(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 September 2019 (26.09.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/183266 A1

(51) International Patent Classification:
A61K 39/395 (2006.01) *A61P 35/00* (2006.01)
C07K 16/28 (2006.01) *A61P 37/06* (2006.01)
A61P 29/00 (2006.01)

(21) International Application Number:
PCT/US2019/023238

(22) International Filing Date:
20 March 2019 (20.03.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/646,210 21 March 2018 (21.03.2018) US

(71) Applicant: **ALX ONCOLOGY INC.** [US/US]; 866 Malcom Road, Suite 100, Burlingame, California 94010 (US).

(72) Inventors: **PONS, Jaume**. **SIM, Bang Janet**. **WAN, Hong**. **KUO, Tracy Chia-Chien**.

(74) Agent: **JONES, Kevin**; Morrison & Foerster LLP, 425 Market Street, San Francisco, California 94105 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: ANTIBODIES AGAINST SIGNAL-REGULATORY PROTEIN ALPHA AND METHODS OF USE

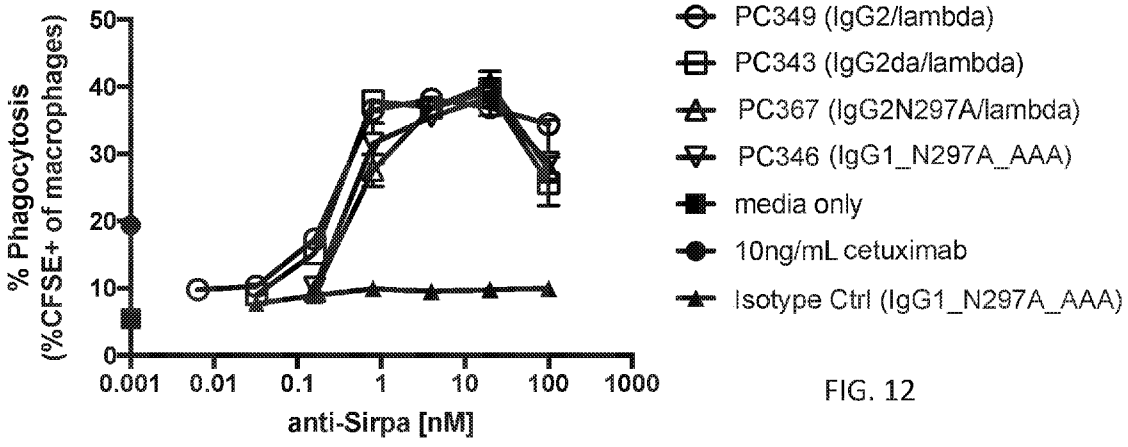

FIG. 12

(57) Abstract: Provided herein, *inter alia*, are isolated, humanized antibodies that bind an extracellular domain of a human SIRP-α polypeptide. Also provided are polynucleotides, vectors, host cells, and methods of production and use related thereto.

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ANTIBODIES AGAINST SIGNAL-REGULATORY PROTEIN ALPHA AND METHODS OF USE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. Provisional Application Serial No. 62/646,210, filed March 21, 2018, which is hereby incorporated by reference in its entirety.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: 757972000640SEQLIST.txt, date recorded: March 19, 2019, size: 230 KB).

FIELD

[0003] The present disclosure relates to isolated, humanized antibodies that bind an extracellular domain (e.g., the D1 domain) of a human SIRP-α polypeptide, as well as polynucleotides, vectors, host cells, and methods related thereto.

BACKGROUND

[0004] Signal-regulatory protein alpha (SIRP-α) is part of a family of cell-surface receptors that plays critical roles in the regulation of the immune system (*see, e.g.*, Barclay, A.N. and Brown, M.H. (2006) *Nat. Rev. Immunol.* 6:457-64). SIRP-α is expressed on the surface of various cells, including leukocytes such as dendritic cells, eosinophils, neutrophils, and macrophages. SIRP-α includes an extracellular domain that interacts with external stimuli such as ligands and an intracellular domain that mediates a variety of intracellular signals.

[0005] One of the major roles of SIRP-α is its regulation of the immune response through interactions with CD47. CD47 is expressed on the surface of a variety of cell types. When the IgSF domain of CD47 binds the extracellular domain (e.g., the D1 domain) of SIRP-α expressed on an immune cell (*e.g.*, a macrophage), this transduces a SIRP-α-mediated signal in the immune cell that prevents phagocytosis of the CD47-expressing cell. Thus, CD47 serves to convey what has been termed a "don't eat me"

signal to the immune system that prevents phagocytosis of healthy cells (*see, e.g.*, WO2015/138600 and Weiskopf, K. *et al.* (2013) *Science* 341:88-91). However, CD47 has also been shown to be highly expressed by a variety of cancers, and its interaction with SIRP-α in this context is thought to allow tumors to mimic the healthy "don't eat me" signal in order to evade immune surveillance and phagocytosis by macrophages (*see, e.g.*, Majeti, R. *et al.* (2009) *Cell* 138:286-99; Zhao, X.W. *et al.* (2011) *Proc. Natl. Acad. Sci.* 108:18342-7). As such, antibodies that block this interaction are highly desirable.

[0006] SIRP-α is known to be a highly polymorphic protein in humans, monkeys, and mice. For example, polymorphic differences have been identified between SIRP-α proteins in the NOD and C57BL/6 mouse strains, and these polymorphisms lead to functional consequences related to CD47 binding and engraftment of human hematopoietic stem cells in these mouse strains. In humans, two prevalent alleles of the *SIRPA* gene have been reported (Treffers, LW. *et al.* (2018), *Eur. J. Immunol.* 48:344-354; Zhao, X. *et al.* (2011), *PNAS.* 108:18342-47; van der Heijden, J. (2014). Genetic variation in human Fc gamma receptors: Functional consequences of polymorphisms and copy number variation (Doctoral dissertation)).

[0007] Due to the importance of the SIRP-α-CD47 interaction in normal immune function and tumorigenesis, the identification of antibodies that bind human SIRP-α is of great interest for development of clinical candidates.

[0008] All references cited herein, including patent applications, patent publications, non-patent literature, and UniProtKB/Swiss-Prot Accession numbers are herein incorporated by reference in their entirety, as if each individual reference were specifically and individually indicated to be incorporated by reference.

SUMMARY

[0009] To meet these and other needs, provided herein, *inter alia*, are isolated antibodies that bind an extracellular domain of a human SIRP-α polypeptide. Advantageously, these antibodies possess one or more of many useful *in vitro* and/or *in vivo* properties, such as binding to multiple SIRP-α polypeptides with high affinity, cross-reactivity against multiple mammalian SIRP-α polypeptides (*e.g.*, human v1, human v2, cynomolgus, and/or multiple murine SIRP-α proteins), the ability to enhance macrophage phagocytosis, the ability to enhance dendritic cell activation, and/or the ability to inhibit *in vivo* growth of a tumor that expresses CD47. In addition, the present disclosure provides

antibodies with variant light chains that have been engineered to remove potential liabilities, such as residues that may be modified by deamidation or glycation, resulting in antibodies with more desirable characteristics for manufacturing, storage, and/or drug development.

[0010] In certain aspects, the present disclosure provides antibodies (*e.g.*, isolated antibodies) that bind an extracellular domain of a human SIRP-α polypeptide, where the antibodies comprise: a heavy chain comprising a heavy chain variable (VH) domain that comprises the amino acid sequence of SEQ ID NO:26; and a light chain comprising a light chain variable (VL) domain that comprises an amino acid sequence according to the formula SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQQKPGQAPVTLIYSDDKRPSNIPERFSGSSSGTTVTLTISGVQAEDEADYYCGGYDQSSYTNPFGX$_1$GTX$_2$X$_3$TVL (SEQ ID NO:71), wherein X$_1$ is G or T; X$_2$ is K, Q, or R; and X$_3$ is L or V, and wherein the VL domain does not comprise the sequence of SEQ ID NO:25.

[0011] In some embodiments, the VL domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:39-41. In some embodiments, the light chain further comprises a light chain constant (CL) domain sequence that comprises an amino acid sequence according to the formula GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSX$_4$X$_5$KYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO:72), wherein X$_4$X$_5$ is ND, DN, DS, or SD. In some embodiments, the light chain further comprises a light chain constant (CL) domain sequence that comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:43-46. In some embodiments, the light chain further comprises a kappa light chain constant (CL) domain. In some embodiments, the light chain comprises the amino acid sequence of SEQ ID NO:36. In some embodiments, the light chain further comprises a lambda light chain constant (CL) domain. In some embodiments, the light chain comprises the amino acid sequence of SEQ ID NO:37 or SEQ ID NO:38. In some embodiments, the light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:48-57.

[0012] In some embodiments, the antibody is a scFv-Fc, single domain antibody, single heavy chain antibody, or single light chain antibody. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody comprises a heavy chain

comprising a heavy chain constant domain that comprises an Fc region. In some embodiments, the Fc region is a human Fc region selected from the group consisting of an IgG1 Fc region, an IgG2 Fc region, and an IgG4 Fc region. In some embodiments, the Fc region is a human IgG1 Fc region. In some embodiments, the Fc region is a human IgG1 Fc region comprising L234A, L235A, and G237A substitutions, amino acid position numbering according to EU. In some embodiments, the Fc region is a human IgG2 Fc region. In some embodiments, the Fc region is a human IgG2 Fc region comprising A330S and P331S substitutions, amino acid position numbering according to EU. In some embodiments, the Fc region further comprises an N297A substitution, amino acid position numbering according to EU. In some embodiments, the Fc region is a human IgG2 Fc region comprising an N297A substitution, amino acid position numbering according to EU. In some embodiments, the Fc region is a human IgG4 Fc region, and wherein the heavy chain comprises an S228P substitution, amino acid position numbering according to EU. In some embodiments, the heavy chain comprises a heavy chain constant domain that comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:31-35. In some embodiments, the heavy chain comprises a heavy chain constant domain that comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:33, 34, and 137. In some embodiments, the heavy chain comprises a heavy chain constant domain that comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:132-139.

[0013] In some embodiments, the heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:58-62. In some embodiments, the heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:60, 61, and 129. In some embodiments, the heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:124-131.

[0014] In certain aspects, the present disclosure provides antibodies (*e.g.*, isolated antibodies) that bind an extracellular domain of a human SIRP-α polypeptide, wherein: the heavy chain comprises the amino acid sequence of SEQ ID NO:62, and the light chain comprises the amino acid sequence of SEQ ID NO:52; the heavy chain comprises the amino acid sequence of SEQ ID NO:62, and the light chain comprises the amino acid sequence of SEQ ID NO:53; the heavy chain comprises the amino acid sequence of SEQ ID NO:62, and the light chain comprises the amino acid sequence of SEQ ID NO:54; the heavy chain comprises the amino acid sequence of SEQ ID NO:62, and the light chain comprises

the amino acid sequence of SEQ ID NO:55; the heavy chain comprises the amino acid sequence of SEQ ID NO:62, and the light chain comprises the amino acid sequence of SEQ ID NO:56; or the heavy chain comprises the amino acid sequence of SEQ ID NO:62, and the light chain comprises the amino acid sequence of SEQ ID NO:57.

[0015] In certain aspects, the present disclosure provides antibodies (*e.g.*, isolated antibodies) that bind an extracellular domain of a human SIRP-α polypeptide, wherein: the heavy chain comprises the amino acid sequence of SEQ ID NO:58, and the light chain comprises the amino acid sequence of SEQ ID NO:52; the heavy chain comprises the amino acid sequence of SEQ ID NO:59, and the light chain comprises the amino acid sequence of SEQ ID NO:52; the heavy chain comprises the amino acid sequence of SEQ ID NO:60, and the light chain comprises the amino acid sequence of SEQ ID NO:52; the heavy chain comprises the amino acid sequence of SEQ ID NO:61, and the light chain comprises the amino acid sequence of SEQ ID NO:52; the heavy chain comprises the amino acid sequence of SEQ ID NO:58, and the light chain comprises the amino acid sequence of SEQ ID NO:53; the heavy chain comprises the amino acid sequence of SEQ ID NO:59, and the light chain comprises the amino acid sequence of SEQ ID NO:53; the heavy chain comprises the amino acid sequence of SEQ ID NO:60, and the light chain comprises the amino acid sequence of SEQ ID NO:53; the heavy chain comprises the amino acid sequence of SEQ ID NO:61, and the light chain comprises the amino acid sequence of SEQ ID NO:53; the heavy chain comprises the amino acid sequence of SEQ ID NO:58, and the light chain comprises the amino acid sequence of SEQ ID NO:54; the heavy chain comprises the amino acid sequence of SEQ ID NO:59, and the light chain comprises the amino acid sequence of SEQ ID NO:54; the heavy chain comprises the amino acid sequence of SEQ ID NO:60, and the light chain comprises the amino acid sequence of SEQ ID NO:54; the heavy chain comprises the amino acid sequence of SEQ ID NO:61, and the light chain comprises the amino acid sequence of SEQ ID NO:54; the heavy chain comprises the amino acid sequence of SEQ ID NO:58, and the light chain comprises the amino acid sequence of SEQ ID NO:55; the heavy chain comprises the amino acid sequence of SEQ ID NO:59, and the light chain comprises the amino acid sequence of SEQ ID NO:55; the heavy chain comprises the amino acid sequence of SEQ ID NO:60, and the light chain comprises the amino acid sequence of SEQ ID NO:55; the heavy chain comprises the amino acid sequence of SEQ ID NO:61, and the light chain comprises the amino acid sequence of SEQ ID NO:55; the heavy chain comprises the amino acid

sequence of SEQ ID NO:58, and the light chain comprises the amino acid sequence of SEQ ID NO:56; the heavy chain comprises the amino acid sequence of SEQ ID NO:59, and the light chain comprises the amino acid sequence of SEQ ID NO:56; the heavy chain comprises the amino acid sequence of SEQ ID NO:60, and the light chain comprises the amino acid sequence of SEQ ID NO:56; the heavy chain comprises the amino acid sequence of SEQ ID NO:61, and the light chain comprises the amino acid sequence of SEQ ID NO:56; the heavy chain comprises the amino acid sequence of SEQ ID NO:58, and the light chain comprises the amino acid sequence of SEQ ID NO:57; the heavy chain comprises the amino acid sequence of SEQ ID NO:59, and the light chain comprises the amino acid sequence of SEQ ID NO:57; the heavy chain comprises the amino acid sequence of SEQ ID NO:60, and the light chain comprises the amino acid sequence of SEQ ID NO:57; the heavy chain comprises the amino acid sequence of SEQ ID NO:61, and the light chain comprises the amino acid sequence of SEQ ID NO:57; the heavy chain comprises the amino acid sequence of SEQ ID NO:60, and the light chain comprises the amino acid sequence of SEQ ID NO:55; the heavy chain comprises the amino acid sequence of SEQ ID NO:61, and the light chain comprises the amino acid sequence of SEQ ID NO:55; the heavy chain comprises the amino acid sequence of SEQ ID NO:129, and the light chain comprises the amino acid sequence of SEQ ID NO:55; the heavy chain comprises the amino acid sequence of SEQ ID NO:124, and the light chain comprises the amino acid sequence of SEQ ID NO:52; or the heavy chain comprises the amino acid sequence of SEQ ID NO:124, and the light chain comprises the amino acid sequence of SEQ ID NO:55.

[0016] In some embodiments, the antibody enhances phagocytosis by a macrophage expressing a human SIRP-α polypeptide. In some embodiments, the antibody enhances activation of a dendritic cell expressing a human SIRP-α polypeptide. In some embodiments, the antibody inhibits *in vivo* growth of a tumor that expresses CD47.

[0017] In some embodiments of any of the above embodiments, the antibody is conjugated to an agent, *e.g.*, a cytotoxic agent, label, or moiety that modules the immune system.

[0018] In some embodiments of any of the above embodiments, the antibody is a bispecific antibody. In some embodiments, the antibody comprises a first antigen binding domain that binds an extracellular domain of a human SIRP-α polypeptide and a second antigen binding domain that binds an antigen expressed by a cancer cell. In some

embodiments, the antigen expressed by the cancer cell is selected from the group consisting of CD19, CD20, CD22, CD30, CD33, CD38, CD52, CD56, CD70, CD74, CD79b, CD123, CD138, CS1/SLAMF7, Trop-2, 5T4, EphA4, BCMA, Mucin 1, Mucin 16, PD-L1, PTK7, STEAP1, Endothelin B Receptor, mesothelin, EGFRvIII, ENPP3, SLC44A4, GNMB, nectin 4, NaPi2b, LIV-1A, Guanylyl cyclase C, DLL3, EGFR, HER2, VEGF, VEGFR, integrin αVβ3, integrin α5β1, MET, IGF1R, TRAILR1, TRAILR2, RANKL, FAP, Tenascin, Le^y, EpCAM, CEA, gpA33, PSMA, TAG72, a mucin, CAIX, EPHA3, folate receptor α, GD2, GD3, and an MHC/peptide complex comprising a peptide from NY-ESO-1/LAGE, SSX-2, a MAGE family protein, MAGE-A3, gp100/pmel17, Melan-A/MART-1, gp75/TRP1, tyrosinase, TRP2, CEA, PSA, TAG-72, immature laminin receptor, MOK/RAGE-1, WT-1, SAP-1, BING-4, EpCAM, MUC1, PRAME, survivin, BRCA1, BRCA2, CDK4, CML66, MART-2, p53, Ras, β-catenin, TGF-βRII, HPV E6, or HPV E7. In some embodiments, the antibody comprises a first antigen binding domain that binds an extracellular domain of a human SIRP-α polypeptide and a second antigen binding domain that binds an antigen expressed by an immune cell. In some embodiments, the antigen expressed by the immune cell is selected from the group consisting of BDCA2, BDCA4, ILT7, LILRB1, LILRB2, LILRB3, LILRB4, LILRB5, Siglec-3, Siglec-7, Siglec-9, Siglec-15, FGL-1, CD200, CD200R, CSF-1R, CD40, CD40L, CD163, CD206, DEC205, CD47, CD123, arginase, IDO, TDO, AhR, EP2, COX-2, CCR2, CCR-7, CXCR1, CX3CR1, CXCR2, CXCR3, CXCR4, CXCR7, TGF-β RI, TGF-β RII, c-Kit, CD244, L-selectin/CD62L, CD11b, CD11c, CD68, 41BB, CTLA4, PD1, PD-L1, PD-L2, TIM-3, BTLA, VISTA, LAG-3, CD28, OX40, GITR, CD137, CD27, HVEM, CCR4, CD25, CD103, KIrg1, Nrp1, CD278, Gpr83, TIGIT, CD154, CD160, TNFR2, PVRIG, DNAM, and ICOS. In some embodiments, the antibody comprises a first antigen binding domain that binds an extracellular domain of a human SIRP-α polypeptide and a second antigen binding domain that binds an antigen expressed by a natural killer (NK) cell. In some embodiments, the antigen expressed by the NK cell is selected from the group consisting of NKR-P1A, CD94, KLRG1, KIR2DL5A, KIR2DL5B, KIR2DL1, KIR2DL2, KIR2DL3, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DS1, KIR2DS1, CD94, NKG2D, CD160, CD16, NKp46, NKp30, NKp44, DNAM1, CRTAM, CD27, NTB-A, PSGL1, CD96, CD100, NKp80, SLAMF7, and CD244.

[0019] In certain aspects, the present disclosure provides a polynucleotide encoding the antibody according to any one of the above embodiments. In certain aspects, the present

disclosure provides a vector comprising the polynucleotide according to any one of the above embodiments. In certain aspects, the present disclosure provides a host cell comprising the polynucleotide or vector according to any one of the above embodiments. In certain aspects, the present disclosure provides a method of producing an antibody, the method comprising culture the host cell according to any one of the above embodiments such that the antibody is produced. In some embodiments, the method further comprises recovering the antibody from the host cell.

[0020] In certain aspects, the present disclosure provides a method of treating or delaying progression of cancer in an individual, the method comprising administering to the individual an effective amount of the antibody according to any one of the above embodiments. In some embodiments, the method further comprises administering to the individual an effective amount of a second antibody that binds an antigen expressed by the cancer. In some embodiments, the antigen expressed by the cancer is selected from the group consisting of CD19, CD20, CD22, CD30, CD33, CD38, CD52, CD56, CD70, CD74, CD79b, CD123, CD138, CS1/SLAMF7, Trop-2, 5T4, EphA4, BCMA, Mucin 1, Mucin 16, PTK7, STEAP1, Endothelin B Receptor, mesothelin, EGFRvIII, ENPP3, SLC44A4, GNMB, nectin 4, NaPi2b, LIV-1A, Guanylyl cyclase C, DLL3, EGFR, HER2, VEGF, VEGFR, integrin αVβ3, integrin α5β1, MET, IGF1R, TRAILR1, TRAILR2, RANKL, FAP, Tenascin, Le^y, EpCAM, CEA, gpA33, PSMA, TAG72, a mucin, CAIX, EPHA3, folate receptor α, GD2, GD3, and an MHC/peptide complex comprising a peptide from NY-ESO-1/LAGE, SSX-2, a MAGE family protein, MAGE-A3, gp100/pmel17, Melan-A/MART-1, gp75/TRP1, tyrosinase, TRP2, CEA, PSA, TAG-72, immature laminin receptor, MOK/RAGE-1, WT-1, SAP-1, BING-4, EpCAM, MUC1, PRAME, survivin, BRCA1, BRCA2, CDK4, CML66, MART-2, p53, Ras, β-catenin, TGF-βRII, HPV E6, or HPV E7. In some embodiments, the method further comprises administering to the individual an effective amount of an immunotherapeutic agent. In some embodiments, the immunotherapeutic agent comprises a second antibody. In some embodiments, the second antibody binds to an antigen selected from the group consisting of BDCA2, BDCA4, ILT7, LILRB1, LILRB2, LILRB3, LILRB4, LILRB5, Siglec-3, Siglec-7, Siglec-9, Siglec-15, FGL-1, CD200, CD200R, CSF-1R, CD40, CD40L, CD163, CD206, DEC205, CD47, CD123, arginase, IDO, TDO, AhR, EP2, COX-2, CCR2, CCR-7, CXCR1, CX3CR1, CXCR2, CXCR3, CXCR4, CXCR7, TGF-β RI, TGF-β RII, c-Kit, CD244, L-selectin/CD62L, CD11b, CD11c, CD68, 41BB, CTLA4, PD1, PD-L1, PD-L2, TIM-3,

BTLA, VISTA, LAG-3, CD28, OX40, GITR, CD137, CD27, HVEM, CCR4, CD25, CD103, KIrg1, Nrp1, CD278, Gpr83, TIGIT, CD154, CD160, TNFR2, PVRIG, DNAM, and ICOS. In some embodiments, the second antibody binds to PD-1. In some embodiments, the second antibody binds to PD-L1. In some embodiments, the immunotherapeutic agent comprises a vaccine, oncolytic virus, adoptive cell therapy, cytokine, or small molecule. In some embodiments, the method further comprises administering to the individual an effective amount of a second antibody that binds an antigen expressed by a natural killer (NK) cell. In some embodiments, the antigen expressed by the NK cell is selected from the group consisting of NKR-P1A, CD94, KLRG1, KIR2DL5A, KIR2DL5B, KIR2DL1, KIR2DL2, KIR2DL3, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DS1, KIR2DS1, CD94, NKG2D, CD160, CD16, NKp46, NKp30, NKp44, DNAM1, CRTAM, CD27, NTB-A, PSGL1, CD96, CD100, NKp80, SLAMF7, and CD244. In some embodiments, the method further comprises administering to the individual an effective amount of a chemotherapeutic agent or small molecule anti-cancer agent. In some embodiments, the method further comprises administering to the individual an effective amount of a targeted small molecule inhibitor. In some embodiments, the targeted small molecule inhibitor is a VEGFR and/or PDGFR inhibitor, EGFR inhibitor, ALK inhibitor, CDK4/6 inhibitor, PARP inhibitor, mTOR inhibitor, KRAS inhibitor, TRK inhibitor, BCL2 inhibitor, IDH inhibitor, PI3K inhibitor, DNA damage response (DDR) inhibitor, or hypomethylation agent.

[0021] In certain aspects, the present disclosure provides a method of treating or delaying progression of an autoimmune disease or an inflammatory disease in an individual, the method comprising administering to the individual an effective amount of the antibody according to any one of the above embodiments. In some embodiments, the autoimmune disease or inflammatory disease is selected from the group consisting of multiple sclerosis, rheumatoid arthritis, a spondyloarthropathy, systemic lupus erythematosus, an antibody-mediated inflammatory or autoimmune disease, graft versus host disease, sepsis, diabetes, psoriasis, psoriatic arthritis, atherosclerosis, Sjogren's syndrome, progressive systemic sclerosis, scleroderma, acute coronary syndrome, ischemic reperfusion, Crohn's Disease, ulcerative colitis, endometriosis, glomerulonephritis, IgA nephropathy, polycystic kidney disease, myasthenia gravis, idiopathic pulmonary fibrosis, fibrotic disease (*e.g.*, pulmonary fibrosis, liver cirrhosis, atrial fibrosis, endomyocardial

fibrosis, myelofibrosis, or retroperitoneal fibrosis), asthma, atopic dermatitis, acute respiratory distress syndrome (ARDS), vasculitis, and inflammatory autoimmune myositis.

[0022] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present invention. These and other aspects of the invention will become apparent to one of skill in the art. These and other embodiments of the invention are further described by the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] **FIG. 1** shows the results of an *in vivo* syngeneic mouse colon carcinoma model to assess single agent activity. MC38 cells were implanted subcutaneously in C57BL/6 mice and randomized into groups (8 mice/group). Mice were treated with vehicle (PBS), CD47 blocking anti-SIRP-α antibody AB27b, CD47 blocking anti-SIRP-α antibody AB25b, or CD47 blocking anti-SIRP-α antibody AB25c. Treatment was initiated when tumors were an average of 60mm^3, day 7 post implant. Mice were dosed intraperitoneally (IP) at 10 mg/kg twice a week for three weeks with anti-SIRPα antibodies. Animals were sacrificed when tumors reached a volume of ~2000mm^3.

[0024] **FIG. 2** compares expression yield and binding affinity of antibodies having the AB21 human heavy chain and the indicated humanized light chains. The antibodies were produced by expression in FreeStyle™ 293-FS cells (Thermo Fisher).

[0025] **FIGS. 3A & 3B** show the results of *in vitro* phagocytosis assays using EGFR(+) DLD-1 cells as the target and M2 macrophages as the phagocytosing cell. Anti-SIRP-α antibodies were tested at the indicated concentrations in combination with the anti-EGFR antibody cetuximab. Phagocytosis was measured by percentage of CFSE+ cells.

[0026] **FIGS. 4A & 4B** show the results of *in vivo* dendritic cell activation assays with the indicated anti-SIRP-α antibodies. Mice were intravenously injected with the indicated antibody at 10 mg/kg, and spleens were harvested five hours after injection. Activation markers CD86, MHCII and CCR7 on CD4+ dendritic cells were measured by flow cytometry.

[0027] **FIG. 5** shows the results of an *in vivo* syngeneic mouse colon carcinoma model to assess activity of combining anti-SIRP-α treatment with PD-L1/PD-1 pathway inhibition. CT26 cells were implanted subcutaneously in BALBc mice and randomized into groups (8 mice/group). Mice were treated with vehicle (PBS), anti-PD-L1 antibody, CD47 blocking anti-SIRP-α antibody AB25b, or AB25b and PD-L1. Treatment was initiated when tumors

were an average of 60mm^3, day 7 post implant. Mice were dosed intraperitoneally (IP) at 10 mg/kg twice a week for three weeks and sacrificed when tumors reach a volume of ~2000mm^3.

[0028] **FIG. 6** shows the results of an *in vivo* syngeneic mouse colon carcinoma model to assess activity of combining anti-SIRP-α treatment with PD-L1/PD-1 pathway inhibition. MC38 cells were implanted subcutaneously in C57BL/6 mice and randomized into groups (8 mice/group). Mice were treated with vehicle (PBS), anti-PD-1 antibody, CD47 blocking anti-SIRP-α antibody AB25b, or AB25b and anti-PD-1. Treatment was initiated when tumors were an average of 60mm^3, day 7 post implant. Mice were dosed intraperitoneally (IP) at 10 mg/kg twice a week for three weeks and sacrificed when tumors reach a volume of ~2000mm^3.

[0029] **FIG. 7** shows the total extracted ion chromatography, area under the curve (XIC, AUC) of the glycated (black) versus unmodified form (grey) of peptides of antibody PC336, as analyzed by mass spectrometry. Sequences shown are SEQ ID NOs:64-66 (left to right).

[0030] **FIG. 8** shows the percentage of peptides modified by deamidation at the indicated residues of the light chain and heavy chain of anti-SIRP-α antibody PC301, as analyzed by mass spectrometry. Amino acid numbering is based on sequential numbering of residues in the light and heavy chain sequences of SEQ ID NOs: 63 and 59, respectively (not Kabat numbering).

[0031] **FIG. 9** shows a fragment of the Hum1 light chain with the K104 residue subject to glycation (SEQ ID NO:67; "Original"), along with 3 variants (SEQ ID NOs:68-70; versions 1-3, respectively) engineered to remove the glycation site. Glycation site is underlined.

[0032] **FIGS. 10 & 11** show an alignment of the original Hum1 light chain (with Hum1 VL and IGCL1 constant domain) with 6 variants. Variants each include 1 of the 3 glycation site variants (v1, v2, and v3) and either DS or SD mutations to replace the N171/N172 deamidation site. Shown from top to bottom are SEQ ID NOs:47 and 52-57, respectively. Asterisks indicate sequence differences. CDRs are depicted in boxes (representing SEQ ID NOs:22, 23, and 24, respectively). CDR delineations are according to Kabat. Amino acid numbering is based on sequential numbering of residues.

[0033] **FIG. 12** shows the results of *in vitro* phagocytosis assays using EGFR(+) DLD-1 cells as the target and M2 macrophages as the phagocytosing cell. Anti-SIRP-α

antibodies or isotype control with the indicated Fc regions were tested in combination with the anti-EGFR antibody cetuximab. Phagocytosis was measured by percentage of CFSE+ cells.

[0034] **FIGS. 13A-13E** show the results of depletion assays testing anti-SIRP-α antibodies with the indicated Fc regions for the ability to deplete various cell types from peripheral blood mononuclear cells (PBMCs). **FIGS. 13A & 13B** show the depletion of lin- HLADR+ dendritic cells (DCs) and represent the results of experiments performed using PBMCs obtained from different donors. **FIG. 13C** shows the lack of depletion of CD3+ T cells by anti-SIRP-α antibodies and isotype controls. **FIG. 13D** shows the lack of depletion of CD14+ monocytes by anti-SIRP-α antibodies and isotype controls. **FIG. 13E** shows the lack of depletion of CD20+ B cells by anti-SIRP-α antibodies and isotype controls.

[0035] **FIGS. 14A & 14B** show the results of *in vitro* phagocytosis assays using EGFR(+) DLD-1 cells as the target and M2 macrophages as the phagocytosing cell. Anti-SIRP-α antibodies with the indicated Fc regions were tested in combination with the anti-EGFR antibody cetuximab. Phagocytosis was measured by percentage of CFSE+ cells.

[0036] **FIGS. 15A & 15B** show the results of *in vitro* phagocytosis assays using EGFR(+) DLD-1 cells as the target and M2 macrophages as the phagocytosing cell. Anti-SIRP-α antibodies with the indicated light chain constant domains (lambda or kappa) were tested in combination with the anti-EGFR antibody cetuximab. Phagocytosis was measured by percentage of CFSE+ cells.

DETAILED DESCRIPTION

[0037] The present disclosure describes antibodies that bind the extracellular domains (e.g., the D1 domains) of one or more human SIRP-α polypeptides and have a variety of SIRP-α binding profiles of potential interest. In addition, the present disclosure describes anti-SIRP-α antibodies with one or more *in vitro* and/or *in vivo* biological properties of interest, such as binding to multiple SIRP-α polypeptides with high affinity and the ability to enhance macrophage phagocytosis, enhance dendritic cell activation, and/or inhibit *in vivo* growth of a tumor that expresses CD47. These antibodies have a light chain derived from chicken, which provides unique opportunities to generate antibodies that cross-react across multiple mammalian SIRP-α polypeptides. Indeed, these antibodies were found to cross-react with human SIRP-α v1 and v2, cynomolgus SIRP-α, and murine SIRP-α from

multiple strains, making them highly advantageous for both pre-clinical testing and clinical application across a large human population. Importantly, these antibodies have also been engineered to remove potential liabilities, such as residues that may be modified by deamidation or glycation, resulting in antibodies with more desirable characteristics for manufacturing, storage, and/or drug development.

[0038] In one aspect, provided herein are isolated antibodies that bind the extracellular domain (e.g., the D1 domain) of a human SIRP-α polypeptide. Further provided herein are polynucleotides and vectors encoding the antibodies of the present disclosure, as well as methods of antibody production related thereto.

[0039] In another aspect, provided herein are methods for treating or delaying progression of cancer in an individual, comprising administering to the individual an effective amount of an antibody of the present disclosure.

[0040] In another aspect, provided herein are methods for treating or delaying progression of an autoimmune or inflammatory disease in an individual, comprising administering to the individual an effective amount of an antibody of the present disclosure.

Definitions

[0041] Before describing the disclosed embodiments in detail, it is to be understood that the present disclosure is not limited to particular compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[0042] As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a molecule" optionally includes a combination of two or more such molecules, and the like.

[0043] The term "about" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se.

[0044] It is understood that aspects and embodiments of the present disclosure include "comprising," "consisting," and "consisting essentially of" aspects and embodiments.

[0045] A "SIRP-α polypeptide" as used herein may refer to any endogenous or naturally occurring SIRP-α polypeptide encoded by a genome from any vertebrate,

including mammals such as humans, monkeys, rodents (*e.g.*, mouse or rat), and birds, such as chickens. The term also includes naturally occurring variants, *e.g.*, alternatively spliced variants, allelic variants, or polymorphisms (*e.g.*, those described herein). The term may further refer to full-length, unprocessed SIRP-α polypeptides as well as SIRP-α polypeptides that result from cellular processing, *e.g.*, removal of a signal sequence, etc. Exemplary SIRP-α polypeptide sequences are described herein. In some embodiments, a human SIRP-α polypeptide is one encoded by a human *SIRPA* gene, *e.g.*, as described by NCBI Gene ID No. 140885. As described herein, SIRP-α polypeptides are highly polymorphic within and among species, including, for example, multiple human variants with amino acid polymorphisms in the extracellular domain.

[0046] SIRP-α polypeptides include an extracellular domain that binds ligands/partners, *e.g.*, CD47. SIRP-α polypeptides comprise 3 highly homologous immunoglobulin (Ig)-like extracellular domains—D1, D2, and D3. The SIRP-α D1 domain ("D1 domain") refers to the membrane distal, extracellular domain of SIRP-α and mediates binding of SIRP-α to CD47 (*see, e.g.*, Hatherley, D. *et al.* (2008) *Mol. Cell* 31:266-77; Hatherley, D. *et al.* (2007) *J. Biol. Chem.* 282:14567-75; Hatherley, D. *et al.* (2009) *J. Biol. Chem.* 284:26613-9; and Lee, W. Y. *et al.* (2010) *J. Biol. Chem.* 285:37953-63). The extracellular domain generally refers to the entire extracellular portion of SIRP-α, *e.g.*, as expressed on a cell surface, and may include distinct SIRP-α domains, such as the D1 domain. The D1 domain contains residues shown to be critical for mediating CD47 binding (*see, e.g.*, Lee, W.Y. *et al.* (2007) *J. Immunol.* 179:7741-50). In some embodiments, an antibody that binds an extracellular domain of a SIRP-α polypeptide binds one or more residues of the D1 domain.

[0047] As used herein, "CD47" (also known as integrin associated protein (IAP), MER6, and OA3) refers to a polypeptide that, among other roles, serves as a binding partner for SIRP-α polypeptides. In some embodiments, CD47 refers to a human CD47 polypeptide, *e.g.*, a polypeptide encoded by a human *CD47* gene, such as that described by NCBI Ref Seq ID No. 961. Exemplary human CD47 amino acid sequences are known (*see, e.g.*, NCBI Reference Sequence Accession No. NP_001768). In particular, the IgSF domain of CD47 refers to the N-terminal extracellular domain of CD47 that is known to be critical for SIRP-α binding (*see, e.g.*, Barclay, A.N. and Brown, M.H. (2006) *Nat. Rev. Immunol.* 6:457-64 and Hatherley, D. *et al.* (2009) *J. Biol. Chem.* 284:26613-9). The term "CD47" may also include modified CD47 polypeptides that are able to bind SIRP-α, *e.g.*, a

polypeptide comprising an IgSF domain of CD47 conjugated to another polypeptide or other moiety, *e.g.*, an Ig Fc region.

[0048] As used herein "modulating SIRP-α signaling" may refer to antagonizing, agonizing, or otherwise interfering with one or more aspects of SIRP-α signaling in a cell expressing a SIRP-α polypeptide. SIRP-α signaling may refer to one or more intracellular signaling events mediated by activation of a SIRP-α polypeptide, including without limitation tyrosine phosphorylation of the intracellular region of SIRP-α, phosphatase (*e.g.*, SHP1) binding, adaptor protein binding (*e.g.*, SCAP2, FYB, and/or GRB2), cytokine production (e.g. IL-10, IL-1β, IFN or TNF), and nitric oxide production; and/or one or more intercellular phenotypes, including without limitation macrophage phagocytosis and other activating or suppressive phenotypes of macrophages, eosinophils, neutrophils, dendritic cells, and myeloid-derived suppressor cells (MDSCs).

[0049] As used herein, the term "antibody" may refer to intact antibodies; antibody fragments (including without limitation Fab, F(ab')2, Fab'-SH, Fv, diabodies, scFv, scFv-Fc, single domain antibodies, single heavy chain antibodies, and single light chain antibodies), provided that they exhibit the desired biological activity (e.g. epitope binding); monoclonal antibodies; polyclonal antibodies; monospecific antibodies; multi-specific antibodies (e.g., bispecific antibodies); and antibody-like proteins.

[0050] As used herein, the term "bispecific" when used in reference to an antibody or antibody fragment includes an antibody or antibody fragment that possesses two different binding specificities. For example, each binding specificity may recognize a different antigen, or each binding specificity may recognize the same antigen with different affinity and/or precise epitope. In some embodiments, each different binding specificity comprises one or more different antibody antigen binding domains (*e.g.*, variable domains), such that the bispecific antibody or antibody fragment comprises at least a first antigen binding domain with a first binding specificity and a second antigen binding domain with a second binding specificity. A variety of exemplary bispecific antibody formats are described herein and known in the art.

[0051] An "isolated" antibody may refer to an antibody that has been separated and/or recovered from a component of its natural environment, *e.g.*, a host cell or organism. In some embodiments, an antibody is purified to a desired purity by weight (*e.g.*, at least 95%); and/or homogeneity by SDS-PAGE using, for example, staining by silver,

Coomassie, etc. In some embodiments, an isolated antibody is obtained following one or more purification steps.

[0052] As is known in the art, "native" antibodies refer to typically heterotetrameric complexes including two identical light (L) chains and two identical heavy (H) chains. Variable numbers of disulfide bonds connect the two heavy chains, and one connects each light chain to a heavy chain, in addition to intrachain disulfide bridges. The heavy chains include a variable domain (VH) followed (N-terminus to C-terminus) by three or four constant domains. The light chains include a variable domain (VL) followed by a constant domain (CL). Typically, mammalian light chains fall into one of two categories based on amino acid sequence: kappa and lambda.

[0053] A "constant domain" may refer to the more conserved portion of the antibody or fragment, *e.g.*, outside the variable domains. The term may include the CL domain as well as heavy chain constant domains CH1, CH2, and CH3, along with the heavy chain hinge region. Optionally, a heavy chain constant domain may further comprise a CH4 heavy chain constant domain.

[0054] Constant domains of the heavy chain can be assigned to one of 5 major types: IgA, IgD, IgE, IgG, and IgM. Several subtypes exist for many of these major types. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known and described generally in, for example, Abbas et al. *Cellular and Mol. Immunology*, 4th ed. (W.B. Saunders, Co., 2000).

[0055] As used herein, the term "antibody variable domain" refers to the portions of the light and heavy chains of an antibody that include the complementary determining regions (CDRs, e.g., CDR L1, CDR L2, CDR L3, CDR H1, CDR H2, and CDR H3) and framework regions (FRs).

[0056] The term "variable" refers to the fact that subsequences of the variable domains differ substantially in sequence between antibodies and are critical to the binding specificity of a particular antibody for its antigen. Variability is concentrated in three hypervariable regions (HVRs) in both VH and VL domains. The more conserved portions of variable domains are called the framework regions (FR) in which the HVRs are interspersed. The variable domains of native heavy and light chains each comprise four FR regions connected by three HVRs that form loops (*see* Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, National Institute of Health, Bethesda, Md. (1991)).

[0057] The term "hypervariable region (HVR)" may refer to the subregions of the VH and VL domains characterized by enhanced sequence variability and/or formation of defined loops. These include three HVRs in the VH domain (H1, H2, and H3) and three HVRs in the VL domain (L1, L2, and L3). H3 is believed to be critical in imparting fine binding specificity, with L3 and H3 showing the highest level of diversity. *See* Johnson and Wu, in *Methods in Molecular Biology* 248:1-25 (Lo, ed., Human Press, Totowa, N.J., 2003).

[0058] A number of HVR delineations are known. The Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). Chothia refers instead to the location of the structural loops (Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)). The AbM HVRs represent a compromise between the Kabat HVRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The "contact" HVRs are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below. "Framework" or "FR" residues are those variable domain residues other than the HVR residues.

Loop	Kabat	AbM	Chothia	Contact
L1	L24-L34	L24-L34	L26-L32	L30-L36
L2	L50-L56	L50-L56	L50-L52	L46-L55
L3	L89-L97	L89-L97	L91-L96	L89-L96
H1	H31-H35B	H26-H35B	H26-H32	H30-H35B (Kabat Numbering)
H1	H31-H35	H26-H35	H26-H32	H30-H35 (Chothia Numbering)
H2	H50-H65	H50-H58	H53-H55	H47-H58
H3	H95-H102	H95-H102	H96-H101	H93-H101

[0059] "Extended" HVRs are also known: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 or 89-96 (L3) in the VL and 26-35 (H1), 50-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3) in the VH (Kabat numbering).

[0060] "Numbering according to Kabat" may refer to the numbering system used for heavy chain variable domains or light chain variable domains of the compilation of antibodies in Kabat et al., *supra*. The actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence. Typically, the Kabat numbering is used when referring to a residue in the variable domains (approximately residues 1-107 of the light chain and residues 1-113 of the heavy chain), whereas the EU numbering system or index (*e.g.*, the EU index as in Kabat, numbering according to EU IgG1) is generally used when referring to a residue in the heavy chain constant region.

[0061] "Full length" or "intact" antibodies typically include heavy chains with an Fc region, *e.g.*, as opposed to an antibody fragment. Antigen-binding "Fab" fragments with a single antigen binding site may be released from the residual Fc fragment by papain digestion. F(ab')2 fragments include two antigen-binding sites produced by pepsin treatment of an antibody. Antibody fragments will, however, include one or more antibody variable regions.

[0062] An "Fv" fragment contains a complete antigen-binding site. A single chain Fv (scFv) can include a VH and a VL domain linked by a peptide linker such that the VH and VL domains associate, *e.g.*, as in an antibody or Fab fragment, such that the HVRs form an antigen binding site. *See* Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York, 1994), pp. 269-315. In some embodiments, the scFv is fused to an antibody Fc domain (*e.g.*, scFv-Fc). While six HVRs typically comprise an antigen binding site, a single variable domain with three HVRs is still capable of binding an antigen, albeit at a lower affinity. *See* Hamers-Casterman et al., *Nature* 363:446-448 (1993); Sheriff et al., *Nature Struct. Biol.* 3:733-736 (1996). Single domain antibodies (*e.g.*, camelid antibodies) typically include a single, monomeric variable domain for antigen binding. Single heavy chain (VHH) and single light chain antibodies are also known. A Fab' fragment typically includes a few more residues at the C-terminal end than a Fab fragment. A Fab'-SH includes cysteine residues with a free thiol. Various chemical couplings of antibody fragments are known in the art.

[0063] A "diabody" includes antibody fragments with two antigen-binding sites. These include a VH and VL domain connected by a linker, which is typically too short to facilitate

pairing of domains in the same chain. Diabodies may be bivalent or bispecific. Tribodies and tetrabodies, or other numbers of VH/VL domains are known. *See* Hudson et al., *Nat. Med.* 9:129-134 (2003).

