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(54) Titre : COMPOSITIONS DE CELLULES ALLOGENIQUES ET METHODES D'UTILISATION
(54) Title: ALLOGENEIC CELL COMPOSITIONS AND METHODS OF USE

(57) Abrégé/Abstract:
Disclosed are chimeric stimulatory receptors (CSRs), cell compositions comprising CSRs, methods of making and methods of using same for the treatment of a disease or disorder in a subject.

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(54) Title: ALLOGENEIC CELL COMPOSITIONS AND METHODS OF USE

(57) Abstract: Disclosed are chimeric stimulatory receptors (CSRs), cell compositions comprising CSRs, methods of making and methods of using same for the treatment of a disease or disorder in a subject.

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LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

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CONTENANT LES PAGES 1 À 161

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JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

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ALLOGENEIC CELL COMPOSITIONS AND METHODS OF USE

CROSS-REFERENCE TO RELATED APPLICATIONS

[01] This application claims the priority to, and benefit of, U.S. Provisional Application No. 62/727,498, filed on September 5, 2018, U.S. Provisional Application No. 62/744,073, filed on October 10, 2018, U.S. Provisional Application No. 62/815,334, filed on March 7, 2019, and U.S. Provisional Application No. 62/815,880, filed on March 8, 2019. The contents of each of these applications are hereby incorporated by reference in their entireties.

FIELD OF THE DISCLOSURE

[02] The disclosure is directed to molecular biology, and more, specifically, to chimeric receptors, allogeneic cell compositions, methods of making and methods of using the same.

INCORPORATION-BY-REFERENCE OF SEQUENCE LISTING

[03] The contents of the file named "POTH-046_001WO_SequenceListing.txt", which was created on September 5, 2019, and is 55.7 MB in size are hereby incorporated by reference in their entirety.

BACKGROUND OF THE INVENTION

[04] There has been a long-felt but unmet need in the art for an allogeneic cell composition that overcomes the challenges presented by eliminating genes involved in a graft versus host response and host versus graft response. The disclosure provides allogeneic cell compositions, methods of making and methods of using these compositions which comprise non-naturally occurring structural improvements to restore responsiveness of allogeneic cells to environmental stimuli as well as reduce or prevent rejection by natural killer cell-mediated cytotoxicity.

SUMMARY OF THE INVENTION

[05] The present disclosure provides a non-naturally occurring chimeric stimulatory receptor (CSR) comprising: (a) an ectodomain comprising a activation component, wherein the activation component is isolated or derived from a first protein; (b) a transmembrane domain; and (c) an

endodomain comprising at least one signal transduction domain, wherein the at least one signal transduction domain is isolated or derived from a second protein; wherein the first protein and the second protein are not identical.

[06] The activation component can comprise a portion of one or more of a component of a T-cell Receptor (TCR), a component of a TCR complex, a component of a TCR co-receptor, a component of a TCR co-stimulatory protein, a component of a TCR inhibitory protein, a cytokine receptor, and a chemokine receptor to which an agonist of the activation component binds. The activation component can comprise a CD2 extracellular domain or a portion thereof to which an agonist binds.

[07] The signal transduction domain can comprise one or more of a component of a human signal transduction domain, T-cell Receptor (TCR), a component of a TCR complex, a component of a TCR co-receptor, a component of a TCR co-stimulatory protein, a component of a TCR inhibitory protein, a cytokine receptor, and a chemokine receptor. The signal transduction domain can comprise a CD3 protein or a portion thereof. The CD3 protein can comprise a CD3ζ protein or a portion thereof.

[08] The endodomain can further comprise a cytoplasmic domain. The cytoplasmic domain can be isolated or derived from a third protein. The first protein and the third protein can be identical. The ectodomain can further comprise a signal peptide. The signal peptide can be derived from a fourth protein. The first protein and the fourth protein can be identical. The transmembrane domain can be isolated or derived from a fifth protein. The first protein and the fifth protein can be identical.

[09] In some aspects, the activation component does not bind a naturally-occurring molecule. In some aspects, the activation component binds a naturally-occurring molecule but the CSR does not transduce a signal upon binding of the activation component to a naturally-occurring molecule. In some aspects, the activation component binds to a non-naturally occurring molecule. In some aspects, the activation component does not bind a naturally-occurring molecule but binds a non-naturally occurring molecule. The CSR can selectively transduces a signal upon binding of the activation component to a non-naturally occurring molecule.

In a preferred aspect, the present disclosure provides a non-naturally occurring chimeric stimulatory receptor (CSR) comprising: (a) an ectodomain comprising a signal peptide and an

activation component, wherein the signal peptide comprises a CD2 signal peptide or a portion thereof and wherein the activation component comprises a CD2 extracellular domain or a portion thereof to which an agonist binds; (b) a transmembrane domain, wherein the transmembrane domain comprises a CD2 transmembrane domain or a portion thereof; and (c) an endodomain comprising a cytoplasmic domain and at least one signal transduction domain, wherein the cytoplasmic domain comprises a CD2 cytoplasmic domain or a portion thereof and wherein the at least one signal transduction domain comprises a CD3ζ protein or a portion thereof. In some aspects, the non-naturally CSR comprises an amino acid sequence at least 80%, at least 90%, at least 95% or at least 99% identical to SEQ ID NO:17062. In a preferred aspect, the non-naturally occurring CSR comprises an amino acid sequence of SEQ ID NO:17062.

[010] The present disclosure also provides a non-naturally occurring chimeric stimulatory receptor (CSR) wherein the ectodomain comprises a modification. The modification can comprise a mutation or a truncation of the amino acid sequence of the activation component or the first protein when compared to a wild type sequence of the activation component or the first protein. The mutation or a truncation of the amino acid sequence of the activation component can comprise a mutation or truncation of a CD2 extracellular domain or a portion thereof to which an agonist binds. The mutation or truncation of the CD2 extracellular domain can reduce or eliminate binding with naturally occurring CD58. In some aspects, the CD2 extracellular domain comprising the mutation or truncation comprises an amino acid sequence at least 80%, at least 90%, at least 95% or at least 99% identical to SEQ ID NO:17119. In a preferred aspect, the CD2 extracellular domain comprising the mutation or truncation comprises an amino acid sequence of SEQ ID NO:17119.

[011] In a preferred aspect, the present disclosure provides non-naturally occurring chimeric stimulatory receptor (CSR) comprising: (a) an ectodomain comprising a signal peptide and an activation component, wherein the signal peptide comprises a CD2 signal peptide or a portion thereof and wherein the activation component comprises a CD2 extracellular domain or a portion thereof to which an agonist binds and wherein the CD2 extracellular domain or a portion thereof to which an agonist binds comprises a mutation or truncation; (b) a transmembrane domain, wherein the transmembrane domain comprises a CD2 transmembrane domain or a portion thereof; and (c) an endodomain comprising a cytoplasmic domain and at least one signal

transduction domain, wherein the cytoplasmic domain comprises a CD2 cytoplasmic domain or a portion thereof and wherein the at least one signal transduction domain comprises a CD3ζ protein or a portion thereof. In some aspects, the non-naturally CSR comprises an amino acid sequence at least 80%, at least 90%, at least 95% or at least 99% identical to SEQ ID NO:17118. In a preferred aspect, the non-naturally occurring CSR comprises an amino acid sequence of SEQ ID NO:17118.

[012] The present disclosure provides a nucleic acid sequence encoding any CSR disclosed herein. The present disclosure provides a vector comprising a nucleic acid sequence encoding any CSR disclosed herein. The present disclosure provides a transposon comprising a nucleic acid sequence encoding any CSR disclosed herein.

[013] The present disclosure provides a cell comprising any CSR disclosed herein. The present disclosure provides a cell comprising a nucleic acid sequence encoding any CSR disclosed herein. The present disclosure provides a cell comprising a vector comprising a nucleic acid sequence encoding any CSR disclosed herein. The present disclosure provides a cell comprising a transposon comprising a nucleic acid sequence encoding any CSR disclosed herein.

[014] A modified cell disclosed herein can be an allogeneic cell or an autologous cell. In some preferred aspects, the modified cell is an allogeneic cell. In some preferred aspects, the modified cell is an allogeneic T-cell or a modified allogeneic CAR T-cell.

[015] The present disclosure provides a composition comprising any CSR disclosed herein. The present disclosure provides a composition comprising a nucleic acid sequence encoding any CSR disclosed herein. The present disclosure provides a composition comprising a vector comprising a nucleic acid sequence encoding any CSR disclosed herein. The present disclosure provides a composition comprising a transposon comprising a nucleic acid sequence encoding any CSR disclosed herein. The present disclosure provides a composition comprising a modified cell disclosed herein or a composition comprising a plurality of modified cells disclosed herein.

[016] The present disclosure provides a modified T lymphocyte (T-cell), comprising: (a) a modification of an endogenous sequence encoding a T-cell Receptor (TCR), wherein the modification reduces or eliminates a level of expression or activity of the TCR; and (b) a chimeric stimulatory receptor (CSR) comprising: (i) an ectodomain comprising an activation component, wherein the activation component is isolated or derived from a first protein; (ii) a

transmembrane domain; and (iii) an endodomain comprising at least one signal transduction domain, wherein the at least one signal transduction domain is isolated or derived from a second protein; wherein the first protein and the second protein are not identical.

[017] The modified T-cell can further comprise an inducible proapoptotic polypeptide. The modified T-cell can further comprise a modification of an endogenous sequence encoding Beta-2-Microglobulin (B2M), wherein the modification reduces or eliminates a level of expression or activity of a major histocompatibility complex (MHC) class I (MHC-I).

[018] The modified T-cell can further comprise a non-naturally occurring polypeptide comprising an HLA class I histocompatibility antigen, alpha chain E (HLA-E) polypeptide. The non-naturally occurring polypeptide comprising a HLA-E polypeptide can further comprise a B2M signal peptide. The non-naturally occurring polypeptide comprising a HLA-E polypeptide can further comprise a B2M polypeptide. The non-naturally occurring polypeptide comprising an HLA-E polypeptide can further comprise a linker, wherein the linker is positioned between the B2M polypeptide and the HLA-E polypeptide. The non-naturally occurring polypeptide comprising an HLA-E polypeptide can further comprise a peptide and a B2M polypeptide. The non-naturally occurring polypeptide comprising an HLA-E can further comprise a first linker positioned between the B2M signal peptide and the peptide, and a second linker positioned between the B2M polypeptide and the peptide encoding the HLA-E.

[019] The modified T-cell can further comprise a non-naturally occurring antigen receptor, a sequence encoding a therapeutic polypeptide, or a combination thereof. The non-naturally occurring antigen receptor can comprise a chimeric antigen receptor (CAR).

[020] The CSR can be transiently expressed in the modified T-cell. The CSR can be stably expressed in the modified T-cell. The polypeptide comprising the HLA-E polypeptide can be transiently expressed in the modified T-cell. The polypeptide comprising the HLA-E polypeptide can be stably expressed in the modified T-cell. The inducible proapoptotic polypeptide can be transiently expressed in the modified T-cell. The inducible proapoptotic polypeptide can be stably expressed in the modified T-cell. The non-naturally occurring antigen receptor or a sequence encoding a therapeutic protein can be transiently expressed in the modified T-cell. The non-naturally occurring antigen receptor or a sequence encoding a therapeutic protein can be stably expressed in the modified T-cell.

[021] The modified T-cell can be an autologous cell. The modified T-cell can be an allogeneic cell. The modified T-cell can be an early memory T cell, a stem cell-like T cell, a stem memory T cell (T_{SCM}), a central memory T cell (T_{CM}) or a stem cell-like T cell.

[022] The present disclosure provides a composition comprising any modified T-cell disclosed herein. The present disclosure also provides a composition comprising a population of modified T lymphocytes (T-cells), wherein a plurality of the modified T-cells of the population comprise the CSR disclosed herein. The present disclosure also provides a composition comprising a population of T lymphocytes (T-cells), wherein a plurality of the T-cells of the population comprise the modified T-cell disclosed herein.

[023] The present disclosure provides methods of treating a disease or disorder comprising administering to a subject in need thereof a therapeutically-effective amount of any composition disclosed herein; or a composition for use in the treatment of a disease or disorder. In one aspect, the composition is a modified T-cell or population of modified T-cells as disclosed herein. The present disclosure also a method of treating a disease or disorder comprising administering to a subject in need thereof a therapeutically-effective amount of a composition disclosed herein and at least one non-naturally occurring molecule that binds the CSR.

[024] The present disclosure provides a method of producing a population of modified T-cells comprising, consisting essential of, or consisting of introducing into a plurality of primary human T-cells a composition comprising the CSR of the present disclosure or a sequence encoding the same to produce a plurality of modified T-cells under conditions that stably express the CSR within the plurality of modified T-cells and preserve desirable stem-like properties of the plurality of modified T-cells. The present disclosure provides a composition comprising a population of modified T-cells produced by the method. In some aspects, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the population comprising the CSR expresses one or more cell-surface marker(s) of a stem memory T cell (T_{SCM}) or a T_{SCM}-like cell; and wherein the one or more cell-surface marker(s) comprise CD45RA and CD62L. some aspects, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at

least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the population expresses one or more cell-surface marker(s) of a central memory T cell (T_{CM}) or a T_{CM}-like cell; and wherein the one or more cell-surface marker(s) comprise CD45RO and CD62L. The composition can be for use in the treatment of a disease or disorder. The present disclosure also provides for use of a composition produced by the method for the treatment of a disease or disorder. The present disclosure further provides a method of treating a disease or disorder comprising administering to a subject in need thereof a therapeutically-effective amount of the composition produced by the method. The method of treating can further comprising administering an activator composition to the subject to activate the population of modified T-cells in vivo, to induce cell division of the population of modified T-cells in vivo, or a combination thereof.

[025] The present disclosure provides a method of producing a population of modified T-cells comprising, consisting essential of, or consisting of introducing into a plurality of primary human T-cells a composition comprising the CSR of the present disclosure or a sequence encoding the same to produce a plurality of modified T-cells under conditions that transiently express the CSR within the plurality of modified T-cells and preserve desirable stem-like properties of the plurality of modified T-cells. The present disclosure provides a composition comprising a population of modified T-cells produced by the method. In some aspects, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the population comprising the CSR expresses one or more cell-surface marker(s) of a stem memory T cell (T_{SCM}) or a T_{SCM}-like cell; and wherein the one or more cell-surface marker(s) comprise CD45RA and CD62L. some aspects, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the population expresses one or more cell-surface marker(s) of a central memory T cell (T_{CM}) or a T_{CM}-like cell; and wherein the one or more cell-surface marker(s)

comprise CD45RO and CD62L. The composition can be for use in the treatment of a disease or disorder. The present disclosure also provides for use of a composition produced by the method for the treatment of a disease or disorder. The present disclosure further provides a method of treating a disease or disorder comprising administering to a subject in need thereof a therapeutically-effective amount of the composition produced by the method. In some aspects, the modified T-cells within the population of modified T-cells administered to the subject no longer express the CSR.

[026] The present disclosure provides a method of expanding a population of modified T-cells comprising introducing into a plurality of primary human T-cells a composition comprising the CSR of the present disclosure or a sequence encoding the same to produce a plurality of modified T-cells under conditions that stably express the CSR within the plurality of modified T-cells and preserve desirable stem-like properties of the plurality of modified T-cells and contacting the cells with an activator composition to produce a plurality of activated modified T-cells, wherein expansion of the plurality of modified T-cells is at least two fold higher than the expansion of a plurality of wild-type T-cells not stably expressing the CSR under the same conditions. In some aspects, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the population comprising the CSR expresses one or more cell-surface marker(s) of a stem memory T cell (T_{SCM}) or a T_{SCM}-like cell; and wherein the one or more cell-surface marker(s) comprise CD45RA and CD62L. some aspects, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the population expresses one or more cell-surface marker(s) of a central memory T cell (T_{CM}) or a T_{CM}-like cell; and wherein the one or more cell-surface marker(s) comprise CD45RO and CD62L. The present disclosure provides a composition comprising a population of modified T-cells expanded by the method. The composition can be for use in the treatment of a disease or disorder. The present disclosure also provides for use of a composition expanded by the method for the treatment of a disease or

disorder. The present disclosure further provides a method of treating a disease or disorder comprising administering to a subject in need thereof a therapeutically-effective amount of the composition expanded by the method. The method of treating can further comprising administering an activator composition to the subject to activate the population of modified T-cells in vivo, to induce cell division of the population of modified T-cells in vivo, or a combination thereof.

[027] The present disclosure provides a method of expanding a population of modified T-cells comprising introducing into a plurality of primary human T-cells a composition comprising the CSR of the present disclosure or a sequence encoding the same to produce a plurality of modified T-cells under conditions that transiently express the CSR within the plurality of modified T-cells and preserve desirable stem-like properties of the plurality of modified T-cells and contacting the cells with an activator composition to produce a plurality of activated modified T-cells, wherein expansion of the plurality of modified T-cells is at least two fold higher than the expansion of a plurality of wild-type T-cells not transiently expressing the CSR under the same conditions. The present disclosure provides a composition comprising a population of modified T-cells expanded by the method. In some aspects, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the population comprising the CSR expresses one or more cell-surface marker(s) of a stem memory T cell (T_{SCM}) or a T_{SCM}-like cell; and wherein the one or more cell-surface marker(s) comprise CD45RA and CD62L. some aspects, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the population expresses one or more cell-surface marker(s) of a central memory T cell (T_{CM}) or a T_{CM}-like cell; and wherein the one or more cell-surface marker(s) comprise CD45RO and CD62L.The composition can be for use in the treatment of a disease or disorder. The present disclosure also provides for use of a composition expanded by the method for the treatment of a disease or disorder. The present disclosure further provides a method of treating a disease or

disorder comprising administering to a subject in need thereof a therapeutically-effective amount of the composition expanded by the method. In some aspects, the modified T-cells within the population of modified T-cells administered to the subject no longer express the CSR.

[028] Any of the above aspects can be combined with any other aspect.

[029] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. In the Specification, the singular forms also include the plural unless the context clearly dictates otherwise; as examples, the terms "a," "an," and "the" are understood to be singular or plural and the term "or" is understood to be inclusive. By way of example, "an element" means one or more element. Throughout the specification the word "comprising," or variations such as "comprises" or "comprising," will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from the context, all numerical values provided herein are modified by the term "about."

[030] Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. The references cited herein are not admitted to be prior art to the claimed invention. In the case of conflict, the present Specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting. Other features and advantages of the disclosure will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[031] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[032] **FIG. 1** is a schematic diagram depicting a T-cell receptor (TCR) and co-receptors CD28 and CD2.

[033] **FIG. 2** is a schematic diagram depicting primary and secondary co-stimulation is delivered to T-cell via binding of agonist mAbs (anti-CD3, anti-CD28, and anti-CD2). Full T-cell activation critically depends on TCR engagement in conjunction with a second signal by co-stimulatory receptors that boost the immune response. Primary and secondary co-stimulation can be delivered to T-cell via treatment with and engagement of surface receptors with agonist mAbs (E.g. anti-CD3, anti-CD28, and anti-CD2).

[034] **FIG. 3** is a schematic diagram showing that, in absence of TCR, only secondary co-stimulation is delivered to T-cell via binding of agonist mAbs. Since full T-cell activation is critically dependent on primary stimulation via CD3ζ in conjunction with a second signal by co-stimulatory receptors, T cell activation and expansion is suboptimal and thus reduced.

[035] **FIG. 4** is a schematic diagram showing that, in absence of TCR, stimulation is enhanced with expression of Chimeric Stimulatory Receptors (CSRs). In the absence of TCR, but in the presence of surface-expressed CSR/s, primary and secondary co-stimulatory signals are delivered when T cell is treated with standard agonist mAbs. Since a fuller T-cell activation is achieved via CSR-mediated stimulatory signals, T cell activation and expansion is enhanced.

[036] **FIG. 5** is a schematic diagram depicting an exemplary CSR CD28z of the disclosure.

[037] **FIG. 6** is a schematic diagram depicting an exemplary CSR CD2z of the disclosure.

[038] **FIG. 7** is a schematic of a strategy for mutation of CSR CD2z to eliminate natural ligand (CD58) binding. A panel of CSR CD2z mutants was designed within the extracellular domain of CD2. The goal of this panel was to identify mutants that no longer bind CD58 but retain their receptivity to being bound by the anti-CD2 activator reagent. This may be desirable for two main reasons: 1) CD58 expression by activated T cells may interact with the wild type (WT) CD2z CSR and possibly interfere with the optimal performance of the CSR, and 2) since the WT CD2z CSR might function as a natural ligand CAR, it is possible that T cells expressing the CSR may mediate cytotoxic activity against CD58-expressing cells, including activated T cells. Thus, a mutant CD2z CSR that cannot interact with CD58 but retains its ability to bind activating anti-CD2 reagent for optimal cell expansion is desired.

[039] **FIG. 8** is a schematic diagram depicting an exemplary CSR CD2z-D111H of the disclosure. A D111H mutation is within the CD2 extracellular domain of the CSR CD2z-D111H construct.

[040] **FIGS. 9A-9B** are a series of plots showing that piggyBac® delivery of CSR enhances the expansion of TCRb/b2M double-knockout CAR-T cells. Pan T cells isolated from normal donor blood were genetically modified using the piggyBac® DNA modification system in combination with the Cas-CLOVER™ gene-editing system. Cells were electroporated in a single reaction with a transposon encoding a CAR, selection gene and a CSR (either CD28z or CD2z), an mRNA encoding the super piggyBac™ transposase enzyme, an mRNA encoding Cas-CLOVER™, and multiple guide RNA (gRNA) targeting TCRb and b2M in order to knockout the TCR and MHCI (double-knockout; DKO). The cells were subsequently stimulated with agonist mAbs anti-CD2, anti-CD3 and anti-CD28, and were later selected for genetic modification over the course of a 16 day culture period. At the end of the initial culture period all T cells expressed the CAR, indicating successful selection for genetically-modified cells (data not shown). In the samples expressing either CD2z or CD28z CSR, a greater degree of expansion of the DKO cells was observed as a greater frequency of the CAR alone DKO cells (FIG. 9A and 9B). In DKO CAR-T cell samples expressing either CD2z or CD28z CSR, at least a two fold expansion of the cells was observed in comparison to DKO CAR-T cells alone.

[041] **FIGS. 10A-10B** are a series of plots showing that CSR CD2z or CD28z in purified DKO CAR-T cells results in enhanced expansion upon re-stimulation. After initial genetic modification and a first round of stimulation and expansion, cells from each group (Mock (WT CAR-T cells), DKO CAR-T cells, DKO CAR-T cells + CD2z CSR, and DKO CAR-T cells + CD28z CSR) were purified for $TCR^{-}MHCI^{-}$ cells using magnetic beads. The purified cells were then re-stimulated using anti-CD2, anti-CD3, and anti-CD28 agonist mAbs. At the end of the 14 day culture period, TCR and MHCI expression (A) as well as magnitude of cell population expansion (B) was determined. After this secondary expansion, all purified DKO cells, including those expressing either CD2z or CD28z CSR, were still extremely pure for DKO cells (>98.8% DKO). DKO CAR-T cells expressing either CD2z or CD28z CSR resulted in enhanced expansion when compared to those not expressing either CSR.

[042] **FIG. 11** is a graph showing that cytokine supplementation can further expand purified DKO CAR-T cells expressing CSR upon re-stimulation. After initial genetic modification and a first round of stimulation and expansion, cells expressing CSRs were purified for DKO cells using magnetic beads. The purified cells were then re-stimulated using anti-CD2, anti-CD3, and anti-CD28 agonist mAbs in the presence exogenous purified recombinant IL7 and IL15. At the end of the 14 day culture period, magnitude of cell population expansion was determined. After a secondary expansion, all purified DKO cells, including those expressing either CD2z or CD28z CSR, were still extremely pure for $TCR^{-}MHCI^{-}$ cells (>98.8% double knockout (data not shown)). In addition, cells grew robustly in the presence of IL7 and IL15, which was greater than that without supplementation. These data demonstrate that exogenous cytokines may be added to further expand WT CAR-T cells expressing CSR.

[043] **FIG. 12** is a graph showing that surface expression of CAR is not significantly affected by co-expression of CSR in DKO cells. After secondary expansion, cells (Mock (WT T cells), WT CAR-T cells, DKO CAR-T cells, DKO CAR-T cells + CD2z CSR, and DKO CAR-T cells + CD28z CSR) were stained for the surface-expression of CAR and compared to control WT CAR-T cells and Mock T cells. Expression of CD2z or CD28z CSR does not have a significant impact on expression of CAR molecule on the surface of T cells.

[044] **FIG. 13** is a graph showing that expression of CSRs does not significantly affect DKO CAR-T cell cytotoxicity *in vitro*. After secondary expansion, cells (Mock (WT T cells), WT CAR-T cells, DKO CAR-T cells, DKO CAR-T cells + CD2z CSR, and DKO CAR-T cells + CD28z CSR) were co-cultured with engineered K562-BCMA-Luciferase (eK562-Luc.BCMA) or negative control line K562-PSMA-Luciferase (eK562-Luc.PSMA) for 48 hours at 10:1, 3:1, or 1:1 E:T ratios. Luciferase signal was measured to determine cytotoxicity. Killing of eK562-Luc.PSMA is shown in dotted lines, while killing of eK562-Luc.BCMA is shown in solid lines. All CAR^{+} T cells expressed an anti-BCMA specific CAR. DKO CAR-T cells exhibit similar *in vitro* cytotoxicity as WT CAR-TCR cells. This activity is not significantly affected by CD2z or CD28z CSR co-expression.

[045] **FIG. 14** is a graph showing that expression of CSRs does not significantly affect DKO CAR-T cell secretion of IFNg *in vitro*. Supernatants from the 48 hour killing assay were assayed for secreted IFNg as a measure of antigen-specific functionality of the BCMA CAR T cells. All

CAR-T cells, either with or without CD2z or CD28z CSR expression secrete IFNg in response to co-culture with target cells expressing BCMA (eK562-Luc.BCMA), but not those expressing an irrelevant target (eK562-Luc.PSMA).

[046] **FIG. 15** is a series of plots showing that expression of CSRs does not significantly affect DKO CAR-T cell proliferation *in vitro*. Mock (WT T-cells), WT CAR-T cells, DKO CAR-T cells, DKO CAR-T cells + CD2z CSR, and DKO CAR-T cells + CD28z CSR cells were labelled with Cell Trace Violet (CTV), which is diluted as cells proliferate. The cells were co-cultured for 5 days with eK562-Luc.PSMA or eK562-Luc.BCMA cells at a 1:2 E:T ratio. All CAR-T cells, either with or without CD2z or CD28z proliferate in response to target cells expressing BCMA (eK562-Luc.BCMA) but not those expressing an irrelevant antigen (eK562-Luc.PSMA).

[047] **FIG. 16** is a pair of graphs showing that the memory phenotype of DKO CAR-T is not significantly affected with CD2z CSR co-expression. WT CAR-T cells, DKO CAR-T cells, DKO CAR-T cells + CD2z, and DKO CAR-T cells + CD28z were stained for expression of surface CD45RA, CD45RO, and CD62L to define Tscm, Tcm, Tem, and Teff cells; Tscm ($CD45RA^+CD45RO^-CD62L^+$), Tcm ($CD45RA^-CD45RO^+CD62L^+$), Tem ($CD45RA^-CD45RO^+CD62L^-$), Teff ($CD45RA^+CD45RO^-CD62L^-$). WT and DKO CAR-T cells with or without CD2z are comprised predominantly of exceptionally high levels of favorable Tscm and Tcm cells. However, when CD28z is expressed in DKO CAR-T cells, the phenotype is significantly more differentiated, favoring Tcm and Tem cells. This phenotype may have a negative impact on the *in vivo* functionality of these CAR T cells since they appear to be more differentiated.

[048] **FIG. 17** is a series of graphs showing that the expression of activation/exhaustion markers in DKO CAR-T is not significantly affected with CD2z CSR co-expression. Mock (WT T cells), WT CAR-T cells, DKO CAR-T cells, DKO CAR-T cells + CD2z, and DKO CAR-T cells + CD28z were examined by flow cytometry for the expression of important exhaustion molecules Lag3, PD1, and Tim3. WT and DKO CAR-T cells with or without CD2z have little to no expression of exhaustion molecules when compared to mock T cells. However, expression of CD28z CSR in DKO CAR-T during the expansion process leads to significant upregulation of exhaustion markers Lag3, PD1, and Tim3. This phenotype may have a negative impact on the *in*

vivo functionality of these CAR T cells since they appear to be more exhausted. By contrast, CD2z expression has little to no effect on the exhaustion phenotype of DKO CAR-T cells while significantly enhancing the expansion capability of the cells.

[049] **FIG. 18** is a graph showing that delivery of CSR enhances the expansion of CAR-T cells. CSRs were delivered to CAR-T cells either transiently by mRNA or stably by piggyBac®. Pan T cells isolated from the blood of a normal donor were genetically modified using the piggyBac® DNA modification system and the standard Poseida process. Cells were co-electroporated in a single reaction with mRNA encoding the Super piggyBac™ transposase enzyme (SPB), a transposon encoding a BCMA CAR and selection gene, along with an additional mRNA encoding a CSR (either CD28z or CD2z; resulting in transient expression) or a CD19 mRNA control, or, with a transposon encoding a BCMA CAR, selection gene and a CSR (either CD28z or CD2z; resulting in stable expression). The cells were subsequently stimulated with agonist mAbs anti-CD2, anti-CD3 and anti-CD28, and were later selected for genetic modification over the course of a 19 day culture period. At the end of the initial culture period all T cells expressed the CAR, indicating successful selection for genetically-modified cells (data not shown). Bars represent total live CAR-T cells in well and numbers indicate fold-enhancement of expansion above CAR-T cells produced in the absence of a CSR or a CD19 mRNA control. In the samples expressing either CD2z or CD28z CSR, either transiently or stably, a greater degree of expansion of the CAR-T cells.

[050] **FIG. 19** is a series of bar graphs showing that expression of CSRs does not significantly affect CAR-T cell cytotoxicity. CSRs were delivered to CAR-T cells either transiently by mRNA or stably by piggyBac®. Pan T cells isolated from the blood of a normal donor were genetically modified using the piggyBac® DNA modification system and the standard Poseida process. Cells were co-electroporated in a single reaction with mRNA encoding the Super piggyBac™ transposase enzyme (SPB), a transposon encoding a BCMA CAR and selection gene, along with an additional mRNA encoding a CSR (either CD28z or CD2z; resulting in transient expression), or, with a transposon encoding a BCMA CAR, selection gene and a CSR (either CD28z or CD2z; resulting in stable expression). The cells were subsequently stimulated with agonist mAbs anti-CD2, anti-CD3 and anti-CD28, and were later selected for genetic modification over the course of a 19 day culture period. At the end of the initial culture period all T cells expressed the

CAR, indicating successful selection for genetically-modified cells (data not shown). To assess CAR-T cell ability to kill, cells were co-cultured with engineered K562-BCMA-Luciferase (eK562-Luc.BCMA) or negative control line K562-Luciferase (eK562-Luc) for 48 hours at 10:1, 3:1, or 1:1 E:T ratios. Luciferase signal was measured to determine cytotoxicity. Killing of eK562-Luc is shown in bar graph on left, while killing of eK562-Luc.BCMA is shown in bar graph on right. All CAR^+ T cells expressed an anti-BCMA specific CAR and exhibited similar *in vitro* cytotoxicity against BCMA+ target cells. In summary, this activity was not significantly affected by transient or stable CSR co-expression.

[051] **FIG. 20** is a schematic diagram showing that, in presence of TCR, stimulation is enhanced with expression of Chimeric Stimulatory Receptors (CSRs). In the presence of surface-expressed CSR/s, either transiently or stably expressed, enhanced primary and secondary co-stimulatory signals are delivered when T cell is treated with reagents displaying agonist mAbs. In one aspect, this schematic diagram represents an autologous cell. Since a fuller T-cell activation is achieved via CSR-mediated stimulatory signals, T cell activation and expansion is enhanced.

[052] **FIG. 21** is a series of graphs showing that CSRs are expressed on the surface of T cells and do not lead to cellular activation in the absence of exogenous stimulation. Pan T cells from normal blood donors were stimulated with anti-CD3/anti-CD28 beads in standard T cell culture media, then rested. These cells were then electroporated (BTX ECM 830 electroporator @ 500V for 700 μs) with 10 μg of mRNA encoding either CD28 CSR, CD2 CSR, or wild-type CD19 control. Two days later the electroporated cells were examined by flow cytometry for surface-expression of each molecule and data are shown as stacked histograms. In addition, cell size (FSC-A) and CD69 expression was evaluated as a possible indication of cellular activation above the Mock electroporated control cells. Increased surface expression of CD28, CD2, and CD19 were detected in T cells electroporated either with CD28z CSR, CD2z CSR or CD19, respectively. Expression of these molecules on the surface of T cells did not intrinsically activate the cells in the absence of exogenous stimulation.

[053] **FIG. 22** is a series of line graphs showing that CSR molecules can be delivered transiently during manufacturing for the enhanced expansion of CAR-T cells. Pan T cells isolated from healthy donor blood were genetically modified using the piggyBac® DNA

modification system in combination with the Cas-CLOVER™ gene-editing system (CC) for the production of allogeneic (Allo) CAR-T cells, or without CC gene-editing for the production of autologous (Auto) CAR-T cells; auto CAR-T cells were produced by nucleofection of an mRNA encoding the super piggyBac® transposase enzyme (SPB) and a transposon encoding a CAR, selection gene and a safety switch. For production of Allo CAR-T, cells were electroporated (EP) in a single reaction with an mRNA encoding the SPB enzyme, an mRNA encoding CC, multiple guide RNAs (gRNA) targeting TCRb and b2M for the knockout of TCR and MHCI, and a transposon encoding either a CAR, selection gene and the CSR CD2z, or a transposon encoding a CAR, selection gene and a safety switch that did not encode a CSR. For CAR-T cells that did not receive a CSR encoded in the transposon for stable integration, the CD2z CSR was provided to the cells transiently as an mRNA only once in the initial EP reaction, at varying amounts of 5 µg, 10 µg, and 20 µg of mRNA in a 100 µl EP reaction. Following EP, all cells were subsequently stimulated with a cocktail of agonist mAbs anti-CD2, anti-CD3 and anti-CD28, and were later selected for genetic modification over the course of a 19-day culture period using the selection gene. At the end of the initial culture period, all T cells expressed the CAR, indicating successful selection for genetically-modified cells (data not shown). Data for each is shown in line graph at various days of production. In the samples where the CD2z CSR was provided stably (as encoded in the transposon (Stable)) or transiently (as encoded in mRNA (mRNA)), a greater degree of expansion of the CAR-T cells was observed as compared to the CAR-T cells produced without a CSR. These data show that the CSR can be delivered transiently as mRNA during manufacturing for enhanced expansion of both autologous and allogeneic CAR-T products.

[054] **FIG. 23A** is a bar graph showing CSR CD2z mutant staining data. A panel of CSR CD2z mutants was designed, constructed, and tested for surface expression and binding to several anti-CD2 antibody reagents. To do so, each mutant was synthesized, subcloned into an in-house mRNA production vector, and then high-quality mRNA was produced for each. K562 cells were electroporated with 9 µg of mRNA, and surface-expression of each molecule was analyzed by flow cytometry the next day and data are shown as bar graphs. Each molecule was stained with anti-CD2 activator reagent, anti-CD2 monoclonal antibody (clone TS1/8), or anti-

CD2 polyclonal antibody reagent (goat anti-human CD2). Variable binding was observed for each construct and data are summarized in FIG. 23C.

[055] **FIG. 23B** is a series of bar graphs showing CSR CD2z mutant degranulation data. The panel of CSR CD2z mutants was tested for the capability of mediating degranulation against CD58-positive cell targets. T cell degranulation is a surrogate of T cell killing that can be measured by FACS staining for intracellular CD107a expression following coculture with target cell lines expressing target antigen. Specifically, pan T cells from normal blood donors were stimulated with anti-CD3/anti-CD28 beads in standard T cell culture media, then rested. These cells were then electroporated (BTX ECM 830 electroporator @ 500V for 700 μs) with 9 μg of mRNA expressing CSR CD2z mutants and cultured overnight. The next day, the cells were cocultured for 4-6 hours in the presence of various target cell lines. Positive target cell lines included K562 cells or Rat2 cells that were electroporated or lipofected, respectively, with mRNA encoding human CD58, while negative controls were either Rat2 cells that were not electroporated or CSR CD2z mutant expressing T cells alone. Only T cells expressing CSR CD2z mutants that recognized surface-expressed human CD58 were capable of degranulating at levels above background. Little reactivity was observed for the D111H, K67R/Y110D, K67R/Q70K/Y110D/D111H, Delta K106-120, CD3z deletion and mock control, and data are summarized in FIG. 23C.

[056] **FIG. 23C** is a summary of staining and degranulation data. Data from surface-expression and binding studies, as well as those from degranulation experiments for each CSR CD2z mutant is summarized in the table. Two candidates that are expressed on the surface and/or retain binding to the anti-CD2 activator reagent that do not mediate anti-CD58 degranulation activity are the D111H and K67R/Y110D CSR CD2z mutants. Only the D111H mutant is strongly bound by all staining reagents on the cell surface while completely abrogating anti-CD58 degranulation activity.

[057] **FIG. 23D** is a series of flow cytometry plots showing the expression of CD48, CD58 or CD59 on K562 and Rat2 cells. To confirm possible ligands for the CSR WT CD2z molecule, a panel of known and suspected ligands including human CD48, CD58, and CD59 were tested. Degranulation of engineered T cells was evaluated against the cell lines K562 and Rat2 that were made to overexpress the target ligands and confirmed for expression by FACS staining. Red

histograms are unstained cells and blue histograms are cells that were electroporated/lipofected with mRNA and then stained for expression of the respective marker by FACS.

[058] **FIG. 23E** is a bar graph showing that CSR CD2z recognizes human CD58, but not CD48 or CD59. To confirm possible ligands for the CSR WT CD2z molecule, a panel of known and suspected ligands including human CD48, CD58, and CD59 were tested. Degranulation of engineered T cells was evaluated against the cell lines K562 and Rat2 that were made to overexpress the target ligands and confirmed for expression by FACS staining. Cells were electroporated/lipofected with mRNA and then stained for expression of the respective marker by FACS. As a control, a BCMA CAR was included as well as a K562 cell line overexpressing BCMA. In addition, T cells transfected with GFP were also included as a control. T cell degranulation is a surrogate of T cell killing that can be measured by FACS staining for intracellular CD107a expression following coculture with target cell lines expressing target antigen. Pan T cells from normal blood donors were stimulated with anti-CD3/anti-CD28 beads in standard T cell culture media, then rested. These T cells were then electroporated with mRNA expressing CSR WT CD2z, BCMA CAR, or GFP and cultured overnight. The next day, the cells were cocultured for 4-6 hours in the presence of the various target cell lines that were electroporate/lipofected with mRNA encoding human CD48, CD58 or CD59, while negative controls were either K562 or Rat2 cells that were not electroporated/lipofected, or each of the electroporated T cells alone. T cells expressing either the CSR WT CD2z or BCMA CAR were capable of degranulating at levels above background when cocultured with cell lines overexpressing human CD58 or BCMA, respectively, and not against human CD48 or CD59. Little reactivity was observed for the T cells expressing GFP.

[059] **FIG. 24A** is a bar graph showing that the delivery of CSR CD2z-D111H mutant enhances the expansion of Allo CAR-T cells. Pan T cells isolated from healthy donor blood were genetically modified using the piggyBac® DNA modification system in combination with the Cas-CLOVER™ gene-editing system (CC) for the production of allogeneic (Allo) CAR-T cells, or without CC gene-editing, as a control, for the production of autologous (Auto) CAR-T without a CSR (No CSR); auto CAR-T cells were produced by nucleofection of an mRNA encoding the super piggyBac™ transposase enzyme (SPB) and a transposon encoding a CAR, selection gene and a safety switch. For production of Allo CAR-T, cells were electroporated

(EP) in a single reaction with an mRNA encoding the SPB enzyme, an mRNA encoding CC, multiple guide RNAs (gRNA) targeting TCRb and b2M for the knockout of TCR and MHCI, and a transposon encoding either a CAR, selection gene and either the WT or mutant (D111H) CSR CD2z, or a transposon encoding a CAR, selection gene and a safety switch that did not encode a CSR. For the latter, Allo CAR-T cells that did not receive a CSR encoded in the transposon for stable integration, the WT or mutant (D111H) CSR CD2z was provided to the cells transiently as an mRNA only once in the initial EP reaction. Following EP, all cells were subsequently stimulated with a cocktail of agonist mAbs anti-CD2, anti-CD3 and anti-CD28, and were later selected for genetic modification over the course of up to a 15-day culture period using the selection gene. At the end of the initial culture period, all T cells expressed the CAR, indicating successful selection for genetically-modified cells (data not shown), and then all non-edited TCR-positive cells were depleted via negative selection to yield a population of Allo CAR-T cells that were >99% TCR-negative (data not shown). All samples were performed in duplicate, except the Auto (No CSR) control, and data for peak expansion for each (day of peak expansion is displayed) is shown in bar graph where error bars represent standard deviation. In the samples where either the WT or mutant (D111H) CD2z was provided stably (as encoded in the transposon (Stable)) or transiently (as encoded in mRNA (mRNA)), a greater degree of expansion of the Allo CAR-T cells was observed as compared to the Allo CAR-T cells produced without a CSR.

[060] **FIG. 24B** is a series of bar graphs showing that the delivery of CSR CD2z-D111H mutant does not inhibit gene editing. Pan T cells isolated from healthy donor blood were genetically modified using the piggyBac® DNA modification system in combination with the Cas-CLOVER™ gene-editing system (CC) to produce allogeneic (Allo) CAR-T cells. Cells were electroporated (EP) in a single reaction with an mRNA encoding the SPB enzyme, an mRNA encoding CC, multiple guide RNA (gRNA) targeting TCRb and b2M for the knockout of TCR and MHCI, and a transposon encoding either a CAR, selection gene and either the WT or mutant (D111H) CSR CD2z, or a transposon encoding a CAR, selection gene and a safety switch that did not encode a CSR. For the latter, cells that did not receive a CSR encoded in the transposon for stable integration, the WT or mutant (D111H) CSR CD2z was provided transiently as an mRNA only once in the initial EP reaction. Following EP, all cells were

subsequently stimulated with a cocktail of agonist mAbs anti-CD2, anti-CD3 and anti-CD28, and were later selected for genetic modification over the course of up to a 14-day culture period using the selection gene. At the end of the initial culture period, all T cells expressed the CAR, indicating successful selection for genetically-modified cells (data not shown). All samples were performed in duplicate, and data is shown in bar graph where error bars represent standard deviation. In the samples where either the WT or mutant (D111H) CD2z was provided stably (as encoded in the transposon (Stable)) or transiently (as encoded in mRNA (mRNA)), a similar or greater degree of gene editing of the Allo CAR-T cells was observed as compared to the Allo CAR-T cells produced without a CSR.

[061] **FIG. 24C** is a bar graph showing that the memory phenotype of Allo CAR-T is not significantly affected by delivery of CD2z CSRs. Allo CAR-T cells with no CSR and Allo CAR-Ts with CSR that was delivered either stably or transiently were stained for expression of surface CD45RA, CD45RO, and CD62L to define Tscm, Tcm, Tem, and Teff cells; Tscm ($CD45RA^+CD45RO^-CD62L^+$), Tcm ($CD45RA^-CD45RO^+CD62L^+$), Tem ($CD45RA^-CD45RO^+CD62L^-$), Teff ($CD45RA^+CD45RO^-CD62L^-$). All samples were performed in duplicate, and data is shown in bar graph where error bars represent standard deviation. Delivery of CSRs did not dramatically affect the levels of favorable Tscm and Tcm cells in the products.

[062] **FIG. 25** is a schematic diagram depicting an exemplary HLA-bGBE composition of the disclosure.

[063] **FIG. 26** is a schematic diagram depicting an exemplary HLA-gBE composition of the disclosure.

[064] **FIG. 27** is a pair of graphs showing that expression of single-chain HLA-E diminishes NK cell-mediated cytotoxicity against HLA-deficient T cells. B2M and TCRαβ was knocked-out of T cells (Jurkat) using CRISPR. B2M/TCRαβ double-knockout (DKO) T cells were electroporated with mRNA encoding an HLA-E molecule (HLA-bGBE), expressed on a single chain with B2M and the peptide VMAPRETLIL (SEQ ID NO: 17127) (B2M/peptide/HLA-E). DKO T cells electroporated with varying amounts of mRNA encoding single chain HLA-E were used as targets for artificial antigen presenting cell (aAPC)-expanded NK cells in a 3 hour co-culture. % cytotoxicity was calculated based on the number of target cells remaining after 3

hours compared to target cells alone. These data demonstrate that surface expression of HLA-E in DKO T cells reduces the total level of cell killing by NK cells in a dose-dependent manner.

[065] **FIG. 28** is a listing of gRNA sequences (from top to bottom) and primer sequences (from top to bottom)

[066] **FIG. 29** is a series of flow cytometry plots showing that targeted knockout of endogenous HLA-ABC, but not HLA-E. Since we showed that surface expression of HLA-E in MHCI KO T cells can increase their resistance to NK cell-mediated cytotoxicity, we explored additional strategies beyond introduction of a single-chain HLA-E gene. To do so, multiple guide RNA (gRNA) were designed to disrupt the expression of the main targets of host versus graft (HvG), HLA-A, HLA-B and HLA-C, while minimizing disruption of endogenous HLA-E. Specifically, guides were designed to target a conserved region occurring in all the three MHCI protein targets, but not in HLA-E. Pan human T cells were electroporated with mRNA encoding CRISPR Cas9 in combination with various gRNAs and efficiency of MHCI knockout was measured by surface HLA-A and HLA-E expression. FACS analysis of HLA-A and HLA-E expression was performed after a single round of T cell expansion and data are displayed below. These data demonstrate that gene-editing technology can be used to target disruption of MHCI while retaining levels of endogenous HLA-E on the surface of gene-edited T cells.

[067] **FIG. 30** is a schematic diagram of the missing-self hypothesis of natural killer mediated toxicity towards MHCI-KO cells.

[068] **FIG. 31** is a schematic depiction of the Csy4-T2A-Clo051-G4Slinker-dCas9 construct map (Embodiment 2).

[069] **FIG. 32** is a schematic depiction of the pRT1-Clo051-dCas9 Double NLS construct map (Embodiment 1).

[070] **FIG. 33** is a schematic diagram showing an exemplary method for the production of allogeneic CAR-Ts of the disclosure.

[071] **FIG. 34A** is a graph showing high efficiency gene editing of endogenous TCRa in proliferating Jurkat cells and in resting primary human pan T cells as an exemplary method for the production of allogeneic and universal CAR-Ts using Cas-CLOVER™ (an RNA-guided fusion protein comprising a dCas9-Clo051). Cas-CLOVER system disrupted TCRα expression in rapidly proliferating Jurkat T cells and non-dividing resting T cells at comparably high levels.

[072] **FIG. 34B** is a series of flow cytometry graphs showing efficient gene editing of endogenous TCRa, TCRb, and B2M in resting primary human pan T cells using Cas-CLOVER™. Critical targets TCRa, TCRB, and B2M that mediate alloreactivity were efficiently edited by Cas-CLOVER in resting human T cells.

[073] **FIG. 35** is a series of flow cytometry plots showing that Cas-CLOVER can be multiplexed by co-delivering reagents for TCRβ and β2M into primary human T cells. TCRβ/β2M double knock-out (DKO) cells were further enriched using antibody-beads based purification, and purified cells were analyzed by FACS for downregulation of surface expressed CD3 and β2M.

[074] **FIG. 36** is a series of graphs demonstrating reduced alloreactivity after KO of TCR and MHCI. Alloreactivities of WT or DKO (TCR and MHCI) CAR-T cells was analyzed by mixed lymphocyte reaction (MLR) and IFNγ by ELISpot assay. On the left, WT or gene-edited DKO CAR-T cells were labeled with celltrace violet (CTV) and mixed at 1:1 ratio with irradiated peripheral blood mononuclear cells (PBMC)s and incubated for 12 days or 20 hr before analysis of proliferation or activation-induced secretion of IFNγ by ELISpot assay, respectively. WT or DKO CAR-T cells were incubated with PBMCs from either allogenic (Donor #1 PBMC and Donor #2 PBMC) or autologous (Autologous PBMC) donors at 1:1 ratio. After 12 days, CTV dye dilution was assessed by FACS and results showed significant proliferation of WT CAR-T cells when incubated with allogeneic PBMCs; proliferative rates of 40% and 39% by WT CAR-T cells was observed when cultured with allogeneic PBMCs from two different donors in comparison to only 2% when WT CAR-T cells were incubated with autologous PBMCs. On the other hand, DKO CAR-T cells did not proliferate when incubated with allogeneic PBMCs, demonstrating that KO of TCR and MHCI resulted in the elimination of graft-versus-host alloreactivity. This was also true in the short-term IFNγ by ELISpot assay (lower left) which showed that only WT CAR-T cells became activated and secreted IFNγ when incubated with allogeneic PBMCs, but not the DKO CAR-T cells. On the right, irradiated WT or DKO CAR-T cells were mixed at 1:1 ratio with PBMCs labeled with CFSE and incubated for 12 days or 20hr before analysis of proliferation or activation-induced secretion of IFNγ by ELISpot assay, respectively. After 12 days, CFSE dye dilution was assessed by FACS and showed significant proliferation of PBMCs (most likely T cells) when incubated with allogeneic CAR-T cells; 37%

and 9% of PBMCs proliferated in comparison to only 2% when incubated with autologous CAR-T cells. On the other hand, PBMCs did not proliferate above background when incubated with allogeneic CAR-T cells, demonstrating that KO of TCR and MHCI resulted in the elimination of host-versus-graft alloreactivity. This was also true in the short-term IFNγ by ELISpot assay (lower left) which showed that only WT CAR-T cells caused activation and secretion of IFNγ by PBMCs when incubated with allogeneic CAR-Ts, not the DKO CAR-T cells.

[075] **FIG. 37** is a series of graphs showing that DKO and WT CAR-Ts have similar CAR-expression and stem-like phenotypes. Gene editing does not affect CAR-T cell phenotype. BCMA CAR-expressing TCRβ/β2M DKO and WT T cells were analyzed for phenotype. CAR expression was comparable in WT and DKO. WT and DKO CAR-T cells were analyzed by FACS for expression of CD45RA and CD62L, markers for T stem cell memory (TSCM). These data demonstrate that gene editing of allo CAR-Ts does not significantly reduce the composition of memory CAR-T cells, retaining the exceptionally high and predominantly Tscm phenotype.

[076] **FIG. 38** is a series of graphs showing that DKO CAR-Ts are highly functional. Gene editing does not affect CAR-T cell functionality. BCMA CAR-expressing TCRβ/β2M DKO and WT T cells were analyzed for function. Proliferation against H929 (BCMA+) tumor lines was assessed by mixing CAR-T cells with H929 cells, incubated for 7 days, and analyzed for tumor-specific proliferation by FACS. Cytotoxicity and IFNg secretion against H929 (BCMA+) tumor lines was assessed by mixing CAR-T cells with H929 cells at various ratios, incubated for 24hrs and analyzed for tumor-specific killing by FACS. Cytotoxicity data are normalized to the tumor cell only sample. These data show that gene editing to produce DKO CAR-T cells does not significantly affect their functional capacity.

[077] **FIG. 39A** is a schematic diagram showing preclinical evaluation of the P-PSMA-101 transposon when delivered by a full-length plasmid (FLP) versus a nanotransposon (NT) at 'stress' doses using the Murine Xenograft Model. The murine xenograft model using a luciferase-expressing LNCaP cell line (LNCaP.luc) injected subcutaneously (SC) into NSG mice was utilized to assess *in vivo* anti-tumor efficacy of the P-PSMA-101 transposon as delivered by a full-length plasmid (FLP) or a nanotransposon (NT) at two different 'stress' doses (2.5x10^6 or 4x10^6) of total CAR-T cells from two different normal donors. All CAR-T cells were produced using piggyBac® (PB) delivery of P-PSMA-101 transposon using either FLP or NT delivery.

Mice were injected in the axilla with LNCaP and treated when tumors were established (100-200 mm^3 by caliper measurement). Mice were treated with two different 'stress' doses (2.5x10^6 or 4x10^6) of P-PSMA-101 CAR-Ts by IV injection for greater resolution in detecting possible functional differences in efficacy between transposon delivery by the FLP and the NT.

[078] **FIG. 39B** are a series of graphs showing the tumor volume assessment of mice treated as described in FIG. 34A. Tumor volume assessment by caliper measurement for control mice (black), Donor #1 FLP mice (red), Donor #1 NT mice (blue), Donor #2 FLP mice (orange), and Donor #2 NT mice (green) as displayed as group averages with error bars (top) and individual mice (bottom). The y-axis shows the tumor volume (mm^3) assessed by caliper measurement. The x-axis shows the number of days post T cell treatment. Delivered by NT, P-PSMA-101 transposon at a 'stress' dose demonstrated enhanced anti-tumor efficacy as measured by caliper in comparison to the FLP and control mice against established SC LNCaP.luc solid tumors.

DETAILED DESCRIPTION OF THE INVENTION

[079] The present disclosure provides a non-naturally occurring chimeric stimulatory receptor (CSR) comprising, consisting essential of, or consisting of: (a) an ectodomain comprising a activation component, wherein the activation component is isolated or derived from a first protein; (b) a transmembrane domain; and (c) an endodomain comprising at least one signal transduction domain, wherein the at least one signal transduction domain is isolated or derived from a second protein, wherein the first protein and the second protein are not identical.

[080] The activation component can comprise, consist essential of, or consist of: one or more of a component of a human transmembrane receptor, a human cell-surface receptor, a T-cell Receptor (TCR), a component of a TCR complex, a component of a TCR co-receptor, a component of a TCR co-stimulatory protein, a component of a TCR inhibitory protein, a cytokine receptor, or a chemokine receptor. The activation component can comprise, consist essential of, or consist of: a portion of one or more of a component of a T-cell Receptor (TCR), a component of a TCR complex, a component of a TCR co-receptor, a component of a TCR co-stimulatory protein, a component of a TCR inhibitory protein, a cytokine receptor, or a chemokine receptor to which an agonist of the activation component binds.

[081] The ectodomain can comprise, consist essential of, or consist of: a CD2 extracellular domain or a portion thereof to which an agonist binds or the ectodomain can comprise, consist

essential of, or consist of: a CD28 extracellular domain or a portion thereof to which an agonist binds. The activation component can comprise, consist essential of, or consist of: a CD2 extracellular domain or a portion thereof to which an agonist binds or the activation component can comprise, consist essential of, or consist of: a CD28 extracellular domain or a portion thereof to which an agonist binds. The CD2 extracellular domain to which an agonist binds comprises, consists essential of, or consists of the amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 17111. The CD2 extracellular domain to which an agonist binds comprises, consists essential of, or consists of the amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO: 17111. The CD2 extracellular domain to which an agonist binds comprises, consists essential of, or consists of the amino acid sequence of SEQ ID NO: 17111. The CD28 extracellular domain to which an agonist binds comprises, consists essential of, or consists of the amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 17099. The CD28 extracellular domain to which an agonist binds comprises, consists essential of, or consists of the amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO: 17099. The CD28 extracellular domain to which an agonist binds comprises, consists essential of, or consists of the amino acid sequence of SEQ ID NO: 17099.

[082] The signal transduction domain can comprise, consist essential of, or consist of: one or more of a component of a human signal transduction domain, T-cell Receptor (TCR), a component of a TCR complex, a component of a TCR co-receptor, a component of a TCR co-stimulatory protein, a component of a TCR inhibitory protein, a cytokine receptor, or a chemokine receptor. The second protein can comprise, consist essential of, or consist of: a CD3 protein or a portion thereof. The signal transduction domain can comprise, consist essential of, or consist of a CD3 protein or a portion thereof. The CD3 protein can comprise, consist essential of, or consist of a CD3ζ protein or a portion thereof. The CD3ζ protein comprises, consists essential of, or consists of the amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 17102. The CD3ζ protein comprises, consists essential of, or consists of the amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO: 17102. The CD3ζ protein comprises, consists essential of, or consists of the amino acid sequence of SEQ ID NO: 17102.

[083] The endodomain of a CSR of the present disclosure can further comprise, consist essential of, or consist of a cytoplasmic domain. The cytoplasmic domain can be isolated or derived from a third protein. In some aspects, the first protein and the third protein of a CSR of the present disclosure are identical. The cytoplasmic domain can comprise, consist essential of, or consist of: a CD2 cytoplasmic domain or a portion thereof or the cytoplasmic domain can comprise, consist essential of, or consist of: a CD28 cytoplasmic domain or a portion thereof.

[084] The CD2 cytoplasmic domain comprises, consists essential of, or consists of the amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 17113. The CD2 cytoplasmic domain comprises, consists essential of, or consists of the amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO: 17113. The CD2 cytoplasmic domain comprises, consists essential of, or consists of the amino acid sequence of SEQ ID NO: 17113. The CD28 cytoplasmic domain comprises, consists essential of, or consists of the amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 17101. The CD28 cytoplasmic domain comprises, consists essential of, or consists of the amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO: 17101. The CD28 cytoplasmic domain comprises, consists essential of, or consists of the amino acid sequence of SEQ ID NO: 17101.

[085] The endodomain of a CSR of the present disclosure can further comprise, consist essential of, or consist of a signal peptide. The signal peptide can be isolated or derived from a fourth protein. In some aspects, the first protein and the fourth protein of a CSR of the present disclosure are identical. The signal peptide can comprise, consist essential of, or consist of: a CD2 signal peptide or a portion thereof; the signal peptide can comprise, consist essential of, or consist of: a CD28 signal peptide or a portion thereof or the signal peptide can comprise, consist essential of, or consist of: a CD8a signal peptide or a portion thereof. The CD2 signal peptide comprises, consists essential of, or consists of the amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 17110. The CD2 signal peptide comprises, consists essential of, or consists of the amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO: 17110. The CD2 signal peptide comprises, consists essential of, or consists of the amino acid sequence of SEQ ID NO: 17110. The CD28 signal peptide comprises, consists essential of, or consists of the amino acid sequence at least 95% identical to the amino

acid sequence of SEQ ID NO: 17098. The CD28 signal peptide comprises, consists essential of, or consists of the amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO: 17098. The CD28 signal peptide comprises, consists essential of, or consists of the amino acid sequence of SEQ ID NO: 17098. The CD8a signal peptide comprises, consists essential of, or consists of the amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 17037. The CD8a signal peptide comprises, consists essential of, or consists of the amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO: 17037. The CD8a signal peptide comprises, consists essential of, or consists of the amino acid sequence of SEQ ID NO: 17037.

[086] The transmembrane domain of a CSR of the present disclosure can be isolated or derived from a fifth protein. In some aspects, the first protein and the fifth protein of a CSR of the present disclosure are identical. The transmembrane domain can comprise, consist essential of, or consist of: a CD2 transmembrane domain or a portion thereof or the transmembrane domain can comprise, consist essential of, or consist of: a CD28 transmembrane domain or a portion thereof. The CD2 transmembrane domain comprises, consists essential of, or consists of the amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 17112. The CD2 transmembrane domain comprises, consists essential of, or consists of the amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO: 17112. The CD2 transmembrane domain comprises, consists essential of, or consists of the amino acid sequence of SEQ ID NO: 17112. The CD28 transmembrane domain comprises, consists essential of, or consists of the amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 17100. The CD28 transmembrane domain comprises, consists essential of, or consists of the amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO: 17100. The CD28 transmembrane domain comprises, consists essential of, or consists of the amino acid sequence of SEQ ID NO: 17100.

[087] In some aspects, the activation component of the CSR of the present disclosure does not bind or is incapable of binding a naturally-occurring molecule. In some aspects, the activation component of the CSR of the present disclosure binds or is capable of binding a naturally-occurring molecule and the CSR transduces a signal upon binding of the activation component to the naturally-occuring molecule. In other aspects, the activation component of the CSR of the

present disclosure can bind a naturally-occurring molecule but the CSR does not transduce a signal upon binding of the activation component to a naturally-occurring molecule. In preferred aspects, the activation component of the CSR of the present disclosure binds or is capable of binding to a non-naturally occurring molecule. The activation component of the CSR of the present disclosure selectively transduces a signal upon binding of a non-naturally occurring molecule to the activation component. In one aspect, the naturally occurring molecule is an naturally occurring agonist/activating agent for the activation component of the CSR. The naturally occurring agonist/activating agent that can bind a CSR activation component can be any naturally occurring antibody or antibody fragment. The naturally occurring antibody or antibody fragment can be a naturally occurring anti-CD3 antibody or fragment thereof, an anti-CD2 antibody or fragment thereof, an anti-CD28 antibody or fragment thereof, or any combination thereof. In some aspects, the naturally occurring agonist/activating agent that can bind a CSR activation component can be one or more of an anti-human CD3 monospecific tetrameric antibody complex, an anti-human CD2 monospecific tetrameric antibody complex, an anti-human CD28 monospecific tetrameric antibody complex, or a combination thereof. In one aspect, the non-naturally occurring molecule is an non-naturally occurring agonist/activating agent for the activation component of the CSR. The non-naturally occurring agonist/activating agent that can bind a CSR activation component can be any non-naturally occurring antibody or antibody fragment. The non-naturally occurring antibody or antibody fragment can be a non-naturally occurring anti-CD3 antibody or fragment thereof, an anti-CD2 antibody or fragment thereof, an anti-CD28 antibody or fragment thereof, or any combination thereof. In some aspects, the non-naturally occurring agonist/activating agent that can bind a CSR activation component can be one or more of an anti-human CD3 monospecific tetrameric antibody complex, an anti-human CD2 monospecific tetrameric antibody complex, an anti-human CD28 monospecific tetrameric antibody complex, or a combination thereof. In some aspects, the non-naturally occurring agonist/activating agent that can bind a CSR activation component can be selected from the group consisting of anti-CD2 monoclonal antibody, BTI-322 (Przepiorka et al., Blood 92(11):4066-4071, 1998) and humanized anti-CD2 monoclonal antibody clone AFC-TAB-104 (Siplizumab)(Bissonnette et al. Arch. Dermatol. Res. 301(6):429-442, 2009).

[088] In some aspects, the ectodomain of the CSR of the present disclosure can comprise a modification. The modification can comprise a mutation or a truncation in the amino acid sequence of the activation component or the first protein when compared to a wild type amino acid sequence of the activation component or the first protein. The mutation or a truncation in the amino acid sequence of the activation component or the first protein can comprise a mutation or truncation of a CD2 extracellular domain or a portion thereof to which an agonist binds. The mutation or truncation of the CD2 extracellular domain reduces or eliminates binding with naturally occurring CD58.

[089] A reduction in binding is when at least 50%, at least 75%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the binding ability of the mutated or truncated CD2 extracellular domain is reduced when compared to the naturally occurring wild-type counterpart. An elimination in binding is when 100% of the binding ability of the mutated or truncated CD2 extracellular domain is reduced when compared to the naturally occurring wild-type CD2 extracellular domain.

[090] The mutated or truncated CD2 extracellular domain binds anti-CD2 activating agonists and anti-CD2 activating molecules but does not bind naturally occurring CD58. The mutated or truncated CD2 extracellular domain comprises, consists essential of, or consists of the amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NO: 17119. The mutated or truncated CD2 extracellular domain comprises, consists essential of, or consists of the amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO: 17119. The mutated or truncated CD2 extracellular domain comprises, consists essential of, or consists of the amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO: 17119. The mutated or truncated CD2 extracellular domain comprises, consists essential of, or consists of the amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 17119. The mutated or truncated CD2 extracellular domain comprises, consists essential of, or consists of the amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO: 17119. The mutated or truncated CD2 extracellular domain comprises, consists essential of, or consists of the amino acid sequence of SEQ ID NO: 17119 . The CSR comprising the mutated or truncated CD2 extracellular domain comprises, consists essential of, or consists of the amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:

17118. The CSR comprising the mutated or truncated CD2 extracellular domain comprises, consists essential of, or consists of the amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 17118. The CSR comprising the mutated or truncated CD2 extracellular domain comprises, consists essential of, or consists of the amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO: 17118. The CSR comprising the mutated or truncated CD2 extracellular domain comprises, consists essential of, or consists of the amino acid sequence of SEQ ID NO: 17118.

[091] The present disclosure also provides a non-naturally occurring chimeric stimulatory receptor (CSR) comprising, consisting essential of, or consisting of: (a) an ectodomain comprising a activation component, wherein the activation component is isolated or derived from a first protein and wherein the activation component binds to a non-naturally occurring molecule but does not bind a naturally-occurring molecule; (b) a transmembrane domain; and (c) an endodomain comprising at least one signal transduction domain, wherein the at least one signal transduction domain is isolated or derived from a second protein; wherein the first protein and the second protein are not identical.

[092] The present disclosure also provides a non-naturally occurring chimeric stimulatory receptor (CSR) comprising, consisting essential of, or consisting of: (a) an ectodomain comprising a activation component, wherein the activation component is isolated or derived from a first protein; (b) a transmembrane domain; and (c) an endodomain comprising at least one signal transduction domain, wherein the at least one signal transduction domain is isolated or derived from a second protein; wherein the first protein and the second protein are not identical and wherein the CSR does not transduce a signal upon binding of a naturally-occurring molecule to the activation component.

[093] The present disclosure also provides a non-naturally occurring chimeric stimulatory receptor (CSR) comprising, consisting essential of, or consisting of: (a) an ectodomain comprising a activation component, wherein the activation component is isolated or derived from a first protein; (b) a transmembrane domain; and (c) an endodomain comprising at least one signal transduction domain, wherein the at least one signal transduction domain is isolated or derived from a second protein; wherein the first protein and the second protein are not identical

and wherein the CSR transduces a signal upon binding of a non-naturally-occurring molecule to the activation component.

[094] The present disclosure also provides a non-naturally occurring chimeric stimulatory receptor (CSR) comprising, consisting essential of, or consisting of: (a) an ectodomain comprising a signal peptide and an activation component, wherein the signal peptide comprises a CD2 signal peptide or a portion thereof and wherein the activation component comprises a CD2 extracellular domain or a portion thereof to which an agonist binds; (b) a transmembrane domain, wherein the transmembrane domain comprises a CD2 transmembrane domain or a portion thereof; and (c) an endodomain comprising a cytoplasmic domain and at least one signal transduction domain, wherein the cytoplasmic domain comprises a CD2 cytoplasmic domain or a portion thereof and wherein the at least one signal transduction domain comprises a CD3ζ protein or a portion thereof.

[095] The present disclosure also provides a non-naturally occurring chimeric stimulatory receptor (CSR) comprising, consisting essential of, or consisting of: (a) an ectodomain comprising a signal peptide comprising the amino acid sequence of SEQ ID NO: 17110 and an activation component comprising the amino acid sequence of SEQ ID NO: 17111; (b) a transmembrane domain of SEQ ID NO: 17112; and (c) an endodomain comprising a cytoplasmic domain comprising the amino acid sequence of SEQ ID NO: 17113 and at least one signal transduction domain comprising the amino acid sequence of SEQ ID NO: 17102. The non-naturally occurring chimeric stimulatory receptor (CSR) can comprise, consist essential of, or consist of an amino acid sequence at least 80% identical to SEQ ID NO:17062. The non-naturally occurring chimeric stimulatory receptor (CSR) can comprise, consist essential of, or consist of an amino acid sequence at least 85% identical to SEQ ID NO:17062. The non-naturally occurring chimeric stimulatory receptor (CSR) can comprise, consist essential of, or consist of an amino acid sequence at least 90% identical to SEQ ID NO:17062. The non-naturally occurring chimeric stimulatory receptor (CSR) can comprise, consist essential of, or consist of an amino acid sequence at least 95% identical to SEQ ID NO:17062. The non-naturally occurring chimeric stimulatory receptor (CSR) can comprise, consist essential of, or consist of an amino acid sequence at least 99% identical to SEQ ID NO:17062. The non-

naturally occurring chimeric stimulatory receptor (CSR) can comprise, consist essential of, or consist of an amino acid sequence of SEQ ID NO:17062.

[096] The present disclosure further provides a non-naturally occurring chimeric stimulatory receptor (CSR) comprising, consisting essential of, or consisting of: (a) an ectodomain comprising a signal peptide and an activation component, wherein the signal peptide comprises a CD2 signal peptide or a portion thereof and wherein the activation component comprises a mutation or truncation of a wild-type CD2 extracellular domain or a portion thereof to which an agonist binds; (b) a transmembrane domain, wherein the transmembrane domain comprises a CD2 transmembrane domain or a portion thereof; and (c) an endodomain comprising a cytoplasmic domain and at least one signal transduction domain, wherein the cytoplasmic domain comprises a CD2 cytoplasmic domain or a portion thereof and wherein the at least one signal transduction domain comprises a CD3ζ protein or a portion thereof. In one aspect, the mutation or truncation of the CD2 extracellular domain reduces or eliminates binding with naturally occurring CD58. In another aspect, the mutated or truncated CD2 extracellular domain binds anti-CD2 activating agonists and anti-CD2 activating molecules but does not bind naturally occurring CD58.

[097] The present disclosure further provides a non-naturally occurring chimeric stimulatory receptor (CSR) comprising, consisting essential of, or consisting of: (a) an ectodomain comprising a signal peptide comprising the amino acid sequence of SEQ ID NO: 17110 and a activation component comprising the amino acid sequence of SEQ ID NO: 17119; (b) a transmembrane domain of SEQ ID NO: 17112; and (c) an endodomain comprising a cytoplasmic domain comprising the amino acid sequence of SEQ ID NO: 17113 and at least one signal transduction domain comprising the amino acid sequence of SEQ ID NO: 17102. The non-naturally occurring chimeric stimulatory receptor (CSR) can comprise, consist essential of, or consist of an amino acid sequence at least 80% identical to SEQ ID NO: 17118. The non-naturally occurring chimeric stimulatory receptor (CSR) can comprise, consist essential of, or consist of an amino acid sequence at least 85% identical to SEQ ID NO: 17118. The non-naturally occurring chimeric stimulatory receptor (CSR) can comprise, consist essential of, or consist of an amino acid sequence at least 90% identical to SEQ ID NO: 17118. The non-naturally occurring chimeric stimulatory receptor (CSR) can comprise, consist essential of, or

consist of an acid sequence at least 95% identical to SEQ ID NO: 17118. The non-naturally occurring chimeric stimulatory receptor (CSR) can comprise, consist essential of, or consist of an acid sequence at least 99% identical to SEQ ID NO: 17118. The non-naturally occurring chimeric stimulatory receptor (CSR) can comprise, consist essential of, or consist of an acid sequence of SEQ ID NO: 17118.

