating lipopeptide-2 synthetic, Nafarelin acetate, Nesiritide, NGR-hTNF, Octreotide acetate, Oritavancin, Oxytocin, Pasireotide, Peginesatide, Pentagastrin, Pentetreotide (plus indium-111), Phenylephrine, Pleurocidin, Pramlintide, Protirelin, thyrotropin releasing hormone, TRH, TRF, Salmon calcitonin, Saralasin acetate, Secretin (human),
25 Secretin (porcine), Semaglutide, Seractide acetate, ACTH, corticotropin, Sermorelin acetate, GRF 1-29, Sinapultide, KL4 in lucinactant, Sincalide, Somatostatin acetate, GHRH, GHRF, GRF, Somatostatin acetate, Spaglumet magnesium (or sodium) salt, Substance P, Taltirelin hydrate, Teduglutide, Teicoplanin, Telavancin, Teriparatide, Terlipressin acetate, Tetracosactide, Thymalfasin, thymosin a-1, Thymopentin, Trebananib, Triptorelin, Triptorelin
30 pamoate, Tyrosine, Ularitide, Vancomycin, Vapreotide acetate, Vasoactive intestinal peptide acetate, Vx-001c, TERT572Y, Ziconotide acetate, α 5- α 6 Bax peptide, and β -defensin.

In some embodiments, the peptide is any peptide which would assist in achieving a desired purpose with the composition. For example, in some embodiments, the peptide is any

peptide that will facilitate treatment of any type of disease and/or disorder (e.g., peripheral ischemia, cancer, inflammatory disorders, genetic disorders, etc.).

In some embodiments, the biomacromolecule agent is a nucleic acid. Such embodiments encompass any type of nucleic acid molecule including, but not limited to, RNA, siRNA, 5 microRNA, interference RNA, mRNA, replicon mRNA, RNA-analogues, and DNA.

In certain embodiments, the present invention provides methods for treating conditions, disorders and/or diseases with such compositions comprising a nanoparticle (as described herein), wherein any kind of biomacromolecule is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the nanoparticle.

10 The present invention is not limited to specific types of conditions, disorders and/or diseases.

In some embodiments, the condition, disorder and/or disease is peripheral ischemia, cancer, an inflammatory disorder, a genetic disorder, etc.

In some embodiments, the condition, disorder and/or disease is selected from 15 erythropoietic porphyries, T2 diabetes, antifibrinolytic, central diabetes insipidus, delaying the birth in case of threat of premature birth, antibiotic, cystic fibrosis, angina, anticoagulant in patients with unstable angina undergoing PTCA or PCI, systemic lupus erythematosus, hypercalcemia, osteoporosis, pagets disease, carbetocin works as an oxytocic, antihemorrhagic and uterotonic drug in the peripheral nervous system, prevention of uterine atony, induction, and 20 control postpartum bleeding or haemorrhage, stimulant of the gastric secretion, for treat hormone-sensitive cancers of the prostate and breast, inhibition of premature LH surges in women undergoing controlled ovarian stimulation, immunosuppression in organ transplantation to prevent rejection, peritumoral brain edema, diagnosis of ACTHdependent Cushing's syndrome, allergies, ankylosing spondylitis, psoriasis, chorioiditis, erythema, keratitis, sclerosis, 25 dermatomyositis, rheumatoid arthritis, Stevens-Johnson Syndrome, ulcerative colitis, diagnosis of adrenocortical insufficiency, antibiotic, systemic infections caused by gram positive organisms, nocturnal enuresis, nocturia, and stoppage of bleeding or haemorrhage in haemophilia A patients, acute hereditary angioderma, postmenopausal osteoporosis, anti-parathyroid, Paget's disease, hypercalcaemia, hypertension, AIDS/HIV-1 infection, acute 30 coronary syndrome, unstable angina undergoing PCI, Alzheimer's and Parkinson's disease, inhibition of premature LH surges in women undergoing controlled ovarian hyperstimulation, Relapsing- Remitting Multiple Sclerosis, hepatic insufficiency, wound healing, inflammation of respiratory tract, asthenia, release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary, stimulate the secretion of gonadotropin during

disturbances fertility, and diagnosis of the functional capacity and response of the gonadotropes of the anterior pituitary, for skin lesions, surface wounds and eye infections, postmenopausal osteoporosis, Paget's disease, hypercalcaemia, hereditary angioedema, immune system related diseases, acromegaly, anticoagulant, fibroids and endometriosis, central diabetes insipidus, Cushing's syndrome, diabetic foot ulcers, treatment of central precocious puberty, uterine fibroids and endometriosis, vasodilatory, natriuretic, diuretic and neurohormonal effects, acromegaly, carcinoid syndrome, acute bacterial skin and skin structure infections, initiation or improvement of uterine contractions, and control postpartum bleeding or haemorrhage, hematide Chronic kidney disease associated anemia, stomatitis, pharyngitis, diagnostic assessment of thyroid function, postmenopausal osteoporosis, hypercalcaemia, diagnosis of pancreatic exocrine dysfunction, and gastrinoma, Zollinger-Ellison syndrome, prevention of RDS in premature infants, and meconium aspiration syndrome, acute variceal bleeding, allergic rhinitis and conjunctivitis, spinocerebellar degeneration/ataxia, Short Bowel Syndrome, antibiotic, bactericidal, teriparatide is the only anabolic (i.e., bone growing) agent indicated for use in postmenopausal women with osteoporosis, Cortrosyn Analogue of adrenocorticotrophic hormone (ACTH) used for diagnostic purposes, treatment of adrenal insufficiency, epilepsy, Chronic hepatitis B, chronic hepatitis C, primary and secondary immune deficiencies, acute decompensated heart failure, colitis, esophageal variceal bleeding in patients with cirrhotic liver disease and AIDS-related diarrhea, sarcoidosis and acute lung injury, and severe chronic pain.

In some embodiments, the peptide and condition, disorder and/or disease to be treated is shown in each row of Table 1. Table 1 provides a list of therapeutic peptides and conditions, disorders, and/or diseases targeted by such therapeutic peptides (e.g., each row presents a therapeutic peptide and the condition, disorder and/or disease targeted by the respective therapeutic peptide).

Table 1.

Therapeutic Peptide	Target Condition, Disorder and/or Disease
177Lu-DOTA0-Tyr3-Octreotate	Midgut carcinoid tumors
Abarelix acetate	Advanced prostate cancer
ADH-1	Malignant melanoma (FDA orphan drug status Phase II)
Afamelanotide, or melanotan-1, or CUV1647	Erythropoietic porphyries (EMEA and FDA orphan drug status Phase III)
Albiglutide	T2 diabetes
Aprotinin	Antifibrinolytic
Argipressin	Central diabetes insipidus, and BOV

Atosiban acetate	Delaying the birth in case of threat of premature birth
Bacitracin	Antibiotic
Bentiromide	Approved by FDA for screening test of exocrine pancreatic insufficiency in patients with cystic fibrosis
BH3 domain	Cancer: apoptosis
Bivalirudin	Direct thrombin inhibitor
Bivalirudin	Angina
Bivalirudin trifluoroacetate hydrate	Anticoagulant in patients with unstable angina undergoing PTCA or PCI
Blisibimod	Systemic lupus erythematosus
Bortezomib	Multiple myeloma, and refractory, mantle cell lymphoma
Buserelin	Treatment of prostate and breast cancer
Buserelin acetate	Advanced prostate cancer
Calcitonin	Hypercalcemia, osteoporosis, pagets disease
Carbetocin	Carbetocin works as an oxytocic, antihemorrhagic and uterotonic drug in the peripheral nervous system
Carbetocin acetate	Prevention of uterine atony, induction, and control postpartum bleeding or haemorrhage
Cecropin A and B	Leukemia; Bladder cancer
Ceruletide	Potent cholecystokinetic agent with a direct spasmogenic effect on the gallbladder muscle and bile ducts in humans and animals
Ceruletide diethylamine	Diagnosis of the functional state of the gallbladder and pancreas, and stimulant of the gastric secretion
Cetrorelix	GnRH antagonist, used to treat hormone-sensitive cancers of the prostate and breast
Cetrorelix acetate	Inhibition of premature LH surges in women undergoing controlled ovarian stimulation
Ciclosporine	An immunosuppressant drug widely used in organ transplantation to prevent rejection
Cilengitidec, or EMD121974	GBM (EMA and FDA orphan drug status – Phase III)
Corticotropin acetate injection, or hCRF	Peritumoral brain edema (FDA orphan drug status – Phase III)
Corticotropin ovine trifluoroacetate, or corticotropin trifluoroacetate	Diagnosis of ACTHdependent Cushing's syndrome
Corticotropin	Allergies, ankylosing spondylitis, psoriasis, chorioiditis, erythema, keratitis, sclerosis, dermatomyositis, rheumatoid arthritis, Stevens-Johnson Syndrome, Systemic Lupus Erythematosus, Ulcerative Colitis
Cosyntropin, or ACTH 1-24, or tetracosactide hexaacetate	Diagnosis of adrenocortical insufficiency

Dalbavancin	antibiotic
Daptomycin	Systemic infections caused by gram positive organisms
Degarelix acetate	Treatment of prostate cancer
Depreotide trifluoroacetate (plus sodium pertechnetate)	Diagnosis (scintigraphic imaging) of lung tumours
Desmopressin acetate	insipidus, nocturnal enuresis, nocturia, and stoppage of bleeding or haemorrhage in haemophilia A patients
Desmopressin DDAVP	To treat nocturnal enuresis (bedwetting)
Dulaglutide	Type 2 diabetes mellitus
Ecallantide	Acute hereditary angioderma
Edotreotide (plus yttrium-90)	Gastro-entero-pancreatic neuroendocrine tumours (FDA orphan drug status – Phase II)
Elcatonin acetate	Postmenopausal osteoporosis, anti-parathyroid, Paget's disease, hypercalcaemia
Enalapril maleate (or 2-butanedioate)	Hypertension
Enfuvirtide	AIDS/HIV-1 infection
Eptifibatide	Acute coronary syndrome, unstable angina undergoing PCI
Exenatide	Type 2 diabetes. Preclinical Studies also revealed its neuroprotective role in Alzheimer's and Parkinson's disease
Ganirelix acetate	Inhibition of premature LH surges in women undergoing controlled ovarian hyperstimulation
Glatiramer acetate	Reduction of the frequency of relapses in patients with Relapsing- Remitting Multiple Sclerosis
Glutathion	Hepatic insufficiency, wound healing, inflammation of respiratory tract, asthenia
Gonadorelin	Release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary
Gonadorelin acetate, or GnRH, or LHRH	Stimulate the secretion of gonadotropin during disturbances fertility, and diagnosis of the functional capacity and response of the gonadotropes of the anterior pituitary
Goserelin	Breast cancer and prostate cancer
Goserelin acetate	Advanced prostate cancer, breast cancer
Gramicidin	Used to treat skin lesions, surface wounds and eye infections
Histreltin acetate	Treatment of prostate and breast cancer
Human calcitonin	Postmenopausal osteoporosis, Paget's disease, hypercalcaemia
Icatibant	Hereditary angioedema
Icatibant acetate	Hereditary angioedema
IM862, or oglufanide disodium	Immune system related diseases (FDA orphan drug status for ovarian cancer – Phase II)
KLAKLAK	glioblastoma

Lanreotide acetate	Acromegaly
Lepirudin	Anticoagulant
Leuprolide	Treatment of prostate and breast cancer, and to treat fibroids and endometriosis, Alzheimer disease
Leuprolide acetate, or leuprorelin	Advanced prostate cancer, breast cancer, central precocious puberty
Liraglutide	Type 2 diabetes
Lisinopril	Hypertension, congestive heart failure
Lixisenatide	Treatment of Diabetes
Lypressin	Central diabetes insipidus, Cushing's syndrome
Magainin2	Bladder cancer; Diabetic foot ulcers
MALP-2Sc, or macrophage-activating lipopeptide-2 synthetic	Pancreatic cancer (EMA orphan drug status – Phase II)
Nafarelin acetate	Treatment of central precocious puberty, uterine fibroids and endometriosis
Nesiritide	Vasodilatory, natriuretic, diuretic and neurohormonal effects
NGR-hTNF	Mesothelioma
Octreotide acetate	Acromegaly, carcinoid syndrome
Oritavancin	Acute bacterial skin and skin structure infections
Oxytocin	Initiation or improvement of uterine contractions, and control postpartum bleeding or haemorrhage
Pasireotide	Cushing's disease and Acromegaly
Peginesatide	Hemate Chronic kidney disease associated anemia
Pentagastrin	Diagnosis of the gastric secretion
Pentetreotide (plus indium-111)	Diagnosis (scintigraphic imaging) of primary and metastatic neuroendocrine tumours
Phenylephrine	Stomatitis, pharyngitis
Pleurocidin	Breast cancer
Pramlintide	Diabetes
Protirelin, or thyroliberin, or TRH, or TRF	Diagnostic assessment of thyroid function
Salmon calcitonin	Postmenopausal osteoporosis, Paget's disease, hypercalcaemia
Saralasin acetate	Hypertension
Secretin (human)	Diagnosis of pancreatic exocrine dysfunction, and gastrinoma, Zollinger-Ellison syndrome
Secretin (porcine)	Diagnosis of pancreatic exocrine dysfunction, and gastrinoma, Zollinger-Ellison syndrome
Semaglutide	T2 diabetes
Seractide acetate, or ACTH, or corticotropin	Diagnosis of adrenocortical insufficiency
Sermorelin acetate or GRF 1-29	Growth hormone deficiency, diagnosis evaluation of pituitary function
Sinapultide, or KL4 in lucinactant	Prevention of RDS in premature infants, and meconium aspiration syndrome
Sincalide	Diagnosis of the functional state of the

	gallbladder and pancreas, and stimulant of the gastric secretion
Somatorelin acetate, or GHRH, or GHRF, or GRF	Diagnosis of somatotropic function of the anterior pituitary gland in cases of suspected growth hormone deficiency (hypophysic and hypothalamic disorders)
Somatostatin acetate	Acute variceal bleeding
Spaglumat magnesium (or sodium) salt	Allergic rhinitis and conjunctivitis
Taltirelin hydrate	Spinocerebellar degeneration/ataxia
Teduglutide	Short Bowel Syndrome (EMA and FDA orphan drug status – Phase III)
Teicoplanin	Antibiotic
Telavancin	bactericidal
Teriparatide	Teriparatide is the only anabolic (i.e., bone growing) agent indicated for use in postmenopausal women with osteoporosis
Terlipressin acetate	BOV
Tetracosactide	Cortrosyn Analogue of adrenocorticotrophic hormone (ACTH) used for diagnostic purposes, treatment of adrenal insufficiency, different types of drug resistant epilepsy
Thymalfasin, or thymosin a-1	Chronic hepatitis B, chronic hepatitis C
Thymopentin	Primary and secondary immune deficiencies, autoimmunity, infections, cancer
Trebananib	Ovarian, peritoneal or fallopian tube cancers
Triptorelin	Trelstar Treatment of prostate and breast cancer
Triptorelin pamoate	Advanced prostate cancer, central precocious puberty, endometriosis, uterine fibroids, ovarian stimulation in in vitro fecundation
Tyroserleutide	Hepatocellular carcinoma
Ularitide	Acute decompensated heart failure
Vancomycin	Treat colitis (inflammation of the intestine caused by certain bacteria) that may occur after antibiotic treatment
Vapreotide acetate	Treatment of esophageal variceal bleeding in patients with cirrhotic liver disease and AIDS-related diarrhea
Vasoactive intestinal peptide acetate	Sarcoidosis and acute lung injury (EMA and FDA orphan drug status Phase II)
Vx-001c, or TERT572Y	NSCLC (EMA and FDA orphan drug status – Phase II)
Ziconotide acetate	Severe chronic pain
α 5- α 6 Bax peptide	Cancer: apoptosis
β -defensin	Antimicrobial

Additional embodiments will be apparent to persons skilled in the relevant art based on the teachings contained herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1: (A) TEM picture and size distribution of sHDL; (B) Biodistribution of DiI-labeled sHDL in mice; (C) Cellular uptake of DiO-sHDL by SR-BI negative or positive cells without or with excess blank sHDL; (D) schematic of HDL-siRNA; (E) GPC assay of sHDL loaded with different concentrations of PCSK9 siRNA; (F) The western blot showed that PCSK9 siRNA-sHDL was better able to knockdown PCSK9 than the free PCSK9 Cho-siRNA in HepG2 cells.

FIG. 2: (A) Schematic of antigens and adjuvants-loaded sHDL; (B) Addition of antigens to functional lipids containing sHDL led to the formation of lipid-antigen conjugates as measured by HPLC; (C) The Cho-CpG could be quantitatively incorporated into sHDL as measured by GPC; (D) Co-localized delivery of antigens (Ag) and adjuvants (CpG) by sHDL led to more potent cellular response than the mixture of antigens and adjuvants in montanide.

FIG. 3 shows a schematic of the synthesis of sHDL-CSSSIINFEK(FITC)L/CpG(SEQ ID NO:386).

FIG. 4 shows homogenous particle size of sHDL-Ag/CpG as analyzed by cryoEM and dynamic light scattering.

FIGs. 5A and 5B show that compared with free antigen form, antigen delivery via sHDL significantly prolongs antigen presentation by dendritic cells.

FIG. 6 shows that sHDL-Ag/CpG significantly enhances elicitation of antigen-specific CD8⁺ T cells, compared with vaccination with free antigen mixed with conventional adjuvants.

FIG. 7 shows sHDL-Ag/CpG vaccination elicits strong CD8⁺ T cell responses in tumor-bearing mice and reduces tumor growth.

FIG. 8 shows that compared with free soluble form, alpha-GalCer delivered via sHDL significantly enhanced CD1d presentation of antigen-presenting cells.

FIG. 9 shows that lyophilization offers a convenient method of large-scale synthesis of sHDL loaded with alpha-GalCer.

FIG. 10 presents a schematic of the lyophilization method for rapid preparation of sHDL comprising encapsulated siRNA.

FIG. 11 shows a schematic of using sHDL to regulate PCSK9 for LDL-C management. As shown, (A) LDL is cleared by LDLR through endocytosis; (B) Binding of PCSK9 to LDLR leads to the degradation of LDLR in lysosomes and prevents the recycling of LDLR; (C) Knockdown of PCSK9 can upregulate LDLR and reduce LDL-C. (D) PCSK9 antibody induced by PCSK9 vaccine can block the interaction between PCSK9 and LDLR, thus upregulating

LDLR and reducing LDL-C.

FIG. 12: Design of sHDL nanodisc platform for “personalized” cancer vaccines. a, sHDL nanodiscs, composed of phospholipids and apolipoprotein-1 mimetic peptides (22A), are engineered for co-delivery of antigen (Ag) peptides and adjuvants. Pre-formed sHDL nanodiscs displaying 4 mol% DOPE-PDP (insert) are mixed with cysteine-modified Ag peptides, including tumor-associated antigens (TAAs) and tumor-specific mutated neo-antigens identified via tumor exome DNA sequencing, and subsequent incubation with cholesterol-modified immunostimulatory molecules (Cho-CpG) leads to formation of sHDL nanodiscs co-loaded with Ag and CpG (sHDL-Ag/CpG). b, Upon administration, sHDL nanodiscs efficiently co-deliver Ag and CpG to draining lymph nodes, promote strong and durable Ag presentation by dendritic cells (DCs) (Signal 1), and induce DC maturation (Signal 2), resulting in elicitation of robust Ag-specific CD8 α ⁺ cytotoxic T lymphocyte (CTL) responses. Activated CTLs recognize and kill their target cancer cells in peripheral tissues and exert strong anti-tumor efficacy.

FIG. 13: Effect of 22A variants and lipids on the formation of sHDL nanodisc. a, DMPC (containing 4% mol DOPE-PDP) and different 22A mutants were used to prepare sHDL. In addition to 22A that we have used throughout this study, several other 22A variants, including 22A composed of D-amino acids, formed homogeneous sHDL nanodiscs (as analyzed by dynamic light scattering) that remained stable up to one month at 4^oC. N.D., not determined due to aggregation. b, Synthesis of sHDL requires phospholipids with high transition temperature (T_m) and ApoA-mimetic peptides. DPPC and DMPC (T_m = 41^oC and 24^oC, respectively) but not POPC or DOPC (T_m = -2^oC and -17^oC, respectively), formed homogeneous sHDL in the presence of 22A and 4 mol% DOPE-PDP.

FIG. 14: Synthesis of functional lipid DOPE-PDP. a, DOPE, SPDP (succinimidyl 3-(2-pyridyldithio) propionate) and triethylamine (1:1:1.5 molar ratio) were dissolved in chloroform and allowed to react in dark with stirring for 5 h. b, The reaction progress was monitored by thin layer chromatography (TLC), using the following mixture as the developing solvent: chloroform/methanol/water = 65/25/4 (volume ratio). c-d, The reaction mixture was purified using a silica gel column, and the purity was assessed by c, TLC and d, HPLC using the condition described in Example VI.

FIG. 15: Preparation and characterization of sHDL-CSSSIINFEKL/CpG (SEQ ID NO:384), sHDL-gp100/CpG, and sHDL-Adpgk/CpG. CSSSIINFEKL (SEQ ID NO:384), CSS-gp 100 or CSS-Adpgk were incubated with sHDL-PDP, followed by insertion of Cho-CpG to sHDL-CSSSIINFEKL (SEQ ID NO:384), sHDLgp100 or sHDL-Adpgk. Shown are HPLC chromatograms confirming the conjugation of a, CSSSIINFEKL (SEQ ID NO:384), c, gp 100,

or e, Adpgk to sHDL-PDP. GPC of b, sHDL-CSSSIINFEKL/CpG (SEQ ID NO:384), d, sHDL-gp100/CpG, and f, sHDL-Adpgk/CpG showed homogeneity of all formulations and efficient loading of Cho-CpG in sHDL nanodiscs.

FIG. 16: Strong and durable Ag presentation mediated by sHDL nanodiscs. a, Dynamic light scattering analysis and b, transmission electron microscopy imaging showed uniform sHDL-Ag/CpG (10.5 nm \pm 0.5 average diameter) with nanodisc-like morphology. c, Homogeneity of nanodiscs was maintained after sterile-filtration (0.22 μ m), and long-term storage (8 weeks) at -20°C, followed by thawing at 37°C. d-e, BMDCs were incubated with vaccine formulations for d, 24 h or e, indicated lengths of time, and Ag presentation was quantified by flow-cytometry analysis of DCs stained with 25-D1.16 mAb that recognizes SIINFEKL-H-2K^b (SEQ ID NO:385) complex. f-g, Confocal microscopy images of JAWSII cells (immature DCs). f, JAWSII cells were incubated with free Ag+CpG or sHDL-Ag/CpG for 24 h and stained with 25-D1.16 mAb. Scale bars = 20 μ m. g, JAWSII cells were incubated with free CSSSIINFEK_(FITC)L(SEQ ID NO:386) + CpG or sHDL-CSSSIINFEK_(FITC)L(SEQ ID NO:386)/CpG for 6, 24, or 48 h, followed by staining with Hoechst and LysoTracker. Scale bars = 10 μ m. h, BMDCs were incubated with different concentrations of indicated formulations: low dose = 20 nM SIINFEKL (SEQ ID NO:385) and 3 nM CpG; medium dose = 100 nM SIINFEKL (SEQ ID NO:385) and 15 nM CpG; and high dose = 500 nM SIINFEKL (SEQ ID NO:385) and 75 nM CpG. After incubation for 24 h or 48 h, BMDCs were co-cultured with SIINFEKL-specific B3Z T-cell hybridoma for another 24 h, followed by assessment of T cell activation. The data show mean \pm SD from a representative experiment (n = 3) from 2-4 independent experiments. **** $p < 0.0001$, analyzed by two-way ANOVA with Tukey's HSD post-test.

FIG. 17: Strong and durable Ag presentation mediated by sHDL-Ag/CpG. BMDCs were incubated with vaccine formulations for a-b, 24 h, or c, indicated lengths of time, and Ag presentation was quantified by flow-cytometry analysis of DCs stained with 25-D1.16 mAb that recognizes SIINFEKL-H-2K^b (SEQ ID NO:385) complex. Shown are a, the percent of antigen presenting BMDCs at the 24 h time point, b, representative histograms, and c, the percent of antigen presenting BMDCs over 48 h. The data show mean \pm SD from a representative experiment (n = 3) from 2-4 independent experiments. **** $p < 0.0001$, analyzed by two-way ANOVA with Tukey's HSD post-test.

FIG. 18: Ag delivery and presentation mediated by sHDL-Ag/CpG (broader view). JAWSII cells were incubated with free CSSSIINFEK(SEQ ID NO:386)(FITC)L + CpG or sHDL-CSSSIINFEK(SEQ ID NO:386) (FITC)L/CpG for 6, 24, or 48 h, and stained with

Hochest and LysoTracker. Scale bar = 50 μ m.

FIG. 19: Intracellular delivery of sHDL (broader view). JAWSII cells were incubated for 24 h with sHDL containing either Rhodamine-labeled DOPE (DOPE-Rhod) or Texas Red-labeled 22A and stained with Hochest and LysoTracker. Scale bar = 50 μ m.

5 FIG. 20: Stimulation of bone marrow-derived dendritic cells (BMDCs) by CpG-containing formulations. BMDCs were incubated with blank sHDL or 75 nM CpG formulations for 24 h. The expression levels of CD40, CD80, and CD86 were measured by flow cytometry after staining with corresponding fluorophore-labeled antibodies. The data show mean \pm SD from a representative experiment (n = 3) from 3 independent experiments.

10 FIG. 21: Vaccine nanodiscs for LN-targeting of Ag and adjuvants and elicitation of CTL responses. a-b, C57BL/6 mice were administered subcutaneously at tail base with a, 31 nmol FITC-tagged Ag (CSSSIINFEK_(FITC)L (SEQ ID NO:386)) or b, 2.3 nmol Cho-CpG (20% labeled by Cy5) in free soluble or sHDL form, and fluorescence signal in the draining inguinal LNs were quantified with IVIS after 24 h. c-f, C57BL/6 mice were immunized with the indicated
15 formulations (15.5 nmol Ag peptide and 2.3 nmol CpG) on days 0, 21, and 42. c, The frequency of SIINFEKL-specific CD8 α ⁺ T-cells in peripheral blood was measured 7 days post each immunization by flow-cytometry analysis of tetramer⁺ CD8 α ⁺ T-cells, and d, their representative scatter plots on day 49 are shown. e-f, On day 50, pre-vaccinated animals were challenged with subcutaneous flank injection of 2×10^5 B16OVA cells. e, Tumor growth and f, overall survival are shown. g-h, C57BL/6 mice were immunized with the indicated formulations
20 in a biweekly interval. Shown are g, percent of SIINFEKL-specific CD8 α ⁺ T-cells among PBMCs and h, ELISPOT analysis of IFN- γ spot-forming cells among splenocytes after *ex vivo* restimulation with SIINFEKL (SEQ ID NO:385) on day 42. The data show mean \pm SD from a representative experiment (n = 4-5) from 2-3 independent experiments. * $p < 0.05$, ** $p < 0.01$,
25 *** $p < 0.001$, and **** $p < 0.0001$, analyzed by (a-b) two-tailed unpaired Student's *t* test, (c,e,g) two-way ANOVA with Tukey's HSD post-test, or (f) log-rank (Mantel-Cox) test. Asterisks in panel e indicate statistically significant differences between sHDL-Ag/CpG and SIINFEKL+CpG+Montanide.

FIG. 22: Colocalization of antigen peptides and sHDL in dLNs after subcutaneous
30 administration. sHDL-CSSSIINFEK (SEQ ID NO:386) (FITC)L nanodiscs incorporated with Cy5-labeled 22A were injected subcutaneously (31 nmol antigen peptides/mouse) at the tail base of C57BL/6 mice. After 24 h, draining inguinal lymph nodes were harvested and frozen sections were prepared for confocal microscopy. The confocal images showed antigen peptides and 22A were colocalized in the lymph nodes (indicated by white arrows). Scale bar = 50 μ m.

FIG. 23: Elicitation of CTL responses with sHDL-Ag/CpG vaccination. C57BL/6 mice were immunized with the indicated formulations in a biweekly interval. Shown are representative scatter plots for SIINFEKL-specific CD8⁺ T-cells among PBMCs on day 35 and their effector CD8⁺ T-cell phenotype as analyzed by CD44 and CD62L staining.

5 FIG. 24: Therapeutic vaccination against melanoma with sHDL-Ag/CpG. C57BL/6 mice (n = 5) were inoculated subcutaneously with 2×10⁵ B16OVA cells and vaccinated on days 4 and 11 with the indicated formulations (equivalent to 15.5 nmol Ag peptide and 2.3 nmol CpG). a, Shown are the frequency of SIINFEKL-specific CD8⁺ T-cells among PBMCs as measured by tetramer staining; b, their representative scatter plots on day 17; c, B16OVA tumor growth; and
10 d, animal survival. The data show mean ± SD from a representative experiment (n = 5) from 2-3 independent experiments. * $p < 0.05$, and **** $p < 0.0001$, analyzed by (a,c) two-way ANOVA with Tukey's HSD post-test or (d) log-rank (Mantel-Cox) test. Asterisks in panels c indicate statistically significant differences between sHDL-Ag/CpG and all other groups.

FIG. 25: Nanodisc vaccination with tumor-associated antigens and tumor-specific neo-
15 antigens for treatment of melanoma and colon adenocarcinoma. a-c, C57BL/6 mice were inoculated subcutaneously with 2×10⁵ non-immunogenic B16F10 melanoma cells and vaccinated on days 4 and 11 with the indicated formulations (equivalent to 15.5 nmol Ag peptide and 2.3 nmol CpG). a, Shown are the frequency of gp100-specific CD8⁺ T-cells among PBMCs; b, B16F10 tumor growth; and c, animal survival. d, Mutation of Adpgk in MC-38
20 murine colon adenocarcinoma cells was confirmed by sequencing cDNA of Adpgk. e-h, C57BL/6 mice were inoculated subcutaneously with 10⁵ MC-38 tumor cells and vaccinated with the indicated formulations (equivalent to 15.5 nmol mutated Adpgk peptide and 2.3 nmol CpG) on days 10, 17, and 24. Shown are e, the frequencies of Adpgk-specific CD8⁺ T-cells among PBMCs and representative scatter plots of Adpgk-tetramer⁺ CD8⁺ T-cells on day 23; f, the
25 percentages of intracellular IFN- γ ⁺, TNF- α ⁺, and IFN- γ ⁺TNF- α ⁺ CD8⁺ T-cells among PBMCs on day 30 after *ex vivo* restimulation with the mutated Adpgk Ag and their representative scatter plots; g, growth of MC-38 tumor masses; and h, animal survival. The data show mean ± SD from a representative experiment (n = 5-8) from 2-3 independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$, analyzed by (a,b,e,g) two-way or (f) one-way
30 ANOVA with Tukey's HSD post-test or (c,h) log-rank (Mantel-Cox) test. Asterisks in (b,g) indicate statistically significant differences between sHDL-Ag/CpG and all other groups.

FIG. 26: Therapeutic vaccination against melanoma with sHDLAg/CpG. C57BL/6 mice were inoculated subcutaneously with 2×10⁵ B16F10 cells and vaccinated on days 4 and 11 with the indicated formulations (equivalent to 15.5 nmol Ag peptide and 2.3 nmol CpG). Shown are

the representative scatter plots for gp100-specific CD8 α ⁺ T-cells among PBMCs in B16F10 tumor-bearing mice on day 17.

FIGs. 27A-C: cDNA sequencing of MC-38 cells for mutated Adpgk neoantigen. Two different lengths (485 bp and 250 bp) of cDNA for the neoantigen Adpgk mRNA (amino acid sequence ASMTNMELM (SEQ ID NO:383)) were prepared by using two different sets of primers. The sequence of cDNA was analyzed by DNA sequencing. Shown are A, two different lengths of cDNA bands on agarose gel and the results of Sanger DNA sequencing for B, 485 bp cDNA and C, 250 bp cDNA. Arrows indicate the mutation of G \rightarrow T.

FIG. 28: Nanodisc-based vaccination with multivalent neo-antigen peptides elicited strong CD4⁺ and CD8⁺ T cell responses. (a) PBMCs from mice vaccinated with sHDL-M30/M27/CpG showed strong IFN gamma secretion from CD4⁺ T cells upon restimulation by M30 peptide. (b) PBMCs from mice vaccinated with sHDL-M30/M27/CpG showed strong IFN gamma secretion from CD8⁺ T cells upon restimulation by M27 peptide. Data represent mean \pm SD (n = 3 - 4).

FIG. 29: Nanoparticle formulations improve CD8⁺ T cell responses and therapeutic effect of neo-antigen peptide vaccination. C57BL/6 mice were inoculated with tumor cells (1×10^5 MC38 cells per mouse) on the right flank by subcutaneous injection on day 0. Mice were vaccinated on days 10 and 17 with 15.5 nmol of ASMTNMELM (SEQ ID NO:383) and 2.3 nmol of CpG in either soluble or liposomal forms. AuNP (gold nanoparticles) groups were immunized on day 10 and exposed to laser or not on day 11, followed by tetramer staining on day 17. (a) Percent of antigen specific CD8⁺ T cells among PBMCs elicited by different formulations on day 7 post last vaccination. (b) Tumor growth curves for indicated formulations. Data represent mean \pm SD (n = 3 - 5).

Fig. 30 describes a cell culture process for recombinant production of apolipoproteins.

Fig. 31 describes a process for the purification of apolipoproteins.

Fig. 32 describes a process for the purification of ApoA-I from plasma.

Fig. 33 describes a process for chemical synthesis of apolipoprotein-mimetic peptides.

Fig. 34 describes a process for apolipoprotein-mimetic peptide synthesis.

Fig. 35 describes the characterization of HDL made with full length Apolipoprotein A-I.

Fig. 36 describes the characterization of HDL made with full length Apolipoprotein A-I, and in particular, dynamic light scattering data of HDL (with intensity averaged).

Fig. 37A and B describes the characterization of HDL made with full length Apolipoprotein A-I made by cholate/bio-bead method.

Fig. 38 describes a 22A-sHDL prepared by co-lyophilization, and analyzed with GPC.

Fig. 39 describes a 22A-sHDL prepared by co-lyophilization, and analyzed with DLS.

Fig. 40 describes experiments wherein C57BL/6 mice were immunized with sHDL-CpG (equivalent to 2.3 nmol CpG per dose) for 3 times in an 1-week interval. Fig. 40 shows the percent of 22A-specific CD4⁺ T cells (a), 22A-specific CD8⁺ T cells (b) among PBMCs one week after the third vaccination, and (c) the titers of IgG antibody against 22A one week after the third vaccination. Data represent mean \pm SD from a representative experiment (n = 3) from 2 independent experiments. NS, non-statistically significant.

Fig. 41: Nanodisc-based neo-antigen vaccination combined with immune checkpoint blockade for treatment of colon adenocarcinoma. a, C57BL/6 mice were inoculated subcutaneously with 10⁵ MC-38 tumor cells and vaccinated with the indicated formulations (equivalent to 15.5 nmol mutated Adpgk peptide and 2.3 nmol CpG) on days 10, 17, and 24. Shown are the percent of intracellular IFN- γ ⁺, TNF- α ⁺, and IFN- γ ⁺TNF- α ⁺ CD8 α ⁺ T-cells in peripheral blood on day 30 after *ex vivo* restimulation with the mutated Adpgk Ag. Average and individual MC-38 tumor growth curves are shown with fraction of complete tumor regression (CR). b, C57BL/6 mice were inoculated subcutaneously with 10⁵ MC-38 tumor cells and vaccinated with the indicated formulations (equivalent to 15.5 nmol mutated Adpgk peptide and 2.3 nmol CpG) on days 10 and 17. On days 1 and 4 after each vaccination, mice were administered intraperitoneally with α PD-1 (100 μ g/mouse). Average and individual MC-38 tumor growth curves are shown. The data show mean \pm SD from a representative experiment (n = 5-10) from 2-3 independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

Fig. 42: Nanodisc-based neo-antigen vaccination combined with immune checkpoint blockade for treatment of melanoma. a-d, C57BL/6 mice were inoculated subcutaneously with 10⁵ melanoma B16F10 cells and vaccinated on days 4, 11, and 18 with indicated formulations (10 nmol of each antigen peptide and 2.3 nmol of CpG). For the combination immunotherapy, on days 1 and 4 after each vaccination, α PD-1 and α CTLA-4 (100 μ g/mouse each) were administered intraperitoneally. Shown are a, the percent of IFN- γ ⁺ CD8 α ⁺ or CD4⁺ T cells in peripheral blood measured by intracellular cytokine staining, and b-d, average and individual B16F10 tumor growth curves. The data show mean \pm SD from a representative experiment (n = 5-10) from 2-3 independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

DEFINITIONS

To facilitate an understanding of the present invention, a number of terms and phrases are defined below:

As used here, the term “lipids” or “lipid molecules” refer to fatty substances that are insoluble in water and include fats, oils, waxes, and related compounds. They may be either
5 made in the blood (endogenous) or ingested in the diet (exogenous). Lipids are essential for normal body function and whether produced from an exogenous or endogenous source, they must be transported and then released for use by the cells. The production, transportation and release of lipids for use by the cells is referred to as lipid metabolism. While there are several classes of lipids, two major classes are cholesterol and triglycerides. Cholesterol may be
10 ingested in the diet and manufactured by the cells of most organs and tissues in the body, primarily in the liver. Cholesterol can be found in its free form or, more often, combined with fatty acids as what is called cholesterol esters. As used herein, “lipid” or “lipid molecule” refers to any lipophilic compound. Non-limiting examples of lipid compounds include fatty acids, cholesterol, phospholipids, complex lipids, and derivatives or analogs thereof. They are usually
15 divided into at least three classes: (1) “simple lipids,” which include fats and oils as well as waxes; (2) “compound lipids,” which include phospholipids and glycolipids; and (3) “derived lipids” such as steroids. Lipids or lipid molecules suitable for use in the present invention include both membrane-forming lipids and non-membrane-forming lipids.

As used herein the term, “lipoproteins” refer to spherical compounds that are structured
20 so that water-insoluble lipids are contained in a partially water-soluble shell. Depending on the type of lipoprotein, the contents include varying amounts of free and esterified cholesterol, triglycerides and apoproteins or apolipoproteins. There are five major types of lipoproteins, which differ in function and in their lipid and apoprotein content and are classified according to increasing density: (i) chylomicrons and chylomicron remnants, (ii) very low density
25 lipoproteins (“VLDL”), (iii) intermediate-density lipoproteins (“IDL”), (iv) low-density lipoproteins (“LDL”), and (v) high-density lipoproteins (“HDL”). Cholesterol circulates in the bloodstream as particles associated with lipoproteins.

As used herein, the term “HDL” or “high density lipoprotein” refers to high-density lipoprotein. HDL comprises a complex of lipids and proteins in approximately equal amounts
30 that functions as a transporter of cholesterol in the blood. HDL is mainly synthesized in and secreted from the liver and epithelial cells of the small intestine. Immediately after secretion, HDL is in a form of a discoidal particle containing apolipoprotein A-I (also called apoA-I) and phospholipid as its major constituents, and also called nascent HDL. This nascent HDL receives, in blood, free cholesterol from cell membranes of peripheral cells or produced in the hydrolysis

course of other lipoproteins, and forms mature spherical HDL while holding, at its hydrophobic center, cholesterol ester converted from said cholesterol by the action of LCAT (lecithin cholesterol acyltransferase). HDL plays an extremely important role in a lipid metabolism process called "reverse cholesterol transport", which takes, in blood, cholesterol out of peripheral tissues and transports it to the liver. High levels of HDL are associated with a decreased risk of atherosclerosis and coronary heart disease (CHD) as the reverse cholesterol transport is considered one of the major mechanisms for HDL's prophylactic action on atherosclerosis.

As used herein, the terms "synthetic HDL," "sHDL," "reconstituted HDL", or "rHDL" refer to a particle structurally analogous to native HDL, composed of a lipid or lipids in association with at least one of the proteins of HDL, preferably Apo A-I or a mimetic thereof. Typically, the components of sHDL may be derived from blood, or produced by recombinant technology.

As used herein, the term "complexed" as used herein relates to the non-covalent interaction of a biomacromolecule agent (e.g., antigen, adjuvant, etc) with a nanoparticle and/or microparticle.

As used herein, the term "conjugated" as used herein indicates a covalent bond association between a biomacromolecule agent (e.g., antigen, adjuvant, etc) and a nanoparticle and/or microparticle.

As used herein, the term "encapsulated" refers to the location of a biomacromolecule agent (e.g., antigen, adjuvant, etc) that is enclosed or completely contained within the inside of a nanoparticle and/or microparticle.

As used herein, the term "absorbed" refers to a biomacromolecule agent (e.g., antigen, adjuvant, etc) that is taken into and stably retained in the interior, that is, internal to the outer surface, of a nanoparticle and/or microparticle.

As used herein, the term "adsorbed" refers to the attachment of a biomacromolecule agent (e.g., antigen, adjuvant, etc) to the external surface of a nanoparticle and/or microparticle. Such adsorption preferably occurs by electrostatic attraction. Electrostatic attraction is the attraction or bonding generated between two or more oppositely charged or ionic chemical groups. Generally, the adsorption is typically reversible.

As used herein, the term "admixed" refers to a biomacromolecule agent (e.g., antigen, adjuvant, etc) that is dissolved, dispersed, or suspended in a nanoparticle and/or microparticle. In some cases, the biomacromolecule agent may be uniformly admixed in the nanoparticle and/or microparticle.

As used herein, the terms “biological biomacromolecule” or “biomacromolecule” or “biomacromolecule agent” as used herein refer to a molecule with a molecular mass exceeding 1 kDa which can be isolated from an organism or from cellular culture, e.g., eukaryotic (e.g., mammalian) cell culture or prokaryotic (e.g., bacterial) cell culture. In some embodiments, the use of the term refers to polymers, e.g., biopolymers such as nucleic acids (including, but not limited to, RNA, siRNA, microRNA, interference RNA, mRNA, replicon mRNA, RNA-analogues, DNA, etc.), polypeptides (such as proteins), carbohydrates, and lipids. In some embodiments, the term “biomacromolecule” refers to a protein. In some embodiments, the term “biomacromolecule” refers to a recombinant protein or a fusion protein. In some embodiments, the protein is soluble. In some embodiments, the biomacromolecule is an antibody, e.g., a monoclonal antibody. In some embodiments, the biomacromolecule is an adjuvant, an antigen, a therapeutic agent, an imaging agent, etc.

As used herein, the term “antigen” is defined herein as a molecule which contains one or more epitopes that will stimulate a hosts immune system to make a cellular antigen-specific immune response, and/or a humoral antibody response. Antigens can be peptides, proteins, polysaccharides, saccharides, lipids, nucleic acids, and combinations thereof. The antigen can be derived from a virus, bacterium, parasite, plant, protozoan, fungus, tissue or transformed cell such as a cancer or leukemic cell and can be a whole cell or immunogenic component thereof, e.g., cell wall components. An antigen may be an oligonucleotide or polynucleotide which expresses an antigen. Antigens can be natural or synthetic antigens, for example, haptens, polyepitopes, flanking epitopes, and other recombinant or synthetically derived antigens (see, e.g., Bergmann, et al., *Eur. J. Immunol.*, 23:2777-2781 (1993); Bergmann, et al., *J. Immunol.*, 157:3242-3249 (1996); Suhrbier, *Immunol. and Cell Biol.*, 75:402-408 (1997)).

As used herein, the term “neo-antigen” or “neo-antigenic” means a class of tumor antigens that arises from a tumor-specific mutation(s) which alters the amino acid sequence of genome encoded proteins.

As used herein, the term “tumor-specific antigen” is defined herein as an antigen that is unique to tumor cells and does not occur in or on other cells in the body.

As used herein, the term “tumor-associated antigen” is defined herein as an antigen that is not unique to a tumor cell and is also expressed in or on a normal cell under conditions that fail to induce an immune response to the antigen.

As used herein, the term “adjuvant” is defined herein as a substance increasing the immune response to other antigens when administered with other antigens. Adjuvants are also referred to herein as “immune potentiators” and “immune modulators”.

As used herein, the term “antigen-presenting cells” are defined herein as highly specialized cells that can process antigens and display their peptide fragments on the cell surface together with molecules required for lymphocyte activation. The major antigen-presenting cells for T cells are dendritic cells, macrophages and B cells. The major antigen-presenting cells for B cells are follicular dendritic cells.

As used herein, the term “cross-presentation” is defined herein as the ability of antigen-presenting cells to take up, process and present extracellular antigens with MHC class I molecules to CD8 T cells (cytotoxic T cells). This process induces cellular immunity against most tumors and against viruses that do not infect antigen-presenting cells. Cross-presentation is also required for induction of cytotoxic immunity by vaccination with protein antigens, for example in tumor vaccination.

As used herein, the terms “immunologic”, “immunological” or “immune” response is the development of a humoral and/or a cellular response directed against an antigen.

As used herein, the term "kit" refers to any delivery system for delivering materials. In the context of the sHDL nanoparticles as described herein (e.g., compositions comprising a sHDL nanoparticle encapsulating siRNA) (e.g., compositions comprising an sHDL nanoparticle configured to activate an immune responses), such delivery systems include systems that allow for the storage, transport, or delivery of such compositions and/or supporting materials (e.g., written instructions for using the materials, etc.) from one location to another. For example, kits include one or more enclosures (e.g., boxes) containing the necessary agents and/or supporting materials. As used herein, the term "fragmented kit" refers to delivery systems comprising two or more separate containers that each contain a subportion of the total kit components. The containers may be delivered to the intended recipient together or separately. For example, a first container may contain a composition comprising an sHDL nanoparticle or the ingredients necessary to synthesize such an sHDL nanoparticle, while a second container contains a second agent (e.g., siRNA, an antigen, an adjuvant) (e.g., an antibiotic or spray applicator). Indeed, any delivery system comprising two or more separate containers that each contains a subportion of the total kit components are included in the term "fragmented kit." In contrast, a "combined kit" refers to a delivery system containing all of the components necessary to synthesize and utilize any of the sHDL nanoparticles as described (e.g., in a single box housing each of the desired components). The term "kit" includes both fragmented and combined kits.

As used herein, the term "subject" refers to any animal (e.g., a mammal), including, but not limited to, humans, non-human primates, rodents, and the like, which is to be the recipient of

a particular treatment. Typically, the terms "subject" and "patient" are used interchangeably herein in reference to a human subject.

As used herein, the term "sample" is used in its broadest sense. In one sense, it is meant to include a specimen or culture obtained from any source, as well as biological and
5 environmental samples. Biological samples may be obtained from animals (including humans) and encompass fluids, solids, tissues, and gases. Biological samples include blood products, such as plasma, serum and the like. Environmental samples include environmental material such as surface matter, soil, water, crystals and industrial samples. Such examples are not however to be construed as limiting the sample types applicable to the present invention.

10 As used herein, the term "*in vitro*" refers to an artificial environment and to processes or reactions that occur within an artificial environment. *In vitro* environments can consist of, but are not limited to, test tubes and cell culture. The term "*in vivo*" refers to the natural environment (*e.g.*, an animal or a cell) and to processes or reaction that occur within a natural environment.

15 As used herein, the term "drug" or "therapeutic agent" is meant to include any molecule, molecular complex or substance administered to an organism for diagnostic or therapeutic purposes, including medical imaging, monitoring, contraceptive, cosmetic, nutraceutical, pharmaceutical and prophylactic applications. The term "drug" is further meant to include any such molecule, molecular complex or substance that is chemically modified and/or operatively
20 attached to a biologic or biocompatible structure.

As used herein, the term "solvent" refers to a medium in which a reaction is conducted. Solvents may be liquid but are not limited to liquid form. Solvent categories include but are not limited to nonpolar, polar, protic, and aprotic.

25 **DETAILED DESCRIPTION OF THE INVENTION**

The present invention relates to nanoparticles associated with (*e.g.*, complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) biomacromolecule agents configured for treating, preventing or ameliorating various types of disorders, and methods of synthesizing the same. In particular, the present invention is directed to compositions comprising
30 nanoparticles (*e.g.*, synthetic high density lipoprotein (sHDL)) associated with (*e.g.*, complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) biomacromolecule agents (*e.g.*, nucleic acid, peptides, glycolipids, etc.), methods for synthesizing such nanoparticles, as well as systems and methods utilizing such nanoparticles (*e.g.*, in diagnostic and/or therapeutic settings).

Nanoparticles

The present invention is not limited to specific types or kinds of nanoparticles associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) biomacromolecule agents configured for treating, preventing or ameliorating various types of disorders.

Examples of nanoparticles include, but are not limited to, fullerenes (a.k.a. C₆₀, C₇₀, C₇₆, C₈₀, C₈₄), endohedral metallofullerenes (EMI's) buckyballs, which contain additional atoms, ions, or clusters inside their fullerene cage), trimetallic nitride templated endohedral metallofullerenes (TNT EMEs, high-symmetry four-atom molecular cluster endohedrals, which are formed in a trimetallic nitride template within the carbon cage), single-walled and multi-walled carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods, single-walled and multi-walled boron/nitrate nanotubes, carbon nanotube peapods (nanotubes with internal metallo-fullerenes and/or other internal chemical structures), carbon nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, quantum dots, superparamagnetic nanoparticles, nanorods, and cellulose nanoparticles. The particle embodiment can also include microparticles with the capability to enhance effectiveness or selectivity. Other non-limiting exemplary nanoparticles include glass and polymer micro- and nano-spheres, biodegradable PLGA micro- and nano-spheres, gold, silver, carbon, and iron nanoparticles.

In some embodiments, the nanoparticle is a modified micelle. In these embodiments, the modified micelle comprises polyol polymers modified to contain a hydrophobic polymer block. The term "hydrophobic polymer block" as used in the present disclosure indicates a segment of the polymer that on its own would be hydrophobic. The term "micelle" as used herein refers to an aggregate of molecules dispersed in a liquid. A typical micelle in aqueous solution forms an aggregate with the hydrophilic "head" regions in contact with surrounding solvent, sequestering the hydrophobic single tail regions in the micelle centre. In some embodiments the head region may be, for example, a surface region of the polyol polymer while the tail region may be, for example, the hydrophobic polymer block region of the polyol polymer.

The invention further encompasses use of particles on the micrometer scale in addition to the nanometer scale. Where microparticles are used, it is preferred that they are relatively small, on the order of 1-50 micrometers. For ease of discussion, the use herein of "nanoparticles" encompasses true nanoparticles (sizes of from about 1 nm to about 1000 nm), microparticles (e.g., from about 1 micrometer to about 50 micrometers), or both.

Examples of nanoparticles include, by way of example and without limitation,

paramagnetic nanoparticles, superparamagnetic nanoparticles, metal nanoparticles, fullerene-like materials, inorganic nanotubes, dendrimers, dendrimers with covalently attached metal chelates, nanofibers, nanohorns, nano-onions, nanorods, nanoropes and quantum dots. In some embodiments, a nanoparticle is a metal nanoparticle (for example, a nanoparticle of gold, palladium, platinum, silver, copper, nickel, cobalt, iridium, or an alloy of two or more thereof). Nanoparticles can include a core or a core and a shell, as in core-shell nanoparticles.

In some embodiments, the nanoparticles are sHDL nanoparticles. Generally, sHDL nanoparticles are composed of a mixture of HDL apolipoprotein and an amphipathic lipid.

The present invention is not limited to use of a particular type or kind of HDL apolipoprotein. HDL apolipoproteins include, for example apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), and apolipoprotein E (apo E). In some embodiments, the HDL apolipoprotein is selected from preproapolipoprotein, preproApoA-I, proApoA-I, ApoA-I, preproApoA-II, proApoA-II, ApoA-II, preproApoA-IV, proApoA-IV, ApoA-IV, ApoA-V, preproApoE, proApoE, ApoE, preproApoA-IMilano, proApoA-IMilano ApoA-IMilano preproApoA-IParis, proApoA-IParis, and ApoA-IParis and peptide mimetics of these proteins mixtures thereof. Preferably, the carrier particles are composed of Apo A-I or Apo A-II, however the use of other lipoproteins including apolipoprotein A4, apolipoprotein Cs or apolipoprotein E may be used alone or in combination to formulate carrier particle mixtures for delivery of therapeutic agents. In some embodiments, mimetics of such HDL apolipoproteins are used.

ApoA-I is synthesized by the liver and small intestine as preproapolipoprotein which is secreted as a proprotein that is rapidly cleaved to generate a mature polypeptide having 243 amino acid residues. ApoA-I consists mainly of 6 to 8 different 22 amino acid repeats spaced by a linker moiety which is often proline, and in some cases consists of a stretch made up of several residues. ApoA-I forms three types of stable complexes with lipids: small, lipid-poor complexes referred to as pre-beta-1 HDL; flattened discoidal particles containing polar lipids (phospholipid and cholesterol) referred to as pre-beta-2 HDL; and spherical particles containing both polar and nonpolar lipids, referred to as spherical or mature HDL (HDL₃ and HDL₂). Most HDL in the circulating population contain both ApoA-I and ApoA-II (the second major HDL protein).

In some embodiments, ApoA-I agonists or mimetics are provided. In some embodiments, such ApoA-I mimetics are capable of forming amphipathic α -helices that mimic the activity of ApoA-I, and have specific activities approaching or exceeding that of the native molecule. In some, the ApoA-I mimetics are peptides or peptide analogues that form amphipathic helices (in the presence of lipids), bind lipids, form pre- β -like or HDL-like

complexes, activate lecithin:cholesterol acyltransferase (LCAT), increase serum levels of HDL fractions, and promote cholesterol efflux.

The present invention is not limited to use of a particular ApoA-I mimetic. In some embodiments, any of the ApoA-I mimetics described in Srinivasa, et al., 2014 Curr. Opinion Lipidology Vol. 25(4): 304-308 are utilized. In some embodiments, any of the ApoA-I mimetics
5 described in U.S. Patent Application Publication Nos. 20110046056 and 20130231459 are utilized.

In some embodiments, the “22A” ApoA-I mimetic is used (PVLDLFRELLNELLEALKQKCLK) (SEQ ID NO: 4) (see, Examples I-IV) (see, e.g., U.S.
10 Patent No. 7,566,695). In some embodiments, any of the following ApoA-I mimetics shown in Table 2 as described in U.S. Patent No. 7,566,695 are utilized:

Table 2. ApoA-I mimetics

SEQ ID NO	AMINO ACID SEQUENCE
(SEQ ID NO:1)	PVLDLFRELLNELLEZLKQKCLK
(SEQ ID NO:2)	GVLDLFRELLNELLEALKQKCLKK
(SEQ ID NO:3)	PVLDLFRELLNELLEWLKQKCLK
(SEQ ID NO:4)	PVLDLFRELLNELLEALKQKCLK
(SEQ ID NO:5)	pVLDLFRELLNELLEALKQKCLKK
(SEQ ID NO:6)	PVLDLFRELLNEXLEALKQKCLK
(SEQ ID NO:7)	PVLDLFKELLNELLEALKQKCLK
(SEQ ID NO:8)	PVLDLFRELLNEGLEALKQKCLK
(SEQ ID NO:9)	PVLDLFRELGNELLEALKQKCLK
(SEQ ID NO:10)	PVLDLFRELLNELLEAZKQKCLK
(SEQ ID NO:11)	PVLDLFKELLQELLEALKQKCLK
(SEQ ID NO:12)	PVLDLFRELLNELLEAGKQKCLK
(SEQ ID NO:13)	GVLDLFRELLNEGLEALKQKCLK
(SEQ ID NO:14)	PVLDLFRELLNELLEALOQOLO
(SEQ ID NO:15)	PVLDLFRELWNELLEALKQKCLK
(SEQ ID NO:16)	PVLDLLRELLNELLEALKQKCLK
(SEQ ID NO:17)	PVLELFKELLQELLEALKQKCLK
(SEQ ID NO:18)	GVLDLFRELLNELLEALKQKCLK
(SEQ ID NO:19)	pVLDLFRELLNEGLEALKQKCLK
(SEQ ID NO:20)	PVLDLFREGLNELLEALKQKCLK
(SEQ ID NO:21)	pVLDLFRELLNELLEALKQKCLK
(SEQ ID NO:22)	PVLDLFRELLNELLEGLKQKCLK
(SEQ ID NO:23)	PLLELFKELLQELLEALKQKCLK
(SEQ ID NO:24)	PVLDLFRELLNELLEALQKCLK
(SEQ ID NO:25)	PVLDFFRELLNEXLEALKQKCLK

(SEQ ID NO:26)	PVLDLDFRELLNELLELLKQKLLK
(SEQ ID NO:27)	PVLDLDFRELLNELZEALKQKLLK
(SEQ ID NO:28)	PVLDLDFRELLNELWEALKQKLLK
(SEQ ID NO:29)	AVLDLDFRELLNELLEALKQKLLK
(SEQ ID NO:30)	PVLDLDFRELLNELLEALKQKLLK ¹
(SEQ ID NO:31)	PVLDLDFLELLNEXLEALKQKLLK
(SEQ ID NO:32)	XVLDLDFRELLNELLEALKQKLLK
(SEQ ID NO:33)	PVLDLDFREKLNELLEALKQKLLK
(SEQ ID NO:34)	PVLDZFRELLNELLEALKQKLLK
(SEQ ID NO:35)	PVLDWFRELLNELLEALKQKLLK
(SEQ ID NO:36)	PLLELLKELLQELLEALKQKLLK
(SEQ ID NO:37)	PVLDLDFREWLNELLEALKQKLLK
(SEQ ID NO:38)	PVLDLDFRELLNEXLEAWKQKLLK
(SEQ ID NO:39)	PVLDLDFRELLEELLKALKKLLK
(SEQ ID NO:40)	PVLDLDFNELLRELLEALQKLLK
(SEQ ID NO:41)	PVLDLDFWRELLNEXLEALKQKLLK
(SEQ ID NO:42)	PVLDEFREKLNEXWEALKQKLLK
(SEQ ID NO:43)	PVLDEFREKLWEXLEALKQKLLK
(SEQ ID NO:44)	pvldefreklneXlealkqkllk
(SEQ ID NO:45)	PVLDEFREKLNEXLEALKQKLLK
(SEQ ID NO:46)	PVLDLDFREKLNEXLEALKQKLLK
(SEQ ID NO:47)	~VLDLDFRELLNEGLEALKQKLLK
(SEQ ID NO:48)	pvLDLDFRELLNELLEALKQKLLK
(SEQ ID NO:49)	PVLDLDFRNLLLEKLLLEALEQKLLK
(SEQ ID NO:50)	PVLDLDFRELLWEXLEALKQKLLK
(SEQ ID NO:51)	PVLDLDFWELLNEXLEALKQKLLK
(SEQ ID NO:52)	PVWDEFREKLNEXLEALKQKLLK
(SEQ ID NO:53)	VVLDLDFRELLNELLEALKQKLLK
(SEQ ID NO:54)	PVLDLDFRELLNEWLEALKQKLLK
(SEQ ID NO:55)	P~~~LFRELLNELLEALKQKLLK
(SEQ ID NO:56)	PVLDLDFRELLNELLEALKQKLLK
(SEQ ID NO:57)	PVLDLDFRNLLLEELLKALEQKLLK
(SEQ ID NO:58)	PVLDEFREKLNEXLEALKQKLL~
(SEQ ID NO:59)	LVLDLDFRELLNELLEALKQKLLK
(SEQ ID NO:60)	PVLDLDFRELLNELLEALKQ~~~
(SEQ ID NO:61)	PVLDEFRWKLNEXLEALKQKLLK
(SEQ ID NO:62)	PVLDEWREKLNEXLEALKQKLLK
(SEQ ID NO:63)	PVLDLDFREKLNEXLEALKQKLLK
(SEQ ID NO:64)	PWLDEFREKLNEXLEALKQKLLK
(SEQ ID NO:65)	~VLDEFREKLNEXLEALKQKLLK
(SEQ ID NO:66)	PVLDLDFRNLLLEELLEALQKLLK
(SEQ ID NO:67)	~VLDLDFRELLNELLEALKQKLLK

(SEQ ID NO:68)	PVLDEFRELLKEXLEALKQKCLK
(SEQ ID NO:69)	PVLDEFRKKLNEXLEALKQKCLK
(SEQ ID NO:70)	PVLDEFRELLYEXLEALKQKCLK
(SEQ ID NO:71)	PVLDEFREKLNELXEALKQKCLK
(SEQ ID NO:72)	PVLDLFRELLNEXLWALKQKCLK
(SEQ ID NO:73)	PVLDEFWEKLNEXLEALKQKCLK
(SEQ ID NO:74)	PVLDKFREKLNEXLEALKQKCLK
(SEQ ID NO:75)	PVLDEFREKLNEELEALKQKCLK
(SEQ ID NO:76)	PVLDEFRELLFEXLEALKQKCLK
(SEQ ID NO:77)	PVLDEFREKLNKXLEALKQKCLK
(SEQ ID NO:78)	PVLDEFRDKLNEXLEALKQKCLK
(SEQ ID NO:79)	PVLDEFRELLNELLEALKQKCLK
(SEQ ID NO:80)	PVLDLFERLLNELLEALQKCLK
(SEQ ID NO:81)	PVLDEFREKLNWXLEALKQKCLK
(SEQ ID NO:82)	~~LDEFREKLNEXLEALKQKCLK
(SEQ ID NO:83)	PVLDEFREKLNEXLEALWQKCLK
(SEQ ID NO:84)	PVLDEFREKLNELLEALKQKCLK
(SEQ ID NO:85)	P~LDLFRELLNELLEALKQKCLK
(SEQ ID NO:86)	PVLELLEFERLLDELLNALQKCLK
(SEQ ID NO:87)	pllellkellqellealkqclk
(SEQ ID NO:88)	PVLDKFRELLNEXLEALKQKCLK
(SEQ ID NO:89)	PVLDEFREKLNEXLWALKQKCLK
(SEQ ID NO:90)	~~~DEFREKLNEXLEALKQKCLK
(SEQ ID NO:91)	PVLDEFRELLNEXLEALKQKCLK
(SEQ ID NO:92)	PVLDEFRELYNEXLEALKQKCLK
(SEQ ID NO:93)	PVLDEFREKLNEXLKALKQKCLK
(SEQ ID NO:94)	PVLDEFREKLNLEALKQKCLK
(SEQ ID NO:95)	PVLDLFRELLNLXLEALKQKCLK
(SEQ ID NO:96)	pvlldfrellneXlealkqclk
(SEQ ID NO:97)	PVLDLFRELLNELLE~~~~~
(SEQ ID NO:98)	PVLDLFRELLNEELEALKQKCLK
(SEQ ID NO:99)	KLKQKLAELLENLLERFLDLVP
(SEQ ID NO:100)	pvlldfrellnellealkqclk
(SEQ ID NO:101)	PVLDLFRELLNWXLEALKQKCLK
(SEQ ID NO:102)	PVLDLFRELLNLXLEALKEKCLK
(SEQ ID NO:103)	PVLDEFRELLNEELEALKQKCLK
(SEQ ID NO:104)	P~~~~~LLNELLEALKQKCLK
(SEQ ID NO:105)	PAADAFREAAAEAAEAQKQKAK
(SEQ ID NO:106)	PVLDLFREKLNEELEALKQKCLK
(SEQ ID NO:107)	klkqklaellenlflldlvp
(SEQ ID NO:108)	PVLDLFRWLLNEXLEALKQKCLK
(SEQ ID NO:109)	PVLDEFREKLNERLEALKQKCLK

(SEQ ID NO:110)	PVLDEFREKLNEXXEALKQKCLK
(SEQ ID NO:111)	PVLDEFREKLWEXWEALKQKCLK
(SEQ ID NO:112)	PVLDEFREKLNEXSEALKQKCLK
(SEQ ID NO:113)	PVLDEFREKLNEPLEALKQKCLK
(SEQ ID NO:114)	PVLDEFREKLNEXMEALKQKCLK
(SEQ ID NO:115)	PKLDEFREKLNEXLEALKQKCLK
(SEQ ID NO:116)	PHLDEFREKLNEXLEALKQKCLK
(SEQ ID NO:117)	PELDEFREKLNEXLEALKQKCLK
(SEQ ID NO:118)	PVLDEFREKLNEXLEALEQKCLK
(SEQ ID NO:119)	PVLDEFREKLNEELEAXKQKCLK
(SEQ ID NO:120)	PVLDEFREKLNEELEXLKQKCLK
(SEQ ID NO:121)	PVLDEFREKLNEELEALWQKCLK
(SEQ ID NO:122)	PVLDEFREKLNEELEWLKQKCLK
(SEQ ID NO:123)	QVLDFRELLNELLEALKQKCLK
(SEQ ID NO:124)	PVLDFOELLNELLEALOQOLO
(SEQ ID NO:125)	NVLDFRELLNELLEALKQKCLK
(SEQ ID NO:126)	PVLDFRELLNELGEALKQKCLK
(SEQ ID NO:127)	PVLDFRELLNELLELLKQKCLK
(SEQ ID NO:128)	PVLDFRELLNELLEFLKQKCLK
(SEQ ID NO:129)	PVLELFNDLLRELLEALQKCLK
(SEQ ID NO:130)	PVLELFNDLLRELLEALKQKCLK
(SEQ ID NO:131)	PVLELFKELLNELLDALRQKCLK
(SEQ ID NO:132)	PVLDFRELLNLEALQKCLK
(SEQ ID NO:133)	PVLELFERLLEDLLQALNKKCLK
(SEQ ID NO:134)	PVLELFERLLEDLLKALNOKCLK
(SEQ ID NO:135)	DVLDFRELLNELLEALKQKCLK
(SEQ ID NO:136)	PALELFKDLLQELLEALKQKCLK
(SEQ ID NO:137)	PVLDFRELLNEGLEAZKQKCLK
(SEQ ID NO:138)	PVLDFRELLNEGLEWLKQKCLK
(SEQ ID NO:139)	PVLDFRELWNEGLEALKQKCLK
(SEQ ID NO:140)	PVLDFRELLNEGLEALOQOLO
(SEQ ID NO:141)	PVLDFRELLNEGLEALKQKCLK
(SEQ ID NO:142)	PVLELFRELLNEGLEALKQKCLK
(SEQ ID NO:143)	PVLDFRELLNEGLEALKQKCLK*
(SEQ ID NO:144)	pVLELFENLLERLLDALQKCLK
(SEQ ID NO:145)	GVLELFENLLERLLDALQKCLK
(SEQ ID NO:146)	PVLELFENLLERLLDALQKCLK
(SEQ ID NO:147)	PVLELFENLLERLFDALQKCLK
(SEQ ID NO:148)	PVLELFENLLERLGDALQKCLK
(SEQ ID NO:149)	PVLELFENLWERLLDALQKCLK
(SEQ ID NO:150)	PLLELFENLLERLLDALQKCLK
(SEQ ID NO:151)	PVLELFENLGERLLDALQKCLK

(SEQ ID NO:152)	PVFELFENLLERLLDALQKKLK
(SEQ ID NO:153)	AVLELFENLLERLLDALQKKLK
(SEQ ID NO:154)	PVLELFENLLERGLDALQKKLK
(SEQ ID NO:155)	PVLELFLNLWERLLDALQKKLK
(SEQ ID NO:156)	PVLELFLNLLERLLDALQKKLK
(SEQ ID NO:157)	PVLEFFENLLERLLDALQKKLK
(SEQ ID NO:158)	PVLELFLNLLERLLDWLQKKLK
(SEQ ID NO:159)	PVLDLFENLLERLLDALQKKLK
(SEQ ID NO:160)	PVLELFENLLERLLDWLQKKLK
(SEQ ID NO:161)	PVLELFENLLERLLEALQKKLK
(SEQ ID NO:162)	PVLELFENWLERLLDALQKKLK
(SEQ ID NO:163)	PVLELFENLLERLWDALQKKLK
(SEQ ID NO:164)	PVLELFENLLERLLDAWQKKLK
(SEQ ID NO:165)	PVLELFENLLERLLDLLQKKLK
(SEQ ID NO:166)	PVLELFLNLLKLLDALQKKLK
(SEQ ID NO:167)	PVLELFENGLERLLDALQKKLK
(SEQ ID NO:168)	PVLELFEQLLEKLLDALQKKLK
(SEQ ID NO:169)	PVLELFENLLEKLLDALQKKLK
(SEQ ID NO:170)	PVLELFENLLEOLLDALQOOLO
(SEQ ID NO:171)	PVLELFENLLEKLLDLLQKKLK
(SEQ ID NO:172)	PVLELFLNLLERLGDALQKKLK
(SEQ ID NO:173)	PVLDLFDNLLDRLLDLLNKKLK
(SEQ ID NO:174)	pvelfenllerlldalqkkk
(SEQ ID NO:175)	PVLELFENLLERLLELLNKKLK
(SEQ ID NO:176)	PVLELWENLLERLLDALQKKLK
(SEQ ID NO:177)	GVLELFLNLLERLLDALQKKLK
(SEQ ID NO:178)	PVLELFDNLLKLEALQKKLR
(SEQ ID NO:179)	PVLELFDNLLERLLDALQKKLK
(SEQ ID NO:180)	PVLELFDNLLDKLLDALQKKLR
(SEQ ID NO:181)	PVLELFENLLERWLDALQKKLK
(SEQ ID NO:182)	PVLELFENLLEKLEALQKKLK
(SEQ ID NO:183)	PLLELFENLLEKLLDALQKKLK
(SEQ ID NO:184)	PVLELFLNLLERLLDAWQKKLK
(SEQ ID NO:185)	PVLELFENLLERLLDALQOOLO
(SEQ ID NO:186)	PVLELFEQLLERLLDALQKKLK
(SEQ ID NO:187)	PVLELFENLLERLLDALNKKLK
(SEQ ID NO:188)	PVLELFENLLDRLLDALQKKLK
(SEQ ID NO:189)	DVLELFENLLERLLDALQKKLK
(SEQ ID NO:190)	PVLEFWDNLLDKLLDALQKKLR
(SEQ ID NO:191)	PVLDLLRELLEELKQK*KLK*
(SEQ ID NO:192)	PVLDLDFKELLEELKQK*KLK*
(SEQ ID NO:193)	PVLDLDFRELLEELKQK*KLK*

(SEQ ID NO:194)	PVLELFFRELLEELKQK*KLK*
(SEQ ID NO:195)	PVLELFFKELLEELKQK*KLK*
(SEQ ID NO:196)	PVLDLFFRELLEELKKN*KLK*
(SEQ ID NO:197)	PLLDLFFRELLEELKQK*KLK*
(SEQ ID NO:198)	GVLDLFFRELLEELKQK*KLK*
(SEQ ID NO:199)	PVLDLFFRELWEELKQK*KLK*
(SEQ ID NO:200)	NVLDLFFRELLEELKQK*KLK*
(SEQ ID NO:201)	PLLDLFFKELLEELKQK*KLK*
(SEQ ID NO:202)	PALELFFKDLLEELRQK*LR*
(SEQ ID NO:203)	AVLDLFFRELLEELKQK*KLK*
(SEQ ID NO:204)	PVLDLFFRELLEELKQK*KLK*
(SEQ ID NO:205)	PVLDLFFREWLEELKQK*KLK*
(SEQ ID NO:206)	PLLELLKELLEELKQK*KLK*
(SEQ ID NO:207)	PVLELLKELLEELKQK*KLK*
(SEQ ID NO:208)	PALELFFKDLLEELRQK*RLK*
(SEQ ID NO:209)	PVLDLFFRELLNELLQK*KLK
(SEQ ID NO:210)	PVLDLFFRELLEELKQK*KLK
(SEQ ID NO:211)	PVLDLFFRELLEELOQO*LO*
(SEQ ID NO:212)	PVLDLFFOELLEELOQO*LK*
(SEQ ID NO:213)	PALELFFKDLLEEFRQK*RLK*
(SEQ ID NO:214)	pVLDLFFRELLEELKQK*KLK*
(SEQ ID NO:215)	PVLDLFFRELLEEWKQK*KLK*
(SEQ ID NO:216)	PVLELFFKELLEELKQK*KLK
(SEQ ID NO:217)	PVLDLFFRELLELLKQK*KLK
(SEQ ID NO:218)	PVLDLFFRELLNELLQK*KLK*
(SEQ ID NO:219)	PVLDLFFRELLNELWQK*KLK
(SEQ ID NO:220)	PVLDLFFRELLEELQK*KLK
(SEQ ID NO:221)	DVLDLFFRELLEELKQK*KLK*
(SEQ ID NO:222)	PVLDLFFRELLEALLQK*KLK
(SEQ ID NO:223)	PVLDLFFRELLEALAQK*KLK
(SEQ ID NO:224)	PVLDLFFREGWEELKQK*KLK
(SEQ ID NO:225)	PVLDLFFRELAEALAQK*KLK
(SEQ ID NO:226)	PVLDLFFRELGEALLQK*KLK
(SEQ ID NO:227)	PVLDLFFRELGEELKQK*KLK*
(SEQ ID NO:228)	PVLDLFFREGLEELKQK*KLK*
(SEQ ID NO:229)	PVLDLFFRELLEEGKQK*KLK*
(SEQ ID NO:230)	PVLELFFERLLEDLQK*KLK
(SEQ ID NO:231)	PVLDLFFRELLEKLEQK*KLK
(SEQ ID NO:232)	PLLELFFKELLEELKQK*KLK*
(SEQ ID NO:233)	LDDLLQKWAEAFNQLL*KLK
(SEQ ID NO:234)	EWLKAFFYEKVLK*KLKELF*
(SEQ ID NO:235)	EWLEAFYK*KLKVLK*KLKELF*

(SEQ ID NO:236)	DWLKAFYDKVAEKLKEAF*
(SEQ ID NO:237)	DWFKAFYDKVFEKFKEFF
(SEQ ID NO:238)	GIKKFLGSIWKFIKAFVG
(SEQ ID NO:239)	DWFKAFYDKVAEKFKEAF
(SEQ ID NO:240)	DWLKAFYDKVAEKLKEAF
(SEQ ID NO:241)	DWLKAFYDKVFEKFKEFF
(SEQ ID NO:242)	EWLEAFYKKVLEKLELPEL
(SEQ ID NO:243)	DWFKAFYDKFFEKFKEFF
(SEQ ID NO:244)	EWLKAFYEKLVLEKLELPEL
(SEQ ID NO:245)	EWLKAEYEKVEEKLELPEL*
(SEQ ID NO:246)	EWLKAEYEKLVLEKLELPEL*
(SEQ ID NO:247)	EWLKAFYKKVLEKLELPEL*
(SEQ ID NO:248)	PVLDFRELLEKQK*
(SEQ ID NO:249)	PVLDFRELLEELKQK*
(SEQ ID NO:250)	PVLDFRELLEKLEKQK*
(SEQ ID NO:251)	PVLDFRELLEKLEKQK*
(SEQ ID NO:252)	PVLDFRELLEALKQK*
(SEQ ID NO:253)	PVLDFENLLERLEKQK*
(SEQ ID NO:254)	PVLDFRELLNELKQK*

* indicates peptides that are N-terminal acetylated and C-terminal amidated; indicates peptides that are N-terminal dansylated; sp indicates peptides that exhibited solubility problems under the experimental conditions; X is Aib; Z is Nal; O is Orn; He (%) designates percent helicity; mics designates micelles; and ~ indicates deleted amino acids.

In some embodiments, an ApoA-I mimetic having the following sequence as described in U.S. Patent No. 6,743,778 is utilized: Asp Trp Leu Lys Ala Phe Tyr Asp Lys Val Ala Glu Lys
 10 Leu Lys Glu Ala Phe (SEQ ID NO:255).

In some embodiments, any of the following ApoA-I mimetics shown in Table 3 as described in U.S. Patent Application Publication No. 2003/0171277 are utilized:

Table 3.

SEQ ID NO	AMINO ACID SEQUENCE
(SEQ ID NO:256)	D-W-L-K-A-F-Y-D-K-V-A-E-K-L-K-E-A-F
(SEQ ID NO:257)	Ac-D-W-L-K-A-F-Y-D-K-V-A-E-K-L-K-E-A-F-NH ₂
(SEQ ID NO:258)	Ac-D-W-F-K-A-F-Y-D-K-V-A-E-K-L-K-E-A-F-NH ₂
(SEQ ID NO:259)	Ac-D-W-L-K-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH ₂
(SEQ ID NO:260)	Ac-D-W-F-K-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH ₂

(SEQ ID NO:261)	Ac-D-W-L-K-A-F-Y-D-K-V-F-E-K-F-K-E-F-F-NH ₂
(SEQ ID NO:262)	Ac-D-W-L-K-A-F-Y-D-K-F-F-E-K-F-K-E-F-F-NH ₂
(SEQ ID NO:263)	Ac-D-W-F-K-A-F-Y-D-K-F-F-E-K-F-K-E-F-F-NH ₂
(SEQ ID NO:264)	Ac-D-W-L-K-A-F-Y-D-K-V-A-E-K-L-K-E-F-F-NH ₂
(SEQ ID NO:265)	Ac-D-W-L-K-A-F-Y-D-K-V-F-E-K-F-K-E-A-F-NH ₂
(SEQ ID NO:266)	Ac-D-W-L-K-A-F-Y-D-K-V-F-E-K-L-K-E-F-F-NH ₂
(SEQ ID NO:267)	Ac-D-W-L-K-A-F-Y-D-K-V-A-E-K-F-K-E-F-F-NH ₂
(SEQ ID NO:268)	Ac-D-W-L-K-A-F-Y-D-K-V-F-E-K-F-K-E-F-F-NH ₂
(SEQ ID NO:269)	Ac-E-W-L-K-L-F-Y-E-K-V-L-E-K-F-K-E-A-F-NH ₂
(SEQ ID NO:270)	Ac-E-W-L-K-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH ₂
(SEQ ID NO:271)	Ac-E-W-L-K-A-F-Y-D-K-V-A-E-K-L-K-E-F-F-NH ₂
(SEQ ID NO:272)	Ac-E-W-L-K-A-F-Y-D-K-V-F-E-K-F-K-E-A-F-NH ₂
(SEQ ID NO:273)	Ac-E-W-L-K-A-F-Y-D-K-V-F-E-K-L-K-E-F-F-NH ₂
(SEQ ID NO:274)	Ac-E-W-L-K-A-F-Y-D-K-V-A-E-K-F-K-E-F-F-NH ₂
(SEQ ID NO:275)	Ac-E-W-L-K-A-F-Y-D-K-V-F-E-K-F-K-E-F-F-NH ₂
(SEQ ID NO:276)	Ac-A-F-Y-D-K-V-A-E-K-L-K-E-A-F-NH ₂
(SEQ ID NO:277)	Ac-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH ₂
(SEQ ID NO:278)	Ac-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH ₂
(SEQ ID NO:279)	Ac-A-F-Y-D-K-F-F-E-K-F-K-E-F-F-NH ₂
(SEQ ID NO:280)	Ac-A-F-Y-D-K-F-F-E-K-F-K-E-F-F-NH ₂
(SEQ ID NO:281)	Ac-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH ₂
(SEQ ID NO:282)	Ac-A-F-Y-D-K-V-A-E-K-L-K-E-F-F-NH ₂
(SEQ ID NO:283)	Ac-A-F-Y-D-K-V-F-E-K-F-K-E-A-F-NH ₂
(SEQ ID NO:284)	Ac-A-F-Y-D-K-V-F-E-K-L-K-E-F-F-NH ₂
(SEQ ID NO:285)	Ac-A-F-Y-D-K-V-A-E-K-F-K-E-F-F-NH ₂
(SEQ ID NO:286)	Ac-K-A-F-Y-D-K-V-F-E-K-F-K-E-F-NH ₂
(SEQ ID NO:287)	Ac-L-F-Y-E-K-V-L-E-K-F-K-E-A-F-NH ₂
(SEQ ID NO:288)	Ac-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH ₂
(SEQ ID NO:289)	Ac-A-F-Y-D-K-V-A-E-K-L-K-E-F-F-NH ₂
(SEQ ID NO:290)	Ac-A-F-Y-D-K-V-F-E-K-F-K-E-A-F-NH ₂
(SEQ ID NO:291)	Ac-A-F-Y-D-K-V-F-E-K-L-K-E-F-F-NH ₂
(SEQ ID NO:292)	Ac-A-F-Y-D-K-V-A-E-K-F-K-E-F-F-NH ₂
(SEQ ID NO:293)	Ac-A-F-Y-D-K-V-F-E-K-F-K-E-F-F-NH ₂
(SEQ ID NO:294)	Ac-D-W-L-K-A-L-Y-D-K-V-A-E-K-L-K-E-A-L-NH ₂

(SEQ ID NO:295)	Ac-D-W-F-K-A-F-Y-E-K-V-A-E-K-L-K-E-F-F-NH ₂
(SEQ ID NO:296)	Ac-D-W-F-K-A-F-Y-E-K-F-F-E-K-F-K-E-F-F-NH ₂
(SEQ ID NO:297)	Ac-E-W-L-K-A-L-Y-E-K-V-A-E-K-L-K-E-A-L-NH ₂
(SEQ ID NO:298)	Ac-E-W-L-K-A-F-Y-E-K-V-A-E-K-L-K-E-A-F-NH ₂
(SEQ ID NO:299)	Ac-E-W-F-K-A-F-Y-E-K-V-A-E-K-L-K-E-F-F-NH ₂
(SEQ ID NO:300)	Ac-E-W-L-K-A-F-Y-E-K-V-F-E-K-F-K-E-F-F-NH ₂
(SEQ ID NO:301)	Ac-E-W-L-K-A-F-Y-E-K-F-F-E-K-F-K-E-F-F-NH ₂
(SEQ ID NO:302)	Ac-E-W-F-K-A-F-Y-E-K-F-F-E-K-F-K-E-F-F-NH ₂
(SEQ ID NO:303)	Ac-D-F-L-K-A-W-Y-D-K-V-A-E-K-L-K-E-A-W-NH ₂
(SEQ ID NO:304)	Ac-E-F-L-K-A-W-Y-E-K-V-A-E-K-L-K-E-A-W-NH ₂
(SEQ ID NO:305)	Ac-D-F-W-K-A-W-Y-D-K-V-A-E-K-L-K-E-W-W-NH ₂
(SEQ ID NO:306)	Ac-E-F-W-K-A-W-Y-E-K-V-A-E-K-L-K-E-W-W-NH ₂
(SEQ ID NO:307)	Ac-D-K-L-K-A-F-Y-D-K-V-F-E-W-A-K-E-A-F-NH ₂
(SEQ ID NO:308)	Ac-D-K-W-K-A-V-Y-D-K-F-A-E-A-F-K-E-F-L-NH ₂
(SEQ ID NO:309)	Ac-E-K-L-K-A-F-Y-E-K-V-F-E-W-A-K-E-A-F-NH ₂
(SEQ ID NO:310)	Ac-E-K-W-K-A-V-Y-E-K-F-A-E-A-F-K-E-F-L-NH ₂
(SEQ ID NO:311)	Ac-D-W-L-K-A-F-V-D-K-F-A-E-K-F-K-E-A-Y-NH ₂
(SEQ ID NO:312)	Ac-E-K-W-K-A-V-Y-E-K-F-A-E-A-F-K-E-F-L-NH ₂
(SEQ ID NO:313)	Ac-D-W-L-K-A-F-V-Y-D-K-V-F-K-L-K-E-F-F-NH ₂
(SEQ ID NO:314)	Ac-E-W-L-K-A-F-V-Y-E-K-V-F-K-L-K-E-F-F-NH ₂
(SEQ ID NO:315)	Ac-D-W-L-R-A-F-Y-D-K-V-A-E-K-L-K-E-A-F-NH ₂
(SEQ ID NO:316)	Ac-E-W-L-R-A-F-Y-E-K-V-A-E-K-L-K-E-A-F-NH ₂
(SEQ ID NO:317)	Ac-D-W-L-K-A-F-Y-D-R-V-A-E-K-L-K-E-A-F-NH ₂
(SEQ ID NO:318)	Ac-E-W-L-K-A-F-Y-E-R-V-A-E-K-L-K-E-A-F-NH ₂
(SEQ ID NO:319)	Ac-D-W-L-K-A-F-Y-D-K-V-A-E-R-L-K-E-A-F-NH ₂
(SEQ ID NO:320)	Ac-E-W-L-K-A-F-Y-E-K-V-A-E-R-L-K-E-A-F-NH ₂
(SEQ ID NO:321)	Ac-D-W-L-K-A-F-Y-D-K-V-A-E-K-L-R-E-A-F-NH ₂
(SEQ ID NO:322)	Ac-E-W-L-K-A-F-Y-E-K-V-A-E-K-L-R-E-A-F-NH ₂
(SEQ ID NO:323)	Ac-D-W-L-K-A-F-Y-D-R-V-A-E-R-L-K-E-A-F-NH ₂
(SEQ ID NO:324)	Ac-E-W-L-K-A-F-Y-E-R-V-A-E-R-L-K-E-A-F-NH ₂
(SEQ ID NO:325)	Ac-D-W-L-R-A-F-Y-D-K-V-A-E-K-L-R-E-A-F-NH ₂
(SEQ ID NO:326)	Ac-E-W-L-R-A-F-Y-E-K-V-A-E-K-L-R-E-A-F-NH ₂
(SEQ ID NO:327)	Ac-D-W-L-R-A-F-Y-D-R-V-A-E-K-L-K-E-A-F-NH ₂
(SEQ ID NO:328)	Ac-E-W-L-R-A-F-Y-E-R-V-A-E-K-L-K-E-A-F-NH ₂

(SEQ ID NO:329)	Ac-D-W-L-K-A-F-Y-D-K-V-A-E-R-L-R-E-A-F-NH ₂
(SEQ ID NO:330)	Ac-E-W-L-K-A-F-Y-E-K-V-A-E-R-L-R-E-A-F-NH ₂
(SEQ ID NO:331)	Ac-D-W-L-R-A-F-Y-D-K-V-A-E-R-L-K-E-A-F-NH ₂
(SEQ ID NO:332)	Ac-E-W-L-R-A-F-Y-E-K-V-A-E-R-L-K-E-A-F-NH ₂

In some embodiments, an ApoA-I mimetic having the following sequence as described in U.S. Patent Application Publication No. 2006/0069030 is utilized: F-A-E-K-F-K-E-A-V-K-D-Y-F-A-K-F-W-D (SEQ ID NO:333).

5 In some embodiments, an ApoA-I mimetic having the following sequence as described in U.S. Patent Application Publication No. 2009/0081293 is utilized:

DWFKAFYDKVAEKFEAF (SEQ ID NO: 334); DWLKAFYDKVAEKLKEAF (SEQ ID NO: 335); PALEDLRQGLLPVLESFKVFLSALEEYTKKLNTQ (SEQ ID NO: 336).

In some embodiments, an ApoA-I mimetic having one of the following sequences is
 10 utilized: WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341),
 LKLLDNWDSVTSTFSKLEOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLOEMS
 (SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO:
 345), PYLDDFQKKWQEEMELYRQKVE (SEQ ID NO: 346),
 PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDALRTHLA
 15 (SEQ ID NO: 348), PYSDELQRLAARLEALKENGG (SEQ ID NO: 349),
 ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351),
 PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ
 ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352),
 TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDYGKDLME (SEQ ID
 20 NO:355), KVKSPLOAEAKSYFEKSKE (SEQ ID NO:356),
 VLTALVAVAGARAEVSADOVATV (SEQ ID NO:357),
 NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358),
 LPVLVWLSIVLEGPAPAOGTPDVSS (SEQ ID NO:359),
 LPVLVVVLSIVLEGPAPAQGTPDVSS (SEQ ID NO:360), ALDKLKEFGNTLEDKARELIS
 25 (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362),
 HLRKLRKRLLRDADDLQKRLAVYOA (SEQ ID NO:363),
 AQAWGERLRARMEEMGSRTRDR (SEQ ID NO:364), LDEVKEQVAEVRACLEEQAQ
 (SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236),
 DWLKAFYDKVAEKLKEAFPDWAKAAYDKAAEKAKEAA (SEQ ID NO:366),
 30 PVLDLDFRELLNELLEALKQKL (SEQ ID NO:367), PVLDLDFRELLNELLEALKQKLA (SEQ

ID NO:368), PVLDFRELLNELLEALKQKLN (SEQ ID NO:4),
 PVLDFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDFRELLNELLEALKKLLK
 (SEQ ID NO:370), PVLDFRELLNELLEALKKLLA (SEQ ID NO:371),
 PLLDFRELLNELLEALKKLLA (SEQ ID NO:372), and
 5 EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

Amphipathic lipids include, for example, any lipid molecule which has both a hydrophobic and a hydrophilic moiety. Examples include phospholipids or glycolipids. Examples of phospholipids which may be used in the sHDL-TA nanoparticles include but are not limited to dipalmitoylphosphatidylcholine (DPPC), dioleoyl-*sn*-glycero-3-
 10 phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(*p*-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(*p*-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(*p*-maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-
 15 phosphoethanolamine-N-[4-(*p*-maleimidomethyl)cyclohexane-carboxamide], phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and combinations thereof. In some embodiments, the phospholipid is complexed with an imaging agent (e.g., rhodamine (Rhod)-labeled DOPE (DOPE-Rhod)). In some embodiments, the phospholipids are thiol reactive phospholipids such as, for example, Dioleoyl-*sn*-glycero-3-
 20 phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP), 1,2-dihexadecanoyl-*sn*-glycero-3-phosphothioethanol, or N-4-(*p*-maleimidophenyl)butyryl dipalmitoylphosphatidylethanolamine (MPB-DPPE)).

In some embodiments, exemplary phospholipids include, but are not limited to, small alkyl chain phospholipids, egg phosphatidylcholine, soybean phosphatidylcholine,
 25 dipalmitoylphosphatidylcholine, dimyristoylphosphatidylcholine, distearoylphosphatidylcholine 1-myristoyl-2-palmitoylphosphatidylcholine, 1-palmitoyl-2-myristoylphosphatidylcholine, 1-palmitoyl-2-stearoylphosphatidylcholine, 1-stearoyl-2-palmitoylphosphatidylcholine, dioleoylphosphatidylcholine dioleophosphatidylethanolamine, dilauroylphosphatidylglycerol phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol,
 30 phosphatidylglycerols, diphosphatidylglycerols such as dimyristoylphosphatidylglycerol, dipalmitoylphosphatidylglycerol, distearoylphosphatidylglycerol, dioleoylphosphatidylglycerol, dimyristoylphosphatidic acid, dipalmitoylphosphatidic acid, dimyristoylphosphatidylethanolamine, dipalmitoylphosphatidylethanolamine, dimyristoylphosphatidylserine, dipalmitoylphosphatidylserine, brain phosphatidylserine, brain

sphingomyelin, egg sphingomyelin, milk sphingomyelin, palmitoyl sphingomyelin, phytosphingomyelin, dipalmitoylsphingomyelin, distearoylsphingomyelin, dipalmitoylphosphatidylglycerol salt, phosphatidic acid, galactocerebroside, gangliosides, cerebroside, dilaurylphosphatidylcholine, (1,3)-D-mannosyl-(1,3)diglyceride, aminophenylglycoside, 3-cholesteryl-6'-(glycosylthio)hexyl ether glycolipids, and cholesterol and its derivatives. Phospholipid fractions including SM and palmitoylsphingomyelin can optionally include small quantities of any type of lipid, including but not limited to lysophospholipids, sphingomyelins other than palmitoylsphingomyelin, galactocerebroside, gangliosides, cerebroside, glycerides, triglycerides, and cholesterol and its derivatives.

10 In some embodiments, the sHDL nanoparticles have a molar ratio of phospholipid/ HDL apolipoprotein from 2 to 250 (e.g., 10 to 200, 20 to 100, 20 to 50, 30 to 40).

Generally, the sHDL nanoparticles so formed are spherical and have a diameter of from about 5 nm to about 20 nm (e.g., 4 – 75 nm, 4-60 nm, 4-50 nm, 4-22 nm, 6 – 18 nm, 8 – 15 nm, 8- 10 nm, etc.). In some embodiments, the sHDL nanoparticles are subjected to size exclusion chromatography to yield a more homogeneous preparation.

15 The present invention addresses the need for improved stable and targeted delivery (e.g., in vitro or in vivo) of biomacromolecules (e.g., peptides, nucleic acids, glycolipids). Indeed, the present invention addresses such needs through providing synthetic high density lipoprotein (sHDL) nanoparticles for stable and targeted delivery of biomacromolecules, including peptides, nucleic acids, and glycolipids.

20 Compared to other strategies, including conventional nanoparticle vehicles, sHDL nanoparticles have impressive biocompatibility and capacity for cargo loading. For example, the ultrasmall but tunable size (e.g., 10-20 nm) enables the sHDL nanoparticles to effectively drain to lymph nodes and deliver cargo peptide antigens and nucleic acid-based adjuvants to lymph node-resident dendritic cells, thus positioning them as an efficient platform for co-delivery of antigen and adjuvant for tumor immunotherapy. In addition, experiments conducted during the course of developing embodiments for the present invention demonstrated broad applicability of the sHDL-based approach by (1) targeting delivery of siRNA to hepatocytes, which are the natural target cells of endogenous HDL, and (2) targeting immunostimulatory glycolid (alpha-galactosyl ceramide) to antigen presenting cells.

30 Such experiments further demonstrated the engineering of sHDL nanoparticles prepared with phospholipids and Apolipoprotein A-I mimetic peptides and loaded with biomacromolecular drugs. To load peptide drugs on HDL nanodiscs, synthesized thiol-reactive phospholipids were utilized that allowed reduction-sensitive linkage of peptides on the surfaces

of HDL nanodiscs. To load nucleic acids (including CpG motifs and siRNA), nucleic acids were modified with a cholesteryl moiety, which was shown to allow facile insertion of nucleic acids into the sHDL nanoparticles. To load glycolipids into HDL, hydrophobic interactions between glycolipids and HDL were utilized. Such experiments further demonstrated stable delivery of such cargo to target tissues in vitro and in vivo.

RNA Interference

In certain embodiments, the sHDL nanoparticles are used within RNA interference methods and systems.

RNA interference is a highly conserved mechanism triggered by double-stranded RNA (dsRNA) and able to down regulate transcript of genes homologous to the dsRNA. The dsRNA is first processed by Dicer into short duplexes of 21-23 nt, called short interfering RNAs (siRNAs). Incorporated in RNA-induced silencing complex (RISC), they are able to mediate gene silencing through cleavage of the target mRNA. “siRNA” or “small-interfering ribonucleic acid” refers to two strands of ribonucleotides which hybridize along a complementary region under physiological conditions. The siRNA molecules comprise a double-stranded region which is substantially identical to a region of the mRNA of the target gene. A region with 100% identity to the corresponding sequence of the target gene is suitable. This state is referred to as “fully complementary”. However, the region may also contain one, two or three mismatches as compared to the corresponding region of the target gene, depending on the length of the region of the mRNA that is targeted, and as such may be not fully complementary. Methods to analyze and identify siRNAs with sufficient sequence identity in order to effectively inhibit expression of a specific target sequence are known in the art. A suitable mRNA target region would be the coding region. Also suitable are untranslated regions, such as the 5'-UTR, the 3'-UTR, and splice junctions as long as the regions are unique to the mRNA target and not directed to a mRNA poly A tail.

In some embodiments, siRNA encapsulated within sHDL nanoparticles are utilized conducting methods and systems involving RNA interference.

Such embodiments are not limited to a particular size or type of siRNA molecule. The length of the region of the siRNA complementary to the target, for example, may be from 15 to 100 nucleotides, 18 to 25 nucleotides, 20 to 23 nucleotides, or more than 15, 16, 17 or 18 nucleotides. Where there are mismatches to the corresponding target region, the length of the complementary region is generally required to be somewhat longer.

In certain embodiments, it is contemplated that the siRNA delivery approach using

sHDL nanoparticles disclosed herein (e.g., through encapsulation of the siRNA within an sHDL nanoparticle) can be used to inhibit any gene of interest.

The present invention is not limited to particular methods for generating sHDL nanoparticles comprising encapsulated siRNA molecules. For example, in some embodiments, lyophilization methods are used for the preparation of homogenous sHDL. In some
5 embodiments, phospholipids and ApoA mimetic peptides are dissolved in glacial acetic acid and lyophilized. In some embodiments, loading of an siRNA molecule into the sHDL nanoparticle is facilitated through cholesterol modification of the siRNA molecule. For example, the siRNA is modified with cholesterol at the 3' sense strand (e.g., Kuwahara, H.; et al., *Molecular Therapy*
10 2011, 19 (12), 2213-2221) and an intermediate level of chemical modification will be used to stabilize siRNA in the serum without significantly compromising its silencing effect (see, e.g., Behlke, M. A., *Oligonucleotides* 2008, 18 (4), 305-319). In some embodiments, the lyophilized phospholipids and ApoA mimetic peptides are hydrated (e.g., hydrated in PBS (pH 7.4)) and thermocycled above and below the transition temperature (T_m) of phospholipids to form blank
15 sHDL, which are next incubated with the cholesterol modified siRNA at room temperature for an optimal amount of time (e.g., 5, 10, 20, 25, 30, 35, 50, 80, 120, 360 minutes) to form sHDL comprising encapsulated siRNA. Fig. 10 presents a schematic of the lyophilization method for rapid preparation of sHDL comprising encapsulated siRNA.

Such embodiments are not limited to a particular manner of characterizing the sHDL
20 comprising encapsulated siRNA. In some embodiments, the morphology of sHDL is observed by TEM. In some embodiments, the size distribution of sHDL is analyzed by dynamic light scattering (DLS) using a Malven Nanosizer instrument and GPC assay.

Such embodiments are not limited to a particular manner of assessing the delivery profile of the siRNA in vitro and in vivo. In some embodiments, labelling the siRNA molecules with an
25 imaging agent (e.g., fluorescent dye Cy3) permits visualization of the biodistribution of siRNA molecules at the organ level and also the intracellular delivery profile. In some embodiments, RT-PCR and western blot are used to analyze the target protein at the mRNA level and protein level, respectively.

As such, in certain embodiments, the present invention provides methods for inhibiting a
30 target gene in a cell comprising introducing into the cell a siRNA capable of inhibiting the target gene by RNA interference, wherein the siRNA comprises two RNA strands that are complementary to each other, wherein the siRNA is encapsulated within a sHDL nanoparticle. In some embodiments, the siRNA is modified with cholesterol at the 3' sense strand. In some embodiments, the cell is within a human being.

In certain embodiments, sHDL nanoparticles are provided wherein siRNAs specific for proprotein convertase subtilisin/kexin 9 (PCSK9) are encapsulated within the sHDL nanoparticle. Compelling evidence has shown that an elevated plasma level of low-density lipoprotein cholesterol (LDL-C) is a cardinal risk factor for coronary heart disease (CHD) (see, 5 e.g., Law, M. R.; et al., *British Medical Journal* 2003, 326 (7404), 1423-1427; Boekholdt, S. M.; et al., *Jama-Journal of the American Medical Association* 2012, 307 (12), 1302-1309; Sniderman, A. D.; et al., *Circulation-Cardiovascular Quality and Outcomes* 2011, 4 (3), 337-U144). PCSK9 synthesized in the liver performs important roles in regulating LDL-C: PCSK9 can bind to the LDL receptor (LDLR) on hepatocytes and prevent the recycling of LDLR from 10 lysosomes to the cell surface, and this in turn leads to the down-regulation of LDLR and increased levels of LDL-C (see, e.g., Maxwell, K. N.; et al., *Proceedings of the National Academy of Sciences of the United States of America* 2004, 101 (18), 7100-7105; Dadu, R. T.; et al., *Nature Reviews Cardiology* 2014, 11 (10), 563-575; Horton, J. D.; et al., *Trends in Biochemical Sciences* 2007, 32 (2), 71-77). Therefore PCSK9 inhibition can potentially 15 decrease LDL-C (see, e.g., Shen, L.; et al., *Pharmacological Research* 2013, 73, 27-34). Therapeutic approaches under development for PCSK9 inhibition in vivo include siRNA-mediated knockdown of PCSK9 and vaccination against PCSK9 (see, e.g., Fitzgerald, K.; et al., *Lancet* 2014, 383 (9911), 60-68; Galabova, G.; et al., *Circulation* 2013, 128 (22)), but both strategies face the major challenge: how to efficiently deliver therapeutic agents to the target 20 cells, namely hepatocytes and immune cells, respectively, in order to maximize the in vivo efficacy of each strategy.

Previously, PCSK9 siRNA has been delivered to the hepatocytes by lipid nanoparticles (see, e.g., Frank-Kamenetsky, M.; et al., *Proceedings of the National Academy of Sciences of the United States of America* 2008, 105 (33), 11915-11920) or by conjugating siRNA to N- 25 acetylgalactosamine (GalNAc) ligands (see, e.g., Akinc, A.; et al., *Molecular Therapy* 2010, 18 (7), 1357-1364), which allow siRNA to be targeted to hepatocytes passively or through the recognition of Asialoglycoprotein Receptor (ASGPR) on hepatocytes. However, these conventional delivery strategies can subject the siRNA molecules to the intracellular endosomes/lysosomes pathway, in which siRNA cargo can be degraded, leading to suboptimal 30 knockdown of PCSK9. Therefore, developing strategies that can both target the hepatocyte and bypass the endosomes/lysosomes pathway are urgently needed.

Use of sHDL nanoparticles comprising encapsulated PCSK9 siRNA molecules overcomes such limitations. Indeed, sHDL nanoparticles have similar properties to endogenous HDL, which can intrinsically target hepatocytes after i.v. injection, thus permitting direct

delivery of siRNA cargoes to the cytosol of hepatocytes and knockdown of PCSK9 without going through the intracellular endosome/lysosome pathway.

In certain embodiments, sHDL comprising encapsulated PCSK9 siRNA molecules are delivered into the cytosol where they can associate with RNA-induced silencing complex (RISC) to knockdown the PCSK9 proteins (see, e.g., Chendrimada, T. P.; et al., *Nature* 2005, 436 (7051), 740-744; Matranga, C.; et al., *Cell* 2005, 123 (4), 607-620) within SR-BI expressing hepatocytes (see, e.g., Goldstein, J. L.; Brown, M. S., *Arteriosclerosis Thrombosis and Vascular Biology* 2009, 29 (4), 431-438; Wolfrum, C.; et al., *Nature Biotechnology* 2007, 25 (10), 1149-1157).

10 Fig. 11 shows a schematic of using sHDL to regulate PCSK9 for LDL-C management.

The present invention is not limited to use of a particular PCSK9 siRNA sequence. In some embodiments, the PCSK9 siRNA sequence is cross-reactive to murine, rat, nonhuman primate and human PCSK9 mRNA (see, e.g., Frank-Kamenetsky, et al., *Proceedings of the National Academy of Sciences of the United States of America* 2008, 105 (33), 11915-11920).

15 In certain embodiments, the present invention provides methods for inhibiting a PCSK9 gene in a cell comprising introducing into the cell a PCSK9 siRNA capable of inhibiting the PCSK9 gene by RNA interference, wherein the PCSK9 siRNA comprises two RNA strands that are complementary to each other, wherein the PCSK9 siRNA is encapsulated within a sHDL nanoparticle. In some embodiments, the PCSK9 siRNA is modified with cholesterol at the 3' sense strand. In some embodiments, the cell is within a human being.

20 In certain embodiments, the present invention provides methods for reducing serum LDL-C levels in patient (e.g., human patient), comprising administering to the patient a therapeutically effective amount of a pharmaceutical composition comprising a PCSK9 siRNA encapsulated within a sHDL nanoparticle, wherein the PCSK9 siRNA is capable of inhibiting the PCSK9 gene by RNA interference, wherein the PCSK9 siRNA comprises two RNA strands that are complementary to each other, wherein inhibiting of the PCSK9 gene results in reduction of serum LDL-C levels.

25 In certain embodiments, the present invention provides methods for treating coronary heart disease in a patient through reducing serum LDL-C levels in the patient, comprising administering to the patient a therapeutically effective amount of a pharmaceutical composition comprising a PCSK9 siRNA encapsulated within a sHDL nanoparticle, wherein the PCSK9 siRNA is capable of inhibiting the PCSK9 gene by RNA interference, wherein the PCSK9 siRNA comprises two RNA strands that are complementary to each other, wherein inhibiting of the PCSK9 gene results in reduction of serum LDL-C levels.

In certain embodiments, the sHDL nanoparticles are used to activate an immune response. Such embodiments are not limited to a particular manner of activating an immune response.

5 **Biomacromolecule Delivery**

In certain embodiments, the present invention provides compositions comprising a nanoparticle (as described herein), wherein any kind of biomacromolecule agent (e.g., nucleic acid, peptides, glycolipids, etc.) is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the nanoparticle.

10 In some embodiments, the biomacromolecule agent is a peptide.

For example, in some embodiments, the peptide is an antigen. In some embodiments wherein the peptide is an antigen, the composition further comprises an adjuvant (as described herein).

As noted, the peptide is not limited to a particular type of peptide.

