(12) STANDARD PATENT APPLICATION (11) Application No. **AU 2018246235 A2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Truncated NKG2D chimeric receptors and uses thereof in natural killer cell immunotherapy

(51) International Patent Classification(s)

C07K 14/705 (2006.01)	***A61K 38/00*** (2006.01)
A01N 63/00 (2006.01)	***C07K 14/725*** (2006.01)
A61K 35/17 (2015.01)	***C12N 5/0783*** (2010.01)

(21) Application No: **2018246235** (22) Date of Filing: **2018.03.27**

(87) WIPO No: **WO18/183385**

(30) Priority Data

(31) Number	(32) Date	(33) Country
62/477,335	**2017.03.27**	**US**
62/628,774	**2018.02.09**	**US**

(43) Publication Date: **2018.10.04**
(43) Amended Journal Date: **2021.04.08**

(71) Applicant(s)
National University of Singapore;Nkarta, Inc.

(72) Inventor(s)
Leong, Jun Hao;Shimasaki, Noriko;Seow, See Voon;Campana, Dario;Trager, James Barnaby;Lazetic, Alexandra Leida Liana;Guo, Chao;Buren, Luxuan Guo;Masrani, Shyam Sashikant

(74) Agent / Attorney
AJ PARK, GPO Box 2513, Sydney, NSW, 2001, AU

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

(19) **World Intellectual Property Organization**
International Bureau

(43) **International Publication Date**
04 October 2018 (04.10.2018)

WIPO | PCT

(10) **International Publication Number**
WO 2018/183385 A1

(51) **International Patent Classification:**
A61K 35/17 (2015.01) *C07K 14/725* (2006.01)
A61K 38/00 (2006.01) *C07K 14/705* (2006.01)
A01N 63/00 (2006.01) *C12N 5/0783* (2010.01)

(21) **International Application Number:**
PCT/US2018/024650

(22) **International Filing Date:**
27 March 2018 (27.03.2018)

(25) **Filing Language:** English

(26) **Publication Language:** English

(30) **Priority Data:**
62/477,335 27 March 2017 (27.03.2017) US
62/628,774 09 February 2018 (09.02.2018) US

(71) **Applicants: NATIONAL UNIVERSITY OF SINGAPORE** [SG/SG]; 21 Lower Kent Ridge Road, Singapore 119077 (SG). **NKARTA, INC.** [US/US]; 329 Oyster Point Boulevard, 3rd Floor, South San Francisco, CA 94080 (US).

(72) **Inventors: LEONG, Jun, Hao**; c/o National University Of Singapore, 21 Lower Kent Ridge Road, Singapore 119077 (SG). **SHIMASAKI, Noriko**; c/o National University Of Singapore, 21 Lower Kent Ridge Road, Singapore 119077 (SG). **SEOW, See, Voon**; c/o National University Of Singapore, 21 Lower Kent Ridge Road, Singapore 119077 (SG). **CAMPANA, Dario**; c/o National University Of Singapore, 21 Lower Kent Ridge Road, Singapore 119077 (SG). **TRAGER, James, Barnaby**; c/o Nkarta, Inc., 329 Oyster Point Boulevard, 3rd Floor, South San Francisco, CA 94080 (US). **LAZETIC, Alexandra, Leida Liana**; c/o Nkarta, Inc., 329 Oyster Point Boulevard, 3rd Floor, South San Francisco, CA 94080 (US). **GUO, Chao**; c/o Nkarta, Inc., 329 Oyster Point Boulevard, 3rd Floor, South San Francisco, CA 94080 (US). **BUREN, Luxuan, Guo**; c/o Nkarta, Inc., 329 Oyster Point Boulevard, 3rd Floor, South San Francisco, CA 94080 (US). **MASRANI, Shyam, Sashikant**; c/o Nkarta, Inc., 329 Oyster Point Boulevard, 3rd Floor, South San Francisco, CA 94080 (US).

(74) **Agent: CARROLL, Alice, O.** et al.; Hamilton, Brook, Smith & Reynolds, P.C., 530 Virginia Rd., P.O. Box 9133, Concord, MA 01742-9133 (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,

(54) Title: TRUNCATED NKG2D CHIMERIC RECEPTORS AND USES THEREOF IN NATURAL KILLER CELL IMMUNOTHERAPY

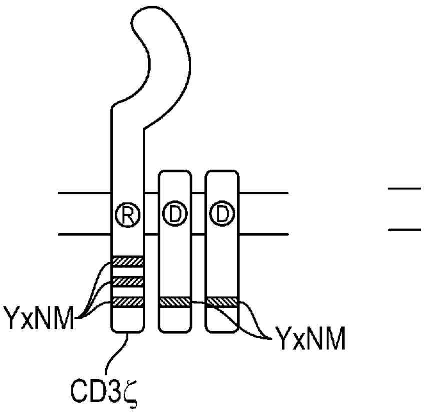

FIG. 1B

(57) **Abstract:** Several embodiments disclosed herein relate to the compositions comprising engineered Natural Killer (NK) cells that express a chimeric receptor, the chimeric receptor imparting to the NK cells an enhanced ability to target specific cells, such as cancerous cells or those affected by an infectious disease. Several embodiments relate to NK cells that target cells expressing natural ligands of NKG2D, where the NK cells comprise transmembrane and/or signaling domains that lead to cytotoxic and/or cytolytic effects when the NK cells bind a target cell. Uses of NK cell compositions to treat diseases are also provided for in several embodiments.

WO 2018/183385 A1

[Continued on next page]

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).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

TRUNCATED NKG2D CHIMERIC RECEPTORS AND USES THEREOF IN NATURAL KILLER CELL IMMUNOTHERAPY

RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/477,335, filed on March 27, 2017 and U.S. Provisional Application No. 62/628,774, filed on February 9, 2018. The entirety of each of the above-listed applications is incorporated by reference herein.

INCORPORATION BY REFERENCE OF MATERIAL IN ASCII TEXT FILE

[0002] This application incorporates by reference the Sequence Listing contained in the following ASCII text file being submitted concurrently herewith:

a) File name: 44591144002SequenceListing.txt; created March 27, 2018, 186 KB in size.

BACKGROUND

[0003] The emergence and persistence of many diseases is characterized by an insufficient immune response to aberrant cells, including malignant and virally infected cells. Immunotherapy is the use and manipulation of the patient's immune system for treatment of various diseases.

SUMMARY

[0004] Immunotherapy presents a new technological advancement in the treatment of disease, wherein immune cells are engineered to express certain targeting and/or effector molecules that specifically identify and react to diseased or damaged cells. This represents a promising advance due, at least in part, to the potential for specifically targeting diseased or damaged cells, as opposed to more traditional approaches, such as chemotherapy, where all cells are impacted, and the desired outcome is that sufficient healthy cells survive to allow the patient to live. One immunotherapy approach is the recombinant expression of chimeric receptors in immune cells to achieve the targeted recognition and destruction of aberrant cells of interest.

[0005] To address this need for specifically targeting and destroying, disabling or otherwise rendering inert diseased or infected cells, there are provided for herein polynucleotides, amino acids, and vectors that encode chimeric receptors that impart enhanced targeting and cytotoxicity to cells, such as natural killer cells. Also provided for are methods for producing the cells, and methods of using the cells to target and destroy diseased or damaged cells. In several embodiments, there is provided a polynucleotide encoding a chimeric receptor comprising an extracellular receptor domain and an effector domain comprising a transmembrane region and an intracellular signaling domain, wherein the extracellular receptor domain comprises a peptide that binds native ligands of Natural Killer Group 2 member D (NKG2D), wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D.

[0006] In several embodiments, there is provided a polynucleotide encoding a chimeric receptor comprising one or both of: (a) an extracellular receptor domain and (b) an effector domain comprising a transmembrane region and an intracellular signaling domain. In several embodiments, the extracellular receptor domain comprises a peptide that binds native ligands of Natural Killer Group 2 member D (NKG2D). In several embodiments, the peptide that binds native ligands of NKG2D is a fragment of NKG2D, for example, a fragment of NKG2D is encoded by a polynucleotide comprising SEQ ID NO. 2. As disclosed, herein, additional NKG2D fragments are also used, depending on the embodiment. In several embodiments, the intracellular signaling domain comprises CD3zeta. In one embodiment, the CD3zeta is encoded by a polynucleotide comprising SEQ ID NO. 13, though, as disclosed herein, sequences that differ from CD3zeta, but share similar function may also be used, depending on the embodiment.

[0007] In several embodiments, the transmembrane region of the effector domain comprises a CD8a transmembrane domain. In one embodiment, the transmembrane region of the effector domain further comprises a CD8a hinge region. In several embodiments, the CD8a hinge region is encoded by a polynucleotide comprising SEQ ID NO: 5. In several embodiments, the intracellular signaling domain further comprises 4-1BB. In one embodiment, the 4-1BB is encoded by a polynucleotide comprising SEQ ID NO. 12, though, as disclosed herein, sequences that differ from 4-1BB, but share similar function may also be used, depending on the embodiment.

[0008] In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to CD8a, 4-1BB and CD3z. In several embodiments, such a chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO. 18. In additional embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO. 108, though, as disclosed herein, sequences that differ from SEQ ID NO. 108, but share similar function may also be used, depending on the embodiment. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO. 19.

[0009] In several embodiments, any of chimeric receptors disclosed herein can also be co-expressed with membrane-bound interleukin 15 (mbIL15). In some embodiments, the mbIL15 is encoded by a polynucleotide comprising SEQ ID NO. 16. In some embodiments, the mbIL15 comprises an amino acid sequence of SEQ ID NO: 17. Other sequences for mbIL15 may also be used, depending on the embodiment. In some embodiments, the mbIL15 is bicistronically expressed on the same polynucleotide as the chimeric receptor. In other embodiments, the mbIL15 is co-expressed on a separate construct. In several embodiments, the intracellular signaling domain is further enhanced by coupling its expression with that of membrane-bound interleukin 15 (mbIL15).

[0010] In several embodiments, the effector domain further comprises an OX-40 domain. In several embodiments, the OX-40 domain is either in place of, or in addition to mbIL15. In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD8a transmembrane domain, the OX-40 domain, and the CD3zeta. In some embodiments, the polynucleotide construct is configured to bicistronically co-express mbIL15. In some such embodiments, the polynucleotide construct comprises one or more cleavage sites (e.g., T2A, P2A, E2A, and/or F2A cleavage site(s)) recognized and cleaved by, for example, a cytosolic protease. In some embodiments, the mbIL15 is coupled to the chimeric receptor by a cytosolic protease cleavage site. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 90 coupled to the mbIL15 encoded by SEQ ID NO. 16 by a cytosolic protease cleavage site. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 109 coupled to the mbIL15 encoded by SEQ ID NO. 16 by a cytosolic protease cleavage site. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 91 and is co-expressed with mbIL15 comprising the amino acid sequence of SEQ ID NO. 17. As disclosed herein, sequences that differ from SEQ ID NOs: 90, 91, 109, 16, and/or 16, but share similar function may also be used, depending on the embodiment.

[0011] In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to a IgG4 hinge, a CD8a transmembrane domain, the OX-40 domain, and the CD3zeta. In some embodiments, the polynucleotide construct is configured to bicistronically co-express mbIL15 with the chimeric receptor. In some such embodiments, the polynucleotide construct comprises one or more cleavage sites (e.g., T2A, P2A, E2A, and/or F2A cleavage site(s)) recognized and cleaved by a cytosolic protease. In some embodiments, the mbIL15 is coupled to the chimeric receptor by a cytosolic protease cleavage site. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 100 coupled to the mbIL15 encoded by SEQ ID NO. 16 by a cytosolic protease cleavage site. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 101 and is co-expressed with mbIL15 comprising the amino acid sequence of SEQ ID NO. 17. As disclosed herein, sequences that differ from SEQ ID NOs: 100, 101 and/or 16, but share similar function may also be used, depending on the embodiment.

[0012] In several embodiments, there are provided methods for treating cancer, comprising administering to a subject having a cancer a composition comprising a Natural Killer (NK) cell expressing the chimeric receptor encoded by the polynucleotides described above, or elsewhere herein.

[0013] In one embodiment, the NK cells are autologous cells isolated from a patient having a cancer or an infectious disease. In additional embodiments, the NK cells are allogeneic cells isolated from a donor.

[0014] Also provided for herein is use of a polynucleotide as described above, or elsewhere herein, in the manufacture of a medicament for enhancing NK cell cytotoxicity in a mammal in need thereof. In several embodiments, there is provided for the use of a polynucleotide as described above, or elsewhere herein, in the manufacture of a medicament for treating or preventing cancer or an infectious disease in a mammal in need thereof.

[0015] According to several embodiments, there is provided a polynucleotide encoding a chimeric receptor, the chimeric receptor comprising an extracellular receptor domain an effector domain comprising a transmembrane region and an intracellular signaling domain. As discussed in more detail herein, the extracellular receptor domain serves to recognize and bind ligands on a target cell. The effector domain serves to transmit signals (upon binding of a target cell by the extracellular domain) that set in motion a signal cascade that leads to cytotoxic activity against the target cell. In accordance with several embodiments, the polynucleotide encodes a chimeric receptor that provides unexpectedly increased cytotoxicity as compared to non-engineered NK cells.

[0016] In several embodiments, the extracellular receptor domain comprises a peptide that binds native ligands of Natural Killer Group 2 member D (NKG2D). According to several embodiments, the peptide that binds native ligands of NKG2D is a functional fragment of NKG2D (e.g., a truncation, fragment or portion of full length NKG2D. As used, herein the terms, "fragment", "truncation", and "portion" shall be given their ordinary meanings and shall also be interchangeable with one another. For example, in several embodiments, the fragment of NKG2D is encoded by a polynucleotide comprising a fragment of the sequence of SEQ ID NO: 1. In several embodiments, the fragment of NKG2D comprises the sequence of SEQ ID NO: 2, while in additional embodiments, the fragment encoding NKG2D is codon optimized, and comprises, for example, the sequence of SEQ ID NO: 3. In additional embodiments, the fragment encoding NKG2D is codon optimized, and comprises, for example, the sequence of SEQ ID NO: 68.

[0017] In several embodiments, the effector domain comprises one or more of CD16, NCR1, NCR2, NCR3, 4-1BB, NKp80, CD3zeta and 2B4. In several embodiments, these effector domains are coupled to CD8 alpha.

[0018] In several embodiments, the chimeric receptor comprises a fragment of NKG2D coupled to CD16. As used herein, coupled shall be given its ordinary meaning and shall also refer to direct (e.g., a first nucleotide followed directly be a second nucleotide) or indirect (e.g., sequences are in frame with one another but separated by intervening nucleotides) linkage of nucleotide sequences in a manner that allows for expression of the nucleotide sequences in, for example, an *in vitro* transcription/translation system, a host cell (e.g., *in vitro* and/or *in vivo*). As used herein, "linked" and "coupled" are used interchangeably. In several embodiments, the NKG2D/CD16 chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 23. In several embodiments, the NKG2D/CD16 chimeric receptor comprises the amino acid sequence of SEQ ID NO: 24. In several embodiments, the chimeric receptor comprises a fragment of NKG2D coupled to NCR1. In several embodiments, such a chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 27. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 28.

[0019] As discussed above, in several embodiments, the NKG2D fragment is coupled to NCR2, and the resultant chimeric receptor comprises at least a portion of the amino acid sequence of SEQ ID NO: 21. Several embodiments provide for a chimeric receptor comprising a fragment of NKG2D coupled to NCR3. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO. 29, and the chimeric receptor comprises the amino acid sequence of SEQ ID NO. 30.

[0020] As discussed in more detail below, combinations of transmembrane and intracellular domains are used in several embodiments and provide for synergistic interactions between the components of the chimeric receptor and yield enhanced cytotoxic effects. In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to a CD16 transmembrane/intracellular domain and 4-1BB. In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD16 transmembrane/intracellular domain and 4-1BB. In several embodiments, such a chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 25. In several embodiments, the resultant chimeric receptor comprises the amino acid sequence of SEQ ID NO: 26.

[0021] In several embodiments, NCR1 is used in conjunction with the NKG2D fragment. In several embodiments, the NKG2D fragment is linked to NCR1 alone. In additional embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to NCR1 and 4-1BB. In some such embodiments, the chimeric receptor comprises the NCR1 amino acid sequence of SEQ ID NO: 20.

[0022] In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to CD8a, 4-1BB and CD3z. In several embodiments, such an NKG2D/CD8a/4-1bb/CD3z chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO. 18. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO. 19.

[0023] In several embodiments, NCR3 is included in the chimeric receptor. For example, an NKG2D/NCR3 construct is provided for in several embodiments. The resultant chimeric receptor thereby comprises the NCR3 amino acid sequence of SEQ ID NO: 22. In several embodiments, the chimeric receptor comprises a NKG2D/NCR2/4-1BB construct or an NKG2D/NCR3/4-1BB construct.

[0024] In several embodiments, linkers, hinges, or other "spacing" elements are provided for in the chimeric receptor constructs. For example, in several embodiments, the effector domain comprises a linker. In several embodiments, the polynucleotides encode a GS linker between the portions of the construct, such as between any of 4-1BB, CD16, NCR1, NCR3, 2B4 or NKp80. In several embodiments, one or more GS linkers are provided for, for example, 1, 2, 3, 4, 5, 6, or more. In several embodiments, there is provided for a chimeric receptor comprising a hinge region. Depending on the location within a particular construct, a hinge region can be synonymous with a linker region, and vice versa. In several embodiments, the hinge region is encoded by the nucleic acid sequence of SEQ ID NO: 5. In some embodiments, the hinge region can be truncated to a desired length and is therefore encoded by a fragment of the nucleic acid sequence of SEQ ID NO: 5. In several embodiments, a glycine-serine motif is used as a hinge. In several embodiments, the hinge region is comprises a glycine-serine repeating motif having the amino acid sequence of (GGGGS)n (SEQ ID NO: 31) where n is the number of repeats. In several embodiments, 9 repeats are used, resulting in a hinge region comprising the amino acid sequence of SEQ ID NO: 33. In several embodiments, 3 repeats are used, resulting in a hinge region comprising the amino acid sequence of SEQ ID NO: 34.

[0025] In several embodiments, two separate molecules can be used as a hinge or linker, such as the amino acid sequence of SEQ ID NO: 32 (CD8a/GS3). In several embodiments, portions of a beta adrenergic receptor are used as a hinge or linker. In several embodiments, portions of the beta-2 adrenergic receptor are used. In one embodiment, an extracellular domain of the beta-2 adrenergic receptor is used, which is encoded by the nucleic acid sequence of SEQ ID NO: 40. In some embodiments, the first transmembrane helix of the beta-2 adrenergic receptor is used, which is encoded by the nucleic acid sequence of SEQ ID NO: 42. Depending on the embodiment, these two beta-2 adrenergic receptor portions are used together in the chimeric receptor. In several embodiments, the extracellular receptor domain further comprises a CD8a signal peptide, wherein the signal peptide comprises the nucleic acid sequence of SEQ ID NO. 4. Other signal peptides are optionally used, depending on the embodiment. Signal peptides may be employed in a multimeric format, according to some embodiments.

[0026] In several embodiments, the effector domain comprises one or more hemi-ITAM sequences. In some such embodiments, the hemi-ITAM comprises the amino acid motif DGYXXL (where X is any amino acid; SEQ ID NO: 14). Multiple hemi-ITAMs are used in some embodiments. In several embodiments, the hemi-ITAM comprises NKp80. In several embodiments, the effector domain comprises one or more ITSM sequences. ITSM sequences are used in conjunction with hemi-ITAM motifs in several embodiments. In several embodiments, the ITSM comprises the amino acid motif S/TXYXXL/I (where X is any amino acid; SEQ ID NO. 15). In several embodiments, the effector comprises a 2B4 domain.

[0027] In several embodiments, the chimeric receptor comprises a fragment of NKG2D coupled to a GS3 linker, a CD8a hinge, a CD16 transmembrane/intracellular domain and 4-1BB. In several embodiments, the chimeric receptor comprises a fragment of NKG2D coupled to a GS3 linker, a CD16 transmembrane/intracellular domain and 4-1BB. In several embodiments, the chimeric receptor comprises a fragment of NKG2D coupled to a CD16 transmembrane/intracellular domain and 4-1BB. In several embodiments, the chimeric receptor comprises a fragment of NKG2D coupled to a CD8a hinge, a CD8a transmembrane domain, 4-1BB, and 2B4. In several embodiments, the chimeric receptor comprises a fragment of NKG2D coupled to a beta-adrenergic extracellular domain, a beta-adrenergic transmembrane domain, 4-1BB, and 2B4. In several embodiments, the chimeric receptor comprises a fragment of NKG2D coupled to a CD8a hinge, a CD8a transmembrane domain, 4-1BB, 2B4, a GS3 linker, and NKp80. In several embodiments, the chimeric receptor comprises a fragment of NKG2D coupled to a CD8a hinge, a CD8a transmembrane domain, 4-1BB, a GS3 linker, and NKp80. In several embodiments, the chimeric receptor comprises a fragment of NKG2D, wherein the fragment is encoded by a sequence that is codon optimized coupled to a GS3 linker, an additional NKG2D fragment, a beta-adrenergic extracellular domain, a beta-adrenergic transmembrane domain, 4-1BB, an additional GS3 linker, and NKp80. In several embodiments, the chimeric receptor comprises a fragment of NKG2D that is codon optimized coupled to a GS3 linker, an additional NKG2D fragment, a CD8a hinge, a CD8a transmembrane domain, 4-1BB, an additional GS3 linker, and NKp80. In several embodiments, the chimeric receptor comprises a fragment of NKG2D that is codon optimized coupled to a GS3 linker, an additional NKG2D fragment, a CD8a hinge, a CD16 transmembrane/intracellular domain, and 4-1BB. In several embodiments, chimeric receptor comprises a fragment of NKG2D coupled to a CD8a hinge, a CD16 transmembrane/intracellular domain, 4-1BB, and 2B4. In several embodiments, the chimeric receptor comprises a fragment of NKG2D coupled to a CD8a hinge, a CD16 transmembrane/intracellular domain, 4-1BB, a GS3 linker, and NKp80. In several embodiments, the chimeric receptor comprises a fragment of NKG2D that is coupled to a CD8a hinge and a CD8a transmembrane domain. In several embodiments, the effector comprises 4-1BB. In some such embodiments the effector comprises 4-1BB optionally in conjunction with one or more of NKp80, 2B4, CD3zeta, Dap10, Dap12, CD28, or other signaling domains provided for herein). In several embodiments, the effector domain further comprises CD3zeta. In several embodiments, the effector domain comprises an intracellular domain of 2B4. In several embodiments, the effector domain further comprises an intracellular domain of DAP10.

[0028] In several embodiments, the chimeric receptor comprises a fragment of NKG2D coupled to a CD8a hinge, a CD8a transmembrane domain, 4-1BB, 2B4, and CD3zeta. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 58. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 59.

[0029] Additionally, any of chimeric receptors disclosed herein can also be co-expressed with membrane-bound interleukin 15 (mbIL15). For example, provided for in several embodiments is a polynucleotide encoding a chimeric receptor comprising an extracellular receptor domain, wherein the extracellular receptor domain comprises a peptide that binds native ligands of NKG2D, wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D, a transmembrane region, an effector domain, the polynucleotide being co-expressed with an additional construct encoding membrane-bound interleukin 15 (mbIL15). In several embodiments, chimeric receptors as discussed herein are co-expressed with mbIL-15. In several embodiments, the effector domain comprises 4-1BB and CD3 zeta, and the transmembrane region comprises CD8a.

[0030] In several embodiments, the chimeric receptors are engineered such that they do not include DNAX-activating protein 10 (DAP10). Additionally, in several embodiments, the chimeric receptors are engineered such that they do not include an ITAM motif.

[0031] In several embodiments, there is provided a polynucleotide encoding a chimeric receptor comprising, one, two, or all of: (a) an extracellular receptor domain comprising a fragment of NKG2D that binds native ligands of NKG2D, (b) a transmembrane region, wherein the transmembrane region comprises CD8a, and (c) an effector domain, wherein the effector domain comprises 4-1BB and the intracellular domain of 2B4 or DAP10. In several embodiments, the effector domain comprises 2B4 followed by 4-1BB. In additional embodiments, the effector domain comprises 4-1BB followed by 2B4. In several embodiments, the effector domain comprises DAP10 followed by 4-1BB. In additional embodiments, the effector domain comprises 4-1BB followed by DAP10. In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD8a transmembrane domain, 4-1BB, and DAP10. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 60. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 61. In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD8a transmembrane domain, 4-1BB, 2B4, and DAP10. In several embodiments, the effector domain comprises 4-1BB, followed by DAP10, followed by 2B4. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 62. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 63. In several embodiments, the effector domain comprises 4-1BB, followed by 2B4, followed by DAP10. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 64. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 65.

[0032] In several embodiments, the chimeric receptor comprises a codon-optimized fragment of NKG2D coupled to an intracellular effector domain. In several embodiments, multiple fragments of NKG2D are employed, for example, an additional NKG2D fragment (optionally codon optimized) is coupled to the first fragment by, for example, a GS3 linker. In several embodiments, such chimeric receptors further comprise a CD8a hinge, a CD8a transmembrane domain, 4-1BB, and CD3zeta. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 66. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 67. In several embodiments, the polynucleotide is co-expressed with an additional construct encoding membrane-bound interleukin 15 (mbIL15).

[0033] In several embodiments, there is provided a polynucleotide encoding a chimeric receptor comprising an extracellular receptor domain, comprising a fragment of NKG2D that binds a native ligand of NKG2D and is encoded by a fragment of SEQ ID NO: 1, a transmembrane region comprising a CD3zeta transmembrane region, and an effector domain. In several embodiments, there is provided a polynucleotide encoding a chimeric receptor comprising an extracellular receptor domain, comprising a fragment of NKG2D that binds a native ligand of NKG2D and is encoded by SEQ ID NO. 2, a transmembrane region comprising a CD3zeta transmembrane region, and an effector domain. In several embodiments, there is provided a polynucleotide encoding a chimeric receptor comprising an extracellular receptor domain, comprising a fragment of NKG2D that binds a native ligand of NKG2D and is encoded by SEQ ID NO. 3, a transmembrane region comprising a CD3zeta transmembrane region, and an effector domain. In several embodiments, there is provided a polynucleotide encoding a chimeric receptor comprising an extracellular receptor domain, comprising a fragment of NKG2D that binds a native ligand of NKG2D and is encoded by SEQ ID NO. 68, a transmembrane region comprising a CD3zeta transmembrane region, and an effector domain. In several embodiments, fragments of the NKG2D encoded by any of SEQ ID NO. 2, 3, or 68 may also be used. In several embodiments, the CD3zeta transmembrane region comprises the amino acid sequence of SEQ ID NO: 69. Fragments of the sequence of SEQ ID NO: 69 are also use, in several embodiments, the fragments retaining the ability to transduce at least about 65%, about 75%, about 85%, or about 95% of the signal transduction of a native CD3 zeta subunit (including dimers). In several embodiments, the extracellular receptor domain further comprises additional resides adjacent to the CD3zeta transmembrane region. In several embodiments, the additional amino acids are extracellular residues of a native CD3zeta sequence. In other embodiments, the additional amino acids are randomly selected. In several embodiments, there are 2, 3, 4, 5, 6, 8, 10, 15, or 20 additional amino acids. In several embodiments, the chimeric receptor domain comprises a hinge region, which in several embodiments, a CD8a hinge encoded by the nucleic acid sequence of SEQ ID NO: 5. In several embodiments, the hinge region is a CD8a hinge encoded by a fragment of the nucleic acid sequence of SEQ ID NO: 5. Depending on the embodiment, the fragment is about 75%, about 80%, about 85%, about 90%, about 95% of the length of the nucleic acid sequence of SEQ ID NO: 5. Depending on the embodiment, the fragment is about 75%, about 80%, about 85%, about 90%, about 95%, about 98%, or about 99% homologous to the nucleic acid sequence of SEQ ID NO: 5. In several embodiments, the extracellular receptor domain further comprises a CD8a signal peptide, which, depending on the embodiment, can comprise the nucleic acid sequence of SEQ ID NO. 4. In several embodiments, the effector domain comprises 4-1BB. In several embodiments, the effector domain comprises a CD16 intracellular domain. In several embodiments, the effector domain comprises 4-1BB and CD16 (with either moiety being “first” vs. “second” in the construct). In several embodiments, repeats of one or more of 4-1BB and/or CD16 are used.

[0034] In several embodiments, the chimeric receptor comprises a fragment of NKG2D that is codon optimized and is coupled to a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising 4-1BB. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 78. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 79.

[0035] In several embodiments, the chimeric receptor comprises a fragment of NKG2D that is codon optimized coupled to a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising CD16 followed by 4-1BB. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 71. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 70.

[0036] In several embodiments, the chimeric receptor comprises a fragment of NKG2D that is codon optimized and coupled to a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising 4-1BB followed by CD16, optionally coupled by a GS3 linker. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 85. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 84.

[0037] In several embodiments, the chimeric receptor comprises a fragment of NKG2D that is codon optimized and is coupled to a GS3 linker, an additional NKG2D fragment, a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising a CD16 and 4-1BB. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 72. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 73.

[0038] In several embodiments, the effector domain includes NKp80. In several embodiments, the effector domain is NKp80. In several embodiments, the chimeric receptor comprises a fragment of NKG2D that is coupled to a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising a CD16, 4-1BB, and NKp80, and optionally including a GS3 linker. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 74. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 75. In several embodiments, the chimeric receptor comprises the fragment of NKG2D that is codon optimized and is coupled to a GS3 linker, an additional NKG2D fragment (optionally codon optimized), a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising a CD16, 4-1BB, and NKp80, and optionally including a GS3 linker. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 76. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 77. In several embodiments, the chimeric receptor comprises a fragment of NKG2D that is codon optimized and is coupled to a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising 4-1BB and NKp80, and optionally including a GS3 linker. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 82. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 83.

[0039] In several embodiments, the effector domain comprises CD3zeta. In several embodiments, the chimeric receptor comprises a fragment of NKG2D that is codon optimized and is coupled to a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising 4-1BB and CD3zeta. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 80. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 81.

[0040] In several embodiments, the effector domain comprises FcRγ. In several embodiments, the chimeric receptor comprises a fragment of NKG2D coupled to a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising 4-1BB and FcRγ. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 86. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 87.

[0041] In several embodiments, the effector domain comprises CD28. In several embodiments, the chimeric receptor comprises a fragment of NKG2D coupled to a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising CD28 and CD3zeta. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 102. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 103.

[0042] In several embodiments, the effector domain comprises a GS linker.

[0043] In several embodiments, the polynucleotides disclosed herein are co-expressed with membrane-bound interleukin 15 (mbIL15).

[0044] In several embodiments, a polynucleotide encoding a chimeric receptor comprising an extracellular receptor domain comprising a fragment of NKG2D that is capable of binding a native ligand of NKG2D and is encoded by a fragment of any one of the sequence of SEQ ID NO: 1, of SEQ ID NO. 2, of SEQ ID NO. 3, or SEQ ID NO. 68, and an effector domain comprising a transmembrane region and an intracellular signaling domain. In several embodiments, there is provided a polynucleotide encoding a chimeric receptor comprising an extracellular receptor domain comprising a fragment of NKG2D that is capable of binding a native ligand of NKG2D and is encoded by (i) a fragment of the sequence of SEQ ID NO: 1, (ii) the sequence of SEQ ID NO. 2, (iii) the sequence of SEQ ID NO. 3, or (iv) the sequence of SEQ ID NO. 68, and an effector domain comprising a transmembrane region and an intracellular signaling domain. In several embodiments, a polynucleotide encoding a chimeric receptor comprising an extracellular receptor domain comprising a fragment of NKG2D that is capable of binding a native ligand of NKG2D and is encoded by the sequence of SEQ ID NO. 2, and an effector domain comprising a transmembrane region and an intracellular signaling domain. In several embodiments, a polynucleotide encoding a chimeric receptor comprising an extracellular receptor domain comprising a fragment of NKG2D that is capable of binding a native ligand of NKG2D and is encoded by the sequence of SEQ ID NO. 3, and an effector domain comprising a transmembrane region and an intracellular signaling domain. In several embodiments, a polynucleotide encoding a chimeric receptor comprising an extracellular receptor domain comprising a fragment of NKG2D that is capable of binding a native ligand of NKG2D and is encoded by a fragment of the sequence of SEQ ID NO. 68, and an effector domain comprising a transmembrane region and an intracellular signaling domain. In several embodiments, the extracellular receptor domain comprises a hinge region. In several embodiments, the hinge region is a CD8a hinge encoded by the nucleic acid sequence of SEQ ID NO: 5, or optionally a fragment of the nucleic acid sequence of SEQ ID NO: 5 (e.g., a fragment having about 75%, about 85%, about 95% homology to SEQ ID NO: 5). In several embodiments, the hinge region is an Immunoglobulin G4 (IgG4) hinge encoded by the nucleic acid sequence of SEQ ID NO: 104. In several embodiments, the hinge region is an Immunoglobulin G4 (IgG4) hinge encoded by a fragment of the nucleic acid sequence of SEQ ID NO: 104 (e.g., a fragment having about 75%, about 85%, about 95% homology to SEQ ID NO: 104). In several embodiments, the extracellular receptor domain further comprises a CD8a signal peptide, wherein the signal peptide comprises the nucleic acid sequence of SEQ ID NO. 4. In several embodiments, the effector domain comprises at least one signaling domains selected from the group consisting of OX40 (CD134), CD3zeta, 4-1BB, CD28 and DAP12. In several embodiments, the chimeric receptor transmembrane domain comprises a CD8 transmembrane domain. In several embodiments, the chimeric receptor comprises IL-15 linked (optionally by a GS3 linker) to the fragment of NKG2D coupled to a CD8a hinge, a CD8a transmembrane domain, 4-1BB, and CD3z. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 88. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 89.

[0045] In several embodiments, the chimeric receptor comprises a fragment of NKG2D coupled to an IgG4 hinge, a CD8a transmembrane domain, 4-1BB, and CD3zeta. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 96. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 97.

[0046] In several embodiments, the effector domain comprises OX40. In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD8a transmembrane domain, OX40, and CD3z. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 90. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 109. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 91. In several embodiments, the chimeric receptor comprises a fragment of NKG2D coupled to an IgG4 hinge, a CD8a transmembrane domain, OX40 and CD3zeta. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 100. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 101.

[0047] In several embodiments, the chimeric receptor comprises a CD28 transmembrane/intracellular domain. In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD28 transmembrane/intracellular domain, and CD3zeta. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 92. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 93.

[0048] In several embodiments, the chimeric receptor comprises a fragment of NKG2D coupled to a CD8a hinge, a CD28 transmembrane/intracellular domain, 4-1BB, and CD3zeta. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 94. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 95.

[0049] In several embodiments, the chimeric receptor comprises a fragment of NKG2D coupled to an IgG4 hinge, a CD28 transmembrane/intracellular domain and CD3zeta. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 98. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 99.

[0050] In several embodiments, the effector domain comprises a GS linker. In several embodiments, the polynucleotides disclosed herein are configured to be co-expressed (either on the same polynucleotide, or another polynucleotide) with membrane-bound interleukin 15 (mbIL15).

[0051] Any of the chimeric receptors can optionally include an extracellular receptor domain that includes a second peptide that binds native ligands of NKG2D. In several embodiments, the second peptide is homologous with NKG2D, while in other embodiments, the second peptide is heterologous with respect to the NKG2D. Whether the chimeric receptor includes a dimerized extracellular receptor domain, the extracellular receptor domains can recognize at least the following native ligands of NKG2D: MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5 or ULBP6.

[0052] As discussed in more detail below, functional variants of the NKG2D ligand binding domains are employed in several embodiments. For example the peptide that binds native ligands of NKG2D has, in several embodiments, at least 80% homology to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 68. In several embodiments, the peptide that binds native ligands of NKG2D has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homology to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 68.

[0053] Additionally provided for herein in several embodiments are vectors for expressing the chimeric receptors. In several embodiments, the polynucleotides provided for herein are mRNA and can include an operable linkage to least one regulatory element for the expression of the chimeric receptor. In several embodiments, the polynucleotides further include one or more internal ribosome entry site (IRES). In several embodiments, the vector is a retrovirus.

[0054] Engineered natural killer cells are also provided for, in several embodiments, that express any of the chimeric receptor constructs disclosed herein, the engineered NK cells exhibiting enhanced cytotoxic effects against target cells. Enhanced cytotoxic effects include, but are not limited to, higher affinity for target (e.g., cancerous) cells as compared to normal (e.g., non-cancerous) cells, a greater killing effect directed against target cells, reduced off-target effects, increased duration of cytotoxic effects, more efficient cytotoxicity, and the like. Such enhanced effects can be identified through the use of various in vitro cytotoxicity assays (e.g., measurement of cytokine production, etc.), measurement of target cell death, or through various clinical outcomes (e.g., reduction in tumor burden). In several embodiments, the engineered NK cells are an autologous cell isolated from a patient. In additional embodiments, the engineered NK cells are generated from allogeneic cells isolated from a donor. Such engineered NK cells as disclosed herein are used, in several embodiments, to enhance NK cell cytotoxicity in a mammal in need thereof, by administering the NK cells. These engineered NK cells are used, in several embodiments for treating or preventing cancer or an infectious disease in a mammal. The polynucleotides encoding, the vectors carrying, and the NK cells expressing the various chimeric receptors disclosed herein can also be used, in several embodiments in the manufacture of a medicament for enhancing NK cell cytotoxicity (e.g., to treat or prevent cancer or an infectious disease). In several embodiments, the chimeric receptor constructs disclosed herein do not significantly increase the cytotoxicity of the engineered NK cells against normal cells and, as described herein, are advantageously improved as compared to non-engineered NK cells.In several embodiments, there is provided a polynucleotide encoding a chimeric receptor comprising an extracellular receptor domain, a transmembrane region, and an effector domain. In several embodiments, the extracellular receptor domain comprises a peptide that binds native ligands of Natural Killer Group 2 member D (NKG2D), wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D. Several embodiments, relate to a polynucleotide encoding a chimeric receptor comprising: (a) an extracellular receptor domain, wherein said extracellular receptor domain comprises a peptide that binds native ligands of Natural Killer Group 2 member D (NKG2D), wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D, wherein the fragment of NKG2D is encoded by a polynucleotide comprising: (i) a fragment of the sequence of SEQ ID NO: 1, (ii) the sequence of SEQ ID NO. 2, (iii) the sequence of SEQ ID NO. 3, or (iv) the sequence of SEQ ID NO. 68, (b) a transmembrane region, and (c) an effector domain.

[0055] In several embodiments, there is provided a polynucleotide encoding a chimeric receptor comprising: (a) an extracellular receptor domain, wherein said extracellular receptor domain comprises a peptide that binds native ligands of Natural Killer Group 2 member D (NKG2D), wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D, wherein the fragment of NKG2D is encoded by a polynucleotide comprising: (i) a fragment of the sequence of SEQ ID NO: 1, (ii) the sequence of SEQ ID NO. 2, (iii) the sequence of SEQ ID NO. 3, (iv) or the sequence of

SEQ ID NO. 68; and (b) an effector domain comprising a transmembrane region and an intracellular signaling domain.

[0056] In several embodiments, the transmembrane region comprises a CD3zeta transmembrane region. In several embodiments, the CD3zeta transmembrane region comprises the amino acid sequence of SEQ ID NO: 69. In several embodiments, the transmembrane region comprises CD8a. In several embodiments, the effector domain comprises 4-1BB, an intracellular domain of 2B4, NKp80, a CD16 intracellular domain, Natural Cytotoxicity Triggering Receptor 1 (NCR1), Natural Cytotoxicity Triggering Receptor 2 (NCR2), Natural Cytotoxicity Triggering Receptor 3 (NCR3), and/or an intracellular domain of DAP10. In one embodiment, the effector domain comprises 4-1BB and CD16. In several embodiments, the effector domain comprises 4-1BB and CD3 zeta. In several embodiments, the effector domain comprises 4-1BB and an intracellular domain of 2B4 or DAP10. In several embodiments, the effector domain comprises 2B4 followed by 4-1BB while in other embodiments the effector domain comprises 4-1BB followed by 2B4. In several embodiments, the effector domain comprises DAP10 followed by 4-1BB. In several embodiments, the effector domain comprises 4-1BB followed by DAP10. In several embodiments, the effector domain further comprises CD3zeta. In several embodiments, the effector domain comprises at least one signaling domain selected from the group consisting of OX40 (CD134), CD3zeta, 4-1BB, CD28 and DAP12. In several embodiments the effector domain comprises one or more hemi-ITAM sequences. In several embodiments, the hemi-ITAM comprises the amino acid sequence of SEQ ID NO. 14. In several embodiments, the hemi-ITAM comprises the amino acid sequence of SEQ ID NO. 37. In several embodiments, the effector domain comprises one or more ITSM sequences. In several embodiments, the ITSM comprises the amino acid sequence of SEQ ID NO. 15 or the amino acid sequence of SEQ ID NO. 35

[0057] In several embodiments, the chimeric receptor comprises a fragment of NKG2D coupled to a CD8a hinge, a CD8a transmembrane domain, 4-1BB, 2B4, and CD3zeta. In one embodiment, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 58. In one embodiment, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 59. In several embodiments, the chimeric receptor comprises a fragment of NKG2D coupled to a CD8a hinge, a CD8a transmembrane domain, 4-1BB, and DAP10. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 60 and comprises the amino acid sequence of SEQ ID NO: 61.

[0058] In several embodiments, the chimeric receptor comprises a fragment of NKG2D coupled to a CD8a hinge, a CD8a transmembrane domain, 4-1BB, 2B4, and DAP10. In several embodiments, the effector domain comprises 4-1BB, followed by DAP10, followed by 2B4. In some embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 62 and the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 63. In several embodiments, the effector domain comprises 4-1BB, followed by 2B4, followed by DAP10. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 64 and the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 65.

[0059] In several embodiments, the chimeric receptor comprises a fragment of NKG2D that is codon optimized coupled to a GS3 linker, an additional NKG2D fragment, a CD8a hinge, a CD8a transmembrane domain, 4-1BB, and CD3zeta. In one embodiment, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 66. In several embodiments, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 67.

[0060] In several embodiments, the chimeric receptor comprises a fragment of NKG2D that is codon optimized coupled to a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising 4-1BB, is encoded by the nucleic acid sequence of SEQ ID NO: 78 and/or comprises the amino acid sequence of SEQ ID NO: 79.

[0061] In several embodiments, the chimeric receptor comprises a fragment of NKG2D that is codon optimized coupled to a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising CD16 followed by 4-1BB. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 70 and/or comprises the amino acid sequence of SEQ ID NO: 71.

[0062] In several embodiments, the chimeric receptor comprises a fragment of NKG2D that is codon optimized coupled to a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising 4-1BB followed by a GS3 linker and CD16. In one embodiment, the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 85 and/or is encoded by the nucleic acid sequence of SEQ ID NO: 84.

[0063] In several embodiments, the chimeric receptor comprises a fragment of NKG2D that is codon optimized coupled to a GS3 linker, an additional NKG2D fragment, a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising a CD16 and 4-1BB. In one embodiment, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 72 and/or comprises the amino acid sequence of SEQ ID NO: 73.

[0064] In several embodiments, the chimeric receptor comprises IL-15 linked by a GS3 linker to the fragment of NKG2D coupled to a CD8a hinge, a CD8a transmembrane domain, 4-1BB, and CD3zeta, is encoded by the nucleic acid sequence of SEQ ID NO: 88 and/or comprises the amino acid sequence of SEQ ID NO: 89.

[0065] In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to a IgG4 hinge, a CD8a transmembrane domain, 4-1BB, and CD3zeta is encoded by the nucleic acid sequence of SEQ ID NO: 96, and/or comprises the amino acid sequence of SEQ ID NO: 97.

[0066] In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD8a transmembrane domain, OX40, and CD3z,is encoded by the nucleic acid sequence of SEQ ID NO: 90, and/or comprises the amino acid sequence of SEQ ID NO: 91.

[0067] In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to an IgG4 hinge, a CD8a transmembrane domain, OX40 and CD3zeta, is encoded by the nucleic acid sequence of SEQ ID NO: 100, and/or comprises the amino acid sequence of SEQ ID NO: 101.

[0068] In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD28 transmembrane/intracellular domain, and CD3zeta, is encoded by the nucleic acid sequence of SEQ ID NO: 92, and/or comprises the amino acid sequence of SEQ ID NO: 93.

[0069] In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD28 transmembrane/intracellular domain, 4-1BB, and CD3zeta, is encoded by the nucleic acid sequence of SEQ ID NO: 94, and/or comprises the amino acid sequence of SEQ ID NO: 95.

[0070] In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to an IgG4 hinge, a CD28 transmembrane/intracellular domain and CD3zeta, is encoded by the nucleic acid sequence of SEQ ID NO: 98, and/or comprises the amino acid sequence of SEQ ID NO: 99.

[0071] In several embodiments, the chimeric receptor comprises a fragment of NKG2D coupled to a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising a CD16, 4-1BB, a GS3 linker, and NKp80. In one embodiment, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 74 and/or comprises the amino acid sequence of SEQ ID NO: 75.

[0072] In several embodiments, the chimeric receptor comprises the fragment of NKG2D that is codon optimized coupled to a GS3 linker, an additional NKG2D fragment, a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising a CD16, 4-1BB, a GS3 linker, and NKp80. In one embodiment, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 76 and/or comprises the amino acid sequence of SEQ ID NO: 77. In several embodiments, the chimeric receptor comprises a fragment of NKG2D that is codon optimized coupled to a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising 4-1BB, a GS3 linker, and NKp80. In one embodiment, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 82 and/or comprises the amino acid sequence of SEQ ID NO: 83.

[0073] In several embodiments, the chimeric receptor comprises a fragment of NKG2D that is codon optimized coupled to a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising 4-1BB and CD3zeta. In one embodiment, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 80 and/or comprises the amino acid sequence of SEQ ID NO: 81.

[0074] Depending on the embodiment, the effector domain may also comprise FcRγ. For example, in several embodiments, the chimeric receptor comprises a fragment of NKG2D coupled to a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising 4-1BB and FcRγ. In one embodiment, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 86 and/or comprises the amino acid sequence of SEQ ID NO: 87.

[0075] Depending on the embodiment, the effector domain may also comprise CD28. For example, in several embodiments, the chimeric receptor comprises a fragment of NKG2D coupled to a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising CD28 and CD3zeta. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 102 and/or comprises the amino acid sequence of SEQ ID NO: 103.

[0076] In several embodiments, the effector domain comprises a GS linker.

[0077] In several embodiments, the extracellular receptor domain further comprises a CD8a signal peptide, wherein the signal peptide comprises the nucleic acid sequence of SEQ ID NO. 4. In several embodiments, the extracellular receptor domain further comprises 2 extracellular residues of CD3zeta directly adjacent to the CD3zeta transmembrane region. In several embodiments, the extracellular receptor domain comprises a CD8a signal peptide, wherein the signal peptide comprises the nucleic acid sequence of SEQ ID NO. 4.

[0078] In several embodiments, the chimeric receptor comprises one or more GS3 linkers. In several embodiments, the chimeric receptor domain comprises a hinge region. In several embodiments, the hinge region is encoded by the nucleic acid sequence of SEQ ID NO: 5, while in some embodiments, the hinge region is encoded by a fragment of the nucleic acid sequence of SEQ ID NO: 5. In several embodiments, the hinge region is a CD8a hinge. In several embodiments, the hinge region comprises a glycine-serine repeating motif having the amino acid sequence of SEQ ID NO: 31. In several embodiments, the hinge region comprises the amino acid sequence of SEQ ID NO: 32 and in some embodiments, the hinge region comprises the amino acid sequence of SEQ ID NO: 33. In additional embodiments, the hinge region is encoded by the nucleic acid sequence of SEQ ID NO: 34. In several embodiments, the hinge region comprises a portion of the beta-adrenergic receptor. In some such embodiments, the hinge region is encoded by the nucleic acid sequence of SEQ ID NO: 40. In additional embodiments, the hinge region is encoded by the nucleic acid sequence of SEQ ID NO: 42. In several embodiments, the hinge region is Immunoglobulin G4 (IgG4) hinge encoded by the nucleic acid sequence of SEQ ID NO: 104. In several embodiments, the hinge region is a Immunoglobulin G4 (IgG4) hinge encoded by a fragment of the nucleic acid sequence of SEQ ID NO: 104. In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge and a CD8a transmembrane domain.

[0079] In one embodiment, the chimeric receptor comprises the fragment of NKG2D coupled to CD16, is encoded by the nucleic acid sequence of SEQ ID NO: 23, and/or comprises the amino acid sequence of SEQ ID NO: 24. In one embodiment, the chimeric receptor comprises the fragment of NKG2D coupled to NCR1. In some such embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 27 and/or comprises the amino acid sequence of SEQ ID NO: 28. In several embodiments, the chimeric receptor comprises at least a portion of the amino acid sequence of SEQ ID NO: 21. In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to NCR3, in several embodiments is encoded by the nucleic acid sequence of SEQ ID NO. 29 and/or comprises the amino acid sequence of SEQ ID NO. 30.

[0080] In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to a CD16 transmembrane/intracellular domain and 4-1BB. In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD16 transmembrane/intracellular domain and 4-1BB, is encoded by the nucleic acid sequence of SEQ ID NO: 25, and/or comprises the amino acid sequence of SEQ ID NO: 26.

[0081] In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to NCR1 and 4-1BB, wherein the chimeric receptor comprises the NCR1 amino acid sequence of SEQ ID NO: 20.

[0082] In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to CD8a, 4-1BB and CD3z, is encoded by the nucleic acid sequence of SEQ ID NO. 18 and/or comprises the amino acid sequence of SEQ ID NO. 19.

[0083] In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to NCR3 and 4-1BB, and wherein the NCR3 comprises the amino acid sequence of SEQ ID NO: 22. In one embodiment, the chimeric receptor comprises one or more of the NCR1 transmembrane/intracellular domain of SEQ ID NO: 20 or the NCR3 transmembrane/intracellular domain of SEQ ID NO: 22.

[0084] In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to a GS3 linker, a CD8a hinge, a CD16 transmembrane/intracellular domain and 4-1BB. In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 43. In several embodiments, the chimeric receptors comprises the fragment of NKG2D coupled to a GS3 linker, a CD16 transmembrane/intracellular domain and 4-1BB. In one embodiment, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 44.

[0085] In several embodiments, the the chimeric receptor comprises the fragment of NKG2D coupled to a CD16 transmembrane/intracellular domain and 4-1BB and is encoded by the nucleic acid sequence of SEQ ID NO: 45.

[0086] In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD8a transmembrane domain, 4-1BB, and 2B4 and is encoded by the nucleic acid sequence of SEQ ID NO: 46.

[0087] In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to a beta-adrenergic extracellular domain, a beta-adrenergic transmembrane domain, 4-1BB, and 2B4 and is encoded by the nucleic acid sequence of SEQ ID NO: 47.

[0088] In several embodiments the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD8a transmembrane domain, 4-1BB, 2B4, a GS3 linker, and NKp80 and is encoded by the nucleic acid sequence of SEQ ID NO: 48.

[0089] In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD8a transmembrane domain, 4-1BB, a GS3 linker, and NKp80 and is encoded by the nucleic acid sequence of SEQ ID NO: 49.

[0090] In several embodiments, the chimeric receptor comprises the fragment of NKG2D that is codon optimized coupled to a GS3 linker, an additional NKG2D fragment, a beta-adrenergic extracellular domain, a beta-adrenergic transmembrane domain, 4-1BB, an additional GS3 linker, and NKp80 and is encoded by the nucleic acid sequence of SEQ ID NO: 50.

[0091] In several embodiments, the chimeric receptor comprises the fragment of NKG2D that is codon optimized coupled to a GS3 linker, an additional NKG2D fragment, a CD8a hinge, a CD8a transmembrane domain, 4-1BB, an additional GS3 linker, and NKp80 and is encoded by the nucleic acid sequence of SEQ ID NO: 51.

[0092] In several embodiments, the chimeric receptor comprises the fragment of NKG2D that is codon optimized coupled to a GS3 linker, an additional NKG2D fragment, a CD8a hinge, a CD16 transmembrane/intracellular domain, and 4-1BB and is encoded by the nucleic acid sequence of SEQ ID NO: 52.

[0093] In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD16 transmembrane/intracellular domain, 4-1BB, and 2B4 and is encoded by the nucleic acid sequence of SEQ ID NO: 53.

[0094] In several embodiments, the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD16 transmembrane/intracellular domain, 4-1BB, a GS3 linker, and NKp80 and is encoded by the nucleic acid sequence of SEQ ID NO: 54.

[0095] In several embodiments, the chimeric receptor constructs are encoded by a polynucleotide that encodes a chimeric receptor wherein the extracellular receptor domain comprises a second peptide that binds native ligands of NKG2D, (e.g., one or more of MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5 or ULBP6. Depending on the embodiment, the peptide that binds native ligands of NKG2D has at least 80% homology to SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO. 3.

[0096] In several embodiments, the polynucleotide is co-expressed with an additional construct encoding membrane-bound interleukin 15 (mbIL15). In several embodiments, the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 18. In several embodiments, the chimeric receptor is encoded by the amino acid sequence of SEQ ID NO: 19.

[0097] According to several embodiments, the chimeric receptor does not comprise DNAX-activating protein 10 (DAP10) and/or the chimeric receptor does not encode an immunoreceptor tyrosine-based activation (ITAM) motif.

[0098] In several embodiments, the polynucleotides disclosed herein are mRNA. Additionally, in several embodiments, the polynucleotide disclosed herein are operably linked to at least one regulatory element for the expression of the chimeric receptor.

[0099] Also provided for herein are vectors that comprise the polynucleotides disclosed herein. In several embodiments, the polynucleotide is operatively linked to at least one regulatory element for expression of the chimeric receptor. In several embodiments, the vector is a retrovirus.

[00100] Also provided for herein are genetically engineered natural killer cells comprising the any one or more of the polynucleotides disclosed herein. In several embodiments, the natural killer cells are for autologous use, while in some embodiments they are for allogeneic use.

[00101] Also provided for herein are methods of enhancing NK cell cytotoxicity in a mammal in need thereof, comprising administering to the mammal NK cells, wherein said NK cells express a chimeric receptor encoded by a polynucleotide disclosed herein.

[00102] Additionally, there are provided methods for treating or preventing cancer or an infectious disease in a mammal in need thereof, said method comprising administering to said mammal a therapeutically effective amount of NK cells, wherein said NK cells express a chimeric receptor encoded by a polynucleotide disclosed herein. As disclosed above, the NK cells can be allogeneic or autologous.

[00103] There is provided a use of a polynucleotide as disclosed herein in the manufacture of a medicament for enhancing NK cell cytotoxicity in a mammal in need thereof. Further there is provided a use of a polynucleotide in the manufacture of a medicament for treating or preventing cancer or an infectious disease in a mammal in need thereof.

[00104] Also provided is the use of a vector comprising a polynucleotide disclosed herein in the manufacture of a medicament for enhancing NK cell cytotoxicity in a mammal in need thereof. Also provided is the use of a vector comprising a polynucleotide disclosed herein in the manufacture of a medicament for treating or preventing cancer or an infectious disease in a mammal in need thereof.

[00105] Also provided is the use of an isolated genetically engineered natural killer cell expressing a chimeric receptor as disclosed herein for enhancing NK cell cytotoxicity in a mammal in need thereof. Also provided is the use of an isolated genetically engineered natural killer cell expressing a chimeric receptor as disclosed herein for treating or preventing cancer or an infectious disease in a mammal in need thereof.

[00106] The compositions and related methods summarized above and set forth in further detail below describe certain actions taken by a practitioner; however, it should be understood that they can also include the instruction of those actions by another party. Thus, actions such as "administering a population of NK cells expressing a chimeric receptor" include "instructing the administration of a population of NK cells expressing a chimeric receptor."

BRIEF DESCRIPTION OF THE DRAWINGS

[00107] The descriptions of the figures below are related to experiments and results that represent non-limiting embodiments of the inventions disclosed herein.

[00108] FIGs. 1A-1C depict schematic representations of the chimeric receptors according to several embodiments disclosed herein. FIG. 1A depicts endogenous NKG2D, FIG. 1B depicts NKG2D-DAP10-CD3ζ, and FIG. 1C depicts NKG2D-41BB-CD3ζ.

[00109] FIGs. 2A-2B depict schematic representations of the chimeric receptors, according to several embodiments disclosed herein. FIG. 2A depicts NKG2D-CD16 and FIG. 2B depicts NKG2D-CD16-41BB.

[00110] FIGs. 3A-3B depict plasmid maps illustrating the point of insertion of certain constructs according to several embodiments into the plasmids, illustrated is a Murine Stem Cell Virus (MSCV) plasmid. FIG. 3A shows gene constructs for NKG2D-DAP10-CD3ζ and NKG2D-41BB-CD3ζ that were inserted into the EcoRI and NotI restriction sites, with removal the IRES-GFP sequence in the vector. FIG. 3B depicts the plasmids for NKG2D-CD16 and NKG2D-CD16-41BB that were inserted into EcoRI and XhoI restriction sites located in the multiple cloning site (MCS). IRES-GFP sequence in the vector allows for the tracing of transduction efficiency.

[00111] FIGs. 4A-4C depict data related to the expression of NKG2D-DAP10-CD3ζ and NKG2D-41BB-CD3ζ in NK cells. FIG. 4A shows flow cytometry data illustrating the percentage of NKG2D-positive NK cells after transduction. FIG. 4B shows a dot plots summarizing the percentage of NKG2D-positive NK cells. FIG. 4C shows data related to the mean fluorescence intensity (MFI) in different group of NK cells after transduction.

[00112] FIGs. 5A-5C depict data related to the cytotoxicity of the various constructs generated from NK cells from Donor 1, Donor 2, and Donor 3 (FIGs. 5A, 5B, and 5C, respectively) against cultured REH cells.

[00113] FIGs. 6A-6C depict data related to the cytotoxicity of the various constructs generated from NK cells from Donor 1, Donor 2, and Donor 3 (FIGs. 6A, 6B, and 6C, respectively) against cultured U-2 OS cells.

[00114] FIGs. 7A-7B depict data related to the production of interferon-gamma by NK cells expressing various NKG2D constructs in the presence and absence of stimulation with REH cells. FIG. 7A depicts the relative amount of IFNγ in the different groups of NK cells with or without stimulation by REH cells. FIG. 7B depicts levels of IFNγ between different groups of NK cells after stimulation (median values represented).

[00115] FIGs. 8A-8C depict data related to the expression of NKG2D-DAP10-CD3ζ and NKG2D-CD16 in NK cells. FIG. 8A shows flow cytometry data illustrating the percentage of NKG2D-positive NK cells after transduction. FIG. 8B shows a dot plots summarizing the percentage of NKG2D-positive NK cells. FIG. 8C shows data related to the mean fluorescence intensity (MFI) in different group of NK cells after transduction.

[00116] FIGs. 9A-9C depict data related to the cytotoxicity of the various constructs generated from NK cells from 3 donors (FIGs. 9A, 9B, and 9C, respectively) against cultured REH cells.

[00117] FIGs. 10A-10C depict data related to the cytotoxicity of the various constructs generated from NK cells from 3 donors (FIGs. 10A, 10B, and 10C, respectively) against cultured U-2 OS cells.

[00118] FIG. 11 depicts data related to the production of interferon-gamma by NK cells expressing various NKG2D constructs in the presence and absence of stimulation with REH cells.

[00119] FIGs. 12A-12B depict data related to expression of NKG2D-DAP10-CD3ζ and NKG2D-CD16-41BB in NK cells. FIG. 12A shows flow cytometry data illustrating the percentage of NKG2D-positive NK cells after transduction. FIG. 12B shows a histogram related to relative amount of surface expression of the various constructs on NK cells.

[00120] FIGs. 13A-13B depict data related to the degree of cytotoxicity of various NKG2d constructs. FIG. 13A depicts the degree of cytotoxicity against cultured REH cells. FIG. 13B depicts the degree of cytotoxicity against cultured U2OS cells.

[00121] FIG. 14 schematically depicts construct maps of several NKG2D constructs according to some embodiments disclosed herein.

[00122] FIG. 15 schematically depicts construct maps of additional NKG2D constructs according to some embodiments disclosed herein.

[00123] FIGs. 16A-16C depict data related to the expression of the various NKG2D constructs in NK cells. FIG. 16A shows data related to the mean fluorescence intensity (MFI) of the various NKG2D constructs in NK cells. FIG. 16B shows flow cytometry data illustrating the percentage of NKG2D-positive and CD56-positive NK cells after transduction of various NKG2D constructs into the NK cells of two donors (505 and 870). FIG. 16C shows data related to the mean fluorescence intensity (MFI) in NK cells from 2 donors seven days after transduction.

[00124] FIG. 17 depicts data related to the cytotoxicity of the various NKG2D constructs 14 days post-transduction into NK cells at a 1:1 E:T ratio.

[00125] FIGs. 18A-18B depicts data related to the expression of the various NKG2D constructs following transduction into NK cells. FIG. 18A shows data related to the mean fluorescence intensity (MFI) in NK cells seven days after transduction. FIG. 18B shows data related to the fold-change in MFI of the various NKG2D constructs relative to the mock-transduced NK cells.

[00126] FIGs. 19A-19B depict data related to the cytotoxicity of the various NKG2D constructs. FIG. 19A shows data related to the cytotoxicity of the various NKG2D constructs transduced into NK cells at a 1:1 E:T ratio. FIG. 19B shows data related to the percent change in cytotoxicity of the various NKG2D constructs relative to the mock-transduced NK cells.

[00127] FIG. 20 depicts data related to the cytotoxicity of the various NKG2D constructs 14 days post-transduction into NK cells at a 1:1 E:T ratio. Prior to analysis NK cells were cultured in media supplemented with 40 IU of IL-2/mL.

[00128] FIG. 21 depicts data related to the cytotoxicity of the various NKG2D constructs 10 days post-transduction into Donor 238 NK cells (with 4 days of culturing in media supplemented with 40 IU of IL-2/mL every two days) against cultured REH cells at 1:1 and 1:2 E:T ratios for two hours.

[00129] FIG. 22 schematically depicts construct maps of additional NKG2D constructs according to embodiments disclosed herein.

[00130] FIGs. 23A-23B depict data related to the persistence of the various NKG2D constructs generated from NK cells from two different donors (Donor 61 and Donor 103 in FIGs. 23A and 23B, respectively). NK cells were cultured in media supplemented with 40 IU of IL-2/mL.

[00131] FIG. 24 depicts data related to the expression of the various NKG2D constructs. NK cells were expanded from peripheral blood mononuclear cells (PBMC) of 4 healthy donors (224, 225, 362 and 363) and transduced with viruses directing the expression of the indicated constructs. Three days following transduction, NK cells were stained with a fluorescently labelled anti-NKG2D antibody and analyzed using flow cytometry. Relative NKG2D expression was assessed by mean fluorescence intensity (MFI) of labeled cells.

