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(54) **Titre : CELLULES INGENIERISEES POUR UNE THERAPIE**
(54) **Title: ENGINEERED CELLS FOR THERAPY**

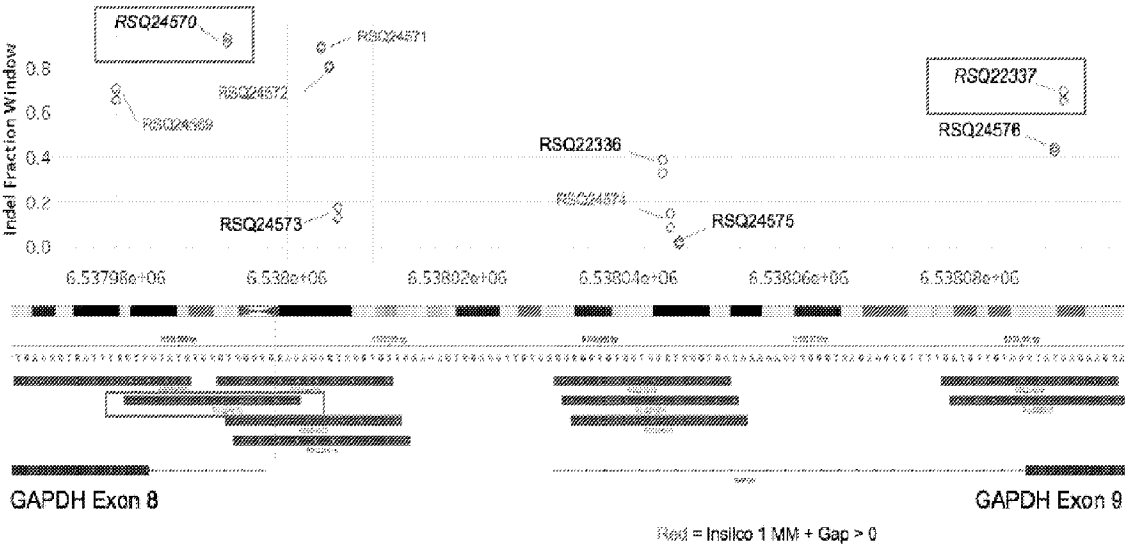


Fig. 1

(57) **Abrégé/Abstract:**
Strategies, systems, compositions, and methods for genetically modifying cells to include one or more loss-of-function modifications and/or to include one or more gain-of-function modifications, as well as modified cells (and compositions of such cells) that include one or more loss-of-function modifications and/or that include one or more gain-of-function modifications, are described. In certain aspects, such modified cells include at least one gain-of-function modification within a coding region of an essential gene.

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Abstract:

Strategies, systems, compositions, and methods for genetically modifying cells to include one or more loss-of-function modifications and/or to include one or more gain-of-function modifications, as well as modified cells (and compositions of such cells) that include one or more loss-of-function modifications and/or that include one or more gain-of-function modifications, are described. In certain aspects, such modified cells include at least one gain-of-function modification within a coding region of an essential gene.

ENGINEERED CELLS FOR THERAPY

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Applications Nos. 63/184,202 filed May 4, 2021, 63/184,453 filed May 5, 2021, 63/228,645 filed August 3, 2021, 63/233,701 filed August 16, 2021, 63/233,690 filed August 16, 2021, 63/233,688 filed August 16, 2021, 63/270,895 filed October 22, 2021, 63/275,269 filed November 3, 2021, 63/297,518 filed January 7, 2022, and 63/321,890 filed March 21, 2022. The entirety of each of the priority applications is incorporated herein by reference.

BACKGROUND

[0002] Various therapeutic approaches for treatment of cancer exist, such as the use of genetically engineered cell therapies. However, engineered cells can exhibit limited tumor cell killing and/or limited persistence. There remains a need for engineered cell therapies for effective treatment of cancer.

SUMMARY

[0003] Some aspects of the present disclosure are based, at least in part, on methods and systems for genetically modifying NK cells and/or pluripotent stem cells (e.g., iPSCs) that are, e.g., differentiated into modified iNK cells, to include one or more gain-of-function modifications (e.g., one or more gain-of-function modifications described herein), and optionally to include one or more loss-of-function modifications (e.g., one or more loss-of-function modifications described herein), as well as modified NK cells and/or modified pluripotent stem cells (e.g., iPSCs) that are, e.g., differentiated into modified iNK cells (and compositions of such cells) that include one or more gain-of-function modifications (e.g., one or more gain-of-function modifications described herein), and optionally that include one or more loss-of-function modifications (e.g., one or more loss-of-function modifications described herein). In certain aspects of the disclosure, such modified NK cells and/or modified pluripotent stem cells (e.g., iPSCs) that are, e.g., differentiated into modified iNK cells, include at least one gain-of-function modification within a coding region of an essential gene (e.g., an essential gene described herein).

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[0004] In one aspect, the disclosure features a Natural Killer (NK) cell (or a progeny or daughter cell of such NK cell, or a population of such NK cells) comprising: (a) one or more genomic edits that results in loss of function of one or more of gene products; and/or (b) a genome comprising an exogenous coding sequence, wherein the exogenous coding sequence is in frame with and downstream (3') of a coding sequence of an essential gene, and wherein at least part of the essential gene comprises an exogenous coding sequence.

[0005] In some embodiments, the one or more genomic edits results in loss of function of one or more of: adenosine A2a receptor (ADORA2A), β-2 microglobulin (B2M), class II major histocompatibility complex transactivator (CIITA), cytokine inducible SH2 containing protein (CISH), two or more human leukocyte antigen (HLA) class II histocompatibility antigen alpha chain genes, two or more HLA class II histocompatibility antigen beta chain genes, natural killer group 2 member A receptor (NKG2A), programmed cell death protein 1 (PD-1), T cell immunoreceptor with Ig and ITIM domains (TIGIT), an agonist of the TGF beta signaling pathway (e.g., transforming growth factor beta receptor II (TGFβRII)), or any combination of two or more thereof.

[0006] In some embodiments, the exogenous coding sequence encodes (i) FcγRIII (CD16) or variant thereof and/or (ii) a membrane bound interleukin 15 (mbIL-15).

[0007] In some embodiments, the genome comprises a first exogenous coding sequence and a second exogenous coding sequence. In some embodiments, the first exogenous coding sequence encodes FcγRIII (CD16) or variant thereof. In some embodiments, the second exogenous coding sequence encodes mbIL-15. In some embodiments, the first exogenous coding sequence encodes FcγRIII (CD16) or variant thereof and the second exogenous coding sequence encodes mbIL-15.

[0008] In some embodiments, the genome comprises: (i) the first exogenous coding sequence and the second exogenous coding sequence at a first allele of the essential gene; and (ii) the first exogenous coding sequence and the second exogenous coding sequence at a second allele of the essential gene.

[0009] In some embodiments, the first exogenous coding sequence is upstream (5') of the second exogenous coding sequence. In some embodiments, the genome comprises: (i) a first regulatory element between the coding sequence of the essential gene and the first exogenous

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coding sequence; and (ii) a second regulatory element between the first exogenous coding sequence and the second exogenous coding sequence. In some embodiments, the first regulatory element is an IRES or 2A element and the second regulatory element is an IRES or 2A element. In some embodiments, the genome comprises a polyadenylation sequence downstream (3') of the second exogenous coding sequence. In some embodiments, the genome comprises a 3' untranslated region (UTR) sequence downstream (3') of the second exogenous coding sequence and upstream (5') of the polyadenylation sequence.

[0010] In some embodiments, the second exogenous coding sequence is upstream (5') of the first exogenous coding sequence. In some embodiments, the genome comprises: (i) a first regulatory element between the coding sequence of the essential gene and the second exogenous coding sequence; and (ii) a second regulatory element between the second exogenous coding sequence and the first exogenous coding sequence. In some embodiments, the first regulatory element is an IRES or 2A element and the second regulatory element is an IRES or 2A element. In some embodiments, the genome comprises a polyadenylation sequence downstream (3') of the first exogenous coding sequence. In some embodiments, the genome comprises a 3' untranslated region (UTR) sequence downstream (3') of the first exogenous coding sequence and upstream (5') of the polyadenylation sequence.

[0011] In some embodiments, the first exogenous coding sequence is or comprises SEQ ID NO: 166. In some embodiments, the second exogenous coding sequence is or comprises SEQ ID NO: 172. In some embodiments, the CD16 is or comprises the amino acid sequence of SEQ ID NO: 184. In some embodiments, the mbIL-15 comprises an IL-15, a linker, a sushi domain, and an IL-15Rα. In some embodiments, the mbIL-15 is or comprises the amino acid sequence of SEQ ID NO: 190.

[0012] In some embodiments, the NK cell is an induced pluripotent stem cell (iPSC)-derived NK (iNK) cell.

[0013] In some embodiments, the essential gene encodes a gene product that is required for survival and/or proliferation of the cell. In some embodiments, the essential gene is a housekeeping gene, e.g., a gene listed in Table 3. In some embodiments, the essential gene encodes glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

[0014] In some embodiments, the NK cell comprises: (i) a genomic edit that results in loss of function of CISH; and (ii) a genomic edit that results in loss of function of TGFβRII.

[0015] In some embodiments, the NK cell is for use as a medicament. In some embodiments, the NK cell is for use in the treatment of a disease, disorder, or condition, e.g., a tumor and/or a cancer.

[0016] In some embodiments, the NK cell or population of NK cells is characterized in that, when contacted with tumor cells, a level of killing of tumor cells by the NK cells is increased (e.g., by at least about 10%, 20%, 40%, 60%, 80%, 100%, 150%, 200%, 300%, or more) relative to a reference level of killing of tumor cells by a reference population of NK cells, e.g., as measured using any known method, e.g., a method described in Example 11 or Example 15.

[0017] In some embodiments, the NK cell or population of NK cells is characterized in that, when contacted with tumor cells and an antibody, a level of antibody-dependent cellular cytotoxicity (ADCC) induced by the NK cells is increased (e.g., by at least about 10%, 20%, 40%, 60%, 80%, 100%, 150%, 200%, 300%, or more) relative to a reference level of ADCC induced by a reference population of NK cells, e.g., as measured using any known method, e.g., a method described in Example 11 or Example 15.

[0018] In some embodiments, a level of persistence of the population of NK cells is increased (e.g., by at least about 10%, 20%, 40%, 60%, 80%, 100%, 150%, 200%, 300%, or more) relative to a reference level of persistence of a reference population of NK cells, e.g., as measured using any known method, e.g., a method described in Example 14 or Example 15. In some embodiments, the level of persistence is measured following contacting with tumor cells.

[0019] In some embodiments, the reference population of NK cells does not comprise NK cells comprising a genome comprising the first exogenous coding sequence and the second exogenous coding sequence. In some embodiments, the reference population of NK cell does not comprise NK cells comprising a genomic edit that results in loss of function of TGFβRII and a genomic edit that results in loss of function of CISH. In some embodiments, the reference population of NK cells does not comprise NK cells comprising a genome comprising the first exogenous coding sequence and the second exogenous coding sequence, and does not comprise

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NK cells comprising a genomic edit that results in loss of function of TGFβRII and a genomic edit that results in loss of function of CISH.

[0020] In some aspects, the disclosure provides a pharmaceutical composition comprising an NK cell, the progeny or daughter cell, or a population of NK cells described herein. In some embodiments, the pharmaceutical composition comprises a pharmaceutically acceptable carrier.

[0021] In another aspect, the disclosure provides methods of treating a condition, disorder, and/or disease, comprising administering to a subject suffering therefrom an NK cell, a progeny or daughter cell, or a population of NK cells described herein, or a pharmaceutical composition described herein. In some embodiments, the subject is suffering from a tumor, e.g., a solid tumor. In some embodiments, the subject is suffering from a cancer.

[0022] In some embodiments, the NK cell, the progeny or daughter cell, or the population of NK cells is allogenic to the subject. In some embodiments, the NK cell, the progeny or daughter cell, or the population of NK cells is autologous to the subject. In some embodiments, the method further comprises administering an antibody to the subject. In some embodiments, the antibody is trastuzumab, rituximab, or cetuximab. In some embodiments, the subject is a human.

[0023] In another aspect, the disclosure features a method, comprising administering to a subject an NK cell, a progeny or daughter cell, or a population of NK cells described herein, or a pharmaceutical composition described herein. In some embodiments, the subject is suffering from a tumor, e.g., a solid tumor. In some embodiments, the subject is suffering from a cancer.

[0024] In some embodiments, the NK cell, the progeny or daughter cell, or the population of NK cells is allogenic to the subject. In some embodiments, the NK cell, the progeny or daughter cell, or the population of NK cells is autologous to the subject. In some embodiments, the method further comprises administering an antibody to the subject. In some embodiments, the antibody is trastuzumab, rituximab, or cetuximab. In some embodiments, the subject is a human.

[0025] In another aspect, the disclosure provides a method of increasing tumor killing ability of a NK cell, the method comprising: (a) knocking-into the genome of the NK cell a first exogenous coding sequence for FcγRIII (CD16) or variant thereof and a second exogenous

coding sequence for a membrane bound interleukin 15 (mbIL-15), wherein the first exogenous coding sequence and the second exogenous coding sequence are knocked-in in frame and downstream (3') of an essential gene; and (b) knocking-out one or more genes of the NK cell, wherein the one or more genes encode adenosine A2a receptor (ADORA2A), β-2 microglobulin (B2M), class II major histocompatibility complex transactivator (CIITA), cytokine inducible SH2 containing protein (CISH), two or more human leukocyte antigen (HLA) class II histocompatibility antigen alpha chain genes, two or more HLA class II histocompatibility antigen beta chain genes, natural killer group 2 member A receptor (NKG2A), programmed cell death protein 1 (PD-1), T cell immunoreceptor with Ig and ITIM domains (TIGIT), an agonist of the TGF beta signaling pathway (e.g., transforming growth factor beta receptor II (TGFβRII)), or any combination of two or more thereof; thereby increasing a level of tumor killing activity of the NK cell (e.g., by at least about 10%, 20%, 40%, 60%, 80%, 100%, 150%, 200%, 300%, or more) relative to a reference level of tumor killing activity of a reference NK cell, e.g., as measured using any known method, e.g., a method described in Example 11 or Example 15.

[0026] In some embodiments, the reference NK cell does not comprise a genome comprising the first exogenous coding sequence and the second exogenous coding sequence. In some embodiments, the reference NK cell does not comprise a genomic edit that results in loss of function of TGFβRII and a genomic edit that results in loss of function of CISH. In some embodiments, the reference NK cell does not comprise a genome comprising the first exogenous coding sequence and the second exogenous coding sequence, and does not comprise a genomic edit that results in loss of function of TGFβRII and a genomic edit that results in loss of function of CISH.

[0027] In another aspect, the disclosure provides a method of increasing antibody-dependent cellular cytotoxicity (ADCC) induced by a NK cell, the method comprising: (a) knocking-into the genome of the NK cell a first exogenous coding sequence for FcγRIII (CD16) or variant thereof and a second exogenous coding sequence for a membrane bound interleukin 15 (mbIL-15), wherein the first exogenous coding sequence and the second exogenous coding sequence are knocked-in in frame and downstream (3') of an essential gene; and (b) knocking-out one or more genes of the NK cell, wherein the one or more genes encode adenosine A2a receptor (ADORA2A), β-2 microglobulin (B2M), class II major histocompatibility complex

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transactivator (CIITA), cytokine inducible SH2 containing protein (CISH), two or more human leukocyte antigen (HLA) class II histocompatibility antigen alpha chain genes, two or more HLA class II histocompatibility antigen beta chain genes, natural killer group 2 member A receptor (NKG2A), programmed cell death protein 1 (PD-1), T cell immunoreceptor with Ig and ITIM domains (TIGIT), an agonist of the TGF beta signaling pathway (e.g., transforming growth factor beta receptor II (TGFβRII)), or any combination of two or more thereof; thereby increasing a level of ADCC induced by the NK cell (e.g., by at least about 10%, 20%, 40%, 60%, 80%, 100%, 150%, 200%, 300%, or more) relative to a reference level of ADCC induced by a reference NK cell, e.g., as measured using any known method, e.g., a method described in Example 11 or Example 15.

[0028] In some embodiments, the reference NK cell does not comprise a genome comprising the first exogenous coding sequence and the second exogenous coding sequence. In some embodiments, the reference NK cell does not comprise a genomic edit that results in loss of function of TGFβRII and a genomic edit that results in loss of function of CISH. In some embodiments, the reference NK cell does not comprise a genome comprising the first exogenous coding sequence and the second exogenous coding sequence, and does not comprise a genomic edit that results in loss of function of TGFβRII and a genomic edit that results in loss of function of CISH.

[0029] In another aspect, the disclosure provides a method of increasing persistence of a NK cell, the method comprising: (a) knocking-into the genome of the NK cell a first exogenous coding sequence for FcγRIII (CD16) or variant thereof and a second exogenous coding sequence for a membrane bound interleukin 15 (mbIL-15), wherein the first exogenous coding sequence and the second exogenous coding sequence are knocked-in in frame and downstream (3') of an essential gene; and (b) knocking-out one or more genes of the NK cell, wherein the one or more genes encode adenosine A2a receptor (ADORA2A), β-2 microglobulin (B2M), class II major histocompatibility complex transactivator (CIITA), cytokine inducible SH2 containing protein (CISH), two or more human leukocyte antigen (HLA) class II histocompatibility antigen alpha chain genes, two or more HLA class II histocompatibility antigen beta chain genes, natural killer group 2 member A receptor (NKG2A), programmed cell death protein 1 (PD-1), T cell immunoreceptor with Ig and ITIM domains (TIGIT), an agonist of the TGF beta signaling

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pathway (e.g., transforming growth factor beta receptor II (TGFβRII)), or any combination of two or more thereof; thereby increasing a level of persistence of the NK cell (e.g., by at least about 10%, 20%, 40%, 60%, 80%, 100%, 150%, 200%, 300%, or more) relative to a reference level of persistence of a reference NK cell, e.g., as measured using any known method, e.g., a method described in Example 14 or Example 15. In some embodiments, the level of persistence is measured following contacting the NK cell with tumor cells.

[0030] In some embodiments, the reference NK cell does not comprise a genome comprising the first exogenous coding sequence and the second exogenous coding sequence. In some embodiments, the reference NK cell does not comprise a genomic edit that results in loss of function of TGFβRII and a genomic edit that results in loss of function of CISH. In some embodiments, the reference NK cell does not comprise a genome comprising the first exogenous coding sequence and the second exogenous coding sequence, and does not comprise a genomic edit that results in loss of function of TGFβRII and a genomic edit that results in loss of function of CISH.

[0031] In another aspect, the disclosure features a method of manufacturing a genetically modified NK cell, the method comprising: (a) knocking-into the genome of an NK cell a first exogenous coding sequence for FcγRIII (CD16) or variant thereof and a second exogenous coding sequence for a membrane bound interleukin 15 (mbIL-15), wherein the first exogenous coding sequence and the second exogenous coding sequence are knocked-in in frame and downstream (3') of an essential gene; and (b) knocking-out one or more genes of the NK cell, wherein the one or more genes encode adenosine A2a receptor (ADORA2A), β-2 microglobulin (B2M), class II major histocompatibility complex transactivator (CIITA), cytokine inducible SH2 containing protein (CISH), two or more human leukocyte antigen (HLA) class II histocompatibility antigen alpha chain genes, two or more HLA class II histocompatibility antigen beta chain genes, natural killer group 2 member A receptor (NKG2A), programmed cell death protein 1 (PD-1), T cell immunoreceptor with Ig and ITIM domains (TIGIT), an agonist of the TGF beta signaling pathway (e.g., transforming growth factor beta receptor II (TGFβRII)), or any combination of two or more thereof.

[0032] In some embodiments, knocking-in comprises contacting the NK cell with: (i) a nuclease that causes a break within an endogenous coding sequence of the essential gene, and (ii)

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a donor template that comprises a knock-in cassette comprising the first exogenous coding sequence and the second exogenous coding sequence in frame with and downstream (3 ') of an exogenous coding sequence or partial coding sequence of the essential gene, wherein the knock-in cassette is integrated into the genome of the cell by homology-directed repair (HDR) of the break.

[0033] In some embodiments, the nuclease is a CRISPR/Cas nuclease and knocking-in further comprises contacting the NK cell with a guide molecule for the CRISPR/Cas nuclease.

[0034] In some embodiments, knocking-out comprises contacting the NK cell with one or more nucleases that cause a break within an endogenous coding sequence of the one or more genes. In some embodiments, the one or more nucleases are CRISPR/Cas nucleases and knocking-out further comprises contacting the NK cell with one or more guide molecules for the CRISPR/Cas nuclease.

[0035] In some embodiments, the NK cell is an induced pluripotent stem cell (iPSC)-derived NK (iNK) cell.

[0036] In some embodiments, the essential gene encodes a gene product that is required for survival and/or proliferation of the NK cell. In some embodiments, the essential gene is a housekeeping gene, e.g., a gene listed in Table 3. In some embodiments, the essential gene encodes glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

[0037] In some embodiments, the method comprises knocking-out a gene encoding CISH and knocking-out a gene encoding TGFβRII.

[0038] In one aspect, the disclosure features an NK cell, a pluripotent human stem cell, or a modified iNK cell differentiated from such stem cell, wherein the cell comprises: (i) one or more genomic edits that results in loss of function of one or more of adenosine A2a receptor (ADORA2A), β-2 microglobulin (B2M), class II major histocompatibility complex transactivator (CIITA), cytokine inducible SH2 containing protein (CISH), two or more human leukocyte antigen (HLA) class II histocompatibility antigen alpha chain genes, two or more HLA class II histocompatibility antigen beta chain genes, natural killer group 2 member A receptor (NKG2A), programmed cell death protein 1 (PD-1), T cell immunoreceptor with Ig and ITIM domains (TIGIT), an agonist of the TGF beta signaling pathway (e.g., transforming growth factor beta receptor II (TGFβRII)), or any combination of two or more thereof; and (ii) a genome

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comprising a first exogenous coding sequence for FcγRIII (CD16) or variant thereof and a second exogenous coding sequence for a membrane bound interleukin 15 (mbIL-15), wherein the first exogenous coding sequence and second exogenous coding sequence are in frame with and downstream (3') of a coding sequence of an essential gene, e.g., the GAPDH gene, wherein at least part of the coding sequence of the essential gene, e.g., the GAPDH gene comprises an exogenous coding sequence.

[0039] In one aspect, the disclosure features an NK cell, a pluripotent human stem cell, or a modified iNK cell differentiated from such stem cell, wherein the cell comprises: (i) a genome a first exogenous coding sequence for FcγRIII (CD16) or variant thereof and a second exogenous coding sequence for a membrane bound interleukin 15 (mbIL-15), wherein the first exogenous coding sequence and second exogenous coding sequence are in frame with and downstream (3') of a coding sequence of an essential gene, e.g., the GAPDH gene, wherein at least part of the coding sequence of the essential gene, e.g., the GAPDH gene, comprises an exogenous coding sequence,; and wherein the cell comprises (ii) one or more genomic edits that results in loss of function of one or more of adenosine A2a receptor (ADORA2A), β-2 microglobulin (B2M), class II major histocompatibility complex transactivator (CIITA), cytokine inducible SH2 containing protein (CISH), two or more human leukocyte antigen (HLA) class II histocompatibility antigen alpha chain genes, two or more HLA class II histocompatibility antigen beta chain genes, natural killer group 2 member A receptor (NKG2A), programmed cell death protein 1 (PD-1), T cell immunoreceptor with Ig and ITIM domains (TIGIT), an agonist of the TGF beta signaling pathway (e.g., transforming growth factor beta receptor II (TGFβRII)), or any combination of two or more thereof.

[0040] In some embodiments, the cell comprises a genomic edit that results in a loss of function of an agonist of the TGF beta signaling pathway and a genomic edit that results in a loss of function of CISH.

[0041] In some embodiments, the cell comprises a genomic edit that results in a loss of function of a TGF beta receptor or a dominant-negative variant of a TGF beta receptor. In some embodiments, the TGF beta receptor is a TGF beta receptor II (TGFβRII).

[0042] In some embodiments, the cell expresses one or more pluripotency markers selected from the group consisting of SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, TRA-2-49/6E, ALP, Sox2, E-cadherin, UTF-1, Oct4, Rex1, and Nanog.

[0043] In some embodiments, the exogenous coding sequence of the GAPDH gene comprises about 2000, 1500, 1000, 750, 500, 400, 300, 200, 100, or 50 base pairs of the coding sequence of the GAPDH gene. In some embodiments, the exogenous coding sequence of the GAPDH gene comprises about 200 base pairs of the coding sequence of the GAPDH gene.

[0044] In some embodiments, the exogenous coding sequence of the GAPDH gene encodes a C-terminal fragment of a protein encoded by the GAPDH gene. In some embodiments, the C-terminal fragment is less than about 500, 250, 150, 125, 100, 75, 50, 25, 20, 15 or 10 amino acids in length. In some embodiments, the C-terminal fragment is less than about 25 amino acids in length. In some embodiments, the C-terminal fragment includes an amino acid sequence that is encoded by a region of the endogenous coding sequence of the GAPDH gene that spans the break.

[0045] In some embodiments, the exogenous coding sequence of the GAPDH gene is less than 100% identical to the corresponding endogenous coding sequence of the GAPDH gene of the cell. In some embodiments, the exogenous coding sequence of the GAPDH gene has been codon optimized relative to the corresponding endogenous coding sequence of the GAPDH gene of the cell to remove a target site of a nuclease, e.g., a Cas. In some embodiments, the nuclease is a Cas (e.g., Cas9, Cas12a, Cas12b, Cas12c, Cas12e, CasX, or CasΦ (Cas12j), or variants thereof), the exogenous coding sequence of the GAPDH gene includes at least one PAM site for the Cas, and the at least one PAM site (or all PAM sites) has been codon optimized or saturated with silent and/or missense mutations.

[0046] In some embodiments, the cell's genome comprises a regulatory element that enables expression of the gene product encoded by the GAPDH gene and the first and second exogenous coding sequences as separate gene products, optionally, wherein at least one of the gene products is a protein and the regulatory element enables expression of that protein separate from the other gene product. In some embodiments, the cell's genome comprises an IRES or 2A element located between the coding sequence of the GAPDH gene and the first exogenous

coding sequence, and/or between the first exogenous coding sequence and the second exogenous coding sequence.

[0047] In some embodiments, the cell's genome does not comprise a reporter gene, e.g., a fluorescent reporter gene or an antibiotic resistance gene.

[0048] In another aspect, the disclosure features an NK cell, a pluripotent human stem cell, or an iNK cell differentiated from such stem cell, comprising a genomic modification, wherein the modification comprises: (i) a genomic edit that results in loss of function of Cytokine Inducible SH2 Containing Protein (CISH) and (ii) a genomic edit that results in a loss of function of an agonist of the TGF beta signaling pathway; and (iii) an insertion of an exogenous knock-in cassette within an endogenous coding sequence of a GAPDH gene in the cell's genome, wherein the knock-in cassette comprises a first exogenous coding sequence for FcγRIII (CD16) or variant thereof and a second exogenous coding sequence for a membrane bound interleukin 15 (mbIL-15), wherein the first exogenous coding sequence and second exogenous coding sequence are in frame with and downstream (3') of an exogenous coding sequence or partial coding sequence encoding GAPDH, or a functional variant thereof, wherein the cell expresses FcγRIII (CD16) or variant thereof, mbIL-15, and GAPDH, or a functional variant thereof, optionally wherein FcγRIII (CD16) or variant thereof, mbIL-15, and GAPDH are expressed from the endogenous GAPDH promoter.

[0049] In another aspect, the disclosure features an NK cell, a pluripotent human stem cell, or an iNK cell differentiated from such stem cell, comprising a genomic modification, wherein the modification comprises: (i) an insertion of an exogenous knock-in cassette within an endogenous coding sequence of a GAPDH gene in the cell's genome, wherein the knock-in cassette comprises a first exogenous coding sequence for FcγRIII (CD16) or variant thereof and a second exogenous coding sequence for a membrane bound interleukin 15 (mbIL-15), wherein the first exogenous coding sequence and second exogenous coding sequence are in frame with and downstream (3') of an exogenous coding sequence or partial coding sequence encoding GAPDH, or a functional variant thereof, wherein the cell expresses FcγRIII (CD16) or variant thereof, mbIL-15, and GAPDH, or a functional variant thereof, optionally wherein FcγRIII (CD16) or variant thereof, mbIL-15, and GAPDH are expressed from the endogenous GAPDH promoter, and wherein the NK cell, pluripotent human stem cell, or iNK cell differentiated from

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such a stem cell further comprises (ii) one or more genomic edits that results in loss of function of one or more of adenosine A2a receptor (ADORA2A), β-2 microglobulin (B2M), class II major histocompatibility complex transactivator (CIITA), cytokine inducible SH2 containing protein (CISH), two or more human leukocyte antigen (HLA) class II histocompatibility antigen alpha chain genes, two or more HLA class II histocompatibility antigen beta chain genes, natural killer group 2 member A receptor (NKG2A), programmed cell death protein 1 (PD-1), T cell immunoreceptor with Ig and ITIM domains (TIGIT), an agonist of the TGF beta signaling pathway (e.g., transforming growth factor beta receptor II (TGFβRII)), or any combination of two or more thereof.

[0050] In some embodiments, the cell comprises a genomic edit that results in a loss of function of an agonist of the TGF beta signaling pathway and a genomic edit that results in a loss of function of CISH.

[0051] In some embodiments, the cell comprises a genomic edit that results in a loss of function of a TGF beta receptor or a dominant-negative variant of a TGF beta receptor. In some embodiments, the TGF beta receptor is a TGF beta receptor II (TGFβRII).

[0052] In some embodiments, the cell expresses one or more pluripotency markers selected from the group consisting of SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, TRA-2-49/6E, ALP, Sox2, E-cadherin, UTF-1, Oct4, Rex1, and Nanog.

[0053] In some embodiments, the exogenous coding sequence or partial coding sequence encoding GAPDH comprises about 2000, 1500, 1000, 750, 500, 400, 300, 200, 100, or 50 base pairs of the coding sequence of the GAPDH gene. In some embodiments, the exogenous coding sequence or partial coding sequence encoding GAPDH comprises about 200 base pairs of the coding sequence of the GAPDH gene.

[0054] In some embodiments, the exogenous coding sequence or partial coding sequence encoding GAPDH encodes a C-terminal fragment of GAPDH. In some embodiments, the C-terminal fragment is less than about 500, 250, 150, 125, 100, 75, 50, 25, 20, 15 or 10 amino acids in length. In some embodiments, the C-terminal fragment is less than about 25 amino acids in length. In some embodiments, the C-terminal fragment includes an amino acid sequence that is encoded by a region of the endogenous coding sequence of the GAPDH gene that spans the break.

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[0055] In some embodiments, the exogenous coding sequence or partial coding sequence encoding GAPDH is less than 100% identical to the corresponding endogenous coding sequence of the GAPDH gene of the cell. In some embodiments, the exogenous coding sequence or partial coding sequence encoding GAPDH has been codon optimized relative to the corresponding endogenous coding sequence of the GAPDH gene of the cell to remove a target site of a nuclease, e.g., a Cas. In some embodiments, the nuclease is a Cas (e.g., Cas9, Cas12a, Cas12b, Cas12c, Cas12e, CasX, CasΦ (Cas12j)), or a variant thereof), the exogenous coding sequence or partial coding sequence encoding GAPDH includes at least one PAM site for the Cas, and the at least one PAM site (or all PAM sites) has been codon optimized or saturated with silent and/or missense mutations.

[0056] In some embodiments, the cell's genome comprises a regulatory element that enables expression of the gene product encoded by the GAPDH gene and the first and second exogenous coding sequences as separate gene products, optionally, wherein at least one of the gene products is a protein and the regulatory element enables expression of that protein separate from the other gene product. In some embodiments, the cell's genome comprises an IRES or 2A element located between the coding sequence of the GAPDH gene and the first exogenous coding sequence and/or between the first exogenous coding sequence and the second exogenous coding sequence.

[0057] In some embodiments, the first exogenous coding sequence is upstream (5') of the second exogenous coding sequence, and the cell's genome comprises a polyadenylation sequence, and optionally a 3' UTR sequence, downstream of the second exogenous coding sequence, and, if a 3'UTR sequence is present, the 3'UTR sequence is positioned 3' of the second exogenous coding sequence and 5' of the polyadenylation sequence.

[0058] In some embodiments, the second exogenous coding sequence is upstream (5') of the first exogenous coding sequence, and the cell's genome comprises a polyadenylation sequence, and optionally a 3' UTR sequence, downstream of the first exogenous coding sequence, and, if a 3'UTR sequence is present, the 3'UTR sequence is positioned 3' of the first exogenous coding sequence and 5' of the polyadenylation sequence.

[0059] In some embodiments, the cell's genome does not comprise a reporter gene, e.g., a fluorescent reporter gene or an antibiotic resistance gene.

[0060] In some embodiments, the knock-in cassette comprises the first exogenous coding sequence, a linker (e.g., T2A, P2A, and/or IRES), and the second exogenous coding sequence. In some embodiments, the genome-edited cell comprises (i) knock-in cassettes at one or both alleles of the GAPDH gene; and (ii) one or more loss-of-function modifications at one or both alleles. In some embodiments, the genome-edited cell expresses FcγRIII (CD16) or variant thereof, mbIL-15, and GAPDH, or a functional variant thereof.

[0061] In some embodiments, the engineered cell comprises (i) one or more loss-of-function modifications at one or both alleles (e.g., at least one genomic edit that results in a loss of function of at least one of: CISH; TGF beta signaling pathway; ADORA2A; T cell immunoreceptor with Ig and ITIM domains (TIGIT); β-2 microglobulin (B2M); programmed cell death protein 1 (PD-1); class II, major histocompatibility complex, transactivator (CIITA); natural killer cell receptor NKG2A (natural killer group 2A); two or more HLA class II histocompatibility antigen alpha chain genes, and/or two or more HLA class II histocompatibility antigen beta chain genes; cluster of differentiation 32B (CD32B, FCGR2B); T cell receptor alpha constant (TRAC); or any combination of two or more thereof) and (ii) multi-cistronic knock-ins (e.g., at one or both alleles of GAPDH gene) of coding sequence for FcγRIII (CD16) or variant thereof and coding sequence for mbIL-15.

[0062] In some embodiments, the engineered cell comprises (i) one or more loss-of-function modifications at one or both alleles (e.g., at least one genomic edit that results in a loss of function of at least one of: CISH; TGF beta signaling pathway; ADORA2A; T cell immunoreceptor with Ig and ITIM domains (TIGIT); β-2 microglobulin (B2M); programmed cell death protein 1 (PD-1); class II, major histocompatibility complex, transactivator (CIITA); natural killer cell receptor NKG2A (natural killer group 2A); two or more HLA class II histocompatibility antigen alpha chain genes, and/or two or more HLA class II histocompatibility antigen beta chain genes; cluster of differentiation 32B (CD32B, FCGR2B); T cell receptor alpha constant (TRAC); or any combination of two or more thereof); and (ii) bi-allelic knock-ins (e.g.,

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the first exogenous coding sequence at a first allele of GAPDH gene, and the second exogenous coding sequence at a second allele of GAPDH gene).

[0063] In some embodiments, the disclosure features a differentiated iNK cell, wherein the differentiated iNK cell is a daughter cell of a pluripotent human stem cell described herein. In some embodiments, the cell does not express endogenous CD3, CD4, and/or CD8.

[0064] In some embodiments, a genomic edit resulting in loss of function of CISH in any of the cells described herein was produced using a guide RNA comprising a targeting domain sequence comprising or consisting of the nucleotide sequence according to any one of SEQ ID NO: 258-364, 1155, 1162, and 1173. In some embodiments, a genomic edit resulting in loss of function of CISH in any of the cells described herein was produced using a guide RNA comprising a targeting domain sequence comprising or consisting of a nucleotide sequence that is identical to, or differs by no more than 1, 2, or 3 nucleotides from, any one of SEQ ID NO: : 258-364, 1155, 1162, and 1173. In some embodiments, a genomic edit resulting in loss of function of CISH in any of the cells described herein was produced using a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO:1155 or 1162, and (ii) a 5' extension sequence depicted in Table 6. In some embodiments, a genomic edit resulting in loss of function of CISH in any of the cells described herein was produced using a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO:1155 or 1162, (ii) a scaffold sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1153 located 5' of the targeting domain sequence, and (iii) the nucleotide sequence of SEQ ID NO:1154 at the 5' of the scaffold sequence.

[0065] In some embodiments, a genomic edit resulting in loss of function of TGFβRII in any of the cells described herein was produced using a guide RNA comprising a targeting domain sequence comprising or consisting of the nucleotide sequence according to any one of SEQ ID NO: 29-257, 1157, 1161, and 1172. In some embodiments, a genomic edit resulting in loss of function of TGFβRII in any of the cells described herein was produced using a guide RNA comprising a targeting domain sequence comprising or consisting of a nucleotide sequence that is identical to, or differs by no more than 1, 2, or 3 nucleotides from, any one of SEQ ID NO: 29-257, 1157, 1161, and 1172. In some embodiments, a genomic edit resulting in loss of

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function of TGFβRII in any of the cells described herein was produced using a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1157 or 1161, and (ii) a 5' extension sequence depicted in Table 6. In some embodiments, a genomic edit resulting in loss of function of TGFβRII in any of the cells described herein was produced using a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1157 or 1161, (ii) a scaffold sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1153 located 5' of the targeting domain sequence, and (iii) the nucleotide sequence of SEQ ID NO:1154 at the 5' of the scaffold sequence.

[0066] In some embodiments, a genomic edit resulting in loss of function of CISH in any of the cells described herein was produced using a ribonucleoprotein (RNP) complex comprising (i) an RNA-guided nuclease (e.g., a Cas12a variant, e.g., a Cas12a variant comprising 1, 2, or 3 of the amino acid substitutions selected from M537R, F870L, and H800A, e.g., a Cas12a variant comprising an amino acid sequence having 90%, 95%, or 100% identity to SEQ ID NO: 62) and (ii) a guide RNA comprising a targeting domain sequence comprising or consisting of the nucleotide sequence according to any one of SEQ ID NO: : 258-364, 1155, 1162, and 1173. In some embodiments, a genomic edit resulting in loss of function of CISH in any of the cells described herein was produced using a ribonucleoprotein (RNP) complex comprising (i) an RNA-guided nuclease (e.g., a Cas12a variant, e.g., a Cas12a variant comprising 1, 2, or 3 of the amino acid substitutions selected from M537R, F870L, and H800A, e.g., a Cas12a variant comprising an amino acid sequence having 90%, 95%, or 100% identity to SEQ ID NO: 62) and (ii) a guide RNA comprising a targeting domain sequence comprising or consisting of a nucleotide sequence that is identical to, or differs by no more than 1, 2, or 3 nucleotides from, any one of SEQ ID NO: 258-364, 1155, 1162, and 1173. In some embodiments, a genomic edit resulting in loss of function of CISH in any of the cells described herein was produced using a ribonucleoprotein (RNP) complex comprising (i) an RNA-guided nuclease (e.g., a Cas12a variant, e.g., a Cas12a variant comprising 1, 2, or 3 of the amino acid substitutions selected from M537R, F870L, and H800A, e.g., a Cas12a variant comprising an amino acid sequence having 90%, 95%, or 100% identity to SEQ ID NO: 62) and (ii) a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO:1155 or

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1162, and (ii) a 5' extension sequence depicted in Table 6. In some embodiments, a genomic edit resulting in loss of function of CISH in any of the cells described herein was produced using a ribonucleoprotein (RNP) complex comprising (i) an RNA-guided nuclease (e.g., a Cas12a variant, e.g., a Cas12a variant comprising 1, 2, or 3 of the amino acid substitutions selected from M537R, F870L, and H800A, e.g., a Cas12a variant comprising an amino acid sequence having 90%, 95%, or 100% identity to SEQ ID NO: 62) and (ii) a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO:1155 or 1162, (ii) a scaffold sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1153 located 5' of the targeting domain sequence, and (iii) the nucleotide sequence of SEQ ID NO:1154 at the 5' of the scaffold sequence.

[0067] In some embodiments, a genomic edit resulting in loss of function of TGFβRII in any of the cells described herein was produced using a ribonucleoprotein (RNP) complex comprising (i) an RNA-guided nuclease (e.g., a Cas12a variant, e.g., a Cas12a variant comprising 1, 2, or 3 of the amino acid substitutions selected from M537R, F870L, and H800A, e.g., a Cas12a variant comprising an amino acid sequence having 90%, 95%, or 100% identity to SEQ ID NO: 62), and (ii) a guide RNA comprising a targeting domain sequence comprising or consisting of the nucleotide sequence according to any one of SEQ ID NO: 29-257, 1157, 1161, and 1172. In some embodiments, a genomic edit resulting in loss of function of TGFβRII in any of the cells described herein was produced using a ribonucleoprotein (RNP) complex comprising (i) an RNA-guided nuclease (e.g., a Cas12a variant, e.g., a Cas12a variant comprising 1, 2, or 3 of the amino acid substitutions selected from M537R, F870L, and H800A, e.g., a Cas12a variant comprising an amino acid sequence having 90%, 95%, or 100% identity to SEQ ID NO: 62) and (ii) a guide RNA comprising a targeting domain sequence comprising or consisting of a nucleotide sequence that is identical to, or differs by no more than 1, 2, or 3 nucleotides from, any one of SEQ ID NO: 29-257, 1157, 1161, and 1172. In some embodiments, a genomic edit resulting in loss of function of TGFβRII in any of the cells described herein was produced using a ribonucleoprotein (RNP) complex comprising (i) an RNA-guided nuclease (e.g., a Cas12a variant, e.g., a Cas12a variant comprising 1, 2, or 3 of the amino acid substitutions selected from M537R, F870L, and H800A, e.g., a Cas12a variant comprising an amino acid sequence having 90%, 95%, or 100% identity to SEQ ID NO: 62), and (ii) a guide RNA comprising (i) a

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targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1157 or 1161, and (ii) a 5' extension sequence depicted in Table 6. In some embodiments, a genomic edit resulting in loss of function of TGFβRII in any of the cells described herein was produced using a ribonucleoprotein (RNP) complex comprising (i) an RNA-guided nuclease (e.g., a Cas12a variant, e.g., a Cas12a variant comprising 1, 2, or 3 of the amino acid substitutions selected from M537R, F870L, and H800A, e.g., a Cas12a variant comprising an amino acid sequence having 90%, 95%, or 100% identity to SEQ ID NO: 62), and (ii) a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1157 or 1161, (ii) a scaffold sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1153 located 5' of the targeting domain sequence, and (iii) the nucleotide sequence of SEQ ID NO:1154 at the 5' of the scaffold sequence.

[0068] In another aspect, the disclosure features a method of making a cell, e.g., a cell described herein, the method comprising (A) contacting an NK cell, a pluripotent human stem cell or human induced pluripotent stem cell, with: an RNA-guided nuclease and a guide RNA comprising a targeting domain sequence comprising or consisting of a nucleotide sequence that is identical to, or differs by no more than 1, 2, or 3 nucleotides from, any one of 258-364, 1155, 1162, and 1173; and an RNA-guided nuclease and a guide RNA comprising a targeting domain sequence comprising or consisting of a nucleotide sequence that is identical to, or differs by no more than 1, 2, or 3 nucleotides from, any one of 29-257, 1157, 1161, and 1172; and (B) contacting the cell with: (i) a nuclease that causes a break within an endogenous coding sequence of a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene in the cell, and (ii) a donor template that comprises a knock-in cassette comprising a first exogenous coding sequence for FcγRIII (CD16) or variant thereof and a second exogenous coding sequence for a membrane bound interleukin 15 (mbIL-15), wherein the first exogenous coding sequence and second exogenous coding sequence are in frame with and downstream (3') of an exogenous coding sequence or partial coding sequence of the GAPDH gene, wherein the knock-in cassette is integrated into the genome of the cell by homology-directed repair (HDR) of the break, resulting in a genome-edited cell that expresses: (a) FcγRIII (CD16) or variant thereof, (b) mbIL-15, and (c) GAPDH, or a functional variant thereof.

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[0069] In some embodiments, the method comprises contacting the cell with: (1) a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO:1155 or 1162, and (ii) a 5' extension sequence depicted in Table 6; and (2) a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1157 or 1161, and (ii) a 5' extension sequence depicted in Table 6.

[0070] In some embodiments, the method comprises contacting the cell with: (1) a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO:1155 or 1162, (ii) a scaffold sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1153 located 5' of the targeting domain sequence, and (iii) the nucleotide sequence of SEQ ID NO:1154 at the 5' of the scaffold sequence; and (2) a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1157 or 1161, (ii) a scaffold sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1153 located 5' of the targeting domain sequence, and (iii) the nucleotide sequence of SEQ ID NO:1154 at the 5' of the scaffold sequence.

[0071] In some embodiments, the RNA-guided nuclease is a Cas12a variant. In some embodiments, the Cas12a variant comprises one or more amino acid substitutions selected from M537R, F870L, and H800A. In some embodiments, the Cas12a variant comprises amino acid substitutions M537R, F870L, and H800A. In some embodiments, the Cas12a variant comprises an amino acid sequence having 90%, 95%, or 100% identity to SEQ ID NO: 62.

[0072] In another aspect, the disclosure features a method of making a cell, e.g., a cell described herein, the method comprising (A) contacting an NK cell, a pluripotent human stem cell or a human induced pluripotent stem cell, with: a ribonucleoprotein (RNP) complex comprising (i) an RNA-guided nuclease (e.g., a Cas12a variant, e.g., a Cas12a variant comprising 1, 2, or 3 of the amino acid substitutions selected from M537R, F870L, and H800A, e.g., a Cas12a variant comprising an amino acid sequence having 90%, 95%, or 100% identity to SEQ ID NO: 62) and (ii) a guide RNA comprising a targeting domain sequence comprising or consisting of a nucleotide sequence that is identical to, or differs by no more than 1, 2, or 3 nucleotides from, any one of SEQ ID NO: 258-364, 1155, 1162, and 1173; and a ribonucleoprotein (RNP) complex comprising (i) an RNA-guided nuclease (e.g., a Cas12a

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variant, e.g., a Cas12a variant comprising 1, 2, or 3 of the amino acid substitutions selected from M537R, F870L, and H800A, e.g., a Cas12a variant comprising an amino acid sequence having 90%, 95%, or 100% identity to SEQ ID NO: 62) and (ii) a guide RNA comprising a targeting domain sequence comprising or consisting of a nucleotide sequence that is identical to, or differs by no more than 1, 2, or 3 nucleotides from, any one of SEQ ID NO: 29-257, 1157, 1161, and 1172; and (B) contacting the cell with: (i) a nuclease that causes a break within an endogenous coding sequence of a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene in the cell, and (ii) a donor template that comprises a knock-in cassette comprising a first exogenous coding sequence for FcγRIII (CD16) or variant thereof and a second exogenous coding sequence for a membrane bound interleukin 15 (mbIL-15), wherein the first exogenous coding sequence and second exogenous coding sequence are in frame with and downstream (3') of an exogenous coding sequence or partial coding sequence of the GAPDH gene, wherein the knock-in cassette is integrated into the genome of the cell by homology-directed repair (HDR) of the break, resulting in a genome-edited cell that expresses: (a) FcγRIII (CD16) or variant thereof, (b) mbIL-15, and (c) GAPDH, or a functional variant thereof.

[0073] In some embodiments, the method comprises contacting the cell with: (1) an RNP comprising a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO:1155 or 1162, and (ii) a 5' extension sequence depicted in Table 6; and (2) an RNP comprising a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1157 or 1161, and (ii) a 5' extension sequence depicted in Table 6.

[0074] In some embodiments, the method comprises contacting the cell with: (1) an RNP comprising a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO:1155 or 1162, (ii) a scaffold sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1153 located 5' of the targeting domain sequence, and (iii) the nucleotide sequence of SEQ ID NO:1154 at the 5' of the scaffold sequence; and (2) an RNP comprising a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1157 or 1161, (ii) a scaffold sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1153 located 5' of

the targeting domain sequence, and (iii) the nucleotide sequence of SEQ ID NO:1154 at the 5' of the scaffold sequence.

[0075] In some embodiments, the RNA-guided nuclease is a Cas12a variant. In some embodiments, the Cas12a variant comprises one or more amino acid substitutions selected from M537R, F870L, and H800A. In some embodiments, the Cas12a variant comprises amino acid substitutions M537R, F870L, and H800A. In some embodiments, the Cas12a variant comprises an amino acid sequence having 90%, 95%, or 100% identity to SEQ ID NO: 62.

[0076] In another aspect, the disclosure features a method of making a cell, e.g., a cell described herein, the method comprising (A) contacting an NK cell, a pluripotent human stem cell or a human induced pluripotent stem cell, with (i) a guide RNA comprising a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1155 or 1162; and a guide RNA comprises a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1157 or 1161; and (ii) an RNA-guided nuclease comprising an amino acid sequence having 90%, 95%, or 100% identity to one of SEQ ID NO:58-66 (or a portion thereof); and (B) contacting the cell with: (i) a nuclease that causes a break within an endogenous coding sequence of a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene in the cell, and (ii) a donor template that comprises a knock-in cassette comprising a first exogenous coding sequence for FcγRIII (CD16) or variant thereof and a second exogenous coding sequence for a membrane bound interleukin 15 (mbIL-15), wherein the first exogenous coding sequence and second exogenous coding sequence are in frame with and downstream (3') of an exogenous coding sequence or partial coding sequence of the GAPDH gene, wherein the knock-in cassette is integrated into the genome of the cell by homology-directed repair (HDR) of the break, resulting in a genome-edited cell that expresses: (a) FcγRIII (CD16) or variant, (b) mbIL-15, and (c) GAPDH, or a functional variant thereof.

[0077] In another aspect, the disclosure features a method of making a cell, e.g., a cell described herein, the method comprising (A) contacting an NK cell, a pluripotent human stem cell or a human induced pluripotent stem cell, with (1) an RNP comprising (i) a guide RNA comprising a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1155 or 1162; and (ii) an RNA-guided nuclease comprising an amino acid sequence having 90%, 95%, or 100% identity to one of SEQ ID NO:58-66 (or a portion thereof);

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and (2) an RNP comprising (i) a guide RNA comprises a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1157 or 1161, and (ii) an RNA-guided nuclease comprising an amino acid sequence having 90%, 95%, or 100% identity to one of SEQ ID NO:58-66 (or a portion thereof); and (B) contacting the cell with: (i) a nuclease that causes a break within an endogenous coding sequence of a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene in the cell, and (ii) a donor template that comprises a knock-in cassette comprising a first exogenous coding sequence for FcγRIII (CD16) or variant thereof and a second exogenous coding sequence for a membrane bound interleukin 15 (mbIL-15), wherein the first exogenous coding sequence and second exogenous coding sequence are in frame with and downstream (3') of an exogenous coding sequence or partial coding sequence of the GAPDH gene, wherein the knock-in cassette is integrated into the genome of the cell by homology-directed repair (HDR) of the break, resulting in a genome-edited cell that expresses: (a) FcγRIII (CD16) or variant thereof, (b) mbIL-15, and (c) GAPDH, or a functional variant thereof.

[0078] In another aspect, the disclosure features a method of making a cell, e.g., a cell described herein, the method comprising (A) contacting an NK cell, a pluripotent human stem cell or a human induced pluripotent stem cell, with (1) a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO:1155 or 1162, and (ii) a 5' extension sequence depicted in Table 6; (2) a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1157 or 1161, and (ii) a 5' extension sequence depicted in Table 6; and (3) an RNA-guided nuclease comprising an amino acid sequence having 90%, 95%, or 100% identity to one of SEQ ID NO:58-66 (or a portion thereof); and (B) contacting the cell with: (i) a nuclease that causes a break within an endogenous coding sequence of a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene in the cell, and (ii) a donor template that comprises a knock-in cassette comprising a first exogenous coding sequence for FcγRIII (CD16) or variant thereof and a second exogenous coding sequence for a membrane bound interleukin 15 (mbIL-15), wherein the first exogenous coding sequence and second exogenous coding sequence are in frame with and downstream (3') of an exogenous coding sequence or partial coding sequence of the GAPDH gene, wherein the knock-in cassette is integrated into the genome of the cell by

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homology-directed repair (HDR) of the break, resulting in a genome-edited cell that expresses: (a) FcγRIII (CD16) or variant thereof, (b) mbIL-15, and (c) GAPDH, or a functional variant thereof.

[0079] In another aspect, the disclosure features a method of making a cell, e.g., a cell described herein, the method comprising (A) contacting an NK cell, a pluripotent human stem cell or a human induced pluripotent stem cell, with (1) an RNP comprising (a) a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO:1155 or 1162, and (ii) a 5' extension sequence depicted in Table 6; and (b) an RNA-guided nuclease comprising an amino acid sequence having 90%, 95%, or 100% identity to one of SEQ ID NO:58-66 (or a portion thereof); and (2) an RNP comprising (a) a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1157 or 1161, and (ii) a 5' extension sequence depicted in Table 6; and (b) an RNA-guided nuclease comprising an amino acid sequence having 90%, 95%, or 100% identity to one of SEQ ID NO:58-66 (or a portion thereof); and (B) contacting the cell with: (i) a nuclease that causes a break within an endogenous coding sequence of a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene in the cell, and (ii) a donor template that comprises a knock-in cassette comprising a first exogenous coding sequence for FcγRIII (CD16) or variant thereof and a second exogenous coding sequence for a membrane bound interleukin 15 (mbIL-15), wherein the first exogenous coding sequence and second exogenous coding sequence are in frame with and downstream (3') of an exogenous coding sequence or partial coding sequence of the GAPDH gene, wherein the knock-in cassette is integrated into the genome of the cell by homology-directed repair (HDR) of the break, resulting in a genome-edited cell that expresses: (a) FcγRIII (CD16) or variant thereof, (b) mbIL-15, and (c) GAPDH, or a functional variant thereof.

[0080] In another aspect, the disclosure features a method of making a cell, e.g., a cell described herein, the method comprising (A) contacting an NK cell, a pluripotent human stem cell or a human induced pluripotent stem cell, with (1) a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO:1155 or 1162, (ii) a scaffold sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1153 located 5' of the targeting domain sequence, and (iii) the nucleotide sequence of SEQ

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ID NO:1154 at the 5' of the scaffold sequence; (2) a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1157 or 1161, (ii) a scaffold sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1153 located 5' of the targeting domain sequence, and (iii) the nucleotide sequence of SEQ ID NO:1154 at the 5' of the scaffold sequence; and (3) an RNA-guided nuclease comprising an amino acid sequence having 90%, 95%, or 100% identity to one of SEQ ID NO:58-66 (or a portion thereof); and (B) contacting the cell with: (i) a nuclease that causes a break within an endogenous coding sequence of a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene in the cell, and (ii) a donor template that comprises a knock-in cassette comprising a first exogenous coding sequence for FcγRIII (CD16) or variant thereof and a second exogenous coding sequence for a membrane bound interleukin 15 (mbIL-15), wherein the first exogenous coding sequence and second exogenous coding sequence are in frame with and downstream (3') of an exogenous coding sequence or partial coding sequence of the GAPDH gene, wherein the knock-in cassette is integrated into the genome of the cell by homology-directed repair (HDR) of the break, resulting in a genome-edited cell that expresses: (a) FcγRIII (CD16) or variant thereof, (b) mbIL-15, and (c) GAPDH, or a functional variant thereof.

[0081] In another aspect, the disclosure features a method of making a cell, e.g., a cell described herein, the method comprising (A) contacting an NK cell, a pluripotent human stem cell or a human induced pluripotent stem cell, with (1) an RNP comprising (a) a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO:1155 or 1162, (ii) a scaffold sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1153 located 5' of the targeting domain sequence, and (iii) the nucleotide sequence of SEQ ID NO:1154 at the 5' of the scaffold sequence; and (b) an RNA-guided nuclease comprising an amino acid sequence having 90%, 95%, or 100% identity to one of SEQ ID NO:1144-1151 (or a portion thereof); and (2) an RNP comprising (a) a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1157 or 1161, (ii) a scaffold sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1153 located 5' of the targeting domain sequence, and (iii) the nucleotide sequence of SEQ ID NO:1154 at the 5' of the scaffold sequence; and (b) an RNA-guided nuclease comprising an amino acid sequence having 90%, 95%, or 100% identity to one

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of SEQ ID NO:1144-1151 (or a portion thereof); and (B) contacting the cell with: (i) a nuclease that causes a break within an endogenous coding sequence of a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene in the cell, and (ii) a donor template that comprises a knock-in cassette comprising a first exogenous coding sequence for FcγRIII (CD16) or variant thereof and a second exogenous coding sequence for a membrane bound interleukin 15 (mbIL-15), wherein the first exogenous coding sequence and second exogenous coding sequence are in frame with and downstream (3') of an exogenous coding sequence or partial coding sequence of the GAPDH gene, wherein the knock-in cassette is integrated into the genome of the cell by homology-directed repair (HDR) of the break, resulting in a genome-edited cell that expresses: (a) FcγRIII (CD16) or variant thereof, (b) mbIL-15, and (c) GAPDH, or a functional variant thereof.

[0082] In another aspect, the disclosure features a method of making a modified cell, e.g., a modified NK cell, a modified pluripotent human stem cell, a modified NK cell differentiated from such a stem cell, the method comprising (A) contacting a cell with: (i) an RNA-guided nuclease and a guide RNA that cause a break within an endogenous coding sequence of an essential gene in the cell, such as, e.g., glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene, and (ii) a donor template that comprises a knock-in cassette comprising a first exogenous coding sequence for FcγRIII (CD16) or variant thereof and a second exogenous coding sequence for a membrane bound interleukin 15 (mbIL-15), wherein the first exogenous coding sequence and second exogenous coding sequence are in frame with and downstream (3') of an exogenous coding sequence or partial coding sequence of the essential gene, e.g., the GAPDH gene, wherein the knock-in cassette is integrated into the genome of the cell by homology-directed repair (HDR) of the break, resulting in a genome-edited cell that expresses: (a) FcγRIII (CD16) or variant thereof, (b) mbIL-15, and (c) the essential gene, e.g., GAPDH, or a functional variant thereof; and, , (B) contacting the cell (e.g., the NK cell or the pluripotent human stem cell or the human induced pluripotent stem cell) with one or more of: at least one RNA-guided nuclease and at least one guide RNA comprising a targeting domain sequence, wherein the RNA-guided nuclease and the guide RNA cause a genomic edit within an endogenous coding sequence of a gene of interest, e.g., a break or genomic edit resulting in a loss of function of the gene of interest, wherein the gene of interest comprises, e.g., adenosine A2a receptor (ADORA2A), β-2

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microglobulin (B2M), class II major histocompatibility complex transactivator (CIITA), cytokine inducible SH2 containing protein (CISH), two or more human leukocyte antigen (HLA) class II histocompatibility antigen alpha chain genes, two or more HLA class II histocompatibility antigen beta chain genes, natural killer group 2 member A receptor (NKG2A), programmed cell death protein 1 (PD-1), T cell immunoreceptor with Ig and ITIM domains (TIGIT), an agonist of the TGF beta signaling pathway (e.g., transforming growth factor beta receptor II (TGFβRII)), or any combination of two or more thereof.

[0083] In some embodiments, the method comprises contacting the cell with: (1) a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO:1155 or 1162, and (ii) a 5' extension sequence depicted in Table 6; and (2) a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1157 or 1161, and (ii) a 5' extension sequence depicted in Table 6.

[0084] In some embodiments, the method comprises contacting the cell with: (1) a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO:1155 or 1162, (ii) a scaffold sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1153 located 5' of the targeting domain sequence, and (iii) the nucleotide sequence of SEQ ID NO:1154 at the 5' of the scaffold sequence; and (2) a guide RNA comprising (i) a targeting domain sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1157 or 1161, (ii) a scaffold sequence comprising or consisting of the nucleotide sequence of SEQ ID NO: 1153 located 5' of the targeting domain sequence, and (iii) the nucleotide sequence of SEQ ID NO:1154 at the 5' of the scaffold sequence.

[0085] In some embodiments, the RNA-guided nuclease is a Cas12a variant. In some embodiments, the Cas12a variant comprises one or more amino acid substitutions selected from M537R, F870L, and H800A. In some embodiments, the Cas12a variant comprises amino acid substitutions M537R, F870L, and H800A. In some embodiments, the Cas12a variant comprises an amino acid sequence having 90%, 95%, or 100% identity to SEQ ID NO: 62.

[0086] In another aspect, the disclosure features a method of making a population of modified cells, e.g., a population of modified NK cells, a population of modified pluripotent human stem cells, a population of modified NK cells differentiated from such stem cells, the

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method comprising (A) contacting a population of cells with: (i) an RNA-guided nuclease and a guide RNA (e.g., configured together as an RNP) that cause a break within an endogenous coding sequence of an essential gene in at least one cell within the population of cells, such as, e.g., glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene, and (ii) a donor template that comprises a knock-in cassette comprising a first exogenous coding sequence for FcγRIII (CD16) or variant thereof and a second exogenous coding sequence for a membrane bound interleukin 15 (mbIL-15), wherein the first exogenous coding sequence and second exogenous coding sequence are in frame with and downstream (3') of an exogenous coding sequence or partial coding sequence of the essential gene, e.g., the GAPDH gene, wherein the knock-in cassette is integrated into the genome of the cell by homology-directed repair (HDR) of the break, resulting in a genome-edited cell that expresses: (a) FcγRIII (CD16) or variant thereof, (b) mbIL-15, and (c) the essential gene, e.g., GAPDH, or a functional variant thereof; and (B) contacting the population of cells (e.g., the population of NK cells or the population of pluripotent human stem cells or the population of induced human induced pluripotent stem cells) with one or more of: at least one RNA-guided nuclease and a guide RNA comprising a targeting domain sequence, wherein the RNA-guided nuclease and the guide RNA cause a genomic edit within an endogenous coding sequence of a gene of interest within at least one cell in the population of cells, e.g., a genomic edit resulting in a break and/or a genomic edit resulting in a loss of function of the gene of interest, wherein the gene of interest comprises, e.g., adenosine A2a receptor (ADORA2A), β-2 microglobulin (B2M), class II major histocompatibility complex transactivator (CIITA), cytokine inducible SH2 containing protein (CISH), two or more human leukocyte antigen (HLA) class II histocompatibility antigen alpha chain genes, two or more HLA class II histocompatibility antigen beta chain genes, natural killer group 2 member A receptor (NKG2A), programmed cell death protein 1 (PD-1), T cell immunoreceptor with Ig and ITIM domains (TIGIT), an agonist of the TGF beta signaling pathway (e.g., transforming growth factor beta receptor II (TGFβRII)), or any combination of two or more thereof. In some embodiments, the population of cells is optionally contacted with at least a first RNA-guided nuclease and a first guide RNA that cause a genomic edit within the endogenous coding sequence of a first gene of interest and a second RNA-guided nuclease and a second guide RNA that cause a genomic edit within the endogenous coding sequence of a second gene of interest;

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and, optionally, wherein the population of cells is contacted with a third, fourth, and/or fifth (or more) RNA-guided nuclease and a third, fourth, and/or fifth (or more) guide RNA that causes a genomic edit within the endogenous coding sequence of a third, fourth, and/or fifth (or more) gene of interest, respectively.

[0087] In some embodiments, the RNA-guided nuclease editing efficiency is high, e.g., wherein the RNA-guided nuclease is capable of editing about 60% to 100% of cells in a population of cells, e.g., at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% oc cells in a population. In some embodiments, the RNA-guided nuclease is configured with a guide RNA to form an RNP, and the RNP causes a break within the essential gene (e.g., within the terminal exon in the locus of any essential gene provided in Table 3, such as, e.g., GAPDH) in at least 60% of the cells in the population of cells (e.g., in at least 60%, in at least 65%, in at least 70%, in at least 75%, in at least 80%, in at least 85%, in at least 90%, in at least 91%, in at least 92%, in at least 93%, in at least 94%, in at least 95%, in at least 96%, in at least 97%, in at least 98%, or in at least 99% of the cells in the population of cells). In some embodiments, the RNA-guided nuclease is configured with a guide RNA to form an RNP, and the RNP induces knock-in cassette integration at the essential gene (e.g., within the terminal exon in the locus of any essential gene provided in Table 3, such as, e.g., GAPDH) in at least 50% of the cells in the population of cells (e.g., in at least 50%, in at least 55%, in at least 60%, in at least 65%, in at least 70%, in at least 75%, in at least 80%, in at least 85%, in at least 90%, in at least 91%, in at least 92%, in at least 93%, in at least 94%, in at least 95%, in at least 96%, in at least 97%, in at least 98%, or in at least 99% of the cells in the population of cells) at between 4 days and 9 days (e.g., at 4 days, 5 days, 6 days, 7 days, 8 days or 9 days) after the population of cells is contacted with the RNA-guided nuclease and the guide RNA (the RNP) and the donor template. In some embodiments, the RNA-guided nuclease comprises Cas9, Cas12a, Cas12b, Cas12c, Cas12e, CasX, or CasΦ (Cas12j), or a variant thereof, e.g., a variant capable of editing about 60% to 100% of cells in a population of cells.

[0088] In some embodiment, at least 50% (e.g., 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 98%, or at least 99%) of the cells in the population of cells comprises the knock-in cassette

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comprising the first and second exogenous coding sequences integrated at the essential gene in the genome at between 4 days and 9 days (e.g., at 4 days, 5 days, 6 days, 7 days, 8 days or 9 days) after the population of cells is contacted with the donor template and the RNA-guided nuclease and the guide RNA (e.g., configured together an an RNP) that cause a break within the endogenous coding sequence of the essential gene.

[0089] In some embodiments, at least 50% (e.g., 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 98%, or at least 99%) of the cells in the population of cells comprises the knock-in cassette comprising the first and second exogenous coding sequences integrated at the essential gene in the genome at between 4 days and 9 days (e.g., at 4 days, 5 days, 6 days, 7 days, 8 days or 9 days) after the population of cells is contacted with the donor template and the RNA-guided nuclease and the guide RNA (e.g., configured together an an RNP) that cause a break within the endogenous coding sequence of the essential gene, and at least 60% of the cells (e.g., 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 98%, or at least 99%) in the population of cells comprises a genomic edit (e.g., a genomic edit resulting in a break and/or a genomic edit resulting in a loss of function) within an endogenous coding sequence of a gene of interest, e.g., at between 4 days and 9 days (e.g., at 4 days, 5 days, 6 days, 7 days, 8 days or 9 days) after the population of cells is contacted with the an RNA-guided nuclease and a guide RNA (e.g., configured together an RNP) that cause a genomic edit within the endogenous coding sequence of the gene of interest.

[0090] In some embodiments, at least 50% (e.g., 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 98%, or at least 99%) of the cells in the population of cells comprise the knock-in cassette comprising the first and second exogenous coding sequences integrated at the essential gene in the genome at between 4 days and 9 days (e.g., at 4 days, 5 days, 6 days, 7 days, 8 days or 9 days) after the population of cells is contacted with the donor template and the RNA-guided nuclease and the guide RNA (e.g., configured together as an RNP) that cause a break within the endogenous coding sequence of the essential gene, and at least 60% of the cells (e.g., 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 98%, or at least 99%) in the population of cells comprise a genomic edit (e.g., a

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genomic edit resulting in a break and/or a genomic edit resulting in a loss of function) within an endogenous coding sequence of a gene of interest, e.g., at between 4 days and 9 days (e.g., at 4 days, 5 days, 6 days, 7 days, 8 days or 9 days) after the population of cells is contacted with an RNA-guided nuclease and a guide RNA (e.g., configured together as an RNP) that cause a genomic edit within the endogenous coding sequence of the gene of interest. In some embodiments, at least 50% (e.g., 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 98%, or at least 99%) of the cells in the population of cells comprise the knock-in cassette comprising the first and second exogenous coding sequences integrated at the essential gene in the genome at between 4 days and 9 days (e.g., at 4 days, 5 days, 6 days, 7 days, 8 days or 9 days) after the population of cells is contacted with the donor template and the RNA-guided nuclease and the guide RNA (e.g., configured together as an RNP) that cause a break within the endogenous coding sequence of the essential gene; and at least 60% of the cells (e.g., 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 98%, or at least 99%) in the population of cells comprise a genomic edit (e.g., a genomic edit resulting in a break and/or a genomic edit resulting in a loss of function) within an endogenous CISH coding sequence, e.g., at between 4 days and 9 days (e.g., at 4 days, 5 days, 6 days, 7 days, 8 days or 9 days) after the population of cells is contacted with an RNA-guided nuclease and a guide RNA (e.g., configured together as an RNP) that cause a genomic edit within the endogenous CISH coding sequence; and at least 60% of the cells (e.g., 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 98%, or at least 99%) in the population of cells comprise a genomic edit (e.g., a genomic edit resulting in a break and/or a genomic edit resulting in a loss of function) within an endogenous TGFβRII coding sequence, e.g., at between 4 days and 9 days (e.g., at 4 days, 5 days, 6 days, 7 days, 8 days or 9 days) after the population of cells is contacted with an RNA-guided nuclease and a guide RNA (e.g., configured together as an RNP) that cause a genomic edit within the endogenous TGFβRII coding sequence.

[0091] In some embodiments, at least 50% (e.g., 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 98%, or at least 99%) of the cells in the population of cells expresses FcγRIII (CD16) or

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variant thereof and mbIL-15, e.g., at between 4 days and 9 days (e.g., at 4 days, 5 days, 6 days, 7 days, 8 days or 9 days) after the population of cells is contacted with the RNA-guided nuclease and the guide RNA (e.g., configured together as an RNP) and the donor template. In some embodiments, at least 50% (e.g., 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 98%, or at least 99%) of the cells in the population of cells expresses FcγRIII (CD16) or variant thereof and mbIL-15, e.g., at between 4 days and 9 days (e.g., at 4 days, 5 days, 6 days, 7 days, 8 days or 9 days) after the population of cells is contacted with the RNA-guided nuclease and the guide RNA (e.g., configured together as an RNP) and the donor template, and at least 60% of the cells (e.g., 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 98%, or at least 99%) in the population of cells do not express CISH or TGFβRII after the population of cells is contacted with an RNA-guided nuclease and guide RNAs (e.g., configured as an RNP) that cause a genomic edit within the endogenous CISH and TGFβRII coding sequences.

BRIEF DESCRIPTION OF THE DRAWING

[0092] The teachings described herein will be more fully understood from the following description of various exemplary embodiments, when read together with the accompanying drawing. It should be understood that the drawing described below is for illustration purposes only and is not intended to limit the scope of the present teachings in any way.

[0093] Fig. 1 shows the locations on the *GAPDH* gene where exemplary AsCpf1 (AsCas12a) guide RNAs bind, and the results of screening the exemplary guide RNAs that target the *GAPDH* gene three days after transfection. Results are from gDNA from living cells.

[0094] Fig. 2 shows results of screening the exemplary AsCpf1 (AsCas12a) guide RNAs that target the *GAPDH* gene, three days after transfection. Results are from gDNA from living cells.

[0095] Fig. 3A shows an exemplary integration strategy that targets an essential gene according to certain embodiments of the present disclosure. In particular embodiments, introducing a double strand break using CRISPR gene editing (e.g., by Cas12a, Cas9, Cas12b, Cas12c, Cas12e, CasX, or CasΦ (Cas12j), or a variant thereof, e.g., a variant with a high editing efficiency, e.g., capable of editing about 60% to 100% of cells in a population of cells) within a

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terminal exon (e.g., within about 500 bp upstream (5′) of the stop codon of the essential gene) and administering a donor plasmid with homology arms designed to mediate homology directed repair (HDR) at the cleavage site, results in a population of viable cells carrying a cargo of interest integrated at the essential gene locus. Those cells that were edited by the CRISPR nuclease, but failed to undergo integration of the cargo at the essential gene locus, do not survive.

[0096] Fig. 3B shows an exemplary integration strategy that targets the *GAPDH* gene according to certain embodiments of the present disclosure. Although Fig. 3B shows a strategy wherein the *GAPDH* gene is modified in an induced pluripotent stem cell (iPSC), this strategy can be applied to a variety of cell types, including primary cells, e.g., T cells, NK cells, stem cells, iPSCs, and cells differentiated from iPSCs, e.g., iPSC-derived T cells or NK cells for treating cancer.

[0097] Fig. 3C shows an exemplary integration strategy that targets the *GAPDH* gene according to certain embodiments of the present disclosure. The diagram shows that the only cells that should survive over time are those cells that underwent targeted integration of a cassette that restores the *GAPDH* locus and includes a cargo of interest, as well as unedited cells. The population of unedited cells following CRISPR editing should be small if the nuclease and guide RNA are highly effective at cleaving the essential gene target site and introduce indels that significantly reduce the function of the essential gene product.

[0098] Fig. 3D shows an exemplary integration strategy that targets an essential gene according to certain embodiments of the present disclosure. In particular embodiments, introducing a double strand break using CRISPR gene editing (e.g., by Cas12a, Cas9, Cas12b, Cas12c, Cas12e, CasX, or CasΦ (Cas12j), or a variant thereof, e.g., a variant with a high editing efficiency, e.g., capable of editing about 60% to 100% of cells in a population of cells) to target a 5′ exon (e.g., within about 500 bp downstream (3′) of a start codon of the essential gene) and administering a donor plasmid with homology arms designed to mediate homology directed repair (HDR) at the cleavage site, results in a population of viable cells carrying a cargo of interest integrated at the essential gene locus. Those cells that were edited by the CRISPR nuclease, but failed to undergo integration of the cargo at the essential gene locus, do not survive.

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[0099] Fig. 4 shows editing efficiency at different concentrations (0.625 μM to 4 μM) of an exemplary AsCpf1 (AsCas12a) guide RNA that targets the *GAPDH* gene.

[0100] Fig. 5 shows the knock-in (KI) efficiency of a CD47 encoding "cargo" in the *GAPDH* gene 4 days post-electroporation when the dsDNA plasmid ("PLA") was also present. Knock-in efficiency was measured with two different concentrations of the plasmid. Knock-in was measured using ddPCR targeting the 3′ positions of the knock-in "cargo".

[0101] Fig. 6 shows the knock-in efficiency of a CD47 encoding "cargo" in the *GAPDH* gene 9 days post-electroporation when the dsDNA plasmid was also present. Knock-in was measured using ddPCR both targeting the 5′ and 3′ positions of the knock-in "cargo", increasing the reliability of the result.

[0102] Fig. 7 shows the efficiency of integration of a knock-in cassette, comprising a GFP protein encoding "cargo" sequence, into the GAPDH locus of iPSCs, measured 7 days following transfection. **(A)** Depicts exemplary microscopy (brightfield and fluorescent) images, and **(B)** depicts exemplary flow cytometry data. Images and flow cytometry data depict insertion rates for cargo transfection alone (PLA1593 or PLA1651) compared to cargo and guide RNA transfections (RSQ22337 + PLA1593 or RSQ24570 + PLA1651), additionally, insertion rates with an exemplary exonic coding region targeting guide RNA with appropriate cargo (RSQ22337 + PLA1593) are compared to insertion rates with an intronic targeting guide RNA with appropriate cargo (RSQ24570 + PLA1651).

[0103] Fig. 8A depicts a schematic representation of a bicistronic knock-in cassette (e.g., comprising two cistrons separated by a linker) for insertion into the GAPDH locus. The leading GAPDH Exon 9 coding region and exogenous sequences encoding proteins of interest are separated by linker sequences, and the second GAPDH allele can comprise a target knock-in cassette insertion, indels, or is wild type (WT).

[0104] Fig. 8B depicts a schematic representation of bi-allelic knock-in cassettes for insertion into the GAPDH locus. Exogenous "cargo" sequences encoding proteins of interest are located on different knock-in cassettes. For each construct, the leading GAPDH Exon 9 coding region is separated from an exogenous sequence encoding a protein of interest by a linker sequence.

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[0105] Fig. 9A depicts a schematic representation of a bicistronic knock-in cassette for insertion into the GAPDH locus, with the leading GAPDH Exon 9 coding region and exogenous sequences encoding GFP and mCherry separated by linker sequences P2A, T2A, and/or IRES.

[0106] Fig. 9B is a panel of exemplary microscopic images (brightfield and fluorescent) of iPSCs nine days following nucleofection of RNPs comprising RSQ22337 (SEQ ID NO: 95) targeting GAPDH and Cas12a (SEQ ID NO: 62) and a bicistronic knock-in cassette comprising "cargo" sequence encoding GFP and mCherry molecules inserted at the GAPDH locus. iPSCs comprising exemplary "cargo" molecules PLA1582 (comprising donor template SEQ ID NO: 41) with linkers P2A and T2A, PLA1583 (comprising donor template SEQ ID NO: 42) with linkers T2A and P2A, and PLA1584 (comprising donor template SEQ ID NO: 43) with linkers T2A and IRES are shown. Results show that at least two different cargos can be inserted in a bicistronic manner and expression is detectable irrespective of linker type used. All images were taken at 2X 100 μm on a Keyence Microscope.

[0107] Fig. 9C depicts expression quantification (Y axis) of exemplary "cargo" molecules GFP and mCherry from various bicistronic molecules comprising the described linker pairs (X axis). mCherry as a sole "cargo" protein was utilized as a relative control.

[0108] Fig. 10A depicts exemplary flow cytometry data for bi-allelic GFP and mCherry knock-in at the *GAPDH* gene.

[0109] Fig. 10B depicts fluorescence imaging of cell populations prior to flow cytometry analysis following bi-allelic GFP and mCherry knock-in at the *GAPDH* gene.

[0110] Fig. 10C are histograms depicting exemplary flow cytometry analysis data for bi-allelic GFP and mCherry knock-in at the *GAPDH* gene. Cells were nucleofected with 0.5 μM RNPs comprising Cas12a (SEQ ID NO: 62) and RSQ22337 (SEQ ID NO: 95), and 2.5 μg (5 trials) or 5 μg (1 trial) GFP and mCherry donor templates.

[0111] Fig. 11A depicts exemplary flow cytometry data for GFP expression in iPSCs seven days after being transfected with a gRNA and an appropriate donor template comprising a knock-in cassette with a "cargo" sequence encoding GFP that was recombined into various loci.

[0112] Fig. 11B depicts the percentage of cells having editing events as measured by Inference of CRISPR Edits (ICE) assays 48 hours after being transfected with the noted gRNA.

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[0113] Fig. 11C depicts relative integrated "cargo" (GFP) expression intensity as determined by flow cytometry conducted with a FITC channel to filter GFP signal for iPSCs transfected with the noted exemplary gRNA and knock-in cassette combinations.

[0114] Fig. 11D depicts relative integrated "cargo" (GFP) expression intensity as determined by flow cytometry conducted with a FITC channel to filter GFP signal for iPSCs transfected with exemplary gRNA targeting the noted essential gene. Knock-in efficiency at each essential gene is denoted by a percentage.

[0115] Fig. 12 depicts exemplary flow cytometry data highlighting the efficiency of integration of a donor template comprising a knock-in cassette comprising a GFP protein encoding "cargo" sequence into the *TBP* locus of iPSCs.

[0116] Fig. 13 is exemplary ddPCR results describing knock-in cassette integration ratios in *GAPDH* or *TBP* alleles in an iPSC population.

[0117] Fig. 14 is a histogram representation of exemplary flow cytometry data for AAV6 mediated knock-in of GFP into T cells using RNPs comprising RSQ22337 targeting GAPDH and Cas12a (SEQ ID NO: 62) at various concentrations of RNP and various AAV6 multiplicity of infection (MOI) rates (vg/cell) measured seven days after electroporation and transduction. The Y axis represents percentage of the cell population expressing GFP, while the X axis depicts AAV6 MOI.

[0118] Fig. 15 is a histogram representation of exemplary flow cytometry data depicting cell viability following AAV6 mediated knock-in of GFP at the *GAPDH* gene in differentiated cells. Depicted is T cell viability four days after AAV6 mediated transduction of a GFP cargo and electroporation with 1 μM RNPs comprising RSQ22337 and Cas12a (SEQ ID NO: 62); the Y axis notes cell viability as a function of total cell population, while the X axis lists various MOIs used to transduce the cells.

[0119] Fig. 16A depicts exemplary flow cytometry charts for a population of T cells transduced by AAV6 comprising a knock-in GFP cargo targeting GAPDH at 5E4 MOI and transformed with 4 μM RNP comprising Cas12a (SEQ NO: 62) and RSQ22337.

[0120] Fig. 16B depicts exemplary control experiment flow cytometry charts for T cells that were not transduced by AAV6, but solely transformed with 4 μM RNP comprising Cas12a (SEQ NO: 62) and RSQ22337.

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[0121] Fig. 17A are histograms depicting exemplary flow cytometry data for AAV6 mediated knock-in of GFP into T cells at either the GAPDH locus using RNPs comprising RSQ22337 and Cas12a (SEQ ID NO: 62), or at the TRAC locus. Integration constructs each comprised homology arms approximately 500bp in length, and T cells were transduced with the same concentration of RNP and AAV MOI. The mean and standard deviation of three independent biological replicates is shown, significant differences in targeted integration were observed ($p = 0.0022$ using unpaired t-test).

[0122] Fig. 17B depicts an exemplary flow cytometry chart for a population of T cells transduced by AAV6 comprising a GFP cargo targeted for knock-in at GAPDH at 5E4 MOI and transformed with 4 μM of RNPs comprising Cas12a (SEQ NO: 62) and RSQ22337.

[0123] Fig. 17C depicts exemplary expansion and viability data for a population of T cells transduced by AAV6 and transformed with RNPs as described in Fig. 17B, and for a population of T cells that did not undergo RNP transfection ("mock").

[0124] Fig. 17D depicts exemplary flow cytometry data for AAV6 mediated knock-in of GFP into T cells at either the GAPDH locus using RNPs comprising RSQ22337 and Cas12a (SEQ ID NO: 62), as described in Fig. 17B, or at the TRAC locus. Integration constructs each comprised homology arms approximately 500bp in length, and T cells were transduced with the same concentration of RNPs and AAV MOI. Three independent biological replicates are shown, significant differences in targeted integration were observed ($p = < 0.001$ using unpaired t-test).

[0125] Fig. 17E depicts exemplary flow cytometry data for AAV6 mediated knock-in of GFP into T cells at either the GAPDH locus (GAPDH KI) using RNPs comprising RSQ22337 and Cas12a (SEQ ID NO: 62), or at the TRAC locus (TRAC KI). Knock-in efficiency was examined at varying concentrations of AAV6. Integration constructs each comprised homology arms approximately 500bp in length. The X-axis quantifies AAV6 concentration (vg/ml), while the Y-axis quantifies the percentage of cells that are expressing GFP as detected by flow cytometry. Three independent biological replicates are shown per each knock-in location at each AAV6 concentration. Significant differences in EC50 for AAV6 concentration were observed. ****$p = < 0.0001$ (unpaired t-test).

[0126] Fig. 18A is a histogram depicting the knock-in efficiency of CD16 encoding "cargo" integrated at the *GAPDH* gene of iPSCs. Targeting integration (TI) was measured at day

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0 and day 19 of bulk edited cell populations using ddPCR targeting the 5′ (5′ assay) and 3′ (3′ assay) positions of the knock-in cargo.

[0127] Fig. 18B is a histogram depicting the genotypes of iPSC clones with CD16 encoding "cargo" integrated at the *GAPDH* gene, measured using ddPCR targeting the 5′ (5′ CDN probe) and 3′ (3′ PolyΛ probe) positions of the knock-in cargo. Shown are results for four exemplary cell lines, two lines were classified as homozygous knock-in with targeted integration (TI) rates of 88.5% (clone 1) and 90.5% (clone 2) respectively, and two lines were classified as heterozygous knock-in with TI rates of 45.6% (clone 1) and 46.5% (clone 2) respectively.

[0128] Fig. 19A depicts exemplary flow cytometry data from day 32 of homozygous clone 1 CD16 knock-in iPSCs differentiated into iNKs. The data highlights the efficiency of integration and high expression (e.g., approximately 98%) of a knock-in cassette comprising a CD16 protein encoding "cargo" sequence into the *GAPDH* gene of iPSCs. In addition, the data shows knock-in of a "cargo" at the *GADPH* gene does not inhibit the differentiation process, as represented by high CD56+CD45+ population proportions.

[0129] Fig. 19B depicts exemplary flow cytometry data from day 32 of homozygous clone 2 CD16 knock-in iPSCs differentiated into iNKs. The data highlights the efficiency of integration and expression of a knock-in cassette comprising a CD16 protein encoding "cargo" sequence into the *GAPDH* gene of iPSCs.

[0130] Fig. 19C depicts exemplary flow cytometry data from day 32 of heterozygous clone 1 CD16 knock-in iPSCs differentiated into iNKs. The data highlights the efficiency of integration and high expression (e.g., approximately 97.8%) of a knock-in cassette comprising a CD16 protein encoding "cargo" sequence into the *GAPDH* gene of iPSCs.

[0131] Fig. 19D depicts exemplary flow cytometry data from day 32 of heterozygous clone 2 CD16 knock-in iPSCs differentiated into iNKs. The data highlights the efficiency of integration and expression of a knock-in cassette comprising a CD16 protein encoding "cargo" sequence into the *GAPDH* gene of iPSCs.

[0132] Fig. 20 is a schematic representation of an exemplary solid tumor cell killing assay, depicting the use of knock-in iPSCs differentiated into iNK cells to kill 3D spheroids created from a cancer cell line (e.g., SK-OV-3 ovarian cancer cells). Antibodies and/or cytokines may optionally be added during the 3D spheroid killing stage.

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[0133] Fig. 21A shows the results of a solid tumor killing assay as described in FIG 20. Homozygous clones comprising CD16 knock-in at the *GAPDH* gene were differentiated into iNK cells and functioned to reduce tumor cell spheroid size, particularly following the addition of an antibody, e.g., 10μg/mL trastuzumab; addition of an antibody promotes antibody dependent cellular cytotoxicity (ΛDCC) and tumor cell killing by iNKs. Control "WT PCS" cells were bulk unedited parental clones that were electroporated without RNPs or plasmids, and at the same stage of iNK cell differentiation as test cells. The Y axis depicts normalized total integrated red object intensity, a proxy for tumor cell abundance, while the X axis depicts the Effector to Target cell (E:T) ratio.

[0134] Fig. 21B shows the results of a solid tumor killing assay as described in FIG 20. Heterozygous clones comprising CD16 knock-in at the *GAPDH* gene were differentiated into iNK cells and functioned to reduce tumor cell spheroid size, particularly following the addition of an antibody, e.g., 10 μg/mL trastuzumab; addition of an antibody promotes ADCC and tumor cell killing by iNKs. Control "WT PCS" cells were bulk unedited parental clones that were electroporated without RNPs or plasmids, and at the same stage of iNK cell differentiation as test cells. The Y axis depicts normalized total integrated red object intensity, a proxy for tumor cell abundance, while the X axis depicts the E:T ratio.

[0135] Fig. 22 shows the results of an *in vitro* serial killing assay, where homozygous or heterozygous clones comprising CD16 knock-in at the *GAPDH* gene were differentiated into iNK cells and were serially challenged with hematological cancer cells (e.g., Raji cells), with or without the addition of antibody (0.1 μg/mL rituximab). The X axis represents time (0-598 hr.) with an additional tumor cell bolus (5,000 cells) being added approximately every 48 hours, and the Y axis represents killing efficacy as measured by normalized total red object area (e.g., presence of tumor cells). Star (*) denotes onset of addition of 0.1 μg/mL rituximab in previously rituximab absent trials. The data shows that edited iNK cells (CD16 knock-in at *GAPDH* gene; clones "Homo_C1", "Homo_C2", "Het_C1", and "Het_C2") continue to kill hematological cancer cells while unedited ("PCS") or control edited iNKs ("GFP Bulk") derived from parental iPSCs lose this function at equivalent time points.

[0136] Fig. 23 depicts a correlation (R^2 of 0.768) between CD16 expression and reduction in tumor spheroid size at an Effector to Target (E:T) ratio of 3.16:1. Shown are

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differentiated iNK cells derived from either iPSC bulk edited cells or iPSC individual clones with CD16 knock-in at the *GAPDH* gene. The Y axis represents normalized tumor cell killing values, while the X axis represents the percentage of a cell population expressing CD16.

[0137] Fig. 24A is a histogram depicting exemplary ddPCR data measured at day 9 post nucleofection of two different iPSC lines with plasmids and 2 μM RNPs comprising RSQ22337 targeting the *GAPDH* gene and Cas12a (SEQ ID NO: 62), for knock-in of CD16 cargo, a CAR cargo, or a biallelic GFP/mCherry cargo into the *GAPDH* gene.

[0138] Fig. 24B depicts exemplary flow cytometry data from iPSC lines edited with plasmids and 2 μM RNPs comprising RSQ22337 targeting the *GAPDH* gene and Cas12a (SEQ ID NO: 62) for knock-in of CXCR2 cargo into the *GAPDH* gene (GAPDH::CXCR2), or control iPSCs transformed with RNP only (Wild-type). CXCR2 expression is noted on the X axis, edited cells expressing CXCR2 were 29.2% of the bulk edited cell population, while surface expression of CXCR2 was 8.53% of the bulk edited cell population.

[0139] Fig. 25 is a histogram depicting the knock-in efficiency of a series of knock-in cassette cargo sequences such as CD16-P2A-CAR, CD16-IRES-CAR, CAR-P2A-CD16, CAR-IRES-CD16, and mbIL-15 into the *GAPDH* gene using RNPs comprising RSQ22337 targeting the *GAPDH* gene and Cas12a (SEQ ID NO: 62), measured on day 0 post-electroporation using ddPCR targeting the 5′ (5′ CDN probe) and 3′ (3′ PolyA probe) positions of the knock-in "cargo".

[0140] Fig. 26 diagrammatically depicts a membrane-bound IL15.IL15Rα (mbIL-15) construct that can be utilized as a knock-in cargo sequence as described herein.

[0141] Fig. 27 is a histogram depicting the TI of mbIL-15 into the *GAPDH* gene when measured as a percentage of a bulk edited population. Shown are TI rates from iPSCs that that are on day 28 of the differentiation to iNK cell process.

[0142] Fig. 28A depicts exemplary flow cytometry data from bulk edited mbIL-15 *GAPDH* gene knock-in iPSC populations at day 39 of differentiation into iNKs.

[0143] Fig. 28B depicts exemplary flow cytometry data from bulk edited mbIL-15 *GAPDH* gene knock-in iPSC populations at day 39 of differentiation into iNKs.

[0144] Fig 28C shows surface expression phenotypes (measured as a percentage of the population) of bulk edited mbIL-15 *GAPDH* gene knock-in iPSC populations being

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differentiated into iNK cells as compared to parental clone cells also being differentiated into iNK cells ("WT") at day 32, day 39, day 42, and day 49 of iPSC differentiation.

[0145] Fig. 29 shows the results from two *in-vitro* tumor cell killing assays. Two biological replicates of bulk edited iPSC populations (S1 and S2) comprising mbIL-15 knock-in at the *GAPDH* gene were differentiated into iNK cells (day 56 of differentiation for S2, and day 63 of differentiation for S1) and functioned to reduce hematological cancer cells (e.g., Raji cells) fluorescence signal when compared to WT parental cells also differentiated into iNK cells, measured in the absence or presence of 10 μg/mL rituximab, E:T ratios of 1 (A) or 2.5 (B); (experiments performed in duplicate, R1 and R2).

[0146] Fig. 30A shows the results of a solid tumor killing assay as described in FIG 20. Two biological replicates of bulk edited iPSC populations (S1 and S2) comprising mbIL-15 knock-in at the *GAPDH* gene were differentiated into iNK cells (day 39 of iPSC differentiation) and functioned to reduce tumor cell spheroid size when compared to WT parental cells also differentiated into iNK cells. Addition of 5 ng/mL exogenous IL-15 increased tumor cell killing by iNKs. The Y axis depicts normalized total integrated red object intensity, a proxy for tumor cell abundance, while the X axis depicts E:T ratio.

[0147] Fig. 30B shows the results of solid tumor killing assays as described in FIG 20. Two biological replicates of bulk edited iPSC populations (S1 and S2) comprising mbIL-15 knock-in at the *GAPDH* gene were differentiated into iNK cells (day 39 of differentiation) and functioned to reduce tumor cell spheroid size when compared to WT parental cells also differentiated into iNK cells at corresponding stages of differentiation and E:T ratios (shown is an E:T ratio of approximately 31.6). Addition of 5 ng/mL exogenous IL-15 was necessary for robust WT iNK cell spheroid reduction, while mbIL-15 KI iNK cells were able to reduce tumor volume without exogenous IL-15. X axis represents time (0-100 hr) while the Y axis represents killing efficacy as measured by normalized total red object area (e.g., presence of tumor cells).

[0148] Fig. 30C shows the results of solid tumor killing assays as described in FIG 20. Two biological replicates of bulk edited populations (S1 and S2) comprising mbIL-15 knock-in at the *GAPDH* gene were differentiated into iNK cells (e.g., at day 39, day 42, day 49, day 56, and day 63 of differentiation) and functioned to reduce tumor cell spheroid size when compared

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to WT parental cells at corresponding stages of iNK cell differentiation (experiments performed in duplicate, R1 and R2).

[0149] Fig. 30D shows the results of solid tumor killing assays as described in FIG 20. Two biological replicates of bulk edited iPSC populations (S1 and S2) comprising mbIL-15 knock-in at the *GAPDH* gene were differentiated into iNK cells (e.g., at day 39, day 42, day 49, day 56, and day 63 of differentiation; in duplicate R1 and R2) and functioned to reduce tumor cell spheroid size when compared to WT parental cells at corresponding stages of iNK cell differentiation (experiments performed in duplicate, R1 and R2). Cell populations were supplemented with exogenous IL-15 (5 ng/mL), leading to more robust iNK cell induced spheroid reduction at each stage of maturation tested when compared to non-supplemented cells (Figure 30C) (experiments performed in duplicate, R1 and R2).

[0150] Fig. 31A shows the results of solid tumor killing assays as described in FIG 20. Two biological replicates of bulk edited iPSC populations (S1 and S2) comprising mbIL-15 knock-in at the *GAPDH* gene were differentiated into iNK cells (day 63 of iPSC differentiation for S1, and day 56 of iPSC differentiation for S2) and functioned to reduce tumor cell spheroid size. The Y axis represents killing efficacy as measured by normalized total red object area (e.g., presence of tumor cells), while the X axis represents the E:T cell ratio; experiments were performed in duplicate or triplicate, R1, R2, and R2.1.

[0151] Fig. 31B shows the results of solid tumor killing assays as described in 31A, but with the addition of 10 μg/mL Herceptin antibody, an addition that triggers ADCC tumor cell killing.

[0152] Fig. 31C shows the results of solid tumor killing assays as described in 31A, but with the addition of 5 ng/mL exogenous IL-15.

[0153] Fig. 31D shows the results of solid tumor killing assays as described in 31A, but with the addition of 5 ng/mL exogenous IL-15 and 10 μg/mL Herceptin antibody, an addition that triggers ADCC tumor cell killing.

[0154] Fig. 32 depicts the cumulative results of two independent sets of cells and 3-5 repeats of solid tumor killing assays as described in FIG 20. Two independent bulk edited populations (S1 and S2) comprising mbIL-15 knock-in at the *GAPDH* gene were differentiated into iNK cells (day 39 and 49 of iPSC differentiation for set 1, and day 42 of iPSC differentiation

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for S2) and functioned to significantly reduce tumor cell spheroid size when compared to differentiated WT parental cell iNKs in the absence of exogenous IL-15 (P=0.034, +/- standard deviation, unpaired t-test); in addition, differentiated knock-in cells trended towards significant reduction of tumor cell spheroid size when compared to differentiated WT parental cells in the presence of 5 ng/mL exogenous IL-15 (P=0.052, +/- standard deviation, unpaired t-test).

[0155] Fig. 33A schematically depicts a knock-in cassette cargo sequence comprising membrane-bound IL15.IL15Rα (mbIL-15) coupled with a GFP sequence, for integration at a target gene as described herein.

[0156] Fig. 33B schematically depicts a knock-in cassette cargo sequence comprising CD16, IL15, and IL15Rα, for integration at a target gene as described herein.

[0157] Fig. 33C schematically depicts a knock-in cassette cargo sequence comprising CD16 and membrane bound IL15.IL15Rα (mbIL-15), for integration at a target gene as described herein.

[0158] Fig. 34A depicts exemplary flow cytometry data from bulk edited iPSC populations seven days after transformation with PLA1829 (see Fig. 33A) comprising a cargo sequence of membrane-bound IL15.IL15Rα (mbIL-15) coupled with a GFP sequence inserted in the *GAPDH* gene using RNPs comprising RSQ22337 targeting the *GAPDH* gene and Cas12a (SEQ ID NO: 62), or control WT cells transformed with RNPs only, measured using ddPCR. Shown on the Y axis is IL-15Rα expression, while GFP expression is shown on the X axis.

[0159] Fig. 34B depicts exemplary flow cytometry data from bulk edited iPSC populations seven days after transformation with PLA1832 or PLA1834 (see Fig. 33B and 33C), comprising a cargo sequence of CD16, IL-15, and IL15Rα, or comprising a cargo sequence of CD16 and membrane-bound IL15.IL15Rα (mbIL-15); inserted in the *GAPDH* gene using RNPs comprising RSQ22337 targeting the *GAPDH* gene and Cas12a (SEQ ID NO: 62), measured using ddPCR. Shown on the Y axis is IL-15Rα expression, X axis is GFP expression.

[0160] Fig. 35A is a histogram depicting the genotypes of individual colonies following transformation as described in Fig. 34A with PLA1829 (5 μg) and 2 μM RNPs comprising RSQ22337 targeting the *GAPDH* gene and Cas12a (SEQ ID NO: 62), measured using ddPCR. Shown are individual homozygous (~100% TI), heterozygous (~50% TI), or wild type (~0% TI) cells.

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[0161] Fig. 35B is a histogram depicting the genotypes of individual colonies following transformation as described in Fig. 34B with PLA1832 (5 μg) and 2 μM RNPs comprising RSQ22337 targeting the *GAPDH* gene and Cas12a (SEQ ID NO: 62), measured using ddPCR. Shown are individual homozygous (~100% TI), heterozygous (~50% TI), or wild type (~0% TI) cells.

[0162] Fig. 35C is a histogram depicting the genotypes of individual colonies following transformation as described in Fig. 34B with PLA1834 (5 μg) and 2 μM RNPs comprising RSQ22337 targeting the *GAPDH* gene and Cas12a (SEQ ID NO: 62), measured using ddPCR. Shown are individual homozygous (~100% TI), heterozygous (~50% TI), or wild type (~0% TI) cells.

[0163] Fig. 36A depicts exemplary flow cytometry data from cells comprising knock-in cargo sequences from PLA1829, PLA1832, or PLA1834 at the *GAPDH* gene (as described in Fig. 34A-34C) measured at day 32 of differentiation into iNKs; "WT" cells were transformed with RNPs only and were also at day 32 of differentiation into iNKs. The data highlights the efficiency of integration and expression of knock-in cassettes comprising an IL-15Rα protein encoding "cargo" sequence. The Y axis quantifies the percentage of cells from the noted population that are expressing IL-15Rα, while the X axis denotes colony genotype.

[0164] Fig. 36B depicts exemplary flow cytometry data from cells comprising knock-in cargo sequences from PLA1829, PLA1832, or PLA1834 at the *GAPDH* gene (as described in Fig. 34A-34C) measured at day 32 of differentiation into iNKs; "WT" cells were transformed with RNPs only and were also at day 32 of differentiation into iNKs. The data highlights the efficiency of integration and expression of knock-in cassettes comprising a CD16 protein encoding "cargo" sequence. The Y axis quantifies the percentage of cells from the noted population that are expressing CD16, while the X axis denotes colony genotype.

[0165] Fig. 36C depicts exemplary flow cytometry data from cells comprising knock-in cargo sequences from PLA1829, PLA1832, or PLA1834 at the *GAPDH* gene (as described in Fig. 34A-34C) measured at day 32 of differentiation into iNKs; "WT" cells were transformed with RNPs only and were also at day 32 of differentiation into iNKs. The data highlights the efficiency of integration and expression of knock-in cassettes comprising an IL-15Rα protein

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encoding "cargo" sequence. The Y axis quantifies the median fluorescence intensity (MFI) of a cell population expressing IL-15Rα, while the X axis denotes colony genotype.

[0166] Fig. 36D depicts exemplary flow cytometry data from cells comprising knock-in cargo sequences from PLA1829, PLA1832, or PLA1834 at the *GAPDH* gene (as described in Fig. 34A-34C) measured at day 32 of differentiation into iNKs; "WT" cells were transformed with RNPs only and were also at day 32 of differentiation into iNKs. The data highlights the efficiency of integration and expression of knock-in cassettes comprising a CD16 protein encoding "cargo" sequence. The Y axis quantifies the median fluorescence intensity (MFI) of a cell population expressing CD16, while the X axis denotes colony genotype.

[0167] Fig. 36E shows exemplary flow cytometry data from unedited (WT) cells or homozygous cells comprising knock-in cargo sequences from PLA1834 at the *GAPDH* locus ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$). The data highlights the efficiency of integration and expression of knock-in cassettes comprising a CD16 and IL-15Rα protein encoding cargo sequence. The Y axis quantifies the percentage of cells from the noted population that are expressing the selected gene, while the X axis denotes whether the selected gene is CD16 or IL-15Rα.

[0168] Fig. 36F depicts exemplary flow cytometry data from iNK cells comprising knock-in cargo sequences from PLA1829 or PLA1834 at the *GAPDH* gene, or from WT cells, before or after cytotoxicity assay in the absence of trastuzumab (Herceptin).

[0169] Fig. 36G depicts exemplary flow cytometry data from iNK cells comprising knock-in cargo sequences from PLA1829 or PLA1834 at the *GAPDH* gene, or from WT cells, before or after cytotoxicity assay in the presence of trastuzumab (Herceptin).

[0170] Fig. 36H depicts CD16 surface expression from two independent flow cytometry analyses of homozygous iNK cells comprising knock-in cargo sequences from PLA1834 at the *GAPDH* gene ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$), or unedited (WT) cells. CD16 surface expression was assessed before or after a 2D cell killing (LDH) assay and in absence or presence of trastuzumab. The Y axis quantifies the percentage of cells from the noted population that are CD56/CD16+, while the X axis denotes whether the sample was before or after the 2D killing assay.

[0171] Fig. 36I depicts percent cytotoxicity demonstrated by homozygous PLA1834-transformed ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) iNK cells or unedited (WT) iNK cells in a 2D cell killing assay (LDH assay). Assays were performed in the presence or absence of 10 μg/ml trastuzumab

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at an E:T ratio of 1 (left) or 2.5 (right). The Y axis quantifies the percent cytotoxicity, while the X axis denotes the presence or absence of trastuzumab. *p<0.05, **p<0.01 (two-way ANOVA).

[0172] Fig. 36J depicts total cell number (left panel) of iNK cells comprising knock-in cargo sequences from PLA1829 or PLA1834 at the *GAPDH* gene, or of unedited (WT) iNK cells, following an *in vitro* persistence assay in the absence of the cytokines, IL-2 and IL-15. Fold change of cells comprising a knock-in from PLA1834 relative to cells comprising a homozygous knock-in from PLA1829 is shown in the top right panel. Fold change of cells comprising a homozygous knock-in from PLA1834 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) relative to unedited (WT) cells is shown in the bottom right panel.

[0173] Fig. 37A shows the results of a solid tumor killing assay as described in Fig. 20. Clones comprising homozygous CD16 knock-in at the *GAPDH* gene were differentiated into iNK cells and functioned to reduce tumor cell spheroid size, particularly following the addition of an antibody, e.g., 10 μg/mL trastuzumab. The addition of an antibody promotes antibody dependent cellular cytotoxicity (ADCC) and tumor cell killing by iNKs. Control "WT" cells were bulk unedited parental clones that were electroporated without RNPs or plasmids and were at the same stage of iNK cell differentiation as test cells. The Y axis depicts normalized total integrated red object intensity, a proxy for tumor cell abundance, while the X axis depicts the Effector to Target cell (E:T) ratio. The IC50 for "WT" cells was an E:T ratio of 3.0, while the IC50 for SLEEK CD16 KI cells was an E:T ratio of 0.5.

[0174] Fig. 37B shows the results of a 3D tumor spheroid killing assay conducted as depicted in Fig. 20. Homozygous PLA1834-transformed ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) iNK cells and unedited (WT) iNK cells were introduced to SK-OV-3 tumor cells at an E:T ratio of 10 in the absence (left panels) or presence (right panels) of 10 μg/ml trastuzumab. The top panels display imaging of the tumor spheroid at 0 hours and 100 hours with visibility of the red object signal used to measure tumor cell abundance. The bottom panels display spheroid size as measured via the integrated red object intensity on the Y axis and time in hours on the X axis.

[0175] Fig. 37C shows the results of 3D tumor spheroid killing assays conducted as depicted in Fig. 20. Unedited (WT) iNK cells, peripheral blood NK cells, and two clones of homozygous PLA1834-transformed ($CD16^{+/-}$/$mbIL\text{-}15^{+/+}$) iNK cells were used against SK-OV-3 tumor cells at varying E:T ratios. In the left panels, 5 ng/ml exogenous IL-15 and 10 μg/ml

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trastuzumab was present. Two independent experiments were performed for each type of cell or clone with the exception of one experiment for the peripheral blood NK cells. IC50 values based on the top left panel are presented in the table in the bottom left panel and highlight the greater efficacy of the $CD16^{+/-}$/mbIL-$15^{+/+}$ iNK cells in killing tumor cells. The right panel displays IC50 values from 3D tumor spheroid killing assays for homozygous PLA1834-transformed ($CD16^{+/+}$/mbIL-$15^{+/+}$) iNK cells and unedited (WT) iNK cells in the absence and presence of 10 μg/ml trastuzumab. *$p<0.05$, **$p<0.01$ (unpaired t-test).

[0176] Fig. 38A depicts percent cytotoxicity demonstrated by mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI iNK cells or unedited (WT) iNK cells in a lactate dehydrogenase (LDH) cytotoxicity assay. Three different clones (A2, A4, C4) of mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI iNK cells were tested. Assays were performed in the presence or absence of 10 μg/ml trastuzumab and at an E:T ratio of 1. The Y axis quantifies the percent cytotoxicity, while the X axis denotes the iNK cells examined. Error bars denote standard deviation.

[0177] Fig. 38B depicts flow cytometry data of unedited (WT) and mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI iNK cells. Two clones (A2, A4) of mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI iNK cells were examined. Cells were pre-gated for living hCD45+ cells and further analyzed for CD16/CD56 expression. Approximately 100% of mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI iNK cells displayed high CD16 expression compared to approximately 50% of WT iNK cells.

[0178] Fig. 38C is a schematic of an *in vivo* tumor killing assay. Mice were intraperitoneally inoculated with 0.25×10^6 SKOV3-luc cells, and following 2-6 days to allow for tumor establishment, mice were randomized into groups. One day later, mice intraperitoneally received 2×10^6 or 5×10^6 mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{-/+}$) DKI iNK cells in combination with 2.5 mpk trastuzumab. In some treatment groups, mice received an additional dose of 2.5 mpk trastuzumab at 35 days (as indicated by the arrowhead) or at 21, 28, and 35 days (as indicated by the arrows) post-introduction of iNK cells. Mice were followed for up to 90 days post-introduction of iNK cells. The X axis represents time since introduction of NK cells.

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[0179] Fig. 38D shows averaged results with standard error of the mean of the *in vivo* tumor killing assay described in Fig. 38C. Groups of mice are represented by each horizontal line. The groups included mice that received mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells (DKI iNK) with trastuzumab, trastuzumab alone, or an isotype control. Doses of trastuzumab are indicated by arrows and arrowheads for groups receiving a total of 4 doses or 2 doses, respectively. The X axis represents time since introduction of NK cells, while the Y axis represents tumor burden as measured by bioluminescent imaging (BLI) using an *in vivo* imaging system (IVIS).

[0180] Fig. 38E shows the survival of mice subjected to the *in vivo* tumor killing assay described in Fig. 38C. Groups of mice are represented by each horizontal line. Mice dosed with mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells in combination with trastuzumab (5M DKI iNK + Tras. x 4, 2M DKI iNK + Tras x 2) had prolonged survival compared to mice dosed with trastuzumab alone. The X axis represents time since introduction of NK cells, while the Y axis represents percent survival of the mice.

[0181] Fig. 38F shows bioluminescent imaging of mice subjected to the *in vivo* tumor killing assay described in Fig. 38C. The treatment groups of the mice are denoted along the top of the panel, while the time since introduction of NK cells is denoted along the left side of the panel. The right color scale represents the radiance ($p/sec/cm^2/sr$) of the bioluminescence (from a minimum of 2.23×10^6 to a maximum of 5.57×10^7) as seen in the images.

[0182] Fig. 38G shows flow cytometry data of cells obtained by peritoneal lavage of mice subjected to the *in vivo* tumor killing assay described in Fig. 38C. The top row shows data following sacrifice at day 90, from the mouse that received 5×10^6 mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells + trastuzumab according to the *in vivo* tumor killing assay as described in Fig. 38C. The bottom row shows data following sacrifice at day 118, from the mouse that received 2×10^6 mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells + trastuzumab according to the *in vivo* tumor killing assay as described in Fig. 38C. iNK cells (inset boxes in top left and bottom left) were identified by flow cytometry using the human CD46 (hCD46) marker and further analyzed for expression of CD16/CD56. The data highlights that the mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells persist *in vivo* for at least 118 days.

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[0183] Fig. 39A is a schematic of an *in vivo* tumor killing assay. Mice were intraperitoneally inoculated with 0.25 x 10^6 SKOV3-luc cells, and following 2-6 days to allow for tumor establishment, mice were randomized into groups. One day later, mice intraperitoneally received 5 x 10^6 (5M) unedited (WT) or mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells. In some treatment groups, mice received a single dose of 2.5 mpk trastuzumab at introduction of the iNK cells (day 0) or multiple doses of 2.5 mpk trastuzumab at 0, 7, and 14 days (as indicated by the arrows) post-introduction of iNK cells.

[0184] Fig. 39B shows tumor burden (median with interquartile range) for the *in vivo* tumor killing assay described in Fig. 39A. Groups of mice are represented by each horizontal line. Each treatment group had 8 mice. The groups included mice that received unedited (WT) iNK cells, mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells (DKI iNK), or an isotype control. The mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK clone (A2) used corresponds to the A2 clone as identified in Fig. 35C, 38A, and 38B. The X axis represents time since introduction of NK cells, while the Y axis represents tumor burden as measured by bioluminescent imaging (BLI) using an *in vivo* imaging system (IVIS).

[0185] Fig. 39C shows tumor burden (median with interquartile range) for the *in vivo* tumor killing assay described in Fig. 39A. Groups of mice are represented by each horizontal line. Each treatment group had 8 mice. The groups included mice that received unedited (WT) iNK cells + trastuzumab, mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells (DKI iNK) + trastuzumab, trastuzumab alone, or an isotype control. The mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK clones (A2, A4) used correspond to the A2 and A4 clones as identified in Fig. 35C, 38A, and 38B. Dosing of trastuzumab on day 0 is indicated by the arrow. The X axis represents time since introduction of NK cells, while the Y axis represents tumor burden as measured by bioluminescent imaging (BLI) using an *in vivo* imaging system (IVIS).

[0186] Fig. 39D shows the survival of mice subjected to the *in vivo* tumor killing assay described in Fig. 39A. Groups of mice are represented by each horizontal line. Mice dosed with mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells in combination with trastuzumab (DKI iNK + Tras. x 1) had significantly prolonged survival compared to mice dosed with trastuzumab alone (Trastuzumab x 1). The X axis represents time since introduction of NK cells, while the Y axis represents percent survival of the mice. ****$p<0.0001$ (Log-rank Mantel-Cox test).

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[0187] Fig. 39E shows tumor burden (median with interquartile range) for the *in vivo* tumor killing assay described in Fig. 39A. Groups of mice are represented by each horizontal line. Each treatment group had 8 mice. The groups included mice that received unedited (WT) iNK cells in combination with trastuzumab (TRA x 3), mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells in combination with trastuzumab (TRA x 3), trastuzumab (TRA x 3) alone, or an isotype control. The mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK clone used corresponds to the A2 clone as identified in, e.g., Fig. 35C, 38A, and 38B. Mice dosed with the mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells + trastuzumab had significantly decreased tumor burden as compared to mice dosed with WT iNK cells + trastuzumab. Doses of trastuzumab on day 0, 7, and 14 are indicated by the arrows. The X axis represents time since introduction of NK cells, while the Y axis represents tumor burden as measured by bioluminescent imaging (BLI) using an *in vivo* imaging system (IVIS). ***$p<0.001$ (unpaired t-test).

[0188] Fig. 39F shows the survival of mice subjected to the *in vivo* tumor killing assay described in Fig. 39A. Groups of mice are represented by each horizontal line. Mice dosed with mbIL-15/CD16 ($CD16^{+/+}$/$mIL\text{-}15^{+/+}$) DKI iNK cells in combination with trastuzumab (x3) had significantly prolonged survival compared to mice dosed with WT iNK cells in combination with trastuzumab (x3). Additionally, mice dosed with either mbIL-15/CD16 ($CD16^{+/+}$/$mIL\text{-}15^{+/+}$) DKI iNK cells + trastuzumab (x3) or WT iNK cells + trastuzumab (x3) had a significantly greater probability of survival as compared to trastuzumab alone (TRA x 3, TRA x 1). The X axis represents time since introduction of NK cells, while the Y axis represents percent survival of the mice. *$p<0.05$ (unpaired t-test).

[0189] Fig. 39G shows measured tumor burden per mouse on day 33 of the *in vivo* tumor killing assay described in Fig. 39A. The left panel depicts data for mice receiving a single dose of trastuzumab (on day 0 post-introduction of iNK cells). The right panel depicts data for mice receiving three doses of trastuzumab (on days 0, 7, and 14 post-introduction of iNK cells). The X axis denotes the treatment group, while the Y axis represents tumor burden as measured by bioluminescent imaging (BLI) using an *in vivo* imaging system (IVIS). **$p<0.01$, ****$p<0.0001$, ns denotes not significant (unpaired t-test).

[0190] Fig. 39H shows measured tumor burden per mouse on day 11 (left panel) and day 54 (right panel) of the *in vivo* tumor killing assay described in Fig. 39A. Mice dosed with mbIL-

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15/CD16 ($CD16^{+/+}$/mIL-$15^{+/+}$) DKI iNK cells in combination with trastuzumab (DKI iNK + Tras. x 1) had significantly reduced tumor burden at day 11 and at day 54 as compared to mice dosed with unedited iNK cells in combination with trastuzumab or trastuzumab alone. The X axis denotes the treatment group, while the Y axis represents tumor burden as measured by bioluminescent imaging (BLI) using an *in vivo* imaging system (IVIS). ***$p<0.001$, ****$p<0.0001$ (Mann-Whitney test).

[0191] Fig. 39I shows representative bioluminescent imaging of mice subjected to the *in vivo* tumor killing assay described in Fig. 39A. The treatment groups of the mice are denoted along the top of the panel, while the time since introduction of NK cells is denoted along the left side of the panel. Each treatment group had 8 mice. The table below the images displays the number of tumor free mice / total mice in the treatment group (from top of panel) at day 40 post-introduction of NK cells. The bottom color scale represents the radiance (p/sec/cm^2/sr) of the bioluminescence (from a minimum of 2.30×10^5 to a maximum of 3.72×10^7) as seen in the images.

[0192] Fig. 39J depicts flow cytometry data of cells obtained by peritoneal lavage of mice subjected to the *in vivo* tumor killing assay described in Fig. 39A. The top row shows representative data following sacrifice at day 144 from mice that received WT iNK cells + trastuzumab (x3) according to the *in vivo* tumor killing assay as described in Fig. 39A. The bottom row shows representative data following sacrifice at day 144 from mice that received mbIL-15/CD16 ($CD16^{+/+}$/mIL-$15^{+/+}$) DKI iNK cells + trastuzumab (x3) according to the *in vivo* tumor killing assay as described in Fig. 39A. iNK cells (inset boxes in top left and bottom left) were identified by flow cytometry using the human CD45 (hCD45) marker and further analyzed for expression of human CD16 (hCD16) and human CD56 (hCD56). The data highlights that the mbIL-15/CD16 ($CD16^{+/+}$/mIL-$15^{+/+}$) DKI iNK cells persist *in vivo* for at least 144 days and almost all of these cells continue to express CD16 on their surface.

[0193] Fig. 40 shows microscopy of cell morphology and flow cytometry of pluripotency markers of human induced pluripotent stem cells (hiPSCs) grown in various media in the absence or presence of Activin A (1 ng/ml or 4 ng/ml ActA).

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[0194] Fig. 41 shows morphology of TGFβRII knockout hiPSCs (clone 7) or CISH/TGFβRII DKO hiPSCs (clone 7) cultured in media with or without Activin A (1 ng/mL, 2 ng/mL, 4 ng/mL, or 10 ng/mL).

[0195] Fig. 42 shows morphology of TGFβRII knockout hiPSCs (clone 9) cultured in media with or without Activin A (1 ng/mL, 2 ng/mL, 4 ng/mL, or 10 ng/mL).

[0196] Fig. 43A shows the bulk editing rates at the CISH and TGFβRII loci for single knockout and double knockout hiPSCs.

[0197] Fig. 43B shows expression of Oct4 and SSEA4 in TGFβRII knockout hiPSCs, CISH knockout hiPSCs, and double knockout hiPSCs cultured in Activin A.

[0198] Fig. 44 shows expression of Nanog and Tra-1-60 in TGFβRII knockout hiPSCs, CISH knockout hiPSCs, and double knockout hiPSCs cultured in Activin A.

[0199] Fig. 45 is a schematic of the procedure related to the STEMdiff™ Trilineage Differentiation Kit (STEMCELL Technologies Inc.).

[0200] Fig. 46A shows expression of differentiation markers of TGFβRII knockout hiPSCs, CISH knockout hiPSCs, and double knockout hiPSCs cultured in Activin A.

[0201] Fig. 46B shows karyotypes of TGFβRII / CISH double knockout hiPSCs cultured in Activin A.

[0202] Fig. 46C shows an expanded Activin A concentration curve performed on an unedited parental PSC line, an edited TGFβRII KO clone (C7), and an additional representative (unedited) cell line designated RUCDR. The minimum concentration of Activin A required to maintain each line varied slightly with the TGFβRII KO clone requiring a higher baseline amount of Activin A as compared to the parental control (0.5 ng/ml vs 0.1 ng/ml).

[0203] Fig. 46D shows the stemness marker expression in an unedited parental PSC line, an edited TGFβRII KO clone (C7), and an unedited RUCDR cell line, when cultured with the base medias alone (no supplemental Activin A). The TGFβRII KO iPSCs did not maintain stemness marker expression while the two unedited lines were able to maintain stemness marker expression in E8.

[0204] Fig. 47A is a schematic representation of an exemplary method for creating edited iPSC clones, followed by the differentiation to and characterization of enhanced CD56+ iNK cells.

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[0205] Fig. 47B is a schematic of an iNK cell differentiation process utilizing STEMDiff APEL2 during the second stage of the differentiation process.

[0206] Fig. 47C is a schematic of an iNK cell differentiation process utilizing NK-MACS with 15% serum during the second stage of the differentiation process.

[0207] Fig. 47D shows the fold-expansion of unedited PCS-derived iNK cells and the percentage of iNK cells expressing CD45 and CD56 at day 39 of differentiation when differentiated using NK-MACS or Apel2 methods as depicted in Fig. 47C and Fig. 47B respectively.

[0208] Fig. 47E shows in the upper panel a heat map of the surface expression phenotypes (measured as a percentage of the population) of differentiated iNK cells derived from unedited PCS iPSCs when differentiated using NK-MACS or APEL2 methods as depicted in Fig. 47C and Fig. 47B respectively. The bottom panel displays representative histogram plots to illustrate the differences in the iNKs generated by the two methods.

[0209] Fig. 47F shows a heat map of the surface expression phenotypes (measured as a percentage of the population) of differentiated edited iNKs (TGFβRII knockout, CISH knockout, and double knockout (DKO)) and unedited parental iPSCs (WT) when differentiated using NK-MACS or APEL2 methods as depicted in Fig. 47C and Fig. 47B respectively.

[0210] Fig. 47G shows unedited iNK cell effector function when differentiated using NK-MACS or APEL2 methods as depicted in Fig. 47C and Fig. 47B respectively.

[0211] Fig. 48 shows differentiation phenotypes of edited clones (TGFβRII knockout, CISH knockout, and double knockout) as compared to parental wild type clones.

[0212] Fig. 49 shows surface expression phenotype of edited iNKs (TGFβRII knockout, CISH knockout, and double knockout) as compared to parental clone iNKs and wild type cells.

[0213] Fig. 50A shows surface expression phenotype of edited iNKs (TGFβRII knockout, CISH knockout, and double knockout) as compared to parental clone iNKs ("WT") and peripheral blood-derived natural killer cells.

[0214] Fig. 50B is a flow cytometry histogram plot that shows the surface expression phenotype of edited iNK cells (TGFβRII/CISH double knockout) as compared to parental clone iNK cells ("unedited iNK cells").

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[0215] Fig. 50C shows surface expression phenotypes (measured as a percentage of the population) of edited iNK cells (TGFβRII/CISH double knockout) as compared to parental clone iNK cells ("unedited iNK cells") at day 25, day 32, and day 39 post-hiPSC differentiation (average values from at least 5 separate differentiations).

[0216] Fig. 50D shows pSTAT3 expression phenotypes (measured as a percentage of the population) of edited CD56+ iNK cells ("CISH KO iNKs") as compared to parental clone CD56+ iNK cells ("unedited iNKs") at 10 minutes and 120 minutes following IL-15 induced activation. Briefly, the day 39 or day 40 iNKs are plated the day before in a cytokine starved condition. The next day the cells are stimulated with 10 ng/ml of IL15 for the length of time indicated. The cells are fixed immediately at the end of the time point, stained for CD56 followed by an intracellular stain. The cells were processed on a NovoCyte Quanteon and the data was analyzed in FlowJo. Data shown is a representative experiment of >3 experiments performed.

[0217] Fig. 50E shows pSMAD2/3 expression phenotypes (measured as a percentage of the population) of edited CD56+ iNK cells (TGFβRII/CISH double knockout, "DKO iNKs") as compared to parental clone CD56+ iNK cells ("unedited iNK cells") at 10 minutes and 120 minutes following IL-15 and TGF-β induced activation. Briefly, the day 39 or day 40 iNKs were plated the day before in a cytokine starved condition. The next day the cells were stimulated with 10 ng/ml of IL-15 and 50 ng/ml of TGF-β for the length of time indicated. The cells were fixed immediately at the end of the time point, stained for CD56 followed by an intracellular stain. The cells were processed on a NovoCyte Quanteon and the data was analyzed in FlowJo. Data shown is a representative experiment of >3 experiments performed.

[0218] Fig. 50F shows IFN-γ expression phenotypes (measured as a percentage of the population) of edited CD56+ iNK cells (TGFβRII/CISH double knockout, "DKO IFNg") as compared to parental clone CD56+ iNK cells (unedited iNKs, "WT IFNg") with or without phorbol myristate acetate (PMA) and ionomycin (IMN) stimulation. The data is representative. It is generated from a single differentiation and each condition in the assay is run with 2 technical replicates. **$p<0.05$ vs unedited iNK cells (paired t test).

[0219] Fig. 50G shows TNF-α expression phenotypes (measured as a percentage of the population) of edited CD56+ iNK cells (TGFβRII/CISH double knockout, "DKO TNF a") as

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compared to parental clone CD56+ iNK cells (unedited iNK cells, "WT TNFa") with or without Phorbol myristate acetate (PMA) and Ionomycin (IMN) stimulation. The data is representative. It is generated from a single differentiation and each condition in the assay is run with 2 technical replicates. **p<0.05 vs unedited iNK cells (paired t test).

[0220] Fig. 51A is a schematic representation of an exemplary solid tumor cell killing assay, depicting the use of edited iNK cells (TGFβRII/CISH double knockout) to kill SK-OV-3 ovarian cells in the presence or absence of IL-15 and TGF-β.

[0221] Fig. 51B shows the results of a solid tumor killing assay as described in Fig. 51A. iNK cells function to reduce tumor cell spheroid size. Certain edited iNK cells (CISH single knockout, "CISH_2, 4, 5, and 8") were not significantly different from the parental clone iNK cells ("WT_2"), while certain edited iNK cells (TGFβRII single knockout, "TGFβRII_7", and TGFβRII/CISH double knockout "DKO") functioned significantly better at effector-target (E:T) ratios of 1 or greater when measured in the presence of TGF- β as compared to parental clone iNK cells ("WT_2"). ****p<0.0001 vs unedited iNK cells (two-way ANOVA, Sidak's multiple comparisons test).

[0222] Fig. 51C shows edited iNK cell effector function as compared to unedited iNK cells.

[0223] Fig. 52 shows the results of an *in-vitro* serial killing assay, where iNK cells are serially challenged with hematological cancer cells (e.g., Nalm6 cells) in the presence of 10 ng/ml of IL-15 and 10 ng/ml of TGF-β; the X axis represents time, with tumor cells being added every 48 hours, while the Y axis represents killing efficacy as measured by normalized total red object area (e.g., presence of tumor cells). The data shows that edited iNK cells (TGFβRII/CISH double knockout) continue to kill hematological cancer cells while unedited iNK cells lose this function at equivalent time points.

[0224] Fig. 53 shows surface expression phenotypes (measured as a percentage of the population) of certain edited iNK clonal cells (CISH single knockout "CISH_C2, C4, C5, and C8", TGFβRII single knockout "TGFβRII-C7", and TGFβRII/CISH double knockout "DKO-C1") as compared to parental clone iNK cells ("WT") at day 25, day 32, and day 39 post-hiPSC differentiation when cultured in the presence of 1 ng/mL or 10 ng/mL IL-15.

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[0225] Fig. 54A is a schematic of an *in-vivo* tumor killing assay. Mice were intraperitoneally inoculated with 1 x 10^6 SKOV3-luc cells, mice are randomized, and 4 days later, 20 x 10^6 iNK cells were introduced intraperitoneally. Mice were followed for up to 60 days post-tumor implantation. The X axis represents time since implantation, while the Y axis represents killing efficacy as measured by total bioluminescence (p/s).

[0226] Fig. 54B shows the results of an *in-vivo* tumor killing assay as described in Fig. 54A. An individual mouse is represented by each horizontal line. The data show that both unedited iNK cells ("unedited iNK") and DKO edited iNK cells (TGFβRII/CISH double knockout) prevent tumor growth better than vehicle, while edited iNK cells kill tumor cells significantly better than vehicle in-vivo. Each experimental group had 9 animals each. ***p<0.001, ****p<0.0001 by a 2-way ANOVA analysis.

[0227] Fig. 54C shows the averaged results with standard error of the mean of the in-vivo tumor killing assay described in Fig. 54B. Populations of mice are represented by each horizontal line. The data show that DKO edited iNK cells (TGFβRII/CISH double knockout) prevent tumor growth and kill tumor cells significantly better than vehicle or unedited iNK cells *in-vivo*. ***p<0.001, ****p<0.0001 by a 2-way ANOVA analysis.

[0228] Fig. 55A shows surface expression phenotypes (measured as a percentage of the population) of bulk edited iNK cells (left panel - ADORA2A single knockout) or certain edited iNK clonal cells (right panel - ADORA2A single knockout) as compared to parental clone iNK cells ("PCS_WT") at day 25, day 32, and day 39 or at day 28, day 36, and day 39 post-hiPSC differentiation. Representative data from multiple differentiations.

[0229] Fig. 55B shows cyclic AMP (cAMP) concentration phenotypes following 5′-(N-Ethylcarboxamido)adenosine ("NECA", adenosine agonist) activation for edited iNK clonal cells (ADORA2A single knockout) as compared to parental clone iNK cells ("unedited iNKs"). The Y axis represents average cAMP concentration in nM (a proxy for ADORA2A activation), while the X axis represents NECA concentration in nM.

[0230] Fig. 55C shows the results of an *in-vitro* serial killing assay, where iNK cells are serially challenged with hematological cancer cells (e.g., Nalm6 cells) in the presence of 100μM NECA, and 10 ng/ml of IL-15; the X axis represents time, with tumor cells being added every 48hours, while the Y axis represents killing efficacy as measured by total red object area (e.g.,

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presence of tumor cells). The data shows that edited iNK cells ("ADORA2A KO iNK") kill hematological cancer cells more effectively than unedited iNK cells ("Ctrl iNK") under conditions that mimic adenosine suppression.

[0231] Fig. 56A shows surface expression phenotypes (measured as a percentage of the population) of certain edited iNK clonal cells (TGFβRII/CISH/ADORA2A triple knockout, "CRA_6" and "CR+A_8") as compared to parental clone iNK cells ("WT_2") at day 25, day 32, and day 39 post-hiPSC differentiation. Data is representative of multiple differentiations.

[0232] Fig. 56B shows cyclic AMP (cAMP) concentration phenotypes following NECA (adenosine agonist) activation for edited iNK clonal cells (TGFβRII/CISH/ADORA2A triple knockout, "TKO iNKs") as compared to parental clone iNK cells ("unedited iNKs"). The Y axis represents average cAMP concentration in nM (a proxy for ADORA2A activation), while the X axis represents NECA concentration in nM.

[0233] Fig. 56C shows the results of a solid tumor killing assay as described in Fig. 51A without IL-15. iNK cells function to reduce tumor cell spheroid size. The Y axis measures total integrated red object (e.g., presence of tumor cells), while the X axis represents the effector to target (E:T) cell ratio. The edited iNK cells (ADORA2A single knockout "ADORA2A", TGFβRII/CISH double knockout "DKO", or TGFβRII/CISH/ADORA2A triple knockout "TKO") had lower EC50 rates when measured in the presence of TGF- β as compared to parental clone iNK cells ("Control") (average values from at least 3 separate differentiations).

[0234] Fig. 57 shows the results of guide RNA selection assays for the loci TGFβRII, CISH, ADORA2A, TIGIT, and NKG2A utilizing in-vitro editing in iPSCs.

[0235] Fig. 58A depicts an exemplary flow cytometry chart for a population of T cells transduced by AAV6 comprising a CD19 CAR cargo targeted for knock-in at GAPDH at 5E4 MOI but without the addition of an RNP.

[0236] Fig. 58B depicts an exemplary flow cytometry chart for a population of T cells transduced by AAV6 comprising a CD19 CAR cargo targeted for knock-in at GAPDH at 5E4 MOI and transformed with 1 μM of RNPs comprising Cas12a (SEQ NO: 62) and RSQ22337.

[0237] Fig. 58C depicts exemplary expansion and viability data for a population of T cells transduced by AAV6 as described in Fig. 58A and Fig. 58B.

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[0238] Fig. 58D depicts an exemplary flow cytometry chart for a population of T cells that have been transformed with RNPs targeting the TRAC locus.

[0239] Fig. 58E depicts an exemplary flow cytometry chart for a population of T cells transduced by AAV6 comprising a CD19 CAR cargo targeted for knock-in at GAPDH at 5E4 MOI and transformed with 4 μM of RNPs comprising Cas12a (SEQ NO: 62) and RSQ22337 and RNPs targeting the TRAC locus.

[0240] Fig. 58F depicts a histogram showing genotype data derived from exemplary flow cytometry experiments on populations of T cells transformed with TRAC targeting RNPs, GAPDH targeting RNPs, and/or transduced with AAV6 comprising a CD19 CAR cargo targeted for knock-in at GAPDH. T cells that have CD19 CAR KI were observed at rates greater than 90% when cells were transformed with GAPDH targeting RNPs and transduced with AAV6 comprising the CD19 CAR cargo targeting GAPDH. T cells that have TRAC KO and CD19 CAR KI were observed at rates greater than 80% when cells were transformed with TRAC targeting RNPs, GAPDH targeting RNPs, and transduced with AAV6 comprising a CD19 CAR cargo targeting GAPDH.

[0241] Fig. 58G depicts an exemplary flow cytometry chart for a population of T cells transduced by AAV6 comprising a CD19 CAR cargo targeted for knock-in at GAPDH at 5E4 MOI and transformed with 4 μM of RNPs comprising Cas12a (SEQ NO: 62) with RSQ22337, TRAC targeting RNPs, and TGFBR2 targeting RNPs.

[0242] Fig. 58H depicts a histogram showing genotype data derived from exemplary flow cytometry experiments on populations of T cells transformed with GAPDH targeting RNPs (comprising Cas12a (SEQ ID NO: 62) and RSQ22337), and transduced with AAV6 comprising a GFP cargo targeted for knock-in at GAPDH, a CD19 CAR cargo targeted for knock-in at GAPDH, or an HLA-E alloshield cargo targeted for knock-in at GAPDH. Transgene integration efficiencies greater than 80% at the GAPDH locus were observed for each population of edited T cells.

[0243] Fig. 58I shows the results of an *in-vitro* tumor cell killing assay, where T cells comprising CD19 CAR knock-in at the GAPDH gene were challenged with hematological cancer cells (e.g., Raji cells). Significant Raji cell cytolysis was observed in test samples when compared to control samples comprising cancer cells only or when compared to T cells

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comprising GFP knock-in at the GAPDH gene that were challenged with Raji cells. N = 4, 1 biological replicate in 4 technical replicates, shown are the mean and standard error of the mean, statistical analysis with one-way ANOVA provides a P value of <0.0001.

[0244] Fig. 58J shows the results of an *in-vitro* tumor cell killing assay, where T cells comprising CD19 CAR knock-in at the GAPDH gene in combination with TRAC and/or TGFBR2 knock-out were challenged with hematological cancer cells (e.g., Raji cells). As compared to T cells comprising GFP knock-in at the GAPDH gene or unedited T cells, significant cytotoxicity was observed with T cells comprising the CD19 CAR knock-in as assessed by LDH release following 24 hours of co-culture at an E:T of 2. Average spontaneous LDH release by Raji cells (dashed horizontal line) and average LDH released upon treatment with lysis buffer (solid horizontal line) provided for comparison. Each filled circle represents data from four technical replicates from one biological sample. The X axis denotes T cell group, while the Y axis quantifies LDH release as relative fluorescence units (RFUs) as detected using a plate reader with an excitation of 560nm and emission of 590nm. Black lines represent means. Not significant (n.s.), ***p<0.001, ****p<0.0001 (unpaired t-test).

[0245] Fig. 59 depicts HLA-E surface expression in T cells modified as described herein. Left panel depicts HLA-E surface expression in T cells transduced with AAV6 comprising a B2M-HLA-E cargo targeted for knock-in at GAPDH at 5E4 MOI and transformed with 1 μM of RNPs comprising Cas12a (SEQ NO: 62) with RSQ22337, compared to mock transduced control cells (no AAV6 transduction). Right panel depicts expansion data for T cells comprising knock-in of the B2M-HLA-E cargo at GAPDH and expansion data for the mock transduced control T cells. Cells were stained with PE anti-human HLA-E antibody clone: 3D12 (1:100 dilution).

[0246] Fig. 60A is a comparison of T cells modified as described herein utilizing either a one-step or a sequential process, wherein a combination of RNPs targeting different loci are administered to the T cells either together (one step) or sequentially. The left panel depicts exemplary flow cytometry data from T cells that have undergone a one-step electroporation for transformation with RNPs targeting TRAC, B2M, and GAPDH (0.5 μM of each type of RNP) combined with transduction with AAV6 comprising a GFP cargo targeted for knock-in at GAPDH at 5E4 MOI. The right panel depicts exemplary flow cytometry data from T cells that have undergone a series of electroporations for transformation wherein RNPs targeting GAPDH

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(at 5 μM) were administered to the cells along with transduction with AAV6 comprising a GFP cargo targeted for knock-in at GAPDH at 5E4 MOI, followed four days later by transformation with RNPs targeting TRAC, and RNPs targeting B2M at 0.5 μM of each RNP. Flow cytometry data assayed the number of cells that had at least TRAC knocked-out, the number of cells that had at least B2M knocked-out, and the number of cells that had both TRAC and B2M knocked-out and also exhibited GFP expression. These results show one-step KO/KI has comparable efficiency when compared to sequential KI and KO processes.

[0247] Fig. 60B depicts the total number of editing events found in T cells modified as described herein using a one-step process comprising transforming a population of T cells with RNPs targeting TRAC, B2M, CIITA, TGFBR2, and GAPDH (comprising Cas12a (SEQ ID NO: 62) and RSQ22337, and transducing the cells with an AAV6 comprising a GFP cargo targeted for knock-in at the *GAPDH* gene. Each editing event (KO or cargo KI) occurred at an individual rate of greater than 80%.

[0248] Fig. 61A depicts an exemplary flow cytometry chart for a population of NK cells transduced by AAV6 comprising a GFP cargo targeted for knock-in at GAPDH at 5E4 MOI but without the addition of an RNP.

[0249] Fig. 61B depicts an exemplary flow cytometry chart for a population of NK cells transduced by AAV6 comprising a GFP cargo targeted for knock-in at GAPDH at 5E4 MOI and transformed with 4 μM of RNPs comprising Cas12a (SEQ NO: 62) and RSQ22337.

[0250] Fig. 61C depicts an exemplary flow cytometry chart for a population of NK cells transduced by AAV6 comprising a CD19 CAR cargo targeted for knock-in at GAPDH at 5E4 MOI but without the addition of an RNP.

[0251] Fig. 61D depicts an exemplary flow cytometry chart for a population of NK cells transduced by AAV6 comprising a CD19 CAR cargo targeted for knock-in at GAPDH at 5E4 MOI and transformed with 4 μM of RNPs comprising Cas12a (SEQ NO: 62) and RSQ22337.

[0252] Fig. 61E depicts a histogram showing genotype data derived from exemplary flow cytometry experiments on populations of NK cells transformed with GAPDH targeting RNPs (comprising Cas12a (SEQ ID NO: 62) and RSQ22337) and transduced with AAV6 comprising either a GFP cargo targeted for knock-in at GAPDH at 5E4 MOI or a CD19 CAR cargo targeted

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for knock-in at GAPDH. Transgene integration efficiencies greater than 80% at the GAPDH locus were observed in each edited NK cell population.

[0253] Fig. 61F shows the results of an *in vitro* tumor cell killing assay, where NK cells comprising CD19 CAR knock-in at the *GAPDH* gene were challenged with hematological cancer cells (Raji cells). Significantly greater Raji cell cytolysis was observed in edited NK cells comprising CD19 CAR KI when compared to control NK cells (unedited). N = 3, 1 biological replicate in 3 technical replicates, shown are the mean and standard error of the mean, statistical analysis with one-way ANOVA provides a P value of <0.05.

[0254] Fig. 61G shows the results of an *in vitro* tumor killing assay, where NK cells comprising CD19 CAR knock-in (KI) or GFP knock-in (KI) at the GAPDH gene were challenged with hematological cancer cells (Nalm6 cells). Significantly greater cytotoxicity was observed with NK cells comprising the CD19 CAR knock-in than the GFP knock-in as assessed by BATDA release following 2 hours of co-culture at an E:T of 1. Average spontaneous BATDA release by Nalm6 cells (dashed horizontal line) and average BATDA released upon treatment with lysis buffer (solid horizontal line) provided for comparison. Each filled circle represents data from eight technical replicates from one biological sample. The X axis denotes NK cell group, while the Y axis quantifies BATDA release as relative fluorescence units (RFUs) as detected by a time-resolved fluorometer. Black horizontal lines represent means. ****$p<0.0001$ (unpaired t-test).

[0255] Fig. 62A shows the results of an *in vitro* persistence assay of mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells and unedited (WT) iNK cells. The X axis represents days since removal of exogenous cytokine support, while the Y axis represents the total number of live cells.

[0256] Fig. 62B shows averaged results of an *in vitro* persistence assay of mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells and CD16/mbIL-15 DKI (DKI) iNK cells. The X axis represents days since removal of exogenous cytokine support, while the Y axis represents the total number of live cells.

[0257] Fig. 63A shows averaged results of an *in vitro* tumor cell killing assay where mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells with or without 10 μg/ml cetuximab (CTX) were added to Detroit-562 (pharyngeal carcinoma) cells at

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various E:T ratios (e.g., 1:1, 5:1, 10:1). The X axis represents time in hours:minutes:seconds from initial seeding of the Detroit-562 cells, while the Y axis represents percent cytolysis as measured by electrical impedance. N = 3, error bars represent standard deviation.

[0258] Fig. 63B shows averaged results of an *in vitro* tumor cell killing assay where mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells with or without 10 μg/ml cetuximab (CTX) were added to FaDu (pharyngeal carcinoma) cells at various E:T ratios (e.g., 1:1, 5:1, 10:1). The X axis represents time in hours:minutes:seconds from initial seeding of the FaDu cells, while the Y axis represents percent cytolysis as measured by electrical impedance. N =3, error bars represent standard deviation.

[0259] Fig. 63C shows averaged results of an *in vitro* tumor cell killing assay where mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells with or without 10 μg/ml cetuximab (CTX) were added to HT29 (colorectal adenocarcinoma) cells at various E:T ratios (e.g., 1:1, 5:1, 10:1). The X axis represents time in hours:minutes:seconds from initial seeding of the HT29 cells, while the Y axis represents percent cytolysis as measured by electrical impedance. N =3, error bars represent standard deviation.

[0260] Fig. 63D shows averaged results of an *in vitro* tumor cell killing assay where mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells with or without 10 μg/ml cetuximab (CTX) were added to HCT116 (colorectal carcinoma) cells at various E:T ratios (e.g., 1:1, 5:1, 10:1). The X axis represents time in hours:minutes:seconds from initial seeding of the HCT116 cells, while the Y axis represents percent cytolysis as measured by electrical impedance. N =3, error bars represent standard deviation.

[0261] Fig. 64A shows averaged results of an *in vitro* tumor cell killing assay where mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells or unedited (WT) iNK cells added to HT29 (colorectal adenocarcinoma) cells at an E:T ratio of 10:1. The X axis represents time in hours:minutes:seconds from initial seeding of the HT29 cells, while the Y axis represents percent cytolysis as measured by electrical impedance. N =3, error bars represent standard deviation.

[0262] Fig. 64B shows results of an *in vitro* persistence assay of mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells and unedited (WT) iNK cells. DKI/DKO or WT iNK cells were co-cultured with HT-29 cells for 4 days at a 10:1

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E:T ratio. The X axis denotes evaluation category (e.g., percentage of live NK cells of all cells, percentage of CD16+ live NK cells), while the Y axis represents the percentage as measured by flow cytometry. Black horizontal lines represent means.

[0263] Fig. 64C depicts exemplary flow cytometry data from before and after an *in vitro* persistence assay of mbIL-15/CD16 ($CD16^{+/-}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells and unedited (WT) iNK cells. DKI/DKO or WT iNK cells were co-cultured with HT-29 cells for 4 days at a 1:1 E:T ratio.

[0264] Fig. 65A shows exemplary flow cytometry data from unedited (WT) iNK cells or mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells. The data highlights the efficiency of integration and expression of knock-in cassettes comprising a CD16 and IL-15Rα protein encoding cargo sequence. The X axis denotes whether the selected gene is CD16 or IL-15Rα, while the Y axis quantifies the percentage of cells from the noted population that are expressing the selected gene. Horizontal lines represent group means. N = 1, ****$p<0.0001$ (two-way ANOVA).

[0265] Fig. 65B shows the results of 3D tumor spheroid killing assays conducted as depicted in Fig. 20. Unedited (WT) iNK cells or mbIL-15/CD16 ($CD16^{-/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells were used against SK-OV-3 tumor cells at varying E:T ratios. DKI/DKO or WT iNK cells were co-cultured with the tumor spheroids and imaged every 2 hours to measure red object intensity (a proxy for tumor cell abundance) for up to 4 days. Data were normalized to the red object intensity at time of iNK cell addition. IC50 values based on the left panel are presented in the table in the right panel and highlight the greater efficacy of the DKI/DKO iNK cells in killing tumor cells. The X axis represents time in hours since addition of iNK cells to the tumor spheroid, while the Y axis represents normalized spheroid size as measured by red object intensity. N = 1, two technical replicates per cell line.

[0266] Fig. 65C shows the results of a 3D tumor spheroid killing assay conducted as depicted in Fig. 20. Unedited (WT) iNK cells or mbIL-15/CD16 ($CD16^{-/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells were used against SK-OV-3 tumor cells at varying E:T ratios and in the presence of either 10 μg/ml trastuzumab or IgG (control). DKI/DKO or WT iNK cells were co-cultured with the tumor spheroids and imaged every 2 hours to measure red object intensity (a proxy for tumor cell abundance) for up to 4 days. DKI/DKO iNK cells

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demonstrate significantly greater antibody-dependent cellular cytotoxicity (ADCC) than WT iNK cells. The X axis represents treatment group, while the Y axis represents the calculated IC50 (e.g., the E:T ratio required to reduce the SK-OV-3 spheroids by 50% after 100 hours of killing). Data represents 11 independent experiments. ****$p<0.0001$ (unpaired t-test).

[0267] Fig. 65D shows the results of an *in vitro* persistence assay of unedited (WT) iNK cells and mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells in the absence of the cytokines IL-2 and IL-15. The X axis represents days in culture since removal of exogenous cytokine support, while the Y axis represents viability as the percentage of live cells. N = 1, two technical replicates per cell line, error bars represent standard deviation.

[0268] Fig. 65E shows the results of an *in vitro* SMAD2/3 phosphorylation assay of unedited (WT) iNK cells and mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells following treatment with TGFβ (TGFb). DKI/DKO iNK cells or WT iNK cells were plated in a cytokine starved condition and 10 ng/ml of TGFβ was added to the iNK cells the following day. Cells were immediately fixed following the time indicated. The X axis represents time in minutes since addition of the TGFβ, while the Y axis represents normalized level of SMAD2/3 phosphorylation. Data represents one independent experiment. Dashed horizontal line represents level of SMAD2/3 phosphorylation following treatment with vehicle.

[0269] Fig. 65F shows the results of a 3D tumor spheroid killing assay conducted as depicted in Fig. 20. Unedited (WT) iNK cells or mbIL-15/CD16 ($CD16^{-/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells were used against SK-OV-3 tumor cells at an E:T ratio of 31.6 and in the presence of either 10 ng/ml TGFβ or IgG (control). DKI/DKO or WT iNK cells were co-cultured with the tumor spheroids and imaged every 2 hours to measure red object intensity (a proxy for tumor cell abundance) for up to 100 days. Results for the DKI/DKO iNK cells are displayed in the left panel, while the results for the WT iNK cells are displayed in the right panel. The X axis represents time in hours since addition of iNK cells to the tumor spheroid, while the Y axis represents normalized spheroid size as measured by red object intensity. N = 1.

[0270] Fig. 65G shows the results of an *in vitro* serial killing assay where unedited (WT) iNK cells or mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells were challenged with Nalm6 tumor cells. At day 0, 10×10^3 Nalm6 tumor cells and 2×10^5

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iNK cells were plated together in the presence of 10 ng/ml TGFβ. At 48 hour intervals, a bolus of 5 x 10^3 Nalm6 tumor cells was added to re-challenge the iNK cell population. The X axis represents the number of challenges that occurred, while the Y axis represents the tumor burden as measured by red object intensity. N = 1, three technical replicates per cell line, error bars represent standard deviation.

[0271] Fig. 66A is a schematic of an *in vivo* tumor killing assay. Mice were intravenously (IV) inoculated with 0.125 x 10^6 (0.125e6) SKOV3-luc cells, and following 19 days to allow for tumor establishment, on day -2, mice were imaged to establish pre-treatment tumor burden and randomized into two groups. Two days later, on day 0, a first group of mice intravenously received 20 x 10^6 (20e6) mbIL-15/CD16 ($CD16^{+/-}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells in combination with 2.5 mpk trastuzumab (Tras) and a second group of mice intraperitoneally received only 2.5 mpk trastuzumab (Tras). Mice were imaged weekly using an *in vivo* imaging system (IVIS) to assess tumor burden over time.

[0272] Fig. 66B shows tumor burden (median with interquartile range) for the *in vivo* tumor killing assay described in Fig. 66A. Groups of mice are represented by each horizontal line. Each treatment group had 4 mice. The groups include mice that received mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells in combination with a single dose of trastuzumab (DKI/DKO iNK + Tras.), a single dose of trastuzumab alone (Tras. Only), or an isotype control. Mice dosed with the DKI/DKO iNK cells in combination with trastuzumab had significantly decreased tumor burden as compared to mice dosed with trastuzumab alone. The dose of trastuzumab on day 0 is indicated by the arrow. The dashed vertical line represents the dose of iNK cells. The X axis represents time in days since introduction of NK cells, while the Y axis represents tumor burden as measured by bioluminescent imaging (BLI) using an *in vivo* imaging system (IVIS).

[0273] Fig. 66C shows representative bioluminescent imaging of mice subjected to the *in vivo* tumor killing assay described in Fig. 66A. The treatment groups of the mice are denoted along the top of the panel, while the time since dosing with iNK cells in combination with trastuzumab or trastuzumab alone is denoted along the left side of the panel. Each treatment group had 4 mice. The color scale at the right represents the radiance (p/sec/cm^2/sr) of the

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bioluminescence (from a minimum of 3.94 x 10^4 to a maximum of 7.02 x 10^5) as seen in the images.

[0274] Fig. 67A is a schematic of an *in vivo* tumor killing assay. Mice were intraperitoneally inoculated with 0.25 x 10^6 SKOV3-luc cells, and following 4 days to allow for tumor establishment, mice were randomized into groups. One day later, some groups of mice intraperitoneally received 5 x 10^6 (5E6) unedited (WT) or mbIL-15/CD16 ($CD16^{+/-}$/mbIL-$15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells. In some treatment groups, mice received a dose of 2.5 mpk trastuzumab at 0, 7, and 14 days (as indicated by the arrows) post-introduction of iNK cells, for a total of 3 doses of trastuzumab. Mice were imaged weekly using an *in vivo* imaging system (IVIS) to assess tumor burden over time.

[0275] Fig. 67B shows tumor burden (median with interquartile range) for the *in vivo* tumor killing assay described in Fig. 67A. Groups of mice are represented by each horizontal line. Each treatment group had 5-6 mice. The groups included mice that received unedited iNK cells (WT iNK), mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI / CISH/TGFβRII DKO iNK cells (DKI/DKO iNK), or an isotype control. The X axis represents time since introduction of NK cells, while the Y axis represents tumor burden as measured by bioluminescent imaging (BLI) using an *in vivo* imaging system (IVIS).

[0276] Fig. 67C shows tumor burden (median with interquartile range) for the *in vivo* tumor killing assay described in Fig. 67A. Groups of mice are represented by each horizontal line. Each treatment group had 5-6 mice. The groups included mice that received unedited (WT) iNK cells in combination with trastuzumab (WT + Tras. x 3), mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells in combination with trastuzumab (DKI DKO + Tras. x 3), trastuzumab alone, or an isotype control. Mice dosed with the DKI/DKO iNK cells in combination with trastuzumab had significantly decreased tumor burden as compared to mice dosed with WT iNK cells in combination with trastuzumab or trastuzumab alone. Doses of trastuzumab on day 0, 7, and 14 are indicated by the arrows. The X axis represents time since introduction of NK cells, while the Y axis represents tumor burden as measured by bioluminescent imaging (BLI) using an *in vivo* imaging system (IVIS).
****$p<0.0001$ (one-way ANOVA).

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[0277] Fig. 67D shows the survival of mice subjected to the *in vivo* tumor killing assay described in Fig. 67A. Groups of mice are represented by each horizontal line. The X axis represents time since introduction of NK cells, while the Y axis represents percent survival of the mice. *$p<0.05$, **$p<0.01$ (Log-rank Mantel-Cox test).

[0278] Fig. 67E shows representative bioluminescent imaging of mice subjected to the *in vivo* tumor killing assay described in Fig. 67A. The treatment groups of the mice are denoted along the top of the panel, while the time since introduction of NK cells is denoted along the left side of the panel. Each treatment group had 5-6 mice. The table below the images displays the number of mice with complete tumor clearance / total mice in the treatment group (from top of panel) at day 31 post-introduction of NK cells.

DETAILED DESCRIPTION

Definitions and Abbreviations

[0279] Unless otherwise specified, each of the following terms have the meaning set forth in this section.

[0280] The indefinite articles "a" and "an" refer to at least one of the associated noun, and are used interchangeably with the terms "at least one" and "one or more." The conjunctions "or" and "and/or" are used interchangeably as non-exclusive disjunctions.

[0281] The term "cancer" (also used interchangeably with the term "neoplastic"), as used herein, refers to cells having the capacity for autonomous growth, i.e., an abnormal state or condition characterized by rapidly proliferating cell growth. Cancerous disease states may be categorized as pathologic, i.e., characterizing or constituting a disease state, e.g., malignant tumor growth, or may be categorized as non-pathologic, i.e., a deviation from normal but not associated with a disease state, e.g., cell proliferation associated with wound repair.

[0282] The terms "CRISPR/Cas nuclease" as used herein refer to any CRISPR/Cas protein with DNA nuclease activity, e.g., a Cas9 or a Cas12 protein that exhibits specific association (or "targeting") to a DNA target site, e.g., within a genomic sequence in a cell in the presence of a guide molecule. The strategies, systems, and methods disclosed herein can use any combination of CRISPR/Cas nuclease disclosed herein, or known to those of ordinary skill in the art. Those of ordinary skill in the art will be aware of additional CRISPR/Cas nucleases and

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variants suitable for use in the context of the present disclosure, and it will be understood that the present disclosure is not limited in this respect.

[0283] The term "differentiation" as used herein is the process by which an unspecialized ("uncommitted") or less specialized cell acquires the features of a specialized cell such as, for example, a blood cell. In some embodiments, a differentiated or differentiation-induced cell is one that has taken on a more specialized ("committed") position within the lineage of a cell. For example, an iPS cell (iPSC) can be differentiated into various more differentiated cell types, for example, a hematopoietic stem cell, a lymphocyte, and other cell types, upon treatment with suitable differentiation factors in the cell culture medium. Suitable methods, differentiation factors, and cell culture media for the differentiation of pluri- and multipotent cell types into more differentiated cell types are well known to those of skill in the art. In some embodiments, the term "committed", is applied to the process of differentiation to refer to a cell that has proceeded through a differentiation pathway to a point where, under normal circumstances, it would or will continue to differentiate into a specific cell type or subset of cell types, and cannot, under normal circumstances, differentiate into a different cell type (other than a specific cell type or subset of cell types) nor revert to a less differentiated cell type.

[0284] The terms "differentiation marker," "differentiation marker gene," or "differentiation gene," as used herein refers to genes or proteins whose expression are indicative of cell differentiation occurring within a cell, such as a pluripotent cell. In some embodiments, differentiation marker genes include, but are not limited to, the following genes: CD34, CD4, CD8, CD3, CD56 (NCAM), CD49, CD45, NK cell receptor (cluster of differentiation 16 (CD16)), natural killer group-2 member D (NKG2D), CD69, NKp30, NKp44, NKp46, CD158b, FOXA2, FGF5, SOX17, XIST, NODAL, COL3A1, OTX2, DUSP6, EOMES, NR2F2, NR0B1, CXCR4, CYP2B6, GAT A3, GATA4, ERBB4, GATA6, HOXC6, INHA, SMAD6, RORA, NIPBL, TNFSF11, CDH11, ZIC4, GAL, SOX3, PITX2, APOA2, CXCL5, CER1, FOXQ1, MLL5, DPP10, GSC, PCDH10, CTCFL, PCDH20, TSHZ1, MEGF10, MYC, DKK1, BMP2, LEFTY2, HES1, CDX2, GNAS, EGR1, COL3A1, TCF4, HEPH, KDR, TOX, FOXA1, LCK, PCDH7, CD1D FOXG1, LEFTY1, TUJ1, T gene (Brachyury), ZIC1, GATA1, GATA2, HDAC4, HDAC5, HDAC7, HDAC9, NOTCH1, NOTCH2, NOTCH4, PAX5, RBPJ, RUNX1, STAT1 and STAT3.

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[0285] The terms "differentiation marker gene profile," or "differentiation gene profile," "differentiation gene expression profile," "differentiation gene expression signature," "differentiation gene expression panel," "differentiation gene panel," or "differentiation gene signature" as used herein refer to expression or levels of expression of a plurality of differentiation marker genes.

[0286] The term "nuclease" as used herein refers to any protein that catalyzes the cleavage of phosphodiester bonds. In some embodiments the nuclease is a DNA nuclease. In some embodiments the nuclease is a "nickase" which causes a single-strand break when it cleaves double-stranded DNA, e.g., genomic DNA in a cell. In some embodiments the nuclease causes a double-strand break when it cleaves double-stranded DNA, e.g., genomic DNA in a cell. In some embodiments the nuclease binds a specific target site within the double-stranded DNA that overlaps with or is adjacent to the location of the resulting break. In some embodiments, the nuclease causes a double-strand break that contains overhangs ranging from 0 (blunt ends) to 22 nucleotides in both 3′ and 5′ orientations. As discussed herein, CRISPR/Cas nucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and meganucleases are exemplary nucleases that can be used in accordance with the strategies, systems, and methods of the present disclosure.

[0287] The term "edited iNK cell" as used herein refers to an iNK cell which has been modified to change at least one expression product of at least one gene at some point in the development of the cell. In some embodiments, a modification can be introduced using, e.g., gene editing techniques such as CRISPR-Cas or, e.g., dominant-negative constructs. In some embodiments, an iNK cell is edited at a time point before it has differentiated into an iNK cell, e.g., at a precursor stage, at a stem cell stage, etc. In some embodiments, an edited iNK cell is compared to a non-edited iNK cell (an NK cell produced by differentiating an iPSC cell, which iPSC cell and/or iNK cell do not have modifications, e.g., genetic modifications).

[0288] The term "embryonic stem cell" as used herein refers to pluripotent stem cells derived from the inner cell mass of the embryonic blastocyst. In some embodiments, embryonic stem cells are pluripotent and give rise during development to all derivatives of the three primary germ layers: ectoderm, endoderm and mesoderm. In some such embodiments, embryonic stem cells do not contribute to the extra-embryonic membranes or the placenta, i.e., are not totipotent.

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[0289] The term "endogenous," as used herein in the context of nucleic acids refers to a native nucleic acid (e.g., a gene, a protein coding sequence) in its natural location, e.g., within the genome of a cell.

[0290] The term "essential gene" as used herein with respect to a cell refers to a gene that encodes at least one gene product that is required for survival and/or proliferation of the cell. An essential gene can be a housekeeping gene that is essential for survival of all cell types or a gene that is required to be expressed in a specific cell type for survival and/or proliferation under particular culture conditions, e.g., for proper differentiation of iPS or ES cells or expansion of iPS- or ES-derived cells. Loss of function of an essential gene results, in some embodiments, in a significant reduction of cell survival, e.g., of the time a cell characterized by a loss of function of an essential gene survives as compared to a cell of the same cell type but without a loss of function of the same essential gene. In some embodiments, loss of function of an essential gene results in the death of the affected cell. In some embodiments, loss of function of an essential gene results in a significant reduction of cell proliferation, e.g., in the ability of a cell to divide, which can manifest in a significant time period the cell requires to complete a cell cycle, or, in some preferred embodiments, in a loss of a cell's ability to complete a cell cycle, and thus to proliferate at all.

[0291] The term "exogenous," as used herein in the context of nucleic acids refers to a nucleic acid (whether native or non-native) that has been artificially introduced into a man-made construct (e.g., a knock-in cassette, or a donor template) or into the genome of a cell using, for example, gene editing or genetic engineering techniques, e.g., HDR based integration techniques.

[0292] The term "genome editing system" refers to any system having RNA-guided DNA editing activity.

[0293] The term "guide molecule" or "guide RNA" or "gRNA" when used in reference to a CRISPR/Cas system is any nucleic acid that promotes the specific association (or "targeting") of a CRISPR/Cas nuclease, e.g., a Cas9 or a Cas12 protein to a DNA target site such as within a genomic sequence in a cell. While guide molecules are typically RNA molecules it is well known in the art that chemically modified RNA molecules including DNA/RNA hybrid molecules can be used as guide molecules.

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[0294] The terms "hematopoietic stem cell," or "definitive hematopoietic stem cell" as used herein, refer to CD34-positive (CD34+) stem cells. In some embodiments, CD34-positive stem cells are capable of giving rise to mature myeloid and/or lymphoid cell types. In some embodiments, the myeloid and/or lymphoid cell types include, for example, T cells, natural killer (NK) cells and/or B cells.

[0295] The terms "induced pluripotent stem cell", "iPS cell" or "iPSC" as used herein to refer to a stem cell obtained from a differentiated somatic (e.g., adult, neonatal, or fetal) cell by a process referred to as reprogramming (e.g., dedifferentiation). In some embodiments, reprogrammed cells are capable of differentiating into tissues of all three germ or dermal layers: mesoderm, endoderm, and ectoderm. iPSCs are not found in nature.

[0296] The terms "iPS-derived NK cell" or "iNK cell" or as used herein refers to a natural killer cell which has been produced by differentiating an iPS cell, which iPS cell may or may not have a genetic modification.

[0297] The terms "iPS-derived T cell" or "iT cell" or as used herein refers to a T which has been produced by differentiating an iPS cell, which iPS cell may or may not have a genetic modification.

[0298] The term "multipotent stem cell" as used herein refers to a cell that has the developmental potential to differentiate into cells of one or more germ layers (ectoderm, mesoderm and endoderm), but not all three germ layers. Thus, in some embodiments, a multipotent cell may also be termed a "partially differentiated cell." Multipotent cells are well-known in the art, and examples of multipotent cells include adult stem cells, such as for example, hematopoietic stem cells and neural stem cells. In some embodiments, "multipotent" indicates that a cell may form many types of cells in a given lineage, but not cells of other lineages. For example, a multipotent hematopoietic cell can form the many different types of blood cells (red, white, platelets, etc.), but it cannot form neurons. Accordingly, in some embodiments, "multipotency" refers to a state of a cell with a degree of developmental potential that is less than totipotent and pluripotent.

[0299] The term "pluripotent" as used herein refers to ability of a cell to form all lineages of the body or soma (i.e., the embryo proper) or a given organism (e.g., human). For example, embryonic stem cells are a type of pluripotent stem cells that are able to form cells from each of

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the three germ layers, the ectoderm, the mesoderm, and the endoderm. Generally, pluripotency may be described as a continuum of developmental potencies ranging from an incompletely or partially pluripotent cell (e.g., an epiblast stem cell or EpiSC), which is unable to give rise to a complete organism to the more primitive, more pluripotent cell, which is able to give rise to a complete organism (e.g., an embryonic stem cell or an induced pluripotent stem cell).

[0300] The term "pluripotency" as used herein refers to a cell that has the developmental potential to differentiate into cells of all three germ layers (ectoderm, mesoderm, and endoderm). In some embodiments, pluripotency can be determined, in part, by assessing pluripotency characteristics of the cells. In some embodiments, pluripotency characteristics include, but are not limited to: (i) pluripotent stem cell morphology; (ii) the potential for unlimited self-renewal; (iii) expression of pluripotent stem cell markers including, but not limited to SSEA1 (mouse only), SSEA3/4, SSEA5, TRA1- 60/81, TRAl-85, TRA2-54, GCTM-2, TG343, TG30, CD9, CD29, CD133/prominin, CD140a, CD56, CD73, CD90, CD105, OCT4 (also known as POU5F1), NANOG, SOX2, CD30 and/or CD50; (iv) ability to differentiate to all three somatic lineages (ectoderm, mesoderm and endoderm); (v) teratoma formation consisting of the three somatic lineages; and (vi) formation of embryoid bodies consisting of cells from the three somatic lineages.

[0301] The term "pluripotent stem cell morphology" as used herein refers to the classical morphological features of an embryonic stem cell. In some embodiments, normal embryonic stem cell morphology is characterized as small and round in shape, with a high nucleus-to-cytoplasm ratio, the notable presence of nucleoli, and typical intercell spacing.

[0302] The term "polycistronic" or "multicistronic" when used herein with reference to a knock-in cassette refers to the fact that the knock-in cassette can express two or more proteins from the same mRNA transcript. Similarly, a "bicistronic" knock-in cassette is a knock-in cassette that can express two proteins from the same mRNA transcript.

[0303] The term "polynucleotide" (including, but not limited to "nucleotide sequence", "nucleic acid", "nucleic acid molecule", "nucleic acid sequence", and "oligonucleotide") as used herein refers to a series of nucleotide bases (also called "nucleotides") and means any chain of two or more nucleotides. In some embodiments, polynucleotides, nucleotide sequences, nucleic acids, etc. can be chimeric mixtures or derivatives or modified versions thereof, single-stranded

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or double-stranded. In some such embodiments, modifications can occur at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, its hybridization parameters, etc. In general, a nucleotide sequence typically carries genetic information, including, but not limited to, the information used by cellular machinery to make proteins and enzymes. In some embodiments, a nucleotide sequence and/or genetic information comprises double- or single-stranded genomic DNA, RNA, any synthetic and genetically manipulated polynucleotide, and/or sense and/or antisense polynucleotides. In some embodiments, nucleic acids contain modified bases.

[0304] Conventional IUPAC notation is used in nucleotide sequences presented herein, as shown in Table 1, below (see also Cornish-Bowden, Nucleic Acids Res. 1985; 13(9):3021-30, incorporated by reference herein). It should be noted, however, that "T" denotes "Thymine or Uracil" in those instances where a sequence may be encoded by either DNA or RNA, for example in certain CRISPR/Cas guide molecule targeting domains.

Table 1: IUPAC nucleic acid notation

Character	Base
A	Adenine
T	Thymine or Uracil
G	Guanine
C	Cytosine
U	Uracil
K	G or T/U
M	A or C
R	A or G
Y	C or T/U
S	C or G
W	A or T/U
B	C, G or T/U
V	A, C or G
H	A, C or T/U
D	A, G or T/U
N	A, C, G or T/U

[0305] The terms "potency" or "developmental potency" as used herein refer to the sum of all developmental options accessible to the cell (i.e., the developmental potency), particularly, for example in the context of cellular developmental potential. In some embodiments, the continuum of cell potency includes, but is not limited to, totipotent cells, pluripotent cells, multipotent cells, oligopotent cells, unipotent cells, and terminally differentiated cells.

[0306] The terms "prevent," "preventing," and "prevention" as used herein with reference to a disease refer to the prevention of the disease in a mammal, e.g., in a human, including (a) avoiding or precluding the disease; (b) affecting the predisposition toward the disease; or (c) preventing or delaying the onset of at least one symptom of the disease.

[0307] The terms "protein," "peptide" and "polypeptide" as used herein are used interchangeably to refer to a sequential chain of amino acids linked together via peptide bonds. The terms include individual proteins, groups or complexes of proteins that associate together, as well as fragments or portions, variants, derivatives and analogs of such proteins. Unless otherwise specified, peptide sequences are presented herein using conventional notation, beginning with the amino or N-terminus on the left, and proceeding to the carboxyl or C-terminus on the right. Standard one-letter or three-letter abbreviations can be used.

[0308] The term "gene product of interest" as used herein can refer to any product encoded by a gene including any polynucleotide or polypeptide. In some embodiments the gene product is a protein which is not naturally expressed by a target cell of the present disclosure. In some embodiments the gene product is a protein which confers a new therapeutic activity to the cell such as, but not limited to, a chimeric antigen receptor (CAR) or antigen-binding fragment thereof, a T cell receptor or antigen-binding portion thereof, a non-naturally occurring variant of FcγRIII (CD16), interleukin 15 (IL-15), interleukin 15 receptor (IL-15R) or a variant thereof, interleukin 12 (IL-12), interleukin-12 receptor (IL-12R) or a variant thereof, human leukocyte antigen G (HLA-G), human leukocyte antigen E (HLA-E), leukocyte surface antigen cluster of differentiation CD47 (CD47), or any combination of two or more thereof. It is to be understood that the methods and cells of the present disclosure are not limited to any particular gene product of interest and that the selection of a gene product of interest will depend on the type of cell and ultimate use of the cells.

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[0309] The term "reporter gene" as used herein refers to an exogenous gene that has been introduced into a cell, e.g., integrated into the genome of the cell, that confers a trait suitable for artificial selection. Common reporter genes are fluorescent reporter genes that encode a fluorescent protein, e.g., green fluorescent protein (GFP) and antibiotic resistance genes that confer antibiotic resistance to cells.

[0310] The terms "reprogramming" or "dedifferentiation" or "increasing cell potency" or "increasing developmental potency" as used herein refer to a method of increasing potency of a cell or dedifferentiating a cell to a less differentiated state. For example, in some embodiments, a cell that has an increased cell potency has more developmental plasticity (i.e., can differentiate into more cell types) compared to the same cell in the non-reprogrammed state. That is, in some embodiments, a reprogrammed cell is one that is in a less differentiated state than the same cell in a non-reprogrammed state. In some embodiments, "reprogramming" refers to de-differentiating a somatic cell, or a multipotent stem cell, into a pluripotent stem cell, also referred to as an induced pluripotent stem cell, or iPSC. Suitable methods for the generation of iPSCs from somatic or multipotent stem cells are well known to those of skill in the art.

[0311] The terms "RNA-guided nuclease" and "RNA-guided nuclease molecule" are used interchangeably herein. In some embodiments, the RNA-guided nuclease is a RNA-guided DNA endonuclease enzyme. In some embodiments, the RNA-guided nuclease is a CRISPR nuclease. Non-limiting examples of RNA-guided nucleases are listed in Table 5 below, and the methods and compositions disclosed herein can use any combination of RNA-guided nucleases disclosed herein, or known to those of ordinary skill in the art. Those of ordinary skill in the art will be aware of additional nucleases and nuclease variants suitable for use in the context of the present disclosure, and it will be understood that the present disclosure is not limited in this respect.

[0312] Additional suitable RNA-guided nucleases, e.g., Cas9 and Cas12 nucleases, will be apparent to the skilled artisan in view of the present disclosure, and the disclosure is not limited by the exemplary suitable nucleases provided herein. In some embodiments, a suitable nuclease is a Cas12a, Cas9, Cas12b, Cas12c, Cas12e, CasX, or CasΦ (Cas12j), or a variant thereof (e.g., a variant with a high editing efficiency, e.g., capable of editing about 60% to 100% of cells in a population of cells) nuclease. In some embodiments, the disclosure also embraces

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nuclease variants, e.g., Cas9, Cpf1 (Cas12a, such as the Mad7 Cas12a variant), Cas12b, Cas12e, CasX, or CasΦ (Cas12j) nuclease variants. In some embodiments, a nuclease is a nuclease variant, which refers to a nuclease comprising an amino acid sequence characterized by one or more amino acid substitutions, deletions, or additions as compared to the wild type amino acid sequence of the nuclease. In some embodiments, a suitable nuclease and/or nuclease variant may also include purification tags (e.g., polyhistidine tags) and/or signaling peptides, e.g., comprising or consisting of a nuclear localization signal sequence. Some non-limiting examples of suitable nucleases and nuclease variants are described in more detail elsewhere herein and also include those described in PCT application PCT/US2019/22374, filed March 14, 2019, and entitled "*Systems and Methods for the Treatment of Hemoglobinopathies*," the entire contents of which are incorporated herein by reference. In some embodiments, the RNA-guided nuclease is an *Acidaminococcus sp.* Cpf1 variant (AsCpf1 variant). In some embodiments, suitable Cpf1 nuclease variants, including suitable AsCpf1 variants will be known or apparent to those of ordinary skill in the art based on the present disclosure, and include, but are not limited to, the Cpf1 variants disclosed herein or otherwise known in the art. For example, in some embodiments, the RNA-guided nuclease is a *Acidaminococcus sp.* Cpf1 RR variant (AsCpf1-RR). In another embodiment, the RNA-guided nuclease is a Cpf1 RVR variant. For example, suitable Cpf1 variants include those having an M537R substitution, an H800A substitution, and/or an F870L substitution, or any combination thereof (numbering scheme according to AsCpf1 wild-type sequence).

[0313] The term "subject" as used herein means a human or non-human animal. In some embodiments a human subject can be any age (e.g., a fetus, infant, child, young adult, or adult). In some embodiments a human subject may be at risk of or suffer from a disease, or may be in need of alteration of a gene or a combination of specific genes. Alternatively, in some embodiments, a subject may be a non-human animal, which may include, but is not limited to, a mammal. In some embodiments, a non-human animal is a non-human primate, a rodent (e.g., a mouse, rat, hamster, guinea pig, etc.), a rabbit, a dog, a cat, and so on. In certain embodiments of this disclosure, the non-human animal subject is livestock, e.g., a cow, a horse, a sheep, a goat, etc. In certain embodiments, the non-human animal subject is poultry, e.g., a chicken, a turkey, a duck, etc.

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[0314] The terms "treatment," "treat," and "treating," as used herein refer to a clinical intervention aimed to reverse, alleviate, delay the onset of, or inhibit the progress, ameliorate, reduce severity of, prevent or delay the recurrence of a disease, disorder, or condition or one or more symptoms thereof, and/or improve one or more symptoms of a disease, disorder, or condition as described herein. In some embodiments, a condition includes an injury. In some embodiments, an injury may be acute or chronic (e.g., tissue damage from an underlying disease or disorder that causes, e.g., secondary damage such as tissue injury). In some embodiments, treatment, e.g., in the form of an iPSC-derived NK cell or a population of iPSC-derived NK cells as described herein, may be administered to a subject after one or more symptoms have developed and/or after a disease has been diagnosed. Treatment may be administered in the absence of symptoms, e.g., to prevent or delay onset of a symptom or inhibit onset or progression of a disease. For example, in some embodiments, treatment may be administered to a susceptible subject prior to the onset of symptoms (e.g., in light of genetic or other susceptibility factors). In some embodiments, treatment may also be continued after symptoms have resolved, for example to prevent or delay their recurrence. In some embodiments, treatment results in improvement and/or resolution of one or more symptoms of a disease, disorder or condition.

[0315] The term "variant" as used herein refers to an entity such as a polypeptide or polynucleotide that shows significant structural identity with a reference entity but differs structurally from the reference entity in the presence or level of one or more chemical moieties as compared with the reference entity. In many embodiments, a variant also differs functionally from its reference entity. In general, whether a particular entity is properly considered to be a "variant" of a reference entity is based on its degree of structural identity with the reference entity. As used herein, the terms "functional variant" refer to a variant that confers the same function as the reference entity, e.g., a functional variant of a gene product of an essential gene is a variant that promotes the survival and/or proliferation of a cell. It is to be understood that a functional variant need not be functionally equivalent to the reference entity as long as it confers the same function as the reference entity.

Target Cells

[0316] Methods of the disclosure can be used to edit the genome of any cell. In certain embodiments, the target cell is a stem cell, e.g., an iPS or ES cell. In certain embodiments, the

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target cell can be an iPS- or ES-derived cell, where the genetic modification is made at any stage during the reprogramming process from donor cell to iPSC, during the iPSC stage, and/or at any stage of the process of differentiating the iPSC or ESC to a specialized cell, or even up to or at the final specialized cell state. In certain embodiments, the target cell can be an iPS-derived NK cell (iNK cell) or iPS-derived T cell (iT cell) where the genetic modification is made at any stage during the reprogramming process from donor cell to iPSC, during the iPSC stage, and/or at any stage of the process of differentiating the iPSC to an iNK or iT state, e.g., at an intermediary state, such as, for example, an iPSC-derived HSC state, or even up to or at the final iNK or iT cell state.

[0317] In certain embodiments, a target cell is one or more of a long-term hematopoietic stem cell, a short term hematopoietic stem cell, a multipotent progenitor cell, a lineage restricted progenitor cell, a lymphoid progenitor cell, a myeloid progenitor cell, a common myeloid progenitor cell, an erythroid progenitor cell, a megakaryocyte erythroid progenitor cell, a retinal cell, a photoreceptor cell, a rod cell, a cone cell, a retinal pigmented epithelium cell, a trabecular meshwork cell, a cochlear hair cell, an outer hair cell, an inner hair cell, a pulmonary epithelial cell, a bronchial epithelial cell, an alveolar epithelial cell, a pulmonary epithelial progenitor cell, a striated muscle cell, a cardiac muscle cell, a muscle satellite cell, a neuron, a neuronal stem cell, a mesenchymal stem cell, an induced pluripotent stem (iPS) cell, an embryonic stem cell, a fibroblast, a monocyte-derived macrophage or dendritic cell, a megakaryocyte, a neutrophil, an eosinophil, a basophil, a mast cell, a reticulocyte, a B cell, e.g., a progenitor B cell, a Pre B cell, a Pro B cell, a memory B cell, a plasma B cell, a gastrointestinal epithelial cell, a biliary epithelial cell, a pancreatic ductal epithelial cell, an intestinal stem cell, a hepatocyte, a liver stellate cell, a Kupffer cell, an osteoblast, an osteoclast, an adipocyte, a preadipocyte, a pancreatic islet cell (e.g., a beta cell, an alpha cell, a delta cell), a pancreatic exocrine cell, a Schwann cell, or an oligodendrocyte. In some embodiments, a target cell is a neuronal progenitor cell. In some embodiments, a target cell is a neuron.

[0318] In some embodiments, a target cell is a circulating blood cell, e.g., a reticulocyte, megakaryocyte erythroid progenitor (MEP) cell, myeloid progenitor cell (CMP/GMP), lymphoid progenitor (LP) cell, hematopoietic stem/progenitor cell (HSC), or endothelial cell (EC). In some embodiments, a target cell is one or more of a bone marrow cell (e.g., a reticulocyte, an

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erythroid cell (e.g., erythroblast), an MEP cell, myeloid progenitor cell (CMP/GMP), LP cell, erythroid progenitor (EP) cell, HSC, multipotent progenitor (MPP) cell, endothelial cell (EC), hemogenic endothelial (HE) cell, or mesenchymal stem cell). In some embodiments, a target cell is one or more of a myeloid progenitor cell (e.g., a common myeloid progenitor (CMP) cell or granulocyte macrophage progenitor (GMP) cell). In some embodiments, a target cell is a lymphoid progenitor cell, e.g., a common lymphoid progenitor (CLP) cell. In some embodiments, a target cell is one or more of an erythroid progenitor cell (e.g., an MEP cell). In some embodiments, a target cell is one or more of a hematopoietic stem/progenitor cell (e.g., a long term HSC (LT-HSC), short term HSC (ST-HSC), MPP cell, or lineage restricted progenitor (LRP) cell). In certain embodiments, the target cell is a $CD34^+$ cell, $CD34^+CD90^+$ cell, $CD34^+CD38^-$ cell, $CD34^+CD90^+CD49f^+CD38^-CD45RA^-$ cell, $CD105^+$ cell, $CD31^+$, or $CD133^+$ cell, or a $CD34^+CD90^+$ $CD133^+$ cell. In some embodiments, a target cell is one or more of an umbilical cord blood $CD34^+$ HSPC, umbilical cord venous endothelial cell, umbilical cord arterial endothelial cell, amniotic fluid $CD34^+$ cell, amniotic fluid endothelial cell, placental endothelial cell, or placental hematopoietic $CD34^+$ cell. In some embodiments, a target cell is one or more of a mobilized peripheral blood hematopoietic $CD34^+$ cell (after the subject is treated with a mobilization agent, e.g., G-CSF or Plerixafor). In some embodiments, a target cell is a peripheral blood endothelial cell. In some embodiments, a target cell is a peripheral blood natural killer cell.

[0319] In certain embodiments, a target cell is a primary cell, e.g., a cell isolated from a human subject. In certain embodiments, a target cell is an immune cell, e.g., a primary immune cell isolated from a human subject. In certain embodiments, a target cell is part of a population of cells isolated from a subject, e.g., a human subject. In some embodiments, the population of cells comprises a population of immune cells isolated from a subject. In some embodiments, the population of cells comprises tumor infiltrating lymphocytes (TILs), e.g., TILs isolated from a human subject. In some embodiments, a target cell is isolated from a healthy subject, e.g., a healthy human donor. In some embodiments, a target cell is isolated from a subject having a disease or illness, e.g., a human patient in need of a treatment.

[0320] In certain embodiments, a target cell is an immune cell, e.g., a primary immune cell, e.g., a $CD8^+$ T cell, a $CD8^+$ naïve T cell, a $CD4^+$ central memory T cell, a $CD8^+$ central

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memory T cell, a $CD4^-$ effector memory T cell, a $CD4^+$ effector memory T cell, a $CD4^+$ T cell, a $CD4^+$ stem cell memory T cell, a $CD8^+$ stem cell memory T cell, a $CD4^+$ helper T cell, a regulatory T cell, a cytotoxic T cell, a natural killer T cell, a CD4+ naïve T cell, a TH17 $CD4^+$ T cell, a TH1 $CD4^+$ T cell, a TH2 $CD4^+$ T cell, a TH9 $CD4^+$ T cell, a $CD4^+$ $Foxp3^+$ T cell, a $CD4^+$ $CD25^+$ $CD127^-$ T cell, or a $CD4^+$ $CD25^+$ $CD127^-$ $Foxp3^+$ T cell. In some embodiments, a target cell is an alpha-beta T cell, a gamma-delta T cell or a Treg. In some embodiments a target cell is macrophage. In some embodiments, a target cell is an innate lymphoid cell. In some embodiments, a target cell is a dendritic cell. In some embodiments, a target cell is a beta cell, e.g., a pancreatic beta cell.

[0321] In some embodiments, a target cell is isolated from a subject having a cancer.

[0322] In some embodiments, a target cell is isolated from a subject having a cancer, including but not limited to, acoustic neuroma; adenocarcinoma; adrenal gland cancer; anal cancer; angiosarcoma (*e.g.*, lymphangiosarcoma, lymphangioendotheliosarcoma, hemangiosarcoma); appendix cancer; benign monoclonal gammopathy; biliary cancer (*e.g.*, cholangiocarcinoma); bile duct cancer; bladder cancer; bone cancer; breast cancer (*e.g.*, adenocarcinoma of the breast, papillary carcinoma of the breast, mammary cancer, medullary carcinoma of the breast); brain cancer (*e.g.*, meningioma, glioblastomas, glioma (*e.g.*, astrocytoma, oligodendroglioma, medulloblastoma); bronchus cancer; carcinoid tumor; cardiac tumor; cervical cancer (*e.g.*, cervical adenocarcinoma); choriocarcinoma; chordoma; craniopharyngioma; colorectal cancer (*e.g.*, colon cancer, rectal cancer, colorectal adenocarcinoma); connective tissue cancer; epithelial carcinoma; ductal carcinoma *in situ*; ependymoma; endotheliosarcoma (*e.g.*, Kaposi's sarcoma, multiple idiopathic hemorrhagic sarcoma); endometrial cancer (*e.g.*, uterine cancer, uterine sarcoma); esophageal cancer (*e.g.*, adenocarcinoma of the esophagus, Barrett's adenocarcinoma); Ewing's sarcoma; eye cancer (*e.g.*, intraocular melanoma, retinoblastoma); familiar hypereosinophilia; gall bladder cancer; gastric cancer (*e.g.*, stomach adenocarcinoma); gastrointestinal stromal tumor (GIST); germ cell cancer; head and neck cancer (*e.g.*, head and neck squamous cell carcinoma, oral cancer (*e.g.*, oral squamous cell carcinoma), throat cancer (*e.g.*, laryngeal cancer, pharyngeal cancer, nasopharyngeal cancer, oropharyngeal cancer); hematopoietic cancer (*e.g.*, lymphomas, primary pulmonary lymphomas, bronchus-associated lymphoid tissue lymphomas, splenic lymphomas,

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nodal marginal zone lymphomas, pediatric B cell non-Hodgkin lymphomas); hemangioblastoma; histiocytosis; hypopharynx cancer; inflammatory myofibroblastic tumors; immunocytic amyloidosis; kidney cancer (*e.g.*, nephroblastoma a.k.a. Wilms' tumor, renal cell carcinoma); liver cancer (*e.g.*, hepatocellular cancer (HCC), malignant hepatoma); lung cancer (*e.g.*, bronchogenic carcinoma, small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC), adenocarcinoma of the lung); leiomyosarcoma (LMS); melanoma; midline tract carcinoma; multiple endocrine neoplasia syndrome; muscle cancer; mesothelioma; nasopharynx cancer; neuroblastoma; neurofibroma (*e.g.*, neurofibromatosis (NF) type 1 or type 2, schwannomatosis); neuroendocrine cancer (*e.g.*, gastroenteropancreatic neuroendocrine tumor (GEP-NET), carcinoid tumor); osteosarcoma (*e.g.*, bone cancer); ovarian cancer (*e.g.*, cystadenocarcinoma, ovarian embryonal carcinoma, ovarian adenocarcinoma); papillary adenocarcinoma; pancreatic cancer (*e.g.*, pancreatic adenocarcinoma, intraductal papillary mucinous neoplasm (IPMN), Islet cell tumors); parathyroid cancer; papillary adenocarcinoma; penile cancer (*e.g.*, Paget's disease of the penis and scrotum); pharyngeal cancer; pinealoma; pituitary cancer; pleuropulmonary blastoma; primitive neuroectodermal tumor (PNT); plasma cell neoplasia; paraneoplastic syndromes; intraepithelial neoplasms; prostate cancer (*e.g.*, prostate adenocarcinoma); rectal cancer; rhabdomyosarcoma; retinoblastoma; salivary gland cancer; skin cancer (*e.g.*, squamous cell carcinoma (SCC), keratoacanthoma (KA), melanoma, basal cell carcinoma (BCC)); small bowel cancer (*e.g.*, appendix cancer); soft tissue sarcoma (*e.g.*, malignant fibrous histiocytoma (MFH), liposarcoma, malignant peripheral nerve sheath tumor (MPNST), chondrosarcoma, fibrosarcoma, myxosarcoma); sebaceous gland carcinoma; stomach cancer; small intestine cancer; sweat gland carcinoma; synovioma; testicular cancer (*e.g.*, seminoma, testicular embryonal carcinoma); thymic cancer; thyroid cancer (*e.g.*, papillary carcinoma of the thyroid, papillary thyroid carcinoma (PTC), medullary thyroid cancer); urethral cancer; uterine cancer; vaginal cancer; vulvar cancer (*e.g.*, Paget's disease of the vulva), or any combination thereof.

[0323] In some embodiments, a target cell is isolated from a subject having a hematological disorder. In some embodiments, a target cell is isolated form a subject having sickle cell anemia. In some embodiments, a target cell is isolated from a subject having β-thalassemia.

Stem Cells

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[0324] Methods of the disclosure can be used with stem cells. Stem cells are typically cells that have the capacity to produce unaltered daughter cells (self-renewal; cell division produces at least one daughter cell that is identical to the parent cell) and to give rise to specialized cell types (potency). Stem cells include, but are not limited to, embryonic stem (ES) cells, embryonic germ (EG) cells, germline stem (GS) cells, human mesenchymal stem cells (hMSCs), adipose tissue-derived stem cells (ADSCs), multipotent adult progenitor cells (MAPCs), multipotent adult germline stem cells (maGSCs) and unrestricted somatic stem cell (USSCs). Generally, stem cells can divide without limit. After division, the stem cell may remain as a stem cell, become a precursor cell, or proceed to terminal differentiation. A precursor cell is a cell that can generate a fully differentiated functional cell of at least one given cell type. Generally, precursor cells can divide. After division, a precursor cell can remain a precursor cell, or may proceed to terminal differentiation.

[0325] Pluripotent stem cells are generally known in the art. The present disclosure provides technologies (e.g., systems, compositions, methods, etc.) related to pluripotent stem cells. In some embodiments, pluripotent stem cells are stem cells that: (a) are capable of inducing teratomas when transplanted in immunodeficient (SCID) mice; (b) are capable of differentiating to cell types of all three germ layers (e.g., can differentiate to ectodermal, mesodermal, and endodermal cell types); and/or (c) express one or more markers of embryonic stem cells (e.g., human embryonic stem cells express Oct-4, alkaline phosphatase, SSEA-3 surface antigen, SSEA-4 surface antigen, nanog, TRA-1-60, TRA-1-81, Sox-2, REX1, etc.). In some aspects, human pluripotent stem cells do not show expression of differentiation markers. In some embodiments, ES cells and/or iPSCs edited using methods of the disclosure maintain their pluripotency, e.g., (a) are capable of inducing teratomas when transplanted in immunodeficient (SCID) mice; (b) are capable of differentiating to cell types of all three germ layers, e.g., can differentiate to ectodermal, mesodermal, and endodermal cell types); and/or (c) express one or more markers of embryonic stem cells.

[0326] In some embodiments, ES cells (e.g., human ES cells) can be derived from the inner cell mass of blastocysts or morulae. In some embodiments, ES cells can be isolated from one or more blastomeres of an embryo, e.g., without destroying the remainder of the embryo. In some embodiments, ES cells can be produced by somatic cell nuclear transfer. In some

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embodiments, ES cells can be derived from fertilization of an egg cell with sperm or DNA, nuclear transfer, parthenogenesis, or by means to generate ES cells, e.g., with homozygosity in the HLA region. In some embodiments, human ES cells can be produced or derived from a zygote, blastomeres, or blastocyst-staged mammalian embryo produced by the fusion of a sperm and egg cell, nuclear transfer, parthenogenesis, or the reprogramming of chromatin and subsequent incorporation of the reprogrammed chromatin into a plasma membrane to produce an embryonic cell. Exemplary human ES cells are known in the art and include, but are not limited to, MAO1, MAO9, ACT-4, No. 3, H1, H7, H9, H14 and ACT30 ES cells. In some embodiments, human ES cells, regardless of their source or the particular method used to produce them, can be identified based on, e.g., (i) the ability to differentiate into cells of all three germ layers, (ii) expression of at least Oct-4 and alkaline phosphatase, and/or (iii) ability to produce teratomas when transplanted into immunocompromised animals. In some embodiments, ES cells have been serially passaged as cell lines.

iPS Cells

[0327] Induced pluripotent stem cells (iPSC) are a type of pluripotent stem cell artificially derived from a non-pluripotent cell, such as an adult somatic cell (e.g., a fibroblast cell or other suitable somatic cell), by inducing expression of certain genes. iPSCs can be derived from any organism, such as a mammal. In some embodiments, iPSCs are produced from mice, rats, rabbits, guinea pigs, goats, pigs, cows, non-human primates or humans. iPSCs are similar to ES cells in many respects, such as the expression of certain stem cell genes and proteins, chromatin methylation patterns, doubling time, embryoid body formation, teratoma formation, viable chimera formation, potency and/or differentiability. Various suitable methods for producing iPSCs are known in the art. In some embodiments, iPSCs can be derived by transfection of certain stem cell-associated genes (such as Oct-3/4 (Pouf51) and Sox-2) into non-pluripotent cells, such as adult fibroblasts. Transfection can be achieved through viral vectors, such as retroviruses, lentiviruses, or adenoviruses. Additional suitable reprogramming methods include the use of vectors that do not integrate into the genome of the host cell, e.g., episomal vectors, or the delivery of reprogramming factors directly via encoding RNA or as proteins has also been described. For example, cells can be transfected with Oct-3/4, Sox-2, Klf4, and/or c-Myc using a retroviral system or with Oct-4, Sox-2, NANOG, and/or LIN28 using a lentiviral

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system. After 3-4 weeks, small numbers of transfected cells begin to become morphologically and biochemically similar to pluripotent stem cells, and can be isolated through morphological selection, doubling time, or through a reporter gene and antibiotic selection. In one example, iPSCs from adult human cells are generated by the method described by Yu et al., Science 2007; 318(5854):1224 or Takahashi et al., Cell 2007; 131:861-72. Numerous suitable methods for reprogramming are known to those of skill in the art, and the present disclosure is not limited in this respect.

[0328] In some embodiments, a target cell for the editing and cargo integration methods described herein is an iPSC, wherein the edited iPSC is then differentiated, e.g., into an iPSC-derived immune cell. In some embodiments, the differentiated cell is an iPSC-derived immune cell. In some embodiments, the differentiated cell is an iPSC-derived iNK cell, an iPSC-derived T cell (e.g., an iPSC-derived alpha-beta T cell, gamma-delta T cell, Treg, CD4+ T cell, or CD8+ T cell), an iPSC-derived dendritic cell, or an iPSC-derived macrophage. In some embodiments, the differentiated cell is an iPSC-derived pancreatic beta cell.

iNK Cells

[0329] In some embodiments, the present disclosure provides methods of generating iNK cells (e.g., genetically modified iNK cells), e.g., derived from a genetically modified stem cell (e.g., iPSC).

[0330] In some embodiments, genetic modifications present in an iNK cell of the present disclosure can be made at any stage during the reprogramming process from donor cell to iPSC, during the iPSC stage, and/or at any stage of the process of differentiating the iPSC to an iNK state, e.g., at an intermediary state, such as, for example, an iPSC-derived HSC state, or even up to or at the final iNK cell state.

[0331] For example, one or more genomic modifications present in a genetically modified iNK cell of the present disclosure may be made at one or more different cell stages (e.g., reprogramming from donor to iPSC, differentiation of iPSC to iNK). In some embodiments, one or more genomic modifications present in a genetically modified iNK cell provided herein is made before reprogramming a donor cell to an iPSC state. In some embodiments, all edits present in a genetically modified iNK cell provided herein are made at the same time, in close temporal proximity, and/or at the same cell stage of the

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reprogramming/differentiation process, e.g., at the donor cell stage, during the reprogramming process, at the iPSC stage, or during the differentiation process, e.g., from iPSC to iNK. In some embodiments, two or more edits present in a genetically modified iNK cell provided herein are made at different times and/or at different cell stages of the reprogramming/differentiation process from donor cell to iPSC to iNK. For example, in some embodiments, a first edit is made at the donor cell stage and a second (different) edit is made at the iPSC stage. In some embodiments, a first edit is made at the reprogramming stage (e.g., donor to iPSC) and a second (different) edit is made at the iPSC stage.

[0332] A variety of cell types can be used as a donor cell that can be subjected to reprogramming, differentiation, and/or genetic engineering strategies described herein. For example, the donor cell can be a pluripotent stem cell or a differentiated cell, e.g., a somatic cell, such as, for example, a fibroblast or a T lymphocyte. In some embodiments, donor cells are manipulated (e.g., subjected to reprogramming, differentiation, and/or genetic engineering) to generate iNK cells described herein.

[0333] A donor cell can be from any suitable organism. For example, in some embodiments, the donor cell is a mammalian cell, e.g., a human cell or a non-human primate cell. In some embodiments, the donor cell is a somatic cell. In some embodiments, the donor cell is a stem cell or progenitor cell. In certain embodiments, the donor cell is not or was not part of a human embryo and its derivation does not involve destruction of a human embryo.

[0334] In some embodiments, a genetically modified iNK cell is derived from an iPSC, which in turn is derived from a somatic donor cell. Any suitable somatic cell can be used in the generation of iPSCs, and in turn, the generation of iNK cells. Suitable strategies for deriving iPSCs from various somatic donor cell types have been described and are known in the art. In some embodiments, a somatic donor cell is a fibroblast cell. In some embodiments, a somatic donor cell is a mature T cell.

[0335] For example, in some embodiments, a somatic donor cell, from which an iPSC, and subsequently an iNK cell is derived, is a developmentally mature T cell (a T cell that has undergone thymic selection). One hallmark of developmentally mature T cells is a rearranged T cell receptor locus. During T cell maturation, the TCR locus undergoes V(D)J rearrangements to generate complete V-domain exons. These rearrangements are retained throughout

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reprogramming of a T cells to an iPSC, and throughout differentiation of the resulting iPSC to a somatic cell.

[0336] In certain embodiments, a somatic donor cell is a $CD8^+$ T cell, a $CD8^+$ naïve T cell, a $CD4^+$ central memory T cell, a $CD8^+$ central memory T cell, a $CD4^+$ effector memory T cell, a $CD4^+$ effector memory T cell, a $CD4^+$ T cell, a $CD4^+$ stem cell memory T cell, a $CD8^+$ stem cell memory T cell, a $CD4^+$ helper T cell, a regulatory T cell, a cytotoxic T cell, a natural killer T cell, a CD4+ naïve T cell, a TH17 $CD4^+$ T cell, a TH1 $CD4^+$ T cell, a TH2 $CD4^+$ T cell, a TH9 $CD4^+$ T cell, a $CD4^+$ $Foxp3^+$ T cell, a $CD4^+$ $CD25^+$ $CD127^-$ T cell, or a $CD4^+$ $CD25^+$ $CD127^-$ $Foxp3^+$ T cell.

[0337] T cells can be advantageous for the generation of iPSCs. For example, T cells can be edited with relative ease, e.g., by CRISPR-based methods or other genetic engineering methods. Additionally, the rearranged TCR locus allows for genetic tracking of individual cells and their daughter cells. For example, if the reprogramming, expansion, culture, and/or differentiation strategies involved in the generation of NK cells a clonal expansion of a single cell, the rearranged TCR locus can be used as a genetic marker unambiguously identifying a cell and its daughter cells. This, in turn, allows for the characterization of a cell population as truly clonal, or for the identification of mixed populations, or contaminating cells in a clonal population. Another potential advantage of using T cells in generating iNK cells carrying multiple edits is that certain karyotypic aberrations associated with chromosomal translocations are selected against in T cell culture. Such aberrations can pose a concern when editing cells by CRISPR technology, and in particular when generating cells carrying multiple edits. Using T cell derived iPSCs as a starting point for the derivation of therapeutic lymphocytes can allow for the expression of a pre-screened TCR in the lymphocytes, e.g., via selecting the T cells for binding activity against a specific antigen, e.g., a tumor antigen, reprogramming the selected T cells to iPSCs, and then deriving lymphocytes from these iPSCs that express the TCR (e.g., T cells). This strategy can allow for activating the TCR in other cell types, e.g., by genetic or epigenetic strategies. Additionally, T cells retain at least part of their "epigenetic memory" throughout the reprogramming process, and thus subsequent differentiation of the same or a closely related cell type, such as iNK cells can be more efficient and/or result in higher quality

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cell populations as compared to approaches using non-related cells, such as fibroblasts, as a starting point for iNK derivation.

[0338] In some embodiments, a donor cell being manipulated, e.g., a cell being reprogrammed and/or undergoing genetic engineering as described herein, is one or more of a long-term hematopoietic stem cell, a short term hematopoietic stem cell, a multipotent progenitor cell, a lineage restricted progenitor cell, a lymphoid progenitor cell, a myeloid progenitor cell, a common myeloid progenitor cell, an erythroid progenitor cell, a megakaryocyte erythroid progenitor cell, a retinal cell, a photoreceptor cell, a rod cell, a cone cell, a retinal pigmented epithelium cell, a trabecular meshwork cell, a cochlear hair cell, an outer hair cell, an inner hair cell, a pulmonary epithelial cell, a bronchial epithelial cell, an alveolar epithelial cell, a pulmonary epithelial progenitor cell, a striated muscle cell, a cardiac muscle cell, a muscle satellite cell, a neuron, a neuronal stem cell, a mesenchymal stem cell, an induced pluripotent stem (iPS) cell, an embryonic stem cell, a fibroblast, a monocyte-derived macrophage or dendritic cell, a megakaryocyte, a neutrophil, an eosinophil, a basophil, a mast cell, a reticulocyte, a B cell, e.g., a progenitor B cell, a Pre B cell, a Pro B cell, a memory B cell, a plasma B cell, a gastrointestinal epithelial cell, a biliary epithelial cell, a pancreatic ductal epithelial cell, an intestinal stem cell, a hepatocyte, a liver stellate cell, a Kupffer cell, an osteoblast, an osteoclast, an adipocyte, a preadipocyte, a pancreatic islet cell (e.g., a beta cell, an alpha cell, a delta cell), a pancreatic exocrine cell, a Schwann cell, or an oligodendrocyte.

[0339] In some embodiments, a donor cell is one or more of a circulating blood cell, e.g., a reticulocyte, megakaryocyte erythroid progenitor (MEP) cell, myeloid progenitor cell (CMP/GMP), lymphoid progenitor (LP) cell, hematopoietic stem/progenitor cell (HSC), or endothelial cell (EC). In some embodiments, a donor cell is one or more of a bone marrow cell (e.g., a reticulocyte, an erythroid cell (e.g., erythroblast), an MEP cell, myeloid progenitor cell (CMP/GMP), LP cell, erythroid progenitor (EP) cell, HSC, multipotent progenitor (MPP) cell, endothelial cell (EC), hemogenic endothelial (HE) cell, or mesenchymal stem cell). In some embodiments, a donor cell is one or more of a myeloid progenitor cell (e.g., a common myeloid progenitor (CMP) cell or granulocyte macrophage progenitor (GMP) cell). In some embodiments, a donor cell is one or more of a lymphoid progenitor cell, e.g., a common lymphoid progenitor (CLP) cell. In some embodiments, a donor cell is one or more of an

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erythroid progenitor cell (e.g., an MEP cell). In some embodiments, a donor cell is one or more of a hematopoietic stem/progenitor cell (e.g., a long term HSC (LT-HSC), short term HSC (ST-HSC), MPP cell, or lineage restricted progenitor (LRP) cell). In certain embodiments, the donor cell is a $CD34^+$ cell, $CD34^+CD90^+$ cell, $CD34^+CD38^-$ cell, $CD34^+CD90^+CD49f^-CD38^-CD45RA^-$ cell, $CD105^+$ cell, $CD31^+$, or $CD133^+$ cell, or a $CD34^-CD90^-$ $CD133^+$ cell. In some embodiments, a donor cell is one or more of an umbilical cord blood $CD34^+$ HSPC, umbilical cord venous endothelial cell, umbilical cord arterial endothelial cell, amniotic fluid $CD34^+$ cell, amniotic fluid endothelial cell, placental endothelial cell, or placental hematopoietic $CD34^+$ cell. In some embodiments, a donor cell is one or more of a mobilized peripheral blood hematopoietic $CD34^-$ cell (after the subject is treated with a mobilization agent, e.g., G-CSF or Plerixafor). In some embodiments, a donor cell is a peripheral blood endothelial cell. In some embodiments, a donor cell is a peripheral blood natural killer cell.

