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(71) Applicant: **NATIONAL UNIVERSITY OF SINGAPORE** [SG/SG]; 21 Lower Kent Ridge Road, Singapore 119077 (SG).

(72) Inventors: **SEOW, See, Voon**; c/o National University of Singapore, Yong Loo Lin School of Medicine, 21 Lower Kent Ridge Road, Singapore 119077 (SG). **CAMPANA, Dario**; c/o National University of Singapore, Yong Loo Lin School of Medicine, 21 Lower Kent Ridge Road, Singapore 119077 (SG).

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(54) **Title:** ACTIVATING CHIMERIC RECEPTORS AND USES THEREOF IN NATURAL KILLER CELL IMMUNOTHERAPY

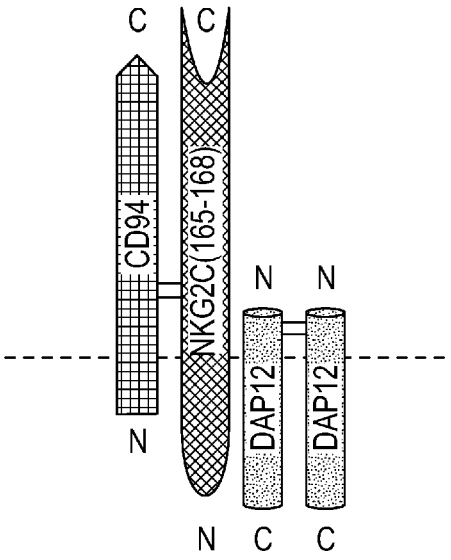

FIG. 1A

(57) **Abstract:** Current invention relates to a polynucleotide encoding activating chimeric receptors comprising engineered Natural Killer Group 2 member C (NKG2C) having enhanced affinity for HLA class I histocompatibility antigen alpha chain E (HLA-E)/peptide complex or an extracellular receptor domain of NKG2A coupled to an effector domain. It also relates to NK cells expressing such constructs and the use of these NK cells to induce cytotoxicity. It further exemplifies that the NK cells expressing polynucleotide encoding NKG2C (SIIS)/CD94/DAP12 or NKG2C/CD94/4-1 BB/CD3z showed enhanced NK cytotoxicity against cancer cells.

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ACTIVATING CHIMERIC RECEPTORS AND USES THEREOF IN NATURAL KILLER CELL IMMUNOTHERAPY

RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Serial No. 62/628,788, filed February 9, 2018 and U.S. Provisional Serial No. 62/736,879, filed September 26, 2018. The entirety of each of these 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: 4459_1147_002_Seq_List.txt; created February 6, 2019, 86.4KB 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 activating 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 activating 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 an activating 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 an immune antigen with high affinity.

[0006] For example, in several embodiments, there is provided a polynucleotide encoding an activating chimeric receptor that comprises an extracellular receptor domain that comprises an engineered variant of Natural Killer Group 2 member C (NKG2C) having higher affinity for an immune ligand of NKG2C. In several embodiments, the polynucleotide is designed to impart to the encoded NKG2C variant an enhanced binding affinity for an immune ligand, such as the HLA-E/peptide complex, relative to native NKG2C, whereas a non-engineered NKG2C has lower binding affinity for HLA-E/peptide complex. In several embodiments, an engineered NKG2C variant is used in the absence of a native or engineered NKG2A variant. In several embodiments, full length, or other native truncated form of, NKG2C is not used.

[0007] In several embodiments, there is provided a polynucleotide encoding an activating chimeric receptor that comprises or an extracellular receptor domain that comprises a fragment of Natural Killer Group 2 member A (NKG2A), the fragment engineered to transduce activation signals, rather than inhibitory signals, upon binding of an immune ligand, such as the HLA-E/peptide complex. In several embodiments, an engineered NKG2A variant is used in the absence of a native or engineered NKG2C variant. In several embodiments, full length, or other native truncated form of, NKG2A is not used.

[0008] In several embodiments, the activating chimeric receptor encoded by the polynucleotide comprises an effector domain that comprises a transmembrane region and an intracellular signaling domain, those domains serving to transduces activation and/or costimulatory signals following binding of the chimeric receptor (with enhanced affinity) to an immune antigen, such as the HLA-E/peptide complex. In several embodiments, the extracellular receptor domain comprises an engineered NKG2C variant coupled to an effector domain comprising a transmembrane region and an intracellular signaling domain. In several embodiments, the engineered NKG2C variant extracellular domain is coupled to a native NKG2C transmembrane region and/or a native NKG2C intracellular signaling domain. In several embodiments, the engineered NKG2C variant comprises the amino acid sequence of SEQ ID NO. 58. In several embodiments, the engineered NKG2C variant is encoded by the nucleic

acid sequence of SEQ ID NO: 63, or a fragment thereof. In some embodiments, the NKG2C variant has at least about 70%, about75%, about 80%, about 85%, about 90%, about 95%, about 98%, or about 99% homology as compared to SEQ ID NO: 63.

[0009] In several embodiments, the activating chimeric receptor comprises a fragment of NKG2A coupled to an effector domain comprising a transmembrane region and an intracellular signaling domain. In several embodiments, the NKG2A fragment is coupled to a native NKG2A transmembrane region and/or a native NKG2A intracellular signaling domain. In several embodiments, the NKG2A fragment is coupled to a native NKG2C transmembrane region and/or a native NKG2C intracellular signaling domain. In several embodiments, the NKG2A fragment has the sequence of SEQ ID NO: 65. In several embodiments, the fragment of NKG2A has at least about 70%, about 75%, about 80%, about 85%, about 90%, about95%, about 98%, or about 99% homology as compared to SEQ ID NO: 65. In one embodiment, the NKG2A fragment is coupled to a CD8a transmembrane domain and an effector domain comprising one or more of 4-1BB and CD3zeta. In several embodiments, such an activating chimeric receptor construct is encoded by the nucleic acid sequence of SEQ ID NO: 61 or comprises the amino acid sequence of SEQ ID NO: 62.

[0010] In several embodiments, other signaling moieties are used within the effector domain. For example, in several embodiments, the polynucleotide further encodes DNAX-activating protein 12 (DAP12) or DAP10. In several embodiments, however, DAP10 is not included in the activating receptor construct and/or is not included in another engineered construct expressed by cells. Likewise, in several embodiments, DAP12 is not included in the activating receptor construct and/or is not included in another engineered construct expressed by cells. In several embodiments, the polynucleotide encodes native CD94 or a chimeric CD94. In several embodiments, the chimeric CD94 comprises an extracellular receptor domain comprising a fragment of CD94 and an effector domain comprising a transmembrane region and an intracellular signaling domain. In additional embodiments, a chimeric CD94 receptor comprises a fragment of CD94 coupled a CD8a transmembrane domain and an effector domain comprising one or more of 4-1BB and CD3zeta. In several embodiments, the chimeric CD94 is encoded by the nucleic acid sequence of SEQ ID NO: 59. In several embodiments, the chimeric CD94 comprises the amino acid sequence of SEQ ID NO: 60. Variants, fragments, or truncations of CD94 are also used in some embodiments. For example, in several embodiments, a CD94 variant having at least about 70%, about75%, about 80%, about 85%, about 90%, about 95%, about 98%, or about 99% homology as compared to SEQ ID NO: 59 or 60 is used. In one

embodiment the fragment of CD94 has at least 80% homology to SEQ ID NO: 67. In one embodiment the fragment of CD94 has at least 80% homology to SEQ ID NO: 68.

[0011] In several embodiments, the effector domain comprises one or more of CD16, NCR1, NCR2, NCR3, 4-1BB, NKp80, DAP10, CD3zeta, 2B4. Actively signaling fragments of any of these signaling moieties are also useful, according to some embodiments.

[0012] In several embodiments, the activating chimeric receptor further comprises a linker and/or a hinge region. In several embodiments, these regions (if included) can function to space apart other portions of the molecule, for example to reduce steric hindrance of the receptor, and/or to reduce or eliminate functional issues that may result upon formation of tertiary structures. In several embodiments, the activating chimeric receptor comprises a GS linker that can optionally be repeated two or more times. For example, in several embodiments the GS linker is repeated 3, 4, 5, 6 or more times. In several embodiments, the hinge region comprises a glycine-serine repeating motif having the amino acid sequence of SEQ ID NO: 31. Repeats need not be in a "head to tail" format serially, but can be spaced apart from one another. In several embodiments, the chimeric receptor comprises a hinge region. Some embodiments employ hinges encoded by nucleic acid sequence of SEQ ID NO: 5. Additional embodiments employ a hinge region encoded by a fragment of the nucleic acid sequence of SEQ ID NO: 5. In several embodiments, the hinge region comprises the amino acid sequence of SEQ ID NO: 32 or SEQ ID NO: 33. In several embodiments, the hinge region is encoded by the nucleic acid sequence of SEQ ID NO: 34. Some embodiments employ portions of the beta-adrenergic receptor as a hinge region. Some embodiments employ a hinge region is encoded by the nucleic acid sequence of SEQ ID NO: 40 or of SEQ ID NO: 42.

[0013] In several embodiments, a signal peptide is also provided. Thus, 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.

[0014] In order to facilitate and, in some embodiments, enhance activating signaling by the chimeric receptors disclosed, the effector domain is configured to include one or more hemi-ITAM sequences. In several embodiments, the hemi-ITAM comprises either the amino acid sequence of SEQ ID NO. 14 or the amino acid sequence of SEQ ID NO. 37. In some embodiments, wherein more than one sequence of hemi-ITAM (or other transmembrane or signaling component) is provided, the ultimate construct encoded by the polynucleotide may utilize a mixture of the sequences (e.g., hetero-tandem signaling domains). In several embodiments, the effector domain comprises one or more ITSM sequences. For example, the

ITSM, in several embodiments, comprises the amino acid sequence of SEQ ID NO. 15 and/or the amino acid sequence of SEQ ID NO. 35

[0015] In several embodiments, the polynucleotide further encodes a chimeric receptor comprising an extracellular receptor domain that binds native ligands of Natural Killer Group 2 member D (NKG2D) and an effector domain comprising a transmembrane region and an intracellular signaling domain that serve to transduce signals to a cell expressing the chimeric receptor upon binding of an NKG2D ligand. In several embodiments, the chimeric receptor does not employ full-length or wild-type NKG2D, but rather a fragment of NKG2D that retains the ability to bind one or more ligands of NKG2D. In several embodiments, the chimeric receptor that binds NKG2D is provided on a separate polynucleotide from that encoding the activating receptor (yet ultimately both can be co-expressed in a single cell).

[0016] In several embodiments, the polynucleotide also encodes a short hairpin RNA (shRNA) that specifically inhibits transcription or translation of native NKG2A. In such embodiment, the shRNA that inhibits transcription or translation of native NKG2A results in a reduced, if not eliminated, expression of NKG2A on cells expressing the polynucleotide. In some embodiments, this reduces the competition for an immune antigen between the higher affinity NKG2A and NKG2C. As a result, the relatively lower affinity NKG2C receptor is able to bind an immune antigen (such as the HLA-E/peptide complex) and transduce activating signals to the cell (such as an NK cell). In one embodiment, the shRNA comprises a nucleotide sequence that hybridizes under stringent conditions to the native NKG2A gene and comprises a sense fragment being substantially identical to a target sequence in the NKG2A gene (that target sequence being absent from the NKG2A fragment), and an antisense fragment, wherein the sense and antisense fragments are separated by a loop fragment. Other mechanisms to reduce native NKG2A receptor expression can also be employed, depending on the embodiment. For example, a separate vector could be used that comprises antisense sequences to disrupt native NKG2A receptor production (but spares production of engineered NKG2A fragments/constructs as disclosed herein). Gene editing techniques can also be employed to selective trim one or more regions of DNA encoding native NKG2A receptors. Immune or mechanical separation techniques (affinity immobilization) can also be used to selectively deplete cell populations, such as immune cells like NK or T cells, of cells expressing NKG2A receptors.

[0017] In several embodiments, the provided polynucleotide encodes, or is co-expressed with an additional construct that encodes, membrane-bound interleukin 15 (mbIL15). Membrane-bound IL15 facilitates, in several embodiments, the expansion of cells expressing the activating chimeric receptors disclosed herein.

[0018] In some embodiments, the polynucleotide is an mRNA. In several embodiments, the polynucleotide is operably linked to at least one regulatory element for the expression of the activating chimeric receptors.

[0019] In addition to polynucleotides, there are provided herein vectors that comprise the polynucleotides, the vectors configured to deliver and facilitate the expression of the protein encoded by the polynucleotide in a cell, such as an immune cell (e.g., a NK cell). In several embodiments, the vector is a retrovirus, such as a lentivirus or HIV. Additional embodiments provide for other vectors, such as adenovirus, adeno-associated virus and even non-viral vectors (e.g., liposomes).

[0020] Additionally provided for herein are genetically engineered cells, such as immune cells, that comprise the polynucleotide(s) disclosed herein and express the activating chimeric receptor(s). Various immune cells are employed depending on the embodiment. In several embodiments, NK cells are used. In some embodiments, autologous cells (e.g., NK cells) engineered to express the activating chimeric receptors are provided. Additional embodiments provide for allogeneic cells (e.g., NK cells) engineered to express the activating chimeric receptors disclosed herein. In several embodiments, the cell populations are enriched for high affinity activating chimeric receptors. In one embodiment, enrichment comprises converting (e.g., by expressing the polynucleotides disclosed herein) inhibitory NKG2A receptors into activating NKG2A receptors. Additionally, this conversion can be coupled with expression of engineered NKG2C receptors having increased affinity for ligands that result in NKG2C transmitting activating signals. Moreover, cell populations can be configured to lack surface expression of native NKG2A, either through genetic modulation of the nucleic acids encoding native NKG2A and/or by selective depletion of cells expressing native NKG2A from a population.

[0021] Also provide for herein is a genetically engineered immune cells, such as a NK cell, comprising an activating chimeric receptor that is either an engineered NKG2C receptor having increased affinity for antigens or an NKG2A variant that has been converted from an inhibitory receptor to an activating receptor. In several embodiments, such cells may also include one or more of a polynucleotide encoding a chimeric receptor configured to bind native ligands of Natural Killer Group 2 member D (NKG2D) through an extracellular receptor domain (and comprising an effector domain comprising a transmembrane region and an intracellular signaling domain), a polynucleotide encoding membrane-bound interleukin 15 (mbIL15), a polynucleotide encoding a short hairpin RNA (shRNA) that specifically inhibits transcription or translation of native NKG2A or combinations thereof.

[0022] Also provided for herein are methods for enhancing immune cell cytotoxicity in a mammal by administering to said mammal immune cell cells, wherein said immune cell cells express an activating chimeric receptor that is either an engineered NKG2C receptor having increased affinity for antigens or an NKG2A variant that has been converted from an inhibitory receptor to an activating receptor. Various immune cells are employed depending on the embodiment. In several embodiments, NK cells are used. In some embodiments, autologous cells (e.g., NK cells) engineered to express the activating chimeric receptors are provided. Additional embodiments provide for allogeneic cells (e.g., NK cells) engineered to express the activating chimeric receptors disclosed herein. In several embodiments, the methods further comprise depleting a population of cells to be administered to a subject of cells expressing native NKG2A. In several embodiments, depletion results in a 20%, 25%, 30%, 40%, or 50% (or more) reduction in cells expression native NKG2A. In several embodiments, the enhanced immune cell-based cytotoxicity is used in treating or preventing cancer or an infectious disease.

[0023] Also provided for herein is the use of a polynucleotide encoding an activating chimeric receptor in the manufacture of a cell-based medicament for enhancing Natural Killer (NK) cell cytotoxicity. As discussed herein, in the generation of the medicament, the activating chimeric receptor can be an engineered NKG2C receptor having increased affinity for antigens or an NKG2A variant that has been converted from an inhibitory receptor to an activating receptor. In several embodiments, both engineered NKG2C and NKG2A variants are used in manufacture of a medicament for the treatment of cancer or an infectious disease.

[0024] 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 an activating chimeric receptor" include "instructing the administration of a population of NK cells expressing an activating chimeric receptor."

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] FIGs. 1A-1B depict an engineered receptor according to several embodiments disclosed herein. FIG. 1A depicts a schematic showing an engineered Natural Killer Group 2 C (NKG2C) variant engineered for high affinity to HLA-E/peptide complex (165-168 SIIS) in a complex with CD94 and a Dap12 dimer (termed "D12(SIIS)2C94" herein), according to several embodiments disclosed herein. FIG. 1B depicts a schematic of a construct comprising NKG2C (165-168 SIIS), Dap12, and CD94 inserted into a MSCV retroviral vector containing green

fluorescence protein (GFP) after an internal ribosomal entry site (IRES), according to several embodiments disclosed herein.

[0026] FIGs. 2A-2F depict flow cytometry data related to the expression of the high affinity activating D12(SIIS)2C94 receptor complex in purified NKG2C(+)NKG2A(-) NK cells. The D12(SIIS)2C94 expression profiles of NK cells transduced with a vector containing GFP only (FIGs. 2A-2C) and NK cells transduced with a vector containing D12(SIIS)2C94 (FIGs. 2D-2F) are depicted.

[0027] FIGs. 3A-3B depict the design and generation of a HLA-G signal peptide containing HLA-E molecule. FIG. 3A depicts the substitution of HLA-E signal peptide with HLA-G signal peptide of HLA-E (designated as GpHLA-E; HLA-G signal peptide carrying HLA-E). FIG. 3B depicts flow cytometry data related to the exogenous expression of GpHLA-E in solid tumor cell lines HT29, U2OS, ES8, and EW8.

[0028] FIGs. 4A-4C depicts data related to 4-hour cytotoxicity assays at the indicated E:T ratios of purified NKG2C(+)NKG2A(-) NK cells that are transduced with D12(SIIS)2C94 against the genetically-modified tumor cell lines HT29-GpHLA-E (FIG. 4A), U2OS-GpHLA-E (FIG. 4B), and SKBR3-GpHLA-E (FIG. 4C).

[0029] FIGs. 5A-5B depict data related to characterization of transduced NK cells. FIG. 5A depicts flow cytometry data related the NKG2A depletion of NK cells to generate a population of NKG2A(-) NK cells. FIG. 5B depicts data related to cytotoxicity assays, at the indicated E:T ratios, of mock transduced NKG2A(-) NK cell populations or D12(SIIS)2C94-transduced NKG2A(-) NK cells against the genetically-modified tumor cell lines (i) HT29-GpHLA-E and (ii) U2OS-GpHLA-E, incubated with or without anti-NKG2A antibody Z199.

[0030] FIGs. 6A-6B depict schematics of an embodiment of a chimeric receptor as disclosed herein. FIG. 6A depicts a schematic showing truncated forms of Natural Killer Group 2 member A (NKG2A) and CD94 wherein there is a deletion of the N-terminus portion of the transmembrane and inhibitory cytoplasmic domain and replacement with the CD8a transmembrane domain and cytoplasmic domains of 4-1BB (CD137) and CD3zeta, according to several embodiments disclosed herein. The activating NKG2A receptor and chimeric CD94 receptor form an activating complex (termed "2A/94BBz" herein), according to several embodiments disclosed herein. FIG. 6B depicts schematics of constructs comprising chimeric CD94 and activating NKG2A receptor inserted into a MSCV retroviral vector containing green fluorescence protein (GFP) after an internal ribosomal entry site (IRES), according to several embodiments disclosed herein.

[0031] FIGs. 7A-7C depict flow cytometry data related to the expression of activating NKG2A/CD94-41BB-CD3z receptor in transduced NKG2A-depleted NK cells (bottom row) and in mock transduced NK cell populations (top row).

[0032] FIGs. 8A-8B depict data related to 4-hour cytotoxicity assays at the indicated E:T ratios of mock transduced NK cell populations or expanded NK cells expressing activating NKG2A/CD94-41BB-CD3z receptor against (a) U2OS-GpHLA-E cells (FIG. 8A) and HT29-GpHLA-E cells (FIG. 8B).

[0033] FIG. 9 provides non-limiting embodiments of constructs and portions thereof according to several embodiments of the invention.

[0034] FIG. 10 depicts data related to a xenograft model of colorectal adenocarcinoma and the anti-tumor activity of NK-cell constructs according to several embodiments disclosed herein.

[0035] FIG. 11 depicts survival data from a xenograft model of colorectal adenocarcinoma after administration of NK-cell constructs according to several embodiments disclosed herein.

DETAILED DESCRIPTION

General

[0036] 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 activating 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 activating 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 activating 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 activating 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

[0037] One immunotherapy approach involves administering to patients T cells engineered to express activating chimeric receptors to elicit a positive immune response. However, a potential 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 activating 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.

[0038] In several embodiments, engagement and activation of a recombinant NK cell (e.g., by binding to a ligand on a target cell) expressing an activating 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 activating 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.).

Activating Chimeric Receptors that Bind HLA-E/peptide Complex

[0039] As mentioned above, in several embodiments, NK cells recognize and destroy aberrant cells, including tumor cells and/or 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 underlying logic is that an inhibitory receptor on an NK cell that interacts with a "self" molecule is not going to be activated and thereby spares the cell bearing the "self" molecule from destruction by the NK cell. In contrast, when an NK cell expressing an activating receptor interacts with a "non-self" ligand, for example on a diseased cell, the NK cell is activated and cytotoxic effects ensue. Thus, an increased engagement of activating receptors relative to inhibitory receptors leads to NK cell activation and target cell lysis.

[0040] Natural Killer Group 2 members A (NKG2A) and C (NKG2C) are C-type lectin receptors expressed predominantly on the surface of NK cells receptors that influence the balance of inhibitory and activating signaling. Both NKG2A and NKG2C are capable of forming heterodimers with, for example, the integral membrane glycoprotein CD94. Further, both NKG2C/CD94 and NKG2A/CD94 bind the nonclassical MHC class I molecule HLA-E/peptide complex in humans. HLA-E has a specialized role in cell recognition by NK cells, as HLA-E binds a restricted subset of peptides derived from signal peptides of classical MHC class I molecules, namely HLA-A, B, C, G. Therefore, NK cells can indirectly monitor the expression of classical MHC class I molecules through the interaction of NKG2C/CD94 and NKG2A/CD94 with HLA-E. NKG2C is an activating receptor and HLA-E/peptide complex binding enables interaction between NKG2C/CD94 and ITAM-bearing adaptor protein DAP12. In contrast, NKG2A is an inhibitory receptor, and NKG2A/CD94 transmits inhibitory signals via an ITIM motif in its cytoplasmic domain.

[0041] The ability of NK cells to recognize and destroy aberrant cells, including tumor cells and/or virally-infected cells, make it a potentially useful component of immunotherapy approaches (including chimeric receptor-based immunotherapy approaches). However, complicating the use of NK cells is the fact that HLA-E is upregulated in multiple tumors and exposure of tumor cells to IFN gamma (which is secreted by immune cells) enhances expression of HLA-E. NKG2C has lower affinity for peptide complexes as compared to NKG2A, so the increased HLA-E expression on target cells can act as a "camouflage" of sorts. This is a result of the higher affinity, but inhibitory, NKG2A being able to outcompete NKG2C for binding to a target peptide, and thus invoke an overall inhibitory effect on NK cell activity.

[0042] One approach to address this is, as provided for in several embodiments, polynucleotides encoding an activating chimeric receptor that binds HLA-E/peptide complex with enhanced affinity. In some embodiments, the activating chimeric receptor comprises a NKG2C variant engineered for enhanced affinity to HLA-E/peptide complex (e.g., as compared to the affinity of a non-modified NKG2C). In some embodiments of the NKG2C variant, residues 165-168 of wild-type NKG2C are replaced with the corresponding residues of NKG2A (SIIS). In several embodiments, the NKG2C variant comprises the amino acid sequence of SEQ ID NO. 58 (SIIS), where SIIS refers to Serine-Isoleucine-Isoleucine-Serine. In such embodiments, the affinity of the original NKG2A receptor is "transplanted" such that the previously lower affinity NKG2C receptor is engineered to have enhanced affinity. In several embodiments, the affinity of mutated NKG2C (as compared to non-mutated NKG2C) is increased by about 20%, by about 30%, by about 40% by about 50%, or more, depending on the embodiment.

[0043] In some embodiments disclosed herein, polynucleotides encoding activating chimeric receptors, such as a mutated NKG2C, are provided wherein the extracellular receptor domain is a fragment of a NKG2C variant that lacks its native transmembrane and/or intracellular domains, which, in several embodiments imparts to the variant still additional enhanced affinity for the HLA-E/peptide complex. In several embodiments, the NKG2C variant fragment is encoded by SEQ ID NO. 63. In several embodiments, the fragment of the NKG2C variant 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 NKG2C. In several embodiments, the fragment may have one or more additional mutations (e.g., insertions, deletions, and/or conservative or non-conservative substitutions) from SEQ ID NO. 63, but retains, or in some embodiments, has enhanced, ligand-binding function. In several embodiments, the NKG2C variant 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 NKG2C variant fragment is optionally fully or partially codon optimized.

[0044] 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 some embodiments, the polynucleotide encodes a NKG2C variant receptor and DAP12. In some embodiments, the polynucleotide encodes a NKG2C variant receptor and CD94. In some embodiments, the polynucleotide encodes a NKG2C variant receptor, CD94, and DAP12. In several embodiments, wherein NKG2C is

employed, the cells are further engineered to alter expression (e.g., reduce or eliminate transcription and/or reduce or eliminate translation) of native NKG2A.

[0045] In some embodiments, NKG2A is engineered to deliver activating signals upon HLA-E/peptide complex binding, as opposed to its normal delivery of inhibitory signals. As used herein, "NKG2A" refers to any variant, derivative, or isoform of the NKG2A gene or encoded protein, including, but not limited to, NKG2B. Thus, according to several embodiments disclosed herein, polynucleotides encoding activating chimeric receptors are provided wherein the extracellular receptor domain is a fragment of NKG2A that lacks its native transmembrane or intracellular domains yet advantageously retains its ability to bind HLA-E/peptide complex. Thus, in several embodiments, the chimeric receptor encoded by the polypeptides disclosed herein does not comprise an ITIM, an ITAM or a hemi-ITAM/hemi-ITAM. In several embodiments, the NKG2A fragment is encoded by SEQ ID NO. 65. In several embodiments, the fragment of NKG2A 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 NKG2A. In several embodiments, the fragment may have one or more additional mutations (e.g., insertions, deletions, and/or conservative or non-conservative substitutions) from SEQ ID NO. 65, but retains, or in some embodiments, has enhanced, ligand-binding function. In several embodiments, the NKG2A 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 NKG2A fragment is optionally fully or partially codon optimized. 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 some embodiments, the polynucleotide encodes an activating NKG2A receptor and a CD94 receptor.

[0046] In some embodiments, CD94 is engineered to deliver activating signals upon HLA-E/peptide complex binding. Thus, according to several embodiments disclosed herein, polynucleotides encoding activating chimeric receptors are provided wherein the extracellular receptor domain is a fragment of CD94 that lacks its native transmembrane or intracellular domains yet advantageously retains its ability to dimerize with NKG2A and NKG2C. In several embodiments, the CD94 fragment is encoded by SEQ ID NO. 67. In several embodiments, the fragment of CD94 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 CD94. In several embodiments, the fragment may have one or more additional mutations (e.g., insertions, deletions, and/or conservative or

non-conservative substitutions) from SEQ ID NO. 67, but retains, or in some embodiments, has enhanced, ligand-binding function. In several embodiments, the CD94 fragment comprises the amino acid sequence of SEQ ID NO. 68. In several embodiments, the fragment of CD94 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 CD94. In several embodiments, the fragment may have one or more additional mutations (e.g., insertions, deletions, and/or conservative or non-conservative substitutions) from SEQ ID NO. 68, but retains, or in some embodiments, has enhanced, ligand-binding function. In several embodiments, the CD94 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 CD94 fragment is optionally fully or partially codon optimized. 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 some embodiments, the polynucleotide encodes a chimeric CD94 receptor and an activating NKG2A receptor. In some embodiments, the polynucleotide encodes an activating NKG2C variant receptor and a chimeric CD94 receptor.

[0047] In some embodiments, polynucleotides encoding the activating chimeric receptors disclosed herein are expressed in NKG2A depleted cell populations (for example a population of NK cells that is depleted, substantially depleted, or completely depleted of cells expressing NKG2A – e.g., immunodepletion methods).

Transmembrane, Signaling and Combination Domains

[0048] As mentioned above, the general activating chimeric 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.

[0049] In several embodiments, a fragment of CD94, a fragment of NKG2A, and/or NKG2C variant 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" (e.g., the portion between the extracellular domain and the intracellular domain) 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, trimers, or repeated sequences of CD8α and CD8β are used.

[0050] In several embodiments, the transmembrane domain comprises CD16, which serves as a signaling domain as well, in some embodiments. 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 is 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 is 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.

[0051] In several embodiments, activating chimeric receptors provided for herein 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 employed as 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.

[0052] In some embodiments, signaling is achieved through DAP10. 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. However, in some embodiments, neither DAP10 nor DAP12 are used, either in an NKG2A, NKG2C, or NKG2D construct.

[0053] 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 immune 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 2B4 provides synergistic stimulation effects, resulting in particularly effective (e.g., cytotoxic) NK cells.

[0054] 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, CD3zeta is not used. 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 activating 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.

[0055] In still further embodiments, the signaling portion of the activating 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 activating 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 one hemi-tam confers enhanced signaling and cytotoxicity to the NK cells comprising an activating chimeric receptor employing the at least one hemi-tam. As discussed in more detail below, in several activating chimeric receptor comprises NKp80, which is one non-limiting example of a hemi-tam.