[00132] FIGs. 25A-25B depict data related to the cytotoxicity of NK cells transduced with various NKG2D constructs. NK cells were expanded from PBMC of 4 donors; Eight days after transduction, NK cytotoxicity against cultured REH and HL60 cells (FIGs. 25A and 25B, respectively) was measured at a 1:1 E:T ratio. NK cells were cultured in media supplemented with 40 IU of IL-2/mL prior to analysis.

[00133] FIGs. 26A-26C depict data related to the production of interferon-gamma (IFNγ), tumor necrosis factor-alpha (TNFα), and granulocyte-macrophage colony-stimulating factor (GM-CSF) by NK cells expressing various NKG2D constructs after overnight stimulation with REH tumor cells. Eight days after transduction with the indicated constructs, 1×10^5 NK cells were stimulated with 1×10^5 REH cells in individual wells of a 96-well round bottom plate; after overnight incubation, supernatants were harvested, and cytokine levels measured against relevant standards using a Meso Scale Discovery device. FIG. 26A depicts the accumulated levels of IFNγ, FIG. 26B depicts the levels of TNFα, and FIG. 26C depicts the levels of GM-CSF in the different groups of NK cells following stimulation. Prior to analysis NK cells were cultured in media supplemented with 40 IU of IL-2/mL.

[00134] FIGs. 27A-27B depict data related to the persistence of NK cells from two donors (donors 224 and 225 in FIGs. 27A and 27B, respectively) expressing the various NKG2D constructs 7, 14, and 21 days post-transduction. Prior to analysis NK cells were cultured in media supplemented with 40 IU of IL-2/mL.

[00135] FIGs. 28A-28B depict data related to the cytotoxicity of NK cells transduced with the indicated NKG2D constructs. NK cytotoxicity was measured against U2OS cells stably transduced to express Red Fluorescent Protein; U2OS cells were cultured with NK cells at a 1:4 and 1:2 E:T ratios (FIGs. 28A and 28B, respectively). Live U2OS cells were counted every 60 minutes for 72 hours using an Incucyte S3 Live-Cell Analysis System. Prior to analysis NK cells were cultured in media supplemented with 40 IU of IL-2/mL.

DETAILED DESCRIPTION

General

[00136] The emergence and persistence of aberrant cells (including virally infected and malignant cells) underlying many diseases is enabled by an insufficient immune response to said aberrant cells. A goal of immunotherapy is to initiate or augment the response of the patient's immune system, for example, to boost the ability of immune cells, such as Natural Killer (NK) cells to damage, kill, or otherwise inhibit damaged or diseased cells. One immunotherapy approach is the recombinant expression of chimeric receptors in immune cells for targeted recognition and destruction of the aberrant cells. In general, chimeric receptors comprise an extracellular receptor domain that recognizes ligands on target cells, an anchoring transmembrane domain, and an effector domain that transduces activating signals upon ligand binding. Some embodiments disclosed herein utilize chimeric receptors having that general structure, or having variations in that general structure. Additionally, in several embodiments, the transmembrane domain and the effector domain are separate peptides fused together. In several other embodiments, the transmembrane and the effector domain are derived from the same peptide. In some such embodiments, the transmembrane and effector domains comprise a single peptide (e.g., one peptide that passes through the membrane and is also poised to initiate a signaling cascade). As discussed in more detail below, truncations, mutations, additional linkers/spacer elements, dimers, and the like are used to generate chimeric receptor constructs that exhibit a desired degree of expression in an immune cell (e.g., an NK cell), induce cytotoxic activity from the NK cell, balanced with a degree of target avidity that avoids adverse effects on non-target cells. The recombinant expression of chimeric receptors as disclosed herein on the surface of immune cells can redirect the targeting of immune cells to aberrant cells of interest as well as augment the immune activation upon engagement.

NK Cells for Immunotherapy

[00137] One immunotherapy approach involves administering to patients T cells engineered to express chimeric receptors to elicit a positive immune response. However, a drawback of this approach is that it necessitates the use of autologous cells to prevent the induction of graft-versus-host-disease in the patient. As is provided in several embodiments disclosed herein, compositions comprising engineered NK cells enjoy several advantages. For example, either autologous or donor-derived allogeneic cells can be employed with an NK cell approach. Additionally, according to several embodiments, the engineered NK cells as provided for herein do not significantly increase cytotoxicity against normal cells. Further, NK cells have a significant cytotoxic effect, once activated. In view of this, it is unexpected that the engineered NK cells as provided for herein, are able to further elevate that cytotoxic effect, thus providing an even more effective means of selectively killing diseased target cells. Accordingly, in several embodiments, there is provided a method of treating or preventing cancer or an infectious disease, comprising administering a therapeutically effective amount of NK cells expressing the chimeric receptors described herein. In one embodiment, the NK cells administered are autologous cells. In a further embodiment, the NK cells administered are donor-derived (allogeneic) cells.

[00138] In several embodiments, engagement and activation of a recombinant NK cell (e.g., by binding to a ligand on a target cell) expressing a chimeric receptor leads to the direct killing of the stressed and/or aberrant cell (e.g., tumor cells, virally-infected cells, etc.) by cytolysis. Accordingly, in several embodiments, there is provided a method of enhancing NK cell cytotoxicity, comprising administering NK cells engineered to express the chimeric receptors described herein. In one embodiment, the NK cells administered are autologous cells. In a further embodiment, the NK cells are donor-derived (allogenic) cells. In several embodiments, engineered NK cells lead to indirect destruction or inhibition of stressed and/or aberrant cell (e.g., tumor cells, virally-infected cells, etc.).

Ligand Binding Domains

[00139] As mentioned above, in several embodiments NK cells recognize and destroy aberrant cells, including tumor cells and virally-infected cells. The cytotoxic activity of these innate immune cells is regulated by the balance of signaling from inhibitory and activating receptors, respectively, that reside on the cell surface. The former bind self-molecules expressed on the surface of healthy cells while the latter bind ligands expressed on aberrant cells. The increased engagement of activating receptors relative to inhibitory receptors leads to NK cell activation and target cell lysis. Natural killer Group 2 member D (NKG2D) is an important NK cell activating receptor that recognizes a number of ligands expressed on stressed and aberrant cells. The surface expression of various NKG2D ligands is generally low in healthy cells but is upregulated upon malignant transformation or viral infection. Non-limiting examples of ligands recognized by NKG2D include, but are not limited to, MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and ULBP6, as well as other molecules expressed on target cells that control the cytolytic or cytotoxic function of NK cells.

[00140] NKG2D's ability to recognize a plurality of surface markers of cell stress and infection make it a potentially useful component of a chimeric receptor-based immunotherapy approach. However, complicating the use of NKG2D as a chimeric receptor is its relationship with partner DAP10. NKG2D is a type II transmembrane glycoprotein that forms homodimers and assembles with two homodimers of DNAX-activating protein 10 (DAP10) to yield hexameric complexes on the membrane surface. This NKG2D-DAP10 association is necessary for both surface membrane expression of endogenous NKG2D as well as for transduction of the activation signal upon ligand binding. In several embodiments, a full length NKG2D is used. In one embodiment, full length NKG2D has the nucleic acid sequence of SEQ ID NO. 1. According to several embodiments disclosed herein, polynucleotides encoding chimeric receptors are provided wherein the extracellular receptor domain is a fragment of NKG2D that lacks its native transmembrane or intracellular domains yet advantageously retains its ability to bind native ligands of NKG2D, as well as transduce activation signals upon ligand binding. Thus, in several embodiments, the chimeric receptor encoded by the polypeptides disclosed herein does not comprise DAP10. In several embodiments, the NKG2D fragment is encoded by SEQ ID NO. 2. In several embodiments, the fragment of NKG2D is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with full-length wild-type NKG2D. In several embodiments, the fragment may have one or more additional mutations from SEQ ID NO. 2, but retains, or in some embodiments, has enhanced, ligand-binding function. In several embodiments, the NKG2D fragment is provided as a dimer, trimer, or other concatameric format, such embodiments providing enhanced ligand-binding activity. In several embodiments, the sequence encoding the NKG2D fragment is optionally fully or partially codon optimized. In one embodiment, a sequence encoding a codon optimized NKG2D fragment comprises the sequence of SEQ ID NO. 3. Additionally, in several embodiments signal peptides are used. The species or sequence of the signal peptide can vary with the construct. However, in several embodiments, a signal peptide derived from CD8 is used. In one embodiment, the signal peptide is from CD8a and has the sequence of SEQ ID NO. 4. In one embodiment, a sequence encoding a codon optimized NKG2D fragment comprises the sequence of SEQ ID NO. 68. In several embodiments, the fragment may have one or more additional mutations from SEQ ID NO. 68, but retains ligand-binding function. In several embodiments, the fragment may have one or more additional mutations from SEQ ID NO. 68, but has improved ligand-binding function.

Transmembrane, Signaling and Combination Domains

[00141] As mentioned above, the general chimeric antigen receptor structure comprises at least one transmembrane domain, linking the ligand binding domain to a signaling domain(s). In several embodiments, however, a transmembrane domain can also serve to provide signaling function.

[00142] In several embodiments, the NKG2D fragment retains at least a portion of its normal transmembrane domain. In several embodiments, the transmembrane domain comprises at least a portion of CD8, which is a transmembrane glycoprotein normally expressed on both T cells and NK cells. In several embodiments, the transmembrane domain comprises CD8α, while in some embodiments CD8β is used. In several embodiments, the "hinge" of CD8α has the sequence of SEQ ID NO. 5. In several embodiments, the CD8α can be truncated or modified, such that it is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% homologous with the CD8α having the sequence of SEQ ID NO. 5. In several embodiments, CD8β has the sequence of SEQ ID NO. 6. In several embodiments, the CD8β can be truncated or modified, such that it is at least 70%, at least 75%, at least 80%, at least 85%, at least

90%, at least 95% homologous with the CD8β having the sequence of SEQ ID NO. 6. In several embodiments, dimers of CD8α and CD8β are used.

[00143] In several embodiments, the transmembrane domain comprises CD16, which serves as a signaling domain as well. CD16 exists in two isoforms, a and b (also known as Fc gamma receptor IIIa and IIIb, respectively). These receptors normally bind to the Fc portion of IgG antibodies that in turn activates NK cells. Accordingly, in several embodiments, the transmembrane domain comprises CD16a, while in some embodiments CD16b is used. In several embodiments, CD16a has the sequence of SEQ ID NO. 7. In several embodiments, the CD16a can be truncated or modified, such that it is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% homologous with the CD16a having the sequence of SEQ ID NO. 7. In several embodiments, CD16b has the sequence of SEQ ID NO. 8. In several embodiments, the CD16b can be truncated or modified, such that it is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% homologous with the CD16b having the sequence of SEQ ID NO. 8. In several embodiments, dimers of CD16a and CD16b are used. In several embodiments the modifications to the CD16 transmembrane domain comprise additional nucleic acid residues to increase the length of the domain. Alternatively, CD16 may be shortened. The modifications to the length of CD16 advantageously can facilitate enhanced ligand-receptor interactions.

[00144] In several embodiments, the chimeric receptor comprises the Natural Killer Receptor 2B4 domain (referred to herein as “2B4”, and also known as CD244), which serves as a signaling domain as well. 2B4 is expressed on NK cells and regulates non-major histocompatibility complex (MHC) restricted killing through interactions between this receptor and its ligands on target cells. In several embodiments, the transmembrane domain comprises 2B4, while in several embodiments the 2B4 domain is an intracellular signaling domain. In several embodiments, 2B4 has the sequence of SEQ ID NO. 9. In several embodiments, the 2B4 can be truncated or modified, such that it is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% homologous with the 2B4 having the sequence of SEQ ID NO. 9. In several embodiments, 2B4 is used as the sole transmembrane/signaling domain in the construct, however, in several embodiments, 2B4 can be used with one or more other domains. For example, combinations of CD16, 4-1BB, and/or 2B4 are used in some embodiments.

[00145] In some embodiments, signaling is achieved through DAP10, as mentioned above. In several embodiments, the fragment of NKG2D associates with DAP10 to provide pro-cytotoxic signals to the NK cell. In several embodiments, dimers of DAP10 are used. In several embodiments, the transmembrane domain comprises DAP10. In several embodiments, DAP10 has the sequence of SEQ ID NO. 10. In several embodiments, DAP10 can be truncated or modified, such that it is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% homologous with the DAP10 having the sequence of SEQ ID NO. 10. Similarly, in some embodiments, DAP12 can be used, as it can also transduce such signals. In several embodiments, DAP12 has the sequence of SEQ ID NO. 11. In several embodiments, DAP12 can be truncated or modified, such that it is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% homologous with the DAP12 having the sequence of SEQ ID NO. 11. In several embodiments, heterodimers of DAP10 and DAP12 are used.

[00146] In several embodiments, signaling is provided through 4-1BB (also known as CD137 and tumor necrosis factor receptor superfamily member 9 (TNFRSF 9)). 4-1BB is a co-stimulatory immune checkpoint molecule, typically functioning as a stimulatory molecule for activated T cells (e.g., crosslinking of 4-1BB enhances T cell proliferation and cytolytic activity). However, in several embodiments, the function of 4-1BB is advantageously used in conjunction with NK cells. In several embodiments, 4-1BB has the sequence of SEQ ID NO. 12. In several embodiments, 4-1BB can be truncated or modified, such that it is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% homologous with the 4-1BB having the sequence of SEQ ID NO. 12. In several embodiments, 4-1BB is the sole signaling domain, but as discussed above, in several embodiments, 4-1BB functions unexpectedly well in combination with one or more of the other transmembrane/signaling domains disclosed herein. For example, in several embodiments, CD16 in conjunction with 4-1BB provides synergistic stimulation effects, resulting in particularly effective (e.g., cytotoxic) NK cells. In several embodiments, DAP10 in conjunction with 4-1BB provides synergistic stimulation effects, resulting in particularly effective (e.g., cytotoxic) NK cells. In several embodiments, DAP10 in conjunction with 4-1BB and/or 2B4 provides synergistic stimulation effects, resulting in particularly effective (e.g., cytotoxic) NK cells. Other improved characteristics result, in several embodiments, such as improved expression, improved persistence, and the like.

[00147] In several embodiments, the signaling domain comprises at least a portion of the CD3 T cell receptor complex. The T cell receptor complex comprises multiple subunits, including the zeta, alpha, beta, gamma, delta, and epsilon subunits. In several embodiments, the NK cells engineered according to several embodiments disclosed herein comprise at least one of these subunits (or a fragment thereof). In several embodiments, the signaling domain comprises the CD3 zeta subunit. In several embodiments, CD3 zeta has the sequence of SEQ ID NO. 13. In several embodiments, CD3 zeta can be truncated or modified, such that it is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% homologous with the CD3 zeta having the sequence of SEQ ID NO. 13. In several embodiments, the CD3 zeta is mutated (e.g., amino acid mutations, insertions, or deletions) such that the domain no longer is consistent with the canonical immunoreceptor tyrosine-based activation motif or ITAM motif. Thus, in several embodiments, the NK cells comprise an engineered receptor that does not contain an ITAM motif. In some embodiments, the resultant engineered NK cells exhibit particularly enhanced cytotoxicity against target cells, with limited or reduced adverse side effects. This, in several embodiments, results from the synergistic interactions of the various portions of the chimeric receptor that are used in that given embodiment. In several embodiments, CD3zeta in conjunction with 4-1BB provides synergistic stimulation effects, resulting in particularly effective (e.g., cytotoxic) NK cells. In several embodiments, CD3zeta in conjunction with 2B4 provides synergistic stimulation effects, resulting in particularly effective (e.g., cytotoxic) NK cells. In several embodiments, CD3zeta in combination with 2B4 and 4-1BB provides synergistic stimulation effects, resulting in particularly effective (e.g., cytotoxic) NK cells. In several embodiments, the chimeric receptors leverage the dimerization of CD3zeta via its transmembrane domain. Thus, in several embodiments, the transmembrane domain comprises the CD3zeta transmembrane domain (or a fragment thereof). In some embodiments, 1, 2, 3, 4, 5, 6 or more extracellular CD3zeta residues (the "juxta-membrane portion") are directly adjacent to the CD3zeta transmembrane domain. In some embodiments, CD3zeta transmembrane domain has the sequence of SEQ ID NO. 69. In several embodiments, the CD3zeta transmembrane domain can be truncated or modified, such that it is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% homologous with the CD3zeta transmembrane domain having the sequence of SEQ ID NO. 69. In several embodiments the modifications to the CD3zeta transmembrane domain comprise additional nucleic acid residues to increase the length of the domain. In several embodiments, the CD3zeta transmembrane domain and CD3zeta juxta-membrane portion recruits full-length CD3zeta molecule to the synapse. In several embodiments, the recruitment of native CD3zeta to the engineered receptor (as compared to a receptor without a CD3zeta transmembrane domain) is increased by about 20%, by about 30%, by about 40% by about 50%, or more, depending on the embodiment. In several embodiments, the CD3zeta transmembrane domain is coupled to an effector domain comprising one or more of CD16, NCR1, NCR2, NCR3, 4-1BB, NKp80, FcRγ, CD3zeta and 2B4.

[00148] In several embodiments, the chimeric receptor comprises a CD28 domain. In several embodiments, the transmembrane domain comprises CD28, while in several embodiments the CD28 domain is an intracellular signaling domain, while in several embodiments the CD28 domain is a transmembrane/intracellular signaling domain. In several embodiments, the CD28 transmembrane domain has the sequence of SEQ ID NO. 105. In several embodiments, the CD28 transmembrane domain can be truncated or modified, such that it is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% homologous with the CD28 having the sequence of SEQ ID NO. 105. In several embodiments, the CD28 intracellular signaling domain has the sequence of SEQ ID NO. 106. In several embodiments, the CD28 intracellular signaling domain can be truncated or modified, such that it is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% homologous with the CD28 having the sequence of SEQ ID NO. 106. In several embodiments, CD28 is used as the sole transmembrane/signaling domain in the construct, however, in several embodiments, CD28 can be used with one or more other domains. For example, combinations of CD28, OX40, 4-1BB, and/or CD3zeta are used in some embodiments.

[00149] In several embodiments, the chimeric receptor comprises an OX40 domain. In several embodiments the OX40 domain is an intracellular signaling domain. In several embodiments, the OX40 intracellular signaling domain has the sequence of SEQ ID NO. 107. In several embodiments, the OX40 intracellular signaling domain can be truncated or modified, such that it is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% homologous with the OX40 having the sequence of SEQ ID NO. 107. In several embodiments, OX40 is used as the sole transmembrane/signaling domain in the construct, however, in several embodiments, OX40 can be used with one or more other domains. For example, combinations of CD28, OX40, 4-1BB, and/or CD3zeta are used in some embodiments.

[00150] In still further embodiments, the signaling portion of the chimeric receptor comprises a portion of an ITAM, for example a hemi-tam. In several embodiments, these portions do not make up the canonical ITAM sequence, but rather comprise a portion that still can convey the signal required for NK cell cytotoxicity. In several embodiments, the hemi-tam has the sequence of SEQ ID NO. 14 (wherein X can be any residue). In several embodiments, the hemi-tam can be truncated or modified, such that it is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% homologous with the hemi-tam having the sequence of SEQ ID NO. 14. In several embodiments, the chimeric receptor construct comprises the hemi-tam of SEQ ID NO. 14. In several embodiments, multiple hemi-tams can be used, for example in a head to tail, tail to head, head to head, or tail to tail configuration. In several embodiments, the presence of at least on hemi-tam confers enhanced signaling and cytotoxicity to the NK cells comprising a chimeric receptor employing the at least one hemi-tam. As discussed in more detail below, in several chimeric receptor comprises NKp80, which is one non-limiting example of a hemi-tam.

[00151] In several embodiments, additional signaling regions are used, including, for example, signaling regions derived from receptors of the signaling lymphocytic activation molecule (SLAM) family. These receptors include, but are not limited to 2B4 (discussed above). Receptors of the SLAM family share a consensus motif that is tyrosine-based, in their cytoplasmic tails. That motif is S/TxYxxL/I, which are referred to as immunoreceptor tyrosine-based switch motifs (ITSM) (SEQ ID NO. 15). These receptors transmit activation signals through the SLAM-associated protein (SAP, encoded by the gene SH2D1A), which recruits the tyrosine kinase Fyn. Thus, according to several embodiments, the signaling region comprise a polypeptide sequence (or the nucleic acid encoding the same) comprising an ITSM motif. In several embodiments, the ITSM motif need not be fully encoded, but the signaling region is able to transmit an activation signal through SAP (or another similar pathway). In several embodiments, the ITSM motif has the sequence of SEQ ID NO. 15 (wherein X can be any amino acid residue). In several embodiments, the ITSM motif can be truncated or modified, such that it is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% homologous with the ITSM motif having the sequence of SEQ ID NO. 15. In several embodiments, the ITSM motif comprises the sequence of SEQ ID NO. 15.

[00152] In addition to these variations in the NKG2D receptor, the transmembrane domain and signaling domain (and the combination transmembrane/signaling domains), additional co-activating molecules can be provided, in several embodiments. For example, in several embodiments, the NK cells are engineered to express membrane-bound interleukin 15 (mbIL15). In such embodiments, the presence of the mbIL15 on the NK cell function to further enhance the cytotoxic effects of the NK cell by synergistically enhancing the proliferation and longevity of the NK cells. In several embodiments, mbIL15 has the nucleic acid sequence of SEQ ID NO. 16. In several embodiments, mbIL15 can be truncated or modified, such that it is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% homologous with the sequence of SEQ ID NO. 16. In several embodiments, the mbIL15 has the amino acid sequence of SEQ ID NO. 17. In conjunction with the chimeric receptors disclosed herein, such embodiments provide particularly effective NK cell compositions for targeting and destroying particular target cells.

Chimeric Receptor Constructs

[00153] In view of the disclosure provided herein, there are a variety of chimeric receptors that can be generated and expressed in NK cells in order to target and destroy particular target cells, such as diseased or cancerous cells. Non-limiting examples of such chimeric receptors are discussed in more detail below.

[00154] As discussed above, portions of the T cell receptor complex, in particular CD3zeta, serve as potent activators of immune signaling cascades. Likewise, the receptor 4-1BB, a tumor necrosis factor superfamily member, activates NK cells upon ligand binding. In several embodiments, these two signaling components act in a synergistic manner to activate NK cells upon binding of a ligand to the chimeric receptor. Thus, in several embodiments, there are provided polynucleotides encoding a NKG2D/CD8a/4-1BB/CD3zeta chimeric receptor, which comprises an NKG2D fragment extracellular receptor domain that binds native ligands of NKG2D, a CD8 transmembrane region, and an effector domain comprising the signaling domains of 4-1BB and CD3zeta. In one embodiment, this chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 18. In one embodiment, this chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 108. In yet another embodiment, the NKG2D-CD8a-4-1BB-CD3zeta chimeric receptor comprises the amino acid sequence of SEQ ID NO: 19. In several embodiments, this construct is particularly efficacious when the NK cells concurrently express mbIL15, the mbIL15 provides a further synergistic effect with respect to the activation and cytotoxic nature of the NK cells. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 18 (such as, for example, SEQ ID NO: 108), but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 18. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 18 (such as, for example, SEQ ID NO: 108), the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function.

[00155] The receptor 2B4 possesses several immunoreceptor tyrosine-based switch motifs (ITSMs) and has the potential to transduce activating signals. Likewise, signaling through the receptor 4-1BB, a tumor necrosis factor superfamily member, also activates NK cells upon ligand binding. Thus, capitalizing on the ability of these signaling molecules to cooperate to generate unexpectedly effectively cytotoxic NK cells, in several embodiments, there are provided polynucleotides encoding a NKG2D/CD8a/2B4/4-1BB chimeric receptor, which comprises an NKG2D fragment extracellular receptor domain that binds native ligands of NKG2D, a CD8a transmembrane region, and an effector domain comprising the signaling domains of 4-1BB and 2B4. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15.

[00156] In several embodiments, combinations of 2B4 with CD3zeta are used with NK cells to generate enhanced cytotoxicity against target cells. Thus, in several embodiments, there are provided polynucleotides encoding a NKG2D/CD8a/2B4/CD3zeta chimeric receptor, which comprises an NKG2D fragment extracellular receptor domain that binds native ligands of NKG2D, a CD8a transmembrane region, and an effector domain comprising the signaling domains of CD3zeta and 2B4. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15. As discussed above, 4-1BB, like CD3zeta and 2B4, can function as a potent activator of immune signaling cascades. In several embodiments, these three signaling components act in a synergistic manner to activate NK cells upon binding of a ligand to the chimeric receptor. Thus, in several embodiments, there are provided polynucleotides encoding a NKG2D/CD8a/4-1BB/2B4/CD3zeta chimeric receptor, which comprises an NKG2D fragment extracellular receptor domain that binds native ligands of NKG2D, a CD8 transmembrane region, and an effector domain comprising the signaling domains of 4-1BB, 2B4 and CD3zeta. In one embodiment, this chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 58. In yet another embodiment, the NKG2D-CD8a-4-1BB-CD3zeta chimeric receptor comprises the amino acid sequence of SEQ ID NO: 59. In several embodiments, this construct is particularly efficacious when the NK cells concurrently express mbIL15, the mbIL15 provides a further synergistic effect with respect to the activation and/or cytotoxic nature of the NK cells. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 58, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 58. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 58, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function.

[00157] In several alternative embodiments, there are provided polynucleotides encoding a NKG2D/CD8a/DAP10/4-1BB chimeric receptor, which comprises an NKG2D fragment extracellular receptor domain that binds native ligands of NKG2D, a CD8a transmembrane region, and an effector domain comprising the signaling domains of 4-1BB and DAP10. In one embodiment, this chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 60. In yet another embodiment, the NKG2D-CD8a-4-1BB-DAP10 chimeric receptor comprises the amino acid sequence of SEQ ID NO: 61. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15. In several embodiments, this construct is particularly efficacious when the NK cells concurrently express mbIL15, the mbIL15 provides a further synergistic effect with respect to the activation and cytotoxic nature of the NK cells. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 60, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 60. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 60, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function. Further, as discussed above, 2B4, like DAP10 and 4-1BB, is a potent activator of immune signaling cascades. In several embodiments, these three signaling components act in a synergistic manner to activate NK cells upon binding of a ligand to the chimeric receptor. Thus, in several embodiments, there are provided polynucleotides encoding a NKG2D/CD8a/4-1BB/DAP10/2B4 chimeric receptor, which comprises an NKG2D fragment extracellular receptor domain that binds native ligands of NKG2D, a CD8 transmembrane region, and an effector domain comprising the signaling domains of 4-1BB, 2B4 and DAP10, wherein 4-1BB is followed by DAP10, and DAP10 is followed by 2B4. In one embodiment, this chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 62. In yet another embodiment, the NKG2D-CD8a-4-1BB-CD3zeta chimeric receptor comprises the amino acid sequence of SEQ ID NO: 63. In several embodiments, this construct is particularly efficacious when the NK cells concurrently express mbIL15, the mbIL15 provides a further synergistic effect with respect to the activation and cytotoxic nature of the NK cells. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 62, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 62. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 62, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function. In several other embodiments, there are provided polynucleotides encoding a NKG2D/CD8a/4-1BB/2B4/DAP10 chimeric receptor, which comprises an NKG2D fragment extracellular receptor domain that binds native ligands of NKG2D, a CD8 transmembrane region, and an effector domain comprising the signaling domains of 4-1BB, 2B4 and DAP10, wherein 4-1BB is followed by 2B4, and 2B4 is followed by DAP10. In one embodiment, this chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 64. In yet another embodiment, the NKG2D-CD8a-4-1BB-CD3zeta chimeric receptor comprises the amino acid sequence of SEQ ID NO: 65. In several embodiments, this construct is particularly efficacious when the NK cells concurrently express mbIL15, the mbIL15 provides a further synergistic effect with respect to the activation and cytotoxic nature of the NK cells. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 64, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 64. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 64, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function.

[00158] In several additional embodiments, transmembrane and effector domains (and associated function) of the chimeric receptor are derived from the same peptide. CD16 is a potent activating receptor expressed on the surface of NK cells. Thus, in several embodiments, polynucleotides are provided encoding a NKG2D/CD16 chimeric receptor, which comprises an NKG2D fragment extracellular receptor domain that binds native ligands of NKG2D and a CD16 peptide comprising both the transmembrane region and intracellular effector domain. In one embodiment, this chimeric receptor comprises the nucleic acid sequence of SEQ ID NO: 23. In yet another embodiment, this chimeric receptor is encoded by the amino acid sequence of SEQ ID NO: 24. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 23, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 23. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 23, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15.

[00159] In several additional embodiments, polynucleotides are provided encoding a NKG2D/CD16/4-1BB chimeric receptor, wherein the signaling domain of 4-1BB acts as a second transducer of activating signals in the effector domain. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15.

[00160] CD3zeta dimerizes via its transmembrane domain. Thus, in several embodiments, chimeric receptors are provided wherein a CD3zeta transmembrane domain recruits full-length CD3zeta molecule to the synapse. In several embodiments, there are provided polynucleotides encoding a chimeric receptor which comprises a NKG2D fragment that binds native ligands of NKG2D, a CD8a hinge, 0, 1, 2, 3, 4, 5, 6 or more extracellular CD3zeta residues (the “juxta-membrane portion”) directly adjacent to a CD3zeta transmembrane domain, and an effector domain comprising one or more of CD16, NCR1, NCR2, NCR3, 4-1BB, NKp80, FcRγ, CD3zeta and 2B4.

[00161] In several embodiments, chimeric receptors are provided wherein a CD3zeta transmembrane domain is coupled to an effector domain comprising one or both of 4-1BB and CD16. Thus, in several embodiments, polynucleotides are provided encoding a NKG2D/CD3zetaTM/4-1BB chimeric receptor, which comprises a fragment of NKG2D that is codon optimized coupled to a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising 4-1BB. In one embodiment, this chimeric receptor comprises the nucleic acid sequence of SEQ ID NO: 78. In yet another embodiment, this chimeric receptor is encoded by the amino acid sequence of SEQ ID NO: 79. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 78, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 78. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 78, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15.

[00162] In several embodiments, polynucleotides are provided encoding a NKG2D/CD3zetaTM/CD16/4-1BB chimeric receptor, which comprises a fragment of NKG2D that is codon optimized coupled to a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising CD16 followed by 4-1BB. In one embodiment, this chimeric receptor comprises the nucleic acid sequence of SEQ ID NO: 70. In yet another embodiment, this chimeric receptor is encoded by the amino acid sequence of SEQ ID NO: 71. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 70, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 70. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 70, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15. Further, in several embodiments, polynucleotides are provided encoding a NKG2D/CD3zetaTM/4-1BB/CD16 chimeric receptor, which comprises a fragment of NKG2D that is codon optimized coupled to a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising 4-1BB followed by CD16. In some embodiments, the effector domain further comprises a GS3 linker. In some embodiments, the GS3 linker is positioned between 4-1BB and CD16. In one embodiment, this chimeric receptor comprises the nucleic acid sequence of SEQ ID NO: 84. In yet another embodiment, this chimeric receptor is encoded by the amino acid sequence of SEQ ID NO: 85. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 84, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 84. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 84, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15. Further, in several embodiments, polynucleotides are provided encoding a NKG2Dx2/CD3zetaTM/CD16/4-1BB chimeric receptor, which comprises the fragment of NKG2D that is codon optimized coupled to a GS3 linker, an additional NKG2D fragment, a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising a CD16 and 4-1BB. In one embodiment, this chimeric receptor comprises the nucleic acid sequence of SEQ ID NO: 72. In yet another embodiment, this chimeric receptor is encoded by the amino acid sequence of SEQ ID NO: 73. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 72, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 72. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 72, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15.

[00163] In several embodiments, chimeric receptors are provided wherein a CD3zeta transmembrane domain is coupled to an effector domain comprising NKp80. Thus, in several embodiments, polynucleotides are provided encoding a NKG2D/CD3zetaTM/CD16/4-1BB/NKp80 chimeric receptor, which chimeric receptor comprises a fragment of NKG2D coupled to a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising a CD16, 4-1BB, and NKp80. In some embodiments, the effector domain further comprises a GS3 linker. In some embodiments, the GS3 linker is positioned between 4-1BB and NKp80. In one embodiment, this chimeric receptor comprises the nucleic acid sequence of SEQ ID NO: 74. In yet another embodiment, this chimeric receptor is encoded by the amino acid sequence of SEQ ID NO: 75. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 74, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 74. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 74, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15. Further, in several embodiments, polynucleotides are provided encoding a 2xNKG2D/CD3zetaTM/ CD16/4-1BB/NKp80 chimeric receptor, which comprises the fragment of NKG2D that is codon optimized coupled to a GS3 linker, an additional NKG2D fragment, a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising a CD16, 4-1BB, and NKp80. In some embodiments, the effector domain further comprises a GS3 linker. In some embodiments, the GS3 linker is positioned between 4-1BB and NKp80. In one embodiment, this chimeric receptor comprises the nucleic acid sequence of SEQ ID NO: 76. In yet another embodiment, this chimeric receptor is encoded by the amino acid sequence of SEQ ID NO: 77. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 76, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 76. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 76, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15. Further, in several embodiments, polynucleotides are provided encoding a NKG2D/CD3zetaTM/4-1BB/NKp80 chimeric receptor, which comprises a fragment of NKG2D that is codon optimized coupled to a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising 4-1BB and NKp80. In some embodiments, the effector domain further comprises a GS3 linker. In some embodiments, the GS3 linker is positioned between 4-1BB and NKp80. In one embodiment, this chimeric receptor comprises the nucleic acid sequence of SEQ ID NO: 82. In yet another embodiment, this chimeric receptor is encoded by the amino acid sequence of SEQ ID NO: 83. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 82, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 82. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 82, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15.

[00164] In several embodiments, chimeric receptors are provided wherein a CD3zeta transmembrane domain is coupled to an effector domain comprising CD3zeta. Thus, in several embodiments, polynucleotides are provided encoding a NKG2D/CD3zetaTM/4-1BB/CD3zeta chimeric receptor, which comprises a fragment of NKG2D that is codon optimized coupled to a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising 4-1BB and CD3zeta. In one embodiment, this chimeric receptor comprises the nucleic acid sequence of SEQ ID NO: 80. In yet another embodiment, this chimeric receptor is encoded by the amino acid sequence of SEQ ID NO: 81. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 80, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 80. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 80, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15.

[00165] In several embodiments, chimeric receptors are provided wherein a CD3zeta transmembrane domain is coupled to an effector domain comprising FcRγ. Thus, in several embodiments, polynucleotides are provided encoding a NKG2D/CD3zetaTM/4-1BB/FcRγ chimeric receptor, which comprises a fragment of NKG2D coupled to a CD8a hinge, a CD3zeta transmembrane region, and an effector domain comprising 4-1BB and FcRγ. In one embodiment, this chimeric receptor comprises the nucleic acid sequence of SEQ ID NO: 86. In yet another embodiment, this chimeric receptor is encoded by the amino acid sequence of SEQ ID NO: 87. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 86, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 86. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 86, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15.

[00166] In several embodiments, chimeric receptors are provided wherein a CD3zeta transmembrane domain is coupled to an effector domain comprising CD28. Thus, in several embodiments, polynucleotides are provided encoding a NKG2D/CD3zetaTM/CD28/CD3zeta chimeric receptor, which comprises an NKG2D fragment extracellular receptor domain that binds native ligands of NKG2D, a CD8a hinge, a CD3zeta transmembrane region, and intracellular effector domain comprising CD28 and CD3zeta. In one embodiment, this chimeric receptor comprises the nucleic acid sequence of SEQ ID NO: 102. In yet another embodiment, this chimeric receptor is encoded by the amino acid sequence of SEQ ID NO: 103. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 102, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 102. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 102, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15.

[00167] In several embodiments, chimeric receptors are provided wherein the extracellular domain comprises a fragment of NKG2D coupled IL15. Thus, in several embodiments, polynucleotides are provided encoding an IL15/NKG2D/CD8a/4-1BB/CD3zeta chimeric receptor, which comprises an NKG2D fragment extracellular receptor domain that binds native ligands of NKG2D linked to IL-15, a CD8a hinge, a CD8a transmembrane domain, and intracellular effector domain comprising 4-1BB and CD3z. In some embodiments, the extracellular domain further comprises a GS3 linker. In some embodiments, the GS3 linker is positioned between IL15 and the NKG2D fragment extracellular receptor domain. In one embodiment, this chimeric receptor comprises the nucleic acid sequence of SEQ ID NO: 88. In yet another embodiment, this chimeric receptor is encoded by the amino acid sequence of SEQ ID NO: 89. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 88, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 88. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 88, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function.

[00168] In several embodiments, chimeric receptors are provided wherein the extracellular domain comprises a fragment of NKG2D coupled to a IgG4 short hinge. Thus, in several embodiments, polynucleotides are provided encoding a NKG2D/IgG4/CD8a/4-1BB/CD3zeta chimeric receptor, which comprises an NKG2D fragment extracellular receptor domain that binds native ligands of NKG2D, an IgG4 short hinge, a CD8a transmembrane domain, and intracellular effector domain comprising 4-1BB, and CD3zeta. In one embodiment, this chimeric receptor comprises the nucleic acid sequence of SEQ ID NO: 96. In yet another embodiment, this chimeric receptor is encoded by the amino acid sequence of SEQ ID NO: 97. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 96, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 96. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 96, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15.

[00169] In several embodiments, chimeric receptors are provided wherein the effector domain comprises OX40. Thus, in several embodiments, polynucleotides are provided encoding a NKG2D/CD8a/OX40/CD3z chimeric receptor, which comprises an NKG2D fragment extracellular receptor domain that binds native ligands of NKG2D, a CD8a hinge, a CD8a transmembrane domain, and an intracellular effector domain comprising OX40, and CD3z. In one embodiment, this chimeric receptor comprises the nucleic acid sequence of SEQ ID NO: 90. In yet another embodiment, this chimeric receptor is encoded by the amino acid sequence of SEQ ID NO: 91. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 90, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 90. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 90, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15. In several embodiments, polynucleotides are provided encoding a NKG2D/IgG4/CD8a/OX40/CD3zeta chimeric receptor, which comprises an NKG2D fragment extracellular receptor domain that binds native ligands of NKG2D, an IgG4 hinge, a CD8a transmembrane domain, and intracellular effector domain comprising OX40 and CD3zeta. In one embodiment, this chimeric receptor comprises the nucleic acid sequence of SEQ ID NO: 100. In yet another embodiment, this chimeric receptor is encoded by the amino acid sequence of SEQ ID NO: 101. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 100, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 100. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 100, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15.

[00170] In several embodiments, chimeric receptors are provided comprising a CD28 peptide comprising both the transmembrane region and intracellular effector domain. Thus, in several embodiments, polynucleotides are provided encoding a NKG2D/CD28/CD3zeta chimeric receptor, which comprises an NKG2D fragment extracellular receptor domain that binds native ligands of NKG2D, a CD8a hinge, a CD28 transmembrane/intracellular domain, and CD3zeta. In one embodiment, this chimeric receptor comprises the nucleic acid sequence of SEQ ID NO: 92. In yet another embodiment, this chimeric receptor is encoded by the amino acid sequence of SEQ ID NO: 93. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 92, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 92. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 92, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15. In further embodiments, polynucleotides are provided encoding a NKG2D/CD28/CD3zeta/4-1BB chimeric receptor, which comprises an NKG2D fragment extracellular receptor domain that binds native ligands of NKG2D, a CD8a hinge, a CD28 transmembrane/intracellular domain, and 4-1BB and CD3zeta. In one embodiment, this chimeric receptor comprises the nucleic acid sequence of SEQ ID NO: 94. In yet another embodiment, this chimeric receptor is encoded by the amino acid sequence of SEQ ID NO: 95. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 94, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 94. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 94, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15. In further embodiments, polynucleotides are provided encoding a NKG2D/IgG4/CD28/CD3zeta chimeric receptor, which comprises an NKG2D fragment extracellular receptor domain that binds native ligands of NKG2D, an IgG4 hinge, a CD28 transmembrane/intracellular domain, and CD3zeta. In one embodiment, this chimeric receptor comprises the nucleic acid sequence of SEQ ID NO: 98. In yet another embodiment, this chimeric receptor is encoded by the amino acid sequence of SEQ ID NO: 99. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 98, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 98. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 98, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15.

[00171] NCR1 (NKp46), NCR2 (NKp44) and NCR3 (NKp30) are receptors on NK cells that transduce activation signals upon ligand binding. Thus, in several embodiments, polynucleotides are provided encoding a NKG2D/NCR1 chimeric receptor, which comprises an NKG2D fragment extracellular receptor domain that binds native ligands of NKG2D and a NCR1 peptide comprising both the transmembrane region and intracellular effector domain. In one embodiment, this chimeric receptor comprises the nucleic acid sequence of SEQ ID NO: 27. In yet another embodiment, this chimeric receptor is encoded by the amino acid sequence of SEQ ID NO: 28. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 30, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 27. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 27, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15.

[00172] In several additional embodiments, polynucleotides are provided encoding a NKG2D/NCR1/4-1BB chimeric receptor, wherein the signaling domain of 4-1BB acts as a second transducer of activating signals in the effector domain, leading to synergistically enhanced NK cell activation and cytotoxicity. In several additional embodiments, polynucleotides are provided encoding a NKG2D/NCR2 chimeric receptor, which comprises an NKG2D fragment extracellular receptor domain that binds native ligands of NKG2D and a NCR2 peptide comprising both the transmembrane region and intracellular effector domain. As with NCR1, in several embodiments these constructs are particularly amenable for use in creating NK cells expressing the chimeric receptor, due to their relatively small size and simplicity on sequence. However, they retain the ability, in several embodiments, to yield highly effective NK cells, despite the apparent simplicity of the construct. Additionally, in several embodiments, these constructs can optionally be co-expressed with mbIL15.

[00173] In several additional embodiments, polynucleotides are provided encoding a NKG2D/NCR3 chimeric receptor, which comprises an NKG2D fragment extracellular receptor domain that binds native ligands of NKG2D and a NCR3 peptide comprising both the transmembrane region and intracellular effector domain. As with NCR1 and or NCR2, in several embodiments these constructs are particularly amenable for use in creating NK cells expressing the chimeric receptor, due to their relatively small size and simplicity on sequence. However, they retain the ability, in several embodiments, to yield highly effective NK cells, despite the apparent simplicity of the construct. In one embodiment, this chimeric receptor comprises the nucleic acid sequence of SEQ ID NO: 29. In yet another embodiment, this chimeric receptor is encoded by the amino acid sequence of SEQ ID NO: 30. In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 29, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 29. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 29, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15.

[00174] In several additional embodiments, polynucleotides are provided encoding a NKG2D/NCR2/4-1BB chimeric receptor, wherein the signaling domain of 4-1BB acts as a second transducer of activating signals in the effector domain, thereby leading to a synergistic effect between the signaling domains, and unexpectedly effectively cytotoxic NK cells. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15.

[00175] In several additional embodiments, polynucleotides are provided encoding a NKG2D/NCR3/4-1BB chimeric receptor, wherein the signaling domain of 4-1BB acts as a second transducer of activating signals in the effector domain, thereby leading to a synergistic effect between the signaling domains, and unexpectedly effectively cytotoxic NK cells. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15.

[00176] In some embodiments the surface expression and efficacy of the chimeric receptors disclosed herein are enhanced by variations in a spacer region (hinge), which, in several embodiments, are located in the extracellular domain between the NKG2D fragment and the transmembrane domain. In some embodiments, the hinge regions can be included between other portions of the chimeric receptor (e.g., between intracellular and transmembrane domains, or between multiple intracellular domains). In some embodiments, domains that serve certain purposes as disclosed elsewhere herein, can serve additional functions. For example, in several embodiments, CD8a is repurposed to serve as a hinge region (encoded, in several embodiments, by the nucleic acid sequence of SEQ ID NO: 5). In yet another embodiment, the hinge region comprises an N-terminal truncated form of CD8a and/or a C-terminal truncated form of CD8a. Depending on the embodiment, these truncations can be at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90% homologous to the hinge encoded by SEQ ID NO. 5. In several additional embodiments, the hinge comprises spans of Glycine and Serine residues (herein termed "GS linkers") where GSn represents the sequence (Gly-Gly-Gly-Gly-Ser)n (SEQ ID NO. 42). In one embodiment, the hinge comprises both CD8a and GS3, and is encoded by the amino acid sequence of SEQ ID NO: 32, for example, where n=3. In additional embodiments, the value of n may be equal to 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or greater depending on the embodiment. In several embodiments, the hinge could also be structured as GSn/CD8a. Alternatively, the GS linker can comprise the entire hinge region. In one such embodiment, the hinge region is encoded by the nucleic acid sequence of SEQ ID NO: 33. In another such embodiment, the hinge region is encoded by the nucleic acid sequence of SEQ ID NO: 34. In several embodiments, IgG4 is repurposed as a hinge region (encoded, in several embodiments, by the nucleic acid sequence of SEQ ID NO: 104). In yet another embodiment, the hinge region comprises an N-terminal truncated form of IgG4 and/or a C-terminal truncated form of IgG4. Depending on the embodiment, these truncations can be at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90% homologous to the hinge encoded by SEQ ID NO. 104.

[00177] In several embodiments, the chimeric receptor constructs employ a 2B4 intracellular signaling domain. In several embodiments, this domain is encoded by the amino acid sequence of SEQ ID NO. 35. In some embodiments, the 2B4 domain is encoded by the nucleic acid sequence of SEQ ID NO. 36. In some embodiments, the sequence of the 2B4 intracellular domain used in a chimeric receptor may vary from SEQ ID NO. 36, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 36. In several embodiments, while the signaling domain of the chimeric receptor may vary from SEQ ID NO. 36, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function. Likewise, in several embodiments an NKp80 intracellular domain is used, in several embodiments. In some embodiments, the NKp80 domain is the sole intracellular signaling domain, while in some embodiments, that domain is used in conjunction with one or more additional domains. In several embodiments, the NKp80 is encoded by the amino acid sequence of SEQ ID NO. 37. In some embodiments, the NKp80 domain is encoded by the nucleic acid sequence of SEQ ID NO. 38. In some embodiments, the sequence of the NKp80 intracellular domain used in a chimeric receptor may vary from SEQ ID NO. 38, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 38. In several embodiments, while the signaling domain of the chimeric receptor may vary from SEQ ID NO. 38, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function.

[00178] In several embodiments, the chimeric receptor uses a portion of a beta-adrenergic receptor as a transmembrane domain. In several embodiments, the portion comprises a portion of the beta-adrenergic extracellular domain. In several embodiments, the portion is a portion of the beta-adrenergic receptor transmembrane domain. In several embodiments, a combination of an extracellular domain and a transmembrane domain of the beta adrenergic receptor is used. Depending on the embodiment the portions are from the beta-1 and/or beta-2 adrenergic receptor. In several embodiments, a portion of the N-terminal extracellular region of the beta-2 adrenergic receptor is used. In several embodiments that portion has the amino acid sequence of SEQ ID NO. 39. In some embodiments, the extracellular beta-2 adrenergic domain is encoded by the nucleic acid sequence of SEQ ID NO. 40. In some embodiments, the sequence of the extracellular beta-2 adrenergic domain used in a chimeric receptor may vary from SEQ ID NO. 39, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 39. In several embodiments, the first transmembrane helix of the beta-2 adrenergic receptor is used, optionally in conjunction with the extracellular beta-2 adrenergic domain. In several embodiments, the first transmembrane helix of the beta-2 adrenergic receptor has the amino acid sequence of SEQ ID NO. 41. In some embodiments, the first transmembrane helix of the beta-2 adrenergic receptor is encoded by the nucleic acid sequence of SEQ ID NO. 42. In some embodiments, the sequence of the first transmembrane helix of the beta-2 adrenergic receptor used in a chimeric receptor may vary from SEQ ID NO. 41, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 41.

[00179] In one embodiment, the chimeric receptor comprises CD8, truncated NKG2D, CD8a, transmembrane domain, a CD16 intracellular domain, and 4-1BB as a costimulatory molecule. In several embodiments, such a construct is encoded by SEQ ID NO. 25. In some embodiments, the chimeric receptor may vary from SEQ ID NO. 25, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 25. In several embodiments, hinge regions surrounding CD8 are increased by way of addition of GS linkers (disclosed herein), such as GS3, by way of non-limiting example. In such embodiments, the construct is encoded by the nucleic acid of SEQ ID NO. 43. In some embodiments, the chimeric receptor may vary from SEQ ID NO. 43, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 43. In several embodiments, hinge regions surrounding CD8 are increased by way of addition of longer GS linkers, such as GS12, or other linker. In several embodiments, hinge regions are decreased by way of truncating CD8. For example, in several embodiments, the N-terminal region of CD8a is truncated by at least 20%, at least 30%, at least 40%, or at least 50%. In several embodiments, the CD8 hinge is replaced with a GS linker. For example, in several embodiments, the hinge region comprises a GS3 linker, thereby the construct comprises NKG2D-GS3-CD16-4-1BB. In one embodiment, such a construct is encoded by the nucleic acid of SEQ ID NO. 44. In some embodiments, the chimeric receptor may vary from SEQ ID NO. 44, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 44. In several embodiments, neither CD8 nor GSn are used. In one embodiment, such a construct is encoded by the nucleic acid of SEQ ID NO. 45. In some embodiments, the chimeric receptor may vary from SEQ ID NO. 45, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 45.

[00180] As discussed above, in several embodiments, codon optimized sequences are employed. For example in several embodiments, codon optimization (full or partial) is performed on the NKG2D domain of a chimeric receptor. In several embodiments, however, codon optimization is not performed. In several embodiments, a chimeric receptor construct is provided with an NKG2D extracellular domain that is not optimized, a CD8a hinge, and a 4-1BB signaling domain. In several embodiments, a chimeric receptor construct is provided with an NKG2D extracellular domain that is not optimized, a CD8a hinge and transmembrane domain, and a 4-1BB signaling domain. In several embodiments, a chimeric receptor construct is provided with an NKG2D extracellular domain that is not optimized, a CD8a hinge and transmembrane domain, a 4-

1BB signaling domain and a 2B4 signaling domain. In several embodiments, such a construct has the nucleic acid sequence of SEQ ID NO. 46. In some embodiments, the chimeric receptor may vary from SEQ ID NO. 46, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 46.

[00181] In several embodiments, a chimeric receptor construct is provided with an NKG2D extracellular domain that is not optimized, a beta-adrenergic derived transmembrane domain, and a 4-1BB signaling domain. In several embodiments, a chimeric receptor construct is provided with an NKG2D extracellular domain that is not optimized, a beta-adrenergic derived transmembrane domain made up of the extracellular region of the beta-2 adrenergic receptor and the first transmembrane helix of the beta-2 adrenergic receptor, and a 4-1BB signaling domain. In several embodiments, a chimeric receptor construct is provided with an NKG2D extracellular domain that is not optimized, a beta-adrenergic derived transmembrane domain made up of the extracellular region of the beta-2 adrenergic receptor and the first transmembrane helix of the beta-2 adrenergic receptor, a 4-1BB signaling domain and a 2B4 signaling domain. In several embodiments, such a construct has the nucleic acid sequence of SEQ ID NO. 47. In some embodiments, the chimeric receptor may vary from SEQ ID NO. 47, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 47.

[00182] In several embodiments, a chimeric receptor construct is provided with an NKG2D extracellular domain that is not optimized, a CD8a hinge, and a 2B4 signaling domain. In several embodiments, a chimeric receptor construct is provided with an NKG2D extracellular domain that is not optimized, a CD8a hinge and transmembrane domain, and both a 2B4 and a 4-1BB signaling domain. In several embodiments, a chimeric receptor construct is provided with an NKG2D extracellular domain that is not optimized, a CD8a hinge and transmembrane domain, a 4-1BB signaling domain and a 2B4 signaling domain, as well as a NKp80 domain. In several embodiments, a GS linker, such as a GS3 linker joins the 2B4 and NKp80 domains. In several embodiments, such a construct has the nucleic acid sequence of SEQ ID NO. 48. In some embodiments, the chimeric receptor may vary from SEQ ID NO. 48, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 48.

[00183] In several embodiments, a chimeric receptor construct is provided with an NKG2D extracellular domain that is not optimized, a CD8a hinge, and a NKp80 signaling domain. In several embodiments, a chimeric receptor construct is provided with an NKG2D extracellular domain that is not optimized, a CD8a hinge and transmembrane domain, and a NKp80 signaling domain. In several embodiments, a chimeric receptor construct is provided with an NKG2D extracellular domain that is not optimized, a CD8a hinge and transmembrane domain, a 4-1BB signaling domain and a NKp80 domain. In several embodiments, a GS linker, such as a GS3 linker joins the 4-1BB and NKp80 domains. In several embodiments, such a construct has the nucleic acid sequence of SEQ ID NO. 49. In some embodiments, the chimeric receptor may vary from SEQ ID NO. 49, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 49.

[00184] In several embodiments, a CD8 transmembrane domain is coupled with a 2B4 intracellular domain. In several embodiments, a CD8 transmembrane domain is replaced with a 2B4 domain that is transmembrane and intracellular. In several embodiments, the CD8 transmembrane domain is replaced with 2B4 and 4-1BB is expressed in a proximal configuration.

[00185] In several embodiments, a CD16 intracellular signaling domain is coupled with a CD3zeta or gamma subunit which are exogenously expressed in *trans* to the chimeric receptors described herein. As discussed above, such constructs can result in unexpectedly enhanced signal transduction, and thus an unexpected increase in cytotoxic effects of the NK cells.

[00186] In several embodiments, the chimeric receptors are configured to dimerize, as discussed in additional detail herein. In several embodiments a truncated NKG2D receptor according to several embodiments disclosed herein is optionally dimerized. Dimerization may comprise homodimers or heterodimers, depending on the embodiment. In several embodiments, dimerization results in a shift of avidity of the chimeric receptor (and hence the NK cells expressing the receptor) to better ligand recognition with a coordinate balance in reduced (or lack) of adverse toxic effects. In still further embodiments, the extracellular receptor domain further comprises a CD8a signal peptide. In several embodiments, the chimeric receptors employ internal dimers, or repeats of one or more component subunits. For example, in several embodiments, the chimeric receptor comprises a NKG2D extracellular domain coupled to a second NKG2D extracellular domain, and a transmembrane/signaling region (or a separate transmembrane region along with a separate signaling region). In several embodiments, one or more of the NKG2D extracellular domains are codon optimized. In several embodiments, the two NKG2D extracellular domains are separated by a linker, for example a GSn linker. In one embodiment, a GS3 linker is used. In several embodiments, the transmembrane domain comprises an extracellular region of the beta-adrenergic receptor. In several embodiments, the transmembrane domain transmembrane domain comprises an extracellular region of the beta-2 adrenergic receptor and further comprises the first transmembrane domain of the beta-2 adrenergic receptor. In several embodiments, the signaling region comprises 4-1BB. In several embodiments, the signaling region comprises NKp80. In several embodiments, the signaling region comprises a CD16 transmembrane-intracellular domain. In several embodiments, the signaling region comprises 4-1BB in conjunction with NKp80 or a CD16 transmembrane-intracellular domain. In several embodiments, the chimeric receptor has the nucleic acid sequence of SEQ ID NO. 50. In some embodiments, the chimeric receptor may vary from SEQ ID NO. 50, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 50. In several embodiments, the chimeric receptor has the nucleic acid sequence of SEQ ID NO. 51. In some embodiments, the chimeric receptor may vary from SEQ ID NO. 51, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 51. In several embodiments, the chimeric receptor has the nucleic acid sequence of SEQ ID NO. 52. In some embodiments, the chimeric receptor may vary from SEQ ID NO. 52, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 52. In several embodiments, the chimeric receptor comprises a hinge region. In several embodiments, CD8a is repurposed to serve as a hinge region (encoded, in several embodiments, by the nucleic acid sequence of SEQ ID NO: 5). In several embodiments, the chimeric receptor comprises a CD8a transmembrane domain. In several embodiments, the signaling region comprises 4-1BB in conjunction with 2B4 and CD3zeta. In some embodiments, the chimeric receptor comprises the fragment of NKG2D that is codon optimized coupled to a GS3 linker, an additional NKG2D fragment, a CD8a hinge, a CD8a transmembrane domain, and an effector domain comprising 4-1BB and CD3zeta. In several embodiments, the chimeric receptor has the nucleic acid sequence of SEQ ID NO. 66. In some embodiments, the chimeric receptor may vary from SEQ ID NO. 66, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 50. In several embodiments, the chimeric receptor chimeric receptor comprises the amino acid sequence of SEQ ID NO: 67. In some embodiments, the chimeric receptor may vary from SEQ ID NO. 66, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 50.

[00187] In several embodiments, the chimeric receptors are configured to be bispecific, as discussed in additional detail herein. In several embodiments, a truncated NKG2D receptor according to several embodiments disclosed herein is bispecific due to a second peptide that binds, for example, non-NKG2D ligands. In several embodiments, bi-specificity results in a shift of the targeting of the chimeric receptor (and hence the NK cells expressing the receptor) to better target cell recognition with a coordinate balance in reduced (or lack) of adverse toxic effects. In still further embodiments, the extracellular receptor domain further comprises a CD8a signal peptide. For example, in several embodiments, the chimeric receptor comprises a NKG2D extracellular domain coupled to a second extracellular domain that binds other (non-NKG2D) ligands, and a transmembrane/signaling region (or a separate transmembrane region along with a separate signaling region). In several embodiments, the two extracellular domains are separated by a linker, for example a GSn linker. In one embodiment, a GS3 linker is used.

[00188] According to several embodiments disclosed herein, additional chimeric receptors employing codon optimized NKG2D domains are provided for (optionally, these constructs can also be replicated with non-optimized or partially optimized domains). For example, in several embodiments, a codon optimized extracellular domain is coupled with a hinge and at least two transmembrane/signaling domains. In several embodiments, the multiple signaling domains provide enhanced cytotoxic efficacy of the NK cells because multiple, non-redundant signal cascades are set in motion. While in some embodiments these multiple pathways may converge on a single signaling molecule (e.g., IFNγ), the overall cytotoxic effect is unexpectedly increased because of the overall magnitude of signaling molecules driving a cytotoxic endpoint. As a non-limiting example, in several embodiments an NKG2D is coupled to a CD8a hinge followed by a CD16 transmembrane-intracellular signaling domain and a 4-1BB signaling domain. In several embodiments, this construct further comprises a 2B4 signaling domains. In several embodiments, such a chimeric receptor has the nucleic acid sequence of SEQ ID NO. 53. In some embodiments, the chimeric receptor may vary from SEQ ID NO. 53, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 53. In additional embodiments, the NKG2D-CD8a-CD16IC/TM construct further comprises a NKp80 signaling domain. In several embodiments, such a construct further comprises a GS3 linker between the 4-1BB and NKp80 domains. In several embodiments, such a chimeric receptor has the nucleic acid sequence of SEQ ID NO. 54. In some embodiments, the chimeric receptor may vary from SEQ ID NO. 54, but remains, depending on the embodiment, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous with SEQ ID NO. 54.

[00189] In still additional embodiments, certain components of a chimeric receptor can be replaced with one or more additional subunits that lead to enhanced efficacy (e.g., activation or cytotoxicity of NK cells). For example, in one embodiment, a CD16 intracellular signaling domain can be replaced with a quad-repeat of DAP10 (e.g., 4xDAP10). In an additional embodiment, a CD16 intracellular signaling domain can be replaced with a Zap70 subunit. Certain such embodiments lead to unexpectedly enhanced NK cell cytotoxicity.

[00190] In several additional embodiments, the effector domain comprises one or more consensus hemi-ITAM sequences to enhance the transduction of activation signaling upon ligand binding. In additional embodiments, the inclusion of a GS linker between the signaling domains of 4-1BB, CD16, NCR1, NCR2 and/or NCR3 enhances signal transduction. Moreover, in several embodiments one or both of CD3ζ and FcRγ are additionally expressed along with the chimeric receptors described herein (either on the same or a separate construct), which results in unexpectedly enhanced signal transduction, and thus an unexpected increase in cytotoxic effects of the NK cells. Depending on the embodiment, the engineered expression of one or more of CD3ζ and FcRγ supplements endogenous expression of these molecules by NK cells, thereby further enhancing the signaling and ultimate cytotoxic potency of the NK cells.

[00191] Optionally, depending on the embodiment, any of the polynucleotides disclosed herein may also encode truncations and/or variants of one or more of the constituent subunits of a chimeric receptor, yet retain their ability to direct NK cells to target cells and in several embodiments unexpectedly enhance cytotoxicity upon binding. In addition, any of the polynucleotides disclosed herein may also optionally include codon-optimized nucleotide sequences encoding the various constituent subunits of a chimeric receptor. As used herein, the terms "fragment" and "truncated" shall be given their ordinary meaning and shall also include N- and C-terminal deletion variants of proteins.

[00192] The polynucleotides encoding the chimeric receptors described herein may be inserted into vectors to achieve recombinant protein expression in NK cells. In one embodiment, the polynucleotide is operably linked to at least one regulatory element for the expression of the chimeric receptor. In specific embodiments, transcriptional regulatory elements heterologous, such as, for example an internal ribosome entry site (IRES) or enhancer element, to the peptides disclosed herein are employed to direct the transcription of the chimeric receptor. In some embodiments, the polynucleotide comprises one or more cytosolic protease cleavage sites. In some embodiments, the cleavage site is recognized and cleaved by a cytosolic protease. In some embodiments, this cleavage site is selected from the group comprising a T2A cleavage site, a P2A cleavage site, an E2A cleavage site, and an F2A cleavage site. Depending on the embodiment, the various constituent parts of a chimeric receptor can be delivered to an NK cell in a single vector, or alternatively in multiple vectors. In some embodiments, a chimeric receptor construct is delivered in a single vector, while another factor that enhances efficacy of the chimeric receptor, such as mbIL15, is delivered in a separate vector. In several embodiments, a chimeric receptor and a factor that enhances efficacy of the chimeric receptor (e.g., mbIL15), is delivered in a single vector. Regardless of the number of vectors used, any polynucleotide may optionally include a tag sequence, allowing identification of the presence of NK cells expressing the construct. For example, in several embodiments a FLAG tag (DYKDDDDK, SEQ ID NO. 55) is used. Also available are other tag sequences, such as a polyhistidine tag (His-tag) (HHHHHH, SEQ ID NO. 56), HA-tag or myc-tag (EQKLISEEDL; SEQ ID NO: 57). Alternatively, green fluorescent protein, or other fluorescent moiety, is used. Combinations of tag types can also be used, to individually recognize sub-components of a chimeric receptor.