[098] The present disclosure also provides a nucleic acid sequence encoding an amino acid sequence of any chimeric stimulatory receptor (CSR) disclosed herein. The present disclosure also provides transposon, a vector, a donor sequence or a donor plasmid comprising, consisting essential of or consisting of a nucleic acid sequence encoding the amino acid sequence of any chimeric stimulatory receptor (CSR) disclosed herein. In one aspect, the vector can be a viral vector. In one aspect, a viral vector can be an an adenoviral vector, adeno-associated viral (AAV) vector, retroviral vector, lentiviral vector or a chimeric viral vector.

[099] The present disclosure also provides a cell comprising, consisting essential of or consisting of any chimeric stimulatory receptor (CSR) disclosed herein. The present disclosure also provides a cell comprising, consisting essential of or consisting of a nucleic acid sequence encoding an amino acid sequence of any chimeric stimulatory receptor (CSR) disclosed herein. The present disclosure also provides a cell comprising, consisting essential of or consisting of a transposon, a vector, a donor sequence or a donor plasmid comprising, consisting essential of or consisting of a nucleic acid sequence encoding the amino acid sequence of any chimeric stimulatory receptor (CSR) disclosed herein. In one aspect, the vector can be a viral vector. In one aspect, a viral vector can be an an adenoviral vector, adeno-associated viral (AAV) vector, retroviral vector, lentiviral vector or a chimeric viral vector. A cell of the present disclosure comprising, consisting essential of or consisting of any chimeric stimulatory receptor (CSR) disclosed herein can be an allogeneic cell or an autologous cell. In some preferred embodiments, the cell is an allogeneic cell.

[0100] The present disclosure also provides a composition comprising, consisting essential of or consisting of any chimeric stimulatory receptor (CSR) disclosed herein. The present disclosure also provides a composition comprising, consisting essential of or consisting of a nucleic acid sequence encoding an amino acid sequence of any chimeric stimulatory receptor (CSR) disclosed herein. The present disclosure also provides a composition comprising,

consisting essential of or consisting of a transposon, a vector, a donor sequence or a donor plasmid comprising, consisting essential of or consisting of a nucleic acid sequence encoding the amino acid sequence of any chimeric stimulatory receptor (CSR) disclosed herein. In one aspect, the vector can be a viral vector. In one aspect, a viral vector can be an an adenoviral vector, adeno-associated viral (AAV) vector, retroviral vector, lentiviral vector or a chimeric viral vector. The present disclosure also provides a composition comprising, consisting essential of or consisting of a cell or a plurality of cells comprising, consisting essential of or consisting of any chimeric stimulatory receptor (CSR) disclosed herein.

[0101] The present disclosure provides a modified cell comprising, consisting essential of, or consisting of a chimeric stimulatory receptor (CSR) comprising, consisting essential of, or consisting of: (i) an ectodomain comprising an activation component, wherein the activation component is isolated or derived from a first protein; (ii) a transmembrane domain; and (iii) an endodomain comprising at least one signal transduction domain, wherein the at least one signal transduction domain is isolated or derived from a second protein; wherein the first protein and the second protein are not identical.

[0102] The present disclosure also provides a modified cell comprising, consisting essential of, or consisting of (a) a chimeric stimulatory receptor (CSR) comprising: (i) an ectodomain comprising an activation component, wherein the activation component is isolated or derived from a first protein; (ii) a transmembrane domain; and (iii) an endodomain comprising at least one signal transduction domain, wherein the at least one signal transduction domain is isolated or derived from a second protein; wherein the first protein and the second protein are not identical; and (b) an inducible proapoptotic polypeptide.

[0103] The present disclosure also provides a modified cell comprising, consisting essential of, or consisting of: (a) a chimeric stimulatory receptor (CSR) comprising: (i) an ectodomain comprising an activation component, wherein the activation component is isolated or derived from a first protein; (ii) a transmembrane domain; and (iii) an endodomain comprising at least one signal transduction domain, wherein the at least one signal transduction domain is isolated or derived from a second protein; wherein the first protein and the second protein are not identical; (b) a sequence encoding an inducible proapoptotic polypeptide; and wherein the cell is a T-cell,

(c) a modification of an endogenous sequence encoding a T-cell Receptor (TCR), wherein the modification reduces or eliminates a level of expression or activity of the TCR.

[0104] The present disclosure provides a modified cell comprising, consisting essential of, or consisting of: (a) a modification of an endogenous sequence encoding Beta-2-Microglobulin (B2M), wherein the modification reduces or eliminates a level of expression or activity of a major histocompatibility complex (MHC) class I (MHC-I); and (b) a non-naturally occurring sequence comprising an HLA class I histocompatibility antigen, alpha chain E (HLA-E) polypeptide.

[0105] The present disclosure provides a modified T lymphocyte (T-cell) comprising, consisting essential of, or consisting of: (a) a modification of an endogenous sequence encoding a T-cell Receptor (TCR), wherein the modification reduces or eliminates a level of expression or activity of the TCR; and (b) chimeric stimulatory receptor (CSR) comprising: (i) an ectodomain comprising an activation component, wherein the activation component is isolated or derived from a first protein; (ii) a transmembrane domain; and (iii) an endodomain comprising at least one signal transduction domain, wherein the at least one signal transduction domain is isolated or derived from a second protein; wherein the first protein and the second protein are not identical.

[0106] The present disclosure provides a modified T lymphocyte (T-cell) comprising, consisting essential of, or consisting of: (a) a modification of an endogenous sequence encoding a T-cell Receptor (TCR), wherein the modification reduces or eliminates a level of expression or activity of the TCR; (b) chimeric stimulatory receptor (CSR) comprising: (i) an ectodomain comprising an activation component, wherein the activation component is isolated or derived from a first protein; (ii) a transmembrane domain; and (iii) an endodomain comprising at least one signal transduction domain, wherein the at least one signal transduction domain is isolated or derived from a second protein; wherein the first protein and the second protein are not identical; and (c) a non-naturally occurring chimeric antigen receptor.

[0107] The present disclosure provides a modified T lymphocyte (T-cell) comprising, consisting essential of, or consisting of: (a) a modification of an endogenous sequence encoding a T-cell Receptor (TCR), wherein the modification reduces or eliminates a level of expression or activity of the TCR; (b) a modification of an endogenous sequence encoding Beta-2-Microglobulin (B2M), wherein the modification reduces or eliminates a level of expression or

activity of a major histocompatibility complex (MHC) class I (MHC-I); and (c) a chimeric stimulatory receptor (CSR) comprising: (i) an ectodomain comprising an activation component, wherein the activation component is isolated or derived from a first protein; (ii) a transmembrane domain; and (iii) an endodomain comprising at least one signal transduction domain, wherein the at least one signal transduction domain is isolated or derived from a second protein; wherein the first protein and the second protein are not identical.

[0108] The present disclosure provides a modified T lymphocyte (T-cell) comprising, consisting essential of, or consisting of: (a) a modification of an endogenous sequence encoding a T-cell Receptor (TCR), wherein the modification reduces or eliminates a level of expression or activity of the TCR; (b) a modification of an endogenous sequence encoding Beta-2-Microglobulin (B2M), wherein the modification reduces or eliminates a level of expression or activity of a major histocompatibility complex (MHC) class I (MHC-I); (c) a chimeric stimulatory receptor (CSR) comprising: (i) an ectodomain comprising an activation component, wherein the activation component is isolated or derived from a first protein; (ii) a transmembrane domain; and (iii) an endodomain comprising at least one signal transduction domain, wherein the at least one signal transduction domain is isolated or derived from a second protein; wherein the first protein and the second protein are not identical; and (d) a non-naturally occurring chimeric antigen receptor.

[0109] The present disclosure also provides a modified T lymphocyte (T-cell) comprising, consisting essential of, or consisting of: (a) a modification of an endogenous sequence encoding a T-cell Receptor (TCR), wherein the modification reduces or eliminates a level of expression or activity of the TCR; (b) a modification of an endogenous sequence encoding Beta-2-Microglobulin (B2M), wherein the modification reduces or eliminates a level of expression or activity of a major histocompatibility complex (MHC) class I (MHC-I); (c) a non-naturally occurring sequence comprising an HLA class I histocompatibility antigen, alpha chain E (HLA-E); and (d) a chimeric stimulatory receptor (CSR) comprising: (i) an ectodomain comprising an activation component, wherein the activation component is isolated or derived from a first protein; (ii) a transmembrane domain; and (iii) an endodomain comprising at least one signal transduction domain, wherein the at least one signal transduction domain is isolated or derived from a second protein; wherein the first protein and the second protein are not identical.

[0110] The present disclosure also provides a modified T lymphocyte (T-cell) comprising, consisting essential of, or consisting of: (a) a modification of an endogenous sequence encoding a T-cell Receptor (TCR), wherein the modification reduces or eliminates a level of expression or activity of the TCR; (b) a modification of an endogenous sequence encoding Beta-2-Microglobulin (B2M), wherein the modification reduces or eliminates a level of expression or activity of a major histocompatibility complex (MHC) class I (MHC-I); (c) a non-naturally occurring sequence comprising an HLA class I histocompatibility antigen, alpha chain E (HLA-E); (d) a chimeric stimulatory receptor (CSR) comprising: (i) an ectodomain comprising an activation component, wherein the activation component is isolated or derived from a first protein; (ii) a transmembrane domain; and (iii) an endodomain comprising at least one signal transduction domain, wherein the at least one signal transduction domain is isolated or derived from a second protein; wherein the first protein and the second protein are not identical; and (e) a non-naturally occurring chimeric antigen receptor.

[0111] The present disclosure also provides a modified T lymphocyte (T-cell), consisting essential of, or consisting of: (a) a modification of an endogenous sequence encoding a T-cell Receptor (TCR), wherein the modification reduces or eliminates a level of expression or activity of the TCR; (b) a modification that reduces or eliminates a level of expression or activity of a HLA class I histocompatibility antigen, alpha chain A (HLA-A), HLA class I histocompatibility antigen, alpha chain B (HLA-B), HLA class I histocompatibility antigen, alpha chain C (HLA-C), or a combination thereof; and (c) a chimeric stimulatory receptor (CSR) comprising: (i) an ectodomain comprising an activation component, wherein the activation component is isolated or derived from a first protein; (ii) a transmembrane domain; and (iii) an endodomain comprising at least one signal transduction domain, wherein the at least one signal transduction domain is isolated or derived from a second protein; wherein the first protein and the second protein are not identical.

[0112] The present disclosure also provides a modified T lymphocyte (T-cell), consisting essential of, or consisting of: (a) a modification of an endogenous sequence encoding a T-cell Receptor (TCR), wherein the modification reduces or eliminates a level of expression or activity of the TCR; (b) a modification that reduces or eliminates a level of expression or activity of a HLA class I histocompatibility antigen, alpha chain A (HLA-A), HLA class I histocompatibility

antigen, alpha chain B (HLA-B), HLA class I histocompatibility antigen, alpha chain C (HLA-C), or a combination thereof; (c) a non-naturally occurring sequence comprising an HLA class I histocompatibility antigen, alpha chain E (HLA-E); and (d) a chimeric stimulatory receptor (CSR) comprising: (i) an ectodomain comprising an activation component, wherein the activation component is isolated or derived from a first protein; (ii) a transmembrane domain; and (iii) an endodomain comprising at least one signal transduction domain, wherein the at least one signal transduction domain is isolated or derived from a second protein; wherein the first protein and the second protein are not identical.

[0113] A modified cell of the present disclosure (preferably a modified T-cell of the present disclosure) can further comprise, consist essential of, or consist of an inducible proapoptotic polypeptide. The inducible proapoptotic polypeptide comprises, consists essential of, or consists of the amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 14641. The inducible proapoptotic polypeptide comprises, consists essential of, or consists of the amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO: 14641. The inducible proapoptotic polypeptide comprises, consists essential of, or consists of the amino acid sequence of SEQ ID NO: 14641.

[0114] A modified cell of the present disclosure (preferably a modified T-cell of the present disclosure) can further comprise, consist essential of, or consist of a modification of an endogenous sequence encoding Beta-2-Microglobulin (B2M), wherein the modification reduces or eliminates a level of expression or activity of a major histocompatibility complex (MHC) class I (MHC-I). A reduction of a level of expression or activity is when at least 50%, at least 75%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the expression of the MHC-I in a cell or the functional activity of the MHC-I in a cell is reduced when compared to the naturally occurring wild-type counterpart of the cell. A reduction of a level of expression or activity is when at least 50%, at least 75%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the expression of the MHC-I in a T-cell or the functional activity of the MHC-I in a T-cell is reduced when compared to a naturally occurring wild-type T-cell. An elimination a level of expression or activity is when 100% of the expression of the MHC-I in a cell or the functional activity of the MHC-I in a cell is

reduced when compared to the naturally occurring wild-type counterpart of the cell. An elimination a level of expression or activity is when 100% of the expression of the MHC-I in a T-cell or the functional activity of the MHC-I in a T-cell is reduced when compared to the naturally occurring wild-type T-cell.

[0115] A modified cell of the present disclosure (preferably a modified T-cell of the present disclosure) can further comprise, consist essential of, or consist of a non-naturally occurring polypeptide comprising an HLA class I histocompatibility antigen, alpha chain E (HLA-E). The HLA-E polypeptide comprises, consists essential of, or consists of the amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 17131. The HLA-E polypeptide comprises, consists essential of, or consists of the amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO: 17131. The HLA-E polypeptide comprises, consists essential of, or consists of the amino acid sequence of SEQ ID NO: 17131.

[0116] The non-naturally occurring polypeptide comprising a HLA-E can further comprise, consist essential of, or consist of a B2M signal peptide. The B2M signal peptide comprises, consists essential of, or consists of the amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 17126. The B2M signal peptide comprises, consists essential of, or consists of the amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO: 17131. The B2M signal peptide comprises, consists essential of, or consists of the amino acid sequence of SEQ ID NO: 17131.

[0117] The non-naturally occurring polypeptide comprising a HLA-E can further comprise, consist essential of, or consist of a B2M polypeptide. The B2M polypeptide comprises, consists essential of, or consists of the amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 17129. The B2M polypeptide comprises, consists essential of, or consists of the amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO: 17129. The B2M polypeptide comprises, consists essential of, or consists of the amino acid sequence of SEQ ID NO: 17129.

[0118] The non-naturally occurring polypeptide comprising a HLA-E can further comprise, consist essential of, or consist of a linker molecule (referred to herein as a linker). The non-naturally occurring polypeptide comprising a HLA-E can further comprise, consist essential of, or consist of a linker, wherein the linker is positioned between the B2M polypeptide and the

HLA-E polypeptide. The linker comprises, consists essential of, or consists of the amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 17130. The linker comprises, consists essential of, or consists of the amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO: 17130. The linker comprises, consists essential of, or consists of the amino acid sequence of SEQ ID NO: 17130.

[0119] The non-naturally occurring polypeptide comprising a HLA-E can further comprise, consist essential of, or consist of a peptide and a B2M polypeptide. The peptide comprises, consists essential of, or consists of the amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 17127. The peptide comprises, consists essential of, or consists of the amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO: 17127. The peptide comprises, consists essential of, or consists of the amino acid sequence of SEQ ID NO: 17127.

[0120] The non-naturally occurring polypeptide comprising a HLA-E can further comprise, consist essential of, or consist of a first linker positioned between the B2M signal peptide and the peptide, and a second linker positioned between the B2M polypeptide and the HLA-E polypeptide. The first linker comprises, consists essential of, or consists of the amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 17128. The first linker comprises, consists essential of, or consists of the amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO: 17128. The first linker comprises, consists essential of, or consists of the amino acid sequence of SEQ ID NO: 17128. The second linker comprises, consists essential of, or consists of the amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 17130. The second linker comprises, consists essential of, or consists of the amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO: 17130. The second linker comprises, consists essential of, or consists of the amino acid sequence of SEQ ID NO: 17130.

[0121] In one aspect, the non-naturally occurring polypeptide comprising an HLA-E comprises, consists essential of, or consists of a B2M signal peptide, a peptide, a first linker, a B2M polypeptide, a second linker and an HLA-E polypeptide. The peptide can be positioned between the B2M signal peptide and the first linker, the B2M polypeptide can be positioned between the first linker and the second linker and the second linker can be positioned between

the B2M polypeptide and the HLA-E polypeptide. The non-naturally occurring polypeptide comprising an HLA-E comprises, consists essential of, or consists of the amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 17064. The non-naturally occurring polypeptide comprising an HLA-E comprises, consists essential of, or consists of the amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO: 17064. The non-naturally occurring polypeptide comprising an HLA-E comprises, consists essential of, or consists of the amino acid sequence of SEQ ID NO: 17064. The non-naturally occurring polypeptide comprising an HLA-E can be encoded by the nucleic acid have the sequence of SEQ ID NO: 17065.

[0122] In one aspect, the non-naturally occurring polypeptide comprising an HLA-E comprises, consists essential of, or consists of a B2M signal peptide, a B2M polypeptide, a linker and an HLA-E polypeptide. The B2M polypeptide can be positioned between the B2M signal peptide and the linker, the linker can be positioned between the B2M polypeptide and the HLA-E polypeptide. The non-naturally occurring polypeptide comprising an HLA-E comprises, consists essential of, or consists of the amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 17066. The non-naturally occurring polypeptide comprising an HLA-E comprises, consists essential of, or consists of the amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO: 17066. The non-naturally occurring polypeptide comprising an HLA-E comprises, consists essential of, or consists of the amino acid sequence of SEQ ID NO: 17066. The non-naturally occurring polypeptide comprising an HLA-E can be encoded by the nucleic acid have the sequence of SEQ ID NO: 17067.

[0123] In one aspect, the non-naturally occurring polypeptide comprising an HLA-E comprises, consists essential of, or consists of a B2M signal peptide and an HLA-E polypeptide. The B2M signal peptide can be positioned before (e.g. 5' in the context of a nucleic acid sequence or amino terminus in the context of an amino acid sequence) HLA-E polypeptide. The non-naturally occurring polypeptide comprising an HLA-E comprises, consists essential of, or consists of the amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 17068. The non-naturally occurring polypeptide comprising an HLA-E comprises, consists essential of, or consists of the amino acid sequence at least 99% identical to the amino acid sequence of SEQ ID NO: 17068. The non-naturally occurring polypeptide comprising an HLA-

E comprises, consists essential of, or consists of the amino acid sequence of SEQ ID NO: 17068. The non-naturally occurring polypeptide comprising an HLA-E can be encoded by the nucleic acid have the sequence of SEQ ID NO: 17069.

[0124] A modified cell of the present disclosure (preferably a modified T-cell of the present disclosure) can further comprise, consist essential of, or consist of a non-naturally occurring antigen receptor, a sequence encoding a therapeutic polypeptide, or a combination thereof. In a preferred aspect, the non-naturally occurring antigen receptor comprises, consists essential of or consists of a chimeric antigen receptor (CAR). The CAR comprise, consist essential of, or consist of (a) an ectodomain comprising an antigen recognition region, (b) a transmembrane domain, and (c) an endodomain comprising at least one costimulatory domain. The ectodomain of the CAR can further comprise, consist essential of, or consist of a signal peptide. The ectodomain of the CAR can further comprise, consist essential of, or consist of a hinge between the antigen recognition region and the transmembrane domain. The endodomain of the CAR can further comprise, consist essential of, or consist of a human CD3ζ endodomain. The at least one costimulatory domain of the CAR can further comprise, consist essential of, or consist of a human 4-1BB, CD28, CD40, ICOS, MyD88, OX-40 intracellular segment, or any combination thereof. In a preferred aspect, at least one costimulatory domain comprises a human CD28 and/or a 4-1BB costimulatory domain.

[0125] A modified cell of the present disclosure can be an immune cell or an immune cell precursor. The immune cell can be a lymphoid progenitor cell, a natural killer (NK) cell, a cytokine induced killer (CIK) cell, a T lymphocyte (T-cell), a B lymphocyte (B-cell) or an antigen presenting cell (APC). In preferred aspects, the immune cell is a T cell, an early memory T cell, a stem cell-like T cell, a stem memory T cell (T_{SCM}), a central memory T cell (T_{CM}) or a stem cell-like T cell. The immune cell precursor can a hematopoietic stem cell (HSC). The modified cell can be a stem cell, a differentiated cell, a somatic cell or an antigen presenting cell (APC). The modified cell can be an autologous cell or an allogeneic cell. In one aspect, the cell is a modified allogeneic T-cell. In another aspect, the cell is modified allogeneic T-cell expressing a chimeric antigen receptor (CAR), a CAR T-cell.

[0126] A modified cell of the present disclosure (preferably a modified T-cell of the present disclosure) can express a CSR of the present disclosure transiently or stably. In one aspect, a

CSR of the present disclosure is transiently expressed in a modified cell of the present disclosure (preferably a modified T-cell of the present disclosure). In one aspect, a CSR of the present disclosure is stably expressed in a modified cell of the present disclosure (preferably a modified T-cell of the present disclosure).

[0127] A modified cell of the present disclosure (preferably a modified T-cell of the present disclosure) can express a non-naturally occurring polypeptide comprising the HLA-E of the present disclosure transiently or stably. In one aspect, a non-naturally occurring polypeptide comprising the HLA-E of the present disclosure is transiently expressed in a modified cell of the present disclosure (preferably a modified T-cell of the present disclosure). In one aspect, a non-naturally occurring polypeptide comprising the HLA-E of the present disclosure is stably expressed in a modified cell of the present disclosure (preferably a modified T-cell of the present disclosure).

[0128] A modified cell of the present disclosure (preferably a modified T-cell of the present disclosure) can express an inducible proapoptotic polypeptide of the present disclosure transiently or stably. In one aspect, an inducible proapoptotic polypeptide of the present disclosure is transiently expressed in a modified cell of the present disclosure (preferably a modified T-cell of the present disclosure). In a preferred aspect, an inducible proapoptotic polypeptide of the present disclosure is stably expressed in a modified cell of the present disclosure (preferably a modified T-cell of the present disclosure).

[0129] A modified cell of the present disclosure (preferably a modified T-cell of the present disclosure) can express a non-naturally occurring antigen receptor or a sequence encoding a therapeutic protein of the present disclosure transiently or stably. In one aspect, a non-naturally occurring antigen receptor or a sequence encoding a therapeutic protein of the present disclosure is transiently expressed in a modified cell of the present disclosure (preferably a modified T-cell of the present disclosure). In a preferred aspect, a non-naturally occurring antigen receptor or a sequence encoding a therapeutic protein of the present disclosure is stably expressed in a modified cell of the present disclosure (preferably a modified T-cell of the present disclosure).

[0130] In one aspect, a CSR of the present disclosure is stably expressed, the inducible proapoptotic polypeptide of the present disclosure is stably expressed and a non-naturally

occurring antigen receptor or a sequence encoding a therapeutic protein is stably expressed in a modified cell of the present disclosure (preferably a modified T-cell of the present disclosure).

[0131] In one aspect, a CSR of the present disclosure is stably expressed, a non-naturally occurring polypeptide comprising the HLA-E of the present disclosure is stably expressed, the inducible proapoptotic polypeptide of the present disclosure is stably expressed and a non-naturally occurring antigen receptor or a sequence encoding a therapeutic protein is stably expressed in a modified cell of the present disclosure (preferably a modified T-cell of the present disclosure).

[0132] In one aspect, a CSR of the present disclosure is stably expressed, a non-naturally occurring polypeptide comprising the HLA-E of the present disclosure is transiently expressed, the inducible proapoptotic polypeptide of the present disclosure is stably expressed and a non-naturally occurring antigen receptor or a sequence encoding a therapeutic protein is stably expressed in a modified cell of the present disclosure (preferably a modified T-cell of the present disclosure).

[0133] In one aspect, a CSR of the present disclosure is transiently expressed, the inducible proapoptotic polypeptide of the present disclosure is stably expressed and the non-naturally occurring antigen receptor or a sequence encoding a therapeutic protein is stably expressed in a modified cell of the present disclosure (preferably a modified T-cell of the present disclosure).

[0134] In one aspect, a CSR of the present disclosure is transiently expressed, a non-naturally occurring polypeptide comprising the HLA-E of the present disclosure is transiently expressed, the inducible proapoptotic polypeptide of the present disclosure is stably expressed and a non-naturally occurring antigen receptor or a sequence encoding a therapeutic protein is stably expressed in a modified cell of the present disclosure (preferably a modified T-cell of the present disclosure).

[0135] In one aspect, a CSR of the present disclosure is transiently expressed, a non-naturally occurring polypeptide comprising the HLA-E of the present disclosure is stably expressed, the inducible proapoptotic polypeptide of the present disclosure is stably expressed and a non-naturally occurring antigen receptor or a sequence encoding a therapeutic protein is stably expressed in a modified cell of the present disclosure (preferably a modified T-cell of the present disclosure).

[0136] The present disclosure provides a modified cell (preferably a modified T-cell comprising, consisting essential of, or consisting of (a) a modification of an endogenous sequence encoding a T-cell Receptor (TCR), wherein the modification reduces or eliminates a level of expression or activity of the TCR; and (b) a sequence encoding a chimeric stimulatory receptor (CSR) comprising: (i) an ectodomain comprising an activation component, wherein the activation component is isolated or derived from a first protein; (ii) a transmembrane domain; and (iii) an endodomain comprising at least one signal transduction domain, wherein the at least one signal transduction domain is isolated or derived from a second protein; wherein the first protein and the second protein are not identical.

[0137] The modified cell further can further comprise, consist essential of or consist of a sequence encoding an inducible proapoptotic polypeptide. The modified cell can further comprise, consist essential of or consist of a sequence encoding a non-naturally occurring antigen receptor, a sequence encoding a therapeutic polypeptide, or a combination thereof. The non-naturally occurring antigen receptor can comprise, consist essential of or consist of a chimeric antigen receptor (CAR).

[0138] A transposon, a vector, a donor sequence or a donor plasmid can comprise, consist essential of or consist of the sequence encoding the CSR, the sequence encoding the inducible proapoptotic polypeptide, or a combination thereof. The transposon, the vector, the donor sequence or the donor plasmid can further comprise, consist essential of or consist of a sequence encoding a non-naturally occurring antigen receptor or a sequence encoding a therapeutic protein. The transposon, the vector, the donor sequence, or the donor plasmid can further comprise, consist essential of or consist of a sequence encoding a selection marker. The transposon can be a piggyBac® transposon, a piggy-Bac® like transposon, a Sleeping Beauty transposon, a Helraiser transposon, a Tol2 transposon or a TcBuster transposon. The sequence encoding the CSR can be transiently expressed in the cell. The sequence encoding the CSR can be stably expressed in the cell. The sequence encoding the inducible proapoptotic polypeptide can be stably expressed in the cell. The sequence encoding a non-naturally occurring antigen receptor or a sequence encoding a therapeutic protein is stably expressed in the cell. In some aspects, the sequence encoding the CSR can be transiently expressed in the cell and the sequence encoding the inducible proapoptotic polypeptide can be stably expressed in the cell. In some

aspects, the sequence encoding the CSR can be stably expressed in the cell and the sequence encoding the inducible proapoptotic polypeptide can be stably expressed in the cell. In some aspects, the sequence encoding the CSR can be transiently expressed in the cell, the sequence encoding the inducible proapoptotic polypeptide can be stably expressed in the cell and sequence encoding a non-naturally occurring antigen receptor or a sequence encoding a therapeutic protein is stably expressed in the cell. In some aspects, the sequence encoding the CSR can be stably expressed in the cell, the sequence encoding the inducible proapoptotic polypeptide can be stably expressed in the cell and sequence encoding a non-naturally occurring antigen receptor or a sequence encoding a therapeutic protein is stably expressed in the cell. In one aspect, the vector can be a viral vector. In one aspect, a viral vector can be an an adenoviral vector, adeno-associated viral (AAV) vector, retroviral vector, lentiviral vector or a chimeric viral vector.

[0139] A first transposon, a first vector, a first donor sequence, or a first donor plasmid can comprise, consist essential of or consist of the sequence encoding the CSR. The first transposon, the first vector, the first donor sequence, or the first donor plasmid can further comprise, consist essential of or consist of a sequence encoding a first selection marker.

[0140] A second transposon, a second vector, a second donor sequence, or a second donor plasmid can comprise, consist essential of or consist of one or more of the sequence encoding the inducible proapoptotic polypeptide, the sequence encoding a non-naturally occurring antigen receptor, and the sequence encoding a therapeutic protein. The second transposon, the second vector, the second donor sequence, or the second donor plasmid can further comprise, consist essential of or consist of a sequence encoding a second selection marker. The first selection marker and the second selection marker are identical. The first selection marker and the second selection marker are not identical. The selection marker can comprise, consist essential of or consist of a cell surface marker. The selection marker can comprise, consist essential of or consist of a protein that is active in dividing cells and not active in non-dividing cells. The selection marker can comprise, consist essential of or consist of a metabolic marker.

[0141] In one aspect, the selection marker can comprise, consist essential of or consist of a dihydrofolate reductase (DHFR) mutein enzyme. The DHFR mutein enzyme can comprise, consist essential of or consist of the amino acid sequence of SEQ ID NO: 17012.

[0142] The DHFR mutein enzyme of SEQ ID NO: 17012 can further comprise, consist essential of or consist of a mutation at one or more of positions 80, 113, or 153. The amino acid sequence of the DHFR mutein enzyme of SEQ ID NO: 17012 can further comprise, consist essential of or consist of one or more of a substitution of a Phenylalanine (F) or a Leucine (L) at position 80; a substitution of a Leucine (L) or a Valine (V) at position 113, and a substitution of a Valine (V) or an Aspartic Acid (D) at position 153.

[0143] A modified cell of the present disclosure (preferably a modified T-cell of the present disclosure) can further comprise, consist essential of or consist of a gene editing composition. The gene editing composition can comprise, consist essential of or consist of a sequence encoding a DNA binding domain and a sequence encoding a nuclease protein or a nuclease domain thereof. The gene editing composition can be expressed transiently by the modified cell. The gene editing composition can be expressed stably by the modified cell.

[0144] The gene editing composition can comprise, consist essential of or consist of a sequence encoding a nuclease protein or a sequence encoding a nuclease domain thereof. The sequence encoding a nuclease protein or the sequence encoding a nuclease domain thereof can comprise, consist essential of or consist of a DNA sequence, an RNA sequence, or a combination thereof. The nuclease or the nuclease domain thereof can comprise, consist essential of or consist of one or more of a CRISPR/Cas protein, a Transcription Activator-Like Effector Nuclease (TALEN), a Zinc Finger Nuclease (ZFN), and an endonuclease. The CRISPR/Cas protein can comprise, consist essential of or consist of a nuclease-inactivated Cas (dCas) protein. The nuclease or the nuclease domain thereof can comprise, consist essential of or consist of a nuclease-inactivated Cas (dCas) protein and an endonuclease. The endonuclease can comprise, consist essential of or consist of a Clo051 nuclease or a nuclease domain thereof. The gene editing composition can comprise, consist essential of or consist of a fusion protein. The fusion protein can comprise, consist essential of or consist of a nuclease-inactivated Cas9 (dCas9) protein and a Clo051 nuclease or a Clo051 nuclease domain. The fusion protein can comprise, consist essential of or consist of the amino acid sequence of SEQ ID NO: 17013. The fusion protein is encoded by a nucleic acid comprising, consisting essential of or consisting of the sequence of SEQ ID NO: 17014. The fusion protein can comprise, consist essential of or consist of the amino acid

sequence of SEQ ID NO: 17058. The fusion protein is encoded by a nucleic acid comprising, consisting essential of or consisting of the sequence of SEQ ID NO: 17059.

[0145] The gene editing composition can further comprise, consist essential of or consist of a guide sequence. The guide sequence can comprise, consist essential of or consist of an RNA sequence. In aspects when the modified cell is a T-cell, the guide RNA can comprise, consist essential of or consist of a sequence complementary to a target sequence encoding an endogenous TCR. The guide RNA can comprise, consist essential of or consist of a sequence complementary to a target sequence encoding a B2M polypeptide. The guide RNA can comprise, consist essential of or consist of a sequence complementary to a target sequence within a safe harbor site of a genomic DNA sequence.

[0146] The transposon, the vector, the donor sequence or the donor plasmid can further comprise, consist essential of or consist of a gene editing composition comprising a guide sequence and a sequence encoding a fusion protein comprising a sequence encoding an inactivated Cas9 (dCas9) and a sequence encoding a Clo051 nuclease or a nuclease domain thereof.

[0147] The first transposon, the first vector, the first donor sequence or the first donor plasmid can further comprise, consist essential of or consist of a gene editing composition comprising a guide sequence and a sequence encoding a fusion protein comprising a sequence encoding an inactivated Cas9 (dCas9) and a sequence encoding a Clo051 nuclease or a nuclease domain thereof.

[0148] The second transposon, the second vector, the second donor sequence or the second donor plasmid can further comprise, consist essential of or consist of a gene editing composition comprising a guide sequence and a sequence encoding a fusion protein comprising a sequence encoding an inactivated Cas9 (dCas9) and a sequence encoding a Clo051 nuclease or a nuclease domain thereof.

[0149] A third transposon, a third vector, a third donor sequence or a third donor plasmid can comprise, consist essential of or consist of a gene editing composition comprising a guide sequence and a sequence encoding a fusion protein comprising a sequence encoding an inactivated Cas9 (dCas9) and a sequence encoding a Clo051 nuclease or a nuclease domain thereof.

[0150] The Clo051 nuclease or a nuclease domain thereof can induce a single or double strand break in a target sequence. The donor sequence or a donor plasmid can integrate at a position of single or double strand break or at a position of cellular repair within a target sequence, or a combination thereof.

[0151] The present disclosure provides a composition comprising, consisting essential of, or consisting of a modified cell of the present disclosure (preferably a modified T-cell of the present disclosure).

[0152] The present disclosure provides a plurality of modified cells comprising any non-naturally occurring chimeric stimulatory receptor (CSR) disclosed herein and provides a plurality of modified cells comprising any modified cell disclosed herein. The plurality of modified cells can comprise, consist essential of, or consist of immune cells or an immune cell precursors. The plurality of immune cells can comprise, consist essential of, or consist of lymphoid progenitor cells, natural killer (NK) cells, cytokine induced killer (CIK) cells, T lymphocytes (T-cells), B lymphocytes (B-cells) or antigen presenting cells (APCs).

[0153] The present disclosure provides a composition comprising a population of modified cells, wherein a plurality of the modified cells of the population comprise any non-naturally occurring chimeric stimulatory receptor (CSR) disclosed herein and provides a composition comprising a population of modified cells, wherein a plurality of the modified cells of the population comprise any modified cell disclosed herein. The population of modified cells can comprise, consist essential of, or consist of immune cells or an immune cell precursors. The population of immune cells can comprise, consist essential of, or consist of lymphoid progenitor cells, natural killer (NK) cells, cytokine induced killer (CIK) cells, T lymphocytes (T-cells), B lymphocytes (B-cells) or antigen presenting cells (APCs). The composition can comprise a pharmaceutically-acceptable carrier.

[0154] The present disclosure provides a composition comprising a population of modified T lymphocytes (T-cells), wherein a plurality of the modified T-cells of the population comprise any non-naturally occurring chimeric stimulatory receptor (CSR) disclosed herein and provides a composition comprising a population of T lymphocytes (T-cells), wherein a plurality of the T-cells of the population comprise any modified T-cell disclosed herein. The composition can comprise a pharmaceutically-acceptable carrier.

[0155] Preferably, the present disclosure provides a composition comprising a population of T lymphocytes (T-cells), wherein a plurality of the T-cells of the population comprise a non-naturally occurring chimeric stimulatory receptor (CSR) comprising, consisting essential of, or consisting of: (a) an ectodomain comprising a activation component, wherein the activation component is isolated or derived from a first protein; (b) a transmembrane domain; and (c) an endodomain comprising at least one signal transduction domain, wherein the at least one signal transduction domain is isolated or derived from a second protein, wherein the first protein and the second protein are not identical. The composition can comprise a pharmaceutically-acceptable carrier. In some aspects, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the population comprise the CSR.

[0156] The plurality of the T-cells of the population can further comprise an inducible proapoptotic polypeptide. In some aspects, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the population comprise the inducible proapoptotic polypeptide.

[0157] The plurality of the T-cells of the population can further comprise a modification of an endogenous sequence encoding a T-cell Receptor (TCR), wherein the modification reduces or eliminates a level of expression or activity of the TCR. In some aspects, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the population comprise the modification of the endogenous sequence encoding the TCR, wherein the modification reduces or eliminates a level of expression or activity of the TCR.

[0158] The plurality of the T-cells of the population can further comprise a modification of an endogenous sequence encoding Beta-2-Microglobulin (B2M), wherein the modification reduces

or eliminates a level of expression or activity of a major histocompatibility complex (MHC) class I (MHC-I). In some aspects, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the population comprise the modification of the endogenous sequence encoding B2M, wherein the modification reduces or eliminates a level of expression or activity of MHC-I.

[0159] The plurality of the T-cells of the population can further comprise a modification of an endogenous sequence encoding a T-cell Receptor (TCR), wherein the modification reduces or eliminates a level of expression or activity of the TCR and a modification of an endogenous sequence encoding Beta-2-Microglobulin (B2M), wherein the modification reduces or eliminates a level of expression or activity of a major histocompatibility complex (MHC) class I (MHC-I).

[0160] In some aspects, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the population comprise both modification of the endogenous sequence encoding the TCR, wherein the modification reduces or eliminates a level of expression or activity of the TCR and the modification of the endogenous sequence encoding B2M, wherein the modification reduces or eliminates a level of expression or activity of MHC-I.

[0161] The plurality of the T-cells of the population can further comprise a non-naturally occurring sequence comprising an HLA class I histocompatibility antigen, alpha chain E (HLA-E) polypeptide. In some aspects, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the population comprise the non-naturally occurring sequence comprising the HLA-E polypeptide.

[0162] The plurality of the T-cells of the population can further comprise a non-naturally occurring antigen receptor, a sequence encoding a therapeutic polypeptide, or a combination thereof. In some aspects, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at

least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the population comprise the non-naturally occurring antigen receptor, the sequence encoding a therapeutic polypeptide, or a combination thereof. In preferred aspects, the non-naturally occurring antigen receptor is a chimeric antigen receptor (CAR).

[0163] The plurality of the T-cells of the population can comprise an early memory T cell, a stem cell-like T cell, a stem memory T cell (T_{SCM}), a central memory T cell (T_{CM}) or a stem cell-like T cell. In some aspects, one or more of a stem cell-like T cell, a stem cell memory T cell (T_{SCM}) and a central memory T cell (T_{CM}) comprise at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the population of modified T-cells.

[0164] In some aspects, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the population comprising the CSR expresses one or more cell-surface marker(s) of a stem memory T cell (T_{SCM}) or a T_{SCM}-like cell; and wherein the one or more cell-surface marker(s) comprise CD45RA and CD62L.

[0165] In some aspects, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the population expresses one or more cell-surface marker(s) of a central memory T cell (T_{CM}) or a T_{CM}-like cell; and wherein the one or more cell-surface marker(s) comprise CD45RO and CD62L.

[0166] In some aspects, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at

least 96%, at least 97%, at least 98%, at least 99%, or 100% of the population expresses one or more of CD127, CD45RO, CD95 and IL-2Rβ cell-surface marker(s).

[0167] The present disclosure provides compositions for use in the treatment of a disease or disorder disclosed herein or the use of a composition for the treatment of any disease or disorder disclosed herein. The present disclosure also provides methods of treating a disease or disorder comprising, consisting essential of, or consisting of administering to a subject in need thereof a therapeutically-effective amount of a composition disclosed herein. The compositions can comprise, consist essential of or consist of any of the modified cells or populations of modified cells disclosed herein. Preferably, any of the modified T-cells or CAR T-cells disclosed herein.

[0168] The present disclosure provides a method of producing a modified T-cell comprising, consisting essential of, or consisting of, introducing into a primary human T-cell a composition comprising a Chimeric Stimulator Receptor (CSR) of the present disclosure or a sequence encoding the same to produce a modified T-cell under conditions that stably express the CSR within the modified T-cell and preserve desirable stem-like properties of the modified T-cell. The primary human T-cell can be a resting primary human T-cell. The present disclosure provides a modified T-cell produced by the disclosed method. The present disclosure provides a method of administering the modified T-cell comprising the stably expressed CSR produced by the disclosed method. The present disclosure provides the method of administering the modified T-cell comprising the stably expressed CSR produced by the disclosed method to treat a disease or disorder.

[0169] The present disclosure provides a method of producing a population of modified T-cells comprising, consisting essential of, or consisting of, introducing into a plurality of primary human T-cells a composition comprising a Chimeric Stimulator Receptor (CSR) of the present disclosure or a sequence encoding the same to produce a plurality of modified T-cells under conditions that stably express the CSR within the plurality of modified T-cells and preserve desirable stem-like properties of the plurality of modified T-cells. The primary human T-cells can comprise resting primary human T-cells. The present disclosure provides a population of modified T-cells produced by the disclosed method. The present disclosure provides a method of administering the population of modified T-cells comprising the stably expressed CSR produced by the disclosed method. The present disclosure provides a method of administering the

population of modified T-cells comprising the stably expressed CSR produced by the disclosed method to treat a disease or disorder.

[0170] The present disclosure provides a method of producing a modified T-cell comprising, consisting essential of, or consisting of, introducing into a primary human T-cell a composition comprising a Chimeric Stimulator Receptor (CSR) of the present disclosure or a sequence encoding the same to produce a modified T-cell under conditions that transiently express the CSR within the modified T-cell and preserve desirable stem-like properties of the modified T-cell. The primary human T-cell can be a resting primary human T-cell. The present disclosure provides a modified T-cell produced by the disclosed method. The present disclosure provides a method of administering the modified T-cell comprising the transiently expressed CSR produced by the disclosed method. In one aspect, the present disclosure provides a method of administering the modified T-cell produced by the disclosed method after the modified T-cell no longer expresses the CSR. The present disclosure provides a method of administering a modified T-cell comprising the transiently expressed CSR produced by the disclosed method to treat a disease or disorder. In one aspect, the present disclosure provides a method of administering the modified T-cell produced by the disclosed method after the modified T-cell no longer expresses the CSR to treat a disease or disorder.

[0171] The present disclosure provides a method of producing a population of modified T-cells comprising, consisting essential of, or consisting of, introducing into a plurality of primary human T-cells a composition comprising a Chimeric Stimulator Receptor (CSR) of the present disclosure or a sequence encoding the same to produce a plurality of modified T-cells under conditions that transiently express the CSR within the plurality of modified T-cells and preserve desirable stem-like properties of the plurality of modified T-cells. The primary human T-cells can comprise resting primary human T-cells. The present disclosure provides a population of modified T-cell produced by the disclosed method. The present disclosure provides a method of administering the population of modified T-cells comprising the transiently expressed CSR produced by the disclosed method. In one aspect, the present disclosure provides a method of administering the population of modified T-cells produced by the disclosed method after the plurality of T-cells no longer express the CSR. The present disclosure provides a method of administering the population of modified T-cells comprising the transiently expressed CSR

produced by the disclosed method to treat a disease or disorder. In one aspect, the present disclosure provides a method of administering the population of modified T-cells produced by the disclosed method after the plurality of modified T-cells no longer express the CSR to treat a disease or disorder.

[0172] The method of producing a modified T-cell or producing a population of modified T-cells can further comprise introducing a modification of an endogenous sequence encoding a T-cell Receptor (TCR), wherein the modification reduces or eliminates a level of expression or activity of the TCR. The method of producing a modified T-cell or producing a population of modified T-cells can further comprise introducing a modification of an endogenous sequence encoding Beta-2-Microglobulin (B2M), wherein the modification reduces or eliminates a level of expression or activity of a major histocompatibility complex (MHC) class I (MHC-1). In some aspects, the method of producing a modified T-cell or producing a population of modified T-cells can further comprising introducing both a modification of an endogenous sequence encoding TCR, wherein the modification reduces or eliminates a level of expression or activity of the TCR and introducing a modification of an endogenous sequence encoding B2M, wherein the modification reduces or eliminates a level of expression or activity of MHC-1.

[0173] The method of producing a modified T-cell or producing a population of modified T-cells can further comprise introducing into the primary human T-cell or plurality of primary human T cells a composition comprising an antigen receptor, a therapeutic protein or a sequence encoding the same. In one aspect, the antigen receptor is a non-naturally occurring antigen receptor. In a preferred aspect, the method of producing a modified T-cell or producing a population of modified T-cells can further comprise introducing into the primary human T-cell or plurality of primary human T cells a composition comprising a Chimeric Antigen Receptor (CAR) or a sequence encoding the same. The method can further comprise introducing into the primary human T-cell or plurality of primary human T cells a composition comprising an inducible proapoptotic polypeptide or a sequence encoding the same. The method of producing a modified T-cell or producing a population of modified T-cells can further comprise introducing into the primary human T-cell or plurality of primary human T cells a composition comprising an antigen receptor, a therapeutic protein or a sequence encoding the same and a composition comprising an inducible proapoptotic polypeptide or a sequence encoding the same.

[0174] The method of producing a modified T-cell or producing a population of modified T-cells can further comprise contacting the modified T-cell or population of modified T-cells with an activator composition. The activator composition can comprise, consist essential of, or consist of one or more agonists or activating agents that can bind a CSR activation component of the modified T-cell or plurality of modified T-cells. The agonist/activating agent can be naturally occurring or non-naturally occurring. In preferred aspects, the agonist/activating agent is an antibody or antibody fragment. The agonist/activating agent can be one or more of an anti-CD3 antibody or fragment thereof, an anti-CD2 antibody or fragment thereof, an anti-CD28 antibody or fragment thereof, or any combination thereof. In some aspects, the agonist/activating agent that can be one or more of an anti-human CD3 monospecific tetrameric antibody complex, an anti-human CD2 monospecific tetrameric antibody complex, an anti-human CD28 monospecific tetrameric antibody complex, or a combination thereof. The agonist/activating can contact the modified T-cell or population of modified T-cells in vitro, ex vivo or in vivo. In a preferred aspect, the agonist/activating activates the modified T-cell or population of modified T-cells, induces cell division in the modified T-cell or population of modified T-cells, increases cell division (e.g., cell doubling time) in the modified T-cell or population of modified T-cells, increases fold expansion in the modified T-cell or population of modified T-cells, or any combination thereof.

[0175] The present disclosure provides a method of expanding a population of modified T-cells comprising, consisting essential of, or consisting of, introducing into a plurality of primary human T-cells a composition comprising a Chimeric Stimulator Receptor (CSR) of the present disclosure or a sequence encoding the same to produce a plurality of modified T-cells under conditions that stably express the CSR within the plurality of modified T-cells and preserve desirable stem-like properties of the plurality of modified T-cells and contacting the cells with an activator composition to produce a plurality of activated modified T-cells, wherein expansion of the plurality of modified T-cells is at least two fold higher than the expansion of a plurality of wild-type T-cells not stably expressing a CSR of the present disclosure under the same conditions. The method wherein the expansion of the plurality of modified T-cells is at least three fold, at least four fold, at least five fold, at least six fold, at least seven fold, at least eight

fold, at least nine fold or at least 10 fold higher than the expansion of a plurality of wild-type T-cells not stably expressing a CSR of the present disclosure under the same conditions.

[0176] The present disclosure provides a method of expanding a population of modified T-cells comprising, consisting essential of, or consisting of, introducing into a plurality of primary human T-cells a composition comprising a Chimeric Stimulator Receptor (CSR) of the present disclosure or a sequence encoding the same to produce a plurality of modified T-cells under conditions that transiently express the CSR within the plurality of modified T-cells and preserve desirable stem-like properties of the plurality of modified T-cells and contacting the cells with an activator composition to produce a plurality of activated modified T-cells, wherein expansion of the plurality of modified T-cells is at least two fold higher than the expansion of a plurality of wild-type T-cells not transiently expressing a CSR of the present disclosure under the same conditions. The method wherein the expansion of the plurality of modified T-cells is at least three fold, at least four fold, at least five fold, at least six fold, at least seven fold, at least eight fold, at least nine fold or at least 10 fold higher than the expansion of a plurality of wild-type T-cells not transiently expressing a CSR of the present disclosure under the same conditions.

[0177] The activator composition of the methods of expanding a population of can comprise, consist essential of, or consist of one or more agonists or activating agents that can bind a CSR activation component of the modified T-cell or plurality of modified T-cells. The agonist/activating agent can be naturally occurring or non-naturally occurring. In preferred aspects, the agonist/activating agent is an antibody or antibody fragment. The agonist/activating agent can be one or more of an anti-CD3 antibody or fragment thereof, an anti-CD2 antibody or fragment thereof, an anti-CD28 antibody or fragment thereof, or any combination thereof. In some aspects, the agonist/activating agent that can be one or more of an anti-human CD3 monospecific tetrameric antibody complex, an anti-human CD2 monospecific tetrameric antibody complex, an anti-human CD28 monospecific tetrameric antibody complex, or a combination thereof.

[0178] The conditions can comprise culturing the modified T-cell or plurality of modified T-cells in a media comprising a sterol; an alkane; phosphorus and one or more of an octanoic acid, a palmitic acid, a linoleic acid, and an oleic acid. The culturing can be in vivo or ex vivo. The modified T-cell can be an allogeneic T-cell or the plurality of modified T-cells can be allogeneic

T-cells. The modified T-cell can be an autologous T-cell or the plurality of modified T-cells can be autologous T-cells.

[0179] In some aspects, the media can comprise one or more of octanoic acid at a concentration of between 0.9 mg/kg to 90 mg/kg, inclusive of the endpoints; palmitic acid at a concentration of between 0.2 mg/kg to 20 mg/kg, inclusive of the endpoints; linoleic acid at a concentration of between 0.2 mg/kg to 20 mg/kg, inclusive of the endpoints; oleic acid at a concentration of 0.2 mg/kg to 20 mg/kg, inclusive of the endpoints; and a sterol at a concentration of about 0.1 mg/kg to 10 mg/kg, inclusive of the endpoints.

[0180] In some aspects, the media can comprise one or more of octanoic acid at a concentration of about 9 mg/kg, palmitic acid at a concentration of about 2 mg/kg, linoleic acid at a concentration of about 2 mg/kg, oleic acid at a concentration of about 2 mg/kg and a sterol at a concentration of about 1 mg/kg.

[0181] In some aspects, the media can comprise one or more of octanoic acid at a concentration of between 6.4 μmol/kg and 640 μmol/kg, inclusive of the endpoints; palmitic acid at a concentration of between 0.7 μmol/kg and 70 μmol/kg, inclusive of the endpoints; linoleic acid at a concentration of between 0.75 μmol/kg and 75 μmol/kg, inclusive of the endpoints; oleic acid at a concentration of between 0.75 μmol/kg and 75 μmol/kg, inclusive of the endpoints; and a sterol at a concentration of between 0.25 μmol/kg and 25 μmol/kg, inclusive of the endpoints.

[0182] In some aspects, the media can comprise one or more of octanoic acid at a concentration of about 64 μmol/kg, palmitic acid at a concentration of about 7 μmol/kg, linoleic acid at a concentration of about 7.5 μmol/kg, oleic acid at a concentration of about 7.5 μmol/kg and a sterol at a concentration of about 2.5 μmol/kg.

[0183] The present disclosure provides compositions comprising any modified T-cell produced by a method dislosed herein. The present disclosure provides compositions comprising any population of modified T-cell produced by a method dislosed herein. The present disclosure provides compositions comprising any modified T-cell expanded by a method dislosed herein. The present disclosure provides compositions comprising any population of modified T-cell expanded by a method dislosed herein.

[0184] The present disclosure provides compositions for use in the treatment of a disease or disorder disclosed herein or the use of a composition for the treatment of any disease or disorder

disclosed herein. The present disclosure also provides methods of treating a disease or disorder comprising, consisting essential of, or consisting of administering to a subject in need thereof a therapeutically-effective amount of a composition disclosed herein and at least one non-naturally occurring molecule which binds to the activation component of a CSR disclosed herein. The compositions can comprise, consist essential of or consist of any of the modified cells or populations of modified cells disclosed herein. Preferably, any of the modified T-cells or CAR T-cells disclosed herein. Any non-naturally occurring molecule capable of binding to the activation component of the CSR of the present disclosure and selectively transducing a signal upon binding can be administered. Preferably, the non-naturally occurring molecule is an non-naturally CSR agonist/activating agent for the activation component. The non-naturally occurring agonist/activating agent that can bind a CSR activation component can be any non-naturally occurring antibody or antibody fragment. The non-naturally occurring antibody or antibody fragment can be a non-naturally occurring anti-CD3 antibody or fragment thereof, an anti-CD2 antibody or fragment thereof, an anti-CD28 antibody or fragment thereof, or any combination thereof. In some aspects, the non-naturally occurring agonist/activating agent that can bind a CSR activation component can be one or more of an anti-human CD3 monospecific tetrameric antibody complex, an anti-human CD2 monospecific tetrameric antibody complex, an anti-human CD28 monospecific tetrameric antibody complex, or a combination thereof. In some aspects, the non-naturally occurring agonist/activating agent that can bind an activation component can be selected from the group consisting of anti-CD2 monoclonal antibody, BTI-322 (Przepiorka et al., Blood 92(11):4066-4071, 1998) and humanized anti-CD2 monoclonal antibody clone AFC-TAB-104 (Siplizumab)(Bissonnette et al. Arch. Dermatol. Res. 301(6):429-442, 2009). In some aspects, administration of non-naturally occurring molecule capable of binding to the activation component of the CSR stimulates cell division of the modified cells in vivo. Thus, the present disclosure provides a method of stimulating cell division of a modified cell of the present disclosure in vivo by administering a non-naturally CSR agonist/activating agent for the activation component to a subject harboring the modified cell of the present disclosure.

[0185] In some aspects, the disease or disorder is a cell proliferation disease or disorder. In some aspects, the cell proliferation disease or disorder is cancer. The cancer can be a solid

tumor cancer or a hematologic cancer. In some aspects, the solid tumor is prostate cancer or breast cancer. In preferred aspects, the prostate cancer is castrate-resistant prostate cancer. In some aspects, the hematologic cancer is multiple myeloma.

[0186] The modified cells or population of modified cells comprised within the disclosed compositions can be cultured in vitro or ex vivo prior to administration to a subject in need thereof. The modified cells can be allogenic modified cells or autologous modified cells. In some aspects, the cells are allogeneic modified T-cells or autologous modified T-cells. In some aspects, the cells are allogeneic modified CAR T-cells or autologous modified CAR T-cells. In some aspects, the cells are allogeneic modified CAR T-cells comprising a CSR of the present disclosure or autologous modified CAR T-cells comprising a CSR of the present disclosure.

[0187] The modified cell compositions or the compositions comprising populations of modified cells can be administered to the patient by any means known in the art. In some aspects, the composition is administered by systemic administration. In some aspects, the composition is administered by intravenous administration. The intravenous administration can be in an intravenous injection or an intravenous infusion. In some aspects, the composition is administered by local administration. In some aspects, the composition is administered by an intraspinal, intracerebroventricular, intraocular or intraosseous injection or infusion.

[0188] The therapeutically effective amount can be a single dose or multiple doses of modified cell compositions or the compositions comprising populations of modified cells. In some aspects, the therapeutically effective dose is a single dose and wherein the allogeneic cells of the composition engraft and/or persist for a sufficient time to treat the disease or disorder. In some aspects, the single dose is one of at least 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or any number of doses in between that are manufactured simultaneously

[0189] In some aspects, the uses and methods for the treatment of a disease or disorder further provide that subjects do not develop graft v host (GvH) disease, host v graft (HvG) disease, or a combination thereof, following administration of modified cell compositions disclosed herein or the compositions comprising populations of modified cells disclosed herein.

[0190] Allogeneic cells of the disclosure are engineered to prevent adverse reactions to engraftment following administration to a subject. Allogeneic cells may be any type of cell.

[0191] In some embodiments of the composition and methods of the disclosure, allogeneic cells are stem cells. In some embodiments, allogeneic cells are derived from stem cells. Exemplary stem cells include, but are not limited to, embryonic stem cells, adult stem cells, induced pluripotent stem cells (iPSCs), multipotent stem cells, pluripotent stem cells, and hematopoetic stem cells (HSCs).

[0192] In some embodiments of the composition and methods of the disclosure, allogeneic cells are differentiated somatic cells.

[0193] In some embodiments of the composition and methods of the disclosure, allogeneic cells are immune cells. In some embodiments, allogeneic cells are T lymphocytes (T cells). In some embodiments, allogeneic cells are T cells that do not express one or more components of a naturally-occurring T-cell Receptor (TCR). In some embodiments, allogeneic cells are T cells that express a non-naturally occurring antigen receptor. Alternatively, or in addition, in some embodiments, allogeneic cells are T cells that express a non-naturally occurring Chimeric Stimulatory Receptor (CSR). In some embodiments, the non-naturally occurring CSR comprises or consists of a switch receptor. In some embodiments, the switch receptor comprises an extracellular domain, a transmembrane domain, and an intracellular domain. In some embodiments, the extracellular domain of the switch receptor binds to a TCR co-stimulatory molecule and transduces a signal to the intracellular space of the allogeneic cell that recapitulates TCR signaling or TCR co-stimulatory signaling.

Chimeric Stimulatory Receptors (CSRs)

[0194] Adoptive cell compositions that are "universally" safe for administration to any patient requires a significant reduction or elimination of alloreactivity.

[0195] Towards this end, allogeneic cells of the disclosure are modified to interrupt expression or function of a T-cell Receptor (TCR) and/or a class of Major Histocompatibility Complex (MHC). The TCR mediates graft vs host (GvH) reactions whereas the MHC mediates host vs graft (HvG) reactions. In preferred embodiments, any expression and/or function of the TCR is eliminated in allogeneic cells of the disclosure to prevent T-cell mediated GvH that could cause death to the subject. Thus, in particularly preferred embodiments, the disclosure provides a pure TCR-negative allogeneic T-cell composition (e.g. each cell of the composition expresses at a level so low as to either be undetectable or non-existent).

[0196] In preferred embodiments, expression and/or function of MHC class I (MHC-I, specifically, HLA-A, HLA-B, and HLA-C) is reduced or eliminated in allogeneic cells of the disclosure to prevent HvG and, consequently, to improve engraftment of allogeneic cells of the disclosure in a subject. Improved engraftment of the allogeneic cells of the disclosure results in longer persistence of the cells, and, therefore, a larger therapeutic window for the subject. Specifically, in the allogeneic cells of the disclosure, expression and/or function of a structural element of MHC-I, Beta-2-Microglobulin (B2M), is reduced or eliminated in allogeneic cells of the disclosure.

[0197] The above strategies for generating an allogeneic cell of the disclosure induce further challenges. T Cell Receptor (TCR) knockout (KO) in T cells results in loss of expression of CD3-zeta (CD3z or CD3ζ), which is part of the TCR complex. The loss of CD3ζ in TCR-KO T-cells dramatically reduces the ability of optimally activating and expanding these cells using standard stimulation/activation reagents, including, but not limited to, agonist anti-CD3 mAb. When the expression or function of any one component of the TCR complex is interrupted, all components of the complex are lost, including TCR-alpha (TCRα), TCR-beta (TCRβ), CD3-gamma (CD3γ), CD3-epsilon (CD3ε), CD3-delta (CD3δ), and CD3-zeta (CD3ζ). Both CD3ε and CD3ζ are required for T cell activation and expansion. Agonist anti-CD3 mAbs typically recognize CD3ε and possibly another protein within the complex which, in turn, signals to CD3ζ. CD3ζ provides the primary stimulus for T cell activation (along with a secondary co-stimulatory signal) for optimal activation and expansion. Under normal conditions, full T-cell activation depends on the engagement of the TCR in conjunction with a second signal mediated by one or more co-stimulatory receptors (e.g. CD28, CD2, 4-1BBL, etc…) that boost the immune response. However, when the TCR is not present, T cell expansion is severely reduced when stimulated using standard activation/stimulation reagents, including agonist anti-CD3 mAb. In fact, T cell expansion is reduced to only 20-40% of the normal level of expansion when stimulated using standard activation/stimulation reagents, including agonist anti-CD3 mAb.

[0198] The disclosure provides a Chimeric Stimulatory Receptor (CSR) to deliver CD3z primary stimulation to allogeneic T cells in the absence of an endogenous TCR (and, consequently, an endogenous CD3ζ) when stimulated using standard activation/stimulation reagents, including agonist anti-CD3 mAb.

[0199] In the absence of an endogenous TCR, Chimeric Stimulatory Receptors (CSRs) of the disclosure provide a CD3ζ stimulus to enhance activation and expansion of allogeneic T cells. In other words, in the absence of an endogenous TCR, Chimeric Stimulatory Receptors (CSRs) of the disclosure rescue the allogeneic cell from an activation-based disadvantage when compared to non-allogeneic T-cells that express an endogenous TCR. In some embodiments, CSRs of the disclosure comprise an agonist mAb epitope extracellularly and a CD3ζ stimulatory domain intracellularly and, functionally, convert an anti-CD28 or anti-CD2 binding event on the surface into a CD3z signaling event in an allogeneic T cell modified to express the CSR. In some embodiments, a CSR comprises a wild type CD28 or CD2 protein and a CD3z intracellular stimulation domain, to produce CD28z CSR and CD2z CSR, respectively. In preferred embodiments, CD28z CSR and/or CD2z CSR further express a non-naturally occurring antigen receptor and/or a therapeutic protein. In preferred embodiments, the non-naturally occurring antigen receptor comprises a Chimeric Antigen Receptor.

[0200] The data provided herein demonstrate that modified allogeneic T cells of the disclosure comprising/expressing a CSR of the disclosure improve or rescue, the expansion of allogeneic T cells that no longer express endogenous TCR when compared to those cells that do not comprise/express a CSR of the disclosure.

[0201] A wildtype/natural human CD28 protein (NCBI: CD28_HUMAN; UniProt/Swiss-Prot: P10747.1) comprises or consists of the amino acid sequence of:

MLRLLLALNLFPSIQVTGNKILVKQSPMLVAYDNAVNLSCKYSYNLFSREFRASLHKGLDSAVE
VCVVYGNYSQQLQVYSKTGFNCDGKLGNESVTFYLQNLYVNQTDIYFCKIEVMYPPPYLDNEKS
NGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYM
NMTPRRPGPTRKHYQPYAPPRDFAAYRS (SEQ ID NO: 17096)

[0202] A nucleotide sequence encoding wildtype/natural CD28 protein (NCBI: CCDS2361.1) comprises or consists of the nucleotide sequence of:

ATGCTCAGGCTGCTCTTGGCTCTCAACTTATTCCCTTCAATTCAAGTAACAGGAAACAAGATTT
TGGTGAAGCAGTCGCCCATGCTTGTAGCGTACGACAATGCGGTCAACCTTAGCTGCAAGTATTC
CTACAATCTCTTCTCAAGGGAGTTCCGGGCATCCCTTCACAAAGGACTGGATAGTGCTGTGGAA
GTCTGTGTTGTATATGGGAATTACTCCCAGCAGCTTCAGGTTTACTCAAAAACGGGGTTCAACT
GTGATGGGAAATTGGGCAATGAATCAGTGACATTCTACCTCCAGAATTTGTATGTTAACCAAAC
AGATATTTACTTCTGCAAAATTGAAGTTATGTATCCTCCTCCTTACCTAGACAATGAGAAGAGC
AATGGAACCATTATCCATGTGAAAGGGAAACACCTTTGTCCAAGTCCCCTATTTCCCGGACCTT
CTAAGCCCTTTTGGGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAAC
AGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATG

AACATGACTCCCCGCCGCCCCGGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCG
ACTTCGCAGCCTATCGCTCCTGA (SEQ ID NO: 17097)

[0203] An exemplary CSR CD28z protein of the disclosure comprises or consists of the amino acid sequence of (**CD28 Signal peptide**, *CD28 Extracellular Domain*, CD28 Transmembrane domain, ***CD28 Cytoplasmic Domain***, CD3z Intracellular Domain):

MLRLLLALNLFPSIQVTG*NKILVKQSPMLVAYDNAVNLSCKYSYNLFSREFRASLHKGLDSAVE VCVVYGNYSQQLQVYSKTGFNCDGKLGNESVTFYLQNLYVNQTDIYFCKIEVMYPPPYLDNEKS NGTIIHVKGKHLCPSPLFPGPSKP*FWVLVVVGGVLACYSLLVTVAFIIFWV***RSKRSRLLHSDYM NMTPRRPGPTRKHYQPYAPPRDFAAYRS***RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLD KRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT YDALHMQALPPR (SEQ ID NO: 17060)

CD28 Signal peptide:
MLRLLLALNLFPSIQVTG (SEQ ID NO: 17098)

CD28 Extracellular Domain:
NKILVKQSPMLVAYDNAVNLSCKYSYNLFSREFRASLHKGLDSAVEVCVVYGNYSQQLQVYSKT GFNCDGKLGNESVTFYLQNLYVNQTDIYFCKIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLF PGPSKP (SEQ ID NO: 17099)

CD28 Transmembrane domain:
FWVLVVVGGVLACYSLLVTVAFIIFWV (SEQ ID NO: 17100)

CD28 Cytoplasmic Domain:
RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS (SEQ ID NO: 17101)

CD3z Intracellular Domain:
RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 17102)

[0204] An exemplary nucleotide sequence encoding a CSR CD28z protein of the disclosure comprises or consists of the nucleotide sequence of (**CD28 Signal peptide**, *CD28 Extracellular Domain*, CD28 Transmembrane domain, ***CD28 Cytoplasmic Domain***, CD3z Intracellular Domain):

ATGCTGAGACTGCTGCTGGCCCTGAATCTGTTCCCCAGCATCCAAGTGACCGGC*AACAAGATCC TGGTCAAGCAGAGCCCTATGCTGGTGGCCTACGACAACGCCGTGAACCTGAGCTGCAAGTACAG CTACAACCTGTTCAGCAGAGAGTTCCGGGCCAGCCTGCACAAAGGACTGGATTCTGCTGTGGAA GTGTGCGTGGTGTACGGCAACTACAGCCAGCAGCTGCAGGTCTACAGCAAGACCGGCTTCAACT GCGACGGCAAGCTGGGCAATGAGAGCGTGACCTTCTACCTGCAAAACCTGTACGTGAACCAGAC CGACATCTATTTCTGCAAGATCGAAGTGATGTACCCGCCTCCTTACCTGGACAACGAGAAGTCC*

AACGGCACCATCATCCACGTGAAGGGCAAGCACCTGTGTCCTTCTCCACTGTTCCCCGGACCTA
*GCAAGCCT*TTCTGGGTGCTCGTTGTTGTTGGCGGCGTGCTGGCCTGTTATAGCCTGCTGGTTAC
AGTGGCCTTCATCATCTTTTGGGTC***CGAAGCAAGCGGAGCCGGCTGCTGCACAGCGACTACATG***
AACATGACCCCTAGACGGCCCGGACCAACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAG
ACTTCGCCGCCTACCGGTCCAGAGTGAAGTTCTCCAGATCCGCCGATGCTCCCGCCTATAAGCA
GGGCCAGAACCAGCTGTACAACGAGCTGAACCTGGGGAGAAGAGAAGAGTACGATGTGCTGGAC
AAGCGGAGAGGCAGAGATCCTGAGATGGGCGGCAAGCCCAGACGGAAGAATCCTCAAGAGGGCC
TGTACAATGAACTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGA
GCGCAGAAGAGGCAAGGGACACGATGGACTGTACCAGGGCCTGAGCACCGCCACCAAGGATACC
TATGATGCCCTGCACATGCAGGCCCTGCCTCCAAGA (SEQ ID NO: 17061)

CD28 Signal peptide:
ATGCTGAGACTGCTGCTGGCCCTGAATCTGTTCCCCAGCATCCAAGTGACCGGC (SEQ ID
NO: 17103)

CD28 Extracellular Domain:
AACAAGATCCTGGTCAAGCAGAGCCCTATGCTGGTGGCCTACGACAACGCCGTGAACCTGAGCT
GCAAGTACAGCTACAACCTGTTCAGCAGAGAGTTCCGGGCCAGCCTGCACAAAGGACTGGATTC
TGCTGTGGAAGTGTGCGTGGTGTACGGCAACTACAGCCAGCAGCTGCAGGTCTACAGCAAGACC
GGCTTCAACTGCGACGGCAAGCTGGGCAATGAGAGCGTGACCTTCTACCTGCAAAACCTGTACG
TGAACCAGACCGACATCTATTTCTGCAAGATCGAAGTGATGTACCCGCCTCCTTACCTGGACAA
CGAGAAGTCCAACGGCACCATCATCCACGTGAAGGGCAAGCACCTGTGTCCTTCTCCACTGTTC
CCCGGACCTAGCAAGCCT (SEQ ID NO: 17104)

CD28 Transmembrane domain:
TTCTGGGTGCTCGTTGTTGTTGGCGGCGTGCTGGCCTGTTATAGCCTGCTGGTTACAGTGGCCT
TCATCATCTTTTGGGTC (SEQ ID NO: 17105)

***CD28 Cytoplasmic Domain*:**
CGAAGCAAGCGGAGCCGGCTGCTGCACAGCGACTACATGAACATGACCCCTAGACGGCCCGGAC
CAACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAGACTTCGCCGCCTACCGGTCC
(SEQ ID NO: 17106)

CD3z Intracellular Domain:
AGAGTGAAGTTCTCCAGATCCGCCGATGCTCCCGCCTATAAGCAGGGCCAGAACCAGCTGTACA
ACGAGCTGAACCTGGGGAGAAGAGAAGAGTACGATGTGCTGGACAAGCGGAGAGGCAGAGATCC
TGAGATGGGCGGCAAGCCCAGACGGAAGAATCCTCAAGAGGGCCTGTACAATGAACTGCAGAAA
GACAAGATGGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGAGCGCAGAAGAGGCAAGGGAC
ACGATGGACTGTACCAGGGCCTGAGCACCGCCACCAAGGATACCTATGATGCCCTGCACATGCA
GGCCCTGCCTCCAAGA (SEQ ID NO: 17107)

[0205] A wildtype/natural human CD2 protein (NCBI: CD2_HUMAN; UniProt/Swiss-Prot: P06729.2) comprises or consists of the amino acid sequence of:

MSFPCKFVASFLLIFNVSSKGAVSKEITNALETWGALGQDINLDIPSFQMSDDIDDIKWEKTSD
KKKIAQFRKEKETFKEKDTYKLFKNGTLKIKHLKTDDQDIYKVSIYDTKGKNVLEKIFDLKIQE
RVSKPKISWTCINTTLTCEVMNGTDPELNLYQDGKHLKLSQRVITHKWTTSLSAKFKCTAGNKV
SKESSVEPVSCPEKGLDIYLIIGICGGGSLLMVFVALLVFYITKRKKQRSRRNDEELETRAHRV
ATEERGRKPHQIPASTPQNPATSQHPPPPPGHRSQAPSHRPPPPGHRVQHQPQKRPPAPSGTQV
HQQKGPPLPRPRVQPKPPHGAAENSLSPSSN (SEQ ID NO: 17108)

[0206] A nucleotide sequence encoding wildtype/natural CD2 protein (NCBI: CCDS889.1) comprises or consists of the nucleotide sequence of:

ATGAGCTTTCCATGTAAATTTGTAGCCAGCTTCCTTCTGATTTTCAATGTTTCTTCCAAAGGTG
CAGTCTCCAAAGAGATTACGAATGCCTTGGAAACCTGGGGTGCCTTGGGTCAGGACATCAACTT
GGACATTCCTAGTTTTCAAATGAGTGATGATATTGACGATATAAAATGGGAAAAAACTTCAGAC
AAGAAAAAGATTGCACAATTCAGAAAAGAGAAAGAGACTTTCAAGGAAAAAGATACATATAAGC
TATTTAAAAATGGAACTCTGAAAATTAAGCATCTGAAGACCGATGATCAGGATATCTACAAGGT
ATCAATATATGATACAAAAGGAAAAAATGTGTTGGAAAAAATATTTGATTTGAAGATTCAAGAG
AGGGTCTCAAAACCAAAGATCTCCTGGACTTGTATCAACACAACCCTGACCTGTGAGGTAATGA
ATGGAACTGACCCCGAATTAAACCTGTATCAAGATGGGAAACATCTAAAACTTTCTCAGAGGGT
CATCACACACAAGTGGACCACCAGCCTGAGTGCAAAATTCAAGTGCACAGCAGGGAACAAAGTC
AGCAAGGAATCCAGTGTCGAGCCTGTCAGCTGTCCAGAGAAAGGTCTGGACATCTATCTCATCA
TTGGCATATGTGGAGGAGGCAGCCTCTTGATGGTCTTTGTGGCACTGCTCGTTTTCTATATCAC
CAAAAGGAAAAAACAGAGGAGTCGGAGAAATGATGAGGAGCTGGAGACAAGAGCCCACAGAGTA
GCTACTGAAGAAAGGGGCCGGAAGCCCCACCAAATTCCAGCTTCAACCCCTCAGAATCCAGCAA
CTTCCCAACATCCTCCTCCACCACCTGGTCATCGTTCCCAGGCACCTAGTCATCGTCCCCCGCC
TCCTGGACACCGTGTTCAGCACCAGCCTCAGAAGAGGCCTCCTGCTCCGTCGGGCACACAAGTT
CACCAGCAGAAAGGCCCGCCCCTCCCCAGACCTCGAGTTCAGCCAAAACCTCCCCATGGGGCAG
CAGAAAACTCATTGTCCCCTTCCTCTAATTAA (SEQ ID NO: 17109)

[0207] An exemplary CSR CD2z protein of the disclosure comprises or consists of the amino acid sequence of (**CD2 Signal peptide**, *CD2 Extracellular Domain*, CD2 Transmembrane domain, ***CD2 Cytoplasmic Domain***, CD3z Intracellular Domain):

MSFPCKFVASFLLIFNVSSKGAVS*KEITNALETWGALGQDINLDIPSFQMSDDIDDIKWEKTSD
KKKIAQFRKEKETFKEKDTYKLFKNGTLKIKHLKTDDQDIYKVSIYDTKGKNVLEKIFDLKIQE
RVSKPKISWTCINTTLTCEVMNGTDPELNLYQDGKHLKLSQRVITHKWTTSLSAKFKCTAGNKV
SKESSVEPVSCPEKGLD*IYLIIGICGGGSLLMVFVALLVFYIT***KRKKQRSRRNDEELETRAHRV
ATEERGRKPHQIPASTPQNPATSQHPPPPPGHRSQAPSHRPPPPGHRVQHQPQKRPPAPSGTQV
HQQKGPPLPRPRVQPKPPHGAAENSLSPSSN**RVKFSRSADAPAYKQGQNQLYNELNLGRREEYD
VLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTAT
KDTYDALHMQALPPR (SEQ ID NO: 17062)

CD2 Signal peptide: MSFPCKFVASFLLIFNVSSKGAVS (SEQ ID NO: 17110)

CD2 Extracellular Domain:
KEITNALETWGALGQDINLDIPSFQMSDDIDDIKWEKTSDKKKIAQFRKEKETFKEKDTYKLFK
NGTLKIKHLKTDDQDIYKVSIYDTKGKNVLEKIFDLKIQERVSKPKISWTCINTTLTCEVMNGT
DPELNLYQDGKHLKLSQRVITHKWTTSLSAKFKCTAGNKVSKESSVEPVSCPEKGLD (SEQ
ID NO: 17111)

CD2 Transmembrane domain: IYLIIGICGGGSLLMVFVALLVFYIT (SEQ ID NO:
17112)

***CD2 Cytoplasmic Domain*:**
KRKKQRSRRNDEELETRAHRVATEERGRKPHQIPASTPQNPATSQHPPPPPGHRSQAPSHRPPP
PGHRVQHQPQKRPPAPSGTQVHQQKGPPLPRPRVQPKPPHGAAENSLSPSSN (SEQ ID NO:
17113)

CD3z Intracellular Domain:
RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK
DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO:
17102)

[0208] The present disclosure provides a non-naturally occurring CSR CD2 protein comprising, consisting essential of, or consisting of an amino acid sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:17062. The present disclosure provides a CD2 signal peptide comprising, consisting essential of, or consisting of an amino acid sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:17110. The present disclosure provides a CD2 extracellular domain comprising, consisting essential of, or consisting of an amino acid sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:17111. The present disclosure provides a CD2 transmembrande domain comprising, consisting essential of, or consisting of an amino acid sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:17112. The present disclosure provides a CD2 cytoplasmic domain comprising, consisting essential of, or consisting of an amino acid sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or

100% identical to SEQ ID NO:17113. The present disclosure provides a CD3z intracellular domain comprising, consisting essential of, or consisting of an amino acid sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:17102.