[0056] 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.

[0057] In addition to these variations in the extracellular receptor domain, 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 activating chimeric receptors disclosed herein, such embodiments provide particularly effective NK cell compositions for targeting and destroying particular target cells.

Chimeric Receptor Constructs

[0058] In view of the disclosure provided herein, there are a variety of activating 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.

[0059] 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 NKG2A/CD8a/4-1BB/CD3zeta chimeric receptor, which comprises an NKG2A fragment extracellular receptor domain that binds HLA-E/peptide complex, 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: 61. In yet another embodiment, the NKG2A/CD8a/4-1BB/CD3zeta chimeric receptor comprises the amino acid sequence of SEQ ID NO: 62. 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. 61, 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. 61. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 61, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function.

[0060] Additionally, in several embodiments, there are provided polynucleotides encoding a CD94/CD8a/4-1BB/CD3zeta chimeric receptor, which comprises a CD94 fragment extracellular receptor domain, 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: 59. In yet another embodiment, the CD94/CD8a/4-1BB/CD3zeta chimeric receptor comprises the amino acid sequence of SEQ ID NO: 60. 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 long-term activation and cytotoxic nature of the NK cells (e.g., in vivo persistence). In some embodiments, the sequence of the chimeric receptor may vary from SEQ ID NO. 59, 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. 59. In several embodiments, while the chimeric receptor may vary from SEQ ID NO. 59, the chimeric receptor retains, or in some embodiments, has enhanced, NK cell activating and/or cytotoxic function.

[0061] 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 an activating chimeric receptor, which comprises an extracellular receptor domain that comprises a fragment of NKG2A, CD94, or NKG2C variant, 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.

[0062] 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 an activating chimeric receptor which comprises an extracellular receptor domain that comprises a fragment of NKG2A, CD94, or NKG2C variant, 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, 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.

[0063] In several embodiments, there are provided polynucleotides encoding an activating chimeric receptor which comprises an extracellular receptor domain that comprises a fragment of NKG2A, CD94, or NKG2C variant, a CD8a transmembrane region, and an effector domain comprising the signaling domains of 4-1BB and DAP10. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15.

[0064] In several additional embodiments, transmembrane and effector domains (and associated function) of the activating 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 NKG2A/CD16 chimeric receptor, NKG2C variant/CD16 chimeric receptor, or CD94/CD16 chimeric receptor, which comprises a fragment of NKG2C variant, CD94, or NKG2A, and a CD16 peptide comprising both the transmembrane region and intracellular effector domain. Additionally, in several embodiments, this construct can optionally be co-expressed with mbIL15.

[0065] In several additional embodiments, polynucleotides are provided encoding a NKG2A/CD16/4-1BB chimeric receptor, CD94/CD16/4-1BB chimeric receptor, or NKG2C variant/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.

[0066] 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 NKG2A/NCR1 chimeric receptor, CD94/NCR1 chimeric receptor, or NKG2C variant/NCR1 chimeric receptor, which comprises an NKG2A, CD94, or NKG2C variant fragment, and a NCR1 peptide comprising both the transmembrane region and intracellular effector domain.

[0067] In several additional embodiments, polynucleotides are provided encoding a NKG2A/NCR1/4-1BB chimeric receptor, CD94/NCR1/4-1BB chimeric receptor, or NKG2C variant/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 NKG2A/NCR2 chimeric receptor, CD94/NCR2 chimeric receptor, NKG2C variant/NCR2 chimeric receptor, which comprises 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.

[0068] In several additional embodiments, polynucleotides are provided encoding a NKG2A/NCR3 chimeric receptor, CD94/NCR3 chimeric receptor, or NKG2C variant/NCR3 chimeric receptor, which comprises an 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 activating 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.

[0069] In several additional embodiments, polynucleotides are provided encoding a NKG2A/NCR2/4-1BB chimeric receptor, CD94/NCR2/4-1BB chimeric receptor, or NKG2C variant/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.

[0070] In several additional embodiments, polynucleotides are provided encoding a NKG2A/NCR3/4-1BB chimeric receptor, CD94/NCR3/4-1BB chimeric receptor, or NKG2C variant/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.

[0071] 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 transmembrane domain and any one of the CD94 fragment, NKG2A fragment, or NKG2C fragment. In some embodiments, the hinge regions can be included between other portions of the chimeric activating 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.

[0072] In several embodiments, the activating chimeric receptor constructs employ a 2B4 intracellular signaling domain. In several embodiments, this domain includes 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 an activating 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 activating chimeric receptor may vary from SEQ ID NO. 36, the activating 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 activating 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.

[0073] In several embodiments, the activating 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.

[0074] 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 extracellular receptor domain comprising a fragment of a CD94, NKG2A, or NKG2C variant. In several embodiments, however, codon optimization is not performed. In several embodiments, a chimeric receptor construct is provided with an extracellular domain comprising a fragment of NKG2A, CD94, or NKG2C variant that is not optimized, a CD8a hinge, and a 4-1BB signaling domain. In several embodiments, a chimeric receptor construct is provided with an extracellular domain comprising a fragment of NKG2A, CD94, or NKG2C variant that is not optimized, a CD8a hinge and transmembrane domain, and a 4-1BB signaling domain.

[0075] In several embodiments, an activating chimeric receptor construct is provided with an extracellular domain comprising a fragment of NKG2A, CD94, or NKG2C variant that is not optimized, a CD8a hinge, and a NKp80 signaling domain. In several embodiments, a chimeric receptor construct is provided with an extracellular domain comprising a fragment of NKG2A, CD94, or NKG2C variant that is not optimized, a CD8a hinge and transmembrane domain, and a NKp80 signaling domain. In several embodiments, a GS linker, such as a GS3 linker joins the 4-1BB and NKp80 domains.

[0076] 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.

[0077] In several embodiments, a CD16 intracellular signaling domain is coupled with a CD3zeta or gamma subunit which are exogenously expressed in trans to the activating 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.

[0078] In several embodiments, the activating chimeric receptors are configured to dimerize, as discussed in additional detail herein. In several embodiments a receptor comprising a fragment of CD94, NKG2A, or NKG2C variant 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 activating 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 activating chimeric receptors employ internal dimers, or repeats of one or more component subunits. For example, in several embodiments, the chimeric receptor comprises a NKG2C variant extracellular domain coupled to a second NKG2C variant 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 extracellular domains are codon optimized. 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. 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.

[0079] According to several embodiments disclosed herein, additional chimeric receptors employing codon optimized extracellular receptor 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 domains (e.g. a transmembrane domain and a signaling domain). 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.

[0080] In still additional embodiments, certain components of an activating 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.

[0081] 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 CD3zeta 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 CD3zeta and FcRγ supplements endogenous expression of these molecules by NK cells, thereby further enhancing the signaling and ultimate cytotoxic potency of the NK cells.

[0082] 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 an activating 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.

[0083] The polynucleotides encoding the activating 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 activating 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 activating chimeric receptor. Depending on the embodiment, the various constituent parts of a activating chimeric receptor can be delivered to an NK cell in a single vector, or alternatively in multiple vectors. In some embodiments, an activating chimeric receptor construct is delivered in a single vector, while another factor that enhances efficacy of the activating chimeric receptor, such as mbIL15, is delivered in a separate vector. In several embodiments, an activating chimeric receptor and a factor that enhances efficacy of the activating 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 an activating chimeric receptor.

[0084] In several embodiments, the polynucleotide encoding the activating 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 activating 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.

[0085] Natural killer cells may be engineered to express the activating chimeric receptors disclosed herein. Activating 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 activating chimeric receptors are transiently expressed in the NK cells. In another embodiment, the activating 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.

[0086] 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 an activating chimeric receptor as disclosed herein, the activating 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, intraarterial, 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 105 cells per kg to about 1012 cells per kg (e.g., 105 – 107, 107- 1010, 1010- 1012 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 106 cells/kg to about 1 x 108 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.

[0087] 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 fungal infections, and such as infections of fungal origin may include, for example, infections with fungi from one or more of the following genera: *Aspergillus*, *Blastomyces*, *Candida*, *Coccidioides*, *Cryptococcus*, and *Histoplasma*, 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.

[0088] 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.

[0089] 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.

[0090] There are provided for herein, according to several embodiments, polynucleotides encoding activating chimeric receptors, comprising an extracellular receptor domain, wherein the extracellular receptor domain comprises a fragment of CD94, NKG2A, or NKG2C variant, an effector domain comprising a transmembrane region and an intracellular signaling domain. 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 CD16 and a fragment of CD94, NKG2A, or NKG2C variant. In several embodiments, the polynucleotide encodes a chimeric receptor made up of NCR1 and fragment of CD94, NKG2A,

or NKG2C variant. In several embodiments, the polynucleotide encodes a chimeric receptor made up of NCR2 and a fragment of CD94, NKG2A, or NKG2C variant. In additional embodiments, the polynucleotide encodes a chimeric receptor made up of a fragment of CD94, NKG2A, or NKG2C variant 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.

[0091] 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.

[0092] 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.

[0093] In several embodiments, the activating chimeric receptors described herein are co-expressed with a chimeric receptor targeting cells that express natural ligands of Natural Killer Group 2 member D (NKG2D), leading to synergistically enhanced NK cell activation and cytotoxicity. Thus, in several embodiments, there is provided also provided a polynucleotide encoding a NKGD chimeric receptor comprising an extracellular receptor domain, wherein the extracellular receptor domain comprises a peptide that binds native NKG2D, wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D, a transmembrane region, and an effector domain. 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 several embodiments, the effector domain comprises one or more of CD16, NCR1, NCR2, NCR3, 4-1BB, CD28, NKp80, DAP10, CD3zeta and 2B4. In several embodiments, these effector domains are coupled to CD8 alpha. In several embodiments, the NKG2D domain is not full-length or wild-type NKG2D and in some such embodiments, DAP10 is not used in the chimeric receptor construct. In still further embodiments, CD3Zeta or an ITAM is not used. As discussed herein, combinations of

transmembrane and intracellular domains are used in several embodiments and provide for synergistic interactions between the components of the NKG2D chimeric receptor and yield enhanced cytotoxic effects. In several embodiments, linkers, hinges, or other "spacing" elements are provided for in the NKG2D 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 NKG2D chimeric receptor construct, such as between any of 4-1BB, CD28, CD16, NCR1, NCR3, CD3zeta, DAP10, 2B4 or NKp80. In several embodiments, the NKG2D chimeric receptor effector domain comprises a linker. In several embodiments, the polynucleotides encode a GS linker between the portions of the NKG2D chimeric receptor construct, such as between any of 4-1BB, CD28, CD16, NCR1, NCR3, 2B4 or NKp80. In several embodiments, there is provided for a chimeric receptor comprising a hinge region. In several embodiments, the NKG2D chimeric receptor effector domain comprises one or more hemi-ITAM sequences. Additionally, any of chimeric receptors disclosed herein can also be co-expressed with membrane-bound interleukin 15 (mbIL15).

[0094] 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 activating 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 or comprising one or more regions or portions from a different receptor or signaling molecule (e.g., another subtype or type of receptor). 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.

[0095] 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 an activating chimeric receptor. In several embodiments, the vector is a retrovirus.

[0096] Further provided herein are engineered natural killer cells comprising the polynucleotide, vector, or chimeric activating 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.

[0097] In several embodiments, the polynucleotides disclosed herein further encode a short hairpin RNA (shRNA) that specifically inhibits transcription or translation of native NKG2A, wherein the shRNA comprises a nucleotide sequence that hybridizes under stringent conditions to the native NKG2A gene, wherein the shRNA comprises a sense fragment, which comprises a nucleotide sequence substantially identical to a target sequence in the NKG2A gene that is absent in the NKG2A fragment, and an antisense fragment, wherein the sense and antisense fragments are separated by a loop fragment. In several embodiments, the polynucleotide is co-expressed with an additional construct encoding membrane-bound interleukin 15 (mbIL15). In several embodiments, the polynucleotide is an mRNA. In several embodiments, the polynucleotide is

operably linked to at least one regulatory element for the expression of the activating chimeric receptor.

[0098] Also provided for herein in several embodiments, in several embodiments is a vector comprising a polynucleotide of the present disclosure, wherein the polynucleotide is operatively linked to at least one regulatory element for expression of the activating chimeric receptor. In several embodiments, the vector is a retrovirus.

[0099] In several embodiments, there is provided a method 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 activating chimeric receptor encoded by a polynucleotide according to the present disclosure. In several embodiments, the NK cells are autologous cells isolated from a patient having a cancer or an infectious disease. In several embodiments, the NK cells are allogenic cells isolated from a donor. In several embodiments, the NK cells lack surface expression of native NKG2A.

[00100] In several embodiments, there is provided the use of a polynucleotide according to the present disclosure in the manufacture of a medicament for treating or preventing cancer or an infectious disease in a mammal in need thereof. In several embodiments, there is provided the use of a vector according the present disclosure in the manufacture of a medicament for enhancing NK cell cytotoxicity in a mammal in need thereof. In several embodiments the vector is used in the manufacture of a medicament for treating or preventing cancer or an infectious disease in a mammal in need thereof.

[00101] In several embodiments, there is provided the use of an isolated genetically engineered natural killer cell according to the present disclosure for enhancing NK cell cytotoxicity in a mammal in need thereof. In several embodiments, there is also provided the use of an isolated genetically engineered natural killer cell according to the present disclosure for treating or preventing cancer or an infectious disease in a mammal in need thereof.

[00102] In several embodiments, there is provided a chimeric Natural Killer Group 2 member C (NKG2C) comprising an extracellular receptor domain, a transmembrane domain, and a cytoplasmic domain, wherein the extracellular receptor domain comprises SEQ ID NO. 58. In several embodiments, there is provided a chimeric receptor comprising a Natural Killer Group 2 member A (NKG2A) extracellular receptor domain, a transmembrane region, and a cytoplasmic domain. In several embodiments, the transmembrane region comprises a CD8 transmembrane region. In several embodiments, the cytoplasmic domain comprises one or more of a 4-1BB cytoplasmic domain and a CD3zeta cytoplasmic domain.

[00103] In several embodiments, there is provided a polynucleotide encoding a chimeric receptor, the chimeric receptor comprising an extracellular domain, a transmembrane domain, and a cytoplasmic domain, wherein the extracellular domain comprises a Natural Killer Group 2 member C (NKG2C) and SEQ ID NO. 58.

[00104] In several embodiments, there is provided a polynucleotide encoding a chimeric receptor, the chimeric receptor comprising a Natural Killer Group 2 member A (NKG2A) extracellular receptor domain, a transmembrane region, and a cytoplasmic domain. In several embodiments, the transmembrane region comprises a CD8 transmembrane region. In several embodiments, the cytoplasmic domain comprises one or more of a 4-1BB cytoplasmic domain and a CD3zeta cytoplasmic domain.

EXAMPLES

Methods

[00105] The following experimental methods and materials were used in the non-limiting experimental examples disclosed below.

Cell Lines and Culture Conditions

[00106] The human tumor cell lines SKBR3, HT-29, U2-OS were purchased from the American Type Culture Collection (ATCC; Rockville, MD). The Ewing sarcoma cell line ES8 and EW8 were from the tissue repository of St. Jude Research Hospital (Memphis, TN). Cell lines were maintained in RPMI-1640 (ThermoFisher, Waltham, MA); media were supplemented with 10% fetal bovine serum (FBS; GE Healthcare, Chicago, IL) and antibiotics. The cell lines were transduced with a murine stem cell virus (MSCV) retroviral vector (from the Vector Development and Production Shared Resource of St. Jude Children's Research Hospital, Memphis, TN) containing either green fluorescence protein (GFP) and luciferase.

Expansion of Human NK Cells and Selection of NK Cell Subsets

[00107] Peripheral blood samples were obtained from discarded anonymized by-products of platelet donations from healthy adult donors at the National University Hospital Blood Bank, Singapore.

[00108] Mononucleated cells were separated by centrifugation on a Lymphoprep density step (Nycomed, Oslo, Norway) and washed twice in RPMI-1640. To expand NK cells, mononucleated cells were co-cultured with the genetically-modified K562-mb15-41BBL cell line. Briefly, peripheral blood mononucleated cells (3×10^6) were cultured in a 6-well tissue

culture plate with 2 x 10^6 irradiated (100 Gy) K562-mb15-41BBL cells in SCGM medium (CellGenix, Freiburg, Germany) containing 10% FBS and 40 IU/mL human interleukin (IL)-2 (Novartis, Basel, Switzerland). Every 2-3 days, fresh tissue culture medium and IL-2 was added. After 7 days of co-culture, residual T cells were removed using Dynabeads CD3 (Thermo Fisher), producing cell populations containing > 90% CD56+ CD3– NK cells. Expanded NK cells were maintained in SCGM with FBS, antibiotics, and 400 IU/mL IL2 before the experiments.

[00109] To deplete NKG2A+ NK cells, expanded NK cells were labeled with anti-NKG2A antibody conjugated to allophycocyanin (APC) and anti-APC microbeads and separated on LD column (all from Miltenyi Biotech).

DNA Plasmids, Production of Retrovirus and Transduction of NK Cells

[00110] Plasmids encoding all constructs were synthesized by Genescript (Nanjing, China). A RD114-pseudotyped MSCV retrovirus containing the constructs was used to transduce NK cells. Retroviral vector-conditioned medium was added to RetroNectin (Takara, Otsu, Japan)-coated polypropylene tubes; after centrifugation and removal of the supernatant, expanded NK cells (3 x 10^5) were added to the tubes and left at 37°C for 12 hours; fresh viral supernatant was added every 12 hours for a total of 6 times. Cells were then maintained in RPMI with FBS, antibiotics and 400 IU/ml of IL-2 until the time of the experiments.

Detection of Activating Receptor Expression by Flow Cytometry

[00111] Surface expression of the anti-NKG2C was detected with an anti-NKG2C-phycoerithrin (PE) antibody (Miltenyi Biotech). Expression of NKG2A was detected with anti-NKG2A antibodies conjugated to PE or APC (Miltenyi Biotech). Expression of CD94 was detected with anti-CD94-APC (BD Biosciences).

Cytotoxicity Assays

[00112] Target cells transduced with GFP/luciferase were suspended in RPMI-1640 with 10% FBS, and plated into 96-well flat bottom plates (Costar, Corning, NY). The plates were placed in an incubator for at least 4 hours to allow for cell attachment before adding NK cells. Expanded NK cells expressing various receptors or GFP alone, suspended in RPMI-1640 with 10% FBS were then added at various effector-to-target (E:T) ratios, and co-cultured with target cells for 4 hours. At the end of the cultures, number of viable cells were measured, after adding BrightGlo (Promega, Fitchburg, WI) to the wells, using a Flx 800 plate reader (BioTek, Winooski, VT).

Example 1 – Activating High Affinity NKG2C Receptor Constructs

[00113] As disclosed herein, various activating chimeric receptor constructs comprising an extracellular receptor domain coupled with various transmembrane domains are provided. The present experiment was conducted to evaluate the expression and cytotoxic activity of an activating Natural Killer Group 2 member C (NKG2C) variant engineered for high affinity to HLA-E/peptide complex. The activating NKG2C receptor construct was 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.

[00114] FIG. 1A depicts a schematic showing an engineered NKG2C variant (165-168 SIIS) engineered for high affinity to HLA-E/peptide complex in a complex with CD94 and a Dap12 dimer (termed "D12(SIIS)2C94" herein). FIG. 1B depicts a schematic of a construct comprising NKG2C (165-168 SIIS), Dap12, and CD94 inserted into a MSCV retroviral vector containing green fluorescence protein (GFP) after an internal ribosomal entry site (IRES).

[00115] The ability of NK cells to effectively express this construct was first assessed. FIG. 2 depicts flow cytometry data demonstrating the robust expression of the high affinity activating D12(SIIS)2C94 receptor complex in purified NKG2C(+)NKG2A(-) NK cells relative to NK cells transduced with a vector containing GFP only. Collectively, these data demonstrate that, in accordance with several embodiments disclosed herein, engineered activating chimeric receptor constructs can successfully be expressed on NK cells.

[00116] 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.

[00117] NKG2C/CD94 and NKG2A/CD94 bind the nonclassical MHC class I molecule HLA-E/peptide complex in humans. HLA-E has a very specialized role in cell recognition by NK cells, and HLA-E binds a restricted subset of peptides derived from signal peptides of classical MHC class I molecules, namely HLA-A, B, C, G. Therefore NK cells can indirectly monitor the expression of classical MHC class I molecules through the interaction of NKG2C/CD94 and NKG2A/CD94 with HLA-E/peptide complex. To evaluate the efficacy of the activating NKG2C receptor constructs and activating NKG2A receptor constructs described herein, cancer cells

were engineered to express HLA-E containing the HLA-G signal peptide. FIG. 3 depicts the design and generation of a HLA-G signal peptide containing HLA-E molecule. FIG. 3A depicts the substitution of HLA-E signal peptide (SEQ ID NO. 69) with HLA-G signal peptide (SEQ ID NO. 70) of HLA-E (designated as GpHLA-E; HLA-G signal peptide carrying HLA-E). FIG. 3B depicts flow cytometry data verifying the exogenous expression of GpHLA-E in solid tumor cell lines HT29, U2OS, ES8, and EW8.

[00118] To evaluate the potency of the populations of transduced NK cells, cytotoxicity assays were performed using cancer cell lines that express GpHLA-E. Purified NKG2C(+) NKG2A(-) NK cells expressing D12(SIIS)2C94 displayed significantly higher cytotoxicity against U2OS-GpHLA-E, SKBR3-GpHLA-E, and HT29-GpHLA-E cells than the control NK cells at all of the E:T ratios tested (FIG. 4). These data provide evidence that NK cells can not only be engineered to express activating chimeric receptor constructs, but those cells that express the activating chimeric receptor are able to be activated and successfully generate enhanced cytotoxic effects against target cells.

[00119] To confirm the depletion of NKG2A(+) cells, presence of which could potentially confound the results of these studies and NK cell-based therapies, flow cytometry analysis was conducted. Following NKG2A depletion, we confirmed that the cell population was comprised of NKG2C(+)NKG2A(-) NK cells. Further, cytotoxicity assays of mock transduced NK cell populations or D12(SIIS)2C94-transduced NKG2C(+)NKG2A(-) NK cells against the genetically-modified tumor cell lines demonstrated the enhanced cytotoxicity of the latter NK cell population, regardless of whether or not cells were incubated with anti-NKG2A antibody Z199 (FIG. 5).

Example 2 – Activating Chimeric NKG2A/CD94 Receptor Constructs

[00120] As disclosed herein, various activating chimeric receptor constructs comprising an extracellular receptor domain coupled with various transmembrane domains are provided. The present experiment was conducted to evaluate the expression and cytotoxic activity of an activating Natural Killer Group 2 member A (NKG2A)/CD94 receptor construct. The activating NKG2A/CD94 receptor construct was 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.

[00121] FIG. 6A depicts a schematic showing a complex comprised of truncated forms of Natural Killer Group 2 member A (NKG2A) and CD94 wherein there is a deletion of the N-terminus portion of the transmembrane and inhibitory cytoplasmic domain. These domains have

been replaced with the CD8a transmembrane domain and cytoplasmic domains of 4-1BB (CD137) and CD3zeta. The activating NKG2A receptor and chimeric CD94 receptor form an activating complex (termed "2A/94BBz" herein). FIG. 6B depicts schematics of constructs comprising chimeric CD94 receptor and activating NKG2A receptor inserted into a MSCV retroviral vector containing green fluorescence protein (GFP) after an internal ribosomal entry site (IRES).

[00122] The ability of NK cells to effectively express these constructs was first assessed. FIG. 7 depicts flow cytometry data demonstrating the robust expression of activating NKG2A/CD94-41BB-CD3z receptor in NKG2A-depleted NK cells relative to mock transduced NK cell populations. Collectively, these data demonstrate that, in accordance with several embodiments disclosed herein, engineered activating chimeric receptor constructs can successfully be expressed on NK cells.

[00123] To evaluate the potency of the populations of transduced NK cells, cytotoxicity assays were performed using cancer cell lines that express GpHLA-E. Expression of activating NKG2A/CD94-41BB-CD3z receptor on expanded NK cells significantly increased cytotoxicity against U2OS-GpHLA-E and HT29-GpHLA-E cells in 4-hour cytotoxicity assays relative to control NK cells. These data provide evidence that NK cells can not only be engineered to express activating chimeric receptor constructs, but those cells that express the activating chimeric receptors are able to be activated and successfully generate enhanced cytotoxic effects against target cells.

Example 3 – Anti-tumor Activity of Activating Chimeric Receptor Constructs

[00124] In order to demonstrate the ability of the activating NK receptor constructs disclosed herein to exert cytotoxic effects against target cells (e.g., tumor cells), a xenograft model of colorectal adenocarcinoma was used. HT-29 cells are an immortalized colorectal adenocarcinoma cell line. HT-29 cells were transduced with both GpHLA-E and luciferase, such that the HT-29 cells express HLA-E, which is a portion of the target recognized by the engineered activating receptors used in this example (the D12(SIIS)2C/94 construct is engineered for high affinity to an HLA-E/peptide complex (165-168 SIIS)). The transduced HT-29 cells were injected intraperitoneally at a dose of 1 x 10^5 cells per mouse into each of 12 NOD-SCID IL2RGnull mice. Subsequently, NK cells (1 x 10^7 cells) were injected on day 3, 6, 10 and 13. NK cells were either transduced with GFP only ("Control") or the D12(SIIS)2C/94 construct. Mice also received IL-2 (20,000 IU) three times per week by intraperitoneal injection. Ventral

and dorsal bioluminescence was measured to assess tumor burden using a Xenogen Spectrum instrument (each symbol is the average of ventral and dorsal readings).

[00125] As can be seen in the left panel of FIG. 10 (no NK cells) the average bioluminescence increased in each mouse over time, which is indicative of increasing tumor burden from the growth/proliferation of the injected HT-29 cells. The central panel (mice injected with NK cells expressing GFP) exhibited generally increasing average bioluminescence, though to a lesser degree than the "No NK" group. In stark contrast, the average bioluminescence in the D12(SIIS)2C/94 shows very limited increase, even to the ~75 day timepoint at which the experiments were concluded. A single mouse had a modest elevation of average bioluminescence between ~40 and 75 days of the experiment. These data indicate that the engineered NK cells expressing an activating chimeric receptor have significant anti-tumor activity, even when compared to NK cells, which have a relatively robust anti-tumor effect on their own.

[00126] Further investigating the effects of the engineered activating receptors disclosed herein, survival curves were generated to determine survival rates of the mice in the various groups. Kaplan Meier survival curves analyzed by log rank test are shown in FIG. 11, with the same experimental groups as described above. As shown (dashed line) the mice receiving HT-29 cells but no NK cells reached 0% survival at ~60 days. Similarly, the mice receiving NK cells expressing GFP (solid gray line) showed a similar survival pattern, reaching 0% survival by ~80 days. Statistical evaluation of these groups showed no statistical difference between the No NK and Control groups (Kaplan Meier survival curve, log rank test, FIG. 11). In contrast to the first two groups (similar to the average bioluminescence data above), the D12(SIIS)2C/94 group showed significantly enhanced survival. Not only did one of the four D12(SIIS)2C/94 mice survive the entire duration of the experiment, the duration of survival of all of the mice in the D12(SIIS)2C/94 was enhanced. Statistical evaluation shows that survival in the D12(SIIS)2C/94 was significant in comparison to the No NK group as well as the NK cell-receiving Control group (Kaplan Meier survival curves, log rank test, p values depicted in FIG. 11). Thus, these data demonstrate that, even in comparison to NK cells (which exhibit relatively high natural cytotoxicity) NK cells engineered to express activating chimeric receptors have unexpectedly elevated cytotoxic effects against target cells. In several embodiments, the expression of activating chimeric receptors on NK cells allows one or more of a reduced frequency of treatment, reduced duration of treatment, reduced required dose of NK cells, and an overall improved outcome (e.g., reduced tumor burden and/or increased survival) of a NK-based

cancer immunotherapy regimen (e.g., for example as compared to treatment frequency, duration etc. using NK cells not engineered to express the high affinity domains as disclosed herein).

[00127] 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.

[00128] 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 10 nanometers" includes "10 nanometers."

WHAT IS CLAIMED IS:

1. A polynucleotide encoding an activating chimeric receptor that binds HLA class I histocompatibility antigen, alpha chain E (HLA-E)/peptide complex, wherein the activating chimeric receptor transduces activation and/or costimulatory signals following binding to the HLA-E/peptide complex, wherein the activating chimeric receptor comprises:

a) an extracellular receptor domain, wherein said extracellular receptor domain comprises an engineered variant of Natural Killer Group 2 member C (NKG2C),

wherein non-engineered NKG2C has low binding affinity for HLA-E/peptide complex,

wherein the engineered NKG2C variant has enhanced binding affinity for HLA-E/peptide complex relative to native NKG2C, and

wherein the engineered NKG2C variant comprises the amino acid sequence of SEQ ID NO. 58; or

b) an extracellular receptor domain, wherein said extracellular receptor domain comprises a fragment of Natural Killer Group 2 member A (NKG2A),

wherein the fragment of NKG2A is coupled to an effector domain comprising a transmembrane region and an intracellular signaling domain,

wherein native NKG2A transduces inhibitory signals following binding to HLA-E/peptide complex, and

wherein the fragment of NKG2A transduces activation and/or costimulatory signals following binding to HLA-E/peptide complex.

