MODIFIED NATURAL KILLER (NK) CELLS FOR IMMUNOTHERAPY

RELATED APPLICATIONS

[1] This application claims priority to U.S. Provisional Application No. 62/806,457, filed on February 15, 2019; U.S. Provisional Application No. 62/841,066, filed on April 30, 2019; U.S. Provisional Application No. 62/841,684, filed on May 1, 2019; and U.S. Provisional Application No. 62/943,649, filed on December 4, 2019, the entire contents of each of which are expressly incorporated herein by reference.

SEQUENCE LISTING

[1.1] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on March 12, 2020, is named 126454-02320_SL.txt and is 408,177 bytes in size.

BACKGROUND

[2] NK cells are useful for immunotherapy approaches, for example, in the context of immuno-oncology. NK cells are a type of cytotoxic innate lymphocyte. NK cells play an important role in tumor immunity, and the cytotoxic activity of NK cells is tightly regulated by a network of activating and inhibitory pathways (see, *e.g.*, Gras Navarro A, Bjorklund AT and Chekenya M (2015) *Front. Immunol.* 6:202; incorporated in its entirety herein by reference).

[3] The use of naturally occurring or modified NK cells in immunotherapy approaches, *e.g.*, via autologous or allogeneic NK cell transfer, has been reported, and while some success has been achieved, such approaches are typically characterized by a suboptimal NK cell response. In the context of immune-oncology, it is believed that this suboptimal response is, at least in part, to tumors harnessing NK cell inhibitory pathways to suppress cytotoxic NK cell activity, limit NK cell invasion, and/or inhibit NK cell proliferation and survival. Thus, application of NK cells in the therapy of solid tumors has seen limited success.

[4] Initial work has been performed in trying to focus NK cell response on specific cells, *e.g.*, by expressing a chimeric antigen receptor in NK cells that targets the NK cells to tumor cells, or by modulating activating or inhibitory NK cell pathways to achieve a stronger and/or more sustained NK cell response. See, *e.g.*, Jing Y, *et al.* (2015) *PLoS ONE* 10(3):e0121788; and Oberschmidt 0, Kloess S and Koehl U (2017) *Front. Immunol*. 8:654; incorporated in their entireties herein by reference.

[5] In pursuit of an off-the shelf allogeneic NK cell therapy that could be used in combination with a therapeutic antibody, an induced pluripotent stem cell line has been developed in which cells express an enhanced version of CD16 (hnCD16), and NK cells have been derived from this iPSC line. See, *e.g.*, Li et al., *Cell Stem Cell.* 2018 Aug 2;23(2):181-192.e5; incorporated in its entirety herein by reference.

[6] However, to date all of these approaches have seen limited success. Therefore, there remains a need for the development of better therapeutic approaches for immunotherapy.

Summary

[6.1] In one aspect, the present disclosure refers to a modified lymphocyte, wherein the modified lymphocyte: (a) does not express endogenous CD3, CD4, and/or CD8; and (b) expresses at least one endogenous gene encoding: (i) CD56 (NCAM), CD49, and/or CD45; (ii) NK cell receptor immunoglobulin gamma Fc region receptor III (FcγRIII, cluster of differentiation 16 (CD16)); (iii) natural killer group-2 member D (NKG2D); (iv) CD69; (v) a natural cytotoxicity receptor; or any combination of two or more thereof; wherein the modified lymphocyte further: (1) comprises at least one exogenous nucleic acid expression construct comprising a nucleic acid sequence encoding: (i) a chimeric antigen receptor (CAR); (ii) a non-naturally occurring variant of FcγRIII (CD16); (iii) interleukin 15 (IL-15); (iv) IL-15 receptor (IL-15R), or a variant thereof; (v) interleukin 12 (IL-12); (vi) IL-12 receptor (IL-12R), or a variant thereof; (vii) human leukocyte antigen G (HLA-G); (viii) human leukocyte antigen E (HLA-E); (ix) leukocyte surface antigen cluster of differentiation CD47 (CD47); or any combination of two or more thereof; and/or (2) exhibits a loss of function of at least one of: (i) transforming growth factor beta receptor 2 (TGFβR2); (ii) adenosine A2a receptor (ADORA2A); (iii) T cell immunoreceptor with Ig and ITIM domains (TIGIT); (iv) β-2 microgobulin (B2M); (v) programmed cell death protein 1 (PD-1); (vi) cytokine inducible SH2 containing protein (CISH); (vii) class II, major histocompatibility complex, transactivator (CIITA); (viii) natural killer cell receptor NKG2A (natural killer group 2A); (ix) two or more HLA class II histocompatibility antigen alpha chain genes, and/or two or more HLA class II histocompatibility antigen beta chain genes; (x) cluster of differentiation 32B (CD32B, FCGR2B); (xi) T cell receptor alpha constant (TRAC); or any combination of two or more thereof.

[6.2] In another aspect, the present disclosure refers to modified cell, wherein the modified cell (1) comprises at least one exogenous nucleic acid expression construct comprising a nucleic acid sequence encoding: (i) a chimeric antigen receptor (CAR); (ii) a non-naturally occurring variant of FcγRIII (CD16); (iii) interleukin 15 (IL-15); (iv) IL-15 receptor (IL-15R), or a variant thereof; (v) interleukin 12 (IL-12); (vi) IL-12 receptor (IL-12R), or a variant thereof; (vii) human leukocyte antigen G (HLA-G); (viii)human leukocyte antigen E (HLA-E); (ix) leukocyte surface antigen cluster of differentiation CD47 (CD47); or any combination of two or more thereof; and/or (2) exhibits a loss of function of at least one of: (i) transforming growth factor beta receptor 2 (TGFβR2); (ii) adenosine A2a receptor (ADORA2A); (iii) T cell immunoreceptor with Ig and ITIM domains (TIGIT); (iv) β-2 microgobulin (B2M); (v) programmed cell death protein 1 (PD-1); (vi) cytokine inducible SH2 containing protein (CISH); (vii) class II, major histocompatibility complex, transactivator (CIITA); (viii) natural killer cell receptor NKG2A (natural killer group 2A); (ix) two or more HLA class II

histocompatibility antigen alpha chain genes, and/or two or more HLA class II histocompatibility antigen beta chain genes; (x) cluster of differentiation 32B (CD32B, FCGR2B); (xi) T cell receptor alpha constant (TRAC); or any combination of two or more thereof.

[6.3] In another aspect, the present disclosure refers to a population of cells comprising the modified lymphocyte as disclosed herein, or the modified cell disclosed herein.

[6.4] In another aspect, the present disclosure refers to a pharmaceutical composition comprising the population of cells as disclosed herein.

[6.5] In another aspect, the present disclosure refers to n isolated population of lymphocytes, wherein the population comprises at least 1x103, at least 1x104, at least 1x105, at least 2x105, at least 3x105, at least 4x105, at least 5x105,at least 1x106, at least 2x106, at least 3x106, at least 4x106, at least 5x106,at least 1x107, at least 1x107, at least 2x107, at least 3x107, at least 4x107, at least 5x107,at least 1x108, at least 2x108, at least 3x108, at least 4x108, at least 5x108,at least 1x109, at least 1x109, at least 2x109, at least 3x109, at least 4x109, at least 5x109,at least 1x1010, at least 2x1010, at least 3x1010, at least 4x1010, at least 5x1010, at least 1x1011, or at least 1x1012 cells, and wherein at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.9%, at least 99.99%, at least 99.999%, or virtually 100% of the lymphocytes in the population: (a) comprise a rearranged T-cell receptor (TCR) locus; (b) do not express endogenous CD3; (c) express endogenous CD56 (NCAM), CD49, and/or CD45; and (d) expresses at least one endogenous gene encoding: (i) FcγRIII (CD16); (ii) natural killer group-2 member D (NKG2D); (iii) CD69; (iv) a natural cytotoxicity receptor; or any combination of two or more thereof; and wherein the modified lymphocyte further: (1) comprises at least one exogenous nucleic acid expression construct comprising a nucleic acid sequence encoding: (i) chimeric antigen receptor (CAR); (ii) non-naturally occurring variant of immunoglobulin gamma Fc region receptor III (FcγRIII, CD16); (iii) interleukin 15 (IL-15); (iv) IL-15 receptor (IL-15R), or a variant thereof; (v) interleukin 12 (IL-12); (vi) IL-12 receptor (IL-12R), or a variant thereof; (vii) human leukocyte antigen G (HLA-G); (viii) human leukocyte antigen E (HLA-E); (ix) leukocyte surface antigen cluster of differentiation CD47 (CD47); or any combination of two or more thereof; and/or (2) exhibits a loss of function of at least one of: (i) transforming growth factor beta receptor 2 (TGFβR2); (ii) adenosine A2a receptor (ADORA2A); (iii) T cell immunoreceptor with Ig and ITIM domains (TIGIT); (iv) β-2 microgobulin (B2M); (v) programmed cell death protein 1 (PD-1); (vi) cytokine inducible SH2 containing protein (CISH); (vii) class II, major histocompatibility complex, transactivator (CIITA); (viii) natural killer cell receptor NKG2A (natural killer group 2A); (ix) two or more HLA class II histocompatibility antigen alpha chain genes, and/or two or more HLA class II histocompatibility antigen beta chain genes; (x) cluster of differentiation 32B (CD32B, FCGR2B); (xi) T cell receptor alpha constant (TRAC); or any combination of two or more thereof.

[6.6] In another aspect, the present disclosure refers to the use of the lymphocyte as disclosed herein, the modified cell as disclosed herein, the population of cells as disclosed herein, the pharmaceutical composition as disclosed herein, or the isolated *in vitro* population of lymphocytes as disclosed herein, in the manufacture of a medicament for treating a proliferative disease.

[6.7] In another aspect, the present disclosure refers to a method of generating the lymphocyte as disclosed herein, the modified cell as disclosed herein, the population of cells as disclosed herein, or the isolated *in vitro* population of lymphocytes as disclosed herein, the method comprising: (a) obtaining an induced pluripotent stem cell (iPSC); (b) modifying the iPSC, or an undifferentiated or differentiated daughter cell thereof, to comprise express at least one exogenous nucleic acid expression construct of (1) and/or to comprise a loss of function in at least one gene of (2); (c) directing differentiation of the iPSC to hematopoietic lineage cells, wherein the hematopoietic lineage cells retain the edited genetic loci comprised in the iPSCs.

[6.8] In another aspect, the present disclosure refers to a method, the method comprising: differentiating a genetically modified pluripotent stem cell into a lymphocyte, wherein the genetically modified pluripotent stem cell comprises: (1) an exogenous nucleic acid expression construct comprising: (i) a nucleic acid sequence encoding a chimeric antigen receptor (CAR); (ii) a nucleic acid sequence encoding a non-naturally occurring variant of FcγRIII (CD16); (iii) a nucleic acid sequence encoding interleukin 15 (IL-15); (iv) a nucleic acid sequence encoding interleukin 15 receptor (IL-15R) or a variant thereof; (v) a nucleic acid sequence encoding interleukin 12 (IL12); (vi) a nucleic acid sequence encoding interleukin-12 receptor (IL-12R) or a variant thereof; (vii) a nucleic acid sequence encoding human leukocyte antigen G (HLA-G); (viii) a nucleic acid sequence encoding human leukocyte antigen E (HLA-E); (ix) a nucleic acid sequence encoding leukocyte surface antigen cluster of differentiation CD47 (CD47); or any combination of two or more thereof; and (2) an indel, or an insertion of an exogenous nucleic acid in one or more of the following genetic loci: (i) transforming growth factor beta receptor 2 (TGFβR2); (ii) adenosine A2a receptor (ADORA2A); (iii) T cell immunoreceptor with Ig and ITIM domains (TIGIT); (iv) β-2 microgobulin (B2M); (v) programmed cell death protein 1 (PD-1, CD279); (vi) cytokine inducible SH2 containing protein (CISH); (vii) class II, major histocompatibility complex, transactivator (CIITA); (viii) natural killer cell receptor NKG2A (natural killer group 2A); (ix) two or more HLA class II histocompatibility antigen alpha chain genes, and/or two or more HLA class II histocompatibility antigen beta chain genes; (x) cluster of differentiation 32B (CD32B, FCGR2B); (xi) T cell receptor alpha constant (TRAC); or any combination of two or more thereof, wherein the indel or insertion results in a loss-of-function of a gene product encoded by the respective genetic locus or loci.

[6.9] In another aspect, the present disclosure refers to a method, the method comprising: reprogramming a donor cell to a pluripotent state; editing a target locus in the donor cell genome; and differentiating the reprogrammed donor cell into a lymphocyte.

[6.10] In another aspect, the present disclosure refers to a modified lymphocyte as disclosed herein, the modified cell as disclosed herein, or the population of cells as disclosed herein for use in therapy.
[6.11] In yet another aspect, the present disclosure refers to the use of the modified lymphocyte as disclosed herein, the modified cell as disclosed herein, or the population of cells as disclosed herein in the manufacture of a medicament for treating a proliferative disease.

[7] Some examples of the present disclosure provide compositions, cells, cell populations, methods, strategies, and treatment modalities that are useful in the context of immunotherapeutic approaches, *e.g.*, immunooncology therapeutic approaches. In some examples, the present disclosure provides modified NK cells (or other lymphocytes) that are useful in NK cell therapy, *e.g.*, in the context of immunotherapeutic approaches. In some examples, the cells and cell populations provided herein are characterized by one or more modifications that enhance their efficacy in immunotherapeutic approaches. For example, in some examples, NK cells are provided that comprise one or more modifications that effect a loss-of-function in a gene or protein associated with inhibition of NK cell function in a therapeutic context, and/or one or more modifications that effect an expression of an exogenous nucleic acid or protein associated with an enhanced NK cell function in a therapeutic context. In some examples, the present disclosure provides modified NK cells that are derived from an induced pluripotent cell (iPSC). IPSC-derived NK cells are also referred to herein as iNK cells. In some examples, modified iNK cells are provided that are derived from a somatic cell, for example, and without limitation, from a fibroblast, a peripheral blood cell, or a developmentally mature T cell (T cell that have undergone thymic selection). In some examples, the NK or iNK cells provided herein comprise one or more genomic edits, *e.g.*, indels or insertions of exogenous nucleic acid constructs resulting from cutting a genomic locus with an RNA-guided nuclease. The use of RNA-guided nuclease technology in the context of the generation of modified NK and iNK cells allows for the engineering of complex alterations with enhanced characteristics relevant for clinical applications.
[8] Some examples of the present disclosure provide complex editing strategies, and resulting NK cells having complex genomic alterations, that allow for the generation of advanced NK cell products for clinical applications, *e.g.*, for immunooncology therapeutic approaches. In some examples, the modified NK cells provided herein can serve as an off-the-shelf clinical solution for patients having, or having been diagnosed with, a hyperproliferative disease, such as, for example, a cancer. In some examples, the modified NK cells exhibit an enhanced survival, proliferation, NK cell response level, NK cell response duration, resistance against NK cell exhaustion, and/or target recognition as compared to non-modified NK cells. For example, the modified NK cells provided herein may comprise genomic edits that result in: expression of a chimeric antigen receptor (CAR) of interest, *e.g.,* a CAR targeting mesothelin, EGFR, HER2 and/or MICA/B; expression of a CD16 variant, *e.g.*, a non-naturally occurring CD16 variant such as, for example, hnCD16 (see, e.g., Zhu et al., Blood 2017,