15 In some embodiments, the peptide is Adrenocorticotrophic Hormone (ACTH), a growth hormone peptide, a Melanocyte Stimulating Hormone (MSH), Oxytocin, Vasopressin, Corticotropin Releasing Factor (CRF), a CRF-related peptide, a Gonadotropin Releasing Hormone Associated Peptide (GAP), Growth Hormone Releasing Factor (GRF), Lutenizing Hormone Release Hormone (LH-RH), an orexin, a Prolactin Releasing Peptide (PRP), a
20 somatostatin, Thyrotropin Releasing Hormone (THR), a THR analog, Calcitonin (CT), a CT-precursor peptide, a Calcitonin Gene Related Peptide (CGRP), a Parathyroid Hormone (PTH), a Parathyroid Hormone Related Protein (PTHrP), Amylin, Glucagon, Insulin, an Insulin-like peptide, NeuroPeptide Y (NPY), a Pancreatic Polypeptide (PP), Peptide YY (PYY), Cholecystokinin (CCK), a CCK-related peptide, Gastrin Releasing Peptide (GRP), Gastrin, a
25 Gastrin-related peptide, a Gastrin inhibitory peptide, Motilin, Secretin, Vasoactive Intestinal Peptide (VIP), a VIP-related peptide, an Atrial-Natriuretic Peptide (ANP), a Brain Natriuretic Peptide (BNP), a C-Type Natriuretic Peptide(CNP), a tachykinin, an angiotensin, a renin substrate, a renin inhibitor, an endothelin, an endothelin-related peptide, an opioid peptide, a thymic peptide, an adrenomedullin peptide, an allostatin peptide, an amyloid beta-protein
30 fragment, an antimicrobial peptide, an antioxidant peptide, an apoptosis related peptide, a Bag Cell Peptide (BCPs), Bombesin, a bone Gla protein peptide, a Cocaine and Amphetamine Related Transcript (CART) peptide, a cell adhesion peptide, a chemotactic peptide, a complement inhibitor, a cortistatin peptide, a fibronectin fragment, a fibrin related peptide, FMRF, a FMRF amide-related peptide (FaRP), Galanin, a Galanin-related peptide, a growth

factor, a growth factor-related peptide, a G-Therapeutic Peptide-Binding Protein fragment, Gualylin, Uroguanylin, an Inhibin peptide, Interleukin (IL), an Interleukin Receptor protein, a laminin fragment, a leptin fragment peptide, a leucokinin, Pituitary Adenylate Cyclase Activating Polypeptide (PAPCAP), Pancreastatin, a polypeptide repetitive chain, a signal
 5 transducing reagent, a thrombin inhibitor, a toxin, a trypsin inhibitor, a virus-related peptide, an adjuvant peptide analog, Alpha Mating Factor, Antiarrhythmic Peptide, Anorexigenic Peptide, Alpha-1 Antitrypsin, Bovine Pineal Antireproductive Peptide, Bursin, C3 Peptide P16, Cadherin Peptide, Chromogranin A Fragment, Contraceptive Tetrapeptide, Conantokin G, Conantokin T, Crustacean Cardioactive Peptide, C-Telopeptide, Cytochrome b588 Peptide,
 10 Decorsin, Delicious Peptide, Delta-Sleep-Inducing Peptide, Diazepam-Binding Inhibitor Fragment, Nitric Oxide Synthase Blocking Peptide, OVA Peptide, Platelet Calpain Inhibitor (P1), Plasminogen Activator Inhibitor 1, Rigin, Schizophrenia Related Peptide, Sodium Potassium Atherapeutic Peptidase Inhibitor-1, Speract, Sperm Activating Peptide, Systemin, a Thrombin receptor agonist, Tuftsin, Adipokinetic Hormone, Uremic Pentapeptide, Antifreeze
 15 Polypeptide, Tumor Necrosis Factor (TNF), Leech [Des Asp10]Decorsin, L-Ornithyltaurine Hydrochloride, P-Aminophenylacetyl Tuftsin, Ac-Glu-Glu-Val-Val-Ala-Cys-pNA, Ac-Ser-Asp-Lys-Pro, Ac-rfwink-NH₂, Cys-Gly-Tyr-Gly-Pro-Lys-Lys-Lys-Arg-Lys-Val-Gly-Gly, D-Ala-Leu, D-D-D-D-D, D-D-D-D-D-D, N-P-N-A-N-P-N-A, V-A-I-T-V-L-V-K, V-G-V-R-V-R, V-I-H-S, V-P-D-P-R, Val-Thr-Cys-Gly, R-S-R, Sea Urchin Sperm Activating Peptide, a SHU-9119
 20 antagonist, a MC3-R antagonist, a MC4-R antagonist, Glaspimod, HP-228, Alpha 2-Plasmin Inhibitor, APC Tumor Suppressor, Early Pregnancy Factor, Gamma Interferon, Glandular Kallikrein N-1, Placental Ribonuclease Inhibitor, Sarcolecine Binding Protein, Surfactant Protein D, Wilms' Tumor Suppressor, GABAB 1b Receptor Peptide, Prion Related Peptide (iPRP13), Choline Binding Protein Fragment, Telomerase Inhibitor, Cardiostatin Peptide, Endostatin
 25 Derived Peptide, Prion Inhibiting Peptide, N-Methyl D-Aspartate Receptor Antagonist, and C-Peptide Analog.

In some embodiments, the peptide is selected from 177Lu-DOTA0-Tyr3-Octreotate, Abarelix acetate, ADH-1, Afamelanotide, melanotan-1, CUV1647, Albiglutide, Aprotinin, Argipressin, Atosiban acetate, Bacitracin, Bentiromide, a BH3 domain, Bivalirudin, Bivalirudin
 30 trifluoroacetate hydrate, Blisibimod, Bortezomib, Buserelin, Buserelin acetate, Calcitonin, Carbetocin, Carbetocin acetate, Cecropin A and B, Ceruletide, Ceruletide diethylamine, Cetrorelix, Cetrorelix acetate, Ciclosporine, Cilengitide, EMD121974, Corticorelin acetate injection, hCRF, Corticorelin ovine triflutate, corticorelin trifluoroacetate, Corticotropin, Cosyntropin, ACTH 1-24, tetracosactide hexaacetate, Dalbavancin, Daptomycin, Degarelix

acetate, Depreotide trifluoroacetate (plus sodium pertechnetate), Desmopressin acetate, Desmopressin DDAVP, Dulaglutide, Ecallantide, Edotreotide (plus yttrium-90), Elcatonin acetate, Enalapril maleate (or 2-butanedioate), Enfuvirtide, Eptifibatide, Exenatide, Ganirelix acetate, Glatiramer acetate, Glutathion, Gonadorelin, Gonadorelin acetate, GnRH, LHRH, 5 Goserelin, Goserelin acetate, Gramicidin, Histrelin acetate, Human calcitonin, Icatibant, Icatibant acetate, IM862, oglufanide disodium, KLAKLAK, Lanreotide acetate, Lepirudin, Leuprolide, Leuprolide acetate, leuprorelin, Liraglutide, Lisinopril, Lixisenatide, Lypressin, Magainin2, MALP-2Sc, macrophage-activating lipopeptide-2 synthetic, Nafarelin acetate, Nesiritide, NGR-hTNF, Octreotide acetate, Oritavancin, Oxytocin, Pasireotide, Peginesatide, 10 Pentagastrin, Pentetreotide (plus indium-111), Phenypressin, Pleurocidin, Pramlintide, Protirelin, thyroliberin, TRH, TRF, Salmon calcitonin, Saralasin acetate, Secretin (human), Secretin (porcine), Semaglutide, Seractide acetate, ACTH, corticotropin, Sermorelin acetate, GRF 1-29, Sinapultide, KL4 in lucinactant, Sincalide, Somatorelin acetate, GHRH, GHRF, GRF, Somatostatin acetate, Spaglumat magnesium (or sodium) salt, Substance P, Taltirelin 15 hydrate, Teduglutide, Teicoplanin, Telavancin, Teriparatide, Terlipressin acetate, Tetracosactide, Thymalfasin, thymosin a-1, Thymopentin, Trebananib, Triptorelin, Triptorelin pamoate, Tyroserleutide, Ularitide, Vancomycin, Vapreotide acetate, Vasoactive intestinal peptide acetate, Vx-001c, TERT572Y, Ziconotide acetate, α 5- α 6 Bax peptide, and β -defensin.

In some embodiments, the peptide is any peptide which would assist in achieving a 20 desired purpose with the composition. For example, in some embodiments, the peptide is any peptide that will facilitate treatment of any type of disease and/or disorder (e.g., peripheral ischemia, cancer, inflammatory disorders, genetic disorders, etc.).

In some embodiments, the biomacromolecule agent is a nucleic acid. Such embodiments encompass any type of nucleic acid molecule including, but not limited to, RNA, siRNA, 25 microRNA, interference RNA, mRNA, replicon mRNA, RNA-analogues, and DNA.

In certain embodiments, the present invention provides methods for treating conditions, disorders and/or diseases with such compositions comprising a nanoparticle (as described herein), wherein any kind of biomacromolecule is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the nanoparticle.

30 The present invention is not limited to specific types of conditions, disorders and/or diseases.

In some embodiments, the condition, disorder and/or disease is peripheral ischemia, cancer, an inflammatory disorder, a genetic disorder, etc.

In some embodiments, the condition, disorder and/or disease is selected from erythropoietic porphyries, T2 diabetes, antifibrinolytic, central diabetes insipidus, delaying the birth in case of threat of premature birth, antibiotic, cystic fibrosis, angina, anticoagulant in patients with unstable angina undergoing PTCA or PCI, systemic lupus erythematosus, hypercalcemia, osteoporosis, pagets disease, carbetocin works as an oxytocic, antihemorrhagic and uterotonic drug in the peripheral nervous system, prevention of uterine atony, induction, and control postpartum bleeding or haemorrhage, stimulant of the gastric secretion, for treat hormone-sensitive cancers of the prostate and breast, inhibition of premature LH surges in women undergoing controlled ovarian stimulation, immunosuppression in organ transplantation to prevent rejection, peritumoral brain edema, diagnosis of ACTHdependent Cushing's syndrome, allergies, ankylosing spondylitis, psoriasis, chorioiditis, erythema, keratitis, sclerosis, dermatomyositis, rheumatoid arthritis, Stevens-Johnson Syndrome, ulcerative colitis, diagnosis of adrenocortical insufficiency, antibiotic, systemic infections caused by gram positive organisms, nocturnal enuresis, nocturia, and stoppage of bleeding or haemorrhage in haemophilia A patients, acute hereditary angioderma, postmenopausal osteoporosis, anti-parathyroid, Paget's disease, hypercalcaemia, hypertension, AIDS/HIV-1 infection, acute coronary syndrome, unstable angina undergoing PCI, Alzheimer's and Parkinson's disease, inhibition of premature LH surges in women undergoing controlled ovarian hyperstimulation, Relapsing- Remitting Multiple Sclerosis, hepatic insufficiency, wound healing, inflammation of respiratory tract, asthenia, release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary, stimulate the secretion of gonadotropin during disturbances fertility, and diagnosis of the functional capacity and response of the gonadotropes of the anterior pituitary, for skin lesions, surface wounds and eye infections, postmenopausal osteoporosis, Paget's disease, hypercalcaemia, hereditary angioedema, immune system related diseases, acromegaly, anticoagulant, fibroids and endometriosis, central diabetes insipidus, Cushing's syndrome, diabetic foot ulcers, treatment of central precocious puberty, uterine fibriods and endometriosis, vasodilatory, natriuretic, diuretic and neurohormonal effects, acromegaly, carcinoid syndrome, acute bacterial skin and skin structure infections, initiation or improvement of uterine contractions, and control postpartum bleeding or haemorrhage, hematide Chronic kidney disease associated anemia, stomatitis, pharyngitis, diagnostic assessment of thyroid function, postmenopausal osteoporosis, hypercalcaemia, diagnosis of pancreatic exocrine dysfunction, and gastrinoma, Zollinger-Ellison syndrome, prevention of RDS in premature infants, and meconium aspiration syndrome, acute variceal bleeding, allergic rhinitis and conjunctivitis, spinocerebellar degeneration/ataxia, Short Bowel Syndrome, antibiotic,

bactericidal, teriparatide is the only anabolic (i.e., bone growing) agent indicated for use in postmenopausal women with osteoporosis, Cortrosyn Analogue of adrenocorticotrophic hormone (ACTH) used for diagnostic purposes, treatment of adrenal insufficiency, epilepsy, Chronic hepatitis B, chronic hepatitis C, primary and secondary immune deficiencies, acute
5 decompensated heart failure, colitis, esophageal variceal bleeding in patients with cirrhotic liver disease and AIDS-related diarrhea, sarcoidosis and acute lung injury, and severe chronic pain.

In some embodiments, the following Table 1 recites a peptide and disorder and/or disease to be treated.

10 **Immune Response Stimulation**

The immune system can be classified into two functional subsystems: the innate and the acquired immune system. The innate immune system is the first line of defense against infections, and most potential pathogens are rapidly neutralized by this system before they can cause, for example, a noticeable infection. The acquired immune system reacts to molecular
15 structures, referred to as antigens, of the intruding organism. There are two types of acquired immune reactions, which include the humoral immune reaction and the cell-mediated immune reaction. In the humoral immune reaction, antibodies secreted by B cells into bodily fluids bind to pathogen-derived antigens, leading to the elimination of the pathogen through a variety of mechanisms, e.g. complement-mediated lysis. In the cell-mediated immune reaction, T-cells
20 capable of destroying other cells are activated. For example, if proteins associated with a disease are present in a cell, they are fragmented proteolytically to peptides within the cell. Specific cell proteins then attach themselves to the antigen or peptide formed in this manner and transport them to the surface of the cell, where they are presented to the molecular defense mechanisms, in particular T-cells, of the body. Cytotoxic T cells recognize these antigens and kill the cells
25 that harbor the antigens.

The molecules that transport and present peptides on the cell surface are referred to as proteins of the major histocompatibility complex (MHC). MHC proteins are classified into two types, referred to as MHC class I and MHC class II. The structures of the proteins of the two MHC classes are very similar; however, they have very different functions. Proteins of MHC
30 class I are present on the surface of almost all cells of the body, including most tumor cells. MHC class I proteins are loaded with antigens that usually originate from endogenous proteins or from pathogens present inside cells, and are then presented to naive or cytotoxic T-lymphocytes (CTLs). MHC class II proteins are present on dendritic cells, B-lymphocytes, macrophages and other antigen-presenting cells. They mainly present peptides, which are

processed from external antigen sources, i.e. outside of the cells, to T-helper (Th) cells. Most of the peptides bound by the MHC class I proteins originate from cytoplasmic proteins produced in the healthy host cells of an organism itself, and do not normally stimulate an immune reaction. Accordingly, cytotoxic T-lymphocytes that recognize such self-peptide-presenting MHC
5 molecules of class I are deleted in the thymus (central tolerance) or, after their release from the thymus, are deleted or inactivated, i.e. tolerized (peripheral tolerance). MHC molecules are capable of stimulating an immune reaction when they present peptides to non-tolerized T-lymphocytes. Cytotoxic T-lymphocytes have both T-cell receptors (TCR) and CD8 molecules on their surface. T-Cell receptors are capable of recognizing and binding peptides complexed
10 with the molecules of MHC class I. Each cytotoxic T-lymphocyte expresses a unique T-cell receptor which is capable of binding specific MHC/peptide complexes.

The peptide antigens attach themselves to the molecules of MHC class I by competitive affinity binding within the endoplasmic reticulum, before they are presented on the cell surface. Here, the affinity of an individual peptide antigen is directly linked to its amino acid sequence
15 and the presence of specific binding motifs in defined positions within the amino acid sequence. If the sequence of such a peptide is known, it is possible to manipulate the immune system against diseased cells using, for example, peptide vaccines.

Peptide-based cancer vaccines have been extensively investigated due to their good safety profiles and ease of manufacturing and quality control. However, their anti-tumor efficacy
20 in clinical trials have been disappointing, a phenomenon that has been attributed to inefficient codelivery of Ag peptides and adjuvants to draining lymph nodes (dLNs), and subsequent immunological tolerance and CTL fratricide (see, e.g., Toes, R.E., et al., Proc. Natl. Acad. Sci. U. S. A. 93, 7855-7860 (1996); Su, M.W., et al., J. Immunol. 151, 658-667 (1993); Melief, C.J. & van der Burg, S.H. Nat. Rev. Cancer 8, 351-360 (2008)). Although depot-forming
25 water-in-oil adjuvant systems can improve immunogenicity (see, e.g., Speiser, D.E. et al. J. Clin. Invest. 115, 739-746 (2005); Fourcade, J. et al. J. Immunother. 31, 781-791 (2008)), booster immunizations can cause T-cell sequestration at the vaccine site, causing T-cell exhaustion and deletion (see, e.g., Rezvani, K. et al. Haematologica 96, 432-440 (2011); Hailemichael, Y. et al. Nat. Med. 19, 465-472 (2013)). To address these issues, various nanoparticle-based vaccine
30 systems have been evaluated in animal models (see, e.g., Reddy, S.T. et al. Nat. Biotechnol. 25, 1159-1164 (2007); Li, A.V. et al. Sci. Transl. Med. 5, 204ra130 (2013); Jeanbart, L. et al. Cancer. Immunol. Res. 2, 436-447 (2014); Xu, Z., et al., ACS Nano 8, 3636-3645 (2014); Liu, H. et al. Nature 507, 519-522 (2014); Fan, Y. & Moon, J.J. Vaccines (Basel) 3, 662-685 (2015)). However, potential safety concerns and scale-up manufacturing of nanoparticles, especially in a

manner suitable for personalized therapeutics with patient-specific neo-antigens, remain as the major challenges.

Experiments conducted during the course of developing embodiments for the present invention developed an alternative, simple strategy where preformed nanoparticles, with an established clinical manufacturing procedure and excellent safety profiles in humans, were mixed with Ag peptides and adjuvants to produce “personalized” cancer vaccines (Fig. 12). The strategy was based on synthetic high density lipoprotein (sHDL) nanodiscs, composed of phospholipids and apolipoprotein A1 (ApoA1)-mimetic peptides. Compared with other HDLs containing 243-amino acid ApoA1 purified from human plasma or produced recombinantly (see, e.g., Wolfrum, C. et al. *Nat. Biotechnol.* 25, 1149-1157 (2007); Diditchenko, S. et al. *Arterioscler. Thromb. Vasc. Biol.* 33, 2202-2211 (2013); Fischer, N.O. et al. *J. Am. Chem. Soc.* 135, 2044-2047 (2013); Tardy, C. et al. *Atherosclerosis* 232, 110-118 (2014); Duivenvoorden, R. et al. *Nat. Commun.* 5, 3065 (2014)), sHDL nanodiscs were synthesized with 22-mer peptides (22A), derived from the repeat α -helix domain of ApoA1 (see, e.g., U.S. Patent No. 6,734,169; U.S. Patent No. 8,378,068; Li, D., Gordon, S., Schwendeman, A. & Remaley, A.T. *Apolipoprotein mimetic peptides for stimulating cholesterol efflux. in Apolipoprotein Mimetics in the Management of Human Disease* (eds. Anantharamaiah, G.M. & Goldberg, D.) 29-42 (Springer, Switzerland, 2015)), with no sequence homology to endogenous ApoA1, thus averting potential trigger of autoimmunity. Importantly, sHDL has been previously manufactured for clinical testing and proven to be safe in humans with the maximum tolerated dose at $\sim 2.2 \text{ g/m}^2$ (see, e.g., Khan, M., et al., *Circulation* 108 (Suppl IV), 563-564 (2003); Miles, J., et al. *Proceedings of Arteriosclerosis Thrombosis and Vascular Biology* 24, E19-E19 (2004), a value one- to two-orders of magnitude greater than most polymeric or inorganic nanoparticles in clinical trials (see, e.g., Alexis, F., et al., *Mol. Pharm.* 5, 505-515 (2008); Anselmo, A.C. & Mitragotri, S. A, *AAPS J* 17, 1041-1054 (2015)).

Experiments conducted during the course of developing embodiments for the present invention developed a nanodisc-based platform for neo-antigen vaccination (Fig. 12). Exploiting the endogenous role of HDL as a nanoparticle for cholesterol, immunostimulatory agent CpG, a strong Toll-like receptor-9 agonist, was modified with cholesterol (Cho-CpG) to enhance its *in vivo* trafficking. It was shown that preformed sHDL nanodiscs can be simply mixed with cholesteryl-CpG and tumor Ag peptides, including neo-antigens identified via tumor DNA sequencing, to produce homogeneous, stable, and ultrasmall nanodiscs in $< 2 \text{ h}$ at room temperature (RT). The nanodiscs efficiently promoted co-delivery of Ag/CpG to dLNs, prolonged Ag presentation on antigen-presenting cells (APCs), and elicited striking levels of

CTL responses with anti-tumor efficacy. Owing to the facile production process, robust therapeutic efficacy, and clinical safety demonstrated previously (see, e.g., Khan, M., et al., Circulation 108 (Suppl IV), 563-564 (2003); Miles, J., et al. Proceedings of Arteriosclerosis Thrombosis and Vascular Biology 24, E19-E19 (2004)), this approach offers an attractive
 5 platform technology for patient-tailored cancer vaccines as well as other bioactive therapeutics.

Accordingly, in certain embodiments, nanoparticles (e.g., sHDL nanoparticles) associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) an antigen are used for inducing an immune response. In some embodiments, the nanoparticles are further associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed,
 10 admixed) an adjuvant (e.g., dendritic cell targeting molecule (DC)). In some embodiments, the nanoparticles are co-administered with an adjuvant. In some embodiments, the antigen is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the adjuvant. In some embodiments, the antigen is not associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the adjuvant. In some embodiments, the antigen is
 15 conjugated with a hydrophobic molecule. In some embodiments, the adjuvant is conjugated with a hydrophobic molecule. In some embodiments, the average size of the nanoparticle is between 6 to 500 nm.

In some embodiments, the hydrophobic molecule is a lipid molecule. In some embodiments, the lipid molecule is a membrane-forming lipid molecule. In some embodiments,
 20 the lipid molecule molecule is a non-membrane-forming lipid molecule.

Examples of lipid molecules applicable with the embodiments of the present invention include, but are not limited to, phospholipids such as lecithin, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, egg sphingomyelin (ESM), cephalin, cardiolipin, phosphatidic acid,
 25 cerebrosides, dicetylphosphate, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoylphosphatidylethanolamine (DOPE), palmitoyloleoyl-phosphatidylcholine (POPC), palmitoyloleoyl-phosphatidylethanolamine (POPE), palmitoyloleoyl-phosphatidylglycerol
 30 (POPG), dioleoylphosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl-phosphatidylethanolamine (DPPE), dimyristoyl-phosphatidylethanolamine (DMPE), distearoyl-phosphatidylethanolamine (DSPE), monomethyl-phosphatidylethanolamine, dimethyl-phosphatidylethanolamine, dielaidoyl-phosphatidylethanolamine (DEPE), stearylloleoyl-phosphatidylethanolamine (SOPE),

lysophosphatidylcholine, dilinoleoylphosphatidylcholine, and mixtures thereof. Other diacylphosphatidylcholine and diacylphosphatidylethanolamine phospholipids can also be used. The acyl groups in these lipids are preferably acyl groups derived from fatty acids having C₁₀-C₂₄ carbon chains, e.g., lauroyl, myristoyl, palmitoyl, stearyl, or oleoyl.

5 Other non-limiting examples of lipid molecules include sterols such as cholesterol and derivatives thereof such as cholestanol, cholestanone, cholestenone, coprostanol, cholesteryl-2'-hydroxyethyl ether, cholesteryl-4'-hydroxybutyl ether, and mixtures thereof.

Other examples of lipid molecules suitable for use in the present invention include nonphosphorous containing lipids such as, e.g., stearylamine, dodecylamine, hexadecylamine, 10 acetyl palmitate, glycerolricinoleate, hexadecyl stearate, isopropyl myristate, amphoteric acrylic polymers, triethanolamine-lauryl sulfate, alkyl-aryl sulfate polyethoxylated fatty acid amides, dioctadecyldimethyl ammonium bromide, ceramide, sphingomyelin, and the like.

Other examples of lipid molecules suitable for use in the present invention include fatty acids and derivatives or analogs thereof. They include oleic acid, lauric acid, capric acid (n- 15 decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprinate, tricaprinate, monoolein (1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monocaprinate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, C₁- 10 alkyl esters thereof (e.g., methyl, isopropyl and t-butyl), and mono- and di-glycerides thereof (i.e., oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, etc.) (Lee et al., Critical 20 Reviews in Therapeutic Drug Carrier Systems, 1991, p. 92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; El Hariri et al., J. Pharm. Pharmacol., 1992, 44, 651-654).

Other examples of lipid molecules suitable for use in the present invention include a lipid molecule modified with PEG (PEG-lipid). Examples of PEG-lipids include, but are not limited 25 to, PEG coupled to dialkyloxypropyls (PEG-DAA) as described in, e.g., PCT Publication No. WO 05/026372, PEG coupled to diacylglycerol (PEG-DAG) as described in, e.g., U.S. Patent Publication Nos. 20030077829 and 2005008689, PEG coupled to phospholipids such as phosphatidylethanolamine (PEG-PE), PEG conjugated to ceramides as described in, e.g., U.S. Pat. No. 5,885,613, PEG conjugated to cholesterol or a derivative thereof, and mixtures thereof. 30 The disclosures of these patent documents are herein incorporated by reference in their entirety for all purposes. Additional PEG-lipids include, without limitation, PEG-C-DOMG, 2 KPEG-DMG, and a mixture thereof.

PEG is a linear, water-soluble polymer of ethylene PEG repeating units with two terminal hydroxyl groups. PEGs are classified by their molecular weights; for example, PEG

2000 has an average molecular weight of about 2,000 daltons, and PEG 5000 has an average molecular weight of about 5,000 daltons. PEGs are commercially available from Sigma Chemical Co. and other companies and include, for example, the following:

monomethoxypolyethylene glycol (MePEG-OH), monomethoxypolyethylene glycol-succinate
5 (MePEG-S), monomethoxypolyethylene glycol-succinimidyl succinate (MePEG-S-NHS),
monomethoxypolyethylene glycol-amine (MePEG-NH₂), monomethoxypolyethylene glycol-
tresylate (MePEG-TRES), and monomethoxypolyethylene glycol-imidazolyl-carbonyl (MePEG-
IM). Other PEGs such as those described in U.S. Pat. Nos. 6,774,180 and 7,053,150 (e.g., mPEG
(20 KDa) amine) are also useful for preparing the PEG-lipid conjugates of the present invention.

10 The disclosures of these patents are herein incorporated by reference in their entirety for all
purposes. In addition, monomethoxypolyethyleneglycolacetic acid (MePEG-CH₂COOH) is
particularly useful for preparing PEG-lipid conjugates including, e.g., PEG-DAA conjugates.

The PEG moiety of the PEG-lipid conjugates described herein may comprise an average
molecular weight ranging from about 550 daltons to about 10,000 daltons. In certain instances,
15 the PEG moiety has an average molecular weight of from about 750 daltons to about 5,000
daltons (e.g., from about 1,000 daltons to about 5,000 daltons, from about 1,500 daltons to about
3,000 daltons, from about 750 daltons to about 3,000 daltons, from about 750 daltons to about
2,000 daltons, etc.). In preferred embodiments, the PEG moiety has an average molecular weight
of about 2,000 daltons or about 750 daltons.

20 In certain instances, the PEG can be optionally substituted by an alkyl, alkoxy, acyl, or
aryl group. The PEG can be conjugated directly to the lipid or may be linked to the lipid via a
linker moiety. Any linker moiety suitable for coupling the PEG to a lipid can be used including,
e.g., non-ester containing linker moieties and ester-containing linker moieties. In a preferred
embodiment, the linker moiety is a non-ester containing linker moiety. As used herein, the term
25 “non-ester containing linker moiety” refers to a linker moiety that does not contain a carboxylic
ester bond (—OC(O)—). Suitable non-ester containing linker moieties include, but are not
limited to, amido (—C(O)NH—), amino (—NR—), carbonyl (—C(O)—), carbamate (—
NHC(O)O—), urea (—NHC(O)NH—), disulphide (—S—S—), ether (—O—), succinyl (—
(O)CCH₂CH₂C(O)—), succinamidyl (—NHC(O)CH₂CH₂C(O)NH—), ether, disulphide, as well
30 as combinations thereof (such as a linker containing both a carbamate linker moiety and an
amido linker moiety). In a preferred embodiment, a carbamate linker is used to couple the PEG
to the lipid.

In other embodiments, an ester containing linker moiety is used to couple the PEG to the lipid. Suitable ester containing linker moieties include, e.g., carbonate (—OC(O)O—), succinoyl, phosphate esters (—O—(O)POH—O—), sulfonate esters, and combinations thereof.

Phosphatidylethanolamines having a variety of acyl chain groups of varying chain lengths and degrees of saturation can be conjugated to PEG to form the lipid conjugate. Such phosphatidylethanolamines are commercially available, or can be isolated or synthesized using conventional techniques known to those of skilled in the art.

Phosphatidylethanolamines containing saturated or unsaturated fatty acids with carbon chain lengths in the range of C_{10} to C_{20} are preferred. Phosphatidylethanolamines with mono- or diunsaturated fatty acids and mixtures of saturated and unsaturated fatty acids can also be used. Suitable phosphatidylethanolamines include, but are not limited to, dimyristoyl-phosphatidylethanolamine (DMPE), dipalmitoyl-phosphatidylethanolamine (DPPE), dioleoylphosphatidylethanolamine (DOPE), and distearoyl-phosphatidylethanolamine (DSPE).

Such embodiments are not limited to particular antigen. Indeed, antigens can be peptides, proteins, polysaccharides, saccharides, lipids, glycolipids, nucleic acids, or combinations thereof. The antigen can be derived from any source, including, but not limited to, a virus, bacterium, parasite, plant, protozoan, fungus, tissue or transformed cell such as a cancer or leukemic cell and can be a whole cell or immunogenic component thereof, e.g., cell wall components or molecular components thereof.

In some embodiments, the antigens are known in the art and are available from commercial government and scientific sources. In some embodiments, the antigens are whole inactivated or attenuated organisms. These organisms may be infectious organisms, such as viruses, parasites and bacteria. These organisms may also be tumor cells. The antigens may be purified or partially purified polypeptides derived from tumors or viral or bacterial sources. Criteria for identifying and selecting effective antigenic peptides (e.g., minimal peptide sequences capable of eliciting an immune response) can be found in the art. The antigens can be recombinant polypeptides produced by expressing DNA encoding the polypeptide antigen in a heterologous expression system. The antigens can be DNA encoding all or part of an antigenic protein. The DNA may be in the form of vector DNA such as plasmid DNA.

Antigens may be provided as single antigens or may be provided in combination. Antigens may also be provided as complex mixtures of polypeptides or nucleic acids.

In some embodiments, the antigen is a self antigen. As used herein, the term “self-antigen” refers to an immunogenic antigen or epitope which is native to a mammal and which may be involved in the pathogenesis of an autoimmune disease.

In some embodiments, the antigen is a viral antigen. Viral antigens can be isolated from any virus including, but not limited to, a virus from any of the following viral families:

Arenaviridae, Arterivirus, Astroviridae, Baculoviridae, Badnavirus, Barnaviridae, Birnaviridae, Bromoviridae, Bunyaviridae, Caliciviridae, Capillovirus, Carlavirus, Caulimovirus,
 5 Circoviridae, Closterovirus, Comoviridae, Coronaviridae (e.g., Coronavirus, such as severe acute respiratory syndrome (SARS) virus), Corticoviridae, Cystoviridae, Deltavirus, Dianthovirus, Enamovirus, Filoviridae (e.g., Marburg virus and Ebola virus (e.g., Zaire, Reston, Ivory Coast, or Sudan strain)), Flaviviridae, (e.g., Hepatitis C virus, Dengue virus 1, Dengue virus 2, Dengue virus 3, and Dengue virus 4), Hepadnaviridae, Herpesviridae (e.g., Human
 10 herpesvirus 1, 3, 4, 5, and 6, and Cytomegalovirus), Hypoviridae, Iridoviridae, Leviviridae, Lipothrixviridae, Microviridae, Orthomyxoviridae (e.g., Influenzavirus A and B and C), Papovaviridae, Paramyxoviridae (e.g., measles, mumps, and human respiratory syncytial virus), Parvoviridae, Picornaviridae (e.g., poliovirus, rhinovirus, hepatovirus, and aphthovirus), Poxviridae (e.g., vaccinia and smallpox virus), Reoviridae (e.g., rotavirus), Retroviridae (e.g.,
 15 lentivirus, such as human immunodeficiency virus (HIV) 1 and HIV 2), Rhabdoviridae (for example, rabies virus, measles virus, respiratory syncytial virus, etc.), Togaviridae (for example, rubella virus, dengue virus, etc.), and Totiviridae. Suitable viral antigens also include all or part of Dengue protein M, Dengue protein E, Dengue D1NS1, Dengue D1NS2, and Dengue D1NS3.

Viral antigens may be derived from a particular strain such as a papilloma virus, a herpes
 20 virus, i.e. herpes simplex 1 and 2; a hepatitis virus, for example, hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), the delta hepatitis D virus (HDV), hepatitis E virus (HEV) and hepatitis G virus (HGV), the tick-borne encephalitis viruses; parainfluenza, varicella-zoster, cytomeglavirus, Epstein-Barr, rotavirus, rhinovirus, adenovirus, coxsackieviruses, equine encephalitis, Japanese encephalitis, yellow fever, Rift Valley fever, and
 25 lymphocytic choriomeningitis.

In some embodiments, the antigen is a bacterial antigen. Bacterial antigens can originate from any bacteria including, but not limited to, *Actinomyces*, *Anabaena*, *Bacillus*, *Bacteroides*, *Bdellovibrio*, *Bordetella*, *Borrelia*, *Campylobacter*, *Caulobacter*, *Chlamydia*, *Chlorobium*, *Chromatium*, *Clostridium*, *Corynebacterium*, *Cytophaga*, *Deinococcus*, *Escherichia*,
 30 *Francisella*, *Halobacterium*, *Heliobacter*, *Haemophilus*, Hemophilus influenza type B (HIB), *Hyphomicrobium*, *Legionella*, *Leptspirosis*, *Listeria*, *Meningococcus* A, B and C, *Methanobacterium*, *Micrococcus*, *Myobacterium*, *Mycoplasma*, *Myxococcus*, *Neisseria*, *Nitrobacter*, *Oscillatoria*, *Prochloron*, *Proteus*, *Pseudomonas*, *Phodospirillum*, *Rickettsia*, *Salmonella*, *Shigella*, *Spirillum*, *Spirochaeta*, *Staphylococcus*, *Streptococcus*, *Streptomyces*,

Sulfolobus, Thermoplasma, Thiobacillus, and Treponema, Vibrio, and Yersinia.

In some embodiments, the antigen is a parasite antigen. Parasite antigens can be obtained from parasites such as, but not limited to, an antigen derived from *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Candida albicans*, *Candida tropicalis*, *Nocardia asteroides*, *Rickettsia rickettsii*, *Rickettsia typhi*, *Mycoplasma pneumoniae*, *Chlamydia psittaci*, *Chlamydia trachomatis*, *Plasmodium falciparum*, *Trypanosoma brucei*, *Entamoeba histolytica*, *Toxoplasma gondii*, *Trichomonas vaginalis* and *Schistosoma mansoni*. These include Sporozoan antigens, Plasmodian antigens, such as all or part of a Circumsporozoite protein, a Sporozoite surface protein, a liver stage antigen, an apical membrane associated protein, or a Merozoite surface protein.

In some embodiments, the antigen is an allergen and environmental antigen, such as, but not limited to, an antigen derived from naturally occurring allergens such as pollen allergens (tree-, herb, weed-, and grass pollen allergens), insect allergens (inhalant, saliva and venom allergens), animal hair and dandruff allergens, and food allergens. Important pollen allergens from trees, grasses and herbs originate from the taxonomic orders of Fagales, Oleales, Pinales and platanaceae including i.a. birch (*Betula*), alder (*Alnus*), hazel (*Corylus*), hornbeam (*Carpinus*) and olive (*Olea*), cedar (*Cryptomeria* and *Juniperus*), Plane tree (*Platanus*), the order of Poales including i.e. grasses of the genera *Lolium*, *Phleum*, *Poa*, *Cynodon*, *Dactylis*, *Holcus*, *Phalaris*, *Secale*, and *Sorghum*, the orders of Asterales and Urticales including i.a. herbs of the genera *Ambrosia*, *Artemisia*, and *Parietaria*. Other allergen antigens that may be used include allergens from house dust mites of the genus *Dermatophagoides* and *Euroglyphus*, storage mite e.g. *Lepidoglyphus*, *Glycyphagus* and *Tyrophagus*, those from cockroaches, midges and fleas e.g. *Blattella*, *Periplaneta*, *Chironomus* and *Ctenocephalides*, those from mammals such as cat, dog and horse, birds, venom allergens including such originating from stinging or biting insects such as those from the taxonomic order of *Hymenoptera* including bees (superfamily Apidae), wasps (superfamily Vespidae), and ants (superfamily Formicoidae). Still other allergen antigens that may be used include inhalation allergens from fungi such as from the genera *Alternaria* and *Cladosporium*.

In some embodiments, the antigen is a tumor antigen. The antigen can be a tumor antigen, including a tumor-associated or tumor-specific antigen, such as, but not limited to, alpha-actinin-4, Bcr-Abl fusion protein, Casp-8, beta-catenin, cdc27, cdk4, cdkn2a, coa-1, dekan fusion protein, EF2, ETV6-AML1 fusion protein, LDLR-fucosyltransferaseAS fusion protein, HLA-A2, HLA-A11, hsp70-2, KIAA0205, Mart2, Mum-1, 2, and 3, neo-PAP, myosin class I, OS-9, pml-RAR α fusion protein, PTPRK, K-ras, N-ras, Triosephosphate isomeras, Bage-

1, Gage 3,4,5,6,7, GnTV, Herv-K-mel, Lage-1, Mage-A1,2,3,4,6,10,12, Mage-C2, NA-88, NY-Eso-1/Lage-2, SP17, SSX-2, and TRP2-Int2, MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15(58), CEA, RAGE, NY-ESO (LAGS), SCP-1, Hom/Mel-40, PRAME, p53, H-Ras, HER-2/neu, BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, β -Catenin, CDK4, Mum-1, p16, TAGE, PSMA, PSCA, CT7, telomerase, 43-9F, 5T4, 791Tgp72, α -fetoprotein, 13HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), human EGFR protein or its fragments, such as human EGFR residues 306–325 (SCVRACGADSYEMEEDGVRK (SEQ ID NO:374)) and residues 897–915 (VWSYGVTVWELMTFGSKPY (SEQ ID NO:375)), HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB\70K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein\cyclophilin C-associated protein), TAAL6, TAG72, TLP, TPS, WT1 (and WT1-derivaed peptide sequences: WT1 126–134 (RMFP NAPYL (SEQ ID NO:376)), WT1 122–140 (SGQARMFPNAPYLPSICLES (SEQ ID NO:377)), and WT1 122–144 (SGQARMFPNAPYLPSICLESQPTI (SEQ ID NO:378))), MUC1 (and MUC1-derived peptides and glycopeptides such as RPAPGS (SEQ ID NO:379), PPAHGVT (SEQ ID NO:380), and PDTRP (SEQ ID NO:381))), LMP2, EGFRvIII, Idiotype, GD2, Ras mutant, p53 mutant, Proteinase3 (PR1), Survivin, hTERT, Sarcoma translocation breakpoints, EphA2, EphA4, LMW-PTP, PAP, ML-IAP, AFP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, ALK, Androgen receptor, Cyclin B1, Polysialic acid, MYCN, RhoC, TRP-2, GD3, Fucosyl GM1, Mesothelin, sLe(animal), CYP1B1, PLAC1, GM3, BORIS, Tn, GloboH, NY-BR-1, RGS5, SART3, STn, Carbonic anhydrase IX, PAX5, OY-TES1, Sperm protein 17, LCK, HMWMAA, AKAP-4, XAGE 1, B7H3, Legumain, Tie 2, Page4, VEGFR2, MAD-CT-1, FAP, PDGFR-alpha, PDGFR- β , MAD-CT-2, Fos-related antigen 1, ERBB2, Folate receptor 1 (FOLR1 or FBP), IDH1, IDO, LY6K, fms-related tyro- sine kinase 1 (FLT1, best known as VEGFR1), KDR, PADRE, TA-CIN (recombinant HPV16 L2E7E6), SOX2, and aldehyde dehydrogenase.

One of the critical barriers to developing curative and tumor- specific immunotherapy is the identification and selection of highly specific and restricted tumor antigens to avoid autoimmunity. Tumor neo-antigens, which arise as a result of genetic change (e.g., inversions, translocations, deletions, missense mutations, splice site mutations, etc.) within malignant cells, represent the most tumor- specific class of antigens.

In some embodiments, the antigen is a neo-antigen. The term neoantigen is used herein

to define any newly expressed antigenic determinant. Neoantigens may arise upon conformational change in a protein, as newly expressed determinants (especially on the surfaces of transformed or infected cells), as the result of complex formation of one or more molecules or as the result of cleavage of a molecule with a resultant display of new antigenic determinants.

5 Thus, as used herein, the term neoantigen covers antigens expressed upon infection (e.g. viral infection, protozoal infection or bacterial infection), in prion-mediated diseases, an on cell transformation (cancer), in which latter case the neoantigen may be termed a tumour-associated antigen.

The present invention is not limited to a particular manner of identifying neo-antigens. In
10 some embodiments, identification of neo-antigens involves identifying all, or nearly all, mutations in the neoplasia/tumor at the DNA level using whole genome sequencing, whole exome (e.g., only captured exons) sequencing, or RNA sequencing of tumor versus matched germline samples from each patient. In some embodiments, identification of neo-antigens involves analyzing the identified mutations with one or more peptide-MHC binding prediction
15 algorithms to generate a plurality of candidate neo-antigen T cell epitopes that are expressed within the neoplasia/tumor and may bind patient HLA alleles. In some embodiments, identification of neo-antigens involves synthesizing the plurality of candidate neo-antigen peptides selected from the sets of all neo open reading frame peptides and predicted binding peptides for use in a cancer vaccine.

20 As such, the present invention is based, at least in part, on the ability to identify all, or nearly all, of the mutations within a neoplasia/tumor (e.g., translocations, inversions, large and small deletions and insertions, missense mutations, splice site mutations, etc.). In particular, these mutations are present in the genome of neoplasia/tumor cells of a subject, but not in normal tissue from the subject. Such mutations are of particular interest if they lead to changes
25 that result in a protein with an altered amino acid sequence that is unique to the patient's neoplasia/tumor (e.g., a neo-antigen). For example, useful mutations may include: (1) non-synonymous mutations leading to different amino acids in the protein; (2) read-through mutations in which a stop codon is modified or deleted, leading to translation of a longer protein with a novel tumor- specific sequence at the C-terminus; (3) splice site mutations that lead to the
30 inclusion of an intron in the mature mRNA and thus a unique tumor- specific protein sequence; (4) chromosomal rearrangements that give rise to a chimeric protein with tumor- specific sequences at the junction of 2 proteins (i.e., gene fusion); (5) frameshift mutations or deletions that lead to a new open reading frame with a novel tumor- specific protein sequence; and the like. Peptides with mutations or mutated polypeptides arising from, for example, splice- site,

frameshift, read-through, or gene fusion mutations in tumor cells may be identified by sequencing DNA, RNA or protein in tumor versus normal cells.

Also within the scope of the present invention is personal neo-antigen peptides derived from common tumor driver genes and may further include previously identified tumor specific mutations.

Preferably, any suitable sequencing-by-synthesis platform can be used to identify mutations. Four major sequencing-by-synthesis platforms are currently available: the Genome Sequencers from Roche/454 Life Sciences, the HiSeq Analyzer from Illumina/Solexa, the SOLiD system from Applied BioSystems, and the Heliscope system from Helicos Biosciences. Sequencing-by-synthesis platforms have also been described by Pacific Biosciences and VisiGen Biotechnologies. Each of these platforms can be used in the methods of the invention. In some embodiments, a plurality of nucleic acid molecules being sequenced is bound to a support (e.g., solid support). To immobilize the nucleic acid on a support, a capture sequence/universal priming site can be added at the 3' and/or 5' end of the template. The nucleic acids may be bound to the support by hybridizing the capture sequence to a complementary sequence covalently attached to the support. The capture sequence (also referred to as a universal capture sequence) is a nucleic acid sequence complementary to a sequence attached to a support that may dually serve as a universal primer.

As an alternative to a capture sequence, a member of a coupling pair (such as, e.g., antibody/antigen, receptor/ligand, or the avidin-biotin pair as described in, e.g., U.S. Patent Application No. 2006/0252077) may be linked to each fragment to be captured on a surface coated with a respective second member of that coupling pair. Subsequent to the capture, the sequence may be analyzed, for example, by single molecule detection/sequencing, e.g., as described in the Examples and in U.S. Patent No. 7,283,337, including template-dependent sequencing-by-synthesis. In sequencing-by-synthesis, the surface-bound molecule is exposed to a plurality of labeled nucleotide triphosphates in the presence of polymerase. The sequence of the template is determined by the order of labeled nucleotides incorporated into the 3' end of the growing chain. This can be done in real time or in a step-and-repeat mode. For real-time analysis, different optical labels to each nucleotide may be incorporated and multiple lasers may be utilized for stimulation of incorporated nucleotides.

Any cell type or tissue may be utilized to obtain nucleic acid samples for use in the sequencing methods described herein. In some embodiments, the DNA or RNA sample is obtained from a neoplasia/tumor or a bodily fluid, e.g., blood, obtained by known techniques (e.g. venipuncture) or saliva. Alternatively, nucleic acid tests can be performed on dry samples

(e.g. hair or skin).

A variety of methods are available for detecting the presence of a particular mutation or allele in an individual's DNA or RNA. Advancements in this field have provided accurate, easy, and inexpensive large-scale SNP genotyping. Most recently, for example, several new
5 techniques have been described including dynamic allele-specific hybridization (DASH), microplate array diagonal gel electrophoresis (MADGE), pyrosequencing, oligonucleotide-specific ligation, the TaqMan system as well as various DNA "chip" technologies such as the Affymetrix SNP chips. These methods require amplification of the target genetic region, typically by PCR. Still other newly developed methods, based on the generation of small signal
10 molecules by invasive cleavage followed by mass spectrometry or immobilized padlock probes and rolling-circle amplification, might eventually eliminate the need for PCR. Several of the methods known in the art for detecting specific single nucleotide polymorphisms are summarized below. The method of the present invention is understood to include all available methods.

15 PCR based detection means may include multiplex amplification of a plurality of markers simultaneously. For example, it is well known in the art to select PCR primers to generate PCR products that do not overlap in size and can be analyzed simultaneously.

Alternatively, it is possible to amplify different markers with primers that are differentially labeled and thus can each be differentially detected. Of course, hybridization based
20 detection means allow the differential detection of multiple PCR products in a sample. Other techniques are known in the art to allow multiplex analyses of a plurality of markers.

Several methods have been developed to facilitate analysis of single nucleotide polymorphisms in genomic DNA or cellular RNA. In one embodiment, the single base polymorphism can be detected by using a specialized exonuclease-resistant nucleotide, as
25 disclosed, e.g., U.S. Patent No. 4,656,127. According to the method, a primer complementary to the allelic sequence immediately 3' to the polymorphic site is permitted to hybridize to a target molecule obtained from a particular animal or human. If the polymorphic site on the target molecule contains a nucleotide that is complementary to the particular exonuclease-resistant nucleotide derivative present, then that derivative will be incorporated onto the end of the
30 hybridized primer. Such incorporation renders the primer resistant to exonuclease, and thereby permits its detection. Since the identity of the exonuclease-resistant derivative of the sample is known, a finding that the primer has become resistant to exonucleases reveals that the nucleotide present in the polymorphic site of the target molecule was complementary to that of the nucleotide derivative used in the reaction. This method has the advantage that it does not require

the determination of large amounts of extraneous sequence data.

In another embodiment of the invention, a solution-based method is used for determining the identity of the nucleotide of a polymorphic site (see, e.g, French Patent No. 2,650,840; PCT Application No. WO1991/02087). As in the method of U.S. Patent No. 4,656,127, a primer may
5 be employed that is complementary to allelic sequences immediately 3' to a polymorphic site. The method determines the identity of the nucleotide of that site using labeled dideoxynucleotide derivatives, which, if complementary to the nucleotide of the polymorphic site, will become incorporated onto the terminus of the primer.

An alternative method, known as Genetic Bit Analysis or GBA® is described in PCT
10 Application No. WO 1992/ 15712). GBA® uses mixtures of labeled terminators and a primer that is complementary to the sequence 3' to a polymorphic site. The labeled terminator that is incorporated is thus determined by, and complementary to, the nucleotide present in the polymorphic site of the target molecule being evaluated. In contrast to the method of Cohen et al. (French Patent 2,650,840; PCT Application No. W01991/02087) the GBA® method is
15 preferably a heterogeneous phase assay, in which the primer or the target molecule is immobilized to a solid phase. Recently, several primer-guided nucleotide incorporation procedures for assaying polymorphic sites in DN A have been described (see, e.g., Komher, J. S. et al., Nucl. Acids. Res. 17:7779- 7784 (1989); Sokolov, B. P., Nucl. Acids Res. 18:3671 (1990); Syvanen, A.-C, et al., Genomics 8:684-692 (1990); Kuppaswamy, M. N. et al., Proc.
20 Natl. Acad. Sci. (U.S.A.) 88: 1143- 1147 (1991); Prezant, T. R. et al., Hum. Mutat. 1: 159-164 (1992); Ugozzoli, L. et al., GATA 9: 107- 112 (1992); Nyren, P. et al., Anal. Biochem. 208: 171-175 (1993)). These methods differ from GBA® in that they all rely on the incorporation of labeled deoxynucleotides to discriminate between bases at a polymorphic site. In such a format, since the signal is proportional to the number of deoxynucleotides incorporated, polymorphisms
25 that occur in runs of the same nucleotide can result in signals that are proportional to the length of the run (see, e.g., Syvanen, A.-C, et al., Amer. J. Hum. Genet. 52:46-59 (1993)).

An alternative method for identifying tumor specific neo-antigens is direct protein sequencing. Protein sequencing of enzymatic digests using multidimensional MS techniques (MSn) including tandem mass spectrometry (MS/MS)) can also be used to identify neo-antigens
30 of the invention. Such proteomic approaches permit rapid, highly automated analysis (see, e.g., K. Gevaert and J. Vandekerckhove, Electrophoresis 21: 1145- 1154 (2000)). It is further contemplated within the scope of the invention that high-throughput methods for de novo sequencing of unknown proteins may be used to analyze the proteome of a patient's tumor to identify expressed neo-antigens. For example, meta shotgun protein sequencing may be used to

identify expressed neo-antigens (see, e.g., Guthals et al. (2012) Shotgun Protein Sequencing with Meta-contig Assembly, *Molecular and Cellular Proteomics* 11(10): 1084-96).

Tumor specific neo-antigens may also be identified using MHC multimers to identify neo-antigen-specific T-cell responses. For example, highthroughput analysis of neo-antigen-specific T-cell responses in patient samples may be performed using MHC tetramer-based screening techniques (see, e.g., Hombrink et al. (2011) High-Throughput Identification of Potential Minor Histocompatibility Antigens by MHC Tetramer-Based Screening: Feasibility and Limitations 6(8): 1-11; Hadrup et al. (2009) Parallel detection of antigen-specific T-cell responses by multidimensional encoding of MHC multimers, *Nature Methods*, 6(7):520-26; van Rooij et al. (2013) Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an Ipilimumab-responsive melanoma, *Journal of Clinical Oncology*, 31: 1-4; and Heemskerk et al. (2013) The cancer antigenome, *EMBO Journal*, 32(2): 194-203). It is contemplated within the scope of the invention that such tetramer-based screening techniques may be used for the initial identification of tumor specific neo-antigens, or alternatively as a secondary screening protocol to assess what neo-antigens a patient may have already been exposed to, thereby facilitating the selection of candidate neo-antigens for the vaccines of the invention.

The invention further includes isolated peptides (e.g., neo-antigenic peptides containing the tumor specific mutations identified by the described methods, peptides that comprise known tumor specific mutations, and mutant polypeptides or fragments thereof identified by the described methods). These peptides and polypeptides are referred to herein as "neo-antigenic peptides" or "neo-antigenic polypeptides." The polypeptides or peptides can be of a variety of lengths and will minimally include the small region predicted to bind to the HLA molecule of the patient (the "epitope") as well as additional adjacent amino acids extending in both the N- and C-terminal directions. The polypeptides or peptides can be either in their neutral (uncharged) forms or in forms which are salts, and either free of modifications such as glycosylation, side chain oxidation, or phosphorylation or containing these modifications, subject to the condition that the modification not destroy the biological activity of the polypeptides as herein described.

In certain embodiments the size of the at least one neo-antigenic peptide molecule may comprise, but is not limited to, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, about 50, about 60, about

70, about 80, about 90, about 100, about 110, about 120 or greater amino molecule residues, and any range derivable therein. In specific embodiments the neo-antigenic peptide molecules are equal to or less than 50 amino acids. In a preferred embodiment, the neo-antigenic peptide molecules are equal to about 20 to about 30 amino acids.

5 As such, the present invention provides nanoparticles (e.g., sHDL nanoparticles) associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) one or more neo-antigenic peptides. In some embodiments, the nanoparticle (e.g., sHDL nanoparticle) is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) one neo-antigenic peptide. In some embodiments, the nanoparticle (e.g., sHDL
10 nanoparticle) is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) two neo-antigenic peptides. In some embodiments, the nanoparticle (e.g., sHDL nanoparticle) is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) at least 5 or more neo-antigenic peptides. In some embodiments, the nanoparticle (e.g., sHDL nanoparticle) is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) at least about 6, about 8, about 10, about 12, about 14, about 16, about 18,
15 or about 20 distinct peptides. In some embodiments, the nanoparticle (e.g., sHDL nanoparticle) is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) at least 20 distinct peptides.

 The neo-antigenic peptides, polypeptides, and analogs can be further modified to contain
20 additional chemical moieties not normally part of the protein. Those derivatized moieties can improve the solubility, the biological half-life, absorption of the protein, or binding affinity. The moieties can also reduce or eliminate any desirable side effects of the proteins and the like. An overview for those moieties can be found in Remington's Pharmaceutical Sciences, 20th ed., Mack Publishing Co., Easton, PA (2000). For example, neo-antigenic peptides and polypeptides
25 having the desired activity may be modified as necessary to provide certain desired attributes, e.g. improved pharmacological characteristics, while increasing or at least retaining substantially all of the biological activity of the unmodified peptide to bind the desired MHC molecule and activate the appropriate T cell. For instance, the neo-antigenic peptide and polypeptides may be subject to various changes, such as substitutions, either conservative or non-conservative, where
30 such changes might provide for certain advantages in their use, such as improved MHC binding. Such conservative substitutions may encompass replacing an amino acid residue with another amino acid residue that is biologically and/or chemically similar, e.g., one hydrophobic residue for another, or one polar residue for another. The effect of single amino acid substitutions may also be probed using D- amino acids. Such modifications may be made using well known

peptide synthesis procedures, as described in e.g., Merrifield, *Science* 232:341-347 (1986), Barany & Merrifield, *The Peptides*, Gross & Meienhofer, eds. (N.Y., Academic Press), pp. 1-284 (1979); and Stewart & Young, *Solid Phase Peptide Synthesis*, (Rockford, Ill., Pierce), 2d Ed. (1984).

5 In some embodiments, the neo-antigenic peptides and polypeptides may be modified with linking agents for purposes of facilitating association with the nanoparticle (e.g., sHDL nanoparticle). The invention is not limited to a particular type or kind of linking agent. In some embodiments, the linking agent is a cysteine-serine-serine (CSS) molecule.

10 In some embodiments wherein the nanoparticle is sHDL and the neo-antigenic peptide or polypeptide is modified with CSS, the sHDL is further modified with dioleoyl-sn-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP) wherein upon mixing, the DOPE-PDP and CSS engage thereby resulting in a complexing (linking) of the CSS-Ag with the sHDL.

15 The neo-antigenic peptide and polypeptides may also be modified by extending or decreasing the compound's amino acid sequence, e.g., by the addition or deletion of amino acids. The neo-antigenic peptides, polypeptides, or analogs can also be modified by altering the order or composition of certain residues. It will be appreciated by the skilled artisan that certain amino acid residues essential for biological activity, e.g., those at critical contact sites or conserved residues, may generally not be altered without an adverse effect on biological activity. The non-
20 critical amino acids need not be limited to those naturally occurring in proteins, such as L-amino acids, or their D-isomers, but may include non-natural amino acids as well, such as β - γ - δ -amino acids, as well as many derivatives of L-amino acids.

25 Typically, a neo-antigen polypeptide or peptide may be optimized by using a series of peptides with single amino acid substitutions to determine the effect of electrostatic charge, hydrophobicity, etc. on MHC binding. For instance, a series of positively charged (e.g., Lys or Arg) or negatively charged (e.g., Glu) amino acid substitutions may be made along the length of the peptide revealing different patterns of sensitivity towards various MHC molecules and T cell receptors. In addition, multiple substitutions using small, relatively neutral moieties such as Ala, Gly, Pro, or similar residues may be employed. The substitutions may be homo-oligomers or
30 hetero-oligomers. The number and types of residues which are substituted or added depend on the spacing necessary between essential contact points and certain functional attributes which are sought (e.g., hydrophobicity versus hydrophilicity). Increased binding affinity for an MHC molecule or T cell receptor may also be achieved by such substitutions, compared to the affinity of the parent peptide. In any event, such substitutions should employ amino acid residues or

other molecular fragments chosen to avoid, for example, steric and charge interference which might disrupt binding. Amino acid substitutions are typically of single residues. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a final peptide.

One of skill in the art will appreciate that there are a variety of ways in which to produce such tumor specific neo-antigens. In general, such tumor specific neo-antigens may be produced either in vitro or in vivo. Tumor specific neo-antigens may be produced in vitro as peptides or polypeptides, which may then be formulated into a personalized neoplasia vaccine and administered to a subject. Such in vitro production may occur by a variety of methods known to one of skill in the art such as, for example, peptide synthesis or expression of a peptide/polypeptide from a DNA or RNA molecule in any of a variety of bacterial, eukaryotic, or viral recombinant expression systems, followed by purification of the expressed peptide/polypeptide.

Alternatively, tumor specific neo-antigens may be produced in vivo by introducing molecules (e.g., DNA, RNA, viral expression systems, and the like) that encode tumor specific neo-antigens into a subject, whereupon the encoded tumor specific neo-antigens are expressed.

Proteins or peptides may be made by any technique known to those of skill in the art, including the expression of proteins, polypeptides or peptides through standard molecular biological techniques, the isolation of proteins or peptides from natural sources, or the chemical synthesis of proteins or peptides. The nucleotide and protein, polypeptide and peptide sequences corresponding to various genes have been previously disclosed, and may be found at computerized databases known to those of ordinary skill in the art. One such database is the National Center for Biotechnology Information's Genbank and GenPept databases located at the National Institutes of Health website. The coding regions for known genes may be amplified and/or expressed using the techniques disclosed herein or as would be known to those of ordinary skill in the art. Alternatively, various commercial preparations of proteins, polypeptides and peptides are known to those of skill in the art.

Peptides can be readily synthesized chemically utilizing reagents that are free of contaminating bacterial or animal substances (Merrifield RB: Solid phase peptide synthesis. I. The synthesis of a tetrapeptide. J. Am. Chem. Soc. 85:2149-54, 1963).

A further aspect of the invention provides a nucleic acid (e.g., a polynucleotide) encoding a neo-antigenic peptide of the invention, which may be used to produce the neo-antigenic peptide in vitro. The polynucleotide may be, e.g., DNA, cDNA, PNA, CNA, RNA, either single- and/or double- stranded, or native or stabilized forms of polynucleotides, such as e.g. polynucleotides with a phosphorothiate backbone, or combinations thereof and it may or

may not contain introns so long as it codes for the peptide. A still further aspect of the invention provides an expression vector capable of expressing a polypeptide according to the invention. Expression vectors for different cell types are well known in the art and can be selected without undue experimentation. Generally, the DNA is inserted into an expression vector, such as a
5 plasmid, in proper orientation and correct reading frame for expression. If necessary, the DNA may be linked to the appropriate transcriptional and translational regulatory control nucleotide sequences recognized by the desired host (e.g., bacteria), although such controls are generally available in the expression vector. The vector is then introduced into the host bacteria for
10 cloning using standard techniques (see, e.g., Sambrook et al. (1989) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.).

The invention further embraces variants and equivalents which are substantially homologous to the identified tumor specific neo-antigens described herein. These can contain, for example, conservative substitution mutations, i.e., the substitution of one or more amino acids by similar amino acids. For example, conservative substitution refers to the substitution of
15 an amino acid with another within the same general class such as, for example, one acidic amino acid with another acidic amino acid, one basic amino acid with another basic amino acid, or one neutral amino acid by another neutral amino acid. What is intended by a conservative amino acid substitution is well known in the art.

The invention also includes expression vectors comprising the isolated polynucleotides,
20 as well as host cells containing the expression vectors. It is also contemplated within the scope of the invention that the neo-antigenic peptides may be provided in the form of RNA or cDNA molecules encoding the desired neo-antigenic peptides. The invention also provides that the one or more neo-antigenic peptides of the invention may be encoded by a single expression vector. The invention also provides that the one or more neo-antigenic peptides of the invention may be
25 encoded and expressed in vivo using a viral based system (e.g., an adenovirus system).

The term "polynucleotide encoding a polypeptide" encompasses a polynucleotide which includes only coding sequences for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequences. The polynucleotides of the invention can be in the form of RNA or in the form of DNA. DNA includes cDNA, genomic DNA, and synthetic
30 DNA; and can be double-stranded or single-stranded, and if single stranded can be the coding strand or non-coding (anti-sense) strand.

In embodiments, the polynucleotides may comprise the coding sequence for the tumor specific neo-antigenic peptide fused in the same reading frame to a polynucleotide which aids, for example, in expression and/or secretion of a polypeptide from a host cell (e.g., a leader

sequence which functions as a secretory sequence for controlling transport of a polypeptide from the cell). The polypeptide having a leader sequence is a preprotein and can have the leader sequence cleaved by the host cell to form the mature form of the polypeptide.

In some embodiments, the polynucleotides can comprise the coding sequence for the tumor specific neo-antigenic peptide fused in the same reading frame to a marker sequence that allows, for example, for purification of the encoded polypeptide, which may then be incorporated into the personalized neoplasia vaccine. For example, the marker sequence can be a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the mature polypeptide fused to the marker in the case of a bacterial host, or the marker sequence can be a hemagglutinin (HA) tag derived from the influenza hemagglutinin protein when a mammalian host (e.g., COS-7 cells) is used. Additional tags include, but are not limited to, Calmodulin tags, FLAG tags, Myc tags, S tags, SBP tags, Softag 1, Softag 3, V5 tag, Xpress tag, Isopeptag, SpyTag, Biotin Carboxyl Carrier Protein (BCCP) tags, GST tags, fluorescent protein tags (e.g., green fluorescent protein tags), maltose binding protein tags, Nus tags, Strep-tag, thioredoxin tag, TC tag, Ty tag, and the like. In embodiments, the polynucleotides may comprise the coding sequence for one or more of the tumor specific neo-antigenic peptides fused in the same reading frame to create a single concatamerized neo-antigenic peptide construct capable of producing multiple neo-antigenic peptides.

In embodiments, the present invention provides isolated nucleic acid molecules having a nucleotide sequence at least 60% identical, at least 65% identical, at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, or at least 96%, 97%, 98% or 99% identical to a polynucleotide encoding a tumor specific neo-antigenic peptide of the present invention.

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence can include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence can be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence can be inserted into the reference sequence. These mutations of the reference sequence can occur at the amino- or carboxy-terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups

within the reference sequence.

As a practical matter, whether any particular nucleic acid molecule is at least 80% identical, at least 85% identical, at least 90% identical, and in some embodiments, at least 95%, 96%, 97%, 98%, or 99% identical to a reference sequence can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). Bestfit uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics* 2:482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

The isolated tumor specific neo-antigenic peptides described herein can be produced in vitro (e.g., in the laboratory) by any suitable method known in the art. Such methods range from direct protein synthetic methods to constructing a DNA sequence encoding isolated polypeptide sequences and expressing those sequences in a suitable transformed host. In some embodiments, a DNA sequence is constructed using recombinant technology by isolating or synthesizing a DNA sequence encoding a wild-type protein of interest. Optionally, the sequence can be mutagenized by site-specific mutagenesis to provide functional analogs thereof. See, e.g. Zoeller et al., *Proc. Nat'l. Acad. Sci. USA* 81:5662-5066 (1984) and U.S. Pat. No. 4,588,585.

In embodiments, a DNA sequence encoding a polypeptide of interest would be constructed by chemical synthesis using an oligonucleotide synthesizer. Such oligonucleotides can be designed based on the amino acid sequence of the desired polypeptide and selecting those codons that are favored in the host cell in which the recombinant polypeptide of interest will be produced. Standard methods can be applied to synthesize an isolated polynucleotide sequence encoding an isolated polypeptide of interest. For example, a complete amino acid sequence can be used to construct a back-translated gene. Further, a DNA oligomer containing a nucleotide sequence coding for the particular isolated polypeptide can be synthesized. For example, several small oligonucleotides coding for portions of the desired polypeptide can be synthesized and then ligated. The individual oligonucleotides typically contain 5' or 3' overhangs for complementary assembly.

Once assembled (e.g., by synthesis, site-directed mutagenesis, or another method), the polynucleotide sequences encoding a particular isolated polypeptide of interest will be inserted

into an expression vector and optionally operatively linked to an expression control sequence appropriate for expression of the protein in a desired host. Proper assembly can be confirmed by nucleotide sequencing, restriction mapping, and expression of a biologically active polypeptide in a suitable host. As well known in the art, in order to obtain high expression levels of a
5 transfected gene in a host, the gene can be operatively linked to transcriptional and translational expression control sequences that are functional in the chosen expression host. Recombinant expression vectors may be used to amplify and express DNA encoding the tumor specific neo-antigenic peptides. Recombinant expression vectors are replicable DNA constructs which have synthetic or cDNA-derived DNA fragments encoding a tumor specific neo-antigenic peptide or
10 a bioequivalent analog operatively linked to suitable transcriptional or translational regulatory elements derived from mammalian, microbial, viral or insect genes. A transcriptional unit generally comprises an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, transcriptional promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate
15 transcription and translation initiation and termination sequences, as described in detail below. Such regulatory elements can include an operator sequence to control transcription. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants can additionally be incorporated. DNA regions are operatively linked when they are functionally related to each other. For example, DNA for a
20 signal peptide (secretory leader) is operatively linked to DNA for a polypeptide if it is expressed as a precursor which participates in the secretion of the polypeptide; a promoter is operatively linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operatively linked to a coding sequence if it is positioned so as to permit translation. Generally, operatively linked means contiguous, and in the case of secretory leaders,
25 means contiguous and in reading frame. Structural elements intended for use in yeast expression systems include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it can include an N-terminal methionine residue. This residue can optionally be subsequently cleaved from the expressed recombinant protein to provide a final product.

30 The choice of expression control sequence and expression vector will depend upon the choice of host. A wide variety of expression host/vector combinations can be employed. Useful expression vectors for eukaryotic hosts, include, for example, vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus and cytomegalovirus. Useful expression vectors for bacterial hosts include known bacterial plasmids, such as plasmids from

Escherichia coli, including pCR 1, pBR322, pMB9 and their derivatives, wider host range plasmids, such as M13 and filamentous single- stranded DNA phages.

Suitable host cells for expression of a polypeptide include prokaryotes, yeast, insect or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram
5 negative or gram positive organisms, for example E. coli or bacilli. Higher eukaryotic cells include established cell lines of mammalian origin. Cell-free translation systems could also be employed. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are well known in the art (see Pouwels et al., Cloning Vectors: A Laboratory Manual, Elsevier, N.Y., 1985).

10 Various mammalian or insect cell culture systems are also advantageously employed to express recombinant protein. Expression of recombinant proteins in mammalian cells can be performed because such proteins are generally correctly folded, appropriately modified and completely functional. Examples of suitable mammalian host cell lines include the COS-7 lines
15 of monkey kidney cells, described by Gluzman (Cell 23: 175, 1981), and other cell lines capable of expressing an appropriate vector including, for example, L cells, C127, 3T3, Chinese hamster ovary (CHO), HeLa and BHK cell lines. Mammalian expression vectors can comprise nontranscribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking nontranscribed sequences, and 5' or
20 3' nontranslated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, Bio/Technology 6:47 (1988).

The proteins produced by a transformed host can be purified according to any suitable method. Such standard methods include chromatography (e.g., ion exchange, affinity and sizing
25 column chromatography, and the like), centrifugation, differential solubility, or by any other standard technique for protein purification. Affinity tags such as hexahistidine, maltose binding domain, influenza coat sequence, glutathione-S-transferase, and the like can be attached to the protein to allow easy purification by passage over an appropriate affinity column. Isolated proteins can also be physically characterized using such techniques as proteolysis, nuclear
30 magnetic resonance and x-ray crystallography.

For example, supernatants from systems which secrete recombinant protein into culture media can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a suitable purification matrix. Alternatively, an anion exchange

resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl
5 or carboxymethyl groups. Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a cancer stem cell protein-Fc composition. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous recombinant protein.

10 Recombinant protein produced in bacterial culture can be isolated, for example, by initial extraction from cell pellets, followed by one or more concentration, salting-out, aqueous ion exchange or size exclusion chromatography steps. High performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of a recombinant protein can be disrupted by any convenient method, including freeze-thaw
15 cycling, sonication, mechanical disruption, or use of cell lysing agents.

As such, in certain embodiments, the present invention relates to personalized strategies for the treatment of disorders (e.g., neoplasia), and more particularly tumors, by administering a therapeutically effective amount of a sHDL molecule associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) one or more neoplasia/tumor specific
20 neo-antigens to a subject (e.g., a mammal such as a human) (e.g., a vaccine composition capable of raising a specific T-cell response). Indeed, in certain embodiments, whole genome/exome sequencing may be used to identify all, or nearly all, mutated neo-antigens that are uniquely present in a neoplasia/tumor of an individual patient, and that this collection of mutated neo-antigens may be analyzed to identify a specific, optimized subset of neo-antigens for use as a
25 personalized cancer vaccine for treatment of the patient's neoplasia/tumor. For example, in some embodiments, a population of neoplasia/tumor specific neo-antigens may be identified by sequencing the neoplasia/tumor and normal DNA of each patient to identify tumor-specific mutations, and determining the patient's HLA allotype. The population of neoplasia/tumor specific neo-antigens and their cognate native antigens may then be subject to bioinformatic
30 analysis using validated algorithms to predict which tumor-specific mutations create epitopes that could bind to the patient's HLA allotype, and in particular which tumor-specific mutations create epitopes that could bind to the patient's HLA allotype more effectively than the cognate native antigen. Based on this analysis, one or more peptides corresponding to a subset of these mutations may be designed and synthesized for each patient, and pooled together for use as a

cancer vaccine in immunizing the patient. The neo-antigens peptides may be combined another anti-neoplastic agent. In some embodiments, such neo-antigens are expected to bypass central thymic tolerance (thus allowing stronger antitumor T cell response), while reducing the potential for autoimmunity (e.g., by avoiding targeting of normal self-antigens).

5 The invention further provides a method of inducing a neoplasia/tumor specific immune response in a subject, vaccinating against a neoplasia/tumor, treating and or alleviating a symptom of cancer in a subject by administering the subject a neo-antigenic peptide or vaccine composition of the invention.

10 According to the invention, the above-described cancer vaccine may be used for a patient that has been diagnosed as having cancer, or at risk of developing cancer. In one embodiment, the patient may have a solid tumor such as breast, ovarian, prostate, lung, kidney, gastric, colon, testicular, head and neck, pancreas, brain, melanoma, and other tumors of tissue organs and hematological tumors, such as lymphomas and leukemias, including acute myelogenous leukemia, chronic myelogenous leukemia, chronic lymphocytic leukemia, T cell lymphocytic
15 leukemia, and B cell lymphomas.

The peptide or composition of the invention is administered in an amount sufficient to induce a CTL response. The neo-antigenic peptide, polypeptide or vaccine composition of the invention can be administered alone or in combination with other therapeutic agents. The therapeutic agent is for example, a chemotherapeutic or biotherapeutic agent, radiation, or
20 immunotherapy. Any suitable therapeutic treatment for a particular cancer may be administered. Examples of chemotherapeutic and biotherapeutic agents include, but are not limited to, aldesleukin, altretamine, amifostine, asparaginase, bleomycin, capecitabine, carboplatin, carmustine, cladribine, cisapride, cisplatin, cyclophosphamide, cytarabine, dacarbazine (DTIC), dactinomycin, docetaxel, doxorubicin, dronabinol, epoetin alpha, etoposide, filgrastim,
25 fludarabine, fluorouracil, gemcitabine, granisetron, hydroxyurea, idarubicin, ifosfamide, interferon alpha, irinotecan, lansoprazole, levamisole, leucovorin, megestrol, mesna, methotrexate, metoclopramide, mitomycin, mitotane, mitoxantrone, omeprazole, ondansetron, paclitaxel (Taxol®), pilocarpine, prochloroperazine, rituximab, tamoxifen, taxol, topotecan hydrochloride, trastuzumab, vinblastine, vincristine and vinorelbine tartrate. For prostate cancer
30 treatment, a preferred chemotherapeutic agent with which anti-CTLA-4 can be combined is paclitaxel (Taxol®).

In addition, the subject may be further administered an anti-immunosuppressive or immuno stimulatory agent. For example, the subject is further administered an anti-CTLA-4 antibody, anti-PD-1, anti-PD-L1, anti-TIM-3, anti-BTLA, anti-VISTA, anti-LAG3, anti-CD25,

anti-CD27, anti-CD28, anti-CD137, anti-OX40, anti-GITR, anti-ICOS, anti-TIGIT, and inhibitors of IDO. Blockade of CTLA-4 or PD-1/PD-L1 by antibodies can enhance the immune response to cancerous cells in the patient. In particular, CTLA-4 blockade has been shown effective when following a vaccination protocol.

5 The optimum amount of each peptide to be included in the vaccine composition and the optimum dosing regimen can be determined by one skilled in the art without undue experimentation. For example, the peptide or its variant may be prepared for intravenous (i.v.) injection, sub-cutaneous (s.c.) injection, intradermal (i.d.) injection, intraperitoneal (i.p.) injection, intramuscular (i.m.) injection. Preferred methods of peptide injection include s.c, i.d.,
10 i.p., i.m., and i.v. Preferred methods of DNA injection include i.d., i.m., s.c, i.p. and i.v. For example, doses of between 1 and 500 mg 50 µg and 1.5 mg, preferably 10 µg to 500 µg, of peptide or DNA may be given and will depend from the respective peptide or DNA. Doses of this range were successfully used in previous trials (Brunsvig P F, et al., Cancer Immunol
15 Immunother. 2006; 55(12): 1553- 1564; M. Staehler, et al., ASCO meeting 2007; Abstract No 3017). Other methods of administration of the vaccine composition are known to those skilled in the art.

 The inventive vaccine may be compiled so that the selection, number and/or amount of peptides present in the composition is/are tissue, cancer, and/or patient- specific. For instance, the exact selection of peptides can be guided by expression patterns of the parent proteins in a
20 given tissue to avoid side effects. The selection may be dependent on the specific type of cancer, the status of the disease, earlier treatment regimens, the immune status of the patient, and, of course, the HLA-haplotype of the patient. Furthermore, the vaccine according to the invention can contain individualized components, according to personal needs of the particular patient. Examples include varying the amounts of peptides according to the expression of the related
25 neoantigen in the particular patient, unwanted side-effects due to personal allergies or other treatments, and adjustments for secondary treatments following a first round or scheme of treatment.

 Such vaccines may be administered to an individual already suffering from cancer. In therapeutic applications, such vaccines are administered to a patient in an amount sufficient to
30 elicit an effective CTL response to the tumor antigen and to cure or at least partially arrest symptoms and/or complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on, e.g., the peptide composition, the manner of administration, the stage and severity of the disease being treated, the weight and general state of health of the patient, and the judgment of the prescribing

physician, but generally range for the initial immunization (that is for therapeutic or prophylactic administration) from about 1.0 μg to about 50,000 μg of peptide for a 70 kg patient, followed by boosting dosages or from about 1.0 μg to about 10,000 μg of peptide pursuant to a boosting regimen over weeks to months depending upon the patient's response and condition and possibly
5 by measuring specific CTL activity in the patient's blood. It should be kept in mind that the peptide and compositions of the present invention may generally be employed in serious disease states, that is, life-threatening or potentially life threatening situations, especially when the cancer has metastasized. For therapeutic use, administration should begin as soon as possible after the detection or surgical removal of tumors. This is followed by boosting doses until at
10 least symptoms are substantially abated and for a period thereafter. The pharmaceutical compositions (e.g., vaccine compositions) for therapeutic treatment are intended for parenteral, topical, nasal, oral or local administration. Preferably, the pharmaceutical compositions are administered parenterally, e.g., intravenously, subcutaneously, intradermally, or intramuscularly. The compositions may be administered at the site of surgical excision to induce a local immune
15 response to the tumor.

Such embodiments are not limited to a particular type of adjuvant. Generally, adjuvants are any substance whose admixture into the vaccine composition increases or otherwise modifies the immune response to the mutant peptide. Carriers are scaffold structures, for example a polypeptide or a polysaccharide, to which the antigenic peptide (e.g., neo-antigenic
20 peptide) is capable of being associated. Optionally, adjuvants are conjugated covalently or non-covalently to the peptides or polypeptides of the invention.

The ability of an adjuvant to increase the immune response to an antigen is typically manifested by a significant increase in immune-mediated reaction, or reduction in disease symptoms. For example, an increase in humoral immunity is typically manifested by a
25 significant increase in the titer of antibodies raised to the antigen, and an increase in T-cell activity is typically manifested in increased cell proliferation, or cellular cytotoxicity, or cytokine secretion. An adjuvant may also alter an immune response, for example, by changing a primarily humoral or Th2 response into a primarily cellular, or Th1 response.

Suitable adjuvants include, but are not limited to 1018 ISS, aluminum salts, Amplivax,
30 AS15, BCG, CP-870,893, CpG7909, CyaA, dSLIM, GM-CSF, IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, OK-432, OM-174, OM-197-MP-EC, ONTAK, PepTel.RTM. vector system, PLG microparticles, resiquimod, SRL172, Virosomes and other Virus-like particles, YF-17D, VEGF trap, R848,

beta-glucan, Pam3Cys, Aquila's QS21 stimulon (Aquila Biotech, Worcester, Mass., USA) which is derived from saponin, mycobacterial extracts and synthetic bacterial cell wall mimics, and other proprietary adjuvants such as Ribi's Detox. Quil or Superfos. Several immunological adjuvants (e.g., MF59) specific for dendritic cells and their preparation have been described
5 previously (Dupuis M, et al., Cell Immunol. 1998; 186(1): 18-27; Allison A C; Dev Biol Stand. 1998; 92:3-11). Also cytokines may be used. Several cytokines have been directly linked to influencing dendritic cell migration to lymphoid tissues (e.g., TNF-alpha), accelerating the maturation of dendritic cells into efficient antigen -presenting cells for T-lymphocytes (e.g., GM- CSF, IL-1 and IL-4) (U.S. Pat. No. 5,849,589, specifically incorporated herein by reference
10 in its entirety) and acting as immunoadjuvants (e.g., IL-12) (Gabrilovich D I, et al., J Immunother Emphasis Tumor Immunol. 1996 (6):414-418). Toll like receptors (TLRs) may also be used as adjuvants, and are important members of the family of pattern recognition receptors (PRRs) which recognize conserved motifs shared by many micro-organisms, termed "pathogen-associated molecular patterns" (PAMPS).

15 Recognition of these "danger signals" activates multiple elements of the innate and adaptive immune system. TLRs are expressed by cells of the innate and adaptive immune systems such as dendritic cells (DCs), macrophages, T and B cells, mast cells, and granulocytes and are localized in different cellular compartments, such as the plasma membrane, lysosomes, endosomes, and endolysosomes. Different TLRs recognize distinct PAMPS. For example, TLR4
20 is activated by LPS contained in bacterial cell walls, TLR9 is activated by unmethylated bacterial or viral CpG DNA, and TLR3 is activated by double stranded RNA. TLR ligand binding leads to the activation of one or more intracellular signaling pathways, ultimately resulting in the production of many key molecules associated with inflammation and immunity (particularly the transcription factor NF- κ B and the Type-I interferons). TLR mediated DC
25 activation leads to enhanced DC activation, phagocytosis, upregulation of activation and co-stimulation markers such as CD80, CD83, and CD86, expression of CCR7 allowing migration of DC to draining lymph nodes and facilitating antigen presentation to T cells, as well as increased secretion of cytokines such as type I interferons, IL-12, and IL-6. All of these downstream events are critical for the induction of an adaptive immune response.

30 Other receptors which may be targeted include the toll-like receptors (TLRs). TLRs recognize and bind to pathogen-associated molecular patterns (PAMPs). PAMPs target the TLR on the surface of the dendritic cell and signals internally, thereby potentially increasing DC antigen uptake, maturation and T-cell stimulatory capacity. PAMPs conjugated to the particle surface or co-encapsulated include unmethylated CpG DNA (bacterial), double-stranded RNA

(viral), lipopolysacharride (bacterial), peptidoglycan (bacterial), lipoarabinomannin (bacterial), zymosan (yeast), mycoplasmal lipoproteins such as MALP-2 (bacterial), flagellin (bacterial) poly(inosinic-cytidylic) acid (bacterial), lipoteichoic acid (bacterial) or imidazoquinolines (synthetic).

5 Among the most promising cancer vaccine adjuvants currently in clinical development are the TLR9 agonist CpG and the synthetic double- stranded RNA (dsRNA) TLR3 ligand poly- ICLC. In preclinical studies poly-ICLC appears to be the most potent TLR adjuvant when compared to LPS and CpG due to its induction of pro-inflammatory cytokines and lack of stimulation of IL-10, as well as maintenance of high levels of co-stimulatory molecules in DCs.
10 Furthermore, poly-ICLC was recently directly compared to CpG in non-human primates (rhesus macaques) as adjuvant for a protein vaccine consisting of human papillomavirus (HPV)16 capsomers (Stahl-Hennig C, Eisenblatter M, Jasny E, et al. Synthetic double- stranded RNAs are adjuvants for the induction of T helper 1 and humoral immune responses to human papillomavirus in rhesus macaques. PLoS pathogens. Apr 2009;5(4)).

15 In some embodiments, the adjuvant is a dendritic cell targeting molecule (DC). DC is potent and is responsible for initiating antigen-specific immune responses. One biological feature of DCs is their ability to sense conditions under which antigen is encountered, initiating a process of “DC maturation”. Using receptors for various microbial and inflammatory products, DCs respond to antigen exposure in different ways depending on the nature of the pathogen
20 (virus, bacteria, protozoan) encountered. This information is transmitted to T cells by altered patterns of cytokine release at the time of antigen presentation in lymph nodes, altering the type of T cell response elicited. Thus, targeting DCs provides the opportunity not only to quantitatively enhance the delivery of antigen and antigen responses in general, but to qualitatively control the nature of the immune response depending on the desired vaccination
25 outcome.

 Dendritic cells express a number of cell surface receptors that can mediate the endocytosis of bound antigen. Targeting exogenous antigens to internalizing surface molecules on systemically-distributed antigen presenting cells facilitates uptake of antigens and thus overcomes a major rate-limiting step in immunization and thus in vaccination.

30 Dendritic cell targeting molecules include monoclonal or polyclonal antibodies or fragments thereof that recognize and bind to epitopes displayed on the surface of dendritic cells. Dendritic cell targeting molecules also include ligands which bind to a cell surface receptor on dendritic cells. One such receptor, the lectin DEC-205, has been used in vitro and in mice to boost both humoral (antibody-based) and cellular (CD8 T cell) responses by 2-4 orders of

magnitude (see, e.g., Hawiger, et al., J. Exp. Med., 194(6):769-79 (2001); Bonifaz, et al., J. Exp. Med., 196(12):1627-38 (2002); Bonifaz, et al., J. Exp. Med., 199(6):815-24 (2004)).

A variety of other endocytic receptors, including a mannose-specific lectin (mannose receptor) and IgG Fc receptors, have also been targeted in this way with similar enhancement of antigen presentation efficiency. Other suitable receptors which may be targeted include, but are not limited to, DC-SIGN, 33D1, SIGLEC-H, DCIR, CD11c, heat shock protein receptors and scavenger receptors.

In some embodiments, the adjuvant is CpG. CpG immuno stimulatory oligonucleotides have also been reported to enhance the effects of adjuvants in a vaccine setting. Without being bound by theory, CpG oligonucleotides act by activating the innate (non- adaptive) immune system via Toll-like receptors (TLR), mainly TLR9. CpG triggered TLR9 activation enhances antigen- specific humoral and cellular responses to a wide variety of antigens, including peptide or protein antigens, live or killed viruses, dendritic cell vaccines, autologous cellular vaccines and polysaccharide conjugates in both prophylactic and therapeutic vaccines. More importantly, it enhances dendritic cell maturation and differentiation, resulting in enhanced activation of Th1 cells and strong cytotoxic T- lymphocyte (CTL) generation, even in the absence of CD4 T-cell help. The Th1 bias induced by TLR9 stimulation is maintained even in the presence of vaccine adjuvants such as alum or incomplete Freund's adjuvant (IFA) that normally promote a Th2 bias. CpG oligonucleotides show even greater adjuvant activity when formulated or co-administered with other adjuvants or in formulations such as microparticles, nano particles, lipid emulsions or similar formulations, which are especially necessary for inducing a strong response when the antigen is relatively weak. They also accelerate the immune response and enabled the antigen doses to be reduced by approximately two orders of magnitude, with comparable antibody responses to the full-dose vaccine without CpG in some experiments (Arthur M. Krieg, Nature Reviews, Drug Discovery, 5, Jun. 2006, 471-484). U.S. Pat. No. 6,406,705 B1 describes the combined use of CpG oligonucleotides, non-nucleic acid adjuvants and an antigen to induce an antigen- specific immune response. A commercially available CpG TLR9 antagonist is dSLIM (double Stem Loop Immunomodulator) by Mologen (Berlin, GERMANY), which is a preferred component of the pharmaceutical composition of the present invention. Other TLR binding molecules such as RNA binding TLR 7, TLR 8 and/or TLR 9 may also be used.

Xanthenone derivatives such as, for example, Vadimezan or AsA404 (also known as 5,6-dimethylxanthenone-4-acetic acid (DMXAA)), may also be used as adjuvants according to embodiments of the invention. Alternatively, such derivatives may also be administered in parallel to the vaccine of the invention, for example via systemic or intratumoral delivery, to

stimulate immunity at the tumor site. Without being bound by theory, it is believed that such xanthenone derivatives act by stimulating interferon (IFN) production via the stimulator of IFN gene (STING) receptor (see e.g., Conlon et al. (2013) Mouse, but not Human STING, Binds and Signals in Response to the Vascular Disrupting Agent 5, 6-Dimethylxanthenone-4- Acetic Acid, *Journal of Immunology*, 190:5216-25 and Kim et al. (2013) Anticancer Flavonoids are Mouse-Selective STING Agonists, *8*: 1396-1401). Other examples of useful adjuvants include, but are not limited to, chemically modified CpGs (e.g. CpR, Idera), Poly(I:C)(e.g. polyi:CI2U), non-CpG bacterial DNA or RNA as well as immunoactive small molecules and antibodies such as cyclophosphamide, sunitinib, bevacizumab, celebrex, NCX-4016, sildenafil, tadalafil, vardenafil, sorafenib, XL-999, CP- 547632, pazopanib, ZD2171, AZD2171, ipilimumab, tremelimumab, and SC58175, which may act therapeutically and/or as an adjuvant. The amounts and concentrations of adjuvants and additives useful in the context of the present invention can readily be determined by the skilled artisan without undue experimentation. Additional adjuvants include colony- stimulating factors, such as Granulocyte Macrophage Colony Stimulating Factor (GM-CSF, sargramostim).

Poly-ICLC is a synthetically prepared double-stranded RNA consisting of polyI and polyC strands of average length of about 5000 nucleotides, which has been stabilized to thermal denaturation and hydrolysis by serum nucleases by the addition of polylysine and carboxymethylcellulose. The compound activates TLR3 and the RNA helicase-domain of MDA5, both members of the PAMP family, leading to DC and natural killer (NK) cell activation and production of a "natural mix" of type I interferons, cytokines, and chemokines. Furthermore, poly-ICLC exerts a more direct, broad host-targeted anti-infectious and possibly antitumor effect mediated by the two IFN-inducible nuclear enzyme systems, the 2' 5' -OAS and the PI/eIF2a kinase, also known as the PKR (4-6), as well as RIG-I helicase and MDA5.

Such methods are not limited to generating sHDL nanoparticles associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) an antigen and an adjuvant (e.g., dendritic cell targeting molecule). In some embodiments, the antigen and adjuvant are conjugated to outer surface of the sHDL nanoparticle.

In some embodiments, the sHDL nanoparticle is synthesized with thiol-reactive phospholipids that permit reduction-sensitive linkage of the antigen and/or adjuvant. In some embodiments, loading of the DC within the sHDL nanoparticle is facilitated through cholesterol modification of the DC molecule. In some embodiments, lyophilization methods are used for the preparation of homogenous sHDL. In some embodiments, phospholipids and ApoA mimetic peptides are dissolved in glacial acetic acid and lyophilized. In some embodiments, antigen

peptides are incubated with sHDL in a buffer (e.g., a sodium phosphate buffer (pH 7.4)) (e.g., at room temperature for 3 hours) to allow for the conjugation of antigen peptides. In some embodiments, the unconjugated antigen peptides are removed using a desalting column (MWCO = 7000 Da). In some embodiments, incorporation of the cholesterol modified DC (Cho-DC) to sHDL involves incubation with sHDL at room temperature for approximately 30 min.

Such embodiments are not limited to a particular manner of characterizing the sHDL conjugated with antigen and DC. In some embodiments, the morphology of sHDL is observed by TEM. In some embodiments, the size distribution of sHDL is analyzed by dynamic light scattering (DLS) using a Malven Nanosizer instrument and GPC assay.

The sHDL nanoparticles configured to activate an immune response (e.g., sHDL- α GalCer) (e.g., Ag/DC-sHDL) are useful for activating T cells in subjects for prophylactic and therapeutic applications. Activation of T cells by nanoparticle vaccine compositions increases their proliferation, cytokine production, differentiation, effector functions and/or survival. Methods for measuring these are well known to those in the art. The T cells activated by the nanoparticle vaccine compositions can be any cell which express the T cell receptor, including α/β and γ/δ T cell receptors. T-cells include all cells which express CD3, including T-cell subsets which also express CD4 and CD8. T-cells include both naive and memory cells and effector cells such as CTL. T-cells also include regulatory cells such as Th1, Tc1, Th2, Tc2, Th3, Treg, and Tr1 cells. T-cells also include NKT-cells and similar unique classes of the T-cell lineage. In some embodiments, the T cells that are activated are CD8⁺ T cells.

In general, compositions comprising the sHDL nanoparticles configured to activate an immune response (e.g., sHDL- α GalCer) (e.g., Ag/DC-sHDL) are useful for treating a subject having or being predisposed to any disease or disorder to which the subject's immune system mounts an immune response. The compositions are useful as prophylactic vaccines, which confer resistance in a subject to subsequent exposure to infectious agents. The compositions are also useful as therapeutic vaccines, which can be used to initiate or enhance a subject's immune response to a pre-existing antigen, such as a tumor antigen in a subject with cancer, or a viral antigen in a subject infected with a virus. The compositions are also useful as desensitizing vaccines, which function to "tolerize" an individual to an environmental antigen, such as an allergen.

The ability to target these compositions to professional antigen-presenting cells such as dendritic cells, and the ability of these compositions to elicit T-cell mediated immune responses by causing cross-presentation of antigens makes these compositions especially useful for eliciting a cell-mediated response to a disease-related antigen in order to attack the disease.

Thus, in some embodiments, the type of disease to be treated or prevented is a malignant tumor or a chronic infectious disease caused by a bacterium, virus, protozoan, helminth, or other microbial pathogen that enters intracellularly and is attacked, i.e., by the cytotoxic T lymphocytes.

5 The desired outcome of a prophylactic, therapeutic or de-sensitized immune response may vary according to the disease, according to principles well known in the art. For example, an immune response against an infectious agent may completely prevent colonization and replication of an infectious agent, affecting “sterile immunity” and the absence of any disease symptoms. However, a vaccine against infectious agents may be considered effective if it
10 reduces the number, severity or duration of symptoms; if it reduces the number of individuals in a population with symptoms; or reduces the transmission of an infectious agent. Similarly, immune responses against cancer, allergens or infectious agents may completely treat a disease, may alleviate symptoms, or may be one facet in an overall therapeutic intervention against a disease. For example, the stimulation of an immune response against a cancer may be coupled
15 with surgical, chemotherapeutic, radiologic, hormonal and other immunologic approaches in order to affect treatment.

 Subjects with or exposed to infectious agents can be treated therapeutically or prophylactically the sHDL nanoparticles configured to activate an immune response (e.g., sHDL- α GalCer) (e.g., Ag/DC-sHDL) as disclosed herein. Infectious agents include bacteria,
20 viruses and parasites. In some instances, the subject can be treated prophylactically, such as when there may be a risk of developing disease from an infectious agent. An individual traveling to or living in an area of endemic infectious disease may be considered to be at risk and a candidate for prophylactic vaccination against the particular infectious agent. Preventative treatment can be applied to any number of diseases where there is a known relationship between
25 the particular disease and a particular risk factor, such as geographical location or work environment.

 Subjects with or at risk for developing malignant tumors can be treated therapeutically or prophylactically the sHDL nanoparticles configured to activate an immune response (e.g., sHDL- α GalCer) (e.g., Ag/DC-sHDL) as disclosed herein. In a mature animal, a balance usually
30 is maintained between cell renewal and cell death in most organs and tissues. The various types of mature cells in the body have a given life span; as these cells die, new cells are generated by the proliferation and differentiation of various types of stem cells. Under normal circumstances, the production of new cells is so regulated that the numbers of any particular type of cell remain constant. Occasionally, though, cells arise that are no longer responsive to normal growth-

control mechanisms. These cells give rise to clones of cells that can expand to a considerable size, producing a tumor or neoplasm. A tumor that is not capable of indefinite growth and does not invade the healthy surrounding tissue extensively is benign. A tumor that continues to grow and becomes progressively invasive is malignant. The term cancer refers specifically to a malignant tumor. In addition to uncontrolled growth, malignant tumors exhibit metastasis. In this process, small clusters of cancerous cells dislodge from a tumor, invade the blood or lymphatic vessels, and are carried to other tissues, where they continue to proliferate. In this way a primary tumor at one site can give rise to a secondary tumor at another site. The sHDL nanoparticles configured to activate an immune response (e.g., sHDL- α GalCer) (e.g., Ag/DC-sHDL) as disclosed herein are useful for treating subjects having malignant tumors.

Malignant tumors which may be treated are classified herein according to the embryonic origin of the tissue from which the tumor is derived. Carcinomas are tumors arising from endodermal or ectodermal tissues such as skin or the epithelial lining of internal organs and glands. A melanoma is a type of carcinoma of the skin for which this invention is particularly useful. Sarcomas, which arise less frequently, are derived from mesodermal connective tissues such as bone, fat, and cartilage. The leukemias and lymphomas are malignant tumors of hematopoietic cells of the bone marrow. Leukemias proliferate as single cells, whereas lymphomas tend to grow as tumor masses. Malignant tumors may show up at numerous organs or tissues of the body to establish a cancer.

The types of cancer that can be treated in with the provided sHDL nanoparticles configured to activate an immune response (e.g., sHDL- α GalCer) (e.g., Ag/DC-sHDL) include, but are not limited to, the following: bladder, brain, breast, cervical, colo-rectal, esophageal, kidney, liver, lung, nasopharyngeal, pancreatic, prostate, skin, stomach, uterine, and the like. Administration is not limited to the treatment of an existing tumor or infectious disease but can also be used to prevent or lower the risk of developing such diseases in an individual, i.e., for prophylactic use. Potential candidates for prophylactic vaccination include individuals with a high risk of developing cancer, i.e., with a personal or familial history of certain types of cancer.

Subjects with or at risk for exposure to allergens can be treated therapeutically or prophylactically the sHDL nanoparticles configured to activate an immune response (e.g., sHDL- α GalCer) (e.g., Ag/DC-sHDL) as disclosed herein. Such sHDL nanoparticles may be administered to subjects for the purpose of preventing and/or attenuating allergic reactions, such as allergic reactions which lead to anaphylaxis. Allergic reactions may be characterized by the T_H2 responses against an antigen leading to the presence of IgE antibodies. Stimulation of T_H1 immune responses and the production of IgG antibodies may alleviate allergic disease. Thus, the

sHDL nanoparticles configured to activate an immune response (e.g., sHDL- α GalCer) (e.g., Ag/DC-sHDL) as disclosed herein are useful for producing antibodies that prevent and/or attenuate allergic reactions in subjects exposed to allergens.

5 Subjects with or at risk for immunosuppressed conditions can be treated therapeutically or prophylactically the sHDL nanoparticles configured to activate an immune response (e.g., sHDL- α GalCer) (e.g., Ag/DC-sHDL) as disclosed herein. The sHDL nanoparticle vaccines disclosed herein can be used for treatment of disease conditions characterized by immunosuppression, including, but not limited to, AIDS or AIDS-related complex, idiopathic immuno suppression, drug induced immunosuppression, other virally or environmentally-
10 induced conditions, and certain congenital immune deficiencies. Such sHDL nanoparticle vaccine compositions can also be employed to increase immune function that has been impaired by the use of radiotherapy of immunosuppressive drugs (e.g., certain chemotherapeutic agents), and therefore can be particularly useful when used in conjunction with such drugs or radiotherapy.

15 Subjects with or at risk for coronary heart disease and/or elevated LDL-C levels can be treated therapeutically or prophylactically the sHDL nanoparticles configured to activate an immune response as disclosed herein. While effectiveness of mAb therapy against PCSK9 has established (see, e.g., Banerjee, Y.; et al., *New England Journal of Medicine* 2012, 366 (25), 2425-2426; Stein, E. A.; et al., *Circulation* 2013, 128 (19), 2113-2120), development of more
20 durable PCSK9 vaccines are needed. In addition, one of the challenges for PCSK9 vaccines is that self antigens, such as PCSK9 peptides, are not immunogenic, unless they are coupled to vaccine/adjuvant systems that can efficiently co-deliver antigens and immunostimulatory molecules to immune cells (see, e.g., Krishnamachari, Y.; et al., *Advanced Drug Delivery Reviews* 2009, 61 (3), 205-217; Hamdy, S.; et al., *Advanced Drug Delivery Reviews* 2011, 63
25 (10-11), 943-955).

Embodiments of the present invention wherein sHDL nanoparticles are conjugated with a PCSK9-antigen and a CpG-adjuvant (PCSK9-Ag/CpG-sHDL) address such needs. Indeed, vaccination against PCSK9 with PCSK9-Ag/CpG-sHDL embodiments effectively inhibits interaction between PCSK9 and LDLR, while avoiding the need for repeated injections of
30 expensive mAb (see, e.g., Fattori, E.; et al., *Journal of Lipid Research* 2012, 53 (8), 1654-1661; Gergana Galabova, et al., *PLOS ONE* 2014, 9 (12)). Moreover, such PCSK9-Ag/CpG-sHDL nanoparticles have a sufficiently small size (e.g., 10-45 nm) permitting efficient drainage to the lymph nodes compared to larger particles (see, e.g., Bachmann, M. F.; et al., *Nature Reviews Immunology* 2010, 10 (11), 787-796).

In general, methods of administering vaccines as disclosed herein (e.g., sHDL nanoparticles configured to activate an immune response (e.g., sHDL- α GalCer) (e.g., Ag/DC-sHDL)) are well known in the art. Any acceptable method known to one of ordinary skill in the art may be used to administer a formulation to the subject. The administration may be localized (i.e., to a particular region, physiological system, tissue, organ, or cell type) or systemic. Vaccines can be administered by a number of routes including, but not limited to: oral, inhalation (nasal or pulmonary), intravenous, intraperitoneal, intramuscular, transdermal, subcutaneous, topical, sublingual, or rectal means. Injections can be e.g., intravenous, intradermal, subcutaneous, intramuscular, or intraperitoneal. In some embodiments, the injections can be given at multiple locations.

Administration of the formulations may be accomplished by any acceptable method which allows an effective amount of the vaccine to reach its target. The particular mode selected will depend upon factors such as the particular formulation, the severity of the state of the subject being treated, and the dosage required to induce an effective immune response. As generally used herein, an "effective amount" is that amount which is able to induce an immune response in the treated subject. The actual effective amounts of vaccine can vary according to the specific antigen or combination thereof being utilized, the particular composition formulated, the mode of administration, and the age, weight, condition of the individual being vaccinated, as well as the route of administration and the disease or disorder.

In certain embodiments, glycolipids encapsulated within sHDL nanoparticles are used as stimulators of natural killer T cell-mediated immune responses.

Natural killer T (NKT) cells are a heterogeneous group of T cells that share properties of both T cells and natural killer cells. Many of these cells recognize the non-polymorphic CD1d molecule, an antigen-presenting molecule that binds self and foreign lipids and glycolipids. NKT cells constitute only approximately 0.1% of all peripheral blood T cells. NKT cells are a subset of T cells that coexpress an $\alpha\beta$ T-cell receptor, but also express a variety of molecular markers that are typically associated with NK cells, such as NK1.1. The best-known NKT cells differ from conventional $\alpha\beta$ T cells in that their T-cell receptors are far more limited in diversity ('invariant' or 'type 1' NKT). They and other CD1d-restricted T cells ('type 2' NKT) recognize lipids and glycolipids presented by CD1d molecules, a member of the CD1 family of antigen-presenting molecules, rather than peptide-major histocompatibility complexes (MHCs). NKT cells include both NK1.1⁺ and NK1.1⁻, as well as CD4⁺, CD4⁻, CD8⁺ and CD8⁻ cells.

In some embodiments the glycolipid is the synthetic glycolipid alpha-galactosylceramide (α GalCer). Dendritic cells presenting antigens in the context of CD1d can lead to rapid innate

and prolonged production of cytokines such as interferon and IL-4 by natural killer T cells (NKT cells). CD1d is a major histocompatibility complex class I-like molecule that presents glycolipid antigens to a subset of NKT cells. Advantageously, α GalCer is not toxic to humans and has been shown to act as an adjuvant, priming both antigen-specific CD4⁺ and CD8⁺ T cell responses.

5 For example, it has been shown that α GalCer in conjunction with a malaria vaccine can lead to cytotoxic responses against infected cells, which is an ideal scenario for vaccines against infectious diseases. In addition to α GalCer, other glycolipids that function as adjuvants to activate NKT cell-mediated immune responses can be used.

The present invention is not limited to particular methods for generating sHDL
10 nanoparticles having encapsulated α GalCer. For example, in some embodiments, lyophilization methods are used for the preparation of homogenous sHDL. In some embodiments, phospholipids and ApoA mimetic peptides are dissolved in glacial acetic acid and lyophilized. In some embodiments, loading of α GalCer into the sHDL nanoparticle is facilitated through hydrophobic interactions between the α GalCer and the sHDL. In some embodiments, the
15 lyophilized phospholipids and ApoA mimetic peptides are hydrated (e.g., hydrated in PBS (pH 7.4)) and thermocycled above and below the transition temperature (T_m) of phospholipids to form blank sHDL, which are next incubated with α GalCer at room temperature for an optimal amount of time (e.g., 5, 10, 20, 25, 30, 35, 50, 80, 120, 360 minutes) to form sHDL comprising encapsulated α GalCer.

20 Such embodiments are not limited to a particular manner of characterizing the sHDL comprising encapsulated α GalCer. In some embodiments, the morphology of sHDL- α GalCer is observed by TEM. In some embodiments, the size distribution of sHDL- α GalCer is analyzed by dynamic light scattering (DLS) using a Malven Nanosizer instrument and GPC assay.

Such embodiments are not limited to a particular manner of assessing the delivery profile
25 of the α GalCer in vitro and in vivo. In some embodiments, labelling the molecules with an imaging agent (e.g., fluorescent dye Cy3) permits visualization of the biodistribution of α GalCer molecules at the organ level and also the intracellular delivery profile.

In certain embodiments, the present invention provides methods for inducing a natural killer T cell-mediated immune response in a cell comprising exposing the cell to a composition
30 comprising an α GalCer glycolipid encapsulated within a sHDL nanoparticle, wherein such exposure results in the induction of a natural killer T cell-mediated immune response. In some embodiments, the cells are in vivo cells. In some embodiments, the cells are in vitro cells. In some embodiments, the cells are ex vivo cells.

In certain embodiments, the present invention provides methods for inducing a natural

killer T cell-mediated immune response in a subject (e.g., a human patient) comprising administering to the patient a pharmaceutical composition comprising an α GalCer glycolipid encapsulated within a sHDL nanoparticle, wherein such administration results in the induction of a natural killer T cell-mediated immune response.

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Additional Embodiments

In certain embodiments, the sHDL nanoparticles as described herein (e.g., configured for RNA Interference) (e.g., configured for activating an immune response) are associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) one or more therapeutic agents. Such embodiments are not limited to particular type or kind of therapeutic agent.