[0340] In some embodiments, a donor cell is a dividing cell. In some embodiments, a donor cell is a non-dividing cell.

[0341] In some embodiments, a genetically modified (e.g., edited) iNK cell resulting from one or more methods and/or strategies described herein, are administered to a subject in need thereof, e.g., in the context of an immuno-oncology therapeutic approach. In some embodiments, donor cells, or any cells of any stage of the reprogramming, differentiating, and/or genetic engineering strategies provided herein, can be maintained in culture or stored (e.g., frozen in liquid nitrogen) using any suitable method known in the art, e.g., for subsequent characterization or administration to a subject in need thereof.

Genetically Modified Cells

Loss-of-Function Modifications

[0342] In some embodiments, a target cell described herein (e.g., an NK cell or a stem cell (e.g., iPSC) described herein) is genetically engineered to introduce a disruption (e.g., a knockout) in one or more targets described herein. For example, in some embodiments, a target cell described herein (e.g., an NK cell or a stem cell (e.g., iPSC) described herein) can be genetically engineered to knockout all or a portion of one or more target gene, introduce a frameshift in one or more target genes, and/or cause a truncation of an encoded gene product (e.g., by introducing a premature stop codon). In some embodiments, a target cell described

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herein (e.g., an NK cell or a stem cell (e.g., iPSC) described herein) can be genetically engineered to knockout all or a portion of a target gene using a gene-editing system, e.g., as described herein. In some such embodiments, a gene-editing system may be or comprise a CRISPR system, a zinc finger nuclease system, a TALEN, and/or a meganuclease.

[0343] In some embodiments, the present disclosure provides methods suitable for high-efficiency knockout (e.g., a high proportion of a cell population comprises a knockout). In some embodiments, high-efficiency knockout results in at least 65% of the cells in a population of cells comprising a knockout (e.g., at least 65%, at least 70%, at least 75%, 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%, or at least 99% of the cells in a population of cells comprise a knockout).

[0344] In certain embodiments, the disclosure provides a genetically engineered target cell described herein (e.g., an NK cell or a stem cell (e.g., iPSC) described herein), and/or progeny cell, comprising a disruption in TGF signaling, e.g., TGF beta signaling. In some embodiments, this is useful, for example, in circumstances where it is desirable to generate a differentiated cell (e.g., an NK cell) from pluripotent stem cell, wherein TGF signaling, e.g., TGF beta signaling is disrupted in the differentiated cell.

[0345] TGF beta signaling inhibits or decreases the survival and/or activity of some differentiated cell types that are useful for therapeutic applications, e.g., TGF beta signaling is a negative regulator of natural killer cells, which can be used in immunotherapeutic applications. In some embodiments, it is desirable to generate a clinically effective number of natural killer cells comprising a genetic modification that disrupts TGF beta signaling, thus avoiding the negative effect of TGF beta on the clinical effectiveness of such cells. It is advantageous, in some embodiments, to source such NK cells from a pluripotent stem cell, instead, for example, from mature NK cells obtained from a donor. Modifying a stem cell instead of a differentiated cell has, among others, the advantage of allowing for clonal derivation, characterization, and/or expansion of a specific genotype, e.g., a specific stem cell clone harboring a specific genetic modification (e.g., a targeted disruption of TGFβRII in the absence of any undesired (e.g., off-target) modifications). In some embodiments, a stem cell, e.g., a human iPSC, is genetically engineered not to express one or more TGFβ receptor, e.g., TGFβRII, or to express a dominant

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negative variant of a TGFβ receptor, e.g., a dominant negative TGFβRII variant. Exemplary sequences of TGFβRII are set forth in KR710923.1, NM_001024847.2, and NM_003242.5. An exemplary dominant negative TGFβRII is disclosed in Immunity. 2000 Feb;12(2):171-81.

[0346] In certain embodiments, the disclosure provides a genetically engineered target cell described herein (e.g., an NK cell or a stem cell (e.g., iPSC) described herein), and/or progeny cell, that additionally or alternatively comprises a disruption in interleukin signaling, e.g., IL-15 signaling. IL-15 is a cytokine with structural similarity to Interleukin-2 (IL-2), which binds to and signals through a complex composed of IL-2/IL-15 receptor beta chain (CD122) and the common gamma chain (gamma-C, CD132). Exemplary sequences of IL-15 are provided in NG_029605.2. Disruption of IL-15 signaling may be useful, for example, in circumstances where it is desirable to generate a differentiated cell from a pluripotent stem cell, but with certain signaling pathways (e.g., IL-15) disrupted in the differentiated cell. IL-15 signaling can inhibit or decrease survival and/or activity of some types of differentiated cells, such as cells that may be useful for therapeutic applications. For example, IL-15 signaling is a negative regulator of natural killer (NK) cells.

[0347] CISH (encoded by the CISH gene) is downstream of the IL-15 receptor and can act as a negative regulator of IL-15 signaling in NK cells. As used herein, the term "CISH" refers to the Cytokine Inducible SH2 Containing Protein (see, e.g., Delconte et al., Nat Immunol. 2016 Jul;17(7):816-24; exemplary sequences for CISH are set forth as NG_023194.1). In some embodiments, disruption of CISH regulation may increase activation of Jak/STAT pathways, leading to increased survival, proliferation and/or effector functions of NK cells. Thus, in some embodiments, genetically engineered NK cells (e.g., iNK cells, e.g., generated from genetically engineered hiPSCs comprising a disruption of CISH regulation) exhibit greater responsiveness to IL-15-mediated signaling than non-genetically engineered NK cells. In some such embodiments, genetically engineered NK cells exhibit greater effector function relative to non-genetically engineered NK cells.

[0348] In some embodiments, a genetically engineered NK cell, stem cell and/or progeny cell, additionally or alternatively, comprises a disruption and/or loss of function in one or more of B2M, NKG2A, PD1, TIGIT, ADORA2a, CIITA, HLA class II histocompatibility antigen alpha chain genes, HLA class II histocompatibility antigen beta chain genes, CD32B, or TRAC.

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[0349] As used herein, the term "B2M" (β2 microglobulin) refers to a serum protein found in association with the major histocompatibility complex (MHC) class I heavy chain on the surface of nearly all nucleated cells. Exemplary sequences for B2M are set forth as NG_012920.2.

[0350] As used herein, the term "NKG2A" (natural killer group 2A) refers to a protein belonging to the killer cell lectin-like receptor family, also called NKG2 family, which is a group of transmembrane proteins preferentially expressed in NK cells. This family of proteins is characterized by the type II membrane orientation and the presence of a C-type lectin domain. See, e.g., Kamiya-T et al., J Clin Invest 2019 https://doi.org/10.1172/JCI123955. Exemplary sequences for NKG2A are set forth as AF461812.1.

[0351] As used herein, the term "PD1" (Programmed cell death protein 1), also known CD279 (cluster of differentiation 279), refers to a protein found on the surface of cells that has a role in regulating the immune system's response to the cells of the human body by down-regulating the immune system and promoting self-tolerance by suppressing T cell inflammatory activity. PD1 is an immune checkpoint and guards against autoimmunity. Exemplary sequences for PD1 are set forth as NM_005018.3.

[0352] As used herein, the term "TIGIT" (T cell immunoreceptor with Ig and ITIM domains) refers to a member of the PVR (poliovirus receptor) family of immunoglobulin proteins. The product of this gene is expressed on several classes of T cells including follicular B helper T cells (TFH). Exemplary sequences for TIGIT are set forth in NM_173799.4.

[0353] As used herein, the term "ADORA2A" refers to the adenosine A2a receptor, a member of the guanine nucleotide-binding protein (G protein)-coupled receptor (GPCR) superfamily, which is subdivided into classes and subtypes. This protein, an adenosine receptor of A2A subtype, uses adenosine as the preferred endogenous agonist and preferentially interacts with the G(s) and G(olf) family of G proteins to increase intracellular cAMP levels. Exemplary sequences of ADORA2a are provided in NG_052804.1.

[0354] As used herein, the term "CIITA" refers to the protein located in the nucleus that acts as a positive regulator of class II major histocompatibility complex gene transcription, and is referred to as the "master control factor" for the expression of these genes. The protein also binds GTP and uses GTP binding to facilitate its own transport into the nucleus. Mutations in

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this gene have been associated with bare lymphocyte syndrome type II (also known as hereditary MHC class II deficiency or HLA class II-deficient combined immunodeficiency), increased susceptibility to rheumatoid arthritis, multiple sclerosis, and possibly myocardial infarction. See, e.g., Chang et al., J Exp Med 180:1367-1374; and Chang et al., Immunity. 1996 Feb;4(2):167-78, the entire contents of each of which are incorporated by reference herein. An exemplary sequence of CIITA is set forth as NG_009628.1.

[0355] In some embodiments, two or more HLA class II histocompatibility antigen alpha chain genes and/or two or more HLA class II histocompatibility antigen beta chain genes are disrupted, e.g., knocked out, e.g., by genomic editing. For example, in some embodiments, two or more HLA class II histocompatibility antigen alpha chain genes selected from HLA-DQA1, HLA-DRA, HLA-DPA1, HLA-DMA, HLA-DQA2, and HLA-DOA are disrupted, e.g., knocked out. For another example, in some embodiments, two or more HLA class II histocompatibility antigen beta chain genes selected from HLA-DMB, HLA-DOB, HLA-DPB1, HLA-DQB1, HLA-DQB3, HLA-DQB2, HLA-DRB1, HLA-DRB3, HLA-DRB4, and HLA-DRB5 are disrupted, e.g., knocked out. See, e.g., Crivello et al., J Immunol January 2019, ji1800257; DOI: https://doi.org/10.4049/jimmunol.1800257, the entire contents of which are incorporated herein by reference.

[0356] As used herein, the term "CD32B" (cluster of differentiation 32B) refers to a low affinity immunoglobulin gamma Fc region receptor II-b protein that, in humans, is encoded by the FCGR2B gene. See, e.g., Rankin-CT et al., Blood 2006 108(7):2384-91, the entire contents of which are incorporated herein by reference.

[0357] As used herein, the term "TRAC" refers to the T-cell receptor alpha subunit (constant), encoded by the TRAC locus.

Gain-of-Function Modifications

[0358] In some embodiments, a target cell described herein (e.g., an NK cell or a stem cell (e.g., iPSC) described herein) can additionally be genetically engineered to comprise a genetic modification that leads to expression of one or more gene products of interest described herein using, e.g., a gene-editing system, e.g., as described herein. In some such embodiments, a gene-editing system may be or comprise a CRISPR system, a zinc finger nuclease system, a TALEN, and/or a meganuclease.

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[0359] In some embodiments, a cell is produced by a method of the present disclosure, e.g., a method that comprises contacting the cell with a nuclease that causes a break within an endogenous coding sequence of an essential gene in the cell wherein the essential gene encodes at least one gene product that is required for survival and/or proliferation of the cell. The cell is also contacted with a donor template that comprises a knock-in cassette comprising an exogenous coding sequence for a gene product of interest in frame with and downstream (3′) of an exogenous coding sequence or partial coding sequence of the essential gene. The knock-in cassette is integrated into the genome of the cell by homology-directed repair (HDR) of the break, resulting in a genome-edited cell that expresses the gene product of interest and the gene product encoded by the essential gene that is required for survival and/or proliferation of the cell, or a functional variant thereof. This is illustrated in Fig. 3 for an exemplary method. In some embodiments, a cell is contacted with a donor template that comprises a knock-in cassette comprising an exogenous coding sequence for a gene product of interest in frame with and upstream (5′) of an exogenous coding sequence or partial coding sequence of the essential gene.

[0360] In some embodiments, the cell comprises a genome with an exogenous coding sequence for a gene product of interest in frame with and downstream (3′) of a coding sequence of an essential gene, wherein the essential gene encodes a gene product that is required for survival and/or proliferation of the cell.

[0361] In some embodiments, the cell comprises a genome with an exogenous coding sequence for a gene product of interest in frame with and upstream (5′) of a coding sequence of an essential gene, wherein the essential gene encodes a gene product that is required for survival and/or proliferation of the cell.

[0362] In some embodiments, the cell comprises a genomic modification, wherein the genomic modification comprises an insertion of an exogenous knock-in cassette within an endogenous coding sequence of an essential gene in the cell's genome, wherein the essential gene encodes a gene product that is required for survival and/or proliferation of the cell, wherein the knock-in cassette comprises an exogenous coding sequence for a gene product of interest in frame with and downstream (3′) of an exogenous coding sequence or partial coding sequence encoding the gene product of the essential gene, or a functional variant thereof, and wherein the cell expresses the gene product of interest and the gene product encoded by the essential gene

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that is required for survival and/or proliferation of the cell, or a functional variant thereof. In some embodiments, the gene product of interest and the gene product encoded by the essential gene are expressed from the endogenous promoter of the essential gene.

Donor template

[0363] In one aspect the present disclosure provides a donor template comprising a knock-in cassette with an exogenous coding sequence for a gene product of interest in frame with and downstream (3′) of an exogenous coding sequence or partial coding sequence of an essential gene, wherein the essential gene encodes a gene product that is required for survival and/or proliferation of the cell.

[0364] In one aspect the present disclosure provides an impetus for designing donor templates comprising a knock-in cassette with an exogenous coding sequence for a gene product of interest in frame with and upstream (5′) of an exogenous coding sequence or partial coding sequence of an essential gene, wherein the essential gene encodes a gene product that is required for survival and/or proliferation of the cell; see e.g., Fig. 3D.

[0365] In some embodiments, the donor template is for use in editing the genome of a cell by homology-directed repair (HDR).

[0366] Donor template design is described in detail in the literature, for instance in PCT Publication No. WO2016/073990A1. Donor templates can be single-stranded or double-stranded and can be used to facilitate HDR-based repair of double-strand breaks (DSBs), and are particularly useful for inserting a new sequence into the target sequence, or replacing the target sequence altogether. In some embodiments, the donor template is a donor DNA template. In some embodiments the donor DNA template is double-stranded.

[0367] Whether single-stranded or double stranded, donor templates generally include regions that are homologous to regions of DNA within or near (e.g., flanking or adjoining) a target sequence to be cleaved. These homologous regions are referred to herein as "homology arms," and are illustrated schematically below relative to the knock-in cassette (which may be separated from one or both of the homology arms by additional spacer sequences that are not shown):

[0368] [5′ homology arm] – [knock-in cassette] – [3′ homology arm].

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[0369] The homology arms can have any suitable length (including 0 nucleotides if only one homology arm is used), and 5′ and 3′ homology arms can have the same length, or can differ in length. The selection of appropriate homology arm lengths can be influenced by a variety of factors, such as the desire to avoid homologies or microhomologies with certain sequences such as Alu repeats or other very common elements. For example, a 5′ homology arm can be shortened to avoid a sequence repeat element. In other embodiments, a 3′ homology arm can be shortened to avoid a sequence repeat element. In some embodiments, both the 5′ and the 3′ homology arms can be shortened to avoid including certain sequence repeat elements.

[0370] A donor template can be a nucleic acid vector, such as a viral genome or circular double-stranded DNA, e.g., a plasmid. Nucleic acid vectors comprising donor templates can include other coding or non-coding elements. For example, a donor template nucleic acid can be delivered as part of a viral genome (e.g., in an AAV, adenoviral, Sendai virus, or lentiviral genome) that includes certain genomic backbone elements (e.g., inverted terminal repeats, in the case of an AAV genome). In some embodiments, a donor template is comprised in a plasmid that has not been linearized. In some embodiments, a donor template is comprised in a plasmid that has been linearized. In some embodiments, a donor template is comprised within a linear dsDNA fragment. In some embodiments, a donor template nucleic acid can be delivered as part of an AAV genome. In some embodiments, a donor template nucleic acid can be delivered as a single stranded oligo donor (ssODN), for example, as a long multi-kb ssODN derived from m13 phage synthesis, or alternatively, short ssODNs, e.g., that comprise small genes of interest, tags, and/or probes. In some embodiments, a donor template nucleic acid can be delivered as a Doggybone™ DNA (dbDNA™) template. In some embodiments, a donor template nucleic acid can be delivered as a DNA minicircle. In some embodiments, a donor template nucleic acid can be delivered as an Integration-deficient Lentiviral Particle (IDLV). In some embodiments, a donor template nucleic acid can be delivered as a MMLV-derived retrovirus. In some embodiments, a donor template nucleic acid can be delivered as a piggyBac™ sequence. In some embodiments, a donor template nucleic acid can be delivered as a replicating EBNA1 episome.

[0371] In certain embodiments, the 5′ homology arm may be about 25 to about 1,000 base pairs in length, e.g., at least about 100, 200, 400, 600, or 800 base pairs in length. In certain embodiments, the 5′ homology arm comprises about 50 to 800 base pairs, e.g., 100 to 800, 200 to

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800, 400 to 800, 400 to 600, or 600 to 800 base pairs. In certain embodiments, the 3′ homology arm may be about 25 to about 1,000 base pairs in length, e.g., at least about 100, 200, 400, 600, or 800 base pairs in length. In certain embodiments, the 3′ homology arm comprises about 50 to 800 base pairs, e.g., 100 to 800, 200 to 800, 400 to 800, 400 to 600, or 600 to 800 base pairs. In certain embodiments, the 5′ and 3′ homology arms are symmetrical in length. In certain embodiments, the 5′ and 3′ homology arms are asymmetrical in length.

[0372] In certain embodiments, a 5′ homology arm is less than about 3,000 base pairs, less than about 2,900 base pairs, less than about 2,800 base pairs, less than about 2,700 base pairs, less than about 2,600 base pairs, less than about 2,500 base pairs, less than about 2,400 base pairs, less than about 2,300 base pairs, less than about 2,200 base pairs, less than about 2,100 base pairs, less than about 2,000 base pairs, less than about 1,900 base pairs, less than about 1,800 base pairs, less than about 1,700 base pairs, less than about 1,600 base pairs, less than about 1,500 base pairs, less than about 1,400 base pairs, less than about 1,300 base pairs, less than about 1,200 base pairs, less than about 1,100 base pairs, less than about 1,000 base pairs, less than about 900 base pairs, less than about 800 base pairs, less than about 700 base pairs, less than about 600 base pairs, less than about 500 base pairs, or less than about 400 base pairs.

[0373] In certain embodiments, e.g., where a viral vector is utilized to introduce a knock-in cassette through a method described herein, a 5′ homology arm is less than about 1,000 base pairs, less than about 900 base pairs, less than about 800 base pairs, is less than about 700 base pairs, less than about 600 base pairs, less than about 500 base pairs, less than about 400 base pairs, or less than about 300 base pairs. In certain embodiments, e.g., where a viral vector is utilized to introduce a knock-in cassette through a method described herein, a 5′ homology arm is about 400-600 base pairs, e.g., about 500 base pairs.

[0374] In certain embodiments, a 3′ homology arm is less than about 3,000 base pairs, less than about 2,900 base pairs, less than about 2,800 base pairs, less than about 2,700 base pairs, less than about 2,600 base pairs, less than about 2,500 base pairs, less than about 2,400 base pairs, less than about 2,300 base pairs, less than about 2,200 base pairs, less than about 2,100 base pairs, less than about 2,000 base pairs, less than about 1,900 base pairs, less than about 1,800 base pairs, less than about 1,700 base pairs, less than about 1,600 base pairs, less

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than about 1,500 base pairs, less than about 1,400 base pairs, less than about 1,300 base pairs, less than about 1,200 base pairs, less than about 1,100 base pairs, less than 1,000 base pairs, less than about 900 base pairs, less than about 800 base pairs, less than about 700 base pairs, less than about 600 base pairs, less than about 500 base pairs, or less than about 400 base pairs.

[0375] In certain embodiments, e.g., where a viral vector is utilized to introduce a knock-in cassette through a method described herein, a 3′ homology arm is less than about 1,000 base pairs, less than about 900 base pairs, less than about 800 base pairs, less than about 700 base pairs, less than about 600 base pairs, less than about 500 base pairs, less than about 400 base pairs, or less than about 300 base pairs. In certain embodiments, e.g., where a viral vector is utilized to introduce a knock-in cassette through a method described herein, a 3′ homology arm is about 400-600 base pairs, e.g., about 500 base pairs.

[0376] In certain embodiments, the 5′ and 3′ homology arms flank the break and are less than 100, 75, 50, 25, 15, 10 or 5 base pairs away from an edge of the break. In certain embodiments, the 5′ and 3′ homology arms flank an endogenous stop codon. In certain embodiments, the 5′ and 3′ homology arms flank a break located within about 500 base pairs (e.g., about 500 base pairs, about 450 base pairs, about 400 base pairs, about 350 base pairs, about 300 base pairs, about 250 base pairs, about 200 base pairs, about 150 base pairs, about 100 base pairs, about 50 base pairs, or about 25 base pairs) upstream (5′) of an endogenous stop codon, e.g., the stop codon of an essential gene. In certain embodiments, the 5′ homology arm encompasses an edge of the break.

Knock-in cassette

[0377] In some embodiments, the knock-in cassette within the donor template comprises an exogenous coding sequence for the gene product of interest in frame with and downstream (3′) of an exogenous coding sequence or partial coding sequence of the essential gene. In some embodiments, a knock-in cassette within a donor template comprises an exogenous coding sequence for the gene product of interest in frame with and upstream (5′) of an exogenous coding sequence or partial coding sequence of an essential gene. In some embodiments, the knock-in cassette is a polycistronic knock-in cassette. In some embodiments, the knock-in cassette is a bicistronic knock-in cassette. In some embodiment the knock-in cassette does not comprise a reporter gene, e.g., a fluorescent reporter gene or an antibiotic resistance gene.

[0378] In some embodiments, a single essential gene locus will be targeted by two knock-in cassettes comprising different "cargo" sequences. In some embodiments, one allele will

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incorporate one knock-in cassette, while the other allele will incorporate the other knock-in cassette. In some embodiments, a gRNA utilized to generate an appropriate DNA break may be the same for each of the two different knock-in cassettes. In some embodiments, gRNAs utilized to generate appropriate DNA breaks for each of the two different knock-in cassettes may be different, such that the "cargo" sequence is incorporated at a different position for each allele. In some embodiments, such a different position for each allele may still be within the ultimate exons coding region. In some embodiments, such a different position for each allele may be within the penultimate exon (second to last), and/or ultimate (last) exons coding region. In some embodiments, such a different position for at least one of the alleles may be within the first exon. In some embodiments, such a different position for at least one of the alleles may be within the first or second exon.

[0379] In order to properly restore the essential gene coding region in the genetically modified cell (so that a functioning gene product is produced) the knock-in cassette does not need to comprise an exogenous coding sequence that corresponds to the entire coding sequence of the essential gene. Indeed, depending on the location of the break in the endogenous coding sequence of the essential gene it may be possible to restore the essential gene by providing a knock-in cassette that comprises a partial coding sequence of the essential gene, e.g., that corresponds to a portion of the endogenous coding sequence of the essential gene that spans the break and the entire region downstream of the break (minus the stop codon), and/or that corresponds to a portion of the endogenous coding sequence of the essential gene that spans the break and the entire region upstream of the break (up to and optionally including the start codon).

[0380] In order to minimize the size of the knock-in cassette it may in fact be advantageous, in some embodiments, to have the break located within the last 1500, 1000, 750, 500, 400, 300, 200, 100, or 50 base pairs of the endogenous coding sequence of the essential gene, i.e., towards the 3′ end of the coding sequence. In some embodiments, a base pair's location in a coding sequence may be defined 3′-to-5′ from an endogenous translational stop signal (e.g., a stop codon). In some embodiments, as used herein, an "endogenous coding sequence" can include both exonic and intronic base pairs, and refers to gene sequence occurring 5′ to an endogenous functional translational stop signal. In some embodiments, a break within an

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endogenous coding sequence comprises a break within one DNA strand. In some embodiments, a break within an endogenous coding sequence comprises a break within both DNA strands. In some embodiments, a break is located within the last 1000 base pairs of the endogenous coding sequence. In some embodiments, a break is located within the last 750 base pairs of the endogenous coding sequence. In some embodiments, a break is located within the last 600 base pairs of the endogenous coding sequence. In some embodiments, a break is located within the last 500 base pairs of the endogenous coding sequence. In some embodiments, a break is located within the last 400 base pairs of the endogenous coding sequence. In some embodiments, a break is located within the last 300 base pairs of the endogenous coding sequence. In some embodiments, a break is located within the last 250 base pairs of the endogenous coding sequence. In some embodiments, a break is located within the last 200 base pairs of the endogenous coding sequence. In some embodiments, a break is located within the last 150 base pairs of the endogenous coding sequence. In some embodiments, a break is located within the last 100 base pairs of the endogenous coding sequence. In some embodiments, a break is located within the last 75 base pairs of the endogenous coding sequence. In some embodiments, a break is located within the last 50 base pairs of the endogenous coding sequence. In some embodiments, a break is located within the last 21 base pairs of the endogenous coding sequence.

[0381] In some embodiments, the exogenous partial coding sequence of the essential gene in the knock-in cassette encodes a C-terminal fragment of a protein encoded by the essential gene, e.g., a fragment that is less than 500, 250, 150, 125, 100, 75, 50, 25, 20, 15 or 10 amino acids in length. In some embodiments, the exogenous partial coding sequence of the essential gene in the knock-in cassette is codon optimized. In some embodiments, the exogenous partial coding sequence of the essential gene in the knock-in cassette is codon optimized to eliminate at least one PAM site. In some embodiments, the exogenous partial coding sequence of the essential gene in the knock-in cassette is codon optimized to eliminate more than one PAM site. In some embodiments, the exogenous partial coding sequence of the essential gene in the knock-in cassette is codon optimized to eliminate all relevant nuclease specific PAM sites. In some embodiments, a C-terminal fragment of a protein encoded by the essential gene is about 140 amino acids in length. In some embodiments, a C-terminal fragment of a protein encoded by the essential gene is about 130 amino acids in length. In some embodiments, a C-terminal

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fragment of a protein encoded by the essential gene is about 120 amino acids in length. In some embodiments, the C-terminal fragment includes an amino acid sequence that is encoded by a region of the endogenous coding sequence of the essential gene that spans the break. In some embodiments, a C-terminal fragment includes an amino acid sequence that is encoded by a region of the endogenous coding sequence within 1 exon of the essential gene. In some embodiments, a C-terminal fragment includes an amino acid sequence that is encoded by a region of the endogenous coding sequence within 2 exons of the essential gene. In some embodiments, a C-terminal fragment includes an amino acid sequence that is encoded by a region of the endogenous coding sequence within 3 exons of the essential gene. In some embodiments, a C-terminal fragment includes an amino acid sequence that is encoded by a region of the endogenous coding sequence within 4 exons of the essential gene. In some embodiments, a C-terminal fragment includes an amino acid sequence that is encoded by a region of the endogenous coding sequence within 5 exons of the essential gene.

[0382] In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette encodes a C-terminal fragment of a protein encoded by an essential gene, e.g., a fragment that is less than 500, 250, 150, 125, 100, 75, 50, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, or 7 amino acids in length. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette encodes a 20 amino acid C-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette encodes a 19 amino acid C-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette encodes an 18 amino acid C-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette encodes a 17 amino acid C-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette encodes a 16 amino acid C-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette encodes a 1 amino acid C-terminal fragment of a protein encoded by an essential gene.

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[0383] In some embodiments, e.g., when the essential gene includes many exons as shown in the exemplary method of Fig. 3A, it may be advantageous to have the break within the last exon of the essential gene. In some embodiments, e.g., when the essential gene includes many exons as shown in the exemplary method of Fig. 3A, it may be advantageous to have the break within the penultimate exon of the essential gene. It is to be understood however that the present disclosure is not limited to any particular location for the break and that the available positions will vary depending on the nature and length of the essential gene and the length of the exogenous coding sequence for the gene product of interest. For example, for essential genes that include a few exons or when the gene product of interest is small it may be possible to locate the break in an upstream exon.

[0384] In order to minimize the size of the knock-in cassette it may in fact be advantageous, in some embodiments, to have the break located within the first 1500, 1000, 750, 500, 400, 300, 200, 100, or 50 base pairs of an endogenous coding sequence of the essential gene, i.e., starting from the 5′ end of a coding sequence. In some embodiments, a base pair's location in a coding sequence may be defined 5′-to-3′ from an endogenous translational start signal (e.g., a start codon). In some embodiments, as used herein, an "endogenous coding sequence" can include both exonic and intronic base pairs, and refers to gene sequence occurring 3′ to an endogenous functional translational start signal. In some embodiments, a break within an endogenous coding sequence comprises a break within one DNA strand. In some embodiments, a break within an endogenous coding sequence comprises a break within both DNA strands. In some embodiments, a break is located within the first 1000 base pairs of the endogenous coding sequence. In some embodiments, a break is located within the first 750 base pairs of an endogenous coding sequence. In some embodiments, a break is located within the first 600 base pairs of the endogenous coding sequence. In some embodiments, a break is located within the first 500 base pairs of the endogenous coding sequence. In some embodiments, a break is located within the first 400 base pairs of the endogenous coding sequence. In some embodiments, a break is located within the first 300 base pairs of the endogenous coding sequence. In some embodiments, a break is located within the first 250 base pairs of the endogenous coding sequence. In some embodiments, a break is located within the first 200 base pairs of the endogenous coding sequence. In some embodiments, a break is located within the first 150 base

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pairs of the endogenous coding sequence. In some embodiments, a break is located within the first 100 base pairs of the endogenous coding sequence. In some embodiments, a break is located within the first 75 base pairs of the endogenous coding sequence. In some embodiments, a break is located within the first 50 base pairs of the endogenous coding sequence. In some embodiments, a break is located within the first 21 base pairs of the endogenous coding sequence.

[0385] In some embodiments, the exogenous partial coding sequence of the essential gene in the knock-in cassette encodes an N-terminal fragment of a protein encoded by the essential gene, e.g., a fragment that is less than 500, 250, 150, 125, 100, 75, 50, 25, 20, 15 or 10 amino acids in length. In some embodiments, an N-terminal fragment of a protein encoded by the essential gene is about 140 amino acids in length. In some embodiments, an N-terminal fragment of a protein encoded by the essential gene is about 130 amino acids in length. In some embodiments, an N-terminal fragment of a protein encoded by the essential gene is about 120 amino acids in length. In some embodiments, an N-terminal fragment includes an amino acid sequence that is encoded by a region of the endogenous coding sequence of the essential gene that spans the break. In some embodiments, an N-terminal fragment includes an amino acid sequence that is encoded by a region of the endogenous coding sequence within 1 exon of the essential gene. In some embodiments, an N-terminal fragment includes an amino acid sequence that is encoded by a region of the endogenous coding sequence within 2 exons of the essential gene. In some embodiments, an N-terminal fragment includes an amino acid sequence that is encoded by a region of the endogenous coding sequence within 3 exons of the essential gene. In some embodiments, an N-terminal fragment includes an amino acid sequence that is encoded by a region of the endogenous coding sequence within 4 exons of the essential gene. In some embodiments, an N-terminal fragment includes an amino acid sequence that is encoded by a region of the endogenous coding sequence within 5 exons of the essential gene.

[0386] In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette encodes an N-terminal fragment of a protein encoded by an essential gene, e.g., a fragment that is less than 500, 250, 150, 125, 100, 75, 50, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, or 7 amino acids in length. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette encodes a 20 amino acid N-terminal

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fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette encodes a 19 amino acid N-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette encodes an 18 amino acid N-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette encodes a 17 amino acid N-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette encodes a 16 amino acid N-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette encodes a 1 amino acid N-terminal fragment of a protein encoded by an essential gene.

[0387] In some embodiments, the exogenous coding sequence or partial coding sequence of the essential gene in the knock-in cassette is less than 100% identical to the corresponding endogenous coding sequence of the essential gene of the cell, e.g., less than 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55% or less than 50% (i.e., when the two sequences are aligned using a standard pairwise sequence alignment tool that maximizes the alignment between the corresponding sequences). For example, in some embodiments, the exogenous coding sequence or partial coding sequence of the essential gene in the knock-in cassette is codon optimized relative to the corresponding endogenous coding sequence of the essential gene of the cell, e.g., to prevent further binding of a nuclease to the target site. Alternatively or additionally it may be codon optimized to reduce the likelihood of recombination after integration of the knock-in cassette into the genome of the cell and/or to increase expression of the gene product of the essential gene and/or the gene product of interest after integration of the knock-in cassette into the genome of the cell.

[0388] In some embodiments, a knock-in cassette comprises one or more nucleotides or base pairs that differ (e.g., are mutations) relative to an endogenous knock-in site. In some embodiments, such mutations in a knock-in cassette provide resistance to cutting by a nuclease. In some embodiments, such mutations in a knock-in cassette prevent a nuclease from cutting the target loci following homologous recombination. In some embodiments, such mutations in a knock-in cassette occur within one or more coding and/or non-coding regions of a target gene. In

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some embodiments, such mutations in a knock-in cassette are silent mutations. In some embodiments, such mutations in a knock-in cassette are silent and/or missense mutations.

[0389] In some embodiments, such mutations in a knock-in cassette occur within a target protospacer motif and/or a target protospacer adjacent motif (PAM) site. In some embodiments, a knock-in cassette includes a target protospacer motif and/or a PAM site that are saturated with silent mutations. In some embodiments, a knock-in cassette includes a target protospacer motif and/or a PAM site that are approximately 30%, 40%, 50%, 60%, 70%, 80%, or 90% saturated with silent mutations. In some embodiments, a knock-in cassette includes a target protospacer motif and/or a PAM site that are saturated with silent and/or missense mutations. In some embodiments, a knock-in cassette includes a target protospacer motif and/or a PAM site that comprise at least one mutation, at least 2 mutations, at least 3 mutations, at least 4 mutations, at least 5 mutations, at least 6 mutations, at least 7 mutations, at least 8 mutations, at least 9 mutations, at least 10 mutations, at least 11 mutations, at least 12 mutations, at least 13 mutations, at least 14 mutations, or at least 15 mutations.

[0390] In some embodiments, certain codons encoding certain amino acids in a target site cannot be mutated through codon-optimization without losing some portion of an endogenous proteins natural function. In some embodiments, certain codons encoding certain amino acids in a target site cannot be mutated through codon-optimization.

[0391] In some embodiments, the knock-in cassette is codon optimized in only a portion of the coding sequence. For example, in some embodiments, a knock-in cassette encodes a C-terminal fragment of a protein encoded by an essential gene, e.g., a fragment that is less than 500, 250, 150, 125, 100, 75, 50, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, or 7 amino acids in length. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 20 amino acid C-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 19 amino acid C-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes an 18 amino acid C-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of

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an essential gene in a knock-in cassette that has been codon optimized encodes a 17 amino acid C-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 16 amino acid C-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 15 amino acid C-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 14 amino acid C-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 13 amino acid C-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 12 amino acid C-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 11 amino acid C-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 10 amino acid C-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 9 amino acid C-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes an 8 amino acid C-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 7 amino acid C-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 6 amino acid C-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 5 amino acid C-terminal

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fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes an amino acid C-terminal fragment that is less than 5 amino acids of a protein encoded by an essential gene.

[0392] In some embodiments, the knock-in cassette is codon optimized in only a portion of the coding sequence. For example, in some embodiments, a knock-in cassette encodes an N-terminal fragment of a protein encoded by an essential gene, e.g., a fragment that is less than 500, 250, 150, 125, 100, 75, 50, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, or 7 amino acids in length. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 20 amino acid N-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 19 amino acid N-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes an 18 amino acid N-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 17 amino acid N-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 16 amino acid N-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 15 amino acid N-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 14 amino acid N-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 13 amino acid N-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 12 amino acid N-terminal fragment of a protein encoded by an essential gene. In some embodiments, the

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exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 11 amino acid N-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 10 amino acid N-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 9 amino acid N-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes an 8 amino acid N-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 7 amino acid N-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 6 amino acid N-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes a 5 amino acid N-terminal fragment of a protein encoded by an essential gene. In some embodiments, the exogenous partial coding sequence of an essential gene in a knock-in cassette that has been codon optimized encodes an amino acid N-terminal fragment that is less than 5 amino acids of a protein encoded by an essential gene.

[0393] In some embodiments, the knock-in cassette comprises one or more sequences encoding a linker peptide, e.g., between an exogenous coding sequence or partial coding sequence of the essential gene and a "cargo" sequence and/or a regulatory element described herein. Such linker peptides are known in the art, any of which can be included in a knock-in cassette described herein. In some embodiments, the linker peptide comprises the amino acid sequence GSG.

[0394] In some embodiments, the knock-in cassette comprises other regulatory elements such as a polyadenylation sequence, and optionally a 3′ UTR sequence, downstream of the exogenous coding sequence for the gene product of interest. If a 3′UTR sequence is present, the

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3'UTR sequence is positioned 3' of the exogenous coding sequence and 5' of the polyadenylation sequence.

[0395] In some embodiments, the knock-in cassette comprises other regulatory elements such as a 5' UTR and a start codon, upstream of the exogenous coding sequence for the gene product of interest. If a 5'UTR sequence is present, the 5'UTR sequence is positioned 5' of the "cargo" sequence and/or exogenous coding sequence.

Exemplary Homology Arms (HA)

[0396] In certain embodiments, a donor template comprises a 5' and/or 3' homology arm homologous to region of a *GAPDH* locus. In some embodiments, a donor template comprises a 5' homology arm comprising or consisting of the sequence of SEQ ID NO: 1, 2, or 3. In some embodiments, a 5' homology arm comprises or consists of a sequence that is at least 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO: 1, 2, or 3. In some embodiments, a donor template comprises a 3' homology arm comprising or consisting of the sequence of SEQ ID NO:4 or 5. In certain embodiments, a 3' homology arm comprises or consists of a sequence that is at least 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO: 4 or 5.

[0397] In some embodiments, a donor template comprises a 5' homology arm comprising SEQ ID NO: 1, and a 3' homology arm comprising SEQ ID NO: 4. In some embodiments, a donor template comprises a 5' homology arm comprising SEQ ID NO: 2, and a 3' homology arm comprising SEQ ID NO: 4. In some embodiments, a donor template comprises a 5' homology arm comprising SEQ ID NO: 3, and a 3' homology arm comprising SEQ ID NO: 5.

[0398] In some embodiments, a stretch of sequence flanking a nuclease cleavage site may be duplicated in both a 5' and 3' homology arm. In some embodiments, such a duplication is designed to optimize HDR efficiency. In some embodiments, one of the duplicated sequences may be codon optimized, while the other sequence is not codon optimized. In some embodiments, both of the duplicated sequences may be codon optimized. In some embodiments, codon optimization may remove a target PAM site. In some embodiments, a duplicated sequence may be no more than: 100 bp in length, 90 bp in length, 80 bp in length, 70 bp in length, 60 bp in length, 50 bp in length, 40 bp in length, 30 bp in length, or 20 bp in length.

SEQ ID NO: 1 - exemplary 5' HA for knock-in cassette insertion at *GAPDH* locus

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GAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATC
ATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATCCCTGAGCTGAACGGGAAGC
TCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCT
AGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTC
AAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACT
CCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTG
GTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCCTCTGGTGGCTG
GCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATGACAACGAGTTCGGATATAG
CAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAG

SEQ ID NO: 2 - exemplary 5′ HA for knock-in cassette insertion at *GAPDH* locus

GAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATC
ATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATCCCTGAGCTGAACGGGAAGC
TCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCT
AGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTC
AAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACT
CCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTG
GTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCCTCTGGTGGCTG
GCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATGACAACGAGTTCGGATATAG
CAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGGAAGCGGAGCTACTAACTTC
AGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCT

SEQ ID NO: 3 - exemplary 5′ HA for knock-in cassette insertion at *GAPDH* locus

GGCTTTCCCATAATTTCCTTTCAAGGTGGGGAGGGAGGTAGAGGGGTGATGTGGGGAGTACGCT
GCAGGGCCTCACTCCTTTTGCAGACCACAGTCCATGCCATCACTGCCACCCAGAAGACTGTGGA
TGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATCATCCCTGCCTCT
ACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATCCCTGAGCTGAACGGGAAGCTCACTGGCATGG
CCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCTAGAAAAACCTGC
CAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTCAAGGGCATCCTG
GGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACTCCTCCACCTTTG
ACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATCTCTTGGTACGACAATGA
GTTCGGATATAGCAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAG

SEQ ID NO: 4 - exemplary 3′ HA for knock-in cassette insertion at *GAPDH* locus

ATTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATGGCCTCCAAGGAGTAAGACCCC
TGGACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGACCCTCACTGCTGGGGAGTCCCT
GCCACACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCACAGTTGCCATGTAGACCCCTTGA
AGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACCATCAATAAAGTACCCTGTGCTCAA
CCAGTTACTTGTCCTGTCTTATTCTAGGGTCTGGGGCAGAGGGGAGGGAAGCTGGGCTTGTGTC
AAGGTGAGACATTCTTGCTGGGGAGGGACCTGGTATGTTCTCCTCAGACTGAGGGTAGGGCCTC
CAAACAGCCTTGCTTGCTTCGAGAACCATTTGCTTCCCGCTCAGACGTCTTGAGTGCTACAGGA
AGCTGGCACCACTACTTCAGAGAACAAGGCCTTTTCCTCTCCTCGCTCCAGT

SEQ ID NO: 5 - exemplary 3′ HA for knock-in cassette insertion at *GAPDH* locus

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AGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCCTCTGGTGGCTGGCTCAGAAAAAGGGCCCT
GACAACTCTTTTCATCTTCTAGGTATGACAACGAATTTGGCTACAGCAACAGGGTGGTGGACCT
CATGGCCCACATGGCCTCCAAGGAGTAAGACCCCTGGACCACCAGCCCCAGCAAGAGCACAAGA
GGAAGAGAGAGACCCTCACTGCTGGGGAGTCCCTGCCACACTCAGTCCCCCACCACACTGAATC
TCCCCTCCTCACAGTTGCCATGTAGACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTT
GTCATGTACCATCAATAAAGTACCCTGTGCTCAACCAGTTACTTGTCCTGTCTTATTCTAGGGT
CTGGGGCAGAGGGGAGGGAAGCTGGGCTTGTGTCAAGGTGAGACATTCTTGCTGGGGAGGGACC
TGGTATGTTCTCCTCAGACTGAGGGTAGGGCCTCCAAACAGCCTTGCTTGCT

[0399] In some embodiments, a donor template comprises a 5′ and/or 3′ homology arm homologous to a region of a *TBP* locus. In some embodiments, a donor template comprises a 5′ homology arm comprising or consisting of the sequence of SEQ ID NO:6, 7, or 8. In some embodiments, a 5′ homology arm comprises or consists of a sequence that is at least 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO: 6, 7, or 8. In some embodiments, a donor template comprises a 3′ homology arm comprising or consisting of the sequence of SEQ ID NO:9, 10, or 11. In certain embodiments, a 3′ homology arm comprises or consists of a sequence that is at least 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO: 9, 10, or 11.

[0400] In some embodiments, a donor template comprises a 5′ homology arm comprising SEQ ID NO: 6, and a 3′ homology arm comprising SEQ ID NO: 9. In some embodiments, a donor template comprises a 5′ homology arm comprising SEQ ID NO: 7, and a 3′ homology arm comprising SEQ ID NO: 10. In some embodiments, a donor template comprises a 5′ homology arm comprising SEQ ID NO: 8, and a 3′ homology arm comprising SEQ ID NO: 11.

SEQ ID NO: 6 - exemplary 5′ HA for knock-in cassette insertion at *TBP* locus

GCAGACTTCCATTTACAGTGAGGAGGTGAGCATTGCATTGAACAAAAGATGGCGTTTTCACTTG
GAATTAGTTATCTGAAGCTTTAGGATTCCTCAGCAATATGATTATGAGACAAGAAAGGAAGATT
CAGAAATGAGTCTAGTTGAAGGCAGCAATTCAGAGAAGAAGATTCAGTTGTTATCATTGCCGTC
CTGCTTGGTTTATGGCCTGGTTCAGGACCAAGGAGAGAAGTGTGAATACATGCCTCTTGAGCTA
TAGAATGAGACGCTGGAGTCACTAAGATGATTTTTTAAAAGTATTGTTTTATAAACAAAAATAA
GATTGTGACAAGGGATTCCACTATTAATGTTTTCATGCCTGTGCCTTAATCTGACTGGGTATGG
TGAGAATTGTGCTTGCAGCTTTAAGGTAAGAATTTTACCATCTTAATATGTTAAGAAGTGCCAT
TTCAGTCTCTCATCTCTACTCCAACTTGTCTTCTTAGGTGCTAAAGTCAGAGCCGAAATCTACG
AGGCCTTCGAGAACATCTACCCCATCCTGAAGGGCTTCAGAAAGACCACC

SEQ ID NO: 7 - exemplary 5′ HA for knock-in cassette insertion at *TBP* locus

CTGACCACAGCTCTGCAAGCAGACTTCCATTTACAGTGAGGAGGTGAGCATTGCATTGAACAAA
AGATGGCGTTTTCACTTGGAATTAGTTATCTGAAGCTTTAGGATTCCTCAGCAATATGATTATG
AGACAAGAAAGGAAGATTCAGAAATGAGTCTAGTTGAAGGCAGCAATTCAGAGAAGAAGATTCA

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GTTGTTATCATTGCCGTCCTGCTTGGTTTATGGCCTGGTTCAGGACCAAGGAGAGAAGTGTGAA
TACATGCCTCTTGAGCTATAGAATGAGACGCTGGAGTCACTAAGATGATTTTTTAAAAGTATTG
TTTTATAAACAAAAATAAGATTGTGACAAGGGATTCCACTATTAATGTTTTCATGCCTGTGCCT
TAATCTGACTGGGTATGGTGAGAATTGTGCTTGCAGCTTTAAGGTAAGAATTTTACCATCTTAA
TATGTTAAGAAGTGCCATTTCAGTCTCTCATCTCTACTCCAACTTGTCTTCTTAGGGGCTAAAG
TGCGGGCCGAGATCTACGAGGCCTTCGAGAATATCTACCCCATCCTGAAGGGCTTCAGAAAGAC
CACC

SEQ ID NO: 8 - exemplary 5′ HA for knock-in cassette insertion at *TBP* locus

ACAAAAGATGGCGTTTTCACTTGGAATTAGTTATCTGAAGCTTTAGGATTCCTCAGCAATATGA
TTATGAGACAAGAAAGGAAGATTCAGAAATGAGTCTAGTTGAAGGCAGCAATTCAGAGAAGAAG
ATTCAGTTGTTATCATTGCCGTCCTGCTTGGTTTATGGCCTGGTTCAGGACCAAGGAGAGAAGT
GTGAATACATGCCTCTTGAGCTATAGAATGAGACGCTGGAGTCACTAAGATGATTTTTTAAAAG
TATTGTTTTATAAACAAAAATAAGATTGTGACAAGGGATTCCACTATTAATGTTTTCATGCCTG
TGCCTTAATCTGACTGGGTATGGTGAGAATTGTGCTTGCAGCTTTAAGGTAAGAATTTTACCAT
CTTAATATGTTAAGAAGTGCCATTTCAGTCTCTCATCTCTACTCCAACTTGTCTTCTTAGGTGC
TAAAGTCAGAGCAGAAATTTATGAAGCATTCGAGAACATCTACCCTATTCTAAAGGGATTCAGG
AAGACGACG

SEQ ID NO: 9 - exemplary 3′ HA for knock-in cassette insertion at *TBP* locus

CAGAAATTTATGAAGCATTTGAAAACATCTACCCTATTCTAAAGGGATTCAGGAAGACGACGTA
ATGGCTCTCATGTACCCTTGCCTCCCCCACCCCCTTCTTTTTTTTTTTTTAAACAAATCAGTTT
GTTTTGGTACCTTTAAATGGTGGTGTTGTGAGAAGATGGATGTTGAGTTGCAGGGTGTGGCACC
AGGTGATGCCCTTCTGTAAGTGCCCACCGCGGGATGCCGGGAAGGGGCATTATTTGTGCACTGA
GAACACCGCGCAGCGTGACTGTGAGTTGCTCATACCGTGCTGCTATCTGGGCAGCGCTGCCCAT
TTATTTATATGTAGATTTTAAACACTGCTGTTGACAAGTTGGTTTGAGGGAGAAAACTTTAAGT
GTTAAAGCCACCTCTATAATTGATTGGACTTTTTAATTTTAATGTTTTTCCCCATGAACCACAG
TTTTTATATTTCTACCAGAAAAGTAAAAATCTTTTTTAAAAGTGTTGTTTTT

SEQ ID NO: 10 - exemplary 3′ HA for knock-in cassette insertion at *TBP* locus

TAGGTGCTAAAGTCAGAGCAGAAATTTATGAAGCATTTGAAAACATCTACCCTATTCTAAAGGG
ATTCAGGAAGACGACGTAATGGCTCTCATGTACCCTTGCCTCCCCCACCCCCTTCTTTTTTTTT
TTTTAAACAAATCAGTTTGTTTTGGTACCTTTAAATGGTGGTGTTGTGAGAAGATGGATGTTGA
GTTGCAGGGTGTGGCACCAGGTGATGCCCTTCTGTAAGTGCCCACCGCGGGATGCCGGGAAGGG
GCATTATTTGTGCACTGAGAACACCGCGCAGCGTGACTGTGAGTTGCTCATACCGTGCTGCTAT
CTGGGCAGCGCTGCCCATTTATTTATATGTAGATTTTAAACACTGCTGTTGACAAGTTGGTTTG
AGGGAGAAAACTTTAAGTGTTAAAGCCACCTCTATAATTGATTGGACTTTTTAATTTTAATGTT
TTTCCCCATGAACCACAGTTTTTATATTTCTACCAGAAAAGTAAAAATCTTT

SEQ ID NO: 11 - exemplary 3′ HA for knock-in cassette insertion at *TBP* locus

AAGGGATTCAGGAAGACGACGTAATGGCTCTCATGTACCCTTGCCTCCCCCACCCCCTTCTTTT
TTTTTTTTTAAACAAATCAGTTTGTTTTGGTACCTTTAAATGGTGGTGTTGTGAGAAGATGGAT
GTTGAGTTGCAGGGTGTGGCACCAGGTGATGCCCTTCTGTAAGTGCCCACCGCGGGATGCCGGG
AAGGGGCATTATTTGTGCACTGAGAACACCGCGCAGCGTGACTGTGAGTTGCTCATACCGTGCT

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GCTATCTGGGCAGCGCTGCCCATTTATTTATATGTAGATTTTAAACACTGCTGTTGACAAGTTG
GTTTGAGGGAGAAAACTTTAAGTGTTAAAGCCACCTCTATAATTGATTGGACTTTTTAATTTTA
ATGTTTTTCCCCATGAACCACAGTTTTTATATTTCTACCAGAAAAGTAAAAATCTTTTTTAAAA
GTGTTGTTTTTCTAATTTATAACTCCTAGGGGTTATTTCTGTGCCAGACACA

[0401] In some embodiments, a donor template comprises a 5′ and/or 3′ homology arm homologous to a region of a *G6PD* locus. In some embodiments, a donor template comprises a 5′ homology arm comprising or consisting of the sequence of SEQ ID NO:12. In some embodiments, a 5′ homology arm comprises or consists of a sequence that is at least 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO: 12. In some embodiments, a donor template comprises a 3′ homology arm comprising or consisting of the sequence of SEQ ID NO:13. In certain embodiments, a 3′ homology arm comprises or consists of a sequence that is at least 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO:13.

[0402] In some embodiments, a donor template comprises a 5′ homology arm comprising SEQ ID NO: 12, and a 3′ homology arm comprising SEQ ID NO: 13.

SEQ ID NO: 12 - exemplary 5′ HA for knock-in cassette insertion at *G6PD* locus

GGCCCGGGGGACTCCACATGGTGGCAGGCAGTGGCATCAGCAAGACACTCTCTCCCTCACAGAA
CGTGAAGCTCCCTGACGCCTATGAGCGCCTCATCCTGGACGTCTTCTGCGGGAGCCAGATGCAC
TTCGTGCGCAGGTGAGGCCCAGCTGCCGGCCCCTGCATACCTGTGGGCTATGGGGTGGCCTTTG
CCCTCCCTCCCTGTGTGCCACCGGCCTCCCAAGCCATACCATGTCCCCTCAGCGACGAGCTCCG
TGAGGCCTGGCGTATTTTCACCCCACTGCTGCACCAGATTGAGCTGGAGAAGCCCAAGCCCATC
CCCTATATTTATGGCAGGTGAGGAAAGGGTGGGGGCTGGGGACAGAGCCCAGCGGGCAGGGGCG
GGGTGAGGGTGGAGCTACCTCATGCCTCTCCTCCACCCGTCACTCTCCAGCCGAGGCCCCACGG
AGGCAGACGAGCTGATGAAGAGAGTGGGCTTCCAGTACGAGGGAACCTACAAATGGGTCAACCC
TCACAAGCTG

SEQ ID NO: 13 - exemplary 3′ HA for knock-in cassette insertion at *G6PD* locus

GTGGGTGAACCCCCACAAGCTCTGAGCCCTGGGCACCCACCTCCACCCCCGCCACGGCCACCCT
CCTTCCCGCCGCCCGACCCCGAGTCGGGAGGACTCCGGGACCATTGACCTCAGCTGCACATTCC
TGGCCCCGGGCTCTGGCCACCCTGGCCCGCCCCTCGCTGCTGCTACTACCCGAGCCCAGCTACA
TTCCTCAGCTGCCAAGCACTCGAGACCATCCTGGCCCCTCCAGACCCTGCCTGAGCCCAGGAGC
TGAGTCACCTCCTCCACTCACTCCAGCCCAACAGAAGGAAGGAGGAGGGCGCCCATTCGTCTGT
CCCAGAGCTTATTGGCCACTGGGTCTCACTCCTGAGTGGGGCCAGGGTGGGAGGGAGGGACGAG
GGGGAGGAAAGGGGCGAGCACCCACGTGAGAGAATCTGCCTGTGGCCTTGCCCGCCAGCCTCAG
TGCCACTTGACATTCCTTGTCACCAGCAACATCTCGAGCCCCCTGGATGTCC

[0403] In some embodiments, a donor template comprises a 5′ and/or 3′ homology arm homologous to a region of a *E2F4* locus. In some embodiments, a donor template comprises a 5′ homology arm comprising or consisting of the sequence of SEQ ID NO: 14, 15, or 16. In some

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embodiments, a 5′ homology arm comprises or consists of a sequence that is at least 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO: 14, 15, or 16. In some embodiments, a donor template comprises a 3′ homology arm comprising or consisting of the sequence of SEQ ID NO: 17, 18, or 19. In certain embodiments, a 3′ homology arm comprises or consists of a sequence that is at least 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO: 17, 18, or 19.

[0404] In some embodiments, a donor template comprises a 5′ homology arm comprising SEQ ID NO: 14, and a 3′ homology arm comprising SEQ ID NO: 17. In some embodiments, a donor template comprises a 5′ homology arm comprising SEQ ID NO: 15, and a 3′ homology arm comprising SEQ ID NO: 18. In some embodiments, a donor template comprises a 5′ homology arm comprising SEQ ID NO: 16, and a 3′ homology arm comprising SEQ ID NO: 19.

SEQ ID NO: 14 - exemplary 5′ HA for knock-in cassette insertion at *E2F4* locus

CCAGGGGGCTGTAGTGGGGCCAGGCTGGACCTCTGTGCCCTGAGCATGGCTTTCTTGTTTTTCA
GTTTTGGAACTCCCCAAAGAGCTGTCAGAAATCTTTGATCCCACACGAGGTAGGCTGCTGCATT
CCTCCCTGAGGCTAGGGGTAAGGGACACAGCTCATTGGGTCCTATGGCTGTTTTCTTGCCCTTT
TGAGGACCTTGTTGTGGCGCTTATGGTAACTGGGGCAAAGGGTGAAGTTCCTGATGGGCAGGTG
GGGTTCCCTTTCCTGGGCTTTGGTGGGTGGAGAGGTGGGAGCTGGAATGTTAGTAACTGAGCTC
CCTCCATTCCCACACTGCATCACCTCCGACCTGCTGCACGACTTCATGTCCTCACAACGTCGCT
GGCCCTGGAAGGTGGGAGTGGGTGTGGGCAGGGGTTGGGCTGCTGCTAGGGGAGCCCTGGCCCA
GGGCCTGAGACTAGTGCTCTCTGCAGTGTTCGCCCCTCTGCTGAGACTTTCTCCTCCTCCTGGC
GACCACGACTACATCTACAACCTGGACGAGAGCGAGGGCGTGTGCGACCTGTTTGATGTGCCCG
TGCTGAACCTG

SEQ ID NO: 15 - exemplary 5′ HA for knock-in cassette insertion at *E2F4* locus

CCAGGCTGGACCTCTGTGCCCTGAGCATGGCTTTCTTGTTTTTCAGTTTTGGAACTCCCCAAAG
AGCTGTCAGAAATCTTTGATCCCACACGAGGTAGGCTGCTGCATTCCTCCCTGAGGCTAGGGGT
AAGGGACACAGCTCATTGGGTCCTATGGCTGTTTTCTTGCCCTTTTGAGGACCTTGTTGTGGCG
CTTATGGTAACTGGGGCAAAGGGTGAAGTTCCTGATGGGCAGGTGGGGTTCCCTTTCCTGGGCT
TTGGTGGGTGGAGAGGTGGGAGCTGGAATGTTAGTAACTGAGCTCCCTCCATTCCCAGAGTGCA
TGAGCTCGGAGCTGCTGGAGGAGTTGATGTCCTCAGAAGGTGGGTGGCCCTGGAAGGTGGGAGT
GGGTGTGGGCAGGGGTTGGGCTGCTGCTAGGGGAGCCCTGGCCCAGGGCCTGAGACTAGTGCTC
TCTGCAGTGTTTGCCCCTCTGCTTCGTCTTAGTCCTCCTCCGGGCGACCACGACTACATCTACA
ACCTGGACGAGAGCGAGGGCGTGTGCGACCTGTTTGATGTGCCCGTGCTGAACCTG

SEQ ID NO: 16 - exemplary 5′ HA for knock-in cassette insertion at *E2F4* locus

GTCAGAAATCTTTGATCCCACACGAGGTAGGCTGCTGCATTCCTCCCTGAGGCTAGGGGTAAGG
GACACAGCTCATTGGGTCCTATGGCTGTTTTCTTGCCCTTTTGAGGACCTTGTTGTGGCGCTTA
TGGTAACTGGGGCAAAGGGTGAAGTTCCTGATGGGCAGGTGGGGTTCCCTTTCCTGGGCTTTGG
TGGGTGGAGAGGTGGGAGCTGGAATGTTAGTAACTGAGCTCCCTCCATTCCCAGAGTGCATGAG

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CTCGGAGCTGCTGGAGGAGTTGATGTCCTCAGAAGGTGGGTGGCCCTGGAAGGTGGGAGTGGGT
GTGGGCAGGGGTTGGGCTGCTGCTAGGGGAGCCCTGGCCCAGGGCCTGAGACTAGTGCTCTCTG
CAGTGTTTGCCCCTCTGCTTCGTCTTTCTCCACCCCCGGGAGACCACGATTATATCTACAACCT
GGACGAGAGTGAAGGTGTCTGTGACCTCTTCGACGTGCCCGTGCTCAACCTC

SEQ ID NO: 17 - exemplary 3′ HA for knock-in cassette insertion at *E2F4* locus

CCACCCCCGGGAGACCACGATTATATCTACAACCTGGACGAGAGTGAAGGTGTCTGTGACCTCT
TTGATGTGCCTGTTCTCAACCTCTGACTGACAGGGACATGCCCTGTGTGGCTGGGACCCAGACT
GTCTGACCTGGGGGTTGCCTGGGGACCTCTCCCACCCGACCCCTACAGAGCTTGAGAGCCACAG
ACGCCTGGCTTCTCCGGCCTCCCCTCACCGCACAGTTCTGGCCACAGCTCCCGCTCCTGTGCTG
GCACTTCTGTGCTCGCAGAGCAGGGGAACAGGACTCAGCCCCCATCACCGTGGAGCCAAAGTGT
TTGCTTCTCCCTTTCTGCGGCCTTCGCCAGCCCAGGCTCGGCTGCCACCCAGTGGCACAGAACC
GAGGAGCTGCCATTACCCCCCATAGGGGGCAGTGTCTTGTTCCTGCCAGCCTCAGTGTCTTGCT
TCTGCCAGCTCCTTCCCCTAGGAGGGAAGGGTGGGGTGGAACTGGGCACATG

SEQ ID NO: 18 - exemplary 3′ HA for knock-in cassette insertion at *E2F4* locus

ATTATATCTACAACCTGGACGAGAGTGAAGGTGTCTGTGACCTCTTTGATGTGCCTGTTCTCAA
CCTCTGACTGACAGGGACATGCCCTGTGTGGCTGGGACCCAGACTGTCTGACCTGGGGGTTGCC
TGGGGACCTCTCCCACCCGACCCCTACAGAGCTTGAGAGCCACAGACGCCTGGCTTCTCCGGCC
TCCCCTCACCGCACAGTTCTGGCCACAGCTCCCGCTCCTGTGCTGGCACTTCTGTGCTCGCAGA
GCAGGGGAACAGGACTCAGCCCCCATCACCGTGGAGCCAAAGTGTTTGCTTCTCCCTTTCTGCG
GCCTTCGCCAGCCCAGGCTCGGCTGCCACCCAGTGGCACAGAACCGAGGAGCTGCCATTACCCC
CCATAGGGGGCAGTGTCTTGTTCCTGCCAGCCTCAGTGTCTTGCTTCTGCCAGCTCCTTCCCCT
AGGAGGGAAGGGTGGGGTGGAACTGGGCACATGCCAGCACCACTTCTAGCTT

SEQ ID NO: 19 - exemplary 3′ HA for knock-in cassette insertion at *E2F4* locus

TGACTGACAGGGACATGCCCTGTGTGGCTGGGACCCAGACTGTCTGACCTGGGGGTTGCCTGGG
GACCTCTCCCACCCGACCCCTACAGAGCTTGAGAGCCACAGACGCCTGGCTTCTCCGGCCTCCC
CTCACCGCACAGTTCTGGCCACAGCTCCCGCTCCTGTGCTGGCACTTCTGTGCTCGCAGAGCAG
GGGAACAGGACTCAGCCCCCATCACCGTGGAGCCAAAGTGTTTGCTTCTCCCTTTCTGCGGCCT
TCGCCAGCCCAGGCTCGGCTGCCACCCAGTGGCACAGAACCGAGGAGCTGCCATTACCCCCCAT
AGGGGGCAGTGTCTTGTTCCTGCCAGCCTCAGTGTCTTGCTTCTGCCAGCTCCTTCCCCTAGGA
GGGAAGGGTGGGGTGGAACTGGGCACATGCCAGCACCACTTCTAGCTTCCTTCGCTATCCCCCA
CCCCCTGACCCTCCAGCTCCTCCTGGCCCTCTCACGTGCCCACTTCTGCTGG

[0405] In some embodiments, a donor template comprises a 5′ and/or 3′ homology arm homologous to a region of a *KIF11* locus. In some embodiments, a donor template comprises a 5′ homology arm comprising or consisting of the sequence of SEQ ID NO: 20, 21, or 22. In some embodiments, a 5′ homology arm comprises or consists of a sequence that is at least 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO: 20, 21, or 22. In some embodiments, a donor template comprises a 3′ homology arm comprising or consisting of the

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sequence of SEQ ID NO: 23, 24, or 25. In certain embodiments, a 3′ homology arm comprises or consists of a sequence that is at least 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NO: 23, 24, or 25.

[0406] In some embodiments, a donor template comprises a 5′ homology arm comprising SEQ ID NO: 20, and a 3′ homology arm comprising SEQ ID NO: 23. In some embodiments, a donor template comprises a 5′ homology arm comprising SEQ ID NO: 21, and a 3′ homology arm comprising SEQ ID NO: 24. In some embodiments, a donor template comprises a 5′ homology arm comprising SEQ ID NO: 22, and a 3′ homology arm comprising SEQ ID NO: 25.

SEQ ID NO: 20 - exemplary 5′ HA for knock-in cassette insertion at *KIF11* locus

AGAGCAGGGTTTCTTGACAGCAGTGCTATTGGCATTTTAAACTGGATAATTCTTTGTTGTGATG
GGCTTTCCTGTGGACTGTACTATGTTGGTACACAAGAAAAACAGTGTACTATGTGAATACTCAC
TCAAAGCCAGTAGCACTCCCTGATTGTAACACCAAAAAAGTCTCTCAGCATTGCCAAATGTCCC
CTGTGGCAGCAGAATCACTCCCTGATGAGAACCACTACCCTGGAGTAAAATCTATAACTATGTC
TTAGAAAATAACACAGAAAATTAATATTTCTTTCACTCTACTCCTTCCATTAGTGATCAAATAA
AGAAGGCATTTGGCGCTACTTGCCAAATTGTTGGCTCAAACTTGTGCTGAACCTTTTTTGGTTT
TCTACACTTAAGTTTTTTTGCCTATAACCCAGAGAACTTTGAAAATAGAGTGTAGTTAATGTGT
ATCTAATGTTACTTTGTATTGACTTAATTTACCGGCCTTTAATCCACAGCATAAGAAGTCCCAC
GGCAAGGACAAAGAGAACCGGGGCATCAACACACTGGAACGGTCCAAGGTCGAGGAAACAACCG
AGCACCTGGTCACCAAGAGCAGACTGCCTCTGAGAGCCCAGATCAACCTG

SEQ ID NO: 21 - exemplary 5′ HA for knock-in cassette insertion at *KIF11* locus

TTCCTGTGGACTGTACTATGTTGGTACACAAGAAAAACAGTGTACTATGTGAATACTCACTCAA
AGCCAGTAGCACTCCCTGATTGTAACACCAAAAAAGTCTCTCAGCATTGCCAAATGTCCCCTGT
GGCAGCAGAATCACTCCCTGATGAGAACCACTACCCTGGAGTAAAATCTATAACTATGTCTTAG
AAAATAACACAGAAAATTAATATTTCTTTCACTCTACTCCTTCCATTAGTGATCAAATAAAGAA
GGCATTTGGCGCTACTTGCCAAATTGTTGGCTCAAACTTGTGCTGAACCTTTTTTGGTTTTCTA
CACTTAAGTTTTTTTGCCTATAACCCAGAGAACTTTGAAAATAGAGTGTAGTTAATGTGTATCT
AATGTTACTTTGTATTGACTTAATTTTCCCGCCTTAAATCCACAGCATAAAAAATCACATGGAA
AAGACAAAGAAAACAGAGGCATTAACACACTGGAGAGGTCTAAAGTGGAAGAAACAACCGAGCA
CCTGGTCACCAAGAGCAGACTGCCTCTGAGAGCCCAGATCAACCTG

SEQ ID NO: 22 - exemplary 5′ HA for knock-in cassette insertion at *KIF11* locus

TTAAACTGGATAATTCTTTGTTGTGATGGGCTTTCCTGTGGACTGTACTATGTTGGTACACAAG
AAAAACAGTGTACTATGTGAATACTCACTCAAAGCCAGTAGCACTCCCTGATTGTAACACCAAA
AAAGTCTCTCAGCATTGCCAAATGTCCCCTGTGGCAGCAGAATCACTCCCTGATGAGAACCACT
ACCCTGGAGTAAAATCTATAACTATGTCTTAGAAAATAACACAGAAAATTAATATTTCTTTCAC
TCTACTCCTTCCATTAGTGATCAAATAAAGAAGGCATTTGGCGCTACTTGCCAAATTGTTGGCT
CAAACTTGTGCTGAACCTTTTTTGGTTTTCTACACTTAAGTTTTTTTGCCTATAACCCAGAGAA
CTTTGAAAATAGAGTGTAGTTAATGTGTATCTAATGTTACTTTGTATTGACTTAATTTTCCCGC
CTTAAATCCACAGCATAAAAAATCACATGGAAAAGACAAAGAAAACAGAGGCATCAACACACTG

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GAACGGTCCAAGGTCGAGGAAACAACCGAGCACCTGGTCACCAAGAGCAGACTGCCTCTGAGAG
CCCAGATCAACCTG

SEQ ID NO: 23 - exemplary 3′ HA for knock-in cassette insertion at *KIF11* locus

AAAAAATCACATGGAAAAGACAAAGAAAACAGAGGCATTAACACACTGGAGAGGTCTAAAGTGG
AAGAAACTACAGAGCACTTGGTTACAAAGAGCAGATTACCTCTGCGAGCCCAGATCAACCTTTA
ATTCACTTGGGGGTTGGCAATTTTATTTTTAAAGAAAACTTAAAAATAAAACCTGAAACCCCAG
AACTTGAGCCTTGTGTATAGATTTTAAAAGAATATATATATCAGCCGGGCGCGGTGGCTCATGC
CTGTAATCCCAGCACTTTGGGAGGCTGAGGCGGGTGGATTGCTTGAGCCCAGGAGTTTGAGACC
AGCCTGGCCAACGTGGCAAAACCTCGTCTCTGTTAAAAATTAGCCGGGCGTGGTGGCACACTCC
TGTAATCCCAGCTACTGGGGAGGCTGAGGCACGAGAATCACTTGAACCCAGGAAGCGGGGTTGC
AGTGAGCCAAAGGTACACCACTACACTCCAGCCTGGGCAACAGAGCAAGACT

SEQ ID NO: 24 - exemplary 3′ HA for knock-in cassette insertion at *KIF11* locus

AACTACAGAGCACTTGGCTACATAGAGCAGATTACCTCTGCGAGCCCAGATCAACCTTTAATTC
ACTTGGGGGTTGGCAATTTTATTTTTAAAGAAAACTTAAAAATAAAACCTGAAACCCCAGAACT
TGAGCCTTGTGTATAGATTTTAAAAGAATATATATATCAGCCGGGCGCGGTGGCTCATGCCTGT
AATCCCAGCACTTTGGGAGGCTGAGGCGGGTGGATTGCTTGAGCCCAGGAGTTTGAGACCAGCC
TGGCCAACGTGGCAAAACCTCGTCTCTGTTAAAAATTAGCCGGGCGTGGTGGCACACTCCTGTA
ATCCCAGCTACTGGGGAGGCTGAGGCACGAGAATCACTTGAACCCAGGAAGCGGGGTTGCAGTG
AGCCAAAGGTACACCACTACACTCCAGCCTGGGCAACAGAGCAAGACTCGGTCTCAAAAACAAA
ATTTAAAAAAGATATAAGGCAGTACTGTAAATTCAGTTGAATTTTGATATCT

SEQ ID NO: 25 - exemplary 3′ HA for knock-in cassette insertion at *KIF11* locus

ATTAACACACTGGAGAGTTCTGAAGTGGAAGAAACTACAGAGCACTTGGTTACAAAGAGCAGAT
TACCTCTGCGAGCCCAGATCAACCTTTAATTCACTTGGGGGTTGGCAATTTTATTTTTAAAGAA
AACTTAAAAATAAAACCTGAAACCCCAGAACTTGAGCCTTGTGTATAGATTTTAAAAGAATATA
TATATCAGCCGGGCGCGGTGGCTCATGCCTGTAATCCCAGCACTTTGGGAGGCTGAGGCGGGTG
GATTGCTTGAGCCCAGGAGTTTGAGACCAGCCTGGCCAACGTGGCAAAACCTCGTCTCTGTTAA
AAATTAGCCGGGCGTGGTGGCACACTCCTGTAATCCCAGCTACTGGGGAGGCTGAGGCACGAGA
ATCACTTGAACCCAGGAAGCGGGGTTGCAGTGAGCCAAAGGTACACCACTACACTCCAGCCTGG
GCAACAGAGCAAGACTCGGTCTCAAAAACAAAATTTAAAAAAGATATAAGGC

Inverted Terminal Repeats (ITRs)

[0407] In certain embodiments, a donor template comprises an AAV derived sequence. In certain embodiments, a donor template comprises AAV derived sequences that are typical of an AAV construct, such as cis-acting 5′ and 3′ inverted terminal repeats (ITRs) (See, e.g., B. J. Carter, in "Handbook of Parvoviruses", ed., P. Tijsser, CRC Press, pp. 155 168 (1990), which is incorporated in its entirety herein by reference). Generally, ITRs are able to form a hairpin. The ability to form a hairpin can contribute to an ITRs ability to self-prime, allowing primase-independent synthesis of a second DNA strand. ITRs also play a role in integration of AAV

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construct (e.g., a coding sequence) into a genome of a target cell. ITRs can also aid in efficient encapsidation of an AAV construct in an AAV particle.

[0408] In some embodiments, a donor template described herein is included within an rAAV particle (e.g., an AAV6 particle). In some embodiments, an ITR is or comprises about 145 nucleic acids. In some embodiments, all or substantially all of a sequence encoding an ITR is used. In some embodiments, an AAV ITR sequence may be obtained from any known AAV, including presently identified mammalian AAV types. In some embodiments an ITR is an AAV6 ITR.

[0409] An example of an AAV construct employed in the present disclosure is a "cis-acting" construct containing a cargo sequence (e.g., a donor template described herein), in which the donor template is flanked by 5′ or "left" and 3′ or "right" AAV ITR sequences. 5′ and left designations refer to a position of an ITR sequence relative to an entire construct, read left to right, in a sense direction. For example, in some embodiments, a 5′ or left ITR is an ITR that is closest to a target loci promoter (as opposed to a polyadenylation sequence) for a given construct, when a construct is depicted in a sense orientation, linearly. Concurrently, 3′ and right designations refer to a position of an ITR sequence relative to an entire construct, read left to right, in a sense direction. For example, in some embodiments, a 3′ or right ITR is an ITR that is closest to a polyadenylation sequence in a target loci (as opposed to a promoter sequence) for a given construct, when a construct is depicted in a sense orientation, linearly. ITRs as provided herein are depicted in 5′ to 3′ order in accordance with a sense strand. Accordingly, one of skill in the art will appreciate that a 5′ or "left" orientation ITR can also be depicted as a 3′ or "right" ITR when converting from sense to antisense direction. Further, it is well within the ability of one of skill in the art to transform a given sense ITR sequence (e.g., a 5′/left AAV ITR) into an antisense sequence (e.g., 3′/right ITR sequence). One of ordinary skill in the art would understand how to modify a given ITR sequence for use as either a 5′/left or 3′/right ITR, or an antisense version thereof.

[0410] For example, in some embodiments an ITR (e.g., a 5′ ITR) can have a sequence according to SEQ ID NO: 158. In some embodiments, an ITR (e.g., a 3′ ITR) can have a sequence according to SEQ ID NO: 159. In some embodiments, an ITR includes one or more modifications, e.g., truncations, deletions, substitutions or insertions, as is known in the art. In

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some embodiments, an ITR comprises fewer than 145 nucleotides, e.g., 127, 130, 134 or 141 nucleotides. For example, in some embodiments, an ITR comprises 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123 ,124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143 144, or 145 nucleotides.

[0411] A non-limiting example of 5′ AAV ITR sequences includes SEQ ID NO: 158. A non-limiting example of 3′ AAV ITR sequences includes SEQ ID NO: 159. In some embodiments, the 5′ and a 3′ AAV ITRs (e.g., SEQ ID NO: 158 and 159) flank a donor template described herein (e.g., a donor template comprising a 5′HA, a knock-in cassette, and a 3′ HA). The ability to modify ITR sequences is within the skill of the art. (See, e.g., texts such as Sambrook et al. "Molecular Cloning. A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory, New York (1989); and K. Fisher et al., J Virol., 70:520 532 (1996), each of which is incorporated in its entirety herein by reference). In some embodiments, a 5′ ITR sequence is at least 85%, 90%, 95%, 98% or 99% identical to a 5′ ITR sequence represented by SEQ ID NO: 158. In some embodiments, a 3′ ITR sequence is at least 85%, 90%, 95%, 98% or 99% identical to a 3′ ITR sequence represented by SEQ ID NO: 159.

SEQ ID NO: 158 - exemplary 5′ ITR for knock-in cassette insertion

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGC
GACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATC
ACTAGGGGTTCCT

SEQ ID NO: 159 - exemplary 3′ ITR for knock-in cassette insertion

AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGG
GCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGC
AGCTGCCTGCAGG

Flanking untranslated regions, 5′ UTRs and 3′ UTRs

[0412] In some embodiments, a knock-in cassette described herein includes all or a portion of an untranslated region (UTR), such as a 5′ UTR and/or a 3′ UTR. UTRs of a gene are transcribed but not translated. A 5′ UTR starts at a transcription start site and continues to the start codon but does not include the start codon. A 3′ UTR starts immediately following the stop codon and continues until the transcriptional termination signal. The regulatory and/or control features of a UTR can be incorporated into any of the knock-in cassettes described herein to

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enhance or otherwise modulate the expression of an essential target gene loci and/or a cargo sequence.

[0413] Natural 5′ UTRs include a sequence that plays a role in translation initiation. In some embodiments, a 5′ UTR comprises sequences, like Kozak sequences, which are commonly known to be involved in the process by which the ribosome initiates translation of many genes. Kozak sequences have the consensus sequence CCR(A/G)CCAUGG, where R is a purine (A or G) three bases upstream of the start codon (AUG), and the start codon is followed by another "G". The 5′ UTRs have also been known to form secondary structures that are involved in elongation factor binding. Non-limiting examples of 5′ UTRs include those from the following genes: albumin, serum amyloid A, Apolipoprotein A/B/E, transferrin, alpha fetoprotein, erythropoietin, and Factor VIII.

[0414] In some embodiments, a UTR may comprise a non-endogenous regulatory region. In some embodiments, a UTR that comprises a non-endogenous regulatory region is a 3' UTR. In some embodiments, a UTR that comprises a non-endogenous regulatory region is a 5' UTR. In some embodiments, a non-endogenous regulatory region may be a target of at least one inhibitory nucleic acid. In some embodiments, an inhibitory nucleic acid inhibits expression and/or activity of a target gene. In some embodiments, an inhibitory nucleic acid is a short interfering RNA (siRNA), a short hairpin RNA (shRNA), a microRNA (miRNA), an antisense oligonucleotide, a guide RNA (gRNA), or a ribozyme. In some embodiments, an inhibitory nucleic acid is an endogenous molecule. In some embodiments, an inhibitory nucleic acid is a non-endogenous molecule. In some embodiments, an inhibitory nucleic acid displays a tissue specific expression pattern. In some embodiments, an inhibitory nucleic acid displays a cell specific expression pattern.

[0415] In some embodiments, a knock-in cassette may comprise more than one non-endogenous regulatory regions, e.g., two, three, four, five, six, seven, eight, nine, or ten regulatory regions. In some embodiments, a knock-in cassette may comprise four non-endogenous regulatory regions. In some embodiments, a construct may comprise more than one non-endogenous regulatory regions, wherein at least one of the more than one non-endogenous regulatory regions are not the same as at least one of the other non-endogenous regulatory regions.

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[0416] In some embodiments, a 3′ UTR is found immediately 3′ to the stop codon of a gene of interest. In some embodiments, a 3′ UTR from an mRNA that is transcribed by a target cell can be included in any knock-in cassette described herein. In some embodiments, a 3′ UTR is derived from an endogenous target loci and may include all or part of the endogenous sequence. In some embodiments, a 3′ UTR sequence is at least 85%, 90%, 95% or 98% identical to the sequence of SEQ ID NO: 26.

SEQ ID NO: 26 - exemplary 3′ UTR for knock-in cassette insertion
GCGGCCGCGTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGA

Polyadenylation Sequences

[0417] In some embodiments, a knock-in cassette construct provided herein can include a polyadenylation (poly(A)) signal sequence. Most nascent eukaryotic mRNAs possess a poly(A) tail at their 3′ end, which is added during a complex process that includes cleavage of the primary transcript and a coupled polyadenylation reaction driven by the poly(A) signal sequence (see, e.g., Proudfoot et al., Cell 108:501-512, 2002, which is incorporated herein by reference in its entirety). A poly(A) tail confers mRNA stability and transferability (Molecular Biology of the Cell, Third Edition by B. Alberts et al., Garland Publishing, 1994, which is incorporated herein by reference in its entirety). In some embodiments, a poly(A) signal sequence is positioned 3′ to a coding sequence.

[0418] As used herein, "polyadenylation" refers to the covalent linkage of a polyadenylyl moiety, or its modified variant, to a messenger RNA molecule. In eukaryotic organisms, most messenger RNA (mRNA) molecules are polyadenylated at the 3′ end. A 3′ poly(A) tail is a long sequence of adenine nucleotides (e.g., 50, 60, 70, 100, 200, 500, 1000, 2000, 3000, 4000, or 5000) added to the pre-mRNA through the action of an enzyme, polyadenylate polymerase. In some embodiments, a poly(A) tail is added onto transcripts that contain a specific sequence, e.g., a polyadenylation (or poly(A)) signal. A poly(A) tail and associated proteins aid in protecting mRNA from degradation by exonucleases. Polyadenylation also plays a role in transcription termination, export of the mRNA from the nucleus, and translation. Polyadenylation typically occurs in the nucleus immediately after transcription of DNA into RNA, but also can occur later in the cytoplasm. After transcription has been terminated, an mRNA chain is cleaved through the action of an endonuclease complex associated with RNA polymerase. A cleavage site is

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usually characterized by the presence of the base sequence AAUAAA near the cleavage site. After the mRNA has been cleaved, adenosine residues are added to the free 3′ end at the cleavage site.

[0419] As used herein, a "poly(A) signal sequence" or "polyadenylation signal sequence" is a sequence that triggers the endonuclease cleavage of an mRNA and the addition of a series of adenosines to the 3′ end of the cleaved mRNA.

[0420] There are several poly(A) signal sequences that can be used, including those derived from bovine growth hormone (bGH) (Woychik et al., Proc. Natl. Acad. Sci. US.A. 81(13):3944-3948, 1984; U.S. Patent No. 5,122,458, each of which is incorporated herein by reference in its entirety), mouse-β-globin, mouse-α-globin (Orkin et al., EMBO J 4(2):453-456, 1985; Thein et al., Blood71(2):313-319, 1988, each of which is incorporated herein by reference in its entirety), human collagen, polyoma virus (Batt et al., Mol. Cell Biol. 15(9):4783-4790, 1995, which is incorporated herein by reference in its entirety), the Herpes simplex virus thymidine kinase gene (HSV TK), IgG heavy-chain gene polyadenylation signal (US 2006/0040354, which is incorporated herein by reference in its entirety), human growth hormone (hGH) (Szymanski et al., Mol. Therapy 15(7):1340-1347, 2007, which is incorporated herein by reference in its entirety), the group comprising a SV40 poly(A) site, such as the SV40 late and early poly(A) site (Schek et al., Mol. Cell Biol. 12(12):5386- 5393, 1992, which is incorporated herein by reference in its entirety).

[0421] The poly(A) signal sequence can be AATAAA. The AATAAA sequence may be substituted with other hexanucleotide sequences with homology to AATAAA and that are capable of signaling polyadenylation, including ATTAAA, AGTAAA, CATAAA, TATAAA, GATAAA, ACTAAA, AATATA, AAGAAA, AATAAT, AAAAAA, AATGAA, AATCAA, AACAAA, AATCAA, AATAAC, AATAGA, AATTAA, or AATAAG (see, e.g., WO 06/12414, which is incorporated herein by reference in its entirety).

[0422] In some embodiments, a poly(A) signal sequence can be a synthetic polyadenylation site (see, e.g., the pCl-neo expression construct of Promega that is based on Levitt et al., Genes Dev. 3(7):1019-1025, 1989, which is incorporated herein by reference in its entirety). In some embodiments, a poly(A) signal sequence is the polyadenylation signal of soluble neuropilin-1 (sNRP) (AAATAAAATACGAAATG) (see, e.g., WO 05/073384, which is

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incorporated herein by reference in its entirety). In some embodiments, a poly(A) signal sequence comprises or consists of the SV40 poly(A) site. In some embodiments, a poly(A) signal sequence comprises or consists of SEQ ID NO: 27. In some embodiments, a poly(A) signal sequence comprises or consists of bGHpA. In some embodiments, a poly(A) signal sequence comprises or consists of SEQ ID NO: 28. Additional examples of poly(A) signal sequences are known in the art. In some embodiments, a poly(A) sequence is at least 85%, 90%, 95%, 98% or 99% identical to the sequence of SEQ ID NOs: 27 or 28.

SEQ ID NO: 27 - exemplary SV40 poly(A) signal sequence

AACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATA
AAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTA

SEQ ID NO: 28 - exemplary bGH poly(A) signal sequence

CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGA
AGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGG
TGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATA
GCAGGCATGCTGGGGATGCGGTGGGCTCTATGG

IRES and 2A Elements

[0423] In some embodiments, the knock-in cassette comprises a regulatory element that enables expression of the gene product encoded by the essential gene and the gene product of interest as separate gene products, e.g., an IRES or 2A element located between the exogenous coding sequence or partial coding sequence of the essential gene and the exogenous coding sequence for the gene product of interest.

[0424] In some embodiments, a knock-in cassette may comprise multiple gene products of interest (e.g., at least two gene products of interest). In some embodiments, gene products of interest may be separated by a regulatory element that enables expression of the at least two gene products of interest as more than one gene product, e.g., an IRES or 2A element located between the at least two coding sequences, facilitating creation of at least two peptide products.

[0425] Internal Ribosome Entry Site (IRES) elements are one type of regulatory element that are commonly used for this purpose. As is well known in the art, IRES elements allow for initiation of translation from an internal region of the mRNA and hence expression of two separate proteins from the same mRNA transcript. IRES was originally discovered in poliovirus RNA, where it promotes translation of the viral genome in eukaryotic cells. Since then, a variety

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of IRES sequences have been discovered – many from viruses, but also some from cellular mRNAs, e.g., see Mokrejs et al., Nucleic Acids Res. 2006; 34(Database issue):D125-D130.

[0426] 2A elements are another type of regulatory element that are commonly used for this purpose. These 2A elements encode so-called "self-cleaving" 2A peptides which are short peptides (about 20 amino acids) that were first discovered in picornaviruses. The term "self-cleaving" is not entirely accurate, as these peptides are thought to function by making the ribosome skip the synthesis of a peptide bond at the C-terminus of a 2A element, leading to separation between the end of the 2A sequence and the next peptide downstream. The "cleavage" occurs between the Glycine (G) and Proline (P) residues found on the C-terminus meaning the upstream cistron, i.e., protein encoded by the essential gene will have a few additional residues from the 2A peptide added to the end, while the downstream cistron, i.e., gene product of interest will start with the Proline (P).

[0427] Table 2 below lists the four commonly used 2A peptides (an optional GSG sequence is sometimes added to the N-terminal end of the peptide to improve cleavage efficiency). There are many potential 2A peptides that may be suitable for methods and compositions described herein (see e.g., Luke et al., Occurrence, function and evolutionary origins of '2A-like' sequences in virus genomes. *J Gen Virol.* 2008). Those skilled in the art know that the choice of specific 2A peptide for a particular knock-in cassette will ultimately depend on a number of factors such as cell type or experimental conditions. Those skilled in the art will recognize that nucleotide sequences encoding specific 2A peptides can vary while still encoding a peptide suitable for inducing a desired cleavage event.

Table 2: Exemplary IRES and 2A peptide and nucleic acid sequences

SEQ ID NO:	2A peptide	Amino acid sequence
29	T2A	EGRGSLLTCGDVEENPGP
30	P2A	ATNFSLLKQAGDVEENPGP
31	E2A	QCTNYALLKLAGDVESNPGP
32	F2A	VKQTLNFDLLKLAGDVESNPGP
33	T2A	GAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAGA ATCCTGGCCCG
34	P2A	GGAAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGCTGGAG ACGTGGAGGAGAACCCTGGACCT

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35	E2A	CAGTGTACTAATTATGCTCTCTTGAAATTGGCTGGAGATGTTG AGAGCAACCCTGGACCT
36	F2A	GTGAAACAGACTTTGAATTTTGACCTTCTCAAGTTGGCGGGAG ACGTGGAGTCCAACCCTGGACCT
37	IRES	CCCCTCTCCCTCCCCCCCCCCTAACGTTACTGGCCGAAGCCGC TTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCA CCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGG CCCTGTCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCCTCTC GCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAG TTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGAC CCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTC TGCGGCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGCGG CACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAG AGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAG GATGCCCAGAAGGTACCCCATTGTATGGGATCTGATCTGGGGC CTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAA ACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCCTTTGA AAAACACGATGATAA

Essential genes

[0428] An essential gene can be any gene that is essential for the survival and/or the proliferation of the cell. In some embodiments, an essential gene is a housekeeping gene that is essential for survival of all cell types, e.g., a gene listed in Table 3. See also other housekeeping genes discussed in Eisenberg, Trends in Gen. 2014; 30(3):119-20 and Moein et al., Adv. Biomed Res. 2017; 6:15. Additional genes that are essential for various cell types, including iPSCs/ESCs, are listed in Table 4 (see also the essential genes discussed in Yilmaz et al., Nat. Cell Biol. 2018; 20:610-619 the entire contents of which are incorporated herein by reference).

[0429] In some embodiments the essential gene is *GAPDH* and the DNA nuclease causes a break in exon 9, e.g., a double-strand break. In some embodiments the essential gene is *TBP* and the DNA nuclease causes a break in exon 7, or exon 8, e.g., a double-strand break. In some embodiments the essential gene is *E2F4* and the DNA nuclease causes a break in exon 10, e.g., a double-strand break. In some embodiments the essential gene is *G6PD* and the DNA nuclease causes a break in exon 13, e.g., a double-strand break. In some embodiments the essential gene is *KIF11* and the DNA nuclease causes a break in exon 22, e.g., a double-strand break.

Table 3: Exemplary housekeeping genes

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Ensembl ID	Gene Symbol	Ensembl ID	Gene Symbol
ENSG00000075624	ACTB	ENSG00000231500	RPS18
ENSG00000116459	ATP5F1	ENSG00000112592	TBP
ENSG00000166710	B2M	ENSG00000072274	TFRC
ENSG00000111640	GAPDH	ENSG00000164924	YWHAZ
ENSG00000169919	GUSB	ENSG00000089157	RPLP0
ENSG00000165704	HPRT1	ENSG00000142541	RPL13A
ENSG00000102144	PGK1	ENSG00000147604	RPL7
ENSG00000196262	PPIA	ENSG00000205250	E2F4
ENSG00000138160	KIF11	ENSG00000160211	G6PD

Table 4: Additional exemplary essential genes

Ensembl ID	Gene Symbol	Ensembl ID	Gene Symbol
ENSG00000111704	NANOG	ENSG00000181449	SOX2
ENSG00000179059	ZFP42	ENSG00000136997	MYC
ENSG00000136826	KLF4	ENSG00000175166	PSMD2
ENSG00000118655	DCLRE1B	ENSG00000070614	NDST1
ENSG00000172409	CLP1	ENSG00000115484	CCT4
ENSG00000082898	XPO1	ENSG00000100890	KIAA0391
ENSG00000114867	EIF4G1	ENSG00000149474	CSRP2BP
ENSG00000115866	DARS	ENSG00000102738	MRPS31
ENSG00000204628	GNB2L1	ENSG00000136104	RNASEH2B
ENSG00000198242	RPL23A	ENSG00000106246	PTCD1
ENSG00000158526	TSR2	ENSG00000248919	ATP5J2-PTCD1
ENSG00000125450	NUP85	ENSG00000138663	COPS4
ENSG00000134371	CDC73	ENSG00000115368	WDR75
ENSG00000164941	INTS8	ENSG00000128564	VGF
ENSG00000055483	USP36	ENSG00000128191	DGCR8
ENSG00000258366	RTEL1	ENSG00000008294	SPAG9
ENSG00000188846	RPL14	ENSG00000131475	VPS25
ENSG00000247626	MARS2	ENSG00000105523	FAM83E
ENSG00000095787	WAC	ENSG00000172269	DPAGT1
ENSG00000108094	CUL2	ENSG00000170312	CDK1
ENSG00000185946	RNPC3	ENSG00000104131	EIF3J
ENSG00000154473	BUB3	ENSG00000150753	CCT5
ENSG00000204394	VARS	ENSG00000140443	IGF1R
ENSG00000103051	COG4	ENSG00000010292	NCAPD2
ENSG00000104738	MCM4	ENSG00000171763	SPATA5L1

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Ensembl ID	Gene Symbol	Ensembl ID	Gene Symbol
ENSG00000117222	RBBP5	ENSG00000180098	TRNAU1AP
ENSG00000082516	GEMIN5	ENSG00000168374	ARF4
ENSG00000100162	CENPM	ENSG00000173812	EIF1
ENSG00000141456	PELP1	ENSG00000100554	ATP6V1D
ENSG00000137807	KIF23	ENSG00000072756	TRNT1
ENSG00000112685	EXOC2	ENSG00000135372	NAT10
ENSG00000125995	ROMO1	ENSG00000178394	HTR1A
ENSG00000136891	TEX10	ENSG00000128272	ATF4
ENSG00000173113	TRMT112	ENSG00000204070	SYS1
ENSG00000075914	EXOSC7	ENSG00000137815	RTF1
ENSG00000119523	ALG2	ENSG00000198026	ZNF335
ENSG00000244038	DDOST	ENSG00000117410	ATP6V0B
ENSG00000108175	ZMIZ1	ENSG00000112739	PRPF4B
ENSG00000129691	ASH2L	ENSG00000129347	KRI1
ENSG00000183207	RUVBL2	ENSG00000221818	EBF2
ENSG00000055044	NOP58	ENSG00000198431	TXNRD1
ENSG00000204315	FKBPL	ENSG00000104979	C19orf53
ENSG00000187522	HSPA14	ENSG00000136709	WDR33
ENSG00000169375	SIN3A	ENSG00000149100	EIF3M
ENSG00000143748	NVL	ENSG00000125835	SNRPB
ENSG00000021776	AQR	ENSG00000116698	SMG7
ENSG00000132467	UTP3	ENSG00000087586	AURKA
ENSG00000087470	DNM1L	ENSG00000169230	PRELID1
ENSG00000130811	EIF3G	ENSG00000143799	PARP1
ENSG00000180198	RCC1	ENSG00000146731	CCT6A
ENSG00000101407	TTI1	ENSG00000163877	SNIP1
ENSG00000116455	WDR77	ENSG00000215421	ZNF407
ENSG00000135763	URB2	ENSG00000197724	PHF2
ENSG00000133316	WDR74	ENSG00000172590	MRPL52
ENSG00000189091	SF3B3	ENSG00000175203	DCTN2
ENSG00000109917	ZNF259	ENSG00000149273	RPS3
ENSG00000130640	TUBGCP2	ENSG00000204822	MRPL53
ENSG00000011376	LARS2	ENSG00000109775	UFSP2
ENSG00000135249	RINT1	ENSG00000165733	BMS1
ENSG00000126883	NUP214	ENSG00000104671	DCTN6
ENSG00000163510	CWC22	ENSG00000175224	ATG13
ENSG00000101138	CSTF1	ENSG00000142541	RPL13A
ENSG00000104221	BRF2	ENSG00000173805	HAP1

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Ensembl ID	Gene Symbol	Ensembl ID	Gene Symbol
ENSG00000125630	POLR1B	ENSG00000115750	TAF1B
ENSG00000083896	YTHDC1	ENSG00000165688	PMPCA
ENSG00000105726	ATP13A1	ENSG00000159720	ATP6V0D1
ENSG00000105618	PRPF31	ENSG00000074201	CLNS1A
ENSG00000117748	RPA2	ENSG00000158417	EIF5B
ENSG00000143294	PRCC	ENSG00000196588	MKL1
ENSG00000156239	N6AMT1	ENSG00000138614	VWA9
ENSG00000143384	MCL1	ENSG00000124571	XPO5
ENSG00000113407	TARS	ENSG00000198000	NOL8
ENSG00000086589	RBM22	ENSG00000181991	MRPS11
ENSG00000133119	RFC3	ENSG00000149823	VPS51
ENSG00000052749	RRP12	ENSG00000151348	EXT2
ENSG00000103047	TANGO6	ENSG00000162396	PARS2
ENSG00000142751	GPN2	ENSG00000204843	DCTN1
ENSG00000101057	MYBL2	ENSG00000177302	TOP3A
ENSG00000176915	ANKLE2	ENSG00000142684	ZNF593
ENSG00000071127	WDR1	ENSG00000074800	ENO1
ENSG00000106344	RBM28	ENSG00000167513	CDT1
ENSG00000100316	RPL3	ENSG00000141101	NOB1
ENSG00000139131	YARS2	ENSG00000047315	POLR2B
ENSG00000182831	C16orf72	ENSG00000131966	ACTR10
ENSG00000167325	RRM1	ENSG00000115875	SRSF7
ENSG00000172262	ZNF131	ENSG00000186141	POLR3C
ENSG00000007168	PAFAH1B1	ENSG00000108424	KPNB1
ENSG00000117174	ZNHIT6	ENSG00000111845	PAK1IP1
ENSG00000196497	IPO4	ENSG00000148832	PAOX
ENSG00000188566	NDOR1	ENSG00000156017	C9orf41
ENSG00000183091	NEB	ENSG00000198901	PRC1
ENSG00000011304	PTBP1	ENSG00000134001	EIF2S1
ENSG00000109805	NCAPG	ENSG00000146918	NCAPG2
ENSG00000123154	WDR83	ENSG00000144713	RPL32
ENSG00000147416	ATP6V1B2	ENSG00000185122	HSF1
ENSG00000163961	RNF168	ENSG00000167658	EEF2
ENSG00000163811	WDR43	ENSG00000164190	NIPBL
ENSG00000143624	INTS3	ENSG00000163902	RPN1
ENSG00000101161	PRPF6	ENSG00000244045	TMEM199
ENSG00000130726	TRIM28	ENSG00000143476	DTL
ENSG00000165494	PCF11	ENSG00000149503	INCENP

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Ensembl ID	Gene Symbol	Ensembl ID	Gene Symbol
ENSG00000053900	ANAPC4	ENSG00000071243	ING3
ENSG00000168255	POLR2J3	ENSG00000186073	C15orf41
ENSG00000129534	MIS18BP1	ENSG00000088836	SLC4A11
ENSG00000164754	RAD21	ENSG00000136273	HUS1
ENSG00000120158	RCL1	ENSG00000005007	UPF1
ENSG00000161016	RPL8	ENSG00000070010	UFD1L
ENSG00000030066	NUP160	ENSG00000106263	EIF3B
ENSG00000099624	ATP5D	ENSG00000213024	NUP62
ENSG00000116120	FARSB	ENSG00000067191	CACNB1
ENSG00000115233	PSMD14	ENSG00000179091	CYC1
ENSG00000086504	MRPL28	ENSG00000113312	TTC1
ENSG00000160752	FDPS	ENSG00000085831	TTC39A
ENSG00000049541	RFC2	ENSG00000118197	DDX59
ENSG00000148688	RPP30	ENSG00000134871	COL4A2
ENSG00000114573	ATP6V1A	ENSG00000088986	DYNLL1
ENSG00000086200	IPO11	ENSG00000138778	CENPE
ENSG00000119720	NRDE2	ENSG00000106244	PDAP1
ENSG00000058262	SEC61A1	ENSG00000177600	RPLP2
ENSG00000073111	MCM2	ENSG00000112081	SRSF3
ENSG00000138160	KIF11	ENSG00000100413	POLR3H
ENSG00000215193	PEX26	ENSG00000172508	CARNS1
ENSG00000161057	PSMC2	ENSG00000147123	NDUFB11
ENSG00000187514	PTMA	ENSG00000119953	SMNDC1
ENSG00000135829	DHX9	ENSG00000111640	GAPDH
ENSG00000058729	RIOK2	ENSG00000117899	MESDC2
ENSG00000110330	BIRC2	ENSG00000075624	ACTB
ENSG00000141759	TXNL4A	ENSG00000163166	IWS1
ENSG00000166986	MARS	ENSG00000114503	NCBP2
ENSG00000153774	CFDP1	ENSG00000198522	GPN1
ENSG00000130177	CDC16	ENSG00000099899	TRMT2A
ENSG00000241553	ARPC4	ENSG00000181544	FANCB
ENSG00000132604	TERF2	ENSG00000136982	DSCC1
ENSG00000114982	KANSL3	ENSG00000068366	ACSL4
ENSG00000213780	GTF2H4	ENSG00000062716	VMP1
ENSG00000139343	SNRPF	ENSG00000111802	TDP2
ENSG00000101189	MRGBP	ENSG00000185627	PSMD13
ENSG00000079246	XRCC5	ENSG00000020426	MNAT1
ENSG00000196943	NOP9	ENSG00000113734	BNIP1

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Ensembl ID	Gene Symbol	Ensembl ID	Gene Symbol
ENSG00000122965	RBM19	ENSG00000102241	HTATSF1
ENSG00000132383	RPA1	ENSG00000160789	LMNA
ENSG00000094880	CDC23	ENSG00000062822	POLD1
ENSG00000213639	PPP1CB	ENSG00000168944	CEP120
ENSG00000109911	ELP4	ENSG00000139718	SETD1B
ENSG00000180957	PITPNB	ENSG00000132792	CTNNBL1
ENSG00000122257	RBBP6	ENSG00000173540	GMPPB
ENSG00000173145	NOC3L	ENSG00000128789	PSMG2
ENSG00000179115	FARSA	ENSG00000196365	LONP1
ENSG00000105171	POP4	ENSG00000160214	RRP1
ENSG00000148303	RPL7A	ENSG00000179041	RRS1
ENSG00000167508	MVD	ENSG00000143106	PSMA5
ENSG00000115541	HSPE1	ENSG00000168411	RFWD3
ENSG00000170445	HARS	ENSG00000073584	SMARCE1
ENSG00000168496	FEN1	ENSG00000175334	BANF1
ENSG00000141367	CLTC	ENSG00000077152	UBE2T
ENSG00000087191	PSMC5	ENSG00000173611	SCAI
ENSG00000163159	VPS72	ENSG00000171720	HDAC3
ENSG00000130741	EIF2S3	ENSG00000182197	EXT1
ENSG00000168495	POLR3D	ENSG00000114346	ECT2
ENSG00000071894	CPSF1	ENSG00000124214	STAU1
ENSG00000058600	POLR3E	ENSG00000126254	RBM42
ENSG00000100726	TELO2	ENSG00000127184	COX7C
ENSG00000165501	LRR1	ENSG00000174276	ZNHIT2
ENSG00000113575	PPP2CA	ENSG00000177971	IMP3
ENSG00000116922	C1orf109	ENSG00000104872	PIH1D1
ENSG00000073712	FERMT2	ENSG00000132155	RAF1
ENSG00000174437	ATP2A2	ENSG00000163872	YEATS2
ENSG00000176407	KCMF1	ENSG00000119906	FAM178A
ENSG00000140525	FANCI	ENSG00000217930	PAM16
ENSG00000101182	PSMA7	ENSG00000197498	RPF2
ENSG00000130204	TOMM40	ENSG00000130348	QRSL1
ENSG00000239306	RBM14	ENSG00000147536	GINS4
ENSG00000248643	RBM14-RBM4	ENSG00000174748	RPL15
ENSG00000172113	NME6	ENSG00000159147	DONSON
ENSG00000136448	NMT1	ENSG00000157593	SLC35B2
ENSG00000186166	CCDC84	ENSG00000181938	GINS3
ENSG00000166233	ARIH1	ENSG00000187446	CHP1

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Ensembl ID	Gene Symbol	Ensembl ID	Gene Symbol
ENSG00000111877	MCM9	ENSG00000070371	CLTCL1
ENSG00000204316	MRPL38	ENSG00000096063	SRPK1
ENSG00000101868	POLA1	ENSG00000141564	RPTOR
ENSG00000107951	MTPAP	ENSG00000108474	PIGL
ENSG00000039650	PNKP	ENSG00000187741	FANCA
ENSG00000123064	DDX54	ENSG00000213465	ARL2
ENSG00000183955	SETD8	ENSG00000117593	DARS2
ENSG00000138107	ACTR1A	ENSG00000171863	RPS7
ENSG00000244005	NFS1	ENSG00000117395	EBNA1BP2
ENSG00000188986	NELFB	ENSG00000111142	METAP2
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ENSG00000141141	DDX52	ENSG00000055950	MRPL43
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ENSG00000089053	ANAPC5	ENSG00000147533	GOLGA7
ENSG00000111602	TIMELESS	ENSG00000064313	TAF2
ENSG00000145592	RPL37	ENSG00000137154	RPS6
ENSG00000106615	RHEB	ENSG00000104886	PLEKHJ1
ENSG00000180817	PPA1	ENSG00000122882	ECD
ENSG00000110172	CHORDC1	ENSG00000184967	NOC4L
ENSG00000137876	RSL24D1	ENSG00000088325	TPX2
ENSG00000104408	EIF3E	ENSG00000183520	UTP11L
ENSG00000143436	MRPL9	ENSG00000179051	RCC2
ENSG00000108883	EFTUD2	ENSG00000157510	AFAP1L1
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ENSG00000163781	TOPBP1	ENSG00000115947	ORC4
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ENSG00000160949	TONSL	ENSG00000178057	NDUFAF3
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ENSG00000110871	COQ5	ENSG00000076201	PTPN23
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ENSG00000134748	PRPF38A	ENSG00000115446	UNC50
ENSG00000070061	IKBKAP	ENSG00000177700	POLR2L
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ENSG00000134440	NARS	ENSG00000132196	HSD17B7
ENSG00000014164	ZC3H3	ENSG00000151849	CENPJ
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ENSG00000108296	CWC25	ENSG00000113593	PPWD1
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ENSG00000160948	VPS28	ENSG00000081248	CACNA1S
ENSG00000065427	KARS	ENSG00000153201	RANBP2
ENSG00000102978	POLR2C	ENSG00000126698	DNAJC8
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ENSG00000053501	USE1	ENSG00000139719	VPS33A
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ENSG00000112159	MDN1	ENSG00000084623	EIF3I
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ENSG00000115946	PNO1	ENSG00000167118	URM1
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ENSG00000113643	RARS	ENSG00000119383	PPP2R4
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ENSG00000187555	USP7	ENSG00000155506	LARP1
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ENSG00000143374	TARS2	ENSG00000179965	ZNF771
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ENSG00000204531	POU5F1	ENSG00000179295	PTPN11
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ENSG00000161981	SNRNP25	ENSG00000136100	VPS36
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ENSG00000164808	SPIDR	ENSG00000128908	INO80
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ENSG00000169045	HNRNPH1	ENSG00000128731	HERC2
ENSG00000087510	TFAP2C	ENSG00000134014	ELP3
ENSG00000105819	PMPCB	ENSG00000181163	NPM1
ENSG00000204351	SKIV2L	ENSG00000148444	COMMD3
ENSG00000160783	PMF1	ENSG00000095319	NUP188
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ENSG00000124228	DDX27	ENSG00000055070	SZRD1
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ENSG00000135476	ESPL1	ENSG00000147596	PRDM14
ENSG00000174177	CTU2	ENSG00000092094	OSGEP
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ENSG00000105401	CDC37	ENSG00000110921	MVK
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ENSG00000114942	EEF1B2	ENSG00000187144	SPATA21
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ENSG00000203814	HIST2H2BF	ENSG00000057935	MTA3
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ENSG00000079313	REXO1	ENSG00000006025	OSBPL7
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ENSG00000108270	AATF	ENSG00000103275	UBE2I
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ENSG00000204619	PPP1R11	ENSG00000103245	NARFL
ENSG00000091651	ORC6	ENSG00000183513	COA5
ENSG00000134480	CCNH	ENSG00000174547	MRPL11
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ENSG00000111445	RFC5	ENSG00000115539	PDCL3
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ENSG00000105220	GPI	ENSG00000168028	RPSA
ENSG00000140521	POLG	ENSG00000213066	FGFR1OP
ENSG00000075856	SART3	ENSG00000143228	NUF2
ENSG00000143742	SRP9	ENSG00000137413	TAF8
ENSG00000163029	SMC6	ENSG00000124207	CSE1L
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ENSG00000100129	EIF3L	ENSG00000132773	TOE1
ENSG00000170348	TMED10	ENSG00000129460	NGDN
ENSG00000182217	HIST2H4B	ENSG00000188613	NANOS1
ENSG00000183941	HIST2H4A	ENSG00000163636	PSMD6
ENSG00000116221	MRPL37	ENSG00000146232	NFKBIE
ENSG00000196235	SUPT5H	ENSG00000135902	CHRND
ENSG00000161920	MED11	ENSG00000143641	GALNT2

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Ensembl ID	Gene Symbol	Ensembl ID	Gene Symbol
ENSG00000134690	CDCA8	ENSG00000073969	NSF
ENSG00000131153	GINS2	ENSG00000041982	TNC
ENSG00000138018	EPT1	ENSG00000108256	NUFIP2
ENSG00000173141	MRP63	ENSG00000198911	SREBF2
ENSG00000154727	GABPA	ENSG00000141385	AFG3L2
ENSG00000120800	UTP20	ENSG00000176108	CHMP6
ENSG00000114767	RRP9	ENSG00000257365	FNTB
ENSG00000174231	PRPF8	ENSG00000186487	MYT1L
ENSG00000137547	MRPL15	ENSG00000127423	AUNIP
ENSG00000146576	C7orf26	ENSG00000112110	MRPL18
ENSG00000065268	WDR18	ENSG00000114650	SCAP
ENSG00000147162	OGT	ENSG00000178104	PDE4DIP
ENSG00000198917	C9orf114	ENSG00000105656	ELL
ENSG00000180822	PSMG4	ENSG00000186393	KRT26
ENSG00000125977	EIF2S2	ENSG00000124541	RRP36
ENSG00000173418	NAA20	ENSG00000182108	DEXI
ENSG00000155561	NUP205	ENSG00000139133	ALG10
ENSG00000173545	ZNF622	ENSG00000082068	WDR70
ENSG00000127993	RBM48	ENSG00000151388	ADAMTS12
ENSG00000197102	DYNC1H1	ENSG00000172172	MRPL13
ENSG00000119392	GLE1	ENSG00000184979	USP18
ENSG00000174444	RPL4	ENSG00000239857	GET4
ENSG00000149716	ORAOV1	ENSG00000069345	DNAJA2
ENSG00000155876	RRAGA	ENSG00000073050	XRCC1
ENSG00000198841	KTI12	ENSG00000070985	TRPM5
ENSG00000056097	ZFR	ENSG00000158715	SLC45A3
ENSG00000227057	WDR46	ENSG00000172062	SMN1
ENSG00000167670	CHAF1A	ENSG00000205571	SMN2
ENSG00000127191	TRAF2	ENSG00000113141	IK
ENSG00000072506	HSD17B10	ENSG00000186105	LRRC70
ENSG00000215021	PHB2	ENSG00000157895	C12orf43
ENSG00000175467	SART1	ENSG00000166441	RPL27A
ENSG00000121073	SLC35B1	ENSG00000106346	USP42
ENSG00000079459	FDFT1	ENSG00000185379	RAD51D
ENSG00000143493	INTS7	ENSG00000116667	C1orf21
ENSG00000141543	EIF4A3	ENSG00000176444	CLK2
ENSG00000174197	MGA	ENSG00000105472	CLEC11A
ENSG00000131269	ABCB7	ENSG00000065613	SLK

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Ensembl ID	Gene Symbol	Ensembl ID	Gene Symbol
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ENSG00000197780	TAF13	ENSG00000125459	MSTO1
ENSG00000036549	ZZZ3	ENSG00000139146	FAM60A
ENSG00000066135	KDM4A	ENSG00000060069	CTDP1
ENSG00000176473	WDR25	ENSG00000130935	NOL11
ENSG00000124614	RPS10	ENSG00000115677	HDLBP
ENSG00000107581	EIF3A	ENSG00000105254	TBCB
ENSG00000084463	WBP11	ENSG00000075539	FRYL
ENSG00000137656	BUD13	ENSG00000196747	HIST1H2AI
ENSG00000183751	TBL3	ENSG00000181513	ACBD4
ENSG00000119537	KDSR	ENSG00000153107	ANAPC1
ENSG00000204220	PFDN6	ENSG00000160211	G6PD
ENSG00000170291	ELP5	ENSG00000111481	COPZ1
ENSG00000198563	DDX39B	ENSG00000070761	C16orf80
ENSG00000077549	CAPZB	ENSG00000168924	LETM1
ENSG00000255529	POLR2M	ENSG00000105058	FAM32A
ENSG00000100034	PPM1F	ENSG00000204569	PPP1R10
ENSG00000196367	TRRAP	ENSG00000153914	SREK1
ENSG00000167258	CDK12	ENSG00000161509	GRIN2C
ENSG00000039123	SKIV2L2	ENSG00000162702	ZNF281
ENSG00000076043	REXO2	ENSG00000004939	SLC4A1
ENSG00000213676	ATF6B	ENSG00000139620	KANSL2
ENSG00000058453	CROCC	ENSG00000025293	PHF20
ENSG00000153575	TUBGCP5	ENSG00000158545	ZC3H18
ENSG00000110700	RPS13	ENSG00000142546	NOSIP
ENSG00000101181	MTG2	ENSG00000143398	PIP5K1A
ENSG00000071539	TRIP13	ENSG00000197958	RPL12
ENSG00000075702	WDR62	ENSG00000067225	PKM
ENSG00000171453	POLR1C	ENSG00000172534	HCFC1
ENSG00000090989	EXOC1	ENSG00000155438	MKI67IP
ENSG00000037897	METTL1	ENSG00000166582	CENPV
ENSG00000095139	ARCN1	ENSG00000145912	NHP2
ENSG00000078142	PIK3C3	ENSG00000180992	MRPL14
ENSG00000141030	COPS3	ENSG00000118705	RPN2
ENSG00000126249	PDCD2L	ENSG00000163161	ERCC3
ENSG00000117408	IPO13	ENSG00000136819	C9orf78
ENSG00000130725	UBE2M	ENSG00000124787	RPP40
ENSG00000175054	ATR	ENSG00000179104	TMTC2

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Ensembl ID	Gene Symbol	Ensembl ID	Gene Symbol
ENSG00000149016	TUT1	ENSG00000140694	PARN
ENSG00000165060	FXN	ENSG00000143751	SDE2
ENSG00000117597	DIEXF	ENSG00000136997	MYC
ENSG00000185085	INTS5	ENSG00000147274	RBMX
ENSG00000113595	TRIM23	ENSG00000084693	AGBL5
ENSG00000040633	PHF23	ENSG00000165271	NOL6
ENSG00000178952	TUFM	ENSG00000221838	AP4M1
ENSG00000120539	MASTL	ENSG00000171444	MCC
ENSG00000103549	RNF40	ENSG00000101882	NKAP
ENSG00000119723	COQ6	ENSG00000186847	KRT14
ENSG00000171311	EXOSC1	ENSG00000014824	SLC30A9
ENSG00000106245	BUD31	ENSG00000166685	COG1
ENSG00000118046	STK11	ENSG00000108349	CASC3
ENSG00000125484	GTF3C4	ENSG00000175216	CKAP5
ENSG00000089094	KDM2B	ENSG00000259494	MRPL46
ENSG00000121621	KIF18A	ENSG00000028310	BRD9
ENSG00000129911	KLF16	ENSG00000136450	SRSF1
ENSG00000102302	FGD1	ENSG00000204859	ZBTB48
ENSG00000135679	MDM2	ENSG00000165209	STRBP
ENSG00000185115	NDNL2	ENSG00000163466	ARPC2
ENSG00000140553	UNC45A	ENSG00000125485	DDX31
ENSG00000129562	DAD1	ENSG00000070778	PTPN21
ENSG00000100138	NHP2L1	ENSG00000126001	CEP250
ENSG00000111641	NOP2	ENSG00000169249	ZRSR2
ENSG00000173660	UQCRH	ENSG00000111011	RSRC2
ENSG00000198677	TTC37	ENSG00000139496	NUPL1
ENSG00000135503	ACVR1B	ENSG00000131746	TNS4
ENSG00000180998	GPR137C	ENSG00000061936	SFSWAP
ENSG00000153187	HNRNPU	ENSG00000196584	XRCC2
ENSG00000106459	NRF1	ENSG00000168286	THAP11
ENSG00000156261	CCT8	ENSG00000119787	ATL2
ENSG00000118363	SPCS2	ENSG00000182446	NPLOC4
ENSG00000164134	NAA15	ENSG00000071462	WBSCR22
ENSG00000060642	PIGV	ENSG00000213397	HAUS7
ENSG00000090889	KIF4A	ENSG00000178028	DMAP1
ENSG00000101361	NOP56	ENSG00000067596	DHX8
ENSG00000167792	NDUFV1	ENSG00000198015	MRPL42
ENSG00000184162	NR2C2AP	ENSG00000133706	LARS

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Ensembl ID	Gene Symbol	Ensembl ID	Gene Symbol
ENSG00000128524	ATP6V1F	ENSG00000149635	OCSTAMP
ENSG00000100387	RBX1	ENSG00000117505	DR1
ENSG00000110906	KCTD10	ENSG00000155868	MED7
ENSG00000147457	CHMP7	ENSG00000129197	RPAIN
ENSG00000124570	SERPINB6	ENSG00000065978	YBX1
ENSG00000186468	RPS23	ENSG00000260238	PMF1-BGLAP
ENSG00000136122	BORA	ENSG00000178988	MRFAP1L1
ENSG00000047249	ATP6V1H	ENSG00000168005	C11orf84
ENSG00000127804	METTL16	ENSG00000162408	NOL9
ENSG00000104412	EMC2	ENSG00000140350	ANP32A
ENSG00000173726	TOMM20	ENSG00000261796	ISY1-RAB43
ENSG00000138777	PPA2	ENSG00000174405	LIG4
ENSG00000170043	TRAPPC1	ENSG00000197414	GOLGA6L1
ENSG00000124486	USP9X	ENSG00000116062	MSH6
ENSG00000105705	SUGP1	ENSG00000116906	GNPAT
ENSG00000223501	VPS52	ENSG00000134597	RBMX2
ENSG00000107815	C10orf2	ENSG00000071994	PDCD2
ENSG00000100109	TFIP11	ENSG00000112742	TTK
ENSG00000136271	DDX56	ENSG00000106636	YKT6
ENSG00000146830	GIGYF1	ENSG00000101773	RBBP8
ENSG00000198382	UVRAG	ENSG00000103061	SLC7A6OS
ENSG00000160285	LSS	ENSG00000140259	MFAP1
ENSG00000137770	CTDSPL2	ENSG00000197077	KIAA1671
ENSG00000116670	MAD2L2	ENSG00000204435	CSNK2B
ENSG00000165280	VCP	ENSG00000055130	CUL1
ENSG00000183963	SMTN	ENSG00000100209	HSCB
ENSG00000164961	KIAA0196	ENSG00000113048	MRPS27
ENSG00000157216	SSBP3	ENSG00000189403	HMGB1
ENSG00000129932	DOHH	ENSG00000173011	TADA2B
ENSG00000167721	TSR1	ENSG00000169836	TACR3
ENSG00000188352	FOCAD	ENSG00000133816	MICAL2
ENSG00000104853	CLPTM1	ENSG00000141452	C18orf8
ENSG00000185883	ATP6V0C	ENSG00000006715	VPS41
ENSG00000100519	PSMC6	ENSG00000136518	ACTL6A
ENSG00000110107	PRPF19	ENSG00000100297	MCM5
ENSG00000184203	PPP1R2	ENSG00000165898	ISCA2
ENSG00000148824	MTG1	ENSG00000156384	SFR1
ENSG00000113810	SMC4	ENSG00000145414	NAF1

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Ensembl ID	Gene Symbol	Ensembl ID	Gene Symbol
ENSG00000121152	NCAPH	ENSG00000101972	STAG2
ENSG00000241127	YAE1D1	ENSG00000112658	SRF
ENSG00000139197	PEX5	ENSG00000162736	NCSTN
ENSG00000101464	PIGU	ENSG00000103266	STUB1
ENSG00000132676	DAP3	ENSG00000008018	PSMB1
ENSG00000135972	MRPS9	ENSG00000149506	ZP1
ENSG00000089157	RPLP0	ENSG00000111530	CAND1
ENSG00000138035	PNPT1	ENSG00000027001	MIPEP
ENSG00000171824	EXOSC10	ENSG00000152266	PTH
ENSG00000153179	RASSF3	ENSG00000154174	TOMM70A
ENSG00000110713	NUP98	ENSG00000164045	CDC25A
ENSG00000100865	CINP	ENSG00000164758	MED30
ENSG00000136045	PWP1	ENSG00000160401	C9orf117
ENSG00000167526	RPL13	ENSG00000155959	VBP1
ENSG00000088766	CRLS1	ENSG00000105409	ATP1A3
ENSG00000103510	KAT8	ENSG00000175106	TVP23C
ENSG00000143368	SF3B4	ENSG00000185950	IRS2
ENSG00000156697	UTP14A	ENSG00000149256	TENM4
ENSG00000176248	ANAPC2	ENSG00000116957	TBCE
ENSG00000188786	MTF1	ENSG00000154719	MRPL39
ENSG00000175756	AURKAIP1	ENSG00000105364	MRPL4
ENSG00000140395	WDR61	ENSG00000198218	QRICH1
ENSG00000113368	LMNB1	ENSG00000013503	POLR3B
ENSG00000060339	CCAR1	ENSG00000126756	UXT
ENSG00000162385	MAGOH	ENSG00000184988	TMEM106A
ENSG00000105372	RPS19	ENSG00000186432	KPNA4
ENSG00000083312	TNPO1	ENSG00000156304	SCAF4
ENSG00000100142	POLR2F	ENSG00000090565	RAB11FIP3
ENSG00000204560	DHX16	ENSG00000163508	EOMES
ENSG00000197771	MCMBP	ENSG00000147003	TMEM27
ENSG00000099817	POLR2E	ENSG00000198730	CTR9
ENSG00000161980	POLR3K	ENSG00000105321	CCDC9
ENSG00000117133	RPF1	ENSG00000120333	MRPS14
ENSG00000125901	MRPS26	ENSG00000121680	PEX16
ENSG00000168827	GFM1	ENSG00000088205	DDX18
ENSG00000161513	FDXR	ENSG00000132432	SEC61G
ENSG00000137818	RPLP1	ENSG00000186329	TMEM212
ENSG00000150990	DHX37	ENSG00000094804	CDC6

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ENSG00000253626	EIF5AL1	ENSG00000146426	TIAM2
ENSG00000231500	RPS18	ENSG00000198925	ATG9A
ENSG00000188076	SCGB1C1	ENSG00000168242	HIST1H2BI
ENSG00000174442	ZWILCH	ENSG00000254772	EEF1G
ENSG00000242028	HYPK	ENSG00000090971	NAT14
ENSG00000124217	MOCS3	ENSG00000144381	HSPD1
ENSG00000134186	PRPF38B	ENSG00000127774	EMC6
ENSG00000105849	TWISTNB	ENSG00000126259	KIRREL2
ENSG00000137337	MDC1	ENSG00000111364	DDX55
ENSG00000132207	SLX1A	ENSG00000100749	VRK1
ENSG00000181625	SLX1B	ENSG00000159063	ALG8
ENSG00000110717	NDUFS8	ENSG00000163795	ZNF513
ENSG00000132341	RAN	ENSG00000068394	GPKOW
ENSG00000014123	UFL1	ENSG00000112659	CUL9
ENSG00000101191	DIDO1	ENSG00000187257	RSBN1L
ENSG00000125952	MAX	ENSG00000172167	MTBP
ENSG00000163714	U2SURP	ENSG00000176177	ENTHD1
ENSG00000253710	ALG11	ENSG00000166783	KIAA0430
ENSG00000104356	POP1	ENSG00000165006	UBAP1
ENSG00000130826	DKC1	ENSG00000188958	UTS2B
ENSG00000198780	FAM169A	ENSG00000136247	ZDHHC4
ENSG00000116688	MFN2	ENSG00000196363	WDR5
ENSG00000166166	TRMT61A	ENSG00000116661	FBXO2
ENSG00000214517	PPME1	ENSG00000113013	HSPA9
ENSG00000077235	GTF3C1	ENSG00000090061	CCNK
ENSG00000152240	HAUS1	ENSG00000051596	THOC3
ENSG00000063177	RPL18	ENSG00000140534	TICRR
ENSG00000087157	PGS1	ENSG00000100216	TOMM22
ENSG00000100567	PSMA3	ENSG00000104613	INTS10
ENSG00000169371	SNUPN	ENSG00000183474	GTF2H2C
ENSG00000197651	CCER1	ENSG00000159128	IFNGR2
ENSG00000198900	TOP1	ENSG00000243725	TTC4
ENSG00000213551	DNAJC9	ENSG00000102898	NUTF2
ENSG00000152464	RPP38	ENSG00000170515	PA2G4
ENSG00000131467	PSME3	ENSG00000117036	ETV3
ENSG00000223510	CDRT15	ENSG00000196262	PPIA

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ENSG00000115053	NCL	ENSG00000153037	SRP19
ENSG00000163041	H3F3A	ENSG00000135801	TAF5L
ENSG00000154813	DPH3	ENSG00000119414	PPP6C
ENSG00000181873	IBA57	ENSG00000141013	GAS8
ENSG00000185591	SP1	ENSG00000113845	TIMMDC1
ENSG00000115355	CCDC88A	ENSG00000175826	CTDNEP1
ENSG00000139350	NEDD1	ENSG00000117543	DPH5
ENSG00000108518	PFN1	ENSG00000204779	FOXD4L5
ENSG00000108264	TADA2A	ENSG00000112249	ASCC3
ENSG00000134809	TIMM10	ENSG00000152256	PDK1
ENSG00000124383	MPHOSPH10	ENSG00000169217	CD2BP2
ENSG00000126067	PSMB2	ENSG00000166246	C16orf71
ENSG00000060688	SNRNP40	ENSG00000184164	CRELD2
ENSG00000042429	MED17	ENSG00000107960	OBFC1
ENSG00000196655	TRAPPC4	ENSG00000102384	CENPI
ENSG00000107185	RGP1	ENSG00000079785	DDX1
ENSG00000124608	AARS2	ENSG00000133858	ZFC3H1
ENSG00000092098	RNF31	ENSG00000184110	EIF3C
ENSG00000143569	UBAP2L	ENSG00000146700	SRCRB4D
ENSG00000233822	HIST1H2BN	ENSG00000163380	LMOD3
ENSG00000171848	RRM2	ENSG00000116273	PHF13
ENSG00000183161	FANCF	ENSG00000178229	ZNF543
ENSG00000166197	NOLC1	ENSG00000109475	RPL34
ENSG00000064703	DDX20	ENSG00000156469	MTERFD1
ENSG00000176102	CSTF3	ENSG00000155827	RNF20
ENSG00000106028	SSBP1	ENSG00000213741	RPS29
ENSG00000143315	PIGM	ENSG00000165792	METTL17
ENSG00000136152	COG3	ENSG00000110844	PRPF40B
ENSG00000134697	GNL2	ENSG00000100842	EFS
ENSG00000159217	IGF2BP1	ENSG00000087495	PHACTR3
ENSG00000080608	KIAA0020	ENSG00000126261	UBA2
ENSG00000267368	UPK3BL	ENSG00000136718	IMP4
ENSG00000130119	GNL3L	ENSG00000091640	SPAG7
ENSG00000178950	GAK	ENSG00000184886	PIGW
ENSG00000205659	LIN52	ENSG00000184313	MROH7
ENSG00000123297	TSFM	ENSG00000163481	RNF25
ENSG00000241370	RPP21	ENSG00000137054	POLR1E
ENSG00000129351	ILF3	ENSG00000213085	CCDC19

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Ensembl ID	Gene Symbol	Ensembl ID	Gene Symbol
ENSG00000174446	SNAPC5	ENSG00000171858	RPS21
ENSG00000132382	MYBBP1A	ENSG00000130822	PNCK
ENSG00000100664	EIF5	ENSG00000145216	FIP1L1
ENSG00000131469	RPL27	ENSG00000147130	ZMYM3
ENSG00000185128	TBC1D3F	ENSG00000008086	CDKL5
ENSG00000111231	GPN3	ENSG00000165282	PIGO
ENSG00000182774	RPS17L	ENSG00000038358	EDC4
ENSG00000184779	RPS17	ENSG00000134684	YARS
ENSG00000186871	ERCC6L	ENSG00000153832	FBXO36
ENSG00000204568	MRPS18B	ENSG00000140006	WDR89
ENSG00000108312	UBTF	ENSG00000104643	MTMR9
ENSG00000167965	MLST8	ENSG00000151779	NBAS
ENSG00000115241	PPM1G	ENSG00000077348	EXOSC5
ENSG00000171103	TRMT61B	ENSG00000131043	AAR2
ENSG00000116586	LAMTOR2	ENSG00000160193	WDR4
ENSG00000105793	GTPBP10	ENSG00000140691	ARMC5
ENSG00000100348	TXN2	ENSG00000141959	PFKL
ENSG00000172757	CFL1	ENSG00000112053	SLC26A8
ENSG00000163634	THOC7	ENSG00000197111	PCBP2
ENSG00000008324	SS18L2	ENSG00000145191	EIF2B5
ENSG00000152404	CWF19L2	ENSG00000140988	RPS2
ENSG00000020129	NCDN	ENSG00000181472	ZBTB2

[0430] The gene symbols used in herein (including in Tables 3 and 4) are based on those found in the Human Gene Naming Committee (HGNC) which is searchable on the world-wide web at www.genenames.org. Ensembl IDs are provided for each gene symbol and are searchable world-wide web at www.ensembl.org.

[0431] The genes provided in Tables 3 and 4 are non-limiting examples of essential genes. Although additional essential genes will be apparent to the skilled artisan based on the knowledge in the art, the suitability of a particular gene for use according to the present disclosure can be determined, e.g., as discussed herein. For example, in some embodiments, a particular essential gene can be selected by analysis of potential off-target sites elsewhere in the genome. In some embodiments, only essential genes with one or more gRNA target sites that are unique in the human genome are selected for methods described herein. In some embodiments,

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only essential genes with one or more gRNA target sites that are found in only one other locus in the human genome are selected for methods described herein. In some embodiments, only essential genes with one or more gRNA target sites found in only two other loci in the human genome are selected for methods described herein.

Gene product of interest

[0432] The methods, systems and cells of the present disclosure enable the integration of a gene of interest at an essential gene of a cell. The gene of interest can encode any gene product of interest. In certain embodiments, a gene product of interest comprises an antibody, an antigen, an enzyme, a growth factor, a receptor (e.g., cell surface, cytoplasmic, or nuclear), a hormone, a lymphokine, a cytokine, a chemokine, a reporter, a functional fragment of any of the above, or a combination of any of the above.

[0433] In some embodiments, sequence for a gene product of interest can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and synthetic DNA sequences. For example, a gene of interest may encode an miRNA, an shRNA, a native polypeptide (i.e. a polypeptide found in nature) or fragment thereof; a variant polypeptide (i.e. a mutant of the native polypeptide having less than 100% sequence identity with the native polypeptide) or fragment thereof; an engineered polypeptide or peptide fragment, a therapeutic peptide or polypeptide, an imaging marker, a selectable marker, a degradation signal, and the like.

[0434] In some embodiments, an exemplary gene product of interest is one that confers therapeutic value, e.g., a new therapeutic activity to the cell. In some embodiments, exemplary gene products of interest are polypeptides such as a chimeric antigen receptor (CAR) or antigen-binding fragment thereof, a T cell receptor or antigen binding fragment thereof, a non-naturally occurring variant of FcγRIII (CD16), interleukin 15 (IL-15), interleukin 15 receptor (IL-15R) or a variant thereof, interleukin 12 (IL-12), interleukin-12 receptor (IL-12R) or a variant thereof, human leukocyte antigen G (HLA-G), human leukocyte antigen E (HLA-E), leukocyte surface antigen cluster of differentiation CD47 (CD47), or any combination of two or more thereof. It is to be understood that the methods and cells of the present disclosure are not limited to any particular gene product of interest and that the selection of a gene product of interest will depend on the type of cell and ultimate use of the cells.

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[0435] In some embodiments, a gene product of interest may be a cytokine. In some embodiments, expression of a cytokine from a modified cell generated using a method as described herein allows for localized dosing of the cytokine *in vivo* (e.g., within a subject in need thereof) and/or avoids a need to systemically administer a high-dose of the cytokine to a subject in need thereof (e.g., a lower dose of the cytokine may be administered). In some embodiments, the risk of dose-limiting toxicities associated with administering a cytokine is reduced while cytokine mediated cell functions are maintained. In some embodiments, to facilitate cell function without the need to additionally administer high-doses of soluble cytokines, a partial or full peptide of one or more of IL2, IL4, IL6, IL7, IL9, IL10, IL11, IL12, IL15, IL18, IL21, IFN-α, IFN-β and/or their respective receptor is introduced to the cell to enable cytokine signaling with or without the expression of the cytokine itself, thereby maintaining or improving cell growth, proliferation, expansion, and/or effector function with reduced risk of cytokine toxicities. In some embodiments, the introduced cytokine and/or its respective native or modified receptor for cytokine signaling are expressed on the cell surface. In some embodiments, the cytokine signaling is constitutively activated. In some embodiments, the activation of the cytokine signaling is inducible. In some embodiments, the activation of the cytokine signaling is transient and/or temporal. In some embodiments, a gene product if interest can be IL2, IL3, IL4, IL6, IL7, IL9, IL10, IL11, IL12, IL13, IL15, IL21, GM-CSF, IFN-a, IFN-b, IFN-g, erythropoietin, and/or the respective cytokine receptor. In some embodiments, a gene product of interest can be CCL3, TNFα, CCL23, IL2RB, IL12RB2, or IRF7.

[0436] In some embodiments, a gene product of interest can be a chemokine and/or the respective chemokine receptor. In some embodiments, a chemokine receptor can be, but is not limited to, CCR2, CCR5, CCR8, CX3C1, CX3CR1, CXCR1, CXCR2, CXCR3A, CXCR3B, or CXCR2. In some embodiments, a chemokine can be, but is not limited to, CCL7, CCL19, or CXL14.

[0437] As used herein, the term "chimeric antigen receptor" or "CAR" refers to a receptor protein that has been modified to give cells expressing the CAR the new ability to target a specific protein. Within the context of the disclosure, a cell modified to comprise a CAR or an antigen binding fragment may be used for immunotherapy to target and destroy cells associated

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with a disease or disorder, *e.g.*, cancer cells. In some embodiments, the CAR can bind to any antigen of interest.

[0438] CARs of interest can include, but are not limited to, a CAR targeting mesothelin, EGFR, HER2 and/or MICA/B. To date, mesothelin-targeted CAR T-cell therapy has shown early evidence of efficacy in a phase I clinical trial of subjects having mesothelioma, non-small cell lung cancer, and breast cancer (NCT02414269). Similarly, CARs targeting EGFR, HER2 and MICA/B have shown promise in early studies (see, *e.g.*, Li et al. (2018), *Cell Death & Disease*, 9(177); Han et al. (2018) Am. J. Cancer Res., 8(1):106-119; and Demoulin 2017) Future Oncology, 13(8); the entire contents of each of which are expressly incorporated herein by reference in their entireties).

[0439] CARs are well-known to those of ordinary skill in the art and include those described in, for example: WO13/063419 (mesothelin), WO15/164594 (EGFR), WO13/063419 (HER2), WO16/154585 (MICA and MICB), the entire contents of each of which are expressly incorporated herein by reference in their entireties. In some embodiments, a gene product of interest is any suitable CAR, NK cell specific CAR (NK-CAR), T cell specific CAR, or other binder that targets a cell, *e.g.*, an NK cell, to a target cell, *e.g.*, a cell associated with a disease or disorder, may be expressed in the modified cells provided herein. Exemplary CARs, and binders, include, but are not limited to, bi-specific antigen binding CARs, switchable CARs, dimerizable CARs, split CARs, multi-chain CARs, inducible CARs, CARs and binders that bind BCMA, androgen receptor, PSMA, PSCA, Muc1, HPV viral peptides (i.e., E7), EBV viral peptides, WT1, CEA, EGFR, EGFRvIII, IL13Rα2, GD2, CA125, EpCAM, Muc16, carbonic anhydrase IX (CAIX), CCR1, CCR4, carcinoembryonic antigen (CEA), CD3, CD5, CD7, CD10, CD19, CD20, CD22, CD23, CD24, CD26, CD30, CD33, CD34, CD35, CD38 CD41, CD44, CD44V6, CD49f, CD56, CD70, CD92, CD99, CD123, CD133, CD135, CD148, CD150, CD261, CD362, CLEC12A, MDM2, CYP1B, livin, cyclin 1, NKp30, NKp46, DNAM1, NKp44, CA9, PD1, PDL1, an antigen of cytomegalovirus (CMV), epithelial glycoprotein-40 (EGP-40), GPRC5D, receptor tyrosine kinases erb-B2,3,4, EGFIR, ERBB folate binding protein (FBP), fetal acetylcholine receptor (AChR), folate receptor-a, ganglioside G3 (GD3) human Epidermal Growth Factor Receptor 2 (HER-2), human telomerase reverse transcriptase (hTERT), ICAM-1, Integrin B7, Interleukin-13 receptor subunit alpha-2 (IL-13Ra2), K-light chain, kinase insert

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domain receptor (KDR), Lewis A (CA19.9), Lewis Y (Le Y), L1 cell adhesion molecule (LI-CAM), LILRB2, melanoma antigen family A 1 (MAGE-Al), MICA/B, Mucin 16 (Muc-16), NKCSI, NKG2D ligands, c-Met, cancer-testis antigen NYES0-1, oncofetal antigen (h5T4), PRAME, prostate stem cell antigen (PSCA), PRAME prostate-specific membrane antigen (PSMΛ), tumor-associated glycoprotein 72 (TΛG-72), TIM-3, TRBCI, TRBC2, vascular endothelial growth factor R2 (VEGF-R2), Wilms tumor protein (WT-1), a pathogen antigen, or any suitable combination thereof.. Additional suitable CARs and binders for use in the modified cells provided herein will be apparent to those of skill in the art based on the present disclosure and the general knowledge in the art. Such additional suitable CARs include those described in Figure 3 of Davies and Maher, *Adoptive T-cell Immunotherapy of Cancer Using Chimeric Antigen Receptor-Grafted T Cells*, Archivum Immunologiae et Therapiae Experimentalis 58(3):165-78 (2010), the entire contents of which are incorporated herein by reference. Additional CARs suitable for methods described herein include: CD171-specific CARs (Park et al., Mol Ther (2007) 15(4):825-833), EGFRvIII-specific CARs (Morgan et al, Hum Gene Ther (2012) 23(10): 1043-1053), EGF-R-specific CARs (Kobold et al, J Natl Cancer Inst (2014) 107(1):364), carbonic anhydrase K-specific CARs (Lamers et al., Biochem Soc Trans (2016) 44(3):951-959), FR-a-specific CARs (Kershaw et al., Clin Cancer Res (2006) 12(20):6106-6015), HER2-specific CARs (Ahmed et al., J Clin Oncol (2015) 33(15)1688-1696; Nakazawa et al., Mol Ther (2011) 19(12):2133-2143; Ahmed et al., Mol Ther (2009) 17(10): 1779-1787; Luo et al., Cell Res (2016) 26(7):850-853; Morgan et al., Mol Ther (2010) 18(4):843-85 1; Grada et al., Mol Ther Nucleic Acids (2013) 9(2):32), CEA- specific CARs (Katz et al., Clin Cancer Res (2015) 21 (14):3149-3159), IL13Ra2-specific CARs (Brown et al., Clin Cancer Res (2015) 21(18):4062-4072), GD2-specific CARs (Louis et al., Blood (2011) 118(23):6050-6056; Caruana et al., Nat Med (2015) 21(5):524-529), ErbB2-specific CARs (Wilkie et al., J Clin Immunol (2012) 32(5): 1059-1070), VEGF-R- specific CARs (Chinnasamy et al., Cancer Res (2016) 22(2):436-447), FAP-specific CARs (Wang et al., Cancer Immunol Res (2014) 2(2): 154-166), MSLN-specific CARs (Moon et al., Clin Cancer Res (2011) 17(14):4719-30), CD19-specific CARs (Axicabtagene ciloleucel (Yescarta®) and Tisagenlecleucel (Kymriah®). See also, Li et al., J Hematol and Oncol (2018) 11(22), reviewing clinical trials of tumor-specific CARs. In some embodiments, a CAR is an anti-EGFR CAR. In some embodiments, a CAR is an anti-

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CD19 CAR. In some embodiments, a CAR is an anti-BCMA CAR. In some embodiments, a CAR is an anti-CD7 CAR.

[0440] As used herein, the term "CD16" refers to a receptor (FcγRIII) for the Fc portion of immunoglobulin G, and it is involved in the removal of antigen-antibody complexes from the circulation, as well as other antibody-dependent responses. In some embodiments, a CD16 protein is an hCD16 variant. In some embodiments an hCD16 variant is a high affinity F158V variant.

[0441] In some embodiments, a gene product of interest comprises a high affinity non-cleavable CD16 (hnCD16) or a variant thereof. In some embodiments, a high affinity non-cleavable CD16 or a variant thereof comprises at least any one of the followings: (a) F176V and S197P in ectodomain domain of CD16 (see e.g., Jing et al., Identification of an ADAM17 Cleavage Region in Human CD16 (FcγRIII) and the Engineering of a Non-Cleavable Version of the Receptor in NK Cells; *PLOS One*, 2015); (b) a full or partial ectodomain originated from CD64; (c) a non-native (or non-CD16) transmembrane domain; (d) a non-native (or non-CD16) intracellular domain; (e) a non-native (or nonCD16) signaling domain; (f) a non-native stimulatory domain; and (g) transmembrane, signaling, and stimulatory domains that are not originated from CD16, and are originated from a same or different polypeptide. In some embodiments, the non-native transmembrane domain is derived from CD3D, CD3E, CD3G, CD3s, CD4, CD5, CD5a, CD5b, CD27, CD2S, CD40, CDS4, CD166, 4-lBB, OX40, ICOS, ICAM-1, CTLA-4, PD-1, LAG-3, 2B4, BTLA, CD16, IL7, IL12, IL15, KIR2DL4, KIR2DS1, NKp30, NKp44, NKp46, NKG2C, NKG2D, or T cell receptor (TCR) polypeptide. In some embodiments, the non-native stimulatory domain is derived from CD27, CD2S, 4-lBB, OX40, ICOS, PD-1, LAG-3, 2B4, BTLA, DAP10, DAP12, CTLA-4, or NKG2D polypeptide. In some other embodiments, the non-native signaling domain is derived from CD3s, 2B4, DAP10, DAP12, DNAM1, CD137 (41BB), IL21, IL7, IL12, IL15, NKp30, NKp44, NKp46, NKG2C, or NKG2D polypeptide. In some particular embodiments of a hnCD16 variant, the non-native transmembrane domain is derived from NKG2D, the non-native stimulatory domain is derived from 2B4, and the non-native signaling domain is derived from CD3s. In some embodiments, a gene product of interest comprises a high affinity cleavable CD16 (hnCD16) or a variant thereof. In some embodiments, a high affinity cleavable CD16 or a variant thereof comprises at least

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F176V. In some embodiments, a high affinity cleavable CD16 or a variant thereof does not comprise an S197P amino acid substitution.

[0442] As used herein, the term "IL-15/IL15RA" or "Interleukin-15" (IL-15) refers to a cytokine with structural similarity to Interleukin-2 (IL-2). Like IL-2, IL-15 binds to and signals through a complex composed of IL-2/IL-15 receptor beta chain (CD122) and the common gamma chain (gamma-C, CD132). IL-15 is secreted by mononuclear phagocytes (and some other cells) following infection by virus(es). This cytokine induces cell proliferation of natural killer cells. IL-15 Receptor alpha (IL15RA) specifically binds IL-15 with very high affinity, and is capable of binding IL-15 independently of other subunits (see e.g., Mishra et al., Molecular pathways: Interleukin-15 signaling in health and in cancer, *Clinical Cancer Research*, 2014). It is suggested that this property allows IL-15 to be produced by one cell, endocytosed by another cell, and then presented to a third party cell. IL15RA is reported to enhance cell proliferation and expression of apoptosis inhibitor BCL2L1/BCL2-XL and BCL2. Exemplary sequences of IL-15 are provided in NG_029605.2, and exemplary sequences of IL-15RA are provided in NM_002189.4. In some embodiments, the IL-15R variant is a constitutively active IL-15R variant. In some embodiments, the constitutively active IL-15R variant is a fusion between IL-15R and an IL-15R agonist, e.g., an IL-15 protein or IL-15R-binding fragment thereof. In some embodiments, the IL-15R agonist is IL-15, or an IL-15R-binding variant thereof. Exemplary suitable IL-15R variants include, without limitation, those described, e.g., in Mortier E et al, 2006; The Journal of Biological Chemistry 2006 281: 1612-1619; or in Bessard-A et al., Mol Cancer Ther. 2009 Sep;8(9):2736-45, the entire contents of each of which are incorporated by reference herein. In some embodiments, membrane bound trans-presentation of IL-15 is a more potent activation pathway than soluble IL-15 (see e.g., Imamura et al., Autonomous growth and increased cytotoxicity of natural killer cells expressing membrane-bound interleukin-15, *Blood*, 2014). In some embodiments, IL-15R expression comprises: IL15 and IL15Ra expression using a self-cleaving peptide; a fusion protein of IL15 andIL15Ra; an IL15/IL15Ra fusion protein with intracellular domain of IL15Ra truncated; a fusion protein of IL15 and membrane bound Sushi domain of IL15Ra; a fusion protein of IL15 and IL15Rβ; a fusion protein of IL15 and common receptor γC, wherein the common receptor γC is native or modified; and/or a homodimer of IL15Rβ.

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[0443] As used herein, the term "IL-12" refers to interleukin-12, a cytokine that acts on T and natural killer cells. In some embodiments, a genetically engineered stem cell and/or progeny cell comprises a genetic modification that leads to expression of one or more of an interleukin 12 (IL12) pathway agonist, e.g., IL-12, interleukin 12 receptor (IL-12R) or a variant thereof (e.g., a constitutively active variant of IL-12R, e.g., an IL-12R fused to an IL-12R agonist (IL-12RA).

[0444] In some embodiments, the gene product of interest comprises a protein or polypeptide whose expression within a cell, e.g., a cell modified as described herein, enables the cell to inhibit or evade immune rejection after transplant or engraftment into a subject. In some embodiments, the gene product of interest is HLA-E, HLA-G, CTL4, CD47, or an associated ligand.

[0445] In some embodiments, the gene product of interest is a T cell receptor (TCR) or an antigen-binding fragment thereof, e.g., a recombinant TCR. In some embodiments, the recombinant TCR can bind to an antigen of interest, e.g., an antigen selected from, but not limited to, CD279, CD2, CD95, CD152, CD223CD272, TIM3, KIR, A2aR, SIRPa, CD200, CD200R, CD300, LPA5, NY-ESO, PD1, PDL1, or MAGE-A3/A6. In some embodiments, the TCR or antigen-binding fragment thereof can bind to a viral antigen, e.g., an antigen from hepatitis A, hepatitis B, hepatitis C (HCV), human papilloma virus (HPV) (e.g., HPV-16 (such as HPV-16 E6 or HPV-16 E7), HPV-18, HPV-31, HPV-33, or HPV-35), Epstein-Barr virus (EBV), human herpes virus 8 (HHV-8), human T-cell leukemia virus01 (HTLV-1), human T-cell leukemia virus-2 (HTLV-2) or a cytomegalovirus (CMV).

[0446] In some embodiments, the gene product of interest comprises a single-chain variable fragment that can bind to CD47, PD1, CTLA4, CD28, OX40, 4-1BB, and ligands thereof.

[0447] As used herein, the term "HLA-G" refers to the HLA non-classical class I heavy chain paralogues. This class I molecule is a heterodimer consisting of a heavy chain and a light chain (beta-2 microglobulin). The heavy chain is anchored in the membrane. HLA-G is expressed on fetal derived placental cells. HLA-G is a ligand for NK cell inhibitory receptor KIR2DL4, and therefore expression of this HLA by the trophoblast defends it against NK cell-mediated death. See e.g., Favier et al., Tolerogenic Function of Dimeric Forms of HLA-G Recombinant Proteins: A Comparative Study In Vivo PLOS One 2011, the entire contents of

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which are incorporated herein by reference. An exemplary sequence of HLA-G is set forth as NG_029039.1.

[0448] As used herein, the term "HLA-E" refers to the HLA class I histocompatibility antigen, alpha chain E, also sometimes referred to as MHC class I antigen E. The HLA-E protein in humans is encoded by the HLA-E gene. The human HLA-E is a non-classical MHC class I molecule that is characterized by a limited polymorphism and a lower cell surface expression than its classical paralogues. This class I molecule is a heterodimer consisting of a heavy chain and a light chain (beta-2 microglobulin). The heavy chain is anchored in the membrane. HLA-E binds a restricted subset of peptides derived from the leader peptides of other class I molecules. HLA-E expressing cells escape allogeneic responses and lysis by NK cells. See e.g., Geornalusse-G et al., Nature Biotechnology 2017 35(8), the entire contents of which are incorporated herein by reference. Exemplary sequences of the HLA-E protein are provided in NM_005516.6.

[0449] As used herein, the term "CD47," also sometimes referred to as "integrin associated protein" (IAP), refers to a transmembrane protein that in humans is encoded by the CD47 gene. CD47 belongs to the immunoglobulin superfamily, partners with membrane integrins, and also binds the ligands thrombospondin-1 (TSP-1) and signal-regulatory protein alpha (SIRPα). CD47 acts as a signal to macrophages that allows CD47-expressing cells to escape macrophage attack. See, e.g., Deuse-T, et al., Nature Biotechnology 2019 37: 252–258, the entire contents of which are incorporated herein by reference.

[0450] In some embodiments, a gene product of interest comprises a chimeric switch receptor (see e.g., WO2018094244A1 - TGFBeta Signal Converter; Ankri et al., Human T cells Engineered to express a programmed death 1/28 costimulatory retargeting molecule display enhanced antitumor activity, *The Journal of Immunology,* October 15, 2013, 191; Roth et al., Pooled knockin targeting for genome engineering of cellular immunotherapies, *Cell.* 2020 Apr 30;181(3):728-744.e21; and Boyerinas et al., A Novel TGF-β2/Interleukin Receptor Signal Conversion Platform That Protects CAR/TCR T Cells from TGF-β2-Mediated Immune Suppression and Induces T Cell Supportive Signaling Networks, *Blood,* 2017). In some embodiments, chimeric switch receptors are engineered cell-surface receptors comprising an extracellular domain from an endogenous cell-surface receptor and a heterologous intracellular

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signaling domain, such that ligand recognition by the extracellular domain results in activation of a different signaling cascade than that activated by the wild type form of the cell-surface receptor. In some embodiments, a chimeric switch receptor comprises an extracellular domain of an inhibitory cell-surface receptor fused to an intracellular domain that leads to the transmission of an activating signal rather than the inhibitory signal normally transduced by the inhibitory cell-surface receptor. In some embodiments, extracellular domains derived from cell-surface receptors known to inhibit immune effector cell activation can be fused to activating intracellular domains. In such an embodiment, engagement of the corresponding ligand may then activate signaling cascades that increase, rather than inhibit, the activation of the immune effector cell. For example, in some embodiments, a gene product of interest is a PD1-CD28 switch receptor, wherein the extracellular domain of PD1 is fused to the intracellular signaling domain of CD28 (See e.g., Liu et al., Cancer Res 76:6 (2016), 1578-1590 and Moon et al., Molecular Therapy 22 (2014), S201). In some embodiments, encoding gene product of interest is or comprises the extracellular domain of CD200R and the intracellular signaling domain of CD28 (See Oda et al., Blood 130:22 (2017), 2410-2419).

[0451] In some embodiments, a gene product of interest is a reporter gene (e.g., GFP, mCherry, etc.). In some embodiments, a reporter gene is utilized to confirm the suitability of a knock-in cassette's expression capacity. In certain embodiments, a gene product of interest may be a colored or fluorescent protein such as: blue/UV proteins, e.g. TagBFP, mTagBFP2, Azurite, EBFP2, mKalamal, Sirius, Sapphire, T-Sapphire; cyan proteins, e.g. ECFP, Cerulean, SCFP3A, mTurquoise, mTurquoise2, monomeric Midoriishi-Cyan, TagCFP, mTFPl; green proteins, e.g. EGFP, Emerald, Superfolder GFP, Monomeric Azami Green, TagGFP2, mUKG, m Wasabi, Clover, mNeonGreen; yellow proteins, e.g. EYFP, Citrine, Venus, SYFP2, TagYFP; orange proteins, e.g. Monomeric Kusabira-Orange, mKOK, mK02, mOrange, m0range2; red proteins, e.g. mRaspberry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mApple, mRuby, mRuby2; far-red proteins, e.g. mPlum, HcRed-Tandem, mKate2, mNeptune, NirFP; near-IR proteins, e.g. TagRFP657, IFPl.4, iRFP; long stokes shift proteins, e.g. mKeima Red, LSS-mKatel, LSS-mKate2, mBeRFP; photoactivatible proteins, e.g. PA-GFP, PAmCherryl, PATagRFP; photoconvertible proteins, e.g. Kaede (green), Kaede (red), KikGRl (green), KikGRl

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(red), PS-CFP2, PS-CFP2, mEos2 (green), mEos2 (red), mEos3.2 (green), mEos3.2 (red), PSmOrange, PSmOrange, photoswitchable proteins, e.g. Dronpa, and combinations thereof.

[0452] In some embodiments, a gene of interest provided herein can optionally include a sequence encoding a destabilizing domain ("a destabilizing sequence") for temporal and/or spatial control of protein expression. Non-limiting examples of destabilizing sequences include sequences encoding a FK506 sequence, a dihydrofolate reductase (DHFR) sequence, or other exemplary destabilizing sequences.

[0453] In the absence of a stabilizing ligand, a protein sequence operatively linked to a destabilizing sequence is degraded by ubiquitination. In contrast, in the presence of a stabilizing ligand, protein degradation is inhibited, thereby allowing the protein sequence operatively linked to the destabilizing sequence to be actively expressed. As a positive control for stabilization of protein expression, protein expression can be detected by conventional means, including enzymatic, radiographic, colorimetric, fluorescence, or other spectrographic assays; fluorescent activating cell sorting (FACS) assays; immunological assays (e.g., enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and immunohistochemistry).

[0454] Additional examples of destabilizing sequences are known in the art. In some embodiments, the destabilizing sequence is a FK506- and rapamycin-binding protein (FKBP12) sequence, and the stabilizing ligand is Shield-1 (Shld1) (Banaszynski et al. (2012) Cell 126(5): 995-1004, which is incorporated in its entirety herein by reference). In some embodiments, a destabilizing sequence is a DHFR sequence, and a stabilizing ligand is trimethoprim (TMP) (Iwamoto et al. (2010) Chem Biol 17:981-988, which is incorporated in its entirety herein by reference). In some embodiments, a destabilizing domain is small molecule-assisted shutoff (SMASh), where a constitutive degron with a protease and its corresponding cleavage site derived from hepatitis C virus are combined. In some embodiments, a destabilizing domain comprises a HaloTag system, dTag system, and/or nanobody (see e.g., Luh et al., Prey for the proteasome: targeted protein degradation - a medicinal chemist's perspective; *Angewandte Chemie*, 2020).

[0455] In some embodiments, a destabilizing sequence can be used to temporally control a cell modified as described herein.

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[0456] In some embodiments, a gene product of interest may be a suicide gene, (see e.g., Zarogoulidis et al., Suicide Gene Therapy for Cancer - Current Strategies; *J Genet Syndr Gene Ther.* 2013). In some embodiments, a suicide gene can use a gene-directed enzyme prodrug therapy (GDEPT) approach, a dimerization inducing approach, and/or therapeutic monoclonal antibody mediated approach. In some embodiments, a suicide gene is biologically inert, has an adequate bio-availability profile, an adequate bio-distribution profile, and can be characterized by intrinsic acceptable and/or absence of toxicity. In some embodiments, a suicide gene codes for a protein able to convert, at a cellular level, a non-toxic prodrug into a toxic product. In some embodiments, a suicide gene may improve the safety profile of a cell described herein (see e.g., Greco et al., Improving the safety of cell therapy with the TK-suicide gene; *Front Pharmacology.* 2015; Jones et al., Improving the safety of cell therapy products by suicide gene transfer; *Frontiers Pharmacology,* 2014). In some embodiments, a suicide gene is a herpes simplex virus thymidine kinase (HSV-TK). In some embodiments, a suicide gene is a cytosine deaminase (CD). In some embodiments, a suicide gene is an apoptotic gene (e.g., a caspase). In some embodiments, a suicide gene is dimerization inducing, e.g., comprising an inducible FAS (iFAS) or inducible Caspase9 (iCasp9)/AP1903 system. In some embodiments, a suicide gene is a CD20 antigen, and cells expressing such an antigen can be eliminated by clinical-grade anti-CD20 antibody administration. In some embodiments, a suicide gene is a truncated human EGFR polypeptide (huEGFRt) which confers sensitivity to a pharmaceutical-grade anti-EGFR monoclonal antibody, e.g., cetuximab. In some embodiments a suicide gene is a c-myc tag, which confers sensitivity to pharmaceutical-grade anti-cmyc antibodies.

SEQ ID NO: 161 - Exemplary DHFR destabilizing amino acid sequence

MISLIAALAVDYVIGMENAMPWNLPADLAWFKRNTLNKPVIMGRHTWESIGRPLPGRKNIILSS
QPSTDDRVTWVKSVDEAIAACGDVPEIMVIGGGRVIEQFLPKAQKLYLTHIDAEVEGDTHFPDY
EPDDWESVFSEFHDADAQNSHSYCFEILERR

SEQ ID NO: 162 - Exemplary DHFR destabilizing nucleotide sequence

GGTACCATCAGTCTGATTGCGGCGTTAGCGGTAGATTACGTTATCGGCATGGAAAACGCCATGC
CGTGGAACCTGCCTGCCGATCTCGCCTGGTTTAAACGCAACACCTTAAATAAACCCGTGATTAT
GGGCCGCCATACCTGGGAATCAATCGGTCGTCCGTTGCCAGGACGCAAAAATATTATCCTCAGC
AGTCAACCGAGTACGGACGATCGCGTAACGTGGGTGAAGTCGGTGGATGAAGCCATCGCGGCGT
GTGGTGACGTACCAGAAATCATGGTGATTGGCGGCGGTCGCGTTATTGAACAGTTCTTGCCAAA
AGCGCAAAAACTGTATCTGACGCATATCGACGCAGAAGTGGAAGGCGACACCCATTTCCCGGAT

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TACGAGCCGGATGACTGGGAATCGGTATTCAGCGAATTCCACGATGCTGATGCGCAGAACTCTC
ACAGCTATTGCTTTGAGATTCTGGAGCGGCGATAA

SEQ ID NO: 163 - Exemplary destabilizing domain

ATCAGTCTGATTGCGGCGTTAGCGGTAGATTACGTTATCGGCATGGAAAACGCCATGCCGTGGA
ACCTGCCTGCCGATCTCGCCTGGTTTAAACGCAACACCTTAAATAAACCCGTGATTATGGGCCG
CCATACCTGGGAATCAATCGGTCGTCCGTTGCCAGGACGCAAAAATATTATCCTCAGCAGTCAA
CCGAGTACGGACGATCGCGTAACGTGGGTGAAGTCGGTGGATGAAGCCATCGCGGCGTGTGGTG
ACGTACCAGAAATCATGGTGATTGGCGGCGGTCGCGTTATTGAACAGTTCTTGCCAAAAGCGCA
AAAACTGTATCTGACGCATATCGACGCAGAAGTGGAAGGCGACACCCATTTCCCGGATTACGAG
CCGGATGACTGGGAATCGGTATTCAGCGAATTCCACGATGCTGATGCGCAGAACTCTCACAGCT
ATTGCTTTGAGATTCTGGAGCGGCGA

SEQ ID NO: 164 - Exemplary FKBP12 destabilizing peptide amino acid sequence

MGVEKQVIRPGNGPKPAPGQTVTVHCTGFGKDGDLSQKFWSTKDEGQKPFSFQIGKGAVIKGWD
EGVIGMQIGEVARLRCSSDYAYGAGGFPAWGIQPNSVLDFEIEVLSVQ

[0457] In some embodiments, a coding sequence for a single gene product of interest may be included in a knock-in cassette. In some embodiments, coding sequences for two gene products of interest may be included in a single knock-in cassette; in some embodiments, this may be referred to as a bicistronic or multicistronic construct. In some embodiments, coding sequences for more than two gene products of interest may be included in a single knock-in cassette; in some embodiments, this may be referred to as a multicistronic construct. In some embodiments, when more than one coding sequence for more than one gene product of interest is included in a knock-in cassette, these sequences may have a linker sequence connecting them. Linker sequences are generally known in the art, an exemplary linker sequence is identified in SEQ ID NO: 164000. In some embodiments, where more than one coding sequence for more than one gene product of interest is included in a knock-in cassette, these sequences may be connected by a linker sequence, an IRES, and/or 2A element.

[0458] In some embodiments, a polynucleotide encoding a gene product of interest comprises or consists of the sequence of any one of SEQ ID NOs: 162-163, 165-182, or 164000. In some embodiments, a polynucleotide encoding a gene product of interest comprises or consists of a sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to any one of SEQ ID NOs: 162-163, 165-182, or 164000. In some embodiments, a polynucleotide encoding a gene product of interest comprises or consists of a functional variant of any one of SEQ ID NOs: 162-163,

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165-182, or 164000. In some embodiments, a polynucleotide encoding a gene product of interest comprises or consists of a nucleotide sequence having 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mutations (e.g., substitutions, insertions, and/or deletions) relative to any one of SEQ ID NOs: 162-163, 165-182, or 164000.

SEQ ID NO: 164000 - exemplary linker sequence

TCTGGCGGAGGAAGCGGAGGCGGAGGATCTGGTGGTGGTGGATCTGGCGGCGGTGGTAGTGGCG
GAGGTTCTCTGCAA

SEQ ID NO: 165 - exemplary CD16 knock-in cassette sequence

ATGTGGCAACTGCTGCTGCCTACAGCTCTGCTGCTTCTGGTGTCTGCCGGCATGAGAACCGAGG
ATCTGCCTAAGGCCGTGGTGTTCCTGGAACCTCAGTGGTACAGAGTGCTGGAAAAGGACAGCGT
GACCCTGAAGTGCCAGGGCGCCTATTCTCCCGAGGACAATAGCACCCAGTGGTTCCACAACGAG
AGCCTGATCAGCAGCCAGGCCAGCAGCTACTTTATCGATGCCGCCACCGTGGACGACAGCGGCG
AGTACAGATGCCAGACCAATCTGAGCACCCTGAGCGACCCTGTGCAGCTGGAAGTGCACATTGG
ATGGTTGCTGCTGCAAGCCCCTAGATGGGTGTTCAAAGAAGAGGACCCCATCCACCTGAGATGC
CACTCTTGGAAGAACACAGCCCTGCACAAAGTGACCTACCTGCAGAACGGCAAGGGCAGAAAGT
ACTTCCACCACAACAGCGACTTCTACATCCCCAAGGCCACACTGAAGGACTCCGGCTCCTACTT
CTGCAGAGGCCTGGTCGGCAGCAAGAACGTGTCCAGCGAGACAGTGAACATCACCATCACACAG
GGCCTCGCCGTGTCTACCATCAGCAGCTTTTTCCCACCTGGCTATCAGGTGTCCTTCTGCCTGG
TCATGGTGCTGCTGTTCGCCGTGGATACCGGCCTGTACTTCAGCGTCAAGACCAACATCCGGTC
CAGCACCAGAGACTGGAAGGACCACAAGTTCAAGTGGCGGAAGGACCCTCAGGACAAGTAA

SEQ ID NO: 166 - exemplary CD16 knock-in cassette sequence

ATGTGGCAGCTGTTGCTGCCGACAGCCCTCCTGTTGCTGGTCTCCGCTGGCATGAGAACCGAGG
ATCTGCCTAAGGCCGTGGTGTTCCTGGAACCTCAGTGGTACAGAGTGCTGGAAAAGGACAGCGT
GACCCTGAAGTGCCAGGGCGCCTATTCTCCCGAGGACAATAGCACCCAGTGGTTCCACAACGAG
AGCCTGATCAGCAGCCAGGCCAGCAGCTACTTTATCGATGCCGCCACCGTGGACGACAGCGGCG
AGTACAGATGCCAGACCAATCTGAGCACCCTGAGCGACCCTGTGCAGCTGGAAGTGCACATTGG
ATGGTTGCTGCTGCAAGCCCCTAGATGGGTGTTCAAAGAAGAGGACCCCATCCACCTGAGATGC
CACTCTTGGAAGAACACAGCCCTGCACAAAGTGACCTACCTGCAGAACGGCAAGGGCAGAAAGT
ACTTCCACCACAACAGCGACTTCTACATCCCCAAGGCCACACTGAAGGACTCCGGCTCCTACTT
CTGCAGAGGCCTGGTCGGCAGCAAGAACGTGTCCAGCGAGACAGTGAACATCACCATCACACAG
GGCCTCGCCGTGTCTACCATCAGCAGCTTTTTCCCACCTGGCTATCAGGTGTCCTTCTGCCTGG
TCATGGTGCTGCTGTTCGCCGTGGATACCGGCCTGTACTTCAGCGTCAAGACCAACATCCGGTC
CAGCACCAGAGACTGGAAGGACCACAAGTTCAAGTGGCGGAAGGACCCTCAGGACAAG

SEQ ID NO: 167 - exemplary CD47 knock-in cassette sequence

ATGTGGCCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTGCTGCGGATCAGCTCAGCTACTAT
TTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTCATTCCATGCTTTGT
TACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGGAAATTTAAAGGAAGAGAT

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ATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCCACTGACTTTAGTAGTGCAAAAA
TTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGAGTGATGCTGTCTC
ACACACAGGAAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAGGTGAAACGATCATCGAG
CTAAAATATCGTGTTGTTTCATGGTTTTCTCCAAATGAAAATATTCTTATTGTTATTTTCCCAA
TTTTTGCTATACTCCTGTTCTGGGGACAGTTTGGTATTAAAACACTTAAATATAGATCCGGTGG
TATGGATGAGAAAACAATTGCTTTACTTGTTGCTGGACTAGTGATCACTGTCATTGTCATTGTT
GGAGCCATTCTTTTCGTCCCAGGTGAATATTCATTAAAGAATGCTACTGGCCTTGGTTTAATTG
TGACTTCTACAGGGATATTAATATTACTTCACTACTATGTGTTTAGTACAGCGATTGGATTAAC
CTCCTTCGTCATTGCCATATTGGTTATTCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTG
AGTCTCTGTATTGCGGCGTGTATACCAATGCATGGCCCTCTTCTGATTTCAGGTTTGAGTATCT
TAGCTCTAGCACAATTACTTGGACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTAT
ACAACCTCCTAGGAAAGCTGTAGAGGAACCCCTTAATGCATTCAAAGAATCAAAAGGAATGATG
AATGATGAATGA

SEQ ID NO: 168 - exemplary IL15 knock-in cassette sequence

AATTGGGTCAACGTGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCAGAGCATGCACATCG
ACGCCACACTGTACACCGAGTCCGATGTGCACCCTAGCTGCAAAGTGACCGCCATGAAGTGCTT
TCTGCTGGAACTGCAAGTGATCAGCCTGGAAAGCGGCGACGCCAGCATCCACGATACCGTGGAA
AACCTGATCATCCTGGCCAACAACAGCCTGAGCAGCAACGGCAATGTGACCGAGAGCGGCTGCA
AAGAGTGCGAGGAACTGGAAGAGAAGAACATCAAAGAGTTCCTCCAGAGCTTCGTCCACATCGT
GCAGATGTTCATCAACACCAGC

SEQ ID NO: 169 - exemplary IgE-IL15 knock-in cassette sequence

ATGGATTGGACCTGGATCCTGTTTCTGGTGGCCGCTGCCACAAGAGTGCACAGCAATTGGGTCA
ACGTGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCAGAGCATGCACATCGACGCCACACT
GTACACCGAGTCCGATGTGCACCCTAGCTGCAAAGTGACCGCCATGAAGTGCTTTCTGCTGGAA
CTGCAAGTGATCAGCCTGGAAAGCGGCGACGCCAGCATCCACGATACCGTGGAAAACCTGATCA
TCCTGGCCAACAACAGCCTGAGCAGCAACGGCAATGTGACCGAGAGCGGCTGCAAAGAGTGCGA
GGAACTGGAAGAGAAGAACATCAAAGAGTTCCTCCAGAGCTTCGTCCACATCGTGCAGATGTTC
ATCAACACCAGC

SEQ ID NO: 170 - exemplary IgE-IL15 pro-peptide cargo sequence

ATGGACTGGACCTGGATTCTGTTCCTGGTCGCGGCTGCAACGCGAGTCCATAGCGGTATCCATG
TTTTTATTCTTGGGTGTTTTTCTGCTGGGCTGCCTAAGACCGAGGCCAACTGGGTAAATGTCAT
CAGTGACCTCAAGAAAATAGAAGACCTTATACAAAGCATGCACATTGATGCTACTCTCTACACT
GAGTCAGATGTACATCCCTCATGCAAAGTGACGGCCATGAAATGTTTCCTCCTCGAACTTCAAG
TCATATCTCTGGAAAGTGGCGACGCGTCCATCCACGACACGGTCGAAAACCTGATAATACTCGC
TAATAATAGTCTCTCTTCAAATGGTAACGTAACCGAGTCAGGTTGCAAAGAGTGCGAAGAGTTG
GAAGAAAAAAACATAAAGGAGTTCCTGCAAAGTTTCGTGCACATTGTGCAGATGTTCATTAATA
CCTCT

SEQ ID NO: 171 - exemplary IL15Rα cargo sequence

ATCACCTGTCCTCCACCTATGAGCGTGGAACACGCCGACATCTGGGTCAAGAGCTACAGCCTGT
ACAGCAGAGAGCGGTACATCTGCAACAGCGGCTTCAAGAGAAAGGCCGGCACAAGCAGCCTGAC

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CGAGTGTGTGCTGAACAAGGCCACAAACGTGGCCCACTGGACCACACCTAGCCTGAAGTGCATC
AGAGATCCCGCTCTGGTTCATCAGAGGCCTGCCCCTCCATCTACAGTGACAACAGCTGGCGTGA
CCCCTCAGCCTGAGTCTCTGTCTCCATCTGGAAAAGAGCCTGCCGCCAGCTCTCCCAGCTCTAA
CAATACTGCTGCCACCACAGCCGCTATCGTGCCTGGATCTCAGCTGATGCCTAGCAAGAGCCCT
AGCACCGGCACAACAGAGATCAGCTCTCACGAGAGCAGCCACGGAACACCTTCTCAGACCACCG
CCAAGAATTGGGAGCTGACAGCCTCTGCCTCTCATCAGCCACCTGGCGTGTACCCACAGGGCCA
CTCTGATACAACAGTGGCCATCAGCACCAGCACCGTTCTGCTGTGTGGCCTGTCTGCTGTTAGC
CTGCTGGCCTGCTACCTGAAGTCTAGACAGACACCTCCTCTGGCCAGCGTGGAAATGGAAGCCA
TGGAAGCTCTGCCTGTCACATGGGGCACCAGCAGCAGAGATGAGGACCTCGAGAATTGCAGCCA
CCACCTG

SEQ ID NO: 172 - exemplary mbIL-15 cargo sequence

ATGGATTGGACCTGGATCCTGTTTCTGGTGGCCGCTGCCACAAGAGTGCACAGCAATTGGGTCA
ACGTGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCAGAGCATGCACATCGACGCCACACT
GTACACCGAGTCCGATGTGCACCCTAGCTGCAAAGTGACCGCCATGAAGTGCTTTCTGCTGGAA
CTGCAAGTGATCAGCCTGGAAAGCGGCGACGCCAGCATCCACGATACCGTGGAAAACCTGATCA
TCCTGGCCAACAACAGCCTGAGCAGCAACGGCAATGTGACCGAGAGCGGCTGCAAAGAGTGCGA
GGAACTGGAAGAGAAGAACATCAAAGAGTTCCTCCAGAGCTTCGTCCACATCGTGCAGATGTTC
ATCAACACCAGCTCTGGCGGAGGAAGCGGAGGCGGAGGATCTGGTGGTGGTGGATCTGGCGGCG
GTGGTAGTGGCGGAGGTTCTCTGCAAATCACCTGTCCTCCACCTATGAGCGTGGAACACGCCGA
CATCTGGGTCAAGAGCTACAGCCTGTACAGCAGAGAGCGGTACATCTGCAACAGCGGCTTCAAG
AGAAAGGCCGGCACAAGCAGCCTGACCGAGTGTGTGCTGAACAAGGCCACAAACGTGGCCCACT
GGACCACACCTAGCCTGAAGTGCATCAGAGATCCCGCTCTGGTTCATCAGAGGCCTGCCCCTCC
ATCTACAGTGACAACAGCTGGCGTGACCCCTCAGCCTGAGTCTCTGTCTCCATCTGGAAAAGAG
CCTGCCGCCAGCTCTCCCAGCTCTAACAATACTGCTGCCACCACAGCCGCTATCGTGCCTGGAT
CTCAGCTGATGCCTAGCAAGAGCCCTAGCACCGGCACAACAGAGATCAGCTCTCACGAGAGCAG
CCACGGAACACCTTCTCAGACCACCGCCAAGAATTGGGAGCTGACAGCCTCTGCCTCTCATCAG
CCACCTGGCGTGTACCCACAGGGCCACTCTGATACAACAGTGGCCATCAGCACCAGCACCGTTC
TGCTGTGTGGCCTGTCTGCTGTTAGCCTGCTGGCCTGCTACCTGAAGTCTAGACAGACACCTCC
TCTGGCCAGCGTGGAAATGGAAGCCATGGAAGCTCTGCCTGTCACATGGGGCACCAGCAGCAGA
GATGAGGACCTCGAGAATTGCAGCCACCACCTG

SEQ ID NO: 173 - exemplary mbIL-15 cargo sequence

ATGGACTGGACCTGGATTCTGTTCCTGGTCGCGGCTGCAACGCGAGTCCATAGCGGTATCCATG
TTTTTATTCTTGGGTGTTTTTCTGCTGGGCTGCCTAAGACCGAGGCCAACTGGGTAAATGTCAT
CAGTGACCTCAAGAAAATAGAAGACCTTATACAAAGCATGCACATTGATGCTACTCTCTACACT
GAGTCAGATGTACATCCCTCATGCAAAGTGACGGCCATGAAATGTTTCCTCCTCGAACTTCAAG
TCATATCTCTGGAAAGTGGCGACGCGTCCATCCACGACACGGTCGAAAACCTGATAATACTCGC
TAATAATAGTCTCTCTTCAAATGGTAACGTAACCGAGTCAGGTTGCAAAGAGTGCGAAGAGTTG
GAAGAAAAAAACATAAAGGAGTTCCTGCAAAGTTTCGTGCACATTGTGCAGATGTTCATTAATA
CCTCTAGCGGCGGAGGATCAGGTGGCGGTGGAAGCGGAGGTGGAGGCTCCGGTGGAGGAGGTAG
TGGCGGAGGTTCTCTTCAAATAACTTGTCCTCCACCGATGTCCGTAGAACATGCGGATATTTGG
GTAAAATCCTATAGCTTGTACAGCCGAGAGCGGTATATCTGCAACAGCGGCTTCAAGCGGAAGG
CCGGCACAAGCAGCCTGACCGAGTGCGTGCTGAACAAGGCCACCAACGTGGCCCACTGGACCAC
CCCTAGCCTGAAGTGCATCAGAGATCCCGCCCTGGTGCATCAGCGGCCTGCCCCTCCAAGCACA

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GTGACAACAGCTGGCGTGACCCCCCAGCCTGAGAGCCTGAGCCCTTCTGGAAAAGAGCCTGCCG
CCAGCAGCCCCAGCAGCAACAATACTGCCGCCACCACAGCCGCCATCGTGCCTGGATCTCAGCT
GATGCCCAGCAAGAGCCCTAGCACCGGCACCACCGAGATCAGCAGCCACGAGTCTAGCCACGGC
ACCCCATCTCAGACCACCGCCAAGAACTGGGAGCTGACAGCCAGCGCCTCTCACCAGCCTCCAG
GCGTGTACCCTCAGGGCCACAGCGATACCACAGTGGCCATCAGCACCTCCACCGTGCTGCTGTG
TGGACTGAGCGCCGTGTCACTGCTGGCCTGCTACCTGAAGTCCAGACAGACCCCTCCACTGGCC
AGCGTGGAAATGGAAGCCATGGAAGCACTGCCCGTGACCTGGGGCACCAGCTCCAGAGATGAGG
ATCTGGAAAACTGCTCCCACCACCTG

SEQ ID NO: 174 - exemplary multicistronic CD16, mbIL-15 cargo sequence

ATGTGGCAGCTGTTGCTGCCGACAGCCCTCCTGTTGCTGGTCTCCGCTGGCATGAGAACCGAGG
ATCTGCCTAAGGCCGTGGTGTTCCTGGAACCTCAGTGGTACAGAGTGCTGGAAAAGGACAGCGT
GACCCTGAAGTGCCAGGGCGCCTATTCTCCCGAGGACAATAGCACCCAGTGGTTCCACAACGAG
AGCCTGATCAGCAGCCAGGCCAGCAGCTACTTTATCGATGCCGCCACCGTGGACGACAGCGGCG
AGTACAGATGCCAGACCAATCTGAGCACCCTGAGCGACCCTGTGCAGCTGGAAGTGCACATTGG
ATGGTTGCTGCTGCAAGCCCCTAGATGGGTGTTCAAAGAAGAGGACCCCATCCACCTGAGATGC
CACTCTTGGAAGAACACAGCCCTGCACAAAGTGACCTACCTGCAGAACGGCAAGGGCAGAAAGT
ACTTCCACCACAACAGCGACTTCTACATCCCCAAGGCCACACTGAAGGACTCCGGCTCCTACTT
CTGCAGAGGCCTGGTCGGCAGCAAGAACGTGTCCAGCGAGACAGTGAACATCACCATCACACAG
GGCCTCGCCGTGTCTACCATCAGCAGCTTTTTCCCACCTGGCTATCAGGTGTCCTTCTGCCTGG
TCATGGTGCTGCTGTTCGCCGTGGATACCGGCCTGTACTTCAGCGTCAAGACCAACATCCGGTC
CAGCACCAGAGACTGGAAGGACCACAAGTTCAAGTGGCGGAAGGACCCTCAGGACAAGGGAAGC
GGAGCCACAAACTTCTCTCTGCTGAAGCAGGCAGGAGATGTTGAAGAAAACCCTGGACCTATGG
ATTGGACCTGGATCCTGTTTCTGGTGGCCGCTGCCACAAGAGTGCACAGCAATTGGGTCAACGT
GATCAGCGACCTGAAGAAGATCGAGGACCTGATCCAGAGCATGCACATCGACGCCACACTGTAC
ACCGAGTCCGATGTGCACCCTAGCTGCAAAGTGACCGCCATGAAGTGCTTTCTGCTGGAACTGC
AAGTGATCAGCCTGGAAAGCGGCGACGCCAGCATCCACGATACCGTGGAAAACCTGATCATCCT
GGCCAACAACAGCCTGAGCAGCAACGGCAATGTGACCGAGAGCGGCTGCAAAGAGTGCGAGGAA
CTGGAAGAGAAGAACATCAAAGAGTTCCTCCAGAGCTTCGTCCACATCGTGCAGATGTTCATCA
ACACCAGCTCTGGCGGAGGAAGCGGAGGCGGAGGATCTGGTGGTGGTGGATCTGGCGGCGGTGG
TAGTGGCGGAGGTTCTCTGCAAATCACCTGTCCTCCACCTATGAGCGTGGAACACGCCGACATC
TGGGTCAAGAGCTACAGCCTGTACAGCAGAGAGCGGTACATCTGCAACAGCGGCTTCAAGAGAA
AGGCCGGCACAAGCAGCCTGACCGAGTGTGTGCTGAACAAGGCCACAAACGTGGCCCACTGGAC
CACACCTAGCCTGAAGTGCATCAGAGATCCCGCTCTGGTTCATCAGAGGCCTGCCCCTCCATCT
ACAGTGACAACAGCTGGCGTGACCCCTCAGCCTGAGTCTCTGTCTCCATCTGGAAAAGAGCCTG
CCGCCAGCTCTCCCAGCTCTAACAATACTGCTGCCACCACAGCCGCTATCGTGCCTGGATCTCA
GCTGATGCCTAGCAAGAGCCCTAGCACCGGCACAACAGAGATCAGCTCTCACGAGAGCAGCCAC
GGAACACCTTCTCAGACCACCGCCAAGAATTGGGAGCTGACAGCCTCTGCCTCTCATCAGCCAC
CTGGCGTGTACCCACAGGGCCACTCTGATACAACAGTGGCCATCAGCACCAGCACCGTTCTGCT
GTGTGGCCTGTCTGCTGTTAGCCTGCTGGCCTGCTACCTGAAGTCTAGACAGACACCTCCTCTG
GCCAGCGTGGAAATGGAAGCCATGGAAGCTCTGCCTGTCACATGGGGCACCAGCAGCAGAGATG
AGGACCTCGAGAATTGCAGCCACCACCTG

SEQ ID NO: 175 - exemplary CD19 CAR cargo sequence

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ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATTCCTCCTGATCC
CAGACATCCAGATGACACAGACTACATCCTCCCTGTCTGCCTCTCTGGGAGACAGAGTCACCAT
CAGTTGCAGGGCAAGTCAGGACATTAGTAAATATTTAAATTGGTATCAGCAGAAACCAGATGGA
ACTGTTAAACTCCTGATCTACCATACATCAAGATTACACTCAGGAGTCCCATCAAGGTTCAGTG
GCAGTGGGTCTGGAACAGATTATTCTCTCACCATTAGCAACCTGGAGCAAGAAGATATTGCCAC
TTACTTTTGCCAACAGGGTAATACGCTTCCGTACACGTTCGGAGGGGGGACTAAGTTGGAAATA
ACAGGCTCCACCTCTGGATCCGGCAAGCCCGGATCTGGCGAGGGATCCACCAAGGGCGAGGTGA
AACTGCAGGAGTCAGGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCGTCACATGCACTGT
CTCAGGGGTCTCATTACCCGACTATGGTGTAAGCTGGATTCGCCAGCCTCCACGAAAGGGTCTG
GAGTGGCTGGGAGTAATATGGGGTAGTGAAACCACATACTATAATTCAGCTCTCAAATCCAGAC
TGACCATCATCAAGGACAACTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCAAACTGA
TGACACAGCCATTTACTACTGTGCCAAACATTATTACTACGGTGGTAGCTATGCTATGGACTAC
TGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAGCGGCCGCAATTGAAGTTATGTATCCTCCTC
CTTACCTAGACAATGAGAAGAGCAATGGAACCATTATCCATGTGAAAGGGAAACACCTTTGTCC
AAGTCCCCTATTTCCCGGACCTTCTAAGCCCTTTTGGGTGCTGGTGGTGGTTGGGGGAGTCCTG
GCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCA
GGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCCGGGCCCACCCGCAAGCATTA
CCAGCCCTATGCCCCACCACGCGACTTCGCAGCCTATCGCTCCAGAGTGAAGTTCAGCAGGAGC
GCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAA
GAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAG
AAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTAC
AGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTC
TCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGCTAA

SEQ ID NO: 176 - exemplary EGFR CAR cargo sequence

ATGGCACTCCCCGTCACCGCCCTTCTCTTGCCCCTCGCCCTGCTGCTGCATGCTGCCAGGCCCA
TGGACGAAGTGCAGCTCGTGGAGTCCGGTGGAGGACTCGTCCAACCGGGCGGATCCCTTCGCTT
GTCCTGCGCCGCATCAGGCTTCAGCTTCACCAACTATGGCGTCCACTGGGTCAGACAGGCCCCC
GGAAAGGGACTGGAATGGGTGTCCGTGATCTGGAGCGGCGGGAACACCGACTACAACACCTCCG
TGAAGGGCCGGTTCACTATTAGCCGCGACAACTCCAAGAACACTCTGTACCTCCAAATGAACTC
CCTGAGGGCCGAAGATACTGCTGTGTACTATTGCGCGAGAGCCCTGACCTACTACGACTACGAG
TTCGCGTACTGGGGCCAGGGGACTCTCGTGACCGTGTCCAGCGGTGGTGGAGGTTCCGGAGGCG
GAGGTTCTGGTGGCGGGGGATCAGAAATCGTGCTGACTCAGTCCCCTGCGACCTTGTCCCTGAG
CCCTGGAGAACGGGCCACCCTGAGCTGTAGAGCCAGCCAGAGCATCGGGACAAATATTCACTGG
TACCAGCAGAAACCCGGACAAGCACCACGGCTGCTGATCTACTACGCCTCCGAGTCGATTTCCG
GAATCCCGGCTCGCTTTTCGGGGTCTGGATCGGGAACGGACTTCACTCTGACCATCTCGTCGCT
GGAACCCGAGGATTTCGCCGTGTACTACTGCCAACAGAACAACAATTGGCCGACCACGTTCGGC
CAGGGCACCAAGCTCGAGATTAAGGGATCACTGGAAGCGGCCGCAACCACAACACCTGCTCCAA
GGCCCCCCACACCCGCTCCAACTATAGCCAGCCAACCATTGAGCCTCAGACCTGAAGCTTGCAG
GCCCGCAGCAGGAGGCGCCGTCCATACGCGAGGCCTGGACTTCGCGTGTGATATTTATATTTGG
GCCCCTTTGGCCGGAACATGTGGGGTGTTGCTTCTCTCCCTTGTGATCACTCTGTATTGTAAGC
GCGGGAGAAAGAAGCTCCTGTACATCTTCAAGCAGCCTTTTATGCGACCTGTGCAAACCACTCA
GGAAGAAGATGGGTGTTCATGCCGCTTCCCCGAGGAGGAAGAAGGAGGGTGTGAACTGAGGGTG
AAATTTTCTAGAAGCGCCGATGCTCCCGCATATCAGCAGGGTCAGAATCAGCTCTACAATGAAT
TGAATCTCGGCAGGCGAGAAGAGTACGATGTTCTGGACAAGAGACGGGGCAGGGATCCCGAGAT
GGGGGGAAAGCCCCGGAGAAAAAATCCTCAGGAGGGGTTGTACAATGAGCTGCAGAAGGACAAG

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ATGGCTGAAGCCTATAGCGAGATCGGAATGAAAGGCGAAAGACGCAGAGGCAAGGGGCATGACG
GTCTGTACCAGGGTCTCTCTACAGCCACCAAGGACACTTATGATGCGTTGCATATGCAAGCCTT
GCCACCCCGCTAA

SEQ ID NO: 177 - exemplary GFP cargo sequence

ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCG
ACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCT
GACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACC
CTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCA
AGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTA
CAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGC
ATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACA
ACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAA
CATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGC
CCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACG
AGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGA
CGAGCTGTACAAGTGA

SEQ ID NO: 178 - exemplary CXCR1 cargo sequence

ATGTCAAATATTACAGATCCACAGATGTGGGATTTTGATGATCTAAATTTCACTGGCATGCCAC
CTGCAGATGAAGATTACAGCCCCTGTATGCTAGAAACTGAGACACTCAACAAGTATGTTGTGAT
CATCGCCTATGCCCTAGTGTTCCTGCTGAGCCTGCTGGGAAACTCCCTGGTGATGCTGGTCATC
TTATACAGCAGGGTCGGCCGCTCCGTCACTGATGTCTACCTGCTGAACCTGGCCTTGGCCGACC
TACTCTTTGCCCTGACCTTGCCCATCTGGGCCGCCTCCAAGGTGAATGGCTGGATTTTTGGCAC
ATTCCTGTGCAAGGTGGTCTCACTCCTGAAGGAAGTCAACTTCTACAGTGGCATCCTGCTGTTG
GCCTGCATCAGTGTGGACCGTTACCTGGCCATTGTCCATGCCACACGCACACTGACCCAGAAGC
GTCACTTGGTCAAGTTTGTTTGTCTTGGCTGCTGGGGACTGTCTATGAATCTGTCCCTGCCCTT
CTTCCTTTTCCGCCAGGCTTACCATCCAAACAATTCCAGTCCAGTTTGCTATGAGGTCCTGGGA
AATGACACAGCAAAATGGCGGATGGTGTTGCGGATCCTGCCTCACACCTTTGGCTTCATCGTGC
CGCTGTTTGTCATGCTGTTCTGCTATGGATTCACCCTGCGTACACTGTTTAAGGCCCACATGGG
GCAGAAGCACCGAGCCATGAGGGTCATCTTTGCTGTCGTCCTCATCTTCCTGCTTTGCTGGCTG
CCCTACAACCTGGTCCTGCTGGCAGACACCCTCATGAGGACCCAGGTGATCCAGGAGAGCTGTG
AGCGCCGCAACAACATCGGCCGGGCCCTGGATGCCACTGAGATTCTGGGATTTCTCCATAGCTG
CCTCAACCCCATCATCTACGCCTTCATCGGCCAAAATTTTCGCCATGGATTCCTCAAGATCCTG
GCTATGCATGGCCTGGTCAGCAAGGAGTTCTTGGCACGTCATCGTGTTACCTCCTACACTTCTT
CGTCTGTCAATGTCTCTTCCAACCTCTGA

SEQ ID NO: 179 - exemplary CXCR3B cargo sequence

ATGGAGTTGAGGAAGTACGGCCCTGGAAGACTGGCGGGGACAGTTATAGGAGGAGCTGCTCAGA
GTAAATCACAGACTAAATCAGACTCAATCACAAAAGAGTTCCTGCCAGGCCTTTACACAGCCCC
TTCCTCCCCGTTCCCGCCCTCACAGGTGAGTGACCACCAAGTGCTAAATGACGCCGAGGTTGCC
GCCCTCCTGGAGAACTTCAGCTCTTCCTATGACTATGGAGAAAACGAGAGTGACTCGTGCTGTA
CCTCCCCGCCCTGCCCACAGGACTTCAGCCTGAACTTCGACCGGGCCTTCCTGCCAGCCCTCTA
CAGCCTCCTCTTTCTGCTGGGGCTGCTGGGCAACGGCGCGGTGGCAGCCGTGCTGCTGAGCCGG
CGGACAGCCCTGAGCAGCACCGACACCTTCCTGCTCCACCTAGCTGTAGCAGACACGCTGCTGG

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TGCTGACACTGCCGCTCTGGGCAGTGGACGCTGCCGTCCAGTGGGTCTTTGGCTCTGGCCTCTG
CAAAGTGGCAGGTGCCCTCTTCAACATCAACTTCTACGCAGGAGCCCTCCTGCTGGCCTGCATC
AGCTTTGACCGCTACCTGAACATAGTTCATGCCACCCAGCTCTACCGCCGGGGGCCCCCGGCCC
GCGTGACCCTCACCTGCCTGGCTGTCTGGGGGCTCTGCCTGCTTTTCGCCCTCCCAGACTTCAT
CTTCCTGTCGGCCCACCACGACGAGCGCCTCAACGCCACCCACTGCCAATACAACTTCCCACAG
GTGGGCCGCACGGCTCTGCGGGTGCTGCAGCTGGTGGCTGGCTTTCTGCTGCCCCTGCTGGTCA
TGGCCTACTGCTATGCCCACATCCTGGCCGTGCTGCTGGTTTCCAGGGGCCAGCGGCGCCTGCG
GGCCATGCGGCTGGTGGTGGTGGTCGTGGTGGCCTTTGCCCTCTGCTGGACCCCCTATCACCTG
GTGGTGCTGGTGGACATCCTCATGGACCTGGGCGCTTTGGCCCGCAACTGTGGCCGAGAAAGCA
GGGTAGACGTGGCCAAGTCGGTCACCTCAGGCCTGGGCTACATGCACTGCTGCCTCAACCCGCT
GCTCTATGCCTTTGTAGGGGTCAAGTTCCGGGAGCGGATGTGGATGCTGCTCTTGCGCCTGGGC
TGCCCCAACCAGAGAGGGCTCCAGAGGCAGCCATCGTCTTCCCGCCGGGATTCATCCTGGTCTG
AGACCTCAGAGGCCTCCTACTCGGGCTTGTGA

SEQ ID NO: 180 - exemplary CXCR3A cargo sequence

ATGGTCCTTGAGGTGAGTGACCACCAAGTGCTAAATGACGCCGAGGTTGCCGCCCTCCTGGAGA
ACTTCAGCTCTTCCTATGACTATGGAGAAAACGAGAGTGACTCGTGCTGTACCTCCCCGCCCTG
CCCACAGGACTTCAGCCTGAACTTCGACCGGGCCTTCCTGCCAGCCCTCTACAGCCTCCTCTTT
CTGCTGGGGCTGCTGGGCAACGGCGCGGTGGCAGCCGTGCTGCTGAGCCGGCGGACAGCCCTGA
GCAGCACCGACACCTTCCTGCTCCACCTAGCTGTAGCAGACACGCTGCTGGTGCTGACACTGCC
GCTCTGGGCAGTGGACGCTGCCGTCCAGTGGGTCTTTGGCTCTGGCCTCTGCAAAGTGGCAGGT
GCCCTCTTCAACATCAACTTCTACGCAGGAGCCCTCCTGCTGGCCTGCATCAGCTTTGACCGCT
ACCTGAACATAGTTCATGCCACCCAGCTCTACCGCCGGGGGCCCCCGGCCCGCGTGACCCTCAC
CTGCCTGGCTGTCTGGGGGCTCTGCCTGCTTTTCGCCCTCCCAGACTTCATCTTCCTGTCGGCC
CACCACGACGAGCGCCTCAACGCCACCCACTGCCAATACAACTTCCCACAGGTGGGCCGCACGG
CTCTGCGGGTGCTGCAGCTGGTGGCTGGCTTTCTGCTGCCCCTGCTGGTCATGGCCTACTGCTA
TGCCCACATCCTGGCCGTGCTGCTGGTTTCCAGGGGCCAGCGGCGCCTGCGGGCCATGCGGCTG
GTGGTGGTGGTCGTGGTGGCCTTTGCCCTCTGCTGGACCCCCTATCACCTGGTGGTGCTGGTGG
ACATCCTCATGGACCTGGGCGCTTTGGCCCGCAACTGTGGCCGAGAAAGCAGGGTAGACGTGGC
CAAGTCGGTCACCTCAGGCCTGGGCTACATGCACTGCTGCCTCAACCCGCTGCTCTATGCCTTT
GTAGGGGTCAAGTTCCGGGAGCGGATGTGGATGCTGCTCTTGCGCCTGGGCTGCCCCAACCAGA
GAGGGCTCCAGAGGCAGCCATCGTCTTCCCGCCGGGATTCATCCTGGTCTGAGACCTCAGAGGC
CTCCTACTCGGGCTTGTGA

SEQ ID NO: 181 - exemplary CCR5 cargo sequence

ATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGAGCCCTGCCAAA
AAATCAATGTGAAGCAAATCGCAGCCCGCCTCCTGCCTCCGCTCTACTCACTGGTGTTCATCTT
TGGTTTTGTGGGCAACATGCTGGTCATCCTCATCCTGATAAACTGCAAAAGGCTGAAGAGCATG
ACTGACATCTACCTGCTCAACCTGGCCATCTCTGACCTGTTTTTCCTTCTTACTGTCCCCTTCT
GGGCTCACTATGCTGCCGCCCAGTGGGACTTTGGAAATACAATGTGTCAACTCTTGACAGGGCT
CTATTTTATAGGCTTCTTCTCTGGAATCTTCTTCATCATCCTCCTGACAATCGATAGGTACCTG
GCTGTCGTCCATGCTGTGTTTGCTTTAAAAGCCAGGACGGTCACCTTTGGGGTGGTGACAAGTG
TGATCACTTGGGTGGTGGCTGTGTTTGCGTCTCTCCCAGGAATCATCTTTACCAGATCTCAAAA
AGAAGGTCTTCATTACACCTGCAGCTCTCATTTTCCATACAGTCAGTATCAATTCTGGAAGAAT
TTCCAGACATTAAAGATAGTCATCTTGGGGCTGGTCCTGCCGCTGCTTGTCATGGTCATCTGCT

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ACTCGGGAATCCTAAAAACTCTGCTTCGGTGTCGAAATGAGAAGAAGAGGCACAGGGCTGTGAG
GCTTATCTTCACCATCATGATTGTTTATTTTCTCTTCTGGGCTCCCTACAACATTGTCCTTCTC
CTGAACACCTTCCAGGAATTCTTTGGCCTGAATAATTGCAGTAGCTCTAACAGGTTGGACCAAG
CTATGCAGGTGACAGAGACTCTTGGGATGACGCACTGCTGCATCAACCCCATCATCTATGCCTT
TGTCGGGGAGAAGTTCAGAAACTACCTCTTAGTCTTCTTCCAAAAGCACATTGCCAAACGCTTC
TGCAAATGCTGTTCTATTTTCCAGCAAGAGGCTCCCGAGCGAGCAAGCTCAGTTTACACCCGAT
CCACTGGGGAGCAGGAAATATCTGTGGGCTTGTGA

SEQ ID NO: 182 - exemplary CCR2 cargo sequence

ATGCTGTCCACATCTCGTTCTCGGTTTATCAGAAATACCAACGAGAGCGGTGAAGAAGTCACCA
CCTTTTTTGATTATGATTACGGTGCTCCCTGTCATAAATTTGACGTGAAGCAAATTGGGGCCCA
ACTCCTGCCTCCGCTCTACTCGCTGGTGTTCATCTTTGGTTTTGTGGGCAACATGCTGGTCGTC
CTCATCTTAATAAACTGCAAAAAGCTGAAGTGCTTGACTGACATTTACCTGCTCAACCTGGCCA
TCTCTGATCTGCTTTTTCTTATTACTCTCCCATTGTGGGCTCACTCTGCTGCAAATGAGTGGGT
CTTTGGGAATGCAATGTGCAAATTATTCACAGGGCTGTATCACATCGGTTATTTTGGCGGAATC
TTCTTCATCATCCTCCTGACAATCGATAGATACCTGGCTATTGTCCATGCTGTGTTTGCTTTAA
AAGCCAGGACGGTCACCTTTGGGGTGGTGACAAGTGTGATCACCTGGTTGGTGGCTGTGTTTGC
TTCTGTCCCAGGAATCATCTTTACTAAATGCCAGAAAGAAGATTCTGTTTATGTCTGTGGCCCT
TATTTTCCACGAGGATGGAATAATTTCCACACAATAATGAGGAACATTTTGGGGCTGGTCCTGC
CGCTGCTCATCATGGTCATCTGCTACTCGGGAATCCTGAAAACCCTGCTTCGGTGTCGAAACGA
GAAGAAGAGGCATAGGGCAGTGAGAGTCATCTTCACCATCATGATTGTTTACTTTCTCTTCTGG
ACTCCCTATAATATTGTCATTCTCCTGAACACCTTCCAGGAATTCTTCGGCCTGAGTAACTGTG
AAAGCACCAGTCAACTGGACCAAGCCACGCAGGTGACAGAGACTCTTGGGATGACTCACTGCTG
CATCAATCCCATCATCTATGCCTTCGTTGGGGAGAAGTTCAGAAGCCTTTTTCACATAGCTCTT
GGCTGTAGGATTGCCCCACTCCAAAAACCAGTGTGTGGAGGTCCAGGAGTGAGACCAGGAAAGA
ATGTGAAAGTGACTACACAAGGACTCCTCGATGGTCGTGGAAAAGGAAAGTCAATTGGCAGAGC
CCCTGAAGCCAGTCTTCAGGACAAAGAAGGAGCCTAG

[0459] In some embodiments, a gene product of interest comprises or consists of an amino acid sequence of any one of SEQ ID NOs: 161, 164, or 183-200. In some embodiments, a gene product of interest comprises or consists of an amino acid sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to any one of SEQ ID NOs: 161, 164, or 183-200. In some embodiments, a gene product of interest comprises or consists of a functional variant of any one of SEQ ID NOs: 161, 164, or 183-200. In some embodiments, a gene product of interest comprises or consists of an amino acid sequence having 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mutations (e.g., substitutions, insertions, and/or deletions) relative to any one of SEQ ID NOs: 161, 164, or 183-200.