130:4452, the contents of which are incorporated herein in their entirety by reference); expression of an IL15/IL15RA fusion; a loss-of-function in TGF beta receptor 2 (TGFbetaR2); and/or expression of a dominant-negative variant of TGFbetaR2; a loss-of-function of ADORA2A; a loss-of-function of B2M; expression of HLA-G: a loss-of-function of a CIITA ; a loss-of-function of a PD1 ; a loss-of-function of TIGIT; and/or a loss-of-function of CISH; or any combination of two or more thereof in the modified NK cell. In one example, the modified NK cell comprises genomic edits that result in a loss-of-function of TGFbetaR2 and a loss-of-function of CISH. In one example, the modified NK cell comprises genomic edits that result in a loss-of-function of TGFbetaR2 and a loss-of-function of TIGIT. In one example, the modified NK cell comprises genomic edits that result in a loss-of-function of TGFbetaR2 and a loss-of-function of ADORA2A. In one example, the modified NK cell comprises genomic edits that result in a loss-of-function of TGFbetaR2 and a loss-of-function of NKG2A. In one example, the modified NK cell comprises genomic edits that result in a loss-of-function of CISH and a loss-of-function of TIGIT. In one example, the modified NK cell comprises genomic edits that result in a loss-of-function of CISH and a loss-of-function of ADORA2A. In one example, the modified NK cell comprises genomic edits that result in a loss-of-function of CISH and a loss-of-function of NKG2A. In one example, the modified NK cell comprises genomic edits that result in a loss-of-function of TIGIT and a loss-of-function of ADORA2A. In one example, the modified NK cell comprises genomic edits that result in a loss-of-function of TIGIT and a loss-of-function of NKG2A. In one example, the modified NK cell comprises genomic edits that result in a loss-of-function of ADORA2A and a loss-of-function of NKG2A. In one example, the modified NK cell comprises genomic edits that result in a loss-of-function of TGFbetaR2, a loss-of-function of CISH, and a loss-of-function of TIGIT. In one example, the modified NK cell comprises genomic edits that result in a loss-of-function of TGFbetaR2, a loss-of-function of CISH, and a loss-of-function of ADORA2A. In one example, the modified NK cell comprises genomic edits that result in a loss-of-function of TGFbetaR2, a loss-of-function of CISH, and a loss-of-function of NKG2A. In one example, the modified NK cell comprises genomic edits that result in a loss-of-function of TGFbetaR2, a loss-of-function of TIGIT, and a loss-of-function of ADORA2A. In one example, the modified NK cell comprises genomic edits that result in a loss-of-function of TGFbetaR2, a loss-of-function of TIGIT, and a loss-of-function of NKG2A. In one example, the modified NK cell comprises genomic edits that result in a loss-of-function of TGFbetaR2, a loss-of-function of ADORA2A, and a loss-of-function of NKG2A. In one example, the modified NK cell comprises genomic edits that result in a loss-of-function of CISH, a loss-of-function of TIGIT, and a loss-of-function of ADORA2A. In one example, the modified NK cell comprises genomic edits that result in a loss-of-function of CISH, a loss-of-function of TIGIT, and a loss-of-function of NKG2A. In one example, the modified NK cell comprises genomic edits that result in a loss-of-function of CISH, a loss-of-function of ADORA2A, and a loss-of-function of NKG2A. In one example, the modified NK cell comprises genomic edits that

result in a loss-of-function of TIGIT, a loss-of-function of ADORA2A, and a loss-of-function of NKG2A.

[9] In some examples, the modified NK cells provided herein may comprise genomic edits that result in: expression of an exogenous a CD16 variant, *e.g.*, hnCD16, expression of an exogenous IL15/IL15RA fusion, expression of an exogenous HLA-G, expression of an exogenous DN-TGFbetaR2, a loss of function in TGFbetaR2, a loss of function in B2M, a loss of function of PD1, a loss of function of TIGIT, and/or a loss of function of ADORA2A.

[10] In some examples, the modified NK cells provided herein may comprise genomic edits that result in: expression of an exogenous a CD16 variant, *e.g.*, hnCD16, expression of an exogenous IL15/IL15RA fusion, expression of an exogenous HLA-G, expression of an exogenous DN-TGFbetaR2, expression of a soluble MICA and/or MICB, a loss of function in TGFbetaR2, a loss of function in B2M, a loss of function of PD1, a loss of function of TIGIT, and/or a loss of function of ADORA2A.

[11] In some examples, the modified NK cells provided herein may comprise genomic edits that result in: expression of an exogenous a CD16 variant, *e.g.*, hnCD16, expression of an exogenous IL15/IL15RA fusion, expression of an exogenous HLA-G, expression of an exogenous DN-TGFbetaR2, expression of a soluble MICA and/or MICB, expression of an exogenous IL-12, expression of an exogenous IL-18, a loss of function in TGFbetaR2, a loss of function in B2M, a loss of function of PD1, a loss of function of TIGIT, and/or a loss of function of ADORA2A.

[12] In some examples, the modified NK cells provided herein may comprise genomic edits that result in: expression of an exogenous a CD16 variant, *e.g.*, hnCD16, expression of an exogenous IL15/IL15RA fusion, expression of an exogenous HLA-G, expression of an exogenous DN-TGFbetaR2, expression of an exogenous IL-12, expression of an exogenous IL-18, a loss of function in TGFbetaR2, a loss of function in B2M, a loss of function of PD1, a loss of function of TIGIT, and/or a loss of function of ADORA2A.

[13] In one example the disclosure features a modified lymphocyte, wherein the modified lymphocyte does not express endogenous CD3, CD4, and/or CD8; and expresses at least one endogenous gene encoding: (i) CD56 (NCAM), CD49, and/or CD45; (ii) NK cell receptor (cluster of differentiation 16 (CD16)); (iii) natural killer group-2 member D (NKG2D); (iv) CD69; (v) a natural cytotoxicity receptor; or any combination of two or more thereof; wherein the modified lymphocyte further: (1) comprises at least one exogenous nucleic acid construct encoding: (i) a chimeric antigen receptor (CAR); (ii) a non-naturally occurring variant of immunoglobulin gamma Fc region receptor III (FcγRIII, CD16); (iii) interleukin 15 (IL-15); (iv) IL-15 receptor (IL-15R), or a variant thereof; (v) interleukin 12 (IL-12); (vi) interleukin-12 receptor (IL-12R), or a variant thereof; (vii) human leukocyte antigen G (HLA-G); (viii) human leukocyte antigen E (HLA-E); (ix) a nucleic acid sequence encoding leukocyte surface antigen cluster of differentiation CD47 (CD47); or any

combination of two or more thereof; and/or (2) exhibits a loss of function of at least one of: (i) transforming growth factor beta receptor 2 (TGFβR2); (ii) adenosine A2a receptor (ADORA2A); (iii) T cell immunoreceptor with Ig and ITIM domains (TIGIT); (iv) β-2 microgobulin (B2M); (v) programmed cell death protein 1 (PD-1); (vi) cytokine inducible SH2 containing protein (CISH); (vii) class II, major histocompatibility complex, transactivator (CIITA); (viii) natural killer cell receptor NKG2A (natural killer group 2A); (ix) two or more HLA class II histocompatibility antigen alpha chain genes, and/or two or more HLA class II histocompatibility antigen beta chain genes; (x) cluster of differentiation 32B (CD32B, FCGR2B); (xi) T cell receptor alpha constant (TRAC); or any combination of two or more thereof. In one example, the modified lymphocyte exhibits a loss of function of TGFβR2 and a loss-of-function of CISH. In one example, the modified lymphocyte exhibits a loss-of-function of TGFbetaR2 and a loss-of-function of TIGIT. In one example, the modified lymphocyte exhibits a loss-of-function of TGFbetaR2 and a loss-of-function of ADORA2A. In one example, the modified lymphocyte exhibits a loss-of-function of TGFbetaR2 and a loss-of-function of NKG2A. In one example, the modified lymphocyte exhibits a loss-of-function of CISH and a loss-of-function of TIGIT. In one example, the modified lymphocyte exhibits a loss-of-function of CISH and a loss-of-function of ADORA2A. In one example, the modified lymphocyte exhibits a loss-of-function of CISH and a loss-of-function of NKG2A. In one example, the modified lymphocyte exhibits a loss-of-function of TIGIT and a loss-of-function of ADORA2A. In one example, the modified lymphocyte exhibits a loss-of-function of TIGIT and a loss-of-function of NKG2A. In one example, the modified lymphocyte exhibits a loss-of-function of ADORA2A and a loss-of-function of NKG2A. In one example, the modified lymphocyte exhibits a loss-of-function of TGFbetaR2, a loss-of-function of CISH, and a loss-of-function of TIGIT. In one example, the modified lymphocyte exhibits a loss-of-function of TGFbetaR2, a loss-of-function of CISH, and a loss-of-function of ADORA2A. In one example, the modified lymphocyte exhibits a loss-of-function of TGFbetaR2, a loss-of-function of CISH, and a loss-of-function of NKG2A. In one example, the modified lymphocyte exhibits a loss-of-function of TGFbetaR2, a loss-of-function of TIGIT, and a loss-of-function of ADORA2A. In one example, the modified lymphocyte exhibits a loss-of-function of TGFbetaR2, a loss-of-function of TIGIT, and a loss-of-function of NKG2A. In one example, the modified lymphocyte exhibits a loss-of-function of TGFbetaR2, a loss-of-function of ADORA2A, and a loss-of-function of NKG2A. In one example, the modified lymphocyte exhibits a loss-of-function of CISH, a loss-of-function of TIGIT, and a loss-of-function of ADORA2A. In one example, the modified lymphocyte exhibits a loss-of-function of CISH, a loss-of-function of TIGIT, and a loss-of-function of NKG2A. In one example, the modified lymphocyte exhibits a loss-of-function of CISH, a loss-of-function of ADORA2A, and a loss-of-function of NKG2A. In one example, the modified lymphocyte exhibits a loss-of-function of TIGIT, a loss-of-function of ADORA2A, and a loss-of-function of NKG2A.

[14] In one example, the modified lymphocyte does not express endogenous CD3, CD4, and/or CD8; and expresses at least one endogenous gene encoding: (i) CD56 (NCAM), CD49, and/or CD45; (ii) NK cell receptor (cluster of differentiation 16 (CD16)); (iii) natural killer group-2 member D (NKG2D); (iv) CD69; (v) a natural cytotoxicity receptor; or any combination of two or more thereof; wherein the modified lymphocyte further: (1) comprises at least one exogenous nucleic acid construct encoding: (i) a chimeric antigen receptor (CAR); (ii) a non-naturally occurring variant of immunoglobulin gamma Fc region receptor III (FcγRIII, CD16); (iii) interleukin 15 (IL-15); (iv) IL-15 receptor (IL-15R), or a variant thereof; (v) interleukin 12 (IL-12); (vi) interleukin-12 receptor (IL-12R), or a variant thereof; (vii) human leukocyte antigen G (HLA-G); (viii) human leukocyte antigen E (HLA-E); (ix) a nucleic acid sequence encoding leukocyte surface antigen cluster of differentiation CD47 (CD47); or any combination of two or more thereof; and/or (2) exhibits a loss of function of transforming growth factor beta receptor 2 (TGFβR2), cytokine inducible SH2 containing protein (CISH), or a combination thereof.

[15] In some examples, the nucleic acid construct is an expression construct comprising a nucleic acid sequence encoding the gene product listed under (1)(i)-(1(ix), or any combination thereof, operably linked to a promoter driving expression of the nucleic acid sequence in a target cell, e.g., in a modified lymphocyte, for example, a modified NK cell provided herein. In some examples, the promoter is specifically expressed in the target cell, e.g., the promoter is a lymphocyte- or NK-cell-specific promoter. In some examples, the promoter is a CD56 (NCAM) promoter. In some examples, the promoter is a CD49 promoter. In some examples, the promoter is a CD45 promoter. In some examples, the promoter is an FcγRIII promoter. In some examples, the promoter is an NKG2D promoter. In some examples, the promoter is a CD69 promoter.

[16] In some examples, the exogenous nucleic acid construct encoding a gene product listed under (1) is knocked into a genomic locus encoding a gene product listed under (2), resulting in a loss-of-function of the gene product listed under (2) and expression of a gene product encoded by the exogenous nucleic acid construct, either driven by a heterologous promoter, or driven by the endogenous promoter of the genomic locus that the exogenous nucleic acid construct is knocked into.

[17] In some examples, the exogenous nucleic acid construct encoding a gene product listed under (1) is knocked into a "safe harbor" locus, e.g., a ROSA26 locus, a collagen locus, or an AAVSI genomic locus.

[18] In some examples, the two or more HLA class II histocompatibility antigen alpha chain genes are selected from HLA-DQA1, HLA-DRA, HLA-DPA1, HLA-DMA, HLA-DQA2, and HLA-DOA. In some examples, the two or more HLA class II histocompatibility antigen beta chain genes are selected from HLA-DMB, HLA-DOB, HLA-DPB1, HLA-DQB1, HLA-DQB3, HLA-DQB2, HLA-DRB1, HLA-DRB3, HLA-DRB4, and HLA-DRB5.

[19] In some examples, the modified lymphocyte comprises a rearranged endogenous T-cell receptor (TCR) locus. In some examples, the rearranged TCR comprises TCRα VJ and/or TCRβ V(D)J section rearrangements and complete V-domain exons.

[20] In some examples, the natural cytotoxicity receptor is NKp30, NKp44, NKp46, and/or CD158b.

[21] In some examples, the IL-15R variant is a constitutively active IL-15R variant. In some examples, 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 examples, 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. Additional suitable variants will be apparent to those of ordinary skill in the art based on the present disclosure and the knowledge in the art. The disclosure is not limited in this respect.

[22] In some examples, the TGFβR2 is a dominant-negative variant of TGFβ receptor II (DN-TGFβR2).

[23] In some examples, the CAR is capable of binding mesothelin, EGFR, HER2, MICA/B, BCMA, CD19, CD22, CD20, CD33, CD123, androgen receptor, PSMA, PSCA, Muc1, HPV viral peptides (ie. E7), EBV viral peptides, CD70, WT1, CEA, EGFRvIII, IL13Rα2, GD2, CA125, CD7, EpCAM, Muc16, and/or CD30,

[24] In some examples, the modified lymphocyte is derived from a pluripotent or multipotent stem cell. In some examples, the multipotent stem cell is a hematopoietic stem cell (HSC). In some examples, the pluripotent stem cell is an induced pluripotent stem cell (iPSC). In some examples, the pluripotent stem cell is an embryonic stem cell (ESC).

[25] In some examples, the modified lymphocyte is derived from a pluripotent or multipotent stem cell that comprises at least one or more exogenous nucleic acid constructs encoding any of (1)(i)-(1)(ix), or any combination thereof; and/or at least one genomic alteration that effects the loss-of-function of any of (2)(i)-(2)(xi), or any combination thereof, in the lymphocyte.

[26] In some examples, the modified lymphocyte is derived from a pluripotent or multipotent stem cell that comprises at least one genomic alteration that effects the loss-of-function of any of (2)(i)-(2)(xi), or any combination thereof, in the lymphocyte.

[27] In some examples, the at least one genomic alteration that effects the loss-of-function of one or more (2)(i)-(2)(xi) in the lymphocyte comprises an insertion of an exogenous nucleic acid construct.

[28] In some examples, the exogenous nucleic acid construct encodes any of (1)(i)-(1)(ix), or any combination thereof.

[29] In some examples, the modified lymphocyte exhibits a loss-of-function in two or more of the genes/proteins listed under (2).

[30] In some examples, the modified lymphocyte comprises an indel or an insertion of an exogenous nucleotide construct into a genomic locus harboring a gene or encoding a protein under (2).

[31] In some examples, the modified lymphocyte comprises an indel or an insertion of an exogenous nucleotide construct into two or more genomic loci harboring a gene or encoding a protein under (2).

[32] In some examples, the modified lymphocyte was obtained by editing a genomic locus with an RNA-guided nuclease. In some examples, the RNA-guided nuclease is a CRISPR/Cas nuclease. In some examples, the RNA-guided nuclease is selected from the group consisting of SpCas9, SaCas9, (KKH) SaCas9, AsCpf1 (AsCas12a), LbCpf1, (LbCas12a), CasX, CasY, Cas12h1, Cas12i1, Cas12c1, Cas12c2, eSpCas9, Cas9-HF1, HypaCas9, dCas9-Fokl, Sniper-Cas9, xCas9, AaCas12b, evoCas9, SpCas9-NG, VRQR, VRER, NmeCas9, CjCas9, BhCas12b, and BhCas12b V4.