[0209] An exemplary nucleotide sequence encoding a CSR CD2z protein of the disclosure comprises or consists of the amino acid sequence of (**CD2 Signal peptide**, *CD2 Extracellular Domain*, CD2 Transmembrane domain, ***CD2 Cytoplasmic Domain***, CD3z Intracellular Domain):

ATGAGCTTCCCTTGCAAGTTCGTGGCCAGCTTCCTGCTGATCTTCAACGTGTCCTCTAAGGGCG
CCGTGTCC*AAAGAGATCACAAACGCCCTGGAAACCTGGGGAGCCCTCGGCCAGGATATTAACCT*
GGACATCCCCAGCTTCCAGATGAGCGACGACATCGATGACATCAAGTGGGAGAAAACCAGCGAC
AAGAAGAAGATCGCCCAGTTCCGGAAAGAGAAAGAGACATTCAAAGAGAAGGACACCTACAAGC
TGTTCAAGAACGGCACCCTGAAGATCAAGCACCTGAAAACCGACGACCAGGACATCTATAAGGT
GTCCATCTACGACACCAAGGGCAAGAACGTGCTGGAAAAGATCTTCGACCTCAAGATCCAAGAG
CGGGTGTCCAAGCCTAAGATCAGCTGGACCTGCATCAACACCACACTGACCTGCGAAGTGATGA
ACGGCACAGACCCCGAGCTGAACCTGTACCAGGATGGCAAACACCTGAAGCTGAGCCAGCGCGT
GATCACCCACAAGTGGACAACAAGCCTGAGCGCCAAGTTCAAGTGCACCGCCGGAAACAAAGTG
*TCTAAAGAGTCCAGCGTCGAGCCCGTGTCTTGCCCTGAAAAAGGACTGGAC*ATCTACCTGATCA
TCGGCATCTGTGGCGGCGGAAGCCTGCTGATGGTGTTTGTGGCTCTGCTGGTGTTCTACATCAC
C***AAGCGGAAGAAGCAGCGGAGCAGACGGAACGACGAGGAACTGGAAACACGGGCCCATAGAGTG***
GCCACCGAGGAAAGAGGCAGAAAGCCCCACCAGATTCCAGCCAGCACACCCCAGAATCCTGCCA
CCTCTCAACACCCTCCACCTCCACCTGGACACAGATCTCAGGCCCCATCTCACAGACCTCCACC
ACCTGGTCATCGGGTGCAGCACCAGCCTCAGAAAAGACCTCCTGCTCCTAGCGGCACACAGGTG
CACCAGCAAAAAGGACCTCCACTGCCTCGGCCTAGAGTGCAGCCTAAACCTCCTCATGGCGCCG
CTGAGAACAGCCTGTCTCCAAGCAGCAACAGAGTGAAGTTCAGCCGCAGCGCCGATGCTCCTGC
CTATAAGCAGGGACAGAACCAGCTGTACAACGAGCTGAATCTGGGGCGCAGAGAAGAGTACGAT
GTGCTGGACAAGCGGAGAGGCAGAGATCCTGAGATGGGCGGCAAGCCCAGACGGAAGAATCCTC
AAGAGGGCCTGTATAATGAGCTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCGGAAT
GAAGGGCGAGCGCAGAAGAGGCAAGGGACACGATGGACTGTATCAGGGCCTGAGCACCGCCACC
AAGGATACCTATGATGCCCTGCACATGCAGGCCCTGCCTCCAAGA (SEQ ID NO: 17063)

CD2 Signal peptide:
ATGAGCTTCCCTTGCAAGTTCGTGGCCAGCTTCCTGCTGATCTTCAACGTGTCCTCTAAGGGCG
CCGTGTCC (SEQ ID NO: 17114)

CD2 Extracellular Domain:
AAAGAGATCACAAACGCCCTGGAAACCTGGGGAGCCCTCGGCCAGGATATTAACCTGGACATCC
CCAGCTTCCAGATGAGCGACGACATCGATGACATCAAGTGGGAGAAAACCAGCGACAAGAAGAA
GATCGCCCAGTTCCGGAAAGAGAAAGAGACATTCAAAGAGAAGGACACCTACAAGCTGTTCAAG
AACGGCACCCTGAAGATCAAGCACCTGAAAACCGACGACCAGGACATCTATAAGGTGTCCATCT
ACGACACCAAGGGCAAGAACGTGCTGGAAAAGATCTTCGACCTCAAGATCCAAGAGCGGGTGTC

CAAGCCTAAGATCAGCTGGACCTGCATCAACACCACACTGACCTGCGAAGTGATGAACGGCACA
GACCCCGAGCTGAACCTGTACCAGGATGGCAAACACCTGAAGCTGAGCCAGCGCGTGATCACCC
ACAAGTGGACAACAAGCCTGAGCGCCAAGTTCAAGTGCACCGCCGGAAACAAAGTGTCTAAAGA
GTCCAGCGTCGAGCCCGTGTCTTGCCCTGAAAAAGGACTGGAC (SEQ ID NO: 17115)

CD2 Transmembrane domain:
ATCTACCTGATCATCGGCATCTGTGGCGGCGGAAGCCTGCTGATGGTGTTTGTGGCTCTGCTGG
TGTTCTACATCACC (SEQ ID NO: 17116)

***CD2 Cytoplasmic Domain*:**
AAGCGGAAGAAGCAGCGGAGCAGACGGAACGACGAGGAACTGGAAACACGGGCCCATAGAGTGG
CCACCGAGGAAAGAGGCAGAAAGCCCCACCAGATTCCAGCCAGCACACCCCAGAATCCTGCCAC
CTCTCAACACCCTCCACCTCCACCTGGACACAGATCTCAGGCCCCATCTCACAGACCTCCACCA
CCTGGTCATCGGGTGCAGCACCAGCCTCAGAAAAGACCTCCTGCTCCTAGCGGCACACAGGTGC
ACCAGCAAAAAGGACCTCCACTGCCTCGGCCTAGAGTGCAGCCTAAACCTCCTCATGGCGCCGC
TGAGAACAGCCTGTCTCCAAGCAGCAAC (SEQ ID NO: 17117)

CD3z Intracellular Domain:
AGAGTGAAGTTCAGCCGCAGCGCCGATGCTCCTGCCTATAAGCAGGGACAGAACCAGCTGTACA
ACGAGCTGAATCTGGGGCGCAGAGAAGAGTACGATGTGCTGGACAAGCGGAGAGGCAGAGATCC
TGAGATGGGCGGCAAGCCCAGACGGAAGAATCCTCAAGAGGGCCTGTATAATGAGCTGCAGAAA
GACAAGATGGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGAGCGCAGAAGAGGCAAGGGAC
ACGATGGACTGTATCAGGGCCTGAGCACCGCCACCAAGGATACCTATGATGCCCTGCACATGCA
GGCCCTGCCTCCAAGA (SEQ ID NO: 17107)

[0210] An exemplary mutant CSR CD2z-D111H protein of the disclosure comprises or consists of the amino acid sequence of (**CD2 Signal peptide**, *CD2 Extracellular domain* with *D111H mutation within the CD2 Extracellular domain*, CD2 Transmembrane domain, ***CD2 Cytoplasmic domain***, CD3z Intracellular domain):

MSFPCKFVASFLLIFNVSSKGAVS*KEITNALETWGALGQDINLDIPSFQMSDDIDDIKWEKTSD*
KKKIAQFRKEKETFKEKDTYKLFKNGTLKIKHLKTDDQDIYKVSIYHTKGKNVLEKIFDLKIQE
RVSKPKISWTCINTTLTCEVMNGTDPELNLYQDGKHLKLSQRVITHKWTTSLSAKFKCTAGNKV
*SKESSVEPVSCPEKGLD*IYLIIGICGGGSLLMVFVALLVFYIT***KRKKQRSRRNDEELETRAHRV***
ATEERGRKPHQIPASTPQNPATSQHPPPPPGHRSQAPSHRPPPPGHRVQHQPQKRPPAPSGTQV
HQQKGPPLPRPRVQPKPPHGAAENSLSPSSNRVKFSRSADAPAYKQGQNQLYNELNLGRREEYD
VLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTAT
KDTYDALHMQALPPR (SEQ ID NO: 17118)

CD2 Signal peptide: MSFPCKFVASFLLIFNVSSKGAVS (SEQ ID NO: 17110)

CD2 Extracellular domain with *D111H mutation within the CD2 Extracellular domain*:
KEITNALETWGALGQDINLDIPSFQMSDDIDDIKWEKTSDKKKIAQFRKEKETFKEKDTYKLFK
NGTLKIKHLKTDDQDIYKVSIYHTKGKNVLEKIFDLKIQERVSKPKISWTCINTTLTCEVMNGT

DPELNLYQDGKHLKLSQRVITHKWTTSLSAKFKCTAGNKVSKESSVEPVSCPEKGL (SEQ ID NO: 17119)

CD2 Transmembrane domain:
IYLIIGICGGGSLLMVFVALLVFYIT (SEQ ID NO: 17112)

***CD2 Cytoplasmic domain*:**
KRKKQRSRRNDEELETRAHRVATEERGRKPHQIPASTPQNPATSQHPPPPPGHRSQAPSHRPPP PGHRVQHQPQKRPPAPSGTQVHQQKGPPLPRPRVQPKPPHGAAENSLSPSSN (SEQ ID NO: 17113)

CD3z Intracellular domain:
RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR(SEQ ID NO: 17102)

[0211] The present disclosure provides a non-naturally occurring CSR CD2 protein comprising, consisting essential of, or consisting of an amino acid sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:17118. The present disclosure provides a CD2 extracellular domain comprising, consisting essential of, or consisting of an amino acid sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:17119.

[0212] An exemplary nucleotide sequence encoding a mutant CSR CD2z-D111H protein of the disclosure comprises or consists of the amino acid sequence of (**CD2 Signal peptide**, *CD2 Extracellular domain* with *D111H mutation within the CD2 Extracellular domain* , CD2 Transmembrane domain, ***CD2 Cytoplasmic domain*,** CD3z Intracellular domain):

ATGAGCTTCCCTTGCAAGTTCGTGGCCAGCTTCCTGCTGATCTTCAACGTGTCCTCTAAGGGCG
CCGTGTCC*AAAGAGATCACAAACGCCCTGGAAACCTGGGGAGCCCTCGGCCAGGATATTAACCT*
GGACATCCCCAGCTTCCAGATGAGCGACGACATCGATGACATCAAGTGGGAGAAAACCAGCGAC
AAGAAGAAGATCGCCCAGTTCCGGAAAGAGAAAGAGACATTCAAAGAGAAGGACACCTACAAGC
TGTTCAAGAACGGCACCCTGAAGATCAAGCACCTGAAAACCGACGACCAGGACATCTATAAGGT
GTCCATCTACCACACCAAGGGCAAGAACGTGCTGGAAAAGATCTTCGACCTCAAGATCCAAGAG
CGGGTGTCCAAGCCTAAGATCAGCTGGACCTGCATCAACACCACACTGACCTGCGAAGTGATGA
ACGGCACAGACCCCGAGCTGAACCTGTACCAGGATGGCAAACACCTGAAGCTGAGCCAGCGCGT
GATCACCCACAAGTGGACAACAAGCCTGAGCGCCAAGTTCAAGTGCACCGCCGGAAACAAAGTG
*TCTAAAGAGTCCAGCGTCGAGCCCGTGTCTTGCCCTGAAAAAGGACTGGAC*ATCTACCTGATCA
TCGGCATCTGTGGCGGCGGAAGCCTGCTGATGGTGTTTGTGGCTCTGCTGGTGTTCTACATCAC

CAAGCGGAAGAAGCAGCGGAGCAGACGGAACGACGAGGAACTGGAAACACGGGCCCATAGAGTG
GCCACCGAGGAAAGAGGCAGAAAGCCCCACCAGATTCCAGCCAGCACACCCCAGAATCCTGCCA
CCTCTCAACACCCTCCACCTCCACCTGGACACAGATCTCAGGCCCCATCTCACAGACCTCCACC
ACCTGGTCATCGGGTGCAGCACCAGCCTCAGAAAAGACCTCCTGCTCCTAGCGGCACACAGGTG
CACCAGCAAAAAGGACCTCCACTGCCTCGGCCTAGAGTGCAGCCTAAACCTCCTCATGGCGCCG
*CTGAGAACAGCCTGTCTCCAAGCAGCAAC*AGAGTGAAGTTCAGCCGCAGCGCCGATGCTCCTGC
CTATAAGCAGGGACAGAACCAGCTGTACAACGAGCTGAATCTGGGGCGCAGAGAAGAGTACGAT
GTGCTGGACAAGCGGAGAGGCAGAGATCCTGAGATGGGCGGCAAGCCCAGACGGAAGAATCCTC
AAGAGGGCCTGTATAATGAGCTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCGGAAT
GAAGGGCGAGCGCAGAAGAGGCAAGGGACACGATGGACTGTATCAGGGCCTGAGCACCGCCACC
AAGGATACCTATGATGCCCTGCACATGCAGGCCCTGCCTCCAAGA (SEQ ID NO: 17120)

CD2 Signal peptide:
ATGAGCTTCCCTTGCAAGTTCGTGGCCAGCTTCCTGCTGATCTTCAACGTGTCCTCTAAGGGCG
CCGTGTCC (SEQ ID NO: 17114)

CD2 Extracellular domain with *D111H mutation within the CD2 Extracellular domain*:
AAAGAGATCACAAACGCCCTGGAAACCTGGGGAGCCCTCGGCCAGGATATTAACCTGGACATCC
CCAGCTTCCAGATGAGCGACGACATCGATGACATCAAGTGGGAGAAAACCAGCGACAAGAAGAA
GATCGCCCAGTTCCGGAAAGAGAAAGAGACATTCAAAGAGAAGGACACCTACAAGCTGTTCAAG
AACGGCACCCTGAAGATCAAGCACCTGAAAACCGACGACCAGGACATCTATAAGGTGTCCATCT
ACCACACCAAGGGCAAGAACGTGCTGGAAAAGATCTTCGACCTCAAGATCCAAGAGCGGGTGTC
CAAGCCTAAGATCAGCTGGACCTGCATCAACACCACACTGACCTGCGAAGTGATGAACGGCACA
GACCCCGAGCTGAACCTGTACCAGGATGGCAAACACCTGAAGCTGAGCCAGCGCGTGATCACCC
ACAAGTGGACAACAAGCCTGAGCGCCAAGTTCAAGTGCACCGCCGGAAACAAAGTGTCTAAAGA
GTCCAGCGTCGAGCCCGTGTCTTGCCCTGAAAAAGGACTGGAC (SEQ ID NO: 17121)

CD2 Transmembrane domain:
ATCTACCTGATCATCGGCATCTGTGGCGGCGGAAGCCTGCTGATGGTGTTTGTGGCTCTGCTGG
TGTTCTACATCACC (SEQ ID NO: 17116)

***CD2 Cytoplasmic domain*:**
AAGCGGAAGAAGCAGCGGAGCAGACGGAACGACGAGGAACTGGAAACACGGGCCCATAGAGTGG
CCACCGAGGAAAGAGGCAGAAAGCCCCACCAGATTCCAGCCAGCACACCCCAGAATCCTGCCAC
CTCTCAACACCCTCCACCTCCACCTGGACACAGATCTCAGGCCCCATCTCACAGACCTCCACCA
CCTGGTCATCGGGTGCAGCACCAGCCTCAGAAAAGACCTCCTGCTCCTAGCGGCACACAGGTGC
ACCAGCAAAAAGGACCTCCACTGCCTCGGCCTAGAGTGCAGCCTAAACCTCCTCATGGCGCCGC
TGAGAACAGCCTGTCTCCAAGCAGCAAC (SEQ ID NO: 17117)

CD3z Intracellular domain:
AGAGTGAAGTTCAGCCGCAGCGCCGATGCTCCTGCCTATAAGCAGGGACAGAACCAGCTGTACA
ACGAGCTGAATCTGGGGCGCAGAGAAGAGTACGATGTGCTGGACAAGCGGAGAGGCAGAGATCC
TGAGATGGGCGGCAAGCCCAGACGGAAGAATCCTCAAGAGGGCCTGTATAATGAGCTGCAGAAA
GACAAGATGGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGAGCGCAGAAGAGGCAAGGGAC

ACGATGGACTGTATCAGGGCCTGAGCACCGCCACCAAGGATACCTATGATGCCCTGCACATGCA GGCCCTGCCTCCAAGA (SEQ ID NO:17107)

Endogenous TCR Knock-out

[0213] Gene editing compositions of the disclosure, including but not limited to, RNA-guided fusion proteins comprising dCas9-Clo051, may be used to target and decrease or eliminate expression of an endogenous T-cell receptor of an allogeneic cell of the disclosure. In preferred embodiments, the gene editing compositions of the disclosure target and delete a gene, a portion of a gene, or a regulatory element of a gene (such as a promoter) encoding an endogenous T-cell receptor of an allogeneic cell of the disclosure.

[0214] Nonlimiting examples of primers (including a T7 promoter, genome target sequence, and gRNA scaffold) for the generation of guide RNA (gRNA) templates for targeting and deleting TCR-alpha (TCR-α) are provided in Table 10.

[0215] Table 10. Target sequences underlined

Name	Sequence	SEQ ID NO:
TCRa-gRNA-WT 1	TAATACGACTCACTATA GCTGGTACACGGCAGGGTCA GTTTTAGAGCTAGAAATAG	16821
TCRa-gRNA-WT 2	TAATACGACTCACTATA GAGAATCAAAATCGGTGAAT	16822
TCRa-gRNA--WT 4	TAATACGACTCACTATA GTGCTAGACATGAGGTCTA	16823
TCRa-gRNA-WT 1-2G	TAATACGACTCACTATAG GCTGGTACACGGCAGGGTCA	16824
TCRa-gRNA-WT 2	TAATACGACTCACTATA GAGAATCAAAATCGGTGAAT GTTTTAGAGCTAGAAATAG	16825
TCRa-gRNA-WT 3	TAATACGACTCACTATA GGATTTAGAGTCTCTCAGC GTTTTAGAGCTAGAAATAG	16826
TCRa-gRNA-WT 4	TAATACGACTCACTATA GTGCTAGACATGAGGTCTA GTTTTAGAGCTAGAAATAG	16827
TCRa-gRNA-WT 5	TAATACGACTCACTATA GACACCTTCTTCCCCAGCCC GTTTTAGAGCTAGAAATAG	16828

TCRa-gRNA-NG1-L	TAATACGACTCACTATA g tggaataatgctgttgttga GTTTTAGAGCTAGAAATAG	16829
TCRa-gRNA-NG2-L	TAATACGACTCACTATA g catcacaggaactttctaaa GTTTTAGAGCTAGAAATAG	16830
TCRa-gRNA-NG3-L	TAATACGACTCACTATA gtaaaaccaagaggccacag GTTTTAGAGCTAGAAATAG	16831
TCRa-gRNA-NG4-L	TAATACGACTCACTATA g acccggccactttcaggagg GTTTTAGAGCTAGAAATAG	16832
TCRa-gRNA-NG5-L	TAATACGACTCACTATA gattaaacccggccactttc GTTTTAGAGCTAGAAATAG	16833
TCRa-gRNA-NG1-R	TAATACGACTCACTATA g agcccaggtaagggcagctt GTTTTAGAGCTAGAAATAG	16834
TCRa-gRNA-NG2-1-R	TAATACGACTCACTATA g agctttgaaacaggtaagac GTTTTAGAGCTAGAAATAG	16835
TCRa-gRNA-NG2-2-R	TAATACGACTCACTATA gctttgaaacaggtaagaca GTTTTAGAGCTAGAAATAG	16836
TCRa-gRNA-NG3-R	TAATACGACTCACTATA g tttcaaaacctgtcagtgat GTTTTAGAGCTAGAAATAG	16837
TCRa-gRNA-NG4-R	TAATACGACTCACTATA g ctgcggctgtggtccagctg GTTTTAGAGCTAGAAATAG	16838
TCRa-gRNA-NG5-1-R	TAATACGACTCACTATA gctgtggtccagctgaggtg GTTTTAGAGCTAGAAATAG	16839
TCRa-gRNA-NG5-2-R	TAATACGACTCACTATA g ctgtggtccagctgaggtga GTTTTAGAGCTAGAAATAG	16840
TCRa-gRNA-NG5-3-R	TAATACGACTCACTATA g tgtggtccagctgaggtgag GTTTTAGAGCTAGAAATAG	16841
TCRa-gRNA-NG5-3-Rb	TAATACGACTCACTATA gtgtggtccagctgaggtgag GTTTTAGAGCTAGAAATAG	16842

[0216] Nonlimiting examples of primers for the generation of guide RNA (gRNA) templates for targeting and deleting TCR-beta (TCR-β) are provided in Table 11.

[0217] Table 11. Target sequences underlined

Name	Sequence	SEQ ID NO:
TCRb-gRNA-WT 1	TAATACGACTCACTATA GGCTGCTCCTTGAGGGGCTG GTTTTAGAGCTAGAAATAG	16843
TCRb-gRNA-WT 2	TAATACGACTCACTATA GGCAGTATCTGGAGTCATTG GTTTTAGAGCTAGAAATAG	16844
TCRb-gRNA-WT 3	TAATACGACTCACTATA GGCCTCGGCGCTGACGATCT	16845
TCRb-gRNA-WT 5	TAATACGACTCACTATA GGCTCTCGGAGAATGACGAG	16846
TCRb-gRNA-WT 3	TAATACGACTCACTATA GGCCTCGGCGCTGACGATCT GTTTTAGAGCTAGAAATAG	16847
TCRb-gRNA-WT 4	TAATACGACTCACTATA GGAGAATGACGAGTGGACCC GTTTTAGAGCTAGAAATAG	16848
TCRb-gRNA-WT 5	TAATACGACTCACTATA GGCTCTCGGAGAATGACGAG GTTTTAGAGCTAGAAATAG	16849
TCRb-gRNA-NG1-L	TAATACGACTCACTATA G CAAACACAGCGACCTCGGGT GTTTTAGAGCTAGAAATAG	16850
TCRb-gRNA-NG2-L	TAATACGACTCACTATA G TGGCTCAAACACAGCGACCT GTTTTAGAGCTAGAAATAG	16851
TCRb-gRNA-NG3-L	TAATACGACTCACTATA G AGGGCGGGCTGCTCCTTGAG GTTTTAGAGCTAGAAATAG	16852
TCRb-gRNA-NG4-L	TAATACGACTCACTATA GTATCTGGAGTCATTGAGGG GTTTTAGAGCTAGAAATAG	16853
TCRb-gRNA-NG5-L	TAATACGACTCACTATA G ACTGGACTTGACAGCGGAAG GTTTTAGAGCTAGAAATAG	16854
TCRb-gRNA-NG1-R	TAATACGACTCACTATA G AGAGATCTCCCACACCCAAA GTTTTAGAGCTAGAAATAG	16855
TCRb-gRNA-NG2-R	TAATACGACTCACTATA G CCACACCCAAAAGGCCACAC GTTTTAGAGCTAGAAATAG	16856
TCRb-gRNA-NG3-R	TAATACGACTCACTATA G ACTGCCTGAGCAGCCGCCTG GTTTTAGAGCTAGAAATAG	16857

TCRb-gRNA-NG4-R	TAATACGACTCACTATA G TGAGGGTCTCGGCCACCTTC GTTTTAGAGCTAGAAATAG	16858
TCRb-gRNA-NG5-R	TAATACGACTCACTATA G ATGACGAGTGGACCCAGGAT GTTTTAGAGCTAGAAATAG	16859
TCRb-gRNA-NG6-L	TAATACGACTCACTATA G TGGCTCAAACACAGCGACCT GTTTTAGAGCTAGAAATAG	16860
TCRb-gRNA-NG6-R	TAATACGACTCACTATA G CCACACCCAAAAGGCCACAC GTTTTAGAGCTAGAAATAG	16861

[0218] Nonlimiting examples of primers for the generation of guide RNA (gRNA) templates for targeting and deleting beta-2-microglobulin (β2M) are provided in Table 12.

[0219] Table 12. Target sequences underlined

Primer No.	Name	Sequence	SEQ ID NO:
1	B2-Prom-NG1-R	TAATACGACTCACTATAG AGACAGGTGACGGTCCCTGC GTTTTAGAGCTAGAAATAG	16862
2	B2-Prom-NG1-L	TAATACGACTCACTATA GCAGTGCCAGGTTAGAGAGA GTTTTAGAGCTAGAAATAG	16863
3	B2-Ex2-NG-R	TAATACGACTCACTATA GAAGTTGACTTACTGAAGAA GTTTTAGAGCTAGAAATAG	16864
4	B2-Ex2-NG-L	TAATACGACTCACTATA G ACCCAGACACATAGCAATTC GTTTTAGAGCTAGAAATAG	16865
5	**B2-Ex2-NG2-R**	TAATACGACTCACTATA G TCACGTCATCCAGCAGAGAA GTTTTAGAGCTAGAAATAG	16866
6	**B2-Ex2-NG2-L**	TAATACGACTCACTATA gatattcctcagGTACTCCA GTTTTAGAGCTAGAAATAG	16867
7	b2MEx1 NG-left	TAATACGACTCACTATA GGCCACGGAGCGAGACATCT GTTTTAGAGCTAGAAATAG	16868
8	b2MEx1 NG-right	TAATACGACTCACTATAG ACTCTCTCTTTCTGGCCTGG GTTTTAGAGCTAGAAATAG	16869

9	b2M-gRNA WT Ex2	TAATACGACTCACTATAG GAGAGAGAATTGAAAAAG GTTTTAGAGCTAGAAATAG	16870

Endogenous MHC Knock-out

[0220] Gene editing compositions of the disclosure, including but not limited to, RNA-guided fusion proteins comprising dCas9-Clo051, may be used to target and decrease or eliminate expression of an endogenous MHCI, MHCII, or MHC activator of an allogeneic cell of the disclosure. In preferred embodiments, the gene editing compositions of the disclosure target and delete a gene, a portion of a gene, or a regulatory element of a gene (such as a promoter) encoding one or more components of an endogenous MHCI, MHCII, or MHC activator of an allogeneic cell of the disclosure.

[0221] Nonlimiting examples of guide RNAs (gRNAs) for targeting and deleting MHC activators are provided in Tables 13 and 14.

[0222] Table 13.

Gene	Reagent/ Type	Left Target Sequence	SEQ ID NO:	Right Target Sequence	SEQ ID NO
C2TA	C2TA exon 4 NG	CATCGCTGTTAAGAAGCTCC	16871	CTACCACTTCTATGACCAGA	16880
	C2TA exon6 NG	GGCCCTCCAGCTGGGAGTCC	16872	CAGTAAGTTTGTGGTGGGTG	16881
RFXANK	RFXANK exon1 NG1	GGGTCTGCTGGGTCTGGATG	16873	GGACCCTGAAGACCCCGGAG	16882
	RFXANK exon1 NG2	GTTCTGAGGCAGGGGTCTGC	16874	CCCGGAGAGGAGGCTGCAGA	16883
RFXAP	RFXAP Exon 1 NG1	CCCGCCCCAACGCTGCCCCC	16875	CTGTGCGAAGGGGCCGGGGA	16884
	RFXAP Exon 1 NG2	CCTTCGCACAGGTACCTAAC	16876	AGAGGAGGCTGGGGAGGACG	16885
RFX5	RFX5 exon 1 NG1	GTCTTGGGGCTCTTAGCATC	16877	CCCAGGTGGTGCTGAGGCTG	16886
	RFX5 exon 2 NG2	ACGGCCTTGCTGTGGGGAAG	16878	GGGATCCTGGTAAGTGTGTT	16887

	RFX5 exon5 NG3	TCTGATGATCTTGCCAAAGT	16879	ATCAAAGCTCGAAGGCTTGG	16888

[0223] **Table 14.**

Gene	Reagent/Type	Exon or region	NG-Left Target Sequence	SEQ ID NO:	NG-Right Target Sequence	SEQ ID NO:	Target sequence (if WT crispr)	SEQ ID NO
Beta2-MG	B2-Promoter-NG1	promoter	GCAGTGCCAGGTTAGAGAGA	16889	AGACAGGTGACGGTCCCTGC	**16913**		
	B2-Promoter-NG2	promoter	CAAGCCAGCGACGCAGTGCC	16890	CCTGCGGGCCTTGTCCTGAT	16914		
	B2-Promoter-NG3	promoter	CCAATCAGGACAAGGCCCGC	16891	TATAAGTGGAGGCGTCGCGC	16915		
	B2-Ex2-NG	exon 2	ACCCAGACACATAGCAATTC	16892	GAAGTTGACTTACTGAAGAA	16916		
	B2-Ex2-NG2	**exon 2**	**gatattcctcagGTACTCCA**	**16893**	**TCACGTCATCCAGCAGAGAA**	16917		
	B2-Ex1-NG	exon 1	GGCCACGGAGCGAGACATCT	16894	ACTCTCTCTTTCTGGCCTGG	16918		
	WT-B2MG-exon2						GGAGAGAGAATTGAAAAG	16937
	WT-B2MG-promoter-4	cuts in promoter region Y					GGGCCTTGTCCTGATTGGC	16938
	WT-B2MG-promoter-5	cuts in promoter region					GGCACTGCGTCGCTGGCT	16939
C2TA	C2TA exon 4 NG	exon 4	CATCGCTGTTAAGAAGCTCC	16895	CTACCACTTCTATGACCAGA	16919		
	C2TA exon4 NG2	**exon 4**	**GGTCCATCTGGTCATAGAAG**	**16896**	**AGATTGAGCTCTACTCAGGT**	16920		
	C2TA exon6 NG	exon6	GGCCCTCCAGCTGGGAGTCC	16897	CAGTAAGTTTGTGGTGGGTG	16921		
	C2TA exon4-WT	exon 4					GGTCCATCTGGTCATAGAAG	16940
	C2TA exon6-WT	exon6					GGAGTCCTGGAAGACATAC	16941
	C2TA exon6 NG2	**exon6**	**CCTTGCTCAGGCCCTCCAGC**	**16898**	**TGTGGTGGGTGGGGAGGTCT**	16922		

RFXANK	RFXANK exon1 NG1	exon 1	GGGTCTGCTGGGTCTGGATG	16899	GGACCCTGAAGACCCCGGAG	16923			
	RFXANK exon1 NG2	exon 1	GTTCTGAGGCAGGGGTCTGC	16900	CCCGGAGAGGAGGCTGCAGA	16924			
	RFXANK exon2 NG1	**exon 2**	**TGAGAGTGGTGGAGTGCTTC**	**16901**	**GAACGAGGTGTCAGCTCTGC**	16925			
	RFXANK Exon2 NG2	**exon 2**	**CTCGTTCCCTCGCTGCCGGT**	**16902**	**GGCCACCCTAGACTGTGAGT**	16926			
	RFXANK-WT-exon1-3	exon 1					GGTCCCCAAGTTCTGAGGC	16942	
	RFXANK-WT-exon1-4	exon1					GGCAGGGGTCTGCTGGGTC	16943	
RFXAP	RFXAP Exon 1 NG1	exon 1	CCCGCCCCAACGCTGCCCCC	16903	CTGTGCGAAGGGGCCGGGGA	16927			
	RFXAP Exon 1 NG2	exon 1	CCTTCGCACAGGTACCTAAC	16904	AGAGGAGGCTGGGGAGGACG	16928			
	RFXAP Exon1 NG3	**exon 1**	**CAGCCGGGGCTAGGGCCGCG**	**16905**	**CTTGGCGCCAGCCTCGGTGG**	16929			
	RFXAP Exon1 NG4	**exon 1**	**GCCGCGGCCGCCACCGAGGC**	**16906**	**CTAGTGATGCAACCCTGTGC**	16930			
	RFXAP Exon1 NG5	**exon 1**	**GCCGCGCTCTCGCCTCCCCC**	**16907**	**GAGGACGAGGAGACTCACTC**	16931			
	WT- RFXAP-ex1-3	exon 1					GGCCCCCGGGGGCAGCGTT	16944	
	WT- RFXAP-ex1-4	exon 1					GGTACCTGTGCGAGGGGGC	16945	
RFX5	RFX5 exon1 NG1	exon 1	GTCTTGGGGCTCTTAGCATC	16908	CCCAGGTGGTGCTGAGGCTG	16932			
	RFX5 exon2 NG2	exon 2	ACGGGCCTTGCTGTGGGGAAG	16909	GGGATCCTGGTAAGTGTGTT	16933			
	RFX5 exon5 NG3	exon5	TCTGATGATCTTTGCCAAAGT	16910	ATCAAAGCTCGAAGGCTTGG	16934			
	RFX5 exon1 NG2		**GTCTTGGGGCTCTTAGCATC**	**16911**	**CCCCAGGTGGTGCTGAGGCT**	16935			
	RFX5 exon1 NG3		**AGGCTCATCTTTCTGCCATCC**	**16912**	**ACTGGGGGAAGGGGCCCCCCC**	16936			

WT- RFX5-ex1-4	Exon1						GGGAAGGGGCCCCCCCAGG	16946
WT- RFX5-ex5-5	Exon 5						GCCTTCGAGCTTTGATGTC	16947

Engineered HLA-E compositions

[0224] MHCI knockout (KO) renders cells resistant to killing by T cells, but also makes them susceptible to natural killer (NK) cell-mediated cytotoxicity ("Missing-self hypothesis") (see FIG. 30). It is hypothesized that NK rejection would reduce the in vivo efficacy and/or persistence of these KO cells in a therapeutic setting, such as allogeneic (allo) CAR-T therapy. Retention of MHCI on the surface of allo CAR-T cells would render them susceptible to killing by host T cells, as observed in the classic mixed lymphocyte reaction (MLR) experiment. It is estimated that up to 10% of a person's T cells are specific to foreign MHC, which would mediate the rejection of foreign cells and tissues. A targeted KO of MHCI, specifically HLA-A, B and C, which can be achieved by targeted KO of B2M, results in a loss of additional HLA molecules including HLA-E. Loss of HLA-E, for example, renders the KO cells more susceptible to NK cell-mediated cytotoxicity due to the "Missing-self Hypothesis". NK-mediated cytotoxicity against missing-self cells is a defense mechanism against pathogens that downregulate MHC on the surface of infected cells to evade detection and killing by cells of the adaptive immune system.

[0225] Two strategies are contemplated by the disclosure for engineering allo (MHCI-neg) T cells (including CAR-T cells) more resistant to NK cell-mediated cytotoxicity. In some embodiments, a sequence encoding a molecule (such as single-chain HLA-E) that reduces or prevents NK killing is introduced or delivered to an allogeneic cell. Alternatively, or in addition, gene editing methods of the disclosure retain certain endogenous HLA molecules (such as endogenous HLA-E). For example, the first approach involves piggyBac® (PB) delivery of a single-chain (sc)HLA-E molecule to B2M KO T cells.

[0226] The second approach uses a gene editing composition with guide RNAs selective for HLA-A, HLA-B and HLA-C, but not, for example, HLA-E or other molecules that are protective against natural-killer cell mediated cytotoxicity for MHCI KO cells.

[0227] Alternative or additional molecules to HLA-E that are protective against NK cell-mediated cytotoxicity include, but are not limited to, CD47, interferon alpha/beta receptor 1 (IFNAR1), human IFNAR1, interferon alpha/beta receptor 2 (IFNAR2), human IFNAR2, HLA-G1, HLA-G2, HLA-G3, HLA-G4, HLA-G5, HLA-G6, HLA-G7, human carcino embryonic antigen-related cell adhesion molecule 1 (CEACAM1), viral hemoagglutinins, CD48, LLT1 (also referred to as C-type lectin domain family 2 member (CLC2D)), ULBP2, ULBP3, and sMICA or a variant thereof.

[0228] An exemplary CD47 protein of the disclosure comprises or consists of the amino acid sequence of (Signal peptide, Extracellular, *TM*, *Cytoplasmic*):

MWPLVAALLLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEVYV
KWKFKGRDIYTFDGALNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNY
TCEVTELTREGETIIELKYRVVSWFSPNENI*LIVIFPIFAILLFWGQFGIKTLKYRSGGM*
DEKTIALLVAGLVITVIVIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYYVFSTAIG
LTSFVIAILVIQVIAYILAVVGLSLCIAACIPMHGPLLISGLSILALAQLLGLVYMKFVAS
NQKTIQPPRKAVEEPLNAFKESKGMMNDE (SEQ ID NO: 17016)

[0229] An exemplary INFAR1 protein of the disclosure comprises or consists of the amino acid sequence of (Signal peptide, Extracellular, *TM*, *Cytoplasmic*):

MMVVLLGATTLVLVAVAPWVLSAAAGGKNLKSPQKVEVDIIDDNFILRWNRSDESVGNVT
FSFDYQKTGMDNWIKLSGCQNITSTKCNFSSLKLNVYEEIKLRIRAEKENTSSWYEVDSF
TPFRKAQIGPPEVHLEAEDKAIVIHISPGTKDSVMWALDGLSFTYSLVIWKNSSGVEERI
ENIYSRHKIYKLSPETTYCLKVKAALLTSWKIGVYSPVHCIKTTVENELPPPENIEVSVQ
NQNYVLKWDYTYANMTFQVQWLHAFLKRNPGNHLYKWKQIPDCENVKTTQCVFPQNVFQK
GIYLLRVQASDGNNTSFWSEEIKFDTEIQAFLLPPVFNIRSLSDSFHIYIGAPKQSGNTP
VIQDYPLIYEIIFWENTSNAERKIIEKKTDVTVPNLKPLTVYCVKARAHTMDEKLNKSSV
FSDAVCEKTKPGNTSK*IWLIVGICIALFALPFVIYAA*KVFLRCINYVFFPSLKPSSSIDE
YFSEQPLKNLLLSTSEEQIEKCFIIENISTIATVEETNQTDEDHKKYSSQTSQDSGNYSN
EDESESKTSEELQQDFV (SEQ ID NO: 17017).

[0230] An exemplary INFAR2 protein of the disclosure comprises or consists of the amino acid sequence of (Signal peptide, Extracellular, *TM*, *Cytoplasmic*):

MLLSQNAFIFRSLNLVLMVYISLVFGISYDSPDYTDESCTFKISLRNFRSILSWELKNHS
IVPTHYTLLYTIMSKPEDLKVVKNCANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLF
SCSHNFWLAIDMSFEPPEFEIVGFTNHINVMVKFPSIVEEELQFDLSLVIEEQSEGIVKK
HKPEIKGNMSGNFTYIIDKLIPNTNYCVSVYLEHSDEQAVIKSPLKCTLLPPGQESESAE
SAK*IGGIITVFLIALVLTSTIVTL*KWIGYICLRNSLPKVLNFHNFLAWPFPNLPPLEAMD
MVEVIYINRKKKVWDYNYDDESDSDTEAAPRTSGGGYTMHGLTVRPLGQASATSTESQLI
DPESEEEPDLPEVDVELPTMPKDSPQQLELLSGPCERRKSPLQDPFPEEDYSSTEGSGGR
ITFNVDLNSVFLRVLDDEDSDDLEAPLMLSSHLEEMVDPEDPDNVQSNHLLASGEGTQPT
FPSPSSEGLWSEDAPSDQSDTSESDVDLGDGYIMR (SEQ ID NO: 17018).

[0231] An exemplary HLA-G1 protein of the disclosure comprises or consists of the amino acid sequence of (**Alpha chain 1**, *Alpha chain 2*, Alpha chain 3):

MVVMAPRTLFLLLSGALTLTETWA**GSHSMRYFSAAVSRPGRGEPRFIAMGYVDDTQFVRFDS**
DSACPRMEPRAPWVEQEGPEYWEEETRNTKAHAQTDRMNLQTLRGYYNQSEA*SSHTLQWMIG*
CDLGSDGRLLRGYEQYAYDGKDYLALNEDLRSWTAADTAAQISKRKCEAANVAEQRRAYLEG
*TCVEWLHRYLENGKEMLQRA*DPPKTHVTHHPVFDYEATLRCWALGFYPAEIILTWQRDGEDQ
TQDVELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQHEGLPEPLMLRWKQSSLPTIPIMG
IVAGLVVLAAVVTGAAVAAVLWRKKSSD (SEQ ID NO: 17019).

[0232] An exemplary HLA-G2 protein of the disclosure comprises or consists of the amino acid sequence of (**Alpha chain 1**, *Alpha chain 2*, Alpha chain 3):

MVVMAPRTLFLLLSGALTLTETWA**GSHSMRYFSAAVSRPGRGEPRFIAMGYVDDTQFVRFDS DSACPRMEPRAPWVEQEGPEYWEEETRNTKAHAQTDRMNLQTLRGYYNQSEA**DPPKTHVTHH PVFDYEATLRCWALGFYPAEIILTWQRDGEDQTQDVELVETRPAGDGTFQKWAAVVVPSGEE QRYTCHVQHEGLPEPLMLRWKQSSLPTIPIMGIVAGLVVLAAVVTGAAVAAVLWRKKSSD (SEQ ID NO: 17020).

[0233] An exemplary HLA-G3 protein of the disclosure comprises or consists of the amino acid sequence of (**Alpha chain 1**, *Alpha chain 2*, Alpha chain 3):

MVVMAPRTLFLLLSGALTLTETWA**GSHSMRYFSAAVSRPGRGEPRFIAMGYVDDTQFVRFDS DSACPRMEPRAPWVEQEGPEYWEEETRNTKAHAQTDRMNLQTLRGYYNQSEA**KQSSLPTIPI MGIVAGLVVLAAVVTGAAVAAVLWRKKSSD (SEQ ID NO: 17021).

[0234] An exemplary HLA-G4 protein of the disclosure comprises or consists of the amino acid sequence of (**Alpha chain 1**, *Alpha chain 2*, Alpha chain 3):

MVVMAPRTLFLLLSGALTLTETWA**GSHSMRYFSAAVSRPGRGEPRFIAMGYVDDTQFVRFDS DSACPRMEPRAPWVEQEGPEYWEEETRNTKAHAQTDRMNLQTLRGYYNQSEA***SSHTLQWMIG CDLGSDGRLLRGYEQYAYDGKDYLALNEDLRSWTAADTAAQISKRKCEAANVAEQRRAYLEG TCVEWLHRYLENGKEMLQRA*KQSSLPTIPIMGIVAGLVVLAAVVTGAAVAAVLWRKKSSD (SEQ ID NO: 17022).

[0235] An exemplary HLA-G5 protein of the disclosure comprises or consists of the amino acid sequence of (**Alpha chain 1**, *Alpha chain 2*, Alpha chain 3, ***intron 4***):

MVVMAPRTLFLLLSGALTLTETWA**GSHSMRYFSAAVSRPGRGEPRFIAMGYVDDTQFVRFDS DSACPRMEPRAPWVEQEGPEYWEEETRNTKAHAQTDRMNLQTLRGYYNQSEA***SSHTLQWMIG CDLGSDGRLLRGYEQYAYDGKDYLALNEDLRSWTAADTAAQISKRKCEAANVAEQRRAYLEG TCVEWLHRYLENGKEMLQRA*DPPKTHVTHHPVFDYEATLRCWALGFYPAEIILTWQRDGEDQ TQDVELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQHEGLPEPLMLRW***SKEGDGGIMSVR ESRSLSEDL*** (SEQ ID NO: 17023).

[0236] An exemplary HLA-G5 protein of the disclosure comprises or consists of the amino acid sequence of (**Alpha chain 1**, *Alpha chain 2*, Alpha chain 3, ***intron 4***):

MVVMAPRTLFLLLSGALTLTETWA**GSHSMRYFSAAVSRPGRGEPRFIAMGYVDDTQFVRFDS DSACPRMEPRAPWVEQEGPEYWEEETRNTKAHAQTDRMNLQTLRGYYNQSEA**DPPKTHVTHH PVFDYEATLRCWALGFYPAEIILTWQRDGEDQTQDVELVETRPAGDGTFQKWAAVVVPSGEE QRYTCHVQHEGLPEPLMLRW***SKEGDGGIMSVRESRSLSEDL*** (SEQ ID NO: 17024).

[0237] An exemplary HLA-G5 protein of the disclosure comprises or consists of the amino acid sequence of (**Alpha chain 1**, *Alpha chain 2*, Alpha chain 3, ***intron 2***):

MVVMAPRTLFLLLSGALTLTETWA**GSHSMRYFSAAVSRPGRGEPRFIAMGYVDDTQFVRFDS DSACPRMEPRAPWVEQEGPEYWEEETRNTKAHAQTDRMNLQTLRGYYNQSEA*SE*** (SEQ ID NO: 17025).

[0238] An exemplary CEACAM1 protein of the disclosure comprises or consists of the amino acid sequence of (**Extracellular**, *TM*, Cytoplasmic):

MGHLSAPLHRVRVPWQGLLLTASLLTFWNPPTTA**QLTTESMPFNVAEGKEVLLLVHNLPQ**
QLFGYSWYKGERVDGNRQIVGYAIGTQQATPGPANSGRETIYPNASLLIQNVTQNDTGFY
TLQVIKSDLVNEEATGQFHVYPELPKPSISSNNSNPVEDKDAVAFTCEPETQDTTYLWWI
NNQSLPVSPRLQLSNGNRTLTLLSVTRNDTGPYECEIQNPVSANRSDPVTLNVTYGPDTP
TISPSDTYYRPGANLSLSCYAASNPPAQYSWLINGTFQQSTQELFIPNITVNNSGSYTCH
ANNSVTGCNRTTVKTIIVTELSPVVAKPQIKASKTTVTGDKDSVNLTCSTNDTGISIRWF
FKNQSLPSSERMKLSQGNTTLSINPVKREDAGTYWCEVFNPISKNQSDPIMLNVNYNALP
QENGLSPG*AIAGIVIGVVALVALIAVALACFL*HFGKTGRASDQRDLTEHKPSVSNHTQDH
SNDPPNKMNEVTYSTLNFEAQQPTQPTSASPSLTATEIIYSEVKKQ (SEQ ID NO: 17026).

[0239] An exemplary viral hemagglutinin protein of the disclosure comprises or consists of the amino acid sequence of (HA for Influenza A virus(A/NewCaledonia/20/1999(H1N1); **TM**):

MKAKLLVLLCTFTATYADTICIGYHANNSTDTVDTVLEKNVTVTHSVNLLEDSHNGKLCL
LKGIAPLQLGNCSVAGWILGNPECELLISKESWSYIVETPNPENGTCYPGYFADYEELRE
QLSSVSSFERFEIFPKESSWPNHTVTGVSASCSHNGKSSFYRNLLWLTGKNGLYPNLSKS
YVNNKEKEVLVLWGVHHPPNIGNQRALYHTENAYVSVVSSHYSRRFTPEIAKRPKVRDQE
GRINYYWTLLEPGDTIIFEANGNLIAPWYAFALSRGFGSGIITSNAPMDECDAKCQTPQG
AINSSLPFQNVHPVTIGECPKYVRSAKLRMVTGLRNIPSIQSRGLFGAIAGFIEGGWTGM
VDGWYGYHHQNEQGSGYAADQKSTQNAINGITNKVNSVIEKMNTQFTAVGKEFNKLERRM
ENLNKKVDDGFLDIWTYNAELLVLLENERTLDFHDSNVKNLYEKVKSQLKNNAKEIGNGC
FEFYHKCNNECMESVKNGTYDYPKYSEESKLNREKIDGVKLESMGVYQI**LAIYSTVASSL**
VLLVSLGAISFWMCSNGSLQCRICI (SEQ ID NO: 17027).

[0240] An exemplary CD48 protein of the disclosure comprises or consists of the amino acid sequence of (Signal peptide, **Chain**, Pro peptide removed in mature form):

MCSRGWDSCLALELLLLPLSLLVTSI**QGHLVHMTVVSGSNVTLNISESLP**
ENYKQLTWFYTFDQKIVEWDSRKSKYFESKFKGRVRLDPQSGALYISKVQ
KEDNSTYIMRVLKKTGNEQEWKIKLQVLDPVPKPVIKIEKIEDMDDNCYL
KLSCVIPGESVNYTWYGDKRPFPKELQNSVLETTLMPHNYSRCYTCQVSN
SVSSKNGTVCLSPPCTLARS*FGVEWIASWLVVTVPTILGLLLT* (SEQ ID NO: 17028).

[0241] An exemplary LLT1 protein of the disclosure comprises or consists of the amino acid sequence of (**Cytoplasmic**, *TM*, Extracellular):

MHDSNNVEKDITPSELPANPGCLHSKEHSIKATLIWRL*FFLIMFLTIIVCGMVAALSAIR*
ANCHQEPSVCLQAACPESWIGFQRKCFYFSDDTKNWTSSQRFCDSQDADLAQVESFQELN
FLLRYKGPSDHWIGLSREQGQPWKWINGTEWTRQFPILGAGECAYLNDKGASSARHYTER
KWICSKSDIHV (SEQ ID NO: 17029).

[0242] An exemplary ULBP2 protein of the disclosure comprises or consists of the amino acid sequence of (also known as NKG2D ligand; Genbank ACCESSION No. AAQ89028):

```
  1 maaaaatkil lclplllls gwsragradp hslcyditvi pkfrpgprwc avqgqvdekt
 61 flhydcgnkt vtpvsplgkk lnvttawkaq npvlrevvdi lteqlrdiql enytpkeplt
121 lqarmsceqk aeghssgswq fsfdgqifll fdsekrmwtt vhpgarkmke kwendkvvam
181 sfhyfsmgdc igwledflmg mdstlepsag aplamssgtt qlratattli lcclliilpc
241 filpgi (SEQ ID NO: 17030).
```

[0243] An exemplary ULBP3 protein of the disclosure comprises or consists of the amino acid sequence of (also known as NKG2D ligand; Genbank ACCESSION No. NP_078794):

```
  1 maaaaspail prlailpyll fdwsgtgrad ahslwynfti ihlprhgqqw cevqsqvdqk
 61 nflsydcgsd kvlsmghlee qlyatdawgk qlemirevgq rlrleladte iedftpsgpl
121 tlqvrmscec eadgyirgsw qfsfdgrkfl lfdsnnrkwt vvhagarrmk ekwekdsglt
181 tffkmvsmrd ckswlrdflm hrkkrlepta pptmapglaq pkaiattlsp wsfliilcfi
241 lpgi (SEQ ID NO: 17031).
```

[0244] An exemplary sMICA protein of the disclosure comprises or consists of the amino acid sequence of (Signal Peptide, **Portion of Extracellular domain**, *TM and cytoplasmic domain*) (Genbank Accession No. Q29983):

```
  1 mglgpvflll agifpfappg aaaephslry nltvlswdgs vqsgfltevh ldgqpflrcd
 61 rqkcrakpqg qwaedvlgnk twdretrdlt gngkdlrmtl ahikdqkegl hslqeirvce
121 ihednstrss qhfyydgelf lsqnletkew tmpqssraqt lamnvrnflk edamktkthy
181 hamhadclqe lrrylksgvv lrrtvppmvn vtrseasegn itvtcrasgf ypwnitlswr
241 qdgvslshdt qqwgdvlpdg ngtyqtwvat ricqgeeqrf tcymehsgnh sthpvpsgkv
301 lvlqshwqtf hvsavaaaai fviiifyvrc ckkktsaaeg pelvslqvld qhpvgtsdhr
361 datqlgfqpl msdlgstgst ega (SEQ ID NO: 17032).
```

[0245] An exemplary sMICA protein of the disclosure comprises or consists of the amino acid sequence of (Alpha-1, **Alpha-2**, *Alpha-3*):

```
  1 mglgpvflll agifpfappg aaaephslry nltvlswdgs vqsgfltevh ldgqpflrcd
 61 rqkcrakpqg qwaedvlgnk twdretrdlt gngkdlrmtl ahikdqkegl hslqeirvce
121 ihednstrss qhfyydgelf lsqnletkew tmpqssraqt lamnvrnflk edamktkthy
181 hamhadclqe lrrylksgvv lrrtvppmvn vtrseasegn itvtcrasgf ypwnitlswr
241 qdgvslshdt qqwgdvlpdg ngtyqtwvat ricqgeeqrf tcymehsgnh sthpvpsgkv
301 lvlqshwqtf hvsavaaaai fviiifyvrc ckkktsaaeg pelvslqvld qhpvgtsdhr
361 datqlgfqpl msdlgstgst ega (SEQ ID NO: 17033).
```

[0246] An exemplary sMICA protein of the disclosure comprises or consists of the amino acid sequence of (***Signal peptide***; Alpha-1, **Alpha-2**, *Alpha-3*):

```
MGGVLLTQRTLLSLVLALLFPSMASM  ephslry nltvlswdgs vqsgfltevh ldgqpflrcd
 61 rqkcrakpqg qwaedvlgnk twdretrdlt gngkdlrmtl ahikdqkegl hslqeirvce
121 ihednstrss qhfyydgelf lsqnletkew tmpqssraqt l                  thy
181 hamhadclqe lrrylksgvv lrrtvppmvn vtrseasegn itvtcrasgf ypwnitlswr
241 qdgvslshdt qqwgdvlpdg ngtyqtwvat ricqgeeqrf tcymehsgnh sthpvpsgkv
301 lvlqshw (SEQ ID NO: 17034).
```

[0247] An exemplary sMICA protein of the disclosure comprises or consists of the amino acid sequence of (***Signal peptide***):

MGGVLLTQRTLLSLVLALLFPSMASMEPHSLRYNLTVLSWDGSVQSGFLTEVHLDGQPFLRC
DRQKCRAKPQGQWAEDVLGNKTWDRETRDLTGNGKLDLRMTLAHIKLDQKEGLHSLQEIRVC
EIHEDNSTRSSQHFYYNGELFLSQNLETKEWTMPQSSRAQTLTHYHAMHADCLQELRRYLKS
GVVLRRTVPPMVDVTRSEASEGNITVTCRASGFYPWNITLSWRQDGVSLSHDTQQWGDVLPD
GNGTYQTWVATRICQGEEQRFTCYMEHSGNHSTHPVPSGKVLVLQSHW (SEQ ID NO:
17035).

[0248] An exemplary bGBE Trimer (**270G** and ***484S***) protein of the disclosure comprises or consists of the amino acid sequence of:

MSRSVALAVLALLSLSGLEAVMAPRTLILGGGGSGGGGSGGGGSIQRTPKIQVYSRHPAENG
KSNFLNCYVSGFHPSDIEVDLLKNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYAC
RVNHVTLSQPKIVKWDRDMGGGGSGGGGSGGGGSGGGGSGSHSLKYFHTSVSRPGRGEPRFI
SVGYVDDTQFVRFDNDAASPRMVPRAPWMEQEGSEYWDRETRSARDTAQIFRVNLRTLRGYY
NQSEAGSHTLQWMHGCELGPD**G**RFLRGYEQFAYDGKDYLTLNEDLRSWTAVDTAAQISEQKS
NDASEAEHQRAYLEDTCVEWLHKYLEKGKETLLHLEPPKTHVTHHPISDHEATLRCWALGFY
PAEITLTWQQDGEGHTQDTELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQHEGLPEPVT
LRWKPASQPTIPIVGIIAGLVLLGSVVSGAVVAAVIWRKKSSGGKGGSY***S***KAEWSDSAQGSE
SHSL* (SEQ ID NO: 16972).

[0249] An exemplary bGBE Trimer (**270G** and ***484S***) protein of the disclosure comprises or consists of the nucleic acid sequence of:

atgtctcgcagcgtggccctggccgtgctggccctgctgtccctgtctggcctggaggccgt
gatggccccccggaccctgatcctgggaggaggaggcagcggcggaggaggctccggaggcg
gcggctctatccagcgcacacctaagatccaggtgtattctcggcacccagccgagaacggc
aagagcaacttcctgaattgctacgtgagcggctttcacccttccgacatcgaggtggatct
gctgaagaatggcgagagaatcgagaaggtggagcactccgacctgagcttctccaaggatt
ggtctttttatctgctgtactataccgagtttacccctacagagaaggacgagtacgcctgt
cgcgtgaaccacgtgacactgtcccagccaaagatcgtgaagtgggaccgggatatgggcgg
cggcggctctggcggcggcggcagcggcggcggcggctccggaggaggcggctctggcagcc
actccctgaagtatttccacacctctgtgagccggccaggcagaggagagccacggttcatc
tctgtgggctacgtggacgatacacagttcgtgaggtttgacaatgatgccgccagcccaag
aatggtgcctagggccccatggatggagcaggagggcagcgagtattgggacagggagaccc
ggagcgccagagacacagcacagattttccgggtgaacctgagaaccctgaggggctactat
aatcagtccgaggccggctctcacacactccagtggatgcacggatgcgagctgggaccaga
t**g**gccgcttcctgcggggctacgagcagtttgcctatgacggcaaggattacctgaccctga
acgaggacctgagatcctggaccgccgtggatacagccgcccagatcagcgagcagaagtcc
aatgacgcatctgaggcagagcaccagagggcatatctggaggatacctgcgtggagtggct
gcacaagtacctggagaagggcaaggagacactgctgcacctggagccccctaagacccacg
tgacacaccacccaatcagcgaccacgaggccaccctgaggtgttgggcactgggcttctat
cccgccgagatcaccctgacatggcagcaggacggagagggacacacccaggatacagagct
ggtggagaccaggcccgccggcgatggcacatttcagaagtgggccgccgtggtggtgcctt
ccggagaggagcagagatacacctgtcacgtgcagcacgagggactgccagagccagtgacc
ctgaggtggaagcctgccagccagcccacaatccctatcgtgggaatcatcgcaggcctggt
gctgctgggctctgtggtgagcggagcagtggtggccgccgtgatctggcggaagaagagca

gcggaggcaagggaggctcctact***c***caaggcagagtggagcgactccgcccagggctctgag
agccactccctgtga (SEQ ID NO: 16973).

[0250] An exemplary bGBE Trimer (**270R** and ***484S***) protein of the disclosure comprises or consists of the amino acid sequence of:

MSRSVALAVLALLSLSGLEAVMAPRTLILGGGGSGGGGSGGGGSIQRTPKIQVYSRHPAENG
KSNFLNCYVSGFHPSDIEVDLLKNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYAC
RVNHVTLSQPKIVKWDRDMGGGGSGGGGSGGGGSGGGGSGSHSLKYFHTSVSRPGRGEPRFI
SVGYVDDTQFVRFDNDAASPRMVPRAPWMEQEGSEYWDRETRSARDTAQIFRVNLRTLRGYY
NQSEAGSHTLQWMHGCELGPD**R**RFLRGYEQFAYDGKDYLTLNEDLRSWTAVDTAAQISEQKS
NDASEAEHQRAYLEDTCVEWLHKYLEKGKETLLHLEPPKTHVTHHPISDHEATLRCWALGFY
PAEITLTWQQDGEGHTQDTELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQHEGLPEPVT
LRWKPASQPTIPIVGIIAGLVLLGSVVSGAVVAAVIWRKKSSGGKGGSY***S***KAEWSDSAQGSE
SHSL* (SEQ ID NO: 16974).

[0251] An exemplary bGBE Trimer (**270R** and ***484S***) protein of the disclosure comprises or consists of the nucleic acid sequence of:

atgtctcgcagcgtggccctggccgtgctggccctgctgtccctgtctggcctggaggccgt
gatggccccccggaccctgatcctgggaggaggaggcagcggcggaggaggctccggaggcg
gcggctctatccagcgcacacctaagatccaggtgtattctcggcacccagccgagaacggc
aagagcaacttcctgaattgctacgtgagcggctttcacccttccgacatcgaggtggatct
gctgaagaatggcgagagaatcgagaaggtggagcactccgacctgagcttctccaaggatt
ggtctttttatctgctgtactataccgagtttacccctacagagaaggacgagtacgcctgt
cgcgtgaaccacgtgacactgtcccagccaaagatcgtgaagtgggaccgggatatgggcgg
cggcggctctggcggcggcggcagcggcggcggcggctccggaggaggcggctctggcagcc
actccctgaagtatttccacacctctgtgagccggccaggcagaggagagccacggttcatc
tctgtgggctacgtggacgatacacagttcgtgaggtttgacaatgatgccgccagcccaag
aatggtgcctagggccccatggatggagcaggagggcagcgagtattgggacagggagaccc
ggagcgccagagacacagcacagattttccgggtgaacctgagaaccctgaggggctactat
aatcagtccgaggccggctctcacacactccagtggatgcacggatgcgagctgggaccaga
t***c***gccgcttcctgcggggctacgagcagtttgcctatgacggcaaggattacctgaccctga
acgaggacctgagatcctggaccgccgtggatacagccgcccagatcagcgagcagaagtcc
aatgacgcatctgaggcagagcaccagagggcatatctggaggatacctgcgtggagtggct
gcacaagtacctggagaagggcaaggagacactgctgcacctggagccccctaagacccacg
tgacacaccacccaatcagcgaccacgaggccaccctgaggtgttgggcactgggcttctat
cccgccgagatcaccctgacatggcagcaggacggagagggacacacccaggatacagagct
ggtggagaccaggcccgccggcgatggcacatttcagaagtgggccgccgtggtggtgcctt
ccggagaggagcagagatacacctgtcacgtgcagcacgagggactgccagagccagtgacc
ctgaggtggaagcctgccagccagcccacaatccctatcgtgggaatcatcgcaggcctggt
gctgctgggctctgtggtgagcggagcagtggtggccgccgtgatctggcggaagaagagca
gcggaggcaagggaggctcctact***c***caaggcagagtggagcgactccgcccagggctctgag
agccactccctgtga (SEQ ID NO: 16975).

[0252] An exemplary gBE Dimer (**R** and ***S***) protein of the disclosure comprises or consists of the amino acid sequence of:

MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEV

DLLKNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDR
DMGGGGSGGGGSGGGGSGGGGSGSHSLKYFHTSVSRPGRGEPRFISVGYVDDTQFVRFDN
DAASPRMVPRAPWMEQEGSEYWDRETRSARDTAQIFRVNLRTLRGYYNQSEAGSHTLQWM
HGCELGPDRRFLRGYEQFAYDGKDYLTLNEDLRSWTAVDTAAQISEQKSNDASEAEHQRA
YLEDTCVEWLHKYLEKGKETLLHLEPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQ
QDGEGHTQDTELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQHEGLPEPVTLRWKPAS
QPTIPIVGIIAGLVLLGSVVSGAVVAAVIWRKKSSGGKGGSY*S*KAEWSDSAQGSESHSL
(SEQ ID NO: 16976).

[0253] An exemplary gBE Dimer (**R** and *S*) protein of the disclosure comprises or consists of the nucleic acid sequence of:

ATGAGCAGATCTGTGGCCCTGGCTGTTCTGGCTCTGCTGTCTCTGTCTGGCCTGGAAGCCAT
CCAGCGGACCCCTAAGATCCAGGTGTACAGCAGACACCCCGCCGAGAACGGCAAGAGCAACT
TCCTGAACTGCTACGTGTCCGGCTTTCACCCCAGCGACATTGAGGTGGACCTGCTGAAGAAC
GGCGAGCGGATCGAGAAGGTGGAACACAGCGATCTGAGCTTCAGCAAGGACTGGTCCTTCTA
CCTGCTGTACTACACCGAGTTCACCCCTACCGAGAAGGACGAGTACGCCTGCAGAGTGAACC
ACGTGACACTGAGCCAGCCTAAGATCGTGAAGTGGGACAGAGATATGGGCGGAGGCGGATCT
GGTGGCGGAGGAAGTGGCGGCGGAGGATCTGGCGGTGGTGGTTCTGGATCTCACAGCCTGAA
GTACTTTCACACCTCCGTGTCCAGACCTGGCAGAGGCGAGCCTAGATTCATCAGCGTGGGCT
ACGTGGACGACACCCAGTTCGTCAGATTCGACAACGACGCCGCCTCTCCTCGGATGGTTCCT
AGAGCACCCTGGATGGAACAAGAGGGCAGCGAGTACTGGGATCGCGAGACAAGAAGCGCCAG
AGACACAGCCCAGATCTTCCGCGTGAACCTGAGAACCCTGCGGGGCTACTACAATCAGTCTG
AGGCCGGCTCTCACACCCTGCAGTGGATGCATGGATGTGAACTGGGCCCCGACAGA**CGG**TTC
CTGAGAGGCTATGAGCAGTTCGCCTACGACGGCAAGGACTACCTGACACTGAACGAGGACCT
GAGAAGCTGGACCGCCGTGGATACAGCCGCTCAGATCAGCGAGCAGAAGTCTAACGACGCCA
GCGAGGCCGAACACCAGAGAGCCTATCTGGAAGATACCTGCGTGGAATGGCTGCACAAGTAC
CTGGAAAAGGGCAAAGAGACACTGCTGCACCTGGAACCTCCAAAGACACATGTGACCCACCA
TCCTATCAGCGACCACGAGGCCACACTGAGATGTTGGGCCCTGGGCTTTTACCCTGCCGAGA
TCACACTGACATGGCAGCAGGATGGCGAGGGCCACACACAGGATACAGAGCTGGTGGAAACA
AGACCTGCCGGCGACGGCACCTTCCAGAAATGGGCTGCTGTGGTTGTGCCCAGCGGCGAGGA
ACAGAGATACACCTGTCACGTGCAGCACGAGGGACTGCCTGAACCTGTGACTCTGAGATGGA
AGCCTGCCAGCCAGCCAACAATCCCCATCGTGGGAATCATTGCCGGCCTGGTGCTGCTGGGA
TCTGTGGTTTCTGGTGCTGTGGTGGCCGCCGTGATTTGGAGAAAGAAGTCCTCTGGCGGCAA
AGGCGGCTCCTAC***TCT***AAGGCCGAGTGGAGCGATTCTGCCCAGGGCTCTGAAAGCCACAGCC
TGTAGATAA (SEQ ID NO: 16977).