[00193] In several embodiments, the polynucleotide encoding the chimeric receptor is an mRNA that may be introduced into NK cells by electroporation. In another embodiment, the vector is a virus, preferably a retrovirus, which may be introduced into NK cells by transduction. In several embodiments, the vector is a Murine Stem Cell Virus (MSCV). In additional embodiments, other vectors may be used, for example lentivirus, adenovirus, adeno-associated virus, and the like may be used. In several embodiments, non-HIV-derived retroviruses are used. The vector chosen will depend upon a variety of factors, including, without limitation, the strength of the transcriptional regulatory elements and the cell to be used to express a protein. The vector can be a plasmid, phagemid, cosmid, viral vector, phage, artificial chromosome, and the like. In additional embodiments, the vectors can be episomal, non-homologously, or homologously integrating vectors, which can be introduced into the appropriate cells by any suitable means (transformation, transfection, conjugation, protoplast fusion, electroporation, calcium phosphate-precipitation, direct microinjection, etc.) to transform them. Other approaches to induce expression of chimeric receptors in NK cells are used in several embodiments, including for example, the SV40 early promoter region, the promoter contained in the 3′ long terminal repeat of Rous sarcoma virus, the herpes thymidine kinase promoter, the regulatory sequences of the metallothionein gene, an adenovirus (ADV) promoter, a cytomegalovirus (CMV) promoter, the bovine papilloma virus (BPV) promoter, the parovirus B19p6 promoter, the beta-lactamase promoter, the tac promoter, the nopaline synthetase promoter region or the cauliflower mosaic virus 35S RNA promoter, the promoter of ribulose biphosphate carboxylase, the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, the PGK (phosphoglycerol kinase) promoter, the synthetic MND promoter containing the U3 region of a modified MoMuLV LTR with the myeloproliferative sarcoma virus enhancer, and the alkaline phosphatase promoter.

[00194] Natural killer cells may be engineered to express the chimeric receptors disclosed herein. Chimeric receptor expression constructs may be introduced into NK cells using any of the techniques known to one of skill in the art. In one embodiment, the chimeric receptors are transiently expressed in the NK cells. In another embodiment, the chimeric receptors are stably expressed in NK cells. In an additional embodiment, the NK cells are autologous cells. In yet another embodiment, the NK cells are donor-derived (allogeneic) cells.

[00195] Further provided herein are methods of treating a subject having cancer or an infectious disease comprising administering to the subject a composition comprising NK cells engineered to express a chimeric receptor as disclosed herein, the chimeric receptor designed to target a marker or ligand expressed differentially on the damaged or diseased cells or tissue (e.g., expressed to a different degree as compared to a normal cell or tissue). As used herein, the terms “express”, “expressed” and “expression” be given their ordinary meaning and shall refer to allowing or causing the information in a gene or polynucleotide sequence to become manifest, for example producing a protein by activating the cellular functions involved in transcription and translation of a corresponding gene or DNA sequence. The expression product itself, e.g., the resulting protein, may also be said to be "expressed" by the cell. An expression product may be characterized as intracellular, extracellular or transmembrane. The term "intracellular" shall be given its ordinary meaning and shall refer to inside a cell. The term "extracellular" shall be given its ordinary meaning and shall refer to outside a cell. The term "transmembrane" shall be given its ordinary meaning and shall refer to at least a portion of a polypeptide is embedded in a cell membrane. The term "cytoplasmic" shall be given its ordinary meaning and shall refer to residing within the cell membrane, outside the nucleus. As used herein, the terms "treat," "treating," and "treatment" in the context of the administration of a therapy to a subject shall be given their ordinary meaning and shall refer to the beneficial effects that a subject derives from a therapy. In certain embodiments, treatment of a subject with a genetically engineered cell(s) described herein achieves one, two, three, four, or more of the following effects, including, for example: (i) reduction or amelioration the severity of disease or symptom associated therewith; (ii) reduction in the duration of a symptom associated with a disease; (iii) protection against the progression of a disease or symptom associated therewith; (iv) regression of a disease or symptom associated therewith; (v) protection against the development or onset of a symptom associated with a disease; (vi) protection against the recurrence of a symptom associated with a disease; (vii) reduction in the hospitalization of a subject; (viii) reduction in the hospitalization length; (ix) an increase in the survival of a subject with a disease; (x) a reduction in the number of symptoms associated with a disease; (xi) an enhancement, improvement, supplementation, complementation, or augmentation of the prophylactic or therapeutic effect(s) of another therapy. Administration can be by a variety of routes, including, without limitation, intravenous, intra-arterial, subcutaneous, intramuscular, intrahepatic, intraperitoneal and/or local delivery to an affected tissue. Doses of NK cells can be readily determined for a given subject based on their body mass, disease type and state, and desired aggressiveness of treatment, but range, depending on the embodiments, from about 10^5 cells per kg to about 10^{12} cells per kg (e.g., $10^5 - 10^7$, $10^7 - 10^{10}$, $10^{10} - 10^{12}$ and overlapping ranges therein). In one embodiment, a dose escalation regimen is used. In several embodiments, a range of NK cells is administered, for example between about 1 x 10^6 cells/kg to about 1 x 10^8 cells/kg. Depending on the embodiment, various types of cancer or infection disease can be treated. Various embodiments provided for herein include treatment or prevention of the following non-limiting examples of cancers including, but not limited to, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), adrenocortical carcinoma, Kaposi sarcoma, lymphoma, gastrointestinal cancer, appendix cancer, central nervous system cancer, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancer, brain tumors (including but not limited to astrocytomas, spinal cord tumors, brain stem glioma, craniopharyngioma, ependymoblastoma, ependymoma, medulloblastoma, medulloepithelioma), breast cancer, bronchial tumors, Burkitt lymphoma, cervical cancer, colon cancer, chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), chronic myeloproliferative disorders, ductal carcinoma, endometrial cancer, esophageal cancer, gastric cancer, Hodgkin lymphoma, non-Hodgkin lymphoma, hairy cell leukemia, renal cell cancer, leukemia, oral cancer, nasopharyngeal cancer, liver cancer, lung cancer (including but not limited to, non-small cell lung cancer, (NSCLC) and small cell lung cancer), pancreatic cancer, bowel cancer, lymphoma, melanoma, ocular cancer, ovarian cancer, pancreatic cancer, prostate cancer, pituitary cancer, uterine cancer, and vaginal cancer.

[00196] Further, various embodiments provided for herein include treatment or prevention of the following non-limiting examples of infectious diseases including, but not limited to, infections of bacterial origin may include, for example, infections with bacteria from one or more of the following genera: *Bordetella*, *Borrelia*, *Brucella*, *Campylobacter*, *Chlamydia* and *Chlamydophila*, *Clostridium*, *Corynebacterium*, *Enterococcus*, *Escherichia*, *Francisella*, *Haemophilus*, *Helicobacter*, *Legionella*, *Leptospira*, *Listeria*, *Mycobacterium*, *Mycoplasma*, *Neisseria*, *Pseudomonas*, *Rickettsia*, *Salmonella*, *Shigella*, *Staphylococcus*, *Streptococcus*, *Treponema*, *Vibrio*, and *Yersinia*, and mutants or combinations thereof. In several embodiments, methods are provided to treat a variety to treat viral infections, such as those caused by one or more viruses, such as adenovirus, Coxsackievirus, Epstein-Barr virus, hepatitis a virus, hepatitis b virus, hepatitis c virus, herpes simplex virus, type 1, herpes simplex virus, type 2, cytomegalovirus, ebola virus, human herpesvirus, type 8, HIV, influenza virus, measles virus, mumps virus, human papillomavirus, parainfluenza virus, poliovirus, rabies virus, respiratory syncytial virus, rubella virus, and varicella-zoster virus.

[00197] In some embodiments, also provided herein are nucleic acid and amino acid sequences that have homology of at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% (and ranges therein) as compared with the respective nucleic acid or amino acid sequences of SEQ ID NOS. 1-68 and that also exhibit one or more of the functions as compared with the respective SEQ ID NOS. 1-68: including but not limited to, (i) enhanced proliferation, (ii) enhanced activation, (iii) enhanced cytotoxic activity against cells presenting ligands to which NK cells harboring receptors encoded by the nucleic acid and amino acid sequences bind, (iv) enhanced homing to tumor or infected sites, (v) reduced off target cytotoxic effects, (vi) enhanced secretion of immunostimulatory cytokines and chemokines (including, but not limited to IFNg, TNFa, IL-22, CCL3, CCL4, and CCL5), (vii) enhanced ability to stimulate further innate and adaptive immune responses, and (viii) combinations thereof.

[00198] Additionally, in several embodiments, there are provided amino acid sequences that correspond to any of the nucleic acids disclosed herein, while accounting for degeneracy of the nucleic acid code. Furthermore, those sequences (whether nucleic acid or amino acid) that vary from those expressly disclosed herein, but have functional similarity or equivalency are also contemplated within the scope of the present disclosure. The foregoing includes mutants, truncations, substitutions, or other types of modifications.

[00199] There are provided for herein, according to several embodiments, polynucleotides encoding chimeric receptors, comprising an extracellular receptor domain, wherein the extracellular receptor domain comprises a peptide that binds native ligands of Natural Killer Group 2 member D (NKG2D), wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D, an effector domain comprising a transmembrane region and an intracellular signaling domain. In several embodiments, the fragment of NKG2D is encoded by a polynucleotide comprising the sequence of SEQ ID NO. 2 or 3 or 68, or functional equivalent thereof. In several embodiments, the polynucleotide encodes an effector domain comprising CD16. In several embodiments, the polynucleotide encodes an effector domain comprising NCR1. In several embodiments, the polynucleotide encodes an effector domain comprising NCR2. In several embodiments, the polynucleotide encodes an effector domain comprising NCR3. In some embodiments, the polynucleotide encodes an additional effector domain portion comprising 4-1BB. In several embodiments, the polynucleotide encodes a chimeric receptor made up of NKG2D and CD16. In several embodiments, the polynucleotide encodes a chimeric receptor made up of NKG2D and NCR1. In several embodiments, the polynucleotide encodes a chimeric receptor made up of NKG2D and NCR2. In additional embodiments, the polynucleotide encodes a chimeric receptor made up of NKG2D coupled to CD16 and optionally 4-1BB. In several embodiments, CD16 is replaced by NCR1, and in some embodiments, by NCR2, or even NCR3, depending on the embodiment. In several embodiments, the effector domain further comprises a GS linker between, for example, 4-1BB and one of CD16, NCR1, NCR2, or NCR3.

[00200] In several embodiments, the extracellular receptor domain further comprises a hinge region. In several embodiments, the hinge region comprises CD8a. However, in additional embodiments, the hinge region further comprises one or more linkers, which in some embodiments, comprise GS9, CD8a/GS3, truncated CD8a, GS3, and the like.

[00201] In several embodiments, the extracellular receptor domain further comprises a CD8a signal peptide. In several embodiments, the effector domain comprises one or more hemi-ITAM sequences. In several embodiments, the chimeric receptor does not comprise DNAX-activating protein 10 (DAP10). In several embodiments, the chimeric receptor does not comprise an ITAM motif, but rather employs an alternative signaling region, such as an ITSM, hemi-tam or other co-stimulatory region.

[00202] In one embodiment, there is provided a polynucleotide encoding a chimeric receptor comprising an extracellular receptor domain, wherein the extracellular receptor domain comprises a peptide that binds native ligands of Natural Killer Group 2 member D (NKG2D), wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D, a transmembrane region, wherein the transmembrane region comprises CD8a, and an effector domain, wherein the effector domain comprises 4-1BB and CD3 zeta, wherein the polynucleotide is co-expressed with an additional construct encoding membrane-bound interleukin 15 (mbIL15).

[00203] There is also provided in several embodiments, a polynucleotide encoding a chimeric receptor comprising an extracellular receptor domain, wherein the extracellular receptor domain comprises a peptide that binds native ligands of Natural Killer Group 2 member D (NKG2D), wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D, a transmembrane region, wherein the transmembrane region comprises CD8a, and an effector domain, wherein the effector domain comprises 4-1BB and the intracellular domain of 2B4 or DAP10. The polynucleotide encoding a chimeric receptor as described herein comprises a second peptide that binds native ligands of NKG2D. In several embodiments, the native ligands of NKG2D include, but are not limited to, MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5 or ULBP6.

In several embodiments, the portion of the chimeric receptor that binds native ligands of NKG2D has at least 80% homology to SEQ ID NO: 1, 2, 3, or 68.

[00204] In several embodiments, the provided polynucleotide is an mRNA. In some embodiments, the polynucleotide is operably linked to at least one regulatory element for the expression of the chimeric receptor. As used herein, the terms “nucleic acid,” “nucleotide,” and “polynucleotide” shall be given their ordinary meanings and shall include deoxyribonucleotides, deoxyribonucleic acids, ribonucleotides, and ribonucleic acids, and polymeric forms thereof, and includes either single- or double-stranded forms. Nucleic acids include naturally occurring nucleic acids, such as deoxyribonucleic acid (“DNA”) and ribonucleic acid (“RNA”) as well as nucleic acid analogs. Nucleic acid analogs include those which include non-naturally occurring bases, nucleotides that engage in linkages with other nucleotides other than the naturally occurring phosphodiester bond or which include bases attached through linkages other than phosphodiester bonds. Thus, nucleic acid analogs include, for example and without limitation, phosphorothioates, phosphorodithioates, phosphorotriesters, phosphoramidates, boranophosphates, methylphosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs), locked-nucleic acids (LNAs), and the like. As used herein, the term “operably linked,” for example in the context of a regulatory nucleic acid sequence being “operably linked” to a heterologous nucleic acid sequence, shall be given its ordinary meaning and shall mean that the regulatory nucleic acid sequence is placed into a functional relationship with the heterologous nucleic acid sequence. In the context of an IRES, “operably linked to” refers to a functional linkage between a nucleic acid sequence containing an internal ribosome entry site and a heterologous coding sequence initiation in the middle of an mRNA sequence resulting in translation of the heterologous coding sequence. As used herein, the term “vector” shall be given its ordinary meaning and shall refer to a vehicle by which a DNA or RNA sequence (e.g., a foreign gene) can be introduced into a genetically engineered cell, so as to transform the genetically engineered cell and promote expression (e.g., transcription and/or translation) of the introduced sequence. Vectors include viruses, plasmids, phages, etc. The term “chimeric receptor” as used herein shall be given its ordinary meaning and shall refer to a cell-surface receptor comprising at least two polypeptide domains not naturally found together on a single protein. The term “chimeric receptor complex” as used herein refers to a first polypeptide, which may comprise at least two polypeptide domains in a combination that are not naturally found together on a single protein, which first polypeptide is associated with a second polypeptide, for example, an adaptor polypeptide, a signaling molecule, or a stimulatory molecule. Additional terms relating to generation and use of chimeric receptors as disclosed here are readily understood by one of ordinary skill in the art and can also be found in International Publication WO 2014/117121 and US Patent No. 7,994,298, each of which are incorporated by reference in their entirety herein.

[00205] Additionally provided, according to several embodiments, is a vector comprising the polynucleotide encoding any of the polynucleotides provided for herein, wherein the polynucleotides are optionally operatively linked to at least one regulatory element for expression of a chimeric receptor. In several embodiments, the vector is a retrovirus.

[00206] Further provided herein are engineered natural killer cells comprising the polynucleotide, vector, or chimeric receptors as disclosed herein. In several embodiments, these NK cells are suitable for use in the treatment of prevention of disease, such as, for example, cancer and/or infectious disease.

EXAMPLES

Methods

[00207] The following experimental methods and materials were used in the non-limiting experimental examples disclosed below.

Cell Lines and Culture Conditions

[00208] The human acute lymphoblastic leukemia cell line REH, human osteosarcoma cell line U-2 OS and human embryonic kidney fibroblast 293T (HEK 293T) cells were obtained from the American Type Culture Collection (ATCC; Manassas, Virginia). REH cells were maintained and grown in Roswell Park Memorial Institute series 1640 (RPMI-1640; Gibco, Carlsbad, California) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, Utah) and 1% penicillin-streptomycin. Both HEK 293T and U-2 OS cells were maintained and grown in Dulbecco's modified Eagles Medium (DMEM; Hyclone) supplemented with 10% FBS and 1% penicillin-streptomycin. All mammalian cells were incubated at 37 °C with 5% CO2.

DNA Plasmids

[00209] A DNA plasmid containing the chimeric receptor NKG2D-DAP10-CD3ζ was made as previously described (see Chang et al. Cancer Research, Vol. 73(6): 2013). Splicing by overlapping extension polymerase chain reaction (SOE-PCR) was used to fuse the individual domains forming the NKG2D-41BB-CD3ζ construct. That construct was then inserted into the Murine Stem Cell Virus (MSCV) retroviral vector (Figure 3A). The constructs for NKG2D-CD16 and NKG2D-CD16-41BB were codon optimized and inserted into the MSCV vector (Figure 3B) by GenScript (Nanjing, China). The sequences of the constructs were verified by DNA sequencing.

Expansion of Human NK Cells

[00210] Human peripheral blood mononuclear cells (PBMCs) were obtained by Ficoll density centrifugation of blood samples from healthy adult donors. To expand the NK cells, PBMCs were cultured with K562 genetically modified with membrane bound IL-15 and 4-1BB ligand (K562-mb15-41BBL). Cells were cultured in Stem Cell Growth Medium (SCGM; Cell Genix, Freiburg, Germany) supplemented with 40IU of IL-2/ml every two days.

[00211] After 7 days of culture, NK cells were T-cell depleted using anti-CD3 Dynabeads (Invitrogen, Carlsbad, California). NK cells were then cultured in SCGM supplemented with 40-200 IU of IL-2/ml every two days.

Production of Retrovirus and Transduction of NK Cells

[00212] Production of retrovirus was carried out by transiently transfecting HEK 293T cells with retroviral packaging plasmids. HEK 293T cells were first seeded to a concentration of 2.5×10^6 cells in 12 ml of DMEM 18 hours before the transfection. The cells were then transfected with 3.5 μg of MSCV vector containing the respective NKG2D chimeric receptors (non-limiting constructs are illustrated schematically in Figures 1B-1C and 2A-2B), 3.5 μg of pEQ-PAM3, and 3.0 μg of pRDF. For control, empty MSCV vector containing GFP was used. X-tremeGENE 9 DNA Transfection Reagent (Roche, Basel, Switzerland) was used for the transfection. DMEM was replaced with conditioned RPMI-1640 24 hours after the transfection.

[00213] Transduction of NKG2D chimeric receptor transgene into NK cells was done 18 hours after the changing of media. NK cells were first suspended at a concentration of 0.25×10^6 cells in 2 ml of conditioned RPMI-1640. Cells were subsequently seeded into RetroNectin (TaKaRa, Otsu, Japan) coated tubes. RPMI-1640 containing the retrovirus (virus supernatant) was harvested from the HEK 293T cell cultures and fresh conditioned medium was added back to the cultures. The viral supernatant was supplemented with 200 IU of IL-2/ml and 3 ml of the viral supernatant was dispensed into each RetroNectin coated tubes (containing the seeded NK cells). In accordance with certain embodiments of producing NK cells, seeded NK cells were transduced six times, once every 12 hours with fresh viral media. Transduced NK cells were then harvested 48 hours after the last transduction, and cultured in SCGM with the addition of 200 IU of IL-2/ml every two days. The transduced NK cells were used for experiments 14 to 28 days after expansion.

Detection of Chimeric Receptor Expression by Flow Cytometry

[00214] Transduced NK cells were washed once with phosphate-buffered saline containing albumin, and 2 µl of rabbit serum was added. The cells were then stained with peridinin chlorophyll (PerCP)-conjugated anti-human NKG2D antibody (clone 149810; R&D Systems, Minneapolis, USA) for 10 minutes in the dark. For controls, the transduced NK cells were stained with the respective PerCP-conjugated IgG isotype antibody. All NK cells were washed again and fixed with 300 µl 0.5% formaldehyde before analysis using Accuri C6 flow cytometer (BD, Franklin Lakes, New Jersey). Data was analyzed using a paired t-test.

Cytotoxicity Assays

[00215] REH cells were stained with calcein AM red-orange (Thermo Fisher Scientific, Waltham, Massachusetts). REH cells were seeded into a 96-well round bottom plate (CoStar, Corning, New York). Transduced NK cells were then added at various effector: target (E:T) ratio. The cell cultures were incubated for four hours at 37 °C and 5% CO_2. Stained viable target cells were counted using the Accuri C6 flow cytometer. U-2 OS cells were seeded into 96-well flat bottom white plate (Costar) and incubated for four hours. Transduced NK cells was then added according to different E:T ratios. Cell cultures were then incubated for another four hours. Prior to analysis, Bright-Glo substrate (Promega, Madison, Wisconsin) was added to the cells. Intensity of luminescence from viable target cells was measured using FLx800 Fluorescence Reader (Bio Tek, Winooski, Vermont). Differences between intensity of luminescence and control were converted to percentage cytotoxicity.

Interferon gamma (IFNγ) Production Assay

[00216] To determine the amount of IFNγ produced by the NK cells, effector and target cells were first cultured with (E:T of 1:1) or without REH in a 96-well round bottom plate. Cells were incubated for one hour before the addition of GolgiPlug (brefeldin A; BD Biosciences). After another 5 hours of culture, cells were labeled with phycoerythrin (PE)-conjugated anti-human CD56 antibody (clone MY31, BD Biosciences). Cells were permeabilized using a proprietary permeabilization reagent and incubated for 40 minutes in the dark. The cells were then washed with a proprietary wash buffer. Intracellular IFNγ was detected with allophycocyanin (APC)-conjugated IFNγ antibody (clone 25723.11; BD Biosciences) for 45 minutes. The cells were then fixed and analyzed using Accuri C6 flow cytometer.

Example 1 – CD3-zeta Containing NKG2D Constructs

[00217] As disclosed herein, various constructs comprising NKG2D and/or NKG2D variants coupled with various transmembrane and/or signaling domains are provided. The present experiment was conducted to evaluate the expression and cytotoxic activity of constructs comprising CD3-zeta signaling domains. Two CD3-zeta constructs were prepared and tested according to the methods and materials described above. Depending on the construct, the methods used can be readily adjusted to account for variations required for generating, expressing and testing a construct. The two constructs were NKG2D-DAP10-CD3ζ and NKG2D-41BB-CD3ζ. For reference Figure 1A schematically depicts an endogenous NKG2D. In NK cells, ionic interactions between the transmembrane region of NKG2D allow association with its adaptor protein DAP10 (Wu et al., 1999). Upon ligand binding, NKG2D signals are transduced through the signaling motif, YxNM, found on DAP10. CD3ζ transduce signals through its immunoreceptor tyrosine-based activation motif (ITAM; Lanier, 2008). The two experimental constructs are illustrated schematically in Figure 1B and 1C, respectively. Figure 1B shows NKG2D-DAP10-CD3ζ, with signaling occurring through both the YxNM and ITAM motifs. Figure 1C shows the NKG2D-41BB-CD3ζ construct, which employs a CD8a hinge region as a transmembrane domain and 4-1BB and CD3ζ as signaling domains.

[00218] The ability of NK cells to effectively express these constructs was first assessed. NK cells expanded from PBMC of healthy adult donors were transduced with one of the two chimeric receptors. Mock-transduced NK cells were used as control (transduced with empty MSCV vector containing GFP only). The presence and relative abundance of the chimeric receptors were determined through staining the NK cells with a Per-CP conjugated anti-NKG2D antibody. Figure 4A depicts representative flow cytometry data related to the percentage of NKG2D-positive NK cells after transduction with Mock (left panel), NKG2D-DAP10-CD3ζ (center panel) or NKG2D-41BB-CD3ζ (right panel) constructs. Mock transduced NK cell showed no NKG2D expression with the antibody used (which does not showing staining above an isotype-matched non-reactive antibody, despite the naturally high NKG2D expression on activated NK cells), while just under 60% of cells transduced with the NKG2D-DAP10-CD3ζ construct exhibited NKG2D expression above the isotype-matched non-reactive antibody control, and over 80% of NK cells transduced with the NKG2D-41BB-CD3ζ. Pooled data for the percentage of NKG2D positive NK cells from all donors is shown in Figure 4B. Both engineered NKG2D constructs result in substantial gain in NKG2D expression compared to Mock, though there is not a significant difference between the percent expression of the two constructs. Figure 4C depicts expression data based on Mean Fluorescence Intensity (MFI), which represents, within the population expressing the NKG2D construct, the degree to which that cell expresses the construct (e.g., multiple copies of the construct per cell would yield a greater MFI). By this measure, the expression of the NKG2D-41BB-CD3ζ is significantly greater than that of the NKG2D-DAP10-CD3ζ construct.

[00219] Collectively, these data demonstrate that, in accordance with several embodiments disclosed herein, engineered constructs can successfully be expressed on NK cells. In several embodiments, enhanced expression of the construct can be achieved by repeated transduction of the NK cells with a particular construct. In several embodiments, the components of the constructs can be delivered to a cell in a single vector, or alternatively using multiple vectors. Depending on the embodiment, the construct itself may lead to enhanced expression, for example a linear or head to tail construct may yield increased expression because of a lesser degree of in-cell assembly that a multiple subunit construct requires.

[00220] Further to successfully expressing NKG2D constructs on NK cells, effective signaling of the NK cells is required to act on target cells. To evaluate the potency of the two populations of transduced NK cells, cytotoxicity assays were performed using to cell lines that are sensitive to NK cell activity, REH (suspension cells) and U-2 OS (adherent cells). Data summarizing the percentage cytotoxicity of the different groups of NK cells against REH cells and across independent donors at two E:T ratios are shown in Figures 5A-5C (error bars represent standard deviation; all experiments are done in triplicates; $n = 3$ ($P < 0.001$)). As depicted in Figures 5A-5C, NK cells expressing either NKG2D chimeric receptor (NKG2D-DAP10-CD3ζ shown with an arrow labeled (a) and NKG2D-41BB-CD3ζ shown with an arrow labeled (b)) had a significantly higher cytotoxicity against REH for all three donors as compared to mock NK cells (shown with an arrow labeled (c)). The mean percentage cytotoxicity of NKG2D-DAP10-CD3ζ-expressing NK cells was 91.8% ± 5.8% (1:1 E:T ratio) and 83.9% ± 5.6% (1:2 E:T ratio). Those NK cells transduced with NKG2D-41BB-CD3ζ showed similar potencies - 87.4% ± 6.1% at a 1:1 E:T ratio and 76.2% ± 4.8% at a 1:2 E:T ratio. Chimeric receptor-expressing NK cells also showed elevated cytotoxicity against U-2 OS when compared to mock-transduced NK cells (See Figures 6A-6C, Figure 6A depicts NKG2D-DAP10-CD3ζ shown with an arrow labeled (a), Figure 6B depicts NKG2D-41BB-CD3ζ shown with an arrow labeled (b) and Figure 6C depicts mock NK cells shown with an arrow labeled (c)).

[00221] These data provide evidence that NK cells can not only be engineered to express chimeric receptor constructs, but those cells that express the chimeric receptors are able to be activated and successfully generate enhanced cytotoxic effects against target cells. Importantly, these data also show that there is only a slight decrease in the potency of the cells when in the presence of a greater number of target cells (doubled in this experiment). This suggests that the desired cytotoxic effects of the engineered NK cells can still be realized, even when the NK cells are present in smaller numbers vis-à-vis target cells, as would likely be the case in clinical use. Moreover, these data indicate that, according to some embodiments, a lesser density or degree of chimeric receptor expression on a given NK cell does not necessarily result in coordinately reduced cytotoxic effects, and can be associated with an unexpected efficacy of the NK cells in view of their lesser construct expression. Additionally, these data embody the unexpectedly enhanced cytotoxicity that is achieved according to several embodiments. While non-engineered NK cells are cytotoxic, and express a significant amount of NKG2D upon activation, it is unexpected that the engineered cells disclosed herein can push the cytotoxic effects significantly beyond what can be considered an already elevated ceiling (e.g., native NK cell cytotoxicity).

[00222] Further to the cytotoxicity data, the mechanism by which the NK cells are exerting these effects was examined, by evaluating the production if interferon-gamma (IFNγ) by the NK cells expressing the various NKG2D constructs. IFNγ is a key cytokine produced and released by NK cells (typically during an innate immune response) that recruits macrophages and has immunostimulatory effects. Figure 7A shows the relative amount of IFNγ production (measured by MFI) in Mock (left panel), NKG2D-DAP10-CD3ζ-expressing NK cells (center panel), and NKG2D-41BB-CD3ζ-expressing NK cells (right panel) with or without stimulation by REH cells. NK cells were stained by APC-conjugated anti-IFNγ antibody for intracellular IFNγ. Data was analyzed by paired t test. These data show that each of the three groups of NK cells were observed to have a similar level of IFNγ production without stimulation, with an increase observed after stimulation by REH cells. As provided for in several embodiments, engineered NK cells expressing NKG2D constructs can lead to robust cytokine production. The presence of a target cell (here, REH cells) to which the engineered NK cells responds sets into motion the biochemical cascade which leads to IFNγ production and ultimately cytotoxic effects. As shown in Figure 7A, the NKG2D-41BB-CD3ζ-expressing NK cells show a robust production of IFNγ in the presence of stimulatory REH cells. Interestingly, the NKG2D-DAP10-CD3ζ-expressing NK cells failed to show a similar degree of response. This is further demonstrated in Figure 7B, where levels of IFNγ between different groups of NK cells after stimulation with REH cells (median values were represented; data was analyzed by unpaired t test) are evaluated. All IFNγ experiments were conducted in triplicates, with three independent donors, $n = 9$. Figure 7B shows that IFNγ production by NKG2D-DAP10-CD3ζ-expressing NK cells was not significantly different from mock-transduced NK cells. In contrast, the NKG2D-41BB-CD3ζ-expressing NK cells show a significant increase in IFNγ production as compared to mock-transduced NK cells. These data are interesting because they demonstrate that, as discussed herein, signaling by a chimeric receptor in response to ligand binding is an essential step in generating cytotoxic effects against a target cell of interest. However, there is not a singular pathway through which the various constructs signal, as NK cells transduced with two different chimeric receptors both exhibit relatively similar cytotoxicity, but without mirroring levels of IFNγ production. Thus, according to some embodiments, constructs are provided that achieve cytotoxic effects through an elevated production of IFNγ, or other immunostimulatory cytokine, as compared to normal NK cells. However, in several embodiments, increased production of IFNγ is not necessarily achieved or detected, rather another immunostimulatory pathway can be exploited by a given chimeric construct to achieve elevated cytotoxic effects.

Example 2 – CD16 and CD16-4-1BB Containing NKG2D Constructs

[00223] Additional constructs were generated to evaluate expression, cytotoxicity and cytokine production. As provided for herein, several embodiments relate to constructs comprising a truncated NKG2D (in some embodiments codon optimized), that employ a CD16 transmembrane and/or signaling domain. The constructs generated for evaluation in this experiment are schematically shown in Figures 2A-2B, which show the structure of A) NKG2D-CD16 and B) NKG2D-CD16-41BB chimeric receptors. Both chimeric receptors rely on the transmembrane region of CD16 to associate with either

CD3ζ or FcRγ. The plasmids used to generate these constructs are shown in Figure 3B. As discussed above, in several embodiments, the constructs employed rely on endogenous expression of CD3ζ or FcRγ, however, in several embodiments the plasmid encoding the chimeric receptor (or a separate plasmid) is configured to elevate expression of CD3ζ and/or FcRγ by the NK cell, thereby enhancing the potency of the cells.

[00224] As above, expression levels of the constructs were evaluated. Figure 8A depicts representative flow cytometry data for mock (left panel), NKG2D-DAP10-CD3ζ-expressing NK cells (center panel), and NKG2D-CD16-expressing NK cells (Experiments were conducted using cells from three independent donors represented by different symbols. Data was analyzed by paired t test). Figure 8B shows summary data relating to the percentage of cells that that express NKG2D (and hence the constructs). As expected, mock-transfected NK cells show low levels of NKG2D expression with the antibody used. In contrast, both of the engineered constructs exhibited significantly enhanced expression, with NKG2D-CD16-transduced NK cells expressing 35.8% ± 6.9% greater expression as compared to mock-transduced NK cells. Additionally, as evaluated by MFI (Figure 8C), NKG2D-CD16-transduced NK cells also exhibited increased expression of the construct. These data are important to demonstrate that the constructs can effectively be introduced into NK cells and are expressed.

[00225] Having established expression of the constructs, their ability to exhibit cytotoxic effects was evaluated. As discussed above, NK cells from three donors were tested for cytotoxic effects against REH cells and U-2 OS cells, each at three E:T ratios (all experiments were done in triplicate, n=3). Interestingly, the enhanced expression of the NKG2D-CD16 construct as compared to mock NK cells did not result in increased cytotoxicity (see Figure 9A-9C, error bars represent standard deviations). As with the prior example, NKG2D-DAP10-CD3ζ-expressing NK cells (shown with an arrow labeled (a)) did exhibit an increased cytotoxicity. With respect to cytotoxicity against U-2 OS cells, the NKG2D-CD16 (shown with an arrow labeled (b)) did exhibit an increased cytotoxicity as compared to mock NK cells (shown with an arrow labeled (c)) (see Figures 10A-10C). These data indicate that the degree of cytotoxic impact on a particular given target cell type may vary with the NK construct used. In some embodiments, a particular construct may not be as effective, however, in several embodiments, combinations of populations of NK cells can be used and exhibit synergistic effects. In other words, a population of NK cells, with a portion expressing NKG2D-CD16 and a portion expressing NKG2D-DAP10-CD3ζ (or other combination of any of the constructs disclosed herein), may exhibit unexpectedly enhanced cytotoxicity as compared to either sub-population alone.

[00226] Interferon-γ production was measured next, in order to confirm the mechanism of action of the transfected NK cells. The NK cells expressing the various constructs were either stimulated by REH cells, or not, and the production of IFNγ was measured. These data are shown in Figure 11 (data was analyzed by paired t test). All groups of NK cells had similar level of IFNγ without stimulation, and an increase after incubation with REH cells. The NKG2D-CD16-expressing NK cells exhibited an increase in IFNγ production of 634 ± 211 MFI, which was greater than the increase exhibited by the mock-transduced NK cells (423 ± 70 MFI). However, the increase was lower than that observed for NKG2D-DAP10-CD3ζ-expressing NK cells, which increased 2041 ± 411 MFI. In line with data, according to several embodiments the production of IFNγ is correlated with the cytotoxic effects that NK cells expressing certain constructs exhibit.

[00227] In accordance with several embodiments disclosed herein, multiple signaling regions may be used. Additional experiments were conducts to evaluate the expression of a NKG2D-CD16-41BB in expanded NK cells (experiments were conducted using cells from one donor). The expression data is shown in Figures 12A-12B. Figure 12A shows raw flow cytometry data that demonstrate that the addition of the 4-1BB signaling region does not significantly impair the expression of the construct by NK cells, as compared to the NKG2D-CD16 construct. This is also reflected in the summary histogram of Figure 12B that shows the relative amount of NKG2D receptors on the surface of each of the NK cell groups tested. The NKG2D-CD16-41BB shows slightly reduced MFI as compared to NKG2D-CD16, but both constructs show elevated expression versus mock.

[00228] Cytotoxic effects were evaluated as described above, using both REH and U-2 OS cells as targets. Figures 13A-13B depict the resultant data (error bars represent standard deviations; all experiments were conducted in triplicates, n = 3). Figure 13A shows the cytotoxic effects of the constructs against REH cells. Similar to the experiment above, the NKG2D-CD16-expressing cells shown with an arrow labeled (b)) did not show significantly elevated cytotoxic effects as compared to mock NK cells shown with an arrow labeled (a). In contrast, NK cells expressing NKG2D-CD16-41BB (shown with an arrow labeled (c)) showed enhanced cytotoxicity against REH cells. With respect to efficacy against U-2 OS cells, both the NKG2D-CD16 and NKG2D-

CD16-41BB expressing cells showed enhanced cytotoxicity, with the NKG2D-CD16-41BB expressing cells exhibiting a more robust cytotoxic effect. This demonstrates that, in accordance with several embodiments, use of a combination of signaling domains can result in unexpected enhancements in the efficacy of a transduced NK cell. Thus, as described above, several embodiments employ two or more transmembrane/signaling domains that work synergistically together to yield enhanced cytotoxicity against target cells.

Example 3 – Additional NKG2D Constructs

[00229] Additional constructs with varying extracellular domains, transmembrane domains, and intracellular effector domains were generated to evaluate their expression and cytotoxicity. The 12 constructs generated for evaluation in this experiment are schematically shown in Figure 14. Some of these variant chimeric receptors rely on a CD16 transmembrane region to associate with either CD3ζ or FcRγ. As discussed above, in several embodiments, the constructs employed rely on endogenous expression of CD3ζ or FcRγ, however, in several embodiments the plasmid encoding the chimeric receptor (or a separate plasmid) is configured to elevate expression of CD3ζ and/or FcRγ by the NK cell, thereby enhancing the potency of the cells. As above, expression levels of the constructs were evaluated. Mock-transfected NK cells show low levels of NKG2D expression as evaluated by MFI (Figure 16A). In contrast, NK cells transduced with the variant NKG2D constructs described above showed varying levels of NKG2D expression, with engineered variant constructs 4 and 9 exhibiting significantly enhanced expression in NK cells. Figure 16B depicts representative flow cytometry data for variant NKG2D constructs 1, 4, 8, 9 after transduction into the NK cells of two donors. Relative to mock-transduced NK cells, Variant 8- and 9-transduced NK cells showed particularly strong expression of the chimeric receptor. Variant construct expression persisted in the NK cells of two donors 7 days following transduction, with Variants 8 and 9 showing particularly elevated levels as evaluated by MFI (Figure 16C). These data are important to demonstrate that the constructs can effectively be introduced into NK cells and are expressed. Having established expression of the constructs, their ability to deliver cytotoxic effects in transduced NK cells was also evaluated. The cytotoxicity of the NKG2D variant constructs 4, 8, and 9 were evaluated 14 days post-transduction into NK cells at a 1:1 E:T ratio (Figure 17).

[00230] Further variant constructs were generated and are schematically shown in Figure 15, which show the structure of chimeric receptors comprising various extracellular domains, transmembrane domains, and intracellular effector domains. Some of these variant chimeric receptors rely on an effector domain comprising CD3zeta and/or another signaling domain to transduce signaling upon ligand binding, while other variant chimeric receptors comprise a CD3zeta transmembrane domain that recruits full-length CD3zeta molecule to the synapse via dimerization. As above, expression levels of the constructs were evaluated. As evaluated by MFI (Figures 18A-B), NK cells transduced with engineered constructs exhibited increased expression of the chimeric receptor relative to mock transduced cells. Cytotoxic effects were evaluated as described above using an effector: target ratio of 1:1. As depicted in Figures 19A-B, NK cells transduced with engineered constructs (particularly variant 18) have enhanced cytotoxicity relative to the mock control.

[00231] As variant 18 exhibited robust expression in NK cells that was accompanied by enhanced cytotoxic effects, a series of variant NKG2D constructs comprising a CD3zeta transmembrane domain were generated. These variants are termed “NK39” and are schematically shown in Figure 15. Fourteen days following transfection into donor NK cells (with 4 days of culturing in low IL-2 conditions), the cytotoxicity of the transduced NK cells were evaluated. Figure 21 shows the cytotoxic effects of the constructs against cultured REH cells at 1:1 and 1:2 E:T ratios. All the of the NK cells expressing engineered NK39 constructs showed significantly elevated cytotoxic effects as compared to control NK cells at a 1:1 E:T ratio. When evaluated at a 1:2 E:T ratio, chimeric constructs 16-7, 39-1, 39-2, 39-3, and 39-5 each enhanced the cytotoxic effects of their respective transduced NK cells relative to the mock control. As exogenous expression of activating receptors can lead to NK cell anergy and cell death, the engineered constructs were transduced into two donor NK cells and survival was evaluated after 21 days. As depicted in Figures 23A-B, NK39-5 and NK39-10 transduced cells show better survival than NK16 in two tested donors.

Example 4 – Evaluation of NK45 NKG2D Constructs

[00232] Additional constructs with varying extracellular domains, hinges, transmembrane domains, and intracellular effector domains according to embodiments disclosed herein are schematically shown in Figure 22. The expression, cytotoxicity, persistence, and cytokine production mediated by these 7 constructs were evaluated in this Example relative to three of the NK39 constructs described in Example 3 (NK39-5, NK39-6, NK39-10) as well as a version of NK16 that bicistronically expresses membrane-bound interleukin 15 (NK26-8). In accordance with several embodiments disclosed herein, multiple signaling regions may be used. Some of these variant chimeric receptors rely on an effector domain comprising CD3zeta and/or another signaling domain (e.g., OX40, CD28, and/or 4-1BB costimulatory domains) to transduce signaling upon ligand binding, while other variant chimeric receptors comprise a CD3zeta transmembrane domain that recruits full-length CD3zeta molecule to the synapse via dimerization. As disclosed herein, these constructs are further configured to co-express membrane-bound IL15.

[00233] As above, the ability of NK cells to effectively express these constructs was first assessed. NK cells expanded from the PBMC of four donors were transduced with the variant constructs (or an empty MSCV control vector containing GFP only) and NKG2D expression was evaluated by MFI after 3 days. As depicted in FIG. 24, mock-transfected NK cells show relatively low levels of NKG2D expression. In contrast, the engineered variant constructs exhibited significantly enhanced expression, with NK45-4 (NKG2D-OX40-CD3ζ) showing surprisingly robust expression in all donors. OX40 is expressed in activated NK cells, but its role has not been well-established. A variant chimeric receptor with an effector domain containing a CD28 costimulatory domain (NK45-2; NKG2D-CD28-CD3ζ) also demonstrated robust expression 3 days post-transduction.

[00234] Having established expression of the variant constructs, their ability to exert cytotoxic effects was evaluated as above using REH and HL60 cells as targets. The potency of NK cells from four donors were examined against REH cells (FIG. 25A) and HL60 cells (FIG. 25B) at 1:1 E:T ratios 14 days post-transduction. As depicted in FIGs. 25A-B, the engineered constructs exerted an enhanced cytotoxicity against both REH and HL60 cells in all four donors as compared to mock NK cells. In addition to its pronounced expression profile, cells expressing NK45-4 (NKG2D-OX40-CD3ζ) also exhibited surprisingly elevated cytotoxicity relative to the mock control and the other constructs tested. NK cells expressing NK45-1 and NK45-2 also demonstrated pronounced cytotoxicity in these assays. These data demonstrate that, in accordance with several embodiments, use of a combination of signaling domains (particularly an OX40 costimulatory domain) can result in unexpected enhancements in the efficacy of a transduced NK cell. FIGs. 28A-B depict the cytotoxic activity against U2OS cells of the NK cells transduced with several of the variant constructs at various E:T ratios (1:2 and

1:4) and assessed over a more extended period of time. Surprisingly, NK cells transduced with the 45-4 construct appear to maintain cytotoxic activity through the time course. Advantageously, these experiments indicate that, according to several embodiments disclosed herein, the NKG2D variant constructs provide unexpectedly enhanced cytotoxicity over an extended period of time, which, depending on the embodiment, can range from 2-3 days, 3-5 days, 5-7, days, 7-8 days, 8-10 days, 10-14 days, 14-21 days, or 21-50 days (and any range in between those listed, including endpoints). In several embodiments, even longer durations of cytotoxic effects are achieved.

[00235] Further to the cytotoxicity data, the mechanism by which the NK cells are exerting these effects was examined by evaluating their production of IFNγ, TNFα, and GM-CSF following stimulation with REH cells. As depicted in FIGs. 26A-C, expression of each of the variant constructs yielded enhanced cytokine secretion relative to the production of IFNγ, TNFα, and GM-CSF exhibited by the GFP-expressing control NK cells. The chimeric receptor NK45-1 consistently mediated high cytokine production, which is surprising because this construct expresses at substantially lower levels than NK26-8 (from which it differs only with regards to the hinge region). Thus, these data demonstrate the unexpected importance of the hinge regions disclosed herein to mediating robust cytokine production in response to stimulation. Additionally, NKG2D-OX40-CD3ζ-expressing NK cells also showed an elevated production of IFNγ, TNFα, and GM-CSF.

[00236] As exogenous expression of activating receptors can lead to NK cell anergy and cell death, the engineered constructs were transduced into two donor NK cells and the total cell count was evaluated 7, 14, and 21 days post-transduction. Surprisingly, the unexpectedly robust expression of NK45-4 does not come at the cost of reduced NK cell persistence in culture, as the total cell count remained at levels comparable to the GFP-expressing control cells (FIGs. 27A and 27B). Likewise, other NK cells expressing variant constructs at high levels continued to proliferate in the 2 donors for at least 3 weeks post-transduction. Collectively, these data demonstrate that, in accordance with several embodiments disclosed herein, engineered constructs can successfully be expressed at high levels in NK cells and mediate cytotoxic effects, and further, that this enhanced expression does not come at the detriment of reduced NK cell proliferation and/or survival.

[00237] It is contemplated that various combinations or subcombinations of the specific features and aspects of the embodiments disclosed above may be made and still fall within one or more of the inventions. Further, the disclosure herein of any particular feature, aspect, method, property, characteristic, quality, attribute, element, or the like in connection with an embodiment can be used in all other embodiments set forth herein. Accordingly, it should be understood that various features and aspects of the disclosed embodiments can be combined with or substituted for one another in order to form varying modes of the disclosed inventions. Thus, it is intended that the scope of the present inventions herein disclosed should not be limited by the particular disclosed embodiments described above. Moreover, while the invention is susceptible to various modifications, and alternative forms, specific examples thereof have been shown in the drawings and are herein described in detail. It should be understood, however, that the invention is not to be limited to the particular forms or methods disclosed, but to the contrary, the invention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the various embodiments described and the appended claims. Any methods disclosed herein need not be performed in the order recited. The methods disclosed herein include certain actions taken by a practitioner; however, they can also include any third-party instruction of those actions, either expressly or by implication. For example, actions such as “administering a population of expanded NK cells” include “instructing the administration of a population of expanded NK cells.” In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[00238] The ranges disclosed herein also encompass any and all overlap, sub-ranges, and combinations thereof. Language such as “up to,” “at least,” “greater than,” “less than,” “between,” and the like includes the number recited. Numbers preceded by a term such as “about” or “approximately” include the recited numbers. For example, “about 90%” includes “90%.” In some embodiments, at least 95% homologous includes 96%, 97%, 98%, 99%, and 100% homologous to the reference sequence. In addition, when a sequence is disclosed as “comprising” a nucleotide or amino acid sequence, such a reference shall also include, unless otherwise indicated, that the sequence “comprises”, “consists of” or “consists essentially of” the recited sequence.

WHAT IS CLAIMED IS:

1. A polynucleotide encoding a chimeric receptor expressed by a cell, comprising:

(a) an extracellular receptor domain,

wherein said extracellular receptor domain comprises a peptide that binds native ligands of Natural Killer Group 2 member D (NKG2D),

wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D,

wherein the fragment of NKG2D is encoded by a polynucleotide: (i) comprising a fragment of SEQ ID NO: 1, (ii) having at least 95% sequence homology to SEQ ID NO. 2, or (iii) having at least 95% sequence homology to SEQ ID NO. 3; and

(b) an effector domain comprising a transmembrane region and an intracellular signaling domain,

wherein the intracellular signaling domain comprises CD3zeta, and

wherein the CD3zeta is encoded by a polynucleotide having at least 95% sequence homology to SEQ ID NO. 13 and

wherein the polynucleotide further encodes a membrane-bound interleukin 15 (mbIL15).

2. The polynucleotide of Claim 1, wherein the transmembrane region of the effector domain comprises a CD8a transmembrane domain.

3. The polynucleotide of Claim 1 or Claim 2, wherein the transmembrane region of the effector domain further comprises a CD8a hinge region.

4. The polynucleotide of Claim 3, wherein the CD8a hinge region is encoded by a polynucleotide having at least 95% sequence homology to SEQ ID NO: 5.

5. The polynucleotide according to any one of Claims 1 to 4, wherein the mbIL15 is encoded by a polynucleotide having at least 95% sequence homology to SEQ ID NO. 16.

6. The polynucleotide according to any one of Claims 1 to 5, wherein the mbIL15 is bicistronically expressed on the same polynucleotide as the chimeric receptor.

7. The polynucleotide according to any one of Claims 1 to 6, wherein mbIL15 comprises an amino acid sequence having at least 95% sequence homology to SEQ ID NO: 17.

8. The polynucleotide according to any one of Claims 1 to 7, wherein the effector domain further comprises an OX-40 domain.

9. The polynucleotide of Claim 8, wherein the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD8a transmembrane domain, the OX-40 domain, and the CD3zeta.

10. The polynucleotide of Claim 9, wherein the chimeric receptor is encoded by a nucleic acid sequence having at least 95% sequence homology to SEQ ID NO: 90 coupled to the nucleic acid sequence encoding mbIL15 and having at least 95% sequence homology to SEQ ID NO. 16.

11. The polynucleotide according to any one of Claims 1 to 10, wherein the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 109 coupled to the mbIL15 comprising the amino acid sequence of SEQ ID NO. 17.

12. The polynucleotide according to any one of Claims 1 to 9, wherein the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 100 coupled to the mbIL15 encoded by SEQ ID NO. 16.

13. The polynucleotide of Claim 12, wherein the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 101 coupled to the mbIL15 comprising the amino acid sequence of SEQ ID NO. 17.

14. A vector comprising the polynucleotide of any one of Claims 1 to 13.

15. The vector of Claim 14, wherein the vector is a viral vector.

16. The vector of Claim 14 or 15, wherein the vector is a retroviral vector.

17. An immune cell comprising the polynucleotide of any one of Claims 1 to 13 or the vector of any one of Claims 14 to 16.

18. A polypeptide encoding a chimeric receptor comprising:

(a) an extracellular receptor domain,

wherein said extracellular receptor domain comprises a peptide that binds native ligands of Natural Killer Group 2 member D (NKG2D),

wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D, and

(b) an effector domain comprising a transmembrane region and an intracellular signaling domain,

wherein the transmembrane region comprises a comprises a CD8a transmembrane domain,

wherein the effector domain comprises an OX40 domain, and

wherein the intracellular signaling domain comprises CD3zeta.

19. The polypeptide of Claim 18, wherein the OX40 domain comprises an amino acid sequence have at least 95% sequence homology to SEQ ID NO. 107.

20. The polypeptide of Claim 18 or 19, wherein the CD3Zzeta domain is encoded by a polynucleotide having at least 95% sequence homology to SEQ ID NO. 13.

21. A polypeptide according to any one of Claims 18 to 20, wherein the chimeric receptor comprises the extracellular receptor domain comprising the fragment of NKG2D coupled to a CD8alpha hinge domain, wherein the CD8alpha hinge domain is coupled to a CD8alpha transmembrane domain, wherein the CD8alpha transmembrane domain is coupled to the OX40 domain, and wherein the OX40 domain is coupled to the CD3zeta domain.

22. A polypeptide according to any one of Claims 18 to 21, wherein the chimeric receptor has at least 95% sequence homology to the amino acid sequence of SEQ ID NO: 109.

23. An immune cell expressing a chimeric receptor targeting ligands of Natural Killer Group 2 member D (NKG2D), the chimeric receptor comprising:

an extracellular receptor domain comprising a fragment of an NKG2D receptor,

wherein the fragment of NKG2D is encoded by a polynucleotide: (i) comprising a fragment of SEQ ID NO: 1, (ii) having at least 95% sequence homology to SEQ ID NO. 2, or (iii) having at least 95% sequence homology to SEQ ID NO. 3;

an effector domain comprising a transmembrane region and an intracellular signaling domain,

wherein the intracellular signaling domain comprises CD3zeta,

wherein the CD3zeta is encoded by a polynucleotide having at least 95% sequence homology to SEQ ID NO. 13; and

wherein the cell expresses membrane-bound interleukin 15 (mbIL15).

24. The immune cell of Claim 23, wherein the transmembrane region of the effector domain comprises a CD8a transmembrane domain.

25. The immune cell of Claim 23 or 24, wherein the transmembrane region of the effector domain further comprises a CD8a hinge region.

26. An immune cell according to any one of Claims 23 to 25, wherein the CD8a hinge region is encoded by a polynucleotide having at least 95% sequence homology to SEQ ID NO: 5.

27. An immune cell according to any one of Claims 23 to 26, wherein the mbIL15 is encoded by a polynucleotide having at least 95% sequence homology to SEQ ID NO. 16.

28. An immune cell according to any one of Claims 23 to 27, wherein the mbIL15 is bicistronically expressed on the same polynucleotide as the chimeric receptor.

29. An immune cell according to any one of Claims 23 to 28, wherein mbIL15 comprises an amino acid sequence having at least 95% sequence homology to SEQ ID NO: 17.

30. An immune cell according to any one of Claims 23 to 29, wherein the effector domain further comprises an OX-40 domain.

31. The immune cell of Claim 30, wherein the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD8a transmembrane domain, the OX-40 domain, and the CD3zeta.

32. An immune cell according to any one of Claims 23 to 31, wherein the chimeric receptor is encoded by a nucleic acid sequence having at least 95% sequence homology to SEQ ID NO: 90 coupled to the nucleic acid sequence encoding mbIL15 and having at least 95% sequence homology to SEQ ID NO. 16.

33. An immune cell according to any one of Claims 23 to 31, wherein the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 109 coupled to the mbIL15 comprising the amino acid sequence of SEQ ID NO. 17.

34. An immune cell according to any one of Claims 23 to 31, wherein the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 100 coupled to the mbIL15 encoded by SEQ ID NO. 16.

35. The immune cell of Claim 34, wherein the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 101 coupled to the mbIL15 comprising the amino acid sequence of SEQ ID NO. 17.

36. An immune cell according to any one of Claims 23 to 35, wherein the immune cell is a Natural Killer (NK) cell.

37. An immune cell according to any one of Claims 23 to 35, wherein the immune cell is a T cell.

38. A method of generating a population of immune cells expressing a chimeric receptor for the treatment of cancer, the method comprising:

delivering to a population of immune cells a vector comprising a polynucleotide encoding the chimeric receptor, the chimeric receptor comprising:

an extracellular receptor domain, wherein said extracellular receptor domain comprises a peptide that binds native ligands of Natural Killer Group 2 member D (NKG2D),

wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D, and

wherein the fragment of NKG2D is encoded by a polynucleotide: (i) comprising a fragment of SEQ ID NO: 1, (ii) having at least 95% sequence homology to SEQ ID NO. 2, or (iii) having at least 95% sequence homology to SEQ ID NO. 3, and

an effector domain comprising a transmembrane region and an intracellular signaling domain, wherein the intracellular signaling domain comprises CD3zeta, thereby generating a population of immune cells expressing the chimeric receptor.

39. A method of treating a cancer using immunotherapy, the method comprising:

administering to a subject having the cancer a population of immune cells that express a chimeric receptor, the chimeric receptor comprising:

an extracellular receptor domain, wherein said extracellular receptor domain comprises a peptide that binds native ligands of Natural Killer Group 2 member D (NKG2D),

wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D, and

wherein the fragment of NKG2D is encoded by a polynucleotide: (i) comprising a fragment of SEQ ID NO: 1, (ii) having at least 95% sequence homology to SEQ ID NO. 2, or (iii) having at least 95% sequence homology to SEQ ID NO. 3, and

an effector domain comprising a transmembrane region and an intracellular signaling domain, wherein the intracellular signaling domain comprises a CD3zeta subdomain and an OX-40 subdomain.

40. The method of Claim 38, further comprising expanding the population of immune cells in culture to a population size suitable for a desired dose for the treatment of cancer.

41. The method of Claim 38, 39 or 40, wherein the CD3zeta is encoded by a polynucleotide having at least 95% sequence homology to SEQ ID NO. 13.

42. A method according to any one of Claims 38 to 41, wherein the polynucleotide further encodes a membrane-bound interleukin 15 (mbIL15) or wherein the immune cells express mbIL15.

43. The method of Claim 42, wherein the mbIL15 is encoded by a sequence having at least 95% sequence homology to SEQ ID NO. 16.

44. A method according to any one of Claims 38 to 43, wherein the fragment of NKG2D is encoded by a polynucleotide having at least 95% sequence homology to SEQ ID NO. 2.

45. A method according to any one of Claims 38 to 44, wherein the intracellular signaling domain further comprises an OX-40 domain.

46. A method according to any one of Claims 38 to 45, wherein the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD8a transmembrane domain, OX40, and CD3zeta.

47. A method according to any one of Claims 38 to 46, wherein the chimeric receptor is encoded by a nucleic acid sequence having at least 95% sequence homology to SEQ ID NO: 90.

48. A method according to any one of Claims 38 to 47, wherein the chimeric receptor comprises an amino acid sequence having at least 95% sequence homology to SEQ ID NO: 109.

49. The method of Claim 38, wherein the vector is a retroviral vector.

50. A method according to any one of Claims 38 to 49, wherein population of immune cells comprises natural killer cells.

51. A method according to any one of Claims 38 to 50, wherein the immune cells are allogeneic with respect to a recipient having cancer.

52. Use of a polynucleotide encoding a chimeric receptor for the treatment of cancer, the chimeric receptor comprising:

(a) an extracellular receptor domain,

wherein said extracellular receptor domain comprises a peptide that binds native ligands of Natural Killer Group 2 member D (NKG2D),

wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D,

wherein the fragment of NKG2D is encoded by a polynucleotide: (i) comprising a fragment of SEQ ID NO: 1, (ii) having at least 95% sequence homology to SEQ ID NO. 2, or (iii) having at least 95% sequence homology to SEQ ID NO. 3; and

(b) an effector domain comprising a transmembrane region and an intracellular signaling domain, and

wherein the intracellular signaling domain comprises CD3zeta, and

wherein the CD3zeta is encoded by a polynucleotide having at least 95% sequence homology to SEQ ID NO. 13.

53. Use of a polynucleotide encoding a chimeric receptor in the manufacture of a medicament for the treatment of cancer, the chimeric receptor comprising:

an extracellular receptor domain, wherein said extracellular receptor domain comprises a peptide that binds native ligands of Natural Killer Group 2 member D (NKG2D),

wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D, and

wherein the fragment of NKG2D is encoded by a polynucleotide: (i) comprising a fragment of SEQ ID NO: 1, (ii) having at least 95% sequence homology to SEQ ID NO. 2, or (iii) having at least 95% sequence homology to SEQ ID NO. 3, and an effector domain comprising a transmembrane region and an intracellular signaling domain, wherein the intracellular signaling domain comprises a CD3zeta subdomain and an OX-40 subdomain.

54. Use of a population of immune cells that express a chimeric receptor in the manufacture of a medicament for the treatment of cancer, the chimeric receptor comprising:

an extracellular receptor domain, wherein said extracellular receptor domain comprises a peptide that binds native ligands of Natural Killer Group 2 member D (NKG2D),

wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D, and

wherein the fragment of NKG2D is encoded by a polynucleotide: (i) comprising a fragment of SEQ ID NO: 1, (ii) having at least 95% sequence homology to SEQ ID NO. 2, or (iii) having at least 95% sequence homology to SEQ ID NO. 3, and

an effector domain comprising a transmembrane region and an intracellular signaling domain, wherein the intracellular signaling domain comprises a CD3zeta subdomain and an OX-40 subdomain.

55. A use according to any one of Claims 52 to 54, wherein the polynucleotide further encodes a membrane-bound interleukin 15 (mbIL15) or the immune cells express mbIL15, wherein the mbIL15 is encoded by a sequence having at least 95% sequence homology to SEQ ID NO. 16.

56. A use according to any one of Claim 52 to 55, wherein the fragment of NKG2D is encoded by a polynucleotide having at least 95% sequence homology to SEQ ID NO. 2.

57. A use according to any one of Claims 52 to 56, wherein the chimeric receptor is encoded by a polynucleotide having at least 95% sequence homology to SEQ ID NO. 90.

58. A use according to any one of Claim 52 to 57, wherein the encoded chimeric receptor has at least 95% sequence homology to SEQ ID NO. 109.