[0064] As used herein, a "monoclonal" antibody refers to an antibody obtained from a population of substantially homogeneous antibodies, *e.g.*, substantially identical but allowing for minor levels of background mutations and/or modifications. "Monoclonal" denotes the substantially homogeneous character of antibodies, and does not require production of the antibody by any particular method. In some embodiments, a monoclonal antibody is selected by its HVR, VH, and/or VL sequences and/or binding properties, *e.g.*, selected from a pool of clones (*e.g.*, recombinant, hybridoma, or phage-derived). A monoclonal antibody may be engineered to include one or more mutations, *e.g.*, to affect binding affinity or other properties of the antibody, create a humanized or chimeric antibody, improve antibody production and/or homogeneity, engineer a multispecific antibody, resultant antibodies of which are still considered to be monoclonal in nature. A population of monoclonal antibodies may be distinguished from polyclonal antibodies as the individual monoclonal antibodies of the population recognize the same antigenic site. A variety of techniques for production of monoclonal antibodies are known; *see, e.g.,* the hybridoma method (e.g., Kohler and Milstein, *Nature*, 256:495-97 (1975); Hongo *et al.*, *Hybridoma*, 14 (3): 253-260 (1995), Harlow *et al.*, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567), phage-display technologies (see, e.g., Clackson *et al.*, *Nature*, 352: 624-628 (1991); Marks *et al.*, *J. Mol. Biol.* 222: 581-597 (1992); Sidhu *et al.*, *J. Mol. Biol.* 338(2): 299-310 (2004); Lee *et al.*, *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee *et al.*, *J. Immunol. Methods* 284(1-2): 119-132 (2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, e.g., WO 1998/24893; WO 1996/34096; WO 1996/33735; WO 1991/10741; Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 2551 (1993); Jakobovits *et al.*, *Nature* 362: 255-258 (1993); Bruggemann *et al.*, *Year in Immunol.* 7:33 (1993); U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016; Marks *et al.*, *Bio/Technology* 10: 779-783 (1992); Lonberg et al., *Nature* 368: 856-859 (1994); Morrison, *Nature* 368: 812-813

(1994); Fishwild *et al.*, *Nature Biotechnol.* 14: 845-851 (1996); Neuberger, *Nature Biotechnol.* 14: 826 (1996); and Lonberg and Huszar, *Intern. Rev. Immunol.* 13: 65-93 (1995).

[0065] "Chimeric" antibodies may refer to an antibody with one portion of the heavy and/or light chain from a particular isotype, class, or organism and another portion from another isotype, class, or organism. In some embodiments, the variable region will be from one source or organism, and the constant region will be from another.

[0066] "Humanized antibodies" may refer to antibodies with predominantly human sequence and a minimal amount of non-human (*e.g.*, mouse or chicken) sequence. In some embodiments, a humanized antibody has one or more HVR sequences (bearing a binding specificity of interest) from an antibody derived from a non-human (*e.g.*, mouse or chicken) organism grafted onto a human recipient antibody framework (FR). In some embodiments, non-human residues are further grafted onto the human framework (not present in either source or recipient antibodies), *e.g.*, to improve antibody properties. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin, and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. *See* Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992).

[0067] A "human" antibody may refer to an antibody having an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581 (1991); preparation of human monoclonal antibodies as described in Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner *et al.*, *J. Immunol.*, 147(1):86-95 (1991); and by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, *e.g.*, immunized xenomice (see, *e.g.*, U.S. Pat. Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology) or chickens with

human immunoglobulin sequence(s) (*see, e.g.*, WO2012162422, WO2011019844, and WO2013059159).

[0068] As used herein, the term "linker" refers to a linkage between two elements, e.g., protein domains. In some embodiments, a linker can be a covalent bond or a spacer. The term "spacer" refers to a moiety (e.g., a polyethylene glycol (PEG) polymer) or an amino acid sequence (e.g., a 1-200 amino acid sequence) occurring between two polypeptides or polypeptide domains to provide space or flexibility (or both space and flexibility) between the two polypeptides or polypeptide domains. In some embodiments, an amino acid spacer is part of the primary sequence of a polypeptide (e.g., joined to the spaced polypeptides or polypeptide domains via the polypeptide backbone).

[0069] The term "cytotoxic agent" as used herein may refer to any agent that inhibits cellular proliferation or induces cell death. Cytotoxic agents include, but are not limited to, chemotherapeutic agents; radioactive isotopes; growth inhibitory agents; and toxins such as small molecule toxins or enzymatically active toxins, including fragments and/or variants thereof. Exemplary cytotoxic agents include without limitation metabolic inhibitors, anti-microtubule agents, platinum containing compounds, alkylating agents, proteasome inhibitors, topoisomerase II inhibitors, antimetabolites, topoisomerase I inhibitors, signal transduction pathway inhibitors, non-receptor tyrosine kinase angiogenesis inhibitors, hormones and hormonal analogues, proapoptotic agents, inhibitors of LDH-A, cell cycle inhibitors, HDAC inhibitors, and antibiotic agents.

[0070] As used herein, a "label" may include any moiety that serves as a detection agent, *e.g.*, of binding between a labeled antibody of the present disclosure and a macromolecule or cell. Exemplary labels include without limitation fluorescent (*e.g.*, compounds or proteins), radioactive, or enzymatic moieties, as well as affinity purification tags.

[0071] As used herein, an antibody may be said to "bind" an antigen with an affinity sufficient to render the antibody useful for *in vitro* and/or *in vivo* manipulation of the antigen. In some embodiments, an antibody that "binds" an antigen has a dissociation constant (K_D) for the antigen that is less than or equal to 1 μM at 25°C.

[0072] As used herein, the term "affinity" or "binding affinity" refers to the strength of the binding interaction between two molecules. Generally, binding affinity refers to the strength of the sum total of non-covalent interactions between a molecule and its binding partner, such as a high affinity SIRP-α D1 variant and CD47. Unless indicated otherwise,

binding affinity refers to intrinsic binding affinity, which reflects a 1:1 interaction between members of a binding pair. The binding affinity between two molecules is commonly described by the dissociation constant (K_D) or the association constant (K_A). Two molecules that have low binding affinity for each other generally bind slowly, tend to dissociate easily, and exhibit a large K_D. Two molecules that have high affinity for each other generally bind readily, tend to remain bound longer, and exhibit a small K_D. In some embodiments, the K_D of two interacting molecules is determined using known methods and techniques, e.g., surface plasmon resonance (SPR). K_D can be calculated as the ratio of k_{off}/k_{on}.

[0073] As used herein, the term "K_D less than" refers to a numerically smaller K_D value and an increasing binding affinity relative to the recited K_D value. As used herein, the term "K_D greater than" refers to a numerically larger K_D value and a decreasing binding affinity relative to the recited K_D value.

[0074] As used herein, "treatment" may refer to therapeutic administration of a molecule, compound, formulation, composition, etc. so as to alter one or more pathological symptoms in an individual or cell being treated. Desirable effects of treatment can include without limitation decelerating disease progression, ameliorating or palliating a pathological symptom or disease state, improving prognosis, and/or achieving disease remission. For example, an individual's cancer is successfully "treated" if one or more symptoms associated with cancer are mitigated or abolished, such as, without limitation, reducing the proliferation of cancer cells, eliminating cancer cells or tumor burden, decreasing symptoms resulting from the cancer, increasing the quality of life of the individual, lessening the dose of other medication(s), and/or prolonging survival of the individual. As another example, an autoimmune or inflammatory disease may be successfully "treated" if one or more symptoms associated with the autoimmune or inflammatory disease are mitigated or abolished, such as, without limitation, reducing autoreactive immune cells and/or inflammatory immune cells or cytokines, decreasing immune activation and/or inflammation, slowing or mitigating organ damage resulting from the disease, decreasing symptoms resulting from the disease, increasing the quality of life of the individual, lessening the dose of other medication(s), and/or prolonging survival of the individual.

[0075] As used herein, "delaying progression" of a disease may refer to slowing, retarding, deferring, postponing development of, stabilizing, or otherwise hindering the pathological course of the disease. In some embodiments, the term may refer to a delay

sufficient to effectively encompass prevention, *e.g.*, in preventing the individual from developing the disease. In some embodiments, *e.g.*, an advanced cancer, delaying progression may include delaying metastasis. One of skill in the art will appreciate that the precise length of delay may depend, *e.g.*, upon the specific disease, condition of the individual, and the like.

[0076] The terms "cancer" and "cancerous" may describe dysregulated or unregulated cell growth/proliferation by a cell or cells in a mammal. Any cancer type known in the art may be included, such as but not limited to carcinoma, sarcoma, lymphoma, leukemia, lymphoma, and blastoma. More particular examples of such cancers include, but are not limited to, lung cancer, squamous cell cancer, brain tumors, glioblastoma, head and neck cancer, hepatocellular cancer, colorectal cancer (*e.g.*, colon or rectal cancers), liver cancer, bladder cancer, gastric or stomach cancer, pancreatic cancer, cervical cancer, ovarian cancer, cancer of the urinary tract, breast cancer, peritoneal cancer, uterine cancer, salivary gland cancer, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, anal carcinoma, penile carcinoma, melanoma, multiple myeloma and B-cell lymphoma (including non-Hodgkin's lymphomas (NHL)); acute lymphoblastic leukemia (ALL); chronic lymphocytic leukemia (CLL); acute myeloid leukemia (AML); Merkel cell carcinoma; hairy cell leukemia; chronic myeloblastic leukemia (CML); and associated metastases.

[0077] As used herein, the term "effective amount" may refer to an amount of an antibody of the present disclosure or a pharmaceutical composition containing an antibody of the present disclosure that is sufficient and effective in achieving a desired therapeutic effect in treating or delaying progression of a patient having a disease, such as a cancer, e.g., solid tumor or hematological cancer. In some embodiments, a therapeutically effective amount will avoid adverse side effects, and/or such side effects will be outweighed by beneficial effects. An effective amount may depend upon the individual being treated, *e.g.*, age, weight, sex, disease state, as well as the ability of the agent to produce a desired response. An effective amount can be administered in one or more administrations. As in the clinical context, an effective amount of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition, such as another therapeutic agent. Thus, an "effective amount" may also be considered in the context of administering one or more additional

therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

[0078] As used herein, the term "pharmaceutical composition" may refer to a medicinal or pharmaceutical formulation that includes an active ingredient as well as excipients or diluents (or both excipients and diluents) and enables the active ingredient to be administered by suitable methods of administration. In some embodiments, the pharmaceutical compositions disclosed herein include pharmaceutically acceptable components that are compatible with one or more antibodies of the present disclosure. In some embodiments, the pharmaceutical composition is in tablet or capsule form for oral administration or in aqueous form for intravenous or subcutaneous administration, for example by injection.

[0079] As used herein, the terms "subject," "individual," and "patient" are used interchangeably to refer to a vertebrate, for example, a mammal. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets.

[0080] As used herein, "in conjunction with" or "in combination with" may refer to administration of one therapeutic in addition to (*e.g.*, before, during, and/or after) another therapeutic.

Antibodies

[0081] Certain aspects of the present disclosure relate to antibodies that bind the extracellular domain (e.g., the D1 domain) of a human SIRP-α polypeptide. In some embodiments, the antibody is a humanized antibody. In some embodiments, the antibody is a monoclonal antibody.

[0082] In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising a VH domain shown in Table 2 and/or a light chain comprising a VL domain that comprises an amino acid sequence according to the formula SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQQKPGQAPVTLIYSDDKRPSNIPERFSGSSSGTTVTLTISGVQAEDEADYYCGGYDQSSYTNPFGX$_1$GTX$_2$X$_3$TVL (SEQ ID NO:71), where X$_1$ is G or T; X$_2$ is K, Q, or R; and X$_3$ is L or V. In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising a VH domain shown in Table 2 and a light chain comprising a VL domain that comprises an amino acid sequence according to the formula SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQQKPGQAPVTLIYSDDKRPSNI

PERFSGSSSGTTVTLTISGVQAEDEADYYCGGYDQSSYTNPFGX$_1$GTX$_2$X$_3$TVL (SEQ ID NO:71), where X$_1$ is G or T; X$_2$ is K, Q, or R; and X$_3$ is L or V. In some embodiments of any of the above embodiments, the VL domain does not comprise the sequence of SEQ ID NO:25. In certain embodiments, an antibody of the present disclosure comprises a heavy chain comprising a VH domain that comprises the amino acid sequence of SEQ ID NO:26 and a light chain comprising a VL domain that comprises an amino acid sequence according to the formula SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQQKPGQAPVTLIYSDDKRPSNI PERFSGSSSGTTVTLTISGVQAEDEADYYCGGYDQSSYTNPFGX$_1$GTX$_2$X$_3$TVL (SEQ ID NO:71), where X$_1$ is G or T; X$_2$ is K, Q, or R; and X$_3$ is L or V. In some embodiments, the VL domain does not comprise the sequence of SEQ ID NO:25. In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising a VH domain shown in Table 2 and/or a light chain comprising a VL domain shown in Table 1. In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising a VH domain shown in Table 2 and a light chain comprising a VL domain shown in Table 1.

[0083] In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising a VH domain that comprises the amino acid sequence of SEQ ID NO:26 and/or a light chain comprising a VL domain that comprises an amino acid sequence selected from SEQ ID NOs:39-41. In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising a VH domain that comprises the amino acid sequence of SEQ ID NO:26 and a light chain comprising a VL domain that comprises an amino acid sequence selected from SEQ ID NOs:39-41.

[0084] In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising a VH domain shown in Table 2 and/or a light chain comprising an amino acid sequence selected from SEQ ID NOs:48-57. In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising a VH domain shown in Table 2 and a light chain comprising an amino acid sequence selected from SEQ ID NOs:48-57. In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising a VH domain that comprises the amino acid sequence of SEQ ID NO:26 and/or a light chain comprising an amino acid sequence selected from SEQ ID NOs:48-57. In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising a VH domain that comprises the amino acid sequence of SEQ ID NO:26 and a light chain comprising an amino acid sequence selected from SEQ ID NOs:48-57. In some

embodiments, an antibody of the present disclosure comprises a heavy chain comprising a VH domain that comprises the amino acid sequence of SEQ ID NO:81 and a light chain comprising an amino acid sequence selected from SEQ ID NOs:48-57. In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising a VH domain that comprises the amino acid sequence of SEQ ID NO:83 and a light chain comprising an amino acid sequence selected from SEQ ID NOs:48-57. In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising a VH domain that comprises an amino acid sequence selected from SEQ ID NOs:77-111 and a light chain comprising an amino acid sequence selected from SEQ ID NOs:48-57. In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising a VH domain that comprises an amino acid sequence selected from SEQ ID NOs:77-111 and a light chain that comprises an amino acid sequence selected from SEQ ID NOs:48-51. In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising a VH domain shown in Table 2 and a constant domain that comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:33, 34, and 137 and/or a light chain comprising an amino acid sequence selected from SEQ ID NOs:48-57. In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:60, 61, and 129 and/or a light chain comprising an amino acid sequence selected from SEQ ID NOs:48-57.

[0085] In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising a VH domain that comprises an amino acid sequence selected from SEQ ID NOs:26, 81, or 83 and a light chain that comprises an amino acid sequence selected from SEQ ID NOs:48-57. In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising a VH domain that comprises an amino acid sequence selected from SEQ ID NOs:26, 81, or 83 and a light chain comprising a VL domain that comprises an amino acid sequence selected from SEQ ID NOs:39-41. In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising a VH domain that comprises the amino acid sequence of SEQ ID NO:26 and a light chain comprising a VL domain that comprises an amino acid sequence selected from SEQ ID NOs:39-41.

[0086] In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising a VH domain that comprises an amino acid sequence selected from SEQ

ID NOs:26, 81, or 83 and a light chain that comprises a VL domain sequence selected from SEQ ID NOs:39-41 and a CL domain comprising a sequence selected from SEQ ID NOs:36-38. In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising a VH domain that comprises the amino acid sequence of SEQ ID NO:26 and a light chain that comprises a VL domain sequence selected from SEQ ID NOs:39-41 and a CL domain comprising a sequence selected from SEQ ID NOs:36-38.

[0087] In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising a VH domain that comprises an amino acid sequence selected from SEQ ID NOs:26, 81, or 83 and a light chain that comprises a VL domain comprising the sequence of SEQ ID NO:25 and a CL domain sequence selected from SEQ ID NOs:43-46. In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising a VH domain that comprises the amino acid sequence of SEQ ID NO:26 and a light chain that comprises a VL domain comprising the sequence of SEQ ID NO:25 and a CL domain sequence selected from SEQ ID NOs:43-46.

[0088] In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises a VH domain comprising the amino acid sequence of SEQ ID NO:83 and a light chain that comprises a VL domain sequence selected from SEQ ID NOs:39-41. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises a VH domain comprising the amino acid sequence of SEQ ID NO:83 and a light chain that comprises a VL domain comprising the amino acid sequence of SEQ ID NO:40. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises a VH domain comprising the amino acid sequence of SEQ ID NO:83 and a light chain that comprises the amino acid sequence of SEQ ID NO:55.

[0089] In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:119-123 and a light chain that comprises a VL domain sequence selected from SEQ ID NOs:39-41. In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:119-123 and a light chain that comprises a VL domain comprising the amino acid sequence of SEQ ID NO:40. In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:119-123 and a light chain that comprises the amino acid sequence of SEQ ID NO:55. In some embodiments, an antibody of the present disclosure

comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:119 and a light chain that comprises the amino acid sequence of SEQ ID NO:55. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:120 and a light chain that comprises the amino acid sequence of SEQ ID NO:55. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:121 and a light chain that comprises the amino acid sequence of SEQ ID NO:55. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:122 and a light chain that comprises the amino acid sequence of SEQ ID NO:55. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:123 and a light chain that comprises the amino acid sequence of SEQ ID NO:55.

[0090] In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises a VH domain comprising the amino acid sequence of SEQ ID NO:26 and a constant domain sequence selected from the group consisting of SEQ ID NOs:132-139; and/or a light chain comprising a VL domain that comprises the amino acid sequence of SEQ ID NO:40. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises a VH domain comprising the amino acid sequence of SEQ ID NO:26 and a constant domain sequence selected from the group consisting of SEQ ID NOs:33,34, and 137; and/or a light chain comprising a VL domain that comprises the amino acid sequence of SEQ ID NO:40. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises a VH domain comprising the amino acid sequence of SEQ ID NO:26 and a constant domain sequence selected from the group consisting of SEQ ID NOs:132-139; and/or a light chain comprising the amino acid sequence of SEQ ID NO:55. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises a VH domain comprising the amino acid sequence of SEQ ID NO:26 and a constant domain sequence selected from the group consisting of SEQ ID NOs:33,34, and 137; and/or a light chain comprising the amino acid sequence of SEQ ID NO:55.

[0091] In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:124-131; and/or a light chain comprising a VL domain that comprises the amino acid sequence of SEQ ID NO:40. In some embodiments, an antibody of the present disclosure

comprises a heavy chain that comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:60, 61, and 129; and/or a light chain comprising a VL domain that comprises the amino acid sequence of SEQ ID NO:40. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:124-131; and/or a light chain comprising the amino acid sequence of SEQ ID NO:55. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:60, 61, and 129; and/or a light chain comprising the amino acid sequence of SEQ ID NO:55. In some embodiments, the heavy chain comprises the amino acid sequence of SEQ ID NO:60, and the light chain comprises the amino acid sequence of SEQ ID NO:55. In some embodiments, the heavy chain comprises the amino acid sequence of SEQ ID NO:61, and the light chain comprises the amino acid sequence of SEQ ID NO:55. In some embodiments, the heavy chain comprises the amino acid sequence of SEQ ID NO:137, and the light chain comprises the amino acid sequence of SEQ ID NO:55.

[0092] In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises an amino acid sequence selected from SEQ ID NOs:58-62 and/or a light chain comprising a VL domain that comprises an amino acid sequence according to the formula SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQQKPGQAPVTLIYSDDKRPSNIPERFSGSSSGTTVTLTISGVQAEDEADYYCGGYDQSSYTNPFGX$_1$GTX$_2$X$_3$TVL (SEQ ID NO:71), where X$_1$ is G or T; X$_2$ is K, Q, or R; and X$_3$ is L or V. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises an amino acid sequence selected from SEQ ID NOs:58-62 and a light chain comprising a VL domain that comprises an amino acid sequence according to the formula SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQQKPGQAPVTLIYSDDKRPSNIPERFSGSSSGTTVTLTISGVQAEDEADYYCGGYDQSSYTNPFGX$_1$GTX$_2$X$_3$TVL (SEQ ID NO:71), where X$_1$ is G or T; X$_2$ is K, Q, or R; and X$_3$ is L or V. In some embodiments of any of the above embodiments, the VL domain does not comprise the sequence of SEQ ID NO:25.

[0093] In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises an amino acid sequence selected from SEQ ID NOs:58-62 and a light chain comprising a VL domain shown in Table 1. In some embodiments, an antibody of

the present disclosure comprises a heavy chain that comprises an amino acid sequence selected from SEQ ID NOs:58-62 and a light chain comprising a VL domain that comprises an amino acid sequence selected from SEQ ID NOs:39-41. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises an amino acid sequence selected from SEQ ID NOs:58-62 and a light chain comprising an amino acid sequence selected from SEQ ID NOs:48-57. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises an amino acid sequence selected from SEQ ID NOs:58-62 and a light chain comprising a VL domain that comprises an amino acid sequence selected from SEQ ID NOs:39-41 and a CL domain that comprises an amino acid sequence selected from SEQ ID NOs:36-38. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises an amino acid sequence selected from SEQ ID NOs:58-62 and a light chain comprising a VL domain that comprises the amino acid sequence of SEQ ID NO:25 and a CL domain that comprises an amino acid sequence selected from SEQ ID NOs:43-46.

[0094] In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:62 and a light chain that comprises the amino acid sequence of SEQ ID NO:52. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:62 and a light chain that comprises the amino acid sequence of SEQ ID NO:53. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:62 and a light chain that comprises the amino acid sequence of SEQ ID NO:54. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:62 and a light chain that comprises the amino acid sequence of SEQ ID NO:55. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:62 and a light chain that comprises the amino acid sequence of SEQ ID NO:56. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:62 and a light chain that comprises the amino acid sequence of SEQ ID NO:57.

[0095] In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:58 and a light chain that comprises the amino acid sequence of SEQ ID NO:52. In some embodiments, an antibody

of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:59 and a light chain that comprises the amino acid sequence of SEQ ID NO:52. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:60 and a light chain that comprises the amino acid sequence of SEQ ID NO:52. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:61 and a light chain that comprises the amino acid sequence of SEQ ID NO:52.

[0096] In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:58 and a light chain that comprises the amino acid sequence of SEQ ID NO:53. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:59 and a light chain that comprises the amino acid sequence of SEQ ID NO:53. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:60 and a light chain that comprises the amino acid sequence of SEQ ID NO:53. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:61 and a light chain that comprises the amino acid sequence of SEQ ID NO:53.

[0097] In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:58 and a light chain that comprises the amino acid sequence of SEQ ID NO:54. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:59 and a light chain that comprises the amino acid sequence of SEQ ID NO:54. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:60 and a light chain that comprises the amino acid sequence of SEQ ID NO:54. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:61 and a light chain that comprises the amino acid sequence of SEQ ID NO:54.

[0098] In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:58 and a light chain that comprises the amino acid sequence of SEQ ID NO:55. In some embodiments, an antibody

of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:59 and a light chain that comprises the amino acid sequence of SEQ ID NO:55. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:60 and a light chain that comprises the amino acid sequence of SEQ ID NO:55. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:61 and a light chain that comprises the amino acid sequence of SEQ ID NO:55.

[0099] In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:58 and a light chain that comprises the amino acid sequence of SEQ ID NO:56. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:59 and a light chain that comprises the amino acid sequence of SEQ ID NO:56. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:60 and a light chain that comprises the amino acid sequence of SEQ ID NO:56. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:61 and a light chain that comprises the amino acid sequence of SEQ ID NO:56.

[0100] In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:58 and a light chain that comprises the amino acid sequence of SEQ ID NO:57. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:59 and a light chain that comprises the amino acid sequence of SEQ ID NO:57. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:60 and a light chain that comprises the amino acid sequence of SEQ ID NO:57. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO:61 and a light chain that comprises the amino acid sequence of SEQ ID NO:57.

[0101] In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises a VH domain comprising a sequence selected from SEQ ID NOs:77-111 and a light chain that comprises a VL domain comprising the sequence of SEQ ID NO:25. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises a

VH domain comprising a sequence selected from SEQ ID NOs:77-111 and a light chain that comprises a VL domain comprising a sequence selected from SEQ ID NOs:39-41. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises a VH domain comprising a sequence selected from SEQ ID NOs:77-111 and a light chain that comprises a VL domain comprising a sequence selected from SEQ ID NOs:39-41 and a CL domain comprising a sequence selected from SEQ ID NOs:36-38. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises a VH domain comprising a sequence selected from SEQ ID NOs:77-111 and a light chain that comprises a VL domain comprising the sequence of SEQ ID NO:25 and a CL domain comprising a sequence selected from SEQ ID NOs:43-46. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises a sequence selected from SEQ ID NOs:114-123 and a light chain that comprises a sequence selected from SEQ ID NOs:47-63. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises a sequence selected from SEQ ID NOs:124-131 and a light chain that comprises a sequence selected from SEQ ID NOs:47-63. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises a sequence selected from SEQ ID NOs:114-131 and a light chain that comprises a sequence selected from SEQ ID NOs:47-63.

[0102] In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises an amino acid sequence selected from SEQ ID NOs:114-118 and a light chain that comprises an amino acid sequence selected from SEQ ID NOs:48-57. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises an amino acid sequence selected from SEQ ID NOs:119-123 and a light chain that comprises an amino acid sequence selected from SEQ ID NOs:48-57. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises an amino acid sequence selected from SEQ ID NOs:114-123 and a light chain that comprises a VL domain comprising a sequence selected from SEQ ID NOs:39-41. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises an amino acid sequence selected from SEQ ID NOs:114-123 and a light chain that comprises a VL domain comprising a sequence selected from SEQ ID NOs:39-41 and a CL domain comprising a sequence selected from SEQ ID NOs:36-38. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises an amino acid sequence selected from SEQ ID NOs:114-123 and a light chain that comprises a VL domain comprising the

sequence of SEQ ID NO:25 and a CL domain comprising a sequence selected from SEQ ID NOs:43-46.

[0103] In some embodiments, an antibody of the present disclosure comprises three CDRs from a VH domain comprising a sequence set forth in Table 2 and/or three CDRs from a VL domain comprising a sequence set forth in Table 1. In some embodiments, an antibody of the present disclosure comprises a VH domain comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a VH domain sequence set forth in Table 2 and optionally three CDRs from a VH domain comprising a sequence set forth in Table 2, and/or a VL domain comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a VL domain sequence set forth in Table 1 and optionally three CDRs from a VL domain comprising a sequence set forth in Table 1. In some embodiments, an antibody of the present disclosure comprises a heavy chain comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a heavy chain sequence set forth in Table 2 and optionally three CDRs from a VH domain comprising a sequence set forth in Table 2, and/or a light chain comprising a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a light chain sequence set forth in Table 1 and optionally three CDRs from a VL domain comprising a sequence set forth in Table 1.

Table 1. Light chain antibody sequences.

Name	Description	SEQ ID NO	Sequence
AB25	HVR-L1	22	SGGSYSSYYYA
	HVR-L2	23	SDDKRPS
	HVR-L3	24	GGYDQSSYTNP
Hum1	VL	25	SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQ QKPGQAPVTLIYSDDKRPSNIPERFSGSSSGTTVTLTI SGVQAEDEADYYCGGYDQSSYTNPFGGGTKLTVL
Hum 1 VL version 1 (v1)	VL	39	SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQ QKPGQAPVTLIYSDDKRPSNIPERFSGSSSGTTVTLTI SGVQAEDEADYYCGGYDQSSYTNPFGTGTKVTVL
Hum1 VL version 2 (v2)	VL	40	SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQ QKPGQAPVTLIYSDDKRPSNIPERFSGSSSGTTVTLTI SGVQAEDEADYYCGGYDQSSYTNPFGGGTQLTVL

Hum1 VL version 3 (v3)	VL	41	SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQ QKPGQAPVTLIYSDDKRPSNIPERFSGSSSGTTVTLTI SGVQAEDEADYYCGGYDQSSYTNPFGGGTRLTVL
Human Kappa CL	CL	36	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
Human Lambda IGLC1	CL	37	GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGA VTVAWKADGSPVKAGVETTKPSKQSNNKYAASSYL SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
Human Lambda IGLC2	CL	38	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAV TVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLT PEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
Human Lambda IGLC1_wt	CL	42	GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGA VTVAWKADGSPVKAGVETTKPSKQSNNKYAASSYL SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
Human Lambda IGLC1_ N172D	CL	43	GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGA VTVAWKADGSPVKAGVETTKPSKQSNDKYAASSYL SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
Human Lambda IGLC1_ N171D	CL	44	GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGA VTVAWKADGSPVKAGVETTKPSKQSDNKYAASSYL SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
Human Lambda_I GLC1_N1 71D, N172S (DS)	CL	45	GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGA VTVAWKADGSPVKAGVETTKPSKQSDSKYAASSYL SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
Human Lambda_I GLC1_N1 71S, N172D (SD)	CL	46	GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGA VTVAWKADGSPVKAGVETTKPSKQSSDKYAASSYL SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
Human LC Original	Light chain	47	SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQ QKPGQAPVTLIYSDDKRPSNIPERFSGSSSGTTVTLTI SGVQAEDEADYYCGGYDQSSYTNPFGGGTKLTVL GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGA VTVAWKADGSPVKAGVETTKPSKQSNNKYAASSYL SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
Human LC Original _ N172D	Light chain	48	SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQ QKPGQAPVTLIYSDDKRPSNIPERFSGSSSGTTVTLTI SGVQAEDEADYYCGGYDQSSYTNPFGGGTKLTVL GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGA VTVAWKADGSPVKAGVETTKPSKQSNDKYAASSYL SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
Human LC Original _ N171D	Light chain	49	SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQQ KPGQAPVTLIYSDDKRPSNIPERFSGSSSGTTVTLTISG VQAEDEADYYCGGYDQSSYTNPFGGGTKLTVL GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGA VTVAWKADGSPVKAGVETTKPSKQSDNKYAASSYL

			SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
Human LC original N171D, N172S	Light chain	50	SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQQ KPGQAPVTLIYSDDKRPSNIPERFSGSSSGTTVTLTISG VQAEDEADYYCGGYDQSSYTNPFGGGTKLTVL GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGA VTVAWKADGSPVKAGVETTKPSKQSDSKYAASSYL SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
Human LC original N171S, N172D	Light chain	51	SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQQ KPGQAPVTLIYSDDKRPSNIPERFSGSSSGTTVTLTISG VQAEDEADYYCGGYDQSSYTNPFGGGTKLTVL GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGA VTVAWKADGSPVKAGVETTKPSKQSSDKYAASSYL SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
Human LC-Version 1 + DS	Light chain	52	SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQ QKPGQAPVTLIYSDDKRPSNIPERFSGSSSGTTVTLTI SGVQAEDEADYYCGGYDQSSYTNPFGTGTKVTVL GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGA VTVAWKADGSPVKAGVETTKPSKQSDSKYAASSYL SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
Human LC-Version 1 + SD	Light chain	53	SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQ QKPGQAPVTLIYSDDKRPSNIPERFSGSSSGTTVTLTI SGVQAEDEADYYCGGYDQSSYTNPFGTGTKVTVL GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGA VTVAWKADGSPVKAGVETTKPSKQSSDKYAASSYL SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
Human LC-Version 2 + DS	Light chain	54	SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQ QKPGQAPVTLIYSDDKRPSNIPERFSGSSSGTTVTLTI SGVQAEDEADYYCGGYDQSSYTNPFGGGTQLTVL GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGA VTVAWKADGSPVKAGVETTKPSKQSDSKYAASSYL SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
Human LC-Version 2 + SD	Light chain	55	SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQQ KPGQAPVTLIYSDDKRPSNIPERFSGSSSGTTVTLTISG VQAEDEADYYCGGYDQSSYTNPFGGGTQLTVL GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGA VTVAWKADGSPVKAGVETTKPSKQSSDKYAASSYL SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
Human LC-Version 3 + DS	Light chain	56	SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQQ KPGQAPVTLIYSDDKRPSNIPERFSGSSSGTTVTLTISG VQAEDEADYYCGGYDQSSYTNPFGGGTRLTVL GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGA VTVAWKADGSPVKAGVETTKPSKQSDSKYAASSYL SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
Human LC-Version 3 + SD	Light chain	57	SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQQ KPGQAPVTLIYSDDKRPSNIPERFSGSSSGTTVTLTISG VQAEDEADYYCGGYDQSSYTNPFGGGTRLTVL GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGA VTVAWKADGSPVKAGVETTKPSKQSSDKYAASSYL SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
Hum1 original in Lambda constant	Light chain	63	SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQ QKPGQAPVTLIYSDDKRPSNIPERFSGSSSGTTVTLTI SGVQAEDEADYYCGGYDQSSYTNPFGGGTKLTVLG QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAV TVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLS

IGLC2			LTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

Table 2. Heavy chain antibody sequences.

Name	Description	SEQ ID NO	Sequence
AB21	HVR-H1	19	SNAMS
	HVR-H2	20	GISAGGSDTYYPASVKG
	HVR-H3	21	ETWNHLFDY
AB21 VH MutAll	VH	26	EVQLVESGGGVVQPGGSLRLSCAASGFTFSSNAMS WVRQAPGKGLEWVAGISAGGSDTYYPASVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARETWNHL FDYWGQGTLVTVSS
AB21_ VH MutAll _IgG1 wt	Heavy chain	58	EVQLVESGGGVVQPGGSLRLSCAASGFTFSSNAMS WVRQAPGKGLEWVAGISAGGSDTYYPASVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARETWNHL FDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG
AB21_ HC mutall_ IgG1A AA dead	Heavy chain	59	EVQLVESGGGVVQPGGSLRLSCAASGFTFSSNAMS WVRQAPGKGLEWVAGISAGGSDTYYPASVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARETWNHL FDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCDKTHTCPPCPAPEAAGAPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG
AB21_ VH MutAll _IgG2 wt	Heavy chain	60	EVQLVESGGGVVQPGGSLRLSCAASGFTFSSNAMSW VRQAPGKGLEWVAGISAGGSDTYYPASVKGRFTISR DNSKNTLYLQMNSLRAEDTAVYYCARETWNHLFDY WGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALG CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKT VERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKP REEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNK GLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPML DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPG
AB21_ VH	Heavy chain	61	EVQLVESGGGVVQPGGSLRLSCAASGFTFSSNAMS WVRQAPGKGLEWVAGISAGGSDTYYPASVKGRFTI

MutAll_IgG2Da			SRDNSKNTLYLQMNSLRAEDTAVYYCARETWNHL FDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSEST AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTK VDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEV HNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKE YKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPG
AB21_VH MutAll_IgG4S228P	Heavy chain	62	EVQLVESGGGVVQPGGSLRLSCAASGFTFSSNAMS WVRQAPGKGLEWVAGISAGGSDTYYPASVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARETWNHL FDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSEST AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTK VDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVE VHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPP SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVF SCSVMHEALHNHYTQKSLSLSLG
AB21_HC_wt	VH	77	DVQLVESGGGVVRPGESLRLSCAASGFTFSSNAMS WVRQAPGKGLEWLAGISAGGSDTYYPASVKGRFTI SRDNSKNTLYLQMNTLTAEDTAVYYCARETWNHL FDYWGLGTLVTVSS
AB25_HC_wt	VH	78	DVQLVESGGGVVRPGESLRLSCEASGFTFSSNAMS WVRQAPGKGLEWVAGISSGSDTYYGDSVKGRLTIS RDNSKNILYLQMNSLTAEDTAVYYCARETWNHLF DYWGLGTLVTVSS
AB27_HC_wt	VH	79	DVQLVESGGGVVRPGESLRLSCAVSGFRFSSYAMS WVRQAPGKGLEWVSGISSGGDTYYVDSVKGRFTIS RDNSKNTLYLQVNSLTAEDTAIYYCARETWNHLFD YWGLGTLVTVSS
AB66_HC_wt	VH	80	DVQLVESGGGVVRPGESLRLSCAASGFTFSSYAMS WVRQAPGKGLEWLAGISAGGSDTYYIDSVKGRFTI SRDNPKNSLYLQMSSLTAEDTAVYYCARETWNHL FDYWGLGTLVTVSS
AB25_HC_Mutall	VH	81	EVQLVESGGGVVQPGGSLRLSCAASGFTFSSNAMS WVRQAPGKGLEWVAGISSGSDTYYGDSVKGRFTIS RDNSKNTLYLQMNSLTAEDTAVYYCARETWNHLF DYWGQGTLVTVSS
AB25 VH MutAll M34V	VH	82	EVQLVESGGGVVQPGGSLRLSCAASGFTFSSNAVS WVRQAPGKGLEWVAGISSGSDTYYGDSVKGRFTIS RDNSKNTLYLQMNSLTAEDTAVYYCARETWNHLF DYWGQGTLVTVSS
AB27_HC_Mutall	VH	83	EVQLVESGGGVVQPGGSLRLSCAASGFRFSSYAMS WVRQAPGKGLEWVSGISSGGDTYYVDSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCARETWNHLF DYWGQGTLVTVSS
AB27 VH	VH	84	EVQLVESGGGVVQPGGSLRLSCAASGFRFSSYAVS WVRQAPGKGLEWVSGISSGGDTYYVDSVKGRFTIS

MutAll M34V			RDNSKNTLYLQMNSLRAEDTAVYYCARETWNHLF DYWGQGTLVTVSS
AB21 VH MutAll M34V	VH	85	EVQLVESGGGVVQPGGSLRLSCAASGFTFSSNAVS WVRQAPGKGLEWVAGISAGGSDTYYPASVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARETWNHL FDYWGQGTLVTVSS
AB21_ HC_M utAll_ M34L	VH	86	EVQLVESGGGVVQPGGSLRLSCAASGFTFSSNALS WVRQAPGKGLEWVAGISAGGSDTYYPASVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARETWNHL FDYWGQGTLVTVSS
AB25_ HC_M utAll_ M34L	VH	87	EVQLVESGGGVVQPGGSLRLSCAASGFTFSSNALS WVRQAPGKGLEWVAGISSGSDTYYGDSVKGRFTIS RDNSKNTLYLQMNSLTAEDTAVYYCARETWNHLF DYWGQGTLVTVSS
AB27_ HC_M utAll_ M34L	VH	88	EVQLVESGGGVVQPGGSLRLSCAASGFRFSSYALS WVRQAPGKGLEWVSGISSGGDTYYVDSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCARETWNHLF DYWGQGTLVTVSS
S16	VH	89	DVQLVESGGGVVRPGESLRLSCAVSGFRFSSYAMSWVR QAPGKGLEWVSGISSGGDTYYVDSVKGRFTISRDNSKNT LYLQVNSLTAEDTAIYYCARETWNHLFDYWGLGTLVTV SS
S17	VH	90	DVQLVESGGAVVRPGESLRLSCAASGFTFSSYAMSWVR QAPGKGLEWLAGISAGGSDTYYIDSVKGRFTISRDNSEN SLYLQMNSLTAEDTAVYYCARETWNHLFDYWGLGTLV TVSS
S22	VH	91	DVQLVESGGGVVRPGESLRLSCAASGFTFSSYAMSWVR QAPGKGLEWLAGISAGGSDTYYIDSVKGRFTISRRQFQE QSLSPNEPALTAEDTAVYYCARETWNHLFDYWGLGTLV TVSS
S23	VH	92	DVQLVESGGGVVRPGESLRLSCAASGFTFSSHAMSWVR QAPGKGLEWLAGISAGGSDTYYIDSVKGRFTISRDNSKS SLYLRMNSLTAEDTAVYYCARETWNHLFDYWGLGTLV TVSS
S24	VH	93	DVQLVESGGGVVRPGESLRLSCAASGFTFSSNAMSWVR QAPGKGLEWLAGISAGGSDTYYPASVKGRFTISRDNPKN TLYLQMNTLTAEDTAVYYCARETWNHLFDYWGLGTLV TVSS
S26	VH	94	DVQLVESGGGVVRPGESLRLSCAASGFTFSTYAMSWVR QAPGKGLEWVSGISASGSGTYYGDSVKGRFTMSRDNSK NTLYLQMNSLTAEDTAVYYCARETWNHLFDYWGLGTL VTVSS
S28	VH	95	DVQLVESGGGVVRPGESLRLSCAASGFSFSSNAMSWVR QAPGKGLEWVAGISASGDTYYSGSMKGRFTISRDNSKN TLYLQMNSLTAEDTAVYYCARETWNHLFDYWGLGTLV TVSS
S29	VH	96	DVQLVESGGGVVRPGESLRLSCAVSGFRFSSYAMSWVR QAPGKGLEWVSGISSDSDAYYVDSVKGRFTISRDNSKNT LYLQVNSLTAEDTAVYYCARETWNHLFDYWGLGTMVT VSS
S30	VH	97	DVQLVESGGGVVRPGESLRLSCEASGFTFSSDAMSWVR QAPGKGLEWVSGISSGSSTYYGGSVKGRFTISRDNSKNT LYLQMNSLTAEDTAVYYCARETWNHLFDYWGLGTLVT VSS
S55	VH	98	DVQLVESGGGVVRPGESLRLSCAVSGFRFSSYAMSWVR QAPGKGLEWVSGISSGGDTYYVDSVKGRFTISRDNSKNT LYLQVNSLTAEDTAIYYCARETWNHLFDYWGLGTLVTV

			SS
S56	VH	99	DVQLVESGGGVVRPGESLRLSCAASGFTFSSYAMSWVR QAPGKGLEWLAGISAGGSDTYYIDSVKGRFTISRDNSKN SLYLQVNSLTAEDTAVYYCARETWNHLFDYWGLGTLV TVSS
S59	VH	100	DVQLVESGGGVVRPGESLRLSCAVSGFRFSSHAMSWVR QAPGKGLEWVSGISSGGDTYYVDSVKGRFTISRDNSKNT LYLQVNSLTAEDTAIYYCARETWNHLFDYWGLGTLVTV SS
S60	VH	101	DVQLVDSGGGVVRPGESLRLSCAASGFTFSSYAMSWVR QAPGKGLEWLAGISAGGSDTYYIDSVKGRFTISRDNSKN SLYLQMNSLTAEDTAVYYCARETWNHLFDYWGLGTLV TVSS
S65	VH	102	DVQLVESGGGVVRPGESLRLSCAASGFTFSSYAMSWVR QAPGKGLEWLAGISAGGSDTYYIDSVKGRFTISRDNSKN SLYLQMNSLTAEDTAVYYCARETWNHLFDYWGLGTLV TVSS
S69	VH	103	DLQLVESGGGVVRPGESLRLSCAASGFTFSSYAMSWVR QAPGKGLEWLAGISAGGSDTYYIDSVKGRFTISRDNSKN SLYLQMNSLTAEDTAVYYCARETWNHLFDYWGLGTLV TVSS
S70	VH	104	DVQLVESGGGVVRPGESLRLSCAASGFTFSSYAMSWVR QAPGKGLEWLAGISAGGSDAYYIDSVKGRFTISRDNSKN SLYLQMNSLTAEDTAVYYCARETWNHLFDYWGLGTLV TVSS
S71	VH	105	DVQLVESGGGVVRPGESLRLSCAASGFTFSSYAMSWVR QAPGKGLEWLAGISAGGSDTYYIDSVKGRFTISRDNSKN SLYLQMNSLTAEDTAVYYCARETWNHLFDYWGLGTLV TVSS
S73	VH	106	DVQLVESGGGVVRPGESLRLSCEASGFTFSSNAMSWAR QAPGKGLEWVAGISSGSDTYYGDSVKGRLTISRDNSKNI LYLQMNSLTAEDTAVYYCARETWNHLFDYWGLGTLVT VSS
S74	VH	107	DVQLVESGGGVVRPGESLRLSCAASGFTFSSNAMSWVR QAPGKGLEWLAGISAGDSDTYYPASVKGRFTISRDNPKN TLYLQMNTLTAEDTAVYYCARETWNHLFDYWGLGTLV TVSS
S76	VH	108	DVQLVESGGGVVRPGESLRLSCAASGFTFSSYAMSWVR QAPGKGLEWLAGISAGGSDTYYIDSVKGRFTISRDNSKN SLYLQMNSLTAEDTAVYYCARETWNHLFDYWGLGTLV TVSS
S201	VH	109	DVQLVESGGAVVRPGETLRLSCTASGFTFSSYAMSWVR QAPGKGLEWVSGISASGSDTYYADSVKGRSTISRDNSKN TLYLRMSSLTAEDTAVYYCARETWNHLFDYWGLGTLV TVSS
S202	VH	110	DVQLVESGGGVVRPGESLRLSCAASGFTFSSYAMSWVR QAPGKGLEWLAGISAGGSDTYYIDSVKGRFTISRDNSKN SLYLQMNSLTAEDTAVYYCARETWNHLFDYWGLGTLV TVSS
S206	VH	111	DVQLVESGGAVVRPGETLRLSCTASGFTFSSYAMSWVR QAPGKGLEWVSGISASGSDTYYADSVKGRSTISRDNSKN TLYLRMSSLTAEDTAVYYCARETWNHLFDYWGLGTLV TLSS
AB25 VH Mutall _IgG1 wt	Heavy chain	114	EVQLVESGGGVVQPGGSLRLSCAASGFTFSSN AMSWVRQAPGKGLEWVAGISSGSDTYYGDSV KGRFTISRDNSKNTLYLQMNSLTAEDTAVYYC ARETWNHLFDYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN

			SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSREEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG
AB25 VH Mutall _IgG1 dead	Heavy chain	115	EVQLVESGGGVVQPGGSLRLSCAASGFTFSSN AMSWVRQAPGKGLEWVAGISSGSDTYYGDSV KGRFTISRDNSKNTLYLQMNSLTAEDTAVYYC ARETWNHLFDYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH TCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYASTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSREEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG
AB25 VH Mutall _IgG2 wt	Heavy chain	116	EVQLVESGGGVVQPGGSLRLSCAASGFTFSSN AMSWVRQAPGKGLEWVAGISSGSDTYYGDSV KGRFTISRDNSKNTLYLQMNSLTAEDTAVYYC ARETWNHLFDYWGQGTLVTVSSASTKGPSVFP LAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNF GTQTYTCNVDHKPSNTKVDKTVERKCCVECPP CPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVQFNWYVDGVEVHNAKTKPRE EQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS NKGLPAPIEKTISKTKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPMLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPG
AB25 VH_ Mutall _IgG2 Da	Heavy chain	117	EVQLVESGGGVVQPGGSLRLSCAASGFTFSSN AMSWVRQAPGKGLEWVAGISSGSDTYYGDSV KGRFTISRDNSKNTLYLQMNSLTAEDTAVYYC ARETWNHLFDYWGQGTLVTVSSASTKGPSVFP LAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNF GTQTYTCNVDHKPSNTKVDKTVERKCCVECPP CPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVQFNWYVDGVEVHNAKTKPRE EQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS NKGLPSSIEKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPMLDSDGSFFLYSKLTVDKSRWQQGN

			VFSCSVMHEALHNHYTQKSLSLSPG
AB25 VH_ Mutall _IgG4 _S228 P	Heavy chain	118	EVQLVESGGGVVQPGGSLRLSCAASGFTFSSN AMSWVRQAPGKGLEWVAGISSGSDTYYGDSV KGRFTISRDNSKNTLYLQMNSLTAEDTAVYYC ARETWNHLFDYWGQGTLVTVSSASTKGPSVFP LAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTKTYTCNVDHKPSNTKVDKRVESKYGPPCPP CPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSQEDPEVQFNWYVDGVEVHNAKTKPRE EQFNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGN VFSCSVMHEALHNHYTQKSLSLSLG
AB27 VH_ Mutall _IgG1 wt	Heavy chain	119	EVQLVESGGGVVQPGGSLRLSCAASGFRFSSY AMSWVRQAPGKGLEWVSGISSGGDTYYVDSV KGRFTISRDNSKNTLYLQMNSLRAEDTAVYYC ARETWNHLFDYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSREEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG
AB27 VH_ Mutall _IgG1 dead	Heavy chain	120	EVQLVESGGGVVQPGGSLRLSCAASGFRFSSY AMSWVRQAPGKGLEWVSGISSGGDTYYVDSV KGRFTISRDNSKNTLYLQMNSLRAEDTAVYYC ARETWNHLFDYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH TCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYASTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSREEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG
AB27 VH_ Mutall _IgG2 wt	Heavy chain	121	EVQLVESGGGVVQPGGSLRLSCAASGFRFSSY AMSWVRQAPGKGLEWVSGISSGGDTYYVDSV KGRFTISRDNSKNTLYLQMNSLRAEDTAVYYC ARETWNHLFDYWGQGTLVTVSSASTKGPSVFP LAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNF

			GTQTYTCNVDHKPSNTKVDKTVERKCCVECPP CPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVQFNWYVDGVEVHNAKTKPRE EQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS NKGLPAPIEKTISKTKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPMLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPG
AB27 VH_ Mutall _IgG2 Da	Heavy chain	122	EVQLVESGGGVVQPGGSLRLSCAASGFRFSSY AMSWVRQAPGKGLEWVSGISSGGDTYYVDSV KGRFTISRDNSKNTLYLQMNSLRAEDTAVYYC ARETWNHLFDYWGQGTLVTVSSASTKGPSVFP LAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNF GTQTYTCNVDHKPSNTKVDKTVERKCCVECPP CPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVQFNWYVDGVEVHNAKTKPRE EQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS NKGLPSSIEKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPMLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG
AB27 VH_ Mutall _IgG4 _S228 P	Heavy chain	123	EVQLVESGGGVVQPGGSLRLSCAASGFRFSSY AMSWVRQAPGKGLEWVSGISSGGDTYYVDSV KGRFTISRDNSKNTLYLQMNSLRAEDTAVYYC ARETWNHLFDYWGQGTLVTVSSASTKGPSVFP LAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTKTYTCNVDHKPSNTKVDKRVESKYGPPCPP CPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSQEDPEVQFNWYVDGVEVHNAKTKPRE EQFNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGN VFSCSVMHEALHNHYTQKSLSLSLG
AB21_ MutAll _IgG1 _AAA	Heavy chain	124	EVQLVESGGGVVQPGGSLRLSCAASGFTFSSN AMSWVRQAPGKGLEWVAGISAGGSDTYYPAS VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYY CARETWNHLFDYWGQGTLVTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

AB21_MutAll_IgG2wildtype_C232S	Heavy chain	125	EVQLVESGGGVVQPGGSLRLSCAASGFTFSSN AMSWVRQAPGKGLEWVAGISAGGSDTYYPAS VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYY CARETWNHLFDYWGQGTLVTVSSASTKGPSV FPLAPCSRSTSESTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS NFGTQTYTCNVDHKPSNTKVDKTVERKSCVE CPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVQFNWYVDGVEVHNAKTK PREEQFNSTFRVVSVLTVVHQDWLNGKEYKC KVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPG
AB21_MutAll_IgG2wildtype_C233S	Heavy chain	126	EVQLVESGGGVVQPGGSLRLSCAASGFTFSSN AMSWVRQAPGKGLEWVAGISAGGSDTYYPAS VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYY CARETWNHLFDYWGQGTLVTVSSASTKGPSV FPLAPCSRSTSESTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS NFGTQTYTCNVDHKPSNTKVDKTVERKCSVE CPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVQFNWYVDGVEVHNAKTK PREEQFNSTFRVVSVLTVVHQDWLNGKEYKC KVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPG
AB21_MutAll_IgG2Da_C232S	Heavy chain	127	EVQLVESGGGVVQPGGSLRLSCAASGFTFSSN AMSWVRQAPGKGLEWVAGISAGGSDTYYPAS VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYY CARETWNHLFDYWGQGTLVTVSSASTKGPSV FPLAPCSRSTSESTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS NFGTQTYTCNVDHKPSNTKVDKTVERKSCVE CPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVQFNWYVDGVEVHNAKTK PREEQFNSTFRVVSVLTVVHQDWLNGKEYKC KVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPG
AB21_MutAll_IgG2Da_C233S	Heavy chain	128	EVQLVESGGGVVQPGGSLRLSCAASGFTFSSN AMSWVRQAPGKGLEWVAGISAGGSDTYYPAS VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYY CARETWNHLFDYWGQGTLVTVSSASTKGPSV FPLAPCSRSTSESTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS NFGTQTYTCNVDHKPSNTKVDKTVERKCSVE CPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVT

			CVVVDVSHEDPEVQFNWYVDGVEVHNAKTK PREEQFNSTFRVVSVLTVVHQDWLNGKEYKC KVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPG
AB21_ MutAll _IgG2 _N297 A	Heavy chain	129	EVQLVESGGGVVQPGGSLRLSCAASGFTFSSN AMSWVRQAPGKGLEWVAGISAGGSDTYYPAS VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYY CARETWNHLFDYWGQGTLVTVSSASTKGPSV FPLAPCSRSTSESTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS NFGTQTYTCNVDHKPSNTKVDKTVERKCCVE CPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVQFNWYVDGVEVHNAKTK PREEQFASTFRVVSVLTVVHQDWLNGKEYKC KVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPG
AB21_ MutAll _IgG2 _N297 A_C23 2S	Heavy chain	130	EVQLVESGGGVVQPGGSLRLSCAASGFTFSSN AMSWVRQAPGKGLEWVAGISAGGSDTYYPAS VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYY CARETWNHLFDYWGQGTLVTVSSASTKGPSV FPLAPCSRSTSESTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS NFGTQTYTCNVDHKPSNTKVDKTVERKSCVE CPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVQFNWYVDGVEVHNAKTK PREEQFASTFRVVSVLTVVHQDWLNGKEYKC KVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPG
AB21_ MutAll _IgG2 _N297 A_C23 3S	Heavy chain	131	EVQLVESGGGVVQPGGSLRLSCAASGFTFSSN AMSWVRQAPGKGLEWVAGISAGGSDTYYPAS VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYY CARETWNHLFDYWGQGTLVTVSSASTKGPSV FPLAPCSRSTSESTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS NFGTQTYTCNVDHKPSNTKVDKTVERKCSVE CPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVQFNWYVDGVEVHNAKTK PREEQFASTFRVVSVLTVVHQDWLNGKEYKC KVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPG

[0104] As described *supra*, various techniques for delineating hypervariable regions (HVRs) or complementarity determining regions (CDRs) are known in the art and can be applied to the variable domain sequences described herein. In some embodiments, an antibody of the present disclosure comprises HVRs as defined by Chothia, Kabat, IMGT, or a combination thereof (*e.g.*, one or more HVRs as defined by one delineation and one or more HVRs as defined by a different delineation). As used herein, unless otherwise specified, the numbering of HVR or CDR residues is defined by Kabat numbering.

[0105] In some embodiments, an antibody of the present disclosure binds an extracellular domain (e.g., the D1 domain) of a human SIRP-α v1 polypeptide comprising the amino acid sequence of EEELQVIQPDKSVLVAAGETATLRCTATSLIPVGPIQWFRGAGPGRELIYNQKEGHFP RVTTVSDLTKRNNMDFSIRIGNITPADAGTYYCVKFRKGSPDDVEFKSGAGTELSVR AKPS (SEQ ID NO:5). In some embodiments, an antibody of the present disclosure binds an extracellular domain (e.g., the D1 domain) of a human SIRP-α v2 polypeptide comprising the amino acid sequence of EEELQVIQPDKSVSVAAGESAILHCTVTSLIPVGPIQWFRGAGPARELIYNQKEGHFPR VTTVSESTKRENMDFSISISNITPADAGTYYCVKFRKGSPDTEFKSGAGTELSVRAKPS (SEQ ID NO:6). In some embodiments, an antibody of the present disclosure binds an extracellular domain (e.g., the D1 domain) of a human SIRP-α v1 polypeptide comprising the amino acid sequence of EEELQVIQPDKSVLVAAGETATLRCTATSLIPVGPIQWFRGAGPGRELIYNQKEGHFP RVTTVSDLTKRNNMDFSIRIGNITPADAGTYYCVKFRKGSPDDVEFKSGAGTELSVR AKPS (SEQ ID NO:5) and an extracellular domain (e.g., the D1 domain) of a human SIRP-α v2 polypeptide comprising the amino acid sequence of EEELQVIQPDKSVSVAAGESAILHCTVTSLIPVGPIQWFRGAGPARELIYNQKEGHFPR VTTVSESTKRENMDFSISISNITPADAGTYYCVKFRKGSPDTEFKSGAGTELSVRAKPS (SEQ ID NO:6).

[0106] In some embodiments, an antibody of the present disclosure binds an extracellular domain (e.g., the D1 domain) of a monkey SIRP-α polypeptide (*e.g.*, the D1 domain of a monkey SIRP-α polypeptide). In some embodiments, an antibody of the present disclosure binds an extracellular domain (e.g., the D1 domain) of a cynomolgus SIRP-α polypeptide (*e.g.*, found in the organism *Macaca fascicularis*). In some embodiments, the antibody binds the extracellular domains (e.g., the D1 domains) of at least two different monkey SIRP-α

variant polypeptides. In some embodiments, the antibody binds the extracellular domains (e.g., the D1 domains) of at least two different cynomolgus SIRP-α variant polypeptides. For example, in some embodiments, the antibody binds an extracellular domain (e.g., the D1 domain) of a cynomolgus SIRP-α polypeptide comprising the amino acid sequence of EEELQVIQPEKSVSVAAGESATLNCTATSLIPVGPIQWFRGVGPGRELIYHQKEGHFP RVTPVSDPTKRNNMDFSIRISNITPADAGTYYCVKFRKGSPDVELKSGAGTELSVRAK PS (SEQ ID NO:11), an extracellular domain (e.g., the D1 domain) of a cynomolgus SIRP-α polypeptide comprising the amino acid sequence of EEELQVIQPEKSVSVAAGDSATLNCTVSSLIPVGPIQWFRGAGPGRELIYNLKEGHFP RVTAVSDPTKRNNMDFSIRISNITPADAGTYYCVKFRKGSPDVELKSGAGTELSVRA KPS (SEQ ID NO:12), or both.

[0107] In some embodiments, an antibody of the present disclosure binds an extracellular domain of a murine or mouse SIRP-α polypeptide (*e.g.*, found in the organism *Mus musculus*; *e.g.*, the D1 domain of a murine or mouse SIRP-α polypeptide). In some embodiments, the antibody binds the extracellular domains (e.g., the D1 domains) of two or more different murine SIRP-α variant polypeptides. A variety of murine SIRP-α variant polypeptides from different mouse strains are known. In some embodiments, the murine SIRP-α variant polypeptide comprises an amino acid sequence selected from KELKVTQPEKSVSVAAGDSTVLNCTLTSLLPVGPIKWYRGVGQSRLLIYSFTGEHFPR VTNVSDATKRNNMDFSIRISNVTPEDAGTYYCVKFQKGPSEPDTEIQSGGGTEVYVL AKPS (SEQ ID NO: 7; from 129 mouse strain), TEVKVIQPEKSVSVAAGDSTVLNCTLTSLLPVGPIRWYRGVGQSRQLIYSFTTEHFPR VTNVSDATKRSNLDFSIRISNVTPEDAGTYYCVKFQRGSPDTEIQSGGGTEVYVLAK (SEQ ID NO:8; from NOD mouse strain), KELKVTQPEKSVSVAAGDSTVLNCTLTSLLPVGPIRWYRGVGPSRLLIYSFAGEYVPR IRNVSDTTKRNNMDFSIRISNVTPADAGIYYCVKFQKGSSEPDTEIQSGGGTEVYVLA K (SEQ ID NO:9; from C57BL/6 mouse strain), and TEVKVTQPEKSVSVAAGDSTILNCTVTSLLPVGPIRWYRGVGQSRLLIYSFTGEHFPRI RNVSDTTKRNNMDFSIRISNVTPEDAGTYYCVKFQRGSSEPDTEIQSGGGTEVYVLA K (SEQ ID NO:10; from BALB/c mouse strain).

[0108] In some embodiments, an antibody of the present disclosure binds an extracellular domain (e.g., the D1 domain) of a human SIRP family protein. In some embodiments, an antibody of the present disclosure binds an extracellular domain (e.g., the D1 domain) of a

human SIRP-β polypeptide. In some embodiments, a human SIRP-β polypeptide refers to a polypeptide encoded by a human *SIRPB1* gene, *e.g.*, as described by NCBI Ref Seq ID No. 10326. In some embodiments, the extracellular domain (e.g., the D1 domain) of the human SIRP-β polypeptide comprises the amino acid sequence of EDELQVIQPEKSVSVAAGESATLRCAMTSLIPVGPIMWFRGAGAGRELIYNQKEGHF PRVTTVSELTKRNNLDFSISISNITPADAGTYYCVKFRKGSPDDVEFKSGAGTELSVR AKPS (SEQ ID NO:13) or EEELQVIQPDKSISVAAGESATLHCTVTSLIPVGPIQWFRGAGPGRELIYNQKEGHFPR VTTVSDLTKRNNMDFSIRISNITPADAGTYYCVKFRKGSPDHVEFKSGAGTELSVRA KPS (SEQ ID NO:14).

[0109] In some embodiments, an antibody of the present disclosure binds an extracellular domain (e.g., the D1 domain) of a human SIRP-γ polypeptide. In some embodiments, a human SIRP-γ polypeptide refers to a polypeptide encoded by a human *SIRPG* gene, *e.g.*, as described by NCBI Ref Seq ID No. 55423. In some embodiments, the extracellular domain (e.g., the D1 domain) of the human SIRP-γ polypeptide comprises the amino acid sequence of EEELQMIQPEKLLLVTVGKTATLHCTVTSLLPVGPVLWFRGVGPGRELIYNQKEGHF PRVTTVSDLTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPENVEFKSGPGTEMALG AKPS (SEQ ID NO:15).

[0110] In some embodiments, an antibody of the present disclosure binds an IgSF domain of CD47 (*e.g.*, human CD47). In some embodiments, an antibody of the present disclosure binds a polypeptide comprising the amino acid sequence of QLLFNKTKSVEFTFSNDTVVIPCFVTNMEAQNTTEVYVKWKFKGRDIYTFDGALNKS TVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVTELTREGETIIELKYRV VS (SEQ ID NO:16).

[0111] In some embodiments, an antibody of the present disclosure modulates SIRP-α signaling in a cell expressing a human SIRP-α polypeptide. In some embodiments, an antibody of the present disclosure antagonizes SIRP-α signaling in a cell expressing a human SIRP-α polypeptide. In some embodiments, an antibody of the present disclosure interferes with SIRP-α signaling in a cell expressing a human SIRP-α polypeptide. In some embodiments, an antibody of the present disclosure agonizes SIRP-α signaling in a cell expressing a human SIRP-α polypeptide. In some embodiments, SIRP-α signaling includes one or more intracellular signaling events mediated by activation of a SIRP-α polypeptide,

including without limitation tyrosine phosphorylation of the intracellular region of SIRP-α, phosphatase (*e.g.*, SHP1) binding, adaptor protein binding (*e.g.*, SCAP2, FYB, and/or GRB2), and nitric oxide production. Various assays for measuring SIRP-α signaling in a cell include without limitation SIRP-α phosphorylation, SHP1 and SHP2 co-immunoprecipitation, PI3-kinase signaling, cytokine production (both inflammatory IL-12, IL-23, TNFα, IFN and suppressive cytokines IL-10, IL-4, IL-13, cell surface markers levels for M1 and M2 macrophage markers) or dendritic cell activation and function; Kharitonenkov, A. *et al.* (1997) *Nature* 386: 181-6; Ochi, F. *et al.* (1997) *Biochem. Biophys. Res. Commun.* 239:483-7; Kim, E.J. *et al.* (2013) *Inflammation Research* 62:377-86; Yi, T. *et al.* (2015) *Immunity* 43:764-75.

[0112] In some embodiments, the cell expressing a human SIRP-α polypeptide is a leukocyte. In some embodiments, the cell is a macrophage, dendritic cell, neutrophil, eosinophil, or myeloid-derived suppressor cell (MDSC). In some embodiments, an antibody of the present disclosure decreases or antagonizes SIRP-α signaling in a cell expressing a human SIRP-α polypeptide by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%, *e.g.*, using one or more of the SIRP-α signaling assays described herein or otherwise known in the art. In some embodiments, an antibody of the present disclosure increases or agonizes SIRP-α signaling in a cell expressing a human SIRP-α polypeptide by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%, *e.g.*, using one or more of the SIRP-α signaling assays described herein or otherwise known in the art.

[0113] In some embodiments, an antibody of the present disclosure modulates an intercellular phenotype mediated by SIRP-α. In some embodiments, an antibody of the present disclosure enhances phagocytosis by a macrophage expressing a human SIRP-α polypeptide. For example, phagocytic activity of a macrophage treated or contacted with an antibody of the present disclosure can be compared with phagocytic activity of a macrophage not treated or contacted with the antibody, or phagocytic activity of a macrophage that expresses a human SIRP-α polypeptide and is treated or contacted with an antibody of the present disclosure can be compared with phagocytic activity of a macrophage that does not express a human SIRP-α polypeptide and is treated or contacted with the antibody. Exemplary phagocytosis assays may be found, *e.g.*, in Wieskopf, K. *et al* (2013) *Science* 341: 88 and Willingham, S.B. *et al.* (2012) *Proc. Natl. Acad. Sci.* 109:6662-7. In some embodiments, an antibody of the present disclosure increases phagocytosis by a macrophage

expressing a human SIRP-α polypeptide by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%, *e.g.*, using one or more of the phagocytosis assays described herein or otherwise known in the art.

[0114] In some embodiments, an antibody of the present disclosure enhances activation of dendritic cell(s) expressing a human SIRP-α polypeptide (*e.g.*, an increased level of activation of individual dendritic cells, or an increased proportion of dendritic cells that are activated within a sample population). For example, activation of dendritic cell(s) treated or contacted with an antibody of the present disclosure can be compared with activation of dendritic cell(s) not treated or contacted with the antibody, or activation of dendritic cell(s) that express a human SIRP-α polypeptide and are treated or contacted with an antibody of the present disclosure can be compared with activation of dendritic cell(s) that do not express a human SIRP-α polypeptide and are treated or contacted with the antibody. Exemplary dendritic cell activation assays are described herein. In some embodiments, an antibody of the present disclosure increases dendritic cell (*e.g.*, expressing a human SIRP-α polypeptide) activation by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%, *e.g.*, using one or more of the dendritic cell activation assays described herein or otherwise known in the art.

[0115] In some embodiments, an antibody of the present disclosure inhibits *in vivo* growth of a tumor that expresses CD47. For example, *in vivo* growth of a tumor that expresses CD47 and is treated with an antibody of the present disclosure can be compared against *in vivo* growth of a tumor that expresses CD47 and is not treated with an antibody of the present disclosure. Exemplary *in vivo* tumor growth assays are described herein. In some embodiments, an antibody of the present disclosure inhibits *in vivo* growth of a tumor that expresses CD47 by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%, *e.g.*, using one or more of the *in vivo* tumor growth assays described herein or otherwise known in the art.

[0116] In some embodiments, an antibody of the present disclosure blocks binding between an extracellular domain (e.g., the D1 domain) of a human SIRP-α polypeptide and an IgSF domain of a human CD47 polypeptide (*e.g.*, a "blocking" antibody). For example, the antibody and the CD47 polypeptide may "compete" for the same SIRP-α epitope, and/or antibody binding to SIRP-α may be mutually exclusive with CD47 binding to SIRP-α. The binding interface between SIRP-α and CD47, as well as residues of both proteins that participate in binding, are known; *see* Hatherley, D. *et al.* (2007) *J. Biol. Chem.* 282:14567-

75 and Nakaishi, A. *et al.* (2008) *J. Mol. Biol.* 375:650-60. In some embodiments, an antibody of the present disclosure blocks binding between an extracellular domain (e.g., the D1 domain) of a human SIRP-α polypeptide and an IgSF domain of a human CD47 polypeptide in an *in vitro* assay such as an ELISA or SPR assay, *e.g.*, using purified SIRP-α and/or CD47 polypeptides.

Antibody production and other antibody properties

[0117] An antibody of the present disclosure may be produced by any means known in the art. Exemplary techniques for antibody production are described below; however these exemplary techniques are provided for illustrative purposes only and are not intended to be limiting. In addition, exemplary antibody properties contemplated for use with the antibodies described herein are further described.

[0118] In some embodiments, an antibody that "binds" an antigen has a dissociation constant (K_D) for the antigen that is less than or equal to 1μM at 25°C. In some embodiments, an antibody of the present disclosure has a dissociation constant (K_D) for human v1 and/or v2 SIRP-α polypeptides that is less than or equal to 1μM at 25°C, less than or equal to 500 nM at 25°C, less than or equal to 400 nM at 25°C, less than or equal to 300 nM at 25°C, less than or equal to 250 nM at 25°C, less than or equal to 200 nM at 25°C, less than or equal to 200 nM at 25°C, less than or equal to 100 nM at 25°C, or less than or equal to 50 nM at 25°C. In some embodiments, an antibody that binds a human SIRP-α polypeptide and one or more non-human SIRP-α polypeptides binds the human SIRP-α polypeptide at a higher affinity (*e.g.*, 10-fold or 100-fold higher) than the non-human SIRP-α polypeptide, though it still considered to "bind" both polypeptides. In some embodiments, an antibody that binds a non-human SIRP-α polypeptide and one or more human SIRP-α polypeptides binds the non-human SIRP-α polypeptide at a higher affinity (*e.g.*, 10-fold or 100-fold higher) than the human SIRP-α polypeptide, though it still considered to "bind" both polypeptides. Assays for determining binding affinity are known in the art and include without limitation surface plasmon resonance (SPR), *e.g.*, as described herein; radiolabeled antigen binding assay (RIA), *e.g.*, using a Fab version of an antibody and its antigen; and the like.

[0119] To prepare an antigen, the antigen may be purified or otherwise obtained from a natural source, or it may be expressed using recombinant techniques. In some embodiments, the antigen may be used as a soluble protein. In some embodiments, the antigen may be

conjugate to another polypeptide or other moiety, *e.g.*, to increase its immunogenicity. For example, an antigen described herein may be coupled with an Fc region. In some embodiments, a cell expressing the antigen on its cell surface may be used as the antigen.

[0120] Polyclonal antibodies can be raised in an animal by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the antigen and an adjuvant. For example, descriptions of chicken immunization are described herein. In some embodiments, the antigen is conjugated with an immunogenic protein, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent. Exemplary methods for immunization of chickens are provided herein. Relevant methods suitable for a variety of other organisms, such as mammals, are well known in the art.

[0121] As described *supra*, monoclonal antibodies may be produced by a variety of methods. In some embodiments, a monoclonal antibody of the present disclosure is made using the hybridoma method first described by Kohler *et al.*, *Nature*, 256:495 (1975), and further described in Hongo *et al.*, *Hybridoma*, 14 (3): 253-260 (1995); Harlow *et al.*, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); and Hammerling *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981). Human hybridoma technology (Trioma technology) is described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005). A culture medium in which hybridoma cells are grown may be screened for the presence of an antibody of interest, *e.g.*, by *in vitro* binding assay, immunoprecipitation, ELISA, RIA, etc.; and the binding affinity may be determined, *e.g.*, by Scatchard analysis. A hybridoma that produces an antibody with desired binding properties can be subcloned and grown using known culture techniques, grown *in vivo* as ascites tumors in an animal, and the like.

[0122] In some embodiments, a monoclonal antibody is made using a library method, such as a phage display library. *See, e.g.*, Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001). In some embodiments, repertoires of VH and VL genes are cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which are then screened for antigen-binding phage, *e.g.*, as described in Winter et al., *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Alternatively, the naive repertoire can be cloned (*e.g.*, from human) to

provide a single source of antibodies to a wide range of non-self and also self-antigens without any immunization as described by Griffiths et al., *EMBO J,* 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992).

[0123] In some embodiments, an antibody of the present disclosure is a chicken antibody. Chicken antibodies can be produced using various techniques known in the art; *see, e.g.,* US Pat. Nos. 6,143,559; 8,592,644; and 9,380,769.

[0124] In some embodiments, an antibody of the present disclosure is a chimeric antibody. *See, e.g.,* U.S. Patent No. 4,816,567 and Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984). In some embodiments, a chimeric antibody comprises a non-human variable region (*e.g.*, a variable region derived from a chicken, mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In some embodiments, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

[0125] In some embodiments, a chimeric antibody is a humanized antibody. A non-human antibody can be humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, *e.g.,* CDRs, (or portions thereof) are derived from a non-human antibody (*e.g.*, a chicken antibody), and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (*e.g.*, the antibody from which the HVR residues are derived), *e.g.*, to restore or improve antibody specificity or affinity. Humanized antibodies and methods of making them are reviewed, *e.g.*, in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008). Methods of humanizing a chicken antibody have also been described, *e.g.*, in WO2005014653.

[0126] Human framework regions useful for humanization include but are not limited to: framework regions selected using the "best-fit" method; framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain

variable regions; human somatically mutated framework regions or human germline framework regions; and framework regions derived from screening FR libraries. *See, e.g.*, Sims et al. *J. Immunol.* 151:2296 (1993) ; Carter et al. *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992) ; Presta et al. *J. Immunol.*, 151:2623 (1993); Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008); and Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997).

[0127] In some embodiments, an antibody of the present disclosure is a human antibody. Human antibodies can be produced using various techniques known in the art. In some embodiments, the human antibody is produced by a non-human animal, such as the genetically engineered chickens (*see, e.g.*, US Pat. Nos. 8,592,644; and 9,380,769) and/or mice described herein. Human antibodies are described generally in Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008).

[0128] In some embodiments, an antibody of the present disclosure is generated by or derived from a chicken, *e.g.*, using the methods described herein.

[0129] In some embodiments, an antibody of the present disclosure is an antibody fragment, including without limitation a Fab, F(ab')2, Fab'-SH, Fv, or scFv fragment, or a single domain, single heavy chain, or single light chain antibody. Antibody fragments can be generated, *e.g.*, by enzymatic digestion or by recombinant techniques. In some embodiments, Proteolytic digestion of an intact antibody is used to generate an antibody fragment, *e.g.*, as described in Morimoto et al., *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992) and Brennan et al., *Science*, 229:81 (1985). In some embodiments, an antibody fragment is produced by a recombinant host cell. For example, Fab, Fv and ScFv antibody fragments are expressed by and secreted from *E. coli*. Antibody fragments can alternatively be isolated from an antibody phage library.

[0130] Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab')_2$ fragments. *See* Carter et al., *Bio/Technology* 10:163-167 (1992). $F(ab')_2$ fragments can also be isolated directly from a recombinant host cell culture. Fab and $F(ab')_2$ fragment with increased *in vivo* half-life comprising salvage receptor binding epitope residues are described in U.S. Pat. No. 5,869,046.

[0131] In some embodiments, an antibody is a single chain Fv fragment (scFv). *See* WO 93/16185 and U.S. Pat. Nos. 5,571,894 and 5,587,458. scFv fusion proteins can be constructed to produce a fusion of an effector protein at either the amino or the carboxy terminus of an scFv. The antibody fragment may also be a "linear antibody", e.g., as

described in U.S. Pat. No. 5,641,870, for example. Such linear antibodies may be monospecific or bispecific.

[0132] In some embodiments, an antibody of the present disclosure is a multispecific antibody. Multispecific antibodies possess binding specificities against more than one antigen (*e.g.*, having two, three, or more binding specificities). In some embodiments, the antibody is a bispecific antibody. In some embodiments, a bispecific antibody comprises two different binding specificities for the same antigen (*e.g.*, having different binding affinity and/or specific epitope of the same antigen). In some embodiments, a bispecific antibody comprises binding specificities for two distinct antigens. In some embodiments, the bispecific antibody is a full-length or intact antibody. In some embodiments, the bispecific antibody is an antibody fragment of the present disclosure.

[0133] Bispecific or multispecific antibodies with a variety of combinations of binding specificities are contemplated herein. In some embodiments, the bispecific antibody has a first binding specificity for one or more SIRP-α polypeptides as described herein. In some embodiments, the bispecific antibody has a second binding specificity for an antigen expressed by a cancer cell, *e.g.*, on the cell surface. Exemplary such antigens include without limitation CD19, CD20, CD22, CD30, CD33, CD38, CD52, CD56, CD70, CD74, CD79b, CD123, CD138, CS1/SLAMF7, Trop-2, 5T4, EphA4, BCMA, Mucin 1, Mucin 16, PTK7, PD-L1, STEAP1, Endothelin B Receptor, mesothelin, EGFRvIII, ENPP3, SLC44A4, GNMB, nectin 4, NaPi2b, LIV-1A, Guanylyl cyclase C, DLL3, EGFR, HER2, VEGF, VEGFR, integrin αVβ3, integrin α5β1, MET, IGF1R, TRAILR1, TRAILR2, RANKL, FAP, Tenascin, Le^y, EpCAM, CEA, gpA33, PSMA, TAG72, a mucin, CAIX, EPHA3, folate receptor α, GD2, GD3, and an MHC/peptide complex comprising a peptide from NY-ESO-1/LAGE, SSX-2, a MAGE family protein, MAGE-A3, gp100/pmel17, Melan-A/MART-1, gp75/TRP1, tyrosinase, TRP2, CEA, PSA, TAG-72, immature laminin receptor, MOK/RAGE-1, WT-1, SAP-1, BING-4, EpCAM, MUC1, PRAME, survivin, BRCA1, BRCA2, CDK4, CML66, MART-2, p53, Ras, β-catenin, TGF-βRII, HPV E6, or HPV E7. Without wishing to be bound to theory, it is thought that combining such a binding specificity with a binding specificity against a SIRP-α is particularly advantageous, *e.g.*, to direct FcR-expressing leukocytes to target a tumor cell with the second binding specificity while also inhibiting the responsiveness of SIRP-α expressed by the leukocyte to any CD47 expressed by the tumor cell with the first binding specificity.

[0134] In some embodiments, the bispecific antibody has a second binding specificity for an antigen expressed by an immune cell, *e.g.*, on the cell surface. Exemplary such antigens include without limitation BDCA2, BDCA4, ILT7, LILRB1, LILRB2, LILRB3, LILRB4, CSF-1R, CD40, CD40L, CD163, CD206, DEC205, CD47, CD123, IDO, TDO, 41BB, CTLA4, PD1, PD-L1, PD-L2, TIM-3, BTLA, VISTA, LAG-3, CD28, OX40, GITR, CD137, CD27, HVEM, CCR4, CD25, CD103, KIrg1, Nrp1, CD278, Gpr83, TIGIT, CD154, CD160, PVRIG, DNAM, and ICOS. In some embodiments, the antigen is expressed on a myeloid cell. Such antigens can include without limitation BDCA2, BDCA4, ILT7, LILRB1, LILRB2, LILRB3, LILRB4, CSF-1R, CD40, CD40L, CD163, CD206, DEC205, CD47, CD123, IDO, and TDO. In some embodiments, the antigen is expressed on a T cell. Such antigens can include without limitation 41BB, CTLA4, PD1, PD-L1, PD-L2, TIM-3, BTLA, VISTA, LAG-3, CD28, OX40, GITR, CD137, CD27, HVEM, CCR4, CD25, CD103, KIrg1, Nrp1, CD278, Gpr83, TIGIT, CD154, CD160, TNFR2, PVRIG, DNAM, and ICOS.

[0135] In some embodiments, the bispecific antibody has a second binding specificity for an antigen expressed by an NK cell, *e.g.*, on the cell surface. Exemplary such antigens include without limitation NKR-P1A (KLRB1), CD94 (NKG2A), KLRG1, KIR2DL5A, KIR2DL5B, KIR2DL1, KIR2DL2, KIR2DL3, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DS1, KIR2DS1, CD94 (NKG2C/E), NKG2D, CD160 (BY55), CD16 (FcγRIIIA), NKp46 (NCR1), NKp30 (NCR3), NKp44 (NCR2), DNAM1 (CD226), CRTAM, CD27, NTB-A (SLAMF6), PSGL1, CD96 (Tactile), CD100 (SEMA4D), NKp80 (KLRF1, CLEC5C), SLAMF7 (CRACC, CS1, CD319), and CD244 (2B4, SLAMF4).

[0136] Various methods are known in the art for generating and purifying a bispecific antibody. Numerous approaches have been described. One approach is the "knobs-into-holes" or "protuberance-into-cavity" approach (see, *e.g.*, US Pat. No. 5,731,168). In some embodiments, heterodimerization of Fc domain monomers is promoted by introducing different, but compatible, substitutions in the two Fc domain monomers, such as "knob-into-hole" residue pairs and charge residue pairs. The knob and hole interaction favors heterodimer formation, whereas the knob-knob and the hole-hole interaction hinder homodimer formation due to steric clash and deletion of favorable interactions. A hole refers to a void that is created when an original amino acid in a protein is replaced with a different amino acid having a smaller side-chain volume. A knob refers to a bump that is created when an original amino acid in a protein is replaced with a different amino acid having a larger side-chain volume. For example, in some embodiments, an amino acid being replaced is in

the CH3 antibody constant domain of an Fc domain monomer and involved in the dimerization of two Fc domain monomers. In some embodiments, a hole in one CH3 antibody constant domain is created to accommodate a knob in another CH3 antibody constant domain, such that the knob and hole amino acids act to promote or favor the heterodimerization of the two Fc domain monomers. In some embodiments, a hole in one CH3 antibody constant domain is created to better accommodate an original amino acid in another CH3 antibody constant domain. In some embodiments, a knob in one CH3 antibody constant domain is created to form additional interactions with original amino acids in another CH3 antibody constant domain.

[0137] In some embodiments, a hole is constructed by replacing amino acids having larger side chains such as tyrosine or tryptophan with amino acids having smaller side chains such as alanine, valine, or threonine, for example a Y407V mutation in the CH3 antibody constant domain. Similarly, in some embodiments, a knob is constructed by replacing amino acids having smaller side chains with amino acids having larger side chains, for example a T366W mutation in the CH3 antibody constant domain. In some embodiments, one Fc domain monomer includes the knob mutation T366W and the other Fc domain monomer includes hole mutations T366S, L358A, and Y407V. In some embodiments, a polypeptide of the disclosure including a high affinity SIRP-α D1 variant is fused to an Fc domain monomer including the knob mutation T366W to limit unwanted knob-knob homodimer formation. Examples of knob-into-hole amino acid pairs are included, without limitation, in Table 3.

Table 3. Knob-into-hole amino acid pairs.

Fc domain monomer 1	Y407T	Y407A	F405A	T394S	T366S L358A Y407V	T394W Y407T	T394S Y407A	T366W T394S
Fc domain monomer 2	T366Y	T366W	T394W	F405W	T366W	T366Y F405A	T366W F405W	F405W Y407A

[0138] Another approach uses antibody variable domains with the desired binding specificities (antibody-antigen combining sites) fused to immunoglobulin constant domain sequences, *e.g.*, with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. In some embodiments, the bispecific antibody has a hybrid immunoglobulin heavy chain with a first binding specificity in one arm and a hybrid

immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. *See* WO 94/04690. Another approach uses cross-linking (*see, e.g.,* US Pat No. 4,676,980) to produce a heterconjugate antibody. In some embodiments, bispecific antibodies can be prepared using chemical linkage (*see, e.g.,* Brennan et al., *Science*, 229: 81 (1985)) to proteolytically cleave an intact antibody into $F(ab')_2$ fragments that are reduced in the presence of a dithiol complexing agent and converted to thionitrobenzoate (TNB) derivatives, one of which is reconverted to the Fab'-thiol by reduction and mixed with the other Fab'-TNB derivative to form the bispecific antibody. In some embodiments, Fab'-SH fragments are chemically coupled. In some embodiments, bispecific antibody fragments are produced in cell culture using leucine zippers, as in Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992). For other bispecific antibody formats, see, *e.g.*, Spiess, C. *et al.* (2015) *Mol. Immunol.* 67:95-106.

[0139] In some embodiments, an antibody of the present disclosure is a diabody. *See, e.g.*, Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993). In a diabody, the V_H and V_L domains of one fragment pair with complementary V_L and V_H domains of another fragment, thus forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. *See* Gruber et al, *J. Immunol*, 152:5368 (1994).

[0140] In some embodiments, an antibody of the present disclosure is a single-domain antibody. A single-domain antibody refers to a single polypeptide chain comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (*see, e.g.*, U.S. Pat. No. 6,248,516 B1). In one embodiment, a single-domain antibody includes all or a portion of the heavy chain variable domain of an antibody. Camelid antibodies are also known.

[0141] Antibodies can be produced using recombinant methods. For recombinant production of an anti-antigen antibody, nucleic acid encoding the antibody is isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the antibody may be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Many vectors are available. The vector components generally include, but are not limited to, one or more of the

following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

[0142] An antibody of the present disclosure can be produced recombinantly as a fusion polypeptide with a heterologous polypeptide, *e.g.*, a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. The heterologous signal sequence selected can be one that is recognized and processed (*e.g.*, cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process a native antibody signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the native signal sequence may be substituted by, *e.g.*, the yeast invertase leader, a factor leader (including *Saccharomyces* and *Kluyveromyces* α-factor leaders), or acid phosphatase leader, the *C. albicans* glucoamylase leader, etc.. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the herpes simplex gD signal, are available.

[0143] Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells, *e.g.*, to allow the vector to replicate independently of the host chromosomal DNA. This sequence can include origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may be used because it contains the early promoter).

[0144] Expression and cloning vectors can contain a selection gene or selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media. Examples of dominant selection use the drugs neomycin, mycophenolic acid and hygromycin. Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up antibody-encoding nucleic acid, such as DHFR, glutamine synthetase (GS), thymidine kinase, metallothionein-I and -II, preferably primate metallothionein genes, adenosine deaminase, ornithine decarboxylase, and the like. For example, a Chinese hamster ovary (CHO) cell line deficient in endogenous DHFR activity transformed with the DHFR gene is identified by culturing the transformants in a culture medium containing methotrexate (Mtx), a competitive antagonist of DHFR.

[0145] Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding an antibody of interest, wild-type DHFR gene, and another selectable marker such as aminoglycoside 3'-phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, *e.g.*, kanamycin, neomycin, or G418.

[0146] Expression and cloning vectors generally contain a promoter that is recognized by the host organism and is operably linked to nucleic acid encoding an antibody. Promoters suitable for use with prokaryotic hosts include the *pho*A promoter, β-lactamase and lactose promoter systems, alkaline phosphatase promoter, a tryptophan (trp) promoter system, and hybrid promoters such as the tac promoter. However, other known bacterial promoters are suitable. Promoter sequences are known for eukaryotes. Yeast promoters are well known in the art and can include inducible promoters/enhancers regulated by growth conditions. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Examples include without limitation the promoters for 3-phosphoglycerate kinase or other glycolytic enzymes, such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Antibody transcription from vectors in mammalian host cells can be controlled, for example, by promoters obtained from the genomes of viruses. The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. Alternatively, the Rous Sarcoma Virus long terminal repeat can be used as the promoter.

[0147] Transcription of a DNA encoding an antibody of the present disclosure by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus.

[0148] Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA.

[0149] Suitable host cells for cloning or expressing the DNA in the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes for this purpose include eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *Escherichia*, *e.g.*, *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, *e.g.*, *Salmonella typhimurium*, *Serratia*, *e.g.*, *Serratia marcescans*, and *Shigella*, etc. In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. Certain fungi and yeast strains may be selected in which glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. *See*, *e.g.*, Li et al., *Nat. Biotech.* 24:210-215 (2006).

[0150] Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, duckweed (*Leninaceae*), alfalfa (*M. truncatula*), and tobacco can also be utilized as hosts.

[0151] Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* have been identified.

[0152] Vertebrate cells may be used as hosts, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); mouse sertoli cells (TM4, Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, *Annals N.Y. Acad. Sci.* 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2). Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR$^-$ CHO cells (Urlaub *et al.*, *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell

lines such as NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki and Wu, *Methods in Molecular Biology,* Vol. 248 (B. K. C. Lo, ed., Humana Press, Totowa, N.J., 2003), pp. 255-268.

[0153] The host cells of the present disclosure may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham *et al.*, *Meth. Enz.* 58:44 (1979), Barnes *et al.*, *Anal. Biochem.* 102:255 (1980), U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Pat. Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCIN™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to one of skill in the art.

[0154] When using recombinant techniques, the antibody can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, are removed, for example, by centrifugation or ultrafiltration. Carter *et al.*, *Bio/Technology* 10:163-167 (1992) describe a procedure for isolating antibodies which are secreted to the periplasmic space of *E. coli.*

[0155] The antibody composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, hydrophobic interaction chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being among one of the typically preferred purification steps.

[0156] In some embodiments, an antibody of the present disclosure comprises a light chain comprising a light chain constant (CL) domain sequence that comprises an amino acid sequence according to the formula

GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTK

PSKQSX$_4$X$_5$KYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO:72), where X$_4$X$_5$ is ND, DN, DS, or SD. In certain embodiments, the CL domain comprises an amino acid sequence selected from SEQ ID NOs:43-46.

[0157] In some embodiments, an antibody of the present disclosure comprises a light chain variable (VL) domain comprising one, two, three, or four IGLV3 framework sequences, including without limitation SYELTQPPSVSVSPGQTARITC (SEQ ID NO:27), WYQQKPGQAPVTLIY (SEQ ID NO:28), NIPERFSGSSSGTTVTLTISGVQAEDEADYYC (SEQ ID NO:29), and FGGGTKLTVL (SEQ ID NO:30). In some embodiments, an antibody of the present disclosure comprises a light chain variable (VL) domain comprising the structure FW1—HVR-L1—FW2—HVR-L2—FW3—HVR-L3—FW4, wherein FW1 comprises the sequence SYELTQPPSVSVSPGQTARITC (SEQ ID NO:27), FW2 comprises the sequence WYQQKPGQAPVTLIY (SEQ ID NO:28), FW3 comprises the sequence NIPERFSGSSSGTTVTLTISGVQAEDEADYYC (SEQ ID NO:29), and FW4 comprises the sequence FGGGTKLTVL (SEQ ID NO:30).

[0158] In some embodiments, an antibody of the present disclosure comprises a light chain comprising a kappa or lambda light chain constant (CL) domain. In some embodiments, an antibody of the present disclosure comprises a light chain comprising a light chain constant domain comprising the amino acid sequence of one of SEQ ID NOs:36-38. In some embodiments, an antibody of the present disclosure comprises a light chain comprising a light chain constant domain comprising the amino acid sequence of one of SEQ ID NOs:43-46. In some embodiments, an antibody of the present disclosure comprises a light chain comprising a light chain constant domain sequence shown in Table 1. In some embodiments, an antibody of the present disclosure comprises a light chain that comprises a VL domain comprising the sequence of SEQ ID NO:25 and a CL domain sequence selected from SEQ ID NOs:36-38 and 43-46. In some embodiments, an antibody of the present disclosure comprises a light chain that comprises a VL domain comprising the sequence of SEQ ID NO:25 and a CL domain sequence selected from SEQ ID NOs:43-46. In some embodiments, an antibody of the present disclosure comprises a light chain that comprises an amino acid sequence selected from SEQ ID NOs:48-51. In some embodiments, an antibody of the present disclosure comprises a light chain that comprises a VL domain sequence selected from SEQ ID NOs:39-41 and a CL domain comprising a sequence selected from SEQ ID

NOs:36-38. Any of the above light chains can be combined with a heavy chain shown in Table 2 or an antibody heavy chain comprising a VH domain shown in Table 2.