[33] In some examples, the modified lymphocyte is obtained by editing two or more genomic loci harboring genes encoding any of the proteins under (2). In some examples, at least two of the two or more genomic loci harboring genes encoding any of the proteins under (2) have been edited by a different RNA-guided nuclease. In some examples, at least one of the two or more genomic loci harboring genes encoding any of the proteins under (2) has been edited by Cas9, and wherein at least one of the loci has been edited by Cpf1.

[34] In some examples, the modified lymphocyte expresses endogenous CD56, CD49, and CD45.

[35] In some examples, the modified lymphocyte is a natural killer (NK) cell.

[36] In another example the disclosure features a modified cell, wherein the modified cell (1) comprises at least one exogenous nucleic acid construct encoding: (i) a chimeric antigen receptor (CAR); (ii) a non-naturally occurring variant of immunoglobulin gamma Fc region receptor III (FcγRIII, cluster of differentiation 16 ((CD16); (iii) interleukin 15 (IL-15); (iv) IL-15 receptor (IL-15R), or a variant thereof; (v) interleukin 12 (IL-12); (vi) IL-12 receptor (IL-12R), or a variant thereof; (vii) human leukocyte antigen G (HLA-G); (viii) human leukocyte antigen E (HLA-E); (ix) leukocyte surface antigen cluster of differentiation CD47 (CD47); or any combination of two or more thereof; and/or (2) exhibits a loss of function of at least one of: (i) transforming growth factor beta receptor 2 (TGFβR2); (ii) adenosine A2a receptor (ADORA2A); (iii) T cell immunoreceptor with Ig and ITIM domains (TIGIT); (iv) β-2 microgobulin (B2M); (v) programmed cell death protein 1 (PD-1); (vi) cytokine inducible SH2 containing protein (CISH); (vii) class II, major histocompatibility complex, transactivator (CIITA); (viii) natural killer cell receptor NKG2A (natural killer group 2A); (ix) two or more HLA class II histocompatibility antigen alpha chain genes, and/or two or more HLA class II histocompatibility antigen beta chain genes; (x) cluster of differentiation 32B (CD32B, FCGR2B); (xi) T cell receptor alpha constant (TRAC); or any combination of two or more thereof. In one example, the modified cell exhibits a loss of function of TGFβR2 and a loss-of-function of CISH. In one example, the modified cell exhibits a loss-of-function of TGFbetaR2 and a loss-of-function of TIGIT. In one example, the modified cell exhibits a loss-of-function of TGFbetaR2 and a loss-of-function of ADORA2A. In one example, the modified cell exhibits a loss-of-function of TGFbetaR2 and a loss-of-function of NKG2A. In one example, the modified cell exhibits a loss-of-function of CISH and a loss-of-function of TIGIT. In one example, the modified cell exhibits a loss-of-function of CISH and a loss-of-function of ADORA2A. In one example, the modified cell exhibits a loss-of-function of CISH and a loss-of-function of NKG2A. In one example, the modified cell exhibits a loss-

of-function of TIGIT and a loss-of-function of ADORA2A. In one example, the modified cell exhibits a loss-of-function of TIGIT and a loss-of-function of NKG2A. In one example, the modified cell exhibits a loss-of-function of ADORA2A and a loss-of-function of NKG2A. In one example, the modified cell exhibits a loss-of-function of TGFbetaR2, a loss-of-function of CISH, and a loss-of-function of TIGIT. In one example, the modified cell exhibits a loss-of-function of TGFbetaR2, a loss-of-function of CISH, and a loss-of-function of ADORA2A. In one example, the modified cell exhibits a loss-of-function of TGFbetaR2, a loss-of-function of CISH, and a loss-of-function of NKG2A. In one example, the modified cell exhibits a loss-of-function of TGFbetaR2, a loss-of-function of TIGIT, and a loss-of-function of ADORA2A. In one example, the modified cell exhibits a loss-of-function of TGFbetaR2, a loss-of-function of TIGIT, and a loss-of-function of NKG2A. In one example, the modified cell exhibits a loss-of-function of TGFbetaR2, a loss-of-function of ADORA2A, and a loss-of-function of NKG2A. In one example, the modified cell exhibits a loss-of-function of CISH, a loss-of-function of TIGIT, and a loss-of-function of ADORA2A. In one example, the modified cell exhibits a loss-of-function of CISH, a loss-of-function of TIGIT, and a loss-of-function of NKG2A. In one example, the modified cell exhibits a loss-of-function of CISH, a loss-of-function of ADORA2A, and a loss-of-function of NKG2A. In one example, the modified cell exhibits a loss-of-function of TIGIT, a loss-of-function of ADORA2A, and a loss-of-function of NKG2A.

[37] In one example, the modified cell (1) comprises at least one exogenous nucleic acid construct encoding: (i) a chimeric antigen receptor (CAR); (ii) a non-naturally occurring variant of immunoglobulin gamma Fc region receptor III (FcγRIII, cluster of differentiation 16 ((CD16); (iii) interleukin 15 (IL-15); (iv) IL-15 receptor (IL-15R), or a variant thereof; (v) interleukin 12 (IL-12); (vi) IL-12 receptor (IL-12R), or a variant thereof; (vii) human leukocyte antigen G (HLA-G); (viii) human leukocyte antigen E (HLA-E); (ix) leukocyte surface antigen cluster of differentiation CD47 (CD47); or any combination of two or more thereof; and/or (2) exhibits a loss of function of transforming growth factor beta receptor 2 (TGFβR2), cytokine inducible SH2 containing protein (CISH), or a combination thereof.

[38] In some examples of modified cells comprising an exogenous nucleic acid construct, e.g., modified lymphocytes provided herein, the exogenous nucleic acid construct is an expression construct comprising a nucleic acid sequence encoding the gene product listed under (1)(i)-(1(x), or any combination thereof, operably linked to a promoter driving expression of the nucleic acid sequence in a target cell, e.g., in a modified lymphocyte, for example, a modified NK cell provided herein. In some examples, the promoter is specifically expressed in the target cell, e.g., the promoter is a lymphocyte- or NK-cell-specific promoter. In some examples, the promoter is a CD56 (NCAM) promoter. In some examples, the promoter is a CD49 promoter. In some examples, the promoter is a CD45 promoter. In some examples, the promoter is an FcγRIII promoter. In some examples, the promoter is an NKG2D promoter. In some examples, the promoter is a CD69 promoter.

[39] In some examples of modified cells, e.g., modified lymphocytes provided herein, the exogenous nucleic acid construct encoding a gene product listed under (1) is knocked into a genomic locus encoding a gene product listed under (2), resulting in a loss-of-function of the gene product listed under (2) and

expression of a gene product encoded by the exogenous nucleic acid construct, either driven by a heterologous promoter, or driven by the endogenous promoter of the genomic locus that the exogenous nucleic acid construct is knocked into.

[40] In some examples of modified cells, e.g., modified lymphocytes provided herein, comprising a loss of function in two or more HLA class II histocompatibility antigen alpha chain genes, and/or two or more HLA class II histocompatibility antigen beta chain genes, the two or more HLA class II histocompatibility antigen alpha chain genes are selected from HLA-DQA1, HLA-DRA, HLA-DPA1, HLA-DMA, HLA-DQA2, and HLA-DOA. In some examples, the two or more HLA class II histocompatibility antigen beta chain genes are selected from HLA-DMB, HLA-DOB, HLA-DPB1, HLA-DQB1, HLA-DQB3, HLA-DQB2, HLA-DRB1, HLA-DRB3, HLA-DRB4, and HLA-DRB5.

[41] In some examples, the modified cell is an immune cell. In some examples, the immune cell is a lymphocyte. In some examples, the lymphocyte is an NK cell. In some examples, the lymphocyte is an iNK cell.

[42] In some examples, the modified cell is a multipotent or pluripotent stem cell, e.g., an iPS cell, or a hematopoietic stem cell, or a differentiated cell derived from such a multipotent or pluripotent stem cell, e.g., an iNK cell.

[43] In some examples, the modified cell does not express an endogenous T-cell co-receptor.

[44] In some examples, the lymphocyte is a T cell.

[45] In some examples, the modified cell comprises a rearranged endogenous TCR locus, wherein the rearranged TCR comprises TCRα VJ and/or TCRβ V(D)J section rearrangements and complete V-domain exons.

[46] In some examples, the modified cell expresses at least one endogenous gene encoding: (i) CD56 (NCAM), CD49, and/or CD45; (ii) NK cell receptor (cluster of differentiation 16 (CD16)); (iii) natural killer group-2 member D (NKG2D); (iv) CD69; (v) a natural cytotoxicity receptor; or any combination of two or more thereof.

[47] In some examples, the natural cytotoxicity receptor is NKp30, NKp44, NKp46, and/or CD158b.

[48] In some examples, the modified cell expresses at least one NK cell biomarker. In some examples, the NK cell biomarker is CD56, CD49, and/or CD45.

[49] In one example, disclosed herein is a population of cells comprising the modified lymphocyte described herein, or the modified cell described herein.

[50] In one example, disclosed herein is a pharmaceutical composition comprising the population of cells disclosed herein.

[51] In another example, the disclosure provides an isolated population of lymphocytes, wherein the population comprises at least $1x10^3$, at least $1x10^4$, at least $1x10^5$, at least $2x10^5$, at least $3x10^5$, at least $4x10^5$, at least $5x10^5$,at least $1x10^6$, at least $2x10^6$, at least $3x10^6$, at least $4x10^6$, at least $5x10^6$,at least $1x10^7$, at least $1x10^7$, at least $2x10^7$, at least $3x10^7$, at least $4x10^7$, at least $5x10^7$,at least $1x10^8$, at least $2x10^8$, at least $3x10^8$, at least $4x10^8$, at least $5x10^8$,at least $1x10^9$, at least $1x10^9$, at least $2x10^9$, at least

$3x10^9$, at least $4x10^9$, at least $5x10^9$,at least $1x10^{10}$, at least $2x10^{10}$, at least $3x10^{10}$, at least $4x10^{10}$, at least $5x10^{10}$, at least $1x10^{11}$, or at least $1x10^{12}$ cells, and wherein at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.9%, at least 99.99%, at least 99.999%, or virtually 100% of the lymphocytes in the population: (a) comprise a rearranged T-cell receptor (TCR) locus; (b) do not express endogenous CD3; (c) express endogenous CD56 (NCAM), CD49, and/or CD45; and (d) expresses at least endogenous gene encoding: (i) NK cell receptor (cluster of differentiation 16 (CD16)); (ii) natural killer group-2 member D (NKG2D); (iii) CD69; (iv) a natural cytotoxicity receptor; or any combination of two or more thereof; and wherein the modified lymphocyte further: (1) comprises at least one exogenous nucleic acid construct encoding: (i) chimeric antigen receptor (CAR); (ii) non-naturally occurring variant of immunoglobulin gamma Fc region receptor III (FcγRIII, CD16); (iii) interleukin 15 (IL-15); (iv) IL-15 receptor (IL-15R), or a variant thereof; (v) interleukin 12 (IL-12); (vi) IL-12 receptor (IL-12R), or a variant thereof; (vii) human leukocyte antigen G (HLA-G); (viii) human leukocyte antigen E (HLA-E); (ix) leukocyte surface antigen cluster of differentiation CD47 (CD47); or any combination of two or more thereof; and/or (2) exhibits a loss of function of at least one of: (i) transforming growth factor beta receptor 2 (TGFβR2); (ii) adenosine A2a receptor (ADORA2A); (iii) T cell immunoreceptor with Ig and ITIM domains (TIGIT); (iv) β-2 microgobulin (B2M); (v) programmed cell death protein 1 (PD-1); (vi) cytokine inducible SH2 containing protein (CISH); (vii) class II, major histocompatibility complex, transactivator (CIITA); (viii) natural killer cell receptor NKG2A (natural killer group 2A); (ix) two or more HLA class II histocompatibility antigen alpha chain genes, and/or two or more HLA class II histocompatibility antigen beta chain genes; (x) cluster of differentiation 32B (CD32B, FCGR2B); (xi) T cell receptor alpha constant (TRAC); or any combination of two or more thereof. In one example, the modified lymphocyte exhibits a loss of function of TGFβR2 and a loss-of-function of CISH. In one example, the modified lymphocyte exhibits a loss-of-function of TGFbetaR2 and a loss-of-function of TIGIT. In one example, the modified lymphocyte exhibits a loss-of-function of TGFbetaR2 and a loss-of-function of ADORA2A. In one example, the modified lymphocyte exhibits a loss-of-function of TGFbetaR2 and a loss-of-function of NKG2A. In one example, the modified lymphocyte exhibits a loss-of-function of CISH and a loss-of-function of TIGIT. In one example, the modified lymphocyte exhibits a loss-of-function of CISH and a loss-of-function of ADORA2A. In one example, the modified lymphocyte exhibits a loss-of-function of CISH and a loss-of-function of NKG2A. In one example, the modified lymphocyte exhibits a loss-of-function of TIGIT and a loss-of-function of ADORA2A. In one example, the modified lymphocyte exhibits a loss-of-function of TIGIT and a loss-of-function of NKG2A. In one example, the modified lymphocyte exhibits a loss-of-function of ADORA2A and a loss-of-function of NKG2A. In one example, the modified lymphocyte exhibits a loss-of-function of TGFbetaR2, a loss-of-function of CISH, and a loss-of-function of TIGIT. In one example, the modified lymphocyte exhibits a loss-of-function of TGFbetaR2, a loss-of-function of CISH, and a loss-of-function of ADORA2A. In one example, the modified lymphocyte exhibits a loss-of-function of TGFbetaR2, a loss-of-function of CISH, and a loss-of-function of NKG2A. In one example, the modified lymphocyte exhibits a loss-of-function of TGFbetaR2, a loss-of-function of TIGIT, and a loss-of-function of ADORA2A. In

one example, the modified lymphocyte exhibits a loss-of-function of TGFbetaR2, a loss-of-function of TIGIT, and a loss-of-function of NKG2A. In one example, the modified lymphocyte exhibits a loss-of-function of TGFbetaR2, a loss-of-function of ADORA2A, and a loss-of-function of NKG2A. In one example, the modified lymphocyte exhibits a loss-of-function of CISH, a loss-of-function of TIGIT, and a loss-of-function of ADORA2A. In one example, the modified lymphocyte exhibits a loss-of-function of CISH, a loss-of-function of TIGIT, and a loss-of-function of NKG2A. In one example, the modified lymphocyte exhibits a loss-of-function of CISH, a loss-of-function of ADORA2A, and a loss-of-function of NKG2A. In one example, the modified lymphocyte exhibits a loss-of-function of TIGIT, a loss-of-function of ADORA2A, and a loss-of-function of NKG2A.

[52] In one example, the isolated population of myphocytes comprises at least $1x10^3$, at least $1x10^4$, at least $1x10^5$, at least $2x10^5$, at least $3x10^5$, at least $4x10^5$, at least $5x10^5$,at least $1x10^6$, at least $2x10^6$, at least $3x10^6$, at least $4x10^6$, at least $5x10^6$,at least $1x10^7$, at least $1x10^7$, at least $2x10^7$, at least $3x10^7$, at least $4x10^7$, at least $5x10^7$,at least $1x10^8$, at least $2x10^8$, at least $3x10^8$, at least $4x10^8$, at least $5x10^8$,at least $1x10^9$, at least $1x10^9$, at least $2x10^9$, at least $3x10^9$, at least $4x10^9$, at least $5x10^9$,at least $1x10^{10}$, at least $2x10^{10}$, at least $3x10^{10}$, at least $4x10^{10}$, at least $5x10^{10}$, at least $1x10^{11}$, or at least $1x10^{12}$ cells, and wherein at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.9%, at least 99.99%, at least 99.999%, or virtually 100% of the lymphocytes in the population: (a) comprise a rearranged T-cell receptor (TCR) locus; (b) do not express endogenous CD3; (c) express endogenous CD56 (NCAM), CD49, and/or CD45; and (d) expresses at least endogenous gene encoding: (i) NK cell receptor (cluster of differentiation 16 (CD16)); (ii) natural killer group-2 member D (NKG2D); (iii) CD69; (iv) a natural cytotoxicity receptor; or any combination of two or more thereof; and wherein the modified lymphocyte further: (1) comprises at least one exogenous nucleic acid construct encoding: (i) chimeric antigen receptor (CAR); (ii) non-naturally occurring variant of immunoglobulin gamma Fc region receptor III (FcγRIII, CD16); (iii) interleukin 15 (IL-15); (iv) IL-15 receptor (IL-15R), or a variant thereof; (v) interleukin 12 (IL-12); (vi) IL-12 receptor (IL-12R), or a variant thereof; (vii) human leukocyte antigen G (HLA-G); (viii) human leukocyte antigen E (HLA-E); (ix) leukocyte surface antigen cluster of differentiation CD47 (CD47); or any combination of two or more thereof; and/or (2) exhibits a loss of function of transforming growth factor beta receptor 2 (TGFβR2), cytokine inducible SH2 containing protein (CISH), or a combination thereof.