[0254] An exemplary gBE Dimer (**G** and *S*) protein of the disclosure comprises or consists of the amino acid sequence of:

DLLKNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDR
DMGGGGSGGGGSGGGGSGGGGSGSHSLKYFHTSVSRPGRGEPRFISVGYVDDTQFVRFDN
DAASPRMVPRAPWMEQEGSEYWDRETRSARDTAQIFRVNLRTLRGYYNQSEAGSHTLQWM
HGCELGPD**G**RFLRGYEQFAYDGKDYLTLNEDLRSWTAVDTAAQISEQKSNDASEAEHQRA
YLEDTCVEWLHKYLEKGKETLLHLEPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQ
QDGEGHTQDTELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQHEGLPEPVTLRWKPAS
QPTIPIVGIIAGLVLLGSVVSGAVVAAVIWRKKSSGGKGGSY*S*KAEWSDSAQGSESHSL
(SEQ ID NO: 16978)

[0255] An exemplary gBE Dimer (**G** and ***S***) protein of the disclosure comprises or consists of the amino acid sequence of:

ATGAGCAGATCTGTGGCCCTGGCTGTTCTGGCTCTGCTGTCTCTGTCTGGCCTGGAAGCCAT
CCAGCGGACCCCTAAGATCCAGGTGTACAGCAGACACCCCGCCGAGAACGGCAAGAGCAACT
TCCTGAACTGCTACGTGTCCGGCTTTCACCCCAGCGACATTGAGGTGGACCTGCTGAAGAAC
GGCGAGCGGATCGAGAAGGTGGAACACAGCGATCTGAGCTTCAGCAAGGACTGGTCCTTCTA
CCTGCTGTACTACACCGAGTTCACCCCTACCGAGAAGGACGAGTACGCCTGCAGAGTGAACC
ACGTGACACTGAGCCAGCCTAAGATCGTGAAGTGGGACAGAGATATGGGCGGAGGCGGATCT
GGTGGCGGAGGAAGTGGCGGCGGAGGATCTGGCGGTGGTGGTTCTGGATCTCACAGCCTGAA
GTACTTTCACACCTCCGTGTCCAGACCTGGCAGAGGCGAGCCTAGATTCATCAGCGTGGGCT
ACGTGGACGACACCCAGTTCGTCAGATTCGACAACGACGCCGCCTCTCCTCGGATGGTTCCT
AGAGCACCCTGGATGGAACAAGAGGGCAGCGAGTACTGGGATCGCGAGACAAGAAGCGCCAG
AGACACAGCCCAGATCTTCCGCGTGAACCTGAGAACCCTGCGGGGCTACTACAATCAGTCTG
AGGCCGGCTCTCACACCCTGCAGTGGATGCATGGATGTGAACTGGGCCCCGACAGA**CAG**TTC
CTGAGAGGCTATGAGCAGTTCGCCTACGACGGCAAGGACTACCTGACACTGAACGAGGACCT
GAGAAGCTGGACCGCCGTGGATACAGCCGCTCAGATCAGCGAGCAGAAGTCTAACGACGCCA
GCGAGGCCGAACACCAGAGAGCCTATCTGGAAGATACCTGCGTGGAATGGCTGCACAAGTAC
CTGGAAAAGGGCAAAGAGACACTGCTGCACCTGGAACCTCCAAAGACACATGTGACCCACCA
TCCTATCAGCGACCACGAGGCCACACTGAGATGTTGGGCCCTGGGCTTTTACCCTGCCGAGA
TCACACTGACATGGCAGCAGGATGGCGAGGGCCACACACAGGATACAGAGCTGGTGGAAACA
AGACCTGCCGGCGACGGCACCTTCCAGAAATGGGCTGCTGTGGTTGTGCCCAGCGGCGAGGA
ACAGAGATACACCTGTCACGTGCAGCACGAGGGACTGCCTGAACCTGTGACTCTGAGATGGA
AGCCTGCCAGCCAGCCAACAATCCCCATCGTGGGAATCATTGCCGGCCTGGTGCTGCTGGGA
TCTGTGGTTTCTGGTGCTGTGGTGGCCGCCGTGATTTGGAGAAAGAAGTCCTCTGGCGGCAA
AGGCGGCTCCTAC***TCT***AAGGCCGAGTGGAGCGATTCTGCCCAGGGCTCTGAAAGCCACAGCC
TGTAGATAA (SEQ ID NO: 16979)

[0256] A wildtype/natural human HLA-E protein (NCBI: HLAE_HUMAN; UniProt/Swiss-Prot: P13747.4) comprises or consists of the amino acid sequence of:

MVDGTLLLLLSEALALTQTWAGSHSLKYFHTSVSRPGRGEPRFISVGYVDDTQFVRFDNDAA
SPRMVPRAPWMEQEGSEYWDRETRSARDTAQIFRVNLRTLRGYYNQSEAGSHTLQWMHGCEL
GPDGRFLRGYEQFAYDGKDYLTLNEDLRSWTAVDTAAQISEQKSNDASEAEHQRAYLEDTCV
EWLHKLEKGKETLLHLEPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQQDGEGHTQDT
ELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQHEGLPEPVTLRWKPASQPTIPIVGIIAG
LVLLGSVVSGAVVAAVIWRKKSSGGKGGSYSKAEWSDSAQGSESHSL (SEQ ID NO: 17122)

[0257] A nucleotide sequence encoding wildtype/natural HLA-E protein (NCBI: CCDS34379.1) comprises or consists of the nucleotide sequence of:

ATGGTAGATGGAACCCTCCTTTTACTCCTCTCGGAGGCCCTGGCCCTTACCCAGACCTGGGC
GGGCTCCCACTCCTTGAAGTATTTCCACACTTCCGTGTCCCGGCCCGGCCGCGGGGAGCCCC
GCTTCATCTCTGTGGGCTACGTGGACGACACCCAGTTCGTGCGCTTCGACAACGACGCCGCG
AGTCCGAGGATGGTGCCGCGGGCGCCGTGGATGGAGCAGGAGGGGTCAGAGTATTGGGACCG
GGAGACACGGAGCGCCAGGGACACCGCACAGATTTTCCGAGTGAATCTGCGGACGCTGCGCG
GCTACTACAATCAGAGCGAGGCCGGGTCTCACACCCTGCAGTGGATGCATGGCTGCGAGCTG
GGGCCCGACGGGCGCTTCCTCCGCGGGTATGAACAGTTCGCCTACGACGGCAAGGATTATCT

CACCCTGAATGAGGACCTGCGCTCCTGGACCGCGGTGGACACGGCGGCTCAGATCTCCGAGC
AAAAGTCAAATGATGCCTCTGAGGCGGAGCACCAGAGAGCCTACCTGGAAGACACATGCGTG
GAGTGGCTCCACAAATACCTGGAGAAGGGGAAGGAGACGCTGCTTCACCTGGAGCCCCCAAA
GACACACGTGACTCACCACCCCATCTCTGACCATGAGGCCACCCTGAGGTGCTGGGCCCTGG
GCTTCTACCCTGCGGAGATCACACTGACCTGGCAGCAGGATGGGGAGGGCCATACCCAGGAC
ACGGAGCTCGTGGAGACCAGGCCTGCAGGGGATGGAACCTTCCAGAAGTGGGCAGCTGTGGT
GGTGCCTTCTGGAGAGGAGCAGAGATACACGTGCCATGTGCAGCATGAGGGGCTACCCGAGC
CCGTCACCCTGAGATGGAAGCCGGCTTCCCAGCCCACCATCCCCATCGTGGGCATCATTGCT
GGCCTGGTTCTCCTTGGATCTGTGGTCTCTGGAGCTGTGGTTGCTGCTGTGATATGGAGGAA
GAAGAGCTCAGGTGGAAAAGGAGGGAGCTACTCTAAGGCTGAGTGGAGCGACAGTGCCCAGG
GGTCTGAGTCTCACAGCTTGTAA (SEQ ID NO: 17123)

[0258] An exemplary WT HLA-E Monomer (**R** and ***S***) protein of the disclosure comprises or consists of the amino acid sequence of:

MSRSVALAVLALLSLSGLEAGSHSLKYFHTSVSRPGRGEPRFISVGYVDDTQFVRF
DNDAASPRMVPRAPWMEQEGSEYWDRETRSARDTAQIFRVNLRTLRGYYNQSEAGSHTLQ
WMHGCELGPD**R**RFLRGYEQFAYDGKDYLTLNEDLRSWTAVDTAAQISEQKSNDASEAEHQ
RAYLEDTCVEWLHKYLEKGKETLLHLEPPKTHVTHHPISDHEATLRCWALGFYPAEITLT
WQQDGEGHTQDTELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQHEGLPEPVTLRWKP
ASQPTIPIVGIIAGLVLLGSVVSGAVVAAVIWRKKSSGGKGGSY***S***KAEWSDSAQGSESHS
L (SEQ ID NO: 16980)

[0259] An exemplary WT HLA-E Monomer (**R** and ***S***) protein of the disclosure comprises or consists of the nucleic acid sequence of:

ATGAGCAGATCTGTGGCCCTGGCTGTTCTGGCTCTGCTGTCTCTGTCTGGACTGGAAGCCGG
CAGCCACAGCCTGAAGTACTTTCACACCAGCGTGTCCAGACCTGGCAGAGGCGAGCCTAGAT
TCATCAGCGTGGGCTACGTGGACGACACCCAGTTCGTCAGATTCGACAACGACGCCGCCTCT
CCTCGGATGGTTCCTAGAGCACCCTGGATGGAACAAGAGGGCAGCGAGTACTGGGACAGAGA
GACAAGAAGCGCCAGAGACACAGCCCAGATCTTCAGAGTGAACCTGCGGACCCTGCGGGGCT
ACTACAATCAGTCTGAAGCCGGCTCTCACACCCTGCAGTGGATGCACGGATGTGAACTGGGC
CCCGAC**AGA**AGATTCCTGAGAGGCTACGAGCAGTTCGCCTACGACGGCAAGGACTACCTGAC
ACTGAACGAGGACCTGAGAAGCTGGACCGCCGTGGATACAGCCGCTCAGATCAGCGAGCAGA
AGTCTAACGACGCCTCTGAGGCCGAACACCAGAGAGCCTACCTGGAAGATACCTGCGTGGAA
TGGCTGCACAAGTACCTGGAAAAGGGCAAAGAGACACTGCTGCACCTGGAACCTCCAAAGAC
ACACGTGACCCACCATCCTATCAGCGACCACGAGGCCACACTGAGATGTTGGGCCCTGGGCT
TTTACCCCGCCGAGATCACACTGACATGGCAGCAGGATGGCGAGGGCCACACACAGGATACA
GAGCTGGTGGAAACAAGACCTGCCGGCGACGGCACCTTCCAGAAATGGGCTGCTGTGGTGGT
TCCCAGCGGCGAGGAACAGAGATACACCTGTCACGTGCAGCACGAGGGACTGCCTGAACCTG
TGACACTGAGGTGGAAGCCTGCCAGCCAGCCTACAATCCCCATCGTGGGAATCATTGCCGGC
CTGGTGCTGCTGGGATCTGTGGTTTCTGGTGCAGTGGTGGCCGCCGTGATCTGGCGGAAAAA
AAGCTCAGGCGGCAAAGGCGGCTCCTAC***TCC***AAAGCCGAGTGGAGCGATTCTGCCCAGGGCT
CTGAAAGCCACTCTCTGTAGATAA (SEQ ID NO: 16981).

[0260] An exemplary WT HLA-E Monomer (**G** and ***S***) protein of the disclosure comprises or consists of the nucleic acid sequence of:

MSRSVALAVLALLSLSGLEAGSHSLKYFHTSVSRPGRGEPRFISVGYVDDTQFVRF

DNDAASPRMVPRAPWMEQEGSEYWDRETRSARDTAQIFRVNLRTLRGYYNQSEAGSHTLQ
WMHGCELGPD**G**RFLRGYEQFAYDGKDYLTLNEDLRSWTAVDTAAQISEQKSNDASEAEHQ
RAYLEDTCVEWLHKYLEKGKETLLHLEPPKTHVTHHPISDHEATLRCWALGFYPAEITLT
WQQDGEGHTQDTELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQHEGLPEPVTLRWKP
ASQPTIPIVGIIAGLVLLGSVVSGAVVAAVIWRKKSSGGKGGSY***S***KAEWSDSAQGSESHS
L (SEQ ID NO: 16982).

[0261] An exemplary WT HLA-E Monomer (**G** and ***S***) protein of the disclosure comprises or consists of the nucleic acid sequence of:

ATGAGCAGATCTGTGGCCCTGGCTGTTCTGGCTCTGCTGTCTCTGTCTGGACTGGAAGCCGG
CAGCCACAGCCTGAAGTACTTTCACACCAGCGTGTCCAGACCTGGCAGAGGCGAGCCTAGAT
TCATCAGCGTGGGCTACGTGGACGACACCCAGTTCGTCAGATTCGACAACGACGCCGCCTCT
CCTCGGATGGTTCCTAGAGCACCCTGGATGGAACAAGAGGGCAGCGAGTACTGGGACAGAGA
GACAAGAAGCGCCAGAGACACAGCCCAGATCTTCAGAGTGAACCTGCGGACCCTGCGGGGCT
ACTACAATCAGTCTGAAGCCGGCTCTCACACCCTGCAGTGGATGCACGGATGTGAACTGGGC
CCCGAC**GGA**AGATTCCTGAGAGGCTACGAGCAGTTCGCCTACGACGGCAAGGACTACCTGAC
ACTGAACGAGGACCTGAGAAGCTGGACCGCCGTGGATACAGCCGCTCAGATCAGCGAGCAGA
AGTCTAACGACGCCTCTGAGGCCGAACACCAGAGAGCCTACCTGGAAGATACCTGCGTGGAA
TGGCTGCACAAGTACCTGGAAAAGGGCAAAGAGACACTGCTGCACCTGGAACCTCCAAAGAC
ACACGTGACCCACCATCCTATCAGCGACCACGAGGCCACACTGAGATGTTGGGCCCTGGGCT
TTTACCCCGCCGAGATCACACTGACATGGCAGCAGGATGGCGAGGGCCACACACAGGATACA
GAGCTGGTGGAAACAAGACCTGCCGGCGACGGCACCTTCCAGAAATGGGCTGCTGTGGTGGT
TCCCAGCGGCGAGGAACAGAGATACACCTGTCACGTGCAGCACGAGGGACTGCCTGAACCTG
TGACACTGAGGTGGAAGCCTGCCAGCCAGCCTACAATCCCCATCGTGGGAATCATTGCCGGC
CTGGTGCTGCTGGGATCTGTGGTTTCTGGTGCAGTGGTGGCCGCCGTGATCTGGCGGAAAAA
AAGCTCAGGCGGCAAAGGCGGCTCCTAC***TCC***AAAGCCGAGTGGAGCGATTCTGCCCAGGGCT
CTGAAAGCCACTCTCTGTAGATAA (SEQ ID NO: 16983).

[0262] A wildtype/natural human B2M protein (NCBI: B2MG_HUMAN; UniProt/Swiss-Prot: P61769.1) comprises or consists of the amino acid sequence of:

MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLLKN
GERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM (SEQ
ID NO: 17124)

[0263] A nucleotide sequence encoding wildtype/natural B2M protein (NCBI: CCDS10113.1) comprises or consists of the nucleotide sequence of:

ATGTCTCGCTCCGTGGCCTTAGCTGTGCTCGCGCTACTCTCTCTTTCTGGCCTGGAGGCTAT
CCAGCGTACTCCAAAGATTCAGGTTTACTCACGTCATCCAGCAGAGAATGGAAAGTCAAATT
TCCTGAATTGCTATGTGTCTGGGTTTCATCCATCCGACATTGAAGTTGACTTACTGAAGAAT
GGAGAGAGAATTGAAAAAGTGGAGCATTCAGACTTGTCTTTCAGCAAGGACTGGTCTTTCTA
TCTCTTGTACTACACTGAATTCACCCCCACTGAAAAAGATGAGTATGCCTGCCGTGTGAACC
ATGTGACTTTGTCACAGCCCAAGATAGTTAAGTGGGATCGAGACATGTAA (SEQ ID NO:
17125)

[0264] An exemplary HLA-bGBE (Single Chain Trimer) protein of the disclosure comprises or consists of the amino acid sequence of (**B2M Signal peptide**, *peptide*, Linker, ***B2M domain***, Linker, **HLA-E peptide**):

MSRSVALAVLALLSLSGLEA*VMAPRTLIL*GGGGSGGGGSGGGGS*IQRTPKIQVYSRHPAENG KSNFLNCYVSGFHPSDIEVDLLKNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYAC RVNHVTLSQPKIVKWDRDM*GGGGSGGGGSGGGGSGGGGS**GSHSLKYFHTSVSRPGRGEPRFI SVGYVDDTQFVRFDNDAASPRMVPRAPWMEQEGSEYWDRETRSARDTAQIFRVNLRTLRGYY NQSEAGSHTLQWMHGCELGPDGRFLRGYEQFAYDGKDYLTLNEDLRSWTAVDTAAQISEQKS NDASEAEHQRAYLEDTCVEWLHKYLEKGKETLLHLEPPKTHVTHHPISDHEATLRCWALGFY PAEITLTWQQDGEGHTQDTELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQHEGLPEPVT LRWKPASQPTIPIVGIIAGLVLLGSVVSGAVVAAVIWRKKSSGGKGGSYSKAEWSDSAQGSE SHSL** (SEQ ID NO: 17064)

B2M Signal peptide: MSRSVALAVLALLSLSGLEA (SEQ ID NO: 17126)

Peptide: VMAPRTLIL (SEQ ID NO: 17127)

Linker: GGGGSGGGGSGGGGS (SEQ ID NO: 17128)

***B2M domain*:**
IQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGERIEKVEHSDLSFSKDWSF YLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM (SEQ ID NO: 17129)

Linker: GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 17130)

HLA-E peptide:
GSHSLKYFHTSVSRPGRGEPRFISVGYVDDTQFVRFDNDAASPRMVPRAPWMEQEGSEYWDR ETRSARDTAQIFRVNLRTLRGYYNQSEAGSHTLQWMHGCELGPDGRFLRGYEQFAYDGKDYL TLNEDLRSWTAVDTAAQISEQKSNDASEAEHQRAYLEDTCVEWLHKYLEKGKETLLHLEPPK THVTHHPISDHEATLRCWALGFYPAEITLTWQQDGEGHTQDTELVETRPAGDGTFQKWAAVV VPSGEEQRYTCHVQHEGLPEPVTLRWKPASQPTIPIVGIIAGLVLLGSVVSGAVVAAVIWRK KSSGGKGGSYSKAEWSDSAQGSESHSL(SEQ ID NO: 17131)

[0265] An exemplary nucleotide sequence encoding a HLA-bGBE (Single Chain Trimer) protein of the disclosure comprises or consists of the nucleotide sequence of (**B2M Signal peptide**, *peptide*, Linker, ***B2M domain***, Linker, **HLA-E peptide**):

ATGTCTCGCAGCGTGGCCCTGGCCGTGCTGGCCCTGCTGTCCCTGTCTGGCCTGGAGGCC*GT GATGGCCCCCCCGGACCCTGATCCTG*GGAGGAGGAGGCAGCGGCGGAGGAGGCTCCGGAGGCG GCGGCTCT***ATCCAGCGCACACCTAAGATCCAGGTGTATTCTCGGCACCCAGCCGAGAACGGC AAGAGCAACTTCCTGAATTGCTACGTGAGCGGCTTTCACCCTTCCGACATCGAGGTGGATCT GCTGAAGAATGGCGAGAGAATCGAGAAGGTGGAGCACTCCGACCTGAGCTTCTCCAAGGATT GGTCTTTTTATCTGCTGTACTATACCGAGTTTACCCCTACAGAGAAGGACGAGTACGCCTGT CGCGTGAACCACGTGACACTGTCCCAGCCAAAGATCGTGAAGTGGGACCGGGATATG***GGCGG CGGCGGCTCTGGCGGCGGCGGCAGCGGCGGCGGCGGCTCCGGAGGAGGCGGCTCT**GGCAGCC ACTCCCTGAAGTATTTCCACACCTCTGTGAGCCGGCCAGGCAGAGGAGAGCCACGGTTCATC TCTGTGGGCTACGTGGACGATACACAGTTCGTGAGGTTTGACAATGATGCCGCCAGCCCAAG

AATGGTGCCTAGGGCCCCATGGATGGAGCAGGAGGGCAGCGAGTATTGGGACAGGGAGACCC
GGAGCGCCAGAGACACAGCACAGATTTTCCGGGTGAACCTGAGAACCCTGAGGGGCTACTAT
AATCAGTCCGAGGCCGGCTCTCACACACTCCAGTGGATGCACGGATGCGAGCTGGGACCAGA
TGGCCGCTTCCTGCGGGGCTACGAGCAGTTTGCCTATGACGGCAAGGATTACCTGACCCTGA
ACGAGGACCTGAGATCCTGGACCGCCGTGGATACAGCCGCCCAGATCAGCGAGCAGAAGTCC
AATGACGCATCTGAGGCAGAGCACCAGAGGGCATATCTGGAGGATACCTGCGTGGAGTGGCT
GCACAAGTACCTGGAGAAGGGCAAGGAGACACTGCTGCACCTGGAGCCCCCTAAGACCCACG
TGACACACCACCCAATCAGCGACCACGAGGCCACCCTGAGGTGTTGGGCACTGGGCTTCTAT
CCCGCCGAGATCACCCTGACATGGCAGCAGGACGGAGAGGGACACACCCAGGATACAGAGCT
GGTGGAGACCAGGCCCGCCGGCGATGGCACATTTCAGAAGTGGGCCGCCGTGGTGGTGCCTT
CCGGAGAGGAGCAGAGATACACCTGTCACGTGCAGCACGAGGGACTGCCAGAGCCAGTGACC
CTGAGGTGGAAGCCTGCCAGCCAGCCCACAATCCCTATCGTGGGAATCATCGCAGGCCTGGT
GCTGCTGGGCTCTGTGGTGAGCGGAGCAGTGGTGGCCGCCGTGATCTGGCGGAAGAAGAGCA
GCGGAGGCAAGGGAGGCTCCTACTCCAAGGCAGAGTGGAGCGACTCCGCCCAGGGCTCTGAG
AGCCACTCCCTGTGA (SEQ ID NO: 17065)

B2M Signal peptide:
ATGTCTCGCAGCGTGGCCCTGGCCGTGCTGGCCCTGCTGTCCCTGTCTGGCCTGGAGGCC
(SEQ ID NO: 17132)

Peptide: GTGATGGCCCCCCGGACCCTGATCCTG (SEQ ID NO: 17133)

Linker: GGAGGAGGAGGCAGCGGCGGAGGAGGCTCCGGAGGCGGCGGCTCT (SEQ ID NO: 17134)

***B2M domain*:**
ATCCAGCGCACACCTAAGATCCAGGTGTATTCTCGGCACCCAGCCGAGAACGGCAAGAGCAA
CTTCCTGAATTGCTACGTGAGCGGCTTTCACCCTTCCGACATCGAGGTGGATCTGCTGAAGA
ATGGCGAGAGAATCGAGAAGGTGGAGCACTCCGACCTGAGCTTCTCCAAGGATTGGTCTTTT
TATCTGCTGTACTATACCGAGTTTACCCCTACAGAGAAGGACGAGTACGCCTGTCGCGTGAA
CCACGTGACACTGTCCCAGCCAAAGATCGTGAAGTGGGACCGGGATATG (SEQ ID NO: 17135)

Linker:
GGCGGCGGCGGCTCTGGCGGCGGCGGCAGCGGCGGCGGCGGCTCCGGAGGAGGCGGCTCT
(SEQ ID NO: 17136)

HLA-E peptide:
GGCAGCCACTCCCTGAAGTATTTCCACACCTCTGTGAGCCGGCCAGGCAGAGGAGAGCCACG
GTTCATCTCTGTGGGCTACGTGGACGATACACAGTTCGTGAGGTTTGACAATGATGCCGCCA
GCCCAAGAATGGTGCCTAGGGCCCCATGGATGGAGCAGGAGGGCAGCGAGTATTGGGACAGG
GAGACCCGGAGCGCCAGAGACACAGCACAGATTTTCCGGGTGAACCTGAGAACCCTGAGGGG
CTACTATAATCAGTCCGAGGCCGGCTCTCACACACTCCAGTGGATGCACGGATGCGAGCTGG
GACCAGATGGCCGCTTCCTGCGGGGCTACGAGCAGTTTGCCTATGACGGCAAGGATTACCTG
ACCCTGAACGAGGACCTGAGATCCTGGACCGCCGTGGATACAGCCGCCCAGATCAGCGAGCA
GAAGTCCAATGACGCATCTGAGGCAGAGCACCAGAGGGCATATCTGGAGGATACCTGCGTGG
AGTGGCTGCACAAGTACCTGGAGAAGGGCAAGGAGACACTGCTGCACCTGGAGCCCCCTAAG
ACCCACGTGACACACCACCCAATCAGCGACCACGAGGCCACCCTGAGGTGTTGGGCACTGGG
CTTCTATCCCGCCGAGATCACCCTGACATGGCAGCAGGACGGAGAGGGACACACCCAGGATA

CAGAGCTGGTGGAGACCAGGCCCGCCGGCGATGGCACATTTCAGAAGTGGGCCGCCGTGGTG
GTGCCTTCCGGAGAGGAGCAGAGATACACCTGTCACGTGCAGCACGAGGGACTGCCAGAGCC
AGTGACCCTGAGGTGGAAGCCTGCCAGCCAGCCCACAATCCCTATCGTGGGAATCATCGCAG
GCCTGGTGCTGCTGGGCTCTGTGGTGAGCGGAGCAGTGGTGGCCGCCGTGATCTGGCGGAAG
AAGAGCAGCGGAGGCAAGGGAGGCTCCTACTCCAAGGCAGAGTGGAGCGACTCCGCCCAGGG
CTCTGAGAGCCACTCCCTGTGA (SEQ ID NO: 17137)

[0266] An exemplary HLA-gBE (Single Chain Dimer) protein of the disclosure comprises or consists of the amino acid sequence of (**B2M Signal peptide**, *B2M domain*, Linker, HLA-E peptide):

MSRSVALAVLALLSLSGLEA*IQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLLKN*
*GERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM*GGGGS
GGGGSGGGGSGGGGSGSHSLKYFHTSVSRPGRGEPRFISVGYVDDTQFVRFDNDAASPRMVP
RAPWMEQEGSEYWDRETRSARDTAQIFRVNLRTLRGYYNQSEAGSHTLQWMHGCELGPDRRF
LRGYEQFAYDGKDYLTLNEDLRSWTAVDTAAQISEQKSNDASEAEHQRAYLEDTCVEWLHKY
LEKGKETLLHLEPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQQDGEGHTQDTELVET
RPAGDGTFQKWAAVVVPSGEEQRYTCHVQHEGLPEPVTLRWKPASQPTIPIVGIIAGLVLLG
SVVSGAVVAAVIWRKKSSGGKGGSYYKAEWSDSAQGSESHSL (SEQ ID NO: 17066)

B2M Signal peptide: MSRSVALAVLALLSLSGLEA (SEQ ID NO: 17126)

B2M domain:
IQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGERIEKVEHSDLSFSKDWSF
YLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM (SEQ ID NO: 17129)

Linker: GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 17130)

HLA-E peptide:
GSHSLKYFHTSVSRPGRGEPRFISVGYVDDTQFVRFDNDAASPRMVPRAPWMEQEGSEYWDR
ETRSARDTAQIFRVNLRTLRGYYNQSEAGSHTLQWMHGCELGPDRRFLRGYEQFAYDGKDYL
TLNEDLRSWTAVDTAAQISEQKSNDASEAEHQRAYLEDTCVEWLHKYLEKGKETLLHLEPPK
THVTHHPISDHEATLRCWALGFYPAEITLTWQQDGEGHTQDTELVETRPAGDGTFQKWAAVV
VPSGEEQRYTCHVQHEGLPEPVTLRWKPASQPTIPIVGIIAGLVLLGSVVSGAVVAAVIWRK
KSSGGKGGSYYKAEWSDSAQGSESHSL (SEQ ID NO: 17131)

[0267] An exemplary nucleotide sequence encoding a HLA-gBE (Single Chain Dimer) protein of the disclosure comprises or consists of the nucleotide sequence of (**B2M Signal peptide**, *B2M domain*, Linker, HLA-E peptide):

ATGAGCAGATCTGTGGCCCTGGCTGTTCTGGCTCTGCTGTCTCTGTCTGGCCTGGAAGCC*AT*
CCAGCGGACCCCTAAGATCCAGGTGTACAGCAGACACCCCGCCGAGAACGGCAAGAGCAACT
TCCTGAACTGCTACGTGTCCGGCTTTCACCCCAGCGACATTGAGGTGGACCTGCTGAAGAAC
GGCGAGCGGATCGAGAAGGTGGAACACAGCGATCTGAGCTTCAGCAAGGACTGGTCCTTCTA
CCTGCTGTACTACACCGAGTTCACCCCTACCGAGAAGGACGAGTACGCCTGCAGAGTGAACC
*ACGTGACACTGAGCCAGCCTAAGATCGTGAAGTGGGACAGAGATATG*GGCGGAGGCGGATCT
GGTGGCGGAGGAAGTGGCGGCGGAGGATCTGGCGGTGGTGGTTCTGGATCTCACAGCCTGAA
GTACTTTCACACCTCCGTGTCCAGACCTGGCAGAGGCGAGCCTAGATTCATCAGCGTGGGCT

ACGTGGACGACACCCAGTTCGTCAGATTCGACAACGACGCCGCCTCTCCTCGGATGGTTCCT
AGAGCACCCTGGATGGAACAAGAGGGCAGCGAGTACTGGGATCGCGAGACAAGAAGCGCCAG
AGACACAGCCCAGATCTTCCGCGTGAACCTGAGAACCCTGCGGGGCTACTACAATCAGTCTG
AGGCCGGCTCTCACACCCTGCAGTGGATGCATGGATGTGAACTGGGCCCCGACAGACGGTTC
CTGAGAGGCTATGAGCAGTTCGCCTACGACGGCAAGGACTACCTGACACTGAACGAGGACCT
GAGAAGCTGGACCGCCGTGGATACAGCCGCTCAGATCAGCGAGCAGAAGTCTAACGACGCCA
GCGAGGCCGAACACCAGAGAGCCTATCTGGAAGATACCTGCGTGGAATGGCTGCACAAGTAC
CTGGAAAAGGGCAAAGAGACACTGCTGCACCTGGAACCTCCAAAGACACATGTGACCCACCA
TCCTATCAGCGACCACGAGGCCACACTGAGATGTTGGGCCCTGGGCTTTTACCCTGCCGAGA
TCACACTGACATGGCAGCAGGATGGCGAGGGCCACACACAGGATACAGAGCTGGTGGAAACA
AGACCTGCCGGCGACGGCACCTTCCAGAAATGGGCTGCTGTGGTTGTGCCCAGCGGCGAGGA
ACAGAGATACACCTGTCACGTGCAGCACGAGGGACTGCCTGAACCTGTGACTCTGAGATGGA
AGCCTGCCAGCCAGCCAACAATCCCCATCGTGGGAATCATTGCCGGCCTGGTGCTGCTGGGA
TCTGTGGTTTCTGGTGCTGTGGTGGCCGCCGTGATTTGGAGAAAGAAGTCCTCTGGCGGCAA
AGGCGGCTCCTACTATAAGGCCGAGTGGAGCGATTCTGCCCAGGGCTCTGAAAGCCACAGCC
TGTGA (SEQ ID NO: 17067)

B2M Signal peptide:
ATGAGCAGATCTGTGGCCCTGGCTGTTCTGGCTCTGCTGTCTCTGTCTGGCCTGGAAGCC
(SEQ ID NO: 17132)

B2M domain:
ATCCAGCGGACCCCTAAGATCCAGGTGTACAGCAGACACCCCGCCGAGAACGGCAAGAGCAA
CTTCCTGAACTGCTACGTGTCCGGCTTTCACCCCAGCGACATTGAGGTGGACCTGCTGAAGA
ACGGCGAGCGGATCGAGAAGGTGGAACACAGCGATCTGAGCTTCAGCAAGGACTGGTCCTTC
TACCTGCTGTACTACACCGAGTTCACCCCTACCGAGAAGGACGAGTACGCCTGCAGAGTGAA
CCACGTGACACTGAGCCAGCCTAAGATCGTGAAGTGGGACAGAGATATG (SEQ ID NO:
17135)

Linker:
GGCGGAGGCGGATCTGGTGGCGGAGGAAGTGGCGGCGGAGGATCTGGCGGTGGTGGTTCT
(SEQ ID NO: 17136)

HLA-E peptide:
GGATCTCACAGCCTGAAGTACTTTCACACCTCCGTGTCCAGACCTGGCAGAGGCGAGCCTAG
ATTCATCAGCGTGGGCTACGTGGACGACACCCAGTTCGTCAGATTCGACAACGACGCCGCCT
CTCCTCGGATGGTTCCTAGAGCACCCTGGATGGAACAAGAGGGCAGCGAGTACTGGGATCGC
GAGACAAGAAGCGCCAGAGACACAGCCCAGATCTTCCGCGTGAACCTGAGAACCCTGCGGGG
CTACTACAATCAGTCTGAGGCCGGCTCTCACACCCTGCAGTGGATGCATGGATGTGAACTGG
GCCCCGACAGACGGTTCCTGAGAGGCTATGAGCAGTTCGCCTACGACGGCAAGGACTACCTG
ACACTGAACGAGGACCTGAGAAGCTGGACCGCCGTGGATACAGCCGCTCAGATCAGCGAGCA
GAAGTCTAACGACGCCAGCGAGGCCGAACACCAGAGAGCCTATCTGGAAGATACCTGCGTGG
AATGGCTGCACAAGTACCTGGAAAAGGGCAAAGAGACACTGCTGCACCTGGAACCTCCAAAG
ACACATGTGACCCACCATCCTATCAGCGACCACGAGGCCACACTGAGATGTTGGGCCCTGGG
CTTTTACCCTGCCGAGATCACACTGACATGGCAGCAGGATGGCGAGGGCCACACACAGGATA
CAGAGCTGGTGGAAACAAGACCTGCCGGCGACGGCACCTTCCAGAAATGGGCTGCTGTGGTT
GTGCCCAGCGGCGAGGAACAGAGATACACCTGTCACGTGCAGCACGAGGGACTGCCTGAACC
TGTGACTCTGAGATGGAAGCCTGCCAGCCAGCCAACAATCCCCATCGTGGGAATCATTGCCG
GCCTGGTGCTGCTGGGATCTGTGGTTTCTGGTGCTGTGGTGGCCGCCGTGATTTGGAGAAAG

AAGTCCTCTGGCGGCAAAGGCGGCTCCTACTATAAGGCCGAGTGGAGCGATTCTGCCCAGGG
CTCTGAAAGCCACAGCCTGTGA (SEQ ID NO: 17137)

[0268] An exemplary HLA-bE (Monomer) protein of the disclosure comprises or consists of the amino acid sequence of (**B2M Signal peptide**, HLA-E peptide):

MSRSVALAVLALLSLSGLEAGSHSLKYFHTSVSRPGRGEPRFISVGYVDDTQFVRFDNDAAS
PRMVPRAPWMEQEGSEYWDRETRSARDTAQIFRVNLRTLRGYYNQSEAGSHTLQWMHGCELG
PDRRFLRGYEQFAYDGKDYLTLNEDLRSWTAVDTAAQISEQKSNDASEAEHQRAYLEDTCVE
WLHKYLEKGKETLLHLEPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQQDGEGHTQDT
ELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQHEGLPEPVTLRWKPASQPTIPIVGIIAG
LVLLGSVVSGAVVAAVIWRKKSSGGKGGSYYKAEWSDSAQGSESHSL (SEQ ID NO:
17068)

B2M Signal peptide: MSRSVALAVLALLSLSGLEA (SEQ ID NO: 17126)

HLA-E peptide:
GSHSLKYFHTSVSRPGRGEPRFISVGYVDDTQFVRFDNDAASPRMVPRAPWMEQEGSEYWDR
ETRSARDTAQIFRVNLRTLRGYYNQSEAGSHTLQWMHGCELGPDRRFLRGYEQFAYDGKDYL
TLNEDLRSWTAVDTAAQISEQKSNDASEAEHQRAYLEDTCVEWLHKYLEKGKETLLHLEPPK
THVTHHPISDHEATLRCWALGFYPAEITLTWQQDGEGHTQDTELVETRPAGDGTFQKWAAVV
VPSGEEQRYTCHVQHEGLPEPVTLRWKPASQPTIPIVGIIAGLVLLGSVVSGAVVAAVIWRK
KSSGGKGGSYYKAEWSDSAQGSESHSL (SEQ ID NO: 17131)

[0269] An exemplary nucleotide sequence encoding a HLA-bE (Monomer) protein of the disclosure comprises or consists of the nucleotide sequence of (**B2M Signal peptide**, HLA-E peptide):

ATGTCTCGCAGCGTGGCCCTGGCCGTGCTGGCCCTGCTGTCCCTGTCTGGCCTGGAGGCCGG
CAGCCACTCCCTGAAGTATTTCCACACCTCTGTGAGCCGGCCAGGCAGAGGAGAGCCACGGT
TCATCTCTGTGGGCTACGTGGACGATACACAGTTCGTGAGGTTTGACAATGATGCCGCCAGC
CCAAGAATGGTGCCTAGGGCCCCATGGATGGAGCAGGAGGGCAGCGAGTATTGGGACAGGGA
GACCCGGAGCGCCAGAGACACAGCACAGATTTTCCGGGTGAACCTGAGAACCCTGAGGGGCT
ACTATAATCAGTCCGAGGCCGGCTCTCACACACTCCAGTGGATGCACGGATGCGAGCTGGGA
CCAGATCGCCGCTTCCTGCGGGGCTACGAGCAGTTTGCCTATGACGGCAAGGATTACCTGAC
CCTGAACGAGGACCTGAGATCCTGGACCGCCGTGGATACAGCCGCCCAGATCAGCGAGCAGA
AGTCCAATGACGCATCTGAGGCAGAGCACCAGAGGGCATATCTGGAGGATACCTGCGTGGAG
TGGCTGCACAAGTACCTGGAGAAGGGCAAGGAGACACTGCTGCACCTGGAGCCCCCTAAGAC
CCACGTGACACACCACCCAATCAGCGACCACGAGGCCACCCTGAGGTGTTGGGCACTGGGCT
TCTATCCCGCCGAGATCACCCTGACATGGCAGCAGGACGGAGAGGGACACACCCAGGATACA
GAGCTGGTGGAGACCAGGCCCGCCGGCGATGGCACATTTCAGAAGTGGGCCGCCGTGGTGGT
GCCTTCCGGAGAGGAGCAGAGATACACCTGTCACGTGCAGCACGAGGGACTGCCAGAGCCAG
TGACCCTGAGGTGGAAGCCTGCCAGCCAGCCCACAATCCCTATCGTGGGAATCATCGCAGGC
CTGGTGCTGCTGGGCTCTGTGGTGAGCGGAGCAGTGGTGGCCGCCGTGATCTGGCGGAAGAA
GAGCAGCGGAGGCAAGGGAGGCTCCTACTATAAGGCAGAGTGGAGCGACTCCGCCCAGGGCT
CTGA (SEQ ID NO: 17069)

B2M Signal peptide:
ATGTCTCGCAGCGTGGCCCTGGCCGTGCTGGCCCTGCTGTCCCTGTCTGGCCTGGAGGCC
(SEQ ID NO: 17132)

HLA-E peptide:
GGCAGCCACTCCCTGAAGTATTTCCACACCTCTGTGAGCCGGCCAGGCAGAGGAGAGCCACG
GTTCATCTCTGTGGGCTACGTGGACGATACACAGTTCGTGAGGTTTGACAATGATGCCGCCA
GCCCAAGAATGGTGCCTAGGGCCCCATGGATGGAGCAGGAGGGCAGCGAGTATTGGGACAGG
GAGACCCGGAGCGCCAGAGACACAGCACAGATTTTCCGGGTGAACCTGAGAACCCTGAGGGG
CTACTATAATCAGTCCGAGGCCGGCTCTCACACACTCCAGTGGATGCACGGATGCGAGCTGG
GACCAGATCGCCGCTTCCTGCGGGGCTACGAGCAGTTTGCCTATGACGGCAAGGATTACCTG
ACCCTGAACGAGGACCTGAGATCCTGGACCGCCGTGGATACAGCCGCCCAGATCAGCGAGCA
GAAGTCCAATGACGCATCTGAGGCAGAGCACCAGAGGGCATATCTGGAGGATACCTGCGTGG
AGTGGCTGCACAAGTACCTGGAGAAGGGCAAGGAGACACTGCTGCACCTGGAGCCCCCTAAG
ACCCACGTGACACACCACCCAATCAGCGACCACGAGGCCACCCTGAGGTGTTGGGCACTGGG
CTTCTATCCCGCCGAGATCACCCTGACATGGCAGCAGGACGGAGAGGGACACACCCAGGATA
CAGAGCTGGTGGAGACCAGGCCCGCCGGCGATGGCACATTTCAGAAGTGGGCCGCCGTGGTG
GTGCCTTCCGGAGAGGAGCAGAGATACACCTGTCACGTGCAGCACGAGGGACTGCCAGAGCC
AGTGACCCTGAGGTGGAAGCCTGCCAGCCAGCCCACAATCCCTATCGTGGGAATCATCGCAG
GCCTGGTGCTGCTGGGCTCTGTGGTGAGCGGAGCAGTGGTGGCCGCCGTGATCTGGCGGAAG
AAGAGCAGCGGAGGCAAGGGAGGCTCCTACTATAAGGCAGAGTGGAGCGACTCCGCCCAGGG
CTCTGA (SEQ ID NO: 17137)

Immune and Immune Precursor Cells

[0270] In certain embodiments, immune cells of the disclosure comprise lymphoid progenitor cells, natural killer (NK) cells, T lymphocytes (T-cell), stem memory T cells (T_{SCM} cells), central memory T cells (T_{CM}), stem cell-like T cells, B lymphocytes (B-cells), myeloid progenitor cells, neutrophils, basophils, eosinophils, monocytes, macrophages, platelets, erythrocytes, red blood cells (RBCs), megakaryocytes or osteoclasts.

[0271] In certain embodiments, immune precursor cells comprise any cells which can differentiate into one or more types of immune cells. In certain embodiments, immune precursor cells comprise multipotent stem cells that can self renew and develop into immune cells. In certain embodiments, immune precursor cells comprise hematopoietic stem cells (HSCs) or descendants thereof. In certain embodiments, immune precursor cells comprise precursor cells that can develop into immune cells. In certain embodiments, the immune precursor cells comprise hematopoietic progenitor cells (HPCs).

Hematopoietic Stem Cells (HSCs)

[0272] Hematopoietic stem cells (HSCs) are multipotent, self-renewing cells. All differentiated blood cells from the lymphoid and myeloid lineages arise from HSCs. HSCs

can be found in adult bone marrow, peripheral blood, mobilized peripheral blood, peritoneal dialysis effluent and umbilical cord blood.

[0273] HSCs of the disclosure may be isolated or derived from a primary or cultured stem cell. HSCs of the disclosure may be isolated or derived from an embryonic stem cell, a multipotent stem cell, a pluripotent stem cell, an adult stem cell, or an induced pluripotent stem cell (iPSC).

[0274] Immune precursor cells of the disclosure may comprise an HSC or an HSC descendent cell. Exemplary HSC descendent cells of the disclosure include, but are not limited to, multipotent stem cells, lymphoid progenitor cells, natural killer (NK) cells, T lymphocyte cells (T-cells), B lymphocyte cells (B-cells), myeloid progenitor cells, neutrophils, basophils, eosinophils, monocytes, and macrophages.

[0275] HSCs produced by the methods of the disclosure may retain features of "primitive" stem cells that, while isolated or derived from an adult stem cell and while committed to a single lineage, share characteristics of embryonic stem cells. For example, the "primitive" HSCs produced by the methods of the disclosure retain their "stemness" following division and do not differentiate. Consequently, as an adoptive cell therapy, the "primitive" HSCs produced by the methods of the disclosure not only replenish their numbers, but expand in vivo. "Primitive" HSCs produced by the methods of the disclosure may be therapeutically-effective when administered as a single dose. In some embodiments, primitive HSCs of the disclosure are CD34+. In some embodiments, primitive HSCs of the disclosure are CD34+ and CD38-. In some embodiments, primitive HSCs of the disclosure are CD34+, CD38- and CD90+. In some embodiments, primitive HSCs of the disclosure are CD34+, CD38-, CD90+ and CD45RA-. In some embodiments, primitive HSCs of the disclosure are CD34+, CD38-, CD90+, CD45RA-, and CD49f+. In some embodiments, the most primitive HSCs of the disclosure are CD34+, CD38-, CD90+, CD45RA-, and CD49f+.

[0276] In some embodiments of the disclosure, primitive HSCs, HSCs, and/or HSC descendent cells may be modified according to the methods of the disclosure to express an exogenous sequence (e.g. a chimeric antigen receptor or therapeutic protein). In some embodiments of the disclosure, modified primitive HSCs, modified HSCs, and/or modified HSC descendent cells may be forward differentiated to produce a modified immune cell including, but not limited to, a modified T cell, a modified natural killer cell and/or a modified B-cell of the disclosure.

T Cells

[0277] Modified T cells of the disclosure may be derived from modified hematopoietic stem and progenitor cells (HSPCs) or modified HSCs.

[0278] Unlike traditional biologics and chemotherapeutics, modified-T cells of the disclosure possess the capacity to rapidly reproduce upon antigen recognition, thereby potentially obviating the need for repeat treatments. To achieve this, in some embodiments, modified-T cells of the disclosure not only drive an initial response, but also persist in the patient as a stable population of viable memory T cells to prevent potential relapses. Alternatively, in some embodiments, when it is not desired, modified-T cells of the disclosure do not persist in the patient.

[0279] Intensive efforts have been focused on the development of antigen receptor molecules that do not cause T cell exhaustion through antigen-independent (tonic) signaling, as well as of a modified-T cell product containing early memory T cells, especially stem cell memory (T_{SCM}) or stem cell-like T cells. Stem cell-like modified-T cells of the disclosure exhibit the greatest capacity for self-renewal and multipotent capacity to derive central memory (T_{CM}) T cells or T_{CM} like cells, effector memory (T_{EM}) and effector T cells (T_E), thereby producing better tumor eradication and long-term modified-T cell engraftment. A linear pathway of differentiation may be responsible for generating these cells: Naïve T cells (T_N) > T_{SCM} > T_{CM} > T_{EM} > T_E > T_{TE}, whereby T_N is the parent precursor cell that directly gives rise to T_{SCM}, which then, in turn, directly gives rise to T_{CM}, etc. Compositions of T cells of the disclosure may comprise one or more of each parental T cell subset with T_{SCM} cells being the most abundant (e.g. T_{SCM} > T_{CM} > T_{EM} > T_E > T_{TE}).

[0280] In some embodiments of the methods of the disclosure, the immune cell precursor is differentiated into or is capable of differentiating into an early memory T cell, a stem cell like T-cell, a Naïve T cells (T_N), a T_{SCM}, a T_{CM}, a T_{EM}, a T_E, or a T_{TE}. In some embodiments, the immune cell precursor is a primitive HSC, an HSC, or a HSC descendent cell of the disclosure.

[0281] In some embodiments of the methods of the disclosure, the immune cell is an early memory T cell, a stem cell like T-cell, a Naïve T cells (T_N), a T_{SCM}, a T_{CM}, a T_{EM}, a T_E, or a T_{TE}.

[0282] In some embodiments of the methods of the disclosure, the immune cell is an early memory T cell.

[0283] In some embodiments of the methods of the disclosure, the immune cell is a stem cell like T-cell.

[0284] In some embodiments of the methods of the disclosure, the immune cell is a T_{SCM}.

[0285] In some embodiments of the methods of the disclosure, the immune cell is a T_{CM}.

[0286] In some embodiments of the methods of the disclosure, the methods modify and/or the methods produce a plurality of modified T cells, wherein at least 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or any percentage in between of the plurality of modified T cells expresses one or more cell-surface marker(s) of an early memory T cell. In certain embodiments, the plurality of modified early memory T cells comprises at least one modified stem cell-like T cell. In certain embodiments, the plurality of modified early memory T cells comprises at least one modified T_{SCM}. In certain embodiments, the plurality of modified early memory T cells comprises at least one modified T_{CM}.

[0287] In some embodiments of the methods of the disclosure, the methods modify and/or the methods produce a plurality of modified T cells, wherein at least 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or any percentage in between of the plurality of modified T cells expresses one or more cell-surface marker(s) of a stem cell-like T cell. In certain embodiments, the plurality of modified stem cell-like T cells comprises at least one modified T_{SCM}. In certain embodiments, the plurality of modified stem cell-like T cells comprises at least one modified T_{CM}.

[0288] In some embodiments of the methods of the disclosure, the methods modify and/or the methods produce a plurality of modified T cells, wherein at least 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or any percentage in between of the plurality of modified T cells expresses one or more cell-surface marker(s) of a stem memory T cell (T_{SCM}). In certain embodiments, the cell-surface markers comprise CD62L and CD45RA. In certain embodiments, the cell-surface markers comprise one or more of CD62L, CD45RA, CD28, CCR7, CD127, CD45RO, CD95, CD95 and IL-2Rβ. In certain embodiments, the cell-surface markers comprise one or more of CD45RA, CD95, IL-2Rβ, CCR7, and CD62L.

[0289] In some embodiments of the methods of the disclosure, the methods modify and/or the methods produce a plurality of modified T cells, wherein at least 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or any percentage in between of the plurality of modified T cells expresses one or more cell-surface marker(s) of a central memory T cell (T_{CM}). In certain embodiments, the cell-surface markers comprise one or more of CD45RO, CD95, IL-2Rβ, CCR7, and CD62L.

[0290] In some embodiments of the methods of the disclosure, the methods modify and/or the methods produce a plurality of modified T cells, wherein at least 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or any percentage in between of the plurality of modified T cells expresses one or more cell-surface marker(s) of a naïve T cell (T_N). In certain embodiments, the cell-surface markers comprise one or more of CD45RA, CCR7 and CD62L.

[0291] In some embodiments of the methods of the disclosure, the methods modify and/or the methods produce a plurality of modified T cells, wherein at least 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or any percentage in between of the plurality of modified T cells expresses one or more cell-surface marker(s) of an effector T-cell (modified T_{EFF}). In certain embodiments, the cell-surface markers comprise one or more of CD45RA, CD95, and IL-2Rβ.

[0292] In some embodiments of the methods of the disclosure, the methods modify and/or the methods produce a plurality of modified T cells, wherein at least 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or any percentage in between of the plurality of modified T cells expresses one or more cell-surface marker(s) of a stem cell-like T cell, a stem memory T cell (T_{SCM}) or a central memory T cell (T_{CM}).

[0293] In some embodiments of the methods of the disclosure, a buffer comprises the immune cell or precursor thereof. The buffer maintains or enhances a level of cell viability and/or a stem-like phenotype of the immune cell or precursor thereof, including T-cells. In certain embodiments, the buffer maintains or enhances a level of cell viability and/or a stem-like phenotype of the primary human T cells prior to the nucleofection. In certain embodiments, the buffer maintains or enhances a level of cell viability and/or a stem-like phenotype of the primary human T cells during the nucleofection. In certain embodiments, the buffer maintains or enhances a level of cell viability and/or a stem-like phenotype of the primary human T cells following the nucleofection. In certain embodiments, the buffer comprises one or more of KCl, $MgCl_2$, ClNa, Glucose and $Ca(NO_3)_2$ in any absolute or relative abundance or concentration, and, optionally, the buffer further comprises a supplement selected from the group consisting of HEPES, Tris/HCl, and a phosphate buffer. In certain embodiments, the buffer comprises 5 mM KCl, 15 mM $MgCl_2$, 90 mM ClNa, 10 mM Glucose and 0.4 mM $Ca(NO_3)_2$. In certain embodiments, the buffer comprises 5 mM KCl, 15 mM $MgCl_2$, 90 mM ClNa, 10 mM Glucose and 0.4 mM $Ca(NO_3)_2$ and a supplement

comprising 20 mM HEPES and 75 mM Tris/HCl. In certain embodiments, the buffer comprises 5 mM KCl, 15 mM $MgCl_2$, 90 mM ClNa, 10 mM Glucose and 0.4 mM $Ca(NO_3)_2$ and a supplement comprising 40 mM Na_2HPO_4/NaH_2PO_4 at pH 7.2. In certain embodiments, the composition comprising primary human T cells comprises 100 µl of the buffer and between $5x10^6$ and $25x10^6$ cells. In certain embodiments, the composition comprises a scalable ratio of $250x10^6$ primary human T cells per milliliter of buffer or other media during the introduction step.

[0294] In some embodiments of the methods of the disclosure, the methods comprise contacting an immune cell of the disclosure, including a T cell of the disclosure, and a T-cell expansion composition. In some embodiments of the methods of the disclosure, the step of introducing a transposon and/or transposase of the disclosure into an immune cell of the disclosure may further comprise contacting the immune cell and a T-cell expansion composition. In some embodiments, including those in which the introducing step of the methods comprises an electroporation or a nucleofection step, the electroporation or a nucleofection step may be performed with the immune cell contacting T-cell expansion composition of the disclosure.

[0295] In some embodiments of the methods of the disclosure, the T-cell expansion composition comprises, consists essentially of or consists of phosphorus; one or more of an octanoic acid, a palmitic acid, a linoleic acid, and an oleic acid; a sterol; and an alkane.

[0296] In certain embodiments of the methods of producing a modified T cell of the disclosure, the expansion supplement comprises one or more cytokine(s). The one or more cytokine(s) may comprise any cytokine, including but not limited to, lymphokines. Exemplary lympokines include, but are not limited to, interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-15 (IL-15), interleukin-21 (IL-21), granulocyte-macrophage colony-stimulating factor (GM-CSF) and interferon-gamma (INFγ). The one or more cytokine(s) may comprise IL-2.

[0297] In some embodiments of the methods of the disclosure, the T-cell expansion composition comprises human serum albumin, recombinant human insulin, human transferrin, 2-Mercaptoethanol, and an expansion supplement. In certain embodiments of this method, the T-cell expansion composition further comprises one or more of octanoic acid, nicotinamide, 2,4,7,9-tetramethyl-5-decyn-4,7-diol (TMDD), diisopropyl adipate (DIPA), n-butyl-benzenesulfonamide, 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester, palmitic

acid, linoleic acid, oleic acid, stearic acid hydrazide, oleamide, a sterol and an alkane. In certain embodiments of this method, the T-cell expansion composition further comprises one or more of octanoic acid, palmitic acid, linoleic acid, oleic acid and a sterol. In certain embodiments of this method, the T-cell expansion composition further comprises one or more of octanoic acid at a concentration of between 0.9 mg/kg to 90 mg/kg, inclusive of the endpoints; palmitic acid at a concentration of between 0.2 mg/kg to 20 mg/kg, inclusive of the endpoints; linoleic acid at a concentration of between 0.2 mg/kg to 20 mg/kg, inclusive of the endpoints; oleic acid at a concentration of 0.2 mg/kg to 20 mg/kg, inclusive of the endpoints; and a sterol at a concentration of about 0.1 mg/kg to 10 mg/kg, inclusive of the endpoints. In certain embodiments of this method, the T-cell expansion composition further comprises one or more of octanoic acid at a concentration of about 9 mg/kg, palmitic acid at a concentration of about 2 mg/kg, linoleic acid at a concentration of about 2 mg/kg, oleic acid at a concentration of about 2 mg/kg and a sterol at a concentration of about 1 mg/kg. In certain embodiments of this method, the T-cell expansion composition further comprises one or more of octanoic acid at a concentration of between 6.4 μmol/kg and 640 μmol/kg, inclusive of the endpoints; palmitic acid at a concentration of between 0.7 μmol/kg and 70 μmol/kg, inclusive of the endpoints; linoleic acid at a concentration of between 0.75 μmol/kg and 75 μmol/kg, inclusive of the endpoints; oleic acid at a concentration of between 0.75 μmol/kg and 75 μmol/kg, inclusive of the endpoints; and a sterol at a concentration of between 0.25 μmol/kg and 25 μmol/kg, inclusive of the endpoints. In certain embodiments of this method, the T-cell expansion composition further comprises one or more of octanoic acid at a concentration of about 64 μmol/kg, palmitic acid at a concentration of about 7 μmol/kg, linoleic acid at a concentration of about 7.5 μmol/kg, oleic acid at a concentration of about 7.5 μmol/kg and a sterol at a concentration of about 2.5 μmol/kg.

[0298] In certain embodiments, the T-cell expansion composition comprises one or more of human serum albumin, recombinant human insulin, human transferrin, 2-Mercaptoethanol, and an expansion supplement to produce a plurality of expanded modified T-cells, wherein at least 2% of the plurality of modified T-cells expresses one or more cell-surface marker(s) of an early memory T cell, a stem cell-like T cell, a stem memory T cell (T_{SCM}) and/or a central memory T cell (T_{CM}). In certain embodiments, the T-cell expansion composition comprises or further comprises one or more of octanoic acid, nicotinamide, 2,4,7,9-tetramethyl-5-decyn-4,7-diol (TMDD), diisopropyl adipate (DIPA), n-butyl-benzenesulfonamide, 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester, palmitic acid, linoleic acid, oleic acid,

stearic acid hydrazide, oleamide, a sterol and an alkane. In certain embodiments, the T-cell expansion composition comprises one or more of octanoic acid, palmitic acid, linoleic acid, oleic acid and a sterol (e.g. cholesterol). In certain embodiments, the T-cell expansion composition comprises one or more of octanoic acid at a concentration of between 0.9 mg/kg to 90 mg/kg, inclusive of the endpoints; palmitic acid at a concentration of between 0.2 mg/kg to 20 mg/kg, inclusive of the endpoints; linoleic acid at a concentration of between 0.2 mg/kg to 20 mg/kg, inclusive of the endpoints; oleic acid at a concentration of 0.2 mg/kg to 20 mg/kg, inclusive of the endpoints; and a sterol at a concentration of about 0.1 mg/kg to 10 mg/kg, inclusive of the endpoints (wherein mg/kg = parts per million). In certain embodiments, the T-cell expansion composition comprises one or more of octanoic acid at a concentration of about 9 mg/kg, palmitic acid at a concentration of about 2 mg/kg, linoleic acid at a concentration of about 2 mg/kg, oleic acid at a concentration of about 2 mg/kg, and a sterol at a concentration of about 1 mg/kg (wherein mg/kg = parts per million). In certain embodiments, the T-cell expansion composition comprises one or more of octanoic acid at a concentration of 9.19 mg/kg, palmitic acid at a concentration of 1.86 mg/kg, linoleic acid at a concentration of about 2.12 mg/kg, oleic acid at a concentration of about 2.13 mg/kg, and a sterol at a concentration of about 1.01 mg/kg (wherein mg/kg = parts per million). In certain embodiments, the T-cell expansion composition comprises octanoic acid at a concentration of 9.19 mg/kg, palmitic acid at a concentration of 1.86 mg/kg, linoleic acid at a concentration of 2.12 mg/kg, oleic acid at a concentration of about 2.13 mg/kg, and a sterol at a concentration of 1.01 mg/kg (wherein mg/kg = parts per million). In certain embodiments, the T-cell expansion composition comprises one or more of octanoic acid at a concentration of between 6.4 µmol/kg and 640 µmol/kg, inclusive of the endpoints; palmitic acid at a concentration of between 0.7 µmol/kg and 70 µmol/kg, inclusive of the endpoints; linoleic acid at a concentration of between 0.75 µmol/kg and 75 µmol/kg, inclusive of the endpoints; oleic acid at a concentration of between 0.75 µmol/kg and 75 µmol/kg, inclusive of the endpoints; and a sterol at a concentration of between 0.25 µmol/kg and 25 µmol/kg, inclusive of the endpoints. In certain embodiments, the T-cell expansion composition comprises one or more of octanoic acid at a concentration of about 64 µmol/kg, palmitic acid at a concentration of about 7 µmol/kg, linoleic acid at a concentration of about 7.5 µmol/kg, oleic acid at a concentration of about 7.5 µmol/kg and a sterol at a concentration of about 2.5 µmol/kg. In certain embodiments, the T-cell expansion composition comprises one or more of octanoic acid at a concentration of about 63.75 µmol/kg, palmitic acid at a concentration of about 7.27

μmol/kg, linoleic acid at a concentration of about 7.57 μmol/kg, oleic acid at a concentration of about 7.56 μmol/kg and a sterol at a concentration of about 2.61 μmol/kg. In certain embodiments, the T-cell expansion composition comprises octanoic acid at a concentration of about 63.75 μmol/kg, palmitic acid at a concentration of about 7.27 μmol/kg, linoleic acid at a concentration of about 7.57 μmol/kg, oleic acid at a concentration of 7.56 μmol/kg and a sterol at a concentration of 2.61 μmol/kg.

[0299] As used herein, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of human serum albumin, recombinant human insulin, human transferrin, 2-Mercaptoethanol, and an expansion supplement at 37°C. Alternatively, or in addition, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of phosphorus, an octanoic fatty acid, a palmitic fatty acid, a linoleic fatty acid and an oleic acid. In certain embodiments, the media comprises an amount of phosphorus that is 10-fold higher than may be found in, for example, Iscove's Modified Dulbecco's Medium ((IMDM); available at ThermoFisher Scientific as Catalog number 12440053).

[0300] As used herein, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of human serum albumin, recombinant human insulin, human transferrin, 2-Mercaptoethanol, Iscove's MDM, and an expansion supplement at 37°C. Alternatively, or in addition, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of the following elements: boron, sodium, magnesium, phosphorus, potassium, and calcium. In certain embodiments, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of the following elements present in the corresponding average concentrations: boron at 3.7 mg/L, sodium at 3000 mg/L, magnesium at 18 mg/L, phosphorus at 29 mg/L, potassium at 15 mg/L and calcium at 4 mg/L.

[0301] As used herein, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of human serum albumin, recombinant human insulin, human transferrin, 2-Mercaptoethanol, and an expansion supplement at 37°C. Alternatively, or in addition, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used

interchangeably with a media comprising one or more of the following components: octanoic acid (CAS No. 124-07-2), nicotinamide (CAS No. 98-92-0), 2,4,7,9-tetramethyl-5-decyn-4,7-diol (TMDD) (CAS No. 126-86-3), diisopropyl adipate (DIPA) (CAS No. 6938-94-9), n-butyl-benzenesulfonamide (CAS No. 3622-84-2), 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester (CAS No. 84-69-5), palmitic acid (CAS No. 57-10-3), linoleic acid (CAS No. 60-33-3), oleic acid (CAS No. 112-80-1), stearic acid hydrazide (CAS No. 4130-54-5), oleamide (CAS No. 3322-62-1), sterol (e.g., cholesterol) (CAS No. 57-88-5), and alkanes (e.g., nonadecane) (CAS No. 629-92-5). In certain embodiments, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of the following components: octanoic acid (CAS No. 124-07-2), nicotinamide (CAS No. 98-92-0), 2,4,7,9-tetramethyl-5-decyn-4,7-diol (TMDD) (CAS No. 126-86-3), diisopropyl adipate (DIPA) (CAS No. 6938-94-9), n-butyl-benzenesulfonamide (CAS No. 3622-84-2), 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester (CAS No. 84-69-5), palmitic acid (CAS No. 57-10-3), linoleic acid (CAS No. 60-33-3), oleic acid (CAS No. 112-80-1), stearic acid hydrazide (CAS No. 4130-54-5), oleamide (CAS No. 3322-62-1), sterol (e.g., cholesterol) (CAS No. 57-88-5), alkanes (e.g., nonadecane) (CAS No. 629-92-5), and phenol red (CAS No. 143-74-8). In certain embodiments, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of the following components: octanoic acid (CAS No. 124-07-2), nicotinamide (CAS No. 98-92-0), 2,4,7,9-tetramethyl-5-decyn-4,7-diol (TMDD) (CAS No. 126-86-3), diisopropyl adipate (DIPA) (CAS No. 6938-94-9), n-butyl-benzenesulfonamide (CAS No. 3622-84-2), 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester (CAS No. 84-69-5), palmitic acid (CAS No. 57-10-3), linoleic acid (CAS No. 60-33-3), oleic acid (CAS No. 112-80-1), stearic acid hydrazide (CAS No. 4130-54-5), oleamide (CAS No. 3322-62-1), phenol red (CAS No. 143-74-8) and lanolin alcohol.

[0302] In certain embodiments, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of human serum albumin, recombinant human insulin, human transferrin, 2-Mercaptoethanol, and an expansion supplement at 37°C. Alternatively, or in addition, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of the following ions: sodium, ammonium, potassium, magnesium, calcium, chloride, sulfate and phosphate.

[0303] As used herein, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of human serum albumin, recombinant human insulin, human transferrin, 2-Mercaptoethanol, and an expansion supplement at 37°C. Alternatively, or in addition, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of the following free amino acids: histidine, asparagine, serine, glutamate, arginine, glycine, aspartic acid, glutamic acid, threonine, alanine, proline, cysteine, lysine, tyrosine, methionine, valine, isoleucine, leucine, phenylalanine and tryptophan. In certain embodiments, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of the following free amino acids in the corresponding average mole percentages: histidine (about 1%), asparagine (about 0.5%), serine (about 1.5%), glutamine (about 67%), arginine (about 1.5%), glycine (about 1.5%), aspartic acid (about 1%), glutamic acid (about 2%), threonine (about 2%), alanine (about 1%), proline (about 1.5%), cysteine (about 1.5%), lysine (about 3%), tyrosine (about 1.5%), methionine (about 1%), valine (about 3.5%), isoleucine (about 3%), leucine (about 3.5%), phenylalanine (about 1.5%) and tryptophan (about 0.5%). In certain embodiments, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of the following free amino acids in the corresponding average mole percentages: histidine (about .78%), asparagine (about 0.4%), serine (about 1.6%), glutamine (about 67.01%), arginine (about 1.67%), glycine (about 1.72%), aspartic acid (about 1.00%), glutamic acid (about 1.93%), threonine (about 2.38%), alanine (about 1.11%), proline (about 1.49%), cysteine (about 1.65%), lysine (about 2.84%), tyrosine (about 1.62%), methionine (about 0.85%), valine (about 3.45%), isoleucine (about 3.14%), leucine (about 3.3%), phenylalanine (about 1.64%) and tryptophan (about 0.37%).

[0304] As used herein, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of human serum albumin, recombinant human insulin, human transferrin, 2-Mercaptoethanol, Iscove's MDM, and an expansion supplement at 37°C. Alternatively, or in addition, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of phosphorus, an octanoic fatty acid, a palmitic fatty acid, a linoleic fatty acid and an oleic acid. In certain embodiments, the

media comprises an amount of phosphorus that is 10-fold higher than may be found in, for example, Iscove's Modified Dulbecco's Medium ((IMDM); available at ThermoFisher Scientific as Catalog number 12440053).

[0305] In certain embodiments, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of octanoic acid, palmitic acid, linoleic acid, oleic acid and a sterol (e.g. cholesterol). In certain embodiments, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of octanoic acid at a concentration of between 0.9 mg/kg to 90 mg/kg, inclusive of the endpoints; palmitic acid at a concentration of between 0.2 mg/kg to 20 mg/kg, inclusive of the endpoints; linoleic acid at a concentration of between 0.2 mg/kg to 20 mg/kg, inclusive of the endpoints; oleic acid at a concentration of 0.2 mg/kg to 20 mg/kg, inclusive of the endpoints; and a sterol at a concentration of about 0.1 mg/kg to 10 mg/kg, inclusive of the endpoints (wherein mg/kg = parts per million). In certain embodiments, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of octanoic acid at a concentration of about 9 mg/kg, palmitic acid at a concentration of about 2 mg/kg, linoleic acid at a concentration of about 2 mg/kg, oleic acid at a concentration of about 2 mg/kg, and a sterol at a concentration of about 1 mg/kg (wherein mg/kg = parts per million). In certain embodiments, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of octanoic acid at a concentration of 9.19 mg/kg, palmitic acid at a concentration of 1.86 mg/kg, linoleic acid at a concentration of about 2.12 mg/kg, oleic acid at a concentration of about 2.13 mg/kg, and a sterol at a concentration of about 1.01 mg/kg (wherein mg/kg = parts per million). In certain embodiments, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of octanoic acid at a concentration of 9.19 mg/kg, palmitic acid at a concentration of 1.86 mg/kg, linoleic acid at a concentration of 2.12 mg/kg, oleic acid at a concentration of about 2.13 mg/kg, and a sterol at a concentration of 1.01 mg/kg (wherein mg/kg = parts per million). In certain embodiments, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of octanoic acid at a concentration of between 6.4 μmol/kg and 640 μmol/kg, inclusive of the endpoints; palmitic acid at a concentration of between 0.7 μmol/kg and 70

μmol/kg, inclusive of the endpoints; linoleic acid at a concentration of between 0.75 μmol/kg and 75 μmol/kg, inclusive of the endpoints; oleic acid at a concentration of between 0.75 μmol/kg and 75 μmol/kg, inclusive of the endpoints; and a sterol at a concentration of between 0.25 μmol/kg and 25 μmol/kg, inclusive of the endpoints. In certain embodiments, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of octanoic acid at a concentration of about 64 μmol/kg, palmitic acid at a concentration of about 7 μmol/kg, linoleic acid at a concentration of about 7.5 μmol/kg, oleic acid at a concentration of about 7.5 μmol/kg and a sterol at a concentration of about 2.5 μmol/kg.