2. The polynucleotide of claim 1, wherein the extracellular receptor domain comprises an engineered NKG2C variant coupled to an effector domain comprising a transmembrane region and an intracellular signaling domain.

3. The polynucleotide of claim 1, wherein the extracellular receptor domain comprises an engineered NKG2C variant coupled to a native NKG2C transmembrane region and a native NKG2C intracellular signaling domain.

4. The polynucleotide of Claim 1, wherein the engineered NKG2C variant is encoded by the nucleic acid sequence of SEQ ID NO: 63, or a fragment thereof.

5. The polynucleotide of Claim 1, the polynucleotide further encoding DNAX-activating protein 12 (DAP12).

6. The polynucleotide according of Claim 1, the polynucleotide further encoding native CD94 or a chimeric CD94.

7. The polynucleotide of claim 6, wherein the chimeric CD94 comprises

(a) an extracellular receptor domain,

wherein said extracellular receptor domain comprises a fragment of CD94; and

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

8. The polynucleotide of Claim 7, wherein the effector domain comprises one or more of CD16, NCR1, NCR2, NCR3, 4-1BB, NKp80, DAP10, CD3zeta, 2B4, and fragments thereof.

9. The polynucleotide of Claim 7, wherein the chimeric CD94 receptor comprises a fragment of CD94 coupled a CD8a transmembrane domain and an effector domain comprising 4-1BB and CD3zeta.

10. The polynucleotide of claim 9, wherein the chimeric CD94 is encoded by the nucleic acid sequence of SEQ ID NO: 59.

11. The polynucleotide of claim 9, wherein the chimeric CD94 comprises the amino acid sequence of SEQ ID NO: 60.

12. The polynucleotide of Claim 1, wherein the activating chimeric receptor comprises a fragment of NKG2A coupled to a CD8a transmembrane domain and an effector domain comprising 4-1BB and CD3zeta.

13. The polynucleotide of claim 12, wherein the activating chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 61.

14. The polynucleotide of claim 12, wherein the activating chimeric receptor comprises the amino acid sequence of SEQ ID NO: 62.

15. The polynucleotide of any one of claims 1-14, wherein the activating chimeric receptor further comprises a GS linker.

16. The polynucleotide of any one of claims 1-14, wherein the chimeric receptor comprises a hinge region.

17. The polynucleotide of claim 16, wherein the hinge region is encoded by the nucleic acid sequence of SEQ ID NO: 5.

18. The polynucleotide of claim 16, wherein the hinge region is encoded by a fragment of the nucleic acid sequence of SEQ ID NO: 5.

19. The polynucleotide of claim 16, wherein the hinge region is comprises a glycine-serine repeating motif having the amino acid sequence of SEQ ID NO: 31.

20. The polynucleotide of claim 16, wherein the hinge region comprises the amino acid sequence of SEQ ID NO: 32.

21. The polynucleotide of claim 16, wherein the hinge region comprises the amino acid sequence of SEQ ID NO: 33.

22. The polynucleotide of claim 16, wherein the hinge region is encoded by the nucleic acid sequence of SEQ ID NO: 34.

23. The polynucleotide of claim 16, wherein the hinge region comprises a portion of the beta-adrenergic receptor.

24. The polynucleotide of claim 23, wherein the hinge region is encoded by the nucleic acid sequence of SEQ ID NO: 40.

25. The polynucleotide of claim 23, wherein the hinge region is encoded by the nucleic acid sequence of SEQ ID NO: 42.

26. The polynucleotide of any one of claims 1 to 14, wherein the extracellular receptor domain further comprises a CD8a signal peptide, wherein the signal peptide comprises the nucleic acid sequence of SEQ ID NO. 4.

27. The polynucleotide of any one of claims 1 to 14, wherein the effector domain comprises one or more hemi-ITAM sequences.

28. The polynucleotide of claim 27, wherein the hemi-ITAM comprises the amino acid sequence of SEQ ID NO. 14.

29. The polynucleotide of claim 27, wherein the hemi-ITAM comprises the amino acid sequence of SEQ ID NO. 37.

30. The polynucleotide of any one of claims 1 to 14, wherein the effector domain comprises one or more ITSM sequences.

31. The polynucleotide of claim 30, wherein the ITSM comprises the amino acid sequence of SEQ ID NO. 15.

32. The polynucleotide of claim 30, wherein the ITSM comprises the amino acid sequence of SEQ ID NO. 35

33. The polynucleotide of any one of claims 1 to 14, wherein the NKG2C variant has at least 80% homology to SEQ ID NO: 63.

34. The polynucleotide of any one of claims 1 to 14, wherein the fragment of NKG2A has at least 80% homology to SEQ ID NO: 65.

35. The polynucleotide of any one of claims 1 to 14, wherein the fragment of CD94 has at least 80% homology to SEQ ID NO: 67.

36. The polynucleotide of any one of claims 1 to 14, wherein the polynucleotide further encodes 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); and (b) an effector domain comprising a transmembrane region and an intracellular signaling domain.

37. The polynucleotide of any one of claims 1 to 14, wherein the polynucleotide further encodes a short hairpin RNA (shRNA) that specifically inhibits transcription or translation of native NKG2A,

wherein the shRNA comprises a nucleotide sequence that hybridizes under stringent conditions to the native NKG2A gene, wherein the shRNA comprises a sense fragment, which comprises a nucleotide sequence substantially identical to a target

sequence in the NKG2A gene that is absent in the NKG2A fragment, and an antisense fragment, wherein the sense and antisense fragments are separated by a loop fragment.

38. The polynucleotide of any of the preceding claims, wherein the polynucleotide is co-expressed with an additional construct encoding membrane-bound interleukin 15 (mbIL15).

39. A genetically engineered natural killer cell comprising the polynucleotide of any one of Claims 1 to 14.

40. The genetically engineered natural killer cell of claim 39, which is an autologous cell isolated from a patient.

41. The genetically engineered natural killer cell of claim 39, which is an allogeneic cell isolated from a donor.

42. The genetically engineered natural killer cell of claim 39, wherein the NK cells lack surface expression of native NKG2A.

43. A genetically engineered natural killer cell comprising,

the polynucleotide of any one of Claims 1 to 14, and an additional polynucleotide selected from:

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

(ii) a polynucleotide encoding membrane-bound interleukin 15 (mbIL15);

(iii) a polynucleotide encoding a short hairpin RNA (shRNA) that specifically inhibits transcription or translation of native NKG2A; and

(iv) any combination of (i), (ii), and (iii).

44. A method for enhancing NK cell cytotoxicity in a mammal in need thereof, said method comprising administering to said mammal NK cells, wherein said NK cells express an activating chimeric receptor encoded by a polynucleotide of any one of Claims 1-14.

45. The method of claim 44, wherein said NK cells are autologous cells isolated from a patient.

46. The method of claim 44, wherein said NK cells are allogeneic cells isolated from a donor.

47. The method of claim 44, wherein the NK cells lack surface expression of native NKG2A.

48. Use of a polynucleotide according to any one of Claims 1-14 in the manufacture of a medicament for enhancing NK cell cytotoxicity in a mammal in need thereof.

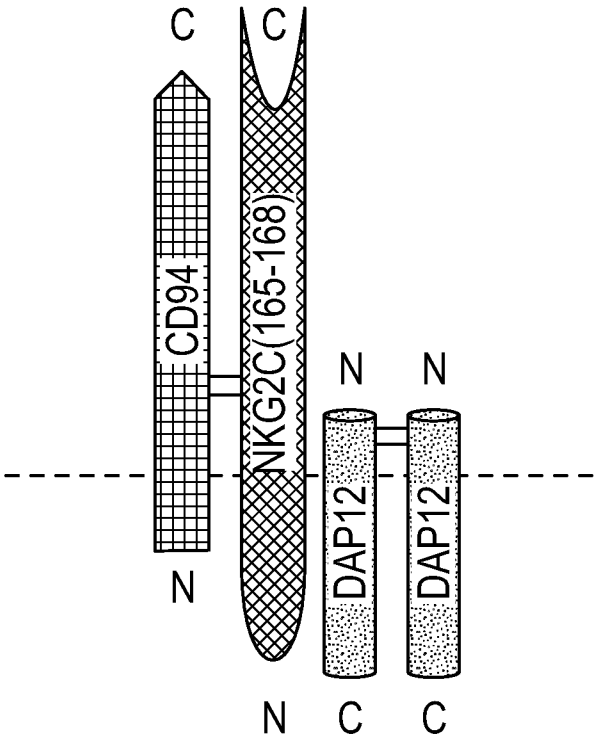


FIG. 1A

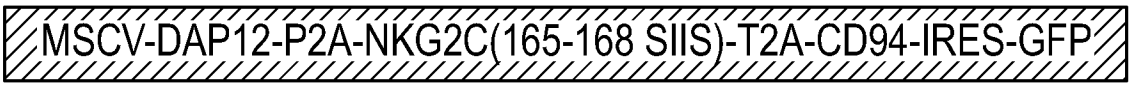
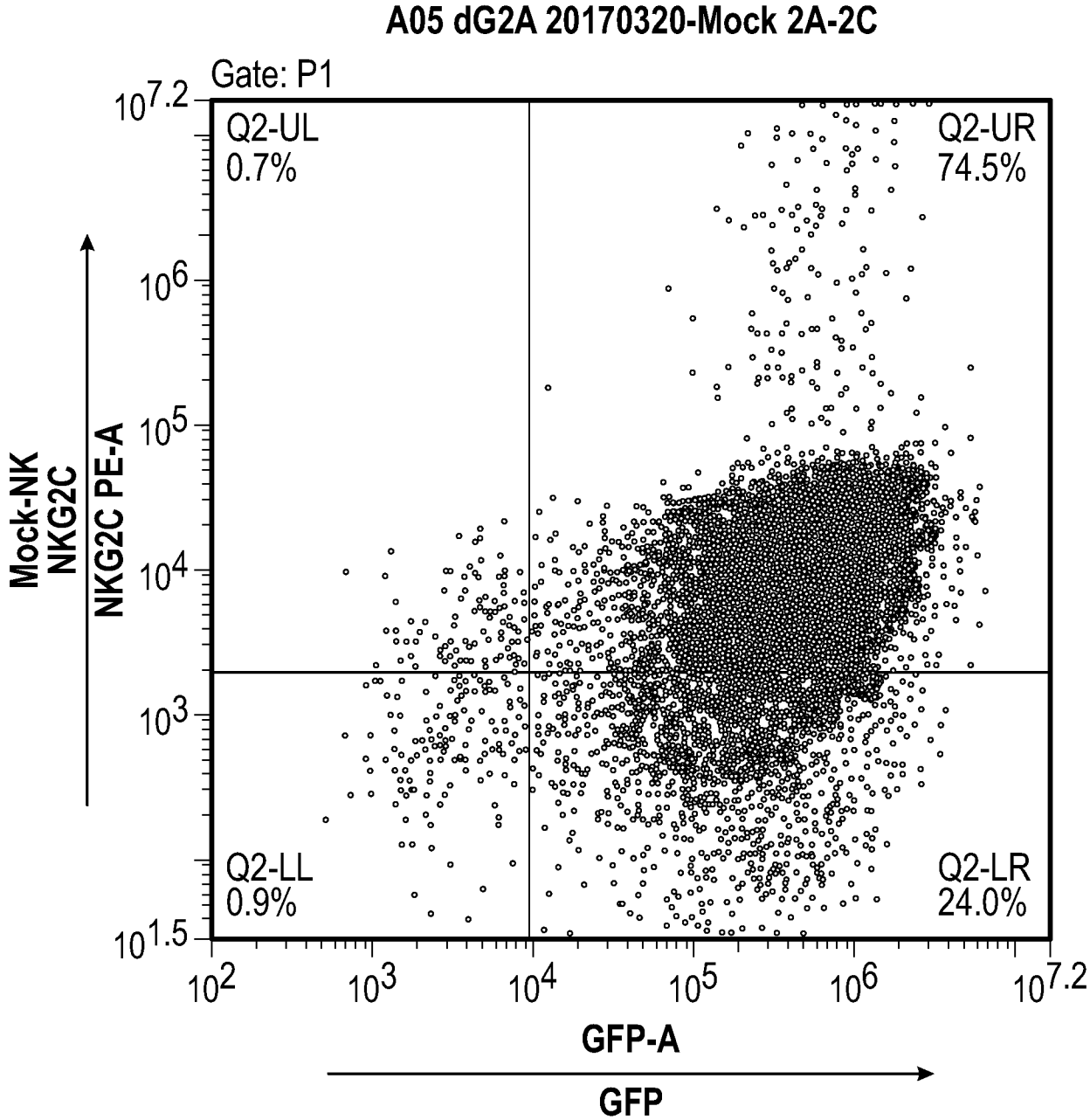


FIG. 1B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Gate: P1
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Q2-UR
74.5%
Q2-LL
0.9%
Q2-LR
24.0%
Mock-NK
NKG2C
NKG2C PE-A
GFP-A
GFP
10^7.2
10^6
10^5
10^4
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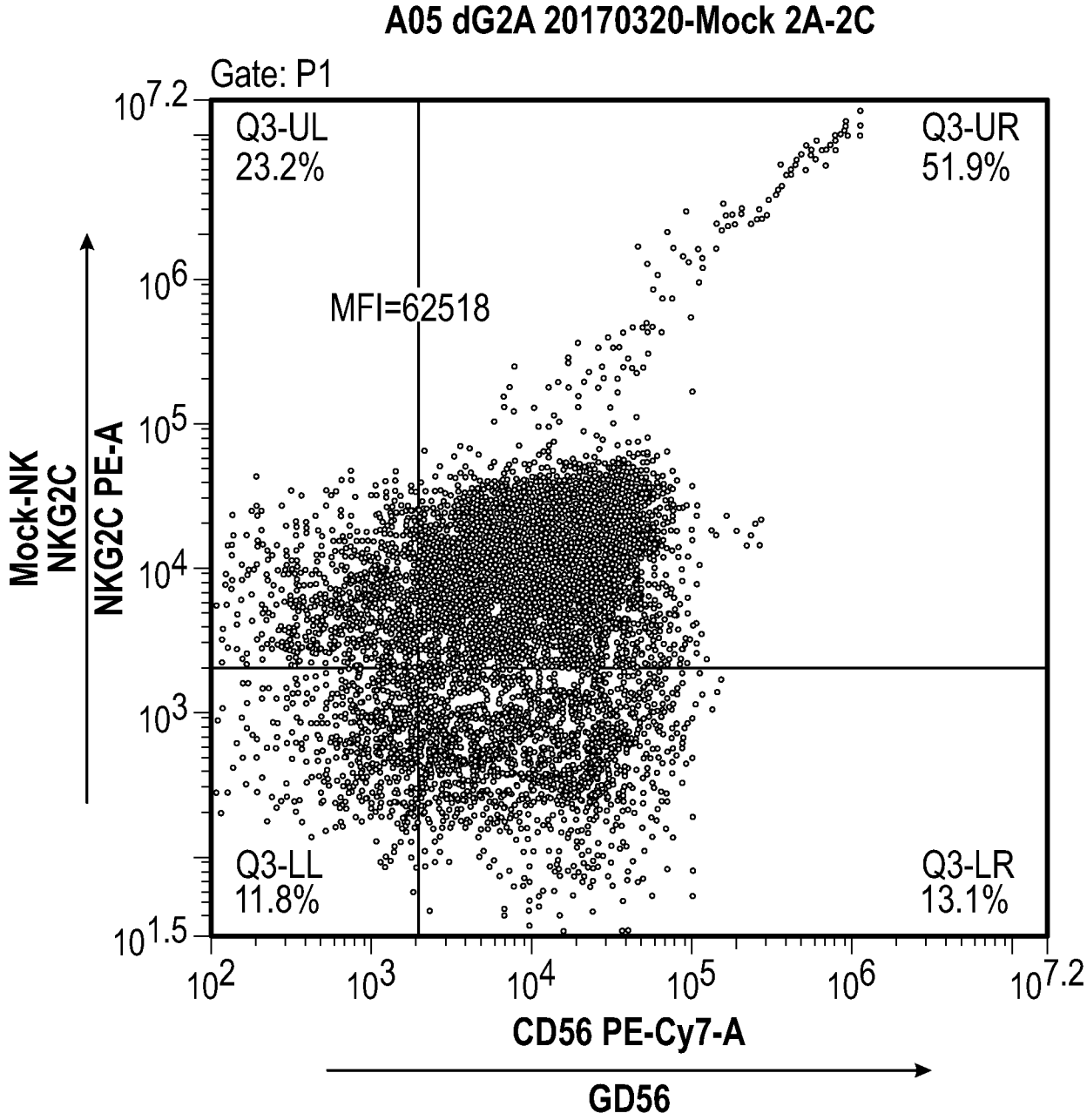
10^6
10^7.2

FIG. 2A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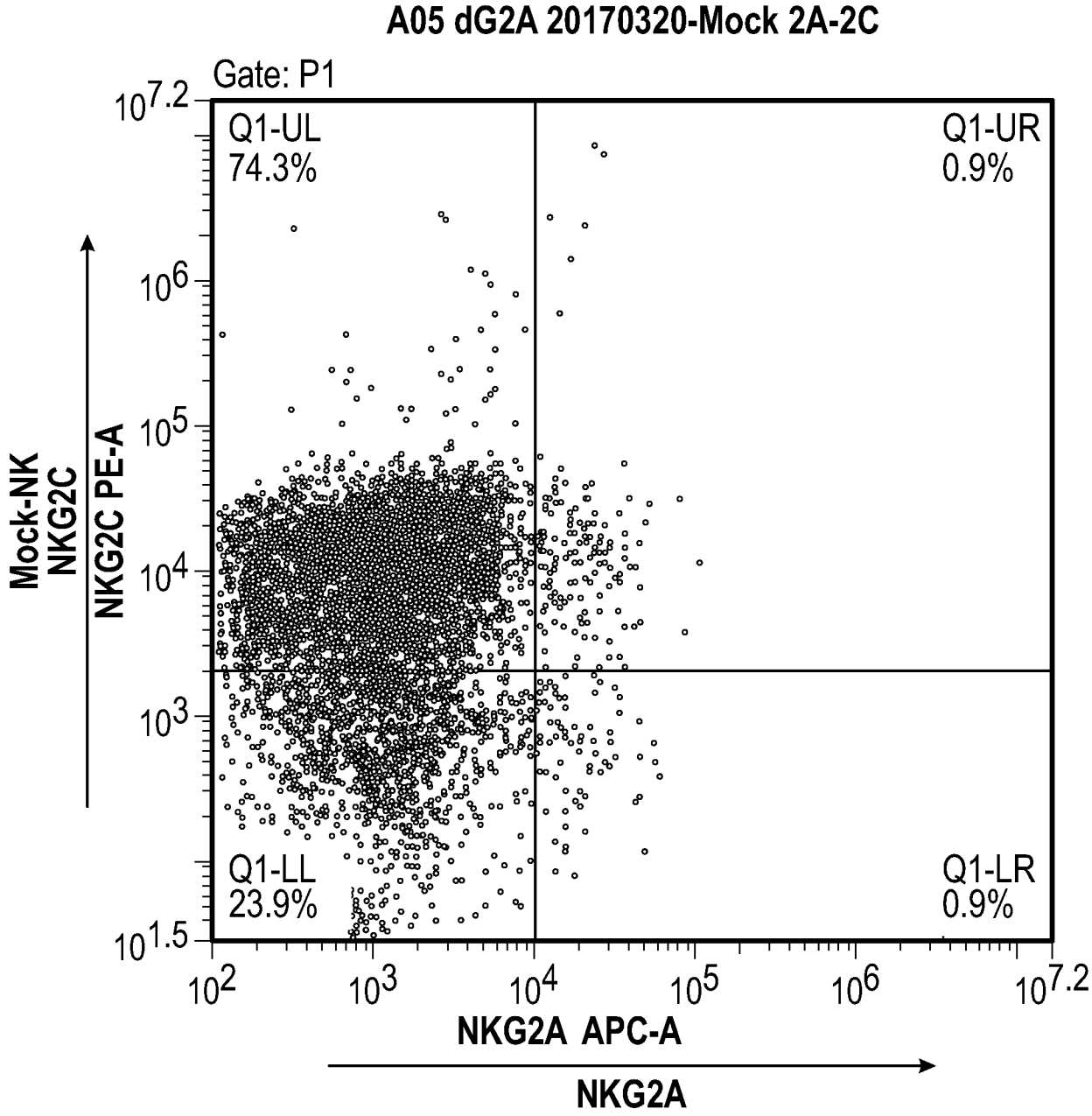
Gate: P1
Q3-UL
23.2%
Q3-UR
51.9%
MFI=62518
Q3-LL
11.8%
Q3-LR
13.1%
Mock-NK
NKG2C
NKG2C PE-A
CD56 PE-Cy7-A
GD56

FIG. 2B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Gate: P1
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Q1-UR
0.9%
Q1-LL
23.9%
Q1-LR
0.9%
Mock-NK
NKG2C
NKG2C PE-A
NKG2A APC-A
NKG2A
$10^{7.2}$
10^{6}
10^{5}
10^{4}
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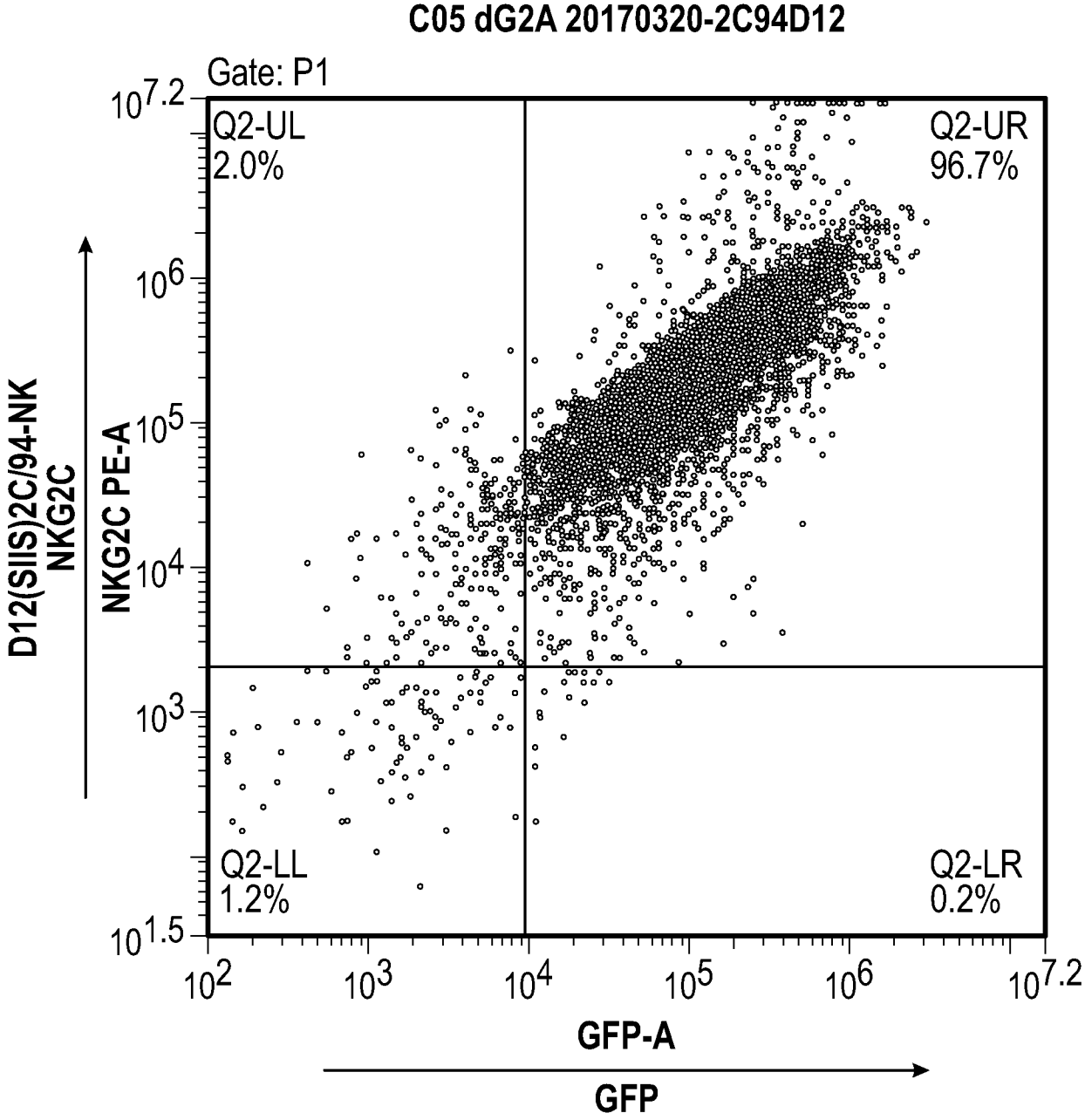
$10^{7.2}$

FIG. 2C


C05 dG2A 20170320-2C94D12
Gate: P1
Q2-UL
2.0%
Q2-UR
96.7%
Q2-LL
1.2%
Q2-LR
0.2%
D12(SIIS)2C/94-NK NKG2C
NKG2C PE-A
GFP-A
GFP
$10^{7.2}$
10^{6}
10^{5}
10^{4}
10^{3}
$10^{1.5}$
10^{2}
10^{3}
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10^{5}
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$10^{7.2}$

FIG. 2D

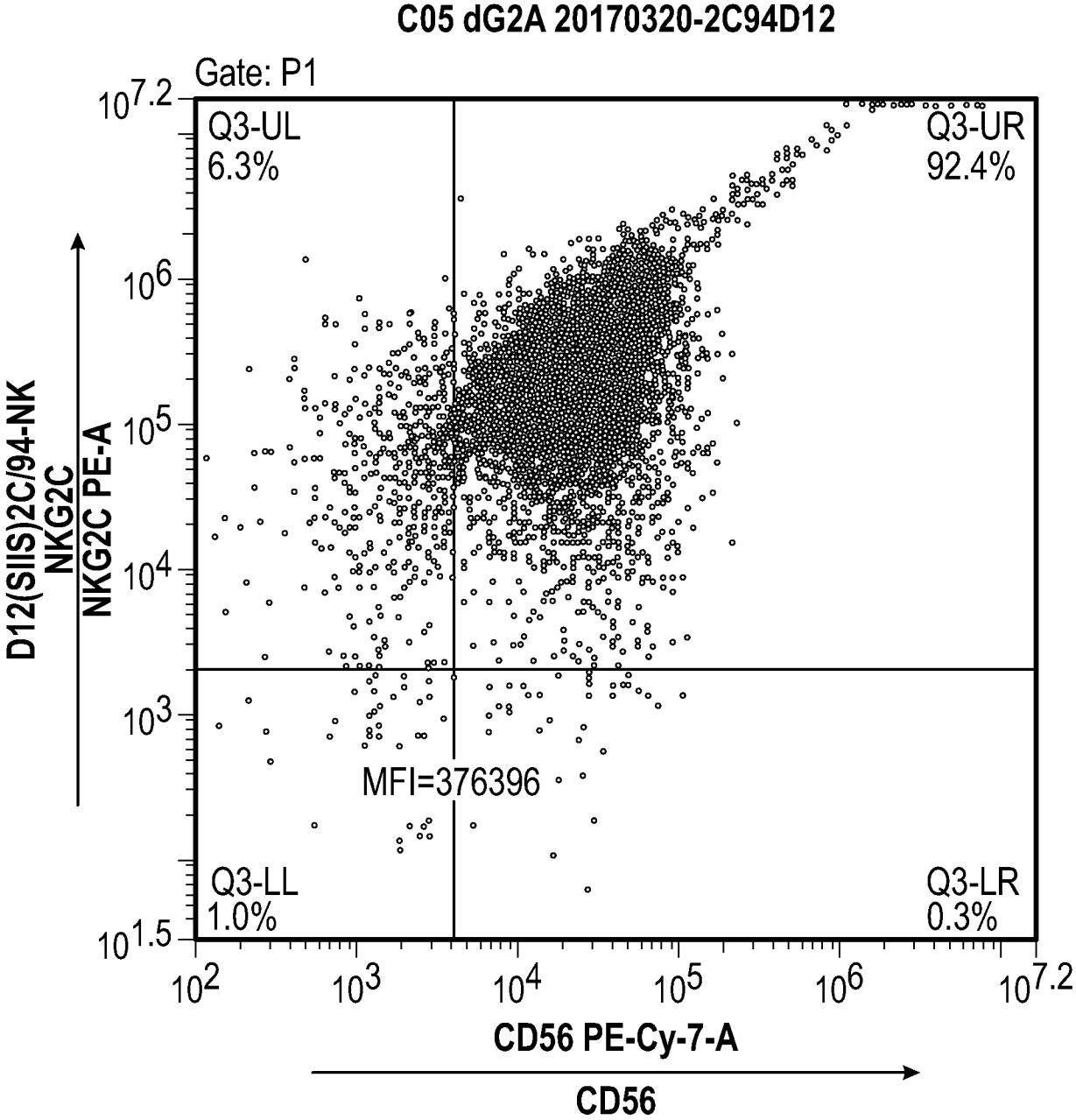
C05 dG2A 20170320-2C94D12
Gate: P1
Q3-UL
6.3%
Q3-UR
92.4%
Q3-LL
1.0%
Q3-LR
0.3%
MFI=376396
D12(SIIS)2C/94-NK
NKG2C
NKG2C PE-A
CD56 PE-Cy-7-A
CD56
$10^{7.2}$
10^{6}
10^{5}
10^{4}
10^{3}
$10^{1.5}$
10^{2}
10^{3}
10^{4}
10^{5}
10^{6}
$10^{7.2}$