SEQ ID NO: 183- exemplary linker amino acid sequence

SGGGSGGGGSGGGGSGGGGSGGGSLQ

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SEQ ID NO: 184 - exemplary CD16 amino acid sequence

MWQLLLPTALLLLVSAGMRTEDLPKAVVFLEPQWYRVLEKDSVTLKCQGAYSPEDNSTQWFHNE
SLISSQASSYFIDAATVDDSGEYRCQTNLSTLSDPVQLEVHIGWLLLQAPRWVFKEEDPIHLRC
HSWKNTALHKVTYLQNGKGRKYFHHNSDFYIPKATLKDSGSYFCRGLVGSKNVSSETVNITITQ
GLAVSTISSFFPPGYQVSFCLVMVLLFAVDTGLYFSVKTNIRSSTRDWKDHKFKWRKDPQDK

SEQ ID NO: 185 - exemplary CD47 amino acid sequence

MWPLVAALLLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEVYVKWKFKGRD
IYTFDGALNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVTELTREGETIIE
LKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVIV
GAILFVPGEYSLKNATGLGLIVTSTGILILLHYYVFSTAIGLTSFVIAILVIQVIAYILAVVGL
SLCIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMM
NDE

SEQ ID NO: 186 - exemplary IL15 amino acid sequence

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVE
NLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS

SEQ ID NO: 187 - exemplary IgE-IL15 amino acid sequence

MDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLE
LQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMF
INTS

SEQ ID NO: 188 - exemplary IgE-IL15 pro-peptide amino acid sequence

MDWTWILFLVAAATRVHSGIHVFILGCFSAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYT
ESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEEL
EEKNIKEFLQSFVHIVQMFINTS

SEQ ID NO: 189 - exemplary IL15Rα amino acid sequence

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCI
RDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTAATTAAIVPGSQLMPSKSP
STGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTTVAISTSTVLLCGLSAVS
LLACYLKSRQTPPLASVEMEAMEALPVTWGTSSRDEDLENCSHHL

SEQ ID NO: 190 - exemplary mbIL-15 amino acid sequence

MDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLE
LQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMF
INTSSGGGSGGGGSGGGGSGGGGSGGGSLQITCPPPMSVEHADIWVKSYSLYSRERYICNSGFK
RKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKE
PAASSPSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQ
PPGVYPQGHSDTTVAISTSTVLLCGLSAVSLLACYLKSRQTPPLASVEMEAMEALPVTWGTSSR
DEDLENCSHHL

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SEQ ID NO: 191 - exemplary mbIL-15 amino acid sequence

MDWTWILFLVAAATRVHSGIHVFILGCFSAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYT
ESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEEL
EEKNIKEFLQSFVHIVQMFINTSSGGGSGGGGSGGGGSGGGGSGGGSLQITCPPPMSVEHADIW
VKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPST
VTTAGVTPQPESLSPSGKEPAASSPSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHG
TPSQTTAKNWELTASASHQPPGVYPQGHSDTTVAISTSTVLLCGLSAVSLLACYLKSRQTPPLA
SVEMEAMEALPVTWGTSSRDEDLENCSHHL

SEQ ID NO: 192 - exemplary multicistronic CD16, mbIL-15 amino acid sequence

MWQLLLPTALLLLVSAGMRTEDLPKAVVFLEPQWYRVLEKDSVTLKCQGAYSPEDNSTQWFHNE
SLISSQASSYFIDAATVDDSGEYRCQTNLSTLSDPVQLEVHIGWLLLQAPRWVFKEEDPIHLRC
HSWKNTALHKVTYLQNGKGRKYFHHNSDFYIPKATLKDSGSYFCRGLVGSKNVSSETVNITITQ
GLAVSTISSFFPPGYQVSFCLVMVLLFAVDTGLYFSVKTNIRSSTRDWKDHKFKWRKDPQDKGS
GATNFSLLKQAGDVEENPGPMDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLY
TESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEE
LEEKNIKEFLQSFVHIVQMFINTSSGGGSGGGGSGGGGSGGGGSGGGSLQITCPPPMSVEHADI
WVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPS
TVTTAGVTPQPESLSPSGKEPAASSPSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSH
GTPSQTTAKNWELTASASHQPPGVYPQGHSDTTVAISTSTVLLCGLSAVSLLACYLKSRQTPPL
ASVEMEAMEALPVTWGTSSRDEDLENCSHHL

SEQ ID NO: 193 - exemplary CD19 CAR amino acid sequence

MLLLVTSLLLCELPHPAFLLIPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDG
TVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEI
TGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGL
EWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDY
WGQGTSVTVSSAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVL
ACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRS
ADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAY
SEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 194 - exemplary EGFR CAR amino acid sequence

MALPVTALLLPLALLLHAARPMDEVQLVESGGGLVQPGGSLRLSCAASGFSFTNYGVHWVRQAP
CKGLEWVSVIWSGGNTDYNTSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARALTYYDYE
FAYWGQGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASQSIGTNIHW
YQQKPGQAPRLLIYYASESISGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQNNNWPTTFG
QGTKLEIKGSLEAAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIW
APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRV
KFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK
MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 195 - exemplary GFP amino acid sequence

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MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTT
LTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKG
IDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDG
PVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK

SEQ ID NO: 196 - exemplary CXCR1 amino acid sequence

MSNITDPQMWDFDDLNFTGMPPADEDYSPCMLETETLNKYVVIIAYALVFLLSLLGNSLVMLVI
LYSRVGRSVTDVYLLNLALADLLFALTLPIWAASKVNGWIFGTFLCKVVSLLKEVNFYSGILLL
ACISVDRYLAIVHATRTLTQKRHLVKFVCLGCWGLSMNLSLPFFLFRQAYHPNNSSPVCYEVLG
NDTAKWRMVLRILPHTFGFIVPLFVMLFCYGFTLRTLFKAHMGQKHRAMRVIFAVVLIFLLCWL
PYNLVLLADTLMRTQVIQESCERRNNIGRALDATEILGFLHSCLNPIIYAFIGQNFRHGFLKIL
AMHGLVSKEFLARHRVTSYTSSSVNVSSNL

SEQ ID NO: 197 - exemplary CXCR3B amino acid sequence

MELRKYGPGRLAGTVIGGAAQSKSQTKSDSITKEFLPGLYTAPSSPFPPSQVSDHQVLNDAEVA
ALLENFSSSYDYGENESDSCCTSPPCPQDFSLNFDRAFLPALYSLLFLLGLLGNGAVAAVLLSR
RTALSSTDTFLLHLAVADTLLVLTLPLWAVDAAVQWVFGSGLCKVAGALFNINFYAGALLLACI
SFDRYLNIVHATQLYRRGPPARVTLTCLAVWGLCLLFALPDFIFLSAHHDERLNATHCQYNFPQ
VGRTALRVLQLVAGFLLPLLVMAYCYAHILAVLLVSRGQRRLRAMRLVVVVVVAFALCWTPYHL
VVLVDILMDLGALARNCGRESRVDVAKSVTSGLGYMHCCLNPLLYAFVGVKFRERMWMLLLRLG
CPNQRGLQRQPSSSRRDSSWSETSEASYSGL

SEQ ID NO: 198 - exemplary CXCR3A amino acid sequence

MVLEVSDHQVLNDAEVAALLENFSSSYDYGENESDSCCTSPPCPQDFSLNFDRAFLPALYSLLF
LLGLLGNGAVAAVLLSRRTALSSTDTFLLHLAVADTLLVLTLPLWAVDAAVQWVFGSGLCKVAG
ALFNINFYAGALLLACISFDRYLNIVHATQLYRRCPPARVTLTCLAVWGLCLLFALPDFIFLSA
HHDERLNATHCQYNFPQVGRTALRVLQLVAGFLLPLLVMAYCYAHILAVLLVSRGQRRLRAMRL
VVVVVVAFALCWTPYHLVVLVDILMDLGALARNCGRESRVDVAKSVTSGLGYMHCCLNPLLYAF
VGVKFRERMWMLLLRLGCPNQRGLQRQPSSSRRDSSWSETSEASYSGL

SEQ ID NO: 199 - exemplary CCR5 amino acid sequence

MDYQVSSPIYDINYYTSEPCQKINVKQIAARLLPPLYSLVFIFGFVGNMLVILILINCKRLKSM
TDIYLLNLAISDLFFLLTVPFWAHYAAAQWDFGNTMCQLLTGLYFIGFFSGIFFIILLTIDRYL
AVVHAVFALKARTVTFGVVTSVITWVVAVFASLPGIIFTRSQKEGLHYTCSSHFPYSQYQFWKN
FQTLKIVILGLVLPLLVMVICYSGILKTLLRCRNEKKRHRAVRLIFTIMIVYFLFWAPYNIVLL
LNTFQEFFGLNNCSSSNRLDQAMQVTETLGMTHCCINPIIYAFVGEKFRNYLLVFFQKHIAKRF
CKCCSIFQQEAPERASSVYTRSTGEQEISVGL

SEQ ID NO: 200 - exemplary CCR2 cargo sequence

MLSTSRSRFIRNTNESGEEVTTFFDYDYGAPCHKFDVKQIGAQLLPPLYSLVFIFGFVGNMLVV
LILINCKKLKCLTDIYLLNLAISDLLFLITLPLWAHSAANEWVFGNAMCKLFTGLYHIGYFGGI
FFIILLTIDRYLAIVHAVFALKARTVTFGVVTSVITWLVAVFASVPGIIFTKCQKEDSVYVCGP
YFPRGWNNFHTIMRNILGLVLPLLIMVICYSGILKTLLRCRNEKKRHRAVRVIFTIMIVYFLFW

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TPYNIVILLNTFQEFFGLSNCESTSQLDQATQVTETLGMTHCCINPIIYAFVGEKFRSLFHIAL
GCRIAPLQKPVCGGPGVRPGKNVKVTTQGLLDGRGKGKSIGRAPEASLQDKEGA

AAV Capsids

[0460] In some embodiments, the present disclosure provides one or more polynucleotide constructs (e.g., knock-in cassettes) packaged into an AAV capsid. In some embodiments, an AAV capsid is from or derived from an AAV capsid of an AAV2, 3, 4, 5, 6, 7, 8, 9, or 10 serotype, or one or more hybrids thereof. In some embodiments, an AAV capsid is from an AAV ancestral serotype. In some embodiments, an AAV capsid is an ancestral (Anc) AAV capsid. An Anc capsid is created from a construct sequence that is constructed using evolutionary probabilities and evolutionary modeling to determine a probable ancestral sequence. In some embodiments, an AAV capsid has been modified in a manner known in the art (see e.g., Büning and Srivastava, Capsid modifications for targeting and improving the efficacy of AAV vectors, *Mol Ther Methods Clin Dev.* 2019)

[0461] In some embodiments, as provided herein, any combination of AAV capsids and AAV constructs (e.g., comprising AAV ITRs) may be used in recombinant AAV (rAAV) particles of the present disclosure. In some embodiments, an AAV ITR is from or derived from an AAV ITR of AAV2, 3, 4, 5, 6, 7, 8, 9, or 10. For example, wild-type or variant AA6 ITRs and AAV6 capsid, wild-type or variant AAV2 ITRs and AAV6 capsid, etc. In some embodiments of the present disclosure, an AAV particle is wholly comprised of AAV6 components (e.g., capsid and ITRs are AAV6 serotype). In some embodiments, an AAV particle is an AAV6/2, AAV6/8 or AAV6/9 particle (e.g., an AAV2, AAV8 or AAV9 capsid with an AAV construct having AAV6 ITRs).

Exemplary AAV Constructs

[0462] In some embodiments, a donor template is included within an AAV construct. In some embodiments, an AAV construct sequence comprises or consists of the sequence of any one of SEQ ID NO: 201-204. In some embodiments, an exemplary AAV construct is represented by SEQ ID NO:201. In some embodiments, an exemplary AAV construct is represented by SEQ ID NO: 202. In some embodiments, an exemplary AAV construct is represented by SEQ ID NO: 203. In some embodiments, an exemplary AAV construct is represented by SEQ ID NO: 204. In

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some embodiments, an exemplary AAV construct is at least 80%, 85%, 90%, 95%, 98%, or 99% identical to a sequence represented by SEQ ID NO: 201-204.

SEQ ID NO: 201 - exemplary AAV construct for donor template insertion at *GAPDH* locus

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGC
GACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATC
ACTAGGGGTTCCTGTCGACGAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCG
CGGGGCTCTCCAGAACATCATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATC
CCTGAGCTGAACGGGAAGCTCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGG
TGGACCTGACCTGCCGTCTAGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCA
GGCGTCGGAGGGCCCCCTCAAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGAC
TTCAACAGCGACACCCACTCCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACT
TTGTCAAGCTCATTTCCTGGTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTC
TGGCGCCCTCTGGTGGCTGGCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATG
ACAACGAGTTCGGATATAGCAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGG
AAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCT
ATGTGGCAACTGCTGCTGCCTACAGCTCTGCTGCTTCTGGTGTCTGCCGGCATGAGAACCGAGG
ATCTGCCTAAGGCCGTGGTGTTCCTGGAACCTCAGTGGTACAGAGTGCTGGAAAAGGACAGCGT
GACCCTGAAGTGCCAGGGCGCCTATTCTCCCGAGGACAATAGCACCCAGTGGTTCCACAACGAG
AGCCTGATCAGCAGCCAGGCCAGCAGCTACTTTATCGATGCCGCCACCGTGGACGACAGCGGCG
AGTACAGATGCCAGACCAATCTGAGCACCCTGAGCGACCCTGTGCAGCTGGAAGTGCACATTGG
ATGGTTGCTGCTGCAAGCCCCTAGATGGGTGTTCAAAGAAGAGGACCCCATCCACCTGAGATGC
CACTCTTGGAAGAACACAGCCCTGCACAAAGTGACCTACCTGCAGAACGGCAAGGGCAGAAAGT
ACTTCCACCACAACAGCGACTTCTACATCCCCAAGGCCACACTGAAGGACTCCGGCTCCTACTT
CTGCAGAGGCCTGGTCGGCAGCAAGAACGTGTCCAGCGAGACAGTGAACATCACCATCACACAG
GGCCTCGCCGTGTCTACCATCAGCAGCTTTTTCCCACCTGGCTATCAGGTGTCCTTCTGCCTGG
TCATGGTGCTGCTGTTCGCCGTGGATACCGCCTCTACTTCACCGTCAAGACCAACATCCGGTC
CAGCACCAGAGACTGGAAGGACCACAAGTTCAAGTGGCGGAAGGACCCTCAGGACAAGTAAGCG
GCCGCGTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTG
CCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACT
GTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGG
GGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGA
TGCGGTGGGCTCTATGGATTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATGGCCT
CCAAGGAGTAAGACCCCTGGACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGACCCTC
ACTGCTGGGGAGTCCCTGCCACACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCACAGTTG
CCATGTAGACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACCATCAATA
AAGTACCCTGTGCTCAACCAGTTACTTGTCCTGTCTTATTCTAGGGTCTGGGGCAGAGGGGAGG
GAAGCTGGGCTTGTGTCAAGGTGAGACATTCTTGCTGGGGAGGGACCTGGTATGTTCTCCTCAG
ACTGAGGGTAGGGCCTCCAAACAGCCTTGCTTGCTTCGAGAACCATTTGCTTCCCGCTCAGACG
TCTTGAGTGCTACAGGAAGCTGGCACCACTACTTCAGAGAACAAGGCCTTTTCCTCTCCTCGCT
CCAGTAGATCTAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTC
ACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCG
AGCGAGCGCGCAGCTGCCTGCAGG

SEQ ID NO: 202 - exemplary AAV construct for donor template insertion at *GAPDH* locus

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CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGC
GACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATC
ACTAGGGGTTCCTGTCGACGAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCG
CGGGGCTCTCCAGAACATCATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATC
CCTGAGCTGAACGGGAAGCTCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGG
TGGACCTGACCTGCCGTCTAGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCA
GGCGTCGGAGGGCCCCCTCAAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGAC
TTCAACAGCGACACCCACTCCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACT
TTGTCAAGCTCATTTCCTGGTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTC
TGGCGCCCTCTGGTGGCTGGCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATG
ACAACGAGTTCGGATATAGCAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGG
AAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCT
ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCG
ACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCT
GACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACC
CTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCA
AGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTA
CAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGC
ATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACA
ACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAA
CATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGC
CCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACG
AGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGA
CGAGCTGTACAAGTGAGCGGCCGCGTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCT
CGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCT
GGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGT
AGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACA
ATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGATTTGGCTACAGCAACAGGGTGGTGGAC
CTCATGGCCCACATGGCCTCCAAGGAGTAAGACCCCTGGACCACCAGCCCCAGCAAGAGCACAA
GAGGAAGAGAGAGACCCTCACTGCTGGGGAGTCCCTGCCACACTCAGTCCCCCACCACACTGAA
TCTCCCCTCCTCACAGTTGCCATGTAGACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACC
TTGTCATGTACCATCAATAAAGTACCCTGTGCTCAACCAGTTACTTGTCCTGTCTTATTCTAGG
GTCTGGGGCAGAGGGGAGGGAAGCTGGGCTTGTGTCAAGGTGAGACATTCTTGCTGGGGAGGGA
CCTGGTATGTTCTCCTCAGACTGAGGGTAGGGCCTCCAAACAGCCTTGCTTGCTTCGAGAACCA
TTTGCTTCCCGCTCAGACGTCTTGAGTGCTACAGGAAGCTGGCACCACTACTTCAGAGAACAAG
GCCTTTTCCTCTCCTCGCTCCAGTAGATCTAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTC
TCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCC
CGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG

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CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGC
GACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATC
ACTAGGGGTTCCTGTCGACGAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCG
CGGGGCTCTCCAGAACATCATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATC
CCTGAGCTGAACGGGAAGCTCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGG
TGGACCTGACCTGCCGTCTAGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCA

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GGCGTCGGAGGGCCCCCTCAAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGAC
TTCAACAGCGACACCCACTCCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACT
TTGTCAAGCTCATTTCCTGGTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTC
TGGCGCCCTCTGGTGGCTGGCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATG
ACAACGAGTTCGGATATAGCAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGG
AAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCT
ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATTCCTCCTGATCC
CAGACATCCAGATGACACAGACTACATCCTCCCTGTCTGCCTCTCTGGGAGACAGAGTCACCAT
CAGTTGCAGGGCAAGTCAGGACATTAGTAAATATTTAAATTGGTATCAGCAGAAACCAGATGGA
ACTGTTAAACTCCTGATCTACCATACATCAAGATTACACTCAGGAGTCCCATCAAGGTTCAGTG
GCAGTGGGTCTGGAACAGATTATTCTCTCACCATTAGCAACCTGGAGCAAGAAGATATTGCCAC
TTACTTTTGCCAACAGGGTAATACGCTTCCGTACACGTTCGGAGGGGGGACTAAGTTGGAAATA
ACAGGCTCCACCTCTGGATCCGGCAAGCCCGGATCTGGCGAGGGATCCACCAAGGGCGAGGTGA
AACTGCAGGAGTCAGGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCGTCACATGCACTGT
CTCAGGGGTCTCATTACCCGACTATGGTGTAAGCTGGATTCGCCAGCCTCCACGAAAGGGTCTG
GAGTGGCTGGGAGTAATATGGGGTAGTGAAACCACATACTATAATTCAGCTCTCAAATCCAGAC
TGACCATCATCAAGGACAACTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCAAACTGA
TGACACAGCCATTTACTACTGTGCCAAACATTATTACTACGGTGGTAGCTATGCTATGGACTAC
TGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAGCGGCCGCAATTGAAGTTATGTATCCTCCTC
CTTACCTAGACAATGAGAAGAGCAATGGAACCATTATCCATGTGAAAGGGAAACACCTTTGTCC
AAGTCCCCTATTTCCCGGACCTTCTAAGCCCTTTTGGGTGCTGGTGGTGGTTGGGGGAGTCCTG
GCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCA
GGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCCGGGCCCACCCGCAAGCATTA
CCAGCCCTATGCCCCACCACGCGACTTCGCAGCCTATCGCTCCAGAGTGAAGTTCAGCAGGAGC
GCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAA
GAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAG
AAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTAC
AGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTC
TCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGCTAAAG
CGGCCGCGTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGT
TGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCA
CTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCT
GGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGG
GATGCGGTGGGCTCTATGGATTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATGGC
CTCCAAGGAGTAAGACCCCTGGACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGACCC
TCACTGCTGGGGAGTCCCTGCCACACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCACAGT
TGCCATGTAGACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACCATCAA
TAAAGTACCCTGTGCTCAACCAGTTACTTGTCCTGTCTTATTCTAGGGTCTGGGGCAGAGGGGA
GGGAAGCTGGGCTTGTGTCAAGGTGAGACATTCTTGCTGGGGAGGGACCTGGTATGTTCTCCTC
AGACTGAGGGTAGGGCCTCCAAACAGCCTTGCTTGCTTCGAGAACCATTTGCTTCCCGCTCAGA
CGTCTTGAGTGCTACAGGAAGCTGGCACCACTACTTCAGAGAACAAGGCCTTTTCCTCTCCTCG
CTCCAGTAGATCTAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGC
TCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAG
CGAGCGAGCGCGCAGCTGCCTGCAGG

SEQ ID NO: 204- exemplary AAV construct for donor template insertion at *GAPDH* locus

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CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGC
GACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATC
ACTAGGGGTTCCTGTCGACGAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCG
CGGGGCTCTCCAGAACATCATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATC
CCTGAGCTGAACGGGAAGCTCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGG
TGGACCTGACCTGCCGTCTAGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCA
GGCGTCGGAGGGCCCCCTCAAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGAC
TTCAACAGCGACACCCACTCCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACT
TTGTCAAGCTCATTTCCTGGTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTC
TGGCGCCCTCTGGTGGCTGGCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATG
ACAACGAGTTCGGATATAGCAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGG
AAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCT
ATGGCACTCCCCGTCACCGCCCTTCTCTTGCCCCTCGCCCTGCTGCTGCATGCTGCCAGGCCCA
TGGACGAAGTGCAGCTCGTGGAGTCCGGTGGAGGACTCGTCCAACCGGGCGGATCCCTTCGCTT
GTCCTGCGCCGCATCAGGCTTCAGCTTCACCAACTATGGCGTCCACTGGGTCAGACAGGCCCCC
GGAAAGGGACTGGAATGGGTGTCCGTGATCTGGAGCGGCGGGAACACCGACTACAACACCTCCG
TGAAGGGCCGGTTCACTATTAGCCGCGACAACTCCAAGAACACTCTGTACCTCCAAATGAACTC
CCTGAGGGCCGAAGATACTGCTGTGTACTATTGCGCGAGAGCCCTGACCTACTACGACTACGAG
TTCGCGTACTGGGGCCAGGGGACTCTCGTGACCGTGTCCAGCGGTGGTGGAGGTTCCGGAGGCG
GAGGTTCTGGTGGCGGGGGATCAGAAATCGTGCTGACTCAGTCCCCTGCGACCTTGTCCCTGAG
CCCTGGAGAACGGGCCACCCTGAGCTGTAGAGCCAGCCAGAGCATCGGGACAAATATTCACTGG
TACCAGCAGAAACCCGGACAAGCACCACGGCTGCTGATCTACTACGCCTCCGAGTCGATTTCCG
GAATCCCGGCTCGCTTTTCGGGGTCTGGATCGGGAACGGACTTCACTCTGACCATCTCGTCGCT
GGAACCCGAGGATTTCGCCGTGTACTACTGCCAACAGAACAACAATTGGCCGACCACGTTCGGC
CAGGGCACCAAGCTCGAGATTAAGGGATCACTGGAAGCGGCCGCAACCACAACACCTGCTCCAA
GGCCCCCCACACCCGCTCCAACTATAGCCAGCCAACCATTGAGCCTCAGACCTGAAGCTTGCAG
GCCCGCAGCAGGAGGCGCCGTCCATACGCGAGGCCTGGACTTCGCGTGTGATATTTATATTTGG
GCCCCTTTGGCCGGAACATGTGGGGTGTTGCTTCTCTCCCTTGTGATCACTCTGTATTGTAAGC
GCGGGAGAAAGAAGCTCCTGTACATCTTCAAGCAGCCTTTTATGCGACCTGTGCAAACCACTCA
GGAAGAAGATGGGTGTTCATGCCGCTTCCCCGAGGAGGAAGAAGGAGGGTGTGAACTGAGGGTG
AAATTTTCTAGAAGCGCCGATGCTCCCGCATATCAGCAGGGTCAGAATCAGCTCTACAATGAAT
TGAATCTCGGCAGGCGAGAAGAGTACGATGTTCTGGACAAGAGACGGGGCAGGGATCCCGAGAT
GGGGGGAAAGCCCCGGAGAAAAAATCCTCAGGAGGGGTTGTACAATGAGCTGCAGAAGGACAAG
ATGGCTGAAGCCTATAGCGAGATCGGAATGAAAGGCGAAAGACGCAGAGGCAAGGGGCATGACG
GTCTGTACCAGGGTCTCTCTACAGCCACCAAGGACACTTATGATGCGTTGCATATGCAAGCCTT
GCCACCCCGCTAAAGCGGCCGCGTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCG
ACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGG
AAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAG
GTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAAT
AGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGATTTGGCTACAGCAACAGGGTGGTGGACCT
CATGGCCCACATGGCCTCCAAGGAGTAAGACCCCTGGACCACCAGCCCCAGCAAGAGCACAAGA
GGAAGAGAGAGACCCTCACTGCTGGGGAGTCCCTGCCACACTCAGTCCCCCACCACACTGAATC
TCCCCTCCTCACAGTTGCCATGTAGACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTT
GTCATGTACCATCAATAAAGTACCCTGTGCTCAACCAGTTACTTGTCCTGTCTTATTCTAGGGT
CTGGGGCAGAGGGGAGGGAAGCTGGGCTTGTGTCAAGGTGAGACATTCTTGCTGGGGAGGGACC
TGGTATGTTCTCCTCAGACTGAGGGTAGGGCCTCCAAACAGCCTTGCTTGCTTCGAGAACCATT

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TGCTTCCCGCTCAGACGTCTTGAGTGCTACAGGAAGCTGGCACCACTACTTCAGAGAACAAGGC
CTTTTCCTCTCCTCGCTCCAGTAGATCTAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTC
TGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCG
GGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG

Exemplary Donor Template Sequences

[0463] In some embodiments, a donor template comprises in 5′ to 3′ order, a target sequence 5′ homology arm (which optionally comprises an optimized sequence that is not a wild type sequence), a second regulatory element that enables expression of a cargo sequence as a separate translational product (e.g., an IRES sequence and/or a 2A element), a cargo sequence (e.g., a gene product of interest), optionally a second regulatory element that enables expression of a cargo sequence as a separate translational product (e.g., an IRES sequence and/or a 2A element), optionally a second cargo sequence (e.g., a gene product of interest), optionally a 3′ UTR, a poly adenylation signal (e.g., a BGHpA signal), and a target sequence 3′ homology arm (which optionally comprises an optimized sequence that is not a wild type sequence).

[0464] In some embodiments, a donor template comprises or consists of the sequence of any one of SEQ ID NOs: 38-57 and 205-218. In some embodiments, a donor template comprises or consists of a sequence that is at least 85%, 90%, 95%, 98% or 99% identical to any one of SEQ ID NOs: 38-57 and 205-218.

SEQ ID NO: 38 – exemplary donor template for insertion at *GAPDH* locus

GAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATC
ATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATCCCTGAGCTGAACGGGAAGC
TCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCT
AGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTC
AAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACT
CCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTG
GTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCCTCTGGTGGCTG
GCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATGACAACGAGTTCGGATATAG
CAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGAGGGCAGAGGAAGTCTTCTA
ACATGCGGTGACGTGGAGGAGAATCCTGGCCCGATGGTGAGCAAGGGCGAGGAGCTGTTCACCG
GGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGG
CGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAG
CTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCT
ACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGA
GCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGC
GACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGG
GGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAA
CGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGAC

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CACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGA
GCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTT
CGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGGAGGGCAGAGGAAGTCTT
CTAACATGCGGTGACGTGGAGGAGAATCCTGGCCCGATGGTGAGCAAGGGCGAGGAGGATAACA
TGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGA
GTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAG
GTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCT
CCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGG
CTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCC
TCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACG
GCCCCGTAATGCAGAAGAAGACAATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGA
CGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCT
GAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACA
TCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGA
GGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTAAGCGGCCGCGTCGAGTCTAGAG
GGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTG
CCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAAT
GAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGG
ACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGA
TTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATGGCCTCCAAGGAGTAAGACCCCT
GGACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGACCCTCACTGCTGGGGAGTCCCTG
CCACACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCACAGTTGCCATGTAGACCCCTTGAA
GAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACCATCAATAAAGTACCCTGTGCTCAAC
CAGTTACTTGTCCTGTCTTATTCTAGGGTCTGGGGCAGAGGGGAGGGAAGCTGGGCTTGTGTCA
AGGTGAGACATTCTTGCTGGGGAGGGACCTGGTATGTTCTCCTCAGACTGAGGGTAGGGCCTCC
AAACAGCCTTGCTTGCTTCGAGAACCATTTGCTTCCCGCTCAGACGTCTTGAGTGCTACAGGAA
GCTGGCACCACTACTTCAGAGAACAAGGCCTTTTCCTCTCCTCGCTCCAGT

SEQ ID NO: 39 – exemplary donor template for insertion at *GAPDH* locus

GAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATC
ATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATCCCTGAGCTGAACGGGAAGC
TCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCT
AGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTC
AAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACT
CCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTG
GTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCCTCTGGTGGCTG
GCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATGACAACGAGTTCGGATATAG
CAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGGAAGCGGAGCTACTAACTTC
AGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGGTGAGCAAGGGCGAGG
AGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTT
CAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGC
ACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGT
GCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGG
CTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTG
AAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACG
GCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGA

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CAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTG
CAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACA
ACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGT
CCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAACCC
CTCTCCCTCCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTT
GTCTATATGTTATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCC
CTGTCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTT
GAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACC
CTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTAT
AAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAG
AGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCCAT
TGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAA
AACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCCTTTGAAAAACACGATGATAAATGGT
GAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATG
GAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGG
GCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCT
GTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTAC
TTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCG
TGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCG
CGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACAATGGGCTGGGAGGCCTCC
TCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGA
AGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCT
GCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATC
GTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGT
AAGCGGCCGCGTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCT
AGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTC
CCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTAT
TCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCT
GGGGATGCGGTGGGCTCTATGGATTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACAT
GGCCTCCAAGGAGTAAGACCCCTGGACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGA
CCCTCACTGCTGGGGAGTCCCTGCCACACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCAC
AGTTGCCATGTAGACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACCAT
CAATAAAGTACCCTGTGCTCAACCAGTTACTTGTCCTGTCTTATTCTAGGGTCTGGGGCAGAGG
GGAGGGAAGCTGGGCTTGTGTCAAGGTGAGACATTCTTGCTGGGGAGGGACCTGGTATGTTCTC
CTCAGACTGAGGGTAGGGCCTCCAAACAGCCTTGCTTGCTTCGAGAACCATTTGCTTCCCGCTC
AGACGTCTTGAGTGCTACAGGAAGCTGGCACCACTACTTCAGAGAACAAGGCCTTTTCCTCTCC
TCGCTCCAGT

SEQ ID NO: 40 - exemplary donor template for insertion at *GAPDH* locus

GAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATC
ATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATCCCTGAGCTGAACGGGAAGC
TCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCT
AGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTC
AAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACT
CCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTG
GTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCCTCTGGTGGCTG

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GCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATGACAACGAGTTCGGATATAG
CAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGGAAGCGGAGCTACTAACTTC
AGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGGTGAGCAAGGGCGAGG
AGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTT
CAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGC
ACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGT
GCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGG
CTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTG
AAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACG
GCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGA
CAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTG
CAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACA
ACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGT
CCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGGGAAGC
GGAGCTACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGG
TGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACAT
GGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAG
GGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCC
TGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTA
CTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGC
GTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGC
GCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACAATGGGCTGGGAGGCCTC
CTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTG
AAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGC
TGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCAT
CGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAG
TAAGCGGCCGCGTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTC
TAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACT
CCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTA
TTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGC
TGGGGATGCGGTGGGCTCTATGGATTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACA
TGGCCTCCAAGGAGTAAGACCCCTGGACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAG
ACCCTCACTGCTGGGGAGTCCCTGCCACACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCA
CAGTTGCCATGTAGACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACCA
TCAATAAAGTACCCTGTGCTCAACCAGTTACTTGTCCTGTCTTATTCTAGGGTCTGGGGCAGAG
GGGAGGGAAGCTGGGCTTGTGTCAAGGTGAGACATTCTTGCTGGGGAGGGACCTGGTATGTTCT
CCTCAGACTGAGGGTAGGGCCTCCAAACAGCCTTGCTTGCTTCGAGAACCATTTGCTTCCCGCT
CAGACGTCTTGAGTGCTACAGGAAGCTGGCACCACTACTTCAGAGAACAAGGCCTTTTCCTCTC
CTCGCTCCAGT

SEQ ID NO: 41 - exemplary donor template for insertion at *GAPDH* locus

GAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATC
ATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATCCCTGAGCTGAACGGGAAGC
TCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCT
AGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTC
AAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACT

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CCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTG
GTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCCTCTGGTGGCTG
GCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATGACAACGAGTTCGGATATAG
CAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGGAAGCGGAGCTACTAACTTC
AGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGGTGAGCAAGGGCGAGG
AGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTT
CAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGC
ACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGT
GCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGG
CTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTG
AAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACG
GCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGA
CAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTG
CAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACA
ACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGT
CCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGGAGGGC
AGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAGAATCCTGGCCCGATGGTGAGCAAGGGCG
AGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGT
GAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACC
GCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGT
TCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTC
CTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTG
ACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACT
TCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACAATGGGCTGGGAGGCCTCCTCCGAGCGGAT
GTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGC
CACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCT
ACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTA
CGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTAAGCGGCCGCG
TCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCC
ATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTT
TCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTG
GGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGT
GGGCTCTATGGATTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATGGCCTCCAAGG
AGTAAGACCCCTGGACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGACCCTCACTGCT
GGGGAGTCCCTGCCACACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCACAGTTGCCATGT
AGACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACCATCAATAAAGTAC
CCTGTGCTCAACCAGTTACTTGTCCTGTCTTATTCTAGGGTCTGGGGCAGAGGGGAGGGAAGCT
GGGCTTGTGTCAAGGTGAGACATTCTTGCTGGGGAGGGACCTGGTATGTTCTCCTCAGACTGAG
GGTAGGGCCTCCAAACAGCCTTGCTTGCTTCGAGAACCATTTGCTTCCCGCTCAGACGTCTTGA
GTGCTACAGGAAGCTGGCACCACTACTTCAGAGAACAAGGCCTTTTCCTCTCCTCGCTCCAGT

SEQ ID NO: 42 – exemplary donor template for insertion at *GAPDH* locus

GAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATC
ATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATCCCTGAGCTGAACGGGAAGC
TCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCT
AGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTC

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AAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACT
CCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTG
GTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCCTCTGGTGGCTG
GCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATGACAACGAGTTCGGATATAG
CAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGAGGGCAGAGGAAGTCTTCTA
ACATGCGGTGACGTGGAGGAGAATCCTGGCCCGATGGTGAGCAAGGGCGAGGAGCTGTTCACCG
GGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGG
CGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAG
CTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCT
ACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGA
GCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGC
GACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGG
GGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAA
CGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGAC
CACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGA
GCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTT
CGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGGGAAGCGGAGCTACTAAC
TTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGGTGAGCAAGGGCG
AGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGT
GAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACC
GCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGT
TCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTC
CTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTG
ACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACT
TCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACAATGGGCTGGGAGGCCTCCTCCGAGCGGAT
GTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGC
CACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCT
ACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTA
CGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTAAGCGGCCGCG
TCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCC
ATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTT
TCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTG
GGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGT
GGGCTCTATGGATTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATGGCCTCCAAGG
AGTAAGACCCCTGGACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGACCCTCACTGCT
GGGGAGTCCCTGCCACACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCACAGTTGCCATGT
AGACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACCATCAATAAAGTAC
CCTGTGCTCAACCAGTTACTTGTCCTGTCTTATTCTAGGGTCTGGGGCAGAGGGGAGGGAAGCT
GGGCTTGTGTCAAGGTGAGACATTCTTGCTGGGGAGGGACCTGGTATGTTCTCCTCAGACTGAG
GGTAGGGCCTCCAAACAGCCTTGCTTGCTTCGAGAACCATTTGCTTCCCGCTCAGACGTCTTGA
GTGCTACAGGAAGCTGGCACCACTACTTCAGAGAACAAGGCCTTTTCCTCTCCTCGCTCCAGT

SEQ ID NO: 43 – exemplary donor template for insertion at *GAPDH* locus

GAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATC
ATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATCCCTGAGCTGAACGGGAAGC
TCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCT

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AGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTC
AAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACT
CCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTG
GTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCCTCTGGTGGCTG
GCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATGACAACGAGTTCGGATATAG
CAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGAGGGCAGAGGAAGTCTTCTA
ACATGCGGTGACGTGGAGGAGAATCCTGGCCCGATGGTGAGCAAGGGCGAGGAGCTGTTCACCG
GGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGG
CGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAG
CTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCT
ACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGA
GCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGC
GACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGG
GGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAA
CGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGAC
CACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGA
GCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTT
CGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAACCCCTCTCCCTCCCC
CCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTA
TTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGA
CGAGCATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAA
GGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAG
CGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCTG
CAAAGGCGGCACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCT
CTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCCATTGTATGGGATCT
GATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAAACGTCTAGGCC
CCCCGAACCACGGGGACGTGGTTTTCCTTTGAAAAACACGATGATAAATGGTGAGCAAGGGCGA
GGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTG
AACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCG
CCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTT
CATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCC
TTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGA
CCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTT
CCCCTCCGACGGCCCCGTAATGCAGAAGAAGACAATGGGCTGGGAGGCCTCCTCCGAGCGGATG
TACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCC
ACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTA
CAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTAC
GAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTAAGCGGCCGCGT
CGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCA
TCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTT
CCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGG
GGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTG
GGCTCTATGGATTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATGGCCTCCAAGGA
GTAAGACCCCTGGACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGACCCTCACTGCTG
GGGAGTCCCTGCCACACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCACAGTTGCCATGTA
GACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACCATCAATAAAGTACC

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CTGTGCTCAACCAGTTACTTGTCCTGTCTTATTCTAGGGTCTGGGGCAGAGGGGAGGGAAGCTG
GGCTTGTGTCAAGGTGAGACATTCTTGCTGGGGAGGGACCTGGTATGTTCTCCTCAGACTGAGG
GTAGGGCCTCCAAACAGCCTTGCTTGCTTCGAGAACCATTTGCTTCCCGCTCAGACGTCTTGAG
TGCTACAGGAAGCTGGCACCACTACTTCAGAGAACAAGGCCTTTTCCTCTCCTCGCTCCAGT

SEQ ID NO: 44 - exemplary donor template for insertion at *GAPDH* locus

GAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATC
ATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATCCCTGAGCTGAACGGGAAGC
TCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCT
AGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTC
AAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACT
CCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTG
GTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCCTCTGGTGGCTG
GCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATGACAACGAGTTCGGATATAG
CAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGGAAGCGGAGCTACTAACTTC
AGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGGTGAGCAAGGGCGAGG
AGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTT
CAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGC
ACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGT
GCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGG
CTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTG
AAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACG
GCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGA
CAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTG
CAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACA
ACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGT
CCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTGAGCG
GCCGCGTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTG
CCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACT
GTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGG
GGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGA
TGCGGTGGGCTCTATGGATTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATGGCCT
CCAAGGAGTAAGACCCCTGGACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGACCCTC
ACTGCTGGGGAGTCCCTGCCACACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCACAGTTG
CCATGTAGACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACCATCAATA
AAGTACCCTGTGCTCAACCAGTTACTTGTCCTGTCTTATTCTAGGGTCTGGGGCAGAGGGGAGG
GAAGCTGGGCTTGTGTCAAGGTGAGACATTCTTGCTGGGGAGGGACCTGGTATGTTCTCCTCAG
ACTGAGGGTAGGGCCTCCAAACAGCCTTGCTTGCTTCGAGAACCATTTGCTTCCCGCTCAGACG
TCTTGAGTGCTACAGGAAGCTGGCACCACTACTTCAGAGAACAAGGCCTTTTCCTCTCCTCGCT
CCAGT

SEQ ID NO: 45 - exemplary donor template for insertion at *GAPDH* locus

GAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATC
ATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATCCCTGAGCTGAACGGGAAGC
TCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCT

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AGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTC
AAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACT
CCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTG
GTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCCTCTGGTGGCTG
GCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATGACAACGAGTTCGGATATAG
CAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGGAAGCGGAGCTACTAACTTC
AGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGGATTGGACCTGGATCC
TGTTTCTGGTGGCCGCTGCCACAAGAGTGCACAGCAATTGGGTCAACGTGATCAGCGACCTGAA
GAAGATCGAGGACCTGATCCAGAGCATGCACATCGACGCCACACTGTACACCGAGTCCGATGTG
CACCCTAGCTGCAAAGTGACCGCCATGAAGTGCTTTCTGCTGGAACTGCAAGTGATCAGCCTGG
AAAGCGGCGACGCCAGCATCCACGATACCGTGGAAAACCTGATCATCCTGGCCAACAACAGCCT
GAGCAGCAACGGCAATGTGACCGAGAGCGGCTGCAAAGAGTGCGAGGAACTGGAAGAGAAGAAC
ATCAAAGAGTTCCTCCAGAGCTTCGTCCACATCGTGCAGATGTTCATCAACACCAGCTCTGGCG
GAGGAAGCGGAGGCGGAGGATCTGGTGGTGGTGGATCTGGCGGCGGTGGTAGTGGCGGAGGTTC
TCTGCAAATCACCTGTCCTCCACCTATGAGCGTGGAACACGCCGACATCTGGGTCAAGAGCTAC
AGCCTGTACAGCAGAGAGCGGTACATCTGCAACAGCGGCTTCAAGAGAAAGGCCGGCACAAGCA
GCCTGACCGAGTGTGTGCTGAACAAGGCCACAAACGTGGCCCACTGGACCACACCTAGCCTGAA
GTGCATCAGAGATCCCGCTCTGGTTCATCAGAGGCCTGCCCCTCCATCTACAGTGACAACAGCT
GGCGTGACCCCTCAGCCTGAGTCTCTGTCTCCATCTGGAAAAGAGCCTGCCGCCAGCTCTCCCA
GCTCTAACAATACTGCTGCCACCACAGCCGCTATCGTGCCTGGATCTCAGCTGATGCCTAGCAA
GAGCCCTAGCACCGGCACAACAGAGATCAGCTCTCACGAGAGCAGCCACGGAACACCTTCTCAG
ACCACCGCCAAGAATTGGGAGCTGACAGCCTCTGCCTCTCATCAGCCACCTGGCGTGTACCCAC
AGGGCCACTCTGATACAACAGTGGCCATCAGCACCAGCACCGTTCTGCTGTGTGGCCTGTCTGC
TGTTAGCCTGCTGGCCTGCTACCTGAAGTCTAGACAGACACCTCCTCTGGCCAGCGTGGAAATG
GAAGCCATGGAAGCTCTGCCTGTCACATGGGGCACCAGCAGCAGAGATGAGGACCTCGAGAATT
GCAGCCACCACCTGTAGGCGGCCGCGTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCC
TCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCC
TGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAG
TAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGAC
AATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGATTTGGCTACAGCAACAGGGTGGTGGA
CCTCATGGCCCACATGGCCTCCAAGGAGTAAGACCCCTGGACCACCAGCCCCAGCAAGAGCACA
AGAGGAAGAGAGAGACCCTCACTGCTGGGGAGTCCCTGCCACACTCAGTCCCCCACCACACTGA
ATCTCCCCTCCTCACAGTTGCCATGTAGACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCAC
CTTGTCATGTACCATCAATAAAGTACCCTGTGCTCAACCAGTTACTTGTCCTGTCTTATTCTAG
GGTCTGGGGCAGAGGGGAGGGAAGCTGGGCTTGTGTCAAGGTGAGACATTCTTGCTGGGGAGGG
ACCTGGTATGTTCTCCTCAGACTGAGGGTAGGGCCTCCAAACAGCCTTGCTTGCTTCGAGAACC
ATTTGCTTCCCGCTCAGACGTCTTGAGTGCTACAGGAAGCTGGCACCACTACTTCAGAGAACAA
GGCCTTTTCCTCTCCTCGCTCCAGT

SEQ ID NO: 46 – exemplary donor template for insertion at *GAPDH* locus

GGCTTTCCCATAATTTCCTTTCAAGGTGGGGAGGGAGGTAGAGGGGTGATGTGGGGAGTACGCT
GCAGGGCCTCACTCCTTTTGCAGACCACAGTCCATGCCATCACTGCCACCCAGAAGACTGTGGA
TGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATCATCCCTGCCTCT
ACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATCCCTGAGCTGAACGGGAAGCTCACTGGCATGG
CCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCTAGAAAAACCTGC
CAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTCAAGGGCATCCTG

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GGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACTCCTCCACCTTTG
ACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATCTCTTGGTACGACAATGA
GTTCGGATATAGCAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGGAAGCGGA
GCTACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGGTGA
GCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAA
CGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTG
AAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCT
ACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGC
CATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACC
CGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACT
TCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTA
TATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAG
GACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGC
TGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCG
CGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTG
TACAAGTGAGCGGCCGCGTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGT
GCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGT
GCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTC
ATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAG
GCATGCTGGGGATGCGGTGGGCTCTATGGAGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCC
TCTGGTGGCTGGCTCAGAAAAAGGGCCCTGACAACTCTTTTCATCTTCTAGGTATGACAACGAA
TTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATGGCCTCCAAGGAGTAAGACCCCT
GGACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGACCCTCACTGCTGGGGAGTCCCTG
CCACACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCACAGTTGCCATGTAGACCCCTTGAA
GAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACCATCAATAAAGTACCCTGTGCTCAAC
CAGTTACTTGTCCTGTCTTATTCTAGGGTCTGGGGCAGAGGGGAGGGAAGCTGGGCTTGTGTCA
AGGTGAGACATTCTTGCTGGGGAGGGACCTGGTATGTTCTCCTCAGACTGAGGGTAGGGCCTCC
AAACAGCCTTGCTTGCT

SEQ ID NO: 47 – exemplary donor template for insertion at *TBP* locus

GCAGACTTCCATTTACAGTGAGGAGGTGAGCATTGCATTGAACAAAAGATGGCGTTTTCACTTG
GAATTAGTTATCTGAAGCTTTAGGATTCCTCAGCAATATGATTATGAGACAAGAAAGGAAGATT
CAGAAATGAGTCTAGTTGAAGGCAGCAATTCAGAGAAGAAGATTCAGTTGTTATCATTGCCGTC
CTGCTTGGTTTATGGCCTGGTTCAGGACCAAGGAGAGAAGTGTGAATACATGCCTCTTGAGCTA
TAGAATGAGACGCTGGAGTCACTAAGATGATTTTTTAAAAGTATTGTTTTATAAACAAAAATAA
GATTGTGACAAGGGATTCCACTATTAATGTTTTCATGCCTGTGCCTTAATCTGACTGGGTATGG
TGAGAATTGTGCTTGCAGCTTTAAGGTAAGAATTTTACCATCTTAATATGTTAAGAAGTGCCAT
TTCAGTCTCTCATCTCTACTCCAACTTGTCTTCTTAGGTGCTAAAGTCAGAGCCGAAATCTACG
AGGCCTTCGAGAACATCTACCCCATCCTGAAGGGCTTCAGAAAGACCACCGGAAGCGGAGCTAC
TAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGGTGAGCAAG
GGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCC
ACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTT
CATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGC
GTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGC
CCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGC
CGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAG

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GAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCA
TGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGG
CAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTG
CCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATC
ACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAA
GTGAGCGGCCGCGTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTT
CTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCAC
TCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCT
ATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATG
CTGGGGATGCGGTGGGCTCTATGGCAGAAATTTATGAAGCATTTGAAAACATCTACCCTATTCT
AAAGGGATTCAGGAAGACGACGTAATGGCTCTCATGTACCCTTGCCTCCCCCACCCCCTTCTTT
TTTTTTTTTTAAACAAATCAGTTTGTTTTGGTACCTTTAAATGGTGGTGTTGTGAGAAGATGGA
TGTTGAGTTGCAGGGTGTGGCACCAGGTGATGCCCTTCTGTAAGTGCCCACCGCGGGATGCCGG
GAAGGGGCATTATTTGTGCACTGAGAACACCGCGCAGCGTGACTGTGAGTTGCTCATACCGTGC
TGCTATCTGGGCAGCGCTGCCCATTTATTTATATGTAGATTTTAAACACTGCTGTTGACAAGTT
GGTTTGAGGGAGAAAACTTTAAGTGTTAAAGCCACCTCTATAATTGATTGGACTTTTTAATTTT
AATGTTTTTCCCCATGAACCACAGTTTTTATATTTCTACCAGAAAAGTAAAAATCTTTTTTAAA
AGTGTTGTTTTT

SEQ ID NO: 49 - exemplary donor template for insertion at *TBP* locus

CTGACCACAGCTCTGCAAGCAGACTTCCATTTACAGTGAGGAGGTGAGCATTGCATTGAACAAA
AGATGGCGTTTTCACTTGGAATTAGTTATCTGAAGCTTTAGGATTCCTCAGCAATATGATTATG
AGACAAGAAAGGAAGATTCAGAAATGAGTCTAGTTGAAGGCAGCAATTCAGAGAAGAAGATTCA
GTTGTTATCATTGCCGTCCTGCTTGGTTTATGGCCTGGTTCAGGACCAAGGAGAGAAGTGTGAA
TACATGCCTCTTGAGCTATAGAATGAGACGCTGGAGTCACTAAGATGATTTTTTAAAAGTATTG
TTTTATAAACAAAAATAAGATTGTGACAAGGGATTCCACTATTAATGTTTTCATGCCTGTGCCT
TAATCTGACTGGGTATGGTGAGAATTGTGCTTGCAGCTTTAAGGTAAGAATTTTACCATCTTAA
TATGTTAAGAAGTGCCATTTCAGTCTCTCATCTCTACTCCAACTTGTCTTCTTAGGGGCTAAAG
TGCGGGCCGAGATCTACGAGGCCTTCGAGAATATCTACCCCATCCTGAAGGGCTTCAGAAAGAC
CACCGGAAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCT
GGACCTATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGG
ACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGG
CAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTG
ACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACT
TCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGG
CAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTG
AAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACA
GCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCG
CCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGC
GACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACC
CCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGG
CATGGACGAGCTGTACAAGTGAGCGGCCGCGTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGAT
CAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTT
GACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGT
CTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGG
AAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGTAGGTGCTAAAGTCAGAGCAGA

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AATTTATGAAGCATTTGAAAACATCTACCCTATTCTAAAGGGATTCAGGAAGACGACGTAATGG
CTCTCATGTACCCTTGCCTCCCCCACCCCCTTCTTTTTTTTTTTTTTAAACAAATCAGTTTGTTT
TGGTACCTTTAAATGGTGGTGTTGTGAGAAGATGGATGTTGAGTTGCAGGGTGTGGCACCAGGT
GATGCCCTTCTGTAAGTGCCCACCGCGGGATGCCGGGAAGGGGCATTATTTGTGCACTGAGAAC
ACCGCGCAGCGTGACTGTGAGTTGCTCATACCGTGCTGCTATCTGGGCAGCGCTGCCCATTTAT
TTATATGTAGATTTTAAACACTGCTGTTGACAAGTTGGTTTGAGGGAGAAAACTTTAAGTGTTA
AAGCCACCTCTATAATTGATTGGACTTTTTAATTTTAATGTTTTTCCCCATGAACCACAGTTTT
TATATTTCTACCAGAAAAGTAAAAATCTTT

SEQ ID NO: 50 – exemplary donor template for insertion at *TBP* locus

ACAAAAGATGGCGTTTTCACTTGGAATTAGTTATCTGAAGCTTTAGGATTCCTCAGCAATATGA
TTATGAGACAAGAAAGGAAGATTCAGAAATGAGTCTAGTTGAAGGCAGCAATTCAGAGAAGAAG
ATTCAGTTGTTATCATTGCCGTCCTGCTTGGTTTATGGCCTGGTTCAGGACCAAGGAGAGAAGT
GTGAATACATGCCTCTTGAGCTATAGAATGAGACGCTGGAGTCACTAAGATGATTTTTTAAAAG
TATTGTTTTATAAACAAAAATAAGATTGTGACAAGGGATTCCACTATTAATGTTTTCATGCCTG
TGCCTTAATCTGACTGGGTATGGTGAGAATTGTGCTTGCAGCTTTAAGGTAAGAATTTTACCAT
CTTAATATGTTAAGAAGTGCCATTTCAGTCTCTCATCTCTACTCCAACTTGTCTTCTTAGGTGC
TAAAGTCAGAGCAGAAATTTATGAAGCATTCGAGAACATCTACCCTATTCTAAAGGGATTCAGG
AAGACGACGGGAAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGA
ACCCTGGACCTATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGA
GCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACC
TACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCC
TCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCA
CGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGAC
GACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCG
AGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTA
CAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAG
ATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCA
TCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAA
AGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACT
CTCGGCATGGACGAGCTGTACAAGTGAGCGGCCGCGTCGAGTCTAGAGGGCCCGTTTAAACCCG
CTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCT
TCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGC
ATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGA
TTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGAAGGGATTCAGGAAGAC
GACGTAATGGCTCTCATGTACCCTTGCCTCCCCCACCCCCTTCTTTTTTTTTTTTTTAAACAAAT
CAGTTTGTTTTGGTACCTTTAAATGGTGGTGTTGTGAGAAGATGGATGTTGAGTTGCAGGGTGT
GGCACCAGGTGATGCCCTTCTGTAAGTGCCCACCGCGGGATGCCGGGAAGGGGCATTATTTGTG
CACTGAGAACACCGCGCAGCGTGACTGTGAGTTGCTCATACCGTGCTGCTATCTGGGCAGCGCT
GCCCATTTATTTATATGTAGATTTTAAACACTGCTGTTGACAAGTTGGTTTGAGGGAGAAAACT
TTAAGTGTTAAAGCCACCTCTATAATTGATTGGACTTTTTAATTTTAATGTTTTTCCCCATGAA
CCACAGTTTTTATATTTCTACCAGAAAAGTAAAAATCTTTTTTAAAAGTGTTGTTTTTCTAATT
TATAACTCCTAGGGGTTATTTCTGTGCCAGACACA

SEQ ID NO: 51 – exemplary donor template for insertion at *G6PD* locus

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GGCCCGGGGGACTCCACATGGTGGCAGGCAGTGGCATCAGCAAGACACTCTCTCCCTCACAGAA
CGTGAAGCTCCCTGACGCCTATGAGCGCCTCATCCTGGACGTCTTCTGCGGGAGCCAGATGCAC
TTCGTGCGCAGGTGAGGCCCAGCTGCCGGCCCCTGCATACCTGTGGGCTATGGGGTGGCCTTTG
CCCTCCCTCCCTGTGTGCCACCGGCCTCCCAAGCCATACCATGTCCCCTCAGCGACGAGCTCCG
TGAGGCCTGGCGTATTTTCACCCCACTGCTGCACCAGATTGAGCTGGAGAAGCCCAAGCCCATC
CCCTATATTTATGGCAGGTGAGGAAAGGGTGGGGGCTGGGGACAGAGCCCAGCGGGCAGGGGCG
GGGTGAGGGTGGAGCTACCTCATGCCTCTCCTCCACCCGTCACTCTCCAGCCGAGGCCCCACGG
AGGCAGACGAGCTGATGAAGAGAGTGGGCTTCCAGTACGAGGGAACCTACAAATGGGTCAACCC
TCACAAGCTGGGAAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAG
AACCCTGGACCTATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCG
AGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCAC
CTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACC
CTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGC
ACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGA
CGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATC
GAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACT
ACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAA
GATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCC
ATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCA
AAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCAC
TCTCGGCATGGACGAGCTGTACAAGTGAGCGGCCGCGTCGAGTCTAGAGGGCCCGTTTAAACCC
GCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCC
TTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCG
CATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGG
ATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGGTGGGTGAACCCCCAC
AAGCTCTGAGCCCTGGGCACCCACCTCCACCCCCGCCACGGCCACCCTCCTTCCCGCCGCCCGA
CCCCGAGTCGGGAGGACTCCGGGACCATTGACCTCAGCTGCACATTCCTGGCCCCGGGCTCTGG
CCACCCTGGCCCGCCCCTCGCTGCTGCTACTACCCGAGCCCAGCTACATTCCTCAGCTGCCAAG
CACTCGAGACCATCCTGGCCCCTCCAGACCCTGCCTGAGCCCAGGAGCTGAGTCACCTCCTCCA
CTCACTCCAGCCCAACAGAAGGAAGGAGGAGGGCGCCCATTCGTCTGTCCCAGAGCTTATTGGC
CACTGGGTCTCACTCCTGAGTGGGGCCAGGGTGGGAGGGAGGGACGAGGGGGAGGAAAGGGGCG
AGCACCCACGTGAGAGAATCTGCCTGTGGCCTTGCCCGCCAGCCTCAGTGCCACTTGACATTCC
TTGTCACCAGCAACATCTCGAGCCCCCTGGATGTCC

SEQ ID NO: 52 - exemplary donor template for insertion at *E2F4* locus

CCAGGGGGCTGTAGTGGGGCCAGGCTGGACCTCTGTGCCCTGAGCATGGCTTTCTTGTTTTTCA
GTTTTGGAACTCCCCAAAGAGCTGTCAGAAATCTTTGATCCCACACGAGGTAGGCTGCTGCATT
CCTCCCTGAGGCTAGGGGTAAGGGACACAGCTCATTGGGTCCTATGGCTGTTTTCTTGCCCTTT
TGAGGACCTTGTTGTGGCGCTTATGGTAACTGGGGCAAAGGGTGAAGTTCCTGATGGGCAGGTG
GGGTTCCCTTTCCTGGGCTTTGGTGGGTGGAGAGGTGGGAGCTGGAATGTTAGTAACTGAGCTC
CCTCCATTCCCAGAGTGCATGAGCTCGGAGCTGCTGGAGGAGTTGATGTCCTCAGAAGGTGGGT
GGCCCTGGAAGGTGGGAGTGGGTGTGGGCAGGGGTTGGGCTGCTGCTAGGGGAGCCCTGGCCCA
GGGCCTGAGACTAGTGCTCTCTGCAGTGTTCGCCCCTCTGCTGAGACTTTCTCCTCCTCCTGGC
GACCACGACTACATCTACAACCTGGACGAGAGCGAGGGCGTGTGCGACCTGTTTGATGTGCCCG
TGCTGAACCTGGGAAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGA
GAACCCTGGACCTATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTC

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GAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCA
CCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCAC
CCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAG
CACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGG
ACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCAT
CGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAAC
TACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCA
AGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCC
CATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGC
AAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCA
CTCTCGGCATGGACGAGCTGTACAAGTGAGCGGCCGCGTCGAGTCTAGAGGGCCCGTTTAAACC
CGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGC
CTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATC
GCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAG
GATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGCCACCCCCGGGAGAC
CACGATTATATCTACAACCTGGACGAGAGTGAAGGTGTCTGTGACCTCTTTGATGTGCCTGTTC
TCAACCTCTGACTGACAGGGACATGCCCTGTGTGGCTGGGACCCAGACTGTCTGACCTGGGGGT
TGCCTGGGGACCTCTCCCACCCGACCCCTACAGAGCTTGAGAGCCACAGACGCCTGGCTTCTCC
GGCCTCCCCTCACCGCACAGTTCTGGCCACAGCTCCCGCTCCTGTGCTGGCACTTCTGTGCTCG
CAGAGCAGGGGAACAGGACTCAGCCCCCATCACCGTGGAGCCAAAGTGTTTGCTTCTCCCTTTC
TGCGGCCTTCGCCAGCCCAGGCTCGGCTGCCACCCAGTGGCACAGAACCGAGGAGCTGCCATTA
CCCCCCATAGGGGGCAGTGTCTTGTTCCTGCCAGCCTCAGTGTCTTGCTTCTGCCAGCTCCTTC
CCCTAGGAGGGAAGGGTGGGGTGGAACTGGGCACATG

SEQ ID NO: 53 - exemplary donor template for insertion at *E2F4* locus

CCAGGCTGGACCTCTGTGCCCTGAGCATGGCTTTCTTGTTTTTCAGTTTTGGAACTCCCCAAAG
AGCTGTCAGAAATCTTTGATCCCACACGAGGTAGGCTGCTGCATTCCTCCCTGAGGCTAGGGGT
AAGGGACACAGCTCATTGGGTCCTATGGCTGTTTTCTTGCCCTTTTGAGGACCTTGTTGTGGCG
CTTATGGTAACTGGGGCAAAGGGTGAAGTTCCTGATGGGCAGGTGGGGTTCCCTTTCCTGGGCT
TTGGTGGGTGGAGAGGTGGGAGCTGGAATGTTAGTAACTGAGCTCCCTCCATTCCCAGAGTGCA
TGAGCTCGGAGCTGCTGGAGGAGTTGATGTCCTCAGAAGGTGGGTGGCCCTGGAAGGTGGGAGT
GGGTGTGGGCAGGGGTTGGGCTGCTGCTAGGGGAGCCCTGGCCCAGGGCCTGAGACTAGTGCTC
TCTGCAGTGTTTGCCCCTCTGCTTCGTCTTAGTCCTCCTCCGGGCGACCACGACTACATCTACA
ACCTGGACGAGAGCGAGGGCGTGTGCGACCTGTTTGATGTGCCCGTGCTGAACCTGGGAAGCGG
AGCTACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGGTG
AGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAA
ACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCT
GAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACC
TACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCG
CCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGAC
CCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGAC
TTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCT
ATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGA
GGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTG
CTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGC
GCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCT

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GTACAAGTGAGCGGCCGCGTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTG
TGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGG
TGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGT
CATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCA
GGCATGCTGGGGATGCGGTGGGCTCTATGGATTATATCTACAACCTGGACGAGAGTGAAGGTGT
CTGTGACCTCTTTGATGTGCCTGTTCTCAACCTCTGACTGACAGGGACATGCCCTGTGTGGCTG
GGACCCAGACTGTCTGACCTGGGGGTTGCCTGGGGACCTCTCCCACCCGACCCCTACAGAGCTT
GAGAGCCACAGACGCCTGGCTTCTCCGGCCTCCCCTCACCGCACAGTTCTGGCCACAGCTCCCG
CTCCTGTGCTGGCACTTCTGTGCTCGCAGAGCAGGGGAACAGGACTCAGCCCCCATCACCGTGG
AGCCAAAGTGTTTGCTTCTCCCTTTCTGCGGCCTTCGCCAGCCCAGGCTCGGCTGCCACCCAGT
GGCACAGAACCGAGGAGCTGCCATTACCCCCCATAGGGGGCAGTGTCTTGTTCCTGCCAGCCTC
AGTGTCTTGCTTCTGCCAGCTCCTTCCCCTAGGAGGGAAGGGTGGGGTGGAACTGGGCACATGC
CAGCACCACTTCTAGCTT

SEQ ID NO: 54 – exemplary donor template for insertion at *E2F4* locus

GTCAGAAATCTTTGATCCCACACGAGGTAGGCTGCTGCATTCCTCCCTGAGGCTAGGGGTAAGG
GACACAGCTCATTGGGTCCTATGGCTGTTTTCTTGCCCTTTTGAGGACCTTGTTGTGGCGCTTA
TGGTAACTGGGGCAAAGGGTGAAGTTCCTGATGGGCAGGTGGGGTTCCCTTTCCTGGGCTTTGG
TGGGTGGAGAGGTGGGAGCTGGAATGTTAGTAACTGAGCTCCCTCCATTCCCAGAGTGCATGAG
CTCGGAGCTGCTGGAGGAGTTGATGTCCTCAGAAGGTGGGTGGCCCTGGAAGGTGGGAGTGGGT
GTGGGCAGGGGTTGGGCTGCTGCTAGGGGAGCCCTGGCCCAGGGCCTGAGACTAGTGCTCTCTG
CAGTGTTTGCCCCTCTGCTTCGTCTTTCTCCACCCCCGGGAGACCACGATTATATCTACAACCT
GGACGAGAGTGAAGGTGTCTGTGACCTCTTCGACGTGCCCGTGCTCAACCTCGGAAGCGGAGCT
ACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGGTGAGCA
AGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGG
CCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAG
TTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACG
GCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCAT
GCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGC
GCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCA
AGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATAT
CATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGAC
GGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGC
TGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGA
TCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTAC
AAGTGAGCGGCCGCGTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCC
TTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCC
ACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATT
CTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCA
TGCTGGGGATGCGGTGGGCTCTATGGTGACTGACAGGGACATGCCCTGTGTGGCTGGGACCCAG
ACTGTCTGACCTGGGGGTTGCCTGGGGACCTCTCCCACCCGACCCCTACAGAGCTTGAGAGCCA
CAGACGCCTGGCTTCTCCGGCCTCCCCTCACCGCACAGTTCTGGCCACAGCTCCCGCTCCTGTG
CTGGCACTTCTGTGCTCGCAGAGCAGGGGAACAGGACTCAGCCCCCATCACCGTGGAGCCAAAG
TGTTTGCTTCTCCCTTTCTGCGGCCTTCGCCAGCCCAGGCTCGGCTGCCACCCAGTGGCACAGA
ACCGAGGAGCTGCCATTACCCCCCATAGGGGGCAGTGTCTTGTTCCTGCCAGCCTCAGTGTCTT
GCTTCTGCCAGCTCCTTCCCCTAGGAGGGAAGGGTGGGGTGGAACTGGGCACATGCCAGCACCA

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CTTCTAGCTTCCTTCGCTATCCCCCACCCCCTGACCCTCCAGCTCCTCCTGGCCCTCTCACGTG
CCCACTTCTGCTGG

SEQ ID NO: 55 – exemplary donor template for insertion at *KIF11* locus

AGAGCAGGGTTTCTTGACAGCAGTGCTATTGGCATTTTAAACTGGATAATTCTTTGTTGTGATG
GGCTTTCCTGTGGACTGTACTATGTTGGTACACAAGAAAAACAGTGTACTATGTGAATACTCAC
TCAAAGCCAGTAGCACTCCCTGATTGTAACACCAAAAAAGTCTCTCAGCATTGCCAAATGTCCC
CTGTGGCAGCAGAATCACTCCCTGATGAGAACCACTACCCTGGAGTAAAATCTATAACTATGTC
TTAGAAAATAACACAGAAAATTAATATTTCTTTCACTCTACTCCTTCCATTAGTGATCAAATAA
AGAAGGCATTTGGCGCTACTTGCCAAATTGTTGGCTCAAACTTGTGCTGAACCTTTTTTGGTTT
TCTACACTTAAGTTTTTTTGCCTATAACCCAGAGAACTTTGAAAATAGAGTGTAGTTAATGTGT
ATCTAATGTTACTTTGTATTGACTTAATTTACCGGCCTTTAATCCACAGCATAAGAAGTCCCAC
GGCAAGGACAAAGAGAACCGGGGCATCAACACACTGGAACGGTCCAAGGTCGAGGAAACAACCG
AGCACCTGGTCACCAAGAGCAGACTGCCTCTGAGAGCCCAGATCAACCTGGGAAGCGGAGCTAC
TAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGGTGAGCAAG
GGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCC
ACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTT
CATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGC
GTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGC
CCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGC
CGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAG
GAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCA
TGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGG
CAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTG
CCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATC
ACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAA
GTGAGCGGCCGCGTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTT
CTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCAC
TCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCT
ATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATG
CTGGGGATGCGGTGGGCTCTATGGAAAAAATCACATGGAAAAGACAAAGAAAACAGAGGCATTA
ACACACTGGAGAGGTCTAAAGTGGAAGAAACTACAGAGCACTTGGTTACAAAGAGCAGATTACC
TCTGCGAGCCCAGATCAACCTTTAATTCACTTGGGGGTTGGCAATTTTATTTTTAAAGAAAACT
TAAAAATAAAACCTGAAACCCCAGAACTTGAGCCTTGTGTATAGATTTTAAAAGAATATATATA
TCAGCCGGGCGCGGTGGCTCATGCCTGTAATCCCAGCACTTTGGGAGGCTGAGGCGGGTGGATT
GCTTGAGCCCAGGAGTTTGAGACCAGCCTGGCCAACGTGGCAAAACCTCGTCTCTGTTAAAAAT
TAGCCGGGCGTGGTGGCACACTCCTGTAATCCCAGCTACTGGGGAGGCTGAGGCACGAGAATCA
CTTGAACCCAGGAAGCGGGGTTGCAGTGAGCCAAAGGTACACCACTACACTCCAGCCTGGGCAA
CAGAGCAAGACT

SEQ ID NO: 56 - exemplary donor template for insertion at *KIF11* locus

TTCCTGTGGACTGTACTATGTTGGTACACAAGAAAAACAGTGTACTATGTGAATACTCACTCAA
AGCCAGTAGCACTCCCTGATTGTAACACCAAAAAAGTCTCTCAGCATTGCCAAATGTCCCCTGT
GGCAGCAGAATCACTCCCTGATGAGAACCACTACCCTGGAGTAAAATCTATAACTATGTCTTAG
AAAATAACACAGAAAATTAATATTTCTTTCACTCTACTCCTTCCATTAGTGATCAAATAAAGAA

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GGCATTTGGCGCTACTTGCCAAATTGTTGGCTCAAACTTGTGCTGAACCTTTTTTGGTTTTCTA
CACTTAAGTTTTTTTGCCTATAACCCAGAGAACTTTGAAAATAGAGTGTAGTTAATGTGTATCT
AATGTTACTTTGTATTGACTTAATTTTCCCGCCTTAAATCCACAGCATAAAAAATCACATGGAA
AAGACAAAGAAAACAGAGGCATTAACACACTGGAGAGGTCTAAAGTGGAAGAAACAACCGAGCA
CCTGGTCACCAAGAGCAGACTGCCTCTGAGAGCCCAGATCAACCTGGGAAGCGGAGCTACTAAC
TTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGGTGAGCAAGGGCG
AGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAA
GTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATC
TGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGC
AGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGA
AGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAG
GTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGG
ACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGC
CGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGC
GTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCG
ACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACAT
GGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTGA
GCGGCCGCGTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAG
TTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCC
ACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTC
TGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGG
GGATGCGGTGGGCTCTATGGAACTACAGAGCACTTGGCTACATAGAGCAGATTACCTCTGCGAG
CCCAGATCAACCTTTAATTCACTTGGGGGTTGGCAATTTTATTTTTAAAGAAAACTTAAAAATA
AAACCTGAAACCCCAGAACTTGAGCCTTGTGTATAGATTTTAAAAGAATATATATATCAGCCGG
GCGCGGTGGCTCATGCCTGTAATCCCAGCACTTTGGGAGGCTGAGGCGGGTGGATTGCTTGAGC
CCAGGAGTTTGAGACCAGCCTGGCCAACGTGGCAAAACCTCGTCTCTGTTAAAAATTAGCCGGG
CGTGGTGGCACACTCCTGTAATCCCAGCTACTGGGGAGGCTGAGGCACGAGAATCACTTGAACC
CAGGAAGCGGGGTTGCAGTGAGCCAAAGGTACACCACTACACTCCAGCCTGGGCAACAGAGCAA
GACTCGGTCTCAAAAACAAAATTTAAAAAAGATATAAGGCAGTACTGTAAATTCAGTTGAATTT
TGATATCT

SEQ ID NO: 57- exemplary donor template for insertion at *KIF11* locus

TTAAACTGGATAATTCTTTGTTGTGATGGGCTTTCCTGTGGACTGTACTATGTTGGTACACAAG
AAAAACAGTGTACTATGTGAATACTCACTCAAAGCCAGTAGCACTCCCTGATTGTAACACCAAA
AAAGTCTCTCAGCATTGCCAAATGTCCCCTGTGGCAGCAGAATCACTCCCTGATGAGAACCACT
ACCCTGGAGTAAAATCTATAACTATGTCTTAGAAAATAACACAGAAAATTAATATTTCTTTCAC
TCTACTCCTTCCATTAGTGATCAAATAAAGAAGGCATTTGGCGCTACTTGCCAAATTGTTGGCT
CAAACTTGTGCTGAACCTTTTTTGGTTTTCTACACTTAAGTTTTTTTGCCTATAACCCAGAGAA
CTTTGAAAATAGAGTGTAGTTAATGTGTATCTAATGTTACTTTGTATTGACTTAATTTTCCCGC
CTTAAATCCACAGCATAAAAAATCACATGGAAAAGACAAAGAAAACAGAGGCATCAACACACTG
GAACGGTCCAAGGTCGAGGAAACAACCGAGCACCTGGTCACCAAGAGCAGACTGCCTCTGAGAG
CCCAGATCAACCTGGGAAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGA
GGAGAACCCTGGACCTATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTG
GTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATG
CCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCC
CACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAG

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CAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCA
AGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCG
CATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTAC
AACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACT
TCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACAC
CCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTG
AGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGA
TCACTCTCGGCATGGACGAGCTGTACAAGTGAGCGGCCGCGTCGAGTCTAGAGGGCCCGTTTAA
ACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCG
TGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGC
ATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGG
GAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGATTAACACACTG
GAGAGTTCTGAAGTGGAAGAAACTACAGAGCACTTGGTTACAAAGAGCAGATTACCTCTGCGAG
CCCAGATCAACCTTTAATTCACTTGGGGGTTGGCAATTTTATTTTTAAAGAAAACTTAAAAATA
AAACCTGAAACCCCAGAACTTGAGCCTTGTGTATAGATTTTAAAAGAATATATATATCAGCCGG
GCGCGGTGGCTCATGCCTGTAATCCCAGCACTTTGGGAGGCTGAGGCGGGTGGATTGCTTGAGC
CCAGGAGTTTGAGACCAGCCTGGCCAACGTGGCAAAACCTCGTCTCTGTTAAAAATTAGCCGGG
CGTGGTGGCACACTCCTGTAATCCCAGCTACTGGGGAGGCTGAGGCACGAGAATCACTTGAACC
CAGGAAGCGGGGTTGCAGTGAGCCAAAGGTACACCACTACACTCCAGCCTGGGCAACAGAGCAA
GACTCGGTCTCAAAAACAAAATTTAAAAAAGATATAAGGC

SEQ ID NO: 48 - exemplary donor template for insertion at *GAPDH* locus

GAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATC
ATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATCCCTGAGCTGAACGGGAAGC
TCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCT
AGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTC
AAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACT
CCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTG
GTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCCTCTGGTGGCTG
GCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATGACAACGAGTTCGGATATAG
CAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGGAAGCGGAGCTACTAACTTC
AGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGTGGCCCCTGGTAGCGG
CGCTGTTGCTGGGCTCGGCGTGCTGCGGATCAGCTCAGCTACTATTTAATAAAACAAAATCTGT
AGAATTCACGTTTTGTAATGACACTGTCGTCATTCCATGCTTTGTTACTAATATGGAGGCACAA
AACACTACTGAAGTATACGTAAAGTGGAAATTTAAAGGAAGAGATATTTACACCTTTGATGGAG
CTCTAAACAAGTCCACTGTCCCCACTGACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACT
AAAAGGAGATGCCTCTTTGAAGATGGATAAGAGTGATGCTGTCTCACACACAGGAAACTACACT
TGTGAAGTAACAGAATTAACCAGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTTGTTT
CATGGTTTTCTCCAAATGAAAATATTCTTATTGTTATTTTCCCAATTTTTGCTATACTCCTGTT
CTGGGGACAGTTTGGTATTAAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATT
GCTTTACTTGTTGCTGGACTAGTGATCACTGTCATTGTCATTGTTGGAGCCATTCTTTTCGTCC
CAGGTGAATATTCATTAAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATT
AATATTACTTCACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCATTGCCATA
TTGGTTATTCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGT
GTATACCAATGCATGGCCCTCTTCTGATTTCAGGTTTGAGTATCTTAGCTCTAGCACAATTACT
TGGACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCT

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GTAGAGGAACCCCTTAATGCATTCAAAGAATCAAAAGGAATGATGAATGATGAATGAGCGGCCG
CGTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAG
CCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCC
TTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGG
TGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCG
GTGGGCTCTATGGATTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATGGCCTCCAA
GGAGTAAGACCCCTGGACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGACCCTCACTG
CTGGGGAGTCCCTGCCACACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCACAGTTGCCAT
GTAGACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACCATCAATAAAGT
ACCCTGTGCTCAACCAGTTACTTGTCCTGTCTTATTCTAGGGTCTGGGGCAGAGGGGAGGGAAG
CTGGGCTTGTGTCAAGGTGAGACATTCTTGCTGGGGAGGGACCTGGTATGTTCTCCTCAGACTG
AGGGTAGGGCCTCCAAACAGCCTTGCTTGCTTCGAGAACCATTTGCTTCCCGCTCAGACGTCTT
GAGTGCTACAGGAAGCTGGCACCACTACTTCAGAGAACAAGGCCTTTTCCTCTCCTCGCTCCAG
T

SEQ ID NO: 205 - exemplary donor template for insertion at *GAPDH* locus

GAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATC
ATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATCCCTGAGCTGAACGGGAAGC
TCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCT
AGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTC
AAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACT
CCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTG
GTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCCTCTGGTGGCTG
GCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATGACAACGAGTTCGGATATAG
CAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGGAAGCGGAGCTACTAACTTC
AGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGTGGCAACTGCTGCTGC
CTACAGCTCTGCTGCTTCTGGTGTCTGCCGGCATGAGAACCGAGGATCTGCCTAAGGCCGTGGT
GTTCCTGGAACCTCAGTGGTACAGAGTGCTGGAAAAGGACAGCGTGACCCTGAAGTGCCAGGGC
GCCTATTCTCCCGAGGACAATAGCACCCAGTGGTTCCACAACGAGAGCCTGATCAGCAGCCAGG
CCAGCAGCTACTTTATCGATGCCGCCACCGTGGACGACAGCGGCGAGTACAGATGCCAGACCAA
TCTGAGCACCCTGAGCGACCCTGTGCAGCTGGAAGTGCACATTGGATGGTTGCTGCTGCAAGCC
CCTAGATGGGTGTTCAAAGAAGAGGACCCCATCCACCTGAGATGCCACTCTTGGAAGAACACAG
CCCTGCACAAAGTGACCTACCTGCAGAACGGCAAGGGCAGAAAGTACTTCCACCACAACAGCGA
CTTCTACATCCCCAAGGCCACACTGAAGGACTCCGGCTCCTACTTCTGCAGAGGCCTGGTCGGC
AGCAAGAACGTGTCCAGCGAGACAGTGAACATCACCATCACACAGGGCCTCGCCGTGTCTACCA
TCAGCAGCTTTTTCCCACCTGGCTATCAGGTGTCCTTCTGCCTGGTCATGGTGCTGCTGTTCGC
CGTGGATACCGGCCTGTACTTCAGCGTCAAGACCAACATCCGGTCCAGCACCAGAGACTGGAAG
GACCACAAGTTCAAGTGGCGGAAGGACCCTCAGGACAAGTAAGCGGCCGCGTCGAGTCTAGAGG
GCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGC
CCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATG
AGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGA
CAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGAT
TTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATGGCCTCCAAGGAGTAAGACCCCTG
GACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGACCCTCACTGCTGGGGAGTCCCTGC
CACACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCACAGTTGCCATGTAGACCCCTTGAAG
AGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACCATCAATAAAGTACCCTGTGCTCAACC

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AGTTACTTGTCCTGTCTTATTCTAGGGTCTGGGGCAGAGGGGAGGGAAGCTGGGCTTGTGTCAA
GGTGAGACATTCTTGCTGGGGAGGGACCTGGTATGTTCTCCTCAGACTGAGGGTAGGGCCTCCA
AACAGCCTTGCTTGCTTCGAGAACCATTTGCTTCCCGCTCAGACGTCTTGAGTGCTACAGGAAG
CTGGCACCACTACTTCAGAGAACAAGGCCTTTTCCTCTCCTCGCTCCAGT

SEQ ID NO: 206 - exemplary donor template for insertion at *GAPDH* locus

GTCGACGAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAG
AACATCATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATCCCTGAGCTGAACG
GGAAGCTCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTG
CCGTCTAGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGC
CCCCTCAAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACA
CCCACTCCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCAT
TTCCTGGTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCCTCTGG
TGGCTGGCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATGACAACGAGTTCGG
ATATAGCAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGGAAGCGGAGCTACT
AACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGCTTCTCCTGG
TGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGCATTCCTCCTGATCCCAGACATCCAGAT
GACACAGACTACATCCTCCCTGTCTGCCTCTCTGGGAGACAGAGTCACCATCAGTTGCAGGGCA
AGTCAGGACATTAGTAAATATTTAAATTGGTATCAGCAGAAACCAGATGGAACTGTTAAACTCC
TGATCTACCATACATCAAGATTACACTCAGGAGTCCCATCAAGGTTCAGTGGCAGTGGGTCTGG
AACAGATTATTCTCTCACCATTAGCAACCTGGAGCAAGAAGATATTGCCACTTACTTTTGCCAA
CAGGGTAATACGCTTCCGTACACGTTCGGAGGGGGGACTAAGTTGGAAATAACAGGCTCCACCT
CTGGATCCGGCAAGCCCGGATCTGGCGAGGGATCCACCAAGGGCGAGGTGAAACTGCAGGAGTC
AGGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCGTCACATGCACTGTCTCAGGGGTCTCA
TTACCCGACTATGGTGTAAGCTGGATTCGCCAGCCTCCACGAAAGGGTCTGGAGTGGCTGGGAG
TAATATGGGGTAGTGAAACCACATACTATAATTCAGCTCTCAAATCCAGACTGACCATCATCAA
GGACAACTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCAAACTGATGACACAGCCATT
TACTACTGTGCCAAACATTATTACTACGGTGGTAGCTATGCTATGGACTACTGGGGTCAAGGAA
CCTCAGTCACCGTCTCCTCAGCGGCCGCAATTGAAGTTATGTATCCTCCTCCTTACCTAGACAA
TGAGAAGAGCAATGGAACCATTATCCATGTGAAAGGGAAACACCTTTGTCCAAGTCCCCTATTT
CCCGGACCTTCTAAGCCCTTTTGGGTGCTGGTGGTGGTTGGGGGAGTCCTGGCTTGCTATAGCT
TGCTAGTAACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCAGGCTCCTGCACAG
TGACTACATGAACATGACTCCCCGCCGCCCCGGGCCCACCCGCAAGCATTACCAGCCCTATGCC
CCACCACGCGACTTCGCAGCCTATCGCTCCAGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCG
CGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGA
TGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCT
CAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGA
TGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCAC
CAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGCTAAAGCGGCCGCGTCGAG
TCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTG
TTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTA
ATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTG
GGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCT
CTATGGATTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATGGCCTCCAAGGAGTAA
GACCCCTGGACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGACCCTCACTGCTGGGGA
GTCCCTGCCACACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCACAGTTGCCATGTAGACC

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CCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACCATCAATAAAGTACCCTGT
GCTCAACCAGTTACTTGTCCTGTCTTATTCTAGGGTCTGGGGCAGAGGGGAGGGAAGCTGGGCT
TGTGTCAAGGTGAGACATTCTTGCTGGGGAGGGACCTGGTATGTTCTCCTCAGACTGAGGGTAG
GGCCTCCAAACAGCCTTGCTTGCTTCGAGAACCATTTGCTTCCCGCTCAGACGTCTTGAGTGCT
ACAGGAAGCTGGCACCACTACTTCAGAGAACAAGGCCTTTTCCTCTCCTCGCTCCAGT

SEQ ID NO: 207 - exemplary donor template for insertion at *GAPDH* locus

GTCGACGAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAG
AACATCATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATCCCTGAGCTGAACG
GGAAGCTCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTG
CCGTCTAGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGC
CCCCTCAAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACA
CCCACTCCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCAT
TTCCTGGTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCCTCTGG
TGGCTGGCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATGACAACGAGTTCGG
ATATAGCAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGGAAGCGGAGCTACT
AACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGGCACTCCCCG
TCACCGCCCTTCTCTTGCCCCTCGCCCTGCTGCTGCATGCTGCCAGGCCCATGGACGAAGTGCA
GCTCGTGGAGTCCGGTGGAGGACTCGTCCAACCGGGCGGATCCCTTCGCTTGTCCTGCGCCGCA
TCAGGCTTCAGCTTCACCAACTATGGCGTCCACTGGGTCAGACAGGCCCCCGGAAAGGGACTGG
AATGGGTGTCCGTGATCTGGAGCGGCGGGAACACCGACTACAACACCTCCGTGAAGGGCCGGTT
CACTATTAGCCGCGACAACTCCAAGAACACTCTGTACCTCCAAATGAACTCCCTGAGGGCCGAA
GATACTGCTGTGTACTATTGCGCGAGAGCCCTGACCTACTACGACTACGAGTTCGCGTACTGGG
GCCAGGGGACTCTCGTGACCGTGTCCAGCGGTGGTGGAGGTTCCGGAGGCGGAGGTTCTGGTGG
CGGGGGATCAGAAATCGTGCTGACTCAGTCCCCTGCGACCTTGTCCCTGAGCCCTGGAGAACGG
GCCACCCTGAGCTGTAGAGCCAGCCAGAGCATCGGGACAAATATTCACTGGTACCAGCAGAAAC
CCGGACAAGCACCACGGCTGCTGATCTACTACGCCTCCGAGTCGATTTCCGGAATCCCGGCTCG
CTTTTCGGGGTCTGGATCGGGAACGGACTTCACTCTGACCATCTCGTCGCTGGAACCCGAGGAT
TTCGCCGTGTACTACTGCCAACAGAACAACAATTGGCCGACCACGTTCGGCCAGGGCACCAAGC
TCGAGATTAAGGGATCACTGGAAGCGGCCGCAACCACAACACCTGCTCCAAGGCCCCCCACACC
CGCTCCAACTATAGCCAGCCAACCATTGAGCCTCAGACCTGAAGCTTGCAGGCCCGCAGCAGGA
GGCGCCGTCCATACGCGAGGCCTGGACTTCGCGTGTGATATTTATATTTGGGCCCCTTTGGCCG
GAACATGTGGGGTGTTGCTTCTCTCCCTTGTGATCACTCTGTATTGTAAGCGCGGGAGAAAGAA
GCTCCTGTACATCTTCAAGCAGCCTTTTATGCGACCTGTGCAAACCACTCAGGAAGAAGATGGG
TGTTCATGCCGCTTCCCCGAGGAGGAAGAAGGAGGGTGTGAACTGAGGGTGAAATTTTCTAGAA
GCGCCGATGCTCCCGCATATCAGCAGGGTCAGAATCAGCTCTACAATGAATTGAATCTCGGCAG
GCGAGAAGAGTACGATGTTCTGGACAAGAGACGGGGCAGGGATCCCGAGATGGGGGGAAAGCCC
CGGAGAAAAAATCCTCAGGAGGGGTTGTACAATGAGCTGCAGAAGGACAAGATGGCTGAAGCCT
ATAGCGAGATCGGAATGAAAGGCGAAAGACGCAGAGGCAAGGGGCATGACGGTCTGTACCAGGG
TCTCTCTACAGCCACCAAGGACACTTATGATGCGTTGCATATGCAAGCCTTGCCACCCCGCTAA
AGCGGCCGCGTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTA
GTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCC
CACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATT
CTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTG
GGGATGCGGTGGGCTCTATGGATTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATG
GCCTCCAAGGAGTAAGACCCCTGGACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGAC

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CCTCACTGCTGGGGAGTCCCTGCCACACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCACA
GTTGCCATGTAGACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACCATC
AATAAAGTACCCTGTGCTCAACCAGTTACTTGTCCTGTCTTATTCTAGGGTCTGGGGCAGAGGG
GAGGGAAGCTGGGCTTGTGTCAAGGTGAGACATTCTTGCTGGGGAGGGACCTGGTATGTTCTCC
TCAGACTGAGGGTAGGGCCTCCAAACAGCCTTGCTTGCTTCGAGAACCATTTGCTTCCCGCTCA
GACGTCTTGAGTGCTACAGGAAGCTGGCACCACTACTTCAGAGAACAAGGCCTTTTCCTCTCCT
CGCTCCAGT

SEQ ID NO: 208 - exemplary donor template for insertion at *GAPDH* locus

GAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATC
ATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATCCCTGAGCTGAACGGGAAGC
TCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCT
AGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTC
AAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACT
CCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTG
GTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCCTCTGGTGGCTG
GCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATGACAACGAGTTCGGATATAG
CAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGGAAGCGGAGCTACTAACTTC
AGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGGATTGGACCTGGATCC
TGTTTCTGGTGGCCGCTGCCACAAGAGTGCACAGCAATTGGGTCAACGTGATCAGCGACCTGAA
GAAGATCGAGGACCTGATCCAGAGCATGCACATCGACGCCACACTGTACACCGAGTCCGATGTG
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CTGCTGCCACCACAGCCGCTATCGTGCCTGGATCTCAGCTGATGCCTAGCAAGAGCCCTAGCAC
CGGCACAACAGAGATCAGCTCTCACGAGAGCAGCCACGGAACACCTTCTCAGACCACCGCCAAG
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GGCCTGCTACCTGAAGTCTAGACAGACACCTCCTCTGGCCAGCGTGGAAATGGAAGCCATGGAA
GCTCTGCCTGTCACATGGGGCACCAGCAGCAGAGATGAGGACCTCGAGAATTGCAGCCACCACC
TGGGAAGCGGAGCCACAAACTTCTCTCTGCTGAAGCAGGCAGGAGATGTTGAAGAAAACCCTGG
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AGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGAC
CACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTC
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CACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCC

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ACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGA
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TGGACGAGCTGTACAAGTAAGCGGCCGCGTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCA
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TGAATCTCCCCTCCTCACAGTTGCCATGTAGACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCG
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TAGGGTCTGGGGCAGAGGGGAGGGAAGCTGGGCTTGTGTCAAGGTGAGACATTCTTGCTGGGGA
GGGACCTGGTATGTTCTCCTCAGACTGAGGGTAGGGCCTCCAAACAGCCTTGCTTGCTTCGAGA
ACCATTTGCTTCCCGCTCAGACGTCTTGAGTGCTACAGGAAGCTGGCACCACTACTTCAGAGAA
CAAGGCCTTTTCCTCTCCTCGCTCCAGT

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GAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATC
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TCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCT
AGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTC
AAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACT
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GTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCCTCTGGTGGCTG
GCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATGACAACGAGTTCGGATATAG
CAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGGAAGCGGAGCTACTAACTTC
AGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGTGGCAGCTGTTGCTGC
CGACAGCCCTCCTGTTGCTGGTCTCCGCTGGCATGAGAACCGAGGATCTGCCTAAGGCCGTGGT
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CCTAGATGGGTGTTCAAAGAAGAGGACCCCATCCACCTGAGATGCCACTCTTGGAAGAACACAG
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CGGCGACGCCAGCATCCACGATACCGTGGAAAACCTGATCATCCTGGCCAACAACAGCCTGAGC
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AAGAGTTCCTCCAGAGCTTCGTCCACATCGTGCAGATGTTCATCAACACCAGCGGAAGCGGAGC
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GCTCTGGTTCATCAGAGGCCTGCCCCTCCATCTACAGTGACAACAGCTGGCGTGACCCCTCAGC
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GGGATGCGGTGGGCTCTATGGATTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATG
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GAGGGAAGCTGGGCTTGTGTCAAGGTGAGACATTCTTGCTGGGGAGGGACCTGGTATGTTCTCC
TCAGACTGAGGGTAGGGCCTCCAAACAGCCTTGCTTGCTTCGAGAACCATTTGCTTCCCGCTCA
GACGTCTTGAGTGCTACAGGAAGCTGGCACCACTACTTCAGAGAACAAGGCCTTTTCCTCTCCT
CGCTCCAGT

SEQ ID NO: 210 - exemplary donor template for insertion at *GAPDH* locus

GAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATC
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TCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCT
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CAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGGAAGCGGAGCTACTAACTTC
AGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGGATTGGACCTGGATCC
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GAGGAAGCGGAGGCGGAGGATCTGGTGGTGGTGGATCTGGCGGCGGTGGTAGTGGCGGAGGTTC
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AGCCTGTACAGCAGAGAGCGGTACATCTGCAACAGCGGCTTCAAGAGAAAGGCCGGCACAAGCA
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GCTCTAACAATACTGCTGCCACCACAGCCGCTATCGTGCCTGGATCTCAGCTGATGCCTAGCAA
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AGGGCCACTCTGATACAACAGTGGCCATCAGCACCAGCACCGTTCTGCTGTGTGGCCTGTCTGC
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GCAGCCACCACCTGGGAAGCGGAGCCACAAACTTCTCTCTGCTGAAGCAGGCAGGAGATGTTGA
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GCTTCCCGCTCAGACGTCTTGAGTGCTACAGGAAGCTGGCACCACTACTTCAGAGAACAAGGCC
TTTTCCTCTCCTCGCTCCAGT

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GAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATC
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CCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTG
GTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCCTCTGGTGGCTG
GCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATGACAACGAGTTCGGATATAG

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CAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGGAAGCGGAGCTACTAACTTC
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GCGTGACCCTGAAGTGCCAGGGCGCCTATTCTCCCGAGGACAATAGCACCCAGTGGTTCCACAA
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GCCTCCAAGGAGTAAGACCCCTGGACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGAC
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TCAGACTGAGGGTAGGGCCTCCAAACAGCCTTGCTTGCTTCGAGAACCATTTGCTTCCCGCTCA
GACGTCTTGAGTGCTACAGGAAGCTGGCACCACTACTTCAGAGAACAAGGCCTTTTCCTCTCCT
CGCTCCAGT

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GAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATC
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TGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATA
GCAGGCATGCTGGGGATGCGGTGGGCTCTATGGATTTGGCTACAGCAACAGGGTGGTGGACCTC
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TCATGTACCATCAATAAAGTACCCTGTGCTCAACCAGTTACTTGTCCTGTCTTATTCTAGGGTC
TGGGGCAGAGGGGAGGGAAGCTGGGCTTGTGTCAAGGTGAGACATTCTTGCTGGGGAGGGACCT
GGTATGTTCTCCTCAGACTGAGGGTAGGGCCTCCAAACAGCCTTGCTTGCTTCGAGAACCATTT
GCTTCCCGCTCAGACGTCTTGAGTGCTACAGGAAGCTGGCACCACTACTTCAGAGAACAAGGCC
TTTTCCTCTCCTCGCTCCAGT

SEQ ID NO: 213 - exemplary donor template for insertion at *GAPDH* locus

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TCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCT
AGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTC
AAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACT
CCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTG
GTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCCTCTGGTGGCTG
GCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATGACAACGAGTTCGGATATAG
CAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGGAAGCGGAGCTACTAACTTC
AGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGTGGCAGCTGTTGCTGC
CGACAGCCCTCCTGTTGCTGGTCTCCGCTGGCATGAGAACCGAGGATCTGCCTAAGGCCGTGGT
GTTCCTGGAACCTCAGTGGTACAGAGTGCTGGAAAAGGACAGCGTGACCCTGAAGTGCCAGGGC
GCCTATTCTCCCGAGGACAATAGCACCCAGTGGTTCCACAACGAGAGCCTGATCAGCAGCCAGG
CCAGCAGCTACTTTATCGATGCCGCCACCGTGGACGACAGCGGCGAGTACAGATGCCAGACCAA
TCTGAGCACCCTGAGCGACCCTGTGCAGCTGGAAGTGCACATTGGATGGTTGCTGCTGCAAGCC
CCTAGATGGGTGTTCAAAGAAGAGGACCCCATCCACCTGAGATGCCACTCTTGGAAGAACACAG
CCCTGCACAAAGTGACCTACCTGCAGAACGGCAAGGGCAGAAAGTACTTCCACCACAACAGCGA
CTTCTACATCCCCAAGGCCACACTGAAGGACTCCGGCTCCTACTTCTGCAGAGGCCTGGTCGGC
AGCAAGAACGTGTCCAGCGAGACAGTGAACATCACCATCACACAGGGCCTCGCCGTGTCTACCA
TCAGCAGCTTTTTCCCACCTGGCTATCAGGTGTCCTTCTGCCTGGTCATGGTGCTGCTGTTCGC
CGTGGATACCGGCCTGTACTTCAGCGTCAAGACCAACATCCGGTCCAGCACCAGAGACTGGAAG
GACCACAAGTTCAAGTGGCGGAAGGACCCTCAGGACAAGGGAAGCGGAGCCACAAACTTCTCTC
TGCTGAAGCAGGCAGGAGATGTTGAAGAAAACCCTGGACCTATGGATTGGACCTGGATCCTGTT
TCTGGTGGCCGCTGCCACAAGAGTGCACAGCAATTGGGTCAACGTGATCAGCGACCTGAAGAAG
ATCGAGGACCTGATCCAGAGCATGCACATCGACGCCACACTGTACACCGAGTCCGATGTGCACC
CTAGCTGCAAAGTGACCGCCATGAAGTGCTTTCTGCTGGAACTGCAAGTGATCAGCCTGGAAAG
CGGCGACGCCAGCATCCACGATACCGTGGAAAACCTGATCATCCTGGCCAACAACAGCCTGAGC
AGCAACGGCAATGTGACCGAGAGCGGCTGCAAAGAGTGCGAGGAACTGGAAGAGAAGAACATCA
AAGAGTTCCTCCAGAGCTTCGTCCACATCGTGCAGATGTTCATCAACACCAGCTCTGGCGGAGG
AAGCGGAGGCGGAGGATCTGGTGGTGGTGGATCTGGCGGCGGTGGTAGTGGCGGAGGTTCTCTG
CAAATCACCTGTCCTCCACCTATGAGCGTGGAACACGCCGACATCTGGGTCAAGAGCTACAGCC
TGTACAGCAGAGAGCGGTACATCTGCAACAGCGGCTTCAAGAGAAAGGCCGGCACAAGCAGCCT
GACCGAGTGTGTGCTGAACAAGGCCACAAACGTGGCCCACTGGACCACACCTAGCCTGAAGTGC
ATCAGAGATCCCGCTCTGGTTCATCAGAGGCCTGCCCCTCCATCTACAGTGACAACAGCTGGCG

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TGACCCCTCAGCCTGAGTCTCTGTCTCCATCTGGAAAAGAGCCTGCCGCCAGCTCTCCCAGCTC
TAACAATACTGCTGCCACCACAGCCGCTATCGTGCCTGGATCTCAGCTGATGCCTAGCAAGAGC
CCTAGCACCGGCACAACAGAGATCAGCTCTCACGAGAGCAGCCACGGAACACCTTCTCAGACCA
CCGCCAAGAATTGGGAGCTGACAGCCTCTGCCTCTCATCAGCCACCTGGCGTGTACCCACAGGG
CCACTCTGATACAACAGTGGCCATCAGCACCAGCACCGTTCTGCTGTGTGGCCTGTCTGCTGTT
AGCCTGCTGGCCTGCTACCTGAAGTCTAGACAGACACCTCCTCTGGCCAGCGTGGAAATGGAAG
CCATGGAAGCTCTGCCTGTCACATGGGGCACCAGCAGCAGAGATGAGGACCTCGAGAATTGCAG
CCACCACCTGTAAGCGGCCGCGTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGA
CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGA
AGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGG
TGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATA
GCAGGCATGCTGGGGATGCGGTGGGCTCTATGGATTTGGCTACAGCAACAGGGTGGTGGACCTC
ATGGCCCACATGGCCTCCAAGGAGTAAGACCCCTGGACCACCAGCCCCAGCAAGAGCACAAGAG
GAAGAGAGAGACCCTCACTGCTGGGGAGTCCCTGCCACACTCAGTCCCCCACCACACTGAATCT
CCCCTCCTCACAGTTGCCATGTAGACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTTG
TCATGTACCATCAATAAAGTACCCTGTGCTCAACCAGTTACTTGTCCTGTCTTATTCTAGGGTC
TGGGGCAGAGGGGAGGGAAGCTGGGCTTGTGTCAAGGTGAGACATTCTTGCTGGGGAGGGACCT
GGTATGTTCTCCTCAGACTGAGGGTAGGGCCTCCAAACAGCCTTGCTTGCTTCGAGAACCATTT
GCTTCCCGCTCAGACGTCTTGAGTGCTACAGGAAGCTGGCACCACTACTTCAGAGAACAAGGCC
TTTTCCTCTCCTCGCTCCAGT

SEQ ID NO: 214 - exemplary donor template for insertion at *GAPDH* locus

GAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATC
ATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATCCCTGAGCTGAACGGGAAGC
TCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCT
AGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTC
AAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACT
CCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTG
GTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCCTCTGGTGGCTG
GCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATGACAACGAGTTCGGATATAG
CAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGGAAGCGGAGCTACTAACTTC
AGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGTCAAATATTACAGATC
CACAGATGTGGGATTTTGATGATCTAAATTTCACTGGCATGCCACCTGCAGATGAAGATTACAG
CCCCTGTATGCTAGAAACTGAGACACTCAACAAGTATGTTGTGATCATCGCCTATGCCCTAGTG
TTCCTGCTGAGCCTGCTGGGAAACTCCCTGGTGATGCTGGTCATCTTATACAGCAGGGTCGGCC
GCTCCGTCACTGATGTCTACCTGCTGAACCTGGCCTTGGCCGACCTACTCTTTGCCCTGACCTT
GCCCATCTGGGCCGCCTCCAAGGTGAATGGCTGGATTTTTGGCACATTCCTGTGCAAGGTGGTC
TCACTCCTGAAGGAAGTCAACTTCTACAGTGGCATCCTGCTGTTGGCCTGCATCAGTGTGGACC
GTTACCTGGCCATTGTCCATGCCACACGCACACTGACCCAGAAGCGTCACTTGGTCAAGTTTGT
TTGTCTTGGCTGCTGGGGACTGTCTATGAATCTGTCCCTGCCCTTCTTCCTTTTCCGCCAGGCT
TACCATCCAAACAATTCCAGTCCAGTTTGCTATGAGGTCCTGGGAAATGACACAGCAAAATGGC
GGATGGTGTTGCGGATCCTGCCTCACACCTTTGGCTTCATCGTGCCGCTGTTTGTCATGCTGTT
CTGCTATGGATTCACCCTGCGTACACTGTTTAAGGCCCACATGGGGCAGAAGCACCGAGCCATG
AGGGTCATCTTTGCTGTCGTCCTCATCTTCCTGCTTTGCTGGCTGCCCTACAACCTGGTCCTGC
TGGCAGACACCCTCATGAGGACCCAGGTGATCCAGGAGAGCTGTGAGCGCCGCAACAACATCGG
CCGGGCCCTGGATGCCACTGAGATTCTGGGATTTCTCCATAGCTGCCTCAACCCCATCATCTAC

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GCCTTCATCGGCCAAAATTTTCGCCATGGATTCCTCAAGATCCTGGCTATGCATGGCCTGGTCA
GCAAGGAGTTCTTGGCACGTCATCGTGTTACCTCCTACACTTCTTCGTCTGTCAATGTCTCTTC
CAACCTCTGAATTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATGGCCTCCAAGGA
GTAAGACCCCTGGACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGACCCTCACTGCTG
GGGAGTCCCTGCCACACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCACAGTTGCCATGTA
GACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACCATCAATAAAGTACC
CTGTGCTCAACCAGTTACTTGTCCTGTCTTATTCTAGGGTCTGGGGCAGAGGGGAGGGAAGCTG
GGCTTGTGTCAAGGTGAGACATTCTTGCTGGGGAGGGACCTGGTATGTTCTCCTCAGACTGAGG
GTAGGGCCTCCAAACAGCCTTGCTTGCTTCGAGAACCATTTGCTTCCCGCTCAGACGTCTTGAG
TGCTACAGGAAGCTGGCACCACTACTTCAGAGAACAAGGCCTTTTCCTCTCCTCGCTCCAGT

SEQ ID NO: 215 - exemplary donor template for insertion at *GAPDH* locus

GAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATC
ATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATCCCTGAGCTGAACGGGAAGC
TCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCT
AGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTC
AAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACT
CCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTG
GTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCCTCTGGTGGCTG
GCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATGACAACGAGTTCGGATATAG
CAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGGAAGCGGAGCTACTAACTTC
AGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGGAGTTGAGGAAGTACG
GCCCTGGAAGACTGGCGGGGACAGTTATAGGAGGAGCTGCTCAGAGTAAATCACAGACTAAATC
AGACTCAATCACAAAAGAGTTCCTGCCAGGCCTTTACACAGCCCCTTCCTCCCCGTTCCCGCCC
TCACAGGTGAGTGACCACCAAGTGCTAAATGACGCCGAGGTTGCCGCCCTCCTGGAGAACTTCA
GCTCTTCCTATGACTATGGAGAAAACGAGAGTGACTCGTGCTGTACCTCCCCGCCCTGCCCACA
GGACTTCAGCCTGAACTTCGACCGGGCCTTCCTGCCAGCCCTCTACAGCCTCCTCTTTCTGCTG
GGGCTGCTGGGCAACGGCGCGGTGGCAGCCGTGCTGCTGAGCCGGCGGACAGCCCTGAGCAGCA
CCGACACCTTCCTGCTCCACCTAGCTGTAGCAGACACGCTGCTGGTGCTGACACTGCCGCTCTG
GGCAGTGGACGCTGCCGTCCAGTGGGTCTTTGGCTCTGGCCTCTGCAAAGTGGCAGGTGCCCTC
TTCAACATCAACTTCTACGCAGGAGCCCTCCTGCTGGCCTGCATCAGCTTTGACCGCTACCTGA
ACATAGTTCATGCCACCCAGCTCTACCGCCGGGGGCCCCCGGCCCGCGTGACCCTCACCTGCCT
GGCTGTCTGGGGGCTCTGCCTGCTTTTCGCCCTCCCAGACTTCATCTTCCTGTCGGCCCACCAC
GACGAGCGCCTCAACGCCACCCACTGCCAATACAACTTCCCACAGGTGGGCCGCACGGCTCTGC
GGGTGCTGCAGCTGGTGGCTGGCTTTCTGCTGCCCCTGCTGGTCATGGCCTACTGCTATGCCCA
CATCCTGGCCGTGCTGCTGGTTTCCAGGGGCCAGCGGCGCCTGCGGGCCATGCGGCTGGTGGTG
GTGGTCGTGGTGGCCTTTGCCCTCTGCTGGACCCCCTATCACCTGGTGGTGCTGGTGGACATCC
TCATGGACCTGGGCGCTTTGGCCCGCAACTGTGGCCGAGAAAGCAGGGTAGACGTGGCCAAGTC
GGTCACCTCAGGCCTGGGCTACATGCACTGCTGCCTCAACCCGCTGCTCTATGCCTTTGTAGGG
GTCAAGTTCCGGGAGCGGATGTGGATGCTGCTCTTGCGCCTGGGCTGCCCCAACCAGAGAGGGC
TCCAGAGGCAGCCATCGTCTTCCCGCCGGGATTCATCCTGGTCTGAGACCTCAGAGGCCTCCTA
CTCGGGCTTGTGAATTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATGGCCTCCAA
GGAGTAAGACCCCTGGACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGACCCTCACTG
CTGGGGAGTCCCTGCCACACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCACAGTTGCCAT
GTAGACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACCATCAATAAAGT
ACCCTGTGCTCAACCAGTTACTTGTCCTGTCTTATTCTAGGGTCTGGGGCAGAGGGGAGGGAAG

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CTGGGCTTGTGTCAAGGTGAGACATTCTTGCTGGGGAGGGACCTGGTATGTTCTCCTCAGACTG
AGGGTAGGGCCTCCAAACAGCCTTGCTTGCTTCGAGAACCATTTGCTTCCCGCTCAGACGTCTT
GAGTGCTACAGGAAGCTGGCACCACTACTTCAGAGAACAAGGCCTTTTCCTCTCCTCGCTCCAG
T

SEQ ID NO: 216 - exemplary donor template for insertion at *GAPDH* locus

GAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATC
ATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATCCCTGAGCTGAACGGGAAGC
TCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCT
AGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTC
AAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACT
CCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTG
GTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCCTCTGGTGGCTG
GCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATGACAACGAGTTCGGATATAG
CAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGGAAGCGGAGCTACTAACTTC
AGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGGTCCTTGAGGTGAGTG
ACCACCAAGTGCTAAATGACGCCGAGGTTGCCGCCCTCCTGGAGAACTTCAGCTCTTCCTATGA
CTATGGAGAAAACGAGAGTGACTCGTGCTGTACCTCCCCGCCCTGCCCACAGGACTTCAGCCTG
AACTTCGACCGGGCCTTCCTGCCAGCCCTCTACAGCCTCCTCTTTCTGCTGGGGCTGCTGGGCA
ACGGCGCGGTGGCAGCCGTGCTGCTGAGCCGGCGGACAGCCCTGAGCAGCACCGACACCTTCCT
GCTCCACCTAGCTGTAGCAGACACGCTGCTGGTGCTGACACTGCCGCTCTGGGCAGTGGACGCT
GCCGTCCAGTGGGTCTTTGGCTCTGGCCTCTGCAAAGTGGCAGGTGCCCTCTTCAACATCAACT
TCTACGCAGGAGCCCTCCTGCTGGCCTGCATCAGCTTTGACCGCTACCTGAACATAGTTCATGC
CACCCAGCTCTACCGCCGGGGGCCCCCGGCCCGCGTGACCCTCACCTGCCTGGCTGTCTGGGGG
CTCTGCCTGCTTTTCGCCCTCCCAGACTTCATCTTCCTGTCGGCCCACCACGACGAGCGCCTCA
ACGCCACCCACTGCCAATACAACTTCCCACAGGTGGGCCGCACGGCTCTGCGGGTGCTGCAGCT
GGTGGCTGGCTTTCTGCTGCCCCTGCTGGTCATGGCCTACTGCTATGCCCACATCCTGGCCGTG
CTGCTGGTTTCCAGGGGCCAGCGGCGCCTGCGGGCCATGCGGCTGGTGGTGGTGGTCGTGGTGG
CCTTTGCCCTCTGCTGGACCCCCTATCACCTGGTGGTGCTGGTGGACATCCTCATGGACCTGGG
CGCTTTGGCCCGCAACTGTGGCCGAGAAAGCAGGGTAGACGTGGCCAAGTCGGTCACCTCAGGC
CTGGGCTACATGCACTGCTGCCTCAACCCGCTGCTCTATGCCTTTGTAGGGGTCAAGTTCCGGG
AGCGGATGTGGATGCTGCTCTTGCGCCTGGGCTGCCCCAACCAGAGAGGGCTCCAGAGGCAGCC
ATCGTCTTCCCGCCGGGATTCATCCTGGTCTGAGACCTCAGAGGCCTCCTACTCGGGCTTGTGA
ATTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATGGCCTCCAAGGAGTAAGACCCC
TGGACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGACCCTCACTGCTGGGGAGTCCCT
GCCACACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCACAGTTGCCATGTAGACCCCTTGA
AGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACCATCAATAAAGTACCCTGTGCTCAA
CCAGTTACTTGTCCTGTCTTATTCTAGGGTCTGGGGCAGAGGGGAGGGAAGCTGGGCTTGTGTC
AAGGTGAGACATTCTTGCTGGGGAGGGACCTGGTATGTTCTCCTCAGACTGAGGGTAGGGCCTC
CAAACAGCCTTGCTTGCTTCGAGAACCATTTGCTTCCCGCTCAGACGTCTTGAGTGCTACAGGA
AGCTGGCACCACTACTTCAGAGAACAAGGCCTTTTCCTCTCCTCGCTCCAGT

SEQ ID NO: 217 - exemplary donor template for insertion at *GAPDH* locus

GAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATC
ATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATCCCTGAGCTGAACGGGAAGC

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TCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCT
AGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTC
AAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACT
CCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTG
GTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCCTCTGGTGGCTG
GCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATGACAACGAGTTCGGATATAG
CAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGGAAGCGGAGCTACTAACTTC
AGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGGATTATCAAGTGTCAA
GTCCAATCTATGACATCAATTATTATACATCGGAGCCCTGCCAAAAAATCAATGTGAAGCAAAT
CGCAGCCCGCCTCCTGCCTCCGCTCTACTCACTGGTGTTCATCTTTGGTTTTGTGGGCAACATG
CTGGTCATCCTCATCCTGATAAACTGCAAAAGGCTGAAGAGCATGACTGACATCTACCTGCTCA
ACCTGGCCATCTCTGACCTGTTTTTCCTTCTTACTGTCCCCTTCTGGGCTCACTATGCTGCCGC
CCAGTGGGACTTTGGAAATACAATGTGTCAACTCTTGACAGGGCTCTATTTTATAGGCTTCTTC
TCTGGAATCTTCTTCATCATCCTCCTGACAATCGATAGGTACCTGGCTGTCGTCCATGCTGTGT
TTGCTTTAAAAGCCAGGACGGTCACCTTTGGGGTGGTGACAAGTGTGATCACTTGGGTGGTGGC
TGTGTTTGCGTCTCTCCCAGGAATCATCTTTACCAGATCTCAAAAAGAAGGTCTTCATTACACC
TGCAGCTCTCATTTTCCATACAGTCAGTATCAATTCTGGAAGAATTTCCAGACATTAAAGATAG
TCATCTTGGGGCTGGTCCTGCCGCTGCTTGTCATGGTCATCTGCTACTCGGGAATCCTAAAAAC
TCTGCTTCGGTGTCGAAATGAGAAGAAGAGGCACAGGGCTGTGAGGCTTATCTTCACCATCATG
ATTGTTTATTTTCTCTTCTGGGCTCCCTACAACATTGTCCTTCTCCTGAACACCTTCCAGGAAT
TCTTTGGCCTGAATAATTGCAGTAGCTCTAACAGGTTGGACCAAGCTATGCAGGTGACAGAGAC
TCTTGGGATGACGCACTGCTGCATCAACCCCATCATCTATGCCTTTGTCGGGGAGAAGTTCAGA
AACTACCTCTTAGTCTTCTTCCAAAAGCACATTGCCAAACGCTTCTGCAAATGCTGTTCTATTT
TCCAGCAAGAGGCTCCCGAGCGAGCAAGCTCAGTTTACACCCGATCCACTGGGGAGCAGGAAAT
ATCTGTGGGCTTGTGAATTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATGGCCTC
CAAGGAGTAAGACCCCTGGACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGACCCTCA
CTGCTGGGGAGTCCCTGCCACACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCACAGTTGC
CATGTAGACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACCATCAATAA
AGTACCCTGTGCTCAACCAGTTACTTGTCCTGTCTTATTCTAGGGTCTGGGGCAGAGGGGAGGG
AAGCTGGGCTTGTGTCAAGGTGAGACATTCTTGCTGGGGAGGGACCTGGTATGTTCTCCTCAGA
CTGAGGGTAGGGCCTCCAAACAGCCTTGCTTGCTTCGAGAACCATTTGCTTCCCGCTCAGACGT
CTTGAGTGCTACAGGAAGCTGGCACCACTACTTCAGAGAACAAGGCCTTTTCCTCTCCTCGCTC
CAGT

SEQ ID NO: 218 - exemplary donor template for insertion at *GAPDH* locus

GAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATC
ATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATCCCTGAGCTGAACGGGAAGC
TCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCT
AGAAAAACCTGCCAAATATGATGACATCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTC
AAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACT
CCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTG
GTATGTGGCTGGGGCCAGAGACTGGCTCTTAAAAAGTGCAGGGTCTGGCGCCCTCTGGTGGCTG
GCTCAGAAAAAGGGCCCTGACAACTCTTTACATCTTCTAGGTATGACAACGAGTTCGGATATAG
CAATAGAGTGGTCGATCTGATGGCTCATATGGCTAGCAAAGAGGGAAGCGGAGCTACTAACTTC
AGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGCTGTCCACATCTCGTT
CTCGGTTTATCAGAAATACCAACGAGAGCGGTGAAGAAGTCACCACCTTTTTTGATTATGATTA

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CGGTGCTCCCTGTCATAAATTTGACGTGAAGCAAATTGGGGCCCAACTCCTGCCTCCGCTCTAC
TCGCTGGTGTTCATCTTTGGTTTTGTGGGCAACATGCTGGTCGTCCTCATCTTAATAAACTGCA
AAAAGCTGAAGTGCTTGACTGACATTTACCTGCTCAACCTGGCCATCTCTGATCTGCTTTTTCT
TATTACTCTCCCATTGTGGGCTCACTCTGCTGCAAATGAGTGGGTCTTTGGGAATGCAATGTGC
AAATTATTCACAGGGCTGTATCACATCGGTTATTTTGGCGGAATCTTCTTCATCATCCTCCTGA
CAATCGATAGATACCTGGCTATTGTCCATGCTGTGTTTGCTTTAAAAGCCAGGACGGTCACCTT
TGGGGTGGTGACAAGTGTGATCACCTGGTTGGTGGCTGTGTTTGCTTCTGTCCCAGGAATCATC
TTTACTAAATGCCAGAAAGAAGATTCTGTTTATGTCTGTGGCCCTTATTTTCCACGAGGATGGA
ATAATTTCCACACAATAATGAGGAACATTTTGGGGCTGGTCCTGCCGCTGCTCATCATGGTCAT
CTGCTACTCGGGAATCCTGAAAACCCTGCTTCGGTGTCGAAACGAGAAGAAGAGGCATAGGGCA
GTGAGAGTCATCTTCACCATCATGATTGTTTACTTTCTCTTCTGGACTCCCTATAATATTGTCA
TTCTCCTGAACACCTTCCAGGAATTCTTCGGCCTGAGTAACTGTGAAAGCACCAGTCAACTGGA
CCAAGCCACGCAGGTGACAGAGACTCTTGGGATGACTCACTGCTGCATCAATCCCATCATCTAT
GCCTTCGTTGGGGAGAAGTTCAGAAGCCTTTTTCACATAGCTCTTGGCTGTAGGATTGCCCCAC
TCCAAAAACCAGTGTGTGGAGGTCCAGGAGTGAGACCAGGAAAGAATGTGAAAGTGACTACACA
AGGACTCCTCGATGGTCGTGGAAAAGGAAAGTCAATTGGCAGAGCCCCTGAAGCCAGTCTTCAG
GACAAAGAAGGAGCCTAGATTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATGGCC
TCCAAGGAGTAAGACCCCTGGACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGACCCT
CACTGCTGGGGAGTCCCTGCCACACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCACAGTT
GCCATGTAGACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACCATCAAT
AAAGTACCCTGTGCTCAACCAGTTACTTGTCCTGTCTTATTCTAGGGTCTGGGGCAGAGGGGAG
GGAAGCTGGGCTTGTGTCAAGGTGAGACATTCTTGCTGGGGAGGGACCTGGTATGTTCTCCTCA
GACTGAGGGTAGGGCCTCCAAACAGCCTTGCTTGCTTCGAGAACCATTTGCTTCCCGCTCAGAC
GTCTTGAGTGCTACAGGAAGCTGGCACCACTACTTCAGAGAACAAGGCCTTTTCCTCTCCTCGC
TCCAGT

Methods of Editing the Genome of a Cell for Gain-of-Function Modifications

[0465] In one aspect, the present disclosure provides methods of editing the genome of a cell. In certain embodiments, the method comprises contacting the cell with a nuclease that causes a break within an endogenous coding sequence of an essential gene in the cell wherein the essential gene encodes at least one gene product that is required for survival and/or proliferation of the cell. The cell is also contacted with (i) a donor template that comprises a knock-in cassette comprising an exogenous coding sequence for a gene product of interest in frame with and downstream (3′) of an exogenous coding sequence or partial coding sequence of the essential gene and/or (ii) a donor template that comprises a knock-in cassette comprising an exogenous coding sequence for a gene product of interest in frame with and upstream (5′) of an exogenous coding sequence or partial coding sequence of the essential gene (Fig. 3D). The knock-in cassette is integrated into the genome of the cell by homology-directed repair (HDR) of the break, resulting in a genome-edited cell that expresses the gene product of interest and the gene product

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encoded by the essential gene that is required for survival and/or proliferation of the cell, or a functional variant thereof. The genetically modified "knock-in" cell survives and proliferates to produce progeny cells with genomes that also include the exogenous coding sequence for the gene product of interest. This is illustrated in Fig. 3A for an exemplary method.

[0466] If the knock-in cassette is not properly integrated into the genome of the cell, undesired editing events that result from the break, e.g., NHEJ-mediated creation of indels, may produce a non-functional, e.g., out of frame, version of the essential gene. This produces a "knock-out" cell when the editing efficiency of the nuclease is high enough to disrupt both alleles. In certain embodiments, this produces a "knock-out" cell when the editing efficiency of the nuclease is high enough to disrupt one allele. Without sufficient functional copies of the essential gene these "knock-out" cells are unable to survive and do not produce any progeny cells.

[0467] In some embodiments, the present disclosure provides methods of editing the genome of a cell. In certain embodiments, the method comprises contacting the cell with a nuclease that causes a break within an endogenous non-coding sequence of an essential gene in the cell wherein the essential gene encodes at least one gene product that is required for survival and/or proliferation of the cell. In some embodiments, such a break within an endogenous non-coding sequence alters a functional region of an essential gene that influences post-transcriptional modification patterns, e.g., mRNA splicing, RNA stability, RNA editing, RNA interference, *etc.* In some embodiments, such a break within an endogenous non-coding sequence occurs in a functional region of the essential gene, for example, but not limited to: a splicesome target site (*e.g.*, a 5′ splice donor site, an intron branch point sequence, a 3′ splice acceptor site, and/or a polypyrimidine tract), an intronic splicing silencer, an intronic splicing enhancer, an exonic splicing silencer, an exonic splicing enhancer, an endogenous RNA interference binding site (*e.g.*, micro RNA, small interfering RNA, *etc.*), an endogenous RNA editing machinery binding site (*e.g.*, a binding site for adenosine deaminases, cytidine deaminases, *etc.*), or combinations thereof. In some embodiments, the nuclease causes a break at or near where an intron borders an exon in an essential gene, reducing or disrupting the function of the essential gene.

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[0468] Since the "knock-in" cells survive and the "knock-out" cells do not survive, the method automatically selects for the "knock-in" cells when it is applied to a population of starting cells. Significantly, in certain embodiments, the method does not require high knock-in efficiencies because of this automatic selection aspect. It is therefore particularly suitable for methods where the donor template is a dsDNA (e.g., a plasmid) where knock-in efficiencies are often below 5%. As noted in the exemplary method of Fig. 3C, in some embodiments some of the cells in the population of starting cells may remain unedited, i.e., unaffected by the nuclease. These cells would also survive and produce progeny with genomes that do not include the exogenous coding sequence for the gene product of interest. When the nuclease editing efficiency is high, e.g., about 60-90%, or higher the percentage of unedited cells will be relatively low as compared to the percentage of genetically modified cells. In some embodiments, high nuclease editing efficiencies (e.g., greater than 65%, greater than 70%, greater than 75%, greater than 80%, greater than 85%, greater than 90%, or greater than 95%) facilitates efficient population wide transgene integration, as the percentage of unedited cells will be relatively low as compared to the percentage of genetically modified cells. In some embodiments of the methods disclosed herein, at least about 65% of the cells (e.g., about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% of the cells) are edited by a nuclease, e.g., a Cas12a, Cas9, Cas12b, Cas12c, Cas12e, CasX, or CasΦ (Cas12j), or a variant thereof (e.g., a variant with a high editing efficiency). In some embodiments, an RNP containing a CRISPR nuclease (e.g., Cas12a, Cas9, Cas12b, Cas12c, Cas12e, CasX, or CasΦ (Cas12j), or a variant thereof (e.g., a variant with a high editing efficiency)) and a guide are capable of cleaving the locus of an essential gene (e.g., a terminal exon in the locus of any essential gene provided in Table 3) in at least 65% of the cells in a population of cells (e.g., at least 65%, at least 70%, at least 75%, 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%, or at least 99% of the cells in a population of cells). In some embodiments, an RNP containing a CRISPR nuclease (e.g., Cas12a, Cas9, Cas12b, Cas12c, Cas12e, CasX, or CasΦ (Cas12j), or a variant thereof (e.g., a variant with a high editing efficiency)) and a guide are capable of inducing knock-in cassette integration at a locus of an essential gene (e.g., a terminal exon in the locus of any essential gene

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provided in Table 3) in at least 65% of the cells in a population of cells (e.g., at least 65%, at least 70%, at least 75%, 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%, or at least 99% of the cells in a population of cells), e.g., at between 4 days and 10 days (e.g., 4 days, 5 days, 6 days, 7 days, 8 days, 9 days or 10 days) after the cells in the population of cells is contacted with the RNP containing a CRISPR nuclease. In some embodiments, editing efficiency is determined prior to target cell die off, e.g., at day 1 and/or day 2 post transfection or transduction. In some embodiments, editing efficiency measured at day 1 and/or day 2 post transfection or transduction may not capture the complete proportion of cells for which editing occurred, as in some embodiments, certain editing events may result in near immediate and/or swift cell death. In some embodiments, near immediate and/or swift cell death may be any period of time less than 48 hours post transfection or transduction, for example, less than 48 hours, less than 44 hours, less than 40 hours, less than 36 hours, less than 32 hours, less than 28 hours, less than 24 hours, less than 20 hours, less than 16 hours, less than 15 hours, less than 14 hours, less than 13 hours, less than 12 hours, less than 11 hours, less than 10 hours, less than 9 hours, less than 8 hours, less than 7 hours, less than 6 hours, less than 5 hours, less than 4 hours, less than 3 hours, less than 2 hours, or less than 1 hour after transfection or transduction.

[0469] In some embodiments, the nuclease causes a double-strand break. In some embodiments the nuclease causes a single-strand break, e.g., in some embodiments the nuclease is a nickase. In some embodiments the nuclease is a prime editor which comprises a nickase domain fused to a reverse transcriptase domain. In some embodiments the nuclease is an RNA-guided prime editor and the gRNA comprises the donor template. In some embodiments a dual-nickase system is used which causes a double-strand break via two single-strand breaks on opposing strands of a double-stranded DNA, e.g., genomic DNA of the cell.

[0470] In some embodiments, the present disclosure provides methods suitable for high-efficiency knock-in (e.g., a high proportion of a cell population comprises a knock-in allele), overcoming a major manufacturing challenge. In some embodiments, high-efficiency knock-in results in at least 65% of the cells in a population of cells comprising a knock-in allele (e.g., at least 65%, at least 70%, at least 75%, 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%, or at

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least 99% of the cells in a population of cells comprise a knock-in allele). Historically, gene of interest knock-in using plasmid vectors results in efficiencies typically between 0.1 and 5% (see e.g., Zhu et al., CRISPR/Cas-Mediated Selection-free Knockin Strategy in Human Embryonic Stem Cells. *Stem Cell Reports*. 2015;4(6):1103-1111), this low knock-in efficiency can result in a need for extensive time and resources devoted to screening potentially edited clones.

[0471] In some embodiments, a gene of interest knocked into a cell may have a role in effector function, specificity, stealth, persistence, homing/chemotaxis, and/or resistance to certain chemicals (see for example, Saetersmoen et al., Seminars in Immunopathology, 2019).

[0472] In certain embodiments, the present disclosure provides methods for creation of knock-in cells that maintain high levels of expression regardless of age, differentiation status, and/or exogenous conditions. For example, in some embodiments, an integrated cargo is expressed at an optimal level with a desired subcellular localization as a function of an insertion site. In some embodiments, the present disclosure provides such cells.

Systems for Editing the Genome of a Cell

[0473] In one aspect the present disclosure provides systems for editing the genome of a cell. In some embodiments, the system comprises the cell, a nuclease that causes a break within an endogenous coding sequence of an essential gene of the cell, wherein the essential gene encodes a gene product that is required for survival and/or proliferation of the cell, and a donor template that comprises a knock-in cassette comprising an exogenous coding sequence for a gene product of interest in frame with and downstream (3′) of an exogenous coding sequence or partial coding sequence of the essential gene.

[0474] In some embodiments, the nuclease causes a double-strand break. In some embodiments the nuclease causes a single-strand break, e.g., in some embodiments the nuclease is a nickase. In some embodiments the nuclease is a prime editor which comprises a nickase domain fused to a reverse transcriptase domain. In some embodiments the nuclease is an RNA-guided prime editor and the gRNA comprises the donor template. In some embodiments a dual-nickase system is used which causes a double-strand break via two single-strand breaks on opposing strand of a double-stranded DNA, e.g., genomic DNA of the cell.

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[0475] In one aspect, genome editing systems of the present disclosure may be used, for example, to edit stem cells. In some embodiments, genome editing systems of the present disclosure include at least two components adapted from naturally occurring CRISPR systems: a guide RNA (gRNA) and an RNA-guided nuclease. These two components form a complex that is capable of associating with a specific nucleic acid sequence and editing the DNA in or around that nucleic acid sequence, for instance by making one or more of a single-strand break (an SSB or nick), a double-strand break (a DSB) and/or a point mutation.

[0476] Naturally occurring CRISPR systems are organized evolutionarily into two classes and five types (Makarova et al. Nat Rev Microbiol. 2011 Jun; 9(6): 467–477 ("Makarova")), and while genome editing systems of the present disclosure may adapt components of any type or class of naturally occurring CRISPR system, the embodiments presented herein are generally adapted from Class 2, and type II or V CRISPR systems. Class 2 systems, which encompass types II and V, are characterized by relatively large, multidomain RNA-guided nuclease proteins (e.g., Cas9 or Cpf1) and one or more guide RNAs (e.g., a crRNA and, optionally, a tracrRNA) that form ribonucleoprotein (RNP) complexes that associate with (i.e., target) and cleave specific loci complementary to a targeting (or spacer) sequence of the crRNA. Genome editing systems according to the present disclosure similarly target and edit cellular DNA sequences, but differ significantly from CRISPR systems occurring in nature. For example, the unimolecular guide RNAs described herein do not occur in nature, and both guide RNAs and RNA-guided nucleases according to this disclosure may incorporate any number of non-naturally occurring modifications.

[0477] Genome editing systems can be implemented (e.g., administered or delivered to a cell or a subject) in a variety of ways, and different implementations may be suitable for distinct applications. For instance, a genome editing system is implemented, in certain embodiments, as a protein/RNA complex (a ribonucleoprotein, or RNP), which can be included in a pharmaceutical composition that optionally includes a pharmaceutically acceptable carrier and/or an encapsulating agent, such as a lipid or polymer micro- or nano-particle, micelle, liposome, etc. In certain embodiments, a genome editing system is implemented as one or more nucleic acids encoding the RNA-guided nuclease and guide RNA components described above (optionally with one or more additional components); in certain embodiments, the genome

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editing system is implemented as one or more vectors comprising such nucleic acids, for instance a viral vector such as an adeno-associated virus; and in certain embodiments, the genome editing system is implemented as a combination of any of the foregoing. Additional or modified implementations that operate according to the principles set forth herein will be apparent to the skilled artisan and are within the scope of this disclosure.

[0478] It should be noted that the genome editing systems of the present disclosure can be targeted to a single specific nucleotide sequence, or may be targeted to — and capable of editing in parallel — two or more specific nucleotide sequences through the use of two or more guide RNAs. The use of multiple gRNAs is referred to as "multiplexing" throughout this disclosure, and can be employed to target multiple, unrelated target sequences of interest, or to form multiple SSBs or DSBs within a single target domain and, in some cases, to generate specific edits within such target domain. For example, International Patent Publication No. WO 2015/138510 by Maeder et al. ("Maeder") describes a genome editing system for correcting a point mutation (C.2991+1655A to G) in the human CEP290 gene that results in the creation of a cryptic splice site, which in turn reduces or eliminates the function of the gene. The genome editing system of Maeder utilizes two guide RNAs targeted to sequences on either side of (i.e., flanking) the point mutation, and forms DSBs that flank the mutation. This, in turn, promotes deletion of the intervening sequence, including the mutation, thereby eliminating the cryptic splice site and restoring normal gene function.

[0479] As another example, WO 2016/073990 by Cotta-Ramusino, et al. ("Cotta-Ramusino") describes a genome editing system that utilizes two gRNAs in combination with a Cas9 nickase (a Cas9 that makes a single strand nick such as *S. pyogenes* D10A), an arrangement termed a "dual-nickase system." The dual-nickase system of Cotta-Ramusino is configured to make two nicks on opposite strands of a sequence of interest that are offset by one or more nucleotides, which nicks combine to create a double strand break having an overhang (5' in the case of Cotta-Ramusino, though 3' overhangs are also possible). The overhang, in turn, can facilitate homology directed repair events in some circumstances. And, as another example, WO 2015/070083 by Palestrant et al. ("Palestrant") describes a gRNA targeted to a nucleotide sequence encoding Cas9 (referred to as a "governing RNA"), which can be included in a genome editing system comprising one or more additional gRNAs to permit transient expression of a

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Cas9 that might otherwise be constitutively expressed, for example in some virally transduced cells. These multiplexing applications are intended to be exemplary, rather than limiting, and the skilled artisan will appreciate that other applications of multiplexing are generally compatible with the genome editing systems described here.

[0480] Genome editing systems can, in some instances, form double strand breaks that are repaired by cellular DNA double-strand break mechanisms such as NHEJ or HDR. These mechanisms are described throughout the literature, for example by Davis & Maizels, PNAS, 111(10):E924-932, March 11, 2014 ("Davis") (describing Alt-HDR); Frit et al. DNA Repair 17(2014) 81-97 ("Frit") (describing Alt-NHEJ); and Iyama and Wilson III, DNA Repair (Amst.) 2013-Aug; 12(8): 620-636 ("Iyama") (describing canonical HDR and NHEJ pathways generally).

[0481] Where genome editing systems operate by forming DSBs, such systems optionally include one or more components that promote or facilitate a particular mode of double-strand break repair or a particular repair outcome. For instance, Cotta-Ramusino also describes genome editing systems in which a single stranded oligonucleotide "donor template" is added; the donor template is incorporated into a target region of cellular DNA that is cleaved by the genome editing system, and can result in a change in the target sequence.

[0482] In certain embodiments, genome editing systems modify a target sequence, or modify expression of a target gene in or near the target sequence, without causing single- or double-strand breaks. For example, a genome editing system may include an RNA-guided nuclease fused to a functional domain that acts on DNA, thereby modifying the target sequence or its expression. As one example, an RNA-guided nuclease can be connected to (e.g., fused to) a cytidine deaminase functional domain, and may operate by generating targeted C-to-A substitutions. Exemplary nuclease/deaminase fusions are described in Komor et al. Nature 533, 420–424 (19 May 2016) ("Komor"). Alternatively, a genome editing system may utilize a cleavage-inactivated (i.e., a "dead") nuclease, such as a dead Cas9 (dCas9), and may operate by forming stable complexes on one or more targeted regions of cellular DNA, thereby interfering with functions involving the targeted region(s) including, without limitation, mRNA transcription, chromatin remodeling, etc.

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Nuclease

[0483] Any nuclease that causes a break within an endogenous genomic sequence, e.g., a coding sequence of an essential gene of the cell can be used in the methods of the present disclosure. In some embodiments the nuclease is a DNA nuclease. In some embodiments the nuclease causes a single-strand break (SSB) within an endogenous coding sequence of an essential gene of the cell, e.g., in a "prime editing" system. In some embodiments the nuclease causes a double-strand break (DSB) within an endogenous coding sequence of an essential gene of the cell. In some embodiments the double-strand break is caused by a single nuclease. In some embodiments the double-strand break is caused by two nucleases that each cause a single-strand break on opposing strands, e.g., a dual "nickase" system. In some embodiments the nuclease is a CRISPR/Cas nuclease and the method further comprises contacting the cell with one or more guide molecules for the CRISPR/Cas nuclease. Exemplary CRISPR/Cas nucleases and guide molecules are described in more detail herein. It is to be understood that the nuclease (including a nickase) is not limited in any manner and can also be a zinc finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN), a meganuclease, or other nuclease known in the art (or a combination thereof). Methods for designing zinc finger nucleases (ZFNs) are well known in the art, e.g., see Urnov et al., Nature Reviews Genetics 2010; 11:636-640 and Paschon et al., Nat. Commun. 2019; 10(1):1133 and references cited therein. Methods for designing transcription activator-like effector nucleases (TALENs) are well known in the art, e.g., see Joung and Sander, Nat. Rev. Mol. Cell Biol. 2013; 14(1):49-55 and references cited therein. Methods for designing meganucleases are also well known in the art, e.g., see Silva et al., Curr. Gene Ther. 2011; 11(1):11-27 and Redel and Prather, Toxicol. Pathol. 2016; 44(3):428-433.

[0484] In some embodiments, a nuclease suitable for methods described herein can have an editing efficiency that is greater than about 50%. In some embodiments, a nuclease suitable for methods described herein can have an editing efficiency that is greater than about 55%. In some embodiments, a nuclease suitable for methods described herein can have an editing efficiency that is greater than about 60%. In some embodiments, a nuclease suitable for methods described herein can have an editing efficiency that is greater than about 65%. In some embodiments, a nuclease suitable for methods described herein can have an editing efficiency

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that is greater than about 70%. In some embodiments, a nuclease suitable for methods described herein can have an editing efficiency that is greater than about 75%. In some embodiments, a nuclease suitable for methods described herein can have an editing efficiency that is greater than about 80%. In some embodiments, a nuclease suitable for methods described herein can have an editing efficiency that is greater than about 85%. In some embodiments, a nuclease suitable for methods described herein can have an editing efficiency that is greater than about 90%. In some embodiments, a nuclease suitable for methods described herein can have an editing efficiency that is greater than about 95%. In some embodiments, a nuclease suitable for methods described herein can have an editing efficiency that is greater than about 96%. In some embodiments, a nuclease suitable for methods described herein can have an editing efficiency that is greater than about 97%. In some embodiments, a nuclease suitable for methods described herein can have an editing efficiency that is greater than about 98%. In some embodiments, a nuclease suitable for methods described herein can have an editing efficiency that is greater than about 99%.

[0485] In general, the nuclease can be delivered to the cell as a protein or a nucleic acid encoding the protein, e.g., a DNA molecule or mRNA molecule. The protein or nucleic acid can be combined with other delivery agents, e.g., lipids or polymers in a lipid or polymer nanoparticle and targeting agents such as antibodies or other binding agents with specificity for the cell. The DNA molecule can be a nucleic acid vector, such as a viral genome or circular double-stranded DNA, e.g., a plasmid. Nucleic acid vectors encoding a nuclease can include other coding or non-coding elements. For example, a nuclease can be delivered as part of a viral genome (e.g., in an AAV, adenoviral or lentiviral genome) that includes certain genomic backbone elements (e.g., inverted terminal repeats, in the case of an AAV genome).

[0486] A CRISPR/Cas nuclease can be delivered to the cell as a protein or a nucleic acid encoding the protein, e.g., a DNA molecule or mRNA molecule. The guide molecule can be delivered as an RNA molecule or encoded by a DNA molecule. A CRISPR/Cas nuclease can also be delivered with a guide molecule as a ribonucleoprotein (RNP) and introduced into the cell via nucleofection (electroporation).

CRISPR/Cas nucleases

[0487] CRISPR/Cas nucleases according to the present disclosure include, but are not limited to, naturally-occurring Class 2 CRISPR nucleases such as Cas9, and Cpf1 (Cas12a), as well as other Cas12 nucleases and nucleases derived or obtained therefrom. In functional terms,

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CRISPR/Cas nucleases are defined as those nucleases that: (a) interact with (e.g., complex with) a gRNA; and (b) together with the gRNA, associate with, and optionally cleave or modify, a target region of a DNA that includes (i) a sequence complementary to the targeting domain of the gRNA and, optionally, (ii) an additional sequence referred to as a "protospacer adjacent motif," or "PAM," which is described in greater detail below. As the following examples will illustrate, CRISPR/Cas nucleases can be defined, in broad terms, by their PAM specificity and cleavage activity, even though variations may exist between individual CRISPR/Cas nucleases that share the same PAM specificity or cleavage activity. Skilled artisans will appreciate that some aspects of the present disclosure relate to systems and methods that can be implemented using any suitable CRISPR/Cas nuclease having a certain PAM specificity and/or cleavage activity. For this reason, unless otherwise specified, the term CRISPR/Cas nuclease should be understood as a generic term, and not limited to any particular type (e.g., Cas9 vs. Cpf1), species (e.g., *S. pyogenes* vs. *S. aureus*) or variation (e.g., full-length vs. truncated or split; naturally-occurring PAM specificity vs. engineered PAM specificity, etc.) of CRISPR/Cas nuclease.

[0488] The PAM sequence takes its name from its sequential relationship to the "protospacer" sequence that is complementary to gRNA targeting domains (or "spacers"). Together with protospacer sequences, PAM sequences define target regions or sequences for specific CRISPR/Cas nuclease and gRNA combinations.

[0489] Various CRISPR/Cas nucleases may require different sequential relationships between PAMs and protospacers. In general, Cas9s recognize PAM sequences that are 3′ of the protospacer. Cpf1 (Cas12a), on the other hand, generally recognizes PAM sequences that are 5′ of the protospacer.

[0490] In addition to recognizing specific sequential orientations of PAMs and protospacers, CRISPR/Cas nucleases can also recognize specific PAM sequences. *S. aureus* Cas9, for instance, recognizes a PAM sequence of NNGRRT or NNGRRV, wherein the N residues are immediately 3′ of the region recognized by the gRNA targeting domain. *S. pyogenes* Cas9 recognizes NGG PAM sequences. *F. novicida* Cpf1 recognizes a TTN PAM sequence. PAM sequences have been identified for a variety of CRISPR/Cas nucleases, and a strategy for identifying novel PAM sequences has been described by Shmakov et al., Molecular Cell 2015; 60:385-397. It should also be noted that engineered CRISPR/Cas nucleases can have

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PAM specificities that differ from the PAM specificities of reference molecules (for instance, in the case of an engineered CRISPR/Cas nuclease, the reference molecule may be the naturally occurring variant from which the CRISPR/Cas nuclease is derived, or the naturally occurring variant having the greatest amino acid sequence homology to the engineered CRISPR/Cas nuclease).

[0491] In addition to their PAM specificity, CRISPR/Cas nucleases can be characterized by their DNA cleavage activity: naturally-occurring CRISPR/Cas nucleases typically form double-strand breaks (DSBs) in target nucleic acids, but engineered variants called "nickases" have been produced that generate only single-strand breaks (SSBs), e.g., those discussed in Ran et al., Cell 2013; 154(6):1380-1389 ("Ran"), or that that do not cut at all.

Cas9

[0492] Crystal structures have been determined for *S. pyogenes* Cas9 (Jinek et al., Science 2014; 343(6176):1247997 ("Jinek 2014"), and for *S. aureus* Cas9 in complex with a unimolecular guide RNA and a target DNA. See Nishimasu et al., Cell 1024; 156:935-949 ("Nishimasu 2014"); Nishimasu et al., Cell 2015; 162:1113-1126 ("Nishimasu 2015"); and Anders et al., Nature 2014; 513(7519):569-73 ("Anders 2014").

[0493] A naturally occurring Cas9 protein comprises two lobes: a recognition (REC) lobe and a nuclease (NUC) lobe; each of which comprise particular structural and/or functional domains. The REC lobe comprises an arginine-rich bridge helix (BH) domain, and at least one REC domain (e.g., a REC1 domain and, optionally, a REC2 domain). The REC lobe does not share structural similarity with other known proteins, indicating that it is a unique functional domain. While not wishing to be bound by any theory, mutational analyses suggest specific functional roles for the BH and REC domains: the BH domain appears to play a role in gRNA:DNA recognition, while the REC domain is thought to interact with the repeat:anti-repeat duplex of the gRNA and to mediate the formation of the Cas9/gRNA complex.

[0494] The NUC lobe comprises a RuvC domain, an HNH domain, and a PAM-interacting (PI) domain. The RuvC domain shares structural similarity to retroviral integrase superfamily members and cleaves the non-complementary (i.e., bottom) strand of the target nucleic acid. It may be formed from two or more split RuvC motifs (such as RuvC I, RuvCII, and RuvCIII in *S. pyogenes* and *S. aureus*). The HNH domain, meanwhile, is structurally similar

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to HNN endonuclease motifs, and cleaves the complementary (i.e., top) strand of the target nucleic acid. The PI domain, as its name suggests, contributes to PAM specificity.

[0495] While certain functions of Cas9 are linked to (but not necessarily fully determined by) the specific domains set forth above, these and other functions may be mediated or influenced by other Cas9 domains, or by multiple domains on either lobe. For instance, in *S. pyogenes* Cas9, as described in Nishimasu 2014, the repeat:antirepeat duplex of the gRNA falls into a groove between the REC and NUC lobes, and nucleotides in the duplex interact with amino acids in the BH, PI, and REC domains. Some nucleotides in the first stem loop structure also interact with amino acids in multiple domains (PI, BH and REC1), as do some nucleotides in the second and third stem loops (RuvC and PI domains).

Cpf1

[0496] The crystal structure of *Acidaminococcus sp.* Cpf1 in complex with crRNA and a dsDNA target including a TTTN PAM sequence has been solved by Yamano et al., Cell. 2016; 165(4):949–962 ("Yamano"). Cpf1, like Cas9, has two lobes: a REC (recognition) lobe, and a NUC (nuclease) lobe. The REC lobe includes REC1 and REC2 domains, which lack similarity to any known protein structures. The NUC lobe, meanwhile, includes three RuvC domains (RuvC-I, -II and -III) and a BH domain. However, in contrast to Cas9, the Cpf1 REC lobe lacks an HNH domain, and includes other domains that also lack similarity to known protein structures: a structurally unique PI domain, three Wedge (WED) domains (WED-I, -II and -III), and a nuclease (Nuc) domain.

[0497] While Cas9 and Cpf1 share similarities in structure and function, it should be appreciated that certain Cpf1 activities are mediated by structural domains that are not analogous to any Cas9 domains. For instance, cleavage of the complementary strand of the target DNA appears to be mediated by the Nuc domain, which differs sequentially and spatially from the HNH domain of Cas9. Additionally, the non-targeting portion of Cpf1 gRNA (the handle) adopts a pseudoknot structure, rather than a stem loop structure formed by the repeat:antirepeat duplex in Cas9 gRNAs.

Nuclease variants

[0498] The CRISPR/Cas nucleases described herein have activities and properties that can be useful in a variety of applications, but the skilled artisan will appreciate that CRISPR/Cas

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nucleases can also be modified in certain instances, to alter cleavage activity, PAM specificity, or other structural or functional features.

[0499] Turning first to modifications that alter cleavage activity, mutations that reduce or eliminate the activity of domains within the NUC lobe have been described above. Exemplary mutations that may be made in the RuvC domains, in the Cas9 HNH domain, or in the Cpf1 Nuc domain are described in Ran, Yamano and PCT Publication No. WO 2016/073990A1, the entire contents of each of which are incorporated herein by reference. In general, mutations that reduce or eliminate activity in one of the two nuclease domains result in CRISPR/Cas nucleases with nickase activity, but it should be noted that the type of nickase activity varies depending on which domain is inactivated. As one example, inactivation of a RuvC domain or of a Cas9 HNH domain results in a nickase. Exemplary nickase variants include Cas9 D10A and Cas9 H840A (numbering scheme according to SpCas9 wild-type sequence). Additional suitable nickase variants, including Cas12a variants, will be apparent to the skilled artisan based on the present disclosure and the knowledge in the art. The present disclosure is not limited in this respect. In some embodiments a nickase may be fused to a reverse transcriptase to produce a prime editor (PE), e.g., as described in Anzalone et al., Nature 2019; 576:149-157, the entire contents of which are incorporated herein by reference.

[0500] Modifications of PAM specificity relative to naturally occurring Cas9 reference molecules has been described for both *S. pyogenes* (Kleinstiver et al., Nature 2015; 523(7561):481-5); and *S. aureus* (Kleinstiver et al., Nat Biotechnol. 2015; 33(12):1293-1298). Modifications that improve the targeting fidelity of Cas9 have also been described (Kleinstiver et al., Nature 2016; 529:490-495). Each of these references is incorporated by reference herein.

[0501] CRISPR/Cas nucleases have also been split into two or more parts, as described by Zetsche et al., Nat Biotechnol. 2015; 33(2):139-42, incorporated by reference, and by Fine et al., Sci Rep. 2015; 5:10777, incorporated by reference.

[0502] CRISPR/Cas nucleases can be, in certain embodiments, size-optimized or truncated, for instance via one or more deletions that reduce the size of the nuclease while still retaining gRNA association, target and PAM recognition, and cleavage activities. In certain embodiments, RNA guided nucleases are bound, covalently or non-covalently, to another polypeptide, nucleotide, or other structure, optionally by means of a linker. Exemplary bound

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nucleases and linkers are described by Guilinger et al., Nature Biotech. 2014; 32:577-582, which is incorporated by reference herein.

[0503] CRISPR/Cas nucleases also optionally include a tag, such as, but not limited to, a nuclear localization signal, to facilitate movement of CRISPR/Cas nuclease protein into the nucleus. In certain embodiments, the CRISPR/Cas nuclease can incorporate C- and/or N-terminal nuclear localization signals. Nuclear localization sequences are known in the art.

[0504] The foregoing list of modifications is intended to be exemplary in nature, and the skilled artisan will appreciate, in view of the instant disclosure, that other modifications may be possible or desirable in certain applications. For brevity, therefore, exemplary systems, methods and compositions of the present disclosure are presented with reference to particular CRISPR/Cas nucleases, but it should be understood that the CRISPR/Cas nucleases used may be modified in ways that do not alter their operating principles. Such modifications are within the scope of the present disclosure.

[0505] Exemplary suitable nuclease variants include, but are not limited to, AsCpf1 (AsCas12a) variants comprising an M537R substitution, an H800A substitution, and/or an F870L substitution, or any combination thereof (numbering scheme according to AsCpf1 wild-type sequence). In some embodiments, a nuclease variant is a Cas12a variant, e.g., a Cas12a variant comprising 1, 2, or 3 of the amino acid substitutions selected from M537R, F870L, and H800A. In some embodiments, a Cas12a variant comprises an amino acid sequence having at least about 90%, 95%, or 100% identity to an AsCpf1 sequence described herein.

[0506] Other suitable modifications of the AsCpf1 amino acid sequence are known to those of ordinary skill in the art. Some exemplary sequences of wild-type AsCpf1 and AsCpf1 variants are provided below:

SEQ ID NO: 58 - His-AsCpf1-sNLS-sNLS H800A amino acid sequence

MGHHHHHHGSTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKPIID
RIYKTYADQCLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIGRTDNLTD
AINKRHAEIYKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYENRKNVFSAE
DISTAIPHRIVQDNFPKFKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIEEVFSFPFYN
QLLTQTQIDLYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPHRFIPLFKQILS
DRNTLSFILEEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNELNSIDLTHIFISHKKLETIS
SALCDHWDTLRNALYERRISELTGKITKSAKEKVQRSLKHEDINLQEIISAAGKELSEAFKQKT
SEILSHAHAALDQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAVDESNEVDPEFSARLTGI
KLEMEPSLSFYNKARNYATKKPYSVEKFKLNFQMPTLASGWDVNKEKNNGAILFVKNGLYYLGI
MPKQKGRYKALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCSTQLKAVTAHFQTHTTPILLSNN

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FIEPLEITKEIYDLNNPEKEPKKFQTAYAKKTGDQKGYREALCKWIDFTRDFLSKYTKTTSIDL
SSLRPSSQYKDLGEYYAELNPLLYHISFQRIAEKEIMDAVETGKLYLFQIYNKDFAKGHHGKPN
LHTLYWTGLFSPENLAKTSIKLNGQAELFYRPKSRMKRMAARLGEKMLNKKLKDQKTPIPDTLY
QELYDYVNHRLSHDLSDEARALLPNVITKEVSHEIIKDRRFTSDKFFFHVPITLNYQAANSPSK
FNQRVNAYLKEHPETPIIGIDRGERNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLDNREKER
VAARQAWSVVGTIKDLKQGYLSQVIHEIVDLMIHYQAVVVLENLNFGFKSKRTGIAEKAVYQQF
EKMLIDKLNCLVLKDYPAEKVGGVLNPYQLTDQFTSFAKMGTQSGFLFYVPAPYTSKIDPLTGF
VDPFVWKTIKNHESRKHFLEGFDFLHYDVKTGDFILHFKMNRNLSFQRGLPGFMPAWDIVFEKN
ETQFDAKGTPFIAGKRIVPVIENHRFTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLEND
DSHAIDTMVALIRSVLQMRNSNAATGEDYINSPVRDLNGVCFDSRFQNPEWPMDADANGAYHIA
LKGQLLLNHLKESKDLKLQNGISNQDWLAYIQELRNGSPKKKRKVGSPKKKRKV

SEQ ID NO: 59 - Cpf1 variant 1 amino acid sequence

MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKPIIDRIYKTYADQ
CLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIGRTDNLTDAINKRHAEI
YKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYENRKNVFSAEDISTAIPHR
IVQDNFPKFKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIEEVFSFPFYNQLLTQTQID
LYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPHRFIPLFKQILSDRNTLSFIL
EEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNELNSIDLTHIFISHKKLETISSALCDHWDT
LRNALYERRISELTGKITKSAKEKVQRSLKHEDINLQEIISAAGKELSEAFKQKTSEILSHAHA
ALDQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAVDESNEVDPEFSARLTGIKLEMEPSLS
FYNKARNYATKKPYSVEKFKLNFQRPTLASGWDVNKEKNNGAILFVKNGLYYLGIMPKQKGRYK
ALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCSTQLKAVTAHFQTHTTPILLSNNFIEPLEITK
EIYDLNNPEKEPKKFQTAYAKKTGDQKGYREALCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQY
KDLGEYYAELNPLLYHISFQRIAEKEIMDAVETGKLYLFQIYNKDFAKGHHGKPNLHTLYWTGL
FSPENLAKTSIKLNGQAELFYRPKSRMKRMAHRLGEKMLNKKLKDQKTPIPDTLYQELYDYVNH
RLSHDLSDEARALLPNVITKEVSHEIIKDRRFTSDKFLFHVPITLNYQAANSPSKFNQRVNAYL
KEHPETPIIGIDRGERNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSV
VGTIKDLKQGYLSQVIHEIVDLMIHYQAVVVLENLNFGFKSKRTGIAEKAVYQQFEKMLIDKLN
CLVLKDYPAEKVGGVLNPYQLTDQFTSFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTI
KNHESRKHFLEGFDFLHYDVKTGDFILHFKMNRNLSFQRGLPGFMPAWDIVFEKNETQFDAKGT
PFIAGKRIVPVIENHRFTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTMV
ALIRSVLQMRNSNAATGEDYINSPVRDLNGVCFDSRFQNPEWPMDADANGAYHIALKGQLLLNH
LKESKDLKLQNGISNQDWLAYIQELRNGRSSDDEATADSQHAAPPKKKRKVGGSGGSGGSGGSG
GSGGSGGSGGSLEHHHHHH

SEQ ID NO: 60 - Cpf1 variant 2 amino acid sequence

MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKPIIDRIYKTYADQ
CLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIGRTDNLTDAINKRHAEI
YKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYENRKNVFSAEDISTAIPHR
IVQDNFPKFKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIEEVFSFPFYNQLLTQTQID
LYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPHRFIPLFKQILSDRNTLSFIL
EEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNELNSIDLTHIFISHKKLETISSALCDHWDT
LRNALYERRISELTGKITKSAKEKVQRSLKHEDINLQEIISAAGKELSEAFKQKTSEILSHAHA
ALDQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAVDESNEVDPEFSARLTGIKLEMEPSLS

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FYNKARNYATKKPYSVEKFKLNFQMPTLASGWDVNKEKNNGAILFVKNGLYYLGIMPKQKGRYK
ALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCSTQLKAVTAHFQTHTTPILLSNNFIEPLEITK
EIYDLNNPEKEPKKFQTAYAKKTGDQKGYREALCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQY
KDLGEYYAELNPLLYHISFQRIAEKEIMDAVETGKLYLFQIYNKDFAKGHHGKPNLHTLYWTGL
FSPENLAKTSIKLNGQAELFYRPKSRMKRMAHRLGEKMLNKKLKDQKTPIPDTLYQELYDYVNH
RLSHDLSDEARALLPNVITKEVSHEIIKDRRFTSDKFFFHVPITLNYQAANSPSKFNQRVNAYL
KEHPETPIIGIDRGERNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSV
VGTIKDLKQGYLSQVIHEIVDLMIHYQAVVVLENLNFGFKSKRTGIAEKAVYQQFEKMLIDKLN
CLVLKDYPAEKVGGVLNPYQLTDQFTSFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTI
KNHESRKHFLEGFDFLHYDVKTGDFILHFKMNRNLSFQRGLPGFMPAWDIVFEKNETQFDAKGT
PFIAGKRIVPVIENHRFTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTMV
ALIRSVLQMRNSNAATGEDYINSPVRDLNGVCFDSRFQNPEWPMDADANGAYHIALKGQLLLNH
LKESKDLKLQNGISNQDWLAYIQELRNGRSSDDEATADSQHAAPPKKKRKVGGSGGSGGSGGSG
GSGGSGGSGGSLEHHHHHH

SEQ ID NO: 61 - Cpf1 variant 3 amino acid sequence

MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKPIIDRIYKTYADQ
CLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIGRTDNLTDAINKRHAEI
YKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYENRKNVFSAEDISTAIPHR
IVQDNFPKFKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIEEVFSFPFYNQLLTQTQID
LYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPHRFIPLFKQILSDRNTLSFIL
EEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNELNSIDLTHIFISHKKLETISSALCDHWDT
LRNALYERRISELTGKITKSAKEKVQRSLKHEDINLQEIISAAGKELSEAFKQKTSEILSHAHA
ALDQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAVDESNEVDPEFSARLTGIKLEMEPSLS
FYNKARNYATKKPYSVEKFKLNFQRPTLASGWDVNKEKNNGAILFVKNGLYYLGIMPKQKGRYK
ALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCSTQLKAVTAHFQTHTTPILLSNNFIEPLEITK
EIYDLNNPEKEPKKFQTAYAKKTGDQKGYREALCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQY
KDLGEYYAELNPLLYHISFQRIAEKEIMDAVETGKLYLFQIYNKDFAKGHHGKPNLHTLYWTGL
FSPENLAKTSIKLNGQAELFYRPKSRMKRMAARLGEKMLNKKLKDQKTPIPDTLYQELYDYVNH
RLSHDLSDEARALLPNVITKEVSHEIIKDRRFTSDKFLFHVPITLNYQAANSPSKFNQRVNAYL
KEHPETPIIGIDRGERNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSV
VGTIKDLKQGYLSQVIHEIVDLMIHYQAVVVLENLNFGFKSKRTGIAEKAVYQQFEKMLIDKLN
CLVLKDYPAEKVGGVLNPYQLTDQFTSFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTI
KNHESRKHFLEGFDFLHYDVKTGDFILHFKMNRNLSFQRGLPGFMPAWDIVFEKNETQFDAKGT
PFIAGKRIVPVIENHRFTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTMV
ALIRSVLQMRNSNAATGEDYINSPVRDLNGVCFDSRFQNPEWPMDADANGAYHIALKGQLLLNH
LKESKDLKLQNGISNQDWLAYIQELRNGRSSDDEATADSQHAAPPKKKRKVGGSGGSGGSGGSG
GSGGSGGSGGSLEHHHHHH

SEQ ID NO: 62 - Cpf1 variant 4 amino acid sequence

MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKPIIDRIYKTYADQ
CLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIGRTDNLTDAINKRHAEI
YKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYENRKNVFSAEDISTAIPHR
IVQDNFPKFKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIEEVFSFPFYNQLLTQTQID
LYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPHRFIPLFKQILSDRNTLSFIL

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EEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNELNSIDLTHIFISHKKLETISSALCDHWDT
LRNALYERRISELTGKITKSAKEKVQRSLKHEDINLQEIISAAGKELSEAFKQKTSEILSHAHA
ALDQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAVDESNEVDPEFSARLTGIKLEMEPSLS
FYNKARNYATKKPYSVEKFKLNFQRPTLASGWDVNKEKNNGAILFVKNGLYYLGIMPKQKGRYK
ALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCSTQLKAVTAHFQTHTTPILLSNNFIEPLEITK
EIYDLNNPEKEPKKFQTAYAKKTGDQKGYREALCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQY
KDLGEYYAELNPLLYHISFQRIAEKEIMDAVETGKLYLFQIYNKDFAKGHHGKPNLHTLYWTGL
FSPENLAKTSIKLNGQAELFYRPKSRMKRMAARLGEKMLNKKLKDQKTPIPDTLYQELYDYVNH
RLSHDLSDEARALLPNVITKEVSHEIIKDRRFTSDKFLFHVPITLNYQAANSPSKFNQRVNAYL
KEHPETPIIGIDRGERNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSV
VGTIKDLKQGYLSQVIHEIVDLMIHYQAVVVLENLNFGFKSKRTGIAEKAVYQQFEKMLIDKLN
CLVLKDYPAEKVGGVLNPYQLTDQFTSFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTI
KNHESRKHFLEGFDFLHYDVKTGDFILHFKMNRNLSFQRGLPGFMPAWDIVFEKNETQFDAKGT
PFIAGKRIVPVIENHRFTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTMV
ALIRSVLQMRNSNAATGEDYINSPVRDLNGVCFDSRFQNPEWPMDADANGAYHIALKGQLLLNH
LKESKDLKLQNGISNQDWLAYIQELRNGRSSDDEATADSQHAAPPKKKRKV

SEQ ID NO: 63 - Cpf1 variant 5 amino acid sequence

MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKPIIDRIYKTYADQ
CLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIGRTDNLTDAINKRHAEI
YKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYENRKNVFSAEDISTAIPHR
IVQDNFPKFKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIEEVFSFPFYNQLLTQTQID
LYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPHRFIPLFKQILSDRNTLSFIL
EEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNELNSIDLTHIFISHKKLETISSALCDHWDT
LRNALYERRISELTGKITKSAKEKVQRSLKHEDINLQEIISAAGKELSEAFKQKTSEILSHAHA
ALDQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAVDESNEVDPEFSARLTGIKLEMEPSLS
FYNKARNYATKKPYSVEKFKLNFQRPTLASGWDVNKEKNNGAILFVKNGLYYLGIMPKQKGRYK
ALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCSTQLKAVTAHFQTHTTPILLSNNFIEPLEITK
EIYDLNNPEKEPKKFQTAYAKKTGDQKGYREALCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQY
KDLGEYYAELNPLLYHISFQRIAEKEIMDAVETGKLYLFQIYNKDFAKGHHGKPNLHTLYWTGL
FSPENLAKTSIKLNGQAELFYRPKSRMKRMAHRLGEKMLNKKLKDQKTPIPDTLYQELYDYVNH
RLSHDLSDEARALLPNVITKEVSHEIIKDRRFTSDKFLFHVPITLNYQAANSPSKFNQRVNAYL
KEHPETPIIGIDRGERNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSV
VGTIKDLKQGYLSQVIHEIVDLMIHYQAVVVLENLNFGFKSKRTGIAEKAVYQQFEKMLIDKLN
CLVLKDYPAEKVGGVLNPYQLTDQFTSFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTI
KNHESRKHFLEGFDFLHYDVKTGDFILHFKMNRNLSFQRGLPGFMPAWDIVFEKNETQFDAKGT
PFIAGKRIVPVIENHRFTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTMV
ALIRSVLQMRNSNAATGEDYINSPVRDLNGVCFDSRFQNPEWPMDADANGAYHIALKGQLLLNH
LKESKDLKLQNGISNQDWLAYIQELRNGRSSDDEATADSQHAAPPKKKRKV

SEQ ID NO: 64 - Cpf1 variant 6 amino acid sequence

MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKPIIDRIYKTYADQ
CLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIGRTDNLTDAINKRHAEI
YKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYENRKNVFSAEDISTAIPHR
IVQDNFPKFKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIEEVFSFPFYNQLLTQTQID

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LYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPHRFIPLFKQILSDRNTLSFIL
EEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNELNSIDLTHIFISHKKLETISSALCDHWDT
LRNALYERRISELTGKITKSAKEKVQRSLKHEDINLQEIISAAGKELSEAFKQKTSEILSHAHA
ALDQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAVDESNEVDPEFSARLTGIKLEMEPSLS
FYNKARNYATKKPYSVEKFKLNFQRPTLASGWDVNKEKNNGAILFVKNGLYYLGIMPKQKGRYK
ALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCSTQLKAVTAHFQTHTTPILLSNNFIEPLEITK
EIYDLNNPEKEPKKFQTAYAKKTGDQKGYREALCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQY
KDLGEYYAELNPLLYHISFQRIAEKEIMDAVETGKLYLFQIYNKDFAKGHHGKPNLHTLYWTGL
FSPENLAKTSIKLNGQAELFYRPKSRMKRMAHRLGEKMLNKKLKDQKTPIPDTLYQELYDYVNH
RLSHDLSDEARALLPNVITKEVSHEIIKDRRFTSDKFLFHVPITLNYQAANSPSKFNQRVNAYL
KEHPETPIIGIDRGERNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSV
VGTIKDLKQGYLSQVIHEIVDLMIHYQAVVVLENLNFGFKSKRTGIAEKAVYQQFEKMLIDKLN
CLVLKDYPAEKVGGVLNPYQLTDQFTSFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTI
KNHESRKHFLEGFDFLHYDVKTGDFILHFKMNRNLSFQRGLPGFMPAWDIVFEKNETQFDAKGT
PFIAGKRIVPVIENHRFTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTMV
ALIRSVLQMRNSNAATGEDYINSPVRDLNGVCFDSRFQNPEWPMDADANGAYHIALKGQLLLNH
LKESKDLKLQNGISNQDWLAYIQELRNGRSSDDEATADSQHAAPPKKKRKVGGSGGSGGSGGSG
GSGGSGGSGGSLEHHHHHH

SEQ ID NO: 65 - Cpf1 variant 7 amino acid sequence

MGRDPGKPIPNPLLGLDSTAPKKKRKVGIHGVPAATQFEGFTNLYQVSKTLRFELIPQGKTLKH
IQEQGFIEEDKARNDHYKELKPIIDRIYKTYADQCLQLVQLDWENLSAAIDSYRKEKTEETRNA
LIEEQATYRNAIHDYFIGRTDNLTDAINKRHAEIYKGLFKAELFNGKVLKQLGTVTTTEHENAL
LRSFDKFTTYFSGFYENRKNVFSAEDISTAIPHRIVQDNFPKFKENCHIFTRLITAVPSLREHF
ENVKKAIGIFVSTSIEEVFSFPFYNQLLTQTQIDLYNQLLGGISREAGTEKIKGLNEVLNLAIQ
KNDETAHIIASLPHRFIPLFKQILSDRNTLSFILEEFKSDEEVIQSFCKYKTLLRNENVLETAE
ALFNELNSIDLTHIFISHKKLETISSALCDHWDTLRNALYERRISELTGKITKSAKEKVQRSLK
HEDINLQEIISAAGKELSEAFKQKTSEILSHAHAALDQPLPTTLKKQEEKEILKSQLDSLLGLY
HLLDWFAVDESNEVDPEFSARLTGIKLEMEPSLSFYNKARNYATKKPYSVEKFKLNFQMPTLAS
GWDVNKEKNNGAILFVKNGLYYLGIMPKQKGRYKALSFEPTEKTSEGFDKMYYDYFPDAAKMIP
KCSTQLKAVTAHFQTHTTPILLSNNFIEPLEITKEIYDLNNPEKEPKKFQTAYAKKTGDQKGYR
EALCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQYKDLGEYYAELNPLLYHISFQRIAEKEIMDA
VETGKLYLFQIYNKDFAKGHHGKPNLHTLYWTGLFSPENLAKTSIKLNGQAELFYRPKSRMKRM
AHRLGEKMLNKKLKDQKTPIPDTLYQELYDYVNHRLSHDLSDEARALLPNVITKEVSHEIIKDR
RFTSDKFFFHVPITLNYQAANSPSKFNQRVNAYLKEHPETPIIGIDRGERNLIYITVIDSTGKI
LEQRSLNTIQQFDYQKKLDNREKERVAARQAWSVVGTIKDLKQGYLSQVIHEIVDLMIHYQAVV
VLENLNFGFKSKRTGIAEKAVYQQFEKMLIDKLNCLVLKDYPAEKVGGVLNPYQLTDQFTSFAK
MGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTIKNHESRKHFLEGFDFLHYDVKTGDFILHFK
MNRNLSFQRGLPGFMPAWDIVFEKNETQFDAKGTPFIAGKRIVPVIENHRFTGRYRDLYPANEL
IALLEEKGIVFRDGSNILPKLLENDDSHAIDTMVALIRSVLQMRNSNAATGEDYINSPVRDLNG
VCFDSRFQNPEWPMDADANGAYHIALKGQLLLNHLKESKDLKLQNGISNQDWLAYIQELRNPKK
KRKVKLAAALEHHHHHH

SEQ ID NO: 66 - Exemplary AsCpf1 wild-type amino acid sequence

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MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKPIIDRIYKTYADQ
CLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIGRTDNLTDAINKRHAEI
YKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYENRKNVFSAEDISTAIPHR
IVQDNFPKFKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIEEVFSFPFYNQLLTQTQID
LYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPHRFIPLFKQILSDRNTLSFIL
EEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNELNSIDLTHIFISHKKLETISSALCDHWDT
LRNALYERRISELTGKITKSAKEKVQRSLKHEDINLQEIISAAGKELSEAFKQKTSEILSHAHA
ALDQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAVDESNEVDPEFSARLTGIKLEMEPSLS
FYNKARNYATKKPYSVEKFKLNFQMPTLASGWDVNKEKNNGAILFVKNGLYYLGIMPKQKGRYK
ALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCSTQLKAVTAHFQTHTTPILLSNNFIEPLEITK
EIYDLNNPEKEPKKFQTAYAKKTGDQKGYREALCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQY
KDLGEYYAELNPLLYHISFQRIAEKEIMDAVETGKLYLFQIYNKDFAKGHHGKPNLHTLYWTGL
FSPENLAKTSIKLNGQAELFYRPKSRMKRMAHRLGEKMLNKKLKDQKTPIPDTLYQELYDYVNH
RLSHDLSDEARALLPNVITKEVSHEIIKDRRFTSDKFFFHVPITLNYQAANSPSKFNQRVNAYL
KEHPETPIIGIDRGERNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSV
VGTIKDLKQGYLSQVIHEIVDLMIHYQAVVVLENLNFGFKSKRTGIAEKAVYQQFEKMLIDKLN
CLVLKDYPAEKVGGVLNPYQLTDQFTSFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTI
KNHESRKHFLEGFDFLHYDVKTGDFILHFKMNRNLSFQRGLPGFMPAWDIVFEKNETQFDAKGT
PFIAGKRIVPVIENHRFTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTMV
ALIRSVLQMRNSNAATGEDYINSPVRDLNGVCFDSRFQNPEWPMDADANGAYHIALKGQLLLNH
LKESKDLKLQNGISNQDWLAYIQELRN

[0507] Additional suitable nucleases and nuclease variants will be apparent to the skilled artisan based on the present disclosure in view of the knowledge in the art. Exemplary suitable nucleases may include, but are not limited to those provided in Table 5.

Table 5: Exemplary Suitable CRISPR/Cas Nucleases

Nuclease	Length (A.A.)	PAM	Reference
SpCas9	1368	NGG	Cong et al., Science 2013; 339(6121):819-23
SaCas9	1053	NNGRRT	Ran et al., Nature 2015; 520(7546):186-91.
(KKH) SaCas9	1067	NNNRRT	Kleinstiver et al., Nat Biotechnol. 2015; 33(12):1293-1298
AsCpf1 (AsCas12a)	1353	TTTV	Zetsche et al., Nat Biotechnol. 2017; 35(1):31-34.
LbCpf1 (LbCas12a)	1274	TTTV	Zetsche et al., Cell 2015; 163(3):759-71.
CasX	980	TTC	Burstein et al., Nature 2017; 542(7640):237-241.

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Nuclease	Length (A.A.)	PAM	Reference
CasY	1200	TA	Burstein et al., Nature 2017; 542(7640):237-241.
Cas12h1	870	RTR	Yan et al., Science 2019; 363(6422):88-91.
Cas12i1	1093	TTN	Yan et al., Science 2019; 363(6422):88-91.
Cas12c1	unknown	TG	Yan et al., Science 2019; 363(6422):88-91.
Cas12c2	unknown	TN	Yan et al., Science 2019; 363(6422):88-91.
eSpCas9	1423	NGG	Chen et al., Nature 2017; 550(7676):407-410.
Cas9-HF1	1367	NGG	Chen et al., Nature 2017; 550(7676):407-410.
HypaCas9	1404	NGG	Chen et al., Nature 2017; 550(7676):407-410.
dCas9-Fokl	1623	NGG	U.S. Patent No. 9,322,037
Sniper-Cas9	1389	NGG	Lee et al., Nat Commun. 2018; 9(1):3048.
xCas9	1786	NGG, NG, GAA, GAT	Hu et al., Nature. 2018 Apr 5;556(7699):57-63.
AaCas12b	1129	TTN	Teng et al., Cell Discov. 2018; 4:63.
evoCas9	1423	NGG	Casini et al., Nat Biotechnol. 2018; 36(3):265-271.
SpCas9-NG	1423	NG	Nishimasu et al., Science 2018; 361(6408):1259-1262.
VRQR	1368	NGA	Li et al., The CRISPR Journal, 2018; 01:01
VRER	1372	NGCG	Kleinstiver et al., Nature 2016; 529(7587):490-5.
NmeCas9	1082	NNNNGATT	Amrani et al., Genome Biol. 2018; 19(1):214.
CjCas9	984	NNNNRYAC	Kim et al., Nat Commun. 2017; 8:14500.
BhCas12b	1108	ATTN	Strecker et al., Nat Commun. 2019; 10(1):212.
BhCas12b V4	1108	ATTN	Pausch *et al.,* Science 2020;369(6501):333-337.
CasΦ	700-800	TBN (where B is G, T, or C)	Pausch *et al.,* Science 2020;369(6501):333-337.

Guide RNA (gRNA) molecules

[0508] Guide RNAs (gRNAs) of the present disclosure may be unimolecular (comprising a single RNA molecule, and referred to alternatively as chimeric), or modular (comprising more

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than one, and typically two, separate RNA molecules, such as a crRNA and a tracrRNA, which are usually associated with one another, for instance by duplexing). gRNAs and their component parts are described throughout the literature, for instance in Briner et al., Molecular Cell 2014; 56(2):333-339 ("Briner"), and in PCT Publication No. WO2016/073990A1.

[0509] In bacteria and archaea, type II CRISPR systems generally comprise an CRISPR/Cas nuclease protein such as Cas9, a CRISPR RNA (crRNA) that includes a 5′ region that is complementary to a foreign sequence, and a trans-activating crRNA (tracrRNA) that includes a 5′ region that is complementary to, and forms a duplex with, a 3′ region of the crRNA. While not intending to be bound by any theory, it is thought that this duplex facilitates the formation of — and is necessary for the activity of — the Cas9/gRNA complex. As type II CRISPR systems were adapted for use in gene editing, it was discovered that the crRNA and tracrRNA could be joined into a single unimolecular or chimeric guide RNA, in one non-limiting example, by means of a four nucleotide (e.g., GAAA) "tetraloop" or "linker" sequence bridging complementary regions of the crRNA (at its 3′ end) and the tracrRNA (at its 5′ end). See Mali et al., Science 2013; 339(6121):823–826 ("Mali"); Jiang et al., Nat Biotechnol. 2013; 31(3):233–239 ("Jiang"); and Jinek et al., Science 2012; 337(6096):816-821 ("Jinek 2012").

[0510] Guide RNAs, whether unimolecular or modular, include a "targeting domain" that is fully or partially complementary to a target domain within a target sequence, such as a DNA sequence in the genome of a cell where editing is desired. Targeting domains are referred to by various names in the literature, including without limitation "guide sequences" (Hsu et al., Nat Biotechnol. 2013; 31(9):827–832, ("Hsu")), "complementarity regions" (PCT Publication No. WO2016/073990A1), "spacers" (Briner) and generically as "crRNAs" (Jiang). Irrespective of the names they are given, targeting domains are typically 10-30 nucleotides in length, and in certain embodiments are 16-24 nucleotides in length (for instance, 16, 17, 18, 19, 20, 21, 22, 23 or 24 nucleotides in length), and are at or near the 5′ terminus of in the case of a Cas9 gRNA, and at or near the 3′ terminus in the case of a Cpf1 gRNA.

[0511] In addition to the targeting domains, gRNAs typically (but not necessarily, as discussed below) include a plurality of domains that may influence the formation or activity of gRNA/Cas9 complexes. For instance, as mentioned above, the duplexed structure formed by first and secondary complementarity domains of a gRNA (also referred to as a repeat:anti-repeat

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duplex) interacts with the recognition (REC) lobe of Cas9 and can mediate the formation of Cas9/gRNA complexes. See Nishimasu 2014 and 2015. It should be noted that the first and/or second complementarity domains may contain one or more poly-A tracts, which can be recognized by RNA polymerases as a termination signal. The sequence of the first and second complementarity domains are, therefore, optionally modified to eliminate these tracts and promote the complete in vitro transcription of gRNAs, for instance through the use of A-G swaps as described in Briner, or A-U swaps. These and other similar modifications to the first and second complementarity domains are within the scope of the present disclosure.

[0512] Along with the first and second complementarity domains, Cas9 gRNAs typically include two or more additional duplexed regions that are involved in nuclease activity in vivo but not necessarily in vitro. See Nishimasu 2015. A first stem-loop one near the 3′ portion of the second complementarity domain is referred to variously as the "proximal domain," (PCT Publication No. WO2016/073990A1) "stem loop 1" (Nishimasu 2014 and 2015) and the "nexus" (Briner). One or more additional stem loop structures are generally present near the 3′ end of the gRNA, with the number varying by species: *S. pyogenes* gRNAs typically include two 3′ stem loops (for a total of four stem loop structures including the repeat:anti-repeat duplex), while *S. aureus* and other species have only one (for a total of three stem loop structures). A description of conserved stem loop structures (and gRNA structures more generally) organized by species is provided in Briner.

[0513] While the foregoing description has focused on gRNAs for use with Cas9, it should be appreciated that other CRISPR/Cas nucleases have been (or may in the future be) discovered or invented which utilize gRNAs that differ in some ways from those described to this point. For instance, Cpf1 ("CRISPR from Prevotella and Franciscella 1") which is also called Cas12a is a CRISPR/Cas nuclease that does not require a tracrRNA to function (see Zetsche et al., Cell 2015; 163:759–771 ("Zetsche I")). A gRNA for use in a Cpf1 genome editing system generally includes a targeting domain and a complementarity domain (alternately referred to as a "handle"). It should also be noted that, in gRNAs for use with Cpf1, the targeting domain is usually present at or near the 3′ end, rather than the 5′ end as described above in connection with Cas9 gRNAs (the handle is at or near the 5′ end of a Cpf1 gRNA).

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[0514] Those of skill in the art will appreciate, however, that although structural differences may exist between gRNAs from different prokaryotic species, or between Cpf1 and Cas9 gRNAs, the principles by which gRNAs operate are generally consistent. Because of this consistency of operation, gRNAs can be defined, in broad terms, by their targeting domain sequences, and skilled artisans will appreciate that a given targeting domain sequence can be incorporated in any suitable gRNA, including a unimolecular or chimeric gRNA, or a gRNA that includes one or more chemical modifications and/or sequential modifications (substitutions, additional nucleotides, truncations, etc.). Thus, for economy of presentation in this disclosure, gRNAs may be described solely in terms of their targeting domain sequences.

[0515] More generally, skilled artisans will appreciate that some aspects of the present disclosure relate to systems, methods and compositions that can be implemented using multiple CRISPR/Cas nucleases. For this reason, unless otherwise specified, the term gRNA should be understood to encompass any suitable gRNA that can be used with any CRISPR/Cas nuclease, and not only those gRNAs that are compatible with a particular species of Cas9 or Cpf1. By way of illustration, the term gRNA can, in certain embodiments, include a gRNA for use with any CRISPR/Cas nuclease occurring in a Class 2 CRISPR system, such as a type II or type V or CRISPR system, or an CRISPR/Cas nuclease derived or adapted therefrom.

[0516] In some embodiments a method or system of the present disclosure may use more than one gRNA. In some embodiments, two or more gRNAs may be used to create two or more double strand breaks in the genome of a cell. In some embodiments, a multiplexed editing strategy may be used that targets two or more essential genes at the same time with two or more knock-in cassettes. In some such embodiments, the two or more knock-in cassettes may comprise different exogenous cargo sequences, e.g., different knock-in cassettes may encode different gene products of interest and thus the edited cells will express a plurality of gene products of interest from different knock-in cassettes targeted to different loci.

[0517] In some embodiments using more than one gRNA, a double-strand break may be caused by a dual-gRNA paired "nickase" strategy. In some embodiments for selecting gRNAs, including the determination for which gRNAs can be used for the dual-gRNA paired "nickase" strategy, gRNA pairs should be oriented on the DNA such that PAMs are facing out and cutting with the D10A Cas9 nickase will result in 5′ overhangs.

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[0518] In some embodiments, a method or system of the present disclosure may use a prime editing gRNA (pegRNA) in conjunction with a prime editor (PE). As is well known in the art, a pegRNA is substantially larger than standard gRNAs, e.g., in some embodiments longer than 50, 100, 150 or 250 nucleotides, e.g., as described in Anzalone et al., Nature 2019; 576:149-157, the entire contents of which are incorporated herein by reference. The pegRNA is a gRNA with a primer binding sequence (PBS) and a donor template containing the desired RNA sequence added at one of the termini, e.g., the 3′ end. The PE:pegRNA complex binds to the target DNA, and the nickase domain of the prime editor nicks only one strand, generating a flap. The PBS, located on the pegRNA, binds to the DNA flap and the edited RNA sequence is reverse transcribed using the reverse transcriptase domain of the prime editor. The edited strand is incorporated into the DNA at the end of the nicked flap, and the target DNA is repaired with the new reverse transcribed DNA. The original DNA segment is removed by a cellular endonuclease. This leaves one strand edited, and one strand unedited. In the newest PE systems, e.g., PE3 and PE3b, the unedited strand can be corrected to match the newly edited strand by using an additional standard gRNA. In this case, the unedited strand is nicked by a nickase and the newly edited strand is used as a template to repair the nick, thus completing the edit.

gRNA design

[0519] Methods for selection and validation of target sequences as well as off-target analyses have been described previously, e.g., in Mali; Hsu; Fu et al., Nat Biotechnol 2014; 32(3):279-84, Heigwer et al., Nat methods 2014; 11(2):122-3; Bae et al., Bioinformatics 2014; 30(10):1473-5; and Xiao et al. Bioinformatics 2014; 30(8):1180-1182. As a non-limiting example, gRNA design may involve the use of a software tool to optimize the choice of potential target sequences corresponding to a user's target sequence, e.g., to minimize total off-target activity across the genome. While off-target activity is not limited to cleavage, the cleavage efficiency at each off-target sequence can be predicted, e.g., using an experimentally-derived weighting scheme. These and other guide selection methods are described in detail in PCT Publication No. WO2016/073990A1.

[0520] For example, methods for selection and validation of target sequences as well as off-target analyses can be performed using cas-offinder (Bae et al., Bioinformatics 2014;

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30:1473–5). Cas-offinder is a tool that can quickly identify all sequences in a genome that have up to a specified number of mismatches to a guide sequence.

[0521] As another example, methods for scoring how likely a given sequence is to be an off-target (e.g., once candidate target sequences are identified) can be performed. An exemplary score includes a Cutting Frequency Determination (CFD) score, as described by Doench et al., Nat Biotechnol. 2016; 34:184–91.

gRNA modifications

[0522] In certain embodiments, gRNAs as used herein may be modified or unmodified gRNAs. In certain embodiments, a gRNA may include one or more modifications. In certain embodiments, the one or more modifications may include a phosphorothioate linkage modification, a phosphorodithioate (PS2) linkage modification, a 2'-O-methyl modification, or combinations thereof. In certain embodiments, the one or more modifications may be at the 5′ end of the gRNA, at the 3′ end of the gRNA, or combinations thereof.

[0523] In certain embodiments, a gRNA modification may comprise one or more phosphorodithioate (PS2) linkage modifications.

[0524] In some embodiments, a gRNA used herein includes one or more or a stretch of deoxyribonucleic acid (DNA) bases, also referred to herein as a "DNA extension." In some embodiments, a gRNA used herein includes a DNA extension at the 5′ end of the gRNA, the 3′ end of the gRNA, or a combination thereof. In certain embodiments, the DNA extension may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 DNA bases long. For example, in certain embodiments, the DNA extension may be 1, 2, 3, 4, 5, 10, 15, 20, or 25 DNA bases long. In certain embodiments, the DNA extension may include one or more DNA bases selected from adenine (A), guanine (G), cytosine (C), or thymine (T). In certain embodiments, the DNA extension includes the same DNA bases. For example, the DNA extension may include a stretch of adenine (A) bases. In certain embodiments, the DNA extension may include a stretch of thymine (T) bases. In certain embodiments, the DNA extension includes a combination of different DNA bases.

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[0525] Exemplary suitable 5′ extensions for Cpf1 guide RNAs are provided in Table 6 below:

Table 6: Exemplary Cpf1 gRNA 5′ Extensions

SEQ ID NO:	5′ extension sequence	5′ modification
N/A	rCrUrUrUrU	+5 RNA
67	rArArGrArCrCrUrUrUrU	+10 RNA
68	rArUrGrUrGrUrUrUrUrUrGrUrCrArArArArGrArCrCrUrUrUrU	+25 RNA
69	rArGrGrCrCrArGrCrUrUrGrCrCrGrGrUrUrUrUrUrUrArGrUrCrGrUrGrCrUrGrCrUrUrCrArUrGrUrGrUrUrUrUrGrUrCrArArArArGrArCrCrUrUrUrU	+60 RNA
N/A	CTTTT	+5 DNA
70	AAGACCTTTT	+10 DNA
71	ATGTGTTTTTGTCAAAAGACCTTTT	+25 DNA
72	AGGCCAGCTTGCCGGTTTTTTAGTCGTGCTGCTTCATGTGTTTTTGTCAAAAGACCTTTT	+60 DNA
73	TTTTTGTCAAAAGACCTTTT	+20 DNA
74	GCTTCATGTGTTTTTGTCAAAAGACCTTTT	+30 DNA
75	GCCGGTTTTTTAGTCGTGCTGCTTCATGTGTTTTTGTCAAAAGACCTTTT	+50 DNA
76	TAGTCGTGCTGCTTCATGTGTTTTTGTCAAAAGACCTTTT	+40 DNA
77	C*C*GAAGTTTTCTTCGGTTTT	+20 DNA + 2xPS
78	T*T*TTTCCGAAGTTTTCTTCGGTTTT	+25 DNA + 2xPS
79	A*A*CGCTTTTTCCGAAGTTTTCTTCGGTTTT	+30 DNA + 2xPS
80	G*C*GTTGTTTTCAACGCTTTTTCCGAAGTTTTCTTCGGTTTT	+41 DNA + 2xPS
81	G*G*CTTCTTTTGAAGCCTTTTTGCGTTGTTTTCAACGCTTTTTCCGAAGTTTTCTTCGGTTTT	+62 DNA + 2xPS
82	A*T*GTGTTTTTGTCAAAAGACCTTTT	+25 DNA + 2xPS
83	AAAAAAAAAAAAAAAAAAAAAAAAA	+25 A
84	TTTTTTTTTTTTTTTTTTTTTTTTT	+25 T
85	mA*mU*rGrUrGrUrUrUrUrGrUrCrArArArArGrArCrCrUrUrUrU	+25 RNA + 2xPS
86	mA*mA*rArArArArArArArArArArArArArArArArArArArA	PolyA RNA + 2xPS
87	mU*mU*rUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrU	PolyU RNA + 2xPS

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[0526] In certain embodiments, a gRNA used herein includes a DNA extension as well as a chemical modification, e.g., one or more phosphorothioate linkage modifications, one or more phosphorodithioate (PS2) linkage modifications, one or more 2'-O-methyl modifications, or one or more additional suitable chemical gRNA modification disclosed herein, or combinations thereof. In certain embodiments, the one or more modifications may be at the 5′ end of the gRNA, at the 3′ end of the gRNA, or combinations thereof.

[0527] Without wishing to be bound by theory, it is contemplated that any DNA extension may be used with any gRNA disclosed herein, so long as it does not hybridize to the target nucleic acid being targeted by the gRNA and it also exhibits an increase in editing at the target nucleic acid site relative to a gRNA which does not include such a DNA extension.

[0528] In some embodiments, a gRNA used herein includes one or more or a stretch of ribonucleic acid (RNA) bases, also referred to herein as an "RNA extension." In some embodiments, a gRNA used herein includes an RNA extension at the 5′ end of the gRNA, the 3′ end of the gRNA, or a combination thereof. In certain embodiments, the RNA extension may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 RNA bases long. For example, in certain embodiments, the RNA extension may be 1, 2, 3, 4, 5, 10, 15, 20, or 25 RNA bases long. In certain embodiments, the RNA extension may include one or more RNA bases selected from adenine (rA), guanine (rG), cytosine (rC), or uracil (rU), in which the "r" represents RNA, 2'-hydroxy. In certain embodiments, the RNA extension includes the same RNA bases. For example, the RNA extension may include a stretch of adenine (rA) bases. In certain embodiments, the RNA extension includes a combination of different RNA bases. In certain embodiments, a gRNA used herein includes an RNA extension as well as one or more phosphorothioate linkage modifications, one or more phosphorodithioate (PS2) linkage modifications, one or more 2'-O-methyl modifications, one or more additional suitable gRNA modification, e.g., chemical modification, disclosed herein, or combinations thereof. In certain embodiments, the one or more modifications may be at the 5′ end of the gRNA, at the 3′ end of

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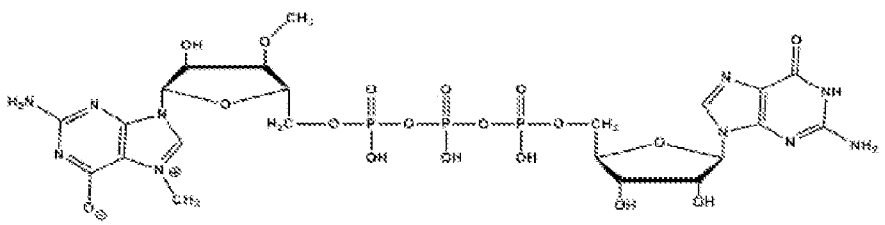
the gRNA, or combinations thereof. In certain embodiments, a gRNA including a RNA extension may comprise a sequence set forth herein.

[0529] It is contemplated that gRNAs used herein may also include an RNA extension and a DNA extension. In certain embodiments, the RNA extension and DNA extension may both be at the 5′ end of the gRNA, the 3′ end of the gRNA, or a combination thereof. In certain embodiments, the RNA extension is at the 5′ end of the gRNA and the DNA extension is at the 3′ end of the gRNA. In certain embodiments, the RNA extension is at the 3′ end of the gRNA and the DNA extension is at the 5′ end of the gRNA.

[0530] In some embodiments, a gRNA which includes a modification, e.g., a DNA extension at the 5′ end and/or a chemical modification as disclosed herein, is complexed with a CRISPR/Cas nuclease, e.g., an AsCpf1 nuclease, to form an RNP, which is then employed to edit a target cell, e.g., a pluripotent stem cell or a progeny thereof.

[0531] Certain exemplary modifications discussed in this section can be included at any position within a gRNA sequence including, without limitation at or near the 5′ end (e.g., within 1-10, 1-5, or 1-2 nucleotides of the 5′ end) and/or at or near the 3′ end (e.g., within 1-10, 1-5, or 1-2 nucleotides of the 3′ end). In some cases, modifications are positioned within functional motifs, such as the repeat-anti-repeat duplex of a Cas9 gRNA, a stem loop structure of a Cas9 or Cpf1 gRNA, and/or a targeting domain of a gRNA.

[0532] As one example, the 5′ end of a gRNA can include a eukaryotic mRNA cap structure or cap analog (e.g., a G(5′)ppp(5′)G cap analog, a m7G(5′)ppp(5′)G cap analog, or a 3′-O-Me-m7G(5′)ppp(5′)G anti reverse cap analog (ARCA)), as shown below:



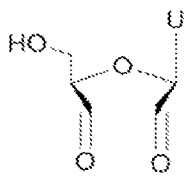
The cap or cap analog can be included during either chemical or enzymatic synthesis of the gRNA.

[0533] Along similar lines, the 5′ end of the gRNA can lack a 5′ triphosphate group. For instance, *in vitro* transcribed gRNAs can be phosphatase-treated (e.g., using calf intestinal alkaline phosphatase) to remove a 5′ triphosphate group.

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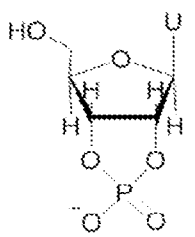
[0534] Another common modification involves the addition, at the 3′ end of a gRNA, of a plurality (e.g., 1-10, 10-20, or 25-200) of adenine (A) residues referred to as a polyA tract. The polyA tract can be added to a gRNA during chemical or enzymatic synthesis, using a polyadenosine polymerase (e.g., *E. coli* Poly(A)Polymerase).

[0535] Guide RNAs can be modified at a 3′ terminal U ribose. For example, the two terminal hydroxyl groups of the U ribose can be oxidized to aldehyde groups and a concomitant opening of the ribose ring to afford a modified nucleoside as shown below:



wherein "U" can be an unmodified or modified uridine.

[0536] The 3′ terminal U ribose can be modified with a 2'3′ cyclic phosphate as shown below:



wherein "U" can be an unmodified or modified uridine.

[0537] Guide RNAs can contain 3′ nucleotides that can be stabilized against degradation, e.g., by incorporating one or more of the modified nucleotides described herein. In certain embodiments, uridines can be replaced with modified uridines, e.g., 5-(2-amino)propyl uridine, and 5-bromo uridine, or with any of the modified uridines described herein; adenosines and guanosines can be replaced with modified adenosines and guanosines, e.g., with modifications at the 8-position, e.g., 8-bromo guanosine, or with any of the modified adenosines or guanosines described herein.

[0538] In certain embodiments, sugar-modified ribonucleotides can be incorporated into a gRNA, e.g., wherein the 2' OH-group is replaced by a group selected from H, -OR, -R (wherein R can be, e.g., alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar), halo, -SH, -SR (wherein R can be, e.g., alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar), amino (wherein

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amino can be, e.g., NH_2, alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroarylamino, diheteroarylamino, or amino acid); or cyano (-CN). In certain embodiments, the phosphate backbone can be modified as described herein, e.g., with a phosphothioate (PhTx) group. In certain embodiments, one or more of the nucleotides of the gRNA can each independently be a modified or unmodified nucleotide including, but not limited to 2'-sugar modified, such as, 2'-O-methyl, 2'-O-methoxyethyl, or 2'-Fluoro modified including, e.g., 2'-F or 2'-O-methyl, adenosine (A), 2'-F or 2'-O-methyl, cytidine (C), 2'-F or 2'-O-methyl, uridine (U), 2'-F or 2'-O-methyl, thymidine (T), 2'-F or 2'-O-methyl, guanosine (G), 2'-O-methoxyethyl-5-methyluridine (Teo), 2'-O-methoxyethyladenosine (Aeo), 2'-O-methoxyethyl-5-methylcytidine (m5Ceo), and any combinations thereof.

[0539] Guide RNAs can also include "locked" nucleic acids (LNA) in which the 2' OH-group can be connected, e.g., by a C1-6 alkylene or C1-6 heteroalkylene bridge, to the 4' carbon of the same ribose sugar. Any suitable moiety can be used to provide such bridges, including without limitation methylene, propylene, ether, or amino bridges; O-amino (wherein amino can be, e.g., NH_2, alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroarylamino, or diheteroarylamino, ethylenediamine, or polyamino) and aminoalkoxy or $O(CH_2)_n$-amino (wherein amino can be, e.g., NH_2, alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroarylamino, or diheteroarylamino, ethylenediamine, or polyamino).

[0540] In certain embodiments, a gRNA can include a modified nucleotide which is multicyclic (e.g., tricyclo; and "unlocked" forms, such as glycol nucleic acid (GNA) (e.g., R-GNA or S-GNA, where ribose is replaced by glycol units attached to phosphodiester bonds), or threose nucleic acid (TNA, where ribose is replaced with α-L-threofuranosyl-(3'→2')).

[0541] Generally, gRNAs include the sugar group ribose, which is a 5-membered ring having an oxygen. Exemplary modified gRNAs can include, without limitation, replacement of the oxygen in ribose (e.g., with sulfur (S), selenium (Se), or alkylene, such as, e.g., methylene or ethylene); addition of a double bond (e.g., to replace ribose with cyclopentenyl or cyclohexenyl); ring contraction of ribose (e.g., to form a 4-membered ring of cyclobutane or oxetane); ring expansion of ribose (e.g., to form a 6- or 7-membered ring having an additional carbon or heteroatom, such as for example, anhydrohexitol, altritol, mannitol, cyclohexanyl, cyclohexenyl, and morpholino that also has a phosphoramidate backbone). Although the majority of sugar

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analog alterations are localized to the 2' position, other sites are amenable to modification, including the 4' position. In certain embodiments, a gRNA comprises a 4'-S, 4'-Se or a 4'-C-aminomethyl-2'-O-Me modification.

[0542] In certain embodiments, deaza nucleotides, e.g., 7-deaza-adenosine, can be incorporated into a gRNA. In certain embodiments, O- and N-alkylated nucleotides, e.g., N6-methyl adenosine, can be incorporated into a gRNA. In certain embodiments, one or more or all of the nucleotides in a gRNA are deoxynucleotides.

[0543] Guide RNAs can also include one or more cross-links between complementary regions of the crRNA (at its 3′ end) and the tracrRNA (at its 5′ end) (e.g., within a "tetraloop" structure and/or positioned in any stem loop structure occurring within a gRNA). A variety of linkers are suitable for use. For example, guide RNAs can include common linking moieties including, without limitation, polyvinylether, polyethylene, polypropylene, polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyglycolide (PGA), polylactide (PLA), polycaprolactone (PCL), and copolymers thereof.

[0544] In some embodiments, a bifunctional cross-linker is used to link a 5′ end of a first gRNA fragment and a 3′ end of a second gRNA fragment, and the 3′ or 5′ ends of the gRNA fragments to be linked are modified with functional groups that react with the reactive groups of the cross-linker. In general, these modifications comprise one or more of amine, sulfhydryl, carboxyl, hydroxyl, alkene (e.g., a terminal alkene), azide and/or another suitable functional group. Multifunctional (e.g. bifunctional) cross-linkers are also generally known in the art, and may be either heterofunctional or homofunctional, and may include any suitable functional group, including without limitation isothiocyanate, isocyanate, acyl azide, an NHS ester, sulfonyl chloride, tosyl ester, tresyl ester, aldehyde, amine, epoxide, carbonate (e.g., Bis(p-nitrophenyl) carbonate), aryl halide, alkyl halide, imido ester, carboxylate, alkyl phosphate, anhydride, fluorophenyl ester, HOBt ester, hydroxymethyl phosphine, O-methylisourea, DSC, NHS carbamate, glutaraldehyde, activated double bond, cyclic hemiacetal, NHS carbonate, imidazole carbamate, acyl imidazole, methylpyridinium ether, azlactone, cyanate ester, cyclic imidocarbonate, chlorotriazine, dehydroazepine, 6-sulfo-cytosine derivatives, maleimide, aziridine, TNB thiol, Ellman's reagent, peroxide, vinylsulfone, phenylthioester, diazoalkanes, diazoacetyl, epoxide, diazonium, benzophenone, anthraquinone, diazo derivatives, diazirine

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derivatives, psoralen derivatives, alkene, phenyl boronic acid, etc. In some embodiments, a first gRNA fragment comprises a first reactive group and the second gRNA fragment comprises a second reactive group. For example, the first and second reactive groups can each comprise an amine moiety, which are crosslinked with a carbonate-containing bifunctional crosslinking reagent to form a urea linkage. In other instances, (a) the first reactive group comprises a bromoacetyl moiety and the second reactive group comprises a sulfhydryl moiety, or (b) the first reactive group comprises a sulfhydryl moiety and the second reactive group comprises a bromoacetyl moiety, which are crosslinked by reacting the bromoacetyl moiety with the sulfhydryl moiety to form a bromoacetyl-thiol linkage. These and other cross-linking chemistries are known in the art, and are summarized in the literature, including by Greg T. Hermanson, Bioconjugate Techniques, 3rd Ed. 2013, published by Academic Press.