[0306] In certain embodiments, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of octanoic acid at a concentration of about 63.75 μmol/kg, palmitic acid at a concentration of about 7.27 μmol/kg, linoleic acid at a concentration of about 7.57 μmol/kg, oleic acid at a concentration of about 7.56 μmol/kg and a sterol at a concentration of about 2.61 μmol/kg. In certain embodiments, the terms "supplemented T-cell expansion composition" or "T-cell expansion composition" may be used interchangeably with a media comprising one or more of octanoic acid at a concentration of about 63.75 μmol/kg, palmitic acid at a concentration of about 7.27 μmol/kg, linoleic acid at a concentration of about 7.57 μmol/kg, oleic acid at a concentration of 7.56 μmol/kg and a sterol at a concentration of 2.61 μmol/kg.

[0307] In certain embodiments of the methods of producing a modified T cell (e.g. a stem cell-like T cell, a T_{SCM} and/or a T_{CM}) of the disclosure, the method comprises contacting a modified T cell and an inhibitor of the P13K-Akt-mTOR pathway. Modified T-cells of the disclosure, including modified stem cell-like T cells, T_{SCM} and/or T_{CM} of the disclosure, may be incubated, cultured, grown, stored, or otherwise, combined at any step in the methods of the procedure with a growth medium comprising one or more inhibitors a component of a PI3K pathway. Exemplary inhibitors a component of a PI3K pathway include, but are not limited to, an inhibitor of GSK3β such as TWS119 (also known as GSK 3B inhibitor XII; CAS Number 601514-19-6 having a chemical formula $C_{18}H_{14}N_4O_2$). Exemplary inhibitors of a component of a PI3K pathway include, but are not limited to, bb007 (BLUEBIRDBIO™). Additional Exemplary inhibitors of a component of a PI3K pathway include, but are not limited to, an allosteric Akt inhibitor VIII (also referred to as Akti-1/2 having Compound number 10196499), ATP competitive inhibitors (Orthosteric inhibitors targeting the ATP-

binding pocket of the protein kinase B (Akt)), Isoquinoline-5-sulfonamides (H-8, H-89, and NL-71-101), Azepane derivatives (A series of structures derived from (−)-balanol), Aminofurazans (GSK690693), Heterocyclic rings (7-azaindole, 6-phenylpurine derivatives, pyrrolo[2,3-d]pyrimidine derivatives, CCT128930, 3-aminopyrrolidine, anilinotriazole derivatives, spiroindoline derivatives, AZD5363, ipatasertib (GDC-0068, RG7440), A-674563, and A-443654), Phenylpyrazole derivatives (AT7867 and AT13148), Thiophenecarboxamide derivatives (Afuresertib (GSK2110183), 2-pyrimidyl-5-amidothiophene derivative (DC120), uprosertib (GSK2141795)), Allosteric inhibitors (Superior to orthosteric inhibitors providing greater specificity, reduced side-effects and less toxicity), 2,3-diphenylquinoxaline analogues (2,3-diphenylquinoxaline derivatives, triazolo[3,4-f][1,6]naphthyridin-3(2H)-one derivative (MK-2206)), Alkylphospholipids (Edelfosine (1-O-octadecyl-2-O-methyl-rac-glycero-3-phosphocholine, ET-18-OCH_3) ilmofosine (BM 41.440), miltefosine (hexadecylphosphocholine, HePC), perifosine (D-21266), erucylphosphocholine (ErPC), erufosine (ErPC3, erucylphosphohomocholine), Indole-3-carbinol analogues (Indole-3-carbinol, 3-chloroacetylindole, diindolylmethane, diethyl 6-methoxy-5,7-dihydroindolo [2,3-b]carbazole-2,10-dicarboxylate (SR13668), OSU-A9), Sulfonamide derivatives (PH-316 and PHT-427), Thiourea derivatives (PIT-1, PIT-2, DM-PIT-1, N-[(1-methyl-1H-pyrazol-4-yl)carbonyl]-N′-(3-bromophenyl)-thiourea), Purine derivatives (Triciribine (TCN, NSC 154020), triciribine mono-phosphate active analogue (TCN-P), 4-amino-pyrido[2,3-d]pyrimidine derivative API-1, 3-phenyl-3H-imidazo[4,5-b]pyridine derivatives, ARQ 092), BAY 1125976, 3-methyl-xanthine, quinoline-4-carboxamide and 2-[4-(cyclohexa-1,3-dien-1-yl)-1H-pyrazol-3-yl]phenol, 3-oxo-tirucallic acid, 3α- and 3β-acetoxy-tirucallic acids, acetoxy-tirucallic acid, and irreversible inhibitors (antibiotics, Lactoquinomycin, Frenolicin B, kalafungin, medermycin, Boc-Phe-vinyl ketone, 4-hydroxynonenal (4-HNE), 1,6-naphthyridinone derivatives, and imidazo-1,2-pyridine derivatives).

[0308] In certain embodiments of the methods of producing a modified T cell (e.g. a stem cell-like T cell, a T_{SCM} and/or a T_{CM}) of the disclosure, the method comprises contacting a modified T cell and an inhibitor of T cell effector differentiation. Exemplary inhibitors of T cell effector differentiation include, but are not limited to, a BET inhibitor (e.g. JQ1, a hienotriazolodiazepine) and/or an inhibitor of the BET family of proteins (e.g. BRD2, BRD3, BRD4, and BRDT).

[0309] In certain embodiments of the methods of producing a modified T cell (e.g. a stem cell-like T cell, a T_{SCM} and/or a T_{CM}) of the disclosure, the method comprises contacting a modified T cell and an agent that reduces nucleo-cytoplasmic Acetyl-CoA. Exemplary agents that reduce nucleo-cytoplasmic Acetyl-CoA include, but are not limited to, 2-hydroxy-citrate (2-HC) as well as agents that increase expression of *Acss1*.

[0310] In certain embodiments of the methods of producing a modified T cell (e.g. a stem cell-like T cell, a TSCM and/or a TCM) of the disclosure, the method comprises contacting a modified T cell and a composition comprising a histone deacetylase (HDAC) inhibitor. In some embodiments, the composition comprising an HDAC inhibitor comprises or consists of valproic acid, Sodium Phenylbutyrate (NaPB) or a combination thereof. In some embodiments, the composition comprising an HDAC inhibitor comprises or consists of valproic acid. In some embodiments, the composition comprising an HDAC inhibitor comprises or consists of Sodium Phenylbutyrate (NaPB).

[0311] In certain embodiments of the methods of producing a modified T cell (e.g. a stem cell-like T cell, a T_{SCM} and/or a T_{CM}) of the disclosure, the activation supplement may comprise one or more cytokine(s). The one or more cytokine(s) may comprise any cytokine, including but not limited to, lymphokines. Exemplary lympokines include, but are not limited to, interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-15 (IL-15), interleukin-21 (IL-21), granulocyte-macrophage colony-stimulating factor (GM-CSF) and interferon-gamma (INFγ). The one or more cytokine(s) may comprise IL-2.

[0312] In certain embodiments of the methods of producing a modified T cell (e.g. a stem cell-like T cell, a T_{SCM} and/or a T_{CM}) of the disclosure, the activation supplement may comprise one or more activator complexes. Exemplary and nonlimiting activator complexes may comprise a monomeric, dimeric, trimeric or tetrameric antibody complex that binds one or more of CD3, CD28, and CD2. In some embodiments, the activation supplement comprises or consists of an activator complex that comprises a human, a humanized or a recombinant or a chimeric antibody. In some embodiments, the activation supplement comprises or consists of an activator complex that binds CD3 and CD28. In some embodiments, the activation supplement comprises or consists of an activator complex that binds CD3, CD28 and CD2.

Natural Killer (NK) cells

[0313] In certain embodiments, the modified immune or immune precursor cells of the disclosure are natural killer (NK) cells. In certain embodiments, NK cells are cytotoxic lymphocytes that differentiate from lymphoid progenitor cells.

[0314] Modified NK cells of the disclosure may be derived from modified hematopoietic stem and progenitor cells (HSPCs) or modified HSCs.

[0315] In certain embodiments, non-activated NK cells are derived from CD3-depleted leukopheresis (containing CD14/CD19/CD56+ cells).

[0316] In certain embodiments, NK cells are electroporated using a Lonza 4D nucleofector or BTX ECM 830 (500V, 700 usec pulse length, 0.2 mm electrode gap, one pulse). All Lonza 4D nucleofector programs are contemplated as within the scope of the methods of the disclosure.

[0317] In certain embodiments, 5x10E6 cells were electroporated per electroporation in 100 µL P3 buffer in cuvettes. However, this ratio of cells per volume is scalable for commercial manufacturing methods.

[0318] In certain embodiments, NK cells were stimulated by co-culture with an additional cell line. In certain embodiments, the additional cell line comprises artificial antigen presenting cells (aAPCs). In certain embodiments, stimulation occurs at day 1, 2, 3, 4, 5, 6, or 7 following electroporation. In certain embodiments, stimulation occurs at day 2 following electroporation.

[0319] In certain embodiments, NK cells express CD56.

B cells

[0320] In certain embodiments, the modified immune or immune precursor cells of the disclosure are B cells. B cells are a type of lymphocyte that express B cell receptors on the cell surface. B cell receptors bind to specific antigens.

[0321] Modified B cells of the disclosure may be derived from modified hematopoietic stem and progenitor cells (HSPCs) or modified HSCs.

[0322] In certain embodiments, HSPCs are modified using the methods of the disclosure, and then primed for B cell differentiation in presence of human IL-3, Flt3L, TPO, SCF, and G-CSF for at least 3 days, at least 4 days, at least 5 days, at least 6 days or at least 7 days. In certain embodiments, HSPCs are modified using the methods of the disclosure, and then primed for B cell differentiation in presence of human IL-3, Flt3L, TPO, SCF, and G-CSF for 5 days.

[0323] In certain embodiments, following priming, modified HSPC cells are transferred to a layer of feeder cells and fed bi-weekly, along with transfer to a fresh layer of feeders once per week. In certain embodiments, the feeder cells are MS-5 feeder cells.

[0324] In certain embodiments, modified HSPC cells are cultured with MS-5 feeder cells for at least 7, 14, 21, 28, 30, 33, 35, 42 or 48 days. In certain embodiments, modified HSPC cells were cultured with MS-5 feeder cells for 33 days.

Inducible Proapoptotic Polypeptides

[0325] Inducible proapoptotic polypeptides of the disclosure are superior to existing inducible polypeptides because the inducible proapoptotic polypeptides of the disclosure are far less immunogenic. While inducible proapoptotic polypeptides of the disclosure are recombinant polypeptides, and, therefore, non-naturally occurring, the sequences that are recombined to produce the inducible proapoptotic polypeptides of the disclosure do not comprise non-human sequences that the host human immune system could recognize as "non-self" and, consequently, induce an immune response in the subject receiving an inducible proapoptotic polypeptide of the disclosure, a cell comprising the inducible proapoptotic polypeptide or a composition comprising the inducible proapoptotic polypeptide or the cell comprising the inducible proapoptotic polypeptide.

[0326] The disclosure provides inducible proapoptotic polypeptides comprising a ligand binding region, a linker, and a proapoptotic peptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments, the non-human sequence comprises a restriction site. In certain embodiments, the proapoptotic peptide is a caspase polypeptide. In certain embodiments, the caspase polypeptide is a caspase 9 polypeptide. In certain embodiments, the caspase 9 polypeptide is a truncated caspase 9 polypeptide. Inducible proapoptotic polypeptides of the disclosure may be non-naturally occurring.

[0327] Caspase polypeptides of the disclosure include, but are not limited to, caspase 1, caspase 2, caspase 3, caspase 4, caspase 5, caspase 6, caspase 7, caspase 8, caspase 9, caspase 10, caspase 11, caspase 12, and caspase 14. Caspase polypeptides of the disclosure include, but are not limited to, those caspase polypeptides associated with apoptosis including caspase 2, caspase 3, caspase 6, caspase 7, caspase 8, caspase 9, and caspase 10. Caspase polypeptides of the disclosure include, but are not limited to, those caspase polypeptides that initiate apoptosis, including caspase 2, caspase 8, caspase 9, and caspase 10. Caspase

polypeptides of the disclosure include, but are not limited to, those caspase polypeptides that execute apoptosis, including caspase 3, caspase 6, and caspase 7.

[0328] Caspase polypeptides of the disclosure may be encoded by an amino acid or a nucleic acid sequence having one or more modifications compared to a wild type amino acid or a nucleic acid sequence. The nucleic acid sequence encoding a caspase polypeptide of the disclosure may be codon optimized. The one or more modifications to an amino acid and/or nucleic acid sequence of a caspase polypeptide of the disclosure may increase an interaction, a cross-linking, a cross-activation, or an activation of the caspase polypeptide of the disclosure compared to a wild type amino acid or a nucleic acid sequence. Alternatively, or in addition, the one or more modifications to an amino acid and/or nucleic acid sequence of a caspase polypeptide of the disclosure may decrease the immunogenicity of the caspase polypeptide of the disclosure compared to a wild type amino acid or a nucleic acid sequence.

[0329] Caspase polypeptides of the disclosure may be truncated compared to a wild type caspase polypeptide. For example, a caspase polypeptide may be truncated to eliminate a sequence encoding a Caspase Activation and Recruitment Domain (CARD) to eliminate or minimize the possibility of activating a local inflammatory response in addition to initiating apoptosis in the cell comprising an inducible caspase polypeptide of the disclosure. The nucleic acid sequence encoding a caspase polypeptide of the disclosure may be spliced to form a variant amino acid sequence of the caspase polypeptide of the disclosure compared to a wild type caspase polypeptide. Caspase polypeptides of the disclosure may be encoded by recombinant and/or chimeric sequences. Recombinant and/or chimeric caspase polypeptides of the disclosure may include sequences from one or more different caspase polypeptides. Alternatively, or in addition, recombinant and/or chimeric caspase polypeptides of the disclosure may include sequences from one or more species (e.g. a human sequence and a non-human sequence). Caspase polypeptides of the disclosure may be non-naturally occurring.

[0330] The ligand binding region of an inducible proapoptotic polypeptide of the disclosure may include any polypeptide sequence that facilitates or promotes the dimerization of a first inducible proapoptotic polypeptide of the disclosure with a second inducible proapoptotic polypeptide of the disclosure, the dimerization of which activates or induces cross-linking of the proapoptotic polypeptides and initiation of apoptosis in the cell.

[0331] The ligand-binding ("dimerization") region may comprise any polypeptide or functional domain thereof that will allow for induction using an endogenous or non-naturally

occurring ligand (i.e. and induction agent), for example, a non-naturally occurring synthetic ligand. The ligand-binding region may be internal or external to the cellular membrane, depending upon the nature of the inducible proapoptotic polypeptide and the choice of ligand (i.e. induction agent). A wide variety of ligand-binding polypeptides and functional domains thereof, including receptors, are known. Ligand-binding regions of the disclosure may include one or more sequences from a receptor. Of particular interest are ligand-binding regions for which ligands (for example, small organic ligands) are known or may be readily produced. These ligand-binding regions or receptors may include, but are not limited to, the FKBPs and cyclophilin receptors, the steroid receptors, the tetracycline receptor, and the like, as well as "non-naturally occurring" receptors, which can be obtained from antibodies, particularly the heavy or light chain subunit, mutated sequences thereof, random amino acid sequences obtained by stochastic procedures, combinatorial syntheses, and the like. In certain embodiments, the ligand-binding region is selected from the group consisting of a FKBP ligand-binding region, a cyclophilin receptor ligand-binding region, a steroid receptor ligand-binding region, a cyclophilin receptors ligand-binding region, and a tetracycline receptor ligand-binding region.

[0332] The ligand-binding regions comprising one or more receptor domain(s) may be at least about 50 amino acids, and fewer than about 350 amino acids, usually fewer than 200 amino acids, either as the endogenous domain or truncated active portion thereof. The binding region may, for example, be small (< 25 kDa, to allow efficient transfection in viral vectors), monomeric, nonimmunogenic, have synthetically accessible, cell permeable, nontoxic ligands that can be configured for dimerization.

[0333] The ligand-binding regions comprising one or more receptor domain(s) may be intracellular or extracellular depending upon the design of the inducible proapoptotic polypeptide and the availability of an appropriate ligand (i.e. induction agent). For hydrophobic ligands, the binding region can be on either side of the membrane, but for hydrophilic ligands, particularly protein ligands, the binding region will usually be external to the cell membrane, unless there is a transport system for internalizing the ligand in a form in which it is available for binding. For an intracellular receptor, the inducible proapoptotic polypeptide or a transposon or vector comprising the inducible proapoptotic polypeptide may encode a signal peptide and transmembrane domain 5′ or 3′ of the receptor domain sequence or may have a lipid attachment signal sequence 5′ of the receptor domain sequence. Where

the receptor domain is between the signal peptide and the transmembrane domain, the receptor domain will be extracellular.

[0334] Antibodies and antibody subunits, e.g., heavy or light chain, particularly fragments, more particularly all or part of the variable region, or fusions of heavy and light chain to create high-affinity binding, can be used as a ligand binding region of the disclosure. Antibodies that are contemplated include ones that are an ectopically expressed human product, such as an extracellular domain that would not trigger an immune response and generally not expressed in the periphery (i.e., outside the CNS/brain area). Such examples, include, but are not limited to low affinity nerve growth factor receptor (LNGFR), and embryonic surface proteins (i.e., carcinoembryonic antigen). Yet further, antibodies can be prepared against haptenic molecules, which are physiologically acceptable, and the individual antibody subunits screened for binding affinity. The cDNA encoding the subunits can be isolated and modified by deletion of the constant region, portions of the variable region, mutagenesis of the variable region, or the like, to obtain a binding protein domain that has the appropriate affinity for the ligand. In this way, almost any physiologically acceptable haptenic compound can be employed as the ligand or to provide an epitope for the ligand. Instead of antibody units, endogenous receptors can be employed, where the binding region or domain is known and there is a useful or known ligand for binding.

[0335] For multimerizing the receptor, the ligand for the ligand-binding region/receptor domains of the inducible proapoptotic polypeptides may be multimeric in the sense that the ligand can have at least two binding sites, with each of the binding sites capable of binding to a ligand receptor region (i.e. a ligand having a first binding site capable of binding the ligand-binding region of a first inducible proapoptotic polypeptide and a second binding site capable of binding the ligand-binding region of a second inducible proapoptotic polypeptide, wherein the ligand-binding regions of the first and the second inducible proapoptotic polypeptides are either identical or distinct). Thus, as used herein, the term "multimeric ligand binding region" refers to a ligand-binding region of an inducible proapoptotic polypeptide of the disclosure that binds to a multimeric ligand. Multimeric ligands of the disclosure include dimeric ligands. A dimeric ligand of the disclosure may have two binding sites capable of binding to the ligand receptor domain. In certain embodiments, multimeric ligands of the disclosure are a dimer or higher order oligomer, usually not greater than about tetrameric, of small synthetic organic molecules, the individual molecules typically being at least about 150 Da and less than about 5 kDa, usually less than about 3 kDa. A variety of pairs of synthetic ligands and

receptors can be employed. For example, in embodiments involving endogenous receptors, dimeric FK506 can be used with an FKBP12 receptor, dimerized cyclosporin A can be used with the cyclophilin receptor, dimerized estrogen with an estrogen receptor, dimerized glucocorticoids with a glucocorticoid receptor, dimerized tetracycline with the tetracycline receptor, dimerized vitamin D with the vitamin D receptor, and the like. Alternatively, higher orders of the ligands, e.g., trimeric can be used. For embodiments involving non-naturally occurring receptors, e.g., antibody subunits, modified antibody subunits, single chain antibodies comprised of heavy and light chain variable regions in tandem, separated by a flexible linker, or modified receptors, and mutated sequences thereof, and the like, any of a large variety of compounds can be used. A significant characteristic of the units comprising a multimeric ligand of the disclosure is that each binding site is able to bind the receptor with high affinity, and preferably, that they are able to be dimerized chemically. Also, methods are available to balance the hydrophobicity/hydrophilicity of the ligands so that they are able to dissolve in serum at functional levels, yet diffuse across plasma membranes for most applications.

[0336] Activation of inducible proapoptotic polypeptides of the disclosure may be accomplished through, for example, chemically induced dimerization (CID) mediated by an induction agent to produce a conditionally controlled protein or polypeptide. Proapoptotic polypeptides of the disclosure not only inducible, but the induction of these polypeptides is also reversible, due to the degradation of the labile dimerizing agent or administration of a monomeric competitive inhibitor.

[0337] In certain embodiments, the ligand binding region comprises a FK506 binding protein 12 (FKBP12) polypeptide. In certain embodiments, the ligand binding region comprises a FKBP12 polypeptide having a substitution of valine (V) for phenylalanine (F) at position 36 (F36V). In certain embodiments, in which the ligand binding region comprises a FKBP12 polypeptide having a substitution of valine (V) for phenylalanine (F) at position 36 (F36V), the induction agent may comprise AP1903, a synthetic drug (CAS Index Name: 2-Piperidinecarboxylic acid, 1-[(2S)-1-oxo-2-(3,4,5-trimethoxyphenyl)butyl]-, 1,2-ethanediylbis[imino(2-oxo-2,1-ethanediyl)oxy-3,1-phenylene[(1R)-3-(3,4-dimethoxyphenyl)propylidene]]ester, [2S-[1(R*),2R*[S*[S*[1(R*),2R*]]]]]-(9Cl) CAS Registry Number: 195514-63-7; Molecular Formula: C78H98N4O20; Molecular Weight: 1411.65)). In certain embodiments, in which the ligand binding region comprises a FKBP12 polypeptide having a substitution of valine (V) for phenylalanine (F) at position 36 (F36V),

the induction agent may comprise AP20187 (CAS Registry Number: 195514-80-8 and Molecular Formula: C82H107N5O20). In certain embodiments, the induction agent is an AP20187 analog, such as, for example, AP1510. As used herein, the induction agents AP20187, AP1903 and AP1510 may be used interchangeably.

[0338] AP1903 API is manufactured by Alphora Research Inc. and AP1903 Drug Product for Injection is made by Formatech Inc. It is formulated as a 5 mg/mL solution of AP1903 in a 25% solution of the non-ionic solubilizer Solutol HS 15 (250 mg/mL, BASF). At room temperature, this formulation is a clear, slightly yellow solution. Upon refrigeration, this formulation undergoes a reversible phase transition, resulting in a milky solution. This phase transition is reversed upon re-warming to room temperature. The fill is 2.33 mL in a 3 mL glass vial (approximately 10 mg AP1903 for Injection total per vial). Upon determining a need to administer AP1903, patients may be, for example, administered a single fixed dose of AP1903 for Injection (0.4 mg/kg) via IV infusion over 2 hours, using a non-DEHP, non-ethylene oxide sterilized infusion set. The dose of AP1903 is calculated individually for all patients, and is not be recalculated unless body weight fluctuates by ≧10%. The calculated dose is diluted in 100 mL in 0.9% normal saline before infusion. In a previous Phase I study of AP1903, 24 healthy volunteers were treated with single doses of AP1903 for Injection at dose levels of 0.01, 0.05, 0.1, 0.5 and 1.0 mg/kg infused IV over 2 hours. AP1903 plasma levels were directly proportional to dose, with mean Cmax values ranging from approximately 10-1275 ng/mL over the 0.01-1.0 mg/kg dose range. Following the initial infusion period, blood concentrations demonstrated a rapid distribution phase, with plasma levels reduced to approximately 18, 7, and 1% of maximal concentration at 0.5, 2 and 10 hours post-dose, respectively. AP1903 for Injection was shown to be safe and well tolerated at all dose levels and demonstrated a favorable pharmacokinetic profile. Iuliucci J D, et al., J Clin Pharmacol. 41: 870-9, 2001.

[0339] The fixed dose of AP1903 for injection used, for example, may be 0.4 mg/kg intravenously infused over 2 hours. The amount of AP1903 needed in vitro for effective signaling of cells is 10-100 nM (1600 Da MW). This equates to 16-160 µg/L or ~0.016-1.6 µg/kg (1.6-160 µg/kg). Doses up to 1 mg/kg were well-tolerated in the Phase I study of AP1903 described above. Therefore, 0.4 mg/kg may be a safe and effective dose of AP1903 for this Phase I study in combination with the therapeutic cells.

[0340] The amino acid and/or nucleic acid sequence encoding ligand binding of the disclosure may contain sequence one or more modifications compared to a wild type amino

acid or nucleic acid sequence. For example, the amino acid and/or nucleic acid sequence encoding ligand binding region of the disclosure may be a codon-optimized sequence. The one or more modifications may increase the binding affinity of a ligand (e.g. an induction agent) for the ligand binding region of the disclosure compared to a wild type polypeptide. Alternatively, or in addition, the one or more modifications may decrease the immunogenicity of the ligand binding region of the disclosure compared to a wild type polypeptide. Ligand binding regions of the disclosure and/or induction agents of the disclosure may be non-naturally occurring.

[0341] Modified cells, transposons and/or vectors of the disclosure may comprise an inducible proapoptotic polypeptide comprising (a) a ligand binding region, (b) a linker, and (c) a proapoptotic polypeptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments, the non-human sequence comprises a restriction site. In certain embodiments, the ligand binding region may be a multimeric ligand binding region. Inducible proapoptotic polypeptides of the disclosure may also be referred to as an "iC9 safety switch". In certain embodiments, modified cells and/or transposons of the disclosure may comprise an inducible caspase polypeptide comprising (a) a ligand binding region, (b) a linker, and (c) a caspase polypeptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments, modified cells and/or transposons of the disclosure may comprise an inducible caspase polypeptide comprising (a) a ligand binding region, (b) a linker, and (c) a caspase polypeptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments, transposons of the disclosure may comprise an inducible caspase polypeptide comprising (a) a ligand binding region, (b) a linker, and (c) a truncated caspase 9 polypeptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments of the inducible proapoptotic polypeptides, inducible caspase polypeptides or truncated caspase 9 polypeptides of the disclosure, the ligand binding region may comprise a FK506 binding protein 12 (FKBP12) polypeptide. In certain embodiments, the amino acid sequence of the ligand binding region that comprise a FK506 binding protein 12 (FKBP12) polypeptide may comprise a modification at position 36 of the sequence. The modification may be a substitution of valine (V) for phenylalanine (F) at position 36 (F36V).

[0342] In certain embodiments, the FKBP12 polypeptide is encoded by an amino acid sequence comprising

GVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKVDSSRDRNKPFKFMLGKQEVI RGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLE (SEQ ID NO: 14635).

[0343] In certain embodiments, the FKBP12 polypeptide is encoded by a nucleic acid sequence comprising GGGGTCCAGGTCGAGACTATTTCACCAGGGGATGGGCGAACATTTCCAAAAAGG GGCCAGACTTGCGTCGTGCATTACACCGGGATGCTGGAGGACGGGAAGAAAGTG GACAGCTCCAGGGATCGCAACAAGCCCTTCAAGTTCATGCTGGGAAAGCAGGAA GTGATCCGAGGATGGGAGGAAGGCGTGGCACAGATGTCAGTCGGCCAGCGGGC CAAACTGACCATTAGCCCTGACTACGCTTATGGAGCAACAGGCCACCCAGGGAT CATTCCCCCTCATGCCACCCTGGTCTTCGAT GTGGAACTGCTGAAGCTGGAG (SEQ ID NO: 14636). In certain embodiments, the induction agent specific for the ligand binding region may comprise a FK506 binding protein 12 (FKBP12) polypeptide having a substitution of valine (V) for phenylalanine (F) at position 36 (F36V) comprises AP20187 and/or AP1903, both synthetic drugs.

[0344] In certain embodiments of the inducible proapoptotic polypeptides, inducible caspase polypeptides or truncated caspase 9 polypeptides of the disclosure, the linker region is encoded by an amino acid comprising GGGGS (SEQ ID NO: 14637) or a nucleic acid sequence comprising GGAGGAGGAGGATCC (SEQ ID NO: 14638). In certain embodiments, the nucleic acid sequence encoding the linker does not comprise a restriction site.

[0345] In certain embodiments of the truncated caspase 9 polypeptides of the disclosure, the truncated caspase 9 polypeptide is encoded by an amino acid sequence that does not comprise an arginine (R) at position 87 of the sequence. Alternatively, or in addition, in certain embodiments of the inducible proapoptotic polypeptides, inducible caspase polypeptides or truncated caspase 9 polypeptides of the disclosure, the truncated caspase 9 polypeptide is encoded by an amino acid sequence that does not comprise an alanine (A) at position 282 the sequence. In certain embodiments of the inducible proapoptotic polypeptides, inducible caspase polypeptides or truncated caspase 9 polypeptides of the disclosure, the truncated caspase 9 polypeptide is encoded by an amino acid comprising GFGDVGALESLRGNADLAYILSMEPCGHCLIINNVNFCRESGLRTRTGSNIDCEKLRR RFSSLHFMVEVKGDLTAKKMVLALLELAQQDHGALDCCVVVILSHGCQASHLQFPG AVYGTDGCPVSVEKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKDHGFEVASTSPEDE

SPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIFVSYSTFPGFVSWRDPKSGSWYVE
TLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCFNFLRKKLFFKTS (SEQ ID
NO: 14639) or a nucleic acid sequence comprising
TTTGGGGACGTGGGGGCCCTGGAGTCTCTGCGAGGAAATGCCGATCTGGCTTAC
ATCCTGAGCATGGAACCCTGCGGCCACTGTCTGATCATTAACAATGTGAACTTCT
GCAGAGAAAGCGGACTGCGAACACGGACTGGCTCCAATATTGACTGTGAGAAGC
TGCGGAGAAGGTTCTCTAGTCTGCACTTTATGGTCGAAGTGAAAGGGGATCTGAC
CGCCAAGAAAATGGTGCTGGCCCTGCTGGAGCTGGCTCAGCAGGACCATGGAGC
TCTGGATTGCTGCGTGGTCGTGATCCTGTCCCACGGGTGCCAGGCTTCTCATCTG
CAGTTCCCCGGAGCAGTGTACGGAACAGACGGCTGTCCTGTCAGCGTGGAGAAG
ATCGTCAACATCTTCAACGGCACTTCTTGCCCTAGTCTGGGGGGAAAGCCAAAAC
TGTTCTTTATCCAGGCCTGTGGCGGGGAACAGAAAGATCACGGCTTCGAGGTGG
CCAGCACCAGCCCTGAGGACGAATCACCAGGGAGCAACCCTGAACCAGATGCAA
CTCCATTCCAGGAGGGACTGAGGACCTTTGACCAGCTGGATGCTATCTCAAGCCT
GCCCACTCCTAGTGACATTTTCGTGTCTTACAGTACCTTCCCAGGCTTTGTCTCAT
GGCGCGATCCCAAGTCAGGGAGCTGGTACGTGGAGACACTGGACGACATCTTTG
AACAGTGGGCCCATTCAGAGGACCTGCAGAGCCTGCTGCTGCGAGTGGCAAACG
CTGTCTCTGTGAAGGGCATCTACAAACAGATGCCCGGGTGCTTCAATTTTCTGAG
AAAGAAACTGTTCTTTAAGACTTCC (SEQ ID NO: 14640).

[0346] In certain embodiments of the inducible proapoptotic polypeptides, wherein the polypeptide comprises a truncated caspase 9 polypeptide, the inducible proapoptotic polypeptide is encoded by an amino acid sequence comprising
GVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKVDSSRDRNKPFKFMLGKQEVI
RGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLEGGGGS
GFGDVGALESLRGNADLAYILSMEPCGHCLIINNVNFCRESGLRTRTGSNIDCEKLRR
RFSSLHFMVEVKGDLTAKKMVLALLELAQQDHGALDCCVVVILSHGCQASHLQFPG
AVYGTDGCPVSVEKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKDHGFEVASTSPEDE
SPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIFVSYSTFPGFVSWRDPKSGSWYVE
TLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCFNFLRKKLFFKTS (SEQ ID
NO: 14641) or the nucleic acid sequence comprising
ggggtccaggtcgagactatttcaccaggggatgggcgaacatttccaaaaaggggccagacttgcgtcgtgcattacaccgggatg
ctggaggacgggaagaaagtggacagctccagggatcgcaacaagcccttcaagttcatgctgggaaagcaggaagtgatccgag
gatgggaggaaggcgtggcacagatgtcagtcggccagcgggccaaactgaccattagccctgactacgcttatggagcaacagg

ccacccagggatcattccccctcatgccaccctggtcttcgatgtggaactgctgaagctggagggaggaggaggatccggatttgg
ggacgtgggggccctggagtctctgcgaggaaatgccgatctggcttacatcctgagcatggaaccctgcggccactgtctgatcatt
aacaatgtgaacttctgcagagaaagcggactgcgaacacggactggctccaatattgactgtgagaagctgcggagaaggttctcta
gtctgcactttatggtcgaagtgaaaggggatctgaccgccaagaaaatggtgctggccctgctggagctggctcagcaggaccatg
gagctctggattgctgcgtggtcgtgatcctgtcccacgggtgccaggcttctcatctgcagttccccggagcagtgtacggaacaga
cggctgtcctgtcagcgtggagaagatcgtcaacatcttcaacggcacttcttgccctagtctgggggggaaagccaaaactgttctttat
ccaggcctgtggcggggaacagaaagatcacggcttcgaggtggccagcaccagccctgaggacgaatcaccagggagcaaccc
tgaaccagatgcaactccattccaggagggactgaggacctttgaccagctggatgctatctcaagcctgcccactcctagtgacatttt
cgtgtcttacagtaccttcccaggctttgtctcatggcgcgatcccaagtcagggagctggtacgtggagacactggacgacatctttga
acagtgggcccattcagaggacctgcagagcctgctgctgcgagtggcaaacgctgtctctgtgaagggcatctacaaacagatgcc
cgggtgcttcaattttctgagaaagaaactgttctttaagacttcc (SEQ ID NO: 14642).

[0347] Inducible proapoptotic polypeptides of the disclosure may be expressed in a cell under the transcriptional regulation of any promoter capable of initiating and/or regulating the expression of an inducible proapoptotic polypeptide of the disclosure in that cell. The term "promoter" as used herein refers to a promoter that acts as the initial binding site for RNA polymerase to transcribe a gene. For example, inducible proapoptotic polypeptides of the disclosure may be expressed in a mammalian cell under the transcriptional regulation of any promoter capable of initiating and/or regulating the expression of an inducible proapoptotic polypeptide of the disclosure in a mammalian cell, including, but not limited to native, endogenous, exogenous, and heterologous promoters. Preferred mammalian cells include human cells. Thus, inducible proapoptotic polypeptides of the disclosure may be expressed in a human cell under the transcriptional regulation of any promoter capable of initiating and/or regulating the expression of an inducible proapoptotic polypeptide of the disclosure in a human cell, including, but not limited to, a human promoter or a viral promoter. Exemplary promoters for expression in human cells include, but are not limited to, a human cytomegalovirus (CMV) immediate early gene promoter, a SV40 early promoter, a Rous sarcoma virus long terminal repeat, β-actin promoter, a rat insulin promoter and a glyceraldehyde-3-phosphate dehydrogenase promoter, each of which may be used to obtain high-level expression of an inducible proapoptotic polypeptide of the disclosure. The use of other viral or mammalian cellular or bacterial phage promoters which are well known in the art to achieve expression of an inducible proapoptotic polypeptide of the disclosure is contemplated as well, provided that the levels of expression are sufficient for initiating apoptosis in a cell. By employing a promoter with well-known properties, the level and

pattern of expression of the protein of interest following transfection or transformation can be optimized.

[0348] Selection of a promoter that is regulated in response to specific physiologic or synthetic signals can permit inducible expression of the inducible proapoptotic polypeptide of the disclosure. The ecdysone system (Invitrogen, Carlsbad, Calif.) is one such system. This system is designed to allow regulated expression of a gene of interest in mammalian cells. It consists of a tightly regulated expression mechanism that allows virtually no basal level expression of a transgene, but over 200-fold inducibility. The system is based on the heterodimeric ecdysone receptor of *Drosophila*, and when ecdysone or an analog such as muristerone A binds to the receptor, the receptor activates a promoter to turn on expression of the downstream transgene high levels of mRNA transcripts are attained. In this system, both monomers of the heterodimeric receptor are constitutively expressed from one vector, whereas the ecdysone-responsive promoter, which drives expression of the gene of interest, is on another plasmid. Engineering of this type of system into a vector of interest may therefore be useful. Another inducible system that may be useful is the Tet-Off™ or Tet-On™ system (Clontech, Palo Alto, Calif.) originally developed by Gossen and Bujard (Gossen and Bujard, Proc. Natl. Acad. Sci. USA, 89:5547-5551, 1992; Gossen et al., Science, 268:1766-1769, 1995). This system also allows high levels of gene expression to be regulated in response to tetracycline or tetracycline derivatives such as doxycycline. In the Tet-On™ system, gene expression is turned on in the presence of doxycycline, whereas in the Tet-Off™ system, gene expression is turned on in the absence of doxycycline. These systems are based on two regulatory elements derived from the tetracycline resistance operon of *E. coli*: the tetracycline operator sequence (to which the tetracycline repressor binds) and the tetracycline repressor protein. The gene of interest is cloned into a plasmid behind a promoter that has tetracycline-responsive elements present in it. A second plasmid contains a regulatory element called the tetracycline-controlled transactivator, which is composed, in the Tet-Off™ system, of the VP16 domain from the herpes simplex virus and the wild-type tetracycline repressor. Thus, in the absence of doxycycline, transcription is constitutively on. In the Tet-On™ system, the tetracycline repressor is not wild type and in the presence of doxycycline activates transcription. For gene therapy vector production, the Tet-Off™ system may be used so that the producer cells could be grown in the presence of tetracycline or doxycycline and prevent expression of a potentially toxic transgene, but when the vector is introduced to the patient, the gene expression would be constitutively on.

[0349] In some circumstances, it is desirable to regulate expression of a transgene in a gene therapy vector. For example, different viral promoters with varying strengths of activity are utilized depending on the level of expression desired. In mammalian cells, the CMV immediate early promoter is often used to provide strong transcriptional activation. The CMV promoter is reviewed in Donnelly, J. J., et al., 1997. Annu. Rev. Immunol. 15:617-48. Modified versions of the CMV promoter that are less potent have also been used when reduced levels of expression of the transgene are desired. When expression of a transgene in hematopoietic cells is desired, retroviral promoters such as the LTRs from MLV or MMTV are often used. Other viral promoters that are used depending on the desired effect include SV40, RSV LTR, HIV-1 and HIV-2 LTR, adenovirus promoters such as from the E1A, E2A, or MLP region, AAV LTR, HSV-TK, and avian sarcoma virus.

[0350] In other examples, promoters may be selected that are developmentally regulated and are active in particular differentiated cells. Thus, for example, a promoter may not be active in a pluripotent stem cell, but, for example, where the pluripotent stem cell differentiates into a more mature cell, the promoter may then be activated.

[0351] Similarly tissue specific promoters are used to effect transcription in specific tissues or cells so as to reduce potential toxicity or undesirable effects to non-targeted tissues. These promoters may result in reduced expression compared to a stronger promoter such as the CMV promoter, but may also result in more limited expression, and immunogenicity (Bojak, A., et al., 2002. Vaccine. 20:1975-79; Cazeaux, N., et al., 2002. Vaccine 20:3322-31). For example, tissue specific promoters such as the PSA associated promoter or prostate-specific glandular kallikrein, or the muscle creatine kinase gene may be used where appropriate.

[0352] Examples of tissue specific or differentiation specific promoters include, but are not limited to, the following: B29 (B cells); CD14 (monocytic cells); CD43 (leukocytes and platelets); CD45 (hematopoietic cells); CD68 (macrophages); desmin (muscle); elastase-1 (pancreatic acinar cells); endoglin (endothelial cells); fibronectin (differentiating cells, healing tissues); and Flt-1 (endothelial cells); GFAP (astrocytes).

[0353] In certain indications, it is desirable to activate transcription at specific times after administration of the gene therapy vector. This is done with such promoters as those that are hormone or cytokine regulatable. Cytokine and inflammatory protein responsive promoters that can be used include K and T kininogen (Kageyama et al., (1987) J. Biol. Chem., 262, 2345-2351), c-fos, TNF-alpha, C-reactive protein (Arcone, et al., (1988) Nucl. Acids Res., 16(8), 3195-3207), haptoglobin (Oliviero et al., (1987) EMBO J., 6, 1905-1912), serum

amyloid A2, C/EBP alpha, IL-1, IL-6 (Poli and Cortese, (1989) Proc. Nat'l Acad. Sci. USA, 86, 8202-8206), Complement C3 (Wilson et al., (1990) Mol. Cell. Biol., 6181-6191), IL-8, alpha-1 acid glycoprotein (Prowse and Baumann, (1988) Mol Cell Biol, 8, 42-51), alpha-1 antitrypsin, lipoprotein lipase (Zechner et al., Mol. Cell. Biol., 2394-2401, 1988), angiotensinogen (Ron, et al., (1991) Mol. Cell. Biol., 2887-2895), fibrinogen, c-jun (inducible by phorbol esters, TNF-alpha, UV radiation, retinoic acid, and hydrogen peroxide), collagenase (induced by phorbol esters and retinoic acid), metallothionein (heavy metal and glucocorticoid inducible), Stromelysin (inducible by phorbol ester, interleukin-1 and EGF), alpha-2 macroglobulin and alpha-1 anti-chymotrypsin. Other promoters include, for example, SV40, MMTV, Human Immunodeficiency Virus (MV), Moloney virus, ALV, Epstein Barr virus, Rous Sarcoma virus, human actin, myosin, hemoglobin, and creatine.

[0354] It is envisioned that any of the above promoters alone or in combination with another can be useful depending on the action desired. Promoters, and other regulatory elements, are selected such that they are functional in the desired cells or tissue. In addition, this list of promoters should not be construed to be exhaustive or limiting; other promoters that are used in conjunction with the promoters and methods disclosed herein.

Antigen Receptors

[0355] In some embodiments of the compositions and methods of the disclosure, a modified autologous cell of the disclosure comprises an antigen receptor.

[0356] In some embodiments of the compositions and methods of the disclosure, a vector comprises a sequence encoding a chimeric antigen receptor or a portion thereof. Exemplary vectors of the disclosure include, but are not limited to, viral vectors, non-viral vectors, plasmids, nanoplasmids, minicircles, transposition systems, liposomes, polymersomes, micelles, and nanoparticles.

[0357] In some embodiments of the compositions and methods of the disclosure, a transposon comprises a sequence encoding a chimeric antigen receptor or a portion thereof. In some embodiments, the transposon is integrated onto a genomic sequence of an autologous cell by a transposase.

[0358] In some embodiments of the compositions and methods of the disclosure, a donor oligonucleotide or a donor plasmid comprises a sequence encoding a chimeric antigen receptor or a portion thereof. In some embodiments, the donor oligonucleotide or the donor plasmid are entirely or partially integrated into a chromosomal sequence of an autologous cell following a single or double-strand break and, optionally, cell-mediated repair.

[0359] Exemplary antigen receptors include non-naturally occurring transmembrane proteins that bind an antigen at a site in an extacellular domain and transduce or induce an intracellular signal through an intracellular domain.

[0360] In some embodiments, non-naturally occurring antigen receptors include, but are not limited to, recombinant, variant, chimeric, or synthetic T-cell Receptors (TCRs). In some embodiments, variant TCRs contain one or more sequence variations in either a nucleotide or amino acid sequence encoding the TCR when compared to a wild type TCR. In some embodiments, a synthetic TCR comprises at least one synthetic or modified nucleic acid or amino acid encoding the TCR. In some embodiments, a recombinant and/or chimeric TCR is encoded by a nucleic acid or amino acid sequence that either across its entire length or a portion thereof, is non-naturally occurring because the sequence is isolated or derived from one or more source sequences.

[0361] In some embodiments, non-naturally occurring antigen receptors include, but are not limited to, chimeric antigen receptors.

Chimeric Antigen Receptors

[0362] In some embodiments of the compositions and methods of the disclosure, a modified autologous cell of the disclosure comprises a chimeric antigen receptor.

[0363] In some embodiments of the compositions and methods of the disclosure, a transposon comprises a sequence encoding a chimeric antigen receptor or a portion thereof.

[0364] Chimeric antigen receptors (CARs) of the disclosure may comprise (a) an ectodomain comprising an antigen recognition region, (b) a transmembrane domain, and (c) an endodomain comprising at least one costimulatory domain. In certain embodiments, the ectodomain may further comprise a signal peptide. Alternatively, or in addition, in certain embodiments, the ectodomain may further comprise a hinge between the antigen recognition region and the transmembrane domain. In certain embodiments of the CARs of the disclosure, the signal peptide may comprise a sequence encoding a human CD2, CD3δ, CD3ε, CD3γ, CD3ζ, CD4, CD8α, CD19, CD28, 4-1BB or GM-CSFR signal peptide. In certain embodiments of the CARs of the disclosure, the signal peptide may comprise a sequence encoding a human CD8α signal peptide. In certain embodiments, the transmembrane domain may comprise a sequence encoding a human CD2, CD3δ, CD3ε, CD3γ, CD3ζ, CD4, CD8α, CD19, CD28, 4-1BB or GM-CSFR transmembrane domain. In certain embodiments of the CARs of the disclosure, the transmembrane domain may comprise a sequence encoding a human CD8α transmembrane domain. In certain

embodiments of the CARs of the disclosure, the endodomain may comprise a human CD3ζ endodomain.

[0365] In certain embodiments of the CARs of the disclosure, the at least one costimulatory domain may comprise a human 4-1BB, CD28, CD40, ICOS, MyD88, OX-40 intracellular segment, or any combination thereof. In certain embodiments of the CARs of the disclosure, the at least one costimulatory domain may comprise a CD28 and/or a 4-1BB costimulatory domain. In certain embodiments of the CARs of the disclosure, the hinge may comprise a sequence derived from a human CD8α, IgG4, and/or CD4 sequence. In certain embodiments of the CARs of the disclosure, the hinge may comprise a sequence derived from a human CD8α sequence.

[0366] The CD28 costimulatory domain may comprise an amino acid sequence comprising RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALP PR (SEQ ID NO: 14477) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALP PR (SEQ ID NO: 14477). The CD28 costimulatory domain may be encoded by the nucleic acid sequence comprising cgcgtgaagtttagtcgatcagcagatgccccagcttacaaacagggacagaaccagctgtataacgagctgaatctgggccgccga gaggaatatgacgtgctggataagcggagaggacgcgaccccgaaatgggaggcaagcccaggcgcaaaaaccctcaggaagg cctgtataacgagctgcagaaggacaaaatggcagaagcctattctgagatcggcatgaaggggagcgacggagaggcaaagg gcacgatgggctgtaccagggactgagcaccgccacaaaggacacctatgatgctctgcatatgcaggcactgcctccaagg (SEQ ID NO: 14478). The 4-1BB costimulatory domain may comprise an amino acid sequence comprising KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO: 14479) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO: 14479) . The 4-1BB costimulatory domain may be encoded by the nucleic acid sequence comprising aagagaggcaggaagaaactgctgtatattttcaaacagcccttcatgcgccccgtgcagactacccaggaggaagacgggtgctcc tgtcgattccctgaggaagaggaaggcgggtgtgagctg (SEQ ID NO: 14480). The 4-1BB costimulatory domain may be located between the transmembrane domain and the CD28 costimulatory domain.

[0367] In certain embodiments of the CARs of the disclosure, the hinge may comprise a sequence derived from a human CD8α, IgG4, and/or CD4 sequence. In certain embodiments of the CARs of the disclosure, the hinge may comprise a sequence derived from a human CD8α sequence. The hinge may comprise a human CD8α amino acid sequence comprising TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 14481) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 14481). The human CD8α hinge amino acid sequence may be encoded by the nucleic acid sequence comprising actaccacaccagcacctagaccaccaactccagctccaaccatcgcgagtcagcccctgagtctgagacctgaggcctgcaggcc agctgcaggaggagctgtgcacaccaggggcctggacttcgcctgcgac (SEQ ID NO: 14482).

ScFv

[0368] The disclosure provides single chain variable fragment (scFv) compositions and methods for use of these compositions to recognize and bind to a specific target protein. ScFv compositions comprise a heavy chain variable region and a light chain variable region of an antibody. ScFv compositions may be incorporated into an antigen recognition region of a chimeric antigen receptor of the disclosure. ScFvs are fusion proteins of the variable regions of the heavy (VH) and light (VL) chains of immunoglobulins, and the VH and VL domains are connected with a short peptide linker. ScFvs retain the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of the linker. An exemplary linker comprises a sequence of GGGGSGGGGSGGGGS (SEQ ID NO: 14483).

Centyrins

[0369] Centyrins of the disclosure specifically bind to an antigen. Chimeric antigen receptors of the disclosure comprising one or more Centyrins that specifically bind an antigen may be used to direct the specificity of a cell, (e.g. a cytotoxic immune cell) towards the specific antigen.

[0370] Centyrins of the disclosure may comprise a protein scaffold, wherein the scaffold is capable of specifically binding an antigen. Centyrins of the disclosure may comprise a protein scaffold comprising a consensus sequence of at least one fibronectin type III (FN3) domain, wherein the scaffold is capable of specifically binding an antigen. The at least one fibronectin type III (FN3) domain may be derived from a human protein. The human protein may be Tenascin-C. The consensus sequence may comprise

LPAPKNLVVSEVTEDSLRLSWTAPDAAFDSFLIQYQESEKVGEAINLTVPGSERSYDL TGLKPGTEYTVSIYGVKGGHRSNPLSAEFTT (SEQ ID NO: 14488) or MLPAPKNLVVSEVTEDSLRLSWTAPDAAFDSFLIQYQESEKVGEAINLTVPGSERSY DLTGLKPGTEYTVSIYGVKGGHRSNPLSAEFTT (SEQ ID NO: 14489). The consensus sequence may comprise an amino sequence at least 74% identical to LPAPKNLVVSEVTEDSLRLSWTAPDAAFDSFLIQYQESEKVGEAINLTVPGSERSYDL TGLKPGTEYTVSIYGVKGGHRSNPLSAEFTT (SEQ ID NO: 14488) or MLPAPKNLVVSEVTEDSLRLSWTAPDAAFDSFLIQYQESEKVGEAINLTVPGSERSY DLTGLKPGTEYTVSIYGVKGGHRSNPLSAEFTT (SEQ ID NO: 14489). The consensus sequence may encoded by a nucleic acid sequence comprising atgctgcctgcaccaaagaacctggtggtgtctcatgtgacagaggatagtgccagactgtcatggactgctcccgacgcagccttcg atagttttatcatcgtgtaccgggagaacatcgaaaccggcgaggccattgtcctgacagtgccagggtccgaacgctcttatgacctg acagatctgaagcccggaactgagtactatgtgcagatcgccggcgtcaaaggaggcaatatcagcttccctctgtccgcaatcttcac caca (SEQ ID NO: 14490). The consensus sequence may be modified at one or more positions within (a) a A-B loop comprising or consisting of the amino acid residues TEDS (SEQ ID NO: 14491) at positions 13-16 of the consensus sequence; (b) a B-C loop comprising or consisting of the amino acid residues TAPDAAF (SEQ ID NO: 14492) at positions 22-28 of the consensus sequence; (c) a C-D loop comprising or consisting of the amino acid residues SEKVGE (SEQ ID NO: 14493) at positions 38-43 of the consensus sequence; (d) a D-E loop comprising or consisting of the amino acid residues GSER (SEQ ID NO: 14494) at positions 51-54 of the consensus sequence; (e) a E-F loop comprising or consisting of the amino acid residues GLKPG (SEQ ID NO: 14495) at positions 60-64 of the consensus sequence; (f) a F-G loop comprising or consisting of the amino acid residues KGGHRSN (SEQ ID NO: 14496) at positions 75-81 of the consensus sequence; or (g) any combination of (a)-(f). Centyrins of the disclosure may comprise a consensus sequence of at least 5 fibronectin type III (FN3) domains, at least 10 fibronectin type III (FN3) domains or at least 15 fibronectin type III (FN3) domains. The scaffold may bind an antigen with at least one affinity selected from a K_D of less than or equal to 10^{-9}M, less than or equal to 10^{-10}M, less than or equal to 10^{-11}M, less than or equal to 10^{-12}M, less than or equal to 10^{-13}M, less than or equal to 10^{-14}M, and less than or equal to 10^{-15}M. The K_D may be determined by surface plasmon resonance.

[0371] The term "antibody mimetic" is intended to describe an organic compound that specifically binds a target sequence and has a structure distinct from a naturally-occurring

antibody. Antibody mimetics may comprise a protein, a nucleic acid, or a small molecule. The target sequence to which an antibody mimetic of the disclosure specifically binds may be an antigen. Antibody mimetics may provide superior properties over antibodies including, but not limited to, superior solubility, tissue penetration, stability towards heat and enzymes (e.g. resistance to enzymatic degradation), and lower production costs. Exemplary antibody mimetics include, but are not limited to, an affibody, an afflilin, an affimer, an affitin, an alphabody, an anticalin, and avimer (also known as avidity multimer), a DARPin (Designed Ankyrin Repeat Protein), a Fynomer, a Kunitz domain peptide, and a monobody.

[0372] Affibody molecules of the disclosure comprise a protein scaffold comprising or consisting of one or more alpha helix without any disulfide bridges. Preferably, affibody molecules of the disclosure comprise or consist of three alpha helices. For example, an affibody molecule of the disclosure may comprise an immunoglobulin binding domain. An affibody molecule of the disclosure may comprise the Z domain of protein A.

[0373] Affilin molecules of the disclosure comprise a protein scaffold produced by modification of exposed amino acids of, for example, either gamma-B crystallin or ubiquitin. Affilin molecules functionally mimic an antibody's affinity to antigen, but do not structurally mimic an antibody. In any protein scaffold used to make an affilin, those amino acids that are accessible to solvent or possible binding partners in a properly-folded protein molecule are considered exposed amino acids. Any one or more of these exposed amino acids may be modified to specifically bind to a target sequence or antigen.

[0374] Affimer molecules of the disclosure comprise a protein scaffold comprising a highly stable protein engineered to display peptide loops that provide a high affinity binding site for a specific target sequence. Exemplary affimer molecules of the disclosure comprise a protein scaffold based upon a cystatin protein or tertiary structure thereof. Exemplary affimer molecules of the disclosure may share a common tertiary structure of comprising an alpha-helix lying on top of an anti-parallel beta-sheet.

[0375] Affitin molecules of the disclosure comprise an artificial protein scaffold, the structure of which may be derived, for example, from a DNA binding protein (e.g. the DNA binding protein Sac7d). Affitins of the disclosure selectively bind a target sequence, which may be the entirety or part of an antigen. Exemplary affitins of the disclosure are manufactured by randomizing one or more amino acid sequences on the binding surface of a DNA binding protein and subjecting the resultant protein to ribosome display and selection. Target sequences of affitins of the disclosure may be found, for example, in the genome or on

the surface of a peptide, protein, virus, or bacteria. In certain embodiments of the disclosure, an affitin molecule may be used as a specific inhibitor of an enzyme. Affitin molecules of the disclosure may include heat-resistant proteins or derivatives thereof.

[0376] Alphabody molecules of the disclosure may also be referred to as Cell-Penetrating Alphabodies (CPAB). Alphabody molecules of the disclosure comprise small proteins (typically of less than 10 kDa) that bind to a variety of target sequences (including antigens). Alphabody molecules are capable of reaching and binding to intracellular target sequences. Structurally, alphabody molecules of the disclosure comprise an artificial sequence forming single chain alpha helix (similar to naturally occurring coiled-coil structures). Alphabody molecules of the disclosure may comprise a protein scaffold comprising one or more amino acids that are modified to specifically bind target proteins. Regardless of the binding specificity of the molecule, alphabody molecules of the disclosure maintain correct folding and thermostability.

[0377] Anticalin molecules of the disclosure comprise artificial proteins that bind to target sequences or sites in either proteins or small molecules. Anticalin molecules of the disclosure may comprise an artificial protein derived from a human lipocalin. Anticalin molecules of the disclosure may be used in place of, for example, monoclonal antibodies or fragments thereof. Anticalin molecules may demonstrate superior tissue penetration and thermostability than monoclonal antibodies or fragments thereof. Exemplary anticalin molecules of the disclosure may comprise about 180 amino acids, having a mass of approximately 20 kDa. Structurally, anticalin molecules of the disclosure comprise a barrel structure comprising antiparallel beta-strands pairwise connected by loops and an attached alpha helix. In preferred embodiments, anticalin molecules of the disclosure comprise a barrel structure comprising eight antiparallel beta-strands pairwise connected by loops and an attached alpha helix.

[0378] Avimer molecules of the disclosure comprise an artificial protein that specifically binds to a target sequence (which may also be an antigen). Avimers of the disclosure may recognize multiple binding sites within the same target or within distinct targets. When an avimer of the disclosure recognize more than one target, the avimer mimics function of a bi-specific antibody. The artificial protein avimer may comprise two or more peptide sequences of approximately 30-35 amino acids each. These peptides may be connected via one or more linker peptides. Amino acid sequences of one or more of the peptides of the avimer may be derived from an A domain of a membrane receptor. Avimers have a rigid structure that may

optionally comprise disulfide bonds and/or calcium. Avimers of the disclosure may demonstrate greater heat stability compared to an antibody.

[0379] DARPins (Designed Ankyrin Repeat Proteins) of the disclosure comprise genetically-engineered, recombinant, or chimeric proteins having high specificity and high affinity for a target sequence. In certain embodiments, DARPins of the disclosure are derived from ankyrin proteins and, optionally, comprise at least three repeat motifs (also referred to as repetitive structural units) of the ankyrin protein. Ankyrin proteins mediate high-affinity protein-protein interactions. DARPins of the disclosure comprise a large target interaction surface.

[0380] Fynomers of the disclosure comprise small binding proteins (about 7 kDa) derived from the human Fyn SH3 domain and engineered to bind to target sequences and molecules with equal affinity and equal specificity as an antibody.

[0381] Kunitz domain peptides of the disclosure comprise a protein scaffold comprising a Kunitz domain. Kunitz domains comprise an active site for inhibiting protease activity. Structurally, Kunitz domains of the disclosure comprise a disulfide-rich alpha+beta fold. This structure is exemplified by the bovine pancreatic trypsin inhibitor. Kunitz domain peptides recognize specific protein structures and serve as competitive protease inhibitors. Kunitz domains of the disclosure may comprise Ecallantide (derived from a human lipoprotein-associated coagulation inhibitor (LACI)).

[0382] Monobodies of the disclosure are small proteins (comprising about 94 amino acids and having a mass of about 10 kDa) comparable in size to a single chain antibody. These genetically engineered proteins specifically bind target sequences including antigens. Monobodies of the disclosure may specifically target one or more distinct proteins or target sequences. In preferred embodiments, monobodies of the disclosure comprise a protein scaffold mimicking the structure of human fibronectin, and more preferably, mimicking the structure of the tenth extracellular type III domain of fibronectin. The tenth extracellular type III domain of fibronectin, as well as a monobody mimetic thereof, contains seven beta sheets forming a barrel and three exposed loops on each side corresponding to the three complementarity determining regions (CDRs) of an antibody. In contrast to the structure of the variable domain of an antibody, a monobody lacks any binding site for metal ions as well as a central disulfide bond. Multispecific monobodies may be optimized by modifying the loops BC and FG. Monobodies of the disclosure may comprise an adnectin.

VHH

[0383] In certain embodiments, the CAR comprises a single domain antibody (SdAb). In certain embodiments, the SdAb is a VHH.

[0384] The disclosure provides chimeric antigen receptors (CARs) comprising at least one VHH (a VCAR). Chimeric antigen receptors of the disclosure may comprise more than one VHH. For example, a bi-specific VCAR may comprise two VHHs that specifically bind two distinct antigens.

[0385] VHH proteins of the disclosure specifically bind to an antigen. Chimeric antigen receptors of the disclosure comprising one or more VHHs that specifically bind an antigen may be used to direct the specificity of a cell, (e.g. a cytotoxic immune cell) towards the specific antigen.

[0386] At least one VHH protein or VCAR of the disclosure can be optionally produced by a cell line, a mixed cell line, an immortalized cell or clonal population of immortalized cells, as well known in the art. See, e.g., Ausubel, et al., ed., Current Protocols in Molecular Biology, John Wiley & Sons, Inc., NY, N.Y. (1987-2001); Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor, N.Y. (1989); Harlow and Lane, Antibodies, a Laboratory Manual, Cold Spring Harbor, N.Y. (1989); Colligan, et al., eds., Current Protocols in Immunology, John Wiley & Sons, Inc., NY (1994-2001); Colligan et al., Current Protocols in Protein Science, John Wiley & Sons, NY, N.Y., (1997-2001).

[0387] Amino acids from a VHH protein can be altered, added and/or deleted to reduce immunogenicity or reduce, enhance or modify binding, affinity, on-rate, off-rate, avidity, specificity, half-life, stability, solubility or any other suitable characteristic, as known in the art.

[0388] Optionally, VHH proteins can be engineered with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, the VHH proteins can be optionally prepared by a process of analysis of the parental sequences and various conceptual engineered products using three-dimensional models of the parental and engineered sequences. Three-dimensional models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate sequences and can measure possible immunogenicity (e.g., Immunofilter program of Xencor, Inc. of Monrovia, Calif.). Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate sequence, i.e., the analysis of residues that influence the ability of the candidate VHH protein to bind its antigen. In this way, residues

can be selected and combined from the parent and reference sequences so that the desired characteristic, such as affinity for the target antigen(s), is achieved. Alternatively, or in addition to, the above procedures, other suitable methods of engineering can be used.

[0389] Screening VHH for specific binding to similar proteins or fragments can be conveniently achieved using nucleotide (DNA or RNA display) or peptide display libraries, for example, in vitro display. This method involves the screening of large collections of peptides for individual members having the desired function or structure. The displayed nucleotide or peptide sequences can be from 3 to 5000 or more nucleotides or amino acids in length, frequently from 5-100 amino acids long, and often from about 8 to 25 amino acids long. In addition to direct chemical synthetic methods for generating peptide libraries, several recombinant DNA methods have been described. One type involves the display of a peptide sequence on the surface of a bacteriophage or cell. Each bacteriophage or cell contains the nucleotide sequence encoding the particular displayed peptide sequence. The VHH proteins of the disclosure can bind human or other mammalian proteins with a wide range of affinities (KD). In a preferred embodiment, at least one VHH of the present disclosure can optionally bind to a target protein with high affinity, for example, with a KD equal to or less than about 10^{-7} M, such as but not limited to, 0.1-9.9 (or any range or value therein) X 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11}, 10^{-12}, 10^{-13}, 10^{-14}, 10^{-15} or any range or value therein, as determined by surface plasmon resonance or the Kinexa method, as practiced by those of skill in the art.

[0390] The affinity or avidity of a VHH or a VCAR for an antigen can be determined experimentally using any suitable method. (See, for example, Berzofsky, et al., "Antibody-Antigen Interactions," In Fundamental Immunology, Paul, W. E., Ed., Raven Press: New York, N.Y. (1984); Kuby, Janis Immunology, W.H. Freeman and Company: New York, N.Y. (1992); and methods described herein). The measured affinity of a particular VHH-antigen or VCAR-antigen interaction can vary if measured under different conditions (e.g., salt concentration, pH). Thus, measurements of affinity and other antigen-binding parameters (e.g., KD, Kon, Koff) are preferably made with standardized solutions of VHH or VCAR and antigen, and a standardized buffer, such as the buffer described herein.

[0391] Competitive assays can be performed with the VHH or VCAR of the disclosure in order to determine what proteins, antibodies, and other antagonists compete for binding to a target protein with the VHH or VCAR of the present disclosure and/or share the epitope region. These assays as readily known to those of ordinary skill in the art evaluate competition between antagonists or ligands for a limited number of binding sites on a protein.

The protein and/or antibody is immobilized or insolubilized before or after the competition and the sample bound to the target protein is separated from the unbound sample, for example, by decanting (where the protein/antibody was preinsolubilized) or by centrifuging (where the protein/antibody was precipitated after the competitive reaction). Also, the competitive binding may be determined by whether function is altered by the binding or lack of binding of the VHH or VCAR to the target protein, e.g., whether the VCAR molecule inhibits or potentiates the enzymatic activity of, for example, a label. ELISA and other functional assays may be used, as well known in the art.

VH

[0392] In certain embodiments, the CAR comprises a single domain antibody (SdAb). In certain embodiments, the SdAb is a VH.

[0393] The disclosure provides chimeric antigen receptors (CARs) comprising a single domain antibody (VCARs). In certain embodiments, the single domain antibody comprises a VH. In certain embodiments, the VH is isolated or derived from a human sequence. In certain embodiments, VH comprises a human CDR sequence and/or a human framework sequence and a non-human or humanized sequence (e.g. a rat Fc domain). In certain embodiments, the VH is a fully humanized VH. In certain embodiments, the VH s neither a naturally occurring antibody nor a fragment of a naturally occurring antibody. In certain embodiments, the VH is not a fragment of a monoclonal antibody. In certain embodiments, the VH is a UniDab™ antibody (TeneoBio).

[0394] In certain embodiments, the VH is fully engineered using the UniRat™ (TeneoBio) system and "NGS-based Discovery" to produce the VH. Using this method, the specific VH are not naturally-occurring and are generated using fully engineered systems. The VH are not derived from naturally-occurring monoclonal antibodies (mAbs) that were either isolated directly from the host (for example, a mouse, rat or human) or directly from a single clone of cells or cell line (hybridoma). These VHs were not subsequently cloned from said cell lines. Instead, VH sequences are fully-engineered using the UniRat™ system as transgenes that comprise human variable regions (VH domains) with a rat Fc domain, and are thus human/rat chimeras without a light chain and are unlike the standard mAb format. The native rat genes are knocked out and the only antibodies expressed in the rat are from transgenes with VH domains linked to a Rat Fc (UniAbs). These are the exclusive Abs expressed in the UniRat. Next generation sequencing (NGS) and bioinformatics are used to identify the full antigen-specific repertoire of the heavy-chain antibodies generated by UniRat™ after

immunization. Then, a unique gene assembly method is used to convert the antibody repertoire sequence information into large collections of fully-human heavy-chain antibodies that can be screened *in vitro* for a variety of functions. In certain embodiments, fully humanized VH are generated by fusing the human VH domains with human Fcs *in vitro* (to generate a non-naturally occurring recombinant VH antibody). In certain embodiments, the VH are fully humanized, but they are expressed *in vivo* as human/rat chimera (human VH, rat Fc) without a light chain. Fully humanized VHs are expressed *in vivo* as human/rat chimera (human VH, rat Fc) without a light chain are about 80kDa (vs 150 kDa).

[0395] VCARs of the disclosure may comprise at least one VH of the disclosure. In certain embodiments, the VH of the disclosure may be modified to remove an Fc domain or a portion thereof. In certain embodiments, a framework sequence of the VH of the disclosure may be modified to, for example, improve expression, decrease immunogenicity or to improve function.

[0396] As used throughout the disclosure, the singular forms "a," "and," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a method" includes a plurality of such methods and reference to "a dose" includes reference to one or more doses and equivalents thereof known to those skilled in the art, and so forth.

[0397] The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, e.g., the limitations of the measurement system. For example, "about" can mean within 1 or more standard deviations. Alternatively, "about" can mean a range of up to 20%, or up to 10%, or up to 5%, or up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated the term "about" meaning within an acceptable error range for the particular value should be assumed.

[0398] The disclosure provides isolated or substantially purified polynucleotide or protein compositions. An "isolated" or "purified" polynucleotide or protein, or biologically active portion thereof, is substantially or essentially free from components that normally accompany or interact with the polynucleotide or protein as found in its naturally occurring environment. Thus, an isolated or purified polynucleotide or protein is substantially free of other cellular material or culture medium when produced by recombinant techniques, or substantially free

of chemical precursors or other chemicals when chemically synthesized. Optimally, an "isolated" polynucleotide is free of sequences (optimally protein encoding sequences) that naturally flank the polynucleotide (i.e., sequences located at the 5' and 3' ends of the polynucleotide) in the genomic DNA of the organism from which the polynucleotide is derived. For example, in various embodiments, the isolated polynucleotide can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, or 0.1 kb of nucleotide sequence that naturally flank the polynucleotide in genomic DNA of the cell from which the polynucleotide is derived. A protein that is substantially free of cellular material includes preparations of protein having less than about 30%, 20%, 10%, 5%, or 1% (by dry weight) of contaminating protein. When the protein of the disclosure or biologically active portion thereof is recombinantly produced, optimally culture medium represents less than about 30%, 20%, 10%, 5%, or 1% (by dry weight) of chemical precursors or non-protein-of-interest chemicals.

[0399] The disclosure provides fragments and variants of the disclosed DNA sequences and proteins encoded by these DNA sequences. As used throughout the disclosure, the term "fragment" refers to a portion of the DNA sequence or a portion of the amino acid sequence and hence protein encoded thereby. Fragments of a DNA sequence comprising coding sequences may encode protein fragments that retain biological activity of the native protein and hence DNA recognition or binding activity to a target DNA sequence as herein described. Alternatively, fragments of a DNA sequence that are useful as hybridization probes generally do not encode proteins that retain biological activity or do not retain promoter activity. Thus, fragments of a DNA sequence may range from at least about 20 nucleotides, about 50 nucleotides, about 100 nucleotides, and up to the full-length polynucleotide of the disclosure.

[0400] Nucleic acids or proteins of the disclosure can be constructed by a modular approach including preassembling monomer units and/or repeat units in target vectors that can subsequently be assembled into a final destination vector. Polypeptides of the disclosure may comprise repeat monomers of the disclosure and can be constructed by a modular approach by preassembling repeat units in target vectors that can subsequently be assembled into a final destination vector. The disclosure provides polypeptide produced by this method as well nucleic acid sequences encoding these polypeptides. The disclosure provides host organisms and cells comprising nucleic acid sequences encoding polypeptides produced this modular approach.

[0401] The term "antibody" is used in the broadest sense and specifically covers single monoclonal antibodies (including agonist and antagonist antibodies) and antibody compositions with polyepitopic specificity. It is also within the scope hereof to use natural or synthetic analogs, mutants, variants, alleles, homologs and orthologs (herein collectively referred to as "analogs") of the antibodies hereof as defined herein. Thus, according to one embodiment hereof, the term "antibody hereof" in its broadest sense also covers such analogs. Generally, in such analogs, one or more amino acid residues may have been replaced, deleted and/or added, compared to the antibodies hereof as defined herein.