FIG. 2E

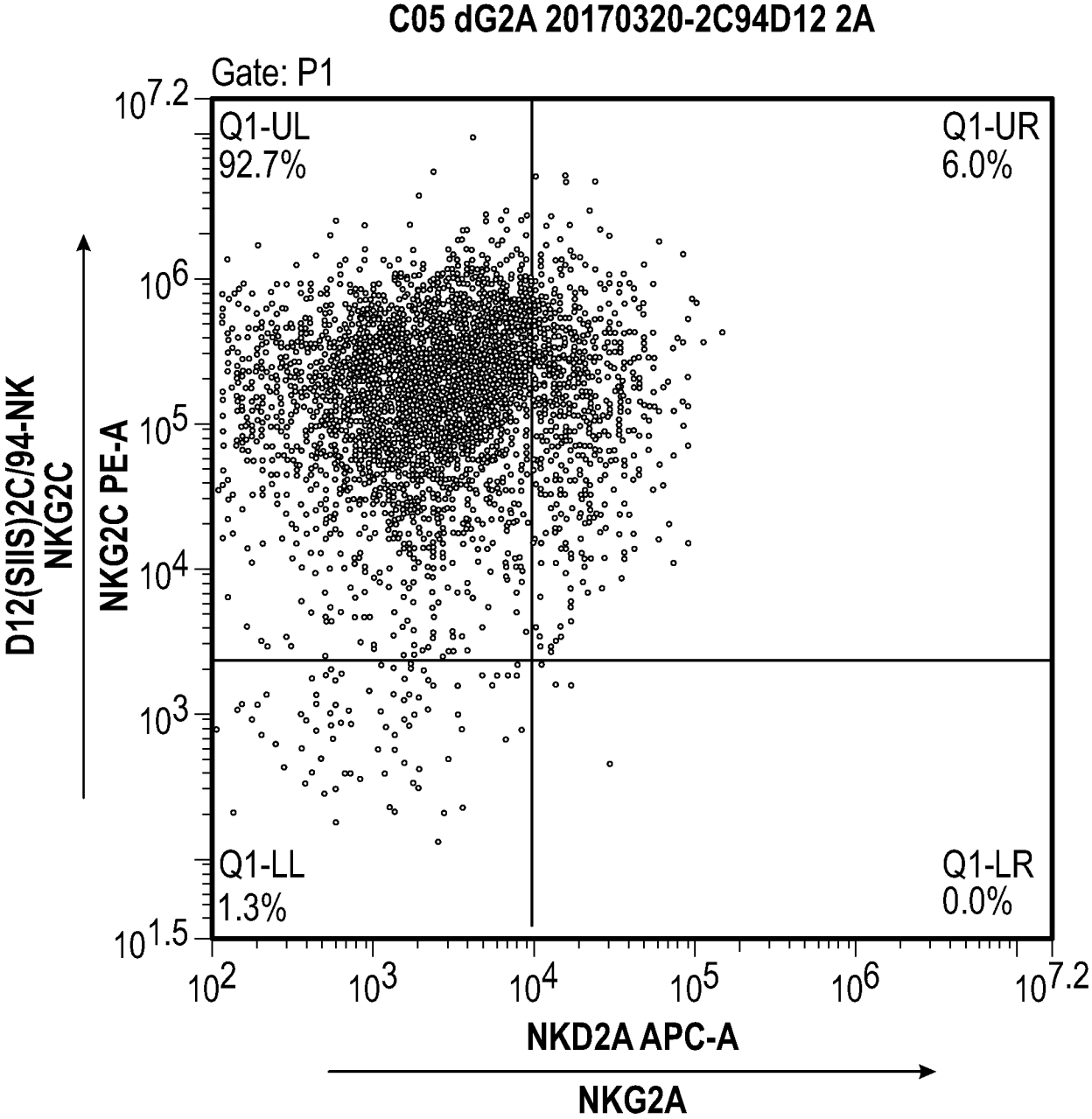
C05 dG2A 20170320-2C94D12 2A
Gate: P1
Q1-UL
92.7%
Q1-UR
6.0%
Q1-LL
1.3%
Q1-LR
0.0%
D12(SIIS)2C/94-NK
NKG2C
NKG2C PE-A
NKD2A APC-A
NKG2A
10^7.2
10^6
10^5
10^4
10^3
10^1.5
10^2
10^3
10^4
10^5
10^6
10^7.2

FIG. 2F

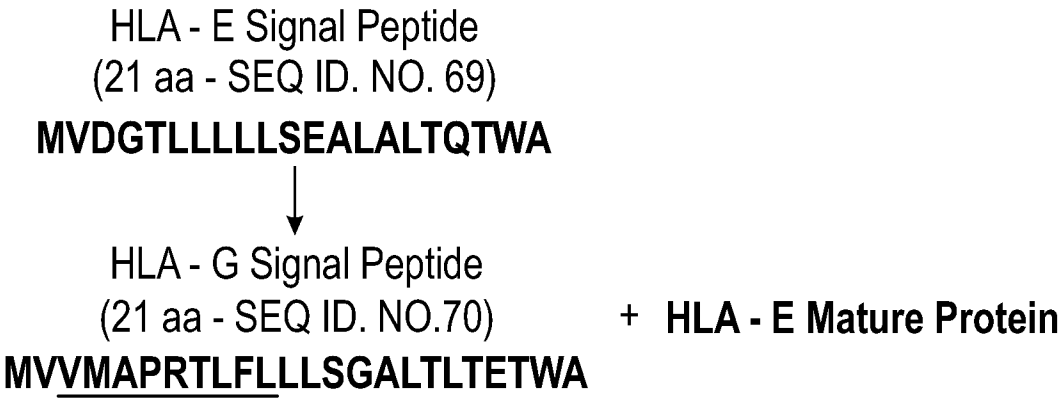
HLA - E Signal Peptide
(21 aa - SEQ ID. NO. 69)
MVDGTLLLLLSEALALTQTWA
HLA - G Signal Peptide
(21 aa - SEQ ID. NO.70)
MVVMAPRTLFLLLSGALTLTETWA
+ HLA - E Mature Protein

FIG. 3A

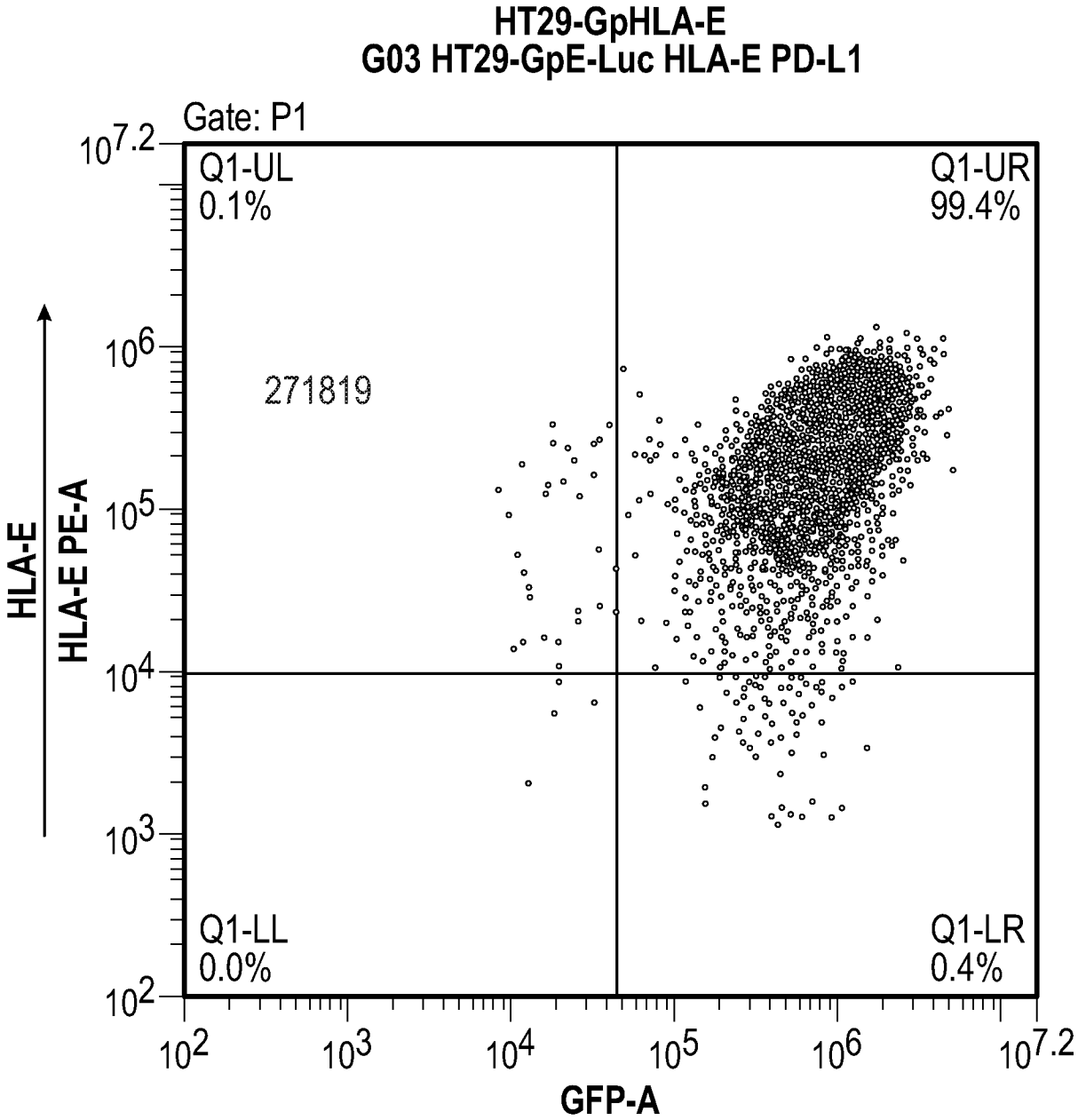
HT29-GpHLA-E
G03 HT29-GpE-Luc HLA-E PD-L1
Gate: P1
Q1-UL
0.1%
Q1-UR
99.4%
271819
Q1-LL
0.0%
Q1-LR
0.4%
HLA-E
HLA-E PE-A
GFP-A
$10^{7.2}$
10^6
10^5
10^4
10^3
10^2
10^2
10^3
10^4
10^5
10^6
$10^{7.2}$

FIG. 3B

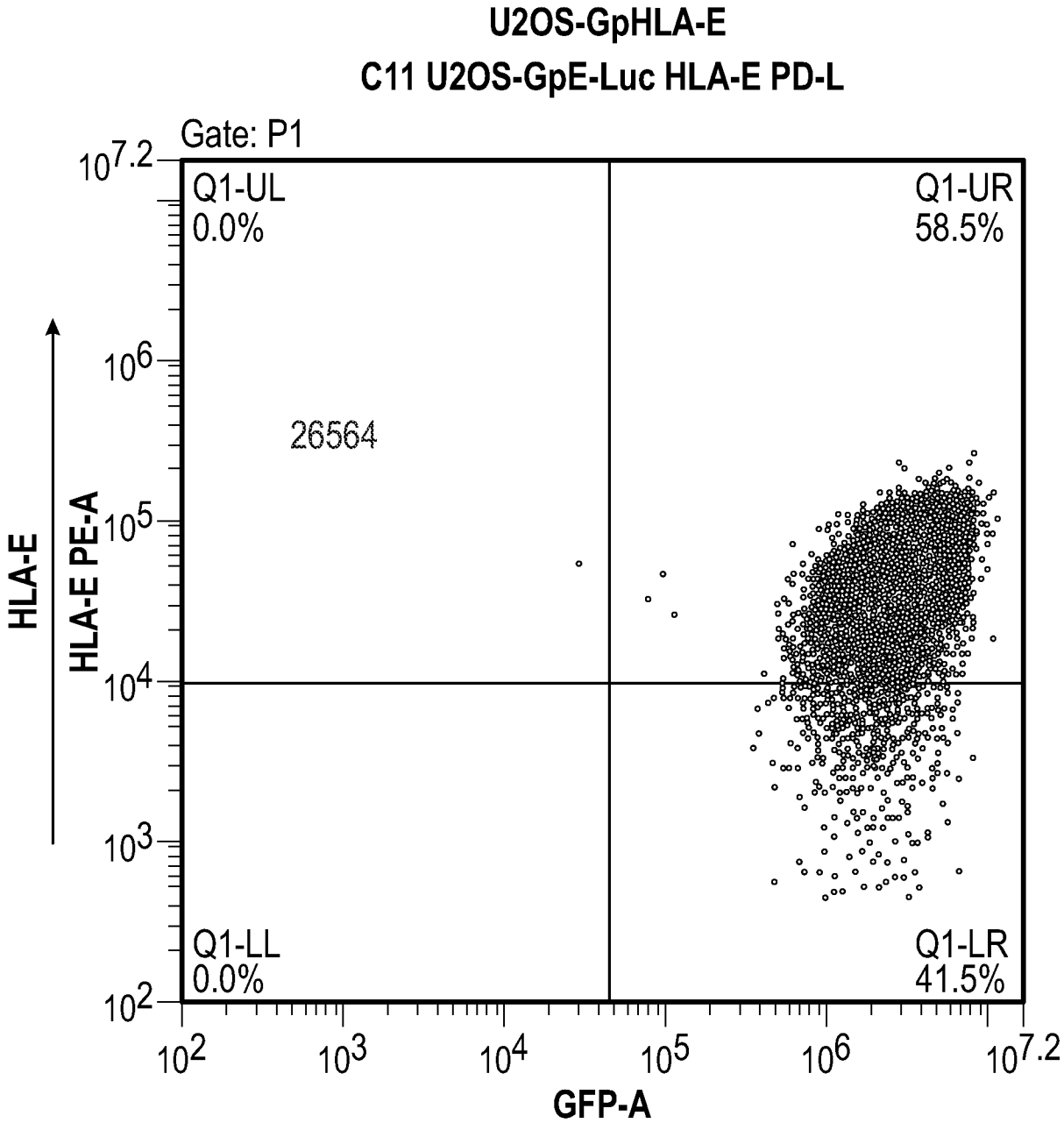

FIG. 3B
(Continued)

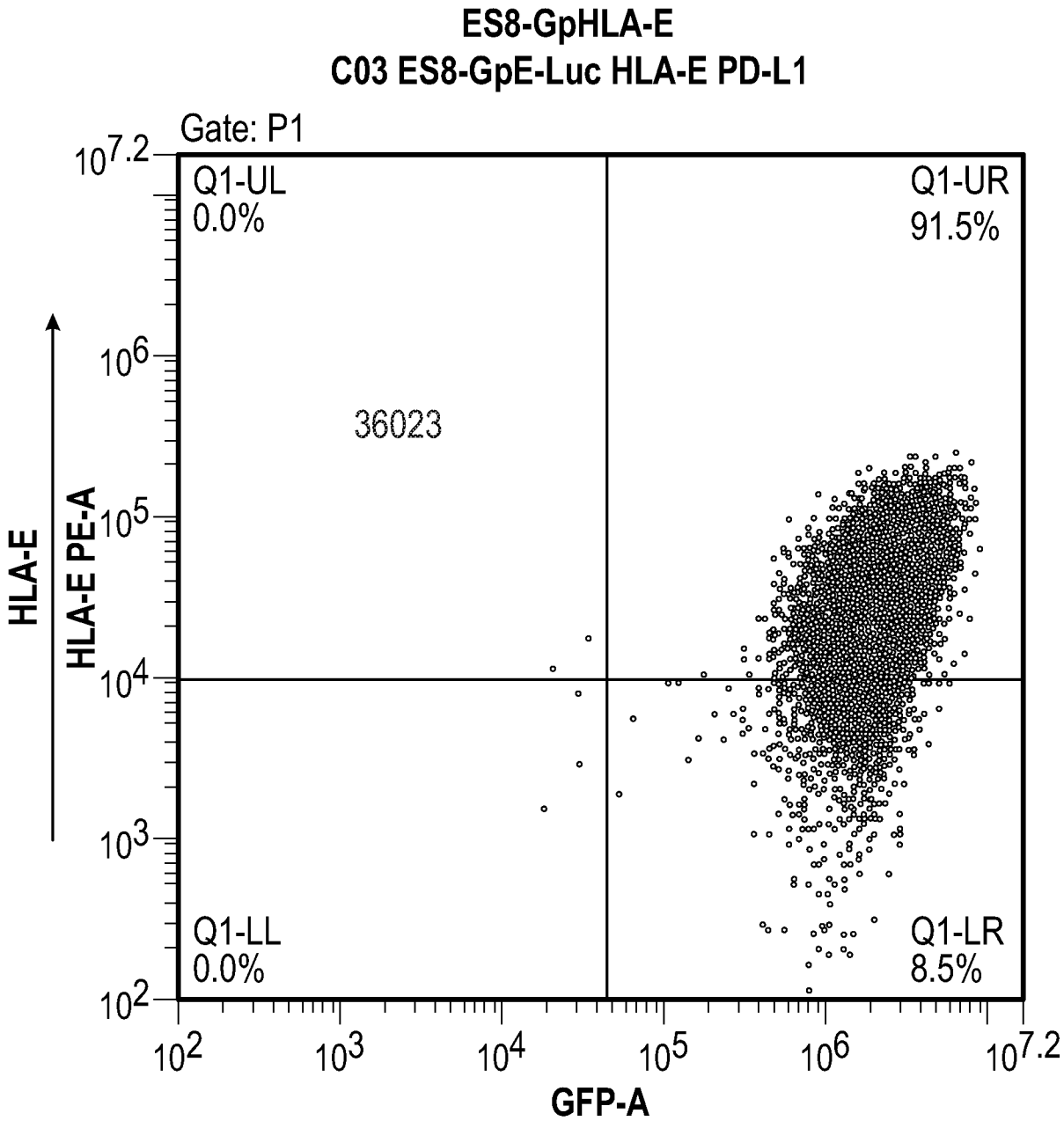

FIG. 3B
(Continued)

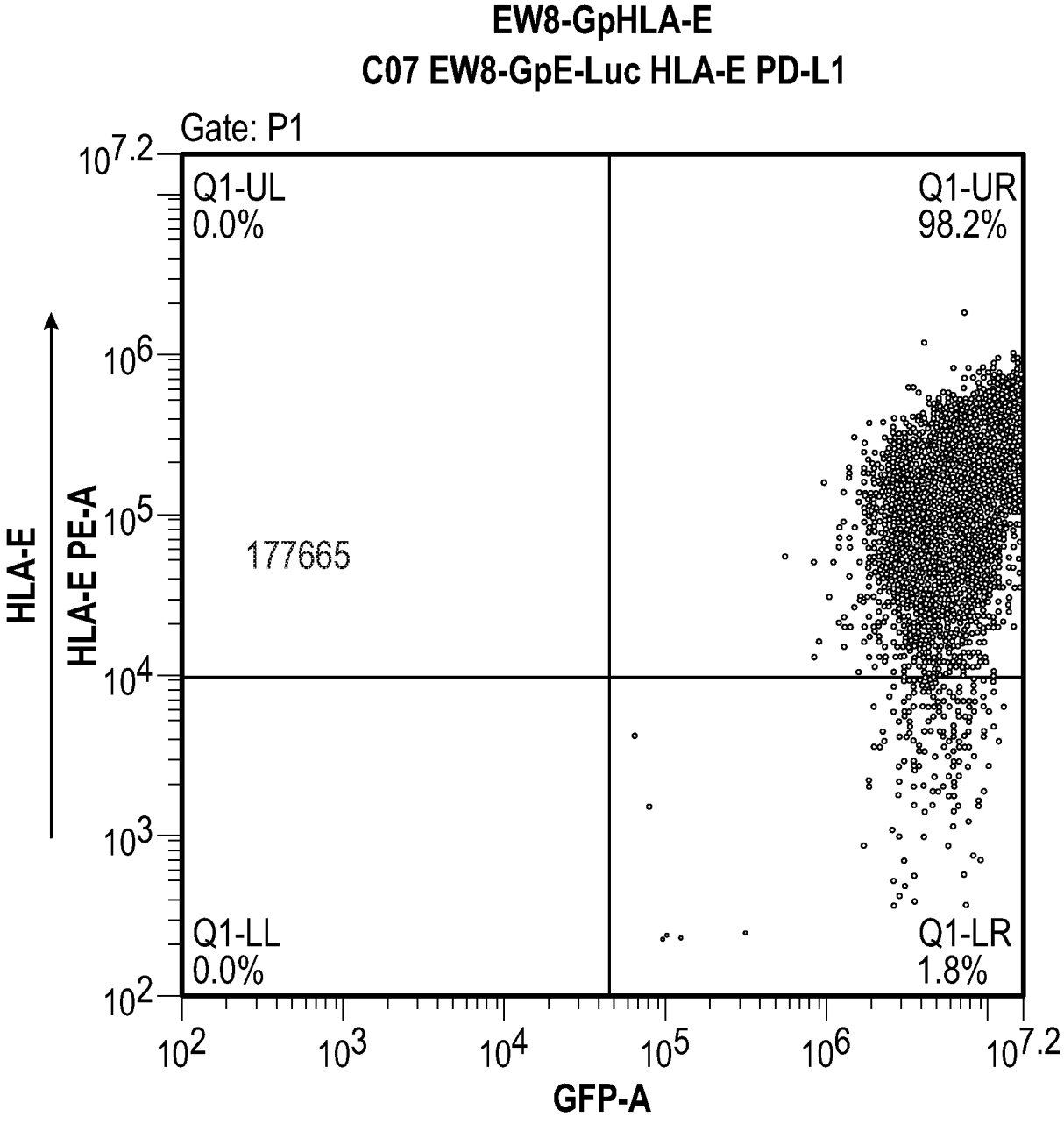

FIG. 3B
(Continued)

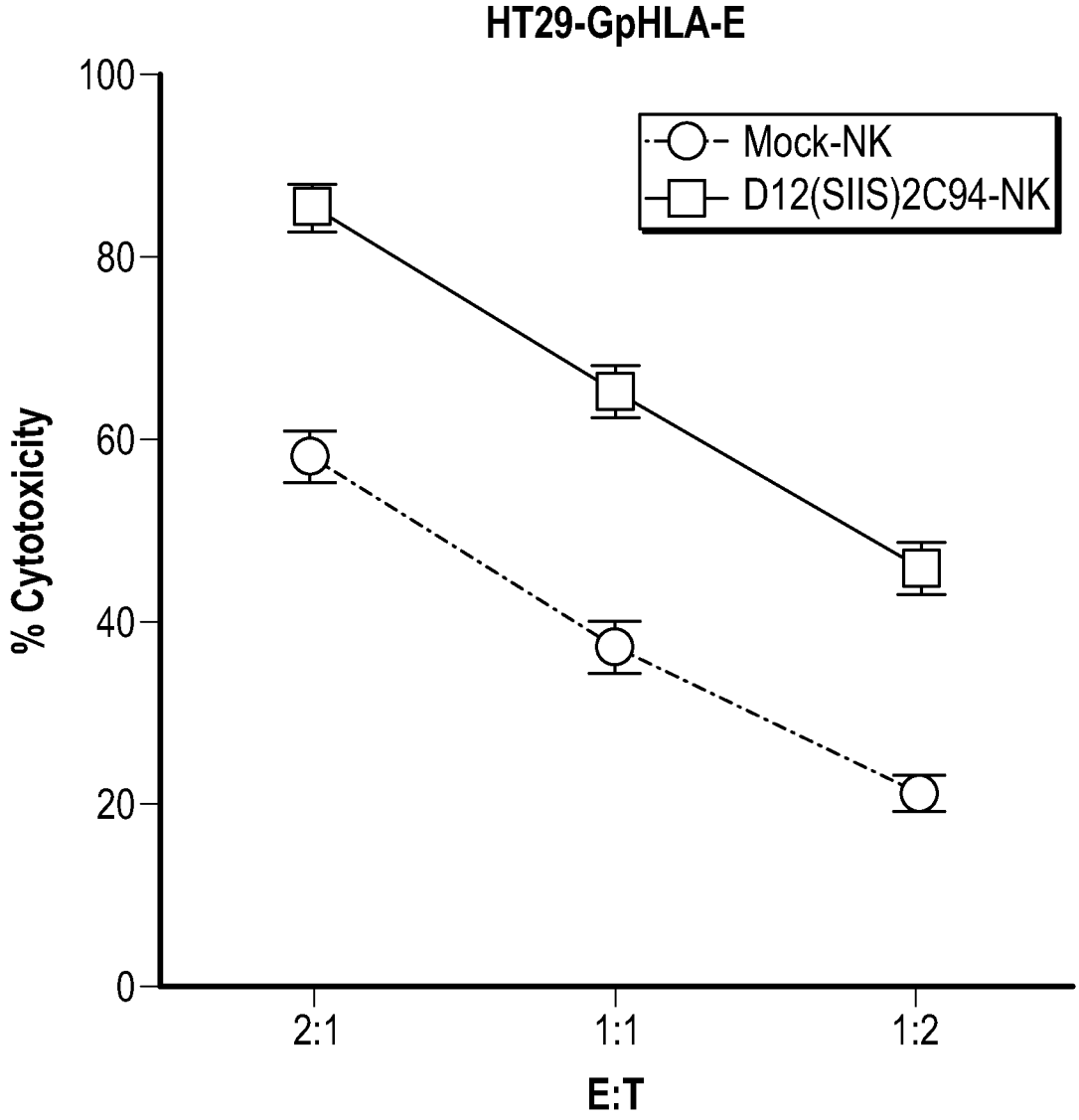
HT29-GpHLA-E
Mock-NK
D12(SIIS)2C94-NK
% Cytotoxicity
100
80
60
40
20
0
2:1
1:1
1:2
E:T

FIG. 4A

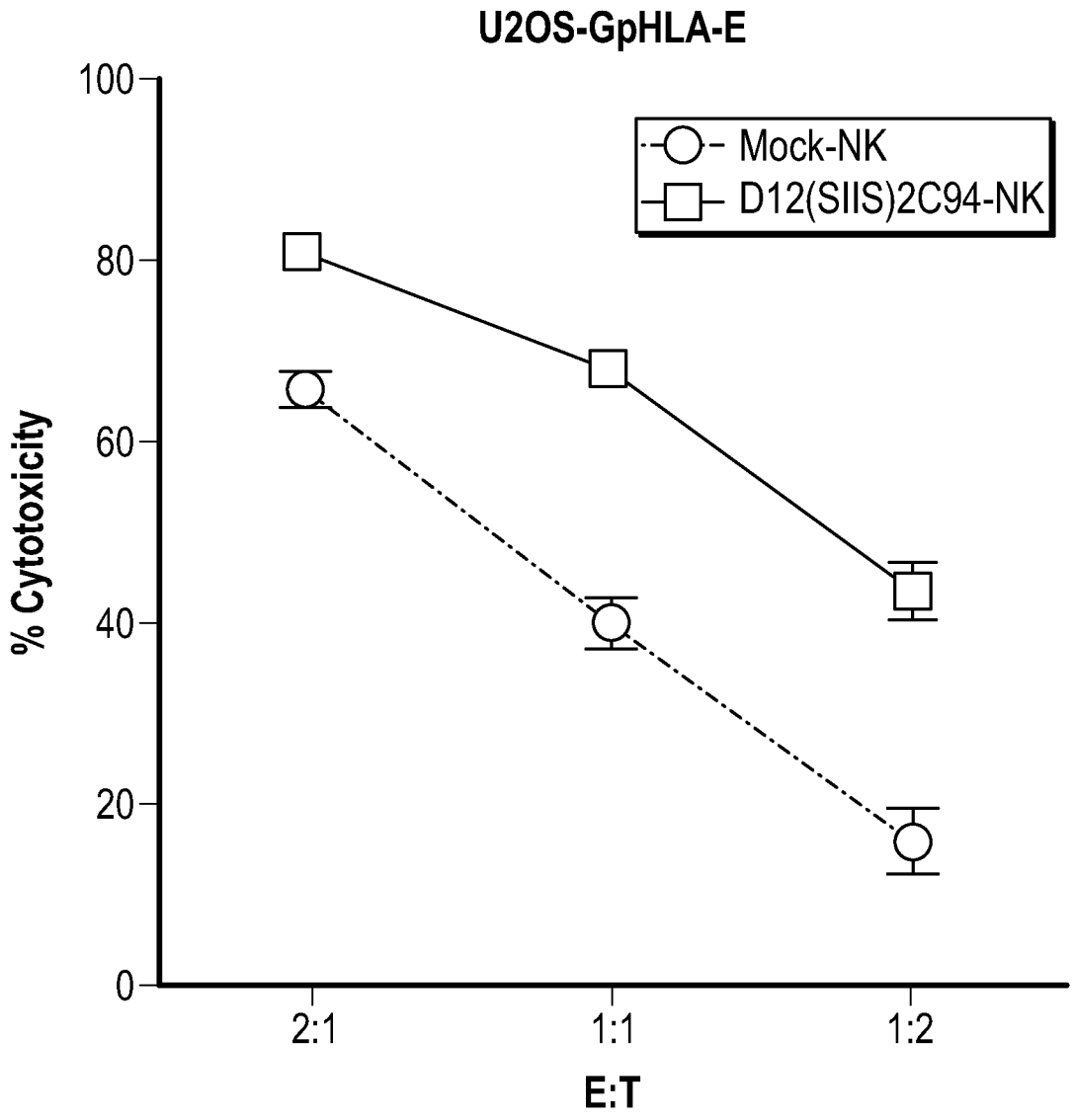
U2OS-GpHLA-E
Mock-NK
D12(SIIS)2C94-NK
% Cytotoxicity
100
80
60
40
20
0
2:1
1:1
1:2
E:T