[53] In some examples, the rearranged TCR locus comprises of TCRα VJ and/or TCRβ V(D)J section rearrangements and complete V-domain exons. In some examples, the rearranged endogenous TCR locus consists of no more than two rearranged alleles.

[54] In some examples, the natural cytotoxicity receptor is NKp30, NKp44, NKp46, and/or CD158b.

[55] In some examples, the *in vitro* population of lymphocytes does not comprise more than 1%, more than 0.1%, more than 0.001%, more than 0.0001%, more than 0.00001%, more than 0.000001%,

more than 0.0000001%, more than 0.00000001%, more than 0.000000001%, more than 0.0000000001%, or more than more than 0.00000000001% of cells expressing a reprogramming factor from an exogenous nucleic acid construct.

[56] In some examples, the *in vitro* population of lymphocytes does not comprise a cell expressing a reprogramming factor from an exogenous nucleic acid construct. In some examples, the reprogramming factor is Oct-4 and/or Sox-2.

[57] In some examples, the *in vitro* population of lymphocytes does not comprise cells harboring episomal expression constructs encoding a reprogramming factor.

[58] In some examples, each cell in *in vitro* population of lymphocytes comprises the same combination of an exogenous nucleic acid construct listed under (1) and a loss of function listed (2).

[59] In some examples, the *in vitro* population of lymphocytes comprises less than 0.001%, less than 0.002%, less than 0.003%, less than 0.004%, less than 0.005%, less than 0.006%, less than 0.007%, less than 0.008%, less than 0.009%, less than 0.01%, less than 0.02%, less than 0.03%, less than 0.04%, less than 0.05%, less than 0.06%, less than 0.07%, less than 0.08%, less than 0.09%, less than 0.1%, less than 0.2%, less than 0.3%, less than 0.4%, less than 0.5%, less than 0.6%, less than 0.7%, less than 0.8%, less than 0.9%, less than 1%, less than 2%, less than 3%, less than 4%, less than 5%, less than 6%, less than 7%, less than 8%, less than 9%, or less than 10% cell that harbor a chromosomal translocation.

[60] In another example, the disclosure provides a method of treating a subject, the method comprising administering any modified lymphocyte, any modified cell, any pharmaceutical composition, or the isolated *in vitro* population of cells as described in the disclosure, to a subject in need thereof. In some examples, the subject has, or is diagnosed with, a proliferative disease. In some examples, the proliferative disease is cancer. In some examples, the cancer is breast cancer, colorectal cancer, gastric cancer, renal cell carcinoma (RCC), or non-small cell lung cancer (NSCLC), solid tumors, 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, soft tissue sarcomas, and hematological cancers like ALL, CLL, NHL, DLBCL, AML, CML, multiple myeloma (MM).

[61] In some examples, the method of generating the modified lymphocyte, the modified cell, the population of cells, or the isolated *in vitro* population of lymphocytes of the disclosure comprises: (a) obtaining an induced pluripotent stem cell (iPSC); (b) modifying the iPSC, or an undifferentiated or differentiated daughter cell thereof, to comprise express at least one exogenous gene of (1) and/or to comprise a loss of function in at least one gene of (2); (c) directing differentiation of the iPSC to hematopoietic lineage cells, wherein the hematopoietic lineage cells retain the edited genetic loci comprised in the iPSCs.

[62] In some examples, directing differentiation comprises: (i) contacting iPSCs with a composition comprising a BMP pathway activator, and optionally bFGF, to obtain mesodermal cells; and (ii) contacting the mesodermal cells with a composition comprising a BMP pathway activator, bFGF, and a WNT pathway activator, to obtain mesodermal cells having definitive hemogenic endothelium (HE) potential, wherein the mesodermal cells having definitive hemogenic endothelium (HE) potential are capable of providing hematopoietic lineage cells; wherein mesodermal cells and mesodermal cells having definitive HE potential are obtained in steps (i) and (ii) without the step of forming embryoid bodies; wherein the hematopoietic lineage cells comprise definitive hemogenic endothelium cells, hematopoietic stem and progenitor cells (HSC), hematopoietic multipotent progenitor cell (MPP), pre-T cell progenitor cells, pre-NK cell progenitor cells, T cell progenitor cells, NK cell progenitor cells, T cells, NK cells, NKT cells, or B cells.

[63] In some examples, the method of directing differentiation of iPSCs to hematopoietic lineage cells further comprises: contacting the mesodermal cells having definitive HE potential with a composition comprising bFGF and a ROCK inhibitor to obtain definitive HE cells.

[64] In some examples, the method of directing differentiation further comprises: contacting the definitive HE cells with a composition comprising a BMP activator, and optionally a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6, Flt3L and IL11 to obtain hematopoietic multipotent progenitor cells (MPP).

[65] In some examples, the method of directing differentiation further comprises: contacting the definitive HE cells with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, and IL7; and optionally one or more of a BMP activator, a ROCK inhibitor, TPO, VEGF and bFGF to obtain pre-T cell progenitors, T cell progenitors, and/or T cells.

[66] In some examples, the method of directing differentiation further comprises: contacting the definitive HE cells with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, TPO, IL7 and IL15, and optionally one or more of a BMP activator, a ROCK inhibitor, VEGF and bFGF to obtain pre-NK cell progenitors, NK cell progenitors, and/or NK cells.

[67] In some examples, the method of generating the modified lymphocyte, the modified cell, the population of cells, or the isolated *in vitro* population of lymphocytes of the disclosure further comprises: prior to step c), contacting the pluripotent stem cells with a composition comprising a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, to seed and expand the cells.

[68] In some examples, the method of generating the modified lymphocyte, the modified cell, the population of cells, or the isolated *in vitro* population of lymphocytes of the disclosure further comprises: detecting a rearranged T-cell receptor (TCR) locus in the hematopoietic lineage cells. In

some examples, the method further comprises selecting the hematopoietic lineage cells comprising the rearranged TCR locus based on the TCR encoded by the rearranged TCR locus binding an antigen of interest. In some examples, the antigen of interest is a tumor antigen.

[69] In another example, the disclosure provides a method, the method comprising: reprogramming a donor cell to a pluripotent state; editing a target locus in the donor cell genome; and differentiating the reprogrammed donor cell into a lymphocyte. In some examples, the editing is performed before or during the step of reprogramming of the donor cell to a pluripotent state. In some examples, the donor cell is a fibroblast, a peripheral blood cell, a lymphocyte, or a T cell.

[70] In another example, the disclosure provides a method, the method comprising: differentiating a genetically modified pluripotent stem cell into a lymphocyte, wherein the genetically modified pluripotent stem cell comprises: (1) an exogenous nucleic acid comprising: (i) a nucleic acid encoding a chimeric antigen receptor (CAR); (ii) a nucleic acid encoding a non-naturally occurring variant of immunoglobulin gamma Fc region receptor III (FcγRIII, CD16); (iii) a nucleic acid encoding interleukin 15 (IL-15); (iv) a nucleic acid encoding IL-15R, or a variant thereof;(v) a nucleic acid encoding interleukin 12 (IL-12); (vi) a nucleic acid encoding IL-12R, or a variant thereof; (vii) a nucleic acid encoding human leukocyte antigen G (HLA-G); (viii) human leukocyte antigen E (HLA-E); (ix) leukocyte surface antigen cluster of differentiation CD47 (CD47); or any combination of two or more thereof; and (2) an indel, or an insertion of an exogenous nucleic acid in one or more of the following genetic loci:(i) transforming growth factor beta receptor 2 (TGFβR2); (ii) adenosine A2a receptor (ADORA2A); (iii) T cell immunoreceptor with Ig and ITIM domains (TIGIT); (iv) β-2 microgobulin (B2M); (v) programmed cell death protein 1 (PD-1, CD279); (vi) cytokine inducible SH2 containing protein (CISH); (vii) class II, major histocompatibility complex, transactivator (CIITA); (viii) natural killer cell receptor NKG2A (natural killer group 2A); (ix) two or more HLA class II histocompatibility antigen alpha chain genes, and/or two or more HLA class II histocompatibility antigen beta chain genes; (x) cluster of differentiation 32B (CD32B, FCGR2B); (xi) T cell receptor alpha constant (TRAC); or any combination of two or more thereof, wherein the indel or insertion results in a loss-of-function of a gene product encoded by the respective genetic locus or loci. In one example, the method comprises differentiating a genetically modified pluripotent stem cell into a lymphocyte, wherein the genetically modified pluripotent stem cell comprises an indel, or an insertion of an exogenous nucleic acid in TGFβR2 and CISH, wherein the indel or insertion results in a loss-of-function of a gene product encoded by TGFβR2 and/or CISH. In one example, the method comprises differentiating a genetically modified pluripotent stem cell into a lymphocyte, wherein the genetically modified pluripotent stem cell comprises an indel, or an insertion of an exogenous nucleic acid in TGFβR2 and TIGIT, wherein the indel or insertion results in a loss-of-function of a gene product encoded by TGFβR2 and/or TIGIT. In one example, the method comprises differentiating a genetically modified pluripotent stem cell into a lymphocyte, wherein the genetically

modified pluripotent stem cell comprises an indel, or an insertion of an exogenous nucleic acid in TGFβR2 and ADORA2A, wherein the indel or insertion results in a loss-of-function of a gene product encoded by TGFβR2 and/or ADORA2A. In one example, the method comprises differentiating a genetically modified pluripotent stem cell into a lymphocyte, wherein the genetically modified pluripotent stem cell comprises an indel, or an insertion of an exogenous nucleic acid in TGFβR2 and NKG2A, wherein the indel or insertion results in a loss-of-function of a gene product encoded by TGFβR2 and/or NKG2A. In one example, the method comprises differentiating a genetically modified pluripotent stem cell into a lymphocyte, wherein the genetically modified pluripotent stem cell comprises an indel, or an insertion of an exogenous nucleic acid in CISH and TIGIT, wherein the indel or insertion results in a loss-of-function of a gene product encoded by CISH and/or TIGIT. In one example, the method comprises differentiating a genetically modified pluripotent stem cell into a lymphocyte, wherein the genetically modified pluripotent stem cell comprises an indel, or an insertion of an exogenous nucleic acid in CISH and ADORA2A, wherein the indel or insertion results in a loss-of-function of a gene product encoded by CISH and/or ADORA2A. In one example, the method comprises differentiating a genetically modified pluripotent stem cell into a lymphocyte, wherein the genetically modified pluripotent stem cell comprises an indel, or an insertion of an exogenous nucleic acid in CISH and NKG2A, wherein the indel or insertion results in a loss-of-function of a gene product encoded by CISH and/or NKG2A. In one example, the method comprises differentiating a genetically modified pluripotent stem cell into a lymphocyte, wherein the genetically modified pluripotent stem cell comprises an indel, or an insertion of an exogenous nucleic acid in TIGIT and ADORA2A, wherein the indel or insertion results in a loss-of-function of a gene product encoded by TIGIT and/or ADORA2A. In one example, the method comprises differentiating a genetically modified pluripotent stem cell into a lymphocyte, wherein the genetically modified pluripotent stem cell comprises an indel, or an insertion of an exogenous nucleic acid in TIGIT and NKG2A, wherein the indel or insertion results in a loss-of-function of a gene product encoded by TIGIT and/or NKG2A. In one example, the method comprises differentiating a genetically modified pluripotent stem cell into a lymphocyte, wherein the genetically modified pluripotent stem cell comprises an indel, or an insertion of an exogenous nucleic acid in ADORA2A and NKG2A, wherein the indel or insertion results in a loss-of-function of a gene product encoded by ADORA2A and/or NKG2A. In one example, the method comprises differentiating a genetically modified pluripotent stem cell into a lymphocyte, wherein the genetically modified pluripotent stem cell comprises an indel, or an insertion of an exogenous nucleic acid in TGFβR2, CISH and TIGIT, wherein the indel or insertion results in a loss-of-function of a gene product encoded by TGFβR2, CISH and/or TIGIT. In one example, the method comprises differentiating a genetically modified pluripotent stem cell into a lymphocyte, wherein the genetically modified pluripotent stem cell comprises an indel, or an insertion of an exogenous nucleic acid in TGFβR2, CISH and ADORA2A,

wherein the indel or insertion results in a loss-of-function of a gene product encoded by TGFβR2, CISH and/or ADORA2A. In one example, the method comprises differentiating a genetically modified pluripotent stem cell into a lymphocyte, wherein the genetically modified pluripotent stem cell comprises an indel, or an insertion of an exogenous nucleic acid in TGFβR2, CISH and NKG2A, wherein the indel or insertion results in a loss-of-function of a gene product encoded by TGFβR2, CISH and/or NKG2A. In one example, the method comprises differentiating a genetically modified pluripotent stem cell into a lymphocyte, wherein the genetically modified pluripotent stem cell comprises an indel, or an insertion of an exogenous nucleic acid in TGFβR2, TIGIT and ADORA2A, wherein the indel or insertion results in a loss-of-function of a gene product encoded by TGFβR2, TIGIT and/or ADORA2A. In one example, the method comprises differentiating a genetically modified pluripotent stem cell into a lymphocyte, wherein the genetically modified pluripotent stem cell comprises an indel, or an insertion of an exogenous nucleic acid in TGFβR2, TIGIT and NKG2A, wherein the indel or insertion results in a loss-of-function of a gene product encoded by TGFβR2, TIGIT and/or NKG2A. In one example, the method comprises differentiating a genetically modified pluripotent stem cell into a lymphocyte, wherein the genetically modified pluripotent stem cell comprises an indel, or an insertion of an exogenous nucleic acid in TGFβR2, ADORA2A and NKG2A, wherein the indel or insertion results in a loss-of-function of a gene product encoded by TGFβR2, ADORA2A and/or NKG2A. In one example, the method comprises differentiating a genetically modified pluripotent stem cell into a lymphocyte, wherein the genetically modified pluripotent stem cell comprises an indel, or an insertion of an exogenous nucleic acid in CISH, TIGIT and ADORA2A, wherein the indel or insertion results in a loss-of-function of a gene product encoded by CISH, TIGIT and/or ADORA2A. In one example, the method comprises differentiating a genetically modified pluripotent stem cell into a lymphocyte, wherein the genetically modified pluripotent stem cell comprises an indel, or an insertion of an exogenous nucleic acid in CISH, TIGIT and NKG2A, wherein the indel or insertion results in a loss-of-function of a gene product encoded by CISH, TIGIT and/or NKG2A. In one example, the method comprises differentiating a genetically modified pluripotent stem cell into a lymphocyte, wherein the genetically modified pluripotent stem cell comprises an indel, or an insertion of an exogenous nucleic acid in CISH, ADORA2A and NKG2A, wherein the indel or insertion results in a loss-of-function of a gene product encoded by CISH, ADORA2A and/or NKG2A. In one example, the method comprises differentiating a genetically modified pluripotent stem cell into a lymphocyte, wherein the genetically modified pluripotent stem cell comprises an indel, or an insertion of an exogenous nucleic acid in TIGIT, ADORA2A and NKG2A, wherein the indel or insertion results in a loss-of-function of a gene product encoded by TIGIT, ADORA2A and/or NKG2A.

[71] In some examples, the exogenous nucleic acid of (2) is the exogenous nucleic acid of (1). In some examples, the pluripotent stem cell is an iPS cell. In some examples, the differentiating

comprises contacting the pluripotent stem cell with a differentiation medium or a sequence of differentiation media.