[0402] "Antibody fragment", and all grammatical variants thereof, as used herein are defined as a portion of an intact antibody comprising the antigen binding site or variable region of the intact antibody, wherein the portion is free of the constant heavy chain domains (i.e. CH2, CH3, and CH4, depending on antibody isotype) of the Fc region of the intact antibody. Examples of antibody fragments include Fab, Fab', Fab'- SH, $F(ab')_2$, and Fv fragments; diabodies; any antibody fragment that is a polypeptide having a primary structure consisting of one uninterrupted sequence of contiguous amino acid residues (referred to herein as a "single-chain antibody fragment" or "single chain polypeptide"), including without limitation (l) single-chain Fv (scFv) molecules (2) single chain polypeptides containing only one light chain variable domain, or a fragment thereof that contains the three CDRs of the light chain variable domain, without an associated heavy chain moiety and (3) single chain polypeptides containing only one heavy chain variable region, or a fragment thereof containing the three CDRs of the heavy chain variable region, without an associated light chain moiety; and multispecific or multivalent structures formed from antibody fragments. In an antibody fragment comprising one or more heavy chains, the heavy chain(s) can contain any constant domain sequence (e.g. CHI in the IgG isotype) found in a non-Fc region of an intact antibody, and/or can contain any hinge region sequence found in an intact antibody, and/or can contain a leucine zipper sequence fused to or situated in the hinge region sequence or the constant domain sequence of the heavy chain(s). The term further includes single domain antibodies ("sdAB") which generally refers to an antibody fragment having a single monomeric variable antibody domain, (for example, from camelids). Such antibody fragment types will be readily understood by a person having ordinary skill in the art.

[0403] "Binding" refers to a sequence-specific, non-covalent interaction between macromolecules (e.g., between a protein and a nucleic acid). Not all components of a binding

interaction need be sequence-specific (e.g., contacts with phosphate residues in a DNA backbone), as long as the interaction as a whole is sequence-specific.

[0404] The term "comprising" is intended to mean that the compositions and methods include the recited elements, but do not exclude others. "Consisting essentially of" when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination when used for the intended purpose. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants or inert carriers. "Consisting of shall mean excluding more than trace elements of other ingredients and substantial method steps. Embodiments defined by each of these transition terms are within the scope of this disclosure.

[0405] The term "epitope" refers to an antigenic determinant of a polypeptide. An epitope could comprise three amino acids in a spatial conformation, which is unique to the epitope. Generally, an epitope consists of at least 4, 5, 6, or 7 such amino acids, and more usually, consists of at least 8, 9, or 10 such amino acids. Methods of determining the spatial conformation of amino acids are known in the art, and include, for example, x-ray crystallography and two-dimensional nuclear magnetic resonance.

[0406] As used herein, "expression" refers to the process by which polynucleotides are transcribed into mRNA and/or the process by which the transcribed mRNA is subsequently being translated into peptides, polypeptides, or proteins. If the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA in a eukaryotic cell.

[0407] "Gene expression" refers to the conversion of the information, contained in a gene, into a gene product. A gene product can be the direct transcriptional product of a gene (e.g., mRNA, tRNA, rRNA, antisense RNA, ribozyme, shRNA, micro RNA, structural RNA or any other type of RNA) or a protein produced by translation of an mRNA. Gene products also include RNAs which are modified, by processes such as capping, polyadenylation, methylation, and editing, and proteins modified by, for example, methylation, acetylation, phosphorylation, ubiquitination, ADP-ribosylation, myristilation, and glycosylation.

[0408] "Modulation" or "regulation" of gene expression refers to a change in the activity of a gene. Modulation of expression can include, but is not limited to, gene activation and gene repression.

[0409] The term "operatively linked" or its equivalents (e.g., "linked operatively") means two or more molecules are positioned with respect to each other such that they are capable of interacting to affect a function attributable to one or both molecules or a combination thereof.

[0410] Non-covalently linked components and methods of making and using non-covalently linked components, are disclosed. The various components may take a variety of different forms as described herein. For example, non-covalently linked (i.e., operatively linked) proteins may be used to allow temporary interactions that avoid one or more problems in the art. The ability of non-covalently linked components, such as proteins, to associate and dissociate enables a functional association only or primarily under circumstances where such association is needed for the desired activity. The linkage may be of duration sufficient to allow the desired effect.

[0411] A method for directing proteins to a specific locus in a genome of an organism is disclosed. The method may comprise the steps of providing a DNA localization component and providing an effector molecule, wherein the DNA localization component and the effector molecule are capable of operatively linking via a non-covalent linkage.

[0412] The term "scFv" refers to a single-chain variable fragment. scFv is a fusion protein of the variable regions of the heavy (VH) and light chains (VL) of immunoglobulins, connected with a linker peptide. The linker peptide may be from about 5 to 40 amino acids or from about 10 to 30 amino acids or about 5, 10, 15, 20, 25, 30, 35, or 40 amino acids in length. Single-chain variable fragments lack the constant Fc region found in complete antibody molecules, and, thus, the common binding sites (e.g., Protein G) used to purify antibodies. The term further includes a scFv that is an intrabody, an antibody that is stable in the cytoplasm of the cell, and which may bind to an intracellular protein.

[0413] The term "single domain antibody" means an antibody fragment having a single monomeric variable antibody domain which is able to bind selectively to a specific antigen. A single-domain antibody generally is a peptide chain of about 110 amino acids long, comprising one variable domain (VH) of a heavy-chain antibody, or of a common IgG, which generally have similar affinity to antigens as whole antibodies, but are more heat-resistant and stable towards detergents and high concentrations of urea. Examples are those derived from camelid or fish antibodies. Alternatively, single-domain antibodies can be made from common murine or human IgG with four chains.

Methods of Gene Delivery

[0414] In some embodiments of the methods of the disclosure, a composition comprises a scalable ratio of $250x10^6$ primary human T cells per milliliter of buffer or other media during a delivery or an introduction step.

[0415] In some embodiments of the methods of the disclosure, a composition is delivered or introduced to a cell by electroporation or nucleofection. In some embodiments, a delivery or introduction step comprises electroporation or nucleofection.

[0416] In some embodiments of the methods of the disclosure, a composition is delivered or introduced to a cell by a method other than electroporation or nucleofection.

[0417] In some embodiments of the methods of the disclosure, a composition is delivered or introduced by one or more of topical delivery, adsorption, absorption, electroporation, spin-fection, co-culture, transfection, mechanical delivery, sonic delivery, vibrational delivery, magnetofection or by nanoparticle-mediated delivery. In some embodiments, a delivery or introduction step comprises one or more of topical delivery, adsorption, absorption, electroporation, spin-fection, co-culture, transfection, mechanical delivery, sonic delivery, vibrational delivery, magnetofection or by nanoparticle-mediated delivery.

[0418] In some embodiments of the methods of the disclosure, a composition is delivered or introduced by liposomal transfection, calcium phosphate transfection, fugene transfection, and dendrimer-mediated transfection. In some embodiments, a delivery or introduction step comprises one or more of liposomal transfection, calcium phosphate transfection, fugene transfection, and dendrimer-mediated transfection.

[0419] In some embodiments of the methods of the disclosure, a composition is delivered or introduced by mechanical transfection comprises cell squeezing, cell bombardment, or gene gun techniques. In some embodiments, a delivery or introduction step comprises one or more of mechanical transfection comprises cell squeezing, cell bombardment, or gene gun techniques.

[0420] In some embodiments of the methods of the disclosure, a composition is delivered or introduced by nanoparticle-mediated transfection comprises liposomal delivery, delivery by micelles, and delivery by polymerosomes. In some embodiments, a delivery or introduction step comprises one or more of liposomal delivery, delivery by micelles, and delivery by polymerosomes.

Construction of Nucleic Acids

[0421] The isolated nucleic acids of the disclosure can be made using (a) recombinant methods, (b) synthetic techniques, (c) purification techniques, and/or (d) combinations thereof, as well-known in the art.

[0422] The nucleic acids can conveniently comprise sequences in addition to a polynucleotide of the present disclosure. For example, a multi-cloning site comprising one or

more endonuclease restriction sites can be inserted into the nucleic acid to aid in isolation of the polynucleotide. Also, translatable sequences can be inserted to aid in the isolation of the translated polynucleotide of the disclosure. For example, a hexa-histidine marker sequence provides a convenient means to purify the proteins of the disclosure. The nucleic acid of the disclosure, excluding the coding sequence, is optionally a vector, adapter, or linker for cloning and/or expression of a polynucleotide of the disclosure.

[0423] Additional sequences can be added to such cloning and/or expression sequences to optimize their function in cloning and/or expression, to aid in isolation of the polynucleotide, or to improve the introduction of the polynucleotide into a cell. Use of cloning vectors, expression vectors, adapters, and linkers is well known in the art. (See, e.g., Ausubel, supra; or Sambrook, supra).

Recombinant Methods for Constructing Nucleic Acids

[0424] The isolated nucleic acid compositions of this disclosure, such as RNA, cDNA, genomic DNA, or any combination thereof, can be obtained from biological sources using any number of cloning methodologies known to those of skill in the art. In some embodiments, oligonucleotide probes that selectively hybridize, under stringent conditions, to the polynucleotides of the present disclosure are used to identify the desired sequence in a cDNA or genomic DNA library. The isolation of RNA, and construction of cDNA and genomic libraries are well known to those of ordinary skill in the art. (See, e.g., Ausubel, supra; or Sambrook, supra).

Nucleic Acid Screening and Isolation Methods

[0425] A cDNA or genomic library can be screened using a probe based upon the sequence of a polynucleotide of the disclosure. Probes can be used to hybridize with genomic DNA or cDNA sequences to isolate homologous genes in the same or different organisms. Those of skill in the art will appreciate that various degrees of stringency of hybridization can be employed in the assay; and either the hybridization or the wash medium can be stringent. As the conditions for hybridization become more stringent, there must be a greater degree of complementarity between the probe and the target for duplex formation to occur. The degree of stringency can be controlled by one or more of temperature, ionic strength, pH and the presence of a partially denaturing solvent, such as formamide. For example, the stringency of hybridization is conveniently varied by changing the polarity of the reactant solution through, for example, manipulation of the concentration of formamide within the range of 0% to 50%. The degree of complementarity (sequence identity) required for detectable binding will vary

in accordance with the stringency of the hybridization medium and/or wash medium. The degree of complementarity will optimally be 100%, or 70-100%, or any range or value therein. However, it should be understood that minor sequence variations in the probes and primers can be compensated for by reducing the stringency of the hybridization and/or wash medium.

[0426] Methods of amplification of RNA or DNA are well known in the art and can be used according to the disclosure without undue experimentation, based on the teaching and guidance presented herein.

[0427] Known methods of DNA or RNA amplification include, but are not limited to, polymerase chain reaction (PCR) and related amplification processes (see, e.g., U.S. Pat. Nos. 4,683,195, 4,683,202, 4,800,159, 4,965,188, to Mullis, et al.; 4,795,699 and 4,921,794 to Tabor, et al; 5,142,033 to Innis; 5,122,464 to Wilson, et al.; 5,091,310 to Innis; 5,066,584 to Gyllensten, et al; 4,889,818 to Gelfand, et al; 4,994,370 to Silver, et al; 4,766,067 to Biswas; 4,656,134 to Ringold) and RNA mediated amplification that uses anti-sense RNA to the target sequence as a template for double-stranded DNA synthesis (U.S. Pat. No. 5,130,238 to Malek, et al, with the tradename NASBA), the entire contents of which references are incorporated herein by reference. (See, e.g., Ausubel, supra; or Sambrook, supra.)

[0428] For instance, polymerase chain reaction (PCR) technology can be used to amplify the sequences of polynucleotides of the disclosure and related genes directly from genomic DNA or cDNA libraries. PCR and other in vitro amplification methods can also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of the desired mRNA in samples, for nucleic acid sequencing, or for other purposes. Examples of techniques sufficient to direct persons of skill through in vitro amplification methods are found in Berger, supra, Sambrook, supra, and Ausubel, supra, as well as Mullis, et al., U.S. Pat. No. 4,683,202 (1987); and Innis, et al., PCR Protocols A Guide to Methods and Applications, Eds., Academic Press Inc., San Diego, Calif. (1990). Commercially available kits for genomic PCR amplification are known in the art. See, e.g., Advantage-GC Genomic PCR Kit (Clontech). Additionally, e.g., the T4 gene 32 protein (Boehringer Mannheim) can be used to improve yield of long PCR products.

Synthetic Methods for Constructing Nucleic Acids

[0429] The isolated nucleic acids of the disclosure can also be prepared by direct chemical synthesis by known methods (see, e.g., Ausubel, et al., supra). Chemical synthesis generally

produces a single-stranded oligonucleotide, which can be converted into double-stranded DNA by hybridization with a complementary sequence, or by polymerization with a DNA polymerase using the single strand as a template. One of skill in the art will recognize that while chemical synthesis of DNA can be limited to sequences of about 100 or more bases, longer sequences can be obtained by the ligation of shorter sequences.

Recombinant Expression Cassettes

[0430] The disclosure further provides recombinant expression cassettes comprising a nucleic acid of the disclosure. A nucleic acid sequence of the disclosure, for example, a cDNA or a genomic sequence encoding a CARTyrin of the disclosure, can be used to construct a recombinant expression cassette that can be introduced into at least one desired host cell. A recombinant expression cassette will typically comprise a polynucleotide of the disclosure operably linked to transcriptional initiation regulatory sequences that will direct the transcription of the polynucleotide in the intended host cell. Both heterologous and non-heterologous (i.e., endogenous) promoters can be employed to direct expression of the nucleic acids of the disclosure.

[0431] In some embodiments, isolated nucleic acids that serve as promoter, enhancer, or other elements can be introduced in the appropriate position (upstream, downstream or in the intron) of a non-heterologous form of a polynucleotide of the disclosure so as to up or down regulate expression of a polynucleotide of the disclosure. For example, endogenous promoters can be altered in vivo or in vitro by mutation, deletion and/or substitution.

Vectors and Host Cells

[0432] The disclosure also relates to vectors that include isolated nucleic acid molecules of the disclosure, host cells that are genetically engineered with the recombinant vectors, and the production of at least one sequence by recombinant techniques, as is well known in the art. See, e.g., Sambrook, et al., supra; Ausubel, et al., supra, each entirely incorporated herein by reference.

[0433] For example, the PB-EF1a vector may be used. The vector comprises the following nucleotide sequence:

tgtacatagattaaccctagaaagataatcatattgtgacgtacgttaaagataatcatgcgtaaaattgacgcatgtgttttatcggtctgt
atatcgaggtttatttattaatttgaatagatattaagttttattatatttacacttacatactaataataaattcaacaaacaatttatttatgtttatt
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ttggccattagccatattattcattggttatatagcataaatcaatattggctattggccattgcatacgttgtatctatatcataata (SEQ
ID NO: 17036)

[0434] The polynucleotides can optionally be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it can be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

[0435] The DNA insert should be operatively linked to an appropriate promoter. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation initiating at the beginning and a termination codon (e.g., UAA, UGA or UAG) appropriately positioned at the end of the mRNA to be translated, with UAA and UAG preferred for mammalian or eukaryotic cell expression.

[0436] Expression vectors will preferably but optionally include at least one selectable marker. Such markers include, e.g., but are not limited to, ampicillin, zeocin (*Sh bla* gene), puromycin (*pac* gene), hygromycin B (*hygB* gene), G418/Geneticin (*neo* gene),

mycophenolic acid, or glutamine synthetase (GS, U.S. Pat. Nos. 5,122,464; 5,770,359; 5,827,739), blasticidin (*bsd* gene), resistance genes for eukaryotic cell culture as well as ampicillin, zeocin (*Sh bla* gene), puromycin (*pac* gene), hygromycin B (*hygB* gene), G418/Geneticin (*neo* gene), kanamycin, spectinomycin, streptomycin, carbenicillin, bleomycin, erythromycin, polymyxin B, or tetracycline resistance genes for culturing in E. coli and other bacteria or prokaryotics (the above patents are entirely incorporated hereby by reference). Appropriate culture mediums and conditions for the above-described host cells are known in the art. Suitable vectors will be readily apparent to the skilled artisan. Introduction of a vector construct into a host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other known methods. Such methods are described in the art, such as Sambrook, supra, Chapters 1-4 and 16-18; Ausubel, supra, Chapters 1, 9, 13, 15, 16.

[0437] Expression vectors will preferably but optionally include at least one selectable cell surface marker for isolation of cells modified by the compositions and methods of the disclosure. Selectable cell surface markers of the disclosure comprise surface proteins, glycoproteins, or group of proteins that distinguish a cell or subset of cells from another defined subset of cells. Preferably the selectable cell surface marker distinguishes those cells modified by a composition or method of the disclosure from those cells that are not modified by a composition or method of the disclosure. Such cell surface markers include, e.g., but are not limited to, "cluster of designation" or "classification determinant" proteins (often abbreviated as "CD") such as a truncated or full length form of CD19, CD271, CD34, CD22, CD20, CD33, CD52, or any combination thereof. Cell surface markers further include the suicide gene marker RQR8 (Philip B et al. Blood. 2014 Aug 21; 124(8):1277-87).

[0438] Expression vectors will preferably but optionally include at least one selectable drug resistance marker for isolation of cells modified by the compositions and methods of the disclosure. Selectable drug resistance markers of the disclosure may comprise wild-type or mutant Neo, TYMS, FRANCF, RAD51C, GCS, MDR1, ALDH1, NKX2.2, or any combination thereof.

[0439] At least one sequence of the disclosure can be expressed in a modified form, such as a fusion protein, and can include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, can be added to the N-terminus of sequence to improve stability and persistence

in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties can be added to a sequence of the disclosure to facilitate purification. Such regions can be removed prior to final preparation of a sequence or at least one fragment thereof. Such methods are described in many standard laboratory manuals, such as Sambrook, supra, Chapters 17.29-17.42 and 18.1-18.74; Ausubel, supra, Chapters 16, 17 and 18.

[0440] Those of ordinary skill in the art are knowledgeable in the numerous expression systems available for expression of a nucleic acid encoding a protein of the disclosure. Alternatively, nucleic acids of the disclosure can be expressed in a host cell by turning on (by manipulation) in a host cell that contains endogenous DNA of the disclosure. Such methods are well known in the art, e.g., as described in U.S. Pat. Nos. 5,580,734, 5,641,670, 5,733,746, and 5,733,761, entirely incorporated herein by reference.

[0441] Illustrative of cell cultures useful for the production of the proteins, specified portions or variants thereof, are bacterial, yeast, and mammalian cells as known in the art. Mammalian cell systems often will be in the form of monolayers of cells although mammalian cell suspensions or bioreactors can also be used. A number of suitable host cell lines capable of expressing intact glycosylated proteins have been developed in the art, and include the COS-1 (e.g., ATCC CRL 1650), COS-7 (e.g., ATCC CRL-1651), HEK293, BHK21 (e.g., ATCC CRL-10), CHO (e.g., ATCC CRL 1610) and BSC-1 (e.g., ATCC CRL-26) cell lines, Cos-7 cells, CHO cells, hep G2 cells, P3X63Ag8.653, SP2/0-Ag14, 293 cells, HeLa cells and the like, which are readily available from, for example, American Type Culture Collection, Manassas, Va. (www.atcc.org). Preferred host cells include cells of lymphoid origin, such as myeloma and lymphoma cells. Particularly preferred host cells are P3X63Ag8.653 cells (ATCC Accession Number CRL-1580) and SP2/0-Ag14 cells (ATCC Accession Number CRL-1851). In a particularly preferred embodiment, the recombinant cell is a P3X63Ab8.653 or an SP2/0-Ag14 cell.

[0442] Expression vectors for these cells can include one or more of the following expression control sequences, such as, but not limited to, an origin of replication; a promoter (e.g., late or early SV40 promoters, the CMV promoter (U.S. Pat. Nos. 5,168,062; 5,385,839), an HSV tk promoter, a pgk (phosphoglycerate kinase) promoter, an EF-1 alpha promoter (U.S. Pat. No. 5,266,491), at least one human promoter; an enhancer, and/or processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites (e.g., an SV40 large T Ag poly A addition site), and transcriptional terminator sequences. See, e.g., Ausubel et al., supra; Sambrook, et al., supra. Other cells

useful for production of nucleic acids or proteins of the present disclosure are known and/or available, for instance, from the American Type Culture Collection Catalogue of Cell Lines and Hybridomas (www.atcc.org) or other known or commercial sources.

[0443] When eukaryotic host cells are employed, polyadenlyation or transcription terminator sequences are typically incorporated into the vector. An example of a terminator sequence is the polyadenlyation sequence from the bovine growth hormone gene. Sequences for accurate splicing of the transcript can also be included. An example of a splicing sequence is the VP1 intron from SV40 (Sprague, et al., J. Virol. 45:773-781 (1983)). Additionally, gene sequences to control replication in the host cell can be incorporated into the vector, as known in the art.

Amino Acid Codes

[0444] The amino acids that make up compositions of the disclosure are often abbreviated. The amino acid designations can be indicated by designating the amino acid by its single letter code, its three letter code, name, or three nucleotide codon(s) as is well understood in the art (see Alberts, B., et al., Molecular Biology of The Cell, Third Ed., Garland Publishing, Inc., New York, 1994). A CARTyrin of the disclosure can include one or more amino acid substitutions, deletions or additions, from spontaneous or mutations and/or human manipulation, as specified herein. Amino acids in a composition of the disclosure that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (e.g., Ausubel, supra, Chapters 8, 15; Cunningham and Wells, Science 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity, such as, but not limited to, at least one neutralizing activity. Sites that are critical for CSR or CAR binding can also be identified by structural analysis, such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith, et al., J. Mol. Biol. 224:899-904 (1992) and de Vos, et al., Science 255:306-312 (1992)).

[0445] As those of skill will appreciate, the disclosure includes at least one biologically active protein of the disclosure. Biologically active protein have a specific activity at least 20%, 30%, or 40%, and, preferably, at least 50%, 60%, or 70%, and, most preferably, at least 80%, 90%, or 95%-99% or more of the specific activity of the native (non-synthetic), endogenous or related and known protein. Methods of assaying and quantifying measures of enzymatic activity and substrate specificity are well known to those of skill in the art.

[0446] In another aspect, the disclosure relates to Centyrins and fragments, as described herein, which are modified by the covalent attachment of an organic moiety. Such modification can produce a protein fragment with improved pharmacokinetic properties (e.g., increased in vivo serum half-life). The organic moiety can be a linear or branched hydrophilic polymeric group, fatty acid group, or fatty acid ester group. In particular embodiments, the hydrophilic polymeric group can have a molecular weight of about 800 to about 120,000 Daltons and can be a polyalkane glycol (e.g., polyethylene glycol (PEG), polypropylene glycol (PPG)), carbohydrate polymer, amino acid polymer or polyvinyl pyrolidone, and the fatty acid or fatty acid ester group can comprise from about eight to about forty carbon atoms.

[0447] The modified sequence and fragments of the disclosure can comprise one or more organic moieties that are covalently bonded, directly or indirectly, to the antibody. Each organic moiety that is bonded to a sequence or fragment thereof of the disclosure can independently be a hydrophilic polymeric group, a fatty acid group or a fatty acid ester group. As used herein, the term "fatty acid" encompasses mono-carboxylic acids and di-carboxylic acids. A "hydrophilic polymeric group," as the term is used herein, refers to an organic polymer that is more soluble in water than in octane. For example, polylysine is more soluble in water than in octane. Thus, a sequence modified by the covalent attachment of polylysine is encompassed by the disclosure. Hydrophilic polymers suitable for modifying sequences of the disclosure can be linear or branched and include, for example, polyalkane glycols (e.g., PEG, monomethoxy-polyethylene glycol (mPEG), PPG and the like), carbohydrates (e.g., dextran, cellulose, oligosaccharides, polysaccharides and the like), polymers of hydrophilic amino acids (e.g., polylysine, polyarginine, polyaspartate and the like), polyalkane oxides (e.g., polyethylene oxide, polypropylene oxide and the like) and polyvinyl pyrolidone. Preferably, the hydrophilic polymer that modifies a sequence of the disclosure has a molecular weight of about 800 to about 150,000 Daltons as a separate molecular entity. For example, PEG5000 and PEG 20,000, wherein the subscript is the average molecular weight of the polymer in Daltons, can be used. The hydrophilic polymeric group can be substituted with one to about six alkyl, fatty acid or fatty acid ester groups. Hydrophilic polymers that are substituted with a fatty acid or fatty acid ester group can be prepared by employing suitable methods. For example, a polymer comprising an amine group can be coupled to a carboxylate of the fatty acid or fatty acid ester, and an activated

carboxylate (e.g., activated with N,N-carbonyl diimidazole) on a fatty acid or fatty acid ester can be coupled to a hydroxyl group on a polymer.

T Cell Isolation from a Leukapheresis Product

[0448] A leukapheresis product or blood may be collected from a subject at clinical site using a closed system and standard methods (e.g., a COBE Spectra Apheresis System). Preferably, the product is collected according to standard hospital or institutional Leukapheresis procedures in standard Leukapheresis collection bags. For example, in preferred embodiments of the methods of the disclosure, no additional anticoagulants or blood additives (heparin, etc.) are included beyond those normally used during leukapheresis.

[0449] Alternatively, white blood cells (WBC)/Peripheral Blood Mononuclear Cells (PBMC) (using Biosafe Sepax 2 (Closed/Automated)) or T cells (using CliniMACS® Prodigy (Closed/Automated)) may be isolated directly from whole blood. However, in certain subjects (e.g. those diagnosed and/or treated for cancer), the WBC/PBMC yield may be significantly lower when isolated from whole blood than when isolated by leukapheresis.

[0450] Either the leukapheresis procedure and/or the direct cell isolation procedure may be used for any subject of the disclosure.

[0451] The leukapheresis product, blood, WBC/PBMC composition and/or T-cell composition should be packed in insulated containers and should be kept at controlled room temperature (+19°C to +25°C) according to standard hospital of institutional blood collection procedures approved for use with the clinical protocol. The leukapheresis product, blood, WBC/PBMC composition and/or T-cell composition should not be refrigerated.

[0452] The cell concentration leukapheresis product, blood, WBC/PBMC composition and/or T-cell composition should not exceed $0.2x10^9$ cells per mL during transportation. Intense mixing of the leukapheresis product, blood, WBC/PBMC composition and/or T-cell composition should be avoided.

[0453] If the leukapheresis product, blood, WBC/PBMC composition and/or T-cell composition has to be stored, e.g. overnight, it should be kept at controlled room temperature (same as above). During storage, the concentration of the leukapheresis product, blood, WBC/PBMC composition and/or T-cell composition should never exceed $0.2x10^9$ cell per mL.

[0454] Preferably, cells of the leukapheresis product, blood, WBC/PBMC composition and/or T-cell composition should be stored in autologous plasma. In certain embodiments, if the cell concentration of the leukapheresis product, blood, WBC/PBMC composition and/or

T-cell composition is higher than $0.2x10^9$ cell per mL, the product should be diluted with autologous plasma.

[0455] Preferably, the leukapheresis product, blood, WBC/PBMC composition and/or T-cell composition should not be older than 24 hours when starting the labeling and separation procedure. The leukapheresis product, blood, WBC/PBMC composition and/or T-cell composition may be processed and/or prepared for cell labeling using a closed and/or automated system (e.g., CliniMACS Prodigy).

[0456] An automated system may perform additional buffy coat isolation, possibly by ficolation, and/or washing of the cellular product (e.g., the leukapheresis product, blood, WBC/PBMC composition and/or T cell composition).

[0457] A closed and/or automated system may be used to prepare and label cells for T-Cell isolation (from, for example, the leukapheresis product, blood, WBC/PBMC composition and/or T cell composition).

[0458] Although WBC/PBMCs may be nucleofected directly (which is easier and saves additional steps), the methods of the disclosure may include first isolating T cells prior to nucleofection. The easier strategy of directly nucleofecting PBMC requires selective expansion of modified cells that is mediated via CSR or CAR signaling, which by itself is proving to be an inferior expansion method that directly reduces the *in vivo* efficiency of the product by rendering T cells functionally exhausted. The product may be a heterogeneous composition of modified cells including T cells, NK cells, NKT cells, monocytes, or any combination thereof, which increases the variability in product from patient to patient and makes dosing and CRS management more difficult. Since T cells are thought to be the primary effectors in tumor suppression and killing, T cell isolation for the manufacture of an autologous product may result in significant benefits over the other more heterogeneous composition.

[0459] T cells may be isolated directly, by enrichment of labeled cells or depletion of labeled cells in a one-way labeling procedure or, indirectly, in a two-step labeling procedure. According to certain enrichment strategies of the disclosure, T cells may be collected in a Cell Collection Bag and the non-labeled cells (non-target cells) in a Negative Fraction Bag. In contrast to an enrichment strategy of the disclosure, the non-labeled cells (target cells) are collected in a Cell Collection Bag and the labeled cells (non-target cells) are collected in a Negative Fraction Bag or in the Non-Target Cell Bag, respectively. Selection reagents may include, but are not limited to, antibody-coated beads. Antibody-coated beads may either be

removed prior to a modification and/or an expansion step, or, retained on the cells prior to a modification and/or an expansion step. One or more of the following non-limiting examples of cellular markers may be used to isolate T-cells: CD3, CD4, CD8, CD25, anti-biotin, CD1c, CD3/CD19, CD3/CD56, CD14, CD19, CD34, CD45RA, CD56, CD62L, CD133, CD137, CD271, CD304, IFN-gamma, TCR alpha/beta, and/or any combination thereof. Methods for the isolation of T-cells may include one or more reagents that specifically bind and/or detectably-label one or more of the following non-limiting examples of cellular markers may be used to isolate T-cells: CD3, CD4, CD8, CD25, anti-biotin, CD1c, CD3/CD19, CD3/CD56, CD14, CD19, CD34, CD45RA, CD56, CD62L, CD133, CD137, CD271, CD304, IFN-gamma, TCR alpha/beta, and/or any combination thereof. These reagents may or may not be "Good Manufacturing Practices" ("GMP") grade. Reagents may include, but are not limited to, Thermo DynaBeads and Miltenyi CliniMACS products. Methods of isolating T-cells of the disclosure may include multiple iterations of labeling and/or isolation steps. At any point in the methods of isolating T-cells of the disclosure, unwanted cells and/or unwanted cell types may be depleted from a T cell product composition of the disclosure by positively or negatively selecting for the unwanted cells and/or unwanted cell types. A T cell product composition of the disclosure may contain additional cell types that may express CD4, CD8, and/or another T cell marker(s).

[0460] Methods of the disclosure for nucleofection of T cells may eliminate the step of T cell isolation by, for example, a process for nucleofection of T cells in a population or composition of WBC/PBMCs that, following nucleofection, includes an isolation step or a selective expansion step via TCR signaling.

[0461] Certain cell populations may be depleted by positive or negative selection before or after T cell enrichment and/or sorting. Examples of cell compositions that may be depleted from a cell product composition may include myeloid cells, CD25+ regulatory T cells (T Regs), dendritic cells, macrophages, red blood cells, mast cells, gamma-delta T cells, natural killer (NK) cells, a Natural Killer (NK)-like cell (e.g. a Cytokine Induced Killer (CIK) cell), induced natural killer (iNK) T cells, NK T cells, B cells, or any combination thereof.

[0462] T cell product compositions of the disclosure may include CD4+ and CD8+ T-Cells. CD4+ and CD8+ T-Cells may be isolated into separate collection bags during an isolation or selection procedure. CD4+ T cells and CD8+ T cells may be further treated separately, or treated after reconstitution (combination into the same composition) at a particular ratio.

[0463] The particular ratio at which CD4+ T cells and CD8+ T cells may be reconstituted may depend upon the type and efficacy of expansion technology used, cell medium, and/or growth conditions utilized for expansion of T-cell product compositions. Examples of possible CD4+: CD8+ ratios include, but are not limited to, 50%:50%, 60%:40%, 40%:60% 75%:25% and 25%:75%.

[0464] CD8+ T cells exhibit a potent capacity for tumor cell killing, while CD4+ T cells provide many of the cytokines required to support CD8+ T cell proliferative capacity and function. Because T cells isolated from normal donors are predominantly CD4+, the T-cell product compositions are artificially adjusted in vitro with respect to the CD4+:CD8+ ratio to improve upon the ratio of CD4+ T cells to CD8+ T cells that would otherwise be present in vivo. An optimized ratio may also be used for the ex vivo expansion of the autologous T- cell product composition. In view of the artificially adjusted CD4+:CD8+ ratio of the T-cell product composition, it is important to note that the product compositions of the disclosure may be significantly different and provide significantly greater advantage than any endogenously-occurring population of T-cells.

[0465] Preferred methods for T cell isolation may include a negative selection strategy for yielding untouched pan T cell, meaning that the resultant T-cell composition includes T-cells that have not been manipulated and that contain an endogenously-occurring variety/ratio of T-cells.

[0466] Reagents that may be used for positive or negative selection include, but are not limited to, magnetic cell separation beads. Magnetic cell separation beads may or may not be removed or depleted from selected populations of CD4+ T cells, CD8+ T cells, or a mixed population of both CD4+ and CD8+ T cells before performing the next step in a T-cell isolation method of the disclosure.

[0467] T cell compositions and T cell product compositions may be prepared for cryopreservation, storage in standard T Cell Culture Medium, and/or genetic modification.

[0468] T cell compositions, T cell product compositions, unstimulated T cell compositions, resting T cell compositions or any portion thereof may be cryopreserved using a standard cryopreservation method optimized for storing and recovering human cells with high recovery, viability, phenotype, and/or functional capacity. Commercially-available cryopreservation media and/or protocols may be used. Cryopreservation methods of the disclosure may include a DMSO free cryopreservant (e.g. CryoSOfree™ DMSO-free Cryopreservation Medium) reduce freezing-related toxicity.

[0469] T cell compositions, T cell product compositions, unstimulated T cell compositions, resting T cell compositions or any portion thereof may be stored in a culture medium. T cell culture media of the disclosure may be optimized for cell storage, cell genetic modification, cell phenotype and/or cell expansion. T cell culture media of the disclosure may include one or more antibiotics. Because the inclusion of an antibiotic within a cell culture media may decrease transfection efficiency and/or cell yield following genetic modification via nucleofection, the specific antibiotics (or combinations thereof) and their respective concentration(s) may be altered for optimal transfection efficiency and/or cell yield following genetic modification via nucleofection.

[0470] T cell culture media of the disclosure may include serum, and, moreover, the serum composition and concentration may be altered for optimal cell outcomes. Human AB serum is preferred over FBS/FCS for culture of T cells because, although contemplated for use in T cell culture media of the disclosure, FBS/FCS may introduce xeno-proteins. Serum may be isolated form the blood of the subject for whom the T-cell composition in culture is intended for administration, thus, a T cell culture medium of the disclosure may comprise autologous serum. Serum-free media or serum-substitute may also be used in T-cell culture media of the disclosure. In certain embodiments of the T-cell culture media and methods of the disclosure, serum-free media or serum-substitute may provide advantages over supplementing the medium with xeno-serum, including, but not limited to, healthier cells that have greater viability, nucleofect with higher efficiency, exhibit greater viability post-nucleofection, display a more desirable cell phenotype, and/or greater/faster expansion upon addition of expansion technologies.

[0471] T cell culture media may include a commercially-available cell growth media. Exemplary commercially-available cell growth media include, but are not limited to, PBS, HBSS, OptiMEM, DMEM, RPMI 1640, AIM-V, X-VIVO 15, CellGro DC Medium, CTS OpTimizer T Cell Expansion SFM, TexMACS Medium, PRIME-XV T Cell Expansion Medium, ImmunoCult-XF T Cell Expansion Medium, or any combination thereof.

[0472] T cell compositions, T cell product compositions, unstimulated T cell compositions, resting T cell compositions or any portion thereof may be prepared for genetic modification. Preparation of T cell compositions, T cell product compositions, unstimulated T cell compositions, resting T cell compositions or any portion thereof for genetic modification may include cell washing and/or resuspension in a desired nucleofection buffer. Cryopreserved T-cell compositions may be thawed and prepared for genetic modification by

nucleofection. Cryopreserved cells may be thawed according to standard or known protocols. Thawing and preparation of cryopreserved cells may be optimized to yield cells that have greater viability, nucleofect with higher efficiency, exhibit greater viability post-nucleofection, display a more desirable cell phenotype, and/or greater/faster expansion upon addition of expansion technologies. For example, Grifols Albutein (25% human albumin) may be used in the thawing and/or preparation process.

Modification of an autologous T cell product composition

[0473] T cell compositions, T cell product compositions, unstimulated T cell compositions, resting T cell compositions or any portion thereof may be modified using, for example, a nucleofection strategy such as electroporation. The total number of cells to be nucleofected, the total volume of the nucleofection reaction, and the precise timing of the preparation of the sample may be optimized to yield cells that have greater viability, nucleofect with higher efficiency, exhibit greater viability post-nucleofection, display a more desirable cell phenotype, and/or greater/faster expansion upon addition of expansion technologies.

[0474] Nucleofection and/or electroporation may be accomplished using, for example, Lonza Amaxa, MaxCyte PulseAgile, Harvard Apparatus BTX, and/or Invitrogen Neon. Non-metal electrode systems, including, but not limited to, plastic polymer electrodes, may be preferred for nucleofection.

[0475] Prior to modification by nucleofection, T cell compositions, T cell product compositions, unstimulated T cell compositions, resting T cell compositions or any portion thereof may be resuspended in a nucleofection buffer. Nucleofection buffers of the disclosure include commercially-available nucleofection buffers. Nucleofection buffers of the disclosure may be optimized to yield cells that have greater viability, nucleofect with higher efficiency, exhibit greater viability post-nucleofection, display a more desirable cell phenotype, and/or greater/faster expansion upon addition of expansion technologies. Nucleofection buffers of the disclosure may include, but are not limited to, PBS, HBSS, OptiMEM, BTXpress, Amaxa Nucleofector, Human T cell nucleofection buffer and any combination thereof. Nucleofection buffers of the disclosure may comprise one or more supplemental factors to yield cells that have greater viability, nucleofect with higher efficiency, exhibit greater viability post-nucleofection, display a more desirable cell phenotype, and/or greater/faster expansion upon addition of expansion technologies. Exemplary supplemental factors include, but are not limited to, recombinant human cytokines, chemokines, interleukins and any combination thereof. Exemplary cytokines,

chemokines, and interleukins include, but are not limited to, IL2, IL7, IL12, IL15, IL21, IL1, IL3, IL4, IL5, IL6, IL8, CXCL8, IL9, IL10, IL11, IL13, IL14, IL16, IL17, IL18, IL19, IL20, IL22, IL23, IL25, IL26, IL27, IL28, IL29, IL30, IL31, IL32, IL33, IL35, IL36, GM-CSF, IFN-gamma, IL-1 alpha/IL-1F1, IL-1 beta/IL-1F2, IL-12 p70, IL-12/IL-35 p35, IL-13, IL-17/IL-17A, IL-17A/F Heterodimer, IL-17F, IL-18/IL-1F4, IL-23, IL-24, IL-32, IL-32 beta, IL-32 gamma, IL-33, LAP (TGF-beta 1), Lymphotoxin-alpha/TNF-beta, TGF-beta, TNF-alpha, TRANCE/TNFSF11/RANK L and any combination thereof. Exemplary supplemental factors include, but are not limited to, salts, minerals, metabolites or any combination thereof. Exemplary salts, minerals, and metabolites include, but are not limited to, HEPES, Nicotinamide, Heparin, Sodium Pyruvate, L-Glutamine, MEM Non-Essential Amino Acid Solution, Ascorbic Acid, Nucleosides, FBS/FCS, Human serum, serum-substitute, anti-biotics, pH adjusters, Earle's Salts, 2-Mercaptoethanol, Human transferrin, Recombinant human insulin, Human serum albumin, Nucleofector PLUS Supplement, KCL, MgCl2, Na2HPO4, NAH2PO4, Sodium lactobionate, Manitol, Sodium succinate, Sodium Chloride, CINa, Glucose, Ca(NO3)2, Tris/HCl, K2HPO4, KH2PO4, Polyethylenimine, Poly-ethylene-glycol, Poloxamer 188, Poloxamer 181, Poloxamer 407, Poly-vinylpyrrolidone, Pop313, Crown-5, and any combination thereof. Exemplary supplemental factors include, but are not limited to, media such as PBS, HBSS, OptiMEM, DMEM, RPMI 1640, AIM-V, X-VIVO 15, CellGro DC Medium, CTS OpTimizer T Cell Expansion SFM, TexMACS Medium, PRIME-XV T Cell Expansion Medium, ImmunoCult-XF T Cell Expansion Medium and any combination thereof. Exemplary supplemental factors include, but are not limited to, inhibitors of cellular DNA sensing, metabolism, differentiation, signal transduction, the apoptotic pathway and combinations thereof. Exemplary inhibitors include, but are not limited to, inhibitors of TLR9, MyD88, IRAK, TRAF6, TRAF3, IRF-7, NF-KB, Type 1 Interferons, pro-inflammatory cytokines, cGAS, STING, Sec5, TBK1, IRF-3, RNA pol III, RIG-1, IPS-1, FADD, RIP1, TRAF3, AIM2, ASC, Caspase1, Pro-IL1B, PI3K, Akt, Wnt3A, inhibitors of glycogen synthase kinase-3β (GSK-3 β) (e.g. TWS119), Bafilomycin, Chloroquine, Quinacrine, AC-YVAD-CMK, Z-VAD-FMK, Z-IETD-FMK and any combination thereof. Exemplary supplemental factors include, but are not limited to, reagents that modify or stabilize one or more nucleic acids in a way to enhance cellular delivery, enhance nuclear delivery or transport, enhance the facilitated transport of nucleic acid into the nucleus, enhance degradation of epi-chromosomal nucleic acid, and/or decrease DNA-mediated toxicity. Exemplary reagents that modify or stabilize one or more nucleic acids

include, but are not limited to, pH modifiers, DNA-binding proteins, lipids, phospholipids, CaPO4, net neutral charge DNA binding peptides with or without NLS sequences, TREX1 enzyme, and any combination thereof.

[0476] Transposition reagents, including a transposon and a transposase, may be added to a nucleofection reaction of the disclosure prior to, simultaneously with, or after an addition of cells to a nucleofection buffer (optionally, contained within a nucleofection reaction vial or cuvette). Transposons of the disclosure may comprise plasmid DNA, linearized plasmid DNA, a PCR product, nanoplasmid, DOGGYBONE™ DNA, an mRNA template, a single or double-stranded DNA, a protein-nucleic acid combination or any combination thereof. Transposons of the disclosure may comprised one or more sequences that encode one or more TTAA site(s), one or more inverted terminal repeat(s) (ITRs), one or more long terminal repeat(s) (LTRs), one or more insulator(s), one or more promotor(s), one or more full-length or truncated gene(s), one or more polyA signal(s), one or more self-cleaving 2A peptide cleavage site(s), one or more internal ribosome entry site(s) (IRES), one or more enhancer(s), one or more regulator(s), one or more replication origin(s), and any combination thereof.

[0477] Transposons of the disclosure may comprise one or more sequences that encode one or more full-length or truncated gene(s). Full-length and/or truncated gene(s) introduced by transposons of the disclosure may encode one or more of a signal peptide, a hinge, a transmembrane domain, a costimulatory domain, a chimeric antigen receptor (CAR), a chimeric T-cell receptor (CAR-T, a CARTyrin or a VCAR), a receptor, a ligand, a cytokine, a drug resistance gene, a tumor antigen, an allo or auto antigen, an enzyme, a protein, a peptide, a poly-peptide, a fluorescent protein, a mutein or any combination thereof.

[0478] Transposons of the disclosure may be prepared in water, TAE, TBE, PBS, HBSS, media, a supplemental factor of the disclosure or any combination thereof.

[0479] Transposons of the disclosure may be designed to optimize clinical safety and/or improve manufacturability. As a non-limiting example, transposons of the disclosure may be designed to optimize clinical safety and/or improve manufacturability by eliminating unnecessary sequences or regions and/or including a non-antibiotic selection marker. Transposons of the disclosure may or may not be GMP grade.

[0480] Transposase enzymes of the disclosure may be encoded by one or more sequences of plasmid DNA, mRNA, protein, protein-nucleic acid combination or any combination thereof.

[0481] Transposase enzymes of the disclosure may be prepared in water, TAE, TBE, PBS, HBSS, media, a supplemental factor of the disclosure or any combination thereof. Transposase enzymes of the disclosure or the sequences/constructs encoding or delivering them may or may not be GMP grade.

[0482] Transposons and transposase enzymes of the disclosure may be delivered to a cell by any means.

[0483] Although compositions and methods of the disclosure include delivery of a transposon and/or transposase of the disclosure to a cell by plasmid DNA (pDNA), the use of a plasmid for delivery may allow the transposon and/or transposase to be integrated into the chromosomal DNA of the cell, which may lead to continued transposase expression. Accordingly, transposon and/or transposase enzymes of the disclosure may be delivered to a cell as either mRNA or protein to remove any possibility for chromosomal integration.

[0484] Transposons and transposases of the disclosure may be pre-incubated alone or in combination with one another prior to the introduction of the transposon and/or transposase into a nucleofection reaction. The absolute amounts of each of the transposon and the transposase, as well as the relative amounts, e.g., a ratio of transposon to transposase may be optimized.

[0485] Following preparation of nucleofection reaction, optionally, in a vial or cuvette, the reaction may be loaded into a nucleofector apparatus and activated for delivery of an electric pulse according to the manufacturer's protocol. Electric pulse conditions used for delivery of a transposon and/or a transposase of the disclosure (or a sequence encoding a transposon and/or a transposase of the disclosure) to a cell may be optimized for yielding cells with enhanced viability, higher nucleofection efficiency, greater viability post-nucleofection, desirable cell phenotype, and/or greater/faster expansion upon addition of expansion technologies. When using Amaxa nucleofector technology, each of the various nucleofection programs for the Amaxa 2B or 4D nucleofector are contemplated.

[0486] Following a nucleofection reaction of the disclosure, cells may be gently added to a cell medium. For example, when T cells undergo the nucleofection reaction, the T cells may be added to a T cell medium. Post-nucleofection cell media of the disclosure may comprise any one or more commercially-available media. Post-nucleofection cell media of the disclosure (including post-nucleofection T cell media of the disclosure) may be optimized to yield cells with greater viability, higher nucleofection efficiency, exhibit greater viability post-nucleofection, display a more desirable cell phenotype, and/or greater/faster expansion

upon addition of expansion technologies. Post-nucleofection cell media of the disclosure (including post-nucleofection T cell media of the disclosure) may comprise PBS, HBSS, OptiMEM, DMEM, RPMI 1640, AIM-V, X-VIVO 15, CellGro DC Medium, CTS OpTimizer T Cell Expansion SFM, TexMACS Medium, PRIME-XV T Cell Expansion Medium, ImmunoCult-XF T Cell Expansion Medium and any combination thereof. Post-nucleofection cell media of the disclosure (including post-nucleofection T cell media of the disclosure) may comprise one or more supplemental factors of the disclosure to enhance viability, nucleofection efficiency, viability post-nucleofection, cell phenotype, and/or greater/faster expansion upon addition of expansion technologies. Exemplary supplemental factors include, but are not limited to, recombinant human cytokines, chemokines, interleukins and any combination thereof. Exemplary cytokines, chemokines, and interleukins include, but are not limited to, IL2, IL7, IL12, IL15, IL21, IL1, IL3, IL4, IL5, IL6, IL8, CXCL8, IL9, IL10, IL11, IL13, IL14, IL16, IL17, IL18, IL19, IL20, IL22, IL23, IL25, IL26, IL27, IL28, IL29, IL30, IL31, IL32, IL33, IL35, IL36, GM-CSF, IFN-gamma, IL-1 alpha/IL-1F1, IL-1 beta/IL-1F2, IL-12 p70, IL-12/IL-35 p35, IL-13, IL-17/IL-17A, IL-17A/F Heterodimer, IL-17F, IL-18/IL-1F4, IL-23, IL-24, IL-32, IL-32 beta, IL-32 gamma, IL-33, LAP (TGF-beta 1), Lymphotoxin-alpha/TNF-beta, TGF-beta, TNF-alpha, TRANCE/TNFSF11/RANK L and any combination thereof. Exemplary supplemental factors include, but are not limited to, salts, minerals, metabolites or any combination thereof. Exemplary salts, minerals, and metabolites include, but are not limited to, HEPES, Nicotinamide, Heparin, Sodium Pyruvate, L-Glutamine, MEM Non-Essential Amino Acid Solution, Ascorbic Acid, Nucleosides, FBS/FCS, Human serum, serum-substitute, anti-biotics, pH adjusters, Earle's Salts, 2-Mercaptoethanol, Human transferrin, Recombinant human insulin, Human serum albumin, Nucleofector PLUS Supplement, KCL, MgCl2, Na2HPO4, NAH2PO4, Sodium lactobionate, Manitol, Sodium succinate, Sodium Chloride, CINa, Glucose, Ca(NO3)2, Tris/HCl, K2HPO4, KH2PO4, Polyethylenimine, Poly-ethylene-glycol, Poloxamer 188, Poloxamer 181, Poloxamer 407, Poly-vinylpyrrolidone, Pop313, Crown-5, and any combination thereof. Exemplary supplemental factors include, but are not limited to, media such as PBS, HBSS, OptiMEM, DMEM, RPMI 1640, AIM-V, X-VIVO 15, CellGro DC Medium, CTS OpTimizer T Cell Expansion SFM, TexMACS Medium, PRIME-XV T Cell Expansion Medium, ImmunoCult-XF T Cell Expansion Medium and any combination thereof. Exemplary supplemental factors include, but are not limited to, inhibitors of cellular DNA sensing, metabolism, differentiation, signal transduction, the

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LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

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What is claimed is:

1. A non-naturally occurring chimeric stimulatory receptor (CSR) comprising:

(a) an ectodomain comprising a activation component, wherein the activation component is isolated or derived from a first protein;

(b) a transmembrane domain; and

(c) an endodomain comprising at least one signal transduction domain, wherein the at least one signal transduction domain is isolated or derived from a second protein;

wherein the first protein and the second protein are not identical.

2. The CSR of claim 1, wherein the activation component comprises a portion of one or more of a component of a T-cell Receptor (TCR), a component of a TCR complex, a component of a TCR co-receptor, a component of a TCR co-stimulatory protein, a component of a TCR inhibitory protein, a cytokine receptor, and a chemokine receptor to which an agonist of the activation component binds.

3. The CSR of claim 1, wherein the activation component comprises a CD2 extracellular domain or a portion thereof to which an agonist binds.

4. The CSR of claim 1, wherein the signal transduction domain comprises one or more of a component of a human signal transduction domain, T-cell Receptor (TCR), a component of a TCR complex, a component of a TCR co-receptor, a component of a TCR co-stimulatory protein, a component of a TCR inhibitory protein, a cytokine receptor, and a chemokine receptor.

5. The CSR of claim 1, wherein the signal transduction domain comprises a CD3 protein or a portion thereof.

6. The CSR of claim 5, wherein the CD3 protein comprises a CD3ζ protein or a portion thereof.

7. The CSR of claim 1, wherein the endodomain further comprises a cytoplasmic domain.

8. The CSR of claim 7, wherein the cytoplasmic domain is isolated or derived from a third protein.

9. The CSR of claim 8, wherein the first protein and the third protein are identical.

10. The CSR of claim 1, wherein the ectodomain further comprises a signal peptide.

11. The CSR of claim 10, wherein the signal peptide is derived from a fourth protein.

12. The CSR of claim 11, wherein the first protein and the fourth protein are identical.

13. The CSR of claim 1, wherein the transmembrane domain is isolated or derived from a fifth protein.

14. The CSR of claim 13, wherein the first protein and the fifth protein are identical.

15. The CSR of claim 1, wherein the activation component does not bind a naturally-occurring molecule.

16. The CSR of claim 1, wherein the CSR does not transduce a signal upon binding of the activation component to a naturally-occurring molecule.

17. The CSR of claim 1, wherein the activation component binds to a non-naturally occurring molecule.

18. The CSR of claim 1, wherein the CSR selectively transduces a signal upon binding of the activation component to a non-naturally occurring molecule.

19. A non-naturally occurring chimeric stimulatory receptor (CSR) comprising:

(a) an ectodomain comprising a signal peptide and an activation component, wherein the signal peptide comprises a CD2 signal peptide or a portion thereof and wherein the

activation component comprises a CD2 extracellular domain or a portion thereof to which an agonist binds;

(b) a transmembrane domain, wherein the transmembrane domain comprises a CD2 transmembrane domain or a portion thereof; and

(c) an endodomain comprising a cytoplasmic domain and at least one signal transduction domain, wherein the cytoplasmic domain comprises a CD2 cytoplasmic domain or a portion thereof and wherein the at least one signal transduction domain comprises a CD3ζ protein or a portion thereof.

20. The CSR of claim 19 comprising an amino acid sequence at least 80% identical to SEQ ID NO:17062.

21. The CSR of claim 19 comprising an amino acid sequence at least 90% identical to SEQ ID NO:17062.

22. The CSR of claim 19 comprising an amino acid sequence at least 95% identical to SEQ ID NO:17062.

23. The CSR of claim 19 comprising an amino acid sequence at least 99% identical to SEQ ID NO:17062.

24. The CSR of claim 19 comprising an amino acid sequence of SEQ ID NO:17062.

25. The CSR of claim 1, wherein the ectodomain comprises a modification.

26. The CSR of claim 25, wherein the modification comprises a mutation or a truncation of the amino acid sequence of the activation component or the first protein when compared to a wild type sequence of the activation component or the first protein.

27. The CSR of claim 26, wherein the mutation or a truncation of the amino acid sequence of the activation component comprises a mutation or truncation of a CD2 extracellular domain or a portion thereof to which an agonist binds.

28. The CSR of claim 27, wherein the CSR comprising a mutation or truncation of a CD2 extracellular domain or a portion thereof to which an agonist binds does not bind CD58.

29. The CSR of claim 27, wherein the CD2 extracellular cellular domain comprising the mutation or truncation comprises an amino acid sequence at least 80% identical to SEQ ID NO:17119.

30. The CSR of claim 27, wherein the CD2 extracellular cellular domain comprising the mutation or truncation comprises an amino acid sequence at least 90% identical to SEQ ID NO:17119.

31. The CSR of claim 27, wherein the CD2 extracellular cellular domain comprising the mutation or truncation comprises an amino acid sequence at least 95% identical to SEQ ID NO:17119.

32. The CSR of claim 27, wherein the CD2 extracellular cellular domain comprising the mutation or truncation comprises an amino acid sequence at least 99% identical to SEQ ID NO:17119.

33. The CSR of claim 27, wherein the CD2 extracellular cellular domain comprising the mutation or truncation comprises an amino acid sequence of SEQ ID NO: 17119.

34. A non-naturally occurring chimeric stimulatory receptor (CSR) comprising:

(a) an ectodomain comprising a signal peptide and an activation component, wherein the signal peptide comprises a CD2 signal peptide or a portion thereof and wherein the activation component comprises a CD2 extracellular domain or a portion thereof to which an agonist binds and wherein the CD2 extracellular domain or a portion thereof to which an agonist binds comprises a mutation or truncation;

(b) a transmembrane domain, wherein the transmembrane domain comprises a CD2 transmembrane domain or a portion thereof; and

(c) an endodomain comprising a cytoplasmic domain and at least one signal transduction domain, wherein the cytoplasmic domain comprises a CD2 cytoplasmic domain

or a portion thereof and wherein the at least one signal transduction domain comprises a CD3ζ protein or a portion thereof.

35. The CSR of claim 34 comprising an amino acid sequence at least 80% identical to SEQ ID NO:17118.

36. The CSR of claim 34 comprising an amino acid sequence at least 90% identical to SEQ ID NO: 17118.

37. The CSR of claim 34 comprising an amino acid sequence at least 95% identical to SEQ ID NO: 17118.

38. The CSR of claim 34 comprising an amino acid sequence at least 99% identical to SEQ ID NO:17118.

39. The CSR of claim 34 comprising an amino acid sequence of SEQ ID NO: 17118.

40. A nucleic acid sequence encoding the CSR of any one of claims 1-39.

41. A vector comprising the nucleic acid sequence of claim 40.

42. A transposon comprising the nucleic acid sequence of claim 40.

43. A cell comprising the CSR of any one of claims 1-39.

44. A cell comprising the nucleic acid of claim 40.

45. A cell comprising the vector of claim 41.

46. A cell comprising the transposon of claim 42.

47. The cell of any one of claims 43-46, wherein the cell is an allogeneic cell.

48. The cell of any one of claims 43-46, wherein the cell is an autologous cell.

49. A composition comprising the CSR of any one of claims 1-39.

50. A composition comprising the nucleic acid sequence of claim 40.

51. A composition comprising the vector of claim 41.

52. A composition comprising the transposon of claim 42.

53. A composition comprising the cell of any one of claims 43-46.

54. A composition comprising a plurality of cells of any one of claims 43-46.

55. A modified T lymphocyte (T-cell), comprising:

(a) a modification of an endogenous sequence encoding a T-cell Receptor (TCR), wherein the modification reduces or eliminates a level of expression or activity of the TCR; and

(b) a chimeric stimulatory receptor (CSR) comprising:

(i) an ectodomain comprising an activation component, wherein the activation component is isolated or derived from a first protein;

(ii) a transmembrane domain; and

(iii) an endodomain comprising at least one signal transduction domain, wherein the at least one signal transduction domain is isolated or derived from a second protein; wherein the first protein and the second protein are not identical.

56. The modified T-cell of claim 55, further comprising an inducible proapoptotic polypeptide.

57. The modified T-cell of claim 55, further comprising a modification of an endogenous sequence encoding Beta-2-Microglobulin (B2M), wherein the modification reduces or eliminates a level of expression or activity of a major histocompatibility complex (MHC) class I (MHC-I).

58. The modified T-cell of claim 55, further comprising a non-naturally occurring polypeptide comprising an HLA class I histocompatibility antigen, alpha chain E (HLA-E) polypeptide.

59. The modified T-cell of claim 58, wherein the non-naturally occurring polypeptide comprising a HLA-E further comprises a B2M signal peptide.

60. The modified T-cell of claim 59, wherein the non-naturally occurring polypeptide comprising an HLA-E further comprises a B2M polypeptide.

61. The modified T-cell of claim 60, wherein the non-naturally occurring polypeptide comprising an HLA-E further comprises a linker, wherein the linker is positioned between the B2M polypeptide and the HLA-E polypeptide.

62. The modified T-cell of claim 61, wherein the non-naturally occurring polypeptide comprising an HLA-E further comprises a peptide and a B2M polypeptide.

63. The modified T-cell of claim 62, wherein the non-naturally occurring polypeptide comprising an HLA-E further comprises

a first linker positioned between the B2M signal peptide and the peptide, and

a second linker positioned between the B2M polypeptide and the peptide encoding the HLA-E.

64. The modified T-cell of claim 55, further comprising a non-naturally occurring antigen receptor, a sequence encoding a therapeutic polypeptide, or a combination thereof.

65. The modified T-cell of claim 64, wherein the non-naturally occurring antigen receptor comprises a chimeric antigen receptor (CAR).

66. The modified T-cell of claim 55, wherein the CSR is transiently expressed in the modified T-cell.

67. The modified T-cell of claim 55, wherein the CSR is stably expressed in the modified T-cell.

68. The modified T-cell of claim 58, wherein the polypeptide comprising the HLA-E polypeptide is transiently expressed in the modified T-cell.

69. The modified T-cell of claim 58, wherein the polypeptide comprising the HLA-E polypeptide is stably expressed in the modified T-cell.

70. The modified T-cell of claim 56, wherein the inducible proapoptotic polypeptide is stably expressed in the modified T-cell.

71. The modified T-cell of claim 64, wherein the non-naturally occurring antigen receptor or a sequence encoding a therapeutic protein is stably expressed in the modified T-cell.

72. The modified T-cell of claim 55, wherein the modified T-cell is an allogeneic cell.

73. The modified T-cell of claim 55, wherein the modified T-cell is an autologous cell.

74. The modified T-cell of claim 55, wherein the modified T-cell is an early memory T cell, a stem cell-like T cell, a stem memory T cell (T_{SCM}), a central memory T cell (T_{CM}) or a stem cell-like T cell.

75. A composition comprising a modified T-cell according to any one of claims 55-74.

76. A composition comprising a population of modified T-cells, wherein a plurality of the modified T-cells of the population comprise the CSR according to any one of claims 1-39.

77. A composition comprising a population of modified T-cells, wherein a plurality of the modified T-cells of the population comprise the modified T-cell according to any one of claims 55-74.

78. The composition of claim 76 or 77, wherein at least 25% of the plurality of modified T-cells of the population expresses one or more cell-surface marker(s) of a stem memory T cell (T_{SCM}) or a T_{SCM}-like cell; and wherein the one or more cell-surface marker(s) comprise CD45RA and CD62L.

79. The composition of claim 76 or 77, wherein at least 50% of the plurality of modified T-cells of the population expresses one or more cell-surface marker(s) of a central memory T cell (T_{CM}) or a T_{CM}-like cell; and wherein the one or more cell-surface marker(s) comprise CD45RO and CD62L.

80. The composition of claim 76 or 77, wherein at least 75% of the plurality of modified T-cells of the population expresses one or more cell-surface marker(s) of a central memory T cell (T_{CM}) or a T_{CM}-like cell; and wherein the one or more cell-surface marker(s) comprise CD45RO and CD62L.

81. The composition according to any one of claims 76 or 77 for use in the treatment of a disease or disorder.

82. The use of a composition according to any one of claims 76 or 77 for the treatment of a disease or disorder.

83. A method of treating a disease or disorder comprising administering to a subject in need thereof a therapeutically-effective amount of a composition according to any one of claims 76 or 77.

84. A method of treating a disease or disorder comprising administering to a subject in need thereof a therapeutically-effective amount of a composition according to any one of claims 76 or 77 and at least one non-naturally occurring molecule that binds the CSR.

85 A method of producing a population of modified T-cells comprising introducing into a plurality of primary human T-cells a composition comprising the CSR of claims 1-39 or a sequence encoding the same to produce a plurality of modified T-cells under conditions that

stably express the CSR within the plurality of modified T-cells and preserve desirable stem-like properties of the plurality of modified T-cells.

86. The method of claim 85, wherein at least 25% of the plurality of modified T-cells of the population expresses one or more cell-surface marker(s) of a stem memory T cell (T_{SCM}) or a T_{SCM}-like cell; and wherein the one or more cell-surface marker(s) comprise CD45RA and CD62L.

87. The method of claim 85, wherein at least 50% of the plurality of modified T-cells of the population expresses one or more cell-surface marker(s) of a central memory T cell (T_{CM}) or a T_{CM}-like cell; and wherein the one or more cell-surface marker(s) comprise CD45RO and CD62L.

88. The method of claim 85, wherein at least 75% of the plurality of modified T-cells of the population expresses one or more cell-surface marker(s) of a central memory T cell (T_{CM}) or a T_{CM}-like cell; and wherein the one or more cell-surface marker(s) comprise CD45RO and CD62L.

89. A composition comprising a population of modified T-cells produced by the method of claim 85.

90. The composition of claim 89 for use in the treatment of a disease or disorder.

91. The use of a composition of claim 89 for the treatment of a disease or disorder.

92. A method of treating a disease or disorder comprising administering to a subject in need thereof a therapeutically-effective amount of the composition of claim 89.

93. The method of claim 92, further comprising administering an activator composition to the subject to activate the population of modified T-cells in vivo, to induce cell division of the population of modified T-cells in vivo, or a combination thereof.

94 A method of producing a population of modified T-cells comprising introducing into a plurality of primary human T-cells a composition comprising the CSR of claims 1-39 or a sequence encoding the same to produce a plurality of modified T-cells under conditions that transiently express the CSR within the plurality of modified T-cells and preserve desirable stem-like properties of the plurality of modified T-cells.

95. The method of claim 94, wherein at least 25% of the plurality of modified T-cells of the population expresses one or more cell-surface marker(s) of a stem memory T cell (T_{SCM}) or a T_{SCM}-like cell; and wherein the one or more cell-surface marker(s) comprise CD45RA and CD62L.

96. The method of claim 94, wherein at least 50% of the plurality of modified T-cells of the population expresses one or more cell-surface marker(s) of a central memory T cell (T_{CM}) or a T_{CM}-like cell; and wherein the one or more cell-surface marker(s) comprise CD45RO and CD62L.

97. The method of claim 94, wherein at least 75% of the plurality of modified T-cells of the population expresses one or more cell-surface marker(s) of a central memory T cell (T_{CM}) or a T_{CM}-like cell; and wherein the one or more cell-surface marker(s) comprise CD45RO and CD62L.

98. A composition comprising a population of modified T-cells produced by the method of claim 94.

99. The composition of claim 98 for use in the treatment of a disease or disorder.

100. The use of a composition of claim 98 for the treatment of a disease or disorder.

101. A method of treating a disease or disorder comprising administering to a subject in need thereof a therapeutically-effective amount of the composition of claim 98.

102. A method of claim 101, wherein the modified T-cells within the population of modified T-cells administered to the subject no longer express the CSR.

103. A method of expanding a population of modified T-cells comprising introducing into a plurality of primary human T-cells a composition comprising the CSR of claims 1-39 or a sequence encoding the same to produce a plurality of modified T-cells under conditions that stably express the CSR within the plurality of modified T-cells and preserve desirable stem-like properties of the plurality of modified T-cells and contacting the cells with an activator composition to produce a plurality of activated modified T-cells, wherein expansion of the plurality of modified T-cells is at least two fold higher than the expansion of a plurality of wild-type T-cells not stably expressing the CSR under the same conditions.

104. The method of claim 103, wherein at least 25% of the plurality of modified T-cells of the population expresses one or more cell-surface marker(s) of a stem memory T cell (T_{SCM}) or a T_{SCM}-like cell; and wherein the one or more cell-surface marker(s) comprise CD45RA and CD62L.

105. The method of claim 103, wherein at least 50% of the plurality of modified T-cells of the population expresses one or more cell-surface marker(s) of a central memory T cell (T_{CM}) or a T_{CM}-like cell; and wherein the one or more cell-surface marker(s) comprise CD45RO and CD62L.

106. The method of claim 103, wherein at least 75% of the plurality of modified T-cells of the population expresses one or more cell-surface marker(s) of a central memory T cell (T_{CM}) or a T_{CM}-like cell; and wherein the one or more cell-surface marker(s) comprise CD45RO and CD62L.

107. A composition comprising a population of modified T-cells expanded by the method of claim 103.

108. The composition of claim 107 for use in the treatment of a disease or disorder.

109. The use of a composition of claim 107 for the treatment of a disease or disorder.

110. A method of treating a disease or disorder comprising administering to a subject in need thereof a therapeutically-effective amount of the composition of claim 107.

111. The method of claim 110, further comprising administering an activator composition to the subject to activate the population of modified T-cells in vivo, to induce cell division of the population of modified T-cells in vivo, or a combination thereof.

112. A method of expanding a population of modified T-cells comprising introducing into a plurality of primary human T-cells a composition comprising the CSR of claims 1-39 or a sequence encoding the same to produce a plurality of modified T-cells under conditions that transiently express the CSR within the plurality of modified T-cells and preserve desirable stem-like properties of the plurality of modified T-cells and contacting the cells with an activator composition to produce a plurality of activated modified T-cells, wherein expansion of the plurality of modified T-cells is at least two fold higher than the expansion of a plurality of wild-type T-cells not transiently expressing the CSR under the same conditions.

113. The method of claim 112, wherein at least 25% of the plurality of modified T-cells of the population expresses one or more cell-surface marker(s) of a stem memory T cell (T_{SCM}) or a T_{SCM}-like cell; and wherein the one or more cell-surface marker(s) comprise CD45RA and CD62L.

114. The method of claim 112, wherein at least 50% of the plurality of modified T-cells of the population expresses one or more cell-surface marker(s) of a central memory T cell (T_{CM}) or a T_{CM}-like cell; and wherein the one or more cell-surface marker(s) comprise CD45RO and CD62L.

115. The method of claim 112, wherein at least 75% of the plurality of modified T-cells of the population expresses one or more cell-surface marker(s) of a central memory T cell (T_{CM}) or a T_{CM}-like cell; and wherein the one or more cell-surface marker(s) comprise CD45RO and CD62L.

116. A composition comprising a population of modified T-cells expanded by the method of claim 112.

17 The composition of claim 116 for use in the treatment of a disease or disorder.

18. The use of a composition of claim 116 for the treatment of a disease or disorder.

19. A method of treating a disease or disorder comprising administering to a subject in eed thereof a therapeutically-effective amount of the composition of claim 116.

20. A method of claim 119, wherein the modified T-cells within the population of iodified T-cells administered to the subject no longer express the CSR.