[0545] Additional suitable gRNA modifications will be apparent to those of ordinary skill in the art based on the present disclosure. Suitable gRNA modifications include, for example, those described in PCT Publication No. WO2019070762A1 entitled "MODIFIED CPF1 GUIDE RNA;" in PCT Publication No. WO2016089433A1 entitled "GUIDE RNA WITH CHEMICAL MODIFICATIONS;" in PCT Publication No. WO2016164356A1 entitled "CHEMICALLY MODIFIED GUIDE RNAS FOR CRISPR/CAS-MEDIATED GENE REGULATION;" and in PCT Publication No. WO2017053729A1 entitled "NUCLEASE-MEDIATED GENOME EDITING OF PRIMARY CELLS AND ENRICHMENT THEREOF;" the entire contents of each of which are incorporated herein by reference.

Exemplary gRNAs

[0546] Non-limiting examples of guide RNAs suitable for certain embodiments embraced by the present disclosure are provided herein, for example, in the Tables below. Those of ordinary skill in the art will be able to envision suitable guide RNA sequences for a specific nuclease, e.g., a Cas9 or Cpf1 nuclease, from the disclosure of the targeting domain sequence, either as a DNA or RNA sequence. For example, a guide RNA comprising a targeting sequence consisting of RNA nucleotides would include the RNA sequence corresponding to the targeting domain sequence provided as a DNA sequence, and this contain uracil instead of thymidine nucleotides. For example, a guide RNA comprising a targeting domain sequence consisting of RNA nucleotides, and described by the DNA sequence TCTGCAGAAATGTTCCCCGT (SEQ

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ID NO: 88) would have a targeting domain of the corresponding RNA sequence UCUGCAGAAAUGUUCCCCGU (SEQ ID NO: 89). As will be apparent to the skilled artisan, such a targeting sequence would be linked to a suitable guide RNA scaffold, e.g., a crRNA scaffold sequence or a chimeric crRNA/tracrRNA scaffold sequence. Suitable gRNA scaffold sequences are known to those of ordinary skill in the art. For AsCpf1, for example, a suitable scaffold sequence comprises the sequence UAAUUUCUACUCUUGUAGAU (SEQ ID NO: 90), added to the 5′-terminus of the targeting domain. In the example above, this would result in a Cpf1 guide RNA of the sequence UAAUUUCUACUCUUGUAGAUUCUGCAGAAAUGUUCCCCGU (SEQ ID NO: 91). Those of skill in the art would further understand how to modify such a guide RNA, e.g., by adding a DNA extension (e.g., in the example above, adding a 25-mer DNA extension as described herein would result, for example, in a guide RNA of the sequence ATGTGTTTTTGTCAAAAGACCTTTTrUrArArUrUrUrCrUrArCrUrCrUrUrGrUrArGrArUrUr CrUrGrCrArGrArArArUrGrUrUrCrCrCrCrGrU (SEQ ID NO: 92)). It will be understood that the exemplary targeting sequences provided herein are not limiting, and additional suitable sequences, e.g., variants of the specific sequences disclosed herein, will be apparent to the skilled artisan based on the present disclosure in view of the general knowledge in the art.

[0547] In some embodiments the gRNA for use in the disclosure is a gRNA targeting TGFβRII (TGFβRII gRNA). In some embodiments, the gRNA targeting TGFβRII is one or more of the gRNAs described in Table 7.

Table 7: Exemplary TGFβRII gRNAs

Name	gRNA Targeting Domain Sequence (DNA)	Length	Enzyme	SEQ ID NO:
TGFBR24326	CAGGACGATGTGCAGCGGCC	20	AsCpf1 RR	29
TGFBR24327	ACCGCACGTTCAGAAGTCGG	20	AsCpf1 RR	30
TGFBR24328	ACAACTGTGTAAATTTTGTG	20	AsCpf1 RR	31
TGFBR24329	CAACTGTGTAAATTTTGTGA	20	AsCpf1 RR	32
TGFBR24330	ACCTGTGACAACCAGAAATC	20	AsCpf1 RR	33
TGFBR24331	CCTGTGACAACCAGAAATCC	20	AsCpf1 RR	34
TGFBR24332	TGTGGCTTCTCACAGATGGA	20	AsCpf1 RR	35
TGFBR24333	TCTGTGAGAAGCCACAGGAA	20	AsCpf1 RR	36
TGFBR24334	AAGCTCCCCTACCATGACTT	20	AsCpf1 RR	37
TGFBR24335	GAATAAAGTCATGGTAGGGG	20	AsCpf1 RR	38

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TGFBR24336	AGAATAAAGTCATGGTAGGG	20	AsCpf1 RR	39
TGFBR24337	CTACCATGACTTTATTCTGG	20	AsCpf1 RR	40
TGFBR24338	TACCATGACTTTATTCTGGA	20	AsCpf1 RR	41
TGFBR24339	TAATGCACTTTGGAGAAGCA	20	AsCpf1 RR	42
TGFBR24340	TTCATAATGCACTTTGGAGA	20	AsCpf1 RR	43
TGFBR24341	AAGTGCATTATGAAGGAAAA	20	AsCpf1 RR	44
TGFBR24342	TGTGTTCCTGTAGCTCTGAT	20	AsCpf1 RR	45
TGFBR24343	TGTAGCTCTGATGAGTGCAA	20	AsCpf1 RR	46
TGFBR24344	AGTGACAGGCATCAGCCTCC	20	AsCpf1 RR	47
TGFBR24345	AGTGGTGGCAGGAGGCTGAT	20	AsCpf1 RR	48
TGFBR24346	AGGTTGAACTCAGCTTCTGC	20	AsCpf1 RR	49
TGFBR24347	CAGGTTGAACTCAGCTTCTG	20	AsCpf1 RR	50
TGFBR24348	ACCTGGGAAACCGGCAAGAC	20	AsCpf1 RR	51
TGFBR24349	CGTCTTGCCGGTTTCCCAGG	20	AsCpf1 RR	52
TGFBR24350	GCGTCTTGCCGGTTTCCCAG	20	AsCpf1 RR	53
TGFBR24351	TGAGCTTCCGCGTCTTGCCG	20	AsCpf1 RR	54
TGFBR24352	GCGAGCACTGTGCCATCATC	20	AsCpf1 RR	55
TGFBR24353	GGATGATGGCACAGTGCTCG	20	AsCpf1 RR	56
TGFBR24354	AGGATGATGGCACAGTGCTC	20	AsCpf1 RR	57
TGFBR24355	CGTGTGCCAACAACATCAAC	20	AsCpf1 RR	58
TGFBR24356	GCTCAATGGGCAGCAGCTCT	20	AsCpf1 RR	59
TGFBR24357	ACCAGGGTGTCCAGCTCAAT	20	AsCpf1 RR	60
TGFBR24358	CACCAGGGTGTCCAGCTCAA	20	AsCpf1 RR	61
TGFBR24359	CCACCAGGGTGTCCAGCTCA	20	AsCpf1 RR	62
TGFBR24360	GCTTGGCCTTATAGACCTCA	20	AsCpf1 RR	63
TGFBR24361	GAGCAGTTTGAGACAGTGGC	20	AsCpf1 RR	64
TGFBR24362	AGAGGCATACTCCTCATAGG	20	AsCpf1 RR	65
TGFBR24363	CTATGAGGAGTATGCCTCTT	20	AsCpf1 RR	66
TGFBR24364	AAGAGGCATACTCCTCATAG	20	AsCpf1 RR	67
TGFBR24365	TATGAGGAGTATGCCTCTTG	20	AsCpf1 RR	68
TGFBR24366	GATTGATGTCTGAGAAGATG	20	AsCpf1 RR	69
TGFBR24367	CTCCTCAGCCGTCAGGAACT	20	AsCpf1 RR	70
TGFBR24368	GTTCCTGACGGCTGAGGAGC	20	AsCpf1 RR	71
TGFBR24369	GCTCCTCAGCCGTCAGGAAC	20	AsCpf1 RR	72
TGFBR24370	TGACGGCTGAGGAGCGGAAG	20	AsCpf1 RR	73
TGFBR24371	TCTTCCGCTCCTCAGCCGTC	20	AsCpf1 RR	74
TGFBR24372	AACTCCGTCTTCCGCTCCTC	20	AsCpf1 RR	75
TGFBR24373	CAACTCCGTCTTCCGCTCCT	20	AsCpf1 RR	76
TGFBR24374	CCAACTCCGTCTTCCGCTCC	20	AsCpf1 RR	77
TGFBR24375	ACGCCAAGGGCAACCTACAG	20	AsCpf1 RR	78

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TGFBR24376	CGCCAAGGGCAACCTACAGG	20	AsCpf1 RR	79
TGFBR24377	AGCTGATGACATGCCGCGTC	20	AsCpf1 RR	80
TGFBR24378	GGGCGAGGGAGCTGCCCAGC	20	AsCpf1 RR	81
TGFBR24379	CGGGCGAGGGAGCTGCCCAG	20	AsCpf1 RR	82
TGFBR24380	CCGGGCGAGGGAGCTGCCCA	20	AsCpf1 RR	83
TGFBR24381	TCGCCCGGGGGATTGCTCAC	20	AsCpf1 RR	84
TGFBR24382	ACATGGAGTGTGATCACTGT	20	AsCpf1 RR	85
TGFBR24383	CAGTGATCACACTCCATGTG	20	AsCpf1 RR	86
TGFBR24384	TGTGGGAGGCCCAAGATGCC	20	AsCpf1 RR	87
TGFBR24385	TGTGCACGATGGGCATCTTG	20	AsCpf1 RR	88
TGFBR24386	CGAGGATATTGGAGCTCTTG	20	AsCpf1 RR	89
TGFBR24387	ATATCCTCGTGAAGAACGAC	20	AsCpf1 RR	90
TGFBR24388	GACGCAGGGAAAGCCCAAAG	20	AsCpf1 RR	91
TGFBR24389	CTGCGTCTGGACCCTACTCT	20	AsCpf1 RR	92
TGFBR24390	TGCGTCTGGACCCTACTCTG	20	AsCpf1 RR	93
TGFBR24391	CAGACAGAGTAGGGTCCAGA	20	AsCpf1 RR	94
TGFBR24392	GCCAGCACGATCCCACCGCA	20	AsCpf1 RVR	95
TGFBR24393	AAGGAAAAAAAAAAGCCTGG	20	AsCpf1 RVR	96
TGFBR24394	ACACCAGCAATCCTGACTTG	20	AsCpf1 RVR	97
TGFBR24395	ACTAGCAACAAGTCAGGATT	20	AsCpf1 RVR	98
TGFBR24396	GCAACTCCCAGTGGTGGCAG	20	AsCpf1 RVR	99
TGFBR24397	TGTCATCATCATCTTCTACT	20	AsCpf1 RVR	100
TGFBR24398	GACCTCAGCAAAGCGACCTT	20	AsCpf1 RVR	101
TGFBR24399	AGGCCAAGCTGAAGCAGAAC	20	AsCpf1 RVR	102
TGFBR24400	AGGAGTATGCCTCTTGGAAG	20	AsCpf1 RVR	103
TGFBR24401	CCTCTTGGAAGACAGAGAAG	20	AsCpf1 RVR	104
TGFBR24402	TTCTCATGCTTCAGATTGAT	20	AsCpf1 RVR	105
TGFBR24403	CTCGTGAAGAACGACCTAAC	20	AsCpf1 RVR	106
TGFbR2036	GGCCGCTGCACATCGTCCTG	20	SpyCas9	107
TGFbR2037	GCGGGGTCTGCCATGGGTCG	20	SpyCas9	108
TGFbR2038	AGTTGCTCATGCAGGATTTC	20	SpyCas9	109
TGFbR2039	CCAGAATAAAGTCATGGTAG	20	SpyCas9	110
TGFbR2040	CCCCTACCATGACTTTATTC	20	SpyCas9	111
TGFbR2041	AAGTCATGGTAGGGGAGCTT	20	SpyCas9	112
TGFbR2042	AGTCATGGTAGGGGAGCTTG	20	SpyCas9	113
TGFbR2043	ATTGCACTCATCAGAGCTAC	20	SpyCas9	114
TGFbR2044	CCTAGAGTGAAGAGATTCAT	20	SpyCas9	115
TGFbR2045	CCAATGAATCTCTTCACTCT	20	SpyCas9	116
TGFbR2046	AAAGTCATGGTAGGGGAGCT	20	SpyCas9	117
TGFbR2047	GTGAGCAATCCCCCGGGCGA	20	SpyCas9	118

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TGFbR2048	GTCGTTCTTCACGAGGATAT	20	SpyCas9	119
TGFbR2049	GCCGCGTCAGGTACTCCTGT	20	SpyCas9	120
TGFbR2050	GACGCGGCATGTCATCAGCT	20	SpyCas9	121
TGFbR2051	GCTTCTGCTGCCGGTTAACG	20	SpyCas9	122
TGFbR2052	GTGGATGACCTGGCTAACAG	20	SpyCas9	123
TGFbR2053	GTGATCACACTCCATGTGGG	20	SpyCas9	124
TGFbR2054	GCCCATTGAGCTGGACACCC	20	SpyCas9	125
TGFbR2055	GCGGTCATCTTCCAGGATGA	20	SpyCas9	126
TGFbR2056	GGGAGCTGCCCAGCTTGCGC	20	SpyCas9	127
TGFbR2057	GTTGATGTTGTTGGCACACG	20	SpyCas9	128
TGFbR2058	GGCATCTTGGGCCTCCCACA	20	SpyCas9	129
TGFbR2059	GCGGCATGTCATCAGCTGGG	20	SpyCas9	130
TGFbR2060	GCTCCTCAGCCGTCAGGAAC	20	SpyCas9	131
TGFbR2061	GCTGGTGTTATATTCTGATG	20	SpyCas9	132
TGFbR2062	CCGACTTCTGAACGTGCGGT	20	SpyCas9	133
TGFbR2063	TGCTGGCGATACGCGTCCAC	20	SpyCas9	134
TGFbR2064	CCCGACTTCTGAACGTGCGG	20	SpyCas9	135
TGFbR2065	CCACCGCACGTTCAGAAGTC	20	SpyCas9	136
TGFbR2066	TCACCCGACTTCTGAACGTG	20	SpyCas9	137
TGFbR2067	CCCACCGCACGTTCAGAAGT	20	SpyCas9	138
TGFbR2068	CGAGCAGCGGGGTCTGCCAT	20	SpyCas9	139
TGFbR2069	ACGAGCAGCGGGGTCTGCCA	20	SpyCas9	140
TGFbR2070	AGCGGGGTCTGCCATGGGTC	20	SpyCas9	141
TGFbR2071	CCTGAGCAGCCCCCGACCCA	20	SpyCas9	142
TGFbR2072	CCATGGGTCGGGGGCTGCTC	20	SpyCas9	143
TGFbR2073	AACGTGCGGTGGGATCGTGC	20	SpyCas9	144
TGFbR2074	GGACGATGTGCAGCGGCCAC	20	SpyCas9	145
TGFbR2075	GTCCACAGGACGATGTGCAG	20	SpyCas9	146
TGFbR2076	CATGGGTCGGGGGCTGCTCA	20	SpyCas9	147
TGFbR2077	CAGCGGGGTCTGCCATGGGT	20	SpyCas9	148
TGFbR2078	ATGGGTCGGGGGCTGCTCAG	20	SpyCas9	149
TGFbR2079	CGGGGTCTGCCATGGGTCGG	20	SpyCas9	150
TGFbR2080	AGGAAGTCTGTGTGGCTGTA	20	SpyCas9	151
TGFbR2081	CTCCATCTGTGAGAAGCCAC	20	SpyCas9	152
TGFbR2082	ATGATAGTCACTGACAACAA	20	SpyCas9	153
TGFbR2083	GATGCTGCAGTTGCTCATGC	20	SpyCas9	154
TGFbR2084	ACAGCCACACAGACTTCCTG	20	SpyCas9	155
TGFbR2085	GAAGCCACAGGAAGTCTGTG	20	SpyCas9	156
TGFbR2086	TTCCTGTGGCTTCTCACAGA	20	SpyCas9	157
TGFbR2087	CTGTGGCTTCTCACAGATGG	20	SpyCas9	158

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TGFbR2088	TCACAAAATTTACACAGTTG	20	SpyCas9	159
TGFbR2089	GACAACATCATCTTCTCAGA	20	SpyCas9	160
TGFbR2090	TCCAGAATAAAGTCATGGTA	20	SpyCas9	161
TGFbR2091	GGTAGGGGAGCTTGGGGTCA	20	SpyCas9	162
TGFbR2092	TTCTCCAAAGTGCATTATGA	20	SpyCas9	163
TGFbR2093	CATCTTCCAGAATAAAGTCA	20	SpyCas9	164
TGFbR2094	CACATGAAGAAAGTCTCACC	20	SpyCas9	165
TGFbR2095	TTCCAGAATAAAGTCATGGT	20	SpyCas9	166
TGFbR2096	TTTTCCTTCATAATGCACTT	20	SpyCas9	167
TGFBR24024	CACAGTTGTGGAAACTTGAC	20	AsCpf1	168
TGFBR24039	CCCAACTCCGTCTTCCGCTC	20	AsCpf1	169
TGFBR24040	GGCTTTCCCTGCGTCTGGAC	20	AsCpf1	170
TGFBR24036	CTGAGGTCTATAAGGCCAAG	20	AsCpf1	171
TGFBR24026	TGATGTGAGATTTTCCACCT	20	AsCpf1	172
TGFBR24038	CCTATGAGGAGTATGCCTCT	20	AsCpf1	173
TGFBR24033	AAGTGACAGGCATCAGCCTC	20	AsCpf1	174
TGFBR24028	CCATGACCCCAAGCTCCCCT	20	AsCpf1	175
TGFBR24031	CTTCATAATGCACTTTGGAG	20	AsCpf1	176
TGFBR24032	TTCATGTGTTCCTGTAGCTC	20	AsCpf1	177
TGFBR24029	TTCTGGAAGATGCTGCTTCT	20	AsCpf1	178
TGFBR24035	CCCACCAGGGTGTCCAGCTC	20	AsCpf1	179
TGFBR24037	AGACAGTGGCAGTCAAGATC	20	AsCpf1	180
TGFBR24041	CCTGCGTCTGGACCCTACTC	20	AsCpf1	181
TGFBR24025	CACAACTGTGTAAATTTTGT	20	AsCpf1	182
TGFBR24030	GAGAAGCAGCATCTTCCAGA	20	AsCpf1	183
TGFBR24027	TGGTTGTCACAGGTGGAAAA	20	AsCpf1	184
TGFBR24034	CCAGGTTGAACTCAGCTTCT	20	AsCpf1	185
TGFBR24043	ATCACAAAATTTACACAGTTG	21	SauCas9	186
TGFBR24065	GGCATCAGCCTCCTGCCACCA	21	SauCas9	187
TGFBR24110	GTTAGCCAGGTCATCCACAGA	21	SauCas9	188
TGFBR24099	GCTGGGCAGCTCCCTCGCCCG	21	SauCas9	189
TGFBR24064	CAGGAGGCTGATGCCTGTCAC	21	SauCas9	190
TGFBR24094	GAGGAGCGGAAGACGGAGTTG	21	SauCas9	191
TGFBR24108	CGTCTGGACCCTACTCTGTCT	21	SauCas9	192
TGFBR24058	TTTTTCCTTCATAATGCACTT	21	SauCas9	193
TGFBR24075	CCATTGAGCTGGACACCCTGG	21	SauCas9	194
TGFBR24057	CTTCTCCAAAGTGCATTATGA	21	SauCas9	195
TGFBR24103	GCCCAAGATGCCCATCGTGCA	21	SauCas9	196
TGFBR24060	TCATGTGTTCCTGTAGCTCTG	21	SauCas9	197
TGFBR24048	GTGATGCTGCAGTTGCTCATG	21	SauCas9	198

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TGFBR24087	TCTCATGCTTCAGATTGATGT	21	SauCas9	199
TGFBR24081	TCCCTATGAGGAGTATGCCTC	21	SauCas9	200
TGFBR24044	CATCACAAAATTTACACAGTT	21	SauCas9	201
TGFBR24077	ATTGAGCTGGACACCCTGGTG	21	SauCas9	202
TGFBR24080	CAGTCAAGATCTTTCCCTATG	21	SauCas9	203
TGFBR24046	AGGATTTCTGGTTGTCACAGG	21	SauCas9	204
TGFBR24101	TCCACAGTGATCACACTCCAT	21	SauCas9	205
TGFBR24079	AGCAGAACACTTCAGAGCAGT	21	SauCas9	206
TGFBR24072	CCGGCAAGACGCGGAAGCTCA	21	SauCas9	207
TGFBR24074	GATGTCAGAGCGGTCATCTTC	21	SauCas9	208
TGFBR24062	TCATTGCACTCATCAGAGCTA	21	SauCas9	209
TGFBR24054	CTTCCAGAATAAAGTCATGGT	21	SauCas9	210
TGFBR24045	AGATTTTCCACCTGTGACAAC	21	SauCas9	211
TGFBR24049	ACTGCAGCATCACCTCCATCT	21	SauCas9	212
TGFBR24098	AGCTGGGCAGCTCCCTCGCCC	21	SauCas9	213
TGFBR24090	TGACGGCTGAGGAGCGGAAGA	21	SauCas9	214
TGFBR24076	CATTGAGCTGGACACCCTGGT	21	SauCas9	215
TGFBR24078	AGCAAAGCGACCTTTCCCCAC	21	SauCas9	216
TGFBR24067	CGCGTTAACCGGCAGCAGAAG	21	SauCas9	217
TGFBR24063	GAAATATGACTAGCAACAAGT	21	SauCas9	218
TGFBR24107	AGACAGAGTAGGGTCCAGACG	21	SauCas9	219
TGFBR24047	CAGGATTTCTGGTTGTCACAG	21	SauCas9	220
TGFBR24096	CTCCTGTAGGTTGCCCTTGGC	21	SauCas9	221
TGFBR24105	ACAGAGTAGGGTCCAGACGCA	21	SauCas9	222
TGFBR24056	GCTTCTCCAAAGTGCATTATG	21	SauCas9	223
TGFBR24068	GCAGCAGAAGCTGAGTTCAAC	21	SauCas9	224
TGFBR24093	TGAGGAGCGGAAGACGGAGTT	21	SauCas9	225
TGFBR24055	CTTTGGAGAAGCAGCATCTTC	21	SauCas9	226
TGFBR24053	CTCCCCTACCATGACTTTATT	21	SauCas9	227
TGFBR24106	GACAGAGTAGGGTCCAGACGC	21	SauCas9	228
TGFBR24092	CTGAGGAGCGGAAGACGGAGT	21	SauCas9	229
TGFBR24102	GGGCATCTTGGGCCTCCCACA	21	SauCas9	230
TGFBR24082	CCAAGAGGCATACTCCTCATA	21	SauCas9	231
TGFBR24051	AGAATGACGAGAACATAACAC	21	SauCas9	232
TGFBR24097	CCTGACGCGGCATGTCATCAG	21	SauCas9	233
TGFBR24073	AGCGAGCACTGTGCCATCATC	21	SauCas9	234
TGFBR24104	GCAGGTTAGGTCGTTCTTCAC	21	SauCas9	235
TGFBR24050	ACCTCCATCTGTGAGAAGCCA	21	SauCas9	236
TGFBR24052	TAAAGTCATGGTAGGGGAGCT	21	SauCas9	237
TGFBR24061	TCAGAGCTACAGGAACACATG	21	SauCas9	238

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TGFBR24086	TCTCAGACATCAATCTGAAGC	21	SauCas9	239
TGFBR24066	CATCAGCCTCCTGCCACCACT	21	SauCas9	240
TGFBR24089	CGCTCCTCAGCCGTCAGGAAC	21	SauCas9	241
TGFBR24071	AACCTGGGAAACCGGCAAGAC	21	SauCas9	242
TGFBR24095	TCCACGCCAAGGGCAACCTAC	21	SauCas9	243
TGFBR24100	GAGGTGAGCAATCCCCCGGGC	21	SauCas9	244
TGFBR24069	CAGCAGAAGCTGAGTTCAACC	21	SauCas9	245
TGFBR24083	TCCAAGAGGCATACTCCTCAT	21	SauCas9	246
TGFBR24070	AGCAGAAGCTGAGTTCAACCT	21	SauCas9	247
TGFBR24088	CCAGTTCCTGACGGCTGAGGA	21	SauCas9	248
TGFBR24085	AGGAGTATGCCTCTTGGAAGA	21	SauCas9	249
TGFBR24084	TTCCAAGAGGCATACTCCTCA	21	SauCas9	250
TGFBR24042	CAACTGTGTAAATTTTGTGAT	21	SauCas9	251
TGFBR24059	TGAAGGAAAAAAAAAAAGCCTG	21	SauCas9	252
TGFBR24091	CGTCTTCCGCTCCTCAGCCGT	21	SauCas9	253
TGFBR24109	CCAGGTCATCCACAGACAGAG	21	SauCas9	254
TGFBR2736	GCCTAGAGTGAAGAGATTCAT	21	SpyCas9	255
TGFBR2737	GTTCTCCAAAGTGCATTATGA	21	SpyCas9	256
TGFBR2738	GCATCTTCCAGAATAAAGTCA	21	SpyCas9	257
TGFBR2739	TGATGTGAGATTTTCCACCTG	21	Cas12a	1172

[0548] In some embodiments the gRNA for use in the disclosure is a gRNA targeting CISH (CISH gRNA). In some embodiments, the gRNA targeting CISH is one or more of the gRNAs described in Table 8.

Table 8: Exemplary CISH gRNAs

Name	gRNA Targeting Domain Sequence (DNA)	Length	Enzyme	SEQ ID NO:
CISH0873	CAACCGTCTGGTGGCCGACG	20	SpyCas9	258
CISH0874	CAGGATCGGGGCTGTCGCTT	20	SpyCas9	259
CISH0875	TCGGGCCTCGCTGGCCGTAA	20	SpyCas9	260
CISH0876	GAGGTAGTCGGCCATGCGCC	20	SpyCas9	261
CISH0877	CAGGTGTTGTCGGGCCTCGC	20	SpyCas9	262
CISH0878	GGAGGTAGTCGGCCATGCGC	20	SpyCas9	263
CISH0879	GGCATACTCAATGCGTACAT	20	SpyCas9	264
CISH0880	CCGCCTTGTCATCAACCGTC	20	SpyCas9	265
CISH0881	AGGATCGGGGCTGTCGCTTC	20	SpyCas9	266
CISH0882	CCTTGTCATCAACCGTCTGG	20	SpyCas9	267
CISH0883	TACTCAATGCGTACATTGGT	20	SpyCas9	268

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CISH0884	GGGTTCCATTACGGCCAGCG	20	SpyCas9	269
CISH0885	GGCACTGCTTCTGCGTACAA	20	SpyCas9	270
CISH0886	GGTTGATGACAAGGCGGCAC	20	SpyCas9	271
CISH0887	TGCTGGGGCCTTCCTCGAGG	20	SpyCas9	272
CISH0888	TTGCTGGCTGTGGAGCGGAC	20	SpyCas9	273
CISH0889	TTCTCCTACCTTCGGGAATC	20	SpyCas9	274
CISH0890	GACTGGCTTGGGCAGTTCCA	20	SpyCas9	275
CISH0891	CATGCAGCCCTTGCCTGCTG	20	SpyCas9	276
CISH0892	AGCAAAGGACGAGGTCTAGA	20	SpyCas9	277
CISH0893	GCCTGCTGGGGCCTTCCTCG	20	SpyCas9	278
CISH0894	CAGACTCACCAGATTCCCGA	20	SpyCas9	279
CISH0895	ACCTCGTCCTTTGCTGGCTG	20	SpyCas9	280
CISH0896	CTCACCAGATTCCCGAAGGT	20	SpyCas9	281
CISH7048	TACGCAGAAGCAGTGCCCGC	20	AsCpf1	282
CISH7049	AGGTGTACAGCAGTGGCTGG	20	AsCpf1	283
CISH7050	GGTGTACAGCAGTGGCTGGT	20	AsCpf1	284
CISH7051	CGGATGTGGTCAGCCTTGTG	20	AsCpf1	285
CISH7052	CACTGACAGCGTGAACAGGT	20	AsCpf1	286
CISH7053	ACTGACAGCGTGAACAGGTA	20	AsCpf1	287
CISH7054	GCTCACTCTCTGTCTGGGCT	20	AsCpf1	288
CISH7055	CTGGCTGTGGAGCGGACTGG	20	AsCpf1	289
CISH7056	GCTCTGACTGTACGGGGCAA	20	AsCpf1 RR	290
CISH7057	AGCTCTGACTGTACGGGGCA	20	AsCpf1 RR	291
CISH7058	ACAGTACCCCTTCCAGCTCT	20	AsCpf1 RR	292
CISH7059	CGTCGGCCACCAGACGGTTG	20	AsCpf1 RR	293
CISH7060	CCAGCCACTGCTGTACACCT	20	AsCpf1 RR	294
CISH7061	ACCCCGGCCCTGCCTATGCC	20	AsCpf1 RR	295
CISH7062	GGTATCAGCAGTGCAGGAGG	20	AsCpf1 RR	296
CISH7063	GATGTGGTCAGCCTTGTGCA	20	AsCpf1 RR	297
CISH7064	GGATGTGGTCAGCCTTGTGC	20	AsCpf1 RR	298
CISH7065	GGCCACGCATCCTGGCCTTT	20	AsCpf1 RR	299
CISH7066	GAAAGGCCAGGATGCGTGGC	20	AsCpf1 RR	300
CISH7067	ACTGCTTGTCCAGGCCACGC	20	AsCpf1 RR	301
CISH7068	TCTGGACTCCAACTGCTTGT	20	AsCpf1 RR	302
CISH7069	GTCTGGACTCCAACTGCTTG	20	AsCpf1 RR	303
CISH7070	GCTTCCGTCTGGACTCCAAC	20	AsCpf1 RR	304
CISH7071	GACGGAAGCTGGAGTCGGCA	20	AsCpf1 RR	305
CISH7072	CGCTGTCAGTGAAAACCACT	20	AsCpf1 RR	306
CISH7073	CTGACAGCGTGAACAGGTAG	20	AsCpf1 RR	307
CISH7074	TTACGGCCAGCGAGGCCCGA	20	AsCpf1 RR	308

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CISH7075	ATTACGGCCAGCGAGGCCCG	20	AsCpf1 RR	309
CISH7076	GGAATCTGGTGAGTCTGAGG	20	AsCpf1 RR	310
CISH7077	CCCTCAGACTCACCAGATTC	20	AsCpf1 RR	311
CISH7078	CGAAGGTAGGAGAAGGTCTT	20	AsCpf1 RR	312
CISH7079	GAAGGTAGGAGAAGGTCTTG	20	AsCpf1 RR	313
CISH7080	GCACCTTTGGCTCACTCTCT	20	AsCpf1 RR	314
CISH7081	TCGAGGAGGTGGCAGAGGGT	20	AsCpf1 RR	315
CISH7082	TGGAACTGCCCAAGCCAGTC	20	AsCpf1 RR	316
CISH7083	AGGGACGGGGCCCACAGGGG	20	AsCpf1 RR	317
CISH7084	GGGACGGGGCCCACAGGGGC	20	AsCpf1 RR	318
CISH7085	CTCCACAGCCAGCAAAGGAC	20	AsCpf1 RR	319
CISH7086	CAGCCAGCAAAGGACGAGGT	20	AsCpf1 RR	320
CISH7087	CTGCCTTCTAGACCTCGTCC	20	AsCpf1 RR	321
CISH7088	CCTAAGGAGGATGCGCCTAG	20	AsCpf1 RVR	322
CISH7089	TGGCCTCCTGCACTGCTGAT	20	AsCpf1 RVR	323
CISH7090	AGCAGTGCAGGAGGCCACAT	20	AsCpf1 RVR	324
CISH7091	CCGACTCCAGCTTCCGTCTG	20	AsCpf1 RVR	325
CISH7092	GGGGTTCCATTACGGCCAGC	20	AsCpf1 RVR	326
CISH7093	CACAGCAGATCCTCCTCTGG	20	AsCpf1 RVR	327
CISH7094	ATTGCCCCGTACAGTCAGAG	20	SauCas9	328
CISH7095	CCCGTACAGTCAGAGCTGGA	20	SauCas9	329
CISH7096	TGGTGGAGGAGCAGGCAGTG	20	SauCas9	330
CISH7097	TCCTTAGGCATAGGCAGGGC	20	SauCas9	331
CISH7098	CGGCCCTGCCTATGCCTAAG	20	SauCas9	332
CISH7099	TAGGCATAGGCAGGGCCGGG	20	SauCas9	333
CISH7100	AGGCAGGGCCGGGGTGGGAG	20	SauCas9	334
CISH7101	GCAGGATCGGGGCTGTCGCT	20	SauCas9	335
CISH7102	CTGCACAAGGCTGACCACAT	20	SauCas9	336
CISH7103	TGCACAAGGCTGACCACATC	20	SauCas9	337
CISH7104	CTGACCACATCCGGAAAGGC	20	SauCas9	338
CISH7105	GGCCACGCATCCTGGCCTTT	20	SauCas9	339
CISH7106	GCGTGGCCTGGACAAGCAGT	20	SauCas9	340
CISH7107	GACAAGCAGTTGGAGTCCAG	20	SauCas9	341
CISH7108	GTTGGAGTCCAGACGGAAGC	20	SauCas9	342
CISH7109	ATGCGTACATTGGTGGGGCC	20	SauCas9	343
CISH7110	TGGCCCCACCAATGTACGCA	20	SauCas9	344
CISH7111	GCTACCTGTTCACGCTGTCA	20	SauCas9	345
CISH7112	TGACAGCGTGAACAGGTAGC	20	SauCas9	346
CISH7113	GTCGGGCCTCGCTGGCCGTA	20	SauCas9	347
CISH7114	GCACTTGCCTAGGCTGGTAT	20	SauCas9	348

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CISH7115	GGGAATCTGGTGAGTCTGAG	20	SauCas9	349
CISH7116	CTCACCAGATTCCCGAAGGT	20	SauCas9	350
CISH7117	CTCCTACCTTCGGGAATCTG	20	SauCas9	351
CISH7118	CAAGACCTTCTCCTACCTTC	20	SauCas9	352
CISH7119	CCAAGACCTTCTCCTACCTT	20	SauCas9	353
CISH7120	GCCAAGACCTTCTCCTACCT	20	SauCas9	354
CISH7121	TATGCACAGCAGATCCTCCT	20	SauCas9	355
CISH7122	CAAAGGTGCTGGACCCAGAG	20	SauCas9	356
CISH7123	GGCTCACTCTCTGTCTGGGC	20	SauCas9	357
CISH7124	AGGGTACCCCAGCCCAGACA	20	SauCas9	358
CISH7125	AGAGGGTACCCCAGCCCAGA	20	SauCas9	359
CISH7126	GTACCCTCTGCCACCTCCTC	20	SauCas9	360
CISH7127	CCTTCCTCGAGGAGGTGGCA	20	SauCas9	361
CISH7128	ATGACTGGCTTGGGCAGTTC	20	SauCas9	362
CISH7129	GGCCCCTGTGGGCCCCGTCC	20	SauCas9	363
CISH7130	AGGACGAGGTCTAGAAGGCA	20	SauCas9	364
CISH7131	ACTGACAGCGTGAACAGGTAG	21	Cas12a	1173

[0549] In some embodiments, the gRNA for use in the disclosure is a gRNA targeting B2M (B2M gRNA). In some embodiments, the gRNA targeting B2M is one or more of the gRNAs described in Table 9.

Table 9: Exemplary B2M gRNAs

gRNA name	gRNA Targeting Domain Target sequence (DNA)	Length	Enzyme	SEQ ID NO:
B2M1	TATAAGTGGAGGCGTCGCGC	20	SpyCas9	365
B2M2	GGGCACGCGTTTAATATAAG	20	SpyCas9	366
B2M3	ACTCACGCTGGATAGCCTCC	20	SpyCas9	367
B2M4	GGCCGAGATGTCTCGCTCCG	20	SpyCas9	368
B2M5	CACGCGTTTAATATAAGTGG	20	SpyCas9	369
B2M6	AAGTGGAGGCGTCGCGCTGG	20	SpyCas9	370
B2M7	GAGTAGCGCGAGCACAGCTA	20	SpyCas9	371
B2M8	AGTGGAGGCGTCGCGCTGGC	20	SpyCas9	372
B2M9	GCCCGAATGCTGTCAGCTTC	20	SpyCas9	373
B2M10	CGCGAGCACAGCTAAGGCCA	20	SpyCas9	374
B2M11	CTCGCGCTACTCTCTCTTTC	20	SpyCas9	375
B2M12	GGCCACGGAGCGAGACATCT	20	SpyCas9	376
B2M13	CGTGAGTAAACCTGAATCTT	20	SpyCas9	377

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B2M14	AGTCACATGGTTCACACGGC	20	SpyCas9	378
B2M15	AAGTCAACTTCAATGTCGGA	20	SpyCas9	379
B2M16	CAGTAAGTCAACTTCAATGT	20	SpyCas9	380
B2M17	ACCCAGACACATAGCAATTC	20	SpyCas9	381
B2M18	GCATACTCATCTTTTTCAGT	20	SpyCas9	382
B2M19	ACAGCCCAAGATAGTTAAGT	20	SpyCas9	383
B2M20	GGCATACTCATCTTTTTCAG	20	SpyCas9	384
B2M21	TTCCTGAAGCTGACAGCATT	20	SpyCas9	385
B2M22	TCACGTCATCCAGCAGAGAA	20	SpyCas9	386
B2M23	CAGCCCAAGATAGTTAAGTG	20	SpyCas9	387
B2M-c1	AAUUCUCUCUCCAUUCUU	18	AsCpf1	388
B2M-c2	AAUUCUCUCUCCAUUCUUC	19	AsCpf1	389
B2M-c3	AAUUCUCUCUCCAUUCUUCA	20	AsCpf1	390
B2M-c4	AAUUCUCUCUCCAUUCUUCAG	21	AsCpf1	391
B2M-c5	AAUUCUCUCUCCAUUCUUCAGU	22	AsCpf1	392
B2M-c6	AAUUCUCUCUCCAUUCUUCAGUA	23	AsCpf1	393
B2M-c7	AAUUCUCUCUCCAUUCUUCAGUAA	24	AsCpf1	394
B2M-c8	ACUUUCCAUUCUCUGCUG	18	AsCpf1	395
B2M-c9	ACUUUCCAUUCUCUGCUGG	19	AsCpf1	396
B2M-c10	ACUUUCCAUUCUCUGCUGGA	20	AsCpf1	397
B2M-c11	ACUUUCCAUUCUCUGCUGGAU	21	AsCpf1	398
B2M-c12	ACUUUCCAUUCUCUGCUGGAUG	22	AsCpf1	399
B2M-c13	ACUUUCCAUUCUCUGCUGGAUGA	23	AsCpf1	400
B2M-c14	ACUUUCCAUUCUCUGCUGGAUGAC	24	AsCpf1	401
B2M-c15	AGCAAGGACUGGUCUUUC	18	AsCpf1	402
B2M-c16	AGCAAGGACUGGUCUUUCU	19	AsCpf1	403
B2M-c17	AGCAAGGACUGGUCUUUCUA	20	AsCpf1	404
B2M-c18	AGCAAGGACUGGUCUUUCUAU	21	AsCpf1	405
B2M-c19	AGCAAGGACUGGUCUUUCUAUC	22	AsCpf1	406
B2M-c20	AGCAAGGACUGGUCUUUCUAUCU	23	AsCpf1	407
B2M-c21	AGCAAGGACUGGUCUUUCUAUCUC	24	AsCpf1	408
B2M-c22	AGUGGGGGUGAAUUCAGU	18	AsCpf1	409
B2M-c23	AGUGGGGGUGAAUUCAGUG	19	AsCpf1	410
B2M-c24	AGUGGGGGUGAAUUCAGUGU	20	AsCpf1	411
B2M-c25	AGUGGGGGUGAAUUCAGUGUA	21	AsCpf1	412
B2M-c26	AGUGGGGGUGAAUUCAGUGUAG	22	AsCpf1	413
B2M-c27	AGUGGGGGUGAAUUCAGUGUAGU	23	AsCpf1	414
B2M-c28	AGUGGGGGUGAAUUCAGUGUAGUA	24	AsCpf1	415

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B2M-c29	AUCCAUCCGACAUUGAAG	18	AsCpf1	416
B2M-c30	AUCCAUCCGACAUUGAAGU	19	AsCpf1	417
B2M-c31	AUCCAUCCGACAUUGAAGUU	20	AsCpf1	418
B2M-c32	AUCCAUCCGACAUUGAAGUUG	21	AsCpf1	419
B2M-c33	AUCCAUCCGACAUUGAAGUUGA	22	AsCpf1	420
B2M-c34	AUCCAUCCGACAUUGAAGUUGAC	23	AsCpf1	421
B2M-c35	AUCCAUCCGACAUUGAAGUUGACU	24	AsCpf1	422
B2M-c36	CAAUUCUCUCUCCAUUCU	18	AsCpf1	423
B2M-c37	CAAUUCUCUCUCCAUUCUU	19	AsCpf1	424
B2M-c38	CAAUUCUCUCUCCAUUCUUC	20	AsCpf1	425
B2M-c39	CAAUUCUCUCUCCAUUCUUCA	21	AsCpf1	426
B2M-c40	CAAUUCUCUCUCCAUUCUUCAG	22	AsCpf1	427
B2M-c41	CAAUUCUCUCUCCAUUCUUCAGU	23	AsCpf1	428
B2M-c42	CAAUUCUCUCUCCAUUCUUCAGUA	24	AsCpf1	429
B2M-c43	CAGUGGGGGUGAAUUCAG	18	AsCpf1	430
B2M-c44	CAGUGGGGGUGAAUUCAGU	19	AsCpf1	431
B2M-c45	CAGUGGGGGUGAAUUCAGUG	20	AsCpf1	432
B2M-c46	CAGUGGGGGUGAAUUCAGUGU	21	AsCpf1	433
B2M-c47	CAGUGGGGGUGAAUUCAGUGUA	22	AsCpf1	434
B2M-c48	CAGUGGGGGUGAAUUCAGUGUAG	23	AsCpf1	435
B2M-c49	CAGUGGGGGUGAAUUCAGUGUAGU	24	AsCpf1	436
B2M-c50	CAUUCUCUGCUGGAUGAC	18	AsCpf1	437
B2M-c51	CAUUCUCUGCUGGAUGACG	19	AsCpf1	438
B2M-c52	CAUUCUCUGCUGGAUGACGU	20	AsCpf1	439
B2M-c53	CAUUCUCUGCUGGAUGACGUG	21	AsCpf1	440
B2M-c54	CAUUCUCUGCUGGAUGACGUGA	22	AsCpf1	441
B2M-c55	CAUUCUCUGCUGGAUGACGUGAG	23	AsCpf1	442
B2M-c56	CAUUCUCUGCUGGAUGACGUGAGU	24	AsCpf1	443
B2M-c57	CCCGAUAUUCCUCAGGUA	18	AsCpf1	444
B2M-c58	CCCGAUAUUCCUCAGGUAC	19	AsCpf1	445
B2M-c59	CCCGAUAUUCCUCAGGUACU	20	AsCpf1	446
B2M-c60	CCCGAUAUUCCUCAGGUACUC	21	AsCpf1	447
B2M-c61	CCCGAUAUUCCUCAGGUACUCC	22	AsCpf1	448
B2M-c62	CCCGAUAUUCCUCAGGUACUCCA	23	AsCpf1	449
B2M-c63	CCCGAUAUUCCUCAGGUACUCCAA	24	AsCpf1	450
B2M-c64	CCGAUAUUCCUCAGGUAC	18	AsCpf1	451
B2M-c65	CCGAUAUUCCUCAGGUACU	19	AsCpf1	452
B2M-c66	CCGAUAUUCCUCAGGUACUC	20	AsCpf1	453

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B2M-c67	CCGAUAUUCCUCAGGUACUCC	21	AsCpf1	454
B2M-c68	CCGAUAUUCCUCAGGUACUCCA	22	AsCpf1	455
B2M-c69	CCGAUAUUCCUCAGGUACUCCAA	23	AsCpf1	456
B2M-c70	CCGAUAUUCCUCAGGUACUCCAAA	24	AsCpf1	457
B2M-c71	CUCACGUCAUCCAGCAGA	18	AsCpf1	458
B2M-c72	CUCACGUCAUCCAGCAGAG	19	AsCpf1	459
B2M-c73	CUCACGUCAUCCAGCAGAGA	20	AsCpf1	460
B2M-c74	CUCACGUCAUCCAGCAGAGAA	21	AsCpf1	461
B2M-c75	CUCACGUCAUCCAGCAGAGAAU	22	AsCpf1	462
B2M-c76	CUCACGUCAUCCAGCAGAGAAUG	23	AsCpf1	463
B2M-c77	CUCACGUCAUCCAGCAGAGAAUGG	24	AsCpf1	464
B2M-c78	CUGAAUUGCUAUGUGUCU	18	AsCpf1	465
B2M-c79	CUGAAUUGCUAUGUGUCUG	19	AsCpf1	466
B2M-c80	CUGAAUUGCUAUGUGUCUGG	20	AsCpf1	467
B2M-c81	CUGAAUUGCUAUGUGUCUGGG	21	AsCpf1	468
B2M-c82	CUGAAUUGCUAUGUGUCUGGGU	22	AsCpf1	469
B2M-c83	CUGAAUUGCUAUGUGUCUGGGUU	23	AsCpf1	470
B2M-c84	CUGAAUUGCUAUGUGUCUGGGUUU	24	AsCpf1	471
B2M-c85	GAGUACCUGAGGAAUAUC	18	AsCpf1	472
B2M-c86	GAGUACCUGAGGAAUAUCG	19	AsCpf1	473
B2M-c87	GAGUACCUGAGGAAUAUCGG	20	AsCpf1	474
B2M-c88	GAGUACCUGAGGAAUAUCGGG	21	AsCpf1	475
B2M-c89	GAGUACCUGAGGAAUAUCGGGA	22	AsCpf1	476
B2M-c90	GAGUACCUGAGGAAUAUCGGGAA	23	AsCpf1	477
B2M-c91	GAGUACCUGAGGAAUAUCGGGAAA	24	AsCpf1	478
B2M-c92	UAUCUCUUGUACUACACU	18	AsCpf1	479
B2M-c93	UAUCUCUUGUACUACACUG	19	AsCpf1	480
B2M-c94	UAUCUCUUGUACUACACUGA	20	AsCpf1	481
B2M-c95	UAUCUCUUGUACUACACUGAA	21	AsCpf1	482
B2M-c96	UAUCUCUUGUACUACACUGAAU	22	AsCpf1	483
B2M-c97	UAUCUCUUGUACUACACUGAAUU	23	AsCpf1	484
B2M-c98	UAUCUCUUGUACUACACUGAAUUC	24	AsCpf1	485
B2M-c99	UCAAUUCUCUCUCCAUUC	18	AsCpf1	486
B2M-c100	UCAAUUCUCUCUCCAUUCU	19	AsCpf1	487
B2M-c101	UCAAUUCUCUCUCCAUUCUU	20	AsCpf1	488
B2M-c102	UCAAUUCUCUCUCCAUUCUUC	21	AsCpf1	489
B2M-c103	UCAAUUCUCUCUCCAUUCUUCA	22	AsCpf1	490
B2M-c104	UCAAUUCUCUCUCCAUUCUUCAG	23	AsCpf1	491

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B2M-c105	UCAAUUCUCUCUCCAUUCUUCAGU	24	AsCpf1	492
B2M-c106	UCACAGCCCAAGAUAGUU	18	AsCpf1	493
B2M-c107	UCACAGCCCAAGAUAGUUA	19	AsCpf1	494
B2M-c108	UCACAGCCCAAGAUAGUUAA	20	AsCpf1	495
B2M-c109	UCACAGCCCAAGAUAGUUAAG	21	AsCpf1	496
B2M-c110	UCACAGCCCAAGAUAGUUAAGU	22	AsCpf1	497
B2M-c111	UCACAGCCCAAGAUAGUUAAGUG	23	AsCpf1	498
B2M-c112	UCACAGCCCAAGAUAGUUAAGUGG	24	AsCpf1	499
B2M-c113	UCAGUGGGGGUGAAUUCA	18	AsCpf1	500
B2M-c114	UCAGUGGGGGUGAAUUCAG	19	AsCpf1	501
B2M-c115	UCAGUGGGGGUGAAUUCAGU	20	AsCpf1	502
B2M-c116	UCAGUGGGGGUGAAUUCAGUG	21	AsCpf1	503
B2M-c117	UCAGUGGGGGUGAAUUCAGUGU	22	AsCpf1	504
B2M-c118	UCAGUGGGGGUGAAUUCAGUGUA	23	AsCpf1	505
B2M-c119	UCAGUGGGGGUGAAUUCAGUGUAG	24	AsCpf1	506
B2M-c120	UGGCCUGGAGGCUAUCCA	18	AsCpf1	507
B2M-c121	UGGCCUGGAGGCUAUCCAG	19	AsCpf1	508
B2M-c122	UGGCCUGGAGGCUAUCCAGC	20	AsCpf1	509
B2M-c123	UGGCCUGGAGGCUAUCCAGCG	21	AsCpf1	510
B2M-c124	UGGCCUGGAGGCUAUCCAGCGU	22	AsCpf1	511
B2M-c125	UGGCCUGGAGGCUAUCCAGCGUG	23	AsCpf1	512
B2M-c126	UGGCCUGGAGGCUAUCCAGCGUGA	24	AsCpf1	513
B2M-c127	AUAGAUCGAGACAUGUAA	18	AsCpf1	514
B2M-c128	AUAGAUCGAGACAUGUAAG	19	AsCpf1	515
B2M-c129	AUAGAUCGAGACAUGUAAGC	20	AsCpf1	516
B2M-c130	AUAGAUCGAGACAUGUAAGCA	21	AsCpf1	517
B2M-c131	AUAGAUCGAGACAUGUAAGCAG	22	AsCpf1	518
B2M-c132	AUAGAUCGAGACAUGUAAGCAGC	23	AsCpf1	519
B2M-c133	AUAGAUCGAGACAUGUAAGCAGCA	24	AsCpf1	520
B2M-c134	CAUAGAUCGAGACAUGUA	18	AsCpf1	521
B2M-c135	CAUAGAUCGAGACAUGUAA	19	AsCpf1	522
B2M-c136	CAUAGAUCGAGACAUGUAAG	20	AsCpf1	523
B2M-c137	CAUAGAUCGAGACAUGUAAGC	21	AsCpf1	524
B2M-c138	CAUAGAUCGAGACAUGUAAGCA	22	AsCpf1	525
B2M-c139	CAUAGAUCGAGACAUGUAAGCAG	23	AsCpf1	526
B2M-c140	CAUAGAUCGAGACAUGUAAGCAGC	24	AsCpf1	527
B2M-c141	CUCCACUGUCUUUUUCAU	18	AsCpf1	528
B2M-c142	CUCCACUGUCUUUUUCAUA	19	AsCpf1	529

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B2M-c143	CUCCACUGUCUUUUUCAUAG	20	AsCpf1	530
B2M-c144	CUCCACUGUCUUUUUCAUAGA	21	AsCpf1	531
B2M-c145	CUCCACUGUCUUUUUCAUAGAU	22	AsCpf1	532
B2M-c146	CUCCACUGUCUUUUUCAUAGAUC	23	AsCpf1	533
B2M-c147	CUCCACUGUCUUUUUCAUAGAUCG	24	AsCpf1	534
B2M-c148	UCAUAGAUCGAGACAUGU	18	AsCpf1	535
B2M-c149	UCAUAGAUCGAGACAUGUA	19	AsCpf1	536
B2M-c150	UCAUAGAUCGAGACAUGUAA	20	AsCpf1	537
B2M-c151	UCAUAGAUCGAGACAUGUAAG	21	AsCpf1	538
B2M-c152	UCAUAGAUCGAGACAUGUAAGC	22	AsCpf1	539
B2M-c153	UCAUAGAUCGAGACAUGUAAGCA	23	AsCpf1	540
B2M-c154	UCAUAGAUCGAGACAUGUAAGCAG	24	AsCpf1	541
B2M-c155	UCCACUGUCUUUUUCAUA	18	AsCpf1	542
B2M-c156	UCCACUGUCUUUUUCAUAG	19	AsCpf1	543
B2M-c157	UCCACUGUCUUUUUCAUAGA	20	AsCpf1	544
B2M-c158	UCCACUGUCUUUUUCAUAGAU	21	AsCpf1	545
B2M-c159	UCCACUGUCUUUUUCAUAGAUC	22	AsCpf1	546
B2M-c160	UCCACUGUCUUUUUCAUAGAUCG	23	AsCpf1	547
B2M-c161	UCCACUGUCUUUUUCAUAGAUCGA	24	AsCpf1	548
B2M-c162	UCUCCACUGUCUUUUUCA	18	AsCpf1	549
B2M-c163	UCUCCACUGUCUUUUUCAU	19	AsCpf1	550
B2M-c164	UCUCCACUGUCUUUUUCAUA	20	AsCpf1	551
B2M-c165	UCUCCACUGUCUUUUUCAUAG	21	AsCpf1	552
B2M-c166	UCUCCACUGUCUUUUUCAUAGA	22	AsCpf1	553
B2M-c167	UCUCCACUGUCUUUUUCAUAGAU	23	AsCpf1	554
B2M-c168	UCUCCACUGUCUUUUUCAUAGAUC	24	AsCpf1	555
B2M-c169	UUCUCCACUGUCUUUUUC	18	AsCpf1	556
B2M-c170	UUCUCCACUGUCUUUUUCA	19	AsCpf1	557
B2M-c171	UUCUCCACUGUCUUUUUCAU	20	AsCpf1	558
B2M-c172	UUCUCCACUGUCUUUUUCAUA	21	AsCpf1	559
B2M-c173	UUCUCCACUGUCUUUUUCAUAG	22	AsCpf1	560
B2M-c174	UUCUCCACUGUCUUUUUCAUAGA	23	AsCpf1	561
B2M-c175	UUCUCCACUGUCUUUUUCAUAGAU	24	AsCpf1	562
B2M-c176	UUUCUCCACUGUCUUUUU	18	AsCpf1	563
B2M-c177	UUUCUCCACUGUCUUUUUC	19	AsCpf1	564
B2M-c178	UUUCUCCACUGUCUUUUUCA	20	AsCpf1	565
B2M-c179	UUUCUCCACUGUCUUUUUCAU	21	AsCpf1	566
B2M-c180	UUUCUCCACUGUCUUUUUCAUA	22	AsCpf1	567

B2M-c181	UUUCUCCACUGUCUUUUUCAUAG	23	AsCpf1	568
B2M-c182	UUUCUCCACUGUCUUUUUCAUAGA	24	AsCpf1	569
B2M-c183	UUUUCUCCACUGUCUUUU	18	AsCpf1	570
B2M-c184	UUUUCUCCACUGUCUUUUU	19	AsCpf1	571
B2M-c185	UUUUCUCCACUGUCUUUUUC	20	AsCpf1	572
B2M-c186	UUUUCUCCACUGUCUUUUUCA	21	AsCpf1	573
B2M-c187	UUUUCUCCACUGUCUUUUUCAU	22	AsCpf1	574
B2M-c188	UUUUCUCCACUGUCUUUUUCAUA	23	AsCpf1	575
B2M-c189	UUUUCUCCACUGUCUUUUUCAUAG	24	AsCpf1	576

[0550] In some embodiments, the gRNA for use in the disclosure is a gRNA targeting PD1. gRNAs targeting B2M and PD1 for use in the disclosure are further described in WO2015161276 and WO2017152015 by Welstead et al.; both incorporated in their entirety herein by reference.

[0551] In some embodiments, the gRNA for use in the disclosure is a gRNA targeting NKG2A (NKG2A gRNA). In some embodiments, the gRNA targeting NKG2A is one or more of the gRNAs described in Table 10.

Table 10: Exemplary NKG2A gRNAs

Name	gRNA Targeting Domain Sequence (DNA)	Length	Enzyme	SEQ ID NO:
NKG2A55	GAGGTAAAGCGTTTGCATTTG	21	AsCpf1	577
NKG2A56	CCTCTAAAGCTTATGCTTACA	21	AsCpf1	578
NKG2A57	AGTCGATTTACTTGTAGCACT	21	AsCpf1	579
NKG2A58	CTTGTAGCACTGCACAGTTAA	21	AsCpf1	580
NKG2A59	TCCATTACAGGATAAAAGACT	21	AsCpf1	581
NKG2A60	CTCCATTACAGGATAAAAGAC	21	AsCpf1	582
NKG2A61	TCTCCATTACAGGATAAAAGA	21	AsCpf1	583
NKG2A62	ATCCTGTAATGGAGAAAAATC	21	AsCpf1	584
NKG2A63	TCCTGTAATGGAGAAAAATCC	21	AsCpf1	585
NKG2A136	AAACATGAGTAAGTTGTTTTG	21	AsCpf1	586
NKG2A137	GCTTTCAAACATGAGTAAGTT	21	AsCpf1	587
NKG2A138	AAAGCCAAACCATTCATTGTC	21	AsCpf1	588
NKG2A139	GTAACAGCAGTCATCATCCAT	21	AsCpf1	589

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NKG2A140	ACCATCCTCATGGATTGGTGT	21	AsCpf1	590
NKG2A141	TGTCCATCATTTCACCATCCT	21	AsCpf1	591
NKG2A142	GAAATTTCTGTCCATCATTTC	21	AsCpf1	592
NKG2A143	AGAAATTTCTGTCCATCATTT	21	AsCpf1	593
NKG2A144	TTTTAGAAATTTCTGTCCATC	21	AsCpf1	594
NKG2A145	CTTTTAGAAATTTCTGTCCAT	21	AsCpf1	595
NKG2A146	TTTTCTTTTAGAAATTTCTGT	21	AsCpf1	596
NKG2A147	TAAAAGAAAAGAAAGAATTTT	21	AsCpf1	597
NKG2A270	AAACATTTACATCTTACCATT	21	AsCpf1	598
NKG2A271	CATCTTACCATTTCTTCTTCA	21	AsCpf1	599
NKG2A272	TATAGATAATGAAGAAGAAAT	21	AsCpf1	600
NKG2A273	TTCTTCATTATCTATAGAAAG	21	AsCpf1	601
NKG2A274	CTGGCCTGTACTTCGAAGAAC	21	AsCpf1	602
NKG2A275	CTTACCAATGTAGTAACAACT	21	AsCpf1	603
NKG2A276	GCACGTCATTGTGGCCATTGT	21	AsCpf1	604
NKG2A277	TTTAGCACGTCATTGTGGCCA	21	AsCpf1	605
NKG2A414	CCATCAGCTCCAGAGAAGCTC	21	AsCpf1	606
NKG2A415	TCTCCCTGCAGATTTACCATC	21	AsCpf1	607
NKG2A437	AAATGCTTTACCTTTGCAGTG	21	AsCpf1	608
NKG2A438	AATGCTTTACCTTTGCAGTGA	21	AsCpf1	609
NKG2A439	CCTTTGCAGTGATAGGTTTTG	21	AsCpf1	610
NKG2A440	CAGTGATAGGTTTTGTCATTC	21	AsCpf1	611
NKG2A441	AAGGGAATGACAAAACCTATC	21	AsCpf1	612
NKG2A442	CAAGGGAATGACAAAACCTAT	21	AsCpf1	613
NKG2A443	GTCATTCCCTTGAAAATCCTG	21	AsCpf1	614
NKG2A444	TCATTCCCTTGAAAATCCTGA	21	AsCpf1	615
NKG2A445	TGAAGGTTTAATTCCGCATAG	21	AsCpf1	616
NKG2A446	GAAGGTTTAATTCCGCATAGG	21	AsCpf1	617
NKG2A447	AAGGTTTAATTCCGCATAGGT	21	AsCpf1	618
NKG2A448	ATTCCGCATAGGTTATTTCCT	21	AsCpf1	619
NKG2A449	GCAACTGAACAGGAAATAACC	21	AsCpf1	620
NKG2A450	AGCAACTGAACAGGAAATAAC	21	AsCpf1	621
NKG2A451	CTGTTCAGTTGCTAAAATGGA	21	AsCpf1	622
NKG2A452	TATTGCCTTTAGGTTTTCGTT	21	AsCpf1	623
NKG2A453	ATTGCCTTTAGGTTTTCGTTG	21	AsCpf1	624
NKG2A454	TTGCCTTTAGGTTTTCGTTGC	21	AsCpf1	625
NKG2A455	GGTTTTCGTTGCTGCCTCTTT	21	AsCpf1	626
NKG2A456	CGTTGCTGCCTCTTTGGGTTT	21	AsCpf1	627
NKG2A457	GTTGCTGCCTCTTTGGGTTTG	21	AsCpf1	628

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NKG2A458	GGTTTGGGGGCAGATTCAGGT	21	AsCpf1	629
NKG2A459	GGGGCAGATTCAGGTCTGAGT	21	AsCpf1	630
NKG2A460	GCAACTGAACAGGAAATAACC	21	Cas12a	1176

[0552] In some embodiments, the gRNA for use in the disclosure is a gRNA targeting TIGIT (TIGIT gRNA). In some embodiments, the gRNA targeting TIGIT is one or more of the gRNAs described in Table 11.

Table 11. Exemplary TIGIT gRNAs

Name	gRNA Targeting Domain Sequence (DNA)	Length	Enzyme	SEQ ID NO:
TIGIT4170	TCTGCAGAAATGTTCCCCGT	20	AsCpf1	631
TIGIT4171	TGCAGAGAAAGGTGGCTCTA	20	AsCpf1	632
TIGIT4172	TAATGCTGACTTGGGGTGGC	20	AsCpf1	633
TIGIT4173	TAGGACCTCCAGGAAGATTC	20	AsCpf1	634
TIGIT4174	TAGTCAACGCGACCACCACG	20	AsCpf1	635
TIGIT4175	TCCTGAGGTCACCTTCCACA	20	AsCpf1	636
TIGIT4176	TATTGTGCCTGTCATCATTC	20	AsCpf1	637
TIGIT4177	TGACAGGCACAATAGAAACAA	21	SauCas9	638
TIGIT4178	GACAGGCACAATAGAAACAAC	21	SauCas9	639
TIGIT4179	AAACAACGGGGAACATTTCTG	21	SauCas9	640
TIGIT4180	ACAACGGGGAACATTTCTGCA	21	SauCas9	641
TIGIT4181	TGATAGAGCCACCTTTCTCTG	21	SauCas9	642
TIGIT4182	GGGTCACTTGTGCCGTGGTGG	21	SauCas9	643
TIGIT4183	GGCACAAGTGACCCAGGTCAA	21	SauCas9	644
TIGIT4184	GTCCTGCTGCTCCCAGTTGAC	21	SauCas9	645
TIGIT4185	TGGCCATTTGTAATGCTGACT	21	SauCas9	646
TIGIT4186	TGGCACATCTCCCCATCCTTC	21	SauCas9	647
TIGIT4187	CATCTCCCCATCCTTCAAGGA	21	SauCas9	648
TIGIT4188	CCACTCGATCCTTGAAGGATG	21	SauCas9	649
TIGIT4189	GGCCACTCGATCCTTGAAGGA	21	SauCas9	650
TIGIT4190	CCTGGGGCCACTCGATCCTTG	21	SauCas9	651
TIGIT4191	GACTGGAGGGTGAGGCCCAGG	21	SauCas9	652
TIGIT4192	ATCGTTCACGGTCAGCGACTG	21	SauCas9	653
TIGIT4193	GTCGCTGACCGTGAACGATAC	21	SauCas9	654
TIGIT4194	CGCTGACCGTGAACGATACAG	21	SauCas9	655
TIGIT4195	GCATCTATCACACCTACCCTG	21	SauCas9	656
TIGIT4196	CCTACCCTGATGGGACGTACA	21	SauCas9	657
TIGIT4197	TACCCTGATGGGACGTACACT	21	SauCas9	658

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TIGIT4198	CCCTGATGGGACGTACACTGG	21	SauCas9	659
TIGIT4199	TTCTCCCAGTGTACGTCCCAT	21	SauCas9	660
TIGIT4200	GGAGAATCTTCCTGGAGGTCC	21	SauCas9	661
TIGIT4201	CATGGCTCCAAGCAATGGAAT	21	SauCas9	662
TIGIT4202	CGCGGCCATGGCTCCAAGCAA	21	SauCas9	663
TIGIT4203	TCGCGGCCATGGCTCCAAGCA	21	SauCas9	664
TIGIT4204	CATCGTGGTGGTCGCGTTGAC	21	SauCas9	665
TIGIT4205	AAAGCCCTCAGAATCCATTCT	21	SauCas9	666
TIGIT4206	CATTCTGTGGAAGGTGACCTC	21	SauCas9	667
TIGIT4207	TTCTGTGGAAGGTGACCTCAG	21	SauCas9	668
TIGIT4208	CCTGAGGTCACCTTCCACAGA	21	SauCas9	669
TIGIT4209	TTCTCCTGAGGTCACCTTCCA	21	SauCas9	670
TIGIT4210	AGGAGAAAATCAGCTGGACAG	21	SauCas9	671
TIGIT4211	GGAGAAAATCAGCTGGACAGG	21	SauCas9	672
TIGIT4212	GCCCCAGTGCTCCCTCACCCC	21	SauCas9	673
TIGIT4213	TGGACACAGCTTCCTGGGGGT	21	SauCas9	674
TIGIT4214	TCTGCCTGGACACAGCTTCCT	21	SauCas9	675
TIGIT4215	AGCTGCACCTGCTGGGCTCTG	21	SauCas9	676
TIGIT4216	GCTGGGCTCTGTGGAGAGCAG	21	SauCas9	677
TIGIT4217	TGGGCTCTGTGGAGAGCAGCG	21	SauCas9	678
TIGIT4218	CTGCATGACTACTTCAATGTC	21	SauCas9	679
TIGIT4219	AATGTCCTGAGTTACAGAAGC	21	SauCas9	680
TIGIT4220	TGGGTAACTGCAGCTTCTTCA	21	SauCas9	681
TIGIT4221	GACAGGCACAATAGAAACAA	20	SpyCas9	682
TIGIT4222	ACAGGCACAATAGAAACAAC	20	SpyCas9	683
TIGIT4223	CAGGCACAATAGAAACAACG	20	SpyCas9	684
TIGIT4224	GGGAACATTTCTGCAGAGAA	20	SpyCas9	685
TIGIT4225	AACATTTCTGCAGAGAAAGG	20	SpyCas9	686
TIGIT4226	ATGTCACCTCTCCTCCACCA	20	SpyCas9	687
TIGIT4227	CTTGTGCCGTGGTGGAGGAG	20	SpyCas9	688
TIGIT4228	GGTCACTTGTGCCGTGGTGG	20	SpyCas9	689
TIGIT4229	CACCACGGCACAAGTGACCC	20	SpyCas9	690
TIGIT4230	CTGGGTCACTTGTGCCGTGG	20	SpyCas9	691
TIGIT4231	GACCTGGGTCACTTGTGCCG	20	SpyCas9	692
TIGIT4232	CACAAGTGACCCAGGTCAAC	20	SpyCas9	693
TIGIT4233	ACAAGTGACCCAGGTCAACT	20	SpyCas9	694
TIGIT4234	CCAGGTCAACTGGGAGCAGC	20	SpyCas9	695
TIGIT4235	CTGCTGCTCCCAGTTGACCT	20	SpyCas9	696
TIGIT4236	CCTGCTGCTCCCAGTTGACC	20	SpyCas9	697
TIGIT4237	GGAGCAGCAGGACCAGCTTC	20	SpyCas9	698

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TIGIT4238	CATTACAAATGGCCAGAAGC	20	SpyCas9	699
TIGIT4239	GGCCATTTGTAATGCTGACT	20	SpyCas9	700
TIGIT4240	GCCATTTGTAATGCTGACTT	20	SpyCas9	701
TIGIT4241	CCATTTGTAATGCTGACTTG	20	SpyCas9	702
TIGIT4242	TTTGTAATGCTGACTTGGGG	20	SpyCas9	703
TIGIT4243	CCCCAAGTCAGCATTACAAA	20	SpyCas9	704
TIGIT4244	GCACATCTCCCCATCCTTCA	20	SpyCas9	705
TIGIT4245	CCCATCCTTCAAGGATCGAG	20	SpyCas9	706
TIGIT4246	CACTCGATCCTTGAAGGATG	20	SpyCas9	707
TIGIT4247	CCACTCGATCCTTGAAGGAT	20	SpyCas9	708
TIGIT4248	GCCACTCGATCCTTGAAGGA	20	SpyCas9	709
TIGIT4249	TTCAAGGATCGAGTGGCCCC	20	SpyCas9	710
TIGIT4250	TGGGGCCACTCGATCCTTGA	20	SpyCas9	711
TIGIT4251	GATCGAGTGGCCCCAGGTCC	20	SpyCas9	712
TIGIT4252	AGTGGCCCCAGGTCCCGGCC	20	SpyCas9	713
TIGIT4253	GTGGCCCCAGGTCCCGGCCT	20	SpyCas9	714
TIGIT4254	GAGGCCCAGGCCGGGACCTG	20	SpyCas9	715
TIGIT4255	TGAGGCCCAGGCCGGGACCT	20	SpyCas9	716
TIGIT4256	GTGAGGCCCAGGCCGGGACC	20	SpyCas9	717
TIGIT4257	TGGAGGGTGAGGCCCAGGCC	20	SpyCas9	718
TIGIT4258	CTGGAGGGTGAGGCCCAGGC	20	SpyCas9	719
TIGIT4259	GCGACTGGAGGGTGAGGCCC	20	SpyCas9	720
TIGIT4260	CGGTCAGCGACTGGAGGGTG	20	SpyCas9	721
TIGIT4261	GTTCACGGTCAGCGACTGGA	20	SpyCas9	722
TIGIT4262	CGTTCACGGTCAGCGACTGG	20	SpyCas9	723
TIGIT4263	TATCGTTCACGGTCAGCGAC	20	SpyCas9	724
TIGIT4264	TCGCTGACCGTGAACGATAC	20	SpyCas9	725
TIGIT4265	CGCTGACCGTGAACGATACA	20	SpyCas9	726
TIGIT4266	GCTGACCGTGAACGATACAG	20	SpyCas9	727
TIGIT4267	GTACTCCCCTGTATCGTTCA	20	SpyCas9	728
TIGIT4268	ATCTATCACACCTACCCTGA	20	SpyCas9	729
TIGIT4269	TCTATCACACCTACCCTGAT	20	SpyCas9	730
TIGIT4270	TACCCTGATGGGACGTACAC	20	SpyCas9	731
TIGIT4271	ACCCTGATGGGACGTACACT	20	SpyCas9	732
TIGIT4272	AGTGTACGTCCCATCAGGGT	20	SpyCas9	733
TIGIT4273	TCCCAGTGTACGTCCCATCA	20	SpyCas9	734
TIGIT4274	CTCCCAGTGTACGTCCCATC	20	SpyCas9	735
TIGIT4275	GTACACTGGGAGAATCTTCC	20	SpyCas9	736
TIGIT4276	CACTGGGAGAATCTTCCTGG	20	SpyCas9	737
TIGIT4277	CTGAGCTTTCTAGGACCTCC	20	SpyCas9	738

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TIGIT4278	AGGTTCCAGATTCCATTGCT	20	SpyCas9	739
TIGIT4279	AAGCAATGGAATCTGGAACC	20	SpyCas9	740
TIGIT4280	GATTCCATTGCTTGGAGCCA	20	SpyCas9	741
TIGIT4281	TGGCTCCAAGCAATGGAATC	20	SpyCas9	742
TIGIT4282	GCGGCCATGGCTCCAAGCAA	20	SpyCas9	743
TIGIT4283	TGGAGCCATGGCCGCGACGC	20	SpyCas9	744
TIGIT4284	AGCCATGGCCGCGACGCTGG	20	SpyCas9	745
TIGIT4285	GACCACCAGCGTCGCGGCCA	20	SpyCas9	746
TIGIT4286	GCAGATGACCACCAGCGTCG	20	SpyCas9	747
TIGIT4287	CATCTGCACAGCAGTCATCG	20	SpyCas9	748
TIGIT4288	CTGCACAGCAGTCATCGTGG	20	SpyCas9	749
TIGIT4289	AGCCCTCAGAATCCATTCTG	20	SpyCas9	750
TIGIT4290	CTCAGAATCCATTCTGTGGA	20	SpyCas9	751
TIGIT4291	TTCCACAGAATGGATTCTGA	20	SpyCas9	752
TIGIT4292	CTTCCACAGAATGGATTCTG	20	SpyCas9	753
TIGIT4293	ATTCTGTGGAAGGTGACCTC	20	SpyCas9	754
TIGIT4294	TGAGGTCACCTTCCACAGAA	20	SpyCas9	755
TIGIT4295	GACCTCAGGAGAAAATCAGC	20	SpyCas9	756
TIGIT4296	CAGGAGAAAATCAGCTGGAC	20	SpyCas9	757
TIGIT4297	GTCCAGCTGATTTTCTCCTG	20	SpyCas9	758
TIGIT4298	GAGAAAATCAGCTGGACAGG	20	SpyCas9	759
TIGIT4299	AATCAGCTGGACAGGAGGAA	20	SpyCas9	760
TIGIT4300	CCCAGTGCTCCCTCACCCCC	20	SpyCas9	761
TIGIT4301	CTGGGGGTGAGGGAGCACTG	20	SpyCas9	762
TIGIT4302	CCTGGGGGTGAGGGAGCACT	20	SpyCas9	763
TIGIT4303	TCCTGGGGGTGAGGGAGCAC	20	SpyCas9	764
TIGIT4304	ACACAGCTTCCTGGGGGTGA	20	SpyCas9	765
TIGIT4305	GACACAGCTTCCTGGGGGTG	20	SpyCas9	766
TIGIT4306	ACCCCCAGGAAGCTGTGTCC	20	SpyCas9	767
TIGIT4307	GCCTGGACACAGCTTCCTGG	20	SpyCas9	768
TIGIT4308	TGCCTGGACACAGCTTCCTG	20	SpyCas9	769
TIGIT4309	CTGCCTGGACACAGCTTCCT	20	SpyCas9	770
TIGIT4310	TCTGCCTGGACACAGCTTCC	20	SpyCas9	771
TIGIT4311	CAGGCAGAAGCTGCACCTGC	20	SpyCas9	772
TIGIT4312	AGGCAGAAGCTGCACCTGCT	20	SpyCas9	773
TIGIT4313	CAGCAGGTGCAGCTTCTGCC	20	SpyCas9	774
TIGIT4314	GCTGCACCTGCTGGGCTCTG	20	SpyCas9	775
TIGIT4315	TGCTCTCCACAGAGCCCAGC	20	SpyCas9	776
TIGIT4316	CTGGGCTCTGTGGAGAGCAG	20	SpyCas9	777
TIGIT4317	TGGGCTCTGTGGAGAGCAGC	20	SpyCas9	778

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TIGIT4318	GGGCTCTGTGGAGAGCAGCG	20	SpyCas9	779
TIGIT4319	CTGTGGAGAGCAGCGGGGAG	20	SpyCas9	780
TIGIT4320	ATTGAAGTAGTCATGCAGCT	20	SpyCas9	781
TIGIT4321	TGTCCTGAGTTACAGAAGCC	20	SpyCas9	782
TIGIT4322	GTCCTGAGTTACAGAAGCCT	20	SpyCas9	783
TIGIT4323	TACCCAGGCTTCTGTAACTC	20	SpyCas9	784
TIGIT4324	TGAAGAAGCTGCAGTTACCC	20	SpyCas9	785
TIGIT4325	TGCAGCTTCTTCACAGAGAC	20	SpyCas9	786
TIGIT5053	GTTGTTTCTATTGTGCCTGT	20	AsCpf1 RR	787
TIGIT5054	CGTTGTTTCTATTGTGCCTG	20	AsCpf1 RR	788
TIGIT5055	CCGTTGTTTCTATTGTGCCT	20	AsCpf1 RR	789
TIGIT5056	CCACGGCACAAGTGACCCAG	20	AsCpf1 RR	790
TIGIT5057	AGTTGACCTGGGTCACTTGT	20	AsCpf1 RR	791
TIGIT5058	AAGTCAGCATTACAAATGGC	20	AsCpf1 RR	792
TIGIT5059	CATCCTTCAAGGATCGAGTG	20	AsCpf1 RR	793
TIGIT5060	ATCCTTCAAGGATCGAGTGG	20	AsCpf1 RR	794
TIGIT5061	AGGATCGAGTGGCCCCAGGT	20	AsCpf1 RR	795
TIGIT5062	AGGTCCCGGCCTGGGCCTCA	20	AsCpf1 RR	796
TIGIT5063	GGCCTGGGCCTCACCCTCCA	20	AsCpf1 RR	797
TIGIT5064	CGGTCAGCGACTGGAGGGTG	20	AsCpf1 RR	798
TIGIT5065	GTCGCTGACCGTGAACGATA	20	AsCpf1 RR	799
TIGIT5066	TGTATCGTTCACGGTCAGCG	20	AsCpf1 RR	800
TIGIT5067	CTGTATCGTTCACGGTCAGC	20	AsCpf1 RR	801
TIGIT5068	ATCAGGGTAGGTGTGATAGA	20	AsCpf1 RR	802
TIGIT5069	AGTGTACGTCCCATCAGGGT	20	AsCpf1 RR	803
TIGIT5070	GGAAGATTCTCCCAGTGTAC	20	AsCpf1 RR	804
TIGIT5071	TGGAGGTCCTAGAAAGCTCA	20	AsCpf1 RR	805
TIGIT5072	AGCAATGGAATCTGGAACCT	20	AsCpf1 RR	806
TIGIT5073	AGATTCCATTGCTTGGAGCC	20	AsCpf1 RR	807
TIGIT5074	GATTCCATTGCTTGGAGCCA	20	AsCpf1 RR	808
TIGIT5075	ATTGCTTGGAGCCATGGCCG	20	AsCpf1 RR	809
TIGIT5076	TTGCTTGGAGCCATGGCCGC	20	AsCpf1 RR	810
TIGIT5077	CAGAATGGATTCTGAGGGCT	20	AsCpf1 RR	811
TIGIT5078	ACAGAATGGATTCTGAGGGC	20	AsCpf1 RR	812
TIGIT5079	TTCTGTGGAAGGTGACCTCA	20	AsCpf1 RR	813
TIGIT5080	GCTGATTTTCTCCTGAGGTC	20	AsCpf1 RR	814
TIGIT5081	TCCTGTCCAGCTGATTTTCT	20	AsCpf1 RR	815
TIGIT5082	TTCCTCCTGTCCAGCTGATT	20	AsCpf1 RR	816
TIGIT5083	TGGGGGTGAGGGAGCACTGG	20	AsCpf1 RR	817
TIGIT5084	AGTGCTCCCTCACCCCCAGG	20	AsCpf1 RR	818

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TIGIT5085	TCACCCCCAGGAAGCTGTGT	20	AsCpf1 RR	819
TIGIT5086	CAGGAAGCTGTGTCCAGGCA	20	AsCpf1 RR	820
TIGIT5087	AGGAAGCTGTGTCCAGGCAG	20	AsCpf1 RR	821
TIGIT5088	GGCAGAAGCTGCACCTGCTG	20	AsCpf1 RR	822
TIGIT5089	CAGAGCCCAGCAGGTGCAGC	20	AsCpf1 RR	823
TIGIT5090	GCTGCTCTCCACAGAGCCCA	20	AsCpf1 RR	824
TIGIT5091	CGCTGCTCTCCACAGAGCCC	20	AsCpf1 RR	825
TIGIT5092	ATGTCCTGAGTTACAGAAGC	20	AsCpf1 RR	826
TIGIT5093	TGCAGAGAAAGGTGGCTCTAT	21	Cas12a	1175

[0553] In some embodiments the gRNA for use in the disclosure is a gRNA targeting ADORA2a (ADORA2a gRNA). In some embodiments, the gRNA targeting ADORA2a is one or more of the gRNAs described in Table 12.

Table 12. Exemplary ADORA2a gRNAs

Name	gRNA Targeting Domain Sequence (DNA)	Length	Enzyme	SEQ ID NO:
ADORA2A337	GAGCACACCCACTGCGATGT	20	SpyCas9	827
ADORA2A338	GATGGCCAGGAGACTGAAGA	20	SpyCas9	828
ADORA2A339	CTGCTCACCGGAGCGGGATG	20	SpyCas9	829
ADORA2A340	GTCTGTGGCCATGCCCATCA	20	SpyCas9	830
ADORA2A341	TCACCGGAGCGGGATGCGGA	20	SpyCas9	831
ADORA2A342	GTGGCAGGCAGCGCAGAACC	20	SpyCas9	832
ADORA2A343	AGCACACCAGCACATTGCCC	20	SpyCas9	833
ADORA2A344	CAGGTTGCTGTTGAGCCACA	20	SpyCas9	834
ADORA2A345	CTTCATTGCCTGCTTCGTCC	20	SpyCas9	835
ADORA2A346	GTACACCGAGGAGCCCATGA	20	SpyCas9	836
ADORA2A347	GATGGCAATGTAGCGGTCAA	20	SpyCas9	837
ADORA2A348	CTCCTCGGTGTACATCACGG	20	SpyCas9	838
ADORA2A349	CGAGGAGCCCATGATGGGCA	20	SpyCas9	839
ADORA2A350	GGGCTCCTCGGTGTACATCA	20	SpyCas9	840
ADORA2A351	CTTTGTGGTGTCACTGGCGG	20	SpyCas9	841
ADORA2A352	CCGCTCCGGTGAGCAGGGCC	20	SpyCas9	842
ADORA2A353	GGGTTCTGCGCTGCCTGCCA	20	SpyCas9	843
ADORA2A354	GGACGAAGCAGGCAATGAAG	20	SpyCas9	844
ADORA2A355	GTGCTGATGGTGATGGCAAA	20	SpyCas9	845
ADORA2A356	AGCGCAGAACCCGGTGCTGA	20	SpyCas9	846
ADORA2A357	GAGCTCCATCTTCAGTCTCC	20	SpyCas9	847

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ADORA2A358	TGCTGATGGTGATGGCAAAG	20	SpyCas9	848
ADORA2A359	GGCGGCGGCCGACATCGCAG	20	SpyCas9	849
ADORA2A360	AATGAAGAGGCAGCCGTGGC	20	SpyCas9	850
ADORA2A361	GGGCAATGTGCTGGTGTGCT	20	SpyCas9	851
ADORA2A362	CATGCCCATCATGGGCTCCT	20	SpyCas9	852
ADORA2A363	AATGTAGCGGTCAATGGCGA	20	SpyCas9	853
ADORA2A364	AGTAGTTGGTGACGTTCTGC	20	SpyCas9	854
ADORA2A365	AGCGGTCAATGGCGATGGCC	20	SpyCas9	855
ADORA2A366	CGCATCCCGCTCCGGTGAGC	20	SpyCas9	856
ADORA2A367	GCATCCCGCTCCGGTGAGCA	20	SpyCas9	857
ADORA2A368	TGGGCAATGTGCTGGTGTGC	20	SpyCas9	858
ADORA2A369	CAACTACTTTGTGGTGTCAC	20	SpyCas9	859
ADORA2A370	CGCTCCGGTGAGCAGGGCCG	20	SpyCas9	860
ADORA2A371	GATGGTGATGGCAAAGGGGA	20	SpyCas9	861
ADORA2A372	GGTGTACATCACGGTGGAGC	20	SpyCas9	862
ADORA2A373	GAACGTCACCAACTACTTTG	20	SpyCas9	863
ADORA2A374	CAGTGACACCACAAAGTAGT	20	SpyCas9	864
ADORA2A375	GGCCATCCTGGGCAATGTGC	20	SpyCas9	865
ADORA2A376	CCCGGCCCTGCTCACCGGAG	20	SpyCas9	866
ADORA2A377	CACCAGCACATTGCCCAGGA	20	SpyCas9	867
ADORA2A378	TTTGCCATCACCATCAGCAC	20	SpyCas9	868
ADORA2A379	CTCCACCGTGATGTACACCG	20	SpyCas9	869
ADORA2A380	GGAGCTGGCCATTGCTGTGC	20	SpyCas9	870
ADORA2A381	CAGGATGGCCAGCACAGCAA	20	SpyCas9	871
ADORA2A382	GAACCCGGTGCTGATGGTGA	20	SpyCas9	872
ADORA2A383	TGGAGCTCTGCGTGAGGACC	20	SpyCas9	873
ADORA2A384	CCCGCTCCGGTGAGCAGGGC	20	SpyCas9	874
ADORA2A385	AGGCAATGAAGAGGCAGCCG	20	SpyCas9	875
ADORA2A386	CCGGCCCTGCTCACCGGAGC	20	SpyCas9	876
ADORA2A387	GCGGCGGCCGACATCGCAGT	20	SpyCas9	877
ADORA2A388	GGTGCTGATGGTGATGGCAA	20	SpyCas9	878
ADORA2A389	CTACTTTGTGGTGTCACTGG	20	SpyCas9	879
ADORA2A390	TACACCGAGGAGCCCATGAT	20	SpyCas9	880
ADORA2A391	TCTGTGGCCATGCCCATCAT	20	SpyCas9	881
ADORA2A392	ATTGCTGTGCTGGCCATCCT	20	SpyCas9	882
ADORA2A393	CGTGAGGACCAGGACGAAGC	20	SpyCas9	883
ADORA2A394	TTGCCATCACCATCAGCACC	20	SpyCas9	884
ADORA2A395	GGATGCGGATGGCAATGTAG	20	SpyCas9	885
ADORA2A396	TTGCCATCCGCATCCCGCTC	20	SpyCas9	886
ADORA2A397	TGAAGATGGAGCTCTGCGTG	20	SpyCas9	887

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ADORA2A398	CATTGCTGTGCTGGCCATCC	20	SpyCas9	888
ADORA2A399	TGCTGGTGTGCTGGGCCGTG	20	SpyCas9	889
ADORA2A820	GGCTCCTCGGTGTACATCACG	21	SauCas9	890
ADORA2A821	GAGCTCTGCGTGAGGACCAGG	21	SauCas9	891
ADORA2A822	GATGGAGCTCTGCGTGAGGAC	21	SauCas9	892
ADORA2A823	CCAGCACACCAGCACATTGCC	21	SauCas9	893
ADORA2A824	AGGACCAGGACGAAGCAGGCA	21	SauCas9	894
ADORA2A825	TGCCATCCGCATCCCGCTCCG	21	SauCas9	895
ADORA2A826	GTGTGGCTCAACAGCAACCTG	21	SauCas9	896
ADORA2A827	AGCTCCACCGTGATGTACACC	21	SauCas9	897
ADORA2A828	GTAGCGGTCAATGGCGATGGC	21	SauCas9	898
ADORA2A829	CGGTGCTGATGGTGATGGCAA	21	SauCas9	899
ADORA2A830	CCCTGCTCACCGGAGCGGGAT	21	SauCas9	900
ADORA2A831	GTGACGTTCTGCAGGTTGCTG	21	SauCas9	901
ADORA2A832	GCTCCACCGTGATGTACACCG	21	SauCas9	902
ADORA2A833	ACTGAAGATGGAGCTCTGCGT	21	SauCas9	903
ADORA2A834	CCAGCTCCACCGTGATGTACA	21	SauCas9	904
ADORA2A835	CCTTTGCCATCACCATCAGCA	21	SauCas9	905
ADORA2A836	CCGGTGCTGATGGTGATGGCA	21	SauCas9	906
ADORA2A837	CCTGGGCAATGTGCTGGTGTG	21	SauCas9	907
ADORA2A838	AGGCAGCCGTGGCAGGCAGCG	21	SauCas9	908
ADORA2A839	GCGATGGCCAGGAGACTGAAG	21	SauCas9	909
ADORA2A840	CGATGGCCAGGAGACTGAAGA	21	SauCas9	910
ADORA2A841	TCCCGCTCCGGTGAGCAGGGC	21	SauCas9	911
ADORA2A842	TGCTTCGTCCTGGTCCTCACG	21	SauCas9	912
ADORA2A843	ACCAGGACGAAGCAGGCAATG	21	SauCas9	913
ADORA2A844	ATGTACACCGAGGAGCCCATG	21	SauCas9	914
ADORA2A845	TCGTCTGTGGCCATGCCCATC	21	SauCas9	915
ADORA2A846	TCAATGGCGATGGCCAGGAGA	21	SauCas9	916
ADORA2A847	GGTGCTGATGGTGATGGCAAA	21	SauCas9	917
ADORA2A848	TAGCGGTCAATGGCGATGGCC	21	SauCas9	918
ADORA2A849	TCCGCATCCCGCTCCGGTGAG	21	SauCas9	919
ADORA2A850	CTGGCGGCGGCCGACATCGCA	21	SauCas9	920
ADORA2A851	GCCATTGCTGTGCTGGCCATC	21	SauCas9	921
ADORA2A852	ATCCCGCTCCGGTGAGCAGGG	21	SauCas9	922
ADORA2A853	AGACTGAAGATGGAGCTCTGC	21	SauCas9	923
ADORA2A854	CCCCGGCCCTGCTCACCGGAG	21	SauCas9	924
ADORA2A855	ATGGTGATGGCAAAGGGGATG	21	SauCas9	925
ADORA2A856	GCTCCTCGGTGTACATCACGG	21	SauCas9	926
ADORA2A248	TGTCGATGGCAATAGCCAAG	20	SpyCas9	927

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ADORA2A249	AGAAGTTGGTGACGTTCTGC	20	SpyCas9	928
ADORA2A250	TTCGCCATCACCATCAGCAC	20	SpyCas9	929
ADORA2A251	GAAGAAGAGGCAGCCATGGC	20	SpyCas9	930
ADORA2A252	CACAAGCACGTTACCCAGGA	20	SpyCas9	931
ADORA2A253	CAACTTCTTCGTGGTATCTC	20	SpyCas9	932
ADORA2A254	CAGGATGGCCAGCACAGCAA	20	SpyCas9	933
ADORA2A255	AATTCCACTCCGGTGAGCCA	20	SpyCas9	934
ADORA2A256	AGCGCAGAAGCCAGTGCTGA	20	SpyCas9	935
ADORA2A257	GTGCTGATGGTGATGGCGAA	20	SpyCas9	936
ADORA2A258	GGAGCTGGCCATTGCTGTGC	20	SpyCas9	937
ADORA2A259	AATAGCCAAGAGGCTGAAGA	20	SpyCas9	938
ADORA2A260	CTCCTCGGTGTACATCATGG	20	SpyCas9	939
ADORA2A261	GGACAAAGCAGGCGAAGAAG	20	SpyCas9	940
ADORA2A262	TCTGGCGGCGGCTGACATCG	20	SpyCas9	941
ADORA2A263	TGGGTAACGTGCTTGTGTGC	20	SpyCas9	942
ADORA2A264	GATGTACACCGAGGAGCCCA	20	SpyCas9	943
ADORA2A265	TAACCCCTGGCTCACCGGAG	20	SpyCas9	944
ADORA2A266	TCACCGGAGTGGAATTCGGA	20	SpyCas9	945
ADORA2A267	GCGGCGGCTGACATCGCGGT	20	SpyCas9	946
ADORA2A268	GATGGTGATGGCGAATGGGA	20	SpyCas9	947
ADORA2A269	GGCTTCTGCGCTGCCTGCCA	20	SpyCas9	948
ADORA2A270	ATTCCACTCCGGTGAGCCAG	20	SpyCas9	949
ADORA2A271	GGTGTACATCATGGTGGAGC	20	SpyCas9	950
ADORA2A272	ATTGCTGTGCTGGCCATCCT	20	SpyCas9	951
ADORA2A273	CTCCACCATGATGTACACCG	20	SpyCas9	952
ADORA2A274	GGCGGCGGCTGACATCGCGG	20	SpyCas9	953
ADORA2A275	TACACCGAGGAGCCCATGGC	20	SpyCas9	954
ADORA2A276	GGGTAACGTGCTTGTGTGCT	20	SpyCas9	955
ADORA2A277	CAGGTTGCTGTTGATCCACA	20	SpyCas9	956
ADORA2A278	TGAAGATGGAACTCTGCGTG	20	SpyCas9	957
ADORA2A279	GATGGCGATGTATCTGTCGA	20	SpyCas9	958
ADORA2A280	CTTCTTCGCCTGCTTTGTCC	20	SpyCas9	959
ADORA2A281	AGGCGAAGAAGAGGCAGCCA	20	SpyCas9	960
ADORA2A282	TGCTTGTGTGCTGGGCCGTG	20	SpyCas9	961
ADORA2A283	GAAGCCAGTGCTGATGGTGA	20	SpyCas9	962
ADORA2A284	CGTGAGGACCAGGACAAAGC	20	SpyCas9	963
ADORA2A285	TGGAACTCTGCGTGAGGACC	20	SpyCas9	964
ADORA2A286	CATTGCTGTGCTGGCCATCC	20	SpyCas9	965
ADORA2A287	TTCTCCCGCCATGGGCTCCT	20	SpyCas9	966
ADORA2A288	TGGCTCACCGGAGTGGAATT	20	SpyCas9	967

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ADORA2A289	TGCTGATGGTGATGGCGAAT	20	SpyCas9	968
ADORA2A290	CTTCGTGGTATCTCTGGCGG	20	SpyCas9	969
ADORA2A291	AGCACACAAGCACGTTACCC	20	SpyCas9	970
ADORA2A292	GGGCTCCTCGGTGTACATCA	20	SpyCas9	971
ADORA2A293	GTACACCGAGGAGCCCATGG	20	SpyCas9	972
ADORA2A294	GAACGTCACCAACTTCTTCG	20	SpyCas9	973
ADORA2A295	TCGCCATCCGAATTCCACTC	20	SpyCas9	974
ADORA2A296	GAGTTCCATCTTCAGCCTCT	20	SpyCas9	975
ADORA2A297	GAATTCCACTCCGGTGAGCC	20	SpyCas9	976
ADORA2A298	CAGAGATACCACGAAGAAGT	20	SpyCas9	977
ADORA2A299	CTTCTTCGTGGTATCTCTGG	20	SpyCas9	978
ADORA2A695	CAGTGCTGATGGTGATGGCGA	21	SauCas9	979
ADORA2A696	CGAATTCCACTCCGGTGAGCC	21	SauCas9	980
ADORA2A697	CCGAATTCCACTCCGGTGAGC	21	SauCas9	981
ADORA2A698	GCTGAAGATGGAACTCTGCGT	21	SauCas9	982
ADORA2A699	CGTGCTTGTGTGCTGGGCCGT	21	SauCas9	983
ADORA2A700	GTGAGGACCAGGACAAAGCAG	21	SauCas9	984
ADORA2A701	TCGATGGCAATAGCCAAGAGG	21	SauCas9	985
ADORA2A702	CATCGACAGATACATCGCCAT	21	SauCas9	986
ADORA2A703	GTACACCGAGGAGCCCATGGC	21	SauCas9	987
ADORA2A704	GCTCCACCATGATGTACACCG	21	SauCas9	988
ADORA2A705	AAGCCAGTGCTGATGGTGATG	21	SauCas9	989
ADORA2A706	CACCGCGATGTCAGCCGCCGC	21	SauCas9	990
ADORA2A707	AGGCTGAAGATGGAACTCTGC	21	SauCas9	991
ADORA2A708	GCCGCCGCCAGAGATACCACG	21	SauCas9	992
ADORA2A709	AGCTCCACCATGATGTACACC	21	SauCas9	993
ADORA2A710	AGGCAGCCATGGCAGGCAGCG	21	SauCas9	994
ADORA2A711	CCTGGCTCACCGGAGTGGAAT	21	SauCas9	995
ADORA2A712	CCAGCTCCACCATGATGTACA	21	SauCas9	996
ADORA2A713	ACCAGGACAAAGCAGGCGAAG	21	SauCas9	997
ADORA2A714	CCTGGGTAACGTGCTTGTGTG	21	SauCas9	998
ADORA2A715	AGGACCAGGACAAAGCAGGCG	21	SauCas9	999
ADORA2A716	TCAGCCGCCGCCAGAGATACC	21	SauCas9	1000
ADORA2A717	GGCTCCTCGGTGTACATCATG	21	SauCas9	1001
ADORA2A718	CTGGCGGCGGCTGACATCGCG	21	SauCas9	1002
ADORA2A719	GATGGAACTCTGCGTGAGGAC	21	SauCas9	1003
ADORA2A720	GCTCCTCGGTGTACATCATGG	21	SauCas9	1004
ADORA2A721	TGTACACCGAGGAGCCCATGG	21	SauCas9	1005
ADORA2A722	GCCATTGCTGTGCTGGCCATC	21	SauCas9	1006
ADORA2A723	CAATAGCCAAGAGGCTGAAGA	21	SauCas9	1007

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ADORA2A724	ATGGTGATGGCGAATGGGATG	21	SauCas9	1008
ADORA2A725	ATGTACACCGAGGAGCCCATG	21	SauCas9	1009
ADORA2A726	GTGTGGATCAACAGCAACCTG	21	SauCas9	1010
ADORA2A727	TGCTTTGTCCTGGTCCTCACG	21	SauCas9	1011
ADORA2A728	GTAACCCCTGGCTCACCGGAG	21	SauCas9	1012
ADORA2A729	CCAGCACACAAGCACGTTACC	21	SauCas9	1013
ADORA2A730	TATCTGTCGATGGCAATAGCC	21	SauCas9	1014
ADORA2A731	GCAATAGCCAAGAGGCTGAAG	21	SauCas9	1015
ADORA2A732	AGTGCTGATGGTGATGGCGAA	21	SauCas9	1016
ADORA2A733	ACACCGAGGAGCCCATGGCGG	21	SauCas9	1017
ADORA2A734	CGCCATCCGAATTCCACTCCG	21	SauCas9	1018
ADORA2A4111	TGGTGTCACTGGCGGCGGCC	20	AsCpf1	1019
ADORA2A4112	CCATCACCATCAGCACCGGG	20	AsCpf1	1020
ADORA2A4113	CCATCGGCCTGACTCCCATG	20	AsCpf1	1021
ADORA2A4114	GCTGACCGCAGTTGTTCCAA	20	AsCpf1	1022
ADORA2A4115	AGGATGTGGTCCCCATGAAC	20	AsCpf1	1023
ADORA2A4116	CCTGTGTGCTGGTGCCCCTG	20	AsCpf1	1024
ADORA2A4117	CGGATCTTCCTGGCGGCGCG	20	AsCpf1	1025
ADORA2A4118	CCCTCTGCTGGCTGCCCCTA	20	AsCpf1	1026
ADORA2A4119	TTCTGCCCCGACTGCAGCCA	20	AsCpf1	1027
ADORA2A4120	AAGGCAGCTGGCACCAGTGC	20	AsCpf1	1028
ADORA2A4121	TAAGGGCATCATTGCCATCTG	21	SauCas9	1029
ADORA2A4122	CGGCCTGACTCCCATGCTAGG	21	SauCas9	1030
ADORA2A4123	GCAGTTGTTCCAACCTAGCAT	21	SauCas9	1031
ADORA2A4124	CCGCAGTTGTTCCAACCTAGC	21	SauCas9	1032
ADORA2A4125	CAAGAACCACTCCCAGGGCTG	21	SauCas9	1033
ADORA2A4126	CTTGGCCCTCCCCGCAGCCCT	21	SauCas9	1034
ADORA2A4127	CACTTGGCCCTCCCCGCAGCC	21	SauCas9	1035
ADORA2A4128	GGCCAAGTGGCCTGTCTCTTT	21	SauCas9	1036
ADORA2A4129	TTCATGGGGACCACATCCTCA	21	SauCas9	1037
ADORA2A4130	TGAAGTACACCATGTAGTTCA	21	SauCas9	1038
ADORA2A4131	CTGGTGCCCCTGCTGCTCATG	21	SauCas9	1039
ADORA2A4132	GCTCATGCTGGGTGTCTATTT	21	SauCas9	1040
ADORA2A4133	CTTCAGCTGTCGTCGCGCCGC	21	SauCas9	1041
ADORA2A4134	CGCGACGACAGCTGAAGCAGA	21	SauCas9	1042
ADORA2A4135	GATGGAGAGCCAGCCTCTGCC	21	SauCas9	1043
ADORA2A4136	GCGTGGCTGCAGTCGGGGCAG	21	SauCas9	1044
ADORA2A4137	ACGATGGCCAGGTACATGAGC	21	SauCas9	1045
ADORA2A4138	CTCTCCCACACCAATTCGGTT	21	SauCas9	1046
ADORA2A4139	GATTCACAACCGAATTGGTGT	21	SauCas9	1047

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ADORA2A4140	GGGATTCACAACCGAATTGGT	21	SauCas9	1048
ADORA2A4141	CGTAGATGAAGGGATTCACAA	21	SauCas9	1049
ADORA2A4142	GGATACGGTAGGCGTAGATGA	21	SauCas9	1050
ADORA2A4143	TCATCTACGCCTACCGTATCC	21	SauCas9	1051
ADORA2A4144	CGGATACGGTAGGCGTAGATG	21	SauCas9	1052
ADORA2A4145	GCGGAAGGTCTGGCGGAACTC	21	SauCas9	1053
ADORA2A4146	AATGATCTTGCGGAAGGTCTG	21	SauCas9	1054
ADORA2A4147	GACGTGGCTGCGAATGATCTT	21	SauCas9	1055
ADORA2A4148	TTGCTGCCTCAGGACGTGGCT	21	SauCas9	1056
ADORA2A4149	CAAGGCAGCTGGCACCAGTGC	21	SauCas9	1057
ADORA2A4150	CGGGCACTGGTGCCAGCTGCC	21	SauCas9	1058
ADORA2A4151	CTTGGCAGCTCATGGCAGTGA	21	SauCas9	1059
ADORA2A4152	CCGTCTCAACGGCCACCCGCC	21	SauCas9	1060
ADORA2A4153	CACACTCCTGGCGGGTGGCCG	21	SauCas9	1061
ADORA2A4154	TGCCGTTGGCCCACACTCCTG	21	SauCas9	1062
ADORA2A4155	CCATTGGGCCTCCGCTCAGGG	21	SauCas9	1063
ADORA2A4156	CATAGCCATTGGGCCTCCGCT	21	SauCas9	1064
ADORA2A4157	AATGGCTATGCCCTGGGGCTG	21	SauCas9	1065
ADORA2A4158	ATGCCCTGGGGCTGGTGAGTG	21	SauCas9	1066
ADORA2A4159	GCCCTGGGGCTGGTGAGTGGA	21	SauCas9	1067
ADORA2A4160	TGGTGAGTGGAGGGAGTGCCC	21	SauCas9	1068
ADORA2A4161	GAGGGAGTGCCCAAGAGTCCC	21	SauCas9	1069
ADORA2A4162	AGGGAGTGCCCAAGAGTCCCA	21	SauCas9	1070
ADORA2A4163	GTCTGGGAGGCCCGTGTTCCC	21	SauCas9	1071
ADORA2A4164	CATGGCTAAGGAGCTCCACGT	21	SauCas9	1072
ADORA2A4165	GAGCTCCTTAGCCATGAGCTC	21	SauCas9	1073
ADORA2A4166	GCTCCTTAGCCATGAGCTCAA	21	SauCas9	1074
ADORA2A4167	GGCCTAGATGACCCCCTGGCC	21	SauCas9	1075
ADORA2A4168	CCCCCTGGCCCAGGATGGAGC	21	SauCas9	1076
ADORA2A4169	CTCCTGCTCCATCCTGGGCCA	21	SauCas9	1077
ADORA2A4416	CCGTGATGTACACCGAGGAG	20	AsCpf1 RR	1078
ADORA2A4417	CTTTGCCATCACCATCAGCA	20	AsCpf1 RR	1079
ADORA2A4418	TTTGCCATCACCATCAGCAC	20	AsCpf1 RR	1080
ADORA2A4419	TTGCCTGCTTCGTCCTGGTC	20	AsCpf1 RR	1081
ADORA2A4420	TCCTGGTCCTCACGCAGAGC	20	AsCpf1 RR	1082
ADORA2A4421	TCTTCAGTCTCCTGGCCATC	20	AsCpf1 RR	1083
ADORA2A4422	GTCTCCTGGCCATCGCCATT	20	AsCpf1 RR	1084
ADORA2A4423	ACCTAGCATGGGAGTCAGGC	20	AsCpf1 RR	1085
ADORA2A4424	AACCTAGCATGGGAGTCAGG	20	AsCpf1 RR	1086
ADORA2A4425	ATGCTAGGTTGGAACAACTG	20	AsCpf1 RR	1087

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ADORA2A4426	GCAGCCCTGGGAGTGGTTCT	20	AsCpf1 RR	1088
ADORA2A4427	CGCAGCCCTGGGAGTGGTTC	20	AsCpf1 RR	1089
ADORA2A4428	AGGGCTGCGGGGAGGGCCAA	20	AsCpf1 RR	1090
ADORA2A4429	TGGGGACCACATCCTCAAAG	20	AsCpf1 RR	1091
ADORA2A4430	CATGAACTACATGGTGTACT	20	AsCpf1 RR	1092
ADORA2A4431	ATGAACTACATGGTGTACTT	20	AsCpf1 RR	1093
ADORA2A4432	ACTTCTTTGCCTGTGTGCTG	20	AsCpf1 RR	1094
ADORA2A4433	TGCTGCTCATGCTGGGTGTC	20	AsCpf1 RR	1095
ADORA2A4434	CAAATAGACACCCAGCATGA	20	AsCpf1 RR	1096
ADORA2A4435	GCTGTCGTCGCGCCGCCAGG	20	AsCpf1 RR	1097
ADORA2A4436	TGGCGGCGCGACGACAGCTG	20	AsCpf1 RR	1098
ADORA2A4437	TCTGCTTCAGCTGTCGTCGC	20	AsCpf1 RR	1099
ADORA2A4438	GGCAGAGGCTGGCTCTCCAT	20	AsCpf1 RR	1100
ADORA2A4439	CGGCAGAGGCTGGCTCTCCA	20	AsCpf1 RR	1101
ADORA2A4440	CCGGCAGAGGCTGGCTCTCC	20	AsCpf1 RR	1102
ADORA2A4441	CACTGCAGAAGGAGGTCCAT	20	AsCpf1 RR	1103
ADORA2A4442	TGCTGCCAAGTCACTGGCCA	20	AsCpf1 RR	1104
ADORA2A4443	ACAATGATGGCCAGTGACTT	20	AsCpf1 RR	1105
ADORA2A4444	TACACATCATCAACTGCTTC	20	AsCpf1 RR	1106
ADORA2A4445	CTTTCTTCTGCCCCGACTGC	20	AsCpf1 RR	1107
ADORA2A4446	GACTGCAGCCACGCCCCTCT	20	AsCpf1 RR	1108
ADORA2A4447	TCTCTGGCTCATGTACCTGG	20	AsCpf1 RR	1109
ADORA2A4448	CAACCGAATTGGTGTGGGAG	20	AsCpf1 RR	1110
ADORA2A4449	ACACCAATTCGGTTGTGAAT	20	AsCpf1 RR	1111
ADORA2A4450	GTTGTGAATCCCTTCATCTA	20	AsCpf1 RR	1112
ADORA2A4451	TTCATCTACGCCTACCGTAT	20	AsCpf1 RR	1113
ADORA2A4452	TCTACGCCTACCGTATCCGC	20	AsCpf1 RR	1114
ADORA2A4453	CGAGTTCCGCCAGACCTTCC	20	AsCpf1 RR	1115
ADORA2A4454	GCCAGACCTTCCGCAAGATC	20	AsCpf1 RR	1116
ADORA2A4455	CCAGACCTTCCGCAAGATCA	20	AsCpf1 RR	1117
ADORA2A4456	GCAAGATCATTCGCAGCCAC	20	AsCpf1 RR	1118
ADORA2A4457	CAAGATCATTCGCAGCCACG	20	AsCpf1 RR	1119
ADORA2A4458	CAGCCACGTCCTGAGGCAGC	20	AsCpf1 RR	1120
ADORA2A4459	AGGCAGCTGGCACCAGTGCC	20	AsCpf1 RR	1121
ADORA2A4460	TCACTGCCATGAGCTGCCAA	20	AsCpf1 RR	1122
ADORA2A4461	TCTCAACGGCCACCCGCCAG	20	AsCpf1 RR	1123
ADORA2A4462	CTCAGGGTGGGGAGCACTGC	20	AsCpf1 RR	1124
ADORA2A4463	CACCCTGAGCGGAGGCCCAA	20	AsCpf1 RR	1125
ADORA2A4464	ACCCTGAGCGGAGGCCCAAT	20	AsCpf1 RR	1126
ADORA2A4465	AGGGCATAGCCATTGGGCCT	20	AsCpf1 RR	1127

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ADORA2A4466	CTCACCAGCCCCAGGGCATA	20	AsCpf1 RR	1128
ADORA2A4467	TCCACTCACCAGCCCCAGGG	20	AsCpf1 RR	1129
ADORA2A4468	TGGGACTCTTGGGCACTCCC	20	AsCpf1 RR	1130
ADORA2A4469	CTGGGACTCTTGGGCACTCC	20	AsCpf1 RR	1131
ADORA2A4470	CCTGGGACTCTTGGGCACTC	20	AsCpf1 RR	1132
ADORA2A4471	AGGGGAACACGGGCCTCCCA	20	AsCpf1 RR	1133
ADORA2A4472	CGTCTGGGAGGCCCGTGTTC	20	AsCpf1 RR	1134
ADORA2A4473	AGACGTGGAGCTCCTTAGCC	20	AsCpf1 RR	1135
ADORA2A4474	TTGAGCTCATGGCTAAGGAG	20	AsCpf1 RR	1136
ADORA2A4475	CTGGCCTAGATGACCCCCTG	20	AsCpf1 RR	1137
ADORA2A4476	TGGCCTAGATGACCCCCTGG	20	AsCpf1 RR	1138
ADORA2A4477	TCCTGGGCCAGGGGGTCATC	20	AsCpf1 RR	1139
ADORA2A4478	CTGGCCCAGGATGGAGCAGG	20	AsCpf1 RR	1140
ADORA2A4479	TGGCCCAGGATGGAGCAGGA	20	AsCpf1 RR	1141
ADORA2A4480	CGCGAGTTCCGCCAGACCTT	20	AsCpf1 RVR	1142
ADORA2A4481	CCCTGGGGCTGGTGAGTGGA	20	AsCpf1RVR	1143
ADORA2A4482	CCATCGGCCTGACTCCCATGC	21	Cas12a	1174

[0554] It will be understood that the exemplary gRNAs disclosed herein are provided to illustrate non-limiting embodiments embraced by the present disclosure. Additional suitable gRNA sequences will be apparent to the skilled artisan based on the present disclosure, and the disclosure is not limited in this respect.

Methods of Characterization

[0555] Methods of characterizing cells including characterizing cellular phenotype are known to those of skill in the art. In some embodiments, one or more such methods may include, but not be limited to, for example, morphological analyses and flow cytometry. Cellular lineage and identity markers are known to those of skill in the art. One or more such markers may be combined with one or more characterization methods to determine a composition of a cell population or phenotypic identity of one or more cells. For example, in some embodiments, cells of a particular population will be characterized using flow cytometry (for example, see Ye Li et al., Cell Stem Cell. 2018 Aug 2; 23(2): 181-192.e5). In some such embodiments, a sample of a population of cells will be evaluated for presence and proportion of one or more cell surface markers and/or one or more intracellular markers. As will be understood by those of skill in the art, such cell surface markers may be representative of different lineages. For example, pluripotent cells may be identified by one or more of any number of markers known to be

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associated with such cells, such as, for example, CD34. Further, in some embodiments, cells may be identified by markers that indicate some degree of differentiation. Such markers will be known to one of skill in the art. For example, in some embodiments, markers of differentiated cells may include those associated with differentiated hematopoietic cells such as, e.g., CD43, CD45 (differentiated hematopoietic cells). In some embodiments, markers of differentiated cells may be associated with NK cell phenotypes such as, e.g., CD56, NK cell receptor immunoglobulin gamma Fc region receptor III (FcγRIII, cluster of differentiation 16 (CD16)), natural killer group-2 member D (NKG2D), CD69, a natural cytotoxicity receptor, etc. In some embodiments, markers may be T cell markers (e.g., CD3, CD4, CD8, etc.).

Methods of Use

[0556] A variety of diseases, disorders and/or conditions may be treated through use of cells provided by the present disclosure. For example, in some embodiments, a disease, disorder and/or condition may be treated by introducing genetically modified or engineered cells as described herein (e.g., genetically modified NK or iNK cells) to a subject. Examples of diseases that may be treated include, but are not limited to, cancer, e.g., solid tumors, e.g., of the brain, prostate, breast, lung, colon, uterus, skin, liver, bone, pancreas, ovary, testes, bladder, kidney, head, neck, stomach, cervix, rectum, larynx, or esophagus; and hematological malignancies, e.g., acute and chronic leukemias, lymphomas, multiple myeloma and myelodysplastic syndromes.

[0557] In some embodiments, the present disclosure provides methods of treating a subject in need thereof by administering to the subject a composition comprising any of the cells described herein. In some embodiments, a therapeutic agent or composition may be administered before, during, or after the onset of a disease, disorder, or condition (including, e.g., an injury). In some embodiments, the present disclosure provides any of the cells described herein for use in the preparation of a medicament. In some embodiments, the present disclosure provides any of the cells described herein for use in the treatment of a disease, disorder, or condition, that can be treated by a cell therapy.

[0558] In particular embodiments, the subject has a disease, disorder, or condition, that can be treated by a cell therapy. In some embodiments, a subject in need of cell therapy is a subject with a disease, disorder and/or condition, whereby a cell therapy, e.g., a therapy in which a composition comprising a cell described herein, is administered to the subject, whereby the cell

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therapy treats at least one symptom associated with the disease, disorder, and/or condition. In some embodiments, a subject in need of cell therapy includes, but is not limited to, a candidate for bone marrow or stem cell transplant, a subject who has received chemotherapy or irradiation therapy, a subject who has or is at risk of having cancer, e.g., a cancer of hematopoietic system, a subject having or at risk of developing a tumor, e.g., a solid tumor, and/or a subject who has or is at risk of having a viral infection or a disease associated with a viral infection.

Pharmaceutical Compositions

[0559] In some embodiments, the present disclosure provides pharmaceutical compositions comprising one or more genetically modified or engineered cells described herein, e.g., a genetically modified NK or iNK cell described herein. In some embodiments, a pharmaceutical composition further comprises a pharmaceutically acceptable excipient. In some embodiments, a pharmaceutical composition comprises isolated pluripotent stem cell-derived hematopoietic lineage cells comprising at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% T cells, NK cells, NKT cells, CD34+ HE cells or HSCs, e.g., genetically modified (e.g., edited) T cells, NK cells, NKT cells, CD34+ HE cells or HSCs. In some embodiments, a pharmaceutical composition comprises isolated pluripotent stem cell-derived hematopoietic lineage cells comprising about 95% to about 100% T cells, NK cells, NKT cells, CD34+ HE cells or HSCs, e.g., genetically modified (e.g., edited) T cells, NK cells, NKT cells, CD34+ HE cells or HSCs.

[0560] In some embodiments, a pharmaceutical composition of the present disclosure comprises an isolated population of pluripotent stem cell-derived hematopoietic lineage cells, wherein the isolated population has less than about 0.1%, 0.5%, 1%, 2%, 5%, 10%, 15%, 20%, 25%, or 30% T cells, NK cells, NKT cells, CD34+ HE cells or HSCs, e.g., genetically modified (e.g., edited) T cells, NK cells, NKT cells, CD34+ HE cells or HSCs. In some embodiments, an isolated population of pluripotent stem cell-derived hematopoietic lineage cells has more than about 0.1%, 0.5%, 1%, 2%, 5%, 10%, 15%, 20%, 25%, or 30% T cells, NK cells, NKT cells, CD34+ HE cells or HSCs, e.g., genetically modified (e.g., edited) T cells, NK cells, NKT cells, CD34+ HE cells or HSCs. In some embodiments, an isolated population of pluripotent stem cell-derived hematopoietic lineage cells has about 0.1% to about 1%, about 1% to about 3%, about 3% to about 5%, about 10%-15%, about 15%-20%, about 20%-25%, about 25%-30%, about 30%-35%, about 35%-40%, about 40%-45%, about 45%-50%, about 60%-70%, about

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70%-80%, about 80%-90%, about 90%-95%, or about 95% to about 100% T cells, NK cells, NKT cells, CD34+ HE cells or HSCs, e.g., genetically modified (e.g., edited) T cells, NK cells, NKT cells, CD34+ HE cells or HSCs.

[0561] In some embodiments, an isolated population of pluripotent stem cell-derived hematopoietic lineage cells comprises about 0.1%, about 1%, about 3%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98%, about 99%, or about 100% T cells, NK cells, NKT cells, CD34+ HE cells or HSCs, e.g., genetically modified (e.g., edited) T cells, NK cells, NKT cells, CD34+ HE cells or HSCs.

[0562] As one of ordinary skill in the art would understand, both autologous and allogeneic cells can be used in adoptive cell therapies. Autologous cell therapies generally have reduced infection, low probability for GVHD, and rapid immune reconstitution relative to other cell therapies. Allogeneic cell therapies generally have an immune mediated graft-versus-malignancy (GVM) effect, and low rate of relapse relative to other cell therapies. Based on the specific condition(s) of the subject in need of the cell therapy, one of ordinary skill in the art would be able to determine which specific type of therapy(ies) to administer.

[0563] In some embodiments, a pharmaceutical composition comprises pluripotent stem cell-derived hematopoietic lineage cells that are allogeneic to a subject. In some embodiments, a pharmaceutical composition comprises pluripotent stem cell-derived hematopoietic lineage cells that are autologous to a subject. For autologous transplantation, the isolated population of pluripotent stem cell-derived hematopoietic lineage cells can be either a complete or partial HLA-match with the subject being treated. In some embodiments, the pluripotent stem cell-derived hematopoietic lineage cells are not HLA-matched to a subject.

[0564] In some embodiments, pluripotent stem cell-derived hematopoietic lineage cells can be administered to a subject without being expanded ex vivo or in vitro prior to administration. In particular embodiments, an isolated population of derived hematopoietic lineage cells is modulated and treated ex vivo using one or more agents to obtain immune cells with improved therapeutic potential. In some embodiments, the modulated population of derived hematopoietic lineage cells can be washed to remove the treatment agent(s), and the improved population can be administered to a subject without further expansion of the population in vitro.

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In some embodiments, an isolated population of derived hematopoietic lineage cells is expanded prior to modulating the isolated population with one or more agents.

[0565] In some embodiments, an isolated population of derived hematopoietic lineage cells can be genetically modified according to the methods of the present disclosure to express a recombinant TCR, CAR or other gene product of interest. For genetically engineered derived hematopoietic lineage cells that express a recombinant TCR or CAR, whether prior to or after genetic modification of the cells, the cells can be activated and expanded using methods as described, for example, in U.S. Pat. Nos. 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041; and U.S. Patent Application Publication No. 20060121005.

Cancers

[0566] Any cancer can be treated using a cell or pharmaceutical composition described herein. Exemplary therapeutic targets of the present disclosure include cancer cells from the bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, eye, gastrointestinal system, gum, head, kidney, liver, lung, nasopharynx, neck, ovary, prostate, skin, stomach, testis, tongue, or uterus. In addition, a cancer may specifically be of the following non-limiting histological type: neoplasm, malignant; carcinoma; carcinoma, undifferentiated; giant and spindle cell carcinoma; small cell carcinoma; papillary carcinoma; squamous cell carcinoma; lymphoepithelial carcinoma; basal cell carcinoma; pilomatrix carcinoma; transitional cell carcinoma; papillary transitional cell carcinoma; adenocarcinoma; gastrinoma, malignant; cholangiocarcinoma; hepatocellular carcinoma; combined hepatocellular carcinoma and cholangiocarcinoma; trabecular adenocarcinoma; adenoid cystic carcinoma; adenocarcinoma in adenomatous polyp; adenocarcinoma, familial polyposis coli; solid carcinoma; carcinoid tumor, malignant; branchiolo-alveolar adenocarcinoma; papillary adenocarcinoma; chromophobe carcinoma; acidophil carcinoma; oxyphilic adenocarcinoma; basophil carcinoma; clear cell adenocarcinoma; granular cell carcinoma; follicular adenocarcinoma; papillary and follicular adenocarcinoma; nonencapsulating sclerosing carcinoma; adrenal cortical carcinoma; endometroid carcinoma; skin appendage carcinoma; apocrine adenocarcinoma; sebaceous adenocarcinoma; ceruminous adenocarcinoma; mucoepidermoid carcinoma; cystadenocarcinoma; papillary cystadenocarcinoma; papillary serous cystadenocarcinoma;

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mucinous cystadenocarcinoma; mucinous adenocarcinoma; signet ring cell carcinoma; infiltrating duct carcinoma; medullary carcinoma; lobular carcinoma; inflammatory carcinoma; Paget's disease, mammary; acinar cell carcinoma; adenosquamous carcinoma; adenocarcinoma w/squamous metaplasia; thymoma, malignant; ovarian stromal tumor, malignant; thecoma, malignant; granulosa cell tumor, malignant; androblastoma, malignant; sertoli cell carcinoma; Leydig cell tumor, malignant; lipid cell tumor, malignant; paraganglioma, malignant; extra-mammary paraganglioma, malignant; pheochromocytoma; glomangiosarcoma; malignant melanoma; amelanotic melanoma; superficial spreading melanoma; malig melanoma in giant pigmented nevus; epithelioid cell melanoma; blue nevus, malignant; sarcoma; fibrosarcoma; fibrous histiocytoma, malignant; myxosarcoma; liposarcoma; leiomyosarcoma; rhabdomyosarcoma; embryonal rhabdomyosarcoma; alveolar rhabdomyosarcoma; stromal sarcoma; mixed tumor, malignant; mullerian mixed tumor; nephroblastoma; hepatoblastoma; carcinosarcoma; mesenchymoma, malignant; brenner tumor, malignant; phyllodes tumor, malignant; synovial sarcoma; mesothelioma, malignant; dysgerminoma; embryonal carcinoma; teratoma, malignant; struma ovarii, malignant; choriocarcinoma; mesonephroma, malignant; hemangiosarcoma; hemangioendothelioma, malignant; Kaposi sarcoma; hemangiopericytoma, malignant; lymphangiosarcoma; osteosarcoma; juxtacortical osteosarcoma; chondrosarcoma; chondroblastoma, malignant; mesenchymal chondrosarcoma; giant cell tumor of bone; Ewing sarcoma; odontogenic tumor, malignant; ameloblastic odontosarcoma; ameloblastoma, malignant; ameloblastic fibrosarcoma; pinealoma, malignant; chordoma; glioma, malignant; ependymoma; astrocytoma; protoplasmic astrocytoma; fibrillary astrocytoma; astroblastoma; glioblastoma; oligodendroglioma; oligodendroblastoma; primitive neuroectodermal; cerebellar sarcoma; ganglioneuroblastoma; neuroblastoma; retinoblastoma; olfactory neurogenic tumor; meningioma, malignant; neurofibrosarcoma; neurilemmoma, malignant; granular cell tumor, malignant; malignant lymphoma; Hodgkin's disease; Hodgkin's lymphoma; paragranuloma; malignant lymphoma, small lymphocytic; malignant lymphoma, large cell, diffuse; malignant lymphoma, follicular; mycosis fungoides; other specified non-Hodgkin's lymphomas; malignant histiocytosis; multiple myeloma; mast cell sarcoma; immunoproliferative small intestinal disease; leukemia; lymphoid leukemia; plasma cell leukemia; erythroleukemia; lymphosarcoma cell leukemia; myeloid leukemia; basophilic leukemia; eosinophilic leukemia; monocytic

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leukemia; mast cell leukemia; megakaryoblastic leukemia; myeloid sarcoma; and hairy cell leukemia.

[0567] In some embodiments, the cancer is a breast cancer. In some embodiments, the cancer is colorectal cancer (*e.g.*, colon cancer). In some embodiments, the cancer is gastric cancer. In some embodiments, the cancer is RCC. In some embodiments, the cancer is non-small cell lung cancer (NSCLC). In some embodiments, the cancer is head and neck cancer.

[0568] In some embodiments, solid cancer indications that can be treated with cells described herein (e.g., cells modified using methods of the disclosure, e.g., genetically modified iNK cells), either alone or in combination with one or more additional cancer treatment modality, include: bladder cancer, hepatocellular carcinoma, prostate cancer, ovarian/uterine cancer, pancreatic cancer, mesothelioma, melanoma, glioblastoma, HPV-associated and/or HPV-positive cancers such as cervical and HPV+ head and neck cancer, oral cavity cancer, cancer of the pharynx, thyroid cancer, gallbladder cancer, and soft tissue sarcomas. In some embodiments, hematological cancer indications that can be treated with cells described herein (e.g., cells modified using methods of the disclosure, e.g., genetically modified iNK cells), either alone or in combination with one or more additional cancer treatment modalities, include: ALL, CLL, NHL, DLBCL, AML, CML, and multiple myeloma (MM).

[0569] In some embodiments, examples of cellular proliferative and/or differentiative disorders of the lung that can be treated with cells described herein (e.g., cells modified using methods of the disclosure) include, but are not limited to, tumors such as bronchogenic carcinoma, including paraneoplastic syndromes, bronchioloalveolar carcinoma, neuroendocrine tumors, such as bronchial carcinoid, miscellaneous tumors, metastatic tumors, and pleural tumors, including solitary fibrous tumors (pleural fibroma) and malignant mesothelioma.

[0570] In some embodiments, examples of cellular proliferative and/or differentiative disorders of the breast that can be treated with cells described herein (e.g., cells modified using methods of the disclosure) include, but are not limited to, proliferative breast disease including, e.g., epithelial hyperplasia, sclerosing adenosis, and small duct papillomas; tumors, e.g., stromal tumors such as fibroadenoma, phyllodes tumor, and sarcomas, and epithelial tumors such as large duct papilloma; carcinoma of the breast including in situ (noninvasive) carcinoma that includes ductal carcinoma in situ (including Paget's disease) and lobular carcinoma in situ, and

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invasive (infiltrating) carcinoma including, but not limited to, invasive ductal carcinoma, invasive lobular carcinoma, medullary carcinoma, colloid (mucinous) carcinoma, tubular carcinoma, and invasive papillary carcinoma, and miscellaneous malignant neoplasms. Disorders in the male breast include, but are not limited to, gynecomastia and carcinoma.

[0571] In some embodiments, examples of cellular proliferative and/or differentiative disorders involving the colon that can be treated with cells described herein (e.g., cells modified using methods of the disclosure) include, but are not limited to, tumors of the colon, such as non-neoplastic polyps, adenomas, familial syndromes, colorectal carcinogenesis, colorectal carcinoma, and carcinoid tumors.

[0572] In some embodiments, examples of cancers or neoplastic conditions, in addition to the ones described above that can be treated with cells described herein (e.g., cells modified using methods of the disclosure), include, but are not limited to, a fibrosarcoma, myosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, gastric cancer, esophageal cancer, rectal cancer, pancreatic cancer, ovarian cancer, prostate cancer, uterine cancer, cancer of the head and neck, skin cancer, brain cancer, squamous cell carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular cancer, small cell lung carcinoma, non-small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma, leukemia, lymphoma, or Kaposi sarcoma.

[0573] In some embodiments, cells described herein (e.g., cells modified using methods of the disclosure) are used in combination with one or more cancer treatment modalities. In some embodiments, other cancer treatment modalities include, but are not limited to: chemotherapeutic agents include alkylating agents such as thiotepa and CYTOXAN® cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including

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altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfanide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gamma1I and calicheamicin omegal1 (see, e.g., Agnew, Chem. Intl. Ed. Engl., 1994; 33:183-186); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, caminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including ADRIAMYCIN®, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, doxorubicin HCl liposome injection (DOXIL®) and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate, gemcitabine (GEMZAR®), tegafur (UFTORAL®), capecitabine (XELODA®), an epothilone, and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals

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such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2′,2″-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoids, e.g., paclitaxel (TAXOL®), albumin-engineered nanoparticle formulation of paclitaxel (ABRAXANET™), and doxetaxel (TAXOTERE®); chloranbucil; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine (VELBAN®); platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine (ONCOVIN®); oxaliplatin; leucovovin; vinorelbine (NAVELBINE®); novantrone; edatrexate; daunomycin; aminopterin; cyclosporine, sirolimus, rapamycin, rapalogs, ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone, and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU, leucovovin; anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX® tamoxifen), raloxifene (EVISTA®), droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (FARESTON®); anti-progesterones; estrogen receptor down-regulators (ERDs); estrogen receptor antagonists such as fulvestrant (FASLODEX®); agents that function to suppress or shut down the ovaries, for example, leutinizing hormone-releasing hormone (LHRH) agonists such as leuprolide acetate (LUPRON® and ELIGARD®), goserelin acetate, buserelin acetate and tripterelin; other anti-androgens such as flutamide, nilutamide and bicalutamide; and aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, megestrol acetate (MEGASE®), exemestane

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(AROMASIN®), formestanie, fadrozole, vorozole (RIVISOR®), letrozole (FEMARA®), and anastrozole (ARIMIDEX®); bisphosphonates such as clodronate (for example, BONEFOS® or OSTAC®), etidronate (DIDROCAL®), NE-58095, zoledronic acid/zoledronate (ZOMETA®), alendronate (FOSAMAX®), pamidronate (AREDIA®), tiludronate (SKELID®), or risedronate (ACTONEL®); troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); aptamers, described for example in U.S. Pat. No. 6,344,321, which is herein incorporated by reference in its entirety; anti HGF monoclonal antibodies (e.g., AV299 from Aveo, AMG102, from Amgen); truncated mTOR variants (e.g., CGEN241 from Compugen); protein kinase inhibitors that block mTOR induced pathways (e.g., ARQ197 from Arqule, XL880 from Exelexis, SGX523 from SGX Pharmaceuticals, MP470 from Supergen, PF2341066 from Pfizer); vaccines such as THERATOPE® vaccine and gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; topoisomerase 1 inhibitor (e.g., LURTOTECAN®); rmRH (e.g., ABARELIX®); lapatinib ditosylate (an ErbB-2 and EGFR dual tyrosine kinase small-molecule inhibitor also known as GW572016); COX-2 inhibitors such as celecoxib (CELEBREX®; 4-(5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl) benzenesulfonamide; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0574] In some embodiments, cells described herein (e.g., cells modified using methods of the disclosure) are used in combination with one or more cancer treatment modalities that facilitate the induction of antibody dependent cellular cytotoxicity (ADCC) (see e.g., Janeway's Immunobiology by K. Murphy and C. weaver). In some embodiments, such a cancer treatment modality is an antibody. In some embodiments, such an antibody is Trastuzumab. In some embodiments, such an antibody is Rituximab. In some embodiments, such an antibody is Rituximab, Palivizumab, Infliximab, Trastuzumab, Alemtuzumab, Adalimumab, Ibritumomab tiuxetan, Omalizumab, Cetuximab, Bevacizumab, Natalizumab, Panitumumab, Ranibizumab, Certolizumab pegol, Ustekinumab, Canakinumab, Golimumab, Ofatumumab, Tocilizumab, Denosumab, Belimumab, Ipilimumab, Brentuximab vedotin, Pertuzumab, Trastuzumab emtansine, Obinutuzumab, Siltuximab, Ramucirumab, Vedolizumab, Blinatumomab, Nivolumab, Pembrolizumab, Idarucizumab, Necitumumab, Dinutuximab, Secukinumab, Mepolizumab, Alirocumab, Evolocumab, Daratumumab, Elotuzumab, Ixekizumab, Reslizumab,

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Olaratumab, Bezlotoxumab, Atezolizumab, Obiltoxaximab, Inotuzumab ozogamicin, Brodalumab, Guselkumab, Dupilumab, Sarilumab, Avelumab, Ocrelizumab, Emicizumab, Benralizumab, Gemtuzumab ozogamicin, Durvalumab, Burosumab, Lanadelumab, Mogamulizumab, Erenumab, Galcanezumab, Tildrakizumab, Cemiplimab, Emapalumab, Fremanezumab, Ibalizumab, Moxetumomab pasudodox, Ravulizumab, Romosozumab, Risankizumab, Polatuzumab vedotin, Brolucizumab, or any combination thereof (see e.g., Lu et al., Development of therapeutic antibodies for the treatment of diseases. *Journal of Biomedical Science,* 2020). In some embodiments, cells described herein (e.g., cells modified using methods of the disclosure) are used in combination with one or more cancer treatment modalities that facilitate the induction of antibody dependent cellular cytotoxicity (ADCC), wherein the cancer treatment modality is an antibody or appropriate fragment thereof targeting CD20, TNFα, HER2, CD52, IgE, EGFR, VEGF-A, ITGA4, CTLA-4, CD30, VEGFR2, α4β7 integrin, CD19, CD3, PD-1, GD2, CD38, SLAMF7, PDGFRα, PD-L1, CD22, CD33, IFNγ, CD79β, or any combination thereof.

[0575] In some embodiments, cells described herein are utilized in combination with checkpoint inhibitors. Examples of suitable combination therapy checkpoint inhibitors include, but are not limited to, antagonists of PD-l (Pdcdl, CD279), PDL-l (CD274), TIM-3 (Havcr2), TIGIT (WUCAM and Vstm3), LAG-3 (Lag3, CD223), CTLA-4 (Ctla4, CD152), 2B4 (CD244), 4-1BB (CD137), 4-1BBL (CD137L), A2aR, BATE, BTLA, CD39 (Entpdl), CD47, CD73 (NT5E), CD94, CD96, CD160, CD200, CD200R, CD274, CEACAM1, CSF-1R, Foxpl, GARP, HVEM, IDO, EDO, TDO, LAIR-l, MICA/B, NR4A2, MAFB, OCT-2 (Pou2f2), retinoic acid receptor alpha (Rara), TLR3, VISTA, NKG2A/HLA-E, inhibitory KIR (for example, 2DL1, 2DL2, 2DL3, 3DL1, and3DL2), or any suitable combination thereof.

[0576] In some embodiments, the antagonist inhibiting any of the above checkpoint molecules is an antibody. In some embodiments, the checkpoint inhibitory antibodies may be murine antibodies, human antibodies, humanized antibodies, a camel Ig, a shark heavychain-only antibody (VNAR), Ig NAR, chimeric antibodies, recombinant antibodies, or antibody fragments thereof. Non-limiting examples of antibody fragments include Fab, Fab', F(ab)'2, F(ab)'3, Fv, single chain antigen binding fragments (scFv), (scFv)2, disulfide stabilized Fv (dsFv), minibody, diabody, triabody, tetrabody, single-domain antigen binding fragments (sdAb, Nanobody),

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recombinant heavy-chain-only antibody (VHH), and other antibody fragments that maintain the binding specificity of the whole antibody, which may be more cost-effective to produce, more easily used, or more sensitive than the whole antibody. In some embodiments, the one, or two, or three, or more checkpoint inhibitors comprise at least one of atezolizumab (anti-PDLl mAb), avelumab (anti-PDLl mAb), durvalumab (anti-PDLl mAb), tremelimumab (anti-CTLA4 mAb), ipilimumab (anti-CTLA4 mAb), IPH4102 (anti-KIR), IPH43 (anti-MICA), IPH33 (anti-TLR3), lirimumab (anti-KIR), monalizumab (anti-NKG2A), nivolumab (anti-PD1 mAb), pembrolizumab (anti -PD 1 mAb), and any derivatives, functional equivalents, or biosimilars thereof.

[0577] In some embodiments, the antagonist inhibiting any of the above checkpoint molecules is microRNA-based, as many miRNAs are found as regulators that control the expression of immune checkpoints (Dragomir et al., Cancer Biol Med. 2018, 15(2): 103-115). In some embodiments, the checkpoint antagonistic miRNAs include, but are not limited to, miR-28, miR-15/16, miR-138, miR-342, miR-20b, miR-21, miR-130b, miR-34a, miR-197, miR-200c, miR-200, miR-17-5p, miR-570, miR-424, miR-155, miR-574-3p, miR-513, miR-29c, and/or any suitable combination thereof.

[0578] In some embodiments, cells described herein (e.g., cells modified using methods of the disclosure) are used in combination with one or more cancer treatment modalities such as exogenous interleukin (IL) dosing. In some embodiments, an exogenous IL provided to a patient is IL-15. In some embodiments, systemic IL-15 dosing when used in combination with cells described herein is reduced when compared to standard dosing concentrations (see e.g., Waldmann et al., IL-15 in the Combination Immunotherapy of Cancer. *Front. Immunology,* 2020).

[0579] Other compounds that are effective in treating cancer are known in the art and described herein that are suitable for use with the compositions and methods of the present disclosure as additional cancer treatment modalities are described, for example, in the "Physicians' Desk Reference, 62nd edition. Oradell, N.J.: Medical Economics Co., 2008", Goodman & Gilman's "The Pharmacological Basis of Therapeutics, Eleventh Edition. McGraw-Hill, 2005", "Remington: The Science and Practice of Pharmacy, 20th Edition. Baltimore, Md.: Lippincott Williams & Wilkins, 2000," and "The Merck Index, Fourteenth Edition. Whitehouse

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Station, N.J.: Merck Research Laboratories, 2006", incorporated herein by reference in relevant parts.

[0580] All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

[0581] Throughout this specification, unless the context requires otherwise, the words "comprise", "comprises" and "comprising" will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements. By "consisting of is meant including, and limited to, whatever follows the phrase "consisting of:" Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory, and that no other elements may be present. By "consisting essentially of" is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase "consisting essentially" of indicates that the listed elements are required or mandatory, but that no other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

[0582] These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

[0583] The various embodiments described above can be combined to provide further embodiments. All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet are incorporated herein by reference, in their entirety. The contents of database entries, e.g., NCBI nucleotide or protein database entries provided herein, are incorporated herein in their entirety. Where database entries are subject to change over time, the contents as of the filing date of the present application are incorporated herein by reference. Aspects of the embodiments can be modified,

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if necessary to employ concepts of the various patents, applications and publications to provide yet further embodiments.

[0584] The disclosure is further illustrated by the following examples. The examples are provided for illustrative purposes only. They are not to be construed as limiting the scope or content of the disclosure in any way.

EXAMPLES

Example 1: Screening of Guide RNAs for *GAPDH*

[0585] This example describes the screening of AsCpf1 (AsCas12a) guide RNAs that target the housekeeping gene *GAPDH*. *GAPDH* encodes Glyceraldehyde-3-Phosphate Dehydrogenase, an essential protein that catalyzes oxidative phosphorylation of glyceraldehyde-3-phosphate in the presence of inorganic phosphate and nicotinamide adenine dinucleotide (NAD), an important energy-yielding step in carbohydrate metabolism. The guide RNAs used in this analysis were all 41-mer RNA molecules with the following design: 5′-UAAUUUCUACUCUUGUAGAU-[21-mer targeting domain sequence]-3′ (SEQ ID NO: 90). For example, the guide RNA denoted RSQ22337 had the following sequence: 5′-UAAUUUCUACUCUUGUAGAUAUCUUCUAGGUAUGACAACGA-3′ (SEQ ID NO: 93) where the 21-mer targeting domain sequence is underlined. The guide RNAs with the targeting domain sequences shown in Table 13 were tested to determine how effective they were at editing *GAPDH*. Cas12a RNPs (RNPs having an engineered Cas12a (SEQ ID NO: 62)), containing each of these guide RNAs were transfected into iPSCs, and then editing levels were assayed three days after transfection (see e.g., Wong, K.G. et al. CryoPause: A New Method to Immediately Initiate Experiments after Cryopreservation of Pluripotent Stem Cells. *Stem Cell Reports* 9, 355-365 (2017)). The results are shown in Fig. 1 and Fig. 2. RSQ24570, RSQ24582, RSQ24589, RSQ24585, and RSQ22337 exhibited the greatest levels of measurable editing out of the *GAPDH* guides tested, editing approximately 70% or more of cells (about 92%, 89%, 88%, 87%, and 70%, respectively). It was observed that cells transfected with gRNAs targeting certain exonic regions yielded much lower amounts of isolatable genomic DNA (gDNA) for analyzing editing efficiency (at day 3 after transfection) when compared to cells transfected with gRNAs targeting intronic regions, indicating that that RNPs with certain exon-targeting gRNAs

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were cytotoxic to the cells. This suggested that cells edited with gRNAs targeting exonic regions could result in significant cell death due to the introduction of indels within GAPDH leading to expression of a non-functional GAPDH protein or a protein with insufficient function. It was postulated that it might be possible to use a rescue plasmid to repair the gRNA-mediated cleavage site in GAPDH while also knocking in a gene cargo of interest in frame with the repaired GAPDH via HDR, thereby rescuing those cells in which GAPDH is repaired and the cargo of interest is successfully integrated (as shown in Fig. 1 and Fig. 2). Those transfected cells that are edited (the majority of transfected cells, if a highly effective RNA-guided nucleases is used) but do not undergo HDR repair of GAPDH and do not integrate the cargo of interest die over time because they do not have a functioning GAPDH gene. Those cells carrying the cargo of interest would have an advantage due to a fully functioning GAPDH gene as the cells grow and divide, and these cells would be selected for over time. The expected end result would be a population of cells with a very high rate of cargo knock-in within the GAPDH locus.

[0586] The data in Fig. 2 suggested that while Cas12a RNP comprising RSQ22337 resulted in an editing level of approximately 70% at 3 days post-transfection, it caused slightly higher levels of toxicity than other exonic guides (RSQ24570, RSQ24582, RSQ24589, and RSQ24585) (see Fig. 2, only about 3.9 ng/μL of gDNA was isolated from edited cells). Thus, the actual editing efficiency was very likely significantly higher than 70%, as many cells had already died by 3 days post-transfection due to the lack of available rescue constructs and NHEJ forming toxic indels. As a result, RSQ22337 was chosen for further testing.

Table 13: Guide RNA sequences

SEQ ID NO:	Name	gRNA targeting domain sequence (RNA)	Location
94	RSQ22336	UGAGCCAGCCACCAGAGGGCG	Intron 8
95	RSQ22337	AUCUUCUAGGUAUGACAACGA	Intron 8/ Exon 9 (cut site in exon 9)
96	RSQ22338	GCUACAGCAACAGGGUGGUGG	Exon 9
97	RSQ24559	CCAUAAUUUCCUUUCAAGGUG	Intron 7
98	RSQ24560	CUUUCAAGGUGGGGAGGGAGG	Intron 7
99	RSQ24561	AAGGUGGGGAGGGAGGUAGAG	Intron 7
100	RSQ24562	GCAGACCACAGUCCAUGCCAU	Exon 8
101	RSQ24563	CAGACCACAGUCCAUGCCAUC	Exon 8
102	RSQ24564	CCGGAGGGGCCAUCCACAGUC	Exon 8

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SEQ ID NO:	Name	gRNA targeting domain sequence (RNA)	Location
103	RSQ24565	UAGACGGCAGGUCAGGUCCAC	Exon 8
104	RSQ24566	CUAGACGGCAGGUCAGGUCCA	Exon 8
105	RSQ24567	UCUAGACGGCAGGUCAGGUCC	Exon 8
106	RSQ24568	GCAGGUUUUUCUAGACGGCAG	Exon 8
107	RSQ24569	UCAAGCUCAUUUCCUGGUAUG	Exon 8
108	RSQ24570	CUGGUAUGUGGCUGGGGCCAG	Exon 8/ Intron 8 (cut site in intron 8)
109	RSQ24571	AGAGCCAGUCUCUGGCCCCAG	Intron 8
110	RSQ24572	AAGAGCCAGUCUCUGGCCCCA	Intron 8
111	RSQ24573	UAAGAGCCAGUCUCUGGCCCC	Intron 8
112	RSQ24574	CUGAGCCAGCCACCAGAGGGC	Intron 8
113	RSQ24575	UCUGAGCCAGCCACCAGAGGG	Intron 8
114	RSQ24576	CAUCUUCUAGGUAUGACAACG	Exon 9
115	RSQ24578	UUGAUGGUACAUGACAAGGUG	1kb_downstream
116	RSQ24579	GAGGCCCUACCCUCAGUCUGA	1kb_downstream
117	RSQ24580	CCUCUCCUCGCUCCAGUCCUA	1kb_downstream
118	RSQ24581	CUCUCCUCGCUCCAGUCCUAG	1kb_downstream
119	RSQ24582	GCCAACAGCAGAUAGCCUAGG	1kb_downstream
120	RSQ24583	UGUGCCCUCGUGUCUUAUCUG	1kb_downstream
121	RSQ24584	CCUAGAUGAAUCCUGCUUGAA	1kb_downstream
122	RSQ24585	GGUACUUGGUUUACCUAGAUG	1kb_downstream
123	RSQ24586	AGGUACUUGGUUUACCUAGAU	1kb_downstream
124	RSQ24587	AAACAUUAUAUAGUCCUUACC	1kb_downstream
125	RSQ24588	UAAACAUUAUAUAGUCCUUAC	1kb_downstream
126	RSQ24589	CCGAUUUUUAAACAUUAUAUA	1kb_downstream
127	RSQ24590	ACCGAUUUUUAAACAUUAUAU	1kb_downstream
128	RSQ24591	UACCGAUUUUUAAACAUUAUA	1kb_downstream
129	RSQ24592	AAAAUCGGUAAAAAUGCCCAC	1kb_downstream
130	RSQ24593	GAGGAAGAUGAACUGAGAUGU	1kb_downstream
131	RSQ24594	AGGAAGAUGAACUGAGAUGUG	1kb_downstream

Example 2: Rescue of *GAPDH* Knock-out Through Targeted Integration

[0587] To test the feasibility of the exemplary selection system illustrated in Figs. 3A, 3B, and 3C, the essential gene *GAPDH* was targeted in iPSCs using an RNP comprising AsCpf1 (SEQ ID NO: 62), and a guide RNA (RSQ22337 (SEQ ID NO: 95)), resulting in a double-strand break towards the 5′ end of the last exon of *GAPDH* (exon 9). While iPSCs were tested for the

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purposes of this experiment, the described methods could be applied to other cell types. RSQ22337 was determined to be highly specific to *GAPDH* and have minimal off-target sites in the genome (data not shown). *GAPDH* was thus considered a good exemplary candidate target gene for the cargo integration and selection methods described herein, at least in part because there was at least one highly specific gRNA targeting a terminal exon capable of mediating highly efficient RNA-guided cleavage.

[0588] The CRISPR/Cas nuclease and guide RNA were introduced into cells by nucleofection (electroporation) of a ribonucleoprotein (RNP) according to known methods. The cells were also contacted with a double stranded DNA donor template (e.g., a dsDNA plasmid) that included a knock-in cassette comprising in 5′-to-3′ order, a 5′ homology arm approximately 500bp in length (comprising a portion of exon 8, intron 8, and a 5′ codon-optimized coding portion of exon 9 optimized to prevent further binding of the gRNA targeting domain sequence of the guide RNA (RSQ22337)), an in-frame sequence encoding the P2A self-cleaving peptide ("P2A"), an in-frame coding sequence for CD47 ("Cargo"), a stop codon and polyA signal sequence, and a 3′ homology arm approximately 500bp in length (comprising a coding portion of exon 9 including a stop codon, the 3′ exonic region of exon 9, and a portion of the downstream intergenic sequence) (as shown in Figure 3B). The 5′ and 3′ homology arms flanking the knock-in cassette were designed to correspond to sequences surrounding the RNP cleavage site.

[0589] As shown schematically in Fig. 3C, NHEJ-mediated creation of indels in cells that are edited by the DNA nuclease but not successfully targeted by the DNA donor template, produce a non-functional version of *GAPDH* which is lethal to the cells. This knock-out is "rescued" in cells that are successfully targeted by the DNA donor template by correct integration of the knock-in cassette, which restores the *GAPDH* coding region so that a functioning gene product is produced, and positions the P2A-Cargo sequence in frame with and downstream (3′) of the *GAPDH* coding sequence. These cells survive and continue to proliferate. Cells that are not edited by the DNA nuclease also continue to proliferate but are expected to represent a very small percentage of the overall cell population, if, as in this case, the editing efficiency of the nuclease in combination with the gRNA is high (see Example 1) and results in creation of a non-functional protein. The editing results for RSQ22337 likely

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underestimate the actual editing efficiency of the guide due to cell death within the population of edited cells.

[0590] The editing efficiency of RNPs containing RSQ22337 were tested at different concentrations (4 μM, 1 μM, 0.25 μM, or 0.0625 μM of RNP) in the absence of double stranded DNA donor template) was first measured at 48 after nucleofection of iPSCs (a time point prior to cell death due to loss of *GAPDH* gene function). The results show that a concentration of 4 μM resulted in the highest editing levels (see Fig. 4).

[0591] Figures 5 and 6 show that a protein-encoding cargo gene can be knocked into a housekeeping gene, such as *GAPDH*, at high efficiency using the selection systems described herein. Fig. 5 shows the knock-in (KI) efficiency of the CD47-encoding "cargo" in *GAPDH* at 4 days post-electroporation when RNP was present at a concentration of 4 μM and the dsDNA plasmid ("PLA") encoding CD47 was also present. Knock-in efficiency was measured with two different concentrations of the plasmid (0.5 μg and 2.5 μg of plasmid) and found to be dose responsive. Knock-in was measured using ddPCR targeting the 3′ position of the knock-in "cargo". Control cells electroporated with RNP alone or PLA alone exhibited much lower knock-in rates than electroporation of RNP and PLA (at a concentration of 2.5 μg).

[0592] Fig. 6 shows the knock-in efficiency of the CD47-encoding "cargo" in *GAPDH* at 9 days post-electroporation of the cells with the RNP and dsDNA plasmid encoding CD47. The percentage knock-in was similar when either the 5′ end or the 3′ end of the cargo was assayed by ddPCR, using a primer specific for the 5′ of the gRNA target site or 3′ of the site in the poly A region, increasing the reliability of the result. The knock-in efficiency of the cargo was significantly higher at 9 days compared to at 4 days post-transfection (compare Figs. 5 and 6), consistent with the expectation that there would be substantial cell death in RNP-induced *GAPDH* knock-out cells that lacked a functional *GAPDH* gene as a result of unsuccessful cargo knock-in and rescue at *GAPDH*.

[0593] An experiment was then conducted to test the mechanism of the selection system described above by confirming that edited cells containing a successfully knocked-in cargo gene would be more efficiently selected for using a gRNA targeting a protein-coding exonic portion of *GAPDH* rather than a gRNA targeting an intron. Fig. 7 compares the knock-in efficiency of a GFP-encoding "cargo" knock-in cassette at the GAPDH locus when using a gRNA that mediates

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cleavage within an intron (RSQ24570 (SEQ ID NO: 108) binds to the exon 8-intron 9 junction, leading to Cas12a-mediated cleavage within intron 8) relative to a gRNA specific for an exon (RSQ22337 (SEQ ID NO: 95), targeting the intron 8-exon 9 junction, leading to Cas12a-mediated cleavage within exon 9). Rescue dsDNA plasmid PLA1593 comprising the reporter "cargo" GFP was nucleofected into iPSCs with an RNP (Cas12a and RSQ22337) targeting *GAPDH* as described above, while dsDNA plasmid PLA1651 comprising a donor template sequence as depicted in SEQ ID NO: 46 was nucleofected with an RNP comprising Cas12a and RSQ24570. The homology arms of each plasmid were designed to mediate HDR based on the target site of each gRNA. Knock-in was visualized using microscopy (Fig. 7A) and was measured using flow cytometry (Fig. 7B). Knock-in efficiency was significantly higher when using a gRNA and associated knock-in cassette that cleaves at an exonic coding region (exon 9) when compared to an intronic region (intron 8). Fig. 7B shows that 95.6% of cells electroporated with RSQ22337 and the GFP-encoding "cargo" knock-in cassette (e.g., PLA1593; comprising donor template SEQ ID NO: 44) expressed GFP compared to only 2.1% of cells electroporated with RSQ24570 and a GFP-encoding "cargo" knock-in cassette (PLA1651; comprising donor template SEQ ID NO: 46). The results depicted in Figure 7 are striking, as while the measured editing efficiency (as determined by indel generation frequency 72 hours post-transfection as discussed above in Example 1, see Fig. 2) of RSQ24570 is higher than that of RSQ22337, the proportion of cells rescued by the knock-in construct targeting the coding exonic region are significantly higher.

[0594] In an additional set of experiments, iPS cells were contacted with an RNP containing AsCas12a (SEQ ID NO: 62), and RSQ22337 (SEQ ID NO: 95) or RSQ24570 (SEQ ID NO: 108), along with either the PLA1593 (comprising donor template SEQ ID NO: 44) or the PLA1651 (comprising donor template SEQ ID NO: 46) double stranded DNA donor template plasmid, respectively, as described above. Flow cytometry was performed 7 days following nucleofection to detect GFP expression and help determine to what extent each plasmid mediated donor template and knock-in cassette was integrated successfully at its respective *GAPDH* target site. The GAPDH results in Fig. 11A show that cells nucleofected with the RNP containing RSQ22337 exhibited a much higher amount of GFP expression relative to cells nucleofected with RSQ24750, showing that most cells express GFP at day 7 following electroporation. This

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suggests that the GFP-encoding knock-in cassette integrated successfully at high levels within the RSQ22337-transfected cells. Cells nucleofected with RNPs containing RSQ24750 displayed much lower GFP expression, indicating that the knock-in cassette did not integrate successfully in most of these cells (Fig. 11A). The *GAPDH* results of Fig. 11B show that use of RSQ22337 resulted in about 80% editing as measured using genomic DNA 48 hours following RNP transfection, while RSQ24570 resulted in about 75% editing as measured using genomic DNA 48 hours following RNP transfection. The high editing of RSQ22337 correlated well with the high GFP expression level depicted in Fig. 11A; however, the high editing of RSQ24750 correlated poorly with the low GFP expression level depicted in Fig. 11A. Fig. 11C and Fig. 11D (representing an additional experiment where RSQ22337 was again used for editing at the GAPDH locus) show the relative integrated "cargo" (GFP) expression intensity of the edited cells. Finally, a ddPCR assay was conducted to determine the percentage of knock-in integration events in *GAPDH* alleles in the cells nucleofected with RNPs containing RSQ22337 and the PLA1593 donor plasmid. Fig. 13 shows by ddPCR that over 60% of alleles had a GFP-encoding cassette knocked-in successfully.

Example 3: Rescue of *GAPDH* Knock-out Through Targeted Integration of Multiple Cargos

[0595] In some cases, it is desirable to use the selection and cargo knock in strategies disclosed herein to efficiently produce and isolate an edited cell containing two or more different exogenous coding sequences, e.g., two or more different exogenous genes, integrated into a single essential gene locus, such as, e.g., the GAPDH locus. Fig. 8 shows two strategies for introducing two or more different exogenous coding regions into an essential gene locus. Fig. 8A shows a first exemplary strategy wherein a multi-cistronic knock-in cassette, e.g., a bi-cistronic knock-in cassette containing two or more coding regions (GFP and mCherry in Fig. 8A), separated by linkers (e.g., T2A, P2A, and/or IRES; see SEQ ID NO: 29-32 and 33-37), is inserted into one or both of the alleles of the essential gene, e.g., GAPDH. Fig. 8B shows a second exemplary strategy (a bi-allelic insertion strategy) wherein two knock-in cassettes comprising different cargo sequences (e.g., different exogenous genes, such as GFP and mCherry in Fig. 8B) are inserted into separate alleles of the essential gene locus, e.g., GAPDH.

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[0596] Experiments were conducted to test the integration strategy depicted in Fig. 8A, and to determine whether the use of different combinations of linkers in the knock-in cassette could affect the expression of the cargo sequences. An RNP containing Cas12a and RSQ22337 (targeting the GAPDH locus, as described in Examples 1 and 2) was nucleofected into iPSCs with one of six different plasmids (PLA) containing a bi-cistronic knock-in cassette comprising "cargo" sequences encoding GFP and mCherry (PLA1573, PLA1574, PLA1575, PLA1582, PLA1583, and PLA1584, as depicted in Fig. 9A; comprising donor templates SEQ ID NOs: 38-43). GFP was the first cargo and mCherry was the second cargo in each of these constructs. Each of the tested plasmids contained a different combination of linkers between the coding sequences (Linkers 1 and 2, as depicted in Fig. 9A). PLA1573 (comprising donor template SEQ ID NO: 38) contained T2A and T2A as linkers 1 and 2, respectively; PLA1574 (comprising donor template SEQ ID NO: 39) contained P2A and IRES as linkers 1 and 2, respectively; PLA1575 (comprising donor template SEQ ID NO: 40) contained P2A and P2A as linkers 1 and 2, respectively; PLA1582 (comprising donor template SEQ ID NO: 41) contained P2A and T2A as linkers 1 and 2, respectively; PLA1583 (comprising donor template SEQ ID NO: 42) contained T2A and P2A as linkers 1 and 2, respectively; and PLA1584 (comprising donor template SEQ ID NO: 43) contained T2A and IRES as linkers 1 and 2, respectively. Fig. 9B and Fig. 9C shows the results of various knock-in cassette integration events at the GAPDH locus. Fig. 9B depicts exemplary microscopy images (brightfield and fluorescent microscopy at 2X on a Keyence microscope) of edited iPSCs nine days following nucleofection with exemplary plasmids PLA1582, PLA1583, and PLA1584, each of which exhibited detectable GFP and mCherry expression.

[0597] Fig. 9C quantifies the fluorescence levels of GFP and mCherry in the iPSCs nucleofected with the various plasmids described in Fig. 9A containing the bi-cistronic knock-in cassettes with the different described linker pairs (PLA1575, PLA1582, PLA1574, PLA1583, PLA1573, and PLA1584). In each of these bi-cistronic constructs, GFP was always the first cargo and mCherry was always the second cargo. A plasmid containing a knock-in cassette with mCherry as a sole "cargo" (as depicted in Fig. 9C) was also tested as a control. The data show that the expression levels of GFP, as the first cargo, were similar between bicistronic constructs and consistently higher than the expression levels of mCherry, the second cargo. Cells containing

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the control knock-in cassette containing mCherry as the sole cargo exhibited the highest mCherry expression, suggesting that it is possible to vary (e.g., reduce) expression of a cargo by placing it as the second cargo in a bicistronic cassette. In addition, Fig. 9C shows that placement of an IRES linker immediately prior to the second cargo coding sequence resulted in lower expression of the second cargo when compared to the placement of a P2A or T2A linker prior to the second cargo coding sequence. Thus, the results show that it is possible to differentially modulate (i.e., increase or decrease) the expression of two cargo coding sequences from a multicistronic knock-in cassette by varying the order of the cargos in the cassette (placing a cargo as the first cargo for higher expression, or as the second cargo for lower expression) and by placing particular linkers (P2A or T2A for higher expression; IRES for lower expression) upstream of each of the cargos.

[0598] An experiment was conducted to test the bi-allelic integration strategy depicted in Fig. 8B. An RNP containing Cas12a and RSQ22337 (targeting the GAPDH locus, as described in Examples 1 and 2) was nucleofected into iPSCs with two different plasmids. One plasmid contained a knock-in cassette containing a GFP coding sequence as the cargo, and the second plasmid contained a knock-in cassette containing an mCherry coding sequence as the cargo (as depicted in Fig. 8B). Fig. 10A shows exemplary flow cytometry data for the nucleofected iPSCs. Gating showed that a high percentage, approximately 15%, of the nucleofected cells expressed GFP and mCherry, suggesting that the GFP knock-in cassette and the mCherry knock-in cassette were each integrated into an allele of GAPDH. Approximately 41% of the nucleofected cells expressed mCherry and approximately 36% of the nucleofected cells expressed GFP.

[0599] An additional experiment was conducted to test biallelic insertion of GFP and mCherry in populations of iPSCs. The iPSC populations were transformed as described. The cells were nucleofected with 0.5 μM RNPs comprising Cas12a and RSQ22337 (targeting the *GAPDH* locus, as described in Examples 1 and 2), and 2.5 μg of donor template (5 trials) or 5 μg of donor template (1 trial), and then sorted 3 or 9 days following nucleofection. An exemplary image of the edited cell populations that were analyzed by flow cytometry analysis is depicted in Fig. 10B. Fig. 10C provides the flow cytometry analysis results from these trials. The larger bar at each time point (day 3 or day 9) in Fig. 10C represents the total percentage of the cells in each

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population that positively express at least one cargo, e.g., at least one allele of GFP and/or at least one allele of mCherry cargo. The smaller bar at each time point shows the percentage of cells in each population that express both GFP and mCherry and therefore represents cells with GFP/mCherry biallelic integration. These results showed that approximately 8-15% percent of the transformed cells in each population displayed a biallelic GFP/mCherry insertion phenotype at nine days following transformation.

Example 4: Rescue of *B2M* Knock-out Through Targeted Integration

[0600] The approach described in Example 2 is used to target the *B2M* gene in NK cells (e.g., by targeting NK cells such as iPS-derived NK cells directly or iPS cells that are then differentiated into NK cells). NK cells that lack a functional *B2M* gene will not be able to recognize MHC Class I on the surface of one another and will attack each other, depleting the population in a phenomenon known as fratricide. By knocking-out the *B2M* gene and knocking-in a "cargo" sequence that also restores a functional *B2M* gene one automatically enriches for the knock-in cell type.

Example 5: Rescue of *TBP* Knock-out Through Targeted Integration

[0601] The knock-in integration and selection approach described in Example 2 was used to target the *TBP* gene in iPSCs. While iPSCs were tested for the purposes of this experiment, the described methods could be applied to other cell types. The *TBP* gene encodes TATA-box binding protein, a transcriptional regulator that plays a key role in the transcription initiation apparatus. AsCpf1 (AsCas12a) guide RNAs that target terminal exons of the *TBP* gene are shown in Table 14 below. The guide RNAs are all 41-mer RNA molecules with the following design: 5′-UAAUUUCUACUCUUGUAGAU-[21-mer targeting domain sequence]-3′ (SEQ ID NO: 90).

Table 14: Guide RNA sequences

Name	Target Site	gRNA targeting domain sequence (RNA)	Location	Plasmid
TBP-1	RSQ33502 (SEQ ID NO: 148)	AAAUGCUUCAUAAAUUUCUGC	Isoform 1 exon 8; isoform 2 exon 7	PLA1615

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Name	Target Site	gRNA targeting domain sequence (RNA)	Location	Plasmid
TBP-2	RSQ33503 (SEQ ID NO: 149)	UGCUCUGACUUUAGCACCUAA	Isoform 1 exon 8; isoform 2 exon 7	PLA1616
TBP-3	RSQ33504 (SEQ ID NO: 150)	AAAACAUCUACCCUAUUCUAA	Isoform 1 exon 8; isoform 2 exon 7	PLA1617

[0602] RSQ33502, RSQ33503, and RSQ33504 (SEQ ID NO: 148-150) described in Table 14 were each determined to be highly specific to *TBP* and have minimal off-target sites in the genome (data not shown). The *TBP* gene was thus considered a good candidate gene target for the cargo integration and selection methods described herein at least in part because there are gRNAs available capable of very specifically targeting a terminal exon (mRNA isoform 1 exon 8, or mRNA isoform 2 exon 7 respectively). However, for any of these gRNAs to be highly suitable for the methods described herein, they need to be highly effective at introducing indels at a location in the *TBP* locus that would knock out and/or severely reduce gene function.

[0603] Each of these gRNAs was then tested to determine whether it could be used to knock-in a cassette comprising a portion of *TBP* and an in-frame cargo sequence encoding GFP into a terminal exon of the *TBP* gene of cells, in the process rescuing the lethal phenotype that would otherwise result by introducing RNP-induced indels into the coding region of this essential gene. If the tested gRNA was effective at introducing indels at a location of TBP important for function at a high frequency, then transfected cells that do not undergo HDR to incorporate the knock-in cassette would be expected to die, resulting in a large population of the cells expressing GFP from the *TBP* locus. Specifically, iPSC cells were contacted with an RNP containing AsCas12a (SEQ ID NO: 62), and RSQ33502, RSQ33503 or RSQ33504 (SEQ ID NOs: 148-150), along with a double stranded DNA donor template (dsDNA plasmid) designed to mediate HDR at each respective gRNA target binding site. The double stranded DNA donor templates included a knock-in cassette with a coding sequence for GFP ("Cargo") in frame with and downstream (3′) of a codon optimized version of a portion of the final *TBP* exon coding sequence (mRNA isoform 1 exon 8, or mRNA isoform 2 exon 7 respectively) and a sequence encoding the P2A self-cleaving peptide ("P2A"), similar to the dsDNA plasmid described in Example 2 for GAPDH. The *TBP* sequence in the double stranded DNA donor templates

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(PLA1615, PLA1616, or PLA1617; comprising donor template SEQ ID NOs: 47, 49, or 50) was codon optimized to prevent further binding by the accompanying guide RNA molecule (RSQ33502, RSQ33503 or RSQ33504). The knock-in cassette also included 3′ UTR and polyA signal sequences downstream of the Cargo sequence. An RNP containing RSQ33502 was administered with PLA1615 (comprising donor template SEQ ID NO: 47); RSQ33503 was administered with PLA1616 (comprising donor template SEQ ID NO: 49); and RSQ33504 was administered with PLA1617 (comprising donor template SEQ ID NO: 50). Each particular dsDNA plasmid (PLA) contained a donor template with homology arms and a knock-in cassette designed to specifically encompass and render ineffective the particular gRNA target site following knock-in cassette integration.

[0604] Flow cytometry was performed 7 days following nucleofection and was used to help determine to what extent each plasmid based knock-in cassette was integrated successfully at its respective *TBP* target site. Fig. 11A shows that cells nucleofected with RNPs containing RSQ33503 exhibited the greatest amounts of GFP expression relative to cells nucleofected with the other RNPs, suggesting that the GFP-encoding knock-in cassette integrated successfully at high levels within these cells. Fig. 12 shows that approximately 76% of the cells nucleofected with RNPs containing RSQ33503 (SEQ ID NO: 149) and the PLA1616 (comprising donor template SEQ ID NO: 49) plasmid expressed GFP compared to only about 1% of cells nucleofected with the PLA1616 plasmid alone (no RNP control). Cells nucleofected with RNPs containing RSQ33504 (SEQ ID NO: 150) also exhibited high levels of GFP expression, also suggesting higher knock-in cassette integration levels (Fig. 11A). Cells nucleofected with RNPs containing RSQ33502 (SEQ ID NO: 148) displayed much lower GFP expression, indicating that the knock-in cassette did not integrate successfully in most of these cells (Fig. 11A). Fig. 11B shows that use of the RNP containing RSQ33503 (SEQ ID NO: 149) resulted in about 80% editing, which correlated with the higher GFP expression level depicted in Fig. 11A. The percentage editing was measured two days following transfection and was determined by ICE assays (as described in Hsiau et al., Inference of CRISPR Edits from Sanger Trace Data. BioRxiv, 251082, August 2019). Use of the RNP containing RSQ33502 (SEQ ID NO: 148) resulted in a relatively low editing percentage, which correlated with the low GFP expression in Fig. 11A. Fig. 11C and Fig. 11D (representing an additional experiment where RSQ33503 was

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again used for editing at the TBP locus) show the relative integrated "cargo" (GFP) expression intensity of the edited cells. Finally, a ddPCR assay was conducted to determine the percent knock-in of the GFP cargo into the *TBP* alleles of the cells nucleofected with RNPs containing RSQ33503 (SEQ ID NO: 149) and the PLA1616 donor plasmid (comprising donor template SEQ ID NO: 49). Fig. 13 shows by ddPCR that over 40% of the *TBP* alleles had the GFP-encoding cassette successfully knocked-in.

Example 6: Rescue of *E2F4* Knock-out Through Targeted Integration

[0605] The knock-in integration and selection approach described in Example 2 was used to target the *E2F4* gene in iPSCs. While iPSCs were tested for the purposes of this experiment, the described methods could be applied to other cell types. The *E2F4* gene encodes E2F Transcription Factor 4. This transcriptional regulator plays a key role in cell cycle regulation. AsCpf1 (AsCas12a) guide RNAs that target terminal exons of the *E2F4* gene are shown in Table 15 below. The guide RNAs are all 41-mer RNA molecules with the following design: 5′-UAAUUUCUACUCUUGUAGAU-[21-mer targeting domain sequence]-3′ (SEQ ID NO: 90).

Table 15: Guide RNA sequences

Name	Target Site	gRNA targeting domain sequence (RNA)	Location	Plasmid
E2F4-1	RSQ33505 (SEQ ID NO: 151)	CCCCUCUGCUUCGUCUUUCUC	Exon 10	PLA1626
E2F4-2	RSQ33506 (SEQ ID NO: 152)	UCCACCCCCGGGAGACCACGA	Exon 10	PLA1627
E2F4-3	RSQ33507 (SEQ ID NO: 153)	AUGUGCCUGUUCUCAACCUCU	Exon 10	PLA1628

[0606] RSQ33505, RSQ33506, and RSQ33507 (SEQ ID NOs: 151-153) were each determined to be highly specific to *E2F4* and have minimal off-target sites in the genome (data not shown). The *E2F4* gene was thus considered a good candidate gene target for the cargo integration and selection methods described herein at least in part because there are gRNAs available that are capable of very specifically targeting a terminal exon (exon 10). However, for any of these gRNAs to be highly suitable for the methods described herein, they need to be highly effective at introducing indels at a location in the *E2F4* locus that would knock out or severely reduce gene function.

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[0607] The gRNAs RSQ33505, RSQ33506, and RSQ33507 (SEQ ID NOs: 151-153) were then tested to determine whether they could be used to knock-in a cassette comprising a portion of *E2F4* and a cargo sequence encoding GFP into a terminal exon of the *E2F4* locus of cells, in the process rescuing the lethal phenotype that would otherwise result by introducing RNP-induced indels into the coding region of this essential gene at a high frequency. Specifically, iPSCs were contacted with an RNP containing AsCas12a (SEQ ID NO: 62), and RSQ33505, RSQ33506, or RSQ33507 (SEQ ID NOs: 151-153) along with a double stranded DNA donor template (dsDNA plasmid) designed to mediate HDR at each respective gRNA target binding site. The double stranded DNA donor templates included a knock-in cassette with a coding sequence for GFP ("Cargo") in frame with and downstream (3′) of a codon optimized version of the final *E2F4* exon coding sequence (exon 10) and a sequence encoding the P2A self-cleaving peptide ("P2A"), similar to the dsDNA plasmid described in Example 2 for GAPDH. The *E2F4* sequence in the double stranded DNA donor templates (PLA1626, PLA1627, or PLA1628; comprising donor template SEQ ID NOs: 52-54) was codon optimized to prevent further binding by the accompanying guide RNA molecule (RSQ33505, RSQ33506 or RSQ33507; SEQ ID NOs: 151-153). The knock-in cassette also included 3′ UTR and polyA signal sequences downstream of the Cargo sequence. An RNP containing RSQ33505 (SEQ ID NO: 151) was administered with PLA1626 (comprising donor template SEQ ID NO: 52); RSQ33506 (SEQ ID NO: 152) was administered with PLA1627 (comprising donor template SEQ ID NO: 53); and RSQ33507 (SEQ ID NO: 153) was administered with PLA1628 (comprising donor template SEQ ID NO: 54). Each particular dsDNA plasmid (PLA) contained a donor template with homology arms and a knock-in cassette designed to specifically encompass and render ineffective the particular gRNA target site following integration.

[0608] Flow cytometry was performed 7 days following nucleofection and was used to help determine to what extent each plasmid based knock-in cassette was integrated successfully at its respective *E2F4* target site. Fig. 11A shows that cells nucleofected with RNPs containing RSQ33505 (SEQ ID NO: 151) exhibited the greatest amount of GFP expression relative to cells nucleofected with the other RNPs targeting *E2F4*, suggesting that the GFP-encoding knock-in cassette integrated successfully in many of these cells. Cells nucleofected with RNPs containing RSQ33506 or RSQ33507 (SEQ ID NOs: 152 and 153) displayed much lower GFP expression,

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indicating that the knock-in cassette did not integrate successfully in most of these cells (Fig. 11A). Fig. 11B shows that use of RNP containing RSQ33505 (SEQ ID NO: 151) or RSQ33506 (SEQ ID NO: 152) resulted in approximately 15% and approximately 20% editing rates respectively, when measured 48 hours after RNP transfection. The relatively lower observed editing rate for RSQ33505 (SEQ ID NO: 151) may be considered to unexpectedly correlate with a relatively high level of GFP integration in *E2F4* (as observed in Fig. 11A), and could partially be the result of significant death within the population of edited cells at 48 hours. The percentage editing was measured two days following transfection and was determined by ICE assays (as described in Hsiau et al., August 2019). Fig. 11C shows the relative integrated "cargo" (GFP) expression intensity of the edited cells.

Example 7: Rescue of *G6PD* Knock-out Through Targeted Integration

[0609] The knock-in integration and selection approach described in Example 2 was used to target the *G6PD* gene in iPSCs. While iPSCs were tested for the purposes of this experiment, the described methods could be applied to other cell types. The *G6PD* gene encodes Glucose-6-Phosphate Dehydrogenase. This metabolic enzyme plays a key role in glycolysis and NADPH production. An AsCpf1 (AsCas12a) guide RNA that targets terminal exons of the *G6PD* gene is shown in Table 16 below.

Table 16: Guide RNA sequences

Name	Target Site	gRNA targeting domain sequence (RNA)	Location	Plasmid
G6PD-1	RSQ33508 (SEQ ID NO: 154)	CAGUAUGAGGGCACCUACAAG	Exon 13	PLA1618

[0610] RSQ33508 (SEQ ID NO: 154) was determined to be highly specific to *G6PD* and has minimal off-target sites in the genome (data not shown). The *G6PD* gene was thus considered a good candidate gene target for the cargo integration and selection methods described herein at least in part because there are gRNAs available that are capable of specifically targeting a terminal exon (exon 13).

[0611] The gRNA RSQ33508 (SEQ ID NO: 154) was then tested to determine whether it could be used to knock-in a cassette comprising a portion of *G6PD* and a cargo sequence encoding GFP into a terminal exon of the *G6PD* locus of cells, in the process rescuing the lethal

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phenotype that would otherwise result by introducing RNP-induced indels into the coding region of this essential gene at a high frequency. Specifically, iPSCs were contacted with an RNP containing AsCas12a (SEQ ID NO: 62), and RSQ33508 (SEQ ID NO: 154) along with a double stranded DNA donor template (dsDNA plasmid) designed to mediate HDR at the gRNA target binding site. The double stranded DNA donor templates included a knock-in cassette with a coding sequence for GFP ("Cargo") in frame with and downstream (3′) of a codon optimized version of the final *G6PD* exon coding sequence (exon 13) and a sequence encoding the P2A self-cleaving peptide ("P2A"), similar to the dsDNA plasmid described in Example 2 for GAPDH. The *G6PD* sequence in the double stranded DNA donor templates (PLA1618; comprising donor template SEQ ID NO: 51) was codon optimized to prevent further binding by the accompanying guide RNA molecule (RSQ33508). The knock-in cassette also included 3′ UTR and polyA signal sequences downstream of the Cargo sequence. An RNP containing RSQ33508 (SEQ ID NO: 154) was administered with PLA1618 (comprising donor template SEQ ID NO: 51). The dsDNA plasmid (PLA) contained a donor template with homology arms and a knock-in cassette designed to specifically encompass and render ineffective the accompanying gRNA target site following integration.

[0612] Flow cytometry was performed 7 days following nucleofection and was used to help determine to what extent the plasmid based knock-in cassette was integrated successfully at its *G6PD* target site. Fig. 11A shows that cells nucleofected with RNPs containing RSQ33508 (SEQ ID NO: 154) exhibited GFP expression in approximately 10% of assayed cells, suggesting that the GFP-encoding knock-in cassette integrated at relatively low levels within these cells. Fig. 11C shows the relative integrated "cargo" (GFP) expression intensity of the edited cells.

Example 8: Rescue of *KIF11* Knock-out Through Targeted Integration

[0613] The knock-in integration and selection approach described in Example 2 was used to target the *KIF11* gene in iPSCs. While iPSCs were tested for the purposes of this experiment, the described methods could be applied to other cell types. The *KIF11* gene encodes Kinesin Family Member 11. This enzyme plays a key role in vesicle movement along intracellular microtubules and chromosome positioning during mitosis. AsCpf1 (AsCas12a) guide RNAs that target terminal exons of the *KIF11* gene are shown in Table 17 below.

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Table 17: Guide RNA sequences

Name	Target Site	gRNA targeting domain sequence (RNA)	Location	Plasmid
KIF11-1	RSQ33509 (SEQ ID NO: 155)	CCGCCUUAAAUCCACAGCAUA	Intron 21 / Exon 22	PLA1629
KIF11-2	RSQ33510 (SEQ ID NO: 156)	UAACCAAGUGCUCUGUAGUUU	Exon 22	PLA1630
KIF11-3	RSQ33511 (SEQ ID NO: 157)	GACCUCUCCAGUGUGUUAAUG	Exon 22	PLA1631

[0614] RSQ33509, RSQ33510, and RSQ33511 (SEQ ID NOs: 155-157) were each determined to be highly specific to *KIF11* and have minimal off-target sites in the genome (data not shown). The *KIF11* gene was thus considered a good candidate gene target for the cargo integration and selection methods described herein at least in part because there are gRNAs available that are capable of very specifically targeting a terminal exon available (exon 22). However, for any of these gRNAs to be highly suitable for the methods described herein, they need to be highly effective at introducing indels at a location in the *KIF11* locus that would knock out or severely reduce gene function.

[0615] Each of these gRNAs was then tested to determine whether it could be used to knock-in a cassette comprising a portion of *KIF11* and a cargo sequence encoding GFP into a terminal exon of the *KIF11* locus of cells, in the process rescuing the lethal phenotype that would otherwise result by introducing RNP-induced indels into the coding region of this essential gene at a high frequency. Specifically, iPSC cells were contacted with an RNP containing AsCas12a (SEQ ID NO: 62), and RSQ33509, RSQ33510, or RSQ33511 (SEQ ID NOs: 155-157), along with a double stranded DNA donor template (dsDNA plasmid) designed to mediate HDR at each respective gRNA target binding site. The double stranded DNA donor templates included a knock-in cassette with a coding sequence for GFP ("Cargo") in frame with and downstream (3′) of a codon optimized version of the final *KIF11* exon coding sequence (exon 22) and a sequence encoding the P2A self-cleaving peptide ("P2A"), similar to the dsDNA plasmid described in Example 2 for GAPDH. The *KIF11* sequence in the double stranded DNA donor templates (PLA1629, PLA1630, or PLA1631; comprising donor template SEQ ID NOs: 55-57) was codon optimized to prevent further binding by the accompanying guide RNA molecule (RSQ33509, RSQ33510, or RSQ33511; SEQ ID NOs: 155-157). The knock-in cassette also included 3′ UTR

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and polyA signal sequences downstream of the Cargo sequence. An RNP containing RSQ33509 (SEQ ID NO: 155) was administered with the PLA1629 plasmid (comprising donor template SEQ ID NO: 55); RSQ33510 (SEQ ID NO: 156) was administered with PLA1630 (comprising donor template SEQ ID NO: 56); and RSQ33511 (SEQ ID NO: 157) was administered with PLA1631 (comprising donor template SEQ ID NO: 57). Each particular dsDNA plasmid (PLA) contained a donor template with homology arms and a knock-in cassette designed to specifically encompass and render ineffective the particular gRNA target site following integration.

[0616] Flow cytometry was performed 7 days following nucleofection and was used to help determine to what extent each plasmid knock-in cassette was integrated successfully at its respective *KIF11* target site. Fig. 11A shows that cells nucleofected with RNPs containing RSQ33509 (SEQ ID NO: 155) exhibited the greatest amount of GFP expression relative to cells nucleofected with the other RNPs targeting *KIF11*, suggesting that the GFP-encoding knock-in cassette integrated successfully in many of these cells. Cells nucleofected with RNPs containing RSQ33510 or RSQ33511 (SEQ ID NO: 156 or 157) also exhibited some GFP expression (Fig. 11A). Fig. 11B shows that use of the RNPs containing RSQ33509 (SEQ ID NO: 155) resulted in about 40% editing at 48 hours following transfection (the lower level possibly a result of significant cell death in the cell population at this time), correlating with the GFP expression levels depicted in Fig. 11A. Interestingly, Fig. 11B shows that use of RNPs containing RSQ33510 (SEQ ID NO: 156) resulted in about 90% observed editing rates, while RNPs containing RSQ33511 (SEQ ID NO: 157) resulted in about 65% observed editing rates, yet the GFP expression in cells transfected with these guides was relatively low when compared to RSQ33509 (SEQ ID NO: 155) transfected cells. These results suggest that the RSQ33510 or RSQ33511 (SEQ ID NO: 156 or 157) guides may not have been generating sufficiently deleterious indels in *KIF11*, allowing a high proportion of cells to be viable despite high editing efficiencies, such that transfected cells were not dying in large enough numbers to allow for effective selection of transfected cells with successful cargo knocked in. Thus, although the RSQ33510 and RSQ33511 (SEQ ID NO: 156 or 157) gRNAs are highly specific for their *KIF11* target sites (with minimal off-targets) and exhibit high editing levels, they may still not be suitable gRNAs for the selection mechanisms described herein as they may not induce toxic indels that result in sufficient malfunction of *KIF11*, which in turn would lead to cell death if

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homologous recombination of a rescue knock-in cassette does not occur. The percentage editing was measured two days following transfection and was determined by ICE assays (as described in Hsiau et al., August 2019). Fig. 11C and Fig. 11D (representing an additional experiment where RSQ33509 was again used for editing at the KIF11 locus) show the relative integrated "cargo" (GFP) expression intensity of the edited cells.

Example 9: Knock-in of Cargo at Essential Gene Loci Using a Viral Vector

[0617] The present example describes use of the gene editing methods described herein comprising viral vector transduction of a cell population.

[0618] The target cells described herein are collected from a donor subject or a subject in need to therapy (e.g., a patient). Following an appropriate sorting, culturing, and/or differentiation process, target cells are transduced with at least one AAV vector comprising a nucleotide sequence comprising a gRNA, a suitable nuclease, and/or a suitable rescue construct. Cells are sorted using flow cytometry to determine successful transduction, editing, integration, and/or expression events.

[0619] A population of hematopoietic stem cells are transduced with an AAV vector (e.g., AAV6) comprising *GAPDH* targeting RNP (including Cas12a of SEQ ID NO: 62 and gRNA RSQ22337 of SEQ ID NO: 95) and PLA1593 (comprising donor template SEQ ID NO: 44). Successful transduction, editing, knock-in cassette integration, and/or expression events are determined using flow cytometry, as described herein. Following AAV transduction, a large proportion of the cells are edited at the *GAPDH* locus by the RNP and have integrated the knock-in cassette via HDR. A population of hematopoietic stem cells are transduced with an AAV vector (e.g., AAV6) comprising *TBP* targeting RNP (including Cas12a of SEQ ID NO: 62 and gRNA RSQ33503 of SEQ ID NO: 149) and PLA1616 (comprising donor template SEQ ID NO: 49). Successful transduction, editing, integration, and/or expression events are determined using flow cytometry, as described herein. Following AAV transduction, a large proportion of the cells are edited at the *TBP* locus by the RNP and have integrated the knock-in cassette via HDR.

[0620] A population of T cells are transduced with an AAV vector (e.g., AAV6) comprising *GAPDH* targeting RNP (including Cas12a of SEQ ID NO: 62 and gRNA RSQ22337 of SEQ ID NO: 95) and PLA1593 (comprising donor template SEQ ID NO: 44). Successful

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transduction, editing, knock-in cassette integration, and/or expression events are determined using flow cytometry, as described herein. Following AAV transduction, a large proportion of the cells are edited at the *GAPDH* locus by the RNP and have integrated the knock-in cassette via HDR. A population of T cells are transduced with an AAV vector (e.g., AAV6) comprising *TBP* targeting RNP (including Cas12a of SEQ ID NO: 62 and gRNA RSQ33503 of SEQ ID NO: 149) and PLA1616 (comprising donor template SEQ ID NO: 49). Successful transduction, editing, integration, and/or expression events are determined using flow cytometry, as described herein. Following AAV transduction, a large proportion of the cells are edited at the *TBP* locus by the RNP and have integrated the knock-in cassette via HDR.

[0621] A population of NK cells are transduced with an AAV vector (e.g., AAV6) comprising *GAPDH* targeting RNP (including Cas12a of SEQ ID NO: 62 and gRNA RSQ22337 of SEQ ID NO: 95) and PLA1593 (comprising donor template SEQ ID NO: 44). Successful transduction, editing, knock-in cassette integration, and/or expression events are determined using flow cytometry, as described herein. Following AAV transduction, a large proportion of the cells are edited at the *GAPDH* locus by the RNP and have integrated the knock-in cassette via HDR. A population of NK cells are transduced with an AAV vector (e.g., AAV6) comprising *TBP* targeting RNP (including Cas12a of SEQ ID NO: 62 and gRNA RSQ33503 of SEQ ID NO: 149) and PLA1616 (comprising donor template SEQ ID NO: 49). Successful transduction, editing, knock-in cassette integration, and/or expression events are determined using flow cytometry, as described herein. Following AAV transduction, a large proportion of the cells are edited at the *TBP* locus by the RNP and have integrated the knock-in cassette via HDR.

[0622] A population of tumor-infiltrating lymphocytes (TILs) are transduced with an AAV vector (e.g., AAV6) comprising *GAPDH* targeting RNP (including Cas12a of SEQ ID NO: 62 and gRNA RSQ22337 of SEQ ID NO: 95) and PLA1593 (comprising donor template SEQ ID NO: 44). Successful transduction, editing, knock-in cassette integration, and/or expression events are determined using flow cytometry, as described herein. Following AAV transduction, a large proportion of the cells are edited at the *GAPDH* locus by the RNP and have integrated the knock-in cassette via HDR. A population of tumor-infiltrating lymphocytes (TILs) are transduced with an AAV vector (e.g., AAV6) comprising *TBP* targeting RNP (including Cas12a

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of SEQ ID NO: 62 and gRNA RSQ33503 of SEQ ID NO: 149) and PLA1616 (comprising donor template SEQ ID NO: 49). Successful transduction, editing, knock-in cassette integration, and/or expression events are determined using flow cytometry, as described herein. Following AAV transduction, a large proportion of the cells are edited at the *TBP* locus by the RNP and have integrated the knock-in cassette via HDR.

[0623] A population of neurons are transduced with an AAV vector (e.g., AAV6) comprising *GAPDH* targeting RNP (including Cas12a of SEQ ID NO: 62 and gRNA RSQ22337 of SEQ ID NO: 95) and PLA1593 (comprising donor template SEQ ID NO: 44). Successful transduction, editing, knock-in cassette integration, and/or expression events are determined using flow cytometry, as described herein. Following AAV transduction, a large proportion of the cells are edited at the *GAPDH* locus by the RNP and have integrated the knock-in cassette via HDR. A population of neurons are transduced with an AAV vector (e.g., AAV6) comprising *TBP* targeting RNP (including Cas12a of SEQ ID NO: 62 and gRNA RSQ33503 of SEQ ID NO: 149) and PLA1616 (comprising donor template SEQ ID NO: 49). Successful transduction, editing, knock-in cassette integration, and/or expression events are determined using flow cytometry, as described herein. Following AAV transduction, a large proportion of the cells are edited at the *TBP* locus by the RNP and have integrated the knock-in cassette via HDR.

Example 10: Knock-in of Cargo at Essential Gene Loci Using a Viral Vector

[0624] The present example describes gene editing of populations of T cells using methods described herein comprising viral vector transduction of populations of T cells. The methods described herein can be applied to other cell types as well, such as other immune cells.

[0625] T cells were thawed in a bead bath as known in the art and were removed from the bath on day two. Cells were electroporated on day four after thawing, in brief 250,000 T cells per well in a Lonza 96-well cuvette were suspended in buffer P2 and electroporated using pulse code CA-137 with varying concentrations of RNP comprising gRNA RSQ22337 (SEQ ID NO: 95) and Cas12a (SEQ ID NO: 62) targeting the *GAPDH* gene (4μM RNP, 2μM RNP, 1μM RNP, or 0.5μM RNP). Appropriate media was added to cells immediately after electroporation and cells were allowed to recover for 15 minutes. AAV6 viral particles comprising a donor plasmid construct containing a knock-in cassette with a GFP cargo were then added to T cells at varying

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multiplicity of infection (MOI) concentrations (5E4, 2.5E4, 1.25E4, 6.25E3, 3.13E3, 1.56E3, or 7.81E2). The donor plasmid was designed as described in Example 2, with a 5′ codon-optimized coding portion of GAPDH exon 9 optimized to prevent further binding of the gRNA targeting domain sequence of the guide RNA (RSQ22337)), an in-frame sequence encoding the P2A self-cleaving peptide ("P2A"), an in-frame coding sequence for GFP ("Cargo"), a stop codon and polyA signal sequence. T cells were split two days later, and then every 48 hours until they were analyzed by flow cytometry. T cells were sorted using flow cytometry seven days post electroporation to determine successful transduction, transformation, editing, knock-in cassette integration, and/or expression events (see Fig. 14, Fig 15, Fig. 16A, and Fig. 16B). As shown in Fig. 14, populations of T cells were transduced with 4 μM RNP, 2 μM RNP, 1 μM RNP, or 0.5 μM RNP, at various AAV6 multiplicity of infection (MOI) (5E4, 2.5E4, 1.25E4, 6.25E3, 3.13E3, 1.56E3, or 7.81E2). High proportions of GFP integration at the *GAPDH* gene were observed in T cell populations transduced/transformed with all RNP concentrations at 5E4 AAV6 MOI and were observed with RNP concentrations greater than 1 μM when cells were transduced with AAV6 MOI as low as 1.25E4 (see Fig. 14 and 16A). Control experiments with no AAV transduction resulted in T cell populations that displayed no GFP integration events (see Fig. 16B). T cell viability was measured four days after cells were transformed with RNPs and AAV6 at various MOI (Fig. 15).

[0626] Furthermore, knock-in efficiencies using methods described herein were compared to optimized versions of methods known in the art. In brief, T cell populations were transduced with AAV6 vector comprising a donor template suitable for knock-in of GFP at the *GAPDH* gene as described herein, and were transformed with gRNA RSQ22337 (SEQ ID NO: 95) and Cas12a (SEQ ID NO: 62) as described above; alternatively, T cell populations were subject to highly optimized GFP knock-in at the TRAC locus using AAV6 vector transduction (see e.g., Vakulskas et al. A high-fidelity Cas9 mutant delivered as a ribonucleoprotein complex enables efficient gene editing in human hematopoietic stem and progenitor cells. Nat Med. 2018;24(8):1216-1224). Flow cytometry was utilized to measure knock-in efficiency (determined by percentage of T cell population expressing GFP, measured 7 days post-electroporation). Knock-in rates at the TRAC locus were high (~50%) when compared to publicly described integration frequencies for similar methodologies, however, knock-in

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efficiency at the *GAPDH* gene using methods described herein facilitated by AAV6 transduction were significantly ($p = 0.0022$ using unpaired t-test) higher (~68%) (see Fig. 17A). The same RNP concentration, AAV6 MOI, and homology arm lengths were utilized in both experiments, averaged results from three independent biological replicates are shown (see Fig. 17A). Thus, the methods described herein can be used to isolate a population of modified cells, such as immune cells like T cells, that highly express a gene of interest relative to other gene knock-in methods.

Example 11: CD16 Knock-In iPSCs Give Rise To Edited iNKs With Enhanced Function

[0627] The present example describes use of gene editing methods described herein to create modified immune cells suitable for killing cancer cells.

[0628] PSCs were edited using the exemplary system illustrated in Figs. 3A, 3B, and 3C, and described in Example 2. In brief, the *GAPDH* gene was targeted in iPSCs using AsCpf1 (SEQ ID NO: 62), and a guide RNA (RSQ22337) (SEQ ID NO: 95), resulting in a double-strand break towards the 5′ end of the last exon of *GAPDH* (exon 9). The CRISPR/Cas nuclease and guide RNA were introduced into cells by nucleofection (electroporation) of a ribonucleoprotein (RNP) according to known methods. The cells were also contacted with a double stranded DNA donor template (dsDNA plasmid, comprising donor template SEQ ID NO: 205) that included a donor template comprising in 5′-to-3′ order, a 5′ homology arm approximately 500bp in length (comprising a 3′ portion of exon 8, intron 8, and a 5′ codon-optimized coding portion of exon 9 optimized to prevent further binding of the gRNA targeting domain sequence of the guide RNA (RSQ22337)), an in-frame sequence encoding the P2A self-cleaving peptide ("P2A"), an in-frame coding sequence for CD16 ("Cargo") (a non-cleavable CD 16; SEQ ID NO: 165), a stop codon and polyA signal sequence, and a 3′ homology arm approximately 500bp in length (comprising a coding portion of exon 9 including a stop codon, the 3′ non-coding exonic region of exon 9, and a portion of the downstream intergenic sequence) (as shown in Figure 3B).

[0629] The cargo gene CD16 was successfully integrated into the *GAPDH* gene of iPSCs at high efficiencies using the selection systems described herein. Fig. 18A shows the efficiency of CD16-encoding "cargo" integration in the *GAPDH* gene at 0 days post-electroporation and at 19 days post-electroporation in iPSCs transformed with RNPs at a concentration of 4 μM and the

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dsDNA plasmid encoding CD16, or in "unedited cells" that were not transformed with the dsDNA plasmid. Knock-in was measured in bulk edited CD16 KI cells using ddPCR targeting the 5′ or 3′ position of the knock-in "cargo" using a primer in the 5′ of the gRNA target site or a primer in the 3′ of the site in the poly A region, increasing the reliability of the result. As shown in Fig. 18A, CD16 was stably knocked-in and present in bulk edited cell populations more than two weeks following electroporation and targeted integration of the knock-in cassette.

[0630] From bulk edited cell populations, single cells were propagated to homogenize genotypes. Shown in Fig. 18B are four edited cell populations: homozygous clone 1, homozygous clone 2, heterozygous clone 3, and heterozygous clone 4. The homozygous clones contained two alleles of the *GAPDH* gene that comprised CD16 knock-in, while heterozygous clones contained one allele of the *GAPDH* gene that comprised CD16 knock-in (measured using ddPCR of the 5′ and 3′ positions of the knock-in cargo).

[0631] Following confirmation of CD16-encoding "cargo" integration at the *GAPDH* gene, homogenized cell lines were differentiated into Natural Killer (NK) immune cells using spin embryoid body methods as known in the art. In brief, iPSCs were placed in an ultra-low attachment 96-well plate at 5,000 to 6,000 cells per well in order to form embryoid bodies (EBs). On day 11 EBs were transferred to a flask where they remain for the remainder of the experiment (see Ye Li et al., Cell Stem Cell. 2018 Aug 2; 23(2): 181-192.e5). At day 32 of the differentiation process, cells were analyzed using flow cytometry methods known in the art. Following standard control gating experiments (see Ye Li et al., Cell Stem Cell. 2018 Aug 2; 23(2): 181-192.e5), the differentiation process was analyzed using expression of markers CD56 and CD45, following this, co-expression of markers CD56 and CD16 was measured. As shown in Fig. 19A-19D, in general, cells that were positive for CD56 expression were also positive for CD16 expression (98%, 99%, 97.8%, and 99.9% respectively), indicating that both homozygous and heterozygous TI clones had stable and robust CD16 expression levels.

[0632] These differentiated iNK cells comprising knock-in of the gene of interest (CD16) at the *GAPDH* gene were then subject to challenge by various cancer cell lines to determine their cytotoxic capacity. An exemplary 3D solid tumor killing assay is depicted in Fig. 20. In brief, spheroids were formed by seeding 5,000 NucLight Red labeled SK-OV-3 cells in 96 well ultra-low attachment plates. Spheroids were incubated at 37°C before addition of effector cells (at

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different E:T ratios) and any optional agents (e.g., cytokines, antibodies, etc.), spheroids were subsequently imaged every 2 hours using the Incucyte S3 system for up to 600 hours. Data shown are normalized to the red object intensity at time of effector addition. Normalization of spheroid curves maintains the same efficacy patterns observed in non-normalized data. Using this assay, the cytotoxicity of iNKs differentiated from iPSCs comprising knock-in of CD16 at the *GAPDH* gene was measured.

[0633] As shown in Fig. 21A and 21B, both homozygous edited iNK lines and both heterozygous edited iNK lines comprising CD16 knocked-in at the *GAPDH* gene were capable of reducing the size of SK-OV-3 spheroids more effectively than unedited iNK control cells (WT PCS) or control cells with GFP knocked-in to the *GAPDH* gene (WT GFP KI) (averaged data from 2 assays). The edited homozygous and heterozygous iNK cells comprising CD16 at *GAPDH* also reduced the size of SK-OV-3 spheroids more effectively than control cells with GFP knocked-in to the *GAPDH* gene (data not shown). Introduction of 10μg/mL of the antibody trastuzumab greatly enhanced the killing capacity of the CD16 KI iNKs when compared to control cells, likely as a function of increased antibody dependent cellular cytotoxicity (ADCC) due to increased FcγRIII (CD16) expression levels (see Fig. 37A). The results of a number of solid tumor killing assays were plotted against the CD16 expression levels of CD16 KI edited iNKs (derived from bulk edited iPSCs or singled edited iPSCs). At an E:T ratio of 3.16:1, there is a correlation shown between the percentage of a cell population expressing CD16, and the amount of cell killing that occurred (see Fig. 23).

[0634] To further elucidate the functionality of the edited iNKs, the cells were subjected to repeated exposure to tumor cells, and the ability of the edited iNKs to kill tumor targets repeatedly over a multiday period was analyzed in an *in vitro* serial killing assay. Results of this experiment are depicted in Fig. 22. At day 0 of the assay, 10×10^6 Raji tumor cells (a lymphoblast-like cell line of hematopoietic origin) and 2×10^5 iNKs were plated in each well of a 96-well plate in the presence or absence of 0.1μg/mL of the antibody rituximab. At approximately 48 hour intervals, a bolus of 5×10^3 Raji tumor cells was added to re-challenge the iNK population. As shown in Figure 22, the edited iNK cells (CD16 KI iNK heterozygous or homozygous) exhibited continued killing of Raji cells after multiple challenges with Raji tumor cells (up to 598 hours), whereas unedited iNK cells were limited in their serial killing effect. The

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data show that iNK cells comprising homozygous or heterozygous CD16 KI at *GAPDH* results in prolonged and enhanced tumor cell killing. Furthermore, the efficacy of heterozygous CD16 KI iNKs highlights the potential for biallelic insertion of two different knock-in cassettes, e.g., comprising CD16 in one allele and a different gene of interest in the other allele of a suitable essential gene (e.g., *GAPDH*, *TBP*, *KIF11*, etc.).

Example 12: Knock-in of Immunologically Relevant Sequences at a Suitable Essential Gene Locus (Monocistronic or Bicistronic)

[0635] Positive targeted integration events at the *GAPDH* gene and cellular phenotypes were noted for integration of GFP, CD47, or CD16 as described above in Example 2 and Example 11. Additional or alternative cargo sequences may be incorporated into the *GAPDH* gene or other suitable essential genes as described herein with high integration rates. The essential gene *GAPDH* was targeted in iPSC cells using an RNP containing AsCpf1 (SEQ ID NO: 62) and a guide RNA (RSQ22337; SEQ ID NO: 95), resulting in a double-strand break towards the 5′ end of the last exon of *GAPDH* (exon 9), as described in Example 2. A donor plasmid containing a knock-in cassette with the cargo of interest was also electroporated with the RNP. As shown in Fig. 24A, the targeted integration (TI) rates at the *GAPDH* gene for cargos such as a) CD16, b) a CAR suitable for expression in NK cells, or c) biallelic GFP/mCherry, were all greater than 40% when assayed in two independent iPSC clonal lines when measured using ddPCR. As shown in Fig. 24B, the targeted TI rates at the *GAPDH* gene for a CXCR2 cargo was at least 29.2% of bulk edited iPSCs (expression determined using flow cytometry), while surface expression of CXCR2 was observed in approximately 8.5% of the bulk edited iPSCs (expression determined using flow cytometry). By contrast, unedited iPSCs very small amounts of CXCR2 (approximately 1%) by flow cytometry (data not shown).