59. A use according to any one of Claims 52 to 58, wherein the cancer is a leukemia.

60. The use of Claim 59, wherein the leukemia is acute myelogenous leukemia.

61. A use according to any one of Claims 52 to 58, wherein the cancer is a liver cancer.

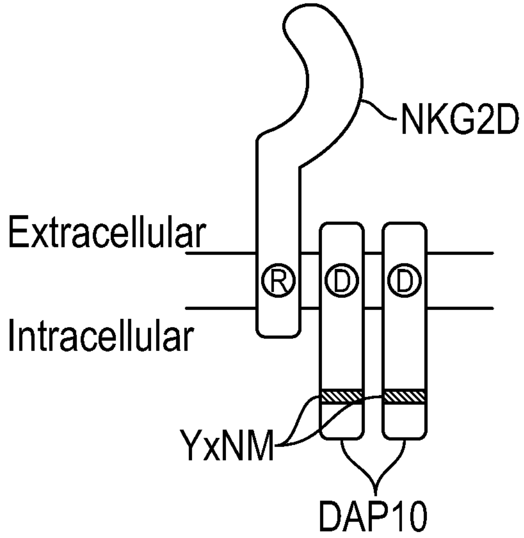

FIG. 1A

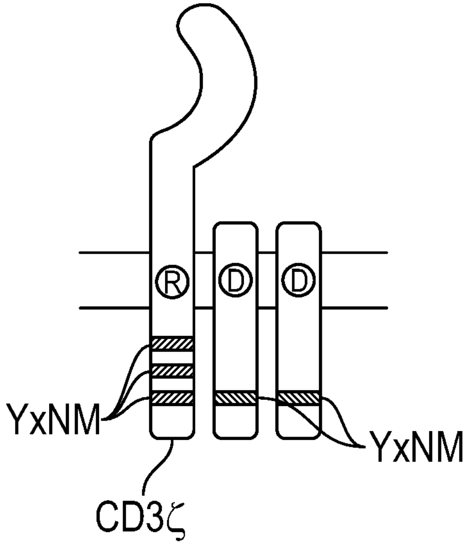

FIG. 1B

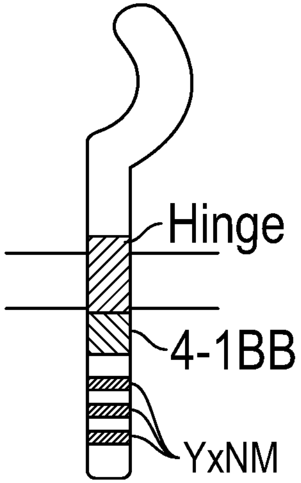

FIG. 1C

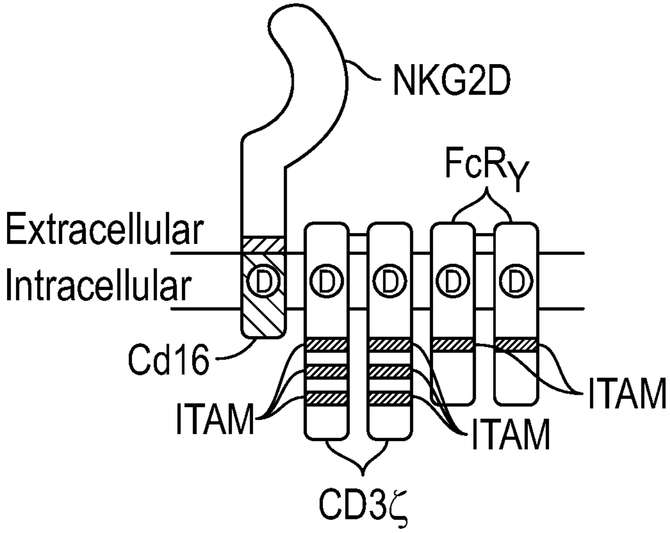

FIG. 2A

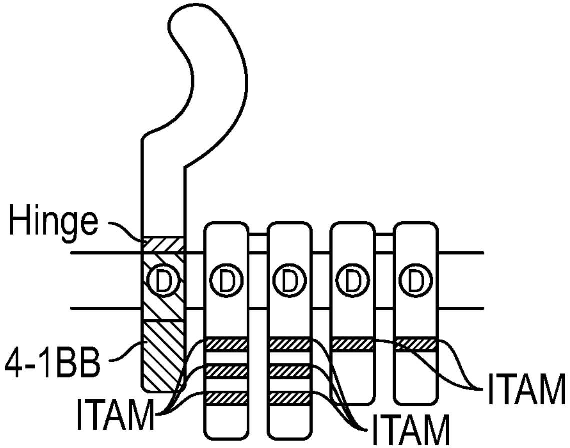

FIG. 2B

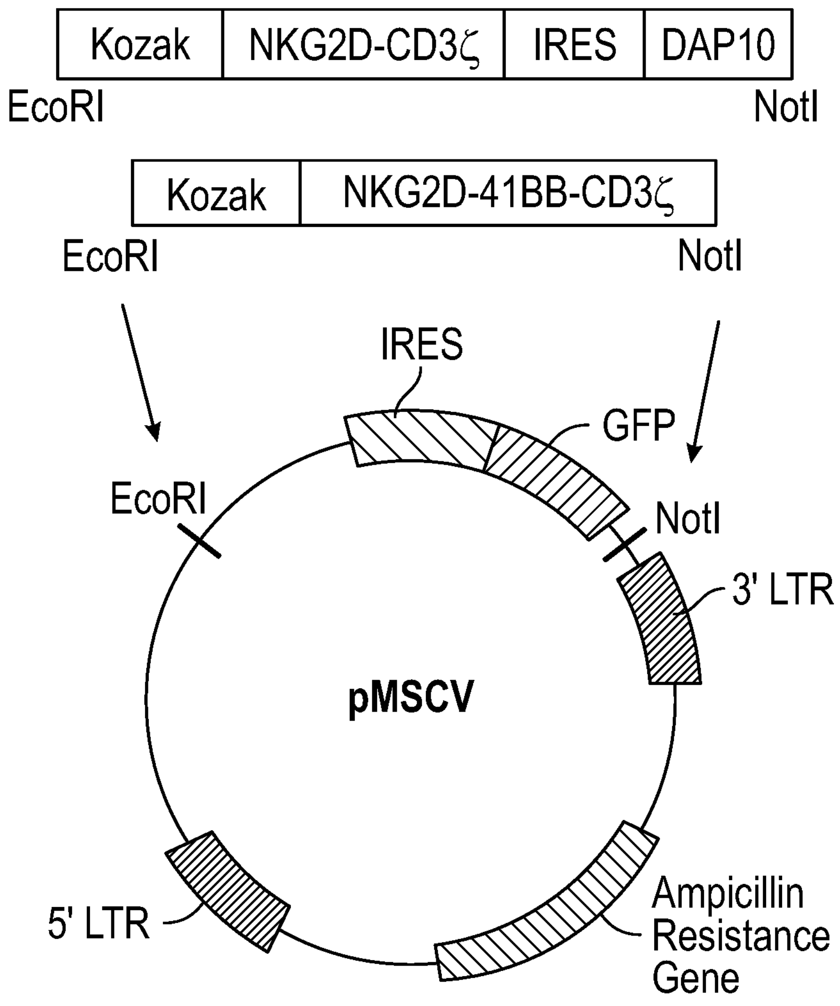
Kozak
NKG2D-CD3ζ
IRES
DAP10
EcoRI
NotI
Kozak
NKG2D-41BB-CD3ζ
EcoRI
NotI
IRES
GFP
EcoRI
NotI
3' LTR
pMSCV
5' LTR
Ampicillin Resistance Gene

FIG. 3A

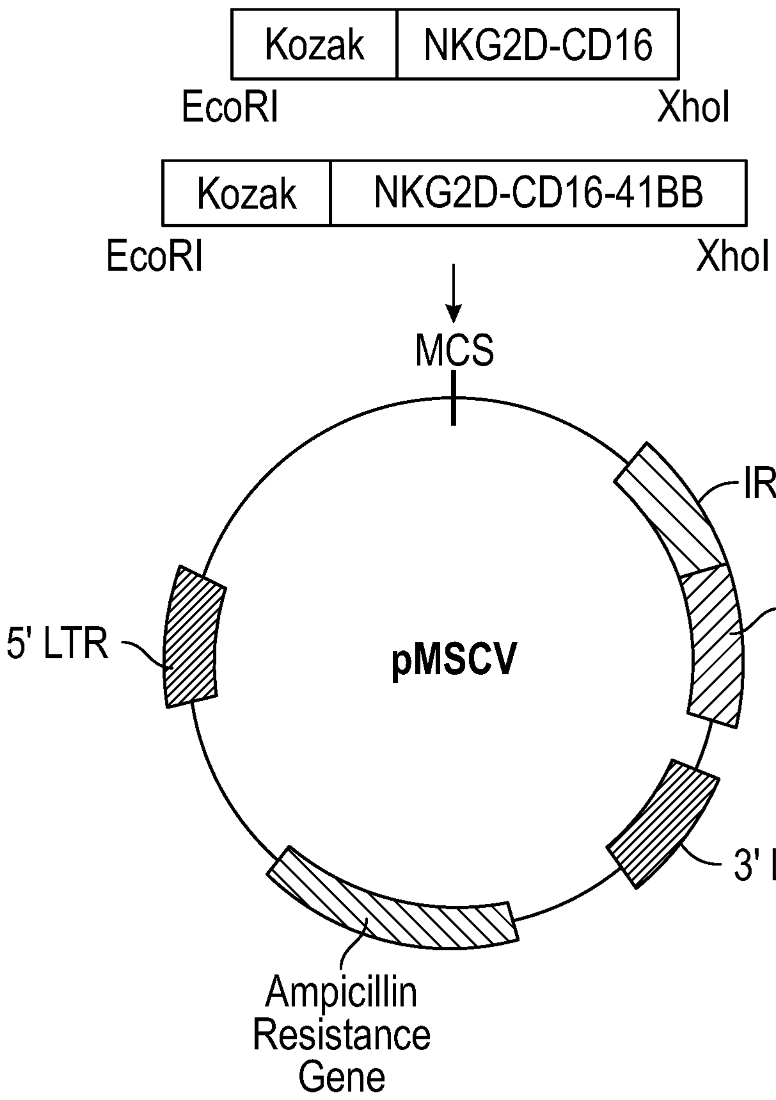
Kozak
NKG2D-CD16
EcoRI
XhoI
Kozak
NKG2D-CD16-41BB
EcoRI
XhoI
MCS
IRES
GFP
5' LTR
pMSCV
3' LTR
Ampicillin Resistance Gene

FIG. 3B

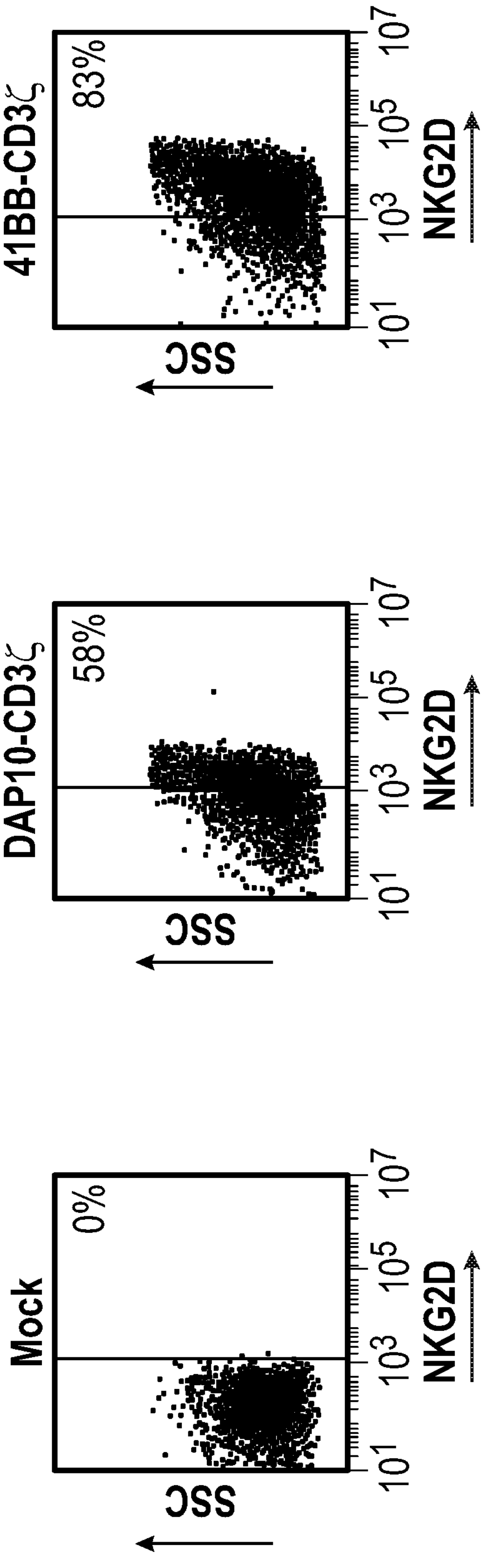
Mock
0%
NKG2D-DAP10-CD3ζ
58%
NKG2D-41BB-CD3ζ
83%
SSC
NKG2D
10^1
10^3
10^5
10^7

FIG. 4A

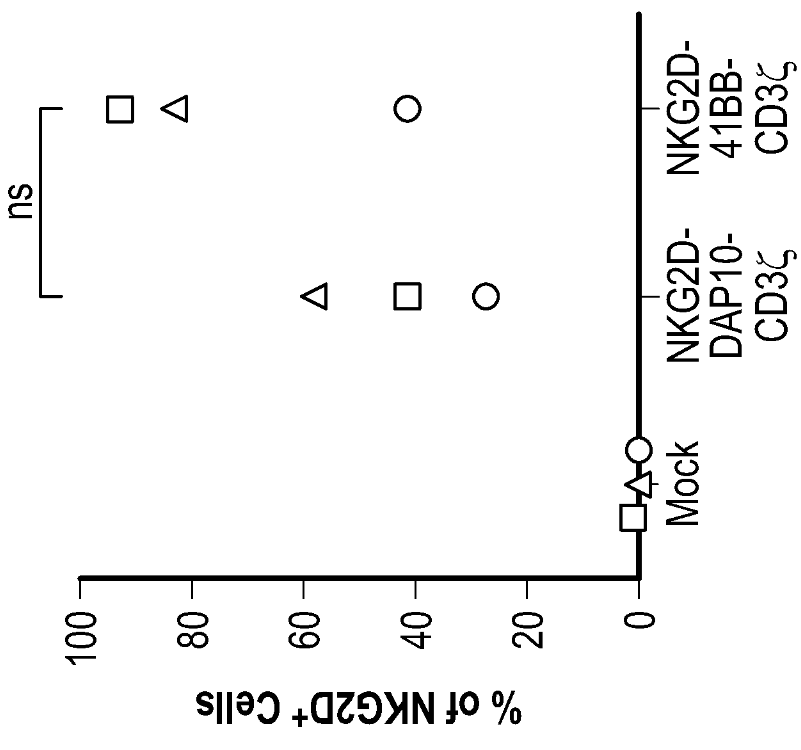
% of NKG2D+ Cells
100
80
60
40
20
0
Mock
NKG2D-DAP10-CD3ζ
NKG2D-41BB-CD3ζ
ns

FIG. 4B

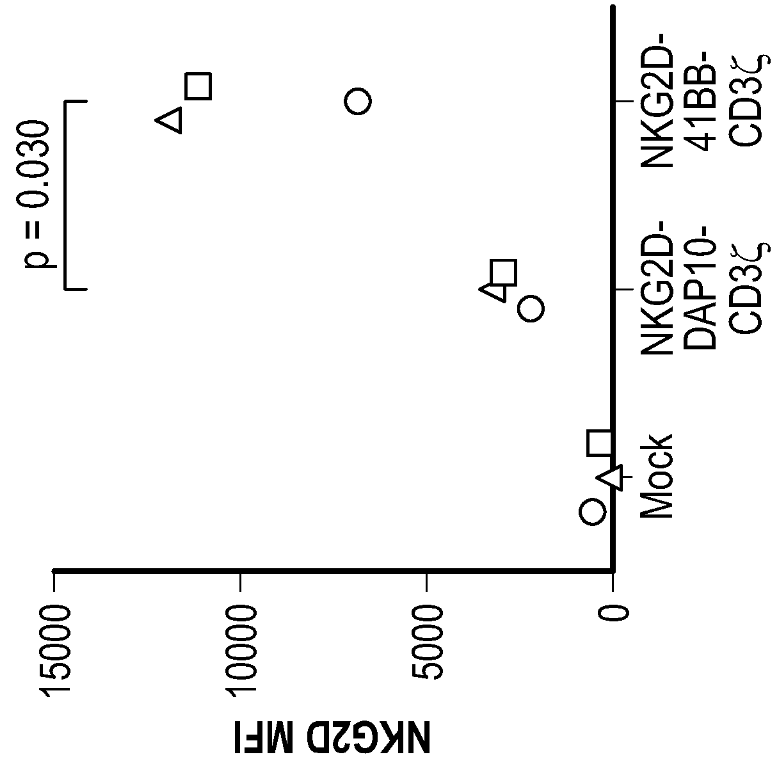
NKG2D MFI
15000
10000
5000
0
Mock
NKG2D-DAP10-CD3ζ
NKG2D-41BB-CD3ζ
p = 0.030

FIG. 4C

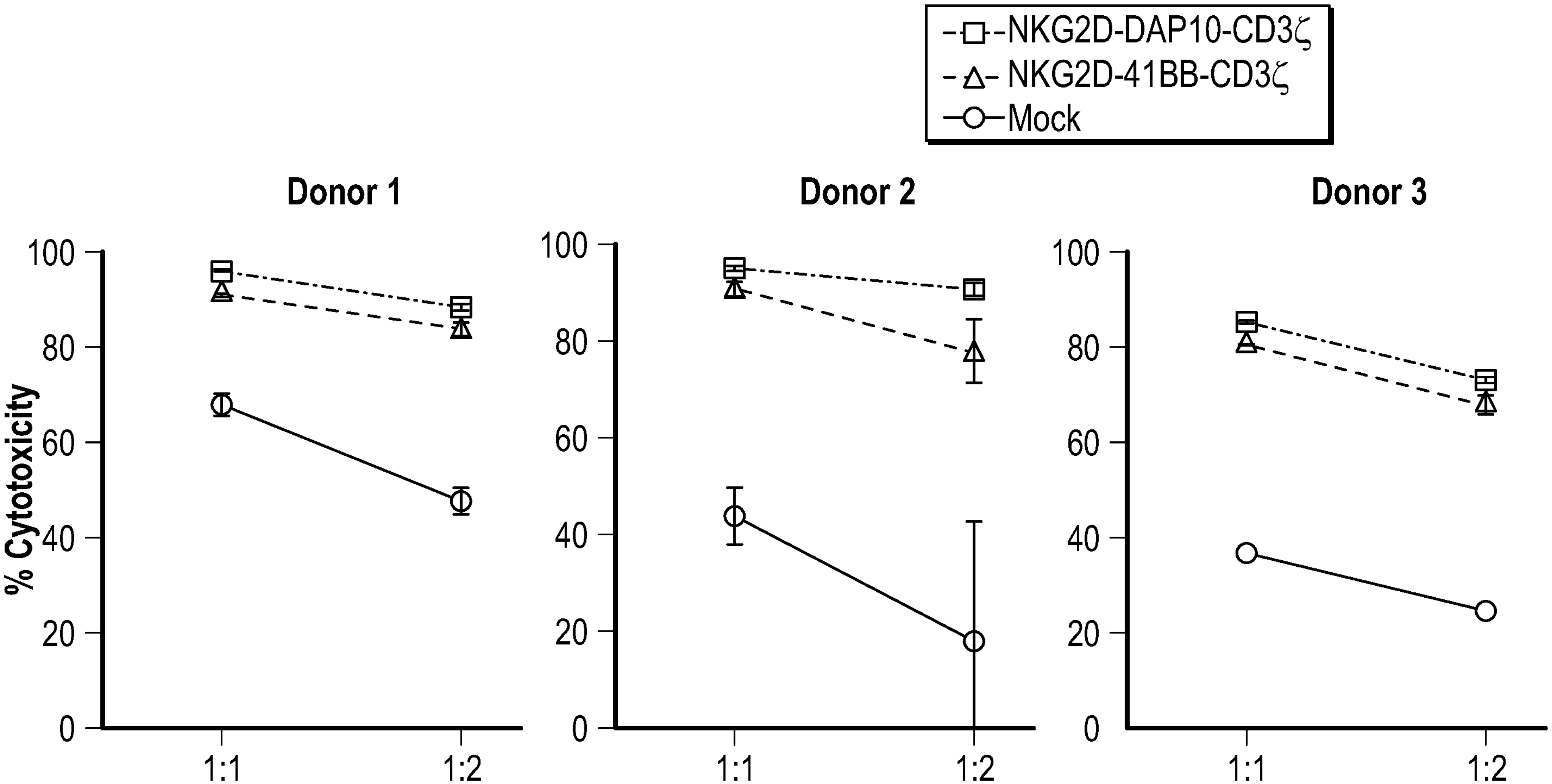

FIG. 5A

FIG. 5B

FIG. 5C

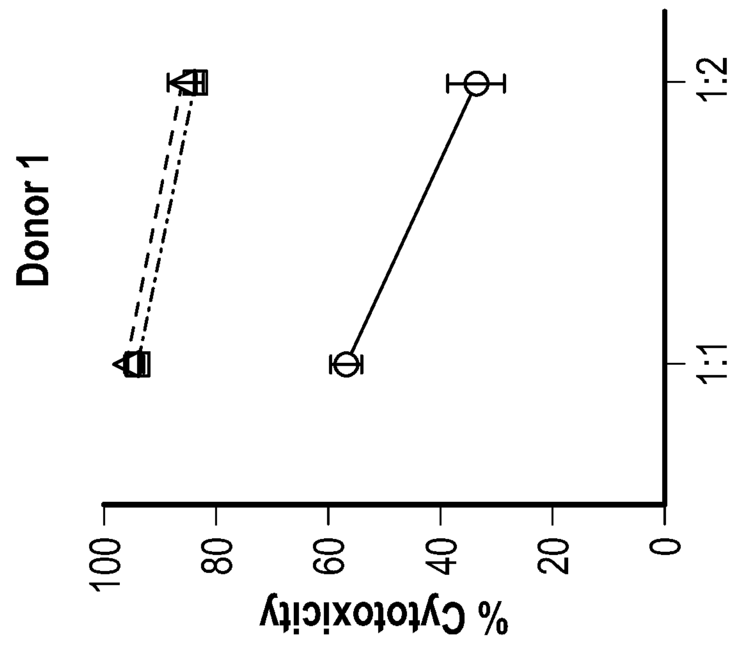

FIG. 6A

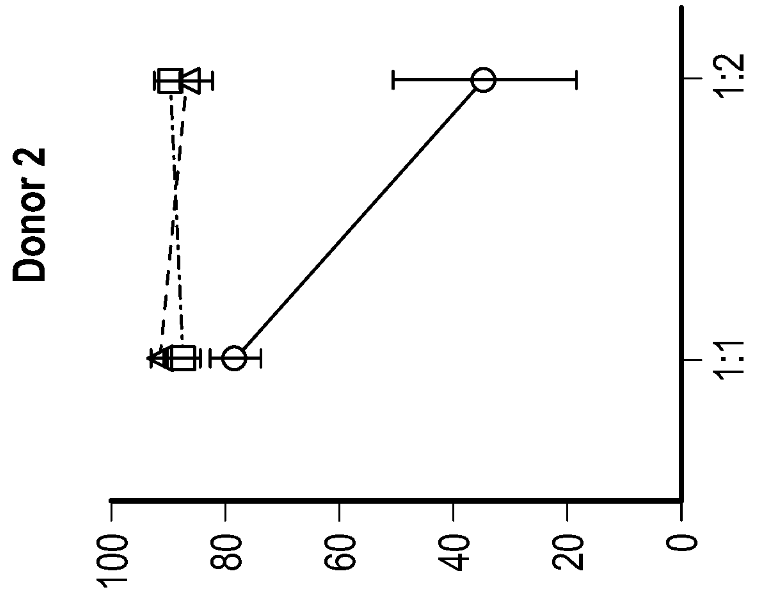

FIG. 6B

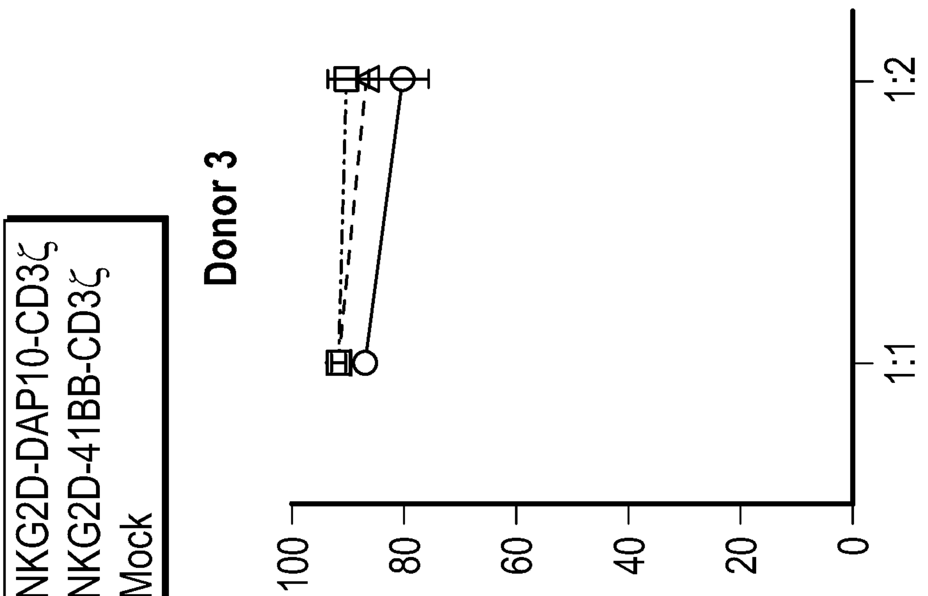

FIG. 6C

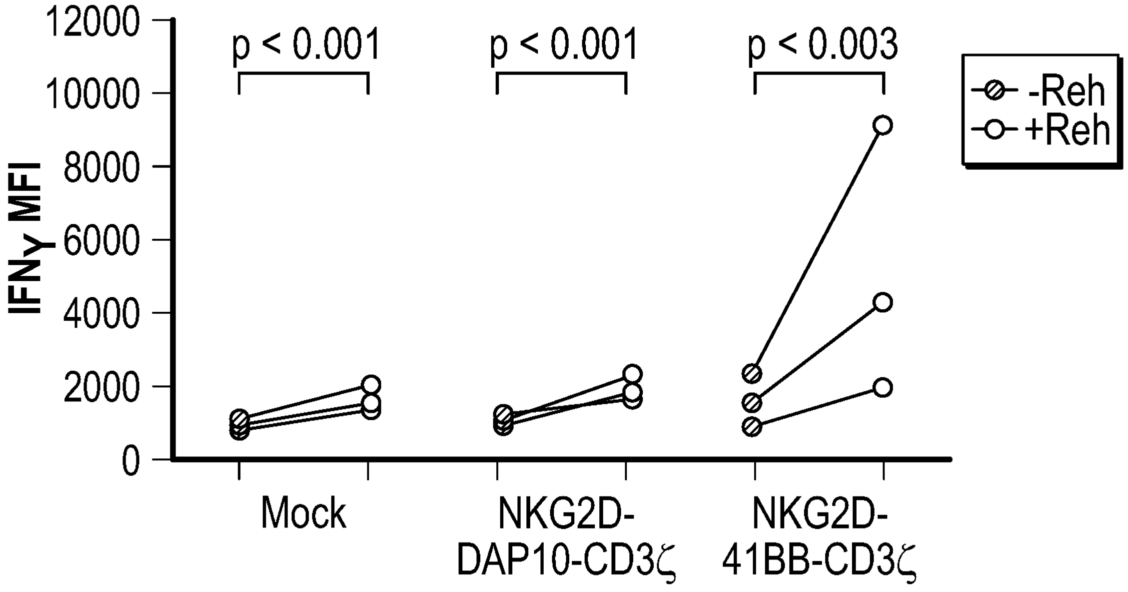
IFNγ MFI
12000
10000
8000
6000
4000
2000
0
p < 0.001
p < 0.001
p < 0.003
-Reh
+Reh
Mock
NKG2D-DAP10-CD3ζ
NKG2D-41BB-CD3ζ

FIG. 7A

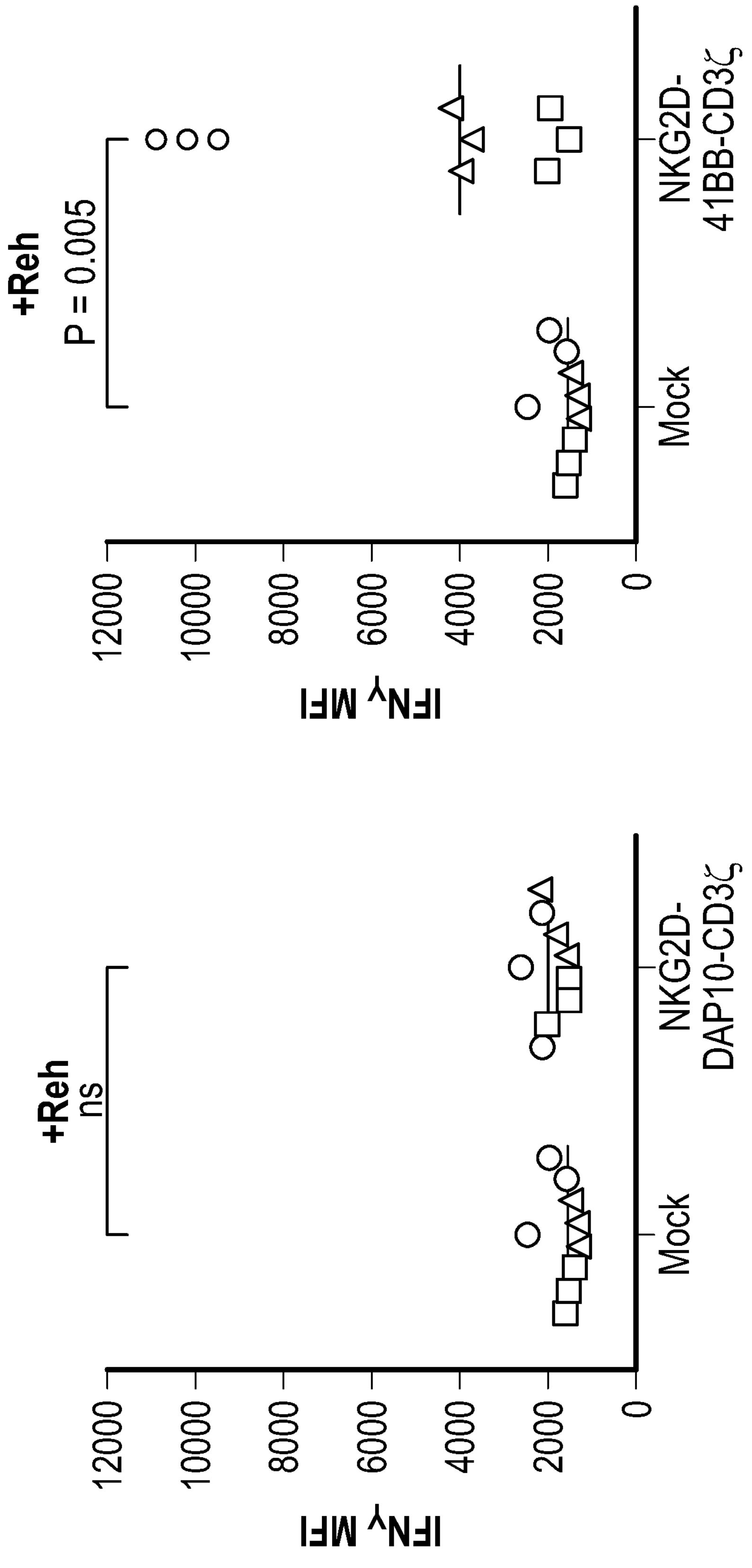
+Reh
P = 0.005
IFNγ MFI
12000
10000
8000
6000
4000
2000
0
Mock
NKG2D-41BB-CD3ζ
+Reh
ns
IFNγ MFI
12000
10000
8000
6000
4000
2000
0
Mock
NKG2D-DAP10-CD3ζ

FIG. 7B

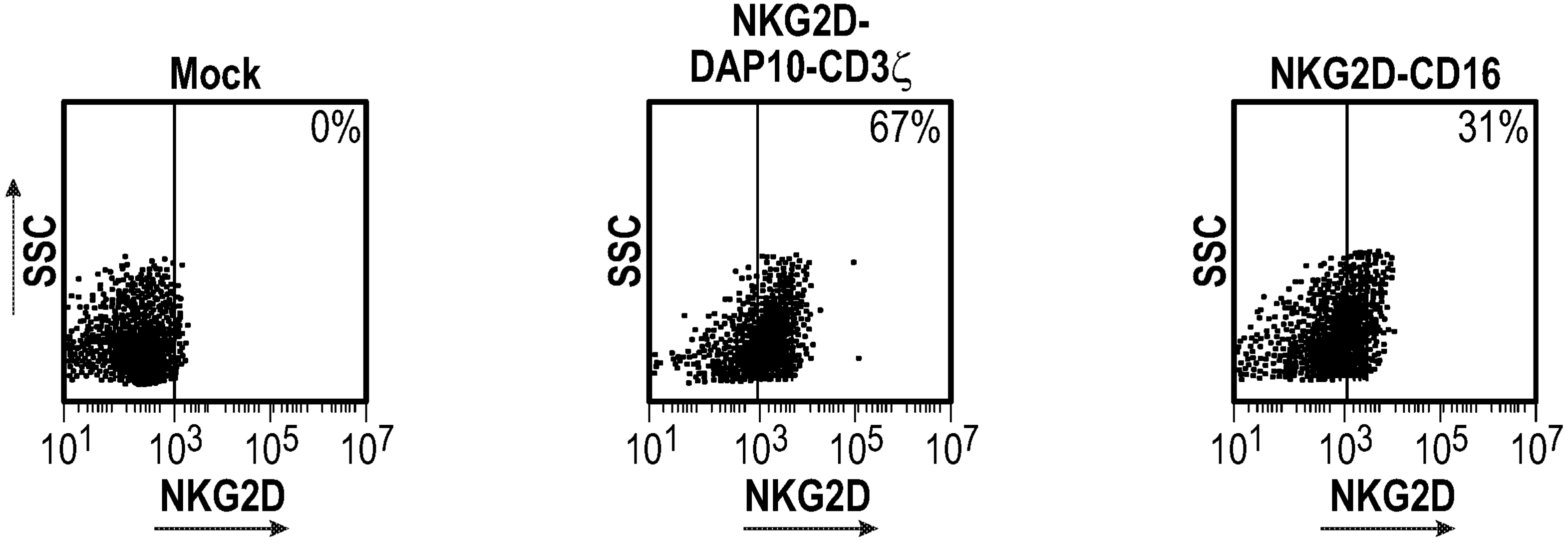
Mock
0%
SSC
10^1
10^3
10^5
10^7
NKG2D
NKG2D-
DAP10-CD3ζ
67%
SSC
10^1
10^3
10^5
10^7
NKG2D
NKG2D-CD16
31%
SSC
10^1
10^3
10^5
10^7
NKG2D

FIG. 8A

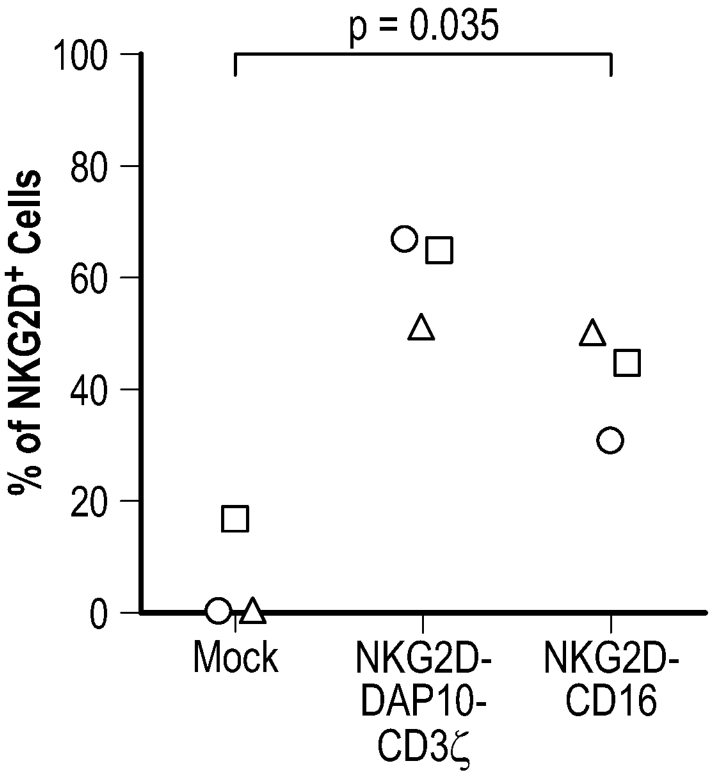
p = 0.035
% of NKG2D⁺ Cells
100
80
60
40
20
0
Mock
NKG2D-DAP10-CD3ζ
NKG2D-CD16

FIG. 8B

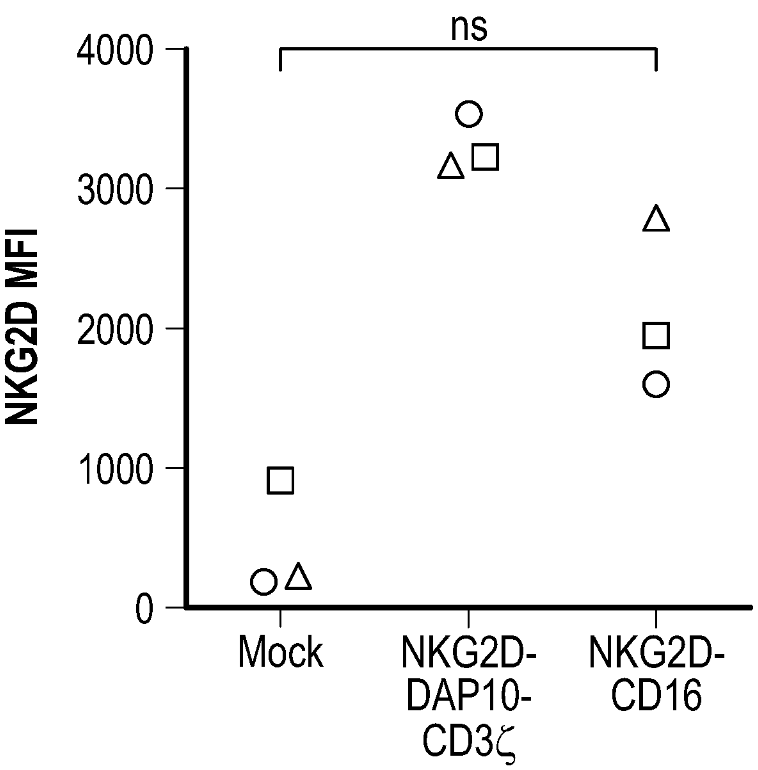
ns
NKG2D MFI
4000
3000
2000
1000
0
Mock
NKG2D-DAP10-CD3ζ
NKG2D-CD16

FIG. 8C

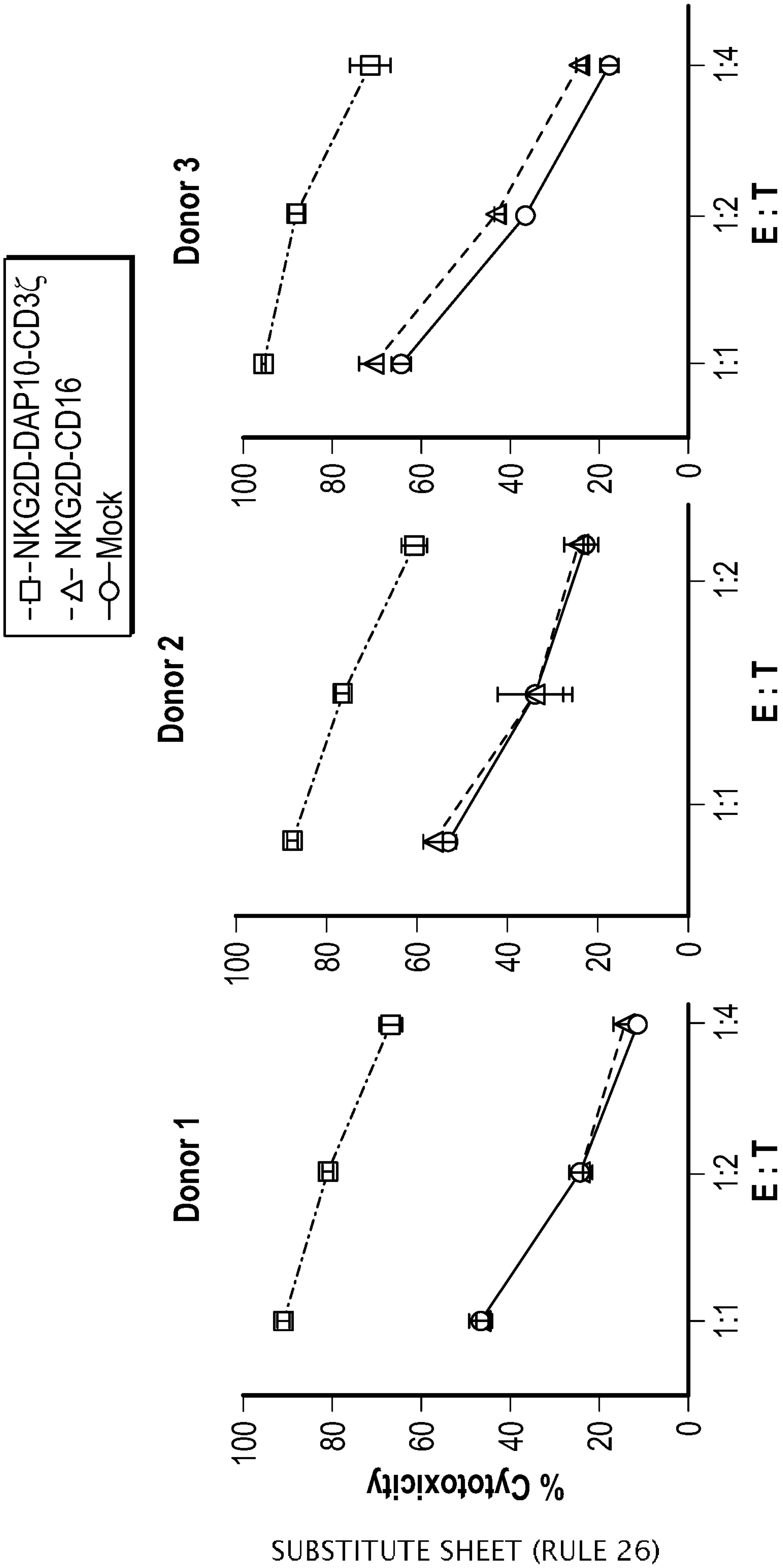

FIG. 9A

FIG. 9B

FIG. 9C

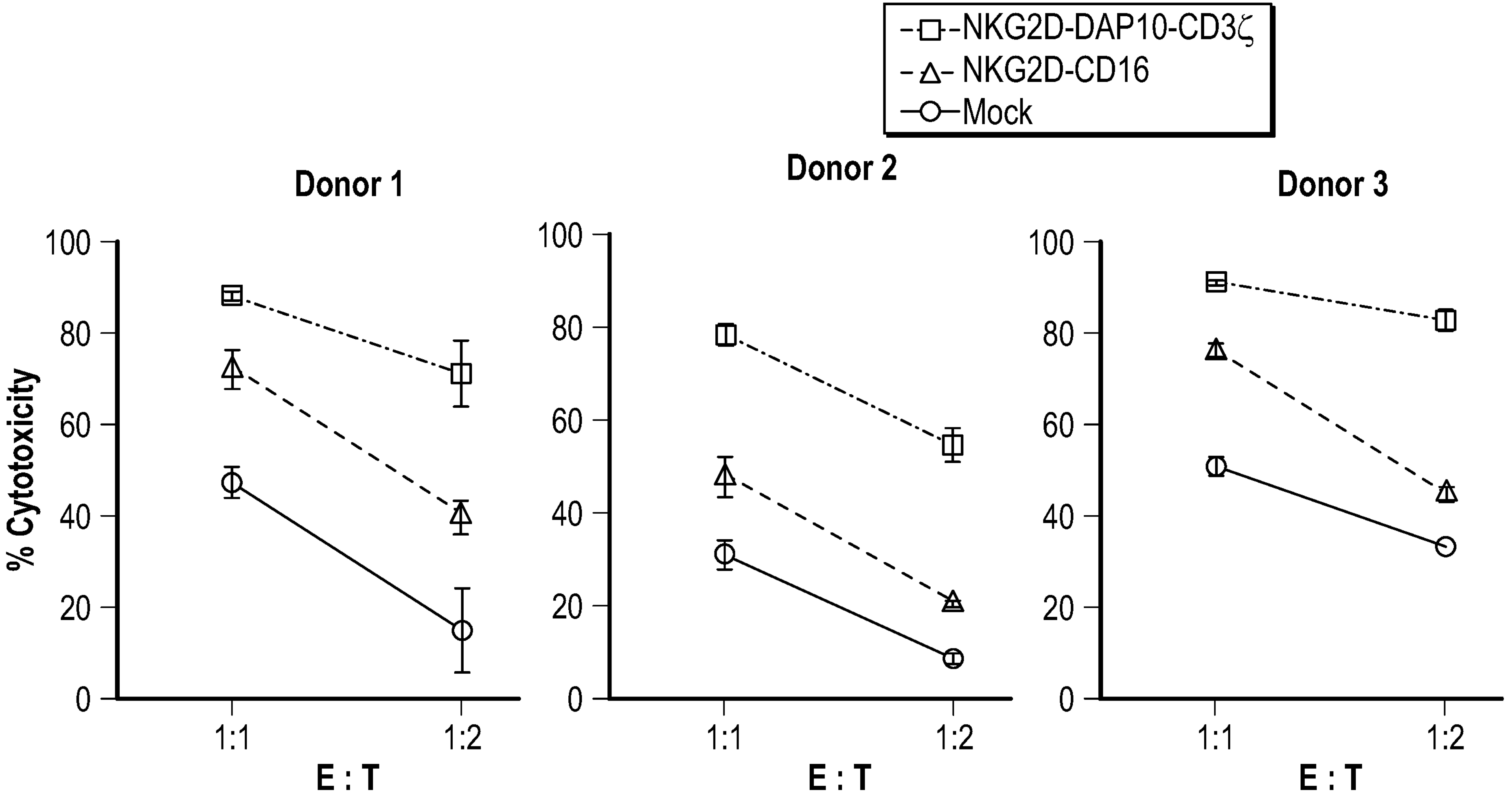

FIG. 10A

FIG. 10B

FIG. 10C

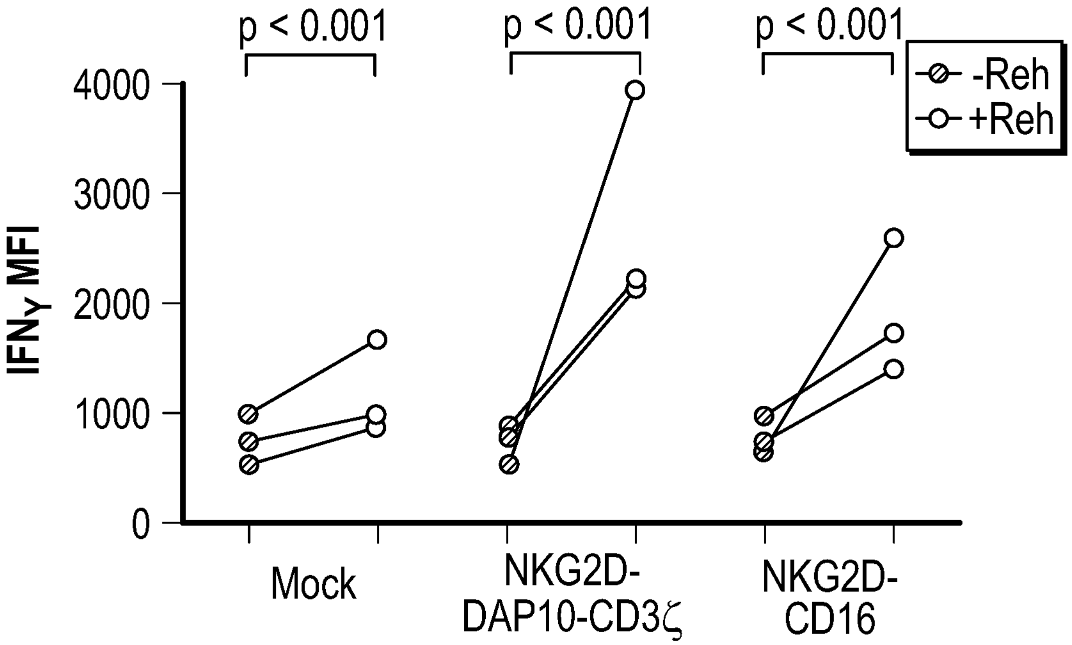
p < 0.001
p < 0.001
p < 0.001
-Reh
+Reh
IFNγ MFI
4000
3000
2000
1000
0
Mock
NKG2D-DAP10-CD3ζ
NKG2D-CD16

FIG. 11

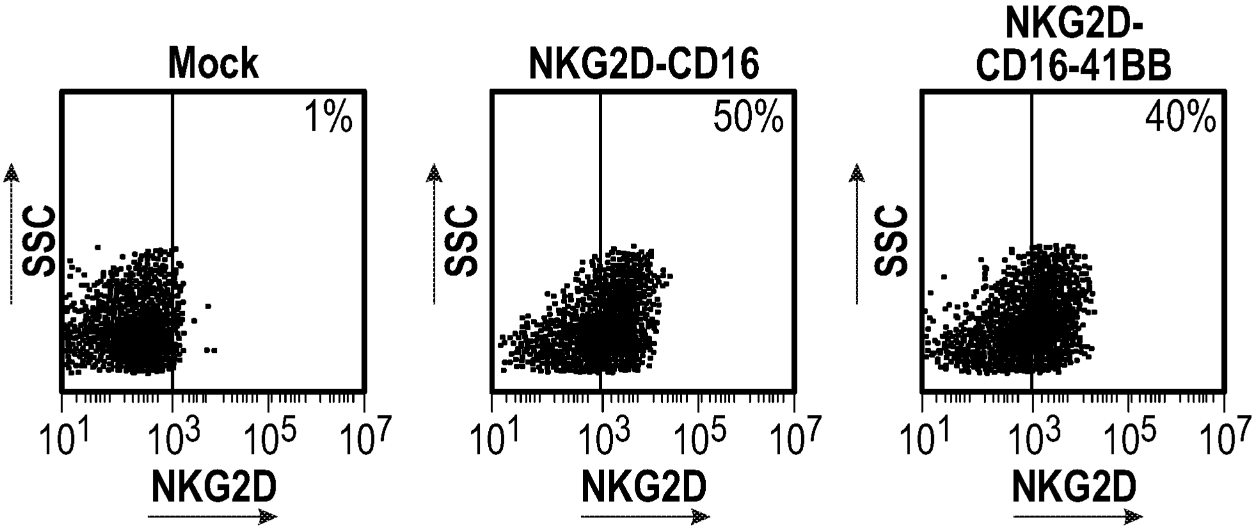

FIG. 12A

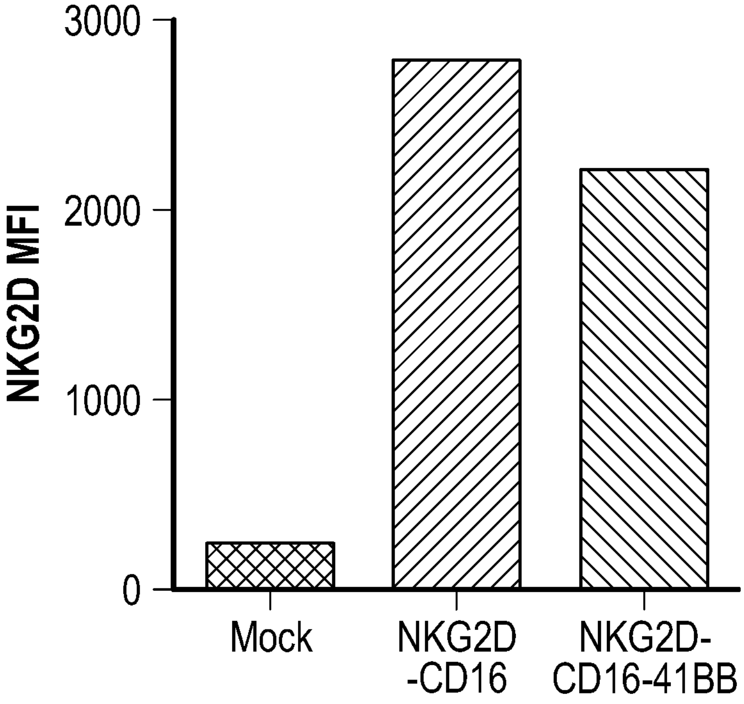

FIG. 12B

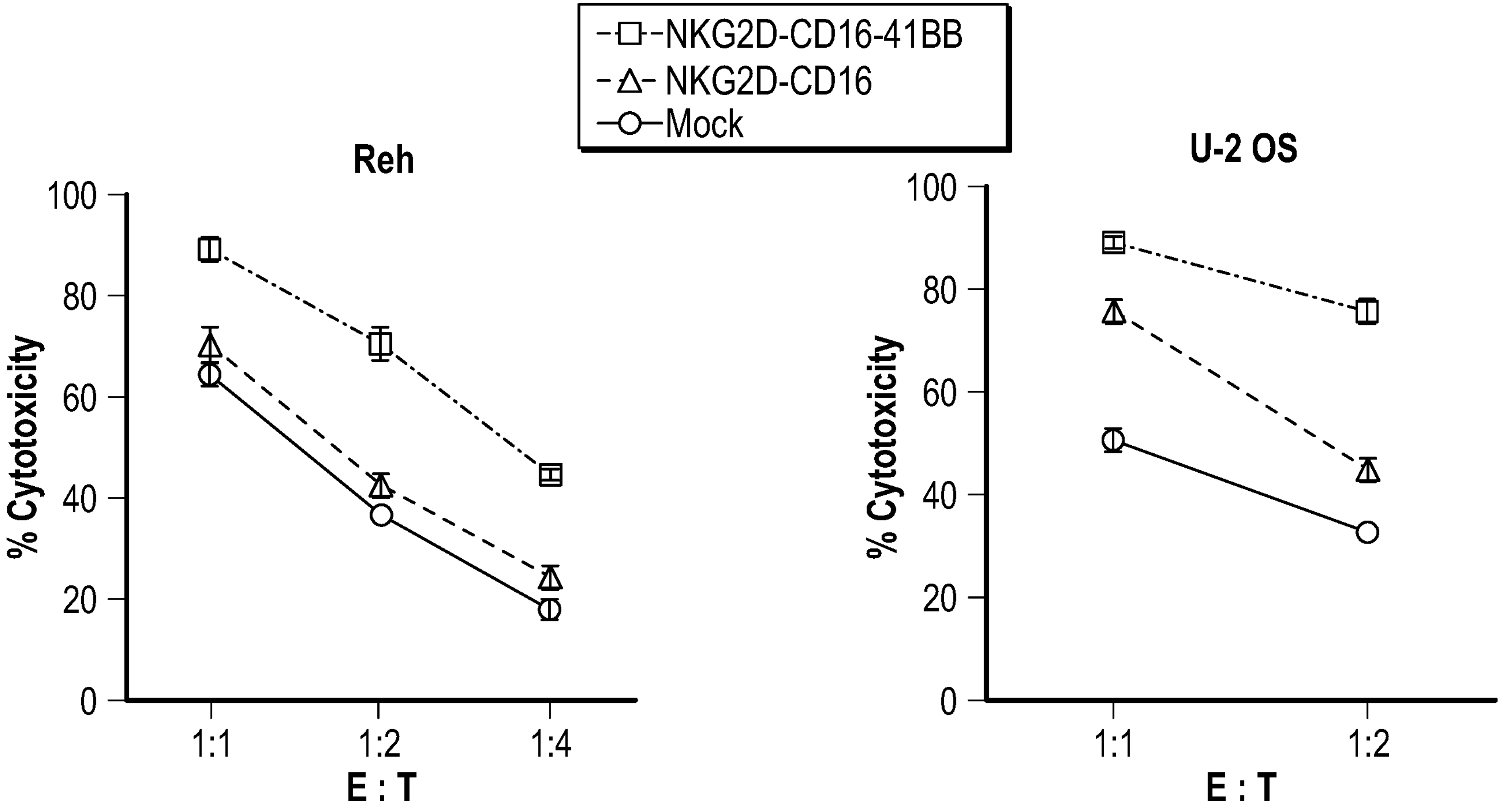

FIG. 13A

FIG. 13B

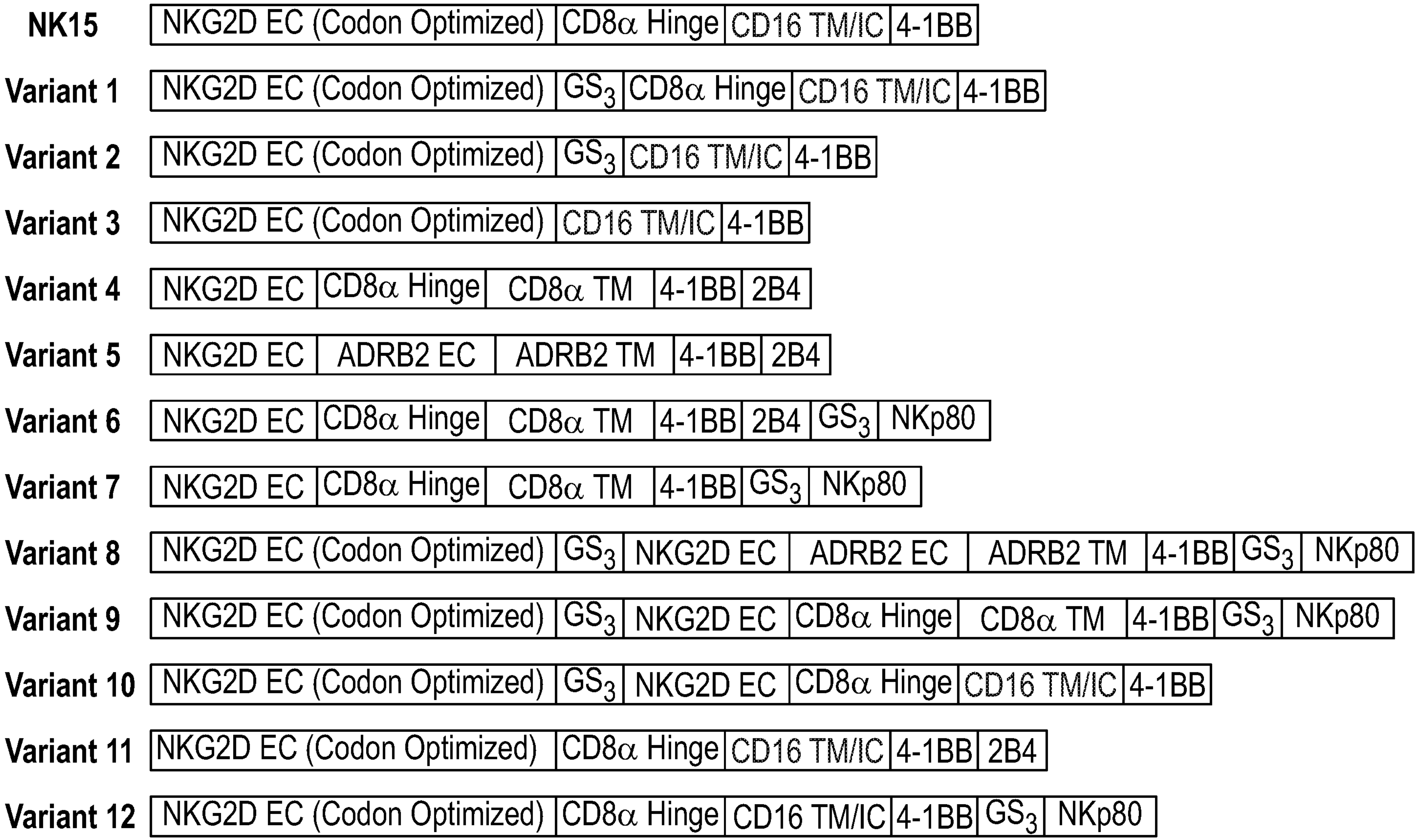
NK15 | NKG2D EC (Codon Optimized) | CD8α Hinge | CD16 TM/IC | 4-1BB
Variant 1 | NKG2D EC (Codon Optimized) | GS$_3$ | CD8α Hinge | CD16 TM/IC | 4-1BB
Variant 2 | NKG2D EC (Codon Optimized) | GS$_3$ | CD16 TM/IC | 4-1BB
Variant 3 | NKG2D EC (Codon Optimized) | CD16 TM/IC | 4-1BB
Variant 4 | NKG2D EC | CD8α Hinge | CD8α TM | 4-1BB | 2B4
Variant 5 | NKG2D EC | ADRB2 EC | ADRB2 TM | 4-1BB | 2B4
Variant 6 | NKG2D EC | CD8α Hinge | CD8α TM | 4-1BB | 2B4 | GS$_3$ | NKp80
Variant 7 | NKG2D EC | CD8α Hinge | CD8α TM | 4-1BB | GS$_3$ | NKp80
Variant 8 | NKG2D EC (Codon Optimized) | GS$_3$ | NKG2D EC | ADRB2 EC | ADRB2 TM | 4-1BB | GS$_3$ | NKp80
Variant 9 | NKG2D EC (Codon Optimized) | GS$_3$ | NKG2D EC | CD8α Hinge | CD8α TM | 4-1BB | GS$_3$ | NKp80
Variant 10 | NKG2D EC (Codon Optimized) | GS$_3$ | NKG2D EC | CD8α Hinge | CD16 TM/IC | 4-1BB
Variant 11 | NKG2D EC (Codon Optimized) | CD8α Hinge | CD16 TM/IC | 4-1BB | 2B4
Variant 12 | NKG2D EC (Codon Optimized) | CD8α Hinge | CD16 TM/IC | 4-1BB | GS$_3$ | NKp80

FIG. 14

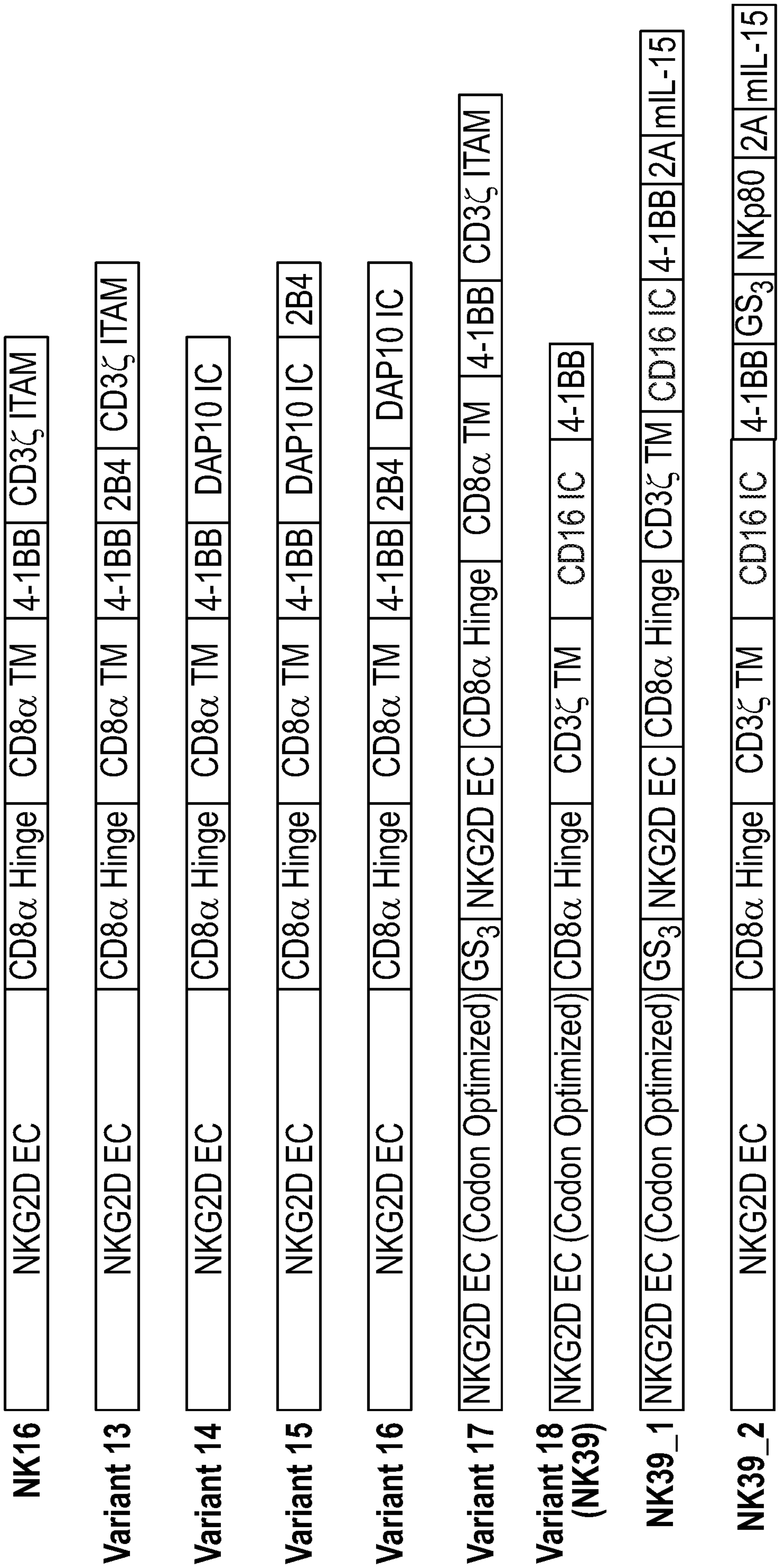
NK16
NKG2D EC
CD8α Hinge
CD8α TM
4-1BB
CD3ζ ITAM
Variant 13
NKG2D EC
CD8α Hinge
CD8α TM
4-1BB
2B4
CD3ζ ITAM
Variant 14
NKG2D EC
CD8α Hinge
CD8α TM
4-1BB
DAP10 IC
Variant 15
NKG2D EC
CD8α Hinge
CD8α TM
4-1BB
DAP10 IC
2B4
Variant 16
NKG2D EC
CD8α Hinge
CD8α TM
4-1BB
2B4
DAP10 IC
Variant 17
NKG2D EC (Codon Optimized)
GS_3
NKG2D EC
CD8α Hinge
CD8α TM
4-1BB
CD3ζ ITAM
Variant 18 (NK39)
NKG2D EC (Codon Optimized)
CD8α Hinge
CD3ζ TM
CD16 IC
4-1BB
NK39_1
NKG2D EC (Codon Optimized)
GS_3
NKG2D EC
CD8α Hinge
CD3ζ TM
CD16 IC
4-1BB
2A
mIL-15
NK39_2
NKG2D EC
CD8α Hinge
CD3ζ TM
CD16 IC
4-1BB
GS_3
NKp80
2A
mIL-15