[0159] In some embodiments, an antibody of the present disclosure includes a heavy chain comprising a heavy chain constant domain that comprises an Fc region. For example, in some embodiments, the Fc region is a human Fc region, *e.g.*, IgG1, IgG2, or IgG4 and subtypes thereof. Exemplary and non-limiting Fc regions are provided within the heavy chain constant domains comprising the amino acid sequences of SEQ ID NOs:31-35 and 132-139, as shown in Table 4. In some embodiments, an Fc region within one or more of the heavy chain constant domain amino acid sequences of SEQ ID NOs:31-35 and 132-139 comprises one or more of the mutations described herein, *e.g., infra*. In some embodiments, an antibody of the present disclosure comprises a heavy chain that comprises a heavy chain chain constant domain sequence shown in Table 2.

Table 4. Exemplary constant region sequences

Name	SEQ ID NO	Sequence
IgG1 wildtype	31	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
IgG1_AAA_N297A	32	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGAPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
IgG2	33	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVD HKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKP REEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIE KTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPG
IgG2Da	34	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVD HKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKP REEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPSSIE

		KTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPG
IgG4_S228P	35	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVD HKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKP REEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ EGNVFSCSVMHEALHNHYTQKSLSLSLG
Human Kappa	36	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
Human Lambda IGLC1	37	GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWK ADGSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSY SCQVTHEGSTVEKTVAPTECS
Human Lambda IGLC2	38	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWK ADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSY SCQVTHEGSTVEKTVAPTECS
IgG1_AAA	132	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAA GAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPG
IgG2 C232S	133	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQ TYTCNVDHKPSNTKVDKTVERKSCVECPPCPAPPVAGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLN GKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPG
IgG2 C233S	134	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQ TYTCNVDHKPSNTKVDKTVERKCSVECPPCPAPPVAGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLN GKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPG
IgG2Da C232S	135	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQ TYTCNVDHKPSNTKVDKTVERKSCVECPPCPAPPVAGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWY

		VDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLN GKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPG
IgG2Da C233S	136	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQ TYTCNVDHKPSNTKVDKTVERKCSVECPPCPAPPVAGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLN GKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPG
IgG2 N297A	137	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQ TYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWY VDGVEVHNAKTKPREEQFASTFRVVSVLTVVHQDWLN GKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPG
IgG2 N297A C232S	138	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQ TYTCNVDHKPSNTKVDKTVERKSCVECPPCPAPPVAGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWY VDGVEVHNAKTKPREEQFASTFRVVSVLTVVHQDWLN GKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPG
IgG2 N297A C233S	139	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQ TYTCNVDHKPSNTKVDKTVERKCSVECPPCPAPPVAGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWY VDGVEVHNAKTKPREEQFASTFRVVSVLTVVHQDWLN GKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPG

[0160] In some embodiments, the Fc region includes one or more mutations that influence one or more antibody properties, such as stability, pattern of glycosylation or other modifications, effector cell function, pharmacokinetics, and so forth. In some embodiments, an antibody of the present disclosure has reduced or minimal glycosylation. In some embodiments, an antibody of the present disclosure has ablated or reduced effector function.

In some embodiments, an antibody of the present disclosure has improved stability (*e.g.*, improved stability of the hinge domain and/or reduced monomer exchange of IgG4 antibodies through use of the S228P mutation).

[0161] Exemplary Fc mutations (*e.g.*, that influence one or more of the properties described *supra*) include without limitation (i) a human IgG1 Fc region mutations L234A, L235A, G237A, and optionally N297A; (ii) a human IgG2 Fc region mutations A330S, P331S and optionally N297A; and (iii) a human IgG4 Fc region mutations S228P and optionally E233P, F234V, L235A, delG236, and N297A (EU numbering). In some embodiments, the human IgG1 Fc region comprises L234A, L235A, and G237A mutations. In some embodiments, the human IgG1 Fc region comprises L234A, L235A, G237A, and N297A mutations. In some embodiments, the human IgG2 Fc region comprises A330S and P331S mutations. In some embodiments, the human IgG2 Fc region comprises A330S, P331S, and N297A mutations. In some embodiments, the human IgG4 Fc region comprises an S288P mutation. In some embodiments, the human IgG4 Fc region comprises S288P and L235E mutations.

[0162] Antibodies that target cell surface antigens can trigger immunostimulatory and effector functions that are associated with Fc receptor (FcR) engagement on immune cells. There are a number of Fc receptors that are specific for particular classes of antibodies, including IgG (gamma receptors), IgE (eta receptors), IgA (alpha receptors) and IgM (mu receptors). Binding of the Fc region to Fc receptors on cell surfaces can trigger a number of biological responses including phagocytosis of antibody-coated particles (antibody-dependent cell-mediated phagocytosis, or ADCP), clearance of immune complexes, lysis of antibody-coated cells by killer cells (antibody-dependent cell-mediated cytotoxicity, or ADCC) and, release of inflammatory mediators, placental transfer, and control of immunoglobulin production. Additionally, binding of the C1 component of complement to antibodies can activate the complement system. Activation of complement can be important for the lysis of cellular pathogens. However, the activation of complement can also stimulate the inflammatory response and can also be involved in autoimmune hypersensitivity or other immunological disorders. Variant Fc regions with reduced or ablated ability to bind certain Fc receptors are useful for developing therapeutic antibodies and Fc-fusion polypeptide constructs which act by targeting, activating, or neutralizing ligand functions while not damaging or destroying local cells or tissues.

[0163] In some embodiments, a Fc domain monomer refers to a polypeptide chain that includes second and third antibody constant domains (e.g., CH2 and CH3). In some embodiments, an Fc domain monomer also includes a hinge domain. In some embodiments, the Fc domain monomer is of any immunoglobulin antibody isotype, including IgG, IgE, IgM, IgA, and IgD. Additionally, in some embodiments, an Fc domain monomer is of any IgG subtype (e.g., IgG1, IgG2, IgG2a, IgG2b, IgG2c, IgG3, and IgG4). In some embodiments, Fc domain monomers include as many as ten changes from a wild-type Fc domain monomer sequence (e.g., 1-10, 1-8, 1-6, 1-4 amino acid substitutions, additions or insertions, deletions, or combinations thereof) that alter the interaction between an Fc domain and an Fc receptor.

[0164] In some embodiments, an Fc domain monomer of an immunoglobulin or a fragment of an Fc domain monomer is capable of forming an Fc domain with another Fc domain monomer. In some embodiments, an Fc domain monomer of an immunoglobulin or a fragment of an Fc domain monomer is not capable of forming an Fc domain with another Fc domain monomer. In some embodiments, an Fc domain monomer or a fragment of an Fc domain is fused to a polypeptide of the disclosure to increase serum half-life of the polypeptide. In some embodiments, an Fc domain monomer or a fragment of an Fc domain monomer fused to a polypeptide of the disclosure dimerizes with a second Fc domain monomer to form an Fc domain which binds an Fc receptor, or alternatively, an Fc domain monomer binds to an Fc receptor. In some embodiments, an Fc domain or a fragment of the Fc domain fused to a polypeptide to increase serum half-life of the polypeptide does not induce any immune system-related response. An Fc domain includes two Fc domain monomers that are dimerized by the interaction between the CH3 antibody constant domains.

[0165] A wild-type Fc domain forms the minimum structure that binds to an Fc receptor, e.g., FcγRI, FcγRIIa, FcγRIIb, FcγRIIIa, FcγRIIIb, and FcγRIV. In some embodiments, the Fc domain in an antibody of the present disclosure comprises one or more amino acid substitutions, additions or insertions, deletions, or any combinations thereof that lead to decreased effector function such as decreased antibody-dependent cell-mediated cytotoxicity (ADCC), decreased complement-dependent cytolysis (CDC), decreased antibody-dependent cell-mediated phagocytosis (ADCP), or any combinations thereof. For example, an antibody of the present disclosure can exhibit decreased binding (e.g., minimal binding or absence of binding) to a human Fc receptor and decreased binding (e.g., minimal binding or absence of binding) to complement protein C1q; decreased binding (e.g., minimal binding or absence of

binding) to human FcγRI, FcγRIIA, FcγRIIB, FcγRIIIB, FcγRIIIB, or any combinations thereof, and C1q; altered or reduced antibody-dependent effector function, such as ADCC, CDC, ADCP, or any combinations thereof; and so forth. Exemplary mutations include without limitation one or more amino acid substitutions at E233, L234, L235, G236, G237, D265, D270, N297, E318, K320, K322, A327, A330, P331, or P329 (numbering according to the EU index of Kabat (Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)).

[0166] In some embodiments, an antibody of the present disclosure has reduced or ablated binding to CD16a, CD32a, CD32b, CD32c, and CD64 Fcγ receptors. In some embodiments, an antibody with a non-native Fc region described herein exhibits at least a 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater reduction in C1q binding compared to an antibody comprising a wild-type Fc region. In some embodiments, an antibody with a non-native Fc region as described herein exhibit at least a 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater reduction in CDC compared to an antibody comprising a wild-type Fc region.

[0167] In some embodiments, the Fc variants herein are minimally glycosylated or have reduced glycosylation relative to a wild-type sequence. In some embodiments, deglycosylation is accomplished with a mutation of N297A, or by mutating N297 to any amino acid which is not N.

[0168] In some embodiments, variants of antibody IgG constant regions (e.g., Fc variants) possess a reduced capacity to specifically bind Fcγ receptors or have a reduced capacity to induce phagocytosis. In some embodiments, variants of antibody IgG constant regions (e.g., Fc variants) possess a reduced capacity to specifically bind Fcγ receptors and have a reduced capacity to induce phagocytosis. For example, in some embodiments, an Fc domain is mutated to lack effector functions, typical of a "dead" Fc domain. For example, in some embodiments, an Fc domain includes specific amino acid substitutions that are known to minimize the interaction between the Fc domain and an Fcγ receptor. In some embodiments, an Fc domain monomer is from an IgG1 antibody and includes one or more of amino acid substitutions L234A, L235A, G237A, and N297A (amino acid position numbering as designated according to the EU numbering system per Kabat et al., 1991). In some embodiments, one or more additional mutations are included in such IgG1 Fc variant. Non-limiting examples of such additional mutations for human IgG1 Fc variants include E318A and K322A. In some instances, a human IgG1 Fc variant has up to 12, 11, 10, 9, 8, 7,

6, 5 or 4 or fewer mutations in total as compared to wild-type human IgG1 sequence. In some embodiments, one or more additional deletions are included in such IgG1 Fc variant. For example, in some embodiments, the C-terminal lysine of the Fc IgG1 heavy chain constant region is deleted, for example to increase the homogeneity of the polypeptide when the polypeptide is produced in bacterial or mammalian cells. In some instances, a human IgG1 Fc variant has up to 12, 11, 10, 9, 8, 7, 6, 5 or 4 or fewer deletions in total as compared to wild-type human IgG1 sequence.

[0169] In some embodiments, an Fc domain monomer is from an IgG2 antibody and includes amino acid substitutions A330S, P331S, or both A330S and P331S. The aforementioned amino acid positions are defined according to Kabat, et al. (1991). The Kabat numbering of amino acid residues can be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence. In some embodiments, the Fc variant comprises a human IgG2 Fc sequence comprising one or more of A330S, P331S and N297A amino acid substitutions (amino acid position numbering as designated according to the EU numbering system per Kabat, et al. (1991). In some embodiments, one or more additional mutations are included in such IgG2 Fc variants. Non-limiting examples of such additional mutations for human IgG2 Fc variant include V234A, G237A, P238S, V309L and H268A (as designated according to the EU numbering system per Kabat et al. (1991)). In some instances, a human IgG2 Fc variant has up to 12, 11, 10, 9, 8, 7, 6, 5, 4, 3 or fewer mutations in total as compared to wild-type human IgG2 sequence. In some embodiments, one or more additional deletions are included in such IgG2 Fc variant.

[0170] When the Fc variant is an IgG4 Fc variant, in some embodiments, such Fc variant comprises a S228P, E233P, F234V, L235A, L235E, or delG236 mutation (amino acid position numbering as designated according to Kabat, et al. (1991)). In some instances, a human IgG4 Fc variant has up to 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 mutation(s) in total as compared to wild-type human IgG4 sequence.

[0171] In some embodiments, the Fc variant exhibits reduced binding to an Fc receptor of the subject compared to the wild-type human IgG Fc region. In some embodiments, the Fc variant exhibits ablated binding to an Fc receptor of the subject compared to the wild-type human IgG Fc region. In some embodiments, the Fc variant exhibits a reduction of phagocytosis compared to the wild-type human IgG Fc region. In some embodiments, the Fc variant exhibits ablated phagocytosis compared to the wild-type human IgG Fc region.

[0172] Antibody-dependent cell-mediated cytotoxicity, which is also referred to herein as ADCC, refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (e.g., Natural Killer (NK) cells and neutrophils) enabling these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell. Antibody-dependent cell-mediated phagocytosis, which is also referred to herein as ADCP, refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain phagocytic cells (e.g., macrophages) enabling these phagocytic effector cells to bind specifically to an antigen-bearing target cell and subsequently engulf and digest the target cell. Ligand-specific high-affinity IgG antibodies directed to the surface of target cells can stimulate the cytotoxic or phagocytic cells and can be used for such killing. In some embodiments, polypeptide constructs comprising an Fc variant as described herein exhibit reduced ADCC or ADCP as compared to a polypeptide construct comprising a wild-type Fc region. In some embodiments, polypeptide constructs comprising an Fc variant as described herein exhibit at least a 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater reduction in ADCC or ADCP compared to a polypeptide construct comprising a wild-type Fc region. In some embodiments, antibodies comprising an Fc variant as described herein exhibit ablated ADCC or ADCP as compared to a polypeptide construct comprising a wild-type Fc region.

[0173] Complement-directed cytotoxicity, which is also referred to herein as CDC, refers to a form of cytotoxicity in which the complement cascade is activated by the complement component C1q binding to antibody Fc. In some embodiments, polypeptide constructs comprising an Fc variant as described herein exhibit at least a 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater reduction in C1q binding compared to a polypeptide construct comprising a wild-type Fc region. In some cases, polypeptide constructs comprising an Fc variant as described herein exhibit reduced CDC as compared to a polypeptide construct comprising a wild-type Fc region. In some embodiments, polypeptide constructs comprising an Fc variant as described herein exhibit at least a 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater reduction in CDC compared to a polypeptide construct comprising a wild-type Fc region. In some cases, antibodies comprising an Fc variant as described herein exhibit negligible CDC as compared to a polypeptide construct comprising a wild-type Fc region.

[0174] Fc variants herein include those that exhibit reduced binding to an Fcγ receptor compared to the wild-type human IgG Fc region. For example, in some embodiments, an Fc

variant exhibits binding to an Fcγ receptor that is less than the binding exhibited by a wild-type human IgG Fc region to an Fcγ receptor. In some instances, an Fc variant has reduced binding to an Fcγ receptor by a factor of 10%, 20% 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (fully ablated effector function). In some embodiments, the reduced binding is for any one or more Fcγ receptors, e.g., CD16a, CD32a, CD32b, CD32c, or CD64.

[0175] In some instances, the Fc variants disclosed herein exhibit a reduction of phagocytosis compared to its wild-type human IgG Fc region. Such Fc variants exhibit a reduction in phagocytosis compared to its wild-type human IgG Fc region, wherein the reduction of phagocytosis activity is e.g., by a factor of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100%. In some instances, an Fc variant exhibits ablated phagocytosis compared to its wild-type human IgG Fc region.

[0176] In some embodiments, an antibody of the present disclosure is conjugated to an agent. In some embodiments, the agent is a cytotoxic agent, including but not limited to the exemplary cytotoxic agents described herein. In some embodiments, the agent is a label, including but not limited to the exemplary labels described herein.

[0177] In some embodiments, the agent is a moiety that modulates the immune system. For example, the moiety may target and/or modulate the function of a cell expressing SIRP-α on its surface, such as a small molecule that modulates a cellular signaling pathway of the cell expressing SIRP-α, *e.g.*, an IDO/TDO inhibitor, AhR inhibitor, arginase inhibitor, A2a R inhibitor, TLR agonists, STING agonist, or Rig-1 agonist. In some embodiments, the moiety may recruit another macromolecule or cell into proximity with a cell expressing SIRP-α on its surface. In some embodiments, the moiety comprises a cytokine, *e.g.*, IL2, IL7, IL-10, IL15, or IFN. In some embodiments, the moiety (*e.g.*, a small molecule) modulates the activity of a cytokine, *e.g.*, IL2, IL7, IL-10, IL15, or IFN. In some embodiments, the moiety comprises a cancer vaccine (comprising, *e.g.*, DNA, RNA, peptide, or other cellular component(s)). In some embodiments, the moiety comprises an adjuvant. In some embodiments, the moiety comprises a CpG oligonucleotide. In some embodiments, the moiety affects antibody purification, screening, and/or display. In some embodiments, the moiety also affects the degree of binding to Fc receptors or the degree of phagocytosis reduction.

[0178] In some embodiments, fusion partners are linked to the Fc variant sequence via a linker sequence. In some embodiments, the linker sequence generally comprises a small

number of amino acids, such as less than ten amino acids, although longer linkers are also utilized. In some cases, the linker has a length less than 10, 9, 8, 7, 6, or 5 amino acids or shorter. In some cases, the linker has a length of at least 10, 11, 12, 13, 14, 15, 20, 25, 30, or 35 amino acids or longer. Optionally, in some embodiments, a cleavable linker is employed.

[0179] In some embodiments, a fusion partner is a targeting or signal sequence that directs an Fc variant protein and any associated fusion partners to a desired cellular location or to the extracellular media. In some embodiments, certain signaling sequences target a protein to be either secreted into the growth media, or into the periplasmic space, located between the inner and outer membrane of the cell. In some embodiments, a fusion partner is a sequence that encodes a peptide or protein that enables purification or screening. Such fusion partners include, but are not limited to, polyhistidine tags (His-tags) (for example His6 and His10) or other tags for use with Immobilized Metal Affinity Chromatography (IMAC) systems (e.g., Ni+2 affinity columns), GST fusions, MBP fusions, Strep-tag, the BSP biotinylation target sequence of the bacterial enzyme BirA, and epitope tags which are targeted by antibodies (for example c-myc tags, flag-tags, and the like).

[0180] In some embodiments, such tags are useful for purification, for screening, or both. For example, in some embodiments, an Fc variant is purified using a His-tag by immobilizing it to a Ni+2 affinity column, and then after purification the same His-tag is used to immobilize the antibody to a Ni+2 coated plate to perform an ELISA or other binding assay.

[0181] Various fusion partners that enable a variety of selection methods are available. For example, by fusing the members of an Fc variant library to the gene III protein, phage display can be employed. In some embodiments, fusion partners enable Fc variants to be labeled. Alternatively, in some embodiments, a fusion partner binds to a specific sequence on the expression vector, enabling the fusion partner and associated Fc variant to be linked covalently or noncovalently with the nucleic acid that encodes them.

[0182] In some embodiments, when a fusion partner is a therapeutic moiety, the therapeutic moiety is, e.g., a cytotoxic agent, a peptide, a protein, an antibody, a siRNA, or a small molecule.

[0183] In some embodiments, an antibody of the present disclosure is bound to various carriers or labels and used to detect the presence of specific antigen expressing cells. Examples of carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble or insoluble. Various different labels and methods

of labeling are known. Examples of labels include enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, and bio-luminescent compounds. Various techniques for binding labels to antibodies disclosed herein are available. In some embodiments, the antibodies are coupled to low molecular weight haptens. These haptens are then specifically detected by means of a second reaction. For example, in some embodiments, the hapten biotin is used with avidin or the haptens dinitrophenol, pyridoxal, or fluorescein are detected with specific anti-hapten antibodies (e.g., anti-dinitrophenol antibodies, anti-pyridoxal antibodies, and anti-fluorescein antibodies respectively). In some embodiments, the antibodies described herein are utilized *in vitro* for binding assays, such as immune assays. For example, in some embodiments, the antibodies are utilized in liquid phase or bound to a solid phase carrier. In some embodiments, antibodies utilized for immunoassays are detectably labeled in various ways.

Methods of Treatment

[0184] Certain aspects of the present disclosure relate to treating a disease or disorder using an antibody described herein. In some embodiments, the disease is cancer. In some embodiments, the disease is an autoimmune or inflammatory disease.

[0185] For example, provided herein are methods of treating or delaying progression of cancer in an individual by administering an effective amount of an antibody of the present disclosure. Without wishing to be bound to theory, it is thought that the antibodies described herein may be useful in the treatment of cancer, *e.g.*, by abrogating the cancer's ability to inhibit phagocytosis and immune surveillance through the CD47: SIRP-α signaling axis, or by otherwise enhancing activation of the immune system (such as by activation of dendritic cells).

[0186] In some embodiments, an antibody of the present disclosure is administered in combination with a second antibody, *e.g.*, an antibody that binds an antigen expressed by the cancer (*e.g.*, an effective amount of the second antibody, which in some embodiments as described above may be considered in the context of administering an anti-SIRP-α antibody of the present disclosure). Exemplary antigens expressed by cancers are known in the art and include without limitation CD19, CD20, CD22, CD30, CD33, CD38, CD52, CD56, CD70, CD74, CD79b, CD123, CD138, CS1/SLAMF7, Trop-2, 5T4, EphA4, BCMA, Mucin 1, Mucin 16, PTK7, PD-L1, STEAP1, Endothelin B Receptor, mesothelin, EGFRvIII, ENPP3, SLC44A4, GNMB, nectin 4, NaPi2b, LIV-1A, Guanylyl cyclase C, DLL3, EGFR, HER2,

VEGF, VEGFR, integrin αVβ3, integrin α5β1, MET, IGF1R, TRAILR1, TRAILR2, RANKL, FAP, Tenascin, Le^y, EpCAM, CEA, gpA33, PSMA, TAG72, a mucin, CAIX, EPHA3, folate receptor α, GD2, GD3, and an MHC/peptide complex comprising a peptide from NY-ESO-1/LAGE, SSX-2, a MAGE family protein, MAGE-A3, gp100/pmel17, Melan-A/MART-1, gp75/TRP1, tyrosinase, TRP2, CEA, PSA, TAG-72, immature laminin receptor, MOK/RAGE-1, WT-1, SAP-1, BING-4, EpCAM, MUC1, PRAME, survivin, BRCA1, BRCA2, CDK4, CML66, MART-2, p53, Ras, β-catenin, TGF-βRII, HPV E6, or HPV E7. For example, in some embodiments, an antibody of the present disclosure is administered in combination with a monoclonal antibody that binds CD123 (also known as IL-3 receptor alpha), such as talacotuzumab (also known as CSL362 and JNJ-56022473). In some embodiments, an antibody of the present disclosure is administered in combination with a monoclonal antibody that binds EGFR (such as cetuximab). In some embodiments, the second antibody includes one or more effector functions, *e.g.*, effector functions that are associated with Fc receptor (FcR) engagement on immune cells including without limitation ADCC or ADCP, and/or complement-dependent cytotoxicity (CDC). Without wishing to be bound to theory, it is thought that combining such an antibody with an antibody of the present disclosure is particularly advantageous, *e.g.*, to direct FcR-expressing leukocytes to target a tumor cell to which the second antibody is bound while also inhibiting the responsiveness of SIRP-α expressed by the leukocyte to any CD47 expressed by the tumor cell with the SIRP-α antibody.

[0187] In some embodiments, an antibody of the present disclosure is administered in combination with a second antibody that binds an antigen expressed by an NK cell. Exemplary antigens expressed by an NK cell include, without limitation, NKR-P1A (KLRB1), CD94 (NKG2A), KLRG1, KIR2DL5A, KIR2DL5B, KIR2DL1, KIR2DL2, KIR2DL3, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DS1, KIR2DS1, CD94 (NKG2C/E), NKG2D, CD160 (BY55), CD16 (FcγRIIIA), NKp46 (NCR1), NKp30 (NCR3), NKp44 (NCR2), DNAM1 (CD226), CRTAM, CD27, NTB-A (SLAMF6), PSGL1, CD96 (Tactile), CD100 (SEMA4D), NKp80 (KLRF1, CLEC5C), SLAMF7 (CRACC, CS1, CD319), and CD244 (2B4, SLAMF4).

[0188] In some embodiments, an antibody of the present disclosure is administered in combination with an immunotherapeutic agent (*e.g.*, an effective amount of the immunotherapeutic agent, which in some embodiments as described above may be considered in the context of administering an anti-SIRP-α antibody of the present disclosure).

An immunotherapeutic agent may refer to any therapeutic that targets the immune system and promotes a therapeutic redirection of the immune system, such as a modulator of a costimulatory pathway, cancer vaccine, recombinantly modified immune cell, etc. Exemplary and non-limiting immunotherapeutic agents are described *infra*. Without wishing to be bound to theory, it is thought that the antibodies of the present disclosure are suitable for use with immunotherapeutic agents due to complementary mechanisms of action, *e.g.*, in activating both macrophages and other immune cells such as $T_{effector}$ cells to target tumor cells.

[0189] In some embodiments, the immunotherapeutic agent comprises an antibody. Exemplary antigens of immunotherapeutic antibodies are known in the art and include without limitation BDCA2, BDCA4, ILT7, LILRB1, LILRB2, LILRB3, LILRB4, LILRB5, Siglec-3, Siglec-7, Siglec-9, Siglec-15, FGL-1, CD200, CD200R, CSF-1R, CD40, CD40L, CD163, CD206, DEC205, CD47, CD123, arginase, IDO, TDO, AhR, EP2, COX-2, CCR2, CCR-7, CXCR1, CX3CR1, CXCR2, CXCR3, CXCR4, CXCR7, TGF-β RI, TGF-β RII, c-Kit, CD244, L-selectin/CD62L, CD11b, CD11c, CD68, 41BB, CTLA4, PD1, PD-L1, PD-L2, TIM-3, BTLA, VISTA, LAG-3, CD28, OX40, GITR, CD137, CD27, HVEM, CCR4, CD25, CD103, KIrg1, Nrp1, CD278, Gpr83, TIGIT, CD154, CD160, TNFR2, PVRIG, DNAM, and ICOS. Immunotherapeutic agents that are approved or in late-stage clinical testing include, without limitation, ipilimumab, pembrolizumab, nivolumab, atezolizumab, avelumab, durvalumab, and the like. In certain embodiments, an antibody of the present disclosure is administered in combination with an inhibitor of the PD-L1/PD-1 pathway, *e.g.*, an anti-PD-L1 or anti-PD-1 antibody. As demonstrated herein, combined administration of an anti-SIRP-α antibody of the present disclosure and an inhibitor of the PD-L1/PD-1 pathway can result in synergistic anti-tumor activity.

[0190] In some embodiments, the immunotherapeutic agent comprises a vaccine, oncolytic virus, adoptive cell therapy, cytokine, or small molecule immunotherapeutic agent. Examples of such immunotherapeutic agents are known in the art. For example, adoptive cell therapies and therapeutics can include without limitation chimeric antigen receptor T-cell therapy (CAR-T), tumor infiltrating lymphocytes (TILs), TCR engineered NK cell, and macrophage cell products. Vaccines can include without limitation polynucleotide vaccines, polypeptide vaccines, or cell-based (*e.g.*, tumor or dendritic cell-based) vaccines. Various cytokines useful for the treatment of cancer are known and include without limitation IL-2, IL-15, IL-7, IL-10, IL-12, IL21, TNFa, IFNs, GM-CSF, and engineered cytokine mutants.

Small molecule immunotherapeutic agents can include without limitation IDO/TDO inhibitors, AhR inhibitors, arginase inhibitors, A2a R inhibitors, TLR agonists, STING agonists, and Rig-1 agonists.

[0191] In some embodiments, an antibody of the present disclosure is administered in combination with a chemotherapeutic agent or small molecule anti-cancer agent. In some embodiments, an antibody of the present disclosure is administered in combination with an immunotherapeutic agent and a chemotherapeutic agent or small molecule anti-cancer agent. For example, it is thought that kinase inhibitors or other inhibitors of signaling pathways (*e.g.*, PAK4, PI3K, etc.) may be useful in combination with modulation of the immune system for treating cancer. As such, the antibodies of the present disclosure may find use in combination with one or more chemotherapeutic agents and/or small molecules (*e.g.*, kinase inhibitors) for treating cancer. Non-limiting examples of chemotherapeutic agents and/or anti-cancer agents contemplated for use in combination with an antibody of the present disclosure are provided *infra*.

[0192] In some embodiments, an antibody of the present disclosure is administered in combination with a therapeutic agent (*e.g.*, a chemotherapeutic/cytotoxic agent) including and not limited to methotrexate (RHEUMATREX®, Amethopterin) cyclophosphamide (CYTOXAN®), thalidomide (THALIDOMID®), acridine carboxamide, Actimid®, actinomycin, 17-N-allylamino-17- demethoxygeldanamycin, aminopterin, amsacrine, anthra-cycline, antineoplastic, antineoplaston, 5-azacytidine, azathioprine, BL22, bendamustine, biricodar, bleomycin, bortezomib, b ostatin, busulfan, calyculin, camptothecin, capecitabine, carboplatin, cetuximab, chlorambucil, cispla- tin, cladribine, clofarabine, cytarabine, dacarbazine, dasatinib, daunorubicin, decitabine, dichloroacetic acid, discode olide, docetaxel, doxorubicin, epirubicin, epothilone, eribulin, estramustine, etoposide, exatecan, exisulind, ferruginol, floxuridine, fludarabine, fluorouracil, fosfestrol, fotemustine, ganciclovir, gemcitabine, hydroxyurea, IT-101, idarubicin, ifosfamide, imiquimod, irinotecan, irofulven, ixabepilone, laniquidar, lapatinib, lenalidomide, lomustine, lurtotecan, mafosfamide, masoprocol, mechlorethamine, melphalan, mercaptopurine, mitomycin, mitotane, mitoxan- trone, nelarabine, nilotinib, oblimersen, oxaliplatin, PAC-1, paclitaxel, pemetrexed, pentostatin, pipobroman, pixantrone, plicamycin, procarbazine, proteasome inhibitors (e.g., bortezomib), raltitrexed, rebeccamycin, Revlimid®, rubite- can, SN-38, salinosporamide A, satraplatin, streptozotocin, swainsonine, tariquidar, taxane, tegafur-uracil, temozolo- mide, testolactone, thioTEPA, tioguanine, topotecan, tra- bectedin, tretinoin,

triplatin tetranitrate, tris(2-chloroethyl) amine, troxacitabine, uracil mustard, valrubicin, vinblastine, vincristine, vinorelbine, vorinostat, zosuquidar, or the like.

[0193] In some embodiments, an antibody of the present disclosure is administered in combination with a targeted small molecule inhibitor. For example, in some embodiments, an antibody of the present disclosure is administered in combination with a VEGFR/PDGFR inhibitor (*e.g.*, sorafenib, sunitinib, lenvatinib, vandetanib, cabozatinib, apatinib, pazopanib, axitinib, or regorafenib), EGFR inhibitor (*e.g.*, erlotinib, gefitinib, or osimertinib), MEK inhibitor (*e.g.*, trametinib, cobimetinib, binimetinib, or selumetinib), ALK inhibitor (*e.g.*, crizotinib, ceritinib, alectinib, brigatinib, lorlatinib, entrectinib, TSR-011, CEP-37440, or X-396), CDK4/6 inhibitor (*e.g.*, palbociclib, ribociclib, or abemaciclib), PARP inhibitor (*e.g.*, olaparib, rucaparib, niraparib, talazoparib, pamiparib, veliparib, CEP 9722, or E7016), mTOR inhibitor, KRAS inhibitor, TRK inhibitor (*e.g.*, larotrectinib), BCL2 inhibitor (*e.g.*, venetoclax), IDH inhibitor (*e.g.*, ivosidenib or enasidenib), hypomethylation agent (*e.g.*, decitabine or azacitidine), PI3K inhibitor, or DDR (*e.g.*, CHK, ATM, or ATR) inhibitor.

[0194] In some embodiments, an antibody of the present disclosure is administered in combination with a therapeutic agent including and not limited to 3F8, 8H9, Abagovomab, Abciximab, Abituzumab, Abrilumab, Actoxumab, Adalimumab, Adecatumumab, Aducanumab, Afelimomab, Afutuzumab, Alacizumab pegol, ALD518, Alemtuzumab, Alirocumab, Altumomab pentetate, Amatuximab, Anatumomab mafenatox, Anetumab ravtansine, Anifrolumab, kinzumab (IMA-638), Apolizumab, Arcitumomab, Ascrinvacumab, Aselizumab, Atezolizumab, Atinumab, Atlizumab (tocilizumab), Atorolimumab, Bapineuzumab, Basiliximab, Bavituximab, Bectumomab, Begelomab, Belimumab, Benralizumab, Bertilimumab, Besilesomab, Bevacizumab, Bezlotoxumab, Biciromab, Bimagrumab, Bimekizumab, Bivatuzumab mertansine, Blinatumomab, Blosozumab, Bococizumab, Brentuximab vedotin, Briakinumab, Brodalumab, Brolucizumab, Brontictuzumab, Canaki- numab, Cantuzumab mertansine, Cantuzumab ravtansine, Caplacizumab, Capromab pendetide, Carlumab, Catumaxomab, cBR96-doxorubicin immunoconjugate, CC49, Cedelizumab, Certolizumab pegol, Cetuximab, Ch.14.18, Citatuzumab bogatox, Cixutumumab, Clazakizumab, Clenoliximab, Clivatuzumab tetraxetan, Codrituzumab, Coltuximab ravtansine, Conatumumab, Concizumab, Crenezumab, CR6261, Dacetuzumab, Daclizumab, Dalotuzumab, Dapi- rolizumab pegol, Daratumumab, Dectrekumab, Demcizumab, Denintuzumab mafodotin, Denosumab, Derlotuximab biotin, Detumomab, Dinutuximab, Diridavumab, Dorlimomab aritox, Drozitumab, Duligotumab,

Dupilumab, Durvalumab, Dusigitumab, Ecromeximab, Eculizumab, Edobacomab, Edrecolomab, Efalizumab, Engumab, Eldelumab, Elgemtumab, Elotuzumab, Elsilimomab, Emactuzumab, Emibetuzumab, Enavatuzumab, Enfortumab vedotin, Enlimomab pegol, Enoblituzumab, Enokizumab, Enoticumab, Ensituximab, Epitumomab cituxetan, Epratuzumab, Erlizumab, Ertumaxomab, Etaracizumab, Etrolizumab, Evinacumab, Evolocumab, Exbivirumab, Fanolesomab, Faralimomab, Farletuzumab, Fasinumab, FBTA05, Felvizumab, Fezakinumab, Ficlatuzumab, Figitumumab, Firivumab, Flanvotumab, Fletikumab, Fontolizumab, Foralumab, Foravirumab, Fresolimumab, Fulranumab, Futuximab, Galiximab, Ganitumab, Gantenerumab, Gavilimomab, Gemtuzumab ozogamicin, Gevokizumab, Girentuximab, Glembatumumab vedotin, Golimumab, Gomiliximab, Guselkumab, Ibalizumab, Ibritumomab tiuxetan, Icrucumab, Idarucizumab, Igovomab, IMAB362, Imalumab, Imciromab, Imgatuzumab, Inclacumab, Indatuximab ravtansine, Indusatumab vedotin, In iximab, Intetumumab, Inolimomab, Inotuzumab ozogamicin, Ipilimumab, Iratumumab, Isatuximab, Itolizumab, Ixekizumab, Keliximab, Labetuzumab, Lambrolizumab, Lampalizumab, Lebrikizumab, Lemalesomab, Lenzilumab, Lerdelimumab, Lexatumumab, Libivirumab, Lifastuzumab vedotin, Ligelizumab, Lilotomab satetraxetan, Lintuzumab, Lirilumab, Lodelcizumab, Lokivetmab, Lorvotuzumab mertansine, Lucatumumab, Lulizumab pegol, Lumiliximab, Lumretuzumab, Mapatumumab, Margetuximab, Maslimomab, Mavrilimumab, Matuzumab, Mepolizumab, Metelimumab, Milatuzumab, Minretumomab, Mitumomab, Mogamulizumab, Morolimumab, Motavizumab, Moxetumomab pasudotox, Muromonab-CD3, Nacolomab tafenatox, Namilumab, Naptumomab estafenatox, Namatumab, Natalizumab, Nebacumab, Necitumumab, Nemolizumab, Nerelimomab, Nesvacumab, Nimotuzumab, Nivolumab, Nofetumomab me entan, Obiltoxaximab, Ocaratuzumab, Ocrelizumab, Odulimomab, Ofatumumab, Olaratumab, Olokizumab, Omalizumab, Onartuzumab, Ontuxizumab, Opicinumab, Oportuzumab monatox, Oregovomab, Orticumab, Otelixizumab, Otlertuzumab, Oxelumab, Ozanezumab, Ozoralizumab, Pagibaximab, Palivizumab, Panitumumab, Pankomab, Panobacumab, Parsatuzumab, Pascolizumab, Pasotuxizumab, Pateclizumab, Patritumab, Pembrolizumab, Pemtumomab, Perakizumab, Pertuzumab, Pexelizumab, Pidilizumab, Pinatuzumab vedotin, Pintumomab, Placulumab, Polatuzumab vedotin, Ponezumab, Priliximab, Pritoxaximab, Pritumumab, PRO 140, Quilizumab, Racotumomab, Radretumab, Ravirumab, Ralpancizumab, Ramucirumab, Ranibizumab, Raxibacumab, Refanezumab, Regavirumab, Reslizumab, Rilotumumab, Rinucumab, Rituximab, Robatumumab,

Roledumab, Romosozumab, Rontalizumab, Rovelizumab, Ruplizumab, Samalizumab, Sarilumab, Satumomab pendetide, Secukinumab, Seribantumab, Setoxaximab, Sevirumab, Sibrotuzumab, SGN-CD19A, SGN-CD33A, Sifalimumab, Siltuximab, Simtuzumab, Siplizumab, Sirukumab, Sotuzumab vedotin, Solanezumab, Solitomab, Sonepcizumab, Sontuzumab, Stamulumab, Sulesomab, Suvizumab, Tabalumab, Tacatuzumab tetraxetan, Tadocizumab, Talizumab, Tanezumab, Taplitumomab paptox, Tarextumab, Te bazumab, Telimomab aritox, Tenatumomab, Teneliximab, Teplizumab, Teprotumumab, Tesidolumab, TGN1412, Ticilimumab (tremelimumab), Tildrakizumab, Tigatuzumab, TNX650, Tocilizumab (atlizumab), Toralizumab, Tosatoxumab, Tositumomab, Tovetumab, Tralokinumab, Trastuzumab, TRBS07, Tregalizumab, Tremelimumab, Tucotuzumab celmoleukin, Tuvirumab, Ublituximab, Ulocuplumab, Urelumab, Urtoxazumab, Ustekinumab, Vandortuzumab vedotin, Vantictumab, Vanucizumab, Vapaliximab, Varlilumab, Vatelizumab, Vedolizumab, Veltuzumab, Vepalimomab, Vesencumab, Visilizumab, Volociximab, Vorsetuzumab mafodotin, Votumumab, Zalutumumab, Zanolimumab, Zatuximab, Ziralimumab, or Zolimomab aritox.

[0195] Combination treatments comprising an antibody of the present disclosure and multiple additional agents (*e.g.*, as described *supra*) are contemplated. For example, in some embodiments, an antibody of the present disclosure is administered in combination with a chemotherapeutic/cytotoxic agent and an antibody or targeted small molecule inhibitor. In some embodiments, an antibody of the present disclosure is administered in combination with a chemotherapeutic/cytotoxic agent and an immunotherapeutic agent. In some embodiments, an antibody of the present disclosure is administered in combination with an antibody or targeted small molecule inhibitor and an immunotherapeutic agent.

[0196] Any cancer type known in the art may be included, such as but not limited to carcinoma, sarcoma, lymphoma, leukemia, lymphoma, and blastoma. More particular examples of such cancers include, but are not limited to, lung cancer, squamous cell cancer, brain tumors, glioblastoma, head and neck cancer, hepatocellular cancer, colorectal cancer (*e.g.*, colon or rectal cancers), liver cancer, bladder cancer, gastric or stomach cancer, pancreatic cancer, cervical cancer, ovarian cancer, cancer of the urinary tract, breast cancer, peritoneal cancer, uterine cancer, salivary gland cancer, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, anal carcinoma, penile carcinoma, melanoma, multiple myeloma and B-cell lymphoma (including non-Hodgkin's lymphomas (NHL)); acute lymphoblastic leukemia (ALL); chronic lymphocytic leukemia (CLL); acute myeloid

leukemia (AML); Merkel cell carcinoma; hairy cell leukemia; chronic myeloblastic leukemia (CML); and associated metastases.

[0197] In addition to cancer therapies, the antibodies provided herein are useful in therapies in which monoclonal antibodies are administered for the purpose of depleting cells, *e.g.*, in the treatment of inflammatory diseases by depletion immune cells. For such purposes the an antibody provided herein is administered in combination with a second therapeutic antibody, e.g. with rituximab for depletion of B cells in inflammatory diseases and autoimmune conditions; alemtuzumab for multiple sclerosis; OKT3 for immunosuppression; others for bone marrow transplant conditioning; and the like.

[0198] Further provided herein are methods of treating or delaying progression of an autoimmune disease or an inflammatory disease in an individual by administering an effective amount of an antibody of the present disclosure. Autoimmune diseases and inflammatory diseases amenable to treatment according to the disclosure include, but are not limited to, multiple sclerosis, rheumatoid arthritis, a spondyloarthropathy, systemic lupus erythematosus, an antibody-mediated inflammatory or autoimmune disease, graft versus host disease, sepsis, diabetes, psoriasis, atherosclerosis, Sjogren's syndrome, progressive systemic sclerosis, scleroderma, acute coronary syndrome, ischemic reperfusion, Crohn's Disease, endometriosis, glomerulonephritis, myasthenia gravis, idiopathic pulmonary fibrosis, fibrotic diseases (*e.g.*, pulmonary fibrosis, liver cirrhosis, atrial fibrosis, endomyocardial fibrosis, myelofibrosis, or retroperitoneal fibrosis), asthma, acute respiratory distress syndrome (ARDS), vasculitis, and inflammatory autoimmune myositis. In some embodiments, an antibody of the present disclosure is administered in combination with a therapeutic agent, such as an immunosuppressive, anti-inflammatory, or immunomodulatory agent. In some embodiments, an antibody provided herein is used in the treatment of an autoimmune disease or an inflammatory disease, e.g., multiple sclerosis, rheumatoid arthritis, a spondyloarthropathy, systemic lupus erythematosus, an antibody-mediated inflammatory or autoimmune disease, graft versus host disease, sepsis, diabetes, psoriasis, psoriatic arthritis, atherosclerosis, Sjogren's syndrome, progressive systemic sclerosis, scleroderma, acute coronary syndrome, ischemic reperfusion, Crohn's Disease, ulcerative colitis, endometriosis, glomerulonephritis, IgA nephropathy, polycystic kidney disease, myasthenia gravis, idiopathic pulmonary fibrosis, fibrotic disease (*e.g.*, pulmonary fibrosis, liver cirrhosis, atrial fibrosis, endomyocardial fibrosis, myelofibrosis, or retroperitoneal fibrosis),

asthma, atopic dermatitis, acute respiratory distress syndrome (ARDS), vasculitis, or inflammatory autoimmune myositis.

[0199] In some embodiments, an antibody of the present disclosure is part of a pharmaceutical formulation, *e.g.*, including the antibody and one or more pharmaceutically acceptable carriers. Pharmaceutical compositions and formulations as described herein can be prepared by mixing the active ingredients (such as an antibody or a polypeptide) having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). In some embodiments, an antibody of the present disclosure is lyophilized.

[0200] In some embodiments, an individual is administered a dose normalized to the body weight of the individual. In some embodiments, an individual is administered a dose of about 10 μg/kg, about 50 μg/kg, about 100 μg/kg, about 200 μg/kg, about 300 μg/kg, about 400 μg/kg, about 500 μg/kg, about 600 μg/kg, about 700 μg/kg, about 800 μg/kg, about 900 μg/kg, about 1,000 μg/kg, about 1,100 μg/kg, 1,200 μg/kg, 1,300 μg/kg, 1,400 μg/kg, 1,500 μg/kg, 1,600 μg/kg, 1,700 μg/kg, 1,800 μg/kg, 1,900 μg/kg, about 2,000 μg/kg, about 3000 μg/kg, about 4000 μg/kg, about 5000 μg/kg, about 6000 μg/kg, about 7000 μg/kg, about 8000 μg/kg, about 9000 μg/kg, about 10 mg/kg, about 20 mg/kg, about 30 mg/kg, about 40 mg/kg, about 50 mg/kg, about 60 mg/kg, about 70 mg/kg, about 80 mg/kg about 90 mg/kg, about 100 mg/kg, about 200 mg/kg, about 300 mg/kg, about 400 mg/kg, about 500 mg/kg, about 600 mg/kg, about 700 mg/kg, about 800 mg/kg, about 900 mg/kg, or about 1000 mg/kg of an antibody of the present disclosure.