In some embodiments, the therapeutic agent configured for treating and/or preventing cancer. Examples of such therapeutic agents include, but are not limited to, chemotherapeutic agents, anti-oncogenic agents, anti-angiogenic agents, tumor suppressor agents, anti-microbial agents, etc.

In some embodiments, the therapeutic agent is configured for treating and/or preventing autoimmune disorders and/or inflammatory disorders. Examples of such therapeutic agents include, but are not limited to, disease-modifying antirheumatic drugs (e.g., leflunomide, methotrexate, sulfasalazine, hydroxychloroquine), biologic agents (e.g., rituximab, infliximab, etanercept, adalimumab, golimumab), nonsteroidal anti-inflammatory drugs (e.g., ibuprofen, celecoxib, ketoprofen, naproxen, piroxicam, diclofenac), analgesics (e.g., acetaminophen, tramadol), immunomodulators (e.g., anakinra, abatacept), glucocorticoids (e.g., prednisone, methylprednisone), TNF- α inhibitors (e.g., adalimumab, certolizumab pegol, etanercept, golimumab, infliximab), IL-1 inhibitors, and metalloprotease inhibitors. In some embodiments, the therapeutic agents include, but are not limited to, infliximab, adalimumab, etanercept, parenteral gold or oral gold.

In some embodiments, the therapeutic agent is configured for treating and/or preventing cardiovascular related disorders (e.g., atherosclerosis, heart failure, arrhythmia, atrial fibrillation, hypertension, coronary artery disease, angina pectoris, etc.). Examples of therapeutic agents known to be useful in treating and/or preventing cardiovascular related disorders include, angiotensin-converting enzyme (ACE) inhibitors (e.g., benazepril, enalapril, Lisinopril, perindopril, Ramipril), adenosine, alpha blockers (alpha adrenergic antagonist medications) (e.g., clonidine, guanabenz, labetalol, phenoxybenzamine, terazosin, doxazosin, guanfacine, methyl dopa, prazosin), angiotensin II receptor blockers (ARBs) (e.g., candesartan, irbesartan,

olmesartan medoxomil, telmisartan, eprosartan, losartan, tasosartan, valsartan), antiocoagulants (e.g., heparin fondaparinux, warfarin, ardeparin, enoxaparin, reviparin, dalteparin, nadroparin, tinzaparin), antiplatelet agents (e.g., abciximab, clopidogrel, eptifibatide, ticlopidine, cilostazol, dipyridamole, sulfinpyrazone, tirofiban), beta blockers (e.g., acebutolol, betaxolol, carteolol, metoprolol, penbutolol, propranolol, atenolol, bisoprolol, esmolol, nadolol, pindolol, timolol), calcium channel blockers (e.g., amlodipine, felodipine, isradipine, nifedipine, verapamil, diltiazem, nicardipine, nimodipine, nisoldipine), diuretics, aldosterone blockers, loop diuretics (e.g., bumetanide, furosemide, ethacrynic acid, torsemide), potassium-sparing diuretics, thiazide diuretics (e.g., chlorothiazide, chlorthalidone, hydrochlorothiazide, hydroflumethiazide, methyclothiazide, metolazone, polythiazide, quinethazone, trichlormethiazide), inotropics, bile acid sequestrants (e.g., cholestyramine, colestipol, colesevelam), fibrates (e.g., clofibrate, gemfibrozil, fenofibrate), statins (e.g., atorvastatin, lovastatin, simvastatin, fluvastatin, pravastatin), selective cholesterol absorption inhibitors (e.g., ezetimibe), potassium channel blockers (e.g., amilorone, ibutilide, dofetilide), sodium channel blockers (e.g., disopyramide, mexiletine, procainamide, quinidine, flecainide, moricizine, propafenone), thrombolytic agents (e.g., alteplase, reteplase, tenecteplase, anistreplase, streptokinase, urokinase), vasoconstrictors, vasodilators (e.g., hydralazine, minoxidil, mecamlamine, isorbide dinitrate, isorbide mononitrate, nitroglycerin).

Generally, the sHDL nanoparticles so formed are spherical and have a diameter of from about 5 nm to about 20 nm (e.g., 4 – 75 nm, 4-60 nm, 4-50 nm, 4-22 nm, 6 – 18 nm, 8 – 15 nm, 8- 10 nm, etc.). In some embodiments, the sHDL nanoparticles are subjected to size exclusion chromatography to yield a more homogeneous preparation.

In some embodiments, the sHDL nanoparticles are further associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) agents useful for determining the location of administered particles. Agents useful for this purpose include fluorescent tags, radionuclides and contrast agents.

Suitable imaging agents include, but are not limited to, fluorescent molecules such as those described by Molecular Probes (Handbook of fluorescent probes and research products), such as Rhodamine, fluorescein, Texas red, Acridine Orange, Alexa Fluor (various), Allophycocyanin, 7-aminoactinomycin D, BOBO-1, BODIPY (various), Calcien, Calcium Crimson, Calcium green, Calcium Orange, 6-carboxyrhodamine 6G, Cascade blue, Cascade yellow, DAPI, DiA, DID, Di1, DiO, DiR, ELF 97, Eosin, ER Tracker Blue-White, EthD-1, Ethidium bromide, Fluo-3, Fluo4, FM1-43, FM4-64, Fura-2, Fura Red, Hoechst 33258, Hoechst 33342, 7-hydroxy-4-methylcoumarin, Indo-1, JC-1, JC-9, JOE dye, Lissamine rhodamine B,

Lucifer Yellow CH, LysoSensor Blue DND-167, LysoSensor Green, LysoSensor Yellow/Blu,
 LysoTracker Green FM, Magnesium Green, Marina Blue, Mitotracker Green FM, Mitotracker
 Orange CMTMRos, MitoTracker Red CMXRos, Monobromobimane, NBD amines, NeruoTrace
 500/525 green, Nile red, Oregon Green, Pacific Blue. POP-1, Propidium iodide, Rhodamine
 5 110, Rhodamine Red, R-Phycoerythrin, Resorfin, RH414, Rhod-2, Rhodamine Green,
 Rhodamine 123, ROX dye, Sodium Green, SYTO blue (various), SYTO green (Various), SYTO
 orange (various), SYTOX blue, SYTOX green, SYTOX orange, Tetramethylrhodamine B,
 TOT-1, TOT-3, X-rhod-1, YOYO-1, YOYO-3. In some embodiments, ceramides are provided
 as imaging agents. In some embodiments, S1P agonists are provided as imaging agents.

10 Additionally radionuclides can be used as imaging agents. Suitable radionuclides
 include, but are not limited to radioactive species of Fe(III), Fe(II), Cu(II), Mg(II), Ca(II), and
 Zn(II) Indium, Gallium and Technetium. Other suitable contrast agents include metal ions
 generally used for chelation in paramagnetic T1-type MIR contrast agents, and include di- and
 tri-valent cations such as copper, chromium, iron, gadolinium, manganese, erbium, europium,
 15 dysprosium and holmium. Metal ions that can be chelated and used for radionuclide imaging,
 include, but are not limited to metals such as gallium, germanium, cobalt, calcium, indium,
 iridium, rubidium, yttrium, ruthenium, yttrium, technetium, rhenium, platinum, thallium and
 samarium. Additionally metal ions known to be useful in neutron-capture radiation therapy
 include boron and other metals with large nuclear cross-sections. Also suitable are metal ions
 20 useful in ultrasound contrast, and X-ray contrast compositions.

Examples of other suitable contrast agents include gases or gas emitting compounds,
 which are radioopaque.

In some embodiments, the sHDL nanoparticles are further associated with (e.g.,
 complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) a targeting agent. In some
 25 embodiments, targeting agents are used to assist in delivery of the sHDL-TA nanoparticles to
 desired body regions (e.g., bodily regions affected by a cardiovascular related disorder).
 Examples of targeting agents include, but are not limited to, an antibody, receptor ligand,
 hormone, vitamin, and antigen, however, the present invention is not limited by the nature of the
 targeting agent. In some embodiments, the antibody is specific for a disease-specific antigen. In
 30 some embodiments, the receptor ligand includes, but is not limited to, a ligand for CFTR,
 EGFR, estrogen receptor, FGR2, folate receptor, IL-2 receptor, glycoprotein, and VEGFR. In
 some embodiments, the receptor ligand is folic acid.

In some embodiments, the sHDL nanoparticles of the present invention may be delivered
 to local sites in a patient by a medical device. Medical devices that are suitable for use in the

present invention include known devices for the localized delivery of therapeutic agents. Such devices include, but are not limited to, catheters such as injection catheters, balloon catheters, double balloon catheters, microporous balloon catheters, channel balloon catheters, infusion catheters, perfusion catheters, etc., which are, for example, coated with the therapeutic agents or through which the agents are administered; needle injection devices such as hypodermic needles and needle injection catheters; needleless injection devices such as jet injectors; coated stents, bifurcated stents, vascular grafts, stent grafts, etc.; and coated vaso-occlusive devices such as wire coils.

Exemplary devices are described in U.S. Pat. Nos. 5,935,114; 5,908,413; 5,792,105; 5,693,014; 5,674,192; 5,876,445; 5,913,894; 5,868,719; 5,851,228; 5,843,089; 5,800,519; 5,800,508; 5,800,391; 5,354,308; 5,755,722; 5,733,303; 5,866,561; 5,857,998; 5,843,003; and 5,933,145; the entire contents of which are incorporated herein by reference. Exemplary stents that are commercially available and may be used in the present application include the RADIUS (SCIMED LIFE SYSTEMS, Inc.), the SYMPHONY (Boston Scientific Corporation), the Wallstent (Schneider Inc.), the PRECEDENT II (Boston Scientific Corporation) and the NIR (Medinol Inc.). Such devices are delivered to and/or implanted at target locations within the body by known techniques.

In some embodiments, the present invention also provides kits comprising sHDL nanoparticles as described herein. In some embodiments, the kits comprise one or more of the reagents and tools necessary to generate sHDL nanoparticles, and methods of using such sHDL nanoparticles.

The sHDL nanoparticles of the present invention may be characterized for size and uniformity by any suitable analytical techniques. These include, but are not limited to, atomic force microscopy (AFM), electrospray-ionization mass spectroscopy, MALDI-TOF mass spectroscopy, ¹³C nuclear magnetic resonance spectroscopy, high performance liquid chromatography (HPLC) size exclusion chromatography (SEC) (equipped with multi-angle laser light scattering, dual UV and refractive index detectors), capillary electrophoresis and gel electrophoresis. These analytical methods assure the uniformity of the sHDL nanoparticle population and are important in the production quality control for eventual use in in vivo applications.

In some embodiments, gel permeation chromatography (GPC), which can separate sHDL nanoparticles from liposomes and free ApoA-I mimetic peptide, is used to analyze the sHDL-TA nanoparticles. In some embodiments, the size distribution and zeta-potential is determined by dynamic light scattering (DLS) using, for example, a Malven Nanosizer instrument.

Where clinical applications are contemplated, in some embodiments of the present invention, the sHDL nanoparticles are prepared as part of a pharmaceutical composition in a form appropriate for the intended application. Generally, this entails preparing compositions that are essentially free of pyrogens, as well as other impurities that could be harmful to humans or animals. However, in some embodiments of the present invention, a straight sHDL nanoparticle formulation may be administered using one or more of the routes described herein.

In preferred embodiments, the sHDL nanoparticles are used in conjunction with appropriate salts and buffers to render delivery of the compositions in a stable manner to allow for uptake by target cells. Buffers also are employed when the sHDL nanoparticles are introduced into a patient. Aqueous compositions comprise an effective amount of the sHDL nanoparticles to cells dispersed in a pharmaceutically acceptable carrier or aqueous medium. Such compositions also are referred to as inocula. The phrase "pharmaceutically or pharmacologically acceptable" refer to molecular entities and compositions that do not produce adverse, allergic, or other untoward reactions when administered to an animal or a human. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. Except insofar as any conventional media or agent is incompatible with the vectors or cells of the present invention, its use in therapeutic compositions is contemplated. Supplementary active ingredients may also be incorporated into the compositions.

In some embodiments of the present invention, the active compositions include classic pharmaceutical preparations. Administration of these compositions according to the present invention is via any common route so long as the target tissue is available via that route. This includes oral, nasal, buccal, rectal, vaginal or topical. Alternatively, administration may be by orthotopic, intradermal, subcutaneous, intramuscular, intraperitoneal or intravenous injection.

The active sHDL nanoparticles may also be administered parenterally or intraperitoneally or intratumorally. Solutions of the active compounds as free base or pharmacologically acceptable salts are prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol,

and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it may be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active sHDL nanoparticles in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Upon formulation, sHDL nanoparticles are administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug release capsules and the like. For parenteral administration in an aqueous solution, for example, the solution is suitably buffered, if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). In some embodiments of the present invention, the active particles or agents are formulated within a therapeutic mixture to comprise about 0.0001 to 1.0 milligrams, or about 0.001 to 0.1 milligrams, or about 0.1 to 1.0 or even about 10 milligrams per dose or so. Multiple doses may be administered.

Additional formulations that are suitable for other modes of administration include vaginal suppositories and pessaries. A rectal pessary or suppository may also be used. Suppositories are solid dosage forms of various weights and shapes, usually medicated, for

insertion into the rectum, vagina or the urethra. After insertion, suppositories soften, melt or dissolve in the cavity fluids. In general, for suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1%-2%.

5 Vaginal suppositories or pessaries are usually globular or oviform and weighing about 5 g each. Vaginal medications are available in a variety of physical forms, e.g., creams, gels or liquids, which depart from the classical concept of suppositories. The sHDL nanoparticles also may be formulated as inhalants.

The present invention also includes methods involving co-administration of the sHDL
10 nanoparticles as described herein with one or more additional active agents. Indeed, it is a further aspect of this invention to provide methods for enhancing prior art therapies and/or pharmaceutical compositions by co-administering the sHDL nanoparticles of this invention. In co-administration procedures, the agents may be administered concurrently or sequentially. In some embodiments, the sHDL nanoparticles described herein are administered prior to the other
15 active agent(s). The agent or agents to be co-administered depends on the type of condition being treated.

The present disclosure further provides kits comprising compositions comprising sHDL nanoparticles as described herein or the ingredients necessary to synthesize the sHDL nanoparticles as described herein. In some embodiments, the kit includes all of the components
20 necessary, sufficient or useful for administering such sHDL nanoparticles.

EXPERIMENTAL

The following examples are provided in order to demonstrate and further illustrate certain preferred embodiments and aspects of the present invention and are not to be construed
25 as limiting the scope thereof.

Example 1.

This example describes the materials and methods for synthesis of a sHDL loaded with biomacromolecules

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Materials

1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), and rhodamine (Rhod)-labeled DOPE (DOPE-Rhod) were all purchased from Avanti Polar Lipids (Alabaster, AL). Dioleoyl-sn-glycero-3-

phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP) was additionally synthesized. All peptides including HDL mimicking peptide (22A; SEQ ID NO: 4), SIINFEKL (SEQ ID NO: 385), CSSIINFEKL (SEQ ID NO: 384), and FITC labeled CSSIINFEK (SEQ ID NO: 386) (FITC)L used were customized from GenScript. The oligodeoxynucleotide TLR 9 ligand CpG 1826 (5'-tccatgacgttctgacgtt-3' (SEQ ID NO: 382), lower case letters represent phosphorothioate backbone) (SEQ ID NO: 343) and cholesterol modified CpG 1826 (5'-tccatgacgttctgacgtt-3' (SEQ ID NO: 382)-TEG-cholesterol) were ordered from Integrated DNA Technologies. HPLC grade solvents such as methanol and acetonitrile were purchased from Fisher Scientific. Fetal bovine serum (FBS), penicillin-streptomycin, β -mercaptoethanol and ACK lysing buffer were purchased from Life Technologies (Grand Island, NY). Granulocyte macrophage colony stimulating factor (GM-CSF) was the product of PeproTech (Rocky Hill, NJ). Rat anti-mouse CD16/32, CD86-PE, CD40-APC, SIINFEKL H-2K^b-PE and MHC Class II-FITC were from eBioscience (San Diego, CA). Rat anti-mouse CD8-APC, hamster anti-mouse CD11c-PE and streptavidin-Cy7 were from BD Bioscience (San Jose, CA). iTAg tetramer/PE - H-2 Kb OVA (SIINFEKL) was purchased from Beckman Coulter (Brea, CA).

Preparation of sHDL nanoparticles loaded with peptides, nucleic acids, or glycolipids.

DMPC and DOPE-PDP (weight ratio = 4:0.25) were dissolved in chloroform. The mixture was dried with nitrogen flow for 5 min and then put in a vacuum oven for 1 h. The obtained lipid film was hydrated in 10 mM sodium phosphate buffer (0.3117 g/L NaH₂PO₄·H₂O and 2.0747 g/L Na₂HPO₄·7H₂O) and sonicated with a bath sonicator for 10 min, followed by probe sonication for another 2.5 min. 22A peptide dissolved in endotoxin free water was added to the above mixture (22A:lipids = 1:2, weight ratio), which was then subjected to heating (50 °C) for 3 min and cooling (ice water) for 3 min, with 3 cycles in total, to obtain sHDL.

To load tumor antigen peptides to sHDL, cysteine terminated tumor antigen peptides dissolved in endotoxin free water were added to the above sHDL (antigen peptides:DOPE-PDP = 2.5:1, molar ratio) and incubated at room temperature with gentle shaking on an orbital shaker for 3 h. Unreacted tumor antigen peptides were removed by using Zeba Spin Desalting columns with a MWCO = 7000 Da cutoff (Pierce) following the manufacturer's instructions. The conjugation efficiency of tumor antigen peptides was calculated based on the decrease of DOPE-PDP determined by the HPLC. Briefly, 200 μ l sHDL formulations were freeze-dried and reconstituted in 300 μ l methanol. The mixture was filtered by a 220 nm PTFE filter before 20 μ l was injected to a Shimadzu HPLC system equipped with a Vydac 219TP Diphenyl column (4.6 mm \times 250 mm ID). The two solvents used for the HPLC analysis consisted of

water:trifluoroacetic acid =100:0.5 (mobile phase A) and methanol:acetonitrile:trifluoroacetic acid = 50:50:0.05 (mobile phase B). Gradient programming of the solvent system was: 25% mobile phase B was linearly increased to 100% B over 75 min, linearly decreased to 25% B at 80 min, and maintained at 25% during 80-90 min for equilibration before the next analysis. The flow rate was 1 mL/min and the detection wavelength was 220 nm. The loading efficiency of tumor antigen peptides was also determined by using FITC-labeled peptides and measuring the fluorescence intensity of sHDL formulations at Ex = 490 nm and Em=520 nm after dissolving the formulations with 1% Triton X-100 containing PBS.

To load CpG to sHDL, different concentrations of cholesterol modified CpG (Cho-CpG) were incubated with sHDL at room temperature with gentle shaking on an orbital shaker for 30 min. The amount of CpG incorporated into sHDL and free CpG were analyzed by the gel permeation chromatography (GPC). Briefly, the sHDL formulations were diluted by PBS to a concentration of 0.5 mg/mL 22A peptide. The formulations were filtered through a 220 nm filter before 40 μ l samples were injected to a Shimadzu HPLC system equipped with a TSKgel G2000SWxl column (7.8 mm ID \times 30 cm, Tosoh Bioscience LLC). The flow rate of mobile phase PBS (pH 7.4) was set at 0.7 mL/min and detection wavelength was set at 260 nm for CpG.

To load alpha-galactosylceramide (aGC) to sHDL, a lyophilization-based method of producing sHDL was developed. Briefly, phospholipids, aGC and ApoA mimetic peptides were dissolved in glacial acetic acid and lyophilized. The obtained powder was hydrated in PBS (pH 7.4) and cycled above and below the transition temperature (T_m) of phospholipids to form aGC-sHDL. Similar protocol was utilized for loading siRNA into sHDL. Cholesterol-modified PCSK9 siRNA was incubated with blank sHDL at room temperature for 30 min to form PCSK9 siRNA-sHDL.

25 Morphology and size measurement of sHDL

The sHDL formulations were diluted to 0.5 mg/mL 22A with PBS and the sizes were measured by dynamic light scattering (DLS, Zetasizer Nano ZSP, Malvern, UK). The morphology of sHDL was observed by transmission electron microscopy (TEM) after proper dilution of the original samples.

30

Preparation of bone marrow-derived dendritic cells (BMDCs)

BMDCs were prepared. Briefly, femur and tibia of a mouse were harvested, washed and grinded in BMDC culture media (RPMI 1640 supplemented with 10% FBS, 1% penicillin-streptomycin, 50 μ M β -mercaptoethanol, and 20 ng/ml GM-CSF). Cells were collected by

passing the cell suspension through a cell strainer (mesh size = 40 μm), followed by centrifugation. Cells were seeded into non-tissue culture treated petri-dish at a density of 2×10^5 cells/ml, cultured at 37°C with 5% CO_2 . Culture media were refreshed on days 3, 6 and 8, and BMDCs were used during day 8-12.

5

Up-regulation of activation markers on BMDCs

Immature BMDCs were plated at 1×10^6 cells/well in 12-well plates 24 h prior to use. The old media were aspirated and BMDCs were washed once with PBS before incubated with 0.5 $\mu\text{g}/\text{mL}$ different CpG-containing formulations or 0.5 $\mu\text{g}/\text{mL}$ LPS (positive control) for 24 h at 37°C . BMDCs were harvested, washed once with FACS buffer (1% BSA in PBS), incubated with anti-CD16/32 at room temperature for 10 min, and then stained with fluorescent probe-labeled antibodies against CD11c, CD40, CD80, CD86, and MHC class II at room temperature for 30 min. Finally, cells were washed twice by FACS buffer and resuspended in 2 $\mu\text{g}/\text{ml}$ DAPI solution and analyzed by flow cytometry (Cyan 5, Beckman Coulter, USA).

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Antigen presentation by BMDCs

Immature BMDCs were plated at 1×10^6 cells/well in 12-well plates 24 h prior to use. The old media were aspirated and BMDCs were washed once with PBS before incubated with 0.5 $\mu\text{g}/\text{mL}$ CpG and/or 0.5 $\mu\text{g}/\text{mL}$ antigen peptide-containing formulations in complete media for different lengths of time (2, 6, 24, and 48 h) at 37°C . BMDCs were harvested, washed once with FACS buffer, incubated with anti-CD16/32 at room temperature for 10 min, and then stained with PE-tagged anti mouse SIINFEKL H-2K^b monoclonal antibody 25-D1.16 at room temperature for 30 min. Finally, cells were washed twice with FACS buffer and resuspended in 2 $\mu\text{g}/\text{ml}$ DAPI solution and analyzed by flow cytometry (Cyan 5, Beckman Coulter, USA).

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Imaging the intracellular delivery of sHDL-based peptide vaccine with CLSM

1×10^6 cells JAWSII cells in 2mL complete media were seeded in 35 mm petri dishes (MatTek) that have been pre-equilibrated with the same culture media and allowed to settle overnight. To learn the intracellular delivery profile of sHDL itself, DOPE-Rhod was used to label the lipid of sHDL, and 22A peptide of sHDL was labeled by incubating sHDL with Texas Red[®]-X, Succinimidyl Ester (Life Technologies), followed by passing through the desalting column to remove the unreacted dye. These labeled sHDL were incubated with JAWSII cells at 37°C for 24 h. After incubation, cells were washed 3 times with PBS and the incubated with phenol and serum free media containing 500 nM LysoTracker[®] Green DND-26 (Life

30

Technologies) and 2 μ g/mL Hoechst for 30 min at 37 °C to stain the lysosomes and nuclei, respectively, before imaging using a confocal microscope (Nikon A1). To learn the intracellular delivery profile of the antigen peptides, free CSSSIINFEK(SEQ ID NO:386) (FITC)L+CpG or sHDL-CSSSIINFEK(SEQ ID NO:386) (FITC)L/CpG were incubated with JAWSII cells for
5 different lengths of time (6, 24, and 48h). After incubation, cells were washed 3 times with PBS and the incubated with phenol and serum free media containing 50 nM LYSOTRACKER Red DND-99 (Life Technologies) and 2 μ g/mL Hoechst for 30 min at 37 °C to stain the lysosomes and nuclei, respectively, before imaging using a confocal microscope (Nikon A1).

10 B3Z T cell activation in vitro

BMDCs were plated at 5 \times 10⁴ cells/well in a U-bottom 96-well plate and allowed to grow overnight. The old media were aspirated and BMDCs were washed once with PBS before incubated with different concentrations (0.02, 0.1, and 0.5 μ g/mL) of SIINFEKL and CpG containing formulations for 24 h or 48 h at 37 °C. After incubation, cells were carefully washed
15 3 times with PBS, and 10 \times 10⁴ B3Z T cells/well were added and cocultured for another 24 h in RPMI 1640 supplemented with 10% FBS, 2mM L-glutamine, 55 μ M β -mercaptoethanol, 1mM pyruvate and 100 U/mL penicillin and 100 μ g/mL streptomycin. Cells were then pelleted via centrifugation (1500 rcf, 7min). The media were carefully aspirated, and 150 μ L CPRG/lysis buffer (0.15 mM chlorophenol red- β -D-galactopyranoside (CPRG), 0.1% Triton-X 100, 9 mM
20 MgCl₂, 100 μ M mercaptoethanol in PBS) was added. The plates were incubated at 37 °C in the dark for 90 min, after which the absorbance of released chlorophenol red was measured at 570 nm using a plate reader.

Lymph nodes draining of antigen peptides

25 sHDL-CSSSIINFEK(SEQ ID NO:386)(FITC)L was prepared as described above. Female C57BL/6 mice of age 6–8 weeks were purchased from Harlan Laboratories. C57BL/6 mice were subcutaneously injected with free CSSSIINFEK(SEQ ID NO:386) (FITC)L or sHDL-CSSSIINFEK(FITC)L(SEQ ID NO:386). 24 hours after injection, mice were euthanized by carbon dioxide inhalation and axillary lymph nodes and inguinal lymph nodes were harvested
30 and imaged with IVIS optical imaging system (Caliper Lifesciences).

In vivo vaccination and analysis of cytotoxic T cell responses in prophylactic and therapeutic settings of melanoma tumor growth

C57BL/6 mice were immunized with different formulations containing SIINFEKL (15µg/mouse) and CpG (15µg/mouse) by subcutaneous injection at the tail base following the predetermined schedule. The percent of tumor antigen specific CD8⁺ T cells were determined 7 days after each vaccination by the tetramer staining assay. In brief, 100 µl of blood will be drawn from each mouse and the blood samples were lysed with ACK lysing buffer, followed by centrifugation to collect pellets, which were then washed once by FACS buffer and blocked by CD16/32 blocking antibody and incubated with PE labeled SIINFEKL tetramer for 30 min at room temperature. Samples were then incubated with anti-CD8-APC for 20 min on ice. Cells were washed twice with FACS buffer and resuspended in 2 µg/ml DAPI solution for analysis by flow cytometry (Cyan 5, Beckman Coulter, USA). To examine the effect of T cell responses against tumor growth, one day after the last tetramer staining, the mice were challenged by subcutaneous injection of 0.2 million B16.OVA/mouse on the right flank. The tumor development was monitored every other day and the tumor volume was calculated by the following equation: tumor volume = length × width² × 0.52. In order to examine the effect of sHDL vaccination against established tumor, C57BL/6 mice were inoculated with 0.2 million B16.OVA/mouse on the right flank by subcutaneous injection on day 0. On day 4 and 11, the mice were immunized with different formulations containing tumor antigen peptides (15 µg/mouse) and CpG (15µg/mouse). The percent of tumor antigen specific CD8⁺ T cells were determined on day 10 and 17 by the tetramer staining assay as described above. The tumor volume was monitored every other day.

aGC-CD1d presentation assay

JAWSII cells were seeded at a density of 0.2 million/well to 12-well plates. After 48 h, media were replaced with fresh media containing 2000 ng/mL of different formulations of aGC. After 20-24 h incubation with formulations, cells were harvested into FACS tubes by trypsination, washed twice by FACS buffer and then incubated with CD16/32 blocking reagent for 10 min at R.T. Cells were then incubated with anti-mouse aGC-CD1d-PE for 30 min at R.T, washed twice by FACS buffer, and suspended in 0.3 mL FACS buffer containing DAPI for flow cytometry.

Characterization of PCSK9 siRNA-loaded sHDL

To quantify the amount of PCSK9 siRNA molecules that are loaded into sHDL, various concentrations of PCSK9 siRNA was incubated with sHDL, and the concentration of PCSK9

siRNA associated with sHDL versus free form will be measured at 260 nm using the gel permeation chromatography (GPC) assay.

PCSK9 knockdown in HepG2 cells

5 Different formulations of PCSK9 siRNA were incubated with HepG2 cells for 48 h. After incubation, cells were washed twice with PBS and the cell lysate was prepared. The PCSK9 protein level was analyzed by the western blot assay.

Biodistribution of sHDL

10 To study the biodistribution of sHDL, DiD-loaded sHDL was intravenously injected to the C57BL/6 mice. 24 h post injection, the mice were euthanized and the distribution of sHDL in major organs (heart, liver, spleen, lung and kidney) was analyzed using the IVIS optical imaging system.

15 **Example II.**

This example demonstrates that PCSK9 siRNA incorporated into sHDL can efficiently accumulate in the liver, deliver its cargo into SR-BI positive cells, and knockdown PCSK9 in HepG2 cells. Rapid and cheap lyophilization methods for the preparation of homogeneous sHDL nanoparticles were implemented. The homogeneity of the sHDL was confirmed by
20 transmission electron microscopy (TEM), dynamic laser scattering (DLS), and gel permeation chromatography (GPC) (Fig. 1A). When sHDL was labeled by the fluorescent dye DiR and intravenously injected to mice, the majority of DiR signal was detected in the liver, with little or no signal in other organs (Fig. 1B). sHDL also efficiently delivered the fluorescent dye DiO into SR-BI positive cells (BHK-SR-BI), but not SR-BI negative cells (BHK-vector), and the uptake
25 by SR-BI positive cells was blocked by the excess blank sHDL (Fig. 1C). Moreover, the preliminary data showed that the cholesterol modified PCSK9 siRNA (PCSK9 Cho-siRNA) could be quantitatively incorporated into sHDL. Although free PCSK9 Cho-siRNA can knockdown PCSK9 in HepG2 cells due to the increased uptake of siRNA induced by cholesterol conjugation, PCSK9 siRNA-sHDL is still better able to knockdown PCSK9 protein in HepG2
30 cells in vitro (Fig. 1D-F).

Example III.

This example demonstrates that co-localized delivery of antigen and adjuvant by sHDL leads to potent immune response. Fig. 4A presents a schematic of antigens and adjuvants-loaded

sHDL. When a MHC class I antigen peptide (CD8⁺ T cell epitope peptide SIINFEKL derived from ovalbumin) was incubated with functional lipids-containing sHDL, the antigen peptide was quantitatively conjugated to functional lipids of sHDL, as can be seen by the disappearance of functional lipids and appearance of lipid-peptide conjugates (Figure 2B). The cholesterol modified CpG (Cho-CpG) was also shown to be quantitatively incorporated into sHDL (Figure 2C). After 1 primary dose and two booster doses, the antigen and CpG-loaded sHDL (sHDL-Ag/CpG) elicited more potent immune responses than the mixture of antigens and CpG in Montanide (CpG + Montanide is one of the most potent experimental adjuvant currently undergoing clinical evaluations) (Figure 2D).

10 Fig. 3 shows a schematic of the synthesis of sHDL-CSSSIINFEK(FITC)L/CpG(SEQ ID NO:386).

Fig. 4 shows homogenous particle size of sHDL-Ag/CpG as analyzed by cryoEM and dynamic light scattering.

15 Figs. 5A and 5B show that compared with free antigen form, antigen delivery via sHDL significantly prolongs antigen presentation by dendritic cells.

Fig. 6 shows that sHDL-Ag/CpG significantly enhances elicitation of antigen-specific CD8⁺ T cells, compared with vaccination with free antigen mixed with conventional adjuvants.

Fig. 7 shows sHDL-Ag/CpG vaccination elicits strong CD8⁺ T cell responses in tumor-bearing mice and reduces tumor growth.

20

Example IV.

This example demonstrates that sHDL delivering alpha-galactosylceramide, a glycolipid ligand for CD1-d to activate induction of natural killer T cells.

25 Fig. 8 shows that compared with free soluble form, alpha-GalCer delivered via sHDL significantly enhanced CD1d presentation of antigen-presenting cells.

Fig. 9 shows that lyophilization offers a convenient method of large-scale synthesis of sHDL loaded with alpha-GalCer.

Example V.

30 This example demonstrates that preformed high density lipoprotein-mimicking nanodiscs can be readily coupled with antigen (Ag) peptides and adjuvants, producing stable, ultrasmall nanoparticles that markedly improve Ag/adjuvant co-delivery to lymphoid organs and achieve sustained Ag presentation on dendritic cells.

Lipids and peptides conducive to nanodisc formation were first identified. DMPC lipid films were hydrated and added with a series of ApoA1-mimetic peptides, followed by thermal cycling between 50 °C and 4 °C. A subset of peptides, including 22A and D-amino acids of 22A, were identified that produced clear sHDL suspensions, stable for one month when stored at 4 °C (Fig. 13a). In addition, use of phospholipids with transition temperature (T_m) below RT (e.g. POPC and DOPC with T_m = -2°C and -17°C, respectively) produced murky liposomal suspension, whereas lipids with high T_m (e.g. DPPC and DMPC with T_m = 41°C and 24°C, respectively) formed clear sHDL suspensions in the presence of 22A (Fig. 13b), showing flexibility in the materials design. Based on their size, homogeneity, and long-term stability, 22A and DMPC as the key components of nanodisc vaccines were chosen for further investigation.

To achieve intracellular release of Ag within APCs via reduction-sensitive conjugation of Ag on sHDL, we synthesized dioleoyl-sn-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (PDP, Fig. 14) and incorporated PDP into sHDL (4 mol%). When incubated for 30 min at RT with Ag peptides modified with a cysteine-serine-serine (CSS) linker (see, e.g., Hirose, S., et al., Vaccine 28, 7897-7906 (2010)), sHDL nanodiscs were efficiently surface-decorated with various Ag peptides (e.g., OVA₂₅₇₋₂₆₄, a model CD8α⁺ T-cell epitope Ag from ovalbumin; gp100₂₅₋₃₃, melanoma-associated Ag; and Adgpk, neo-antigen in MC-38), and subsequent incubation with Cho-CpG for 30 min at RT led to almost complete (~98%) insertion of CpG into sHDL, producing nanodiscs co-loaded with Ag and CpG (termed sHDL-Ag/CpG, with ~6.5 Ag peptides and ~1 CpG molecule per nanodisc, Fig. 15; Table 4). sHDL-Ag/CpG exhibited uniform disc-like morphology with an average diameter of 10.5 ± 0.5 nm and polydispersity index of 0.20 ± 0.02 (Fig. 16a and 16b). Importantly, sHDL-Ag/CpG could be readily sterile-filtered and stored frozen at -20 °C for at least 8 weeks before thawing at 37 °C, without negatively affecting its homogeneity (Fig. 16c).

Table 4.

Formulations	% of PDP-lipid converted to Ag-lipid	% of Cho-CpG inserted into sHDL	Size (d, nm)	PDI
sHDL-CSSSIINFEKL/CpG	92.0 ± 3.5%	98.5 ± 1.1%	10.5 ± 0.5	0.20 ± 0.02
sHDL-gp100/CpG	91.8 ± 2.7%	98.2 ± 1.5%	10.3 ± 0.5	0.23 ± 0.03
sHDL-Adgpk/CpG	91.1 ± 3.1%	98.5 ± 1.8%	10.8 ± 0.3	0.22 ± 0.02

The impact of nanodiscs on Ag presentation was next examined. Bone marrow derived dendritic cells (BMDCs) pulsed for 24 h with sHDL-CSSSIINFEKL/CpG (SEQ ID NO:384) presented OVA₂₅₇₋₂₆₄ SIINFEKL with a greater efficiency than BMDCs treated with free Ag

peptides admixed with CpG or sHDL-CSSSIINFEKL (SEQ ID NO:384), as determined by staining DCs with the 25-D1.16 mAb directed against SIINFEKL-H-2K^b complexes (Fig. 16d; 17a and 17b). Interestingly, DCs pulsed with free SIINFEKL+CpG efficiently presented Ag for the first 6 h of incubation, but Ag presentation decreased precipitously past 6 h (Fig. 16e and 16f; Fig. 17c), suggesting initial direct Ag binding to MHC-I molecules, followed by rapid Ag degradation or disassociation. In contrast, Ag presentation with sHDL-Ag/CpG gradually increased over time, achieving ~9-fold greater levels at 24 h and maintaining ~4-fold higher levels even at 48 h, compared with free SIINFEKL+CpG.

Intrigued by prolonged Ag presentation, the process of nanodisc uptake and Ag localization using CSS-SIINFEK_(FITC)L was investigated; SIINFEKL modified with FITC at ε-amino group in the lysine residue is known to retain its binding capacity to H-2K^b molecules (see, e.g., Saini, S.K. et al. Proc. Natl. Acad. Sci. U. S. A. 110, 15383-15388 (2013)). JAWSII cells (immortalized immature DCs) incubated with free Ag(FITC)+CpG displayed weak fluorescence signal on the plasma membrane at 6 h, and only dim fluorescence was observed by 24 h (Fig. 16g; Fig. 18). In stark contrast, sHDL-Ag(FITC)/CpG treatment led to strong FITC signal co-localized with endosomes/lysosomes by 6 h, and robust Ag(FITC) signal was detected on cell membranes by 24 h and sustained up to 48 h. In addition, nanodiscs containing Rh-PE or Texas Red-labeled-22A were predominantly found within endosomes/lysosomes, indicating cellular uptake of intact whole nanodiscs (Fig. 19). To assess the impact of prolonged Ag presentation on T-cell cross-priming, BMDCs were treated with free Ag peptides+CpG or sHDL-Ag/CpG for 24 or 48 h, and then added SIINFEKL-specific, H-2K^b-restricted B3Z T-cell hybridomas. BMDCs pulsed with sHDL-Ag/CpG promoted strong B3Z T-cell activation even after 48 h incubation, whereas free Ag peptides+CpG induced minimal B3Z T-cell activation beyond the 24 h period (Fig. 16h). Moreover, sHDL-Ag/CpG potently stimulated DC maturation (Fig. 20). Altogether, whereas free Ag peptide was rapidly loaded and dissociated from MHC-I molecules on cell membranes, nanodiscs facilitated intracellular delivery of Ag/CpG and mediated their sustained release within endosomes/lysosomes, thereby promoting durable Ag presentation, APC maturation, and cross-priming CD8α⁺ T-cells *in vitro*.

The impact of nanodiscs on lymphatic delivery of Ag/CpG and induction of CTL responses *in vivo* (see, e.g., Reddy, S.T. et al. Nat. Biotechnol. 25, 1159-1164 (2007)) was next investigated. C57BL/6 mice injected subcutaneously at tail base with 31 nmol free CSS-SIINFEK_(FITC)L had minimal FITC signal in inguinal dLNs after 1 day (see, e.g., Fig. 21a), potentially due to systemic dissemination of small MW Ag peptide or direct Ag binding on non-APCs at the injection site (see, e.g., Melief, C.J. & van der Burg, S.H. Nat. Rev. Cancer 8, 351-

360 (2008). In contrast, sHDL-Ag group exhibited markedly increased FITC signal in dLNs ($p < 0.01$, Fig. 21a), with Ag(FITC) and Cy5-tagged 22A co-localized within dLNs (Fig. 22). Similarly, injection of 2.3 nmol Cy5-tagged Cho-CpG in sHDL increased its LN accumulation, compared with injection in free soluble form ($p < 0.01$, Fig. 21b). These results showed that

5 sHDL nanodisc promoted co-delivery of Ag and CpG to dLNs. C57BL/6 mice were next immunized with 15.5 nmol Ag and 2.3 nmol CpG (non-fluorophore tagged), and peripheral blood mononuclear cells (PBMCs) were analyzed for the frequency of SIINFEKL–MHC-I tetramer+ CD8 α + T-cells. The mixture of free Ag peptides (SIINFEKL or CSS-SIINFEKL) and CpG induced 1-3% Ag-specific CTLs after the third immunization (Fig. 21c and 21d). As the

10 benchmark, animals with the equivalent doses of Ag and CpG emulsified in water-in-oil Montanide were also vaccinated (see, e.g., Speiser, D.E. et al. J. Clin. Invest. 115, 739-746 (2005); Fourcade, J. et al. J. Immunother. 31, 781-791 (2008)). Ag+CpG+Montanide elicited ~2% Ag-specific CTLs after priming; however, no further T-cell expansion was observed even after the third immunization, consistent with a recent study reporting dysfunction and deletion of

15 high-avidity T-cells after repeated immunizations with a depot-forming water-in-oil adjuvant (see, e.g., Rezvani, K. et al. Haematologica 96, 432-440 (2011); Hailemichael, Y. et al. Nat. Med. 19, 465-472 (2013)). In striking contrast, sHDL-Ag/CpG group elicited a peak frequency of ~21% Ag-specific CD8 α + T-cells after the third vaccination (29-fold greater than soluble SIINFEKL+CpG and 9-fold greater than Ag+CpG+Montanide, $p < 0.0001$, Fig. 21c and 21d).

20 When challenged with 2×10^5 B16OVA cells, mice immunized with sHDL-Ag/CpG had no detectable tumor masses up to 28 days, with 40% of animals surviving for more than 200 days, whereas mice immunized with free Ag peptides+CpG or Ag+CpG+Montanide all succumbed to tumors with marginal survival benefits (Fig. 21e and 2f). Importantly, throughout such experiments, no signs of toxicity or autoimmunity in animals immunized multiple times with

25 sHDL-Ag/CpG were observed.

Experiments were conducted to rule out the possibility that CSS-modified peptides or Cho-CpG dissociated from sHDL-Ag/CpG *in vivo* were responsible for the strong CTL responses. Introducing the CSS linker to SIINFEKL and replacing free CpG with Cho-CpG in free soluble form resulted in minimal T-cell responses, and the physical mixture of Ag, CpG,

30 and sHDL also elicited weak CTL responses (Fig. 21g). In contrast, sHDL-Ag/CpG nanodiscs drastically improved CTL responses, eliciting remarkable 41-fold greater frequency of Ag-specific CD8 α + T-cells than CSSSIINFEKL+Cho-CpG group (day 35, $p < 0.0001$, Fig. 21g), with CTLs primarily exhibiting CD44^{high}CD62L^{low} effector phenotype and robust IFN- γ ⁺ ELISPOT responses (Fig. 21h; Fig. 23).

The anti-tumor efficacy of sHDL in tumor-bearing mice was evaluated. Therapeutic sHDL vaccination in mice bearing B16OVA melanoma led to strong Ag-specific CTL responses with significantly slowed tumor growth and extended animal survival (Fig. 24). Nanodisc vaccines were next tested using non-immunogenic B16F10 melanoma as a more clinically relevant model. After confirming incorporation of gp100₂₅₋₃₃ together with Cho-CpG into nanodiscs (Fig. 15; Table 4), mice were treated with 15.5 nmol Ag and 2.3 nmol CpG on days 4 and 11 post subcutaneous inoculation of B16F10 cells. Vaccinations with sHDL-gp100/CpG elicited robust CTL responses, generating 22-fold higher frequency of gp100-specific CTLs than free gp100+CpG (day 17, $p < 0.0001$, Fig. 25a; Fig. 26), leading to significantly delayed tumor growth and prolonged animal survival, compared with the free gp100+CpG group that had no effects (Fig. 25b and 25c).

Finally, to demonstrate the utility of the platform technology for vaccination against neo-antigens, the murine MC-38 colon carcinoma model recently reported to harbor a single-epitope mutation within Adpgk protein (ASMTNRELM → ASMTNMELM (SEQ ID NO:383)) was employed, with the neo-epitope presented in MHC-I H-2D^b molecules (see, e.g., Yadav, M. et al. Nature 515, 572-576 (2014)). The Adpgk neo-antigen mutation in MC-38 cells was confirmed by cDNA sequencing (Fig. 25d; Fig. 27) and synthesized sHDL-Adpgk/CpG by mixing nanodiscs with the neo-epitope modified with the CSS-linker and Cho-CpG. C57BL/6 mice were inoculated subcutaneously with 10⁵ MC-38 cells and treated with 15.5 nmol Adpgk mutated peptide and 2.3 nmol CpG. Mice treated with free Adpgk Ag+CpG had similar levels of Adpgk-specific CD8α⁺ T-cells as non-immunized, MC-38-bearing mice, whereas sHDL-Adpgk/CpG markedly enhanced CTL responses (day 23, $p < 0.001$, Fig. 25e). In addition, sHDL-Adpgk/CpG induced polyfunctional IFN-γ⁺ and IFN-γ⁺TNF-α⁺ Adpgk-specific CD8α⁺ T-cells (2.5-fold and 7-fold greater than the free Adpgk+CpG group, $p < 0.05$ and $p < 0.001$, respectively, Fig. 25f). Importantly, therapeutic treatments with sHDL-Adpgk/CpG substantially slowed MC-38 tumor growth and extended animal survival, in contrast to the traditional soluble Adpgk+CpG vaccine with no statistically significant effects on tumor growth or survival (median survival: 54 d versus 33 d, respectively, $p < 0.01$, Fig. 25g and Fig. 25h).

30 **Example VI.**

This example pertains to the materials and methods for Example V.

Materials

1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), and rhodamine (Rhod)-labeled DOPE (DOPE-Rhod) were purchased from Avanti Polar Lipids (Alabaster, AL). ApoA1 mimetic peptide (22A), OVA₂₅₇₋₂₆₄ SIINFEKL, CSSSIINFEKL (SEQ ID NO:384), CSSSIINFEK(FITC)L(SEQ ID NO:386),
 5 hgp100₂₅₋₃₃ KVPRNQDWL, CSSSKVPRNQDWL, and *Adpgk* mutant peptide ASMTNMELM (SEQ ID NO:383) were synthesized by GenScript Corp. (Piscataway, NJ). CSSASMENMELM was synthesized by AnaSpec (Fremont, CA). The oligodeoxynucleotide TLR9 ligand CpG 1826 (5'-tccatgacgttctgacgtt-3' (SEQ ID NO:382), lower case letters represent phosphorothioate backbone), CpG 1826 modified with cholesterol at the 3' end (Cho-CpG), and Cy5 modified
 10 Cho-CpG were synthesized by Integrated DNA Technologies (Coralville, IA). HPLC grade methanol and acetonitrile were purchased from Fisher Scientific (Pittsburgh, PA). Fetal bovine serum (FBS), penicillin-streptomycin, β -mercaptoethanol and ACK lysis buffer were purchased from Life Technologies (Grand Island, NY). Granulocyte macrophage colony stimulating factor (GM-CSF) was from GenScript Corp. (Piscataway, NJ). Anti-mouse CD16/32, CD86-PE,
 15 CD40-APC, CD62L-PECy7, and 25-D1.16 mAb-PE against SIINFEKL/H-2K^b were from eBioscience (San Diego, CA). Anti-mouse CD8 α -APC, CD44-FITC, TNF- α -FITC, IFN- γ -PE, and CD11c-PECy7 were from BD Bioscience (San Jose, CA). Tetramer H-2K^b-SIINFEKL-PE and Tetramer H-2D^b-KVPRNQDWL-PE was purchased from Beckman Coulter (Brea, CA). Tetramer/H-2D^b-ASMTNMELM-PE (SEQ ID NO:383) was kindly provided by the NIH
 20 Tetramer Core Facility (Atlanta, GA). We obtained B3Z CD8 α ⁺ T cell hybridoma from Dr. N. Shastri (University of California, Berkeley); B16OVA from Dr. Kenneth Rock (University of Massachusetts, Amherst, MA); and MC-38 cells from Dr. Weiping Zou (University of Michigan, Ann Arbor, MI).

25 **Methods**

Synthesis and characterization of DOPE-PDP

Dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP) was synthesized as reported previously with slight modifications (see, e.g., Kuai, R., et al. Mol. Pharm. 7, 1816-1826 (2010)). Briefly, DOPE, SPDP (succinimidyl 3-(2-pyridyldithio)
 30 propionate) and triethylamine (1:1:1.5 molar ratio) were dissolved in chloroform. The mixture was reacted in the dark for 5 h. The reaction progress was monitored by thin layer chromatography (TLC), using the following mixture as the developing solvent: chloroform/methanol/water = 65/25/4 (volume ratio). After TLC indicated disappearance of the

starting materials and appearance of a faster-running spot, the reaction mixture was dried by rotary evaporation and purified on a silica gel column.

Synthesis of sHDL co-loaded with antigen peptides and CpG

5 DMPC and DOPE-PDP (molar ratio = 96:4) were dissolved in chloroform. The mixture was dried with nitrogen flow and placed under vacuum for at least 1 h. The resulting lipid film was hydrated in 10 mM sodium phosphate buffer (0.3117 g/L NaH₂PO₄·H₂O and 2.0747g/L Na₂HPO₄·7H₂O, pH 7.4) and sonicated in a bath sonicator for 10 min, followed by probe sonication for another 2.5 min. ApoA1 mimetic peptide 22A dissolved in endotoxin free water
10 was added to the above mixture (22A:lipids = 1:7.5 molar ratio), which was then subjected to heating (50 °C) for 3 min and cooling (ice water) for 3 min, with 3 cycles in total, to obtain sHDL.

To conjugate tumor antigen peptides to sHDL, cysteine terminated tumor antigen peptides dissolved in endotoxin free water were added to the above sHDL (antigen
15 peptides:DOPE-PDP = 2.5:1, molar ratio) and incubated at room temperature with gentle shaking on an orbital shaker. Unreacted tumor antigen peptides were removed by using Zeba Spin Desalting columns (Pierce) following the manufacturer's instructions. The conjugation efficiency of tumor antigen peptides was calculated based on the decrease of absorbance signal associated with DOPE-PDP as determined by HPLC. Briefly, 200 µl sHDL formulations were
20 freeze-dried and reconstituted in 300 µl methanol. The mixture was filtered by a 0.22 µm PTFE filter and analyzed with a Shimadzu HPLC system using a Vydac 219TP Diphenyl column (4.6 mm × 250 mm ID). The two solvents used for the HPLC analysis consisted of water:trifluoroacetic acid =100:0.5 (mobile phase A) and methanol:acetonitrile:trifluoroacetic acid = 50:50:0.05 (mobile phase B) (0-75 min, 15-100%). The flow rate was 0.4 mL/min and the
25 detection wavelength was 220 nm. The loading efficiency of tumor antigen peptides in sHDL was confirmed by using FITC-labeled peptides and measuring the fluorescence intensity of sHDL formulations at Ex = 490 nm and Em = 520 nm after dissolving the formulations in PBS containing 1% Triton X-100.

To load CpG in sHDL, different concentrations (0-200 µg/mL) of cholesterol modified
30 CpG (Cho-CpG) were incubated with sHDL at room temperature with gentle shaking on an orbital shaker. The amount of CpG incorporated into sHDL and free CpG was analyzed by gel permeation chromatography (GPC). Briefly, the sHDL formulations were diluted in PBS to a concentration of 0.5 mg/mL 22A peptide. The formulations were filtered through a 0.22 µm filter and analyzed with a Shimadzu HPLC system equipped with a TSKgel G2000SWxl column

(7.8 mm ID × 30 cm, Tosoh Bioscience LLC). The flow rate of mobile phase PBS (pH 7.4) was set at 0.7 mL/min, and the detection wavelength was set at 260 nm for CpG.

Characterization of peptide/CpG-loaded sHDL formulations

5 The sHDL formulations were diluted to 0.5 mg/mL 22A with PBS, and the particle sizes were measured by dynamic light scattering (DLS, Zetasizer Nano ZSP, Malvern, UK). The morphology of sHDL was observed by transmission electron microscopy (TEM) after proper dilution of the original samples. Briefly, 3 μ L of the sample solution was deposited on a carbon film-coated 400 mesh copper grid (Electron Microscopy Sciences) and dried for 1 minute. The
10 samples were then negatively-stained with 5 droplets of 1% uranyl acetate solution, excessive solutions on the grid were blotted, and the grid was dried before TEM observation. All images were acquired on JEM 1200EX electron microscope (JEOL USA, Peabody, MA) equipped with an AMT XR-60 digital camera (Advanced Microscopy Techniques Corp. Woburn, MA).

15 Preparation of BMDCs

 BMDCs were prepared as described previously (see, e.g., Lutz, M.B., et al. J. Immunol. Methods 223, 77-92 (1999)). Briefly, femur and tibia were harvested aseptically from C57BL/6 mice, and the bone marrow was flushed into a petri dish using a 5 mL syringe (26 G needle) loaded with BMDC culture media (RPMI 1640 supplemented with 10% FBS, 100 U/mL
20 penicillin, 100 μ g/ml streptomycin, 50 μ M β -mercaptoethanol, and 20 ng/ml GM-CSF). Cells were collected by passing the cell suspension through a cell strainer (mesh size = 40 μ m), followed by centrifugation. Cells were seeded into non-tissue culture treated petri-dish at a density of 2×10^5 cells/ml, cultured at 37 °C with 5% CO₂. Culture media were refreshed on days 3, 6, 8, and 10, and BMDCs were used for the following assays on days 8-12.

25

Activation of BMDCs

 Immature BMDCs were plated at 1×10^6 cells/well in 12-well plates. After 24 h, BMDCs were washed once with PBS and incubated with 75 nM of CpG in different formulations or 0.5 μ g/mL LPS (positive control) for 24 h at 37 °C with 5% CO₂. BMDCs were harvested, washed
30 with FACS buffer (1% BSA in PBS), incubated with anti-CD16/32 at room temperature for at least 10 min, and then stained with fluorophore-labeled antibodies against CD11c, CD40, CD80, and CD86 at room temperature for 30 min. Finally, cells were washed twice by FACS buffer, resuspended in 2 μ g/ml DAPI solution, and analyzed by flow cytometry (Cyan 5, Beckman Coulter, USA).

Antigen presentation on BMDCs

Immature BMDCs were plated at 1×10^6 cells/well in 12-well plates 24 h prior to use. BMDCs were washed with PBS and incubated with 75 nM CpG and/or 500 nM antigen peptide in various formulations in complete media for different lengths of time (2, 6, 24, and 48 h). BMDCs were then harvested, washed with FACS buffer, incubated with anti-CD16/32 at room temperature for at least 10 min, and stained with PE-conjugated anti-mouse SIINFEKL/H-2K^b mAb 25-D1.16 at room temperature for 30 min. Cells were then washed, resuspended in 2 μ g/ml DAPI solution, and analyzed by flow cytometry (Cyan 5, Beckman Coulter, USA).

10

Confocal microscopy imaging of the intracellular trafficking of sHDL

JAWSII cells (ATCC, Manassas, VA) were seeded at 1×10^6 cells on 35 mm petri dishes (MatTek Corp., Ashland, MA) that have been pre-equilibrated with the complete cell culture media and cultured overnight. To investigate the intracellular delivery profiles of antigen peptides, JAWSII cells were incubated with the physical mixture of free CSSSIINFEK(FITC)L (SEQ ID NO:386) and CpG, or sHDL-CSSSIINFEK(FITC)L/CpG for different lengths of time (6, 24, and 48 h). Cells were then washed 3 times with PBS and incubated for 30 min at 37 °C with 50 nM LysoTracker[®] Red DND-99 (Invitrogen) and 2 μ g/mL Hoechst in phenol/serum-free media to stain lysosomes and nuclei, respectively. In parallel, to study the intracellular delivery profiles of structural components of sHDL, the lipid layers of sHDL were incorporated with DOPE-Rhod by adding 0.5 mol % DOPE-Rhod in the initial lipid film, while 22A peptide of sHDL was labeled by incubating pre-formed sHDL with Texas Red[®]-X succinimidyl ester (Life Technologies) and passing Texas Red-labeled sHDL through a desalting column to remove the unreacted dye. The resulting fluorophore-tagged sHDL formulations were incubated with JAWSII cells at 37 °C with 5% CO₂. After 24 h incubation, cells were washed 3 times with PBS and then incubated for 30 min at 37 °C with 500 nM LysoTracker[®] Green DND-26 (Invitrogen) and 2 μ g/mL Hoechst in phenol/serum-free media to stain lysosomes and nuclei, respectively. JAWSII cells were then imaged using a confocal microscope (Nikon A1).

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30 Activation of B3Z CD8⁺ T hybridoma cells with sHDL

BMDCs were plated at 5×10^4 cells/well in a U-bottom 96-well plate. After overnight culture, BMDCs were washed with PBS and incubated with different formulations of SIINFEKL (20, 100 and 500 nM) and CpG (3, 15, and 75 nM) for 24 h or 48 h at 37 °C. Cells were then carefully washed 3 times with PBS, and 10^5 B3Z CD8⁺ T hybridoma cells/well were added in

RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine, 55 μ M β -mercaptoethanol, 1 mM pyruvate and 100 U/mL penicillin and 100 μ g/mL streptomycin. After 24 hr of incubation, cells were pelleted via centrifugation (1500 rcf, 7 min), the media were carefully aspirated, and 150 μ L CPRG/lysis buffer (0.15 mM chlorophenol red- β -D-galactopyranoside (CPRG), 0.1% Triton-X 100, 9 mM MgCl₂, 100 μ M mercaptoethanol in PBS) was added. The plates were incubated at 37 °C in the dark for 90 min, after which the absorbance of released chlorophenol red was measured at 570 nm using a microplate reader.

In vivo immunization studies

Animals were cared for following federal, state, and local guidelines. All work performed on animals was in accordance with and approved by University Committee on Use and Care of Animals (UCUCA) at University of Michigan, Ann Arbor. Female C57BL/6 mice of age 6–8 weeks (Harlan Laboratories) were immunized with different formulations containing antigen peptides (15.5 nmol/mouse) and CpG (2.3 nmol/mouse) in 100 μ l volume by subcutaneous injection at the tail base on indicated time points. In some studies, antigen peptide and CpG emulsified in Montanide served as a positive control (see, e.g., Speiser, D.E., et al. *J. Clin. Invest.* 115, 739-746 (2005); Fourcade, J., et al. *J. Immunother.* 31, 781-791 (2008); Karbach, J., et al. *Int. J. Cancer* 126, 909-918 (2010)). Briefly, antigen peptide (155 nmol) and CpG (23 nmol) in 0.5 mL PBS were thoroughly emulsified in 0.5 mL Montanide until the mixture was homogeneous.

For lymph node draining studies, C57BL/6 mice were injected with free CSSSIINFEK(FITC)L(SEQ ID NO:386), sHDL-CSSSIINFEK(FITC)L, free Cho-CpG(Cy5), or sHDL-Cho-CpG(Cy5). After 24 h, inguinal lymph nodes were harvested, and FITC or Cy5 fluorescence signal was measured with IVIS optical imaging system (Caliper Life Sciences).

For prophylactic tumor challenge studies, vaccinated animals were challenged on day 8 after last immunization by subcutaneous injection of 2×10^5 B16OVA cells/mouse on the right flank. Tumor growth was monitored every other day, and the tumor volume throughout this study was calculated by the following equation (see, e.g., Gorrin-Rivas, M.J., et al. *Clin. Cancer Res.* 6, 1647-1654 (2000)): tumor volume = length \times width² \times 0.52. Animals were euthanized when the tumor masses reached 1.5 cm in diameter or when animals became moribund with severe weight loss or ulceration.

For therapeutic tumor vaccination studies, C57BL/6 mice were inoculated with tumor cells (2×10^5 B16OVA cells, 2×10^5 B16F10 cells, or 1×10^5 MC38 cells per mouse) on the right flank by subcutaneous injection on day 0. For B16OVA and B16F10 studies, mice were

vaccinated on days 4 and 11 with different formulations containing 15.5 nmol of tumor antigen peptides (SIINFEKL and hgp100, respectively) and 2.3 nmol of CpG. For MC-38 studies, mice were vaccinated on days 10, 17, and 24 with 15.5 nmol of ASMTNMELM (SEQ ID NO:383) and 2.3 nmol of CpG in either sHDL or free soluble form. Tumor growth was monitored as
5 indicated above.

Peptide-MHC tetramer assay

Immunized mice were analyzed for the percentages of tumor antigen-specific CD8 α ⁺ T cells among peripheral blood mononuclear cells (PBMCs) using the tetramer staining assay, as
10 described previously (see, e.g., Ochyl, L.J. & Moon, J.J. *J. Vis. Exp.* e52771 (2015)). In brief, 100 μ l of blood was drawn from each mouse on indicated time points by submandibular bleeding, and red blood cells were lysed with ACK lysis buffer. PBMCs were then washed with FACS buffer and blocked by anti-CD16/32 antibody and incubated with peptide-MHC tetramer tagged with PE (e.g. H-2K^b-restricted SIINFEKL, H-2D^b-restricted KVPRNQDWL, or H-2D^b-
15 restricted ASMTNMELM (SEQ ID NO:383)) for 30 min at room temperature. Samples were then incubated with anti-CD8 α -APC for 20 min on ice. Cells were washed twice with FACS buffer and resuspended in 2 μ g/ml DAPI solution for analysis by flow cytometry (Cyan 5, Beckman Coulter, USA).

20 ELISPOT and intracellular cytokine staining assays

For ELISPOT assay, spleens from immunized mice were harvested aseptically, processed into single cell suspensions for each mouse, and seeded at 3×10^5 splenocytes per well in 96-well PVDF plates (EMD Millipore) pre-incubated overnight with IFN- γ coating Ab (R&D Systems). Splenocytes were co-incubated with antigen peptides (2.5 μ g/ml) or controls for 24
25 hours. Assays were completed using sequential incubations with biotinylated-secondary Ab, streptavidin-alkaline phosphatase (Sigma Chemical), and NBT/BCIP substrate (Surmodics). Developed spots were enumerated using an AID iSpot Reader (Autoimmun Diagnostika GmbH, Germany). For intracellular cytokine staining (ICS) assay, 100-150 μ L peripheral blood collected from vaccinated mice was lysed with ACK lysis buffer, washed with PBS, and were
30 plated at ~ 10 million cells/mL in 50 μ L T cell media (RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine, 55 μ M β -mercaptoethanol, 1 mM pyruvate and 100 U/mL penicillin and 100 μ g/mL streptomycin, HEPES, and non-essential amino acids) in 96-well U bottom plates. Cells were pulsed with 10 μ g/mL antigen peptides for 6 hours with protein transport inhibitor, brefeldin A (BD Biosciences), added during the last 4 h of incubation. Cells were then washed

twice with ice-cold FACS buffer (1% BSA in PBS), followed by incubation with anti-CD16/32 for at least 10 minutes and anti-CD8 α for 20 min on ice. Cells were then fix/permeabilized for 20 min on ice and then stained with anti-IFN- γ -PE and anti-TNF- α -FITC for 30 min on ice. After extensive washing, cells were analyzed by flow cytometry.

5

cDNA sequencing of neo-epitope (Adpgk) in MC-38 cells

Total RNA was extracted from MC-38 cells by the RNeasy[®] mini Kit (QIAGEN) following the manufacturer's instructions. The first-strand cDNA was synthesized using 1 μ g of total RNA with the SuperScript[™] III First-Strand Synthesis SuperMix Kit (Invitrogen). Adpgk cDNA with lengths of 250 bp and 485 bp were selectively amplified by using the following two sets of sequence specific primers. Primer 1: TGCCAACCGCTTCATCTTCT (forward primer) and GGTAGACCAGCGTGTGGAAA (reverse primer); Primer 2: CTCCAACGGGGCCATGAATA (forward primer) and CGTGGGAAAGACCTGCTGAT (reverse primer). The amplification was performed using the SuperScript One Step RT-PCR System (Invitrogen). The final cDNA products were visualized in 1.5% agarose gels with ethidium bromide, and the Adpgk cDNA bands were cut and purified using the PureLink[®] Quick Gel Extraction and PCR Purification Combo Kit (Invitrogen). The purified cDNA was sequenced by the Sanger sequencing method (see, e.g., Sanger, F., Nicklen, S. & Coulson, A.R. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. U. S. A. **74**, 5463-5467 (1977)) at the University of Michigan DNA Sequencing Core.

20

Example VII.

This example describes neo-antigen vaccination using other nanoparticles, including sHDL, liposomes, and gold nanoparticles (Fig. 29), and the generation of multivalent neo-antigen vaccination using multiple neo-antigen peptides (Fig. 28).

25

Preparation of sHDL loaded with multivalent neo-antigens

To prepare nanodisc-based multivalent peptide vaccine, multiple neo-antigen peptides (M30 and M27) modified with CSS linker at N-terminus were conjugated to DOPE-PDP in dimethylformamide at room temperature for 3 hours, followed by dilution with 10X water and lyophilization to obtain lipid-peptide conjugates. The conjugate was mixed with DMPC and 22A in acetic acid and lyophilized. The resulting powder was then subjected to heating (50 $^{\circ}$ C) for 3 min and cooling (ice water) for 3 min, with 3 cycles in total, to obtain sHDL loaded with

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different neo-antigens (sHDL-M30/M27). Alternatively, the conjugate was dissolved in DMSO and incubated with preformed sHDL to obtain sHDL loaded with different neo-antigens (sHDL-M30/M27). Any unincorporated neo-antigen peptides were removed by passing through a desalting column. The loading efficiency was analyzed by HPLC. Cholesterol-CpG was
5 incubated with the above sHDL at room temperature for 30 min to obtain the nanodisc-based multivalent peptide vaccine (sHDL-M30/M27/CpG).

Preparation of liposomes loaded with neo-antigens

To prepare liposome-based neo-antigen vaccines, DMPC and DOPE-PDP (molar ratio =
10 92:8) were dissolved in chloroform. The mixture was dried with nitrogen flow and placed under vacuum for at least 1 h. The resulting lipid film was hydrated in 10 mM sodium phosphate buffer (0.3117 g/L NaH₂PO₄·H₂O and 2.0747g/L Na₂HPO₄·7H₂O, pH 7.4) and sonicated in a bath sonicator for 10 min, followed by probe sonication for another 2.5 min to obtain liposomes. The neo-antigen peptide Adpgk was conjugated to liposomes after incubation of CSS-modified
15 Adpgk peptides with PDP-displaying liposomes, followed by desalting column-based separation of unconjugated peptides. The conjugation efficiency was analyzed by HPLC. Cholesterol-CpG was incubated with the above liposomes at room temperature for 30 min to obtain the liposome-based neo-antigen peptide vaccine (lip-Adpgk/CpG).

20 *Preparation of spiky gold nanoparticle-based neo-antigen peptide vaccine*

To obtain spiky gold nanoparticles (AuNPs), citrate gold nanoparticles were first prepared by boiling HAuCl₄ aqueous solution with sodium citrate. They were sequentially added with HAuCl₄, HCl, AgNO₃, and ascorbic acid at room temperature under vigorous stirring to form AuNPs *via* seed-mediated growth method. As-synthesized AuNPs were purified and
25 concentrated by centrifugation with 0.01% SDS. AuNP-based peptide vaccine was prepared by thiol-mediated surface decoration of neo-antigen peptides on AuNPs followed by polyIC and CpG layer loading through electrostatic complexation. Briefly, peptide vaccine was surface-conjugated to AuNPs by overnight incubation of AuNPs with CSS-modified neo-antigen peptide, CSS-ASMTNMELM (SEQ ID NO:383). Any unreacted peptide was removed from
30 AuNP conjugates by centrifugation. To load polyIC and CpG *via* electrostatic interaction, polyethylene glycol (average Mn 6,000)-modified polyethyleneimine (branched, average Mw ~25,000) (PEG-PEI) was employed. The peptide-conjugated AuNPs were mixed with PEG-PEI for 10 min, purified from excessive PEG-PEG by centrifugation, and added to polyIC and CpG mixture solution in 10 mM NaCl. After 5 min, the mixture was transferred to PEG-PEI solution

in 10 mM NaCl, and the salt concentration was step-wise increased to 150 mM NaCl by the increment of 50 mM every 5 min. Finally, the crude mixture solution was centrifuged with 0.01% tween20 to remove any unbound polyIC and CpG.

5 *Intracellular cytokine staining*

C57BL/6 mice were vaccinated with nanodisc-based multivalent neo-antigen peptide vaccine (sHDL-M30/M27/CpG) on day 0, 7, and 14. Seven days after the last vaccination, 100-150 μ L peripheral blood collected from vaccinated mice was lysed with ACK lysis buffer, washed with PBS, and were plated at \sim 10 million cells/mL in 50 μ L T cell media (RPMI 1640
10 supplemented with 10% FBS, 2 mM L-glutamine, 55 μ M β -mercaptoethanol, 1 mM pyruvate and 100 U/mL penicillin and 100 μ g/mL streptomycin, HEPES, and non-essential amino acids) in 96-well U bottom plates. Cells were co-cultured with 50000 BMDCs/well and pulsed with 20 μ g/mL of M30 or M27 peptide for 6 hours with protein transport inhibitor, brefeldin A (BD Biosciences), added during the last 4 h of incubation. Cells were then washed twice with ice-
15 cold FACS buffer (1% BSA in PBS), followed by incubation with anti-CD16/32 for at least 10 minutes and anti-CD8 α and anti-CD4 for 20 min on ice. Cells were then fix/permeabilized for 20 min on ice and then stained with anti-IFN- γ -PE for 30 min on ice. After extensive washing, cells were analyzed by flow cytometry. The results shown in Fig. 28 indicate that sHDL-M30/M27/CpG generated high frequencies of CD4 $^+$ T-cells against neo-antigen M30 (Fig. 28A)
20 and CD8 $^+$ T-cells against neo-antigen M27 (Fig. 28B).

Therapeutic study

For therapeutic tumor vaccination studies, C57BL/6 mice were inoculated with tumor cells (1×10^5 MC38 cells per mouse) on the right flank by subcutaneous injection on day 0. Mice
25 were vaccinated on days 10 and 17 with 15.5 nmol of ASMTNMELM (SEQ ID NO:383) and 2.3 nmol of CpG (or 15 μ g polyIC/mouse) formulated in either liposomes or soluble forms. For the group of mice immunized with AuNPs, intratumoral administration of AuNPs modified with Adpgk and adjuvants was performed on days 10 (both w/ and w/o laser groups) and 16 (only w/o laser group) with 12 nmol of ASMTNMELM (SEQ ID NO:383), 5.2 nmol of CpG, and 83
30 μ g polyIC per mouse. Laser was directly irradiated to tumor tissues at 1.2 W/cm 2 for 5 min using 808 nm CW diode laser.

On indicated time points, PBMCs were collected and stained for Adpgk-specific CD8 $^+$ T cells among PBMCs via tetramer staining, followed by cytometric analysis. The tetramer staining of PBMCs indicated that Adpgk-containing liposomes and AuNPs all generated

stronger neo-antigen-specific CD8⁺ T cell responses, compared with vaccination with soluble peptide plus adjuvants (Fig. 29A). In addition, tumor growth was monitored every other day, and the tumor volume throughout this study was calculated by the following equation: tumor volume = length × width² × 0.52. Animals were euthanized when the tumor masses reached 1.5
5 cm in diameter or when animals became moribund with severe weight loss or ulceration. The results indicated that Adpgk-containing nanoparticles, including liposomes and AuNPs, slowed tumor progression, compared with vaccination with soluble peptide and CpG (Fig. 29B).

Example VIII.

10 This example describes HDL preparation using full length protein.

Heat/Cool (Lipid Film) Method

First, lipid films were prepared by weighing out and dissolving the desired amount of lipid in chloroform, followed by solvent evaporation under a stream of nitrogen gas. Excess
15 chloroform was subsequently removed by placing vials in a vacuum oven set to room temperature overnight. The following day, the lipid films were hydrated with warm phosphate buffer, followed by vortexing and sonication by both bath and probe sonication. Once translucent, ApoA-I protein solution was warmed and added to the lipid suspension. The resulting protein-lipid mixture was vortexed and thermal-cycled between 50 °C and room
20 temperature (10 minutes each) for 3 cycles. The solution turned clear by the end of the third cycle, indicating the formation of HDL. The HDL suspension was filtered using 0.22 um syringe filters and characterized for purity using gel permeation chromatography (GPC) and size by dynamic light scattering (DLS).

Both DMPC and SM lipids were able to form HDL with ApoA-I using this method. GPC
25 showed ApoA-I-DMPC and ApoA-I-SM HDL were 93.5% and 94.4% pure, respectively, with small amounts of un-complexed lipid (~5.59 min) and ApoA-I (~8.7 min) impurities (see, Fig. 35).

Neither POPC, DMPG:DPPG-NH₄⁺, nor EggPC were able to form HDL with ApoA-I
via this method (Fig. 36). Suspensions remained cloudy even after repeated thermal cycling, and
30 DLS measurements show that the average particle sizes were >1 um in diameter with high polydispersity.

DLS data confirmed the sizes of ApoA-I-DMPC and ApoA-I-SM HDL to be ~8.5 nm in diameter (Fig. 36).

Cholate/BioBeads SM- Second Method

For this method, lipid films of DMPG:DPPG-NH₄⁺ (4:1 mol/mol), POPC, or EggPC were prepared using the method described above. After excess solvent removal under vacuum, lipid films were hydrated with warm ApoA-I solution and vortexed, resulting in a cloudy white suspension. Sodium deoxycholate, dissolved to 20 mg/mL in PBS, was slowly titrated into the cloudy protein-lipid suspension. The mixture was vortexed and incubated at 37°C until the suspension turned clear. Cholate was then removed by incubation with BioBeads SM-2 (~20 mg BioBeads per 1 mL solution) at 37 °C with gentle agitation for 3 hours. The resulting HDL suspension was transferred to a new vial and filtered using 0.22 um syringe filters and characterized for purity using gel permeation chromatography (GPC) and size by dynamic light scattering (DLS).

EggPC, POPC, and DMPG:DPPG-NH₄⁺ lipids were able to form HDL with ApoA-I using this method. GPC analysis showed that particles were only about 85% pure (~7.7 min), however, with excess cholate appearing ~11.6 minutes and very small amounts of uncomplexed lipid (~5.5 min) and protein (~9 min) present (see, Fig. 37A).

Neither increasing the incubation time of HDL with BioBeads nor increasing the amount of BioBeads added resulted in additional cholate removal, but rather started to disrupt the HDL particles themselves (as shown in the last slide, splitting of HDL peak with no decrease in cholate peak) (see, Fig. 37B).

20

Example IX.

This example describes HDL preparation using apolipoprotein mimetics.

In contrast to heterogenous HDL formation by recombinant ApoA-I, homogeneous sHDL nanodiscs using ApoA-I-mimetic peptide and various lipids were produced by the following two methods.

25

Co-lyophilization

Appropriate amount of lipids and ApoA-I mimetic peptide were dissolved in glacial acetic acid, which was removed by freeze-drying overnight. Phosphate-buffered saline (PBS, pH = 7.4) was added to the freeze-dried powder, which was cycled 3 times between 50 °C (3 min) and 20 °C (3 min) with gentle shaking to obtain sHDL. The obtained sHDL nanoparticles were sterilized by passing through 0.22 um syringe filters and characterized for purity using gel permeation chromatography (GPC) and size by dynamic light scattering (DLS).

30

Thin Film or Mixing and Thermal Cycling

Appropriate amount of lipids were dissolved in chloroform, which was removed by putting under nitrogen flow and then in vacuum oven to form a thin lipid film. The lipid film was hydrated with 10 mM sodium phosphate buffer (pH 7.4) using bath sonication. ApoA-I
5 mimetic peptide was added to the lipid dispersion, which was cycled 3 times between 50 °C (3 min) and 20 °C (3 min) with gentle shaking to obtain sHDL nanoparticles. The obtained sHDL nanoparticles were sterilized by passing through 0.22 µm syringe filters and characterized for purity using gel permeation chromatography (GPC) and size by dynamic light scattering (DLS).

A broad range of phospholipids with different chain lengths and transition temperatures,
10 including POPC, DLPC, DMPC, SM, DPPC, HSPC, and DSPC were all able to efficiently complex with ApoA-1 mimetic peptide (22A) to form homogeneous sHDL nanoparticles using either of the above two methods. GPC showed the purity of formed sHDL nanoparticles was over 99%, with little to no free peptide or uncomplexed lipids (see, Fig. 38). DLS further confirmed the good homogeneity of HDL nanoparticles. Depending on the lipid composition,
15 the sizes of sHDL nanoparticles were between 8.3 – 10.8 nm, which were all close to that of endogenous HDL (see, Fig. 39).

Example X

This example describes the lack of immunogenicity or autoimmunity after multiple
20 immunizations with “blank” sHDL nanodiscs (without antigen or adjuvant) thereby indicating safety of the ApoA-I-mimetic peptide-based nanodiscs.

Mice immunized multiple times with sHDL composed of phospholipids and ApoA-I-mimetic peptide 22A did not display any signs of toxicity, autoimmunity, nor immune responses directed against the ApoA1-mimetic peptide 22A. The results indicate that “blank” sHDL
25 nanodiscs are not immunogenic on their own and they don't elicit any detectable autoimmune responses.

Measurement of antibody titers against 22A peptide: ELISA plates were coated with 22A peptide in PBS (1 µg/mL) with 100 µL/well and incubated overnight at 4 °C. Plates were blocked with 1% BSA in PBS for 2 h, and 100 µL of 4-fold serial dilutions of serum was added
30 to each 96-well and incubated for 1 hour at room temperature. Wells were incubated with rabbit anti-mouse IgG-HRP (1:5000 dilution) for 1 h at room temperature, followed by addition of the HRP substrate, TMB. The enzymatic reaction was stopped by adding 2N H₂SO₄, and the absorbance at 450 nm (OD₄₅₀) was measured using a microplate reader. The highest dilution with twice the absorbance of background was considered as the end-point dilution titer.

Additional experiments were conducted wherein C57BL/6 mice were immunized with sHDL-CpG (equivalent to 2.3 nmol CpG per dose) for 3 times in an 1-week interval. Fig. 40 shows the percent of 22A-specific CD4⁺ T cells (a), 22A-specific CD8⁺ T cells (b) among PBMCs one week after the third vaccination, and (c) the titers of IgG antibody against 22A one week after the third vaccination. Data represent mean \pm SD from a representative experiment (n = 3) from 2 independent experiments. NS, non-statistically significant. Importantly, throughout such experiments, signs of toxicity, autoimmunity, immune responses directed against the ApoA1-mimetic peptide 22A in animals immunized multiple times with sHDL-Ag/CpG were not observed (see, Fig. 40).

10

Example XI.

This example shows in vivo data where nanodisc vaccination is combined with immunostimulatory agent (e.g., immune checkpoint inhibitors). In addition, this example demonstrates therapeutic efficacy of combination immunotherapy between nanodisc vaccination and immune checkpoint blockade. In addition, this example demonstrates the use of multiple tumor antigens delivered by “cocktail” of nanodiscs with or without immune checkpoint blockade.

15

Preparation of a single or cocktail of tumor antigens on sHDL nanodiscs

To conjugate tumor antigen peptides to sHDL, cysteine terminated tumor antigen peptides dissolved in endotoxin free water were added to the above sHDL (antigen peptide:DOPE-PDP = 2.5:1, molar ratio) and incubated at room temperature with gentle shaking on an orbital shaker. To construct sHDL nanodiscs with multi-antigens, each antigen peptide was reacted with DOPE-PDP (antigen peptide:DOPE-PDP = 1.5:1, molar ratio) for 1 h in dimethylformamide (DMF), which was removed by freeze-drying after dilution with endotoxin-free water. The lipid-peptide conjugates were added to pre-formed sHDL and incubated for 30 min at room temperature. Unreacted tumor antigen peptides were removed by using Zeba Spin Desalting columns (Pierce) following the manufacturer’s instructions. The conjugation efficiency of tumor antigen peptides was calculated based on the decrease of absorbance signal associated with DOPE-PDP as determined by HPLC.

25

30

Combination immunotherapy between sHDL nanodisc vaccination and immune checkpoint blockade

For therapeutic tumor vaccination studies with MC-38 cells, C57BL/6 mice were inoculated with 1×10^5 MC38 cells per mouse on the right flank by subcutaneous injection on

day 0 and vaccinated on days 10, 17, and 24 with 15.5 nmol of ASMTNMELM (SEQ ID NO:383) and 2.3 nmol of CpG in either sHDL or free soluble form. For the combinatorial immunotherapy against MC-38 tumor, mice were inoculated subcutaneously with 1×10^5 MC38 cells on day 0 and vaccinated on days 10, and 17 with 15.5 nmol of ASMTNMELM (SEQ ID NO:383) and 2.3 nmol of CpG in either sHDL or free soluble form. Anti-mouse PD-1 (100 $\mu\text{g}/\text{mouse}$) was administered intraperitoneally on days 1 and 4 after each vaccination. For B16F10 studies, mice were inoculated subcutaneously with 1×10^5 B16F10 cells on day 0 and vaccinated on days 4, 11, and 18 with indicated formulations (10 nmol of each antigen peptide and 2.3 nmol of CpG). For the combinatorial immunotherapy against B16F10 tumor, anti-mouse PD-1 and anti-mouse CTLA-4 (each 100 $\mu\text{g}/\text{mouse}$) antibodies were administered intraperitoneally on days 1 and 4 after each vaccination. Tumor growth was monitored.

MC-38 treatment with neo-antigen sHDL vaccination combined with anti-PD-1 antibody therapy

Therapeutic vaccination with sHDL-Adpgk/CpG induced polyfunctional $\text{IFN-}\gamma^+$ and $\text{IFN-}\gamma^+\text{TNF-}\alpha^+$ Adpgk-specific $\text{CD8}\alpha^+$ T-cells and substantially slowed MC-38 tumor growth (Fig. 41A), compared with the traditional soluble Adpgk+CpG vaccine. However, no tumor rejection was observed in either vaccine groups, potentially due to immunosuppression within tumor microenvironment, as we detected high expression levels of programmed cell death-1 (PD-1) and its ligand PD-L1 among tumor-infiltrating $\text{CD8}\alpha^+$ T-cells and tumor cells, respectively. In order to block the immunosuppressive PD-1/PD-L1 pathway, experiments combined the vaccines with anti-PD-1 antibodies ($\alpha\text{PD-1}$). Combination immunotherapy with sHDL-Adpgk/CpG and $\alpha\text{PD-1}$ treatment generated robust neoantigen-specific CTL responses and led to complete tumor regression in $\sim 88\%$ mice (Fig. 41B), compared with $\sim 25\%$ rate of tumor regression in the soluble Adpgk+CpG+ $\alpha\text{PD-1}$ group.

B16F10 treatment with a cocktail of neo-antigens on sHDL nanodiscs combined with dual anti-PD-1/CTLA-4 antibody therapy

Experiments were conducted to evaluate the nanodisc platform in a melanoma model with B16F10 cells, as they are highly aggressive, poorly immunogenic, and hence hard to treat with conventional cancer vaccines. To prevent tumor immune escape by loss of a single mutant allele, experiments sought to elicit broad-spectrum T-cell responses by employing multiple antigens (multiAgs), including recently reported B16F10 mutated neo-epitopes (MHC I-restricted M27 and MHC II-restricted M30) as well as MHC I-restricted epitope from

tyrosinase-related protein 2 (TRP2, a melanoma-associated Ag), all loaded in the same nanodiscs. C57BL/6 mice inoculated subcutaneously with 10^5 B16F10 cells were vaccinated with sHDL-multiAgs/CpG, eliciting a total of ~30% Ag-specific, IFN- γ^+ CD8 α^+ and CD4+ T-cells in peripheral blood, compared with only 1-3% induced by the soluble multiAgs+CpG or
5 multiAgs+CpG+Montanide groups ($p < 0.0001$, Fig. 42A). Vaccination with sHDL-multiAgs/CpG significantly inhibited B16F10 tumor growth, compared with the soluble or Montanide vaccines ($p < 0.0001$, Fig. 42B). Notably, removing either M27/M30 or TRP2 from sHDL-multiAgs/CpG compromised its therapeutic efficacy, suggesting the benefits of broad CTL responses against neo-antigens and tumor-associated antigens (Fig. 42C). We evaluated
10 sHDL-multiAgs/CpG combined with dual immune checkpoint inhibitors. Combination immunotherapy with sHDL-multiAgs/CpG and α PD-1/ α CTLA-4 treatment led to an impressive rate of B16F10 tumor rejection with ~90% of mice free of tumor, whereas the soluble multiAgs+CpG+ α PD-1/ α CTLA-4 treatment mediated tumor regression in ~38% of animals (Fig. 42D).

15

INCORPORATION BY REFERENCE

The entire disclosure of each of the patent documents and scientific articles referred to herein is incorporated by reference for all purposes.

EQUIVALENTS

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and
25 all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

CLAIMS

We Claim:

- 5 1. A method for inhibiting a target gene in a cell, comprising introducing into the cell a composition comprising a siRNA encapsulated within a nanoparticle, wherein the siRNA is capable of inhibiting the target gene by RNA interference, wherein the siRNA comprises two RNA strands that are complementary to each other.
- 10 2. The method of Claim 1, wherein the nanoparticle is selected from the group consisting of sHDL nanoparticles, fullerenes, endohedral metallofullerenes buckyballs, trimetallic nitride templated endohedral metallofullerenes, single-walled and mutli-walled carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods, single-walled and multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon nanohorns, carbon
15 nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures, microstructures, or their derivatives formed using layer-by-layer processes, self-assembly processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles, nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable PLGA micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles, iron
20 nanoparticles, and modified micelles.
3. The method of Claim 1, wherein the siRNA is modified with cholesterol at the 3' sense strand.
- 25 4. The method of Claim 1, wherein the cell is in vivo, in vitro, or ex vivo.
5. The method of Claim 1, wherein the cell is within a human being.
6. The method of Claim 1, wherein an imaging agent is encapsulated within the
30 nanoparticle.
7. The method of Claim 1, wherein the nanoparticle is a sHDL nanoparticle, wherein the sHDL nanoparticle comprises a mixture of at least one phospholipid and at least one HDL apolipoprotein or apolipoprotein mimetic.

8. The method of Claim 7,

wherein the HDL apolipoprotein is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), and apolipoprotein E (apo E),

wherein the phospholipid is selected from the group consisting of dipalmitoylphosphatidylcholine (DPPC), dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and combinations thereof.

9. The method of Claim 8, wherein the HDL apolipoprotein mimetic is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A-II xxx (apo A-II-xxx), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), apolipoprotein E (apo E), apolipoprotein A-I milano (apo A-I-milano), apolipoprotein A-I paris (apo A-I-paris), apolipoprotein M (apo M), an HDL apolipoprotein mimetic, preproapolipoprotein, preproApoA-I, proApoA I, preproApoA-II, proApoA II, preproApoA-IV, proApoA-IV, ApoA-V, preproApoE, proApoE, preproApoA I_{Milano}, proApoA-I_{Milano}, preproApoA-I_{Paris}, proApoA-I_{Paris}, and mixtures thereof.

10. The method of Claim 9, wherein the ApoA-I mimetic is described by any of SEQ ID NOs: 1-336 and WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341), LKLLDNWDSVTSTFSKLEOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLOEMS (SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO: 345), PYLDDFQKKWQEEMELYRQKVE (SEQ ID NO: 346), PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDALRTHLA (SEQ ID NO: 348), PYSDELQRLAARLEALKENGG (SEQ ID NO: 349), ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ

ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352),
 TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDYGKDLME (SEQ ID
 NO:355), KVKSPELOAEAKSYFEKSKE (SEQ ID NO:356),
 VLTALVAVAGARAEVSADOVATV (SEQ ID NO:357),
 5 NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358),
 LPVLVWLSIVLEGPAPAOGTPDVSS (SEQ ID NO:359),
 LPVLVVVLSIVLEGPAPAQGTPDVSS (SEQ ID NO:360), ALDKLKEFGNTLEDKARELIS
 (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362),
 HLRKLRKRLLRDADDLQKRLAVYOA (SEQ ID NO:363),
 10 AQAWGERLRARMEEMGSRTRDR (SEQ ID NO:364), LDEVKEQVAEVRACLEEQAQ
 (SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236),
 DWLKAFYDKVAEKLKEAFPDWAKAAYDKAAEKAKEAA (SEQ ID NO:366),
 PVLDFRELLNELLEALKQKL (SEQ ID NO:367), PVLDFRELLNELLEALKQKLA (SEQ
 ID NO:368), PVLDFRELLNELLEALKQKLN (SEQ ID NO:4),
 15 PVLDFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDFRELLNELLEALKKLLK
 (SEQ ID NO:370), PVLDFRELLNELLEALKKLLA (SEQ ID NO:371),
 PLLDFRELLNELLEALKKLLA (SEQ ID NO:372), and
 EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

20 11. The method of Claim 1, wherein the average particle size of the nanoparticle is between
 6 to 500 nm.

12. A method for reducing serum LDL-C levels in patient, comprising administering to the
 patient a therapeutically effective amount of a pharmaceutical composition comprising a PCSK9
 25 siRNA encapsulated within a nanoparticle, wherein the PCSK9 siRNA is capable of inhibiting
 the PCSK9 gene by RNA interference, wherein the PCSK9 siRNA comprises two RNA strands
 that are complementary to each other, wherein inhibiting of the PCSK9 gene results in reduction
 of serum LDL-C levels in the patient.

30 13. The method of Claim 12, wherein the nanoparticle is selected from the group consisting
 of sHDL nanoparticles, fullerenes, endohedral metallofullerenes buckyballs, trimetallic nitride
 templated endohedral metallofullerenes, single-walled and multi-walled carbon nanotubes,
 branched and dendritic carbon nanotubes, gold nanorods, silver nanorods, single-walled and
 multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon nanohorns, carbon

nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures, microstructures, or their derivatives formed using layer-by-layer processes, self-assembly processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles, nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable PLGA
 5 micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles, iron nanoparticles, and modified micelles.

14. The method of Claim 12, wherein the patient is a human patient.

10 15. The method of Claim 12, wherein the PCSK9 siRNA is modified with cholesterol at the 3' sense strand.

16. The method of Claim 12, wherein an imaging agent is encapsulated within the nanoparticle.

15

17. The method of Claim 12, wherein the nanoparticle is a sHDL nanoparticle, wherein the sHDL nanoparticle comprises a mixture of at least one phospholipid and at least one HDL apolipoprotein or apolipoprotein mimetic.

20

18. The method of Claim 17,
 wherein the HDL apolipoprotein is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), and apolipoprotein E (apo E),

25

wherein the phospholipid is selected from the group consisting of
 dipalmitoylphosphatidylcholine (DPPC), dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridylidithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide],
 30 phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and combinations thereof.

19. The method of Claim 18, wherein the HDL apolipoprotein mimetic is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A-II xxx (apo A-II-xxx), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), apolipoprotein E (apo E), apolipoprotein A-I milano (apo A-I-milano), apolipoprotein A-I paris (apo A-I-paris), apolipoprotein M (apo M), an HDL apolipoprotein mimetic, preproapolipoprotein, preproApoA-I, proApoA I, preproApoA-II, proApoA II, preproApoA-IV, proApoA-IV, ApoA-V, preproApoE, proApoE, preproApoA I_{Milano}, proApoA-I_{Milano}, preproApoA-I_{Paris}, proApoA-I_{Paris}, and mixtures thereof.
20. The method of Claim 19, wherein the ApoA-I mimetic is described by any of SEQ ID NOs: 1-336 and WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341), LKLLDNWDSVTSTFSKLREOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLR OEMS (SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO: 345), PYLDDFQKKWQEEMELYRQKVE (SEQ ID NO: 346), PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDALRTHLA (SEQ ID NO: 348), PYSDEL RQRLAARLEALKENGG (SEQ ID NO: 349), ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDY GKDLME (SEQ ID NO:355), KVK SPELOAEAKSYFEKSKE (SEQ ID NO:356), VLTALVAVAGARAEVSADOVATV (SEQ ID NO:357), NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358), LPVLVWLSIVLEGPAPAOGTPDVSS (SEQ ID NO:359), LPVLVVVLSIVLEGPAPAQGTPDVSS (SEQ ID NO:360), ALDKLKEFGNTLEDKARELIS (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362), HLRKLRKRLLRDADDLQKRLAVYOA (SEQ ID NO:363), AQAWGERLRARMEEMGSRTRDR (SEQ ID NO:364), LDEVKEQVAEVR AKLEEQAQ (SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236), DWLKAFYDKVAEKLKEAFPDWAKAAYDKAAEKAKEAA (SEQ ID NO:366), PVLDFRELLNELLEALKQKL (SEQ ID NO:367), PVLDFRELLNELLEALKQKLA (SEQ ID NO:368), PVLDFRELLNELLEALKQKLK (SEQ ID NO:4), PVLDFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDFRELLNELLEALKKLLK (SEQ ID NO:370), PVLDFRELLNELLEALKKLLA (SEQ ID NO:371),

PLDLFRELLNELLEALKKLLA (SEQ ID NO:372), and
EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

21. The method of Claim 12, wherein the average particle size of the nanoparticle is between
5 6 to 500 nm.
22. A method for treating coronary heart disease in a patient through reducing serum LDL-C
levels in the patient, comprising administering to the patient a therapeutically effective amount
of a pharmaceutical composition comprising a PCSK9 siRNA encapsulated within a
10 nanoparticle, wherein the PCSK9 siRNA is capable of inhibiting the PCSK9 gene by RNA
interference, wherein the PCSK9 siRNA comprises two RNA strands that are complementary to
each other, wherein inhibiting of the PCSK9 gene results in reduction of serum LDL-C levels.
23. The method of Claim 22, wherein the nanoparticle is selected from the group consisting
15 of sHDL nanoparticles, fullerenes, endohedral metallofullerenes buckyballs, trimetallic nitride
templated endohedral metallofullerenes, single-walled and mutli-walled carbon nanotubes,
branched and dendritic carbon nanotubes, gold nanorods, silver nanorods, single-walled and
multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon nanohorns, carbon
nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures, microstructures, or
20 their derivatives formed using layer-by-layer processes, self-assembly processes, or
polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles, nanorods,
cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable PLGA
micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles, iron
nanoparticles, and modified micelles.
25
24. The method of Claim 22, wherein the patient is a human patient.
25. The method of Claim 22, wherein the PCSK9 siRNA is modified with cholesterol at the
3' sense strand.
30
26. The method of Claim 22, wherein an imaging agent is encapsulated within the
nanoparticle.
27. The method of Claim 22, wherein the nanoparticle is a sHDL nanoparticle, wherein the

sHDL nanoparticle comprises a mixture of at least one phospholipid and at least one HDL apolipoprotein or apolipoprotein mimetic.

28. The method of Claim 27,

5 wherein the HDL apolipoprotein is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), and apolipoprotein E (apo E),

10 wherein the phospholipid is selected from the group consisting of dipalmitoylphosphatidylcholine (DPPC), dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-
15 phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and combinations thereof.

29. The method of Claim 28, wherein the HDL apolipoprotein mimetic is selected from the
20 group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A-II xxx (apo A-II-xxx), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), apolipoprotein E (apo E), apolipoprotein A-I milano (apo A-I-milano), apolipoprotein A-I paris (apo A-I-paris), apolipoprotein M (apo M), an HDL apolipoprotein mimetic, preproapolipoprotein, preproApoA-I, proApoA I, preproApoA-II, proApoA II, preproApoA-IV, proApoA-IV, ApoA-
25 V, preproApoE, proApoE, preproApoA I_{Milano}, proApoA-I_{Milano}, preproApoA-I_{Paris}, proApoA-I_{Paris}, and mixtures thereof.

30. The method of Claim 29, wherein the ApoA-I mimetic is described by any of SEQ ID NOs: 1-336 and WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341),
30 LKLLDNWDSVTSTFSKLREOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLOEMS (SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO: 345), PYLDDFQKKWQEEMELYRQKVE (SEQ ID NO: 346), PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDALRTHLA (SEQ ID NO: 348), PYSDELQRRLAARLEALKENGG (SEQ ID NO: 349),

ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351),
 PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ
 ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352),
 TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDYGKDLME (SEQ ID
 5 NO:355), KVK SPELOAEAKSYFEKSKE (SEQ ID NO:356),
 VLTALVAVAGARAEVSADOVATV (SEQ ID NO:357),
 NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358),
 LPVLVWLSIVLEGPAPAOGTPDVSS (SEQ ID NO:359),
 LPVLVVVLSIVLEGPAPAQGTPDVSS (SEQ ID NO:360), ALDKLKEFGNTLEDKARELIS
 10 (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362),
 HLRKLRKRLLRDADDLQKRLAVYOA (SEQ ID NO:363),
 AQAWGERLRARMEEMGSRTRDR (SEQ ID NO:364), LDEVKEQVAEVRACLEEQAAQ
 (SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236),
 DWLKAFYDKVAEKLKEAFPDWAKAAYDKAAEKAKEAA (SEQ ID NO:366),
 15 PVLDLFRELLNELLEALKQKL (SEQ ID NO:367), PVLDLFRELLNELLEALKQKLA (SEQ
 ID NO:368), PVLDLFRELLNELLEALKQKLK (SEQ ID NO:4),
 PVLDLFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDLFRELLNELLEALKKLLK
 (SEQ ID NO:370), PVLDLFRELLNELLEALKKLLA (SEQ ID NO:371),
 PLLDLFRELLNELLEALKKLLA (SEQ ID NO:372), and
 20 EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

31. The method of Claim 22, wherein the average particle size of the nanoparticle is between 6 to 500 nm.

25 32. A method for inducing a natural killer T cell-mediated immune response in a cell comprising exposing the cell to a composition comprising an α GalCer glycolipid associated (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) within a nanoparticle, wherein such exposure results in the induction of a natural killer T cell-mediated immune response.

30 33. The method of Claim 32, wherein the nanoparticle is selected from the group consisting of sHDL nanoparticles, fullerenes, endohedral metallofullerenes buckyballs, trimetallic nitride templated endohedral metallofullerenes, single-walled and mutli-walled carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods, single-walled and

multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures, microstructures, or their derivatives formed using layer-by-layer processes, self-assembly processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles, nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable PLGA micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles, iron nanoparticles, and modified micelles.

34. The method of Claim 32, wherein the cell is an in vivo cell, an ex vivo cell, or an in vitro cell.

35. The method of Claim 32, wherein the nanoparticle is a sHDL nanoparticle, wherein the sHDL nanoparticle comprises a mixture of at least one phospholipid and at least one HDL apolipoprotein or apolipoprotein mimetic.

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36. The method of Claim 35, wherein the HDL apolipoprotein is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), and apolipoprotein E (apo E),

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wherein the phospholipid is selected from the group consisting of dipalmitoylphosphatidylcholine (DPPC), dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and combinations thereof.

30

37. The method of Claim 36, wherein the HDL apolipoprotein mimetic is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A-II xxx (apo A-II-xxx), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), apolipoprotein E (apo E), apolipoprotein A-I milano (apo A-I-milano), apolipoprotein A-I paris

(apo A-I-paris), apolipoprotein M (apo M), an HDL apolipoprotein mimetic, preproapolipoprotein, preproApoA-I, proApoA I, preproApoA-II, proApoA II, preproApoA-IV, proApoA-IV, ApoA-V, preproApoE, proApoE, preproApoA I_{Milano}, proApoA-I_{Milano}, preproApoA-I_{Paris}, proApoA-I_{Paris}, and mixtures thereof.

5

38. The method of Claim 37, wherein the ApoA-I mimetic is described by any of SEQ ID NOs: 1-336 and WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341), LKLLDNWDSVTSTFSKLREOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLROEMS (SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO: 10 345), PYLDDDFQKKWQEEMELYRQKVE (SEQ ID NO: 346), PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDALRTHLA (SEQ ID NO: 348), PYSDELQRLAARLEALKENGG (SEQ ID NO: 349), ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), 15 TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDYGKDLME (SEQ ID NO:355), KVKSPELOAEAKSYFEKSKE (SEQ ID NO:356), VLTALVAVAGARAEVSADOVATV (SEQ ID NO:357), NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358), 20 LPVLVWLSIVLEGPAOAGTPDVSS (SEQ ID NO:359), LPVLVVVLSIVLEGPAQAQTPDVSS (SEQ ID NO:360), ALDKLKEFGNTLEDKARELIS (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362), HLRKLRKRLLRDADDLQKRLAVYOA (SEQ ID NO:363), AQAWGERLRARMEEMGSRTRDR (SEQ ID NO:364), LDEVKEQVAEVRACLEEQAQ 25 (SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236), DWLKAFYDKVAEKLKEAFPDWAKAAYDKAAEKAKEAA (SEQ ID NO:366), PVLDFRELLNELLEALKQKL (SEQ ID NO:367), PVLDFRELLNELLEALKQKLA (SEQ ID NO:368), PVLDFRELLNELLEALKQKLK (SEQ ID NO:4), PVLDFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDFRELLNELLEALKKLLK 30 (SEQ ID NO:370), PVLDFRELLNELLEALKKLLA (SEQ ID NO:371), PLLDFRELLNELLEALKKLLA (SEQ ID NO:372), and EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

39. The method of Claim 32, wherein the average particle size of the nanoparticle is between 6 to 500 nm.

40. A method for inducing an immune response to an antigen comprising administering to a subject in need an effective amount of a composition comprising a nanoparticle, wherein the antigen is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the nanoparticle, wherein an adjuvant is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the nanoparticle.

41. The method of Claim 40, wherein the nanoparticle is selected from the group consisting of sHDL nanoparticles, fullerenes, endohedral metallofullerenes buckyballs, trimetallic nitride templated endohedral metallofullerenes, single-walled and multi-walled carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods, single-walled and multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures, microstructures, or their derivatives formed using layer-by-layer processes, self-assembly processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles, nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable PLGA micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles, iron nanoparticles, and modified micelles.

42. The method of Claim 40, wherein the antigen is selected from the group consisting of alpha-actinin-4, Bcr-Abl fusion protein, Casp-8, beta-catenin, cdc27, cdk4, cdkn2a, coa-1, dekan fusion protein, EF2, ETV6-AML1 fusion protein, LDLR-fucosyltransferaseAS fusion protein, HLA-A2, HLA-A11, hsp70-2, KIAA0205, Mart2, Mum-1, 2, and 3, neo-PAP, myosin class I, OS-9, pml-RAR α fusion protein, PTPRK, K-ras, N-ras, Triosephosphate isomeras, Bage-1, Gage 3,4,5,6,7, GnTV, Herv-K-mel, Lage-1, Mage-A1,2,3,4,6,10,12, Mage-C2, NA-88, NY-Eso-1/Lage-2, SP17, SSX-2, and TRP2-Int2, MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15(58), CEA, RAGE, NY-ESO (LAGS), SCP-1, Hom/Mel-40, PRAME, p53, H-Ras, HER-2/neu, BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, β -Catenin, CDK4, Mum-1, p16, TAGE, PSMA, PSCA, CT7, telomerase, 43-9F, 5T4, 791Tgp72, α -

fetoprotein, 13HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), human EGFR protein or its fragments, such as human EGFR residues 306–325 (SCVRACGADSYEMEEDGVRK (SEQ ID NO:374)) and residues 897–915 (VWSYGVTVWELMTFGSKPY (SEQ ID NO:375)),

5 HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB\70K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein\cyclophilin C-associated protein), TAAL6, TAG72, TLP, TPS, WT1 (and WT1-derivaed peptide sequences: WT1 126–134 (RMFP NAPYL (SEQ ID NO:376)), WT1 122–140 (SGQARMFPNAPYLPSCLLES (SEQ ID NO:377)), and WT1 122–144 (SGQARMFPNAPYLPSCLLESQPTI (SEQ ID NO:378)), MUC1 (and MUC1-derived peptides

10 and glycopeptides such as RPAPGS (SEQ ID NO:379), PPAHGVV (SEQ ID NO:380), and PDTRP (SEQ ID NO:381))), LMP2, EGFRvIII, Idiotype, GD2, Ras mutant, p53 mutant, Proteinase3 (PR1), Survivin, hTERT, Sarcoma translocation breakpoints, EphA2, EphA4, LMW-PTP, PAP, ML-IAP, AFP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, ALK, Androgen receptor, Cyclin B1, Polysialic acid, MYCN, RhoC, TRP-2, GD3, Fucosyl GM1,

15 Mesothelin, sLe(animal), CYP1B1, PLAC1, GM3, BORIS, Tn, GloboH, NY-BR-1, RGS5, SART3, STn, Carbonic anhydrase IX, PAX5, OY-TES1, Sperm protein 17, LCK, HMWMAA, AKAP-4, XAGE 1, B7H3, Legumain, Tie 2, Page4, VEGFR2, MAD-CT-1, FAP, PDGFR-alpha, PDGFR- β , MAD-CT-2, Fos-related antigen 1, ERBB2, Folate receptor 1 (FOLR1 or FBP), IDH1, IDO, LY6K, fms-related tyro- sine kinase 1 (FLT1, best known as VEGFR1),

20 KDR, PADRE, TA-CIN (recombinant HPV16 L2E7E6), SOX2, and aldehyde dehydrogenase.

43. The method of Claim 40, wherein the adjuvant is selected from the group consisting of CPG, polyIC, poly-ICLC, 1018 ISS, aluminum salts (for example, aluminum hydroxide, aluminum phosphate), Amplivax, BCG, CP-870,893, CpG7909, CyaA, dSLIM, Cytokines (such

25 as GM-CSF, IL-2, IFN- α , Flt-3L), IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, OK-432, OM-174, OM-197-MP-EC, ONTAK, PepTel.RTM, vector system, PLGA microparticles, imiquimod, resiquimod, gardiquimod, 3M-052, SRL172, Virosomes and other Virus-like particles, YF-17D, VEGF trap,

30 beta-glucan, Pam3Cys, Aquila's QS21 stimulon, vadimezan, AsA404 (DMXAA), 3M MEDI9197, glucopyranosyl lipid adjuvant (GLA), GLA-SE, CD1d ligands (such as C20:2, OCH, AH04-2, α -galatosylceramide, α -C-galatosylceramide, α -mannosylceramide, α -fructosylceramide, β -galatosylceramide, β -mannosylceramide), STING agonists (e.g. cyclic dinucleotides, including Cyclic [G(3',5')pA(3',5')p], Cyclic [G(2',5')pA(3',5')p], Cyclic

[G(2',5')pA(2',5')p], Cyclic diadenylate monophosphate, Cyclic diguanylate monophosphate), CL401, CL413, CL429, Flagellin, RC529, E6020, imidazoquinoline-based small molecule TLR-7/8a (including its lipidated analogues), virosomes, AS01, AS02, AS03, AS04, AS15, IC31, CAF01, ISCOM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), bacterial toxins (such as CT, and LT), any derivative of an adjuvant, and any combination of adjuvant.