BRIEF DESCRIPTION OF THE DRAWINGS

[72] **Fig. 1A** and **1B** depict that robust single and double-gene editing of TGFBR2 and CISH was achieved in NK cells. Both single and simultaneous targeting of TGFBR2 and CISH in NK cells using CRISPR-Cpf1 produced in/dels at both targets in greater than 80% of NK cells, with greater than 90% of edited NK cells viable at 72 hours post-editing.

[73] **Fig. 2A** and **2B** depict that normalization of spheroid curves maintains the same efficacy patterns as observed in the non-normalized data, as analyzed across 3 unique donors and 5 independent experiments. Each single knockout (SKO) NK group was significantly more efficient at reducing the SK-OV-3 spheroid size than the control NK, and the double knockout (DKO) NK group was significantly more efficient at reducing the SK-OV-3 spheroid size than the SKO NK groups. **Fig. 2A** depicts SK-OV-3 spheriod analysis at 10:1 E:T with 10ng/mL TGFbeta (3 donors, 5 independent experiments). **Fig. 2B** has error bars

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.

[153] In some embodiments, a gRNA which includes a modification, e.g., a DNA extension at the 5' end, is complexed with a RNA-guided nuclease, e.g., an AsCpf1 nuclease, to form an RNP, which is then employed to edit a target cell, e.g., an NK cell.

[154] Exemplary suitable 5' extensions for Cpf1 guide RNAs are provided in the table below:

[155] Table 3: gRNA 5' Extensions

5' extension Sequence ID No:	5' extension sequence	5' modification
	rCrUrUrUrU	+5 RNA
1	rArArGrArCrCrUrUrUrU	+10 RNA
2	rArUrGrUrGrUrUrUrUrUrGrUrCrArArArArGrArCrCrUrUrUrU	+25 RNA
3	rArGrGrCrCrArGrCrUrUrGrCrCrGrGrUrUrUrUrUrUrArGrUrCrGrUrGrCrUrGrCrUrUrCrArUrGrUrGrUrUrUrUrUrGrUrCrArArArArGrArCrCrUrUrUrU	+60 RNA
	CTTTT	+5 DNA
4	AAGACCTTTT	+10 DNA
5	ATGTGTTTTTGTCAAAAGACCTTTT	+25 DNA
6	AGGCCAGCTTGCCGGTTTTTTAGTCGTGCTGCTTCATGTGTTTTTGTCAAAAGACCTTTT	+60 DNA
7	TTTTTGTCAAAAGACCTTTT	+20 DNA
8	GCTTCATGTGTTTTTGTCAAAAGACCTTTT	+30 DNA
9	GCCGGTTTTTTAGTCGTGCTGCTTCATGTGTTTTTGTCAAAAGACCTTTT	+50 DNA
10	TAGTCGTGCTGCTTCATGTGTTTTTGTCAAAAGACCTTTT	+40 DNA
11	C*C*GAAGTTTTCTTCGGTTTT	+20 DNA + 2xPS
12	T*T*TTTCCGAAGTTTTCTTCGGTTTT	+25 DNA + 2xPS
13	A*A*CGCTTTTTCCGAAGTTTTCTTCGGTTTT	+30 DNA + 2xPS
14	G*C*GTTGTTTTCAACGCTTTTTCCGAAGTTTTCTTCGGTTTT	+41 DNA + 2xPS
15	G*G*CTTCTTTTGAAGCCTTTTTGCGTTGTTTTCAACGCTTTTTCCGAAGTTTTCTTCGGTTTT	+62 DNA + 2xPS
16	A*T*GTGTTTTTGTCAAAAGACCTTTT	+25 DNA + 2xPS
17	AAAAAAAAAAAAAAAAAAAAAAAAA	+25 A
18	TTTTTTTTTTTTTTTTTTTTTTTTT	+25 T
19	mA*mU*rGrUrGrUrUrUrUrGrUrCrArArArArGrArCrCrUrUrUrU	+25 RNA + 2xPS
20	mA*mA*rArArArArArArArArArArArArArArArArArArArArArArArArA	PolyA RNA + 2xPS
21	mU*mU*rUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrU	PolyU RNA + 2xPS

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 ID NO:22) would have a targeting domain of the corresponding RNA sequence UCUGCAGAAAUGUUCCCCGU (SEQ ID NO:23) . 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/tracerRNA 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:24) , 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:25) . 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 ATGTGTTTTTGTCAAAAGACCTTTTrUrArArUrUrUrCrUrArCrUrCrUrUrGrUrArGrArU rUrCrUrGrCrArGrArArArUrGrUrUrCrCrCrCrGrU (SEQ ID NO:26) . 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.

[160] 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 4.

Table 4: TIGIT gRNAs

Name	gRNA Targeting Domain Sequence (DNA)	SEQ ID NO:	Length	Enzyme
TIGIT4170	TCTGCAGAAATGTTCCCCGT	27	20	AsCpf1
TIGIT4171	TGCAGAGAAAGGTGGCTCTA	28	20	AsCpf1
TIGIT4172	TAATGCTGACTTGGGGTGGC	29	20	AsCpf1
TIGIT4173	TAGGACCTCCAGGAAGATTC	30	20	AsCpf1
TIGIT4174	TAGTCAACGCGACCACCACG	31	20	AsCpf1
TIGIT4175	TCCTGAGGTCACCTTCCACA	32	20	AsCpf1
TIGIT4176	TATTGTGCCTGTCATCATTC	33	20	AsCpf1

TIGIT4177	TGACAGGCACAATAGAAACAA	34	21	SauCas9
TIGIT4178	GACAGGCACAATAGAAACAAC	35	21	SauCas9
TIGIT4179	AAACAACGGGGAACATTTCTG	36	21	SauCas9
TIGIT4180	ACAACGGGGAACATTTCTGCA	37	21	SauCas9
TIGIT4181	TGATAGAGCCACCTTTCTCTG	38	21	SauCas9
TIGIT4182	GGGTCACTTGTGCCGTGGTGG	39	21	SauCas9
TIGIT4183	GGCACAAGTGACCCAGGTCAA	40	21	SauCas9
TIGIT4184	GTCCTGCTGCTCCCAGTTGAC	41	21	SauCas9
TIGIT4185	TGGCCATTTGTAATGCTGACT	42	21	SauCas9
TIGIT4186	TGGCACATCTCCCCATCCTTC	43	21	SauCas9
TIGIT4187	CATCTCCCCATCCTTCAAGGA	44	21	SauCas9
TIGIT4188	CCACTCGATCCTTGAAGGATG	45	21	SauCas9
TIGIT4189	GGCCACTCGATCCTTGAAGGA	46	21	SauCas9
TIGIT4190	CCTGGGGCCACTCGATCCTTG	47	21	SauCas9
TIGIT4191	GACTGGAGGGTGAGGCCCAGG	48	21	SauCas9
TIGIT4192	ATCGTTCACGGTCAGCGACTG	49	21	SauCas9
TIGIT4193	GTCGCTGACCGTGAACGATAC	50	21	SauCas9
TIGIT4194	CGCTGACCGTGAACGATACAG	51	21	SauCas9
TIGIT4195	GCATCTATCACACCTACCCTG	52	21	SauCas9
TIGIT4196	CCTACCCTGATGGGACGTACA	53	21	SauCas9
TIGIT4197	TACCCTGATGGGACGTACACT	54	21	SauCas9
TIGIT4198	CCCTGATGGGACGTACACTGG	55	21	SauCas9
TIGIT4199	TTCTCCCAGTGTACGTCCCAT	56	21	SauCas9
TIGIT4200	GGAGAATCTTCCTGGAGGTCC	57	21	SauCas9
TIGIT4201	CATGGCTCCAAGCAATGGAAT	58	21	SauCas9
TIGIT4202	CGCGGCCATGGCTCCAAGCAA	59	21	SauCas9
TIGIT4203	TCGCGGCCATGGCTCCAAGCA	60	21	SauCas9
TIGIT4204	CATCGTGGTGGTCGCGTTGAC	61	21	SauCas9
TIGIT4205	AAAGCCCTCAGAATCCATTCT	62	21	SauCas9
TIGIT4206	CATTCTGTGGAAGGTGACCTC	63	21	SauCas9
TIGIT4207	TTCTGTGGAAGGTGACCTCAG	64	21	SauCas9
TIGIT4208	CCTGAGGTCACCTTCCACAGA	65	21	SauCas9
TIGIT4209	TTCTCCTGAGGTCACCTTCCA	66	21	SauCas9
TIGIT4210	AGGAGAAAATCAGCTGGACAG	67	21	SauCas9
TIGIT4211	GGAGAAAATCAGCTGGACAGG	68	21	SauCas9
TIGIT4212	GCCCCAGTGCTCCCTCACCCC	69	21	SauCas9
TIGIT4213	TGGACACAGCTTCCTGGGGGT	70	21	SauCas9
TIGIT4214	TCTGCCTGGACACAGCTTCCT	71	21	SauCas9
TIGIT4215	AGCTGCACCTGCTGGGCTCTG	72	21	SauCas9
TIGIT4216	GCTGGGCTCTGTGGAGAGCAG	73	21	SauCas9
TIGIT4217	TGGGCTCTGTGGAGAGCAGCG	74	21	SauCas9
TIGIT4218	CTGCATGACTACTTCAATGTC	75	21	SauCas9
TIGIT4219	AATGTCCTGAGTTACAGAAGC	76	21	SauCas9
TIGIT4220	TGGGTAACTGCAGCTTCTTCA	77	21	SauCas9

TIGIT4221	GACAGGCACAATAGAAACAA	78	20	SpyCas9
TIGIT4222	ACAGGCACAATAGAAACAAC	79	20	SpyCas9
TIGIT4223	CAGGCACAATAGAAACAACG	80	20	SpyCas9
TIGIT4224	GGGAACATTTCTGCAGAGAA	81	20	SpyCas9
TIGIT4225	AACATTTCTGCAGAGAAAGG	82	20	SpyCas9
TIGIT4226	ATGTCACCTCTCCTCCACCA	83	20	SpyCas9
TIGIT4227	CTTGTGCCGTGGTGGAGGAG	84	20	SpyCas9
TIGIT4228	GGTCACTTGTGCCGTGGTGG	85	20	SpyCas9
TIGIT4229	CACCACGGCACAAGTGACCC	86	20	SpyCas9
TIGIT4230	CTGGGTCACTTGTGCCGTGG	87	20	SpyCas9
TIGIT4231	GACCTGGGTCACTTGTGCCG	88	20	SpyCas9
TIGIT4232	CACAAGTGACCCAGGTCAAC	89	20	SpyCas9
TIGIT4233	ACAAGTGACCCAGGTCAACT	90	20	SpyCas9
TIGIT4234	CCAGGTCAACTGGGAGCAGC	91	20	SpyCas9
TIGIT4235	CTGCTGCTCCCAGTTGACCT	92	20	SpyCas9
TIGIT4236	CCTGCTGCTCCCAGTTGACC	93	20	SpyCas9
TIGIT4237	GGAGCAGCAGGACCAGCTTC	94	20	SpyCas9
TIGIT4238	CATTACAAATGGCCAGAAGC	95	20	SpyCas9
TIGIT4239	GGCCATTTGTAATGCTGACT	96	20	SpyCas9
TIGIT4240	GCCATTTGTAATGCTGACTT	97	20	SpyCas9
TIGIT4241	CCATTTGTAATGCTGACTTG	98	20	SpyCas9
TIGIT4242	TTTGTAATGCTGACTTGGGG	99	20	SpyCas9
TIGIT4243	CCCCAAGTCAGCATTACAAA	100	20	SpyCas9
TIGIT4244	GCACATCTCCCCATCCTTCA	101	20	SpyCas9
TIGIT4245	CCCATCCTTCAAGGATCGAG	102	20	SpyCas9
TIGIT4246	CACTCGATCCTTGAAGGATG	103	20	SpyCas9
TIGIT4247	CCACTCGATCCTTGAAGGAT	104	20	SpyCas9
TIGIT4248	GCCACTCGATCCTTGAAGGA	105	20	SpyCas9
TIGIT4249	TTCAAGGATCGAGTGGCCCC	106	20	SpyCas9
TIGIT4250	TGGGGCCACTCGATCCTTGA	107	20	SpyCas9
TIGIT4251	GATCGAGTGGCCCCAGGTCC	108	20	SpyCas9
TIGIT4252	AGTGGCCCCAGGTCCCGGCC	109	20	SpyCas9
TIGIT4253	GTGGCCCCAGGTCCCGGCCT	110	20	SpyCas9
TIGIT4254	GAGGCCCAGGCCGGGACCTG	111	20	SpyCas9
TIGIT4255	TGAGGCCCAGGCCGGGACCT	112	20	SpyCas9
TIGIT4256	GTGAGGCCCAGGCCGGGACC	113	20	SpyCas9
TIGIT4257	TGGAGGGTGAGGCCCAGGCC	114	20	SpyCas9
TIGIT4258	CTGGAGGGTGAGGCCCAGGC	115	20	SpyCas9
TIGIT4259	GCGACTGGAGGGTGAGGCCC	116	20	SpyCas9
TIGIT4260	CGGTCAGCGACTGGAGGGTG	117	20	SpyCas9
TIGIT4261	GTTCACGGTCAGCGACTGGA	118	20	SpyCas9
TIGIT4262	CGTTCACGGTCAGCGACTGG	119	20	SpyCas9
TIGIT4263	TATCGTTCACGGTCAGCGAC	120	20	SpyCas9
TIGIT4264	TCGCTGACCGTGAACGATAC	121	20	SpyCas9

TIGIT4265	CGCTGACCGTGAACGATACA	122	20	SpyCas9
TIGIT4266	GCTGACCGTGAACGATACAG	123	20	SpyCas9
TIGIT4267	GTACTCCCCTGTATCGTTCA	124	20	SpyCas9
TIGIT4268	ATCTATCACACCTACCCTGA	125	20	SpyCas9
TIGIT4269	TCTATCACACCTACCCTGAT	126	20	SpyCas9
TIGIT4270	TACCCTGATGGGACGTACAC	127	20	SpyCas9
TIGIT4271	ACCCTGATGGGACGTACACT	128	20	SpyCas9
TIGIT4272	AGTGTACGTCCCATCAGGGT	129	20	SpyCas9
TIGIT4273	TCCCAGTGTACGTCCCATCA	130	20	SpyCas9
TIGIT4274	CTCCCAGTGTACGTCCCATC	131	20	SpyCas9
TIGIT4275	GTACACTGGGAGAATCTTCC	132	20	SpyCas9
TIGIT4276	CACTGGGAGAATCTTCCTGG	133	20	SpyCas9
TIGIT4277	CTGAGCTTTCTAGGACCTCC	134	20	SpyCas9
TIGIT4278	AGGTTCCAGATTCCATTGCT	135	20	SpyCas9
TIGIT4279	AAGCAATGGAATCTGGAACC	136	20	SpyCas9
TIGIT4280	GATTCCATTGCTTGGAGCCA	137	20	SpyCas9
TIGIT4281	TGGCTCCAAGCAATGGAATC	138	20	SpyCas9
TIGIT4282	GCGGCCATGGCTCCAAGCAA	139	20	SpyCas9
TIGIT4283	TGGAGCCATGGCCGCGACGC	140	20	SpyCas9
TIGIT4284	AGCCATGGCCGCGACGCTGG	141	20	SpyCas9
TIGIT4285	GACCACCAGCGTCGCGGCCA	142	20	SpyCas9
TIGIT4286	GCAGATGACCACCAGCGTCG	143	20	SpyCas9
TIGIT4287	CATCTGCACAGCAGTCATCG	144	20	SpyCas9
TIGIT4288	CTGCACAGCAGTCATCGTGG	145	20	SpyCas9
TIGIT4289	AGCCCTCAGAATCCATTCTG	146	20	SpyCas9
TIGIT4290	CTCAGAATCCATTCTGTGGA	147	20	SpyCas9
TIGIT4291	TTCCACAGAATGGATTCTGA	148	20	SpyCas9
TIGIT4292	CTTCCACAGAATGGATTCTG	149	20	SpyCas9
TIGIT4293	ATTCTGTGGAAGGTGACCTC	150	20	SpyCas9
TIGIT4294	TGAGGTCACCTTCCACAGAA	151	20	SpyCas9
TIGIT4295	GACCTCAGGAGAAAATCAGC	152	20	SpyCas9
TIGIT4296	CAGGAGAAAATCAGCTGGAC	153	20	SpyCas9
TIGIT4297	GTCCAGCTGATTTTCTCCTG	154	20	SpyCas9
TIGIT4298	GAGAAAATCAGCTGGACAGG	155	20	SpyCas9
TIGIT4299	AATCAGCTGGACAGGAGGAA	156	20	SpyCas9
TIGIT4300	CCCAGTGCTCCCTCACCCCC	157	20	SpyCas9
TIGIT4301	CTGGGGGTGAGGGAGCACTG	158	20	SpyCas9
TIGIT4302	CCTGGGGGTGAGGGAGCACT	159	20	SpyCas9
TIGIT4303	TCCTGGGGGTGAGGGAGCAC	160	20	SpyCas9
TIGIT4304	ACACAGCTTCCTGGGGGTGA	161	20	SpyCas9
TIGIT4305	GACACAGCTTCCTGGGGGTG	162	20	SpyCas9
TIGIT4306	ACCCCCAGGAAGCTGTGTCC	163	20	SpyCas9
TIGIT4307	GCCTGGACACAGCTTCCTGG	164	20	SpyCas9
TIGIT4308	TGCCTGGACACAGCTTCCTG	165	20	SpyCas9