[0201] In some embodiments, the period of time that an antibody of the present disclosure is administered to the individual is any suitable period as determined by the stage

of the disease, the patient's medical history and the attending physician's discretion. Examples of such suitable periods include, but are not limited to, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, at least about 12 months, at least about 13 months, at least about 14 months, at least about 15 months, at least about 16 months, at least about 17 months, at least about 18 months, at least about 19 months, at least about 20 months, at least about 21 months, at least about 22 months, at least about 23 months, or at least about 24 months or longer. In particular aspects, the treatment period is continued for longer than 24 months, if desired, such as for 30 months, 31 months, 32 months, 33 months, 34 months, 35 months, 36 months, or longer than 36 months. In some embodiments, the period is 6 months, 1 year or 2 years. In another embodiment, the period of time of dosing for any of the methods described herein is for at least about 2 weeks, at least about 4 weeks, at least about 8 weeks, at least about 16 weeks, at least about 17 weeks, at least about 18 weeks, at least about 19 weeks, at least about 20 weeks, at least about 24 weeks, at least about 28 weeks, at least about 32 weeks, at least about 36 weeks, at least about 40 weeks, at least about 44 weeks, at least about 48 weeks, at least about 52 weeks, at least about 60 weeks, at least about 68 weeks, at least about 72 weeks, at least about 80 weeks, at least about 88 weeks, at least about 96 weeks, or at least about 104 weeks.

EXAMPLES

[0202] The present disclosure will be more fully understood by reference to the following examples. The examples should not, however, be construed as limiting the scope of the present disclosure. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

Example 1: Identification of Antibodies with Novel Binding Specificities to SIRP-α Proteins

Methods

Antibody production

[0203] The following proteins were used for immunization. Each includes a human or mouse SIRP-α peptide fused to a modified Fc region (either a human IgG4 Fc with a hinge

region containing an S228P mutation, or an L234A/L235A/G237A/N297A human IgG1 Fc designated as IgG1_AAA_N297A) for increased immunogenicity.

Table A. Immunogen sequences.

Description	SEQ ID NO	Sequence
Human sirpa v1 (Fusion with Fc of IgG4_S228P)	1	EEELQVIQPDKSVLVAAGETATLRCTATSLIPV GPIQWFRGAGPGRELIYNQKEGHFPRVTTVSD LTKRNNMDFSIRIGNITPADAGTYYCVKFRKG SPDDVEFKSGAGTELSVRAKPSESKYGPPCPP CPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSQEDPEVQFNWYVDGVEVHNAKTKP REEQFNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ EGNVFSCSVMHEALHNHYTQKSLSLSLGK
Human sirpa v2 (Fusion with Fc of IgG4_S228P)	2	EEELQVIQPDKSVSVAAGESAILHCTVTSLIPV GPIQWFRGAGPARELIYNQKEGHFPRVTTVSE STKRENMDFSISISNITPADAGTYYCVKFRKGS PDTEFKSGAGTELSVRAKPSESKYGPPCPPCPA PEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSQEDPEVQFNWYVDGVEVHNAKTKPREE QFNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSRLTVDKSRWQE GNVFSCSVMHEALHNHYTQKSLSLSLGK
Mouse 129 sirpa (Fusion with Fc of IgG1_AAA_N297A)	3	KELKVTQPEKSVSVAAGDSTVLNCTLTSLLPV GPIKWYRGVGQSRLLIYSFTGEHFPRVTNVSD ATKRNNMDFSIRISNVTPEDAGTYYCVKFQKG PSEPDTEIQSGGGTEVYVLAKPSDKTHTCPPCP APEAAGAPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYASTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK
Mouse NOD sirpa (Fusion with Fc of IgG1_AAA_N297A)	4	TEVKVIQPEKSVSVAAGDSTVLNCTLTSLLPV GPIRWYRGVGQSRQLIYSFTTEHFPRVTNVSD ATKRSNLDFSIRISNVTPEDAGTYYCVKFQRG SPDTEIQSGGGTEVYVLAKDKTHTCPPCPAPE AAGAPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YASTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGK

[0204] The above proteins were used to immunize wild-type chickens, SynVH chickens which are transgenic chickens containing VH from human and VL from chicken, or chickens with fully human "HuMAB" immunoglobulin loci (Crystal Bioscience; *see, e.g.*, WO2012162422, WO2011019844, and WO2013059159). Chickens were immunized with varied schedules having alternating doses of antigen. An exemplary immunization schedule is as follows: initial immunization with 100μg dose of antigen having the sequence of SEQ ID NO:1 at week 1, boost of 100μg of antigen having the sequence of SEQ ID NO: 2 at week 3, draw at week 4, boost with 50μg dose of antigen having the sequence of SEQ ID NO:1 at week 5, draw at week 6, boost with 50μg of antigen having the sequence of SEQ ID NO: 2 at week 7, and draw at week 8. Additional descriptions of chicken immunization may be found, *e.g.*, in Mettler Izquierdo, S. *et al.* (2016) *Microscopy (Oxf)* 1-16.

[0205] Compared with the mammalian SIRP-α sequences, the sequence of chicken SIPRα was found to be significantly more divergent. Without wishing to be bound to theory, it was thought that the divergence between mammalian and chicken SIRP-α sequences would provide unique opportunities to generate antibodies that cross-react across multiple mammalian SIRP-α proteins. For example, it may be difficult to generate anti-SIRP-α antibodies that cross-react with the murine sequence from a mouse host due to immune tolerance. Moreover, the greater divergence between the chicken and mammalian immune systems may lead to a greater diversity in antibody production.

[0206] Without wishing to be bound to theory, it is thought that antibodies with cross-reactive binding among human, cynomologus, and/or murine proteins may allow for characterization of antibodies in both animal models and clinical testing. Antibodies with isoform- and/or variant-specific binding may be useful for personalized medicine approaches to specific human populations and/or studies on specific variants of interest.

Determination of K_{off}

[0207] Binding of the antibody clones to various SIRP proteins was determined using surface plasmon resonance (SPR) detection on a ProteOn XPR36 instrument (Bio-Rad, Hercules, CA) using phosphate buffered saline (PBS, pH 7.4) supplemented with 0.01% Tween-20 (PBST) as running buffer. The pre-filtered media containing the secreted antibodies was used directly for the assay. First, anti-Human IgG Fc (BR-1008-39, GE Healthcare) was amine-coupled onto a GLC sensor chip to generate the capture surfaces for the antibodies. About 4000 RU per flow cell of immobilized anti-human IgG Fc is achieved.

Each clone is screened using the same method as follows. The SIRP analytes used for the screen were SIRP-α from various species (human v1, human v2, cynomolgus, mouse 129, BL6, BALBc, NOD), human SIRP-β, and human SIRP-γ; SEQ ID NOs:5, 6, 11, 7, 9, 10, 8, 13, and 15, respectively.

[0208] ~5-10uL of pre-filtered media in 10mM sodium acetate buffer (pH4.5) was injected for 2 mins at 30ul/min, followed by buffer flow for 1 min at 100uL/min. SIRP analyte (100nM) was injected for 1 min at 100uL/min, followed by a dissociation cycle of 10mins. Regeneration of the chip surface was accomplished by flowing 3M Magnesium Chloride for 1 min at 25uL/min in both orientations, followed by buffer flow for 1min at 100uL/min. Biosensor data were double-referenced by subtracting the interspot data (containing no immobilized anti-human IgG Fc) from the reaction spot data (immobilized anti-human IgG Fc) and then subtracting the response of a buffer "blank" analyte injection from that of an analyte injection. Binding was fitted using a 1:1 Langmuir and K_{off} (1/S) values calculated. All SPR assays were performed at 25 °C.

Determination of K_D

[0209] The interactions of anti-SIRPα antibodies with SIRPα from various species (human v1, human v2, cynomolgus, mouse 129, BL6, BALBc, NOD), SIRPβ and SIRPγ were analyzed using two methods, direct immobilization of the antibodies (via GLC chip) or capture via biotinylated Protein A (via NLC chip), according to the following protocols. All experiments were performed at 25°C using a SPR-based ProteOn XPR36 biosensor (BioRad, Inc, Hercules, CA) equipped with GLC or NLC sensor chips. Antibodies were expressed using FreeStyle™ 293-FS cells (Thermo Fisher). Purification was carried out by standard Protein A affinity column chromatography and eluted antibodies were stored in PBS buffer.

[0210] The running buffer was PBS pH 7.4 with 0.01% Tween-20 (PBST+). All analytes were used at their nominal concentrations as determined by A280 Absorbance and using their molar calculated extinction coefficient. Analytes were injected in a "one-shot" kinetic mode as described elsewhere (*see, e.g.*, Bravman, T. *et al.* (2006) *Anal. Biochem.* 358:281-288).

[0211] For the method using GLC chip, the analytes were injected and flowed over anti-SIRPα antibodies immobilized (~1000 RUs) on GLC chips using Proteon Amine Coupling Kit. For the immobilization step, GLC chip was activated with EDAC/Sulpho-NHS 1:1 (Biorad) diluted 1/100 for 300s at 25 μL/min. Anti-SIRPα antibodies were diluted to 80 nM

concentration in 10 mM sodium acetate buffer pH 4.5 and immobilized to the chip at 30 µL/min for 50s. Chip was inactivated with ethanolamine for 300s at 25 µL/min. The analytes (*e.g.*, SIRP-α from different species, SIRP-β, SIRP-γ) were injected in a "one-shot" kinetic mode at nominal concentrations of 100, 33, 11, 3.7, 1.2 and 0 nM. Association times were monitored for 90s at 100µL/min, and dissociation times were monitored for 1200s. The surfaces were regenerated with a 2:1 v/v blend of Pierce IgG elution buffer/4M NaCl.

[0212] Alternatively, K_D determination was performed using antibody capture via an NLC chip. In this case, 15ug/mL biotinylated protein A (Thermofisher) was injected at 30uL/min for 120 s over the NLC chip to obtain an immobilization response of ~1000-1200RUs. Next, anti-SIRPα antibodies (~160 nM) were injected for 80s at 30uL/min. The analytes (SIRPα from different species, SIRP-β and SIPR-γ) were subsequently injected in a "one-shot" kinetic mode at nominal concentrations of 100, 33, 11, 3.7, 1.2 and 0 nM. Association times were monitored for 60s at 25µL/min, and dissociation times were monitored for 120s. The surfaces were regenerated with a 2:1 v/v blend of Pierce IgG elution buffer/4M NaCl.

[0213] Biosensor data were double-referenced by subtracting the interspot data (containing no immobilized protein) from the reaction spot data (immobilized protein) and then subtracting the response of a buffer "blank" analyte injection from that of an analyte injection. Double- referenced data were fit globally to a simple Langmuir model and the K_D value was calculated from the ratio of the apparent kinetic rate constants ($K_D = k_d/k_a$).

Results

[0214] The binding kinetics of various antibody clones to selected mouse SIRP-α proteins were determined. Mouse proteins that were tested include BALBc (SEQ ID NO:10), BL6 (SEQ ID NO:9), NOD (SEQ ID NO:8), and m129 (SEQ ID NO:7) SIRP-α proteins. The results are summarized in Tables B-E below. Variable domain sequences for AB21 and AB25 are shown in Table J1.

[0215] As used herein, antibody clones are referred to by clone ID number. In addition, the notation "S[clone number]" refers to an sc-Fv-Fc format; the notation "AB[clone number]" refers to a full IgG antibody format; the notation "AB[clone number]b" refers to a mouse IgG1 N297A format; and "AB[clone number]c" refers to a mouse IgG2a format.

Table B. Summary of kinetics for binding of selected antibodies to BALBc mouse SIRP-α protein (SEQ ID NO:10)

Antibody	K_{on} (1/Ms)	K_{off} (1/s)	K_D (M)
AB21c	$4.62x10^{5}$	$6.18x10^{-4}$	$1.34x10^{-9}$
AB25c	$3.03x10^{5}$	$2.92x10^{-3}$	$9.64x10^{-9}$

Table C. Summary of kinetics for binding of selected antibodies to BL6 mouse SIRP-α protein (SEQ ID NO:9)

Antibody	K_{on} (1/Ms)	K_{off} (1/s)	K_D (M)
AB21c	$2.76x10^{5}$	$2.41x10^{-4}$	$8.76x10^{-10}$
AB25c	$1.42x10^{5}$	$3.99x10^{-4}$	$2.81x10^{-9}$

Table D. Summary of kinetics for binding of selected antibodies to NOD mouse SIRP-α protein (SEQ ID NO: 8)

Antibody	K_{on} (1/Ms)	K_{off} (1/s)	K_D (M)
AB21c	$7.49x10^{5}$	$4.79x10^{-4}$	$6.40x10^{-10}$
AB25c	$3.66x10^{5}$	$1.43x10^{-3}$	$3.90x10^{-9}$

Table E. Summary of kinetics for binding of selected antibodies to m129 mouse SIRP-α protein (SEQ ID NO: 7)

Antibody	K_{on} (1/Ms)	K_{off} (1/s)	K_D (M)
AB21c	$5.63x10^{5}$	$3.31x10^{-5}$	$5.88x10^{-11}$
AB25c	$3.52x10^{5}$	$2.07x10^{-5}$	$5.87x10^{-11}$

[0216] These results demonstrate that the AB21 and AB25 antibody clones bound to all four mouse SIRP-α proteins, making these antibodies suitable for characterization in *in vivo* mouse models.

Example 2: Functional Properties of anti-SIRP-α antibodies

[0217] The previous Example describes the identification and characterization of anti-SIRP-α antibodies. These antibodies were next examined in animal models to explore SIRP-α's biological effects on tumor growth.

[0218] As noted above, antibody clones labeled as "b" were tested as full-length mouse IgG1 antibodies with an N297A mutation. Antibody clones labeled as "c" were tested as full-length mouse IgG2a antibodies.

Methods

In vivo anti-tumor activity

[0219] For the MC38 syngeneic mouse colon carcinoma model, MC38 cells were implanted subcutaneously in C57BL/6 mice and randomized into groups (8-10mice/group). Treatment groups included vehicle (PBS), AB25b, AB25c, and AB27b. All anti-SIRPα antibodies had a mouse IgG1 Fc region bearing an N297A mutation except for AB25c, which had a mouse IgG2a. Treatment was initiated when tumors were an average of 60-65mm^3, day 7 post implant. Mice were dosed intraperitoneally (IP) at 10 mg/kg twice a week for three weeks for anti-SIRPα. Animals were sacrificed when tumors reached a volume of ~2000mm^3.

Results

[0220] The *in vivo* anti-tumor effects of various anti-SIRP-α antibodies were assayed in a syngeneic mouse colon carcinoma model. The anti-tumor activities of blocking anti-SIRP-α antibodies AB25b, AB25c, and AB27b were examined in an MC38 syngeneic mouse colon carcinoma model to assess their single agent activities. The blocking anti-SIRP-α antibodies AB25b, AB25c, and AB27b delayed tumor formation at 10 mg/kg as compared to vehicle alone (**FIG. 1**) in the MC38 syngeneic mouse model. On day 25, groups treated with anti-SIRPα antibodies had three mice below 600mm^3 for AB25b, four mice below 600mm^3 for AB25c, and 3 mice below 600mm^3 for AB27b, while the vehicle-treated group had only two mice below 600mm^3.

[0221] These results demonstrate the efficacy of anti-SIRP-α antibody treatment in inhibiting tumor growth *in vivo*. Blocking anti-SIRP-α antibodies were found to block *in vivo* tumor growth.

Example 3: Humanization of anti-SIRP-α Antibodies

[0222] The Examples above describe the generation and characterization of anti-SIRP-α antibodies having a fully human heavy chain and a chicken light chain. In order to humanize

the chicken-derived light chains, chicken HVRs of these antibodies were grafted onto various human lambda light chain frameworks.

Methods

Humanization

[0223] Antibodies were humanized using standard techniques. For measuring production yield, equal volume of Expi293 cultures expressing anti-SIRPα antibodies were purified by Protein A affinity chromatography. After buffer exchange into PBS, the protein concentration was determined by A280 and expressed in mg/mL.

Results

[0224] In order to design humanized light chains, each chicken light chain sequence was aligned to the closest human germline framework by IgBLAST (NCBI). Using this analysis, the closest match to the chicken lambda light chain framework is human IGLV3 (*see* SEQ ID NOs:27-30).

[0225] In another approach, a literature search was undertaken to determine the optimal human lambda light chain framework sequences to pair with a human VH3 sequences (the human heavy chain used for these antibodies). Based on these analyses, it was thought that human VH3 would pair well with human IGLV1 and IGLV2. *See* Glanville, J. *et al.* (2009) *Proc. Natl. Acad. Sci.* 106:20216-20221; Lloyd, C. *et al.* (2009) *Protein Eng. Des. Sel.* 22:159-168; and Jayaram, N. *et al.* (2012) *Protein Eng. Des. Sel.* 25:523-529.

[0226] Therefore, six humanized light chains were created: Hum1 (AB25 HVRs + human IGLV3 framework; SEQ ID NO:25), Hum2 (AB25 HVRs + human IGLV1 framework), Hum3 (AB66 HVRs + human IGLV3 framework), Hum4 (AB66 HVRs + human IGLV1 framework), Hum5 (AB25 HVRs + human IGLV2 framework), and Hum6 (AB21 HVRs + human IGLV1 framework).

[0227] Each of the 6 humanized light chains was paired with the AB21 heavy chain. Antibodies were expressed as described above. Surprisingly, human IGLV1 framework sequences resulted in decreased antibody expression regardless of the heavy chain. This refers to the heavy chain pairings with Hum2, Hum4 and Hum6. The results are summarized in **FIG. 2** as "protein yield" (row 1). In contrast, antibodies with human IGLV2 and IGLV3 frameworks (Hum 1, Hum3, Hum5) in the light chain showed higher levels of expression.

[0228] Selected antibodies were also characterized for binding to a variety of SIRP proteins, *e.g.*, to human SIRP-α v1 (SEQ ID NO:5), human SIRP-α v2 (SEQ ID NO:6),

cynomolgus SIRP-α (SEQ ID NO:11), mouse BALB/c SIRP-α (SEQ ID NO:10), and human SIRP-γ (SEQ ID NO:15). These data are also summarized in **FIG. 2**. Selected humanized light chains caused a decrease in binding to one or more antigens. For instance, the human IGLV3 framework (represented by Hum1 and Hum3) was found to allow for superior levels of antibody production without perturbing binding affinity. For example, light chain variable domains with the IGLV3 frameworks and either the antibody 25 or antibody 66 HVR sequences (represented by Hum1 and Hum 3 respectively) combined well with the AB21 heavy chain and showed similar binding to different SIRP-α and SIRP-γ proteins. In contrast, IGLV1 and IGLV2 frameworks (represented by Hum2, Hum4, Hum5 and Hum6) were found to either lower expression and/or decrease binding to SIRP when paired with the AB21 heavy chain. Additional binding data from these experiments are provided in Table L *infra*. The human IGLV3 framework was selected for further testing.

[0229] Additional VL domains Hum9 and Hum8 were generated based on the Hum1 VL domain. Compared to Hum1, Hum9 contains 4 amino acid substitutions near or in HVR-L1 and –L2 that increase the humanness of the light chain to greater than or equal to 85% identity to human light chain sequence. Compared to Hum1, Hum8 contains 5 amino acid substitutions respectively near or in HVR-L1 and –L2 that increase the humanness of the light chain to greater than or equal to 85% identity to human light chain sequence. Hum1, Hum8 and Hum9 VLs when paired with heavy chain VH domain all_mut_AB21 (carrying germline mutations; SEQ ID NO:26) produced anti-SIRP-α antibodies that bind to human SIRP-α v1 with affinity equal or better than 10pM (Table M).

Example 4: Induction of Phagocytosis and Dendritic Cell Activation by anti-SIRP-α Antibodies

[0230] Various anti-SIRP-α antibodies were next examined in phagocytosis and dendritic cell activation assays.

Methods

Tumor cell line culturing

[0231] DLD-1 (human colorectal adenocarcinoma) cells were maintained in growth medium comprised of RPMI (Gibco) supplemented with 10 percent heat-inactivated Fetal Bovine Serum (Gibco), one percent penicillin/streptomycin (Gibco), and one percent Glutamax (Gibco).

Derivation and culture of human monocyte-derived macrophages

[0232] Trima residuals were received from Blood Centers of the Pacific and diluted 1:4 with Phosphate Buffered Saline (PBS, Gibco). Diluted blood was split into four tubes and underlayed with 20 ml Ficoll-Paque Plus (GE Healthcare). Tubes were centrifuged for 30 minutes at 400 x g. PBMCs were collected from the interface and resuspended in FACS buffer (PBS with 0.5 percent Bovine Serum Albumin (Gibco)). $CD14^+$ monocytes were purified by negative selection using the Monocyte Isolation Kit II (Miltenyi Biotec) and LS columns (Miltenyi Biotec) according to the manufacturer's protocol.

[0233] For nonpolarized macrophages, $CD14^+$ monocytes were seeded into 15 cm tissue culture plates (Corning) at 10 million cells per dish in 25 ml IMDM (Gibco) supplemented with 10 percent human AB serum (Corning), one percent penicillin/streptomycin, and one percent Glutamax. Cells were cultured for seven to ten days.

[0234] For M2 polarized macrophages, $CD14^+$ monocytes were seeded into 15 cm tissue culture plates (Corning) at 6 million cells per dish in 25 ml RPMI(Gibco) supplemented with 10 fetal bovine serum (Thermo Fisher), one percent penicillin/streptomycin, and one percent Glutamax, and 50ng/ml M-CSF (Miltenyi). Cells were cultured for seven to ten days.

In vitro *phagocytosis assays*

[0235] DLD-1 cells were detached from culture plates by washing twice with 20 ml PBS and incubation in 10 ml TrypLE Select (Gibco) for 10 minutes at 37°C. Cells were centrifuged, washed in PBS, and resuspended in medium. Cells were labeled with the Celltrace CFSE Cell Proliferation kit (Thermo Fisher) according to the manufacturer's instructions and resuspended in IMDM. Macrophages were detached from culture plates by washing twice with 20 ml PBS and incubation in 10 ml TrypLE Select for 20 minutes at 37°C. Cells were removed with a cell scraper (Corning), washed in PBS, and resuspended in IMDM.

[0236] Phagocytosis assays were assembled in ultra-low attachment U-bottom 96 well plates (Corning) containing 100,000 DLD-1 cells, 50,000 macrophages, five-fold serial dilutions of anti-SIRP-α antibody from 100 nM to 6.4 pM, and cetuximab (Absolute Antibody) at 1 or 0.01 ug/ml or control antibody of the same isotype (Southern Biotech). Plates were incubated two hours at 37°C in a humidified incubator with 5 percent carbon dioxide. Cells were pelleted by centrifugation for five minutes at 400 x g and washed in 250

µl FACS buffer. Macrophages were stained on ice for 15 minutes in 50 µl FACS buffer containing 10 µl human FcR Blocking Reagent (Miltenyi Biotec), 0.5 µl anti-CD33 BV421 (Biolegend), and 0.5 µl anti-CD206 APC-Cy7 (Biolegend). Cells were washed in 200 µl FACS buffer, washed in 250 µl PBS, and stained on ice for 30 minutes in 50 µl Fixable Viability Dye eFluor 506 (ebioscience) diluted 1:1000 in PBS. Cells were washed twice in 250 µl FACS buffer and fixed for 30 minutes on ice in 75 µl Cytofix (BD Biosciences). Cells were washed in 175 µl FACS buffer and resuspended in 75 µl FACS buffer. Cells were analyzed on a FACS Canto II (BD Biosciences), with subsequent data analysis by Flowjo 10.7 (Treestar). Dead cells were excluded by gating on the e506-negative population. Macrophages that had phagocytosed tumor cells were identified as cells positive for CD33, CD206, and CFSE.

Dendritic cell activation assays

[0237] Balb/c mice (n=3/group) were intravenously injected with a human IgG1 control, various anti-SIRP-α antibodies, mouse IgG control, or vehicle (PBS) at 10 mg/kg. Five hours post injection, spleens were harvested and processed into single cell suspension by mechanical dissociation. Activation marker CD86, MHCII and CCR7 level on CD4+ splenic dendritic cells was measured by flow cytometry.

Results

[0238] Humanized antibodies described above were tested for their effects on phagocytosis of EGFR(+) DLD-1 cells by M2 macrophages in combination with the anti-EGFR antibody cetuximab (**FIG. 3A**). All humanized antibodies were found to enhance cetuximab-induced phagocytosis. The humanized antibodies described above (antibody 25 heavy chain variant combined with Hum1 or Hum9 light chain and antibody 27 heavy chain variant combined with Hum1 light chain) were tested for their effects on phagocytosis of EGFR(+) DLD-1 cells by M2 macrophages in combination with the anti-EGFR antibody cetuximab (**FIG. 3B**). All humanized antibodies were found to enhance cetuximab-induced phagocytosis. All the antibodies tested were generated as full-length human IgG1 antibodies with L234A, L235A, G237A, and N297A mutations.

[0239] Next, various anti-SIRP-α antibodies were examined for their effects on *in vivo* dendritic cell activation (**FIGS. 4A & 4B**). Failure to engage mouse SIRP-α receptor on splenic dendritic cells via CD47 binding leads to splenic dendritic cell activation. Anti-SIRP-

α antibodies Hum1/AB21mutall, Hum8/AB21mutall, and Hum9/AB21mutall were tested *in vivo* to determine if it leads to dendritic cell activation. As determined by CD86 and MHCII expression, these anti-SIRP-α blocking antibodies were able to induce activation of dendritic cells.

Example 5: Synergistic anti-tumor effects of combining anti-SIRP-α antibodies with inhibition of the PD-L1/PD-1 pathway

Methods

In vivo anti-tumor activity

[0240] For the CT26 syngeneic mouse colon carcinoma model, CT26 cells were implanted subcutaneously in BALB/c mice and randomized into groups (8-9 mice/group). Treatment groups included vehicle (PBS), AB25b, anti-PD-L1, and AB25b/anti-PD-L1. Anti-PD-L1 is generated by fusing the VH and VL domain of Atezolizumab with mouse IgG1 Fc region bearing an N297A mutation. All anti-SIRP-α antibodies also have a mouse IgG1 Fc region bearing an N297A mutation. Treatment was initiated when tumors were an average of 75-80mm^3, day 7 or 8 post implant. Mice were dosed intraperitoneally (IP) at 3mg/kg or 10 mg/kg twice a week for three weeks for anti-SIRPα antibodies and three doses at 3 mg/kg, five days apart for anti-PD-L1. Animals were sacrificed when tumors reached a volume of ~2000mm^3.

[0241] For the MC38 syngeneic mouse colon carcinoma model, MC38 cells were implanted subcutaneously in C57BL/6 mice and randomized into groups (8-10mice/group). Treatment groups included vehicle (PBS), AB25b, anti-PD1 (clone RMP1-14, BioXCell), and AB25b/anti-PD1. All anti-SIRPα antibodies had a murine IgG1 Fc region bearing an N297A mutation except for AB25c. Treatment was initiated when tumors were an average of 60-65mm^3, day 7 post implant. Mice were dosed intraperitoneally (IP) at 10 mg/kg twice a week for three weeks for anti-SIRPα and three doses at 2 mg/kg for anti-PD1. Animals were sacrificed when tumors reached a volume of ~2000mm^3.

Results

[0242] Anti-tumor activity of the blocking AB25b anti-SIRP-α antibody was tested alone and in combination with an anti-PD-L1 antibody in the CT26 syngeneic mouse colon carcinoma model. As shown in **FIG. 5**, administration of AB25b at 10mg/kg in combination with anti-PD-L1 at 3mg/kg delayed tumor formation when compared to treatment with each

single agent or vehicle control. On day 27 of the study, the combination treatment group had six mice with tumors below 600mm^3 in size, as compared to two, two, and two mice with tumors below 600mm^3 in size in the vehicle, anti-PD-L1 single agent, and anti-SIRP-α single agent treatment groups, respectively.

[0243] Next, the anti-tumor activities of the AB25b anti-SIRP-α antibody were tested alone and in combination with an anti-PD-1 antibody in the MC38 syngeneic mouse colon carcinoma model. As shown in **FIG. 6**, combining AB25b (at 10mg/kg) with anti-PD-1 at 5mg/kg delayed tumor formation when compared to treatment with each single agent or vehicle control. On day 27 of the study, the AB25b/PD-1 combination treatment group had seven mice with tumors below 600mm^3 in size, as compared to one, five, and two mice with tumors below 600mm^3 in size in the vehicle, anti-PD-1 single agent, and AB25b single agent treatment groups, respectively.

[0244] A summary of antibodies described herein and their properties is provided in Table K. Additional binding data are provided in Tables L and N.

Example 6: Novel anti-SIRP-α antibody light chains engineered to remove potential liability hot spots

[0245] Due to the properties of blocking anti-SIRP-α antibodies AB21 and AB25 described above, an antibody comprising the variant AB21 VH domain (SEQ ID NO:26) and the humanized Hum1 VL domain (SEQ ID NO:25) was selected for analysis. The sequence of antibody light chains comprising the Hum1 humanized variable light chain domain described above was analyzed for potential liability hot spots, *e.g.*, deamidation or glycation sites.

[0246] Protein deamidation is a post-translational modification in which the side chain amide of a glutamine or asparagine residue is converted into an acidic carboxylate group. Non-enzymatic deamidation of asparagine is faster than that of glutamine, and hence presents a higher physiological significance and greater potential liability risk in the manufacturing and storage of polypeptide-based therapeutics.

[0247] Glycation refers to the non-enzymatic glycosylation or Mallard reaction of proteins, which primarily occurs at the ε-amino group of lysine, or a free amino group. The side chains of arginine, histidine, tryptophan, and cysteine residues represent additional potential glycation sites. Amadori-modified proteins are an early glycation product and undergo further reactions that give rise to advance glycation end products (AGEs).

[0248] The analyses of the Hum1-containing light chains identified sites where engineering may be desired to limit risk due, *e.g.*, to modifications that may occur during manufacturing, storage, and/or drug development of anti-SIRP-α antibodies. This Example describes the testing and construction of Hum1 variants that remove these potential liabilities.

Methods

Peptide mapping analysis

[0249] For trypsin digests, samples were diluted in 6M Guanidine HCl and 1mM EDTA. 10mM DTT and 10mM iodoacetamide were used to reduce and alkylate the samples, respectively. The buffer was than exchanged to 0.1M Tris-HCl and samples were incubated for 4 hours for digestion. For chymotrypsin digests, samples were diluted in 100mM ammonium bicarbonate. 1% progenta anionic acid labile surfactant was added. Again, 10mM DTT and 10mM iodoacetamide were used to reduce and alkylate the samples respectively. Samples were incubated overnight.

[0250] The mass spectral data were acquired by Waters Acuity UHPLC in line with a Q Exactive Hybrid Quadrupole-Orbitrap (Thermo Scientific, San Jose CA). Column used is Agilent AdvanceBio Peptide Mapping (C18, 1x150mm ID). Reversed phase solvents were used and the gradient used was 2% to 40% buffer over 41 minutes. Full MS scan range was 250-2000m/z. Peptide searches and relative abundance was analysed using Byonic and Byologic from Protein Metrics. Precursor Mass Accuracy of 10ppm and fragment mass accuracy of 20ppm was adopted.

Determination of K_D

[0251] The interactions of anti-SIRPa antibodies with SIPRa from various species (human v1, human v2, cynomolgus, mouse 129, BL6, BALBc, NOD), SIRPb and SIRPg were analyzed using direct immobilization of the antibodies. All experiments were performed at 25°C using a SPR-based ProteOn XPR36 biosensor (BioRad, Inc, Hercules, CA) equipped with GLC sensor chips. Antibodies were expressed using FreeStyle™ 293-FS cells (Thermo Fisher). Purification was carried out by standard Protein A affinity column chromatography and eluted antibodies were stored in PBS buffer.

[0252] The running buffer was PBS pH 7.4 with 0.01% Tween-20 (PBST+). All analytes were used at their nominal concentrations as determined by A280 Absorbance and using their

molar calculated extinction coefficient. Analytes were injected in a "one-shot" kinetic mode as described elsewhere (*see, e.g.*, Bravman, T. *et al.* (2006) *Anal. Biochem.* 358:281-288).

[0253] For immobilization using GLC chip, the analytes were injected and flowed over anti-SIRP-α antibodies immobilized (~1000 RUs) on GLC chips using Proteon Amine Coupling Kit. For the immobilization step, GLC chip was activated with EDAC/Sulpho-NHS 1:1 (Biorad) diluted 1/100 for 300s at 25 μL/min. Anti-SIRP-α antibodies were diluted to 80 nM concentration in 10 mM sodium acetate buffer pH 4.5 and immobilized to the chip at 30 μL/min for 50s. Chip was inactivated with ethanolamine for 300s at 25 μL/min. The analytes (*e.g.*, SIRP-α from different species, SIRP-β, SIRP-γ) were injected in a "one-shot" kinetic mode at nominal concentrations of 100, 33, 11, 3.7, 1.2 and 0 nM. Association times were monitored for 90s at 100uL/min, and dissociation times were monitored for 1200s. The surfaces were regenerated with a 2:1 v/v blend of Pierce IgG elution buffer/4M NaCl.

[0254] Biosensor data were double-referenced by subtracting the interspot data (containing no immobilized protein) from the reaction spot data (immobilized protein) and then subtracting the response of a buffer "blank" analyte injection from that of an analyte injection. Double- referenced data were fit globally to a simple Langmuir model and the K_D value was calculated from the ratio of the apparent kinetic rate constants ($K_D = k_d/k_a$).

Results

[0255] An anti-SIRP-α antibody (antibody PC336) comprising a light chain (SEQ ID NO:47) with the Huml VL domain (SEQ ID NO:25) and a human IGLC1 lambda constant domain (SEQ ID NO:37), and a heavy chain (SEQ ID NO:61) with the AB21 MutAll VH domain (SEQ ID NO:26) and a constant region (SEQ ID NO:34) comprising a human IgG2Da Fc region (comprising A330S and P331S mutations, amino acid position numbering according to EU) was carried out by trypsin and chymotrypsin digestion as described above. Overall sequence coverage of heavy chain was 100% (444 out of 444 amino acids), and light chain was 98.6% (211 out of 214 amino acids). Variable domain and full chain sequences for all antibodies are provided in Tables J1 and J2.

[0256] These analyses revealed three light chain residues that were modified by glycation. Table F shows the glycation observed in the light chain. A total of 3 glycated peptides were isolated. Of these, it was observed that ~48% of the peptide with the sequence of SEQ ID NO:65 was glycated. The glycation modification was assigned to K104 (numbering based on sequential numbering of amino acids for light chain, not Kabat). **FIG.**

7 shows the total extracted ion chromatography, area under the curve (XIC, AUC) of the glycated versus unmodified form of peptides of antibody PC336.

Table F. Glycated peptides from Hum1-containing light chain of antibody PC336.

Sequence of peptide	**Modification / Names**	**Modified AAs**	**AA position**	**Trypsin digested peptide**	**%**
ADGSPVKAGVETTK PSK (SEQ ID NO:64)				Unmodified	99.5
	Hex/162.0528	K	158	Glycated	0.521
FSGSSSGTTVTLTISG VQAEDEADYYCGGY DQSSYTNPFGGGTK (SEQ ID NO:65)				Unmodified	52.1
	Hex/162.0528	K	104	Glycated	47.9
ITCSGGSYSSYYYAW YQQKPGQAPVTLIYS DDKRPSNIPER (SEQ ID NO:66)				Unmodified	99
	Hex/162.0528	K	38	Glycated	1.03

[0257] Peptide mapping of a second anti-SIRP-α antibody (antibody PC333) comprising the same light chain (SEQ ID NO:47) and a heavy chain (SEQ ID NO:58) with the AB21 MutAll VH domain (SEQ ID NO:26) and a constant region (SEQ ID NO:31) comprising a wild-type human IgG1 Fc region was also carried out. Similar to what was observed for antibody 336, the lysine residue at position 104 of the light chain was also observed to be glycated in this antibody (~34% of peptides; *see* Table G).

Table G. Glycated peptide from Hum1-containing light chain of antibody PC333.

Position	**Modification/ Names**	**Modified AAs**	**Peptides**	**XIC**	**%**
104			Native	2.770E+8	66.02
	Hex/162.0528	K	Modified	1.430E+8	33.98

[0258] Peptide mapping also revealed the presence of deamidated residues in the Hum1-containing light chain of antibody PC301, which comprises a light chain (SEQ ID NO:63)

with the Huml VL domain (SEQ ID NO:25) and a human IGLC2 lambda constant domain (SEQ ID NO:38), and a heavy chain (SEQ ID NO:59) with the AB21 MutAll VH domain (SEQ ID NO:26) and a constant region (SEQ ID NO:32) comprising a human IgG1 AAA N297A Fc region (comprising L234A, L235A, G237A, and N297A mutations, amino acid position numbering according to EU). As shown in **FIG. 8**, various sites in the light chain and heavy chain of antibody PC301 were observed to be deamidated. In particular, approximately 50% of peptides containing the N171 and N172 residues of the light chain constant domain were found to be modified by deamidation. From peptide mapping, it was not possible to determine whether N171, N172, or both residues were deamidated, since these two residues are adjacent to each other and isolated in the same peptide.

[0259] Post-translational modifications such as deamidation and glycation are undesirable for drug development. This is due to potential issues with product heterogeneity during drug manufacture. Therefore, it is desirable to limit modifications even though these modifications did not appear to affect binding affinities of the anti-SIRP-α antibodies.

[0260] To remove the potentially deamidated residues (N171/N172), 4 deamidation site variants were first tested. Antibody PC334 contained the original Huml VL+IGLC1 light chain (SEQ ID NO:47). Antibody PC338 contained a light chain with the original Huml VL domain and the N172D variant constant domain (SEQ ID NO:48). Antibody PC339 contained a light chain with the original Huml VL domain and the N171D variant constant domain (SEQ ID NO:49). Antibody PC340 contained a light chain with the original Huml VL domain and the N171D,N172S deamidation site variant constant domain (SEQ ID NO:50). Antibody PC341 contained a light chain with the original Huml VL domain and the N171S,N172D deamidation site variant constant domain (SEQ ID NO:51). All antibodies had a heavy chain comprising the AB21HC mut all VH domain (SEQ ID NO:26) and a constant region (SEQ ID NO:32) comprising the human IgG1 AAA N297A Fc region (full heavy chain sequence as shown in SEQ ID NO:59). The results of the binding assay are shown in Table H. All four mutants bound with equivalent affinity to human SIRP-α v1, as compared with the wildtype antibody.

Table H. Binding of anti-SIRP-α antibodies with deamidation site variant Huml light chains to human SIRP-α v1.

Antibody	**Light Chain**	**Fc**	**K_D (M) for human SIRP v1**

PC334	Hum1_IGLC1	IgG1_AAA_dead*	<1E-12
PC338	Hum1_IGLC1_N172D	IgG1_AAA_dead	<1E-12
PC339	Hum1_IGLC1_N171D	IgG1_AAA_dead	<1E-12
PC340	Hum1_IGLC1_N171D, N172S	IgG1_AAA_dead	<1E-12
PC341	Hum1_IGLC1_N171S, N172D	IgG1_AAA_dead	<1E-12

* IgG1_AAA_dead refers to human IgG1 with L234A, L235A, G237A and N297A substitutions.

[0261] Next, the glycation site variants were tested. The region of the Hum1 VL domain with the glycation site is shown in **FIG. 9**. To remove this glycation site, three variants of this VL domain were created, labeled versions 1, 2, and 3 (v1, v2, and v3 are also used interchangeably herein). These variants mutate residues in and around the K104 glycation site. Versions 1 and 2 use sequences identical to the native human IGLJ1 and IGLJ7 sequence, respectively. Version 3 replaces the lysine with arginine to maintain the positive charge and size of residue. The glycation site variants were tested in the context of the N171S, N172D deamidation variants.

[0262] Antibody PC334 contained the original Hum1 VL+IGLC1 light chain (SEQ ID NO:47). Antibody PC341 contained a light chain with the original Hum1 VL domain and the N171S,N172D (abbreviated "SD") deamidation site variant constant domain (SEQ ID NO:51). Antibodies PC345, PC346, and PC347 also contained the SD deamidation site variant constant domain, but included the Hum1 variant 1, 2, and 3 VL domains, respectively (SEQ ID NOs:53, 55, and 57, respectively). All antibodies had a heavy chain comprising the AB21HC mut all VH domain (SEQ ID NO:26) and a constant region (SEQ ID NO:32) comprising the human IgG1 AAA N297A Fc region (full heavy chain sequence as shown in SEQ ID NO:59). The results of the binding assay are shown in Table 11.

Table 11. Binding of anti-SIRP-α antibodies with glycation site variant Hum1 light chains to human SIRP-α v1.

Antibody	**Light Chain**	**Fc**	**K_D (M) for human SIRP v1**
PC334	Hum1_IGLC1	IgG1_AAA_dead*	<1.0E-12
PC341	Hum1_IGLC1_N171S, N172D	IgG1_AAA_dead	<1.0E-12
PC345	Hum1_IGLC1_version 1	IgG1_AAA_dead	<1.0E-12

	+ SD		
PC346	Huml_IGLC1_version 2 + SD	IgG1_AAA_dead	<1.0E-12
PC347	Huml_IGLC1_version 3 + SD	IgG1_AAA_dead	<1.0E-12

* IgG1_AAA_dead refers to human IgG1 with L234A, L235A, G237A and N297A substitutions.

[0263] As shown in Tables H and I1, all antibodies bound with equivalent affinity to human SIRP-α v1, as compared with the wildtype antibody. These results demonstrate that the variants engineered to remove deamidation and glycation liability hot spots had no effect on binding to SIRP-α.

[0264] Based on the above results, 6 preferred light chain variants were generated, and the alignment of the respective sequences (SEQ ID NOs:52-57) are shown in **FIGS. 10 & 11**. They comprised combining two preferred deamidation site variants (N171D/N172S and N171S/N172D, abbreviated as "DS" and "SD," respectively) and the 3 glycation variants (v1, v2, v3). The original huml VL domain and human IGLC1 lambda constant region (SEQ ID NO:47) are also shown in the alignment.

Example 7: Effect of constant domain sequence on biological activities of anti-SIRP-α antibodies

[0265] Anti-SIRP-α antibodies with different Fc regions and light chain constant domains were tested for their effect on phagocytosis in order to understand how these sequences impact the biological properties of anti-SIRP-α antibodies.

Methods

[0266] For functional depletion of dendritic cells, peripheral blood mononuclear cells (PBMC) were isolated from Trima residuals of healthy individuals with Ficoll-Paque Plus. 500,000 PBMCs were incubated in u-bottom 96 well plates (corning) with anti-SIRP at a concentration of 10 ug/mL for 48 hrs at 37C. For flow cytometry, cells were incubated in human FcR blocking reagent and stained with a cocktail of fluorochrome-labeled antibodies against lin- (CD3, CD14, CD16, CD19, CD56) and HLADR. Fixable viability dye was used to identify live cells. After staining, cells were washed and fixed with 0.5% paraformaldehyde in PBS. Prior to acquisition, absolute counting beads were added and samples were acquired with Canto II flow cytometer and analyzed using FlowJo software.

[0267] Phagocytosis was measured using the *in vitro* assay described in Example 4.

Results

[0268] Humanized anti-SIRP-α antibodies with different Fc regions were tested for their effects on phagocytosis of EGFR(+) DLD-1 cells by M2 macrophages in combination with the anti-EGFR antibody cetuximab (**FIG. 12**). All antibodies had the light chain sequence of SEQ ID NO:55 and the VH domain sequence of SEQ ID NO:26. Fc regions tested included the IgG2 wild-type (as represented in the constant domain sequence of SEQ ID NO:33), IgG2Da Fc region (comprising A330S and P331S mutations, amino acid position numbering according to EU; as represented in the constant domain sequence of SEQ ID NO:34), IgG2 Fc region comprising an N297A mutation (amino acid position numbering according to EU; as represented in the constant domain sequence of SEQ ID NO:137), and IgG1 Fc region comprising N297A, L234A, L235A, and G237A mutations (amino acid position numbering according to EU; as represented in the constant domain sequence of SEQ ID NO:32). All antibodies were found to have approximately equivalent activity in enhancing cetuximab-induced phagocytosis.

[0269] Next, anti-SIRP-α antibodies with different Fc regions (or corresponding isotype controls) were tested for their ability to deplete different cell types from donor PBMCs. All antibodies had the light chain sequence of SEQ ID NO:55 and the VH domain sequence of SEQ ID NO:26. As shown in **FIGS. 13A & 13B**, anti-SIRP-α antibodies with wild-type IgG1, wild-type IgG4, wild-type IgG2, or IgG2Da Fc regions were able to deplete DCs from PBMCs obtained from two different donors. This indicates that these Fc regions have the ability to potentiate some DC depletion in the context of the PBMC assay (DCs are known to express SIRP-α). In contrast, only the L234A/L235A/G237A/N297A IgG1 and IgG2 N297A Fc regions did not show DC depletion. None of the antibodies tested led to significant depletion of T cells, monocytes, or B cells (**FIGS. 13C-13E**). Advantageously, the IgG2 N297A Fc region provides the same lack of depletion as the L234A/L235A/G237A/N297A IgG1 Fc region but with fewer mutations (and therefore potentially less immunogenicity).