44. The method of Claim 40, wherein the antigen is conjugated to the outer surface of the nanoparticle.

45. The method of Claim 40, wherein the adjuvant is conjugated to the outer surface of the nanoparticle.

46. The method of Claim 40, wherein the adjuvant is encapsulated within the nanoparticle.

47. The method of Claim 40, wherein the nanoparticle is a sHDL nanoparticle, wherein the sHDL nanoparticle comprises a mixture of at least one phospholipid and at least one HDL apolipoprotein or apolipoprotein mimetic.

48. The method of Claim 47,

wherein the HDL apolipoprotein is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), and apolipoprotein E (apo E),

wherein the phospholipid is selected from the group consisting of

dipalmitoylphosphatidylcholine (DPPC), dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and combinations thereof.

49. The method of Claim 48,

wherein the HDL apolipoprotein mimetic is an ApoA-I mimetic,
 wherein the thiol-reactive phospholipid is dioleoyl-sn-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP).

- 5 50. The method of Claim 49, wherein the ApoA-I mimetic is described by any of SEQ ID NOs: 1-336 and WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341), LKLLDNWDSVTSTFSKLREOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLR OEMS (SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO: 345), PYLDDFQKKWQEEMEL YRQKVE (SEQ ID NO: 346),
- 10 PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDALRTHLA (SEQ ID NO: 348), PYSDEL RQRLAARLEALKENGG (SEQ ID NO: 349), ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352),
- 15 TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDYGKDLME (SEQ ID NO:355), KVKSP ELOAEAKSYFEKSKE (SEQ ID NO:356), VLTALVAVAGARAEVSADOVATV (SEQ ID NO:357), NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358), LPVLVWLSIVLEGPAPAOGTPDVSS (SEQ ID NO:359),
- 20 LPVLVVVLSIVLEGPAPAQGTPDVSS (SEQ ID NO:360), ALDKLKEFGNTLEDKARELIS (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362), HLRKLRKRLLRDADDLQKRLAVYOA (SEQ ID NO:363), AQAWGERLRARMEEMGSRTRDR (SEQ ID NO:364), LDEVKEQVAEVR AKLEEQAQ (SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236),
- 25 DWLKAFYDKVAEKLKEAFPDWAKAAYDKAAEKAKEAA (SEQ ID NO:366), PVLDFRELLNELLEALKQKL (SEQ ID NO:367), PVLDFRELLNELLEALKQKLA (SEQ ID NO:368), PVLDFRELLNELLEALKQK LK (SEQ ID NO:4), PVLDFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDFRELLNELLEALKKLLK (SEQ ID NO:370), PVLDFRELLNELLEALKKLLA (SEQ ID NO:371),
- 30 PLLDFRELLNELLEALKKLLA (SEQ ID NO:372), and EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

51. The method of Claim 40, wherein the average particle size of the nanoparticle is between 6 to 500 nm.

52. A composition comprising a nanoparticle, wherein an antigen is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the nanoparticle, wherein an adjuvant is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) with the nanoparticle.

53. The composition of Claim 52, wherein the nanoparticle is selected from the group consisting of sHDL nanoparticles, fullerenes, endohedral metallofullerenes buckyballs, trimetallic nitride templated endohedral metallofullerenes, single-walled and multi-walled carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods, single-walled and multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures, microstructures, or their derivatives formed using layer-by-layer processes, self-assembly processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles, nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable PLGA micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles, iron nanoparticles, and modified micelles.

54. The composition of Claim 52, wherein the antigen is selected from the group consisting of alpha-actinin-4, Bcr-Abl fusion protein, Casp-8, beta-catenin, cdc27, cdk4, cdkn2a, coa-1, dek-can fusion protein, EF2, ETV6-AML1 fusion protein, LDLR-fucosyltransferaseAS fusion protein, HLA-A2, HLA-A11, hsp70-2, KIAA0205, Mart2, Mum-1, 2, and 3, neo-PAP, myosin class I, OS-9, pml-RAR α fusion protein, PTPRK, K-ras, N-ras, Triosephosphate isomeras, Bage-1, Gage 3,4,5,6,7, GnTV, Herv-K-mel, Lage-1, Mage-A1,2,3,4,6,10,12, Mage-C2, NA-88, NY-Eso-1/Lage-2, SP17, SSX-2, and TRP2-Int2, MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15(58), CEA, RAGE, NY-ESO (LAGS), SCP-1, Hom/Mel-40, PRAME, p53, H-Ras, HER-2/neu, BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, β -Catenin, CDK4, Mum-1, p16, TAGE, PSMA, PSCA, CT7, telomerase, 43-9F, 5T4, 791Tgp72, α -fetoprotein, 13HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), human EGFR protein or its fragments, such as human EGFR residues 306–325 (SCVRACGADSYEMEEDGVRK (SEQ

ID NO:374)) and residues 897–915 (VWSYGVTVWELMTFGSKPY (SEQ ID NO:375)), HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB\70K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein\cyclophilin C-associated protein), TAAL6, TAG72, TLP, TPS, WT1 (and WT1-derivaed peptide sequences: WT1 126–134 (RMFP NAPYL (SEQ ID NO:376)),
 5 WT1 122–140 (SGQARMFPNAPYLPSCLLES (SEQ ID NO:377)), and WT1 122–144 (SGQARMFPNAPYLPSCLLESQPTI (SEQ ID NO:378)), MUC1 (and MUC1-derived peptides and glycopeptides such as RPAPGS (SEQ ID NO:379), PPAHGVV (SEQ ID NO:380), and PDTRP (SEQ ID NO:381))), LMP2, EGFRvIII, Idiotype, GD2, Ras mutant, p53 mutant, Proteinase3 (PR1), Survivin, hTERT, Sarcoma translocation breakpoints, EphA2, EphA4,
 10 LMW-PTP, PAP, ML-IAP, AFP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, ALK, Androgen receptor, Cyclin B1, Polysialic acid, MYCN, RhoC, TRP-2, GD3, Fucosyl GM1, Mesothelin, sLe(animal), CYP1B1, PLAC1, GM3, BORIS, Tn, GloboH, NY-BR-1, RGS5, SART3, STn, Carbonic anhydrase IX, PAX5, OY-TES1, Sperm protein 17, LCK, HMWMAA, AKAP-4, XAGE 1, B7H3, Legumain, Tie 2, Page4, VEGFR2, MAD-CT-1, FAP, PDGFR-
 15 alpha, PDGFR- β , MAD-CT-2, Fos-related antigen 1, ERBB2, Folate receptor 1 (FOLR1 or FBP), IDH1, IDO, LY6K, fms-related tyro- sine kinase 1 (FLT1, best known as VEGFR1), KDR, PADRE, TA-CIN (recombinant HPV16 L2E7E6), SOX2, and aldehyde dehydrogenase.

55. The composition of Claim 52, wherein the adjuvant is selected from the group consisting
 20 of CPG, polyIC, poly-ICLC, 1018 ISS, aluminum salts (for example, aluminum hydroxide, aluminum phosphate), Amplivax, BCG, CP-870,893, CpG7909, CyaA, dSLIM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, OK-432, OM-174, OM-197-MP-
 25 EC, ONTAK, PepTel.RTM, vector system, PLGA microparticles, imiquimod, resiquimod, gardiquimod, 3M-052, SRL172, Virosomes and other Virus-like particles, YF-17D, VEGF trap, beta-glucan, Pam3Cys, Aquila's QS21 stimulon, vadimezan, AsA404 (DMXAA), 3M MEDI9197, glucopyranosyl lipid adjuvant (GLA), GLA-SE, CD1d ligands (such as C20:2, OCH, AH04-2, α -galatosylceramide, α -C-galatosylceramide, α -mannosylceramide, α -
 30 fructosylceramide, β -galatosylceramide, β -mannosylceramide), STING agonists (e.g. cyclic dinucleotides, including Cyclic [G(3',5')pA(3',5')p], Cyclic [G(2',5')pA(3',5')p], Cyclic [G(2',5')pA(2',5')p], Cyclic diadenylate monophosphate, Cyclic diguanylate monophosphate), CL401, CL413, CL429, Flagellin, RC529, E6020, imidazoquinoline-based small molecule TLR-7/8a (including its lipidated analogues), virosomes, AS01, AS02, AS03, AS04, AS15, IC31,

CAF01, ISCOM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), bacterial toxins (such as CT, and LT), any derivative of an adjuvant, and any combination of adjuvant.

56. The composition of Claim 52, wherein the antigen is conjugated to the outer surface of the nanoparticle or the adjuvant is conjugated to the outer surface of the nanoparticle.

57. The composition of Claim 52, wherein the antigen is derived from a self-antigen.

58. The composition of Claim 52, wherein the adjuvant is encapsulated within the sHDL nanoparticle.

59. The composition of Claim 52, wherein the nanoparticle is a sHDL nanoparticle, wherein the sHDL nanoparticle comprises a mixture of at least one phospholipid and at least one HDL apolipoprotein or apolipoprotein mimetic.

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60. The composition of Claim 59,
wherein the HDL apolipoprotein is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), and apolipoprotein E (apo E),

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wherein the phospholipid is selected from the group consisting of dipalmitoylphosphatidylcholine (DPPC), dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and combinations thereof.

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61. The composition of Claim 60,
wherein the HDL apolipoprotein mimetic is an ApoA-I mimetic,
wherein the thiol-reactive phospholipid is dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP).

62. The composition of Claim 61, wherein the ApoA-I mimetic is described by any of SEQ ID NOs: 1-336 and WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341), LKLLDNWDSVTSTFSKLREOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLR OEMS (SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO: 345), PYLDDFQKKWQEEMEL YRQKVE (SEQ ID NO: 346), PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDALRTHLA (SEQ ID NO: 348), PYSDEL RQRLAARLEALKENGG (SEQ ID NO: 349), ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDYGKDLME (SEQ ID NO:355), KVK SPELOAEAKSYFEKSKE (SEQ ID NO:356), VLTALVAVAGARAEVSADOVATV (SEQ ID NO:357), NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358), LPVLVWLSIVLEGPAPAOGTPDVSS (SEQ ID NO:359), LPVLVVVLSIVLEGPAPAQGTPDVSS (SEQ ID NO:360), ALDKLKEFGNTLEDKARELIS (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362), HLRKLRKRLLRDADDLQKRLAVYOA (SEQ ID NO:363), AQAWGERLRARMEEMGSRTRDR (SEQ ID NO:364), LDEVKEQVAEVR AKLEEQAQ (SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236), DWLKAFYDKVAEKLKEAFPDWAKAAYDKAAEKAKEAA (SEQ ID NO:366), PVLDFRELLNELLEALKQKL (SEQ ID NO:367), PVLDFRELLNELLEALKQKLA (SEQ ID NO:368), PVLDFRELLNELLEALKQK LK (SEQ ID NO:4), PVLDFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDFRELLNELLEALKKLLK (SEQ ID NO:370), PVLDFRELLNELLEALKKLLA (SEQ ID NO:371), PLLDFRELLNELLEALKKLLA (SEQ ID NO:372), and EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

63. The composition of Claim 52, wherein the average particle size of the nanoparticle is between 6 to 500 nm.

64. A composition comprising siRNA encapsulated within a nanoparticle, wherein the siRNA is capable of inhibiting a target gene by RNA interference, wherein the siRNA comprises

two RNA strands that are complementary to each other.

65. The composition of Claim 64, wherein the nanoparticle is selected from the group consisting of sHDL nanoparticles, fullerenes, endohedral metallofullerenes buckyballs,
 5 trimetallic nitride templated endohedral metallofullerenes, single-walled and mutli-walled carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods, single-walled and multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures, microstructures, or their derivatives formed using layer-by-layer processes, self-assembly
 10 processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles, nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable PLGA micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles, iron nanoparticles, and modified micelles.

15 66. The composition of Claim 65, wherein the siRNA is modified with cholesterol at the 3' sense strand.

67. The composition of Claim 65, wherein an imaging agent is encapsulated within the nanoparticle.

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68. The composition of Claim 65, wherein the nanoparticle is a sHDL nanoparticle, wherein the sHDL nanoparticle comprises a mixture of at least one phospholipid and at least one HDL apolipoprotein or apolipoprotein mimetic.

25 69. The composition of Claim 68,
 wherein the HDL apolipoprotein is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), and apolipoprotein E (apo E),

wherein the phospholipid is selected from the group consisting of
 30 dipalmitoylphosphatidylcholine (DPPC), dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-

maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and combinations thereof.

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70. The composition of Claim 69, wherein the HDL apolipoprotein mimetic is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A-II xxx (apo A-II-xxx), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), apolipoprotein E (apo E), apolipoprotein A-I milano (apo A-I-milano), apolipoprotein A-I paris (apo A-I-paris), apolipoprotein M (apo M), an HDL apolipoprotein mimetic, preproapolipoprotein, preproApoA-I, proApoA I, preproApoA-II, proApoA II, preproApoA-IV, proApoA-IV, ApoA-V, preproApoE, proApoE, preproApoA I_{Milano}, proApoA-I_{Milano}, preproApoA-I_{Paris}, proApoA-I_{Paris}, and mixtures thereof.

71. The composition of Claim 70, wherein the ApoA-I mimetic is described by any of SEQ ID NOs: 1-336 and WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341), LKLLDNWDSVTSTFSKLREOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLROEMS (SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO: 345), PYLDDFQKKWQEEMELYRQKVE (SEQ ID NO: 346), PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDALRTHLA (SEQ ID NO: 348), PYSDELRQRLAARLEALKENGG (SEQ ID NO: 349), ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDYGKDLME (SEQ ID NO:355), KVKSPELOAEAKSYFEKSKE (SEQ ID NO:356), VLTLALVAVAGARAEVSADOVATV (SEQ ID NO:357), NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358), LPVLVWLSIVLEGPAOAGTPDVSS (SEQ ID NO:359), LPVLVVVLSIVLEGPAOAGTPDVSS (SEQ ID NO:360), ALDKLKEFGNTLEDKARELIS (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362), HLRKLRKRLLRDADDLQKRLAVYOA (SEQ ID NO:363), AQAWGERLRARMEEMGSRTRDR (SEQ ID NO:364), LDEVKEQVAEVRACLEEQAQ (SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236),

DWLKAFYDKVAEKLKEAFPDWAKAAYDKAAEKAKEAA (SEQ ID NO:366),
 PVLDLFRELLNELLEALKQKL (SEQ ID NO:367), PVLDLFRELLNELLEALKQKLA (SEQ
 ID NO:368), PVLDLFRELLNELLEALKQKLK (SEQ ID NO:4),
 PVLDLFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDLFRELLNELLEALKKLLK
 5 (SEQ ID NO:370), PVLDLFRELLNELLEALKKLLA (SEQ ID NO:371),
 PLLDLFRELLNELLEALKKLLA (SEQ ID NO:372), and
 EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

72. The composition of Claim 65, wherein the average particle size of the nanoparticle is
 10 between 6 to 500 nm.

73. A composition comprising a PCSK9 siRNA encapsulated within a nanoparticle, wherein
 the PCSK9 siRNA is capable of inhibiting the PCSK9 gene by RNA interference, wherein the
 PCSK9 siRNA comprises two RNA strands that are complementary to each other.
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74. The composition of Claim 73, wherein the nanoparticle is selected from the group
 consisting of sHDL nanoparticles, fullerenes, endohedral metallofullerenes buckyballs,
 trimetallic nitride templated endohedral metallofullerenes, single-walled and mutli-walled
 carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods,
 20 single-walled and multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon
 nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures,
 microstructures, or their derivatives formed using layer-by-layer processes, self-assembly
 processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles,
 nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable
 25 PLGA micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles,
 iron nanoparticles, and modified micelles.

75. The composition of Claim 74, wherein the PCSK9 siRNA is modified with cholesterol at
 the 3' sense strand.
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76. The composition of Claim 74, wherein an imaging agent is encapsulated within the
 nanoparticle.

77. The composition of Claim 74, wherein the nanoparticle is a sHDL nanoparticle, wherein

the sHDL nanoparticle comprises a mixture of at least one phospholipid and at least one HDL apolipoprotein or apolipoprotein mimetic.

78. The composition of Claim 77,

5 wherein the HDL apolipoprotein is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), and apolipoprotein E (apo E),

10 wherein the phospholipid is selected from the group consisting of dipalmitoylphosphatidylcholine (DPPC), dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-
15 phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and combinations thereof.

79. The composition of Claim 78, wherein the HDL apolipoprotein mimetic is selected from
20 the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A-II xxx (apo A-II-xxx), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), apolipoprotein E (apo E), apolipoprotein A-I milano (apo A-I-milano), apolipoprotein A-I paris (apo A-I-paris), apolipoprotein M (apo M), an HDL apolipoprotein mimetic, preproapolipoprotein, preproApoA-I, proApoA I, preproApoA-II, proApoA II, preproApoA-IV, proApoA-IV, ApoA-V, preproApoE, proApoE, preproApoA I_{Milano}, proApoA-I_{Milano},
25 preproApoA-I_{Paris}, proApoA-I_{Paris}, and mixtures thereof.

80. The composition of Claim 79, wherein the ApoA-I mimetic is described by any of SEQ
ID NOs: 1-336 and WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341),
30 LKLLDNWDSVTSTFSKLREOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLROEMS (SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO: 345), PYLDDFQKKWQEEMELYRQKVE (SEQ ID NO: 346), PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDALRTHLA (SEQ ID NO: 348), PYSDELQRRLAARLEALKENGG (SEQ ID NO: 349),

ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351),
 PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ
 ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352),
 TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDYGKDLME (SEQ ID
 5 NO:355), KVKSPELOAEAKSYFEKSKE (SEQ ID NO:356),
 VLTALVAVAGARAEVSADOVATV (SEQ ID NO:357),
 NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358),
 LPVLVWLSIVLEGPAPAOGTPDVSS (SEQ ID NO:359),
 LPVLVVVLSIVLEGPAPAQGTPDVSS (SEQ ID NO:360), ALDKLKEFGNTLEDKARELIS
 10 (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362),
 HLRKLRKRLLRDADDLQKRLAVYOA (SEQ ID NO:363),
 AQAWGERLRARMEEMGSRTRDR (SEQ ID NO:364), LDEVKEQVAEVRACLEEQAQ
 (SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236),
 DWLKAFYDKVAEKLKEAFPDWAKAAYDKAAEKAKEAA (SEQ ID NO:366),
 15 PVLDLFRELLNELLEALKQKL (SEQ ID NO:367), PVLDLFRELLNELLEALKQKLA (SEQ
 ID NO:368), PVLDLFRELLNELLEALKQKLK (SEQ ID NO:4),
 PVLDLFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDLFRELLNELLEALKKLLK
 (SEQ ID NO:370), PVLDLFRELLNELLEALKKLLA (SEQ ID NO:371),
 PLLDLFRELLNELLEALKKLLA (SEQ ID NO:372), and
 20 EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

81. The composition of Claim 74, wherein the average particle size of the nanoparticle is between 6 to 500 nm.

25 82. A composition comprising an α GalCer glycolipid associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) a nanoparticle.

83. The composition of Claim 82, wherein the nanoparticle is selected from the group
 consisting of sHDL nanoparticles, fullerenes, endohedral metallofullerenes buckyballs,
 30 trimetallic nitride templated endohedral metallofullerenes, single-walled and mutli-walled
 carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods,
 single-walled and multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon
 nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures,
 microstructures, or their derivatives formed using layer-by-layer processes, self-assembly

processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles, nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable PLGA micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles, iron nanoparticles, and modified micelles.

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84. The composition of Claim 82, wherein the nanoparticle is a sHDL nanoparticle, wherein the sHDL nanoparticle comprises a mixture of at least one phospholipid and at least one HDL apolipoprotein or apolipoprotein mimetic.

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85. The composition of Claim 84, wherein the HDL apolipoprotein is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), and apolipoprotein E (apo E),

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wherein the phospholipid is selected from the group consisting of dipalmitoylphosphatidylcholine (DPPC), dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and combinations thereof.

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86. The composition of Claim 85, wherein the HDL apolipoprotein mimetic is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A-II xxx (apo A-II-xxx), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), apolipoprotein E (apo E), apolipoprotein A-I milano (apo A-I-milano), apolipoprotein A-I paris (apo A-I-paris), apolipoprotein M (apo M), an HDL apolipoprotein mimetic,

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preproapolipoprotein, preproApoA-I, proApoA I, preproApoA-II, proApoA II, preproApoA-IV, proApoA-IV, ApoA-V, preproApoE, proApoE, preproApoA I_{Milano}, proApoA-I_{Milano}, preproApoA-I_{Paris}, proApoA-I_{Paris}, and mixtures thereof.

87. The composition of Claim 86, wherein the ApoA-I mimetic is described by any of SEQ ID NOs: 1-336 and WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341), LKLLDNWDSVTSTFSKLEOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLEOEMS (SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO: 5 345), PYLDDFQKKWQEEMELYRQKVE (SEQ ID NO: 346), PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDALRTHLA (SEQ ID NO: 348), PYSDELQRRLAARLEALKENGG (SEQ ID NO: 349), ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ 10 ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDYGKDLME (SEQ ID NO:355), KVKSPELOAEAKSYFEKSKE (SEQ ID NO:356), VLTALVAVAGARAEVSADOVATV (SEQ ID NO:357), NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358), 15 LPVLVWLSIVLEGPAPAOGTPDVSS (SEQ ID NO:359), LPVLVVVLSIVLEGPAPAQGTPDVSS (SEQ ID NO:360), ALDKLKEFGNTLEDKARELIS (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362), HLRKLRKRLLRDADDLQKRLAVYOA (SEQ ID NO:363), AQAWGERLRARMEEMGSRTRDR (SEQ ID NO:364), LDEVKEQVAEVRACLEEQAQ 20 (SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236), DWLKAFYDKVAEKLKEAFPDWAKAAYDKAAEKAKEAA (SEQ ID NO:366), PVLDFRELLNELLEALKQKL (SEQ ID NO:367), PVLDFRELLNELLEALKQKLA (SEQ ID NO:368), PVLDFRELLNELLEALKQKLK (SEQ ID NO:4), PVLDFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDFRELLNELLEALKKLLK 25 (SEQ ID NO:370), PVLDFRELLNELLEALKKLLA (SEQ ID NO:371), PLLDFRELLNELLEALKKLLA (SEQ ID NO:372), and EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

88. The composition of Claim 82, wherein the average particle size of the nanoparticle is 30 between 6 to 500 nm.

89. A method of making a personalized neoplasia vaccine for a subject diagnosed as having a neoplasia, comprising:

obtaining a biological sample of the neoplasia from the subject;

identifying a plurality of mutations in the neoplasia;

analyzing the plurality of mutations to identify one or more neo-antigenic mutations predicted to encode neo-antigenic peptides, the neo-antigenic mutations selected from the group consisting of missense mutations, neoORF mutations, and any combination thereof; and

5 producing a personalized neoplasia vaccine, wherein the personalized neoplasia vaccine comprises a microparticle or nanoparticle associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) one or more neo-antigenic peptides and/or mRNA encoding neo-antigens specific for the analyzed and identified neo-antigenic mutations predicted to encode neo-antigenic peptides.

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90. The method of claim 89, wherein identifying further comprises:
sequencing the genome, transcriptome, or proteome of the neoplasia.

91. The method of claim 89, wherein the one or more neo-antigenic peptides range from
15 about 5 to about 50 amino acids in length.

92. The method of claim 91, wherein the one or more neo-antigenic peptides range from about 15 to about 35 amino acids in length.

20 93. The method of claim 92, wherein the one or more neo-antigenic peptides range from about 18 to about 30 amino acids in length.

94. The method of claim 93, wherein the one or more neo-antigenic peptides range from about 6 to about 15 amino acids in length.

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95. The method of claim 89, wherein the nanoparticle is further associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) an adjuvant.

96. The method of claim 95, wherein the adjuvant is selected from the group consisting of
30 CPG, polyIC, poly-ICLC, 1018 ISS, aluminum salts (for example, aluminum hydroxide, aluminum phosphate), Amplivax, BCG, CP-870,893, CpG7909, CyaA, dSLIM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, OK-432, OM-174, OM-197-MP-

EC, ONTAK, PepTel.RTM, vector system, PLGA microparticles, imiquimod, resiquimod, gardiquimod, 3M-052, SRL172, Virosomes and other Virus-like particles, YF-17D, VEGF trap, beta-glucan, Pam3Cys, Aquila's QS21 stimulon, vadimezan, AsA404 (DMXAA), 3M MEDI9197, glucopyranosyl lipid adjuvant (GLA), GLA-SE, CD1d ligands (such as C20:2, 5 OCH, AH04-2, α -galatosylceramide, α -C-galatosylceramide, α -mannosylceramide, α -fructosylceramide, β -galatosylceramide, β -mannosylceramide), STING agonists (e.g. cyclic dinucleotides, including Cyclic [G(3',5')pA(3',5')p], Cyclic [G(2',5')pA(3',5')p], Cyclic [G(2',5')pA(2',5')p], Cyclic diadenylate monophosphate, Cyclic diguanylate monophosphate), CL401, CL413, CL429, Flagellin, RC529, E6020, imidazoquinoline-based small molecule TLR- 10 7/8a (including its lipidated analogues), virosomes, AS01, AS02, AS03, AS04, AS15, IC31, CAF01, ISCOM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), bacterial toxins (such as CT, and LT), any derivative of an adjuvant, and any combination of adjuvant.

97. The method of Claim 89, wherein the microparticle or nanoparticle is selected from the 15 group consisting of sHDL nanoparticles, fullerenes, endohedral metallofullerenes buckyballs, trimetallic nitride templated endohedral metallofullerenes, single-walled and multi-walled carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods, single-walled and multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures, 20 microstructures, or their derivatives formed using layer-by-layer processes, self-assembly processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles, nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable PLGA micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles, iron nanoparticles, and modified micelles.

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98. The method of claim 97, wherein the microparticle or nanoparticle is a sHDL nanoparticle, wherein the sHDL nanoparticle comprises a mixture of at least one phospholipid and at least one HDL apolipoprotein or apolipoprotein mimetic.

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99. The method of claim 98, wherein the HDL apolipoprotein is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), and apolipoprotein E (apo E),

wherein the phospholipid is selected from the group consisting of dipalmitoylphosphatidylcholine (DPPC), dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and combinations thereof.

100. The method of claim 99, wherein the HDL apolipoprotein mimetic is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A-II xxx (apo A-II-xxx), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), apolipoprotein E (apo E), apolipoprotein A-I milano (apo A-I-milano), apolipoprotein A-I paris (apo A-I-paris), apolipoprotein M (apo M), an HDL apolipoprotein mimetic, preproapolipoprotein, preproApoA-I, proApoA I, preproApoA-II, proApoA II, preproApoA-IV, proApoA-IV, ApoA-V, preproApoE, proApoE, preproApoA I_{Milano}, proApoA-I_{Milano}, preproApoA-I_{Paris}, proApoA-I_{Paris}, and mixtures thereof.

101. The method of claim 100, wherein the ApoA-I mimetic is described by any of SEQ ID NOs: 1-336 and WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341), LKLLDNWDSVTSTFSKLEOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLOEMS (SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO: 345), PYLDDFQKKWQEEMELYRQKVE (SEQ ID NO: 346), PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDALRTHLA (SEQ ID NO: 348), PYSDELQRRLAARLEALKENGG (SEQ ID NO: 349), ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDYGKDLME (SEQ ID NO:355), KVKSPELOAEAKSYFEKSKE (SEQ ID NO:356), VLTLALVAVAGARAEVSADOVATV (SEQ ID NO:357), NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358),

LPVLVWLSIVLEGPAPAOGTPDVSS (SEQ ID NO:359),
 LPVLVVVLSIVLEGPAPAQGTPDVSS (SEQ ID NO:360), ALDKLKEFGNTLEDKARELIS
 (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362),
 HLRKLRKRLLRDADDLQKRLAVYOA (SEQ ID NO:363),
 5 AQAWGERLRARMEEMGSRTRDR (SEQ ID NO:364), LDEVKEQVAEVRACLEEQAAQ
 (SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236),
 DWLKAFYDKVAEKLKEAFPDWAKAAYDKAAEKAKEAA (SEQ ID NO:366),
 PVLDFRELLNELLEALKQKL (SEQ ID NO:367), PVLDFRELLNELLEALKQKLA (SEQ
 ID NO:368), PVLDFRELLNELLEALKQKLG (SEQ ID NO:4),
 10 PVLDFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDFRELLNELLEALKKLLK
 (SEQ ID NO:370), PVLDFRELLNELLEALKKLLA (SEQ ID NO:371),
 PLLDFRELLNELLEALKKLLA (SEQ ID NO:372), and
 EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

- 15 102. The method of claim 89,
 wherein the average particle size of the nanoparticle is between 6 to 500 nm,
 wherein the average size of the microparticle is between 0.5 to 100 microns.
- 20 103. A method of treating a subject diagnosed as having a neoplasia with a personalized
 neoplasia vaccine, comprising:
 obtaining a biological sample of the neoplasia from the subject;
 identifying a plurality of mutations in the neoplasia;
 analyzing the plurality of mutations to identify one or more neo-antigenic mutations
 predicted to encode expressed neo-antigenic peptides, the neo-antigenic mutations selected from
 25 the group consisting of missense mutations, neoORF mutations, and any combination thereof;
 producing a personalized neoplasia vaccine, wherein the personalized neoplasia vaccine
 comprises a microparticle or nanoparticle associated with (e.g., complexed, conjugated,
 encapsulated, absorbed, adsorbed, admixed) one or more neo-antigenic peptides specific for the
 analyzed and identified neo-antigenic mutations predicted to encode neo-antigenic peptides; and
 30 administering the personalized neoplasia vaccine to the subject, thereby treating the
 neoplasia.
104. The method of claim 103, wherein identifying further comprises:
 sequencing the genome, transcriptome, or proteome of the neoplasia.

105. The method of claim 103, wherein the one or more neo-antigenic peptides range from about 5 to about 50 amino acids in length.

5 106. The method of claim 105, wherein the one or more neo-antigenic peptides range from about 15 to about 35 amino acids in length.

107. The method of claim 106, wherein the one or more neo-antigenic peptides range from about 18 to about 30 amino acids in length.

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108. The method of claim 107, wherein the one or more neo-antigenic peptides range from about 6 to about 15 amino acids in length.

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109. The method of claim 103, wherein the personalized neoplasia vaccine is coadministered with one or more of an adjuvant, a chemotherapy, an anti-immunosuppressive agent, an immuno stimulatory agent, and composition comprising a nanoparticle associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) an antigen, wherein an adjuvant is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the nanoparticle associated with the antigen.

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110. The method of claim 109, wherein the adjuvant is selected from the group consisting of CPG, polyIC, poly-ICLC, 1018 ISS, aluminum salts (for example, aluminum hydroxide, aluminum phosphate), Amplivax, BCG, CP-870,893, CpG7909, CyaA, dSLIM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, OK-432, OM-174, OM-197-MP-EC, ONTAK, PepTel.RTM, vector system, PLGA microparticles, imiquimod, resiquimod, gardiquimod, 3M-052, SRL172, Virosomes and other Virus-like particles, YF-17D, VEGF trap, beta-glucan, Pam3Cys, Aquila's QS21 stimulon, vadimezan, AsA404 (DMXAA), 3M MEDI9197, glucopyranosyl lipid adjuvant (GLA), GLA-SE, CD1d ligands (such as C20:2, OCH, AH04-2, α -galatosylceramide, α -C-galatosylceramide, α -mannosylceramide, α -fructosylceramide, β -galatosylceramide, β -mannosylceramide), STING agonists (e.g. cyclic dinucleotides, including Cyclic [G(3',5')pA(3',5')p], Cyclic [G(2',5')pA(3',5')p], Cyclic [G(2',5')pA(2',5')p], Cyclic diadenylate monophosphate, Cyclic diguanylate monophosphate),

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CL401, CL413, CL429, Flagellin, RC529, E6020, imidazoquinoline-based small molecule TLR-7/8a (including its lipidated analogues), virosomes, AS01, AS02, AS03, AS04, AS15, IC31, CAF01, ISCOM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), bacterial toxins (such as CT, and LT), any derivative of an adjuvant, and any combination of adjuvant.

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111. The method of claim 103, wherein the microparticle or nanoparticle is selected from the group consisting of sHDL nanoparticles, fullerenes, endohedral metallofullerenes buckyballs, trimetallic nitride templated endohedral metallofullerenes, single-walled and multi-walled carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods, single-walled and multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures, microstructures, or their derivatives formed using layer-by-layer processes, self-assembly processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles, nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable PLGA micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles, iron nanoparticles, and modified micelles.

112. The method of claim 111, wherein the microparticle or nanoparticle is a sHDL nanoparticle, wherein the sHDL nanoparticle comprises a mixture of at least one phospholipid and at least one HDL apolipoprotein or apolipoprotein mimetic.

113. The method of claim 112,
wherein the HDL apolipoprotein is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), and apolipoprotein E (apo E),

wherein the phospholipid is selected from the group consisting of dipalmitoylphosphatidylcholine (DPPC), dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide],

phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and combinations thereof.

114. The method of claim 113, wherein the HDL apolipoprotein mimetic is selected from the
 5 group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein
 A-II xxx (apo A-II-xxx), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs),
 apolipoprotein E (apo E), apolipoprotein A-I milano (apo A-I-milano), apolipoprotein A-I paris
 (apo A-I-paris), apolipoprotein M (apo M), an HDL apolipoprotein mimetic, preproapolipoprotein,
 preproApoA-I, proApoA I, preproApoA-II, proApoA II, preproApoA-IV, proApoA-IV, ApoA-
 10 V, preproApoE, proApoE, preproApoA I_{Milano}, proApoA-I_{Milano}, preproApoA-I_{Paris}, proApoA-
 I_{Paris}, and mixtures thereof.

115. The method of claim 114, wherein the ApoA-I mimetic is described by any of SEQ ID
 NOs: 1-336 and WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341),
 15 LKLLDNWDSVTSTFSKLREOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLROEMS
 (SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO:
 345), PYLDDFQKKWQEEMELYRQKVE (SEQ ID NO: 346),
 PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDALRTHLA
 (SEQ ID NO: 348), PYSDELQRLAARLEALKENGG (SEQ ID NO: 349),
 20 ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351),
 PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ
 ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352),
 TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDYGKDLME (SEQ ID
 NO:355), KVKSPELOAEAKSYFEKSKE (SEQ ID NO:356),
 25 VLTALVAVAGARAEVSADOVATV (SEQ ID NO:357),
 NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358),
 LPVLVWLSIVLEGPAOAGTPDVSS (SEQ ID NO:359),
 LPVLVVVLSIVLEGPAQAQTPDVSS (SEQ ID NO:360), ALDKLKEFGNTLEDKARELIS
 (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362),
 30 HLRKLRKRLLRDADDLQKRLAVYOA (SEQ ID NO:363),
 AQAWGERLRARMEEMGSRTRDR (SEQ ID NO:364), LDEVKEQVAEVRACLEEQAQ
 (SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236),
 DWLKAFYDKVAEKLKEAFPDWAKAAYDKAAEKAKEAA (SEQ ID NO:366),
 PVLDFRELLNELLEALKQKL (SEQ ID NO:367), PVLDFRELLNELLEALKQKLA (SEQ

ID NO:368), PVLDLFRELLNELLEALKQKLIK (SEQ ID NO:4),
 PVLDLFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDLFRELLNELLEALKKLLK
 (SEQ ID NO:370), PVLDLFRELLNELLEALKKLLA (SEQ ID NO:371),
 PLLDLFRELLNELLEALKKLLA (SEQ ID NO:372), and
 5 EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

116. The method of claim 103,
 wherein the average particle size of the nanoparticle is between 6 to 500 nm,
 wherein the average size of the microparticle is between 0.5 to 100 microns.

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117. The method of claim 111, wherein the microparticle or nanoparticle is further associated
 with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) an adjuvant.

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118. The method of claim 117, wherein the adjuvant is selected from the group consisting of
 CPG, polyIC, poly-ICLC, 1018 ISS, aluminum salts (for example, aluminum hydroxide,
 aluminum phosphate), Amplivax, BCG, CP-870,893, CpG7909, CyaA, dSLIM, Cytokines (such
 as GM-CSF, IL-2, IFN- α , Flt-3L), IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS,
 ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312,
 Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, OK-432, OM-174, OM-197-MP-
 20 EC, ONTAK, PepTel.RTM, vector system, PLGA microparticles, imiquimod, resiquimod,
 gardiquimod, 3M-052, SRL172, Virosomes and other Virus-like particles, YF-17D, VEGF trap,
 beta-glucan, Pam3Cys, Aquila's QS21 stimulon, vadimezan, AsA404 (DMXAA),
 3M MEDI9197, glucopyranosyl lipid adjuvant (GLA), GLA-SE, CD1d ligands (such as C20:2,
 OCH, AH04-2, α -galatosylceramide, α -C-galatosylceramide, α -mannosylceramide, α -
 25 fructosylceramide, β -galatosylceramide, β -mannosylceramide), STING agonists (e.g. cyclic
 dinucleotides, including Cyclic [G(3',5')pA(3',5')p], Cyclic [G(2',5')pA(3',5')p], Cyclic
 [G(2',5')pA(2',5')p], Cyclic diadenylate monophosphate, Cyclic diguanylate monophosphate),
 CL401, CL413, CL429, Flagellin, RC529, E6020, imidazoquinoline-based small molecule TLR-
 7/8a (including its lipidated analogues), virosomes, AS01, AS02, AS03, AS04, AS15, IC31,
 30 CAF01, ISCOM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), bacterial toxins (such as
 CT, and LT), any derivative of an adjuvant, and any combination of adjuvant.

119. The method of claim 109,

wherein the immuno stimulatory agent is selected from the group consisting of anti-CTLA-4 antibody, anti-PD-1, anti-PD-L1, anti-TIM-3, anti-BTLA, anti-VISTA, anti-LAG3, anti-CD25, anti-CD27, anti-CD28, anti-CD137, anti-OX40, anti-GITR, anti-ICOS, anti-TIGIT, and inhibitors of IDO;

5 wherein the antigen is selected from the group consisting of alpha-actinin-4, Bcr-Abl fusion protein, Casp-8, beta-catenin, cdc27, cdk4, cdkn2a, coa-1, dek-can fusion protein, EF2, ETV6-AML1 fusion protein, LDLR-fucosyltransferaseAS fusion protein, HLA-A2, HLA-A11, hsp70-2, KIAA0205, Mart2, Mum-1, 2, and 3, neo-PAP, myosin class I, OS-9, pml-RAR α fusion protein, PTPRK, K-ras, N-ras, Triosephosphate isomeras, Bage-1, Gage 3,4,5,6,7, GnTV, 10 Herv-K-mel, Lage-1, Mage-A1,2,3,4,6,10,12, Mage-C2, NA-88, NY-Eso-1/Lage-2, SP17, SSX-2, and TRP2-Int2, MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15(58), CEA, RAGE, NY-ESO (LAGS), SCP-1, Hom/Mel-40, PRAME, p53, H-Ras, HER-2/neu, BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and 15 E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, β -Catenin, CDK4, Mum-1, p16, TAGE, PSMA, PSCA, CT7, telomerase, 43-9F, 5T4, 791Tgp72, α -fetoprotein, 13HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), human EGFR protein or its fragments, 20 such as human EGFR residues 306–325 (SCVRACGADSYEMEEDGVRK (SEQ ID NO:374)) and residues 897–915 (VWSYGVTVWELMTFGSKPY (SEQ ID NO:375)), HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB\70K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein\cyclophilin C-associated protein), TAAL6, TAG72, TLP, TPS, WT1 (and WT1-derivaed peptide sequences: WT1 126–134 (RMFP NAPYL (SEQ ID NO:376)), WT1 122–140 25 (SGQARMFPNAPYLPSICLES (SEQ ID NO:377)), and WT1 122–144 (SGQARMFPNAPYLPSICLESQPTI (SEQ ID NO:378)), MUC1 (and MUC1-derived peptides and glycopeptides such as RPAPGS (SEQ ID NO:379), PPAHGVT (SEQ ID NO:380), and PDTRP (SEQ ID NO:381))), LMP2, EGFRvIII, Idiotype, GD2, Ras mutant, p53 mutant, Proteinase3 (PR1), Survivin, hTERT, Sarcoma translocation breakpoints, EphA2, EphA4, 30 LMW-PTP, PAP, ML-IAP, AFP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, ALK, Androgen receptor, Cyclin B1, Polysialic acid, MYCN, RhoC, TRP-2, GD3, Fucosyl GM1, Mesothelin, sLe(animal), CYP1B1, PLAC1, GM3, BORIS, Tn, GloboH, NY-BR-1, RGS5, SART3, STn, Carbonic anhydrase IX, PAX5, OY-TES1, Sperm protein 17, LCK, HMWMAA, AKAP-4, XAGE 1, B7H3, Legumain, Tie 2, Page4, VEGFR2, MAD-CT-1, FAP, PDGFR-

alpha, PDGFR- β , MAD-CT-2, Fos-related antigen 1, ERBB2, Folate receptor 1 (FOLR1 or FBP), IDH1, IDO, LY6K, fms-related tyrosine kinase 1 (FLT1, best known as VEGFR1), KDR, PADRE, TA-CIN (recombinant HPV16 L2E7E6), SOX2, and aldehyde dehydrogenase.

5 120. A composition comprising a microparticle or nanoparticle associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) one or more neo-antigenic peptides, wherein each of the one or more neo-antigenic peptides is specific for a neo-antigenic mutation identified from a neoplasia biological sample obtained from a subject.

10 121. The composition of claim 120, wherein the one or more neo-antigenic peptides range from about 5 to about 50 amino acids in length.

122. The composition of claim 121, wherein the one or more neo-antigenic peptides range from about 15 to about 35 amino acids in length.

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123. The composition of claim 122, wherein the one or more neo-antigenic peptides range from about 18 to about 30 amino acids in length.

124. The composition of claim 123, wherein the one or more neo-antigenic peptides range
20 from about 6 to about 15 amino acids in length.

125. The composition of claim 120, wherein the microparticle or nanoparticle is further associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) an adjuvant.

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126. The composition of claim 125, wherein the adjuvant is selected from the group consisting of CPG, polyIC, poly-ICLC, 1018 ISS, aluminum salts (for example, aluminum hydroxide, aluminum phosphate), Amplivax, BCG, CP-870,893, CpG7909, CyaA, dSLIM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), IC30, IC31, Imiquimod, ImuFact IMP321, IS
30 Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, OK-432, OM-174, OM-197-MP-EC, ONTAK, PepTel.RTM, vector system, PLGA microparticles, imiquimod, resiquimod, gardiquimod, 3M-052, SRL172, Virosomes and other Virus-like particles, YF-17D, VEGF trap, beta-glucan, Pam3Cys, Aquila's QS21 stimulon, vadimezan, AsA404 (DMXAA),

3M MEDI9197, glucopyranosyl lipid adjuvant (GLA), GLA-SE, CD1d ligands (such as C20:2, OCH, AH04-2, α -galatosylceramide, α -C-galatosylceramide, α -mannosylceramide, α -fructosylceramide, β -galatosylceramide, β -mannosylceramide), STING agonists (e.g. cyclic dinucleotides, including Cyclic [G(3',5')pA(3',5')p], Cyclic [G(2',5')pA(3',5')p], Cyclic [G(2',5')pA(2',5')p], Cyclic diadenylate monophosphate, Cyclic diguanylate monophosphate), CL401, CL413, CL429, Flagellin, RC529, E6020, imidazoquinoline-based small molecule TLR-7/8a (including its lipidated analogues), virosomes, AS01, AS02, AS03, AS04, AS15, IC31, CAF01, ISCOM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), bacterial toxins (such as CT, and LT), and any derivative of an adjuvant.

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127. The composition of claim 120, wherein the microparticle or nanoparticle is selected from the group consisting of sHDL nanoparticles, fullerenes, endohedral metallofullerenes buckyballs, trimetallic nitride templated endohedral metallofullerenes, single-walled and multi-walled carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods, single-walled and multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures, microstructures, or their derivatives formed using layer-by-layer processes, self-assembly processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles, nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable PLGA micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles, iron nanoparticles, and modified micelles.

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128. The composition of claim 127, wherein the microparticle or nanoparticle is a sHDL nanoparticle, wherein the sHDL nanoparticle comprises a mixture of at least one phospholipid and at least one HDL apolipoprotein or apolipoprotein mimetic.

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129. The composition of claim 128,

wherein the HDL apolipoprotein is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), and apolipoprotein E (apo E),

30

wherein the phospholipid is selected from the group consisting of dipalmitoylphosphatidylcholine (DPPC), dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-

maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide],
 5 phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and combinations thereof.

130. The composition of claim 129, wherein the HDL apolipoprotein mimetic is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II),
 10 apolipoprotein A-II xxx (apo A-II-xxx), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), apolipoprotein E (apo E), apolipoprotein A-I milano (apo A-I-milano), apolipoprotein A-I paris (apo A-I-paris), apolipoprotein M (apo M), an HDL apolipoprotein mimetic, preproapolipoprotein, preproApoA-I, proApoA I, preproApoA-II, proApoA II, preproApoA-IV, proApoA-IV, ApoA-V, preproApoE, proApoE, preproApoA_I^{Milano}, proApoA-I_{Milano},
 15 preproApoA-I_{Paris}, proApoA-I_{Paris}, and mixtures thereof.

131. The composition of claim 130, wherein the ApoA-I mimetic is described by any of SEQ ID NOs: 1-336 and WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341), LKLLDNWDSVTSTFSKLREOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLRROEMS
 20 (SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO: 345), PYLDDDFQKKWQEEMELYRQKVE (SEQ ID NO: 346), PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDALRTHLA (SEQ ID NO: 348), PYSDELQRRLAARLEALKENGG (SEQ ID NO: 349), ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351),
 25 PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDYGKDLME (SEQ ID NO:355), KVKSPELOAEAKSYFEKSKE (SEQ ID NO:356), VLTALVAVAGARAEVSADOVATV (SEQ ID NO:357),
 30 NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358), LPVLVWLSIVLEGPAPAOGTPDVSS (SEQ ID NO:359), LPVLVVVLSIVLEGPAPAQGTPDVSS (SEQ ID NO:360), ALDKLKEFGNTLEDKARELIS (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362), HLRKLRKRLLRDADDLQKRLAVYOA (SEQ ID NO:363),

AQAWGERLRARMEEMGSRTRDR (SEQ ID NO:364), LDEVKEQVAEVRACLEEQAQ
 (SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236),
 DWLKAFYDKVAEKLKEAFPDWAKAAYDKAAEKAKEAA (SEQ ID NO:366),
 PVLDLFRELLNELLEALKQKL (SEQ ID NO:367), PVLDLFRELLNELLEALKQKLA (SEQ
 5 ID NO:368), PVLDLFRELLNELLEALKQKLK (SEQ ID NO:4),
 PVLDLFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDLFRELLNELLEALKKLLK
 (SEQ ID NO:370), PVLDLFRELLNELLEALKKLLA (SEQ ID NO:371),
 PLLDLFRELLNELLEALKKLLA (SEQ ID NO:372), and
 EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

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132. The composition of claim 120,
 wherein the average particle size of the nanoparticle is between 6 to 500 nm,
 wherein the average size of the microparticle is between 0.5 to 100 microns.

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133. A method for inducing an immune response to one or more antigens comprising
 administering to a subject in need an effective amount of a composition comprising a
 nanoparticle, wherein the one or more antigens is associated with (e.g., complexed, conjugated,
 encapsulated, absorbed, adsorbed, admixed) the nanoparticle, wherein an adjuvant is associated
 with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the nanoparticle.

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134. The method of Claim 133, wherein the nanoparticle is selected from the group consisting
 of sHDL nanoparticles, fullerenes, endohedral metallofullerenes buckyballs, trimetallic nitride
 templated endohedral metallofullerenes, single-walled and mutli-walled carbon nanotubes,
 branched and dendritic carbon nanotubes, gold nanorods, silver nanorods, single-walled and
 25 multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon nanohorns, carbon
 nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures, microstructures, or
 their derivatives formed using layer-by-layer processes, self-assembly processes, or
 polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles, nanorods,
 cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable PLGA
 30 micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles, iron
 nanoparticles, and modified micelles.

135. The method of Claim 133, wherein the one or more antigens is against PCSK9, M30,
 M27, Adpgk, and ASMTNMELM (SEQ ID NO:383).

136. The method of Claim 133, wherein the adjuvant is selected from the group consisting of CPG, polyIC, poly-ICLC, 1018 ISS, aluminum salts (for example, aluminum hydroxide, aluminum phosphate), Amplivax, BCG, CP-870,893, CpG7909, CyaA, dSLIM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, OK-432, OM-174, OM-197-MP-EC, ONTAK, PepTel.RTM, vector system, PLGA microparticles, imiquimod, resiquimod, gardiquimod, 3M-052, SRL172, Virosomes and other Virus-like particles, YF-17D, VEGF trap, beta-glucan, Pam3Cys, Aquila's QS21 stimulon, vadimezan, AsA404 (DMXAA), 3M MEDI9197, glucopyranosyl lipid adjuvant (GLA), GLA-SE, CD1d ligands (such as C20:2, OCH, AH04-2, α -galatosylceramide, α -C-galatosylceramide, α -mannosylceramide, α -fructosylceramide, β -galatosylceramide, β -mannosylceramide), STING agonists (e.g. cyclic dinucleotides, including Cyclic [G(3',5')pA(3',5')p], Cyclic [G(2',5')pA(3',5')p], Cyclic [G(2',5')pA(2',5')p], Cyclic diadenylate monophosphate, Cyclic diguanylate monophosphate), CL401, CL413, CL429, Flagellin, RC529, E6020, imidazoquinoline-based small molecule TLR-7/8a (including its lipidated analogues), virosomes, AS01, AS02, AS03, AS04, AS15, IC31, CAF01, ISCOM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), bacterial toxins (such as CT, and LT), any derivative of an adjuvant, and any combination of adjuvant.

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137. The method of Claim 133, wherein the one or more antigens are conjugated to the outer surface of the nanoparticle.

138. The method of Claim 133, wherein the adjuvant is conjugated to the outer surface of the nanoparticle.

139. The method of Claim 133, wherein the adjuvant is encapsulated within the nanoparticle.

140. The method of Claim 133, wherein the nanoparticle is a sHDL nanoparticle, wherein the sHDL nanoparticle comprises a mixture of at least one phospholipid and at least one HDL apolipoprotein or apolipoprotein mimetic.

141. The method of Claim 140,

wherein the HDL apolipoprotein is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), and apolipoprotein E (apo E),

wherein the phospholipid is selected from the group consisting of
 5 dipalmitoylphosphatidylcholine (DPPC), dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-
 10 maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and combinations thereof.

15 142. The method of Claim 141,
 wherein the HDL apolipoprotein mimetic is an ApoA-I mimetic,
 wherein the thiol-reactive phospholipid is dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP).

20 143. The method of Claim 142, wherein the ApoA-I mimetic is described by any of SEQ ID NOs: 1-336 and WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341), LKLLDNWDSVTSTFSKLEOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLOEMS (SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO: 345), PYLDDFQKKWQEEMELYRQKVE (SEQ ID NO: 346),
 25 PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDALRTHLA (SEQ ID NO: 348), PYSDELQRRLAARLEALKENGG (SEQ ID NO: 349), ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352),
 30 TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDYGKDLME (SEQ ID NO:355), KVKSPLOAEAKSYFEKSKE (SEQ ID NO:356), VLTALVAVAGARAEVSADOVATV (SEQ ID NO:357), NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358), LPVLVWLSIVLEGPAOAGTPDVSS (SEQ ID NO:359),

LPVLVVVLSIVLEGPAPAQGTPDVSS (SEQ ID NO:360), ALDKLKEFGNTLEDKARELIS
 (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362),
 HLRKLRKRLLRDADDLQKRLAVYOA (SEQ ID NO:363),
 AQAWGERLRARMEEMGSRTRDR (SEQ ID NO:364), LDEVKEQVAEVRACLEEQAQ
 5 (SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236),
 DWLKAFYDKVAEKLKEAFPDWAKAAYDKAAEKAKEAA (SEQ ID NO:366),
 PVLDFRELLNELLEALKQKL (SEQ ID NO:367), PVLDFRELLNELLEALKQKLA (SEQ
 ID NO:368), PVLDFRELLNELLEALKQKLK (SEQ ID NO:4),
 PVLDFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDFRELLNELLEALKKLLK
 10 (SEQ ID NO:370), PVLDFRELLNELLEALKKLLA (SEQ ID NO:371),
 PLLDFRELLNELLEALKKLLA (SEQ ID NO:372), and
 EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

144. The method of Claim 133, wherein the average particle size of the nanoparticle is
 15 between 6 to 500 nm.

145. A composition comprising nanoparticle, wherein one or more antigens is associated with
 (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the nanoparticle,
 wherein an adjuvant is associated with (e.g., complexed, conjugated, encapsulated, absorbed,
 20 adsorbed, admixed) with the nanoparticle.

146. The composition of Claim 145, wherein the nanoparticle is selected from the group
 consisting of sHDL nanoparticles, fullerenes, endohedral metallofullerenes buckyballs,
 trimetallic nitride templated endohedral metallofullerenes, single-walled and mutli-walled
 25 carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods,
 single-walled and multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon
 nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures,
 microstructures, or their derivatives formed using layer-by-layer processes, self-assembly
 processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles,
 30 nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable
 PLGA micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles,
 iron nanoparticles, and modified micelles.

147. The composition of Claim 145, wherein the one or more antigens is against PCSK9, M30, M27, Adpgk, and ASMTNMELM (SEQ ID NO:383).

148. The composition of Claim 145, wherein the adjuvant is selected from the group
 5 consisting of CPG, polyIC, poly-ICLC, 1018 ISS, aluminum salts (for example, aluminum hydroxide, aluminum phosphate), Amplivax, BCG, CP-870,893, CpG7909, CyaA, dSLIM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, OK-432, OM-174,
 10 OM-197-MP-EC, ONTAK, PepTel.RTM, vector system, PLGA microparticles, imiquimod, resiquimod, gardiquimod, 3M-052, SRL172, Virosomes and other Virus-like particles, YF-17D, VEGF trap, beta-glucan, Pam3Cys, Aquila's QS21 stimulon, vadimezan, AsA404 (DMXAA), 3M MEDI9197, glucopyranosyl lipid adjuvant (GLA), GLA-SE, CD1d ligands (such as C20:2, OCH, AH04-2, α -galatosylceramide, α -C-galatosylceramide, α -mannosylceramide, α -
 15 fructosylceramide, β -galatosylceramide, β -mannosylceramide), STING agonists (e.g. cyclic dinucleotides, including Cyclic [G(3',5')pA(3',5')p], Cyclic [G(2',5')pA(3',5')p], Cyclic [G(2',5')pA(2',5')p], Cyclic diadenylate monophosphate, Cyclic diguanylate monophosphate), CL401, CL413, CL429, Flagellin, RC529, E6020, imidazoquinoline-based small molecule TLR-7/8a (including its lipidated analogues), virosomes, AS01, AS02, AS03, AS04, AS15, IC31,
 20 CAF01, ISCOM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), bacterial toxins (such as CT, and LT), any derivative of an adjuvant, and any combination of adjuvant.

149. The composition of Claim 145, wherein the one or more antigens is conjugated to the
 25 outer surface of the nanoparticle or the adjuvant is conjugated to the outer surface of the nanoparticle.

150. The composition of Claim 145, wherein the one or more antigens is derived from a self-antigen.

30 151. The composition of Claim 145, wherein the adjuvant is encapsulated within the nanoparticle.

152. The composition of Claim 145, wherein the nanoparticle is a sHDL nanoparticle, wherein the sHDL nanoparticle comprises a mixture of at least one phospholipid and at least one

HDL apolipoprotein or apolipoprotein mimetic.

153. The composition of Claim 152,

wherein the HDL apolipoprotein is selected from the group consisting of apolipoprotein
 5 A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A4 (apo A4), apolipoprotein Cs
 (apo Cs), and apolipoprotein E (apo E),

wherein the phospholipid is selected from the group consisting of
 dipalmitoylphosphatidylcholine (DPPC), dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-
 pyridyldithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-
 10 di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-
 maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-
 maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-
 maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-
 phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide],
 15 phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and
 combinations thereof.

154. The composition of Claim 153,

wherein the HDL apolipoprotein mimetic is an ApoA-I mimetic,

20 wherein the thiol-reactive phospholipid is dioleoyl-*sn*-glycero-3-phosphoethanolamine-
 N-[3-(2-pyridyldithio) propionate] (DOPE-PDP).

155. The composition of Claim 154, wherein the ApoA-I mimetic is described by any of SEQ
 ID NOs: 1-336 and WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341),

25 LKLLDNWDSVTSTFSKLREOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLR OEMS
 (SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO:
 345), PYLDDFQKKWQEEMELYRQKVE (SEQ ID NO: 346),
 PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDA LRTHLA
 (SEQ ID NO: 348), PYSDEL RQRLAARLEALKENGG (SEQ ID NO: 349),
 30 ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351),
 PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ
 ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352),
 TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDY GKDLME (SEQ ID
 NO:355), KVK SPELOAEAKSYFEKSKE (SEQ ID NO:356),

VLTALVAVAGARAEVSADOVATV (SEQ ID NO:357),
 NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358),
 LPVLVWLSIVLEGPAPAOGTPDVSS (SEQ ID NO:359),
 LPVLVVVLSIVLEGPAPAQOGTPDVSS (SEQ ID NO:360), ALDKLKEFGNTLEDKARELIS
 5 (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362),
 HLRKLRKRLLRDADDLQKRLAVYOA (SEQ ID NO:363),
 AQAWGERLRARMEEMGSRTRDR (SEQ ID NO:364), LDEVKEQVAEVRKLEEQAQ
 (SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236),
 DWLKAFYDKVAEKLKEAFPDWAKAAYDKAAEKAKEAA (SEQ ID NO:366),
 10 PVLDFRELLNELLEALKQKL (SEQ ID NO:367), PVLDFRELLNELLEALKQKLA (SEQ
 ID NO:368), PVLDFRELLNELLEALKQKLG (SEQ ID NO:4),
 PVLDFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDFRELLNELLEALKKLLK
 (SEQ ID NO:370), PVLDFRELLNELLEALKKLLA (SEQ ID NO:371),
 PLLDFRELLNELLEALKKLLA (SEQ ID NO:372), and
 15 EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

156. The composition of Claim 145, wherein the average particle size of the nanoparticle is between 6 to 500 nm.

20 157. The method of Claim 40, wherein the antigen is selected from the group consisting of a peptide based antigen, a protein based antigen, a polysaccharide based antigen, a saccharide based antigen, a lipid based antigen, a glycolipid based antigen, a nucleic acid based antigen, an inactivated organism based antigen, an attenuated organism based antigen, a viral antigen, a bacterial antigen, a parasite antigen, an antigen derived from an allergen, and a tumor antigen.

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158. The method of Claim 40, wherein the antigen is a tumor antigen selected from the group consisting of alpha-actinin-4, Bcr-Abl fusion protein, Casp-8, beta-catenin, cdc27, cdk4, cdkn2a, coa-1, dek-can fusion protein, EF2, ETV6-AML1 fusion protein, LDLR-fucosyltransferaseAS fusion protein, HLA-A2, HLA-A11, hsp70-2, KIAAO205, Mart2, Mum-1, 2, and 3, neo-PAP, 30 myosin class I, OS-9, pml-RAR α fusion protein, PTPRK, K-ras, N-ras, Triosephosphate isomeras, Bage-1, Gage 3,4,5,6,7, GnTV, Herv-K-mel, Lage-1, Mage-A1,2,3,4,6,10,12, Mage-C2, NA-88, NY-Eso-1/Lage-2, SP17, SSX-2, and TRP2-Int2, MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15(58), CEA, RAGE, NY-ESO (LAGS), SCP-1, Hom/Mel-40, PRAME, p53, H-Ras, HER-2/neu, BCR-ABL,

E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, β -Catenin, CDK4, Mum-1, p16, TAGE, PSMA, PSCA, CT7, telomerase, 43-9F, 5 5T4, 791Tgp72, α -fetoprotein, 13HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), human EGFR protein or its fragments, such as human EGFR residues 306–325 (SCVRACGADSYEMEEDGVRK (SEQ ID NO:374)) and residues 897–915 (VWSYGVTWELMTFGSKPY (SEQ ID NO:375)), HTgp-175, M344, MA-50, MG7-Ag, 10 MOV18, NB\70K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein\cyclophilin C-associated protein), TAAL6, TAG72, TLP, TPS, WT1 (and WT1-derived peptide sequences: WT1 126–134 (RMFP NAPYL (SEQ ID NO:376)), WT1 122–140 (SGQARMFPNAPYLPSICLES (SEQ ID NO:377)), and WT1 122–144 (SGQARMFPNAPYLPSICLESQPTI (SEQ ID NO:378))), MUC1 (and MUC1-derived peptides 15 and glycopeptides such as RPAPGS (SEQ ID NO:379), PPAHGVV (SEQ ID NO:380), and PDTRP (SEQ ID NO:381))), LMP2, EGFRvIII, Idiotype, GD2, Ras mutant, p53 mutant, Proteinase3 (PR1), Survivin, hTERT, Sarcoma translocation breakpoints, EphA2, EphA4, LMW-PTP, PAP, ML-IAP, AFP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, ALK, Androgen receptor, Cyclin B1, Polysialic acid, MYCN, RhoC, TRP-2, GD3, Fucosyl GM1, 20 Mesothelin, sLe(animal), CYP1B1, PLAC1, GM3, BORIS, Tn, GloboH, NY-BR-1, RGS5, SART3, STn, Carbonic anhydrase IX, PAX5, OY-TES1, Sperm protein 17, LCK, HMWMAA, AKAP-4, XAGE 1, B7H3, Legumain, Tie 2, Page4, VEGFR2, MAD-CT-1, FAP, PDGFR-alpha, PDGFR- β , MAD-CT-2, Fos-related antigen 1, ERBB2, Folate receptor 1 (FOLR1 or FBP), IDH1, IDO, LY6K, fms-related tyro- sine kinase 1 (FLT1, best known as VEGFR1), 25 KDR, PADRE, TA-CIN (recombinant HPV16 L2E7E6), SOX2, and aldehyde dehydrogenase.

159. The method of Claim 40, wherein the composition is co-administered with a chemotherapeutic agent.

30 160. The method of Claim 159, wherein the chemotherapeutic agent is one or more of the following: aldesleukin, altretamine, amifostine, asparaginase, bleomycin, capecitabine, carboplatin, carmustine, cladribine, cisapride, cisplatin, cyclophosphamide, cytarabine, dacarbazine (DTIC), dactinomycin, docetaxel, doxorubicin, dronabinol, epoetin alpha, etoposide, filgrastim, fludarabine, fluorouracil, gemcitabine, granisetron, hydroxyurea,

idarubicin, ifosfamide, interferon alpha, irinotecan, lansoprazole, levamisole, leucovorin, megestrol, mesna, methotrexate, metoclopramide, mitomycin, mitotane, mitoxantrone, omeprazole, ondansetron, paclitaxel, pilocarpine, prochloroperazine, rituximab, tamoxifen, taxol, topotecan hydrochloride, trastuzumab, vinblastine, vincristine and vinorelbine tartrate.

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161. The composition of Claim 52, wherein the antigen is selected from the group consisting of a peptide based antigen, a protein based antigen, a polysaccharide based antigen, a saccharide based antigen, a lipid based antigen, a glycolipid based antigen, a nucleic acid based antigen, an inactivated organism based antigen, an attenuated organism based antigen, a viral antigen, a bacterial antigen, a parasite antigen, an antigen derived from an allergen, and a tumor antigen.

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162. The composition of Claim 52, wherein the antigen is a tumor antigen selected from the group consisting of alpha-actinin-4, Bcr-Abl fusion protein, Casp-8, beta-catenin, cdc27, cdk4, cdkn2a, coa-1, dek-can fusion protein, EF2, ETV6-AML1 fusion protein, LDLR-fucosyltransferaseAS fusion protein, HLA-A2, HLA-A11, hsp70-2, KIAA0205, Mart2, Mum-1, 2, and 3, neo-PAP, myosin class I, OS-9, pml-RAR α fusion protein, PTPRK, K-ras, N-ras, Triosephosphate isomeras, Bage-1, Gage 3,4,5,6,7, GnTV, Herv-K-mel, Lage-1, Mage-A1,2,3,4,6,10,12, Mage-C2, NA-88, NY-Eso-1/Lage-2, SP17, SSX-2, and TRP2-Int2, MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15(58), CEA, RAGE, NY-ESO (LAGS), SCP-1, Hom/Mel-40, PRAME, p53, H-Ras, HER-2/neu, BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, β -Catenin, CDK4, Mum-1, p16, TAGE, PSMA, PSCA, CT7, telomerase, 43-9F, 5T4, 791Tgp72, α -fetoprotein, 13HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), human EGFR protein or its fragments, such as human EGFR residues 306–325 (SCVRACGADSYEMEEDGVRK (SEQ ID NO:374)) and residues 897–915 (VWSYGVTWELMTFGSKPY (SEQ ID NO:375)), HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB\70K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein\cyclophilin C-associated protein), TAAL6, TAG72, TLP, TPS, WT1 (and WT1-derivaed peptide sequences: WT1 126–134 (RMFP NAPYL (SEQ ID NO:376)), WT1 122–140 (SGQARMFPNAPYLPCLES (SEQ ID NO:377)), and WT1 122–144 (SGQARMFPNAPYLPCLESQPTI (SEQ ID NO:378)), MUC1 (and MUC1-derived peptides

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and glycopeptides such as RPAPGS (SEQ ID NO:379), PPAHGVT (SEQ ID NO:380), and PDTRP (SEQ ID NO:381))), LMP2, EGFRvIII, Idiotype, GD2, Ras mutant, p53 mutant, Proteinase3 (PR1), Survivin, hTERT, Sarcoma translocation breakpoints, EphA2, EphA4, LMW-PTP, PAP, ML-IAP, AFP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, ALK, Androgen receptor, Cyclin B1, Polysialic acid, MYCN, RhoC, TRP-2, GD3, Fucosyl GM1, Mesothelin, sLe(animal), CYP1B1, PLAC1, GM3, BORIS, Tn, GloboH, NY-BR-1, RGS5, SART3, STn, Carbonic anhydrase IX, PAX5, OY-TES1, Sperm protein 17, LCK, HMWMAA, AKAP-4, XAGE 1, B7H3, Legumain, Tie 2, Page4, VEGFR2, MAD-CT-1, FAP, PDGFR-alpha, PDGFR-β, MAD-CT-2, Fos-related antigen 1, ERBB2, Folate receptor 1 (FOLR1 or FBP), IDH1, IDO, LY6K, fms-related tyro- sine kinase 1 (FLT1, best known as VEGFR1), KDR, PADRE, TA-CIN (recombinant HPV16 L2E7E6), SOX2, and aldehyde dehydrogenase.

163. A method for inducing an immune response to an antigen comprising administering to a subject in need an effective amount of a composition comprising a nanoparticle, wherein the nanoparticle is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) with an adjuvant, wherein the antigen is conjugated with a lipid molecule, wherein the antigen conjugated with a lipid molecule is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the nanoparticle.

164. The method of Claim 163, wherein the nanoparticle is selected from the group consisting of sHDL nanoparticles, fullerenes, endohedral metallofullerenes buckyballs, trimetallic nitride templated endohedral metallofullerenes, single-walled and mutli-walled carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods, single-walled and multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures, microstructures, or their derivatives formed using layer-by-layer processes, self-assembly processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles, nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable PLGA micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles, iron nanoparticles, and modified micelles.

165. The method of Claim 163, wherein the antigen is selected from the group consisting of alpha-actinin-4, Bcr-Abl fusion protein, Casp-8, beta-catenin, cdc27, cdk4, cdkn2a, coa-1, dek-

can fusion protein, EF2, ETV6-AML1 fusion protein, LDLR-fucosyltransferaseAS fusion protein, HLA-A2, HLA-A11, hsp70-2, KIAA0205, Mart2, Mum-1, 2, and 3, neo-PAP, myosin class I, OS-9, pml-RAR α fusion protein, PTPRK, K-ras, N-ras, Triosephosphate isomeras, Bage-1, Gage 3,4,5,6,7, GnTV, Herv-K-mel, Lage-1, Mage-A1,2,3,4,6,10,12, Mage-C2, NA-88, NY-5
 Eso-1/Lage-2, SP17, SSX-2, and TRP2-Int2, MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15(58), CEA, RAGE, NY-ESO (LAGS), SCP-1, Hom/Mel-40, PRAME, p53, H-Ras, HER-2/neu, BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, 10
 c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, β -Catenin, CDK4, Mum-1, p16, TAGE, PSMA, PSCA, CT7, telomerase, 43-9F, 5T4, 791Tgp72, α -fetoprotein, 13HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), human EGFR protein or its fragments, such as human EGFR residues 306–325 (SCVRACGADSYEMEEDGVRK (SEQ 15
 ID NO:374)) and residues 897–915 (VWSYGVTVWELMTFGSKPY (SEQ ID NO:375)), HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB\70K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein\cyclophilin C-associated protein), TAAL6, TAG72, TLP, TPS, WT1 (and WT1-derivaed peptide sequences: WT1 126–134 (RMFP NAPYL (SEQ ID NO:376)), WT1 122–140 (SGQARMFPNAPYLPSICLES (SEQ ID NO:377)), and WT1 122–144 20
 (SGQARMFPNAPYLPSICLESQPTI (SEQ ID NO:378)), MUC1 (and MUC1-derived peptides and glycopeptides such as RPAPGS (SEQ ID NO:379), PPAHGVV (SEQ ID NO:380), and PDTRP (SEQ ID NO:381))), LMP2, EGFRvIII, Idiotype, GD2, Ras mutant, p53 mutant, Proteinase3 (PR1), Survivin, hTERT, Sarcoma translocation breakpoints, EphA2, EphA4, LMW-PTP, PAP, ML-IAP, AFP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, ALK, 25
 Androgen receptor, Cyclin B1, Polysialic acid, MYCN, RhoC, TRP-2, GD3, Fucosyl GM1, Mesothelin, sLe(animal), CYP1B1, PLAC1, GM3, BORIS, Tn, GloboH, NY-BR-1, RGS5, SART3, STn, Carbonic anhydrase IX, PAX5, OY-TES1, Sperm protein 17, LCK, HMWMAA, AKAP-4, XAGE 1, B7H3, Legumain, Tie 2, Page4, VEGFR2, MAD-CT-1, FAP, PDGFR-alpha, PDGFR- β , MAD-CT-2, Fos-related antigen 1, ERBB2, Folate receptor 1 (FOLR1 or 30
 FBP), IDH1, IDO, LY6K, fms-related tyro- sine kinase 1 (FLT1, best known as VEGFR1), KDR, PADRE, TA-CIN (recombinant HPV16 L2E7E6), SOX2, aldehyde dehydrogenase, and any derivative thereof.

166. The method of Claim 163, wherein the adjuvant is selected from the group consisting of CPG, polyIC, poly-ICLC, 1018 ISS, aluminum salts (for example, aluminum hydroxide, aluminum phosphate), Amplivax, BCG, CP-870,893, CpG7909, CyaA, dSLIM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, OK-432, OM-174, OM-197-MP-EC, ONTAK, PepTel.RTM, vector system, PLGA microparticles, imiquimod, resiquimod, gardiquimod, 3M-052, SRL172, Virosomes and other Virus-like particles, YF-17D, VEGF trap, beta-glucan, Pam3Cys, Aquila's QS21 stimulon, vadimezan, AsA404 (DMXAA), 3M MEDI9197, glucopyranosyl lipid adjuvant (GLA), GLA-SE, CD1d ligands (such as C20:2, OCH, AH04-2, α -galatosylceramide, α -C-galatosylceramide, α -mannosylceramide, α -fructosylceramide, β -galatosylceramide, β -mannosylceramide), STING agonists (e.g. cyclic dinucleotides, including Cyclic [G(3',5')pA(3',5')p], Cyclic [G(2',5')pA(3',5')p], Cyclic [G(2',5')pA(2',5')p], Cyclic diadenylate monophosphate, Cyclic diguanylate monophosphate), CL401, CL413, CL429, Flagellin, RC529, E6020, imidazoquinoline-based small molecule TLR-7/8a (including its lipidated analogues), virosomes, AS01, AS02, AS03, AS04, AS15, IC31, CAF01, ISCOM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), bacterial toxins (such as CT, and LT), any derivative of an adjuvant, and any combination of adjuvant.

167. The method of Claim 163, wherein the nanoparticle is a sHDL nanoparticle, wherein the sHDL nanoparticle comprises a mixture of at least one phospholipid and at least one HDL apolipoprotein or apolipoprotein mimetic.

168. The method of Claim 167,
 wherein the HDL apolipoprotein is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), and apolipoprotein E (apo E), and any derivative thereof,
 wherein the phospholipid is selected from the group consisting of dipalmitoylphosphatidylcholine (DPPC), dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-

phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and combinations thereof.

- 5 169. The method of Claim 168,
 wherein the HDL apolipoprotein mimetic is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A-II xxx (apo A-II-xxx), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), apolipoprotein E (apo E), apolipoprotein A-I milano (apo A-I-milano), apolipoprotein A-I paris (apo A-I-paris),
 10 apolipoprotein M (apo M), an HDL apolipoprotein mimetic, preproapolipoprotein, preproApoA-I, proApoA I, preproApoA-II, proApoA II, preproApoA-IV, proApoA-IV, ApoA-V, preproApoE, proApoE, preproApoA I_{Milano}, proApoA-I_{Milano}, preproApoA-I_{Paris}, proApoA-I_{Paris}, and mixtures thereof.
- 15 170. The method of Claim 169, wherein the ApoA-I mimetic is described by any of SEQ ID NOs: 1-336 and WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341), LKLLDNWDSVTSTFSKLREOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLROEMS (SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO: 345), PYLDDFQKKWQEEMELYRQKVE (SEQ ID NO: 346),
 20 PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDALRTHLA (SEQ ID NO: 348), PYSDELQRRLAARLEALKENGG (SEQ ID NO: 349), ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352),
 25 TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDYGKDLME (SEQ ID NO:355), KVKSPELOAEAKSYFEKSKE (SEQ ID NO:356), VLTALVAVAGARAEVSADOVATV (SEQ ID NO:357), NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358), LPVLVWLSIVLEGPAPAOGTPDVSS (SEQ ID NO:359),
 30 LPVLVVVLSIVLEGPAPAQGTPDVSS (SEQ ID NO:360), ALDKLKEFGNTLEDKARELIS (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362), HLRKLRKRLLRDADDLQKRLAVYOA (SEQ ID NO:363), AQAWGERLRARMEEMGSRTRDR (SEQ ID NO:364), LDEVKEQVAEVRACLEEQAQ (SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236),

DWLKAFYDKVAEKLKEAFPDWAKAAYDKAAEKAKEAA (SEQ ID NO:366),
 PVLDLFRELLNELLEALKQKL (SEQ ID NO:367), PVLDLFRELLNELLEALKQKLA (SEQ
 ID NO:368), PVLDLFRELLNELLEALKQKLK (SEQ ID NO:4),
 PVLDLFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDLFRELLNELLEALKKLLK
 5 (SEQ ID NO:370), PVLDLFRELLNELLEALKKLLA (SEQ ID NO:371),
 PLLDLFRELLNELLEALKKLLA (SEQ ID NO:372), and
 EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

171. The method of Claim 162, wherein the average particle size of the nanoparticle is
 10 between 6 to 500 nm.

172. A composition comprising a nanoparticle, wherein an antigen conjugated with a lipid
 molecule is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed,
 admixed) with the nanoparticle, wherein an adjuvant is associated with (e.g., complexed,
 15 conjugated, encapsulated, absorbed, adsorbed, admixed) the nanoparticle.

173. The composition of Claim 172, wherein the nanoparticle is selected from the group
 consisting of sHDL nanoparticles, fullerenes, endohedral metallofullerenes buckyballs,
 trimetallic nitride templated endohedral metallofullerenes, single-walled and mutli-walled
 20 carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods,
 single-walled and multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon
 nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures,
 microstructures, or their derivatives formed using layer-by-layer processes, self-assembly
 processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles,
 25 nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable
 PLGA micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles,
 iron nanoparticles, and modified micelles.

174. The composition of Claim 173, wherein the antigen is selected from the group consisting
 30 of alpha-actinin-4, Bcr-Abl fusion protein, Casp-8, beta-catenin, cdc27, cdk4, cdkn2a, coa-1,
 dek-can fusion protein, EF2, ETV6-AML1 fusion protein, LDLR-fucosyltransferaseAS fusion
 protein, HLA-A2, HLA-A11, hsp70-2, KIAAO205, Mart2, Mum-1, 2, and 3, neo-PAP, myosin
 class I, OS-9, pml-RAR α fusion protein, PTPRK, K-ras, N-ras, Triosephosphate isomeras, Bage-
 1, Gage 3,4,5,6,7, GnTV, Herv-K-mel, Lage-1, Mage-A1,2,3,4,6,10,12, Mage-C2, NA-88, NY-

Eso-1/Lage-2, SP17, SSX-2, and TRP2-Int2, MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15(58), CEA, RAGE, NY-ESO (LAGS), SCP-1, Hom/Mel-40, PRAME, p53, H-Ras, HER-2/neu, BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, β -Catenin, CDK4, Mum-1, p16, TAGE, PSMA, PSCA, CT7, telomerase, 43-9F, 5T4, 791Tgp72, α -fetoprotein, 13HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), human EGFR protein or its fragments, such as human EGFR residues 306–325 (SCVRACGADSYEMEEDGVRK (SEQ ID NO:374)) and residues 897–915 (VWSYGVTVWELMTFGSKPY (SEQ ID NO:375)), HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB\70K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein\cyclophilin C-associated protein), TAAL6, TAG72, TLP, TPS, WT1 (and WT1-derivaed peptide sequences: WT1 126–134 (RMFP NAPYL (SEQ ID NO:376)), WT1 122–140 (SGQARMFPNAPYLPSICLES (SEQ ID NO:377)), and WT1 122–144 (SGQARMFPNAPYLPSICLESQPTI (SEQ ID NO:378))), MUC1 (and MUC1-derived peptides and glycopeptides such as RPAPGS (SEQ ID NO:379), PPAHGVT (SEQ ID NO:380), and PDTRP (SEQ ID NO:381))), LMP2, EGFRvIII, Idiotype, GD2, Ras mutant, p53 mutant, Proteinase3 (PR1), Survivin, hTERT, Sarcoma translocation breakpoints, EphA2, EphA4, LMW-PTP, PAP, ML-IAP, AFP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, ALK, Androgen receptor, Cyclin B1, Polysialic acid, MYCN, RhoC, TRP-2, GD3, Fucosyl GM1, Mesothelin, sLe(animal), CYP1B1, PLAC1, GM3, BORIS, Tn, GloboH, NY-BR-1, RGS5, SART3, STn, Carbonic anhydrase IX, PAX5, OY-TES1, Sperm protein 17, LCK, HMWMAA, AKAP-4, XAGE 1, B7H3, Legumain, Tie 2, Page4, VEGFR2, MAD-CT-1, FAP, PDGFR-alpha, PDGFR- β , MAD-CT-2, Fos-related antigen 1, ERBB2, Folate receptor 1 (FOLR1 or FBP), IDH1, IDO, LY6K, fms-related tyro- sine kinase 1 (FLT1, best known as VEGFR1), KDR, PADRE, TA-CIN (recombinant HPV16 L2E7E6), SOX2, aldehyde dehydrogenase, and any derivative thereof.

175. The composition of Claim 172, wherein the adjuvant is selected from the group consisting of CPG, polyIC, poly-ICLC, 1018 ISS, aluminum salts (for example, aluminum hydroxide, aluminum phosphate), Amplivax, BCG, CP-870,893, CpG7909, CyaA, dSLIM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide

IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, OK-432, OM-174, OM-197-MP-EC, ONTAK, PepTel.RTM, vector system, PLGA microparticles, imiquimod, resiquimod, gardiquimod, 3M-052, SRL172, Virosomes and other Virus-like particles, YF-17D, VEGF trap, beta-glucan, Pam3Cys, Aquila's QS21 stimulon, vadimezan, AsA404 (DMXAA),
 5 3M MEDI9197, glucopyranosyl lipid adjuvant (GLA), GLA-SE, CD1d ligands (such as C20:2, OCH, AH04-2, α -galatosylceramide, α -C-galatosylceramide, α -mannosylceramide, α -fructosylceramide, β -galatosylceramide, β -mannosylceramide), STING agonists (e.g. cyclic dinucleotides, including Cyclic [G(3',5')pA(3',5')p], Cyclic [G(2',5')pA(3',5')p], Cyclic [G(2',5')pA(2',5')p], Cyclic diadenylate monophosphate, Cyclic diguanylate monophosphate),
 10 CL401, CL413, CL429, Flagellin, RC529, E6020, imidazoquinoline-based small molecule TLR-7/8a (including its lipidated analogues), virosomes, AS01, AS02, AS03, AS04, AS15, IC31, CAF01, ISCOM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), bacterial toxins (such as CT, and LT), any derivative of an adjuvant, and any combination of adjuvant.

15 176. The composition of Claim 172, wherein the nanoparticle is a sHDL nanoparticle, wherein the sHDL nanoparticle comprises a mixture of at least one phospholipid and at least one HDL apolipoprotein or apolipoprotein mimetic.

177. The composition of Claim 172,
 20 wherein the HDL apolipoprotein is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), and apolipoprotein E (apo E), and any derivative thereof,
 wherein the phospholipid is selected from the group consisting of
 dipalmitoylphosphatidylcholine (DPPC), dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-
 25 pyridyldithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-
 di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-
 maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-
 maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-
 maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-
 30 phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide],
 phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and combinations thereof.

178. The composition of Claim 177,

wherein the HDL apolipoprotein mimetic is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A-II xxx (apo A-II-xxx), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), apolipoprotein E (apo E), apolipoprotein A-I milano (apo A-I-milano), apolipoprotein A-I paris (apo A-I-paris),
 5 apolipoprotein M (apo M), an HDL apolipoprotein mimetic, preproapolipoprotein, preproApoA-I, proApoA I, preproApoA-II, proApoA II, preproApoA-IV, proApoA-IV, ApoA-V, preproApoE, proApoE, preproApoA I_{Milano}, proApoA-I_{Milano}, preproApoA-I_{Paris}, proApoA-I_{Paris}, and mixtures thereof.

- 10 179. The composition of Claim 178, wherein the ApoA-I mimetic is described by any of SEQ ID NOs: 1-336 and WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341), LKLLDNWDSVTSTFSKLREOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLOEMS (SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO: 345), PYLDDFQKKWQEEMELYRQKVE (SEQ ID NO: 346),
 15 PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDALRTHLA (SEQ ID NO: 348), PYSDELQRRLAARLEALKENGG (SEQ ID NO: 349), ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352),
 20 TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDYGKDLME (SEQ ID NO:355), KVKSPELOAEAKSYFEKSKE (SEQ ID NO:356), VLTALVAVAGARAEVSADOVATV (SEQ ID NO:357), NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358), LPVLVWLSIVLEGPAOAGTPDVSS (SEQ ID NO:359),
 25 LPVLVVVLSIVLEGPAOAGTPDVSS (SEQ ID NO:360), ALDKLKEFGNTLEDKARELIS (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362), HLRKLRKRLLRDADDLQKRLAVYOA (SEQ ID NO:363), AQAWGERLRARMEEMGSRTRDR (SEQ ID NO:364), LDEVKEQVAEVRACLEEQAQ (SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236),
 30 DWLKAFYDKVAEKLKEAFPDWAKAAYDKAAEKAKEAA (SEQ ID NO:366), PVLDFRELLNELLEALKQKL (SEQ ID NO:367), PVLDFRELLNELLEALKQKLA (SEQ ID NO:368), PVLDFRELLNELLEALKQKLK (SEQ ID NO:4), PVLDFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDFRELLNELLEALKKLLK (SEQ ID NO:370), PVLDFRELLNELLEALKKLLA (SEQ ID NO:371),

PLLDLFRELLNELLEALKKLLA (SEQ ID NO:372), and
 EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

180. The composition of Claim 172, wherein the average particle size of the nanoparticle is
 5 between 6 to 500 nm.

181. A composition comprising a nanoparticle, wherein a biomacromolecule agent is
 associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the
 nanoparticle.

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182. The composition of Claim 181, wherein the nanoparticle is selected from the group
 consisting of sHDL nanoparticles, fullerenes, endohedral metallofullerenes buckyballs,
 trimetallic nitride templated endohedral metallofullerenes, single-walled and mutli-walled
 carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods,
 15 single-walled and multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon
 nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures,
 microstructures, or their derivatives formed using layer-by-layer processes, self-assembly
 processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles,
 nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable
 20 PLGA micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles,
 iron nanoparticles, and modified micelles.

183. The composition of Claim 181 wherein the biomacromolecule agent is a peptide,
 wherein the peptide is an antigen.

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184. The composition of Claim 183, wherein the antigen is selected from the group
 consisting of alpha-actinin-4, Bcr-Abl fusion protein, Casp-8, beta-catenin, cdc27, cdk4, cdkn2a,
 coa-1, dek-can fusion protein, EF2, ETV6-AML1 fusion protein, LDLR-fucosyltransferaseAS
 fusion protein, HLA-A2, HLA-A11, hsp70-2, KIAAO205, Mart2, Mum-1, 2, and 3, neo-PAP,
 30 myosin class I, OS-9, pml-RAR α fusion protein, PTPRK, K-ras, N-ras, Triosephosphate
 isomeras, Bage-1, Gage 3,4,5,6,7, GnTV, Herv-K-mel, Lage-1, Mage-A1,2,3,4,6,10,12, Mage-
 C2, NA-88, NY-Eso-1/Lage-2, SP17, SSX-2, and TRP2-Int2, MelanA (MART-I), gp100 (Pmel
 17), tyrosinase, TRP-1, TRP-2, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15(58), CEA,
 RAGE, NY-ESO (LAGS), SCP-1, Hom/Mel-40, PRAME, p53, H-Ras, HER-2/neu, BCR-ABL,

E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, β -Catenin, CDK4, Mum-1, p16, TAGE, PSMA, PSCA, CT7, telomerase, 43-9F, 5 5T4, 791Tgp72, α -fetoprotein, 13HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), human EGFR protein or its fragments, such as human EGFR residues 306–325 (SCVRACGADSYEMEEEDGVRK (SEQ ID NO:374)) and residues 897–915 (VWSYGVTWELMTFGSKPY (SEQ ID NO:375)), HTgp-175, M344, MA-50, MG7-Ag, 10 MOV18, NB\70K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein\cyclophilin C-associated protein), TAAL6, TAG72, TLP, TPS, WT1 (and WT1-derived peptide sequences: WT1 126–134 (RMFP NAPYL (SEQ ID NO:376)), WT1 122–140 (SGQARMFPNAPYLPSICLES (SEQ ID NO:377)), and WT1 122–144 (SGQARMFPNAPYLPSICLESQPTI (SEQ ID NO:378))), MUC1 (and MUC1-derived peptides 15 and glycopeptides such as RPAPGS (SEQ ID NO:379), PPAHGVT (SEQ ID NO:380), and PDTRP (SEQ ID NO:381))), LMP2, EGFRvIII, Idiotype, GD2, Ras mutant, p53 mutant, Proteinase3 (PR1), Survivin, hTERT, Sarcoma translocation breakpoints, EphA2, EphA4, LMW-PTP, PAP, ML-IAP, AFP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, ALK, Androgen receptor, Cyclin B1, Polysialic acid, MYCN, RhoC, TRP-2, GD3, Fucosyl GM1, 20 Mesothelin, sLe(animal), CYP1B1, PLAC1, GM3, BORIS, Tn, GloboH, NY-BR-1, RGS5, SART3, STn, Carbonic anhydrase IX, PAX5, OY-TES1, Sperm protein 17, LCK, HMWMAA, AKAP-4, XAGE 1, B7H3, Legumain, Tie 2, Page4, VEGFR2, MAD-CT-1, FAP, PDGFR-alpha, PDGFR- β , MAD-CT-2, Fos-related antigen 1, ERBB2, Folate receptor 1 (FOLR1 or FBP), IDH1, IDO, LY6K, fms-related tyro- sine kinase 1 (FLT1, best known as VEGFR1), 25 KDR, PADRE, TA-CIN (recombinant HPV16 L2E7E6), SOX2, and aldehyde dehydrogenase.

185. The composition of Claim 183, wherein the composition further comprises an adjuvant is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) with the nanoparticle.

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186. The composition of Claim 185, wherein the adjuvant is selected from the group consisting of CPG, polyIC, poly-ICLC, 1018 ISS, aluminum salts (for example, aluminum hydroxide, aluminum phosphate), Amplivax, BCG, CP-870,893, CpG7909, CyaA, dSLIM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), IC30, IC31, Imiquimod, ImuFact IMP321, IS

Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, OK-432, OM-174, OM-197-MP-EC, ONTAK, PepTel.RTM, vector system, PLGA microparticles, imiquimod, resiquimod, gardiquimod, 3M-052, SRL172, Virosomes and other Virus-like particles, YF-17D, VEGF trap, beta-glucan, Pam3Cys, Aquila's QS21 stimulon, vadimezan, AsA404 (DMXAA), 3M MEDI9197, glucopyranosyl lipid adjuvant (GLA), GLA-SE, CD1d ligands (such as C20:2, OCH, AH04-2, α -galatosylceramide, α -C-galatosylceramide, α -mannosylceramide, α -fructosylceramide, β -galatosylceramide, β -mannosylceramide), STING agonists (e.g. cyclic dinucleotides, including Cyclic [G(3',5')pA(3',5')p], Cyclic [G(2',5')pA(3',5')p], Cyclic [G(2',5')pA(2',5')p], Cyclic diadenylate monophosphate, Cyclic diguanylate monophosphate), CL401, CL413, CL429, Flagellin, RC529, E6020, imidazoquinoline-based small molecule TLR-7/8a (including its lipidated analogues), virosomes, AS01, AS02, AS03, AS04, AS15, IC31, CAF01, ISCOM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), bacterial toxins (such as CT, and LT), any derivative of an adjuvant, and any combination of adjuvant.

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187. The composition of Claim 185, wherein the antigen is conjugated to the outer surface of the nanoparticle or the adjuvant is conjugated to the outer surface of the nanoparticle.

188. The composition of Claim 183, wherein the antigen is derived from a self-antigen.

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189. The composition of Claim 185, wherein the adjuvant is encapsulated within the sHDL nanoparticle.

190. The composition of Claim 181, wherein the nanoparticle is a sHDL nanoparticle, wherein the sHDL nanoparticle comprises a mixture of at least one phospholipid and at least one HDL apolipoprotein or apolipoprotein mimetic.

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191. The composition of Claim 190,

wherein the HDL apolipoprotein is selected from the group consisting of apolipoprotein

A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), and apolipoprotein E (apo E),

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wherein the phospholipid is selected from the group consisting of dipalmitoylphosphatidylcholine (DPPC), dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-

di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-
maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-
maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-
maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-
5 phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide],
phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and
combinations thereof.

192. The composition of Claim 191,
10 wherein the HDL apolipoprotein mimetic is an ApoA-I mimetic,
wherein the thiol-reactive phospholipid is dioleoyl-*sn*-glycero-3-phosphoethanolamine-
N-[3-(2-pyridyldithio) propionate] (DOPE-PDP).

193. The composition of Claim 192, wherein the ApoA-I mimetic is described by any of SEQ
15 ID NOs: 1-336 and WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341),
LKLLDNWDSVTSTFSKLEOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLOEMS
(SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO:
345), PYLDDFQKKWQEEMELYRQKVE (SEQ ID NO: 346),
PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDALRTHLA
20 (SEQ ID NO: 348), PYSDELQRRLAARLEALKENGG (SEQ ID NO: 349),
ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351),
PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ
ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352),
TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDYGKDLME (SEQ ID
25 NO:355), KVKSPLOAEAKSYFEKSKE (SEQ ID NO:356),
VLTALVAVAGARAEVSADOVATV (SEQ ID NO:357),
NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358),
LPVLVWLSIVLEGPAPAOGTPDVSS (SEQ ID NO:359),
LPVLVVVLSIVLEGPAPAQGTPDVSS (SEQ ID NO:360), ALDKLKEFGNTLEDKARELIS
30 (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362),
HLRKLRLRLLRDADDLQKRLAVYOA (SEQ ID NO:363),
AQAWGERLRARMEEMGSRTDR (SEQ ID NO:364), LDEVKEQVAEVRKLEEQAQ
(SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236),
DWLKAFYDKVAEKLKEAFPDWAKAAYDKAAEKAKEAA (SEQ ID NO:366),

PVLDFRELLNELLEALKQKL (SEQ ID NO:367), PVLDFRELLNELLEALKQKLA (SEQ ID NO:368), PVLDFRELLNELLEALKQKLK (SEQ ID NO:4), PVLDFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDFRELLNELLEALKKLLK (SEQ ID NO:370), PVLDFRELLNELLEALKKLLA (SEQ ID NO:371),
 5 PLLDFRELLNELLEALKKLLA (SEQ ID NO:372), and EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

194. The composition of Claim 181, wherein the average particle size of the nanoparticle is between 6 to 500 nm.

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195. The composition of Claim 181, wherein the biomacromolecule agent is a peptide selected from the group consisting of an Adrenocorticotrophic Hormone (ACTH), a growth hormone peptide, a Melanocyte Stimulating Hormone (MSH), Oxytocin, Vasopressin, Corticotropin Releasing Factor (CRF), a CRF-related peptide, a Gonadotropin Releasing
 15 Hormone Associated Peptide (GAP), Growth Hormone Releasing Factor (GRF), Lutenizing Hormone Release Hormone (LH-RH), an orexin, a Prolactin Releasing Peptide (PRP), a somatostatin, Thyrotropin Releasing Hormone (THR), a THR analog, Calcitonin (CT), a CT-precursor peptide, a Calcitonin Gene Related Peptide (CGRP), a Parathyroid Hormone (PTH), a Parathyroid Hormone Related Protein (PTHrP), Amylin, Glucagon, Insulin, an Insulin-
 20 like peptide, NeuroPeptide Y (NPY), a Pancreatic Polypeptide (PP), Peptide YY (PYY), Cholecystokinin (CCK), a CCK-related peptide, Gastrin Releasing Peptide (GRP), Gastrin, a Gastrin-related peptide, a Gastrin inhibitory peptide, Motilin, Secretin, Vasoactive Intestinal Peptide (VIP), a VIP-related peptide, an Atrial-Natriuretic Peptide (ANP), a Brain Natriuretic Peptide (BNP), a C-Type Natriuretic Peptide(CNP), a tachykinin, an angiotensin, a
 25 renin substrate, a renin inhibitor, an endothelin, an endothelin-related peptide, an opioid peptide, a thymic peptide, an adrenomedullin peptide, an allostatin peptide, an amyloid beta-protein fragment, an antimicrobial peptide, an antioxidant peptide, an apoptosis related peptide, a Bag Cell Peptide (BCPs), Bombesin, a bone Gla protein peptide, a Cocaine and Amphetamine Related Transcript (CART) peptide, a cell adhesion peptide, a chemotactic peptide, a
 30 complement inhibitor, a cortistatin peptide, a fibronectin fragment, a fibrin related peptide, FMRF, a FMRF amide-related peptide (FaRP), Galanin, a Galanin-related peptide, a growth factor, a growth factor-related peptide, a G-Therapeutic Peptide-Binding Protein fragment, Gualylin, Uroguanylin, an Inhibin peptide, Interleukin (IL), an Interleukin Receptor protein, a laminin fragment, a leptin fragment peptide, a leucokinin, Pituitary Adenylate Cyclase

- Activating Polypeptide (PAPCAP), Pancreastatin, a polypeptide repetitive chain, a signal transducing reagent, a thrombin inhibitor, a toxin, a trypsin inhibitor, a virus-related peptide, an adjuvant peptide analog, Alpha Mating Factor, Antiarrhythmic Peptide, Anorexigenic Peptide, Alpha-1 Antitrypsin, Bovine Pineal Antireproductive Peptide, Bursin, C3 Peptide P16,
- 5 Cadherin Peptide, Chromogranin A Fragment, Contraceptive Tetrapeptide, Conantokin G, Conantokin T, Crustacean Cardioactive Peptide, C-Telopeptide, Cytochrome b588 Peptide, Decorsin, Delicious Peptide, Delta-Sleep-Inducing Peptide, Diazepam-Binding Inhibitor Fragment, Nitric Oxide Synthase Blocking Peptide, OVA Peptide, Platelet Calpain Inhibitor (P1), Plasminogen Activator Inhibitor 1, Rigin, Schizophrenia Related Peptide, Sodium
- 10 Potassium Atherapeutic Peptidase Inhibitor-1, Speract, Sperm Activating Peptide, Systemin, a Thrombin receptor agonist, Tuftsin, Adipokinetic Hormone, Uremic Pentapeptide, Antifreeze Polypeptide, Tumor Necrosis Factor (TNF), Leech [Des Asp10]Decorsin, L-Ornithyltaurine Hydrochloride, P-Aminophenylacetyl Tuftsin, Ac-Glu-Glu-Val-Val-Ala-Cys-pNA, Ac-Ser-Asp-Lys-Pro, Ac-rfwink-NH₂, Cys-Gly-Tyr-Gly-Pro-Lys-Lys-Lys-Arg-Lys-Val-Gly-Gly, D-Ala-
- 15 Leu, D-D-D-D-D, D-D-D-D-D-D, N-P-N-A-N-P-N-A, V-A-I-T-V-L-V-K, V-G-V-R-V-R, V-I-H-S, V-P-D-P-R, Val-Thr-Cys-Gly, R-S-R, Sea Urchin Sperm Activating Peptide, a SHU-9119 antagonist, a MC3-R antagonist, a MC4-R antagonist, Glaspimod, HP-228, Alpha 2-Plasmin Inhibitor, APC Tumor Suppressor, Early Pregnancy Factor, Gamma Interferon, Glandular Kallikrein N-1, Placental Ribonuclease Inhibitor, Sarcolecine Binding Protein, Surfactant Protein
- 20 D, Wilms' Tumor Suppressor, GABAB 1b Receptor Peptide, Prion Related Peptide (iPRP13), Choline Binding Protein Fragment, Telomerase Inhibitor, Cardiostatin Peptide, Endostatin Derived Peptide, Prion Inhibiting Peptide, N-Methyl D-Aspartate Receptor Antagonist, and C-Peptide Analog.
- 25 196. The composition of Claim 181, wherein the biomacromolecule agent is a peptide selected from the group consisting of 177Lu-DOTA0-Tyr3-Octreotate, Abarelix acetate, ADH-1, Afamelanotide, melanotan-1, CUV1647, Albiglutide, Aprotinin, Argipressin, Atosiban acetate, Bacitracin, Bentiromide, a BH3 domain, Bivalirudin, Bivalirudin trifluoroacetate hydrate, Blisibimod, Bortezomib, Buserelin, Buserelin acetate, Calcitonin, Carbetocin,
- 30 Carbetocin acetate, Cecropin A and B, Ceruletide, Ceruletide diethylamine, Cetrorelix, Cetrorelix acetate, Ciclosporine, Cilengitide, EMD121974, Corticorelin acetate injection, hCRF, Corticorelin ovine trifluoroacetate, corticorelin trifluoroacetate, Corticotropin, Cosyntropin, ACTH 1-24, tetracosactide hexaacetate, Dalbavancin, Daptomycin, Degarelix acetate, Depreotide trifluoroacetate (plus sodium pertechnetate), Desmopressin acetate, Desmopressin

DDAVP, Dulaglutide, Ecallantide, Edotreotide (plus yttrium-90), Elcatonin acetate, Enalapril maleate (or 2-butanedioate), Enfuvirtide, Eptifibatide, Exenatide, Ganirelix acetate, Glatiramer acetate, Glutathion, Gonadorelin, Gonadorelin acetate, GnRH, LHRH, Goserelin, Goserelin acetate, Gramicidin, Histrelin acetate, Human calcitonin, Icatibant, Icatibant acetate, IM862, 5 oglufanide disodium, KLAKLAK, Lanreotide acetate, Lepirudin, Leuprolide, Leuprolide acetate, leuprorelin, Liraglutide, Lisinopril, Lixisenatide, Lypressin, Magainin2, MALP-2Sc, macrophage-activating lipopeptide-2 synthetic, Nafarelin acetate, Nesiritide, NGR-hTNF, Octreotide acetate, Oritavancin, Oxytocin, Pasireotide, Peginesatide, Pentagastrin, Pentetreotide (plus indium-111), Phenypressin, Pleurocidin, Pramlintide, Protirelin, thyroliberin, TRH, TRF, 10 Salmon calcitonin, Saralasin acetate, Secretin (human), Secretin (porcine), Semaglutide, Seractide acetate, ACTH, corticotropin, Sermorelin acetate, GRF 1-29, Sinapultide, KL4 in lucinactant, Sincalide, Somatostatin acetate, GHRH, GHRF, GRF, Somatostatin acetate, Spaglumet magnesium (or sodium) salt, Substance P, Taltirelin hydrate, Teduglutide, Teicoplanin, Telavancin, Teriparatide, Terlipressin acetate, Tetracosactide, Thymalfasin, 15 thymosin a-1, Thymopentin, Trebananib, Triptorelin, Triptorelin pamoate, Tyroserleutide, Ularitide, Vancomycin, Vapreotide acetate, Vasoactive intestinal peptide acetate, Vx-001c, TERT572Y, Ziconotide acetate, α 5- α 6 Bax peptide, and β -defensin.

197. The composition of Claim 181, wherein the biomacromolecule agent is a nucleic acid 20 molecule.

198. The composition of Claim 197, wherein the nucleic acid molecule is selected from RNA, siRNA, microRNA, interference RNA, mRNA, replicon mRNA, RNA-analogues, and DNA.

25 199. The composition of Claim 198, wherein the composition is used as a vaccine against a pathogen.

200. The composition of Claim 199, wherein the pathogen is selected from erythropoietic porphyries, T2 diabetes, antifibrinolytic, central diabetes insipidus, delaying the birth in case of 30 threat of premature birth, antibiotic, cystic fibrosis, angina, anticoagulant in patients with unstable angina undergoing PTCA or PCI, systemic lupus erythematosus, hypercalcemia, osteoporosis, pagets disease, carbetocin works as an oxytocic, antihemorrhagic and uterotonic drug in the peripheral nervous system, prevention of uterine atony, induction, and control postpartum bleeding or haemorrhage, stimulant of the gastric secretion, for treat hormone-

sensitive cancers of the prostate and breast, inhibition of premature LH surges in women undergoing controlled ovarian stimulation, immunosuppression in organ transplantation to prevent rejection, peritumoral brain edema, diagnosis of ACTHdependent Cushing's syndrome, allergies, ankylosing spondylitis, psoriasis, chorioiditis, erythema, keratitis, sclerosis,

5 dermatomyositis, rheumatoid arthritis, Stevens-Johnson Syndrome, ulcerative colitis, diagnosis of adrenocortical insufficiency, antibiotic, systemic infections caused by gram positive organisms, nocturnal enuresis, nocturia, and stoppage of bleeding or haemorrhage in haemophilia A patients, acute hereditary angioderma, postmenopausal osteoporosis, anti-parathyroid, Paget's disease, hypercalcaemia, hypertension, AIDS/HIV-1 infection, acute

10 coronary syndrome, unstable angina undergoing PCI, Alzheimer's and Parkinson's disease, inhibition of premature LH surges in women undergoing controlled ovarian hyperstimulation, Relapsing- Remitting Multiple Sclerosis, hepatic insufficiency, wound healing, inflammation of respiratory tract, asthenia, release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary, stimulate the secretion of gonadotropin during

15 disturbances fertility, and diagnosis of the functional capacity and response of the gonadotropes of the anterior pituitary, for skin lesions, surface wounds and eye infections, postmenopausal osteoporosis, Paget's disease, hypercalcaemia, hereditary angioedema, immune system related diseases, acromegaly, anticoagulant, fibroids and endometriosis, central diabetes insipidus, Cushing's syndrome, diabetic foot ulcers, treatment of central precocious puberty, uterine

20 fibroids and endometriosis, vasodilatory, natriuretic, diuretic and neurohormonal effects, acromegaly, carcinoid syndrome, acute bacterial skin and skin structure infections, initiation or improvement of uterine contractions, and control postpartum bleeding or haemorrhage, hematide Chronic kidney disease associated anemia, stomatitis, pharyngitis, diagnostic assessment of thyroid function, postmenopausal osteoporosis, hypercalcaemia, diagnosis of pancreatic

25 exocrine dysfunction, and gastrinoma, Zollinger-Ellison syndrome, prevention of RDS in premature infants, and meconium aspiration syndrome, acute variceal bleeding, allergic rhinitis and conjunctivitis, spinocerebellar degeneration/ataxia, Short Bowel Syndrome, antibiotic, bactericidal, teriparatide is the only anabolic (i.e., bone growing) agent indicated for use in postmenopausal women with osteoporosis, Cortrosyn Analogue of adrenocorticotrophic

30 hormone (ACTH) used for diagnostic purposes, treatment of adrenal insufficiency, epilepsy, Chronic hepatitis B, chronic hepatitis C, primary and secondary immune deficiencies, acute decompensated heart failure, colitis, esophageal variceal bleeding in patients with cirrhotic liver disease and AIDS-related diarrhea, sarcoidosis and acute lung injury, and severe chronic pain.