TIGIT4309	CTGCCTGGACACAGCTTCCT	166	20	SpyCas9
TIGIT4310	TCTGCCTGGACACAGCTTCC	167	20	SpyCas9
TIGIT4311	CAGGCAGAAGCTGCACCTGC	168	20	SpyCas9
TIGIT4312	AGGCAGAAGCTGCACCTGCT	169	20	SpyCas9
TIGIT4313	CAGCAGGTGCAGCTTCTGCC	170	20	SpyCas9
TIGIT4314	GCTGCACCTGCTGGGCTCTG	171	20	SpyCas9
TIGIT4315	TGCTCTCCACAGAGCCCAGC	172	20	SpyCas9
TIGIT4316	CTGGGCTCTGTGGAGAGCAG	173	20	SpyCas9
TIGIT4317	TGGGCTCTGTGGAGAGCAGC	174	20	SpyCas9
TIGIT4318	GGGCTCTGTGGAGAGCAGCG	175	20	SpyCas9
TIGIT4319	CTGTGGAGAGCAGCGGGGAG	176	20	SpyCas9
TIGIT4320	ATTGAAGTAGTCATGCAGCT	177	20	SpyCas9
TIGIT4321	TGTCCTGAGTTACAGAAGCC	178	20	SpyCas9
TIGIT4322	GTCCTGAGTTACAGAAGCCT	179	20	SpyCas9
TIGIT4323	TACCCAGGCTTCTGTAACTC	180	20	SpyCas9
TIGIT4324	TGAAGAAGCTGCAGTTACCC	181	20	SpyCas9
TIGIT4325	TGCAGCTTCTTCACAGAGAC	182	20	SpyCas9
TIGIT5053	GTTGTTTCTATTGTGCCTGT	183	20	AsCpf1 RR
TIGIT5054	CGTTGTTTCTATTGTGCCTG	184	20	AsCpf1 RR
TIGIT5055	CCGTTGTTTCTATTGTGCCT	185	20	AsCpf1 RR
TIGIT5056	CCACGGCACAAGTGACCCAG	186	20	AsCpf1 RR
TIGIT5057	AGTTGACCTGGGTCACTTGT	187	20	AsCpf1 RR
TIGIT5058	AAGTCAGCATTACAAATGGC	188	20	AsCpf1 RR
TIGIT5059	CATCCTTCAAGGATCGAGTG	189	20	AsCpf1 RR
TIGIT5060	ATCCTTCAAGGATCGAGTGG	190	20	AsCpf1 RR
TIGIT5061	AGGATCGAGTGGCCCCAGGT	191	20	AsCpf1 RR
TIGIT5062	AGGTCCCGGCCTGGGCCTCA	192	20	AsCpf1 RR
TIGIT5063	GGCCTGGGCCTCACCCTCCA	193	20	AsCpf1 RR
TIGIT5064	CGGTCAGCGACTGGAGGGTG	194	20	AsCpf1 RR
TIGIT5065	GTCGCTGACCGTGAACGATA	195	20	AsCpf1 RR
TIGIT5066	TGTATCGTTCACGGTCAGCG	196	20	AsCpf1 RR
TIGIT5067	CTGTATCGTTCACGGTCAGC	197	20	AsCpf1 RR
TIGIT5068	ATCAGGGTAGGTGTGATAGA	198	20	AsCpf1 RR
TIGIT5069	AGTGTACGTCCCATCAGGGT	199	20	AsCpf1 RR
TIGIT5070	GGAAGATTCTCCCAGTGTAC	200	20	AsCpf1 RR
TIGIT5071	TGGAGGTCCTAGAAAGCTCA	201	20	AsCpf1 RR
TIGIT5072	AGCAATGGAATCTGGAACCT	202	20	AsCpf1 RR
TIGIT5073	AGATTCCATTGCTTGGAGCC	203	20	AsCpf1 RR
TIGIT5074	GATTCCATTGCTTGGAGCCA	204	20	AsCpf1 RR
TIGIT5075	ATTGCTTGGAGCCATGGCCG	205	20	AsCpf1 RR
TIGIT5076	TTGCTTGGAGCCATGGCCGC	206	20	AsCpf1 RR
TIGIT5077	CAGAATGGATTCTGAGGGCT	207	20	AsCpf1 RR
TIGIT5078	ACAGAATGGATTCTGAGGGC	208	20	AsCpf1 RR
TIGIT5079	TTCTGTGGAAGGTGACCTCA	209	20	AsCpf1 RR

TIGIT5080	GCTGATTTTCTCCTGAGGTC	210	20	AsCpf1 RR
TIGIT5081	TCCTGTCCAGCTGATTTTCT	211	20	AsCpf1 RR
TIGIT5082	TTCCTCCTGTCCAGCTGATT	212	20	AsCpf1 RR
TIGIT5083	TGGGGGTGAGGGAGCACTGG	213	20	AsCpf1 RR
TIGIT5084	AGTGCTCCCTCACCCCCAGG	214	20	AsCpf1 RR
TIGIT5085	TCACCCCCAGGAAGCTGTGT	215	20	AsCpf1 RR
TIGIT5086	CAGGAAGCTGTGTCCAGGCA	216	20	AsCpf1 RR
TIGIT5087	AGGAAGCTGTGTCCAGGCAG	217	20	AsCpf1 RR
TIGIT5088	GGCAGAAGCTGCACCTGCTG	218	20	AsCpf1 RR
TIGIT5089	CAGAGCCCAGCAGGTGCAGC	219	20	AsCpf1 RR
TIGIT5090	GCTGCTCTCCACAGAGCCCA	220	20	AsCpf1 RR
TIGIT5091	CGCTGCTCTCCACAGAGCCC	221	20	AsCpf1 RR
TIGIT5092	ATGTCCTGAGTTACAGAAGC	222	20	AsCpf1 RR

[161] 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 5.

Table 5. ADORA2a gRNAs

Name	gRNA Targeting Domain Sequence (DNA)	SEQ ID NO:	Length	Enzyme
ADORA2A337	GAGCACACCCACTGCGATGT	223	20	SpyCas9
ADORA2A338	GATGGCCAGGAGACTGAAGA	224	20	SpyCas9
ADORA2A339	CTGCTCACCGGAGCGGGATG	225	20	SpyCas9
ADORA2A340	GTCTGTGGCCATGCCCATCA	226	20	SpyCas9
ADORA2A341	TCACCGGAGCGGGATGCGGA	227	20	SpyCas9
ADORA2A342	GTGGCAGGCAGCGCAGAACC	228	20	SpyCas9
ADORA2A343	AGCACACCAGCACATTGCCC	229	20	SpyCas9
ADORA2A344	CAGGTTGCTGTTGAGCCACA	230	20	SpyCas9
ADORA2A345	CTTCATTGCCTGCTTCGTCC	231	20	SpyCas9
ADORA2A346	GTACACCGAGGAGCCCATGA	232	20	SpyCas9
ADORA2A347	GATGGCAATGTAGCGGTCAA	233	20	SpyCas9
ADORA2A348	CTCCTCGGTGTACATCACGG	234	20	SpyCas9
ADORA2A349	CGAGGAGCCCATGATGGGCA	235	20	SpyCas9

ADORA2A350	GGGCTCCTCGGTGTACATCA	236	20	SpyCas9
ADORA2A351	CTTTGTGGTGTCACTGGCGG	237	20	SpyCas9
ADORA2A352	CCGCTCCGGTGAGCAGGGCC	238	20	SpyCas9
ADORA2A353	GGGTTCTGCGCTGCCTGCCA	239	20	SpyCas9
ADORA2A354	GGACGAAGCAGGCAATGAAG	240	20	SpyCas9
ADORA2A355	GTGCTGATGGTGATGGCAAA	241	20	SpyCas9
ADORA2A356	AGCGCAGAACCCGGTGCTGA	242	20	SpyCas9
ADORA2A357	GAGCTCCATCTTCAGTCTCC	243	20	SpyCas9
ADORA2A358	TGCTGATGGTGATGGCAAAG	244	20	SpyCas9
ADORA2A359	GGCGGCGGCCGACATCGCAG	245	20	SpyCas9
ADORA2A360	AATGAAGAGGCAGCCGTGGC	246	20	SpyCas9
ADORA2A361	GGGCAATGTGCTGGTGTGCT	247	20	SpyCas9
ADORA2A362	CATGCCCATCATGGGCTCCT	248	20	SpyCas9
ADORA2A363	AATGTAGCGGTCAATGGCGA	249	20	SpyCas9
ADORA2A364	AGTAGTTGGTGACGTTCTGC	250	20	SpyCas9
ADORA2A365	AGCGGTCAATGGCGATGGCC	251	20	SpyCas9
ADORA2A366	CGCATCCCGCTCCGGTGAGC	252	20	SpyCas9
ADORA2A367	GCATCCCGCTCCGGTGAGCA	253	20	SpyCas9
ADORA2A368	TGGGCAATGTGCTGGTGTGC	254	20	SpyCas9
ADORA2A369	CAACTACTTTGTGGTGTCAC	255	20	SpyCas9
ADORA2A370	CGCTCCGGTGAGCAGGGCCG	256	20	SpyCas9
ADORA2A371	GATGGTGATGGCAAAGGGGA	257	20	SpyCas9
ADORA2A372	GGTGTACATCACGGTGGAGC	258	20	SpyCas9
ADORA2A373	GAACGTCACCAACTACTTTG	259	20	SpyCas9
ADORA2A374	CAGTGACACCACAAAGTAGT	260	20	SpyCas9
ADORA2A375	GGCCATCCTGGGCAATGTGC	261	20	SpyCas9
ADORA2A376	CCCGGCCCTGCTCACCGGAG	262	20	SpyCas9

ADORA2A377	CACCAGCACATTGCCCAGGA	263	20	SpyCas9
ADORA2A378	TTTGCCATCACCATCAGCAC	264	20	SpyCas9
ADORA2A379	CTCCACCGTGATGTACACCG	265	20	SpyCas9
ADORA2A380	GGAGCTGGCCATTGCTGTGC	266	20	SpyCas9
ADORA2A381	CAGGATGGCCAGCACAGCAA	267	20	SpyCas9
ADORA2A382	GAACCCGGTGCTGATGGTGA	268	20	SpyCas9
ADORA2A383	TGGAGCTCTGCGTGAGGACC	269	20	SpyCas9
ADORA2A384	CCCGCTCCGGTGAGCAGGGC	270	20	SpyCas9
ADORA2A385	AGGCAATGAAGAGGCAGCCG	271	20	SpyCas9
ADORA2A386	CCGGCCCTGCTCACCGGAGC	272	20	SpyCas9
ADORA2A387	GCGGCGGCCGACATCGCAGT	273	20	SpyCas9
ADORA2A388	GGTGCTGATGGTGATGGCAA	274	20	SpyCas9
ADORA2A389	CTACTTTGTGGTGTCACTGG	275	20	SpyCas9
ADORA2A390	TACACCGAGGAGCCCATGAT	276	20	SpyCas9
ADORA2A391	TCTGTGGCCATGCCCATCAT	277	20	SpyCas9
ADORA2A392	ATTGCTGTGCTGGCCATCCT	278	20	SpyCas9
ADORA2A393	CGTGAGGACCAGGACGAAGC	279	20	SpyCas9
ADORA2A394	TTGCCATCACCATCAGCACC	280	20	SpyCas9
ADORA2A395	GGATGCGGATGGCAATGTAG	281	20	SpyCas9
ADORA2A396	TTGCCATCCGCATCCCGCTC	282	20	SpyCas9
ADORA2A397	TGAAGATGGAGCTCTGCGTG	283	20	SpyCas9
ADORA2A398	CATTGCTGTGCTGGCCATCC	284	20	SpyCas9
ADORA2A399	TGCTGGTGTGCTGGGCCGTG	285	20	SpyCas9
ADORA2A820	GGCTCCTCGGTGTACATCACG	286	21	SauCas9
ADORA2A821	GAGCTCTGCGTGAGGACCAGG	287	21	SauCas9
ADORA2A822	GATGGAGCTCTGCGTGAGGAC	288	21	SauCas9
ADORA2A823	CCAGCACACCAGCACATTGCC	289	21	SauCas9
ADORA2A824	AGGACCAGGACGAAGCAGGCA	290	21	SauCas9
ADORA2A825	TGCCATCCGCATCCCGCTCCG	291	21	SauCas9

ADORA2A826	GTGTGGCTCAACAGCAACCTG	292	21	SauCas9
ADORA2A827	AGCTCCACCGTGATGTACACC	293	21	SauCas9
ADORA2A828	GTAGCGGTCAATGGCGATGGC	294	21	SauCas9
ADORA2A829	CGGTGCTGATGGTGATGGCAA	295	21	SauCas9
ADORA2A830	CCCTGCTCACCGGAGCGGGAT	296	21	SauCas9
ADORA2A831	GTGACGTTCTGCAGGTTGCTG	297	21	SauCas9
ADORA2A832	GCTCCACCGTGATGTACACCG	298	21	SauCas9
ADORA2A833	ACTGAAGATGGAGCTCTGCGT	299	21	SauCas9
ADORA2A834	CCAGCTCCACCGTGATGTACA	300	21	SauCas9
ADORA2A835	CCTTTGCCATCACCATCAGCA	301	21	SauCas9
ADORA2A836	CCGGTGCTGATGGTGATGGCA	302	21	SauCas9
ADORA2A837	CCTGGGCAATGTGCTGGTGTG	303	21	SauCas9
ADORA2A838	AGGCAGCCGTGGCAGGCAGCG	304	21	SauCas9
ADORA2A839	GCGATGGCCAGGAGACTGAAG	305	21	SauCas9
ADORA2A840	CGATGGCCAGGAGACTGAAGA	306	21	SauCas9
ADORA2A841	TCCCGCTCCGGTGAGCAGGGC	307	21	SauCas9
ADORA2A842	TGCTTCGTCCTGGTCCTCACG	308	21	SauCas9
ADORA2A843	ACCAGGACGAAGCAGGCAATG	309	21	SauCas9
ADORA2A844	ATGTACACCGAGGAGCCCATG	310	21	SauCas9
ADORA2A845	TCGTCTGTGGCCATGCCCATC	311	21	SauCas9
ADORA2A846	TCAATGGCGATGGCCAGGAGA	312	21	SauCas9
ADORA2A847	GGTGCTGATGGTGATGGCAAA	313	21	SauCas9
ADORA2A848	TAGCGGTCAATGGCGATGGCC	314	21	SauCas9
ADORA2A849	TCCGCATCCCGCTCCGGTGAG	315	21	SauCas9
ADORA2A850	CTGGCGGCGGCCGACATCGCA	316	21	SauCas9
ADORA2A851	GCCATTGCTGTGCTGGCCATC	317	21	SauCas9
ADORA2A852	ATCCCGCTCCGGTGAGCAGGG	318	21	SauCas9
ADORA2A853	AGACTGAAGATGGAGCTCTGC	319	21	SauCas9
ADORA2A854	CCCCGGCCCTGCTCACCGGAG	320	21	SauCas9
ADORA2A855	ATGGTGATGGCAAAGGGGATG	321	21	SauCas9
ADORA2A856	GCTCCTCGGTGTACATCACGG	322	21	SauCas9
ADORA2A248	TGTCGATGGCAATAGCCAAG	323	20	SpyCas9
ADORA2A249	AGAAGTTGGTGACGTTCTGC	324	20	SpyCas9
ADORA2A250	TTCGCCATCACCATCAGCAC	325	20	SpyCas9
ADORA2A251	GAAGAAGAGGCAGCCATGGC	326	20	SpyCas9
ADORA2A252	CACAAGCACGTTACCCAGGA	327	20	SpyCas9
ADORA2A253	CAACTTCTTCGTGGTATCTC	328	20	SpyCas9
ADORA2A254	CAGGATGGCCAGCACAGCAA	329	20	SpyCas9
ADORA2A255	AATTCCACTCCGGTGAGCCA	330	20	SpyCas9