FIG. 15

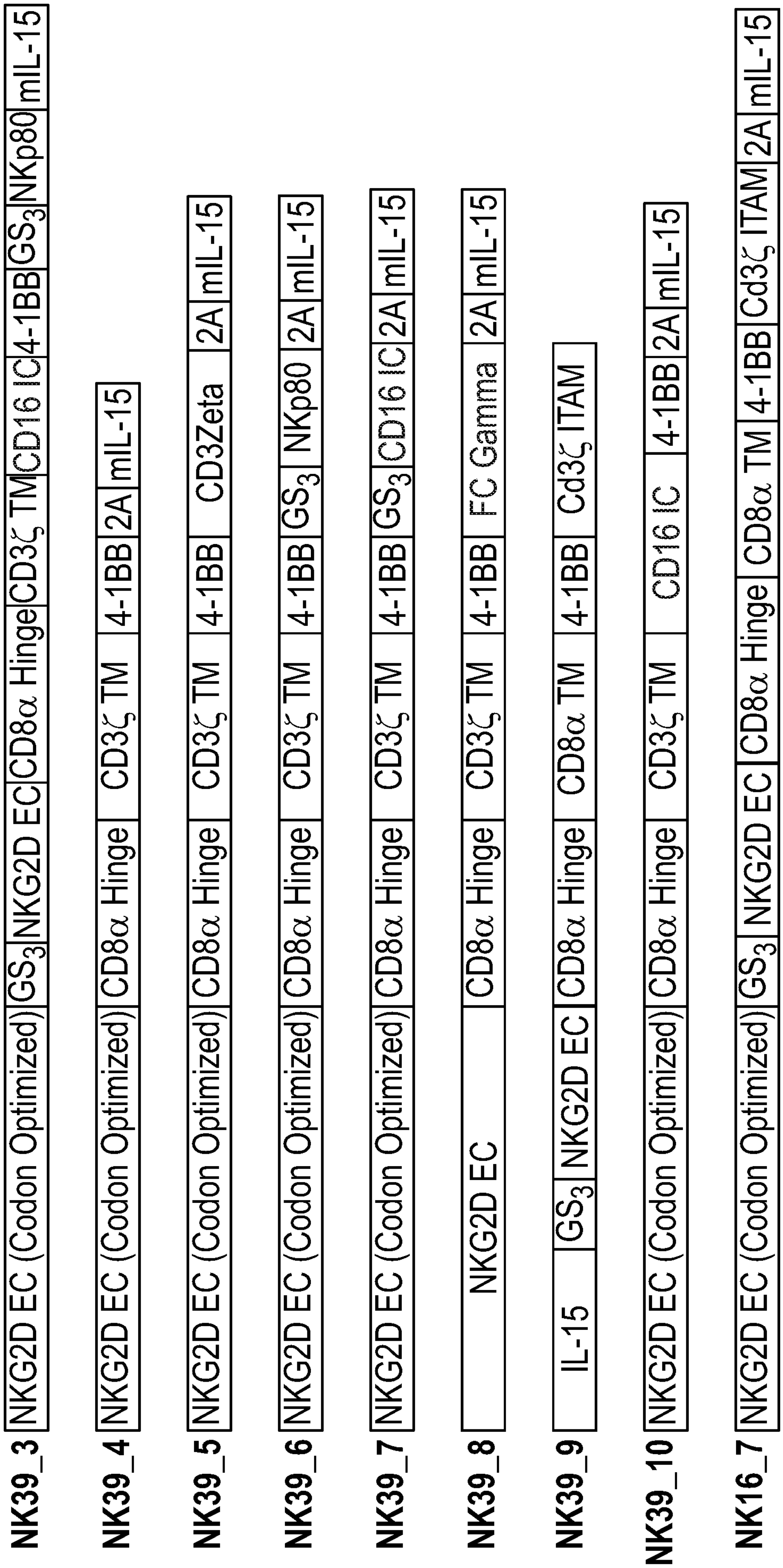

FIG. 15 (Continued)

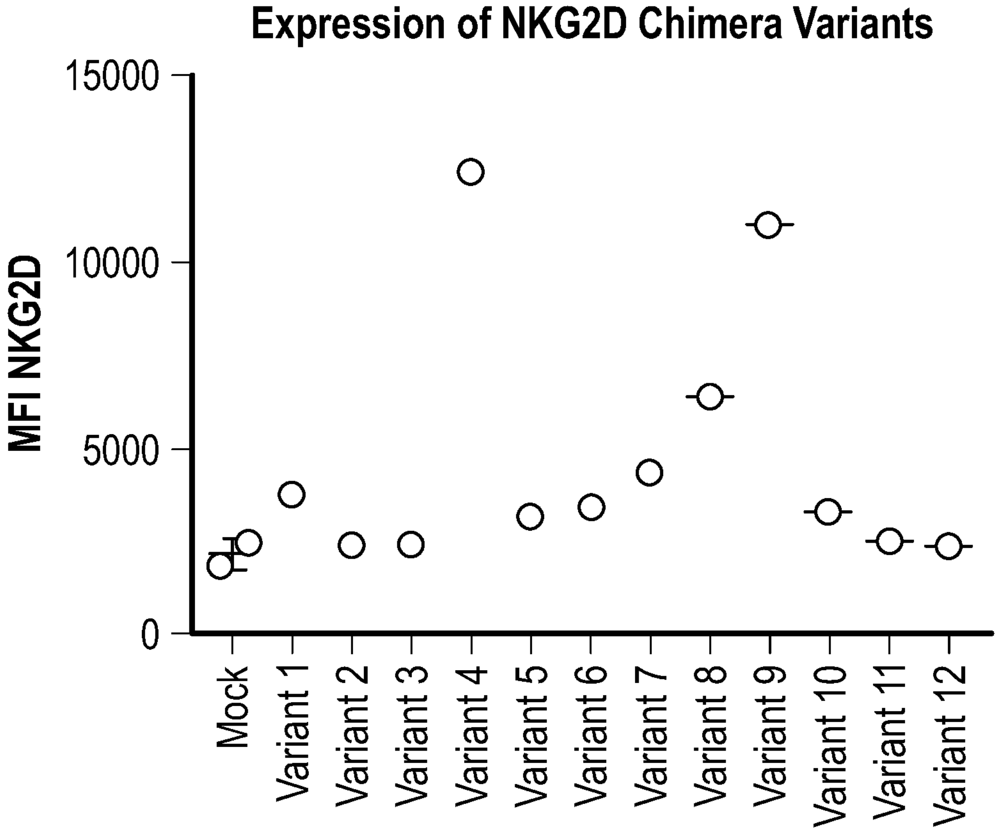
Expression of NKG2D Chimera Variants
15000
10000
5000
0
MFI NKG2D
Mock
Variant 1
Variant 2
Variant 3
Variant 4
Variant 5
Variant 6
Variant 7
Variant 8
Variant 9
Variant 10
Variant 11
Variant 12
Variant

FIG. 16A

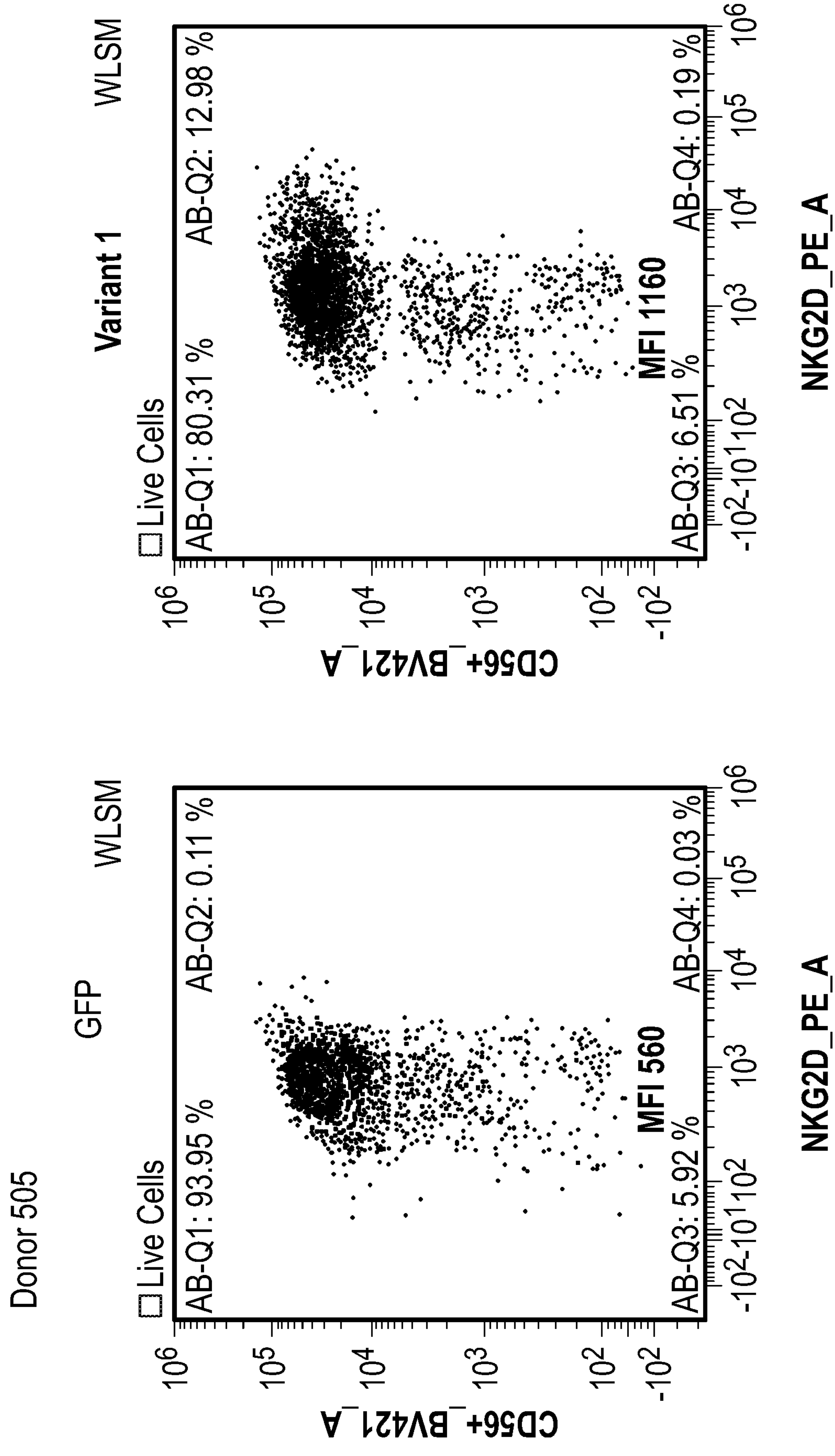
Donor 505
GFP
WLSM
□Live Cells
AB-Q1: 93.95 %
AB-Q2: 0.11 %
AB-Q3: 5.92 %
AB-Q4: 0.03 %
MFI 560
CD56+_BV421_A
NKG2D_PE_A
10^6
10^5
10^4
10^3
10^2
-10^2
-10^1
Variant 1
WLSM
□Live Cells
AB-Q1: 80.31 %
AB-Q2: 12.98 %
AB-Q3: 6.51 %
AB-Q4: 0.19 %
MFI 1160
CD56+_BV421_A
NKG2D_PE_A

FIG. 16B

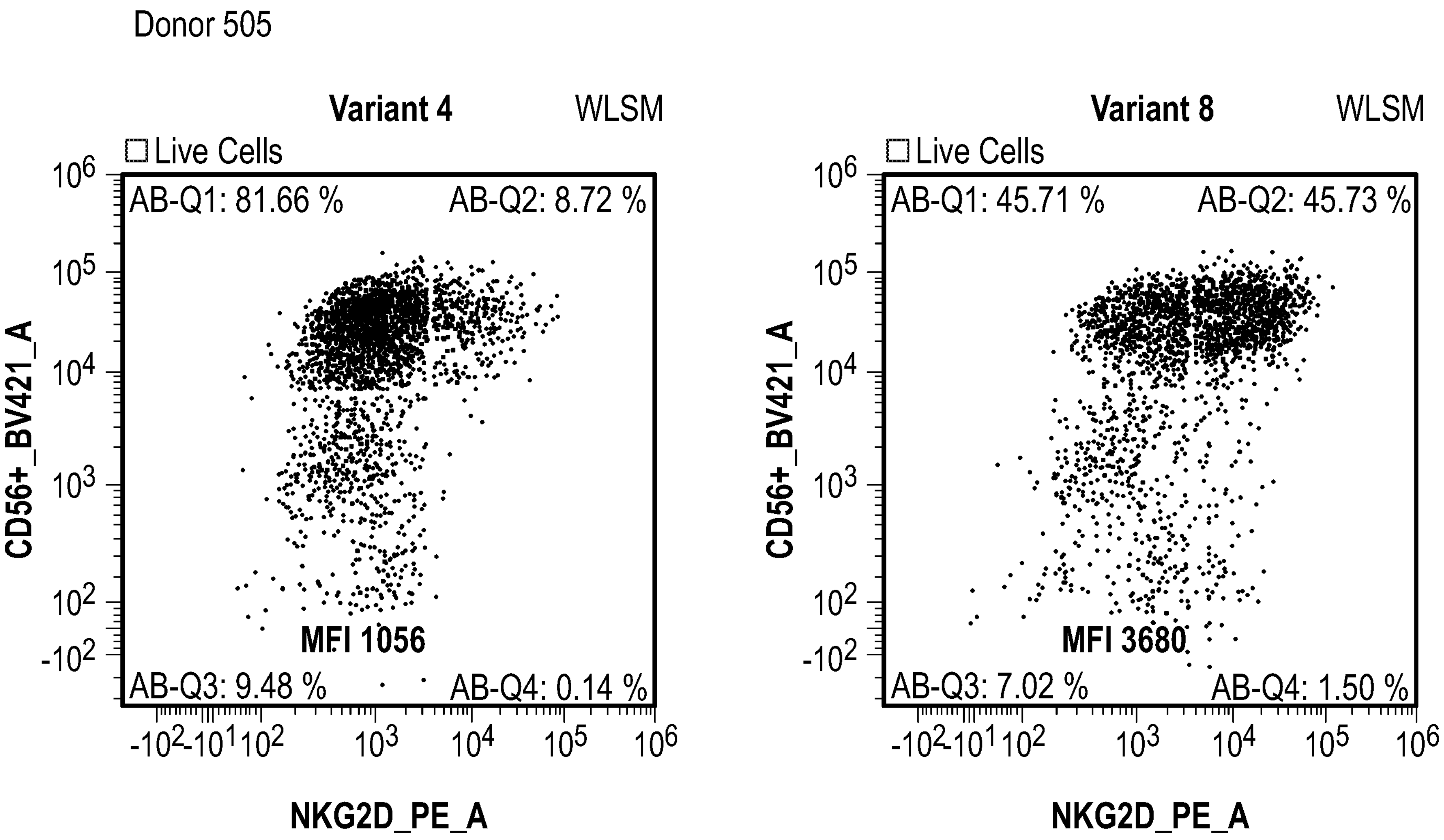

FIG. 16B
(Continued)

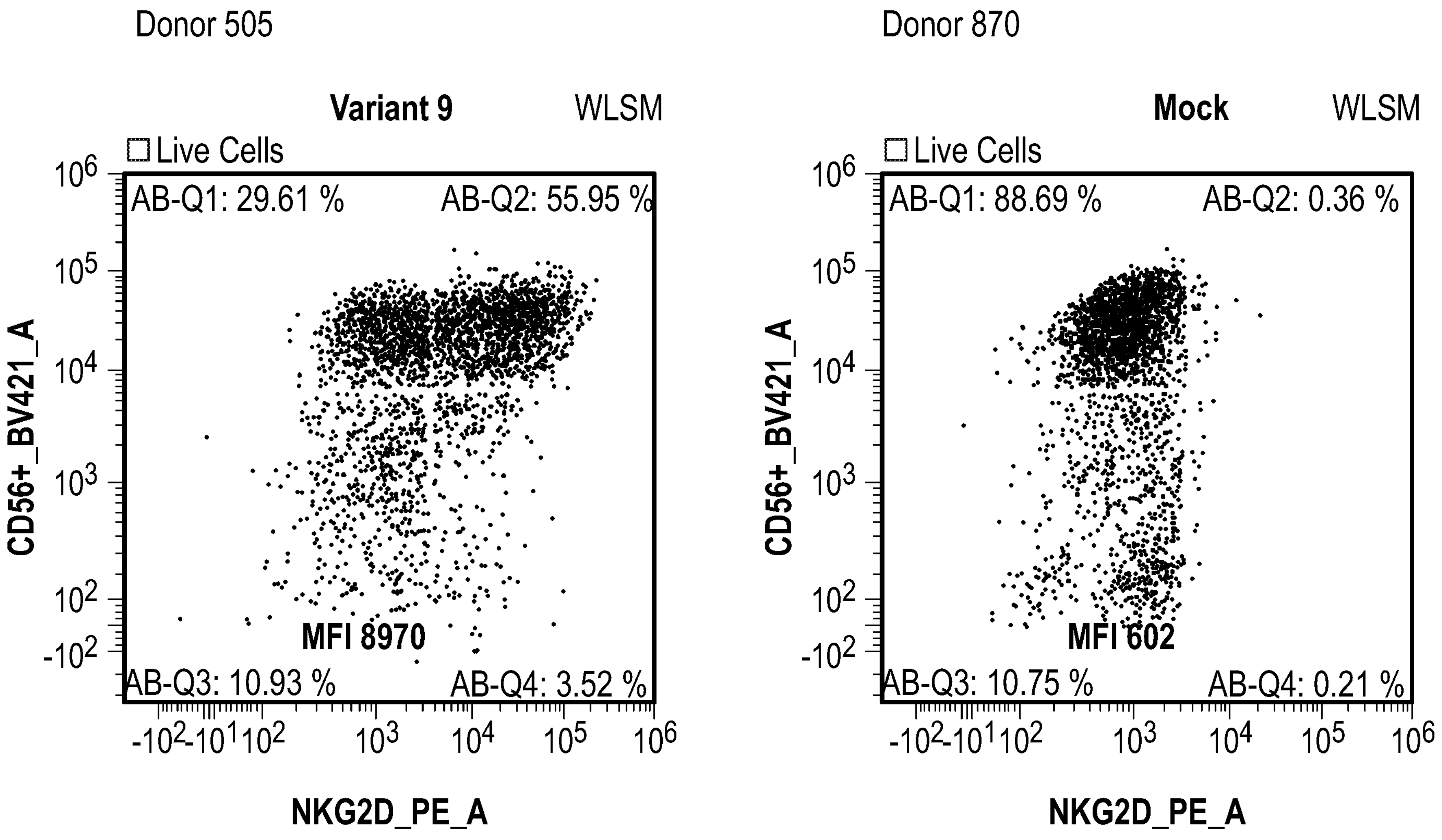

FIG. 16B
(Continued)

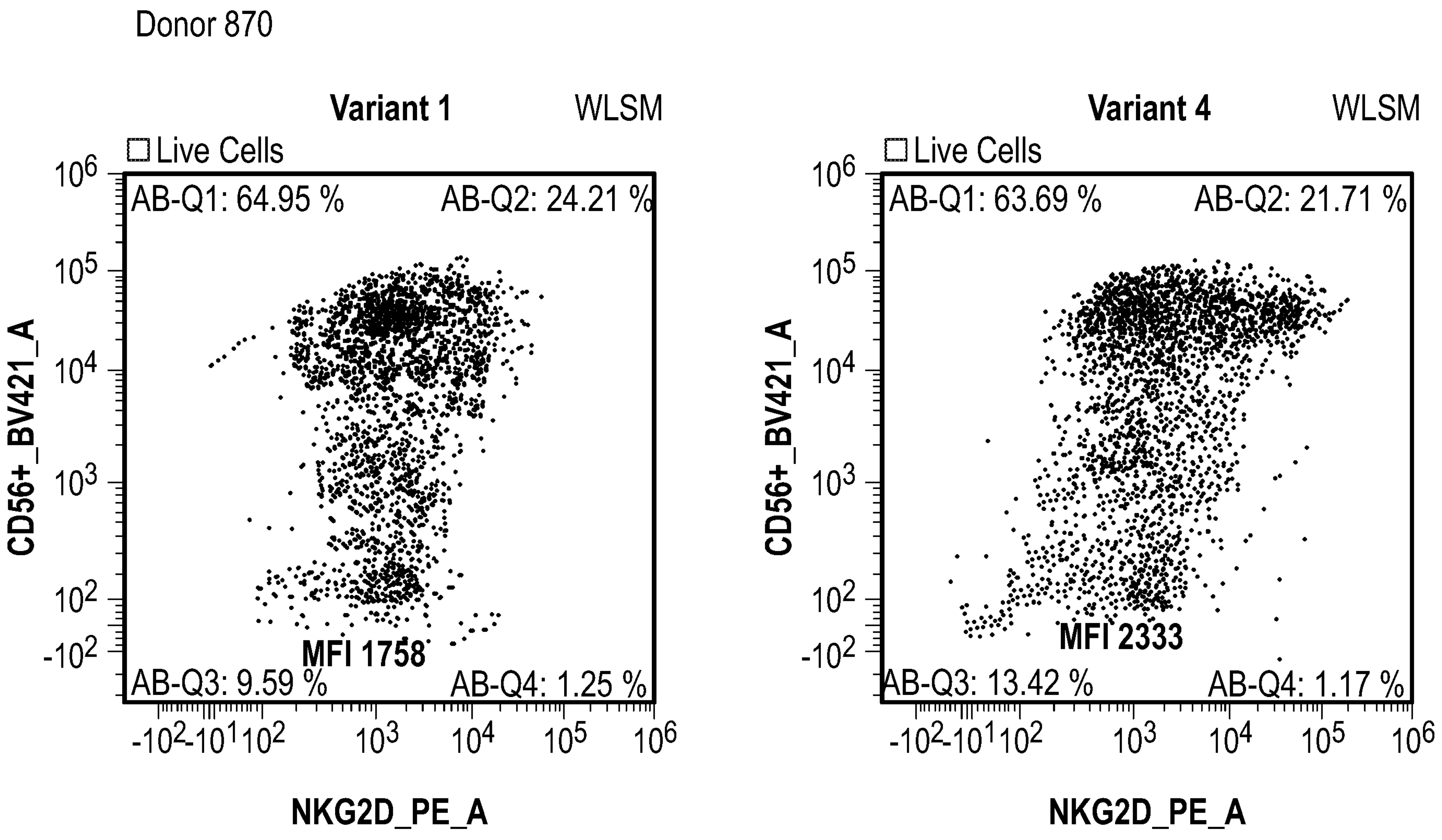

FIG. 16B
(Continued)

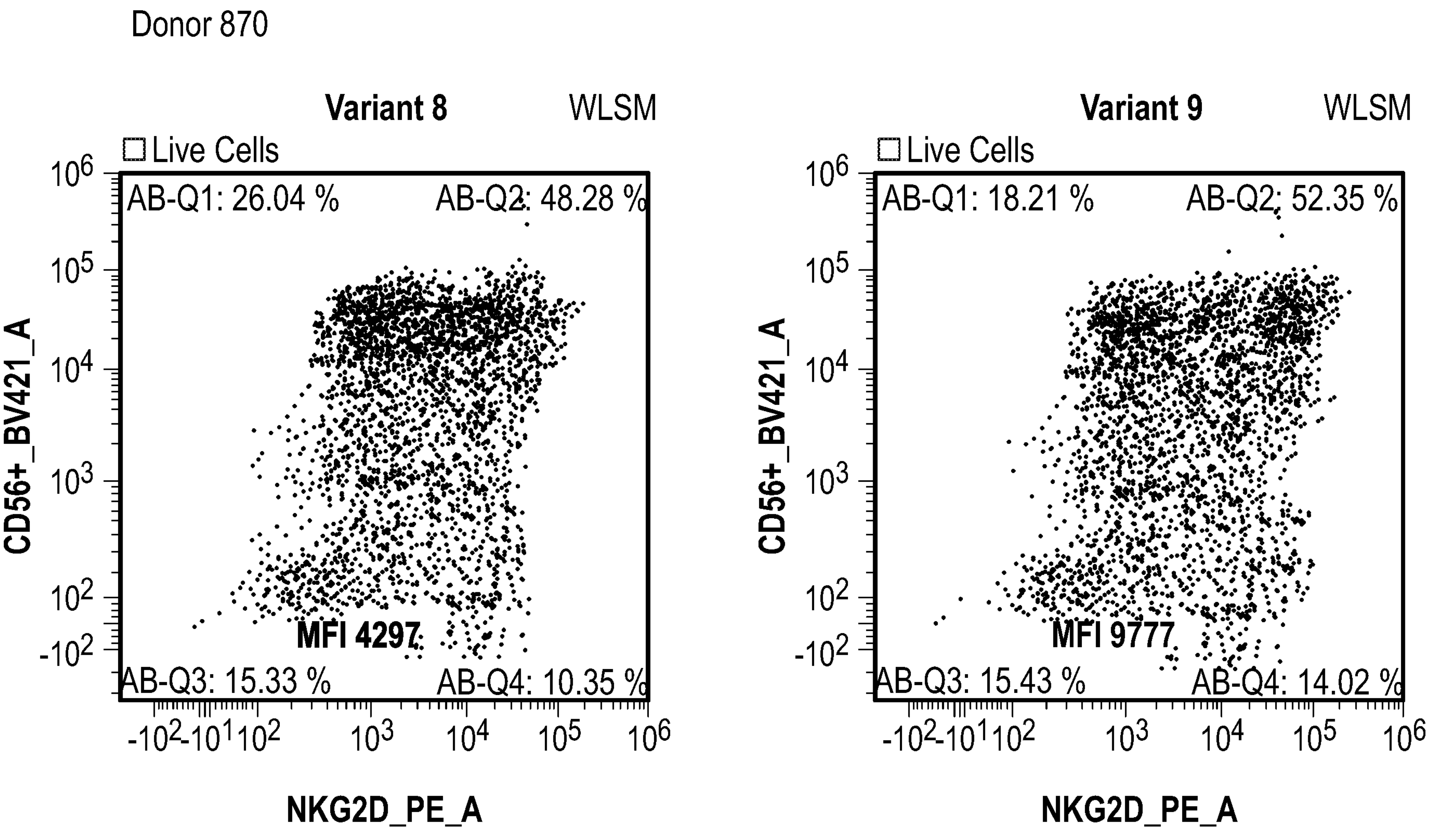

FIG. 16B
(Continued)

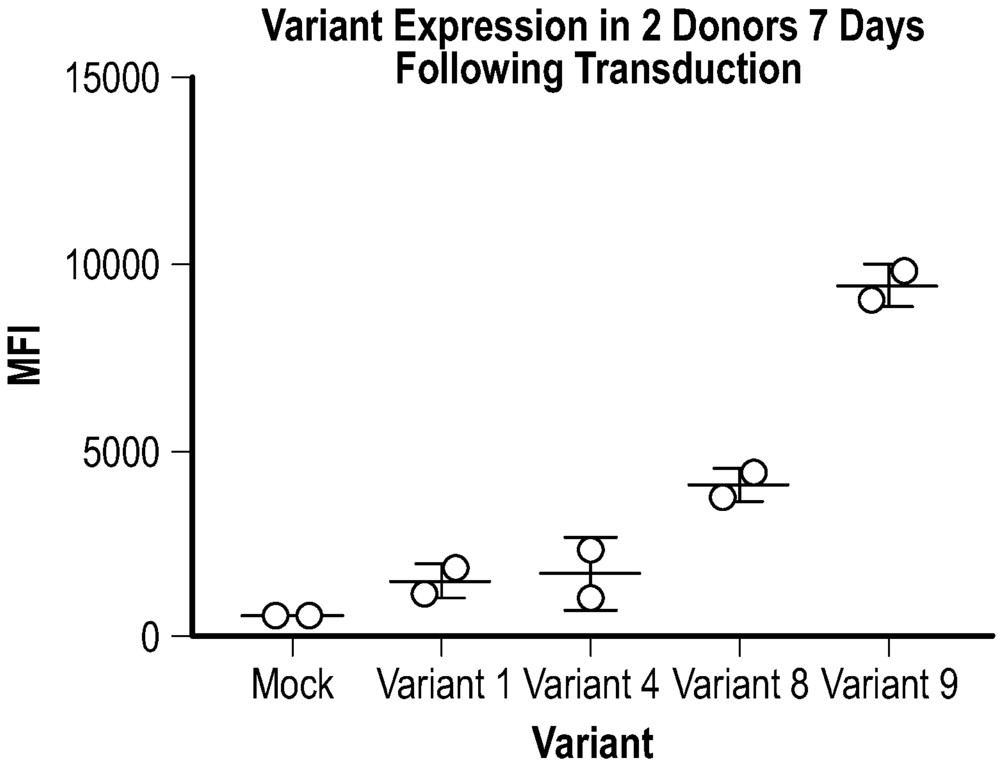
Variant Expression in 2 Donors 7 Days Following Transduction
15000
10000
5000
0
MFI
Mock
Variant 1
Variant 4
Variant 8
Variant 9
Variant

FIG. 16C

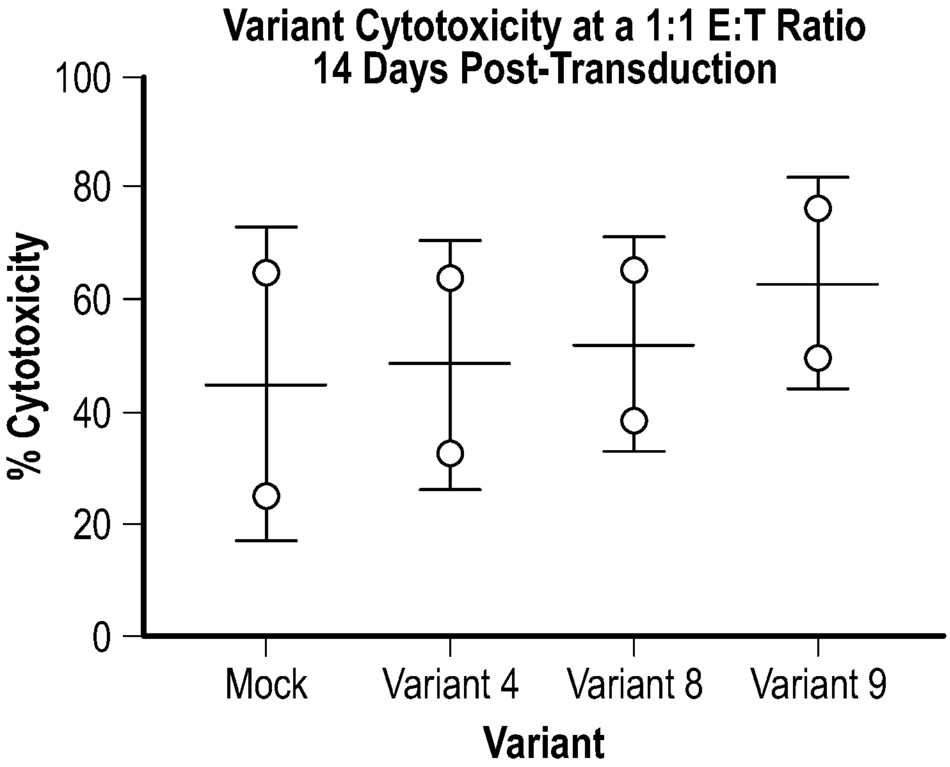
Variant Cytotoxicity at a 1:1 E:T Ratio
14 Days Post-Transduction
% Cytotoxicity
100
80
60
40
20
0
Mock
Variant 4
Variant 8
Variant 9
Variant

FIG. 17

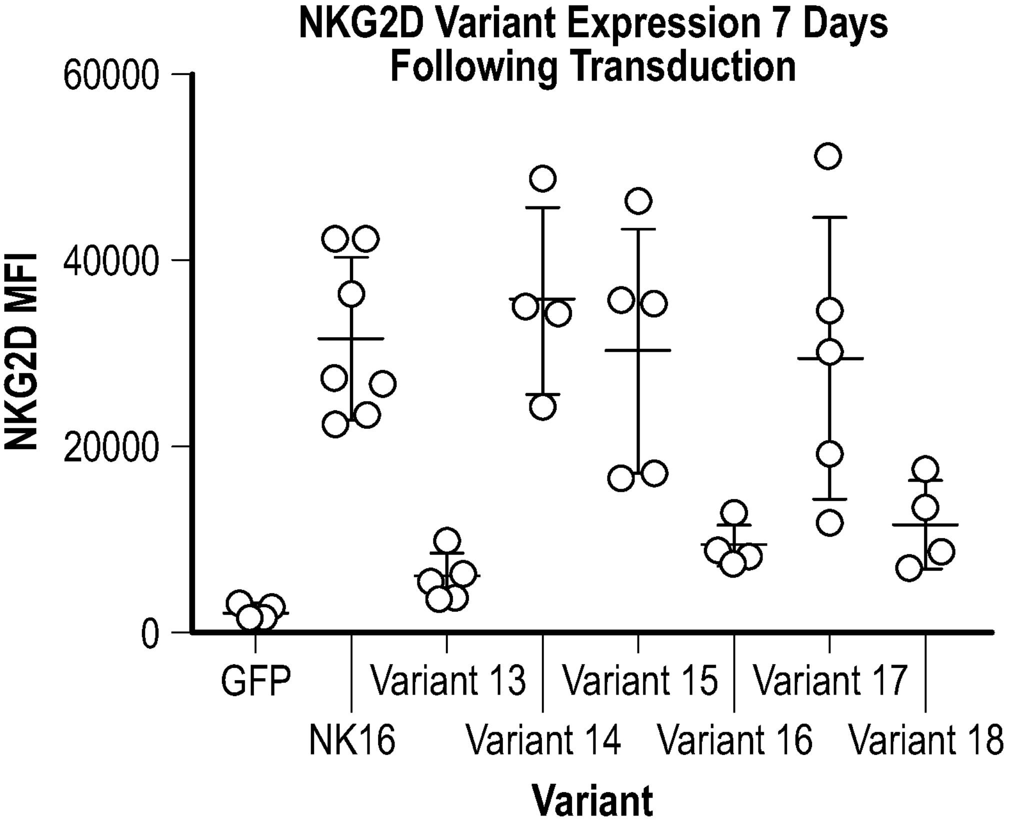

FIG. 18A

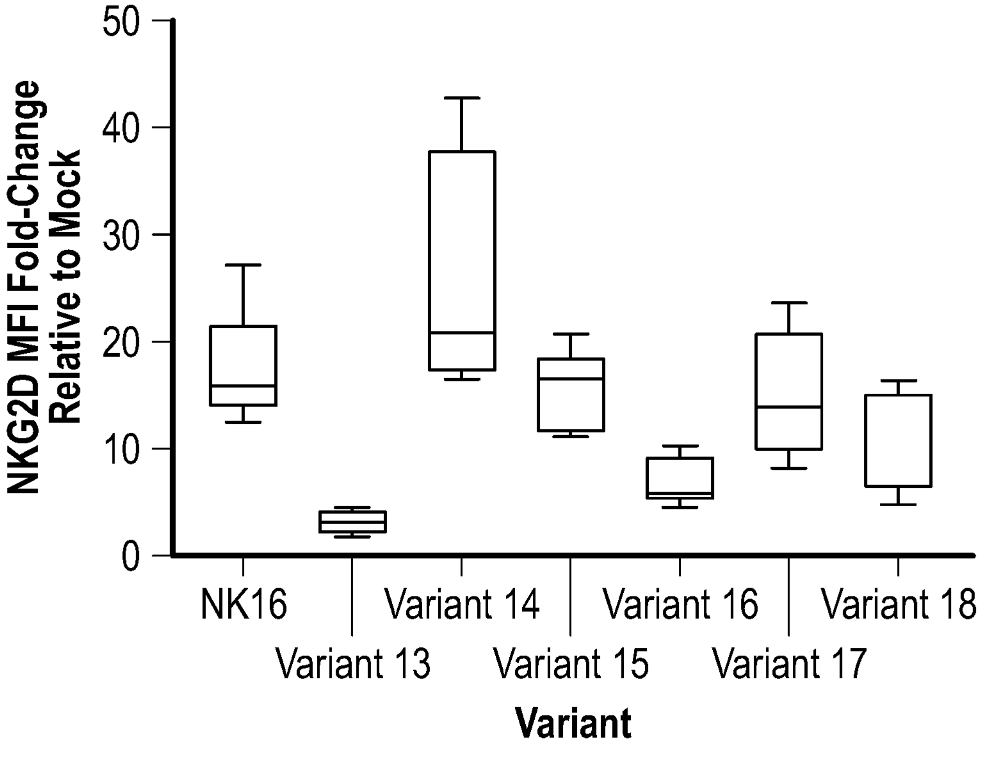

FIG. 18B

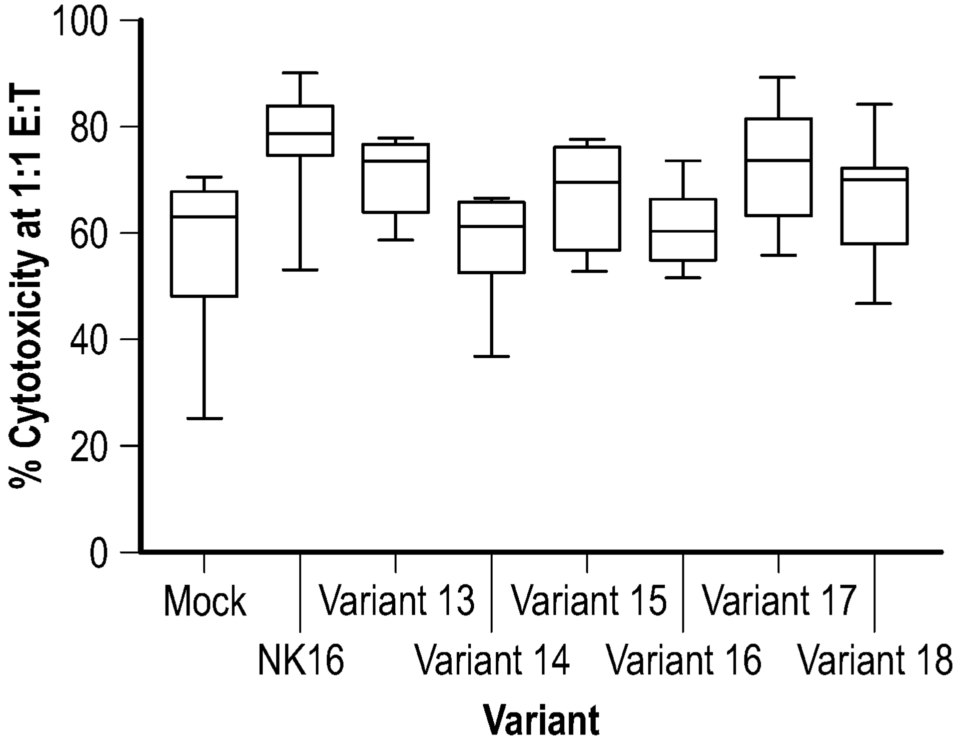

FIG. 19A

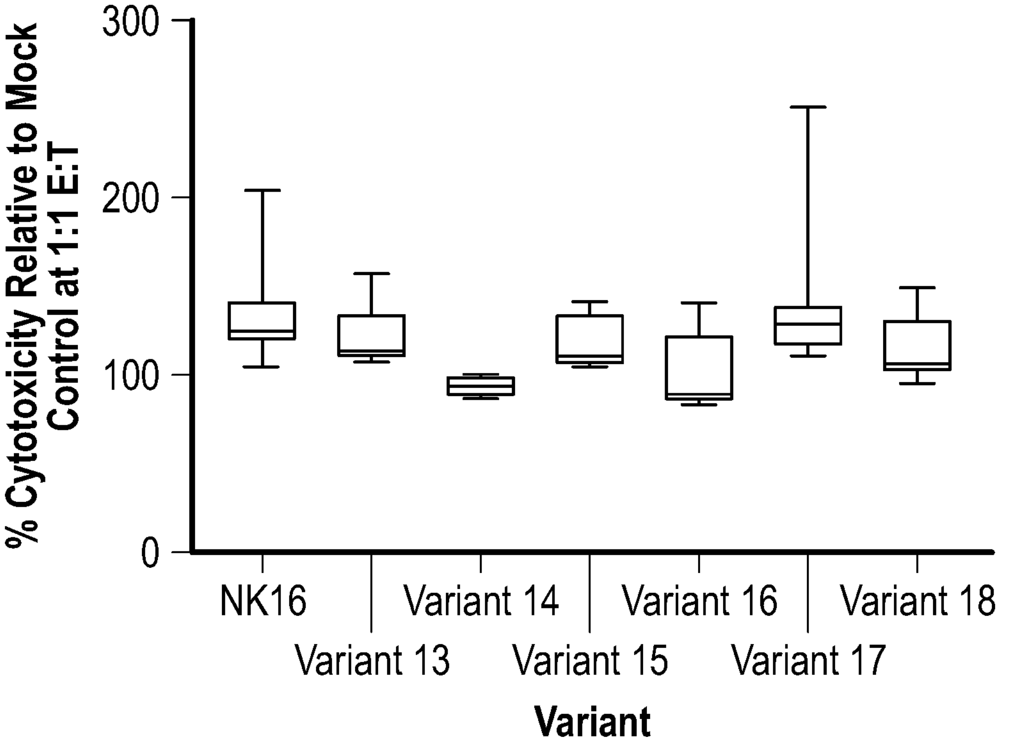

FIG. 19B

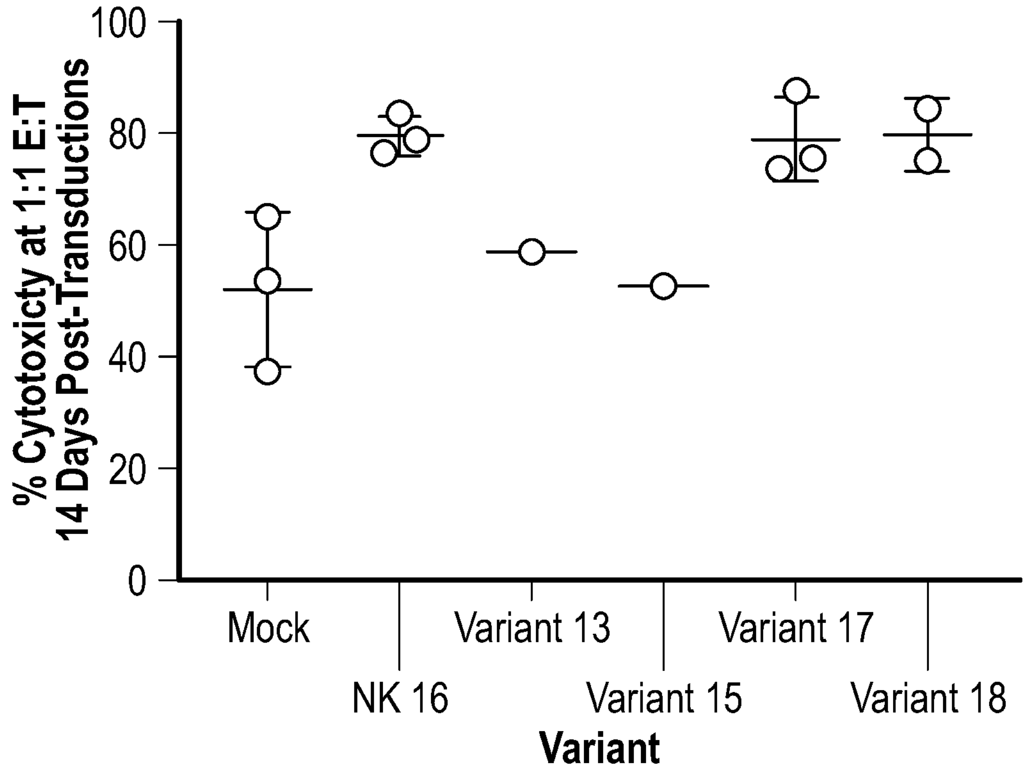
% Cytotoxicty at 1:1 E:T
14 Days Post-Transductions
100
80
60
40
20
0
Mock
NK 16
Variant 13
Variant 15
Variant 17
Variant 18
Variant

FIG. 20

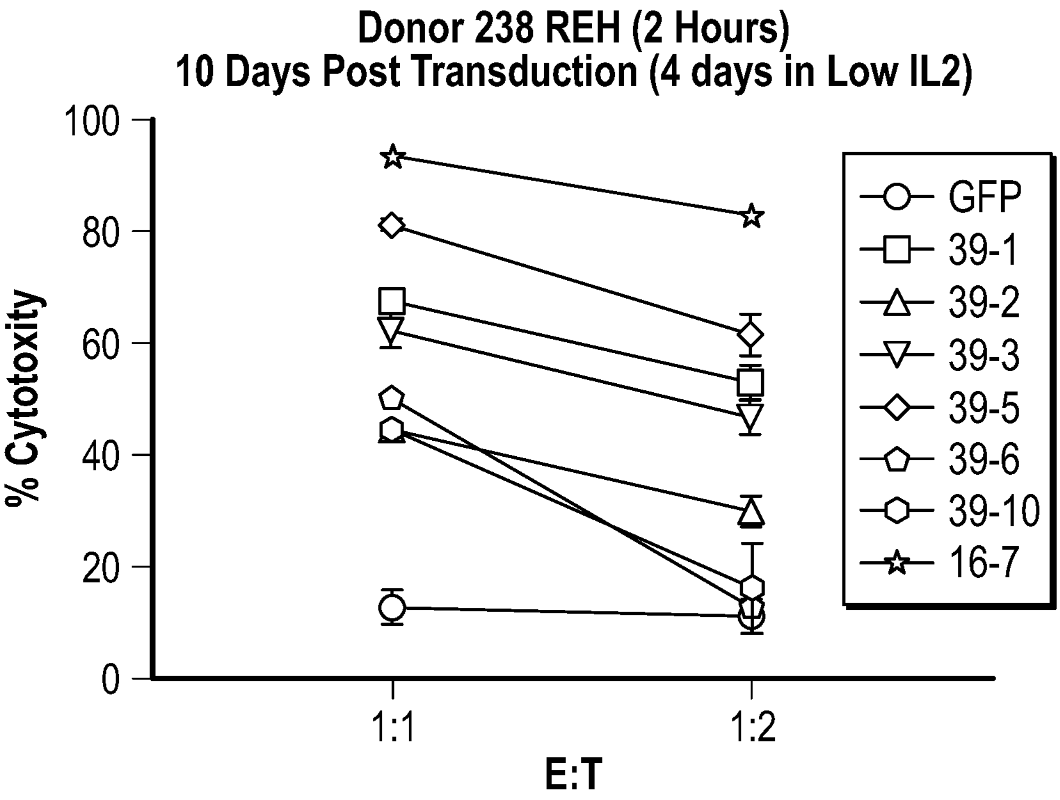
Donor 238 REH (2 Hours)
10 Days Post Transduction (4 days in Low IL2)
% Cytotoxity
100
80
60
40
20
0
1:1
1:2
E:T
GFP
39-1
39-2
39-3
39-5
39-6
39-10
16-7

FIG. 21

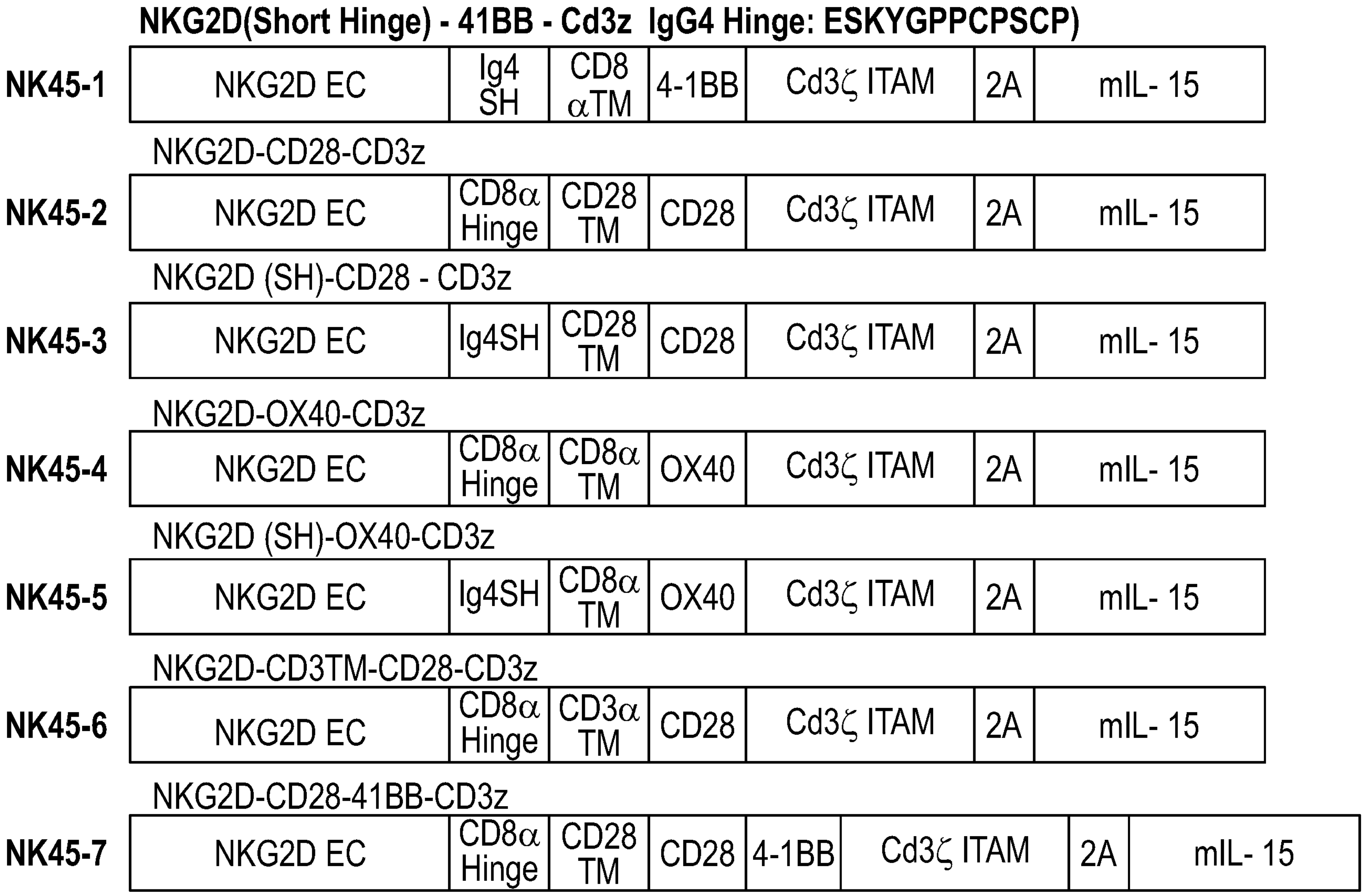
NKG2D(Short Hinge) - 41BB - Cd3z IgG4 Hinge: ESKYGPPCPSCP)
NK45-1
NKG2D EC
Ig4 SH
CD8 αTM
4-1BB
Cd3ζ ITAM
2A
mIL- 15
NKG2D-CD28-CD3z
NK45-2
NKG2D EC
CD8α Hinge
CD28 TM
CD28
Cd3ζ ITAM
2A
mIL- 15
NKG2D (SH)-CD28 - CD3z
NK45-3
NKG2D EC
Ig4SH
CD28 TM
CD28
Cd3ζ ITAM
2A
mIL- 15
NKG2D-OX40-CD3z
NK45-4
NKG2D EC
CD8α Hinge
CD8α TM
OX40
Cd3ζ ITAM
2A
mIL- 15
NKG2D (SH)-OX40-CD3z
NK45-5
NKG2D EC
Ig4SH
CD8α TM
OX40
Cd3ζ ITAM
2A
mIL- 15
NKG2D-CD3TM-CD28-CD3z
NK45-6
NKG2D EC
CD8α Hinge
CD3α TM
CD28
Cd3ζ ITAM
2A
mIL- 15
NKG2D-CD28-41BB-CD3z
NK45-7
NKG2D EC
CD8α Hinge
CD28 TM
CD28
4-1BB
Cd3ζ ITAM
2A
mIL- 15

FIG. 22

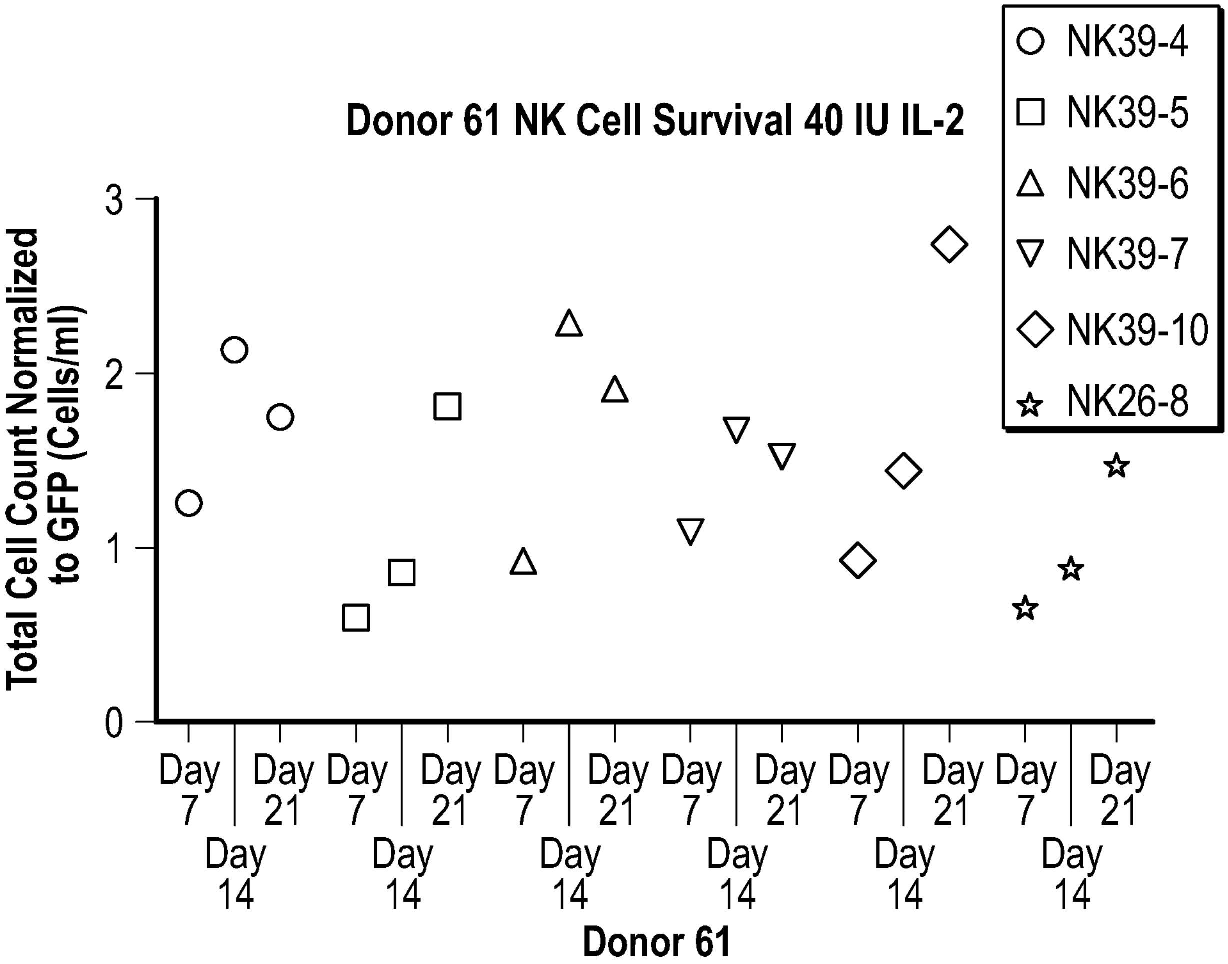
Donor 61 NK Cell Survival 40 IU IL-2
Total Cell Count Normalized to GFP (Cells/ml)
0
1
2
3
○ NK39-4
□ NK39-5
△ NK39-6
▽ NK39-7
◇ NK39-10
☆ NK26-8
Day 7
Day 14
Day 21
Donor 61

FIG. 23A

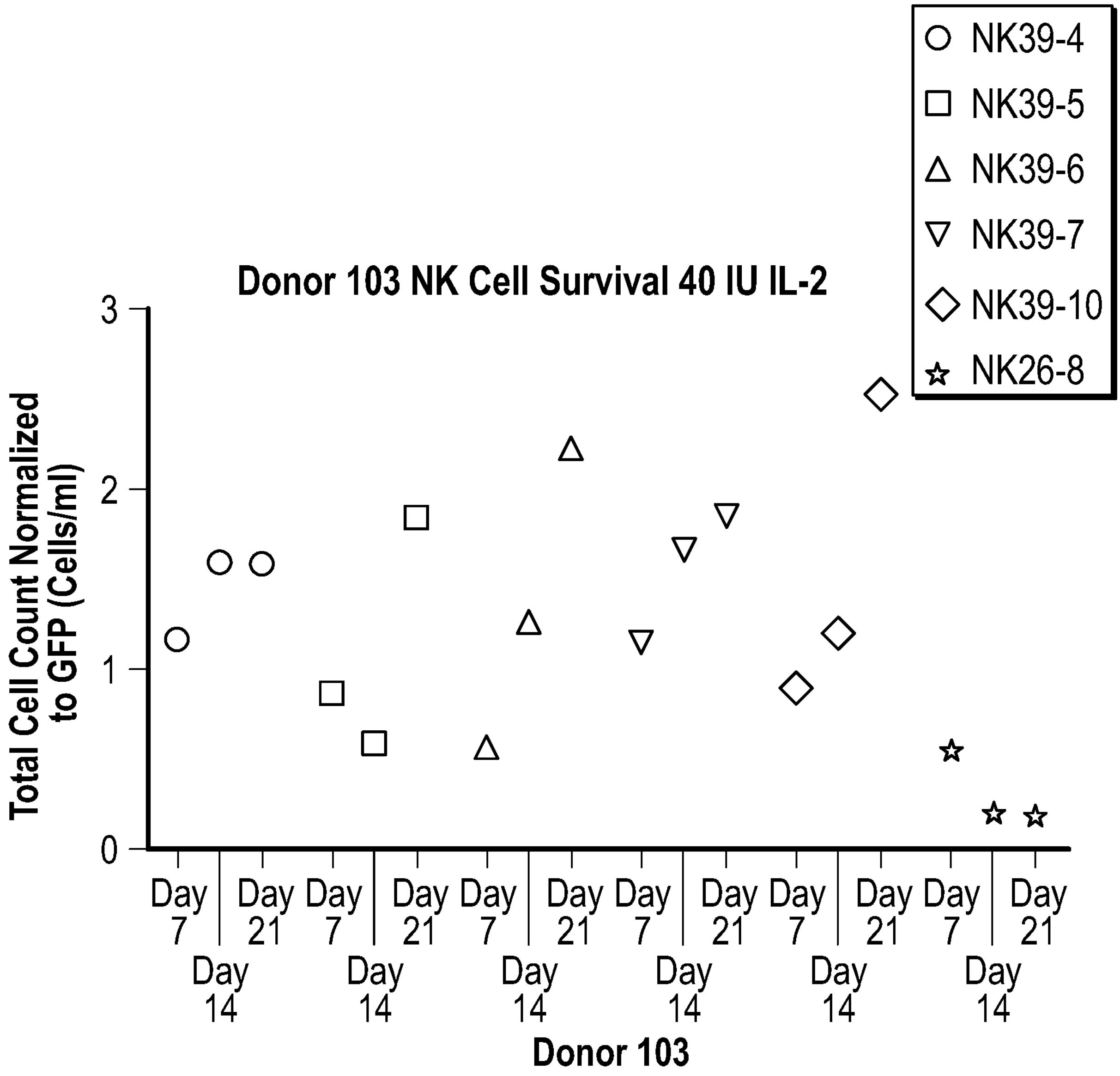
Donor 103 NK Cell Survival 40 IU IL-2
○ NK39-4
□ NK39-5
△ NK39-6
▽ NK39-7
◇ NK39-10
☆ NK26-8
Total Cell Count Normalized to GFP (Cells/ml)
3
2
1
0
Day 7
Day 14
Day 21
Donor 103