[0636] An exemplary ddPCR experiment was used to measure the targeted integration (TI) rates as follows. In brief, TI was measured using a universal set of primers that captures both the 5′ homology arm and 3′ polyA tail for the *GAPDH* terminal exon region, and can detect cargos independent of the particular sequence of the specific cargo. The 5′ CDN primer and 3′ PolyA primer and FAM fluorophore probes are made in combination. An appropriate reference gene probe is a TTC5 HEX probe. For the reaction, probes, genomic DNA, BioRad master mix,

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and 2x control buffer were mixed together in ratios consistent with manufacturer recommendations. First, genomic DNA was placed in the BioRad 96 well plate (9.2 µl total genomic DNA + water), next, master mix with primer probes sets (13.8 µl per well) were added. Water controls comprised a 5′ primer probe set master mix in one well, and a 3′ primer probe set master mix in a different well. For blank well controls, a 50/50 mix of 2x control buffer and water (25 µl total) was added. The auto droplet generator was then prepared and run. Once droplets were generated, the ddPCR plates were sealed at 180°C and then placed in a thermocycler for amplification. 5′ CDN primer: CATCGCATTGTCTGAGTAGGTGTC (SEQ ID NO: 219), 3′ PolyA primer: TGCCCACAGAATAGCTTCTTCC (SEQ ID NO: 220), FAM probe: TCCCCTCCTCACAGTTGCCA (SEQ ID NO: 221), TTC5 reference gene forward primer: GGAGAAAGTGTCCAGGCATAAG (SEQ ID NO: 222), TTC5 reference gene reverse primer: CTCCATCCCACTATGACCATTC, (SEQ ID NO: 223), TTC5 FAM probe: AGTTTGTGTCAGGATGGGTGGT (SEQ ID NO: 224).

[0637] Next, the cargo integration and selection methods described herein were tested using a number of bicistronic knock-in cassettes that contained CD16 and an NK suitable CAR in different 5′-to-3′ orders (e.g., CD16 followed by the CAR, or the CAR followed by CD16) and separated by a P2A or IRES sequence. The essential gene *GAPDH* was targeted in iPSC cells using an RNP containing AsCpf1 (AsCas12a, (SEQ ID NO: 62)) and a guide RNA (RSQ22337; SEQ ID NO: 95), resulting in a double-strand break towards the 5′ end of the last exon of *GAPDH* (exon 9), as described in Example 2. A donor plasmid containing each of the knock-in cassettes depicted in Fig. 25 was also electroporated with the RNP. As shown in Fig. 25, the TI rates for the bicistronic constructs comprising CD16 and the NK suitable CAR ranged from 20-70% when measured in the bulk edited cells using ddPCR at day 0 post-transformation. In addition, a membrane bound IL-15 (mbIL-15) cargo gene (a fusion comprising IL-15 linked to a Sushi domain and a full-length IL-15Rα, as depicted in Fig. 26) was also knocked into the *GAPDH* locus using RNPs comprising (RSQ22337) and Cas12a at a concentration of 4 µM and the dsDNA plasmid encoding mbIL-15 at 5 µg (PLA1632; comprising donor template SEQ ID NO: 45) to determine if additional genes of interest could be integrated into an essential gene at high levels within a population of edited cells. Fig. 25 shows that the mbIL-15 cargo was knocked into the *GAPDH* locus at a percentage TI of greater than 50% as measured by ddPCR

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(day 0 post-transformation). Thus, the methods described herein can be used to isolate populations of edited cells, such as iPSCs, that have very high levels of a gene of interest knocked into an essential gene locus, such as *GAPDH*.

Example 13: IL-15 and/or IL-15/IL15-Rα Knock-In iPSCs Give Rise to Edited iNKs With Enhanced Function.

[0638] The present example describes use of gene editing methods described herein to create modified immune cells suitable for cancer cell killing.

[0639] PSCs were edited using the exemplary system illustrated in Figs. 3A, 3B, and 3C, and described in Example 2. In brief, the *GAPDH* gene was targeted in iPSCs using RNPs containing AsCpf1 (AsCas12a, SEQ ID NO: 62), and a guide RNA (RSQ22337; SEQ ID NO: 95), resulting in a double-strand break towards the 5′ end of the last exon of *GAPDH* (exon 9). The CRISPR/Cas nuclease and guide RNA were introduced into cells by nucleofection (electroporation) of a ribonucleoprotein (RNP) according to known methods. The cells were also contacted with a double stranded DNA donor template (dsDNA plasmid) that included a donor template comprising in 5′-to-3′ order, a 5′ homology arm approximately 500bp in length (comprising a 3′ portion of exon 8, intron 8, and a 5′ codon-optimized coding portion of exon 9 optimized to prevent further binding of the gRNA targeting domain sequence of the guide RNA (RSQ22337)), an in-frame sequence encoding the P2A self-cleaving peptide ("P2A"), an in-frame coding sequence for mbIL-15 as shown in Fig. 32 ("Cargo") (SEQ ID NO: 172), a stop codon and polyA signal sequence, and a 3′ homology arm approximately 500bp in length (comprising a coding portion of exon 9 including a stop codon, the 3′ non-coding exonic region of exon 9, and a portion of the downstream intergenic sequence) (as shown in Figure 3B). The 5′ and 3′ homology arms flanking the cargo coding sequence of the donor template were designed to correspond to sequences located on either side of the endogenous stop codon in the genome of the cell.

[0640] The cargo gene mbIL-15 (as shown in Fig. 26) was successfully integrated into the *GAPDH* gene of iPSCs at high efficiencies using the selection systems described herein (see Example 12). Fig. 25 shows the efficiency of the mbIL-15-encoding "cargo" in *GAPDH* at 0 days post-electroporation in iPSCs transformed with RNPs comprising (RSQ22337) and Cas12a at a concentration of 4 μM and the dsDNA plasmid encoding mbIL-15 at 5 μg (PLA1632;

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comprising donor template SEQ ID NO: 45). Genomic DNA was extracted approximately seven days post nucleofection. After genomic DNA extraction ddPCR was performed.

[0641] Two separate populations of the bulk edited mbIL-15 KI iPSC cells were then differentiated into iNK cells and the TI rates were measured using ddPCR at day 28 of the iNK differentiation process. Fig. 27 shows that TI integrate rates for these edited iNK cell populations ranged from 10-15%. While the TI rates in the iNK populations decreased when compared to the TI at day 0 post-electroporation of iPSCs, the TI integration levels within these cell populations remained significant. At day 32 post-differentiation initiation, flow cytometry was conducted to determine the proportion of cells expressing CD56 and exogenous IL-15Rα in these edited iNK cell populations (see Fig. 28A). The CD56 and CD16 co-expression levels were also determined in these edited iNK cell populations (see Fig. 28B). The bulk edited mbIL-15 KI cell populations were also analyzed for markers of differentiation by flow cytometry on day 32, day 39, day 42, and day 49 post-differentiation initiation (see Fig. 28C).

[0642] At day 39 following the initiation of differentiation from the edited iPSCs into iNKs, cells were challenged in 3D spheroid killing assays as described in Example 11 and depicted in Fig. 20. Using this assay, the cytotoxicity of iNKs differentiated from iPSCs comprising knock-in of mbIL-15 at the *GAPDH* gene was measured (see Fig. 30A). Cells were tested in the presence or absence of 5ng/mL exogenous IL-15. As shown in Table 18 and Fig. 30A, mbIL-15 KI iNK cells (Mb IL-15 S1 and Mb IL-15 S2 populations) exhibited more efficient tumor cell killing when compared to unedited parental cells differentiated into iNKs ("WT" PCS, 1 and 2). Of note, mbIL-15 KI iNK cells exhibited better tumor cell killing in the absence of exogenous IL-15 relative to WT iNK cells in the absence of endogenous IL-15 at lower E:T ratios. The mbIL-15 KI iNK cells also exhibited better tumor cell killing in the presence of low concentrations of exogenous IL-15 (5ng/mL) when compared to unedited WT iNK cells in the presence of the same concentration of exogenous IL-15. In addition, at higher E:T ratios, mbIL-15 KI iNKs outperformed WT iNKs without the addition of exogenous IL-15 (see Fig. 30B). The above described 3D spheroid killing assay was repeated on mbIL-15 KI iNKs and control cells on day 42 and day 49, and for test cells only on day 56, and day 63 post-differentiation initiation, results for these assays in the presence or absence of 5ng/mL IL-15 is

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depicted in Fig. 30C and 30D respectively. These results support the conclusion of mbIL-15 KI iNKs persisting and facilitating tumor cell killing in the absence or presence of exogenous IL-15.

[0643] In addition, mbIL-15 KI iNK cells at later stages of differentiation (day 63 post-differentiation initiation for Set 1 (S1) and day 56 post-differentiation initiation for Set 2 (S2)) were also challenged in 3D spheroid killing assays as described above. Cells were tested in the presence or absence of 10 μg/ml Herceptin and/or 5 ng/mL exogenous IL-15. As shown in Table 19 and Fig. 31A-31D, mbIL-15 KI iNK cells exhibited high tumor cell killing efficiency, particularly when coupled with antibody therapy. At day 63, all mbIL-15 KI iNK cells did not express detectable levels of IL-15Ra; at Day 56, only one mbIL-15 KI iNK cell line (Mb IL-15 S2 R2) expressed detectable levels of IL-15Ra (data not shown).

[0644] The cumulative results of certain 3D spheroid killing assays for mbIL-15 KI iNKs and control WT iNK cells is depicted in Fig. 32. Two independent bulk edited populations of iPSCs (Set 1 (S1) and Set 2 (S2)) comprising mbIL-15 knock-in at the *GAPDH* gene were differentiated into iNK cells (day 39 and 49 of iPSC differentiation for Set 1, and day 42 of iPSC differentiation for Set 2) These iNK cells significantly reduced tumor cell spheroid size when compared to differentiated WT parental cell iNKs in the absence of exogenous IL-15 (P=0.034, +/- standard deviation, unpaired t-test). The differentiated knock-in mbIL-15 iNK cells also trended towards significant reduction of tumor cell spheroid size when compared to differentiated WT parental cells in the presence of 5ng/mL exogenous IL-15 (P=0.052, +/- standard deviation, unpaired t-test). These results show that populations of iNK cells comprising mbIL-15 knock-in at the *GAPDH* locus using the methods described herein perform better in killing tumor cells in the absence of exogenously added IL-15 compared to populations of unedited iNK cells.

Table 18: mbIL-15 KI iNK 3D spheroid killing with IL-15

Cell Line	EC50 with 0ng/mL IL-15	EC50 with 5ng/mL IL-15
Mb IL-15 S1	9.575	1.648
Mb-IL-15 S2	11.05	1.646
WT iNK (PCS) 1	20.71	4.378
WT iNK (PCS) 2	20.99	3.213

Table 19: mbIL-15 KI iNK 3D spheroid killing with Herceptin and/or IL-15

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Cell Line	EC50 with 0µg/mL Herceptin	EC50 with 10µg/mL Herceptin	EC50 with 5ng/mL IL-15 and 0µg/mL Herceptin	EC50 with 5ng/mL IL-15 and 10µg/mL Herceptin
Mb IL-15 Set1 Rep1	2.055	0.6936	0.16515	0.1423
Mb IL-15 Set1 Rep2	1.701	0.5903	0.1794	0.1247
Mb IL-15 Set1 Rep2.1	1.848	0.9570	0.3187	0.1153
Mb IL-15 Set2 Rep1	1.291	1.589	0.2339	0.2096
Mb IL-15 Set2 Rep2	0.8026	0.3783	0.3605	0.2778

[0645] In addition, the mbIL-15 KI iNK cells at later stages of differentiation (day 63 post-differentiation initiation for Set 1 (S1) and day 56 post-differentiation initiation for Set 2 (S2)) were also challenged with hematological cancer cells (e.g., Raji cells). Two biological replicate populations of mbIL-15 KI NK cells (S1 and S2) were tested in the presence or absence of 10µg/ml rituximab. As shown in Fig. 29, mbIL-15 KI iNK cells exhibited high tumor cell killing efficiency, particularly when coupled with antibody therapy. This killing capacity of these cells is significant, as Raji cells are naturally resistant to NK cells, but the mbIL-15 KI iNK cells in combination with antibody were able to find and kill these cells.

Example 14: Knock-in of Multicistronic CD16, IL-15, and/or IL-15Rα Sequences at a Suitable Essential Gene Loci.

[0646] As described above in Example 2, genes of interest (GOI) may be integrated as a cargo sequence into suitable essential gene loci using methods described herein. In certain embodiments, multiple GOIs may be combined into a bicistronic or multicistronic knock-in cargo sequence. Fig. 33A depicts a portion of PLA1829 (comprising donor template SEQ ID NO: 208) comprising a bicistronic knock-in cargo sequence that was utilized for targeted integration at the *GAPDH* gene comprising an IL-15 peptide sequence, an IL-15Rα peptide sequence, and a GFP peptide sequence (SEQ ID NOs: 187, 189, and 195 respectively). Each of these peptide sequences were separate by a P2A sequence. Depicted in Fig. 33B is a portion of PLA1832 (comprising donor template SEQ ID NO: 209) comprising a multicistronic knock-in cargo sequence that was utilized for targeted integration at the *GAPDH* gene comprising a CD16 peptide sequence, an IL-15 peptide sequence, and an IL-15Rα peptide sequence (SEQ ID NOs:184, 187, and 189 respectively). Each of these peptide sequences were separate by a P2A

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sequence. Depicted in Fig. 33C is a portion of PLA1834 (comprising donor template SEQ ID NO: 212) comprising a bicistronic knock-in cargo sequence that was utilized for targeted integration at the *GAPDH* gene comprising a CD16 peptide sequence, and an mbIL-15 peptide sequence (an IL-15 sequence fused to an IL-15Rα sequence as depicted in Fig. 26) (SEQ ID NOs: 184 and 190 respectively) separated by a P2A sequence.

[0647] The knock-in cargo sequences described in Fig. 33A-33C are comprised within Plasmids 1829, 1832, and 1834 respectively (comprising donor template SEQ ID NOs: 208, 209, and 212). PSCs were edited using the exemplary system illustrated in Figs. 3A, 3B, and 3C, and described in Example 2. In brief, the *GAPDH* gene was targeted in iPSCs using AsCpf1 (AsCas12a (SEQ ID NO: 62)) and a guide RNA (RSQ22337 (SEQ ID NO: 95)), resulting in a double-strand break towards the 5′ end of the last exon of *GAPDH* (exon 9). The CRISPR/Cas nuclease and guide RNA were introduced by nucleofection (electroporation) of a ribonucleoprotein (RNP) according to known methods. The cells were also contacted with a double stranded DNA donor template (dsDNA plasmid (PLA1829, PLA1832, or PLA1834 respectively)) that included a donor template (SEQ ID NO: s: 208, 209, and 212) comprising in 5′-to-3′ order, a 5′ homology arm approximately 500bp in length (comprising a 3′ portion of exon 8, intron 8, and a 5′ codon-optimized coding portion of exon 9 optimized to prevent further binding of the gRNA targeting domain sequence of the guide RNA (RSQ22337)), an in-frame sequence encoding the P2A self-cleaving peptide ("P2A"), an in-frame coding sequence as described above ("Cargo"), a stop codon and polyA signal sequence, and a 3′ homology arm approximately 500bp in length (comprising a coding portion of exon 9 including a stop codon, the 3′ non-coding exonic region of exon 9, and a portion of the downstream intergenic sequence) (as shown in Figure 3B). Four unique nucleofection events were conducted (corresponding to RNP and PLA1829, RNP and PLA1832, RNP and PLA1834, and RNP with no plasmid control) and cells were plated at clonal density. Colonies were propagated for analysis of TI using ddPCR.

[0648] Following TI, transformed iPSCs (edited clones) with KI of PLA1829, PLA1832 or PLA1834 cargo sequences, or control WT parental cells transformed with RNP alone, were analyzed using flow cytometry seven days after transformation (see Figs. 34A and 34B). The levels of GFP and IL-15Rα expression were measured in bulk edited iPSC populations. As

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shown in Fig 34A, approximately 57% of cells transformed with PLA1829 expressed both IL-15Rα and GFP, while control cells had no GFP expression and approximately 14.4% IL-15Rα expression levels. As shown in Fig. 34B, approximately 33.1% of cells transformed with PLA1832, and approximately 57.2% of cells transformed with PLA1834 expressed IL-15Rα; neither of these cell populations displayed appreciable GFP levels, as expected as the respective donor templates did not comprise GFP. The expression of these cargo proteins can be used as a proxy for determining successful transformation, editing, and/or integration.

[0649] Fig. 35A-35C depicts the genotypes for 24 of the colonies transformed with PLA1829, PLA1832, or PLA1834 (comprising donor template SEQ ID NOs: 208, 209, and 212) respectively and compared to wild-type cells. Measured with ddPCR, cells with ~85-100% TI are categorized as homozygous, 40-60% are categorized as heterozygous, while those with very low or no signal are categorized as wild type. The colonies were propagated after transformation, and cell populations were then differentiated to iNK cells using a spin embryoid method as known in the art. Shown in Fig. 36A-36D are exemplary flow cytometry results measuring the percentage of cells expressing IL-15Rα and/or CD16, and the median fluorescence intensity (MFI) of IL-15Rα and/or CD16 at day 32 of the iNK differentiation process. As shown in Fig. 36A, transformation with PLA1829, PLA1832, or PLA1834 enabled surface expression of IL-15Rα in heterozygous or homozygous colonies at significantly higher proportions than iNKs differentiated from control WT parental cells. As shown in Fig. 36B, transformation with PLA1832 or PLA1834 enabled surface expression of CD16 in heterozygous or homozygous colonies at significantly higher proportions than iNKs differentiated from control WT parental cells, as cells transformed with the PLA1829 cargo sequence do not comprise a CD16 cargo sequence. As shown in Fig. 36C, transformation with PLA1834 enabled higher MFI of IL-15Rα in heterozygous or homozygous colonies when compared to iNKs differentiated from control WT parental cells, or cells transformed with PLA1829 or PLA1832. As shown in Fig. 36D, transformation with PLA1832 or PLA1834 enabled surface expression of CD16 in heterozygous or homozygous colonies. These data show that the methods described herein can be used to knock-in a multicistronic cargo containing numerous genes of interest into an essential gene such as *GAPDH*, leading to expression of the genes of interest in the edited cells. These data also clearly demonstrate the constitutive nature of cargo expression from the GAPDH locus.

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[0650] The differentiated iNK cells were also used in lactate dehydrogenase (LDH) killing assays, and iNK cells were assessed for surface expression of CD16 by flow cytometry, before and after the cytotoxicity assay (E:T ratio of 1 or 2.5). As shown in Fig. 36F (E:T ratio of 2.5), in the absence of trastuzumab (Herceptin), WT cells and cells transformed with PLA1829 (without CD16 KI) showed small decreases in the surface level expression of CD16 after coming into contact with the SK-OV-3 cells in the LDH assay while cells transformed with PLA1834 (and thus having CD16 KI) showed minimal reduction. In the presence of trastuzumab, cells transformed with PLA1834 demonstrated a similar minimal reduction in the level of CD16 after coming into contact with the SK-OV-3 cells in the LDH assay; however, a marked difference in CD16 surface expression was observed for WT cells and cells transformed with PLA1829 (without CD16 KI) after coming into contact with the SK-OV-3 cells in the LDH assay (Fig. 36G, E:T ratio of 2.5). Further experimental replicates confirmed that homozygous colonies of cells transformed with PLA1834 largely maintained CD16 surface expression after contact with SK-OV-3 cells in the LDH assay, in the absence or presence of trastuzumab, whereas unedited (WT) parental cells displayed substantial decreases in CD16 surface expression after contact with SK-OV-3 cells (Fig. 36H). Overall, the results show that KI of a cleavable CD16 construct at GAPDH can lead to high levels of CD16 surface expression in KI iNK cells, and there is minimal CD16 shedding from the CD16 KI iNK cells after they contact tumor cells. Additionally, as shown in Fig. 36I, homozygous PLA1834-transformed iNK cells exhibited greater cytotoxicity than unedited (WT) iNK cells in the presence and absence of trastuzumab at E:T ratios of 1 and 2.5 in the LDH assay.

[0651] In addition, differentiated iNK cells (unedited (WT) cells) and homozygous colonies of PLA1834-transformed ($CD16^{+/-}$/$mbIL\text{-}15^{+/+}$) cells were used in 3D tumor spheroid killing assays as described in Example 11 and schematized in Fig. 20. Cells were tested for 100 hours at an E:T ratio of 10 and in the absence or presence of 10 μg/ml trastuzumab. $CD16^{+/+}$/$mbIL\text{-}15^{+/+}$ iNK cells elicited greater reduction in tumor spheroid size than unedited iNK cells without or with trastuzumab (Fig. 37B). As shown in Fig. 37C, $CD16^{+/+}$/$mbIL\text{-}15^{+/+}$ iNK cells showed enhanced cytotoxicity in the 3D tumor spheroid assay compared with unedited iNK cells or peripheral blood NK cells across a range of E:T ratios in the presence of 10 μg/ml trastuzumab and 5 ng/ml exogenous IL-15. The average IC50 (as measured via E:T ratio) of the

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$CD16^{+/+}$/mbIL-$15^{+/+}$ iNK cells was significantly lower than the unedited iNK cells in the absence or presence of trastuzumab (Fig. 37C). These 3D tumor spheroid killing assay results further confirm that the $CD16^{+/+}$/mbIL-$15^{-/+}$ (homozygous PLA1834-transformed) iNK cells demonstrate greater cytotoxicity of tumor cells and are more efficient at tumor cell killing than unedited (WT) iNK cells in the presence or absence of trastuzumab or in the presence of the combination of trastuzumab and exogenous IL-15.

[0652] Membrane bound IL-15 also mediated iNK cell survival for a prolonged period of time without the support of homeostatic cytokines. Starting at Day 43 post-differentiation, iNK cells were maintained in the absence of IL-2 or IL-15 for three weeks. As shown in Fig. 36J, in contrast to WT cells, the total number of iNK cells transformed with PLA1834 (heterozygote and homozygote KI cells) remained stable over the three-week assay. These data show that cells transformed with PLA1834 demonstrated superior persistence in the absence of cytokines compared to WT cells and to cells transformed with PLA1829.

Example 15: In Vivo Assay of Bicistronic CD16 and mbIL-15 Sequences at a Suitable Essential Gene Loci

[0653] Plasmid PLA1834 was used to generate iPSC-derived NK (iNK) cells comprising mbIL-15/CD16 double knock-in (DKI), as described in Example 14. From these mbIL-15/CD16 DKI iNK cells, three homozygous ($CD16^{+/+}$/mbIL-$15^{+/+}$) clones (A2, A4, C4) were selected for testing in an *in vitro* lactate dehydrogenase (LDH) release assay to assess cell cytotoxicity against SK-OV-3 tumor cells as described in Example 14. An unedited (WT) iNK cell control was also tested. Cells were tested in the presence and absence of 10 μg/ml trastuzumab and at an E:T ratio of 1. As shown in Figure 38A, mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI iNK cells demonstrated significant increases in average percent cytotoxicity in the presence of trastuzumab as compared to average percentage cytotoxicity seen in the absence trastuzumab, confirming the potent *in vitro* tumor killing activity of these cells described in Example 14. The mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI iNK cells were also examined by flow cytometry for expression of CD16. Samples of unedited (WT) or DKI iNK cells (clones A2 and A4) were pre-gated for alive hCD45+ cells and then examined for CD56 and CD16 expression. As shown in Figure 38B, WT and DKI iNK cells were highly pure CD56+ NK cells. Moreover, both clones of the mbIL-

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15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI iNK cells had approximately 100% of cells expressing high levels of CD16, while approximately half of the WT iNK cells expressed CD16.

[0654] Following confirmation of cytotoxicity in the LDH assay and of high CD16 expression, mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI iNK cells were assayed for their ability to kill tumor targets in an *in vivo* mouse model. Figure 38C depicts a schematic of the assay. Mice were inoculated with 0.25 x 10^6 SK-OV-3 cells engineered to express luciferase (SKOV3-luc). Following 2-6 days to allow for establishment of the tumors, mice were imaged using an *in vivo* imaging system (IVIS) to establish pre-treatment (day -1) tumor burden and then randomized into treatment groups. After 1 additional day (on day 0), mice were injected intraperitoneally (IP) with 2 x 10^6 (2M) or 5 x 10^6 (5M) mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI iNK cells + 2.5 mpk trastuzumab, 2.5 mpk trastuzumab alone, an isotype control, or a vehicle control. As depicted in Figure 38C, one treatment group (mice injected with 5M mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI iNK cells) received an additional dose of trastuzumab on day 35, and another treatment group (mice injected with 2M mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI iNK cells) received additional doses of trastuzumab on days 21, 28, and 35. Following day 0, the mice were imaged weekly using IVIS to assess tumor burden over time. Mice were followed for up to 90 days.

[0655] The average tumor burden over time as measured by bioluminescent imaging (BLI) via IVIS is depicted in Figure 38D, and percent survival over time is depicted in Figure 38E. Representative bioluminescent imaging of the mice at various time points is displayed in Figure 38F. The mouse dosed with 2 x 10^6 mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI iNK cells in combination with trastuzumab (2M DKI iNK + Tras) exhibited complete tumor clearance (Figures 38D and 38F) and prolonged survival (Figure 38E). By contrast, the mice treated with trastuzumab alone or with the isotype control exhibited higher tumor burden and decreased survival. These data demonstrate that mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI iNK cells actively kill tumor cells in an *in vivo* model and that treatment with both the mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI iNK cells and trastuzumab results in better outcomes (e.g., prolonged survival, significantly greater tumor clearance) than dosing with trastuzumab alone.

[0656] The mouse dosed with 5 x 10^6 mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI iNK cells in combination with trastuzumab (5M DKI iNK + Tras) displayed a significant decrease in

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tumor burden by day 14, followed by an increase in tumor burden (Figures 38D and 38F). After sacrificing this mouse at day 90, the rebounded tumor was found to be located subcutaneously and not in the peritoneal cavity as experimentally intended. Thus, the intraperitoneally injected mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells were likely unable to access the tumor to the same extent as mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/-}$) DKI iNK cells administered to mice in other treatment groups. The presence of mbIL-15/CD16 ($CD16^{+/-}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells in the peritoneal cavity of the mouse dosed with 5 x 10^6 DKI iNK cells + trastuzumab was confirmed by flow cytometric analysis of the peritoneal lavage (Figure 38G, top row). Moreover, the mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells expressed high levels of CD56 and CD16, with 100% of the cells expressing high levels of CD16 at day 90 (Figure 38G, top right panel). Thus, the presence of the rebounded tumor in this mouse was unlikely due to a loss of the mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells. The mouse dosed with 2 x 10^6 mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells + trastuzumab was sacrificed at day 118, and presence of mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells in the peritoneal cavity of the mouse was confirmed by flow cytometric analysis of the peritoneal lavage (Figure 38G, bottom row). These cells expressed high levels of CD56 and CD16, with 92% of the cells expressing high levels of CD16 at day 118 (Figure 38G, bottom right panel).

[0657] Figure 38G demonstrates that knock-in of mbIL-15 prolongs the *in vivo* persistence of the iNK cells as compared to short-lived healthy donor-derived WT NK cells (data not shown). Further, the mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells continue to express high levels of CD16 on the cell surface due to the knock-in of CD16 (as described in Example 14), indicating that they retain the ability for ADCC mediated tumor killing in the presence of a therapeutic antibody (such as trastuzumab).

[0658] These data demonstrate that mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells are capable of persisting *in vivo* and maintaining high CD16 expression up to at least 118 days. This is notable, given that unedited NK cells have been reported to have limited persistence (see, e.g., Romee et al., Sci. Trans. Med. 8:357ra123357ra123 (2016)).

[0659] Further testing of the mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells in an *in vivo* mouse model was conducted as depicted in Figure 39A. Mice were inoculated with 0.25 x 10^6 SKOV3-luc cells. Following 2-6 days to allow for establishment of the tumors, mice were

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imaged using an IVIS to establish pre-treatment (day -1) tumor burden and then randomized into treatment groups. After 1 additional day (on day 0), mice were injected intraperitoneally (IP) with 5 x 10^6 unedited (WT) iNK cells, 5 x 10^6 mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI iNK cells (from clone A2 or A4), or no iNK cells for trastuzumab-alone or isotype control. One treatment group ("+ Tras. x 1", "TRA x 1") received an IP injection of 2.5 mpk trastuzumab on day 0. Another treatment group ("+ Tras. x 3", "+ TRA x 3") received IP injections of 2.5 mpk trastuzumab on days 0, 7, and 14. Following day 0, the mice were imaged weekly using an IVIS to assess tumor burden over time.

[0660] The tumor burden over time as measured by bioluminescent imaging (BLI) via IVIS is depicted in Figures 39B, 39C, and 39E. Representative bioluminescent imaging of the mice at various time points is displayed in Figure 39G. As seen in Figure 39B, treatment with the unedited (WT) iNK cells or the mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI iNK cells alone did not lead to tumor reduction *in vivo*. However, mice treated with iNK cells in combination with trastuzumab exhibited greater tumor reduction than mice treated with trastuzumab alone, whether trastuzumab was administered as a single dose (Figure 39C) or as a multi-dose regimen (see Figure 39E). Moreover, the reduction in tumor burden was observed for at least 144 days post-introduction of iNK cells (Figure 39E). As shown in Figure 39G, mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI iNK cells led to significantly greater *in vivo* tumor reduction as compared to the unedited (WT) iNK cells measured at day 33. This was seen with two different mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI clones (A2 and A4) in combination with a single dose of trastuzumab, or with clone A2 in combination with multi-dose regimen of trastuzumab. Furthermore, treatment with mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{-/+}$) DKI iNK cells in combination with a single dose of trastuzumab led to significantly greater *in vivo* tumor reduction as compared to unedited iNK cells in combination with a single dose of trastuzumab or trastuzumab alone as early as at least day 11 and as late as at least day 54 (Figure 39H).

[0661] Mice treated with mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI iNK cells in combination with trastuzumab exhibited significantly prolonged survival as compared to mice treated with trastuzumab alone (Figure 39D and 39F). In addition, mice treated with mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI iNK cells also exhibited significantly prolonged survival as compared to mice treated with unedited (WT) iNK cells (Figure 39F). As shown in Figure 39I,

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treatment with mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells in combination with trastuzumab led to complete tumor clearance in multiple animals, with 50% (4/8) of mice being tumor-free at day 40 following treatment with DKI iNK cells in combination with multiple doses of trastuzumab. Continued monitoring revealed that at day 144, 75% (6/8) of the mice treated with mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/-}$) DKI iNK cells in combination with trastuzumab (x3) showed no detectable tumor by BLI (e.g., were tumor free). Furthermore, histological analysis targeting Her2 (tumor antigen expressed on SKOV3 cells) in the lung tissue of mice revealed that mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells resulted in reduced metastasis compared with unedited iNK cells and completely inhibited tumor metastasis in 86% of mice, compared with only 14% with unedited iNK cells (data not shown). These results confirm that mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells readily kill tumor cells *in vivo* and demonstrate that mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells in combination with trastuzumab produces greater *in vivo* tumor reduction than treatment with either trastuzumab alone or with unedited (WT) iNK cells in combination with trastuzumab.

[0662] Figure 39J demonstrates that knock-in of mbIL-15 at an essential gene (the GAPDH locus) prolongs the *in vivo* persistence of the iNK cells as compared to short-lived WT NK cells. Further, the mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells continue to express high levels of CD16 on the cell surface due to the knock-in of CD16 at the GAPDH locus (as described in Example 14; bottom right plot of Figure 39J), indicating that they retain the ability for ADCC mediated tumor killing in the presence of a therapeutic antibody (such as trastuzumab). Meanwhile, WT iNK cells were not detectable at day 144 (top left and top right plots of Figure 39J). These data demonstrate that mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI iNK cells are capable of persisting *in vivo* and maintaining high CD16 expression up to at least 144 days. This is notable, given that unedited NK cells have been reported to have limited persistence (see, e.g., Romee et al., Sci. Trans. Med. 8:357ra123357ra123 (2016)).

Example 16: Computation Screening of AsCpf1 Guide RNAs Suitable for Selection by Essential-Gene Knock-in

[0663] The present example describes a method for computationally screening for AsCpf1 (AsCas12a; e.g., as represented by SEQ ID NO: 62) guide RNAs (gRNAs) suitable for

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methods described herein that target a number of essential housekeeping genes. The results of this screening are summarized in Table 20, these gRNAs facilitate Cas12a cleavage within the last 500 bp of the DNA coding sequences for the listed essential genes.

[0664] The essential genes in Table 20 selected for this analysis were identified in a pool of essential genes made by combining the essential genes described in Eisenberg et al., (see e.g., Eisenberg and Levanon, Human housekeeping genes, revisited. *Trends Genetics*, 2014) and the genes described in Yilmaz et al., (see e.g., Yilmaz et al., Defining essential genes for human pluripotent stem cells by CRISPR-Cas9 screening in haploid cells. *Nature Cell Biology,* 2018). In brief, essential genes described in Yilmaz et al., with CRISPR Scores less than 0, and FDR of <0.05 were combined with essential genes described in Eisenberg & Levanon to create a list of 4,582 genes in total. These genes were then sorted by their average expression level (mean normalized expression across different tissues, see e.g., RNA consensus tissue gene expression data provided by https://www.proteinatlas.org/download/rna_tissue_consensus.tsv.zip), and the 100 genes with the highest average expression levels across tissues were selected for the analysis. GAPDH was present within this group of genes. TBP, E2F4, G6PD and KIF11 were added to this group, making 104 genes in total, for further analysis.

[0665] Potential gRNA target sequences for each of the genes of interest were generated by searching for nuclease specific PAMs with suitable protospacers mapped to a representative coding region (mRNA -201). Transcripts with its name followed by "-201" were selected as the representative for each gene (e.g., GAPDH-201). Gene information (i.e., coding region) was obtained from GENCODE v.37 gene annotation GTF file. Potential gRNAs were first searched within the genomic regions of target genes in the human reference genome (hg38), and those identified gRNAs with their cut sites within 500bp of the representative coding region's stop site were selected for further analysis. The candidate gRNAs were then aligned to the human reference genome (e.g., hg38) with BWA Aln (maximum mismatch tolerance -n 2). Guides with potential off target binding sites (i.e., aligning to multiple genomic regions; mapping quality MAPQ <30) were filtered out. The resultant gRNAs target highly and/or broadly expressed essential genes within 500 coding base pairs of a representative stop-codon and have no identical off-target binding sites annotated in the human genome. Thus, they are excellent candidate gRNAs for the selection methods described herein.

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Table 20: AsCas12 guide RNAs

SEQ ID NO	Gene	Target Domain Sequence (DNA)	SEQ ID NO	Gene	Target Domain Sequence (DNA)
2250	EIF4G2	AGGCTTTGGCTGGTTCTTTAG	5570	CD63	CCATCTTTCTGTCTCTTATCA
2260	EIF4G2	GCTGGTTCTTTAGTCAGCTTC	5580	CD63	CTCCTGCAGTTTGCCATCTTT
2270	EIF4G2	GTCAGCTTCTTCCTCTGATTC	5590	CD63	TGGGCTGCTGCGGGGCCTGCA
2280	EIF4G2	TAACCAGGTTAGCCACTGATT	5600	RPS24	TGTTTTCAGAACGACACCGTA
2290	EIF4G2	ACAAAAGACTTACCTGGAACA	5610	RPS24	AGAACGACACCGTAACTATCC
2300	EIF4G2	CCGGAAACTCTTGGGTTATAT	5620	RPS24	GGTCATTGATGTCCTTCACCC
2310	EIF4G2	CAAGCCAAGAAAGCTTCTTCT	5630	RPS24	TCATTCAGCATGGCCTGTATG
2320	EIF4G2	CATGTCATAGAAGTGCACAAA	5640	RPS24	CCTCTTCTTCTGGATTACAGA
2330	EIF4G2	GGAAGTTGCTGTTATAGCAGT	5650	RPS24	TAGTGCGGATAGTTACGGTGT
2340	EIF4G2	TGCATTACTGGCTTGAAAGAT	5660	RPS24	CTTAATGAACTATACCTTTTT
2350	EIF4G2	CTGCTCTAACTGTTCTTTGGA	5670	RPS23	GGGCTGTGCCCAAATGAGCTT
2360	EIF4G2	GAAGGAGCAGAGGATGAATCT	5680	RPS23	TTCCAGGAAAATGATGAAGTT
2370	EIF4G2	ATCGCTGGGGGGTTTACTTC	5690	RPS23	TACCCAATGACGGTTGCTTGA
2380	EIF4G2	CTTCACTAGAAATGTACTGTA	5700	RPS23	AGAGGAGTTGAAGCCAAACAG
2390	EIF4G2	TCTACATGAAGTTTGGGAGAG	5710	RPS23	TATTTCAGAGGAGTTGAAGCC
2400	EIF4G2	GGAGAGATGTTATCTTTAATC	5720	RPS23	GGCAAGTGTCGTGGACTTCGT
2410	EIF4G2	TATATGGTTTGAGGGGATGGA	5730	RPS23	ATTTTTAGGCAAGTGTCGTGG
2420	EIF4G2	AGGGGATGGATCCAACTTTAT	5740	EEF2	TCCAGGAAGTTGTCCAGGGCA
2430	EIF4G2	TAGGTGAATCAGTGGCTAACC	5750	EEF2	AGGCCCTTGCGCTTGCGGGTC
2440	EIF4G2	CAAATCTTAATTTATAGGTGA	5760	EEF2	ACCACTGGCAGATCCTGCCCG
2450	EIF4G2	ATTTACAAATCTTAATTTATA	5770	EEF2	TGGTCAAGGCCTATCTGCCCG
2460	EIF4G2	CGGGAAAAGGCAAGGCTTTGT	5780	EEF2	AACAGGAAGCGGGGCCACCTG
2470	EIF4G2	TTGGCTTGGAAAGAAGATATA	5790	EEF2	CCTTCTGGCAGTGTCCAGAGC
2480	EIF4G2	TGCACTTCTATGACATGGAAA	5800	EEF2	TTTCCCTTCTGGCAGTGTCCA
2490	EIF4G2	AGGCATGTTACTTCGCTTTTT	5810	CALR	CTTCTCCCTTCTGCAGGGTGA
2500	EIF4G2	TTCATGATCACGTTGATCTAC	5820	CALR	GCGTGCTGGGCCTGGACCTCT
2510	EIF4G2	AAGCCAGTAATGCAGAAATTT	5830	CALR	ACAACTTCCTCATCACCAACG

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SEQ ID NO	Gene	Target Domain Sequence (DNA)
2520	EIF4G2	TAGTGAAGTAAACCCCCCCAG
2530	EIF4G2	TGTCCAGCTTCTTACAGTACA
2540	EIF4G2	TGAACATCTTAATGACTAGGT
2550	SKP1	AAGACCTTACCTTTTTTAATA
2560	SKP1	CAATGAACTTACCTTCCAACA
2570	SKP1	AGCAGGGCAGAATAAAAACCA
2580	SKP1	TTCATAATTTCAGCAGGGCAG
2590	SKP1	CTTTGTTCATAATTTCAGCAG
2600	SKP1	CAGGCTGCAAACTACTTAGAC
2610	SKP1	TTGTTGTAGGTCATTCAGTGG
2620	SKP1	TTAGATTTGGGAATGGATGAT
2630	SKP1	TTCTGGTTTTCTTAGATTTGG
2640	SKP1	GATGCCTTCAATTAAGTTGCA
2650	SKP1	ATGTCCTTTTTTTTTAGATGC
2660	RPS3	AAGCTTTATGCTGAAAAGGTG
2670	RPS3	AAGGGCCTGCTATGGTGTGCT
2680	RPS3	AAGGAAGCAAGGGATATCCTG
2690	RPS3	AGCATAAAGCTTTAAAGGAAG
2700	RPS3	CCAGACACCACAACCTCGCAG
2710	RPS3	CCAAGCACTCTCAGCTGCTCA
2720	RPS19	TTCTTCCATCTTTTCCCACAG
2730	RPS19	CCACAGGTGGCAGCTGCCAAC
2740	RPS19	TCTGACGTCCCCCATAGATCT
2750	HMGB1	AGCCCTCTTACCTTCCACCTC
2760	HMGB1	TGTTCATTTATTGAAGTTCTA
2770	HMGB1	GTTCGGCCTTCTTCCTCTTCT
2780	HMGB1	TAGACCATGTCTGCTAAAGAG
2790	HMGB1	GAAAAATAACTAAACATGGGC

SEQ ID NO	Gene	Target Domain Sequence (DNA)
5840	CALR	GCAACGAGACGTGGGGCGTAA
5850	CALR	TGGGTGGATCCAAGTGCCCTT
5860	CALR	CTCCAAGTCTCACCTGCCAGA
5870	CALR	TTACGCCCCACGTCTCGTTGC
5880	CALR	TCCTTCATTTGTTTCTCTCCT
5890	CALR	TTGTCTTCTTCCTCCTCCTTA
5900	CALR	TCCTCATCATCCTCCTTGTCC
5910	RPL36AL	TATGCCCAGGGAAGGAGGCGC
5920	SRP14	AGGCTTATTCAAACCTCCTTA
5930	SRP14	AGGTGAGCTCCAAGGAAGTGA
5940	SRP14	CTTCTTTTTCAGGTGAGCTCC
5950	SRP14	CTTCAGATGACGGTCGAACCA
5960	SRP14	CAGAAGTGCCGGACGTCGCGC
5970	SRP14	CAGTTCCTGACGGAGCTGACC
5980	GABARAP	TTTCGGATCTTCTCGCCCTCA
5990	GABARAP	GGATCTTCTCGCCCTCAGAGC
6000	GABARAP	TCTACATTGCCTACAGTGACG
6010	GABARAP	ATCCCAGGAACACCATGAAGA
6020	GABARAP	TGCTTTCATCCCAGGAACACC
6030	GABARAP	TCAACAATGTCATTCCACCCA
6040	GABARAP	TTTGTCAACAATGTCATTCCA
6050	GABARAP	CAGTTGGTCAGTTCTACTTCT
6060	GABARAP	TTGCATCTTGTATCTTTTTGCA
6070	GABARAP	TCAGGTGATAGTAGAAAAGGC
6080	GABARAP	ATCTCTTTATCAGGTGATAGT
6090	RPSA	ATAATCTGCCACTCTTGGCAG
6100	RPSA	TAACCCAGATTGAAAAAGAAG
6110	RPSA	GTATTCTCTTAACAGAAGACT

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SEQ ID NO	Gene	Target Domain Sequence (DNA)	SEQ ID NO	Gene	Target Domain Sequence (DNA)
2800	RPL7	CCCCAAATAGAACCTACCAAG	6120	RPSA	GAGAAGCTTACCTCTTCAGGA
2810	RPL7	ACTTCAGGTACCCCAATCTGA	6130	SET	AATTATTTATTACAGTATTTT
2820	RPL7	CTTTTTCACTTCAGGTACCCC	6140	SET	TTACAGTATTTTGATGAAAAT
2830	RPL7	TGTTTGCTTTTTCACTTCAGG	6150	SET	GGATTTGACGAAACGTTCCAG
2840	RPL7	ACCACAGTATCAATGGAGTGA	6160	SET	ACGAAACGTTCGAGTCAAACG
2850	RPL7	TGGTCCGTTTTCACCACAGTA	6170	SET	AGGTTCCCGATATGGATGATG
2860	RPLP0	AGGTCAAGGCCTTCTTGGCTG	6180	SET	TTTCAGGAGGATGAAGGACAA
2870	RPLP0	ACCACTTCCCCCCTCCTTTCA	6190	SET	AGGAGGATGAAGGAGGAAGATG
2880	G6PD	CTCACCTGCCATAAAATATAGG	6200	SET	TTTTACCTCTCCTTCCTCCCC
2890	G6PD	CAGTATGAGGGCACCTACAAG	6210	SET	GCCAAATTTTCTTTTACCTCT
2900	G6PD	ACCCCACTGCCTGCACCAGATT	6220	GAPDH	CAGACCACAGTCCATGCCATC
2910	G6PD	CGCCACGTAGGGGTGCCCTTC	6230	GAPDH	ATCTTCTAGGTATGACAACGA
2920	RPL4	GCTTGTAGTGCCGCTGCTGCA	6240	RPLP1	TTTGTTGTAGGAGGATAAGAT
2930	RPL4	CCGTGGTGCTCGAAGGGCTCT	6250	RPLP1	TTGTAGGAGGATAAGATCAAT
2940	RPL4	TTGCAGCACAAGCTCCGGGTG	6260	RPLP1	TAGCTGAGGAGAAGAAAGTGG
2950	RPL4	TGCCTAATTTGTTGCAGCACA	6270	RPLP1	CCACCATCACCTTACCTTTGC
2960	RPL4	TAGCAAGAAGATCCATCGCAG	6280	RPLP1	CTACCTGGAGCAGCAGCAGCTG
2970	RPL4	AGTCTTCCCATGCACAAGATG	6290	CFL1	CTCTTAAGGGGCGCAGACTCG
2980	RPL4	CCTTTCAGTCTTCCCATGCAC	6300	CFL1	TAGGGATCAAGCATGAATTGC
2990	EEF1G	TCCCCAGCTGAGTCCAGATTG	6310	CFL1	TTCTTTATAGGGATCAAGCAT
3000	EEF1G	TTCCTCTTAGTACCTTTGTGT	6320	CFL1	TGTCCAGGGCCCCCGAGTCTG
3010	RPL31	GATGGCTCCCGCAAAGAAGGG	6330	RPS15	CTCTTGGTCTCCCGCAGCCCG
3020	RPL31	AATCGTAGGGGCTTCAAGAAG	6340	TPT1	CATTATTTATTTTAACCCACT
3030	RPL31	TTAGGAATGTGCCATACCGAA	6350	TPT1	TTTTAACCCACTTCCTTGTAC
3040	RPL31	CAGATCTACAGACAGTCAATG	6360	TPT1	ACCCACTTCCTTGTACTTACA
3050	RPL31	GCACCTTATTCCTTTGGCCCA	6370	TPT1	CCTGGTAGTTTTTGAAATTAG
3060	RPL31	TGGGATGGAGAACTTACTTTT	6380	TPT1	GAAATGGAAAAATGTGTAAGT
3070	RPL31	ATCTGACGATCAGCGATTAGT	6390	TPT1	CTTCCCAAGTTCTTTATTGGT

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SEQ ID NO	Gene	Target Domain Sequence (DNA)
3080	ITM2B	ACTGTCTTTTTCATATTTTAG
3090	ITM2B	ATATTTTAGGACCCAGATGAT
3100	ITM2B	GGACCCAGATGATGTGGTACC
3110	ITM2B	GACTAGCATTTATGCTTGCAG
3120	ITM2B	TGCTTGCAGGTGTTATTCTAG
3130	ITM2B	TGAATGTAGGCTGGAACCTAT
3140	ITM2B	CCTCAGTCCTATCTGATTCAT
3150	ITM2B	TTTATTTATCGACTGTGTCAT
3160	ITM2B	TTTATCGACTGTGTCATGACA
3170	ITM2B	TCGACTGTGTCATGACAAGGA
3180	ITM2B	CCTCTCCAACAGGTATTCAGA
3190	ITM2B	GCAATTCGGCATTTTGAAAAC
3200	ITM2B	AAAACAAATTTGCCGTGGAAA
3210	ITM2B	CCGTGGAAACTTTAATTTGTT
3220	ITM2B	GCCAACTGGTACCACATCATC
3230	ITM2B	TACAAGTATGCTCCTCCTAGA
3240	ITM2B	CACTTACTTGAAGTGCAAAAT
3250	ITM2B	AATGCGATCAGTAATAACCAT
3260	ITM2B	CTTGTCATGACACAGTCGATA
3270	ITM2B	TAAGTTTCCTTGTCATGACAC
3280	ITM2B	TCTGCGTTGCAGTTTGTAAGT
3290	ITM2B	ATAGTTTCTCTGCGTTGCAGT
3300	ITM2B	AAAAGTATTACCTTTAATAGT
3310	ITM2B	ATATTTAAAAAGTATTACCTT
3320	ITM2B	AAAATGCCGAATTGCGAAACA
3330	ITM2B	TTTTCAAAATGCCGAATTGCG
3340	ITM2B	CACGGCAAATTTGTTTTCAAA
3350	ITM2B	TTGACTGTTCAAGAACAAATT

SEQ ID NO	Gene	Target Domain Sequence (DNA)
6400	TPT1	TTTGCTTCCCAAGTTCTTTAT
6410	TPT1	GAATCAAAGGGAAACTTGAAG
6420	TPT1	TTAATGCAGATGGTCAGTAGG
6430	RPL23	CTACCTTTCATCTCGCCTTTA
6440	RPL23	TTGTTCACTATGACTCCTCCA
6450	RPL23	CTCACCCTTTTTTCTGAGCTC
6460	RPL23	ATGCAGGTTCTGCCATTACAG
6470	RPL23	TTTTTTTAATGCAGGTTCTGC
6480	RPL23	TTCTCTCAGTACATCCAGCAG
6490	RPL34	ACTTTCTAGGTCCCGAACCCC
6500	RPL34	TAGGTCCCGAACCCCTGGTAA
6510	RPL34	TTATGCAGGTTCGTGCTGTAA
6520	RPL34	GTATTTTCCTTTCTAGGATCA
6530	RPL34	CTTTCTAGGATCAAGCGTGCT
6540	RPL34	TAGGATCAAGCGTGCTTTCCT
6550	RPL34	AGAAATACTTACAGCCTACTT
6560	RPL34	ACTTACCTGTCACGAACACAT
6570	RPL34	AGCATTTAACTTACCTGTCAC
6580	COX4I1	TCTTTCAGAATGTTGGCTACC
6590	COX4I1	AGAATGTTGGCTACCAGGCTA
6600	COX4I1	CACCTCTGTGTGTGTACGAGC
6610	COX4I1	TTCAATATGTTTTTCAGAAAG
6620	COX4I1	AGAAAGTGTTGTGAAGAGCGA
6630	COX4I1	GCTCCCAGCTTATATGGATCG
6640	COX4I1	CTGAGATGAACAGGGGCTCGA
6650	COX4I1	ACCGCGCTCGTTATCATGTGG
6660	COX4I1	ACAAAGAGTGGGTGGCCAAGC
6670	COX4I1	TCAAAGCTTTGCGGGAGGGGG

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SEQ ID NO	Gene	Target Domain Sequence (DNA)
3360	RPL23A	CTTTTCTCCCAGCTCCTGCCC
3370	RPL23A	TCCCAGCTCCTGCCCCTCCTA
3380	RPL23A	CCTCTCCCAGGCTTGACCACT
3390	RPL23A	TTTTTCAGATTGGGATCATCT
3400	RPL23A	TAGGAAGGAAACTTACTTTGT
3410	RPL27A	GTCTGGGCTGCCAACATGGTA
3420	RPL27A	TATTCCTGCAGGCAAGCACCG
3430	RPL27A	TCTGTTCTTCTAGGGCTACTA
3440	PCBP2	CCCTCTGACTCTCTCCCAGTC
3450	PCBP2	CTCCTTTTGTAGGCCTATACC
3460	PCBP2	TAGGCCTATACCATTCAAGGA
3470	PCBP2	CTCCTTGCAGTTGACCAAGCT
3480	PCBP2	ACTTGTATCTTAACAGGCATT
3490	PCBP2	GCAGGTTTGGATGCATCTGCT
3500	PCBP2	TTTCTCCCTTAAGTTGATTGG
3510	PCBP2	TCCCTTAAGTTGATTGGCTGC
3520	PCBP2	TGTGTTACAGGCTTTCCTCGG
3530	PCBP2	AGCATGAGCCTGAGGGCTTAC
3540	PCBP2	TTACCTGACCACCTGCAAAGA
3550	PCBP2	ATCATTACCCCAATAGCCTTT
3560	HSPA8	TCTTCCTCAGACTGCTGAGAA
3570	HSPA8	CTAGGCCGTTTGAGCAAGGAA
3580	HSPA8	TTTCCTAGGCCGTTTGAGCAA
3590	HNRNPK	ATCAGCACTGAAACCAACCTG
3600	HNRNPK	AGTTGGCTGGATCTATTATTG
3610	HNRNPK	AAAAATCTTTTCAGTTGGCTG
3620	HNRNPK	AATCAGATTATTCCTATGCAG
3630	HNRNPK	TGTTTTTAGGGTGGCTCCGGA

SEQ ID NO	Gene	Target Domain Sequence (DNA)
6680	COX4I1	GTAGTCCCACTTGGAGGCTAA
6690	RPL27	TCCTTGCTCTCTGCAGAAATG
6700	RPL27	GAACATTGATGATGGCACCTC
6710	RPL27	TCCCCAGGTACTCTGTGGATA
6720	RPL27	CCTTCTAGATACAAGACACGC
6730	RPL27	CGTCCGGAGTAGCGTCCAGCC
6740	RPL27	TCTTTGATCTCTTGGCGATCT
6750	RPL27	ACAAAAGATTTTATCTTTCAT
6760	EDF1	GAGGCTTTGTGTTCATTTCGC
6770	EDF1	TGTTCATTTCGCCCCTAGGCCC
6780	EDF1	GCCCTAGGCCCCCTTCTCGATG
6790	EDF1	CAATGTCCTTTCCCCCGGAGCT
6800	EDF1	CCAAGCACCTGGTTATTGGGT
6810	EDF1	TTGGAAGTCTCCACATCTTCT
6820	EDF1	GCCTGGGCGGCCGTAGGGCCC
6830	EDF1	AGGCCTCAAGCTCCGGGGAAA
6840	EDF1	GAAAATCAATGAGAAGCCACA
6850	EDF1	CCTCACACCGACTCCAGGGGC
6860	EDF1	TAGGCTATCTTAGCGGCACAG
6870	EDF1	TAATTTTCTAGGCTATCTTAG
6880	TMEM59	AAAGAAAAATGCTTAAATTTC
6890	TMEM59	AGAATGAGCAAGATTCACTTT
6900	TMEM59	TAGGTAGAGGCCCTGCTTCTT
6910	TMEM59	GATCTAACAACCACACAAGAGAA
6920	TMEM59	GCTTTTGTTCATTCATAAACT
6930	TMEM59	TTCATTCATAAACTCCAAGTC
6940	TMEM59	CCTCAGAGGGAACATACTGCT
6950	TMEM59	TCCATCTTCAAGAAAATTCCT

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SEQ ID NO	Gene	Target Domain Sequence (DNA)	SEQ ID NO	Gene	Target Domain Sequence (DNA)
3640	HNRNPK	TTTCTGTTTTTAGGGTGGCTC	6960	TMEM59	CTTAGAGATGATTCTCTCAAA
3650	HNRNPK	TCTCTAACAGGTTGGTTTCAG	6970	TMEM59	TAGGCTCCTGCTCCAAATGTG
3660	RPL5	TCTCTTACTATAGATTGCTTA	6980	TMEM59	CGTCATCGGCTTGAAGATAAA
3670	RPL5	CATTGGTTTCTTGAATAGCTT	6990	TMEM59	TGAATGAACAAAAGCTAAACA
3680	RPL5	TTGAATAGCTTCTCAATAGGT	7000	TMEM59	CAGAAGCTGAGTATCTATCGT
3690	UBL5	TGTAGCTCCAGCTAGGATGAT	7010	TMEM59	TTTTGCAGAAGCTGAGTATCT
3700	UBL5	CCTTAACTGCTCTGCGCCCAG	7020	TMEM59	TTGTGCAACTGTTGCTACAGC
3710	UBL5	TTAGGTACACGATTTTTAAGG	7030	TMEM59	GATTTGTTGTGCAACTGTTGC
3720	UBL5	CTTCAGATGAAATCCACGATG	7040	TMEM59	ACTACAACTCTTGTCCTCTCG
3730	CST3	GACAAGGTCATTGTGCCCTGC	7050	TMEM59	CAGTAACTCTGGGTGGATTTT
3740	CST3	AGATGTGGCTGGTCATGGAAG	7060	TMEM59	TTGAAGATGGAGAAAGTGATG
3750	CST3	TTGTACTCGCCGACGGCAAAG	7070	TMEM59	AGCAGATCTGCAAATGAGAAA
3760	CST3	CAGATCTACGCTGTGCCTTGG	7080	TMEM59	AGAGAATCATCTCTAAGCAAA
3770	CST3	ACAGAAAGCATTCTGCTCTTT	7090	TMEM59	GAGCAGGAGCCTACAAATTTG
3780	CST3	CTTTCACAGAAAGCATTCTGC	7100	TMEM59	GTCTAAGCCAGAAATCCAGTA
3790	CST3	ACATGTGTAGATCGTAGCTGG	7110	TMEM59	ATTATTATTTTAGTCTAAGCC
3800	CST3	CCGTCGGCGAGTACACAACAAG	7120	TMEM59	TCTTCAAGCCGATGACGGAAA
3810	RPS29	TCACCAAGAGCGAGAACCCTG	7130	DYNLL1	TCTTTTCCAGGAATTTGACAA
3820	RPS29	TTACAGTCGTGTCTGTTCAAA	7140	DYNLL1	CAGGAATTTGACAAGAAGTAC
3830	RPS10	TACTGTACATGCTTCCTTTTT	7150	DYNLL1	ATGTGTCACATAACTACCGAA
3840	RPS10	CAAATGACATTATCTGAGAGC	7160	NME2	TTTCTTAGGAACATCATTCAT
3850	RPS10	CTCACGTGGCACAGCACTCCG	7170	NME2	TTAGGAACATCATTCATGGCA
3860	RPS10	TGTGGGAACCATACCTTTAGG	7180	TMBIM6	GCTGATGGCAACACCTCATAG
3870	RPS10	TAAAAGGAAGCATGTACAGT	7190	TMBIM6	TGTTTTCTAGGAGTTGGCCTG
3880	RPS10	TCCTATGGCAGGTCCTCATAG	7200	TMBIM6	TAGGAGTTGGCCTGGGCCCTG
3890	RPS10	TAGCTGGTGCCGACAAGAAAG	7210	TMBIM6	TATTGCTGTCAACCCCAGGTA
3900	RPS10	ACTTTCTAGCTGGTGCCGACA	7220	TMBIM6	TAACAGCATCCTTCCCACTGC
3910	RPS10	CATAGGTCTGGAGGGTGAGCG	7230	TMBIM6	ATGGGCACGGCAATGATCTTT

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3920	RPS10	ATTTACATAGGTCTGGAGGGT	7240	TMBIM6	CCTGCTTCACCCTCAGTGCAC
3930	RPS10	TGCCTTACAGTCTCTCAAGTC	7250	TMBIM6	CTGTGTCTTATAGGTATCTTG
3940	RPL6	TTACCAGTCACACAAGTAATAAG	7260	TMBIM6	TCTTCCCTGGGGAATGTTTTC
3950	RPL6	GAAATATGAGATTACGGAGCA	7270	TMBIM6	GATCCATTTGGCTTTTCCAGG
3960	RPL6	TTTAGAAATATGAGATTACGG	7280	TMBIM6	TTAGGCAAACCTGTATGTCGG
3970	RPL6	TCTTTATTTAGAAATATGAGA	7290	TMBIM6	ATACTCAACTCATTATTGAAA
3980	RPL6	ATTTTCTCTTTATTTAGAAAT	7300	TMBIM6	AGGCACTGCATTGATCTCTTC
3990	RPL6	CCCCTTAGGACCTCTGGTCCT	7310	TMBIM6	ATTACTGTCTTCAGAAAACTC
4000	RPL6	ACTTACAGAGGGTGGTTTTCC	7320	TMBIM6	TCCATTTCTAGGATAAGAAGA
4010	RPL6	TTTTTAACTTACAGAGGGTGG	7330	TMBIM6	TAGGATAAGAAGAAAGAGAAG
4020	RPLP2	TGTAGGTATTGGCAAGCTTGC	7340	TMBIM6	ATGGCTATGAGGTGTTGCCAT
4030	ARF1	ACACTGGCTGCCCGGCAGGCC	7350	TMBIM6	TGTTCAGTTTCATGGCTATGA
4040	RPL15	TGTGTAGGTTACGTTATATAT	7360	TMBIM6	CCAGTTCACACTTACCTCCCA
4050	RPL15	CTATTCTAGGAGCGAGCTGGA	7370	TMBIM6	AATAATGAGTTGAGTATCAAA
4060	RPL15	CCTCTGCAACGGACTGAAGGC	7380	TMBIM6	TGAAGACAGTAATGAAATCTA
4070	FAU	CTGGCCGGTCACCTCGAAGGT	7390	TMBIM6	ATTCATGGCCAGGATCATCAT
4080	FAU	CCTGTAGGCTCATGTAGCCTC	7400	TMBIM6	GGTTGTAGGCTAACTAACCTT
4090	FAU	CTCAGTCGCCAATATGCAGCT	7410	RPS7	TTTAGGAAATTGAAGTTGGTG
4100	FAU	TTTACTCAGTCGCCAATATGC	7420	RPS7	GGAAATTGAAGTTGGTGGTGG
4110	RPL36	CCCCCTAGCGTCTGACCAAAC	7430	RPS7	CCTTACAGAGGAGAATTCTGC
4120	RPL36	CCCCGTACGAGCGGCGCGCCA	7440	RPS7	AACTATTCTTTTAGCCGTACT
4130	NACA	CTAGTATACCTCTTCCTCTTC	7450	RPS7	GCCGTACTCTGACAGCTGTGC
4140	NACA	CTCACCTTGGCTTCCCCAAAA	7460	RPS7	TTTTCTTGTAGGTTGAAACTT
4150	NACA	AAATCTTACCTTCCGTGCCTT	7470	RPS7	TTGTAGGTTGAAACTTTTCT
4160	NACA	TCTGTTACAGGAATTAACAAT	7480	RPS7	TGAAACTACTAAAATACTCAC
4170	NACA	CCTCTCATCTCTCAGGTCGAT	7490	ACTB	CTTCCCAGGGCGTGATGGTGG
4180	NACA	TACCCTGTAGATCGAAGATTT	7500	NPM1	ATTTGTAGTGATGATGATGCAT
4190	NACA	GGCTATGTCCAAACTGGGTCT	7510	NPM1	TAATTGCAGTCTATACGACAT

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SEQ ID NO	Gene	Target Domain Sequence (DNA)	SEQ ID NO	Gene	Target Domain Sequence (DNA)
4200	NACA	TCTTCTTTAGGCTATGTCCAA	7520	NPM1	GAAATTCATTTCTTTTTCAGG
4210	NACA	TCTTCTTAGCTGGCGGCAGCA	7530	NPM1	TTTTTCAGGGACAAGAATCCT
4220	PRDX1	GACATCAGGCTTGATGGTATC	7540	NPM1	AGGGACAAGAATCCTTCAAGA
4230	PRDX1	CCATGCTAGATGACAGAAGTG	7550	NPM1	TCTTAATAGGGTGGTTCTCTT
4240	PRDX1	TTAAATTCTTCTGCCCTATCA	7560	NPM1	CAGGCTATTCAAGATCTCTGG
4250	PRDX1	TCTTGCAGTGTGCCCAGCTGG	7570	NPM1	TAAAATCATACTTACTCTTCA
4260	PRDX1	TCATTGATGATAAGGGTATTC	7580	NPM1	CTCACTTTTTCTATACTTGCT
4270	PRDX1	CCAGGGGCCTTTTTATCATTG	7590	RPS6	TTTTTCTTGGTACGCTGCTTC
4280	PRDX1	ATCTCTTTTCCCAGGGGCCTT	7600	RPS6	GGGCCCAGGCGGCGAGGCACT
4290	PRDX1	CTTTCATCTCTTTTCCCAGGG	7610	RPS6	GGAGGCTAAGGAGAAGCGCCA
4300	PRDX1	GTATCAGACCCGAAGCGCACC	7620	RPS6	TTTAGGAGGCTAAGGAGAAGC
4310	PRDX1	CCATAGGGTCAATACACCTAA	7630	RPS6	TTTTGTTTAGGAGGCTAAGGA
4320	PRDX1	CCTTTTGCCATAGGGTCAATA	7640	RPS6	GGTAAGAAACCTAGGACCAAA
4330	PRDX1	AGTGATAGGGCAGAAGAATTT	7650	RPS6	AATTTTTAGGTAAGAAACCTA
4340	PRDX1	CCCTCTTGACTTCACCTTTGT	7660	RPS6	TTCTAAGGAGAGAAGGATATT
4350	PRDX1	CCCCCAGGAAAATATGTTGTG	7670	RPL12	CTTAAAGGAACCATTAAAGAG
4360	ALDOA	CCTTCTCGGTCACATACTGGC	7680	RPL12	TTTACTTAAAGGAACCATTAA
4370	NCL	GCCCAGTCCAAGGTAACTTTA	7690	RPL12	CTCTTCTGCAGTTAAACACAG
4380	NCL	TTTCCATCAATTTCACCGTCT	7700	RPL12	CTGTTTCCTCTTCTGCAGTTA
4390	NCL	CATCAATTTCACCGTCTTCCA	7710	RPL12	TAGTCTCCAAAAAAAGTTCGT
4400	NCL	ACCGTCTTCCATGGCCTCCTT	7720	RPL12	TTTCTAGTCTCCAAAAAAAGT
4410	NCL	GCATCCTCCTCACTGTTGAAG	7730	RPL12	CCCCAGTATACCTGAGGTCCA
4420	NCL	GAGGACCCAGTTTCCCGGTCA	7740	CAPNS1	AACCTGTTACCCACAGACCCT
4430	NCL	CCGGTCAGTAACTATCCTTGC	7750	CAPNS1	GCATTGACACATGTCGCAGCA
4440	NCL	ATGTCTCTTCAGTGGTATCCT	7760	CAPNS1	AGGAATTCAAGTACTTGTCGA
4450	NCL	ACAAACAGAGTTTTGGATGGC	7770	CAPNS1	CAGTAGTGAACTCCCAGGTGC
4460	NCL	GTGGCAGAGGCCGGGGAGGCT	7780	CAPNS1	ATGTTGTTCCACAAGTACTTG
4470	NCL	GAGGACGAGGTGGTGGTAGAG	7790	CAPNS1	TACACACCTGCCACCTTTTGA

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4480	NCL	TAGACTTCAACAGTGAGGAGG	7800	CAPNS1	AGAGGTTTCTACACACCTGCC
4490	NCL	GTTTTGTAGACTTCAACAGTG	7810	CAPNS1	ATCTGAGTAGCGTCGGATGAT
4500	NCL	GTGTTCTAGGTTTGGTTTTGT	7820	CAPNS1	TCAAGAGATTTGAAGGCACCT
4510	NCL	ATTTGGTGTTCTAGGTTTGGT	7830	CAPNS1	TCCAGTGCCATCTTTGTCAAG
4520	NCL	ACGGCTCCGTTCGGGCAAGGA	7840	RPL3	CAGGGTGGCTTTGTCCACTAT
4530	NCL	TCAAAGGCCTGTCTGAGGATA	7850	RPS13	TTTATTAGCTTACCTTTCTTGT
4540	NCL	CTTCCCAGAGCCATCCAAAAC	7860	RPS13	TTAGCTTACCTTTCTTGTTCCT
4550	BTF3	TAGATGAAAGAAACAATCATG	7870	RPS13	AGTGAATCATCTACAGCCTCT
4560	BTF3	CTCTTCTCCCTGACTTTAGGG	7880	RPS13	TTTTTCAGTGAATCATCTACA
4570	BTF3	GGGAACTGCTCGCAGAAAGAA	7890	RPS13	CCCTTTTTTCTTTTTCAGTGA
4580	BTF3	TTTTCTTAATAGGTGAATATG	7900	RPS13	AGGTGTAATCCTGAGAGATTC
4590	BTF3	TTAATAGGTGAATATGTTTAC	7910	RPS13	TATTCCATAACAGTGGTTGAA
4600	BTF3	CATTTCCTTTCATAGCTGTG	7920	RPS21	TCCACAGCTCCGCTAGCAATC
4610	BTF3	CTTTCATAGCTGTGGATGGAA	7930	RPS21	TGACCCTTCTTCTCTTTCTAG
4620	BTF3	ATAGCTGTGGATGGAAAAGCA	7940	RPS21	TAGGTTGACAAGGTCACACGC
4630	BTF3	TACTCTTTTCCTTTTCCTAGA	7950	RPS21	TTAAGGGTGAGTCAGATGATT
4640	BTF3	CTTTTCCTAGATCTTGTGGAG	7960	RPS21	CCCTGGTTCTAGGAACTTTTG
4650	BTF3	CTAGATCTTGTGGAGAATTTT	7970	RPS21	AGACGATGCCATCGGCCTTGG
4660	BTF3	ATACTTGCCTCTTCAATACCA	7980	SERF2	ATTTTCTTTCCTTAGGCGCGTA
4670	E2F4	GGGGCTATCATTGTAGTGAGT	7990	SERF2	TTTCCTTAGGCGGTAACCAGC
4680	E2F4	AGCCCATCAAGGCAGACCCCA	8000	SERF2	CTTAGGCGGTAACCAGCGTGA
4690	E2F4	AGTTTTGGAACTCCCCAAAGA	8010	SERF2	TGCTGCCGCCCGCAAGCAGAG
4700	E2F4	GAACTCCCCAAAGAGCTGTCA	8020	SERF2	ATATTCTTCTGGCGGGCGAGC
4710	E2F4	CCCCTCTGCTTCGTCTTTCTC	8030	SERF2	CCTTAACCGAGTCGCTCTGCT
4720	E2F4	TCCACCCCCGGGAGACCACGA	8040	SERF2	CCTCCCCCTCCCTGGGGCTACC
4730	E2F4	ATGTGCCTGTTCTCAACCTCT	8050	RPL7A	TTTCCCCCTCCTGCCTTTTAGG
4740	E2F4	TGACAGCTCTTTGGGGAGTTC	8060	RPL7A	CCCTCCTGCCTTTTAGGGAAG
4750	KIF11	ACTAAGCTTAATTGCTTTCTG	8070	RPL7A	GGGAAGACAAAGGCGCTTTGG

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4760	KIF11	TGGAACAGGATCTGAAACTGG
4770	KIF11	TACCCATCAACACTGGTAAGA
4780	KIF11	TTCTTTTAGGATGTGGATGTA
4790	KIF11	GGATGTGGATGTAGAAGAGGC
4800	KIF11	CCGCCTTAAATCCACAGCATA
4810	KIF11	ATTAAGTTCTAGATTTTGTGC
4820	KIF11	TGGTTTCATTAAGTTCTAGAT
4830	KIF11	AGATCCTGTTCCAGAAAGCAA
4840	KIF11	AAGTACCTGTTGGGATATCCA
4850	KIF11	TCTTTTAAAGTACCTGTTGGG
4860	KIF11	AGCTGATCAAGGAGATGTTCA
4870	KIF11	CTTTTCAGCTGATCAAGGAGA
4880	KIF11	GCATCATTAACAGCTCAGGCT
4890	KIF11	TGAACAGTTTAGCATCATTAA
4900	KIF11	TTGTTTTCTGAACAGTTTAGC
4910	KIF11	CCGGAATTGTCTCTTCTTTGT
4920	KIF11	AATTTACCGGAATTGTCTCTT
4930	KIF11	TCTTTTCCATGTGATTTTTTA
4940	KIF11	TTTGTCTTTTCCATGTGATTT
4950	KIF11	GACCTCTCCAGTGTGTTAATG
4960	KIF11	TTCCACTTTAGACCTCTCCAG
4970	KIF11	TAACCAAGTGCTCTGTAGTTT
4980	RPL13	TCTTCTAGGTCTATAAGAAGG
4990	RPL13	AGTAAGTGTTCACTTACGTTC
5000	PFDN5	CCTTAATTCTTGCTTCTCAGA
5010	PFDN5	AGCTGAGCAATGGACGTGGAC
5020	PTMA	AAGGACTTAAAGGAGAAGAAG
5030	PTMA	TGTCGAGGAGAATGAGGAAAA

SEQ ID NO	Gene	Target Domain Sequence (DNA)
8080	RPL7A	TCTTTTCAGATCCGCCGTCAC
8090	RPL7A	AGATCCGCCGTCACTGGGCTG
8100	RPL7A	GGGCCAGGCTGTGTACTTACG
8110	RPL7A	GTGTAAAGCTGCCTCTTACCT
8120	HNRNPA2B1	TAAATTACCTCCACCATATGG
8130	HNRNPA2B1	CACTCTTCATTGGACCGTAGT
8140	HNRNPA2B1	CAAAATCATTGTAATTTCCAC
8150	HNRNPA2B1	TTACCTCCTCCATAGTTGTCA
8160	HNRNPA2B1	CACCGCCACCACGTGAATCCC
8170	HNRNPA2B1	GTGGTAGCAGGAACATGGCGG
8180	HNRNPA2B1	GAAATTATAACCAGCAACCTT
8190	HNRNPA2B1	ATAGGAAATTATGGAAGTCGA
8200	HNRNPA2B1	GAGGTAGCCCCGGTTATGCAG
8210	HNRNPA2B1	TAATAGGTGGCAATTTTGCAG
8220	HNRNPA2B1	GGGATGGCTATAATGGGTATG
8230	HNRNPA2B1	GCCCCTAACAGATGGATATGG
8240	HNRNPA2B1	GGACCAGGACCAGGAAGTAAC
8250	HNRNPA2B1	GGGATTCACGTGGTGGCGCTG
8260	HNRNPA2B1	GCTTTGGGATTCACGTGCTG
8270	HNRNPA2B1	TTGTAGGCAACTTTGGCTTTG
8280	HNRNPA2B1	TCTAGACAAGAAATGCAGCAA
8290	RPL13A	TCTAACAGAAAAAGCGGATGG
8300	RPL13A	GCATAGCTCACCTTGTCGTAG
8310	ENO1	AGCAGGAGGCAGTTGCAGCAC
8320	ENO1	TCCTTCCCAAGAATTGAAGAG
8330	ENO1	CCTTTCTCCTTCCCAAGAATT
8340	ENO1	TCCTAGATCAAGACTGGTGCC
8350	ENO1	TTTTCTCCTAGATCAAGACTG

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SEQ ID NO	Gene	Target Domain Sequence (DNA)
5040	PTMA	ATTCTCTCCAGGTGAGGAAGA
5050	PTMA	TCTGCTTAGGATGACGATGTC
5060	RPL11	GCATCCGGAGAAATGAAAAGA
5070	RPL11	TCCACAGGTGCGGGAGTATGA
5080	RPL11	AGCATCGCAGACAAGAAGCGC
5090	RPL11	AGTATGATGGGATCATCCTTC
5100	RPL11	CGGATGCGAAGTTCCCGCATG
5110	RPL11	TCCGGATGCCAAAGGATCTGA
5120	RPL11	ATTTCTCCGGATGCCAAAGGA
5130	RPL11	GACCCTTCTCCAAGATTTCTT
5140	RPL11	TTAACTCATACTCCCGCACCT
5150	RPL11	CCTTCTGCTGGAACCAGCGCA
5160	COX7C	TCTTTTTTTCCAACAGAATTT
5170	COX7C	CAACAGAATTTGCCATTTTCA
5180	RPL8	TTGAGGCCCTCAGCACTAGTT
5190	RPL8	CGGCCAGCAGGGGCATCTCTG
5200	RPL8	TGGGTTACTTACATTCATGGC
5210	RPL8	TCTGCCTGCAGCCTGTGGAGC
5220	RPL10	TTCTCCCTACCTAGCCCTGGA
5230	RPL10	CATTGCTCCTTAGATCCACAT
5240	RPL32	CCTCCCCAAAAGGAAGAGTTC
5250	TBP	CTGCGGTAATCATGAGGATAA
5260	TBP	AGTTCTGGGAAAATGGTGTGC
5270	TBP	CTTTCCCCTAGTGAAGAACAGT
5280	TBP	CCTAGTGAAGAACAGTCCAGA
5290	TBP	CAGCTAAGTTCTTGGACTTCA
5300	TBP	CTATAAGGTTAGAAGGCCTTG
5310	TBP	CAATTTCCTTCTAGTTATGA

SEQ ID NO	Gene	Target Domain Sequence (DNA)
8360	ENO1	CTTAGTGGTGTCTATCGAAGA
8370	PPIA	CTATATGTTGACAGGGTGGTG
8380	PPIA	AAGGTTGGATGGCAAGCATGT
8390	CD81	CCTGTGAGGTGGCCGCCGCCA
8400	CD81	ACCACCTCAGTGCTCAAGAAC
8410	CD81	TGTCCCTCGGGCAGCAACATC
8420	RPL35	TTGACAATGCGCCCCTCAGGC
8430	RPL35	TAGCCGAGTCGTCCGGAAATC
8440	DAD1	TTCTGTGGGTTGATCTGTATT
8450	DAD1	CCAGCACCATCCTGCACCTTG
8460	DAD1	TCTTTGCCAGCACCATCCTGC
8470	DAD1	CTGATTTTCTCTTTGCCAGCA
8480	DAD1	CAAGGCATCTCCCCAGAGCGA
8490	DAD1	CCTGAGAATACAGATCAACCC
8500	DAD1	CTTCTTGTGCAGTTTGCCTGA
8510	DAD1	TGTTTTGCTTCTTTGTGCAGTT
8520	DAD1	TCTCGGGCTTCATCTCTTCTG
8530	DAD1	GCGGTTCTTAGAAGAGTACTT
8540	UBA52	TGAAGACCCTCACTGGCAAAA
8550	UBA52	CCAGTGAGGGTCTTCACAAAG
8560	UBA52	TGGGCAAGCTGGCGGAGACAA
8570	UBA52	ACCTTCTTCTTGGGACGCAGG
8580	RPL30	TAGGTGAAAAGGTTTACTTTT
8590	RPL30	TGATTTAAAAAGCATACCTGG
8600	RPL30	AAAAGCATACCTGGATCAATG
8610	RPL30	GGTGACTCTGACATCATTAGA
8620	RPL30	TTTTTTAGGTGACTCTGACAT
8630	RPL30	TTTTTATTTTTTAGGTGACTC

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SEQ ID NO	Gene	Target Domain Sequence (DNA)
5320	TBP	CTTCTAGTTATGAGCCAGAGT
5330	TBP	CTGGTTTAATCTACAGAATGA
5340	TBP	ATCTACAGAATGATCAAACCC
5350	TBP	TTTCTGGAAAAGTTGTATTAA
5360	TBP	TGGAAAAGTTGTATTAACAGG
5370	TBP	GGTCAAGTTTACAACCAAGAT
5380	TBP	GGGCACGAAGTGCAATGGTCT
5390	TBP	CCAGAACTGAAAATCAGTGCC
5400	TBP	TTACGGCTACCTCTTGGCTCC
5410	TBP	TTGCTGCCAGTCTGGACTGTT
5420	TBP	AGACTTACCTACTAAATTGTT
5430	TBP	ATCATTCTGTAGATTAAACCA
5440	TBP	CAGAACAAAAATAAGGAGAA
5450	TBP	AAATGCTTCATAAATTTCTGC
5460	CD63	CTCAGCCAGCCCCCAATCTTC
5470	CD63	TCCCAATCTGTGTAGTTAGCA
5480	CD63	GGGTAATTCTCCCATCTGCTGC
5490	CD63	GGAATTGTCTCTTTGCCTGCTGC
5500	CD63	CTTCTAGGTTTTGGGAATTGT
5510	CD63	TGCCTGCCACCTTCAGGGCTG
5520	CD63	AACGAGAAGGCGATCCATAAG
5530	CD63	AGTGCTGTGGGGCTGCTAACT
5540	CD63	TTCCCTCCCCCAGTTTAAGTG
5550	CD63	ATAACAACTTCCGGCAGCAGA
5560	CD63	TGTCTCTTATCATGTTGGTGG

SEQ ID NO	Gene	Target Domain Sequence (DNA)
8640	RPL30	GTTCCCAAAGGAAATCTGAAA
8650	RPL30	CCCATTTTGGTTCCCAAAGGA
8660	RPL30	TAGAAAAAGTCGCTGGAGTCG
8670	RPL30	CTTTGTAGAAAAAGTCGCTGG
8680	RPL30	ATGTTTGCTTTGTAGAAAAAG
8690	RNASEK	CGCCTGCCGCCCCCGGATGGG
8700	RNASEK	TCCCACCGCTTTCCGAGCCCG
8710	RNASEK	CGAGCCCGCTTGCACCTCCGC
8720	RNASEK	TGGCGTCGCTCCTGTGCTGTG
8730	RPL38	TGTTGCAGCCTCGGAAAATTG
8740	RPL38	TCTCTTTCCCTCTAGGTTTGG
8750	RPL38	CCTCTAGGTTTGGCAGTGAAG
8760	RPL38	GTCGGGCTGTGAGCAGGAAGT
8770	MYL12B	TTCTTTCTATTGTCTTCCAGG
8780	MYL12B	TATTGTCTTCCAGGCACCATT
8790	MYL12B	GCTAAAGTTCTTTCAGTCATC
8800	PFN1	CCCATCAGCAGGACTAGCCCT
8810	PFN1	CTCCTCCTCCAGCGCTAGTCC
8820	PFN1	TCTTTCCTCCTCCTCCAGCGC
8830	PFN1	GCATGGATCTTCGTACCAAGA
8840	RPS11	TCCTCATAATCTGTAGACTGA
8850	RPS11	TCTTTCCTATCCTTTCAGCCT
8860	RPS11	CTATCCTTTCAGGCTATTGAG
8870	RPS11	AGGCTATTGAGGGCACCTACA
8880	RPS11	TTCTGAGGTTCCCCGCACCTC

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Example 17: Computation Screening of Guide RNAs for Selection by Essential-Gene Knock-in

[0666] The present example describes a method for computationally screening for gRNAs more likely to be suitable for use in targeting essential genes using the selection methods herein that are relevant for different RNA-guided nucleases and variants thereof (e.g., variants of Cas12a, such as Mad7), so long as the RNA-guided nucleases exhibit high cutting efficiency. Cas12b, Cas12e, Cas-Phi, Mad7, and SpyCas9 gRNAs targeting essential genes described preceding examples (GAPDH, TBP, E2F4, G6PD, and KIF11) were selected for this analysis, but a similar process could be applied to identify gRNAs for these RNA-guided nucleases in other essential genes as well. The results of this screening are summarized in Tables 21-25, these gRNAs facilitate DNA cleavage within the last 500 bp of the coding sequences of the listed essential genes.

[0667] Potential target sequences for each of the essential genes in this analysis (GAPDH, TBP, E2F4, G6PD, and KIF11) were generated by searching for nuclease specific PAMs (ATTN, TTCN, TTN, TTN, and NGG for Cas12b, Cas12e, CasΦ, Mad7, and SpyCas9 respectively) with suitable protospacers mapped to a representative coding region (mRNA -201). Transcripts with its name followed by "-201" were selected as the representative for each gene (e.g., GAPDH-201). Gene information (i.e., coding region) was obtained from GENCODE v.37 gene annotation GTF file. Potential gRNAs were first searched within the genomic regions of target genes in the human reference genome (hg38), and those identified gRNAs with their cut sites within 500bp of the representative coding region stop site were selected for further analysis. The candidate gRNAs were then aligned to the human reference genome (e.g., hg38) with BWA Aln (maximum mismatch tolerance -n 2). Guides with potential off target binding sites (i.e., aligning to multiple genomic regions; mapping quality MAPQ <30) were filtered out. The resultant gRNAs target essential genes within 500 coding base pairs of a representative stop-codon and have no identical off-target binding sites annotated in the human genome. Thus, gRNAs in Tables 21-25, corresponding to SEQ ID NOs: 8890-18850, represent excellent candidate gRNAs for applying the selection methods described herein to GAPDH, TBP, E2F4, G6PD, and KIF11.

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Table 21: Cas12b guide RNAs

SEQ ID NO	Gene	Target Domain Sequence (DNA)	SEQ ID NO	Gene	Target Domain Sequence (DNA)
8890	GAPDH	CCCAGCTCTCATACCATGAGTCC	9170	E2F4	CCAGAGTGCATGAGCTCGGAGCT
8900	TBP	TATCCACAGTGAATCTTGGTTGT	9180	E2F4	TATCTACAACCTGGACGAGAGTG
8910	TBP	CACTTCGTGCCCGAAACGCCGAA	9190	E2F4	CCTGGACTTCTGCACTGCCAGGG
8920	TBP	TCTCTGACCATTGTAGCGGTTTG	9200	E2F4	CTGACAGCTCTTTGGGGACTTCC
8930	TBP	TAGCGGTTTGCTGCGGTAATCAT	9210	G6PD	AGCTGGAGAAGCCCAAGCCCATC
8940	TBP	TCAGTTCTGGGAAAATGGTGTGC	9220	G6PD	TCACCCCACTGCTGCACCAGATT
8950	TBP	AGAATATGGTGGGGAGCTGTGAT	9230	KIF11	ATGAAGATAAATTGATAGCACAA
8960	TBP	TCCTTCTAGTTATGAGCCAGAGT	9240	KIF11	ATAGCACAAAATCTAGAACTTAA
8970	TBP	CCTGGTTTAATCTACAGAATGAT	9250	KIF11	GTTTGACTAAGCTTAATTGCTTT
8980	TBP	TTCTCCTTATTTTTGTTTCTGGA	9260	KIF11	CTTTCTGGAACAGGATCTCAAAC
8990	TBP	TTGTTTCTGGAAAAGTTGTATTA	9270	KIF11	ATACCCATCAACACTGGTAAGAA
9000	TBP	ATGAAGCATTTGAAAACATCTAC	9280	KIF11	TTCATCAATTGGCGGGGTTCCAT
9010	TBP	TAAAGGGATTCAGGAAGACGACG	9290	KIF11	GCGGGGTTCCATTTTTCCAGGTA
9020	TBP	GGCGTTTCGGGCACCAAGTGCAA	9300	KIF11	TCCCGCCTTAAAATCCACACCATA
9030	TBP	TATTCGGCGTTTCGGGCACGAAG	9310	KIF11	ACACACTGGAGAGGTCTAAAGTG
9040	TBP	AAATAGATCTAACCTTGGGATTA	9320	KIF11	CCTCTGCGAGCCCAGATCAACCT
9050	TBP	TCCCAGAACTGAAAATCAGTGCC	9330	KIF11	AGTTCTAGATTTTGTGCTATCAA
9060	TBP	CTTACGGCTACCTCTTGGCTCCT	9340	KIF11	TTATGGTTTCATTAAGTTCTAGA
9070	TBP	TCTTGCTGCCAGTCTGGACTGTT	9350	KIF11	AGCTTAGTCAAACCAATTTTTAT
9080	TBP	TGAATCTTGAAGTCCAAGAACTT	9360	KIF11	CTCTTTTAAAGTACCTGTTGGGA
9090	TBP	TTGGTGGGTGAGCACAAGGCCTT	9370	KIF11	TATTTCTCTTTTAAAGTACCTGT
9100	TBP	CAGACTTACCTACTAAATTGTTG	9380	KIF11	ACAGCTCAGGCTGTTTCCTTTTC
9110	TBP	AACCAGGAAATAACTCTGGCTCA	9390	KIF11	TCTCTTCTTTGTTGTTTTCTGAA
9120	TBP	TGTAGATTAAACCAGGAAATAAC	9400	KIF11	ACCGGAATTGTCTCTTCTTTGTT
9130	TBP	TGGGTTTGATCATTCTGTAGATT	9410	KIF11	ATGAACAATCCACACCAGCATCT
9140	TBP	CTGCTCTGACTTTAGCACCTAAG	9420	KIF11	AAGGTTGATCTGGGCTCGCAGAG
9150	TBP	CGTCGTCTTCCTGAATCCCTTTA	9430	KIF11	CCAACCCCCAAGTGAATTAAAGG

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SEQ ID NO	Gene	Target Domain Sequence (DNA)	SEQ ID NO	Gene	Target Domain Sequence (DNA)
9160	E2F4	TAGTGAGTGGCGGCCCTGGGACT	-	-	-

Table 22: Cas12e guide RNAs

SEQ ID NO	Gene	Target Domain Sequence (DNA)	SEQ ID NO	Gene	Target Domain Sequence (DNA)
9440	GAPDH	TCTTCTAGGTATGACAACGAA	9930	E2F4	CTGGACTTCTGCACTGCCAGG
9450	GAPDH	CCAGCTCTCATACCATGAGTC	9940	E2F4	GACAGCTCTTTGGGGAGTTCC
9460	TBP	TGCCCGAAACGCCGAATATAA	9950	E2F4	GAGGACATCAACTCCTCCAGC
9470	TBP	CTCTGACCATTGTAGCGGTTT	9960	E2F4	AGGGCCACCCACCTTCTGAGG
9480	TBP	GTTCTGGGAAAATGGTGTGCA	9970	E2F4	CTCTCGTCCAGGTTGTAGATA
9490	TBP	GGGAAAATGGTGTGCACAGGA	9980	G6PD	CCCACTTGTAGGTGCCCTCAT
9500	TBP	TTTCCCTAGTGAAGAACAGTC	9990	G6PD	TCAGCTCGTCTGCCTCCGTGG
9510	TBP	CTAGTGAAGAACAGTCCAGAC	10000	G6PD	TCACCTGCCATAAATATAGGG
9520	TBP	AGCTAAGTTCTTGGACTTCAA	10010	G6PD	CCAGCTCAATCTGGTGCAGCA
9530	TBP	TGGACTTCAAGATTCAGAATA	10020	G6PD	CTGTAGGGCACCTTGTATCTG
9540	TBP	AGATTCAGAATATGGTGGGGA	10030	G6PD	TGGTCATCATCTTGGTGTACA
9550	TBP	GAATATGGTGGGGAGCTGTGA	10040	G6PD	GGGCCTTGCCGCAGCGCAGGA
9560	TBP	TATAAGGTTAGAAGGCCTTGT	10050	G6PD	AGTATGAGGGCACCTACAAGT
9570	TBP	TTCTAGTTATGAGCCAGAGTT	10060	G6PD	CCCCACTGCTGCACCAGATTG
9580	TBP	AGTTATGAGCCAGAGTTATTT	10070	G6PD	GCGGGAGCCAGATGCACTTCG
9590	TBP	TGGTTTAATCTACAGAATGAT	10080	G6PD	ACCCCGAGGAGTCGGAGCTGG
9600	TBP	CCTTATTTTTGTTTCTGGAAA	10090	G6PD	TCAACCCCGAGGAGTCGGAGC
9610	TBP	GGAAAAGTTGTATTAACAGGT	10100	G6PD	ACCAGCAGTGCAAGCGCAACG
9620	TBP	TAGGTGCTAAAGTCAGAGCAG	10110	G6PD	ATGATGTGGCCGGCGACATCT
9630	TBP	AAAGGGATTCAGGAAGACGAC	10120	G6PD	TCCTGCGCTGCGGCAAGGCCC
9640	TBP	GGCACGAAGTGCAATGGTCTT	10130	G6PD	GCCACGTAGGGGTGCCCTTCA
9650	TBP	GCGTTTCGGGCACGAAGTGCA	10140	KIF11	GGAACAGGATCTGAAACTGGA
9660	TBP	TGGCTCTCTTATCCTCATGAT	10150	KIF11	GAAAACAACAAAGAAGAGACA

SEQ ID NO	Gene	Target Domain Sequence (DNA)
9670	TBP	CAGAACTGAAAATCAGTGCCG
9680	TBP	TACGGCTACCTCTTGGCTCCT
9690	TBP	TGCTGCCAGTCTGGACTGTTC
9700	TBP	GTACAACTCTAGCATATTTTC
9710	TBP	GAATCTTGAAGTCCAAGAACT
9720	TBP	CATCACAGCTCCCCACCATAT
9730	TBP	AACCTTATAGGAAACTTCACA
9740	TBP	GACTTACCTACTAAATTGTTG
9750	TBP	GTAGATTAAACCAGGAAATAA
9760	TBP	GGGTTTGATCATTCTGTAGAT
9770	TBP	AGAAACAAAAATAAGGAGAAC
9780	TBP	TGTTACAACTTACCTGTTAAT
9790	TBP	GCTCTGACTTTAGCACCTAAG
9800	TBP	TAAATTTCTGCTCTGACTTTA
9810	TBP	AATGCTTCATAAATTTCTGCT
9820	TBP	TGAATCCCTTTAGAATAGGGT
9830	E2F4	CTCCCACTGGGCCCAACACA
9840	E2F4	GCCCTGCTGGACAGCAGCAGC
9850	E2F4	TCCGGACCCAACCCTTCTACC
9860	E2F4	ACCTCCTTTGAGCCCATCAAG
9870	E2F4	TGTTTTTCAGTTTTGGAACTC
9880	E2F4	GTTTTGGAACTCCCCAAAGAG
9890	E2F4	CAGAGTGCATGAGCTCGGAGC
9900	E2F4	TCTTTCTCCACCCCCGGGAGA
9910	E2F4	CCACCCCCGGGAGACCACGAT
9920	E2F4	GCACTGCCAGGGACAGCAGTG

SEQ ID NO	Gene	Target Domain Sequence (DNA)
10160	KIF11	TCTTTTAGGATGTGGATGTAG
10170	KIF11	TTTAGGATGTGGATGTAGAAG
10180	KIF11	GGGGCAGTATACTGAAGAACC
10190	KIF11	TCAATTGGCGGGGTTCCATTT
10200	KIF11	CGCCTTAAATCCACAGCATAA
10210	KIF11	AGATTTTGTGCTATCAATTTA
10220	KIF11	TTAAGTTCTAGATTTTGTGCT
10230	KIF11	AGAAAGCAATTAAGCTTAGTC
10240	KIF11	GATCCTGTTCCAGAAAGCAAT
10250	KIF11	CTTTTAAAGTACCTGTTGGGA
10260	KIF11	ATTTCTCTTTTAAAGTACCTG
10270	KIF11	TCTGTGGTGTCGTACCTTTAA
10280	KIF11	TACCAGTGTTGATGGGTATAA
10290	KIF11	GTTCTTACCAGTGTTGATGGG
10300	KIF11	CGTGGTTCAGTTCTTACCAGT
10310	KIF11	GCTGATCAAGGAGATGTTCAC
10320	KIF11	TTTTCAGCTGATCAAGGAGAT
10330	KIF11	GAACAGTTTAGCATCATTAAC
10340	KIF11	TTGTTGTTTTCTGAACAGTTT
10350	KIF11	GTATACTGCCCCAGAACTGCC
10360	KIF11	TCAGTATACTGCCCCAGAACT
10370	KIF11	ATGTGATTTTTTATGCTGTGG
10380	KIF11	TTGTCTTTTCCATGTGATTTT
10390	KIF11	ACTTTAGACCTCTCCAGTGTG
10400	KIF11	TCCACTTTAGACCTCTCCAGT
-	-	-

Table 23: Cas-Phi guide RNAs

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SEQ ID NO	Gene	Target Domain Sequence (DNA)
10410	GAPDH	TGCAGACCACAGTCCATGCCA
10420	GAPDH	GCAGACCACAGTCCATGCCAT
10430	GAPDH	CAGACCACAGTCCATGCCATC
10440	GAPDH	TCATCTTCTAGGTATGACAAC
10450	GAPDH	CATCTTCTAGGTATGACAACG
10460	GAPDH	ATCTTCTAGGTATGACAACGA
10470	GAPDH	TAGGTATGACAACGAATTTGG
10480	GAPDH	CCCAGCTCTCATACCATGAGT
10490	TBP	TATCCACAGTGAATCTTGGTT
10500	TBP	GTTGTAAACTTGACCTAAAGA
10510	TBP	TAAACTTGACCTAAAGACCAT
10520	TBP	ACCTAAAGACCATTGCACTTC
10530	TBP	CACTTCGTGCCCGAAACGCCG
10540	TBP	GTGCCCGAAACGCCGAATATA
10550	TBP	TCTCTGACCATTGTAGCGGTT
10560	TBP	TAGCGGTTTGCTGCGGTAATC
10570	TBP	GCTGCGGTAATCATGAGGATA
10580	TBP	CTGCGGTAATCATGAGGATAA
10590	TBP	TCAGTTCTGGGAAAATGGTGT
10600	TBP	CAGTTCTGGGAAAATGGTGTG
10610	TBP	AGTTCTGGGAAAATGGTGTGC
10620	TBP	TGGGAAAATGGTGTGCACAGG
10630	TBP	TTTCCTTTCCCTAGTGAAGAA
10640	TBP	TTCCTTTCCCTAGTGAAGAAC
10650	TBP	TCCTTTCCCTAGTGAAGAACA
10660	TBP	CCTTTCCCTAGTGAAGAACAG
10670	TBP	CTTTCCCTAGTGAAGAACAGT
10680	TBP	CCCTAGTGAAGAACAGTCCAG

SEQ ID NO	Gene	Target Domain Sequence (DNA)
11920	E2F4	ATGGGCTCAAAGGAGGTAGAA
11930	E2F4	TGACAGCTCTTTGGGGAGTTC
11940	E2F4	CTGACAGCTCTTTGGGGAGTT
11950	E2F4	TGAGGACATCAACTCCTCCAG
11960	E2F4	CAGGGCCACCCACCTTCTGAG
11970	E2F4	TAGATATAATCGTGGTCTCCC
11980	E2F4	ACTCTCGTCCAGGTTGTAGAT
11990	G6PD	TGGGGGTTCACCCACTTGTAG
12000	G6PD	ACCCACTTGTAGGTGCCCTCA
12010	G6PD	TAGGTGCCCTCATACTGGAAA
12020	G6PD	ATCAGCTCGTCTGCCTCCGTG
12030	G6PD	CCTCACCTGCCATAAATATAG
12040	G6PD	CTCACCTGCCATAAATATAGG
12050	G6PD	GGCTTCTCCAGCTCAATCTGG
12060	G6PD	TCCAGCTCAATCTGGTGCAGC
12070	G6PD	TCTGTAGGGCACCTTGTATCT
12080	G6PD	TATCTGTTGCCGTAGGTCAGG
12090	G6PD	CCGTAGGTCAGGTCCAGCTCC
12100	G6PD	AAGAACATGCCCGGCTTCTTG
12110	G6PD	TTGGTCATCATCTTGGTGTAC
12120	G6PD	GTCATCATCTTGGTGTACACG
12130	G6PD	GTGTACACGGCCTCGTTGGGC
12140	G6PD	GGCTGCACGCGGATCACCAGC
12150	G6PD	CGCTTGCACTGCTGGTGGAAG
12160	G6PD	CACTGCTGGTGGAAGATGTCG
12170	G6PD	CGCTCGTTCAGGGCCTTGCCG
12180	G6PD	AGGGCCTTGCCGCAGCGCAGG
12190	G6PD	CCGCAGCGCAGGATGAAGGGC

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SEQ ID NO	Gene	Target Domain Sequence (DNA)
10690	TBP	CCTAGTGAAGAACAGTCCAGA
10700	TBP	TACAGAAGTTGGGTTTTCCAG
10710	TBP	GGTTTTCCAGCTAAGTTCTTG
10720	TBP	TCCAGCTAAGTTCTTGGACTT
10730	TBP	CCAGCTAAGTTCTTGGACTTC
10740	TBP	CAGCTAAGTTCTTGGACTTCA
10750	TBP	TTGGACTTCAAGATTCAGAAT
10760	TBP	GACTTCAAGATTCAGAATATG
10770	TBP	AAGATTCAGAAATATGGTGGGG
10780	TBP	AGAATATGGTGGGGAGCTGTG
10790	TBP	CCTATAAGGTTAGAAGGCCTT
10800	TBP	CTATAAGGTTAGAAGGCCTTG
10810	TBP	TGCTCACCCACCAACAATTTA
10820	TBP	TTGCAATTTTCCTTCTAGTTA
10830	TBP	TGCAATTTTCCTTCTAGTTAT
10840	TBP	GCAATTTTCCTTCTAGTTATG
10850	TBP	CAATTTTCCTTCTAGTTATGA
10860	TBP	TCCTTCTAGTTATGAGCCAGA
10870	TBP	CCTTCTAGTTATGAGCCAGAG
10880	TBP	CTTCTAGTTATGAGCCAGAGT
10890	TBP	TAGTTATGAGCCAGAGTTATT
10900	TBP	TGAGCCAGAGTTATTTCCTGG
10910	TBP	CCTGGTTTAATCTACAGAATG
10920	TBP	CTGGTTTAATCTACAGAATGA
10930	TBP	AATCTACAGAATGATCAAACC
10940	TBP	ATCTACAGAATGATCAAACCC
10950	TBP	TTCTCCTTATTTTTGTTTCTG
10960	TBP	TCCTTATTTTTGTTTCTGGAA

SEQ ID NO	Gene	Target Domain Sequence (DNA)
12200	G6PD	CAGTATGAGGGCACCTACAAG
12210	G6PD	CCAGTATGAGGGCACCTACAA
12220	G6PD	AGCTGGAGAAGCCCAAGCCCA
12230	G6PD	ACCCCACTGCTGCACCAGATT
12240	G6PD	CACCCCACTGCTGCACCAGAT
12250	G6PD	TCACCCCACTGCTGCACCAGA
12260	G6PD	TGCGGGAGCCAGATGCACTTC
12270	G6PD	AACCCCGAGGAGTCGGAGCTG
12280	G6PD	TTCAACCCCGAGGAGTCGGAG
12290	G6PD	CACCAGCAGTGCAAGCGCAAC
12300	G6PD	CATGATGTGGCCGGCGACATC
12310	G6PD	ATCCTGCGCTGCGGCAAGGCC
12320	G6PD	CGCCACGTAGGGGTGCCCTTC
12330	G6PD	CCGCCACGTAGGGGTGCCCTT
12340	KIF11	ATGAAGATAAATTGATAGCAC
12350	KIF11	ATAGCACAAAATCTAGAACTT
12360	KIF11	ATGAAACCATAAAAATTGGTT
12370	KIF11	GTTTGACTAAGCTTAATTGCT
12380	KIF11	GACTAAGCTTAATTGCTTTCT
12390	KIF11	ACTAAGCTTAATTGCTTTCTG
12400	KIF11	ATTGCTTTCTGGAACAGGATC
12410	KIF11	CTTTCTGGAACAGGATCTGAA
12420	KIF11	CTGGAACAGGATCTGAAACTG
12430	KIF11	TGGAACAGGATCTGAAACTGG
12440	KIF11	TCTAATGTCCGTTAAAGGTAC
12450	KIF11	AAGGTACGACACCACAGAGGA
12460	KIF11	TTTATACCCATCAACACTGGT
12470	KIF11	ATACCCATCAACACTGGTAAG

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SEQ ID NO	Gene	Target Domain Sequence (DNA)
10970	TBP	TTTTTGTTTCTGGAAAAGTTG
10980	TBP	TTGTTTCTGGAAAAGTTGTAT
10990	TBP	TGTTTCTGGAAAAGTTGTATT
11000	TBP	GTTTCTGGAAAAGTTGTATTA
11010	TBP	TTTCTGGAAAAGTTGTATTAA
11020	TBP	CTGGAAAAGTTGTATTAACAG
11030	TBP	TGGAAAAGTTGTATTAACAGG
11040	TBP	TCTTCTTAGGTGCTAAAGTCA
11050	TBP	TTAGGTGCTAAAGTCAGAGCA
11060	TBP	GGTGCTAAAGTCAGAGCAGAA
11070	TBP	TAAAGGGATTCAGGAAGACGA
11080	TBP	GGTCAAGTTTACAACCAAGAT
11090	TBP	AGGTCAAGTTTACAACCAAGA
11100	TBP	GGGCACGAAGTGCAATGGTCT
11110	TBP	CGGGCACGAAGTGCAATGGTC
11120	TBP	GGCGTTTCGGGCACGAAGTGC
11130	TBP	TATTCGGCGTTTCGGGCACGA
11140	TBP	GGATTATATTCGGCGTTTCGG
11150	TBP	AAATAGATCTAACCTTGGGAT
11160	TBP	TCCTCATGATTACCGCAGCAA
11170	TBP	GTGGCTCTCTTATCCTCATGA
11180	TBP	CCAGAACTGAAAATCAGTGCC
11190	TBP	CCCAGAACTGAAAATCAGTGC
11200	TBP	TCCCAGAACTGAAAATCAGTG
11210	TBP	GCTCCTGTGCACACCATTTTC
11220	TBP	CGGCTACCTCTTGGCTCCTGT
11230	TBP	TTACGGCTACCTCTTGGCTCC
11240	TBP	CTTACGGCTACCTCTTGGCTC

SEQ ID NO	Gene	Target Domain Sequence (DNA)
12480	KIF11	TACCCATCAACACTGGTAAGA
12490	KIF11	ATCAGCTGAAAAGGAAACAGC
12500	KIF11	ATGATGCTAAACTGTTCAGAA
12510	KIF11	AGAAAACAACAAAGAGAGAC
12520	KIF11	CTTCTTTTAGGATGTGGATGT
12530	KIF11	TTCTTTTAGGATGTGGATGTA
12540	KIF11	TTTTAGGATGTGGATGTAGAA
12550	KIF11	TAGGATGTGGATGTAGAAGAG
12560	KIF11	AGGATGTGGATGTAGAAGAGG
12570	KIF11	GGATGTGGATGTAGAAGAGGC
12580	KIF11	TGGGGCAGTATACTGAAGAAC
12590	KIF11	TTCATCAATTGGCGGGGTTCC
12600	KIF11	ATCAATTGGCGGGGTTCCATT
12610	KIF11	GCGGGGTTCCATTTTTCCAGG
12620	KIF11	TCCCGCCTTAAATCCACAGCA
12630	KIF11	CCCGCCTTAAATCCACAGCAT
12640	KIF11	CCGCCTTAAATCCACAGCATA
12650	KIF11	AATCCACAGCATAAAAAATCA
12660	KIF11	ACACACTGGAGAGGTCTAAAG
12670	KIF11	GTTACAAAGAGCAGATTACCT
12680	KIF11	CAAAGAGCAGATTACCTCTGC
12690	KIF11	CCTCTGCGAGCCCAGATCAAC
12700	KIF11	TAGATTTTGTGCTATCAATTT
12710	KIF11	AGTTCTAGATTTTGTGCTATC
12720	KIF11	ATTAAGTTCTAGATTTTGTGC
12730	KIF11	CATTAAGTTCTAGATTTTGTG
12740	KIF11	TGGTTTCATTAAGTTCTAGAT
12750	KIF11	ATGGTTTCATTAAGTTCTAGA

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SEQ ID NO	Gene	Target Domain Sequence (DNA)
11250	TBP	CTGCCAGTCTGGACTGTTCTT
11260	TBP	TTGCTGCCAGTCTGGACTGTT
11270	TBP	CTTGCTGCCAGTCTGGACTGT
11280	TBP	TCTTGCTGCCAGTCTGGACTG
11290	TBP	TGTACAACTCTAGCATATTTT
11300	TBP	GCTGGAAAACCCAACTTCTGT
11310	TBP	AAGTCCAAGAACTTAGCTGGA
11320	TBP	TGAATCTTGAAGTCCAAGAAC
11330	TBP	ACATCACAGCTCCCCACCATA
11340	TBP	TAACCTTATAGGAAACTTCAC
11350	TBP	GTGGGTGAGCACAAGGCCTTC
11360	TBP	TTGGTGGGTGAGCACAAGGCC
11370	TBP	CCTACTAAATTGTTGGTGGGT
11380	TBP	AGACTTACCTACTAAATTGTT
11390	TBP	CAGACTTACCTACTAAATTGT
11400	TBP	AACCAGGAAATAACTCTGGCT
11410	TBP	TGTAGATTAAACCAGGAAATA
11420	TBP	ATCATTCTGTAGATTAAACCA
11430	TBP	GATCATTCTGTAGATTAAACC
11440	TBP	TGGGTTTGATCATTCTGTAGA
11450	TBP	CAGAAACAAAAATAAGGAGAA
11460	TBP	CCAGAAACAAAAATAAGGAGA
11470	TBP	TCCAGAAACAAAAATAAGGAG
11480	TBP	ATACAACTTTTCCAGAAACAA
11490	TBP	CCTGTTAATACAACTTTTCCA
11500	TBP	CAACTTACCTGTTAATACAAC
11510	TBP	CTGTTACAACTTACCTGTTAA
11520	TBP	TGCTCTGACTTTAGCACCTAA

SEQ ID NO	Gene	Target Domain Sequence (DNA)
12760	KIF11	TATGGTTTCATTAAGTTCTAG
12770	KIF11	TTATGGTTTCATTAAGTTCTA
12780	KIF11	GTCAAACCAATTTTTATGGTT
12790	KIF11	AGCTTAGTCAAACCAATTTTT
12800	KIF11	CAGAAAGCAATTAAGCTTAGT
12810	KIF11	AGATCCTGTTCCAGAAAGCAA
12820	KIF11	CAGATCCTGTTCCAGAAAGCA
12830	KIF11	GGATATCCAGTTTCAGATCCT
12840	KIF11	AAGTACCTGTTGGGATATCCA
12850	KIF11	AAAGTACCTGTTGGGATATCC
12860	KIF11	TAAAGTACCTGTTGGGATATC
12870	KIF11	TCTTTTAAAGTACCTGTTGGG
12880	KIF11	CTCTTTTAAAGTACCTGTTGG
12890	KIF11	TATTTCTCTTTTAAAGTACCT
12900	KIF11	CTCTGTGGTGTCGTACCTTTA
12910	KIF11	CCTCTGTGGTGTCGTACCTTT
12920	KIF11	TCCTCTGTGGTGTCGTACCTT
12930	KIF11	ATGGGTATAAAATAACTTTCC
12940	KIF11	CCAGTGTTGATGGGTATAAAT
12950	KIF11	TTACCAGTGTTGATGGGTATA
12960	KIF11	AGTTCTTACCAGTGTTGATGG
12970	KIF11	ACGTGGTTCAGTTCTTACCAG
12980	KIF11	AGCTGATCAAGGAGATGTTCA
12990	KIF11	CAGCTGATCAAGGAGATGTTC
13000	KIF11	TCAGCTGATCAAGGAGATGTT
13010	KIF11	CTTTTCAGCTGATCAAGGAGA
13020	KIF11	CCTTTTCAGCTGATCAAGGAG
13030	KIF11	ACAGCTCAGGCTGTTTCCTTT

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SEQ ID NO	Gene	Target Domain Sequence (DNA)
11530	TBP	CTGCTCTGACTTTAGCACCTA
11540	TBP	ATAAATTTCTGCTCTGACTTT
11550	TBP	AAATGCTTCATAAATTTCTGC
11560	TBP	CAAATGCTTCATAAATTTCTG
11570	TBP	TCAAATGCTTCATAAATTTCT
11580	TBP	CTGAATCCCTTTAGAATAGGG
11590	TBP	CGTCGTCTTCCTGAATCCCTT
11600	E2F4	GGGGGCTATCATTGTAGTGAG
11610	E2F4	GGGGCTATCATTGTAGTGAGT
11620	E2F4	TAGTGAGTGGCGGCCCTGGGA
11630	E2F4	ACTCCCACTGGGCCCAACAAC
11640	E2F4	TGCCCTGCTGGACAGCAGCAG
11650	E2F4	GTCCGGACCCAACCCTTCTAC
11660	E2F4	TACCTCCTTTGAGCCCATCAA
11670	E2F4	GAGCCCATCAAGGCAGACCCC
11680	E2F4	AGCCCATCAAGGCAGACCCCA
11690	E2F4	CTTGTTTTTCAGTTTTGGAAC
11700	E2F4	TTTTTCAGTTTTGGAACTCCC
11710	E2F4	TTCAGTTTTGGAACTCCCCAA
11720	E2F4	TCAGTTTTGGAACTCCCCAAA
11730	E2F4	CAGTTTTGGAACTCCCCAAAG
11740	E2F4	AGTTTTGGAACTCCCCAAAGA
11750	E2F4	TGGAACTCCCCAAAGAGCTGT
11760	E2F4	GGAACTCCCCAAAGAGCTGTC
11770	E2F4	CCAGAGTGCATGAGCTCGGAG
11780	E2F4	GCCCCTCTGCTTCGTCTTTCT
11790	E2F4	CCCCTCTGCTTCGTCTTTCTC
11800	E2F4	GTCTTTCTCCACCCCCGGGAG

SEQ ID NO	Gene	Target Domain Sequence (DNA)
13040	KIF11	GCATCATTAACAGCTCAGGCT
13050	KIF11	AGCATCATTAACAGCTCAGGC
13060	KIF11	TGAACAGTTTAGCATCATTAA
13070	KIF11	CTGAACAGTTTAGCATCATTA
13080	KIF11	TCTGAACAGTTTAGCATCATT
13090	KIF11	TTTCTGAACAGTTTAGCATC
13100	KIF11	TTGTTTCTGAACAGTTTAGC
13110	KIF11	TTTGTTGTTTCTGAACAGTT
13120	KIF11	TCTCTTCTTTGTTGTTTCTG
13130	KIF11	CCGGAATTGTCTCTTCTTTGT
13140	KIF11	ACCGGAATTGTCTCTTCTTTG
13150	KIF11	AATTTACCGGAATTGTCTCTT
13160	KIF11	AAATTTACCGGAATTGTCTCT
13170	KIF11	AGTATACTGCCCCAGAACTGC
13180	KIF11	TTCAGTATACTGCCCCAGAAC
13190	KIF11	GAGGTTCTTCAGTATACTGCC
13200	KIF11	ACTTAGAGGTTCTTCAGTATA
13210	KIF11	ATGAACAATCCACACCAGCAT
13220	KIF11	TCTGATATGACATACCTGGAA
13230	KIF11	CATGTGATTTTTTATGCTGTG
13240	KIF11	CCATGTGATTTTTTATGCTGT
13250	KIF11	TCCATGTGATTTTTTATGCTG
13260	KIF11	TCTTTTCCATGTGATTTTTTA
13270	KIF11	GTCTTTTCCATGTGATTTTTT
13280	KIF11	TTTGTCTTTTCCATGTGATTT
13290	KIF11	CTTTGTCTTTTCCATGTGATT
13300	KIF11	TCTTTGTCTTTTCCATGTGAT
13310	KIF11	ATGCCTCTGTTTTCTTTGTCT

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SEQ ID NO	Gene	Target Domain Sequence (DNA)	SEQ ID NO	Gene	Target Domain Sequence (DNA)
11810	E2F4	CTCCACCCCCGGGAGACCACG	13320	KIF11	GACCTCTCCAGTGTGTTAATG
11820	E2F4	TCCACCCCCGGGAGACCACGA	13330	KIF11	AGACCTCTCCAGTGTGTTAAT
11830	E2F4	TATCTACAACCTGGACGAGAG	13340	KIF11	CACTTTAGACCTCTCCAGTGT
11840	E2F4	GATGTGCCTGTTCTCAACCTC	13350	KIF11	TTCCACTTTAGACCTCTCCAG
11850	E2F4	ATGTGCCTGTTCTCAACCTCT	13360	KIF11	CTTCCACTTTAGACCTCTCCA
11860	E2F4	TGCACTGCCAGGGACAGCAGT	13370	KIF11	TAACCAAGTGCTCTGTAGTTT
11870	E2F4	CCTGGACTTCTGCACTGCCAG	13380	KIF11	GTAACCAAGTGCTCTGTAGTT
11880	E2F4	CTATCAGTCCCAGGGCCGCCA	13390	KIF11	ATCTGGGCTCGCAGAGGTAAT
11890	E2F4	GGCCCAGTGGGAGTGAACTGA	13400	KIF11	AAGGTTGATCTGGGCTCGCAG
11900	E2F4	TTGGGCCCAGTGGGAGTGAAC	13410	KIF11	CCAACCCCCAAGTGAATTAAA
11910	E2F4	GGTCCGGACGAACTGCTGCTG	-	-	-

Table 24: Mad7 guide RNAs

SEQ ID NO	Gene	Target Domain Sequence (DNA)	SEQ ID NO	Gene	Target Domain Sequence (DNA)
13420	GAPDH	TGCAGACCACAGTCCATGCCA	14890	E2F4	TTGGGCCCAGTGGGAGTGAAC
13430	GAPDH	GCAGACCACAGTCCATGCCAT	14900	E2F4	GGTCCGGACGAACTGCTGCTG
13440	GAPDH	CAGACCACAGTCCATGCCATC	14910	E2F4	ATGGGCTCAAAGGAGGTAGAA
13450	GAPDH	TCATCTTCTAGGTATGACAAC	14920	E2F4	TGACAGCTCTTTGGGGAGTTC
13460	GAPDH	CATCTTCTAGGTATGACAACG	14930	E2F4	CTGACAGCTCTTTGGGGAGTT
13470	GAPDH	ATCTTCTAGGTATGACAACGA	14940	E2F4	TGAGGACATCAACTCCTCCAG
13480	GAPDH	TAGGTATGACAACGAATTTGG	14950	E2F4	CAGGGCCACCCACCTTCTGAG
13490	GAPDH	CCCAGCTCTCATACCATGAGT	14960	E2F4	TAGATATAATCGTGGTCTCCC
13500	TBP	TATCCACAGTGAATCTTGGTT	14970	E2F4	ACTCTCGTCCAGGTTGTAGAT
13510	TBP	GTTGTAAACTTGACCTAAAGA	14980	G6PD	TGGGGGTTCACCCACTTGTAG
13520	TBP	TAAACTTGACCTAAAGACCAT	14990	G6PD	ACCCACTTGTAGGTGCCCTCA
13530	TBP	ACCTAAAGACCATTGCACTTC	15000	G6PD	TAGGTGCCCTCATACTGGAAA
13540	TBP	CACTTCGTGCCCGAAACGCCG	15010	G6PD	ATCAGCTCGTCTGCCTCCGTG

SEQ ID NO	Gene	Target Domain Sequence (DNA)
13550	TBP	GTGCCCGAAACGCCGAATATA
13560	TBP	TCTCTGACCATTGTAGCGGTT
13570	TBP	TAGCGGTTTGCTGCGGTAATC
13580	TBP	GCTGCGGTAATCATGAGGATA
13590	TBP	CTGCGGTAATCATGAGGATAA
13600	TBP	TCAGTTCTGGGAAAATGGTGT
13610	TBP	CAGTTCTGGGAAAATGGTGTG
13620	TBP	AGTTCTGGGAAAATGGTGTGC
13630	TBP	TGGGAAAATGGTGTGCACAGG
13640	TBP	TTTCCTTTCCCTAGTGAAGAA
13650	TBP	TTCCTTTCCCTAGTGAAGAAC
13660	TBP	TCCTTTCCCTAGTGAAGAACA
13670	TBP	CCTTTCCCTAGTGAAGAACAG
13680	TBP	CTTTCCCTAGTGAAGAACAGT
13690	TBP	CCCTAGTGAAGAACAGTCCAG
13700	TBP	CCTAGTGAAGAACAGTCCAGA
13710	TBP	TACAGAAGTTGGGTTTTCCAG
13720	TBP	GGTTTTCCAGCTAAGTTCTTG
13730	TBP	TCCAGCTAAGTTCTTGGACTT
13740	TBP	CCAGCTAAGTTCTTGGACTTC
13750	TBP	CAGCTAAGTTCTTGGACTTCA
13760	TBP	TTGGACTTCAAGATTCAGAAT
13770	TBP	GACTTCAAGATTCAGAATATG
13780	TBP	AAGATTCAGAATATGGTGGGG
13790	TBP	AGAATATGGTGGGGAGCTGTG
13800	TBP	CCTATAAGGTTAGAAGGCCTT
13810	TBP	CTATAAGGTTAGAAGGCCTTG
13820	TBP	TGCTCACCCACCAACAATTTA

SEQ ID NO	Gene	Target Domain Sequence (DNA)
15020	G6PD	CCTCACCTGCCATAAATATAG
15030	G6PD	CTCACCTGCCATAAATATAGG
15040	G6PD	GGCTTCTCCAGCTCAATCTGG
15050	G6PD	TCCAGCTCAATCTGGTGCAGC
15060	G6PD	TCTGTAGGGCACCTTGTATCT
15070	G6PD	TATCTGTTGCCGTAGGTCAGG
15080	G6PD	CCGTAGGTCAGGTCCAGCTCC
15090	G6PD	AAGAACATGCCCGGCTTCTTG
15100	G6PD	TTGGTCATCATCTTGGTGTAC
15110	G6PD	GTCATCATCTTGGTGTACACG
15120	G6PD	GTGTACACGGCCTCGTTGGGC
15130	G6PD	GGCTGCACGCGGATCACCAGC
15140	G6PD	CGCTTGCACTGCTGGTGGAAG
15150	G6PD	CACTGCTGGTGGAAGATGTCG
15160	G6PD	CGCTCGTTCAGGGCCTTGCCG
15170	G6PD	AGGGCCTTGCCGCAGCGCAGG
15180	G6PD	CCGCAGCGCAGGATGAAGGGC
15190	G6PD	CAGTATGAGGGCACCTACAAG
15200	G6PD	CCAGTATGAGGGCACCTACAA
15210	G6PD	AGCTGGAGAAGCCCAAGCCCA
15220	G6PD	ACCCCACTGCTGCACCAGATT
15230	G6PD	CACCCCACTGCTGCACCAGAT
15240	G6PD	TCACCCCACTGCTGCACCAGA
15250	G6PD	TGCGGGAGCCAGATGCACTTC
15260	G6PD	AACCCCGAGGAGTCGGAGCTG
15270	G6PD	TTCAACCCCGAGGAGTCGGAG
15280	G6PD	CACCAGCAGTGCAAGCGCAAC
15290	G6PD	CATGATGTGGCCGGCGACATC

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SEQ ID NO	Gene	Target Domain Sequence (DNA)
13830	TBP	TTGCAATTTTCCTTCTAGTTA
13840	TBP	TGCAATTTTCCTTCTAGTTAT
13850	TBP	GCAATTTTCCTTCTAGTTATG
13860	TBP	CAATTTTCCTTCTAGTTATGA
13870	TBP	TCCTTCTAGTTATGAGCCAGA
13880	TBP	CCTTCTAGTTATGAGCCAGAG
13890	TBP	CTTCTAGTTATGAGCCAGAGT
13900	TBP	TAGTTATGAGCCAGAGTTATT
13910	TBP	TGAGCCAGAGTTATTTCCTGG
13920	TBP	CCTGGTTTAATCTACAGAATG
13930	TBP	CTGGTTTAATCTACAGAATGA
13940	TBP	AATCTACAGAATGATCAAACC
13950	TBP	ATCTACAGAATGATCAAACCC
13960	TBP	TTCTCCTTATTTTTGTTTCTG
13970	TBP	TCCTTATTTTTGTTTCTGGAA
13980	TBP	TTTTTGTTTCTGGAAAAGTTG
13990	TBP	TTGTTTCTGGAAAAGTTGTAT
14000	TBP	TGTTTCTGGAAAAGTTGTATT
14010	TBP	GTTTCTGGAAAAGTTGTATTA
14020	TBP	TTTCTGGAAAAGTTGTATTAA
14030	TBP	CTGGAAAAGTTGTATTAACAG
14040	TBP	TGGAAAAGTTGTATTAACAGG
14050	TBP	TCTTCTTAGGTGCTAAAGTCA
14060	TBP	TTAGGTGCTAAAGTCAGAGCA
14070	TBP	GGTGCTAAAGTCAGAGCAGAA
14080	TBP	TAAAGGGATTCAGGAAGACGA
14090	TBP	GGTCAAGTTTACAACCAAGAT
14100	TBP	AGGTCAAGTTTACAACCAAGA

SEQ ID NO	Gene	Target Domain Sequence (DNA)
15300	G6PD	ATCCTGCGCTGCGGCAAGGCC
15310	G6PD	CGCCACGTAGGGGTGCCCTTC
15320	G6PD	CCGCCACGTAGGGGTGCCCTT
15330	KIF11	ATGAAGATAAATTGATAGCAC
15340	KIF11	ATAGCACAAAATCTAGAACTT
15350	KIF11	ATGAAACCATAAAAATTGGTT
15360	KIF11	GTTTGACTAAGCTTAATTGCT
15370	KIF11	GACTAAGCTTAATTGCTTTCT
15380	KIF11	ACTAAGCTTAATTGCTTTCTG
15390	KIF11	ATTGCTTTCTGGAACAGGATC
15400	KIF11	CTTTCTGGAACAGGATCTGAA
15410	KIF11	CTGGAACAGGATCTGAAACTG
15420	KIF11	TGGAACAGGATCTGAAACTGG
15430	KIF11	TCTAATGTCCGTTAAAGGTAC
15440	KIF11	AAGGTACGACACCACAGAGGA
15450	KIF11	TTTATACCCATCAACACTGGT
15460	KIF11	ATACCCATCAACACTGGTAAG
15470	KIF11	TACCCATCAACACTGGTAAGA
15480	KIF11	ATCAGCTGAAAAGGAAACAGC
15490	KIF11	ATGATGCTAAACTGTTCAGAA
15500	KIF11	AGAAAACAACAAAGAGAGAC
15510	KIF11	CTTCTTTTAGGATGTGGATGT
15520	KIF11	TTCTTTTAGGATGTGGATGTA
15530	KIF11	TTTTAGGATGTGGATGTAGAA
15540	KIF11	TAGGATGTGGATGTAGAAGAG
15550	KIF11	AGGATGTGGATGTAGAAGAGG
15560	KIF11	GGATGTGGATGTAGAAGAGGC
15570	KIF11	TGGGGCAGTATACTGAAGAAC

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SEQ ID NO	Gene	Target Domain Sequence (DNA)
14110	TBP	GGGCACGAAGTGCAATGGTCT
14120	TBP	CGGGCACGAAGTGCAATGGTC
14130	TBP	GGCGTTTCGGGCACGAAGTGC
14140	TBP	TATTCGGCGTTTCGGGCACGA
14150	TBP	GGATTATATTCGGCGTTTCGG
14160	TBP	AAATAGATCTAACCTTGGGAT
14170	TBP	TCCTCATGATTACCGCAGCAA
14180	TBP	GTGGCTCTCTTATCCTCATGA
14190	TBP	CCAGAACTGAAAATCAGTGCC
14200	TBP	CCCAGAACTGAAAATCAGTGC
14210	TBP	TCCCAGAACTGAAAATCAGTG
14220	TBP	GCTCCTGTGCACACCATTTTC
14230	TBP	CGGCTACCTCTTGGCTCCTGT
14240	TBP	TTACGGCTACCTCTTGGCTCC
14250	TBP	CTTACGGCTACCTCTTGGCTC
14260	TBP	CTGCCAGTCTGGACTGTTCTT
14270	TBP	TTGCTGCCAGTCTGGACTGTT
14280	TBP	CTTGCTGCCAGTCTGGACTGT
14290	TBP	TCTTGCTGCCAGTCTGGACTG
14300	TBP	TGTACAACTCTAGCATATTTT
14310	TBP	GCTGGAAAACCCAACTTCTGT
14320	TBP	AAGTCCAAGAACTTAGCTGGA
14330	TBP	TGAATCTTGAAGTCCAAGAAC
14340	TBP	ACATCACAGCTCCCCACCATA
14350	TBP	TAACCTTATAGGAAACTTCAC
14360	TBP	GTGGGTGAGCACAAGGCCTTC
14370	TBP	TTGGTGGGTGAGCACAAGGCC
14380	TBP	CCTACTAAATTGTTGGTGGGT

SEQ ID NO	Gene	Target Domain Sequence (DNA)
15580	KIF11	TTCATCAATTGGCGGGGTTCC
15590	KIF11	ATCAATTGGCGGGGTTCCATT
15600	KIF11	GCGGGGTTCCATTTTTCCAGG
15610	KIF11	TCCCGCCTTAAATCCACAGCA
15620	KIF11	CCCGCCTTAAATCCACAGCAT
15630	KIF11	CCGCCTTAAATCCACAGCATA
15640	KIF11	AATCCACAGCATAAAAAATCA
15650	KIF11	ACACACTGGAGAGGTCTAAAG
15660	KIF11	GTTACAAAGAGCAGATTACCT
15670	KIF11	CAAAGAGCAGATTACCTCTGC
15680	KIF11	CCTCTGCGAGCCCAGATCAAC
15690	KIF11	TAGATTTTGTGCTATCAATTT
15700	KIF11	AGTTCTAGATTTTGTGCTATC
15710	KIF11	ATTAAGTTCTAGATTTTGTGC
15720	KIF11	CATTAAGTTCTAGATTTTGTG
15730	KIF11	TGGTTTCATTAAGTTCTAGAT
15740	KIF11	ATGGTTTCATTAAGTTCTAGA
15750	KIF11	TATGGTTTCATTAAGTTCTAG
15760	KIF11	TTATGGTTTCATTAAGTTCTA
15770	KIF11	GTCAAACCAATTTTTATGGTT
15780	KIF11	AGCTTAGTCAAACCAATTTTT
15790	KIF11	CAGAAAGCAATTAAGCTTAGT
15800	KIF11	AGATCCTGTTCCAGAAAGCAA
15810	KIF11	CAGATCCTGTTCCAGAAAGCA
15820	KIF11	GGATATCCAGTTTCAGATCCT
15830	KIF11	AAGTACCTGTTGGGATATCCA
15840	KIF11	AAAGTACCTGTTGGGATATCC
15850	KIF11	TAAAGTACCTGTTGGGATATC

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SEQ ID NO	Gene	Target Domain Sequence (DNA)	SEQ ID NO	Gene	Target Domain Sequence (DNA)
14390	TBP	AGACTTACCTACTAAATTGTT	15860	KIF11	TCTTTTAAAGTACCTGTTGGG
14400	TBP	CAGACTTACCTACTAAATTGT	15870	KIF11	CTCTTTTAAAGTACCTGTTGG
14410	TBP	AACCAGGAAATAACTCTGGCT	15880	KIF11	TATTTCTCTTTTAAAGTACCT
14420	TBP	TGTAGATTAAACCAGGAAATA	15890	KIF11	ATGGGTATAAATAACTTTTCC
14430	TBP	ATCATTCTGTAGATTAAACCA	15900	KIF11	CCAGTGTTGATGGGTATAAAT
14440	TBP	GATCATTCTGTAGATTAAACC	15910	KIF11	TTACCAGTGTTGATGGGTATA
14450	TBP	TGGGTTTGATCATTCTGTAGA	15920	KIF11	AGTTCTTACCAGTGTTGATGG
14460	TBP	CAGAACAAAAATAAGGAGAA	15930	KIF11	ACGTGGTTCAGTTCTTACCAG
14470	TBP	CCAGAAACAAAAATAAGGAGA	15940	KIF11	AGCTGATCAAGGAGATGTTCA
14480	TBP	TCCAGAAACAAAAATAAGGAG	15950	KIF11	CAGCTGATCAAGGAGATGTTC
14490	TBP	ATACAACTTTTCCAGAAACAA	15960	KIF11	TCAGCTGATCAAGGAGATGTT
14500	TBP	CCTGTTAAATACAACTTTTCCA	15970	KIF11	CTTTTCAGCTGATCAAGGAGA
14510	TBP	CAACTTACCTGTTAAATACAAC	15980	KIF11	CCTTTTCAGCTGATCAAGGAG
14520	TBP	CTGTTACAACTTACCTGTTAA	15990	KIF11	ACAGCTCAGGCTGTTTCCTTT
14530	TBP	ATAAATTTCTGCTCTGACTTT	16000	KIF11	GCATCATTAACAGCTCAGGCT
14540	TBP	AAATGCTTCATAAATTTCTGC	16010	KIF11	AGCATCATTAACAGCTCAGGC
14550	TBP	CAAATGCTTCATAAATTTCTG	16020	KIF11	TGAACAGTTTAGCATCATTAA
14560	TBP	TCAAATGCTTCATAAATTTCT	16030	KIF11	CTGAACAGTTTAGCATCATTA
14570	TBP	CTGAATCCCTTTAGAATAGGG	16040	KIF11	TCTGAACAGTTTAGCATCATT
14580	TBP	CGTCGTCTTCCTGAATCCCTT	16050	KIF11	TTTTCTGAACAGTTTAGCATC
14590	E2F4	GGGGGCTATCATTGTAGTGAG	16060	KIF11	TTGTTTTCTGAACAGTTTAGC
14600	E2F4	GGGGCTATCATTGTAGTGAGT	16070	KIF11	TTTGTTGTTTTCTGAACAGTT
14610	E2F4	TAGTGAGTGGCGGCCCTGGGA	16080	KIF11	TCTCTTCTTTGTTGTTTTCTG
14620	E2F4	ACTCCCACTGGGCCCAACAAC	16090	KIF11	CCGGAATTGTCTCTTCTTTGT
14630	E2F4	TGCCCTGCTGGACAGCAGCAG	16100	KIF11	ACCGGAATTGTCTCTTCTTTG
14640	E2F4	GTCCGGACCCAACCCTTCTAC	16110	KIF11	AATTTACCGGAATTGTCTCTT
14650	E2F4	TACCTCCTTTGAGCCCATCAA	16120	KIF11	AAATTTACCGGAATTGTCTCT
14660	E2F4	GAGCCCATCAAGGCAGACCCC	16130	KIF11	AGTATACTGCCCCAGAACTGC

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SEQ ID NO	Gene	Target Domain Sequence (DNA)	SEQ ID NO	Gene	Target Domain Sequence (DNA)
14670	E2F4	AGCCCATCAAGGCAGACCCCA	16140	KIF11	TTCAGTATACTGCCCCAGAAC
14680	E2F4	CTTGTTTTTCAGTTTTGGAAC	16150	KIF11	GAGGTTCTTCAGTATACTGCC
14690	E2F4	TTTTTCAGTTTTGGAACTCCC	16160	KIF11	ACTTAGAGGTTCTTCAGTATA
14700	E2F4	TTCAGTTTTGGAACTCCCCAA	16170	KIF11	ATGAACAATCCACACCAGCAT
14710	E2F4	TCAGTTTTGGAACTCCCCAAA	16180	KIF11	TCTGATATGACATACCTGGAA
14720	E2F4	CAGTTTTGGAACTCCCCAAAG	16190	KIF11	TCTTTTCCATGTGATTTTTTA
14730	E2F4	AGTTTTGGAACTCCCCAAAGA	16200	KIF11	GTCTTTTCCATGTGATTTTTT
14740	E2F4	TGGAACTCCCCAAAGAGCTGT	16210	KIF11	TTTGTCTTTTCCATGTGATTT
14750	E2F4	GGAACTCCCCAAAGAGCTGTC	16220	KIF11	CTTTGTCTTTTCCATGTGATT
14760	E2F4	CCAGAGTGCATGAGCTCGGAG	16230	KIF11	TCTTTGTCTTTTCCATGTGAT
14770	E2F4	GCCCCTCTGCTTCGTCTTTCT	16240	KIF11	ATGCCTCTGTTTTCTTTGTCT
14780	E2F4	CCCCTCTGCTTCGTCTTTCTC	16250	KIF11	GACCTCTCCAGTGTGTTAATG
14790	E2F4	GTCTTTCTCCACCCCCGGGAG	16260	KIF11	AGACCTCTCCAGTGTGTTAAT
14800	E2F4	CTCCACCCCCGGGAGACCACG	16270	KIF11	CACTTTAGACCTCTCCAGTGT
14810	E2F4	TCCACCCCCGGGAGACCACGA	16280	KIF11	TTCCACTTTAGACCTCTCCAG
14820	E2F4	TATCTACAACCTGGACGAGAG	16290	KIF11	CTTCCACTTTAGACCTCTCCA
14830	E2F4	GATGTGCCTGTTCTCAACCTC	16300	KIF11	TAACCAAGTGCTCTGTAGTTT
14840	E2F4	ATGTGCCTGTTCTCAACCTCT	16310	KIF11	GTAACCAAGTGCTCTGTAGTT
14850	E2F4	TGCACTGCCAGGGACAGCAGT	16320	KIF11	ATCTGGGCTCGCAGAGGTAAT
14860	E2F4	CCTGGACTTCTGCACTGCCAG	16330	KIF11	AAGGTTGATCTGGGCTCGCAG
14870	E2F4	CTATCAGTCCCAGGGCCGCCA	16340	KIF11	CCAACCCCCAAGTGAATTAAA
14880	E2F4	GGCCCAGTGGGAGTGAACTGA	-	-	-

Table 25: SpyCas9 guide RNAs

SEQ ID NO	Gene	Target Domain Sequence (DNA)	SEQ ID NO	Gene	Target Domain Sequence (DNA)
16350	GAPDH	TCTAGGTATGACAACGAATT	17610	G6PD	GTGGGGGTTCACCCACTTGT
16360	GAPDH	AGCCCCAGCGTCAAAGGTGG	17620	G6PD	ACTTGTAGGTGCCCTCATAC

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SEQ ID NO	Gene	Target Domain Sequence (DNA)
16370	TBP	ATTGTATCCACAGTGAATCT
16380	TBP	AAACGCCGAATATAATCCCA
16390	TBP	ACCATTGTAGCGGTTTGCTG
16400	TBP	GGTTTGCTGCGGTAATCATG
16410	TBP	GATAAGAGAGCCACGAACCA
16420	TBP	ACGGCACTGATTTTCAGTTC
16430	TBP	CGGCACTGATTTTCAGTTCT
16440	TBP	GATTTTCAGTTCTGGGAAAA
16450	TBP	TCTGGGAAAATGGTGTGCAC
16460	TBP	TGGTGTGCACAGGAGCCAAG
16470	TBP	TAGTGAAGAACAGTCCAGAC
16480	TBP	TGCTAGAGTTGTACAGAAGT
16490	TBP	GCTAGAGTTGTACAGAAGTT
16500	TBP	GGGTTTTCCAGCTAAGTTCT
16510	TBP	GGACTTCAAGATTCAGAATA
16520	TBP	CTTCAAGATTCAGAATATGG
16530	TBP	TTCAAGATTCAGAATATGGT
16540	TBP	TCAAGATTCAGAATATGGTG
16550	TBP	GTGATGTGAAGTTTCCTATA
16560	TBP	AAGTTTCCTATAAGGTTAGA
16570	TBP	TCACCCACCAACAATTTAGT
16580	TBP	TATGAGCCAGAGTTATTTCC
16590	TBP	GTTCTCCTTATTTTTGTTTC
16600	TBP	TCTGGAAAAGTTGTATTAAC
16610	TBP	AAACATCTACCCTATTCTAA
16620	TBP	ACCCTATTCTAAAGGGATTC
16630	TBP	GATTCAGGAAGACGACGTAA
16640	TBP	CACGAAGTGCAATGGTCTTT

SEQ ID NO	Gene	Target Domain Sequence (DNA)
17630	G6PD	CATCAGCTCGTCTGCCTCCG
17640	G6PD	ATCAGCTCGTCTGCCTCCGT
17650	G6PD	TCAGCTCGTCTGCCTCCGTG
17660	G6PD	CGTCTGCCTCCGTGGGCCT
17670	G6PD	TGCCTCCGTGGGCCTCGGC
17680	G6PD	TCCTCACCTGCCATAAATAT
17690	G6PD	CCTCACCTGCCATAAATATA
17700	G6PD	CTCACCTGCCATAAATATAG
17710	G6PD	CCTGCCATAAATATAGGGGA
17720	G6PD	CTGCCATAAATATAGGGGAT
17730	G6PD	ATAAATATAGGGGATGGGCT
17740	G6PD	TAAATATAGGGGATGGGCTT
17750	G6PD	TGGGCTTCTCCAGCTCAATC
17760	G6PD	AGCTCAATCTGGTGCAGCAG
17770	G6PD	GCTCAATCTGGTGCAGCAGT
17780	G6PD	CTCAATCTGGTGCAGCAGTG
17790	G6PD	CAGTGGGGTGAAAATACGCC
17800	G6PD	TGAAAATACGCCAGGCCTCA
17810	G6PD	CCTCACGGAGCTCGTCGCTG
17820	G6PD	ACCTGCGCACGAAGTGCATC
17830	G6PD	GGCTCCCGCAGAAGACGTCC
17840	G6PD	CGCAGAAGACGTCCAGGATG
17850	G6PD	GTCCAGGATGAGGCGCTCAT
17860	G6PD	ATGAGGCGCTCATAGGCGTC
17870	G6PD	TGAGGCGCTCATAGGCGTCA
17880	G6PD	CACCTTGTATCTGTTGCCGT
17890	G6PD	TGTATCTGTTGCCGTAGGTC
17900	G6PD	CAGGTCCAGCTCCGACTCCT

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SEQ ID NO	Gene	Target Domain Sequence (DNA)
16650	TBP	GTTTCGGGCACGAAGTGCAA
16660	TBP	GGGATTATATTCGGCGTTTC
16670	TBP	TGGGATTATATTCGGCGTTT
16680	TBP	TCTAACCTTGGGATTATATT
16690	TBP	ATTAAAATAGATCTAACCTT
16700	TBP	AAAATCAGTGCCGTGGTTCG
16710	TBP	AGAACTGAAAATCAGTGCCG
16720	TBP	AATTTCTTACGGCTACCTCT
16730	TBP	AGTCTGGACTGTTCTTCACT
16740	TBP	ATATTTTCTTGCTGCCAGTC
16750	TBP	TTGAAGTCCAAGAACTTAGC
16760	TBP	ACAAGGCCTTCTAACCTTAT
16770	TBP	ATTGTTGGTGGGTGAGCACA
16780	TBP	TTACCTACTAAATTGTTGGT
16790	TBP	CTTACCTACTAAATTGTTGG
16800	TBP	AGACTTACCTACTAAATTGT
16810	TBP	ATTAAACCAGGAAATAACTC
16820	TBP	ATCATTCTGTAGATTAAACC
16830	TBP	AAAATAAGGAGAACAATTCT
16840	TBP	CTTTTCCAGAAACAAAAATA
16850	TBP	TCCTGAATCCCTTTAGAATA
16860	TBP	TTCCTGAATCCCTTTAGAAT
16870	E2F4	CTCACTCCCACTGCTGTCCC
16880	E2F4	CCCTGGCAGTGCAGAAGTCC
16890	E2F4	CCTGGCAGTGCAGAAGTCCA
16900	E2F4	CAGTGCAGAAGTCCAGGGAA
16910	E2F4	GCAGAAGTCCAGGGAATGGC
16920	E2F4	GGCCCAGCAGCTGAGATCAC

SEQ ID NO	Gene	Target Domain Sequence (DNA)
17910	G6PD	AGGTCCAGCTCCGACTCCTC
17920	G6PD	GGTCCAGCTCCGACTCCTCG
17930	G6PD	TCGGGGTTGAAGAACATGCC
17940	G6PD	GAAGAACATGCCCCGGCTTCT
17950	G6PD	CGGCTTCTTGGTCATCATCT
17960	G6PD	GGTCATCATCTTGGTGTACA
17970	G6PD	CTTGGTGTACACGGCCTCGT
17980	G6PD	TTGGTGTACACGGCCTCGTT
17990	G6PD	CGGCCTCGTTGGGCTGCACG
18000	G6PD	GCTCGTTGCGCTTGCACTGC
18010	G6PD	CGTTGCGCTTGCACTGCTGG
18020	G6PD	CTGCTGGTGGAAGATGTCGC
18030	G6PD	AGATGTCGCCGGCCACATCA
18040	G6PD	ATGGAACTGCAGCCTCACCT
18050	G6PD	CCTCGGCCTTGCGCTCGTTC
18060	G6PD	CTCGGCCTTGCGCTCGTTCA
18070	G6PD	TCAGGGCCTTGCCGCAGCGC
18080	G6PD	CTTGCCGCAGCGGCAGGATGA
18090	G6PD	TTGCCGCAGCGCAGGATGAA
18100	G6PD	GTATGAGGGCACCTACAAGT
18110	G6PD	AGTATGAGGGCACCTACAAG
18120	G6PD	AGAGTGGGTTTCCAGTATGA
18130	G6PD	GAGAGTGGGTTTCCAGTATG
18140	G6PD	GACGAGCTGATGAAGAGAGT
18150	G6PD	AGACGAGCTGATGAAGAGAG
18160	G6PD	CTCCAGCCGAGGCCCCACGG
18170	G6PD	CACCCGTCACTCTCCAGCCG
18180	G6PD	CCATCCCCTATATTTATGGC

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SEQ ID NO	Gene	Target Domain Sequence (DNA)
16930	E2F4	GGGGCTATCATTGTAGTGAG
16940	E2F4	GCTATCATTGTAGTGAGTGG
16950	E2F4	ATTGTAGTGAGTGGCGGCCC
16960	E2F4	TTGTAGTGAGTGGCGGCCCT
16970	E2F4	CGGCCCTGGGACTGATAGCA
16980	E2F4	GGGACTGATAGCAAGGACAG
16990	E2F4	TGAGCTCAGTTCACTCCCAC
17000	E2F4	GAGCTCAGTTCACTCCCACT
17010	E2F4	CCCACTGGGCCCAACAACAC
17020	E2F4	GCCCAACAACACTGGACACC
17030	E2F4	ACTGCAGTCTTCTGCCCTGC
17040	E2F4	AGTAACAGCAGCAGTTCGTC
17050	E2F4	TACCTCCTTTGAGCCCATCA
17060	E2F4	CCCATCAAGGCAGACCCCAC
17070	E2F4	ATCAAGGCAGACCCCACAGG
17080	E2F4	GAAATCTTTGATCCCACACG
17090	E2F4	TCTTTGATCCCACACGAGGT
17100	E2F4	ATTCCCAGAGTGCATGAGCT
17110	E2F4	GTGCATGAGCTCGGAGCTGC
17120	E2F4	GAGGAGTTGATGTCCTCAGA
17130	E2F4	GAGTTGATGTCCTCAGAAGG
17140	E2F4	AGTTGATGTCCTCAGAAGGT
17150	E2F4	GCTTCGTCTTTCTCCACCCC
17160	E2F4	CTTCGTCTTTCTCCACCCCC
17170	E2F4	CCACGATTATATCTACAACC
17180	E2F4	TACAACCTGGACGAGAGTGA
17190	E2F4	GCACTGCCAGGGACAGCAGT
17200	E2F4	TGCACTGCCAGGGACAGCAG

SEQ ID NO	Gene	Target Domain Sequence (DNA)
18190	G6PD	AAGCCCATCCCCTATATTTA
18200	G6PD	ACTGCTGCACCAGATTGAGC
18210	G6PD	GCGACGAGCTCCGTGAGGCC
18220	G6PD	CCTCAGCGACGAGCTCCGTG
18230	G6PD	GCCAGATGCACTTCGTGCGC
18240	G6PD	TCATCCTGGACGTCTTCTGC
18250	G6PD	CTCATCCTGGACGTCTTCTG
18260	G6PD	CGCCTATGAGCGCCTCATCC
18270	G6PD	GACCTACGGCAACAGATACA
18280	G6PD	TCGGAGCTGGACCTGACCTA
18290	G6PD	CAACCCCGAGGAGTCGGAGC
18300	G6PD	GTTCTTCAACCCCGAGGAGT
18310	G6PD	GGGCATGTTCTTCAACCCCG
18320	G6PD	AAGATGATGACCAAGAAGCC
18330	G6PD	CAAGATGATGACCAAGAAGC
18340	G6PD	GATCCGCGTGCAGCCCAACG
18350	G6PD	GCAGTGCAAGCGCAACGAGC
18360	G6PD	CTGCAGTTCCATGATGTGGC
18370	G6PD	GAGGCTGCAGTTCCATGATG
18380	G6PD	ACGAGCGCAAGGCCGAGGTG
18390	G6PD	CCTGAACGAGCGCAAGGCCG
18400	G6PD	CAAGGCCCTGAACGAGCGCA
18410	G6PD	CTTCATCCTGCGCTGCGGCA
18420	G6PD	GTGCCCTTCATCCTGCGCTG
18430	G6PD	AGAATGAGAGGTGGGATGGT
18440	G6PD	GTGGAGAATGAGAGGTGGGA
18450	KIF11	CTTAATGAAACCATAAAAAT
18460	KIF11	GACTAAGCTTAATTGCTTTC

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SEQ ID NO	Gene	Target Domain Sequence (DNA)
17210	E2F4	CCTGGACTTCTGCACTGCCA
17220	E2F4	CCCTGGACTTCTGCACTGCC
17230	E2F4	CTGCTGGGCCAGCCATTCCC
17240	E2F4	TGTCCTTGCTATCAGTCCCA
17250	E2F4	CTGTCCTTGCTATCAGTCCC
17260	E2F4	CCAGTGTTGTTGGGCCCAGT
17270	E2F4	TCCAGTGTTGTTGGGCCCAG
17280	E2F4	GCCGGGTGTCCAGTGTTGTT
17290	E2F4	GGCCGGGTGTCCAGTGTTGT
17300	E2F4	AGCAGGGCAGAAGACTGCAG
17310	E2F4	GCTGCTGCTGCTGTCCAGCA
17320	E2F4	GGAGGTAGAAGGGTTGGGTC
17330	E2F4	TGGGCTCAAAGGAGGTAGAA
17340	E2F4	ATGGGCTCAAAGGAGGTAGA
17350	E2F4	TGCCTTGATGGGCTCAAAGG
17360	E2F4	GTCTGCCTTGATGGGCTCAA
17370	E2F4	CCTGTGGGGTCTGCCTTGAT
17380	E2F4	ACCTGTGGGGTCTGCCTTGA
17390	E2F4	GCAGGTACTCACCACCTGTG
17400	E2F4	GGCAGGTACTCACCACCTGT
17410	E2F4	GGGCAGGTACTCACCACCTG
17420	E2F4	AGATTTCTGACAGCTCTTTG
17430	E2F4	AAGATTTCTGACAGCTCTTT
17440	E2F4	AAAGATTTCTGACAGCTCTT
17450	E2F4	TGCAGCAGCCTACCTCGTGT
17460	E2F4	ATGCAGCAGCCTACCTCGTG
17470	E2F4	GCTCCGAGCTCATGCACTCT
17480	E2F4	AGCTCCGAGCTCATGCACTC

SEQ ID NO	Gene	Target Domain Sequence (DNA)
18470	KIF11	GCTTAATTGCTTTCTGGAAC
18480	KIF11	TCTGGAACAGGATCTGAAAC
18490	KIF11	CTGAAACTGGATATCCCAAC
18500	KIF11	TTAAAGGTACGACACCACAG
18510	KIF11	TTATTTATACCCATCAACAC
18520	KIF11	ATCTCCTTGATCAGCTGAAA
18530	KIF11	CAACAAAGAAGAGACAATTC
18540	KIF11	TTAGGATGTGGATGTAGAAG
18550	KIF11	GGATGTAGAAGAGGCAGTTC
18560	KIF11	GATGTAGAAGAGGCAGTTCT
18570	KIF11	ATGTAGAAGAGGCAGTTCTG
18580	KIF11	CAAGAGCCATCTGTAGATGC
18590	KIF11	GCCATCTGTAGATGCTGGTG
18600	KIF11	GGTGTGGATTGTTCATCAAT
18610	KIF11	GTGGATTGTTCATCAATTGG
18620	KIF11	TGGATTGTTCATCAATTGGC
18630	KIF11	GGATTGTTCATCAATTGGCG
18640	KIF11	TGGCGGGGTTCCATTTTTCC
18650	KIF11	CCACAGCATAAAAAAATCACA
18660	KIF11	GGAAAAGACAAAGAAAACAG
18670	KIF11	AAACAGAGGCATTAACACAC
18680	KIF11	GAGGCATTAACACACTGGAG
18690	KIF11	CACACTGGAGAGGTCTAAAG
18700	KIF11	GGAAGAAACTACAGAGGCACT
18710	KIF11	CTTAGTCAAACCAATTTTTA
18720	KIF11	TCTCTTTTAAAGTACCTGTT
18730	KIF11	TTCTCTTTTAAAGTACCTGT
18740	KIF11	TATAAATAACTTTTCCTCTG

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SEQ ID NO	Gene	Target Domain Sequence (DNA)	SEQ ID NO	Gene	Target Domain Sequence (DNA)
17490	E2F4	CCAGGGCCACCCACCTTCTG	18750	KIF11	CAGTTCTTACCAGTGTTGAT
17500	E2F4	TGGAGAAAGACGAAGCAGAG	18760	KIF11	TCAGTTCTTACCAGTGTTGA
17510	E2F4	GTGGAGAAAGACGAAGCAGA	18770	KIF11	TGATCAAGGAGATGTTCACG
17520	E2F4	GGTGGAGAAAGACGAAGCAG	18780	KIF11	GTTTCCTTTTTCAGCTGATCA
17530	E2F4	TAATCGTGGTCTCCCGGGGG	18790	KIF11	TTTAGCATCATTAACAGCTC
17540	E2F4	ATATAATCGTGGTCTCCCGG	18800	KIF11	ACAGATGGCTCTTGACTTAG
17550	E2F4	GATATAATCGTGGTCTCCCG	18810	KIF11	TCCACACCAGCATCTACAGA
17560	E2F4	AGATATAATCGTGGTCTCCC	18820	KIF11	ATATGACATACCTGGAAAAA
17570	E2F4	TAGATATAATCGTGGTCTCC	18830	KIF11	AGGTTGATCTGGGCTCGCAG
17580	E2F4	CCAGGTTGTAGATATAATCG	18840	KIF11	AGTGAATTAAAGGTTGATCT
17590	E2F4	AGACACCTTCACTCTCGTCC	18850	KIF11	AAGTGAATTAAAGGTTGATC
17600	E2F4	TGAGAACAGGCACATCAAAG	-	-	-

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Example 18: Generating edited iPSC cells using Cas12a and testing effect of Activin A on pluripotency

[0668] To generate natural killer cells from pluripotent stem cells, a representative induced pluripotent stem cell (iPSC) line was generated and designated "PCS-201". This line was generated by reprogramming adult male human primary dermal fibroblasts purchased from ATCC (ATCC® PCS-201-012) using a commercially available non-modified RNA reprogramming kit (Stemgent/Reprocell, USA). The reprogramming kit contains non-modified reprogramming mRNAs (OCT4, SOX2, KLF4, cMYC, NANOG, and LIN28) with immune evasion mRNAs (E3, K3, and B18R) and double-stranded microRNAs (miRNAs) from the 302/367 clusters. Fibroblasts were seeded in fibroblast expansion medium (DMEM/F12 with 10% FBS). The next day, media was switched to Nutristem medium and daily overnight transfections were performed for 4 days (day 1 to 4). Primary iPSC colonies appeared on day 7 and were picked on day 10-14. Picked colonies were expanded clonally to achieve a sufficient number of cells to establish a master cell bank. The parental line chosen from this process and used for the subsequent experiments passed standard quality controls, including confirmation of stemness marker expression, normal karyotype and pluripotency.

[0669] To generate edited iPSC cells, the PCS-201 (PCS) cells were electroporated with a Cas12a RNP designed to cut at the target gene of interest. Briefly, the cells were treated 24 hours prior to transfection with a ROCK inhibitor (Y27632). On the day of transfection, a single cell solution was generated using accutase and 500,000 PCS iPS cells were resuspended in the appropriate electroporation buffer and Cas12a RNP at a final concentration of 2μM. When two RNPs were added simultaneously, the total RNP concentration was 4 μM (2+2). This solution was electroporated using a Lonza 4D electroporator system. Following electroporation, the cells were plated in 6-well plates in mTESR media containing CloneR (Stemcell Technologies). The cells were allowed to grow for 3-5 days with daily media changes, and the CloneR was removed from the media by 48 hours post electroporation. To pick single colonies, the expanded cells were plated at a low density in 10 cm plates after resuspending them in a single cell suspension. Rock inhibitor was used to support the cells during single cell plating for 3-5 days post plating depending on the size of the colonies on the plate. After 7-10 days, sufficiently sized colonies with acceptable morphology were picked and plated into 24-well plates. The picked colonies were

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expanded to sufficient numbers to allow harvesting of genomic DNA for subsequent analysis and for cell line cryopreservation. Editing was confirmed by NGS and selected clones were expanded further and banked. Ultimately, karyotyping, stemness flow, and differentiation assays were performed on a subset of selected clones.

[0670] Two target genes of interest were CISH and TGFβRII, both of which were hypothesized to enhance natural killer cell function. As the TGFβ:TGFβRII pathway is believed to be involved in the maintenance of pluripotency, it was hypothesized that a functional deletion of TGFβRII in iPSCs could lead to differentiation and prevent generation of TGFβRII edited iPSCs. Due to the convergence of Activin receptor signaling and TGFβRII signaling in regulating SMAD2/3 and other intracellular molecules, it was hypothesized that Activin A could replace TGFβ in commercially available pluripotent stem cell medias to generate edited lines. To test this hypothesis, the pluripotency of unedited and TGFβRII edited iPSCs grown with Activin A was assessed. Several different culture medias were utilized: "E6" (Essential 6™ Medium, #A1516401, ThermoFisher), which lacks TGFβ, "E7", which was E6 supplemented with 100 ng/ml of bFGF (Peprotech, #100-18B), "E8" (Essential 8™ Medium, #A1517001, ThermoFisher), and "E7 + ActA", which was E6 supplemented with 100 ng/ml of bFGF and varying concentrations of Activin A (Peprotech #120-14P). Typically, E6 and E7 medias are typically insufficient to maintain the stemness and pluripotency of PSCs over multiple passages in culture.

[0671] In order to determine whether Activin A could maintain PCS iPSCs in the absence of exogenous TGFβ, unedited PCS iPSCs were plated on a LaminStem™ 521 (Biological Industries) coated 6-well plate and cultured in E6, E7, E8 or E7+ActA (with Activin A at two different concentrations – 1 ng/ml and 4 ng/ml). After 2 passages, the cells were assessed for morphology and stemness marker expression. Morphology was assessed using a standard phase contrast setting on an inverted microscope. Colonies with defined edges and non-differentiated cells typical of iPSC colonies, were deemed to be stem like. To confirm the morphological observations, the expression of standard iPS cell stemness markers was measured using intracellular flow cytometry. Briefly, cells were dissociated, stained for extracellular markers, and then fixed overnight and permeabilized using the reagents and standard protocol from the Foxp3/Transcription Factor Staining Buffer Set (eBioscience™). Cells were stained for flow cytometric analysis with anti-human TRA-1-60-R_AF®488 (Biolegend®; Clone TRA-1-60-R), anti-Human Nanog_AF®647 (BD Pharmingen™; Clone N31-355), and anti-Oct4 (Oct3)_PE (Biolegend®; Clone 3A2A20).

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Cells were recorded on a NovoCyte Quanteon Flow Cytometer (Agilent) and analyzed using FlowJo (FlowJo, LLC). As shown in Figure 46, both 1 ng/mL and 4 ng/ml of Activin A was sufficient to maintain pluripotency with equivalent stemness marker expression to the cells grown in E8. As expected, cells grown in E6 and E7 (which lacked TGFβ) did not maintain stemness gene expression to the same degree as E8, indicating the loss of iPSC stemness in the absence of TGFβ or Activin A. These results suggest that Activin A can supplement iPSC stemness in the absence of TGFβ signaling.

[0672] Given the demonstration that Activin A could support iPSC stemness in the absence of TGFβ, TGFβRII knockout ("KO") iPSCs, CISH KO iPSCs, and TGFβRII/CISH double knockout ("DKO") iPSC lines were generated. Specifically, iPSCs were edited using an RNP having an engineered Cas12a with three amino acid substitutions (M537R, F870L, and H800A (SEQ ID NO: 62)) and a gRNA specific for CISH or TGFβRII. To make CISH/TGFβRII DKO iPSCs, iPSCs were treated with an RNP targeting CISH and an RNP targeting TGFβRII simultaneously. The particular guide RNA sequences of Table 26 were used for editing of CISH and TGFβRII. Both guides were generated with a targeting domain consisting of RNA, an AsCpf1 scaffold of the sequence UAAUUUCUACUCUUGUAGAU (SEQ ID NO: 1153) located 5' of the targeting domain, and a 25-mer DNA extension of the sequence ATGTGTTTTTGTCAAAAGACCTTTT (SEQ ID NO: 1154) at the 5' terminus of the scaffold sequence.

Table 26: Guide RNA sequences

Target	gRNA Targeting Domain Sequence	Full Length gRNA Sequence
CISH 7050	GGUGUACAGCAGUGGCUGGU (SEQ ID NO: 1155)	ATGTGTTTTTGTCAAAAGACCTTTTrUrArArUrUrUrCrUrArCrUrCrUrUrGrUrArGrArUrGrGrUrGrUrArCrArGrCrArGrUrGrGrCrUrGrGrU (SEQ ID NO: 1156)
TGFβRII 24026	UGAUGUGAGAUUUUCCACCU (SEQ ID NO: 1157)	ATGTGTTTTTGTCAAAAGACCTTTTrUrArArUrUrUrCrUrArCrUrCrUrUrGrUrArGrArUrGrUrGrArGrArUrUrUrUrCrCrArCrCrU (SEQ ID NO: 1158)

[0673] The edited clones were generated as described above with a minor modification for the cells treated with TGFβRII RNPs. Briefly, TGFβRII-edited PCS iPSCs and TGFβRII/CISH edited PCS iPSCs were plated after electroporation at the 6-well stage in the mTESR supplemented with 10 ng/ml of Activin A in order to support the generation of

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edited clones. The cells were cultured with 10 ng/ml of Activin A through the cell colony picking and early expansion stages. Colonies assessed as having the correct single KO (CISH KO or TGFβRII KO) or double KO (CISH/TGFβRII DKO) were picked and expanded (clonal selection).

[0674] To determine the optimal concentration of Activin A for culturing of TGFβRII KO and TGFβRII/CISH DKO iPSCs, a slightly expanded concentration curve was tested as shown Figure 41. Similar to the assessment performed previously, the iPSCs were cultured in a Matrigel-treated 6-well plate with concentrations of 1 ng/ml, 2 ng/ml, 4 ng/ml and 10 ng/ml Activin A. As shown in Figure 41, TGFβRII KO or CISH/TGFβRII DKO cells cultured in E7 medium supplemented with 4 ng/mL Activin A for 19 days (over 5 passages) maintained a wild type morphology. Figure 42 shows the morphology of TGFβRII KO PCS-201 hiPSC Clone 9.

[0675] As shown in Figure 43A, the initial editing efficiency of the iPSCs treated simultaneously with the CISH and TGFβRII RNPs (prior to clonal selection) was high, with 95% of the CISH alleles edited and 78% of the TGFβRII alleles edited. Unedited iPSC controls did not have indels at either loci. iPSCs that were treated with CISH or TGFβRII RNPs individually showed 93% and 82% editing rates prior to clone selection (depicted in Figure 43A). The KO cell lines (CISH KO iPSCs, TGFβRII KO iPSCs, and CISH/TGFβRII DKO iPSCs) were subsequently assessed for the presence of pluripotency markers Oct4, SSEA4, Nanog, and Tra-1-60 after culturing in the presence of supplemental Activin A. As shown in Figures 43B and 44, culturing the KO cell lines in Activin A maintained expression of these pluripotency markers.

[0676] The KO iPSC lines cultured in Activin A were next assessed for their capacity to differentiate using the STEMdiff™ Trilineage Differentiation Kit assay (from STEMCELL Technologies Inc., Vancouver, BC, CA) as depicted schematically in Figure 45. As shown in Figure 46A, culturing the single KO (TGFβRII KO iPSCs or CISH KO iPSCs) and DKO (TGFβRII/CISH DKO iPSCs) cell lines in media with supplemental Activin A maintained their ability to differentiate into early progenitors of all 3 germ layers, as shown by expression of ectoderm (OTX2), mesoderm (brachyury), and endoderm (GATA4) markers (Figure 46A). The unedited PCS control cells were also able to express each of these markers.

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[0677] The edited iPSCs were next karyotyped to determine whether the Cas12a editing caused large genetic abnormalities, such as translocations. As shown in Figure 46B, the cells had normal karyotypes with no translocation between the cut sites.

[0678] To further support the results described above, an expanded Activin A concentration curve was performed on the unedited parental PSC line, an edited TGFβRII KO iPSC clone (C7), and an additional representative (unedited) cell line designated RUCDR (RUCDR Infinite Biologics group, Piscaway NJ). At the outset, the iPSCs were seeded at 1e5 cells per well in a 1x LaminStem™ 521 (Biological Industries) coated 12-well plate. Cells were then passaged 10 times over ~40-50 days using 0.5 mM EDTA in 1x PBS dissociation and Y-27632 (Biological Industries) until wells achieved >75% confluency. Cells were cultured in Essential 6™ Medium (Gibco), TeSR™-E7™, and TeSR™-E8™ (StemCell Technologies) for controls and titrated using TeSR™-E7™ supplemented with *E. coli*-derived recombinant human/murine/rat Activin A (PeproTech) spanning a 4-log concentration dosage (0.001 – 10 ng/mL). Following 5 and 10 passages, cells were dissociated and then fixed overnight and permeabilized using the reagents and standard protocol from the Foxp3/Transcription Factor Staining Buffer Set (eBioscience™). Cells were stained for flow cytometric analysis with anti-human TRA-1-60-R AF®488 (Biolegend®; Clone TRA-1-60-R), anti Sox2_PerCP-Cy™5.5 (BD Pharmingen™; Clone O30-678), anti Human Nanog_AF®647 (BD Pharmingen™; Clone N31-355), anti-Oct4 (Oct3)_PE (Biolegend®; Clone 3A2A20), and anti-human SSEA-4_PE/Dazzle™ 594 (Biolegend®; Clone MC-813-70). Cells were recorded on a NovoCyte Quanteon Flow Cytometer (Agilent) and analyzed using FlowJo (FlowJo, LLC). Figure 46C shows the titration curves for the tested iPSC lines. The minimum concentration of Activin A required to maintain each line varied slightly, with the TGFβRII KO iPSCs requiring a higher baseline amount of Activin A as compared to the parental control (0.5 ng/ml vs 0.1 ng/ml). In all 3 cell lines, 4 ng/ml was well above the minimum amount of Activin A necessary to maintain stemness marker expression over an extended culture period. Figure 46D shows the stemness marker expression in the cells culture with the base medias alone (no Activin A). As expected, the TGFβRII KO iPSCs did not maintain expression, while the two unedited lines were able to maintain stemness marker expression in E8.

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Example 19: Differentiation of edited CISH KO, TGFβRII KO, and CISH/TGFβRII DKO iPSCs into iNK cells exhibiting enhanced function

[0679] Figure 47A depicts a schematic of an exemplary workflow for development of a CRISPR-Cas12a-edited iPSC platform for generation of enhanced CD56+ iNK cells. As shown in Figure 47A, the CISH and TGFβRII genes are targeted in iPSCs via delivery of RNPs to the cells using electroporation to generate CISH/TGFβRII DKO iPSCs. iPSCs with the desired edits at both the CISH and TGFβRII genes can then be selected and expanded to create a master iPSC bank. Edited cells from the iPSC master bank can then be differentiated into CD56+ CISH/TGFβRII DKO iNK cells.

[0680] Figure 47B and 47C depict two exemplary schematics of the process of differentiating iPSCs into iNK cells. As shown in Figure 47B and 47C, edited cells (or unedited control cells) were differentiated using a two-phase process. First, in the "hematopoietic differentiation phase," hiPSCs (edited and unedited) were cultured in StemDiff™ APEL2™ medium (StemCell Technologies) with SCF (40 ng/mL), BMP4 (20 ng/mL), and VEGF (20 ng/mL) from days 0-10, to produce spin embryoid bodies (SEBs). As shown in Figure 53B, SEBs were then cultured from days 11-39 in StemDiff™ APEL2™ medium comprising IL-3 (5 ng/mL, only present for the first week of culture), IL-7 (20 ng/mL), IL-15 (10 ng/mL), SCF (20 ng/mL), and Flt3L (10 ng/mL) in an NK cell differentiation phase. CISH KO iPSCs, TGFβRII KO iPSCs, CISH/TGFβRII DKO iPSCs, and unedited wild-type iPSC lines (PCS), were differentiated into iNKs according to the schematic in Figure 47B, and then characterized to assess whether they exhibited a phenotype congruent with NK cells (see Figures 48, 49, and 50A). CISH KO iPSCs, TGFβRII KO iPSCs, CISH/TGFβRII DKO iPSCs, and unedited wild-type iPSC lines, described in Figures 50B, 50C, 51B, 51C, and 52 were also differentiated into iNKs utilizing the alternative method shown in Figure 47C, and then characterized to assess whether they exhibited a phenotype congruent with NK cells (see Figures 50B, 50C, 51B, 51C, and 52).

[0681] Specifically, the CISH KO iNKs, TGFβRII KO iNKs, CISH/TGFβRII DKO iNKs were assessed for exemplary phenotypic markers of (i) stem cells (CD34); and (ii) hematopoietic cells (CD43 and CD45) by flow cytometry. Briefly, two rows of embryoid bodies from a 96-well plate for each genotype were harvested for staining. Once a single cell solution was generated using Trypsin and mechanical disruption, the cells were stained for the human markers CD34, CD45, CD31, CD43, CD235a and CD41. As shown in Figure 48, CISH KO iNKs, TGFβRII KO iNKs, CISH/TGFβRII DKO iNKs, and iNKs derived from

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wild-type parental clones (PCS) exhibited lower levels of CD34 relative to control cells, which were purified CD34+ HSCs. CD34 expression levels were similar across these iNK cell clones indicating that editing of the iPSCs did not affect differentiation to the CD34+ stage. Figure 49 shows that CISH KO iNKs, TGFβRII KO iNKs, CISH/TGFβRII DKO iNKs, and iNKs derived from wild-type parental clones (PCS) exhibited similar surface expression profiles for CD43 and CD45. Thus, iNKs differentiated from edited and unedited iPSCs exhibited similar levels of markers for stem cells and hematopoietic cells, and both differentiated edited and unedited cells exhibited certain NK cell phenotypes based on marker expression profiles.

[0682] CISH KO iNKs, TGFβRII KO iNKs, CISH/TGFβRII DKO iNKs, iNKs derived from wild-type parental clones (WT), and NK cells derived from peripheral blood (PBNKs) were further assayed to determine their surface expression of CD56, a marker for NK cells. Briefly, cells were harvested on day 39 of differentiation, washed and resuspended in a flow staining buffer containing antibodies that recognize human CD56, CD16, NKp80, NKG2A, NKG2D, CD335, CD336, CD337, CD94, CD158. Cells events were recorded on a NovoCyte Quanteon Flow Cytometer (Agilent) and analyzed using FlowJo (FlowJo, LLC). Figure 50A shows that iNK cells derived from edited iPSCs exhibited similar CD56+ surface expression relative to iNK cells derived from unedited iPSC parental clones and PBNK cells (at day 39 in culture). Figure 50B shows that iNK cells derived from edited iPSCs exhibited similar CD56+ and CD16+ surface expression relative to iNKs derived from unedited iPSC parental clones (at day 39 in culture). Figure 50C shows that iNK cells derived from edited iPSCs exhibited similar CD56+, CD54+, KIR+, CD16+, CD94+, NKG2A+, NKG2D+, NCR1+, NCR2+, and NCR3+ surface expression relative to iNKs derived from unedited iPSC parental clones and PBNK cells (at day 39 in culture)

[0683] To confirm cell functionality, cells were assessed using a tumor cell cytotoxicity assay on the xCelligence platform. Briefly, tumor targets, SK-OV-3 tumor cells, were plated and grown to an optimal cell density in 96-well xCelligence plates. iNKs were then added to the tumor targets at different E:T ratios (1:4, 1:2, 1:1, 2:1. 4:1 and 8:1) in the presence of TGFβ. Figure 51C shows that TGFβRII KO and CISH/TGFβRII DKO cells more effectively killed SK-OV-3 cells, as measured by percent cytolysis, relative to unedited iNK cells either in the presence or absence of TGF-β (at E:T ratios of 1:4, 1:2, 1:1, and 2:1).

[0684] While iNK cells generated using the alternative method described in Figure 47B were CD56+ and capable of killing tumor targets in an in vitro cytotoxicity assay, the

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iNKs did not express many of the canonical markers associated with mature NK cells such as CD16, NKG2A, and KIRs. A K562 feeder cell line is typically used to expand and mature iNKs that are generated by similar differentiation methodologies. After expansion on feeders, the iNKs often express CD16, KIRs and other surface markers indicative of a more mature phenotype. In order to identify a feeder free approach to achieve more mature iNKs with enhanced functionality, an alternative media composition was tested for the stage of differentiation between day 11 and day 39. Instead of culturing cells between day 11 and day 39 in APEL2 (as shown in Figure 47B), the spin embryoid bodies (SEBs) were cultured in NK MACS® media (MACS Miltenyi Biotec) with 15% human AB serum in the presence of the same cytokines as mentioned above. This protocol is depicted in Figure 47C. In order to compare the two media compositions, Day 11 SEBs from WT PCS, TGFβRII KO iPSCs, CISH KO iPSCs, and DKO iPSCs were split into two conditions for the second half of the differentiation process, one with APEL2 base and the other with the NKMACS + serum base. At day 39, the cell yield, marker expression, and cytotoxicity levels were assessed. In all cases, the NKMACS + serum condition (depicted in Figure 47C) outperformed the APEL2 condition (depicted in Figure 47B). Figure 47D shows that the NKMACS + serum condition yielded a greater fold expansion at the end of the 39 day process (nearly 300 fold expansion vs 100 fold expansion). When NK marker expression was analyzed by flow cytometry as described above, the iNKs cultured in NKMACS + serum were 34% CD16 positive and exhibited 20% KIR expression while the APEL2 conditions yielded cells that were essentially negative for both markers. This was the case for all genotypes tested. In order to visualize the markers relative to time or condition, flow cytometry data was gated and analyzed in FlowJo and heat maps were constructed (Figures 47E and 47F). Samples were first cleaned by gating for live cells (FSC-H vs. LIVE/DEAD™ Fixable Yellow) followed by immune cells (SSC-A vs. FSC-A), singlets (FSC-H vs. FSC-A) and the natural killer cell population (CD56 vs. CD45). The NK population, defined as CD45+56+ cells, was gated and each marker was analyzed along the X-axis in an analysis synonymous to a histogram/count plot (CD16+, CD94+, NKG2A+, NKG2D+, CD335+, CD336+, CD337+, NKp80+, panKIR+). Statistics for the aforementioned markers are visualized with a double-gradient heat map (GraphPad Prism 8) with the key set to the following parameters: black=0, medium intensity $30<x<50$, maximum intensity=100. Based on this analysis, the expression kinetics and magnitude across all genotypes were improved by the NKMACS + serum condition. The cells were also assessed in a tumor cell cytotoxicity assay as described previously. The iNKs

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generated in the NKMACS + serum conditions were capable of killing at a lower E:T ratio than the cells differentiated in APEL2, indicating that the improved NK maturation had a positive impact on the functionality of the cells (Figure 47G).

[0685] Analysis of additional differentiation markers in NKMACS + serum confirmed the presence of CD16 expression. Figure 50B shows analysis of specific subpopulations (CD45 vs CD56 and CD56 vs CD16) derived from unedited or DKO iPSCs. Additionally, the cell surface marker profile of unedited iNK cells and CISH/TGFβRII DKO iNKs in Figure 50C confirmed that the NK cell marker profile of the edited iNK cells was similar to that of unedited iNK cells. Taken together, these data show that Cas12a-edited single and double KO iPSC clones differentiate into iNK cells in a similar fashion as unedited iPSC clones, as defined by NK cell markers.

[0686] Additionally, certain edited iNK clonal cells (CISH single knockout "CISH_C2, C4, C5, and C8", TGFβRII single knockout "TGFβRII-C7", and TGFβRII/CISH double knockout "DKO-C1"), and parental clone iNK cells ("WT") were cultured in the presence of 1 ng/mL or 10 ng/mL IL-15, and differentiation markers were assessed at day 25, day 32, and day 39 post-hiPSC differentiation. As shown in Figure 53, surface expression phenotypes (measured as a percentage of the population) culturing in 10 ng/mL IL-15 resulted in a higher proportion of surface expression in the single knockouts, double knockouts, and the parental clonal line.

[0687] The edited iNK cells differentiated in NK MACS® medium + serum conditions were assessed for effector function *in vitro* using a range of molecular and functional analyses. First, a phosphoflow cytometry assay was performed to determine the phosphorylated state of STAT3 (pSTAT3) and SMAD2/3 (pSMAD2/3) in the day 39 iNK cells. CISH KO iNKs exhibited increased pSTAT3 upon IL-15 stimulation (Figure 50D), and CISH/TGFβRII DKO iNKs exhibited decreased pSMAD2/3 levels upon TGF-β stimulation as compared to unedited iNK cells (Figure 50E). These data suggest that CISH/TGFβRII DKO iNKs have enhanced sensitivity to IL-15 and resistance to TGF-β mediated immunosuppression. In addition, CISH/TGFβRII DKO iNKs were characterized for IFNγ and TNFα production using a phorbol myristate acetate and Ionomycin (PMA/IMN) stimulation assay. Briefly, cells were treated with 2 ng/ml of PMA and 0.125 μM of Ionomycin along with a protein transport inhibitor for 4 hours. The cells were harvested and stained using a standard intracellular staining protocol. The CISH/TGFβRII DKO iNKs produced significantly higher amounts of IFNγ and TFNα when stimulated with PMA/IMN

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(Figures 50F and 50G), providing evidence of enhanced cytokine production following stimulation relative to unedited control iNKs.

[0688] To test iNK tumor cell killing activity, a 3D solid tumor cell killing assay (depicted schematically in Figure 51A) was utilized. In brief, spheroids were formed by seeding 5,000 NucLight Red labeled SK-OV-3 cells in 96 well ultra-low attachment plates. Spheroids were incubated at 37°C before addition of effector cells (at different E:T ratios) and 10 ng/mL TGF-β, spheroids were subsequently imaged every 2 hours using the Incucyte S3 system for up to 120 hours. Data shown are normalized to the red object intensity at time of effector addition. Normalization of spheroid curves maintains the same efficacy patterns observed in non-normalized data. Using this assay, the cytotoxicity of iNKs differentiated from four CISH KO iPSC clones, two TGFβRII KO iPSC clones and one CISH/TGFβRII DKO iPSC clone were compared to control iNKs derived from the unedited parental iPSCs. As shown in Figure 51B, edited iNK cells were capable of reducing the size of SK-OV-3 spheroids more effectively than unedited iNK control cells (averaged data from 6 assays). In particular the CISH/TGFβRII DKO iNK cells reduced the size of SK-OV-3 spheroids to a greater extent than unedited iNK cells at all E:T ratios greater than 0.01, and significantly at E:T ratios of 1 or higher. The TGFβRII KO clone 7 iNKs also exhibited significantly enhanced killing when compared to unedited iNK cells. While a number of single CISH KO clones did not show significant enhancement of killing at the 10:1 E:T ratio, the majority of clones did display a trend towards increased SK-OV-3 spheroid cell killing, with the greatest differential at the highest E:T ratio. To further elucidate the functionality of the edited iNKs, the cells were pushed to kill tumor targets repeatedly over a multiday period, herein described as an *in vitro* serial killing assay. At day 0 of the assay, 10×10^6 Nalm6 tumor cells (a B cell leukemia cell line) and 2×10^5 iNKs were plated in each well of a 96-well plate in the presence of IL-15 (10 ng/ml) and TGF-β (10ng/ml). At 48 hour intervals, a bolus of 5×10^3 Nalm6 tumor cells (a B cell leukemia cell line) was added to re-challenge the iNK population. As shown in Figure 52, the edited iNK cells (CISH/TGFβRII DKO iNK cells) exhibited continued killing of Nalm6 cells after multiple challenges with Nalm6 tumor cells, whereas unedited iNK cells were limited in their serial killing effect. The data supports the conclusion that the CISH and TGFβRII edits result in prolonged enhancement of cell killing.

[0689] Finally, edited iNK cells (CISH/TGFβRII DKO iNK cells) were assayed for their ability to kill tumor targets in an in vivo model. To this end, an established NOD scid gamma (NSG) xenograft model was utilized in an assay as depicted in Figure 54A. Briefly, 1

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x 10^6 SK-OV-3 cells engineered to express luciferase were injected intraperitoneally (IP) at day 0. On day 3, the inoculated mice were imaged using an In vivo imaging system (IVIS) and randomized into 3 groups. The next day (day 4), 20 x 10^6 unedited iNKs or CISH/TGFβRII DKO iNKs were administered by IP injection, while a third group was injected with vehicle as a control. Following inoculation of the animals with tumor cells, animals were imaged once a week to measure tumor burden over time. Figure 54B depicts the bioluminescence of the tumors in the individual mice in the 3 different groups (n=9 in each group), vehicle, unedited iNKs, and CISH/TGFβRII DKO iNKs. The average tumor burden over time for these same animals is depicted in Figure 54C. A two way anova analysis was performed on the data, and CISH/TGFβRII DKO iNK treated animals had significantly less tumor burden as measured by bioluminescence when compared to animals treated with unedited iNKs (p value: 0.0004). By 10 days post-tumor implantation, mice injected with the CISH/TGFβRII DKO iNKs exhibited a significant reduction in the size of their tumors relative to mice injected with the vehicle controls or the unedited iNKs. The overall reduction in tumor size is seen for several days, and at least until 35 days post-tumor implantation. These data show that the edited DKO iNKs were actively killing tumor cells in this in vivo model.

[0690] Overall, these results demonstrate that unedited and CISH/TGFβRII DKO iPSCs can be differentiated into iNK cells exhibiting canonical NK cell markers. Additionally, CISH/TGFβRII DKO iNK cells demonstrated enhanced anti-tumor activity against tumor cell lines derived from both solid and hematological malignancies.

Example 20: ADORA2A edited iPSCs give rise to edited iNKs with enhanced function

[0691] ADORA2A is another target gene of interest, the loss of which is hypothesized to affect NK cell function in a tumor microenvironment (TME). The ADORA2A gene encodes a receptor that responds to adenosine in the TME, resulting in the production of cAMP which functions to drive a number of inhibitory effects on NK cells. We hypothesized that knocking out the function of ADORA2A could enhance iNK cell function. Utilizing a similar approach to the one described in Examples 18 and 19, the PCS iPSC line was edited using a RNP having an engineered Cas12a with three amino acid substitutions (M537R, F870L, and H800A (SEQ ID NO: 62)) and a gRNA specific to ADORA2A (except that 4 μM RNP was delivered to cells rather than 2 μM RNP). As described in Example 18, the gRNA was generated with a targeting domain consisting of RNA, an AsCpf1 scaffold of

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the sequence UAAUUUCUACUCUUGUAGAU (SEQ ID NO: 1153) located 5' of the targeting domain, and a 25-mer DNA extension of the sequence ATGTGTTTTTGTCAAAAGACCTTTT (SEQ ID NO: 1154) at the 5' terminus of the scaffold sequence. The ADORA2A gRNA sequence is shown in Table 27.

Table 27: Guide RNA sequence

Target	gRNA Targeting Domain Sequence	Full Length gRNA Sequence
ADORA2A 4113	CCAUCGGCCUGACUCCCAUG (SEQ ID NO: 1159)	ATGTGTTTTTGTCAAAAGACCTTTTrUrArArUrUrUrCrUrArCrUrCrUrUrGrUrArGrArUrCrCrArUrCrGrGrCrCrUrGrArCrUrCrCrCrArUrG (SEQ ID NO: 1160)

[0692] The bulk editing rate by the Cas12a RNP prior to clonal selection was 49% as determined by next-generation sequencing (NGS). Nonetheless, several clones that had both ADORA2A alleles edited were identified, expanded and differentiated. To determine whether an ADORA2A edited iPSC could yield CD45+CD56+ iNKs, both bulk and singled ADORA2A KO clones were differentiated using the NKMACS + serum protocol as described in Example 19 (Figure 47C). As shown in Figure 55A, edited iPSCs differentiated to iNKs with similar NK cell marker expression compared to unedited control iPSCs.

[0693] To confirm that Cas12a-mediated ADORA2A editing resulted in a functional deletion of the gene, cAMP accumulation in response to treatment with 5'-N-ethylcarboxamide adenosine ("NECA", a more stable adenosine analog that acts as an ADORA2A agonist) was assessed in both the edited and unedited control iNKs. Edited cells with a functional knockout of ADORA2A would not be expected to accumulate as much cAMP in the cells in response to NECA relative to cells with functional ADORA2A. Briefly, iNK cells were treated with varying concentrations of NECA for 15 minutes. The iNK cells were then lysed, and the cAMP in the lysate was then measured using a CisBio cAMP kit. As shown in Figure 55B, unedited iNKs had increased levels of cAMP accumulation as the concentration of NECA was increased (n=2). Conversely, the ADORA2A ("A2A KOs") KO iNKs showed minimal production of cAMP at increasing concentrations of NECA, indicating that the Cas12a-induced edits functionally knocked out ADORA2A function. The bulk iNKs (top two A2A KO iNK lines in Figure 55B) exhibited slightly higher levels of cAMP than the selected ADORA2A KO clones (lower four A2A KO iNK lines in Figure 55B), as would be expected from the lower editing rates in the bulk population. Based on this molecular

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evidence of functional ablation of ADORA2A, the iNKs would be expected to be resistant to the inhibitory effects of adenosine in a tumor microenvironment.

[0694] The ADORA2A KO iNKs were also tested in an in vitro NALM6 serial killing assay as described in Example 19, with one main difference: 100μM of NECA was added in place of TGFβ. The ADORA2A KO iNKs exhibited enhanced serial killing relative to the wild type iNKs in the presence of NECA, indicating that the ADORA2A KO iNKs were resistant to NECA inhibition (Figure 55C). As a result, the ADORA2A KO iNK cells would be expected to have improved cytotoxicity against tumor cells in the presence of adenosine in the TME relative to unedited iNK cells.

Example 21: Generation of CISH/ TGFβRII /ADORA2A triple edited (TKO) iPSCs and the characterization of differentiated TKO iNKs

[0695] In order to generate CISH, TGFβRII, and ADORA2A triple edited (TKO) iPSCs, two approaches were taken; 1) two step editing in which the CISH/ TGFβRII DKO (CR) iPSC clone described in Examples 18 and 19 was edited at the ADORA2A locus via electroporation with an ADORA2A targeting RNP (as described in Example 20), and 2) simultaneous editing of PCS iPS cells with all 3 RNPs, one for each target gene. Both strategies utilized the editing protocol briefly described in Example 18. In the case of simultaneous editing, the total RNP concentration was 8 μM (Cish:2 μM+ TGFβRII:2 μM+ADORA2A:4μM). Regardless of the approach, cells were plated, expanded and colonies were picked as described above. Using NGS to analyze gDNA harvested from the iPSCs, it was determined that the bulk editing rates were 96.70%, 97.17%, and 90.16% for CISH, TGFβRII and ADORA2A, respectively, when all target genes were edited simultaneously. Picked colonies that had Insertions and/or Deletions (InDels) at all 6 alleles were selected for further analysis.

[0696] Similar to the analysis described in Example 18, unedited iPSCs and the edited iPSCs were differentiated to iNKs using the NK MACS + Serum condition (described in Figure 49C) and assessed by flow cytometry at different time points, including at day 25, day 32, and day 39 in culture. As shown in Figure 56A, analysis of the different NK surface markers revealed no major differences between clones that were generated by the two-step editing method (CR+A 8) or the simultaneous editing method (CRA 6). Both TKO clones (CR+A 8 and CRA 6) showed similar expression profiles to the unedited iNKs (Wt) at each time point. When the TKO iNK cells were analyzed for their responsiveness to NECA (as

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described in Example 20), both TKO iNKs had little to no cAMP accumulation (Figure 56B), demonstrating that ADORA2A was functionally knocked out. By contrast, the unedited iNKs demonstrated a NECA dose dependent increase in cAMP (Figure 56B). These results indicate that the TKO iNKs would be expected to be resistant to the inhibitory effects of adenosine in the TME. Finally, the CISH/TGFβRII/ADORA2A TKO iNKs were assessed alongside CISH/ TGFβRII DKO iNKs, ADORA2A single KO (SKO) iNKs, and unedited iNKs in a 3D tumor cell killing assay. This assay was performed as described in Example 19 with IL-15 and TGFβ but without NECA. Interestingly, both the TKO (CRA6) and DKO (CR) iNKs outperformed the unedited iNKs in killing the tumor cells, indicating that both multiplex edited iNKs have enhanced function over unedited control cells (Figure 56C). These results show that knocking out ADORA2A does not negatively affect the ability of iNKs having CISH and TGFBRII KOs to kill tumor spheroid cells.

Example 22: Selection of CISH, TGFβRII, ADORA2A, TIGIT, and NKG2A targeting gRNAs.

[0697] The cutting efficiency of CISH, TGFBRII, ADORA2A, TIGIT, and NKG2A Cas12a guide RNAs were further tested. Guide RNAs were screened by complexing commercially synthesized gRNAs with Cas12a in vitro and delivering gRNA/Cas12a ribonucleoprotein (RNP) to IPSCs via electroporation. The iPSCs were edited using a RNP having an engineered Cas12a with three amino acid substitutions (M537R, F870L, and H800A (SEQ ID NO: 62)). The gRNAs were generated with a targeting domain consisting of RNA, an AsCpf1 scaffold of the sequence UAAUUUCUACUCUUGUAGAU (SEQ ID NO: 1153) located 5' of the targeting domain, and a 25-mer DNA extension of the sequence ATGTGTTTTTGTCAAAAGACCTTTT (SEQ ID NO: 1154) at the 5' terminus of the scaffold sequence. Table 28 provides the targeting domains of the guide RNAs that were tested for editing activity.

Table 28: guide RNA sequences

Target	gRNA Targeting Domain Sequence
TGFβRII	UGAUGUGAGAUUUUCCACCUG (SEQ ID NO: 1161)
CISH	ACUGACAGCGUGAACAGGUAG (SEQ ID NO: 1162)
ADORA2A	CCAUCGGCCUGACUCCCAUGC (SEQ ID NO: 1163)

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ADORA2A	CCAUCACCAUCAGCACCGGGU (SEQ ID NO: 1164)
ADORA2A	CCUGUGUGCUGGUGCCCCUGC (SEQ ID NO: 1165)
TIGIT	UGCAGAGAAAGGUGGCUCUAU (SEQ ID NO: 1166)
TIGIT	UCUGCAGAAAUGUUCCCCGUU (SEQ ID NO: 1167)
TIGIT	UAGGACCUCCAGGAAGAUUCU (SEQ ID NO: 1168)
NKG2A	GCAACUGAACAGGAAAUAACC (SEQ ID NO: 1169)
NKG2A	GUUGCUGCCUCUUUGGGUUUG (SEQ ID NO: 1170)
NKG2A	AAGGGAAUGACAAAACCUAUC (SEQ ID NO: 1171)

[0698] In brief, 100,000 iPSCs/well were transfected with the RNP of interest, cells were incubated at 37°C for 72 hours, and then harvested for DNA characterization. iPSCs were transfected with gRNA/Cas12a RNPs at various concentrations. The percentage editing events were determined for eight different RNP concentrations ranging from negative control (0 mM) to 8 mM.

[0699] As shown in Figure 57 panel 1, the TGFβRII gRNA (SEQ ID NO: 1161) exhibited an EC50 of ~79nM RNP. As shown in Figure 57 panel 2, the CISH gRNA (SEQ ID NO: 1162) exhibited an EC50 of ~50 nM RNP. As shown in Figure 57 panel 3, an ADORA2A gRNA (SEQ ID NO: 1163) included in RNP2960 exhibited an EC50 of ~63 nM RNP, while an ADORA2A gRNA (SEQ ID NO: 1164) included in RNP3109, or gRNA (SEQ ID NO: 1165) included in RNP3108 exhibited EC50 values of ~493 nM and ~280nM RNP respectively. As shown in Figure 57 panel 4, a TIGIT gRNA (SEQ ID NO: 1166) included in RNP2892 exhibited an EC50 of ~29 nM RNP, while a TIGIT gRNA (SEQ ID NO: 1167) included in RNP3106, or gRNA (SEQ ID NO: 167) included in RNP3107 exhibited EC50 values of ~1146 nM and ~40 nM RNP respectively. As shown in Figure 57 panel 5, a NKG2A gRNA (SEQ ID NO: 1169) included in RNP19142 exhibited an EC50 of ~8 nM RNP, while a NKG2A gRNA (SEQ ID NO: 1170) included in RNP3069, or gRNA (SEQ ID NO: 1171) included in RNP2891 exhibited EC50 values of ~12 nM and ~13 nM RNP respectively.

Example 23: Knock-in of Cargo at Essential Gene Loci in T-cells Using a Viral Vector

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[0700] The present example describes gene editing of populations of T cells using viral vector transduction. Following editing, cells were subjected to various assays such as flow cytometry, ddPCR, next-generation sequencing, or functional tumor killing assays to determine KO/KI efficiency and/or efficacy.

[0701] T cells were thawed in a bead bath as known in the art and were removed from the bath on day two. Cells were electroporated on day four after thawing. Briefly, 250,000 T cells per well in a Lonza 96-well cuvette were suspended in buffer P2 and electroporated with RNP comprising gRNA RSQ22337 (SEQ ID NO: 95) and Cas12a (SEQ ID NO: 62) targeting the *GAPDH* gene (1μM RNP) or with media control, using various pulse codes. Appropriate media was added to cells immediately after electroporation and cells were allowed to recover for 15 minutes. AAV6 viral particles comprising a donor plasmid construct containing a knock-in cassette with a cargo of GFP, CD19 CAR, B2M-HLA-E, or vector control were then added to T cells at varying multiplicity of infection (MOI) concentrations (1E4, 1E5, or 1E6 MOI (vg/cell)). The donor plasmids were designed as described in Example 2, with a 5′ codon-optimized coding portion of GAPDH exon 9 optimized to prevent further binding of the gRNA targeting domain sequence of the guide RNA (RSQ22337)), an in-frame sequence encoding the P2A self-cleaving peptide ("P2A"), an in-frame coding sequence for a cargo sequence (*e.g.*, GFP, CD19 CAR, or B2M-HLA-E) ("Cargo"), a stop codon and polyA signal sequence. T cells were split two days later, and then every 48 hours until they were analyzed by flow cytometry or otherwise utilized. T cells were sorted using flow cytometry seven days post electroporation to determine successful transduction, transformation, editing, knock-in cassette integration, and/or expression events. A very high percentage (94.8%) of cells expressed GFP, indicating that a high proportion of cells had GFP integrated at the *GAPDH* gene in the edited T cell population, and these edited cells exhibited similar viability and ability to expand as control cells that underwent mock transformation (Fig. 17B-17C). Moreover, GFP knock-in at the GAPDH locus generated GFP+ cells at a significantly greater rate than GFP knock-in at the TRAC locus (Fig. 17D). This increase in GFP+ cells produced by GFP knock-in at the GAPDH locus compared to GFP knock-in at the TRAC locus was observed across a range of AAV6 concentrations (Fig. 17E). These results demonstrate that knock-in at an essential gene locus (e.g., GAPDH) can achieve greater knock-in efficiency, including at lower concentrations of the AAV6 donor template, than knock-in at the TRAC locus. A very high percentage (95.8%) of cells expressed CD19 CAR, indicating that a high proportion of cells had CD19 CAR integrated at the *GAPDH* gene in the edited T cell

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population, and these edited cells also exhibited similar viability and ability to expand as control cells that underwent mock transformation (Fig. 58A-58C, and 58H). Additionally, a very high percentage (over 80%) of cells expressed B2M-HLA-E, indicating that a high proportion of cells had B2M-HLA-E integrated at the *GAPDH* gene in the edited T cell population. As shown in Fig. 58H, T cells that were edited to include knock-in of GFP, CD19 CAR, or B2M-HLA-E expressed these transgenes at rates that were greater than 80%. Furthermore, B2M-HLA-E KI cells expressed a higher level of HLA-E when compared to control cells and were viable (see Fig. 59).

[0702] The knock-in efficiencies generated using the methods described herein were compared to the knock-in efficiencies generated using optimized methods known in the art for targeting cargo knock-ins to non-essential genes. In brief, T cell populations were transduced with AAV6 vector comprising a donor template suitable for knock-in of GFP at the *GAPDH* gene as described herein, and were transformed with gRNA RSQ22337 (SEQ ID NO: 95) and Cas12a (SEQ ID NO: 62) as described above. Alternatively, T cell populations were subjected to highly optimized GFP knock-in at the *TRAC* locus using AAV6 vector transduction (see e.g., Vakulskas et al. A high-fidelity Cas9 mutant delivered as a ribonucleoprotein complex enables efficient gene editing in human hematopoietic stem and progenitor cells. Nat Med. 2018;24(8):1216-1224). Flow cytometry was utilized to measure knock-in efficiency (determined by percentage of T cell population expressing GFP, measured 7 days post-electroporation). Knock-in rates at the *TRAC* locus were high (~50%) when compared to publicly described integration frequencies for similar methodologies; however, knock-in efficiency at the *GAPDH* gene using the methods described herein were significantly ($p = <0.001$ using unpaired t-test) higher (~90%) (see Fig. 17D). The same RNP concentration, AAV6 MOI, and homology arm lengths were utilized in both experiments, averaged results from three independent replicates are shown. Thus, the methods described herein can be used to isolate a population of modified cells, such as immune cells like T cells, that more highly express a gene of interest relative to other gene knock-in methods.

[0703] In other experiments, T cells were edited to generate TRAC knock-out cells without (see Fig. 58D) or with (see Fig. 58E) a CD19 CAR KI at the *GAPDH* locus using the methods described above. As shown in Fig. 58E, a very high percentage of edited cells expressed CD19 CAR (87.6%) indicating high levels of CD19 CAR integration at the *GAPDH* gene. By contrast, the control TRAC KO cells did not express CD19 CAR (Fig.

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58D). As shown in Fig. 58F, T cells transformed with TRAC targeting RNPs, GAPDH targeting RNPs, and/or transduced with AAV6 comprising a CD19 cargo targeted for knock-in at GAPDH displayed various phenotypes representative of their respective desired edited genotypes. As determined by flow cytometry, T cells that had CD19 CAR KI were observed at rates greater than 90% when cells were transformed with GAPDH targeting RNPs, and transduced with AAV6 comprising a knock-in CD19 cargo targeting GAPDH. T cells that had TRAC KO and CD19 CAR KI were observed at rates greater than 80% when cells were transformed with TRAC targeting RNPs, GAPDH targeting RNPs, and transduced with AAV6 comprising a CD19 cargo targeted for knock-in at GAPDH. As depicted in Fig. 58I, T cells with CD19 CAR KI at GAPDH were able to destroy hematological cancer cells (CD19+ Raji cells) at rates significantly greater than T cells with GFP KI at GAPDH ("Cells only" refers to unedited T cells). Furthermore, T cells with CD19 CAR KI at GAPDH demonstrated significantly greater cytotoxicity against Raji cells than either T cells with GFP KI at GAPDH or unedited T cells as seen in Fig. 58J. This significant increase in cytotoxicity was also observed with T cells with CD19 CAR KI at GAPDH in combination with TRAC and/or TGFBR2 KO (Fig. 58J).

[0704] In other experiments, a population of T cells were edited to generate KO of TRAC, KO of TGFBR2, and CD19 CAR KI at the *GAPDH* locus using the methods described above, thereby generating triple mutant (TRAC KO, TGFBR2 KO, and CD19 CAR KI) T cells at a high efficiency. A high percentage of the edited T cells (about 73.6%) expressed CD19 CAR (see Fig. 58G).

[0705] As shown in Fig. 60A, highly defined engineered T cells comprising multiple edits can be generated using a one-step electroporation and transformation process in which three RNPs targeting three loci (TRAC, B2M and GAPDH) and an AAV comprising a GFP cargo for knock-in at the GAPDH locus are applied to the T cells (Fig. 60A left panel), or using a sequential electroporation and transformation process in which the same RNPs and AAVs are sequentially applied to the T cells (Fig. 60A right panel). The one-step process generated about the same percentage of cells containing TRAC and B2M knockouts and GFP expression as the sequential process. In addition, T cells were edited to generate multiple knock-outs including at the TRAC locus, B2M locus, CIITA locus, and TGFBR2 locus as well as a GFP cargo knock-in at the *GAPDH* locus using a one-step process wherein five Cas12a (SEQ ID NO:62) RNPs (specific to TRAC, B2M, CIITA, TGFBR2, and GAPDH) and an AAV6 comprising a GFP cargo designed to integrate within the GAPDH locus were

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applied to the cells at once (see Fig. 60B). Each individual editing event occurred within greater than 80% of the total population, while cells that at least comprised three mutations (TRAC KO, B2M KO, and GFP cargo KI at the *GAPDH* locus) occurred at a rate greater than 80%.

[0706] These results show that the methods described herein can produce highly engineered T cell populations with high levels of editing homogeneity for potential use as autologous and/or allogeneic T cell therapies suitable for targeting a variety of tumors and/or cancerous cells.

Example 24: Knock-in of Cargo at Essential Gene Loci in NK Cells Using a Viral Vector

[0707] The present example describes gene editing of populations of NK cells using viral vector transduction.