ADORA2A256	AGCGCAGAAGCCAGTGCTGA	331	20	SpyCas9
ADORA2A257	GTGCTGATGGTGATGGCGAA	332	20	SpyCas9
ADORA2A258	GGAGCTGGCCATTGCTGTGC	333	20	SpyCas9
ADORA2A259	AATAGCCAAGAGGCTGAAGA	334	20	SpyCas9
ADORA2A260	CTCCTCGGTGTACATCATGG	335	20	SpyCas9
ADORA2A261	GGACAAAGCAGGCGAAGAAG	336	20	SpyCas9
ADORA2A262	TCTGGCGGCGGCTGACATCG	337	20	SpyCas9
ADORA2A263	TGGGTAACGTGCTTGTGTGC	338	20	SpyCas9
ADORA2A264	GATGTACACCGAGGAGCCCA	339	20	SpyCas9
ADORA2A265	TAACCCCTGGCTCACCGGAG	340	20	SpyCas9
ADORA2A266	TCACCGGAGTGGAATTCGGA	341	20	SpyCas9
ADORA2A267	GCGGCGGCTGACATCGCGGT	342	20	SpyCas9
ADORA2A268	GATGGTGATGGCGAATGGGA	343	20	SpyCas9
ADORA2A269	GGCTTCTGCGCTGCCTGCCA	344	20	SpyCas9
ADORA2A270	ATTCCACTCCGGTGAGCCAG	345	20	SpyCas9
ADORA2A271	GGTGTACATCATGGTGGAGC	346	20	SpyCas9
ADORA2A272	ATTGCTGTGCTGGCCATCCT	347	20	SpyCas9
ADORA2A273	CTCCACCATGATGTACACCG	348	20	SpyCas9
ADORA2A274	GGCGGCGGCTGACATCGCGG	349	20	SpyCas9
ADORA2A275	TACACCGAGGAGCCCATGGC	350	20	SpyCas9
ADORA2A276	GGGTAACGTGCTTGTGTGCT	351	20	SpyCas9
ADORA2A277	CAGGTTGCTGTTGATCCACA	352	20	SpyCas9
ADORA2A278	TGAAGATGGAACTCTGCGTG	353	20	SpyCas9
ADORA2A279	GATGGCGATGTATCTGTCGA	354	20	SpyCas9
ADORA2A280	CTTCTTCGCCTGCTTTGTCC	355	20	SpyCas9
ADORA2A281	AGGCGAAGAAGAGGCAGCCA	356	20	SpyCas9
ADORA2A282	TGCTTGTGTGCTGGGCCGTG	357	20	SpyCas9

ADORA2A283	GAAGCCAGTGCTGATGGTGA	358	20	SpyCas9
ADORA2A284	CGTGAGGACCAGGACAAAGC	359	20	SpyCas9
ADORA2A285	TGGAACTCTGCGTGAGGACC	360	20	SpyCas9
ADORA2A286	CATTGCTGTGCTGGCCATCC	361	20	SpyCas9
ADORA2A287	TTCTCCCGCCATGGGCTCCT	362	20	SpyCas9
ADORA2A288	TGGCTCACCGGAGTGGAATT	363	20	SpyCas9
ADORA2A289	TGCTGATGGTGATGGCGAAT	364	20	SpyCas9
ADORA2A290	CTTCGTGGTATCTCTGGCGG	365	20	SpyCas9
ADORA2A291	AGCACACAAGCACGTTACCC	366	20	SpyCas9
ADORA2A292	GGGCTCCTCGGTGTACATCA	367	20	SpyCas9
ADORA2A293	GTACACCGAGGAGCCCATGG	368	20	SpyCas9
ADORA2A294	GAACGTCACCAACTTCTTCG	369	20	SpyCas9
ADORA2A295	TCGCCATCCGAATTCCACTC	370	20	SpyCas9
ADORA2A296	GAGTTCCATCTTCAGCCTCT	371	20	SpyCas9
ADORA2A297	GAATTCCACTCCGGTGAGCC	372	20	SpyCas9
ADORA2A298	CAGAGATACCACGAAGAAGT	373	20	SpyCas9
ADORA2A299	CTTCTTCGTGGTATCTCTGG	374	20	SpyCas9
ADORA2A695	CAGTGCTGATGGTGATGGCGA	375	21	SauCas9
ADORA2A696	CGAATTCCACTCCGGTGAGCC	376	21	SauCas9
ADORA2A697	CCGAATTCCACTCCGGTGAGC	377	21	SauCas9
ADORA2A698	GCTGAAGATGGAACTCTGCGT	378	21	SauCas9
ADORA2A699	CGTGCTTGTGTGCTGGGCCGT	379	21	SauCas9
ADORA2A700	GTGAGGACCAGGACAAAGCAG	380	21	SauCas9
ADORA2A701	TCGATGGCAATAGCCAAGAGG	381	21	SauCas9
ADORA2A702	CATCGACAGATACATCGCCAT	382	21	SauCas9
ADORA2A703	GTACACCGAGGAGCCCATGGC	383	21	SauCas9
ADORA2A704	GCTCCACCATGATGTACACCG	384	21	SauCas9
ADORA2A705	AAGCCAGTGCTGATGGTGATG	385	21	SauCas9
ADORA2A706	CACCGCGATGTCAGCCGCCGC	386	21	SauCas9
ADORA2A707	AGGCTGAAGATGGAACTCTGC	387	21	SauCas9
ADORA2A708	GCCGCCGCCAGAGATACCACG	388	21	SauCas9
ADORA2A709	AGCTCCACCATGATGTACACC	389	21	SauCas9
ADORA2A710	AGGCAGCCATGGCAGGCAGCG	390	21	SauCas9

ADORA2A711	CCTGGCTCACCGGAGTGGAAT	391	21	SauCas9
ADORA2A712	CCAGCTCCACCATGATGTACA	392	21	SauCas9
ADORA2A713	ACCAGGACAAAGCAGGCGAAG	393	21	SauCas9
ADORA2A714	CCTGGGTAACGTGCTTGTGTG	394	21	SauCas9
ADORA2A715	AGGACCAGGACAAAGCAGGCG	395	21	SauCas9
ADORA2A716	TCAGCCGCCGCCAGAGATACC	396	21	SauCas9
ADORA2A717	GGCTCCTCGGTGTACATCATG	397	21	SauCas9
ADORA2A718	CTGGCGGCGGCTGACATCGCG	398	21	SauCas9
ADORA2A719	GATGGAACTCTGCGTGAGGAC	399	21	SauCas9
ADORA2A720	GCTCCTCGGTGTACATCATGG	400	21	SauCas9
ADORA2A721	TGTACACCGAGGAGCCCATGG	401	21	SauCas9
ADORA2A722	GCCATTGCTGTGCTGGCCATC	402	21	SauCas9
ADORA2A723	CAATAGCCAAGAGGCTGAAGA	403	21	SauCas9
ADORA2A724	ATGGTGATGGCGAATGGGATG	404	21	SauCas9
ADORA2A725	ATGTACACCGAGGAGCCCATG	405	21	SauCas9
ADORA2A726	GTGTGGATCAACAGCAACCTG	406	21	SauCas9
ADORA2A727	TGCTTTGTCCTGGTCCTCACG	407	21	SauCas9
ADORA2A728	GTAACCCCTGGCTCACCGGAG	408	21	SauCas9
ADORA2A729	CCAGCACACAAGCACGTTACC	409	21	SauCas9
ADORA2A730	TATCTGTCGATGGCAATAGCC	410	21	SauCas9
ADORA2A731	GCAATAGCCAAGAGGCTGAAG	411	21	SauCas9
ADORA2A732	AGTGCTGATGGTGATGGCGAA	412	21	SauCas9
ADORA2A733	ACACCGAGGAGCCCATGGCGG	413	21	SauCas9
ADORA2A734	CGCCATCCGAATTCCACTCCG	414	21	SauCas9
ADORA2A4111	TGGTGTCACTGGCGGCGGCC	415	20	AsCpf1
ADORA2A4112	CCATCACCATCAGCACCGGG	416	20	AsCpf1
ADORA2A4113	CCATCGGCCTGACTCCCATG	417	20	AsCpf1
ADORA2A4114	GCTGACCGCAGTTGTTCCAA	418	20	AsCpf1
ADORA2A4115	AGGATGTGGTCCCCATGAAC	419	20	AsCpf1
ADORA2A4116	CCTGTGTGCTGGTGCCCCTG	420	20	AsCpf1
ADORA2A4117	CGGATCTTCCTGGCGGCGCG	421	20	AsCpf1
ADORA2A4118	CCCTCTGCTGGCTGCCCCTA	422	20	AsCpf1
ADORA2A4119	TTCTGCCCCGACTGCAGCCA	423	20	AsCpf1
ADORA2A4120	AAGGCAGCTGGCACCAGTGC	424	20	AsCpf1
ADORA2A4121	TAAGGGCATCATTGCCATCTG	425	21	SauCas9
ADORA2A4122	CGGCCTGACTCCCATGCTAGG	426	21	SauCas9
ADORA2A4123	GCAGTTGTTCCAACCTAGCAT	427	21	SauCas9
ADORA2A4124	CCGCAGTTGTTCCAACCTAGC	428	21	SauCas9
ADORA2A4125	CAAGAACCACTCCCAGGGCTG	429	21	SauCas9
ADORA2A4126	CTTGGCCCTCCCCGCAGCCCT	430	21	SauCas9
ADORA2A4127	CACTTGGCCCTCCCCGCAGCC	431	21	SauCas9
ADORA2A4128	GGCCAAGTGGCCTGTCTCTTT	432	21	SauCas9
ADORA2A4129	TTCATGGGGACCACATCCTCA	433	21	SauCas9
ADORA2A4130	TGAAGTACACCATGTAGTTCA	434	21	SauCas9

ADORA2A4131	CTGGTGCCCCTGCTGCTCATG	435	21	SauCas9
ADORA2A4132	GCTCATGCTGGGTGTCTATTT	436	21	SauCas9
ADORA2A4133	CTTCAGCTGTCGTCGCGCCGC	437	21	SauCas9
ADORA2A4134	CGCGACGACAGCTGAAGCAGA	438	21	SauCas9
ADORA2A4135	GATGGAGAGCCAGCCTCTGCC	439	21	SauCas9
ADORA2A4136	GCGTGGCTGCAGTCGGGGCAG	440	21	SauCas9
ADORA2A4137	ACGATGGCCAGGTACATGAGC	441	21	SauCas9
ADORA2A4138	CTCTCCCACACCAATTCGGTT	442	21	SauCas9
ADORA2A4139	GATTCACAACCGAATTGGTGT	443	21	SauCas9
ADORA2A4140	GGGATTCACAACCGAATTGGT	444	21	SauCas9
ADORA2A4141	CGTAGATGAAGGGATTCACAA	445	21	SauCas9
ADORA2A4142	GGATACGGTAGGCGTAGATGA	446	21	SauCas9
ADORA2A4143	TCATCTACGCCTACCGTATCC	447	21	SauCas9
ADORA2A4144	CGGATACGGTAGGCGTAGATG	448	21	SauCas9
ADORA2A4145	GCGGAAGGTCTGGCGGAACTC	449	21	SauCas9
ADORA2A4146	AATGATCTTGCGGAAGGTCTG	450	21	SauCas9
ADORA2A4147	GACGTGGCTGCGAATGATCTT	451	21	SauCas9
ADORA2A4148	TTGCTGCCTCAGGACGTGGCT	452	21	SauCas9
ADORA2A4149	CAAGGCAGCTGGCACCAGTGC	453	21	SauCas9
ADORA2A4150	CGGGCACTGGTGCCAGCTGCC	454	21	SauCas9
ADORA2A4151	CTTGGCAGCTCATGGCAGTGA	455	21	SauCas9
ADORA2A4152	CCGTCTCAACGGCCACCCGCC	456	21	SauCas9
ADORA2A4153	CACACTCCTGGCGGGTGGCCG	457	21	SauCas9
ADORA2A4154	TGCCGTTGGCCCACACTCCTG	458	21	SauCas9
ADORA2A4155	CCATTGGGCCTCCGCTCAGGG	459	21	SauCas9
ADORA2A4156	CATAGCCATTGGGCCTCCGCT	460	21	SauCas9
ADORA2A4157	AATGGCTATGCCCTGGGGCTG	461	21	SauCas9
ADORA2A4158	ATGCCCTGGGGCTGGTGAGTG	462	21	SauCas9
ADORA2A4159	GCCCTGGGGCTGGTGAGTGGA	463	21	SauCas9
ADORA2A4160	TGGTGAGTGGAGGGAGTGCCC	464	21	SauCas9
ADORA2A4161	GAGGGAGTGCCCAAGAGTCCC	465	21	SauCas9
ADORA2A4162	AGGGAGTGCCCAAGAGTCCCA	466	21	SauCas9
ADORA2A4163	GTCTGGGAGGCCCGTGTTCCC	467	21	SauCas9
ADORA2A4164	CATGGCTAAGGAGCTCCACGT	468	21	SauCas9
ADORA2A4165	GAGCTCCTTAGCCATGAGCTC	469	21	SauCas9
ADORA2A4166	GCTCCTTAGCCATGAGCTCAA	470	21	SauCas9
ADORA2A4167	GGCCTAGATGACCCCCTGGCC	471	21	SauCas9
ADORA2A4168	CCCCCTGGCCCAGGATGGAGC	472	21	SauCas9
ADORA2A4169	CTCCTGCTCCATCCTGGGCCA	473	21	SauCas9
ADORA2A4416	CCGTGATGTACACCGAGGAG	474	20	AsCpf1 RR
ADORA2A4417	CTTTGCCATCACCATCAGCA	475	20	AsCpf1 RR
ADORA2A4418	TTTGCCATCACCATCAGCAC	476	20	AsCpf1 RR
ADORA2A4419	TTGCCTGCTTCGTCCTGGTC	477	20	AsCpf1