FIG. 1

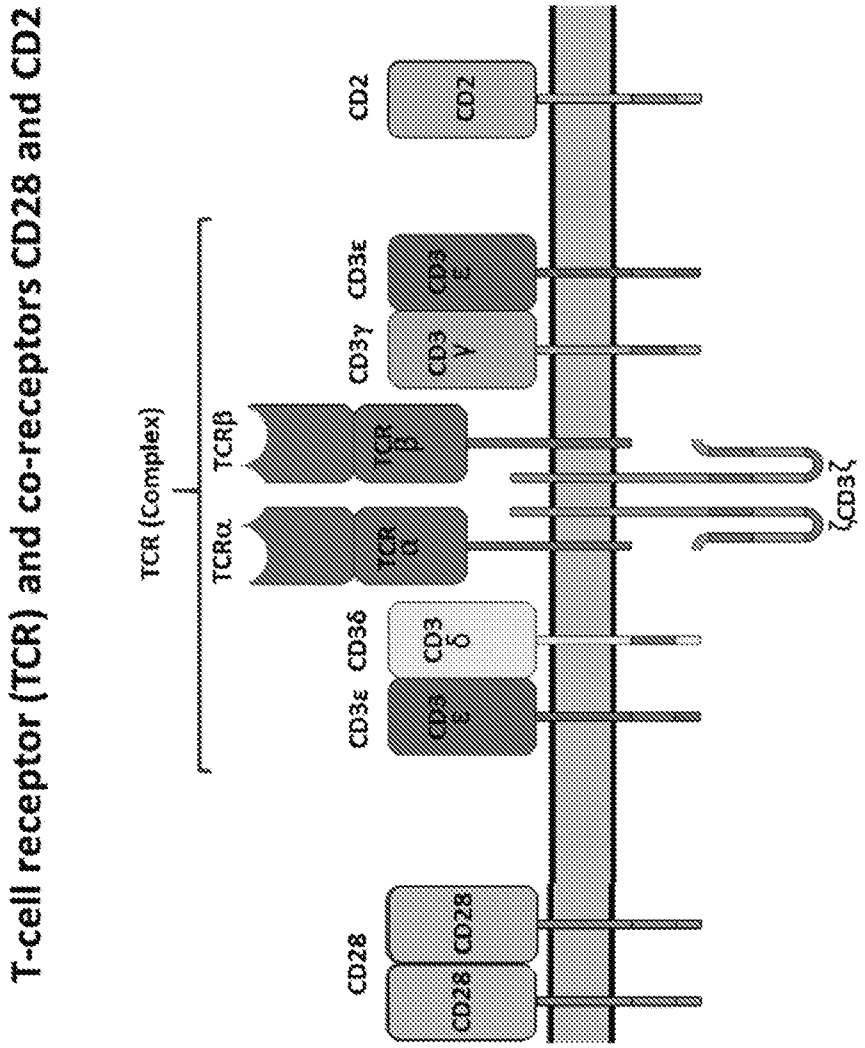

T-cell receptor (TCR) and co-receptors CD28 and CD2
TCR (Complex)
TCRα
TCRβ
CD2
CD3ε
CD3γ
CD36
CD3ε
CD28
CD28
ζCD3ζ

FIG. 2

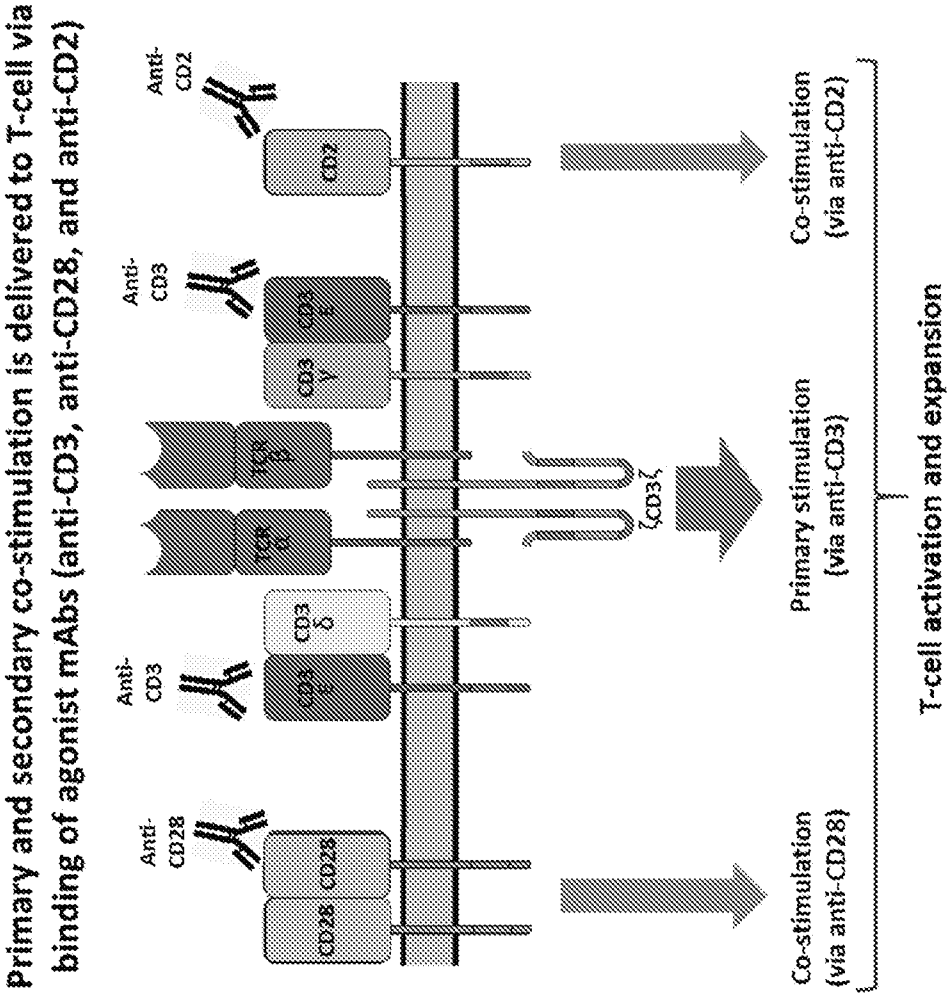

Primary and secondary co-stimulation is delivered to T-cell via binding of agonist mAbs (anti-CD3, anti-CD28, and anti-CD2)
Anti-CD28
CD28
CD28
Anti-CD3
CD3 ε
CD3 δ
TCR α
TCR β
Anti-CD3
CD3 γ
CD3 ε
Anti-CD2
CD2
ζCD3ζ
Co-stimulation (via anti-CD28)
Primary stimulation (via anti-CD3)
Co-stimulation (via anti-CD2)
T-cell activation and expansion

FIG. 3

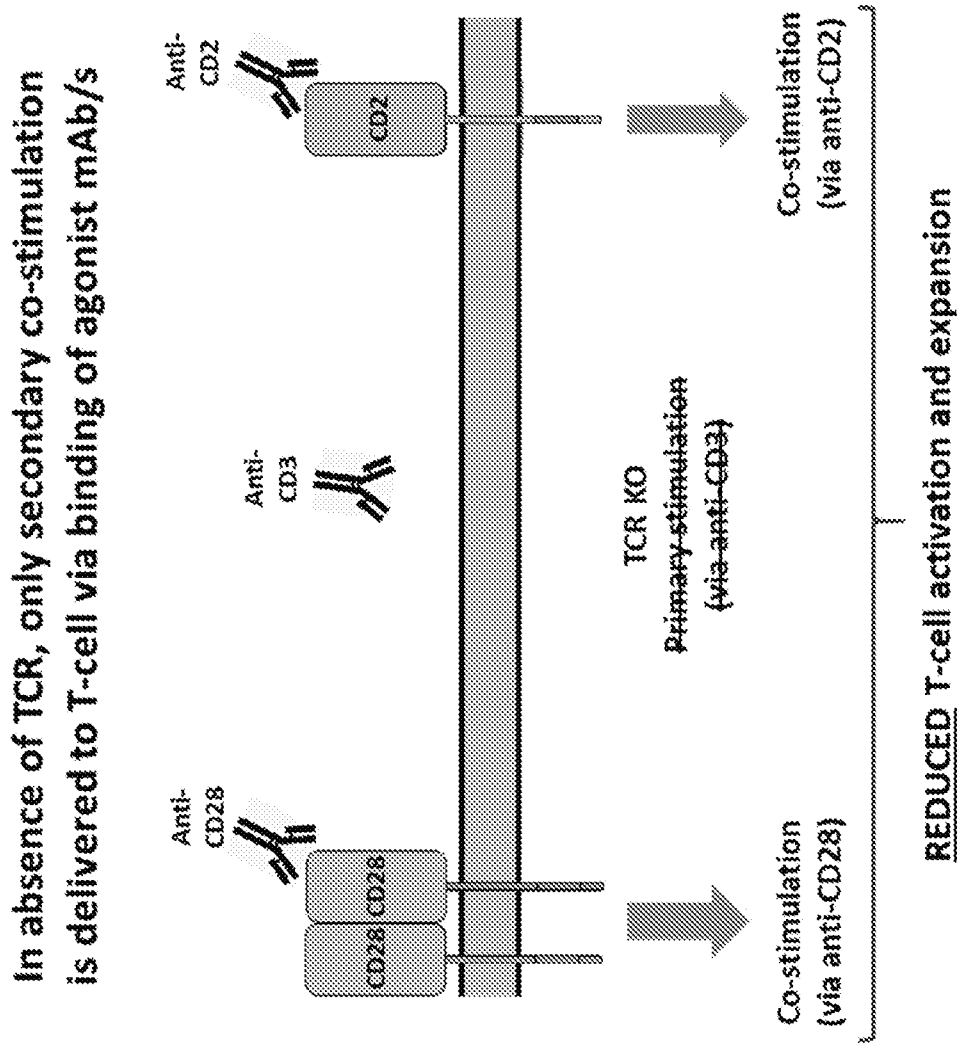

In absence of TCR, only secondary co-stimulation is delivered to T-cell via binding of agonist mAb/s
Anti-CD28
CD28
CD28
Anti-CD3
Anti-CD2
CD2
TCR KO
~~Primary stimulation~~
~~(via anti-CD3)~~
Co-stimulation (via anti-CD28)
Co-stimulation (via anti-CD2)
REDUCED T-cell activation and expansion

FIG. 4

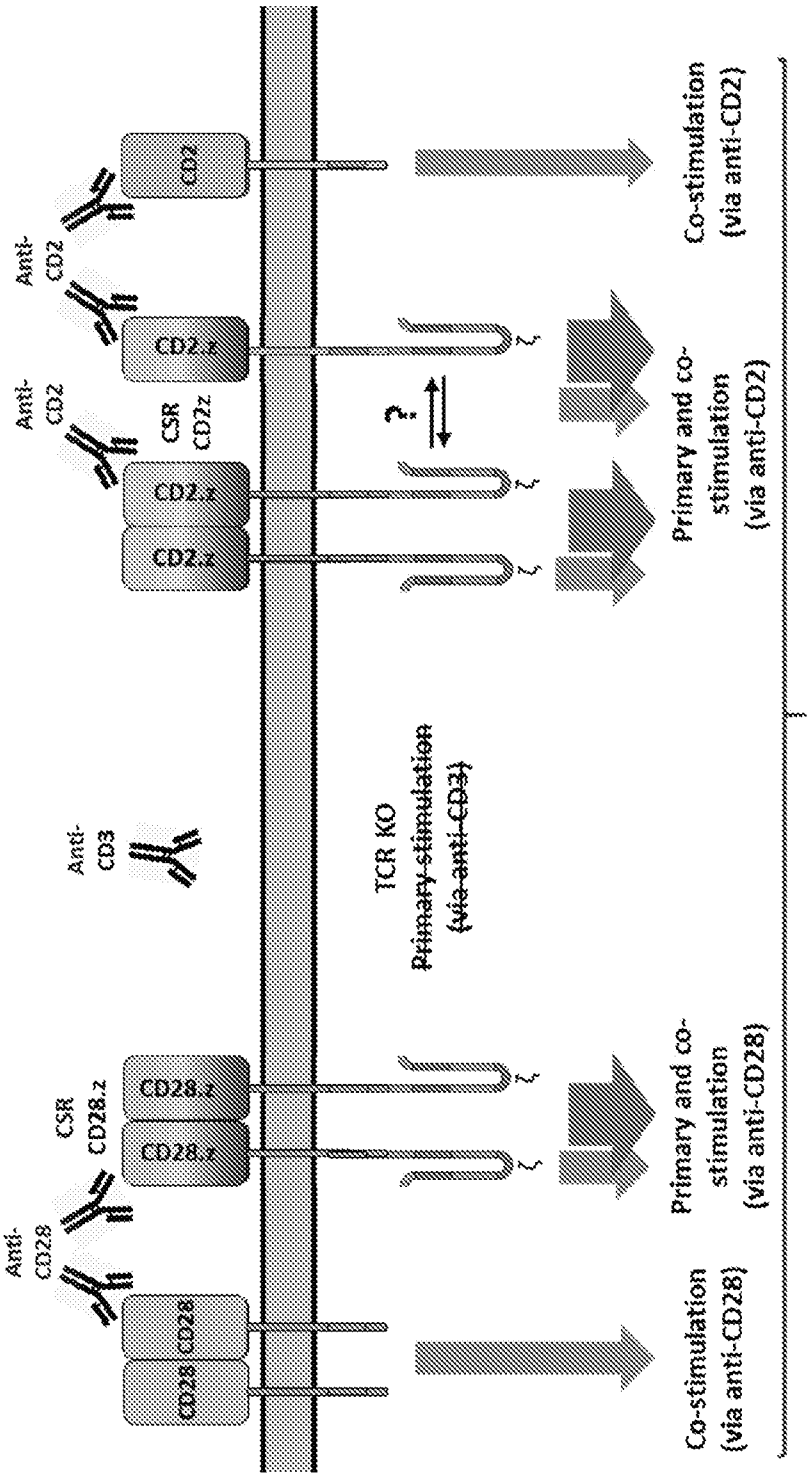

In absence of TCR, stimulation is enhanced with expression of Chimeric Stimulatory Receptors (CSR)s
Anti-CD28
CSR CD28.z
CD28 CD28
CD28.z
CD28.z
Anti-CD3
TCR KO
Primary stimulation (via anti-CD3)
Co-stimulation (via anti-CD28)
Primary and co-stimulation (via anti-CD28)
Anti-CD2
CSR CD2z
CD2.z
CD2.z
CD2.z
CD2
?
Primary and co-stimulation (via anti-CD2)
Co-stimulation (via anti-CD2)
ENHANCED T-cell activation and expansion

FIG. 5

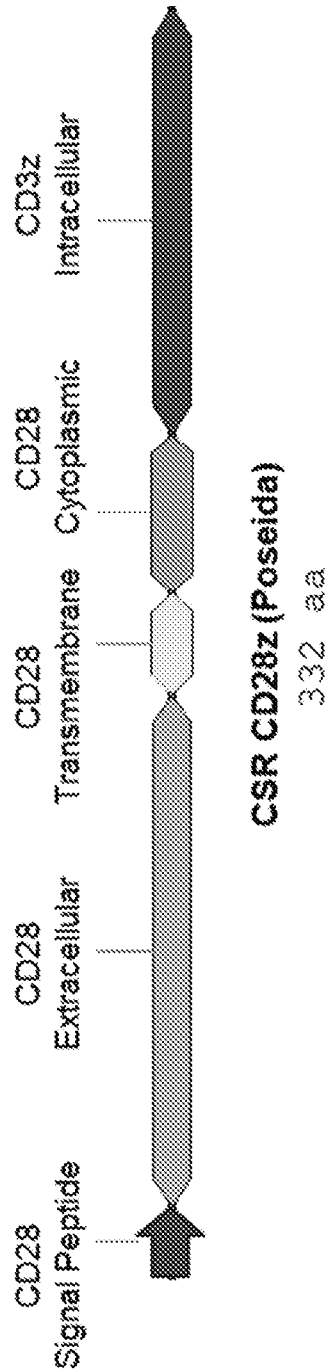

CD28
Signal Peptide
CD28
Extracellular
CD28
Transmembrane
CD28
Cytoplasmic
CD3z
Intracellular
CSR CD28z (Poseida)
332 aa

FIG. 6

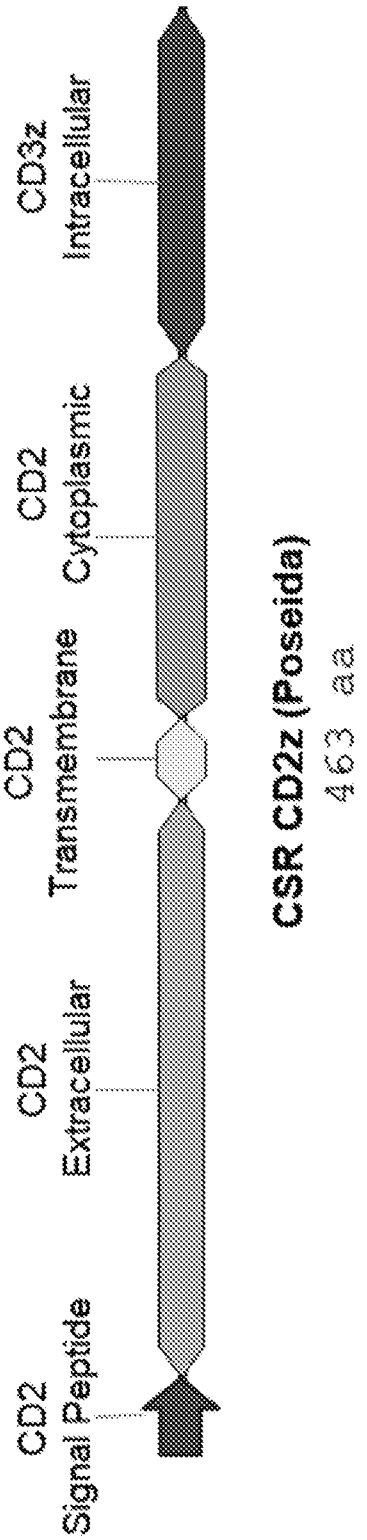

CD2
Signal Peptide
CD2
Extracellular
CD2
Transmembrane
CD2
Cytoplasmic
CD3z
Intracellular
CSR CD2z (Poseida)
463 aa

FIG. 7

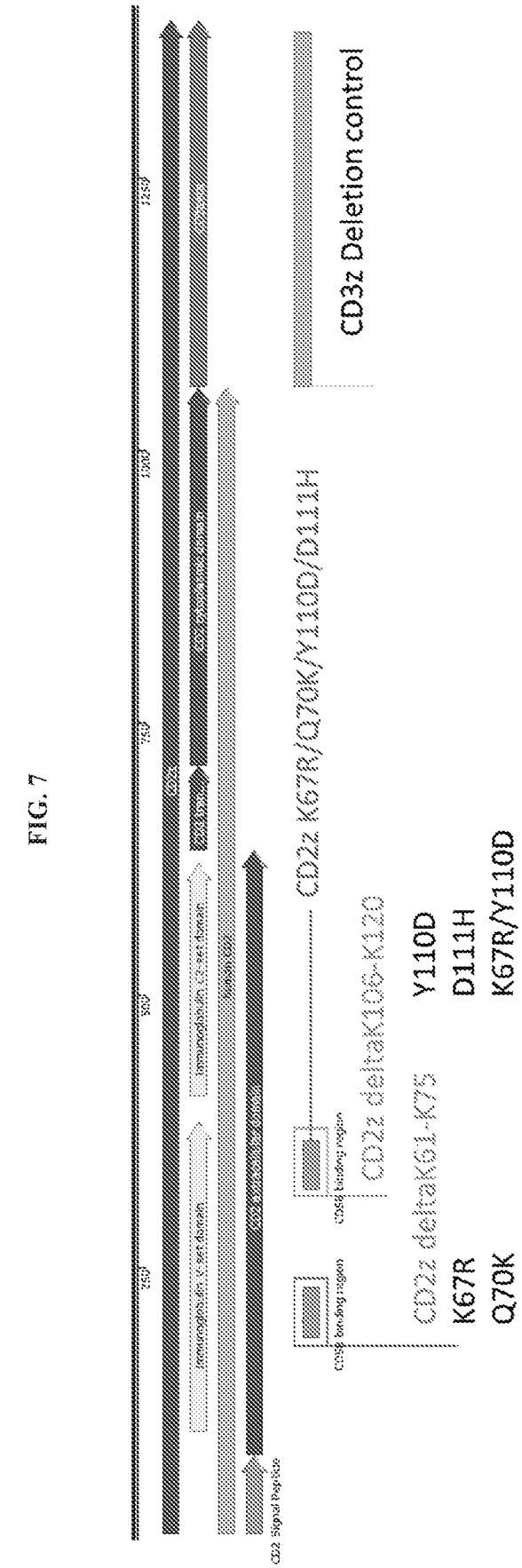
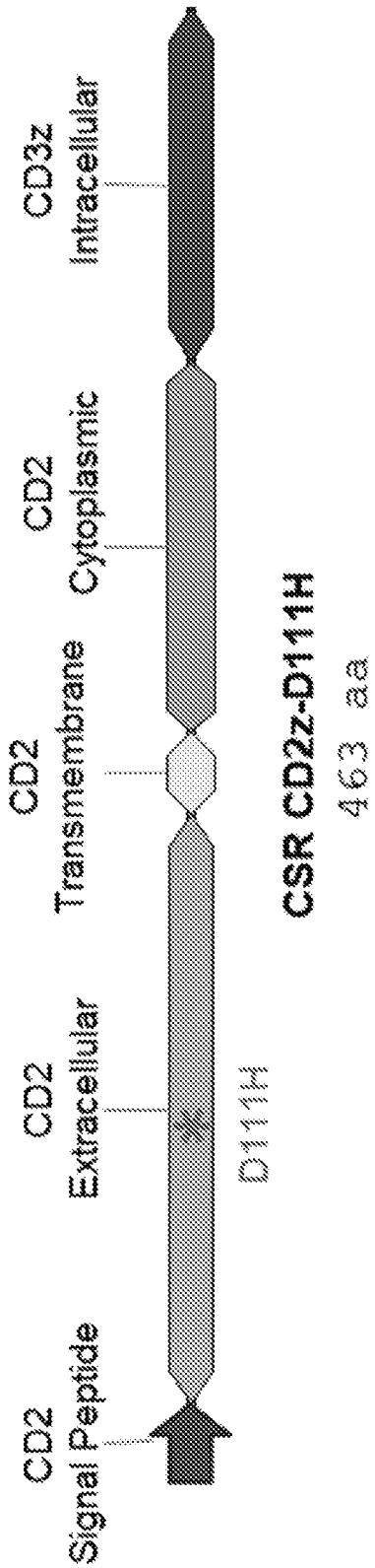

CD2z K67R/Q70K/Y110D/D111H
CD3z Deletion control
CD2z deltaK106-K120
CD2z deltaK61-K75
Y110D
D111H
K67R/Y110D
K67R
Q70K

FIG. 8


CD2
Signal Peptide
CD2
Extracellular
CD2
Transmembrane
CD2
Cytoplasmic
CD3z
Intracellular
D111H
CSR CD2z-D111H
463 aa

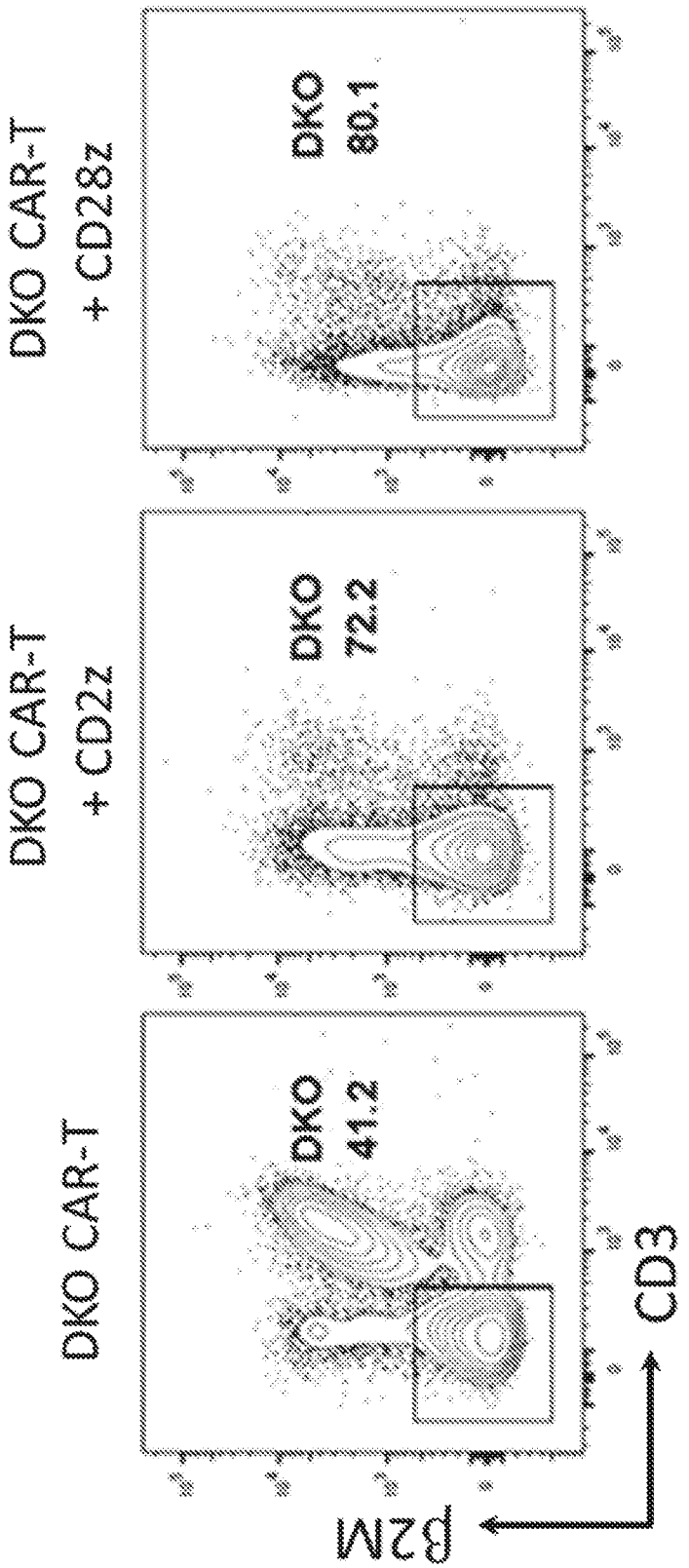
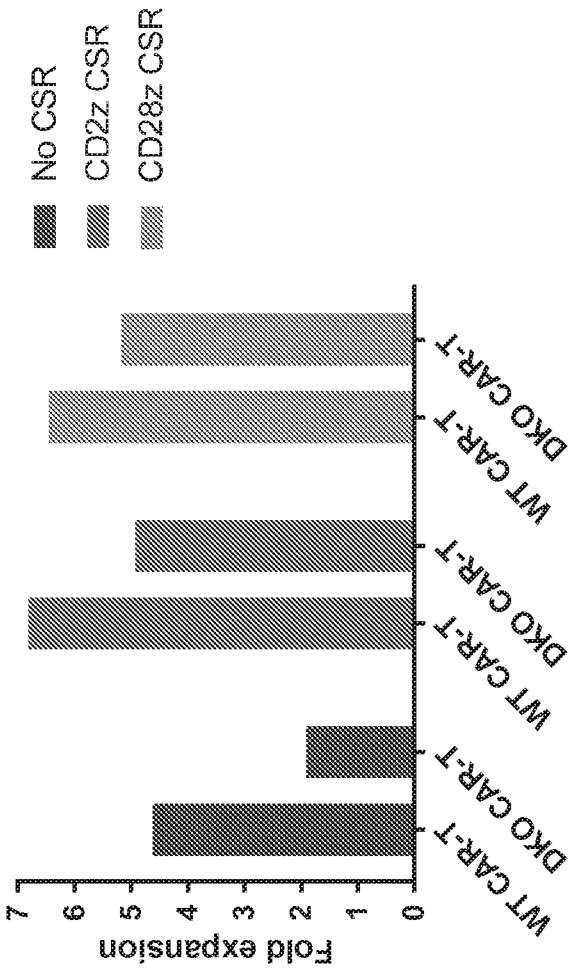

DKO CAR-T
DKO
41.2
DKO CAR-T
+ CD2z
DKO
72.2
DKO CAR-T
+ CD28z
DKO
80.1
β2M
CD3

FIG. 9A

FIG. 9B


No CSR
CD2z CSR
CD28z CSR
Fold expansion
0
1
2
3
4
5
6
7
WT CAR-T
DKO CAR-T
WT CAR-T
DKO CAR-T
WT CAR-T
DKO CAR-T

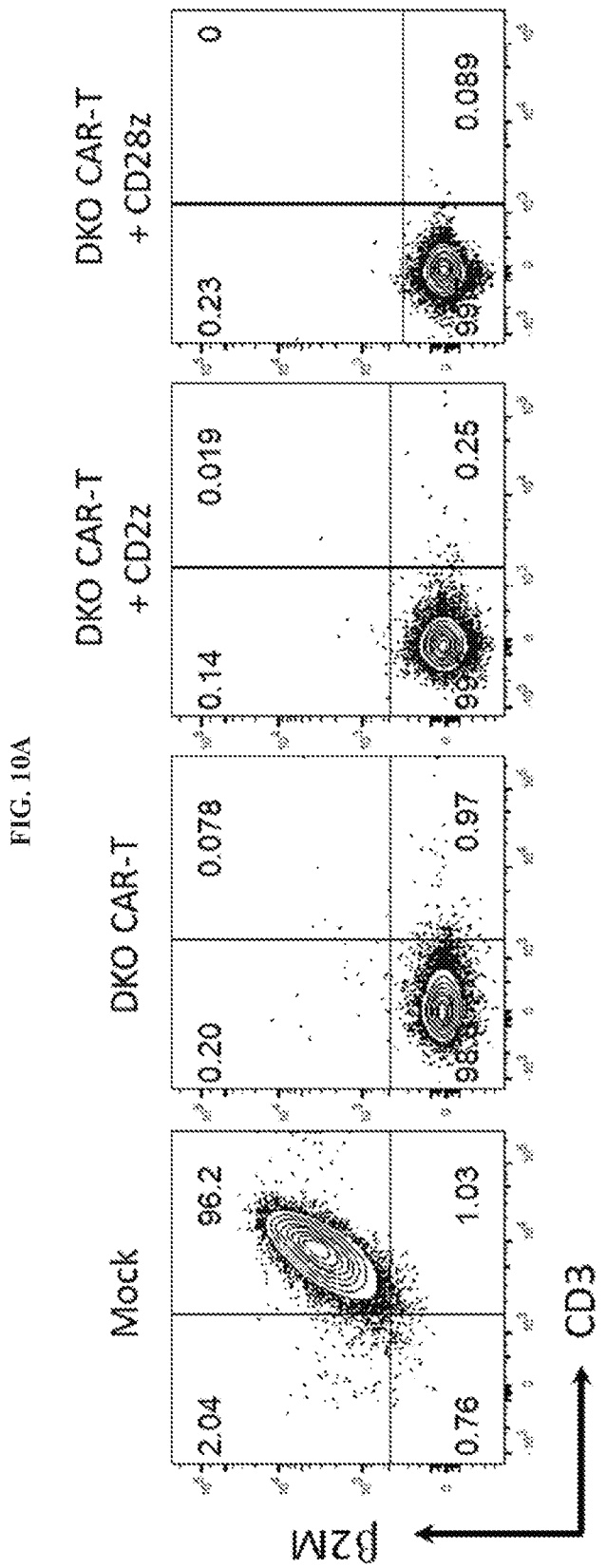

Mock
DKO CAR-T
DKO CAR-T
+ CD2z
DKO CAR-T
+ CD28z
2.04
96.2
0.76
1.03
0.20
0.078
98.9
0.97
0.14
0.019
99.2
0.25
0.23
0
99.7
0.089
β2M
CD3

FIG. 10A

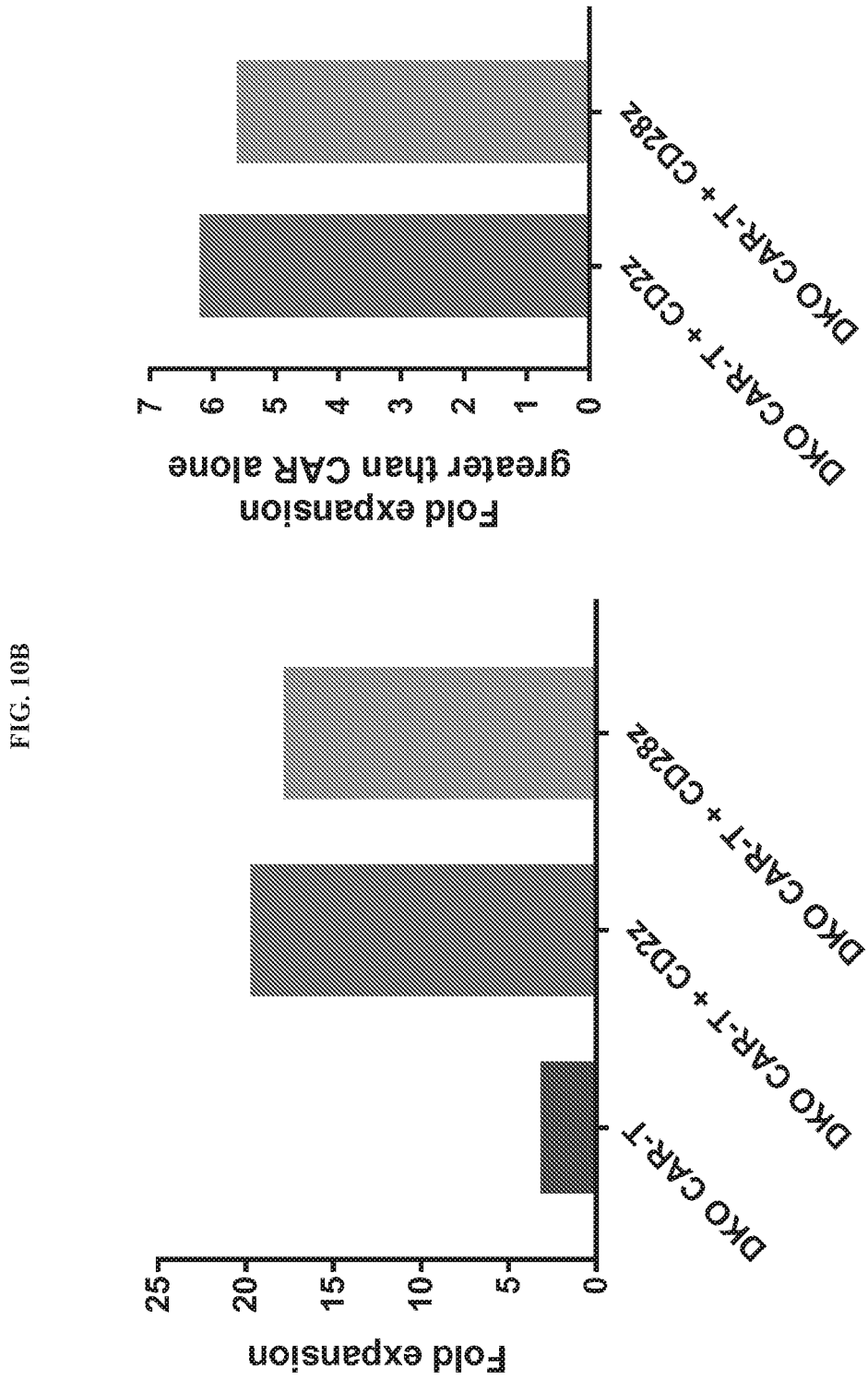

Fold expansion
25
20
15
10
5
0
DKO CAR-T
DKO CAR-T + CD22
DKO CAR-T + CD28z
Fold expansion
greater than CAR alone
7
6
5
4
3
2
1
0
DKO CAR-T + CD22
DKO CAR-T + CD28z

FIG. 10B

FIG. 11

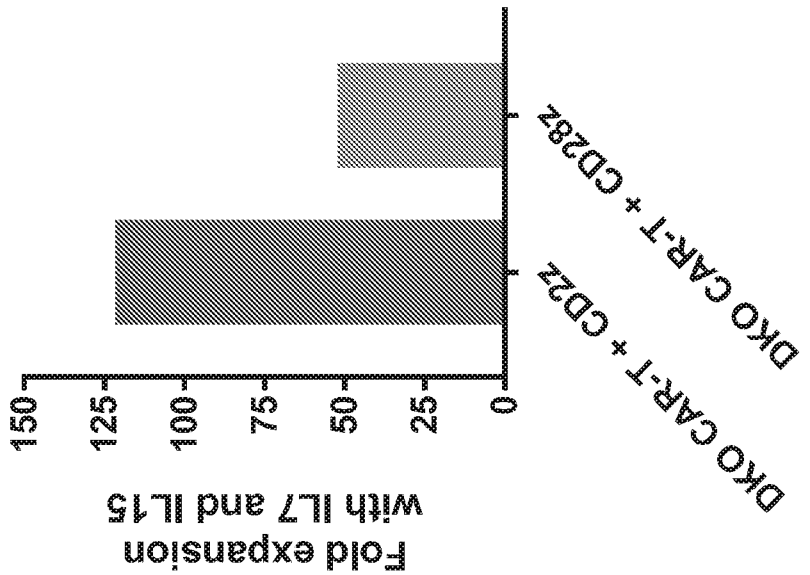

Fold expansion
with IL7 and IL15
150
125
100
75
50
25
0
DKO CAR-T + CD2z
DKO CAR-T + CD28z

FIG. 12

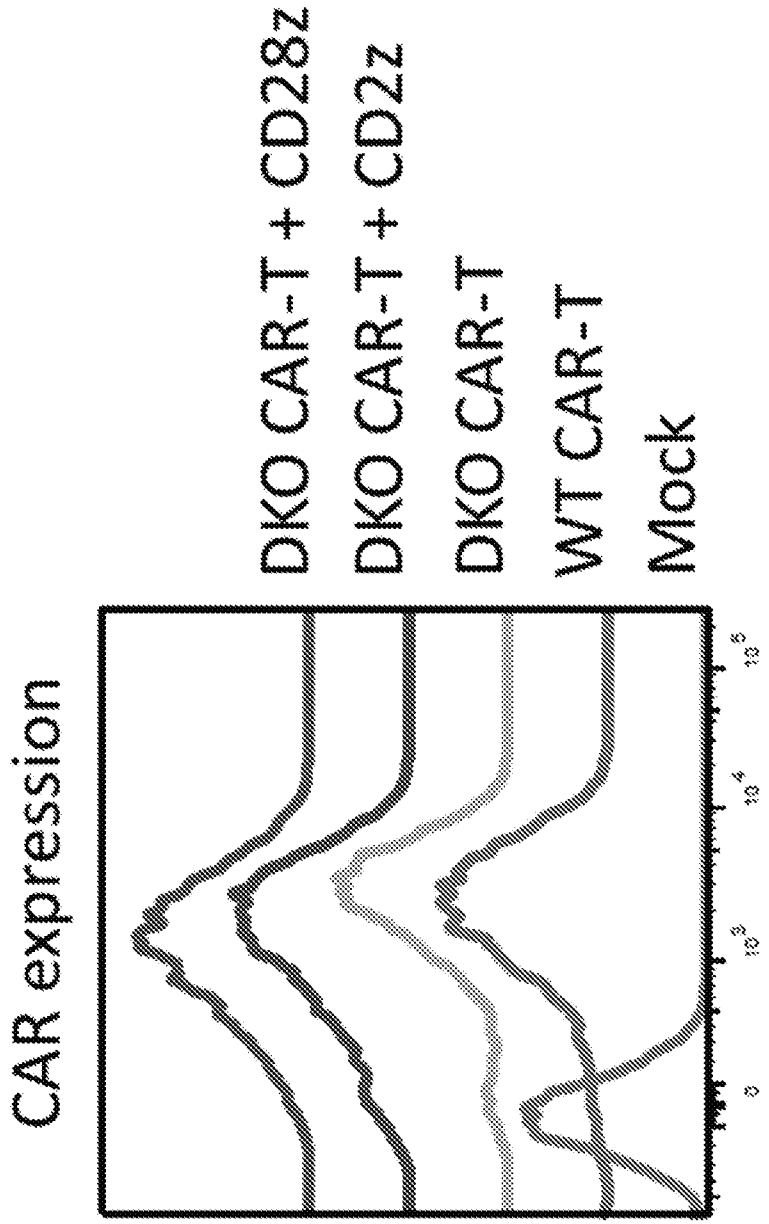

DKO CAR-T + CD28z
DKO CAR-T + CD2z
DKO CAR-T
WT CAR-T
Mock
CAR expression
0
10^3
10^4
10^5

FIG. 13

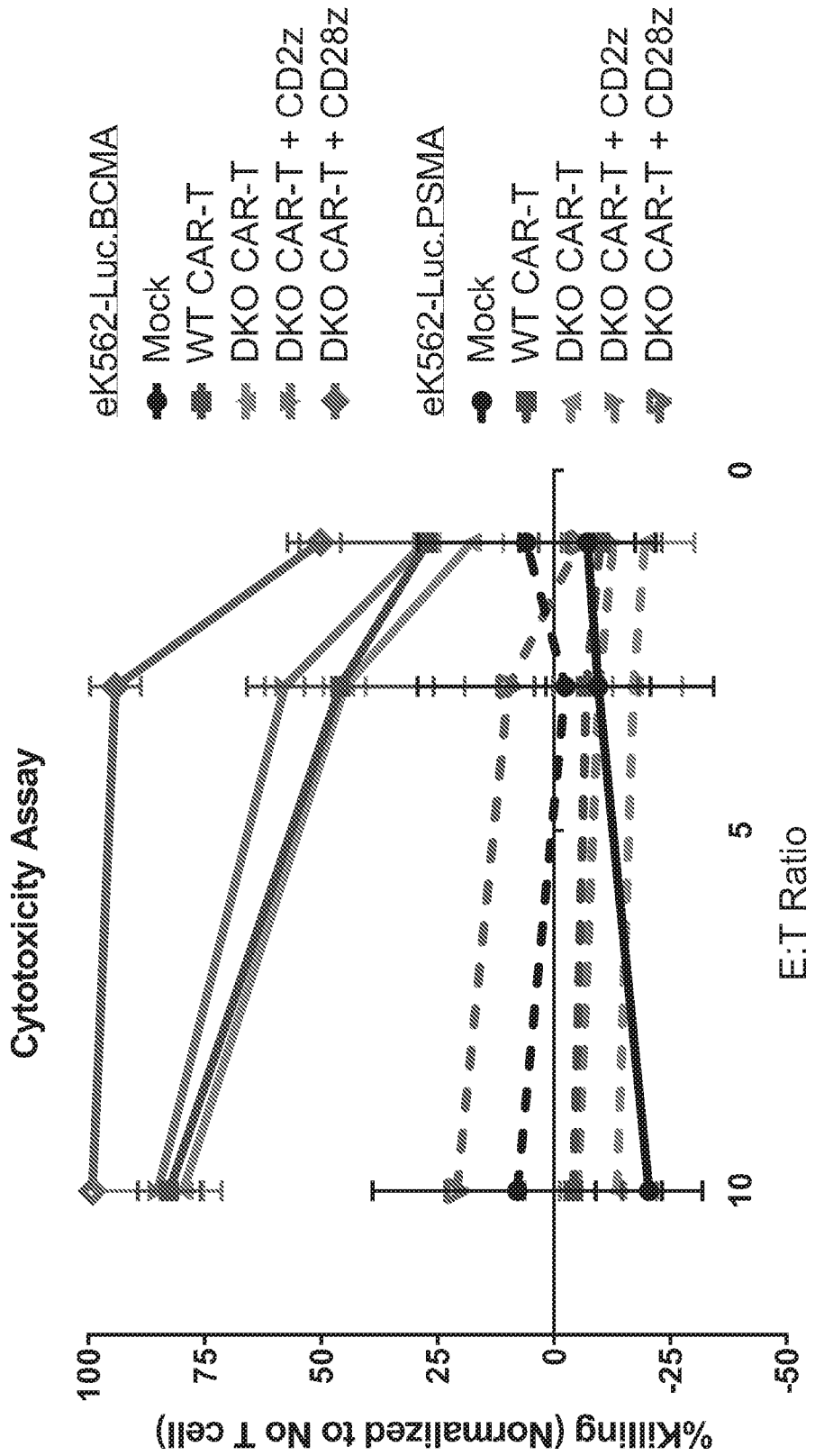

Cytotoxicity Assay
%Killing (Normalized to No T cell)
100
75
50
25
0
-25
-50
0
5
10
E:T Ratio
eK562-Luc.BCMA
Mock
WT CAR-T
DKO CAR-T
DKO CAR-T + CD2z
DKO CAR-T + CD28z
eK562-Luc.PSMA
Mock
WT CAR-T
DKO CAR-T
DKO CAR-T + CD2z
DKO CAR-T + CD28z

FIG. 14

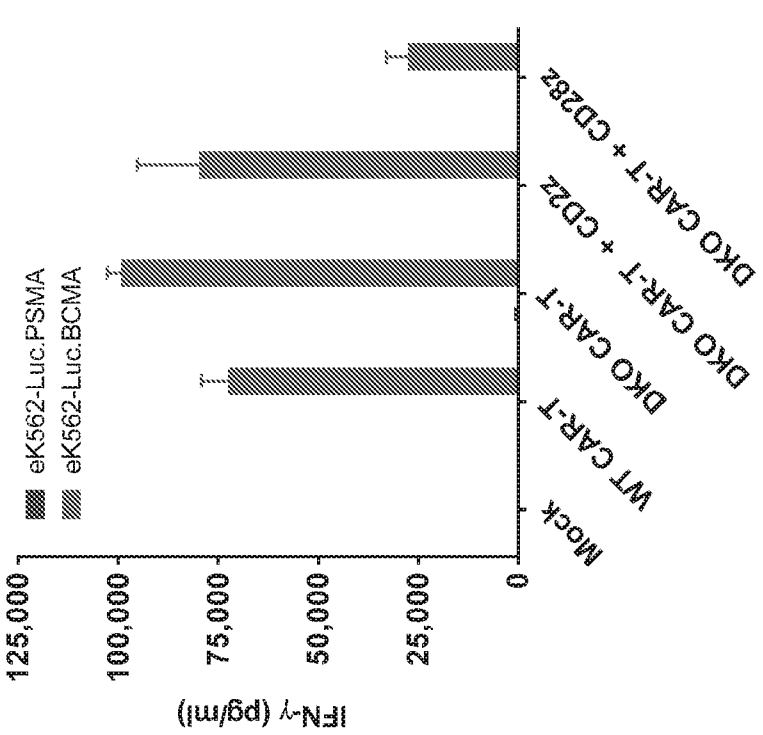
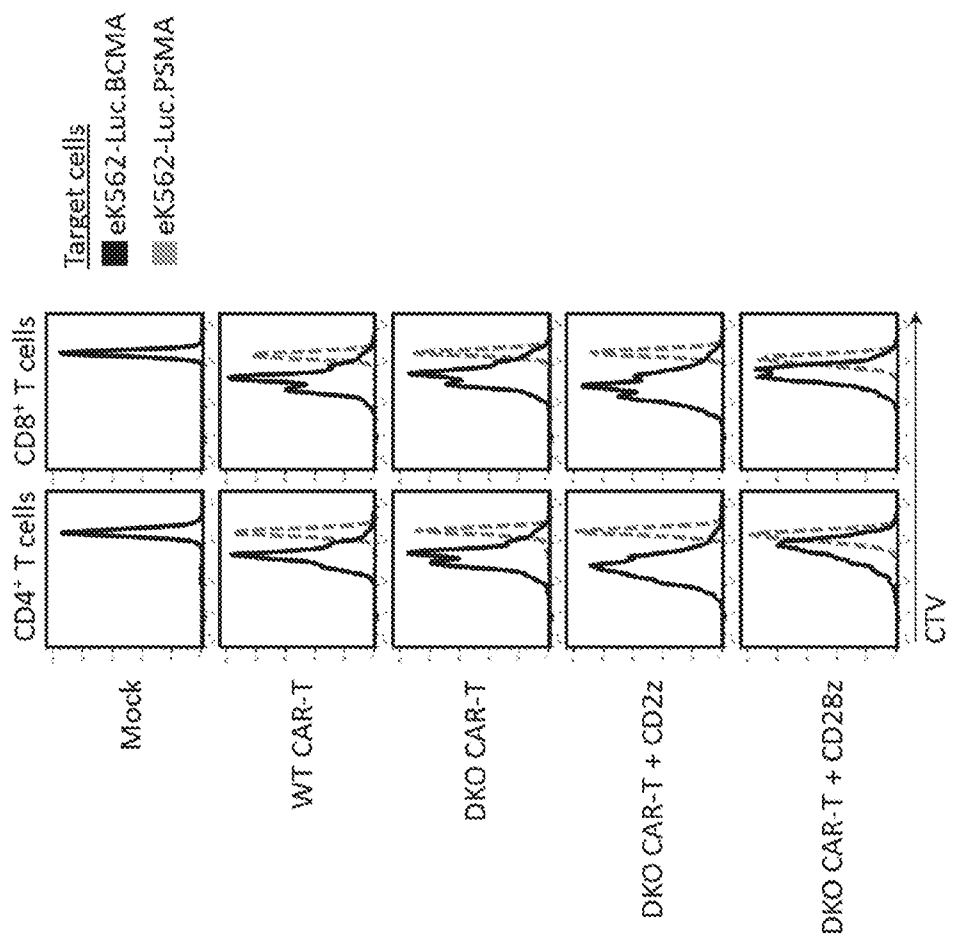

Antigen-Specific Cytokine Secretion
eK562-Luc.PSMA
eK562-Luc.BCMA
IFN-γ (pg/ml)
125,000
100,000
75,000
50,000
25,000
0
Mock
WT CAR-T
DKO CAR-T
DKO CAR-T + CD3ζ
DKO CAR-T + CD28ζ

FIG. 15


Target cells
eK562-Luc.BCMA
eK562-Luc.PSMA
CD4+ T cells
CD8+ T cells
Mock
WT CAR-T
DKO CAR-T
DKO CAR-T + CD2z
DKO CAR-T + CD28z
CTV

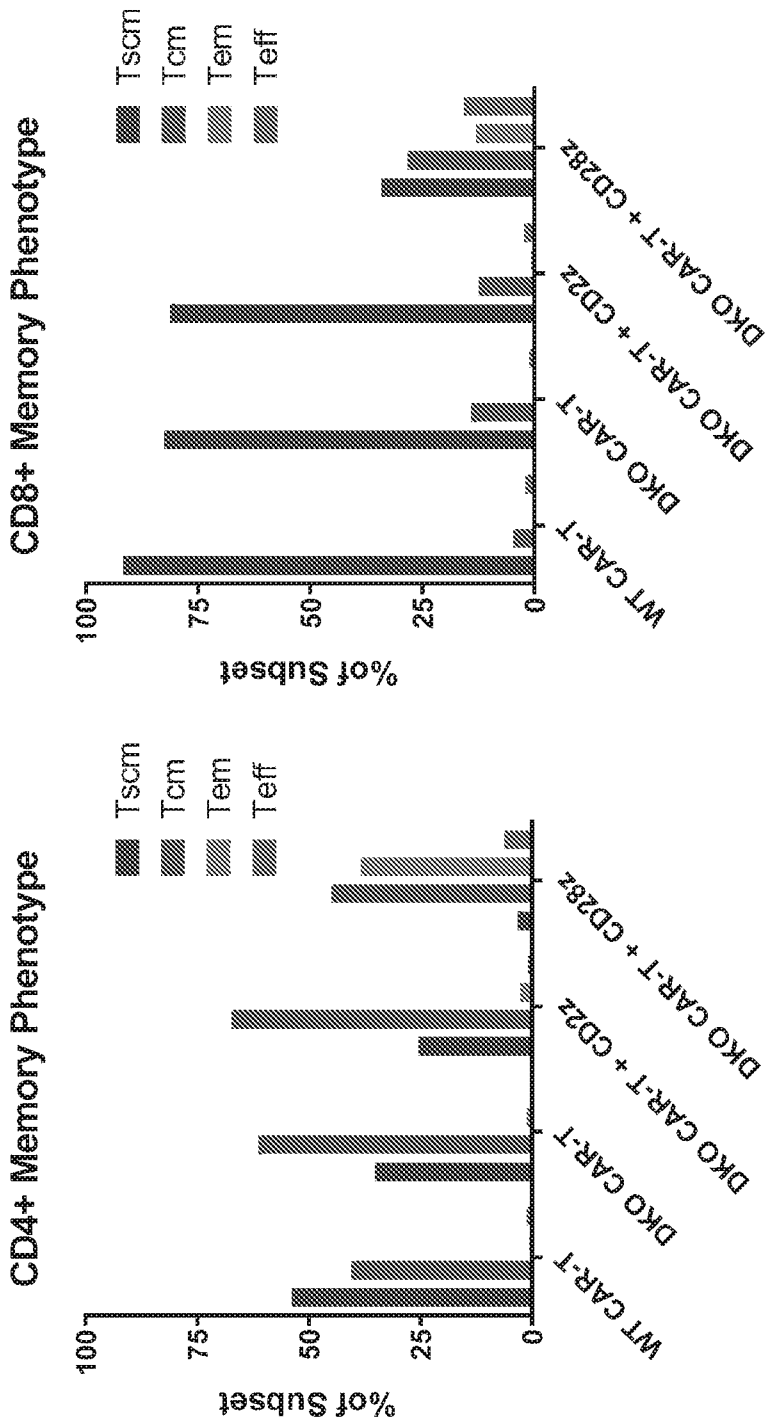

CD8+ Memory Phenotype
Tscm
Tcm
Tem
Teff
%of Subset
100
75
50
25
0
WT CAR-T
DKO CAR-T
DKO CAR-T + CD22
DKO CAR-T + CD28z
CD4+ Memory Phenotype
Tscm
Tcm
Tem
Teff
%of Subset
100
75
50
25
0
WT CAR-T
DKO CAR-T
DKO CAR-T + CD22
DKO CAR-T + CD28z

FIG. 16

FIG. 17

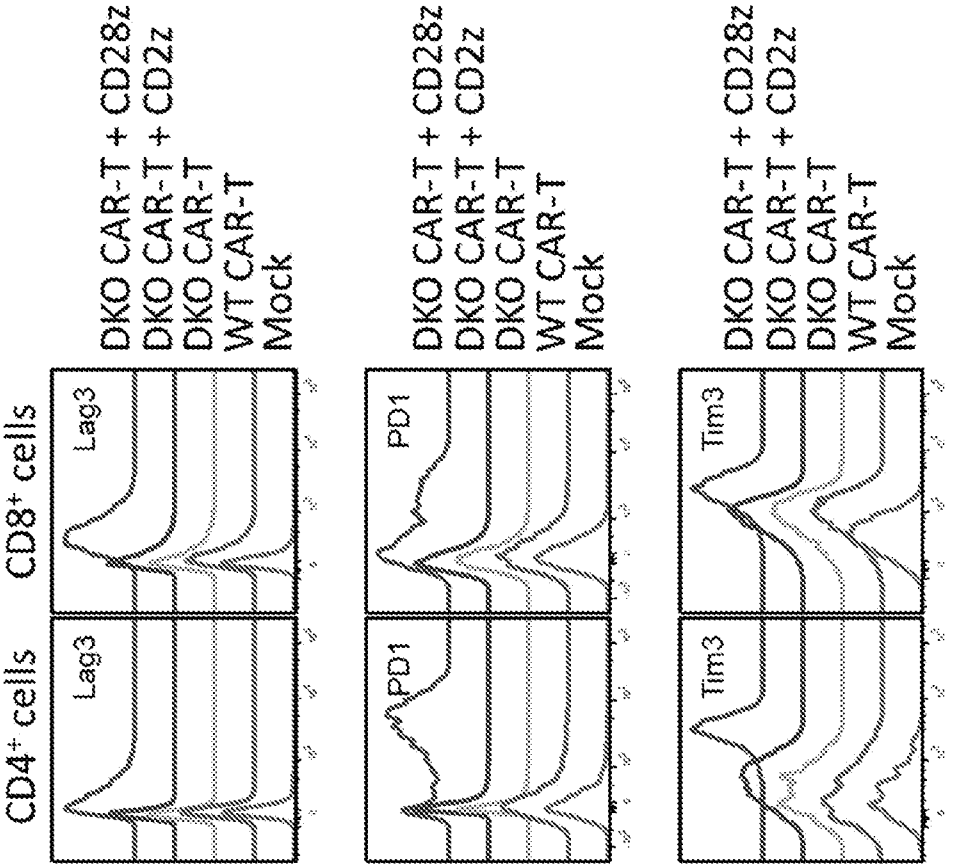

CD4+ cells
CD8+ cells
Lag3
PD1
Tim3
DKO CAR-T + CD28z
DKO CAR-T + CD2z
DKO CAR-T
WT CAR-T
Mock

FIG. 18

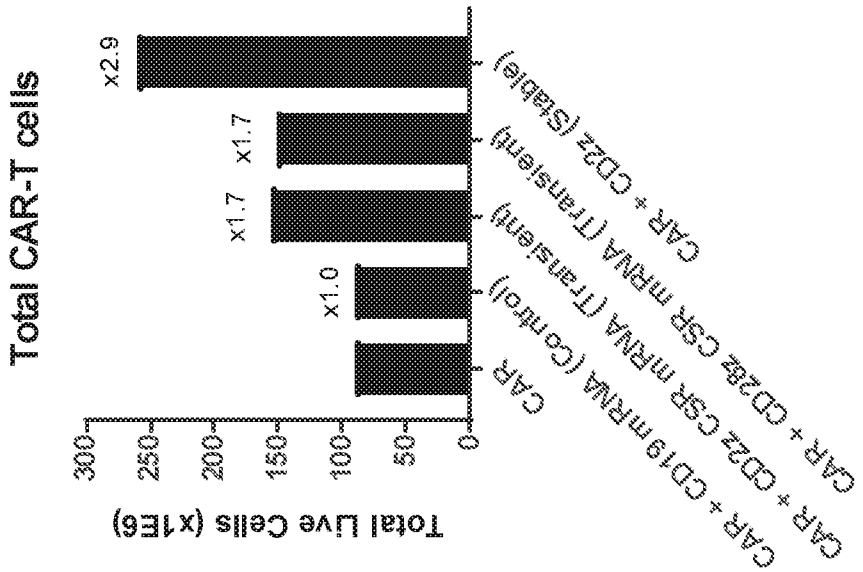

Total CAR-T cells
Total Live Cells (x1E6)
300
250
200
150
100
50
0
x2.9
x1.7
x1.7
x1.0
CAR
CAR + CD19 mRNA (Control)
CAR + CD22 CSR mRNA (Transient)
CAR + CD28z CSR mRNA (Transient)
CAR + CD22 (Stable)

FIG. 19

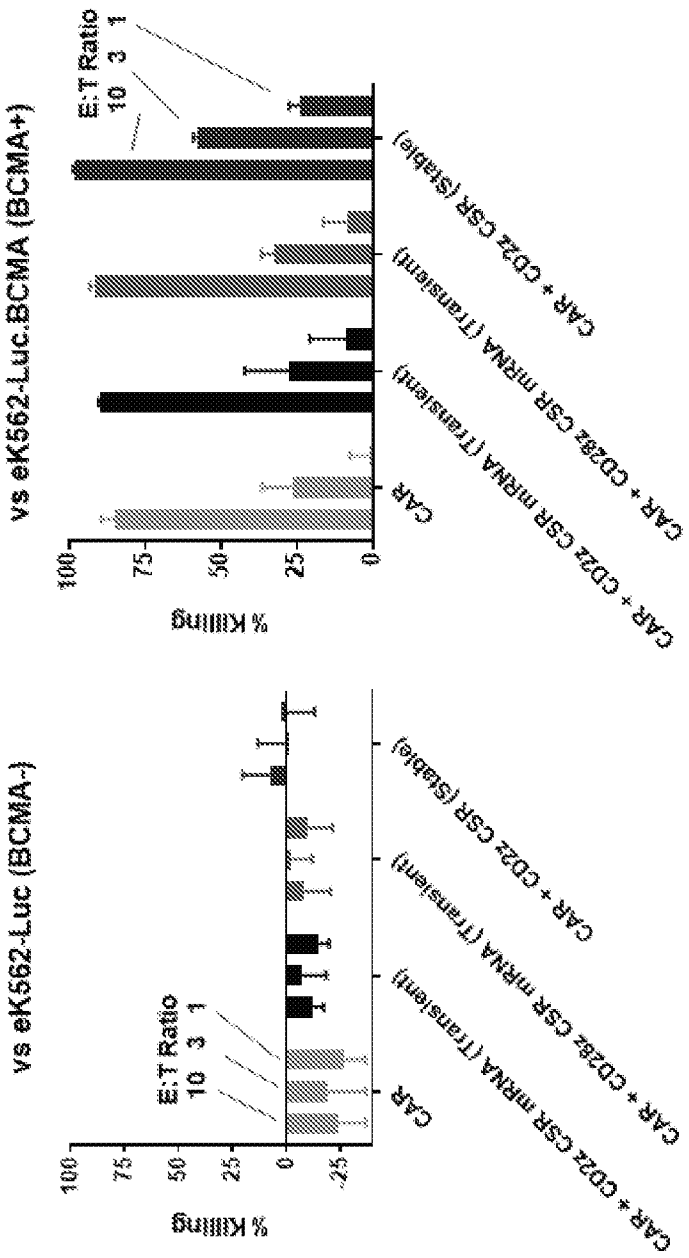

vs eK562-Luc.BCMA (BCMA+)
% Killing
100
75
50
25
0
E:T Ratio
10 3 1
CAR
CAR + CD22 CSR mRNA (Transient)
CAR + CD28z CSR mRNA (Transient)
CAR + CD22 CSR (Stable)
vs eK562-Luc (BCMA-)
% Killing
100
75
50
25
0
25
E:T Ratio
10 3 1
CAR
CAR + CD22 CSR mRNA (Transient)
CAR + CD28z CSR mRNA (Transient)
CAR + CD22 CSR (Stable)

FIG. 20

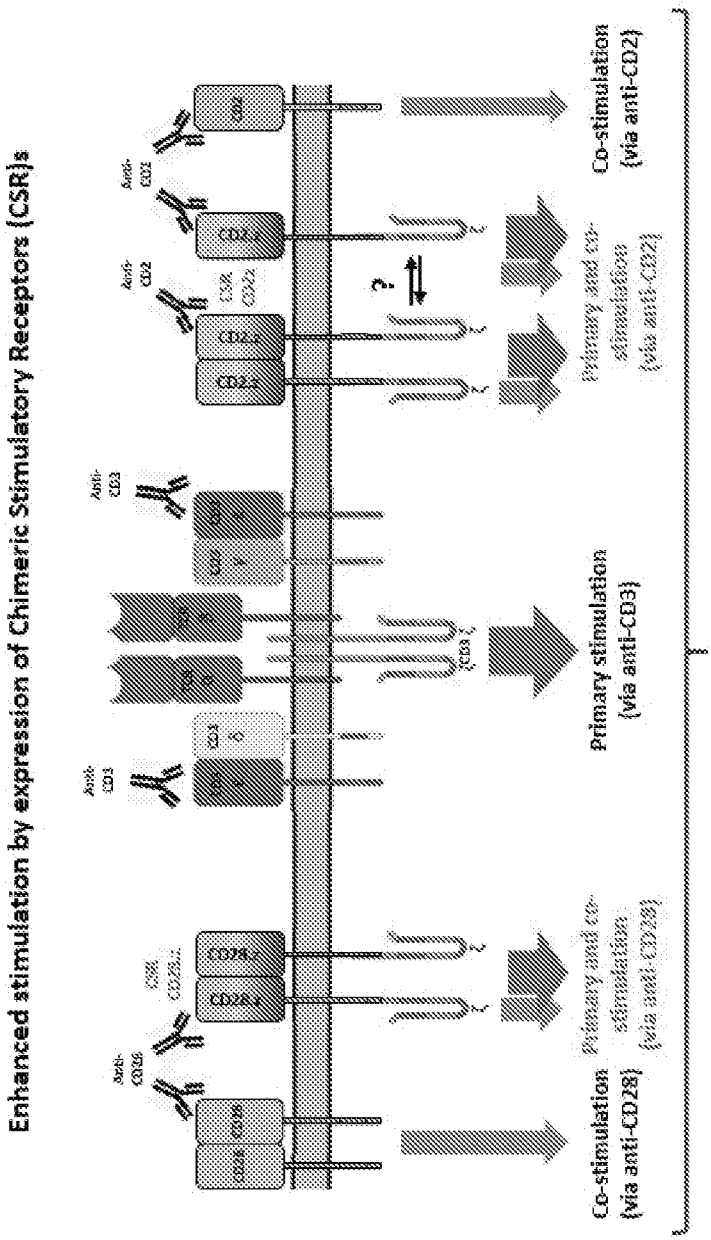

Enhanced stimulation by expression of Chimeric Stimulatory Receptors (CSR)s
Anti-CD28
CSR CD28
CD28
CD28
Anti-CD3
CD3 ζ
CD3 ζ
Anti-CD3
CD3 ζ
CD3 ζ
Anti-CD2
CSR CD2
CD2
CD2
Anti-CD3
CD3 ζ
CD3 ζ
?
Co-stimulation (via anti-CD28)
Primary and co-stimulation (via anti-CD28)
Primary stimulation (via anti-CD3)
Primary and co-stimulation (via anti-CD2)
Co-stimulation (via anti-CD2)
ENHANCED T-cell activation and expansion

FIG. 21

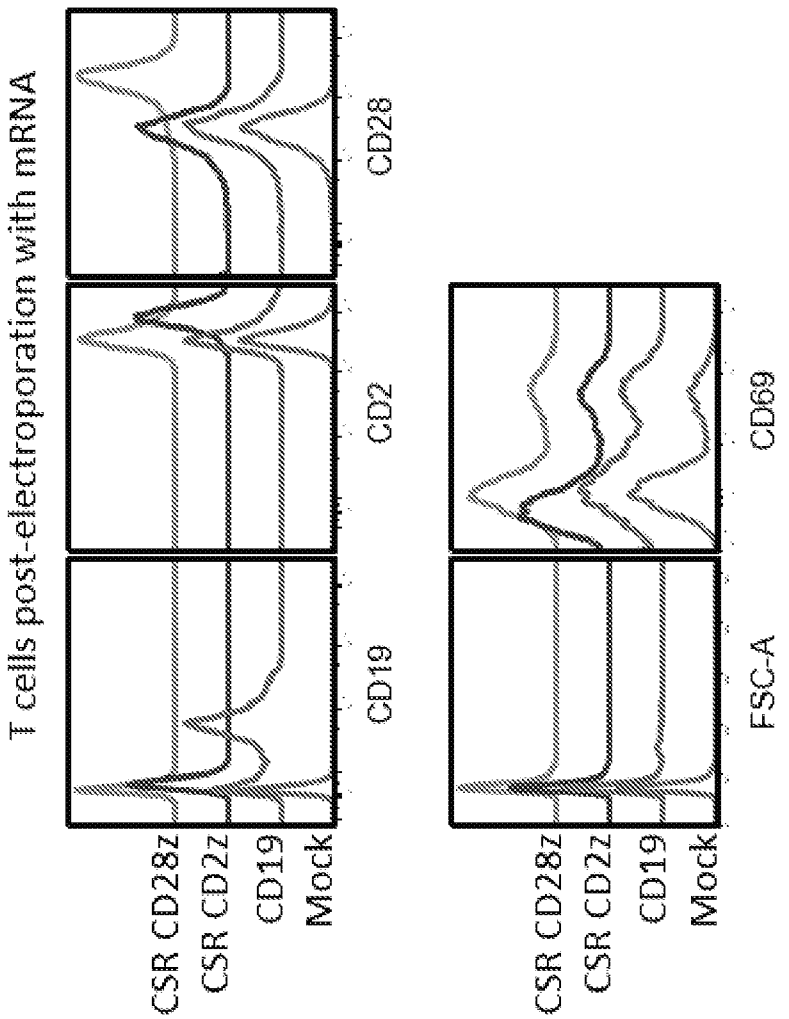

T cells post-electroporation with mRNA
CSR CD28z
CSR CD2z
CD19
Mock
CD19
CD2
CD28
FSC-A
CD69

FIG. 22

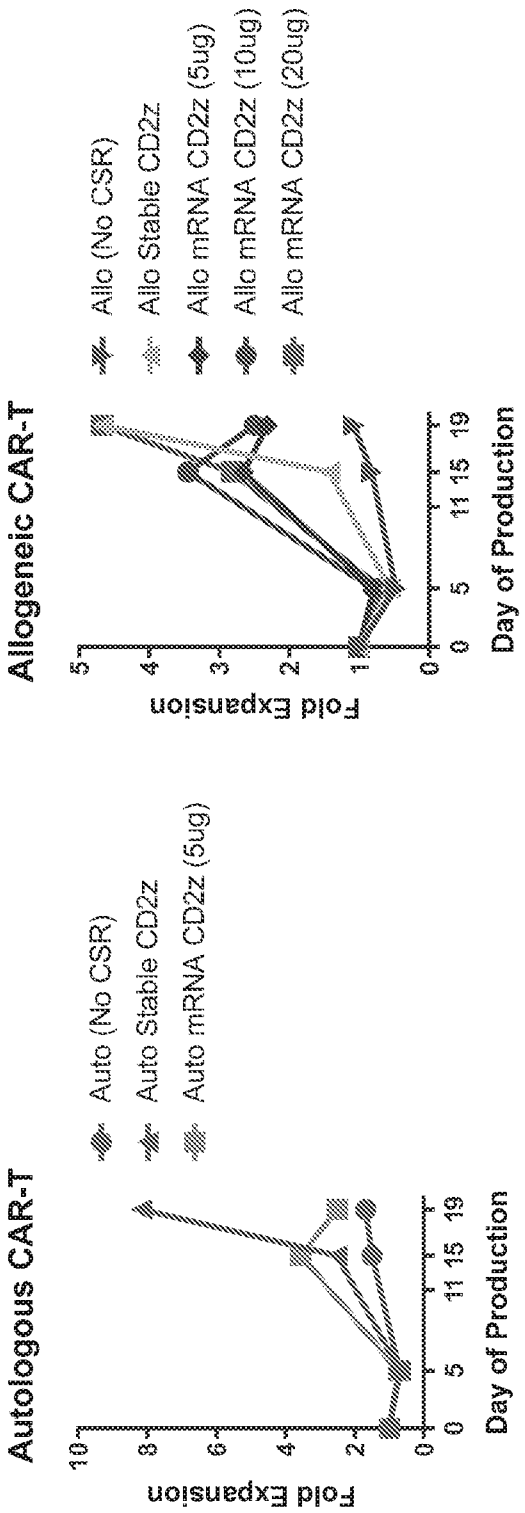

Allogeneic CAR-T
Allo (No CSR)
Allo Stable CD2z
Allo mRNA CD2z (5ug)
Allo mRNA CD2z (10ug)
Allo mRNA CD2z (20ug)
Fold Expansion
0
1
2
3
4
5
0 5 11 15 19
Day of Production
Autologous CAR-T
Auto (No CSR)
Auto Stable CD2z
Auto mRNA CD2z (5ug)
Fold Expansion
0
2
4
6
8
10
0 5 11 15 19
Day of Production

FIG.23A

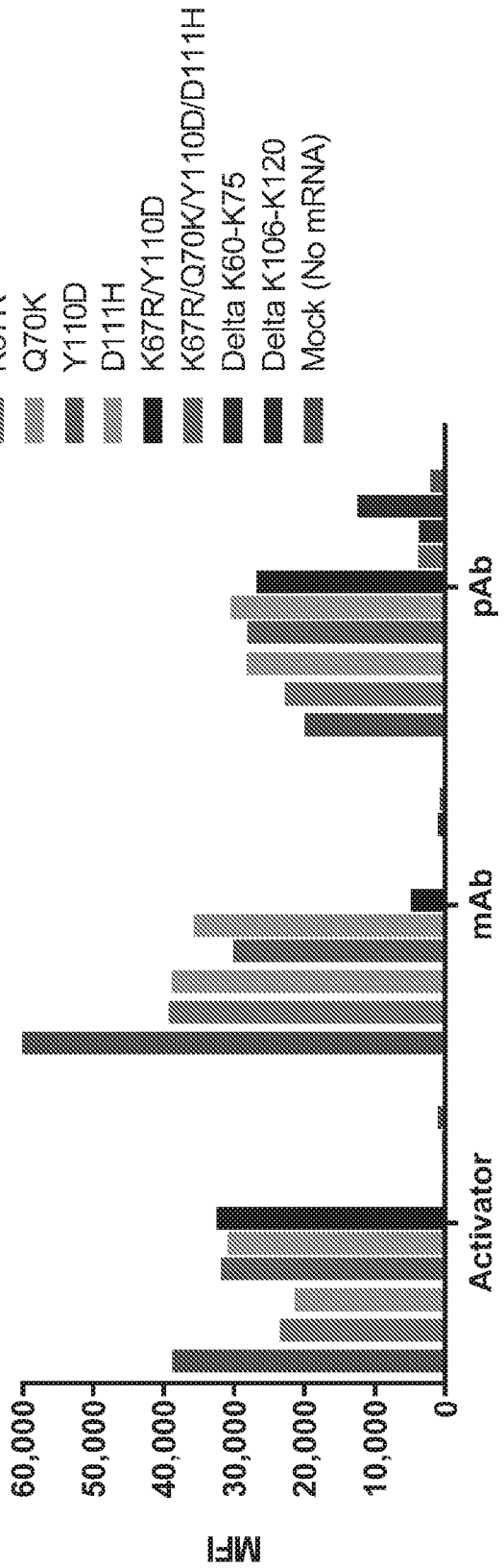

CSR CD2 Mutant Staining
CD2z
K67R
Q70K
Y110D
D111H
K67R/Y110D
K67R/Q70K/Y110D/D111H
Delta K60-K75
Delta K106-K120
Mock (No mRNA)
MFI
60,000
50,000
40,000
30,000
20,000
10,000
0
Activator
mAb
pAb