[0708] NK cells were thawed in a bead bath as known in the art and were removed from the bath on day two. Cells were electroporated on day four after thawing. Briefly, 500,000 NK cells per well in a Lonza 96-well cuvette were suspended in buffer P2 and electroporated with RNP comprising gRNA RSQ22337 (SEQ ID NO: 95) and Cas12a (SEQ ID NO: 62) targeting the *GAPDH* gene (1μM RNP) or media control, using various pulse codes. Appropriate media was added to cells immediately after electroporation and cells were allowed to recover for 15 minutes. AAV6 viral particles comprising a donor plasmid construct containing a knock-in cassette with a cargo of GFP, CD19 CAR, or vector control were then added to NK cells at varying multiplicity of infection (MOI) concentrations (1E4, 1E5, or 1E6 MOI (vg/cell)). The donor plasmids were designed as described in Example 2, with a 5′ codon-optimized coding portion of GAPDH exon 9 optimized to prevent further binding of the gRNA targeting domain sequence of the guide RNA (RSQ22337)), an in-frame sequence encoding the P2A self-cleaving peptide ("P2A"), an in-frame coding sequence for a cargo sequence (*e.g.*, GFP, or CD19 CAR) ("Cargo"), a stop codon and polyA signal sequence. Media was changed 24 hours post electroporation and IL15 was added. Media was changed again at 72 hours post electroporation, cells were split and 10 ng/mL IL15 was added. NK cells were then split every 48 hours until they were analyzed by flow cytometry or otherwise utilized. NK cells were sorted using flow cytometry seven days post electroporation to determine successful transduction, transformation, editing, knock-in cassette integration, and/or expression events. A very high percentage of cells expressed GFP

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(86.6%), indicating that a high proportion of edited cells had GFP integrated at the *GAPDH* gene in the edited NK cell population when compared to a population of control NK cell population that was not transfected with an RNP targeting GAPDH (Fig. 61A and 61B). Additionally, a very high percentage of cells expressed CD19 CAR, indicating that a high proportion of edited cells had CD19 CAR integrated at the *GAPDH* gene in the edited NK cell population when compared to a control NK cell population that was not transfected with an RNP targeting GAPDH (Fig. 61C-61D). The methods described herein produced populations of edited NK cells with knock-in of GFP or CD19 CAR at rates greater than 80% (see Fig. 61E). As depicted in Fig. 61F, NK cells with CD19 CAR KI at GAPDH were able to effectively destroy Raji cells at rates significantly greater than unedited NK cells. Furthermore, NK cells with CD19 CAR KI at GAPDH also demonstrated significantly greater cytotoxicity against Nalm6 cells than NK cells with GFP KI at GAPDH (Fig. 61G). These results show that the methods described herein can produce engineered NK cell populations with high levels of editing homogeneity for potential use as autologous and/or allogeneic NK cell therapies suitable for targeting a variety of tumors and/or cancerous cells.

Example 25: Knock-out of CISH and TGFβRII in combination with Knock-in of Bicistronic CD16 and mbIL-15 Sequences at an Essential Gene Locus

[0709] mbIL-15/CD16 double knock-in (DKI) / CISH/TGFβRII double knock-out (DKO) iPSCs were generated using methods described in Examples 14 and 19. In brief, the CISH/TGFβRII DKO was generated using RNPs having an engineered Cas12a (SEQ ID NO: 62) and a gRNA specific for either CISH or TGFβRII having sequences shown in Table 26. Plasmid PLA1834 was used to generate the mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI, as described in Example 14. Following confirmation of the DKI/DKO genotype using standard sequencing methods known in the art, colonies of DKI/DKO iPSCs were propagated and cell populations were then differentiated to iNK cells using a spin embryoid method. As expected, DKI/DKO iNK cells displayed significantly greater CD16 and IL-15Rα expression as compared to unedited (WT) iNK cells (Figure 65A).

[0710] In an *in vitro* persistence assay, mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO iNK cells maintained a stable cell number across at least 15 days in the absence of exogenous cytokine support (Figure 62A). Unedited (WT) iNK cells displayed a substantial decrease in cell number over the same time period. As shown in Figure 65D,

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DKI/DKO iNK cells displayed stable viability across at least 16 days without exogenous cytokine support, while WT iNK cells displayed a substantial decrease in culture viability over the same time period. Additionally, the DKI/DKO iNK cells showed comparable total live cell count across at least 15 days without exogenous cytokines as compared to mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI iNK cells (Figure 62B). Thus, mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI / CISH/TGFβRII DKO iNK cells demonstrated increased cytokine-independent persistence across at least 15 or 16 days as compared to unedited (WT) iNK cells and similar persistence across this time period as mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI iNK cells.

[0711] Tumor cell killing ability of the mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells was evaluated in a number of *in vitro* tumor cell killing assays. Detroit-562 (pharyngeal carcinoma), FaDu (pharyngeal carcinoma), HT-29 (colorectal adenocarcinoma), or HCT116 (colorectal carcinoma) cells were seeded into Xcelligence plates (ACEA # 5232376001) at 10,000 cells per well and incubated overnight (~20 hours). DKI/DKO iNK cells were added at various Effector:Target (E:T) ratios. For the 1:1 E:T condition, 10 μg/mL anti-EGFR antibody cetuximab (CTX) was also included. Cytolysis, as measured by electrical impedance, was assayed according to the manufacturer's (Xcelligence) protocol. Results are shown in Figures 63A-D (average ± standard deviation; N=3). As depicted, for target Detroit-562, FaDu, HT-29, or HCT116 cells, DKI/DKO iNK cells at 5:1 and 10:1 E:T ratios resulted in significant cytolysis. Additionally, for target HCT117 cells, DKI/DKO iNK cells at a 1:1 E:T ratio also resulted in significant cytolysis. Moreover, for Detroit-562, FaDu, or HCT116 cells, DKI/DKO iNK cells at a 1:1 E:T ratio in combination with cetuximab resulted in significant cytolysis, with the resulting cytolysis greater than the combined effect observed for DKI/DKO iNK cells at a 1:1 E:T ratio alone or cetuximab alone.

[0712] Further tumor cell killing assays were conducted with the mbIL-15/CD16 ($CD16^{+/+}$/mbIL-$15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells. Again, HT-29 (colorectal adenocarcinoma) cells were seeded into Xcelligence plates (ACEA # 5232376001) at 10,000 cells per well and incubated overnight. DKI/DKO iNK cells or unedited (WT) iNK cells were then added at a 10:1 E:T ratio. Cytolysis, as measured by electrical impedance, was assayed according to the manufacturer's (Xcelligence) protocol. Results are shown in Figure 64A (average ± standard deviation; N=3). As depicted, both

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DKI/DKO and WT iNK cells resulted in significant cytolysis. *In vitro* persistence of mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells and unedited (WT) iNK cells was also examined using HT-29 cells. DKI/DKO iNK cells and WT iNK cells were co-cultured with HT-29 cells for 4 days at a 10:1 E:T ratio. Figure 64B depicts viability and CD16 expression, as measured by flow cytometry, at day 4. As depicted, WT iNK cells largely did not survive after killing HT-29 cells, whereas DKI/DKO cells persisted. Additionally, whereas surviving WT iNK cells were <1% CD16+, surviving DKI/DKO iNK cells were >80% CD16+. This *in vitro* persistence assay was repeated at a 1:1 E:T ratio. As shown in Figure 64C, the DKI/DKO cells again demonstrated greater persistence and maintenance CD16 expression following exposure to tumor (HT-29) cells.

[0713] Testing of the mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells in a 3D solid tumor killing assay was also conducted similarly to the depiction in Figure 20. Briefly, spheroids were formed by seeding 5,000 NucLight Red labeled SK-OV-3 cells in 96 well ultra-low attachment plates. Spheroids were incubated at 37°C before addition of mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells at various E:T ratios. Spheroids were subsequently imaged every 2 hours using the Incucyte S3 system for up to 4 days. Data were normalized to the red object intensity at time of effector addition. Results are shown in Fig. 65B (N=1; 2 technical replicates per cell line). As depicted, edited DKI/DKO iNK cells were capable of reducing the size of SK-OV-3 spheroids more effectively than unedited (WT) iNK cells. Further 3D tumor killing assays were conducted similarly to the above using DKI/DKO iNK cells or WT iNK cells in combination with 10 μg/ml trastuzumab or IgG (as a control) (Figure 65C). Potency was determined as IC50, indicating the E:T ratio required to reduce the SK-OV-3 spheroids by 50% after 100 hours of killing. As depicted in Figure 65C, edited DKI/DKO iNK cells reduced the size of SK-OV-3 spheroids more effectively than unedited (WT) iNK cells, both with and without trastuzumab. The results seen in combination with trastuzumab suggest that DKI/DKO iNK cells mediate enhanced antibody-dependent cellular cytotoxicity (ADCC) relative to WT iNK cells.

[0714] A phosphoflow cytometry assay was performed to determine the phosphorylated state of SMAD2/3 (pSMAD2/3) in mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells. Briefly, DKI/DKO iNKs were plated the day before in a cytokine starved condition. The next day, the cells were stimulated with 10

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ng/ml of TGFβ for a set length of time (e.g., 0-60 minutes). The cells were fixed immediately at the end of the time point and stained. The cells were processed on a NovoCyte Quanteon and the data was analyzed in FlowJo. Results are shown in Figure 65E (data represents 1 independent experiment). SMAD2/3 phosphorylation in DKI/DKO iNK cells was unchanged in the presence of TGFβ, while TGFβ increased SMAD2/3 phosphorylation in unedited (WT) iNK cells. Additionally, a 3D solid tumor cell killing assay was performed, similarly as described above, using mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells at an E:T ratio of 31.6 and with or without 10 ng/mL TGFβ. As shown in Figure 65F, DKI/DKO iNK cells were capable of reducing the size of SK-OV-3 spheroids more effectively than unedited iNK control cells after 100 hrs of killing (data represents 1 independent experiment). Moreover, DKI/DKO iNK cell activity was unaffected by the presence of exogenous TGFβ, in contrast to unedited (WT) iNKs. mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells were also pushed to kill tumor targets repeatedly over a multiday period in an *in vitro* serial killing assay. At day 0 of the assay, 10×10^3 Nalm6 tumor cells (a B cell leukemia cell line) and 2×10^5 DKI/DKO iNKs were plated in each well of a 96-well plate in the presence of TGFβ (10 ng/ml). At 48 hour intervals, a bolus of 5×10^3 Nalm6 tumor cells was added to re-challenge the DKI/DKO iNK population. Results are shown in Figure 65G (N=1; 3 technical replicates per cell line; error bars=standard deviation). As depicted, the DKI/DKO iNK cells exhibited continued killing of Nalm6 tumor cells after multiple challenges with Nalm6 tumor cells, even in the presence of TGFβ. In contrast, unedited (WT) iNK cells were limited in their serial killing effect. Taken all together, these data suggest that DKI/DKO iNKs have enhanced resistance to TGFβ-mediated immunosuppression.

[0715] Testing of the mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells in an *in vivo* mouse model was conducted as depicted in Figure 66A. Mice were intravenously (IV) inoculated with 0.125×10^6 SKOV3-luc cells. Following 19 days to allow for establishment of the tumors, mice were imaged using an *in vivo* imaging system (IVIS) to establish pre-treatment (day -2) tumor burden and then randomized into treatment groups. On day 0, mice were injected intravenously with (i) 2.5 mpk trastuzumab, or (ii) 20×10^6 DKI/DKO iNK cells and 2.5 mpk trastuzumab. Following day 0, the mice were imaged using an IVIS to assess tumor burden over time. The tumor burden over time as measured by bioluminescent imaging (BLI) via IVIS is depicted in

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Figures 66B-66C. Tumor burden is displayed in Figure 66B, and representative bioluminescent imaging of the mice at various time points is displayed in Figure 66C. As depicted, mice treated with DKI/DKO iNK cells in combination with trastuzumab exhibited greater tumor reduction than mice treated with trastuzumab alone. Mice treated with DKI/DKO iNK cells in combination with trastuzumab exhibited significant tumor reduction after just 5 days. Furthermore, treatment with the DKI/DKO iNK cells in combination with trastuzumab led to complete tumor clearance in all of the animals in the treatment group at day 5.

[0716] Additional testing of the mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells in an *in vivo* mouse model was conducted as depicted in Figure 67A. Mice were intraperitoneally inoculated with 0.25 x 10^6 SKOV3-luc cells. Following 4 days to allow for establishment of the tumors, mice were imaged using an *in vivo* imaging system (IVIS) to establish pre-treatment (day -1) tumor burden and then randomized into treatment groups. After 1 additional day (on day 0), mice were injected intraperitoneally (IP) with 5 x 10^6 unedited (WT) iNK cells, 5 x 10^6 DKI/DKO iNK cells, or no iNK cells for trastuzumab-alone or the isotype control. Some treatment groups ("Trastuzumab x 3" or "+ Tras. x 3") received IP injections of 2.5 mpk trastuzumab on days 0, 7, and 14. Following day 0, the mice were imaged weekly using an IVIS to assess tumor burden over time. The tumor burden over time as measured by bioluminescent imaging (BLI) via IVIS is depicted in Figures 67B-C. Representative bioluminescent imaging of the mice at various time points is displayed in Figure 67E. As seen in Figure 67B, treatment with the unedited (WT) iNK cells or the DKI/DKO iNK cells alone did not lead to tumor reduction *in vivo*. However, mice treated with iNK cells in combination with trastuzumab exhibited greater tumor reduction than mice treated with trastuzumab alone (Figure 67C). Mice dosed with mbIL-15/CD16 ($CD16^{1/1}$/$mbIL\text{-}15^{1/1}$) DKI / CISH/TGFβRII DKO (DKI/DKO) iNK cells in combination with trastuzumab (DKI/DKO + Tras x 3) also had significantly prolonged survival compared to mice dosed with unedited (WT) iNK cells in combination with trastuzumab (WT + Tras x 3) or trastuzumab alone (Figure 67D). Moreover, the reduction in tumor burden was greater in mice treated with DKI/DKO iNK cells than in mice treated with unedited (WT) iNK cells. Mice treated with DKI/DKO iNK cells in combination with trastuzumab exhibited significant tumor reduction after just 6 days (Figure 67E). Furthermore, as shown in Figure 67E, treatment with the DKI/DKO iNK cells in combination

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with trastuzumab led to complete tumor clearance in two (40%) of the animals in the treatment group at day 31 post-introduction of the NK cells. These results confirm that mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO iNK cells readily kill tumor cells *in vivo* and demonstrate that mbIL-15/CD16 ($CD16^{+/+}$/$mbIL\text{-}15^{+/+}$) DKI / CISH/TGFβRII DKO iNK cells in combination with trastuzumab produces greater *in vivo* tumor reduction than treatment with either trastuzumab alone or with unedited (WT) iNK cells in combination with trastuzumab.

EQUIVALENTS

[0717] It is to be understood that while the disclosure has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the present disclosure, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

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CLAIMS

We claim:

1. A Natural Killer (NK) cell comprising:

(a) one or more genomic edits that results in loss of function of one or more genes, wherein the one or more genes encode adenosine A2a receptor (ADORA2A), β-2 microglobulin (B2M), class II major histocompatibility complex transactivator (CIITA), cytokine inducible SH2 containing protein (CISH), two or more human leukocyte antigen (HLA) class II histocompatibility antigen alpha chain genes, two or more HLA class II histocompatibility antigen beta chain genes, natural killer group 2 member A receptor (NKG2A), programmed cell death protein 1 (PD-1), T cell immunoreceptor with Ig and ITIM domains (TIGIT), an agonist of the TGF beta signaling pathway (e.g., transforming growth factor beta receptor II (TGFβRII)), or any combination of two or more thereof; and

(b) a genome comprising a first exogenous coding sequence for FcγRIII (CD16) or variant thereof and a second exogenous coding sequence for a membrane bound interleukin 15 (mbIL-15), wherein the first exogenous coding sequence and second exogenous coding sequence are in frame with and downstream (3') of a coding sequence of an essential gene, and wherein at least part of the essential gene comprises an exogenous coding sequence.

2. The NK cell of claim 1, wherein the genome comprises:

(i) the first exogenous coding sequence and the second exogenous coding sequence at a first allele of the essential gene; and

(ii) the first exogenous coding sequence and the second exogenous coding sequence at a second allele of the essential gene.

3. The NK cell of claim 1 or 2, wherein the first exogenous coding sequence is upstream (5') of the second exogenous coding sequence.

4. The NK cell of claim 3, wherein the genome comprises:

(i) a first regulatory element between the coding sequence of the essential gene and the first exogenous coding sequence; and

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(ii) a second regulatory element between the first exogenous coding sequence and the second exogenous coding sequence.

5. The NK cell of claim 4, wherein the first regulatory element is an IRES or 2A element and the second regulatory element is an IRES or 2A element.

6. The NK cell of any one of claims 3-5, wherein the genome comprises a polyadenylation sequence downstream (3') of the second exogenous coding sequence.

7. The NK cell of claim 6, wherein the genome comprises a 3' untranslated region (UTR) sequence downstream (3') of the second exogenous coding sequence and upstream (5') of the polyadenylation sequence.

8. The NK cell of claim 1 or 2, wherein the second exogenous coding sequence is upstream (5') of the first exogenous coding sequence.

9. The NK cell of claim 8, wherein the genome comprises:

(i) a first regulatory element between the coding sequence of the essential gene and the second exogenous coding sequence; and

(ii) a second regulatory element between the second exogenous coding sequence and the first exogenous coding sequence.

10. The NK cell of claim 9, wherein the first regulatory element is an IRES or 2A element and the second regulatory element is an IRES or 2A element.

11. The NK cell of any one of claims 8-10, wherein the genome comprises a polyadenylation sequence downstream (3') of the first exogenous coding sequence.

12. The NK cell of claim 11, wherein the genome comprises a 3' untranslated region (UTR) sequence downstream (3') of the first exogenous coding sequence and upstream (5') of the polyadenylation sequence.

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13. The NK cell of any one of the preceding claims, wherein the first exogenous coding sequence is or comprises SEQ ID NO: 166.

14. The NK cell of any one of the preceding claims, wherein the second exogenous coding sequence is or comprises SEQ ID NO: 172.

15. The NK cell of any one of the preceding claims, wherein the CD16 is or comprises the amino acid sequence of SEQ ID NO: 184.

16. The NK cell of any one of the preceding claims, wherein the mbIL-15 comprises an IL-15, a linker, a sushi domain, and an IL-15Rα.

17. The NK cell of claim 16, wherein the mbIL-15 is or comprises the amino acid sequence of SEQ ID NO: 190.

18. The NK cell of any one of the preceding claims, wherein the NK cell is an induced pluripotent stem cell (iPSC)-derived NK (iNK) cell.

19. The NK cell of any one of the preceding claims, wherein the essential gene encodes a gene product that is required for survival and/or proliferation of the cell.

20. The NK cell of any one of the preceding claims, wherein the essential gene is a housekeeping gene, e.g., a gene listed in Table 3.

21. The NK cell of any one of the preceding claims, wherein the essential gene encodes glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

22. The NK cell of any one of the preceding claims, wherein the NK cell comprises:

(i) a genomic edit that results in loss of function of CISH; and

(ii) a genomic edit that results in loss of function of TGFβRII.

23. The NK cell of any one of the preceding claims, for use as a medicament.

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24. The NK cell of any one of the preceding claims, for use in the treatment of a disease, disorder, or condition, e.g., a tumor and/or a cancer.

25. A progeny or daughter cell of the NK cell of any one of claims 1-24.

26. A population of NK cells comprising the NK cell of any one of claims 1-24.

27. The population of NK cells of claim 26, characterized in that, when contacted with tumor cells, a level of killing of tumor cells by the NK cells is increased relative to a reference level of killing of tumor cells by a reference population of NK cells.

28. The population of NK cells of claim 26 or 27, characterized in that, when contacted with tumor cells and an antibody, a level of antibody-dependent cellular cytotoxicity (ADCC) induced by the NK cells is increased relative to a reference level of ADCC induced by a reference population of NK cells.

29. The population of NK cells of any one of claims 26-28, wherein a level of persistence of the population of NK cells is increased relative to a reference level of persistence of a reference population of NK cells.

30. The population of NK cells of claim 29, wherein the level of persistence is measured following contacting with tumor cells.

31. The population of NK cells of any one of claims 26-30, wherein the reference population of NK cells does not comprise NK cells comprising a genome comprising the first exogenous coding sequence and the second exogenous coding sequence.

32. The population of NK cells of claim 31, wherein the reference population of NK cell does not comprise NK cells comprising a genomic edit that results in loss of function of TGFβRII and a genomic edit that results in loss of function of CISH.

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33. A pharmaceutical composition comprising the NK cell, the progeny or daughter cell, or the population of NK cells of any one of claims 1-32.

34. The pharmaceutical composition of claim 33, comprising a pharmaceutically acceptable carrier.

35. A method of treating a condition, disorder, and/or disease, comprising administering to a subject suffering therefrom the NK cell, the progeny or daughter cell, or the population of NK cells of any one of claims 1-32.

36. The method of claim 35, wherein the subject is suffering from a tumor, e.g., a solid tumor.

37. The method of claim 35, wherein the subject is suffering from a cancer.

38. A method, comprising administering to a subject the NK cell, the progeny or daughter cell, or the population of NK cells of any one of claims 1-32.

39. A method of treating a condition, disorder, and/or disease, comprising administering to a subject suffering therefrom the pharmaceutical composition of claim 33 or 34.

40. The method of claim 39, wherein the subject is suffering from a tumor, e.g., a solid tumor.

41. The method of claim 39, wherein the subject is suffering from a cancer.

42. A method, comprising administering to a subject the pharmaceutical composition of claim 33 or 34.

43. The method of any one of claims 35-42, wherein the NK cell, the progeny or daughter cell, or the population of NK cells is allogenic to the subject.

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44. The method of any one of claims 35-42, wherein the NK cell, the progeny or daughter cell, or the population of NK cells is autologous to the subject.

45. The method of any one of claims 35-44, further comprising administering an antibody to the subject.

46. The method of claim 45, wherein the antibody is trastuzumab, rituximab, or cetuximab.

47. The method of any one of claims 35-46, wherein the subject is a human.

48. A method of increasing tumor killing ability of a NK cell, the method comprising:

(a) knocking-into the genome of the NK cell a first exogenous coding sequence for FcγRIII (CD16) or variant thereof and a second exogenous coding sequence for a membrane bound interleukin 15 (mbIL-15), wherein the first exogenous coding sequence and the second exogenous coding sequence are knocked-in in frame and downstream (3') of an essential gene; and

(b) knocking-out one or more genes of the NK cell, wherein the one or more genes encode adenosine A2a receptor (ADORA2A), β-2 microglobulin (B2M), class II major histocompatibility complex transactivator (CIITA), cytokine inducible SH2 containing protein (CISH), two or more human leukocyte antigen (HLA) class II histocompatibility antigen alpha chain genes, two or more HLA class II histocompatibility antigen beta chain genes, natural killer group 2 member A receptor (NKG2A), programmed cell death protein 1 (PD-1), T cell immunoreceptor with Ig and ITIM domains (TIGIT), an agonist of the TGF beta signaling pathway (e.g., transforming growth factor beta receptor II (TGFβRII)), or any combination of two or more thereof;

thereby increasing a level of tumor killing activity of the NK cell relative to a reference level of tumor killing activity of a reference NK cell.

49. A method of increasing antibody-dependent cellular cytotoxicity (ADCC) induced by a NK cell, the method comprising:

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(a) knocking-into the genome of the NK cell a first exogenous coding sequence for FcγRIII (CD16) or variant thereof and a second exogenous coding sequence for a membrane bound interleukin 15 (mbIL-15), wherein the first exogenous coding sequence and the second exogenous coding sequence are knocked-in in frame and downstream (3') of an essential gene; and

(b) knocking-out one or more genes of the NK cell, wherein the one or more genes encode adenosine A2a receptor (ADORA2A), β-2 microglobulin (B2M), class II major histocompatibility complex transactivator (CIITA), cytokine inducible SH2 containing protein (CISH), two or more human leukocyte antigen (HLA) class II histocompatibility antigen alpha chain genes, two or more HLA class II histocompatibility antigen beta chain genes, natural killer group 2 member A receptor (NKG2A), programmed cell death protein 1 (PD-1), T cell immunoreceptor with Ig and ITIM domains (TIGIT), an agonist of the TGF beta signaling pathway (e.g., transforming growth factor beta receptor II (TGFβRII)), or any combination of two or more thereof;

thereby increasing a level of ADCC induced by the NK cell relative to a reference level of ADCC induced by a reference NK cell.

50. A method of increasing persistence of a NK cell, the method comprising:

(a) knocking-into the genome of the NK cell a first exogenous coding sequence for FcγRIII (CD16) or variant thereof and a second exogenous coding sequence for a membrane bound interleukin 15 (mbIL-15), wherein the first exogenous coding sequence and the second exogenous coding sequence are knocked-in in frame and downstream (3') of an essential gene; and

(b) knocking-out one or more genes of the NK cell, wherein the one or more genes encode adenosine A2a receptor (ADORA2A), β-2 microglobulin (B2M), class II major histocompatibility complex transactivator (CIITA), cytokine inducible SH2 containing protein (CISH), two or more human leukocyte antigen (HLA) class II histocompatibility antigen alpha chain genes, two or more HLA class II histocompatibility antigen beta chain genes, natural killer group 2 member A receptor (NKG2A), programmed cell death protein 1 (PD-1), T cell immunoreceptor with Ig and ITIM domains (TIGIT), an agonist of the TGF beta signaling pathway (e.g., transforming growth factor beta receptor II (TGFβRII)), or any combination of two or more thereof;

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thereby increasing a level of persistence of the NK cell relative to a reference level of persistence of a reference NK cell.

51. The method of claim 50, wherein the level of persistence is measured following contacting the NK cell with tumor cells.

52. The method of any one of claims 48-51, wherein the reference NK cell does not comprise a genome comprising the first exogenous coding sequence and the second exogenous coding sequence.

53. The method of any one of claims 48-52, wherein the reference NK cell does not comprise a genomic edit that results in loss of function of TGFβRII and a genomic edit that results in loss of function of CISH.

54. A method of manufacturing a genetically modified NK cell, the method comprising:

(a) knocking-into the genome of an NK cell a first exogenous coding sequence for FcγRIII (CD16) or variant thereof and a second exogenous coding sequence for a membrane bound interleukin 15 (mbIL-15), wherein the first exogenous coding sequence and the second exogenous coding sequence are knocked-in in frame and downstream (3') of an essential gene; and

(b) knocking-out one or more genes of the NK cell, wherein the one or more genes encode adenosine A2a receptor (ADORA2A), β-2 microglobulin (B2M), class II major histocompatibility complex transactivator (CIITA), cytokine inducible SH2 containing protein (CISH), two or more human leukocyte antigen (HLA) class II histocompatibility antigen alpha chain genes, two or more HLA class II histocompatibility antigen beta chain genes, natural killer group 2 member A receptor (NKG2A), programmed cell death protein 1 (PD-1), T cell immunoreceptor with Ig and ITIM domains (TIGIT), an agonist of the TGF beta signaling pathway (e.g., transforming growth factor beta receptor II (TGFβRII)), or any combination of two or more thereof.

55. The method of any one of claims 48-54, wherein knocking-in comprises contacting the NK cell with:

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(i) a nuclease that causes a break within an endogenous coding sequence of the essential gene, and

(ii) a donor template that comprises a knock-in cassette comprising the first exogenous coding sequence and the second exogenous coding sequence in frame with and downstream (3 ') of an exogenous coding sequence or partial coding sequence of the essential gene, wherein the knock-in cassette is integrated into the genome of the cell by homology-directed repair (HDR) of the break.

56. The method of claim 55, wherein the nuclease is a CRISPR/Cas nuclease and knocking-in further comprises contacting the NK cell with a guide molecule for the CRISPR/Cas nuclease.

57. The method of any one of claims 48-56, wherein knocking-out comprises contacting the NK cell with one or more nucleases that cause a break within an endogenous coding sequence of the one or more genes.

58. The method of claim 57, wherein the one or more nucleases are CRISPR/Cas nucleases and knocking-out further comprises contacting the NK cell with one or more guide molecules for the CRISPR/Cas nuclease.

59. The method of any one of claims 48-58, wherein the NK cell is an induced pluripotent stem cell (iPSC)-derived NK (iNK) cell.

60. The method of any one of claims 48-59, wherein the essential gene encodes a gene product that is required for survival and/or proliferation of the NK cell.

61. The method of any one of claims 48-59, wherein the essential gene is a housekeeping gene, e.g., a gene listed in Table 3.

62. The method of any one of claims 48-60, wherein the essential gene encodes glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

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63. The method of any one of claims 48-62, comprising knocking-out a gene encoding CISH and knocking-out a gene encoding TGFβRII.

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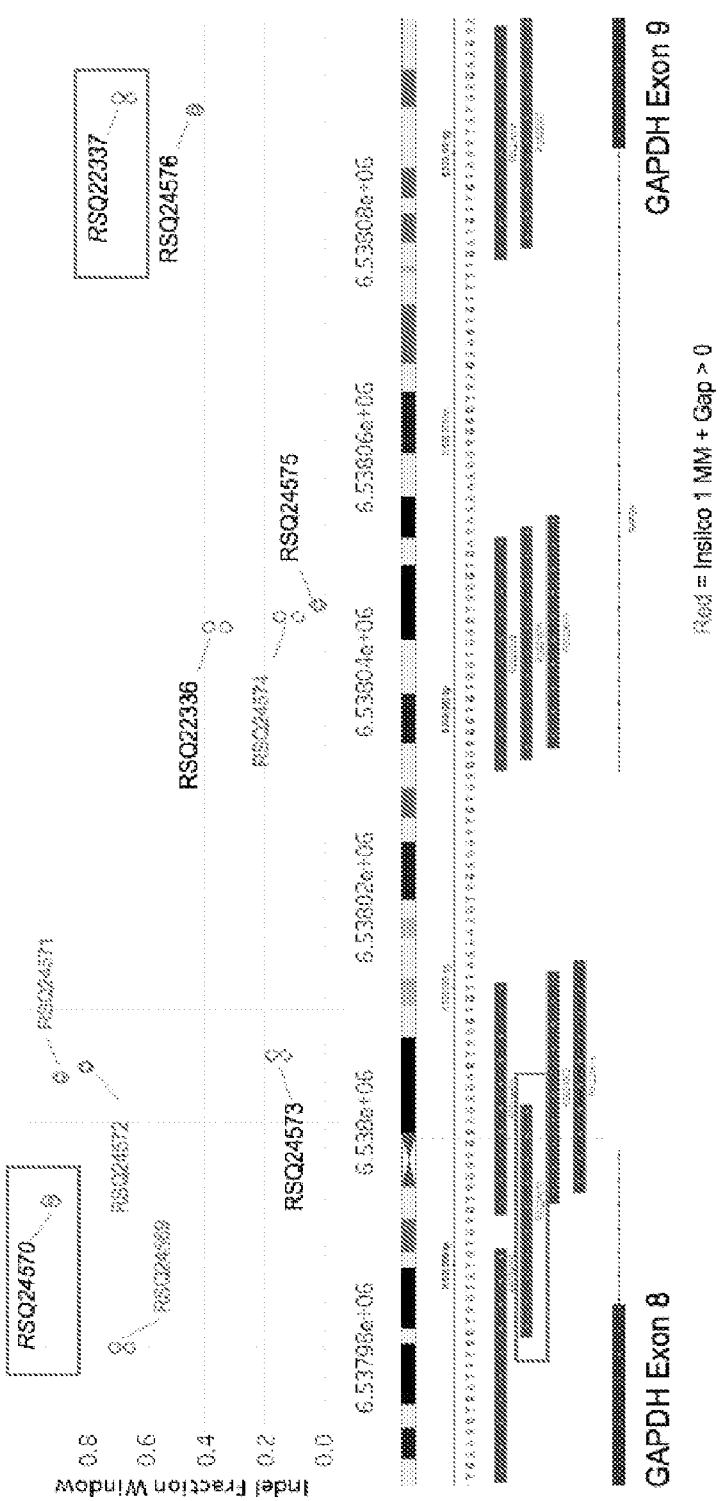

RSQ24570
RSQ24571
RSQ24572
RSQ24569
RSQ24573
RSQ22336
RSQ24574
RSQ24575
RSQ22337
RSQ24576
Indel Fraction Window
0.0
0.2
0.4
0.6
0.8
6.537936e+06
6.538e+06
6.538026e+06
6.538048e+06
6.538068e+06
6.538088e+06
GAPDH Exon 8
GAPDH Exon 8
Read = Insilico 1 MM + Gap > 0

Fig. 1

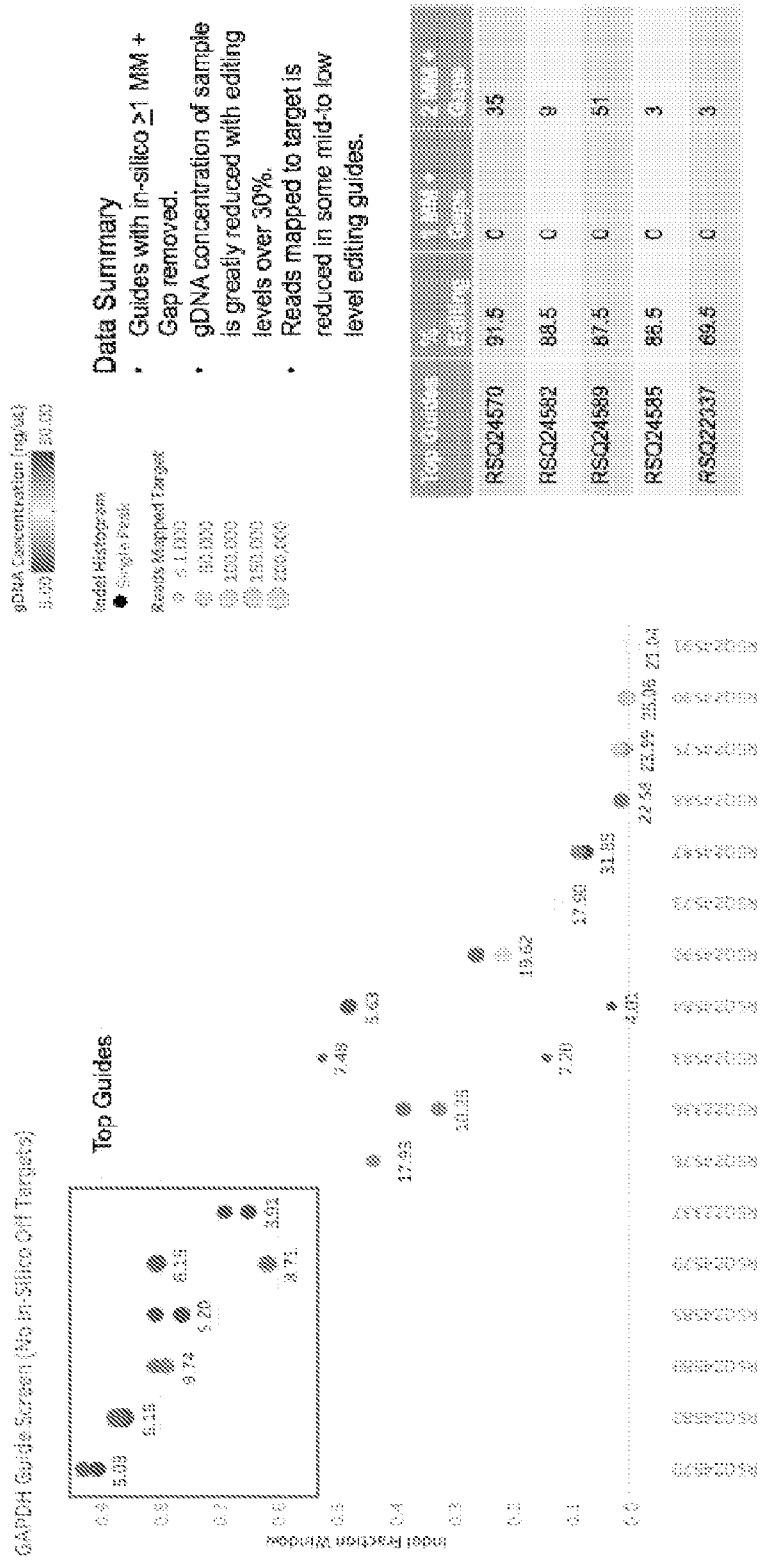

GAPDH Guide Screen (No In-Silico Off Targets)
Top Guides
Indel Fraction Window
gDNA Concentration (ng/mL)
Indel Histogram
Single Peak
Reads Mapped Target
Data Summary
Guides with in-silico ≥1 MM + Gap removed.
gDNA concentration of sample is greatly reduced with editing levels over 30%.
Reads mapped to target is reduced in some mid-to low level editing guides.
RSQ24570 91.5 C 35
RSQ24582 88.5 C 9
RSQ24589 87.5 C 51
RSQ24585 86.5 C 3
RSQ22037 69.5 C 3

Fig. 2

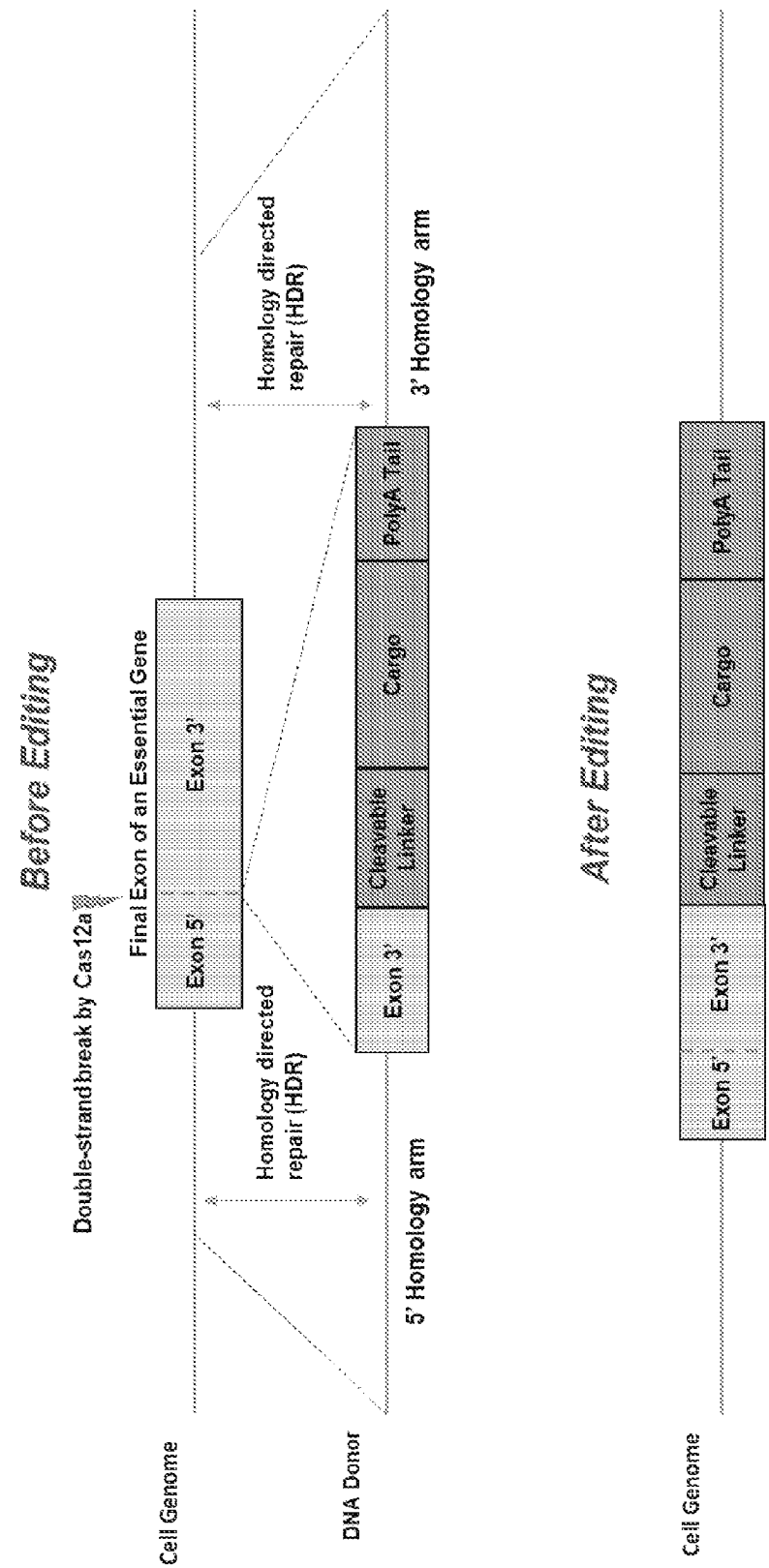

Before Editing
Double-strand break by Cas12a
Final Exon of an Essential Gene
Exon 5'
Exon 3'
Homology directed repair (HDR)
Homology directed repair (HDR)
Cleavable Linker
Cargo
PolyA Tail
Exon 3'
5' Homology arm
3' Homology arm
Cell Genome
DNA Donor
After Editing
Exon 5'
Exon 3'
Cleavable Linker
Cargo
PolyA Tail
Cell Genome

Fig. 3A

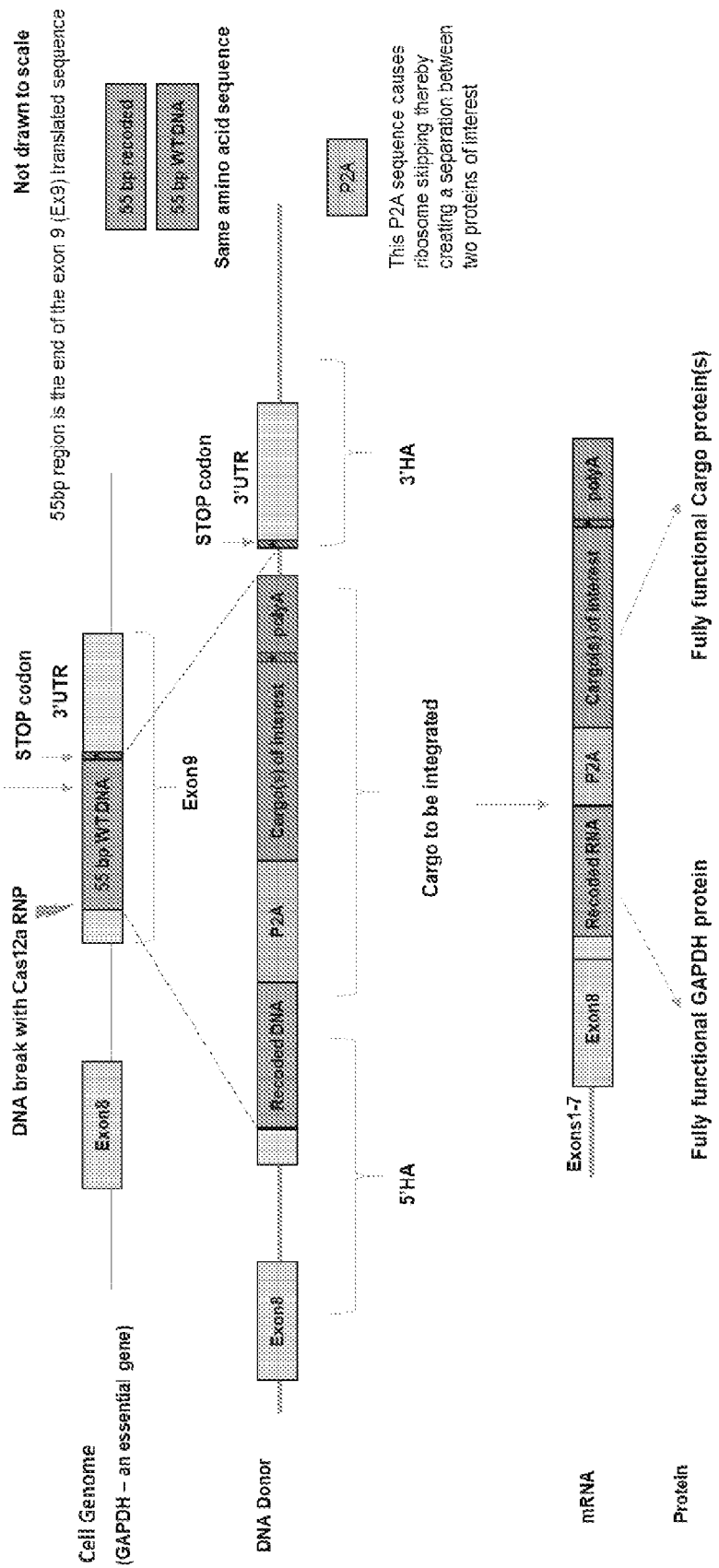

Not drawn to scale
55bp region is the end of the exon 9 (Ex9) translated sequence
55 bp recoded
55 bp WT DNA
Same amino acid sequence
DNA break with Cas12a RNP
STOP codon
3'UTR
55 bp WT DNA
Exon9
STOP codon
3'UTR
P2A
This P2A sequence causes ribosome skipping thereby creating a separation between two proteins of interest
3'HA
Cell Genome
(GAPDH – an essential gene)
Recoded DNA
P2A
Cargo of interest
Poly A
Cargo to be integrated
DNA Donor
Exon8
5'HA
Exons1-7
Exon8
Recoded RNA
P2A
Cargo of interest
poly A
mRNA
Protein
Fully functional GAPDH protein
Fully functional Cargo protein(s)

Fig. 3B

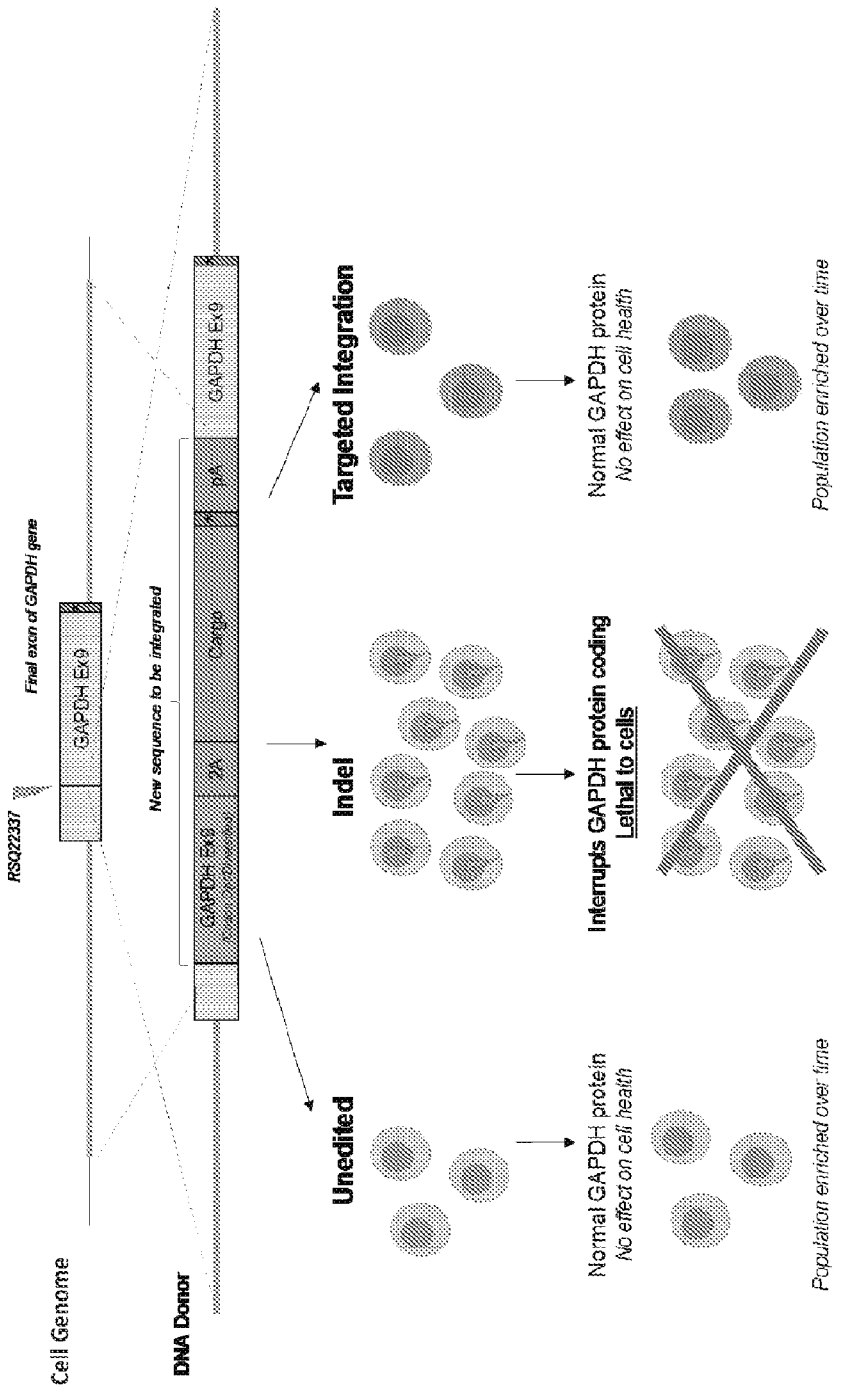

Cell Genome
DNA Donor
RSQ22337
Final exon of GAPDH gene
GAPDH Ex 9
New sequence to be integrated
2A
Cargo
GAPDH Ex 9
Unedited
Indel
Targeted Integration
Normal GAPDH protein
No effect on cell health
Interrupts GAPDH protein coding
Lethal to cells
Normal GAPDH protein
No effect on cell health
Population enriched over time
Population enriched over time

Fig. 3C

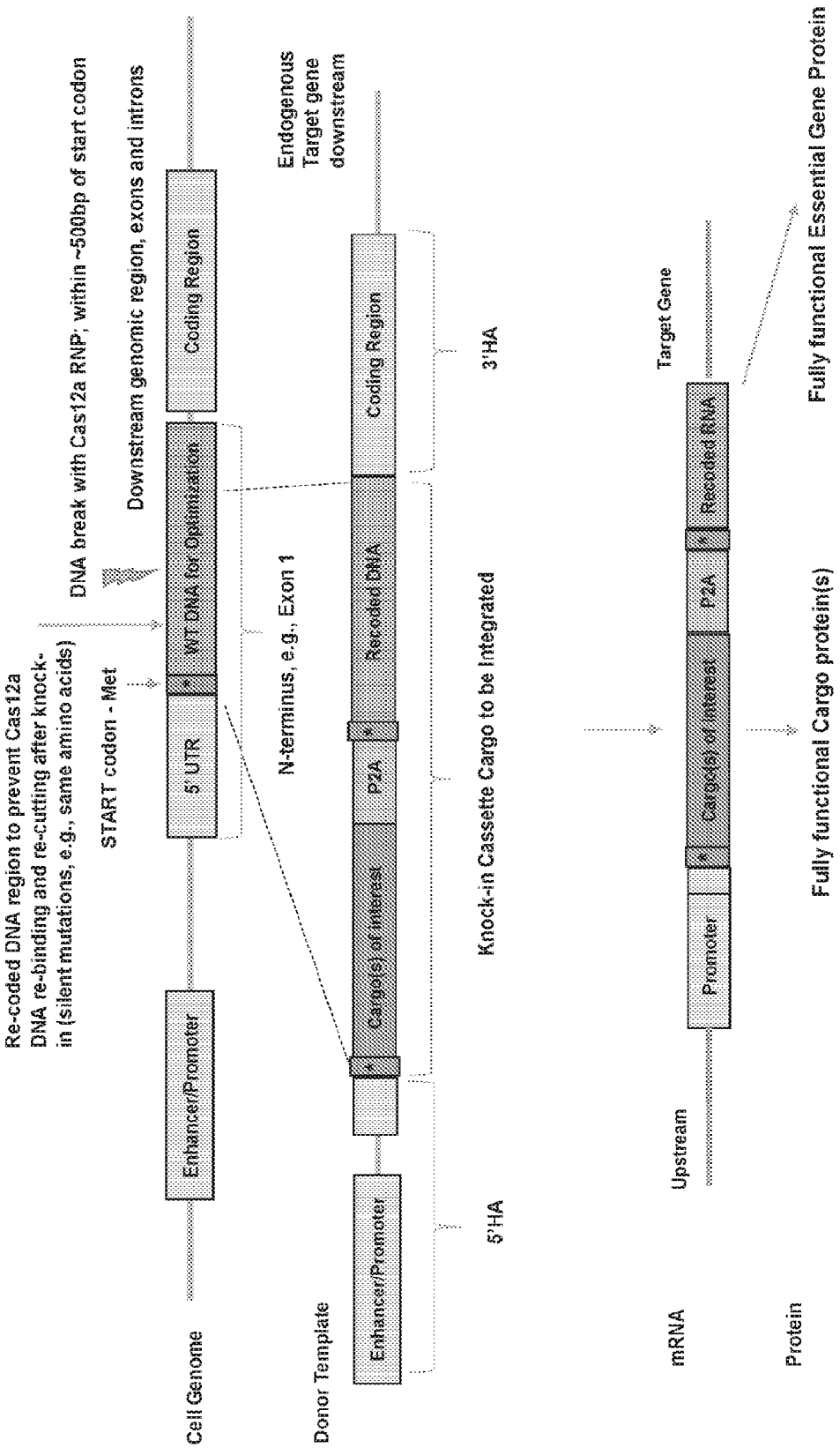

Re-coded DNA region to prevent Cas12a DNA re-binding and re-cutting after knock-in (silent mutations, e.g., same amino acids)
DNA break with Cas12a RNP; within ~500bp of start codon
START codon - Met
Downstream genomic region, exons and introns
Endogenous Target gene downstream
Cell Genome
Enhancer/Promoter
5' UTR
WT DNA for Optimization
Coding Region
N-terminus, e.g., Exon 1
Donor Template
Enhancer/Promoter
Cargo(s) of interest
P2A
Recoded DNA
Coding Region
5'HA
Knock-in Cassette Cargo to be Integrated
3'HA
mRNA
Upstream
Promoter
Cargo(s) of interest
P2A
Recoded RNA
Target Gene
Protein
Fully functional Cargo protein(s)
Fully functional Essential Gene Protein

Fig. 3D

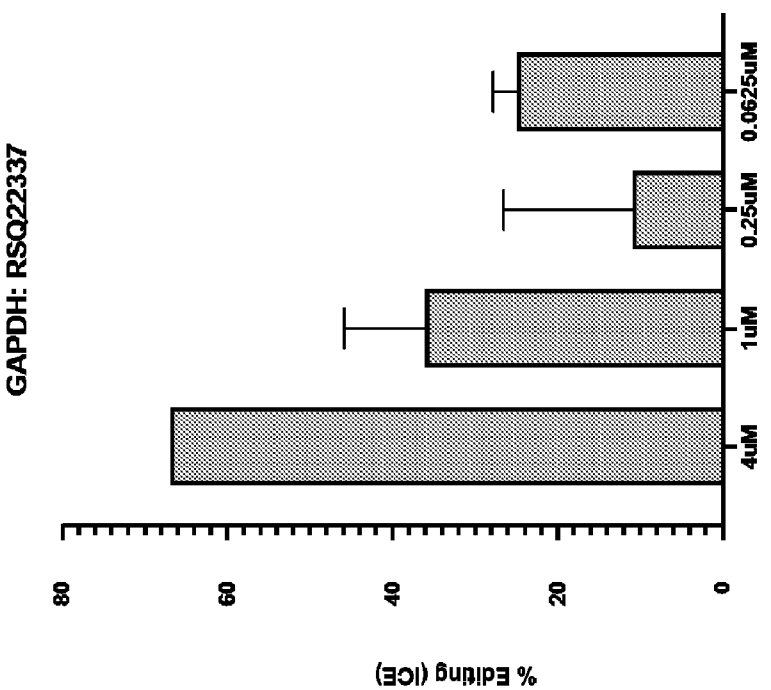

GAPDH: RSQ22337
% Editing (ICE)
0
20
40
60
80
4uM
1uM
0.25uM
0.0625uM

Fig. 4

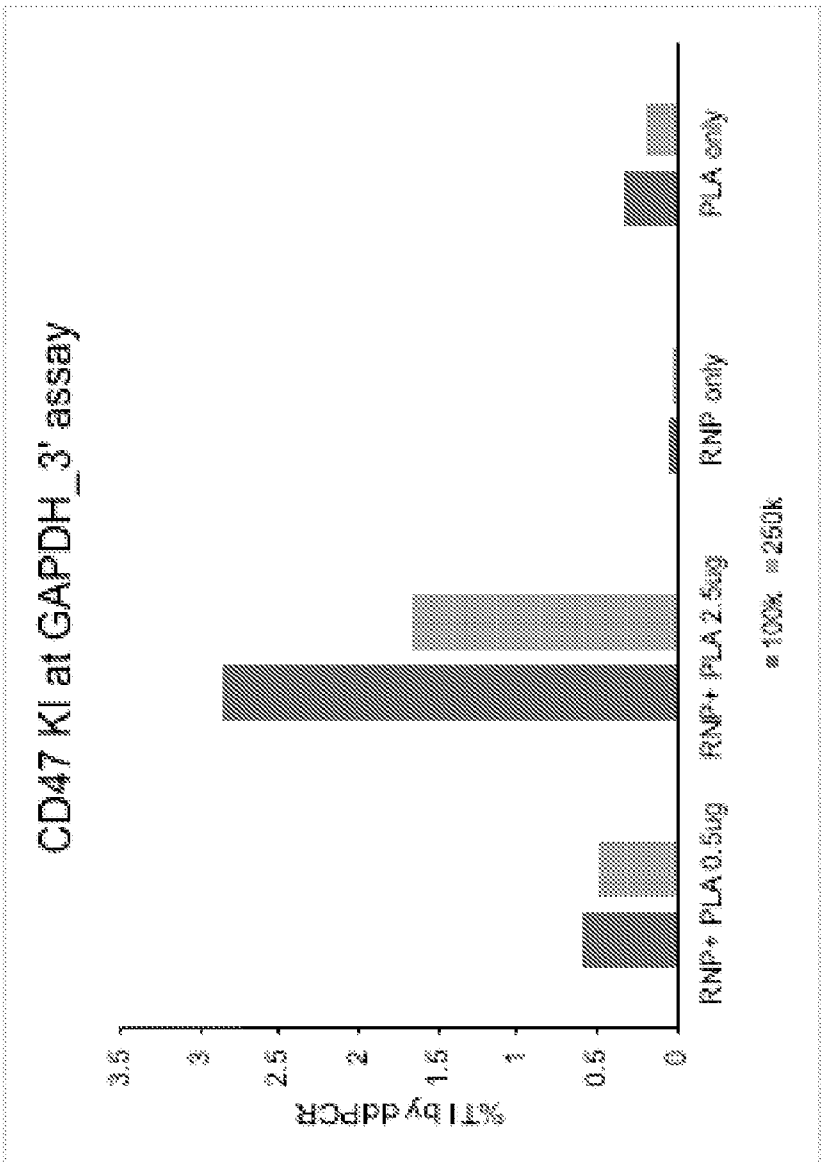

CD47 KI at GAPDH_3' assay
%TI by ddPCR
3.5
3
2.5
2
1.5
1
0.5
0
RNP+ PLA 0.5ug
RNP+ PLA 2.5ug
RNP only
PLA only
= 100% = 250k

Fig. 5

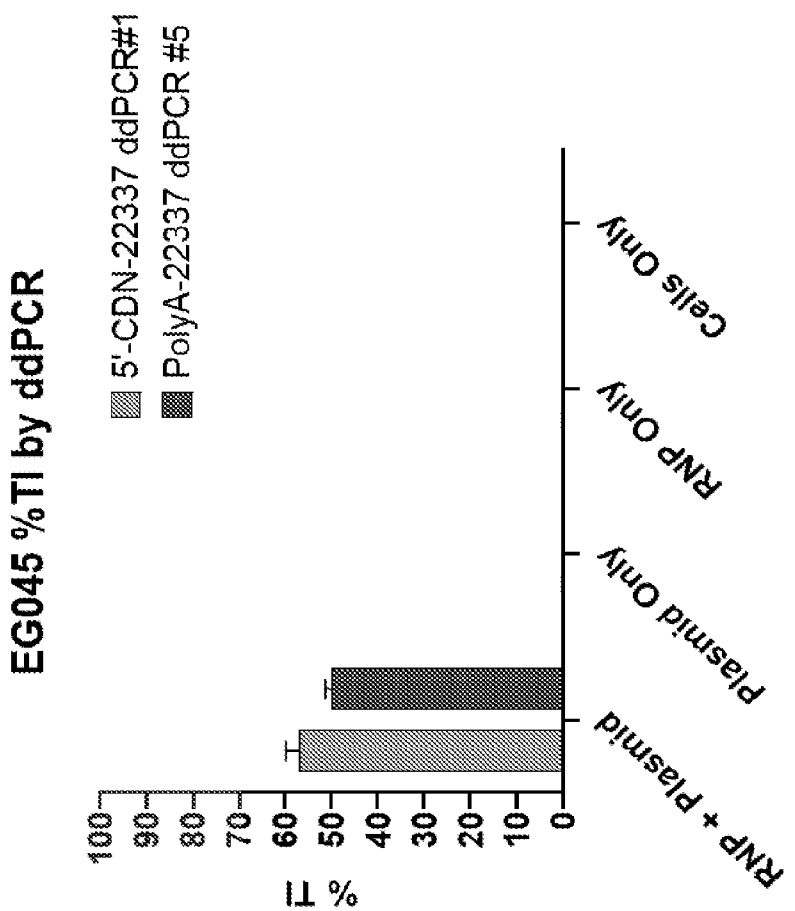

EG045 %TI by ddPCR
5'-CDN-22337 ddPCR#1
PolyA-22337 ddPCR #5
% TI
100
90
80
70
60
50
40
30
20
10
0
RNP + plasmid
plasmid Only
RNP Only
Cells Only

Fig. 6

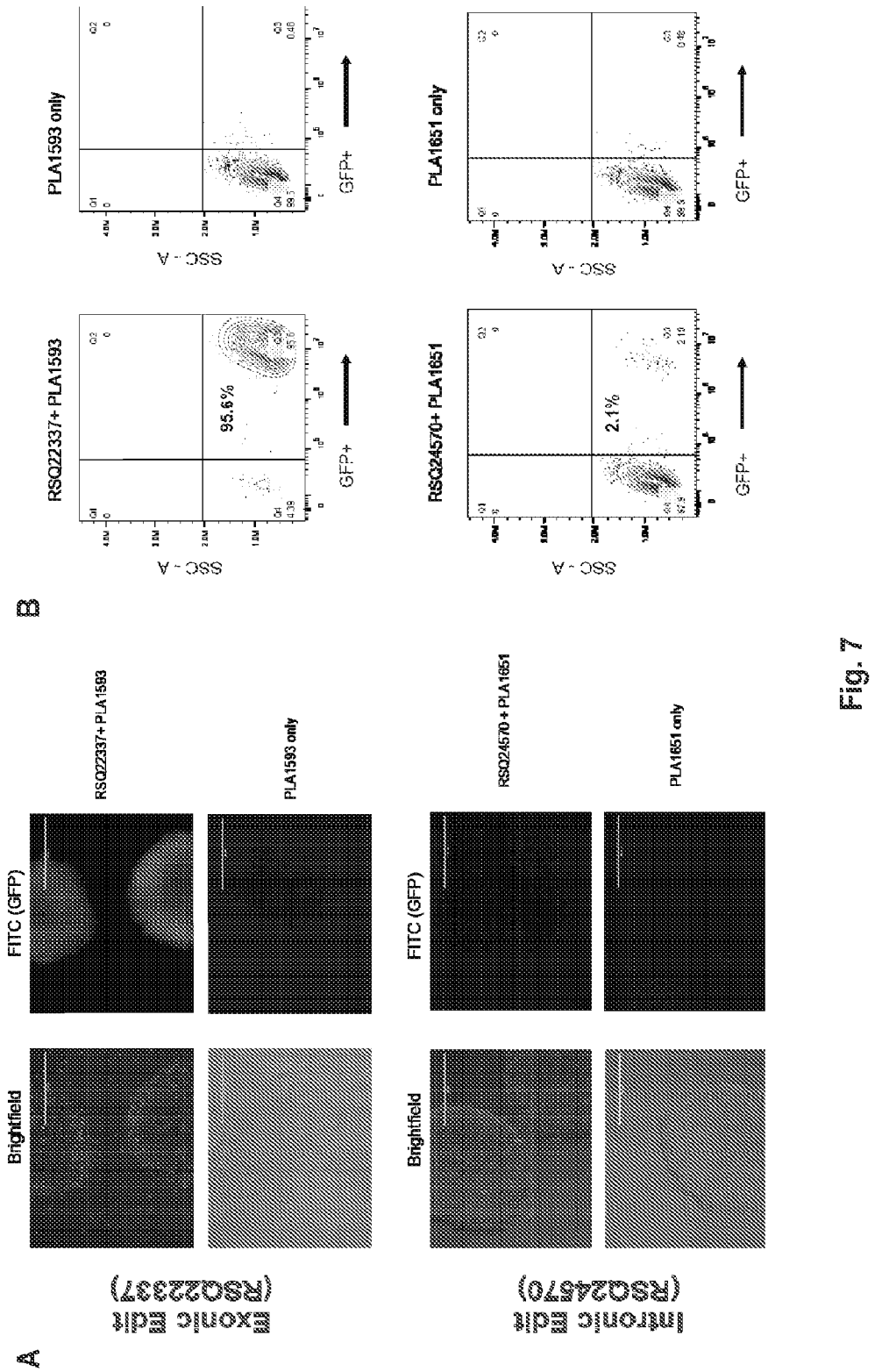

A
Exonic Edit (RSQ22337)
Intronic Edit (RSQ24570)
Brightfield
FITC (GFP)
RSQ22337+ PLA1593
PLA1593 only
RSQ24570 + PLA1651
PLA1651 only
B
RSQ22337+ PLA1593
PLA1593 only
RSQ24570+ PLA1651
PLA1651 only
95.6%
2.1%
SSC - A
GFP+

Fig. 7

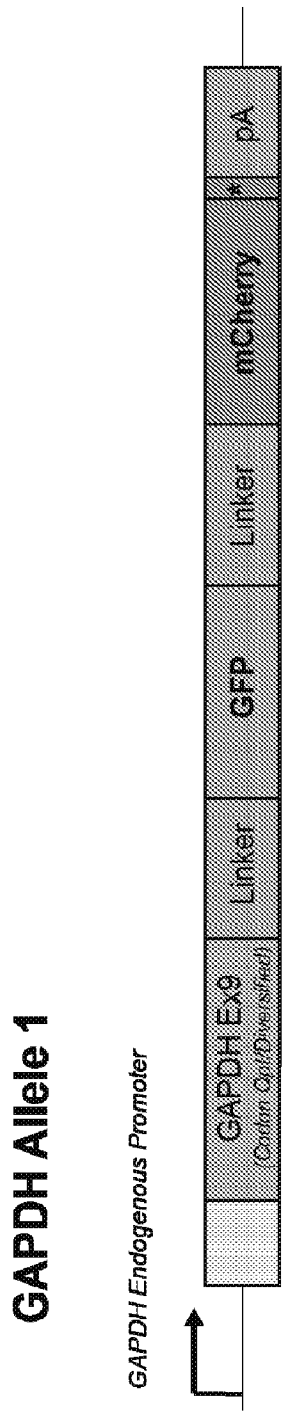

GAPDH Allele 1
GAPDH Endogenous Promoter
GAPDH E3.9
(Codon Out/Downstream)
Linker
GFP
Linker
mCherry
*
pA
GAPDH Allele 2
TI or
Indels or
WT

Fig. 8A

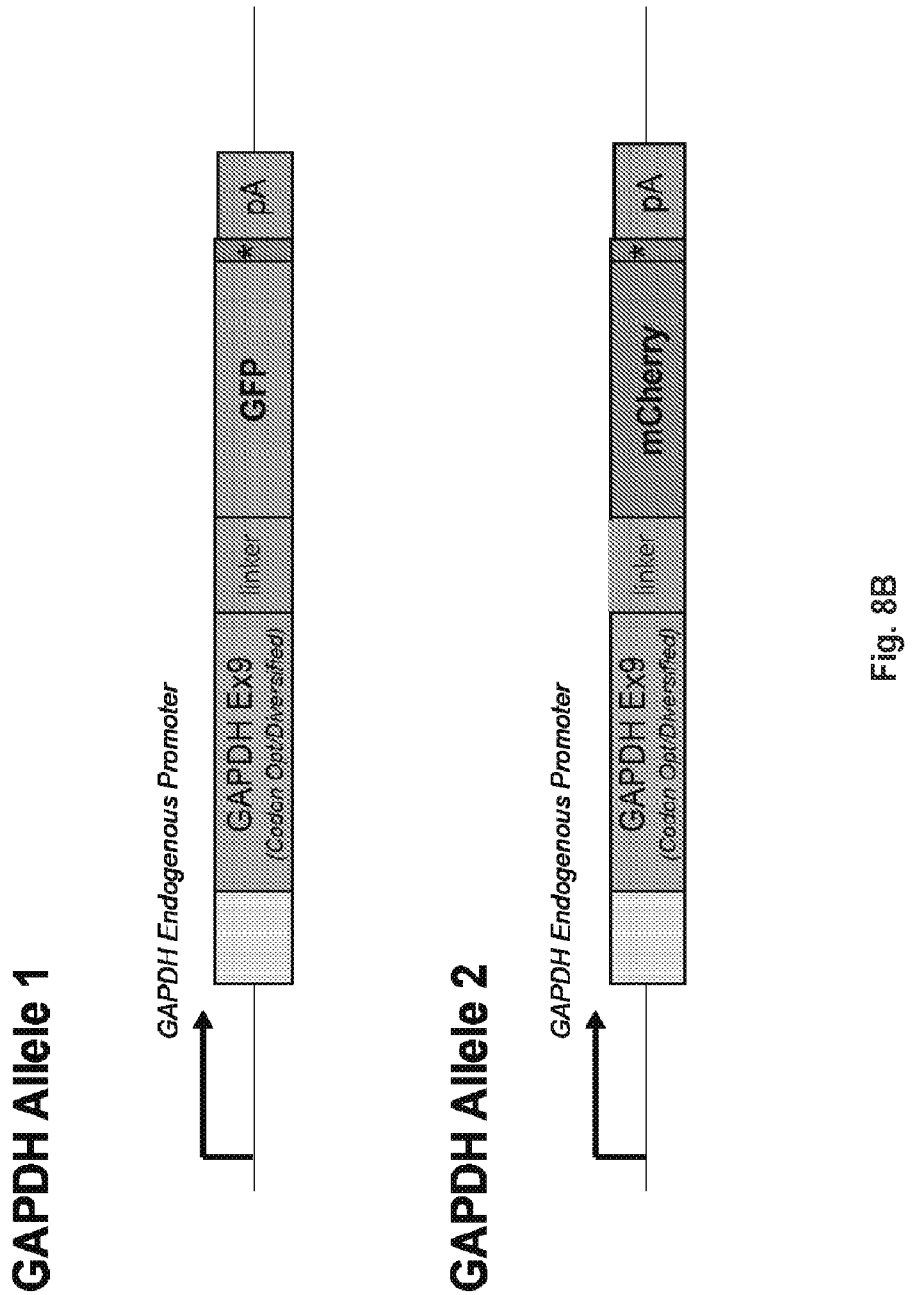

GAPDH Allele 1
GAPDH Endogenous Promoter
GAPDH Ex9
(Codon Opt/Diversified)
linker
GFP
pA
GAPDH Allele 2
GAPDH Endogenous Promoter
GAPDH Ex9
(Codon Opt/Diversified)
linker
mCherry
pA

Fig. 8B



Linkers:
P2A
T2A
IRES

PLA	Linker 1	Linker 2
PLA1575	P2A	P2A
PLA1582	P2A	T2A
PLA1574	P2A	IRES
PLA1583	T2A	P2A
PLA1573	T2A	T2A
PLA1584	T2A	IRES

Fig. 9A

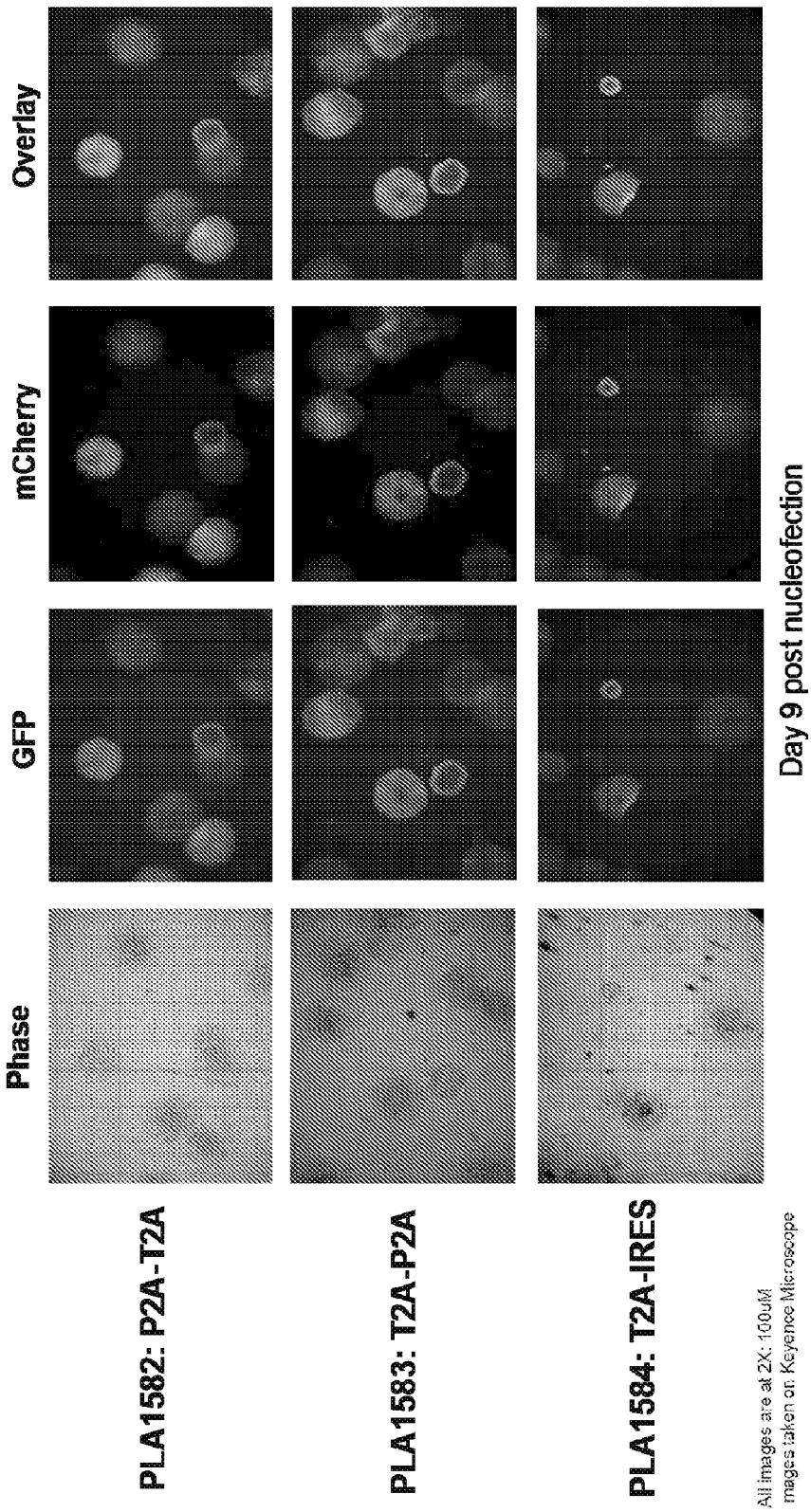

Phase
GFP
mCherry
Overlay
PLA1582: P2A-T2A
PLA1583: T2A-P2A
PLA1584: T2A-IRES
All images are at 2X; 100uM
images taken on Keyence Microscope
Day 9 post nucleofection

Fig. 9B

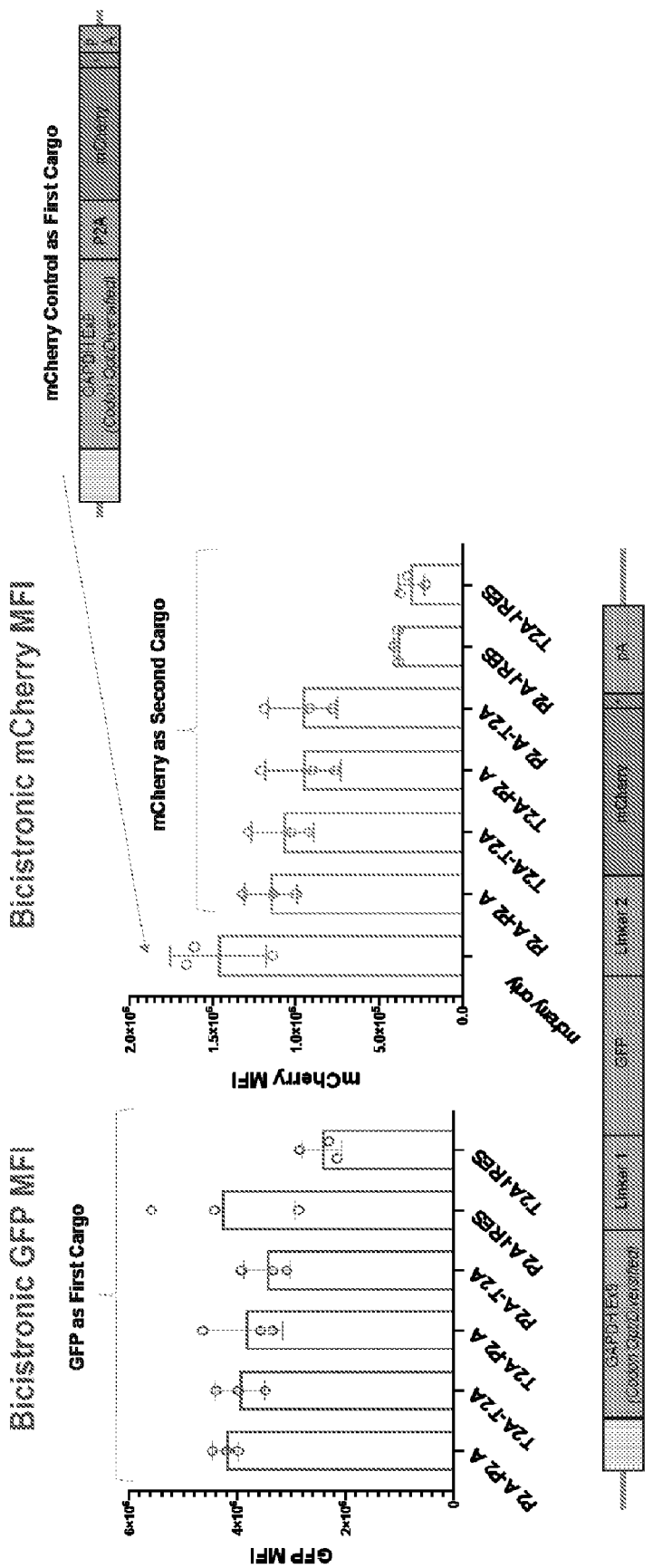

Bicistronic GFP MFI
GFP as First Cargo
GFP MFI
6×10⁶
4×10⁶
2×10⁶
0
P2A-P2A
T2A-T2A
T2A-P2A
P2A-T2A
P2A-IRES
T2A-IRES
Bicistronic mCherry MFI
mCherry as Second Cargo
mCherry MFI
2.0×10⁶
1.5×10⁶
1.0×10⁶
5.0×10⁵
0.0
mCherry only
P2A-P2A
T2A-T2A
T2A-P2A
P2A-T2A
P2A-IRES
T2A-IRES
mCherry Control as First Cargo
CAR/TCR
P2A
mCherry
GFP
Linker 1
Linker 2
TA

Fig. 9C

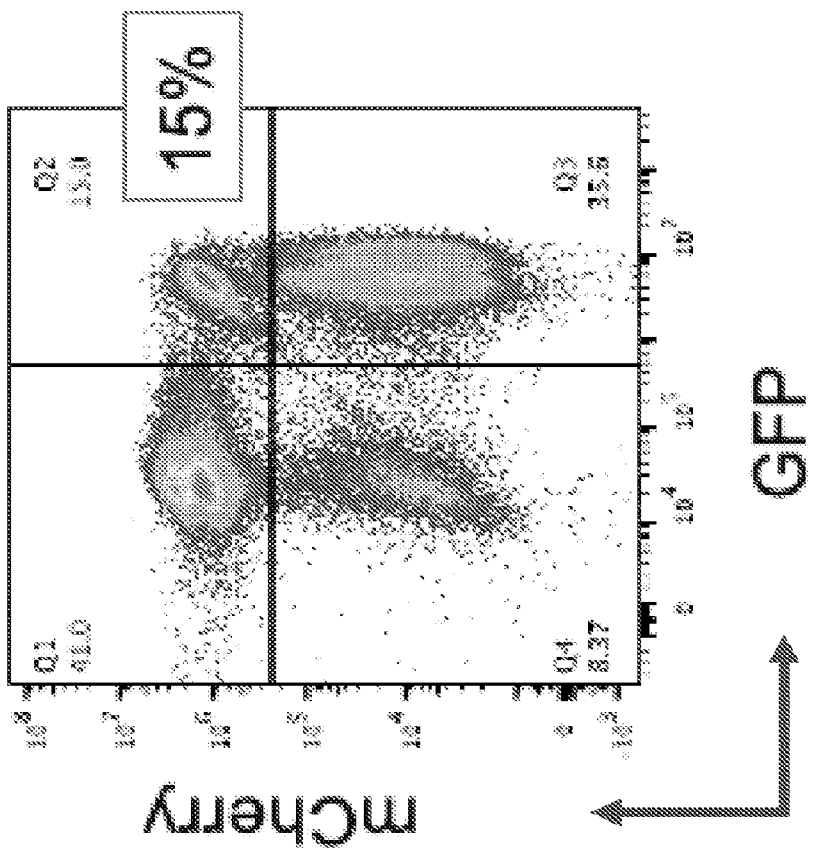


Fig. 10A

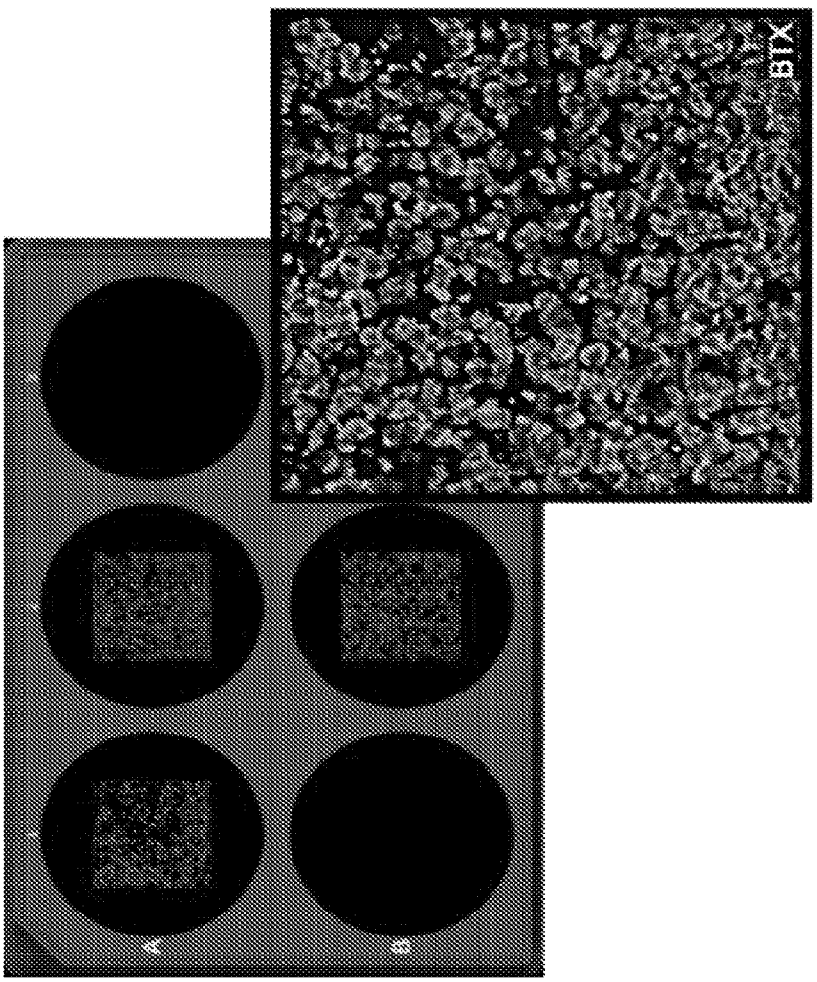


Fig. 10B

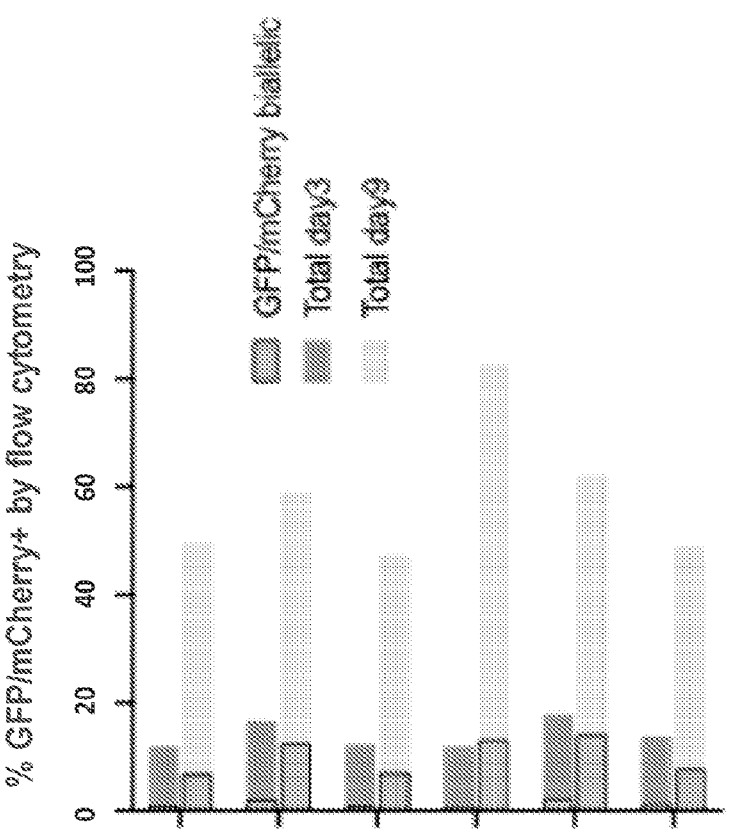

% GFP/mCherry+ by flow cytometry
0
20
40
60
80
100
GFP/mCherry biallelic
Total day3
Total day3
0.5µM RNP + GFP/mCherry 2.5µg (trial 1)
0.5µM RNP + GFP/mCherry 2.5µg (trial 2)
0.5µM RNP + GFP/mCherry 2.5µg (trial 3)
0.5µM RNP + GFP/mCherry 2.5µg (trial 4)
0.5µM RNP + GFP/mCherry 5µg (trial 1)
0.5µM RNP + GFP/mCherry 2.5µg (trial 5)

Fig. 10C

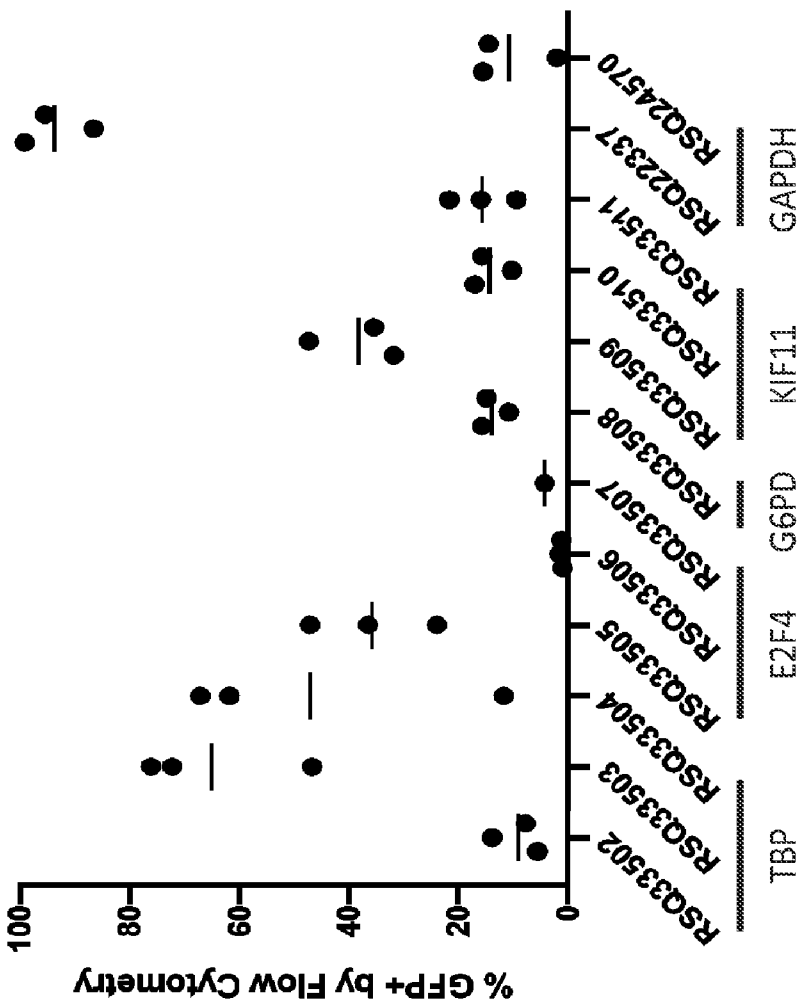

% GFP+ by Flow Cytometry
100
80
60
40
20
0
RSQ33502
RSQ33503
RSQ33504
RSQ33505
RSQ33506
RSQ33507
RSQ33508
RSQ33509
RSQ33510
RSQ33511
RSQ22337
RSQ24570
TBP
E2F4
G6PD
KIF11
GAPDH

Fig. 11A

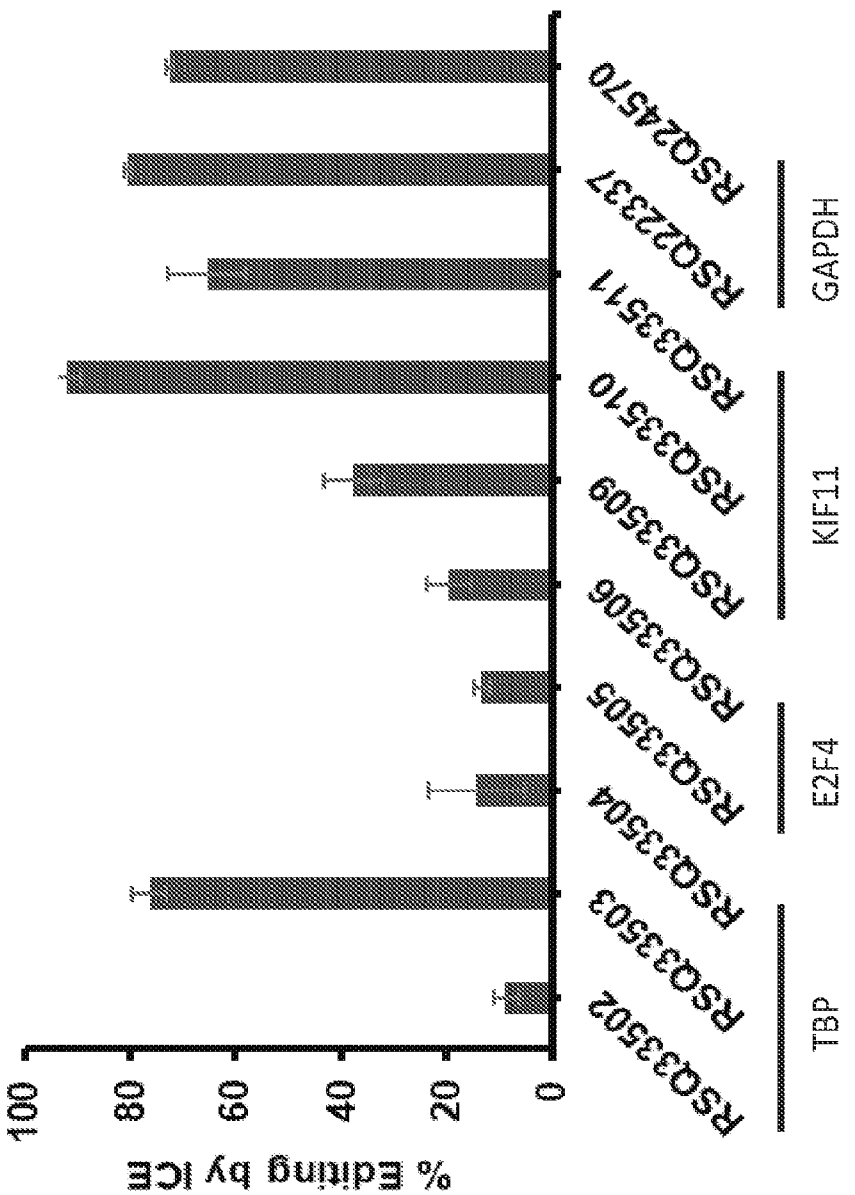

% Editing by ICE
100
80
60
40
20
0
RSQ33502
RSQ33503
RSQ33504
RSQ33505
RSQ33506
RSQ33509
RSQ33510
RSQ33511
RSQ22337
RSQ24570
TBP
E2F4
KIF11
GAPDH

Fig. 11B

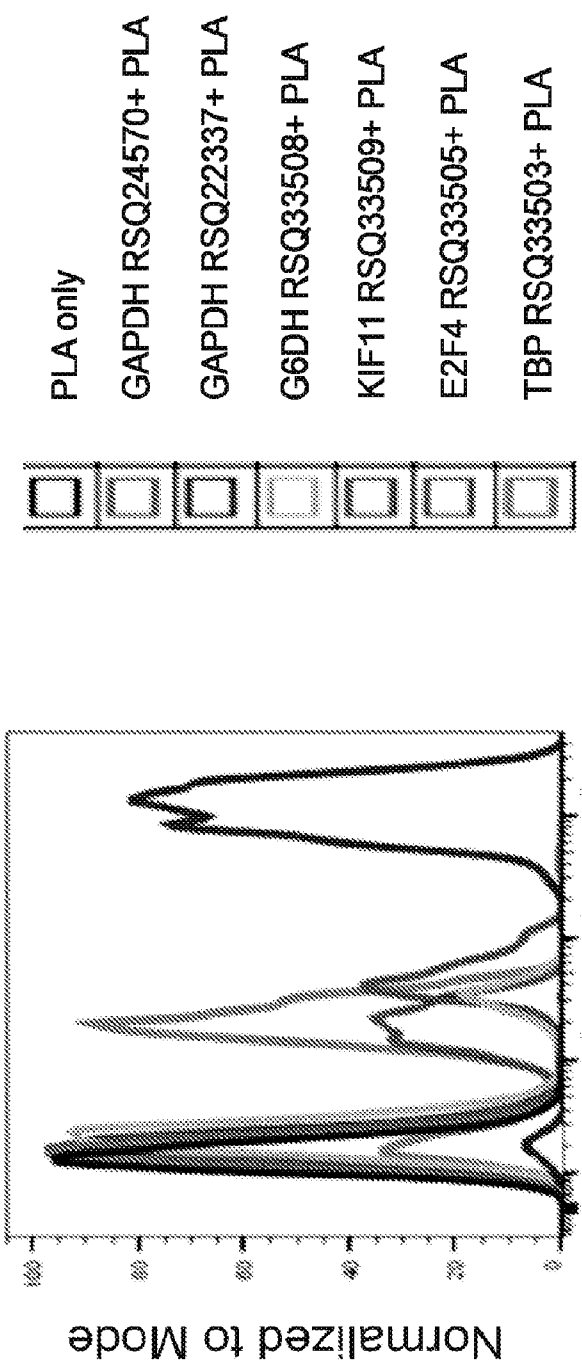

PLA only
GAPDH RSQ24570+ PLA
GAPDH RSQ22337+ PLA
G6DH RSQ33508+ PLA
KIF11 RSQ33509+ PLA
E2F4 RSQ33505+ PLA
TBP RSQ33503+ PLA
Normalized to Mode
GFP+

Fig. 11C

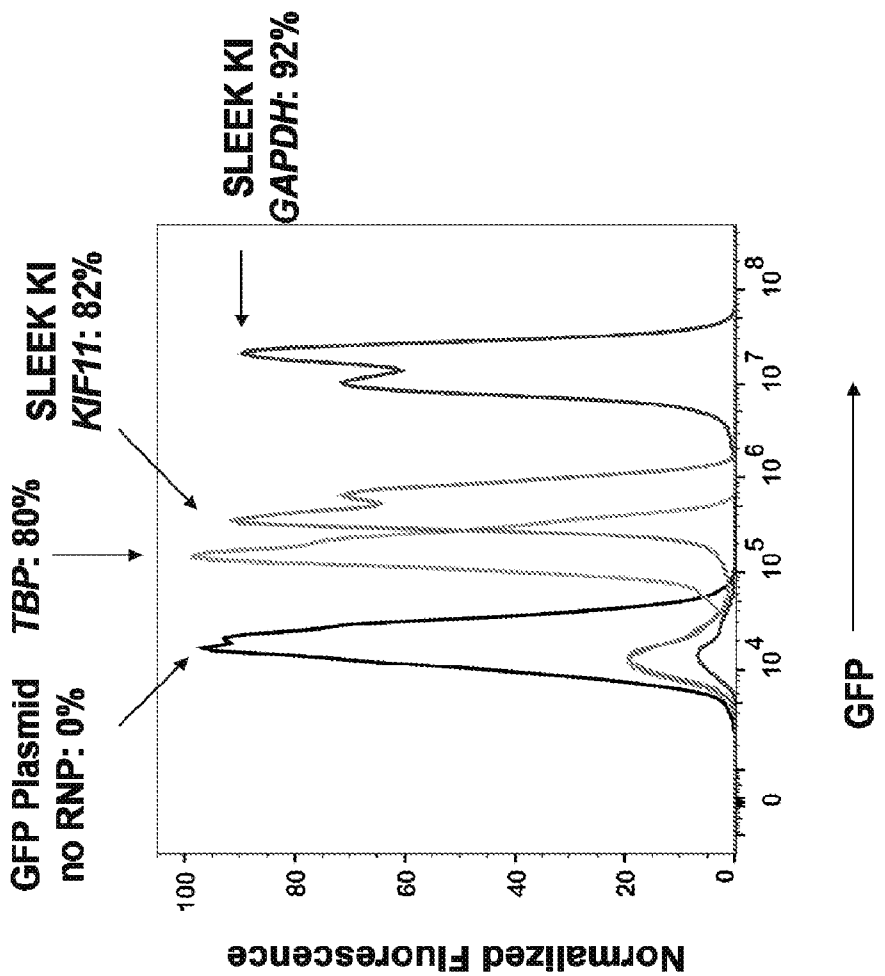

GFP Plasmid
no RNP: 0%
SLEEK KI
TBP: 80%
SLEEK KI
KIF11: 82%
SLEEK KI
GAPDH: 92%
Normalized Fluorescence
0
20
40
60
80
100
0
10^4
10^5
10^6
10^7
10^8
GFP

Fig. 11D

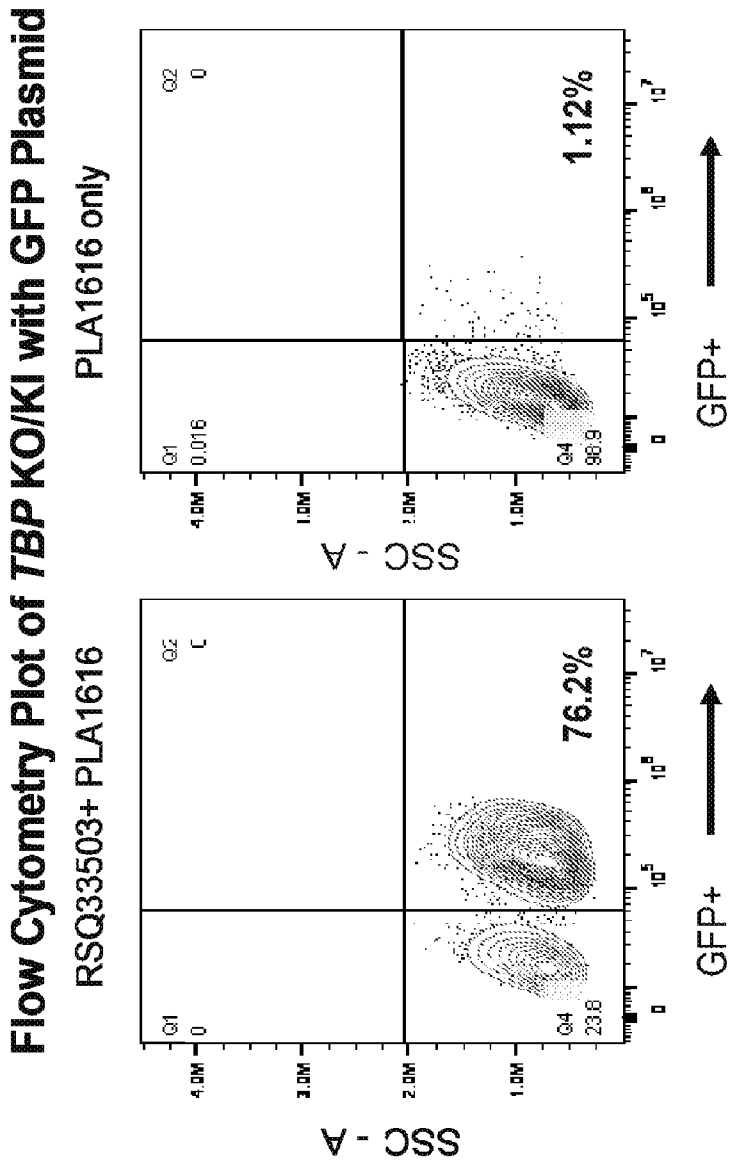

Flow Cytometry Plot of TBP KO/KI with GFP Plasmid
RSQ33503+ PLA1616
PLA1616 only
SSC - A
GFP+
Q1
0
Q2
0
Q4
23.8
76.2%
Q1
0.016
Q2
0
Q4
98.9
1.12%

Fig. 12

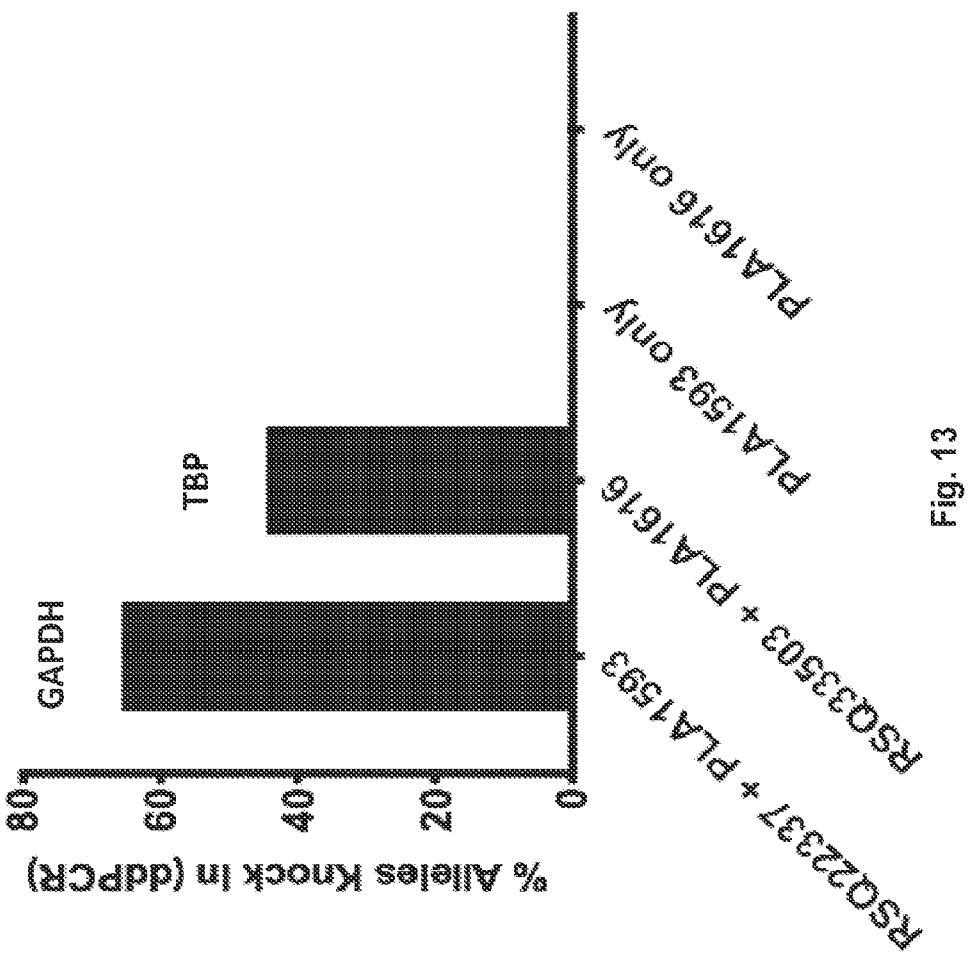

% Alleles Knock In (ddPCR)
0
20
40
60
80
GAPDH
TBP
RSQ22337 + PLA1593
RSQ33503 + PLA1616
PLA1593 only
PLA1616 only

Fig. 13

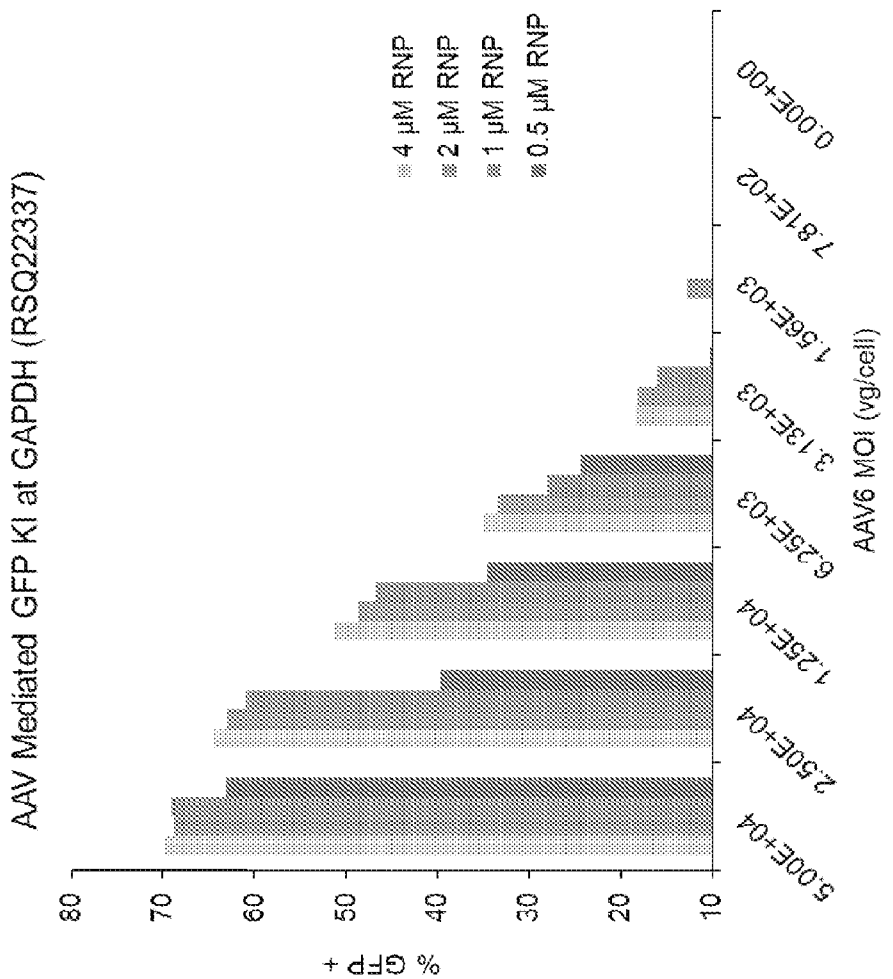

AAV Mediated GFP KI at GAPDH (RSQ22337)
% GFP +
80
70
60
50
40
30
20
10
4 µM RNP
2 µM RNP
1 µM RNP
0.5 µM RNP
5.00E+04
2.50E+04
1.25E+04
6.25E+03
3.13E+03
1.56E+03
7.81E+02
0.00E+00
AAV6 MOI (vg/cell)

Fig. 14

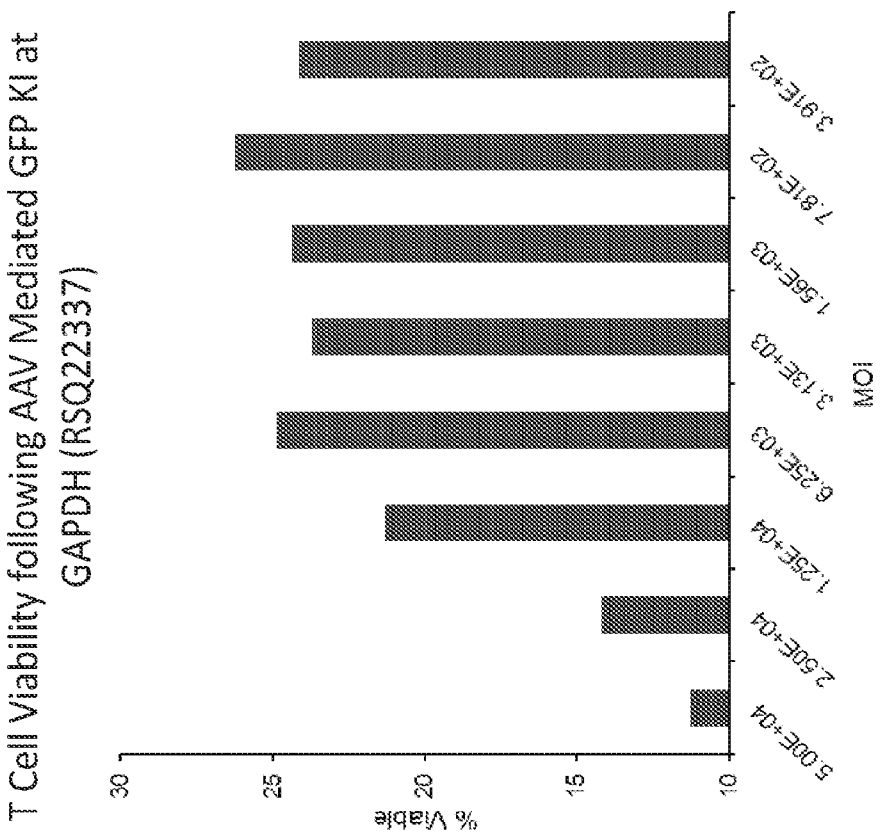

T Cell Viability following AAV Mediated GFP KI at GAPDH (RSQ22337)
% Viable
30
25
20
15
10
5.00E+04
2.50E+04
1.25E+04
6.25E+03
3.13E+03
1.56E+03
7.81E+02
3.91E+02
MOI

Fig. 15

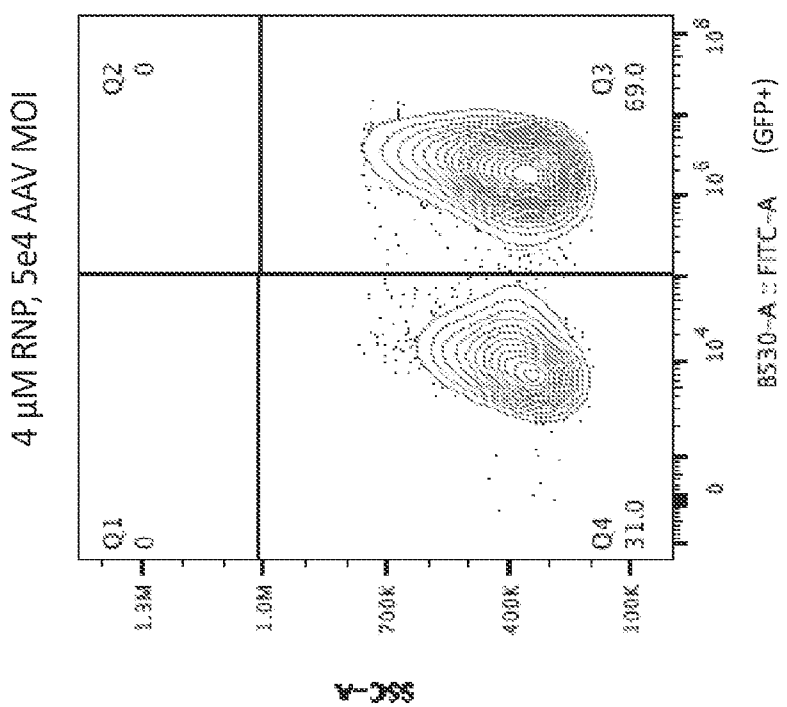

4 µM RNP, 5e4 AAV MOI
Q1
0
Q2
0
Q3
69.0
Q4
31.0
1.3M
1.0M
700K
400K
100K
SSC-A
0
10^4
10^6
10^8
B530-A :: FITC-A (GFP+)

Fig. 16A

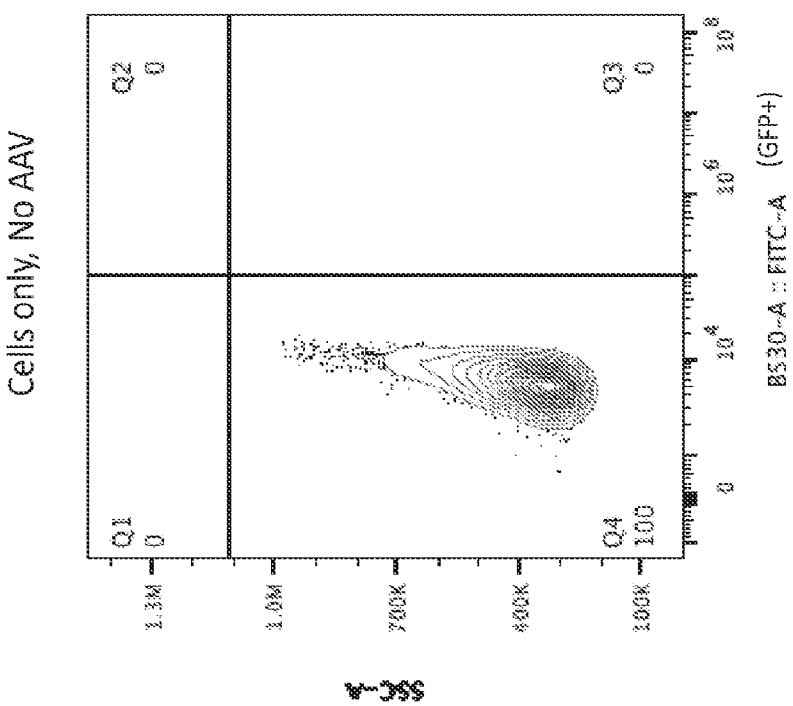

Cells only, No AAV
Q1
0
Q2
0
Q4
100
Q3
0
SSC-A
1.3M
1.0M
700K
400K
100K
0
10^4
10^6
10^8
B530-A :: FITC-A (GFP+)

Fig. 16B

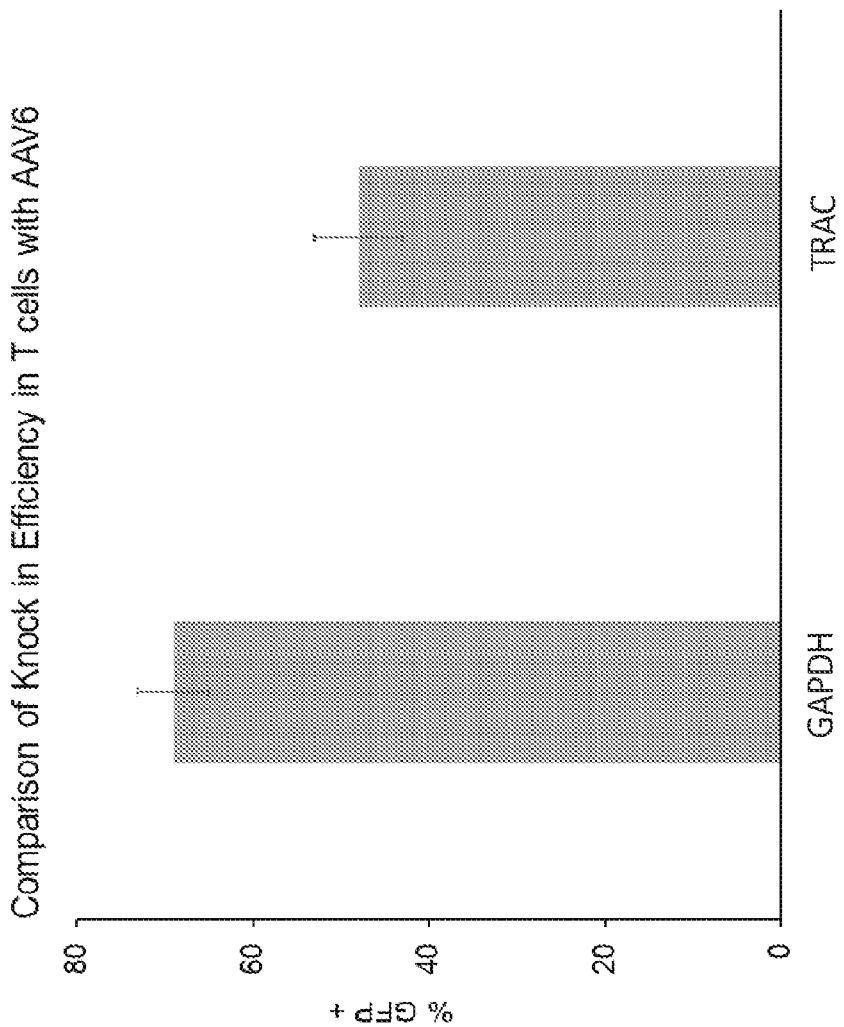

Comparison of Knock in Efficiency in T cells with AAV6
% GFP +
0
20
40
60
80
GAPDH
TRAC

Fig. 17A

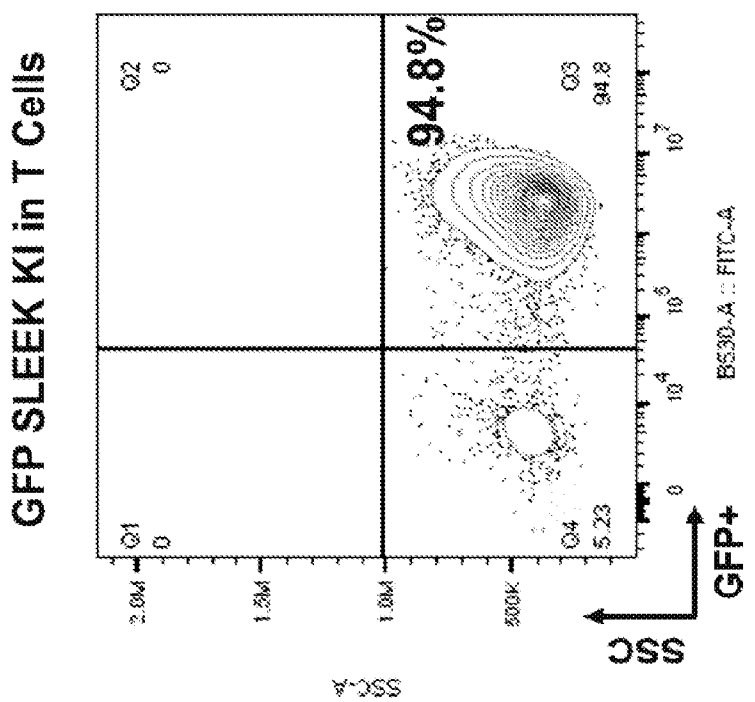

GFP SLEEK KI in T Cells
Q2
0
Q1
0
94.8%
Q3
94.8
Q4
5.23
SSC-A
SSC
GFP+
BS30-A :: FITC-A

Fig. 17B

Expansion and Viability Data

	Mock	RNP + AAV6
Cell count Day 0 (cells/well)	2.5 E+05	2.5 E+05
% Viability Day 0	94	90.5
Cell count Day 7 (cells/well)	6.10 E+05	5.34 E+05
% Viability Day 7	93.5	86.5
Fold Change	2.4	2.1

Fig. 17C

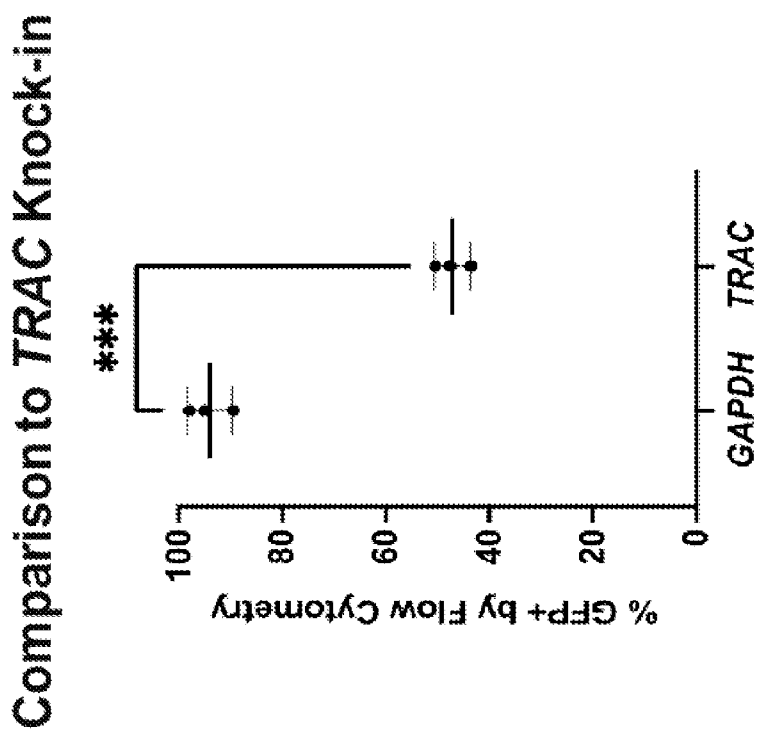

Comparison to TRAC Knock-in
***
% GFP+ by Flow Cytometry
100
80
60
40
20
0
GAPDH
TRAC

Fig. 17D

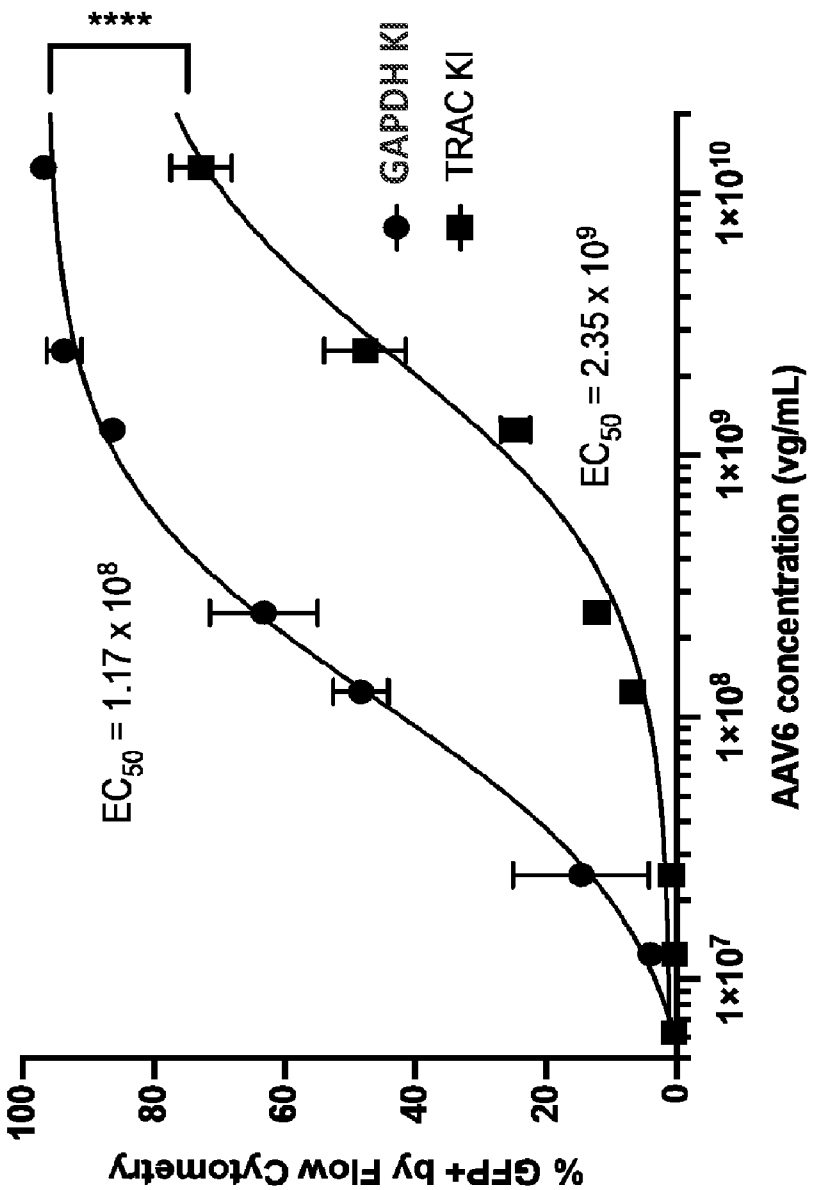

****
GAPDH KI
TRAC KI
EC_{50} = 1.17 x 10^8
EC_{50} = 2.35 x 10^9
% GFP+ by Flow Cytometry
AAV6 concentration (vg/mL)
0
20
40
60
80
100
1×10^7
1×10^8
1×10^9
1×10^10

Fig. 17E

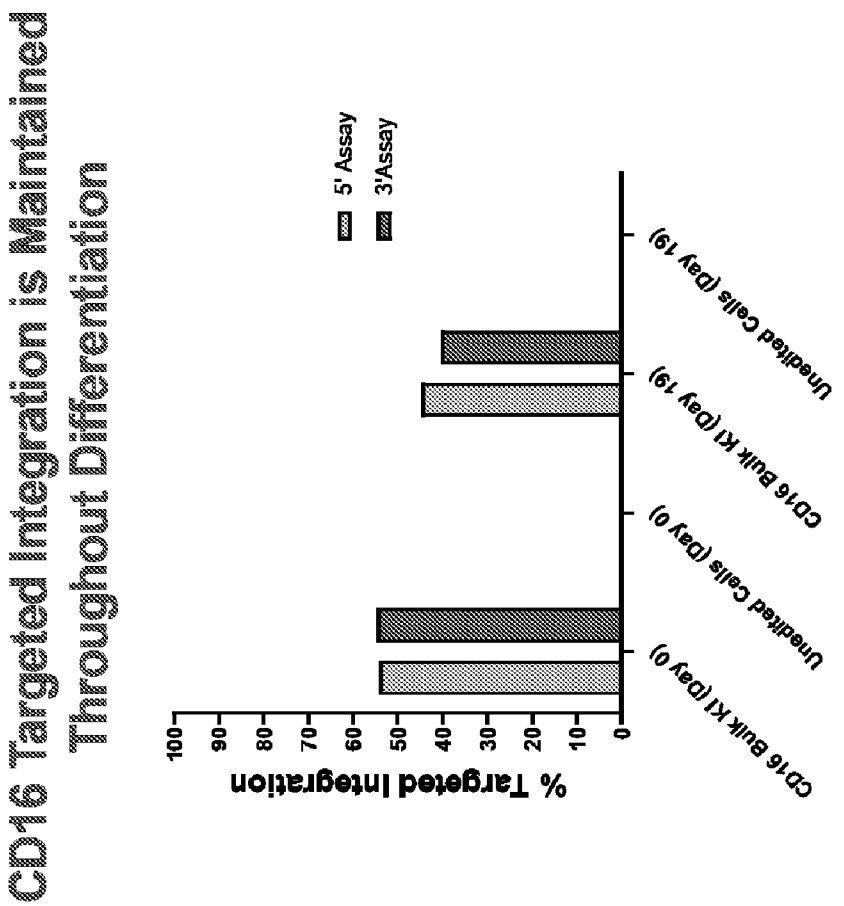

CD16 Targeted Integration is Maintained Throughout Differentiation
% Targeted Integration
100
90
80
70
60
50
40
30
20
10
0
5' Assay
3'Assay
CD16 Bulk KI (Day 0)
Unedited Cells (Day 0)
CD16 Bulk KI (Day 19)
Unedited Cells (Day 19)

Fig. 18A

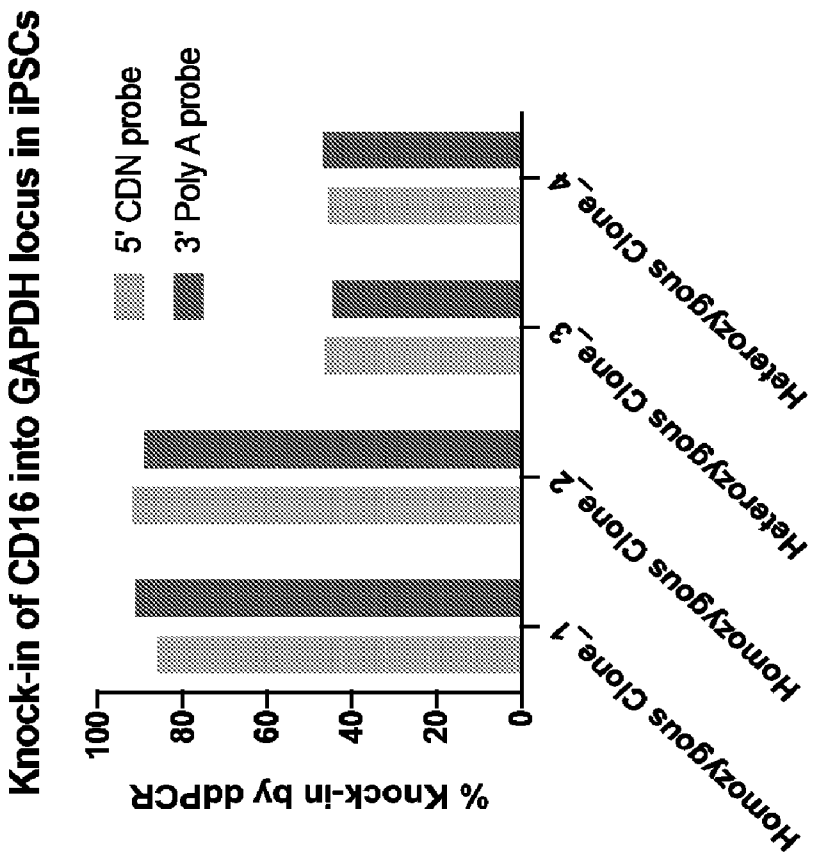

Knock-in of CD16 into GAPDH locus in iPSCs
5' CDN probe
3' Poly A probe
% Knock-in by ddPCR
100
80
60
40
20
0
Homozygous Clone_1
Homozygous Clone_2
Heterozygous Clone_3
Heterozygous Clone_4

Fig. 18B

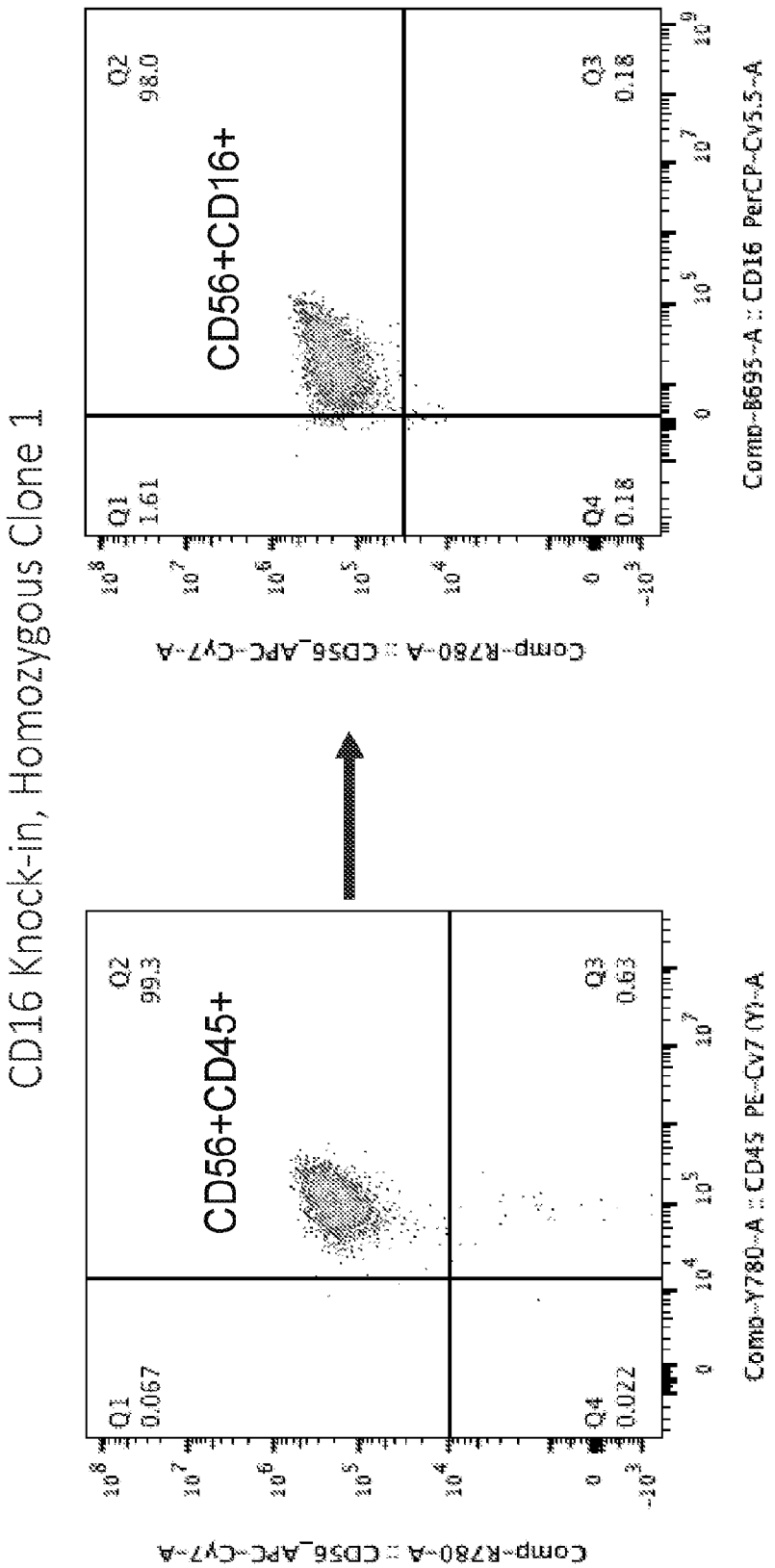

CD16 Knock-in, Homozygous Clone 1
CD56+CD45+
Q1 0.067
Q2 99.3
Q3 0.63
Q4 0.022
Comp-R780-A :: CD56_APC-Cy7-A
Comp-Y780-A :: CD45 PE-Cy7 (Y)-A
CD56+CD16+
Q1 1.61
Q2 98.0
Q3 0.18
Q4 0.18
Comp-R780-A :: CD56_APC-Cy7-A
Comp-B695-A :: CD16 PerCP-Cy5.5-A

Fig. 19A

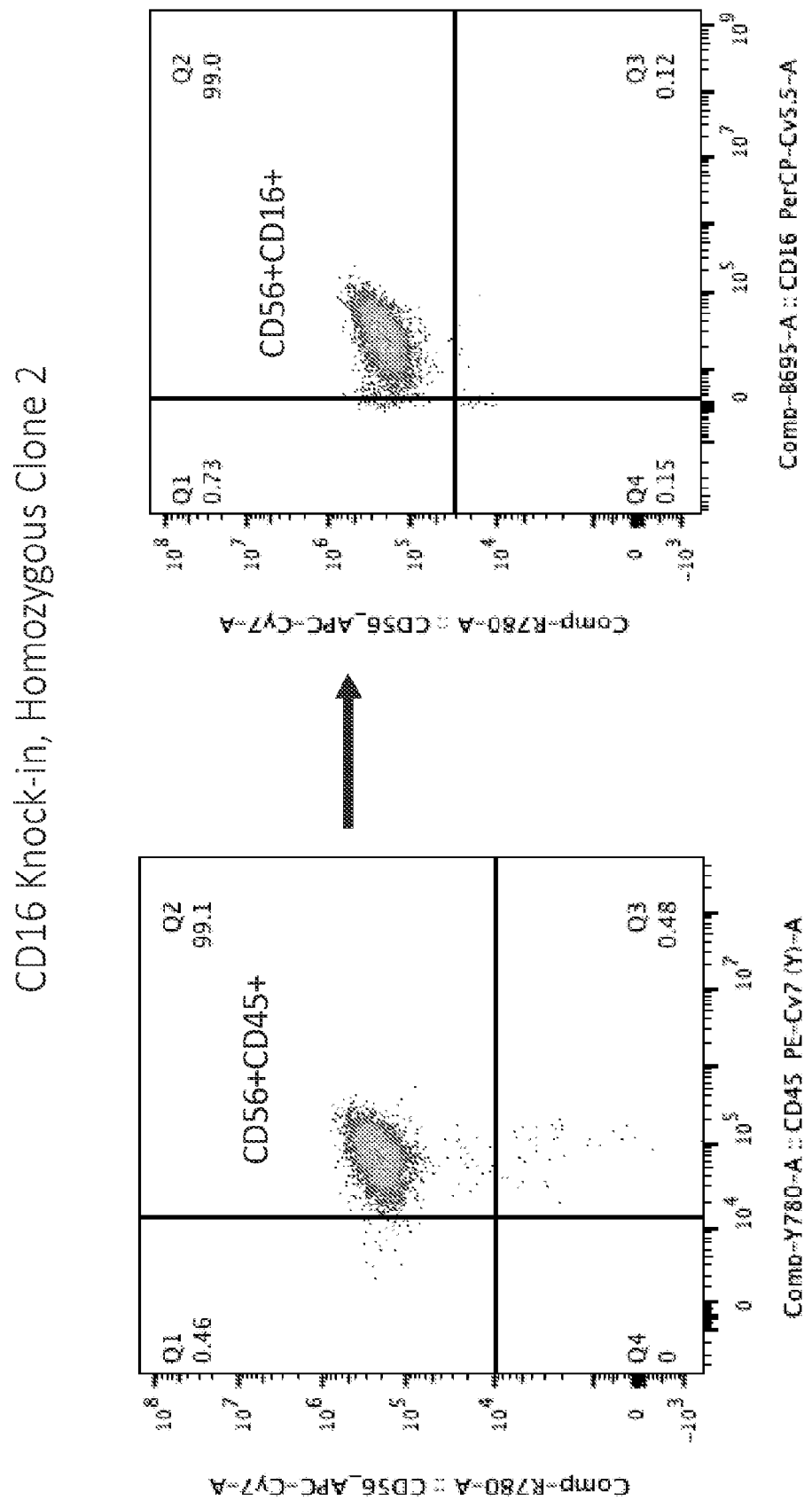

CD16 Knock-in, Homozygous Clone 2
CD56+CD45+
Q1 0.46
Q2 99.1
Q3 0.48
Q4 0
Comp-R780-A :: CD56_APC-Cy7-A
Comp-Y780-A :: CD45 PE-Cy7 (Y)-A
CD56+CD16+
Q1 0.73
Q2 99.0
Q3 0.12
Q4 0.15
Comp-R780-A :: CD56_APC-Cy7-A
Comp-B695-A :: CD16 PerCP-Cy5.5-A

Fig. 19B

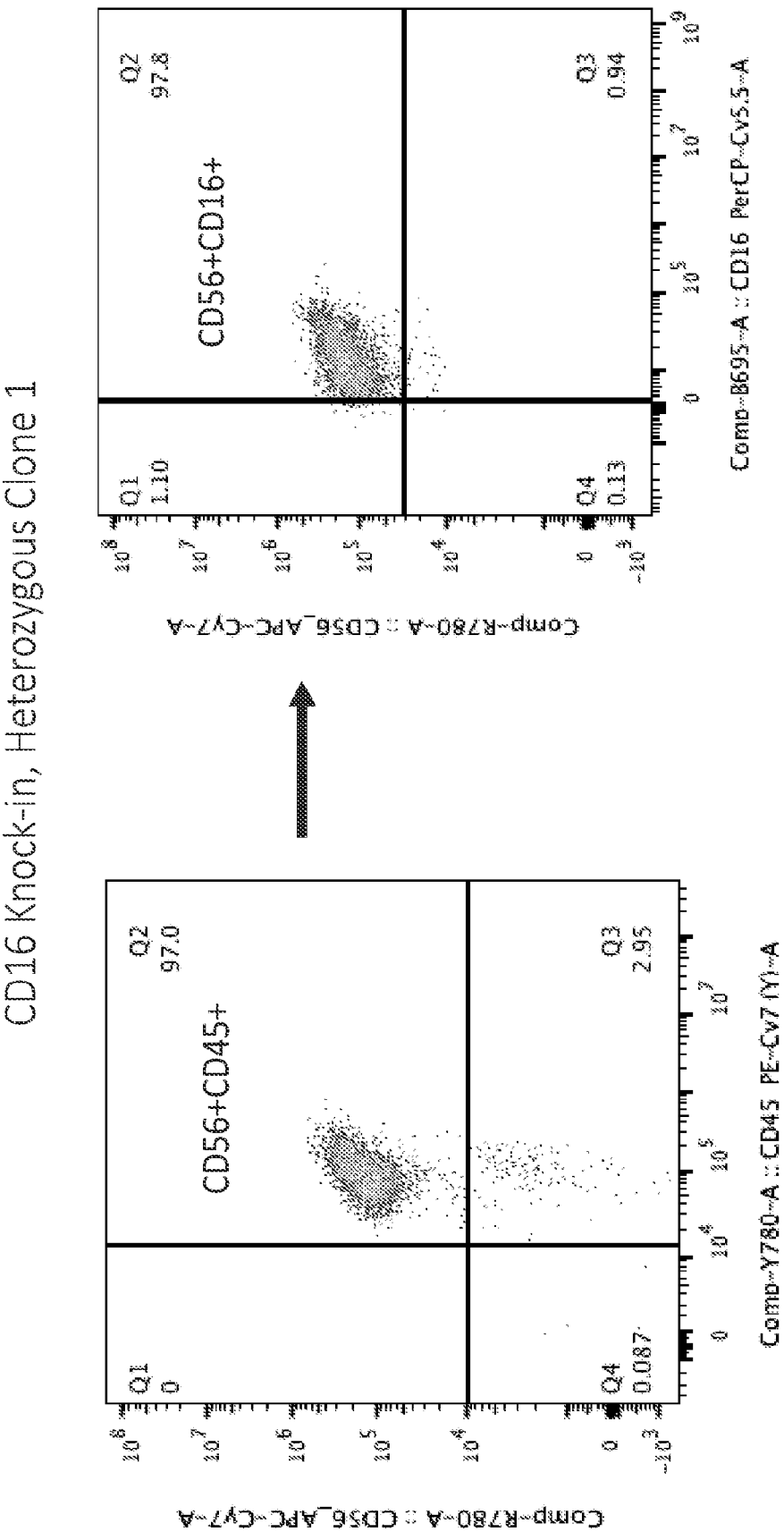

CD16 Knock-in, Heterozygous Clone 1
CD56+CD45+
Q1
0
Q2
97.0
Q3
2.95
Q4
0.087
Comp-Y780-A :: CD45 PE-Cy7 (Y)-A
Comp-R780-A :: CD56_APC-Cy7-A
CD56+CD16+
Q1
1.10
Q2
97.8
Q3
0.94
Q4
0.13
Comp-B695-A :: CD16 PerCP-Cy5.5-A
Comp-R780-A :: CD56_APC-Cy7-A

Fig. 19C

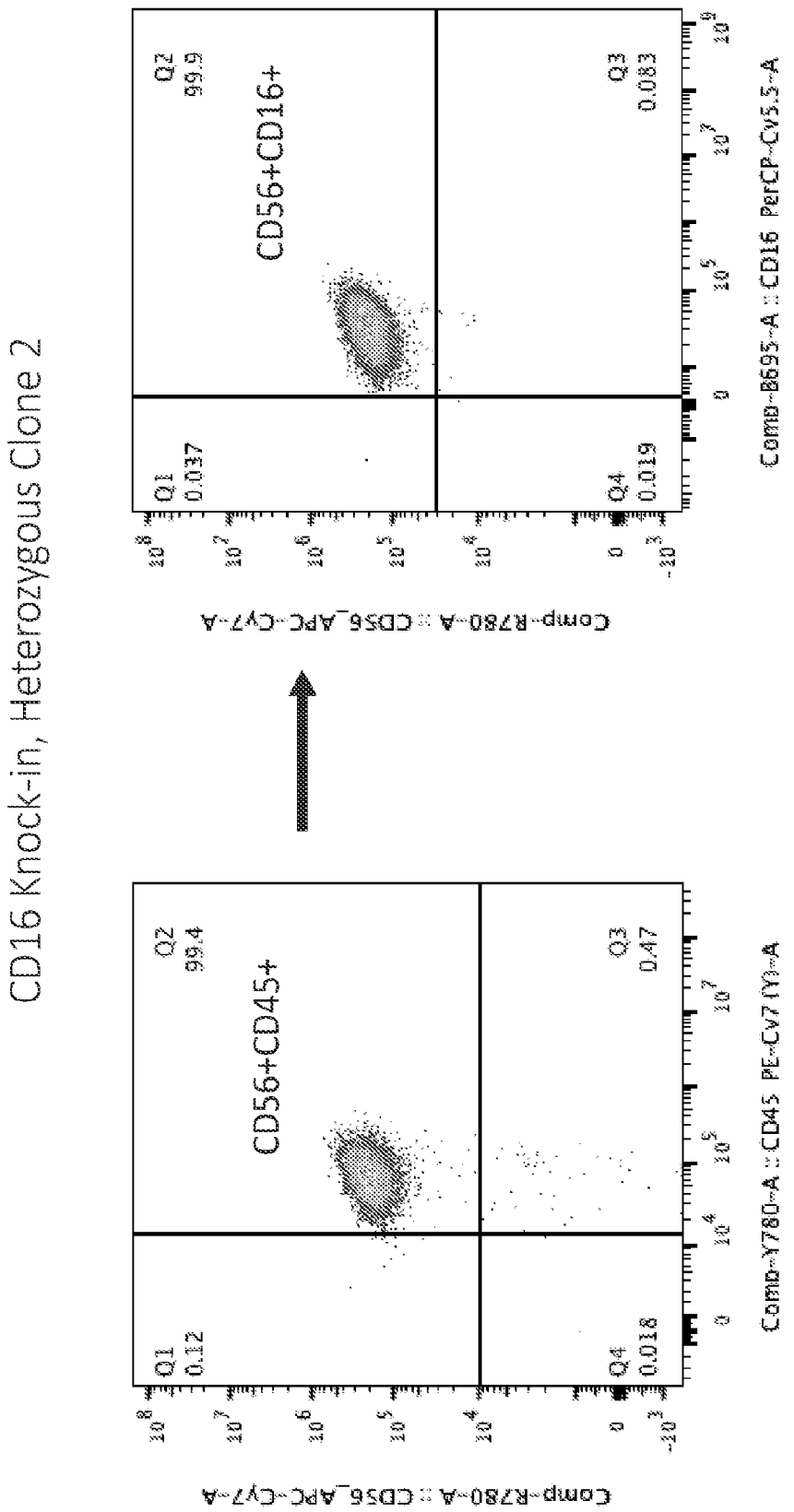

CD16 Knock-in, Heterozygous Clone 2
CD56+CD45+
Q1 0.12
Q2 99.4
Q3 0.47
Q4 0.018
Comp-R780-A :: CD56_APC-Cy7-A
Comp-Y780-A :: CD45 PE-Cy7 (Y)-A
CD56+CD16+
Q1 0.037
Q2 99.9
Q3 0.083
Q4 0.019
Comp-R780-A :: CD56_APC-Cy7-A
Comp-B695-A :: CD16 PerCP-Cy5.5-A

Fig. 19D

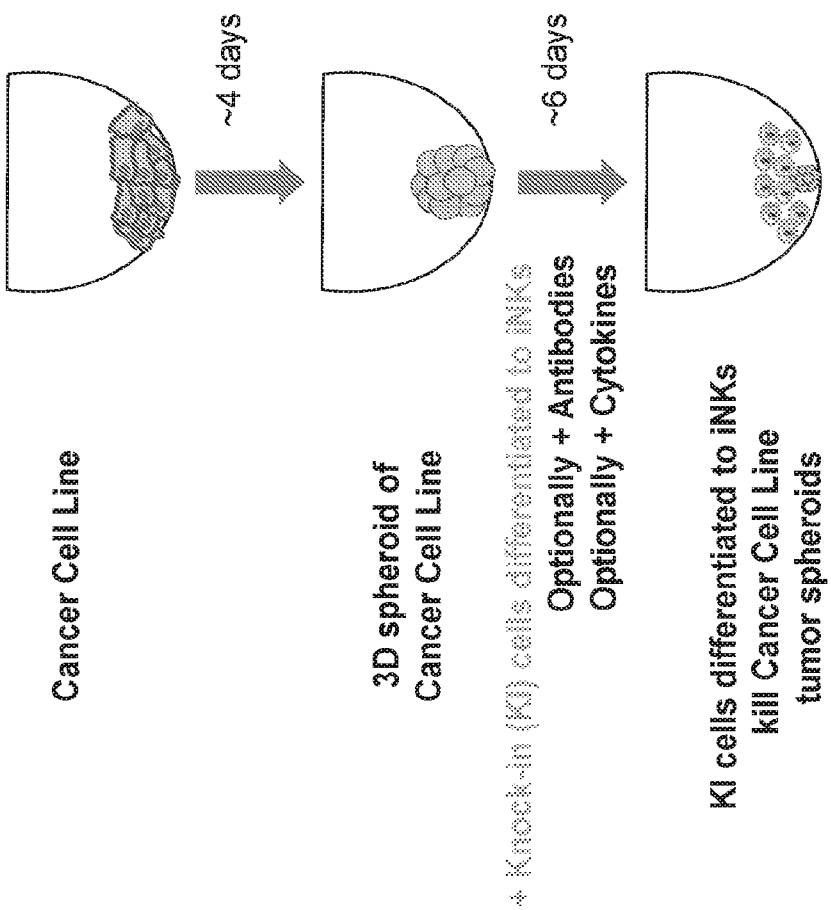

Cancer Cell Line
~4 days
3D spheroid of
Cancer Cell Line
+ Knock-in (KI) cells differentiated to iNKs
Optionally + Antibodies
Optionally + Cytokines
~6 days
KI cells differentiated to iNKs
kill Cancer Cell Line
tumor spheroids

Fig. 20

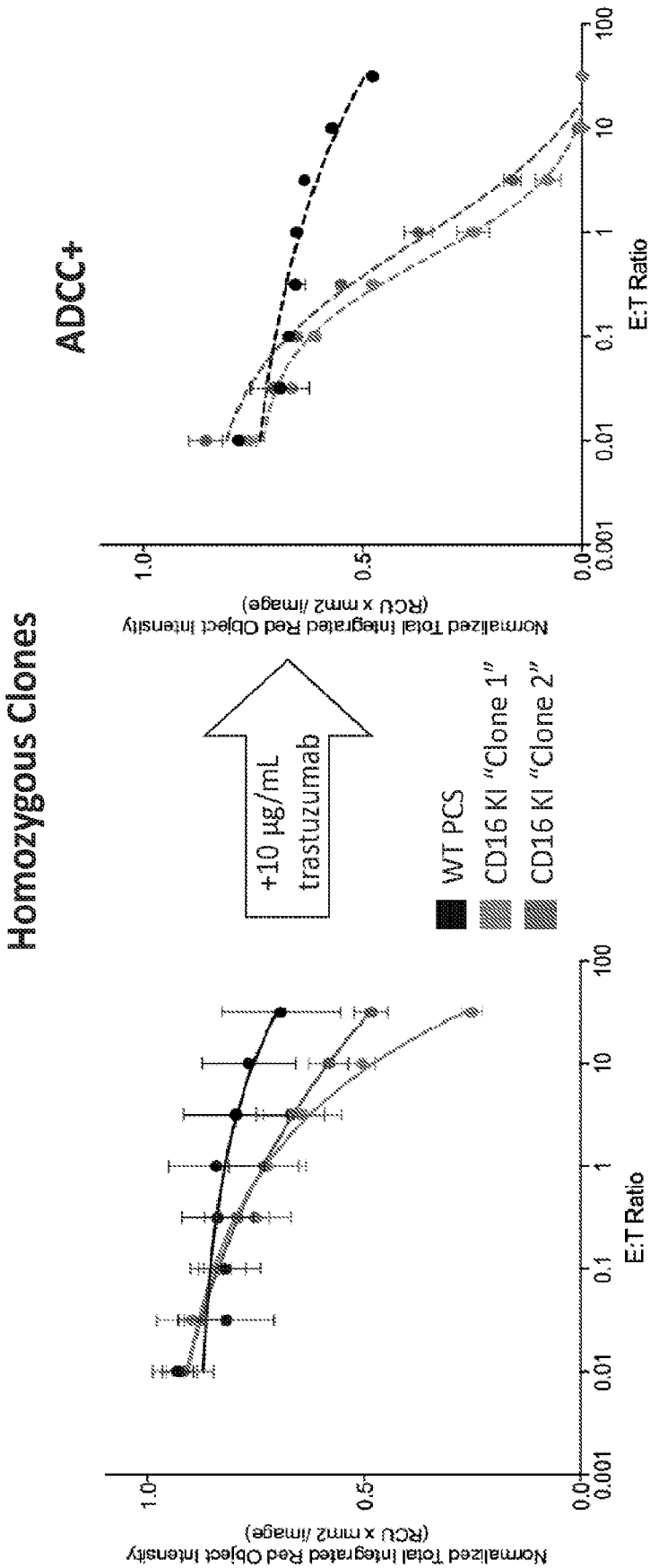

Homozygous Clones
ADCC+
Normalized Total Integrated Red Object Intensity
(RCU x mm2 /image)
1.0
0.5
0.0
0.001
0.01
0.1
1
10
100
E:T Ratio
+10 µg/mL
trastuzumab
WT PCS
CD16 KI "Clone 1"
CD16 KI "Clone 2"

Fig. 21A

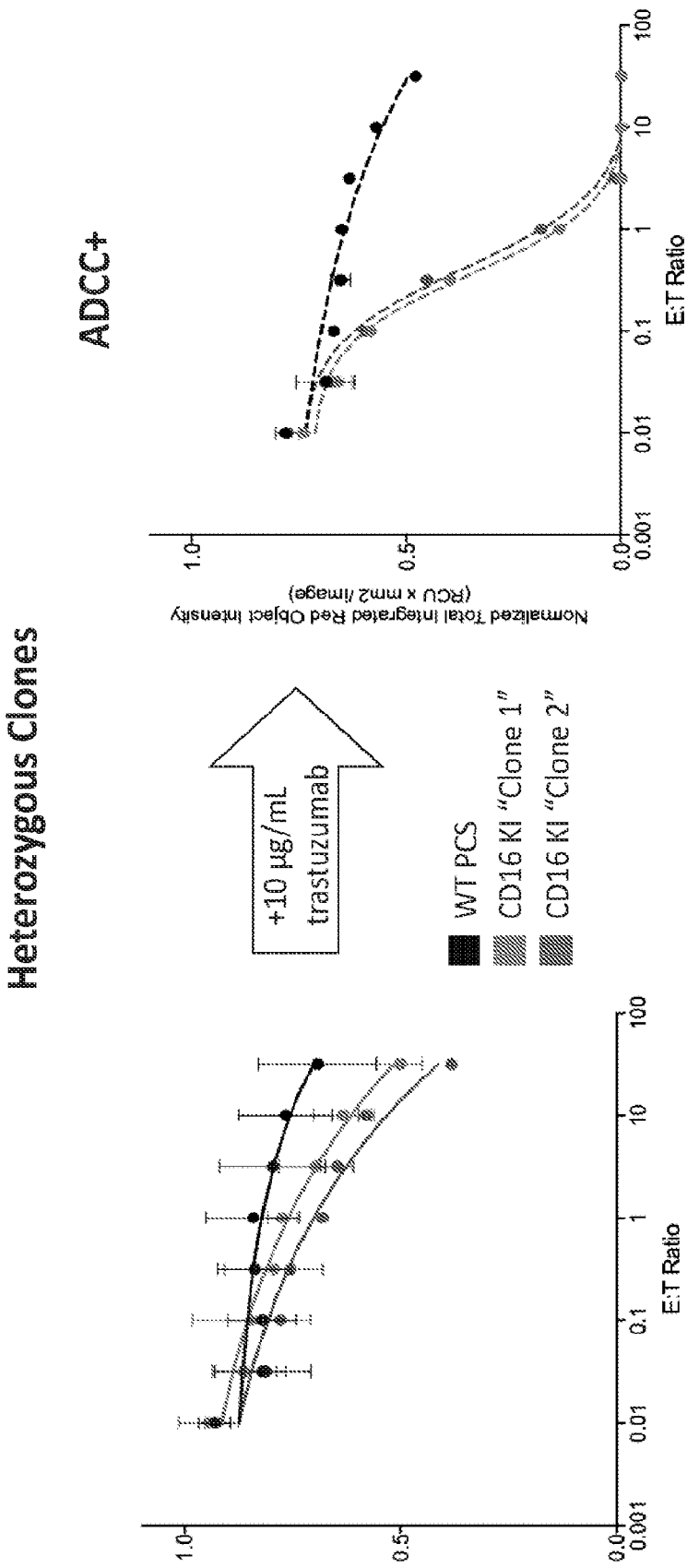

Heterozygous Clones
ADCC+
+10 µg/mL trastuzumab
WT PCS
CD16 KI "Clone 1"
CD16 KI "Clone 2"
Normalized Total Integrated Red Object Intensity (RCU x mm2 /image)
E:T Ratio
0.0
0.5
1.0
0.001
0.01
0.1
1
10
100

Fig. 21B

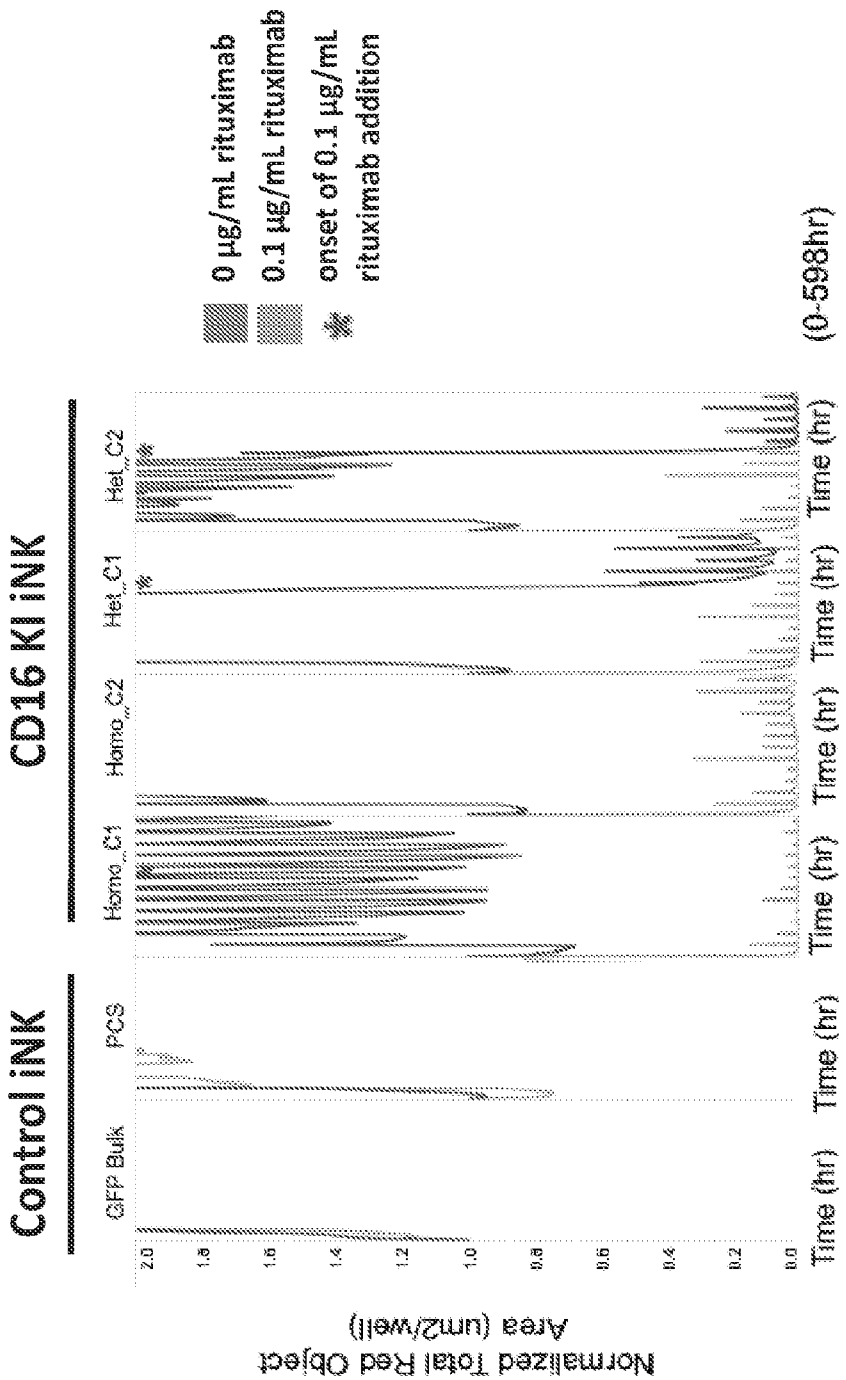

Control iNK
CD16 KI iNK
GFP Bulk
PCS
Homo_C1
Homo_C2
Het_C1
Het_C2
Normalized Total Red Object Area (um2/well)
Time (hr)
0 µg/mL rituximab
0.1 µg/mL rituximab
onset of 0.1 µg/mL rituximab addition
(0-598hr)

Fig. 22

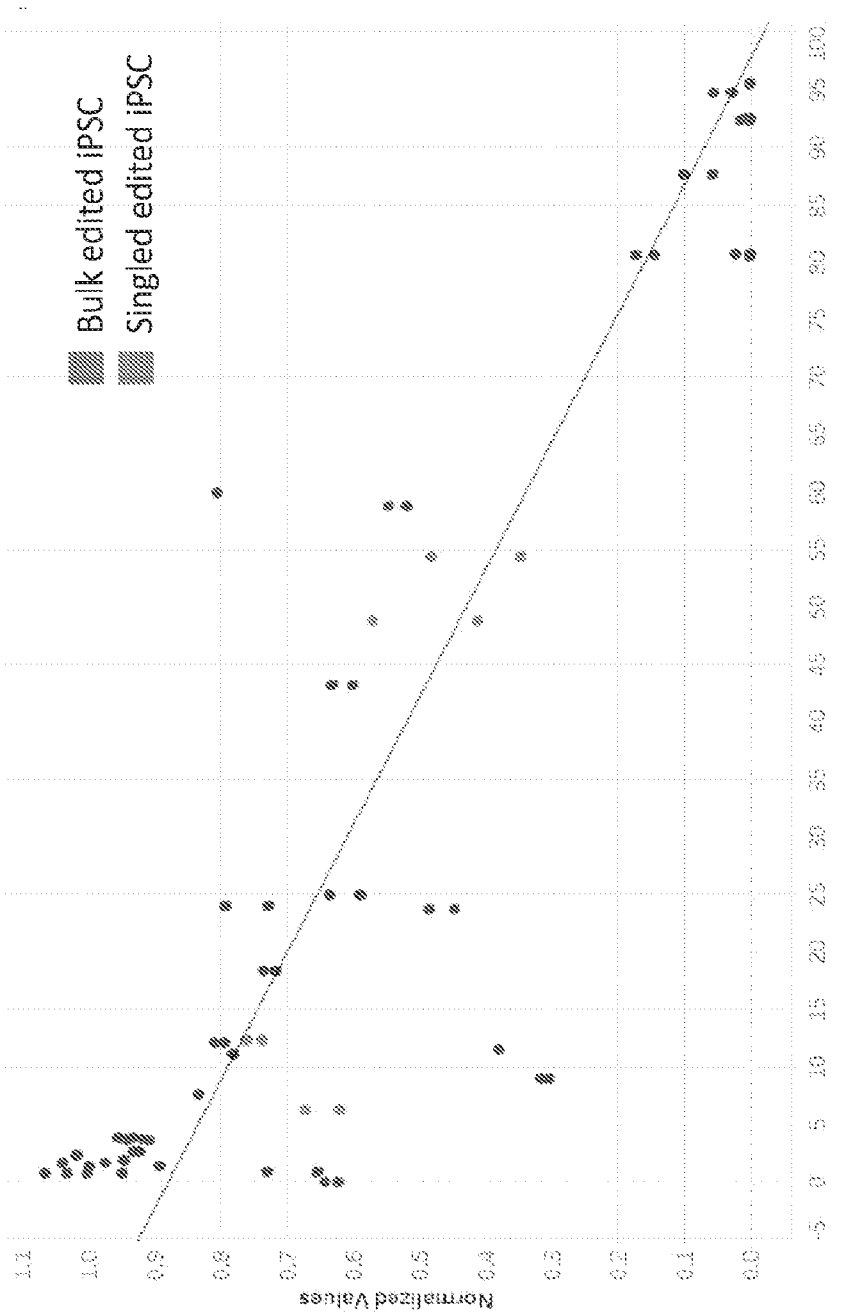

Bulk edited iPSC
Singled edited iPSC
Normalized Values
CD16% Expression
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-5
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100

Fig. 23

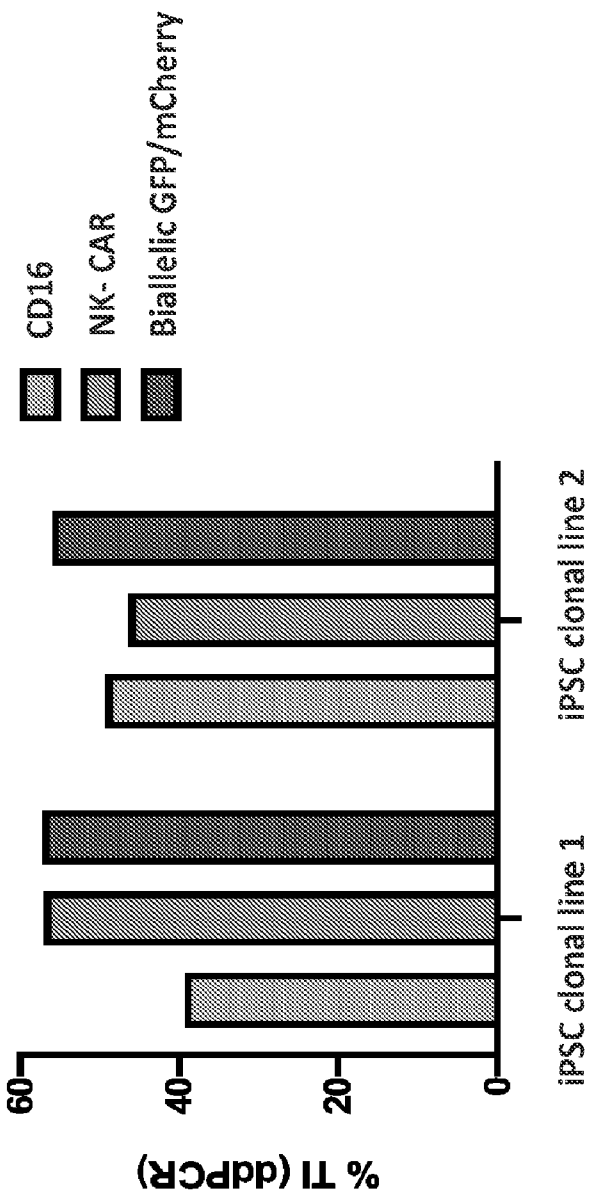

CD16
NK- CAR
Biallelic GFP/mCherry
% TI (ddPCR)
60
40
20
0
iPSC clonal line 1
iPSC clonal line 2

Fig. 24A

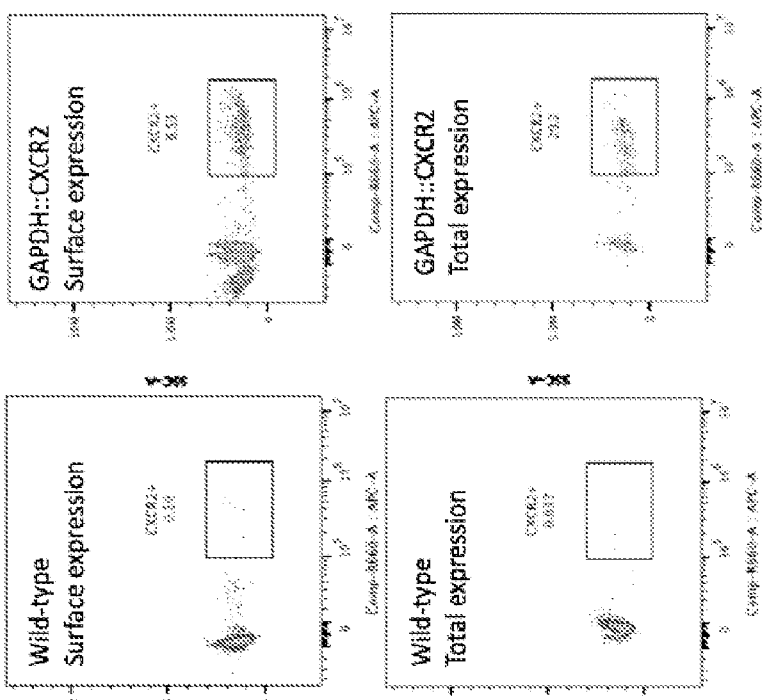

Wild-type
Surface expression
Wild-type
Total expression
GAPDH::CXCR2
Surface expression
GAPDH::CXCR2
Total expression
CXCR4 +

Fig. 24B

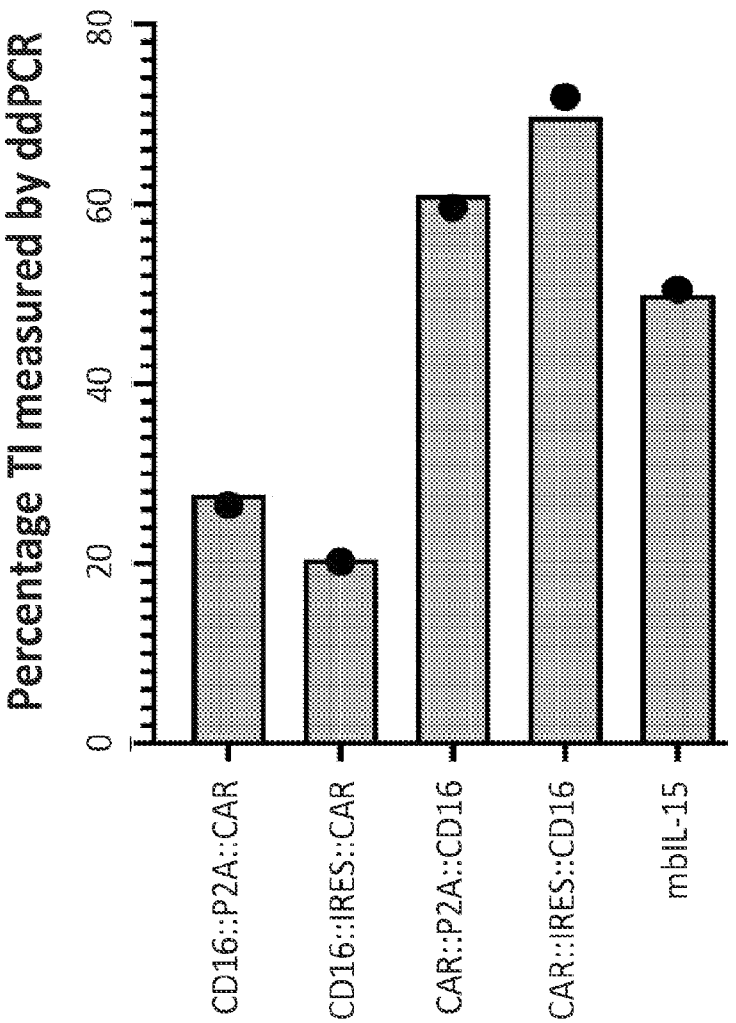

Percentage TI measured by ddPCR
0
20
40
60
80
CD16::P2A::CAR
CD16::IRES::CAR
CAR::P2A::CD16
CAR::IRES::CD16
mbIL-15

Fig. 25

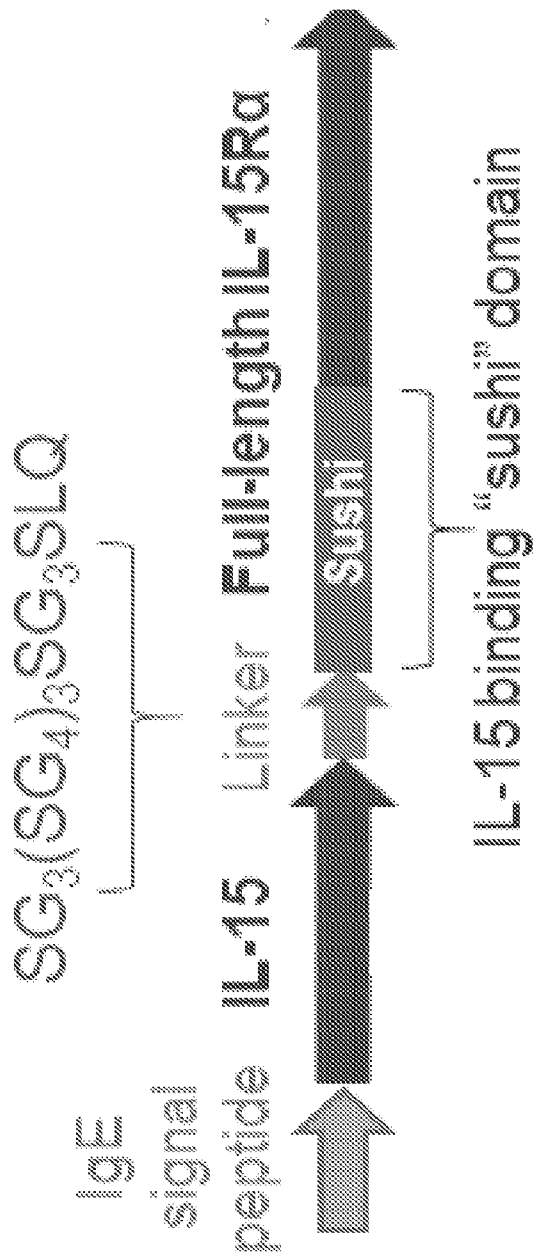

IgE signal peptide
IL-15
Linker
Full-length IL-15Rα
sushi
IL-15 binding "sushi" domain

Fig. 26

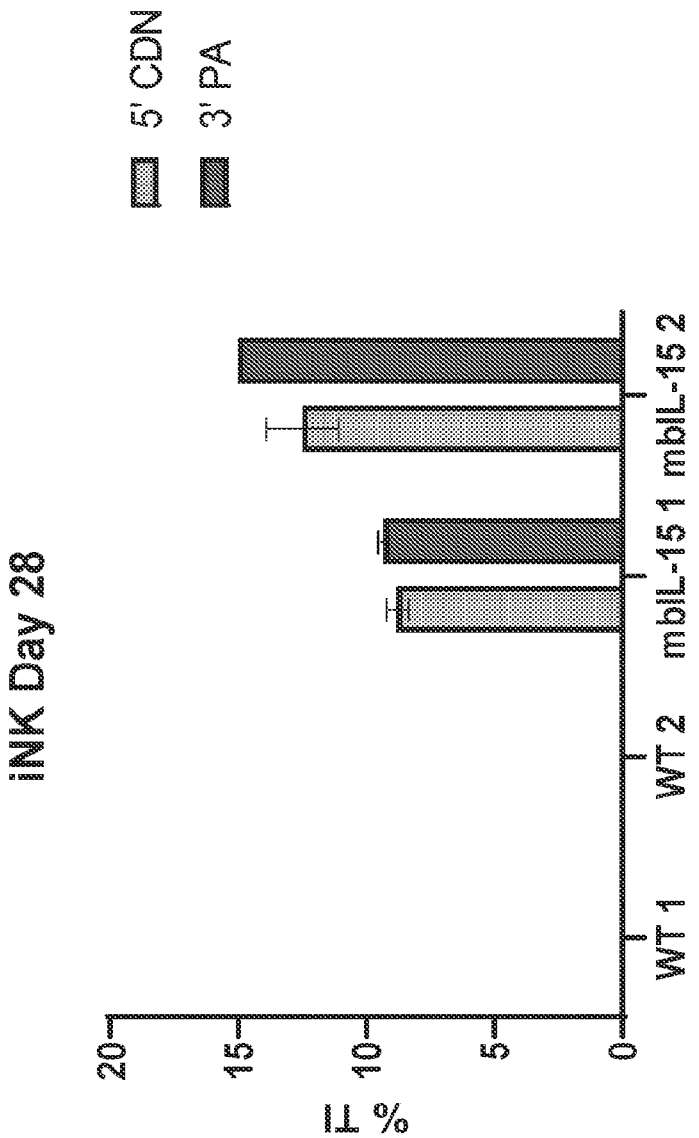


Fig. 27

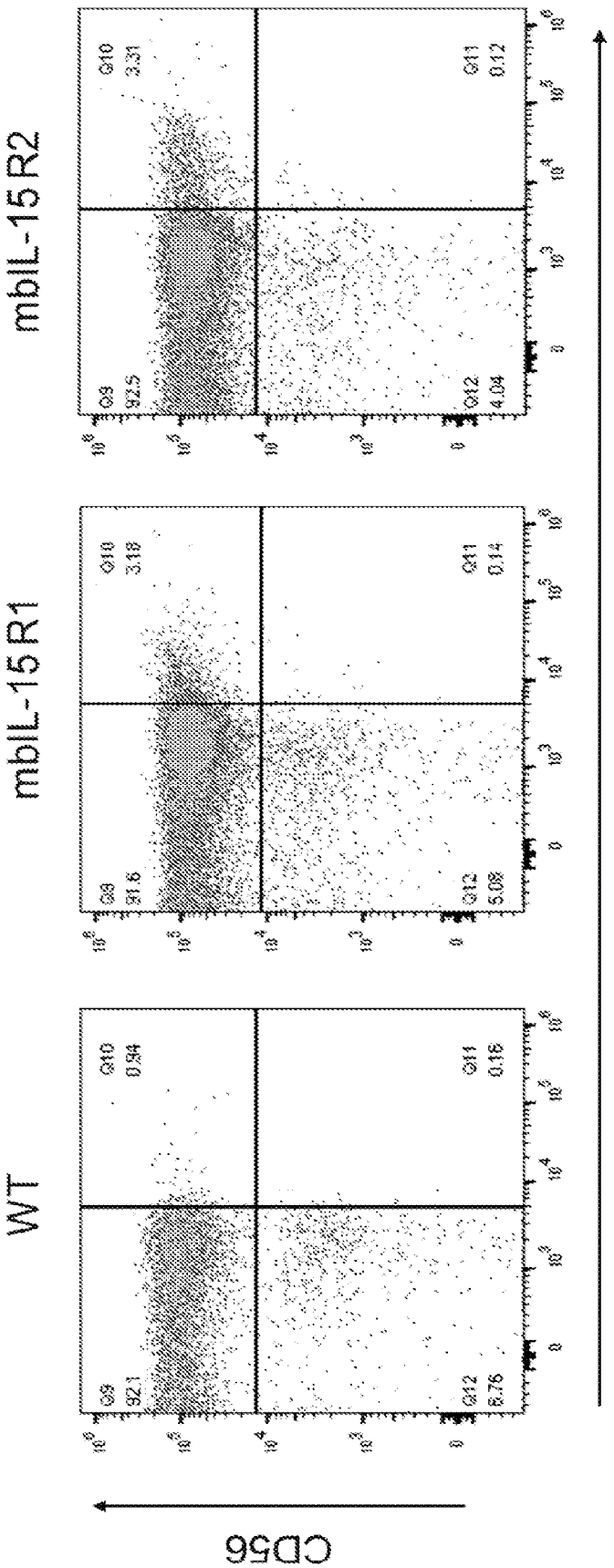

WT
mbIL-15R1
mbIL-15R2
CD56
IL-15Ra
Q9 92.1
Q10 0.94
Q12 6.76
Q11 0.16
Q9 91.6
Q10 3.18
Q12 5.08
Q11 0.14
Q9 92.5
Q10 3.31
Q12 4.04
Q11 0.12

Fig. 28A

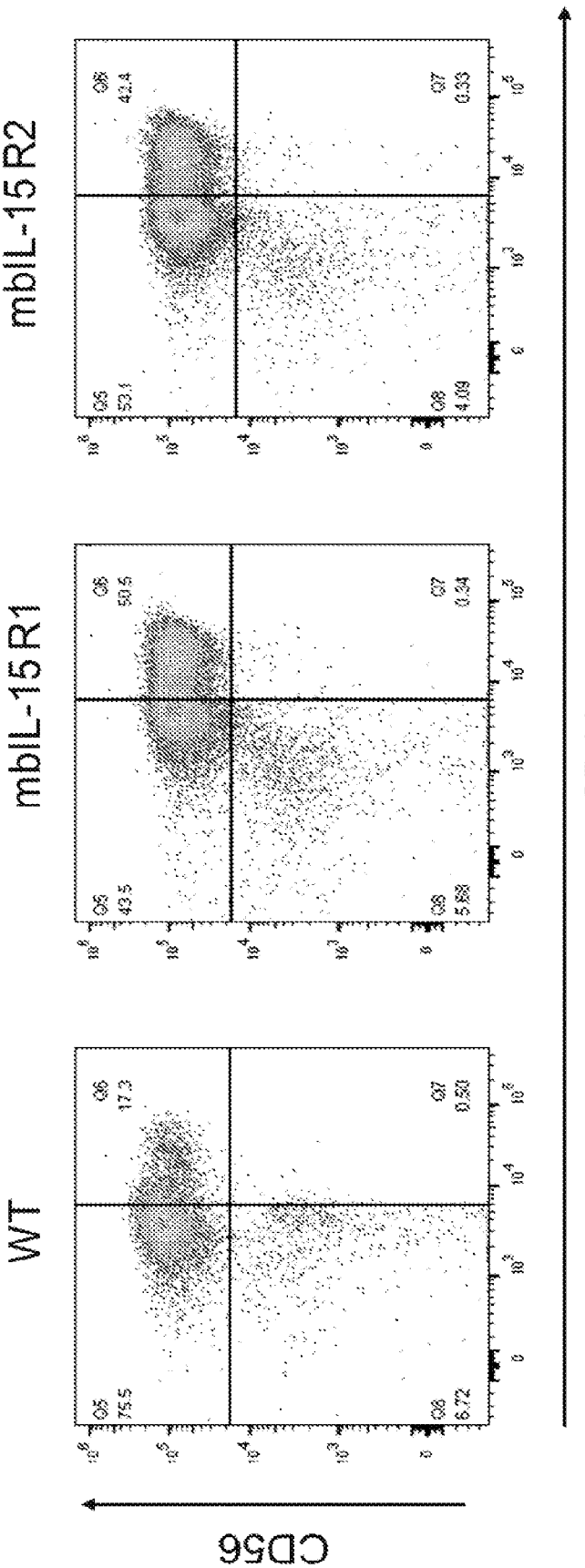

WT
mbIL-15R1
mbIL-15R2
CD56
CD16
Q5 75.5
Q6 17.3
Q7 0.83
Q8 6.72
Q5 43.5
Q6 50.5
Q7 0.34
Q8 5.68
Q5 53.1
Q6 42.4
Q7 0.33
Q8 4.09

Fig. 28B

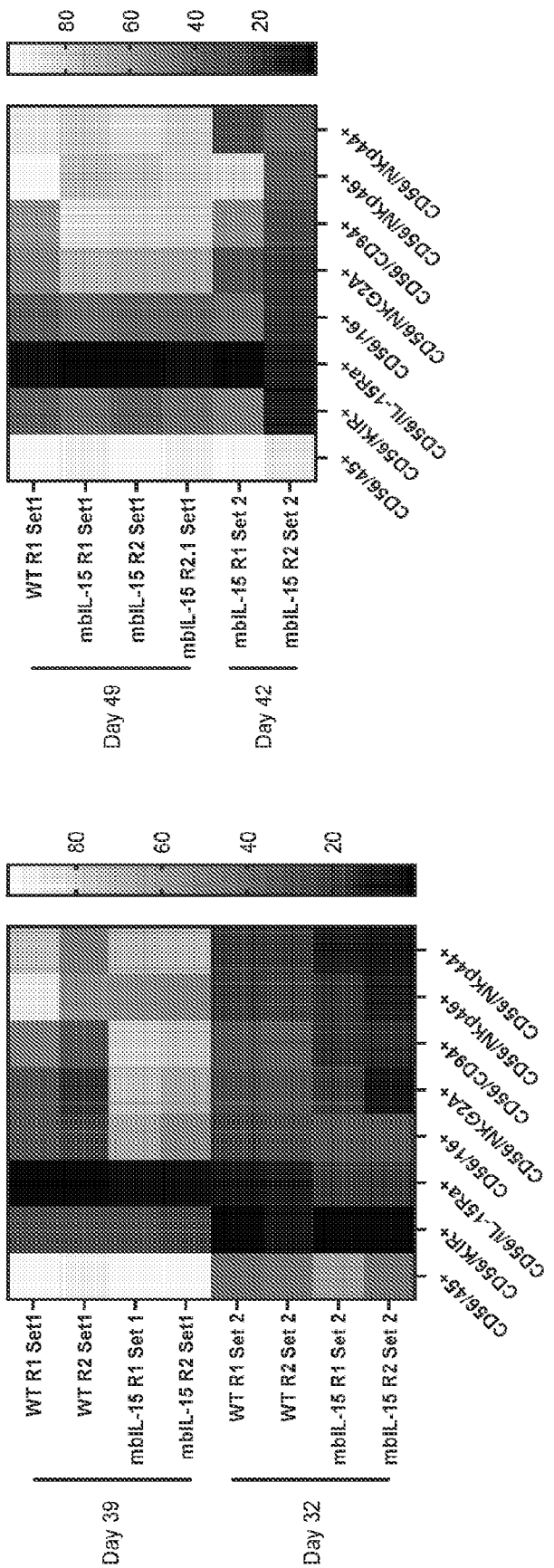

80
60
40
20
WT R1 Set1
mbIL-15 R1 Set1
mbIL-15 R2 Set1
mbIL-15 R2.1 Set1
mbIL-15 R1 Set 2
mbIL-15 R2 Set 2
Day 49
Day 42
CD56/45+
CD56/KIR+
CD56/IL-15Rα+
CD56/16+
CD56/NKG2A+
CD56/CD94+
CD56/NKp46+
CD56/NKp44+
80
60
40
20
WT R1 Set1
WT R2 Set1
mbIL-15 R1 Set 1
mbIL-15 R2 Set1
WT R1 Set 2
WT R2 Set 2
mbIL-15 R1 Set 2
mbIL-15 R2 Set 2
Day 39
Day 32
CD56/45+
CD56/KIR+
CD56/IL-15Rα+
CD56/16+
CD56/NKG2A+
CD56/CD94+
CD56/NKp46+
CD56/NKp44+

Fig. 28C

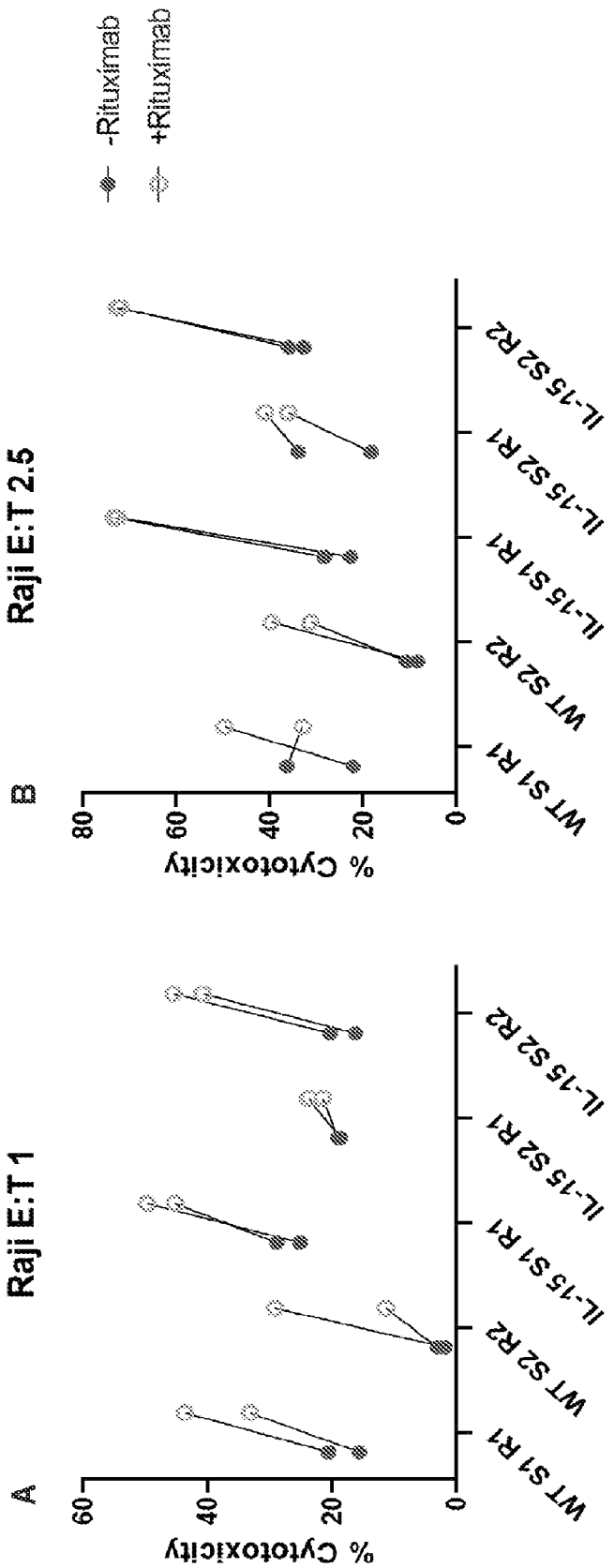

A
Raji E:T 1
% Cytotoxicity
0
20
40
60
WT S1 R1
WT S2 R2
IL-15 S1 R1
IL-15 S2 R1
IL-15 S2 R2
B
Raji E:T 2.5
% Cytotoxicity
0
20
40
60
80
WT S1 R1
WT S2 R2
IL-15 S1 R1
IL-15 S2 R1
IL-15 S2 R2
-Rituximab
+Rituximab

Fig. 29

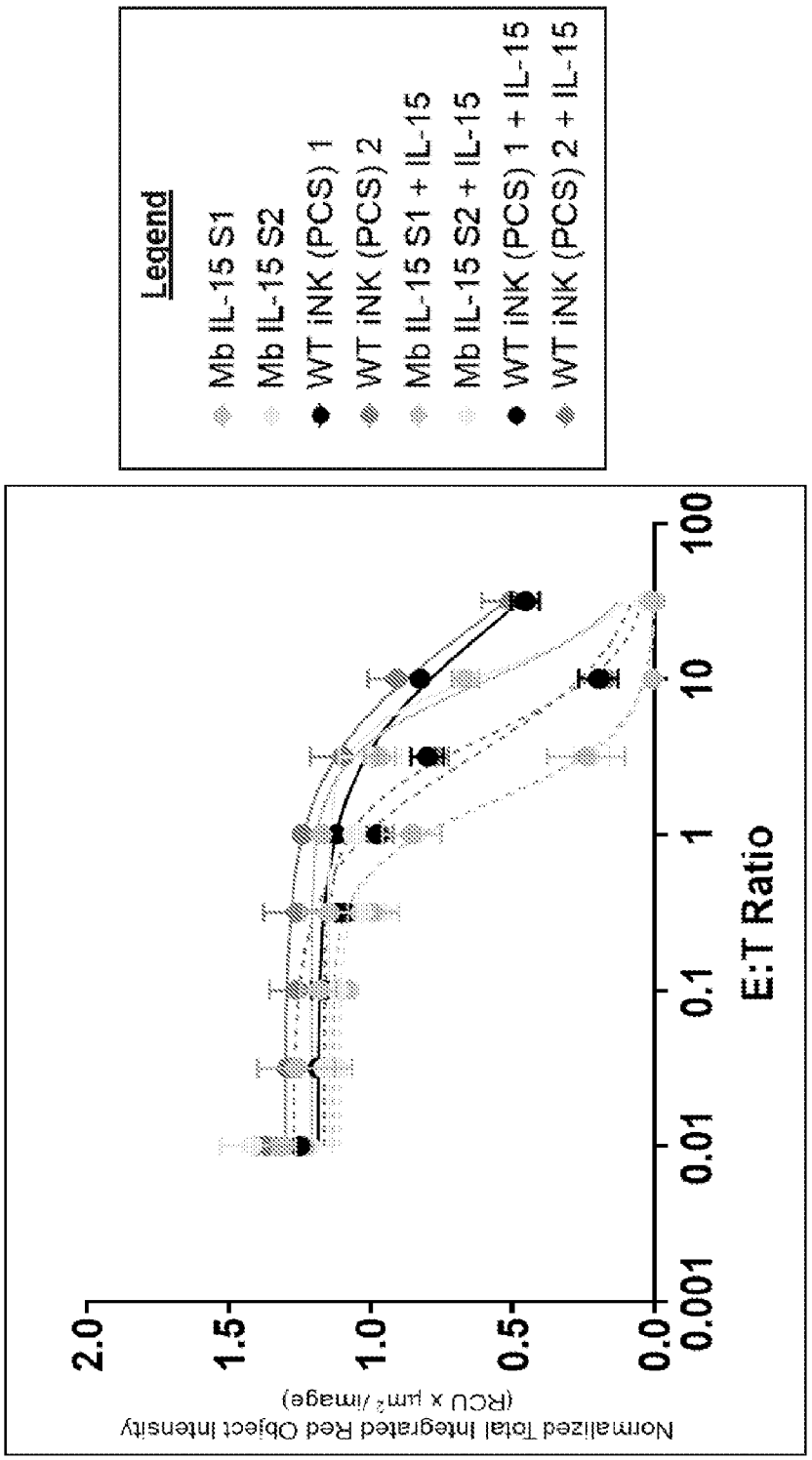

Legend
Mb IL-15 S1
Mb IL-15 S2
WT iNK (PCS) 1
WT iNK (PCS) 2
Mb IL-15 S1 + IL-15
Mb IL-15 S2 + IL-15
WT iNK (PCS) 1 + IL-15
WT iNK (PCS) 2 + IL-15
Normalized Total Integrated Red Object Intensity
(RCU x μm²/Image)
2.0
1.5
1.0
0.5
0.0
0.001
0.01
0.1
1
10
100
E:T Ratio

Fig. 30A

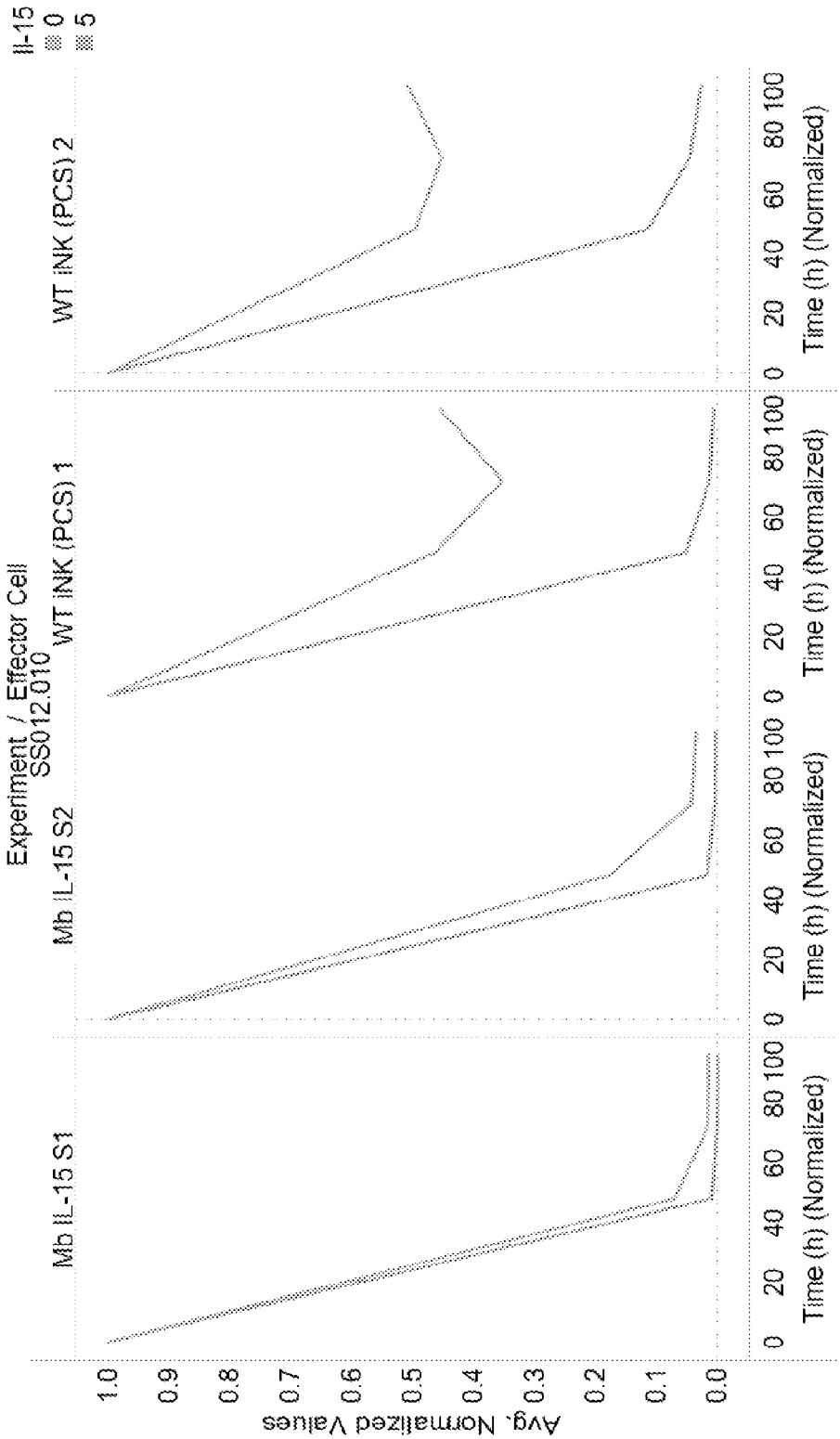

Experiment / Effector Cell
SS012.010
Mb IL-15 S1
Mb IL-15 S2
WT iNK (PCS) 1
WT iNK (PCS) 2
Il-15
0
5
Avg. Normalized Values
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0 20 40 60 80 100
Time (h) (Normalized)

Fig. 30B

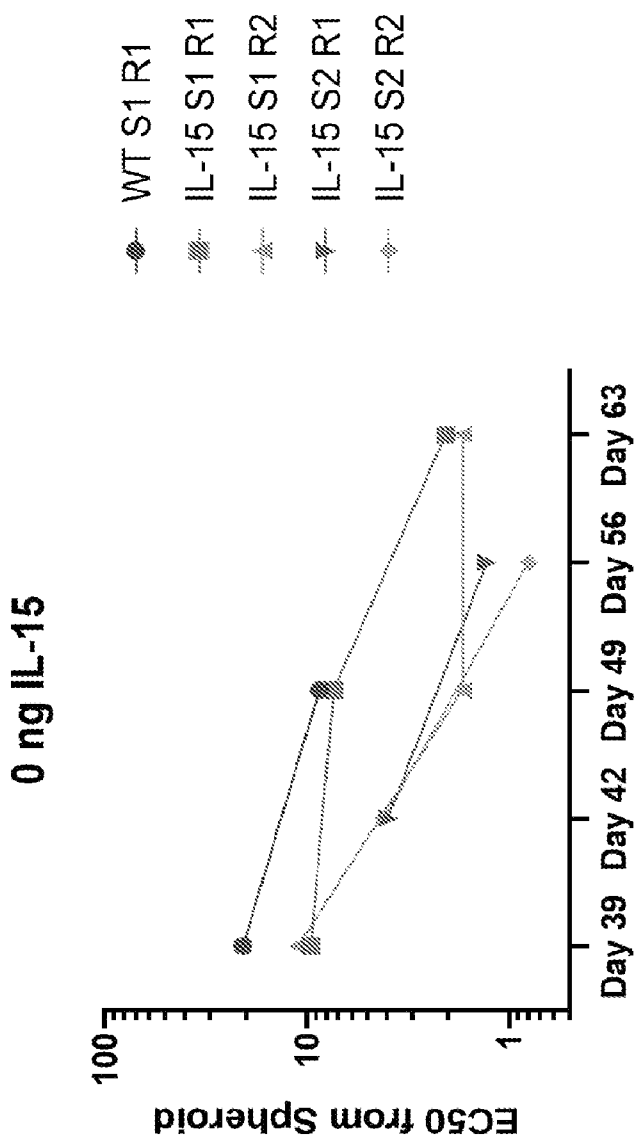

0 ng IL-15
WT S1 R1
IL-15 S1 R1
IL-15 S1 R2
IL-15 S2 R1
IL-15 S2 R2
EC50 from Spheroid
100
10
1
Day 39
Day 42
Day 49
Day 56
Day 63