201. The composition of Claim 198, wherein the composition is used within methods for cancer immunotherapy.

202. A method of delivering a biomacromolecule agent to a subject, comprising administering
5 to the subject a composition as described in Claim 181.

203. A method of treating a condition, disorder and/or disease in a subject, comprising administering a composition as described in Claim 181 to the subject.

10 204. The method of Claim 203, wherein the disorder and/or disease is peripheral ischemia, cancer, an inflammatory disorder, and/or a genetic disorder.

205. The method of Claim 203, wherein the biomacromolecule is a peptide, wherein the peptide and condition, disorder and/or disease is selected from the group consisting of each row
15 in Table 1.

206. The method of Claim 203, wherein the biomacromolecule is a nucleic acid.

207. The method of Claim 206, wherein the nucleic acid molecule is selected from RNA,
20 siRNA, microRNA, interference RNA, mRNA, replicon mRNA, RNA-analogues, and DNA.

208. The method of Claim 207, wherein the condition, disorder and/or disease is selected from erythropoietic porphyries, T2 diabetes, antifibrinolytic, central diabetes insipidus, delaying the birth in case of threat of premature birth, antibiotic, cystic fibrosis, angina, anticoagulant in
25 patients with unstable angina undergoing PTCA or PCI, systemic lupus erythematosus, hypercalcemia, osteoporosis, pagets disease, carbetocin works as an oxytocic, antihemorrhagic and uterotonic drug in the peripheral nervous system, prevention of uterine atony, induction, and control postpartum bleeding or haemorrhage, stimulant of the gastric secretion, for treat hormone-sensitive cancers of the prostate and breast, inhibition of premature LH surges in
30 women undergoing controlled ovarian stimulation, immunosuppression in organ transplantation to prevent rejection, peritumoral brain edema, diagnosis of ACTHdependent Cushing's syndrome, allergies, ankylosing spondylitis, psoriasis, chorioiditis, erythema, keratitis, sclerosis, dermatomyositis, rheumatoid arthritis, Stevens-Johnson Syndrome, ulcerative colitis, diagnosis of adrenocortical insufficiency, antibiotic, systemic infections caused by gram positive

organisms, nocturnal enuresis, nocturia, and stoppage of bleeding or haemorrhage in haemophilia A patients, acute hereditary angioderma, postmenopausal osteoporosis, anti-parathyroid, Paget's disease, hypercalcaemia, hypertension, AIDS/HIV-1 infection, acute coronary syndrome, unstable angina undergoing PCI, Alzheimer's and Parkinson's disease, 5 inhibition of premature LH surges in women undergoing controlled ovarian hyperstimulation, Relapsing- Remitting Multiple Sclerosis, hepatic insufficiency, wound healing, inflammation of respiratory tract, asthenia, release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary, stimulate the secretion of gonadotropin during disturbances fertility, and diagnosis of the functional capacity and response of the gonadotropes 10 of the anterior pituitary, for skin lesions, surface wounds and eye infections, postmenopausal osteoporosis, Paget's disease, hypercalcaemia, hereditary angioedema, immune system related diseases, acromegaly, anticoagulant, fibroids and endometriosis, central diabetes insipidus, Cushing's syndrome, diabetic foot ulcers, treatment of central precocious puberty, uterine fibroids and endometriosis, vasodilatory, natriuretic, diuretic and neurohormonal effects, 15 acromegaly, carcinoid syndrome, acute bacterial skin and skin structure infections, initiation or improvement of uterine contractions, and control postpartum bleeding or haemorrhage, hematide Chronic kidney disease associated anemia, stomatitis, pharyngitis, diagnostic assessment of thyroid function, postmenopausal osteoporosis, hypercalcaemia, diagnosis of pancreatic exocrine dysfunction, and gastrinoma, Zollinger-Ellison syndrome, prevention of RDS in 20 premature infants, and meconium aspiration syndrome, acute variceal bleeding, allergic rhinitis and conjunctivitis, spinocerebellar degeneration/ataxia, Short Bowel Syndrome, antibiotic, bactericidal, teriparatide is the only anabolic (i.e., bone growing) agent indicated for use in postmenopausal women with osteoporosis, Cortrosyn Analogue of adrenocorticotrophic hormone (ACTH) used for diagnostic purposes, treatment of adrenal insufficiency, epilepsy, 25 Chronic hepatitis B, chronic hepatitis C, primary and secondary immune deficiencies, acute decompensated heart failure, colitis, esophageal variceal bleeding in patients with cirrhotic liver disease and AIDS-related diarrhea, sarcoidosis and acute lung injury, and severe chronic pain.

209. The method of Claim 203, wherein the condition, disorder and/or disease is selected 30 from erythropoietic porphyries, T2 diabetes, antifibrinolytic, central diabetes insipidus, delaying the birth in case of threat of premature birth, antibiotic, cystic fibrosis, angina, anticoagulant in patients with unstable angina undergoing PTCA or PCI, systemic lupus erythematosus, hypercalcemia, osteoporosis, pagets disease, carbetocin works as an oxytocic, antihemorrhagic and uterotonic drug in the peripheral nervous system, prevention of uterine atony, induction, and

control postpartum bleeding or haemorrhage, stimulant of the gastric secretion, for treat hormone-sensitive cancers of the prostate and breast, inhibition of premature LH surges in women undergoing controlled ovarian stimulation, immunosuppression in organ transplantation to prevent rejection, peritumoral brain edema, diagnosis of ACTHdependent Cushing's syndrome, allergies, ankylosing spondylitis, psoriasis, chorioiditis, erythema, keratitis, sclerosis, dermatomyositis, rheumatoid arthritis, Stevens-Johnson Syndrome, ulcerative colitis, diagnosis of adrenocortical insufficiency, antibiotic, systemic infections caused by gram positive organisms, nocturnal enuresis, nocturia, and stoppage of bleeding or haemorrhage in haemophilia A patients, acute hereditary angioderma, postmenopausal osteoporosis, anti-parathyroid, Paget's disease, hypercalcaemia, hypertension, AIDS/HIV-1 infection, acute coronary syndrome, unstable angina undergoing PCI, Alzheimer's and Parkinson's disease, inhibition of premature LH surges in women undergoing controlled ovarian hyperstimulation, Relapsing- Remitting Multiple Sclerosis, hepatic insufficiency, wound healing, inflammation of respiratory tract, asthenia, release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary, stimulate the secretion of gonadotropin during disturbances fertility, and diagnosis of the functional capacity and response of the gonadotropes of the anterior pituitary, for skin lesions, surface wounds and eye infections, postmenopausal osteoporosis, Paget's disease, hypercalcaemia, hereditary angioedema, immune system related diseases, acromegaly, anticoagulant, fibroids and endometriosis, central diabetes insipidus, Cushing's syndrome, diabetic foot ulcers, treatment of central precocious puberty, uterine fibroids and endometriosis, vasodilatory, natriuretic, diuretic and neurohormonal effects, acromegaly, carcinoid syndrome, acute bacterial skin and skin structure infections, initiation or improvement of uterine contractions, and control postpartum bleeding or haemorrhage, hematide Chronic kidney disease associated anemia, stomatitis, pharyngitis, diagnostic assessment of thyroid function, postmenopausal osteoporosis, hypercalcaemia, diagnosis of pancreatic exocrine dysfunction, and gastrinoma, Zollinger-Ellison syndrome, prevention of RDS in premature infants, and meconium aspiration syndrome, acute variceal bleeding, allergic rhinitis and conjunctivitis, spinocerebellar degeneration/ataxia, Short Bowel Syndrome, antibiotic, bactericidal, teriparatide is the only anabolic (i.e., bone growing) agent indicated for use in postmenopausal women with osteoporosis, Cortrosyn Analogue of adrenocorticotrophic hormone (ACTH) used for diagnostic purposes, treatment of adrenal insufficiency, epilepsy, Chronic hepatitis B, chronic hepatitis C, primary and secondary immune deficiencies, acute decompensated heart failure, colitis, esophageal variceal bleeding in patients with cirrhotic liver disease and AIDS-related diarrhea, sarcoidosis and acute lung injury, and severe chronic pain.

210. A composition comprising a nanoparticle, wherein an antigen is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the nanoparticle, wherein an adjuvant is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the nanoparticle, wherein the antigen is associated with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) a hydrophobic molecule and/or wherein the adjuvant is conjugated with a hydrophobic molecule.

211. The composition of Claim 210, wherein the hydrophobic molecule is a lipid molecule.

212. The composition of Claim 211, wherein the lipid molecule is a membrane-forming lipid molecule.

213. The composition of Claim 211, wherein the lipid molecule is a non-membrane-forming lipid molecule.

214. The composition of Claim 211, wherein the lipid molecule is selected from the group consisting of phospholipids such as lecithin, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, egg sphingomyelin (ESM), cephalin, cardiolipin, phosphatidic acid, cerebrosides, dicetylphosphate, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoylphosphatidylethanolamine (DOPE), palmitoyloleoyl-phosphatidylcholine (POPC), palmitoyloleoyl-phosphatidylethanolamine (POPE), palmitoyloleoyl-phosphatidylglycerol (POPG), dioleoylphosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl-phosphatidylethanolamine (DPPE), dimyristoyl-phosphatidylethanolamine (DMPE), distearoyl-phosphatidylethanolamine (DSPE), monomethyl-phosphatidylethanolamine, dimethyl-phosphatidylethanolamine, dielaidoyl-phosphatidylethanolamine (DEPE), stearylloleoyl-phosphatidylethanolamine (SOPE), lysophosphatidylcholine, dilinoleoylphosphatidylcholine, sterols such as cholesterol and derivatives thereof such as cholestanol, cholestanone, cholestenone, coprostanol, cholesteryl-2'-hydroxyethyl ether, cholesteryl-4'-hydroxybutyl ether, nonphosphorous containing lipids such as, e.g., stearylamine, dodecylamine, hexadecylamine, acetyl palmitate, glycerolricinoleate, hexadecyl stearate, isopropyl myristate, amphoteric acrylic

polymers, triethanolamine-lauryl sulfate, alkyl-aryl sulfate polyethoxylated fatty acid amides, dioctadecyldimethyl ammonium bromide, ceramide, sphingomyelin, and mixtures thereof.

215. The composition of Claim 211, wherein the lipid molecule is selected from the group
5 consisting of fatty acids and derivatives or analogs thereof including oleic acid, lauric acid, capric acid (n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprinate, tricaprinate, monoolein (1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcamitines, acylcholines, C₁₋₁₀ alkyl esters thereof (e.g., methyl, isopropyl and t-butyl), and
10 mono- and di-glycerides thereof (i.e., oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, etc.).

216. The composition of Claim 211, wherein the lipid molecule is selected from the group
15 consisting of a lipid molecule modified with PEG (PEG-lipid)

217. The composition of Claim 216, wherein the lipid molecule modified with PEG is
selected from the group consisting of PEG coupled to dialkyloxypropyls (PEG-DAA), PEG
coupled to diacylglycerol (PEG-DAG), PEG coupled to phospholipids such as
phosphatidylethanolamine (PEG-PE), PEG conjugated to ceramides, PEG conjugated
20 to cholesterol or a derivative thereof, PEG-C-DOMG, 2 KPEG-DMG, and mixtures thereof.

218. The composition of Claim 211, wherein the nanoparticle is selected from the group
consisting of sHDL nanoparticles, fullerenes, endohedral metallofullerenes buckyballs,
trimetallic nitride templated endohedral metallofullerenes, single-walled and multi-walled
25 carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods, single-walled and multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures, microstructures, or their derivatives formed using layer-by-layer processes, self-assembly processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles,
30 nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable PLGA micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles, iron nanoparticles, and modified micelles.

219. The composition of Claim 211, wherein the antigen is selected from the group consisting of alpha-actinin-4, Bcr-Abl fusion protein, Casp-8, beta-catenin, cdc27, cdk4, cdkn2a, coa-1, dek-can fusion protein, EF2, ETV6-AML1 fusion protein, LDLR-fucosyltransferaseAS fusion protein, HLA-A2, HLA-A11, hsp70-2, KIAA0205, Mart2, Mum-1, 2, and 3, neo-PAP, myosin class I, OS-9, pml-RAR α fusion protein, PTPRK, K-ras, N-ras, Triosephosphate isomeras, Bage-1, Gage 3,4,5,6,7, GnTV, Herv-K-mel, Lage-1, Mage-A1,2,3,4,6,10,12, Mage-C2, NA-88, NY-Eso-1/Lage-2, SP17, SSX-2, and TRP2-Int2, MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15(58), CEA, RAGE, NY-ESO (LAGS), SCP-1, Hom/Mel-40, PRAME, p53, H-Ras, HER-2/neu, BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, β -Catenin, CDK4, Mum-1, p16, TAGE, PSMA, PSCA, CT7, telomerase, 43-9F, 5T4, 791Tgp72, α -fetoprotein, 13HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), human EGFR protein or its fragments, such as human EGFR residues 306--325 (SCVRACGADSYEMEEDGVRK (SEQ ID NO:374)) and residues 897--915 (VWSYGVTVWELMTFGSKPY (SEQ ID NO:375)), HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB\70K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein\cyclophilin C-associated protein), TAAL6, TAG72, TLP, TPS, WT1 (and WT1-derivaed peptide sequences: WT1 126--134 (RMFP NAPYL (SEQ ID NO:376)), WT1 122--140 (SGQARMFPNAPYLPSICLES (SEQ ID NO:377)), and WT1 122--144 (SGQARMFPNAPYLPSICLESQPTI (SEQ ID NO:378)), MUC1 (and MUC1-derived peptides and glycopeptides such as RPAPGS (SEQ ID NO:379), PPAHGVV (SEQ ID NO:380), and PDTRP (SEQ ID NO:381))), LMP2, EGFRvIII, Idiotype, GD2, Ras mutant, p53 mutant, Proteinase3 (PR1), Survivin, hTERT, Sarcoma translocation breakpoints, EphA2, EphA4, LMW-PTP, PAP, ML-IAP, AFP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, ALK, Androgen receptor, Cyclin B1, Polysialic acid, MYCN, RhoC, TRP-2, GD3, Fucosyl GM1, Mesothelin, sLe(animal), CYP1B1, PLAC1, GM3, BORIS, Tn, GloboH, NY-BR-1, RGS5, SART3, STn, Carbonic anhydrase IX, PAX5, OY-TES1, Sperm protein 17, LCK, HMWMAA, AKAP-4, XAGE 1, B7H3, Legumain, Tie 2, Page4, VEGFR2, MAD-CT-1, FAP, PDGFR-alpha, PDGFR- β , MAD-CT-2, Fos-related antigen 1, ERBB2, Folate receptor 1 (FOLR1 or FBP), IDH1, IDO, LY6K, fms-related tyro- sine kinase 1 (FLT1, best known as VEGFR1), KDR, PADRE, TA-CIN (recombinant HPV16 L2E7E6), SOX2, aldehyde dehydrogenase, and any derivative thereof.

220. The composition of Claim 211, wherein the adjuvant is selected from the group consisting of CPG, polyIC, poly-ICLC, 1018 ISS, aluminum salts (for example, aluminum hydroxide, aluminum phosphate), Amplivax, BCG, CP-870,893, CpG7909, CyaA, dSLIM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, OK-432, OM-174, OM-197-MP-EC, ONTAK, PepTel.RTM, vector system, PLGA microparticles, imiquimod, resiquimod, gardiquimod, 3M-052, SRL172, Virosomes and other Virus-like particles, YF-17D, VEGF trap, beta-glucan, Pam3Cys, Aquila's QS21 stimulon, vadimezan, AsA404 (DMXAA), 3M MEDI9197, glucopyranosyl lipid adjuvant (GLA), GLA-SE, CD1d ligands (such as C20:2, OCH, AH04-2, α -galatosylceramide, α -C-galatosylceramide, α -mannosylceramide, α -fructosylceramide, β -galatosylceramide, β -mannosylceramide), STING agonists (e.g. cyclic dinucleotides, including Cyclic [G(3',5')pA(3',5')p], Cyclic [G(2',5')pA(3',5')p], Cyclic [G(2',5')pA(2',5')p], Cyclic diadenylate monophosphate, Cyclic diguanylate monophosphate), CL401, CL413, CL429, Flagellin, RC529, E6020, imidazoquinoline-based small molecule TLR-7/8a (including its lipidated analogues), virosomes, AS01, AS02, AS03, AS04, AS15, IC31, CAF01, ISCOM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), bacterial toxins (such as CT, and LT), any derivative of an adjuvant, and any combination of adjuvant.

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221. The composition of Claim 211, wherein the antigen is conjugated to the outer surface of the nanoparticle or the adjuvant is conjugated to the outer surface of the nanoparticle.

222. The composition of Claim 211, wherein the antigen is derived from a self-antigen.

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223. The composition of Claim 211, wherein the antigen-adjuvant conjugated with the hydrophobic molecule is encapsulated within the sHDL nanoparticle.

224. The composition of Claim 211, wherein the nanoparticle is a sHDL nanoparticle, wherein the sHDL nanoparticle comprises a mixture of at least one phospholipid and at least one HDL apolipoprotein or apolipoprotein mimetic.

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225. The composition of Claim 224,

wherein the HDL apolipoprotein is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), and apolipoprotein E (apo E), and any derivative thereof,

wherein the phospholipid is selected from the group consisting of

- 5 dipalmitoylphosphatidylcholine (DPPC), dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide],
 10 phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and combinations thereof.

- 15 226. The composition of Claim 225,

wherein the HDL apolipoprotein mimetic is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A-II xxx (apo A-II-xxx), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), apolipoprotein E (apo E), apolipoprotein A-I milano (apo A-I-milano), apolipoprotein A-I paris (apo A-I-paris),
 20 apolipoprotein M (apo M), an HDL apolipoprotein mimetic, preproapolipoprotein, preproApoA-I, proApoA I, preproApoA-II, proApoA II, preproApoA-IV, proApoA-IV, ApoA-V, preproApoE, proApoE, preproApoA I_{Milano}, proApoA-I_{Milano}, preproApoA-I_{Paris}, proApoA-I_{Paris}, and mixtures thereof.

- 25 227. The composition of Claim 226, wherein the ApoA-I mimetic is described by any of SEQ ID NOs: 1-336 and WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341), LKLLDNWDSVTSTFSKLREOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLR OEMS (SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO: 345), PYLDDDFQKKWQEEMEL YRQKVE (SEQ ID NO: 346),
 30 PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDALRTHLA (SEQ ID NO: 348), PYSDEL RQRLAARLEALKENGG (SEQ ID NO: 349), ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352),

TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDYGKDLME (SEQ ID
 NO:355), KVKSPPELOAEAKSYFEKSKE (SEQ ID NO:356),
 VLTALVAVAGARAEVSADOVATV (SEQ ID NO:357),
 NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358),
 5 LPVLVWLSIVLEGPAPAOGTPDVSS (SEQ ID NO:359),
 LPVLVVVLSIVLEGPAPAQGTPDVSS (SEQ ID NO:360), ALDKLKEFGNTLEDKARELIS
 (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362),
 HLRKLRKRLLRDADDLQKRLAVYOA (SEQ ID NO:363),
 AQAWGERLRARMEEMGSRTRDR (SEQ ID NO:364), LDEVKEQVAEVRAKLEEQAQ
 10 (SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236),
 DWLKAFYDKVAEKLKEAFPDWAKAAYDKAAEKAKEAA (SEQ ID NO:366),
 PVLDFRELLNELLEALKQKL (SEQ ID NO:367), PVLDFRELLNELLEALKQKLA (SEQ
 ID NO:368), PVLDFRELLNELLEALKQKLG (SEQ ID NO:4),
 PVLDFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDFRELLNELLEALKKLLK
 15 (SEQ ID NO:370), PVLDFRELLNELLEALKKLLA (SEQ ID NO:371),
 PLLDFRELLNELLEALKKLLA (SEQ ID NO:372), and
 EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

228. The composition of Claim 211, wherein the average particle size of the nanoparticle is
 20 between 6 to 500 nm.

229. A method for inducing an immune response to one or more antigens comprising
 administering to a subject in need an effective amount of a composition comprising a
 nanoparticle, wherein the one or more antigens is associated with (e.g., complexed, conjugated,
 25 encapsulated, absorbed, adsorbed, admixed) the nanoparticle, wherein an adjuvant is associated
 with (e.g., complexed, conjugated, encapsulated, absorbed, adsorbed, admixed) the nanoparticle,
 wherein the antigen is associated with (e.g., complexed, conjugated, encapsulated, absorbed,
 adsorbed, admixed) a hydrophobic molecule and/or wherein the adjuvant is conjugated with a
 hydrophobic molecule.

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230. The method of Claim 229, wherein the hydrophobic molecule is a lipid molecule.

231. The method of Claim 229, wherein the lipid molecule is a membrane-forming lipid molecule.

232. The method of Claim 229, wherein the lipid molecule is a non-membrane-forming lipid molecule.

5 233. The method of Claim 229, wherein the lipid molecule is selected from the group consisting of phospholipids such as lecithin, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, egg sphingomyelin (ESM), cephalin, cardiolipin, phosphatidic acid, cerebrosides, dicytlylphosphate, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC),
 10 dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoylphosphatidylethanolamine (DOPE), palmitoyloleoyl-phosphatidylcholine (POPC), palmitoyloleoyl-phosphatidylethanolamine (POPE), palmitoyloleoyl-phosphatidylglycerol (POPG), dioleoylphosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl-
 15 phosphatidylethanolamine (DPPE), dimyristoyl-phosphatidylethanolamine (DMPE), distearoyl-phosphatidylethanolamine (DSPE), monomethyl-phosphatidylethanolamine, dimethyl-phosphatidylethanolamine, dielaidoyl-phosphatidylethanolamine (DEPE), stearylloleoyl-phosphatidylethanolamine (SOPE), lysophosphatidylcholine, dilinoleoylphosphatidylcholine, sterols such as cholesterol and derivatives thereof such as cholestanol, cholestanone,
 20 cholestenone, coprostanol, cholesteryl-2'-hydroxyethyl ether, cholesteryl-4'-hydroxybutyl ether, nonphosphorous containing lipids such as, e.g., stearylamine, dodecylamine, hexadecylamine, acetyl palmitate, glycerolricinoleate, hexadecyl stearate, isopropyl myristate, amphoteric acrylic polymers, triethanolamine-lauryl sulfate, alkyl-aryl sulfate polyethoxylated fatty acid amides, dioctadecyldimethyl ammonium bromide, ceramide, sphingomyelin, and mixtures thereof.

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234. The method of Claim 229, wherein the lipid molecule is selected from the group consisting of fatty acids and derivatives or analogs thereof including oleic acid, lauric acid, capric acid (n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprinate, tricaprinate, monoolein (1-monooleoyl-rac-glycerol), dilaurin,
 30 caprylic acid, arachidonic acid, glycerol 1-monocaprinate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, C₁₋₁₀ alkyl esters thereof (e.g., methyl, isopropyl and t-butyl), and mono- and di-glycerides thereof (i.e., oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, etc.).

235. The method of Claim 229, wherein the lipid molecule is selected from the group consisting of a lipid molecule modified with PEG (PEG-lipid)

236. The method of Claim 235, wherein the lipid molecule modified with PEG is selected from the group consisting of PEG coupled to dialkyloxypropyls (PEG-DAA), PEG coupled to diacylglycerol (PEG-DAG), PEG coupled to phospholipids such as phosphatidylethanolamine (PEG-PE), PEG conjugated to ceramides, PEG conjugated to cholesterol or a derivative thereof, PEG-C-DOMG, 2 KPEG-DMG, and mixtures thereof.

237. The method of Claim 229, wherein the nanoparticle is selected from the group consisting of sHDL nanoparticles, fullerenes, endohedral metallofullerenes buckyballs, trimetallic nitride templated endohedral metallofullerenes, single-walled and multi-walled carbon nanotubes, branched and dendritic carbon nanotubes, gold nanorods, silver nanorods, single-walled and multi-walled boron/nitrate nanotubes, carbon nanotube peapods, carbon nanohorns, carbon nanohorn peapods, liposomes, nanoshells, dendrimers, any nanostructures, microstructures, or their derivatives formed using layer-by-layer processes, self-assembly processes, or polyelectrolytes, microparticles, quantum dots, superparamagnetic nanoparticles, nanorods, cellulose nanoparticles, glass and polymer micro- and nano-spheres, biodegradable PLGA micro- and nano-spheres, gold nanoparticles, silver nanoparticles, carbon nanoparticles, iron nanoparticles, and modified micelles.

238. The method of Claim 229, wherein the antigen is selected from the group consisting of alpha-actinin-4, Bcr-Abl fusion protein, Casp-8, beta-catenin, cdc27, cdk4, cdkn2a, coa-1, dekan fusion protein, EF2, ETV6-AML1 fusion protein, LDLR-fucosyltransferaseAS fusion protein, HLA-A2, HLA-A11, hsp70-2, KIAA0205, Mart2, Mum-1, 2, and 3, neo-PAP, myosin class I, OS-9, pml-RAR α fusion protein, PTPRK, K-ras, N-ras, Triosephosphate isomeras, Bage-1, Gage 3,4,5,6,7, GnTV, Herv-K-mel, Lage-1, Mage-A1,2,3,4,6,10,12, Mage-C2, NA-88, NY-Eso-1/Lage-2, SP17, SSX-2, and TRP2-Int2, MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15(58), CEA, RAGE, NY-ESO (LAGS), SCP-1, Hom/Mel-40, PRAME, p53, H-Ras, HER-2/neu, BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, β -Catenin, CDK4, Mum-1, p16, TAGE, PSMA, PSCA, CT7, telomerase, 43-9F, 5T4, 791Tgp72, α -

fetoprotein, 13HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), human EGFR protein or its fragments, such as human EGFR residues 306–325 (SCVRACGADSYEMEEDGVRK (SEQ ID NO:374)) and residues 897–915 (VWSYGVTVWELMTFGSKPY (SEQ ID NO:375)),

5 HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB\70K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein\cyclophilin C-associated protein), TAAL6, TAG72, TLP, TPS, WT1 (and WT1-derivaed peptide sequences: WT1 126–134 (RMFP NAPYL (SEQ ID NO:376)), WT1 122–140 (SGQARMFPNAPYLPSICLES (SEQ ID NO:377)), and WT1 122–144 (SGQARMFPNAPYLPSICLESQPTI (SEQ ID NO:378)), MUC1 (and MUC1-derived peptides

10 and glycopeptides such as RPAPGS (SEQ ID NO:379), PPAHGVV (SEQ ID NO:380), and PDTRP (SEQ ID NO:381))), LMP2, EGFRvIII, Idiotype, GD2, Ras mutant, p53 mutant, Proteinase3 (PR1), Survivin, hTERT, Sarcoma translocation breakpoints, EphA2, EphA4, LMW-PTP, PAP, ML-IAP, AFP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, ALK, Androgen receptor, Cyclin B1, Polysialic acid, MYCN, RhoC, TRP-2, GD3, Fucosyl GM1,

15 Mesothelin, sLe(animal), CYP1B1, PLAC1, GM3, BORIS, Tn, GloboH, NY-BR-1, RGS5, SART3, STn, Carbonic anhydrase IX, PAX5, OY-TES1, Sperm protein 17, LCK, HMWMAA, AKAP-4, XAGE 1, B7H3, Legumain, Tie 2, Page4, VEGFR2, MAD-CT-1, FAP, PDGFR-alpha, PDGFR- β , MAD-CT-2, Fos-related antigen 1, ERBB2, Folate receptor 1 (FOLR1 or FBP), IDH1, IDO, LY6K, fms-related tyro- sine kinase 1 (FLT1, best known as VEGFR1),

20 KDR, PADRE, TA-CIN (recombinant HPV16 L2E7E6), SOX2, aldehyde dehydrogenase, and any derivative thereof.

239. The method of Claim 229, wherein the adjuvant is selected from the group consisting of CPG, polyIC, poly-ICLC, 1018 ISS, aluminum salts (for example, aluminum hydroxide,

25 aluminum phosphate), Amplivax, BCG, CP-870,893, CpG7909, CyaA, dSLIM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, OK-432, OM-174, OM-197-MP-EC, ONTAK, PepTel.RTM, vector system, PLGA microparticles, imiquimod, resiquimod,

30 gardiquimod, 3M-052, SRL172, Virosomes and other Virus-like particles, YF-17D, VEGF trap, beta-glucan, Pam3Cys, Aquila's QS21 stimulon, vadimezan, AsA404 (DMXAA), 3M MEDI9197, glucopyranosyl lipid adjuvant (GLA), GLA-SE, CD1d ligands (such as C20:2, OCH, AH04-2, α -galatosylceramide, α -C-galatosylceramide, α -mannosylceramide, α -fructosylceramide, β -galatosylceramide, β -mannosylceramide), STING agonists (e.g. cyclic

dinucleotides, including Cyclic [G(3',5')pA(3',5')p], Cyclic [G(2',5')pA(3',5')p], Cyclic [G(2',5')pA(2',5')p], Cyclic diadenylate monophosphate, Cyclic diguanylate monophosphate), CL401, CL413, CL429, Flagellin, RC529, E6020, imidazoquinoline-based small molecule TLR-7/8a (including its lipidated analogues), virosomes, AS01, AS02, AS03, AS04, AS15, IC31, CAF01, ISCOM, Cytokines (such as GM-CSF, IL-2, IFN- α , Flt-3L), bacterial toxins (such as CT, and LT), any derivative of an adjuvant, and any combination of adjuvant.

240. The method of Claim 229, wherein the one or more antigens are conjugated to the outer surface of the nanoparticle.

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241. The method of Claim 229, wherein the adjuvant is conjugated to the outer surface of the nanoparticle.

242. The method of Claim 229, wherein the antigen-adjuvant conjugated with the hydrophobic molecule is encapsulated within the nanoparticle.

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243. The method of Claim 229, wherein the nanoparticle is a sHDL nanoparticle, wherein the sHDL nanoparticle comprises a mixture of at least one phospholipid and at least one HDL apolipoprotein or apolipoprotein mimetic.

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244. The method of Claim 243, wherein the HDL apolipoprotein is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), and apolipoprotein E (apo E), and any derivative thereof,

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wherein the phospholipid is selected from the group consisting of dipalmitoylphosphatidylcholine (DPPC), dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (DOPE-PDP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol, 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide], 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide], phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and combinations thereof.

30

245. The method of Claim 244,

wherein the HDL apolipoprotein mimetic is selected from the group consisting of apolipoprotein A-I (apo A-I), apolipoprotein A-II (apo A-II), apolipoprotein A-II xxx (apo A-II-
 5 xxx), apolipoprotein A4 (apo A4), apolipoprotein Cs (apo Cs), apolipoprotein E (apo E),
 apolipoprotein A-I milano (apo A-I-milano), apolipoprotein A-I paris (apo A-I-paris),
 apolipoprotein M (apo M), an HDL apolipoprotein mimetic, preproapolipoprotein, preproApoA-I,
 proApoA I, preproApoA-II, proApoA II, preproApoA-IV, proApoA-IV, ApoA-V, preproApoE,
 proApoE, preproApoA I_{Milano}, proApoA-I_{Milano}, preproApoA-I_{Paris}, proApoA-I_{Paris}, and mixtures
 10 thereof.

246. The method of Claim 245, wherein the ApoA-I mimetic is described by any of SEQ ID
 NOs: 1-336 and WDRVKDLATVYVDVLKDSGRDYVSQF (SEQ ID NO:341),
 LKLLDNWDSVTSTFSKLREOL (SEQ ID NO:342), PVTOEFWDNLEKETEGLR OEMS
 15 (SEQ ID NO:343), KDLEEVKAKVQ (SEQ ID NO: 344), KDLEEVKAKVO (SEQ ID NO:
 345), PYLDDFQKKWQEEMEL YRQKVE (SEQ ID NO: 346),
 PLRAELQEGARQKLHELOEKLS (SEQ ID NO: 347), PLGEEMRDRARAHVDALRTHLA
 (SEQ ID NO: 348), PYSDEL RQRLAARLEALKENGG (SEQ ID NO: 349),
 ARLAEYHAKATEHLSTLSEKAK (SEQ ID NO: 350), PALEDLROGLL (SEQ ID NO: 351),
 20 PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352), PVLESFVSFLSALEEYTKKLN (SEQ
 ID NO:353), PVLESFKVSFLSALEEYTKKLN (SEQ ID NO:352),
 TVLLLTICSLEGALVRRQAKEPCV (SEQ ID NO: 354) QTVTDY GKDLME (SEQ ID
 NO:355), KVKSP ELOAEAKSYFEKSKE (SEQ ID NO:356),
 VLTLALVAVAGARAEVSADOVATV (SEQ ID NO:357),
 25 NNAKEAVEHLOKSELTOOLNAL (SEQ ID NO:358),
 LPVLVWLSIVLEG PAPAOGTPDVSS (SEQ ID NO:359),
 LPVLVVVLSIVLEG PAPAQGTPDVSS (SEQ ID NO:360), ALDKLKEFGNTLEDKARELIS
 (SEQ ID NO: 361), VVALLALLASARASEAEDASLL (SEQ ID NO:362),
 HLRKLRKRLLRDADDLQKRLAVYOA (SEQ ID NO:363),
 30 AQAWGERLRARMEEMGSRTRDR (SEQ ID NO:364), LDEVKEQVAEVR AKLEEQAQ
 (SEQ ID NO:365), DWLKAFYDKVAEKLKEAF (SEQ ID NO:236),
 DWLKAFYDKVAEKLKEAFPDWAKAAAYDKAAEKAKEAA (SEQ ID NO:366),
 PVLDFRELLNELLEALKQKL (SEQ ID NO:367), PVLDFRELLNELLEALKQKLA (SEQ
 ID NO:368), PVLDFRELLNELLEALKQK LK (SEQ ID NO:4),

PVLDFRELLNELLEALKQKLA (SEQ ID NO:369), PVLDFRELLNELLEALKKLLK (SEQ ID NO:370), PVLDFRELLNELLEALKKLLA (SEQ ID NO:371), PLLDFRELLNELLEALKKLLA (SEQ ID NO:372), and EVRSKLEEWFAAFREFAEEFLARLKS (SEQ ID NO: 373).

5

247. The method of Claim 229, wherein the average particle size of the nanoparticle is between 6 to 500 nm.

REPLACEMENT SHEET

FIG. 1A

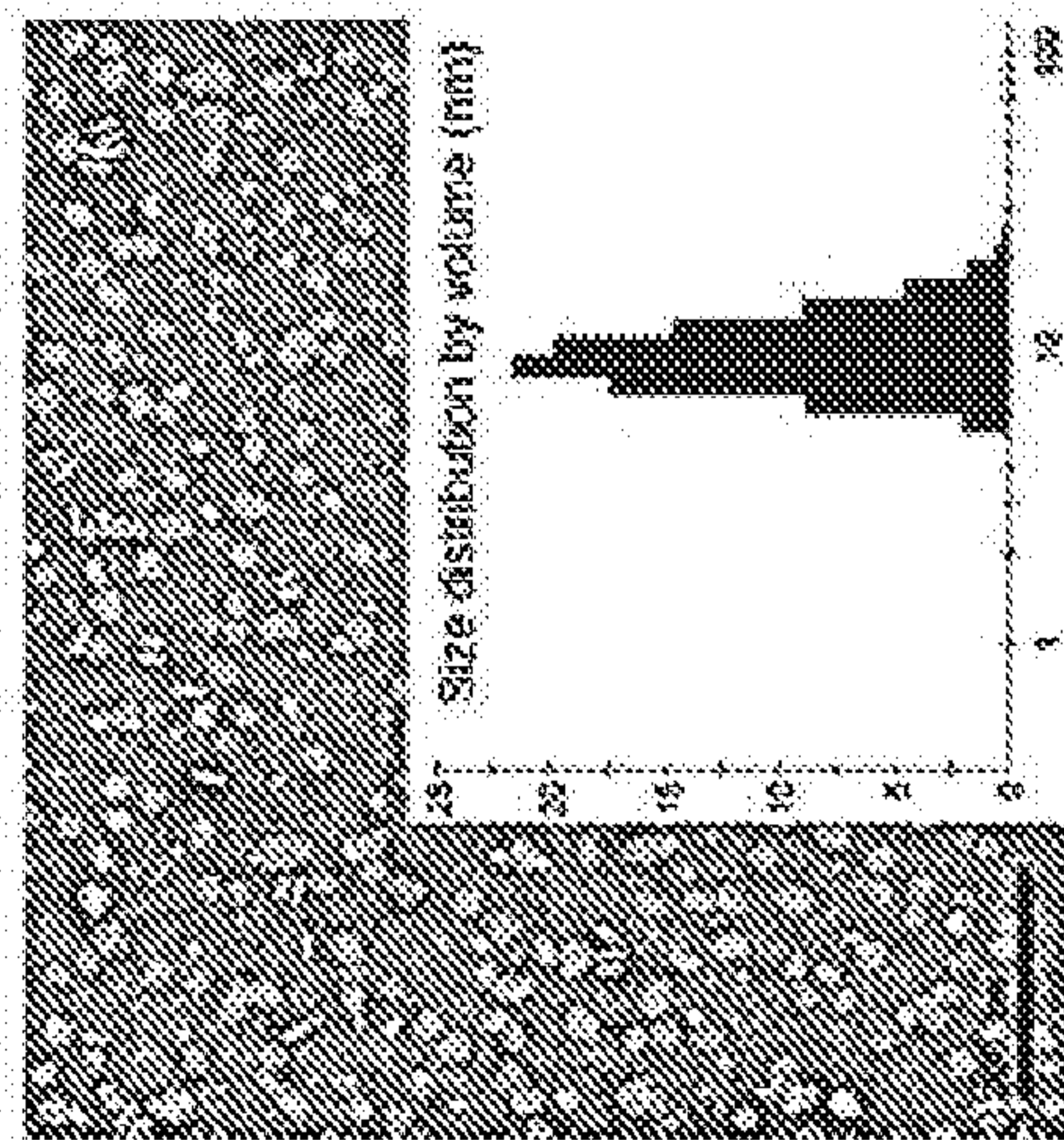


FIG. 1B

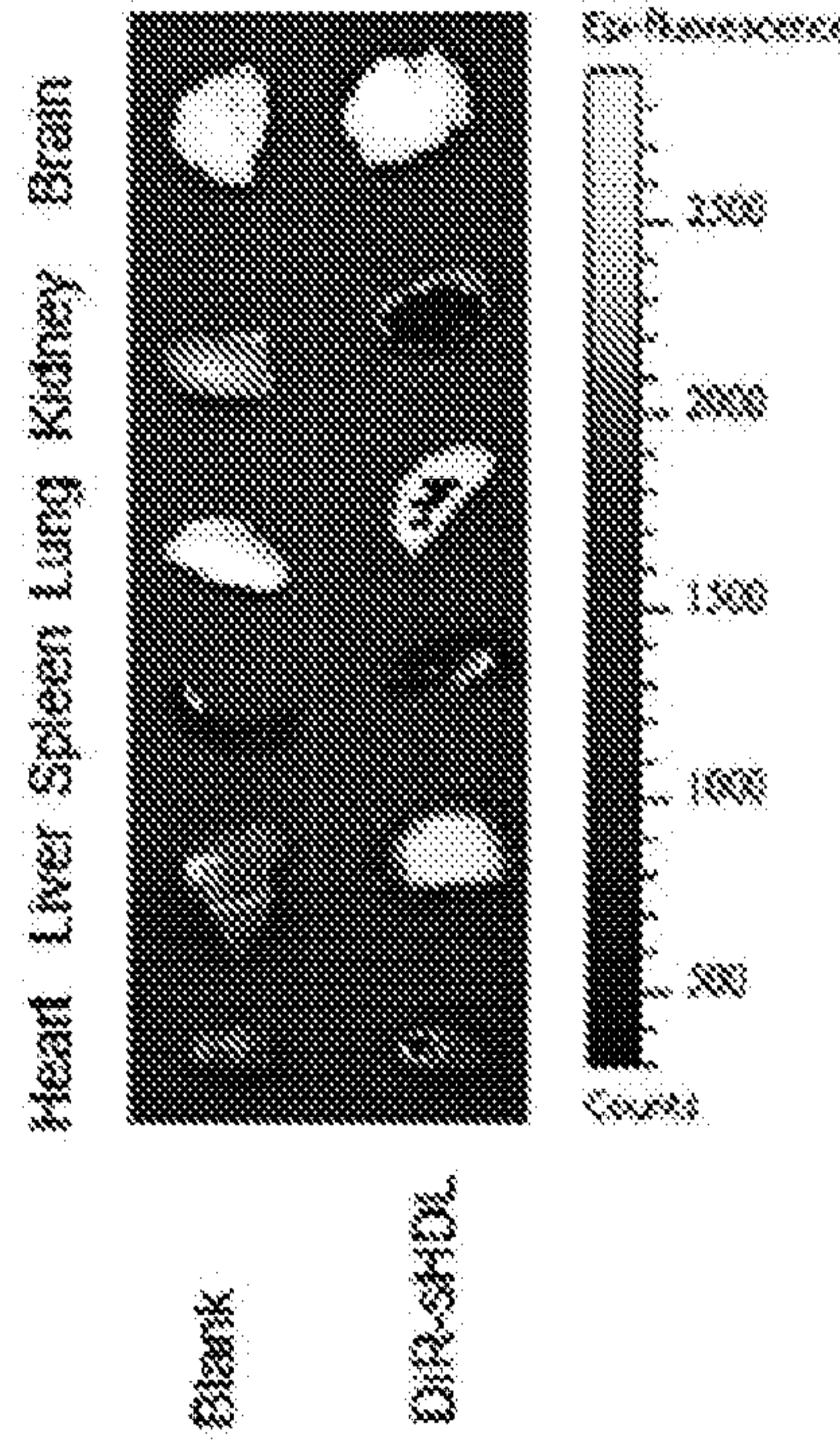
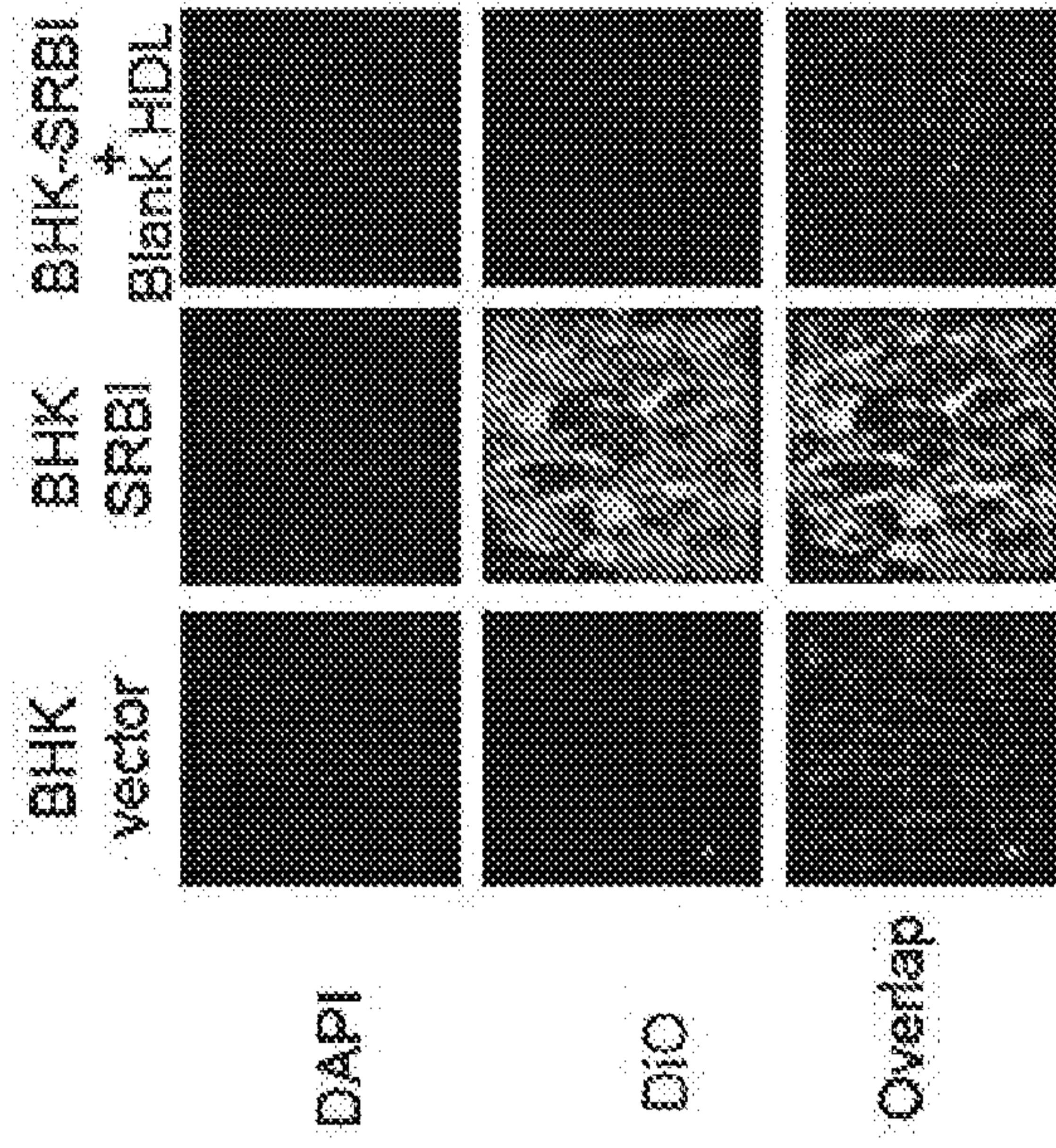


FIG. 1C



REPLACEMENT SHEET

FIG. 1E

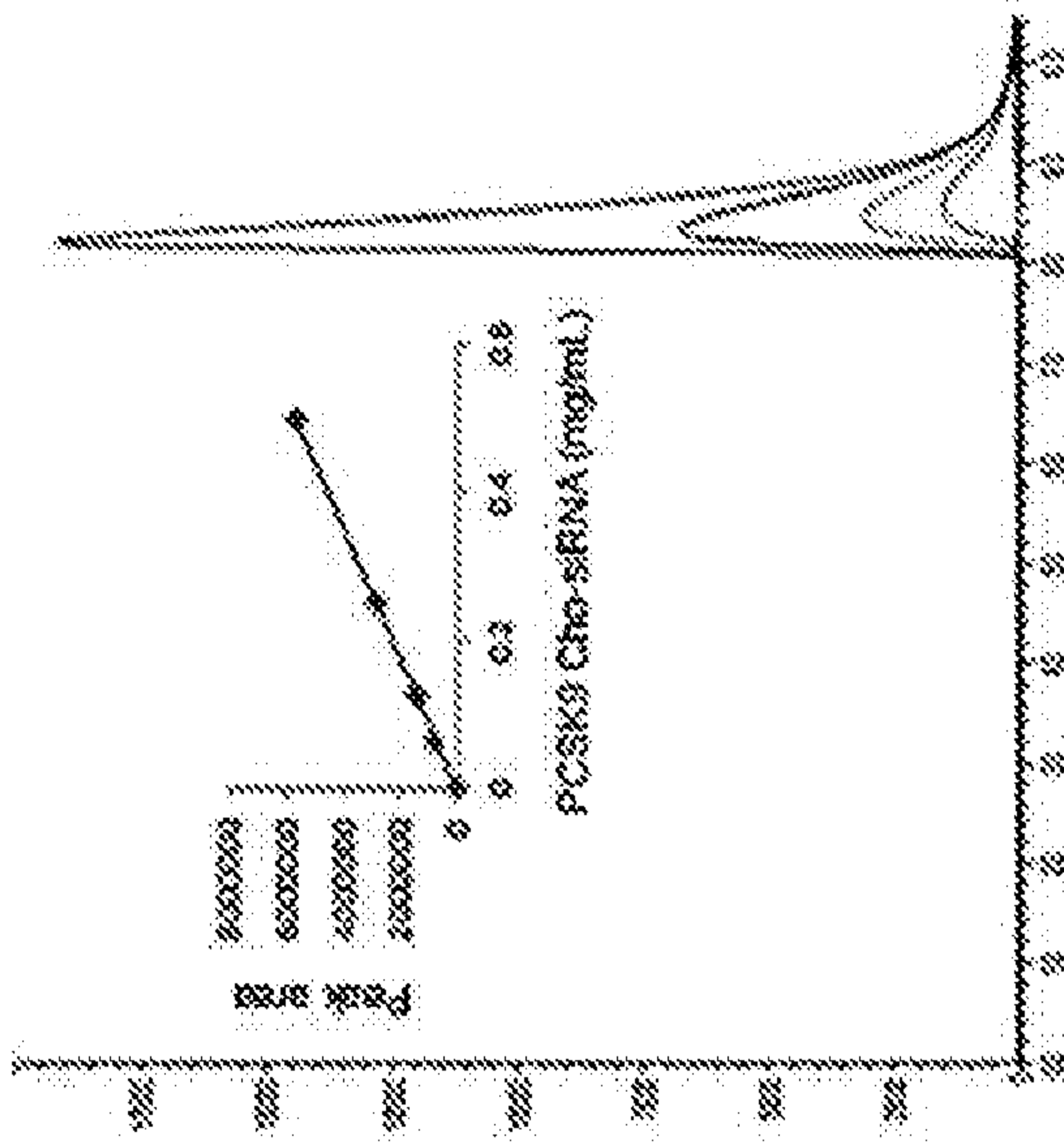


FIG. 1D

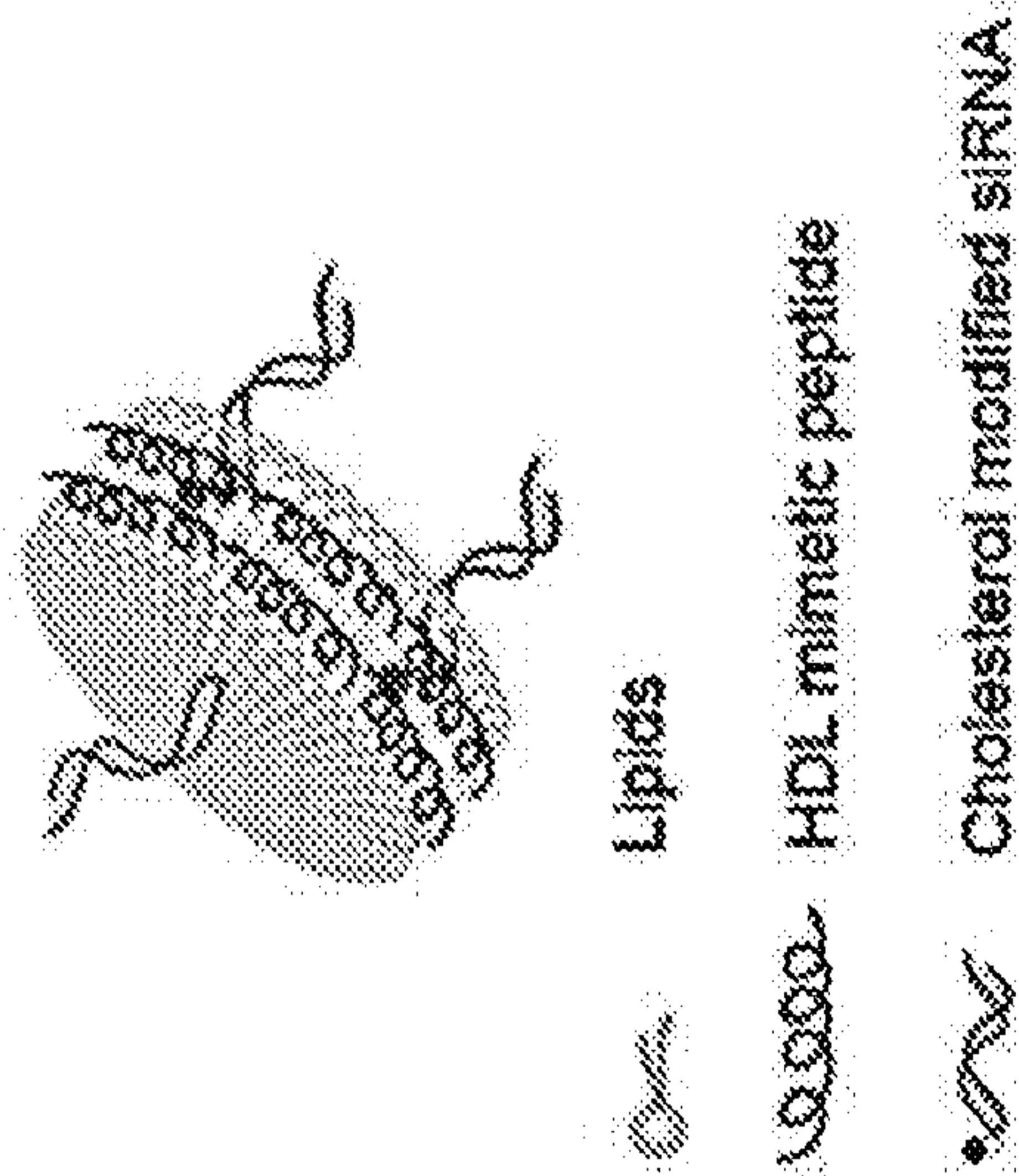
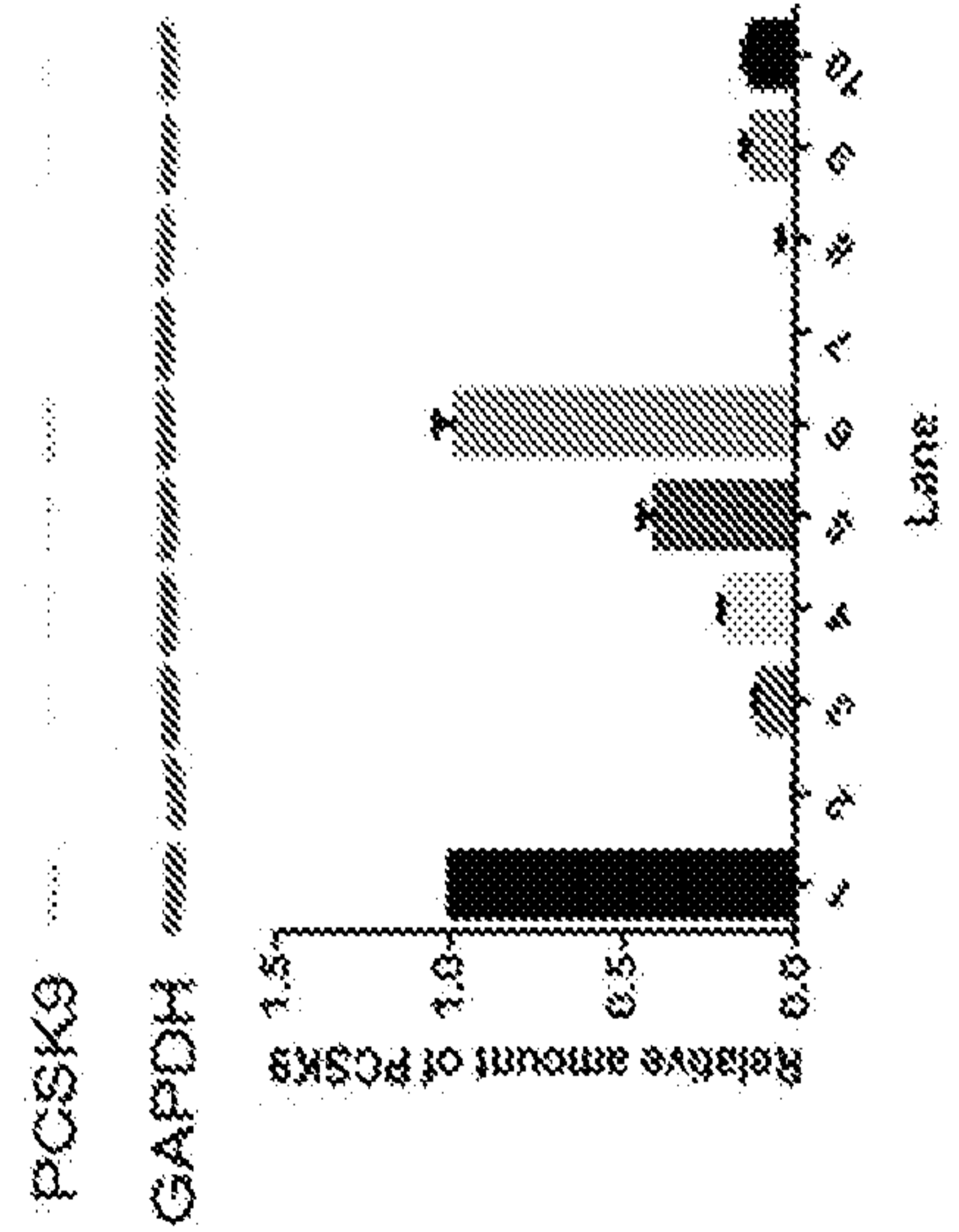


FIG. 1F



REPLACEMENT SHEET

FIG. 2A

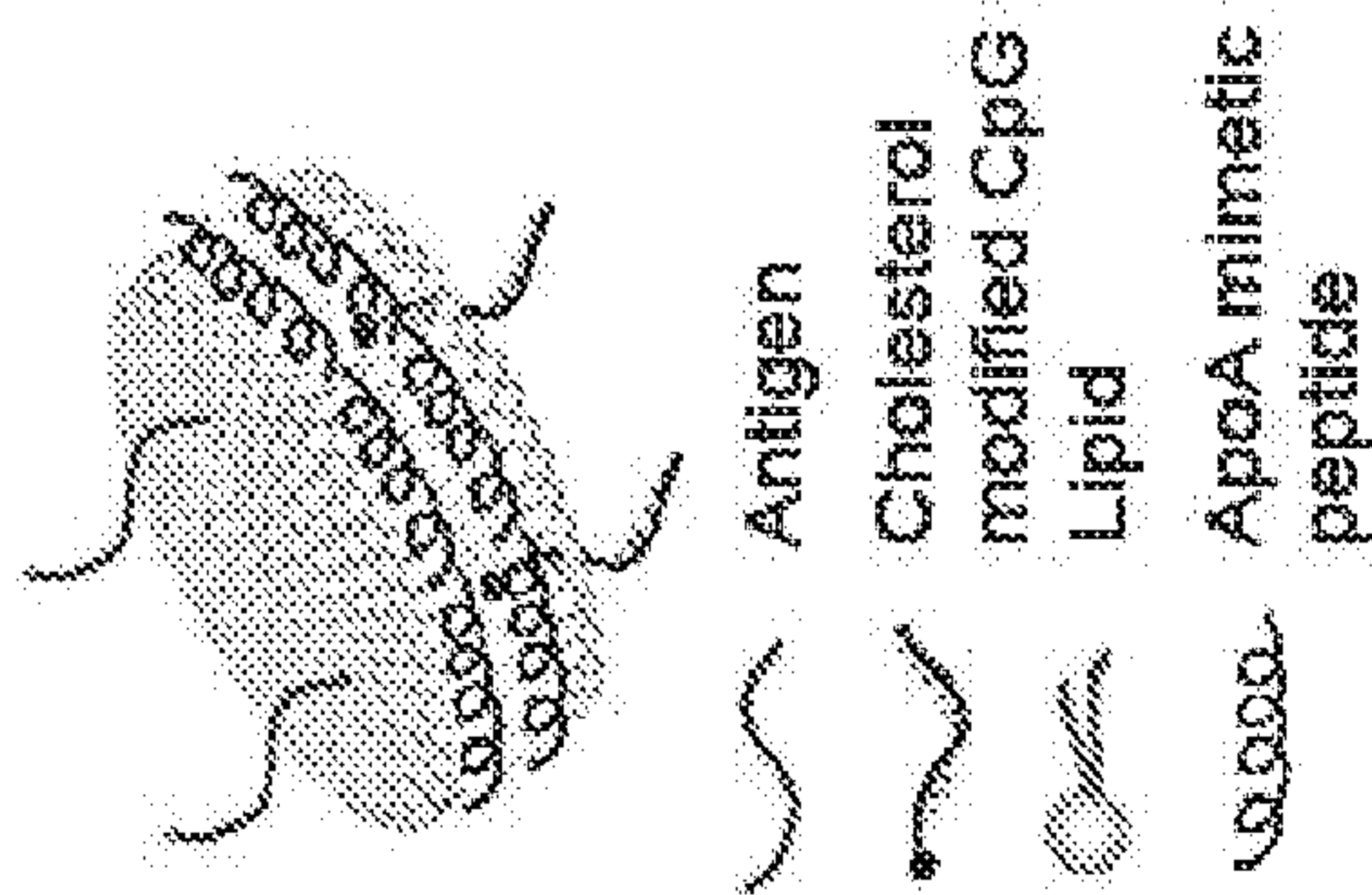


FIG. 2B

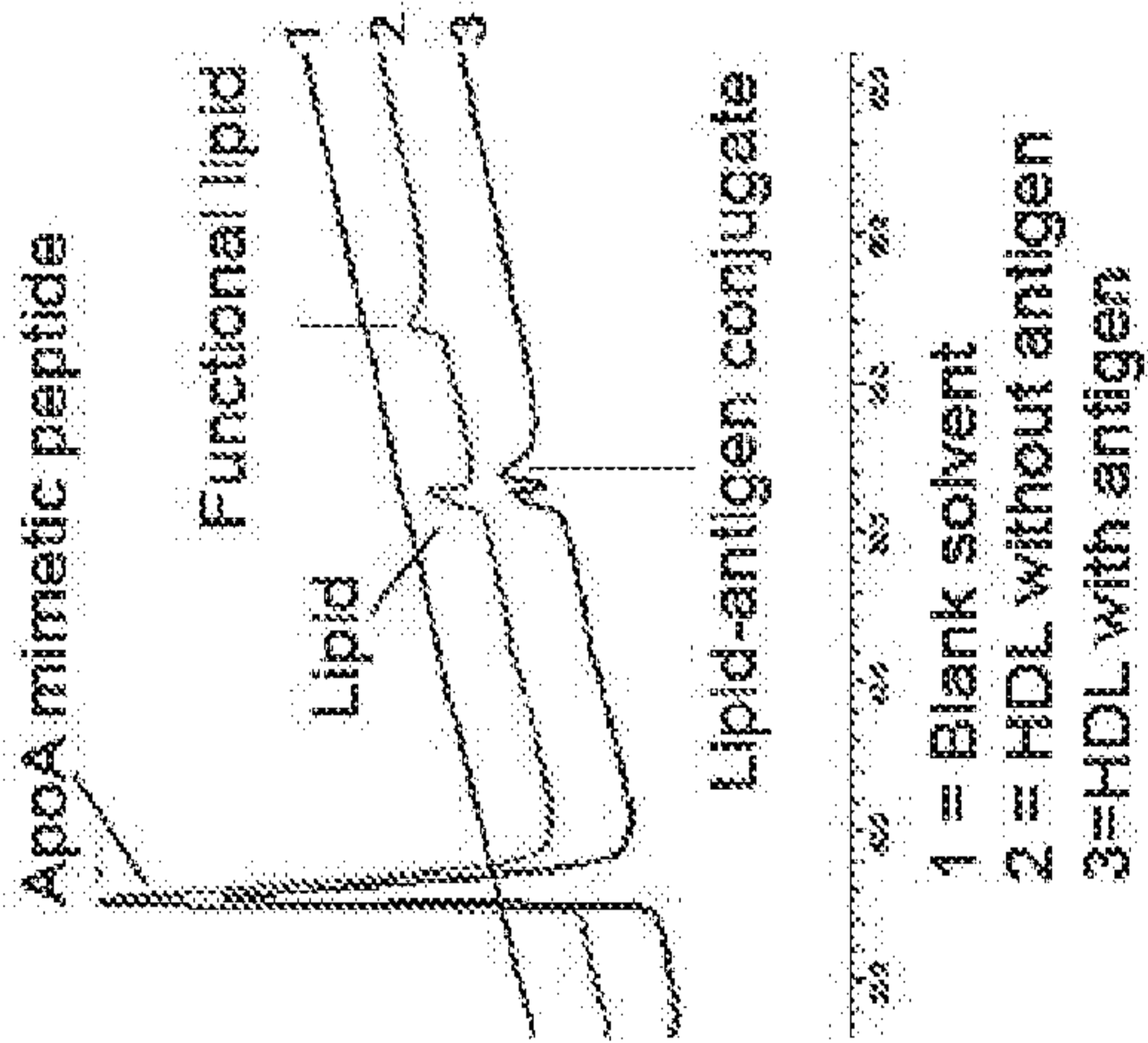


FIG. 2C

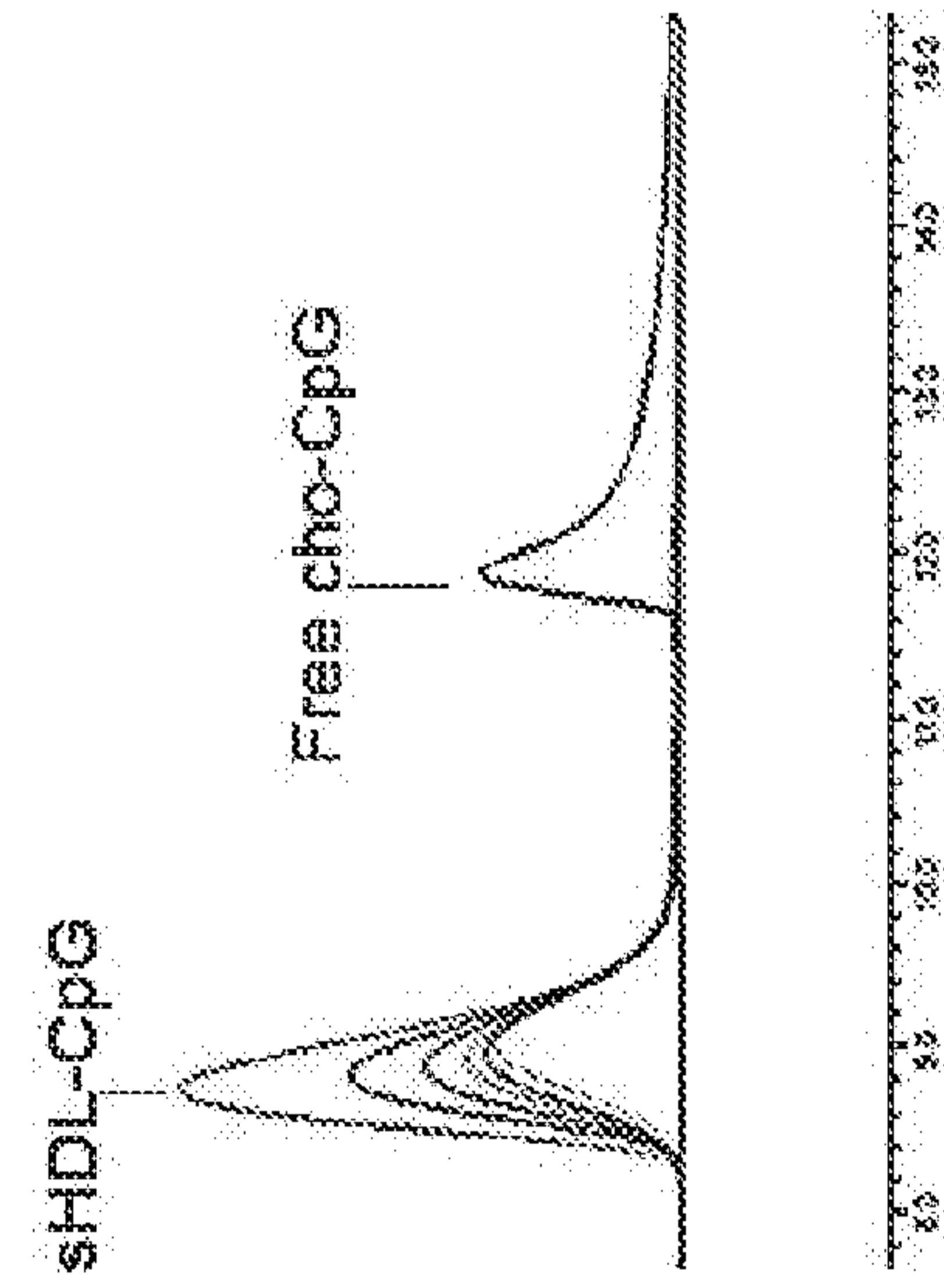


FIG. 2D

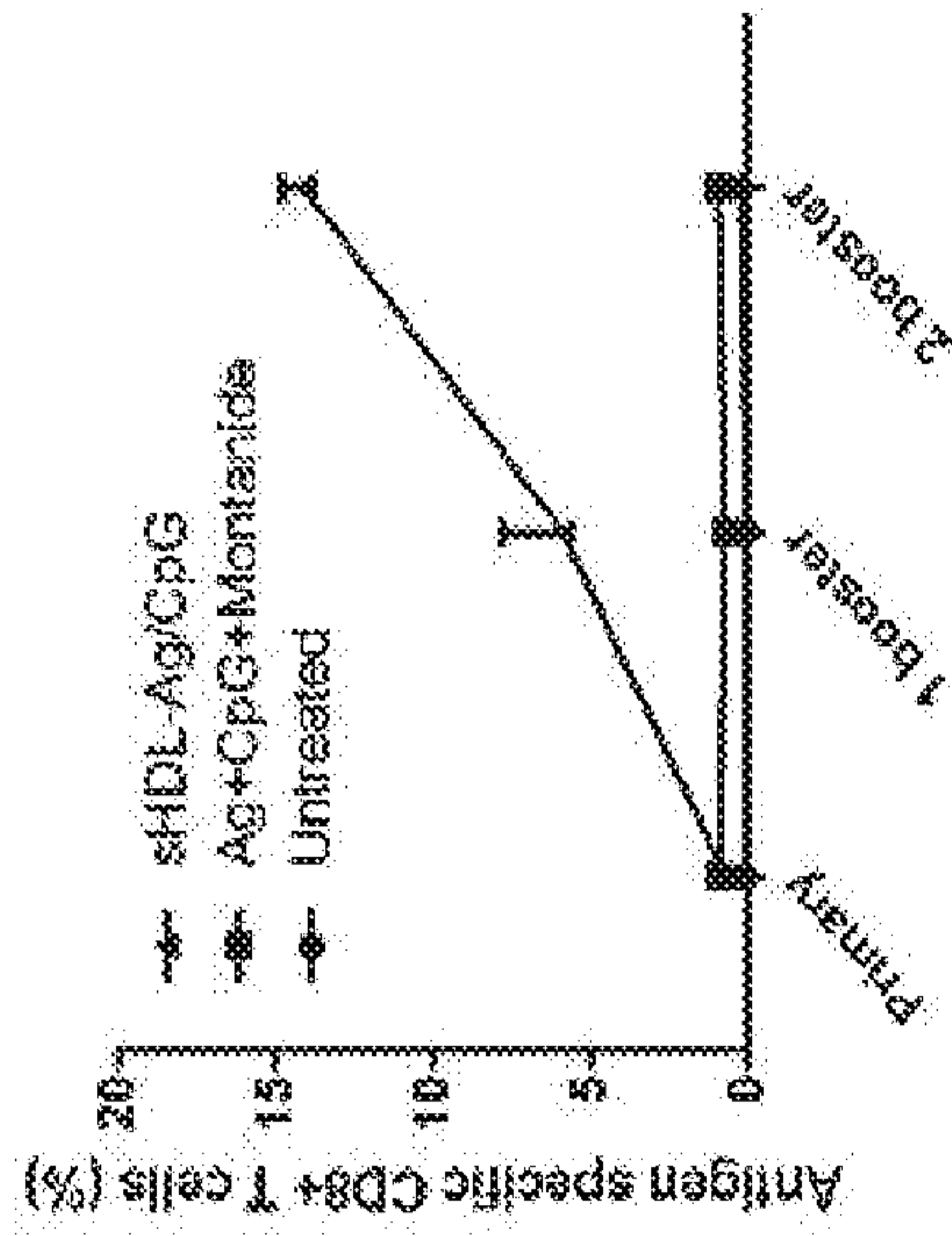


FIG. 3

Synthesis of sHDL loaded with peptide antigen and CpG (sHDL-Ag/CpG)

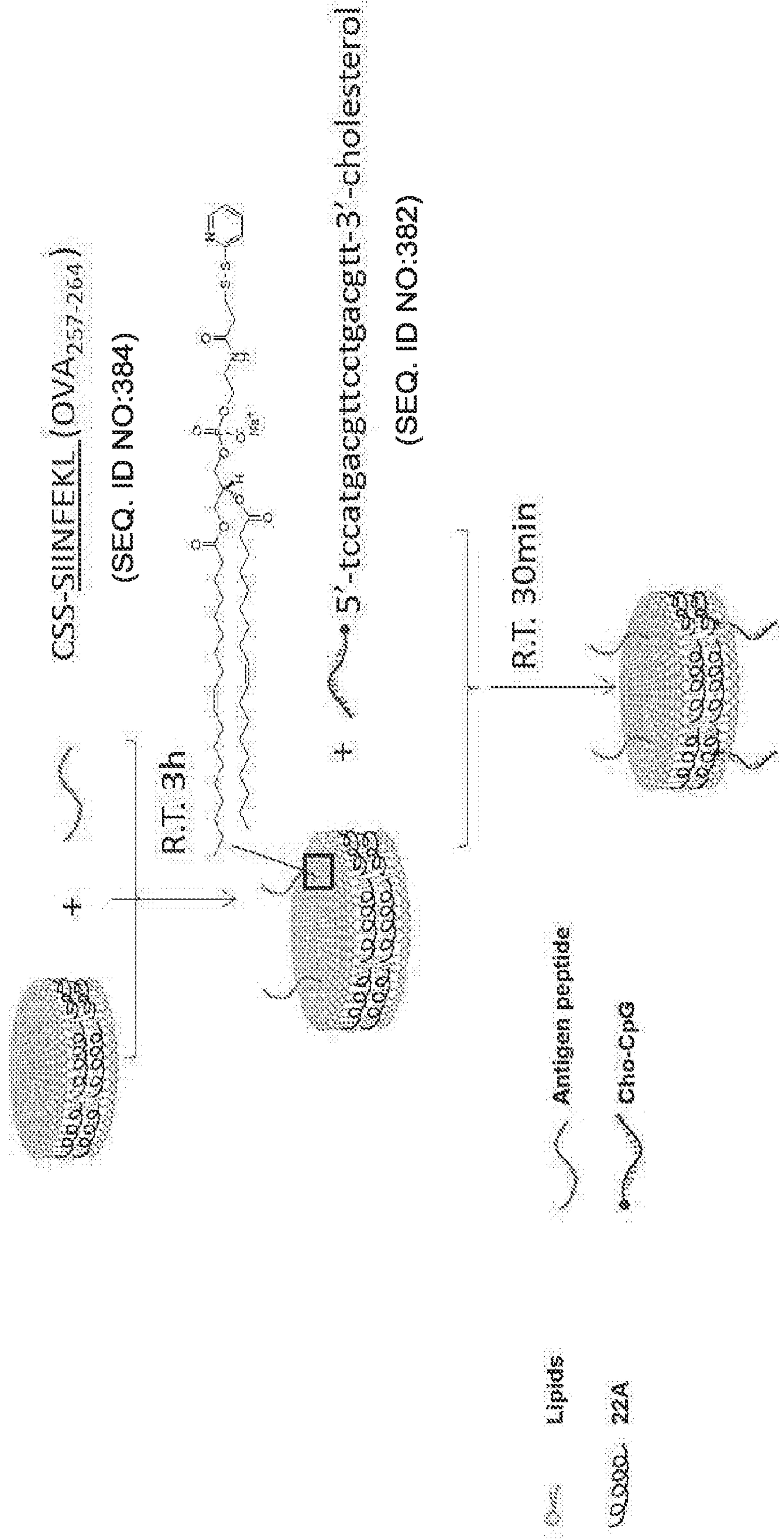
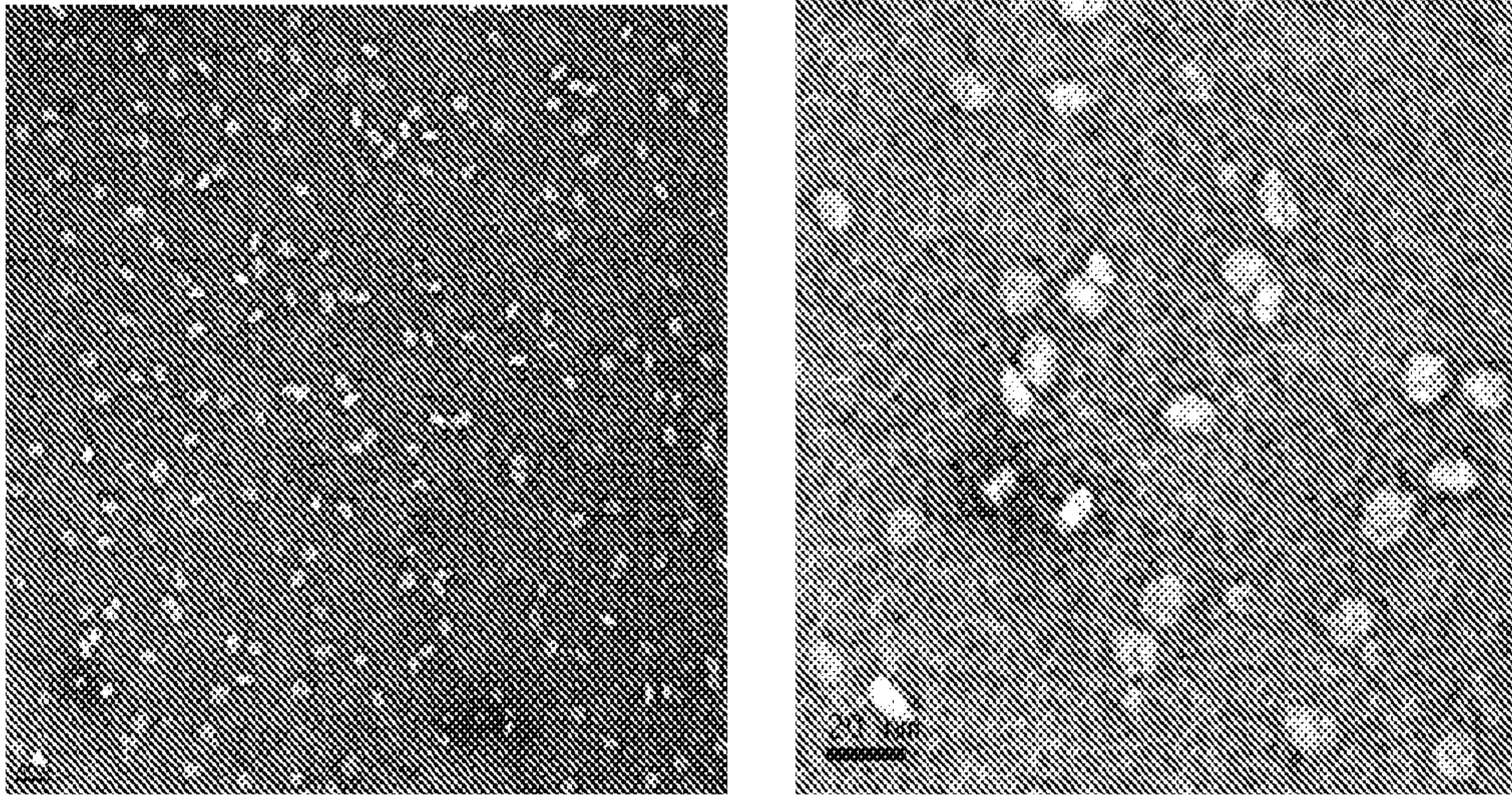
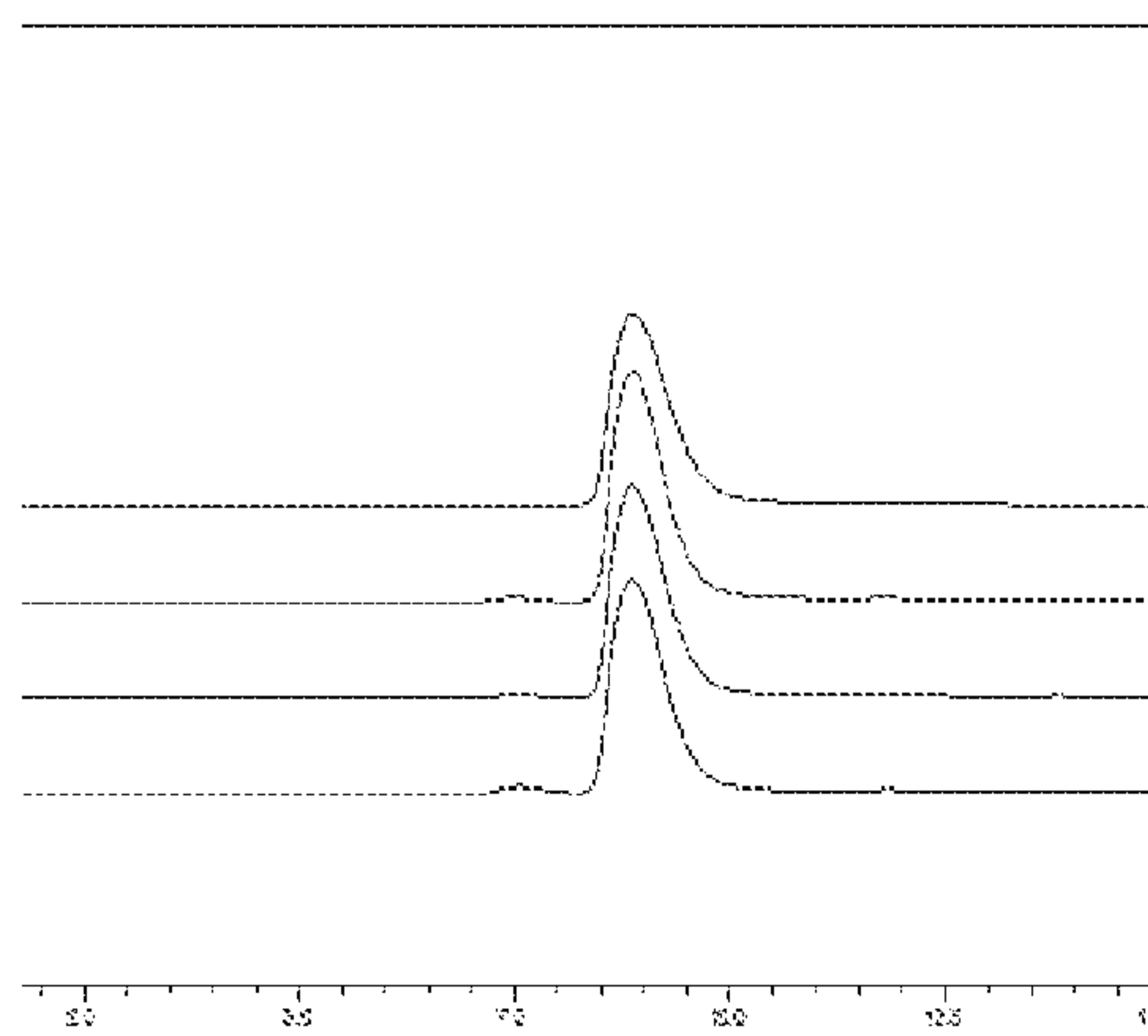
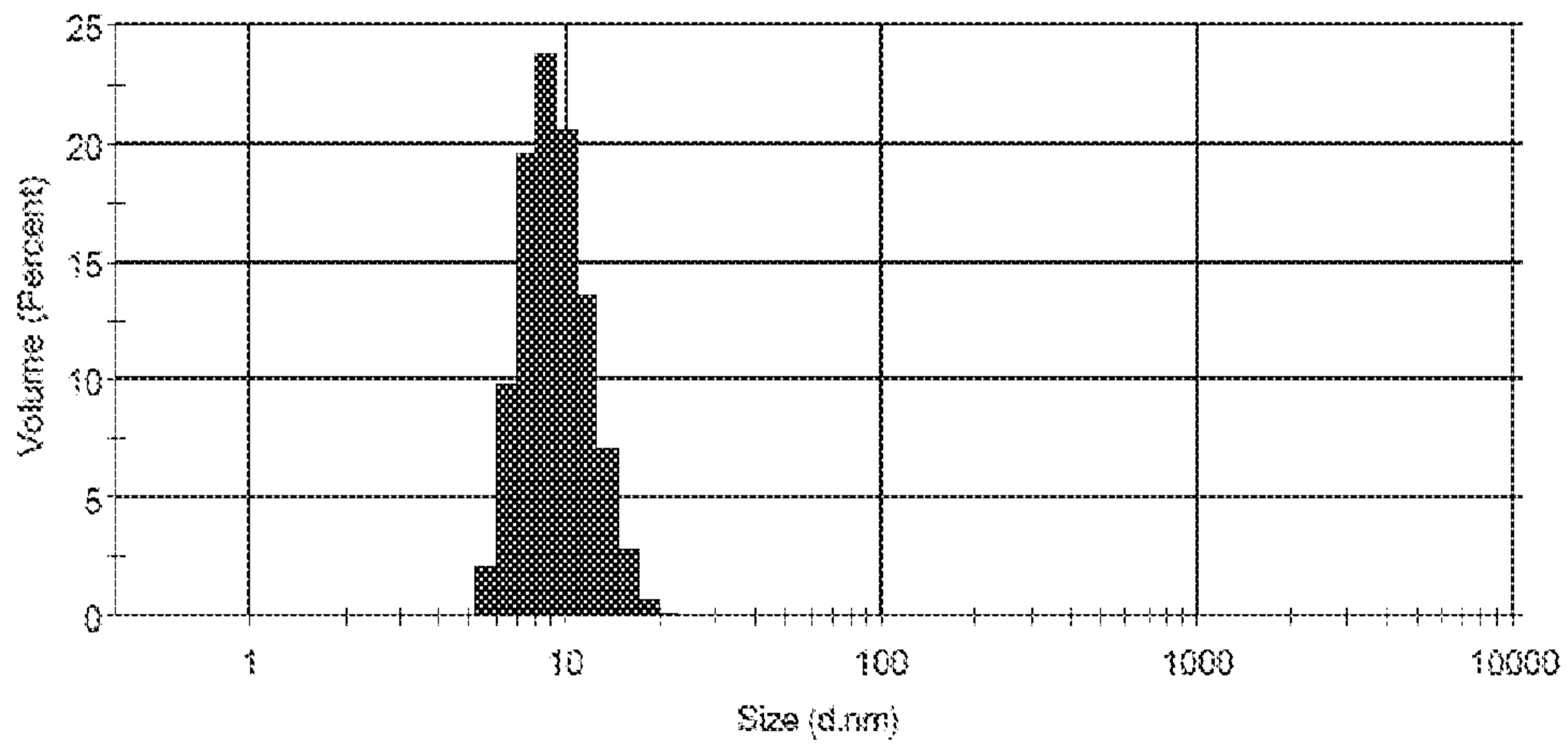


FIG. 4

**Homogeneous particle size of sHDL-Ag/CpG
as analyzed by cryoEM and dynamic light scattering**



Size Distribution by Volume

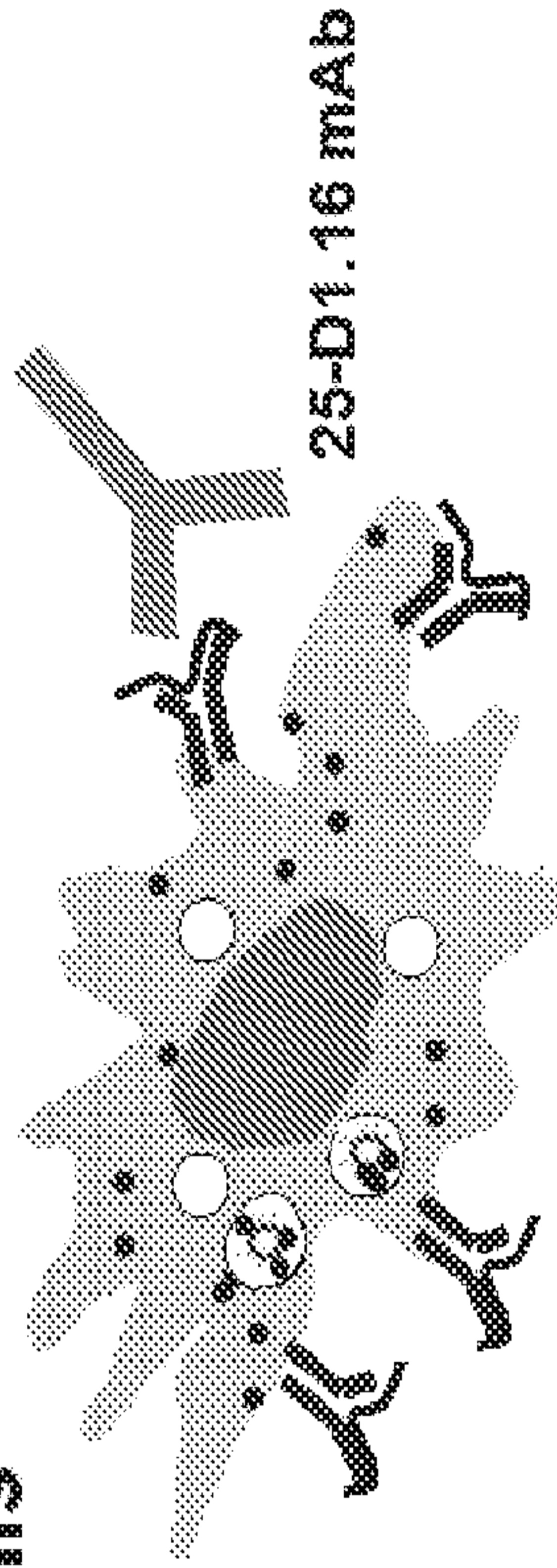


Black: original HDL/CpG
Pink: HDL/CpG+pep2 before column
Blue: HDL/CpG+pep2 after column
Brown: HDL/CpG+pep2 after column and frozen/thawed

REPLACEMENT SHEET

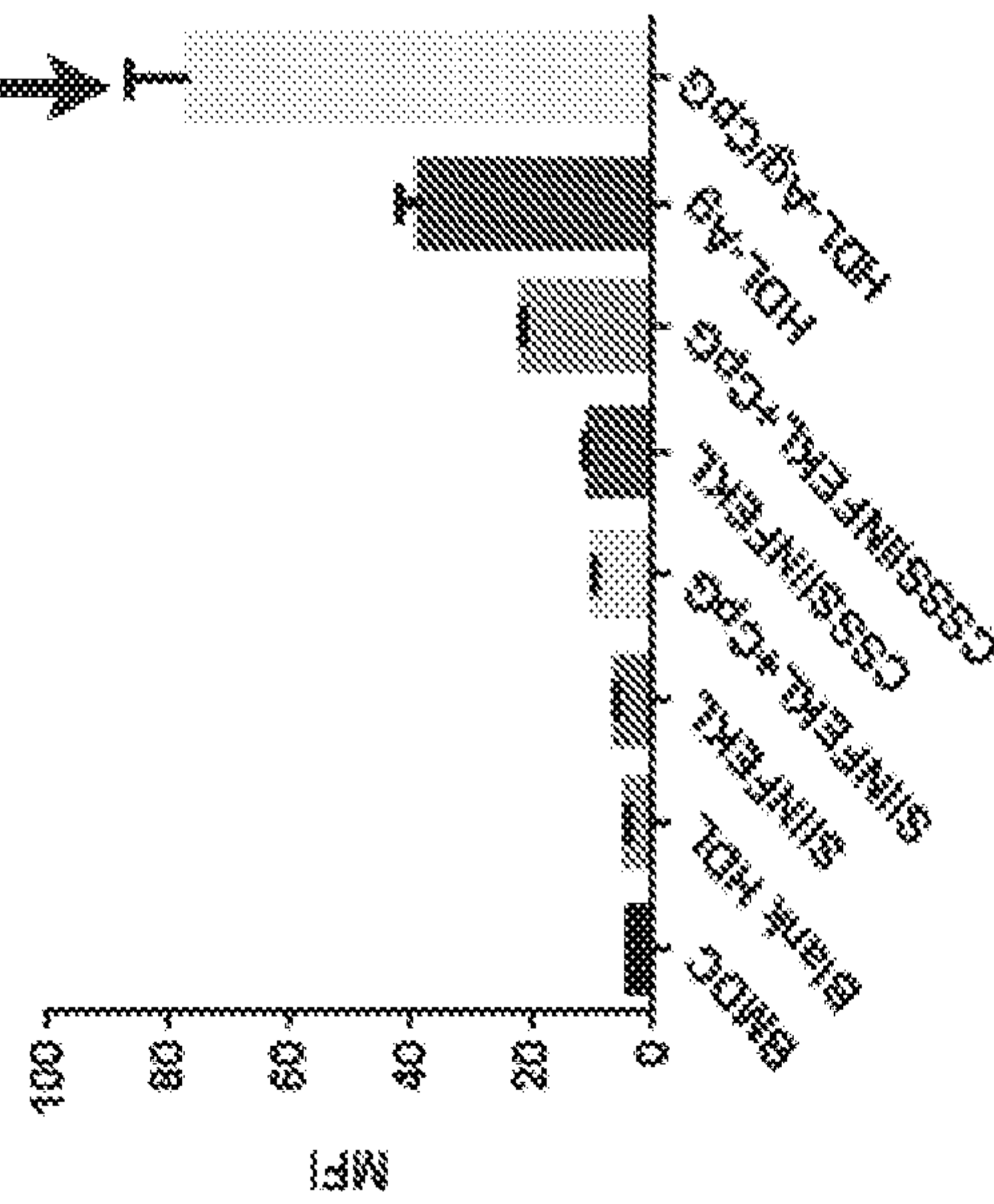
FIG. 5A

Compared with free antigen form, antigen delivery via sHDL significantly prolongs antigen presentation by dendritic cells



HDL-CpG increases antigen presentation

MHC class I - SIINFEKL display



DCs with OVA₂₅₇₋₂₆₄, SIINFEKL-H-Kb complex by prolonging antigen display on MHC-I

Cross presentation kinetics

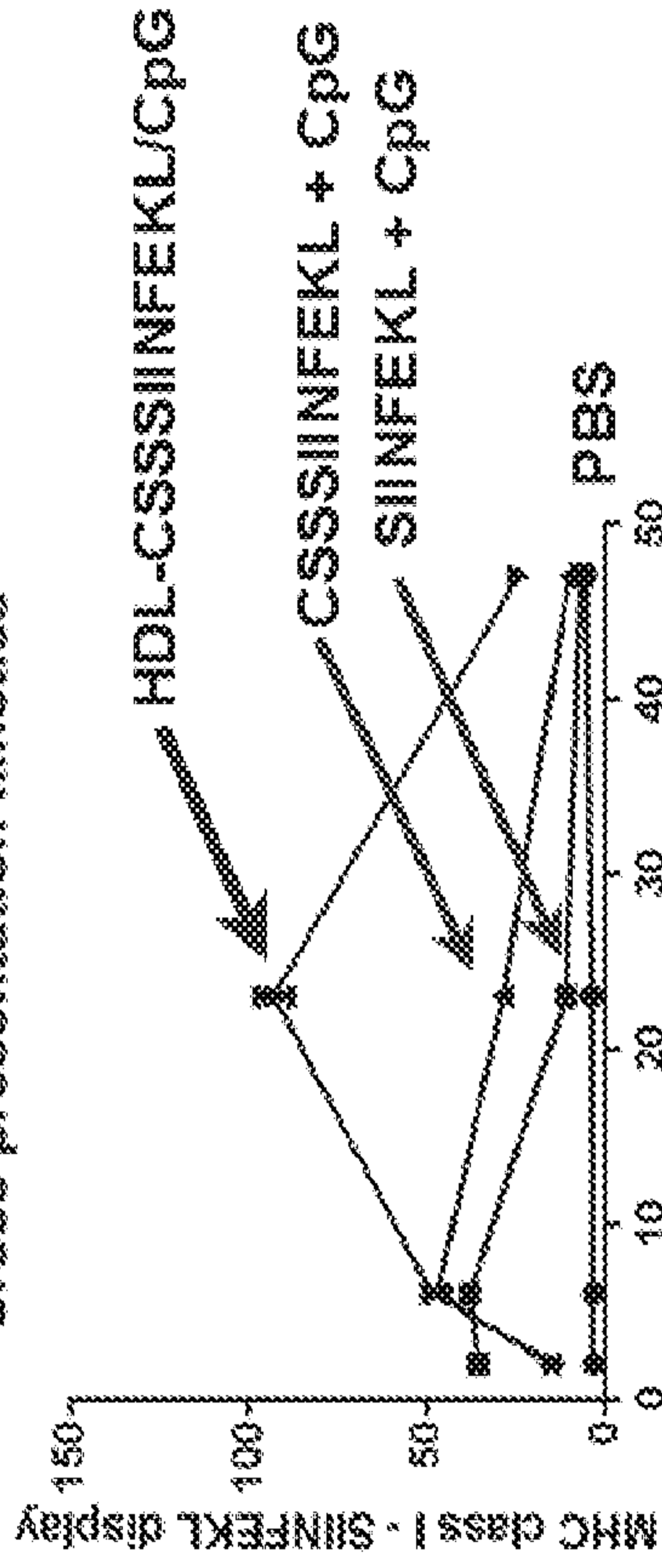


FIG. 5B
Compared with free antigen form,
antigen delivery via sHDL significantly prolongs antigen
presentation by dendritic cells

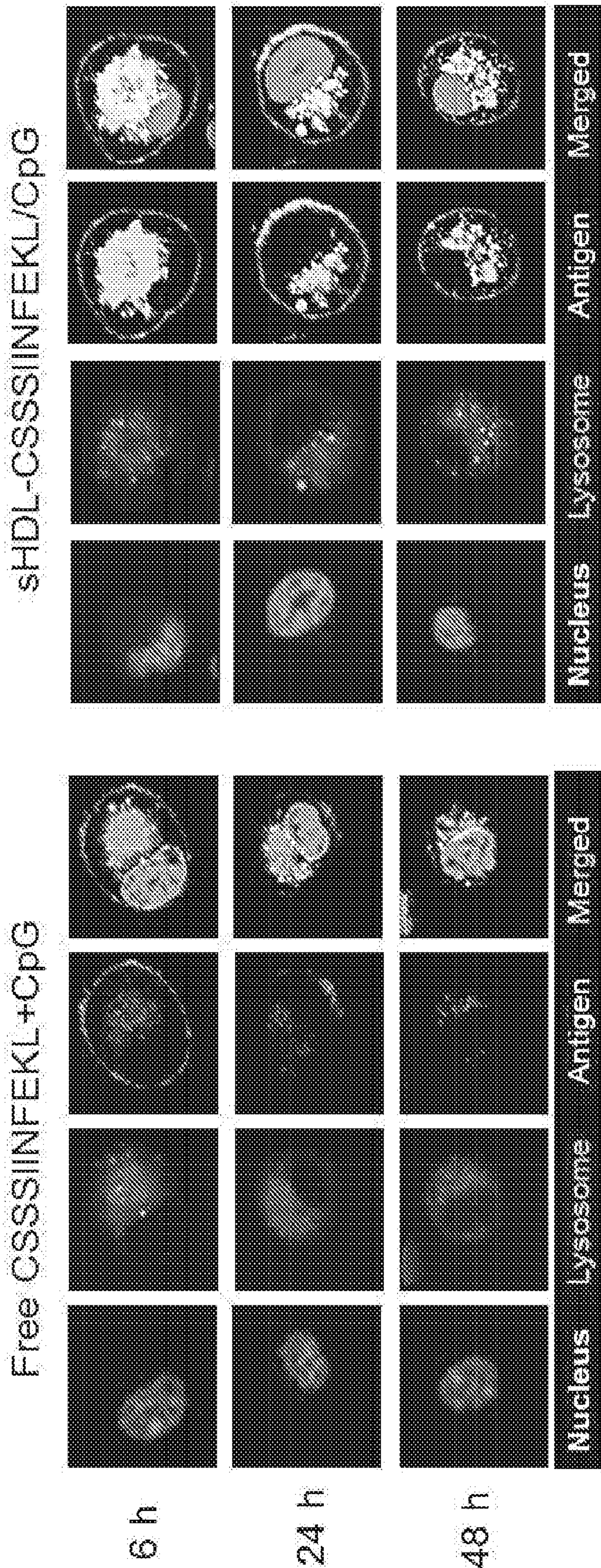
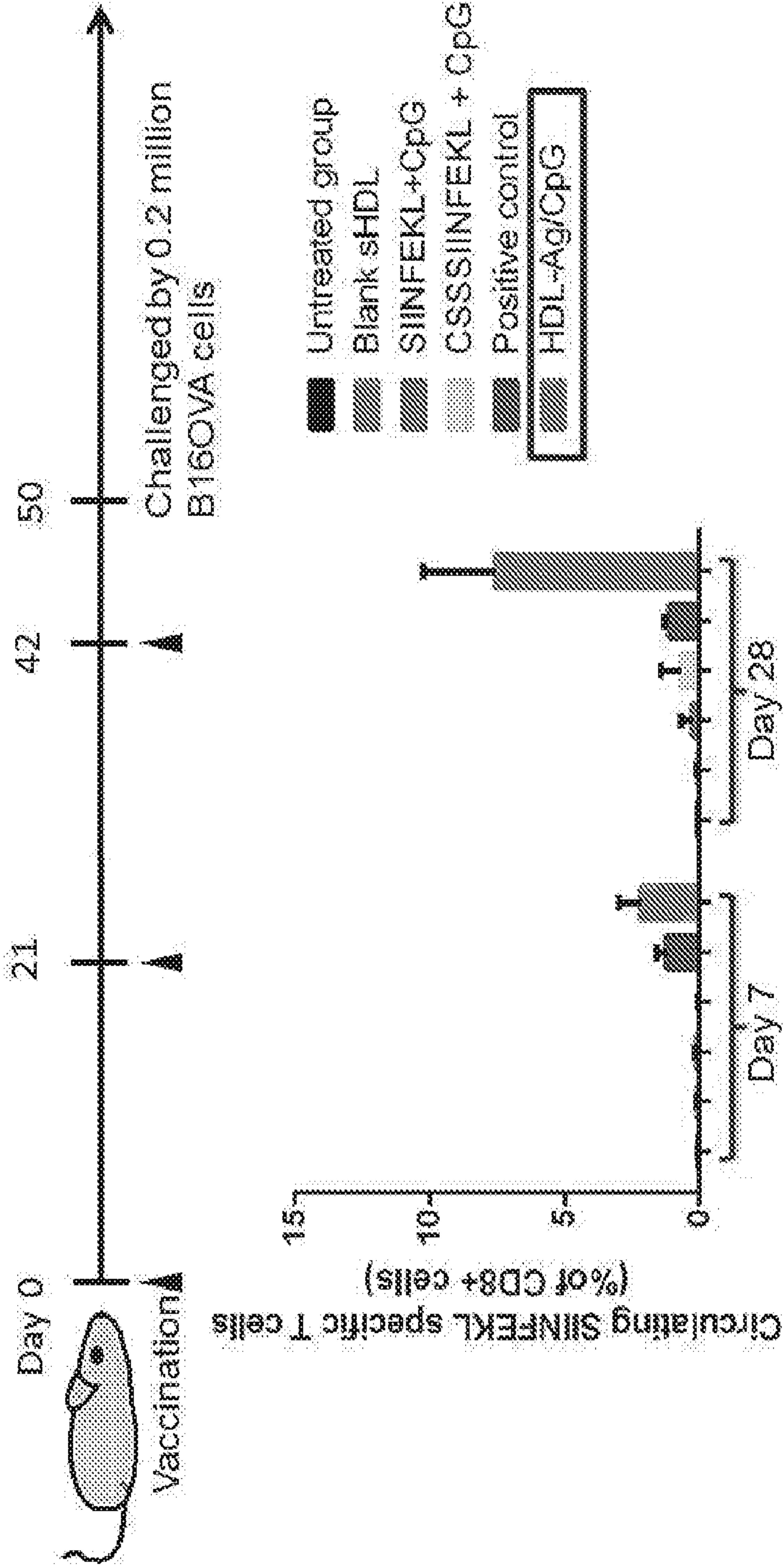


FIG. 6
sHDL-Ag/CpG significantly enhances elicitation of antigen-specific CD8+ T cells, compared with vaccination with free antigen mixed with conventional adjuvants



Positive control = CpG + Montanide, which is a potent adjuvant in Phase I cancer vaccine trials. (Valmori et al., PNAS 2007, 8947-52; Speiser et al., JCI 2005, 739-746.)