FIG. 23B

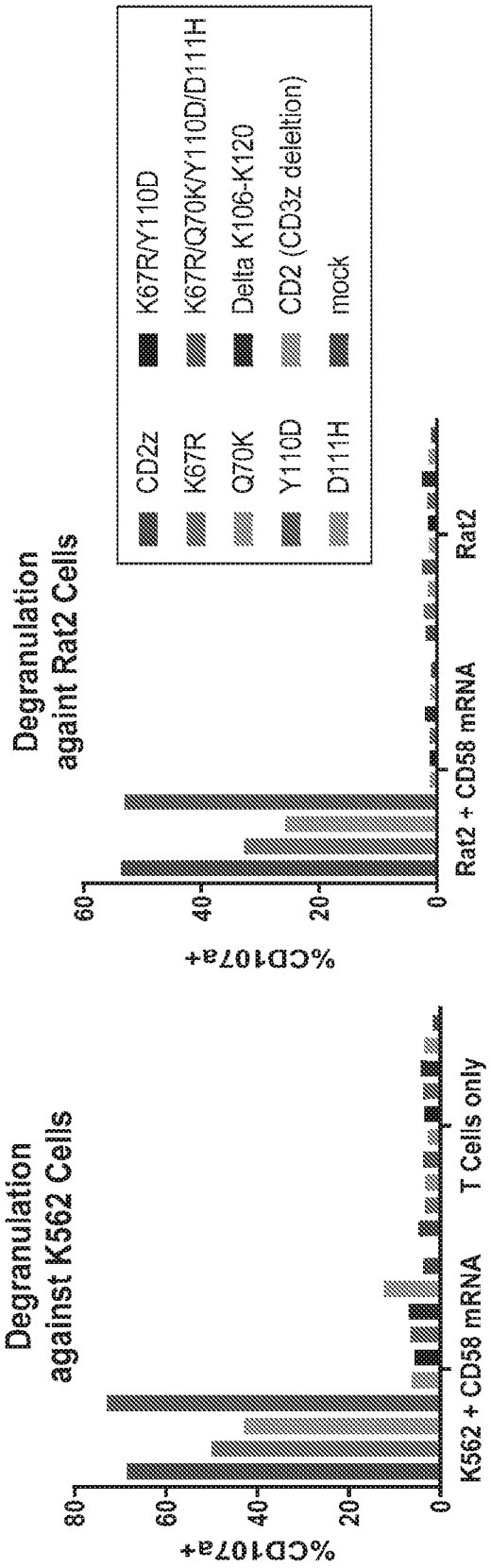
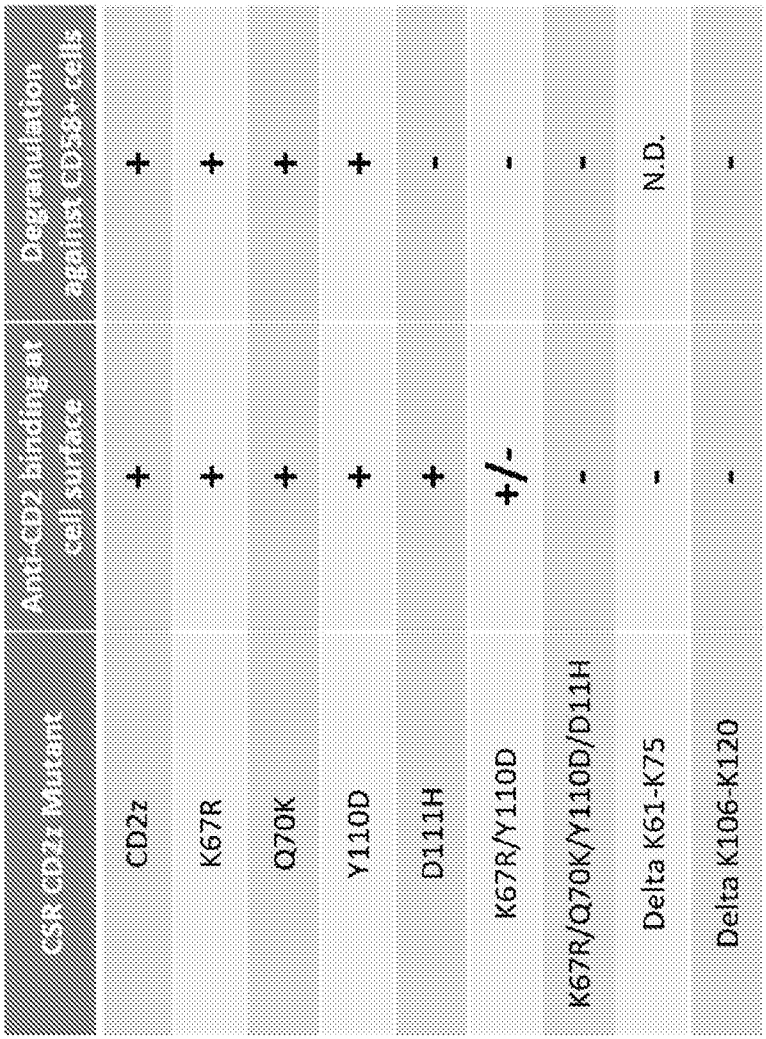

Degranulation
against Rat2 Cells
%CD107a+
0
20
40
60
Rat2 + CD58 mRNA
Rat2
CD2z
K67R
Q70K
Y110D
D111H
K67R/Y110D
K67R/Q70K/Y110D/D111H
Delta K106-K120
CD2 (CD3z deletion)
mock
Degranulation
against K562 Cells
%CD107a+
0
20
40
60
80
K562 + CD58 mRNA
T Cells only

FIG. 23C



CSR CD2z Mutant	Anti-CD2 binding on cell surface	Degranulation against CD58+ cells
CD2z	+	+
K67R	+	+
Q70K	+	+
Y110D	+	+
D111H	+	-
K67R/Y110D	+/-	-
K67R/Q70K/Y110D/D11H	-	-
Delta K61-K75	-	N.D.
Delta K106-K120	-	-

FIG. 23D

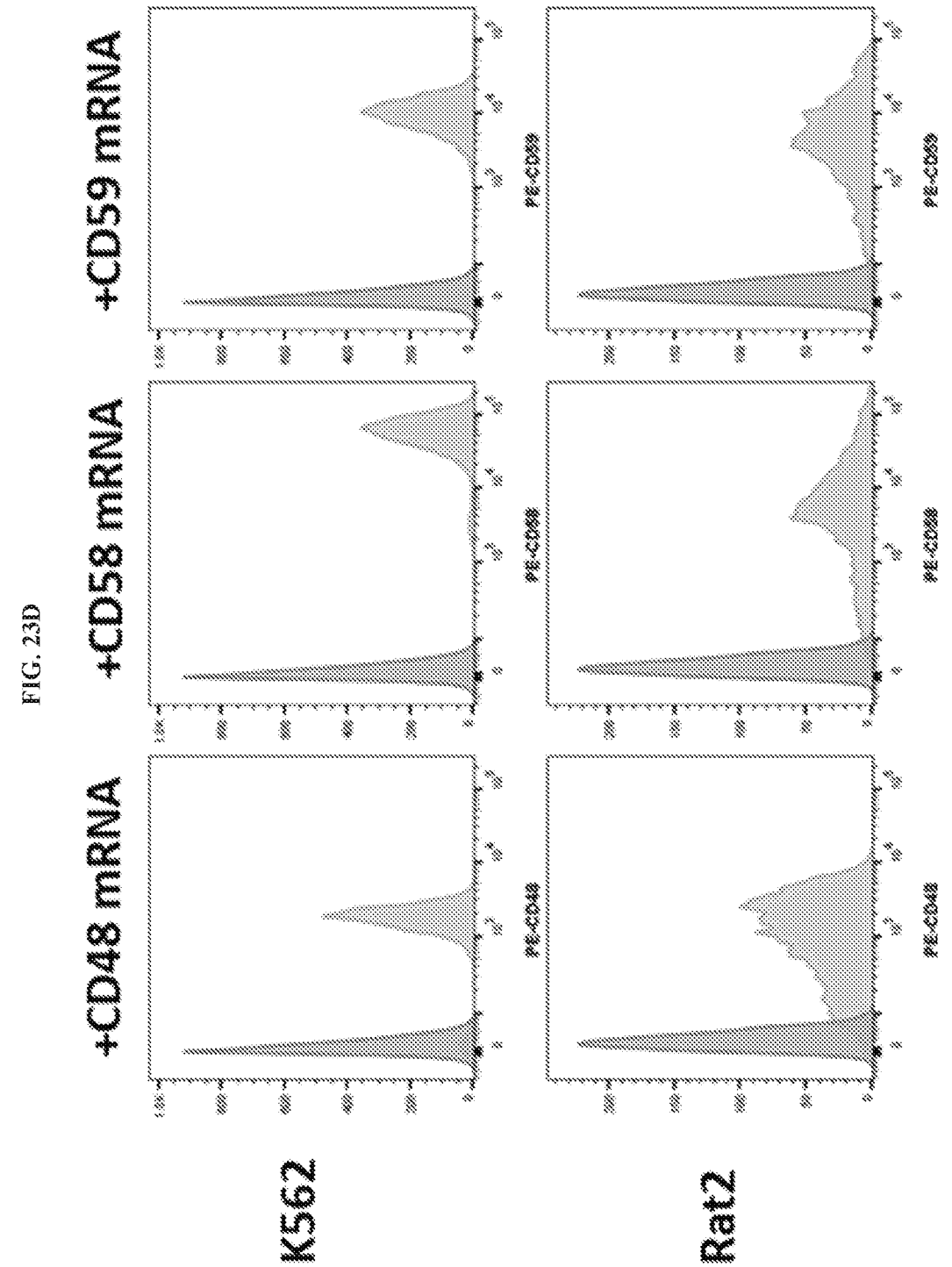
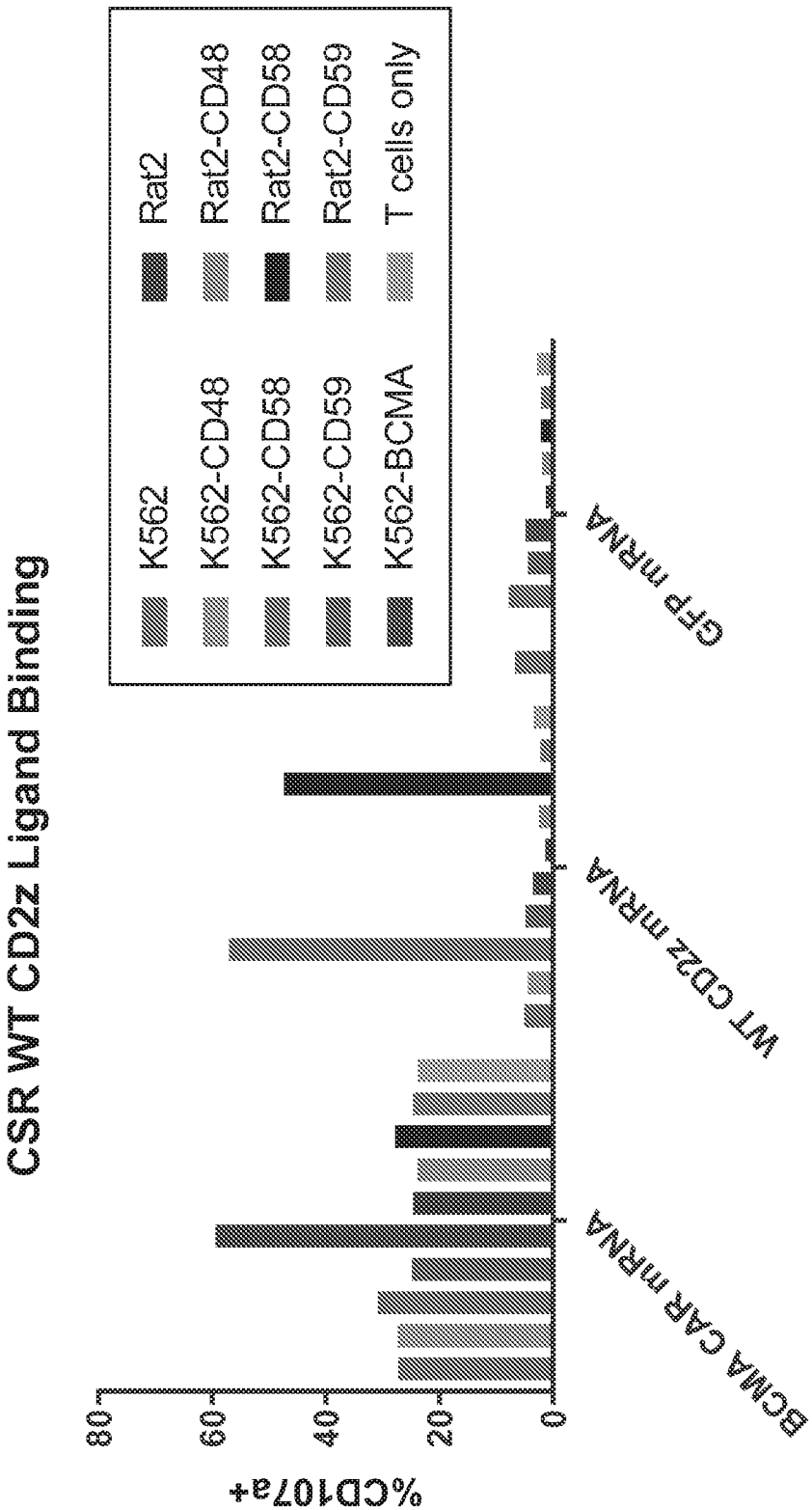

+CD48 mRNA
+CD58 mRNA
+CD59 mRNA
K562
Rat2
PE-CD48
PE-CD58
PE-CD59

FIG. 23E


CSR WT CD2z Ligand Binding
%CD107a+
0
20
40
60
80
BCMA CAR mRNA
WT CD2z mRNA
GFP mRNA
K562
K562-CD48
K562-CD58
K562-CD59
K562-BCMA
Rat2
Rat2-CD48
Rat2-CD58
Rat2-CD59
T cells only

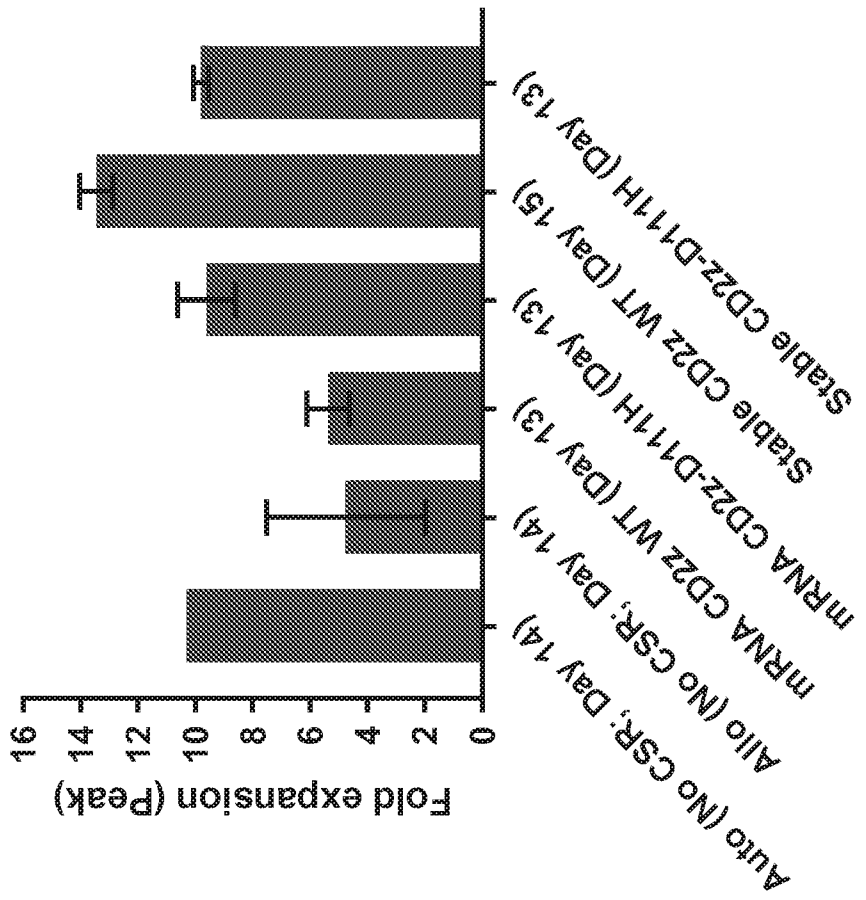

Fold expansion (Peak)
0
2
4
6
8
10
12
14
16
Auto (No CSR; Day 14)
Allo (No CSR; Day 14)
mRNA CD22 WT (Day 13)
mRNA CD22-D114H (Day 13)
Stable CD22 WT (Day 15)
Stable CD22-D114H (Day 13)

FIG. 24A

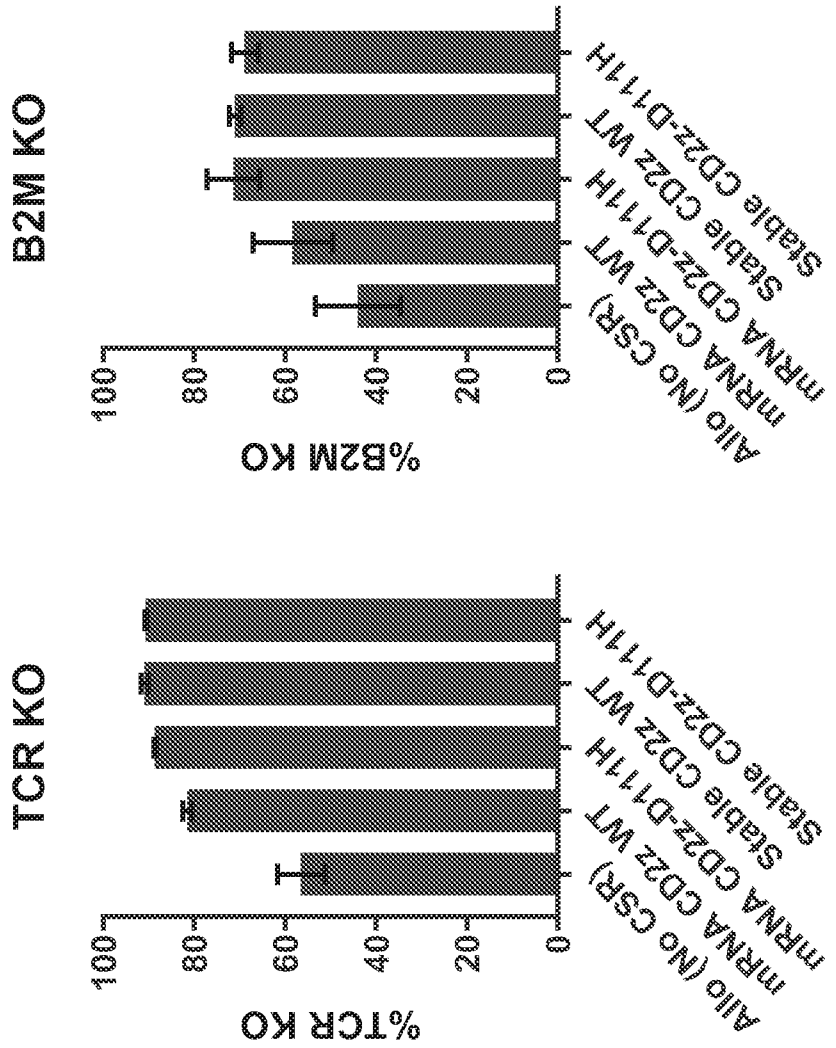

B2M KO
%B2M KO
100
80
60
40
20
0
Allo (No CSR)
mRNA CD22 WT
mRNA CD22-D114H
Stable CD22 WT
Stable CD22-D114H
TCR KO
%TCR KO
100
80
60
40
20
0
Allo (No CSR)
mRNA CD22 WT
mRNA CD22-D114H
Stable CD22 WT
Stable CD22-D114H

FIG. 24B

FIG.24C

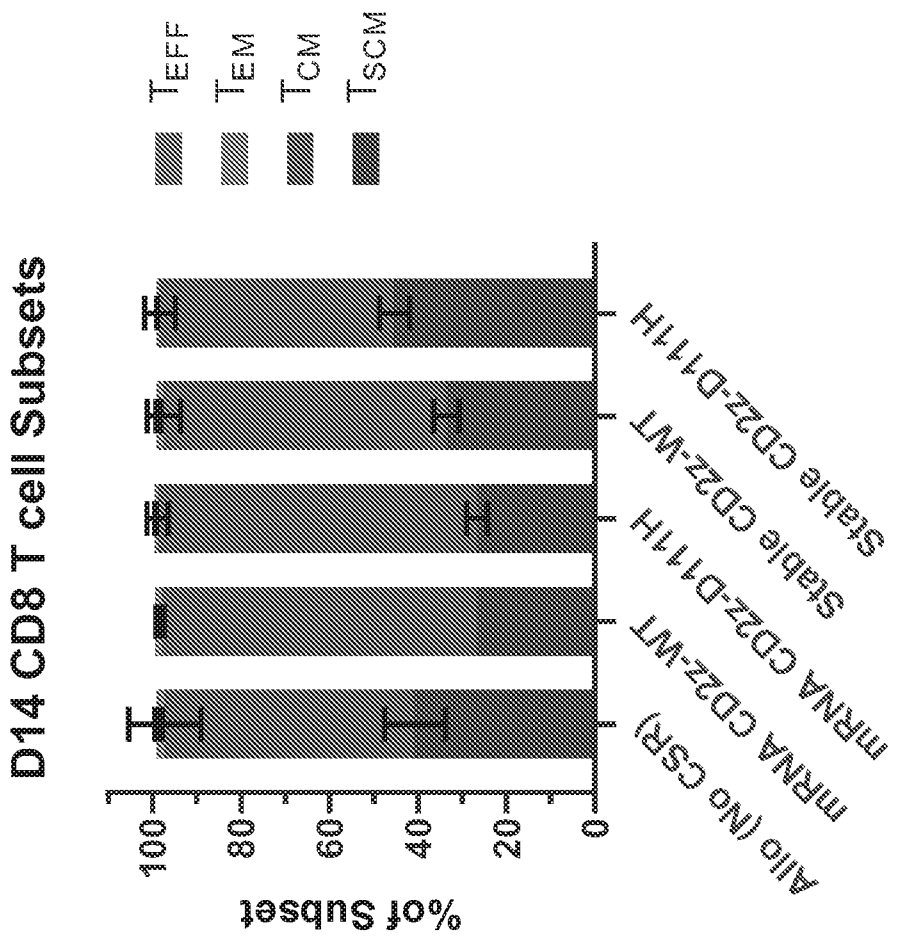

D14 CD8 T cell Subsets
%of Subset
100
80
60
40
20
0
TEFF
TEM
TCM
TSCM
Allo (No CSR)
mRNA CD22-WT
mRNA CD22-D1/1H
Stable CD22-WT
Stable CD22-D1/1H

FIG. 25

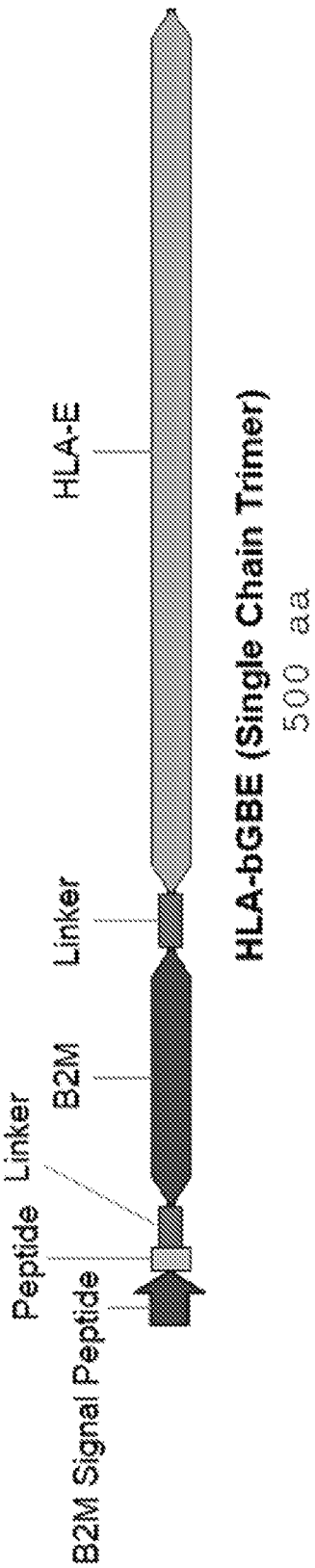

HLA-bGBE (Single Chain Trimer)
500 aa
HLA-E
Linker
B2M
Linker
Peptide
B2M Signal Peptide

FIG. 26

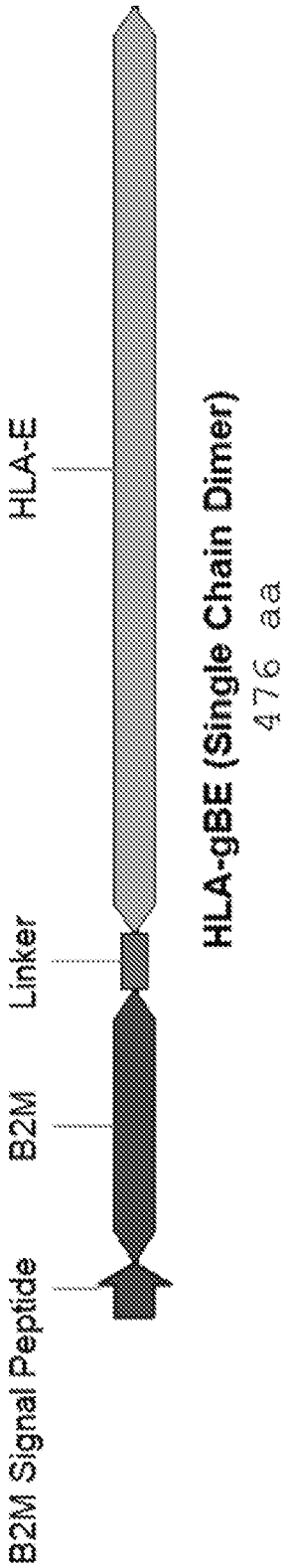

B2M Signal Peptide
B2M
Linker
HLA-E
HLA-gBE (Single Chain Dimer)
476 aa

FIG. 27

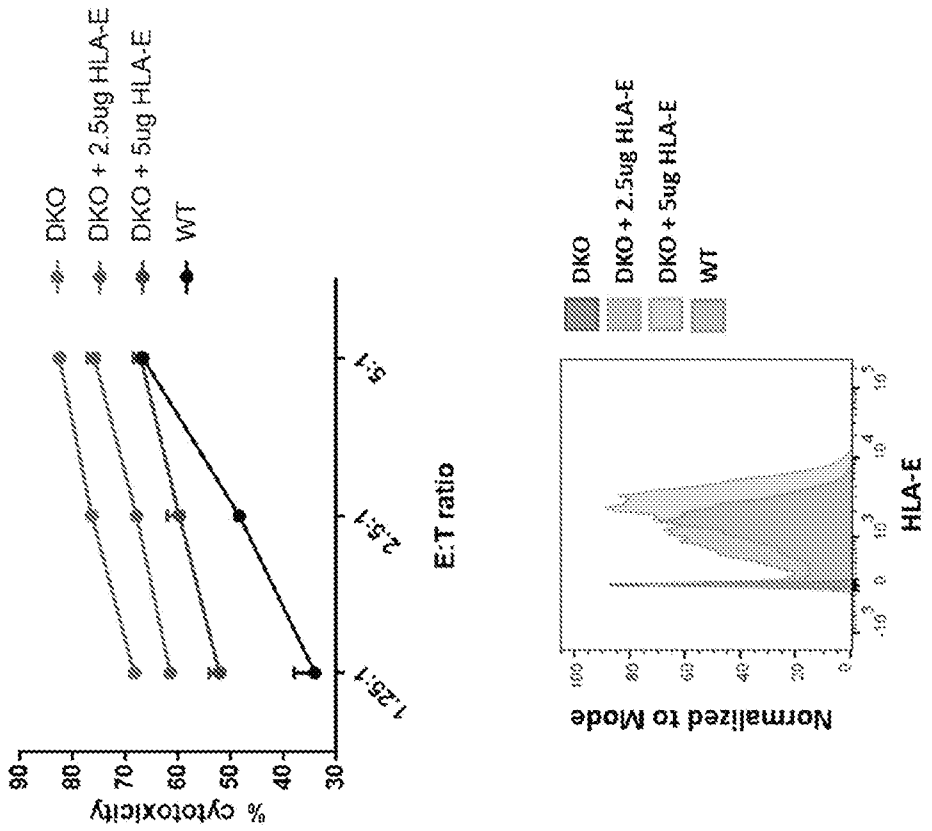

DKO
DKO + 2.5ug HLA-E
DKO + 5ug HLA-E
WT
% cytotoxicity
E:T ratio
1.25:1
2.5:1
5:1
Normalized to Mode
HLA-E

FIG. 28

Guide RNA nucleic acid sequences

WT Cas9 gRNAs for targeting HLA-A, B and C, not E, F, MR1

gRNA1 (HLA-A, B, C, J, L, H), exon 3)
GGATTACATCGCCCTGAACGAGG (SEQ ID NO: 17070)

gRNA2 (HLA-A, C, G), exon 3)
CTACCTGGAGGGCACGTGCGTGG (SEQ ID NO: 17071)

gRNA3 (HLA-A, C, G), exon3)
CACGCACGTGCCCTCCAGGTAGG (SEQ ID NO: 17072)

gRNA4 (B, C, A, H; with one mis-match G J, exon3)
GGAGTGGCTCCGCAGATACCTGG (SEQ ID NO: 17073)

gRNA5 (HLA-A, B, C, H; with one mis-match HLA-G, J, L, exon3)
TCCGCAGATACCTGGAGAACGGG (SEQ ID NO: 17074)

gRNA6 ((HLA-A, B, C, H, G; with one mis-match HLA-A, J, L), exon3)
CAGATACCTGGAGAACGGGAAGG (SEQ ID NO: 17075)

gRNA71 (exon3, 1 mismatch HLA-A, B, C, H, J, L; 2 mismatch G, F)
GGATTACATCGCCCTGAAAGAGG (SEQ ID NO: 17076)

gRNA8 (HLA-A, B, C, G, H, J; 1 mismatch F, L; 2 mismatch E, exon 2)
TACCTCATGGAGTGGGAGCCTGG (SEQ ID NO: 17077)

gRNA9 (HLA-A, B, C, G, J; 1 mismatch at 1st bp of H; 1 mismatch F, exon 2)
CCCCAGGCTCCCACTCCATGAGG (SEQ ID NO: 17078)

gRNA10 (HLA-A, B, C, G, J; 1 mismatch L; 2 mismatch F, H, E, exon 2)
GCAGGAGGGGCCGGAGTATTGGG (SEQ ID NO: 17079)

Primers for PCR amplify gRNA templates

HLA-gRNA1
gaaatTAATACGACTCACTATAGGATTACATCGCCCTGAACGgttttagagctagaaatagcaag
(SEQ ID NO: 17080)

HLA-gRNA2
gaaatTAATACGACTCACTATAGGCTACCTGGAGGGCACGTGCGgttttagagctagaaatagcaag
(SEQ ID NO: 17081)

HLA-gRNA3
gaaatTAATACGACTCACTATAGGCACGCACGTGCCCTCCAGGTgttttagagctagaaatagcaag
(SEQ ID NO: 17082)

HLA-gRNA4
gaaatTAATACGACTCACTATAGGAGTGGCTCCGCAGATACCgttttagagctagaaatagcaag
(SEQ ID NO: 17083)

HLA-gRNA5
gaaatTAATACGACTCACTATAGGTCCGCAGATACCTGGAGAACgttttagagctagaaatagcaag
(SEQ ID NO: 17084)

HLA-gRNA6
gaaatTAATACGACTCACTATAGGCAGATACCTGGAGAACGGGAgttttagagctagaaatagcaag
(SEQ ID NO: 17085)

HLA-gRNA7
gaaatTAATACGACTCACTATAGGATTACATCGCCCTGAACGgttttagagctagaaatagcaag
(SEQ ID NO: 17086)

HLA-gRNA8
gaaatTAATACGACTCACTATAGGTACCTCATGGAGTGGGAGCCgttttagagctagaaatagcaag
(SEQ ID NO: 17087)

HLA-gRNA9
gaaatTAATACGACTCACTATAGGCCCCAGGCTCCCACTCCATGgttttagagctagaaatagcaag
(SEQ ID NO: 17088)

HLA-gRNA10
gaaatTAATACGACTCACTATAGGCAGGAGGGGCCGGAGTATTgttttagagctagaaatagcaag
(SEQ ID NO: 17089)

FIG. 29

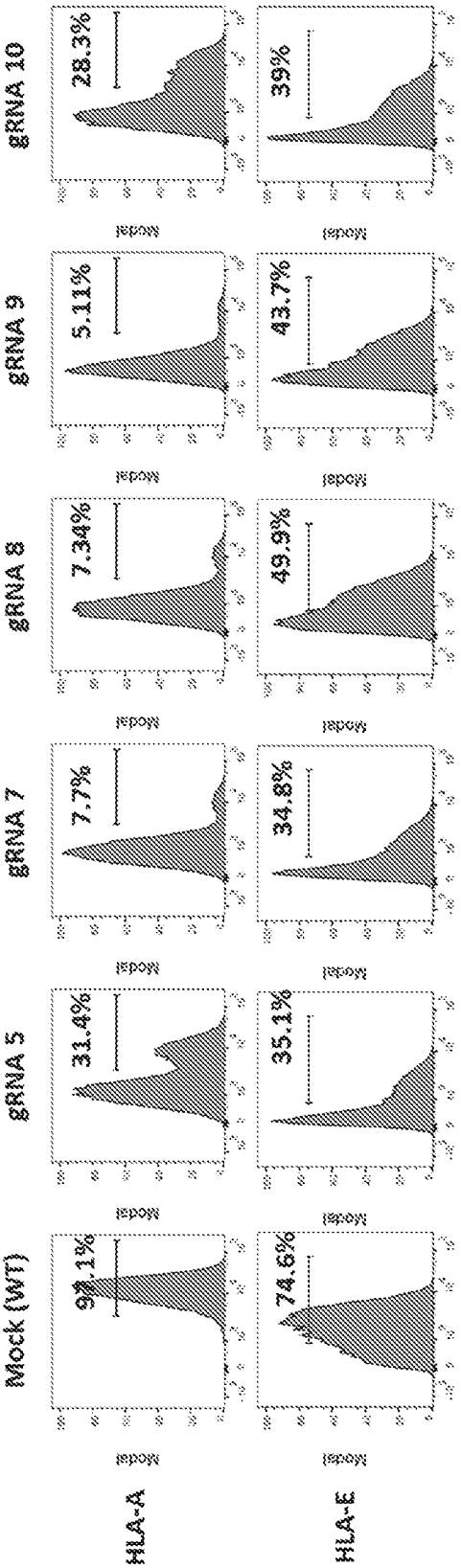

Mock (WT)
gRNA 5
gRNA 7
gRNA 8
gRNA 9
gRNA 10
HLA-A
HLA-E
Modal
31.4%
7.7%
7.34%
5.11%
28.3%
35.1%
34.8%
49.9%
43.7%
39%
74.6%

FIG. 30

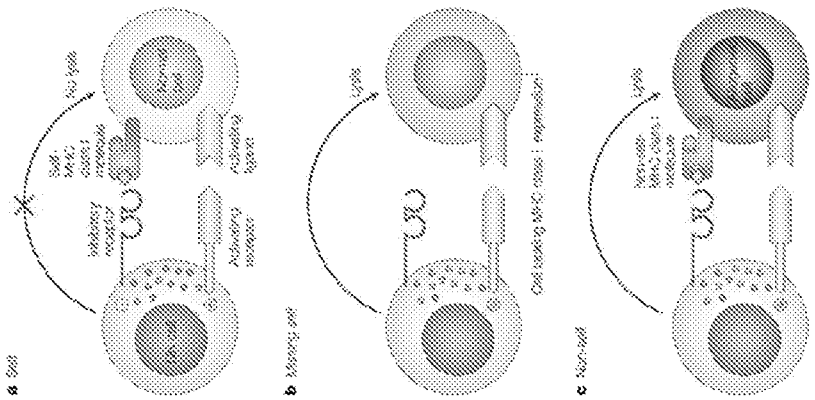


FIG. 31

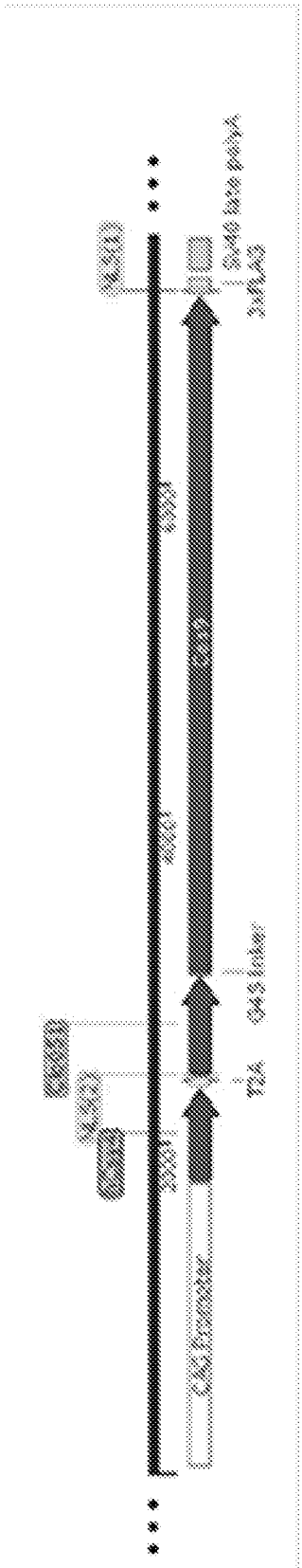


FIG. 32

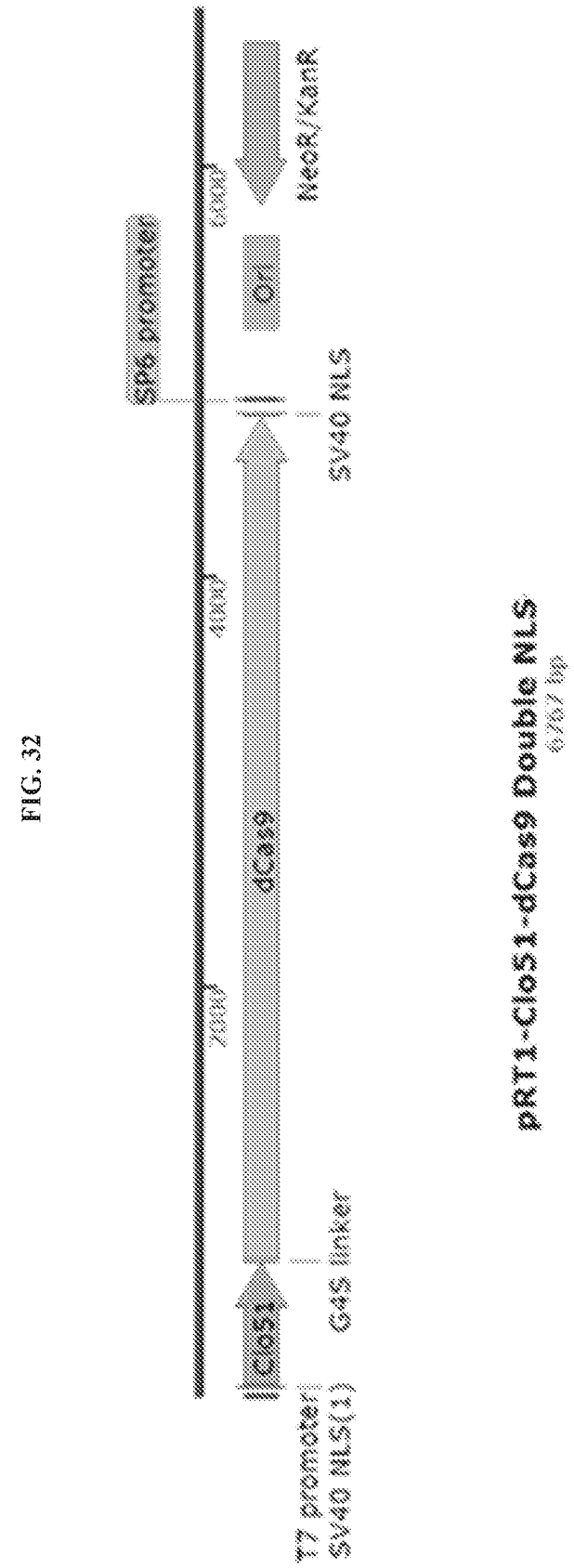

pRT1-Clo51-dCas9 Double NLS
6707 bp
T7 promoter
SV40 NLS(1)
Cas51
G4S linker
dCas9
4002
SV40 NLS
Ori
NeoR/KanR
1422
SP6 promoter
2000

FIG. 33

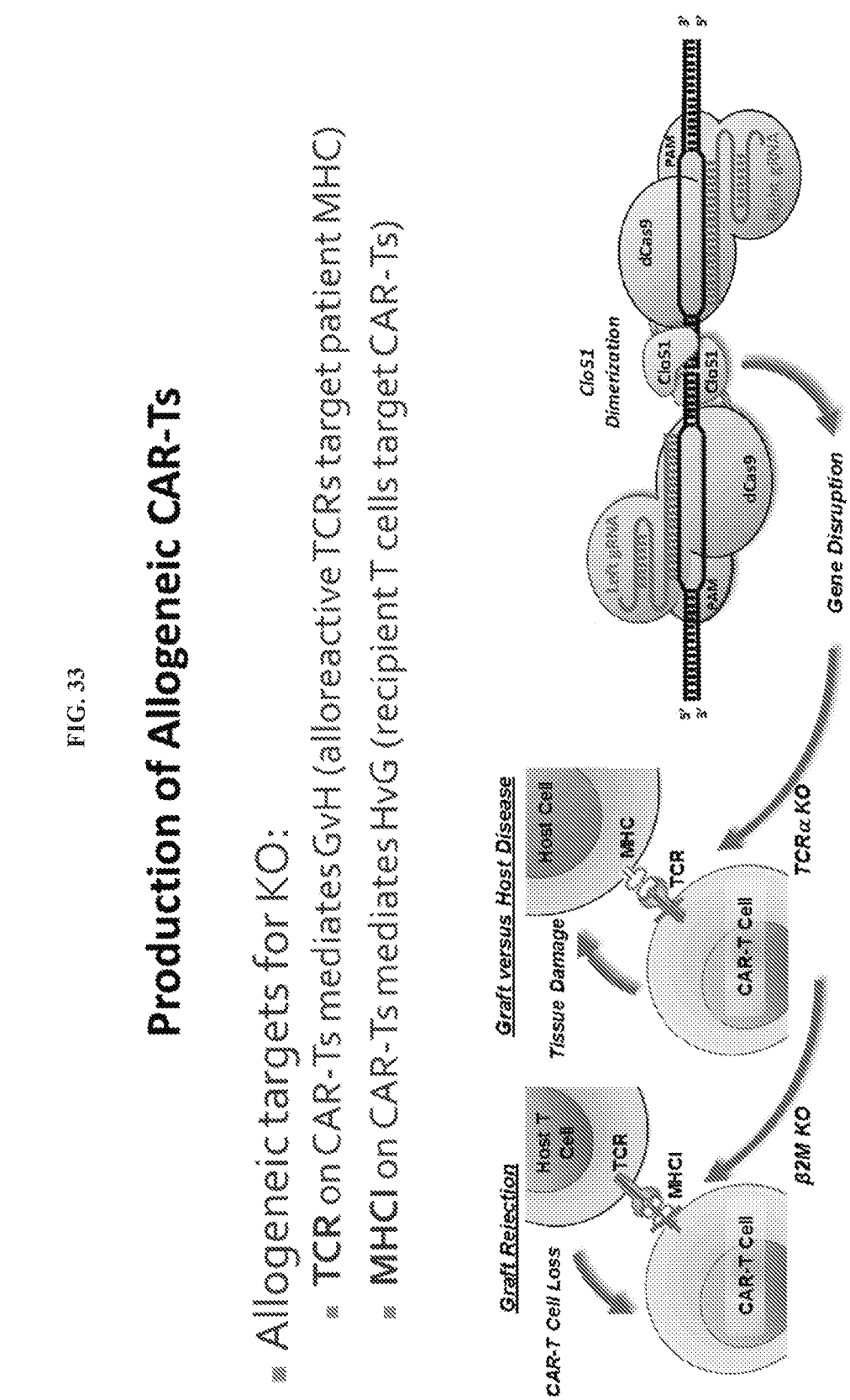
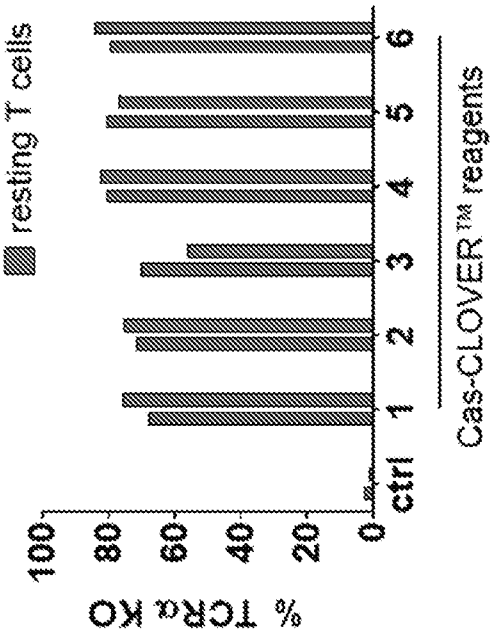

Production of Allogeneic CAR-Ts
Allogeneic targets for KO:
TCR on CAR-Ts mediates GvH (alloreactive TCRs target patient MHC)
MHCI on CAR-Ts mediates HvG (recipient T cells target CAR-Ts)
Graft Reiection
Host T Cell
TCR
MHCI
CAR-T Cell
CAR-T Cell Loss
β2M KO
Graft versus Host Disease
Host Cell
MHC
TCR
CAR-T Cell
Tissue Damage
TCRα KO
ClosI Dimerization
dCas9
ClosI
ClosI
PAM
dCas9
β2M
Gene Disruption

FIG. 34A


Jurkat
resting T cells
% TCRα KO
100
80
60
40
20
0
ctrl
1
2
3
4
5
6
Cas-CLOVER™ reagents

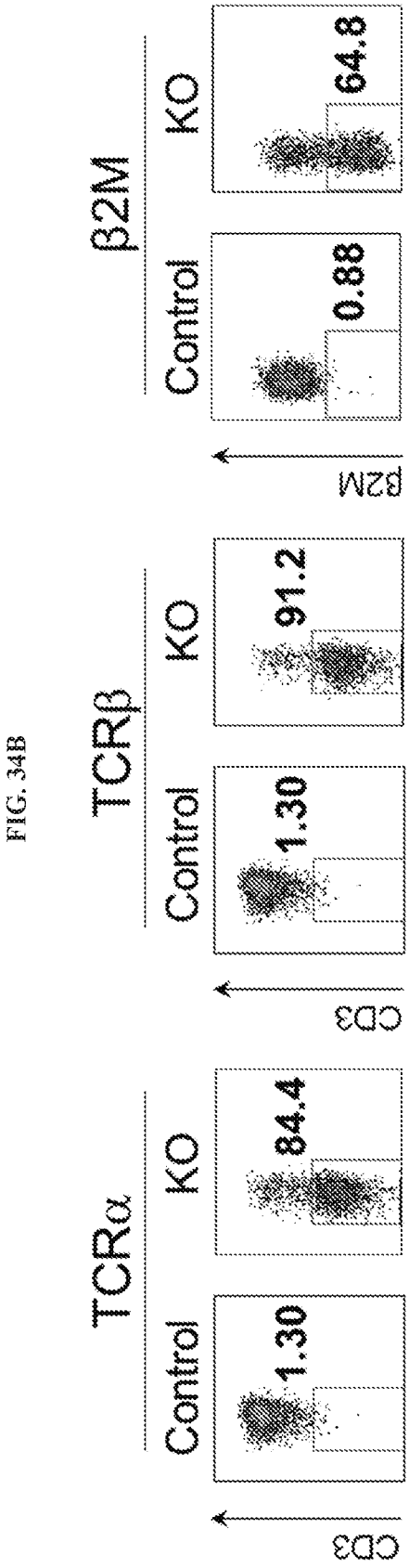

TCRα
Control
KO
1.30
84.4
CD3
TCRβ
Control
KO
1.30
91.2
CD3
β2M
Control
KO
0.88
64.8
β2M

FIG. 34B

FIG. 35

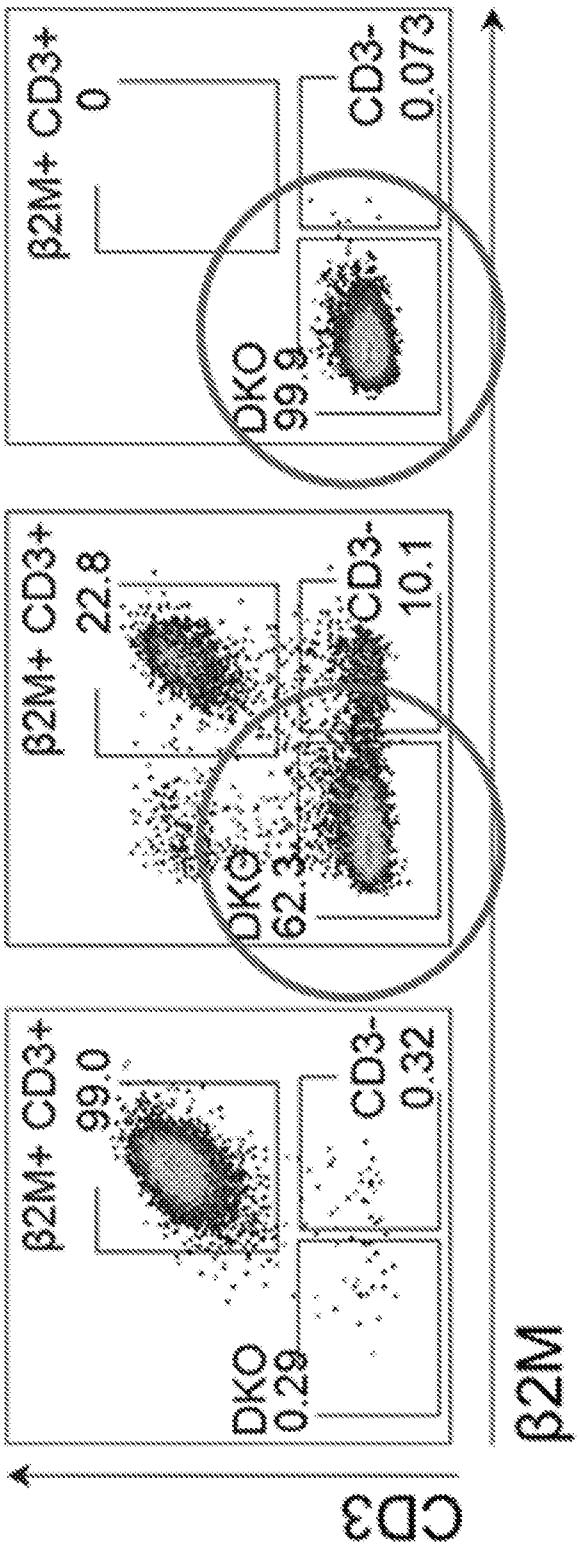

Mock
DKO-non purified
Purified DKO
β2M+ CD3+
99.0
CD3-
0.32
DKO
0.28
β2M+ CD3+
22.8
CD3-
10.1
DKO
62.3
β2M+ CD3+
0
CD3-
0.073
DKO
99.9
CD3
β2M

FIG. 36

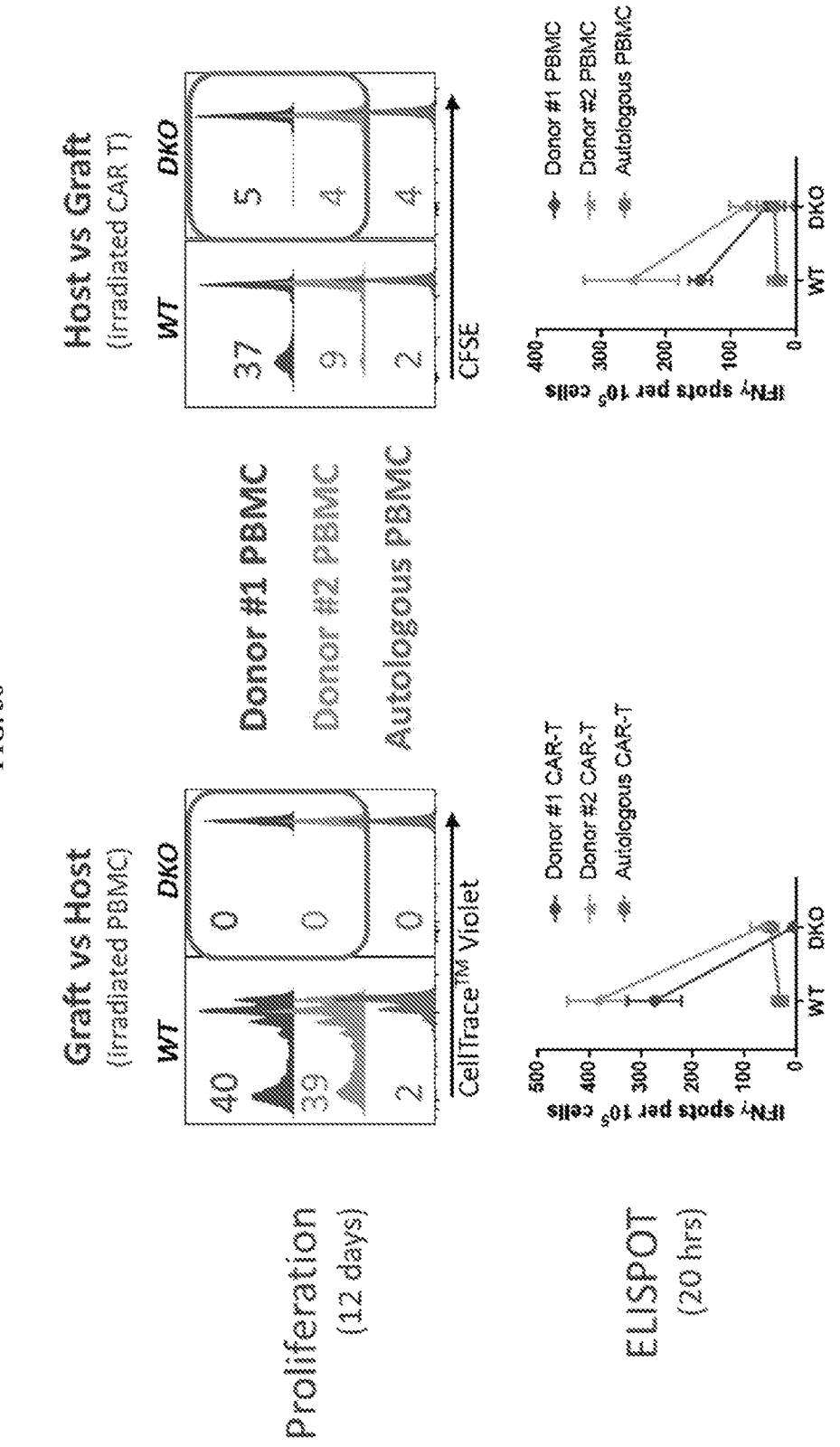

Proliferation
(12 days)
Graft vs Host
(irradiated PBMC)
WT
DKO
40
39
2
0
0
0
CellTrace™ Violet
Donor #1 PBMC
Donor #2 PBMC
Autologous PBMC
Host vs Graft
(irradiated CAR T)
WT
DKO
37
9
2
5
4
4
CFSE
ELISPOT
(20 hrs)
Donor #1 CAR-T
Donor #2 CAR-T
Autologous CAR-T
IFNγ spots per 10^5 cells
500
400
300
200
100
0
WT
DKO
Donor #1 PBMC
Donor #2 PBMC
Autologous PBMC
IFNγ spots per 10^5 cells
400
300
200
100
0
WT
DKO

FIG. 37

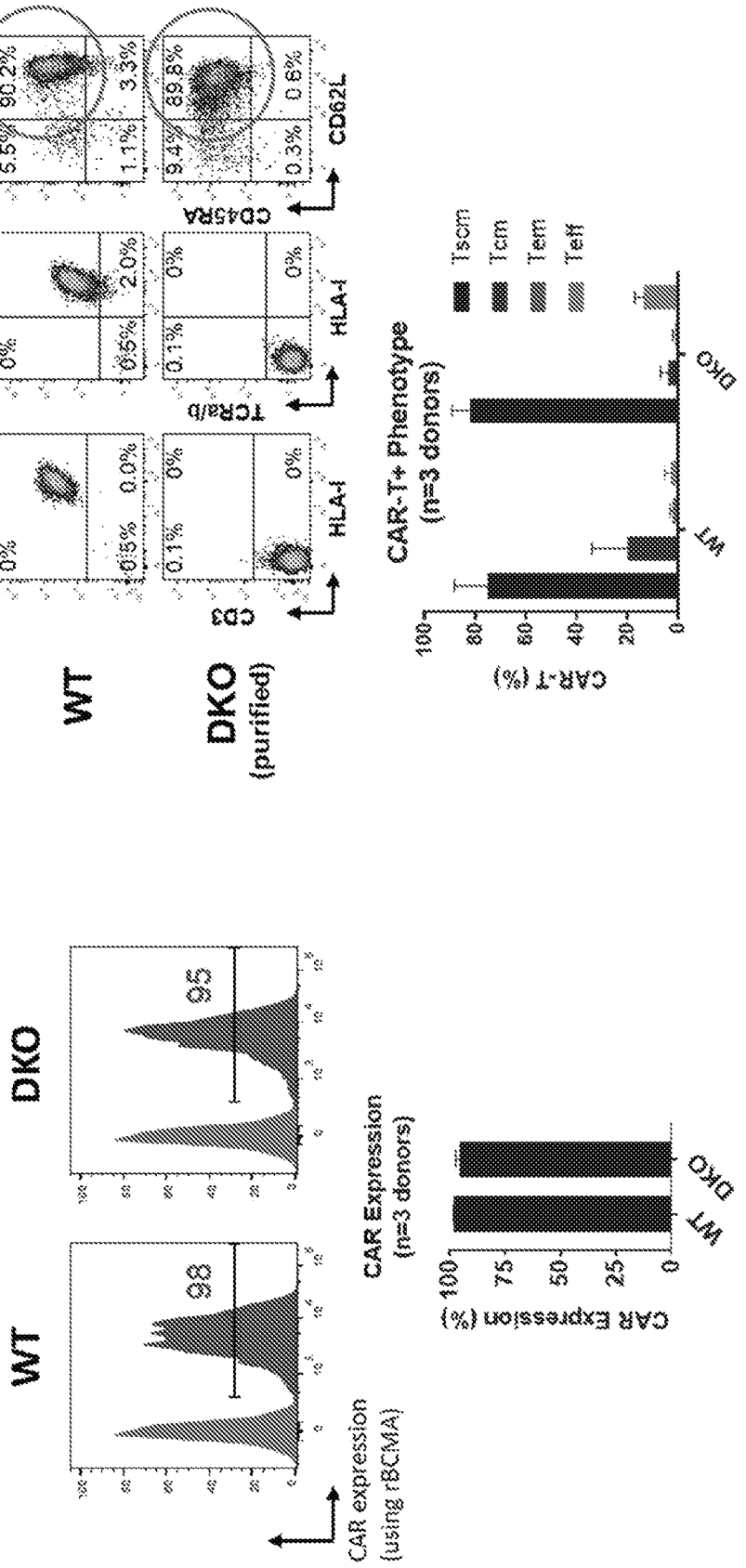

WT
DKO
CAR expression (using rBCMA)
98
95
CAR Expression (n=3 donors)
CAR Expression (%)
100
75
50
25
0
WT
DKO
WT
DKO (purified)
CD3
HLA-I
TCRa/b
HLA-I
CD45RA
CD62L
0%
0.5%
0.0%
0.1%
0%
0%
0%
0.5%
2.0%
0.1%
0%
0%
5.5%
90.2%
1.1%
3.3%
9.4%
89.8%
0.3%
0.6%
CAR-T+ Phenotype (n=3 donors)
Tscm
Tcm
Tem
Teff
CAR-T (%)
100
80
60
40
20
0
WT
DKO

FIG. 38

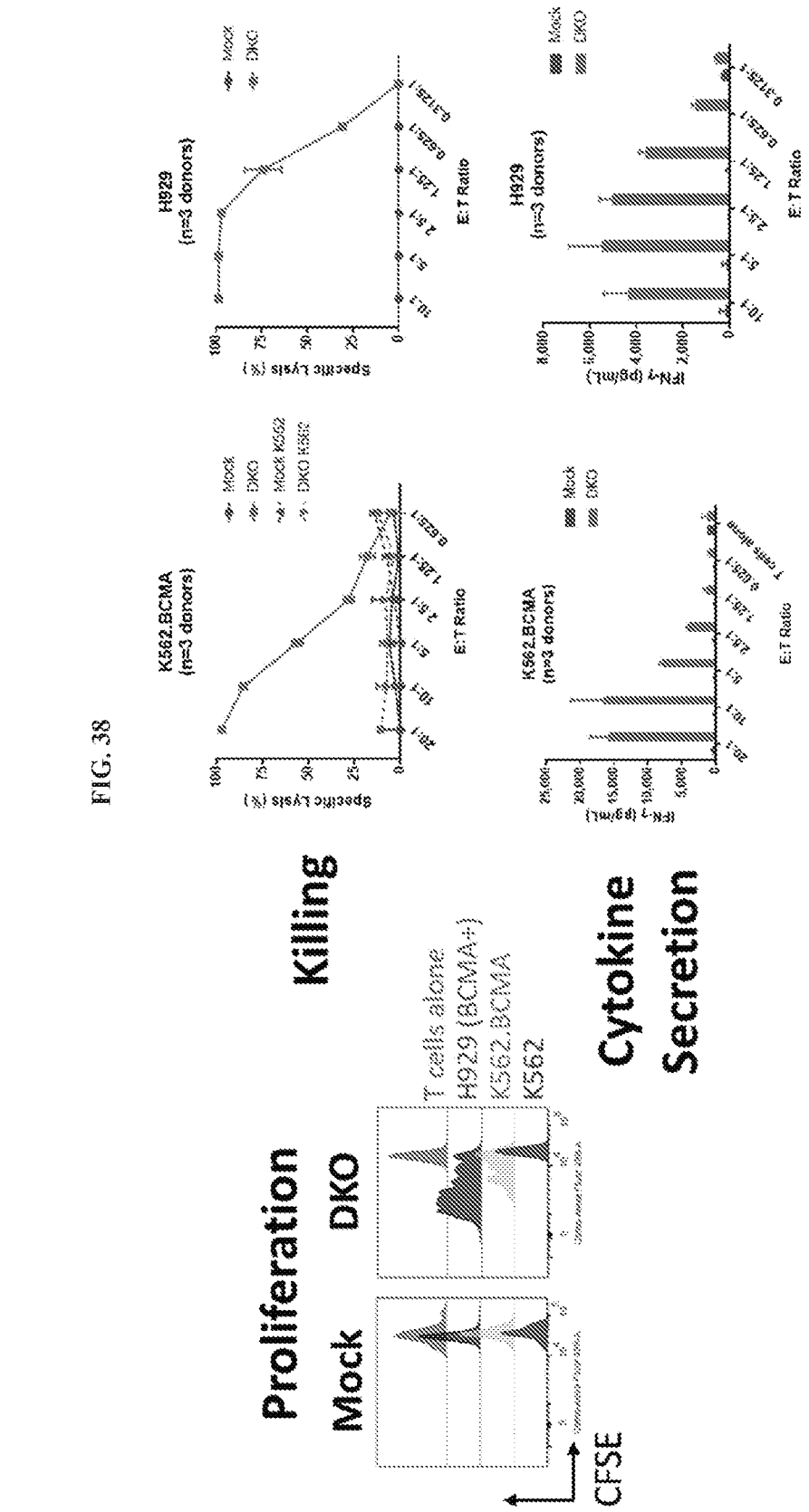
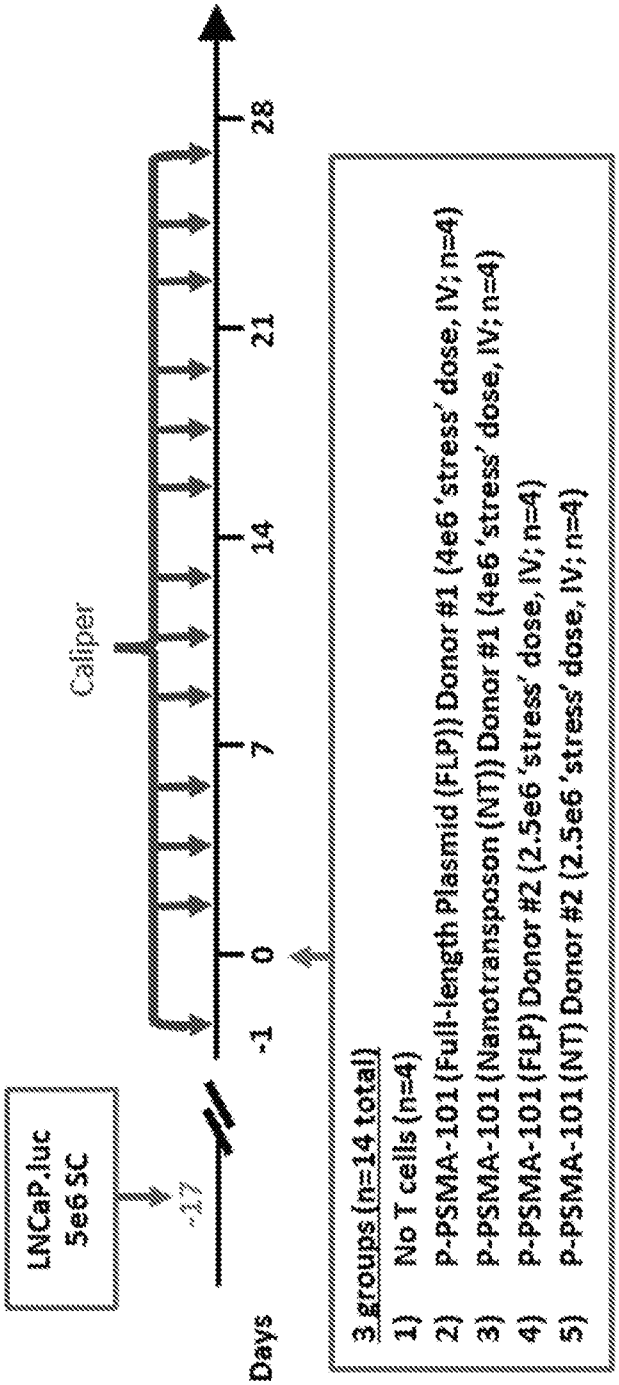
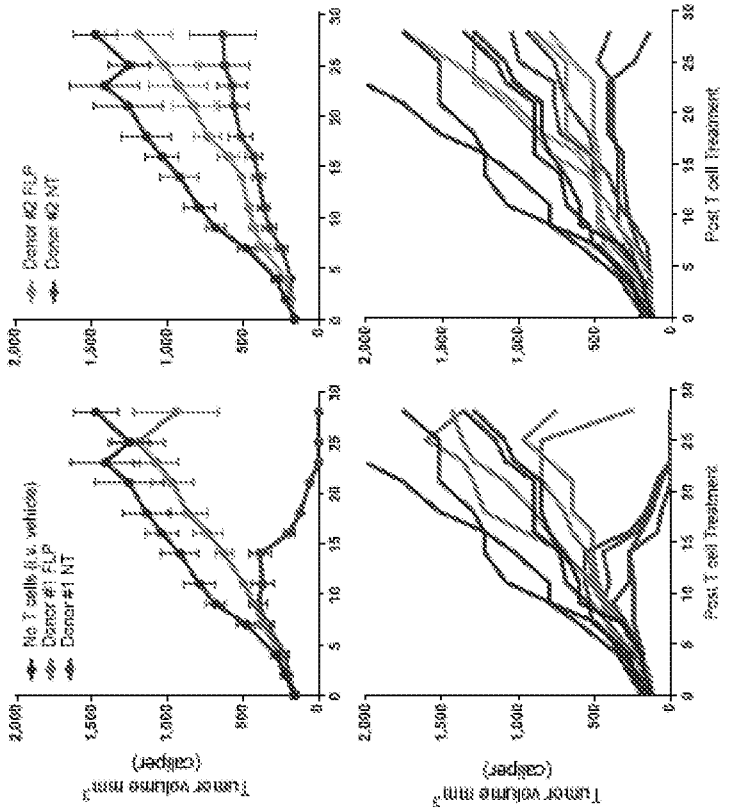

Killing
Proliferation
Mock
DKO
T cells alone
H929 (BCMA+)
K562.BCMA
K562
CFSE
Cytokine Secretion
H929 (n=3 donors)
K562.BCMA (n=3 donors)
Specific Lysis (%)
IFN-γ (pg/mL)
E:T Ratio
Mock
DKO
Mock K562
DKO K562

FIG. 39A



A) Study design

FIG. 39B



B) Caliper Measurements