Fig. 30C

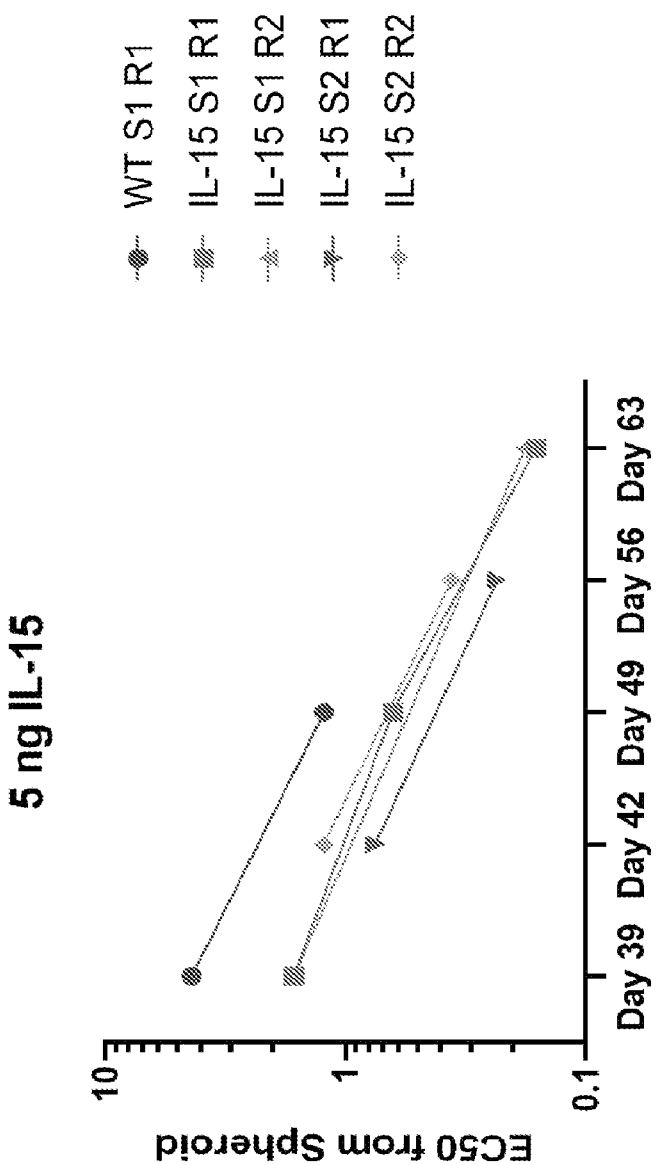

WT S1 R1
IL-15 S1 R1
IL-15 S1 R2
IL-15 S2 R1
IL-15 S2 R2
5 ng IL-15
EC50 from Spheroid
10
1
0.1
Day 39
Day 42
Day 49
Day 56
Day 63

Fig. 30D

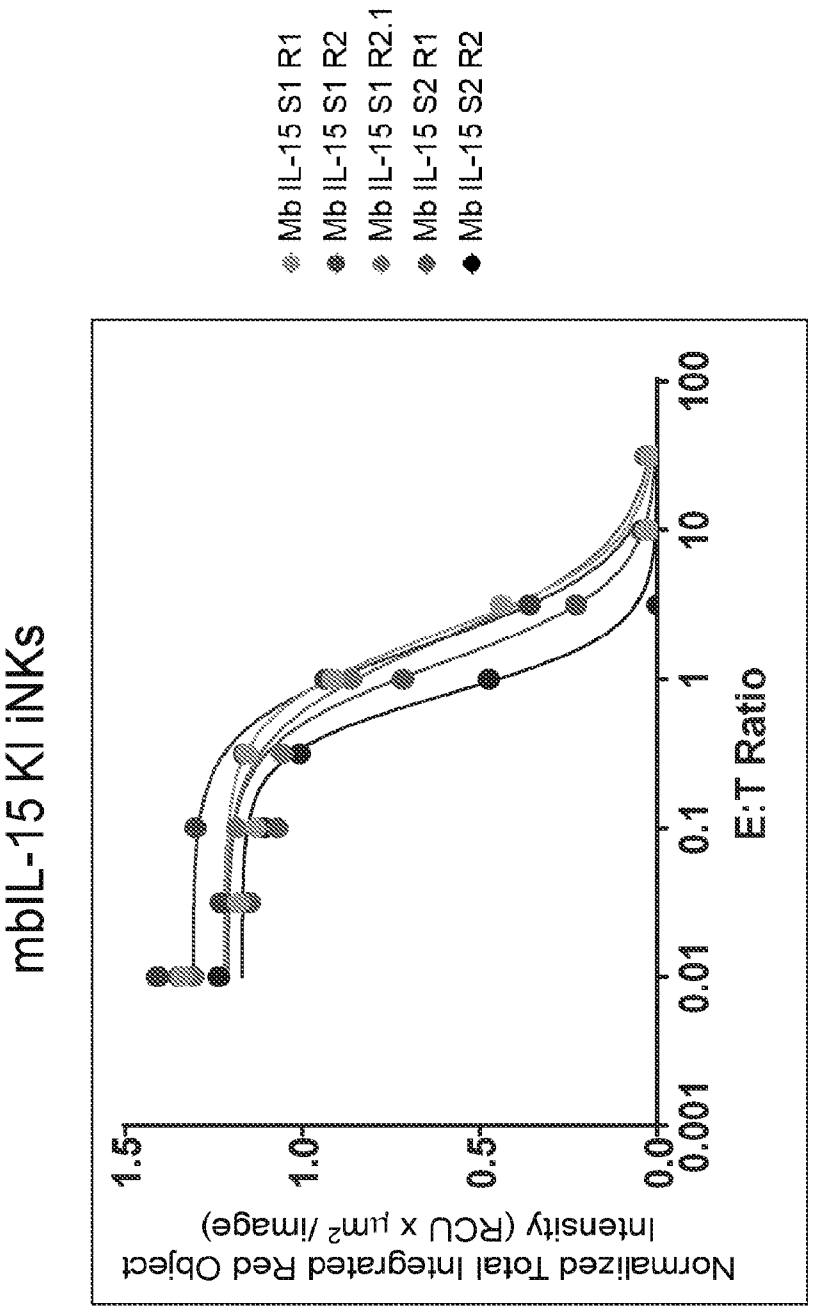

mbIL-15 KI iNKs
Mb IL-15 S1 R1
Mb IL-15 S1 R2
Mb IL-15 S1 R2.1
Mb IL-15 S2 R1
Mb IL-15 S2 R2
Normalized Total Integrated Red Object Intensity (RCU x μm² /image)
1.5
1.0
0.5
0.0
0.001
0.01
0.1
1
10
100
E:T Ratio

Fig. 31A

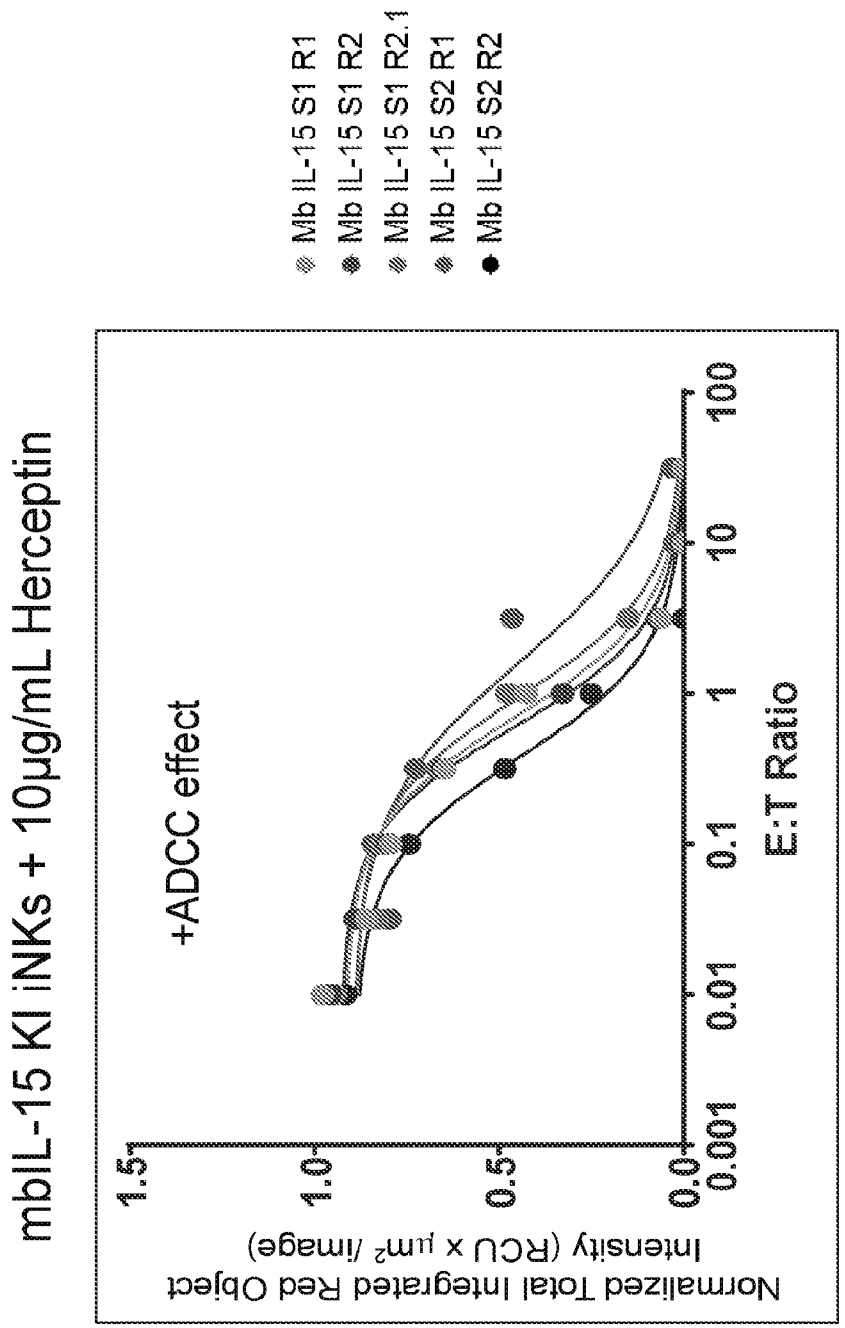

mbIL-15 KI iNKs + 10µg/mL Herceptin
+ADCC effect
Normalized Total Integrated Red Object Intensity (RCU x µm² /image)
1.5
1.0
0.5
0.0
0.001
0.01
0.1
1
10
100
E:T Ratio
Mb IL-15 S1 R1
Mb IL-15 S1 R2
Mb IL-15 S1 R2.1
Mb IL-15 S2 R1
Mb IL-15 S2 R2

Fig. 31B

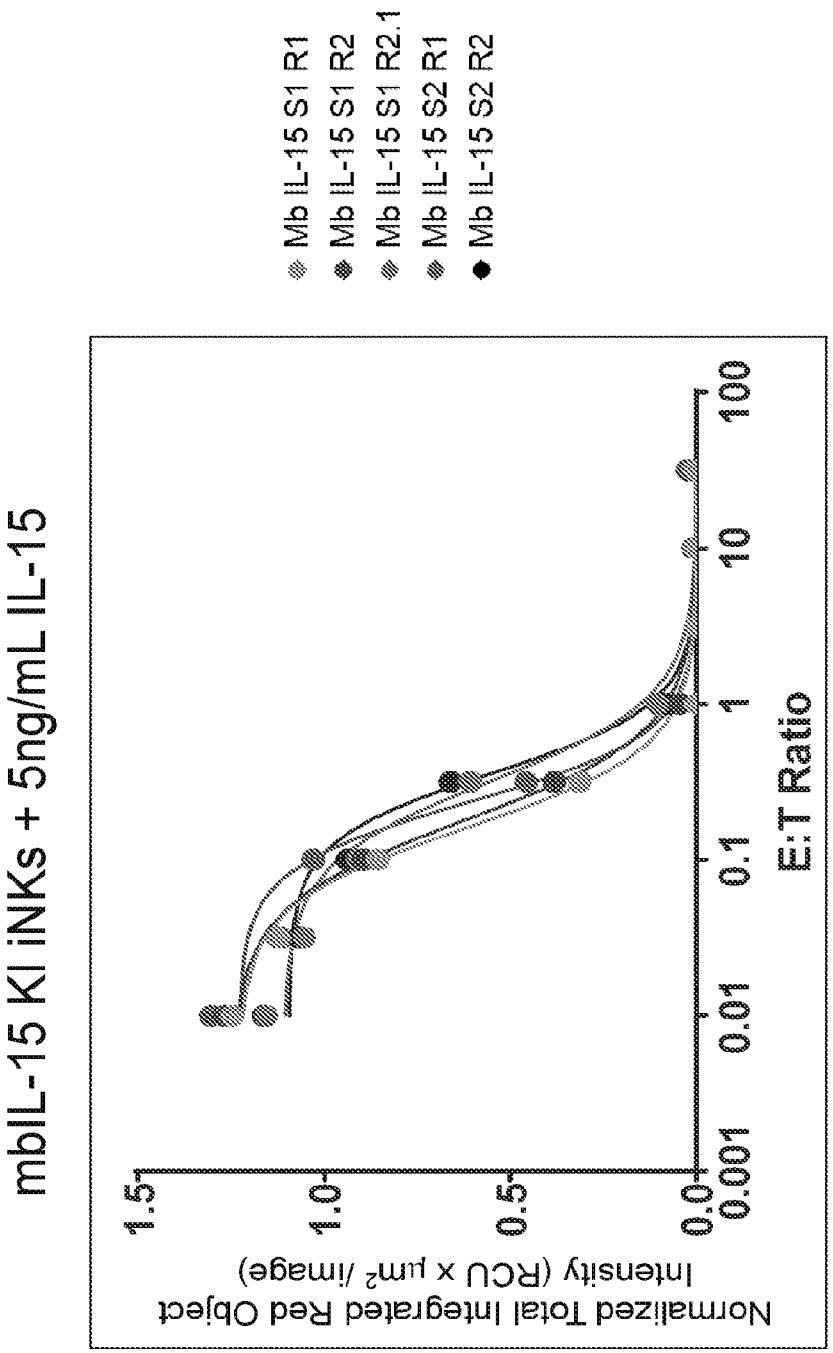

mbIL-15 KI iNKs + 5ng/mL IL-15
Mb IL-15 S1 R1
Mb IL-15 S1 R2
Mb IL-15 S1 R2.1
Mb IL-15 S2 R1
Mb IL-15 S2 R2
Normalized Total Integrated Red Object Intensity (RCU x μm² /image)
1.5
1.0
0.5
0.0
0.001
0.01
0.1
1
10
100
E:T Ratio

Fig. 31C

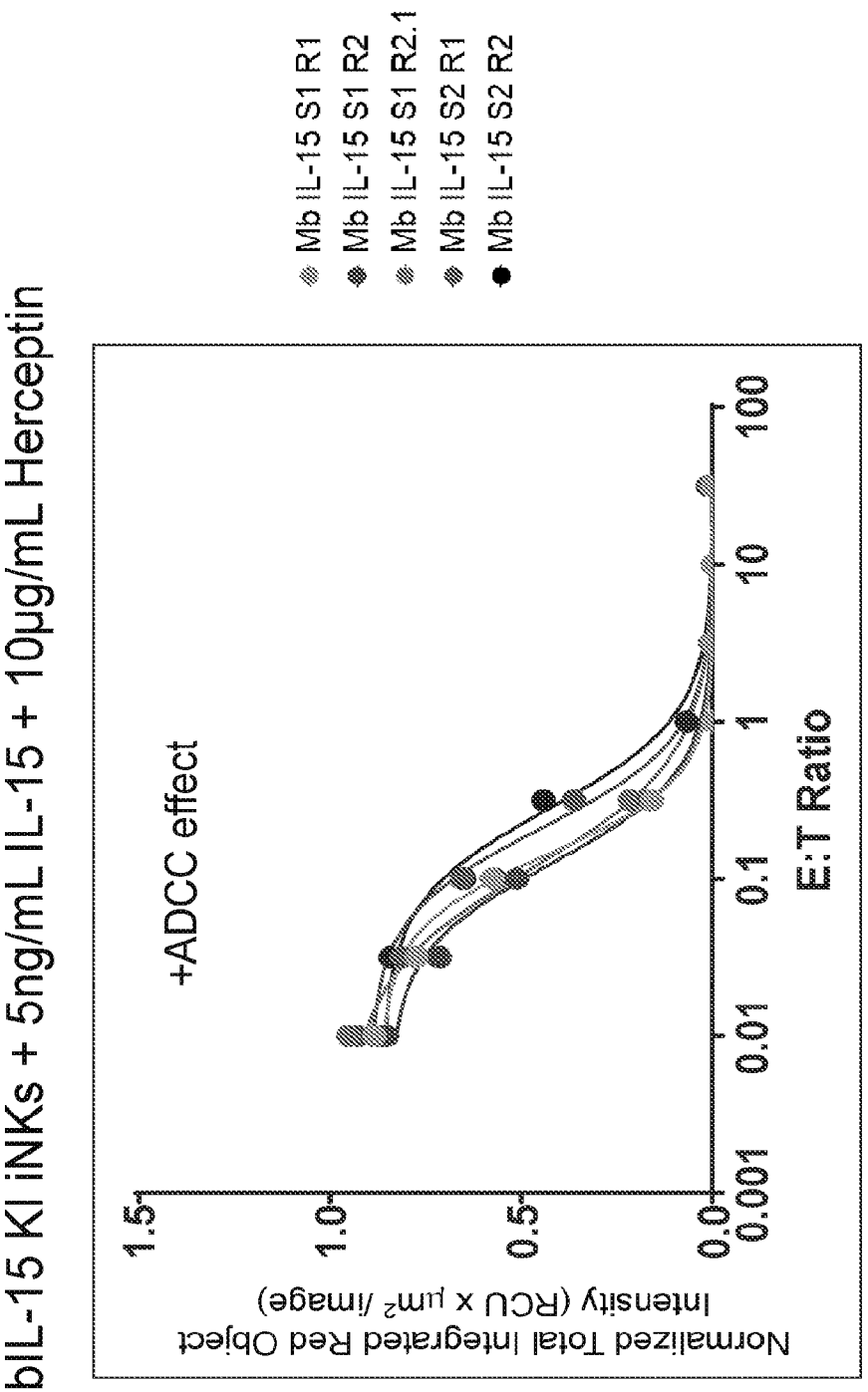


Fig. 31D

CA 03216765 2023-10-25

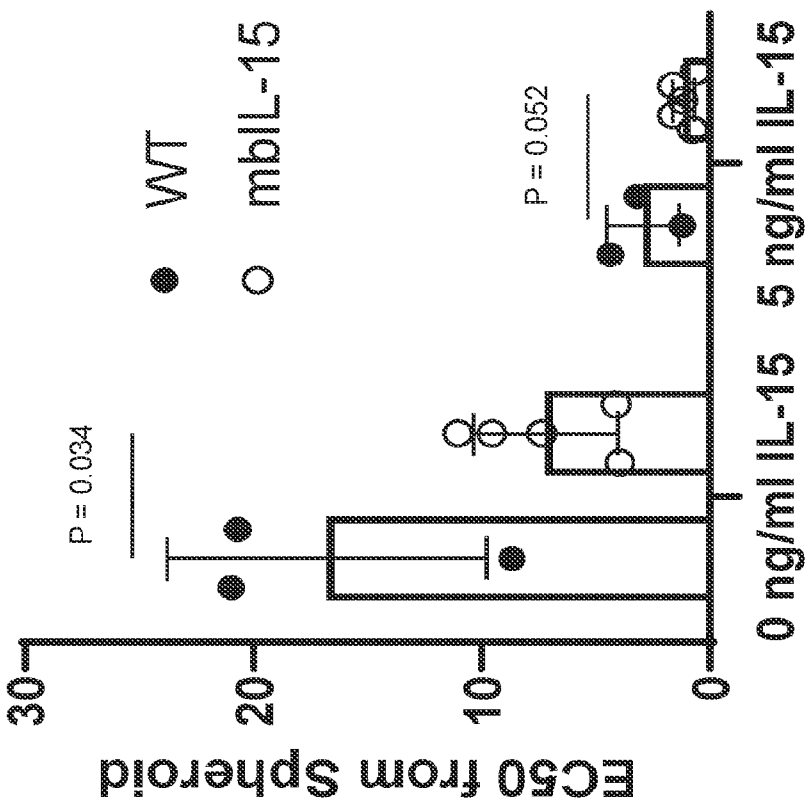

EC50 from Spheroid
30
20
10
0
WT
mbIL-15
P = 0.034
P = 0.052
0 ng/ml IL-15
5 ng/ml IL-15

Fig. 32

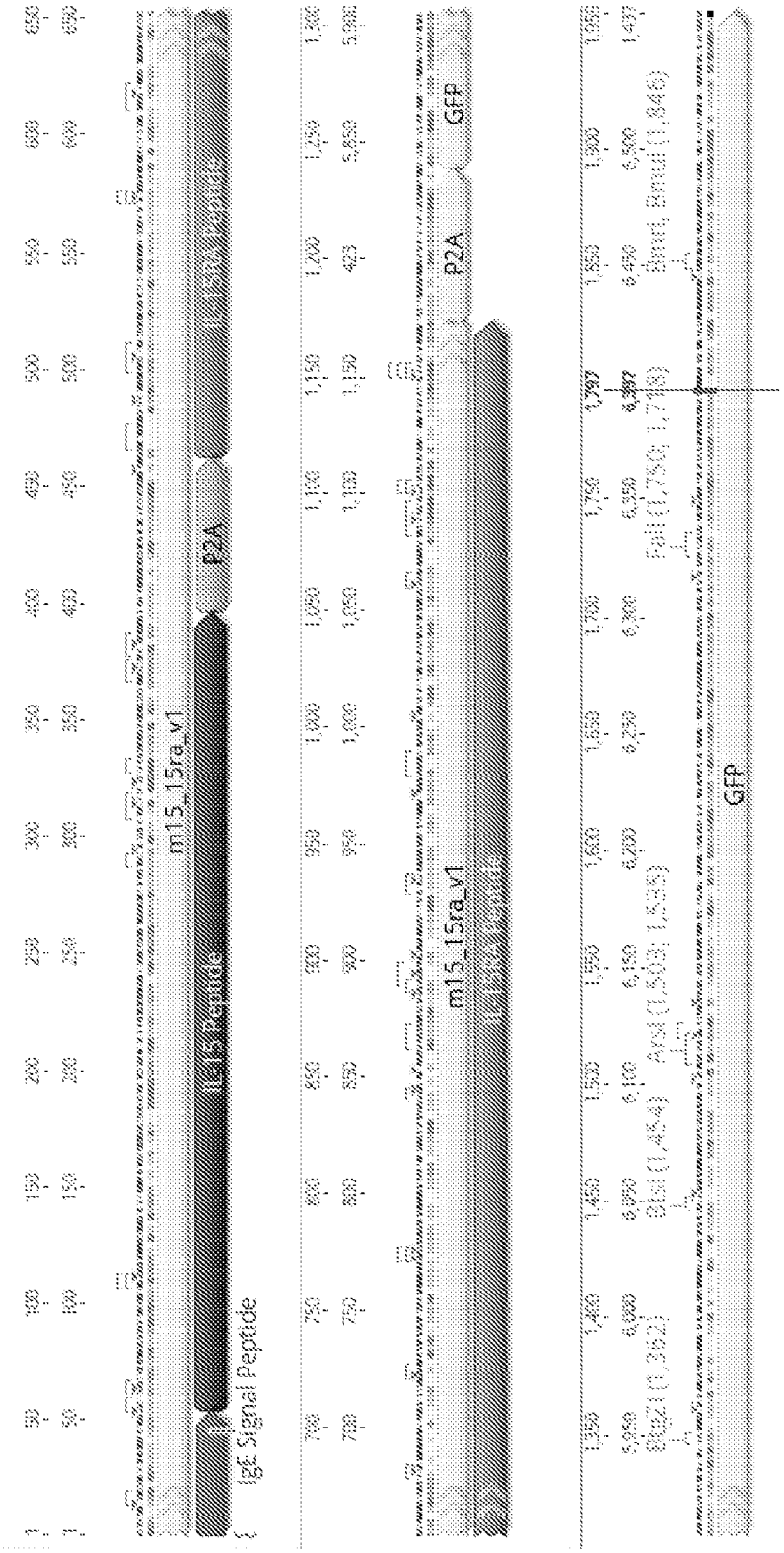

PLA1829
IgE Signal Peptide
m15_15ra_v1
P2A
m15_15ra_v1
P2A
GFP
GFP

Fig. 33A

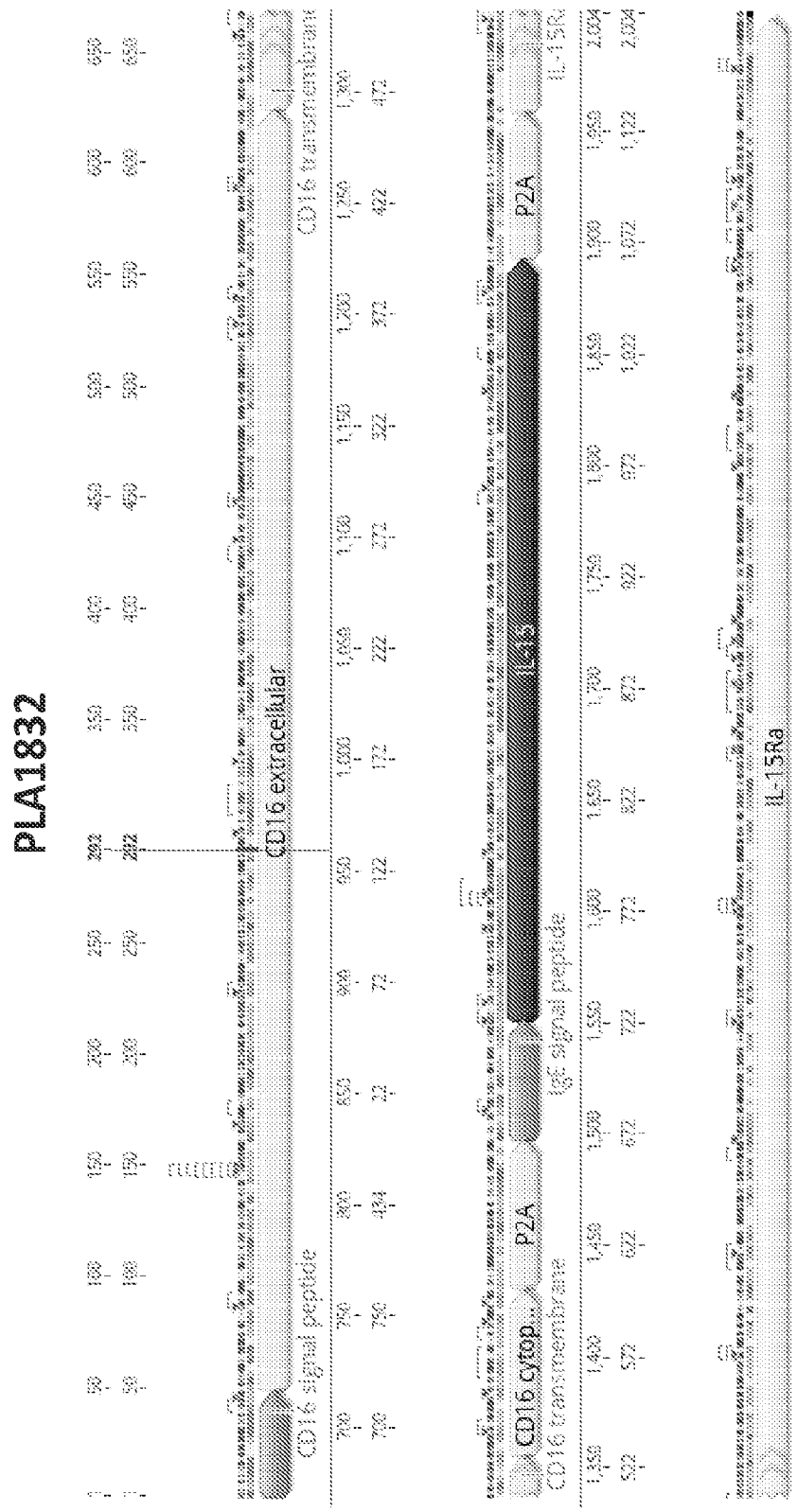

PLA1832
CD16 extracellular
CD16 signal peptide
CD16 transmembrane
CD16 cytop...
P2A
IL-15
IL-15Ra
IgE signal peptide
IL-15R...
P2A

Fig. 33B

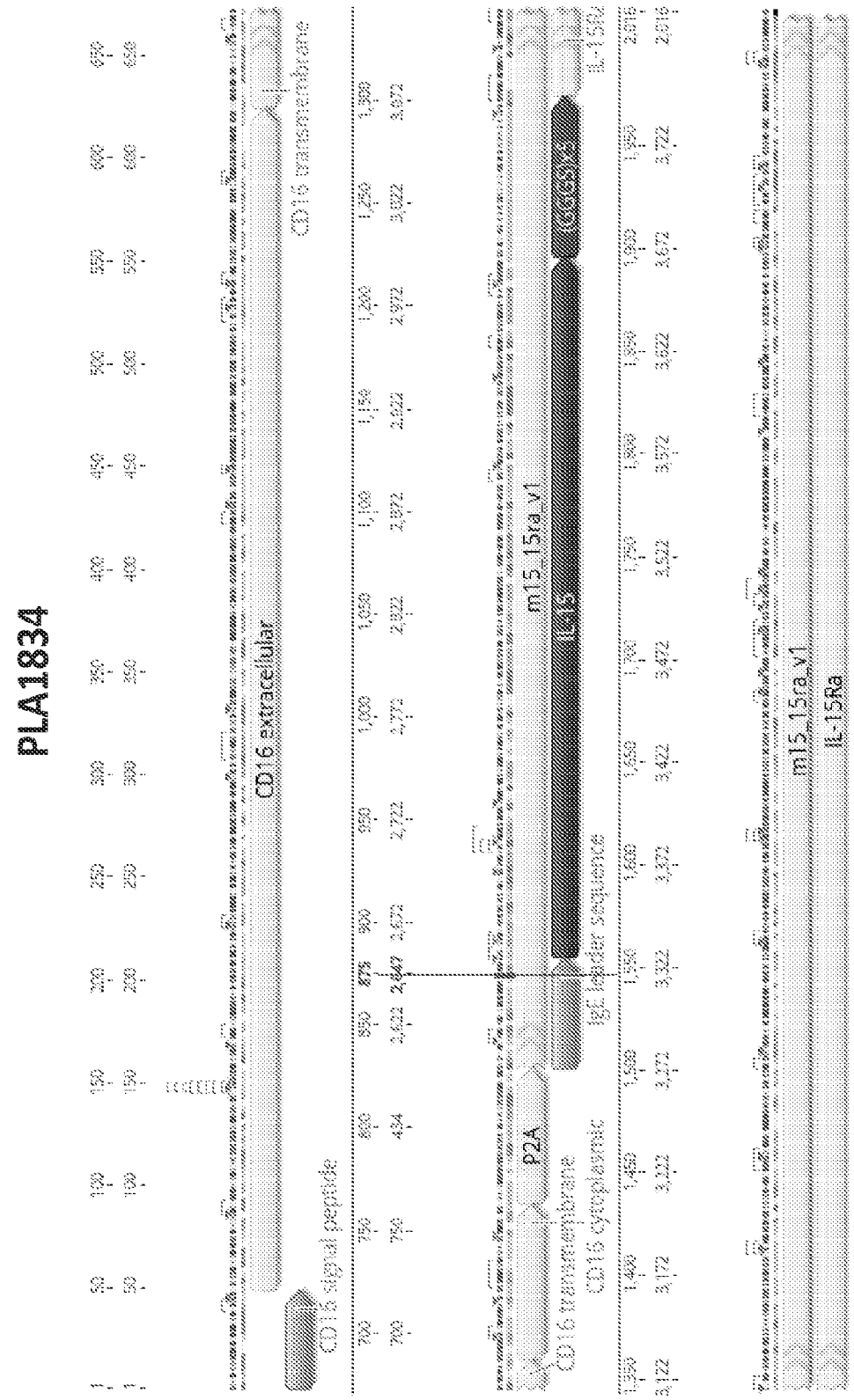

PLA1834
CD16 extracellular
CD16 transmembrane
CD16 signal peptide
CD16 transmembrane
P2A
CD16 cytoplasmic
IgE leader sequence
m15_15ra_v1
IL-15
sIL-15Rα
IL-15
m15_15ra_v1
IL-15Ra

Fig. 33C

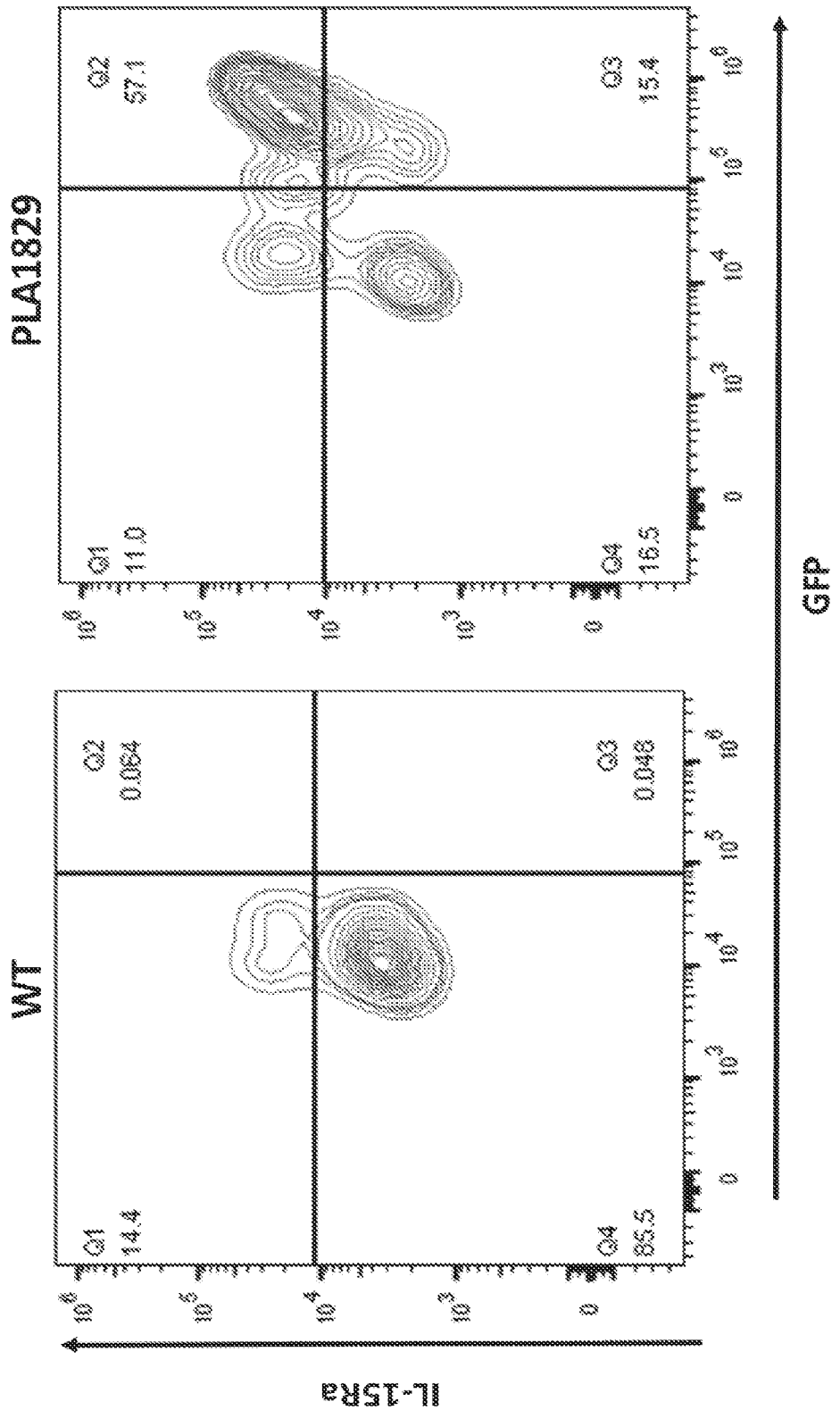

PLA1829
WT
Q1
11.0
Q2
57.1
Q3
15.4
Q4
16.5
Q1
14.4
Q2
0.084
Q3
0.048
Q4
83.5
IL-15Rα
GFP
0
10^3
10^4
10^5
10^6

Fig. 34A

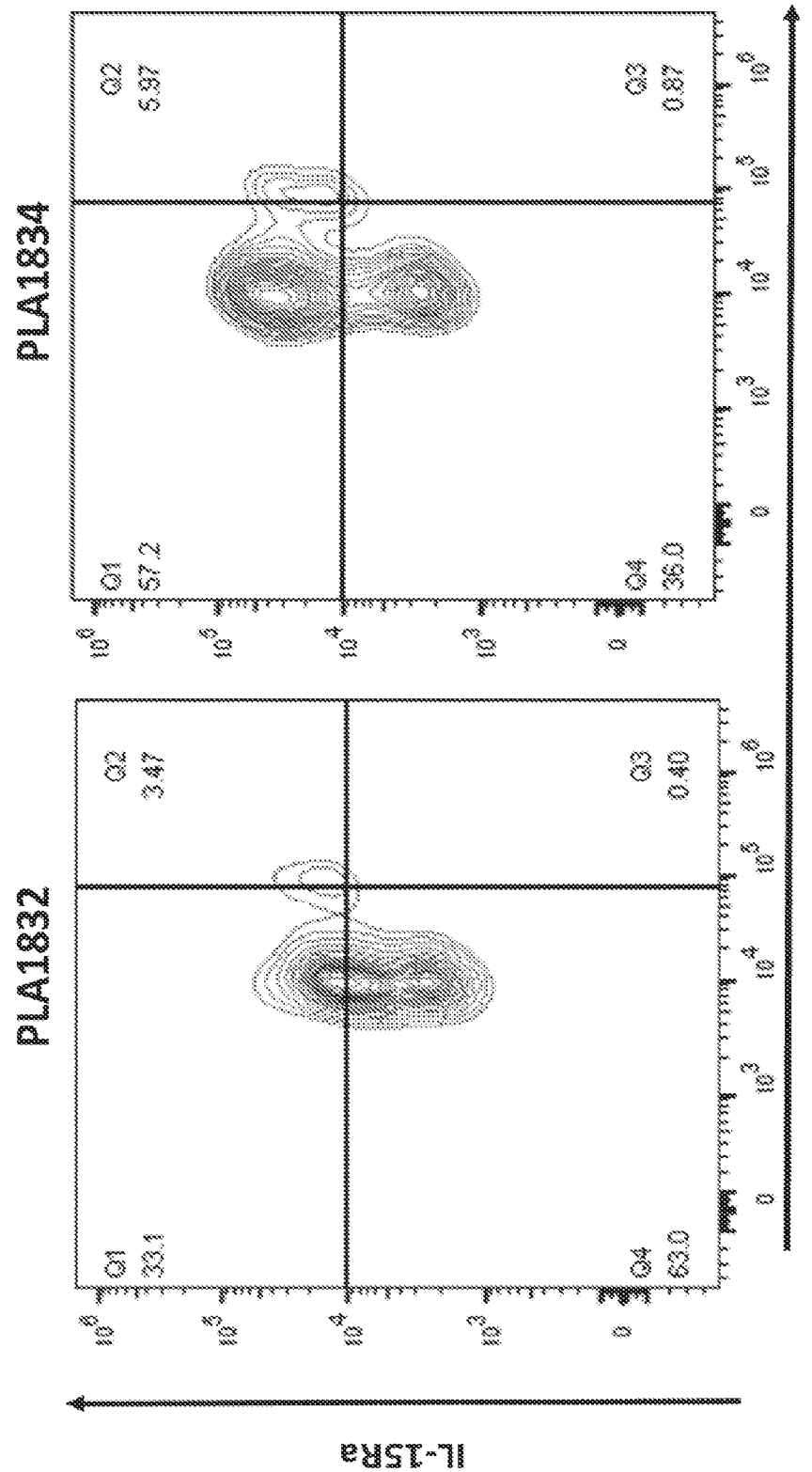

PLA1834
PLA1832
Q1
57.2
Q2
5.97
Q3
0.87
Q4
36.0
Q1
33.1
Q2
3.47
Q3
0.40
Q4
63.0
IL-15Ra
GFP

Fig. 34B

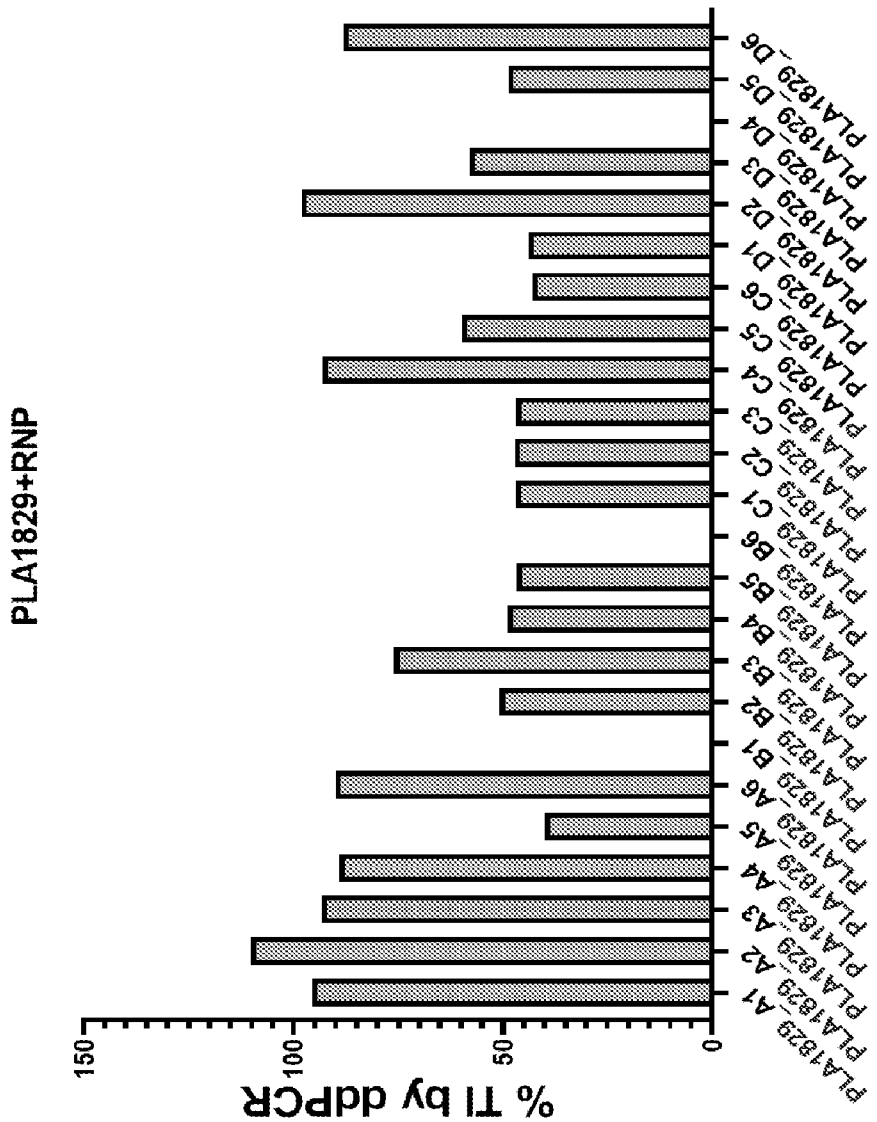

PLA1829+RNP
% TI by ddPCR
150
100
50
0
Clones
PLA1829_A1
PLA1829_A2
PLA1829_A3
PLA1829_A4
PLA1829_A5
PLA1829_A6
PLA1829_B1
PLA1829_B2
PLA1829_B3
PLA1829_B4
PLA1829_B5
PLA1829_B6
PLA1829_C1
PLA1829_C2
PLA1829_C3
PLA1829_C4
PLA1829_C5
PLA1829_C6
PLA1829_D1
PLA1829_D2
PLA1829_D3
PLA1829_D4
PLA1829_D5
PLA1829_D6

Fig. 35A

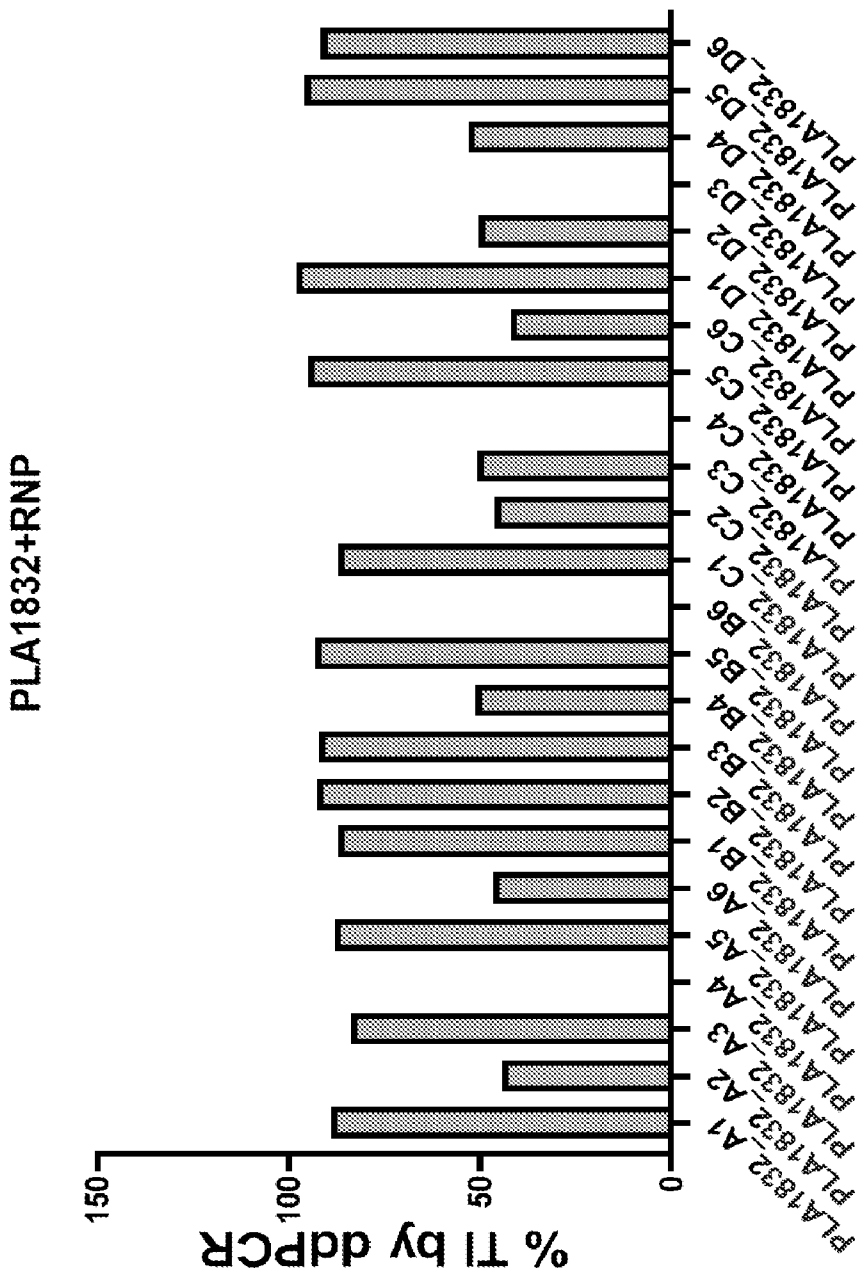

PLA1832+RNP
% TI by ddPCR
150
100
50
0
PLA1832_A7
PLA1832_A2
PLA1832_A3
PLA1832_A4
PLA1832_A5
PLA1832_A6
PLA1832_B1
PLA1832_B2
PLA1832_B3
PLA1832_B4
PLA1832_B5
PLA1832_B6
PLA1832_C1
PLA1832_C2
PLA1832_C3
PLA1832_C4
PLA1832_C5
PLA1832_C6
PLA1832_D1
PLA1832_D2
PLA1832_D3
PLA1832_D4
PLA1832_D5
PLA1832_D6
Clones

Fig. 35B

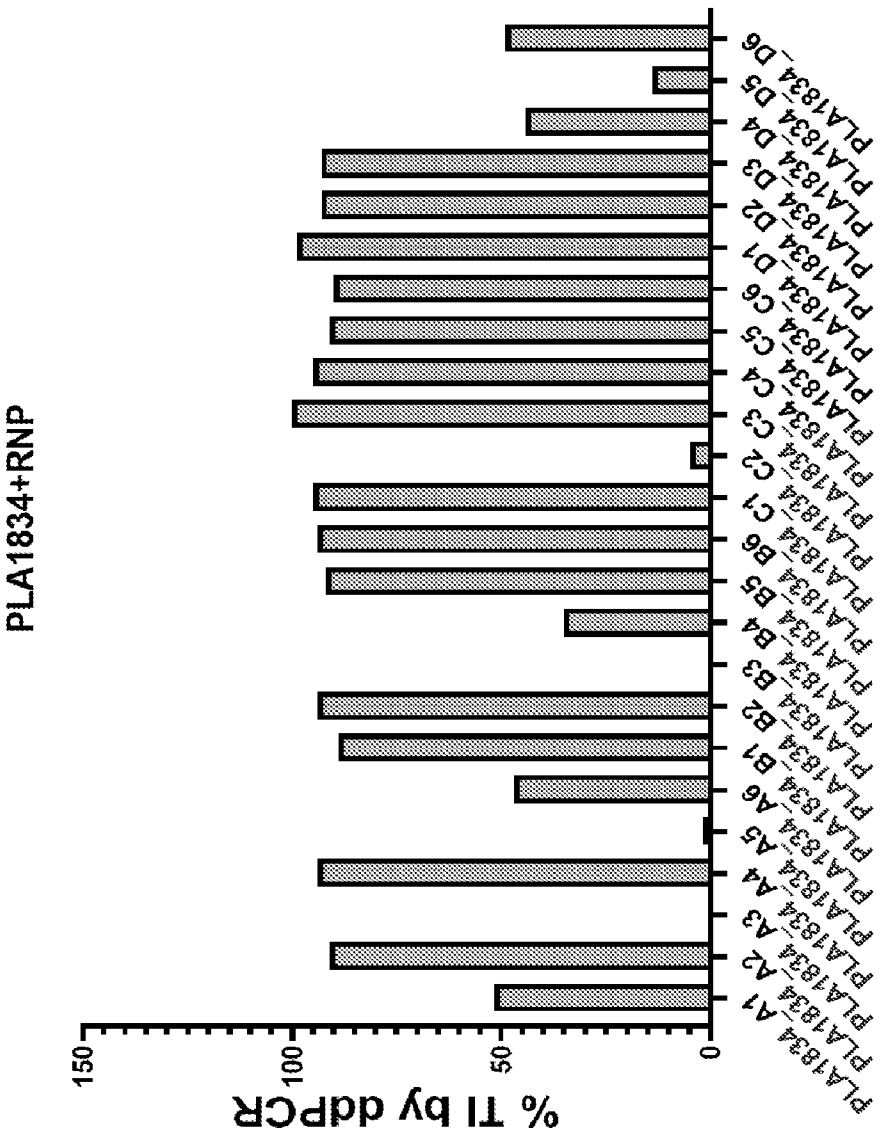

PLA1834+RNP
% TI by ddPCR
150
100
50
0
Clones
PLA1834_A1
PLA1834_A2
PLA1834_A3
PLA1834_A4
PLA1834_A5
PLA1834_A6
PLA1834_B1
PLA1834_B2
PLA1834_B3
PLA1834_B4
PLA1834_B5
PLA1834_B6
PLA1834_C1
PLA1834_C2
PLA1834_C3
PLA1834_C4
PLA1834_C5
PLA1834_C6
PLA1834_D1
PLA1834_D2
PLA1834_D3
PLA1834_D4
PLA1834_D5
PLA1834_D6

Fig. 35C

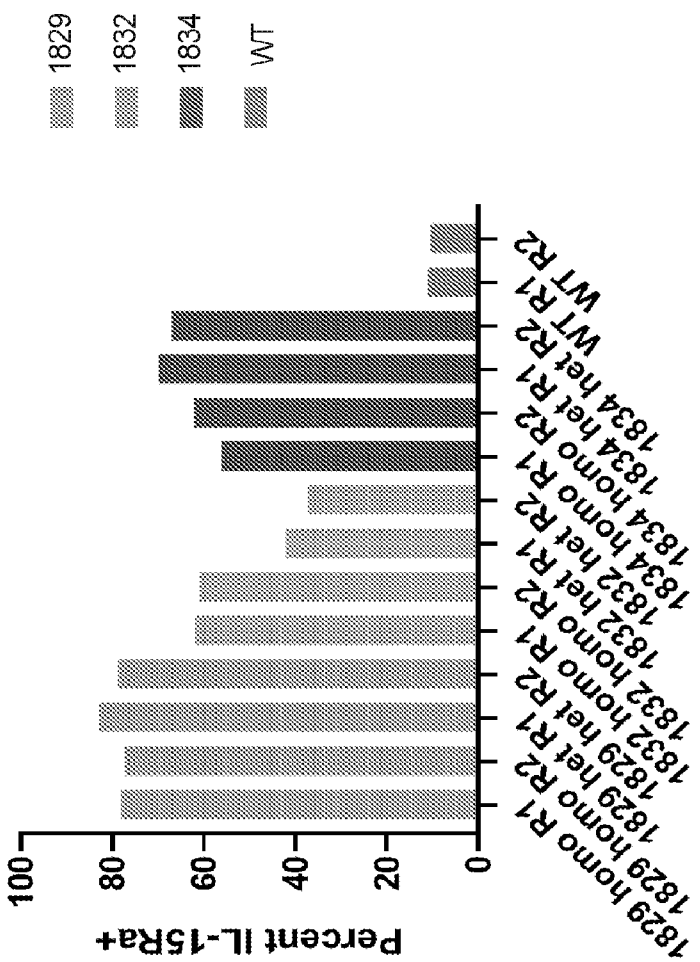

1829
1832
1834
WT
Percent IL-15Rα+
100
80
60
40
20
0
1829 homo R1
1829 homo R2
1829 het R1
1829 het R2
1832 homo R1
1832 homo R2
1832 het R1
1832 het R2
1834 homo R1
1834 homo R2
1834 het R1
1834 het R2
WT R1
WT R2

Fig. 36A

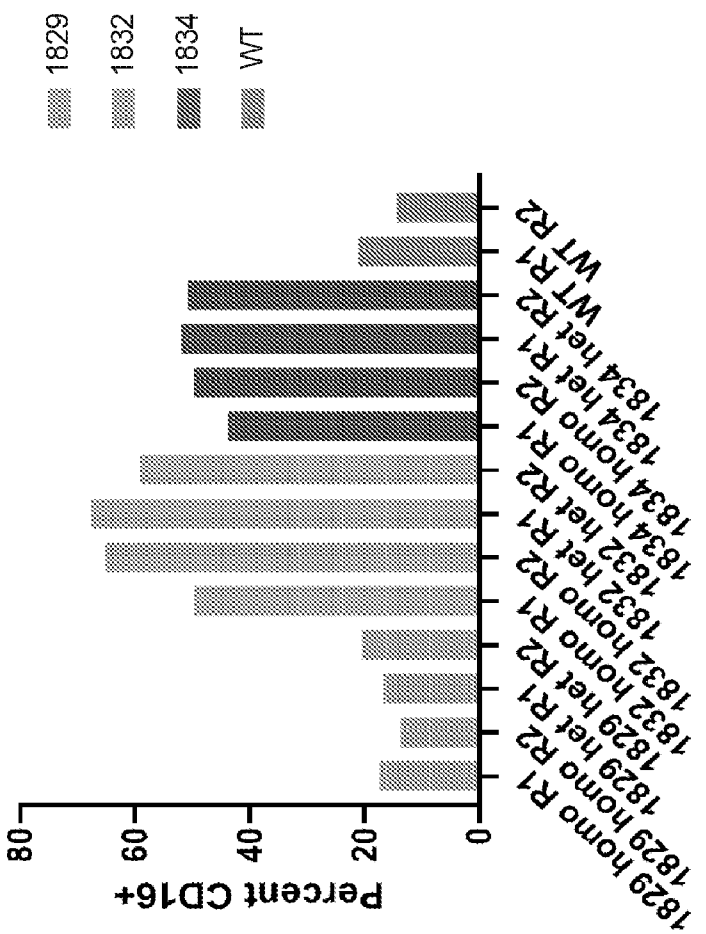

1829
1832
1834
WT
Percent CD16+
0
20
40
60
80
1829 homo R1
1829 homo R2
1829 het R1
1829 het R2
1832 homo R1
1832 homo R2
1832 het R1
1832 het R2
1834 homo R1
1834 homo R2
1834 het R1
1834 het R2
WT R1
WT R2

Fig. 36B

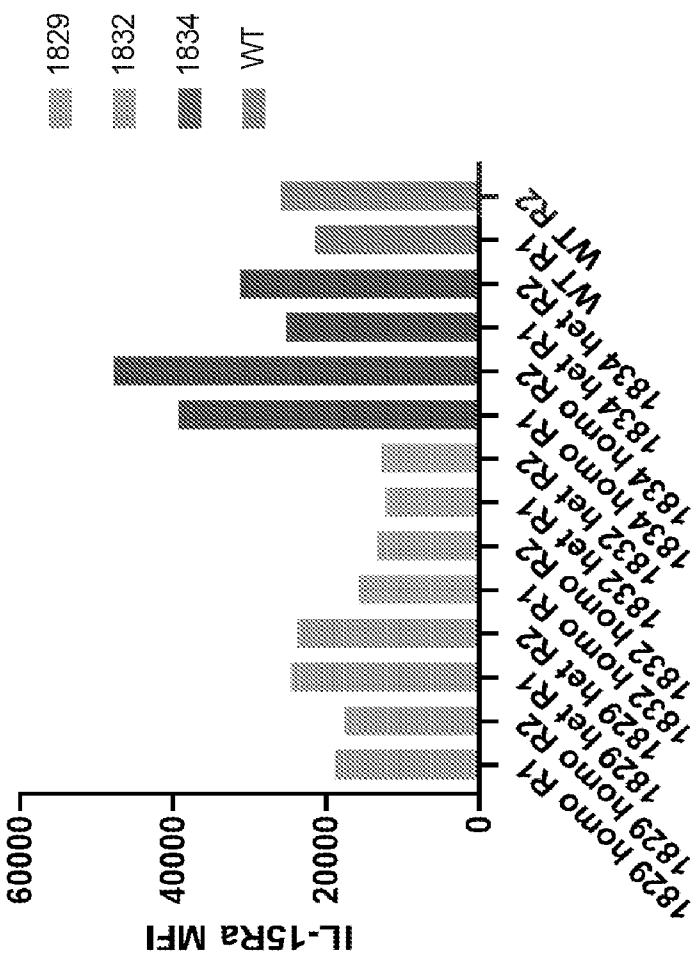

1829
1832
1834
WT
IL-15Rα MFI
60000
40000
20000
0
1829 homo R1
1829 homo R2
1829 het R1
1829 het R2
1832 homo R1
1832 homo R2
1832 het R1
1832 het R2
1834 homo R1
1834 homo R2
1834 het R1
1834 het R2
WT R1
WT R2

Fig. 36C

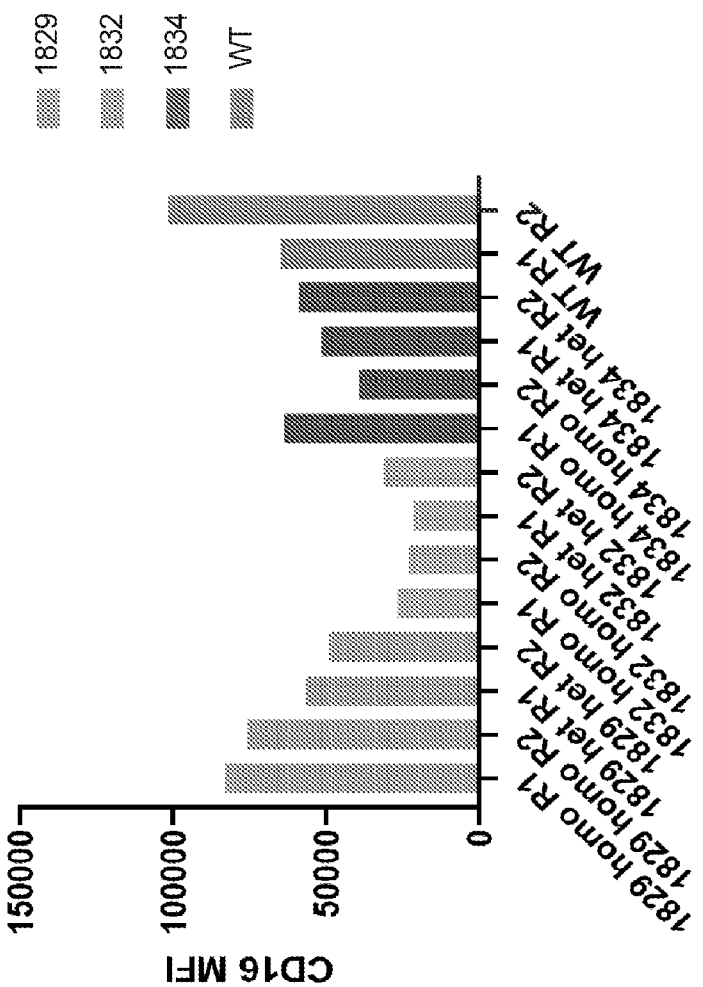

1829
1832
1834
WT
CD16 MFI
150000
100000
50000
0
1829 homo R1
1829 homo R2
1829 het R1
1829 het R2
1832 homo R1
1832 homo R2
1832 het R1
1832 het R2
1834 homo R1
1834 homo R2
1834 het R1
1834 het R2
WT R1
WT R2

Fig. 36D

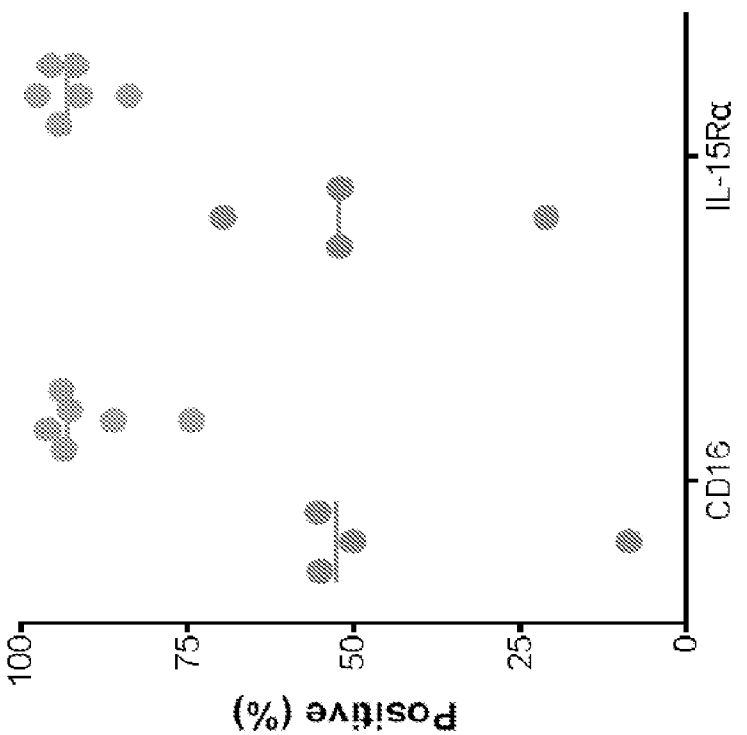


Fig. 36E

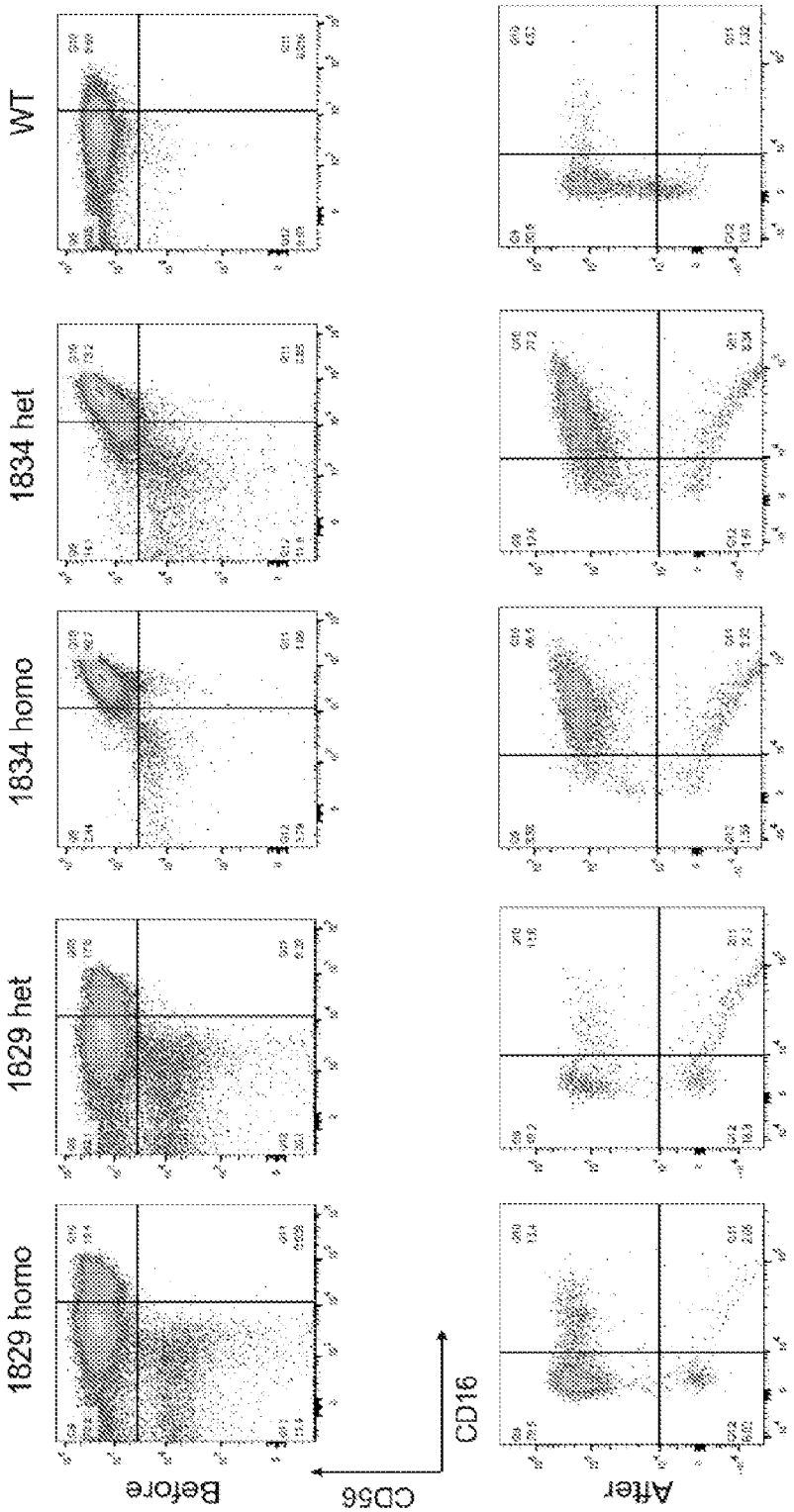

WT
1834 het
1834 homo
1829 het
1829 homo
Before
After
CD56
CD16

Fig. 36F

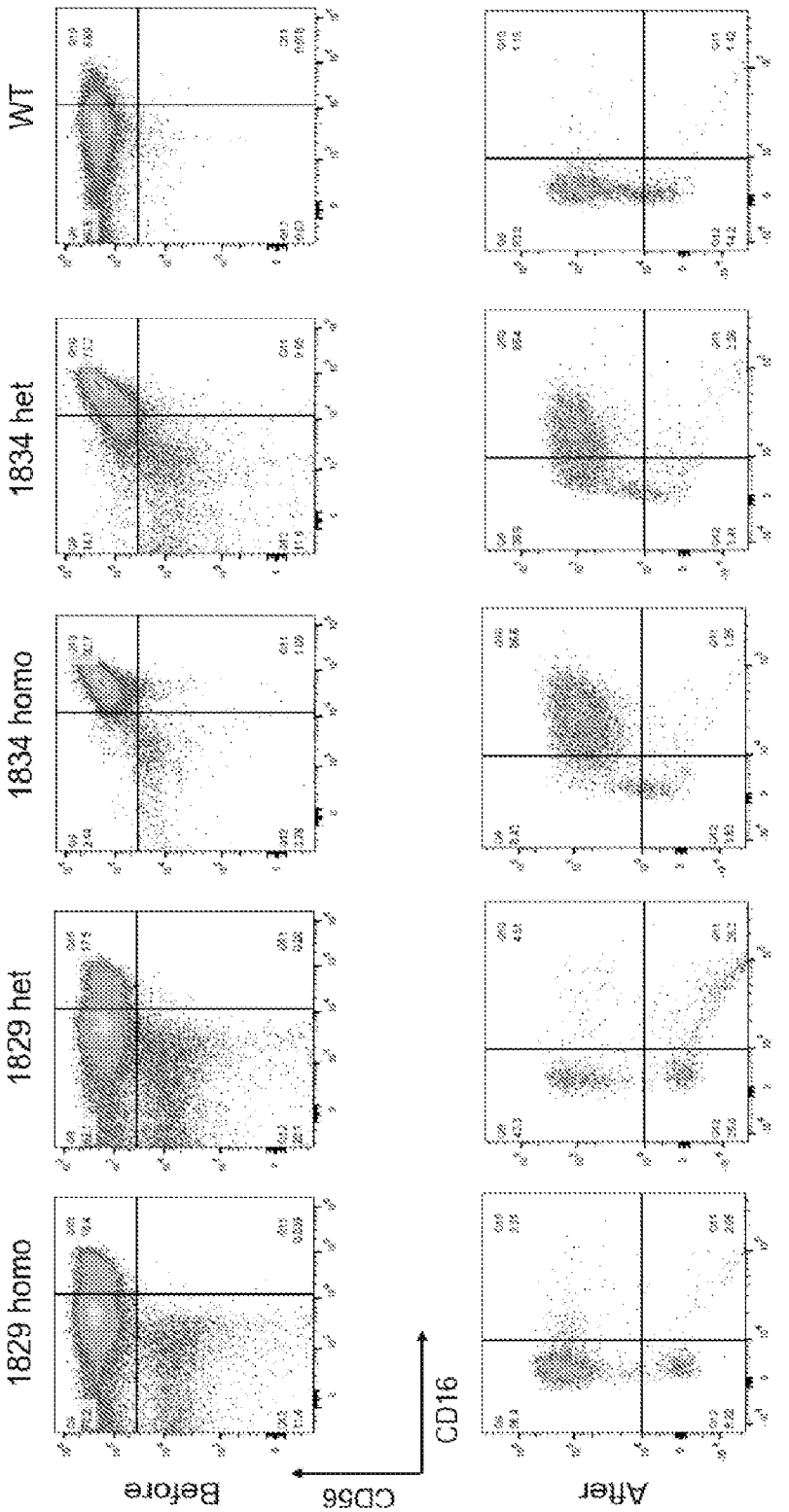

WT
1834 het
1834 homo
1829 het
1829 homo
Before
After
CD56
CD16

Fig. 36G

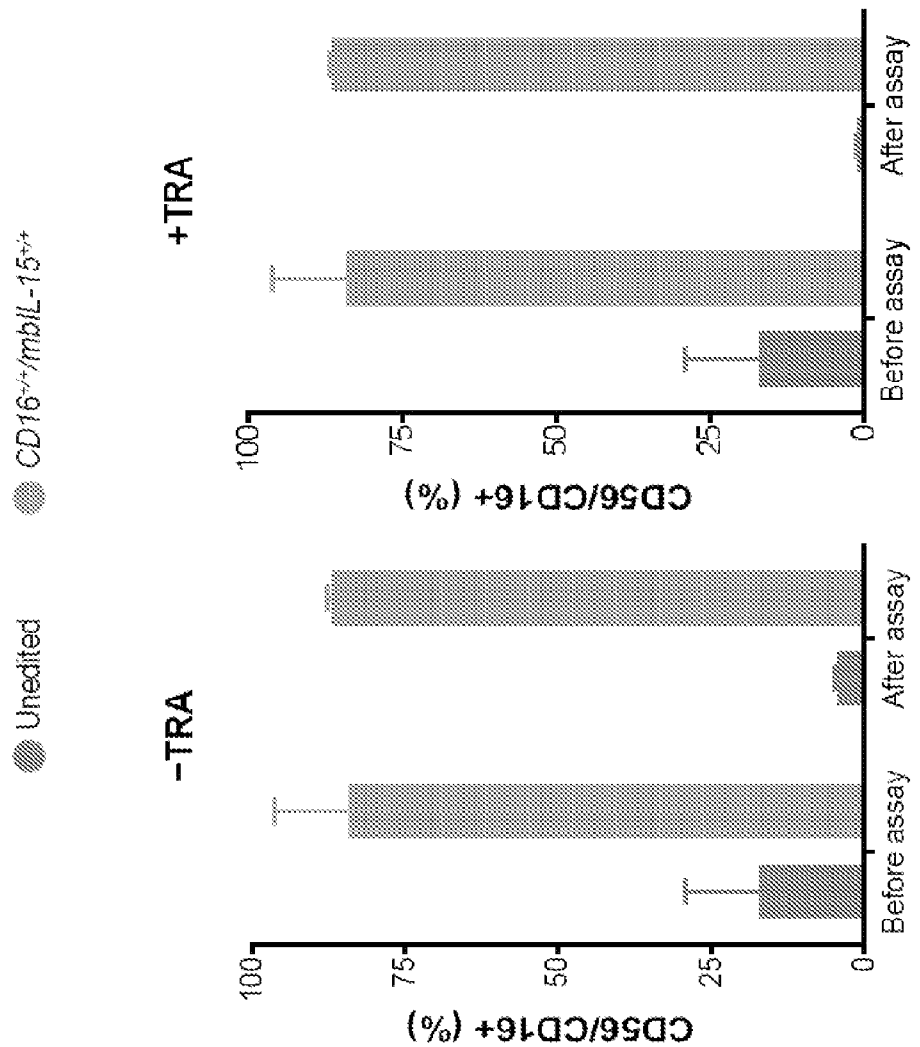

Unedited
CD16-/-/mbIL-15+/+
-TRA
+TRA
CD56/CD16+ (%)
100
75
50
25
0
Before assay
After assay

Fig. 36H

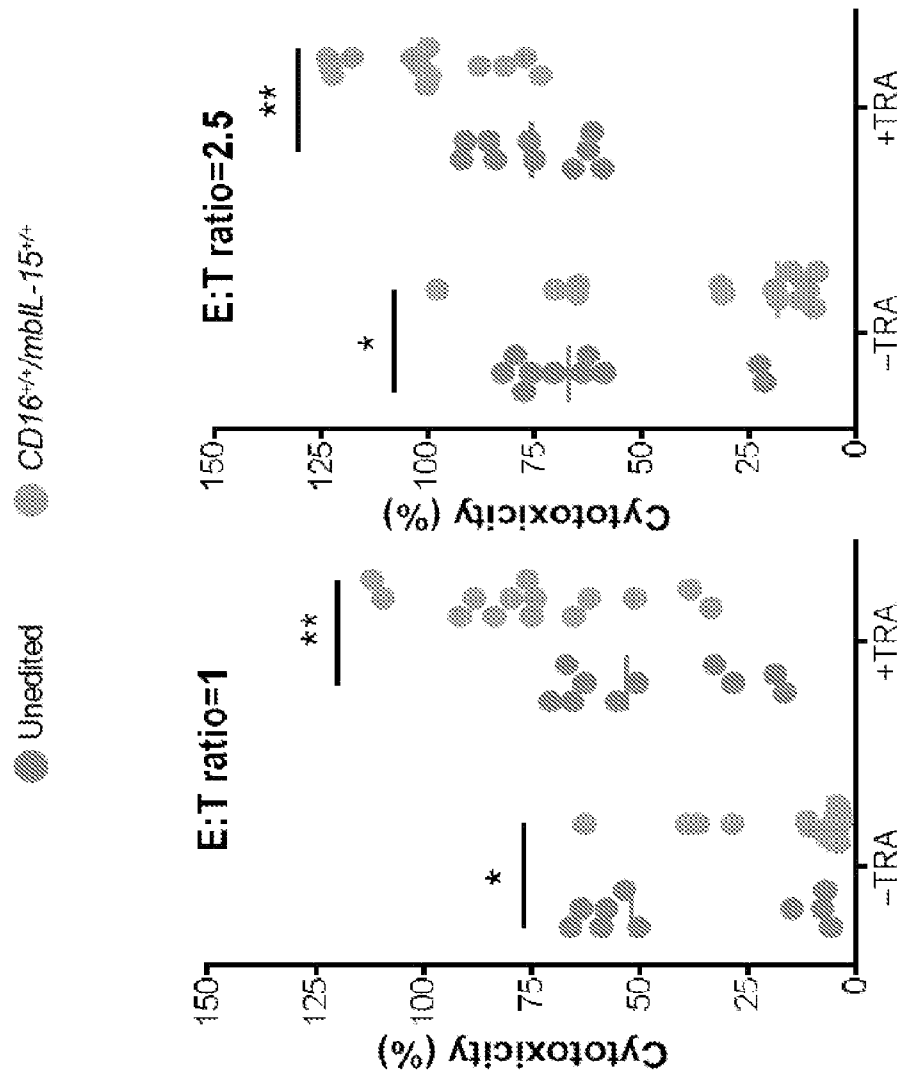

Unedited
CD16+/+/mbIL-15+/+
E:T ratio=1
E:T ratio=2.5
Cytotoxicity (%)
150
125
100
75
50
25
0
*
**
*
**
-TRA
+TRA
-TRA
+TRA

Fig. 36I

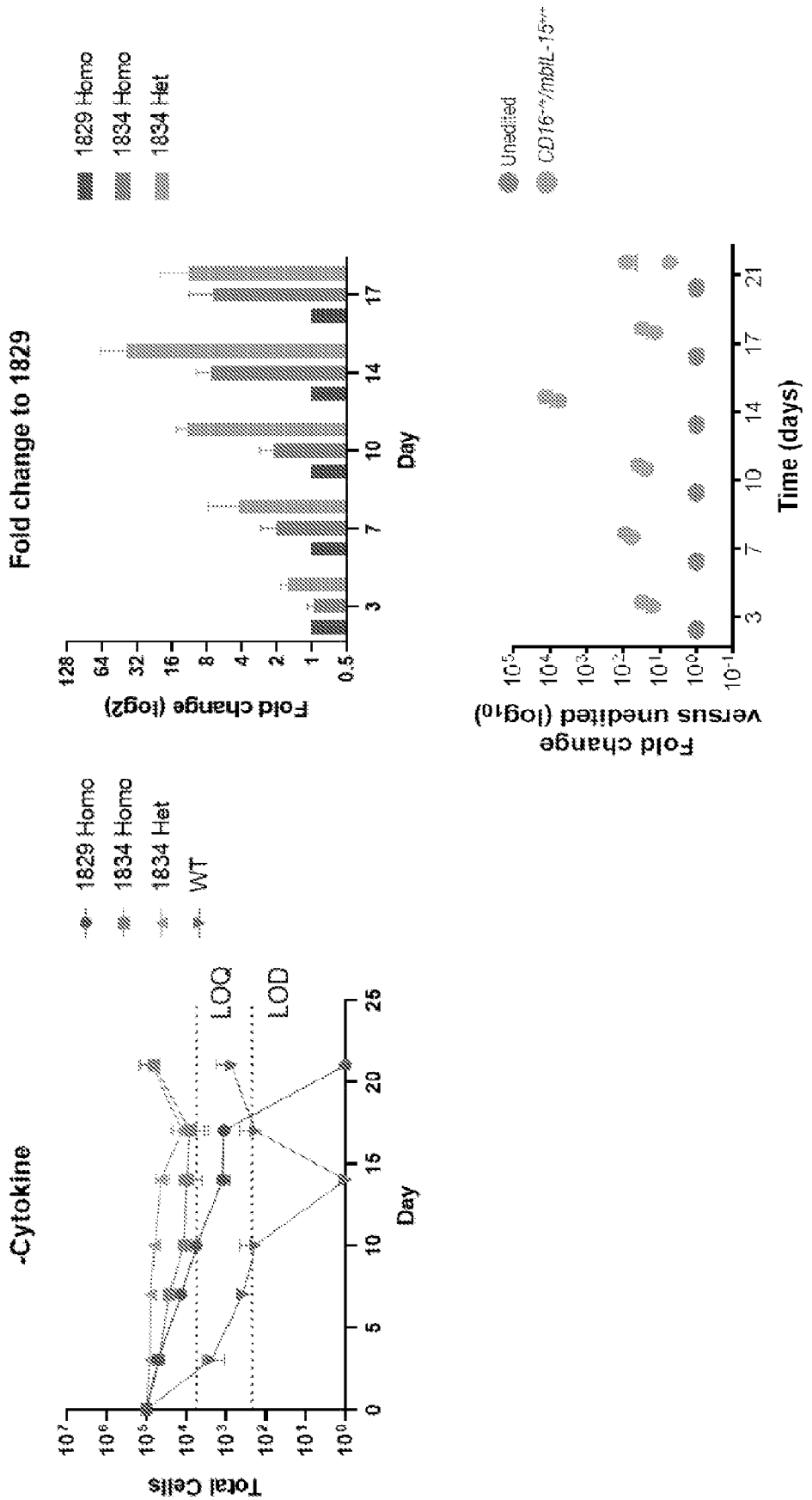

-Cytokine
Total Cells
Day
LOQ
LOD
1829 Homo
1834 Homo
1834 Het
WT
Fold change to 1829
Fold change (log2)
Day
1829 Homo
1834 Homo
1834 Het
Fold change versus unedited (log10)
Time (days)
Unedited
CD16-/mbIL-15

Fig. 36J

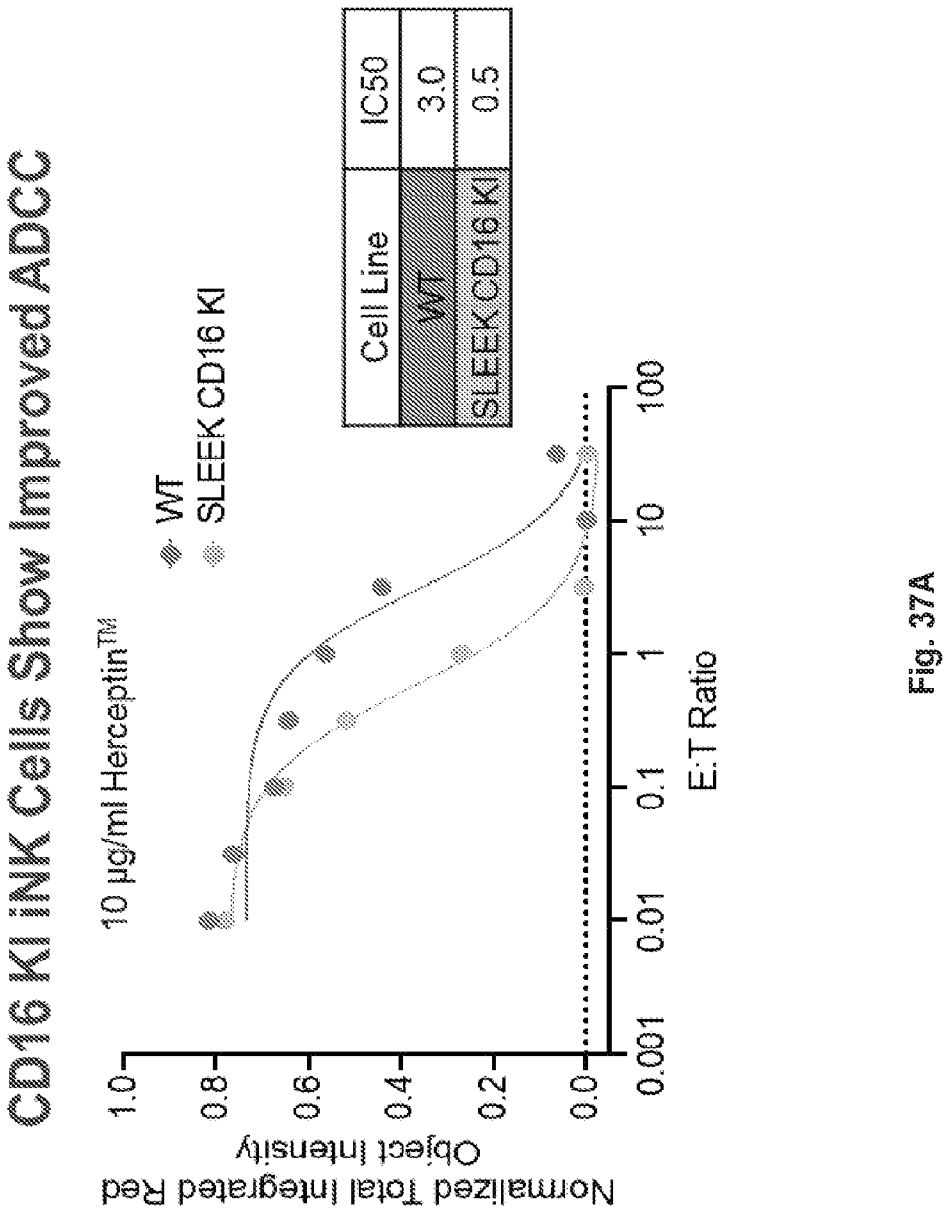

CD16 KI iNK Cells Show Improved ADCC
10 µg/ml Herceptin™
WT
SLEEK CD16 KI
Cell Line
IC50
WT
3.0
SLEEK CD16 KI
0.5
Normalized Total Integrated Red Object Intensity
1.0
0.8
0.6
0.4
0.2
0.0
0.001
0.01
0.1
1
10
100
E:T Ratio

Fig. 37A

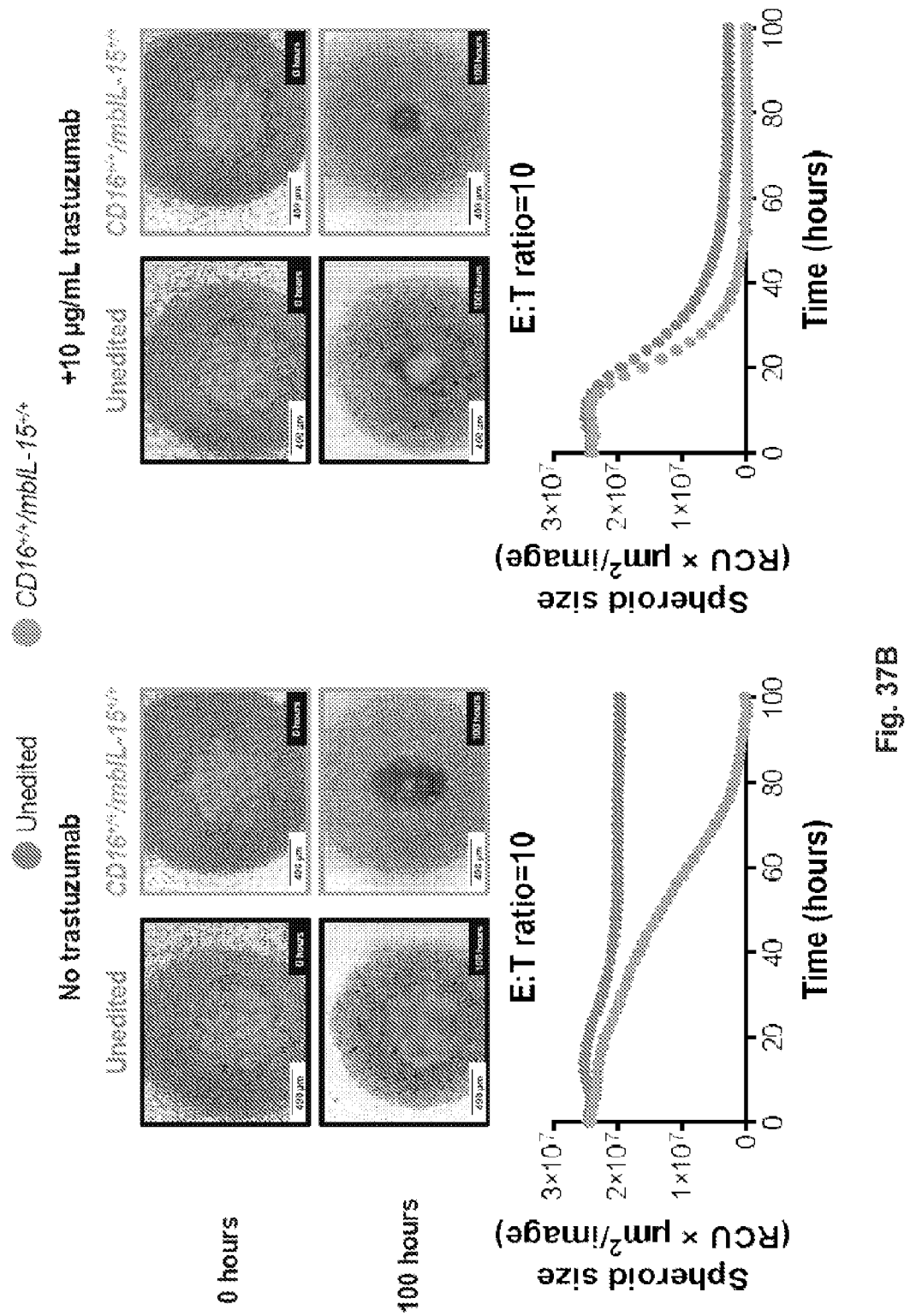

Unedited
CD16+/+/mbIL-15+/+
No trastuzumab
+10 µg/mL trastuzumab
Unedited
CD16-/-/mbIL-15-/-
0 hours
100 hours
E:T ratio=10
Spheroid size
(RCU × µm²/image)
Time (hours)
3×10⁷
2×10⁷
1×10⁷
0
0
20
40
60
80
100

Fig. 37B

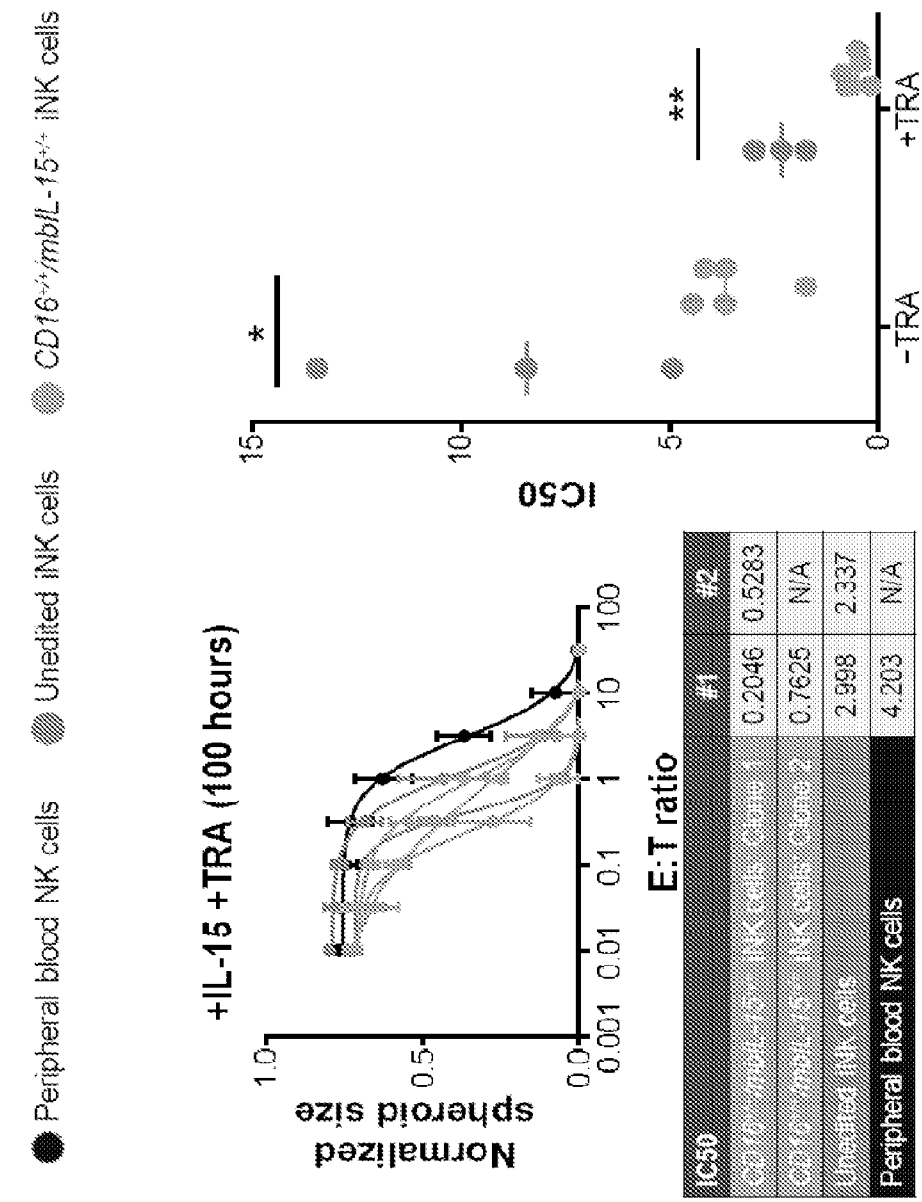

Peripheral blood NK cells
Unedited iNK cells
CD16v1/mbIL-15v1 iNK cells
+IL-15 +TRA (100 hours)
Normalized spheroid size
1.0
0.5
0.0
0.001 0.01 0.1 1 10 100
E:T ratio
IC50
15
10
5
0
*
**
-TRA
+TRA
(E:T)
#1
#2
CD16v1/mbIL-15v1 iNK cells Clone 1
0.2046
0.5283
CD16v1/mbIL-15v1 iNK cells Clone 2
0.7625
N/A
Unedited iNK cells
2.998
2.337
Peripheral blood NK cells
4.203
N/A

Fig. 37C

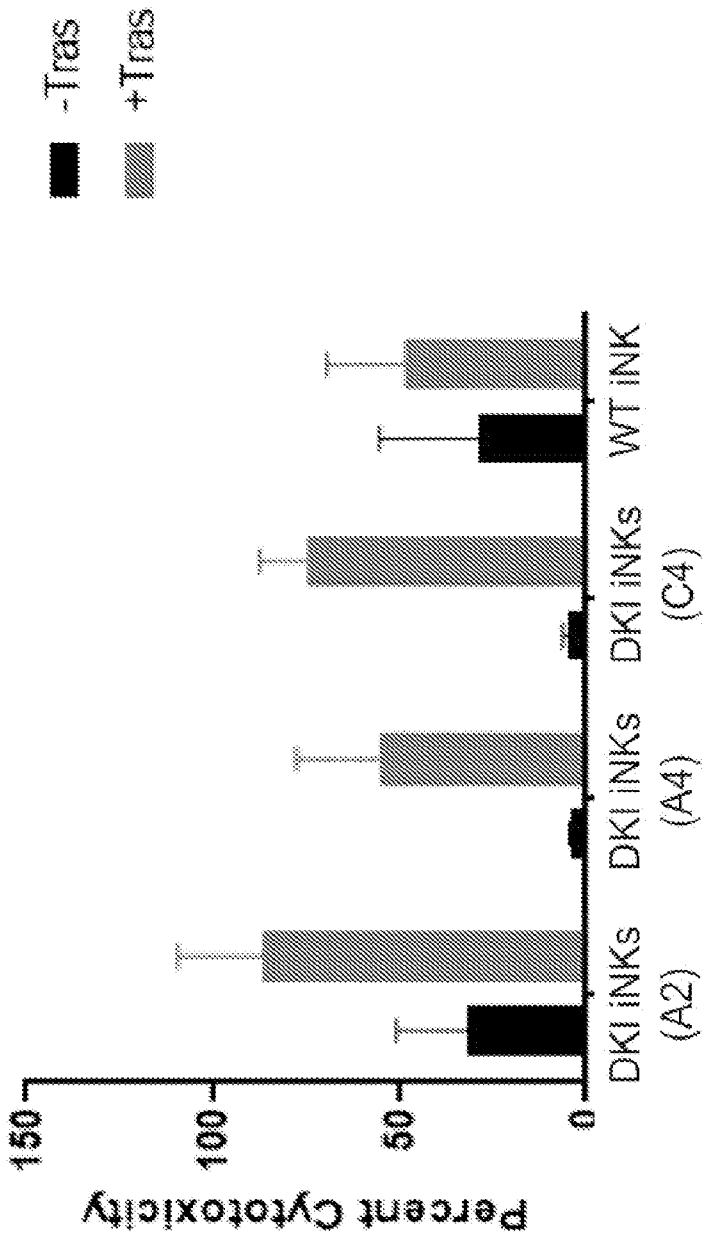

LDH cytotoxicity assay
-Tras
+Tras
Percent Cytotoxicity
150
100
50
0
DKI iNKs (A2)
DKI iNKs (A4)
DKI iNKs (C4)
WT iNK

Fig. 38A

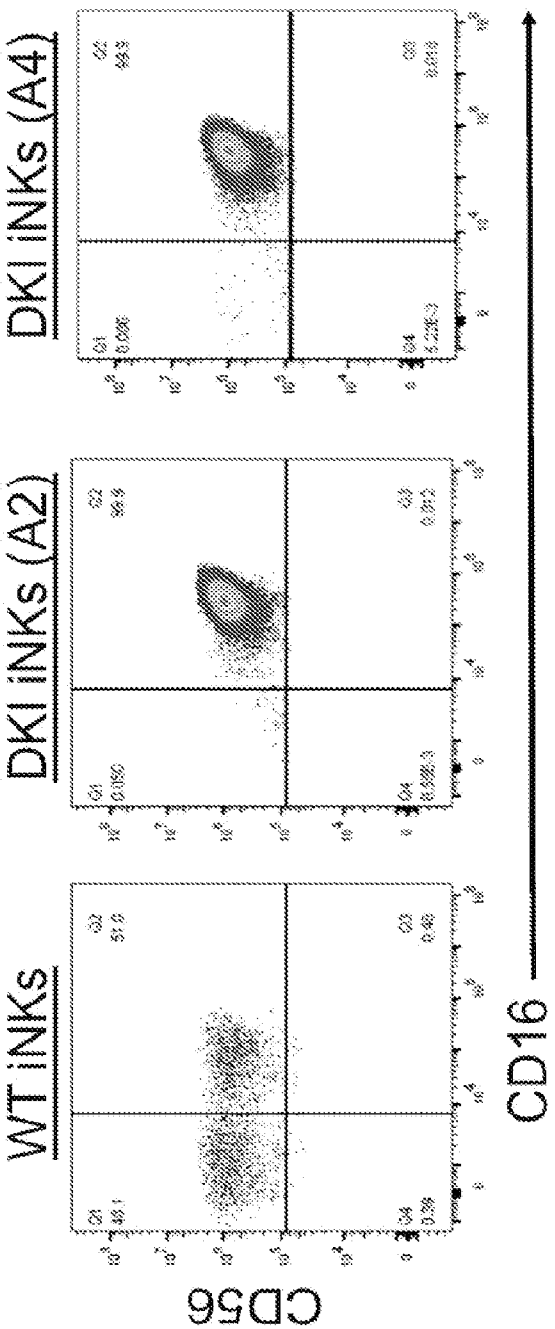


Fig. 38B

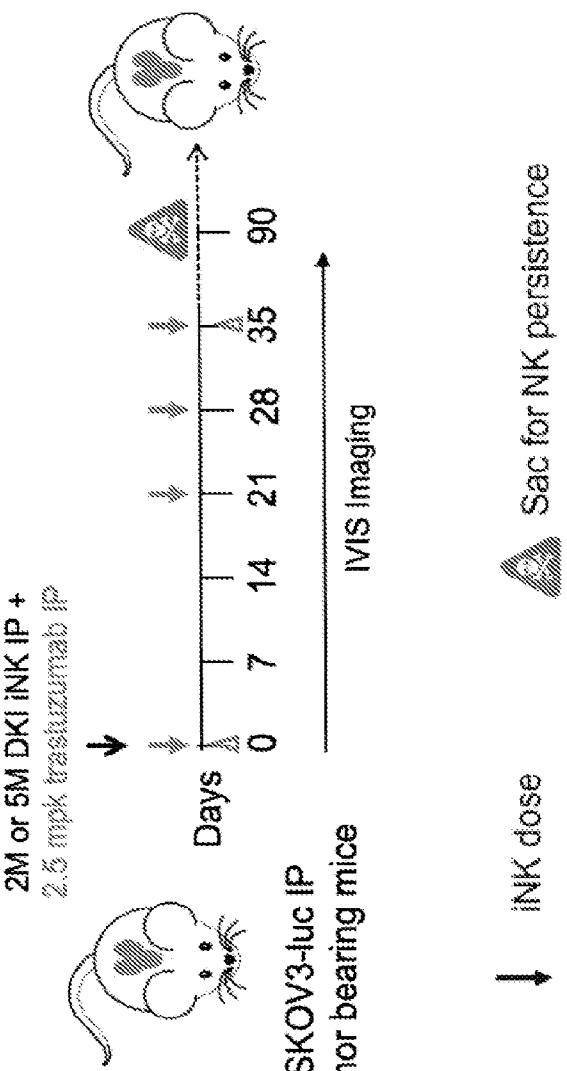

2M or 5M DKI iNK IP +
2.5 mpk trastuzumab IP
SKOV3-luc IP
tumor bearing mice
Days
0
7
14
21
28
35
90
IVIS Imaging
iNK dose
Trastuzumab Dose
Sac for NK persistence

Fig. 38C

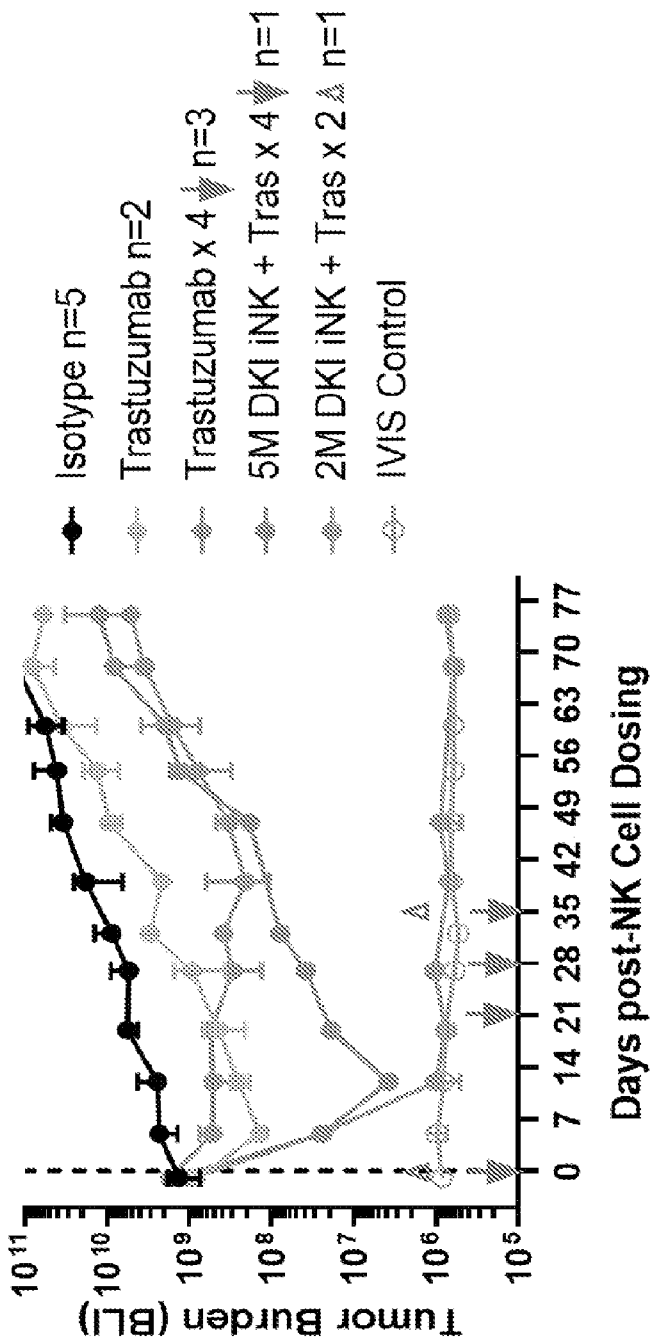

Isotype n=5
Trastuzumab n=2
Trastuzumab x 4 n=3
5M DKI iNK + Tras x 4 n=1
2M DKI iNK + Tras x 2 n=1
IVIS Control
Tumor Burden (BLI)
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
0 7 14 21 28 35 42 49 56 63 70 77
Days post-NK Cell Dosing

Fig. 38D

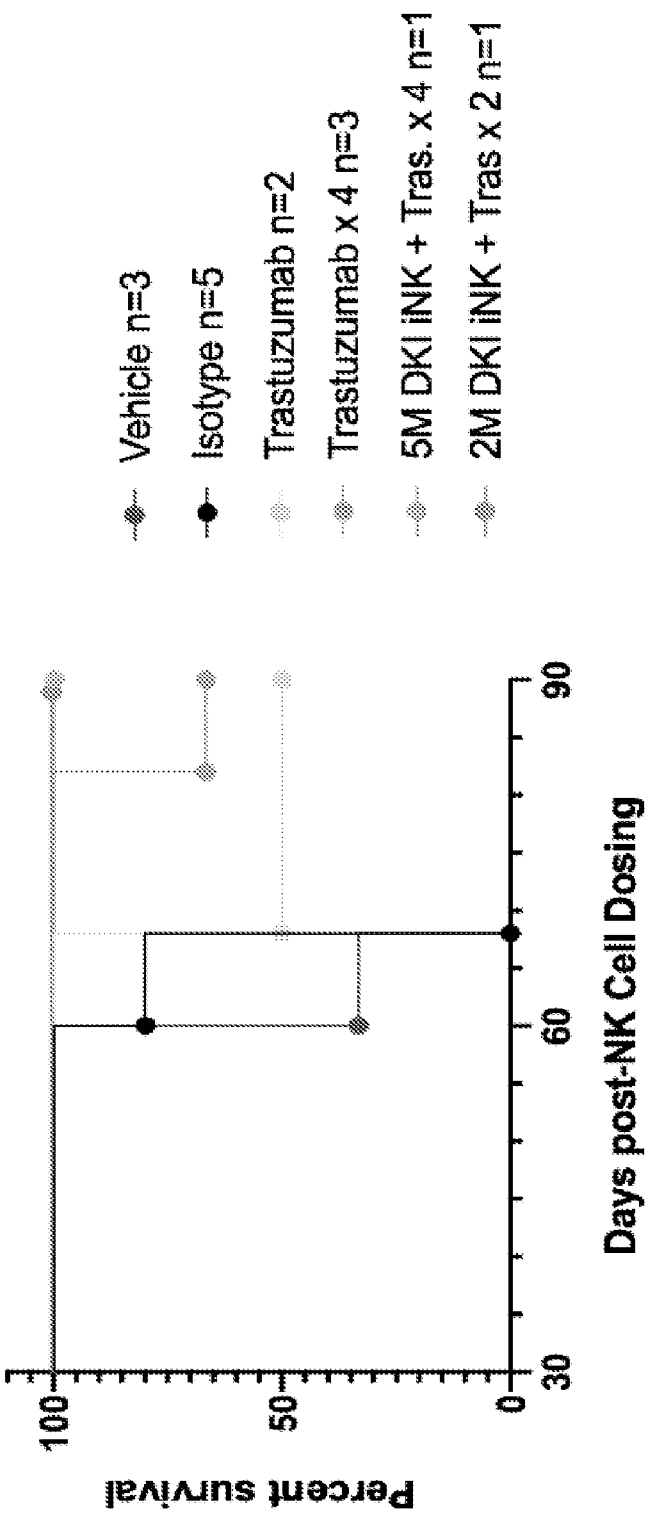

Vehicle n=3
Isotype n=5
Trastuzumab n=2
Trastuzumab x 4 n=3
5M DKI iNK + Tras. x 4 n=1
2M DKI iNK + Tras x 2 n=1
Percent survival
100
50
0
30
60
90
Days post-NK Cell Dosing

Fig. 38E

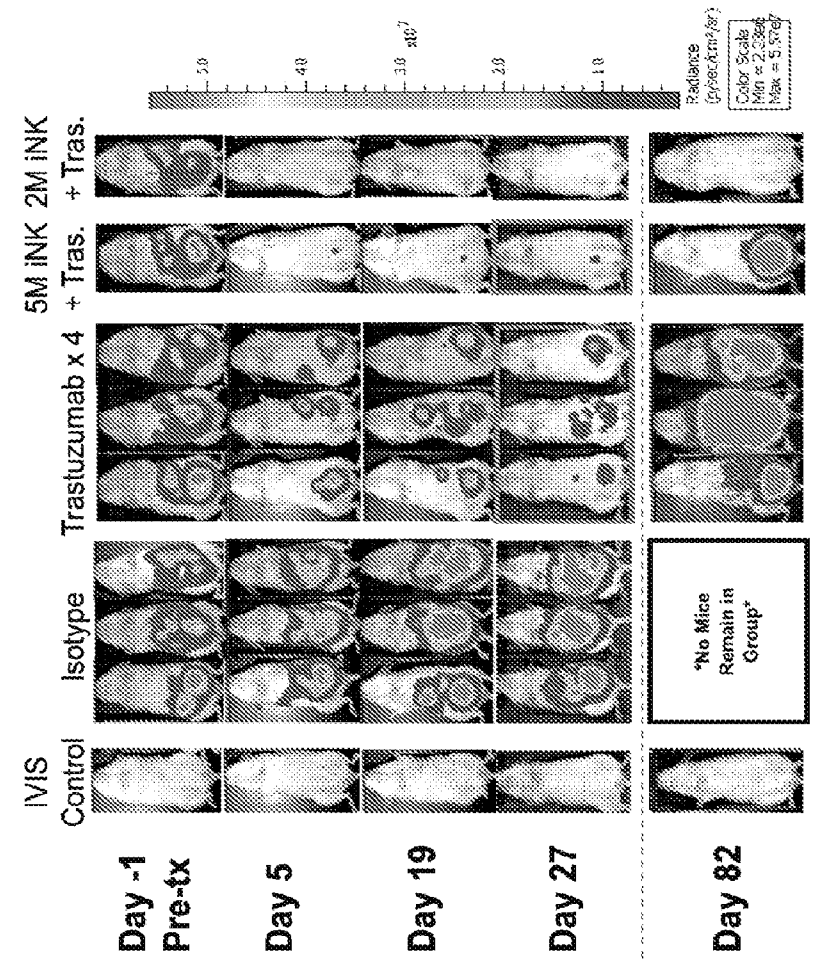

IVIS
Control
Isotype
Trastuzumab x 4
5M iNK + Tras.
2M iNK + Tras.
Day -1 Pre-tx
Day 5
Day 19
Day 27
Day 82
No Mice Remain in Group
Radiance (p/sec/cm²/sr)
Color Scale
Min = 2.03e6
Max = 5.57e7

Fig. 38F

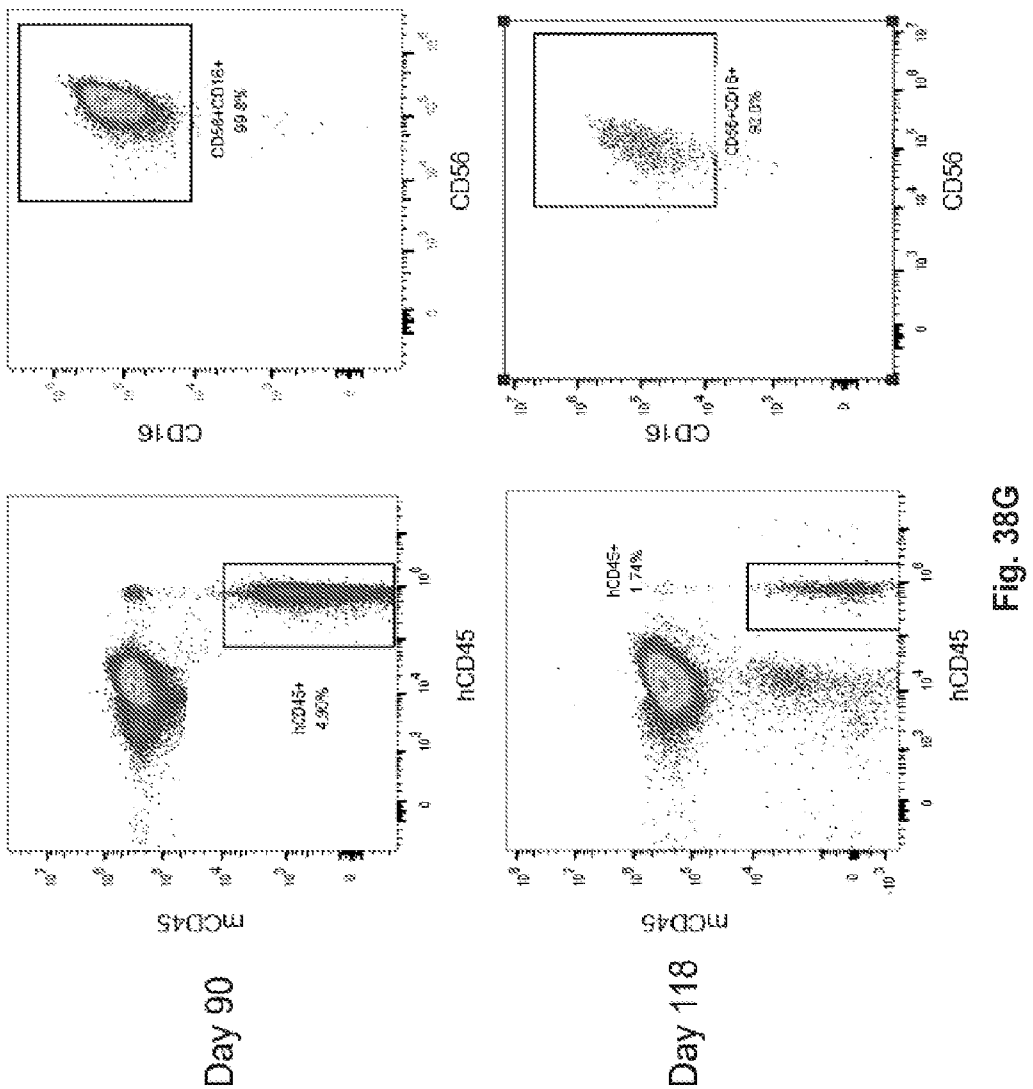

Day 90
mCD45
hCD45
hCD45+
4.90%
CD16
CD56
CD56+CD16+
99.8%
Day 118
mCD45
hCD45
hCD45+
1.74%
CD16
CD56
CD56+CD16+
92.0%

Fig. 38G

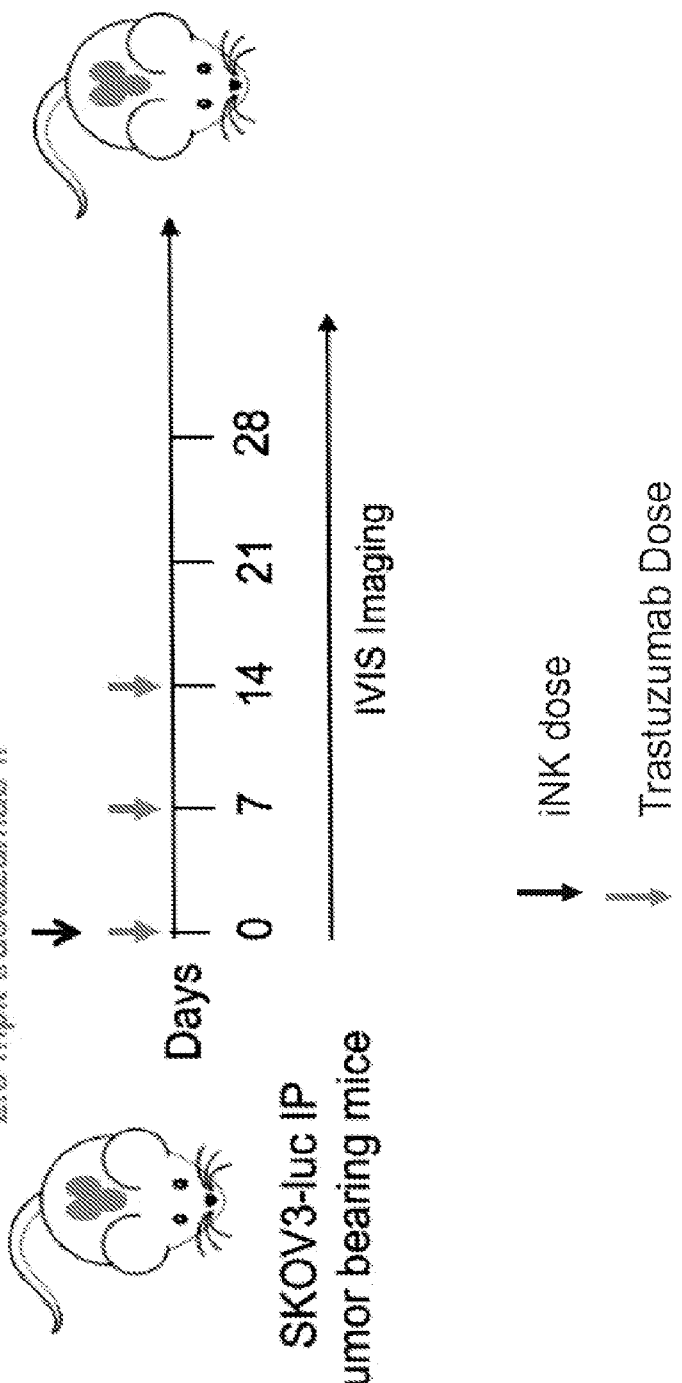

5M WT/DKI iNK IP +
2.5 mpk trastuzumab IP
SKOV3-luc IP
tumor bearing mice
Days
0
7
14
21
28
IVIS Imaging
iNK dose
Trastuzumab Dose

Fig. 39A

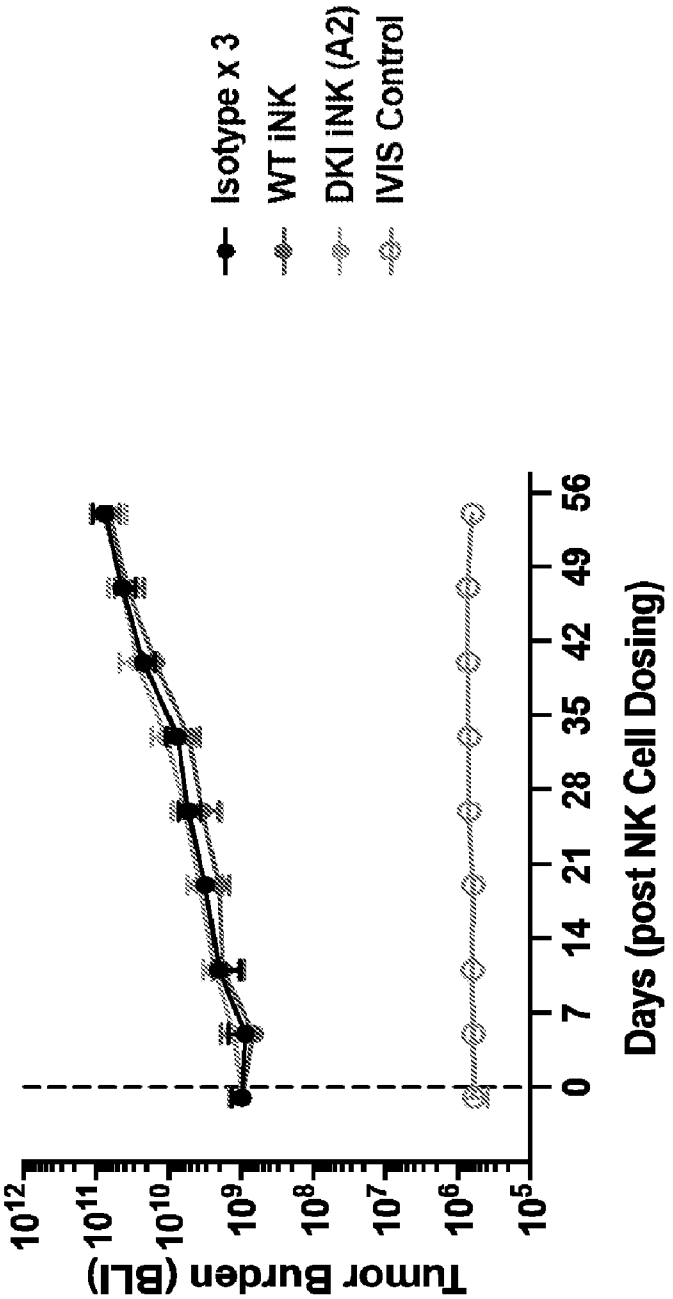

Isotype x 3
WT iNK
DKI iNK (A2)
IVIS Control
Tumor Burden (BLI)
10^{12}
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
0
7
14
21
28
35
42
49
56
Days (post NK Cell Dosing)

Fig. 39B

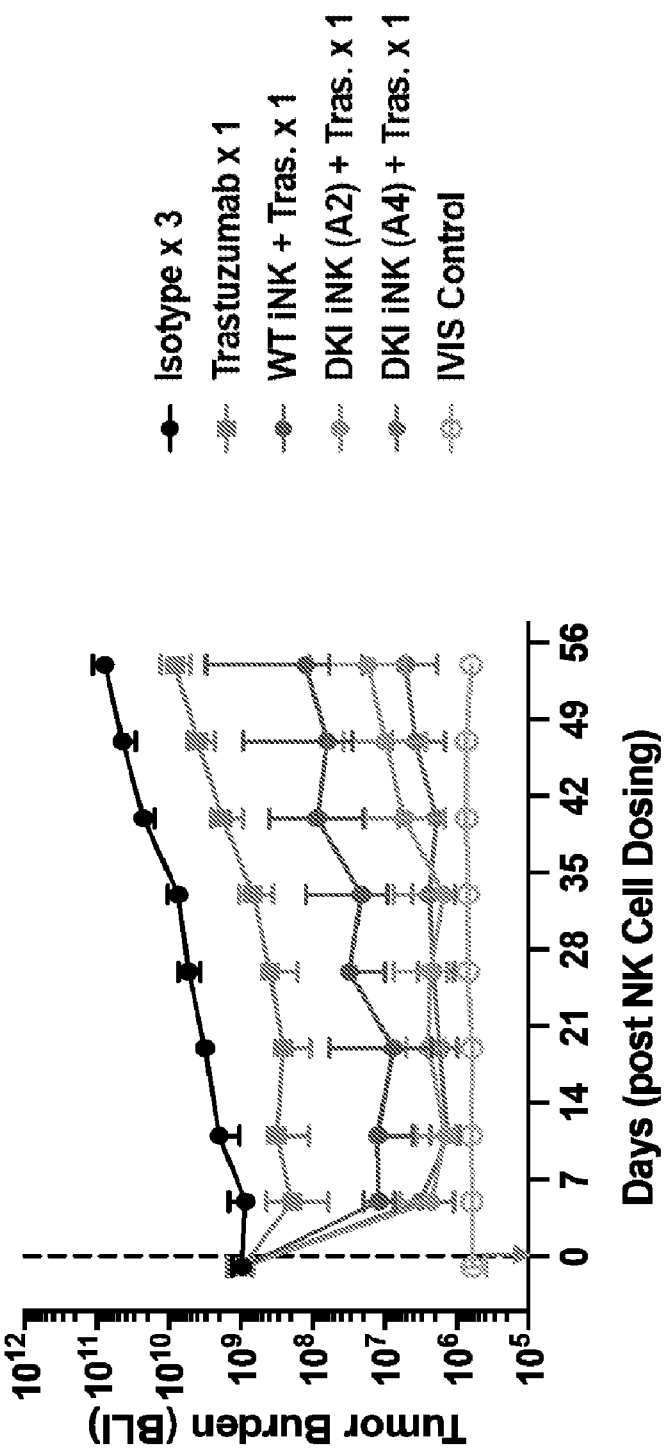

Isotype x 3
Trastuzumab x 1
WT iNK + Tras. x 1
DKI iNK (A2) + Tras. x 1
DKI iNK (A4) + Tras. x 1
IVIS Control
Tumor Burden (BLI)
10^{12}
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
0 7 14 21 28 35 42 49 56
Days (post NK Cell Dosing)

Fig. 39C

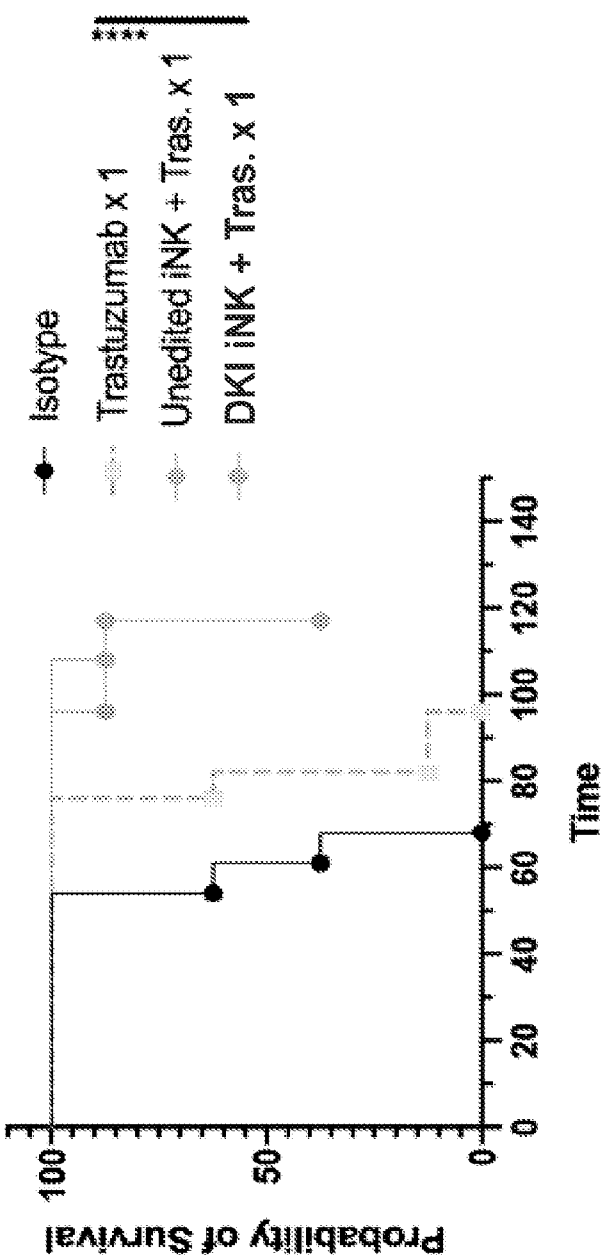

Isotype
Trastuzumab x 1
Unedited iNK + Tras. x 1
DKI iNK + Tras. x 1
***
Probability of Survival
100
50
0
0
20
40
60
80
100
120
140
Time

Fig. 39D

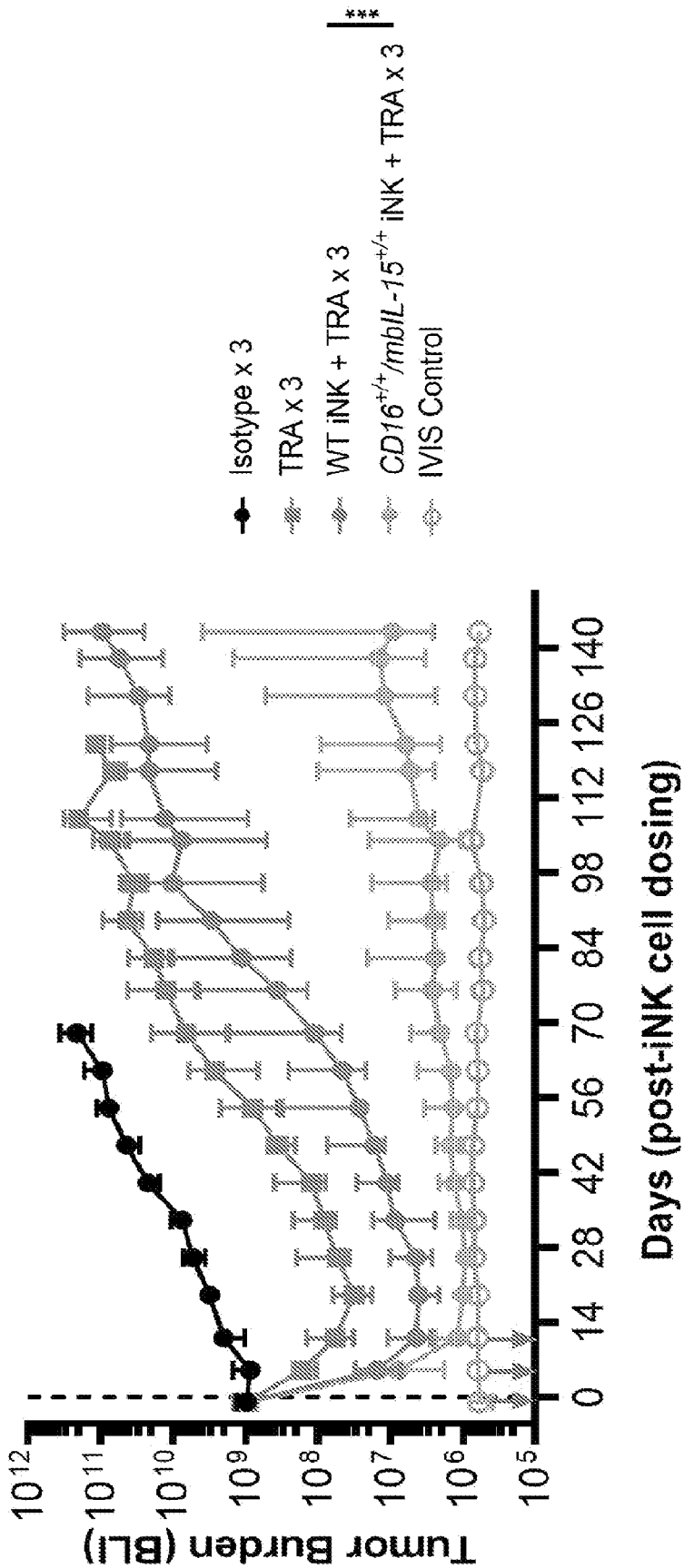

Tumor Burden (BLI)
10^{12}
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
0 14 28 42 56 70 84 98 112 126 140
Days (post-iNK cell dosing)
Isotype x 3
TRA x 3
WT iNK + TRA x 3
CD16$^{+/+}$/mbIL-15$^{+/+}$ iNK + TRA x 3
IVIS Control
***

Fig. 39E

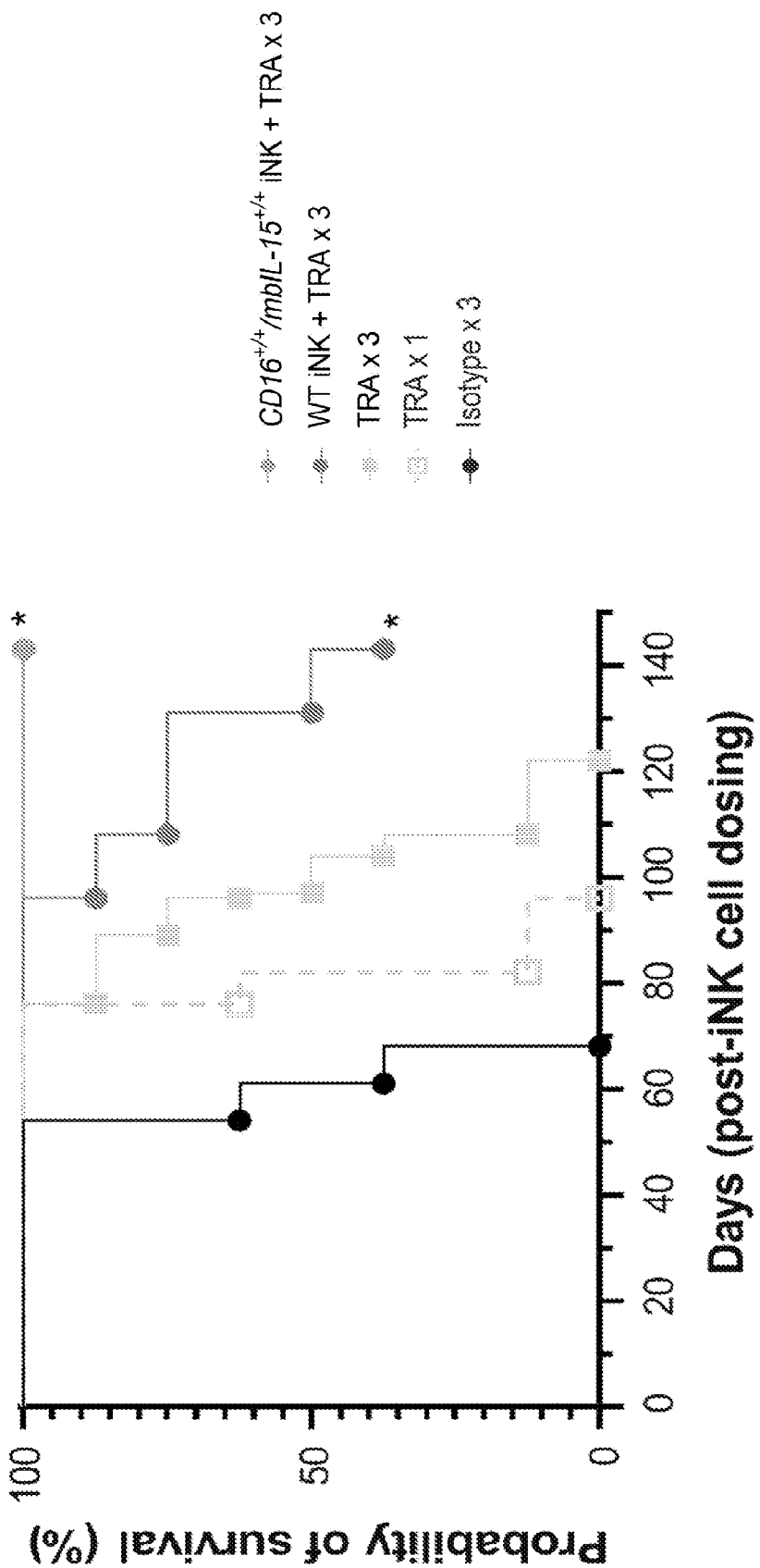

CD16+/+/mbIL-15+/+ iNK + TRA x 3
WT iNK + TRA x 3
TRA x 3
TRA x 1
Isotype x 3
Probability of survival (%)
100
50
0
0
20
40
60
80
100
120
140
Days (post-iNK cell dosing)
*
*

Fig. 39F

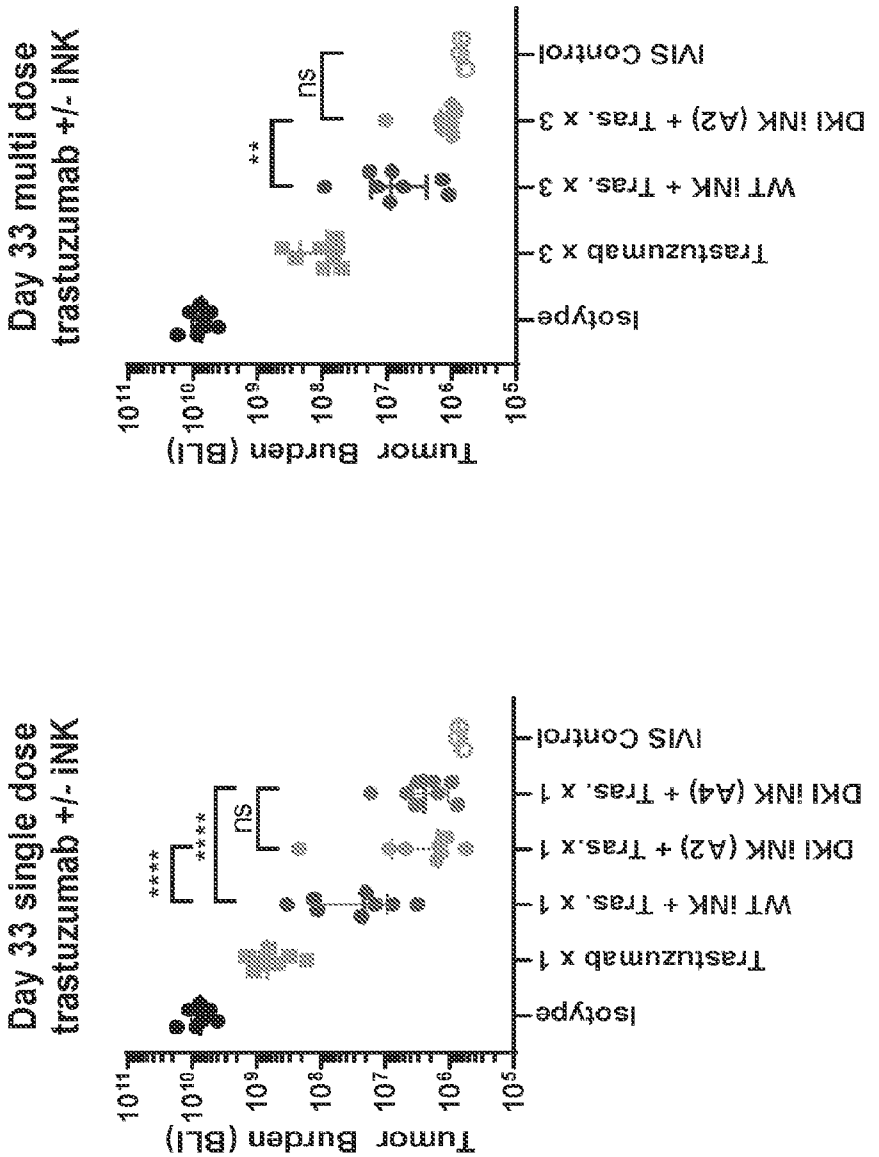

Day 33 multi dose
trastuzumab +/- iNK
Tumor Burden (BLI)
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
**
ns
Isotype
Trastuzumab x 3
WT iNK + Tras. x 3
DKI iNK (A2) + Tras. x 3
IVIS Control
Day 33 single dose
trastuzumab +/- iNK
Tumor Burden (BLI)
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
****
****
ns
Isotype
Trastuzumab x 1
WT iNK + Tras. x 1
DKI iNK (A2) + Tras.x 1
DKI iNK (A4) + Tras. x 1
IVIS Control

Fig. 39G

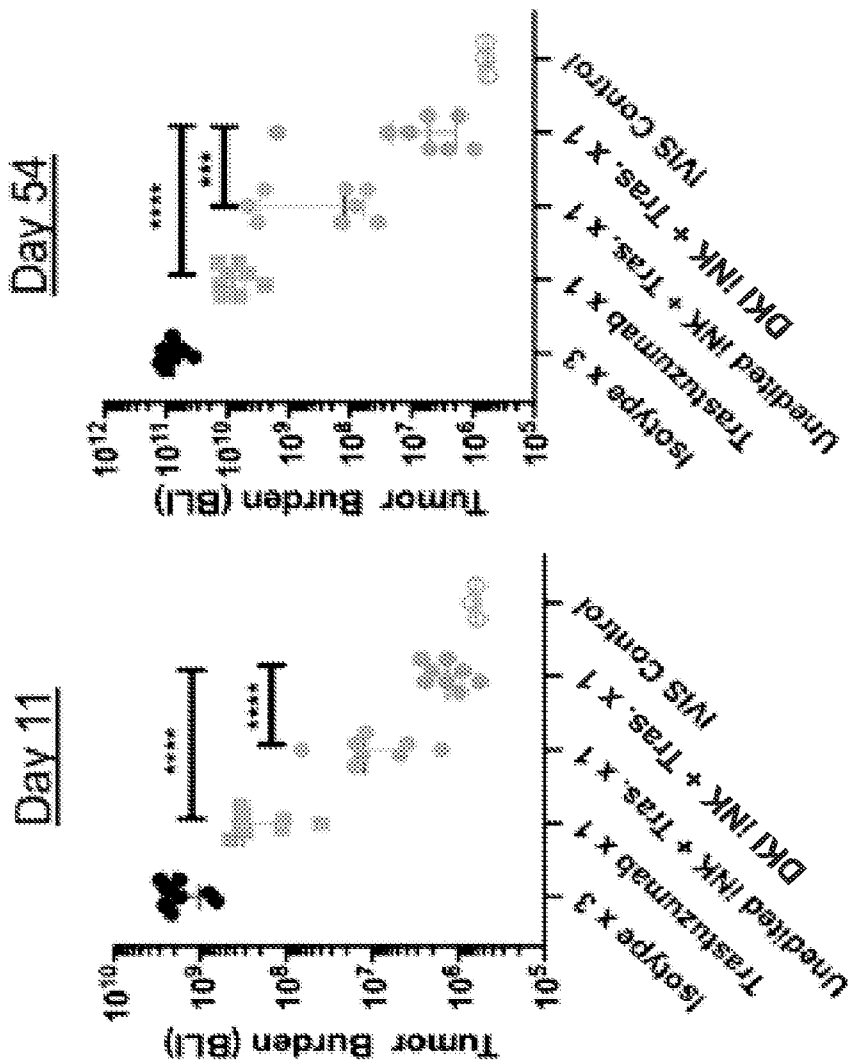

Day 11
Day 54
Tumor Burden (BLI)
Isotype x 3
Trastuzumab x 1
Unedited INK + Tras. x 1
DKI INK + Tras. x 1
IVIS Control
****
***

Fig. 39H

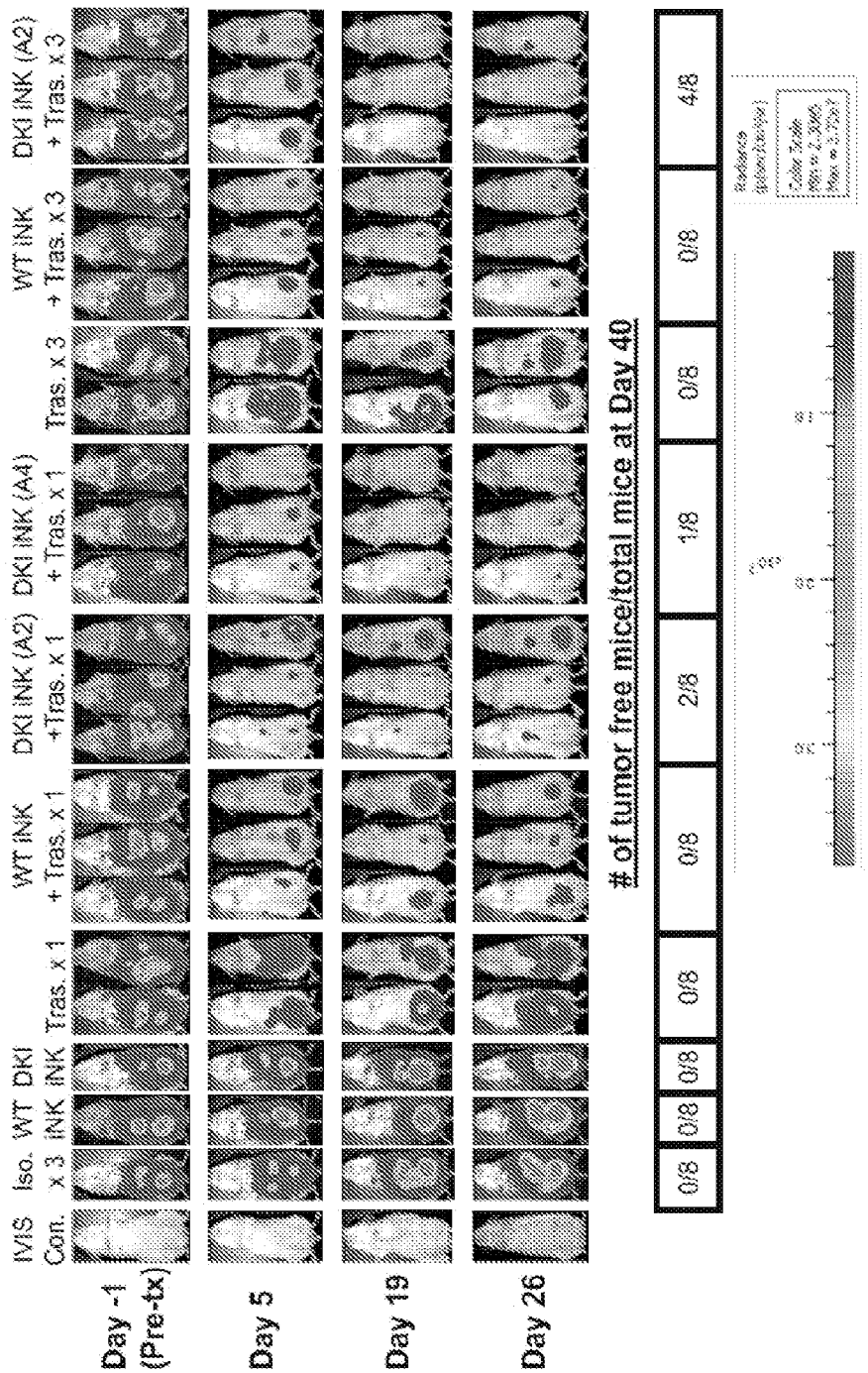

IVIS Con.
Iso. x 3
WT iNK
DKI iNK
Tras. x 1
WT iNK + Tras. x 1
DKI iNK (A2) +Tras. x 1
DKI iNK (A4) + Tras. x 1
Tras. x 3
WT iNK + Tras. x 3
DKI iNK (A2) + Tras. x 3
Day -1 (Pre-tx)
Day 5
Day 19
Day 26
# of tumor free mice/total mice at Day 40
0/8
0/8
0/8
0/8
2/8
1/8
0/8
0/8
4/8

Fig. 39I

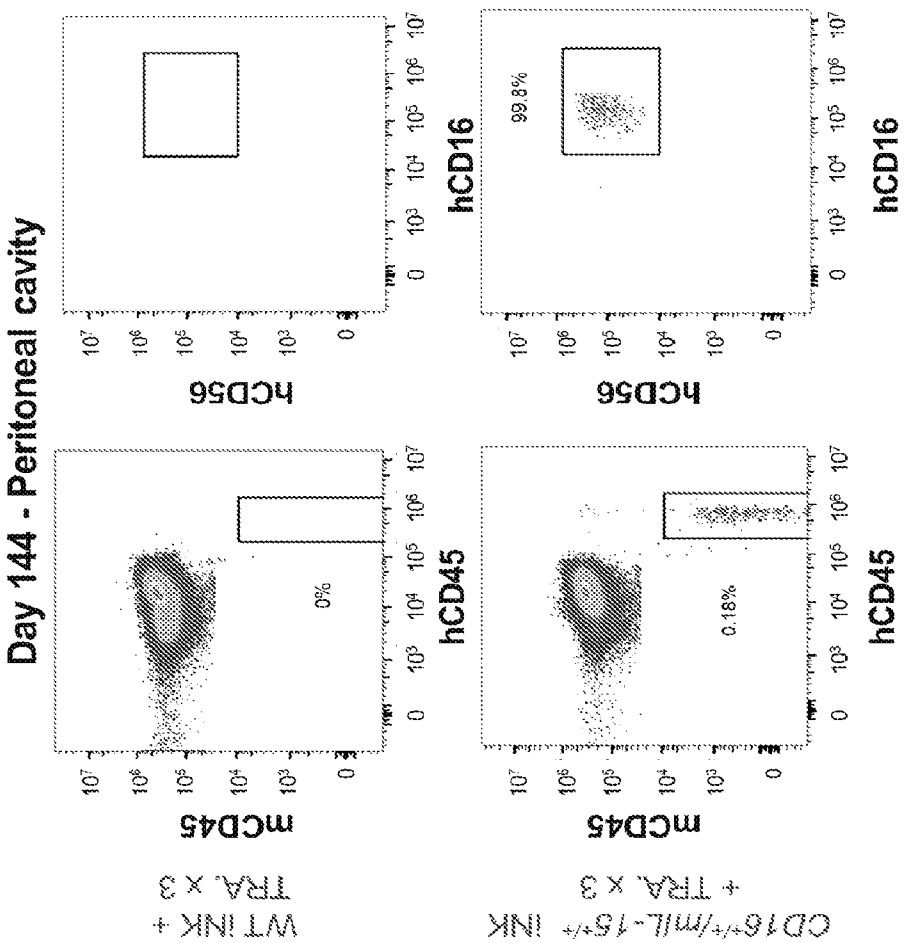

Day 144 - Peritoneal cavity
WT INK + TRA. x 3
CD16+/+/mIL-15+/+ INK + TRA. x 3
mCD45
hCD45
hCD56
hCD16
0%
0.18%
99.8%

Fig. 39J

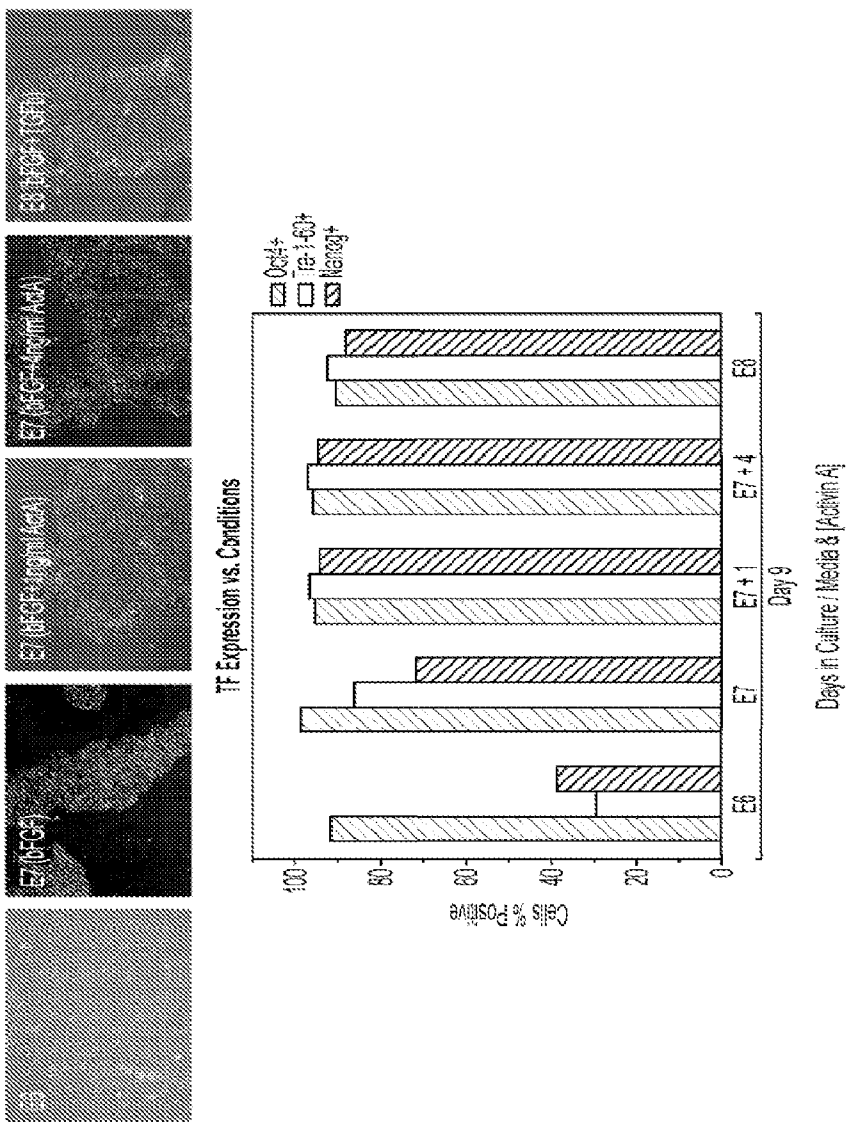

E7 (bFGF)
E7 (bFGF+Activin A)
E7 (bFGF+TGFβ)
TF Expression vs. Conditions
Oct4+
Tra-1-60+
Nanog+
Cells % Positive
100
80
60
40
20
0
E6
E7
E7 + 1
E7 + 4
E8
Day 9
Days in Culture / Media & (Activin A)

Fig. 40

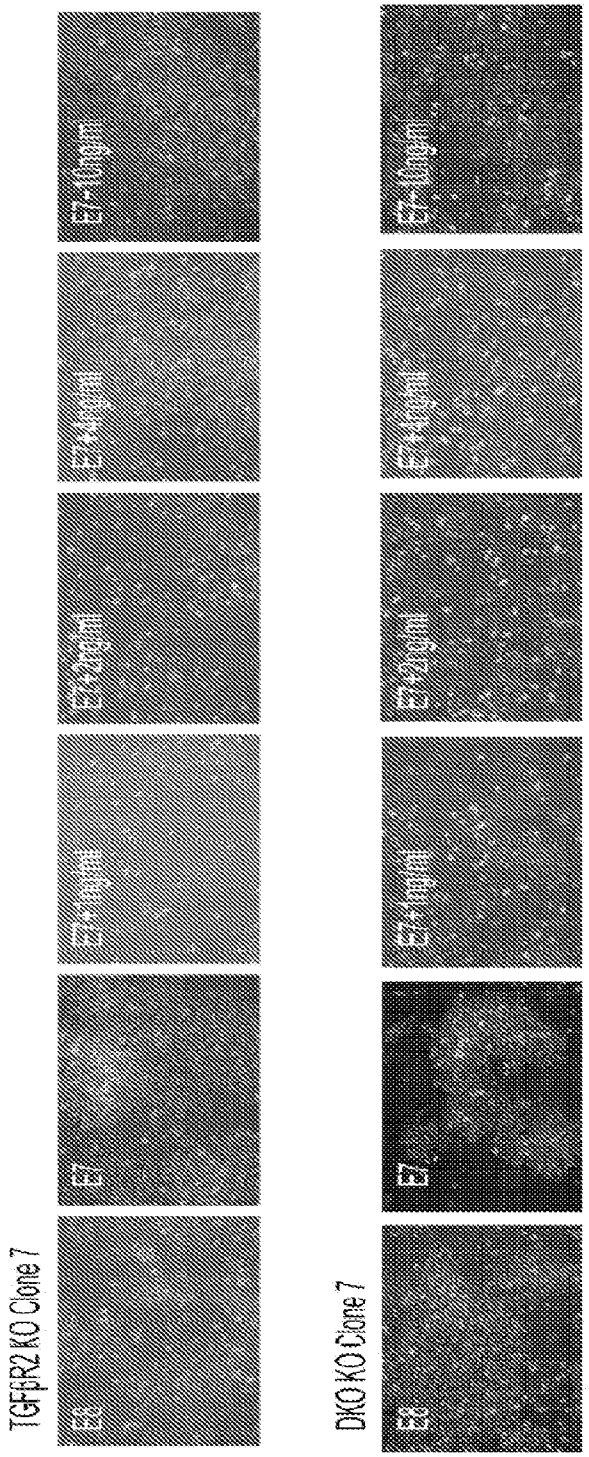

TGFβR2 KO Clone 7
E6
E7
E7-10bpIns
E7-1bpIns
E7-2bpIns
E7-1bpIns
E7-2bpIns
E7-10bpIns
DKO KO Clone 7
E6
E7
E7-1bpIns
E7-2bpIns
E7-1bpIns
E7-2bpIns
E7-10bpIns

Fig. 41

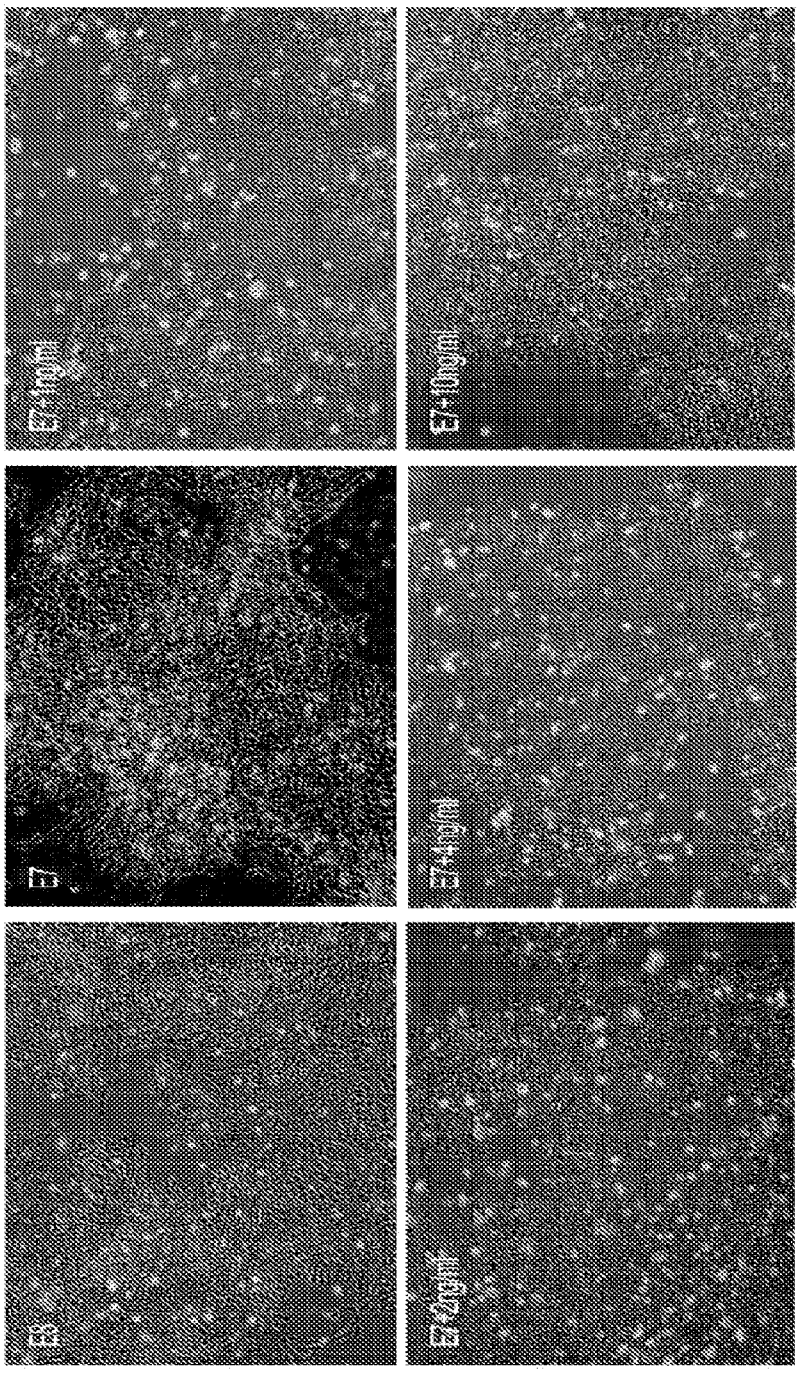


Fig. 42

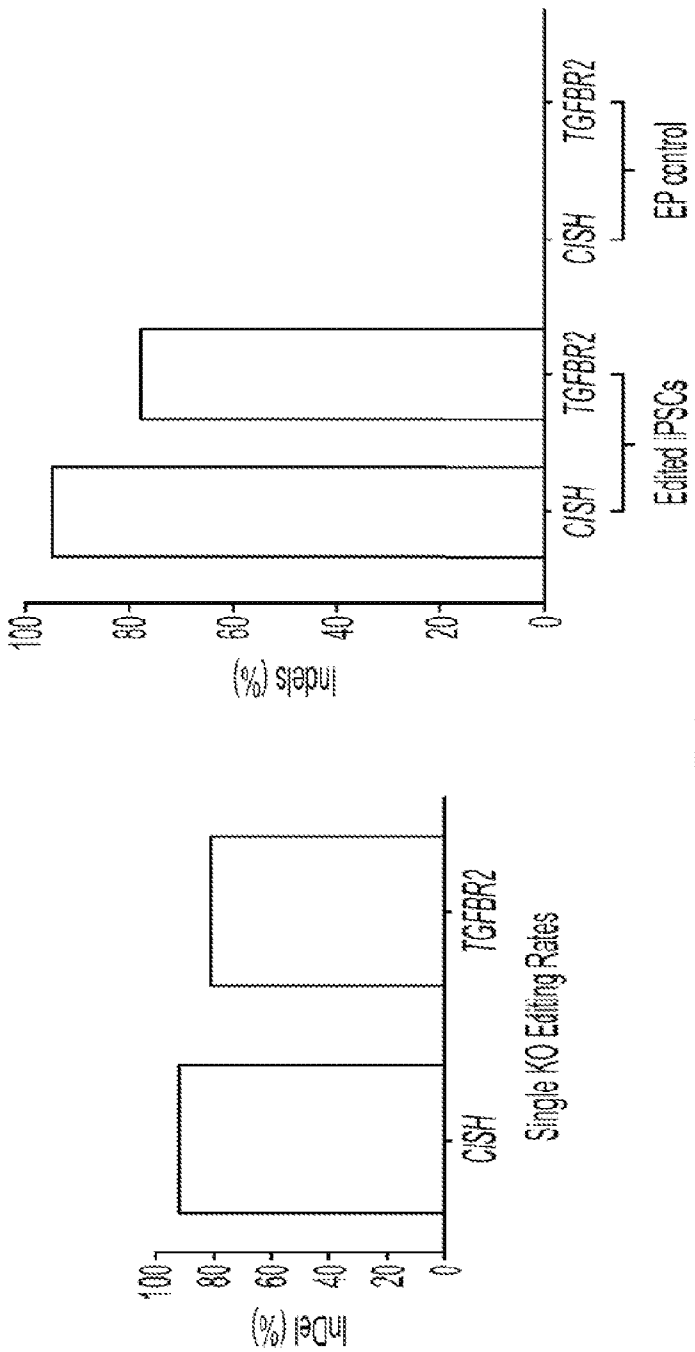

Indels (%)
100
80
60
40
20
0
CISH
TGFBR2
Edited iPSCs
CISH
TGFBR2
EP control
EP: electroporation
Indel (%)
100
80
60
40
20
0
CISH
TGFBR2
Single KO Editing Rates

Fig. 43A

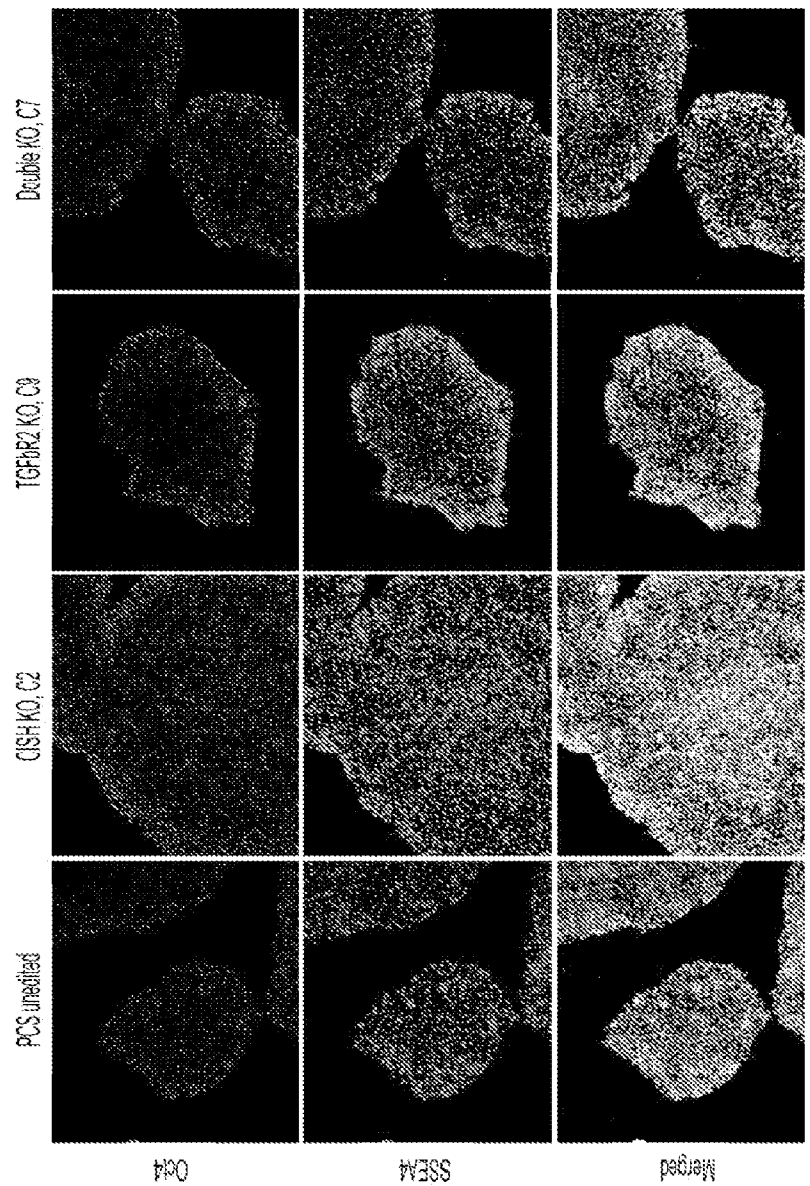

PCS unedited
CISH KO, C2
TGFbR2 KO, C9
Double KO, C7
Oct4
SSEA4
Merged

Fig. 43B

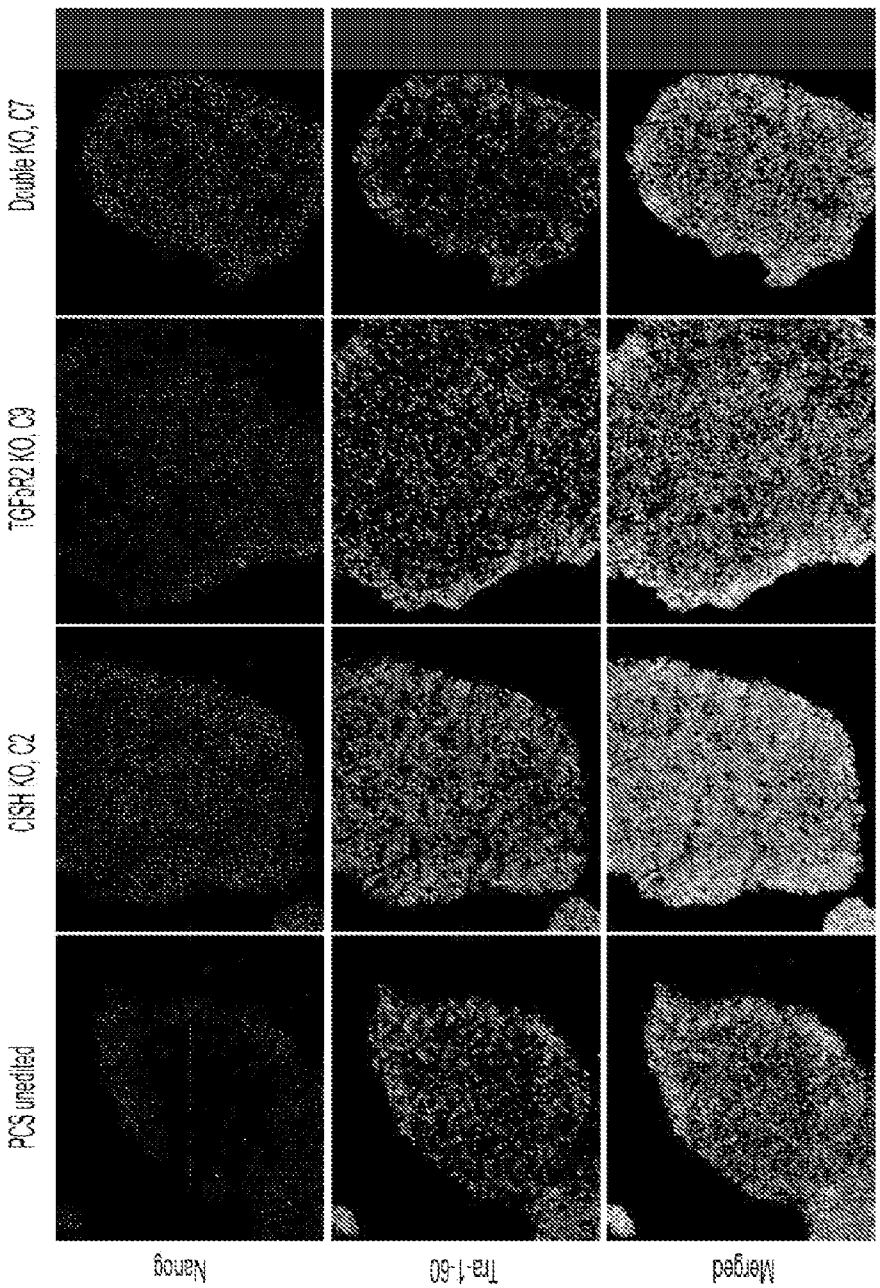
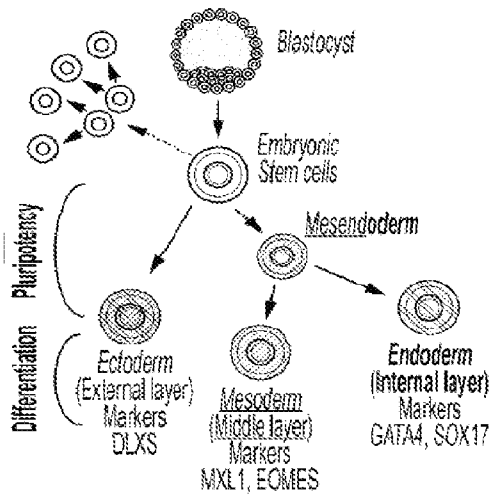

PCS unedited
CISH KO, C2
TGFBR2 KO, C9
Double KO, C7
Nanog
Tra-1-60
Merged

Fig. 44


Blastocyst
Embryonic Stem cells
Pluripotency
Mesendoderm
Differentiation
Ectoderm (External layer) Markers DLX5
Mesoderm (Middle layer) Markers MXL1, EOMES
Endoderm (Internal layer) Markers GATA4, SOX17

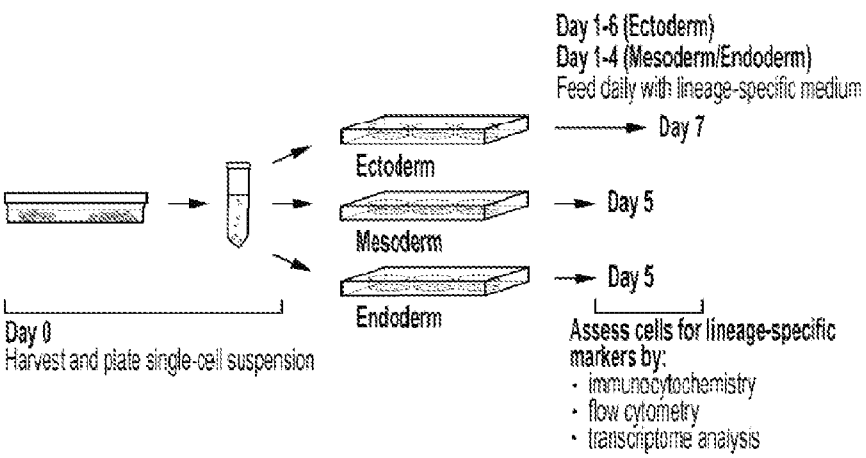

Day 1-6 (Ectoderm)
Day 1-4 (Mesoderm/Endoderm)
Feed daily with lineage-specific medium
Ectoderm
Day 7
Mesoderm
Day 5
Endoderm
Day 5
Day 0
Harvest and plate single-cell suspension
Assess cells for lineage-specific markers by:
• immunocytochemistry
• flow cytometry
• transcriptome analysis

Fig. 45

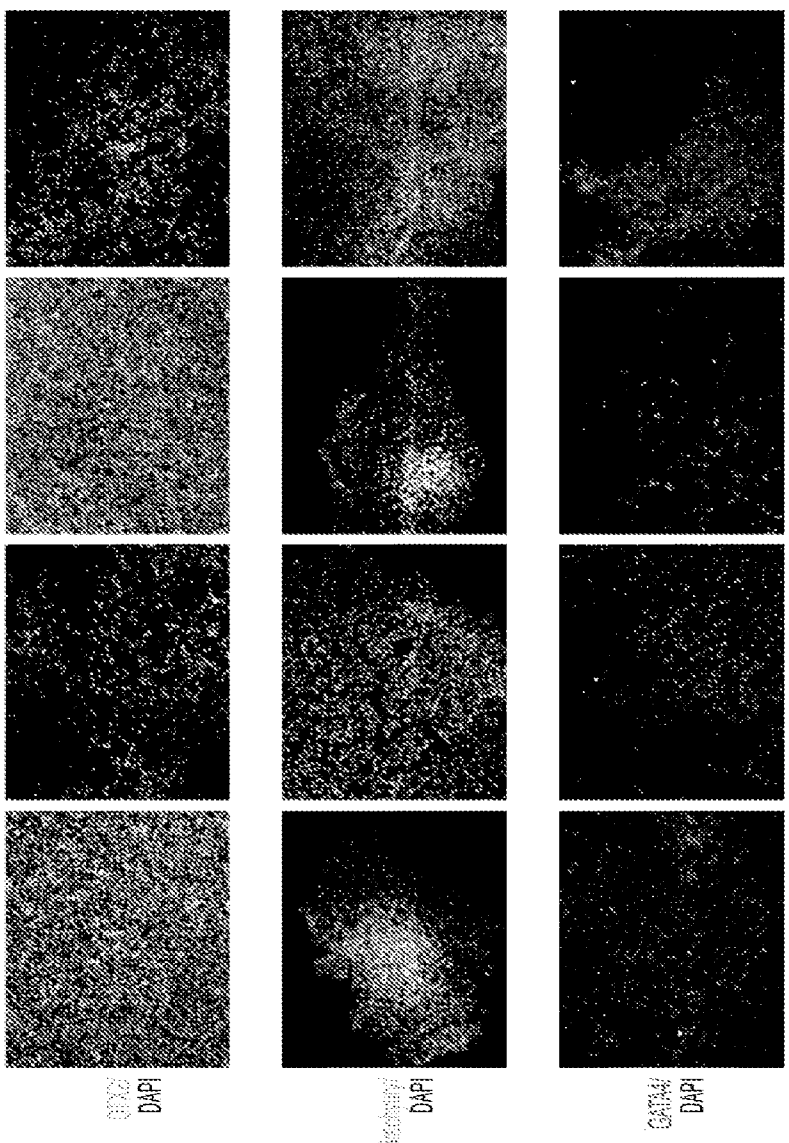

PCS unedited
CISH KO, C2
TGFBR2 KO, C9
Double KO, C1
DAPI
DAPI
DAPI

Fig. 46A

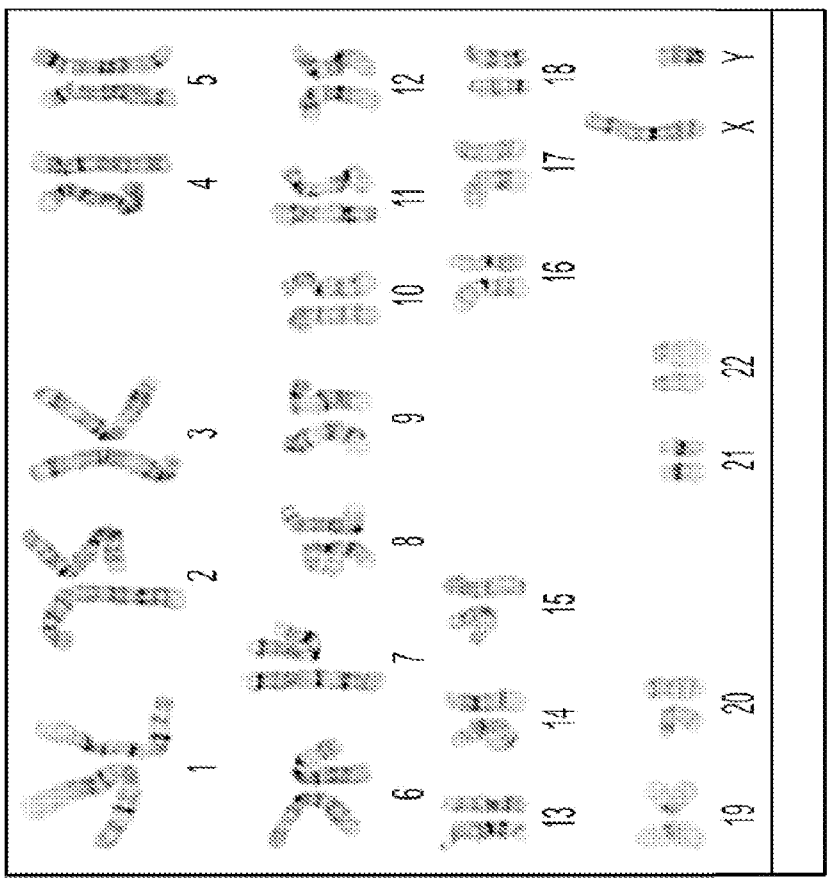


Fig. 46B

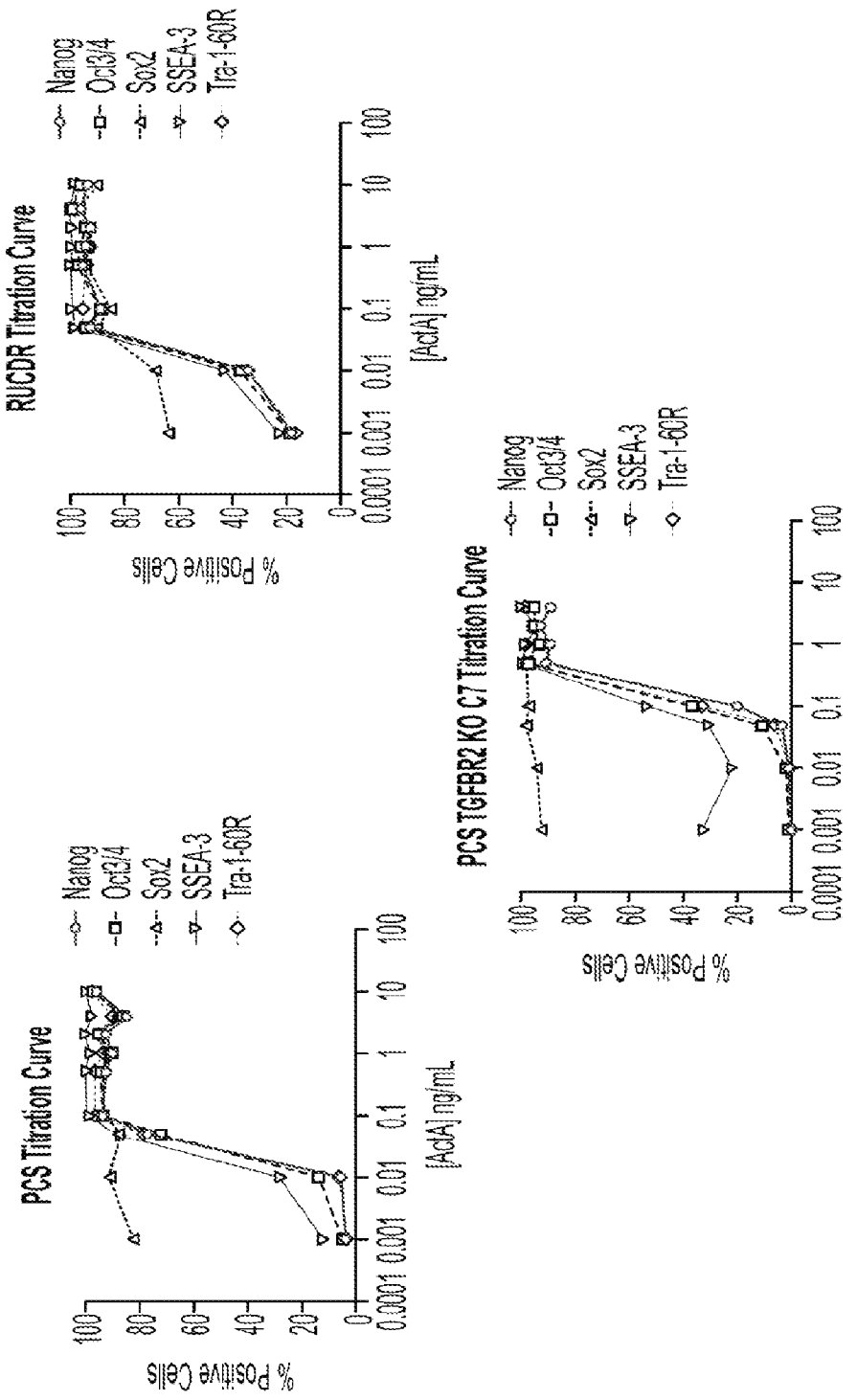

RUCDR Titration Curve
Nanog
Oct3/4
Sox2
SSEA-3
Tra-1-60R
% Positive Cells
[ActA] ng/mL
PCS Titration Curve
PCS TGFBR2 KO C7 Titration Curve

Fig. 46C

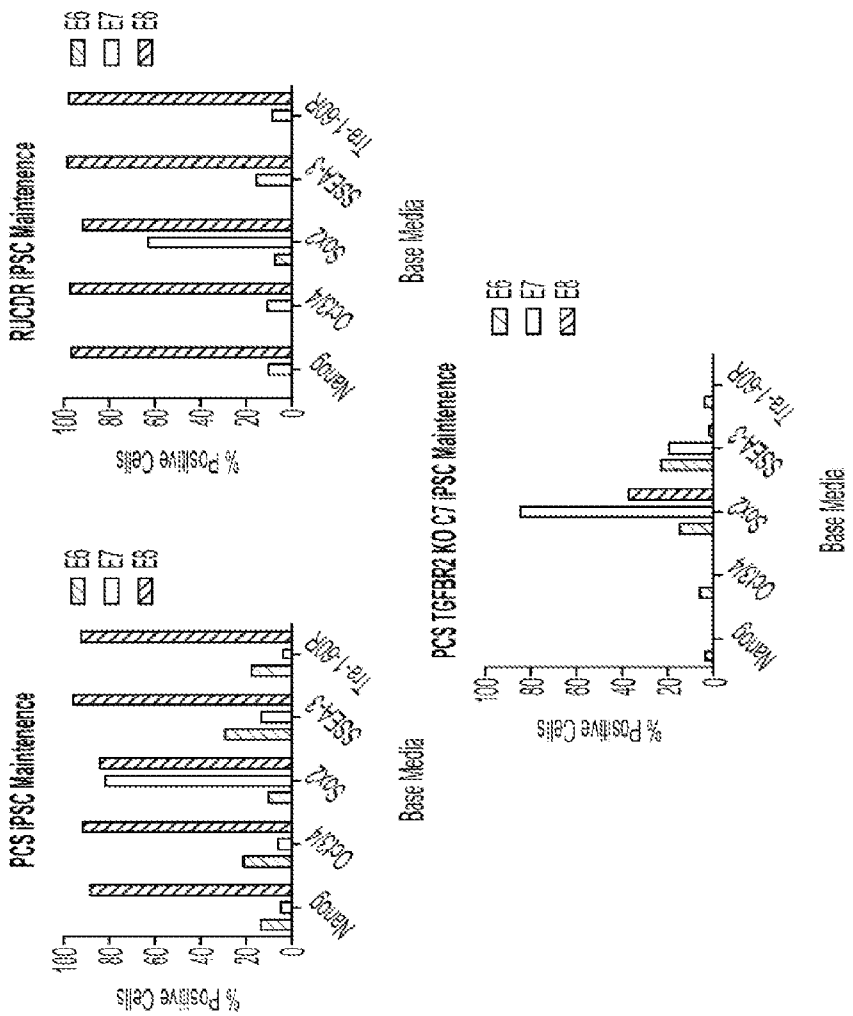

RUCDR iPSC Maintenance
PCS iPSC Maintenance
PCS TGFBR2 KO C7 iPSC Maintenance
% Positive Cells
100
80
60
40
20
0
E6
E7
E8
Nanog
Oct3/4
Sox2
SSEA-3
Tra-1-60R
Base Media

Fig. 46D

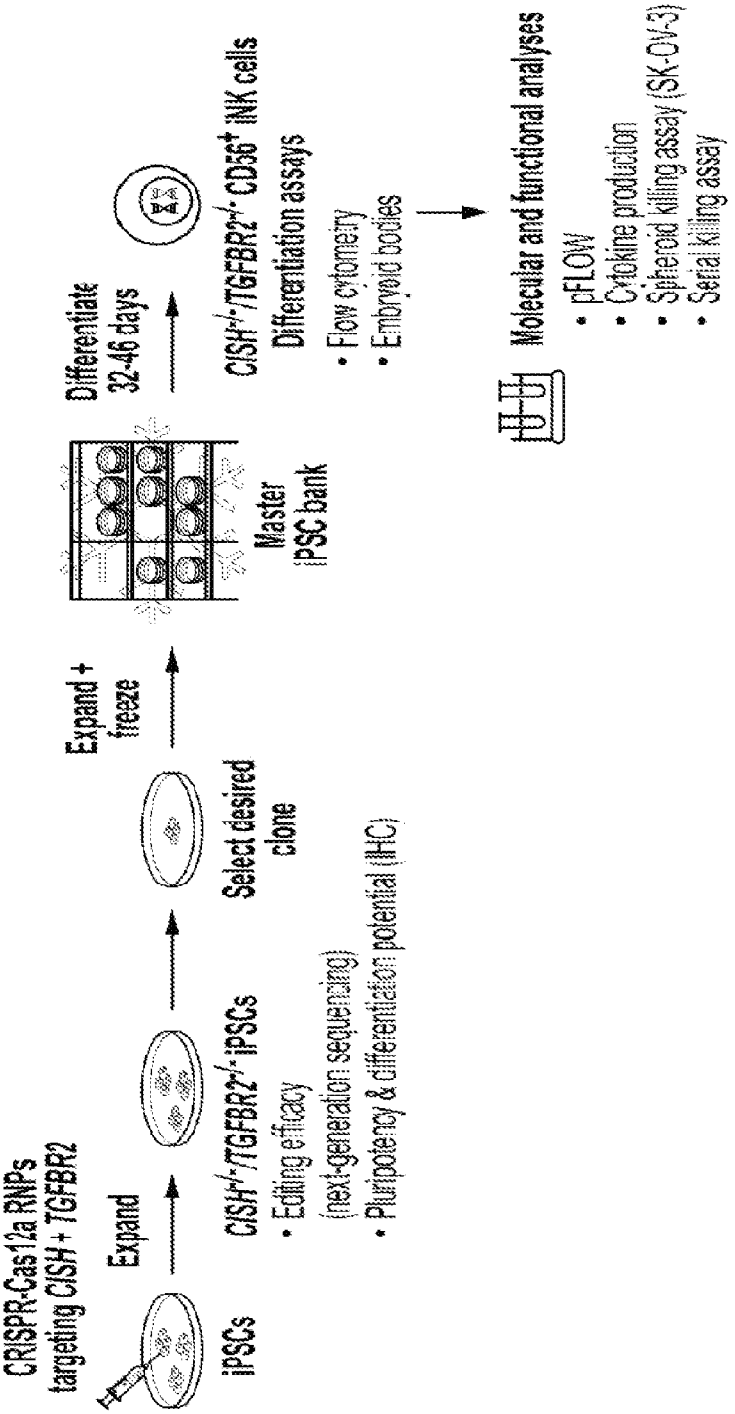

CRISPR-Cas12a RNPs
targeting CISH + TGFBR2
iPSCs
Expand
CISH-/-/TGFBR2-/- iPSCs
• Editing efficacy
(next-generation sequencing)
• Pluripotency & differentiation potential (IHC)
Select desired clone
Expand + freeze
Master iPSC bank
Differentiate 32-46 days
CISH-/-/TGFBR2-/- CD56+ iNK cells
Differentiation assays
• Flow cytometry
• Embryoid bodies
Molecular and functional analyses
• pFLOW
• Cytokine production
• Spheroid killing assay (SK-OV-3)
• Serial killing assay

Fig. 47A

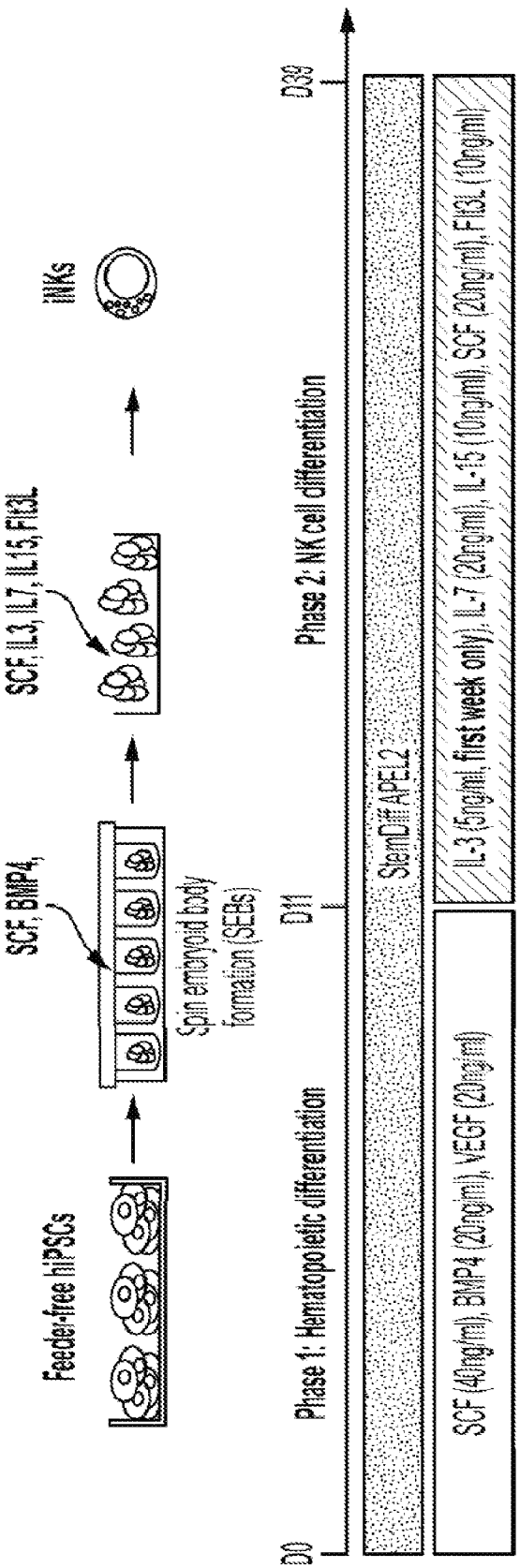

Feeder-free hiPSCs
SCF, BMP4,
Spin embryoid body formation (SEBs)
SCF, IL3, IL7, IL15, FI3L
iNKs
D0
Phase 1: Hematopoietic differentiation
D11
Phase 2: NK cell differentiation
D39
StemDiff APEL2
SCF (40ng/ml), BMP4 (20ng/ml), VEGF (20ng/ml)
IL-3 (5ng/ml, first week only), IL-7 (20ng/ml), IL-15 (10ng/ml), SCF (20ng/ml), FI3L (10ng/ml)

Fig. 47B

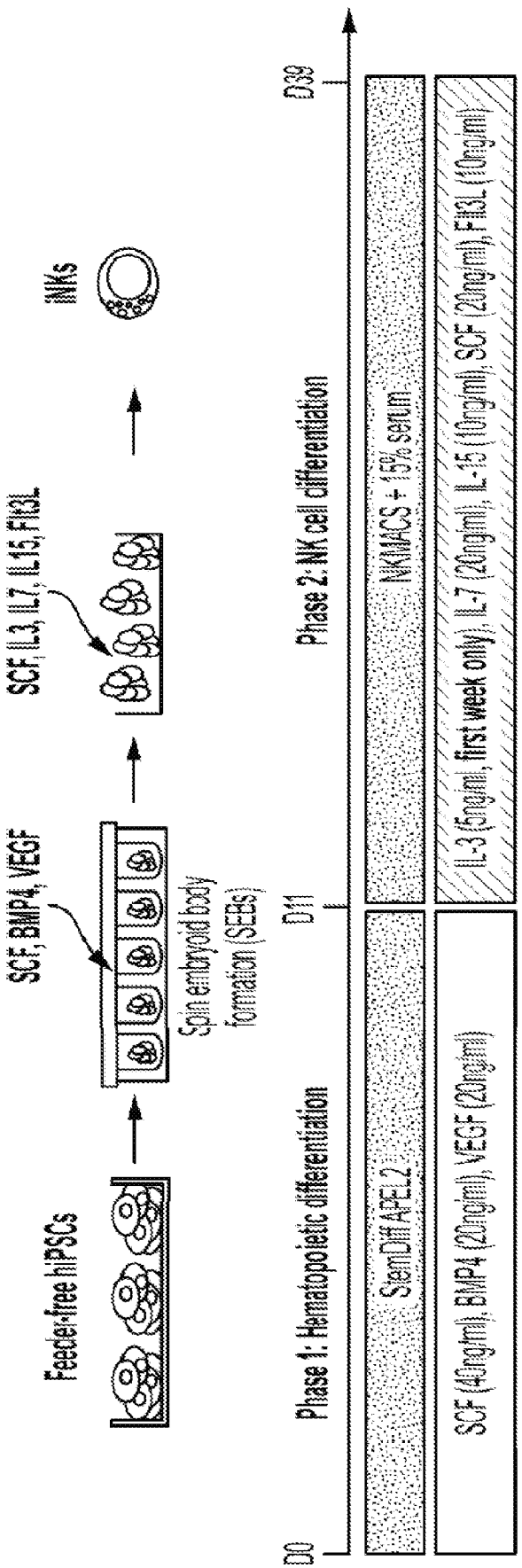

Feeder-free hiPSCs
SCF, BMP4, VEGF
Spin embryoid body formation (SEBs)
SCF, IL3, IL7, IL15, FI3L
iNKs
D0
Phase 1: Hematopoietic differentiation
D11
Phase 2: NK cell differentiation
D39
StemDiff APEL2
NKMACS + 15% serum
SCF (40ng/ml), BMP4 (20ng/ml), VEGF (20ng/ml)
IL-3 (5ng/ml, first week only), IL-7 (20ng/ml), IL-15 (10ng/ml), SCF (20ng/ml), FI3L (10ng/ml)

Fig. 47C

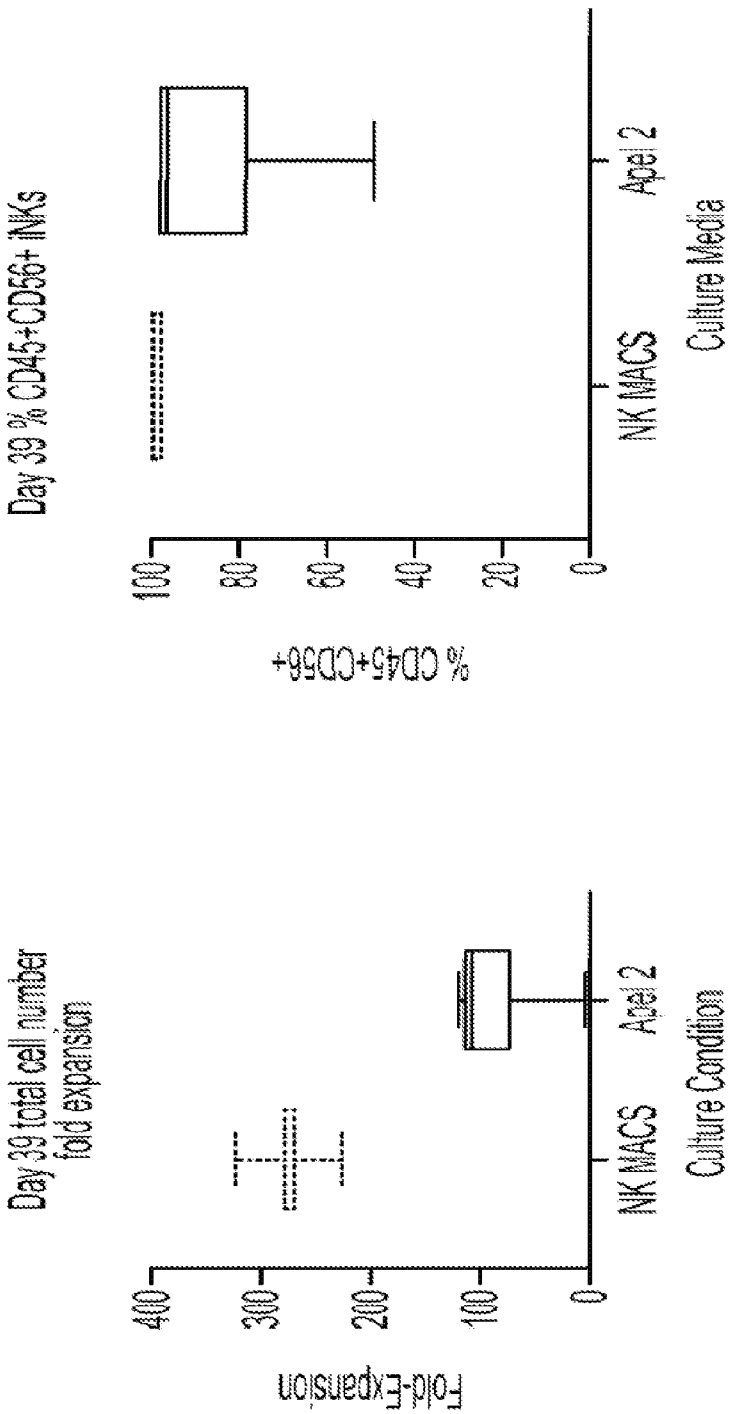

Day 39 % CD45+CD56+ iNKs
% CD45+CD56+
100
80
60
40
20
0
NK MACS
Apel 2
Culture Media
Day 39 total cell number fold expansion
Fold-Expansion
400
300
200
100
0
NK MACS
Apel 2
Culture Condition

Fig. 47D

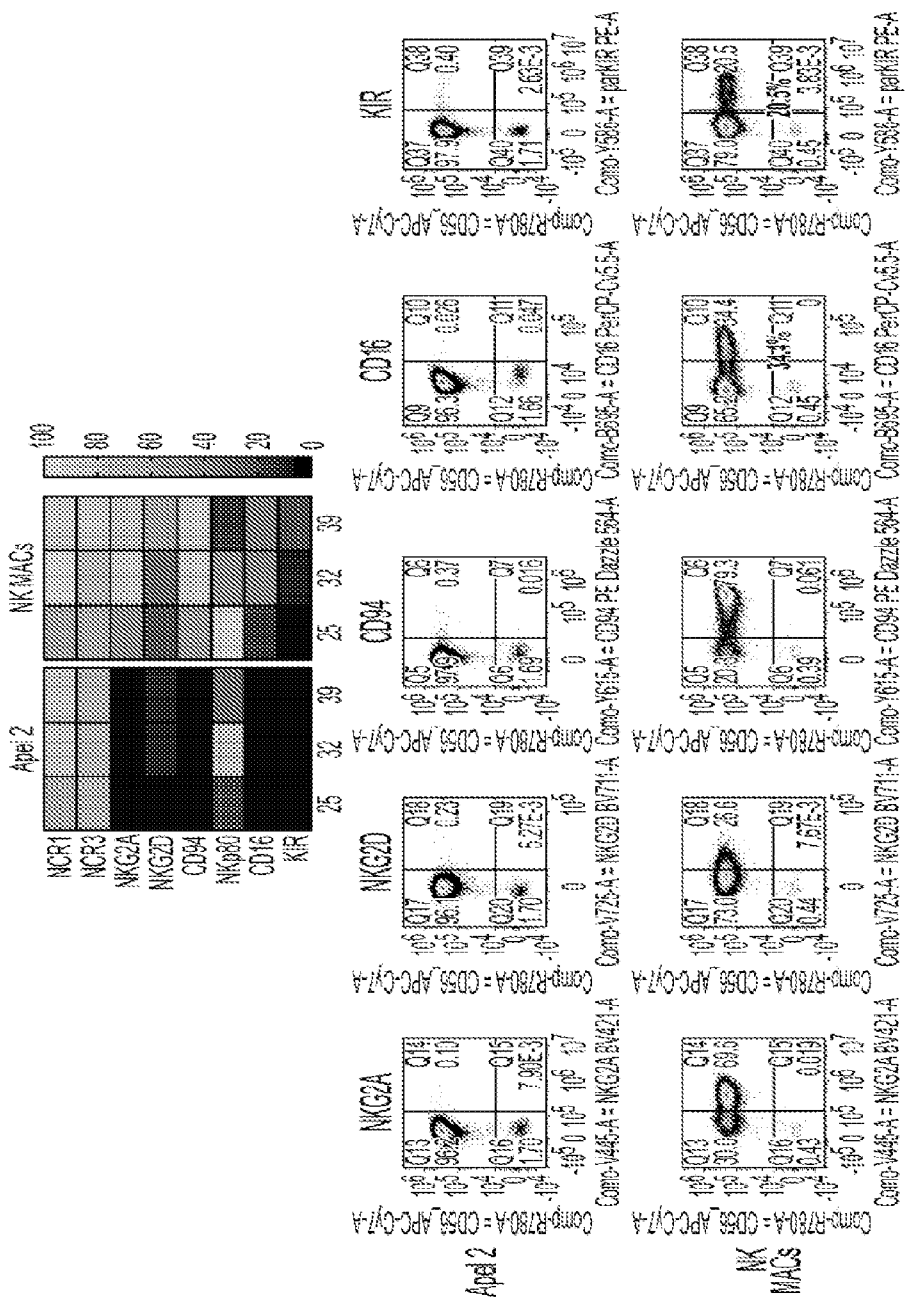

Apel 2
NK MACS
NCR1
NCR3
NKG2A
NKG2D
CD94
NKp80
CD16
KIR
100
80
60
40
20
0
25
32
39
NKG2A
NKG2D
CD94
CD16
KIR
Comp-R780A = CD56_APC-Cy7-A
Comp-V445-A = NKG2A BV421-A
Comp-V726-A = NKG2D BV711-A
Comp-V615-A = CD94 PE Dazzle 594-A
Comp-B695-A = CD16 PerCP-Cy5.5-A
Comp-Y586-A = panKIR PE-A