FIG. 4B

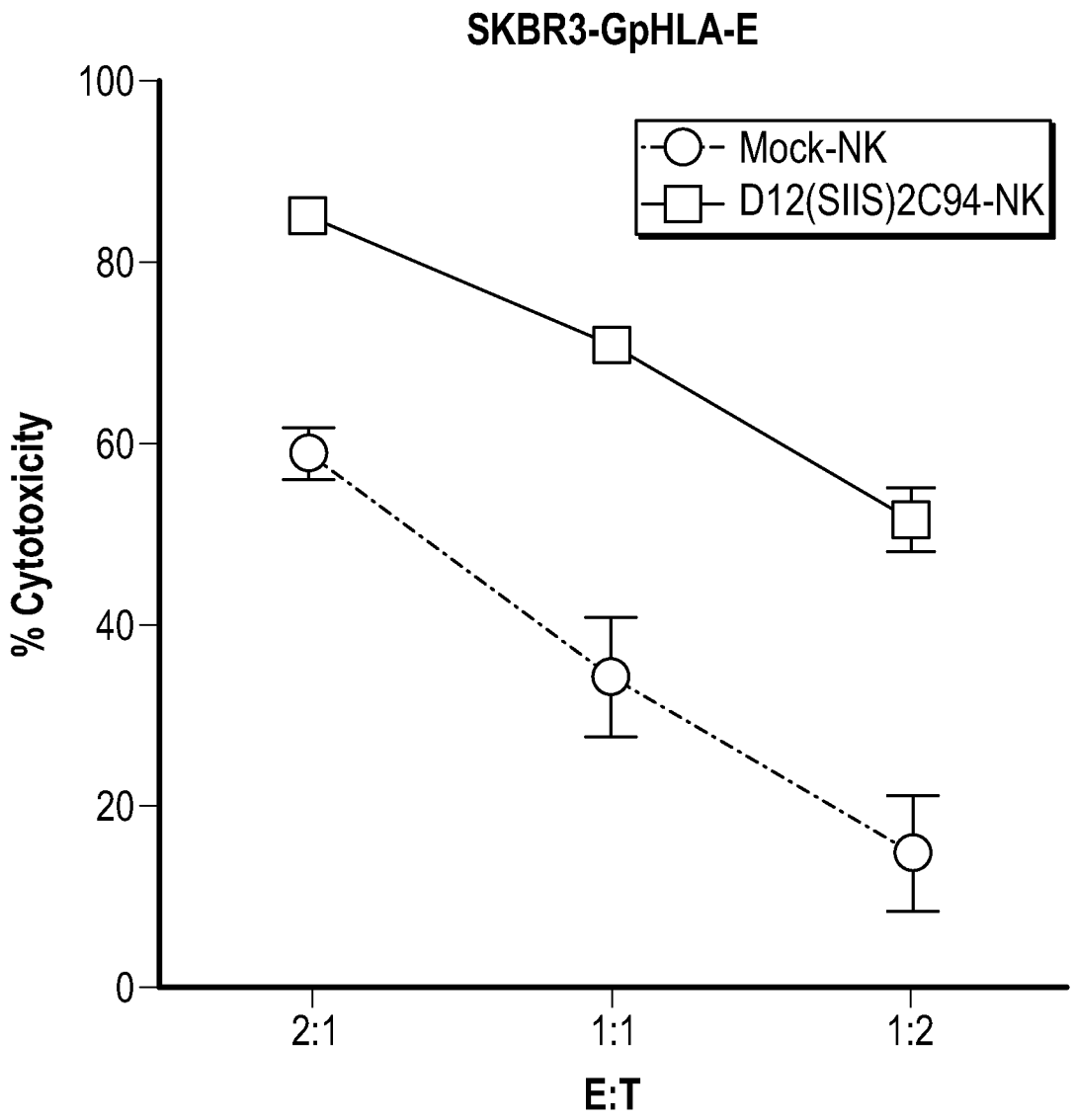
SKBR3-GpHLA-E
100
80
60
40
20
0
% Cytotoxicity
Mock-NK
D12(SIIS)2C94-NK
2:1
1:1
1:2
E:T

FIG. 4C

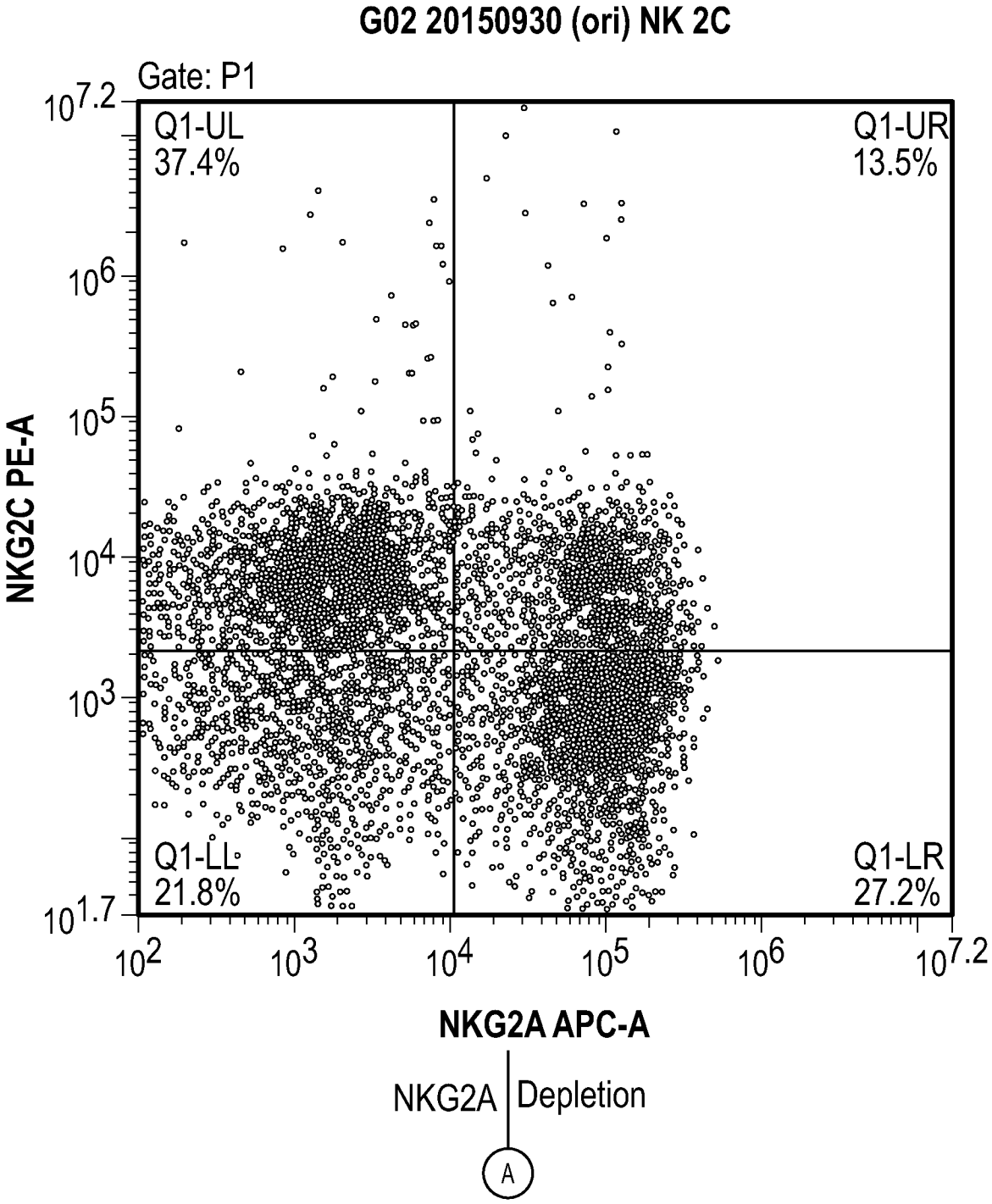
G02 20150930 (ori) NK 2C
Gate: P1
Q1-UL
37.4%
Q1-UR
13.5%
Q1-LL
21.8%
Q1-LR
27.2%
NKG2C PE-A
NKG2A APC-A
$10^{7.2}$
10^{6}
10^{5}
10^{4}
10^{3}
$10^{1.7}$
10^{2}
10^{3}
10^{4}
10^{5}
10^{6}
$10^{7.2}$
NKG2A Depletion
A

FIG. 5A

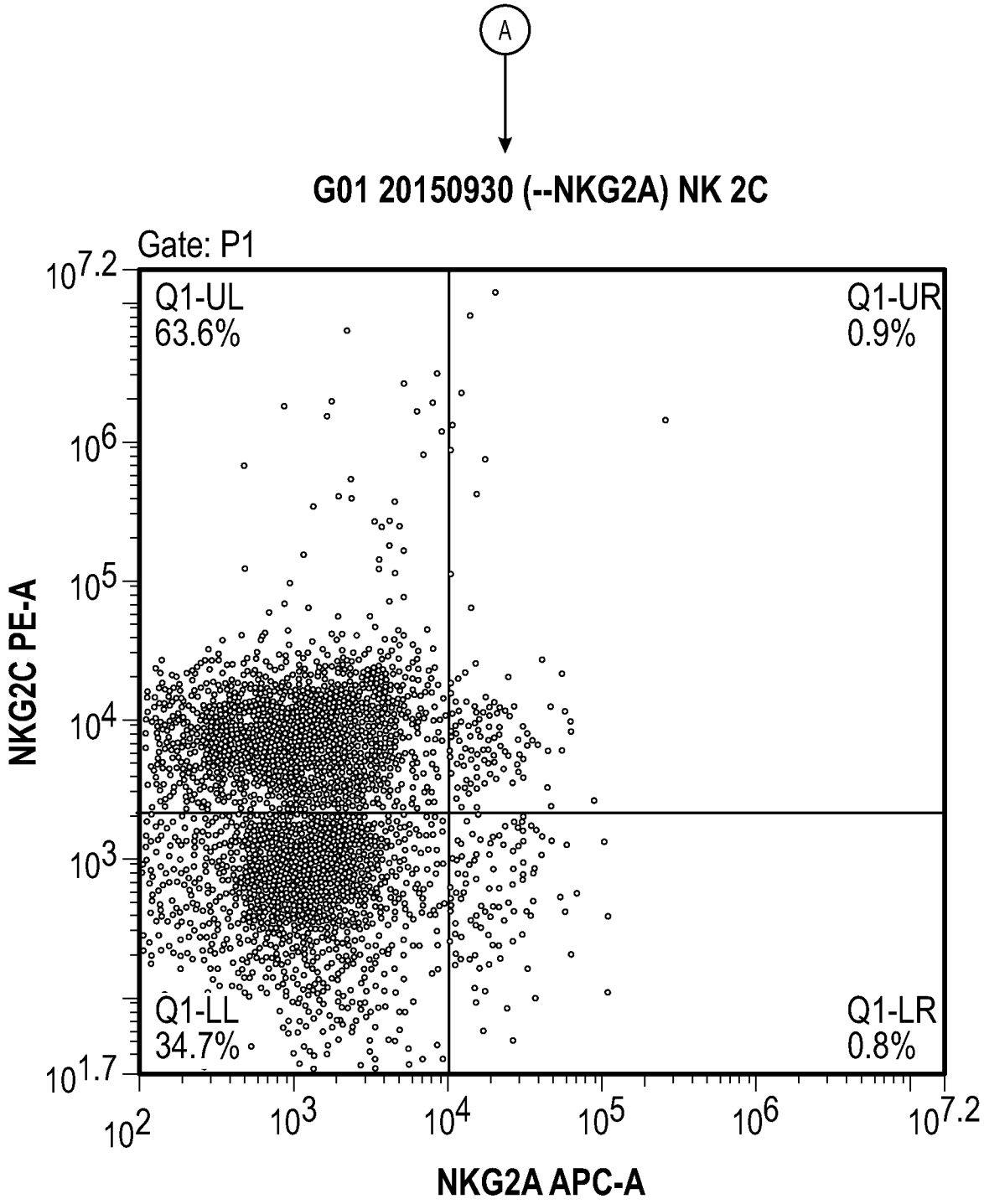

FIG. 5A
(Continued)

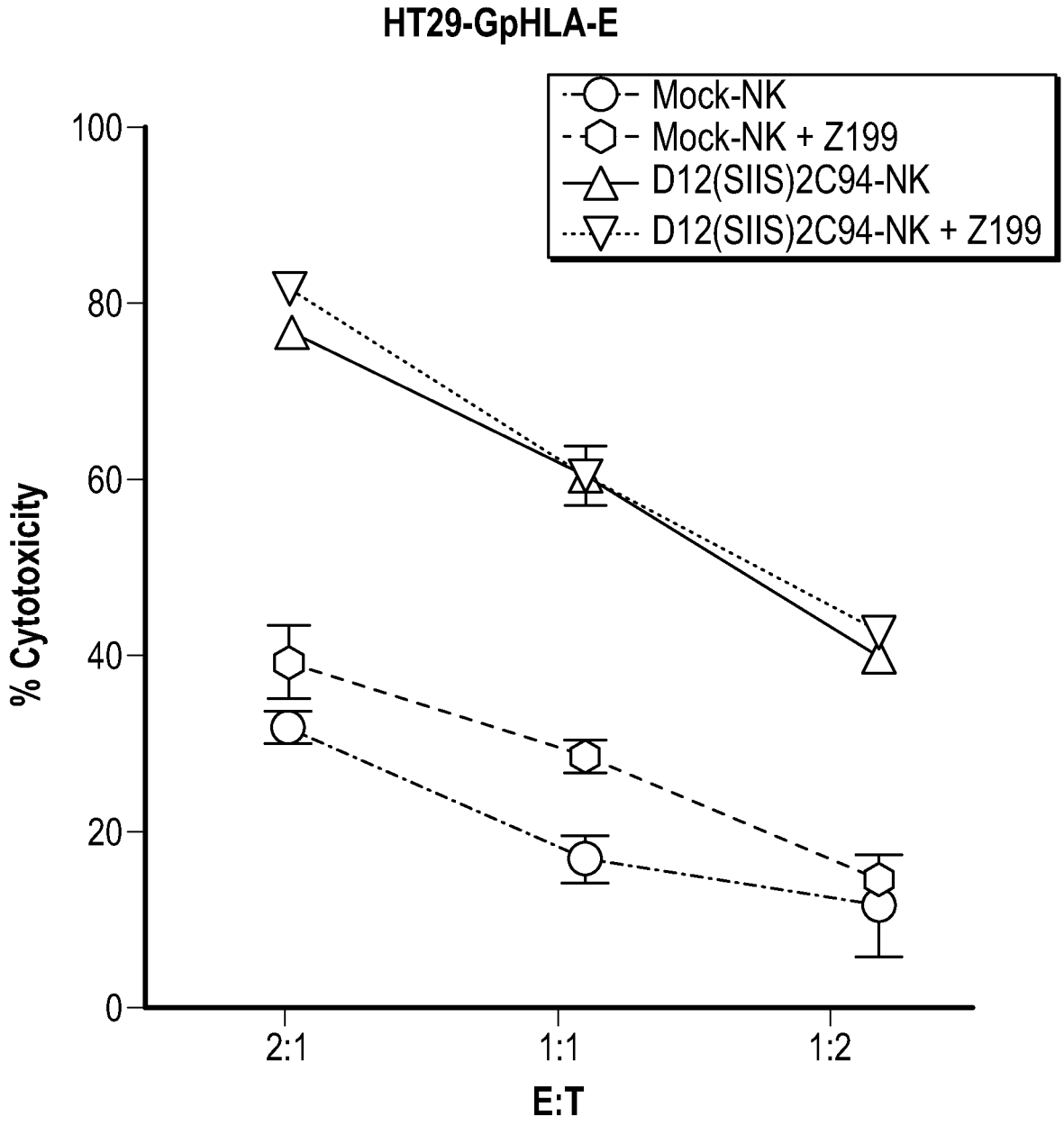
HT29-GpHLA-E
Mock-NK
Mock-NK + Z199
D12(SIIS)2C94-NK
D12(SIIS)2C94-NK + Z199
100
80
60
40
20
0
% Cytotoxicity
2:1
1:1
1:2
E:T

FIG. 5B

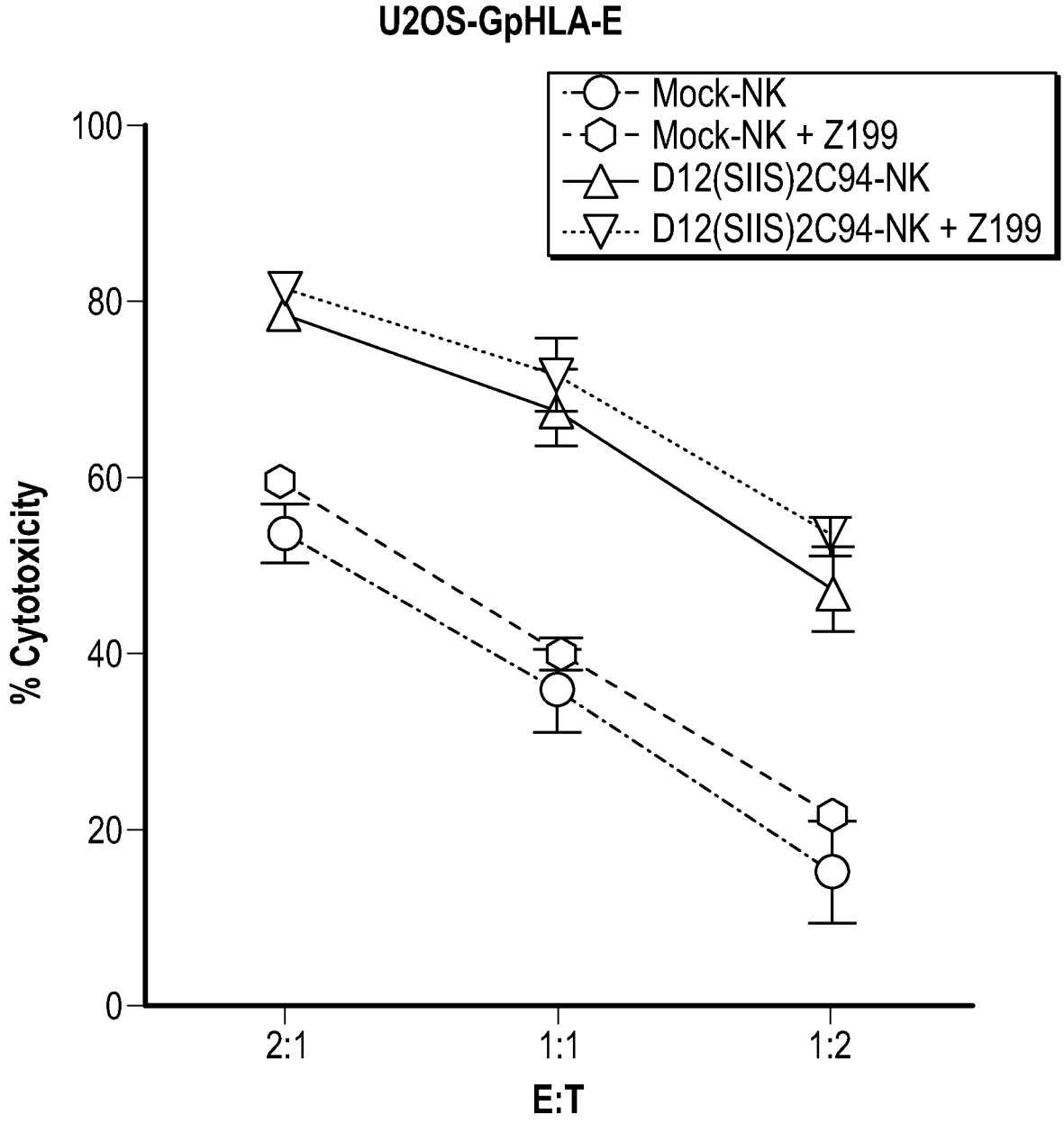

FIG. 5B
(Continued)

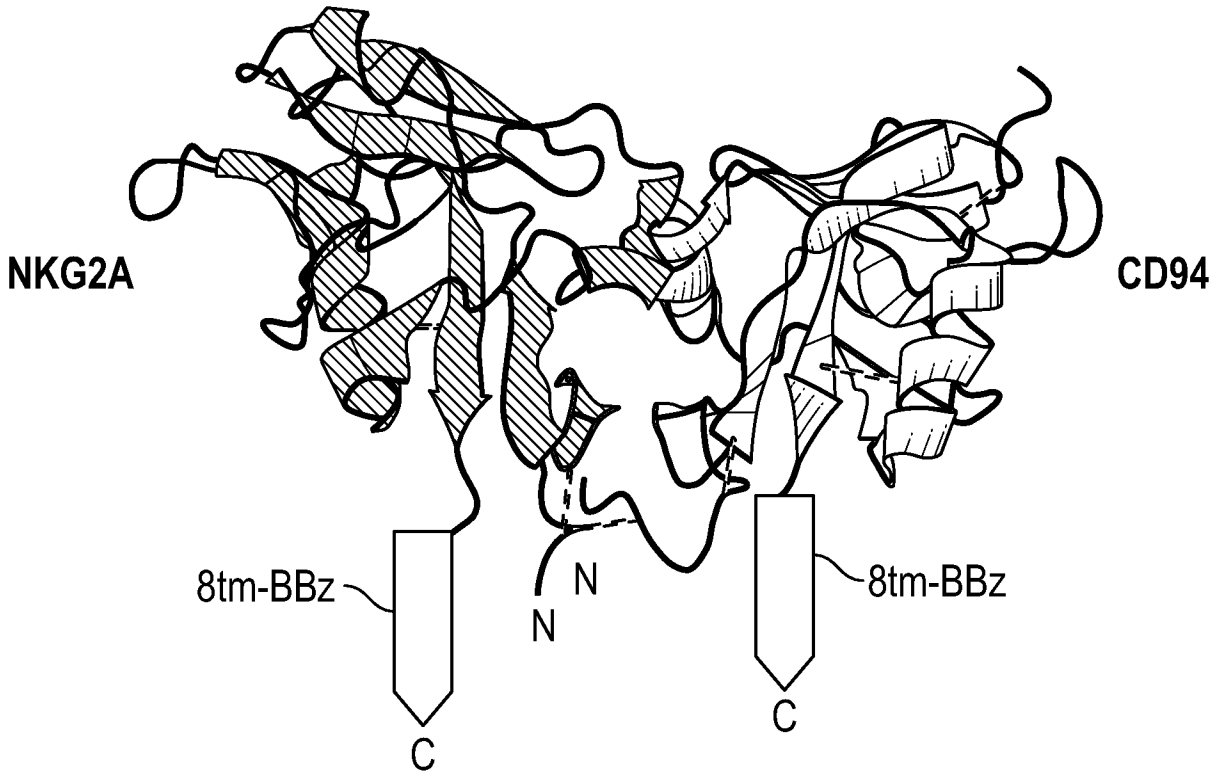

FIG. 6A


MSCV-NKG2A-CD8tm-41BB-CD3ζ-IRES-GFP
MSCV-CD94-CD8tm-41BB-CD3ζ-IRES-GFP

FIG. 6B

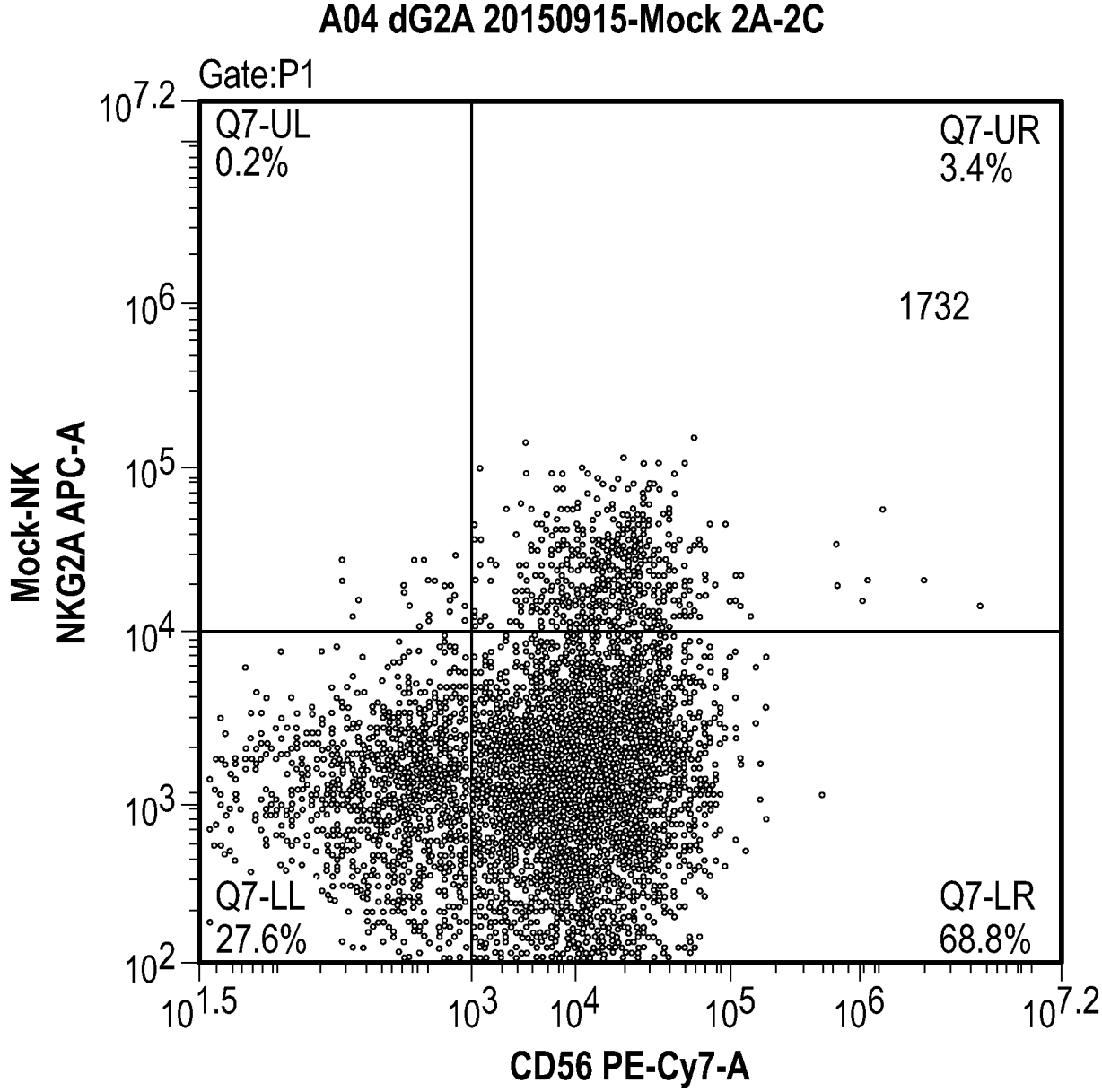
A04 dG2A 20150915-Mock 2A-2C
Gate:P1
Q7-UL
0.2%
Q7-UR
3.4%
1732
Q7-LL
27.6%
Q7-LR
68.8%
Mock-NK
NKG2A APC-A
CD56 PE-Cy7-A
10^7.2
10^6
10^5
10^4
10^3
10^2
10^1.5
10^3
10^4
10^5
10^6
10^7.2