[0270] Anti-SIRP-α antibodies were also tested for binding affinity to human SIRP-α v1; NOD, C57BL/6, and BALBc mouse SIRP-α; human SIRP-β; and human SIRP-γ. Antibodies tested included the following heavy and light chains: PC301: light chain of SEQ ID NO:63 and heavy chain of SEQ ID NO:59; PC334: light chain of SEQ ID NO:47 and heavy chain of

SEQ ID NO:59; and PC367: light chain of SEQ ID NO:55 and heavy chain of SEQ ID NO:129.

Table I2. Binding affinities of anti-SIRP-α antibodies to SIRP proteins (K_D, M).

Protein	hSIRPa v1	hSIRPa v2	Cyno	NOD	C57BL/6	BALBc	hSIRPb	hSIRPg
SEQ ID NO	5	6	11	8	9	10	13	15
PC301	2.06E-12	4.60E-12	3.89E-11	2.93E-09	9.25E-09	9.53E-09	1.22E-11	<1.00E-12
PC334	1.51E-11	4.38E-11	7.74E-11	8.19E-09	3.29E-09	6.75E-09	3.71E-11	4.52E-11
PC367	2.11E-12	8.23E-11	5.01E-11	6.37E-09	1.97E-08	7.87E-09	3.27E-11	3.52E-11

[0271] Human IgG2 antibodies are thought to exist in disulfide-based isoforms (A, B, and AB), and the introduction of C232S or C233S mutations into human IgG2 constant regions has been reported to reduce heterogeneity caused by disulfide shuffling (Lightle, S. *et al.* (2010) *Protein Sci.* 19:753-762). Each of these mutations is thought to force IgG2 antibodies into isoform A. In addition, the lambda light chain is also thought to promote isoform A abundance. Anti-SIRP-α antibodies with wild-type IgG2 (**FIG. 14A**) or IgG2 N297A (**FIG. 14B**) Fc regions comprising C232S or C233S mutations were tested for effects on phagocytosis of EGFR(+) DLD-1 cells by M2 macrophages in combination with the anti-EGFR antibody cetuximab. All antibodies had the light chain sequence of SEQ ID NO:55 and the VH domain sequence of SEQ ID NO:26. Wild-type and N297A Fc regions were tested using antibodies with the lambda light chain to drive isoform A predominance. IgG2 anti-SIRP-α antibodies comprising Fc regions with C232S or C233S mutations showed similar enhancement of phagocytosis, as compared to antibodies with wild-type IgG2 or IgG2 N297A Fc regions.

[0272] The effect of the light chain constant domain on the ability of anti-SIRP-α antibodies to enhance phagocytosis was also examined. All antibodies had the VH domain sequence of SEQ ID NO:26 and the VL domain sequence of SEQ ID NO:18. Anti-SIRP-α antibodies with lambda and kappa light chains showed similar enhancement of phagocytosis, both in the context of a wild-type IgG2 Fc- (**FIG. 15A**) or an IgG2da Fc- (**FIG. 15B**) containing heavy chain.

[0273] Although the foregoing disclosure has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the present disclosure. The

disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

Table J1. Variable domain sequences for selected antibodies.

Antibody	VH Domain	VL Domain
AB21	DVQLVESGGGVVRPGESLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWLAG ISAGGSDTYYPASVKGRFTISRDNSK NTLYLQMNTLTAEDTAVYYCARET WNHLFDYWGLGTLVTVSS (SEQ ID NO:73)	ALTQPASVSANPGETVKIACSGGDYYSY YYGWYQQKAPGSALVTVIYSDDKRPSDI PSRFSGSASGSTATLTITGVRAEDEAVYY CGGYDYSTYANAFGAGTTLTVL (SEQ ID NO:74)
AB25	DVQLVESGGGVVRPGESLRLSCEASG FTFSSNAMSWVRQAPGKGLEWVAGI SSGSDTYYGDSVKGRLTISRDNSKNIL YLQMNSLTAEDTAVYYCARETWNH LFDYWGLGTLVTVSS (SEQ ID NO:75)	ALTQPASVSANPGETVEITCSGGSYSSYY YAWYQQKSPGSAPVTLIYSDDKRPSNIP SRFSGSASGSTATLTITGVRAEDEAVYFC GGYDQSSYTNPFGAGTTLTVL (SEQ ID NO:76)
PC301	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	SYELTQPPSVSVSPGQTARITCSGGSYSS YYYAWYQQKPGQAPVTLIYSDDKRPSNI PERFSGSSSGTTVTLTISGVQAEDEADYY CGGYDQSSYTNPFGGGTKLTVL (SEQ ID NO:25)
PC333	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	SYELTQPPSVSVSPGQTARITCSGGSYSS YYYAWYQQKPGQAPVTLIYSDDKRPSNI PERFSGSSSGTTVTLTISGVQAEDEADYY CGGYDQSSYTNPFGGGTKLTVL (SEQ ID NO:25)
PC334	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	SYELTQPPSVSVSPGQTARITCSGGSYSS YYYAWYQQKPGQAPVTLIYSDDKRPSNI PERFSGSSSGTTVTLTISGVQAEDEADYY CGGYDQSSYTNPFGGGTKLTVL (SEQ ID NO:25)
PC336	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	SYELTQPPSVSVSPGQTARITCSGGSYSS YYYAWYQQKPGQAPVTLIYSDDKRPSNI PERFSGSSSGTTVTLTISGVQAEDEADYY CGGYDQSSYTNPFGGGTKLTVL (SEQ ID NO:25)
PC338	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	SYELTQPPSVSVSPGQTARITCSGGSYSS YYYAWYQQKPGQAPVTLIYSDDKRPSNI PERFSGSSSGTTVTLTISGVQAEDEADYY CGGYDQSSYTNPFGGGTKLTVL (SEQ ID NO:25)
PC339	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	SYELTQPPSVSVSPGQTARITCSGGSYSS YYYAWYQQKPGQAPVTLIYSDDKRPSNI PERFSGSSSGTTVTLTISGVQAEDEADYY CGGYDQSSYTNPFGGGTKLTVL (SEQ ID NO:25)
PC340	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA	SYELTQPPSVSVSPGQTARITCSGGSYSS YYYAWYQQKPGQAPVTLIYSDDKRPSNI

Antibody	VH Domain	VL Domain
	GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	PERFSGSSSGTTVTLTISGVQAEDEADYY CGGYDQSSYTNPFGGGTKLTVL (SEQ ID NO:25)
PC341	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	SYELTQPPSVSVSPGQTARITCSGGSYSS YYYAWYQQKPGQAPVTLIYSDDKRPSNI PERFSGSSSGTTVTLTISGVQAEDEADYY CGGYDQSSYTNPFGGGTKLTVL (SEQ ID NO:25)
PC342	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	SYELTQPPSVSVSPGQTARITCSGGSYSS YYYAWYQQKPGQAPVTLIYSDDKRPSNI PERFSGSSSGTTVTLTISGVQAEDEADYY CGGYDQSSYTNPFGTGTKVTVL (SEQ ID NO:39)
PC343	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	SYELTQPPSVSVSPGQTARITCSGGSYSS YYYAWYQQKPGQAPVTLIYSDDKRPSNI PERFSGSSSGTTVTLTISGVQAEDEADYY CGGYDQSSYTNPFGGGTQLTVL (SEQ ID NO:40)
PC344	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	SYELTQPPSVSVSPGQTARITCSGGSYSS YYYAWYQQKPGQAPVTLIYSDDKRPSNI PERFSGSSSGTTVTLTISGVQAEDEADYY CGGYDQSSYTNPFGGGTRLTVL (SEQ ID NO:41)
PC345	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	SYELTQPPSVSVSPGQTARITCSGGSYSS YYYAWYQQKPGQAPVTLIYSDDKRPSNI PERFSGSSSGTTVTLTISGVQAEDEADYY CGGYDQSSYTNPFGTGTKVTVL (SEQ ID NO:39)
PC346	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	SYELTQPPSVSVSPGQTARITCSGGSYSS YYYAWYQQKPGQAPVTLIYSDDKRPSNI PERFSGSSSGTTVTLTISGVQAEDEADYY CGGYDQSSYTNPFGGGTQLTVL (SEQ ID NO:40)
PC347	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	SYELTQPPSVSVSPGQTARITCSGGSYSS YYYAWYQQKPGQAPVTLIYSDDKRPSNI PERFSGSSSGTTVTLTISGVQAEDEADYY CGGYDQSSYTNPFGGGTRLTVL (SEQ ID NO:41)
AB21_HC_ mutAll + V1	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	SYELTQPPSVSVSPGQTARITCSGGSYSS YYYAWYQQKPGQAPVTLIYSDDKRPSNI PERFSGSSSGTTVTLTISGVQAEDEADYY CGGYDQSSYTNPFGTGTKVTVL (SEQ ID NO:39)
AB21_HC_	EVQLVESGGGVVQPGGSLRLSCAAS	SYELTQPPSVSVSPGQTARITCSGGSYSS

Antibody	VH Domain	VL Domain
mutAll + V2	GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	YYYAWYQQKPGQAPVTLIYSDDKRPSNI PERFSGSSSGTTVTLTISGVQAEDEADYY CGGYDQSSYTNPFGGGTQLTVL (SEQ ID NO:40)
AB21_HC_ mutAll + V3	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	SYELTQPPSVSVSPGQTARITCSGGSYSS YYYAWYQQKPGQAPVTLIYSDDKRPSNI PERFSGSSSGTTVTLTISGVQAEDEADYY CGGYDQSSYTNPFGGGTRLTVL (SEQ ID NO:41)
PC348	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	SYELTQPPSVSVSPGQTARITCSGGSYSS YYYAWYQQKPGQAPVTLIYSDDKRPSNI PERFSGSSSGTTVTLTISGVQAEDEADYY CGGYDQSSYTNPFGGGTQLTVL (SEQ ID NO:40)
PC349	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	SYELTQPPSVSVSPGQTARITCSGGSYSS YYYAWYQQKPGQAPVTLIYSDDKRPSNI PERFSGSSSGTTVTLTISGVQAEDEADYY CGGYDQSSYTNPFGGGTQLTVL (SEQ ID NO:40)
PC350A	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	ALTQPASVSANPGETVEITCSGGSYSSYY YAWYQQKSPGSAPVTLIYSDDKRPSNIP SRFSGSASGSTATLTITGVRAEDEAVYFC GGYDQSSYTNPFGAGTTLTVL (SEQ ID NO:18)
PC350B	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	ALTQPASVSANPGETVEITCSGGSYSSYY YAWYQQKSPGSAPVTLIYSDDKRPSNIP SRFSGSASGSTATLTITGVRAEDEAVYFC GGYDQSSYTNPFGAGTTLTVL (SEQ ID NO:18)
PC363	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	SYELTQPPSVSVSPGQTARITCSGGSYSS YYYAWYQQKPGQAPVTLIYSDDKRPSNI PERFSGSSSGTTVTLTISGVQAEDEADYY CGGYDQSSYTNPFGGGTQLTVL (SEQ ID NO:40)
PC364	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	SYELTQPPSVSVSPGQTARITCSGGSYSS YYYAWYQQKPGQAPVTLIYSDDKRPSNI PERFSGSSSGTTVTLTISGVQAEDEADYY CGGYDQSSYTNPFGGGTQLTVL (SEQ ID NO:40)
PC367	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	SYELTQPPSVSVSPGQTARITCSGGSYSS YYYAWYQQKPGQAPVTLIYSDDKRPSNI PERFSGSSSGTTVTLTISGVQAEDEADYY CGGYDQSSYTNPFGGGTQLTVL (SEQ ID NO:40)

Antibody	VH Domain	VL Domain
PC369	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSS (SEQ ID NO:26)	SYELTQPPSVSVSPGQTARITCSGGSYSS YYYAWYQQKPGQAPVTLIYSDDKRPSNI PERFSGSSSGTTVTLTISGVQAEDEADYY CGGYDQSSYTNPFGGGTQLTVL (SEQ ID NO:40)

Table J2. Full chain sequences for selected antibodies.

Antibody	Heavy chain	Light chain
PC301	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCP PCPAPEAAGAPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVS VLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG (SEQ ID NO:59)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTKL TVLGQPKAAPSVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADSSP VKAGVETTTPSKQSNNKYAASSYLS LTPEQWKSHRSYSCQVTHEGSTVEK TVAPTECS (SEQ ID NO:63)
PC333	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG (SEQ ID NO:58)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTKL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSNNKYAASSYLS LTPEQWKSHRSYSCQVTHEGSTVEK TVAPTECS (SEQ ID NO:47)
PC334	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTKL TVLGQPKANPTVTLFPPSSEELQANK

Antibody	Heavy chain	Light chain
	VFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCP PCPAPEAAGAPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVS VLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG (SEQ ID NO:59)	ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSNNKYAASSYLS LTPEQWKSHRSYSCQVTHEGSTVEK TVAPTECS (SEQ ID NO:47)
PC336	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKCCVECPPCP APPVAGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTFRVVSVLT VVHQDWLNGKEYKCKVSNKGLPSSI EKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPMLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG (SEQ ID NO:61)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTKL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSNNKYAASSYLS LTPEQWKSHRSYSCQVTHEGSTVEK TVAPTECS (SEQ ID NO:47)
PC338	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCP PCPAPEAAGAPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVS VLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG (SEQ ID NO:59)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTKL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSNDKYAASSYLS LTPEQWKSHRSYSCQVTHEGSTVEK TVAPTECS (SEQ ID NO:48)
PC339	EVQLVESGGGVVQPGGSLRLSCAAS	SYELTQPPSVSVSPGQTARITCSGGSY

Antibody	Heavy chain	Light chain
	GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCP PCPAPEAAGAPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVS VLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG (SEQ ID NO:59)	SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTKL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSDNKYAASSYLS LTPEQWKSHRSYSCQVTHEGSTVEK TVAPTECS (SEQ ID NO:49)
PC340	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCP PCPAPEAAGAPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVS VLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG (SEQ ID NO:59)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTKL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSDSKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:50)
PC341	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCP PCPAPEAAGAPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVS VLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFL	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTKL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:51)

Antibody	Heavy chain	Light chain
	YSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG (SEQ ID NO:59)	
PC342	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKCCVECPPCP APPVAGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTFRVVSVLT VVHQDWLNGKEYKCKVSNKGLPSSI EKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPMLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG (SEQ ID NO:61)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGTGTKV TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:53)
PC343	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKCCVECPPCP APPVAGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTFRVVSVLT VVHQDWLNGKEYKCKVSNKGLPSSI EKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPMLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG (SEQ ID NO:61)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTQL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:55)
PC344	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKCCVECPPCP APPVAGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTFRVVSVLT VVHQDWLNGKEYKCKVSNKGLPSSI EKTISKTKGQPREPQVYTLPPSREEM	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTRL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:57)

Antibody	Heavy chain	Light chain
	TKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPMLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG (SEQ ID NO:61)	
PC345	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCP PCPAPEAAGAPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVS VLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG (SEQ ID NO:59)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGTGTKV TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:53)
PC346	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCP PCPAPEAAGAPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVS VLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG (SEQ ID NO:59)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTQL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:55)
PC347	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCP PCPAPEAAGAPSVFLFPPKPKDTLMIS	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTRL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:57)

Antibody	Heavy chain	Light chain
	RTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVS VLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG (SEQ ID NO:59)	
AB21_HC_mut All (IgG1 wt)+ V1+DS	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG (SEQ ID NO:58)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGTGTKV TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSDSKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:52)
AB21_HC_mut All (IgG1 wt) + V1+SD	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG (SEQ ID NO:58)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGTGTKV TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:53)
AB21_HC_mut All (IgG1 wt) + V2+DS	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTQL TVLGQPKANPTVTLFPPSSEELQANK

Antibody	Heavy chain	Light chain
	VFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG (SEQ ID NO:58)	ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSDSKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:54)
AB21_HC_mut All (IgG1 wt) + V2+SD	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG (SEQ ID NO:58)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTQL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:55)
AB21_HC_mut All (IgG1 wt) + V3+DS	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG (SEQ ID NO:58)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTRL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSDSKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:56)

Antibody	Heavy chain	Light chain
AB21_HC_mut All (IgG1 wt) + V3+SD	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG (SEQ ID NO:58)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTRL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:57)
AB21_HC_mut All (IgG1 AAA dead)+ V1+DS	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCP PCPAPEAAGAPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVS VLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG (SEQ ID NO:59)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGTGTKV TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSDSKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:52)
AB21_HC_mut All (IgG1 AAA dead) + V1+SD	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCP PCPAPEAAGAPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVS VLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVE	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGTGTKV TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:53)

Antibody	Heavy chain	Light chain
	WESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG (SEQ ID NO:59)	
AB21_HC_mut All (IgG1 AAA dead) + V2+DS	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCP PCPAPEAAGAPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVS VLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG (SEQ ID NO:59)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTQL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSDSKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:54)
AB21_HC_mut All (IgG1 AAA dead) + V2+SD	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCP PCPAPEAAGAPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVS VLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG (SEQ ID NO:59)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTQL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:55)
AB21_HC_mut All (IgG1 AAA dead) + V3+DS	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCP PCPAPEAAGAPSVFLFPPKPKDTLMIS	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTRL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSDSKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:56)

Antibody	Heavy chain	Light chain
	RTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVS VLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG (SEQ ID NO:59)	
AB21_HC_mut All (IgG1 AAA dead) + V3+SD	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCP PCPAPEAAGAPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVS VLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG (SEQ ID NO:59)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTRL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:57)
AB21_HC_mut All (IgG2 wt)+ V1+DS	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKCCVECPPCP APPVAGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTFRVVSVLT VVHQDWLNGKEYKCKVSNKGLPAPI EKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPMLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG (SEQ ID NO:60)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGTGTKV TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSDSKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:52)
AB21_HC_mut All (IgG2 wt) + V1+SD	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGTGTKV TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP

Antibody	Heavy chain	Light chain
	PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKCCVECPPCP APPVAGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTFRVVSVLT VVHQDWLNGKEYKCKVSNKGLPAPI EKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPMLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG (SEQ ID NO:60)	VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:53)
AB21_HC_mut All (IgG2 wt) + V2+DS	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKCCVECPPCP APPVAGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTFRVVSVLT VVHQDWLNGKEYKCKVSNKGLPAPI EKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPMLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG (SEQ ID NO:60)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTQL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSDSKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:54)
AB21_HC_mut All (IgG2 wt) + V2+SD	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKCCVECPPCP APPVAGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTFRVVSVLT VVHQDWLNGKEYKCKVSNKGLPAPI EKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPMLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG (SEQ ID NO:60)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTQL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:55)
AB21_HC_mut All (IgG2 wt) + V3+DS	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTRL TVLGQPKANPTVTLFPPSSEELQANK

Antibody	Heavy chain	Light chain
	VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKCCVECPPCP APPVAGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTFRVVSVLT VVHQDWLNGKEYKCKVSNKGLPAPI EKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPMLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG (SEQ ID NO:60)	ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSDSKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:56)
AB21_HC_mut All (IgG2 wt) + V3+SD	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKCCVECPPCP APPVAGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTFRVVSVLT VVHQDWLNGKEYKCKVSNKGLPAPI EKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPMLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG (SEQ ID NO:60)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTRL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:57)
AB21_HC_mut All (IgG2 Da)+ V1+DS	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKCCVECPPCP APPVAGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTFRVVSVLT VVHQDWLNGKEYKCKVSNKGLPSSI EKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPMLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG (SEQ ID NO:61)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGTGTKV TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSDSKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:52)
AB21_HC_mut All (IgG2 Da) + V1+SD	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGTGTKV

Antibody	Heavy chain	Light chain
	WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKCCVECPPCP APPVAGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTFRVVSVLT VVHQDWLNGKEYKCKVSNKGLPSSI EKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPMLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG (SEQ ID NO:61)	TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:53)
AB21_HC_mut All (IgG2 Da) + V2+DS	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKCCVECPPCP APPVAGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTFRVVSVLT VVHQDWLNGKEYKCKVSNKGLPSSI EKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPMLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG (SEQ ID NO:61)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTQL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSDSKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:54)
AB21_HC_mut All (IgG2 Da) + V2+SD	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKCCVECPPCP APPVAGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTFRVVSVLT VVHQDWLNGKEYKCKVSNKGLPSSI EKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPMLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG (SEQ ID NO:61)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTQL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:55)
AB21_HC_mut All (IgG2 Da) + V3+DS	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE

Antibody	Heavy chain	Light chain
	KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKCCVECPPCP APPVAGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTFRVVSVLT VVHQDWLNGKEYKCKVSNKGLPSSI EKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPMLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG (SEQ ID NO:61)	DEADYYCGGYDQSSYTNPFGGGTRL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSDSKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:56)
AB21_HC_mut All (IgG2 Da) + V3+SD	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKCCVECPPCP APPVAGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTFRVVSVLT VVHQDWLNGKEYKCKVSNKGLPSSI EKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPMLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG (SEQ ID NO:61)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTRL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:57)
AB21_HC_mut All (IgG4 S228P)+ V1+DS	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTKTYTCNV DHKPSNTKVDKRVESKYGPPCPPCP APEFLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSQEDPEVQFNWYVDG VEVHNAKTKPREEQFNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKGLPSS IEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYS RLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLG (SEQ ID NO:62)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGTGTKV TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSDSKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:52)
AB21_HC_mut All (IgG4	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK

Antibody	Heavy chain	Light chain
S228P) + V1+SD	GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTKTYTCNV DHKPSNTKVDKRVESKYGPPCPPCP APEFLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSQEDPEVQFNWYVDG VEVHNAKTKPREEQFNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKGLPSS IEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYS RLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLG (SEQ ID NO:62)	RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGTGTKV TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:53)
AB21_HC_mut All (IgG4 S228P) + V2+DS	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTKTYTCNV DHKPSNTKVDKRVESKYGPPCPPCP APEFLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSQEDPEVQFNWYVDG VEVHNAKTKPREEQFNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKGLPSS IEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYS RLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLG (SEQ ID NO:62)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTQL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSDSKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:54)
AB21_HC_mut All (IgG4 S228P) + V2+SD	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTKTYTCNV DHKPSNTKVDKRVESKYGPPCPPCP APEFLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSQEDPEVQFNWYVDG VEVHNAKTKPREEQFNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKGLPSS IEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYS RLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLG (SEQ ID NO:62)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTQL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:55)
AB21_HC_mut	EVQLVESGGGVVQPGGSLRLSCAAS	SYELTQPPSVSVSPGQTARITCSGGSY

Antibody	Heavy chain	Light chain
All (IgG4 S228P) + V3+DS	GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTKTYTCNV DHKPSNTKVDKRVESKYGPPCPPCP APEFLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSQEDPEVQFNWYVDG VEVHNAKTKPREEQFNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKGLPSS IEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYS RLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLG (SEQ ID NO:62)	SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTRL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSDSKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:56)
AB21_HC_mut All (IgG4 S228P) + V3+SD	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTKTYTCNV DHKPSNTKVDKRVESKYGPPCPPCP APEFLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSQEDPEVQFNWYVDG VEVHNAKTKPREEQFNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKGLPSS IEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYS RLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLG (SEQ ID NO:62)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTRL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:57)
PC348	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTKTYTCNV DHKPSNTKVDKRVESKYGPPCPPCP APEFLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSQEDPEVQFNWYVDG VEVHNAKTKPREEQFNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKGLPSS IEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYS RLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLG (SEQ ID NO:62)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTQL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:55)

Antibody	Heavy chain	Light chain
PC349	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKCCVECPPCP APPVAGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTFRVVSVLT VVHQDWLNGKEYKCKVSNKGLPAPI EKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPMLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG (SEQ ID NO:60)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTQL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:55)
PC350A	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKCCVECPPCP APPVAGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTFRVVSVLT VVHQDWLNGKEYKCKVSNKGLPSSI EKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPMLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG (SEQ ID NO:61)	ALTQPASVSANPGETVEITCSGGSYSS YYYAWYQQKSPGSAPVTLIYSDDKR PSNIPSRFSGSASGSTATLTITGVRAE DEAVYFCGGYDQSSYTNPFGAGTTL TVLRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSTYSLSSTLTLS KADYEKHKVYACEVTHQGLSSPVTK SFNRGEC (SEQ ID NO:140)
PC350B	EVQLVESGGGVVQPGGSLRLSCAAS GFTFSSNAMSWVRQAPGKGLEWVA GISAGGSDTYYPASVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARET WNHLFDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKCCVECPPCP APPVAGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTFRVVSVLT VVHQDWLNGKEYKCKVSNKGLPAPI EKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPMLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEAL	ALTQPASVSANPGETVEITCSGGSYSS YYYAWYQQKSPGSAPVTLIYSDDKR PSNIPSRFSGSASGSTATLTITGVRAE DEAVYFCGGYDQSSYTNPFGAGTTL TVLRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSTYSLSSTLTLS KADYEKHKVYACEVTHQGLSSPVTK SFNRGEC (SEQ ID NO:140)

Antibody	Heavy chain	Light chain
	HNHYTQKSLSLSPG (SEQ ID NO:60)	
PC363	EVQLVESGGGVVQPGGSLRLSCA ASGFTFSSNAMSWVRQAPGKGLE WVAGISAGGSDTYYPASVKGRFT ISRDNSKNTLYLQMNSLRAEDTA VYYCARETWNHLFDYWGQGTLV TVSSASTKGPSVFPLAPCSRSTSES TAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSV VTVPSSNFGTQTYTCNVDHKPSN TKVDKTVERKSCVECPPCPAPPV AGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVQFNWYVDG VEVHNAKTKPREEQFNSTFRVVS VLTVVHQDWLNGKEYKCKVSNK GLPAPIEKTISKTKGQPREPQVYT LPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPP MLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLS LSPG (SEQ ID NO:125)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTQL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:55)
PC364	EVQLVESGGGVVQPGGSLRLSCA ASGFTFSSNAMSWVRQAPGKGLE WVAGISAGGSDTYYPASVKGRFT ISRDNSKNTLYLQMNSLRAEDTA VYYCARETWNHLFDYWGQGTLV TVSSASTKGPSVFPLAPCSRSTSES TAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSV VTVPSSNFGTQTYTCNVDHKPSN TKVDKTVERKCSVECPPCPAPPV AGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVQFNWYVDG VEVHNAKTKPREEQFNSTFRVVS VLTVVHQDWLNGKEYKCKVSNK GLPAPIEKTISKTKGQPREPQVYT LPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPP MLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLS LSPG (SEQ ID NO:126)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTQL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:55)
PC367	EVQLVESGGGVVQPGGSLRLSCA ASGFTFSSNAMSWVRQAPGKGLE WVAGISAGGSDTYYPASVKGRFT ISRDNSKNTLYLQMNSLRAEDTA VYYCARETWNHLFDYWGQGTLV TVSSASTKGPSVFPLAPCSRSTSES TAALGCLVKDYFPEPVTVSWNSG	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTQL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT

Antibody	Heavy chain	Light chain
	ALTSGVHTFPAVLQSSGLYSLSSV VTVPSSNFGTQTYTCNVDHKPSN TKVDKTVERKCCVECPPCPAPPV AGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVQFNWYVDG VEVHNAKTKPREEQFASTFRVVS VLTVVHQDWLNGKEYKCKVSNK GLPAPIEKTISKTKGQPREPQVYT LPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPP MLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLS LSPG (SEQ ID NO:129)	VAPTECS (SEQ ID NO:55)
PC369	EVQLVESGGGVVQPGGSLRLSCA ASGFTFSSNAMSWVRQAPGKGLE WVAGISAGGSDTYYPASVKGRFT ISRDNSKNTLYLQMNSLRAEDTA VYYCARETWNHLFDYWGQGTLV TVSSASTKGPSVFPLAPCSRSTSES TAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSV VTVPSSNFGTQTYTCNVDHKPSN TKVDKTVERKCSVECPPCPAPPV AGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVQFNWYVDG VEVHNAKTKPREEQFASTFRVVS VLTVVHQDWLNGKEYKCKVSNK GLPAPIEKTISKTKGQPREPQVYT LPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPP MLD (SEQ ID NO:130)	SYELTQPPSVSVSPGQTARITCSGGSY SSYYYAWYQQKPGQAPVTLIYSDDK RPSNIPERFSGSSSGTTVTLTISGVQAE DEADYYCGGYDQSSYTNPFGGGTQL TVLGQPKANPTVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADGSP VKAGVETTKPSKQSSDKYAASSYLSL TPEQWKSHRSYSCQVTHEGSTVEKT VAPTECS (SEQ ID NO:55)

Table K. Anti-SIRP-α antibody summary.

Antibody	Type of Binding	In vitro phago	In vivo mouse	Species Binding (+/-) (Koff)			Human Isoforms (+/-) (Koff)		K_D Human V1 (SEQ ID NO:5)	K_D Human V2 (SEQ ID NO:6)	Heavy Chain	Light Chain
		(+/-)	(+/-)	Human v1 (SEQ ID NO:5)	Cyno (SEQ ID NO:11)	Mouse 129 (SEQ ID NO:7)	Beta (SEQ ID NO:13)	Gamma (SEQ ID NO:15)			(Human/ Chicken)	(Human/ Chicken)
21	Blocker	+	+	+	+	+	+	+	<1.0E-12	<1.0E-12	Human	Chicken
25	Blocker	+	+	+	+	+	+	+	<1.0E-12	<1.0E-12	Human	Chicken

Table L. Anti-SIRP-α antibody humanization summary (round 1).

Antibody Designation	VL	VH	Koff (1/s)					In vitro phago	In vivo mouse
			SEQ ID NO:5	SEQ ID NO:6	SEQ ID NO:11	SEQ ID NO:10	SEQ ID NO:15	(+/-)	(+/-)
			Human V1	Human V2	Cyno	BALBc	SIRPg		
Parental Antibodies									
AB21	Chicken (AB21_LC_wt)	Human (AB21_HC_wt)	7.07E-04	1.92E-03	2.29E-03	2.41E-03	9.02E-04	NT	+
AB25	Chicken (AB25_LC_wt)	Human (AB25_HC_wt)	1.65E-04	3.53E-04	3.94E-04	1.78E-03	2.03E-04	+	+
Humanization of chicken light chain of AB25, AB66 - replaced with human IGLV3 framework									
Hum1 / AB21_HC_wt	Hum1___Humanized (AB25_IGLV3)	Human (AB21_HC_wt)	1.93E-04	3.03E-04	3.95E-04	2.91E-03	1.88E-04	+	
Hum1 / AB25_HC_wt	Hum1___Humanized (AB25_IGLV3)	Human (AB25_HC_wt)	1.33E-04	2.67E-04	3.30E-04	3.74E-03	2.03E-04	+	
Hum3 / AB21_HC_wt	Hum3___Humanized (AB66_IGLV3)	Human (AB21_HC_wt)	1.37E-04	4.38E-04	4.32E-04	2.31E-03	2.10E-04	NT	
Hum3 / AB25_HC_wt	Hum3___Humanized (AB66_IGLV3)	Human (AB25_HC_wt)	5.94E-05	3.53E-04	3.75E-04	2.27E-04	1.69E-04	NT	

NT or blank = not tested.

Table M. Anti-SIRP-α antibody humanization summary (round 2).

Antibody Designation	VL	VH	KD (M)									In vitro phago
			SEQ ID NO:5	SEQ ID NO:6	SEQ ID NO:11	SEQ ID NO:8	SEQ ID NO:9	SEQ ID NO:10	SEQ ID NO: 13	SEQ ID NO: 15	(+/-)
			Human V1	Human V2	Cyno	NOD	BL6	BALBc	SIRPb	sf-4[illegible]584 SIRPg	
Pairing of humanized light chain with heavy chain (Germline mut)											
Hum1 / AB21_HC_Mutall	Hum1_____ Humanized SEQ ID NO:25	Human (AB21_HC _Mutall) SEQ ID NO:26	5.32E-12	4.60E-12	2.91E-11	3.70E-09	9.50E-09	7.91E-09	6.7E-12	>1.0E-12	+
Mutation of humanized light chain to increase % humaness											
Hum8 / AB21_HC_Mutall	Hum8_____ Humanized (AB25_IGL V3) + 5aa in CDR	Human (AB21_HC _Mutall) SEQ ID NO:26	2.01E-11			2.78E-08	4.15E-04	7.12E-08			+
Hum9 / AB21_HC_Mutall	Hum9_____ Humanized (AB25_IGL V3) + 4aa in CDR	Human (AB21_HC _Mutall)	1.19E-11	1.19E-10	2.22E-10	2.41E-08	5.33E-04	1.36E-07	5.69E-11	3.45E-11	+

NT or blank = not tested.

Table N. Anti-SIRP-α antibody binding data summary. Values indicated are tested by SPR (K_{off}, 1/s).

Antibody	CV1-3	v1	v2	cyno1	cyno2	m129	NOD	BL6	SIRPb	SIRPg	
	SEQ ID NO:18	SEQ ID NO:5	SEQ ID NO:6	SEQ ID NO:11	SEQ ID NO:12	SEQ ID NO:7	SEQ ID NO:8	SEQ ID NO:9	SEQ ID NO:13	SEQ ID NO:15	CD47 blocking
S21	1.95E-04	1.80E-04	2.07E-04	2.33E-04	2.52E-04	2.81E-04	2.64E-03	8.06E-04	1.90E-04	1.84E-04	block
S25	1.40E-04	1.12E-04	2.09E-04	2.19E-04	2.12E-04	1.33E-04	7.79E-04	2.90E-04	1.71E-04	1.41E-04	block

CLAIMS

What is claimed is:

1. An isolated antibody that binds an extracellular domain of a human SIRP-α polypeptide, wherein the antibody comprises:

(a) a heavy chain comprising a heavy chain variable (VH) domain that comprises the amino acid sequence of SEQ ID NO:26; and

(b) a light chain comprising a light chain variable (VL) domain that comprises an amino acid sequence according to the formula SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQQKPGQAPVTLIYSDDKRPSNIP ERFSGSSSGTTVTLTISGVQAEDEADYYCGGYDQSSYTNPFGX$_1$GTX$_2$X$_3$TVL (SEQ ID NO:71), wherein X$_1$ is G or T; X$_2$ is K, Q, or R; and X$_3$ is L or V, and wherein the VL domain does not comprise the sequence of SEQ ID NO:25.

2. The antibody of claim 1, wherein the VL domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:39-41.

3. The antibody of claim 1 or claim 2, wherein the light chain further comprises a light chain constant (CL) domain sequence that comprises an amino acid sequence according to the formula GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTK PSKQSX$_4$X$_5$KYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO:72), wherein X$_4$X$_5$ is ND, DN, DS, or SD.

4. The antibody of claim 1 or claim 2, wherein the light chain further comprises a light chain constant (CL) domain sequence that comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:43-46.

5. The antibody of claim 1 or claim 2, wherein the light chain further comprises a kappa light chain constant (CL) domain.

6. The antibody of claim 5, wherein the light chain comprises the amino acid sequence of SEQ ID NO:36.

7. The antibody of claim 1 or claim 2, wherein the light chain further comprises a lambda light chain constant (CL) domain.

8. The antibody of claim 7, wherein the light chain comprises the amino acid sequence of SEQ ID NO:37 or SEQ ID NO:38.

9. The antibody of claim 1, wherein the light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:48-57.

10. The antibody of any one of claims 1-9, wherein the antibody is a scFv-Fc, single domain antibody, single heavy chain antibody, or single light chain antibody.

11. The antibody of any one of claims 1-9, wherein the antibody is a monoclonal antibody.

12. The antibody of any one of claims 1-9, wherein the heavy chain comprises a heavy chain constant domain that comprises an Fc region.

13. The antibody of claim 12, wherein the Fc region is a human Fc region selected from the group consisting of an IgG1 Fc region, an IgG2 Fc region, and an IgG4 Fc region.

14. The antibody of claim 12, wherein the Fc region is a human IgG1 Fc region.

15. The antibody of claim 12, wherein the Fc region is a human IgG1 Fc region comprising L234A, L235A, and G237A substitutions, amino acid position numbering according to EU.

16. The antibody of claim 15, wherein the Fc region further comprises an N297A substitution, amino acid position numbering according to EU.

17. The antibody of claim 12, wherein the Fc region is a human IgG2 Fc region.

18. The antibody of claim 12, wherein the Fc region is a human IgG2 Fc region comprising A330S and P331S substitutions, amino acid position numbering according to EU.

19. The antibody of claim 17 or claim 18, wherein the Fc region further comprises an N297A substitution, amino acid position numbering according to EU.

20. The antibody of claim 12, wherein the Fc region is a human IgG4 Fc region, and wherein the heavy chain comprises an S228P substitution, amino acid position numbering according to EU.

21. The antibody of claim 12, wherein the heavy chain constant domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:31-35.

22. The antibody of claim 12, wherein the heavy chain constant domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:33, 34, and 137.

23. The antibody of claim 12, wherein the heavy chain constant domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:132-139.

24. The antibody of claim 1, wherein the heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:58-62.

25. The antibody of claim 1, wherein the heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:60, 61, and 129.

26. The antibody of claim 1, wherein:

(a) the heavy chain comprises the amino acid sequence of SEQ ID NO:62, and the light chain comprises the amino acid sequence of SEQ ID NO:52;

(b) the heavy chain comprises the amino acid sequence of SEQ ID NO:62, and the light chain comprises the amino acid sequence of SEQ ID NO:53;

(c) the heavy chain comprises the amino acid sequence of SEQ ID NO:62, and the light chain comprises the amino acid sequence of SEQ ID NO:54;

(d) the heavy chain comprises the amino acid sequence of SEQ ID NO:62, and the light chain comprises the amino acid sequence of SEQ ID NO:55;

(e) the heavy chain comprises the amino acid sequence of SEQ ID NO:62, and the light chain comprises the amino acid sequence of SEQ ID NO:56; or

(f) the heavy chain comprises the amino acid sequence of SEQ ID NO:62, and the light chain comprises the amino acid sequence of SEQ ID NO:57.

27. The antibody of claim 1, wherein:

(a) the heavy chain comprises the amino acid sequence of SEQ ID NO:58, and the light chain comprises the amino acid sequence of SEQ ID NO:52;

(b) the heavy chain comprises the amino acid sequence of SEQ ID NO:59, and the light chain comprises the amino acid sequence of SEQ ID NO:52;

(c) the heavy chain comprises the amino acid sequence of SEQ ID NO:60, and the light chain comprises the amino acid sequence of SEQ ID NO:52;

(d) the heavy chain comprises the amino acid sequence of SEQ ID NO:61, and the light chain comprises the amino acid sequence of SEQ ID NO:52;

(e) the heavy chain comprises the amino acid sequence of SEQ ID NO:58, and the light chain comprises the amino acid sequence of SEQ ID NO:53;

(f) the heavy chain comprises the amino acid sequence of SEQ ID NO:59, and the light chain comprises the amino acid sequence of SEQ ID NO:53;

(g) the heavy chain comprises the amino acid sequence of SEQ ID NO:60, and the light chain comprises the amino acid sequence of SEQ ID NO:53;

(h) the heavy chain comprises the amino acid sequence of SEQ ID NO:61, and the light chain comprises the amino acid sequence of SEQ ID NO:53;

(i) the heavy chain comprises the amino acid sequence of SEQ ID NO:58, and the light chain comprises the amino acid sequence of SEQ ID NO:54;

(j) the heavy chain comprises the amino acid sequence of SEQ ID NO:59, and the light chain comprises the amino acid sequence of SEQ ID NO:54;

(k) the heavy chain comprises the amino acid sequence of SEQ ID NO:60, and the light chain comprises the amino acid sequence of SEQ ID NO:54;

(l) the heavy chain comprises the amino acid sequence of SEQ ID NO:61, and the light chain comprises the amino acid sequence of SEQ ID NO:54;

(m) the heavy chain comprises the amino acid sequence of SEQ ID NO:58, and the light chain comprises the amino acid sequence of SEQ ID NO:55;

(n) the heavy chain comprises the amino acid sequence of SEQ ID NO:59, and the light chain comprises the amino acid sequence of SEQ ID NO:55;

(o) the heavy chain comprises the amino acid sequence of SEQ ID NO:60, and the light chain comprises the amino acid sequence of SEQ ID NO:55;

(p) the heavy chain comprises the amino acid sequence of SEQ ID NO:61, and the light chain comprises the amino acid sequence of SEQ ID NO:55;

(q) the heavy chain comprises the amino acid sequence of SEQ ID NO:58, and the light chain comprises the amino acid sequence of SEQ ID NO:56;

(r) the heavy chain comprises the amino acid sequence of SEQ ID NO:59, and the light chain comprises the amino acid sequence of SEQ ID NO:56;

(s) the heavy chain comprises the amino acid sequence of SEQ ID NO:60, and the light chain comprises the amino acid sequence of SEQ ID NO:56;

(t) the heavy chain comprises the amino acid sequence of SEQ ID NO:61, and the light chain comprises the amino acid sequence of SEQ ID NO:56;

(u) the heavy chain comprises the amino acid sequence of SEQ ID NO:58, and the light chain comprises the amino acid sequence of SEQ ID NO:57;

(v) the heavy chain comprises the amino acid sequence of SEQ ID NO:59, and the light chain comprises the amino acid sequence of SEQ ID NO:57;

(w) the heavy chain comprises the amino acid sequence of SEQ ID NO:60, and the light chain comprises the amino acid sequence of SEQ ID NO:57; or

(x) the heavy chain comprises the amino acid sequence of SEQ ID NO:61, and the light chain comprises the amino acid sequence of SEQ ID NO:57.

28. The antibody of claim 1, wherein:

(a) the heavy chain comprises the amino acid sequence of SEQ ID NO:60, and the light chain comprises the amino acid sequence of SEQ ID NO:55;

(b) the heavy chain comprises the amino acid sequence of SEQ ID NO:61, and the light chain comprises the amino acid sequence of SEQ ID NO:55;

(c) the heavy chain comprises the amino acid sequence of SEQ ID NO:129, and the light chain comprises the amino acid sequence of SEQ ID NO:55;

(d) the heavy chain comprises the amino acid sequence of SEQ ID NO:124, and the light chain comprises the amino acid sequence of SEQ ID NO:52; or

(e) the heavy chain comprises the amino acid sequence of SEQ ID NO:124, and the light chain comprises the amino acid sequence of SEQ ID NO:55.

29. The antibody of any one of claims 1-28, wherein the antibody enhances phagocytosis by a macrophage expressing a human SIRP-α polypeptide.

30. The antibody of any one of claims 1-28, wherein the antibody enhances activation of a dendritic cell expressing a human SIRP-α polypeptide.

31. The antibody of any one of claims 1-30, wherein the antibody inhibits *in vivo* growth of a tumor that expresses CD47.

32. The antibody of any one of claims 1-31, wherein the antibody is conjugated to an agent.

33. The antibody of any one of claims 1-31, wherein the antibody is a bispecific antibody.

34. The antibody of claim 33, wherein the antibody comprises a first antigen binding domain that binds an extracellular domain of a human SIRP-α polypeptide and a second antigen binding domain that binds an antigen expressed by a cancer cell.

35. The antibody of claim 34, wherein the antigen expressed by the cancer cell is selected from the group consisting of CD19, CD20, CD22, CD30, CD33, CD38, CD52, CD56, CD70, CD74, CD79b, CD123, CD138, CS1/SLAMF7, Trop-2, 5T4, EphA4, BCMA, Mucin 1, Mucin 16, PD-L1, PTK7, STEAP1, Endothelin B Receptor, mesothelin, EGFRvIII, ENPP3, SLC44A4, GNMB, nectin 4, NaPi2b, LIV-1A, Guanylyl cyclase C, DLL3, EGFR, HER2,

VEGF, VEGFR, integrin αVβ3, integrin α5β1, MET, IGF1R, TRAILR1, TRAILR2, RANKL, FAP, Tenascin, Le^y, EpCAM, CEA, gpA33, PSMA, TAG72, a mucin, CAIX, EPHA3, folate receptor α, GD2, GD3, and an MHC/peptide complex comprising a peptide from NY-ESO-1/LAGE, SSX-2, a MAGE family protein, MAGE-A3, gp100/pmel17, Melan-A/MART-1, gp75/TRP1, tyrosinase, TRP2, CEA, PSA, TAG-72, immature laminin receptor, MOK/RAGE-1, WT-1, SAP-1, BING-4, EpCAM, MUC1, PRAME, survivin, BRCA1, BRCA2, CDK4, CML66, MART-2, p53, Ras, β-catenin, TGF-βRII, HPV E6, or HPV E7.

36. The antibody of claim 33, wherein the antibody comprises a first antigen binding domain that binds an extracellular domain of a human SIRP-α polypeptide and a second antigen binding domain that binds an antigen expressed by an immune cell.

37. The antibody of claim 36, wherein the antigen expressed by the immune cell is selected from the group consisting of BDCA2, BDCA4, ILT7, LILRB1, LILRB2, LILRB3, LILRB4, LILRB5, Siglec-3, Siglec-7, Siglec-9, Siglec-15, FGL-1, CD200, CD200R, CSF-1R, CD40, CD40L, CD163, CD206, DEC205, CD47, CD123, arginase, IDO, TDO, AhR, EP2, COX-2, CCR2, CCR-7, CXCR1, CX3CR1, CXCR2, CXCR3, CXCR4, CXCR7, TGF-β RI, TGF-β RII, c-Kit, CD244, L-selectin/CD62L, CD11b, CD11c, CD68, 41BB, CTLA4, PD1, PD-L1, PD-L2, TIM-3, BTLA, VISTA, LAG-3, CD28, OX40, GITR, CD137, CD27, HVEM, CCR4, CD25, CD103, KIrg1, Nrp1, CD278, Gpr83, TIGIT, CD154, CD160, TNFR2, PVRIG, DNAM, and ICOS.