FIG. 7

sHDL-Ag/CpG vaccination elicits strong CD8+ T cell responses in tumor-bearing mice and reduces tumor growth

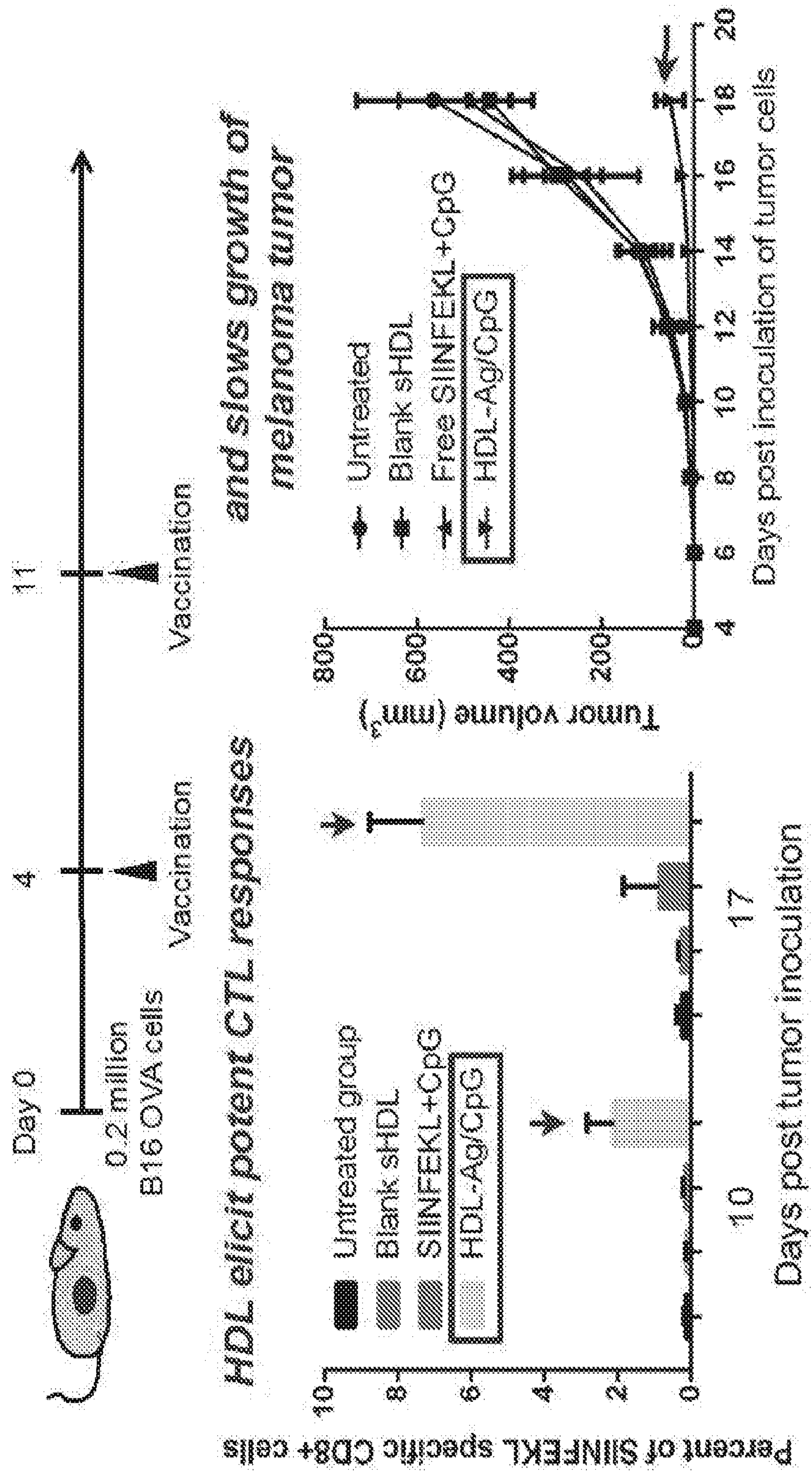


FIG. 8

REPLACEMENT SHEET

Compared with free soluble form, alpha-GalCer delivered via sHDL significantly enhances CD1d presentation on antigen-presenting cells.

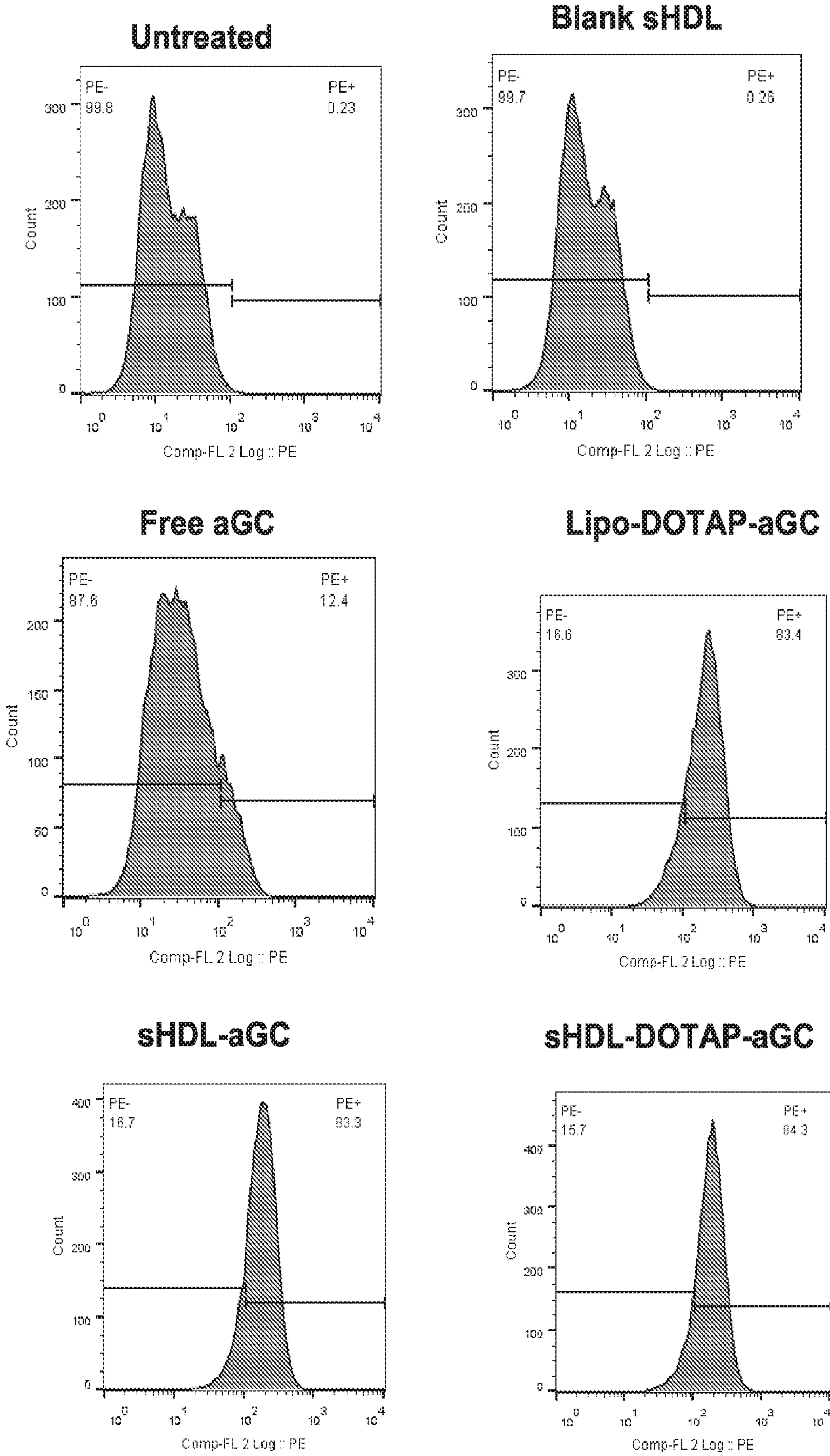
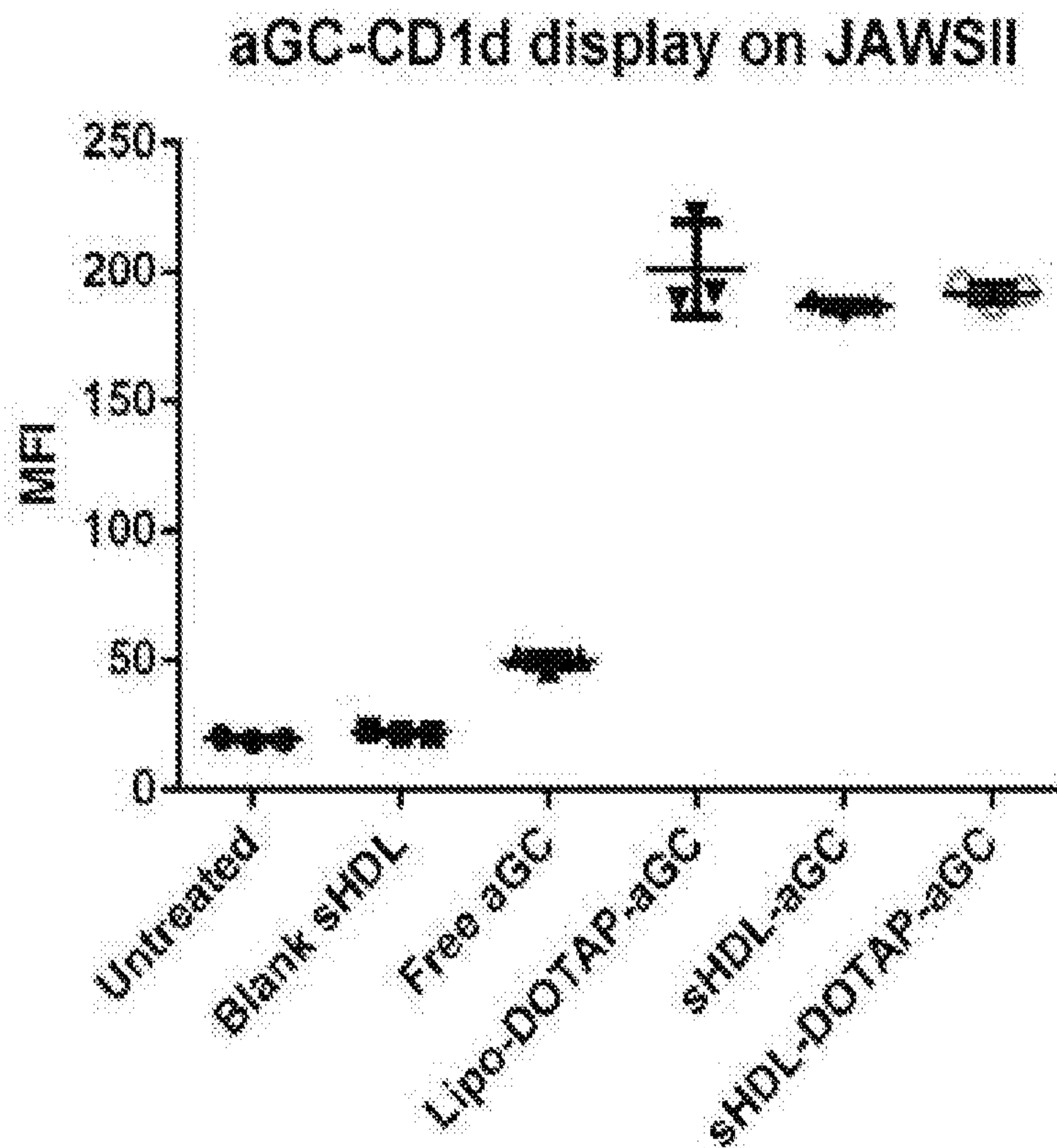


FIG. 8 Cont'd.



sHDL was better than free aGC, but similar to liposomes;

DOTAP didn't significantly affect the result.

FIG. 9

Lyophilization offers a convenient method of large-scale synthesis of sHDL loaded with alpha-GalCer.

Effect of sHDL preparation method on aGC-CD1d level

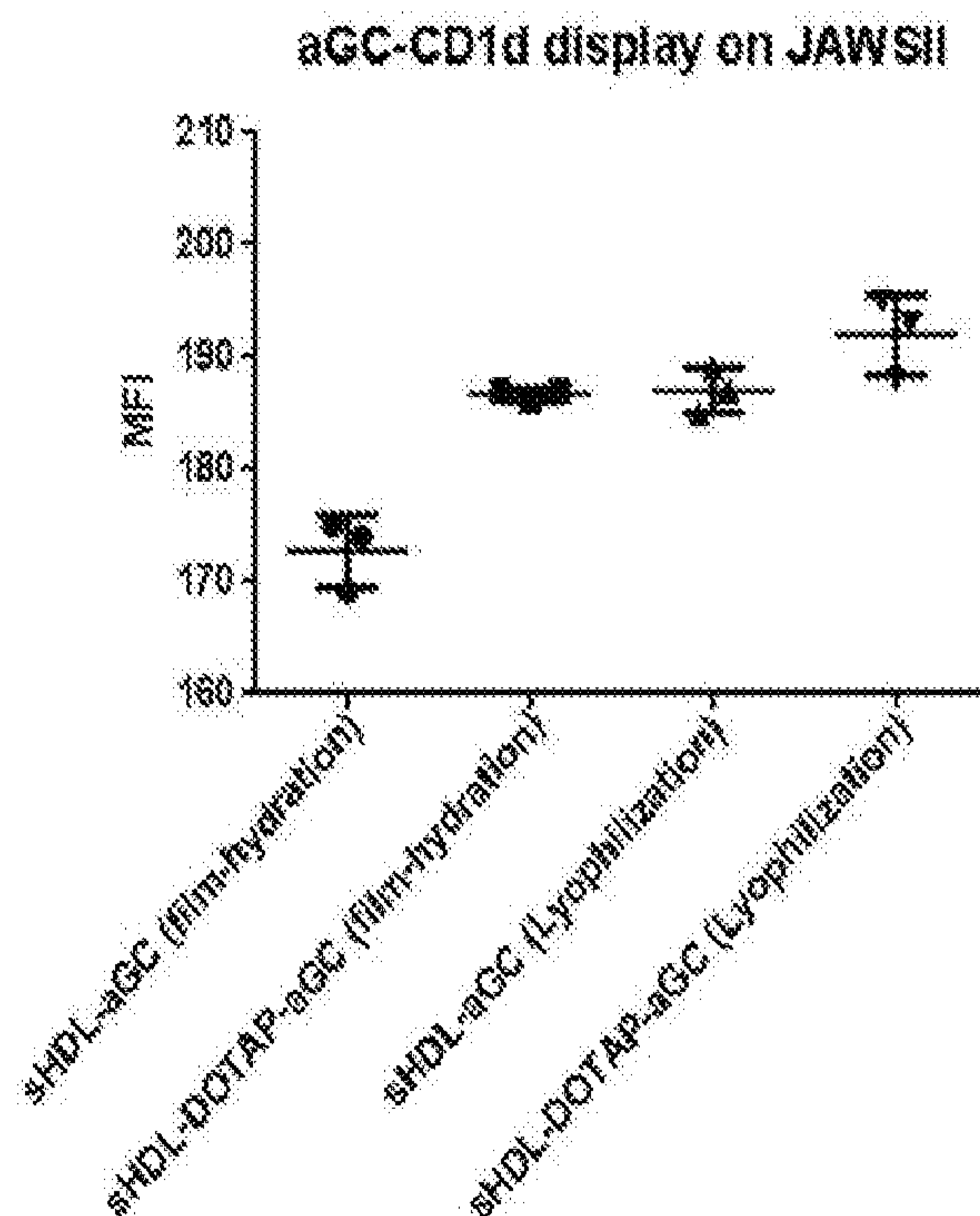
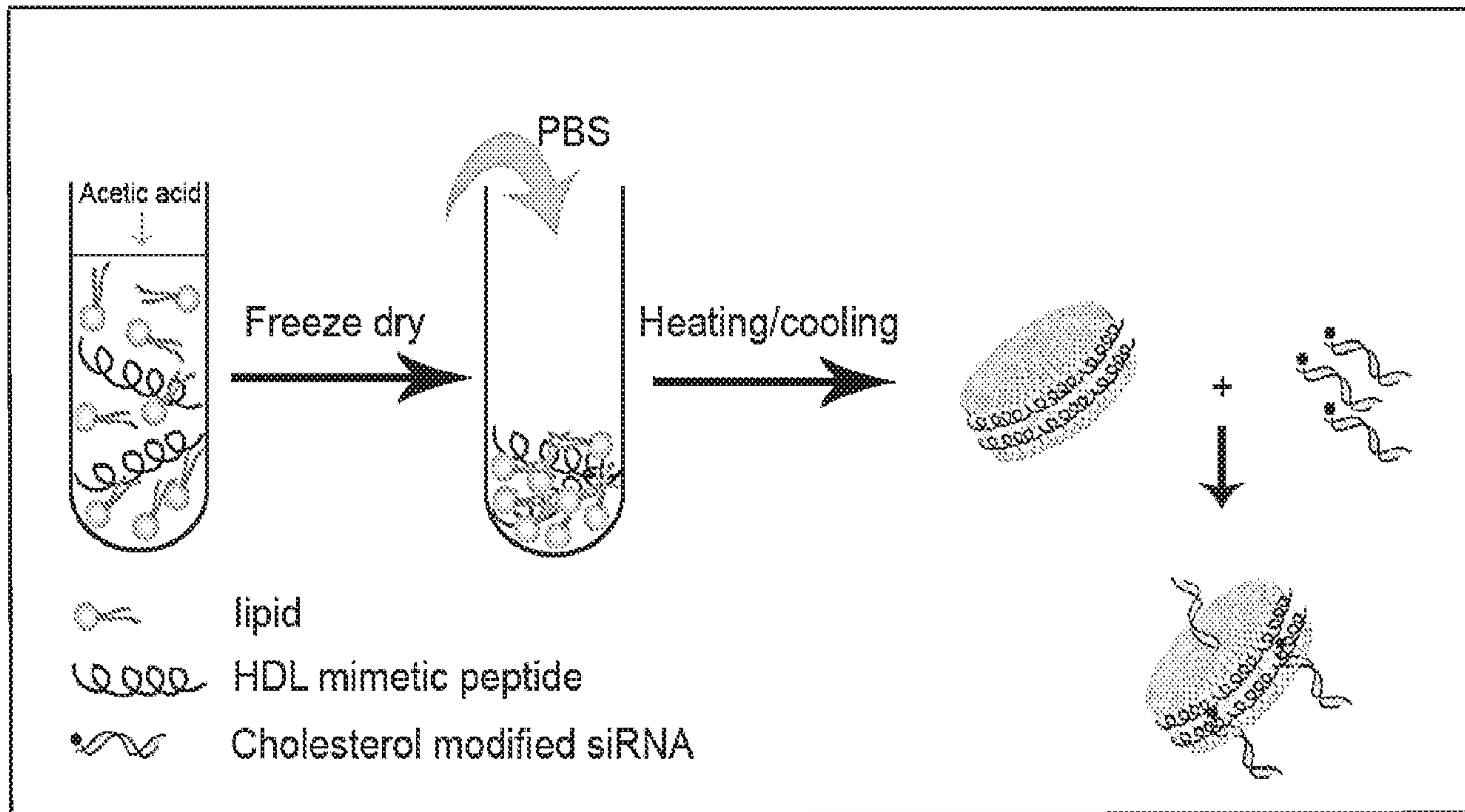


FIG. 10



REPLACEMENT SHEET

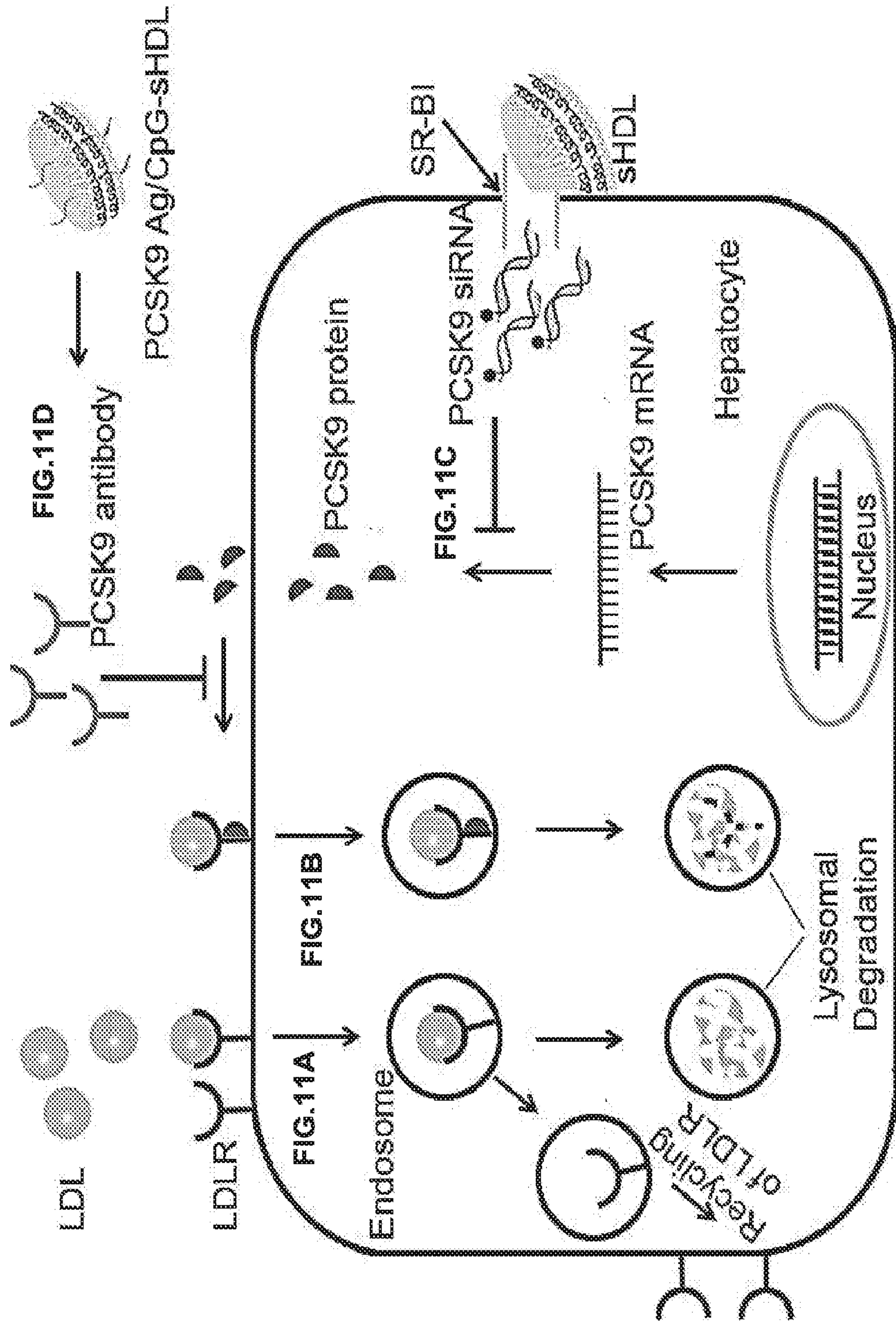


FIG. 12A

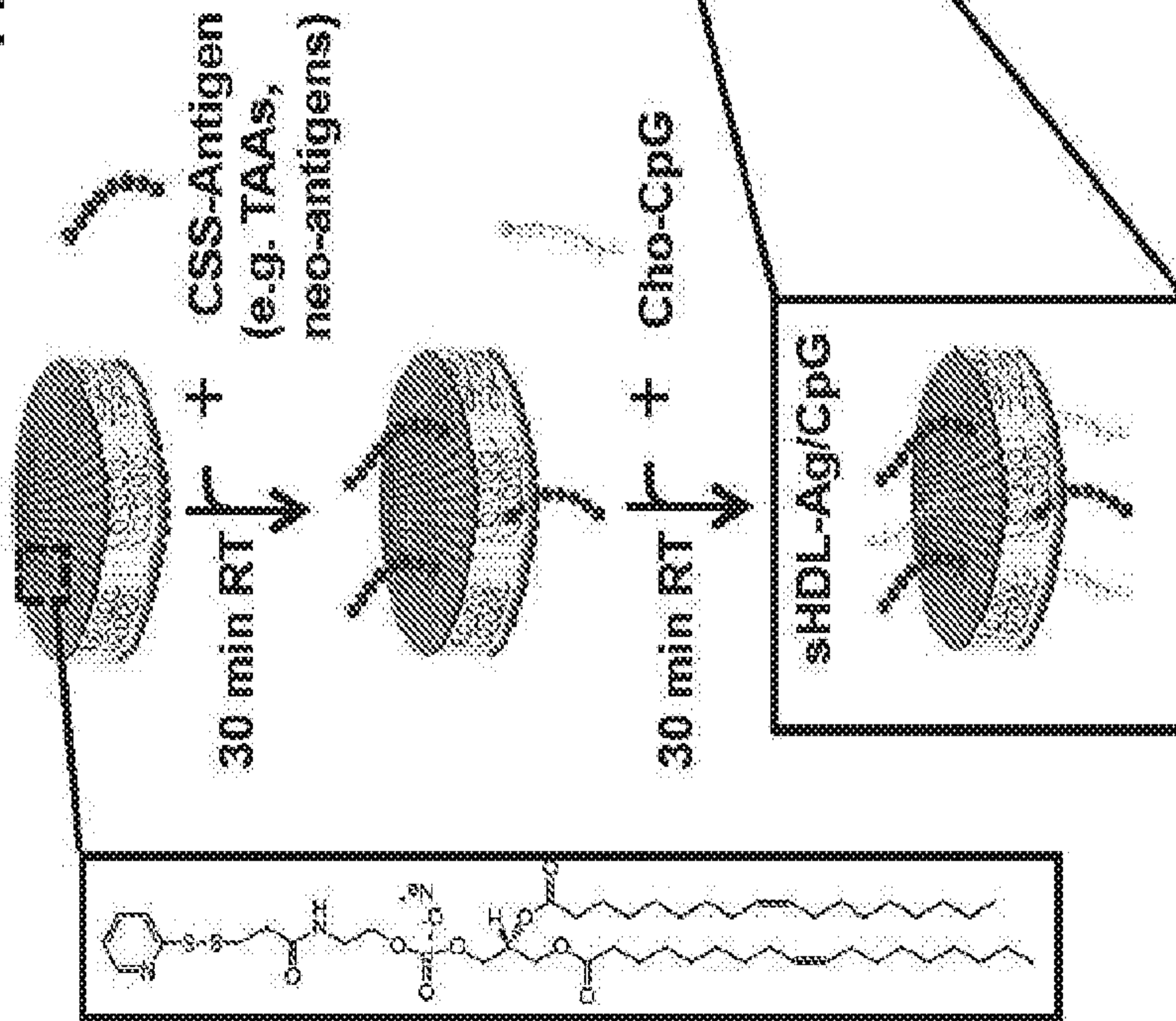


FIG. 12B

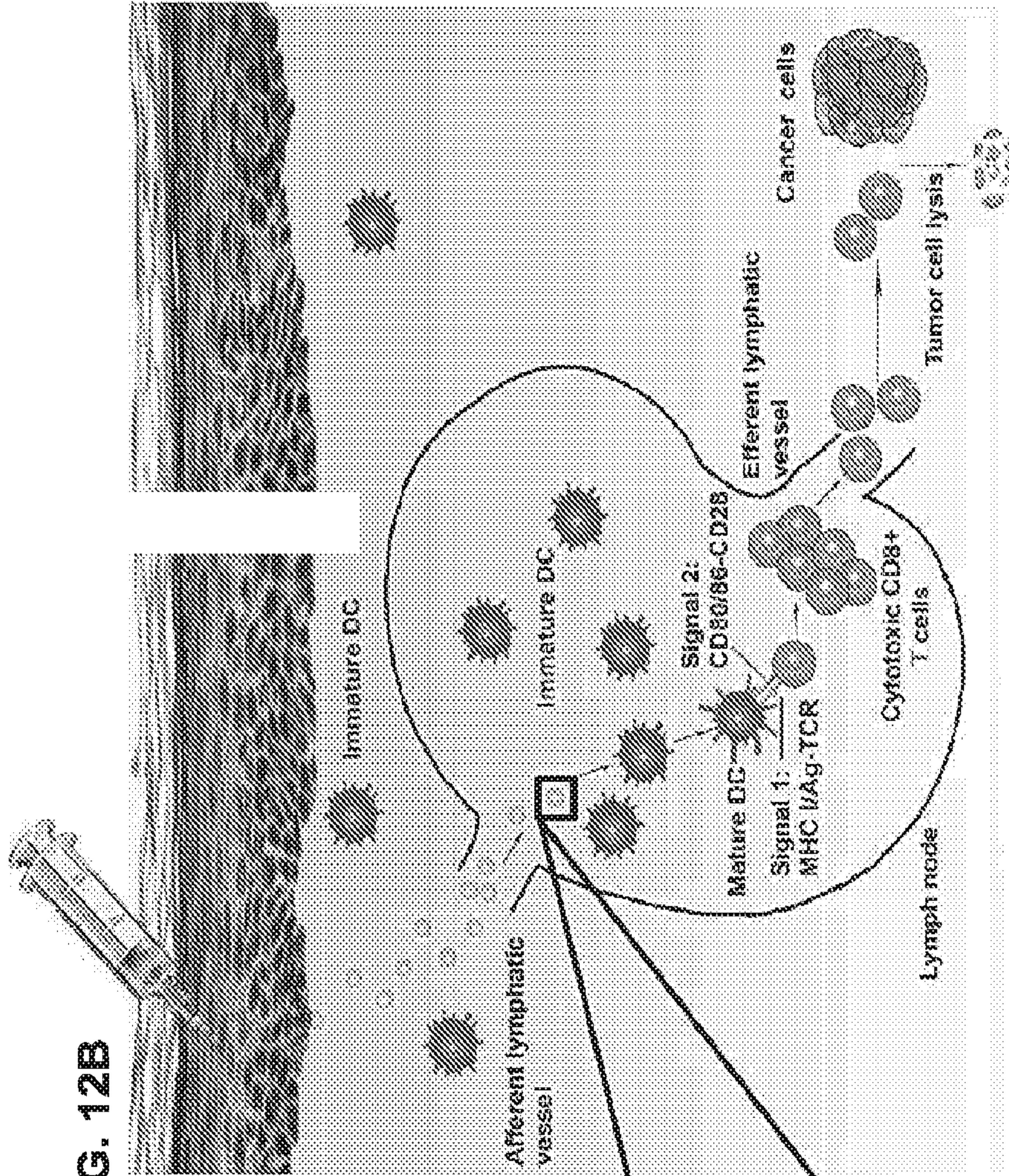


FIG. 13A

	Sample ID	Sequence	Size (d. nm)	PDI
SEQ ID NO: 4	22A	PVLDLFRELLNELLEALKQKLIK	10.6 ± 0.2	0.18 ± 0.01
SEQ ID NO: 75	22A-1	PVLDEFREKLNEELEALKQKLIK	N.D.	N.D.
SEQ ID NO: 1	22A-2	PVLDLFRELLNELLEZLKQKLIK	25.6 ± 2.7	0.28 ± 0.10
SEQ ID NO: 15	22A-3	PVLDLFRELWNELLEALKQKLIK	13.5 ± 0.5	0.25 ± 0.03
SEQ ID NO: 28	22A-4	PVLDLFRELLNELWEALKQKLIK	14.3 ± 0.2	0.29 ± 0.01
SEQ ID NO: 35	22A-5	PVLDWFRELLNELLEALKQKLIK	20.2 ± 2.2	0.27 ± 0.06
SEQ ID NO: 54	22A-6	PVLDLFRELLNEWLEALKQKLIK	13.9 ± 0.2	0.29 ± 0.01
SEQ ID NO: 79	22A-7	PVLDEFRELLNELLEALKQKLIK	13.6 ± 1.8	0.38 ± 0.10
SEQ ID NO: 106	22A-8	PVLDLFREKLNEELEALKQKLIK	N.D.	N.D.
SEQ ID NO: 99	22A-8	KLKQKLAELLENLLERFLDLVP	N.D.	N.D.
SEQ ID NO: 4	22A-10	PVLDLFRELLNELLEALKQKLIK (D-amino acids)	10.8 ± 0.3	0.19 ± 0.01

4°C (stored for one month)



FIG. 13B

	22A	22A-1	22A-2	22A-3	22A-4	22A-5	22A-6	22A-7	22A-8	22A-9	22A-10
DOPC	+	-	-	-	-	-	-	-	-	-	-
POPC	-	+	-	-	-	-	-	-	-	-	-
DMPC	-	-	+	-	-	+	-	-	-	-	-
DPPC	-	-	-	-	+	-	-	+	-	-	-
22A	+	+	+	+	+	-	-	-	-	-	-

FIG. 14A

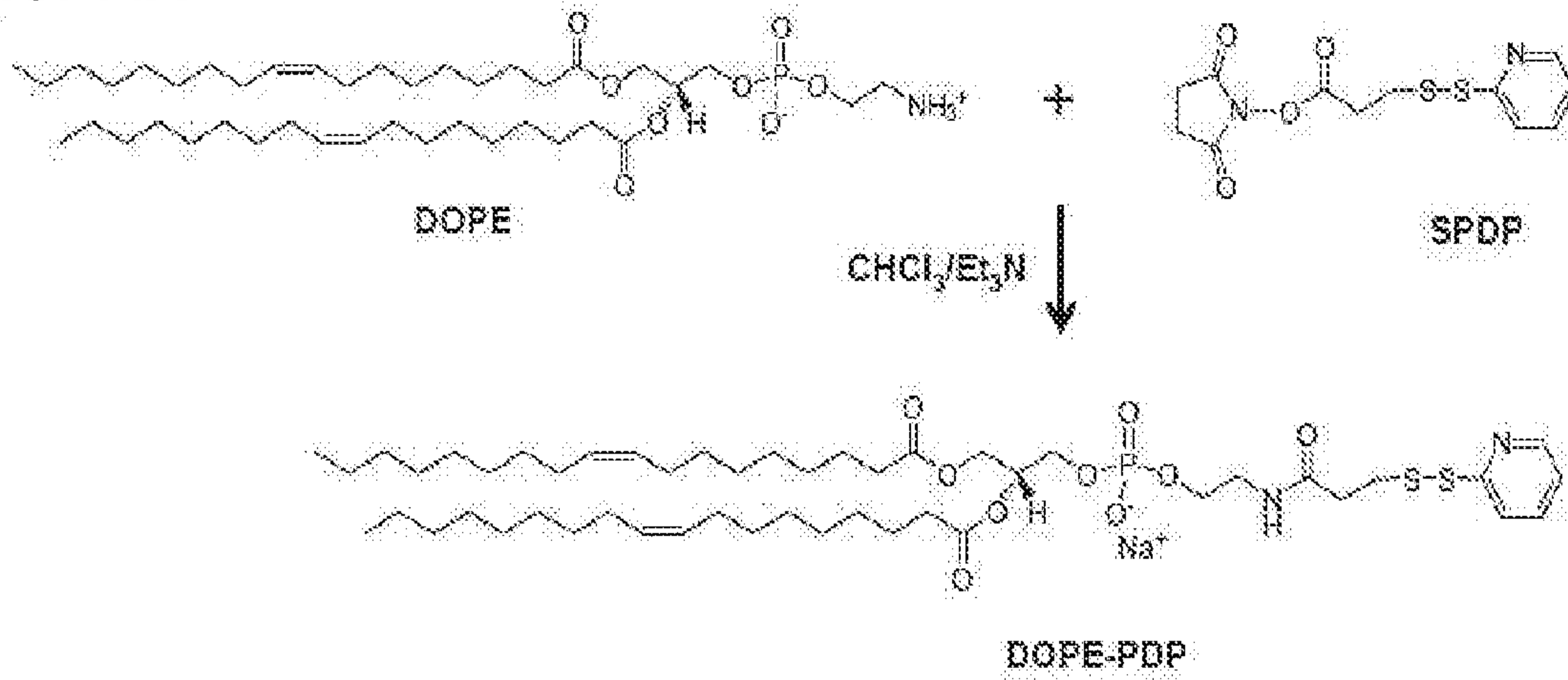
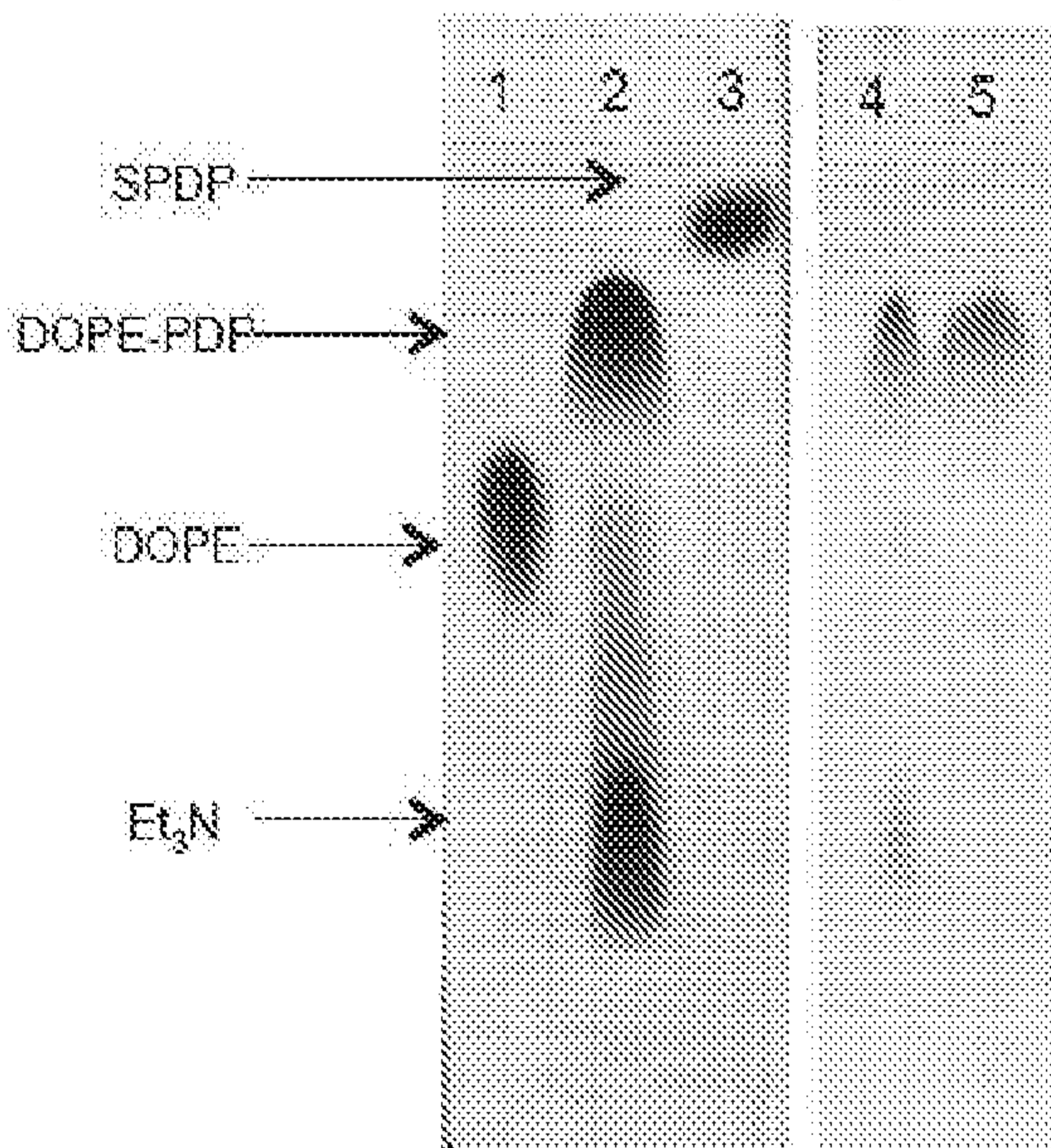


FIG. 14B



- 1 = DOPE
 2 = reaction mixture
 3 = SPDP
 4 = reaction mixture before purification
 5 = reaction mixture after purification

FIG. 14C

FIG. 14D

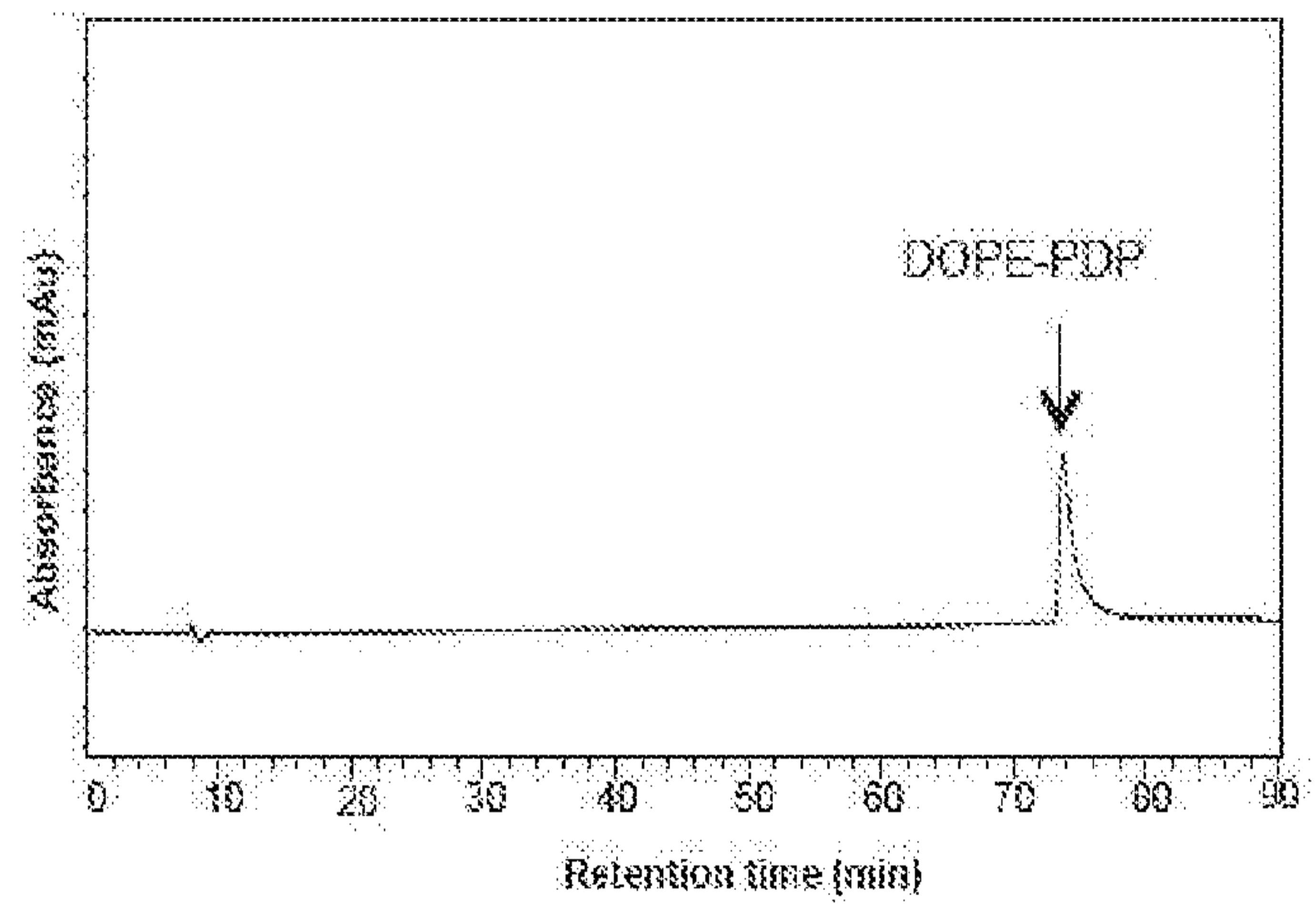


FIG. 15A

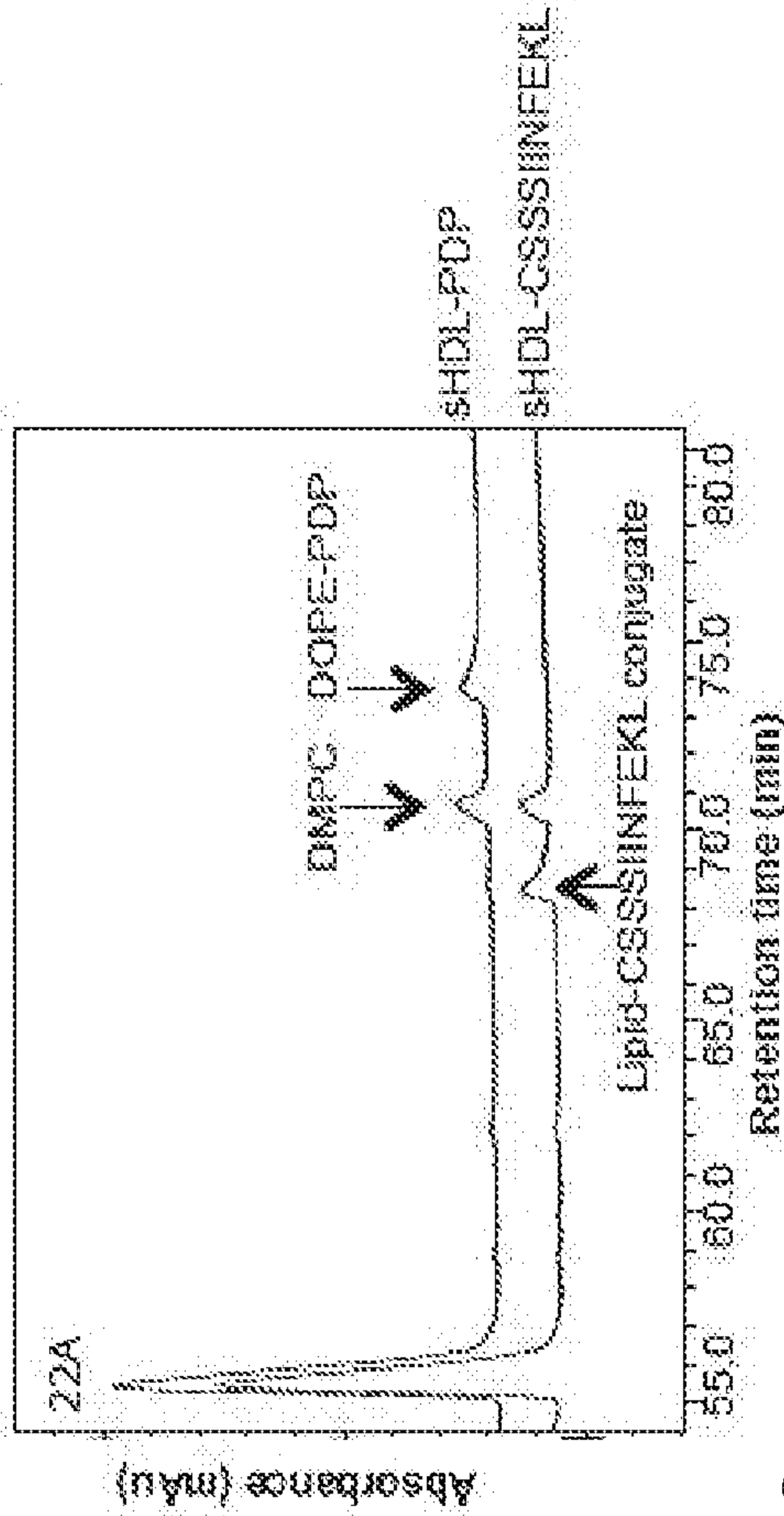


FIG. 15B

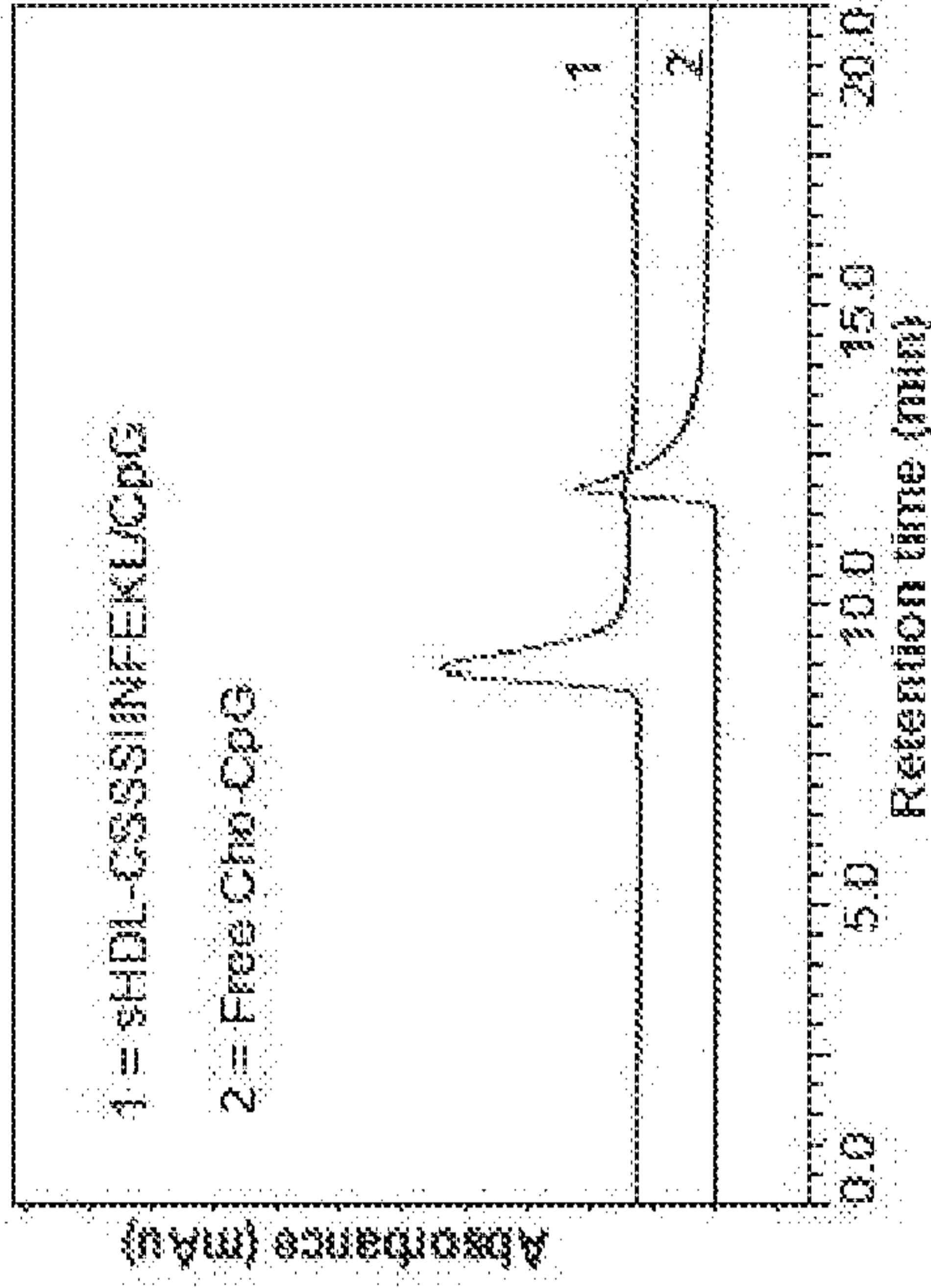


FIG. 15C

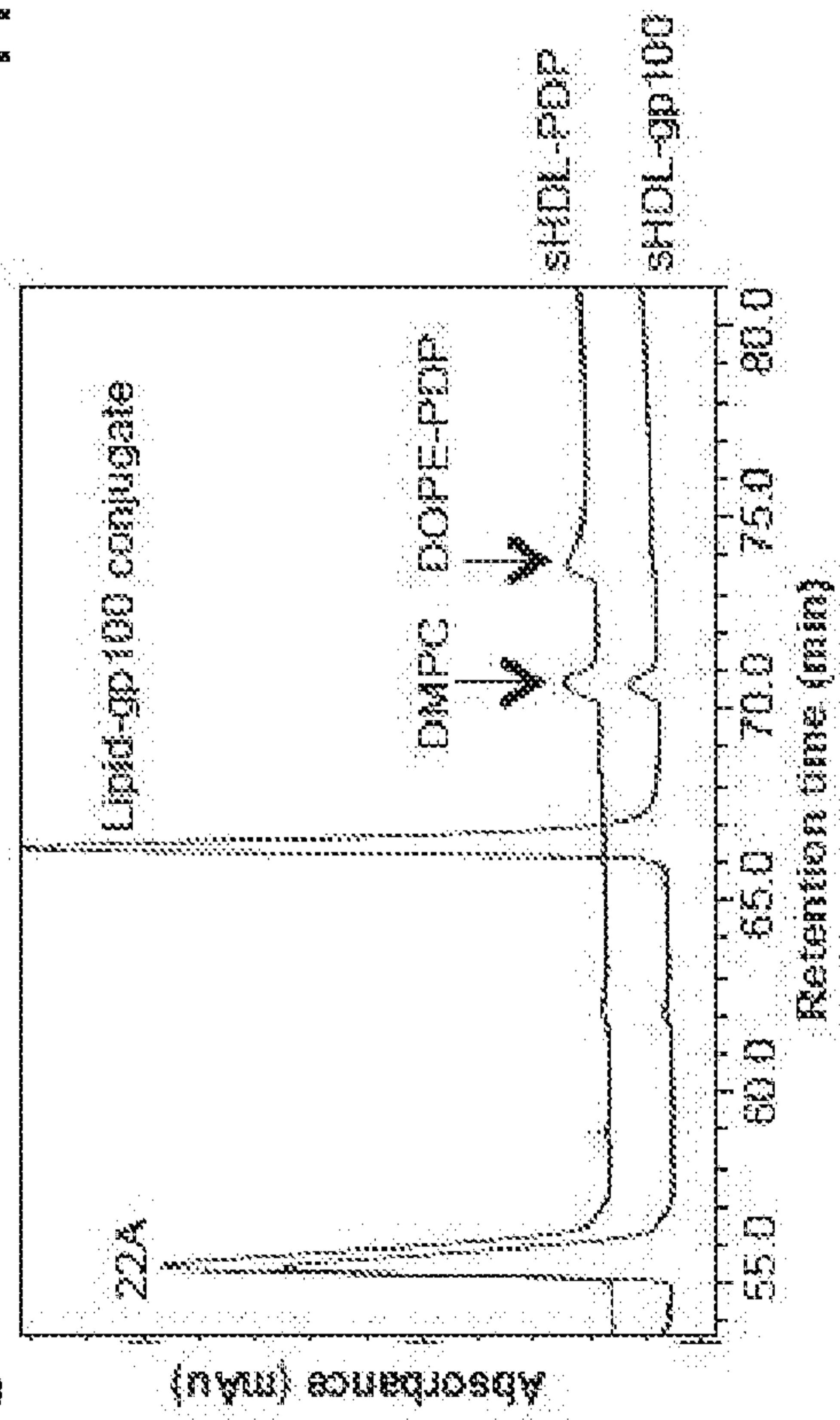
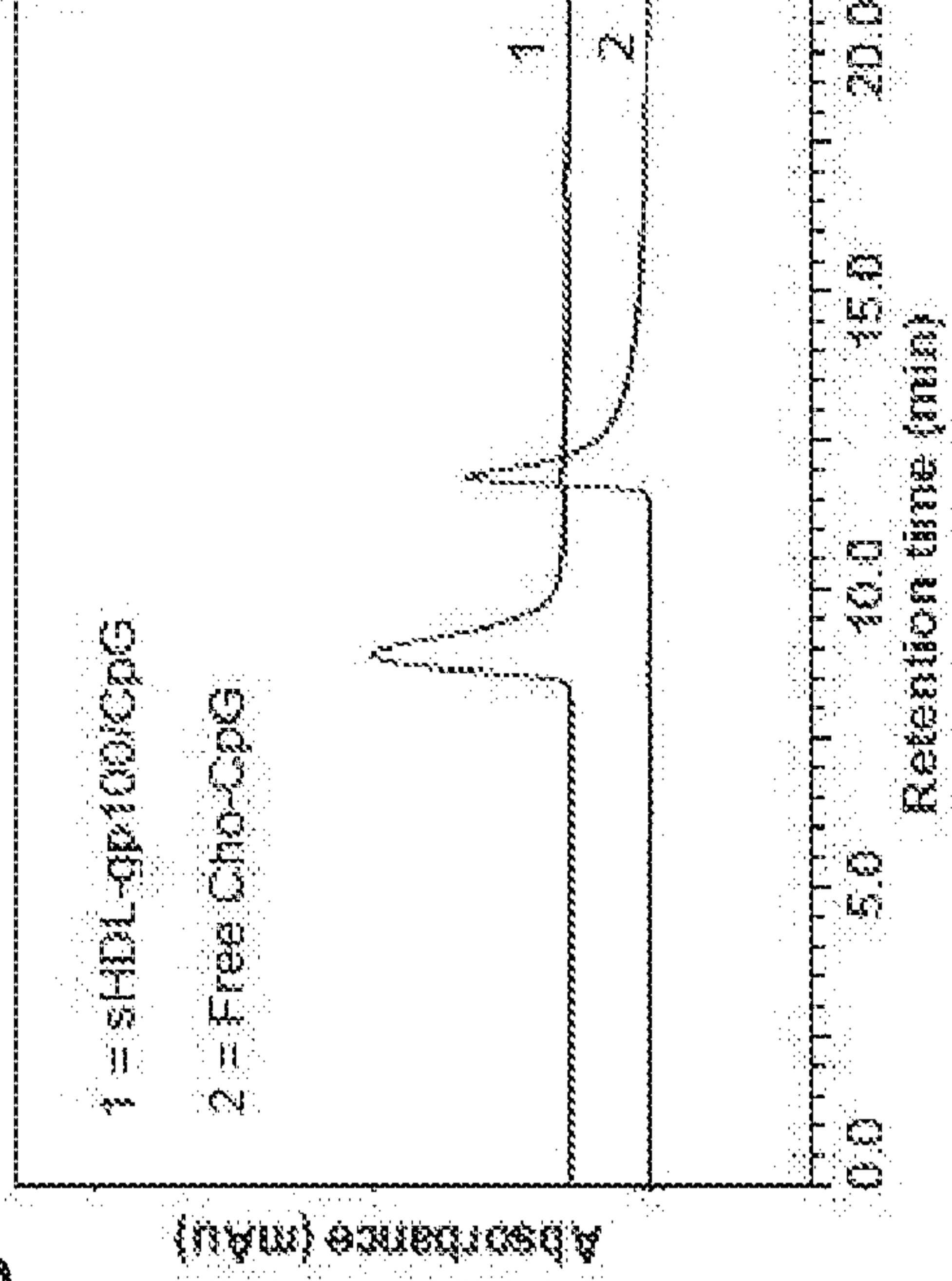


FIG. 15D



REPLACEMENT SHEET

FIG. 15E

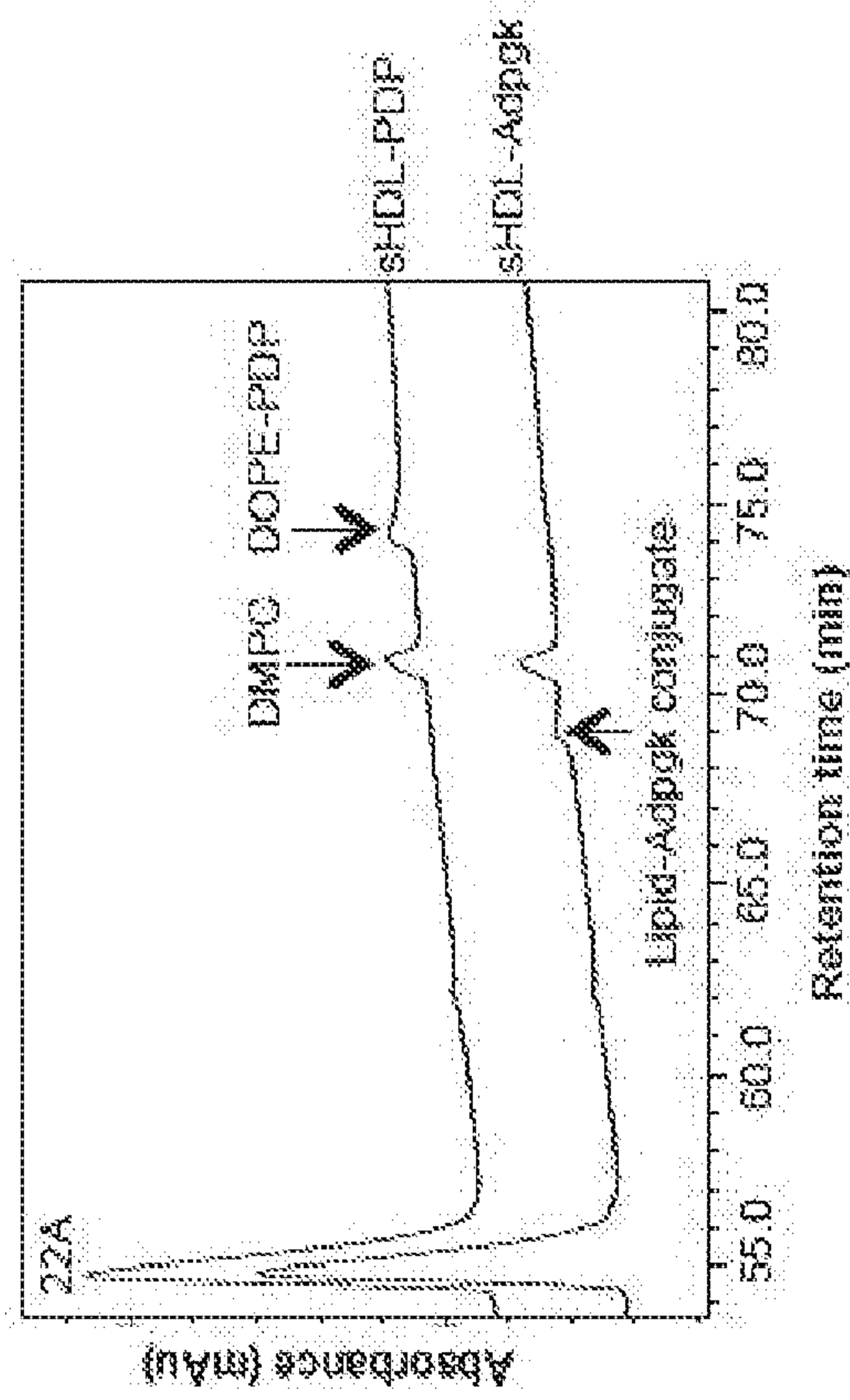


FIG. 15F

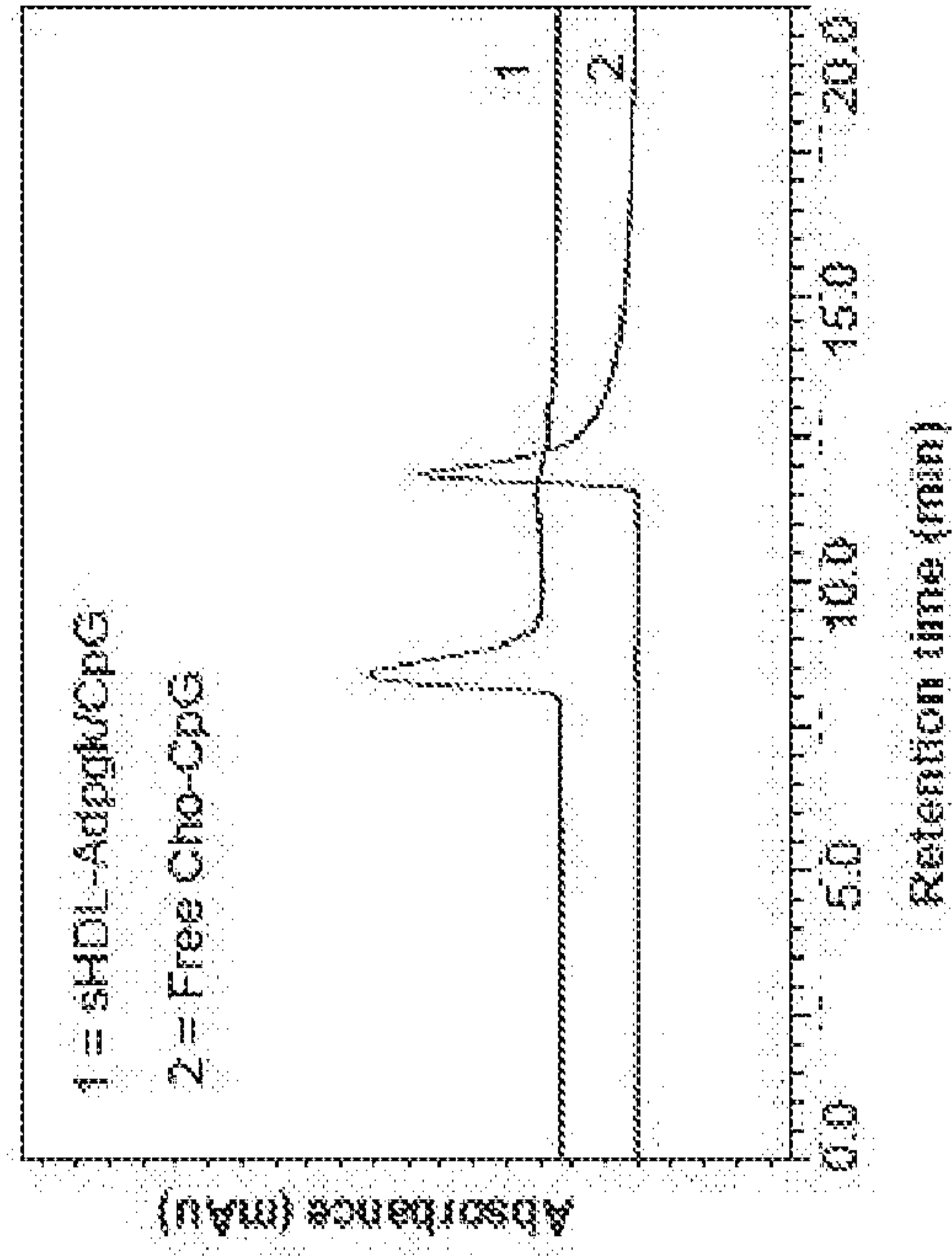


FIG. 16A

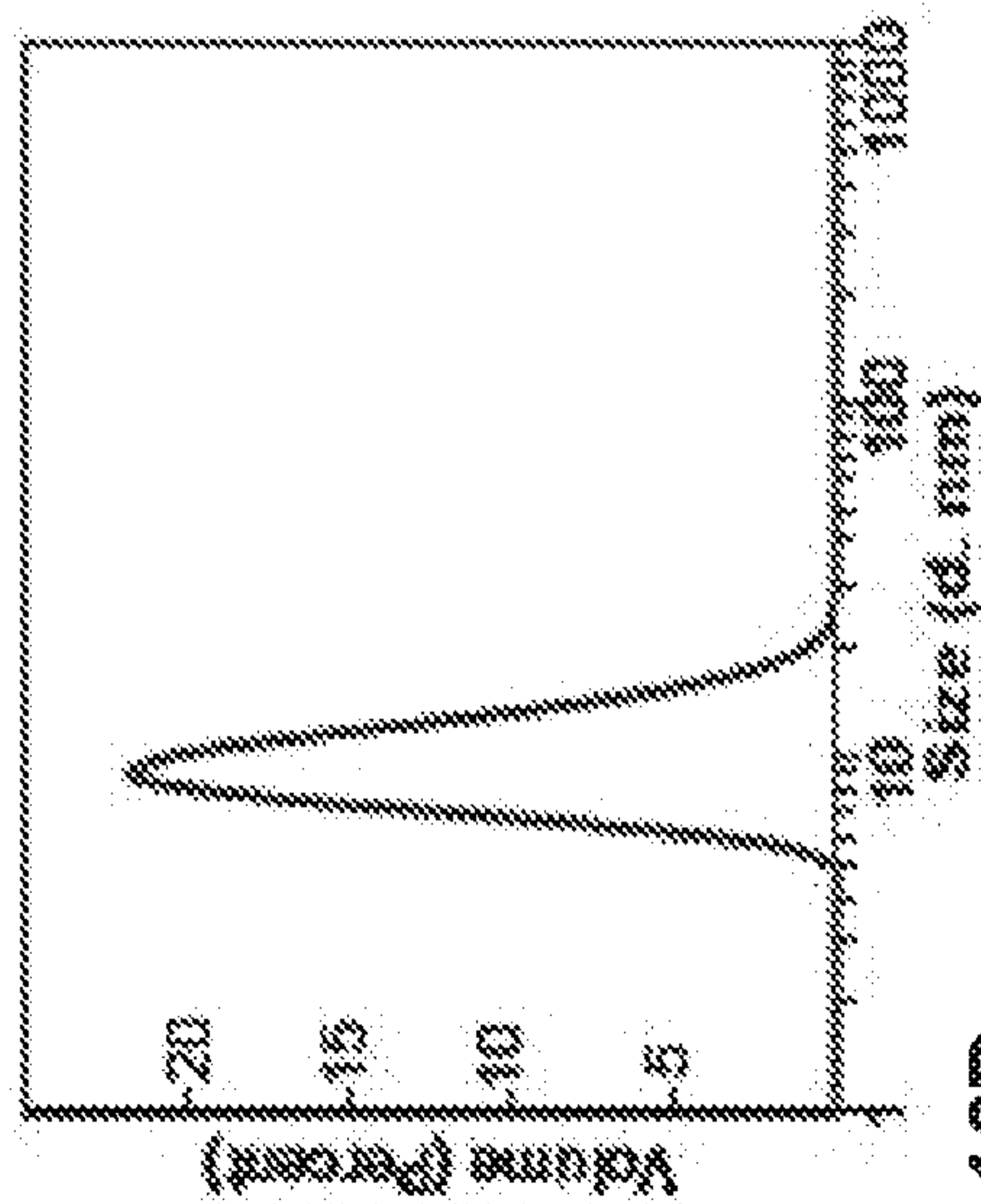


FIG. 16B

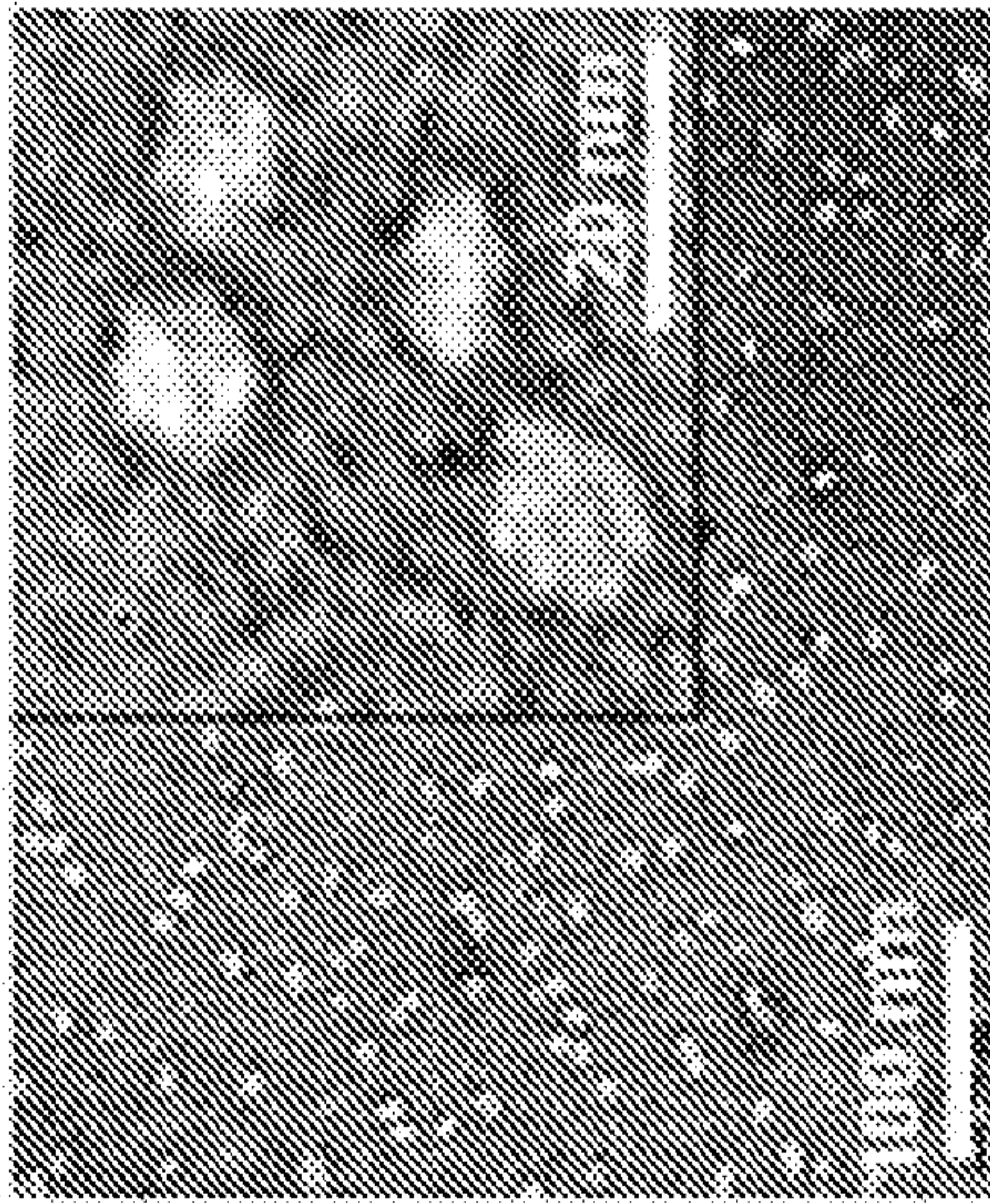


FIG. 16C

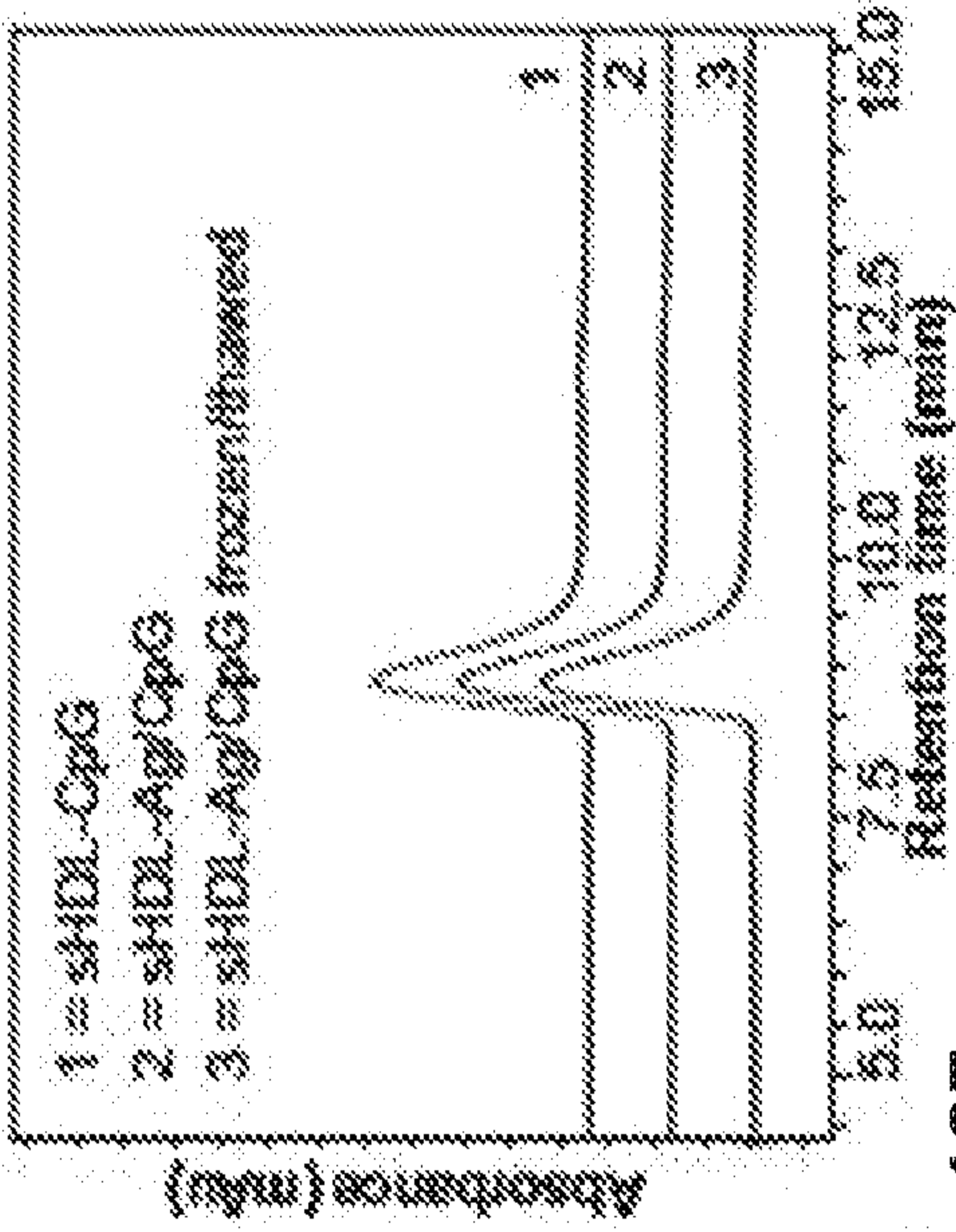


FIG. 16D

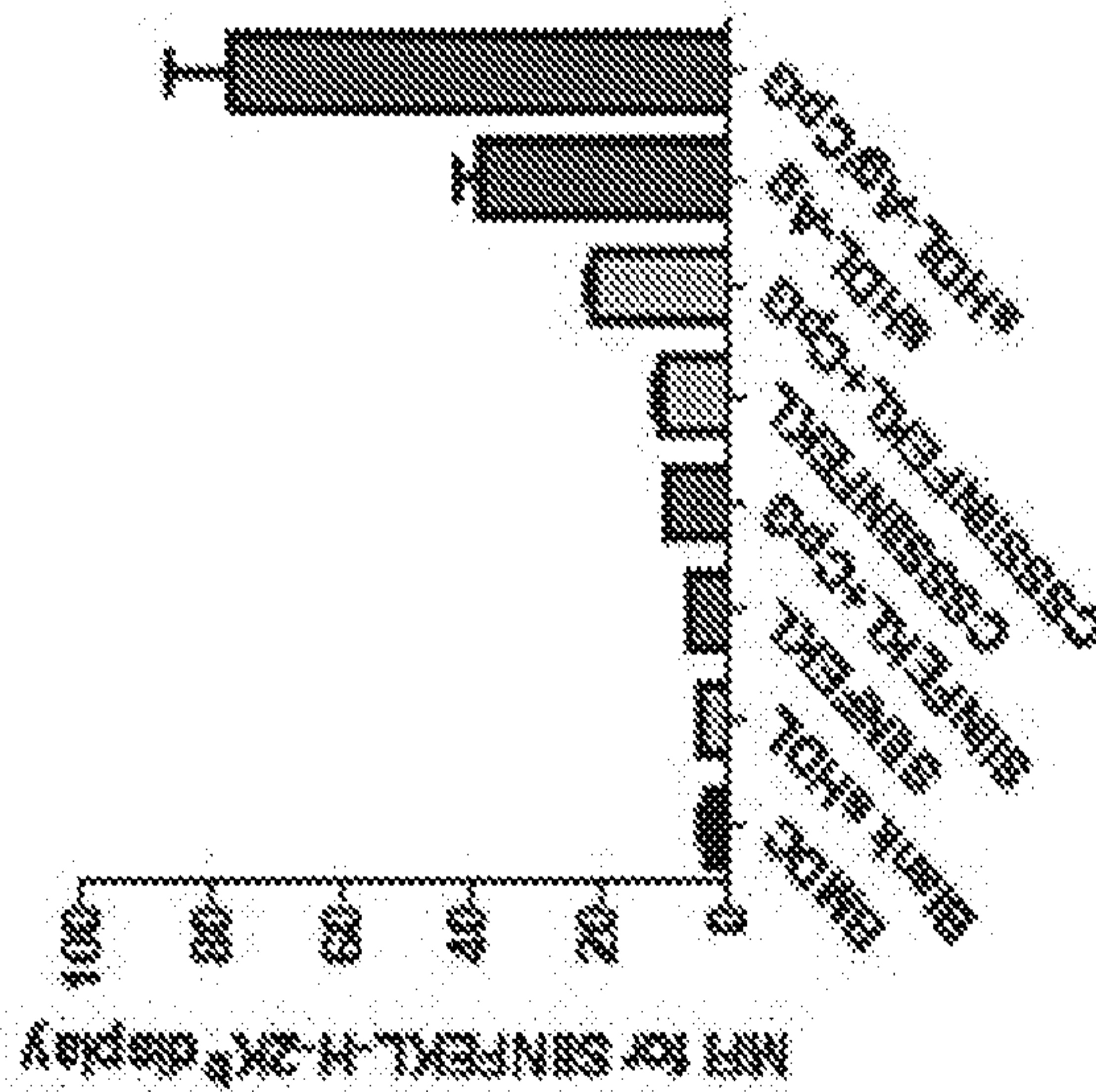


FIG. 16E

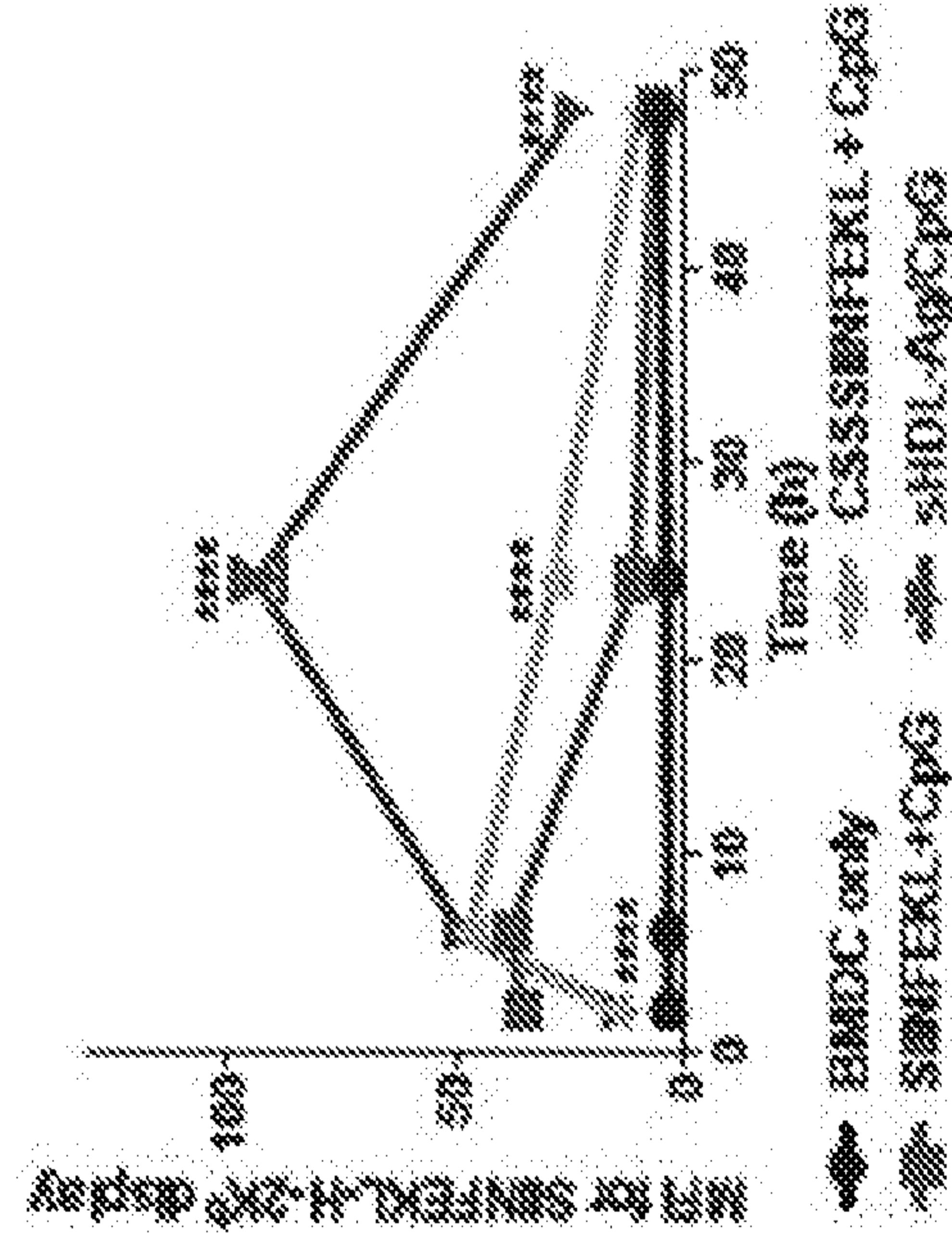
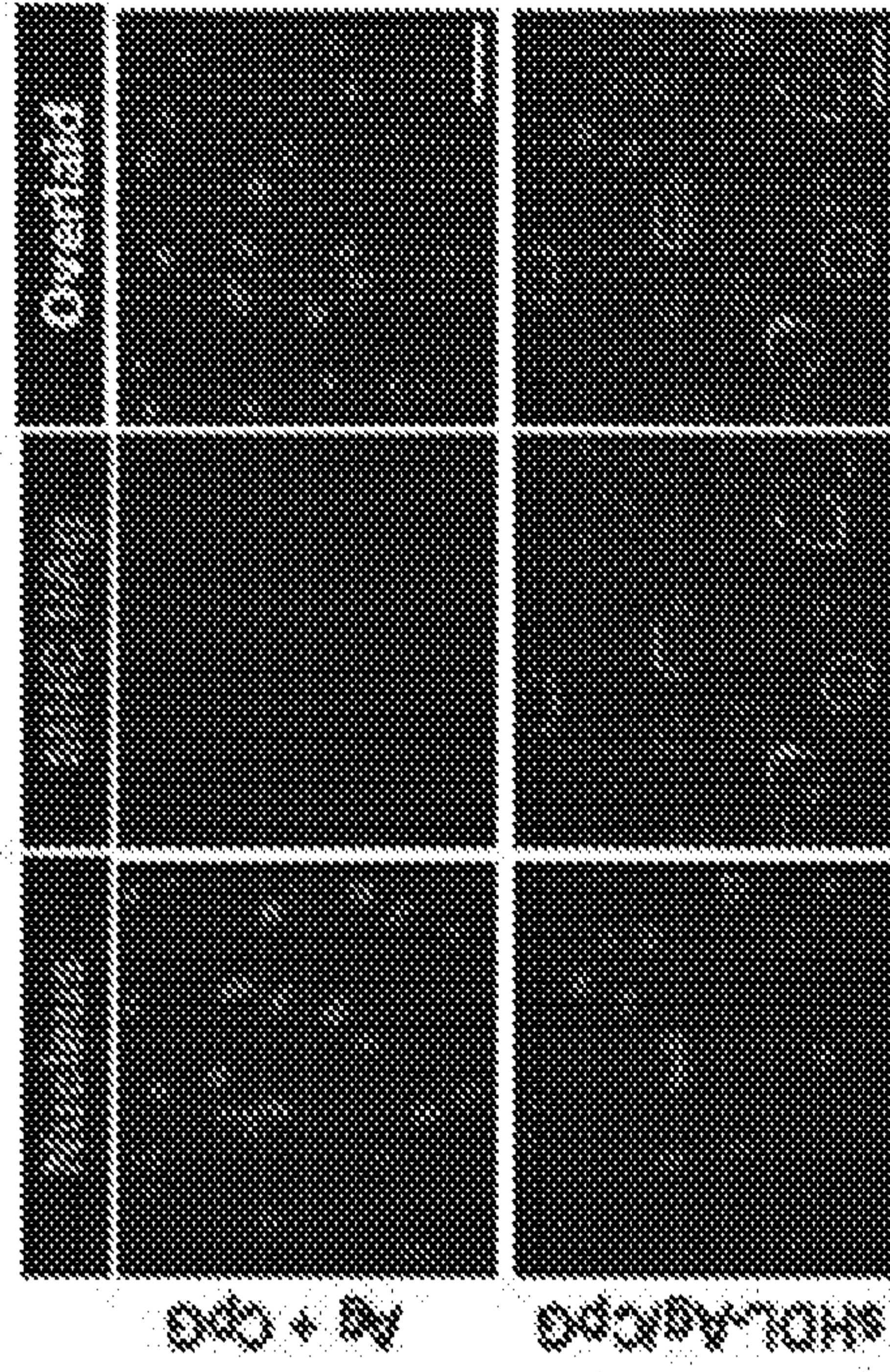


FIG. 16F



REPLACEMENT SHEET

FIG. 16G

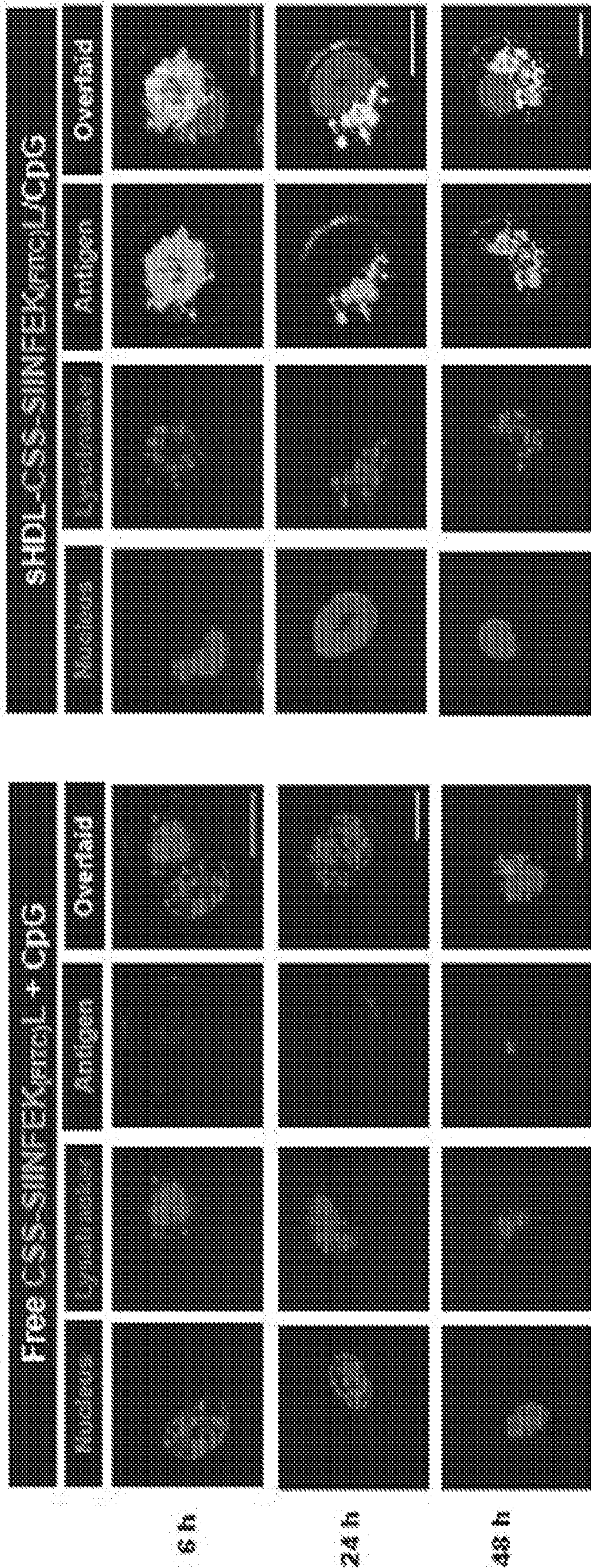
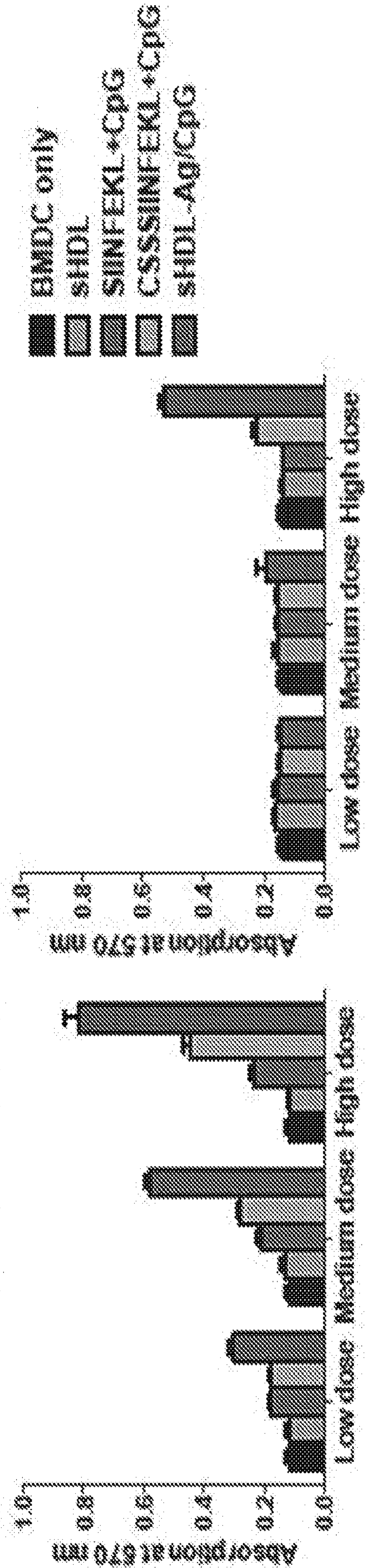


FIG. 16H B3Z T cell activation (24 hr) B3Z T cell activation (48 hr)