FIG. 7A

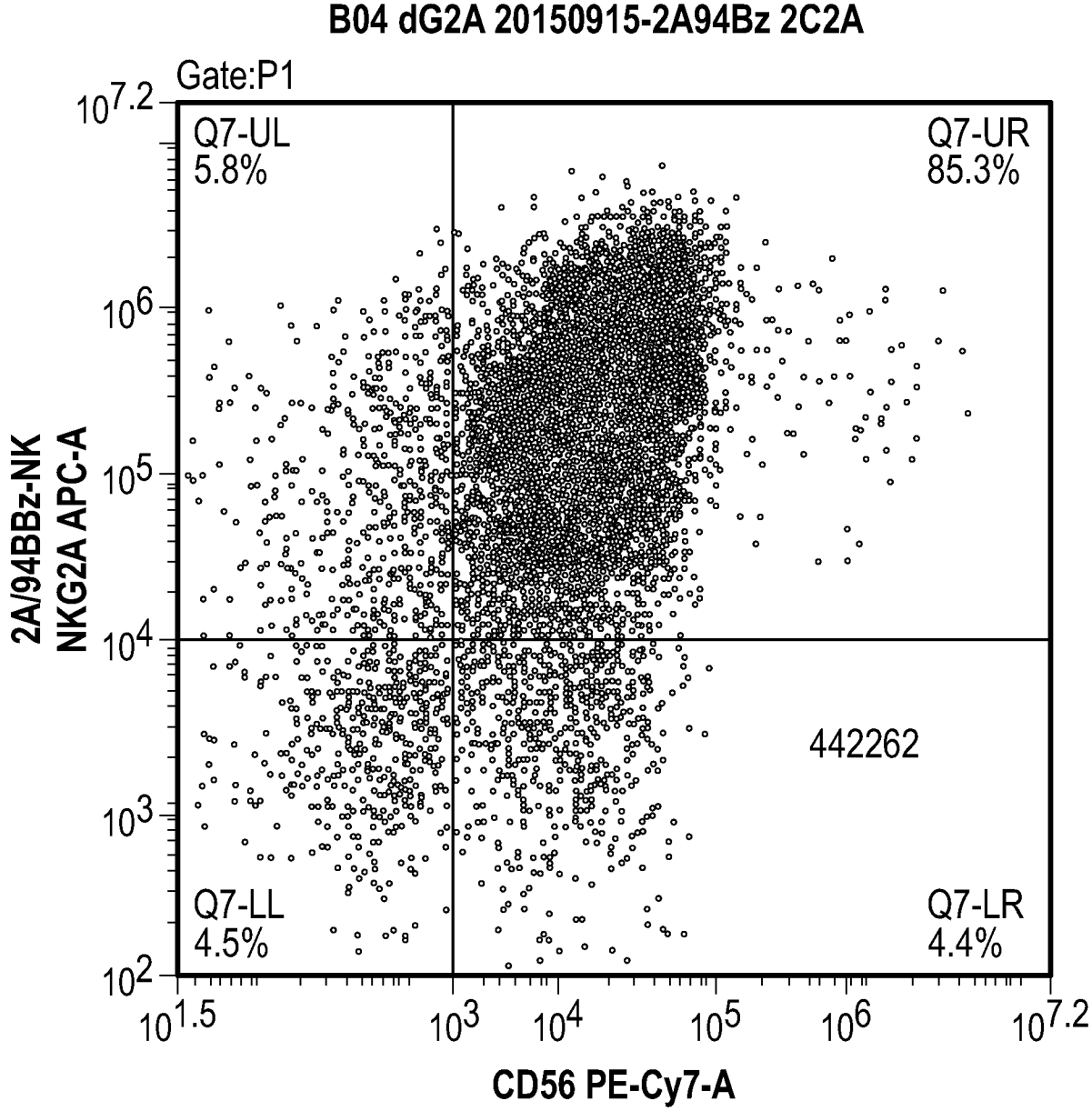

FIG. 7A
(Continued)

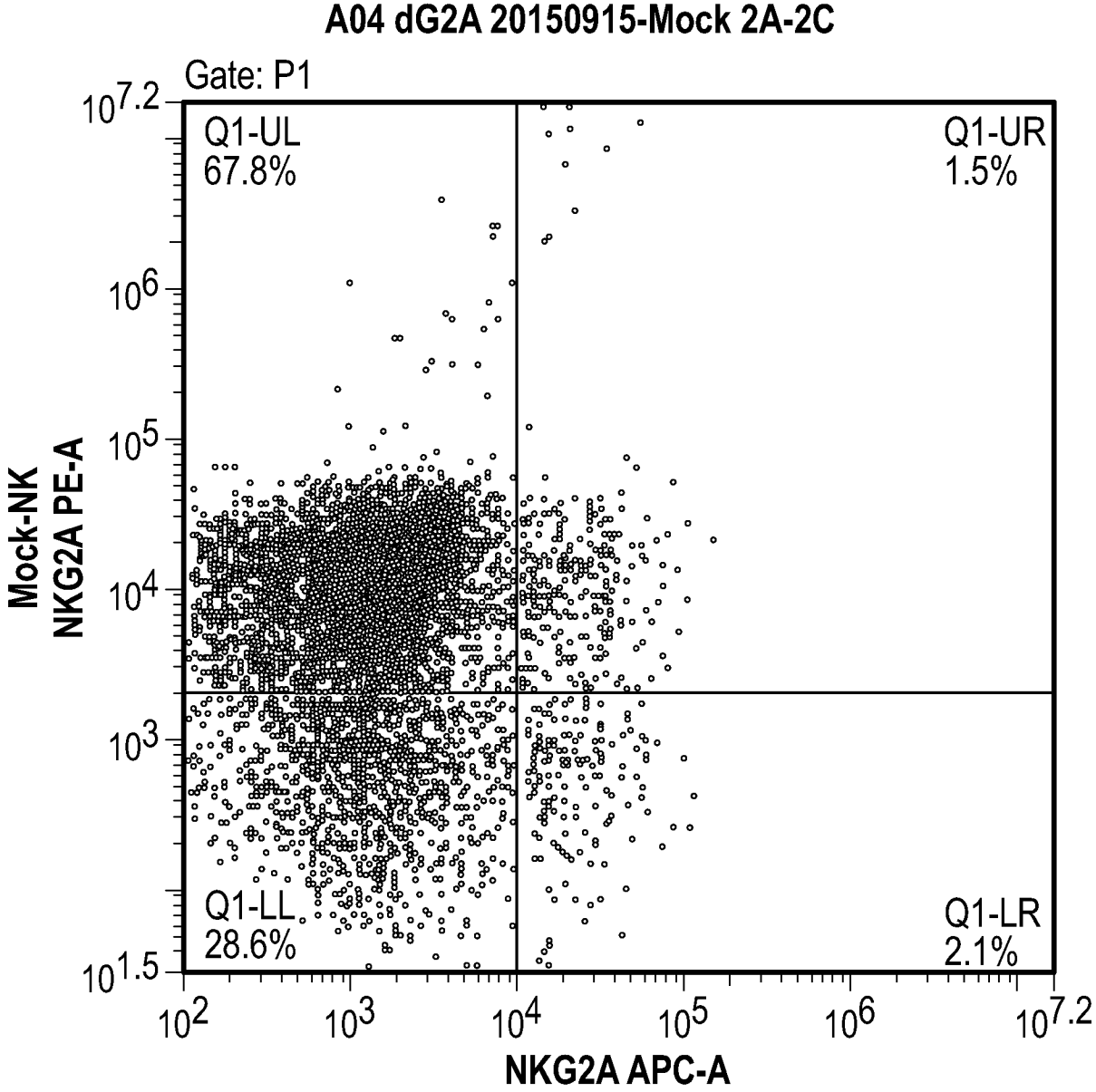

FIG. 7A
(Continued)

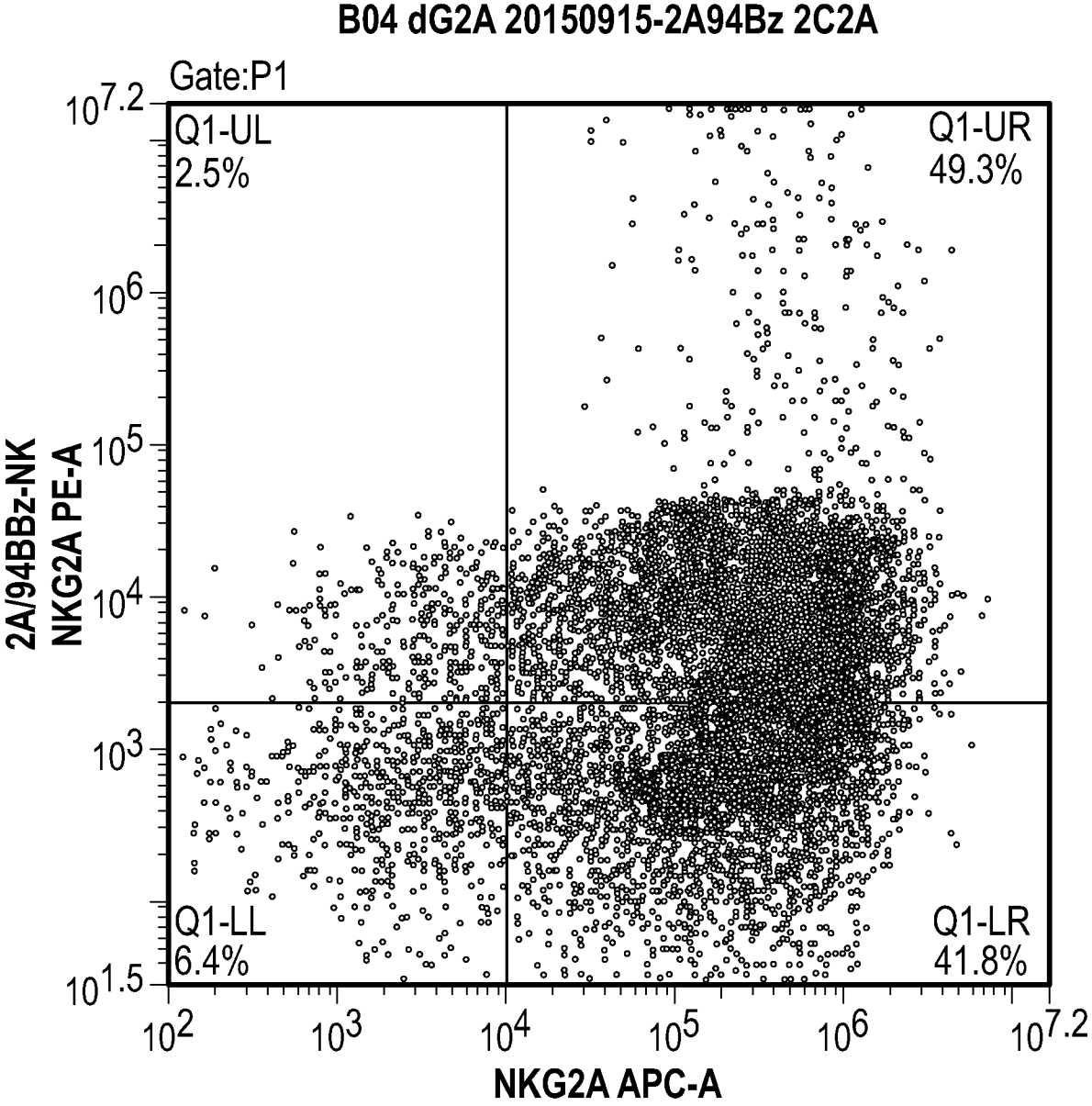

FIG. 7A
(Continued)

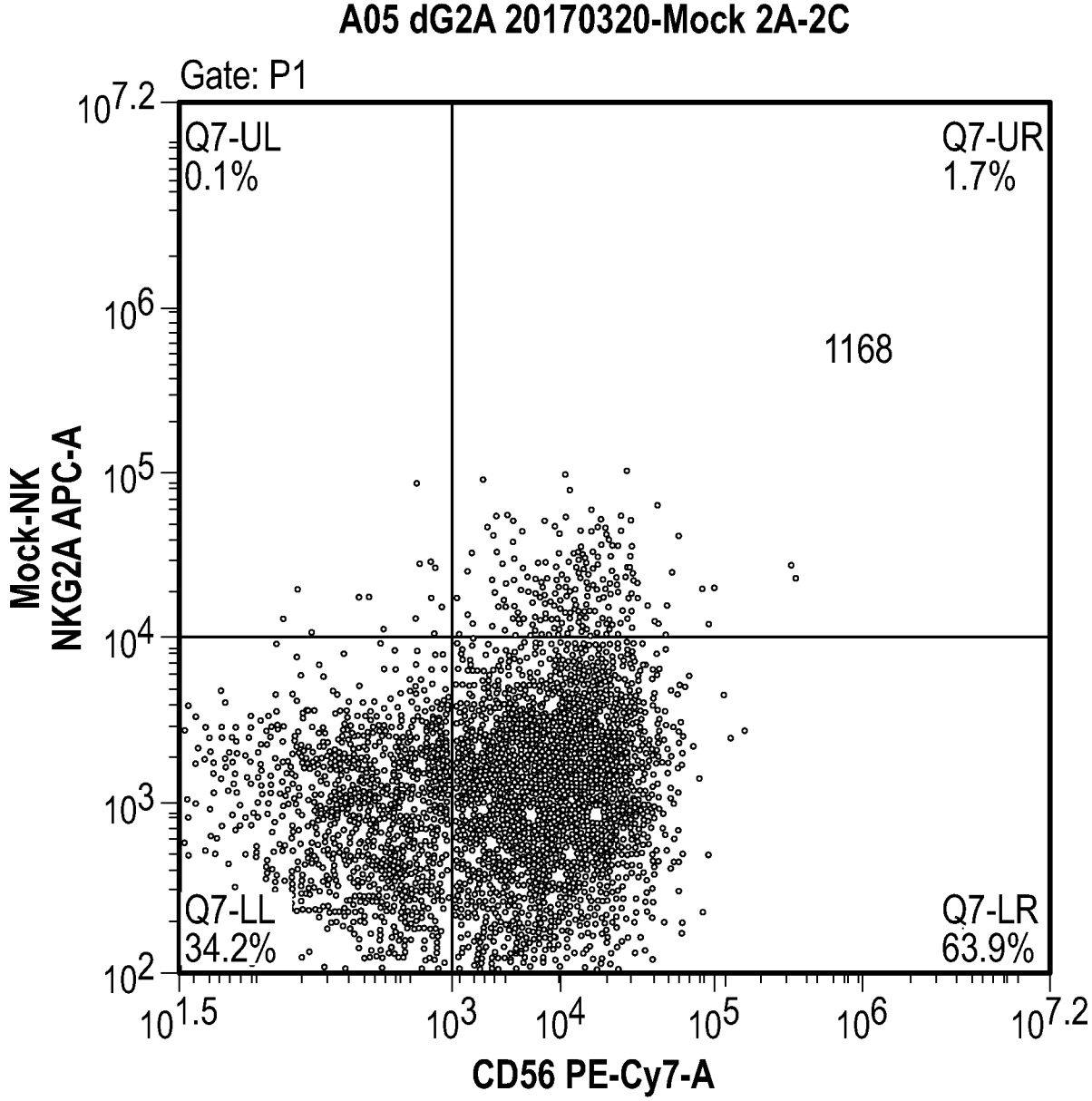
A05 dG2A 20170320-Mock 2A-2C
Gate: P1
Q7-UL
0.1%
Q7-UR
1.7%
1168
Q7-LL
34.2%
Q7-LR
63.9%
Mock-NK
NKG2A APC-A
CD56 PE-Cy7-A
10^7.2
10^6
10^5
10^4
10^3
10^2
10^1.5
10^3
10^4
10^5
10^6
10^7.2

FIG. 7B

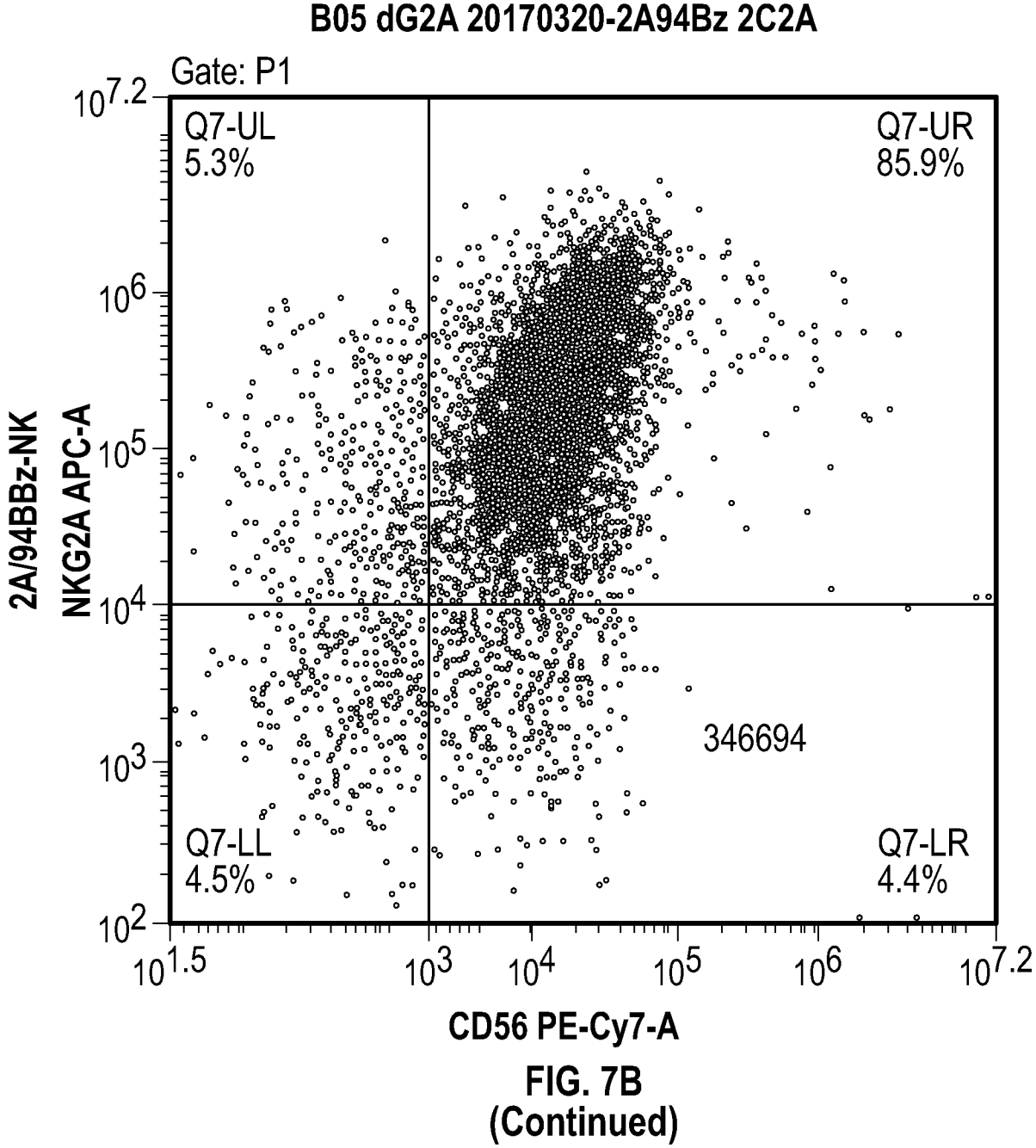

FIG. 7B
(Continued)

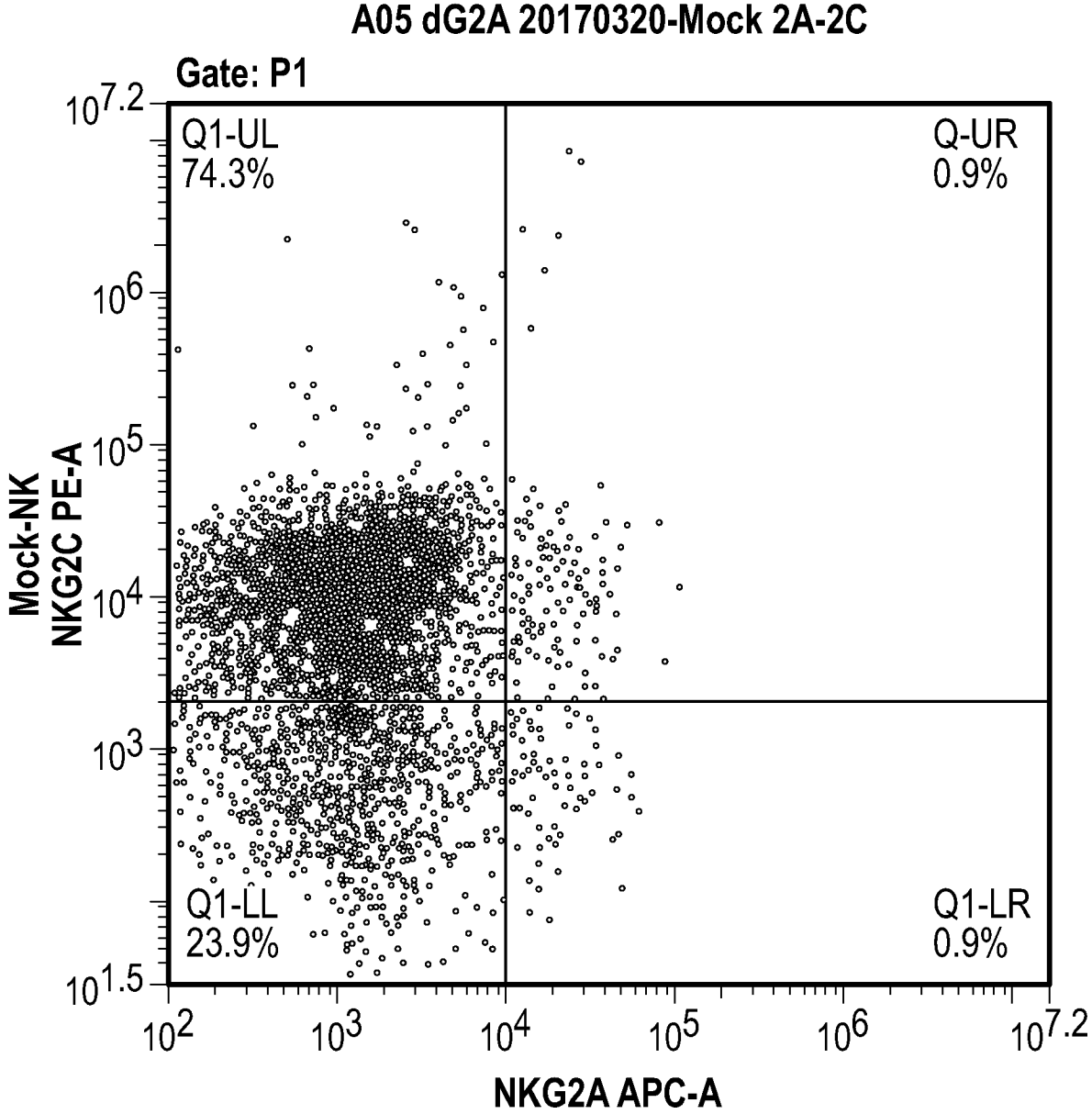

FIG. 7B
(Continued)

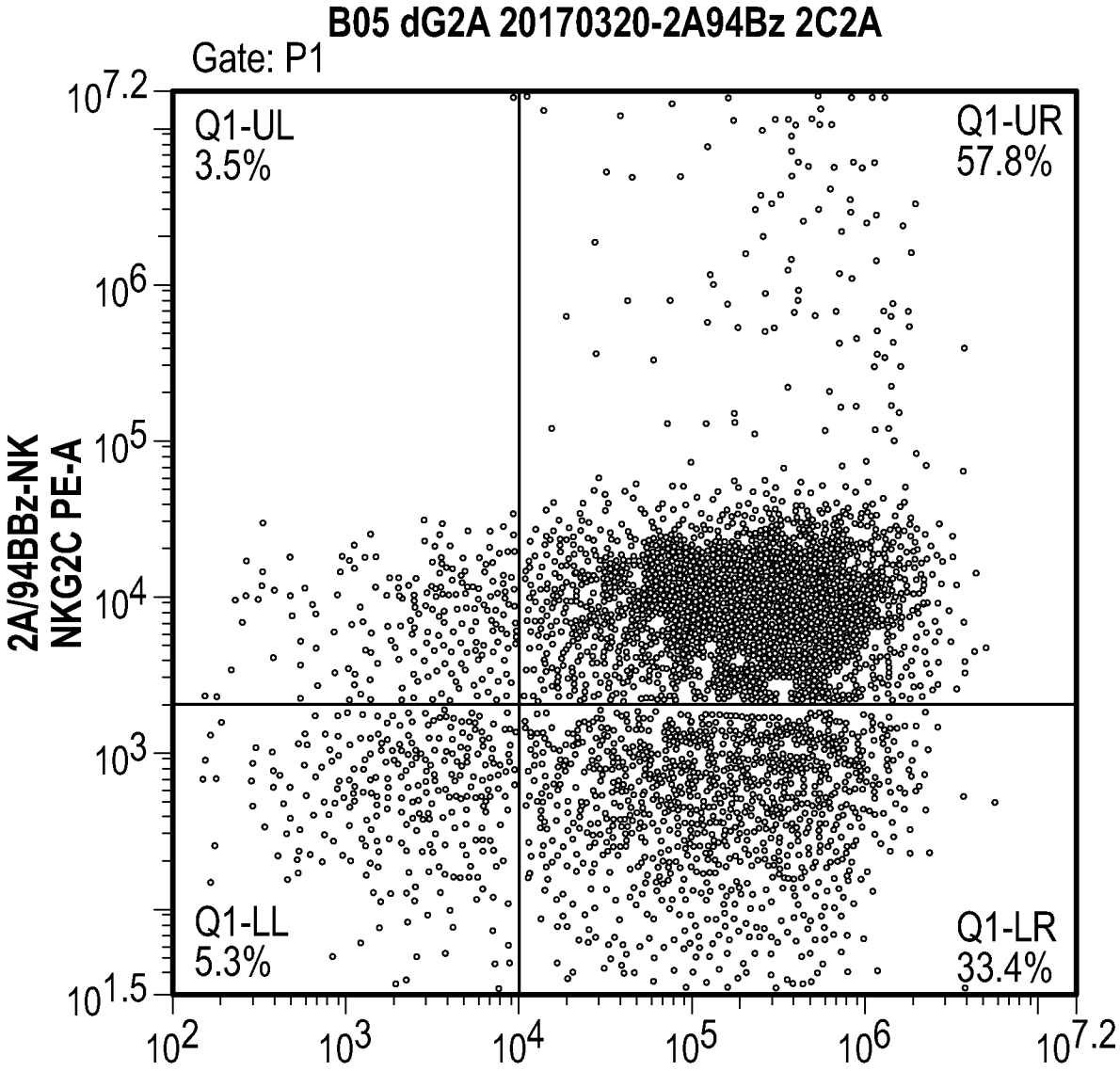

FIG. 7B
(Continued)

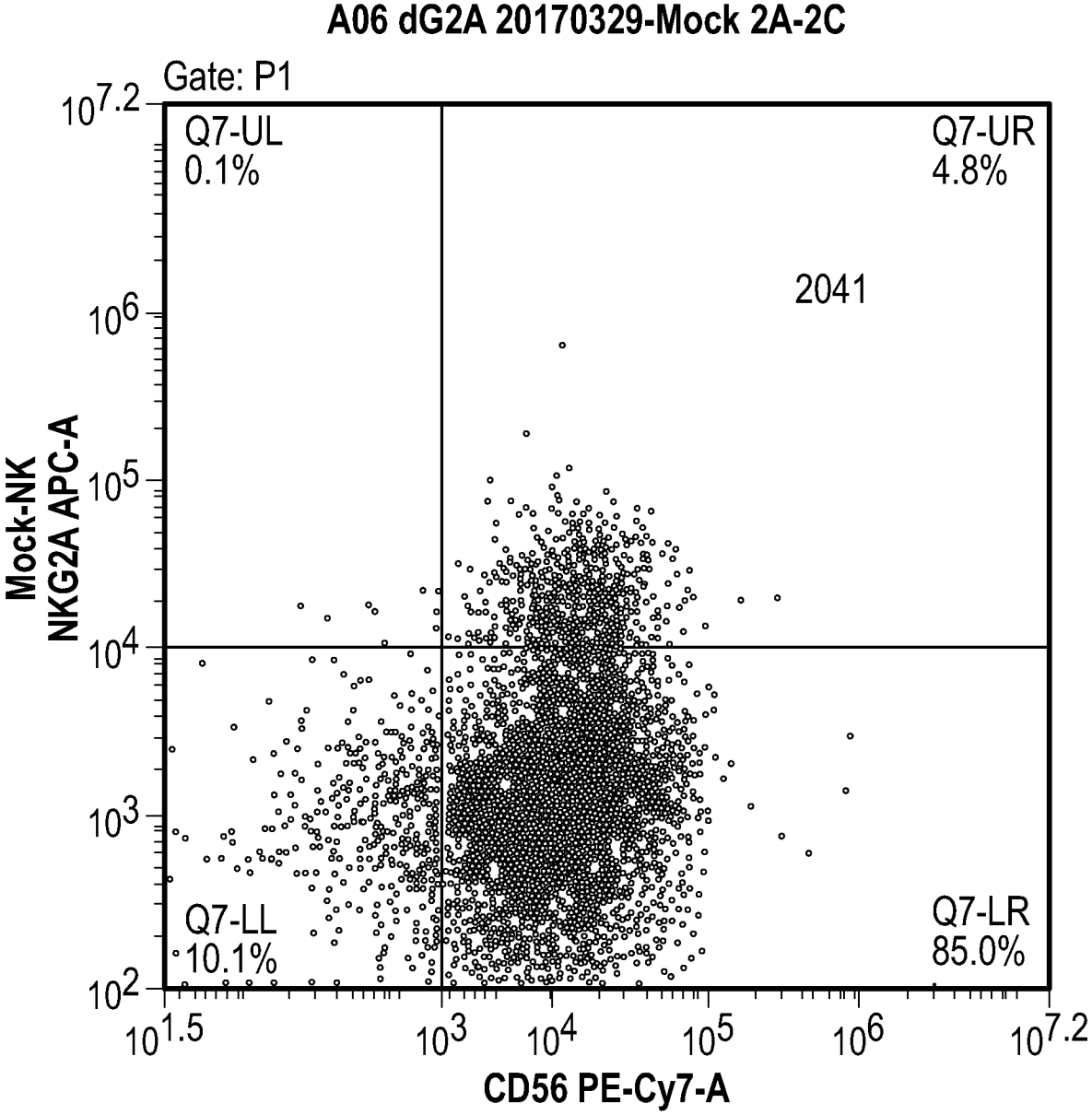
A06 dG2A 20170329-Mock 2A-2C
Gate: P1
Q7-UL
0.1%
Q7-UR
4.8%
2041
Q7-LL
10.1%
Q7-LR
85.0%
Mock-NK
NKG2A APC-A
CD56 PE-Cy7-A
10^7.2
10^6
10^5
10^4
10^3
10^2
10^1.5
10^3
10^4
10^5
10^6
10^7.2