38. The antibody of claim 33, wherein the antibody comprises a first antigen binding domain that binds an extracellular domain of a human SIRP-α polypeptide and a second antigen binding domain that binds an antigen expressed by a natural killer (NK) cell.

39. The antibody of claim 38, wherein the antigen expressed by the NK cell is selected from the group consisting of NKR-P1A, CD94, KLRG1, KIR2DL5A, KIR2DL5B, KIR2DL1, KIR2DL2, KIR2DL3, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DS1, KIR2DS1, CD94, NKG2D, CD160, CD16, NKp46, NKp30, NKp44, DNAM1, CRTAM, CD27, NTB-A, PSGL1, CD96, CD100, NKp80, SLAMF7, and CD244.

40. A polynucleotide encoding the antibody of any one of claims 1-39.

41. A vector comprising the polynucleotide of claim 40.

42. A host cell comprising the polynucleotide of claim 40 or the vector of claim 41.

43. A method of producing an antibody, the method comprising culturing the host cell of claim 42 such that the antibody is produced.

44. The method of claim 43, further comprising recovering the antibody from the host cell.

45. A method of treating or delaying progression of cancer in an individual, the method comprising administering to the individual an effective amount of the antibody of any one of claims 1-39.

46. The method of claim 45, further comprising administering to the individual an effective amount of a second antibody that binds an antigen expressed by the cancer.

47. The method of claim 46, wherein the antigen expressed by the cancer is selected from the group consisting of CD19, CD20, CD22, CD30, CD33, CD38, CD52, CD56, CD70, CD74, CD79b, CD123, CD138, CS1/SLAMF7, Trop-2, 5T4, EphA4, BCMA, Mucin 1, Mucin 16, PTK7, STEAP1, Endothelin B Receptor, mesothelin, EGFRvIII, ENPP3, SLC44A4, GNMB, nectin 4, NaPi2b, LIV-1A, Guanylyl cyclase C, DLL3, EGFR, HER2, VEGF, VEGFR, integrin αVβ3, integrin α5β1, MET, IGF1R, TRAILR1, TRAILR2, RANKL, FAP, Tenascin, Le^y, EpCAM, CEA, gpA33, PSMA, TAG72, a mucin, CAIX, EPHA3, folate receptor α, GD2, GD3, and an MHC/peptide complex comprising a peptide from NY-ESO-1/LAGE, SSX-2, a MAGE family protein, MAGE-A3, gp100/pmel17, Melan-A/MART-1, gp75/TRP1, tyrosinase, TRP2, CEA, PSA, TAG-72, immature laminin receptor, MOK/RAGE-1, WT-1, SAP-1, BING-4, EpCAM, MUC1, PRAME, survivin, BRCA1, BRCA2, CDK4, CML66, MART-2, p53, Ras, β-catenin, TGF-βRII, HPV E6, or HPV E7.

48. The method of any one of claims 45-47, further comprising administering to the individual an effective amount of an immunotherapeutic agent.

49. The method of claim 48, wherein the immunotherapeutic agent comprises a second antibody.

50. The method of claim 49, wherein the second antibody binds to an antigen selected from the group consisting of BDCA2, BDCA4, ILT7, LILRB1, LILRB2, LILRB3, LILRB4,

LILRB5, Siglec-3, Siglec-7, Siglec-9, Siglec-15, FGL-1, CD200, CD200R, CSF-1R, CD40, CD40L, CD163, CD206, DEC205, CD47, CD123, arginase, IDO, TDO, AhR, EP2, COX-2, CCR2, CCR-7, CXCR1, CX3CR1, CXCR2, CXCR3, CXCR4, CXCR7, TGF-β RI, TGF-β RII, c-Kit, CD244, L-selectin/CD62L, CD11b, CD11c, CD68, 41BB, CTLA4, PD1, PD-L1, PD-L2, TIM-3, BTLA, VISTA, LAG-3, CD28, OX40, GITR, CD137, CD27, HVEM, CCR4, CD25, CD103, Klrg1, Nrp1, CD278, Gpr83, TIGIT, CD154, CD160, TNFR2, PVRIG, DNAM, and ICOS.

51. The method of claim 50, wherein the second antibody binds to PD-1.

52. The method of claim 50, wherein the second antibody binds to PD-L1.

53. The method of claim 48, wherein the immunotherapeutic agent comprises a vaccine, oncolytic virus, adoptive cell therapy, cytokine, or small molecule agent.

54. The method of any one of claims 45-53, further comprising administering to the individual an effective amount of a second antibody that binds an antigen expressed by a natural killer (NK) cell.

55. The method of claim 54, wherein the antigen expressed by the NK cell is selected from the group consisting of NKR-P1A, CD94, KLRG1, KIR2DL5A, KIR2DL5B, KIR2DL1, KIR2DL2, KIR2DL3, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DS1, KIR2DS1, CD94, NKG2D, CD160, CD16, NKp46, NKp30, NKp44, DNAM1, CRTAM, CD27, NTB-A, PSGL1, CD96, CD100, NKp80, SLAMF7, and CD244.

56. The method of any one of claims 45-55, further comprising administering to the individual an effective amount of a chemotherapeutic agent or small molecule anti-cancer agent.

57. The method of any one of claims 45-56, further comprising administering to the individual an effective amount of a targeted small molecule inhibitor.

58. The method of claim 57, wherein the targeted small molecule inhibitor is a VEGFR and/or PDGFR inhibitor, EGFR inhibitor, ALK inhibitor, CDK4/6 inhibitor, PARP inhibitor, mTOR inhibitor, KRAS inhibitor, TRK inhibitor, BCL2 inhibitor, IDH inhibitor, PI3K inhibitor, DNA damage response (DDR) inhibitor, or hypomethylation agent.

59. A method of treating or delaying progression of an autoimmune disease or an inflammatory disease in an individual, the method comprising administering to the individual an effective amount of the antibody of any one of claims 1-39.

60. The method of claim 59, wherein the autoimmune disease or inflammatory disease is selected from the group consisting of multiple sclerosis, rheumatoid arthritis, a spondyloarthropathy, systemic lupus erythematosus, an antibody-mediated inflammatory or autoimmune disease, graft versus host disease, sepsis, diabetes, psoriasis, psoriatic arthritis, atherosclerosis, Sjogren's syndrome, progressive systemic sclerosis, scleroderma, acute coronary syndrome, ischemic reperfusion, Crohn's Disease, ulcerative colitis, endometriosis, glomerulonephritis, IgA nephropathy, polycystic kidney disease, myasthenia gravis, idiopathic pulmonary fibrosis, pulmonary fibrosis, liver cirrhosis, atrial fibrosis, endomyocardial fibrosis, myelofibrosis, retroperitoneal fibrosis, asthma, atopic dermatitis, acute respiratory distress syndrome (ARDS), vasculitis, and inflammatory autoimmune myositis.

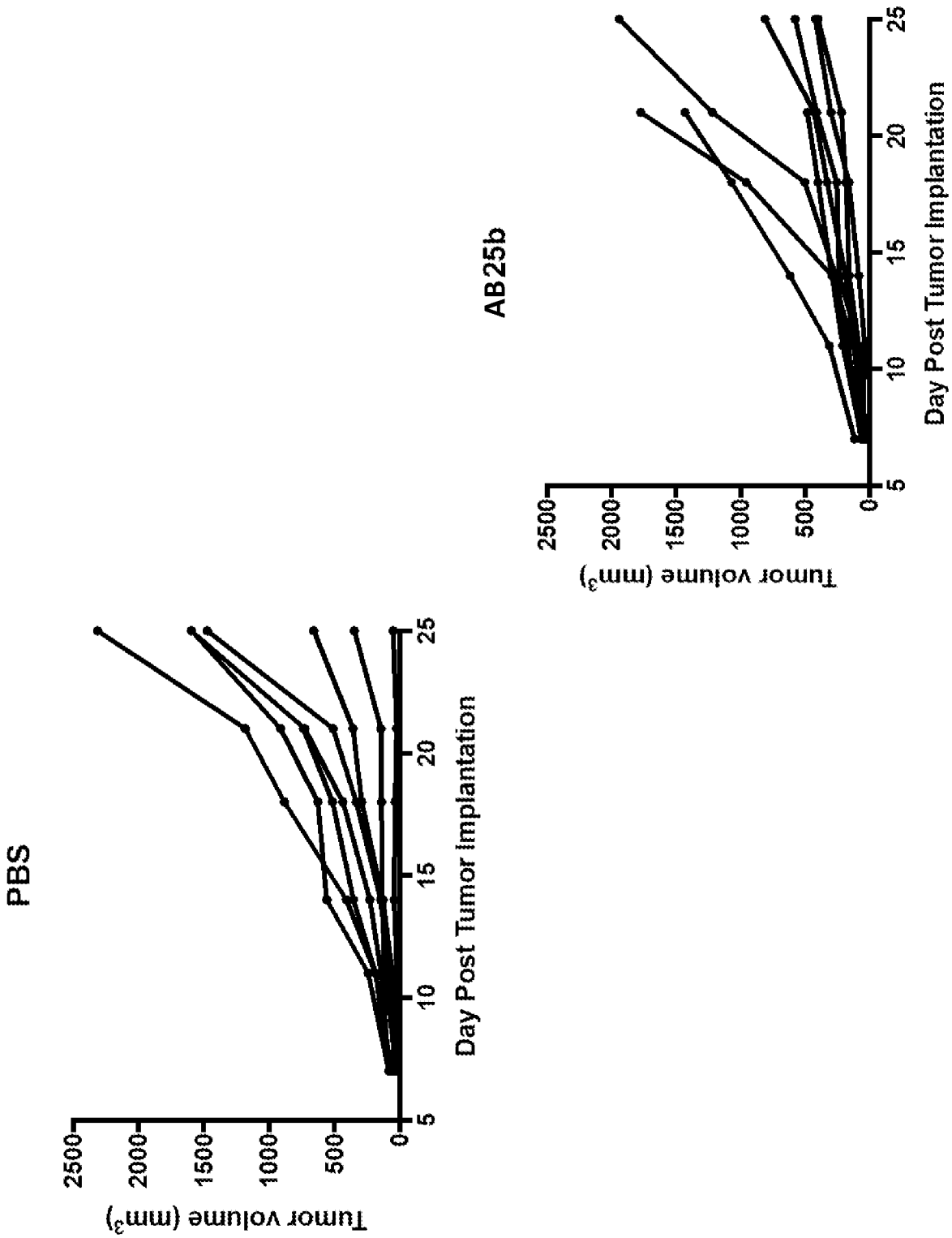

PBS
Tumor volume (mm3)
2500
2000
1500
1000
500
0
5
10
15
20
25
Day Post Tumor Implantation
AB25b
Tumor volume (mm3)
2500
2000
1500
1000
500
0
5
10
15
20
25
Day Post Tumor Implantation

FIG. 1

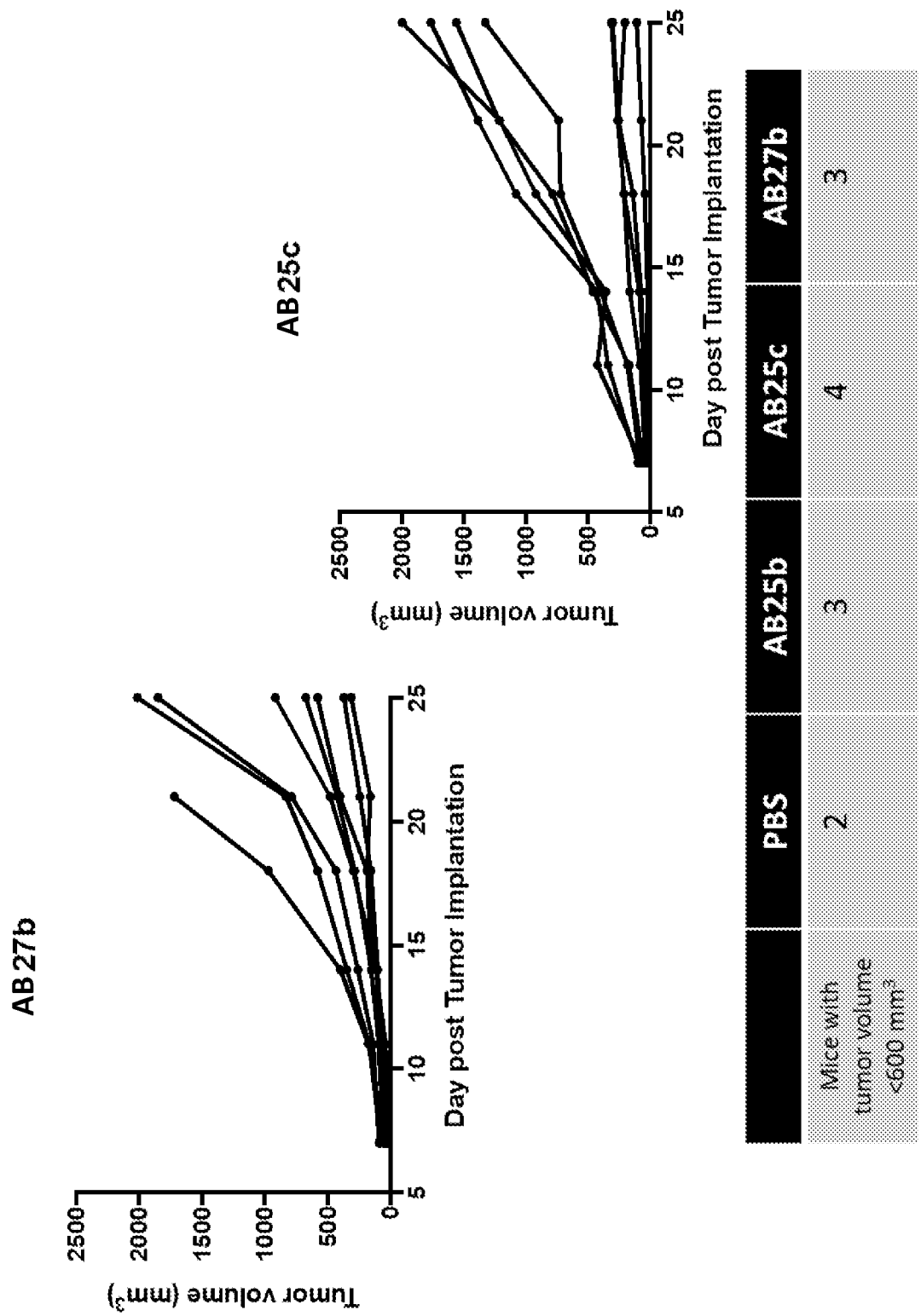

AB 27b
Tumor volume (mm³)
2500
2000
1500
1000
500
0
5
10
15
20
25
Day post Tumor Implantation
AB 25c
Tumor volume (mm³)
2500
2000
1500
1000
500
0
5
10
15
20
25
Day post Tumor Implantation
PBS
AB25b
AB25c
AB27b
Mice with tumor volume <600 mm³
2
3
4
3

FIG. 1 (cont.'d)

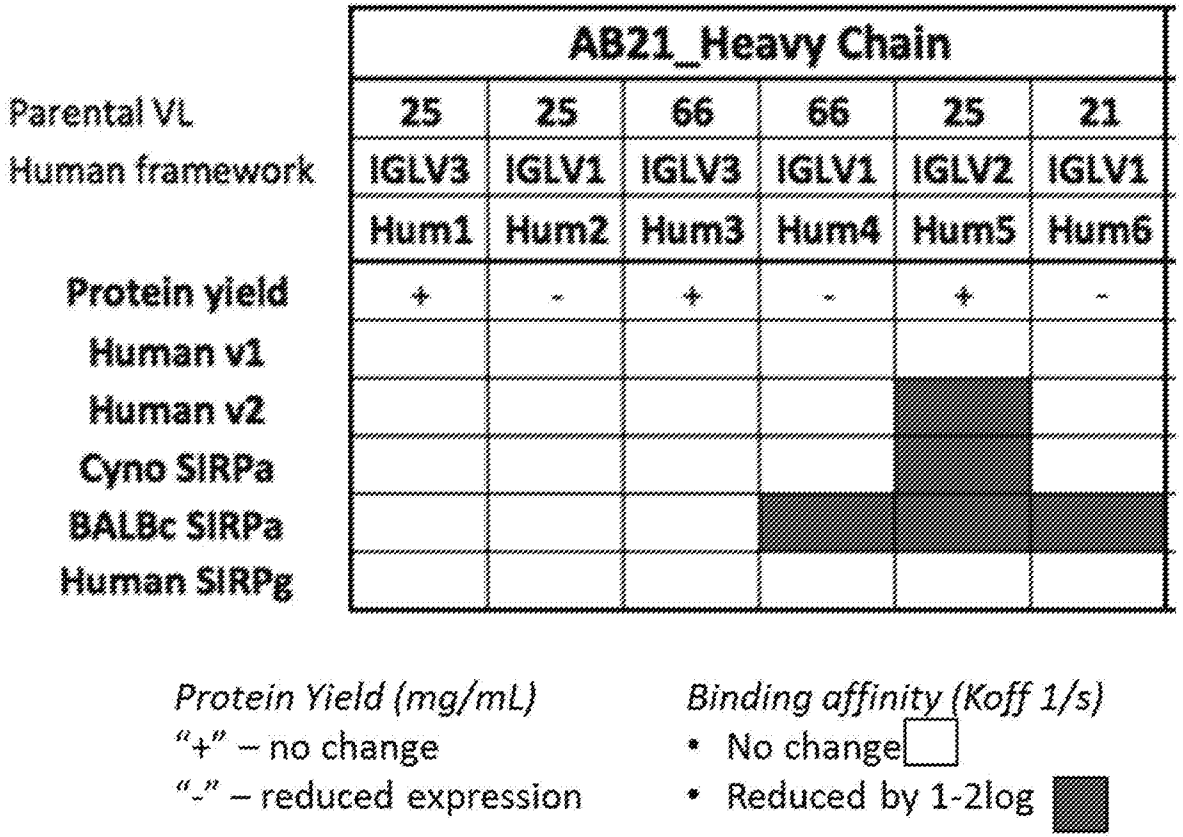

AB21_Heavy Chain
Parental VL
Human framework
25
IGLV3
Hum1
25
IGLV1
Hum2
66
IGLV3
Hum3
66
IGLV1
Hum4
25
IGLV2
Hum5
21
IGLV1
Hum6
Protein yield
Human v1
Human v2
Cyno SIRPa
BALBc SIRPa
Human SIRPg
Protein Yield (mg/mL)
"+" – no change
"-" – reduced expression
Binding affinity (Koff 1/s)
No change
Reduced by 1-2log

FIG. 2

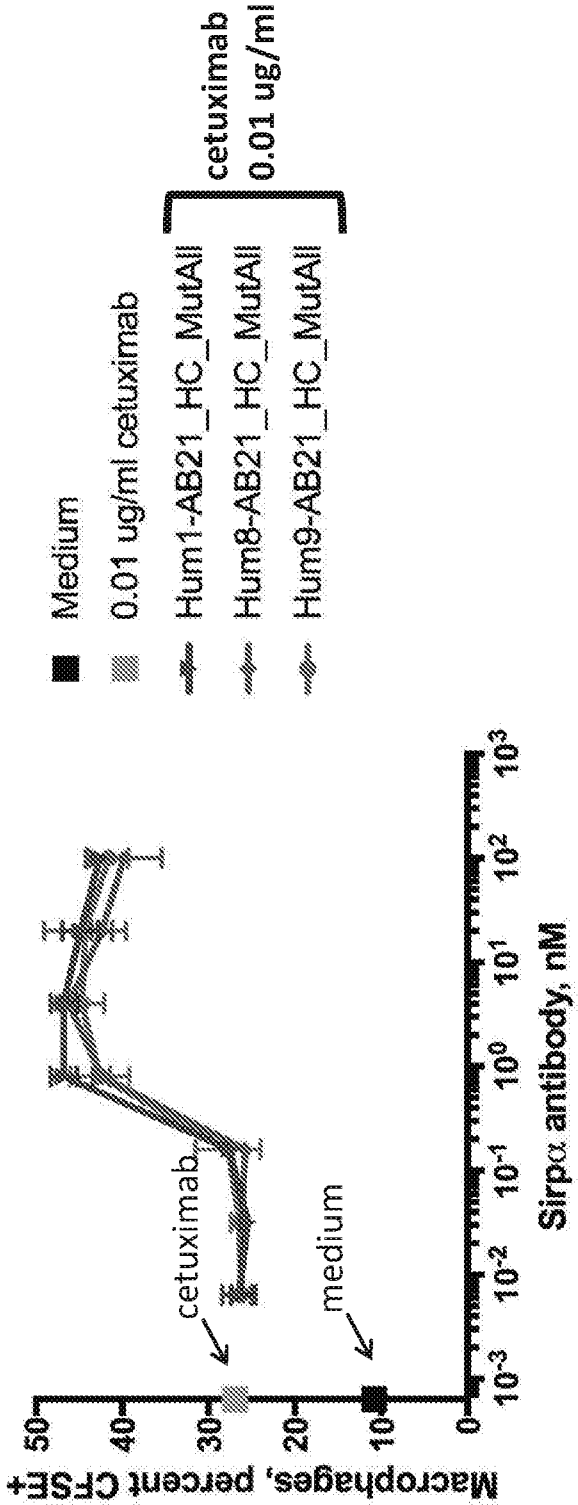

Macrophages, percent CFSE+
Sirpα antibody, nM
Medium
0.01 ug/ml cetuximab
Hum1-AB21_HC_MutAll
Hum8-AB21_HC_MutAll
Hum9-AB21_HC_MutAll
cetuximab
0.01 ug/ml
cetuximab
medium

FIG. 3A

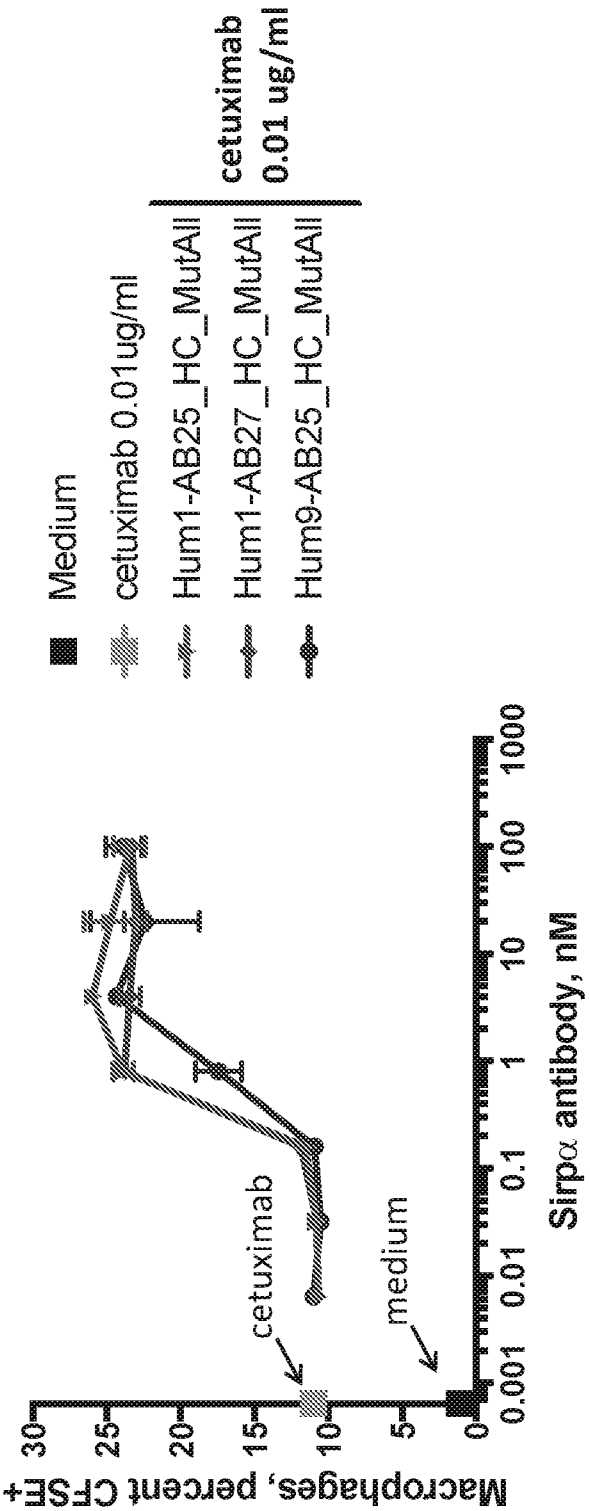

Macrophages, percent CFSE+
30
25
20
15
10
5
0
0.001
0.01
0.1
1
10
100
1000
Sirpα antibody, nM
cetuximab
medium
Medium
cetuximab 0.01ug/ml
Hum1-AB25_HC_MutAll
Hum1-AB27_HC_MutAll
Hum9-AB25_HC_MutAll
cetuximab
0.01 ug/ml

FIG. 3B

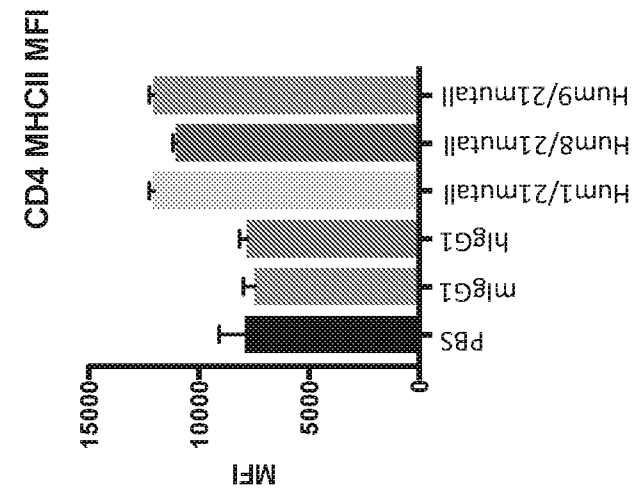

CD4 MHCII MFI
MFI
15000
10000
5000
0
PBS
mIgG1
hIgG1
Hum1/21mutall
Hum8/21mutall
Hum9/21mutall

FIG. 4B

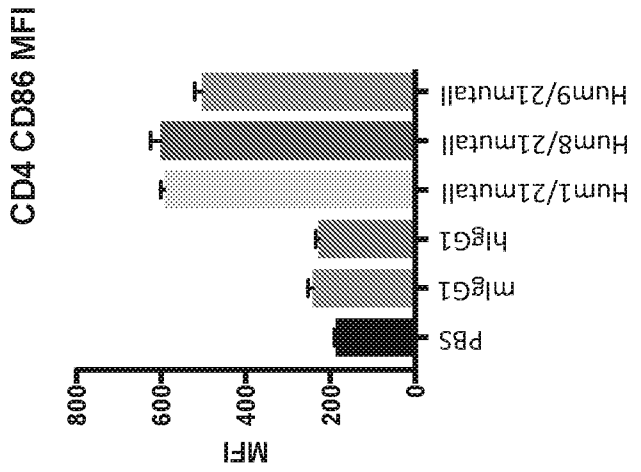

CD4 CD86 MFI
MFI
800
600
400
200
0
PBS
mIgG1
hIgG1
Hum1/21mutall
Hum8/21mutall
Hum9/21mutall

FIG. 4A

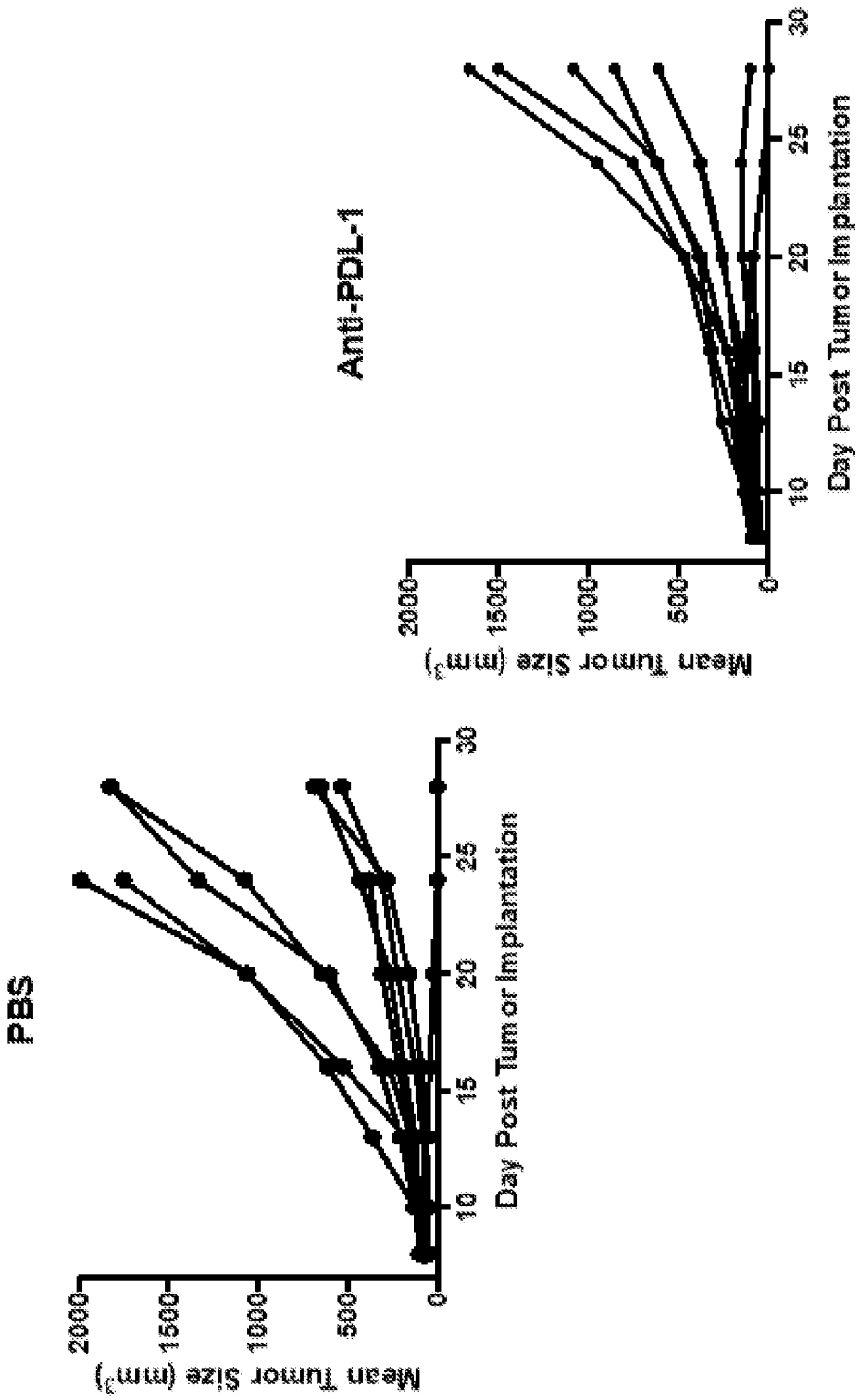
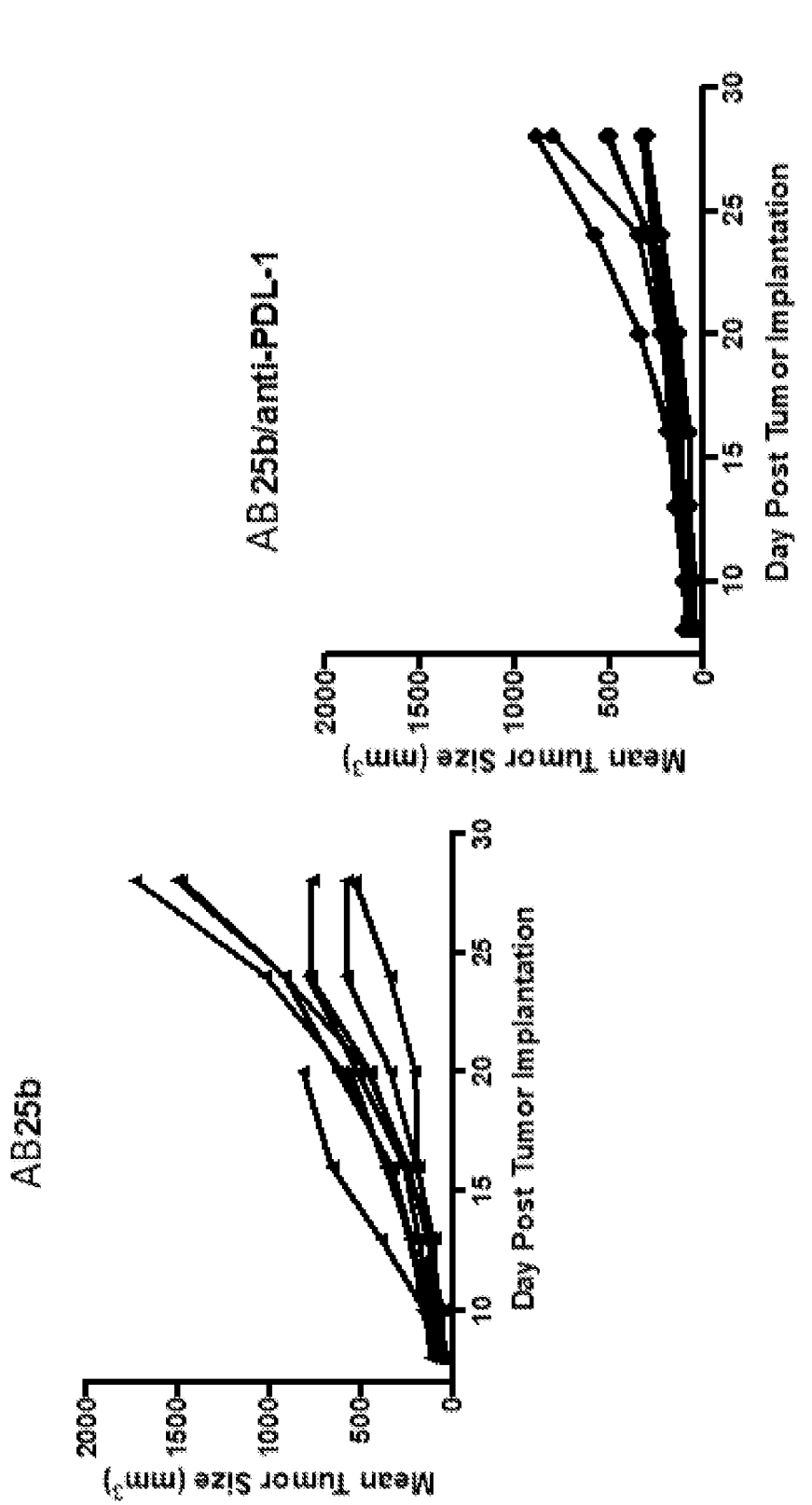

PBS
Anti-PDL-1
Mean Tumor Size (mm³)
0
500
1000
1500
2000
10
15
20
25
30
Day Post Tumor Implantation

FIG. 5



	PBS	Anti-PDL1	AB25b	AB25b/anti-PDL1
Mice with tumor volume <600 mm3	2	2	2	6

FIG. 5 (cont.'d)

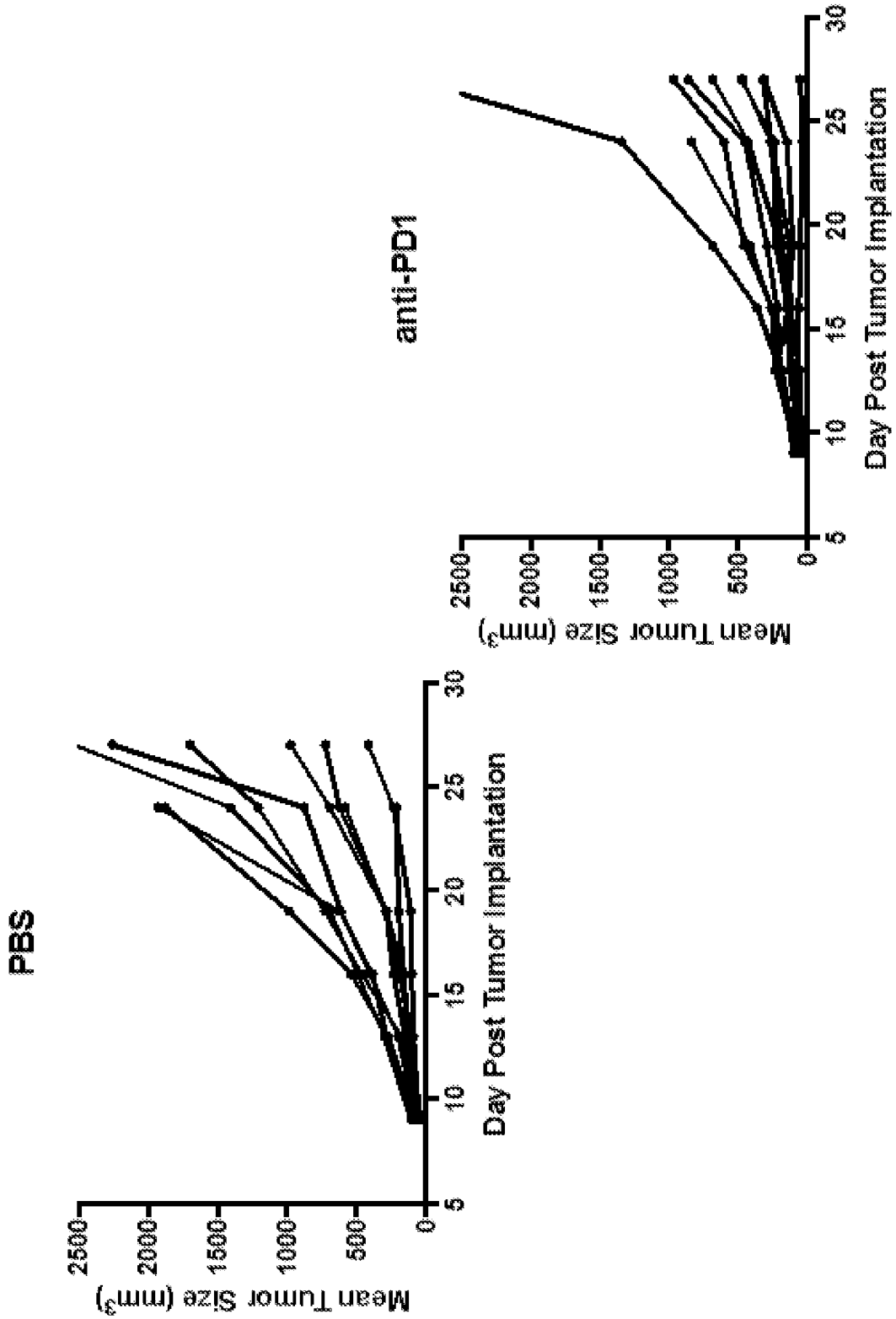

PBS
anti-PD1
Mean Tumor Size (mm³)
Day Post Tumor Implantation
0
500
1000
1500
2000
2500
5
10
15
20
25
30

FIG. 6

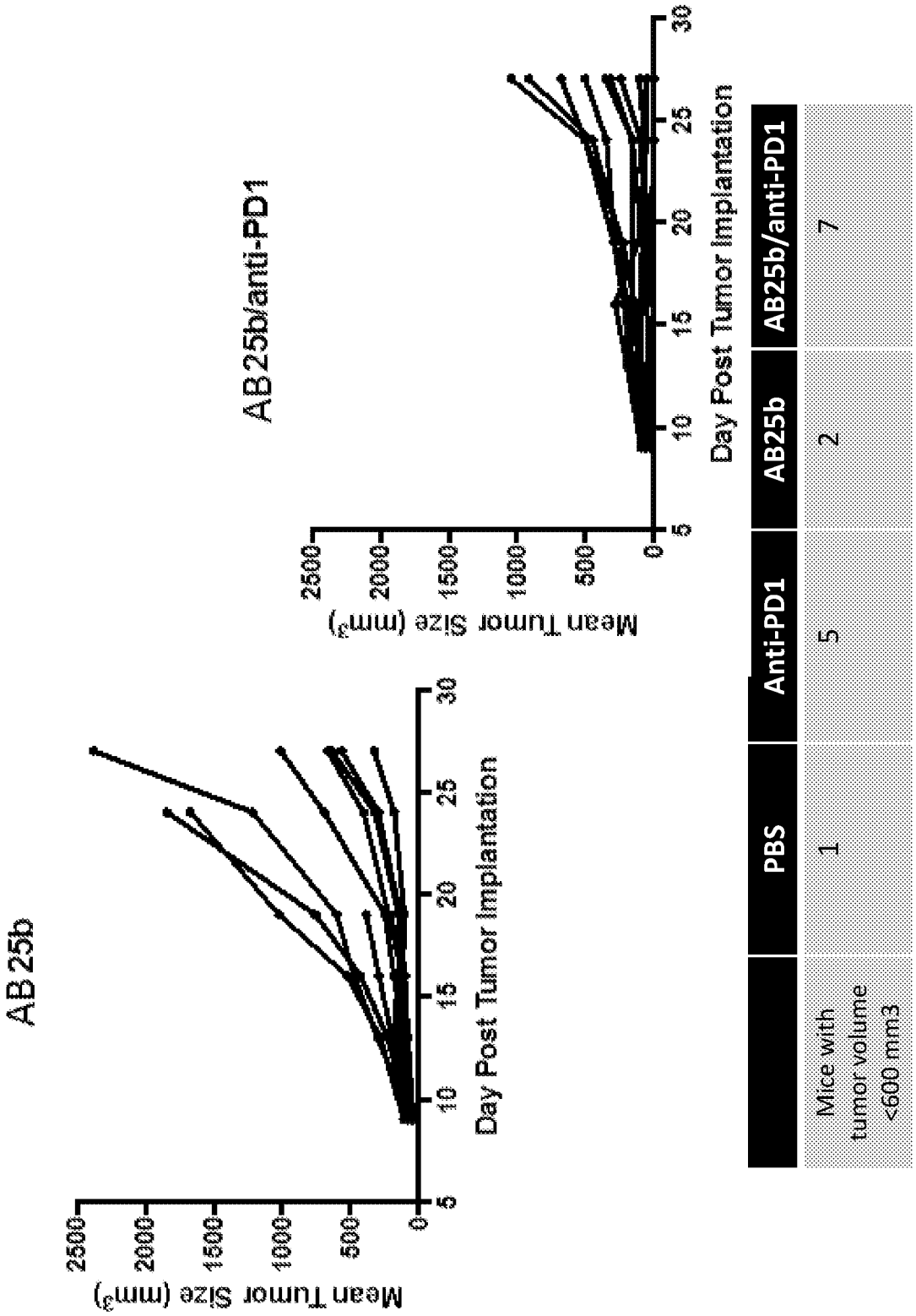

AB25b
Mean Tumor Size (mm³)
2500
2000
1500
1000
500
0
5
10
15
20
25
30
Day Post Tumor Implantation
AB25b/anti-PD1
Mean Tumor Size (mm³)
2500
2000
1500
1000
500
0
5
10
15
20
25
30
Day Post Tumor Implantation
PBS
Anti-PD1
AB25b
AB25b/anti-PD1
Mice with tumor volume <600 mm3
1
5
2
7

FIG. 6 (cont.'d)

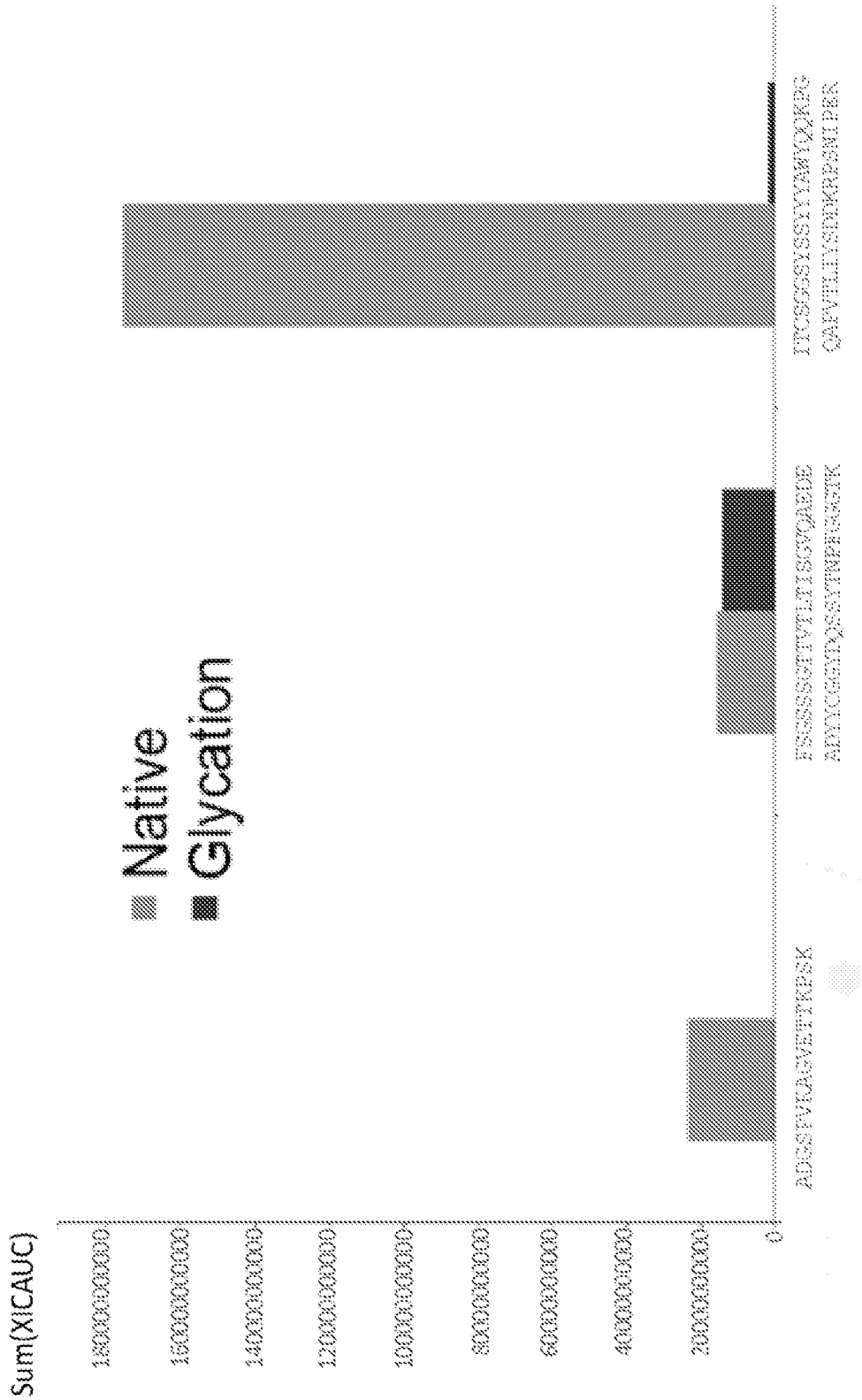

Native
Glycation
Sum(XICAUC)
1800000000
1600000000
1400000000
1200000000
1000000000
800000000
600000000
400000000
200000000
0
ADSSPVKAGVETTKPSK
FSSSSATTVTLTISCTQADE
AMYQGTDSSYTNPFGAGTK
IYCSCGSYSSYYAMYCQKPG
QAPVTLIYSNNKRPSMIPER
Peptide sequence