REPLACEMENT SHEET

FIG. 17C

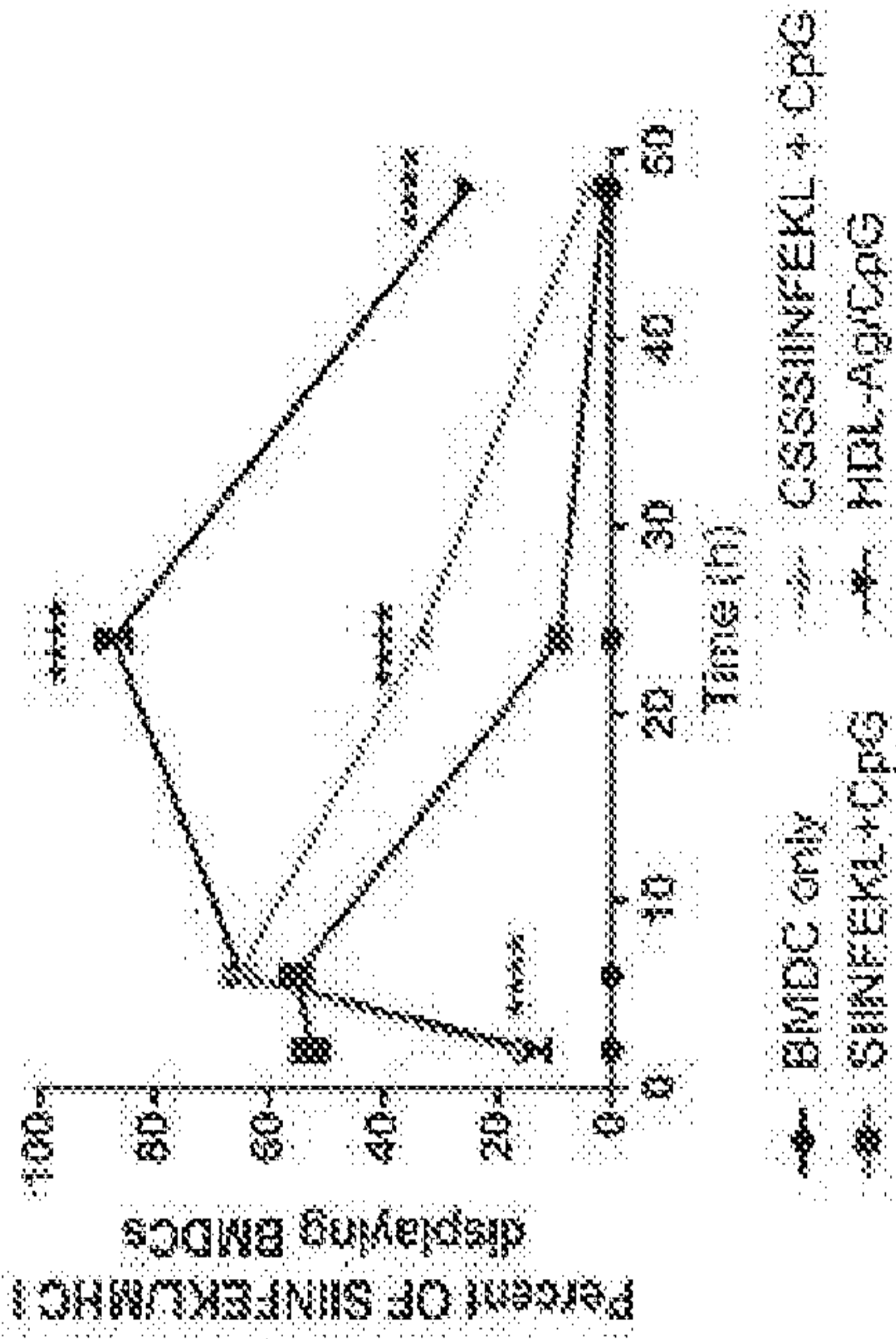


FIG. 17A

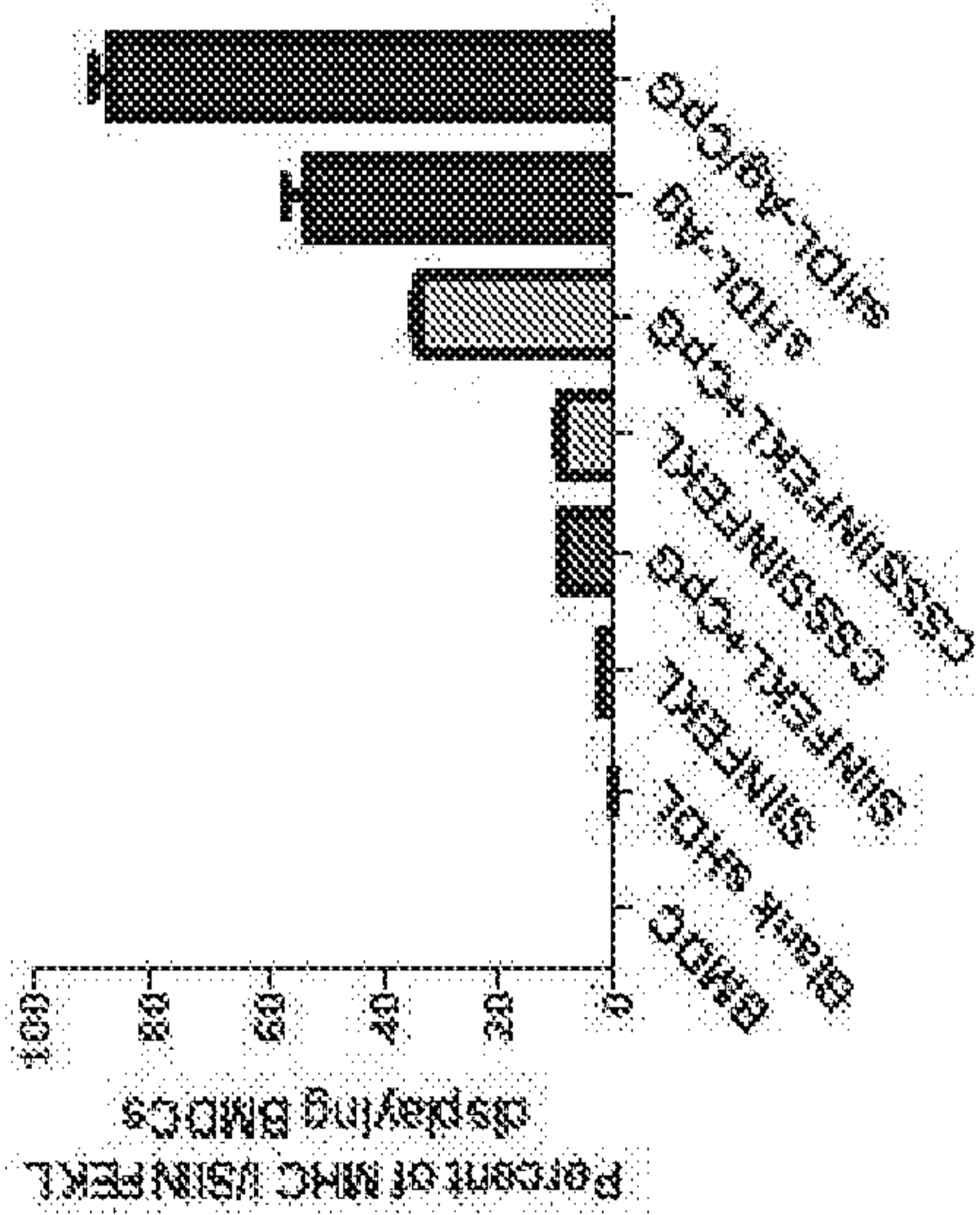


FIG. 17B

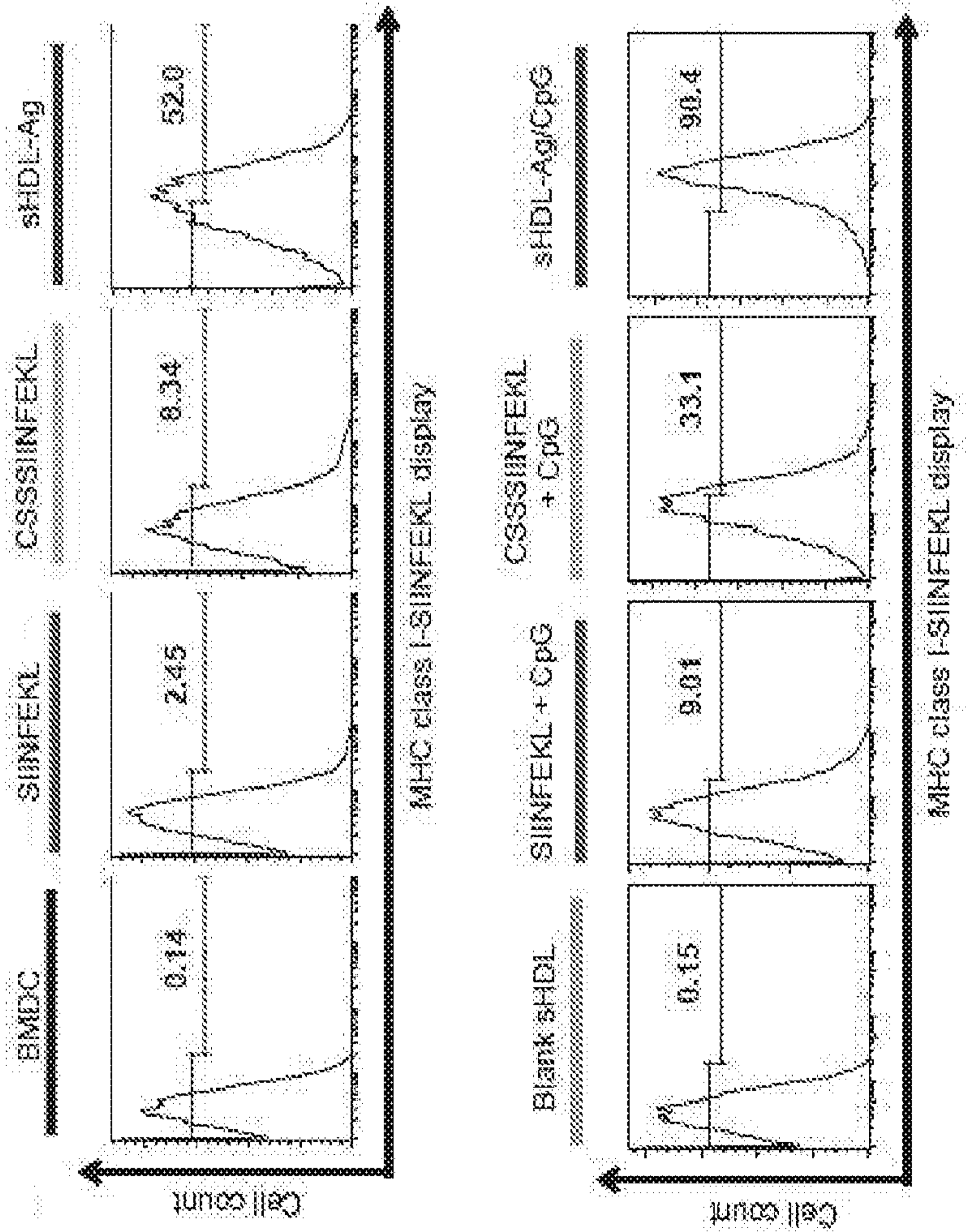


FIG. 18

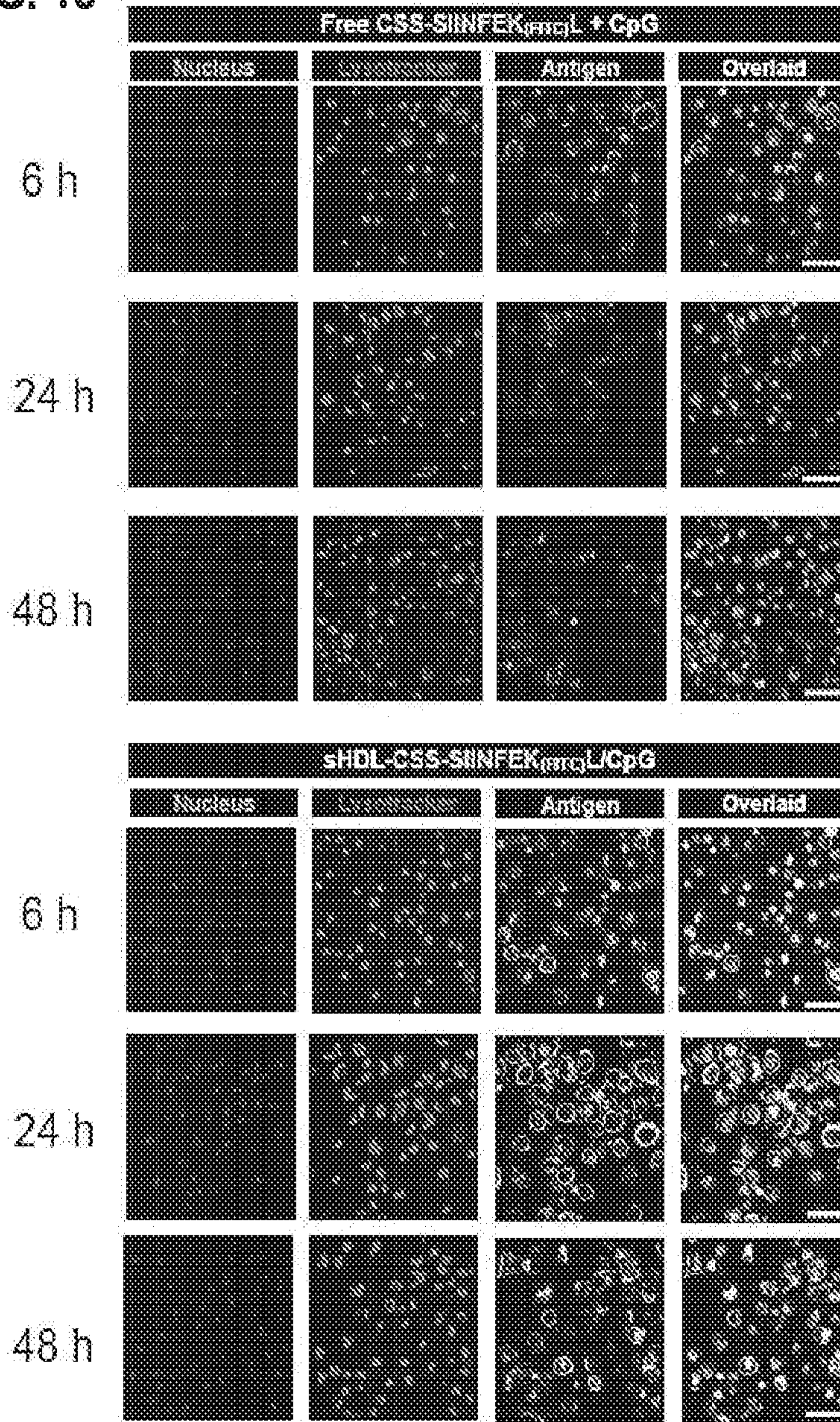


FIG. 19

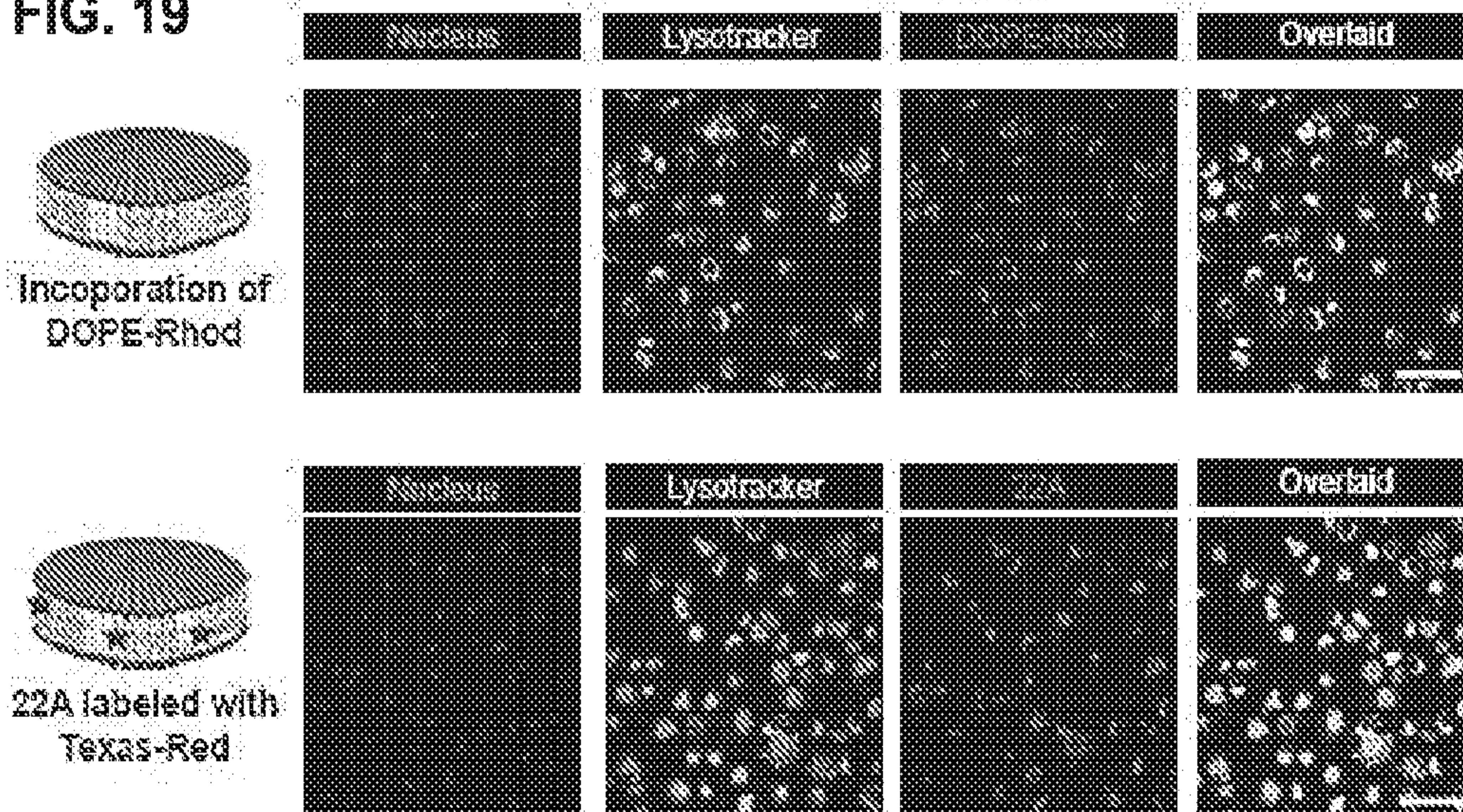


FIG. 20

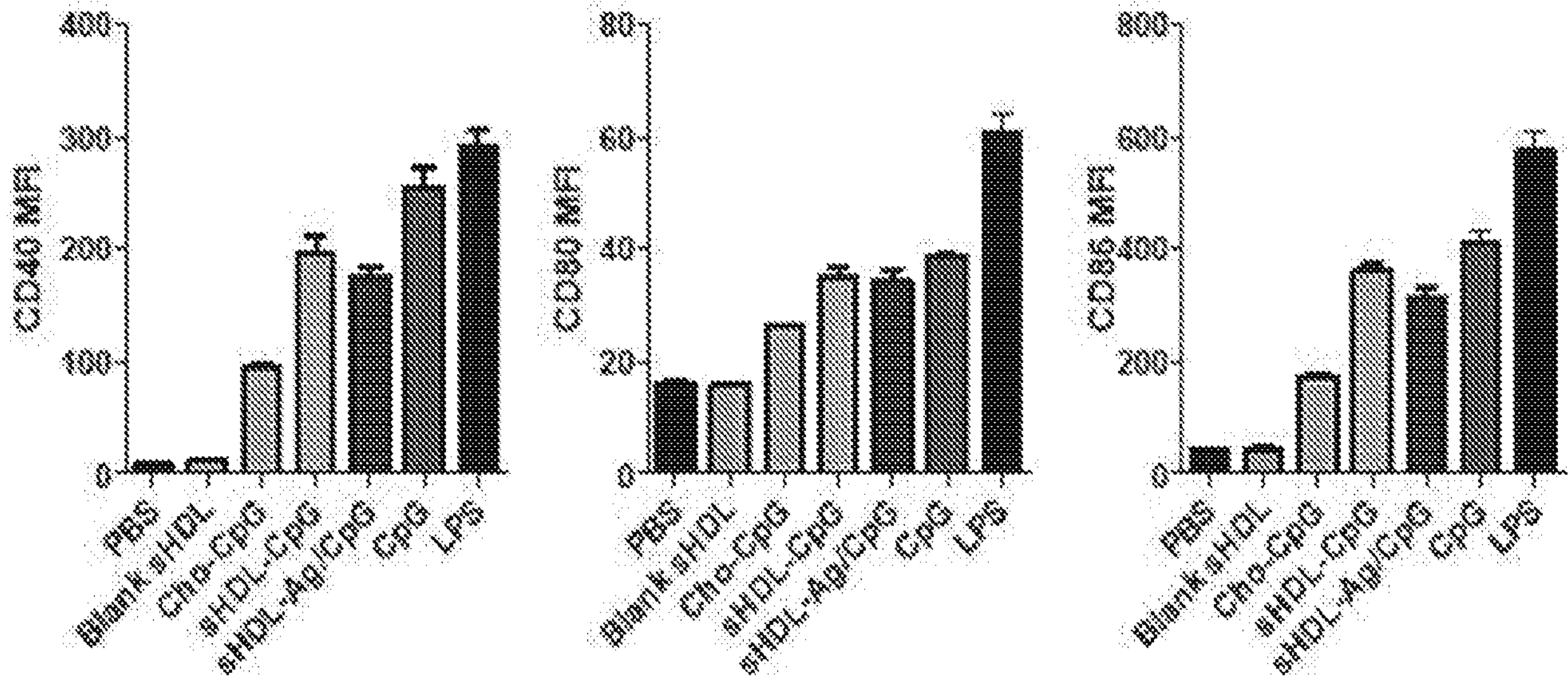


FIG. 21A

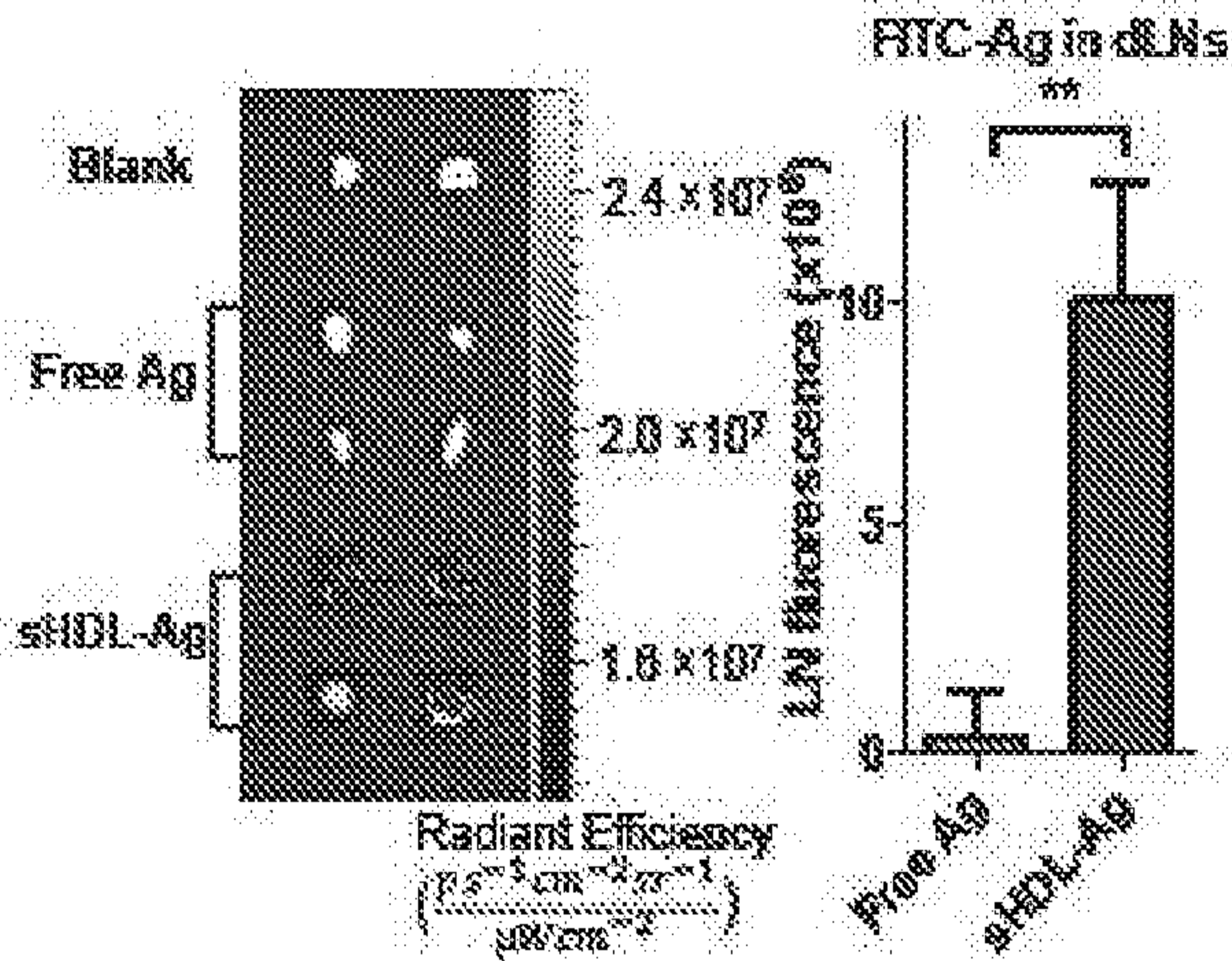


FIG. 21B

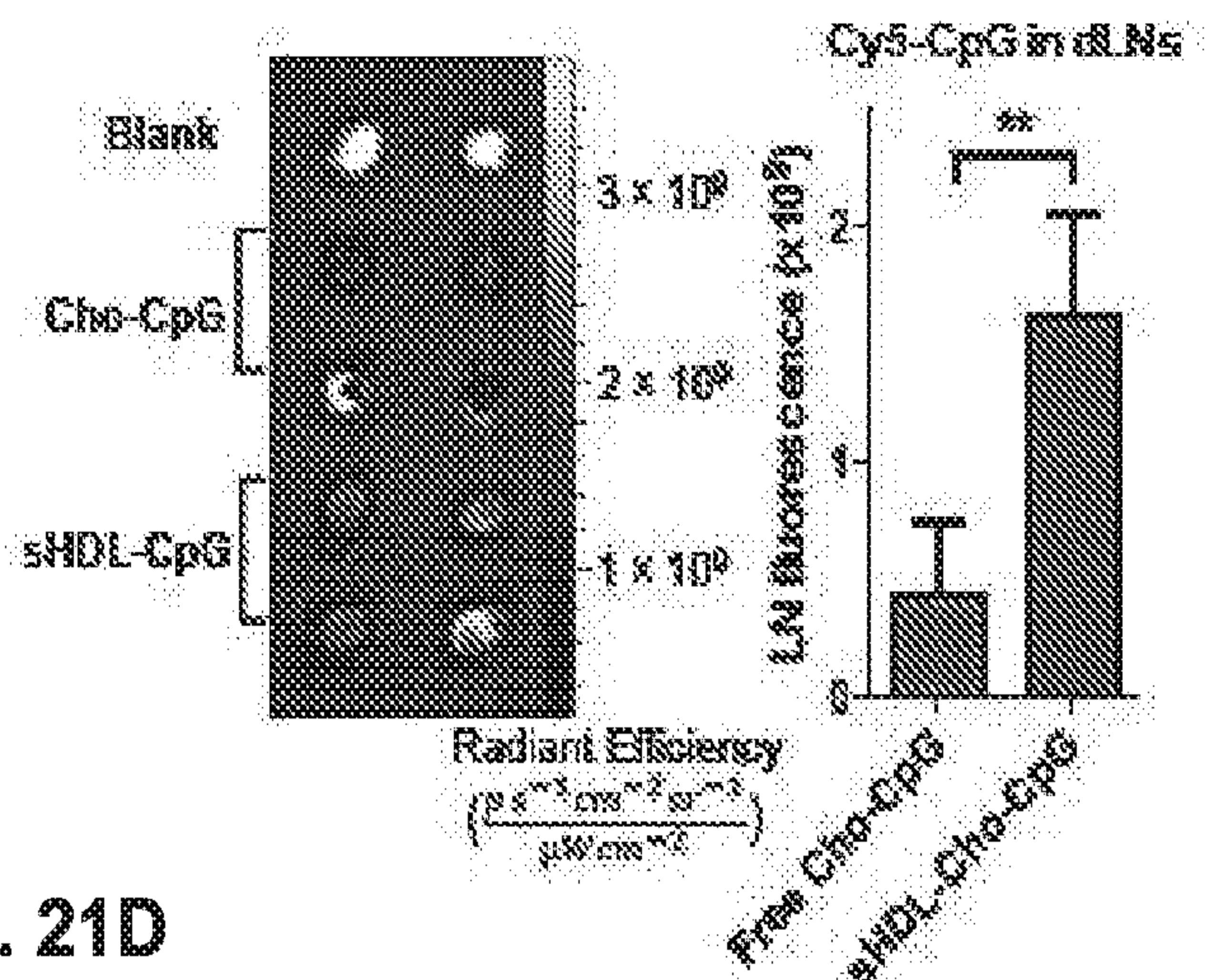


FIG. 21C

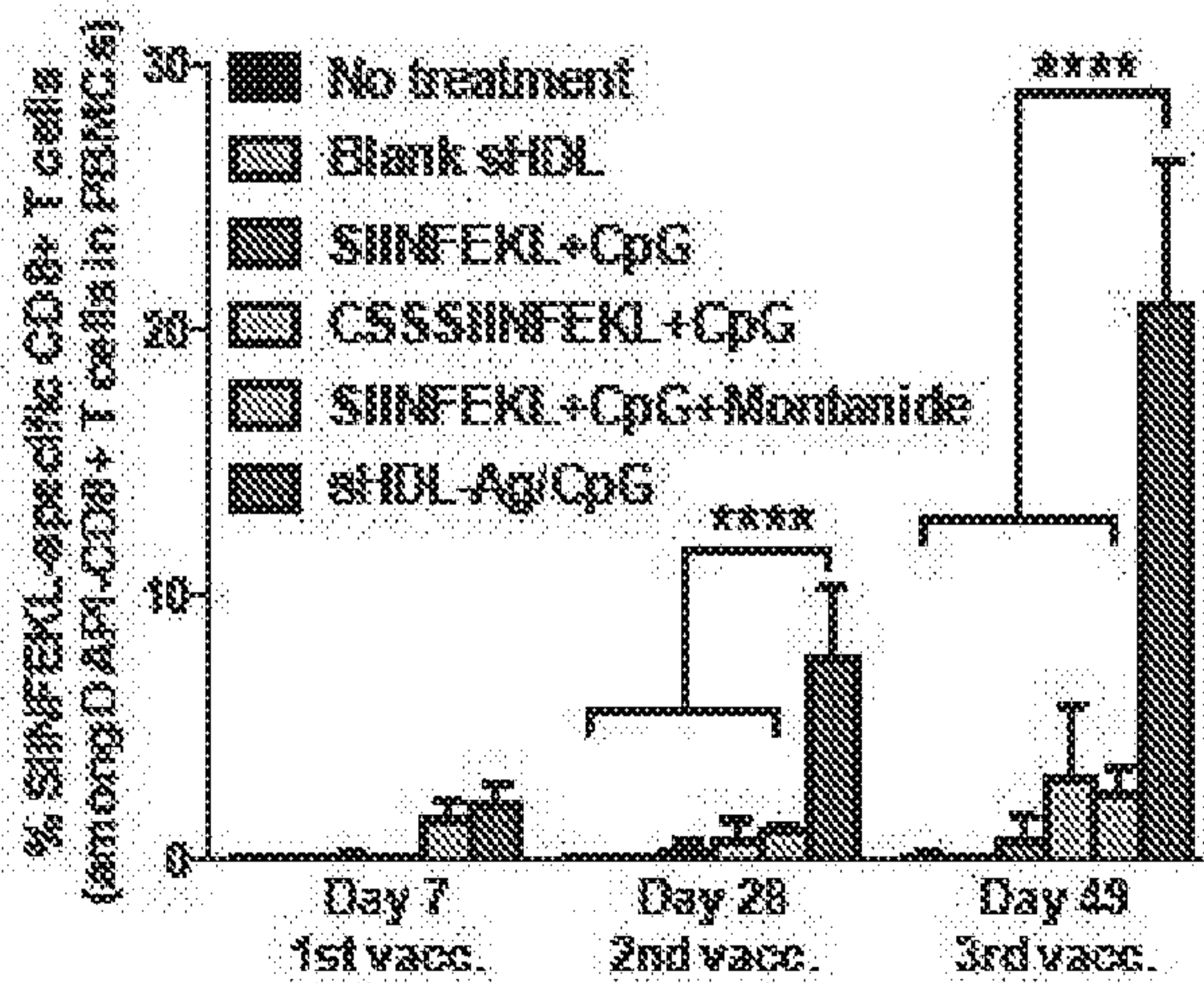


FIG. 21D

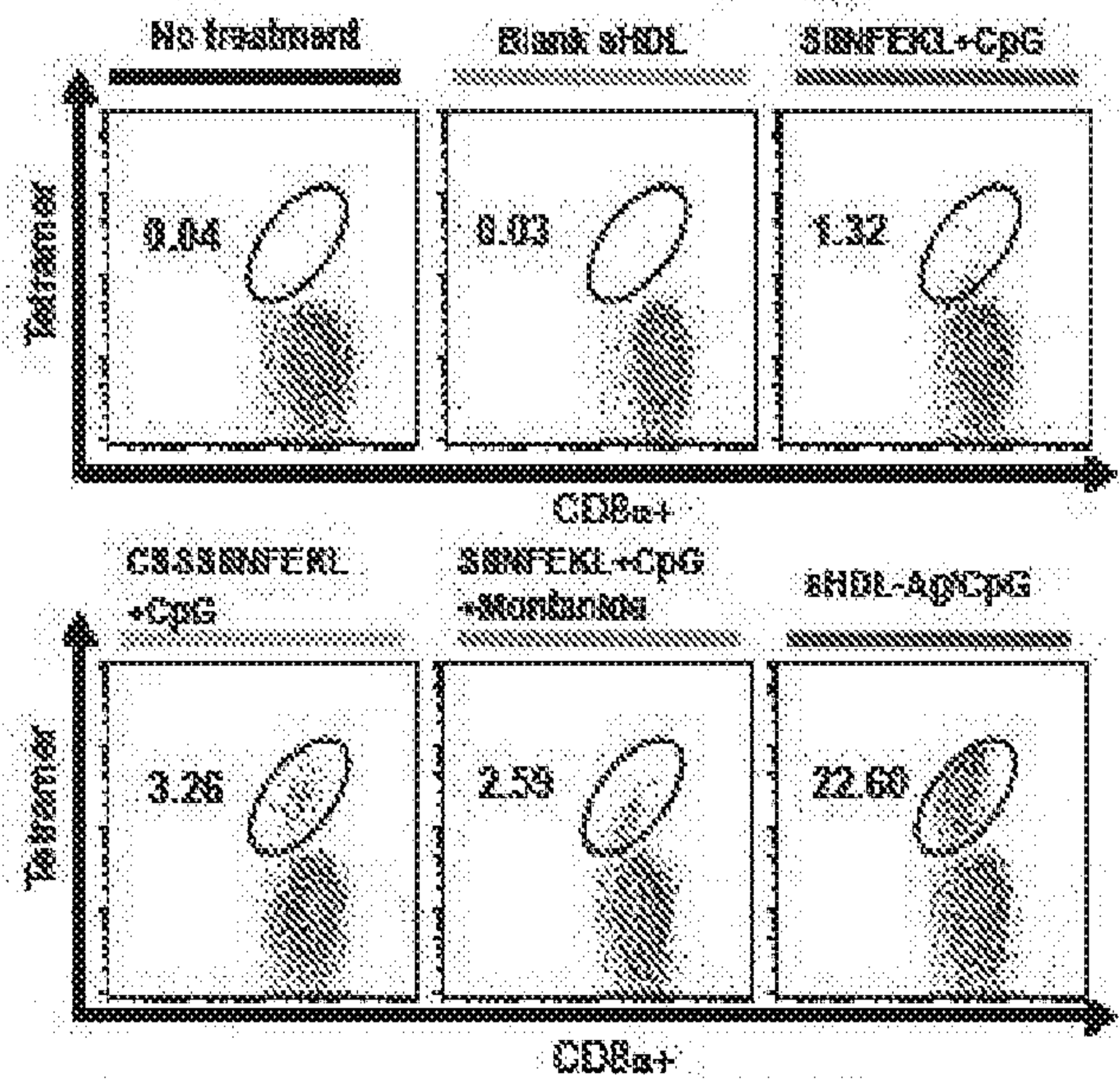


FIG. 21E

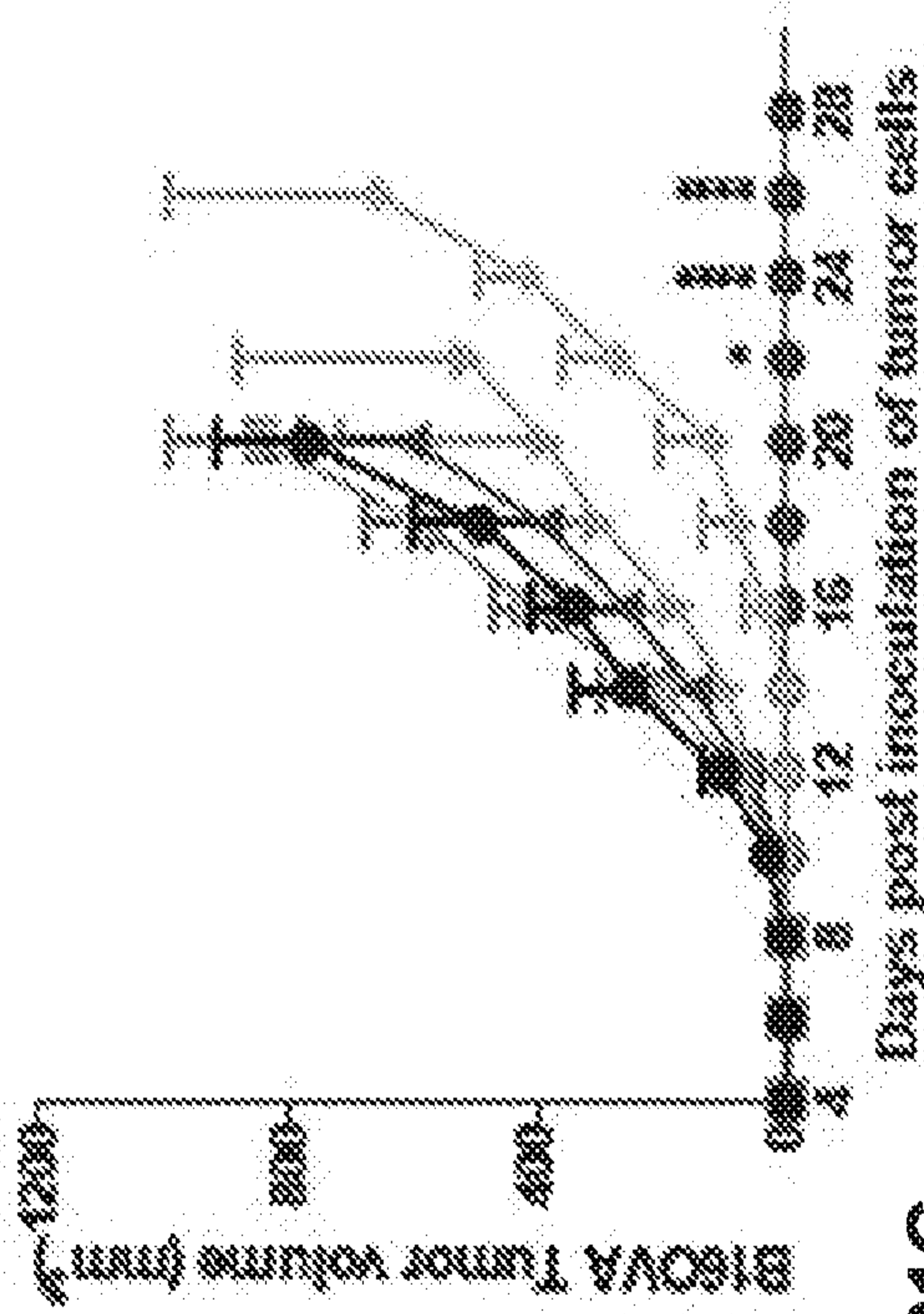


FIG. 21F

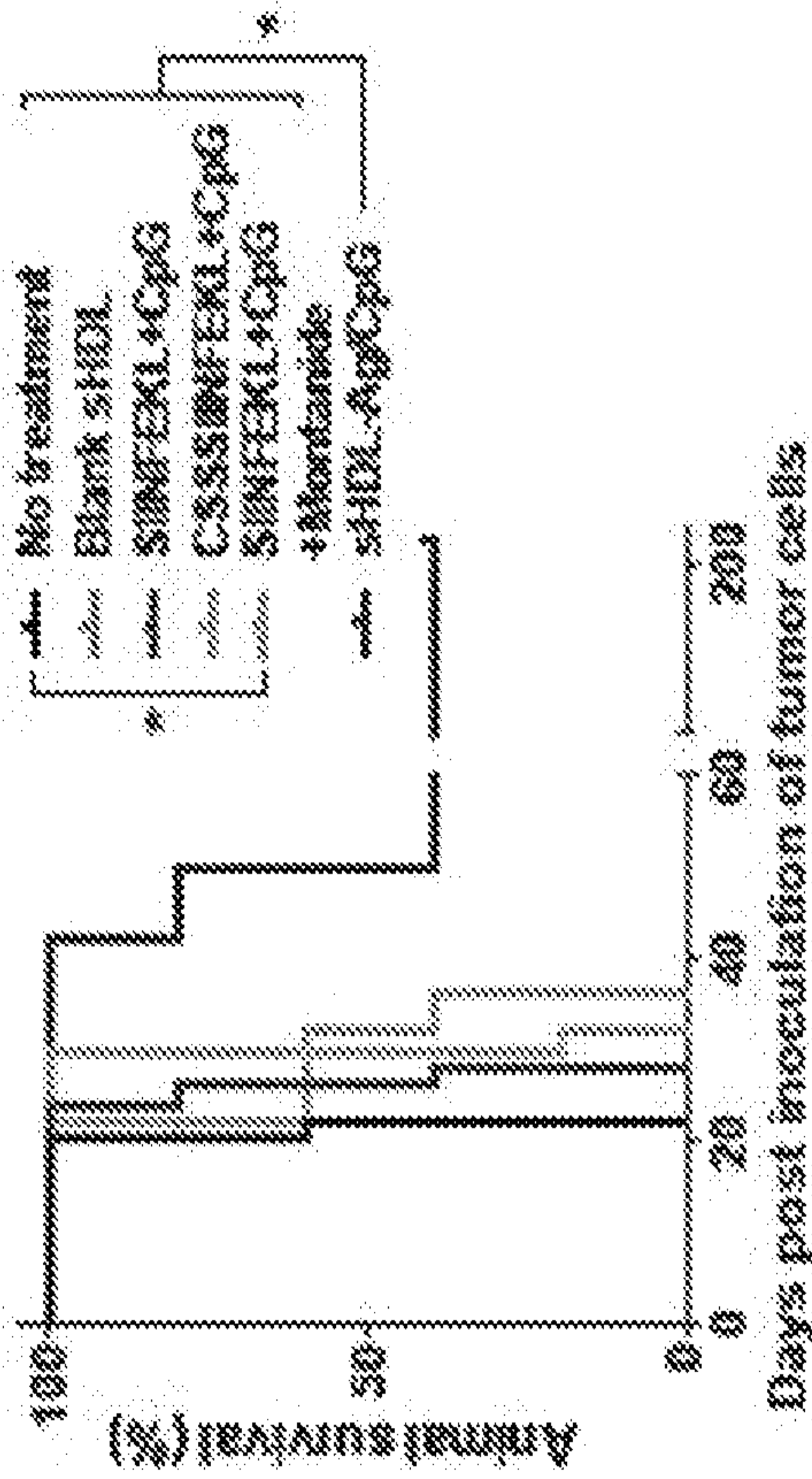


FIG. 21G

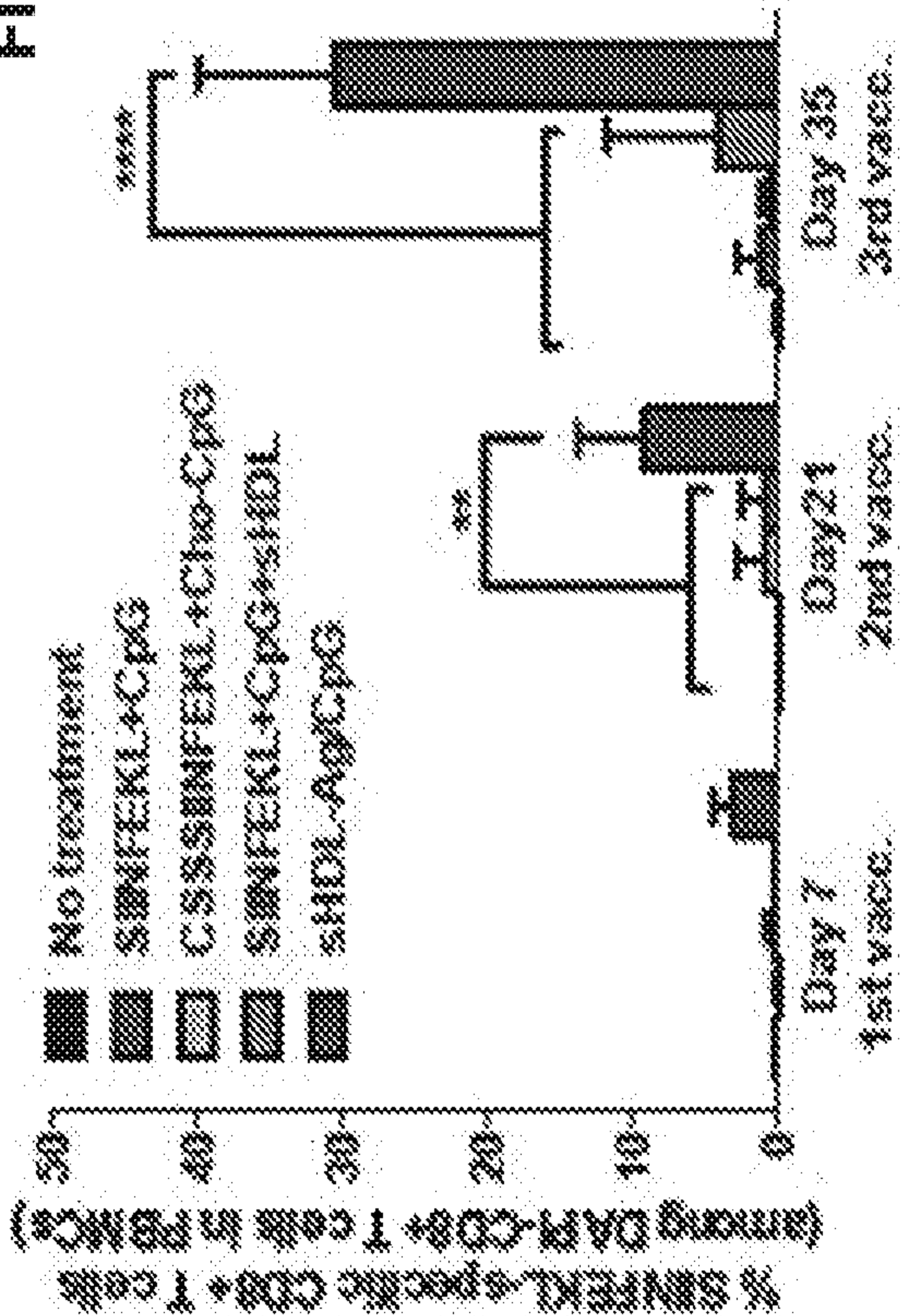
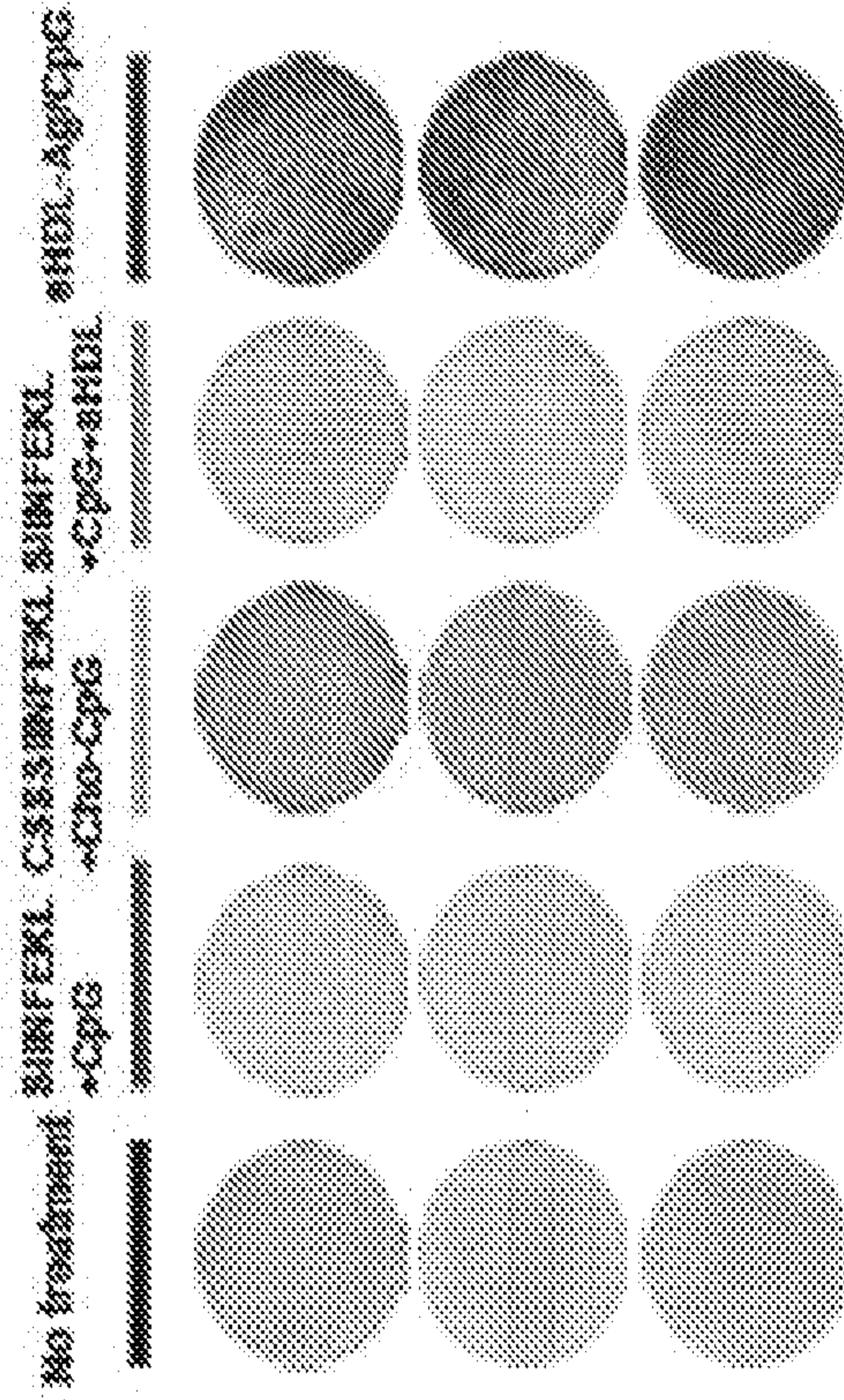


FIG. 21H



REPLACEMENT SHEET

FIG. 22

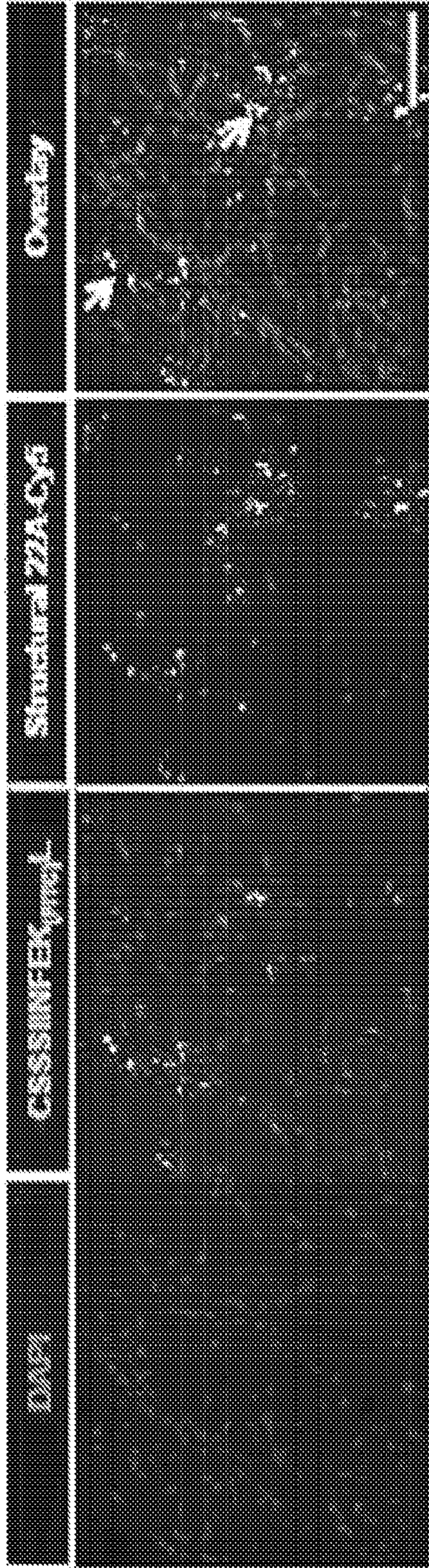


FIG. 23

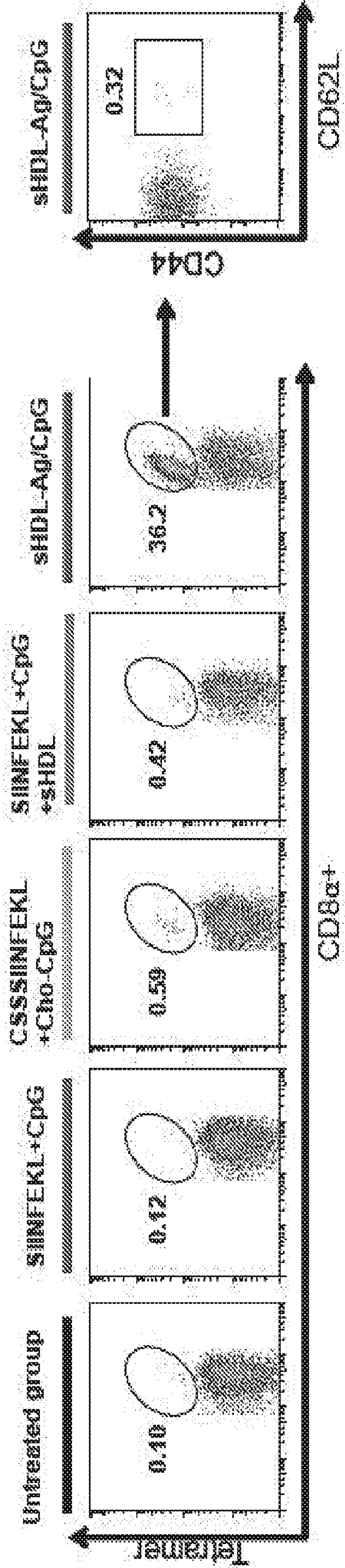


FIG. 24A

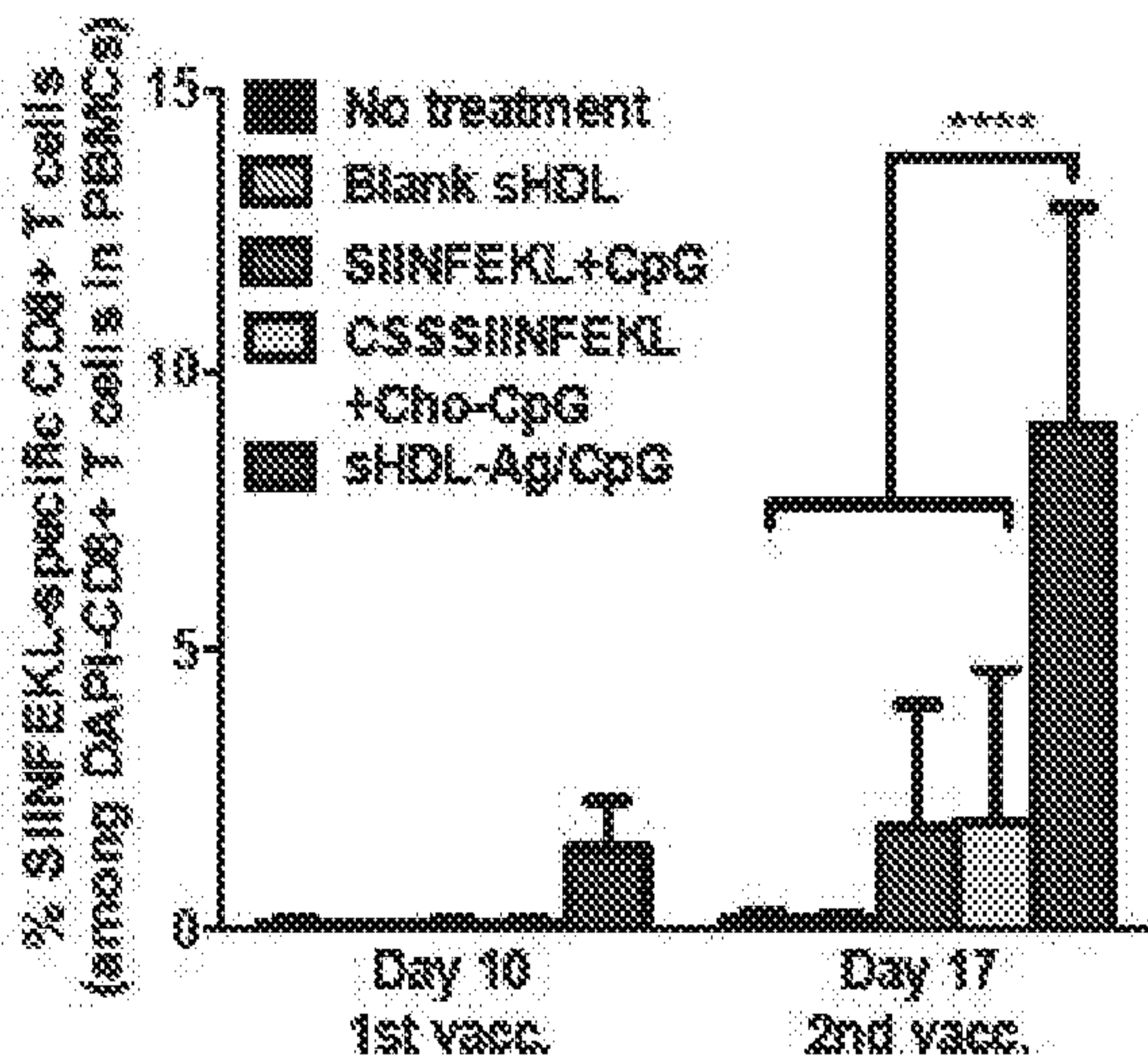


FIG. 24B

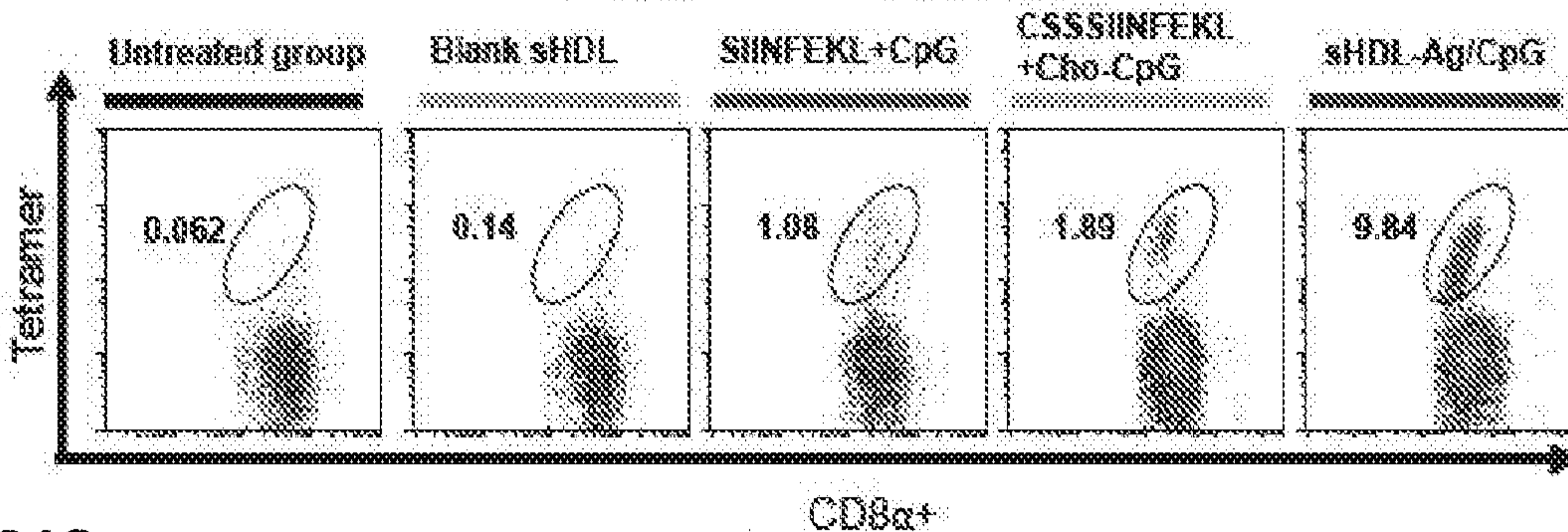


FIG. 24C

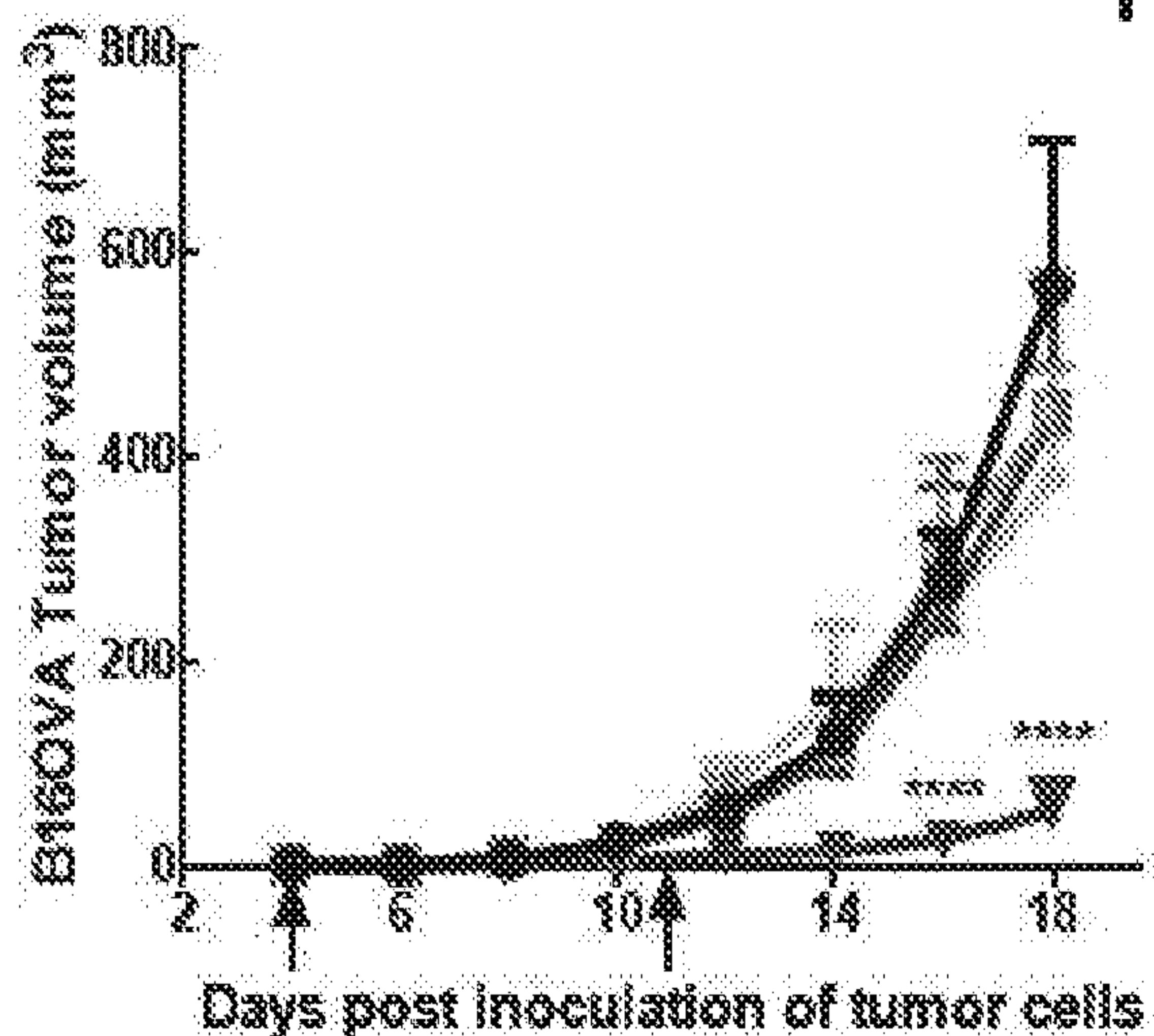


FIG. 24D

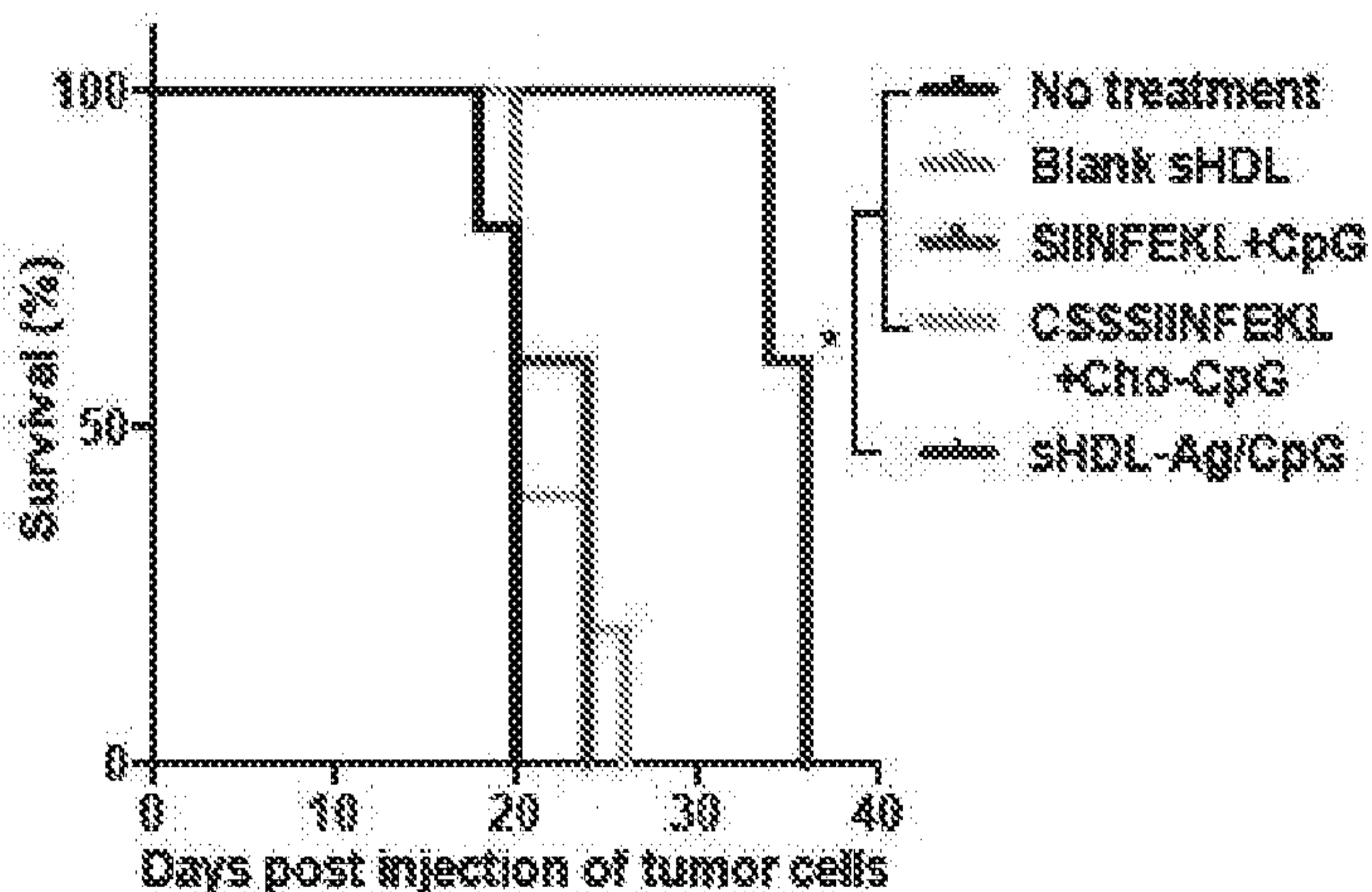


FIG. 25A

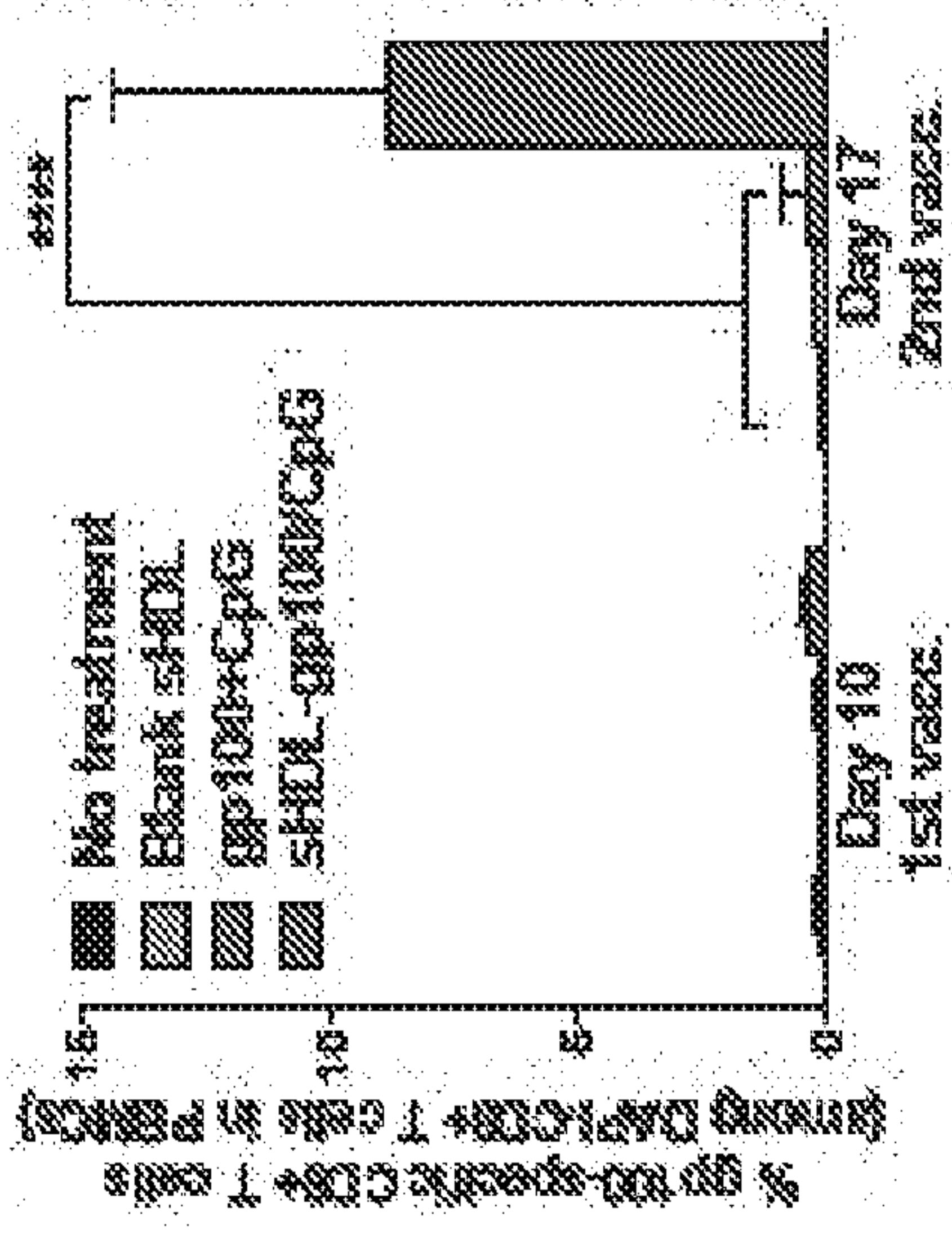


FIG. 25B

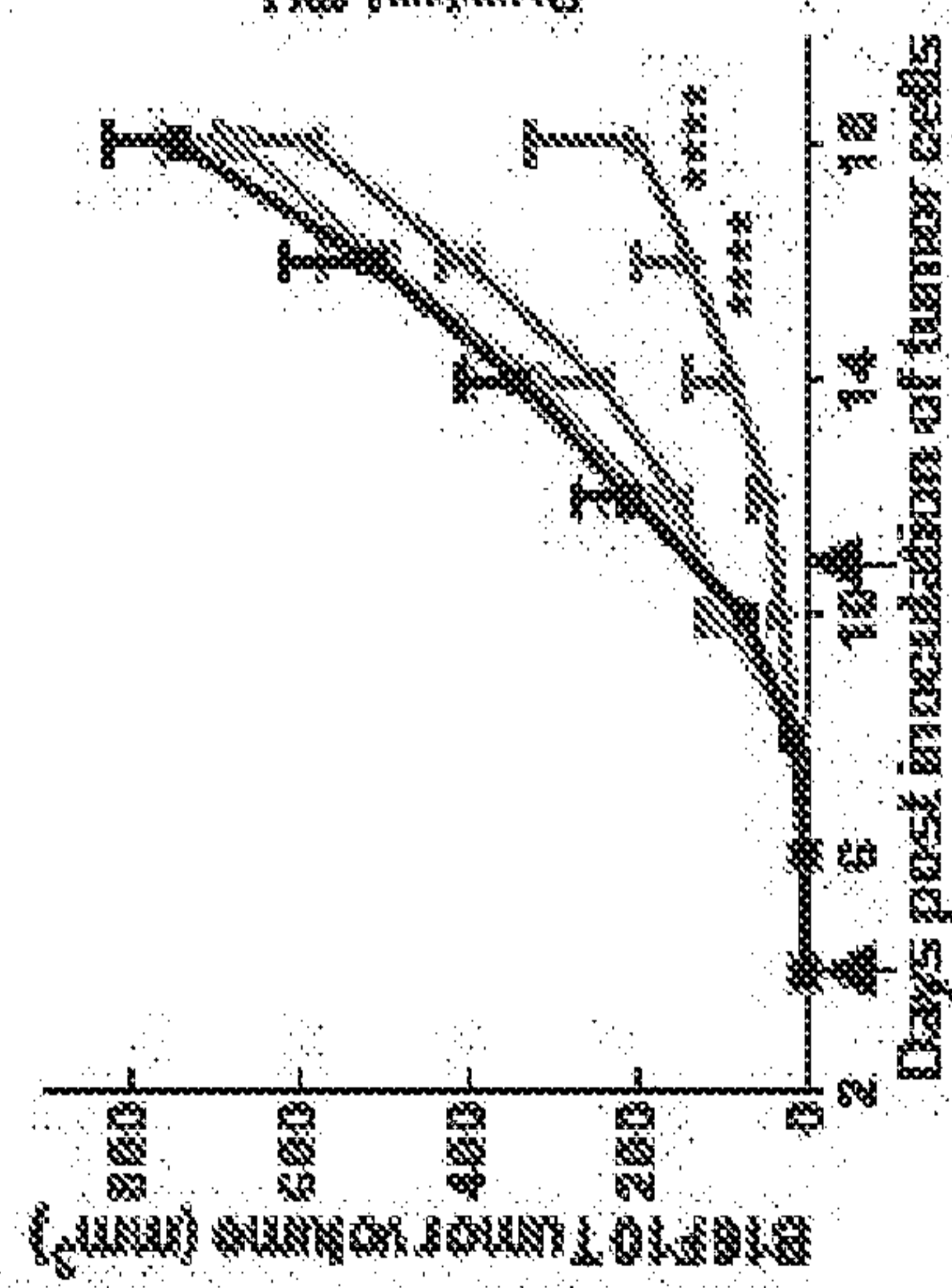


FIG. 25C

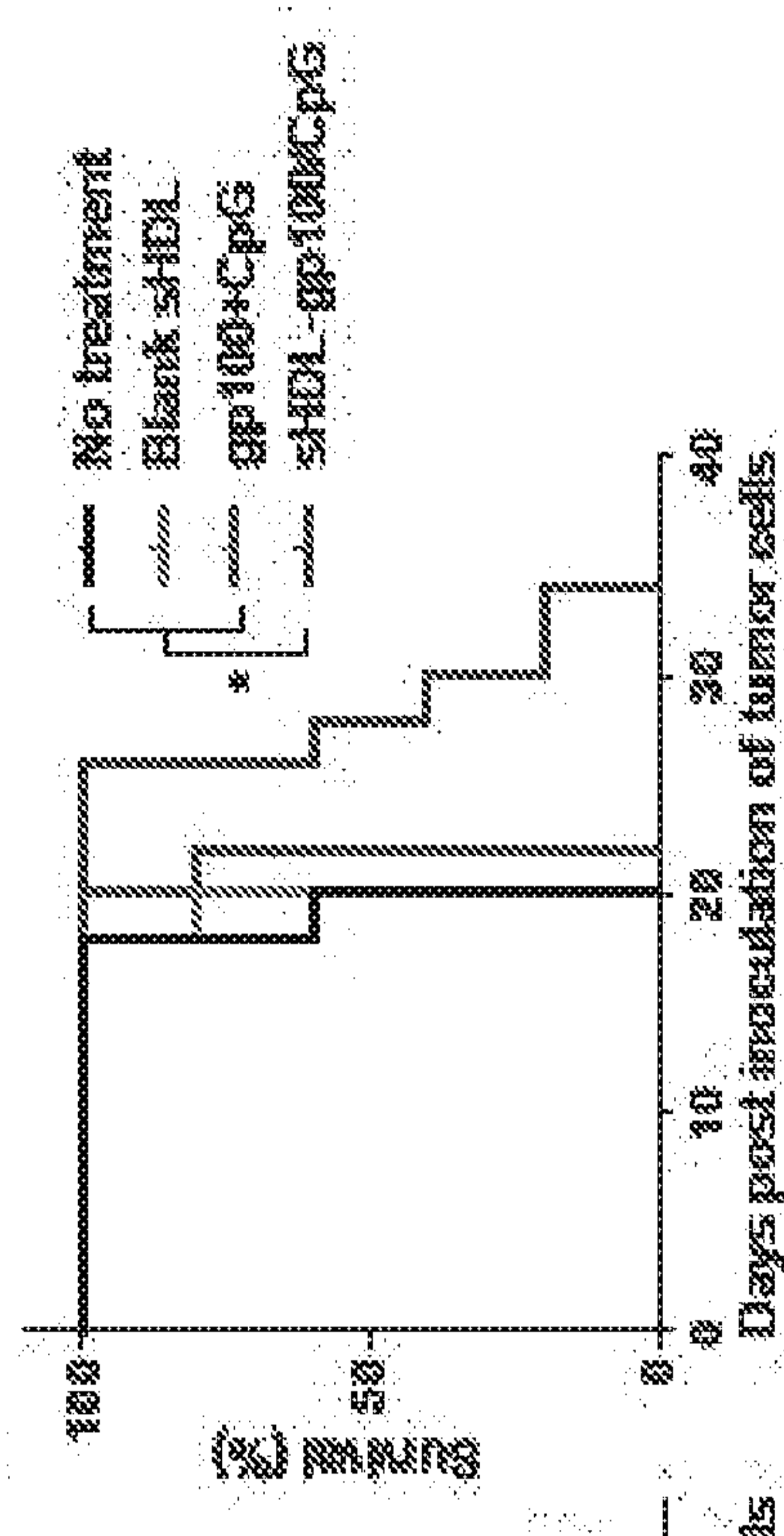
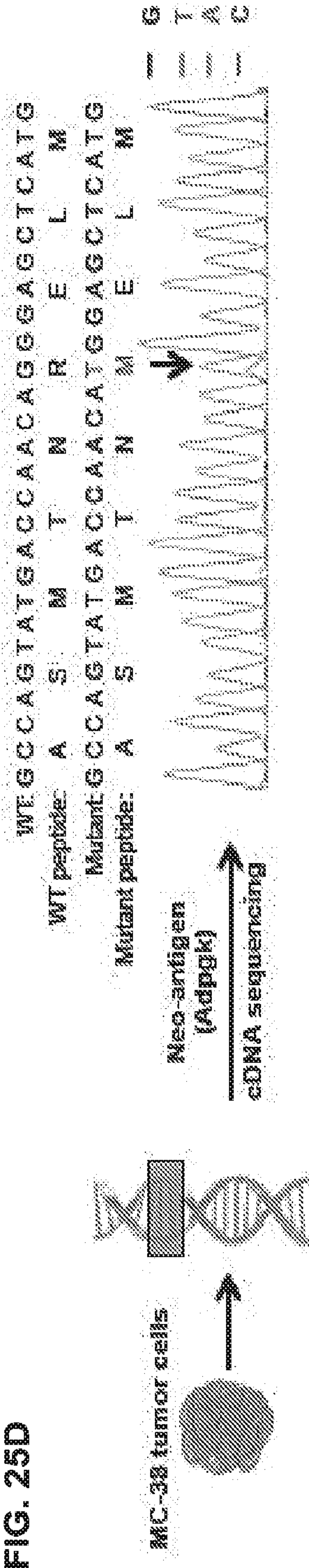


FIG. 25D



WT peptide: A S M T N R E L M
 Mutant peptide: A S M T N E L M

WT sequence: W T G C C A G T A T G A C C A A C A G G G A G C T C A T G
 Mutant sequence: G C C A G T A T G A C C A A C A T G G A G C T C A T G

REPLACEMENT SHEET

FIG. 25E

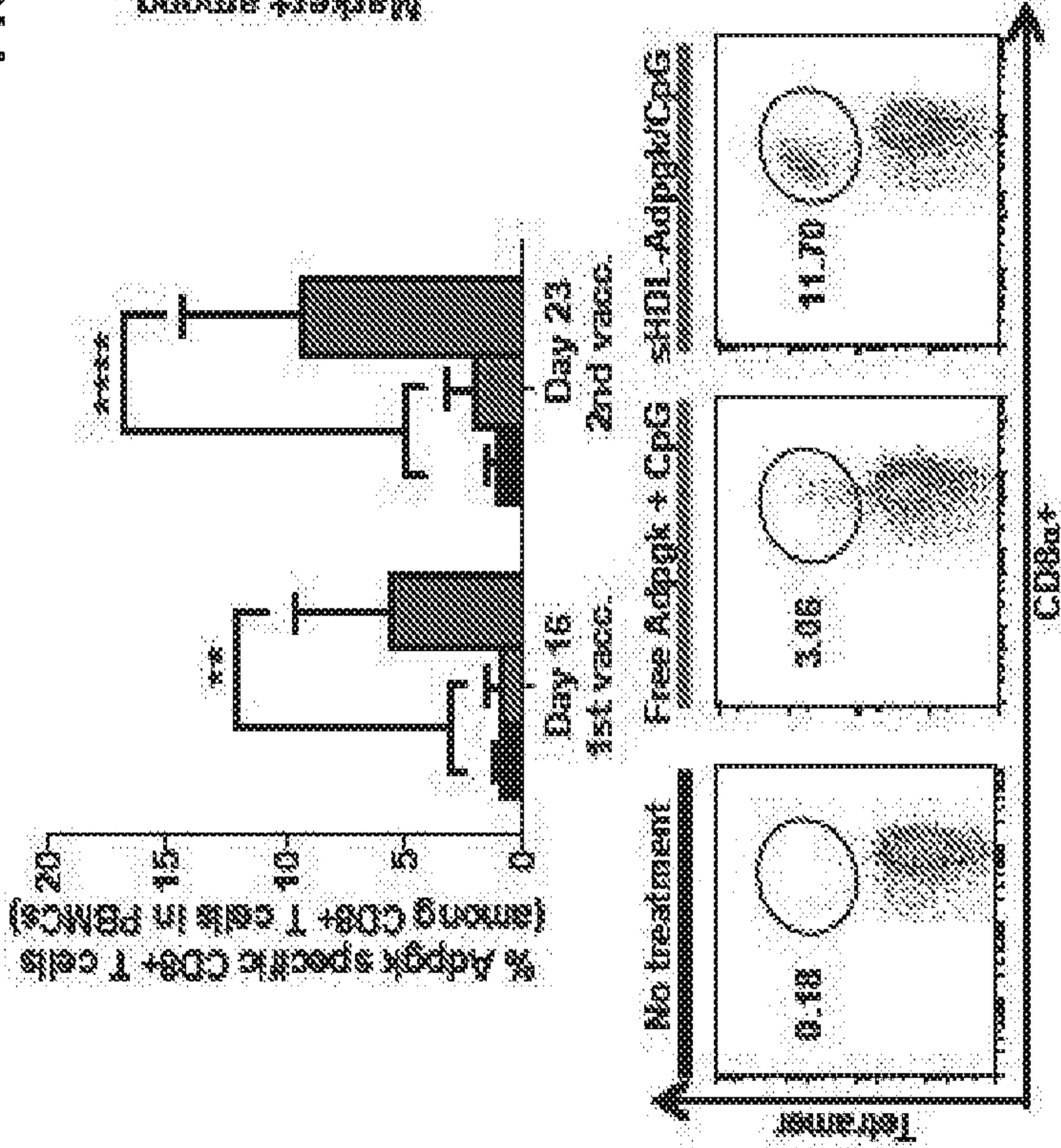


FIG. 25F

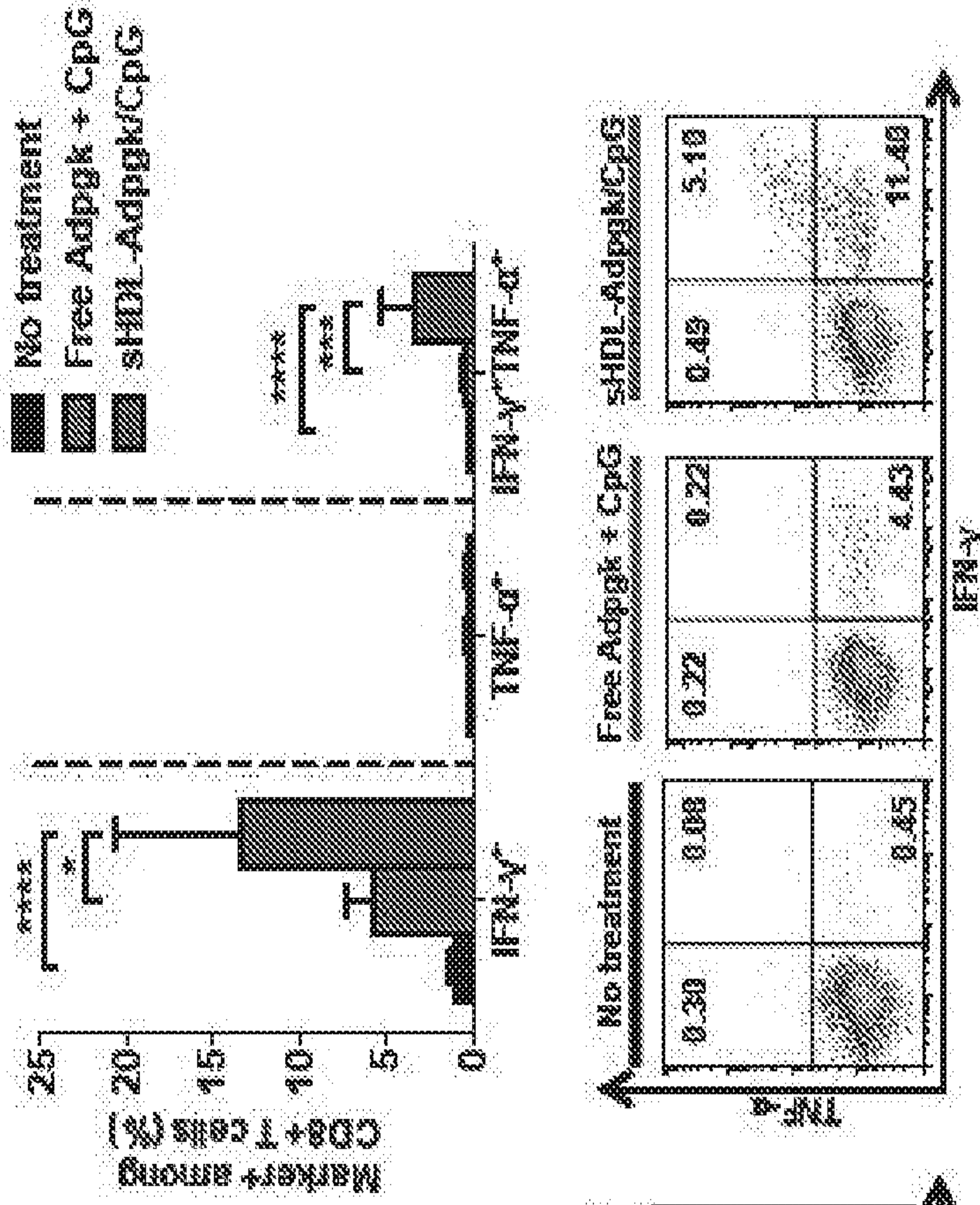


FIG. 25G

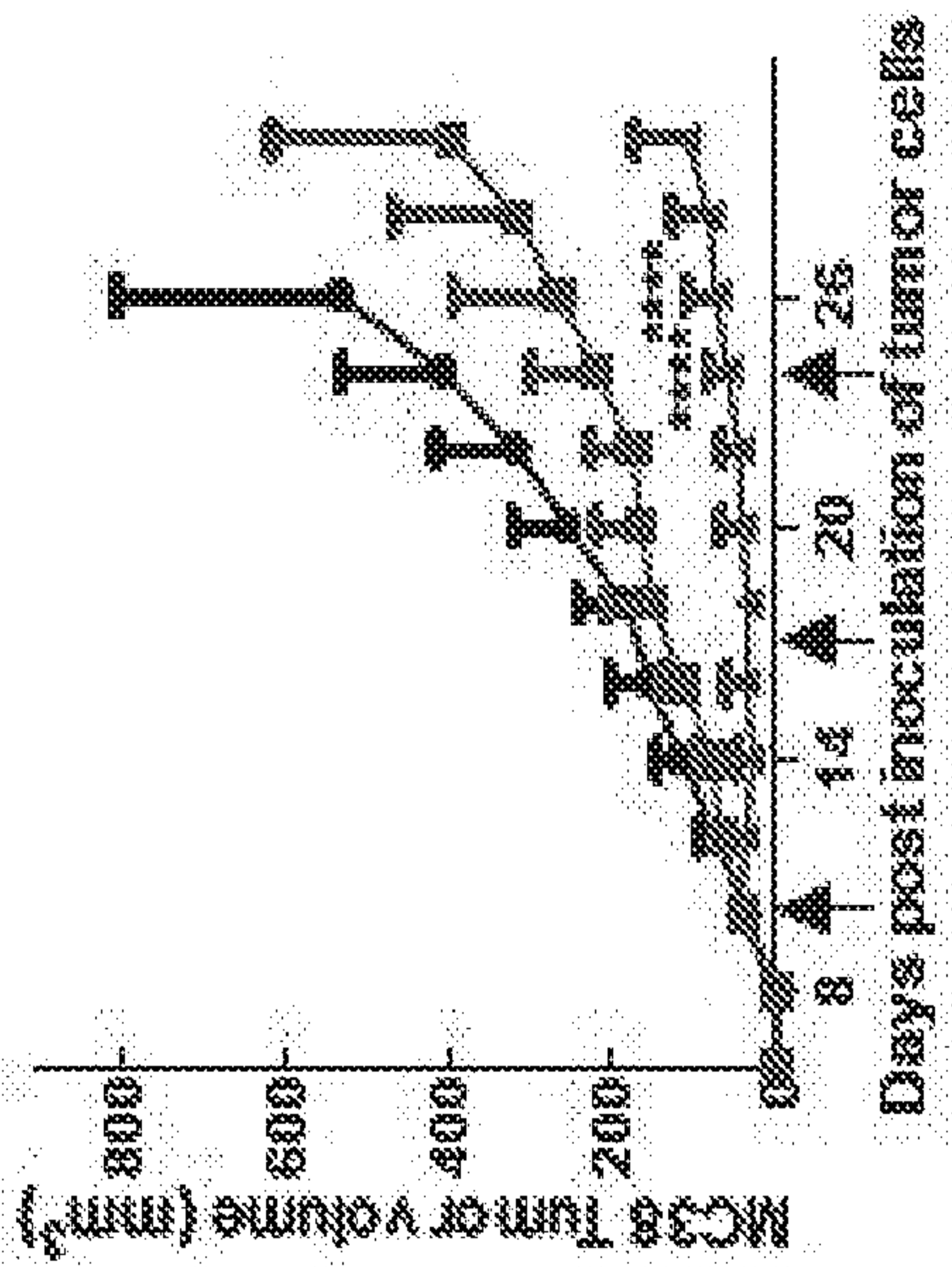


FIG. 25H

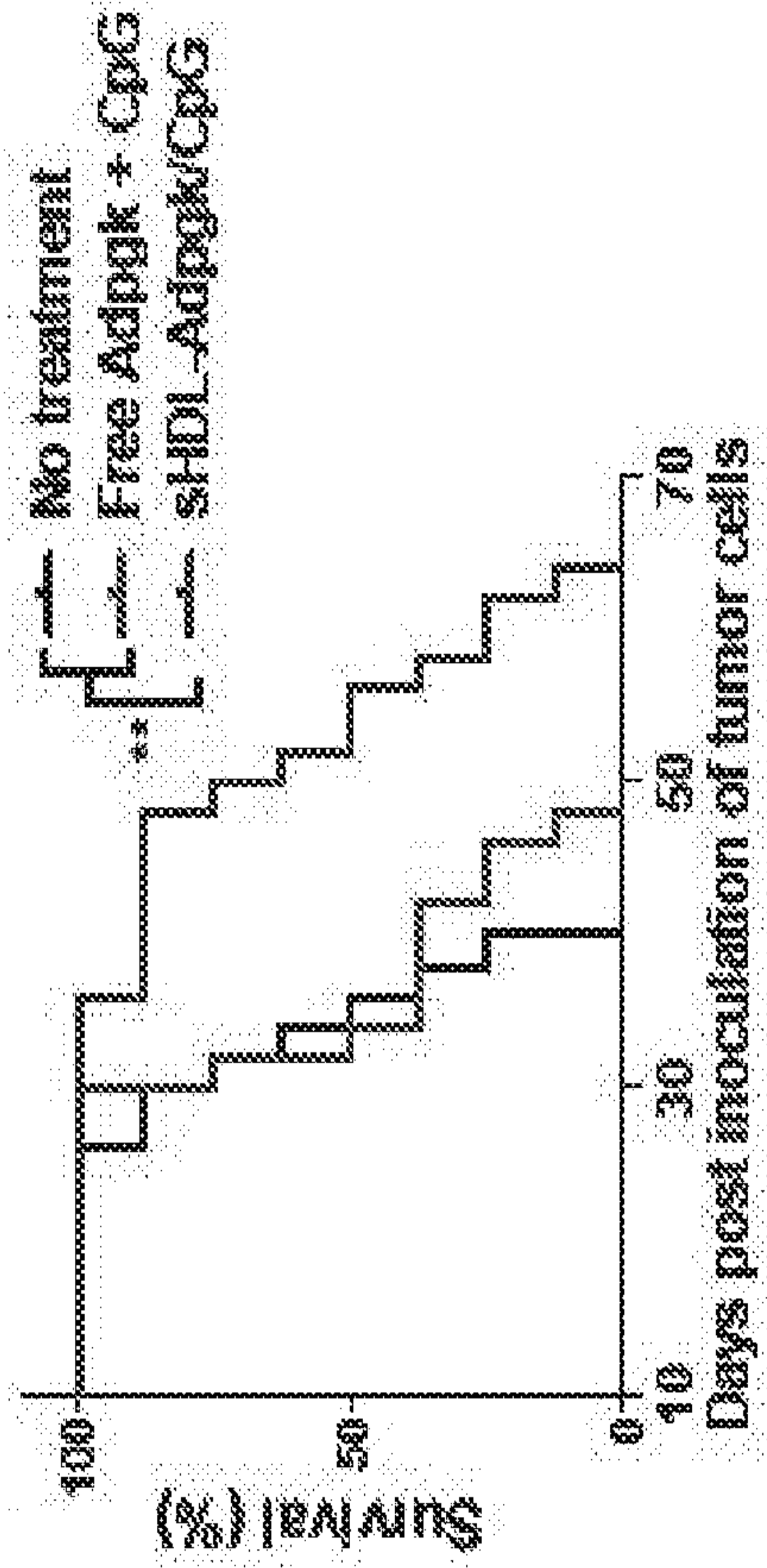
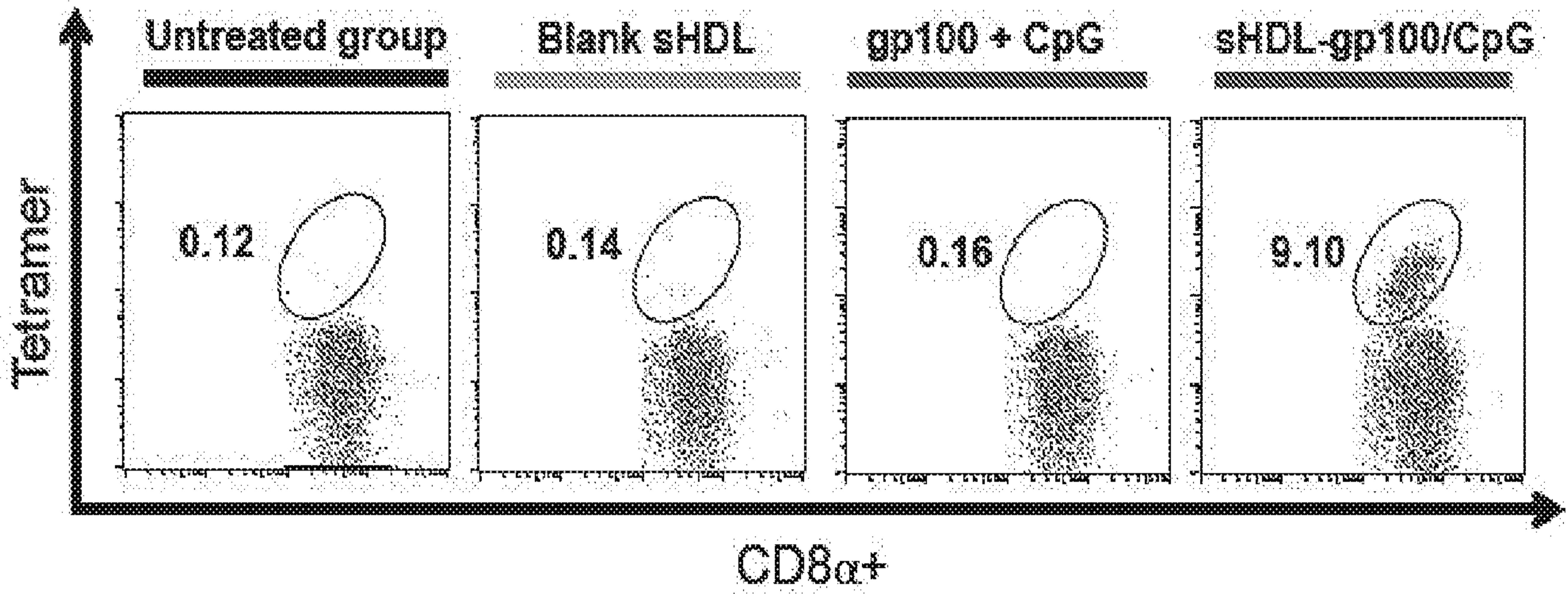


FIG. 26



WT peptide: A S M T N R E L M
 Mutant peptide: A S M T N M E L M

FIG. 27A

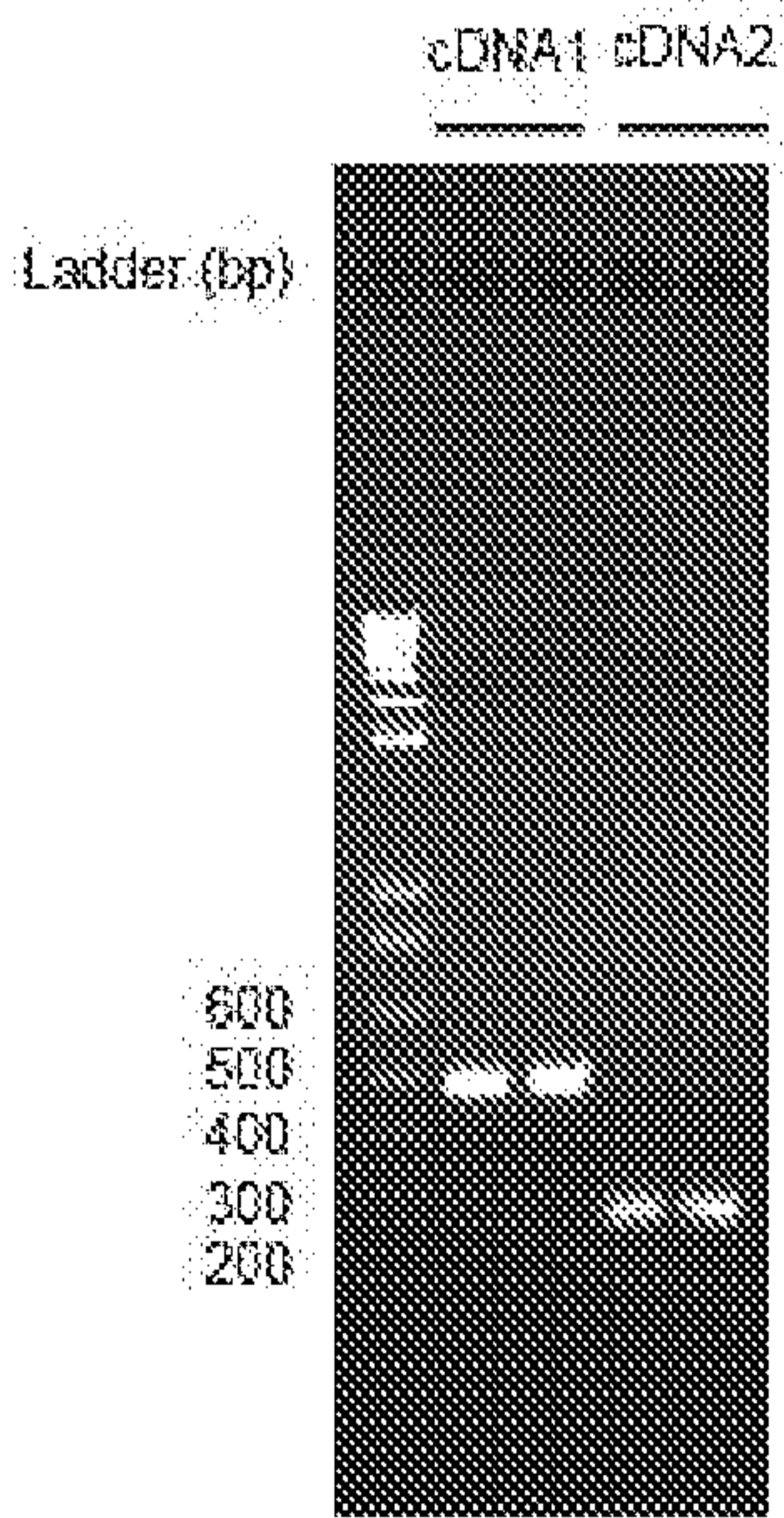


FIG. 27B

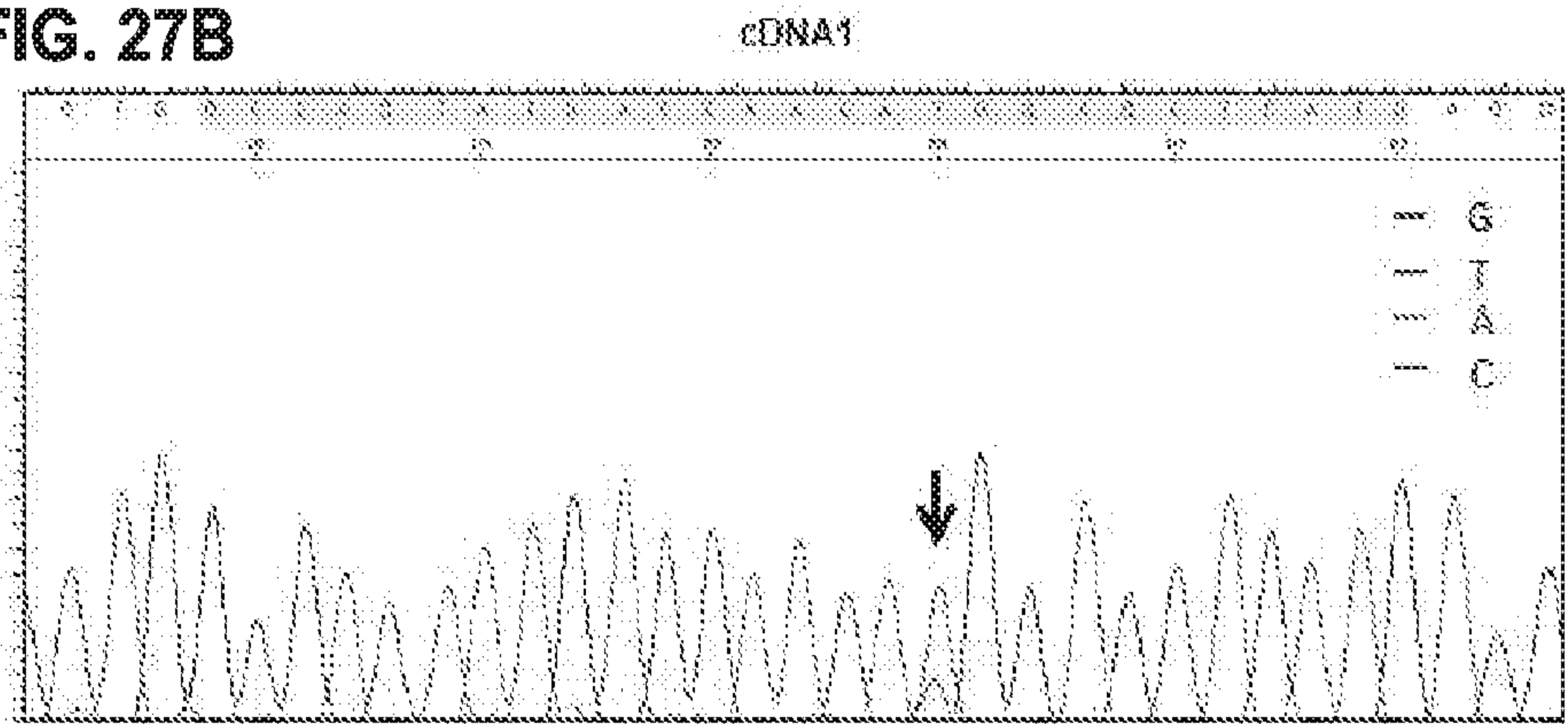
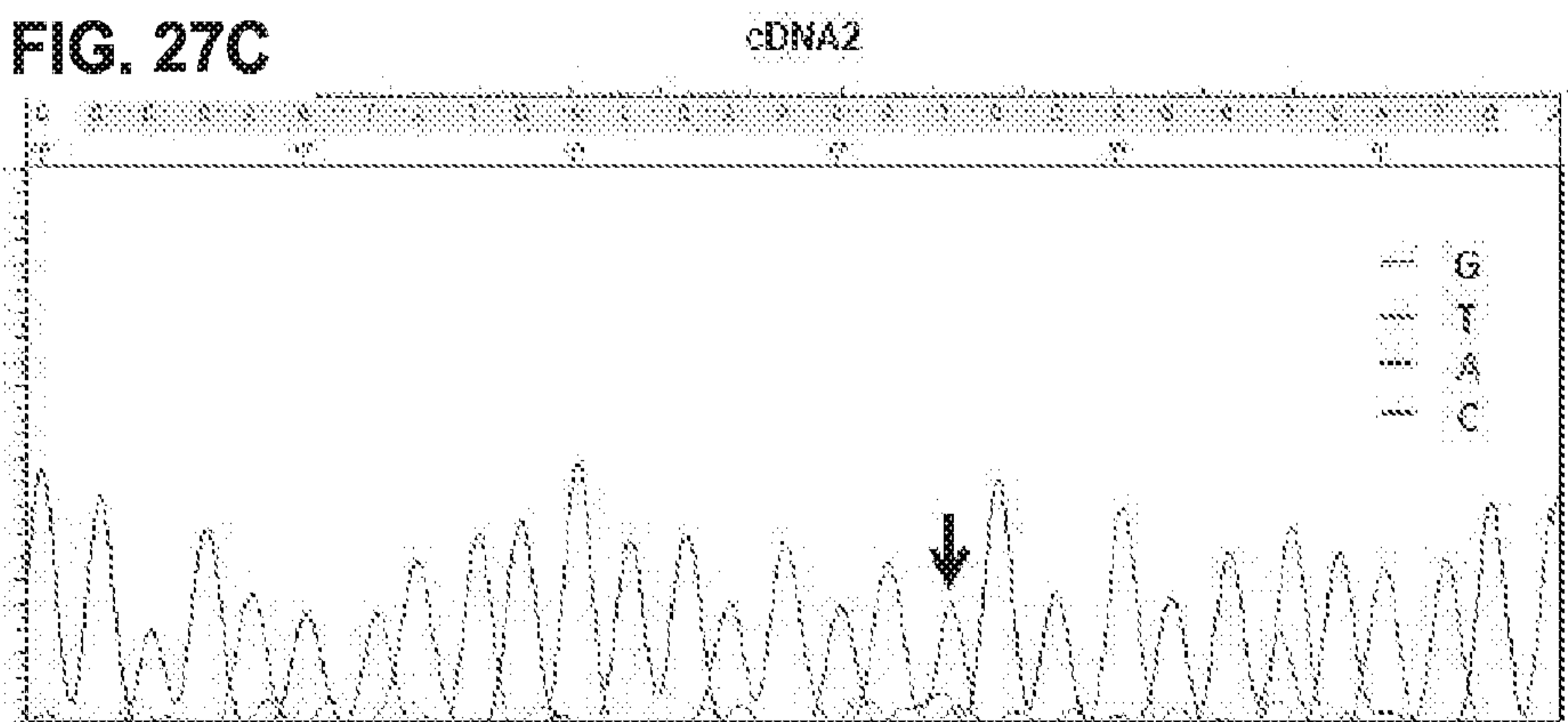