FIG. 7C

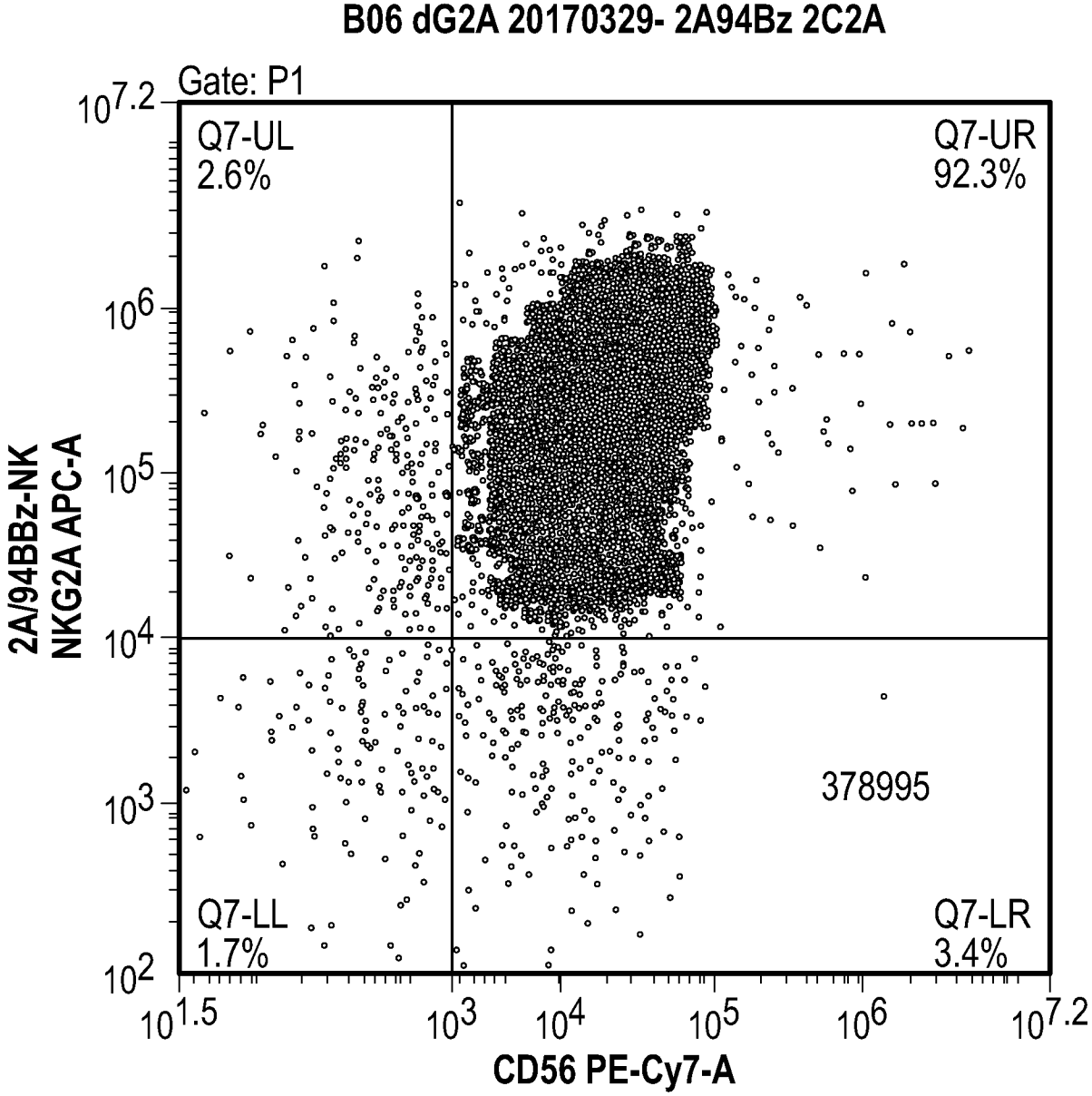

FIG. 7C
(Continued)

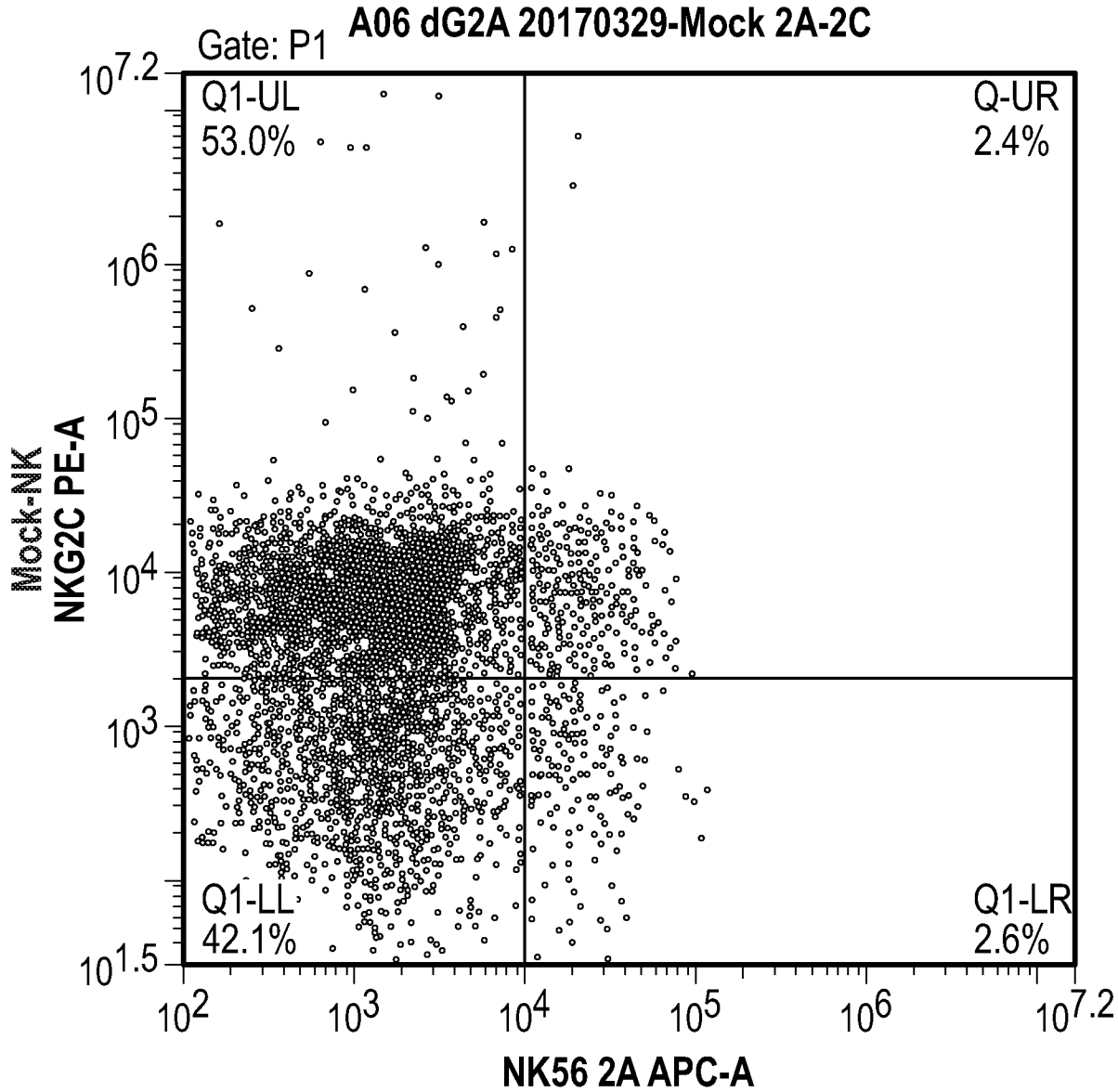

FIG. 7C
(Continued)

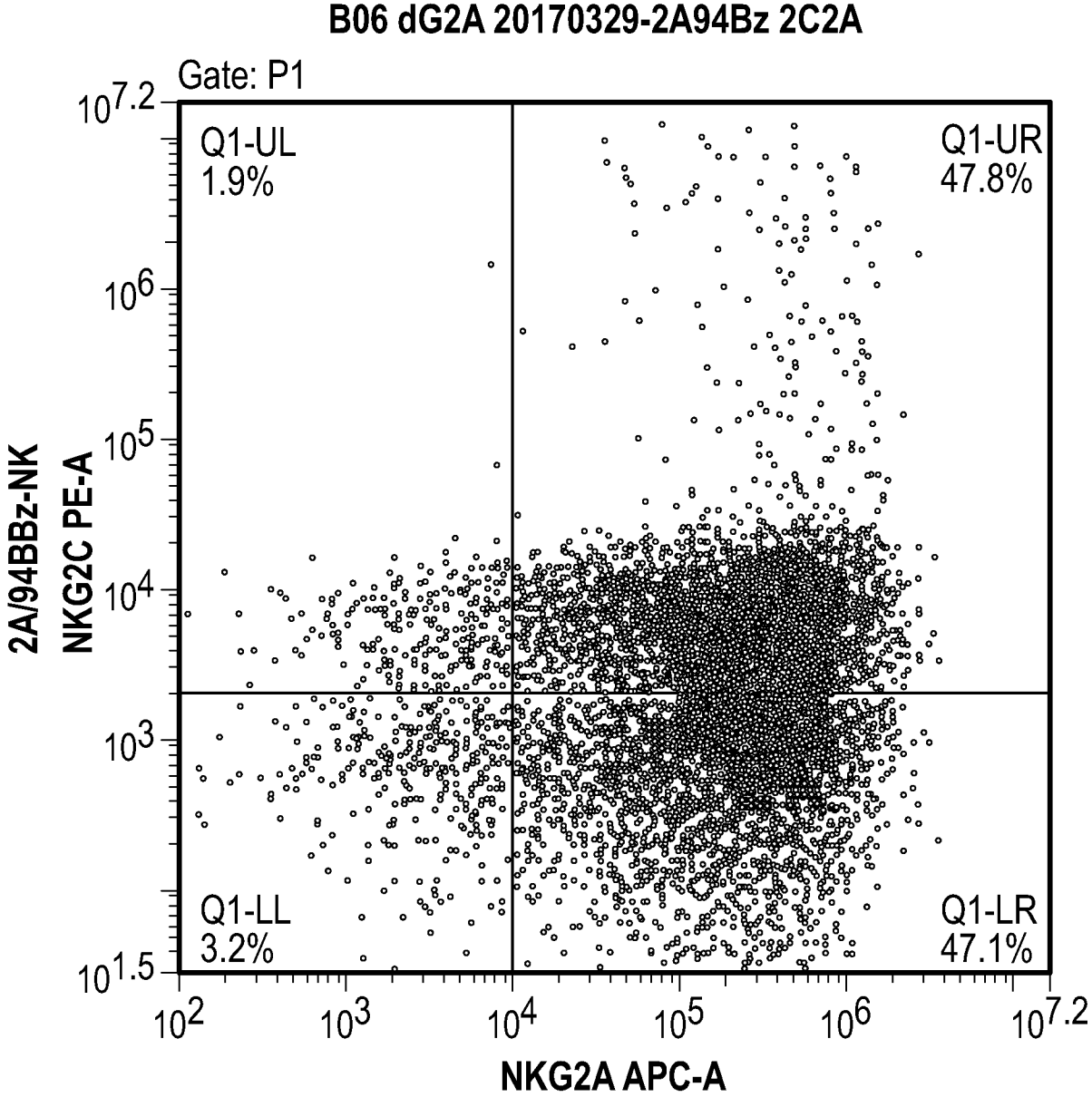

FIG. 7C
(Continued)

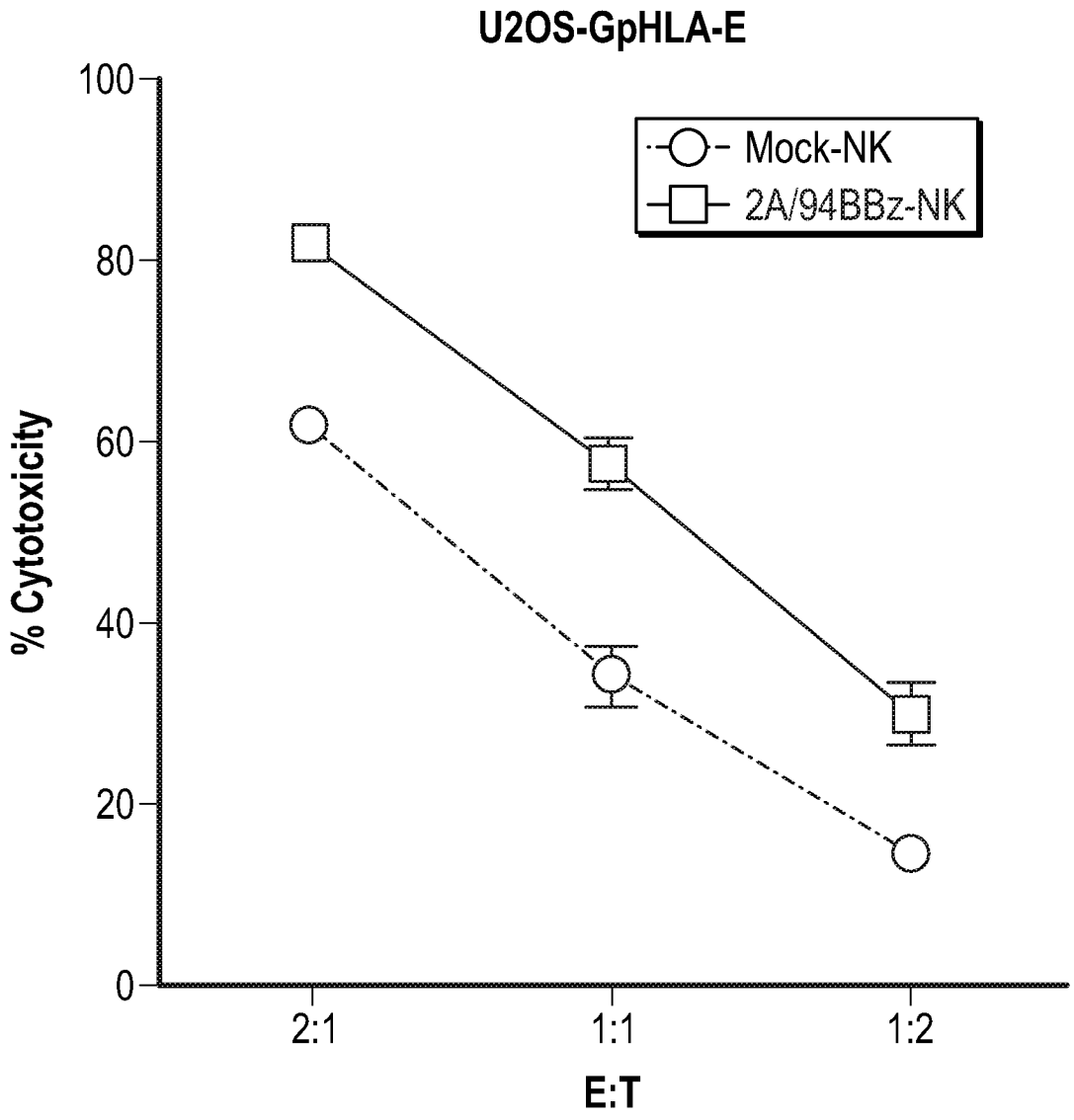
U2OS-GpHLA-E
Mock-NK
2A/94BBz-NK
% Cytotoxicity
100
80
60
40
20
0
2:1
1:1
1:2
E:T

FIG. 8A

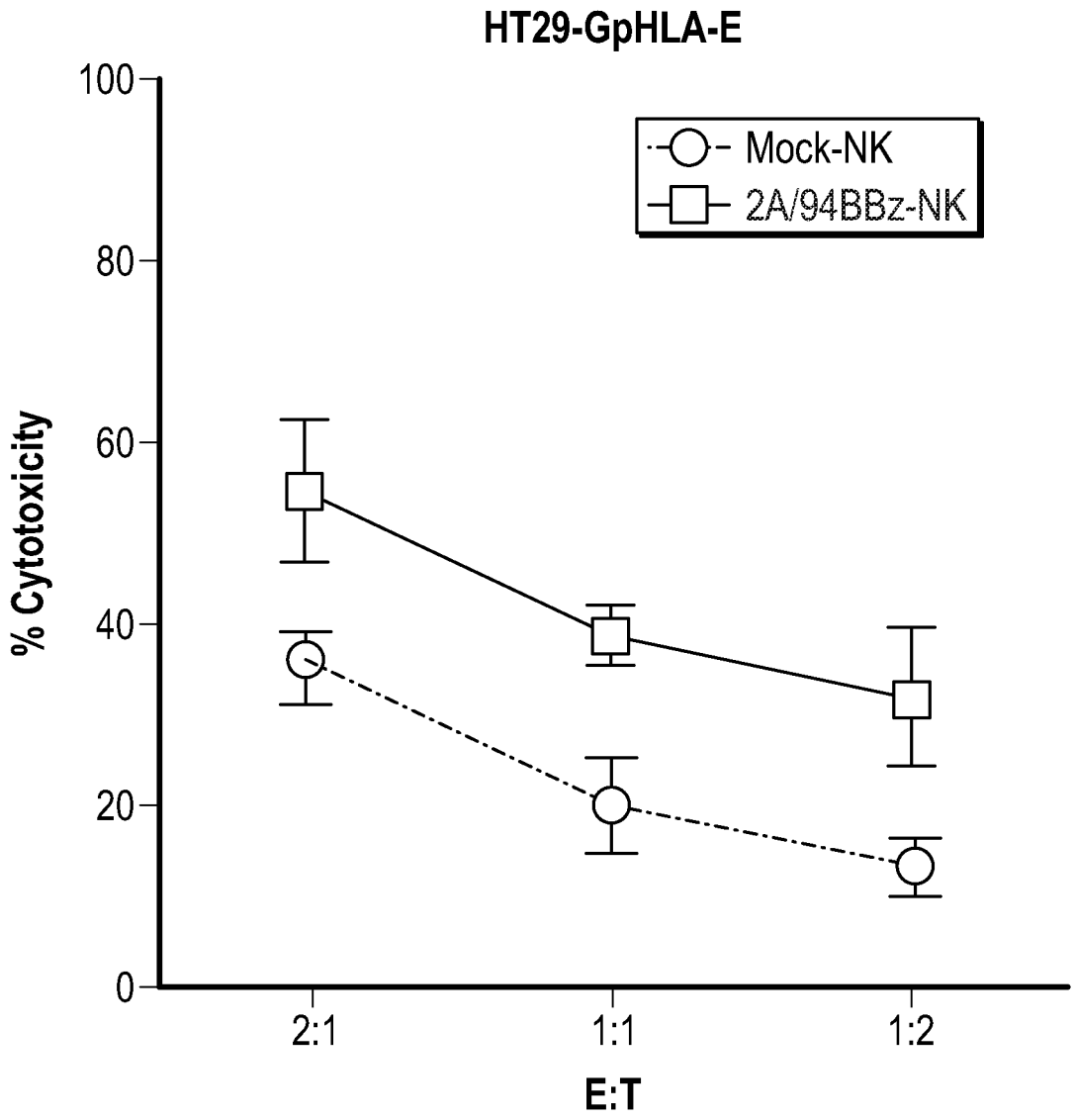
HT29-GpHLA-E
Mock-NK
2A/94BBz-NK
% Cytotoxicity
100
80
60
40
20
0
2:1
1:1
1:2
E:T

FIG. 8B

SEQ ID NO. 1	Full Length NKG2D DNA
SEQ ID NO. 2	Truncated NKG2D DNA
SEQ ID NO. 3	Codon Optimized Truncated NKG2D DNA
SEQ ID NO. 4	CD8 Signaling Sequence DNA
SEQ ID NO. 5	CD8 Alpha Hinge DNA
SEQ ID NO. 6	CD8 Beta DNA
SEQ ID NO. 7	CD16 Alpha DNA
SEQ ID NO. 8	CD16 Beta DNA
SEQ ID NO. 9	2B4 PROTEIN
SEQ ID NO. 10	DAP 10 DNA
SEQ ID NO. 11	DAP 12 DNA
SEQ ID NO. 12	4-1BB DNA
SEQ ID NO. 13	CD3-Zeta DNA
SEQ ID NO. 14	Canonical Hemi-tam PROTEIN
SEQ ID NO. 15	ITSM Motif PROTEIN
SEQ ID NO. 16	Membrane-Bound IL 15 DNA
SEQ ID NO. 17	Membrane-Bound IL 15 PROTEIN
SEQ ID NO. 18	NKG2D/CD8a/4-1BB/CD3z DNA
SEQ ID NO. 19	NKG2D/CD8a/4-1BB/CD3z PROTEIN
SEQ ID NO. 20	NCR1 TM/IC Domains PROTEIN
SEQ ID NO. 21	Full Length NCR2 PROTEIN
SEQ ID NO. 22	NCR3 TM/IC Domains PROTEIN
SEQ ID NO. 23	NKG2D/CD16 DNA
SEQ ID NO. 24	NKG2D/CD16 PROTEIN
SEQ ID NO. 25	CD8 Sig Seq/NKG2D Cod Opt ECD/CD8a Hinge/CD16 Tm/CD16 ICR/4-1BB ICR DNA
SEQ ID NO. 26	CD8 Sig Seq/NKG2D Cod Opt ECD/CD8a Hinge/CD16 Tm/CD16 ICR/4-1BB ICR PROTEIN
SEQ ID NO. 27	NKG2D/NCR1 DNA
SEQ ID NO. 28	NKG2D/NCR1 PROTEIN
SEQ ID NO. 29	NKG2D/NCR3 DNA
SEQ ID NO. 30	NKG2D/NCR3 PROTEIN
SEQ ID NO. 31	Motif for GS Linker PROTEIN
SEQ ID NO. 32	GS_3/CD8a Hinge PROTEIN
SEQ ID NO. 33	GS9 PROTEIN

FIG. 9

SEQ ID NO. 34	GS3 PROTEIN
SEQ ID NO. 35	2B4 ICR PROTEIN
SEQ ID NO. 36	2B4 ICR DNA
SEQ ID NO. 37	NKp80 ICR PROTEIN
SEQ ID NO. 38	NKp80 ICR DNA
SEQ ID NO. 39	B2Ad N-Term ECD PROTEIN
SEQ ID NO. 40	B2 AdR N-Term ECD DNA
SEQ ID NO. 41	B2 AdR First TM Helix PROTEIN
SEQ ID NO. 42	B2 AdR First TM Helix DNA
SEQ ID NO. 43	NK15_1 c/ CD8a Hinge Increase-CD8a Signal Seq/NKG2D Cod Opt/GS3/CD8a Hinge/CD16 TM/CD16 ICR/4-1BB ICR DNA
SEQ ID NO. 44	NK15_2 c/ CD8a Hinge Decrease-CD8a Sig Seq/NKG2D Cod Opt/GS3/CD16 TM/CD16 ICR/4-1BB ICR DNA
SEQ ID NO. 45	NK15_3 c/ CD8a Hinge Decrease-CD8a/NKG2D Cod Opt/CD16 TM/CD16 ICR/4-1BB ICR DNA
SEQ ID NO. 46	CD8a/NKG2D/CD8a Hinge/CD8a TM/4-1BB ICR/2B4 ICR DNA
SEQ ID NO. 47	CD8a/NKG2D/ADRB2 N-Term ECD/ADRB2 First Tm/4-1BB ICR/2B4 ICR DNA
SEQ ID NO. 48	CD8a/NKG2D/CD8a Hinge/CD8a Tm/4-1BB ICR/2B4 ICR/GS/NKp80 ICR DNA
SEQ ID NO. 49	CD8a/NKG2D/CD8a Hinge/CD8a Tm/4-1BB ICR/ NKp80 ICR DNA
SEQ ID NO. 50	CD8a/NKG2D Cod Opt/GS3/NKG2D ECD/ADRB2 EC/ADRB2 Tm/4-1 BB ICR/NKp80 ICR DNA
SEQ ID NO. 51	CD8a/NKG2D Cod Opt/GS3/NKG2D ECD/CD8a Hinge/CD8a Tm/4-1 BB ICR/NKp80 ICR DNA
SEQ ID NO. 52	CD8a Signal Seq/NKG2D Cod Opt ECD/GS3/NKG2D ECD/CD8a Hinge/CD16 TM/CD16 ICR/4-1BB ICR DNA
SEQ ID NO. 53	CD8a Signal Seq/NKG2D Cod Opt ECD/CD8a Hinge/CD16 TM/CD16 ICR/4-1BB ICR/2B4 ICR DNA
SEQ ID NO. 54	CD8a Signal Seq/NKG2D Cod Opt ECD/CD8a Hinge/CD16 TM/CD16 ICR/4-1BB ICR/NKp80 ICR DNA
SEQ ID NO. 55	FLAG Tag PROTEIN
SEQ ID NO. 56	His Tag PROTEIN
SEQ ID NO. 57	Myc Tag PROTEIN

FIG. 9
(Continued)

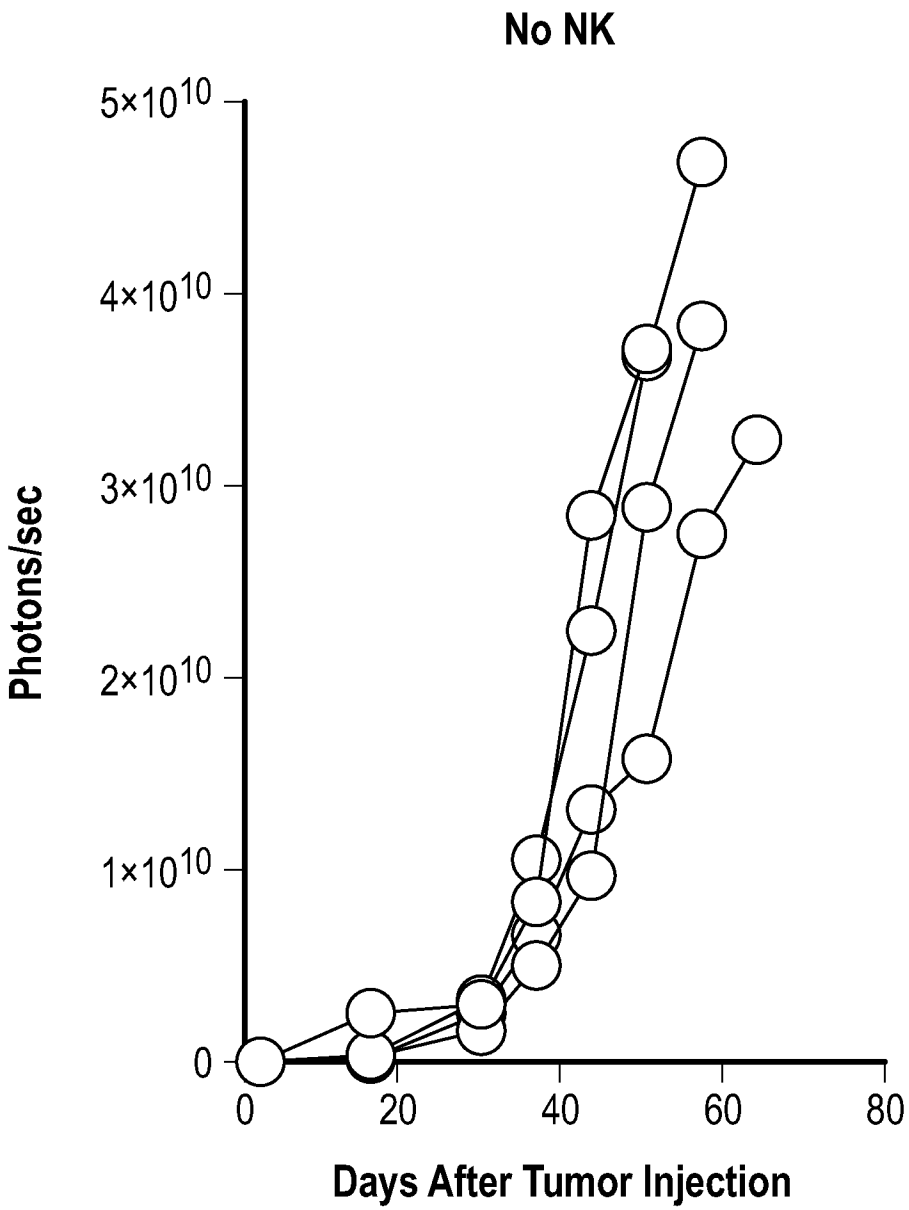
No NK
5×10^10
4×10^10
3×10^10
2×10^10
1×10^10
0
Photons/sec
0
20
40
60
80
Days After Tumor Injection