FIG. 7

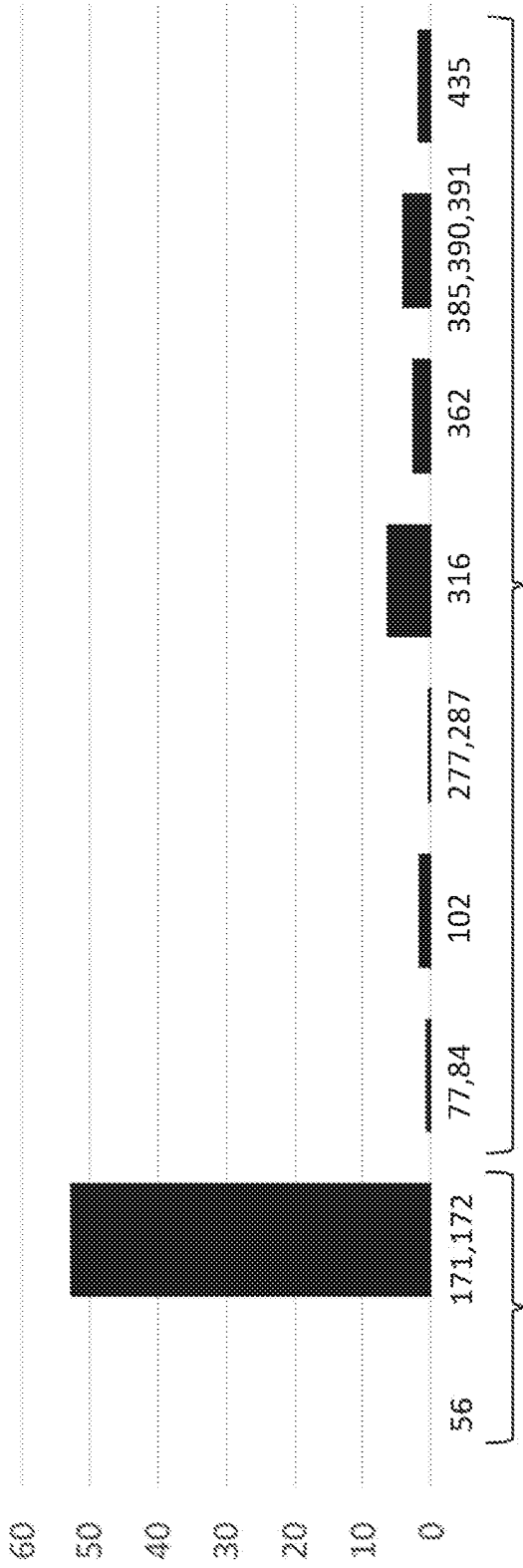

% of deamidated versus unmodified peptide
60
50
40
30
20
10
0
56
171,172
77,84
102
277,287
316
362
385,390,391
435
Light chain
Heavy chain

FIG. 8

Proposed engineering

Original - FGGGTKLTVL identical to IGLJ2
Version 1 - FGTGTKVTVL identical to IGLJ1
Version 2 - FGGGTQLTVL identical to IGLJ7
Version 3 - FGGGTRLTVL

FIG. 9

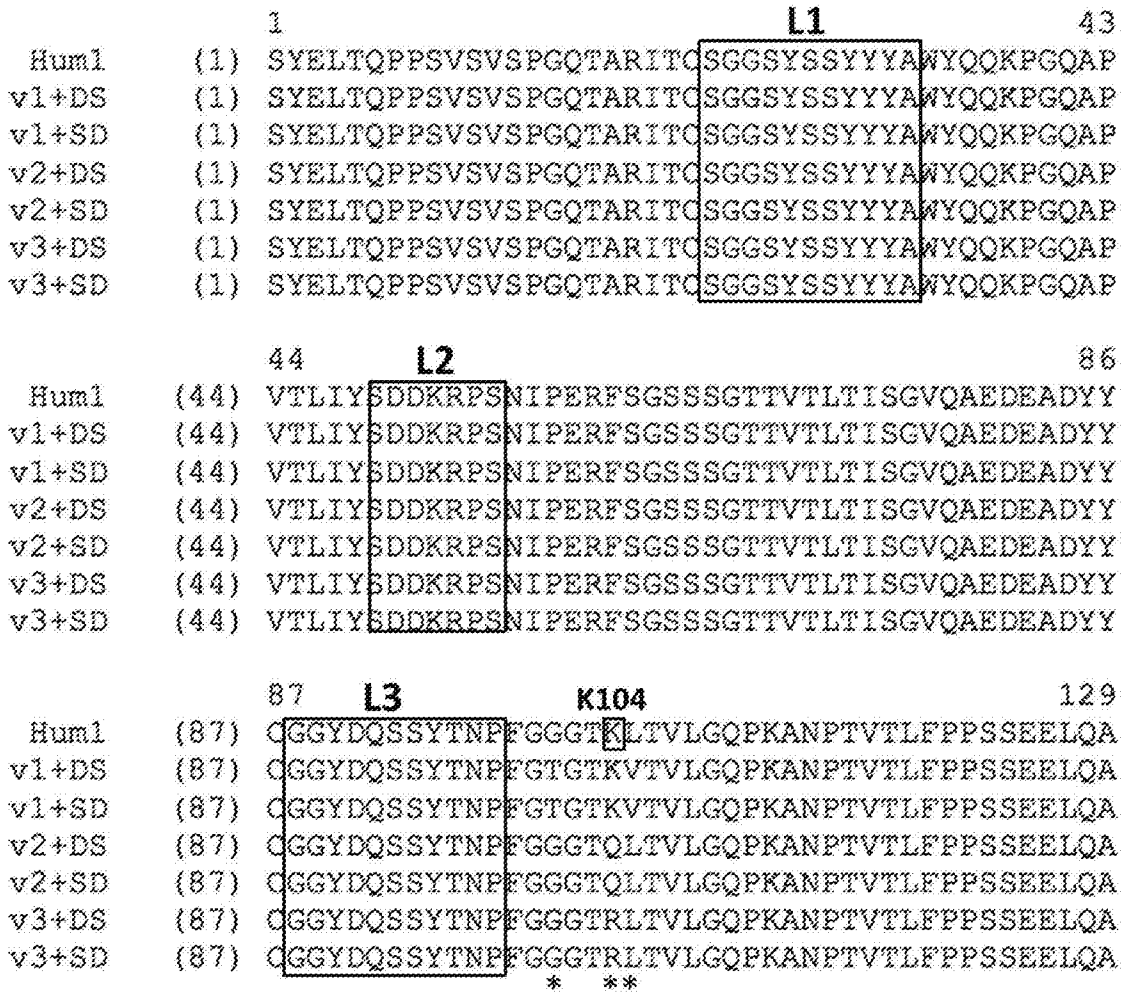

1 L1 43
Huml (1) SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQQKPGQAP
v1+DS (1) SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQQKPGQAP
v1+SD (1) SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQQKPGQAP
v2+DS (1) SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQQKPGQAP
v2+SD (1) SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQQKPGQAP
v3+DS (1) SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQQKPGQAP
v3+SD (1) SYELTQPPSVSVSPGQTARITCSGGSYSSYYYAWYQQKPGQAP
44 L2 86
Huml (44) VTLIYSDDKRPSNIPERFSGSSSGTTVTLTISGVQAEDEADYY
v1+DS (44) VTLIYSDDKRPSNIPERFSGSSSGTTVTLTISGVQAEDEADYY
v1+SD (44) VTLIYSDDKRPSNIPERFSGSSSGTTVTLTISGVQAEDEADYY
v2+DS (44) VTLIYSDDKRPSNIPERFSGSSSGTTVTLTISGVQAEDEADYY
v2+SD (44) VTLIYSDDKRPSNIPERFSGSSSGTTVTLTISGVQAEDEADYY
v3+DS (44) VTLIYSDDKRPSNIPERFSGSSSGTTVTLTISGVQAEDEADYY
v3+SD (44) VTLIYSDDKRPSNIPERFSGSSSGTTVTLTISGVQAEDEADYY
87 L3 K104 129
Huml (87) CGGYDQSSYTNPFGGGTKLTVLGQPKANPTVTLFPPSSEELQA
v1+DS (87) CGGYDQSSYTNPFGTGTKVTVLGQPKANPTVTLFPPSSEELQA
v1+SD (87) CGGYDQSSYTNPFGTGTKVTVLGQPKANPTVTLFPPSSEELQA
v2+DS (87) CGGYDQSSYTNPFGGGTQLTVLGQPKANPTVTLFPPSSEELQA
v2+SD (87) CGGYDQSSYTNPFGGGTQLTVLGQPKANPTVTLFPPSSEELQA
v3+DS (87) CGGYDQSSYTNPFGGGTRLTVLGQPKANPTVTLFPPSSEELQA
v3+SD (87) CGGYDQSSYTNPFGGGTRLTVLGQPKANPTVTLFPPSSEELQA
* **

FIG. 10

```
                130                                                 172
 Huml   (130)  NKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNN
v1+DS   (130)  NKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSDS
v1+SD   (130)  NKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSSD
v2+DS   (130)  NKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSDS
v2+SD   (130)  NKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSSD
v3+DS   (130)  NKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSDS
v3+SD   (130)  NKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSSD
                                                              **
                173                                         214
 Huml   (173)  KYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
v1+DS   (173)  KYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
v1+SD   (173)  KYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
v2+DS   (173)  KYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
v2+SD   (173)  KYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
v3+DS   (173)  KYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
v3+SD   (173)  KYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
```

FIG. 11

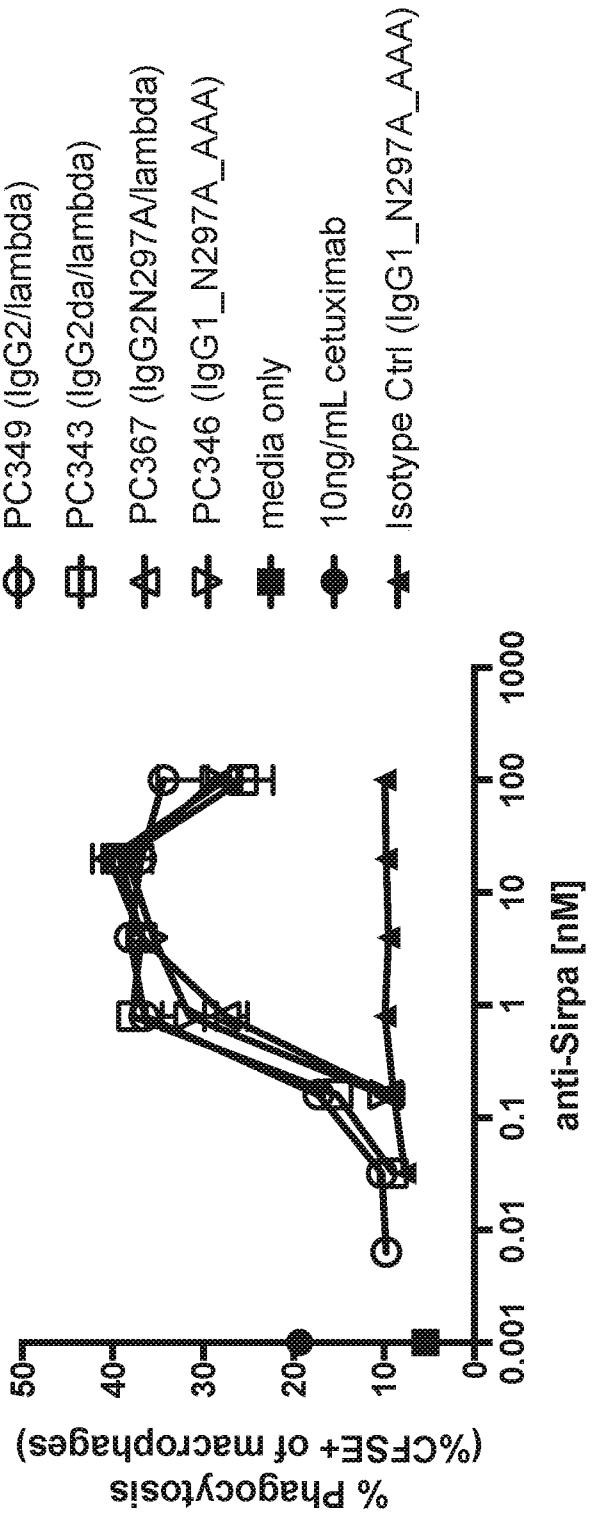

PC349 (IgG2/lambda)
PC343 (IgG2da/lambda)
PC367 (IgG2N297A/lambda)
PC346 (IgG1_N297A_AAA)
media only
10ng/mL cetuximab
Isotype Ctrl (IgG1_N297A_AAA)
% Phagocytosis
(%CFSE+ of macrophages)
50
40
30
20
10
0
0.001
0.01
0.1
1
10
100
1000
anti-Sirpa [nM]

FIG. 12

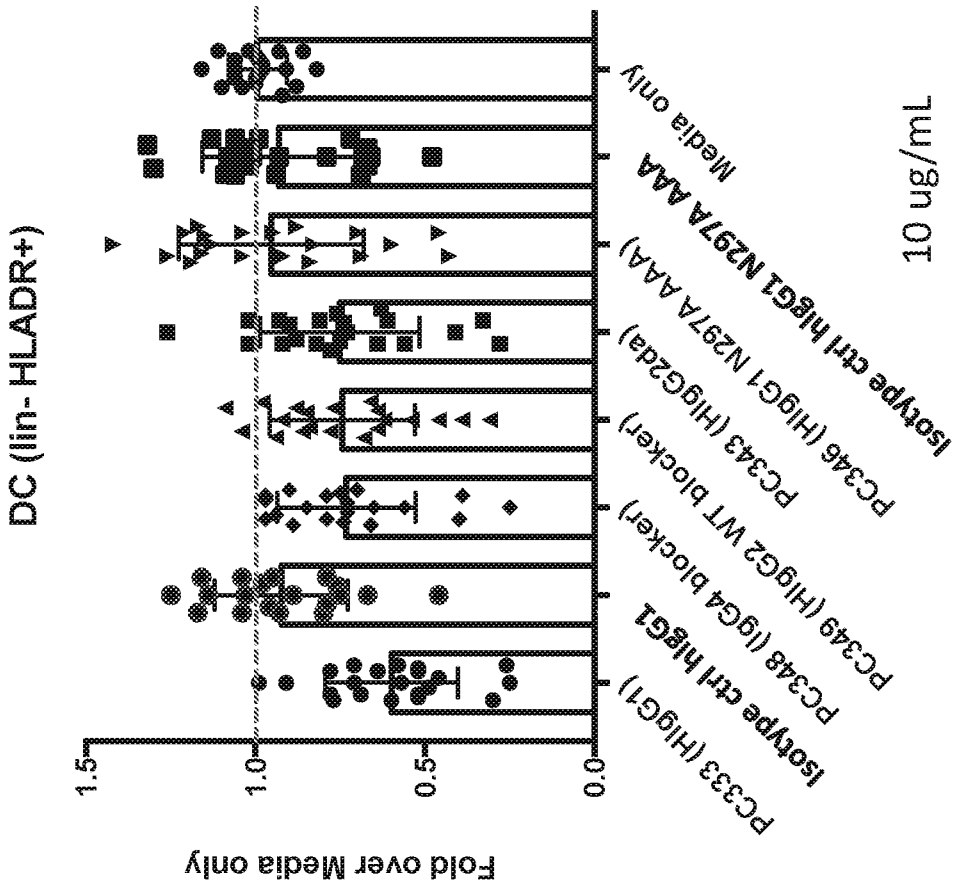

DC (lin- HLADR+)
Fold over Media only
1.5
1.0
0.5
0.0
PC333 (hIgG1)
Isotype ctrl hIgG1
PC348 (IgG4 blocker)
PC349 (hIgG2 WT blocker)
PC343 (hIgG2da)
PC346 (hIgG1 N297A AAA)
Isotype ctrl hIgG1 N297A AAA
Media only
10 ug/mL

FIG. 13A

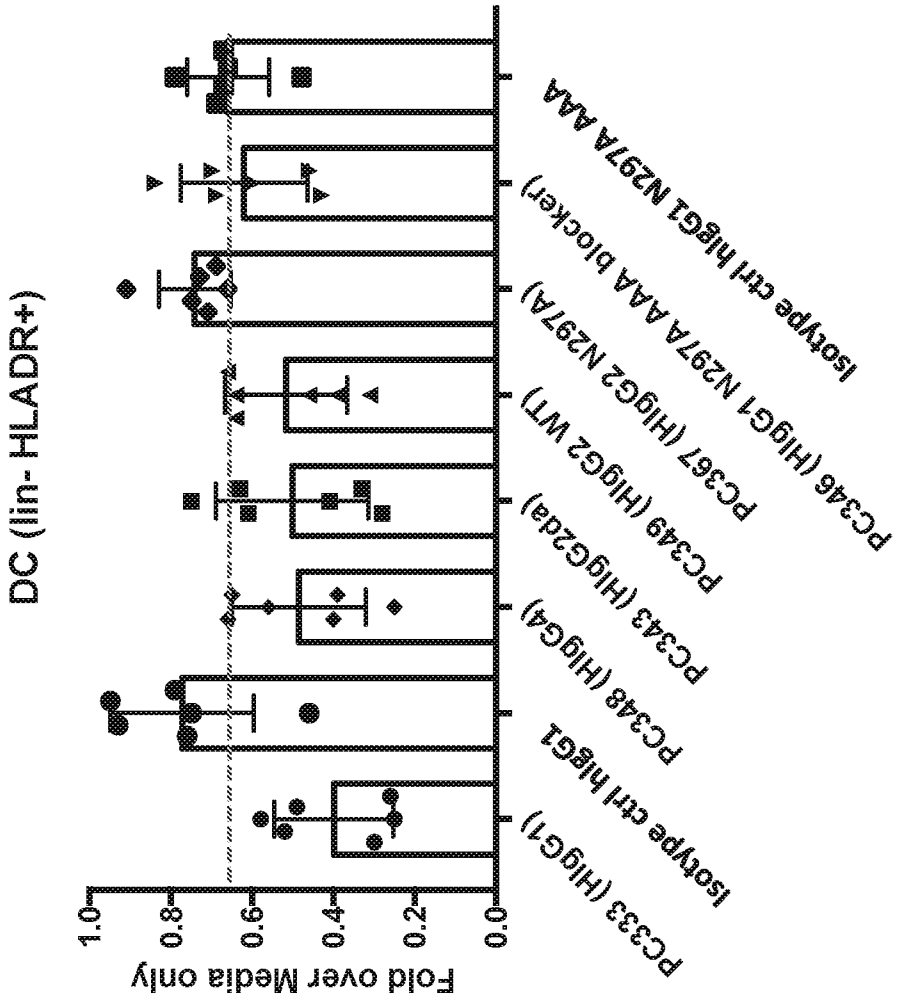

DC (lin- HLADR+)
Fold over Media only
1.0
0.8
0.6
0.4
0.2
0.0
PC333 (hIgG1)
Isotype ctrl hIgG1
PC348 (hIgG4)
PC343 (hIgG2a)
PC349 (hIgG2 WT)
PC367 (hIgG2 N297A)
PC346 (hIgG1 N297A AAA blocker)
Isotype ctrl hIgG1 N297A AAA

FIG. 13B

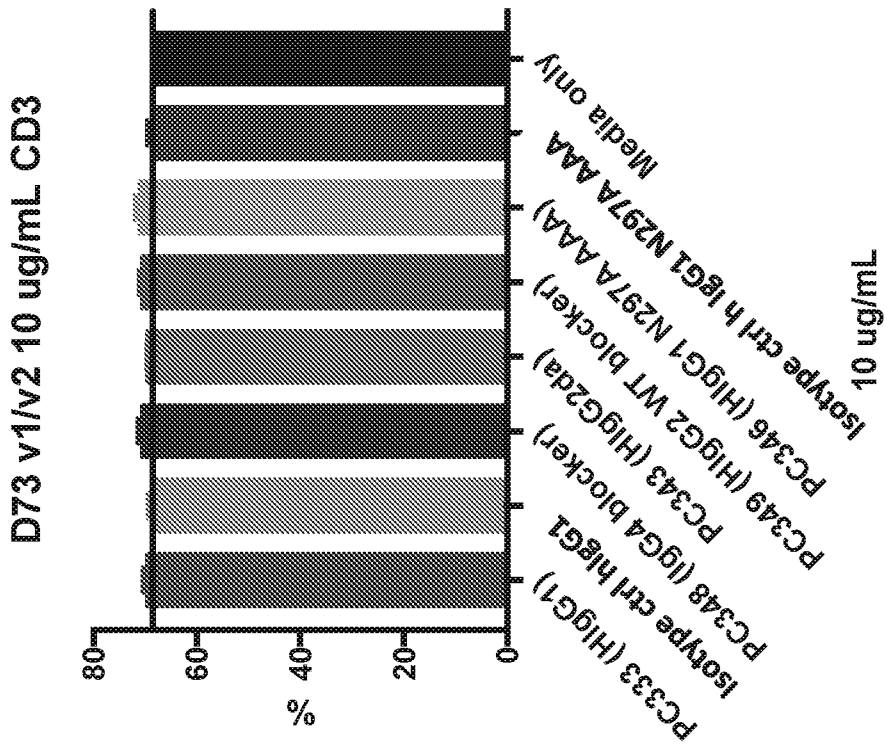

D73 v1/v2 10 ug/mL CD3
%
0
20
40
60
80
PC333 (HIgG1)
Isotype ctrl hIgG1
PC348 (IgG4 blocker)
PC343 (HIgG2da)
PC349 (HIgG2 WT blocker)
PC346 (HIgG1 N297A AAA)
Isotype ctrl h IgG1 N297A AAA
Media only
10 ug/mL

FIG. 13C

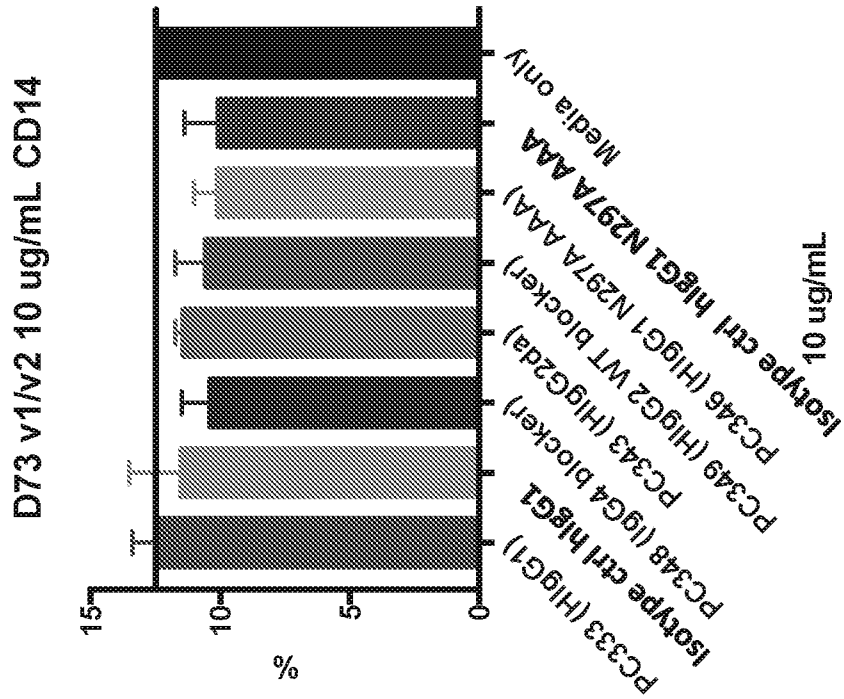

D73 v1/v2 10 ug/mL CD14
%
15
10
5
0
PC333 (HIgG1)
isotype ctrl hIgG1
PC348 (IgG4 blocker)
PC343 (HIgG2da)
PC349 (HIgG2 WT blocker)
PC346 (HIgG1 N297A AAA)
isotype ctrl hIgG1 N297A AAA
Media only
10 ug/mL

FIG. 13D

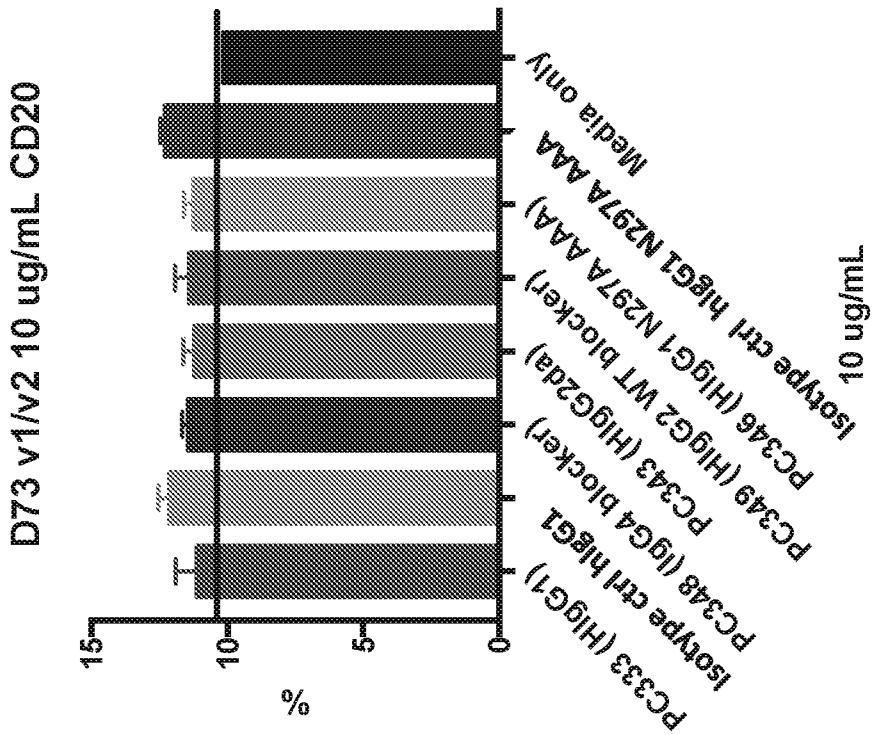

D73 v1/v2 10 ug/mL CD20
15
10
5
0
%
PC333 (hIgG1)
Isotype ctrl hIgG1
PC348 (IgG4 block er)
PC343 (hIgG2da)
PC349 (hIgG2 WT block er)
PC346 (hIgG1 N297A AAA)
Isotype ctrl hIgG1 N297A AAA
Media only
10 ug/mL

FIG. 13E

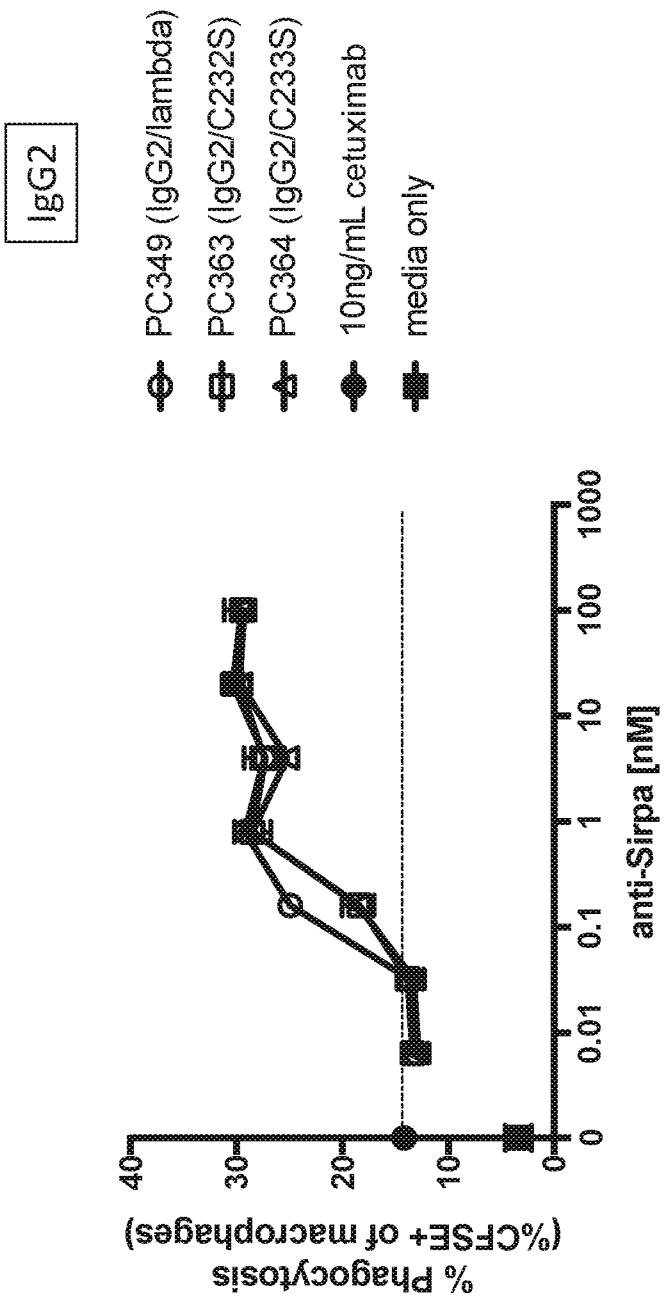

IgG2
PC349 (IgG2/lambda)
PC363 (IgG2/C232S)
PC364 (IgG2/C233S)
10ng/mL cetuximab
media only
% Phagocytosis
(%CFSE+ of macrophages)
40
30
20
10
0
0
0.01
0.1
1
10
100
1000
anti-Sirpa [nM]

FIG. 14A

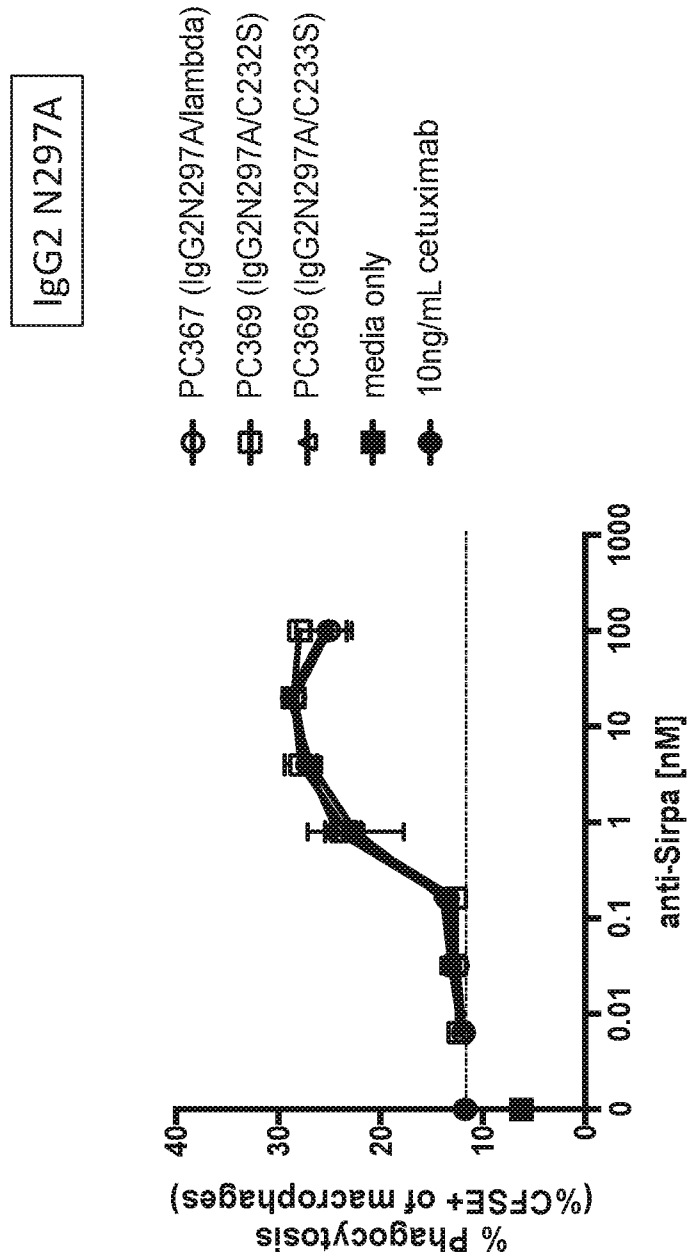

IgG2 N297A
PC367 (IgG2N297A/lambda)
PC369 (IgG2N297A/C232S)
PC369 (IgG2N297A/C233S)
media only
10ng/mL cetuximab
% Phagocytosis
(%CFSE+ of macrophages)
40
30
20
10
0
0
0.01
0.1
1
10
100
1000
anti-Sirpa [nM]

FIG. 14B

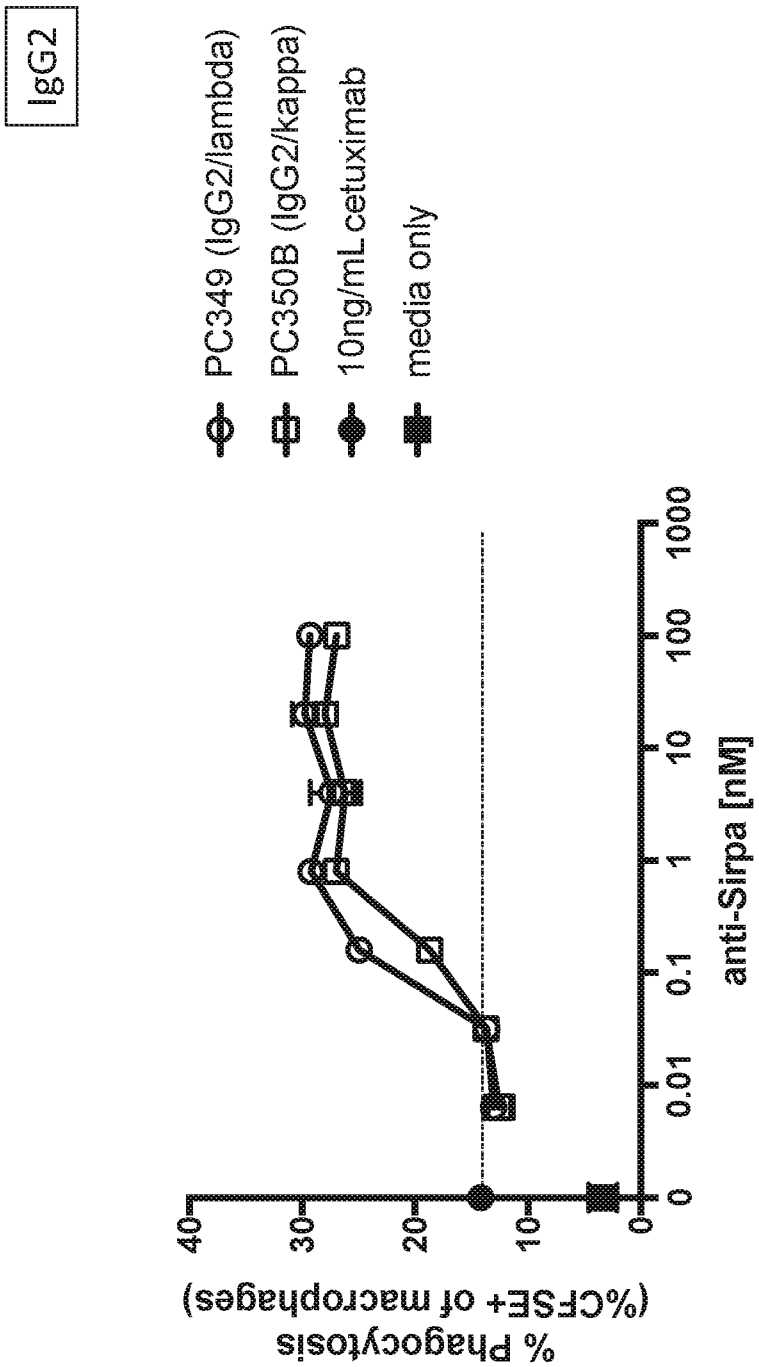

IgG2
PC349 (IgG2/lambda)
PC350B (IgG2/kappa)
10ng/mL cetuximab
media only
% Phagocytosis
(%CFSE+ of macrophages)
40
30
20
10
0
0
0.01
0.1
1
10
100
1000
anti-Sirpa [nM]

FIG. 15A

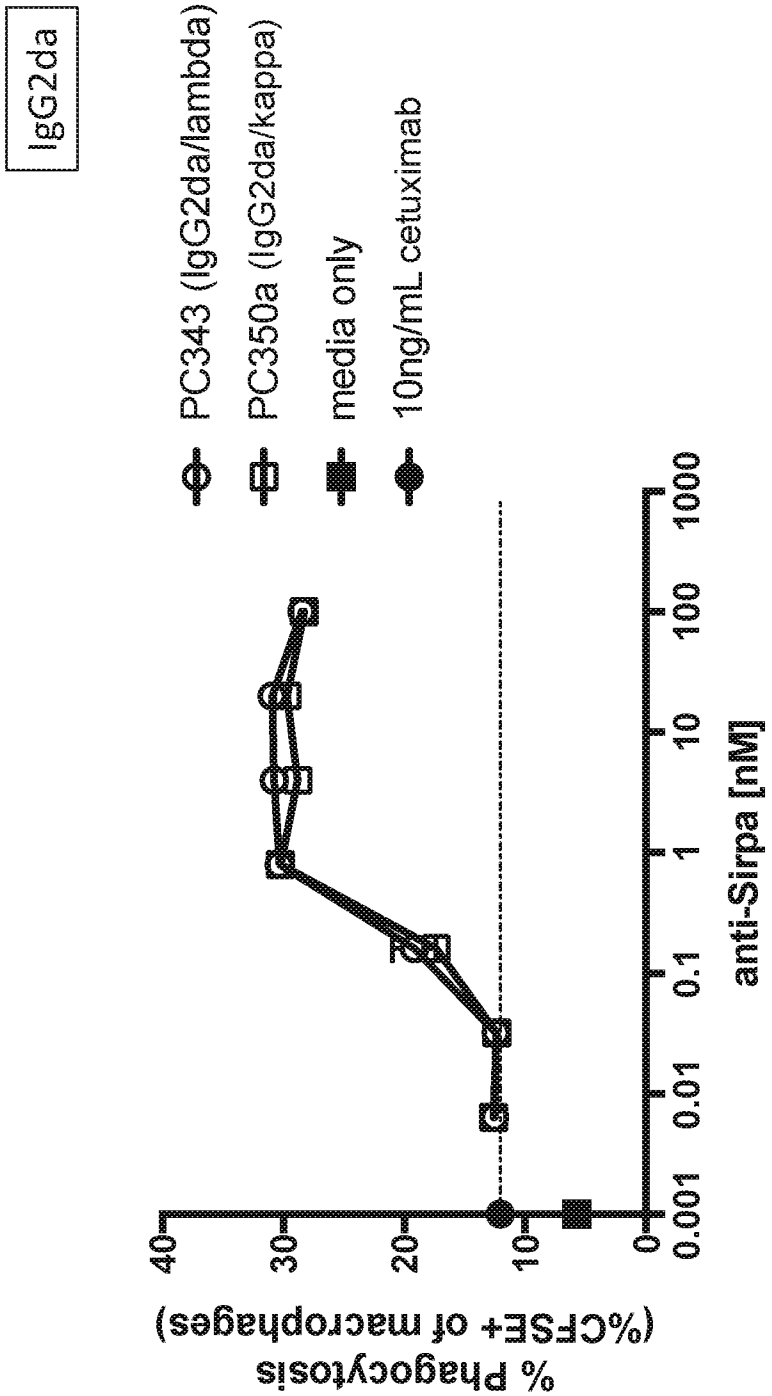

IgG2da
PC343 (IgG2da/lambda)
PC350a (IgG2da/kappa)
media only
10ng/mL cetuximab
% Phagocytosis
(%CFSE+ of macrophages)
40
30
20
10
0
0.001
0.01
0.1
1
10
100
1000
anti-Sirpa [nM]

FIG. 15B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/023238

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/395 (2006.01) C07K 16/28 (2006.01) A61P 29/00 (2006.01) A61P 35/00 (2006.01) A61P 37/06 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPOQUE (PATENW and $COMBI), MEDLINE, CAPLUS, BIOSIS, EMBASE GenomeQuest: SEQ ID NO: 26 and 71, WO2018057669, A61K39/395, C07K16/28, SIRP or signal regulatory protein _α,ALPHA,A, CD_172_A, PTPNS_1 , SHPS_1, Macrophage fusion receptor, antibody, immunoglobulin, extra cellular domain, diabody, triabody, tetrabody, Fab-fragment, Fv-fragment, scFv, Fab, antigen binding protein, cancer, tumo[u]r, neoplasm, lesion, autoimmune, inflammatory and like terms. Applicant/Inventor names searched in external databases: Google Patents, Patentscope, PubMed, Espacenet, in relevant STN Online databases, and in internal databases provided by IP Australia.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 May 2019	20 May 2019
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	PAMPA RAY AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61 2 6283 2466

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	WO 2018/057669 A1 (ALEXO THERAPEUTICS INC.) 29 March 2018 The whole document, in particular claims 89 & 159, paragraphs 308 & 309.	
A	WO 2017/178653 A2 (OSE IMMUNOTHERAPEUTICS) 19 October 2017 The whole document, in particular: claims; page 4, line 30; page 29, lines 21-25; page 36, lines 30-34	
A	WO 2013/056352 A1 (UNIVERSITY HEALTH NETWORK et al.) 25 April 2013 The whole document, in particular: abstract, claims 1-13	
A	Murata, Y. et al. 'The CD47-SIRPα signalling system: its physiological roles and therapeutic application', The Journal of Biochemistry, 2014, vol. 155, no. 6 pages 335-344 The whole document, in particular Figures 1 and 3, last paragraph of page 341	
A	Zhao, X.W. et al. 'CD47-signal regulatory protein-α (SIRPα) interactions form a barrier for antibody-mediated tumor cell destruction', Proceedings of the National Academy of Sciences (USA), 2011, vol. 108, no. 45, pages 18342-18347 The whole document	
A	Seiffert, M. et al. 'Signal-regulatory protein α (SIRPα) but not SIRPβ is involved in T-cell activation, binds to CD47 with high affinity, and is expressed on immature $CD34^{+}CD38^{-}$ haematopoietic cells' Blood, 2001, vol. 97, no. 9 pages 2741-2749 The whole document	

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. PCT/US2019/023238

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2018/057669 A1	29 March 2018	WO 2018057669 A1	29 Mar 2018
		AU 2017332285 A1	14 Feb 2019
		CA 3034480 A1	29 Mar 2018
		CO 2019003809 A2	30 Apr 2019
		US 2018105600 A1	19 Apr 2018
WO 2017/178653 A2	19 October 2017	WO 2017178653 A2	19 Oct 2017
		AU 2017248626 A1	11 Oct 2018
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		CA 3020373 A1	19 Oct 2017
		CL 2018002898 A1	14 Dec 2018
		CN 109071664 A	21 Dec 2018
		CO 2018010855 A2	13 Nov 2018
		EP 3443010 A2	20 Feb 2019
		KR 20180134397 A	18 Dec 2018
		PE 19212018 A1	11 Dec 2018
		PH 12018550160 A1	11 Mar 2019
		SG 11201808465U A	30 Oct 2018
		US 2019127477 A1	02 May 2019
WO 2013/056352 A1	25 April 2013	WO 2013056352 A1	25 Apr 2013
		US 2014242095 A1	28 Aug 2014

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.