FIG. 23B

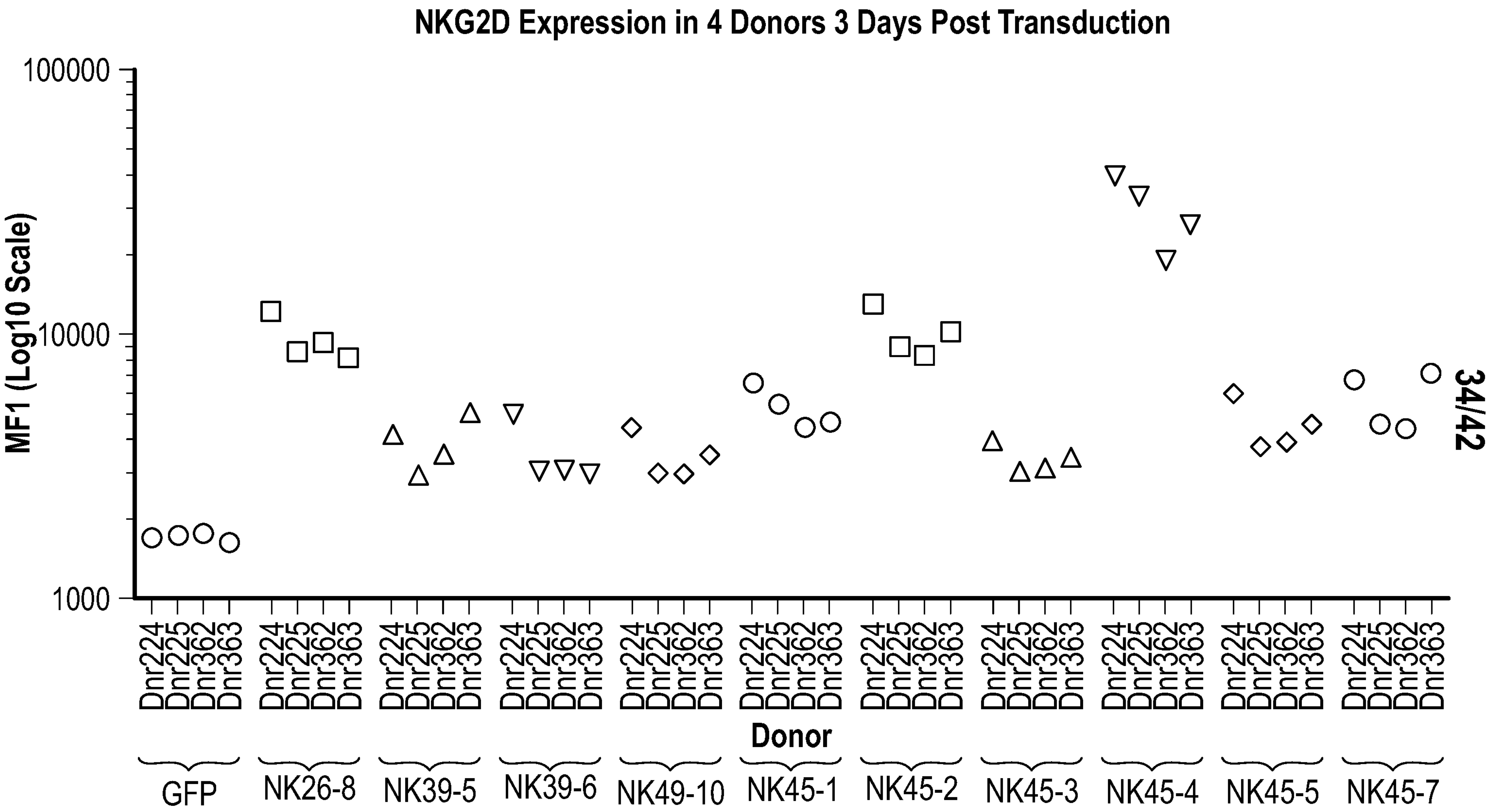
NKG2D Expression in 4 Donors 3 Days Post Transduction

FIG. 24

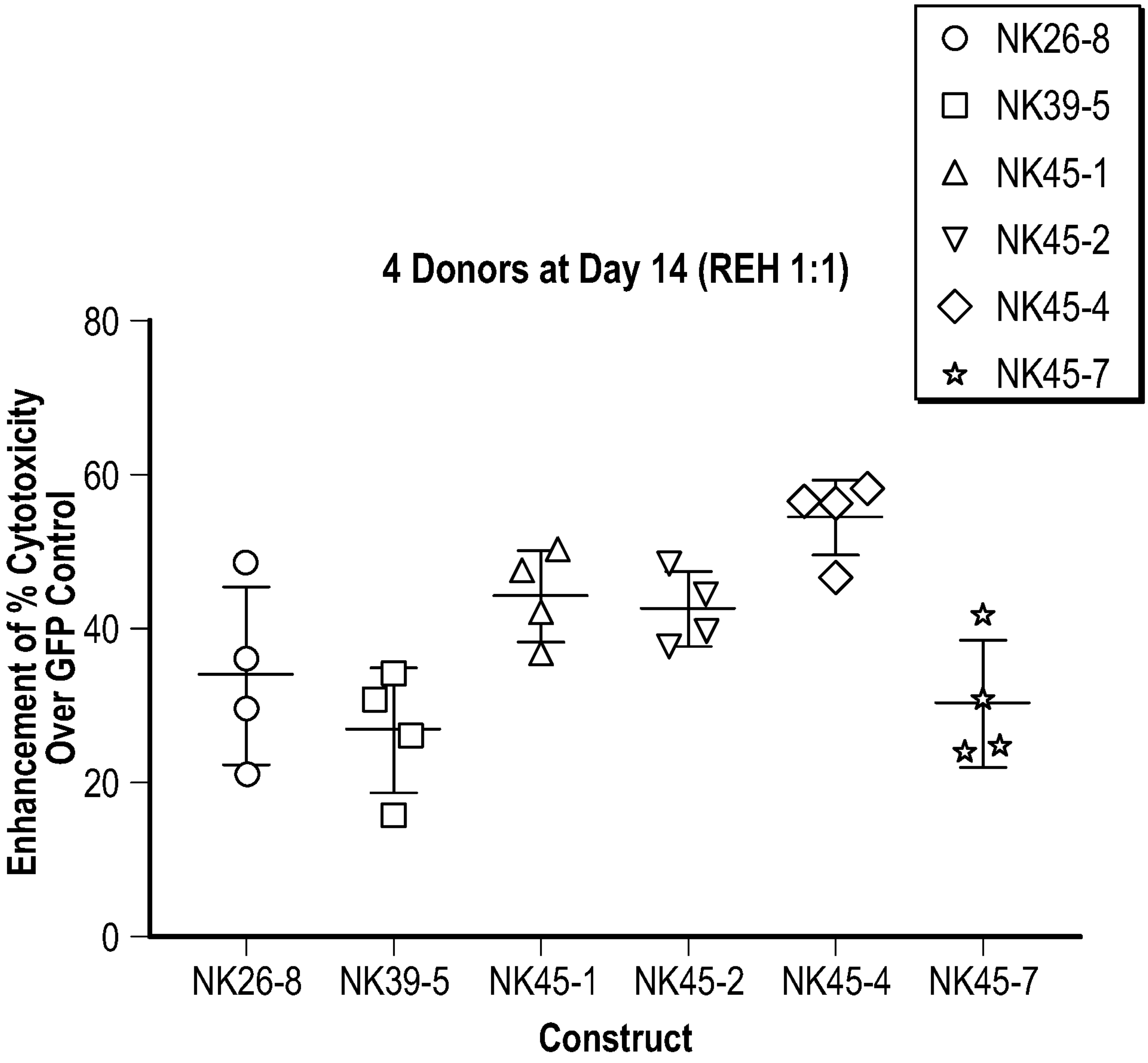
4 Donors at Day 14 (REH 1:1)
Enhancement of % Cytotoxicity Over GFP Control
80
60
40
20
0
NK26-8
NK39-5
NK45-1
NK45-2
NK45-4
NK45-7
Construct
○ NK26-8
□ NK39-5
△ NK45-1
▽ NK45-2
◇ NK45-4
☆ NK45-7

FIG. 25A

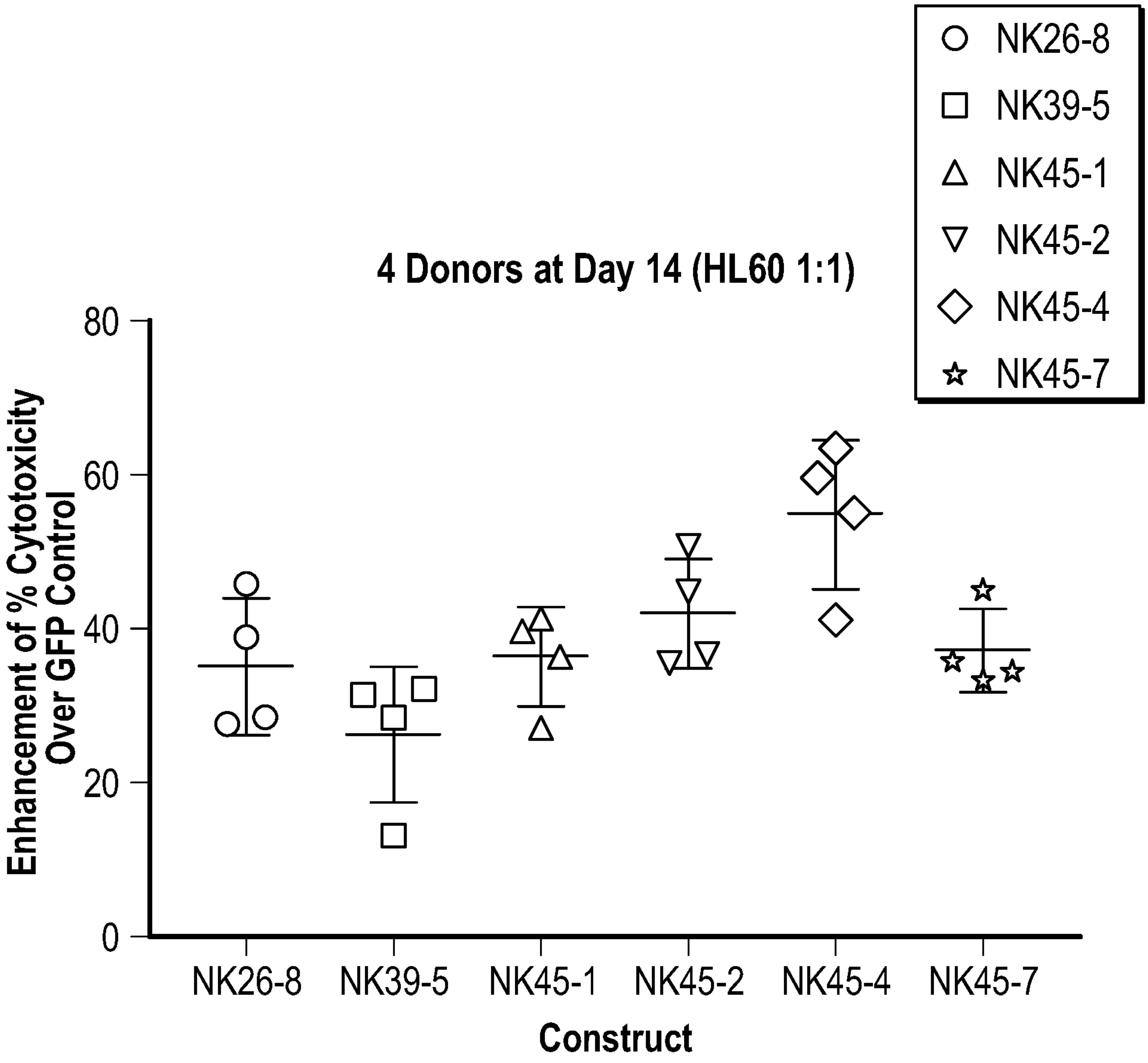
4 Donors at Day 14 (HL60 1:1)
Enhancement of % Cytotoxicity Over GFP Control
80
60
40
20
0
NK26-8
NK39-5
NK45-1
NK45-2
NK45-4
NK45-7
Construct
○ NK26-8
□ NK39-5
△ NK45-1
▽ NK45-2
◇ NK45-4
☆ NK45-7

FIG. 25B

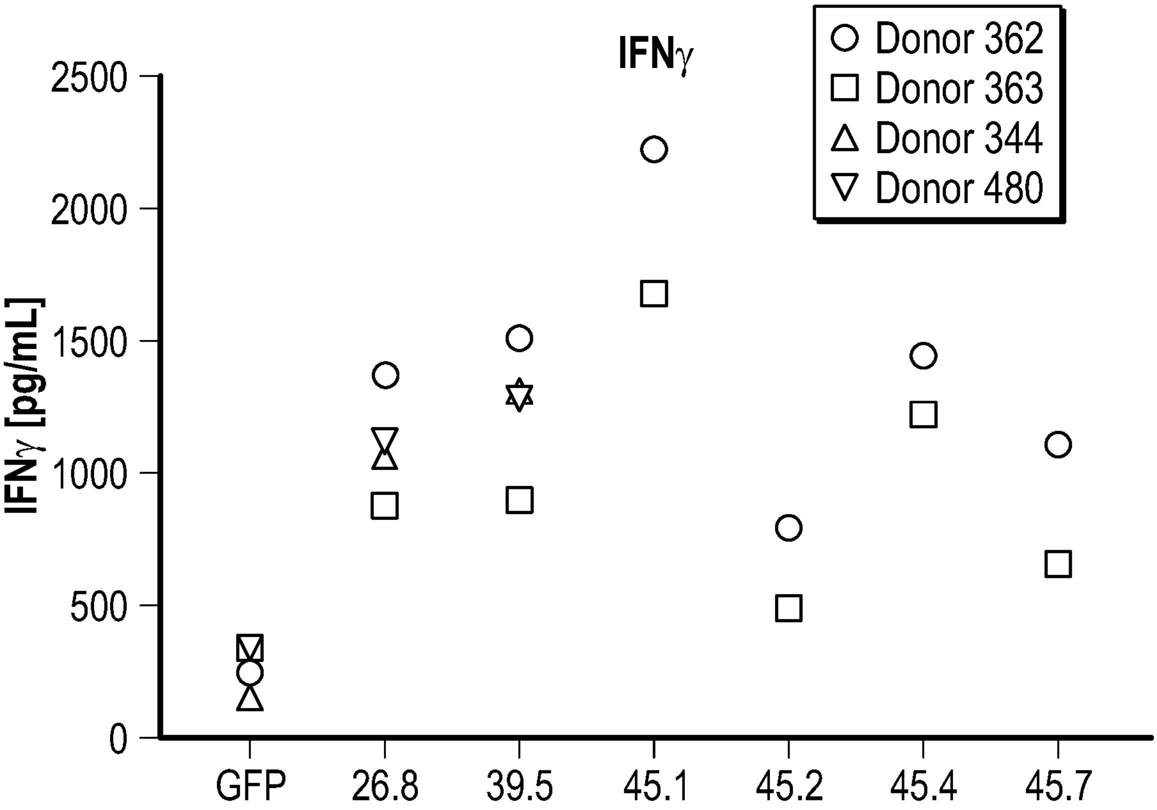

FIG. 26A

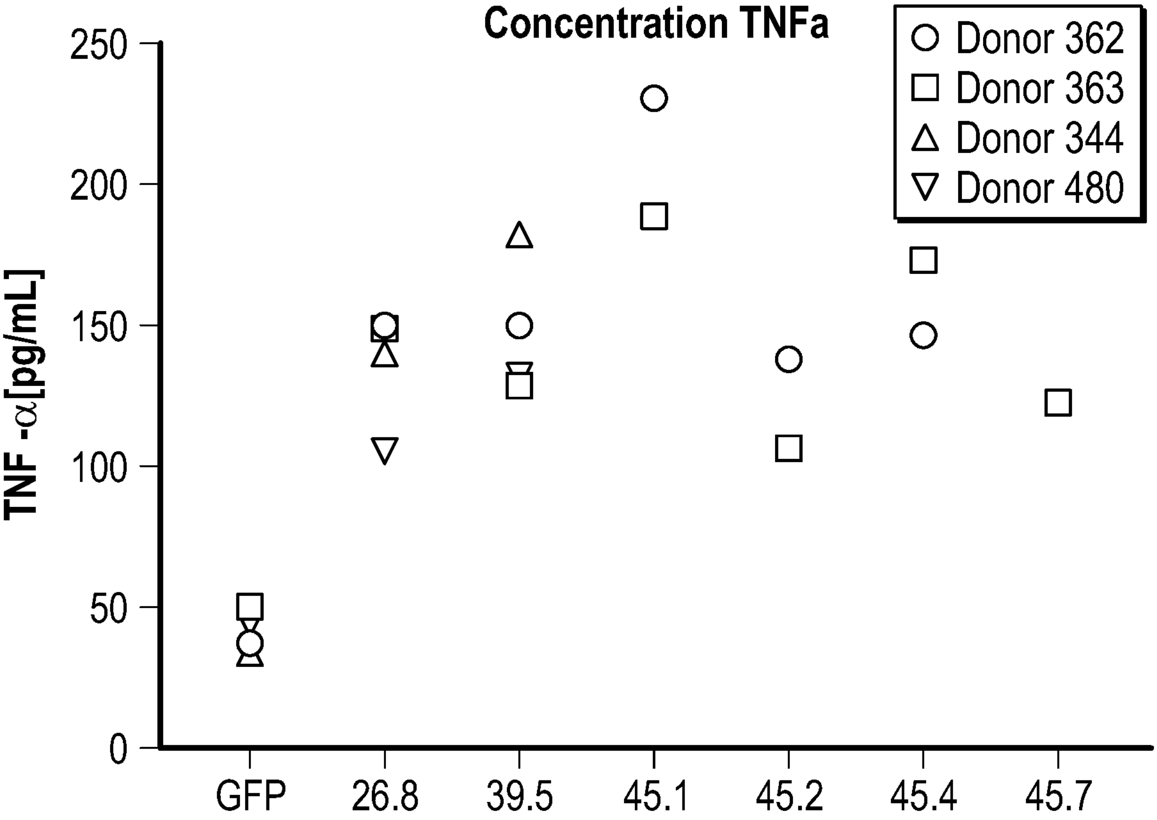

FIG. 26B

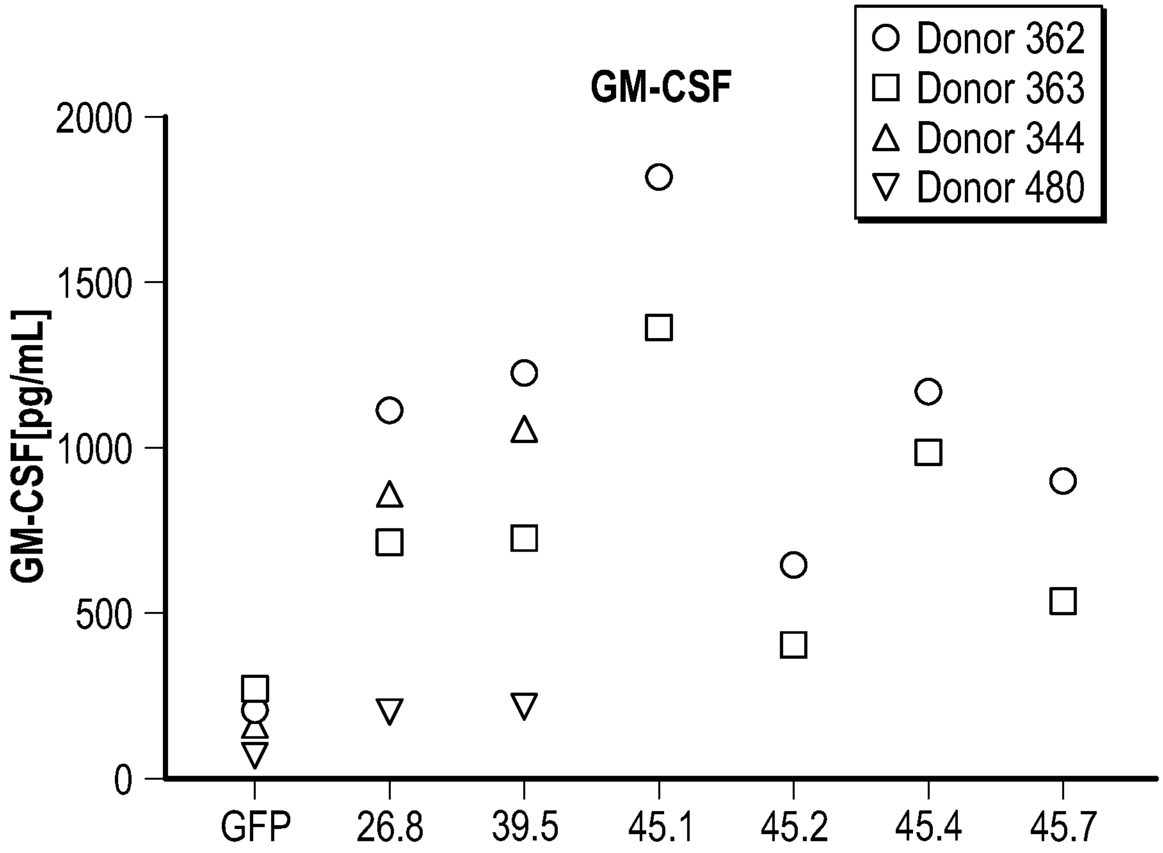
GM-CSF
○ Donor 362
□ Donor 363
△ Donor 344
▽ Donor 480
GM-CSF[pg/mL]
2000
1500
1000
500
0
GFP
26.8
39.5
45.1
45.2
45.4
45.7

FIG. 26C

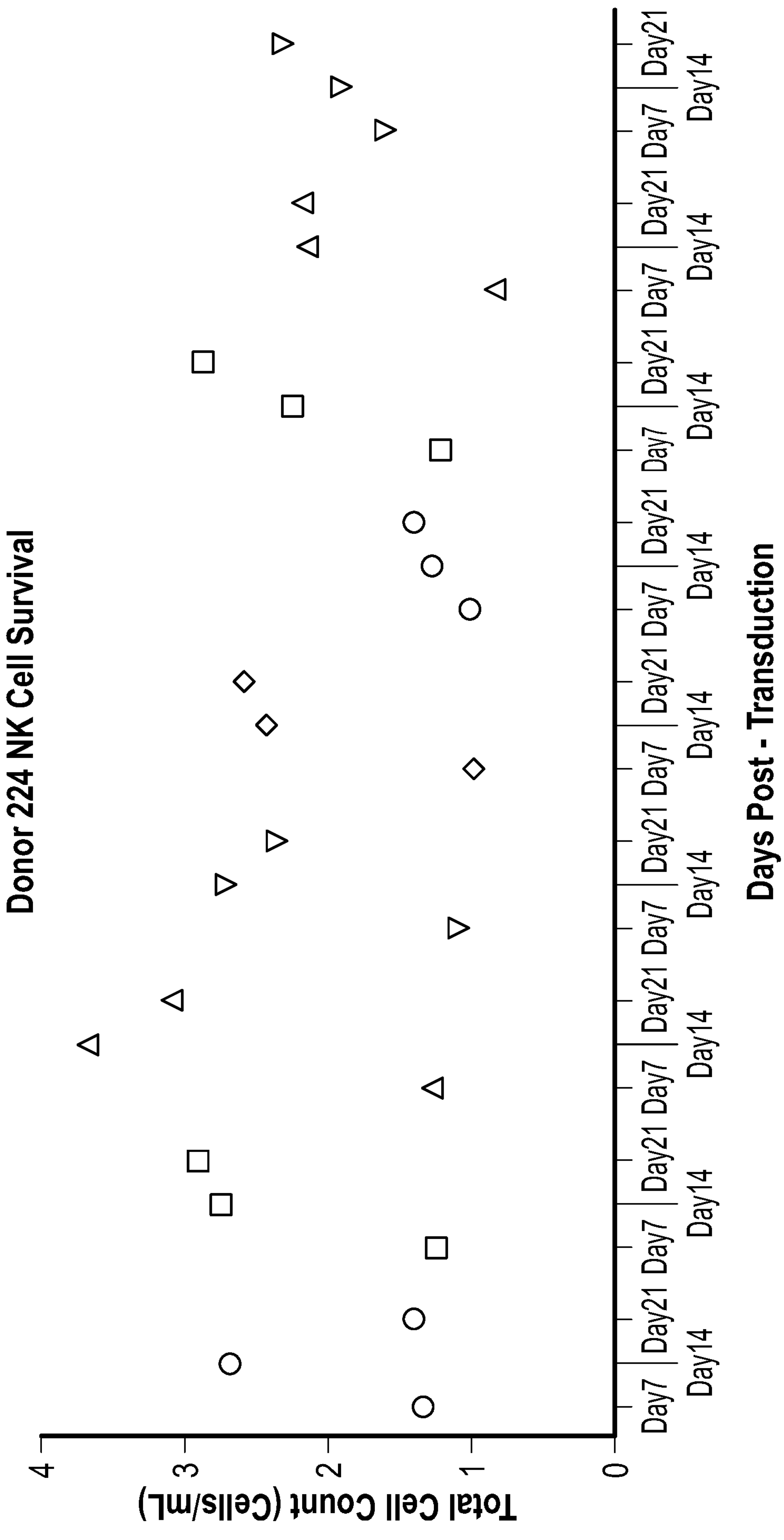
Donor 224 NK Cell Survival
Total Cell Count (Cells/mL)
0
1
2
3
4
Day7
Day14
Day21
GFP
NK26-8
NK39-5
NK45-1
NK45-2
NK45-3
NK45-4
NK45-5
NK45-7
Days Post - Transduction

FIG. 27A

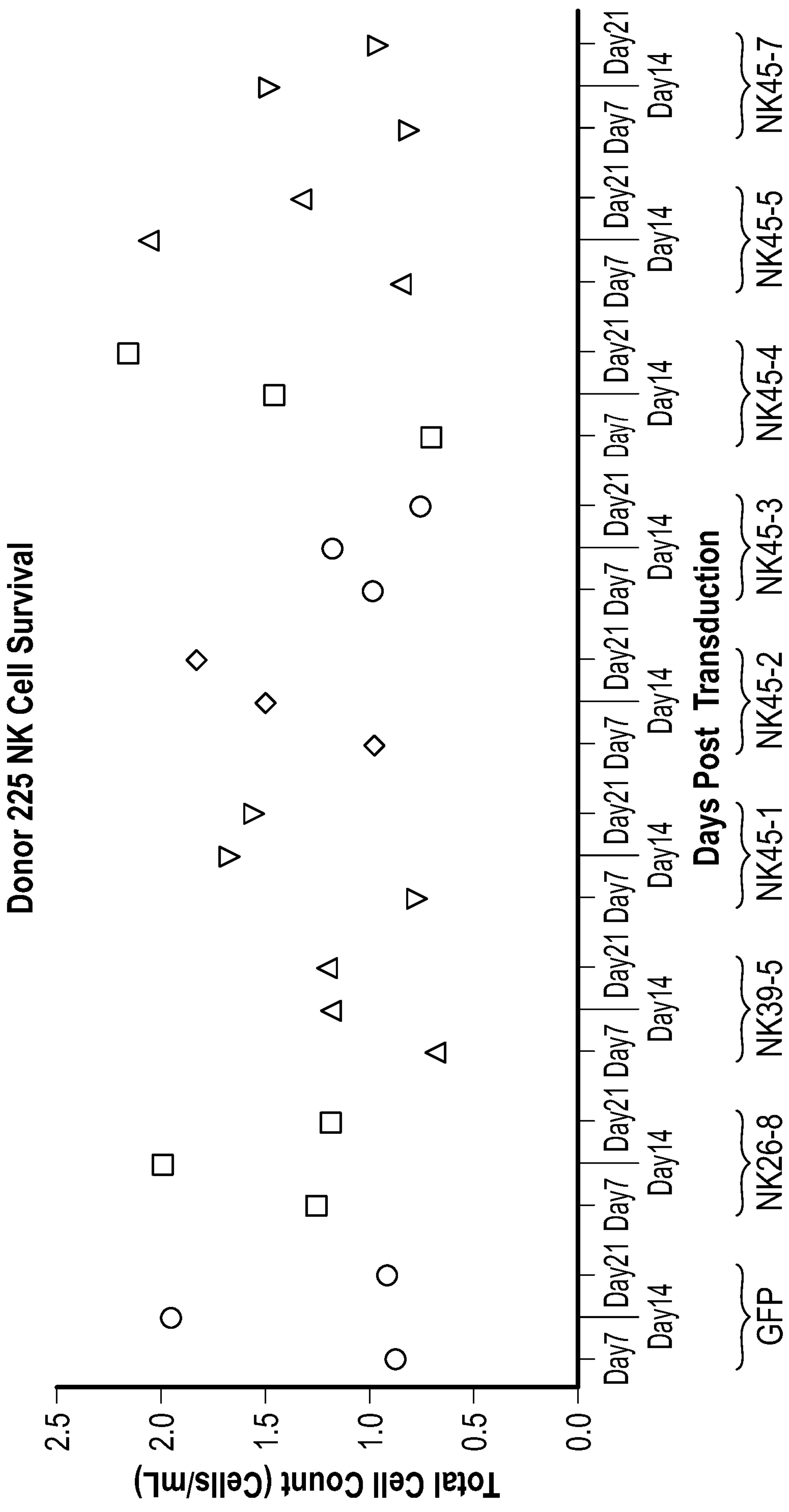
Donor 225 NK Cell Survival
Total Cell Count (Cells/mL)
0.0
0.5
1.0
1.5
2.0
2.5
Day7
Day14
Day21
GFP
NK26-8
NK39-5
NK45-1
NK45-2
NK45-3
NK45-4
NK45-5
NK45-7
Days Post Transduction

FIG. 27B

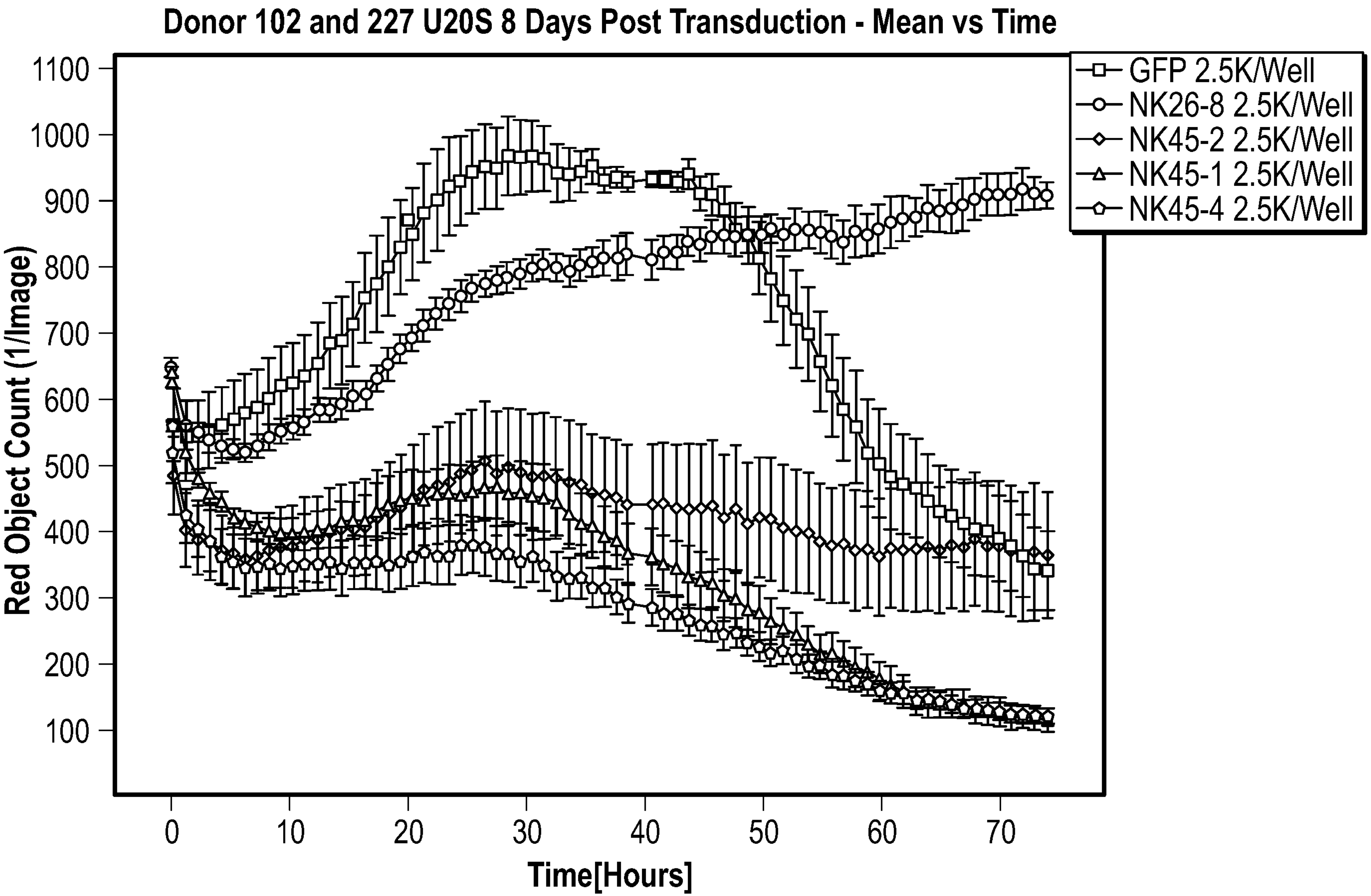
Donor 102 and 227 U20S 8 Days Post Transduction - Mean vs Time
Red Object Count (1/Image)
1100
1000
900
800
700
600
500
400
300
200
100
0
10
20
30
40
50
60
70
Time[Hours]
GFP 2.5K/Well
NK26-8 2.5K/Well
NK45-2 2.5K/Well
NK45-1 2.5K/Well
NK45-4 2.5K/Well

FIG. 28A

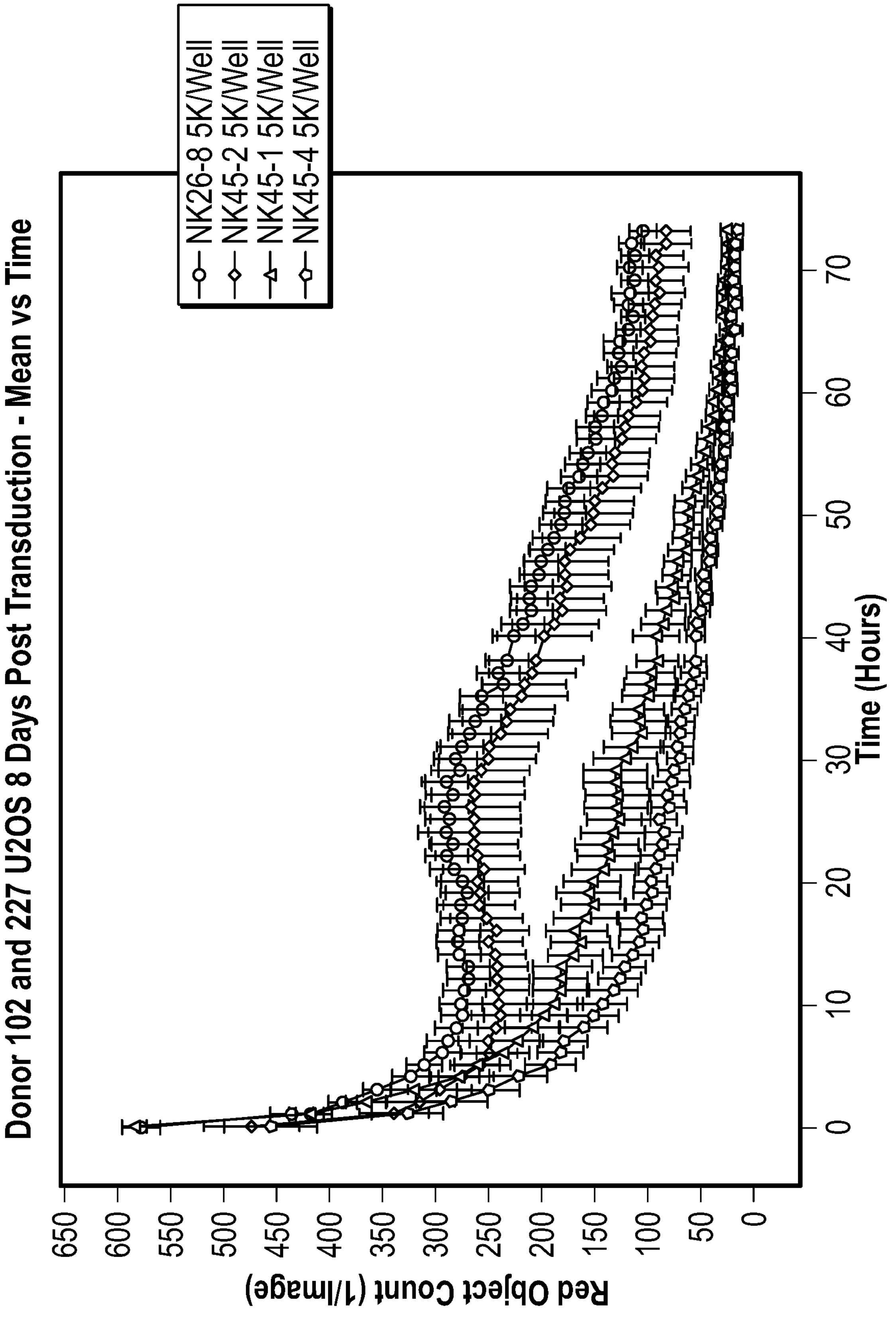
Donor 102 and 227 U2OS 8 Days Post Transduction - Mean vs Time
NK26-8 5K/Well
NK45-2 5K/Well
NK45-1 5K/Well
NK45-4 5K/Well
Red Object Count (1/Image)
650
600
550
500
450
400
350
300
250
200
150
100
50
0
0
10
20
30
40
50
60
70
Time (Hours)

FIG. 28B

SEQUENCE LISTING

```
<110>  National University of Singapore; Nkarta, Inc

<120>  TRUNCATED NKG2D CHIMERIC RECEPTORS AND USES THEREOF IN NATURAL
       KILLER CELL IMMUNOTHERAPY

<130>  4459.1144002

<150>  62/477335
<151>  2017-03-27

<150>  62/628774
<151>  2018-02-09

<160>  109

<170>  PatentIn version 3.5

<210>  1
<211>  645
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  Full length NKG2D

<400>  1
gggtggattc gtggtcggag gtctcgacac agctgggaga tgagtgaatt tcataattat        60

aacttggatc tgaagaagag tgatttttca acacgatggc aaaagcaaag atgtccagta       120

gtcaaaagca aatgtagaga aaatgcatct ccattttttt tctgctgctt catcgctgta       180

gccatgggaa tccgtttcat tattatggta acaatatgga gtgctgtatt cctaaactca       240

ttattcaacc aagaagttca aattcccttg accgaaagtt actgtggccc atgtcctaaa       300

aactggatat gttacaaaaa taactgctac caatttttg atgagagtaa aaactggtat       360

gagagccagg cttcttgtat gtctcaaaat gccagccttc tgaaagtata cagcaaagag       420

gaccaggatt tacttaaact ggtgaagtca tatcattgga tgggactagt acacattcca       480

acaaatggat cttggcagtg ggaagatggc tccattctct cacccaacct actaacaata       540

attgaaatgc agaagggaga ctgtgcactc tatgcctcga gctttaaagg ctatatagaa       600

aactgttcaa ctccaaatac gtacatctgc atgcaaagga ctgtg                      645
```

<210> 2
<211> 405
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> Truncated NKG2D

<400> 2
ttattcaacc aagaagttca aattcccttg accgaaagtt actgtggccc atgtcctaaa 60

aactggatat gttacaaaaa taactgctac caatttttttg atgagagtaa aaactggtat 120

gagagccagg cttcttgtat gtctcaaaat gccagccttc tgaaagtata cagcaaagag 180

gaccaggatt tacttaaact ggtgaagtca tatcattgga tgggactagt acacattcca 240

acaaatggat cttggcagtg ggaagatggc tccattctct cacccaacct actaacaata 300

attgaaatgc agaagggaga ctgtgcactc tatgcctcga gctttaaagg ctatatagaa 360

aactgttcaa ctccaaatac gtacatctgc atgcaaagga ctgtg 405

<210> 3
<211> 405
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> Codon Optimized Truncated NKG2D

<400> 3
ctgttcaatc aggaagtcca gatccccctg acagagtctt actgcggccc atgtcccaag 60

aactggatct gctacaagaa caattgttat cagttctttg acgagagcaa gaactggtat 120

gagtcccagg cctcttgcat gagccagaat gcctctctgc tgaaggtgta cagcaaggag 180

gaccaggatc tgctgaagct ggtgaagtcc tatcactgga tgggcctggt gcacatccct 240

acaaacggct cttggcagtg ggaggacggc tccatcctgt ctccaaatct gctgaccatc 300

atcgagatgc agaagggcga ttgcgccctg tacgccagct ccttcaaggg ctatatcgag 360

aactgctcca cacccaatac ctacatctgt atgcagagga ccgtg 405

<210> 4
<211> 63
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> CD8 Signaling Sequence

<400> 4
atggctctgc ccgtcaccgc actgctgctg cctctggctc tgctgctgca cgccgcacga   60

cca   63

<210> 5
<211> 135
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> CD8 alpha hinge

<400> 5
accacaaccc ctgcaccacg cccccctaca ccagcaccta ccatcgcaag ccagcctctg   60

tccctgcggc cagaggcatg tagaccagca gcaggaggag cagtgcacac aagaggcctg   120

gacttcgcct gcgat   135

<210> 6
<211> 1722
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> CD8 beta

<220>
<221> misc_feature
<222> (674)..(773)
<223> n is a, c, g, or t

<220>
<221> misc_feature

<222> (859)..(958)
<223> n is a, c, g, or t

<400> 6
atctaggtct tgctgcaccc gcacaaccta caaacagcgt cggggccttc tctgcacctc   60

cagttcccag ctcacctccc tcagtgtcac agccggttac ctttccttcc tccctggggg   120

agggcaagac ttggggcttg ctgactccag gcccagccca gcccggggca cccaggagcc   180

cctcaattgc tactcaaaca gacaagaagc ggcccgagtt agtggccagc tccaccatgc   240

actacacatc ctgacctctc tgagcctcta ctgtcactcg gggtcacaac cctttcctga   300

gcacctcccg gggcaggggg cgatgacaca catgcagctg cctgggggag gccggcggtg   360

tcccctcctt tctggaacgc ggagggtcct ggtgggctct ggaaacgcag cccagacctt   420

tgcaatgcta ggaggatgag ggcggagacc tcgcggtccc caacaccaga ctcccgcagc   480

caccgcgccc ggtcccgccc tccccactgc ccccccagct ccccgaccca ggcgccccgc   540

ccggccagct cctcacccac cccagccgcg actgtctccg ccgagccccc ggggccaggt   600

gtcccgggcg cgccacgatg cggccgcggc tgtggctcct cctggccgcg cagctgacag   660

gtaaggcggc ggcnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   720

nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnttgcttt   780

cctcttccag gccggcggag gagagcccgg cttcgtttca tgaaacagta agtgtataac   840

ctgggtgtgg ccttgggann nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   900

nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnct   960

tgctgttgtt ttcagatttt acaaatgagc agagaatacg gttttggtgt cctgctacaa   1020

aaagacatcg gtcagtaacg agcacgatgt ggaaaaatga gagaagggac acattcaacc   1080

ctggagagtt caatggctgc tgaagctgcc tgcttttcac tgctgcaagg cctttctgtg   1140

tgtgacgtgc atgggagcaa cttgttcgtg ggtcatcggg aatactaggg agaaggtttc   1200

attgccccca gggcacttca cagagtgtgc tggaggactg agtaagaaat gctgcccatg   1260

ccaccgcttc cggctcctgt gctttccctg aactgggacc tttagtggtg gccatttagc   1320

caccatcttt gcaggttgct ttgccctggt agggcagtaa cattgggtcc tgggtctttc   1380

atggggtgat gctgggctgg ctccctgttg gtcttcccag gctggggctg accttcctcg   1440 cagagaggcc aggtgcaggt tgggaatgag gcttgctgag aggggctgtc cagttcccag 1500

aaggcatatc agtctctgag ggcttccttt ggggccggga acttgcgggt ttgaggatag 1560

gagttcactt catcttctca gctcccattt ctactcttaa gtttctcagc tcccatttct 1620

actctcccat ggcttaatgc ttctttcatt ttctgtttgt tttatacaaa tgtcttagtt 1680

gtaaaaataa agtcccaggt taaagataac aaacgggtcc tg 1722

<210> 7
<211> 2415
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> CD16 alpha

<400> 7
attcttggtg ctgggtggat ccaaatccag gagatggggc aagcatcctg ggatggctga 60

gggcacactc tggcagattc tgtgtgtgtc ctcagatgct cagccacaga cctttgaggg 120

agtaaagggg gcagacccac ccaccttgcc tccaggctct ttccttcctg gtcctgttct 180

atggtggggc tcccttgcca gacttcagac tgagaagtca gatgaagttt caagaaaagg 240

aaattggtgg gtgacagaga tgggtggagg ggctggggaa aggctgttta cttcctcctg 300

tctagtcggt ttggtccctt tagggctccg gatatctttg gtgacttgtc cactccagtg 360

tggcatcatg tggcagctgc tcctcccaac tgctctgcta cttctagttt cagctggcat 420

gcggactgaa gatctcccaa aggctgtggt gttcctggag cctcaatggt acagggtgct 480

cgagaaggac agtgtgactc tgaagtgcca gggagcctac tcccctgagg acaattccac 540

acagtggttt cacaatgaga gcctcatctc aagccaggcc tcgagctact tcattgacgc 600

tgccacagtc gacgacagtg gagagtacag gtgccagaca aacctctcca ccctcagtga 660

cccggtgcag ctagaagtcc atatcggctg gctgttgctc caggcccctc ggtgggtgtt 720

caaggaggaa gaccctattc acctgaggtg tcacagctgg aagaacactg ctctgcataa 780

ggtcacatat ttacagaatg gcaaaggcag gaagtatttt catcataatt ctgacttcta 840

cattccaaaa gccacactca aagacagcgg ctcctacttc tgcagggggc tttttgggag 900

```
taaaaatgtg tcttcagaga ctgtgaacat caccatcact caaggtttgg cagtgtcaac      960

catctcatca ttctttccac ctgggtacca agtctctttc tgcttggtga tggtactcct     1020

ttttgcagtg gacacaggac tatatttctc tgtgaagaca aacattcgaa gctcaacaag     1080

agactggaag gaccataaat ttaaatggag aaaggaccct caagacaaat gacccccatc     1140

ccatgggggt aataagagca gtagcagcag catctctgaa catttctctg gatttgcaac     1200

cccatcatcc tcaggcctct ctacaagcag caggaaacat agaactcaga gccagatccc     1260

ttatccaact ctcgactttt ccttggtctc cagtggaagg gaaaagccca tgatcttcaa     1320

gcagggaagc cccagtgagt agctgcattc ctagaaattg aagtttcaga gctacacaaa     1380

cactttttct gtcccaaccg ttccctcaca gcaaagcaac aatacaggct agggatggta     1440

atcctttaaa catacaaaaa ttgctcgtgt tataaattac ccagtttaga ggggaaaaaa     1500

aaacaattat tcctaaataa atggataagt agaattaatg gttgaggcag gaccatacag     1560

agtgtgggaa ctgctgggga tctagggaat tcagtgggac caatgaaagc atggctgaga     1620

aatagcaggt agtccaggat agtctaaggg aggtgttccc atctgagccc agagataagg     1680

gtgtcttcct agaacattag ccgtagtgga attaacagga aatcatgagg gtgacgtaga     1740

attgagtctt ccaggggact ctatcagaac tggaccatct ccaagtatat aacgatgagt     1800

cctcttaatg ctaggagtag aaaatggtcc taggaagggg actgaggatt gcggtggggg     1860

gtggggtgga aaagaaagta cagaacaaac cctgtgtcac tgtcccaagt tgctaagtga     1920

acagaactat ctcagcatca gaatgagaaa gcctgagaag aaagaaccaa ccacaagcac     1980

acaggaagga aagcgcagga ggtgaaaatg ctttcttggc cagggtagta agaattagag     2040

gttaatgcag ggactgtaaa accacctttt ctgcttcaat atctaattcc tgtgtagctt     2100

tgttcattgc atttattaaa caaatgttgt ataaccaata ctaaatgtac tactgagctt     2160

cgctgagtta agttatgaaa ctttcaaatc cttcatcatg tcagttccaa tgaggtgggg     2220

atggagaaga caattgttgc ttatgaaaga aagctttagc tgtctctgtt ttgtaagctt     2280

taagcgcaac atttcttggt tccaataaag cattttacaa gatcttgcat gctactctta     2340

gatagaagat gggaaaacca tggtaataaa atatgaatga taaaaaaaaa aaaaaaaaaa     2400

aaaaaaaaaa aaaaa                                                      2415
```

```
<210>  8
<211>  2473
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  CD16 beta

<220>
<221>  misc_feature
<222>  (211)..(310)
<223>  n is a, c, g, or t

<220>
<221>  misc_feature
<222>  (537)..(636)
<223>  n is a, c, g, or t

<220>
<221>  misc_feature
<222>  (968)..(1067)
<223>  n is a, c, g, or t

<400>  8
aaagatgggt ggagggactg gggaaaggct gtttactccc tcctgtctag tcggcttggt        60

ccctttaggg gtccggatat ctttggtgac ttgtccactc cagtgtggca tcatgtggca       120

gctgctcctc ccaactgctc tgctacttct aggtaagtag gatctccctg gttgagggag       180

aagtttgaga tgccttgggt tcagcagaga nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn       240

nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn       300

nnnnnnnnnn aagaggcatg aacagtggaa gaccagagag caggtagcaa ggtttccacc       360

agaaacatcc tgattcttgg gaaaattggg ctcctggggc agaggagggc aggggagttt       420

taaactcact ctatgttcta atcactctga tctctgcccc tactcaatat ttgatttact       480

ctttttttctt gcagtttcag ctggcatgcg gactggtgag tcagcttcat ggtcttnnnn      540

nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn       600

nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnncact gagagctgag ctcccgggcc       660

tggggtgtct ctgtgtcttt caggctggct gttgctccag gcccctcggt gggtgttcaa       720

ggaggaagac cctattcacc tgaggtgtca cagctggaag aacactgctc tgcataaggt       780
```

```
cacatattta cagaatggca aagacaggaa gtattttcat cataattctg acttccacat      840

tccaaaagcc acactcaaag atagcggctc ctacttctgc agggggcttg ttgggagtaa      900

aaatgtgtct tcagagactg tgaacatcac catcactcaa ggtgagacat gtgccaccct      960

ggaatgcnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn     1020

nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnttt ttcatctctc     1080

cacttctcct aataggtttg gcagtgtcaa ccatctcatc attctctcca cctgggtacc     1140

aagtctcttt ctgcttggtg atggtactcc tttttgcagt ggacacagga ctatatttct     1200

ctgtgaagac aaacatttga agctcaacaa gagactggaa ggaccataaa cttaaatgga     1260

gaaaggaccc tcaagacaaa tgacccccat cccatgggag taataagagc agtggcagca     1320

gcatctctga acatttctct ggatttgcaa ccccatcatc ctcaggcctc tctacaagca     1380

gcaggaaaca tagaactcag agccagatcc tttatccaac tctcgatttt tccttggtct     1440

ccagtggaag ggaaaagccc atgatcttca agcagggaag ccccagtgag tagctgcatt     1500

cctagaaatt gaagtttcag agctacacaa acactttttc tgtcccaacc attccctcac     1560

agtaaaacaa caatacaggc tagggatggt aatcctttaa acatacaaaa attgctcgta     1620

ttataaatta cccagtttag accggaaaaa agaaaataat tattcctaaa caaatggata     1680

agtagaatta atgattgagg caggacccta cagagtgtgg gaactgctgg ggatctagag     1740

aattcagtgg gaccaatgaa agcatggctg agaaatagca gggtagtcca ggagagtcta     1800

agggaggtgt tcccatctga gcccagagat aagggtgtct tcctagaaca ttagccgtag     1860

tggaattaac aggaaatcat gagggtgacg tagaattgag tcttccaggg gactctatca     1920

gaactggacc atttccaagt atataacgat gagccctcta atgctaggag tagcaaatgg     1980

tcctaggaag gggactgagg attggggtgg gggtggggtg gaaaagaaag tacagaacaa     2040

accctgtgtc actgtcccaa gttaagctaa gtgaacagaa ctatctcagc atcagaatga     2100

gaaagcctga gaagaaagaa ccaaccacaa gcacacagga aggaaagcgc aggaggtgaa     2160

aatgctttct tggccagggt agtaagaatt agaggttaat gcagggactg taaaaccacc     2220

ttttctgctt caatgtctag ttcctgtata gctttgttca ttgcatttat taaacaaatg     2280

ttgtataacc aatactaaat gtactactga gcttcactga gttacgctgt gaaactttca     2340
```

```
aatccttctt catgtcagtt ccaatgaggt ggggatggag aagacaattg ttgcttatga     2400

aaaaaagctt tagctgtctc tgttttgtaa gctttcagtg caacatttct tggttccaat     2460

aaagcatttt aca                                                        2473


<210>  9
<211>  370
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  2B4

<400>  9

Met Leu Gly Gln Val Val Thr Leu Ile Leu Leu Leu Leu Leu Lys Val
1               5                   10                  15


Tyr Gln Gly Lys Gly Cys Gln Gly Ser Ala Asp His Val Val Ser Ile
            20                  25                  30


Ser Gly Val Pro Leu Gln Leu Gln Pro Asn Ser Ile Gln Thr Lys Val
        35                  40                  45


Asp Ser Ile Ala Trp Lys Lys Leu Leu Pro Ser Gln Asn Gly Phe His
    50                  55                  60


His Ile Leu Lys Trp Glu Asn Gly Ser Leu Pro Ser Asn Thr Ser Asn
65                  70                  75                  80


Asp Arg Phe Ser Phe Ile Val Lys Asn Leu Ser Leu Leu Ile Lys Ala
                85                  90                  95


Ala Gln Gln Gln Asp Ser Gly Leu Tyr Cys Leu Glu Val Thr Ser Ile
            100                 105                 110


Ser Gly Lys Val Gln Thr Ala Thr Phe Gln Val Phe Val Phe Glu Ser
        115                 120                 125
```

```
Leu Leu Pro Asp Lys Val Glu Lys Pro Arg Leu Gln Gly Gln Gly Lys
    130                 135                 140


Ile Leu Asp Arg Gly Arg Cys Gln Val Ala Leu Ser Cys Leu Val Ser
145                 150                 155                 160


Arg Asp Gly Asn Val Ser Tyr Ala Trp Tyr Arg Gly Ser Lys Leu Ile
                165                 170                 175


Gln Thr Ala Gly Asn Leu Thr Tyr Leu Asp Glu Glu Val Asp Ile Asn
            180                 185                 190


Gly Thr His Thr Tyr Thr Cys Asn Val Ser Asn Pro Val Ser Trp Glu
        195                 200                 205


Ser His Thr Leu Asn Leu Thr Gln Asp Cys Gln Asn Ala His Gln Glu
    210                 215                 220


Phe Arg Phe Trp Pro Phe Leu Val Ile Ile Val Ile Leu Ser Ala Leu
225                 230                 235                 240


Phe Leu Gly Thr Leu Ala Cys Phe Cys Val Trp Arg Arg Lys Arg Lys
                245                 250                 255


Glu Lys Gln Ser Glu Thr Ser Pro Lys Glu Phe Leu Thr Ile Tyr Glu
            260                 265                 270


Asp Val Lys Asp Leu Lys Thr Arg Arg Asn His Glu Gln Glu Gln Thr
        275                 280                 285


Phe Pro Gly Gly Gly Ser Thr Ile Tyr Ser Met Ile Gln Ser Gln Ser
    290                 295                 300


Ser Ala Pro Thr Ser Gln Glu Pro Ala Tyr Thr Leu Tyr Ser Leu Ile
305                 310                 315                 320


Gln Pro Ser Arg Lys Ser Gly Ser Arg Lys Arg Asn His Ser Pro Ser
                325                 330                 335
```

```
Phe Asn Ser Thr Ile Tyr Glu Val Ile Gly Lys Ser Gln Pro Lys Ala
            340                 345                 350


Gln Asn Pro Ala Arg Leu Ser Arg Lys Glu Leu Glu Asn Phe Asp Val
        355                 360                 365


Tyr Ser
    370


<210>  10
<211>  279
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  DAP10

<400>  10
atgatccatc tgggtcacat cctcttcctg cttttgctcc cagtggctgc agctcagacg       60

actccaggag agagatcatc actccctgcc ttttaccctg gcacttcagg ctcttgttcc      120

ggatgtgggt ccctctctct gccgctcctg gcaggcctcg tggctgctga tgcggtggca      180

tcgctgctca tcgtgggggc ggtgttcctg tgcgcacgcc cacgccgcag ccccgcccaa      240

gatggcaaag tctacatcaa catgccaggc aggggctga                             279


<210>  11
<211>  575
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  DAP12

<400>  11
agacttcctc cttcacttgc ctggacgctg cgccacatcc caccggccct tacactgtgg       60

tgtccagcag catccggctt catgggggga cttgaaccct gcagcaggct cctgctcctg      120

cctctcctgc tggctgtaag tgattgcagt tgctctacgg tgagcccggg cgtgctggca      180

gggatcgtga tgggagacct ggtgctgaca gtgctcattg ccctggccgt gtacttcctg      240
```

```
ggccggctgg tccctcgggg gcgaggggct gcggaggcag cgacccggaa acagcgtatc      300

actgagaccg agtcgcctta tcaggagctc cagggtcaga ggtcggatgt ctacagcgac      360

ctcaacacac agaggccgta ttacaaatga gcccgaatca tgacagtcag caacatgata      420

cctggatcca gccattcctg aagcccaccc tgcacctcat tccaactcct accgcgatac      480

agacccacag agtgccatcc ctgagagacc agaccgctcc ccaatactct cctaaaataa      540

acatgaagca caaaaacaaa aaaaaaaaaa aaaaa                                 575


<210>  12
<211>  126
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  4-1BB

<400>  12
aaacggggca gaaagaaact cctgtatata ttcaaacaac catttatgag accagtacaa       60

actactcaag aggaagatgg ctgtagctgc cgatttccag aagaagaaga aggaggatgt      120

gaactg                                                                 126


<210>  13
<211>  339
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  CD3-zeta

<400>  13
agagtgaagt tcagcaggag cgcagacgcc cccgcgtacc agcagggcca gaaccagctc       60

tataacgagc tcaatctagg acgaagagag gagtacgatg ttttggacaa gagacgtggc      120

cgggaccctg agatgggggg aaagccgaga aggaagaacc ctcaggaagg cctgtacaat      180

gaactgcaga aagataagat ggcggaggcc tacagtgaga ttgggatgaa aggcgagcgc      240

cggaggggca aggggcacga tggcctttac cagggtctca gtacagccac caaggacacc      300
```

```
tacgacgccc ttcacatgca ggccctgccc cctcgctaa                           339


<210>  14
<211>  6
<212>  PRT
<213>  Homo sapiens



<220>
<221>  MISC_FEATURE
<223>  Canonical hemi-tam

<220>
<221>  MISC_FEATURE
<222>  (4)..(5)
<223>  X = any amino acid

<400>  14

Asp Gly Tyr Xaa Xaa Leu
1               5


<210>  15
<211>  6
<212>  PRT
<213>  Homo sapiens



<220>
<221>  MISC_FEATURE
<223>  ITSM Motif

<220>
<221>  MISC_FEATURE
<222>  (1)..(1)
<223>  N = S or T

<220>
<221>  MISC_FEATURE
<222>  (2)..(2)
<223>  x = any amino acid

<220>
<221>  MISC_FEATURE
<222>  (4)..(5)
<223>  x = any amino acid

<220>
<221>  MISC_FEATURE
```

```
<222>  (6)..(6)
<223>  N = L or I

<400>  15

Asn Xaa Tyr Xaa Xaa Asn
1               5


<210>  16
<211>  614
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  Membrane-bound IL15

<400>  16
atggccttac cagtgaccgc cttgctcctg ccgctggcct tgctgctcca cgccgccagg       60

ccgaactggg tgaatgtaat aagtgatttg aaaaaaattg aagatcttat tcaatctatg      120

catattgatg ctactttata tacggaaagt gatgttcacc ccagttgcaa agtaacagca      180

atgaagtgct ttctcttgga gttacaagtt atttcacttg agtccggaga tgcaagtatt      240

catgatacag tagaaaatct gatcatccta gcaaacaaca gtttgtcttc taatgggaat      300

gtaacagaat ctggatgcaa agaatgtgag gaactggagg aaaaaaatat taaagaattt      360

ttgcagagtt ttgtacatat tgtccaaatg ttcatcaaca cttctaccac gacgccagcg      420

ccgcgaccac caacaccggc gcccaccatc gcgtcgcagc ccctgtccct gcgcccagag      480

gcgtgccggc cagcggcggg gggcgcagtg cacacgaggg ggctggactt cgcctgtgat      540

atctacatct gggcgccctt ggccgggact tgtggggtcc ttctcctgtc actggtatca      600

ccctttactg ctaa                                                        614


<210>  17
<211>  204
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  Membrane-bound IL15
```

```
<400>  17

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15


His Ala Ala Arg Pro Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys
            20                  25                  30


Ile Glu Asp Leu Ile Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr
        35                  40                  45


Glu Ser Asp Val His Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe
    50                  55                  60


Leu Leu Glu Leu Gln Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile
65                  70                  75                  80


His Asp Thr Val Glu Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser
                85                  90                  95


Ser Asn Gly Asn Val Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu
            100                 105                 110


Glu Glu Lys Asn Ile Lys Glu Phe Leu Gln Ser Phe Val His Ile Val
        115                 120                 125


Gln Met Phe Ile Asn Thr Ser Thr Thr Thr Pro Ala Pro Arg Pro Pro
    130                 135                 140


Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu
145                 150                 155                 160


Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp
                165                 170                 175


Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly
            180                 185                 190


Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys
```

        195   200

<210> 18
<211> 1140
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> NKG2D/CD8a/4-1BB/CD3z

<220>
<221> misc_feature
<223> NKG2D/CD8a/4-1BB/CD3z (aka NK16)