FIG. 10

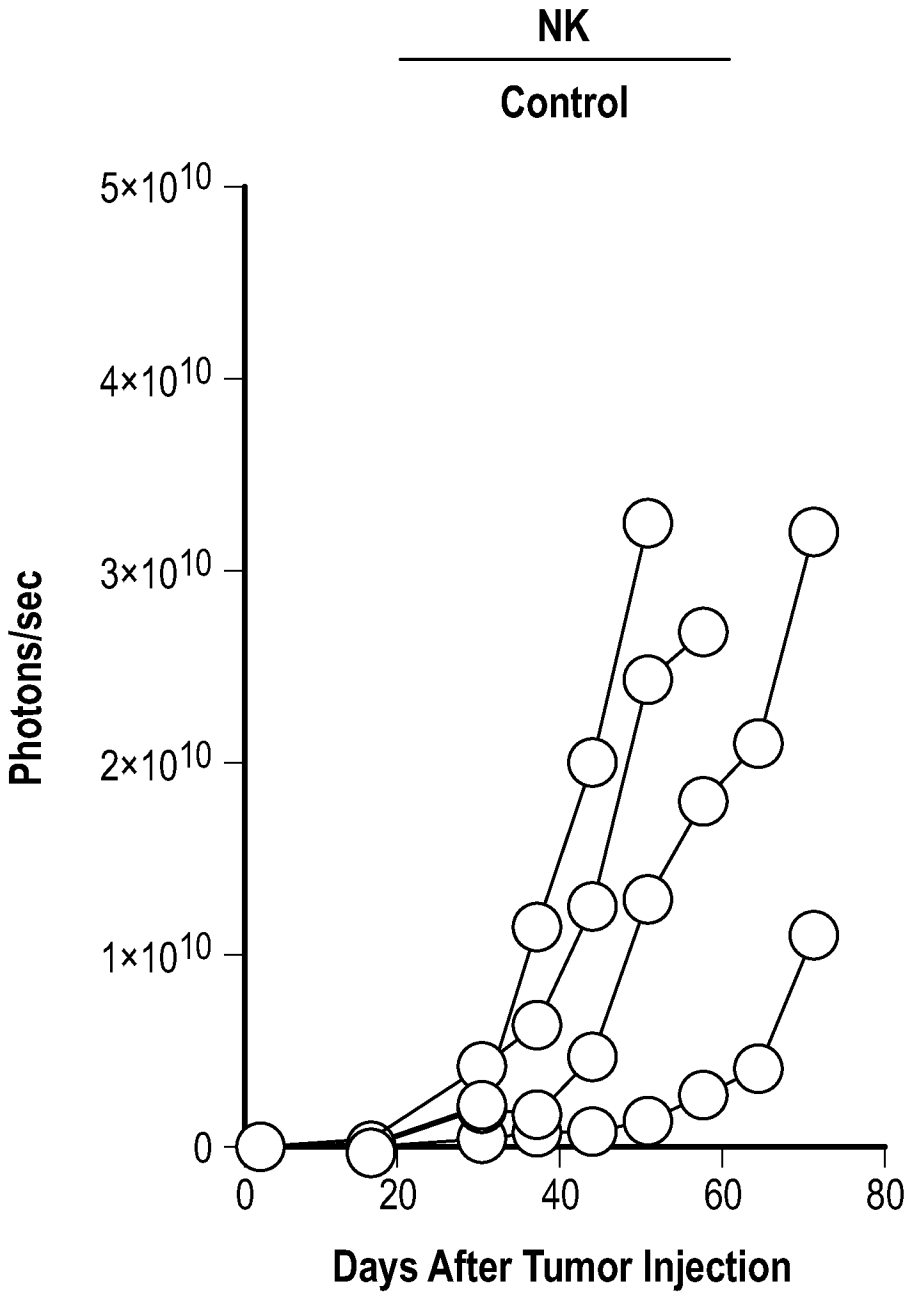

FIG. 10
(Continued)

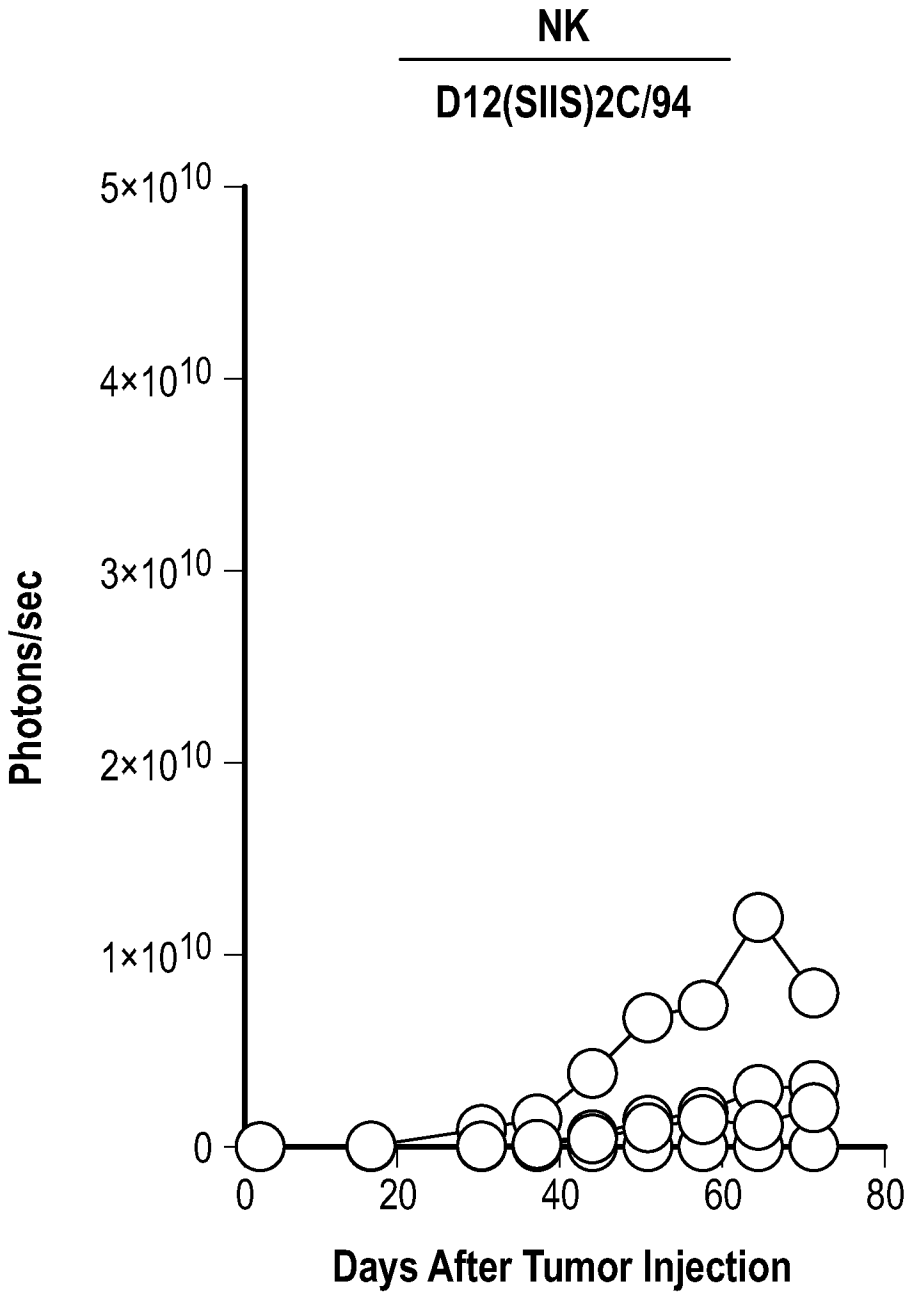

FIG. 10
(Continued)

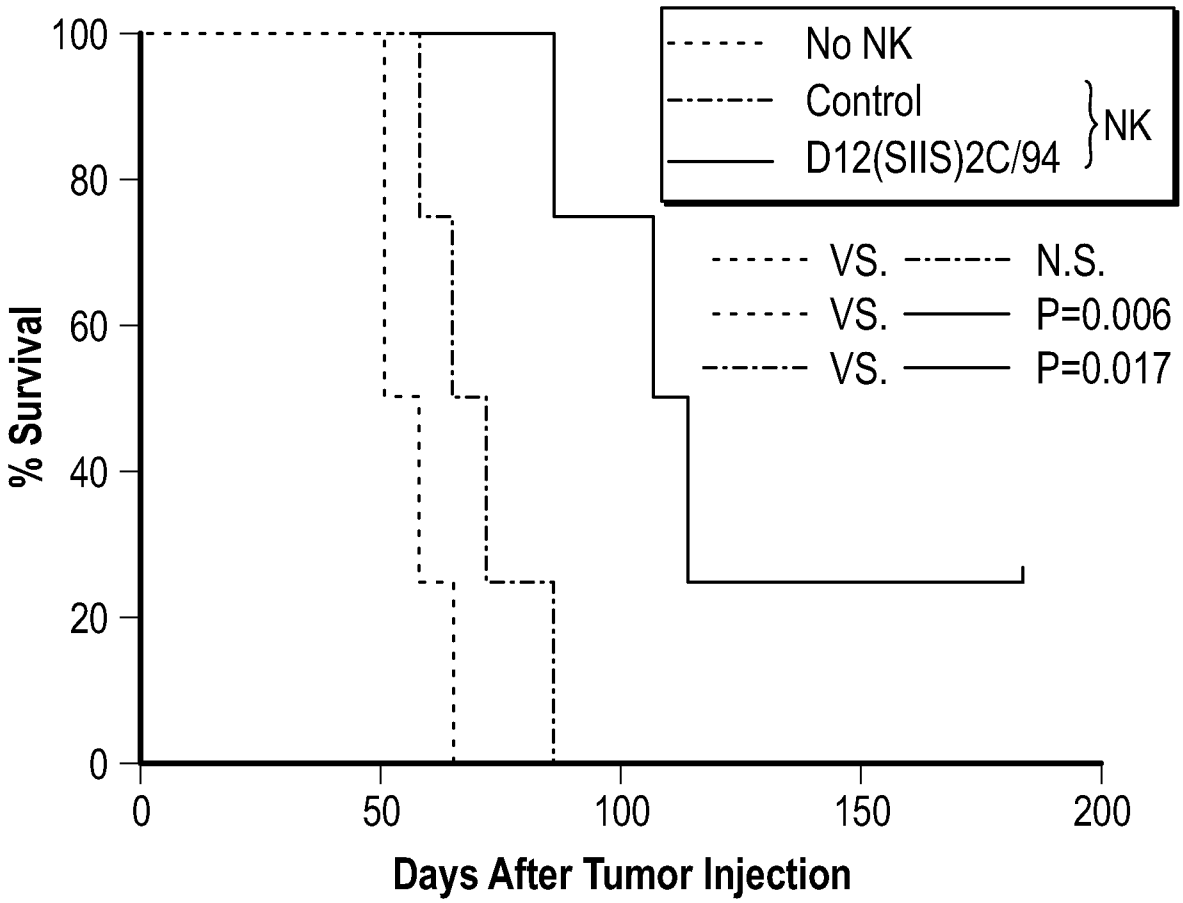
100
80
60
40
20
0
% Survival
0
50
100
150
200
Days After Tumor Injection
No NK
Control
D12(SIIS)2C/94
NK
VS. N.S.
VS. P=0.006
VS. P=0.017

FIG. 11

SEQUENCE LISTING

<110> National University of Singapore

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

<130> 4459.1147002 (NKT.0018WO)

<150> 62/628788
<151> 2018-02-09

<150> 62/736879
<151> 2018-09-26

<160> 70

<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 ccatttttt 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 caatttttg 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
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gaccaggatc tgctgaagct ggtgaagtcc tatcactgga tgggcctggt gcacatccct 240

acaaacggct cttggcagtg ggaggacggc tccatcctgt ctccaaatct gctgaccatc 300

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cca 63

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gacttcgcct gcgat 135

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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

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cagagaggcc aggtgcaggt tgggaatgag gcttgctgag aggggctgtc cagttcccag 1500

aaggcatatc agtctctgag ggcttccttt ggggccggga acttgcgggt ttgaggatag 1560

gagttcactt catcttctca gctcccattt ctactcttaa gtttctcagc tcccatttct 1620

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<213> Homo sapiens

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<223> CD16 alpha

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atggtggggc tcccttgcca gacttcagac tgagaagtca gatgaagttt caagaaaagg 240

aaattggtgg gtgacagaga tgggtggagg ggctggggaa aggctgttta cttcctcctg 300

tctagtcggt ttggtccctt tagggctccg gatatctttg gtgacttgtc cactccagtg 360

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<211> 2473
<212> DNA
<213> Homo sapiens

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<223> CD16 beta

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<223> n is a, c, g, or t

<220>
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<223> n is a, c, g, or t

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<222> (968)..(1067)

<223> n is a, c, g, or t

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aagtttgaga tgccttgggt tcagcagaga nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn 240

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taaactcact ctatgttcta atcactctga tctctgcccc tactcaatat ttgatttact 480

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

<220>
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<223> 2B4

<400> 9

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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
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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
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Gln Asn Pro Ala Arg Leu Ser Arg Lys Glu Leu Glu Asn Phe Asp Val
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Tyr Ser
370

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<213> Homo sapiens

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ggatgtgggt ccctctctct gccgctcctg gcaggcctcg tggctgctga tgcggtggca 180

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gatggcaaag tctacatcaa catgccaggc aggggctga 279

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

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<223> DAP12

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

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

<400> 12
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actactcaag aggaagatgg ctgtagctgc cgatttccag aagaagaaga aggaggatgt 120

gaactg 126

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

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

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cgggaccctg agatgggggg aaagccgaga aggaagaacc ctcaggaagg cctgtacaat 180

gaactgcaga aagataagat ggcggaggcc tacagtgaga ttgggatgaa aggcgagcgc 240

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tacgacgccc ttcacatgca ggccctgccc cctcgctaa 339

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

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<223> X = any amino acid

<400> 14

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<210> 15
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<223> N = S or T

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<223> x = any amino acid

<220>
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<223> x = any amino acid

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<223> N = L or I

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Asn Xaa Tyr Xaa Xaa Asn
1 5

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

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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

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gcgtgccggc cagcggcggg gggcgcagtg cacacgaggg ggctggactt cgcctgtgat 540

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

<220>
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<223> Membrane-bound IL15

<400> 17

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            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
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145 150 155 160

Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp
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Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys
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<210> 18
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ataattgaaa tgcagaaggg agactgtgca ctctatgcct cgagctttaa aggctatata 420

gaaaactgtt caactccaaa tacatacatc 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 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

<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
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Ser Thr Trp Glu Gly 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
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<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
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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
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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 aaggggggctg 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

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

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

<400> 27

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cctctgttca accaggaagt gcagatccct ctgaccgaaa gctattgcgg accttgccct      120

aagaattgga tttgctataa aaacaactgc taccagttct ttgacgagtc taagaattgg      180

tatgagtctc aggccagctg tatgtcccag aacgcctctc tgctgaaggt gtacagcaag      240
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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 ccacccccct 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

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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
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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
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<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 gaaaggggct 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> 4
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> Amino Acid NKG2C high affinity modification

<400> 58

Ser Ile Ile Ser
1

<210> 59
<211> 1137
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> DNA Sequence Chimeric CD94-CD8a TM-4-1BB/CD3zeta

<400> 59
atggccttac cagtgaccgc cttgctcctg ccgctggcct tgctgctcca cgccgccagg 60

ccgccaggac ccaacataga actccagaaa gactctgact gctgttcttg ccaagaaaaa 120

tgggttgggt accggtgcaa ctgttacttc atttccagtg aacagaaaac ttggaacgaa 180

agtcggcatc tctgtgcttc tcagaaatcc agcctgcttc agcttcaaaa cacagatgaa 240

ctggatttta tgagctccag tcaacaattt tactggattg gactctctta cagtgaggag 300

cacaccgcct ggttgtggga gaatggctct gcactctccc agtatctatt tccatcattt 360

gaaactttta atacaaagaa ctgcatagcg tataatccaa atggaaatgc tttagatgaa 420

tcctgtgaag ataaaaatcg ttatatctgt aagcaacagc tcattaccac gacgccagcg 480

ccgcgaccac caacaccggc gcccaccatc gcgtcgcagc ccctgtccct gcgcccagag 540

gcgtgccggc cagcggcggg gggcgcagtg cacacgaggg ggctggactt cgcctgtgat 600

atctacatct gggcgccctt ggccgggact tgtggggtcc ttctcctgtc actggttatc 660

acccttttact gcaaacgggg cagaaagaaa ctcctgtata tattcaaaca accatttatg 720

agaccagtac aaactactca agaggaagat ggctgtagct gccgatttcc agaagaagaa 780

gaaggaggat gtgaactgag agtgaagttc agcaggagcg cagacgcccc cgcgtaccag 840

cagggccaga accagctcta taacgagctc aatctaggac gaagagagga gtacgatgtt 900

ttggacaaga gacgtggccg ggaccctgag atgggggaa agccgagaag gaagaaccct 960

caggaaggcc tgtacaatga actgcagaaa gataagatgg cggaggccta cagtgagatt 1020

gggatgaaag gcgagcgccg gaggggcaag gggcacgatg gcctttacca gggtctcagt 1080

acagccacca aggacaccta cgacgccctt cacatgcagg ccctgccccc tcgctaa 1137

<210> 60
<211> 378
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> Amino Acid Sequence Chimeric CD94-CD8a TM-4-1BB/CD3zeta

<400> 60

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 Pro Gly Pro Asn Ile Glu Leu Gln Lys Asp Ser
20 25 30

Asp Cys Cys Ser Cys Gln Glu Lys Trp Val Gly Tyr Arg Cys Asn Cys
35 40 45

Tyr Phe Ile Ser Ser Glu Gln Lys Thr Trp Asn Glu Ser Arg His Leu
50 55 60

Cys Ala Ser Gln Lys Ser Ser Leu Leu Gln Leu Gln Asn Thr Asp Glu
65 70 75 80

Leu Asp Phe Met Ser Ser Ser Gln Gln Phe Tyr Trp Ile Gly Leu Ser
85 90 95

Tyr Ser Glu Glu His Thr Ala Trp Leu Trp Glu Asn Gly Ser Ala Leu
100 105 110

```
Ser Gln Tyr Leu Phe Pro Ser Phe Glu Thr Phe Asn Thr Lys Asn Cys
        115                 120                 125

Ile Ala Tyr Asn Pro Asn Gly Asn Ala Leu Asp Glu Ser Cys Glu Asp
    130                 135                 140

Lys Asn Arg Tyr Ile Cys Lys Gln Gln Leu Ile Thr Thr Thr Pro Ala
145                 150                 155                 160

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

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

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

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

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

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

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

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

Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg
    290                 295                 300
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Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro
305 310 315 320

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

Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His
340 345 350

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

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

<210> 61
<211> 1119
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> DNA Sequence chimeric NKG2A Receptor - CD8a TM-4-1BB/CD3zeta

<400> 61
atggccttac cagtgaccgc cttgctcctg ccgctggcct tgctgctcca cgccgccagg 60

ccgctgaata caagaactca gaaagcacgt cattgtggcc attgtcctga ggagtggatt 120

acatattcca acagttgtta ctacattggt aaggaaagaa gaacttggga agagagtttg 180

ctggcctgta cttcgaagaa ctccagtctg ctttctatag ataatgaaga agaaatgaaa 240

tttctgtcca tcatttcacc atcctcatgg attggtgtgt ttcgtaacag cagtcatcat 300

ccatgggtga caatgaatgg tttggctttc aaacatgaga taaaagactc agataatgct 360

gaacttaact gtgcagtgct acaagtaaat cgacttaaat cagcccagtg tggatcttca 420

ataatatatc attgtaagca taagcttacc acgacgccag cgccgcgacc accaacaccg 480

gcgcccacca tcgcgtcgca gcccctgtcc ctgcgcccag aggcgtgccg gccagcggcg 540

ggggggcgcag tgcacacgag ggggctggac ttcgcctgtg atatctacat ctgggcgccc 600

ttggccggga cttgtggggt ccttctcctg tcactggtta tcacccttta ctgcaaacgg 660

ggcagaaaga aactcctgta tatattcaaa caaccattta tgagaccagt acaaactact 720

caagaggaag atggctgtag ctgccgattt ccagaagaag aagaaggagg atgtgaactg 780

agagtgaagt tcagcaggag cgcagacgcc cccgcgtacc agcagggcca gaaccagctc 840

tataacgagc tcaatctagg acgaagagag gagtacgatg ttttggacaa gagacgtggc 900

cgggaccctg agatgggggg aaagccgaga aggaagaacc ctcaggaagg cctgtacaat 960

gaactgcaga aagataagat ggcggaggcc tacagtgaga ttgggatgaa aggcgagcgc 1020

cggaggggca aggggcacga tggcctttac cagggtctca gtacagccac caaggacacc 1080

tacgacgccc ttcacatgca ggccctgccc cctcgctaa 1119

<210> 62
<211> 372
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> Amino Acid Sequence chimeric NKG2A Receptor - CD8a TM-4-1BB/CD3zeta

<400> 62

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 Asn Thr Arg Thr Gln Lys Ala Arg His Cys
20 25 30

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

Ile Gly Lys Glu Arg Arg Thr Trp Glu Glu Ser Leu Leu Ala Cys Thr

```
    50                  55                  60

Ser Lys Asn Ser Ser Leu Leu Ser Ile Asp Asn Glu Glu Glu Met Lys
65                  70                  75                  80

Phe Leu Ser Ile Ile Ser Pro Ser Ser Trp Ile Gly Val Phe Arg Asn
                85                  90                  95

Ser Ser His His Pro Trp Val Thr Met Asn Gly Leu Ala Phe Lys His
            100                 105                 110

Glu Ile Lys Asp Ser Asp Asn Ala Glu Leu Asn Cys Ala Val Leu Gln
        115                 120                 125

Val Asn Arg Leu Lys Ser Ala Gln Cys Gly Ser Ser Ile Ile Tyr His
    130                 135                 140

Cys Lys His Lys Leu Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro
145                 150                 155                 160

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

Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala
            180                 185                 190

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

Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys
    210                 215                 220

Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr
225                 230                 235                 240

Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly
```

245 250 255

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

Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg
275 280 285

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

Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn
305 310 315 320

Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met
325 330 335

Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly
340 345 350

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

Leu Pro Pro Arg
370

<210> 63
<211> 690
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> DNA Sequence Engineered NKG2C

<400> 63
aataaacaaa gaggaacctt ctcagaagtg agtctggccc aggacccaaa gcggcagcaa 60

aggaaaccta aaggcaataa aagctccatt tcaggaaccg aacaggaaat attccaagta 120

gaattaaatc ttcaaaatcc ttccctgaat catcaaggga ttgataaaat atatgactgc 180

caaggtttac tgccacctcc agagaagctc actgccgagg tcctaggaat catttgcatt 240

gtcctgatgg ccactgtgtt aaaaacaata gttcttattc ctttcctgga gcagaacaat 300

ttttccccga atacaagaac gcagaaagca cgtcattgtg gccattgtcc tgaggagtgg 360

attacatatt ccaacagttg ttattacatt ggtaaggaaa gaagaacttg ggaagagagt 420

ttgctggcct gtacttcgaa gaactccagt ctgctttcta tagataatga agaagaaatg 480

aaatttctgt ccatcatttc accttcctca tggattggtg tgtttcgtaa cagcagtcat 540

catccatggg tgacaataaa tggtttggct ttcaaacata agataaaaga ctcagataat 600

gctgaactta actgtgcagt gctacaagta aatcgactta aatcagccca gtgtggatct 660

tcaatgatat atcattgtaa gcataagctt 690

<210> 64
<211> 231
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> Amino acid sequence engineered NKG2C

<400> 64

Met Asn Lys Gln Arg Gly Thr Phe Ser Glu Val Ser Leu Ala Gln Asp
1 5 10 15

Pro Lys Arg Gln Gln Arg Lys Pro Lys Gly Asn Lys Ser Ser Ile Ser
20 25 30

Gly Thr Glu Gln Glu Ile Phe Gln Val Glu Leu Asn Leu Gln Asn Pro
35 40 45

Ser Leu Asn His Gln Gly Ile Asp Lys Ile Tyr Asp Cys Gln Gly Leu

```
    50                  55                  60

Leu Pro Pro Pro Glu Lys Leu Thr Ala Glu Val Leu Gly Ile Ile Cys
65                  70                  75                  80

Ile Val Leu Met Ala Thr Val Leu Lys Thr Ile Val Leu Ile Pro Phe
                85                  90                  95

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

His Cys Gly His Cys Pro Glu Glu Trp Ile Thr Tyr Ser Asn Ser Cys
        115                 120                 125

Tyr Tyr Ile Gly Lys Glu Arg Arg Thr Trp Glu Glu Ser Leu Leu Ala
    130                 135                 140

Cys Thr Ser Lys Asn Ser Ser Leu Leu Ser Ile Asp Asn Glu Glu Glu
145                 150                 155                 160

Met Lys Phe Leu Ser Ile Ile Ser Pro Ser Ser Trp Ile Gly Val Phe
                165                 170                 175

Arg Asn Ser Ser His His Pro Trp Val Thr Ile Asn Gly Leu Ala Phe
            180                 185                 190

Lys His Lys Ile Lys Asp Ser Asp Asn Ala Glu Leu Asn Cys Ala Val
        195                 200                 205

Leu Gln Val Asn Arg Leu Lys Ser Ala Gln Cys Gly Ser Ser Met Ile
    210                 215                 220

Tyr His Cys Lys His Lys Leu
225                 230
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<210> 65

<211> 384
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> DNA sequence NKG2A fragment

<400> 65
ctgaatacaa gaactcagaa agcacgtcat tgtggccatt gtcctgagga gtggattaca 60

tattccaaca gttgttacta cattggtaag gaaagaagaa cttgggaaga gagtttgctg 120

gcctgtactt cgaagaactc cagtctgctt tctatagata atgaagaaga aatgaaattt 180

ctgtccatca tttcaccatc ctcatggatt ggtgtgtttc gtaacagcag tcatcatcca 240

tgggtgacaa tgaatggttt ggctttcaaa catgagataa aagactcaga taatgctgaa 300

cttaactgtg cagtgctaca agtaaatcga cttaaatcag cccagtgtgg atcttcaata 360

atatatcatt gtaagcataa gctt 384

<210> 66
<211> 128
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> Amino Acid sequence NKG2A fragment

<400> 66

Leu Asn Thr Arg Thr Gln Lys Ala Arg His Cys Gly His Cys Pro Glu
1 5 10 15

Glu Trp Ile Thr Tyr Ser Asn Ser Cys Tyr Tyr Ile Gly Lys Glu Arg
20 25 30

Arg Thr Trp Glu Glu Ser Leu Leu Ala Cys Thr Ser Lys Asn Ser Ser
35 40 45

```
Leu Leu Ser Ile Asp Asn Glu Glu Glu Met Lys Phe Leu Ser Ile Ile
    50                  55                  60


Ser Pro Ser Ser Trp Ile Gly Val Phe Arg Asn Ser Ser His His Pro
65                  70                  75                  80


Trp Val Thr Met Asn Gly Leu Ala Phe Lys His Glu Ile Lys Asp Ser
                85                  90                  95


Asp Asn Ala Glu Leu Asn Cys Ala Val Leu Gln Val Asn Arg Leu Lys
            100                 105                 110


Ser Ala Gln Cys Gly Ser Ser Ile Ile Tyr His Cys Lys His Lys Leu
        115                 120                 125


<210>  67
<211>  402
<212>  DNA
<213>  Homo sapiens


<220>
<221>  misc_feature
<223>  DNA sequence CD94 fragment

<400>  67
ccaggaccca acatagaact ccagaaagac tctgactgct gttcttgcca agaaaaatgg       60

gttgggtacc ggtgcaactg ttacttcatt tccagtgaac agaaaacttg gaacgaaagt      120

cggcatctct gtgcttctca gaaatccagc ctgcttcagc ttcaaaacac agatgaactg      180

gattttatga gctccagtca acaattttac tggattggac tctcttacag tgaggagcac      240

accgcctggt tgtgggagaa tggctctgca ctctcccagt atctatttcc atcatttgaa      300

acttttaata caaagaactg catagcgtat aatccaaatg gaaatgcttt agatgaatcc      360

tgtgaagata aaaatcgtta tatctgtaag caacagctca tt                         402


<210>  68
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<211> 134
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> Amino acid sequence CD94 fragment

<400> 68

Pro Gly Pro Asn Ile Glu Leu Gln Lys Asp Ser Asp Cys Cys Ser Cys
1 5 10 15

Gln Glu Lys Trp Val Gly Tyr Arg Cys Asn Cys Tyr Phe Ile Ser Ser
20 25 30

Glu Gln Lys Thr Trp Asn Glu Ser Arg His Leu Cys Ala Ser Gln Lys
35 40 45

Ser Ser Leu Leu Gln Leu Gln Asn Thr Asp Glu Leu Asp Phe Met Ser
50 55 60

Ser Ser Gln Gln Phe Tyr Trp Ile Gly Leu Ser Tyr Ser Glu Glu His
65 70 75 80

Thr Ala Trp Leu Trp Glu Asn Gly Ser Ala Leu Ser Gln Tyr Leu Phe
85 90 95

Pro Ser Phe Glu Thr Phe Asn Thr Lys Asn Cys Ile Ala Tyr Asn Pro
100 105 110

Asn Gly Asn Ala Leu Asp Glu Ser Cys Glu Asp Lys Asn Arg Tyr Ile
115 120 125

Cys Lys Gln Gln Leu Ile
130

<210> 69

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

<220>
<221> MISC_FEATURE
<223> HLA-E Signal Peptide

<400> 69

Met Val Asp Gly Thr Leu Leu Leu Leu Leu Ser Glu Ala Leu Ala Leu
1 5 10 15

Thr Gln Thr Trp Ala
20

<210> 70
<211> 24
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> HLA-G Signal Peptide

<400> 70

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

Leu Thr Leu Thr Glu Thr Trp Ala
20