Fig. 47E

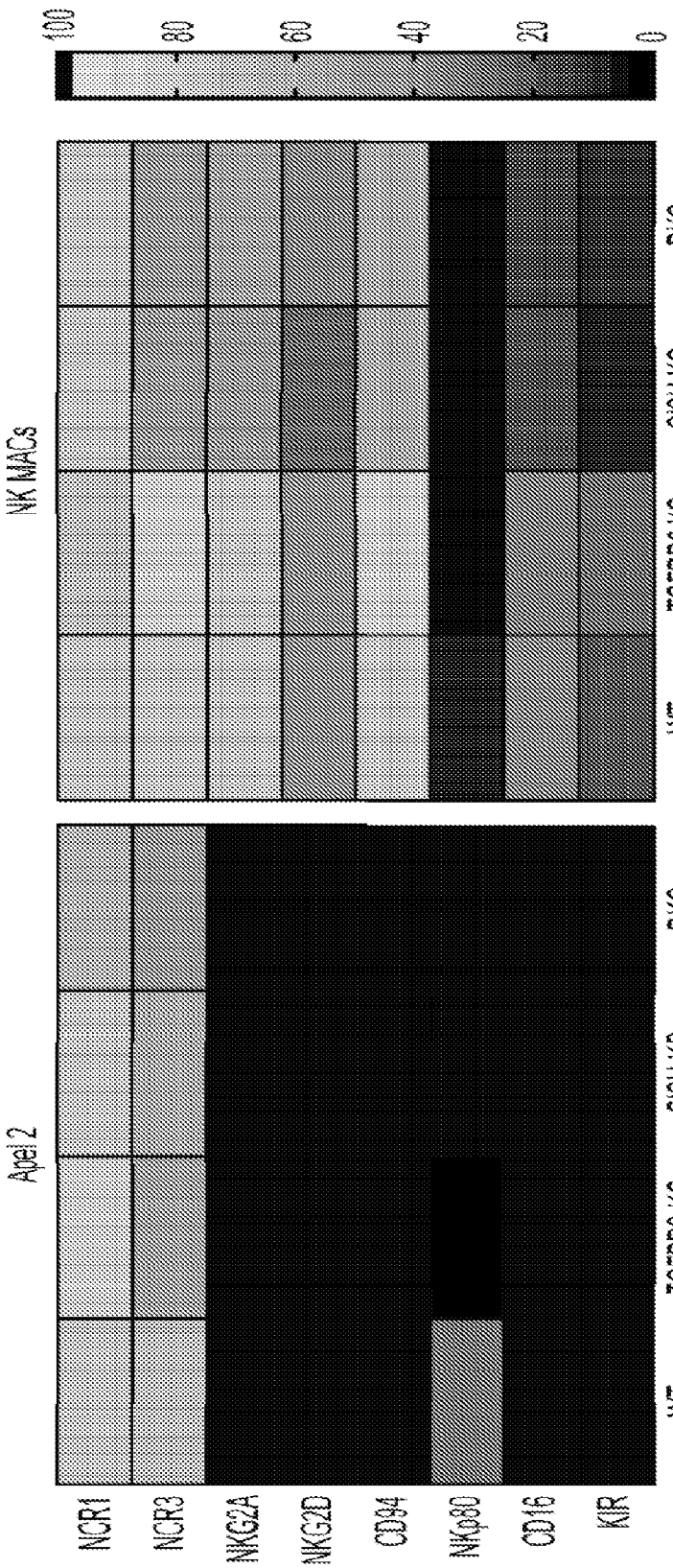
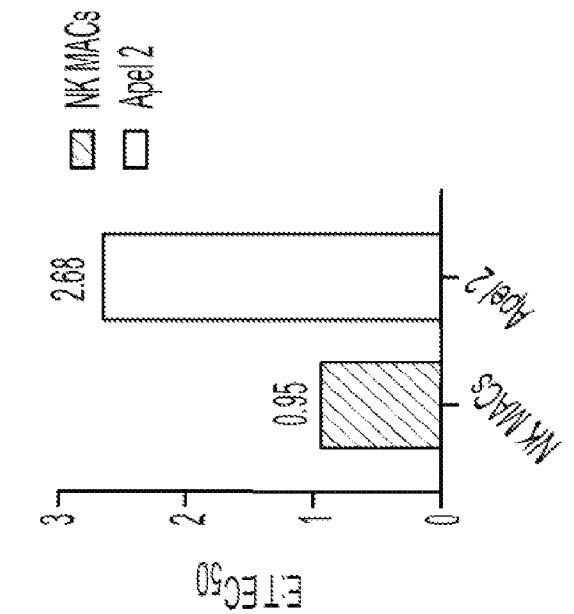

100
80
60
40
20
0
NK MACs
WT
TGFBR2 KO
CISH KO
DKO
Apel 2
WT
TGFBR2 KO
CISH KO
DKO
NCR1
NCR3
NKG2A
NKG2D
CD94
NKp80
CD16
KIR

Fig. 47F


NK MACs
Apel 2
2.68
0.95
Apel 2
NK MACs
ET EC50
3
2
1
0

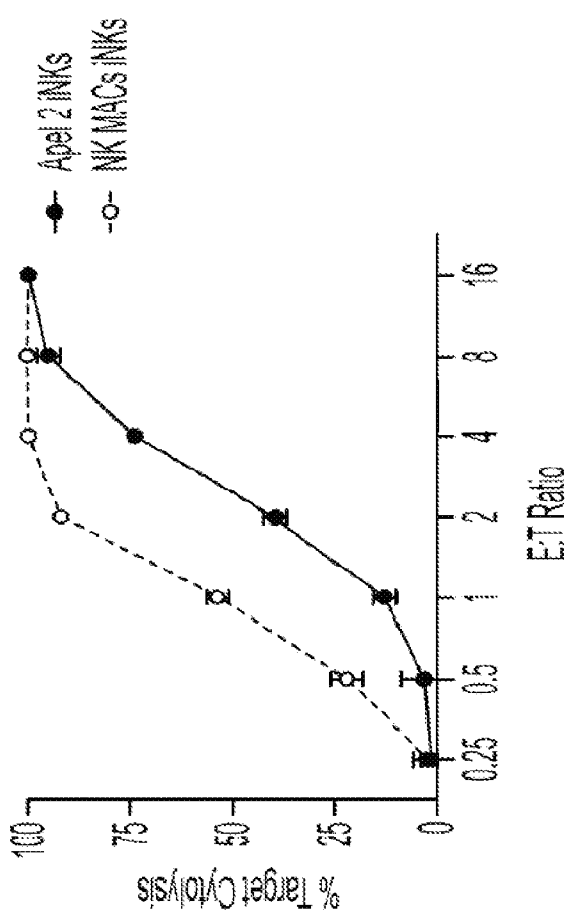

Apel 2 iNKs
NK MACs iNKs
% Target Cytolysis
100
75
50
25
0
0.25
0.5
1
2
4
8
16
E:T Ratio

Fig. 47G

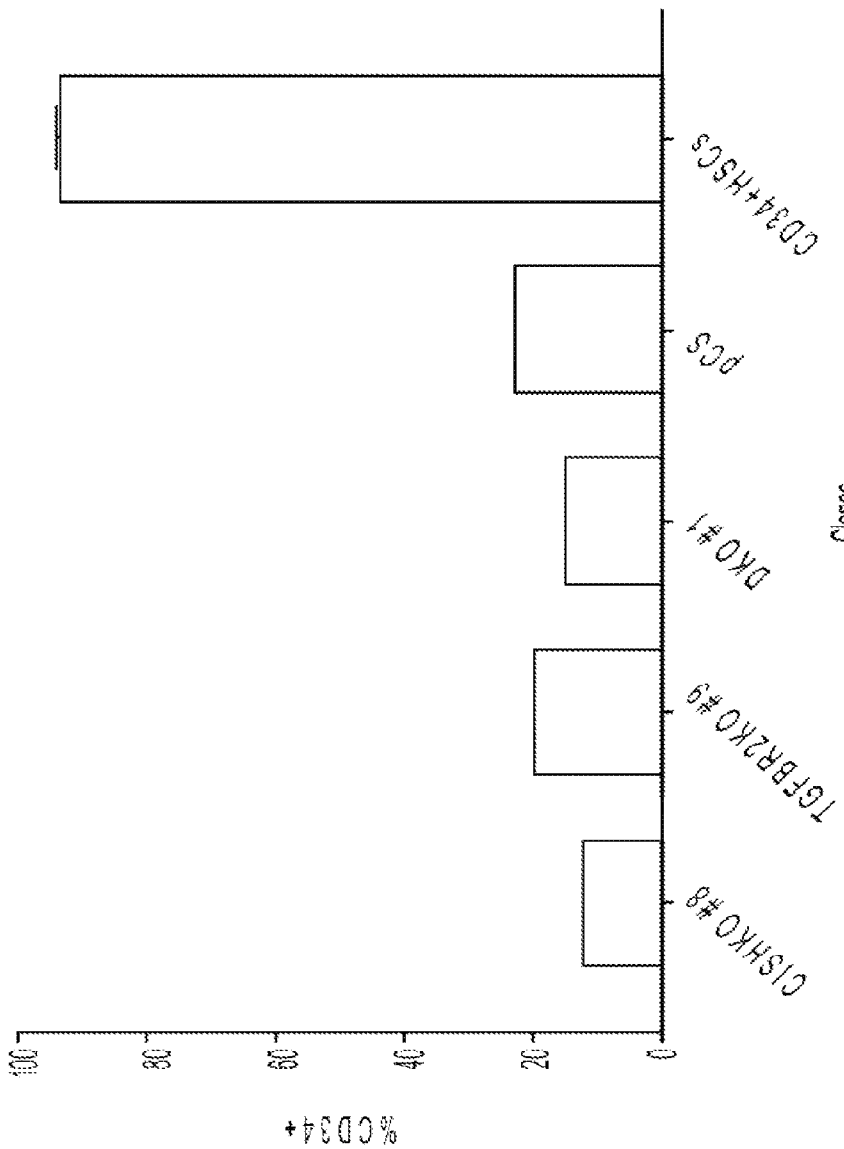

%CD34+
100
80
60
40
20
0
CD34+HSCS
pCS
DKO#1
TGFBR2KO#9
CISHKO#8
Clones

Fig. 48

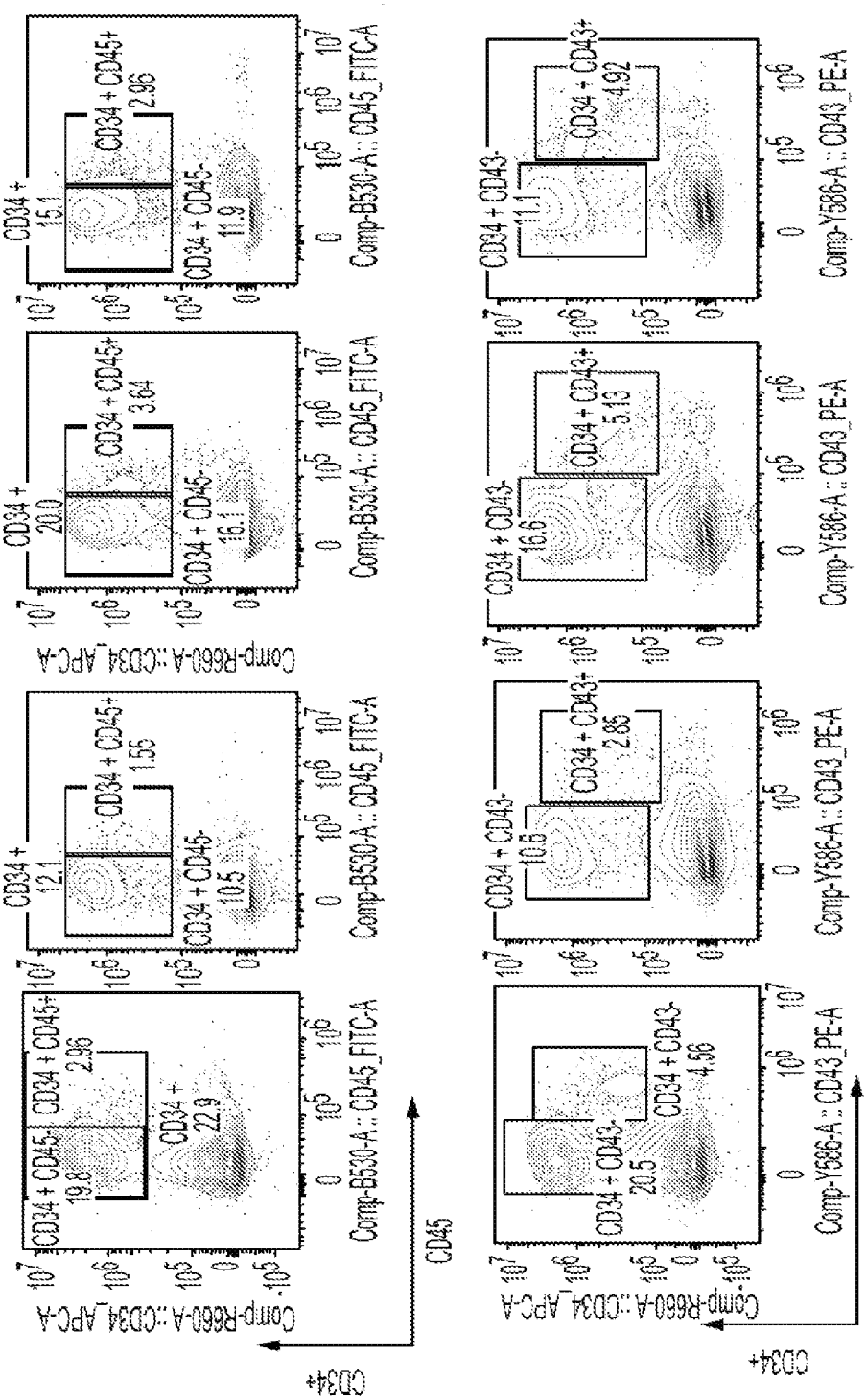

CD34+
CD45
CD43
Comp-R660-A::CD34_APC-A
Comp-B530-A::CD45_FITC-A
Comp-V586-A::CD43_PE-A
CD34 + CD45+
19.8
2.96
CD34 + CD45-
22.9
CD34 + CD45+
12.1
1.56
CD34 + CD45-
10.5
CD34 + CD43-
20.5
CD34 + CD43+
4.56
CD34 + CD43-
10.6
CD34 + CD43+
2.85
CD34 + CD43-
16.6
CD34 + CD43+
5.13
CD34 + CD43-
11.1
CD34 + CD43+
4.92
CD34 + CD45+
20.0
3.64
CD34 + CD45-
16.1
CD34 + CD45+
15.1
2.96
CD34 + CD45-
11.9

Fig. 49

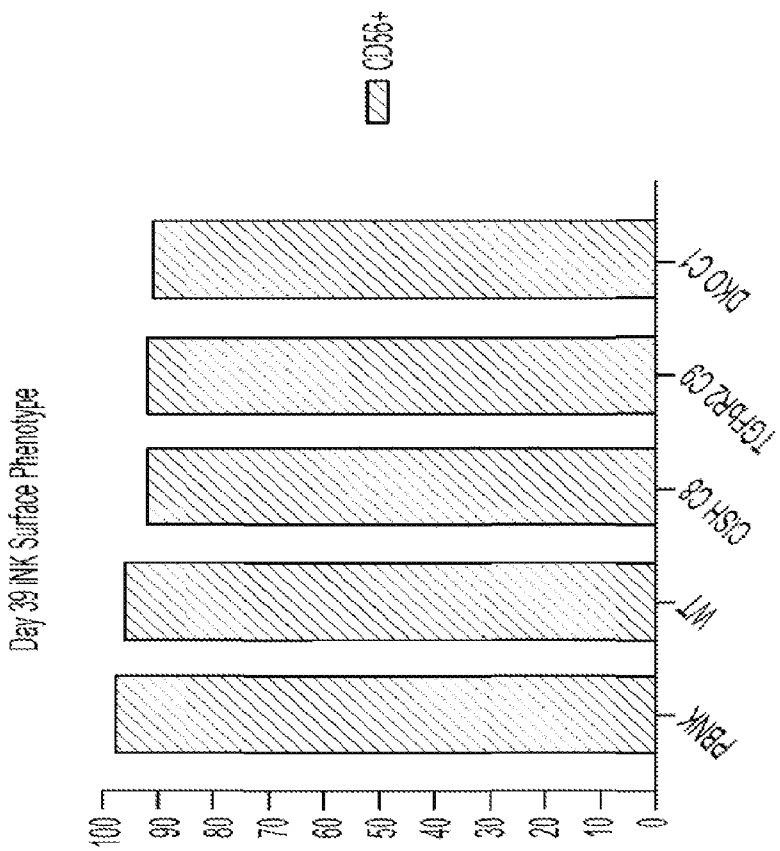

CD56+
Day 39 NK Surface Phenotype
100
90
80
70
60
50
40
30
20
10
0
PBNK
WT
CISH KO
TGFBR2 KO
DKO C1
Cell Line

Fig. 50A

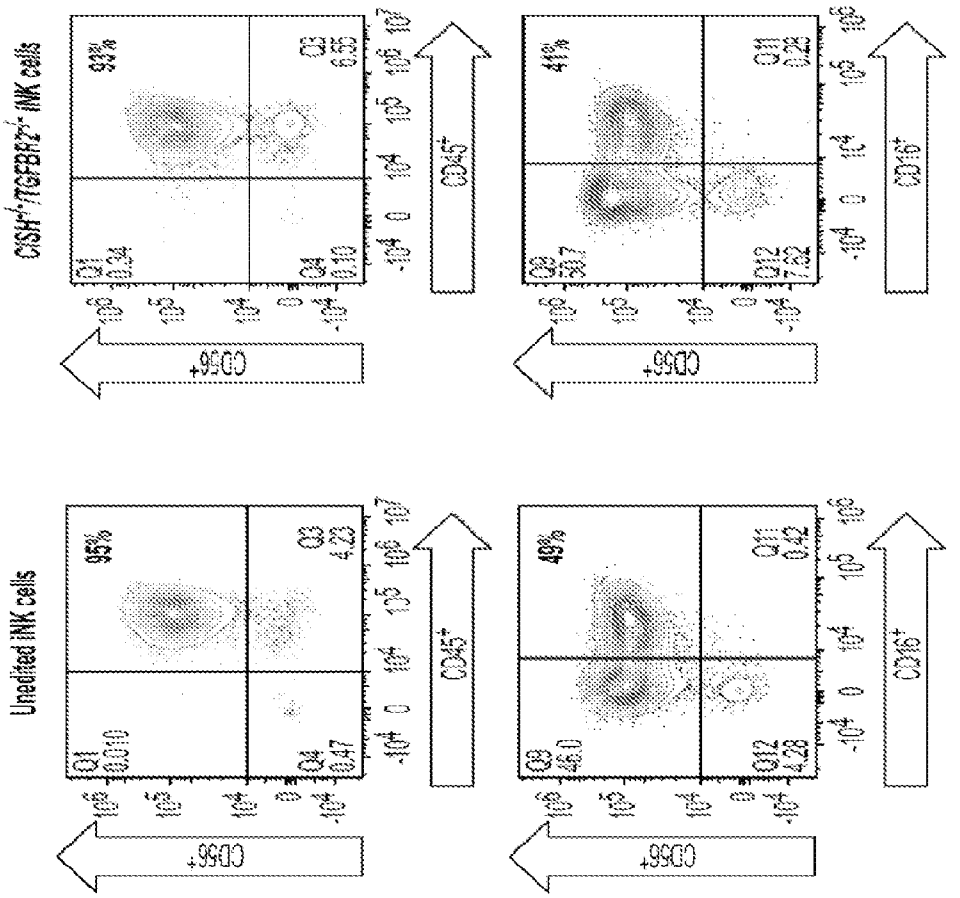

Unedited iNK cells
CISH-/-TGFBR2-/- iNK cells
CD56+
CD45+
CD16+
95%
93%
49%
41%
Q1 0.010
Q4 0.47
Q3 4.23
Q1 0.34
Q4 0.10
Q3 6.55
Q8 46.0
Q12 4.28
Q11 0.42
Q8 50.7
Q12 7.62
Q11 0.28

Fig. 50B

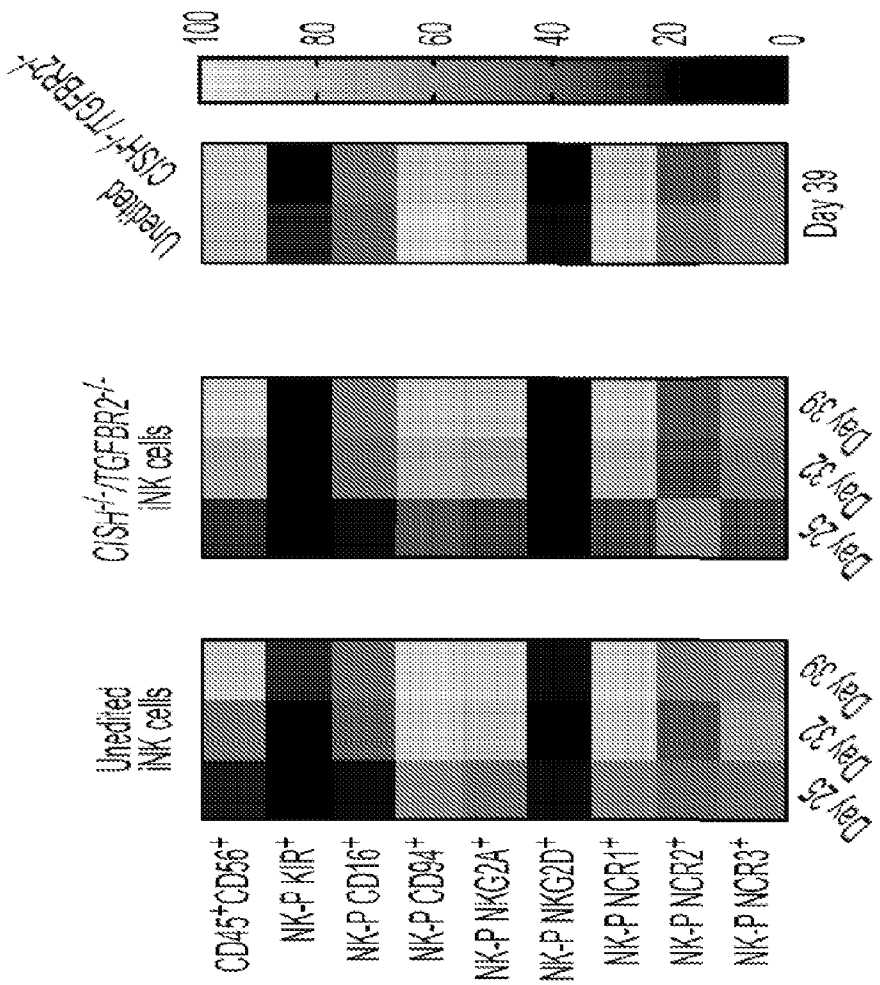

Unedited iNK cells
CISH-/-/TGFBR2-/- iNK cells
Unedited CISH-/-/TGFBR2-/-
Day 25
Day 32
Day 39
Day 39
100
80
60
40
20
0
CD45+CD56+
NK-P KIR+
NK-P CD16+
NK-P CD94+
NK-P NKG2A+
NK-P NKG2D+
NK-P NCR1+
NK-P NCR2+
NK-P NCR3+

Fig. 50C

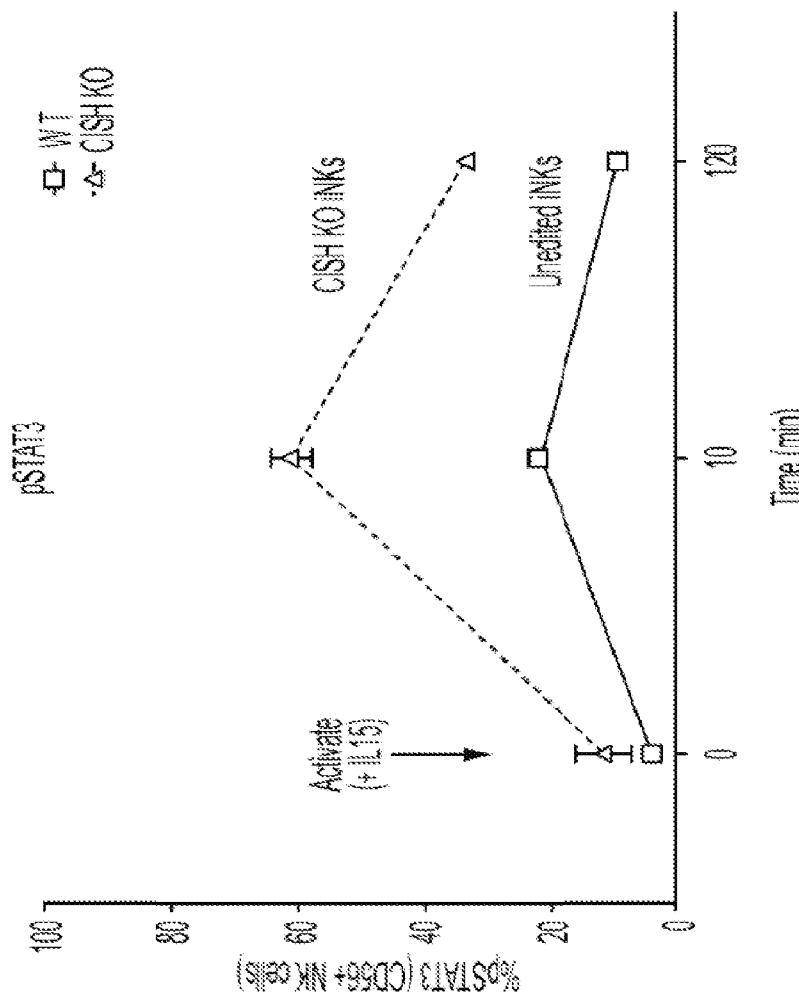

pSTAT3
WT
CISH KO
CISH KO iNKs
Unedited iNKs
Activate (+IL15)
%pSTAT3 (CD56+ NK cells)
100
80
60
40
20
0
0
10
120
Time (min)

Fig. 50D

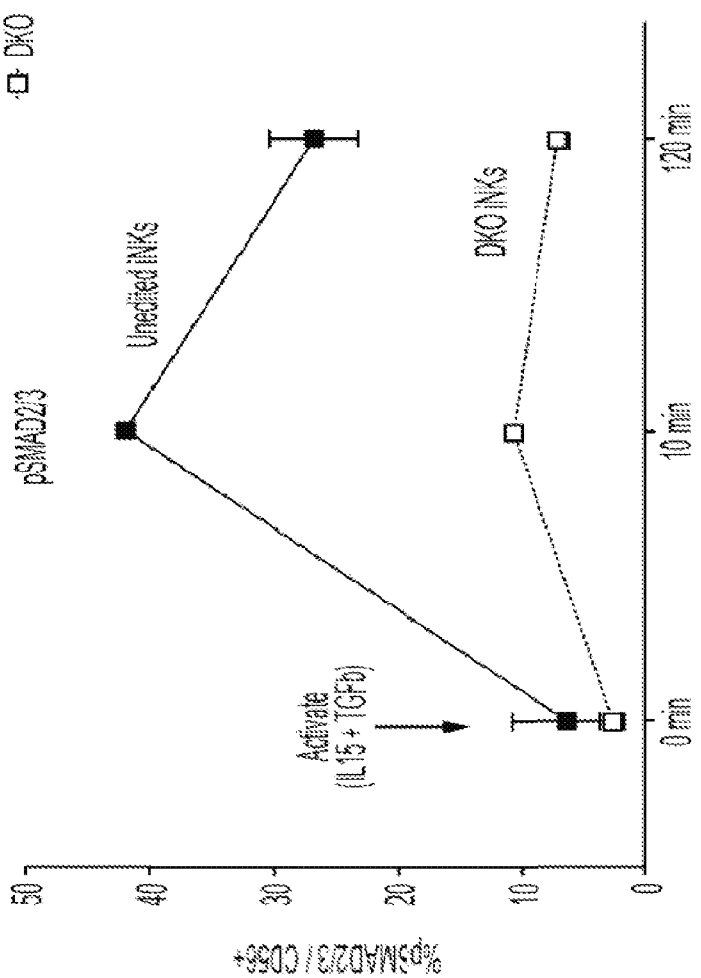

DKO
pSMAD2/3
Unedited iNKs
DKO iNKs
Activate
(IL15 + TGFb)
0 min
10 min
120 min
Time (minutes)
%pSMAD2/3 / CD8+
0
10
20
30
40
50

Fig. 50E

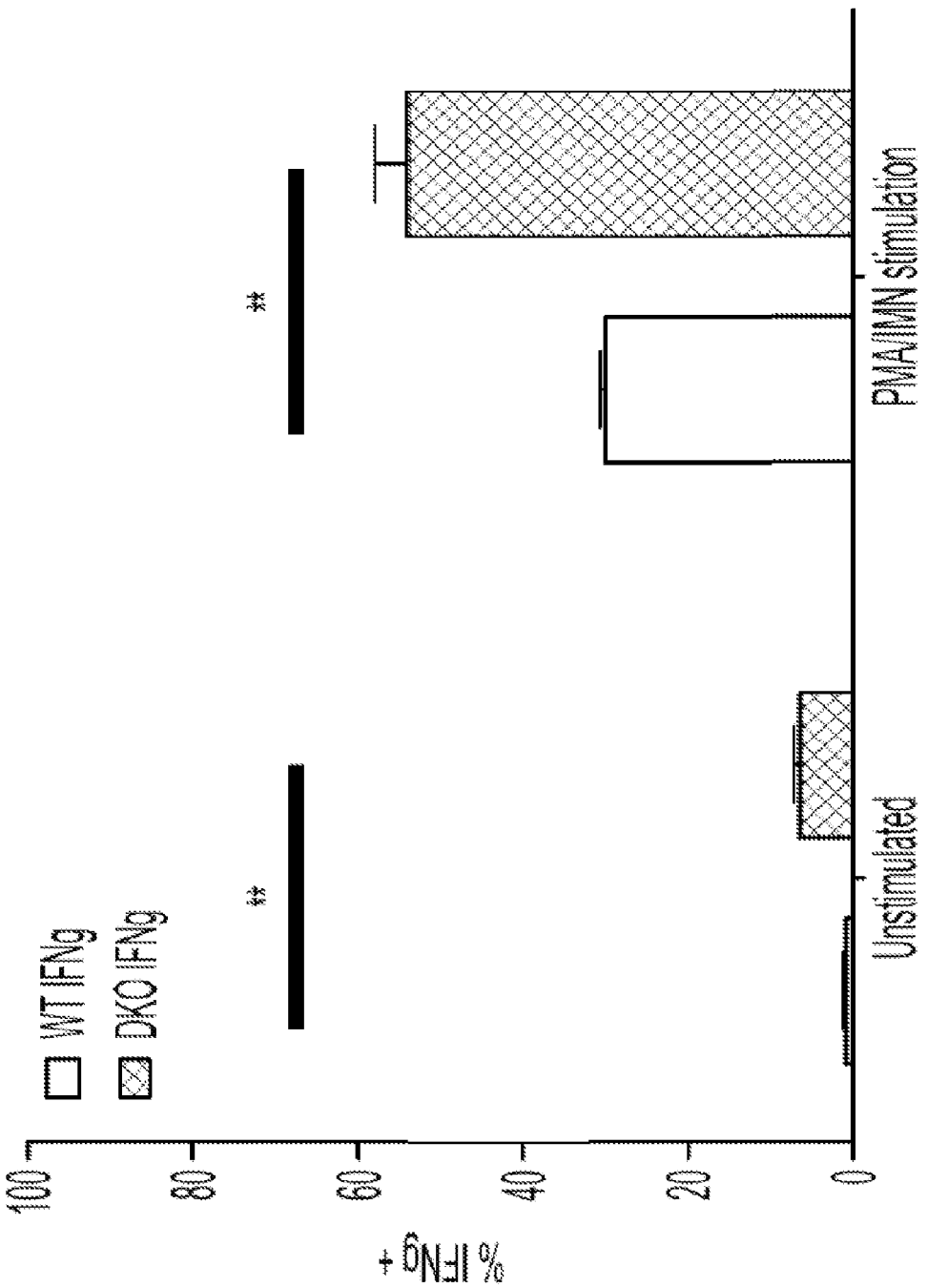

WT IFNg
DKO IFNg
**
**
% IFNg+
100
80
60
40
20
0
Unstimulated
PMA/IMN stimulation

Fig. 50F

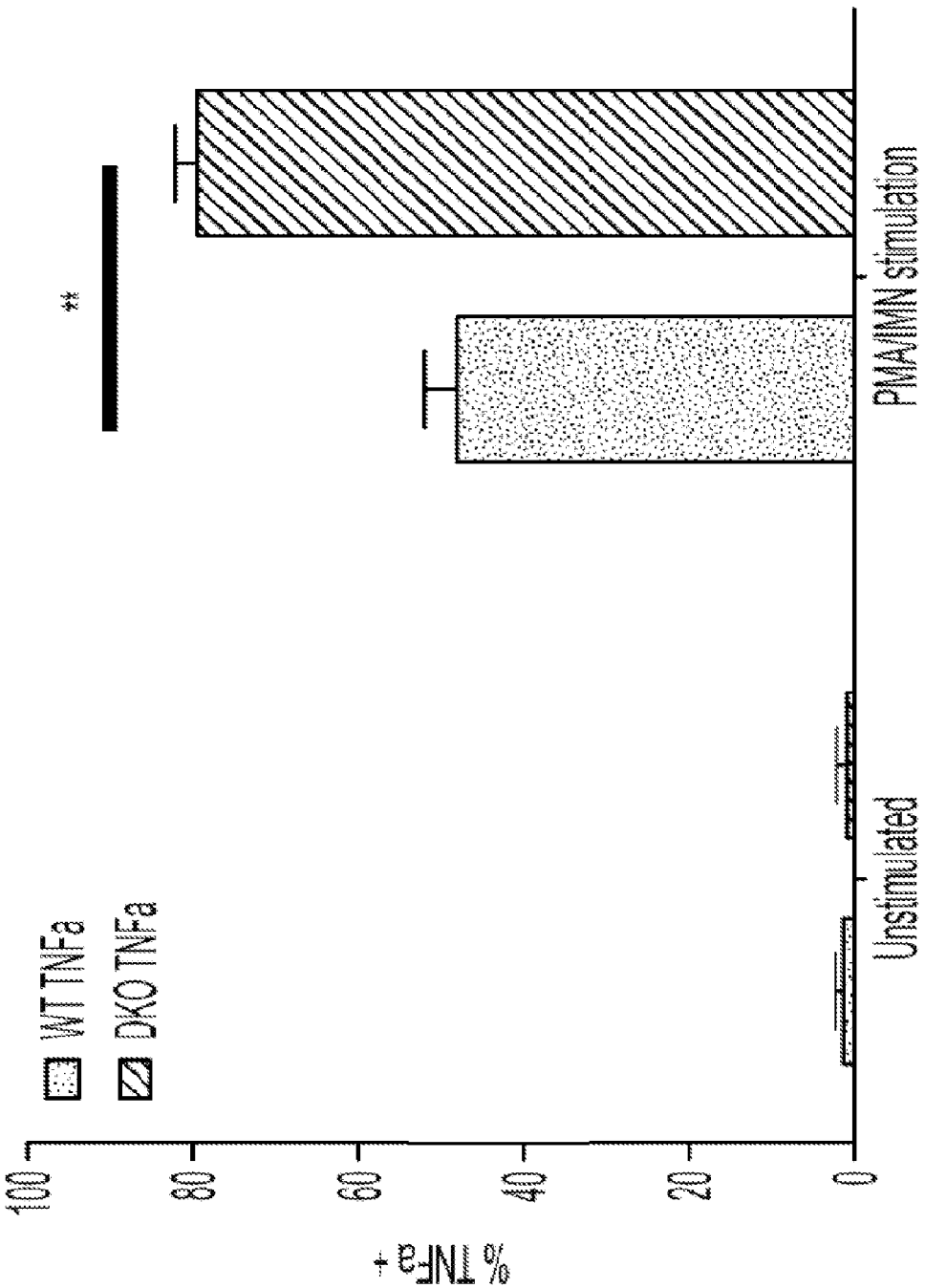

% TNFa +
100
80
60
40
20
0
WT TNFa
DKO TNFa
**
Unstimulated
PMA/IMN stimulation

Fig. 50G

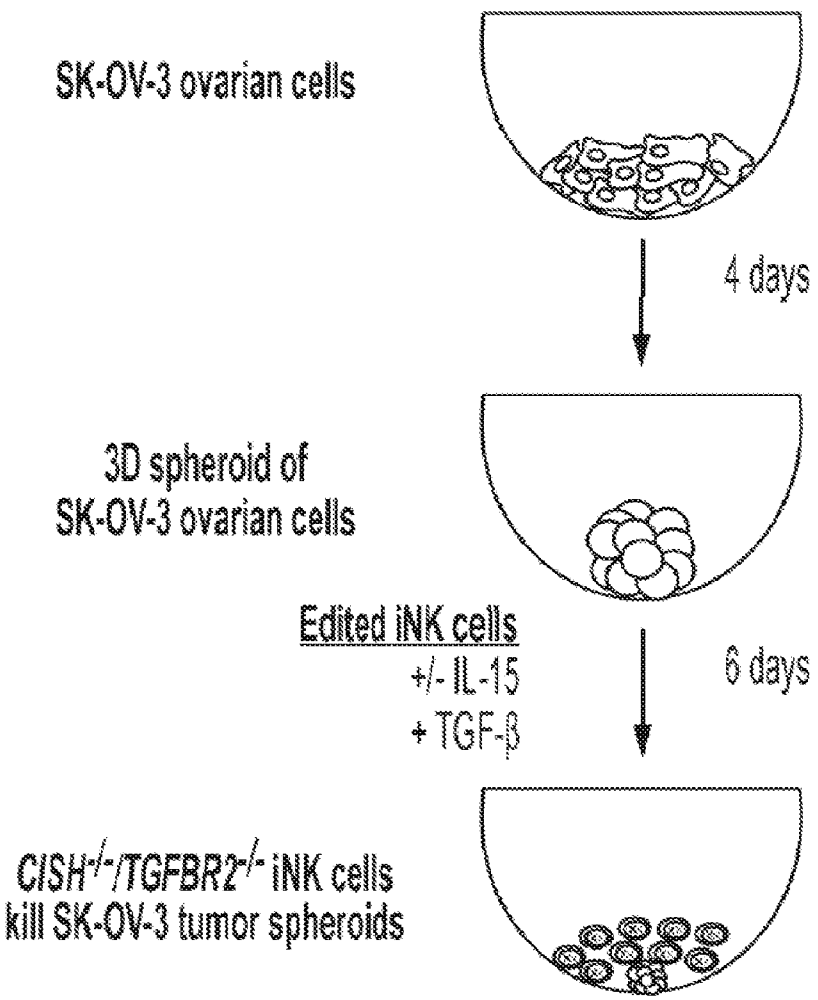

SK-OV-3 ovarian cells
4 days
3D spheroid of
SK-OV-3 ovarian cells
Edited iNK cells
+/- IL-15
+ TGF-β
6 days
CISH-/-/TGFBR2-/- iNK cells
kill SK-OV-3 tumor spheroids

Fig. 51A

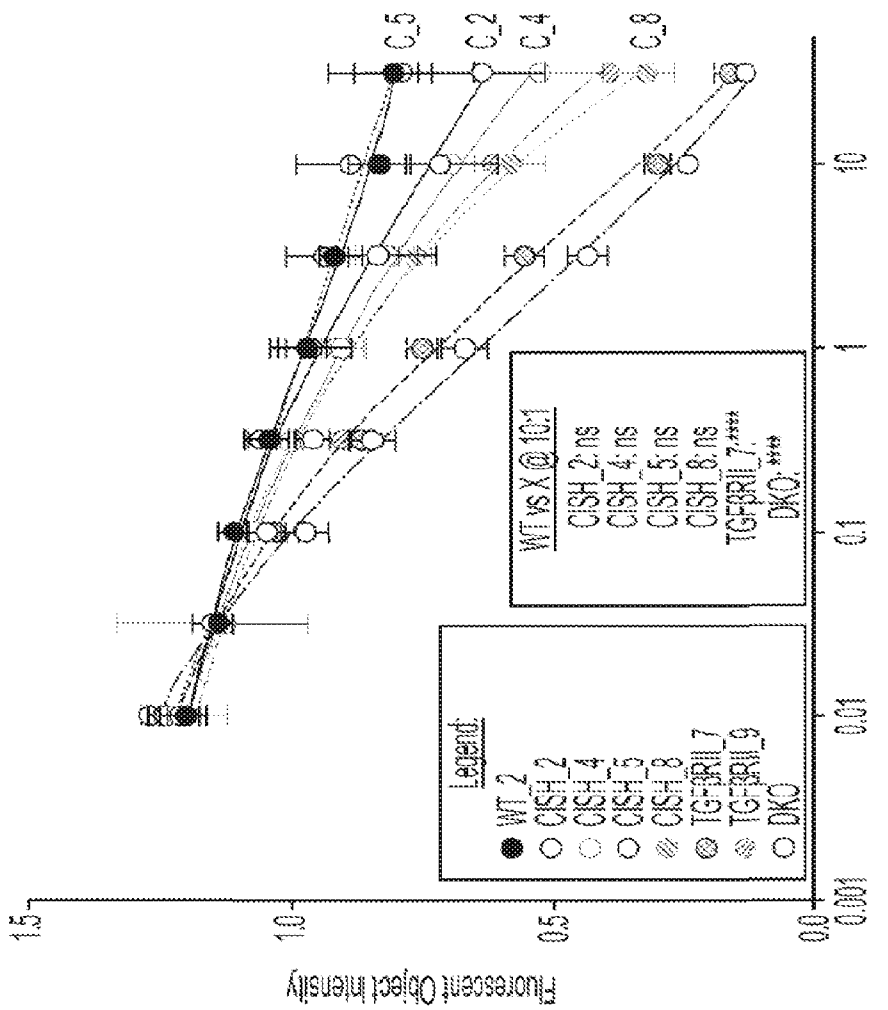

Fluorescent Object Intensity
1.5
1.0
0.5
0.0
0.001
0.01
0.1
1
10
E:T Ratio
C_5
C_2
C_4
C_8
Legend
WT_2
CISH_2
CISH_4
CISH_5
CISH_8
TGFBRII_7
TGFBRII_9
DKO
WT vs X @ 10:1
CISH_2 ns
CISH_4 ns
CISH_5 ns
CISH_8 ns
TGFBRII_7 ****
DKO ***

Fig. 51B

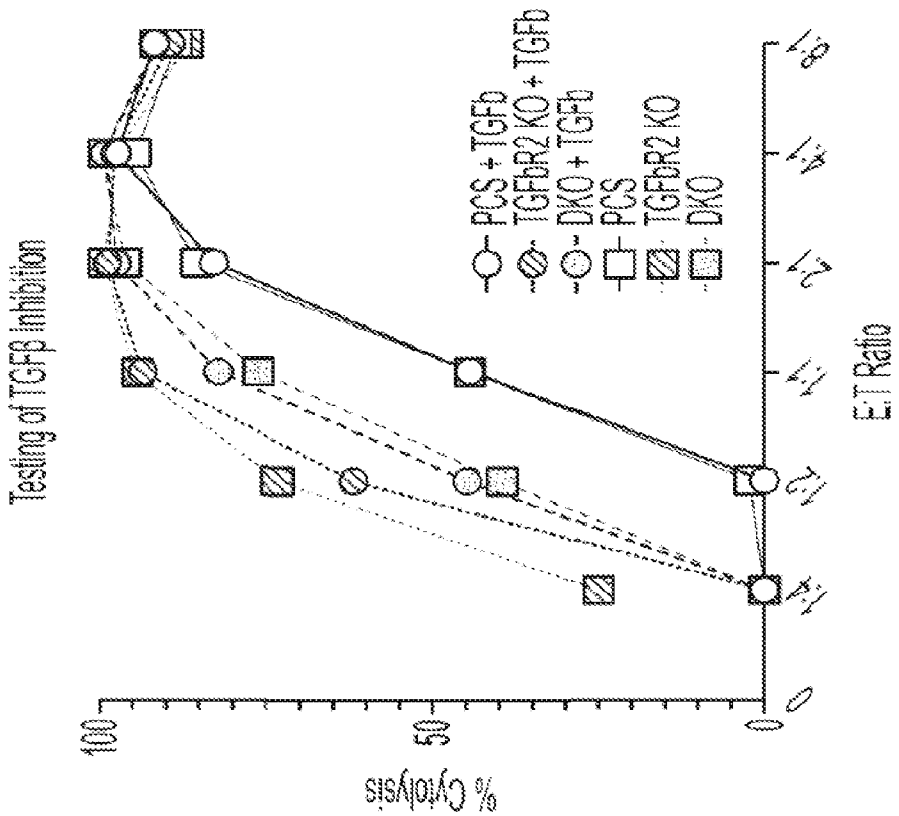

Testing of TGFβ Inhibition
% Cytolysis
100
50
0
1/8
1/4
1/2
1/1
2/1
4/1
0
E:T Ratio
PCS + TGFb
TGFbR2 KO + TGFb
DKO + TGFb
PCS
TGFbR2 KO
DKO

Fig. 51C

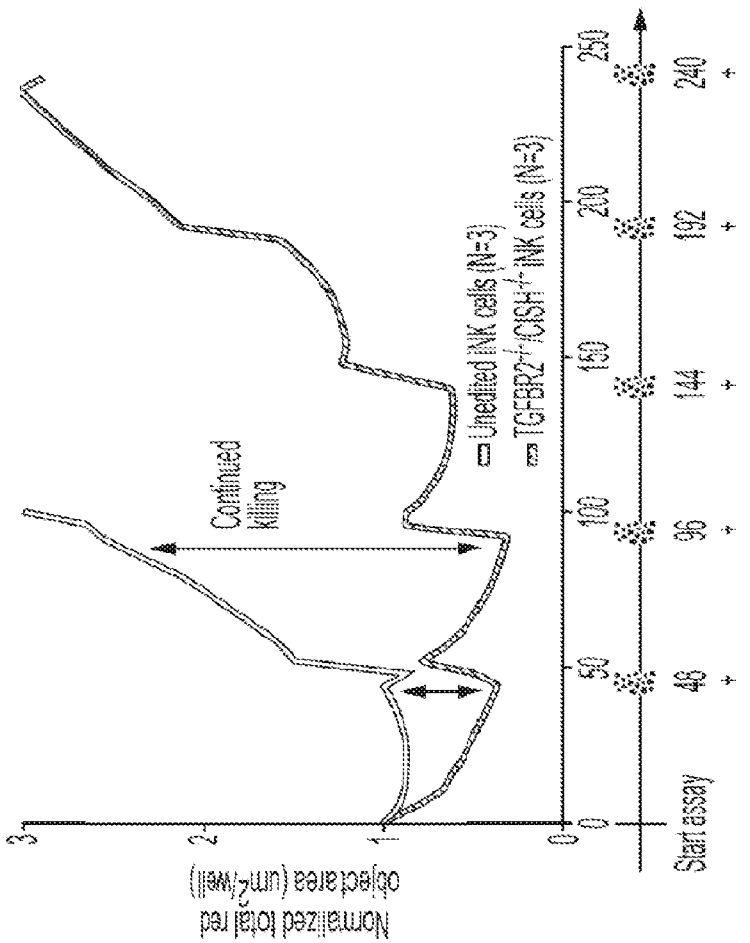

Normalized total red object area (um²/well)
3
2
1
0
Continued killing
Unedited INK cells (N=3)
TGFBR2⁻/⁻/CISH⁻/⁻ INK cells (N=3)
Start assay
0
50
100
150
200
250
46
96
144
192
240
Time (h)
= Tumor cell addition (5x10³ cells)

Fig. 52

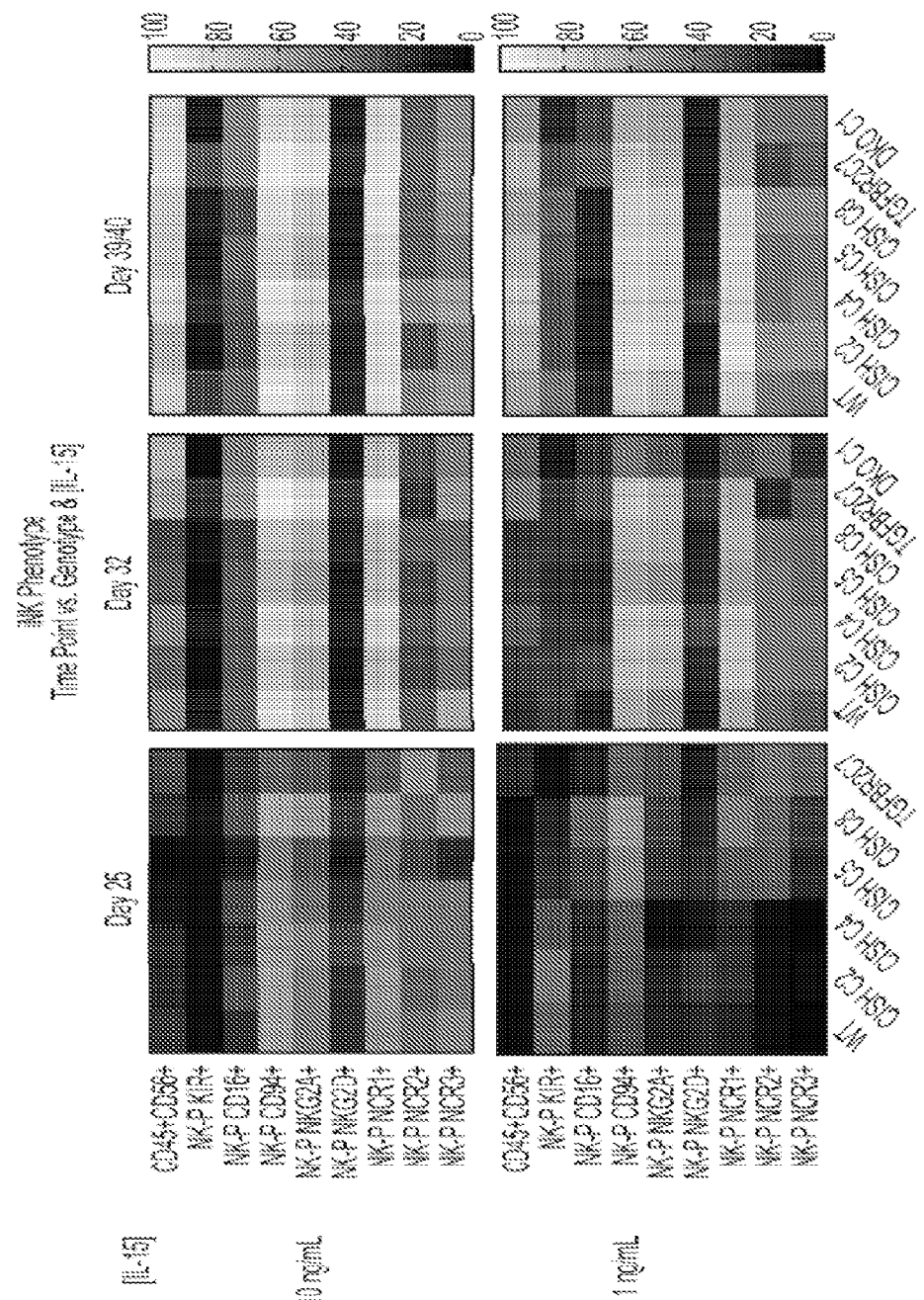

NK Phenotype
Time Point vs. Genotype & [IL-15]
Day 25
Day 32
Day 3940
[IL-15]
10 ng/mL
1 ng/mL
CD45+CD56+
NK-P KIR+
NK-P CD16+
NK-P CD94+
NK-P NKG2A+
NK-P NKG2D+
NK-P NCR1+
NK-P NCR2+
NK-P NCR3+
100
80
60
40
20
0
WT
CISH KO
SOCS1 KO
PTPN2 KO
CD2AP KO
CBLB KO
CBLC KO
DKO

Fig. 53

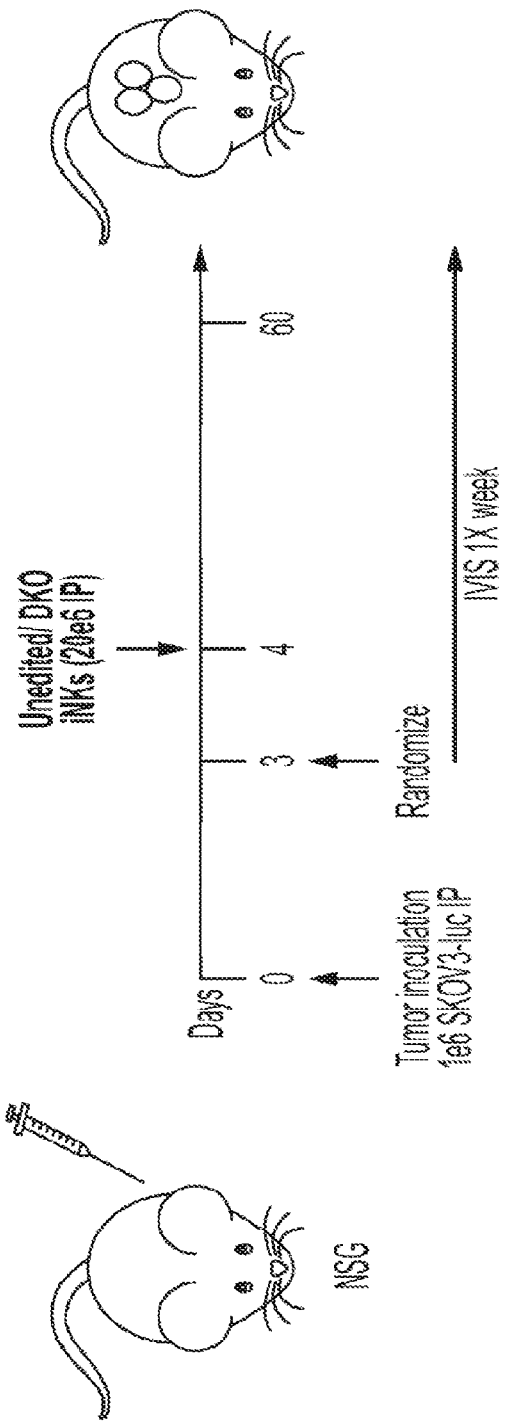

Unedited DKO
iNKs (2e6 IP)
Days
0
3
4
60
Tumor inoculation
1e6 SKOV3-luc IP
Randomize
IVIS 1 X week
NSG

Fig. 54A

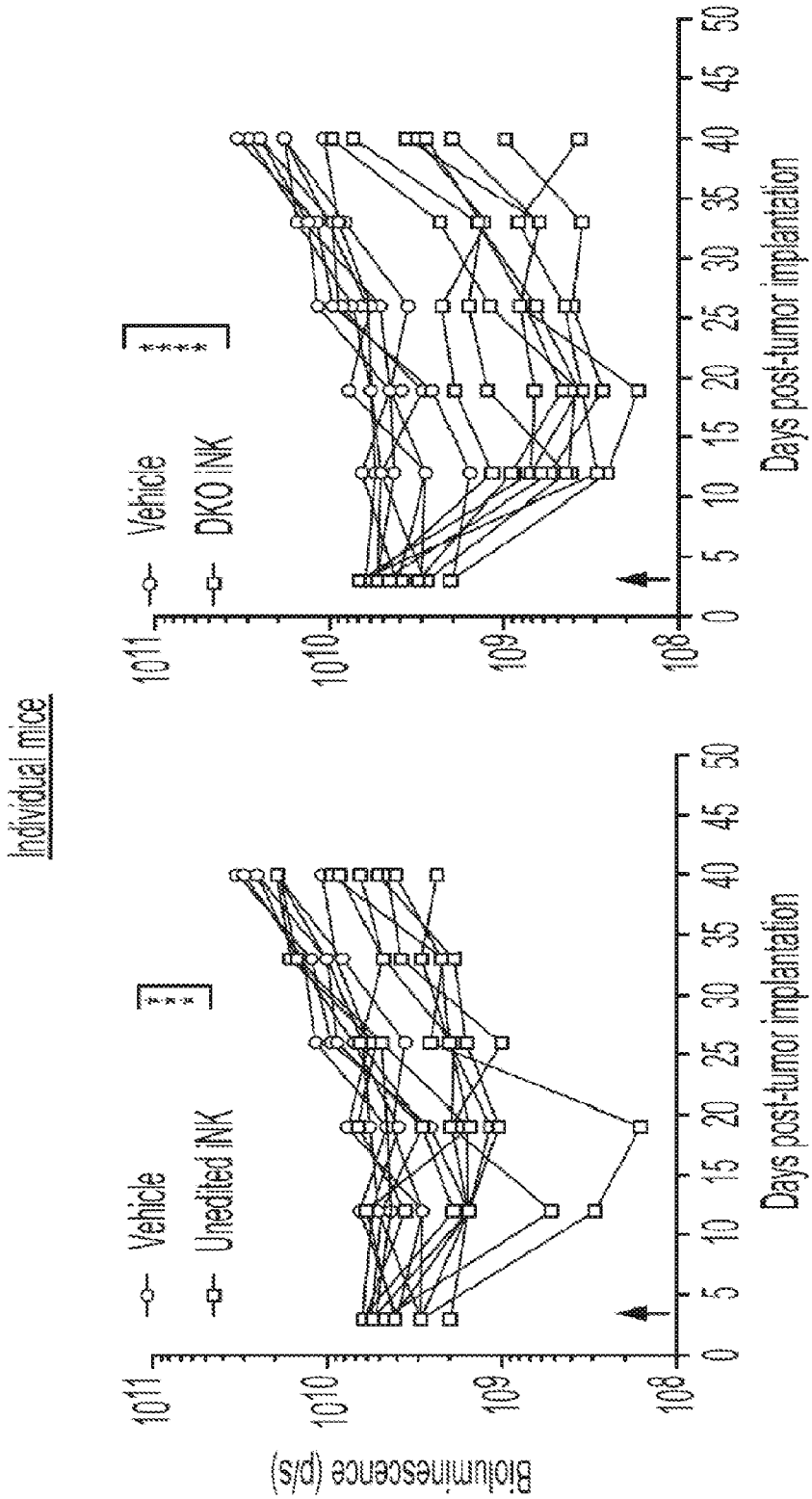

Individual mice
Bioluminescence (p/s)
Vehicle
Unedited iNK
DKO iNK
****
Days post-tumor implantation

Fig. 54B

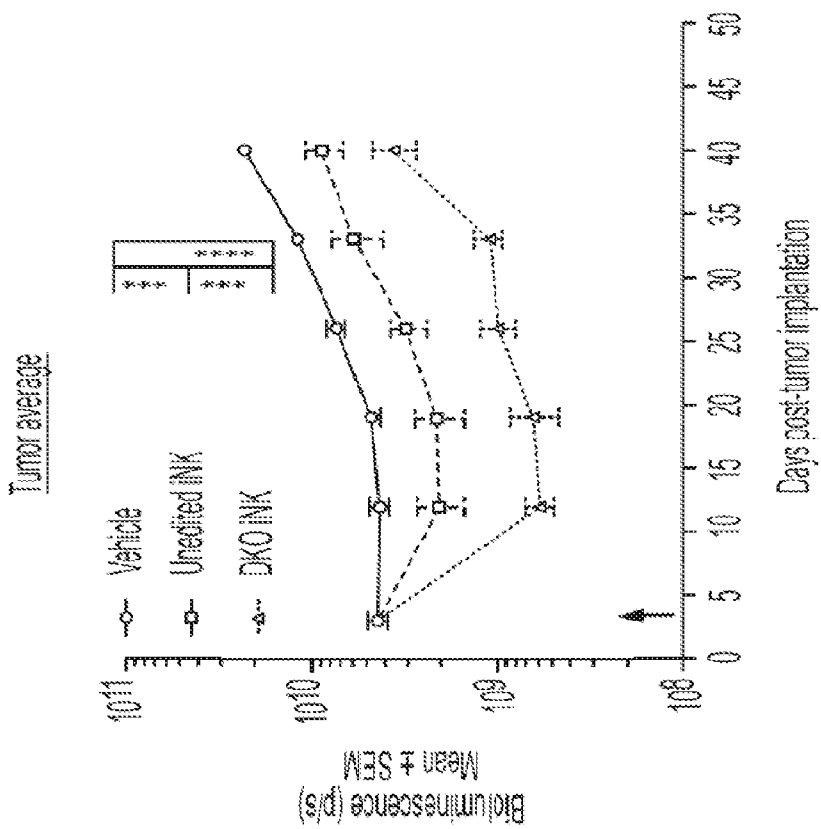

Tumor average
Vehicle
Unedited iNK
DKO iNK
***
***
***
Bioluminescence (p/s)
Mean ± SEM
10^11
10^10
10^9
10^8
0
5
10
15
20
25
30
35
40
45
50
Days post-tumor implantation

Fig. 54C

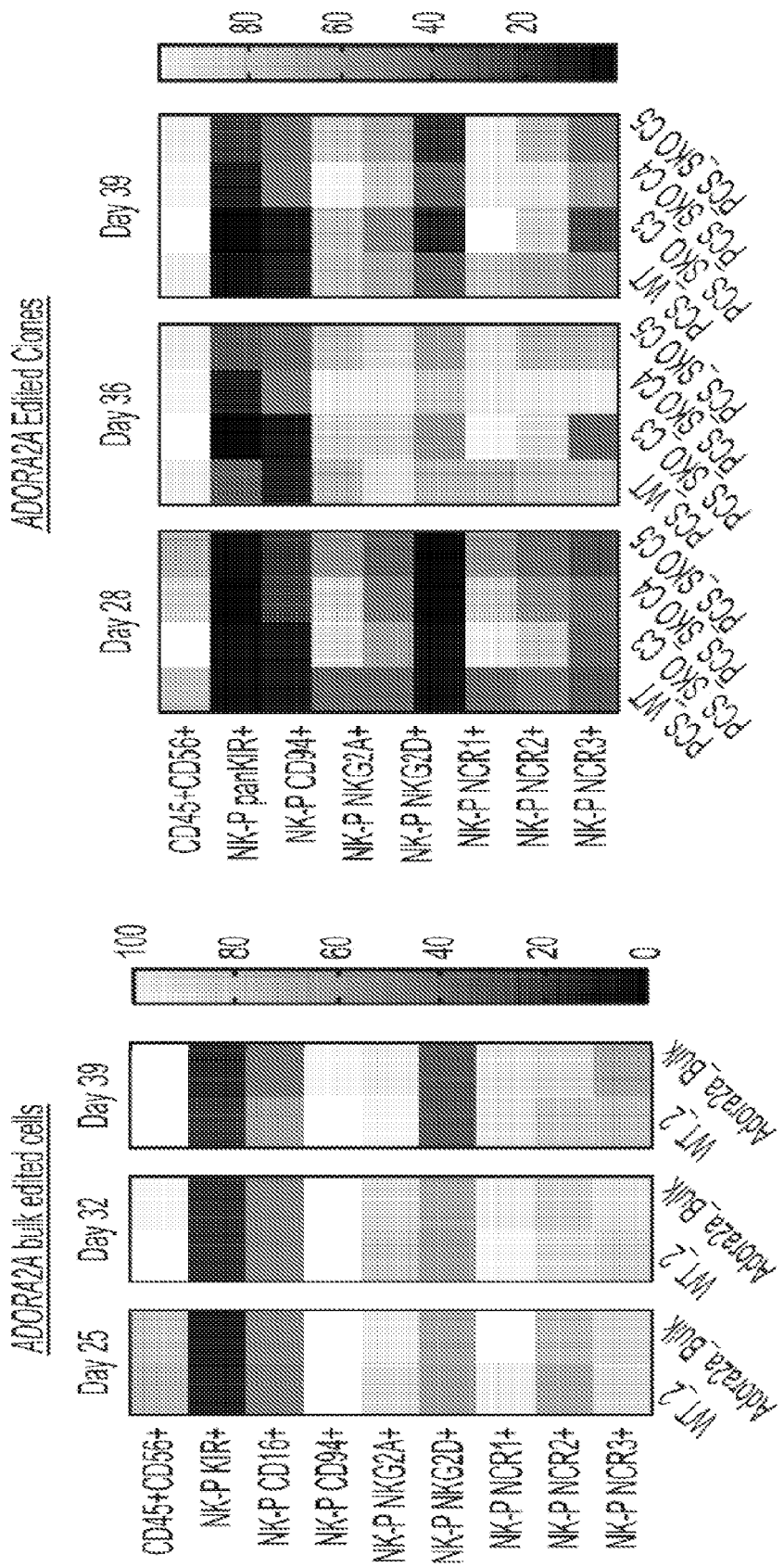

ADORA2A bulk edited cells
Day 25
Day 32
Day 39
CD45+CD56+
NK-P KIR+
NK-P CD16+
NK-P CD94+
NK-P NKG2A+
NK-P NKG2D+
NK-P NCR1+
NK-P NCR2+
NK-P NCR3+
WT_2
Adora2a_Bulk
100
80
60
40
20
0
ADORA2A Edited Clones
Day 28
Day 36
Day 39
CD45+CD56+
NK-P panKIR+
NK-P CD94+
NK-P NKG2A+
NK-P NKG2D+
NK-P NCR1+
NK-P NCR2+
NK-P NCR3+
PCS_WT
PCS_SKO_C3
PCS_SKO_C4
80
60
40
20

Fig. 55A

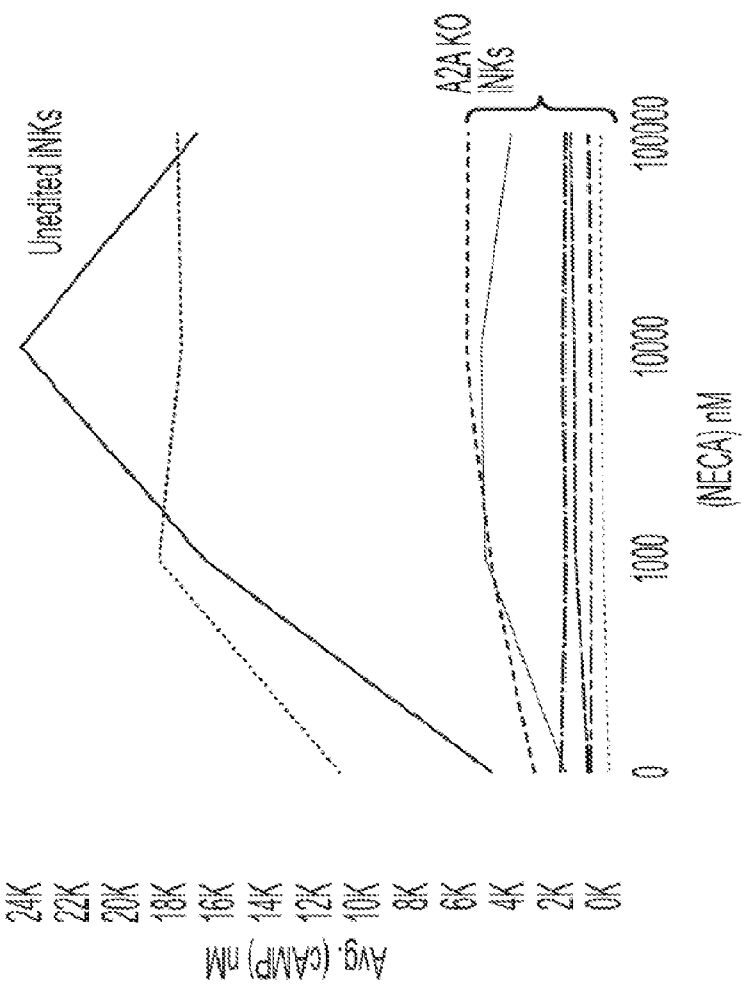

Unedited NKs
A2A KO NKs
Avg. (cAMP) nM
24K
22K
20K
18K
16K
14K
12K
10K
8K
6K
4K
2K
0K
0
1000
10000
100000
(MECA) nM

Fig. 55B

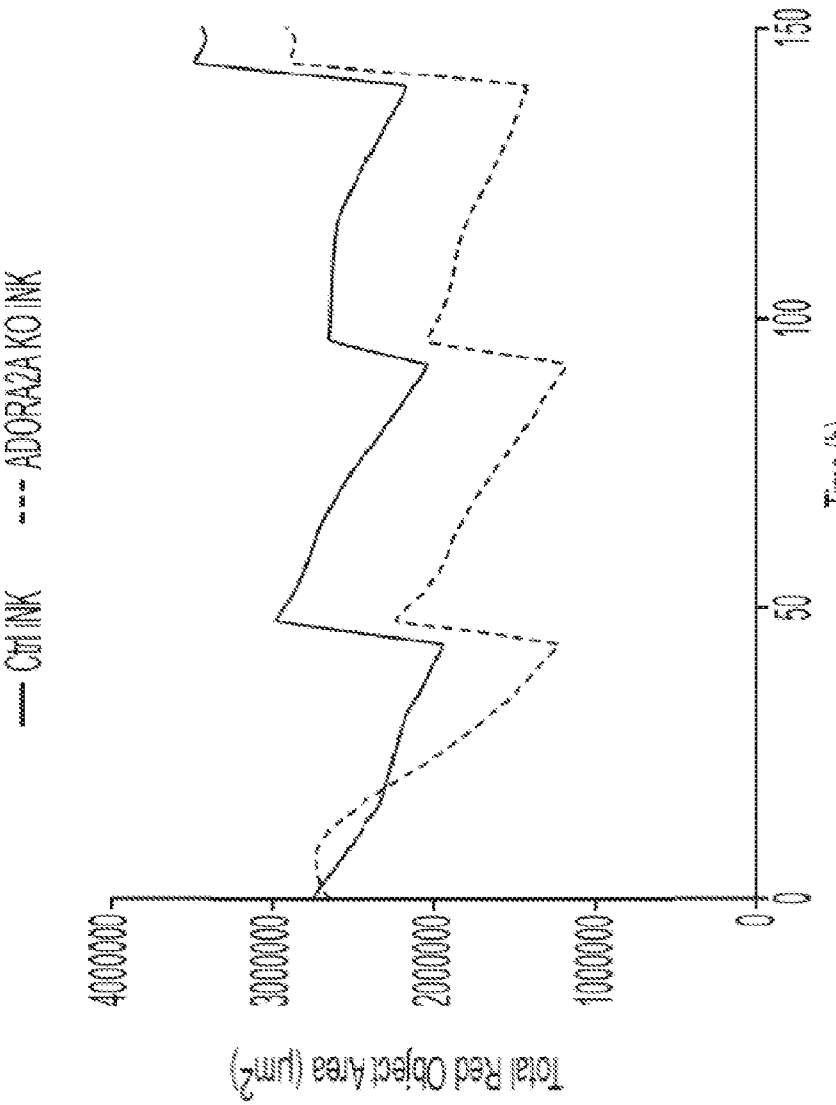

Ctrl INK
ADORA2A KO INK
Total Red Object Area (µm²)
4000000
3000000
2000000
1000000
0
0
50
100
150
Time (h)

Fig. 55C

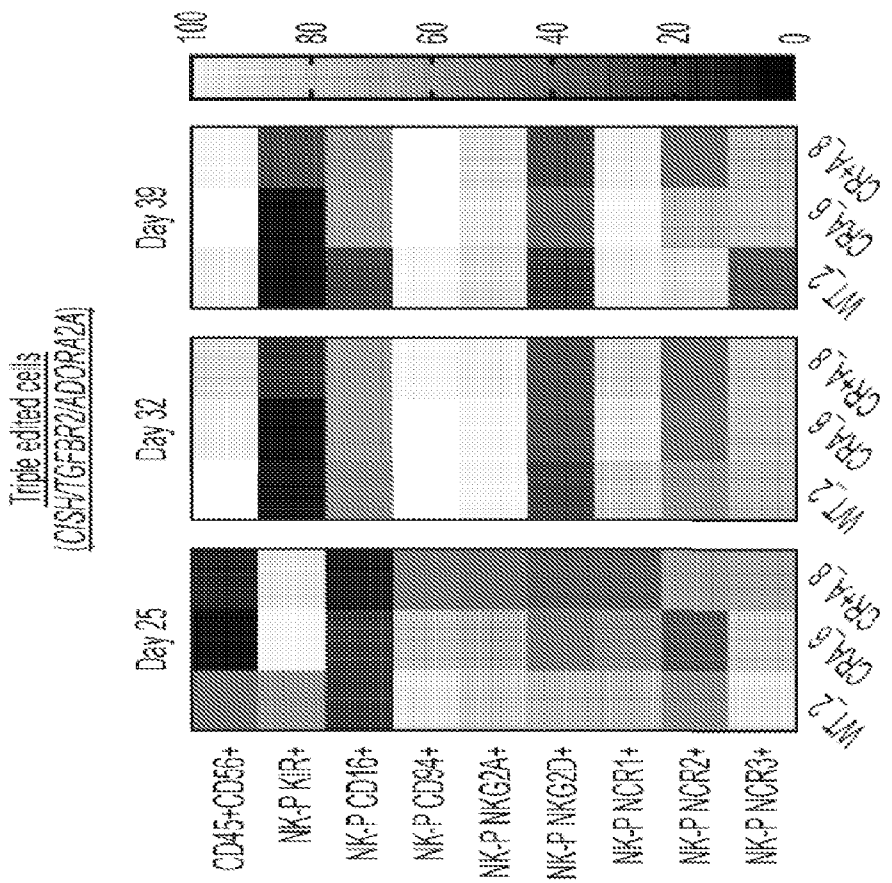

Triple edited cells
(CISH/TGFBR2/ADORA2A)
Day 25
Day 32
Day 39
WT 2
CBA_6
CBA_8
CD45+CD56+
NK-P KIR+
NK-P CD16+
NK-P CD94+
NK-P NKG2A+
NK-P NKG2D+
NK-P NCR1+
NK-P NCR2+
NK-P NCR3+
100
80
60
40
20
0

Fig. 56A

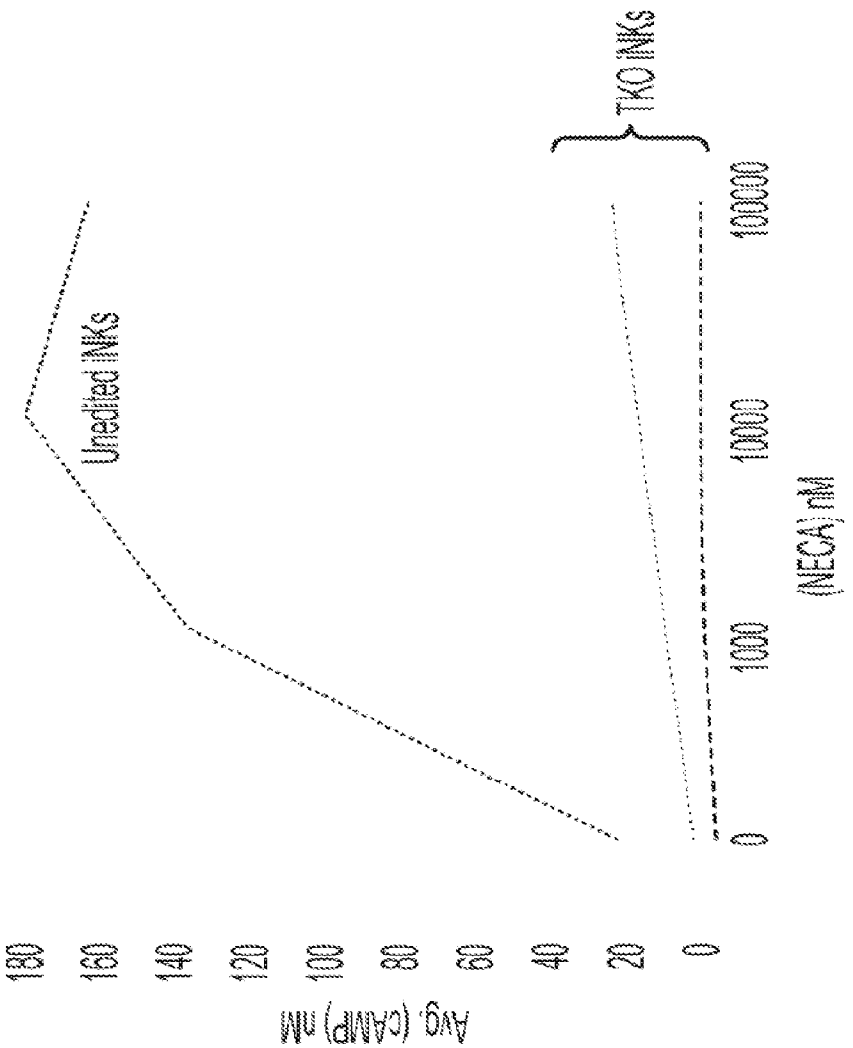

Avg. (cAMP) nM
180
160
140
120
100
80
60
40
20
0
Unedited iNKs
TKO iNKs
0
1000
10000
100000
(NECA) nM

Fig. 56B

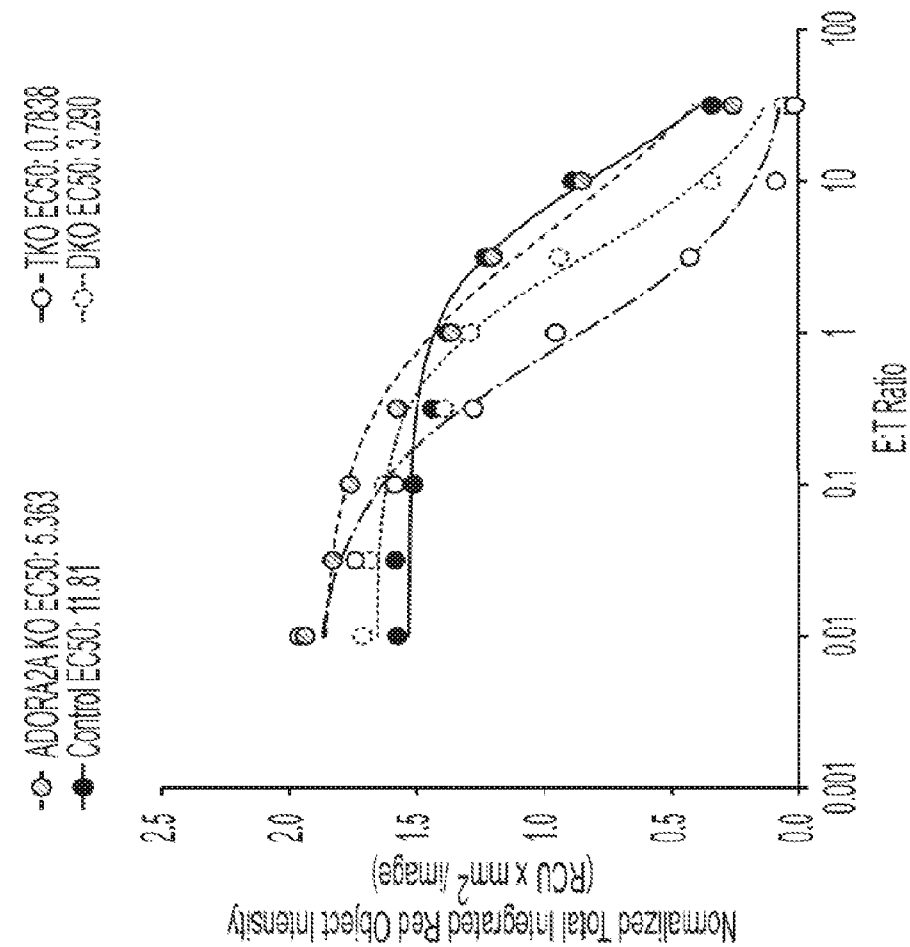

ADORA2A KO EC50: 5.363
Control EC50: 11.81
TKO EC50: 0.7838
DKO EC50: 3.290
Normalized Total Integrated Red Object Intensity
(RCU x mm²/image)
2.5
2.0
1.5
1.0
0.5
0.0
0.001
0.01
0.1
1
10
100
E:T Ratio

Fig. 56C

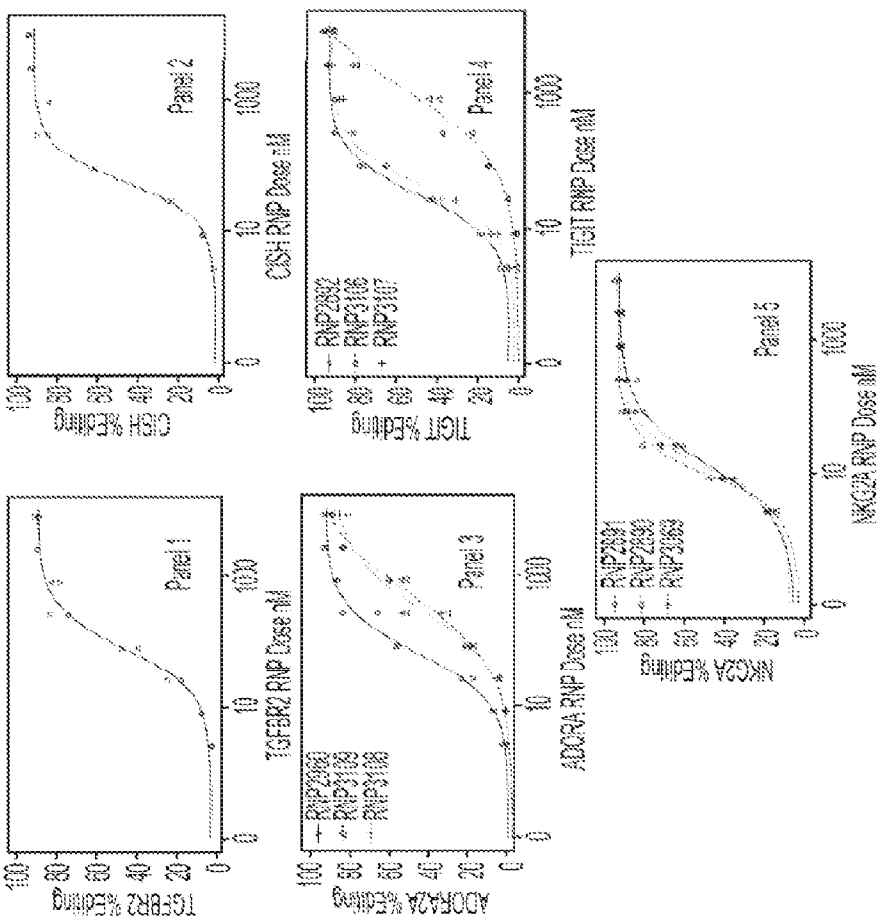

Panel 1
TGFBR2 %Editing
TGFBR2 RNP Dose nM
Panel 2
CISH %Editing
CISH RNP Dose nM
Panel 3
ADORA2A %Editing
ADORA RNP Dose nM
RNP2992
RNP3108
RNP3188
Panel 4
TIGIT %Editing
TIGIT RNP Dose nM
RNP2882
RNP3106
RNP3187
Panel 5
NKG2A %Editing
NKG2A RNP Dose nM
RNP2881
RNP2880
RNP3363

Fig. 57

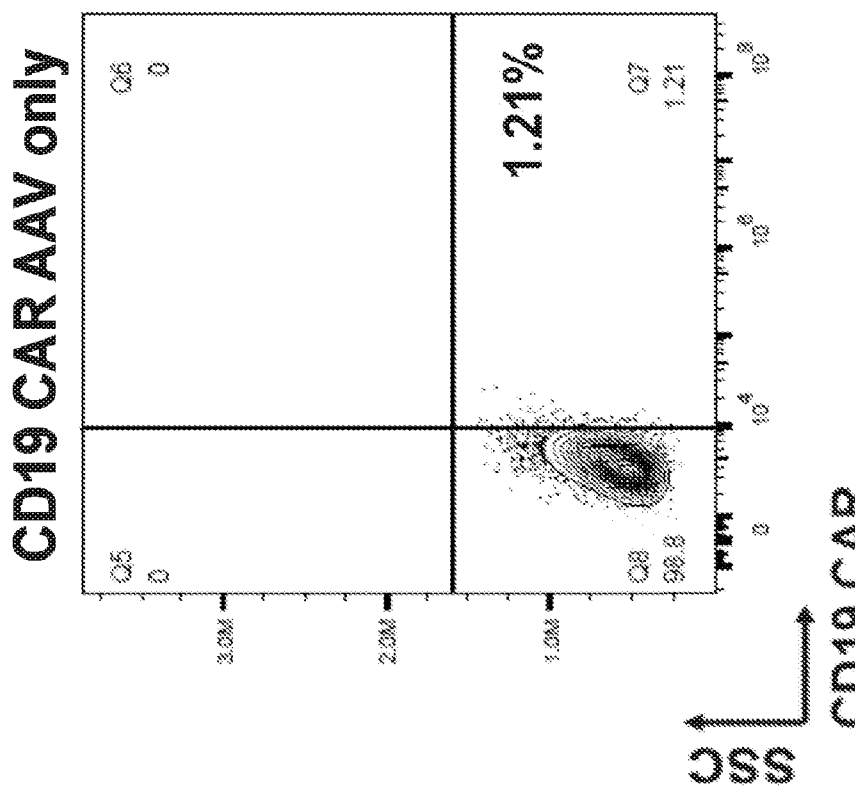


Fig. 58A

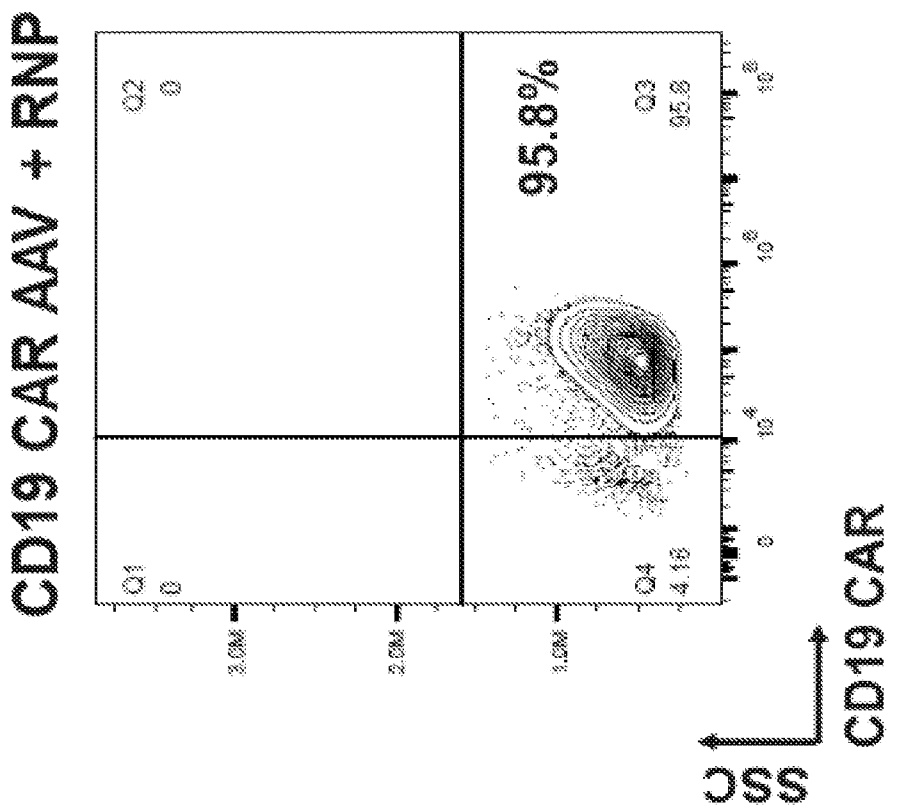


Fig. 58B

T Cell Expansion Following SLEEK KI of CD19 CAR

Sample	Cell count Day 0 (cells/well)	Cell count Day 7 (cells/well)	Fold Change
Mock	2.5 E+05	4.80 E+05	1.92
RNP + AAV6	2.5 E+05	5.58 E+05	2.23

Fig. 58C

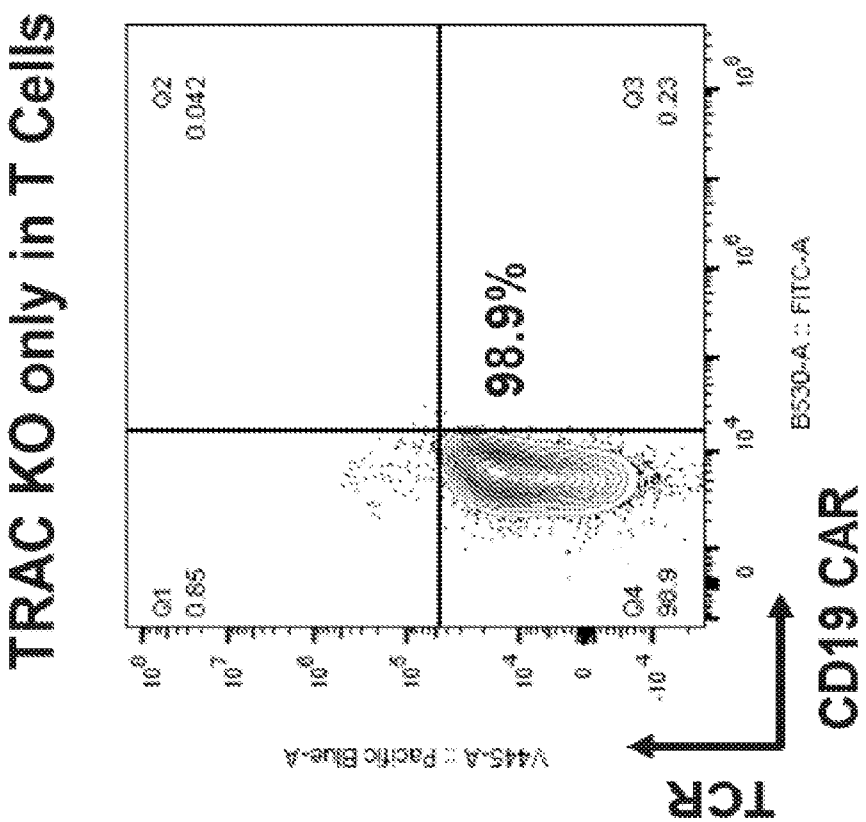

TRAC KO only in T Cells
Q2
0.042
Q3
0.23
98.9%
Q1
0.85
Q4
98.9
V445-A :: Pacific Blue-A
B530-A :: FITC-A
TCR
CD19 CAR

Fig. 58D

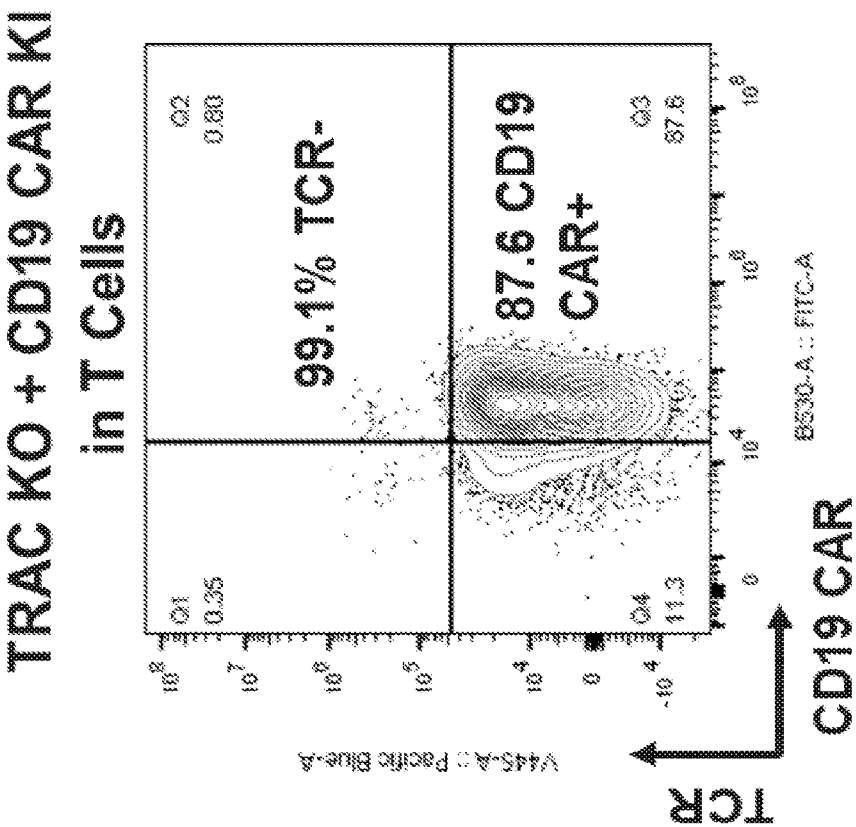

TRAC KO + CD19 CAR KI
in T Cells
99.1% TCR-
87.6 CD19
CAR+
0.2
0.80
0.1
0.35
0.4
11.3
0.3
87.6
TCR
CD19 CAR
V445-A :: Pacific Blue-A
B530-A :: FITC-A

Fig. 58E

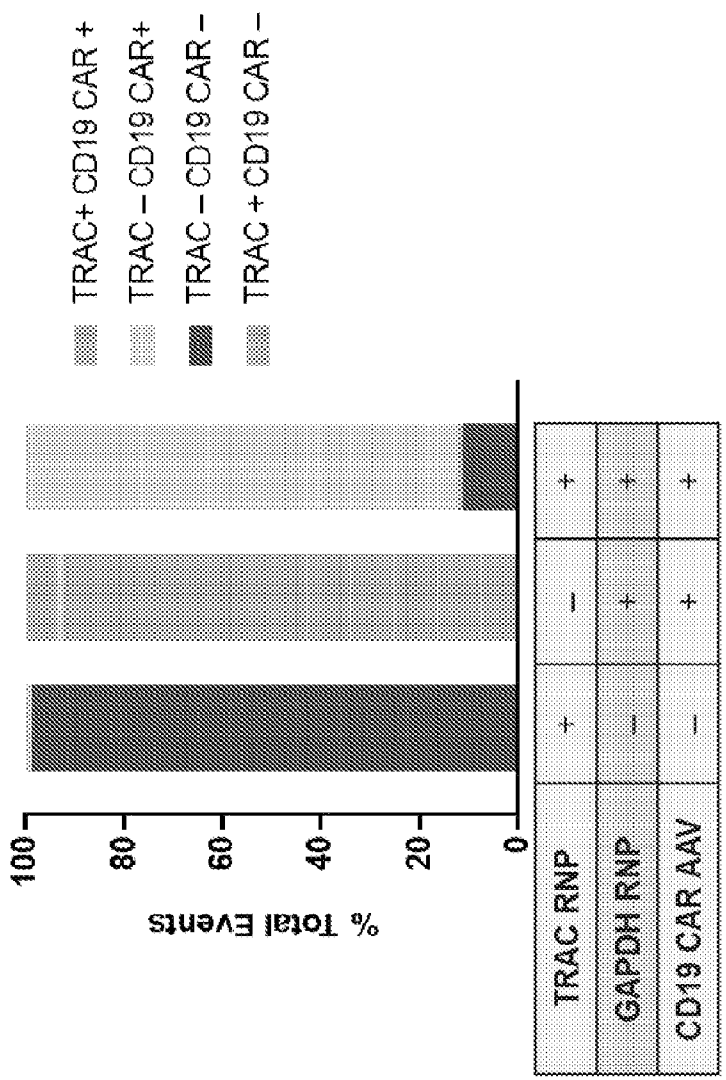

TRAC+ CD19 CAR +
TRAC – CD19 CAR+
TRAC – CD19 CAR –
TRAC + CD19 CAR –
% Total Events
100
80
60
40
20
0
TRAC RNP
GAPDH RNP
CD19 CAR AAV
+
–
+
–
+
+
+
+

Fig. 58F

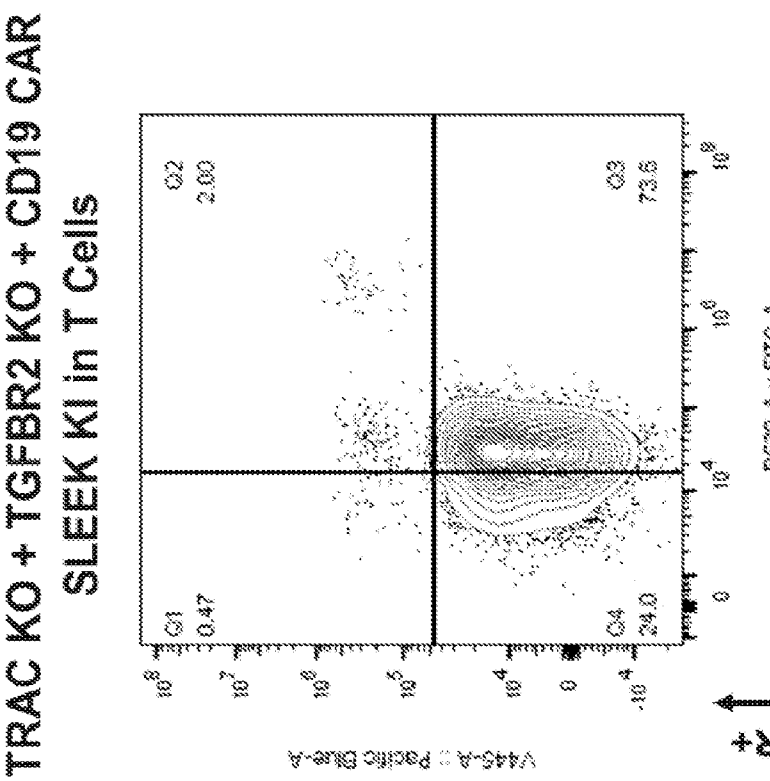

TRAC KO + TGFBR2 KO + CD19 CAR
SLEEK KI in T Cells
Q1
0.47
Q2
2.00
Q3
73.6
Q4
24.0
V450-A :: Pacific Blue-A
B530-A :: FITC-A
TCR+
CD19 CAR+

Fig. 58G

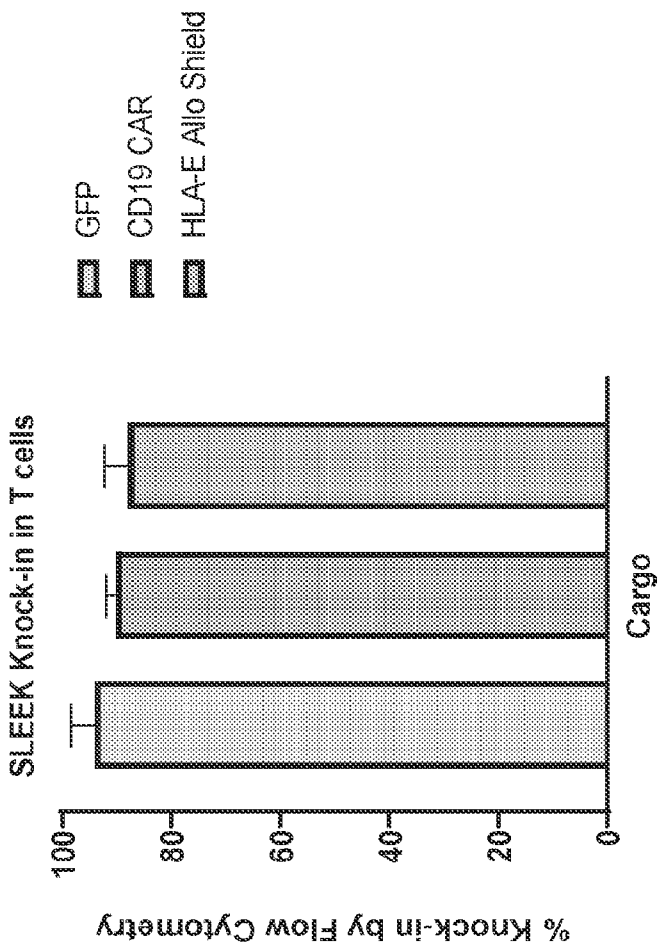

SLEEK Knock-in in T cells
GFP
CD19 CAR
HLA-E Allo Shield
% Knock-in by Flow Cytometry
100
80
60
40
20
0
Cargo

Fig. 58H

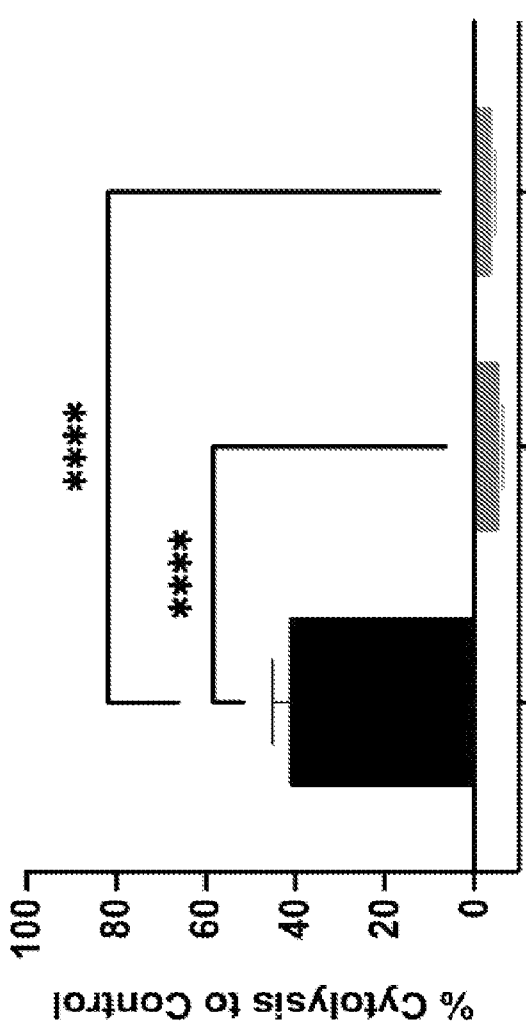

Killing of CD19+ Raji Cells by T Cells
% Cytolysis to Control
100
80
60
40
20
0
****
****
CD19 CAR KI
Cells only
GFP KI
LDH assay after 24 hours, N = 4, 1 biological replicates in 4 technical replicates, +/- SEM, one-way ANOVA, ****, p < 0.0001

Fig. 58I

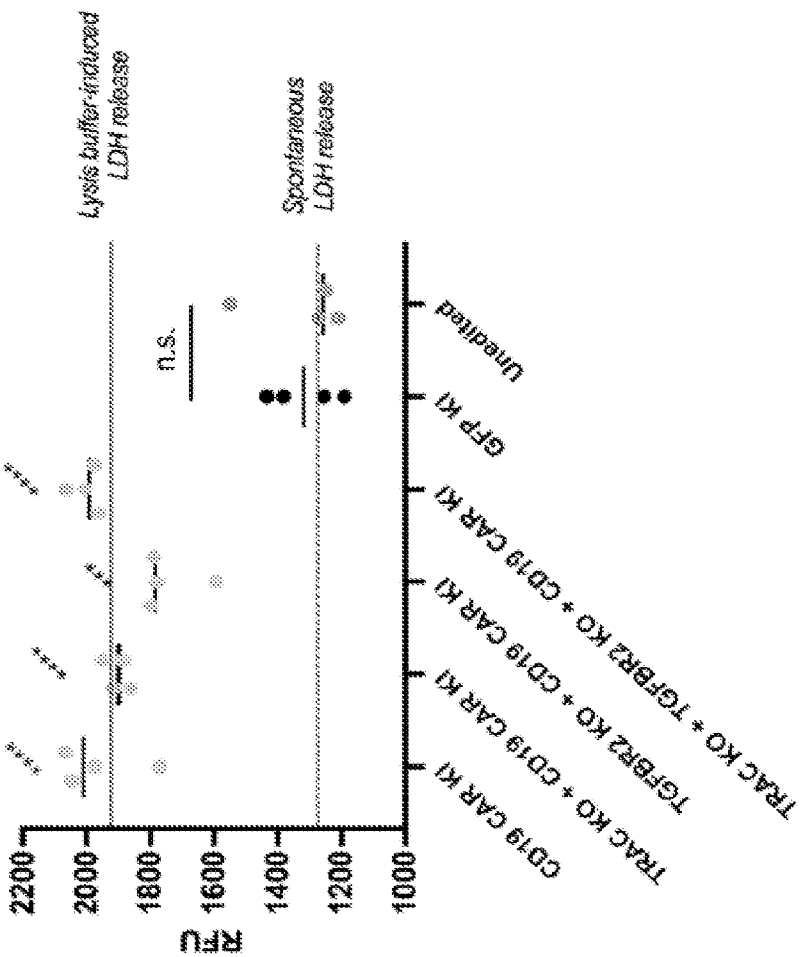
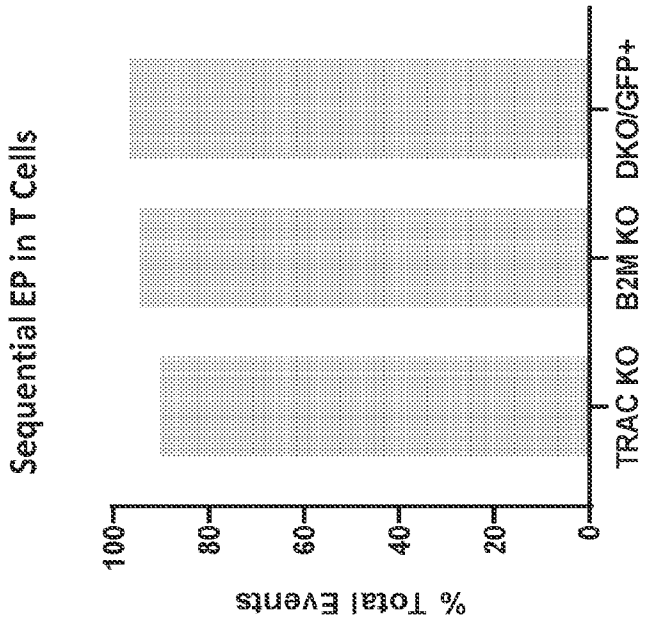

Lysis buffer-induced LDH release
Spontaneous LDH release
n.s.
RFU
2200
2000
1800
1600
1400
1200
1000
Untalted
GFP KI
TRAC KO + TGFBR2 KO + CD19 CAR KI
TGFBR2 KO + CD19 CAR KI
TRAC KO + CD19 CAR KI
CD19 CAR KI

Fig. 58J

T Cell Expansion Following SLEEK KI of B2M-HLA-E

Sample	Cell count Day 0 (cells/well)	Cell count Day 7 (cells/well)	Fold Change
Mock	2.5 E+05	4.10 E+05	1.64
RNP + AAV6	2.5 E+05	4.92 E+05	1.968

Fig. 59


Sequential EP in T Cells
% Total Events
100
80
60
40
20
0
TRAC KO
B2M KO
DKO/GFP+

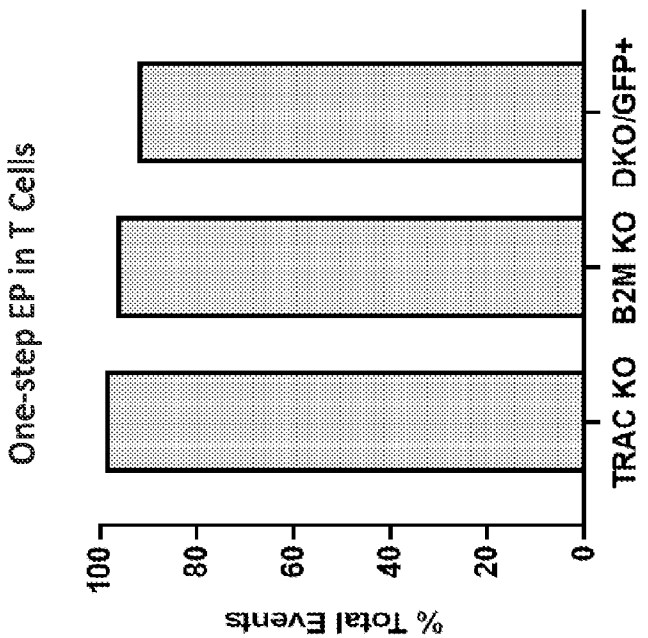

One-step EP in T Cells
% Total Events
100
80
60
40
20
0
TRAC KO
B2M KO
DKO/GFP+

Fig. 60A

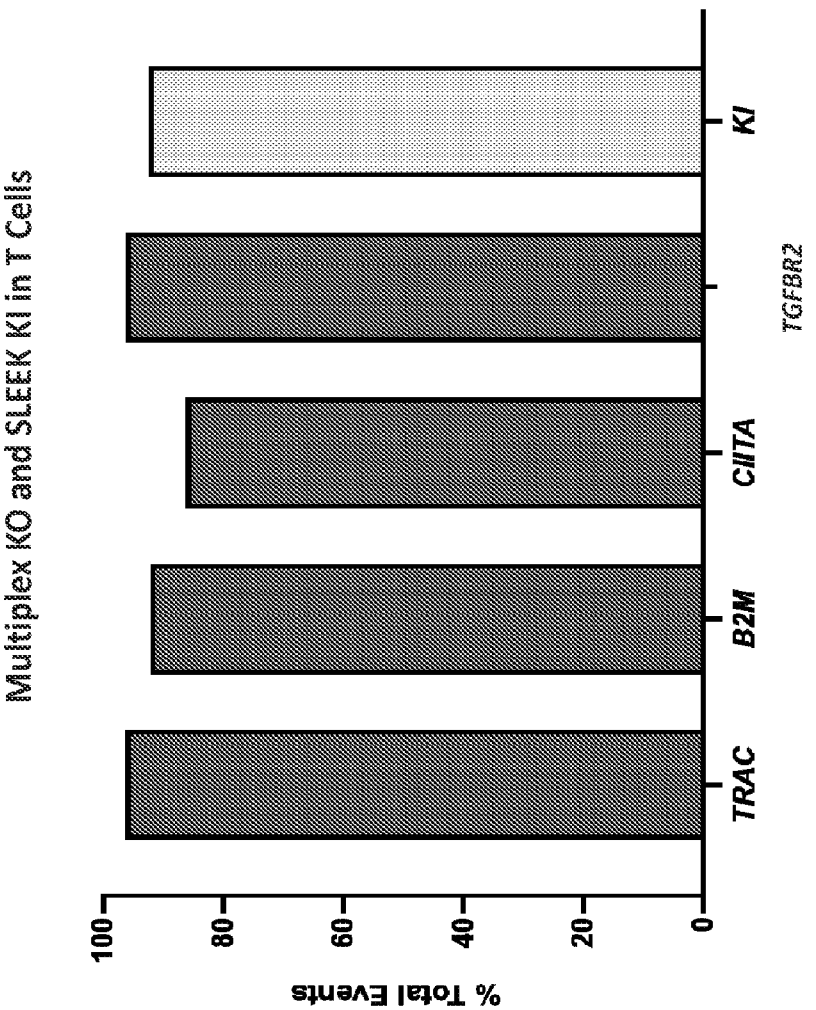

Multiplex KO and SLEEK KI in T Cells
% Total Events
100
80
60
40
20
0
TRAC
B2M
CIITA
TGFBR2
KI

Fig. 60B

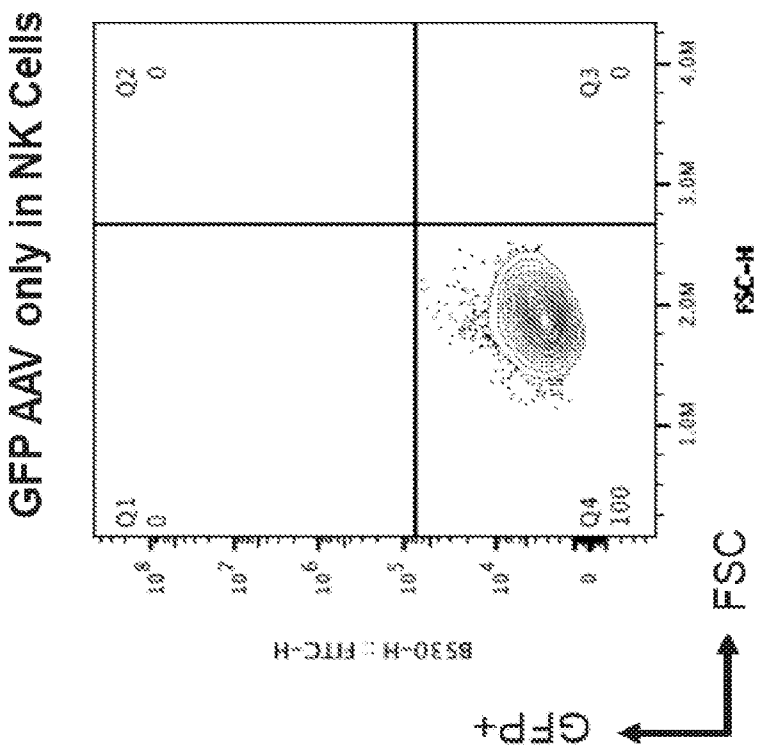

GFP AAV only in NK Cells
Q2
0
Q3
0
Q1
0
Q4
100
B530-H :: FITC-H
FSC-H
GFP+
FSC

Fig. 61A

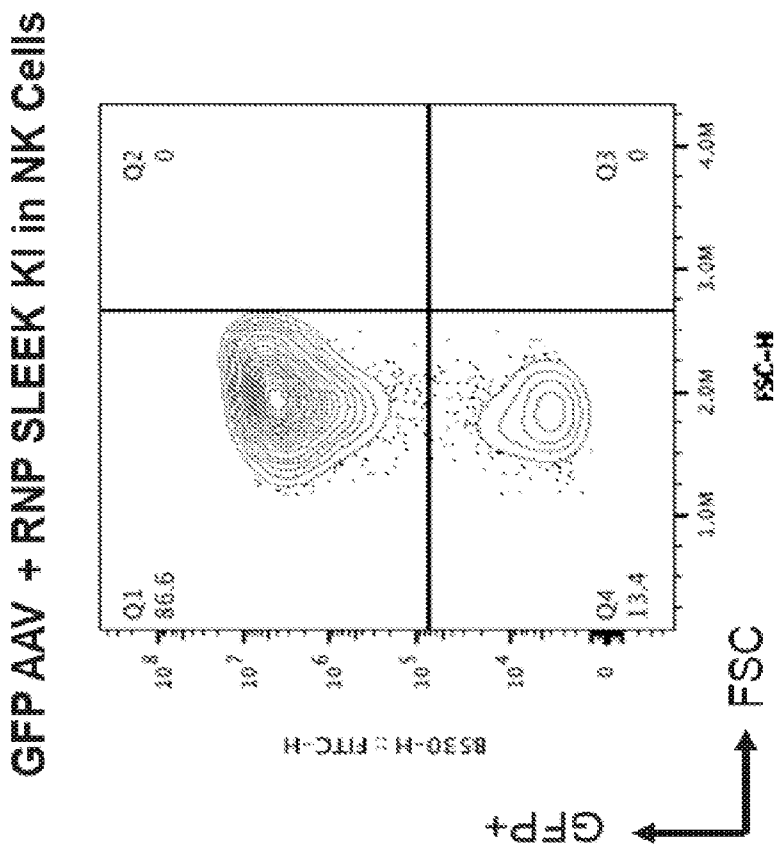

GFP AAV + RNP SLEEK KI in NK Cells
Q2
0
Q3
0
Q1
86.6
Q4
13.4
GFP+
FSC
FSC-H
B530-N :: FITC-N

Fig. 61B

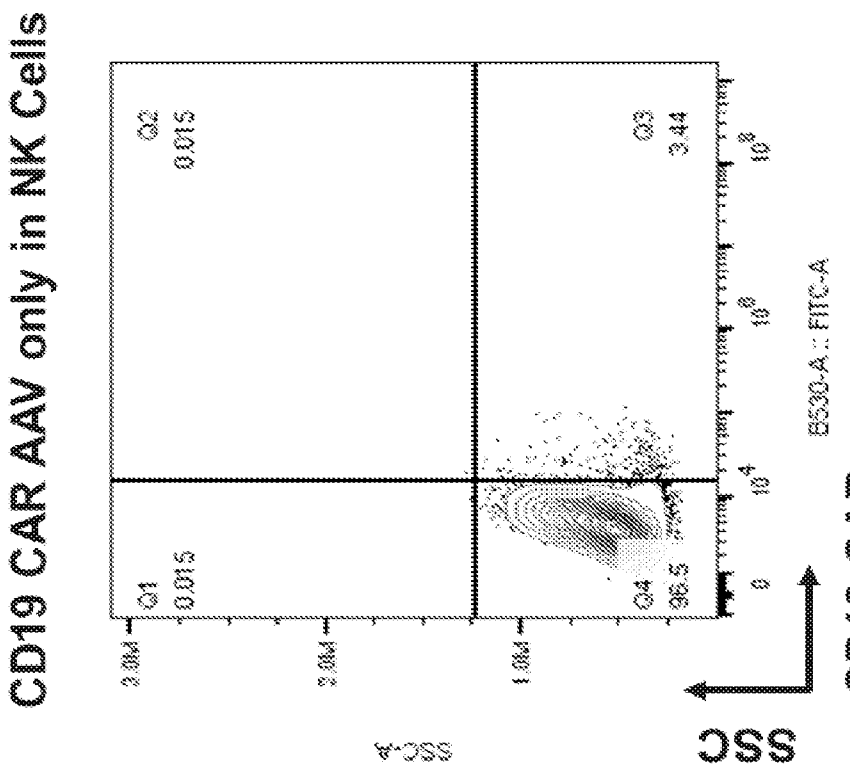

CD19 CAR AAV only in NK Cells
Q1
0.015
Q2
0.015
Q3
3.44
Q4
96.5
SSC-A
SSC
CD19 CAR
B530-A :: FITC-A

Fig. 61C

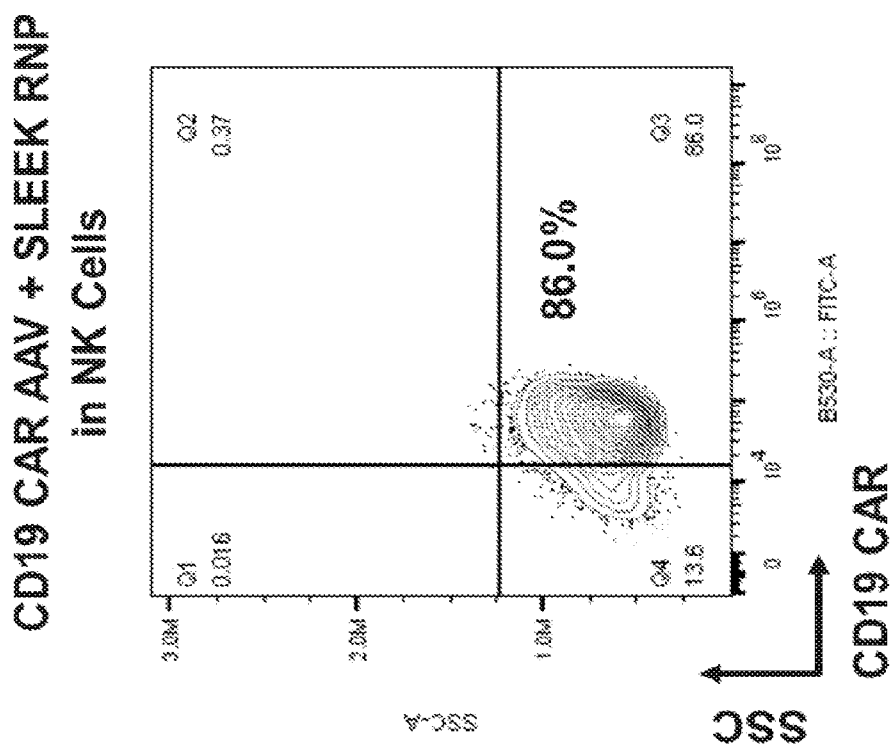

CD19 CAR AAV + SLEEK RNP
in NK Cells
Q2
0.37
Q3
86.0
86.0%
Q1
0.016
Q4
13.6
3.0M
2.0M
1.0M
0
10^4
10^6
10^8
SSC-A
SSC
CD19 CAR
B530-A :: FITC-A

Fig. 61D

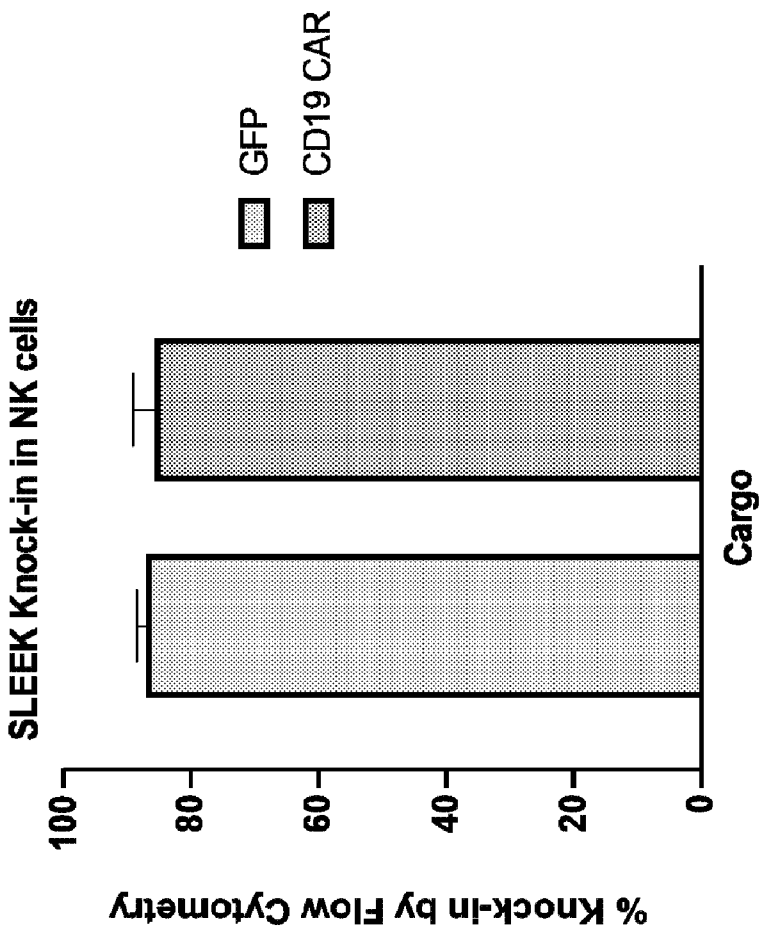

SLEEK Knock-in in NK cells
GFP
CD19 CAR
% Knock-in by Flow Cytometry
100
80
60
40
20
0
Cargo

Fig. 61E

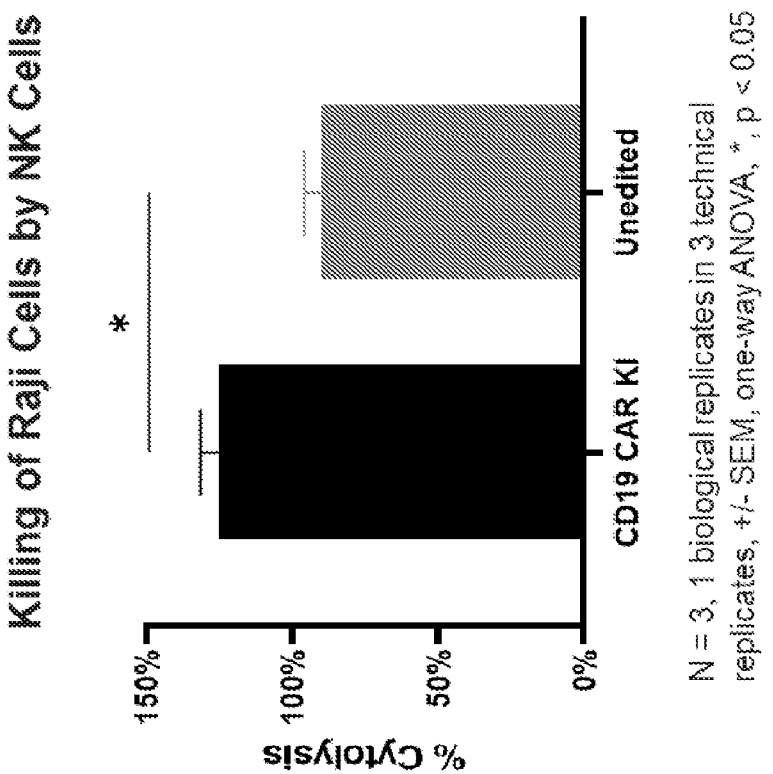

Killing of Raji Cells by NK Cells
*
150%
100%
50%
0%
% Cytolysis
CD19 CAR KI
Unedited
N = 3, 1 biological replicates in 3 technical replicates, +/- SEM, one-way ANOVA, *, p < 0.05

Fig. 61F

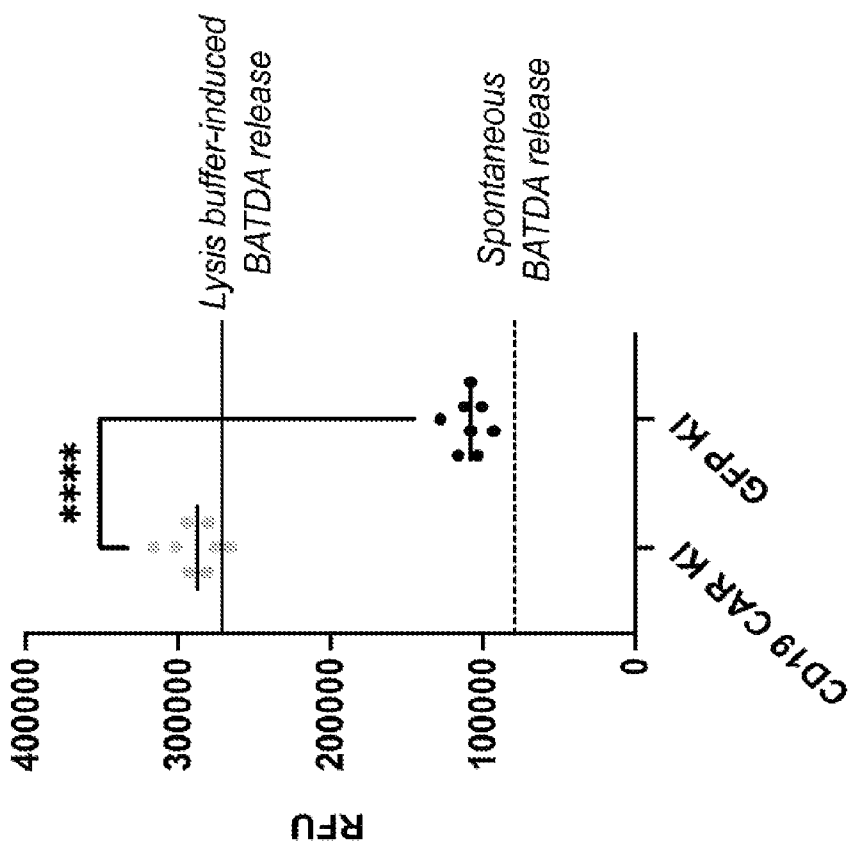

RFU
400000
300000
200000
100000
0
****
Lysis buffer-induced BATDA release
Spontaneous BATDA release
CD19 CAR KI
GFP KI

Fig. 61G

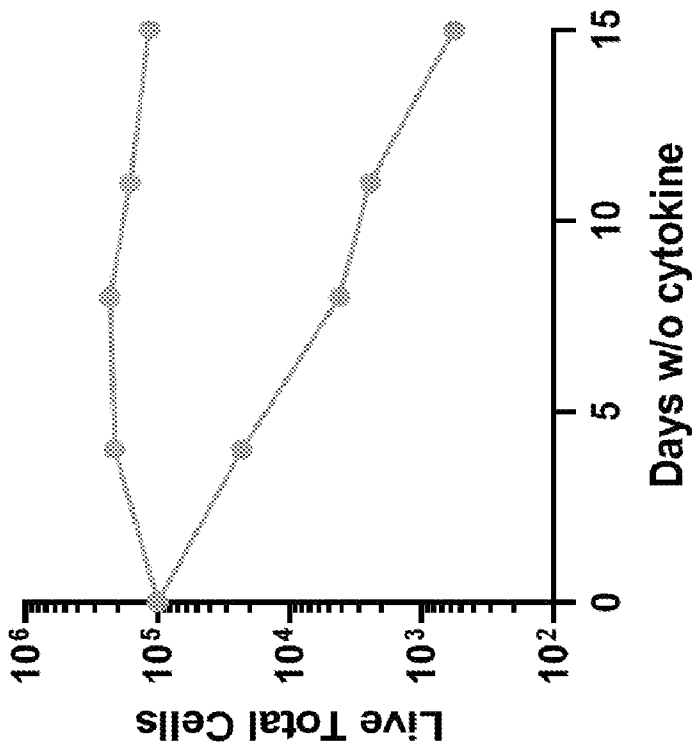

Live Total Cells
10^6
10^5
10^4
10^3
10^2
0
5
10
15
Days w/o cytokine
WT
DKI/DKO

Fig. 62A

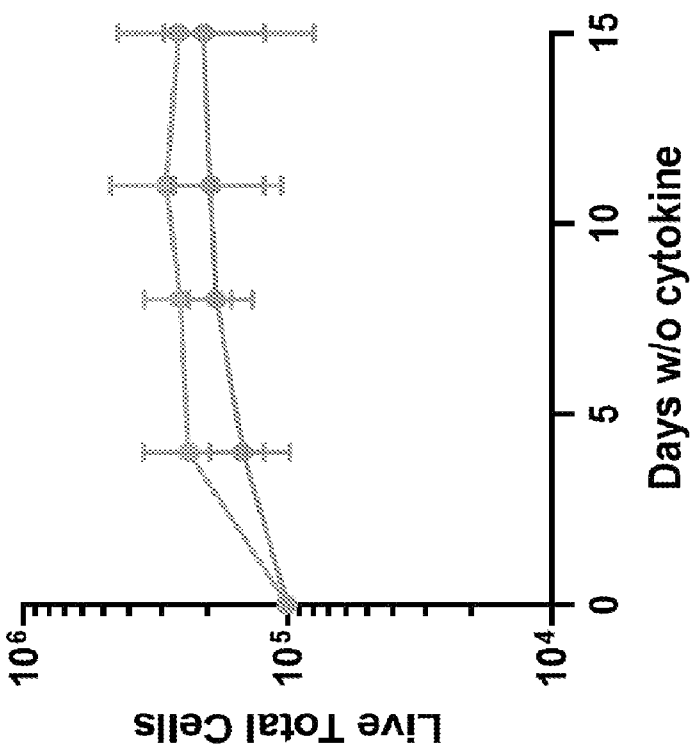

Live Total Cells
10^6
10^5
10^4
0
5
10
15
Days w/o cytokine
DKI
DKI/DKO

Fig. 62B

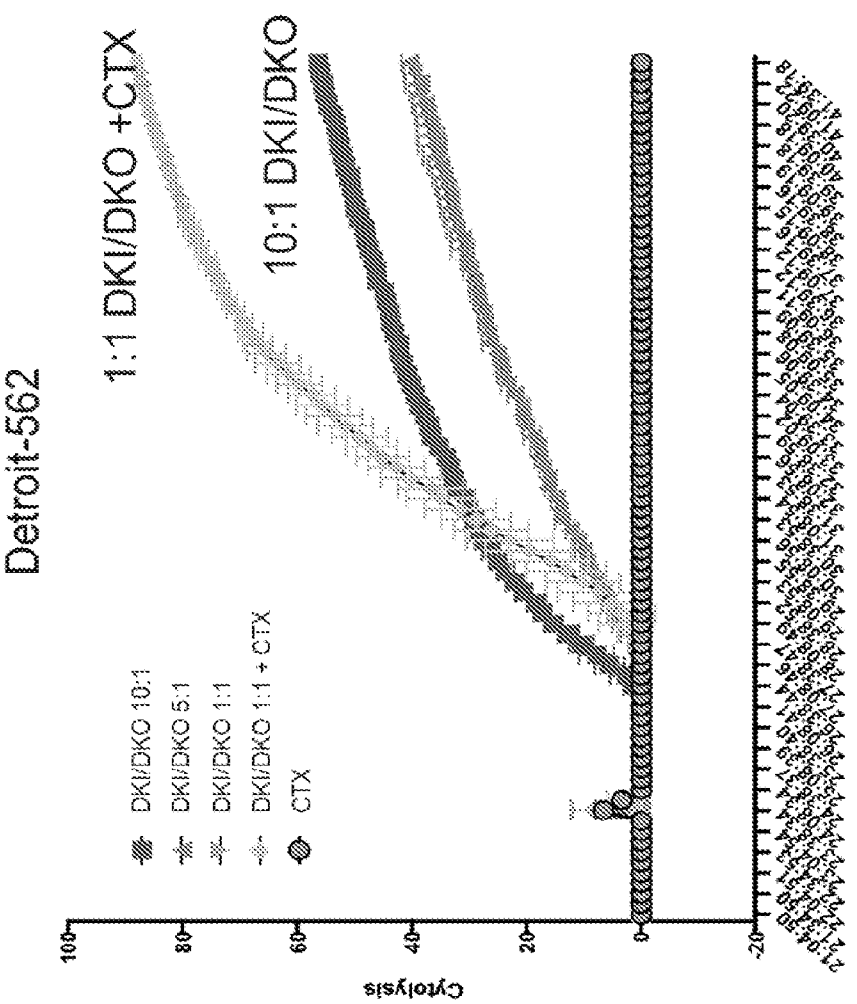

Detroit-562
1:1 DKI/DKO +CTX
10:1 DKI/DKO
DKI/DKO 10:1
DKI/DKO 5:1
DKI/DKO 1:1
DKI/DKO 1:1 + CTX
CTX
Cytolysis
Time
100
80
60
40
20
0
-20

Fig. 63A

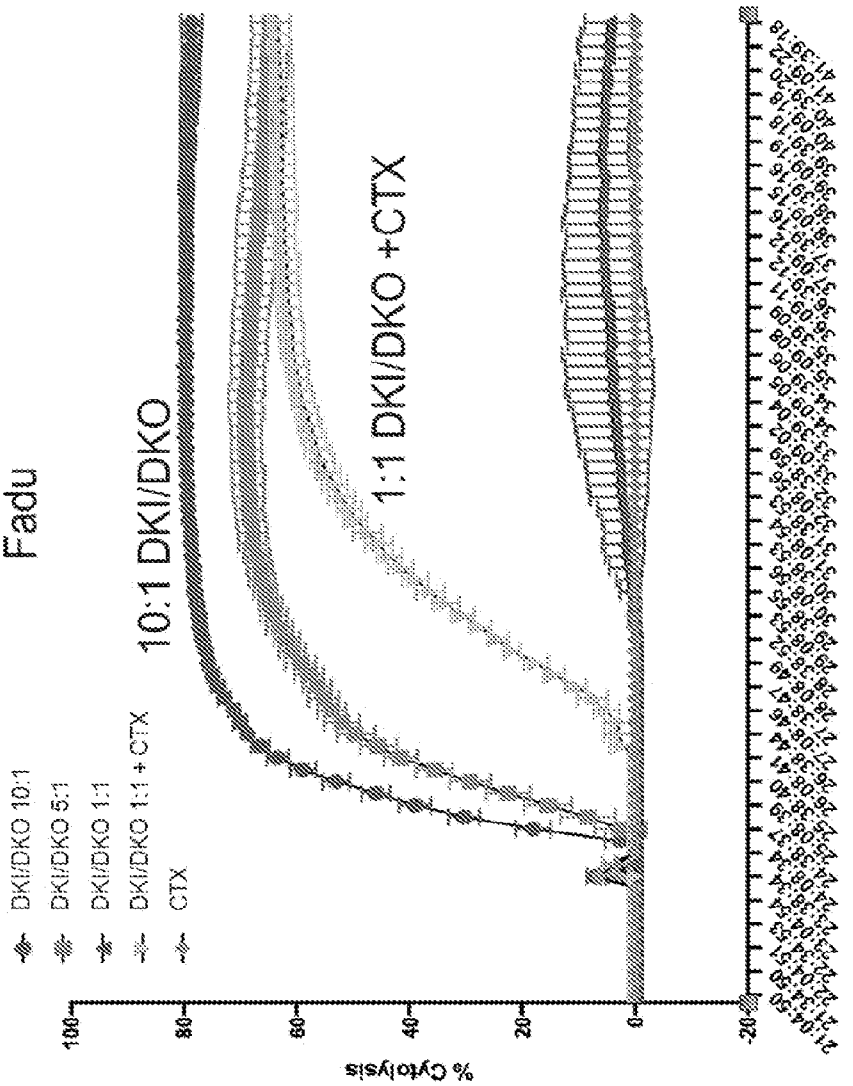

Fadu
10:1 DKI/DKO
1:1 DKI/DKO +CTX
DKI/DKO 10:1
DKI/DKO 5:1
DKI/DKO 1:1
DKI/DKO 1:1 + CTX
CTX
% Cytolysis
100
80
60
40
20
0
-20
Time

Fig. 63B

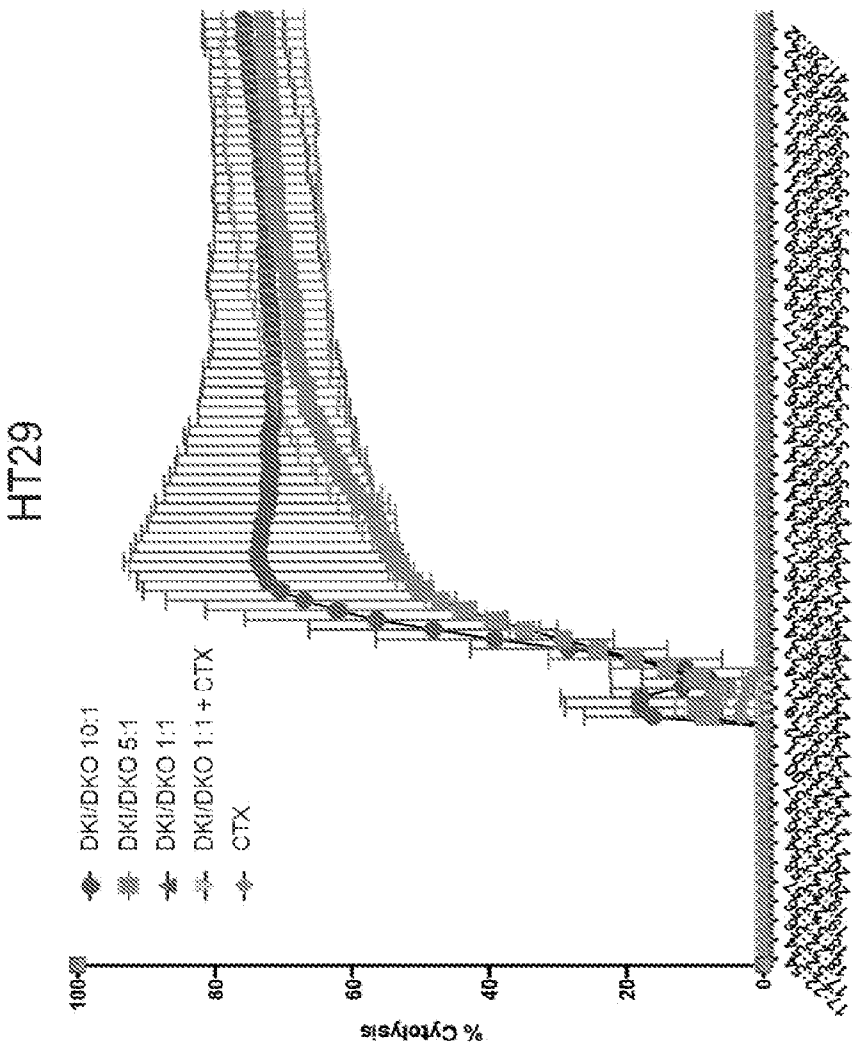

HT29
DKI/DKO 10:1
DKI/DKO 5:1
DKI/DKO 1:1
DKI/DKO 1:1 + CTX
CTX
% Cytolysis
100
80
60
40
20
0
Time

Fig. 63C

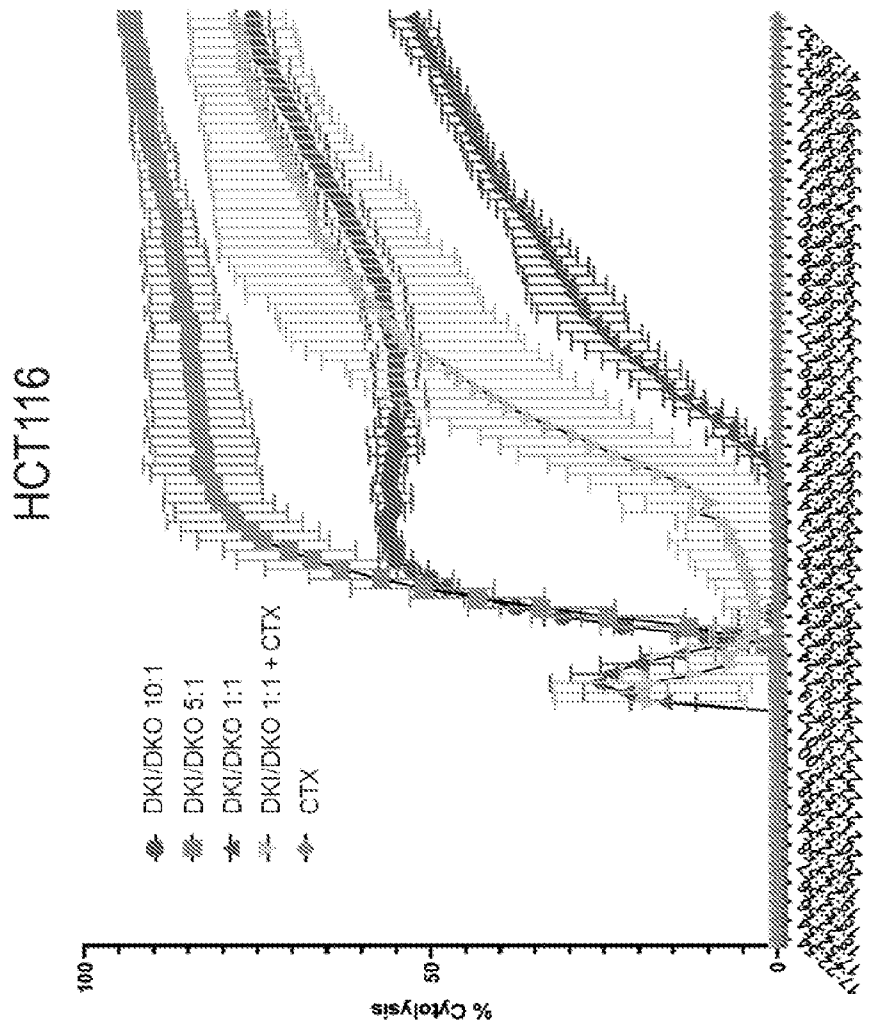

HCT116
DKI/DKO 10:1
DKI/DKO 5:1
DKI/DKO 1:1
DKI/DKO 1:1 + CTX
CTX
% Cytolysis
100
50
0
Time

Fig. 63D

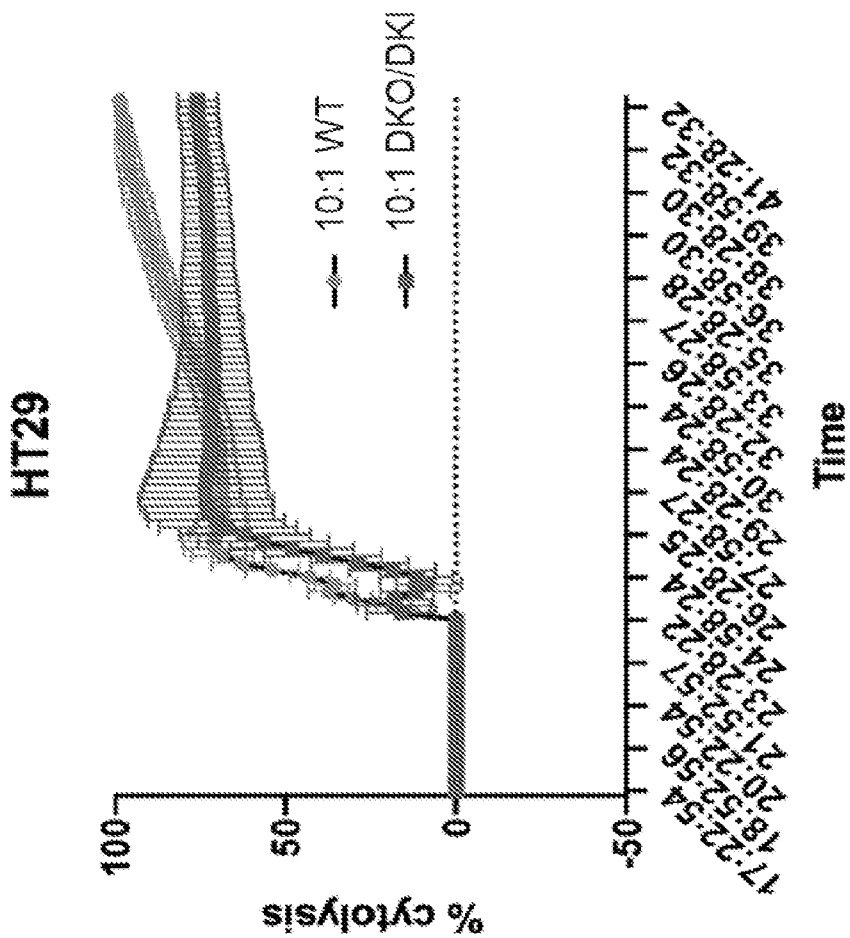

HT29
10:1 WT
10:1 DKO/DKI
% cytolysis
100
50
0
-50
Time
17:23:54:56
18:15:23:59
20:21:53:54
21:12:23:53
24:15:53:57
26:21:28:22
28:27:58:24
29:30:28:25
32:33:58:27
35:36:28:26
38:39:58:27
39:41:28:28
41:28:58:30
28:58:30:32

Fig. 64A

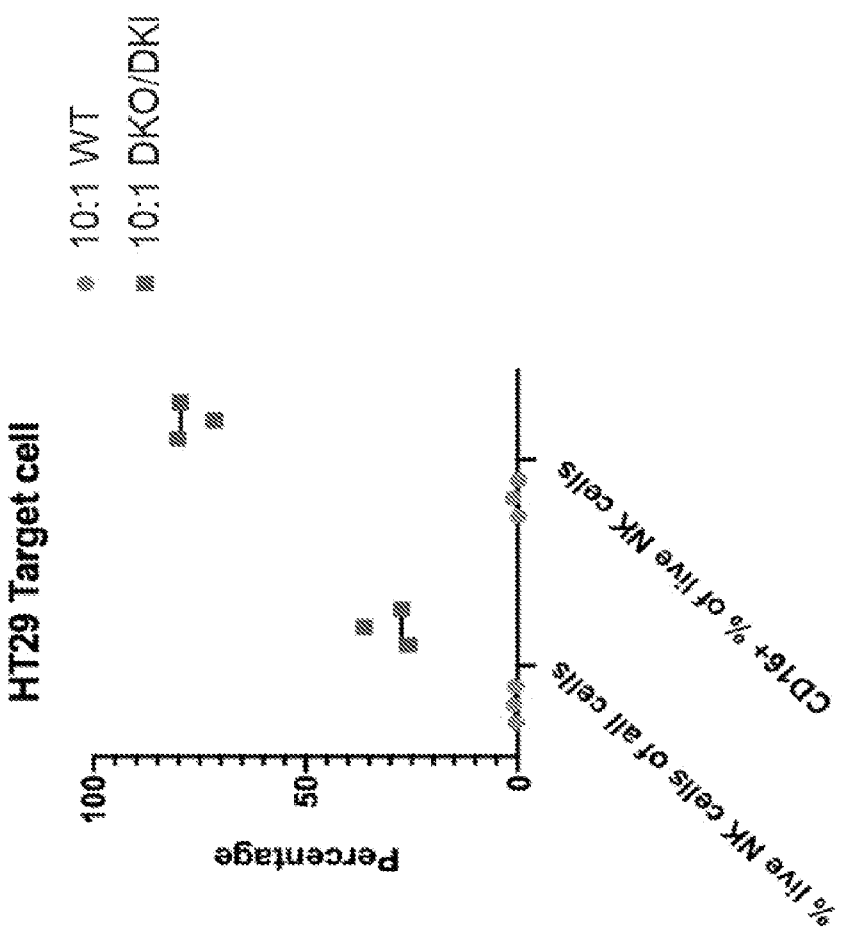

10:1 WT
10:1 DKO/DKI
HT29 Target cell
Percentage
100
50
0
% live NK cells of all cells
CD16+ % of live NK cells

Fig. 64B

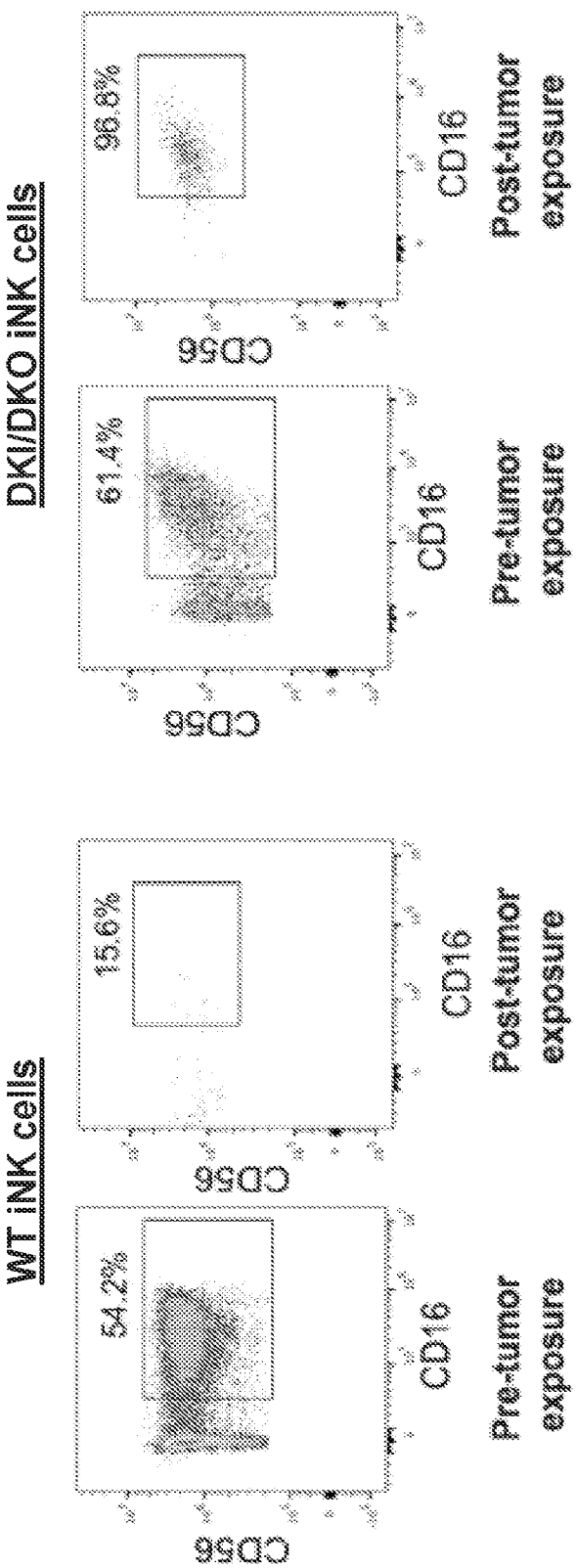

DKI/DKO iNK cells
96.8%
CD56
CD16
Post-tumor exposure
61.4%
CD56
CD16
Pre-tumor exposure
WT iNK cells
15.6%
CD56
CD16
Post-tumor exposure
54.2%
CD56
CD16
Pre-tumor exposure

Fig. 64C

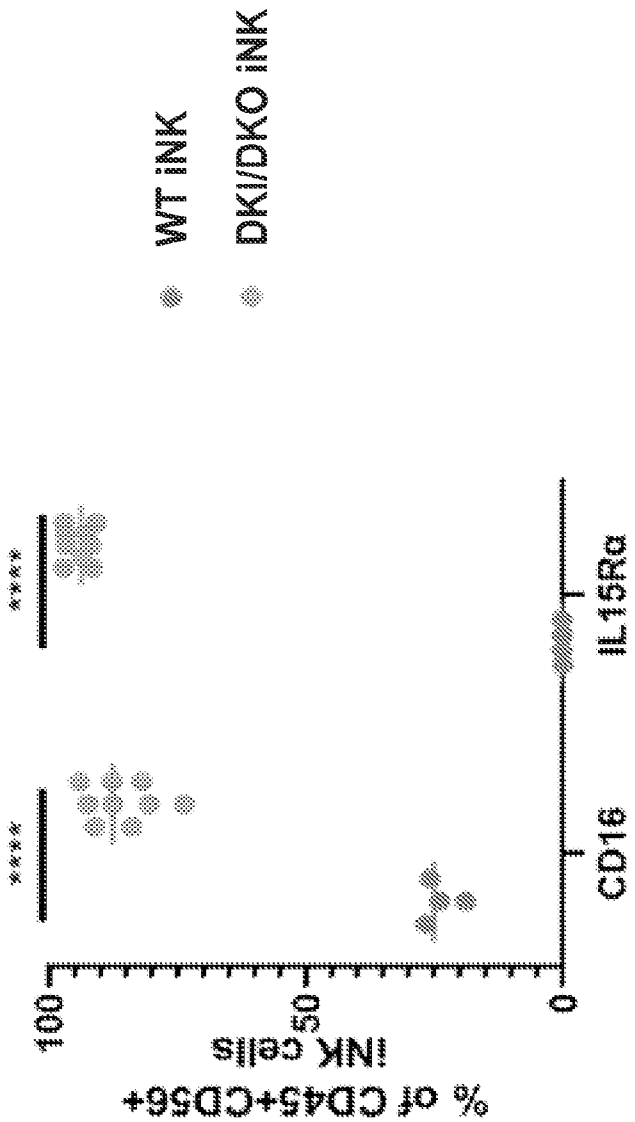


Fig. 65A

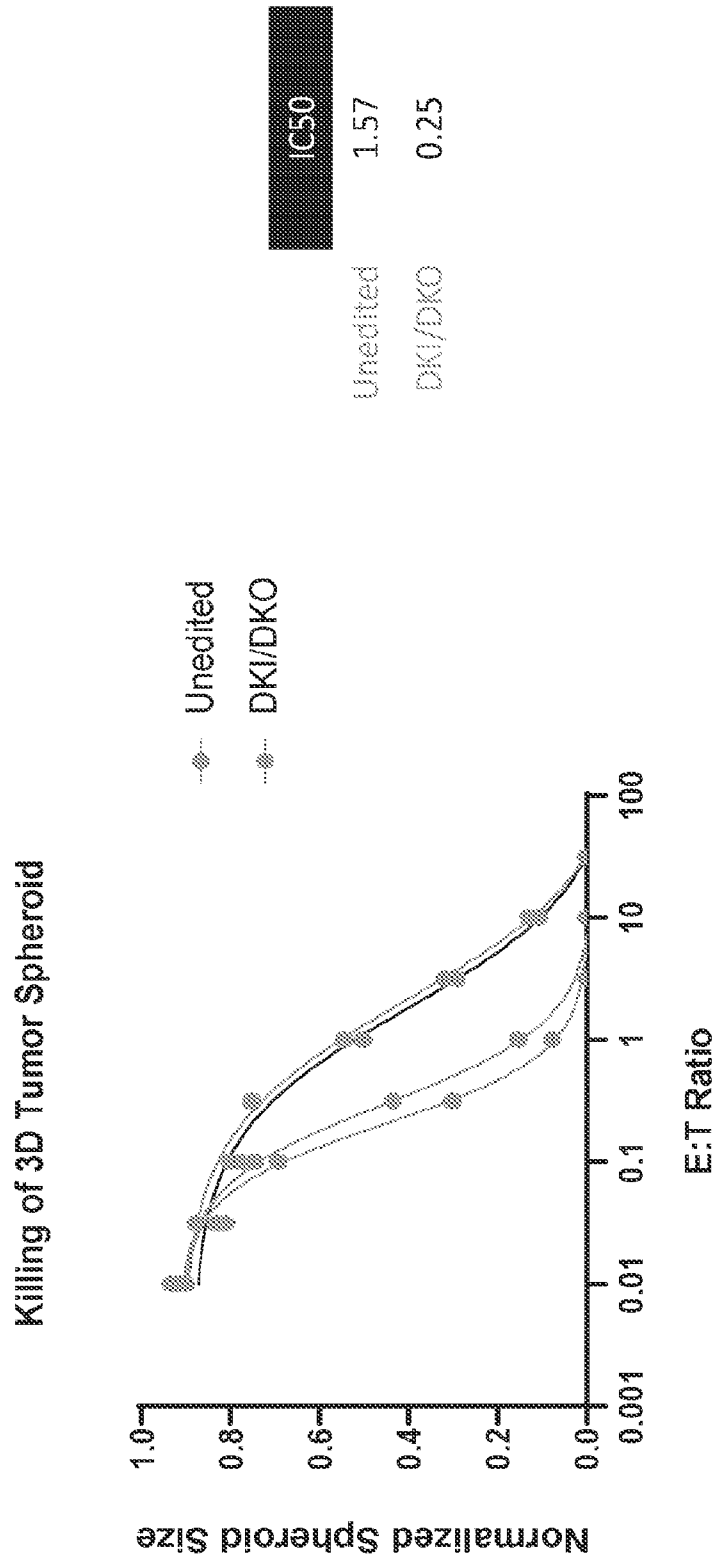

Killing of 3D Tumor Spheroid
Unedited
DKI/DKO
Normalized Spheroid Size
1.0
0.8
0.6
0.4
0.2
0.0
0.001
0.01
0.1
1
10
100
E:T Ratio
IC50
Unedited 1.57
DKI/DKO 0.25

Fig. 65B

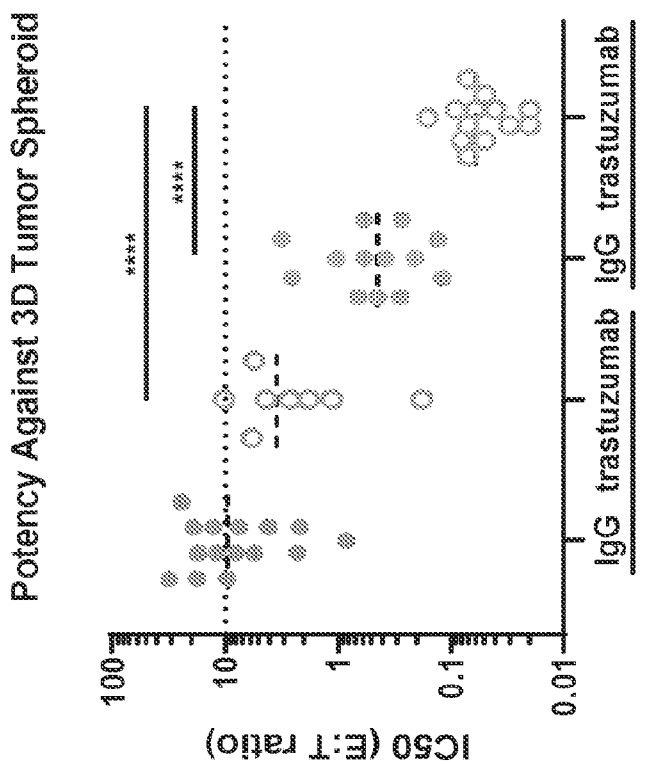

Potency Against 3D Tumor Spheroid
IC50 (E:T ratio)
100
10
1
0.1
0.01
****
****
IgG
trastuzumab
IgG
trastuzumab
Unedited
DKI/DKO

Fig. 65C

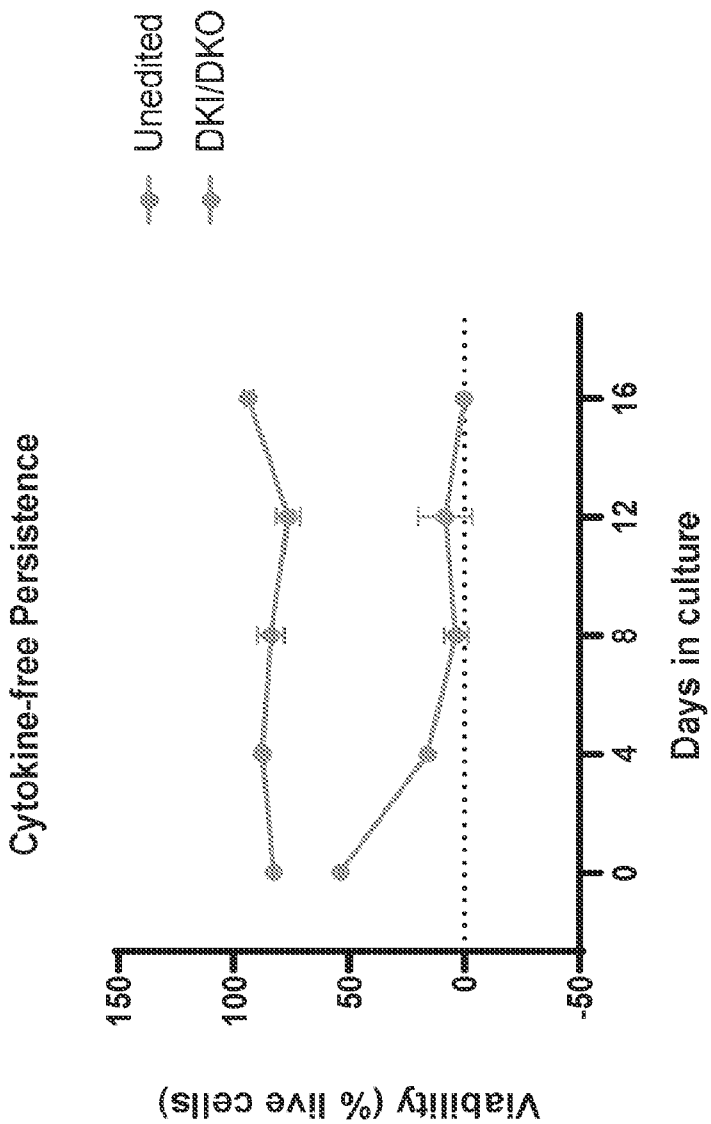

Cytokine-free Persistence
Unedited
DKI/DKO
Viability (% live cells)
150
100
50
0
-50
0
4
8
12
16
Days in culture

Fig. 65D

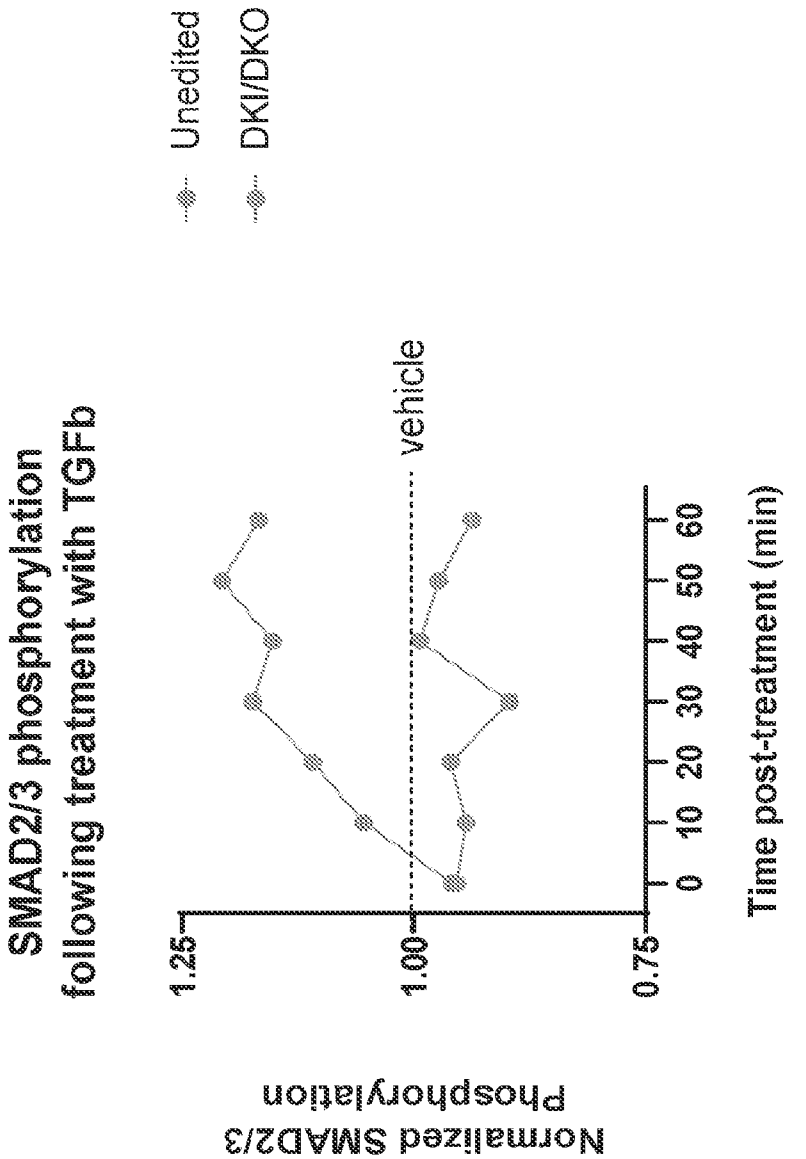

SMAD2/3 phosphorylation
following treatment with TGFb
Unedited
DKI/DKO
vehicle
1.25
1.00
0.75
0
10
20
30
40
50
60
Time post-treatment (min)
Normalized SMAD2/3
Phosphorylation

Fig. 65E

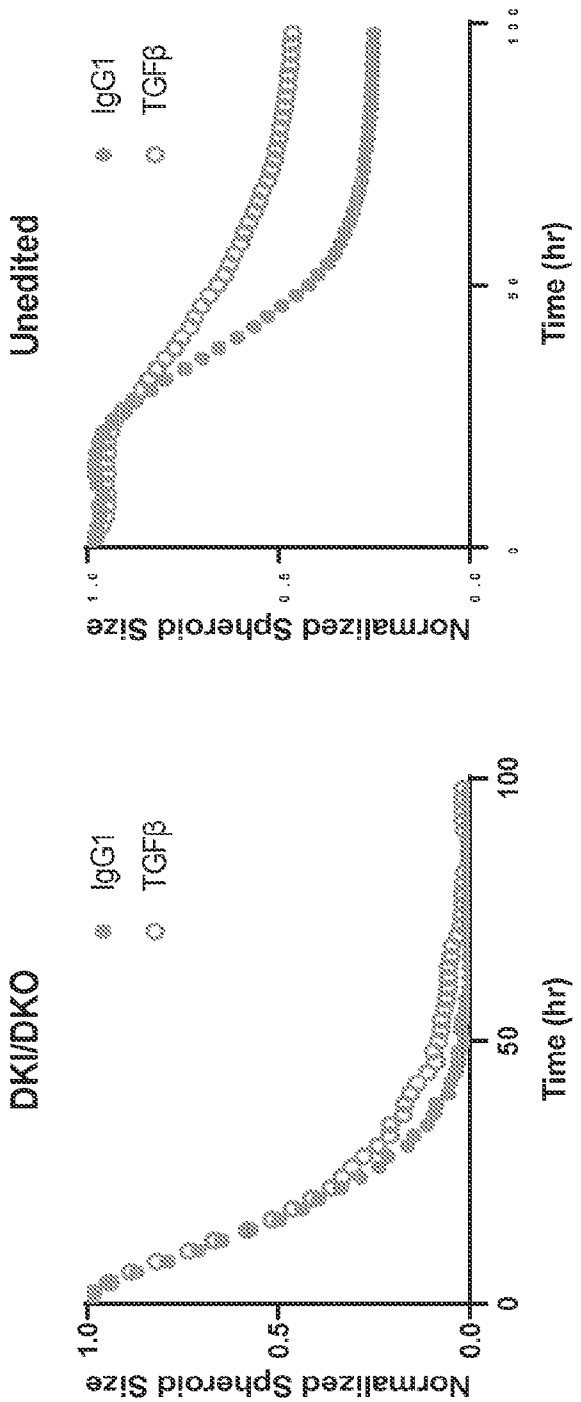

Unedited
IgG1
TGFβ
Normalized Spheroid Size
1.0
0.5
0.0
0
50
100
Time (hr)
DKI/DKO
IgG1
TGFβ
Normalized Spheroid Size
1.0
0.5
0.0
0
50
100
Time (hr)

Fig. 65F

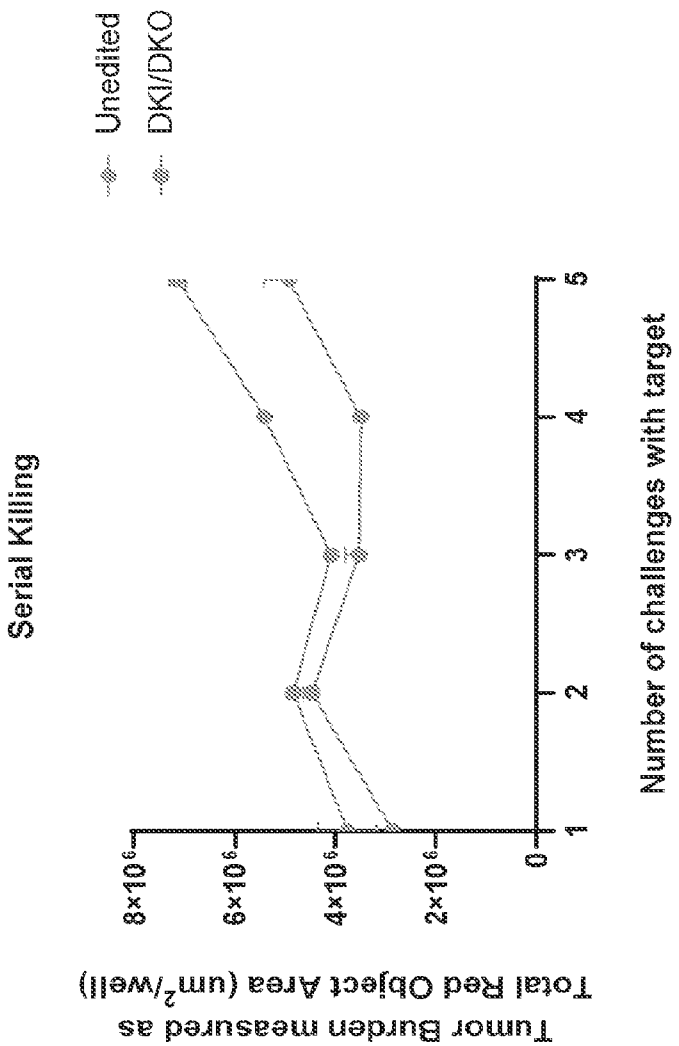

Serial Killing
Unedited
DKI/DKO
Tumor Burden measured as
Total Red Object Area (um²/well)
8×10⁶
6×10⁶
4×10⁶
2×10⁶
0
1
2
3
4
5
Number of challenges with target

Fig. 65G

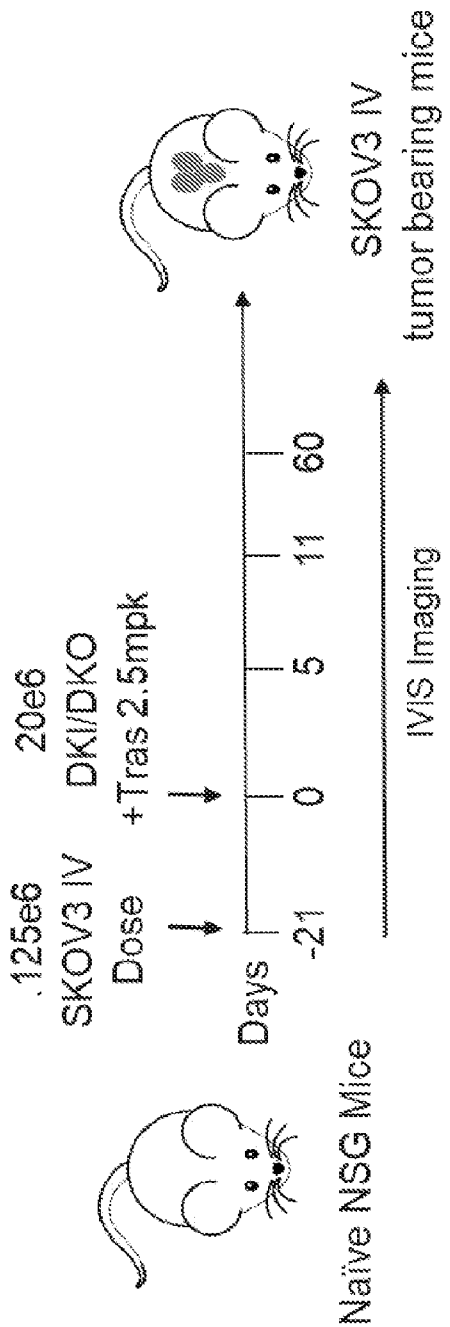

Naïve NSG Mice
.125e6
SKOV3 IV
20e6
DKI/DKO
Dose
+Tras 2.5mpk
Days
-21
0
5
11
60
IVIS Imaging
SKOV3 IV
tumor bearing mice

Fig. 66A

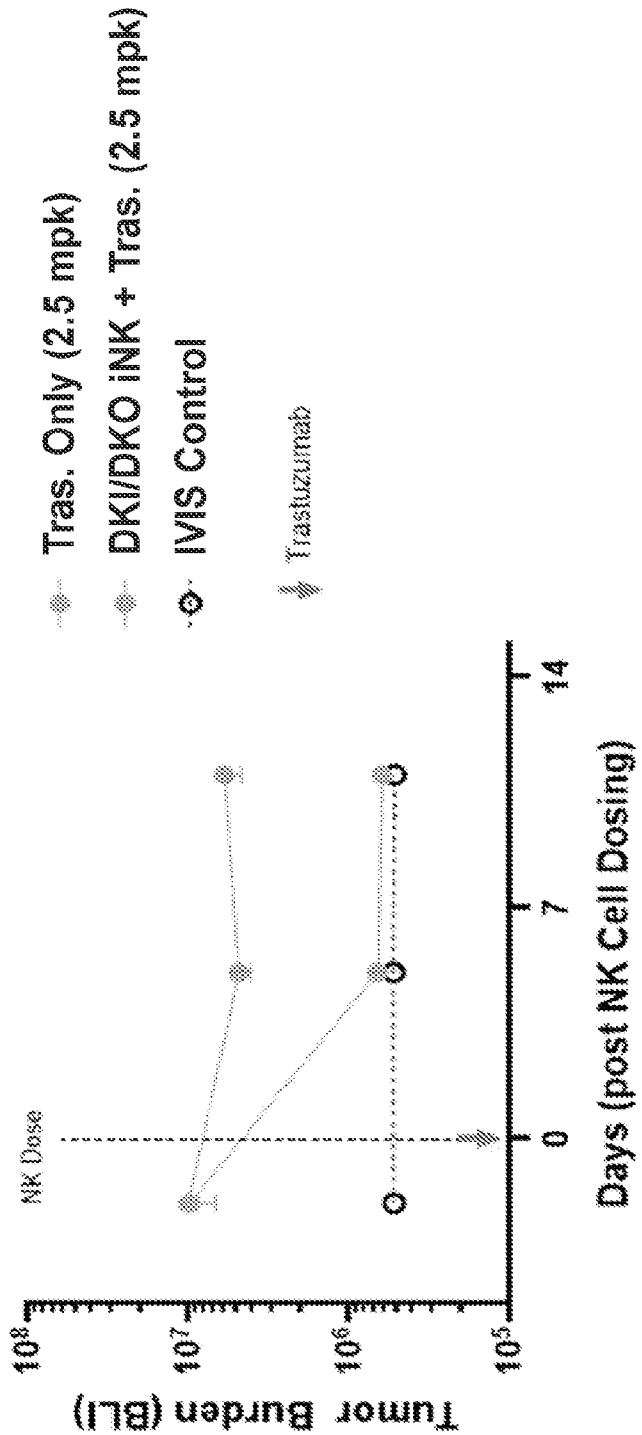

Tras. Only (2.5 mpk)
DKI/DKO iNK + Tras. (2.5 mpk)
IVIS Control
Trastuzumab
NK Dose
Tumor Burden (BLI)
10^8
10^7
10^6
10^5
0
7
14
Days (post NK Cell Dosing)

Fig. 66B

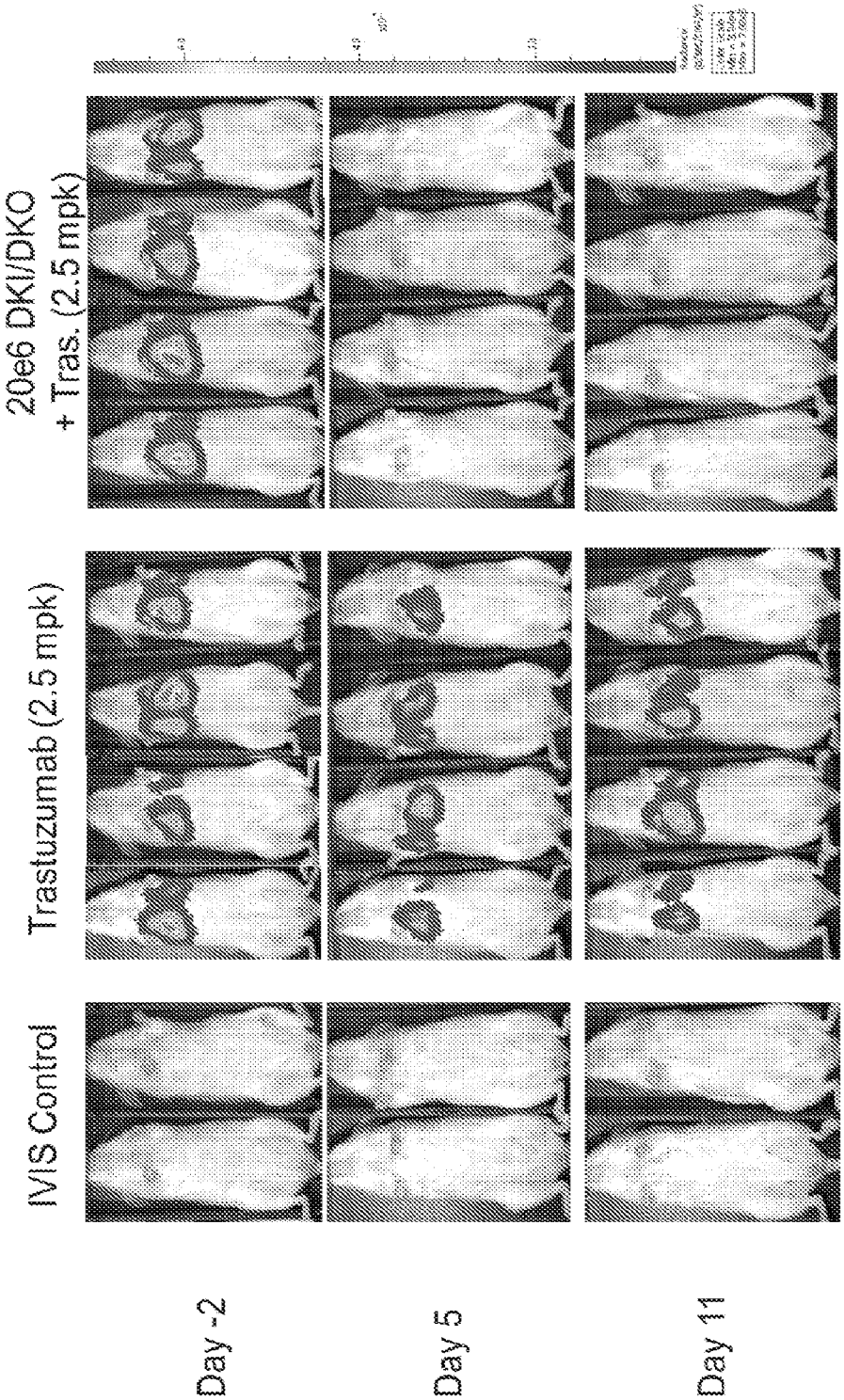

IVIS Control
Trastuzumab (2.5 mpk)
20e6 DKI/DKO
+ Tras. (2.5 mpk)
Day -2
Day 5
Day 11

Fig. 66C

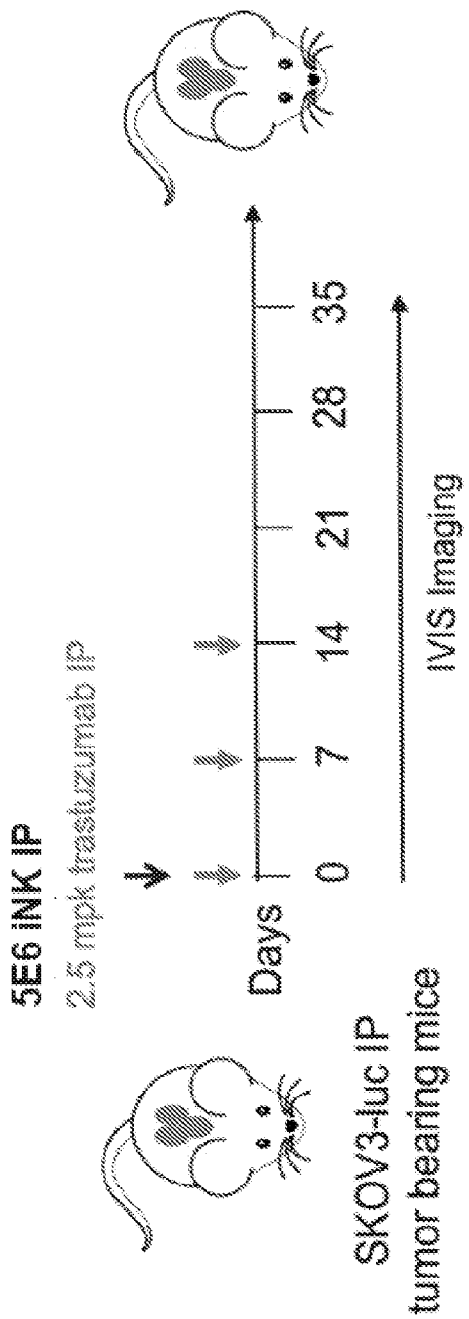

5E6 iNK IP
2.5 mpk trastuzumab IP
Days
0
7
14
21
28
35
IVIS Imaging
SKOV3-luc IP
tumor bearing mice

Fig. 67A

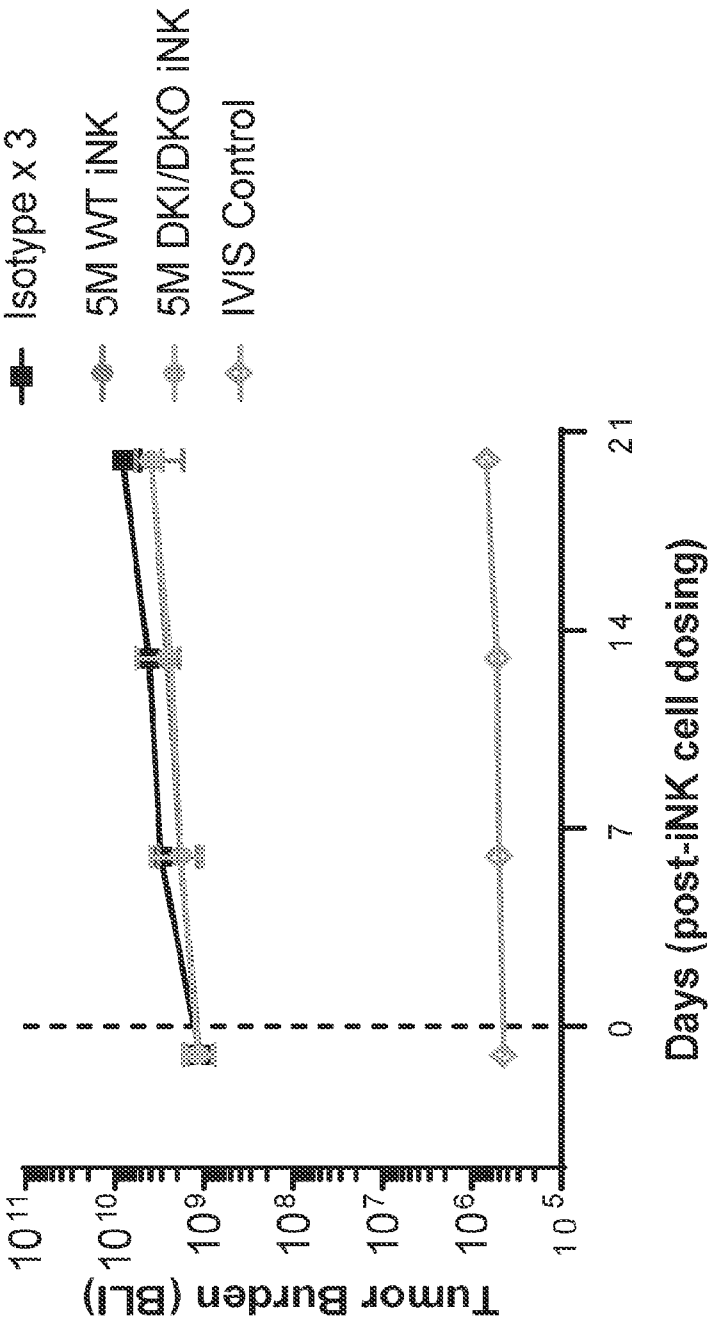

Isotype x 3
5M WT iNK
5M DKI/DKO iNK
IVIS Control
Tumor Burden (BLI)
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
0
7
14
21
Days (post-iNK cell dosing)

Fig. 67B

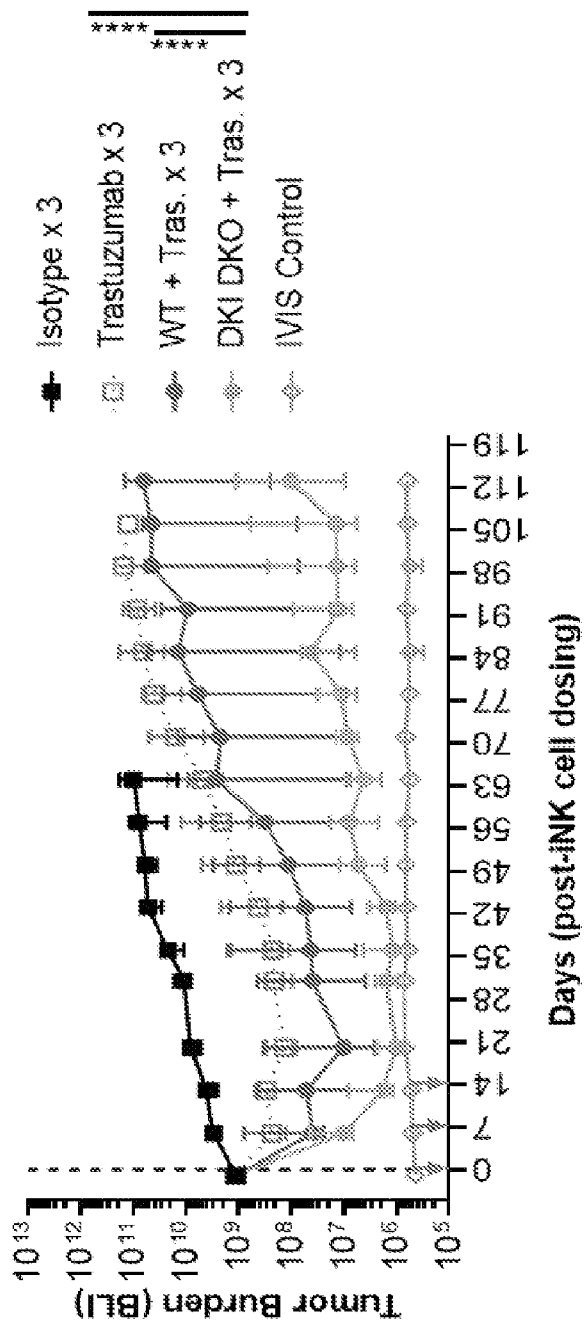

Isotype x 3
Trastuzumab x 3
WT + Tras. x 3
DKI DKO + Tras. x 3
IVIS Control
****
****
Tumor Burden (BLI)
10^{13}
10^{12}
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
0
7
14
21
28
35
42
49
56
63
70
77
84
91
98
105
112
119
Days (post-iNK cell dosing)

Fig. 67C

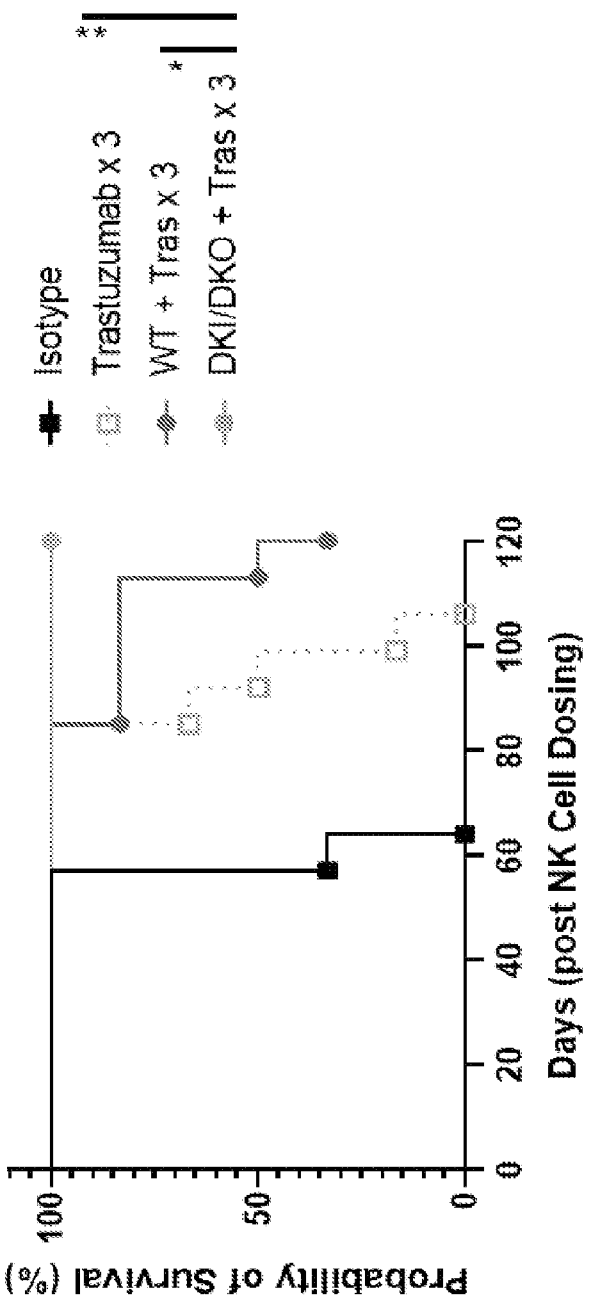

Isotype
Trastuzumab x 3
WT + Tras x 3
DKI/DKO + Tras x 3
**
*
Probability of Survival (%)
100
50
0
0
20
40
60
80
100
120
Days (post NK Cell Dosing)

Fig. 67D

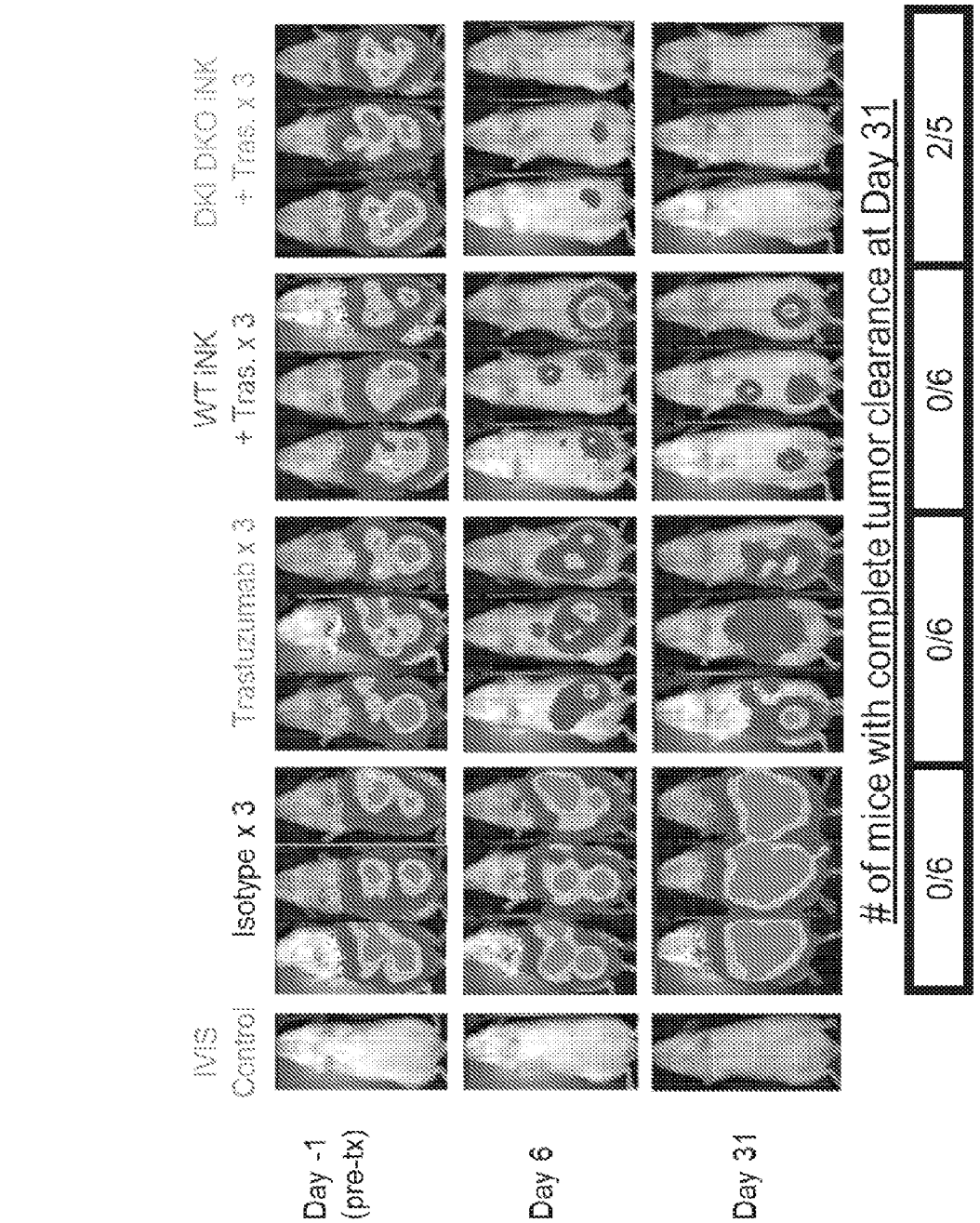

MS Control
Isotype x 3
Trastuzumab x 3
WT iNK + Tras. x 3
DKO iNK + Tras. x 3
Day -1 (pre-tx)
Day 6
Day 31
# of mice with complete tumor clearance at Day 31
0/6
0/6
0/6
2/5

Fig. 67E

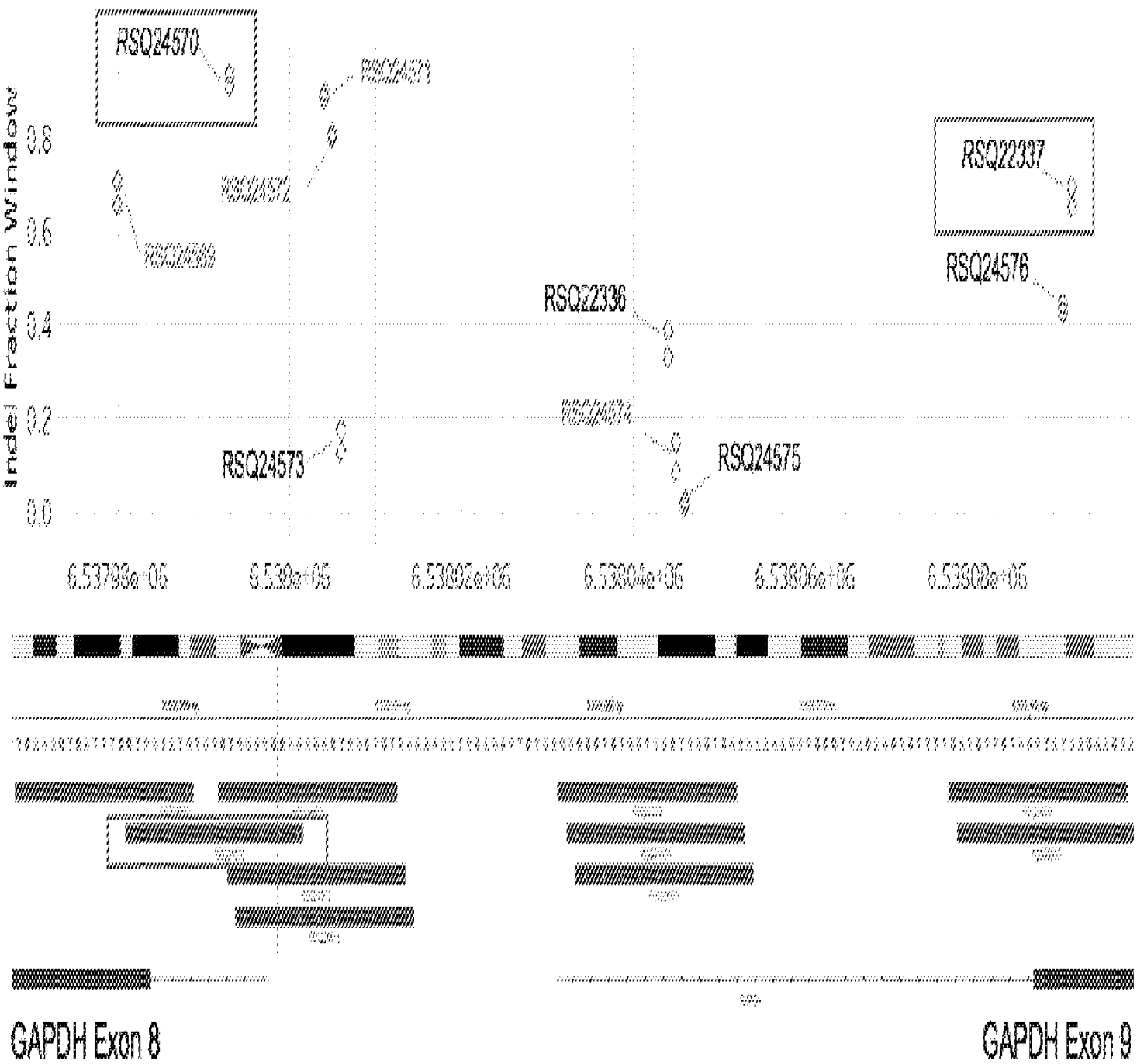

RSQ24570
RSQ24571
RSQ24572
RSQ24569
RSQ22337
RSQ24576
RSQ22336
RSQ24574
RSQ24573
RSQ24575
Indel Fraction Window
0.8
0.6
0.4
0.2
0.0
6.53798e+06
6.538e+06
6.53802e+06
6.53804e+06
6.53806e+06
6.53808e+06
GAPDH Exon 8
GAPDH Exon 9
Red = Insilico 1 MM + Gap > 0

Fig. 1