<400> 18
atggccttac cagtgaccgc cttgctcctg ccgctggcct tgctgctcca cgccgccagg   60

ccgttattca accaagaagt tcaaattccc ttgaccgaaa gttactgtgg cccatgtcct   120

aaaaactgga tatgttacaa aaataactgc taccaatttt ttgatgagag taaaaactgg   180

tatgagagcc aggcttcttg tatgtctcaa aatgccagcc ttctgaaagt atacagcaaa   240

gaggaccagg atttacttaa actggtgaag tcatatcatt ggatgggact agtacacatt   300

ccaacaaatg gatcttggca gtgggaagat ggctccattc tctcacccaa cctactaaca   360

ataattgaaa tgcagaaggg agactgtgca ctctatgcct cgagctttaa aggctatata   420

gaaaactgtt caactccaaa tacatacatc tgcatgcaaa ggactgtgac cacgacgcca   480

gcgccgcgac caccaacacc ggcgcccacc atcgcgtcgc agcccctgtc cctgcgccca   540

gaggcgtgcc ggccagcggc ggggggcgca gtgcacacga gggggctgga cttcgcctgt   600

gatatctaca tctgggcgcc cttggccggg acttgtgggg tccttctcct gtcactggtt   660

atcacccttt actgcaaacg gggcagaaag aaactcctgt atatattcaa acaaccattt   720

atgagaccag tacaaactac tcaagaggaa gatggctgta gctgccgatt tccagaagaa   780

gaagaaggag gatgtgaact gagagtgaag ttcagcagga gcgcagacgc ccccgcgtac   840

cagcagggcc agaaccagct ctataacgag ctcaatctag gacgaagaga ggagtacgat   900

gttttggaca agagacgtgg ccgggaccct gagatggggg gaaagccgag aaggaagaac   960

cctcaggaag gcctgtacaa tgaactgcag aaagataaga tggcggaggc ctacagtgag   1020

```
attgggatga aaggcgagcg ccggaggggc aaggggcacg atggccttta ccagggtctc     1080

agtacagcca ccaaggacac ctacgacgcc cttcacatgc aggccctgcc ccctcgctaa     1140


<210>  19
<211>  379
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  Amino acid sequence of NKG2D/CD8a/4-1BB/CD3z

<220>
<221>  MISC_FEATURE
<223>  Amino acid sequence of NKG2D/CD8a/4-1BB/CD3z (aka NK16)

<400>  19

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15


His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
            20                  25                  30


Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
        35                  40                  45


Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
    50                  55                  60


Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65                  70                  75                  80


Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95


Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110


Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125
```

```
Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140


Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Thr Thr Thr Pro
145                 150                 155                 160


Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
                165                 170                 175


Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
            180                 185                 190


Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu
        195                 200                 205


Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr
    210                 215                 220


Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe
225                 230                 235                 240


Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg
                245                 250                 255


Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser
            260                 265                 270


Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr
        275                 280                 285


Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys
    290                 295                 300


Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn
305                 310                 315                 320


Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu
                325                 330                 335
```

```
Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly
            340                 345                 350


His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr
        355                 360                 365


Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
    370                 375


<210>  20
<211>  46
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  Amino acid for NCR1 TM/IC

<400>  20

Met Gly Leu Ala Phe Leu Val Leu Val Ala Leu Val Trp Phe Leu Val
1               5                   10                  15


Glu Asp Trp Leu Ser Arg Lys Arg Thr Arg Glu Arg Ala Ser Arg Ala
            20                  25                  30


Ser Thr Trp Glu Gly Arg Arg Arg Leu Asn Thr Gln Thr Leu
        35                  40                  45


<210>  21
<211>  276
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  Full length NCR2

<400>  21

Met Ala Trp Arg Ala Leu His Pro Leu Leu Leu Leu Leu Leu Leu Phe
1               5                   10                  15
```

```
Pro Gly Ser Gln Ala Gln Ser Lys Ala Gln Val Leu Gln Ser Val Ala
            20                  25                  30


Gly Gln Thr Leu Thr Val Arg Cys Gln Tyr Pro Pro Thr Gly Ser Leu
        35                  40                  45


Tyr Glu Lys Lys Gly Trp Cys Lys Glu Ala Ser Ala Leu Val Cys Ile
    50                  55                  60


Arg Leu Val Thr Ser Ser Lys Pro Arg Thr Met Ala Trp Thr Ser Arg
65                  70                  75                  80


Phe Thr Ile Trp Asp Asp Pro Asp Ala Gly Phe Phe Thr Val Thr Met
                85                  90                  95


Thr Asp Leu Arg Glu Glu Asp Ser Gly His Tyr Trp Cys Arg Ile Tyr
            100                 105                 110


Arg Pro Ser Asp Asn Ser Val Ser Lys Ser Val Arg Phe Tyr Leu Val
        115                 120                 125


Val Ser Pro Ala Ser Ala Ser Thr Gln Thr Ser Trp Thr Pro Arg Asp
    130                 135                 140


Leu Val Ser Ser Gln Thr Gln Thr Gln Ser Cys Val Pro Pro Thr Ala
145                 150                 155                 160


Gly Ala Arg Gln Ala Pro Glu Ser Pro Ser Thr Ile Pro Val Pro Ser
                165                 170                 175


Gln Pro Gln Asn Ser Thr Leu Arg Pro Gly Pro Ala Ala Pro Ile Ala
            180                 185                 190


Leu Val Pro Val Phe Cys Gly Leu Leu Val Ala Lys Ser Leu Val Leu
        195                 200                 205


Ser Ala Leu Leu Val Trp Trp Gly Asp Ile Trp Trp Lys Thr Met Met
    210                 215                 220
```

```
Glu Leu Arg Ser Leu Asp Thr Gln Lys Ala Thr Cys His Leu Gln Gln
225                 230                 235                 240


Val Thr Asp Leu Pro Trp Thr Ser Val Ser Ser Pro Val Glu Arg Glu
                245                 250                 255


Ile Leu Tyr His Thr Val Ala Arg Thr Lys Ile Ser Asp Asp Asp Asp
            260                 265                 270


Glu His Thr Leu
        275


<210>  22
<211>  66
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  NCR3 TM/IC domains

<400>  22

Ala Gly Thr Val Leu Leu Leu Arg Ala Gly Phe Tyr Ala Val Ser Phe
1               5                   10                  15


Leu Ser Val Ala Val Gly Ser Thr Val Tyr Tyr Gln Gly Lys Cys Leu
            20                  25                  30


Thr Trp Lys Gly Pro Arg Arg Gln Leu Pro Ala Val Val Pro Ala Pro
        35                  40                  45


Leu Pro Pro Pro Cys Gly Ser Ser Ala His Leu Leu Pro Pro Val Pro
    50                  55                  60


Gly Gly
65


<210>  23
<211>  741
<212>  DNA
```

<213> Homo sapiens

<220>
<221> misc_feature
<223> NKG2D/CD16

<400> 23

```
atggccttac cagtgaccgc cttgctcctg ccgctggcct tgctgctcca cgccgcccgc       60

cccctgttca accaggaagt gcagatcccc ctgaccgagt cctattgtgg cccttgccct      120

aagaattgga tttgctataa aaacaactgc taccagttct ttgacgagtc taagaattgg      180

tatgagtccc aggcctcttg tatgagccag aacgcctctc tgctgaaggt gtacagcaag      240

gaggaccagg atctgctgaa gctggtgaag tcctatcact ggatgggcct ggtgcacatc      300

cccacaaacg gctcttggca gtgggaggac ggctccatcc tgtctcctaa tctgctgacc      360

atcatcgaga tgcagaaggg cgattgcgcc ctgtacgcca gctccttcaa gggctatatc      420

gagaactgca gcacacccaa tacctacatc tgtatgcagc ggacagtgac cacaacccca      480

gcacccaggc cccctacacc tgcaccaacc atcgcaagcc agccactgtc cctgaggcct      540

gaggcatgta ggccagcagc aggaggagca gtgcacacac ggggcctgga cttcgcctgc      600

gatgtgagct tttgtctggt catggtgctg ctgttcgccg tggataccgg cctgtatttt      660

tccgtgaaga caaatatccg gtctagcacc agagactgga aggatcacaa gttcaaatgg      720

aggaaggacc cacaggacaa g                                                741
```

<210> 24
<211> 247
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> NKG2D/CD16

<400> 24

```
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15


His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
```

```
            20                  25                  30

Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
        35                  40                  45

Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
    50                  55                  60

Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65                  70                  75                  80

Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95

Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110

Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125

Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140

Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Thr Thr Thr Pro
145                 150                 155                 160

Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
                165                 170                 175

Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
            180                 185                 190

Thr Arg Gly Leu Asp Phe Ala Cys Asp Val Ser Phe Cys Leu Val Met
        195                 200                 205

Val Leu Leu Phe Ala Val Asp Thr Gly Leu Tyr Phe Ser Val Lys Thr
    210                 215                 220

Asn Ile Arg Ser Ser Thr Arg Asp Trp Lys Asp His Lys Phe Lys Trp
```

```
225                 230                 235                 240


Arg Lys Asp Pro Gln Asp Lys
                245


<210>  25
<211>  870
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  CD8/NKG2DOpt/CD8a/CD16 TM/IC/4-1BB

<400>  25
atggctctgc ccgtcaccgc actgctgctg cctctggctc tgctgctgca cgccgcacga        60

ccactgttca atcaggaagt ccagatcccc ctgacagagt cttactgcgg cccatgtccc       120

aagaactgga tctgctacaa gaacaattgt tatcagttct ttgacgagag caagaactgg       180

tatgagtccc aggcctcttg catgagccag aatgcctctc tgctgaaggt gtacagcaag       240

gaggaccagg atctgctgaa gctggtgaag tcctatcact ggatgggcct ggtgcacatc       300

cctacaaacg gctcttggca gtgggaggac ggctccatcc tgtctccaaa tctgctgacc       360

atcatcgaga tgcagaaggg cgattgcgcc ctgtacgcca gctccttcaa gggctatatc       420

gagaactgct ccacacccaa tacctacatc tgtatgcaga ggaccgtgac cacaacccct       480

gcaccacgcc cccctacacc agcacctacc atcgcaagcc agcctctgtc cctgcggcca       540

gaggcatgta gaccagcagc aggaggagca gtgcacacaa gaggcctgga cttcgcctgc       600

gatgtgagct tttgtctggt catggtgctg ctgttcgccg tggataccgg cctgtacttt       660

tccgtgaaga caaatatcag gtctagcacc cgcgactgga aggatcacaa gtttaagtgg       720

cggaaggacc ctcaggataa gaagcggggc agaaagaagc tgctgtatat cttcaagcag       780

cccttcatgc ggcccgtgca gacaacccag gaggaagacg gctgctcatg tagatttcct       840

gaagaagaag aagggggctg tgaactgtaa                                        870


<210>  26
<211>  289
<212>  PRT
```

```
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  CD8/NKG2DOpt/CD8a/CD16 TM/IC/4-1BB

<400>  26

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15


His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
            20                  25                  30


Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
        35                  40                  45


Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
    50                  55                  60


Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65                  70                  75                  80


Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95


Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110


Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125


Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140


Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Thr Thr Thr Pro
145                 150                 155                 160


Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
                165                 170                 175
```

```
Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
            180                 185                 190


Thr Arg Gly Leu Asp Phe Ala Cys Asp Val Ser Phe Cys Leu Val Met
        195                 200                 205


Val Leu Leu Phe Ala Val Asp Thr Gly Leu Tyr Phe Ser Val Lys Thr
    210                 215                 220


Asn Ile Arg Ser Ser Thr Arg Asp Trp Lys Asp His Lys Phe Lys Trp
225                 230                 235                 240


Arg Lys Asp Pro Gln Asp Lys Lys Arg Gly Arg Lys Lys Leu Leu Tyr
                245                 250                 255


Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu
            260                 265                 270


Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu
        275                 280                 285


Leu



<210>  27
<211>  741
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  NKG2D/NCR1

<400>  27
atggccttac cagtgaccgc cttgctcctg ccgctggcct tgctgctcca cgccgcccgc        60

cctctgttca accaggaagt gcagatccct ctgaccgaaa gctattgcgg accttgccct       120

aagaattgga tttgctataa aaacaactgc taccagttct ttgacgagtc taagaattgg       180

tatgagtctc aggccagctg tatgtcccag aacgcctctc tgctgaaggt gtacagcaag       240
```

```
gaggaccagg atctgctgaa gctggtgaag tcctatcact ggatgggcct ggtgcacatc      300

cccacaaacg gctcttggca gtgggaggac ggctctatcc tgagccctaa tctgctgacc      360

atcatcgaga tgcagaaggg cgattgcgcc ctgtacgcca gctccttcaa gggctatatc      420

gagaactgca gcacacccaa tacctacatc tgtatgcaga ggacagtgac cacaacccca      480

gcaccccgcc cccctacacc tgcaccaacc atcgcaagcc agccactgtc cctgcggcct      540

gaggcctgca gaccagcagc aggaggagca gtgcacaccc ggggcctgga cttcgcctgt      600

gatatgggcc tggcctttct ggtgctggtg gccctggtgt ggtttctggt ggaggattgg      660

ctgtcccgga agagaacaag ggagagggcc tcccgggcct ctacctggga aggaagaagg      720

agactgaaca cccagacact g                                                741


<210>  28
<211>  247
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  NKG2D/NCR1

<400>  28

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15


His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
            20                  25                  30


Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
        35                  40                  45


Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
    50                  55                  60


Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65                  70                  75                  80


Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
```

```
                85                  90                  95


Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110


Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125


Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140


Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Thr Thr Thr Pro
145                 150                 155                 160


Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
                165                 170                 175


Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
            180                 185                 190


Thr Arg Gly Leu Asp Phe Ala Cys Asp Met Gly Leu Ala Phe Leu Val
        195                 200                 205


Leu Val Ala Leu Val Trp Phe Leu Val Glu Asp Trp Leu Ser Arg Lys
    210                 215                 220


Arg Thr Arg Glu Arg Ala Ser Arg Ala Ser Thr Trp Glu Gly Arg Arg
225                 230                 235                 240


Arg Leu Asn Thr Gln Thr Leu
                245


<210>  29
<211>  801
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  NKG2D/NCR3
```

<400> 29
atggccttac cagtgaccgc cttgctcctg ccgctggcct tgctgctcca cgccgccaga   60

cccctgttca accaggaggt gcagattccc ctgacagaaa gctattgtgg cccttgccct   120

aaaaattgga tttgctataa aaacaactgc taccagttct ttgacgagtc taagaattgg   180

tatgagtctc aggccagctg tatgtcccag aacgcctctc tgctgaaggt gtacagcaag   240

gaggaccagg atctgctgaa gctggtgaag tcctatcact ggatgggcct ggtgcacatc   300

cctacaaacg gctcttggca gtgggaggac ggctctatcc tgagcccaaa tctgctgacc   360

atcatcgaga tgcagaaggg cgattgcgcc ctgtacgcca gctccttcaa gggctatatc   420

gagaactgca gcacacccaa tacctacatc tgtatgcagc ggacagtgac cacaacccca   480

gcacccagac cccctacacc tgcaccaacc atcgccagcc agccactgtc cctgaggccc   540

gaggcatgca ggcctgcagc aggaggcgcc gtgcacacaa ggggcctgga ctttgcctgt   600

gatgcaggaa ccgtgctgct gctgagagca ggcttctatg ccgtgtcctt tctgtctgtg   660

gccgtgggct ccacagtgta ctatcagggc aagtgcctga cctggaaggg cccacggaga   720

cagctgcccg ccgtggtgcc cgcccctctg ccacccccctt gtggcagtag cgcccacctg   780

ctgccacccg tgcccggagg a   801

<210> 30
<211> 267
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> NKG2D/NCR3

<400> 30

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1   5   10   15

His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
            20   25   30

Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn

```
        35                  40                  45


Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
    50                  55                  60


Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65                  70                  75                  80


Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95


Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110


Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125


Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140


Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Thr Thr Thr Pro
145                 150                 155                 160


Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
                165                 170                 175


Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
            180                 185                 190


Thr Arg Gly Leu Asp Phe Ala Cys Asp Ala Gly Thr Val Leu Leu Leu
        195                 200                 205


Arg Ala Gly Phe Tyr Ala Val Ser Phe Leu Ser Val Ala Val Gly Ser
    210                 215                 220


Thr Val Tyr Tyr Gln Gly Lys Cys Leu Thr Trp Lys Gly Pro Arg Arg
225                 230                 235                 240


Gln Leu Pro Ala Val Val Pro Ala Pro Leu Pro Pro Pro Cys Gly Ser
```

```
                245                 250                 255


Ser Ala His Leu Leu Pro Pro Val Pro Gly Gly
            260                 265



<210>  31
<211>  6
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<222>  (6)..(6)
<223>  N is an integer indicating the number of GGGGS repeated

<400>  31

Gly Gly Gly Gly Ser Asn
1               5


<210>  32
<211>  60
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  GS3/CD8a

<400>  32

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Thr
1               5                   10                  15


Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser
            20                  25                  30


Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly
        35                  40                  45


Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp
    50                  55                  60
```

```
<210>  33
<211>  45
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  GS9

<400>  33

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1               5                   10                  15


Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
            20                  25                  30


Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        35                  40                  45


<210>  34
<211>  15
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  GS3

<400>  34

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1               5                   10                  15


<210>  35
<211>  120
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  2B4 ICR

<400>  35
```

```
Trp Arg Arg Lys Arg Lys Glu Lys Gln Ser Glu Thr Ser Pro Lys Glu
1               5                   10                  15


Phe Leu Thr Ile Tyr Glu Asp Val Lys Asp Leu Lys Thr Arg Arg Asn
            20                  25                  30


His Glu Gln Glu Gln Thr Phe Pro Gly Gly Gly Ser Thr Ile Tyr Ser
        35                  40                  45


Met Ile Gln Ser Gln Ser Ser Ala Pro Thr Ser Gln Glu Pro Ala Tyr
    50                  55                  60


Thr Leu Tyr Ser Leu Ile Gln Pro Ser Arg Lys Ser Gly Ser Arg Lys
65                  70                  75                  80


Arg Asn His Ser Pro Ser Phe Asn Ser Thr Ile Tyr Glu Val Ile Gly
                85                  90                  95


Lys Ser Gln Pro Lys Ala Gln Asn Pro Ala Arg Leu Ser Arg Lys Glu
            100                 105                 110


Leu Glu Asn Phe Asp Val Tyr Ser
        115                 120


<210>  36
<211>  360
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  2B4 ICR

<400>  36
tggaggagga aaaggaagga gaaacagagc gagacctccc ctaaggagtt cctgaccatc       60

tacgaggacg tgaaggacct gaagaccagg aggaaccacg agcaggaaca gacctttcct      120

ggcggaggca gcaccatcta cagcatgatc cagagccaga gcagcgcccc taccagccaa      180

gagcctgcct acaccctgta cagcctgatc cagcccagca ggaaaagcgg ctccaggaag      240

aggaaccaca gccccagctt caacagcacc atctatgagg tgatcggcaa gagccagccc      300
```

```
aaggcccaga accctgccag gctgtccagg aaggagctgg agaacttcga cgtgtacagc      360


<210>  37
<211>  38
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  NKp80 ICR

<400>  37

Met Gln Asp Glu Asp Gly Tyr Met Thr Leu Asn Val Gln Ser Lys Lys
1               5                   10                  15


Arg Ser Ser Ala Gln Thr Ser Gln Leu Thr Phe Lys Asp Tyr Ser Val
            20                  25                  30


Thr Leu His Trp Tyr Lys
        35


<210>  38
<211>  114
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  NKp80 ICR

<400>  38
atgcaggatg aggacggcta tatgaccctg aacgtccagt ccaagaagag gtccagcgct       60

cagaccagcc agctgacctt caaggactac tccgtgaccc tgcactggta caag            114


<210>  39
<211>  30
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
```

```
<223>  B2Ad N-term ECD

<400>  39

Met Gly Gln Pro Gly Asn Gly Ser Ala Phe Leu Leu Ala Pro Asn Arg
1               5                   10                  15


Ser His Ala Pro Asp His Asp Val Thr Gln Gln Arg Asp Glu
            20                  25                  30


<210>  40
<211>  90
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  B2 AdR N-term ECD

<400>  40
atggggcaac ccgggaacgg cagcgccttc ttgctggcac ccaatagaag ccatgcgccg       60

gaccacgacg tcacgcagca aagggacgag                                        90


<210>  41
<211>  33
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  B2 AdR TM helix

<400>  41

Val Trp Val Val Gly Met Gly Ile Val Met Ser Leu Ile Val Leu Ala
1               5                   10                  15


Ile Val Phe Gly Asn Val Leu Val Ile Thr Ala Ile Ala Lys Phe Glu
            20                  25                  30


Arg
```

<210> 42
<211> 99
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> B2AdR TM helix

<400> 42
gtgtgggtgg tgggcatggg catcgtcatg tctctcatcg tcctggccat cgtgtttggc   60

aatgtgctgg tcatcacagc cattgccaag ttcgagcgt   99

<210> 43
<211> 924
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> NK15_1

<400> 43
gccgccacca tggctctgcc cgtcaccgca ctgctgctgc ctctggctct gctgctgcac   60

gccgcacgac cactgttcaa tcaggaagtc cagatccccc tgacagagtc ttactgcggc   120

ccatgtccca agaactggat ctgctacaag aacaattgtt atcagttctt tgacgagagc   180

aagaactggt atgagtccca ggcctcttgc atgagccaga atgcctctct gctgaaggtg   240

tacagcaagg aggaccagga tctgctgaag ctggtgaagt cctatcactg gatgggcctg   300

gtgcacatcc ctacaaacgg ctcttggcag tgggaggacg gctccatcct gtctccaaat   360

ctgctgacca tcatcgagat gcagaagggc gattgcgccc tgtacgccag ctccttcaag   420

ggctatatcg agaactgctc cacacccaat acctacatct gtatgcagag gaccgtgggt   480

ggcggtggct cgggcggtgg tgggtcgggt ggcggcggat ctaccacaac ccctgcacca   540

cgccccccta caccagcacc taccatcgca agccagcctc tgtccctgcg gccagaggca   600

tgtagaccag cagcaggagg agcagtgcac acaagaggcc tggacttcgc ctgcgatgtg   660

agcttttgtc tggtcatggt gctgctgttc gccgtggata ccggcctgta cttttccgtg   720

aagacaaata tcaggtctag cacccgcgac tggaaggatc acaagtttaa gtggcggaag   780 gaccctcagg ataagaagcg gggcagaaag aagctgctgt atatcttcaa gcagcccttc   840

atgcggcccg tgcagacaac ccaggaggaa gacggctgct catgtagatt tcctgaagaa   900

gaagaagggg gctgtgaact gtaa   924

<210> 44
<211> 789
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> NK15_2

<400> 44
gccgccacca tggctctgcc cgtcaccgca ctgctgctgc ctctggctct gctgctgcac   60

gccgcacgac cactgttcaa tcaggaagtc cagatccccc tgacagagtc ttactgcggc   120

ccatgtccca agaactggat ctgctacaag aacaattgtt atcagttctt tgacgagagc   180

aagaactggt atgagtccca ggcctcttgc atgagccaga atgcctctct gctgaaggtg   240

tacagcaagg aggaccagga tctgctgaag ctggtgaagt cctatcactg gatgggcctg   300

gtgcacatcc ctacaaacgg ctcttggcag tgggaggacg gctccatcct gtctccaaat   360

ctgctgacca tcatcgagat gcagaagggc gattgcgccc tgtacgccag ctccttcaag   420

ggctatatcg agaactgctc cacacccaat acctacatct gtatgcagag gaccgtgggt   480

ggcggtggct cgggcggtgg tgggtcgggt ggcggcggat ctgtgagctt ttgtctggtc   540

atggtgctgc tgttcgccgt ggataccggc ctgtactttt ccgtgaagac aaatatcagg   600

tctagcaccc gcgactggaa ggatcacaag tttaagtggc ggaaggaccc tcaggataag   660

aagcggggca gaaagaagct gctgtatatc ttcaagcagc ccttcatgcg gcccgtgcag   720

acaacccagg aggaagacgg ctgctcatgt agatttcctg aagaagaaga agggggctgt   780

gaactgtaa   789

<210> 45
<211> 744
<212> DNA
<213> Homo sapiens

```
<220>
<221>  misc_feature
<223>  NK15_3

<400>  45
gccgccacca tggctctgcc cgtcaccgca ctgctgctgc ctctggctct gctgctgcac        60

gccgcacgac cactgttcaa tcaggaagtc cagatccccc tgacagagtc ttactgcggc       120

ccatgtccca agaactggat ctgctacaag aacaattgtt atcagttctt tgacgagagc       180

aagaactggt atgagtccca ggcctcttgc atgagccaga atgcctctct gctgaaggtg       240

tacagcaagg aggaccagga tctgctgaag ctggtgaagt cctatcactg gatgggcctg       300

gtgcacatcc ctacaaacgg ctcttggcag tgggaggacg gctccatcct gtctccaaat       360

ctgctgacca tcatcgagat gcagaagggc gattgcgccc tgtacgccag ctccttcaag       420

ggctatatcg agaactgctc cacacccaat acctacatct gtatgcagag gaccgtggtg       480

agcttttgtc tggtcatggt gctgctgttc gccgtggata ccggcctgta cttttccgtg       540

aagacaaata tcaggtctag cacccgcgac tggaaggatc acaagtttaa gtggcggaag       600

gaccctcagg ataagaagcg gggcagaaag aagctgctgt atatcttcaa gcagcccttc       660

atgcggcccg tgcagacaac ccaggaggaa gacggctgct catgtagatt tcctgaagaa       720

gaagaagggg gctgtgaact gtaa                                              744


<210>  46
<211>  1164
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  NK15_4

<400>  46
gccgccacca tggccctgcc tgtgacagcc ctgctgctgc ctctggctct gctgctgcac        60

gctgccagac ccttattcaa ccaagaagtt caaattccct tgaccgaaag ttactgtggc       120

ccatgtccta aaaactggat atgttacaaa aataactgct accaattttt tgatgagagt       180

aaaaactggt atgagagcca ggcttcttgt atgtctcaaa atgccagcct tctgaaagta       240
```

tacagcaaag aggaccagga tttacttaaa ctggtgaagt catatcattg gatgggacta 300

gtacacattc caacaaatgg atcttggcag tgggaagatg gctccattct ctcacccaac 360

ctactaacaa taattgaaat gcagaaggga gactgtgcac tctatgcctc gagctttaaa 420

ggctatatag aaaactgttc aactccaaat acgtacatct gcatgcaaag gactgtgacc 480

acaacccccg ctcccagacc tcctacccct gcccctacaa tcgccagcca gcccctgagc 540

ctgagacccg aagcctgtag acctgctgcc ggaggcgctg tgcacacaag aggcctggac 600

ttcgcctgcg atatctatat ctgggcccct ctggctggaa cctgtggcgt gctgctgctg 660

agcctggtga ttaccaagag gggcaggaag aagctgctgt acatcttcaa gcagcctttc 720

atgaggcccg tgcaaaccac ccaggaggag gacggctgca gctgcagatt ccctgaggag 780

gaggagggcg gatgcgagct gtggaggagg aaaaggaagg agaaacagag cgagacctcc 840

cctaaggagt tcctgaccat ctacgaggac gtgaaggacc tgaagaccag gaggaaccac 900

gagcaggaac agacctttcc tggcggaggc agcaccatct acagcatgat ccagagccag 960

agcagcgccc ctaccagcca agagcctgcc tacaccctgt acagcctgat ccagcccagc 1020

aggaaaagcg gctccaggaa gaggaaccac agccccagct tcaacagcac catctatgag 1080

gtgatcggca agagccagcc caaggcccag aaccctgcca ggctgtccag gaaggagctg 1140

gagaacttcg acgtgtacag ctga 1164

<210> 47
<211> 1155
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> NK15_5

<400> 47
gccgccacca tggccctgcc tgtgacagcc ctgctgctgc ctctggctct gctgctgcac 60

gctgccagac ccttattcaa ccaagaagtt caaattccct tgaccgaaag ttactgtggc 120

ccatgtccta aaaactggat atgttacaaa aataactgct accaattttt tgatgagagt 180

aaaaactggt atgagagcca ggcttcttgt atgtctcaaa atgccagcct tctgaaagta 240 tacagcaaag aggaccagga tttacttaaa ctggtgaagt catatcattg gatgggacta 300

gtacacattc caacaaatgg atcttggcag tgggaagatg gctccattct ctcacccaac 360

ctactaacaa taattgaaat gcagaaggga gactgtgcac tctatgcctc gagctttaaa 420

ggctatatag aaaactgttc aactccaaat acgtacatct gcatgcaaag gactgtgatg 480

ggacagcctg gaaacggcag cgccttcctg ctggccccta acagaagcca cgcccccgat 540

cacgatgtga cccagcagag ggacgaggtg tgggtggtgg gcatgggcat cgtgatgagc 600

ctgatcgtgc tggctatcgt gttcggcaac gtgctggtga tcaccgccat cgccaagttc 660

gagaggaaga ggggcaggaa aaagctgctc tacatcttca agcagccctt catgaggccc 720

gtgcagacca cccaggaaga ggatggctgc tcctgtaggt ttcccgagga ggaggagggc 780

ggctgtgagc tgtggaggag aaaaaggaag gagaagcaga gcgagaccag ccccaaggag 840

ttcctgacca tctacgagga cgtgaaggac ctgaagacca ggaggaacca cgagcaggaa 900

cagaccttcc ccggcggagg cagcaccatc tacagcatga tccagagcca gtccagcgcc 960

cccacaagcc aggaacccgc ctacacactg tatagcctga tccagccctc caggaagagc 1020

ggcagcagga agaggaacca cagccccagc ttcaacagca ccatttacga ggtgatcgga 1080

aagagccagc ccaaggctca gaaccccgcc aggctgagca ggaaggagct cgaaaacttc 1140

gacgtgtaca gctga 1155

<210> 48
<211> 1349
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> NK15_6

<400> 48
ggatccgaat tcgccgccac catggccctg cctgtgacag ccctgctgct gcctctggct 60

ctgctgctgc acgctgccag acccttattc aaccaagaag ttcaaattcc cttgaccgaa 120

agttactgtg gcccatgtcc taaaaactgg atatgttaca aaaataactg ctaccaattt 180

tttgatgaga gtaaaaactg gtatgagagc caggcttctt gtatgtctca aaatgccagc 240 cttctgaaag tatacagcaa agaggaccag gatttactta aactggtgaa gtcatatcat 300

tggatgggac tagtacacat tccaacaaat ggatcttggc agtgggaaga tggctccatt 360

ctctcaccca acctactaac aataattgaa atgcagaagg gagactgtgc actctatgcc 420

tcgagcttta aaggctatat agaaaactgt tcaactccaa atacgtacat ctgcatgcaa 480

aggactgtga ccacaacccc tgctcccaga cctcccacac ccgcccctac aatcgcctcc 540

cagcctctga gcctgagacc cgaagcctgt agacctgccg ccggcggagc tgtgcataca 600

agaggcctgg acttcgcctg cgacatctac atctgggccc ctctggctgg cacatgcgga 660

gtcctgctgc tgagcctggt gatcaccaag aggggcagga agaagctgct gtacatcttc 720

aagcagccct tcatgaggcc tgtgcagacc acacaggagg aggacggctg ctcctgcagg 780

ttccctgagg aggaggaggg aggctgcgag ctgtggagga ggaagagaaa ggagaagcag 840

tccgagacct cccccaagga gttcctcacc atttacgagg acgtgaagga cctgaagacc 900

aggagaaacc acgagcagga acaaaccttc cccggcggcg gcagcaccat ctacagcatg 960

atccagagcc agtcctccgc ccctacaagc caggagcctg cctacaccct gtacagcctg 1020

atccagccta gcaggaagag cggctccagg aagaggaacc actcccccag cttcaacagc 1080

accatttatg aggtgatcgg caagtcccag cccaaggccc agaaccctgc cagactgtcc 1140

aggaaggagc tggagaactt cgacgtctac tccggcggcg gcggcagcgg cggaggaggc 1200

tccggaggag gcggcagcat gcaggatgag gacggctata tgaccctgaa cgtccagtcc 1260

aagaagaggt ccagcgctca gaccagccag ctgaccttca aggactactc cgtgaccctg 1320

cactggtaca agtgagcggc cgcgtcgac 1349

<210> 49
<211> 989
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> NK15_7

<400> 49
ggatccgaat tcgccgccac catggccctg cctgtgacag ccctgctgct gcctctggct 60

ctgctgctgc atgccgccag acccttattc aaccaagaag ttcaaattcc cttgaccgaa 120

agttactgtg gcccatgtcc taaaaactgg atatgttaca aaaataactg ctaccaattt 180

tttgatgaga gtaaaaactg gtatgagagc caggcttctt gtatgtctca aaatgccagc 240

cttctgaaag tatacagcaa agaggaccag gatttactta aactggtgaa gtcatatcat 300

tggatgggac tagtacacat tccaacaaat ggatcttggc agtgggaaga tggctccatt 360

ctctcaccca acctactaac aataattgaa atgcagaagg gagactgtgc actctatgcc 420

tcgagcttta aaggctatat agaaaactgt tcaactccaa atacgtacat ctgcatgcaa 480

aggactgtga ccaccacccc tgctcccaga ccccctacac ctgcccctac aatcgccagc 540

cagcccctga gcctgagacc tgaggcctgc agacctgctg ctggaggcgc tgtgcacaca 600

aggggcctcg acttcgcctg cgacatctac atctgggccc ctctggccgg cacatgtgga 660

gtgctgctgc tgtccctggt gatcaccaag aggggcagga agaagctgct gtacatcttc 720

aagcagccct tcatgaggcc cgtgcagacc acccaggagg aggacggctg ctcctgcaga 780

ttccccgagg aggaggaggg cggatgtgaa ctgggcggag gaggcagcgg cggcggcggc 840

agcggcggcg gcggcagcat gcaggatgag gacggctaca tgaccctgaa cgtgcagagc 900

aagaagagga gcagcgccca gaccagccag ctgaccttca aggactacag cgtgaccctg 960

cactggtaca agtgagcggc cgcgtcgac 989

<210> 50
<211> 1430
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> NK15_8

<400> 50
ggatccgaat tcgccgccac catggccctg cccgtgacag ctctgctgct gcctctggcc 60

ctgctgctgc atgccgctag acccctgttc aaccaggagg tgcagatccc cctgaccgaa 120

agctactgcg gcccctgccc caagaactgg atctgttaca agaacaactg ctatcagttc 180

ttcgacgaga gcaagaactg gtacgagagc caggccagct gtatgagcca gaacgccagc 240 ctgctgaaag tgtatagcaa ggaggaccag gacctgctga agctggtgaa gagctaccac 300

tggatgggcc tggtgcacat ccccaccaac ggaagctggc agtgggagga cggcagcatc 360

ctgagcccca acctgctgac catcatcgag atgcagaagg gcgactgcgc cctgtatgcc 420

agcagcttca agggctacat cgagaactgt agcacccccca acacctacat ctgcatgcag 480

aggaccgtgg gcggcggcgg cagcggcgga ggcggctccg gcggcggcgg cagcttattc 540

aaccaagaag ttcaaattcc cttgaccgaa agttactgtg gcccatgtcc taaaaactgg 600

atatgttaca aaaataactg ctaccaattt tttgatgaga gtaaaaactg gtatgagagc 660

caggcttctt gtatgtctca aaatgccagc cttctgaaag tatacagcaa agaggaccag 720

gatttactta aactggtgaa gtcatatcat tggatgggac tagtacacat tccaacaaat 780

ggatcttggc agtgggaaga tggctccatt ctctcaccca acctactaac aataattgaa 840

atgcagaagg gagactgtgc actctatgcc tcgagcttta aaggctatat agaaaactgt 900

tcaactccaa atacgtacat ctgcatgcaa aggactgtga tgggccagcc tggcaacggc 960

agcgcctttc tgctggcccc caacaggagc catgcccctg accacgacgt gacccagcag 1020

agggacgagg tgtgggtggt gggcatgggc atcgtgatga gcctgatcgt gctggccatc 1080

gtgttcggca acgtgctggt gatcaccgcc atcgccaagt tcgagaggaa gaggggcagg 1140

aagaagctgc tgtacatctt caagcagccc ttcatgagac ccgtgcaaac cacccaggag 1200

gaggacggct gcagctgcag gtttcccgag gaggaggagg gcggatgcga actgggaggc 1260

ggaggaagcg gaggaggagg atccggagga ggcggaagca tgcaggacga ggacggctac 1320

atgaccctga acgtccagag caagaagagg agcagcgccc agacctccca gctgaccttc 1380

aaggactact ccgtgaccct gcactggtac aagtgagcgg ccgcgtcgac 1430

<210> 51
<211> 1439
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> NK15_9

<400> 51
ggatccgaat tcgccgccac catggccctg cccgtgacag ctctgctgct gcctctggcc   60

ctgctgctgc atgccgctag acccctgttc aaccaggagg tgcagatccc cctgaccgaa   120

agctactgcg gcccctgccc caagaactgg atctgttaca agaacaactg ctatcagttc   180

ttcgacgaga gcaagaactg gtacgagagc caggccagct gtatgagcca gaacgccagc   240

ctgctgaaag tgtatagcaa ggaggaccag gacctgctga agctggtgaa gagctaccac   300

tggatgggcc tggtgcacat ccccaccaac ggaagctggc agtgggagga cggcagcatc   360

ctgagcccca acctgctgac catcatcgag atgcagaagg gcgactgcgc cctgtatgcc   420

agcagcttca agggctacat cgagaactgt agcacccccca acacctacat ctgcatgcag   480

aggaccgtgg gcggcggcgg cagcggcgga ggcggctccg gcggcggcgg cagcttattc   540

aaccaagaag ttcaaattcc cttgaccgaa agttactgtg gcccatgtcc taaaaactgg   600

atatgttaca aaaataactg ctaccaattt tttgatgaga gtaaaaactg gtatgagagc   660

caggcttctt gtatgtctca aaatgccagc cttctgaaag tatacagcaa agaggaccag   720

gatttactta aactggtgaa gtcatatcat tggatgggac tagtacacat tccaacaaat   780

ggatcttggc agtgggaaga tggctccatt ctctcaccca acctactaac aataattgaa   840

atgcagaagg gagactgtgc actctatgcc tcgagcttta aaggctatat agaaaactgt   900

tcaactccaa atacgtacat ctgcatgcaa aggactgtga ccaccacccc tgctcccaga   960

ccccctacac ctgcccctac aatcgccagc cagcccctga gcctgagacc tgaggcctgc   1020

agacctgctg ctggaggcgc tgtgcacaca aggggcctcg acttcgcctg cgacatctac   1080

atctgggccc ctctggccgg cacatgtgga gtgctgctgc tgtccctggt gatcaccaag   1140

aggggcagga agaagctgct gtacatcttc aagcagccct tcatgaggcc cgtgcagacc   1200

acccaggagg aggacggctg ctcctgcaga ttccccgagg aggaggaggg cggatgtgaa   1260

ctgggcggag gaggcagcgg cggcggcggc agcggcggcg gcggcagcat gcaggatgag   1320

gacggctaca tgaccctgaa cgtgcagagc aagaagagga gcagcgccca gaccagccag   1380

ctgaccttca aggactacag cgtgaccctg cactggtaca agtgagcggc cgcgtcgac   1439

<210> 52
<211> 1329

<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> NK15_10

<400> 52
gccgccacaa tggccctgcc tgtgacagcc ctgctgctgc ctctggccct gctgctgcat   60

gctgccaggc ctctgttcaa ccaggaggtg cagatccctc tgaccgagag ctactgcggc   120

ccctgcccca agaactggat ctgctacaag aacaactgct accagttctt cgacgagagc   180

aagaactggt acgagagcca ggccagctgc atgtcccaga acgctagcct gctgaaggtg   240

tatagcaagg aggaccagga cctgctgaag ctggtgaaga gctaccactg gatgggcctg   300

gtgcacatcc ccaccaacgg ctcctggcag tgggaggacg gcagcatcct gagccctaac   360

ctgctgacca tcatcgagat gcagaaggga gactgcgccc tgtacgccag ctcctttaag   420

ggctacatcg agaactgcag cacccccaac acctacatct gtatgcagag gaccgtggga   480

ggcggcggca gcggcggcgg cggcagcggc ggcggcggca gcttattcaa ccaagaagtt   540

caaattccct tgaccgaaag ttactgtggc ccatgtccta aaaactggat atgttacaaa   600

aataactgct accaattttt tgatgagagt aaaaactggt atgagagcca ggcttcttgt   660

atgtctcaaa atgccagcct tctgaaagta tacagcaaag aggaccagga tttacttaaa   720

ctggtgaagt catatcattg gatgggacta gtacacattc caacaaatgg atcttggcag   780

tgggaagatg gctccattct ctcacccaac ctactaacaa taattgaaat gcagaaggga   840

gactgtgcac tctatgcctc gagctttaaa ggctatatag aaaactgttc aactccaaat   900

acgtacatct gcatgcaaag gactgtgacc accacccctg cccctagacc ccctacacct   960

gcccctacca tcgccagcca gcctctgagc ctgagacccg aggcctgtag acctgctgcc   1020

ggaggagccg tgcacacaag aggcctggac ttcgcctgcg acgtgagctt ctgcctggtg   1080

atggtgctgc tgttcgccgt ggacaccggc ctgtacttca gcgtgaagac caacatcagg   1140

agcagcacca gggactggaa ggaccacaaa ttcaagtgga ggaaggaccc ccaggacaag   1200

aagaggggca ggaagaagct gctgtacatc ttcaagcagc ccttcatgag gcctgtgcag   1260

accacccagg aggaggacgg ctgcagctgc aggttccctg aggaggaaga gggcggctgc   1320 gagctgtga 1329

<210> 53
<211> 1239
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> NK15_11

<400> 53
gccgccacca tggctctgcc cgtcaccgca ctgctgctgc ctctggctct gctgctgcac 60

gccgcacgac cactgttcaa tcaggaagtc cagatccccc tgacagagtc ttactgcggc 120

ccatgtccca agaactggat ctgctacaag aacaattgtt atcagttctt tgacgagagc 180

aagaactggt atgagtccca ggcctcttgc atgagccaga atgcctctct gctgaaggtg 240

tacagcaagg aggaccagga tctgctgaag ctggtgaagt cctatcactg gatgggcctg 300

gtgcacatcc ctacaaacgg ctcttggcag tgggaggacg gctccatcct gtctccaaat 360

ctgctgacca tcatcgagat gcagaagggc gattgcgccc tgtacgccag ctccttcaag 420

ggctatatcg agaactgctc cacacccaat acctacatct gtatgcagag gaccgtgacc 480

acaacccctg caccacgccc ccctacacca gcacctacca tcgcaagcca gcctctgtcc 540

ctgcggccag aggcatgtag accagcagca ggaggagcag tgcacacaag aggcctggac 600

ttcgcctgcg atgtgagctt ttgtctggtc atggtgctgc tgttcgccgt ggataccggc 660

ctgtactttt ccgtgaagac aaatatcagg tctagcaccc gcgactggaa ggatcacaag 720

tttaagtggc ggaaggaccc tcaggataag aagcggggca gaaagaagct gctgtatatc 780

ttcaagcagc ccttcatgcg gcccgtgcag acaacccagg aggaagacgg ctgctcatgt 840

agatttcctg aagaagaaga agggggctgt gaactgtgga ggaggaaaag gaaggagaaa 900

cagagcgaga cctcccctaa ggagttcctg accatctacg aggacgtgaa ggacctgaag 960

accaggagga accacgagca ggaacagacc tttcctggcg gaggcagcac catctacagc 1020

atgatccaga gccagagcag cgcccctacc agccaagagc ctgcctacac cctgtacagc 1080

ctgatccagc ccagcaggaa aagcggctcc aggaagagga accacagccc cagcttcaac 1140

```
agcaccatct atgaggtgat cggcaagagc cagcccaagg cccagaaccc tgccaggctg     1200

tccaggaagg agctggagaa cttcgacgtg tacagctga                            1239


<210>  54
<211>  1064
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  NK15_12

<400>  54
ggatccgaat tcgccgccac catggctctg cccgtcaccg cactgctgct gcctctggct       60

ctgctgctgc acgccgcacg accactgttc aatcaggaag tccagatccc cctgacagag      120

tcttactgcg gcccatgtcc caagaactgg atctgctaca agaacaattg ttatcagttc      180

tttgacgaga gcaagaactg gtatgagtcc caggcctctt gcatgagcca gaatgcctct      240

ctgctgaagg tgtacagcaa ggaggaccag gatctgctga agctggtgaa gtcctatcac      300

tggatgggcc tggtgcacat ccctacaaac ggctcttggc agtgggagga cggctccatc      360

ctgtctccaa atctgctgac catcatcgag atgcagaagg gcgattgcgc cctgtacgcc      420

agctccttca agggctatat cgagaactgc tccacaccca atacctacat ctgtatgcag      480

aggaccgtga ccacaacccc tgcaccacgc ccccctacac cagcacctac catcgcaagc      540

cagcctctgt ccctgcggcc agaggcatgt agaccagcag caggaggagc agtgcacaca      600

agaggcctgg acttcgcctg cgatgtgagc ttttgtctgg tcatggtgct gctgttcgcc      660

gtggataccg gcctgtactt ttccgtgaag acaaatatca ggtctagcac ccgcgactgg      720

aaggatcaca agtttaagtg gcggaaggac cctcaggata agaagcgggg cagaaagaag      780

ctgctgtata tcttcaagca gcccttcatg cggcccgtgc agacaaccca ggaggaagac      840

ggctgctcat gtagatttcc tgaagaagaa gaagggggct gtgaactggg cggaggaggc      900

agcggcggcg gcggcagcgg cggcggcggc agcatgcagg atgaggacgg ctacatgacc      960

ctgaacgtgc agagcaagaa gaggagcagc gcccagacca gccagctgac cttcaaggac     1020

tacagcgtga ccctgcactg gtacaagtga gcggccgcgt cgac                      1064
```

```
<210>  55
<211>  8
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  FLAG tag

<400>  55

Asp Tyr Lys Asp Asp Asp Asp Lys
1               5


<210>  56
<211>  6
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  His tag

<400>  56

His His His His His His
1               5


<210>  57
<211>  10
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  Myc tag

<400>  57

Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu
1               5                   10


<210>  58
<211>  1499
```

<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> DNA Sequence for Variant 13

<400> 58
atggccttac cagtgaccgc cttgctcctg ccgctggcct tgctgctcca cgccgccagg   60

ccgttattca accaagaagt tcaaattccc ttgaccgaaa gttactgtgg cccatgtcct   120

aaaaactgga tatgttacaa aaataactgc taccaatttt ttgatgagag taaaaactgg   180

tatgagagcc aggcttcttg tatgtctcaa aatgccagcc ttctgaaagt atacagcaaa   240

gaggaccagg atttacttaa actggtgaag tcatatcatt ggatgggact agtacacatt   300

ccaacaaatg gatcttggca gtgggaagat ggctccattc tctcacccaa cctactaaca   360

ataattgaaa tgcagaaggg agactgtgca ctctatgcct cgagctttaa aggctatata   420

gaaaactgtt caactccaaa tacgtacatc tgcatgcaaa ggactgtgac cacgacgcca   480

gcgccgcgac caccaacacc ggcgcccacc atcgcgtcgc agcccctgtc cctgcgccca   540

gaggcgtgcc ggccagcggc ggggggcgca gtgcacacga gggggctgga cttcgcctgt   600

gatatctaca tctgggcgcc cttggccggg acttgtgggg tccttctcct gtcactggtt   660

atcacccttt actgcaaacg gggcagaaag aaactcctgt atatattcaa acaaccattt   720

atgagaccag tacaaactac tcaagaggaa gatggctgta gctgccgatt tccagaagaa   780

gaagaaggag gatgtgaact gtggaggagg aaaaggaagg agaaacagag cgagacctcc   840

cctaaggagt tcctgaccat ctacgaggac gtgaaggacc tgaagaccag gaggaaccac   900

gagcaggaac agacctttcc tggcggaggc agcaccatct acagcatgat ccagagccag   960

agcagcgccc ctaccagcca agagcctgcc tacaccctgt acagcctgat ccagcccagc   1020

aggaaaagcg gctccaggaa gaggaaccac agccccagct tcaacagcac catctatgag   1080

gtgatcggca agagccagcc caaggcccag aaccctgcca ggctgtccag gaaggagctg   1140

gagaacttcg acgtgtacag cagagtgaag ttcagcagga gcgcagacgc ccccgcgtac   1200

cagcagggcc agaaccagct ctataacgag ctcaatctag gacgaagaga ggagtacgat   1260

gttttggaca agagacgtgg ccgggaccct gagatggggg gaaagccgag aaggaagaac   1320

```
cctcaggaag gcctgtacaa tgaactgcag aaagataaga tggcggaggc ctacagtgag     1380

attgggatga aaggcgagcg ccggaggggc aaggggcacg atggccttta ccagggtctc     1440

agtacagcca ccaaggacac ctacgacgcc cttcacatgc aggccctgcc ccctcgcta      1499


<210>  59
<211>  499
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  Amino Acid Sequence for Variant 13

<400>  59

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15


His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
            20                  25                  30


Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
        35                  40                  45


Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
    50                  55                  60


Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65                  70                  75                  80


Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95


Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110


Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125
```

```
Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140


Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Thr Thr Thr Pro
145                 150                 155                 160


Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
                165                 170                 175


Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
            180                 185                 190


Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu
        195                 200                 205


Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr
    210                 215                 220


Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe
225                 230                 235                 240


Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg
                245                 250                 255


Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Trp Arg Arg Lys Arg
            260                 265                 270


Lys Glu Lys Gln Ser Glu Thr Ser Pro Lys Glu Phe Leu Thr Ile Tyr
        275                 280                 285


Glu Asp Val Lys Asp Leu Lys Thr Arg Arg Asn His Glu Gln Glu Gln
    290                 295                 300


Thr Phe Pro Gly Gly Gly Ser Thr Ile Tyr Ser Met Ile Gln Ser Gln
305                 310                 315                 320


Ser Ser Ala Pro Thr Ser Gln Glu Pro Ala Tyr Thr Leu Tyr Ser Leu
                325                 330                 335
```

```
Ile Gln Pro Ser Arg Lys Ser Gly Ser Arg Lys Arg Asn His Ser Pro
            340                 345                 350


Ser Phe Asn Ser Thr Ile Tyr Glu Val Ile Gly Lys Ser Gln Pro Lys
        355                 360                 365


Ala Gln Asn Pro Ala Arg Leu Ser Arg Lys Glu Leu Glu Asn Phe Asp
    370                 375                 380


Val Tyr Ser Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr
385                 390                 395                 400


Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg
                405                 410                 415


Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met
            420                 425                 430


Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu
        435                 440                 445


Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys
    450                 455                 460


Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu
465                 470                 475                 480


Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu
                485                 490                 495


Pro Pro Arg



<210>  60
<211>  870
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
```

<223> DNA Sequence Variant 14

<400> 60
atggccttac cagtgaccgc cttgctcctg ccgctggcct tgctgctcca cgccgccagg 60

ccgttattca accaagaagt tcaaattccc ttgaccgaaa gttactgtgg cccatgtcct 120

aaaaactgga tatgttacaa aaataactgc taccaatttt ttgatgagag taaaaactgg 180

tatgagagcc aggcttcttg tatgtctcaa aatgccagcc ttctgaaagt atacagcaaa 240

gaggaccagg atttacttaa actggtgaag tcatatcatt ggatgggact agtacacatt 300

ccaacaaatg gatcttggca gtgggaagat ggctccattc tctcacccaa cctactaaca 360

ataattgaaa tgcagaaggg agactgtgca ctctatgcct cgagctttaa aggctatata 420

gaaaactgtt caactccaaa tacgtacatc tgcatgcaaa ggactgtgac cacgacgcca 480

gcgccgcgac caccaacacc ggcgcccacc atcgcgtcgc agcccctgtc cctgcgccca 540

gaggcgtgcc ggccagcggc gggggggcgca gtgcacacga gggggctgga cttcgcctgt 600

gatatctaca tctgggcgcc cttggccggg acttgtgggg tccttctcct gtcactggtt 660

atcacccttt actgcaaacg gggcagaaag aaactcctgt atatattcaa acaaccattt 720

atgagaccag tacaaactac tcaagaggaa gatggctgta gctgccgatt tccagaagaa 780

gaagaaggag gatgtgaact gctgtgcgca cgcccacgcc gcagccccgc ccaagatggc 840

aaagtctaca tcaacatgcc aggcaggggc 870

<210> 61
<211> 290
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> Amino Acid Sequence Variant 14

<400> 61

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1   5   10   15

His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
            20   25   30

```
Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
        35                  40                  45


Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
    50                  55                  60


Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65                  70                  75                  80


Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95


Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110


Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125


Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140


Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Thr Thr Thr Pro
145                 150                 155                 160


Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
                165                 170                 175


Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
            180                 185                 190


Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu
        195                 200                 205


Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr
    210                 215                 220


Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe
225                 230                 235                 240
```

```
Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg
                245                 250                 255


Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Leu Cys Ala Arg Pro
            260                 265                 270


Arg Arg Ser Pro Ala Gln Asp Gly Lys Val Tyr Ile Asn Met Pro Gly
        275                 280                 285


Arg Gly
    290


<210>  62
<211>  1230
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  DNA Sequence Variant 15

<400>  62
atggccttac cagtgaccgc cttgctcctg ccgctggcct tgctgctcca cgccgccagg       60

ccgttattca accaagaagt tcaaattccc ttgaccgaaa gttactgtgg cccatgtcct      120

aaaaactgga tatgttacaa aaataactgc taccaatttt ttgatgagag taaaaactgg      180

tatgagagcc aggcttcttg tatgtctcaa aatgccagcc ttctgaaagt atacagcaaa      240

gaggaccagg atttacttaa actggtgaag tcatatcatt ggatgggact agtacacatt      300

ccaacaaatg gatcttggca gtgggaagat ggctccattc tctcacccaa cctactaaca      360

ataattgaaa tgcagaaggg agactgtgca ctctatgcct cgagctttaa aggctatata      420

gaaaactgtt caactccaaa tacgtacatc tgcatgcaaa ggactgtgac cacgacgcca      480

gcgccgcgac caccaacacc ggcgcccacc atcgcgtcgc agcccctgtc cctgcgccca      540

gaggcgtgcc ggccagcggc gggggggcgca gtgcacacga gggggctgga cttcgcctgt      600

gatatctaca tctgggcgcc cttggccggg acttgtgggg tccttctcct gtcactggtt      660

atcacccttt actgcaaacg gggcagaaag aaactcctgt atatattcaa acaaccattt      720
```

atgagaccag tacaaactac tcaagaggaa gatggctgta gctgccgatt tccagaagaa   780

gaagaaggag gatgtgaact gctgtgcgca cgcccacgcc gcagccccgc ccaagatggc   840

aaagtctaca tcaacatgcc aggcaggggc tggaggagga aaaggaagga gaaacagagc   900

gagacctccc ctaaggagtt cctgaccatc tacgaggacg tgaaggacct gaagaccagg   960

aggaaccacg agcaggaaca gacctttcct ggcggaggca gcaccatcta cagcatgatc   1020

cagagccaga gcagcgcccc taccagccaa gagcctgcct acaccctgta cagcctgatc   1080

cagcccagca ggaaaagcgg ctccaggaag aggaaccaca gccccagctt caacagcacc   1140

atctatgagg tgatcggcaa gagccagccc aaggcccaga accctgccag gctgtccagg   1200

aaggagctgg agaacttcga cgtgtacagc   1230

<210> 63
<211> 410
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> Amino Acid Sequence Variant 15

<400> 63

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1   5   10   15

His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
            20   25   30

Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
        35   40   45

Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
    50   55   60

Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65   70   75   80

```
Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95


Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110


Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125


Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140


Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Thr Thr Thr Pro
145                 150                 155                 160


Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
                165                 170                 175


Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
            180                 185                 190


Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu
        195                 200                 205


Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr
    210                 215                 220


Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe
225                 230                 235                 240


Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg
                245                 250                 255


Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Leu Cys Ala Arg Pro
            260                 265                 270


Arg Arg Ser Pro Ala Gln Asp Gly Lys Val Tyr Ile Asn Met Pro Gly
        275                 280                 285
```

```
Arg Gly Trp Arg Arg Lys Arg Lys Glu Lys Gln Ser Glu Thr Ser Pro
    290                 295                 300


Lys Glu Phe Leu Thr Ile Tyr Glu Asp Val Lys Asp Leu Lys Thr Arg
305                 310                 315                 320


Arg Asn His Glu Gln Glu Gln Thr Phe Pro Gly Gly Gly Ser Thr Ile
                325                 330                 335


Tyr Ser Met Ile Gln Ser Gln Ser Ser Ala Pro Thr Ser Gln Glu Pro
            340                 345                 350


Ala Tyr Thr Leu Tyr Ser Leu Ile Gln Pro Ser Arg Lys Ser Gly Ser
        355                 360                 365


Arg Lys Arg Asn His Ser Pro Ser Phe Asn Ser Thr Ile Tyr Glu Val
    370                 375                 380


Ile Gly Lys Ser Gln Pro Lys Ala Gln Asn Pro Ala Arg Leu Ser Arg
385                 390                 395                 400


Lys Glu Leu Glu Asn Phe Asp Val Tyr Ser
                405                 410


<210>  64
<211>  1232
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  DNA Sequence Variant 16

<400>  64
atggccttac cagtgaccgc cttgctcctg ccgctggcct tgctgctcca cgccgccagg        60

ccgttattca accaagaagt tcaaattccc ttgaccgaaa gttactgtgg cccatgtcct       120

aaaaactgga tatgttacaa aaataactgc taccaatttt ttgatgagag taaaaactgg       180

tatgagagcc aggcttcttg tatgtctcaa aatgccagcc ttctgaaagt atacagcaaa       240

gaggaccagg atttacttaa actggtgaag tcatatcatt ggatgggact agtacacatt       300
```

ccaacaaatg gatcttggca gtgggaagat ggctccattc tctcacccaa cctactaaca 360

ataattgaaa tgcagaaggg agactgtgca ctctatgcct cgagctttaa aggctatata 420

gaaaactgtt caactccaaa tacgtacatc tgcatgcaaa ggactgtgac cacgacgcca 480

gcgccgcgac caccaacacc ggcgcccacc atcgcgtcgc agcccctgtc cctgcgccca 540

gaggcgtgcc ggccagcggc gggggggcgca gtgcacacga gggggctgga cttcgcctgt 600

gatatctaca tctgggcgcc cttggccggg acttgtgggg tccttctcct gtcactggtt 660

atcacccttt actgcaaacg gggcagaaag aaactcctgt atatattcaa acaaccattt 720

atgagaccag tacaaactac tcaagaggaa gatggctgta gctgccgatt tccagaagaa 780

gaagaaggag gatgtgaact gtggaggagg aaaaggaagg agaaacagag cgagacctcc 840

cctaaggagt tcctgaccat ctacgaggac gtgaaggacc tgaagaccag gaggaaccac 900

gagcaggaac agacctttcc tggcggaggc agcaccatct acagcatgat ccagagccag 960

agcagcgccc ctaccagcca agagcctgcc tacaccctgt acagcctgat ccagcccagc 1020

aggaaaagcg gctccaggaa gaggaaccac agccccagct tcaacagcac catctatgag 1080

gtgatcggca agagccagcc caaggcccag aaccctgcca ggctgtccag gaaggagctg 1140

gagaacttcg acgtgtacag cctgtgcgca cgcccacgcc gcagccccgc ccaagatggc 1200

aaagtctaca tcaacatgcc aggcaggggc tg 1232

<210> 65
<211> 410
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> Amino Acid Sequence Variant 16

<400> 65

```
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15


His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
            20                  25                  30
```

```
Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
        35                  40                  45

Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
    50                  55                  60

Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65                  70                  75                  80

Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95

Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110

Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125

Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140

Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Thr Thr Thr Pro
145                 150                 155                 160

Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
                165                 170                 175

Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
            180                 185                 190

Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu
        195                 200                 205

Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr
    210                 215                 220

Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe
225                 230                 235                 240
```

```
Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg
                245                 250                 255


Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Trp Arg Arg Lys Arg
            260                 265                 270


Lys Glu Lys Gln Ser Glu Thr Ser Pro Lys Glu Phe Leu Thr Ile Tyr
        275                 280                 285


Glu Asp Val Lys Asp Leu Lys Thr Arg Arg Asn His Glu Gln Glu Gln
    290                 295                 300


Thr Phe Pro Gly Gly Gly Ser Thr Ile Tyr Ser Met Ile Gln Ser Gln
305                 310                 315                 320


Ser Ser Ala Pro Thr Ser Gln Glu Pro Ala Tyr Thr Leu Tyr Ser Leu
                325                 330                 335


Ile Gln Pro Ser Arg Lys Ser Gly Ser Arg Lys Arg Asn His Ser Pro
            340                 345                 350


Ser Phe Asn Ser Thr Ile Tyr Glu Val Ile Gly Lys Ser Gln Pro Lys
        355                 360                 365


Ala Gln Asn Pro Ala Arg Leu Ser Arg Lys Glu Leu Glu Asn Phe Asp
    370                 375                 380


Val Tyr Ser Leu Cys Ala Arg Pro Arg Arg Ser Pro Ala Gln Asp Gly
385                 390                 395                 400


Lys Val Tyr Ile Asn Met Pro Gly Arg Gly
                405                 410


<210>  66
<211>  1587
<212>  DNA
<213>  Homo sapiens
```

<220>
<221> misc_feature
<223> DNA Sequence Variant 17

<400> 66
atggccctgc ccgtgacagc tctgctgctg cctctggccc tgctgctgca tgccgctaga   60

cccctgttca accaggaggt gcagatcccc ctgaccgaaa gctactgcgg cccctgcccc   120

aagaactgga tctgttacaa gaacaactgc tatcagttct tcgacgagag caagaactgg   180

tacgagagcc aggccagctg tatgagccag aacgccagcc tgctgaaagt gtatagcaag   240

gaggaccagg acctgctgaa gctggtgaag agctaccact ggatgggcct ggtgcacatc   300

cccaccaacg gaagctggca gtgggaggac ggcagcatcc tgagccccaa cctgctgacc   360

atcatcgaga tgcagaaggg cgactgcgcc ctgtatgcca gcagcttcaa gggctacatc   420

gagaactgta gcacccccaa cacctacatc tgcatgcaga ggaccgtggg cggcggcggc   480

agcggcggag gcggctccgg cggcggcggc agcttattca accaagaagt tcaaattccc   540

ttgaccgaaa gttactgtgg cccatgtcct aaaaactgga tatgttacaa aaataactgc   600

taccaatttt ttgatgagag taaaaactgg tatgagagcc aggcttcttg tatgtctcaa   660

aatgccagcc ttctgaaagt atacagcaaa gaggaccagg atttacttaa actggtgaag   720

tcatatcatt ggatgggact agtacacatt ccaacaaatg gatcttggca gtgggaagat   780

ggctccattc tctcacccaa cctactaaca ataattgaaa tgcagaaggg agactgtgca   840

ctctatgcct cgagctttaa aggctatata gaaaactgtt caactccaaa tacgtacatc   900

tgcatgcaaa ggactgtgac cacgacgcca gcgccgcgac caccaacacc ggcgcccacc   960

atcgcgtcgc agcccctgtc cctgcgccca gaggcgtgcc ggccagcggc gggggggcgca   1020

gtgcacacga gggggctgga cttcgcctgt gatatctaca tctgggcgcc cttggccggg   1080

acttgtgggg tccttctcct gtcactggtt atcacccttt actgcaaacg gggcagaaag   1140

aaactcctgt atatattcaa acaaccattt atgagaccag tacaaactac tcaagaggaa   1200

gatggctgta gctgccgatt tccagaagaa gaagaaggag gatgtgaact gagagtgaag   1260

ttcagcagga gcgcagacgc ccccgcgtac cagcagggcc agaaccagct ctataacgag   1320

ctcaatctag gacgaagaga ggagtacgat gttttggaca agagacgtgg ccgggaccct   1380

gagatggggg gaaagccgag aaggaagaac cctcaggaag gcctgtacaa tgaactgcag   1440

```
aaagataaga tggcggaggc ctacagtgag attgggatga aaggcgagcg ccggaggggc     1500

aaggggcacg atggccttta ccagggtctc agtacagcca ccaaggacac ctacgacgcc     1560

cttcacatgc aggccctgcc ccctcgc                                         1587
```

<210> 67
<211> 529
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> Amino Acid Sequence Variant 17