FIG. 27C



REPLACEMENT SHEET

FIG. 28A

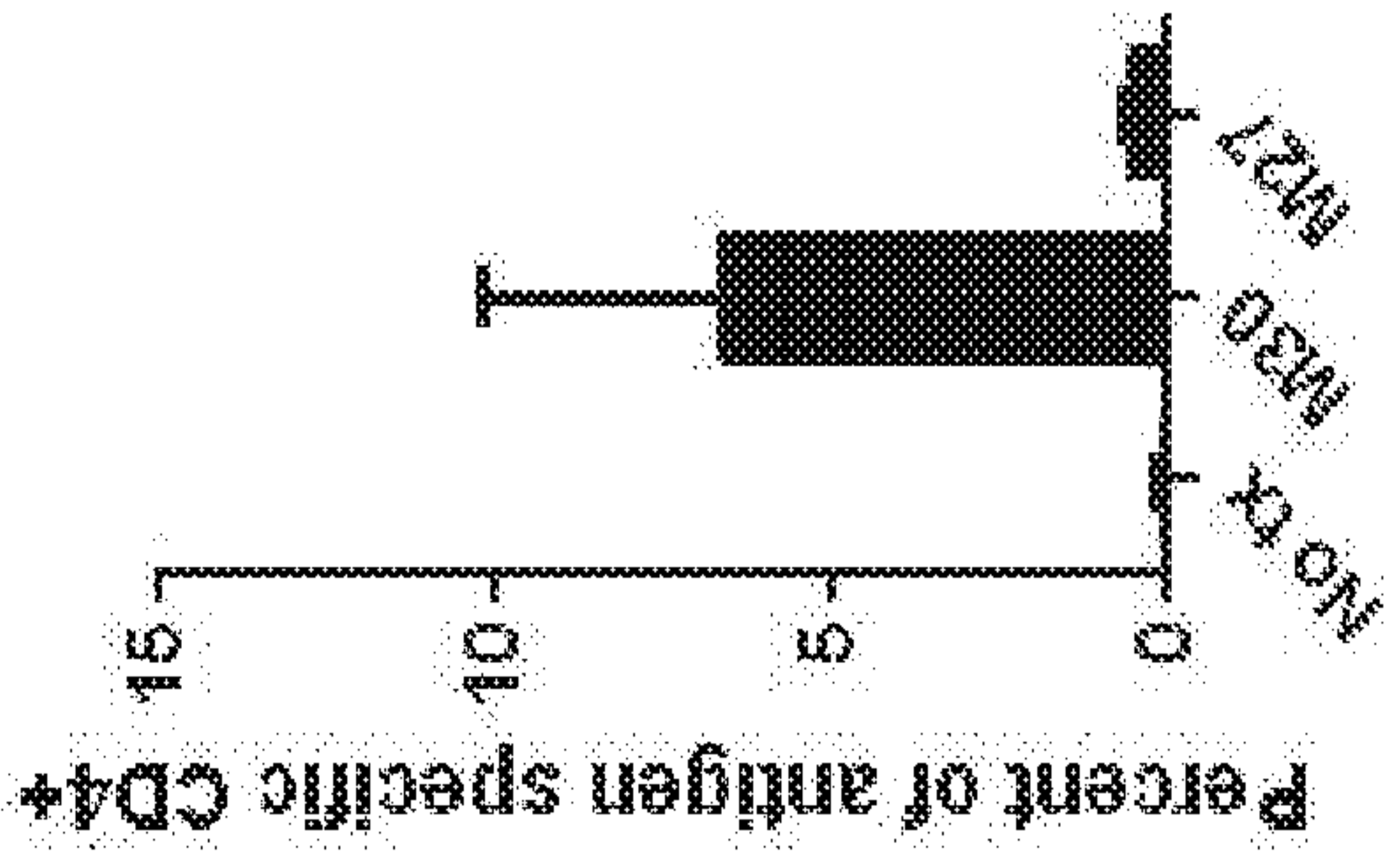
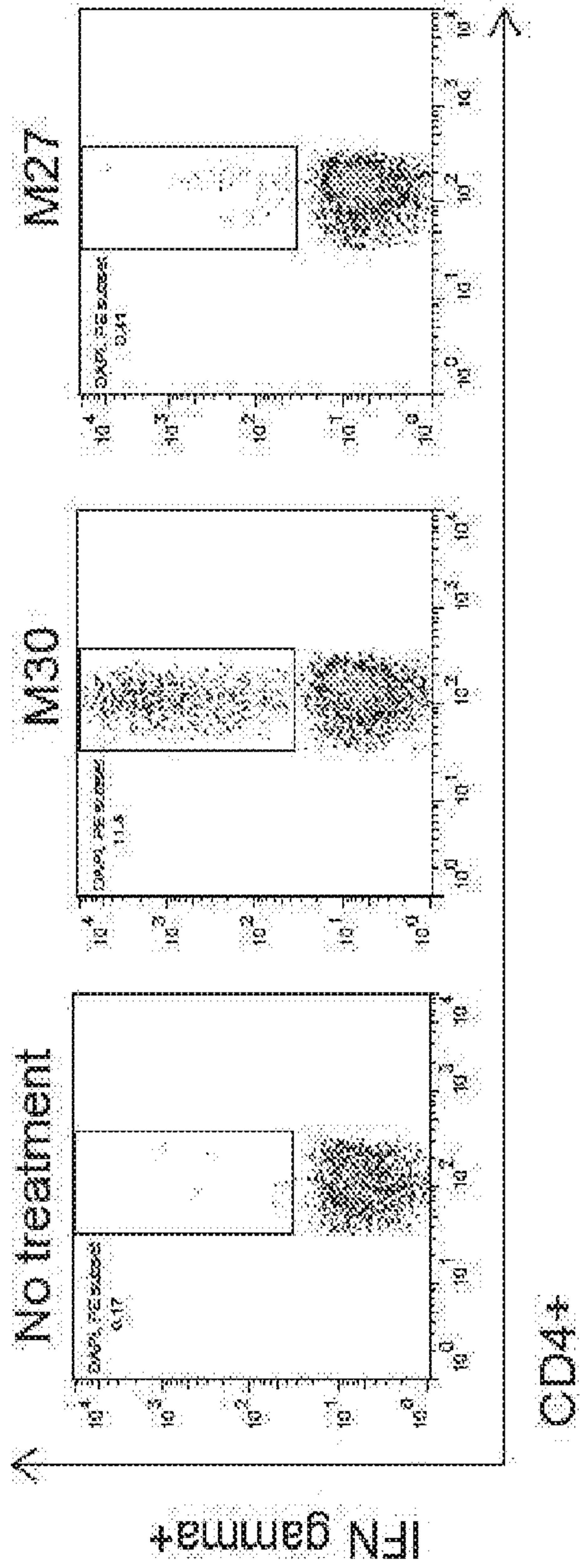
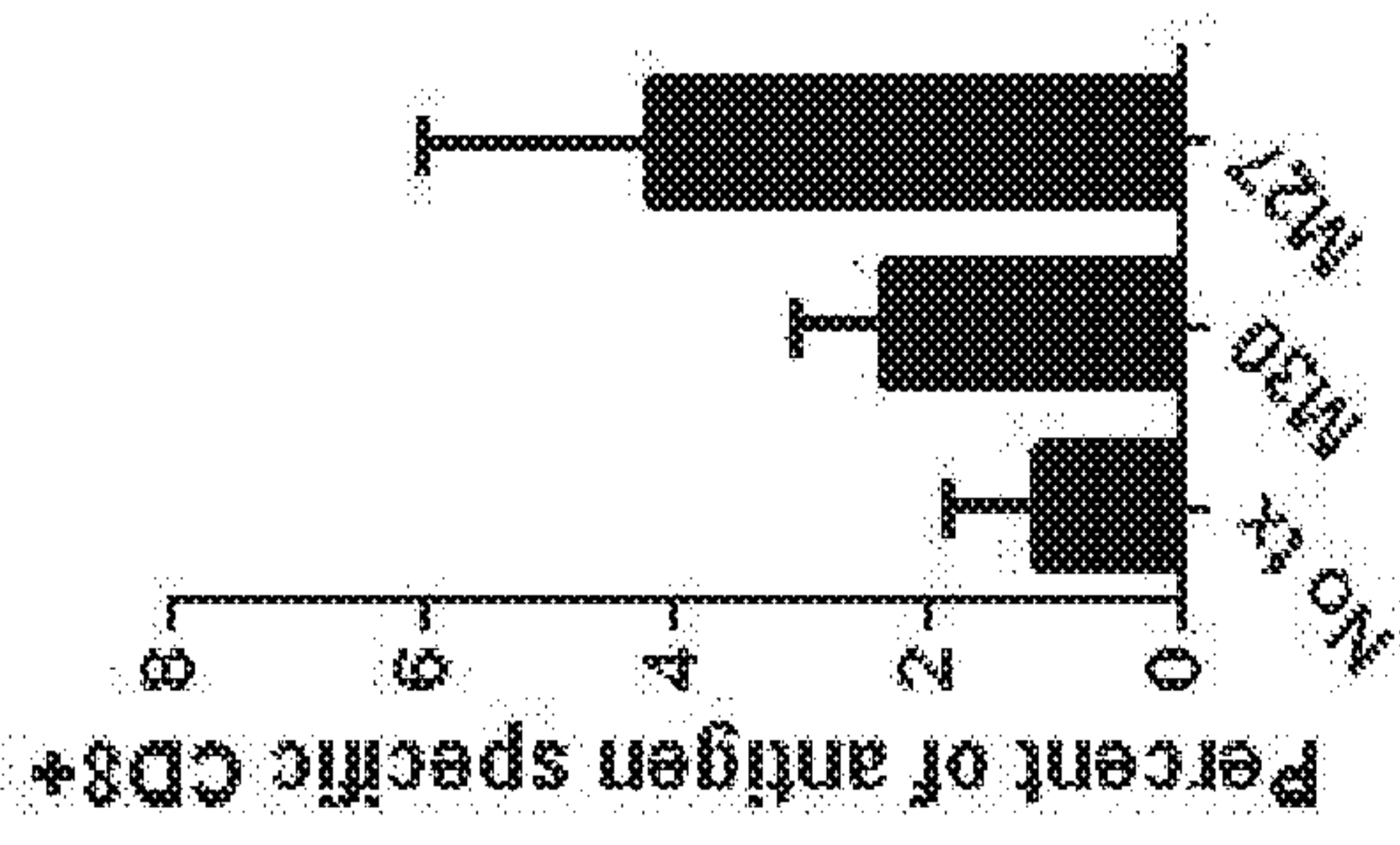
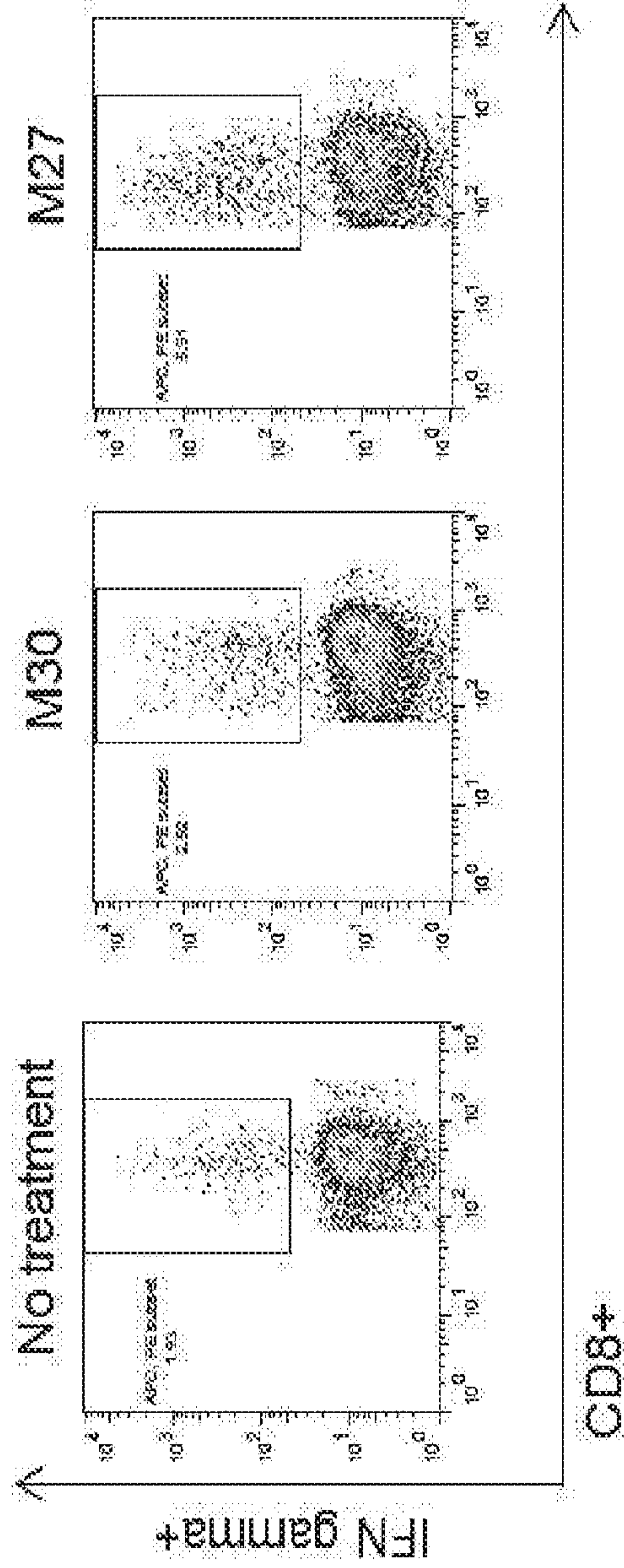


FIG. 28B



REPLACEMENT SHEET

FIG. 29A

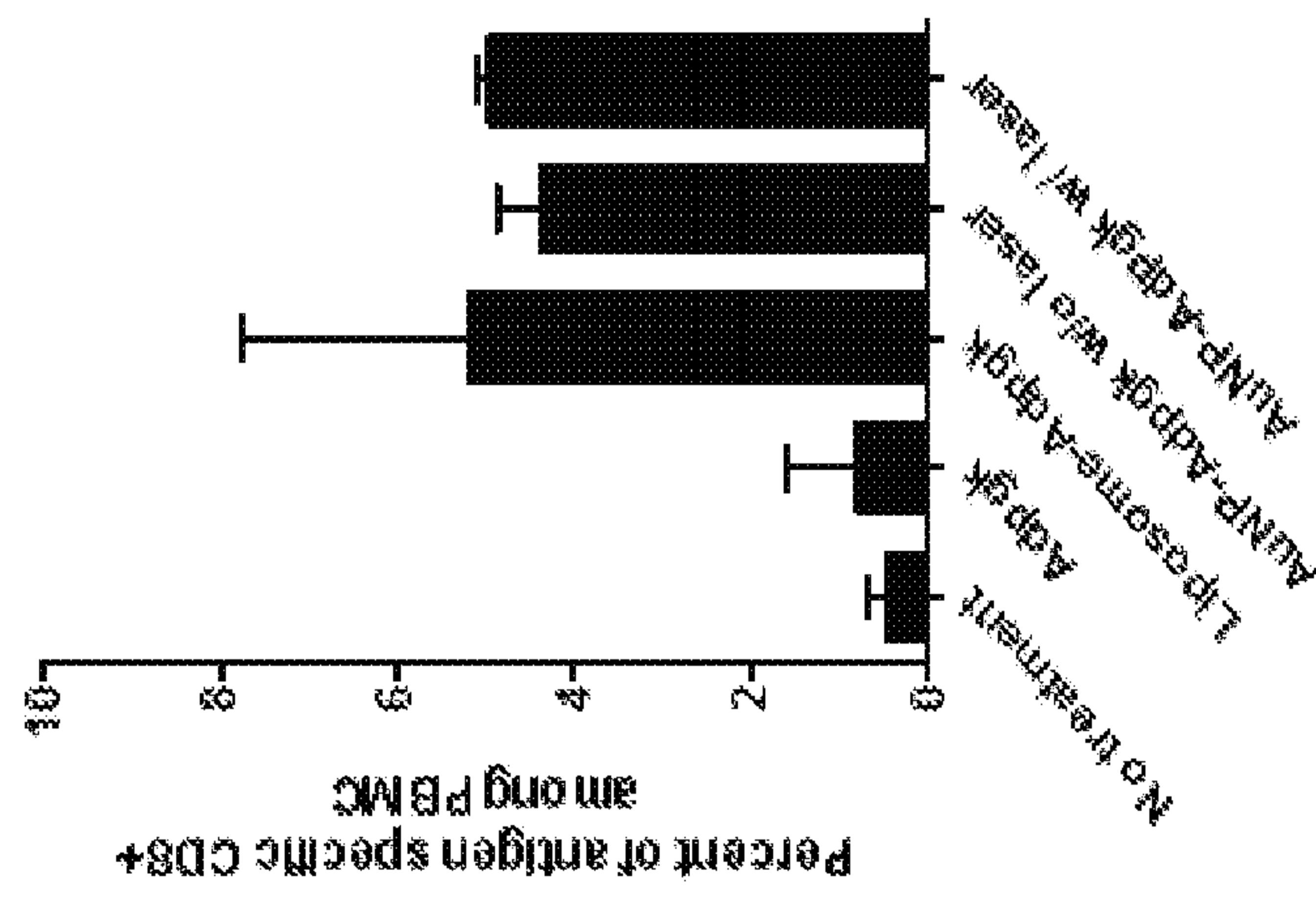
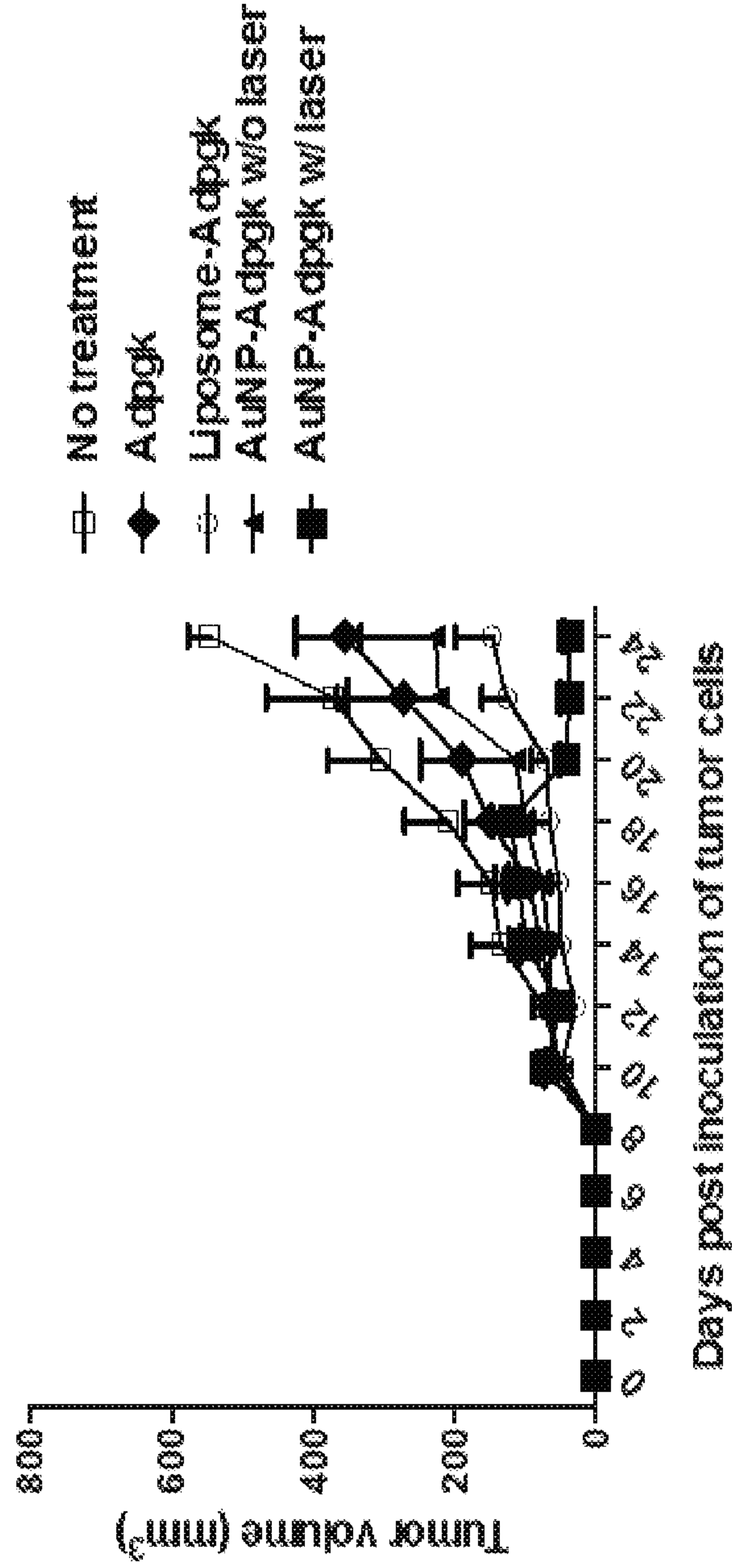


FIG. 29B



REPLACEMENT SHEET

FIG. 31

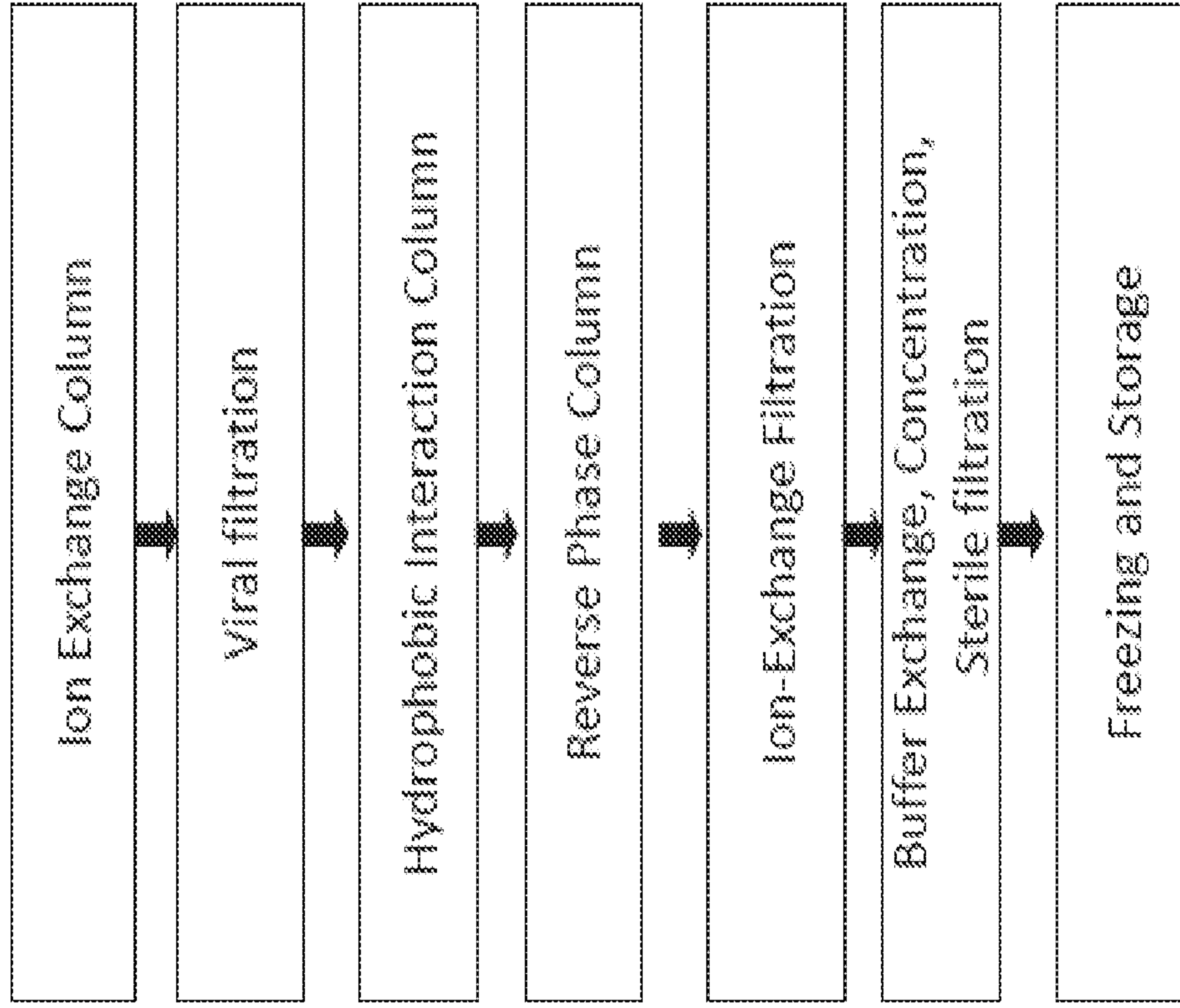
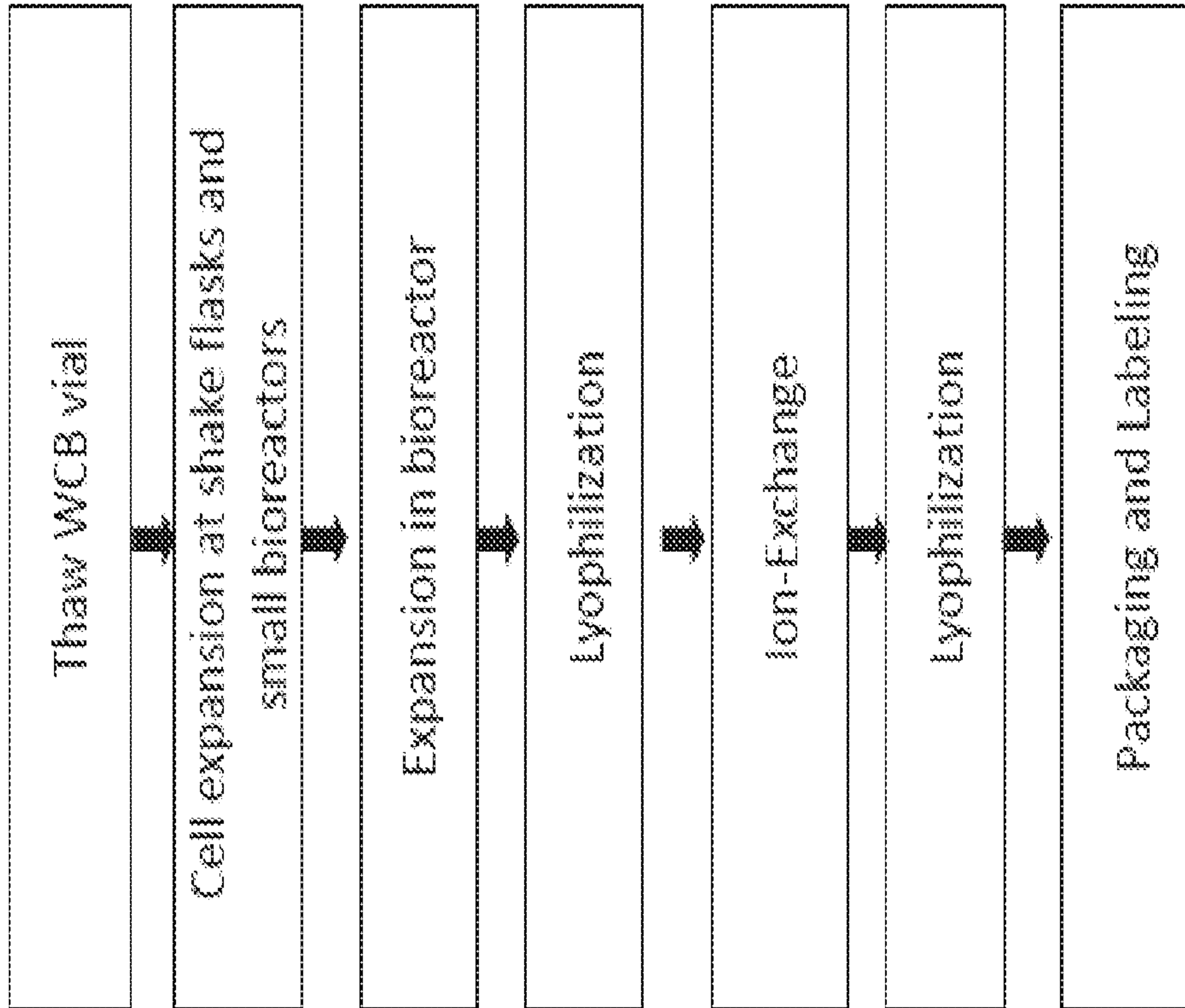


FIG. 30



REPLACEMENT SHEET

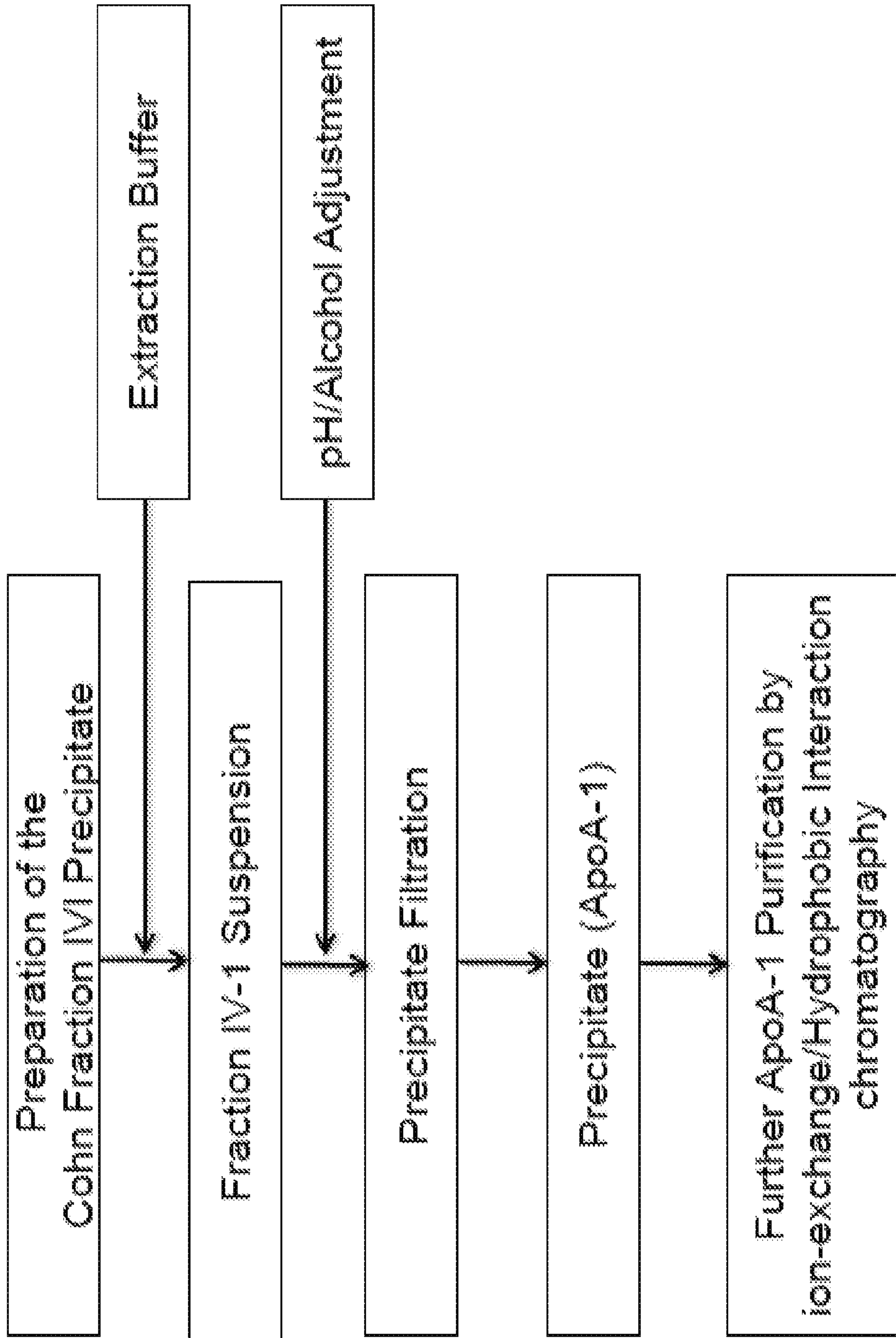
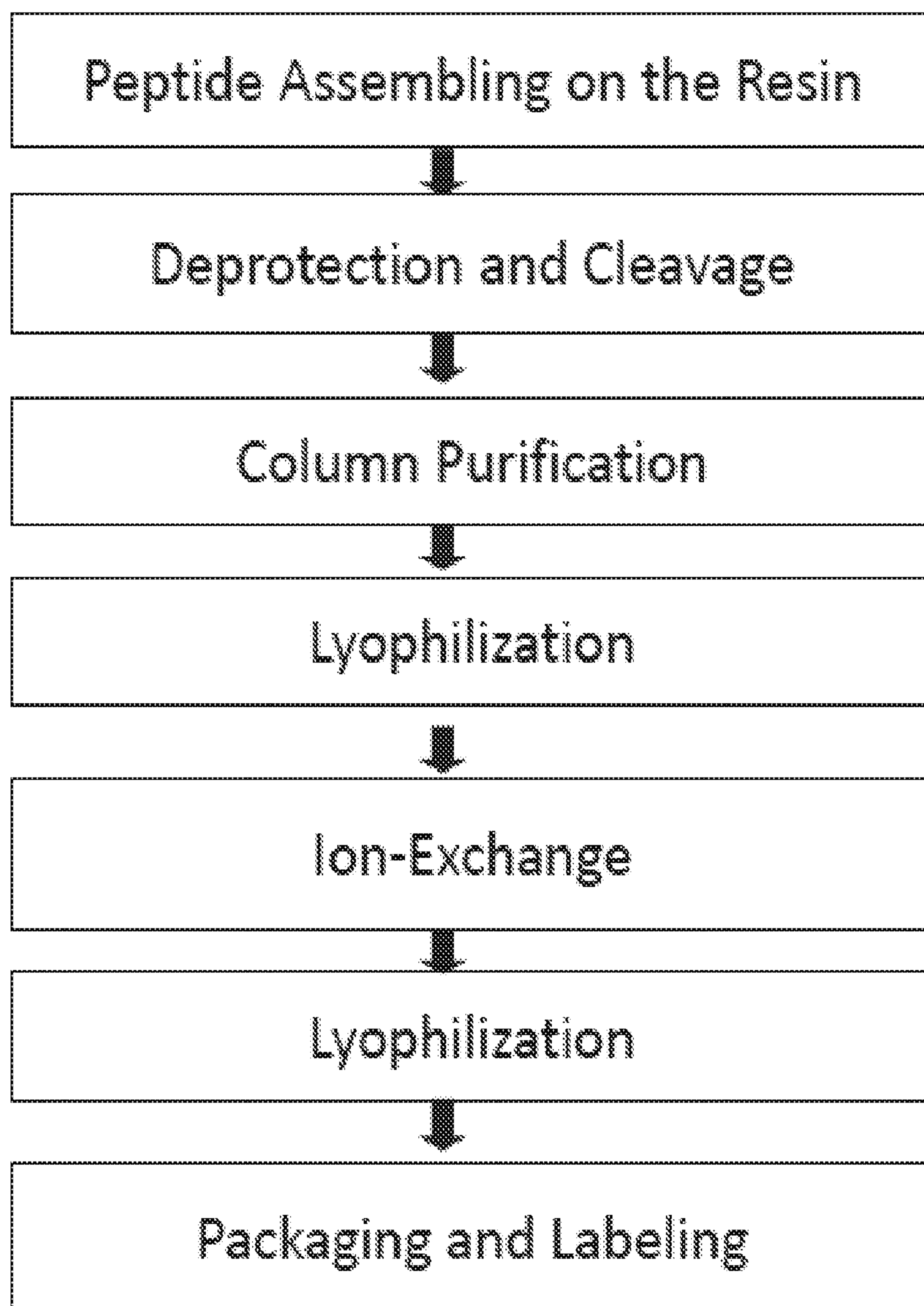


FIG. 32

FIG. 33



REPLACEMENT SHEET

FIG. 34

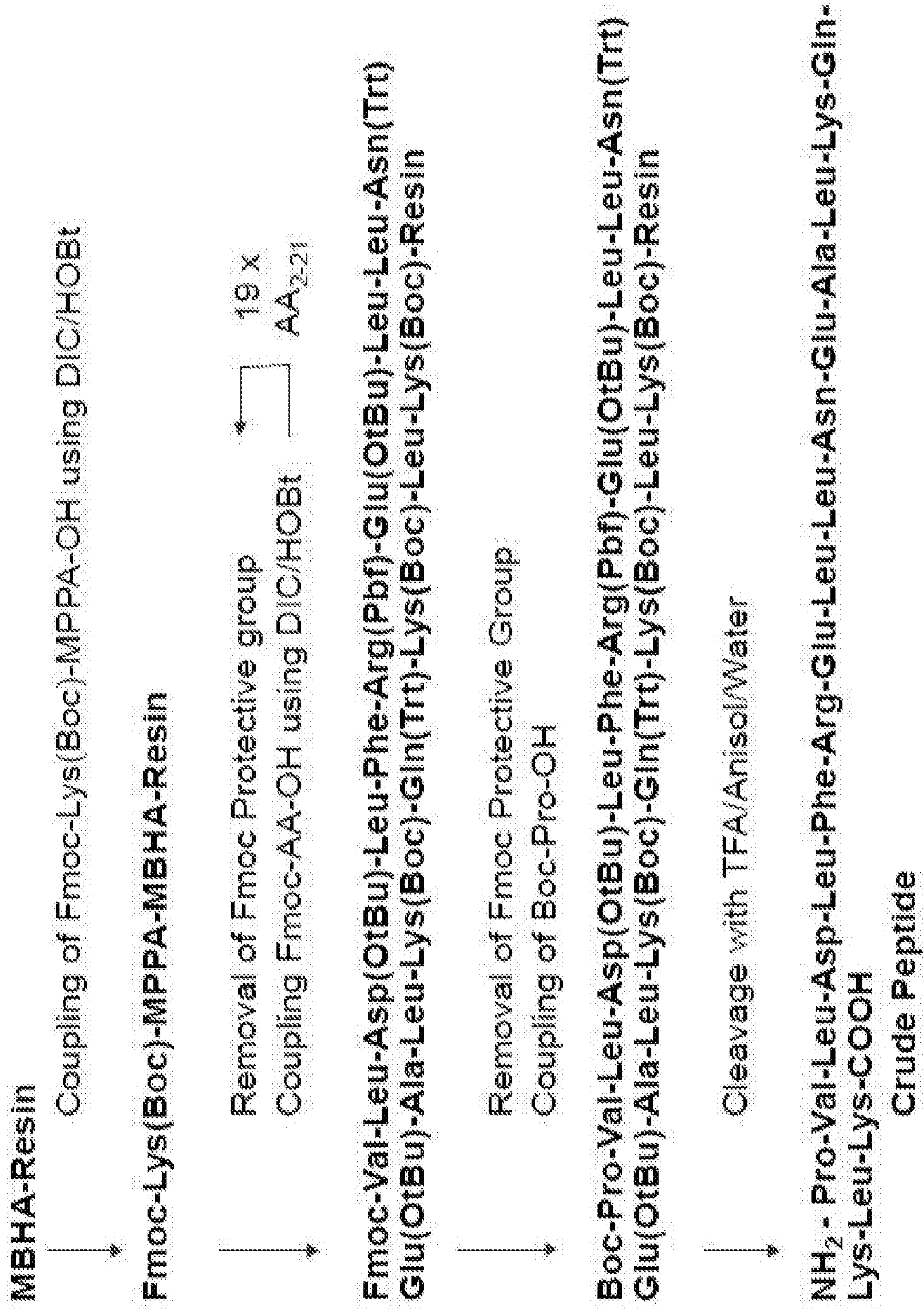
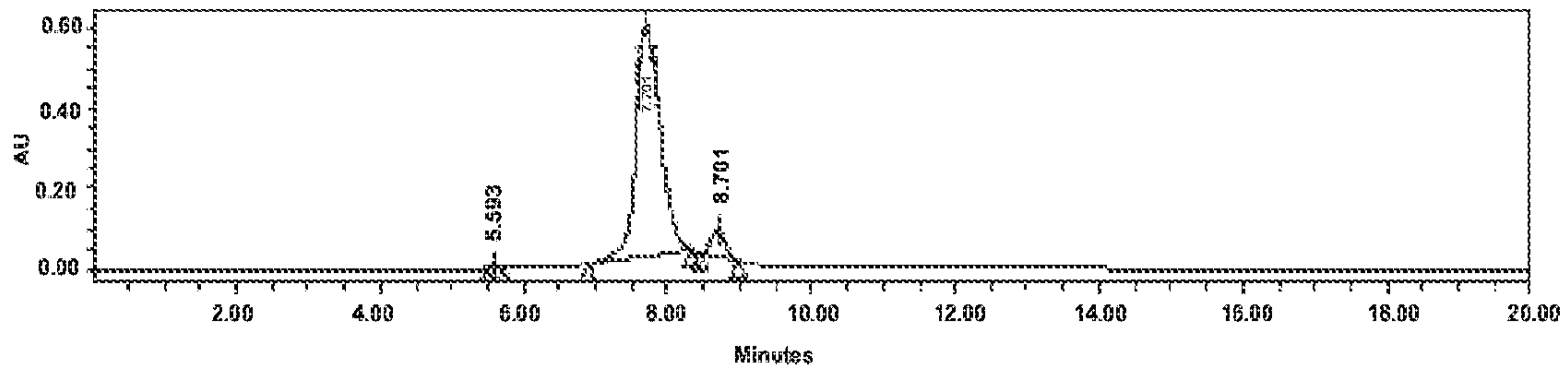


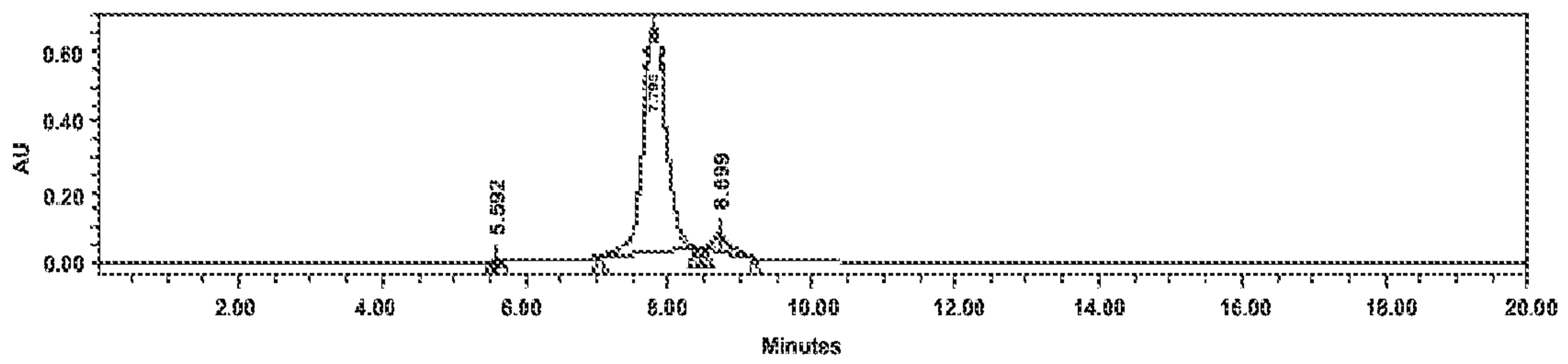
FIG. 35

REPLACEMENT SHEET

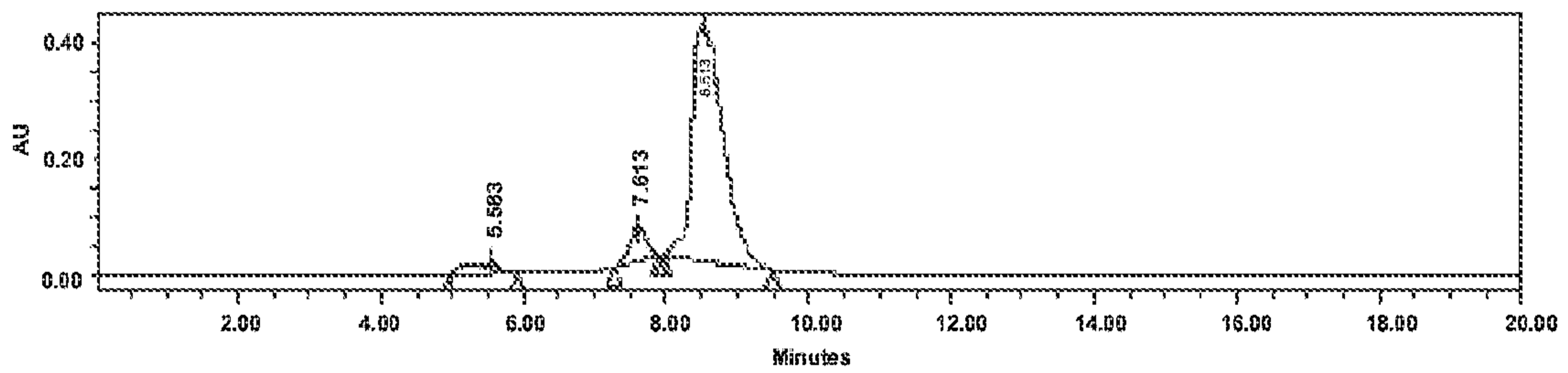
ApoA-1:DMPC 220 nm (purify of HDL: ~94%)



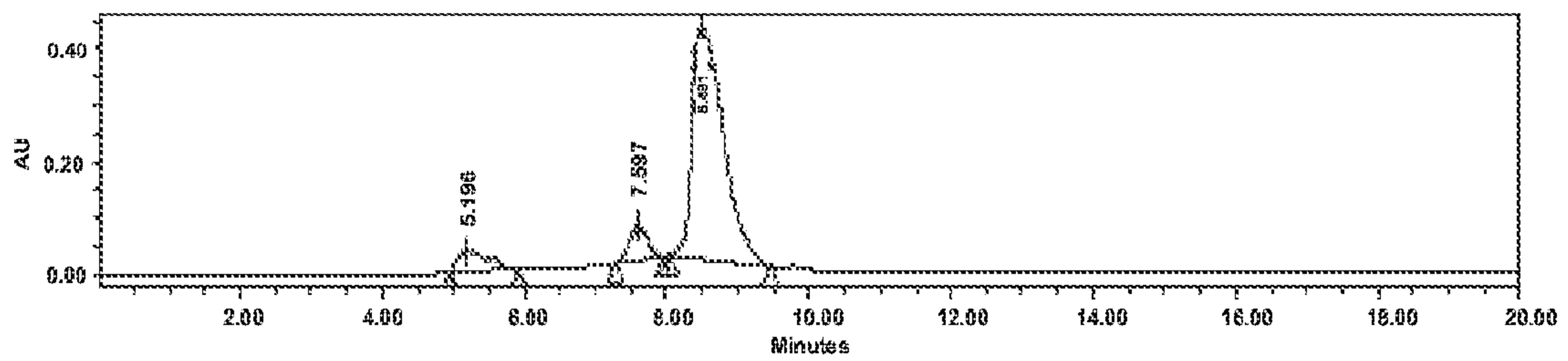
ApoA-1:SM 220 nm (purify of HDL: ~94%)



ApoA-1:POPC 220 nm (purify of HDL: ~17%)



ApoA-1:Egg PC 220 nm (purify of HDL: ~15%)



REPLACEMENT SHEET

FIG. 36

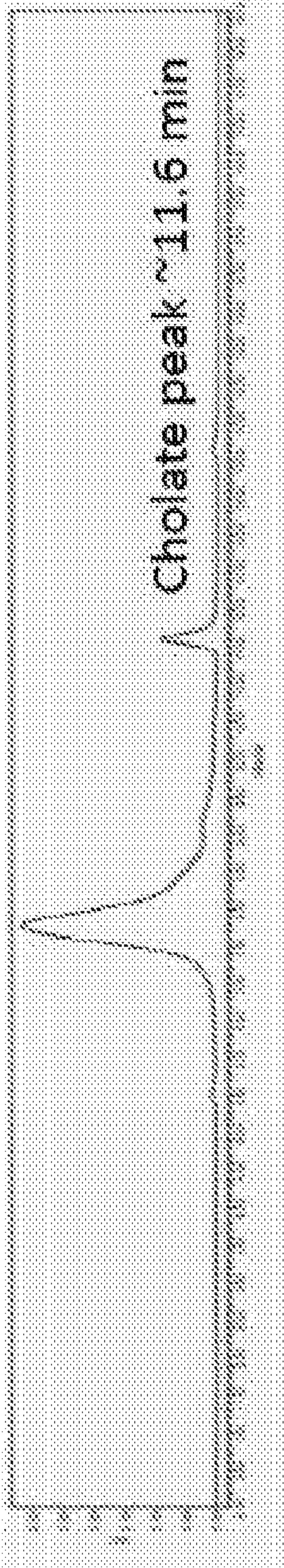
Sample	Run 1 Size nm, (% total area)	Run 2 Size nm, (% total area)	Run 3 Size nm, (% total area)
EggPC:ApoA-1	242.7 (64.3%) 1077 (34.2%)	546.8 (98.5%) 4850 (1.5%)	830.3 (53.1%) 191.5 (46.9%)
POPC:ApoA-1	987.1 (50.9%) 204 (49.1%)	1306 (43.4%) 233.4 (56.6%)	587.6 (96.6%) 4569 (3.4%)
DMPC:ApoA-1	11.28 (100%)	10.96 (100%)	10.98 (100%)
SM:ApoA-1	11.14 (100%)	11.08 (100%)	10.93 (100%)

REPLACEMENT SHEET

FIG. 37A

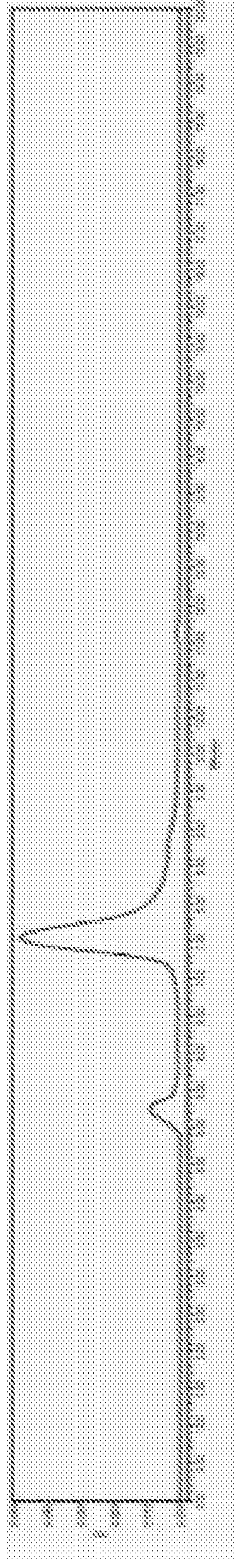
ApoA1:POPC

220 nm:



Retention Time	Area	% Area	Height
1	148246	0.95	8466
2	73370	0.47	4259
3	1307380	84.06	318861
4	1053196	6.77	23951
5	354283	6.70	88525
6	30306	0.32	3524
7	111335	0.72	6378

280 nm:



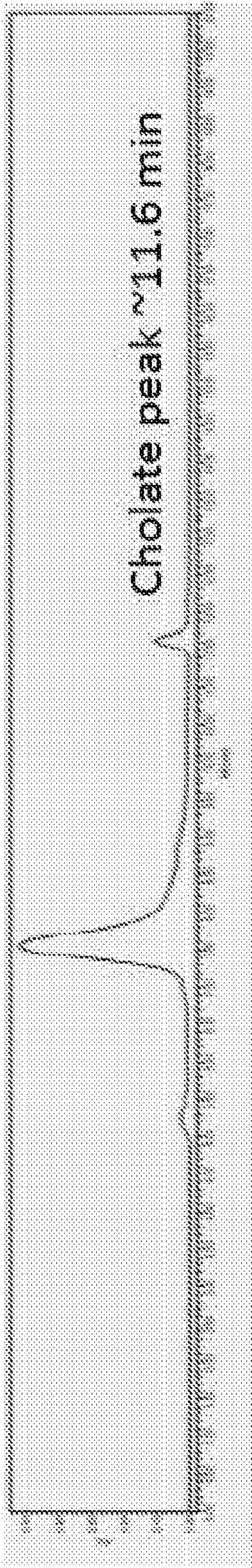
Retention Time	Area	% Area	Height
1	170047	11.16	8637
2	1348334	88.48	46378
3	5541	0.36	599

REPLACEMENT SHEET

FIG. 37B

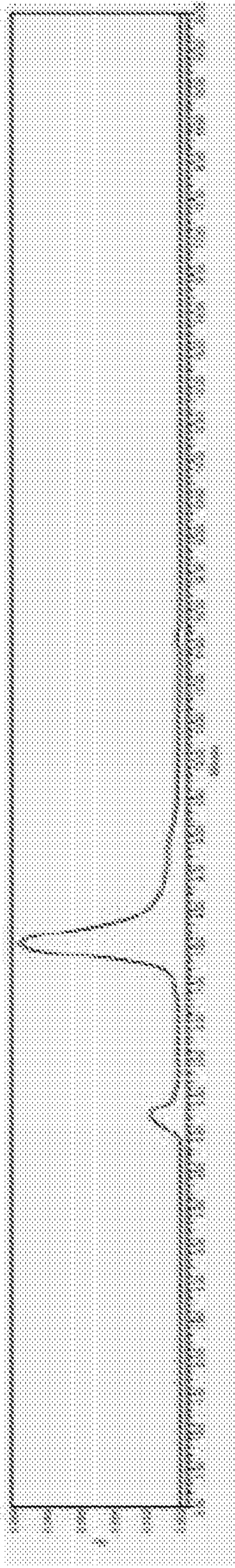
Ap0A1:DMPC:DPPG-NH₄⁺

220 nm:



Retention Time	Area	% Area	Height
1	427289	2.63	24045
2	147567	0.91	10094
3	14637351	90.11	493780
4	1031911	6.35	101191

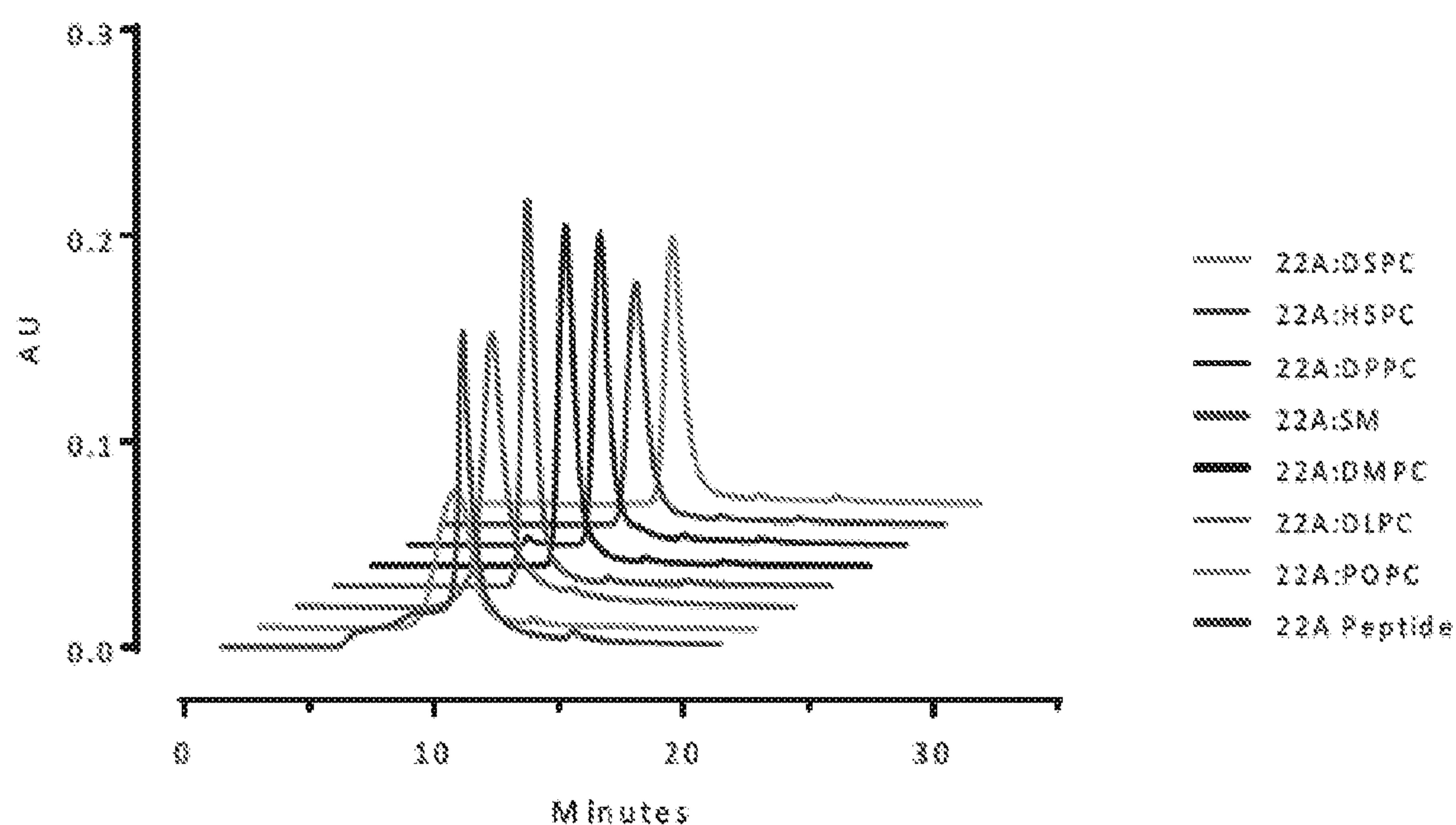
280 nm:



Retention Time	Area	% Area	Height
1	170047	11.16	8837
2	1348334	88.48	46376
3	5541	0.36	599

FIG. 38

sHDL analyzed with GPC

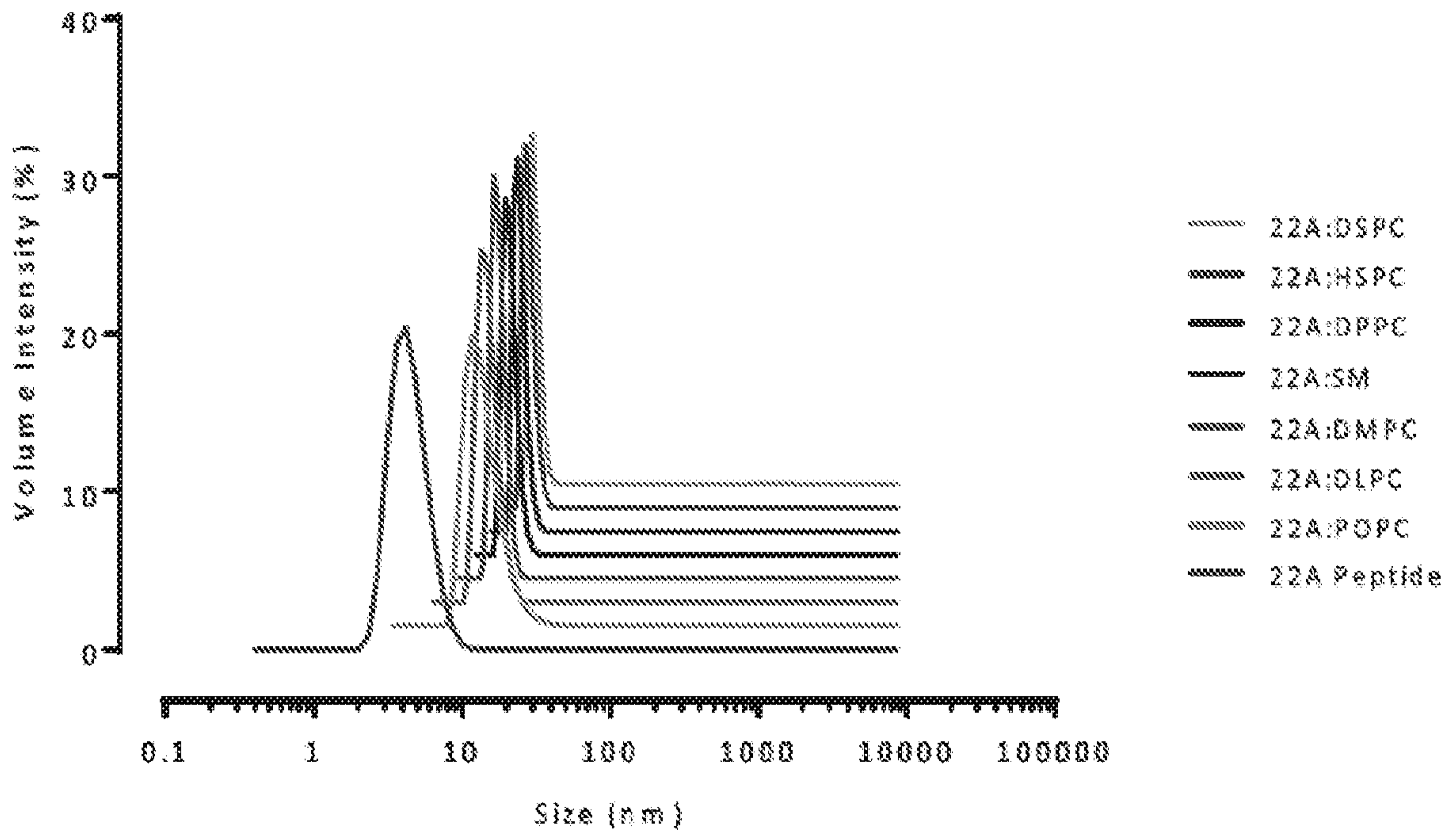


sHDL Composition	Retention Time (min)
22A Peptide	9.656
22A:POPC	7.880
22A:DLPC	7.829
22A:DMPC	7.773
22A: SM	7.754
22A:DPPC	7.649
22A:HSPC	7.579
22A:DSPC	7.558

REPLACEMENT SHEET

FIG.39

sHDL analyzed with DLS



SHDL COMPOSITION	SIZE (NM)
22A PEPTIDE	4.488
22A:POPC	10.84
22A:DLPC	8.663
22A:DMPC	8.307
22A: SM	8.646
22A:DPPC	9.169
22A:HSPC	9.771
22A:DSPC	9.262

REPLACEMENT SHEET

FIG. 40C

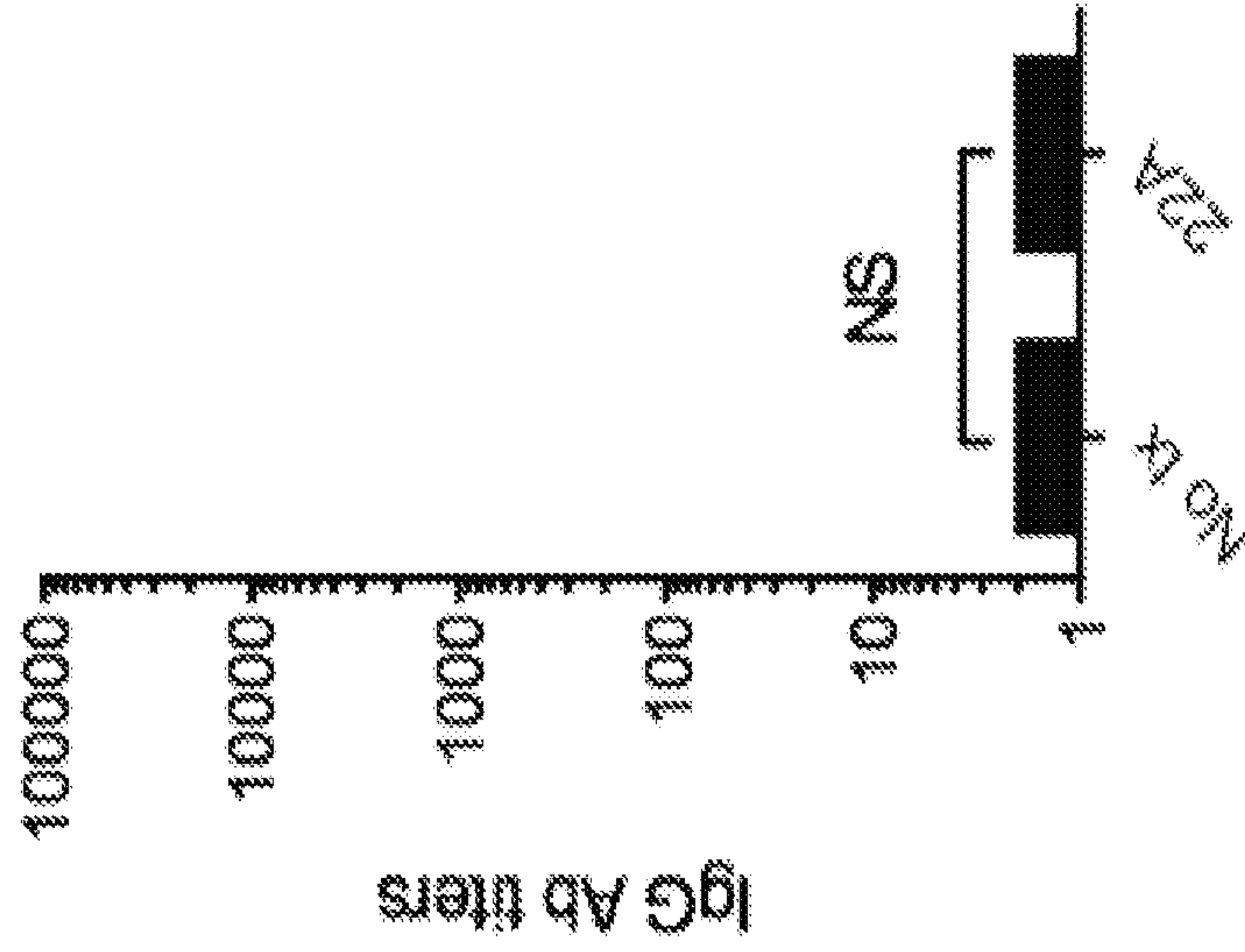


FIG. 40B

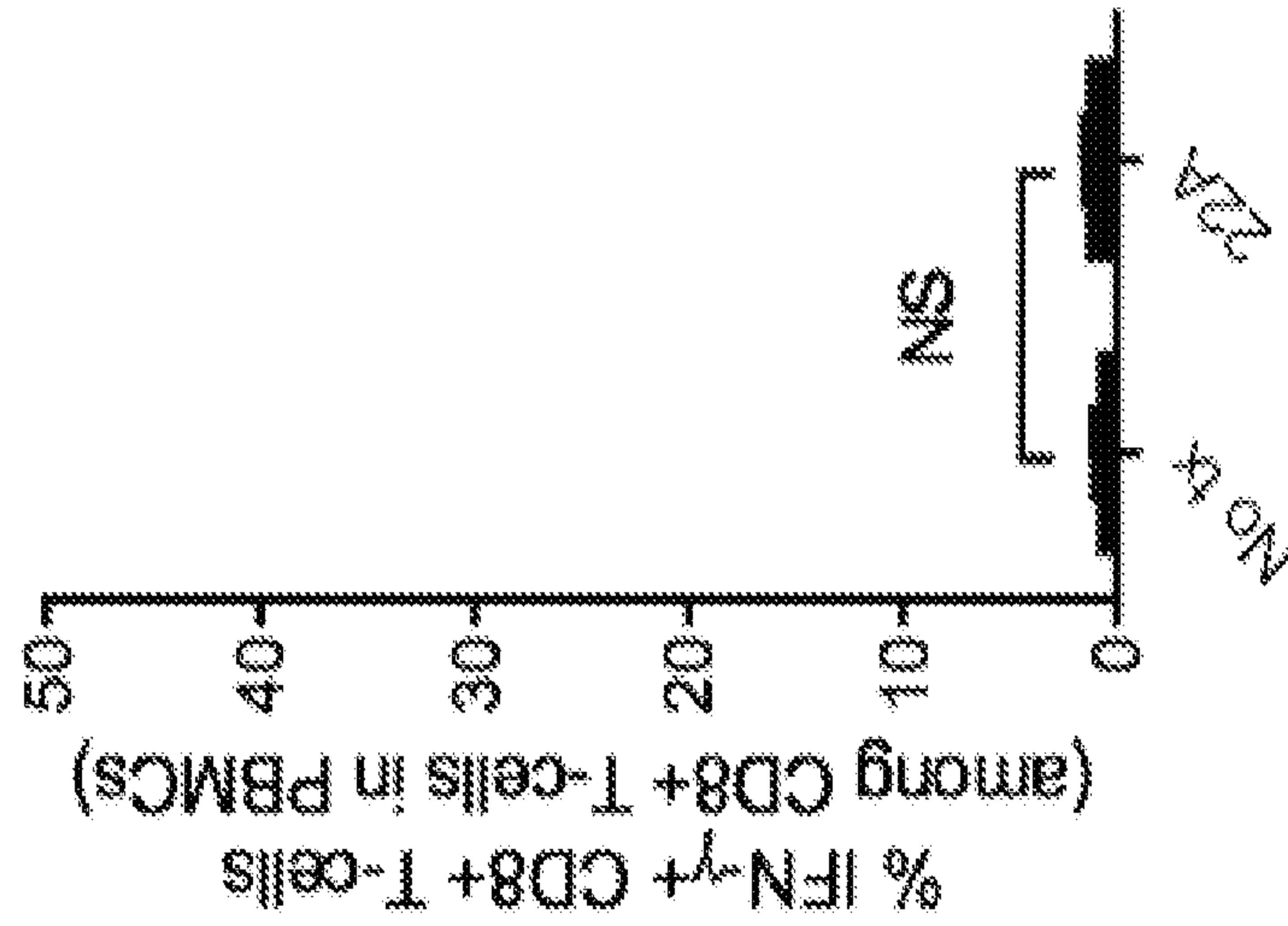
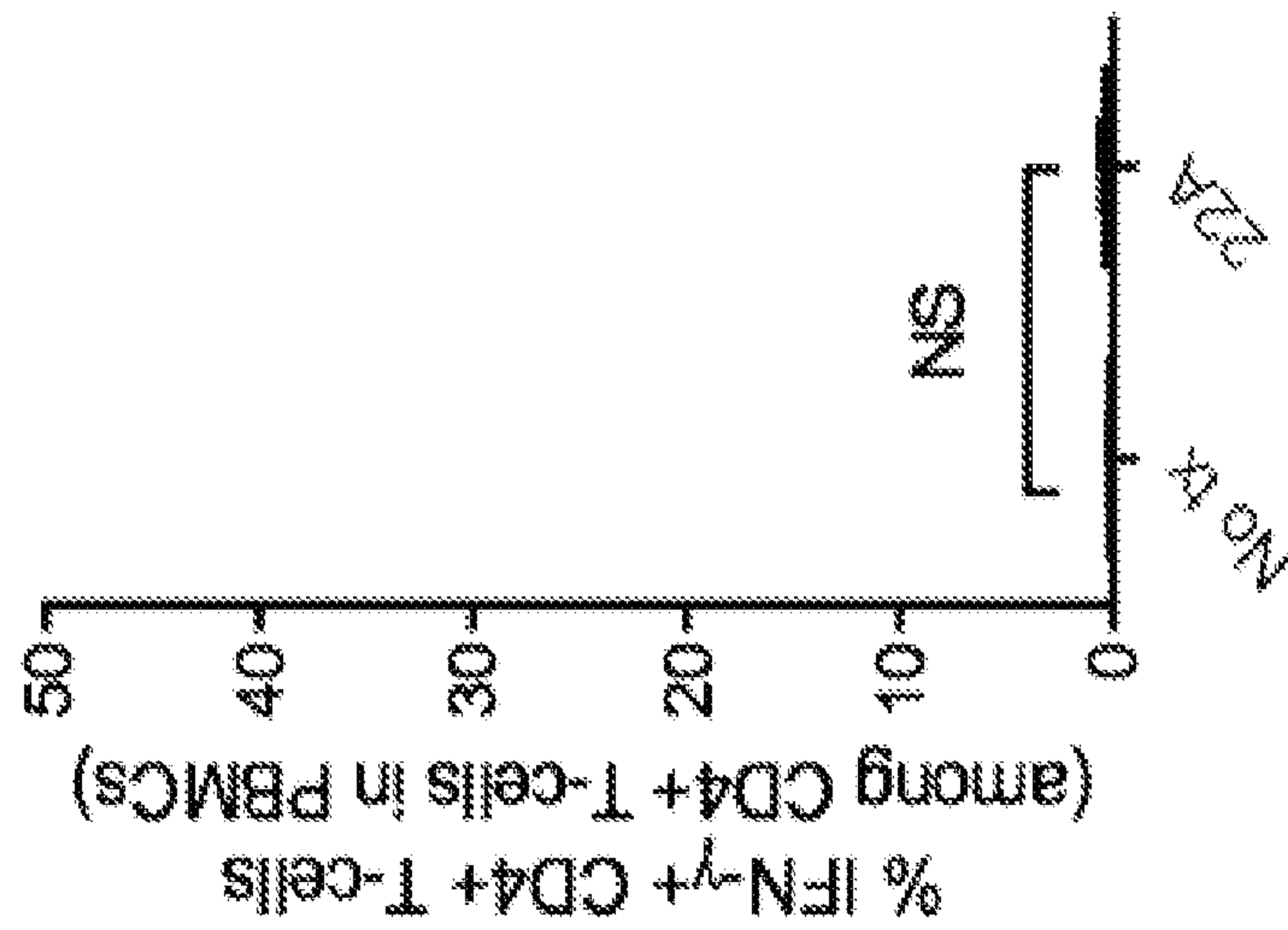


FIG. 40A



REPLACEMENT SHEET

FIG. 41A

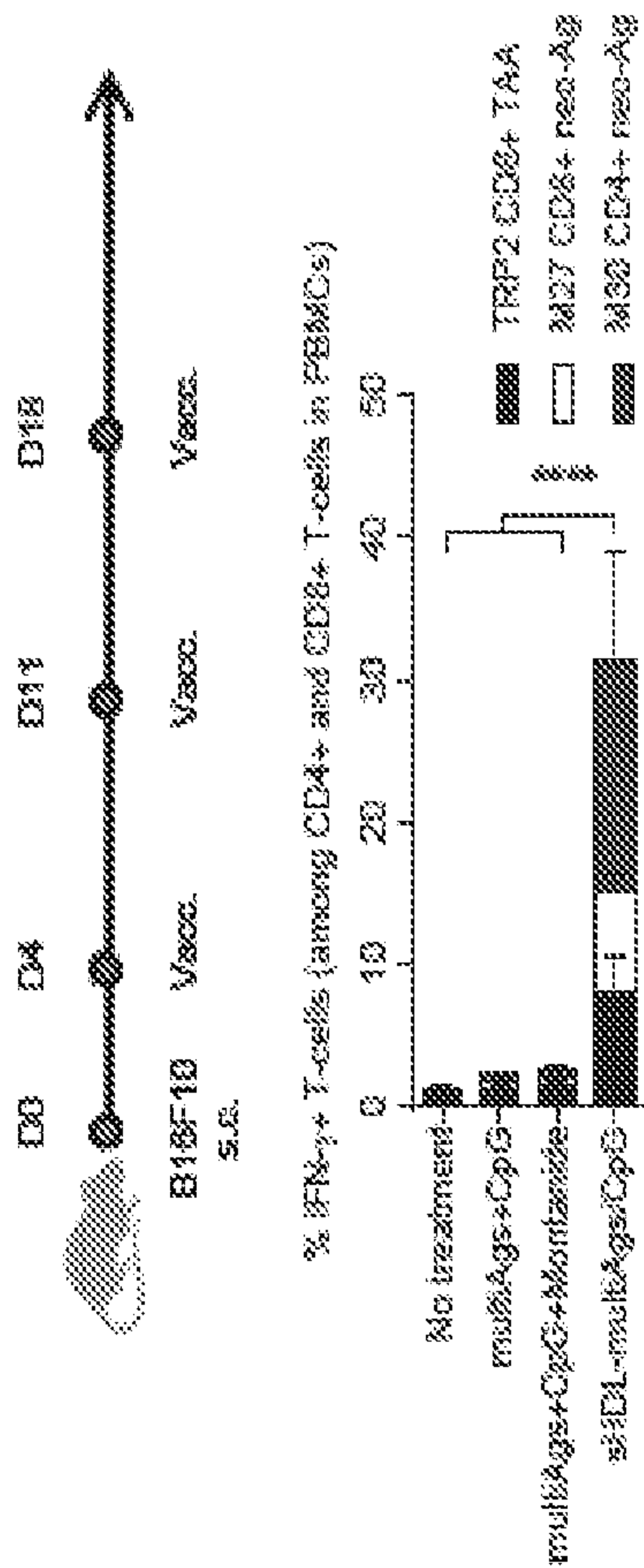


FIG. 41B

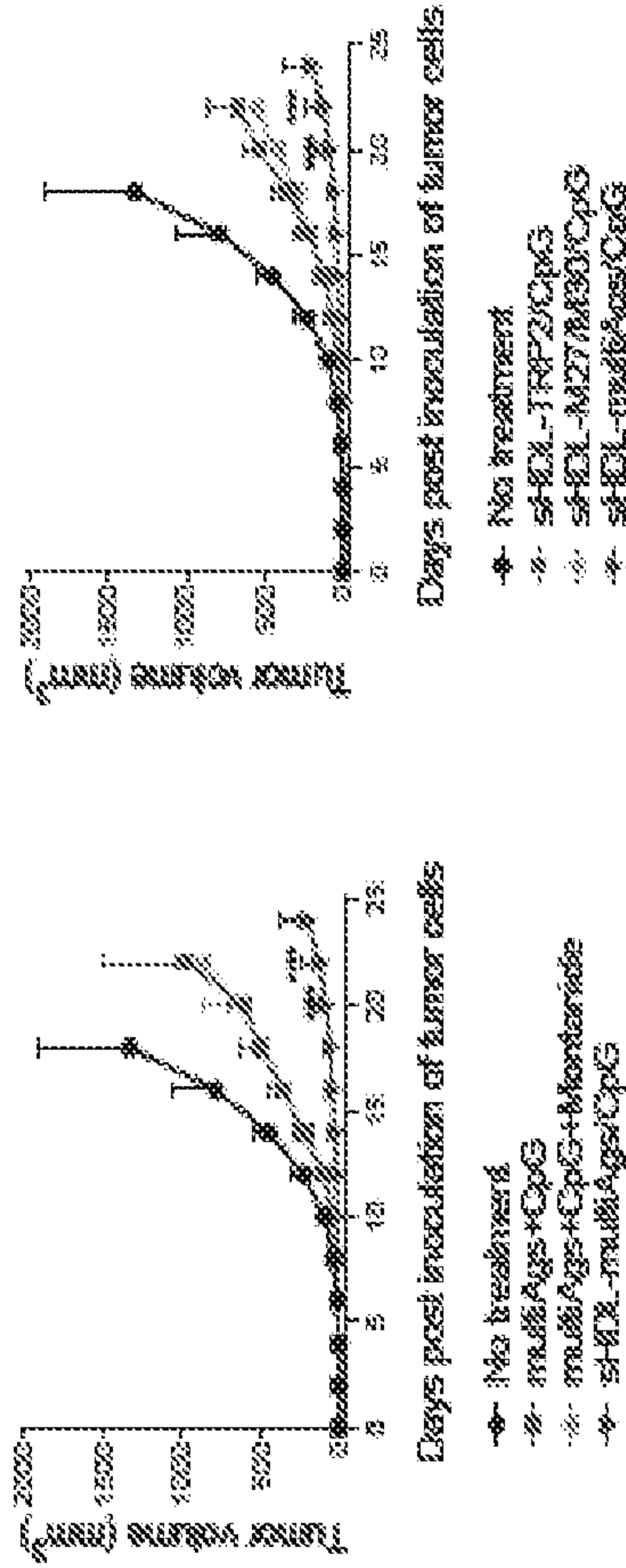
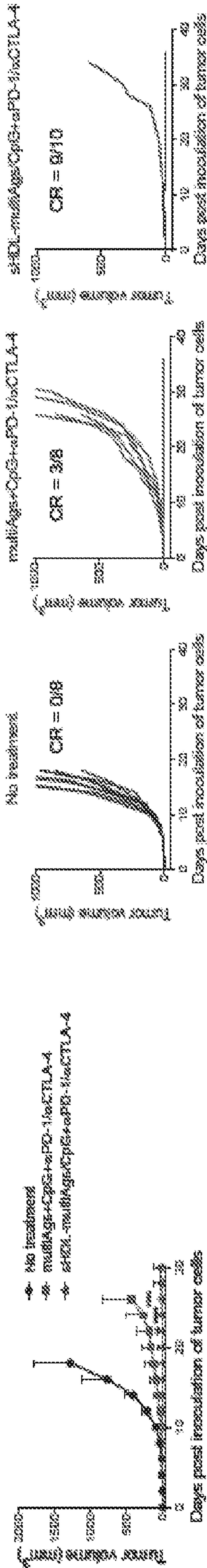


FIG. 41D



REPLACEMENT SHEET

FIG. 42A

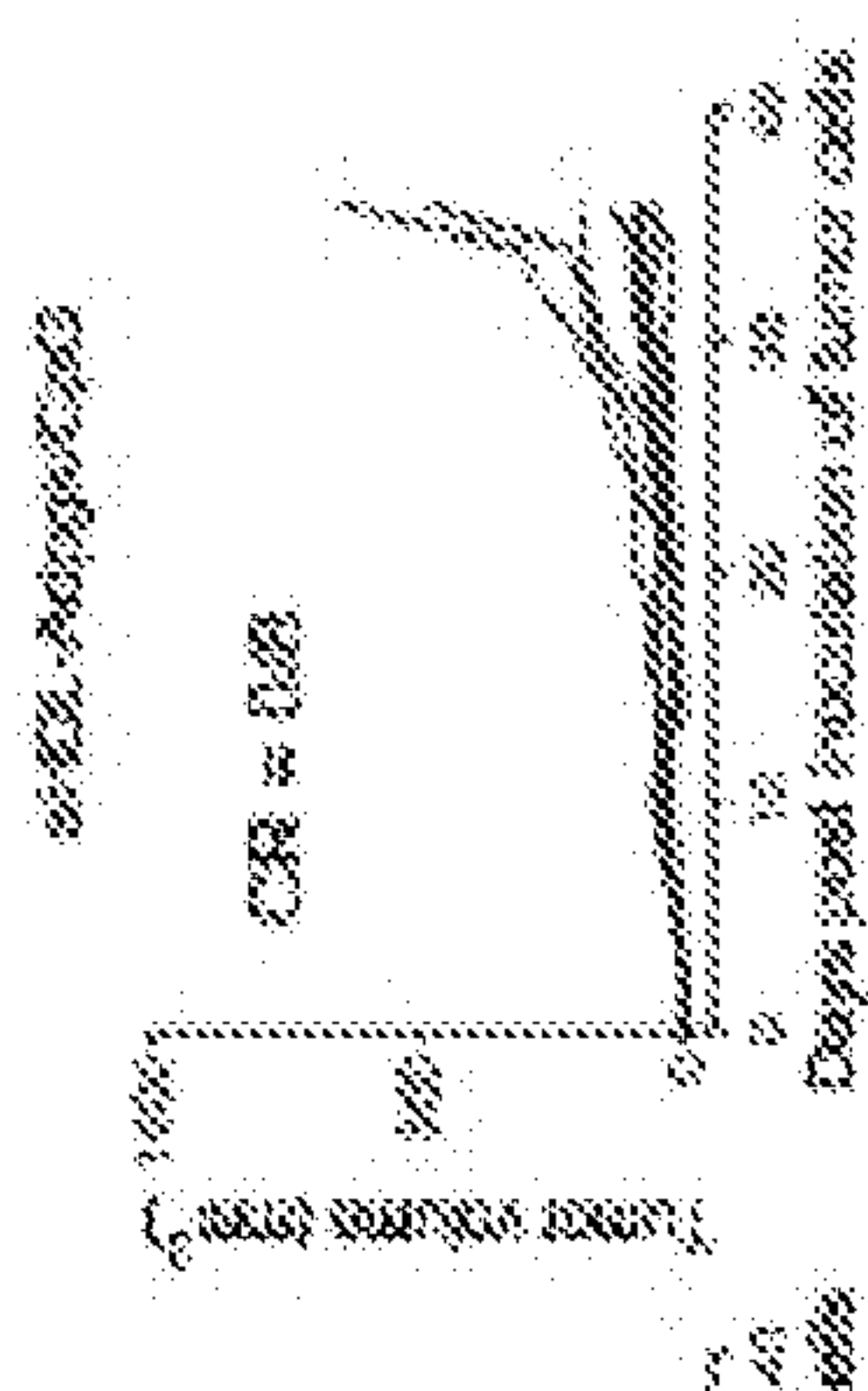
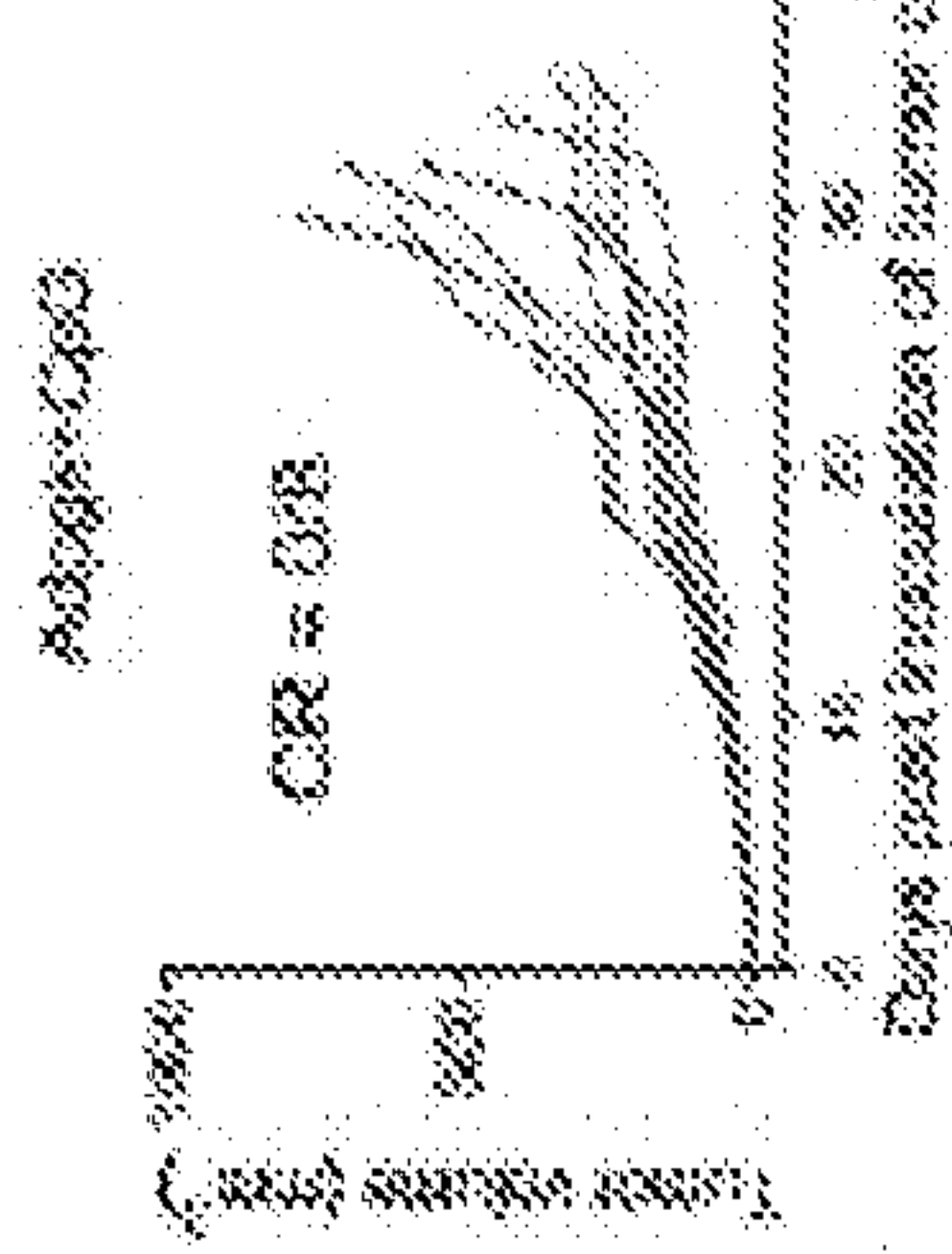
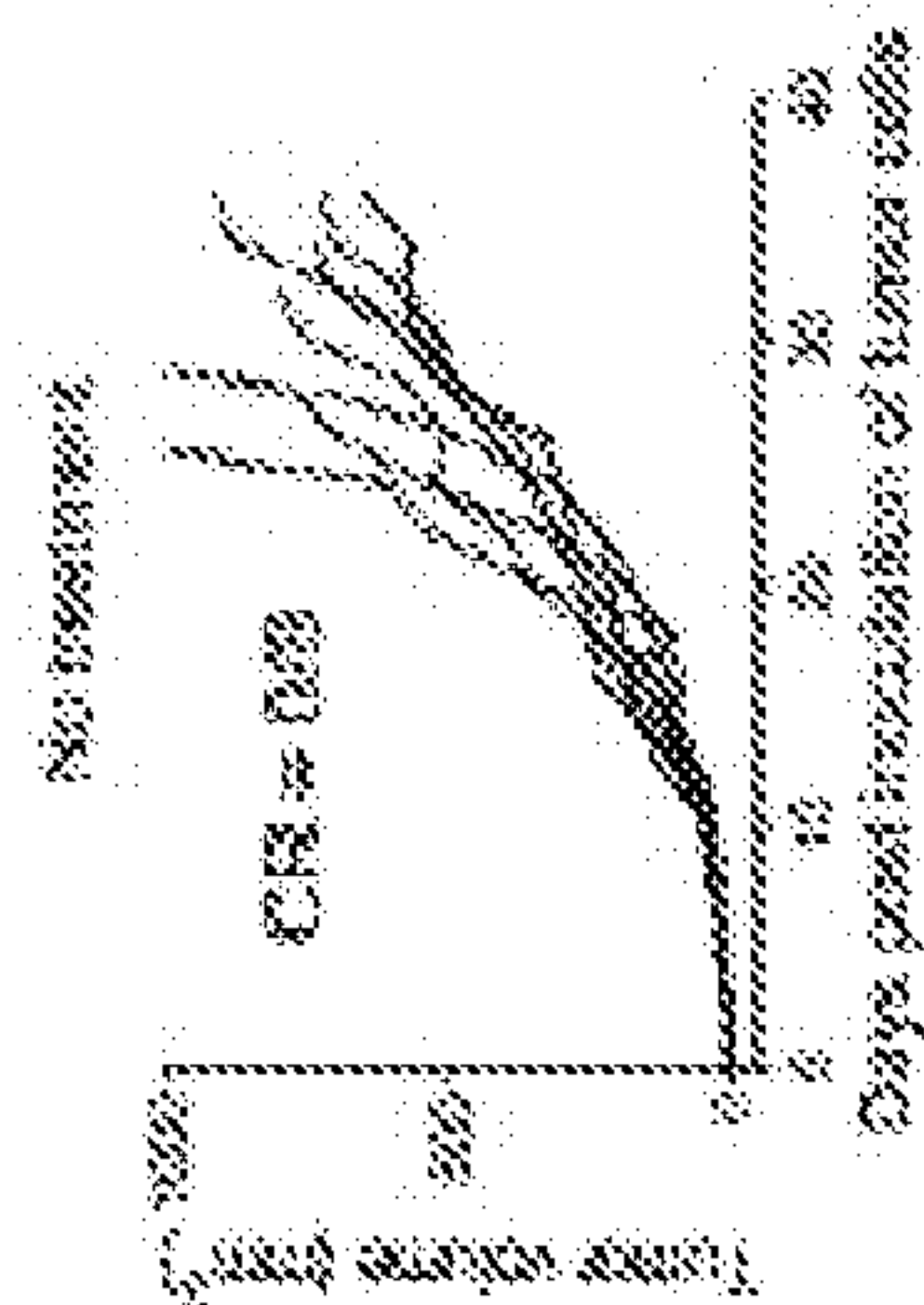
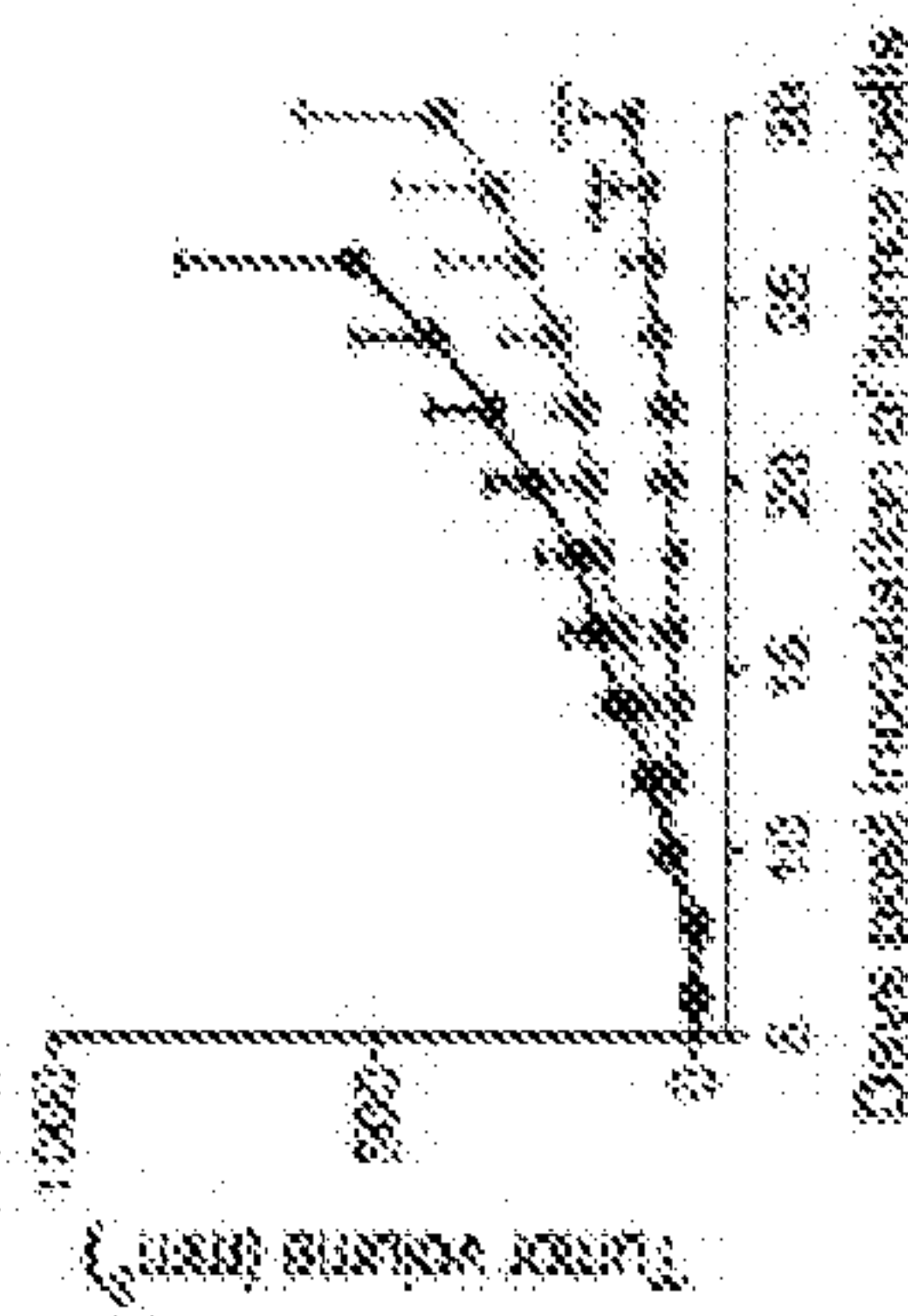
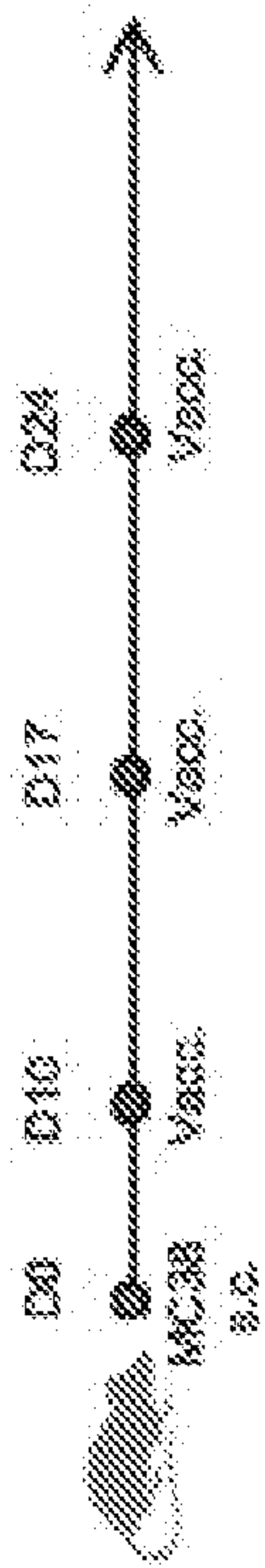


FIG. 42B

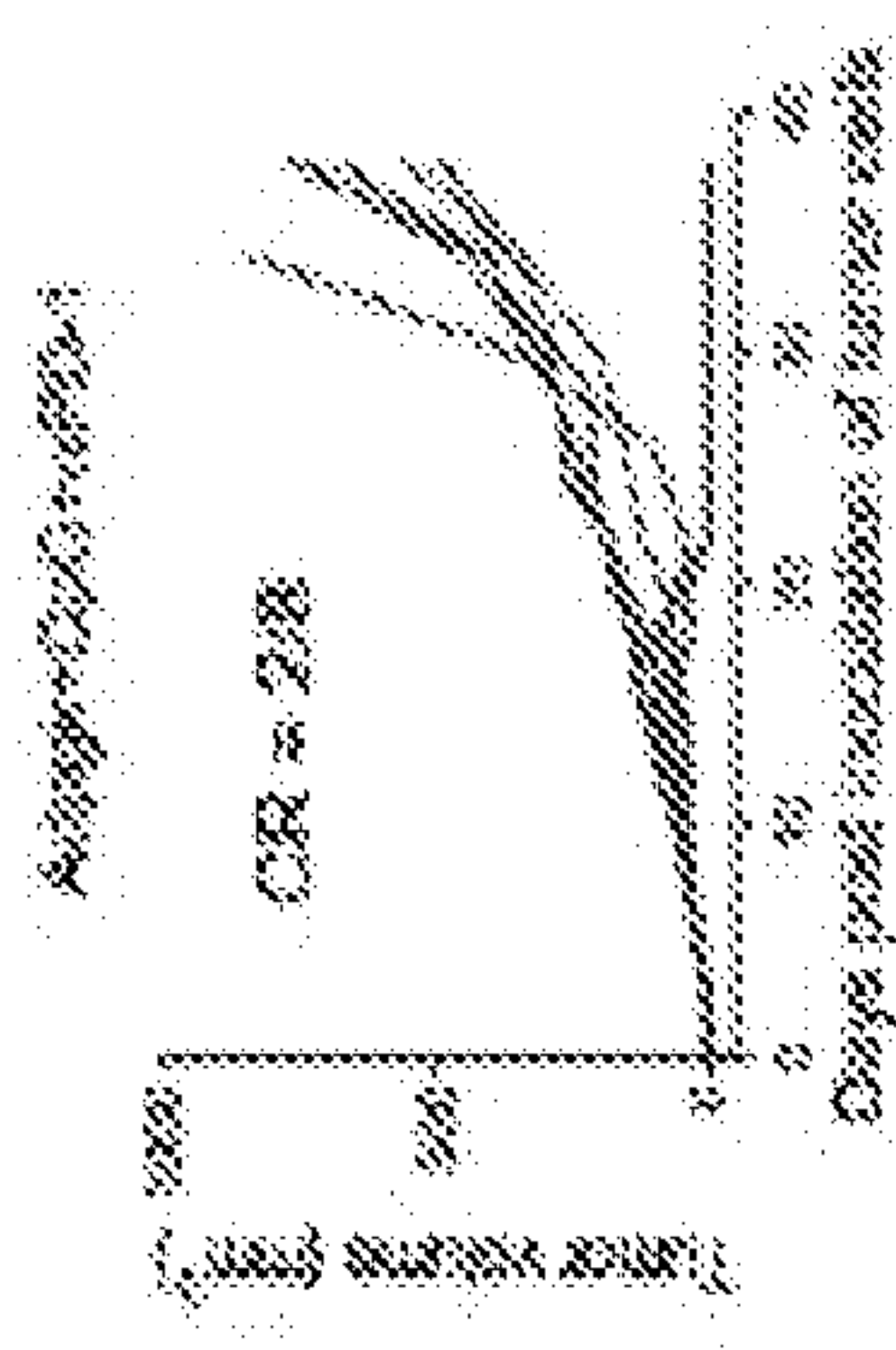
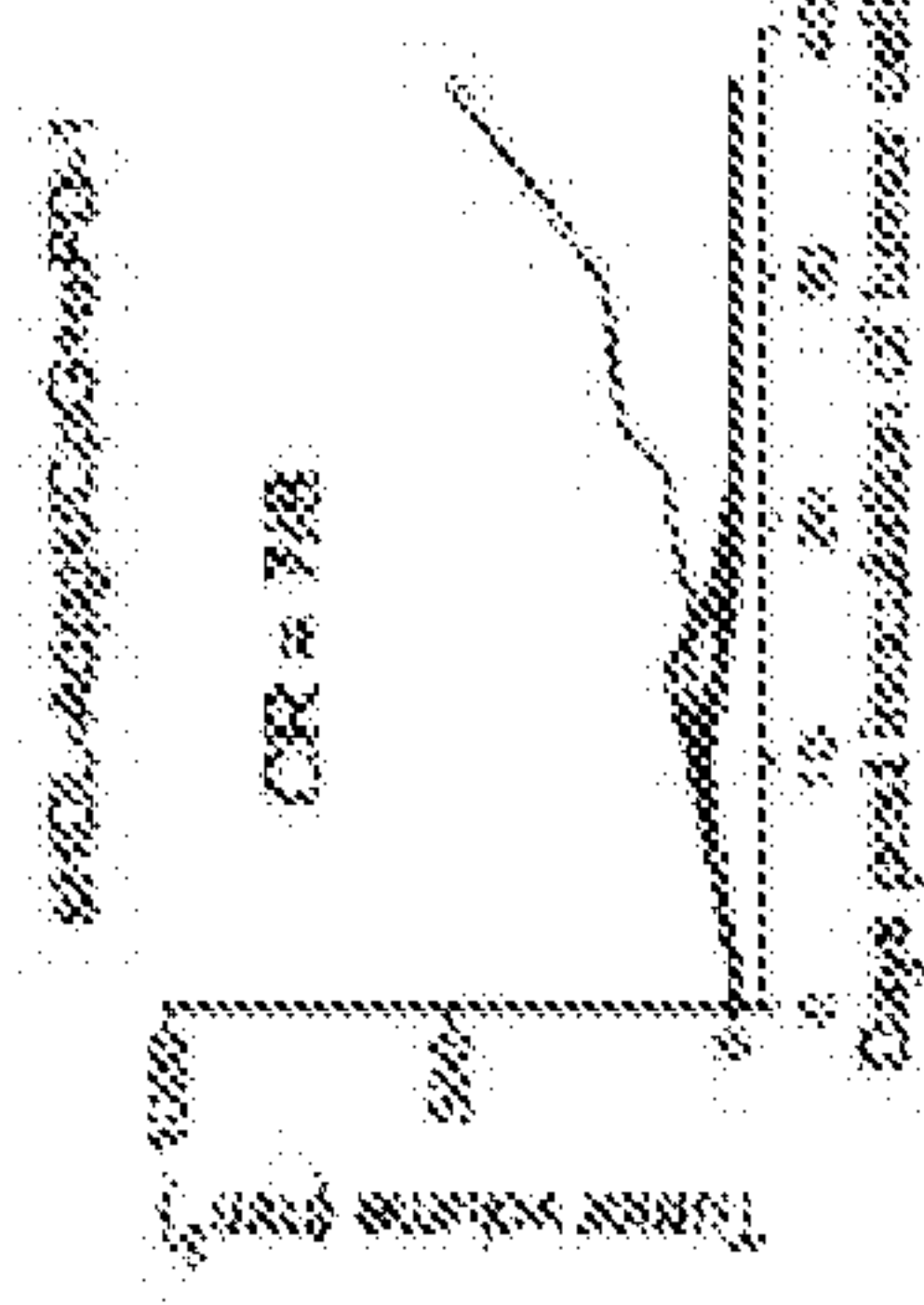
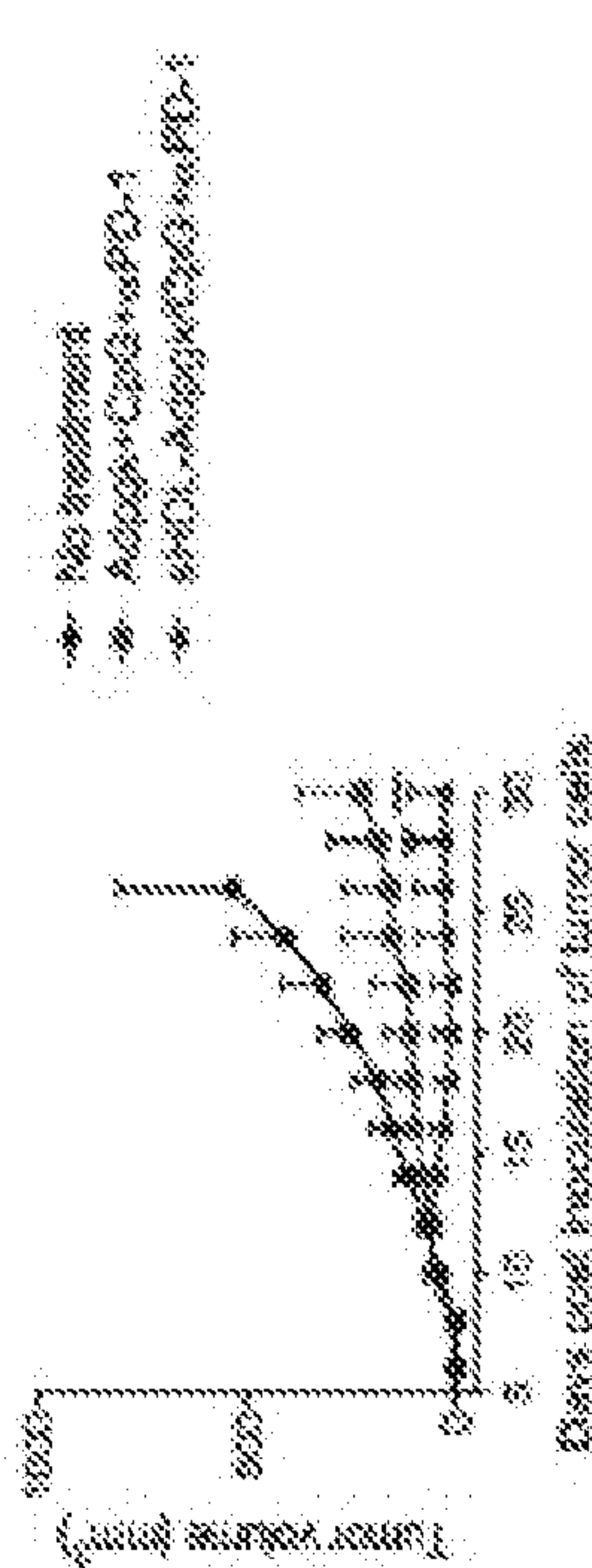
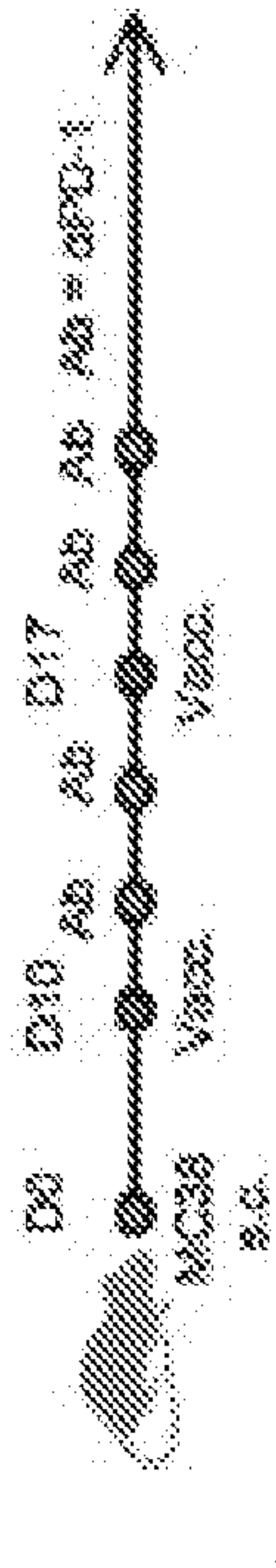


FIG. 1A