<400> 67

```
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15


His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
            20                  25                  30


Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
        35                  40                  45


Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
    50                  55                  60


Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65                  70                  75                  80


Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95


Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110


Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125
```

```
Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140


Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Gly Gly Gly Gly
145                 150                 155                 160


Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Phe Asn Gln Glu
                165                 170                 175


Val Gln Ile Pro Leu Thr Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn
            180                 185                 190


Trp Ile Cys Tyr Lys Asn Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys
        195                 200                 205


Asn Trp Tyr Glu Ser Gln Ala Ser Cys Met Ser Gln Asn Ala Ser Leu
    210                 215                 220


Leu Lys Val Tyr Ser Lys Glu Asp Gln Asp Leu Leu Lys Leu Val Lys
225                 230                 235                 240


Ser Tyr His Trp Met Gly Leu Val His Ile Pro Thr Asn Gly Ser Trp
                245                 250                 255


Gln Trp Glu Asp Gly Ser Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile
            260                 265                 270


Glu Met Gln Lys Gly Asp Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly
        275                 280                 285


Tyr Ile Glu Asn Cys Ser Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg
    290                 295                 300


Thr Val Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr
305                 310                 315                 320


Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala
                325                 330                 335
```

```
Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile
            340                 345                 350


Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser
        355                 360                 365


Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr
    370                 375                 380


Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu
385                 390                 395                 400


Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu
                405                 410                 415


Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln
            420                 425                 430


Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu
        435                 440                 445


Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly
    450                 455                 460


Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln
465                 470                 475                 480


Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu
                485                 490                 495


Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr
            500                 505                 510


Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro
        515                 520                 525


Arg
```

<210> 68
<211> 405
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> DNA Sequence for alternative NKG2D codon-optimized extracellular domain

<400> 68
ctgttcaacc aggaggtgca gatccccctg accgaaagct actgcggccc ctgccccaag 60

aactggatct gttacaagaa caactgctat cagttcttcg acgagagcaa gaactggtac 120

gagagccagg ccagctgtat gagccagaac gccagcctgc tgaaagtgta tagcaaggag 180

gaccaggacc tgctgaagct ggtgaagagc taccactgga tgggcctggt gcacatcccc 240

accaacggaa gctggcagtg ggaggacggc agcatcctga gccccaacct gctgaccatc 300

atcgagatgc agaagggcga ctgcgccctg tatgccagca gcttcaaggg ctacatcgag 360

aactgtagca cccccaacac ctacatctgc atgcagagga ccgtg 405

<210> 69
<211> 21
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> Amino Acid Sequence CD3zeta transmembrane

<400> 69

Leu Cys Tyr Leu Leu Asp Gly Ile Leu Phe Ile Tyr Gly Val Ile Leu
1 5 10 15

Thr Ala Leu Phe Leu
20

<210> 70
<211> 876
<212> DNA
<213> Homo sapiens

```
<220>
<221>  misc_feature
<223>  DNA Sequence Variant 18 (NK39)

<400>  70
atggctctgc ccgtcaccgc actgctgctg cctctggctc tgctgctgca cgccgcacga       60

ccactgttca atcaggaagt ccagatcccc ctgacagagt cttactgcgg cccatgtccc      120

aagaactgga tctgctacaa gaacaattgt tatcagttct ttgacgagag caagaactgg      180

tatgagtccc aggcctcttg catgagccag aatgcctctc tgctgaaggt gtacagcaag      240

gaggaccagg atctgctgaa gctggtgaag tcctatcact ggatgggcct ggtgcacatc      300

cctacaaacg gctcttggca gtgggaggac ggctccatcc tgtctccaaa tctgctgacc      360

atcatcgaga tgcagaaggg cgattgcgcc ctgtacgcca gctccttcaa gggctatatc      420

gagaactgct ccacacccaa tacctacatc tgtatgcaga ggaccgtgac cacaacccct      480

gcaccacgcc cccctacacc agcacctacc atcgcaagcc agcctctgtc cctgcggcca      540

gaggcatgta gaccagcagc aggaggagca gtgcacacaa gaggcctgga cttcgcctgc      600

gatcccaaac tctgctacct gctggatgga atcctcttca tctatggtgt cattctcact      660

gccttgttcc tgaagacaaa tatcaggtct agcacccgcg actggaagga tcacaagttt      720

aagtggcgga aggaccctca ggataagaag cggggcagaa agaagctgct gtatatcttc      780

aagcagccct tcatgcggcc cgtgcagaca acccaggagg aagacggctg ctcatgtaga      840

tttcctgaag aagaagaagg gggctgtgaa ctgtaa                                876


<210>  71
<211>  291
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  Amino Acid Sequence Variant 18 (NK39)

<400>  71

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15
```

```
His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
            20                  25                  30


Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
        35                  40                  45


Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
    50                  55                  60


Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65                  70                  75                  80


Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95


Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110


Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125


Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140


Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Thr Thr Thr Pro
145                 150                 155                 160


Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
                165                 170                 175


Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
            180                 185                 190


Thr Arg Gly Leu Asp Phe Ala Cys Asp Pro Lys Leu Cys Tyr Leu Leu
        195                 200                 205


Asp Gly Ile Leu Phe Ile Tyr Gly Val Ile Leu Thr Ala Leu Phe Leu
    210                 215                 220
```

```
Lys Thr Asn Ile Arg Ser Ser Thr Arg Asp Trp Lys Asp His Lys Phe
225                 230                 235                 240


Lys Trp Arg Lys Asp Pro Gln Asp Lys Lys Arg Gly Arg Lys Lys Leu
                245                 250                 255


Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln
            260                 265                 270


Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly
        275                 280                 285


Cys Glu Leu
    290


<210>  72
<211>  1323
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  DNA Sequence NK39_1

<400>  72
atggccctgc ccgtgacagc tctgctgctg cctctggccc tgctgctgca tgccgctaga        60

cccctgttca accaggaggt gcagatcccc ctgaccgaaa gctactgcgg cccctgcccc       120

aagaactgga tctgttacaa gaacaactgc tatcagttct tcgacgagag caagaactgg       180

tacgagagcc aggccagctg tatgagccag aacgccagcc tgctgaaagt gtatagcaag       240

gaggaccagg acctgctgaa gctggtgaag agctaccact ggatgggcct ggtgcacatc       300

cccaccaacg gaagctggca gtgggaggac ggcagcatcc tgagccccaa cctgctgacc       360

atcatcgaga tgcagaaggg cgactgcgcc ctgtatgcca gcagcttcaa gggctacatc       420

gagaactgta gcacccccaa cacctacatc tgcatgcaga ggaccgtggg cggcggcggc       480

agcggcggag gcggctccgg cggcggcggc agcttattca accaagaagt tcaaattccc       540

ttgaccgaaa gttactgtgg cccatgtcct aaaaactgga tatgttacaa aaataactgc       600
```

```
taccaatttt ttgatgagag taaaaactgg tatgagagcc aggcttcttg tatgtctcaa      660

aatgccagcc ttctgaaagt atacagcaaa gaggaccagg atttacttaa actggtgaag      720

tcatatcatt ggatgggact agtacacatt ccaacaaatg gatcttggca gtgggaagat      780

ggctccattc tctcacccaa cctactaaca ataattgaaa tgcagaaggg agactgtgca      840

ctctatgcct cgagctttaa aggctatata gaaaactgtt caactccaaa tacgtacatc      900

tgcatgcaaa ggactgtgac caccacccct gctcccagac cccctacacc tgcccctaca      960

atcgccagcc agcccctgag cctgagacct gaggcctgca gacctgctgc tggaggcgct     1020

gtgcacacaa ggggcctcga cttcgcctgc gaccccaaac tctgctacct gctggatgga     1080

atcctcttca tctatggtgt cattctcact gccttgttcc tgaagacaaa tatcaggtct     1140

agcacccgcg actggaagga tcacaagttt aagtggcgga aggaccctca ggataagaag     1200

cggggcagaa agaagctgct gtatatcttc aagcagccct tcatgcggcc cgtgcagaca     1260

acccaggagg aagacggctg ctcatgtaga tttcctgaag aagaagaagg gggctgtgaa     1320

ctg                                                                   1323


<210>  73
<211>  441
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  Amino Acid Sequence NK39_1

<400>  73

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15


His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
            20                  25                  30


Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
        35                  40                  45


Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
```

```
    50                  55                  60


Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65                  70                  75                  80


Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95


Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110


Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125


Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140


Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Gly Gly Gly Gly
145                 150                 155                 160


Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Phe Asn Gln Glu
                165                 170                 175


Val Gln Ile Pro Leu Thr Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn
            180                 185                 190


Trp Ile Cys Tyr Lys Asn Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys
        195                 200                 205


Asn Trp Tyr Glu Ser Gln Ala Ser Cys Met Ser Gln Asn Ala Ser Leu
    210                 215                 220


Leu Lys Val Tyr Ser Lys Glu Asp Gln Asp Leu Leu Lys Leu Val Lys
225                 230                 235                 240


Ser Tyr His Trp Met Gly Leu Val His Ile Pro Thr Asn Gly Ser Trp
                245                 250                 255


Gln Trp Glu Asp Gly Ser Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile
```

```
            260                 265                 270

Glu Met Gln Lys Gly Asp Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly
        275                 280                 285

Tyr Ile Glu Asn Cys Ser Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg
    290                 295                 300

Thr Val Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr
305                 310                 315                 320

Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala
                325                 330                 335

Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Pro
            340                 345                 350

Lys Leu Cys Tyr Leu Leu Asp Gly Ile Leu Phe Ile Tyr Gly Val Ile
        355                 360                 365

Leu Thr Ala Leu Phe Leu Lys Thr Asn Ile Arg Ser Ser Thr Arg Asp
    370                 375                 380

Trp Lys Asp His Lys Phe Lys Trp Arg Lys Asp Pro Gln Asp Lys Lys
385                 390                 395                 400

Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg
                405                 410                 415

Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro
            420                 425                 430

Glu Glu Glu Glu Gly Gly Cys Glu Leu
        435                 440


<210>  74
<211>  1032
<212>  DNA
<213>  Homo sapiens
```

<220>
<221> misc_feature
<223> DNA Sequence NK39_2

<400> 74
atggccctgc ccgtgacagc tctgctgctg cctctggccc tgctgctgca tgccgctaga 60

cccttattca accaagaagt tcaaattccc ttgaccgaaa gttactgtgg cccatgtcct 120

aaaaactgga tatgttacaa aaataactgc taccaatttt ttgatgagag taaaaactgg 180

tatgagagcc aggcttcttg tatgtctcaa aatgccagcc ttctgaaagt atacagcaaa 240

gaggaccagg atttacttaa actggtgaag tcatatcatt ggatgggact agtacacatt 300

ccaacaaatg gatcttggca gtgggaagat ggctccattc tctcacccaa cctactaaca 360

ataattgaaa tgcagaaggg agactgtgca ctctatgcct cgagctttaa aggctatata 420

gaaaactgtt caactccaaa tacgtacatc tgcatgcaaa ggactgtgac caccacccct 480

gctcccagac cccctacacc tgcccctaca atcgccagcc agcccctgag cctgagacct 540

gaggcctgca gacctgctgc tggaggcgct gtgcacacaa ggggcctcga cttcgcctgc 600

gaccccaaac tctgctacct gctggatgga atcctcttca tctatggtgt cattctcact 660

gccttgttcc tgaagacaaa tatcaggtct agcacccgcg actggaagga tcacaagttt 720

aagtggcgga aggaccctca ggataagaag cggggcagaa agaagctgct gtatatcttc 780

aagcagccct tcatgcggcc cgtgcagaca acccaggagg aagacggctg ctcatgtaga 840

tttcctgaag aagaagaagg gggctgtgaa ctgggcggag gaggcagcgg cggcggcggc 900

agcggcggcg gcggcagcat gcaggatgag gacggctaca tgaccctgaa cgtgcagagc 960

aagaagagga gcagcgccca gaccagccag ctgaccttca aggactacag cgtgaccctg 1020

cactggtaca ag 1032

<210> 75
<211> 344
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> Amino Acid Sequence NK39_2

```
<400>  75

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15


His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
            20                  25                  30


Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
        35                  40                  45


Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
    50                  55                  60


Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65                  70                  75                  80


Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95


Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110


Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125


Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140


Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Thr Thr Thr Pro
145                 150                 155                 160


Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
                165                 170                 175


Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
            180                 185                 190


Thr Arg Gly Leu Asp Phe Ala Cys Asp Pro Lys Leu Cys Tyr Leu Leu
```

```
        195                 200                 205

Asp Gly Ile Leu Phe Ile Tyr Gly Val Ile Leu Thr Ala Leu Phe Leu
    210                 215                 220

Lys Thr Asn Ile Arg Ser Ser Thr Arg Asp Trp Lys Asp His Lys Phe
225                 230                 235                 240

Lys Trp Arg Lys Asp Pro Gln Asp Lys Lys Arg Gly Arg Lys Lys Leu
                245                 250                 255

Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln
            260                 265                 270

Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly
        275                 280                 285

Cys Glu Leu Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
    290                 295                 300

Gly Ser Met Gln Asp Glu Asp Gly Tyr Met Thr Leu Asn Val Gln Ser
305                 310                 315                 320

Lys Lys Arg Ser Ser Ala Gln Thr Ser Gln Leu Thr Phe Lys Asp Tyr
                325                 330                 335

Ser Val Thr Leu His Trp Tyr Lys
            340


<210>  76
<211>  1416
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  DNA Sequence NK39_3

<400>  76
atggccctgc ccgtgacagc tctgctgctg cctctggccc tgctgctgca tgccgctaga       60
```

ccccctgttca accaggaggt gcagatcccc ctgaccgaaa gctactgcgg cccctgcccc 120

aagaactgga tctgttacaa gaacaactgc tatcagttct tcgacgagag caagaactgg 180

tacgagagcc aggccagctg tatgagccag aacgccagcc tgctgaaagt gtatagcaag 240

gaggaccagg acctgctgaa gctggtgaag agctaccact ggatgggcct ggtgcacatc 300

cccaccaacg gaagctggca gtgggaggac ggcagcatcc tgagccccaa cctgctgacc 360

atcatcgaga tgcagaaggg cgactgcgcc ctgtatgcca gcagcttcaa gggctacatc 420

gagaactgta gcacccccaa cacctacatc tgcatgcaga ggaccgtggg cggcggcggc 480

agcggcggag gcggctccgg cggcggcggc agcttattca accaagaagt tcaaattccc 540

ttgaccgaaa gttactgtgg cccatgtcct aaaaactgga tatgttacaa aaataactgc 600

taccaatttt ttgatgagag taaaaactgg tatgagagcc aggcttcttg tatgtctcaa 660

aatgccagcc ttctgaaagt atacagcaaa gaggaccagg atttacttaa actggtgaag 720

tcatatcatt ggatgggact agtacacatt ccaacaaatg gatcttggca gtgggaagat 780

ggctccattc tctcacccaa cctactaaca ataattgaaa tgcagaaggg agactgtgca 840

ctctatgcct cgagctttaa aggctatata gaaaactgtt caactccaaa tacgtacatc 900

tgcatgcaaa ggactgtgac caccacccct gctcccagac cccctacacc tgcccctaca 960

atcgccagcc agcccctgag cctgagacct gaggcctgca gacctgctgc tggaggcgct 1020

gtgcacacaa ggggcctcga cttcgcctgc gaccccaaac tctgctacct gctggatgga 1080

atcctcttca tctatggtgt cattctcact gccttgttcc tgctttactg caagcggggc 1140

agaaagaagc tgctgtatat cttcaagcag cccttcatgc ggcccgtgca gacaacccag 1200

gaggaagacg gctgctcatg tagatttcct gaagaagaag aagggggctg tgaactgggc 1260

ggaggaggca gcggcggcgg cggcagcggc ggcggcggca gcatgcagga tgaggacggc 1320

tacatgaccc tgaacgtgca gagcaagaag aggagcagcg cccagaccag ccagctgacc 1380

ttcaaggact acagcgtgac cctgcactgg tacaag 1416

<210> 77
<211> 472
<212> PRT
<213> Homo sapiens

```
<220>
<221>  MISC_FEATURE
<223>  Amino Acid Sequence NK39_3

<400>  77

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15


His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
            20                  25                  30


Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
        35                  40                  45


Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
    50                  55                  60


Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65                  70                  75                  80


Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95


Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110


Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125


Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140


Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Gly Gly Gly Gly
145                 150                 155                 160


Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Phe Asn Gln Glu
                165                 170                 175


Val Gln Ile Pro Leu Thr Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn
```

```
            180                 185                 190


Trp Ile Cys Tyr Lys Asn Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys
        195                 200                 205


Asn Trp Tyr Glu Ser Gln Ala Ser Cys Met Ser Gln Asn Ala Ser Leu
    210                 215                 220


Leu Lys Val Tyr Ser Lys Glu Asp Gln Asp Leu Leu Lys Leu Val Lys
225                 230                 235                 240


Ser Tyr His Trp Met Gly Leu Val His Ile Pro Thr Asn Gly Ser Trp
                245                 250                 255


Gln Trp Glu Asp Gly Ser Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile
            260                 265                 270


Glu Met Gln Lys Gly Asp Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly
        275                 280                 285


Tyr Ile Glu Asn Cys Ser Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg
    290                 295                 300


Thr Val Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr
305                 310                 315                 320


Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala
                325                 330                 335


Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Pro
            340                 345                 350


Lys Leu Cys Tyr Leu Leu Asp Gly Ile Leu Phe Ile Tyr Gly Val Ile
        355                 360                 365


Leu Thr Ala Leu Phe Leu Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu
    370                 375                 380


Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln
```

```
385                 390                 395                 400


Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly
                405                 410                 415


Cys Glu Leu Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
            420                 425                 430


Gly Ser Met Gln Asp Glu Asp Gly Tyr Met Thr Leu Asn Val Gln Ser
        435                 440                 445


Lys Lys Arg Ser Ser Ala Gln Thr Ser Gln Leu Thr Phe Lys Asp Tyr
    450                 455                 460


Ser Val Thr Leu His Trp Tyr Lys
465                 470


<210>  78
<211>  807
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  DNA Sequence NK39_4

<400>  78
atggctctgc ccgtcaccgc actgctgctg cctctggctc tgctgctgca cgccgcacga       60

ccactgttca atcaggaagt ccagatcccc ctgacagagt cttactgcgg cccatgtccc      120

aagaactgga tctgctacaa gaacaattgt tatcagttct ttgacgagag caagaactgg      180

tatgagtccc aggcctcttg catgagccag aatgcctctc tgctgaaggt gtacagcaag      240

gaggaccagg atctgctgaa gctggtgaag tcctatcact ggatgggcct ggtgcacatc      300

cctacaaacg gctcttggca gtgggaggac ggctccatcc tgtctccaaa tctgctgacc      360

atcatcgaga tgcagaaggg cgattgcgcc ctgtacgcca gctccttcaa gggctatatc      420

gagaactgct ccacacccaa tacctacatc tgtatgcaga ggaccgtgac cacaacccct      480

gcaccacgcc cccctacacc agcacctacc atcgcaagcc agcctctgtc cctgcggcca      540
```

```
gaggcatgta gaccagcagc aggaggagca gtgcacacaa gaggcctgga cttcgcctgc      600

gatcccaaac tctgctacct gctggatgga atcctcttca tctatggtgt cattctcact      660

gccttgttcc tgctttactg caagcggggc agaaagaagc tgctgtatat cttcaagcag      720

cccttcatgc ggcccgtgca gacaacccag gaggaagacg gctgctcatg tagatttcct      780

gaagaagaag aagggggctg tgaactg                                          807


<210>  79
<211>  269
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  Amino Acid Sequence NK39_4

<400>  79

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15


His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
            20                  25                  30


Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
        35                  40                  45


Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
    50                  55                  60


Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65                  70                  75                  80


Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95


Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110


Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
```

```
        115                 120                 125


Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140


Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Thr Thr Thr Pro
145                 150                 155                 160


Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
                165                 170                 175


Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
            180                 185                 190


Thr Arg Gly Leu Asp Phe Ala Cys Asp Pro Lys Leu Cys Tyr Leu Leu
        195                 200                 205


Asp Gly Ile Leu Phe Ile Tyr Gly Val Ile Leu Thr Ala Leu Phe Leu
    210                 215                 220


Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln
225                 230                 235                 240


Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser
                245                 250                 255


Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu
            260                 265


<210>  80
<211>  1143
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  DNA Sequence NK39_5

<400>  80
atggctctgc ccgtcaccgc actgctgctg cctctggctc tgctgctgca cgccgcacga       60
```

ccactgttca atcaggaagt ccagatcccc ctgacagagt cttactgcgg cccatgtccc 120

aagaactgga tctgctacaa gaacaattgt tatcagttct ttgacgagag caagaactgg 180

tatgagtccc aggcctcttg catgagccag aatgcctctc tgctgaaggt gtacagcaag 240

gaggaccagg atctgctgaa gctggtgaag tcctatcact ggatgggcct ggtgcacatc 300

cctacaaacg gctcttggca gtgggaggac ggctccatcc tgtctccaaa tctgctgacc 360

atcatcgaga tgcagaaggg cgattgcgcc ctgtacgcca gctccttcaa gggctatatc 420

gagaactgct ccacacccaa tacctacatc tgtatgcaga ggaccgtgac cacaacccct 480

gcaccacgcc cccctacacc agcacctacc atcgcaagcc agcctctgtc cctgcggcca 540

gaggcatgta gaccagcagc aggaggagca gtgcacacaa gaggcctgga cttcgcctgc 600

gatcccaaac tctgctacct gctggatgga atcctcttca tctatggtgt cattctcact 660

gccttgttcc tgctttactg caagcggggc agaaagaagc tgctgtatat cttcaagcag 720

cccttcatgc ggcccgtgca gacaacccag gaggaagacg gctgctcatg tagatttcct 780

gaagaagaag aagggggctg tgaactgaga gtgaagttca gcaggagcgc agacgccccc 840

gcgtaccagc agggccagaa ccagctctat aacgagctca atctaggacg aagagaggag 900

tacgatgttt tggacaagag acgtggccgg gaccctgaga tgggggggaaa gccgagaagg 960

aagaaccctc aggaaggcct gtacaatgaa ctgcagaaag ataagatggc ggaggcctac 1020

agtgagattg ggatgaaagg cgagcgccgg aggggcaagg ggcacgatgg cctttaccag 1080

ggtctcagta cagccaccaa ggacacctac gacgcccttc acatgcaggc cctgccccct 1140

cgc 1143

<210> 81
<211> 381
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> Amino Acid Sequence NK39_5

<400> 81

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu

```
1               5                   10                  15

His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
            20                  25                  30

Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
        35                  40                  45

Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
    50                  55                  60

Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65                  70                  75                  80

Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95

Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110

Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125

Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140

Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Thr Thr Thr Pro
145                 150                 155                 160

Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
                165                 170                 175

Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
            180                 185                 190

Thr Arg Gly Leu Asp Phe Ala Cys Asp Pro Lys Leu Cys Tyr Leu Leu
        195                 200                 205

Asp Gly Ile Leu Phe Ile Tyr Gly Val Ile Leu Thr Ala Leu Phe Leu
```

```
    210                 215                 220

Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln
225                 230                 235                 240

Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser
                245                 250                 255

Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys
            260                 265                 270

Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln
        275                 280                 285

Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu
    290                 295                 300

Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg
305                 310                 315                 320

Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met
                325                 330                 335

Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly
            340                 345                 350

Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp
        355                 360                 365

Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
    370                 375                 380
```

<210> 82
<211> 965
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> DNA Sequence NK39_6

```
<400>  82
atggctctgc ccgtcaccgc actgctgctg cctctggctc tgctgctgca cgccgcacga        60

ccactgttca atcaggaagt ccagatcccc ctgacagagt cttactgcgg cccatgtccc       120

aagaactgga tctgctacaa gaacaattgt tatcagttct ttgacgagag caagaactgg       180

tatgagtccc aggcctcttg catgagccag aatgcctctc tgctgaaggt gtacagcaag       240

gaggaccagg atctgctgaa gctggtgaag tcctatcact ggatgggcct ggtgcacatc       300

cctacaaacg gctcttggca gtgggaggac ggctccatcc tgtctccaaa tctgctgacc       360

atcatcgaga tgcagaaggg cgattgcgcc ctgtacgcca gctccttcaa gggctatatc       420

gagaactgct ccacacccaa tacctacatc tgtatgcaga ggaccgtgac cacaacccct       480

gcaccacgcc cccctacacc agcacctacc atcgcaagcc agcctctgtc cctgcggcca       540

gaggcatgta gaccagcagc aggaggagca gtgcacacaa gaggcctgga cttcgcctgc       600

gatcccaaac tctgctacct gctggatgga atcctcttca tctatggtgt cattctcact       660

gccttgttcc tgctttactg caagcggggc agaaagaagc tgctgtatat cttcaagcag       720

cccttcatgc ggcccgtgca gacaacccag gaggaagacg gctgctcatg tagatttcct       780

gaagaagaag aagggggctg tgaactgggc ggaggaggca gcggcggcgg cggcagcggc       840

ggcggcggca gcatgcagga tgaggacggc tacatgaccc tgaacgtgca gagcaagaag       900

aggagcagcg cccagaccag ccagctgacc ttcaaggact acagcgtgac cctgcactgg       960

tacaa                                                                   965


<210>  83
<211>  322
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  Amino Acid Sequence NK39_6

<400>  83

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15
```

```
His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
            20                  25                  30


Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
        35                  40                  45


Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
    50                  55                  60


Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65                  70                  75                  80


Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95


Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110


Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125


Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140


Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Thr Thr Thr Pro
145                 150                 155                 160


Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
                165                 170                 175


Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
            180                 185                 190


Thr Arg Gly Leu Asp Phe Ala Cys Asp Pro Lys Leu Cys Tyr Leu Leu
        195                 200                 205


Asp Gly Ile Leu Phe Ile Tyr Gly Val Ile Leu Thr Ala Leu Phe Leu
    210                 215                 220
```

```
Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln
225                 230                 235                 240


Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser
                245                 250                 255


Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Gly Gly Gly
            260                 265                 270


Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Met Gln Asp Glu
        275                 280                 285


Asp Gly Tyr Met Thr Leu Asn Val Gln Ser Lys Lys Arg Ser Ser Ala
    290                 295                 300


Gln Thr Ser Gln Leu Thr Phe Lys Asp Tyr Ser Val Thr Leu His Trp
305                 310                 315                 320


Tyr Lys



<210>  84
<211>  927
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  DNA Sequence NK39_7

<400>  84
atggctctgc ccgtcaccgc actgctgctg cctctggctc tgctgctgca cgccgcacga       60

ccactgttca atcaggaagt ccagatcccc ctgacagagt cttactgcgg cccatgtccc      120

aagaactgga tctgctacaa gaacaattgt tatcagttct ttgacgagag caagaactgg      180

tatgagtccc aggcctcttg catgagccag aatgcctctc tgctgaaggt gtacagcaag      240

gaggaccagg atctgctgaa gctggtgaag tcctatcact ggatgggcct ggtgcacatc      300

cctacaaacg gctcttggca gtgggaggac ggctccatcc tgtctccaaa tctgctgacc      360
```

atcatcgaga tgcagaaggg cgattgcgcc ctgtacgcca gctccttcaa gggctatatc 420

gagaactgct ccacacccaa tacctacatc tgtatgcaga ggaccgtgac cacaacccct 480

gcaccacgcc cccctacacc agcacctacc atcgcaagcc agcctctgtc cctgcggcca 540

gaggcatgta gaccagcagc aggaggagca gtgcacacaa gaggcctgga cttcgcctgc 600

gatcccaaac tctgctacct gctggatgga atcctcttca tctatggtgt cattctcact 660

gccttgttcc tgctttactg caagcggggc agaaagaagc tgctgtatat cttcaagcag 720

cccttcatgc ggcccgtgca gacaacccag gaggaagacg gctgctcatg tagatttcct 780

gaagaagaag aagggggctg tgaactgggc ggaggaggca gcggcggcgg cggcagcggc 840

ggcggcggca gcaagacaaa tatcaggtct agcacccgcg actggaagga tcacaagttt 900

aagtggcgga aggaccctca ggataag 927

<210> 85
<211> 309
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> Amino Acid Sequence NK39_7

<400> 85

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
20 25 30

Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
35 40 45

Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
50 55 60

Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65 70 75 80

```
Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95


Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110


Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125


Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140


Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Thr Thr Thr Pro
145                 150                 155                 160


Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
                165                 170                 175


Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
            180                 185                 190


Thr Arg Gly Leu Asp Phe Ala Cys Asp Pro Lys Leu Cys Tyr Leu Leu
        195                 200                 205


Asp Gly Ile Leu Phe Ile Tyr Gly Val Ile Leu Thr Ala Leu Phe Leu
    210                 215                 220


Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln
225                 230                 235                 240


Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser
                245                 250                 255


Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Gly Gly Gly
            260                 265                 270


Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Lys Thr Asn Ile
        275                 280                 285
```

Arg Ser Ser Thr Arg Asp Trp Lys Asp His Lys Phe Lys Trp Arg Lys
    290   295   300

Asp Pro Gln Asp Lys
305

<210> 86
<211> 933
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> DNA Sequence NK39_8

<400> 86
atggccctgc ccgtgacagc tctgctgctg cctctggccc tgctgctgca tgccgctaga   60

cccttattca accaagaagt tcaaattccc ttgaccgaaa gttactgtgg cccatgtcct   120

aaaaactgga tatgttacaa aaataactgc taccaatttt ttgatgagag taaaaactgg   180

tatgagagcc aggcttcttg tatgtctcaa aatgccagcc ttctgaaagt atacagcaaa   240

gaggaccagg atttacttaa actggtgaag tcatatcatt ggatgggact agtacacatt   300

ccaacaaatg gatcttggca gtgggaagat ggctccattc tctcacccaa cctactaaca   360

ataattgaaa tgcagaaggg agactgtgca ctctatgcct cgagctttaa aggctatata   420

gaaaactgtt caactccaaa tacgtacatc tgcatgcaaa ggactgtgac caccacccct   480

gctcccagac cccctacacc tgcccctaca atcgccagcc agcccctgag cctgagacct   540

gaggcctgca gacctgctgc tggaggcgct gtgcacacaa ggggcctcga cttcgcctgc   600

gaccccaaac tctgctacct gctggatgga atcctcttca tctatggtgt cattctcact   660

gccttgttcc tgctttactg caagcggggc agaaagaagc tgctgtatat cttcaagcag   720

cccttcatgc ggcccgtgca gacaacccag gaggaagacg gctgctcatg tagatttcct   780

gaagaagaag aagggggctg tgaactgcga ctgaagatcc aagtgcgaaa ggcagctata   840

accagctatg agaaatcaga tggtgtttac acgggcctga gcaccaggaa ccaggagact   900

tacgagactc tgaagcatga gaaaccacca cag   933

```
<210>  87
<211>  311
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  Amino Acid Sequence NK39_8

<400>  87

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15


His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
            20                  25                  30


Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
        35                  40                  45


Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
    50                  55                  60


Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65                  70                  75                  80


Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95


Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110


Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125


Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140


Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Thr Thr Thr Pro
145                 150                 155                 160
```

```
Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
                165                 170                 175


Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
            180                 185                 190


Thr Arg Gly Leu Asp Phe Ala Cys Asp Pro Lys Leu Cys Tyr Leu Leu
        195                 200                 205


Asp Gly Ile Leu Phe Ile Tyr Gly Val Ile Leu Thr Ala Leu Phe Leu
    210                 215                 220


Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln
225                 230                 235                 240


Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser
                245                 250                 255


Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Leu Lys
            260                 265                 270


Ile Gln Val Arg Lys Ala Ala Ile Thr Ser Tyr Glu Lys Ser Asp Gly
        275                 280                 285


Val Tyr Thr Gly Leu Ser Thr Arg Asn Gln Glu Thr Tyr Glu Thr Leu
    290                 295                 300


Lys His Glu Lys Pro Pro Gln
305                 310


<210>  88
<211>  1605
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  DNA Sequence NK39_9

<400>  88
```

atgagaattt cgaaaccaca tttgagaagt atttccatcc agtgctactt gtgtttactt 60

ctaaacagtc attttctaac tgaagctggc attcatgtct tcattttggg ctgtttcagt 120

gcagggcttc ctaaaacaga agccaactgg gtcaacgtga ttagcgattt gaagaaaatc 180

gaggacctta tacagtctat gcatattgac gctacactgt atactgagag tgatgtacac 240

ccgtcctgta aggtaacggc catgaaatgc tttcttctgg agctccaggt catcagcttg 300

gagtctgggg acgcaagcat ccacgatacg gttgaaaacc tcatcatcct tgcgaacaac 360

tctctctcat ctaatggaaa cgttacagag agtgggtgta aggagtgcga agagttggaa 420

gaaaaaaaca tcaaagaatt tcttcaatcc ttcgttcaca tagtgcaaat gttcattaac 480

acgtccggcg gaggaggcag cggcggcggc ggcagcggcg gcggcggcag cttattcaac 540

caagaagttc aaattccctt gaccgaaagt tactgtggcc catgtcctaa aaactggata 600

tgttacaaaa ataactgcta ccaatttttt gatgagagta aaaactggta tgagagccag 660

gcttcttgta tgtctcaaaa tgccagcctt ctgaaagtat acagcaaaga ggaccaggat 720

ttacttaaac tggtgaagtc atatcattgg atgggactag tacacattcc aacaaatgga 780

tcttggcagt gggaagatgg ctccattctc tcacccaacc tactaacaat aattgaaatg 840

cagaagggag actgtgcact ctatgcctcg agctttaaag gctatataga aaactgttca 900

actccaaata cgtacatctg catgcaaagg actgtgacca cgacgccagc gccgcgacca 960

ccaacaccgg cgcccaccat cgcgtcgcag cccctgtccc tgcgcccaga ggcgtgccgg 1020

ccagcggcgg ggggcgcagt gcacacgagg gggctggact tcgcctgtga tatctacatc 1080

tgggcgccct tggccgggac ttgtggggtc cttctcctgt cactggttat caccctttac 1140

tgcaaacggg gcagaaagaa actcctgtat atattcaaac aaccatttat gagaccagta 1200

caaactactc aagaggaaga tggctgtagc tgccgatttc cagaagaaga agaaggagga 1260

tgtgaactga gagtgaagtt cagcaggagc gcagacgccc ccgcgtacca gcagggccag 1320

aaccagctct ataacgagct caatctagga cgaagagagg agtacgatgt tttggacaag 1380

agacgtggcc gggaccctga gatgggggga aagccgagaa ggaagaaccc tcaggaaggc 1440

ctgtacaatg aactgcagaa agataagatg gcggaggcct acagtgagat tgggatgaaa 1500

ggcgagcgcc ggaggggcaa ggggcacgat ggcctttacc agggtctcag tacagccacc 1560

```
aaggacacct acgacgccct tcacatgcag gccctgcccc ctcgc                   1605


<210>  89
<211>  535
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  Amino Acid Sequence NK39_9

<400>  89

Met Arg Ile Ser Lys Pro His Leu Arg Ser Ile Ser Ile Gln Cys Tyr
1               5                   10                  15


Leu Cys Leu Leu Leu Asn Ser His Phe Leu Thr Glu Ala Gly Ile His
            20                  25                  30


Val Phe Ile Leu Gly Cys Phe Ser Ala Gly Leu Pro Lys Thr Glu Ala
        35                  40                  45


Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu Asp Leu Ile
    50                  55                  60


Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp Val His
65                  70                  75                  80


Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu Leu Gln
                85                  90                  95


Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp Thr Val Glu
            100                 105                 110


Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly Asn Val
        115                 120                 125


Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys Asn Ile
    130                 135                 140


Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met Phe Ile Asn
```

```
145                 150                 155                 160


Thr Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
                165                 170                 175


Ser Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr Glu Ser Tyr Cys
            180                 185                 190


Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn Asn Cys Tyr Gln
        195                 200                 205


Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln Ala Ser Cys Met
    210                 215                 220


Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys Glu Asp Gln Asp
225                 230                 235                 240


Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly Leu Val His Ile
                245                 250                 255


Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser Ile Leu Ser Pro
            260                 265                 270


Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp Cys Ala Leu Tyr
        275                 280                 285


Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser Thr Pro Asn Thr
    290                 295                 300


Tyr Ile Cys Met Gln Arg Thr Val Thr Thr Thr Pro Ala Pro Arg Pro
305                 310                 315                 320


Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro
                325                 330                 335


Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu
            340                 345                 350


Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys
```

```
        355                 360                 365


Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly
    370                 375                 380


Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val
385                 390                 395                 400


Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu
                405                 410                 415


Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp
            420                 425                 430


Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn
        435                 440                 445


Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg
    450                 455                 460


Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly
465                 470                 475                 480


Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu
                485                 490                 495


Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu
            500                 505                 510


Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His
        515                 520                 525


Met Gln Ala Leu Pro Pro Arg
    530                 535


<210>  90
<211>  1122
<212>  DNA
<213>  Homo sapiens
```

<220>
<221> misc_feature
<223> DNA Sequence NKG2D-Ox40-CD3z

<400> 90
atggccttac cagtgaccgc cttgctcctg ccgctggcct tgctgctcca cgccgccagg   60

ccgttattca accaagaagt tcaaattccc ttgaccgaaa gttactgtgg cccatgtcct   120

aaaaactgga tatgttacaa aaataactgc taccaatttt ttgatgagag taaaaactgg   180

tatgagagcc aggcttcttg tatgtctcaa aatgccagcc ttctgaaagt atacagcaaa   240

gaggaccagg atttacttaa actggtgaag tcatatcatt ggatgggact agtacacatt   300

ccaacaaatg gatcttggca gtgggaagat ggctccattc tctcacccaa cctactaaca   360

ataattgaaa tgcagaaggg agactgtgca ctctatgcct cgagctttaa aggctatata   420

gaaaactgtt caactccaaa tacgtacatc tgcatgcaaa ggactgtgac cacgacgcca   480

gcgccgcgac caccaacacc ggcgcccacc atcgcgtcgc agcccctgtc cctgcgccca   540

gaggcgtgcc ggccagcggc gggggggcgca gtgcacacga gggggctgga cttcgcctgt   600

gatatctaca tctgggcgcc cttggccggg acttgtgggg tccttctcct gtcactggtt   660

atcacccttt actgccggag ggaccagagg ctgccccccg atgcccacaa gccccctggg   720

ggaggcagtt tccggacccc catccaagag gagcaggccg acgcccactc caccctggcc   780

aagatcagag tgaagttcag caggagcgca gacgcccccg cgtaccagca gggccagaac   840

cagctctata acgagctcaa tctaggacga agagaggagt acgatgtttt ggacaagaga   900

cgtggccggg accctgagat gggggggaaag ccgagaagga agaaccctca ggaaggcctg   960

tacaatgaac tgcagaaaga taagatggcg gaggcctaca gtgagattgg gatgaaaggc   1020

gagcgccgga ggggcaaggg gcacgatggc ctttaccagg gtctcagtac agccaccaag   1080

gacacctacg acgcccttca catgcaggcc ctgccccctc gc   1122

<210> 91
<211> 341
<212> PRT
<213> Homo sapiens

<220>

```
<221>  MISC_FEATURE
<223>  Amino Acid Sequence NKG2D-OX40-CD3z

<400>  91

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15


His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
            20                  25                  30


Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
        35                  40                  45


Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
    50                  55                  60


Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65                  70                  75                  80


Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95


Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110


Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125


Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140


Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Glu Ser Lys Tyr
145                 150                 155                 160


Gly Pro Pro Cys Pro Ser Cys Pro Ile Tyr Ile Trp Ala Pro Leu Ala
                165                 170                 175


Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys
            180                 185                 190
```

```
Arg Arg Asp Gln Arg Leu Pro Pro Asp Ala His Lys Pro Pro Gly Gly
        195                 200                 205


Gly Ser Phe Arg Thr Pro Ile Gln Glu Glu Gln Ala Asp Ala His Ser
    210                 215                 220


Thr Leu Ala Lys Ile Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro
225                 230                 235                 240


Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly
                245                 250                 255


Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro
            260                 265                 270


Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr
        275                 280                 285


Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly
    290                 295                 300


Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln
305                 310                 315                 320


Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln
                325                 330                 335


Ala Leu Pro Pro Arg
            340


<210>  92
<211>  1143
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  DNA Sequence NKG2D-CD28 - CD3z

<400>  92
```

atggccttac cagtgaccgc cttgctcctg ccgctggcct tgctgctcca cgccgccagg 60

ccgttattca accaagaagt tcaaattccc ttgaccgaaa gttactgtgg cccatgtcct 120

aaaaactgga tatgttacaa aaataactgc taccaatttt ttgatgagag taaaaactgg 180

tatgagagcc aggcttcttg tatgtctcaa aatgccagcc ttctgaaagt atacagcaaa 240

gaggaccagg atttacttaa actggtgaag tcatatcatt ggatgggact agtacacatt 300

ccaacaaatg gatcttggca gtgggaagat ggctccattc tctcacccaa cctactaaca 360

ataattgaaa tgcagaaggg agactgtgca ctctatgcct cgagctttaa aggctatata 420

gaaaactgtt caactccaaa tacgtacatc tgcatgcaaa ggactgtgac cacgacgcca 480

gcgccgcgac caccaacacc ggcgcccacc atcgcgtcgc agcccctgtc cctgcgccca 540

gaggcgtgcc ggccagcggc ggggggcgca gtgcacacga gggggctgga cttcgcctgt 600

gatttttggg tgctggtggt ggttggtgga gtcctggctt gctatagctt gctagtaaca 660

gtggccttta ttattttctg ggtgaggagt aagaggagca ggctcctgca cagtgactac 720

atgaacatga ctccccgccg ccccgggccc acccgcaagc attaccagcc ctatgcccca 780

ccacgcgact tcgcagccta tcgctccaga gtgaagttca gcaggagcgc agacgccccc 840

gcgtaccagc agggccagaa ccagctctat aacgagctca atctaggacg aagagaggag 900

tacgatgttt tggacaagag acgtggccgg gaccctgaga tgggggggaaa gccgagaagg 960

aagaaccctc aggaaggcct gtacaatgaa ctgcagaaag ataagatggc ggaggcctac 1020

agtgagattg ggatgaaagg cgagcgccgg aggggcaagg ggcacgatgg cctttaccag 1080

ggtctcagta cagccaccaa ggacacctac gacgcccttc acatgcaggc cctgcccccct 1140

cgc 1143

<210> 93
<211> 381
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> Amino Acid Sequence NKG2D-CD28 - CD3z

<400> 93

```
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15


His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
            20                  25                  30


Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
        35                  40                  45


Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
    50                  55                  60


Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65                  70                  75                  80


Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95


Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110


Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125


Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140


Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Thr Thr Thr Pro
145                 150                 155                 160


Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
                165                 170                 175


Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
            180                 185                 190


Thr Arg Gly Leu Asp Phe Ala Cys Asp Phe Trp Val Leu Val Val Val
        195                 200                 205
```

```
Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile
    210                 215                 220


Ile Phe Trp Val Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr
225                 230                 235                 240


Met Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln
                245                 250                 255


Pro Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser Arg Val Lys
            260                 265                 270


Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln
        275                 280                 285


Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu
    290                 295                 300


Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg
305                 310                 315                 320


Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met
                325                 330                 335


Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly
            340                 345                 350


Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp
        355                 360                 365


Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
    370                 375                 380


<210>  94
<211>  1269
<212>  DNA
<213>  Homo sapiens


<220>
```

<221> misc_feature
<223> DNA Sequence NKG2D - CD28 - 41BB - CD3z

<400> 94
atggccttac cagtgaccgc cttgctcctg ccgctggcct tgctgctcca cgccgccagg 60

ccgttattca accaagaagt tcaaattccc ttgaccgaaa gttactgtgg cccatgtcct 120

aaaaactgga tatgttacaa aaataactgc taccaatttt ttgatgagag taaaaactgg 180

tatgagagcc aggcttcttg tatgtctcaa aatgccagcc ttctgaaagt atacagcaaa 240

gaggaccagg atttacttaa actggtgaag tcatatcatt ggatgggact agtacacatt 300

ccaacaaatg gatcttggca gtgggaagat ggctccattc tctcacccaa cctactaaca 360

ataattgaaa tgcagaaggg agactgtgca ctctatgcct cgagctttaa aggctatata 420

gaaaactgtt caactccaaa tacgtacatc tgcatgcaaa ggactgtgac cacgacgcca 480

gcgccgcgac caccaacacc ggcgcccacc atcgcgtcgc agcccctgtc cctgcgccca 540

gaggcgtgcc ggccagcggc ggggggcgca gtgcacacga gggggctgga cttcgcctgt 600

gatttttggg tgctggtggt ggttggtgga gtcctggctt gctatagctt gctagtaaca 660

gtggccttta ttattttctg ggtgaggagt aagaggagca ggctcctgca cagtgactac 720

atgaacatga ctccccgccg ccccgggccc acccgcaagc attaccagcc ctatgcccca 780

ccacgcgact tcgcagccta tcgctccaaa cggggcagaa agaaactcct gtatatattc 840

aaacaaccat ttatgagacc agtacaaact actcaagagg aagatggctg tagctgccga 900

tttccagaag aagaagaagg aggatgtgaa ctgagagtga agttcagcag gagcgcagac 960

gcccccgcgt accagcaggg ccagaaccag ctctataacg agctcaatct aggacgaaga 1020

gaggagtacg atgttttgga caagagacgt ggccgggacc ctgagatggg gggaaagccg 1080

agaaggaaga accctcagga aggcctgtac aatgaactgc agaaagataa gatggcggag 1140

gcctacagtg agattgggat gaaaggcgag cgccggaggg gcaaggggca cgatggcctt 1200

taccagggtc tcagtacagc caccaaggac acctacgacg cccttcacat gcaggccctg 1260

ccccctcgc 1269

<210> 95
<211> 423
<212> PRT

```
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  Amino Acid Sequence NKG2D - CD28 - 41BB - CD3z

<400>  95

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15


His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
            20                  25                  30


Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
        35                  40                  45


Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
    50                  55                  60


Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65                  70                  75                  80


Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95


Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110


Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125


Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140


Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Thr Thr Thr Pro
145                 150                 155                 160


Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
                165                 170                 175
```

```
Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
            180                 185                 190

Thr Arg Gly Leu Asp Phe Ala Cys Asp Phe Trp Val Leu Val Val Val
        195                 200                 205

Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile
    210                 215                 220

Ile Phe Trp Val Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr
225                 230                 235                 240

Met Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln
                245                 250                 255

Pro Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser Lys Arg Gly
            260                 265                 270

Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val
        275                 280                 285

Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu
    290                 295                 300

Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp
305                 310                 315                 320

Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn
                325                 330                 335

Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg
            340                 345                 350

Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly
        355                 360                 365

Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu
    370                 375                 380
```

```
Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu
385                 390                 395                 400


Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His
                405                 410                 415


Met Gln Ala Leu Pro Pro Arg
            420


<210>  96
<211>  1038
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  DNA Sequence NKG2D(short hinge) - 41BB - CD3z

<400>  96
atggccttac cagtgaccgc cttgctcctg ccgctggcct tgctgctcca cgccgccagg       60

ccgttattca accaagaagt tcaaattccc ttgaccgaaa gttactgtgg cccatgtcct      120

aaaaactgga tatgttacaa aaataactgc taccaatttt ttgatgagag taaaaactgg      180

tatgagagcc aggcttcttg tatgtctcaa aatgccagcc ttctgaaagt atacagcaaa      240

gaggaccagg atttacttaa actggtgaag tcatatcatt ggatgggact agtacacatt      300

ccaacaaatg gatcttggca gtgggaagat ggctccattc tctcacccaa cctactaaca      360

ataattgaaa tgcagaaggg agactgtgca ctctatgcct cgagctttaa aggctatata      420

gaaaactgtt caactccaaa tacgtacatc tgcatgcaaa ggactgtgga gtccaaatat      480

ggtcccccat gcccatcatg cccaatctac atctgggcgc ccttggccgg gacttgtggg      540

gtccttctcc tgtcactggt tatcaccctt tactgcaaac ggggcagaaa gaaactcctg      600

tatatattca aacaaccatt tatgagacca gtacaaacta ctcaagagga agatggctgt      660

agctgccgat ttccagaaga agaagaagga ggatgtgaac tgagagtgaa gttcagcagg      720

agcgcagacg cccccgcgta ccagcagggc cagaaccagc tctataacga gctcaatcta      780

ggacgaagag aggagtacga tgttttggac aagagacgtg gccgggaccc tgagatgggg      840
```

```
ggaaagccga gaaggaagaa ccctcaggaa ggcctgtaca atgaactgca gaaagataag      900

atggcggagg cctacagtga gattgggatg aaaggcgagc gccggagggg caaggggcac      960

gatggccttt accagggtct cagtacagcc accaaggaca cctacgacgc ccttcacatg     1020

caggccctgc cccctcgc                                                   1038


<210>  97
<211>  346
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  Amino Acid Sequence NKG2D(short hinge) - 41BB - CD3z

<400>  97

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15


His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
            20                  25                  30


Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
        35                  40                  45


Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
    50                  55                  60


Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65                  70                  75                  80


Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95


Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110


Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125
```

```
Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140


Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Glu Ser Lys Tyr
145                 150                 155                 160


Gly Pro Pro Cys Pro Ser Cys Pro Ile Tyr Ile Trp Ala Pro Leu Ala
                165                 170                 175


Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys
            180                 185                 190


Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met
        195                 200                 205


Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe
    210                 215                 220


Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg
225                 230                 235                 240


Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn
                245                 250                 255


Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg
            260                 265                 270


Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro
        275                 280                 285


Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala
    290                 295                 300


Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His
305                 310                 315                 320


Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp
                325                 330                 335
```

```
Ala Leu His Met Gln Ala Leu Pro Pro Arg
            340                 345


<210>  98
<211>  1044
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  DNA Sequence NKG2D (SH)-CD28 - CD3z

<400>  98
atggccttac cagtgaccgc cttgctcctg ccgctggcct tgctgctcca cgccgccagg       60

ccgttattca accaagaagt tcaaattccc ttgaccgaaa gttactgtgg cccatgtcct      120

aaaaactgga tatgttacaa aaataactgc taccaatttt ttgatgagag taaaaactgg      180

tatgagagcc aggcttcttg tatgtctcaa aatgccagcc ttctgaaagt atacagcaaa      240

gaggaccagg atttacttaa actggtgaag tcatatcatt ggatgggact agtacacatt      300

ccaacaaatg gatcttggca gtgggaagat ggctccattc tctcacccaa cctactaaca      360

ataattgaaa tgcagaaggg agactgtgca ctctatgcct cgagctttaa aggctatata      420

gaaaactgtt caactccaaa tacgtacatc tgcatgcaaa ggactgtgga gtccaaatat      480

ggtccccccat gcccatcatg cccattttgg gtgctggtgg tggttggtgg agtcctggct      540

tgctatagct tgctagtaac agtggccttt attattttct gggtgaggag taagaggagc      600

aggctcctgc acagtgacta catgaacatg actccccgcc gccccgggcc cacccgcaag      660

cattaccagc cctatgcccc accacgcgac ttcgcagcct atcgctccag agtgaagttc      720

agcaggagcg cagacgcccc cgcgtaccag cagggccaga accagctcta taacgagctc      780

aatctaggac gaagagagga gtacgatgtt ttggacaaga gacgtggccg ggaccctgag      840

atgggggggaa agccgagaag gaagaaccct caggaaggcc tgtacaatga actgcagaaa      900

gataagatgg cggaggccta cagtgagatt gggatgaaag gcgagcgccg gaggggcaag      960

gggcacgatg gcctttacca gggtctcagt acagccacca aggacaccta cgacgccctt     1020

cacatgcagg ccctgccccc tcgc                                            1044
```

```
<210>  99
<211>  348
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  Amino Acid Sequence NKG2D (SH)-CD28 - CD3z

<400>  99

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15


His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
            20                  25                  30


Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
        35                  40                  45


Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
    50                  55                  60


Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65                  70                  75                  80


Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95


Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110


Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125


Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140


Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Glu Ser Lys Tyr
145                 150                 155                 160
```

```
Gly Pro Pro Cys Pro Ser Cys Pro Phe Trp Val Leu Val Val Val Gly
                165                 170                 175


Gly Val Leu Ala Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile Ile
            180                 185                 190


Phe Trp Val Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met
        195                 200                 205


Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro
    210                 215                 220


Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser Arg Val Lys Phe
225                 230                 235                 240


Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu
                245                 250                 255


Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp
            260                 265                 270


Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys
        275                 280                 285


Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala
    290                 295                 300


Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys
305                 310                 315                 320


Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr
                325                 330                 335


Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
            340                 345


<210>  100
<211>  1023
<212>  DNA
```

<213> Homo sapiens

<220>
<221> misc_feature
<223> DNA Sequence NKG2D (SH) - OX40 - CD3z

<400> 100
```
atggccttac cagtgaccgc cttgctcctg ccgctggcct tgctgctcca cgccgccagg       60

ccgttattca accaagaagt tcaaattccc ttgaccgaaa gttactgtgg cccatgtcct      120

aaaaactgga tatgttacaa aaataactgc taccaatttt ttgatgagag taaaaactgg      180

tatgagagcc aggcttcttg tatgtctcaa aatgccagcc ttctgaaagt atacagcaaa      240

gaggaccagg atttacttaa actggtgaag tcatatcatt ggatgggact agtacacatt      300

ccaacaaatg gatcttggca gtgggaagat ggctccattc tctcacccaa cctactaaca      360

ataattgaaa tgcagaaggg agactgtgca ctctatgcct cgagctttaa aggctatata      420

gaaaactgtt caactccaaa tacgtacatc tgcatgcaaa ggactgtgga gtccaaatat      480

ggtcccccat gcccatcatg cccaatctac atctgggcgc ccttggccgg gacttgtggg      540

gtccttctcc tgtcactggt tatcaccctt tactgccgga gggaccagag gctgcccccc      600

gatgcccaca agccccctgg gggaggcagt ttccggaccc ccatccaaga ggagcaggcc      660

gacgcccact ccaccctggc caagatcaga gtgaagttca gcaggagcgc agacgccccc      720

gcgtaccagc agggccagaa ccagctctat aacgagctca atctaggacg aagagaggag      780

tacgatgttt tggacaagag acgtggccgg gaccctgaga tgggggggaaa gccgagaagg      840

aagaaccctc aggaaggcct gtacaatgaa ctgcagaaag ataagatggc ggaggcctac      900

agtgagattg ggatgaaagg cgagcgccgg aggggcaagg ggcacgatgg cctttaccag      960

ggtctcagta cagccaccaa ggacacctac gacgcccttc acatgcaggc cctgcccccт     1020

cgc                                                                 1023
```

<210> 101
<211> 346
<212> PRT
<213> Homo sapiens

<220>

```
<221>  MISC_FEATURE
<223>  Amino Acid Seqeunce NKG2D (SH) - OX40 - CD3z

<400>  101

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15


His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
            20                  25                  30


Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
        35                  40                  45


Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
    50                  55                  60


Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65                  70                  75                  80


Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95


Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110


Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125


Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140


Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Glu Ser Lys Tyr
145                 150                 155                 160


Gly Pro Pro Cys Pro Ser Cys Pro Phe Trp Val Leu Val Val Val Gly
                165                 170                 175


Gly Val Leu Ala Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile Ile
            180                 185                 190
```

```
Phe Trp Val Arg Ser Arg Arg Asp Gln Arg Leu Pro Pro Asp Ala His
        195                 200                 205


Lys Pro Pro Gly Gly Gly Ser Phe Arg Thr Pro Ile Gln Glu Glu Gln
    210                 215                 220


Ala Asp Ala His Ser Thr Leu Ala Lys Ile Arg Val Lys Phe Ser Arg
225                 230                 235                 240


Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn
                245                 250                 255


Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg
            260                 265                 270


Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro
        275                 280                 285


Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala
    290                 295                 300


Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His
305                 310                 315                 320


Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp
                325                 330                 335


Ala Leu His Met Gln Ala Leu Pro Pro Arg
            340                 345


<210>  102
<211>  1125
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  DNA Sequence NKG2D-CD3TM -CD28 - CD3z

<400>  102
```

atggccttac cagtgaccgc cttgctcctg ccgctggcct tgctgctcca cgccgccagg 60

ccgttattca accaagaagt tcaaattccc ttgaccgaaa gttactgtgg cccatgtcct 120

aaaaactgga tatgttacaa aaataactgc taccaatttt ttgatgagag taaaaactgg 180

tatgagagcc aggcttcttg tatgtctcaa aatgccagcc ttctgaaagt atacagcaaa 240

gaggaccagg atttacttaa actggtgaag tcatatcatt ggatgggact agtacacatt 300

ccaacaaatg gatcttggca gtgggaagat ggctccattc tctcacccaa cctactaaca 360

ataattgaaa tgcagaaggg agactgtgca ctctatgcct cgagctttaa aggctatata 420

gaaaactgtt caactccaaa tacgtacatc tgcatgcaaa ggactgtgac cacgacgcca 480

gcgccgcgac caccaacacc ggcgcccacc atcgcgtcgc agcccctgtc cctgcgccca 540

gaggcgtgcc ggccagcggc gggggggcgca gtgcacacga gggggctgga cttcgcctgt 600

gatcccaaac tctgctacct gctggatgga atcctcttca tctatggtgt cattctcact 660

gccttgttcc tgaagaggag caggctcctg cacagtgact acatgaacat gactccccgc 720

cgccccgggc ccacccgcaa gcattaccag ccctatgccc caccacgcga cttcgcagcc 780

tatcgctcca gagtgaagtt cagcaggagc gcagacgccc ccgcgtacca gcagggccag 840

aaccagctct ataacgagct caatctagga cgaagagagg agtacgatgt tttggacaag 900

agacgtggcc gggaccctga gatgggggga aagccgagaa ggaagaaccc tcaggaaggc 960

ctgtacaatg aactgcagaa agataagatg gcggaggcct acagtgagat tgggatgaaa 1020

ggcgagcgcc ggaggggcaa ggggcacgat ggcctttacc agggtctcag tacagccacc 1080

aaggacacct acgacgccct tcacatgcag gccctgcccc ctcgc 1125

<210> 103
<211> 375
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> Amino Acid Sequence NKG2D-CD3TM -CD28 - CD3z

<400> 103

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu

```
1               5                   10                  15

His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
            20                  25                  30

Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
        35                  40                  45

Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
    50                  55                  60

Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65                  70                  75                  80

Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly
                85                  90                  95

Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110

Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125

Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140

Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Thr Thr Thr Pro
145                 150                 155                 160

Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
                165                 170                 175

Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
            180                 185                 190

Thr Arg Gly Leu Asp Phe Ala Cys Asp Pro Lys Leu Cys Tyr Leu Leu
        195                 200                 205

Asp Gly Ile Leu Phe Ile Tyr Gly Val Ile Leu Thr Ala Leu Phe Leu
```

```
    210                 215                 220


Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg
225                 230                 235                 240


Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg
                245                 250                 255


Asp Phe Ala Ala Tyr Arg Ser Arg Val Lys Phe Ser Arg Ser Ala Asp
            260                 265                 270


Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn
        275                 280                 285


Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg
    290                 295                 300


Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly
305                 310                 315                 320


Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu
                325                 330                 335


Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu
            340                 345                 350


Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His
        355                 360                 365


Met Gln Ala Leu Pro Pro Arg
    370                 375


<210>  104
<211>  36
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  DNA Sequence IgG 4 hinge
```

```
<400>  104
gagtccaaat atggtccccc atgcccatca tgccca                                 36


<210>  105
<211>  29
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  Amino Acid Sequence CD28 Transmembrane domain

<400>  105

Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu
1               5                   10                  15


Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg Ser
            20                  25


<210>  106
<211>  39
<212>  PRT
<213>  Homo sapiens


<220>
<221>  MISC_FEATURE
<223>  Amino Acid Sequence CD28 IC domain

<400>  106

Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg
1               5                   10                  15


Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg
            20                  25                  30


Asp Phe Ala Ala Tyr Arg Ser
        35


<210>  107
<211>  37
<212>  PRT
```

<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> Amino Acid Sequence OX40 IC Domain

<400> 107

Arg Arg Asp Gln Arg Leu Pro Pro Asp Ala His Lys Pro Pro Gly Gly
1   5   10   15

Gly Ser Phe Arg Thr Pro Ile Gln Glu Glu Gln Ala Asp Ala His Ser
            20   25   30

Thr Leu Ala Lys Ile
        35

<210> 108
<211> 1140
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> NKG2D-P-frag/CD8a/4-1BB/CD3z

<400> 108
atggccttac cagtgaccgc cttgctcctg ccgctggcct tgctgctcca cgccgccagg   60

ccgttattca accaagaagt tcaaattccc ttgaccgaaa gttactgtgg cccatgtcct   120

aaaaactgga tatgttacaa aaataactgc taccaatttt ttgatgagag taaaaactgg   180

tatgagagcc aggcttcttg tatgtctcaa aatgccagcc ttctgaaagt atacagcaaa   240

gaggaccagg atttacttaa actggtgaag tcatatcatt ggatgggact agtacacatt   300

ccaacaaatg gatcttggca gtgggaagat ggctccattc tctcacccaa cctactaaca   360

ataattgaaa tgcagaaggg agactgtgca ctctatgcct cgagctttaa aggctatata   420

gaaaactgtt caactccaaa tacgtacatc tgcatgcaaa ggactgtgac cacgacgcca   480

gcgccgcgac caccaacacc ggcgcccacc atcgcgtcgc agcccctgtc cctgcgccca   540

gaggcgtgcc ggccagcggc ggggggcgca gtgcacacga gggggctgga cttcgcctgt   600 gatatctaca tctgggcgcc cttggccggg acttgtgggg tccttctcct gtcactggtt 660

atcacccttt actgcaaacg gggcagaaag aaactcctgt atatattcaa acaaccattt 720

atgagaccag tacaaactac tcaagaggaa gatggctgta gctgccgatt tccagaagaa 780

gaagaaggag gatgtgaact gagagtgaag ttcagcagga gcgcagacgc ccccgcgtac 840

cagcagggcc agaaccagct ctataacgag ctcaatctag gacgaagaga ggagtacgat 900

gttttggaca agagacgtgg ccgggaccct gagatggggg gaaagccgag aaggaagaac 960

cctcaggaag gcctgtacaa tgaactgcag aaagataaga tggcggaggc ctacagtgag 1020

attgggatga aaggcgagcg ccggaggggc aaggggcacg atggccttta ccagggtctc 1080

agtacagcca ccaaggacac ctacgacgcc cttcacatgc aggccctgcc ccctcgctaa 1140

<210> 109
<211> 374
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> NKG2D-V2-OX40-CD3z

<400> 109

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr
20 25 30

Glu Ser Tyr Cys Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn
35 40 45

Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln
50 55 60

Ala Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys
65 70 75 80

Glu Asp Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly

```
                85                  90                  95


Leu Val His Ile Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser
            100                 105                 110


Ile Leu Ser Pro Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp
        115                 120                 125


Cys Ala Leu Tyr Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser
    130                 135                 140


Thr Pro Asn Thr Tyr Ile Cys Met Gln Arg Thr Val Thr Thr Thr Pro
145                 150                 155                 160


Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
                165                 170                 175


Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
            180                 185                 190


Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu
        195                 200                 205


Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr
    210                 215                 220


Cys Arg Arg Asp Gln Arg Leu Pro Pro Asp Ala His Lys Pro Pro Gly
225                 230                 235                 240


Gly Gly Ser Phe Arg Thr Pro Ile Gln Glu Glu Gln Ala Asp Ala His
                245                 250                 255


Ser Thr Leu Ala Lys Ile Arg Val Lys Phe Ser Arg Ser Ala Asp Ala
            260                 265                 270


Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu
        275                 280                 285


Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp
```

```
    290                 295                 300


Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu
305                 310                 315                 320


Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile
                325                 330                 335


Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr
            340                 345                 350


Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met
        355                 360                 365


Gln Ala Leu Pro Pro Arg
    370
```