				RR
ADORA2A4420	TCCTGGTCCTCACGCAGAGC	478	20	AsCpf1 RR
ADORA2A4421	TCTTCAGTCTCCTGGCCATC	479	20	AsCpf1 RR
ADORA2A4422	GTCTCCTGGCCATCGCCATT	480	20	AsCpf1 RR
ADORA2A4423	ACCTAGCATGGGAGTCAGGC	481	20	AsCpf1 RR
ADORA2A4424	AACCTAGCATGGGAGTCAGG	482	20	AsCpf1 RR
ADORA2A4425	ATGCTAGGTTGGAACAACTG	483	20	AsCpf1 RR
ADORA2A4426	GCAGCCCTGGGAGTGGTTCT	484	20	AsCpf1 RR
ADORA2A4427	CGCAGCCCTGGGAGTGGTTC	485	20	AsCpf1 RR
ADORA2A4428	AGGGCTGCGGGGAGGGCCAA	486	20	AsCpf1 RR
ADORA2A4429	TGGGGACCACATCCTCAAAG	487	20	AsCpf1 RR
ADORA2A4430	CATGAACTACATGGTGTACT	488	20	AsCpf1 RR
ADORA2A4431	ATGAACTACATGGTGTACTT	489	20	AsCpf1 RR
ADORA2A4432	ACTTCTTTGCCTGTGTGCTG	490	20	AsCpf1 RR
ADORA2A4433	TGCTGCTCATGCTGGGTGTC	491	20	AsCpf1 RR
ADORA2A4434	CAAATAGACACCCAGCATGA	492	20	AsCpf1 RR
ADORA2A4435	GCTGTCGTCGCGCCGCCAGG	493	20	AsCpf1 RR
ADORA2A4436	TGGCGGCGCGACGACAGCTG	494	20	AsCpf1 RR
ADORA2A4437	TCTGCTTCAGCTGTCGTCGC	495	20	AsCpf1 RR
ADORA2A4438	GGCAGAGGCTGGCTCTCCAT	496	20	AsCpf1 RR
ADORA2A4439	CGGCAGAGGCTGGCTCTCCA	497	20	AsCpf1 RR
ADORA2A4440	CCGGCAGAGGCTGGCTCTCC	498	20	AsCpf1 RR
ADORA2A4441	CACTGCAGAAGGAGGTCCAT	499	20	AsCpf1 RR
ADORA2A4442	TGCTGCCAAGTCACTGGCCA	500	20	AsCpf1 RR
ADORA2A4443	ACAATGATGGCCAGTGACTT	501	20	AsCpf1 RR
ADORA2A4444	TACACATCATCAACTGCTTC	502	20	AsCpf1 RR
ADORA2A4445	CTTTCTTCTGCCCCGACTGC	503	20	AsCpf1 RR

ADORA2A4446	GACTGCAGCCACGCCCCTCT	504	20	AsCpf1 RR
ADORA2A4447	TCTCTGGCTCATGTACCTGG	505	20	AsCpf1 RR
ADORA2A4448	CAACCGAATTGGTGTGGGAG	506	20	AsCpf1 RR
ADORA2A4449	ACACCAATTCGGTTGTGAAT	507	20	AsCpf1 RR
ADORA2A4450	GTTGTGAATCCCTTCATCTA	508	20	AsCpf1 RR
ADORA2A4451	TTCATCTACGCCTACCGTAT	509	20	AsCpf1 RR
ADORA2A4452	TCTACGCCTACCGTATCCGC	510	20	AsCpf1 RR
ADORA2A4453	CGAGTTCCGCCAGACCTTCC	511	20	AsCpf1 RR
ADORA2A4454	GCCAGACCTTCCGCAAGATC	512	20	AsCpf1 RR
ADORA2A4455	CCAGACCTTCCGCAAGATCA	513	20	AsCpf1 RR
ADORA2A4456	GCAAGATCATTCGCAGCCAC	514	20	AsCpf1 RR
ADORA2A4457	CAAGATCATTCGCAGCCACG	515	20	AsCpf1 RR
ADORA2A4458	CAGCCACGTCCTGAGGCAGC	516	20	AsCpf1 RR
ADORA2A4459	AGGCAGCTGGCACCAGTGCC	517	20	AsCpf1 RR
ADORA2A4460	TCACTGCCATGAGCTGCCAA	518	20	AsCpf1 RR
ADORA2A4461	TCTCAACGGCCACCCGCCAG	519	20	AsCpf1 RR
ADORA2A4462	CTCAGGGTGGGGAGCACTGC	520	20	AsCpf1 RR
ADORA2A4463	CACCCTGAGCGGAGGCCCAA	521	20	AsCpf1 RR
ADORA2A4464	ACCCTGAGCGGAGGCCCAAT	522	20	AsCpf1 RR
ADORA2A4465	AGGGCATAGCCATTGGGCCT	523	20	AsCpf1 RR
ADORA2A4466	CTCACCAGCCCCAGGGCATA	524	20	AsCpf1 RR
ADORA2A4467	TCCACTCACCAGCCCCAGGG	525	20	AsCpf1 RR
ADORA2A4468	TGGGACTCTTGGGCACTCCC	526	20	AsCpf1 RR
ADORA2A4469	CTGGGACTCTTGGGCACTCC	527	20	AsCpf1 RR
ADORA2A4470	CCTGGGACTCTTGGGCACTC	528	20	AsCpf1 RR
ADORA2A4471	AGGGGAACACGGGCCTCCCA	529	20	AsCpf1 RR
ADORA2A4472	CGTCTGGGAGGCCCGTGTTC	530	20	AsCpf1 RR

ADORA2A4473	AGACGTGGAGCTCCTTAGCC	531	20	AsCpf1 RR
ADORA2A4474	TTGAGCTCATGGCTAAGGAG	532	20	AsCpf1 RR
ADORA2A4475	CTGGCCTAGATGACCCCCTG	533	20	AsCpf1 RR
ADORA2A4476	TGGCCTAGATGACCCCCTGG	534	20	AsCpf1 RR
ADORA2A4477	TCCTGGGCCAGGGGGTCATC	535	20	AsCpf1 RR
ADORA2A4478	CTGGCCCAGGATGGAGCAGG	536	20	AsCpf1 RR
ADORA2A4479	TGGCCCAGGATGGAGCAGGA	537	20	AsCpf1 RR
ADORA2A4480	CGCGAGTTCCGCCAGACCTT	538	20	AsCpf1 RVR
ADORA2A4481	CCCTGGGGCTGGTGAGTGGA	539	20	AsCpf1 RVR

[162] In some embodiments the gRNA for use in the disclosure is a gRNA targeting TGFbetaR2 (TGFbetaR2 gRNA). In some embodiments, the gRNA targeting TGFbetaR2 is one or more of the gRNAs described in Table 6.

Table 6. TGFbetaR2 gRNAs

Name	gRNA Targeting Domain Sequence (DNA)	SEQ ID NO:	Length	Enzyme
TGFBR24326	CAGGACGATGTGCAGCGGCC	540	20	AsCpf1 RR
TGFBR24327	ACCGCACGTTCAGAAGTCGG	541	20	AsCpf1 RR
TGFBR24328	ACAACTGTGTAAATTTTGTG	542	20	AsCpf1 RR
TGFBR24329	CAACTGTGTAAATTTTGTGA	543	20	AsCpf1 RR
TGFBR24330	ACCTGTGACAACCAGAAATC	544	20	AsCpf1 RR
TGFBR24331	CCTGTGACAACCAGAAATCC	545	20	AsCpf1 RR
TGFBR24332	TGTGGCTTCTCACAGATGGA	546	20	AsCpf1 RR
TGFBR24333	TCTGTGAGAAGCCACAGGAA	547	20	AsCpf1 RR
TGFBR24334	AAGCTCCCCTACCATGACTT	548	20	AsCpf1 RR
TGFBR24335	GAATAAAGTCATGGTAGGGG	549	20	AsCpf1 RR
TGFBR24336	AGAATAAAGTCATGGTAGGG	550	20	AsCpf1 RR
TGFBR24337	CTACCATGACTTTATTCTGG	551	20	AsCpf1 RR
TGFBR24338	TACCATGACTTTATTCTGGA	552	20	AsCpf1 RR
TGFBR24339	TAATGCACTTTGGAGAAGCA	553	20	AsCpf1 RR
TGFBR24340	TTCATAATGCACTTTGGAGA	554	20	AsCpf1 RR
TGFBR24341	AAGTGCATTATGAAGGAAAA	555	20	AsCpf1 RR
TGFBR24342	TGTGTTCCTGTAGCTCTGAT	556	20	AsCpf1 RR
TGFBR24343	TGTAGCTCTGATGAGTGCAA	557	20	AsCpf1 RR
TGFBR24344	AGTGACAGGCATCAGCCTCC	558	20	AsCpf1 RR
TGFBR24345	AGTGGTGGCAGGAGGCTGAT	559	20	AsCpf1 RR

TGFBR24346	AGGTTGAACTCAGCTTCTGC	560	20	AsCpf1 RR
TGFBR24347	CAGGTTGAACTCAGCTTCTG	561	20	AsCpf1 RR
TGFBR24348	ACCTGGGAAACCGGCAAGAC	562	20	AsCpf1 RR
TGFBR24349	CGTCTTGCCGGTTTCCCAGG	563	20	AsCpf1 RR
TGFBR24350	GCGTCTTGCCGGTTTCCCAG	564	20	AsCpf1 RR
TGFBR24351	TGAGCTTCCGCGTCTTGCCG	565	20	AsCpf1 RR
TGFBR24352	GCGAGCACTGTGCCATCATC	566	20	AsCpf1 RR
TGFBR24353	GGATGATGGCACAGTGCTCG	567	20	AsCpf1 RR
TGFBR24354	AGGATGATGGCACAGTGCTC	568	20	AsCpf1 RR
TGFBR24355	CGTGTGCCAACAACATCAAC	569	20	AsCpf1 RR
TGFBR24356	GCTCAATGGGCAGCAGCTCT	570	20	AsCpf1 RR
TGFBR24357	ACCAGGGTGTCCAGCTCAAT	571	20	AsCpf1 RR
TGFBR24358	CACCAGGGTGTCCAGCTCAA	572	20	AsCpf1 RR
TGFBR24359	CCACCAGGGTGTCCAGCTCA	573	20	AsCpf1 RR
TGFBR24360	GCTTGGCCTTATAGACCTCA	574	20	AsCpf1 RR
TGFBR24361	GAGCAGTTTGAGACAGTGGC	575	20	AsCpf1 RR
TGFBR24362	AGAGGCATACTCCTCATAGG	576	20	AsCpf1 RR
TGFBR24363	CTATGAGGAGTATGCCTCTT	577	20	AsCpf1 RR
TGFBR24364	AAGAGGCATACTCCTCATAG	578	20	AsCpf1 RR
TGFBR24365	TATGAGGAGTATGCCTCTTG	579	20	AsCpf1 RR
TGFBR24366	GATTGATGTCTGAGAAGATG	580	20	AsCpf1 RR
TGFBR24367	CTCCTCAGCCGTCAGGAACT	581	20	AsCpf1 RR
TGFBR24368	GTTCCTGACGGCTGAGGAGC	582	20	AsCpf1 RR
TGFBR24369	GCTCCTCAGCCGTCAGGAAC	583	20	AsCpf1 RR
TGFBR24370	TGACGGCTGAGGAGCGGAAG	584	20	AsCpf1 RR
TGFBR24371	TCTTCCGCTCCTCAGCCGTC	585	20	AsCpf1 RR
TGFBR24372	AACTCCGTCTTCCGCTCCTC	586	20	AsCpf1 RR
TGFBR24373	CAACTCCGTCTTCCGCTCCT	587	20	AsCpf1 RR
TGFBR24374	CCAACTCCGTCTTCCGCTCC	588	20	AsCpf1 RR
TGFBR24375	ACGCCAAGGGCAACCTACAG	589	20	AsCpf1 RR
TGFBR24376	CGCCAAGGGCAACCTACAGG	590	20	AsCpf1 RR
TGFBR24377	AGCTGATGACATGCCGCGTC	591	20	AsCpf1 RR
TGFBR24378	GGGCGAGGGAGCTGCCCAGC	592	20	AsCpf1 RR
TGFBR24379	CGGGCGAGGGAGCTGCCCAG	593	20	AsCpf1 RR
TGFBR24380	CCGGGCGAGGGAGCTGCCCA	594	20	AsCpf1 RR
TGFBR24381	TCGCCCGGGGGATTGCTCAC	595	20	AsCpf1 RR
TGFBR24382	ACATGGAGTGTGATCACTGT	596	20	AsCpf1 RR
TGFBR24383	CAGTGATCACACTCCATGTG	597	20	AsCpf1 RR
TGFBR24384	TGTGGGAGGCCCAAGATGCC	598	20	AsCpf1 RR
TGFBR24385	TGTGCACGATGGGCATCTTG	599	20	AsCpf1 RR
TGFBR24386	CGAGGATATTGGAGCTCTTG	600	20	AsCpf1 RR
TGFBR24387	ATATCCTCGTGAAGAACGAC	601	20	AsCpf1 RR
TGFBR24388	GACGCAGGGAAAGCCCAAAG	602	20	AsCpf1 RR
TGFBR24389	CTGCGTCTGGACCCTACTCT	603	20	AsCpf1 RR

TGFBR24390	TGCGTCTGGACCCTACTCTG	604	20	AsCpf1 RR
TGFBR24391	CAGACAGAGTAGGGTCCAGA	605	20	AsCpf1 RR
TGFBR24392	GCCAGCACGATCCCACCGCA	606	20	AsCpf1 RVR
TGFBR24393	AAGGAAAAAAAAAAGCCTGG	607	20	AsCpf1 RVR
TGFBR24394	ACACCAGCAATCCTGACTTG	608	20	AsCpf1 RVR
TGFBR24395	ACTAGCAACAAGTCAGGATT	609	20	AsCpf1 RVR
TGFBR24396	GCAACTCCCAGTGGTGGCAG	610	20	AsCpf1 RVR
TGFBR24397	TGTCATCATCATCTTCTACT	611	20	AsCpf1 RVR
TGFBR24398	GACCTCAGCAAAGCGACCTT	612	20	AsCpf1 RVR
TGFBR24399	AGGCCAAGCTGAAGCAGAAC	613	20	AsCpf1 RVR
TGFBR24400	AGGAGTATGCCTCTTGGAAG	614	20	AsCpf1 RVR
TGFBR24401	CCTCTTGGAAGACAGAGAAG	615	20	AsCpf1 RVR
TGFBR24402	TTCTCATGCTTCAGATTGAT	616	20	AsCpf1 RVR
TGFBR24403	CTCGTGAAGAACGACCTAAC	617	20	AsCpf1 RVR
TGFbR2036	GGCCGCTGCACATCGTCCTG	618	20	SpyCas9
TGFbR2037	GCGGGGTCTGCCATGGGTCG	619	20	SpyCas9
TGFbR2038	AGTTGCTCATGCAGGATTTC	620	20	SpyCas9
TGFbR2039	CCAGAATAAAGTCATGGTAG	621	20	SpyCas9
TGFbR2040	CCCCTACCATGACTTTATTC	622	20	SpyCas9
TGFbR2041	AAGTCATGGTAGGGGAGCTT	623	20	SpyCas9
TGFbR2042	AGTCATGGTAGGGGAGCTTG	624	20	SpyCas9
TGFbR2043	ATTGCACTCATCAGAGCTAC	625	20	SpyCas9
TGFbR2044	CCTAGAGTGAAGAGATTCAT	626	20	SpyCas9
TGFbR2045	CCAATGAATCTCTTCACTCT	627	20	SpyCas9
TGFbR2046	AAAGTCATGGTAGGGGAGCT	628	20	SpyCas9
TGFbR2047	GTGAGCAATCCCCCGGGCGA	629	20	SpyCas9
TGFbR2048	GTCGTTCTTCACGAGGATAT	630	20	SpyCas9
TGFbR2049	GCCGCGTCAGGTACTCCTGT	631	20	SpyCas9
TGFbR2050	GACGCGGCATGTCATCAGCT	632	20	SpyCas9
TGFbR2051	GCTTCTGCTGCCGGTTAACG	633	20	SpyCas9
TGFbR2052	GTGGATGACCTGGCTAACAG	634	20	SpyCas9
TGFbR2053	GTGATCACACTCCATGTGGG	635	20	SpyCas9
TGFbR2054	GCCCATTGAGCTGGACACCC	636	20	SpyCas9
TGFbR2055	GCGGTCATCTTCCAGGATGA	637	20	SpyCas9
TGFbR2056	GGGAGCTGCCCAGCTTGCGC	638	20	SpyCas9
TGFbR2057	GTTGATGTTGTTGGCACACG	639	20	SpyCas9
TGFbR2058	GGCATCTTGGGCCTCCCACA	640	20	SpyCas9
TGFbR2059	GCGGCATGTCATCAGCTGGG	641	20	SpyCas9
TGFbR2060	GCTCCTCAGCCGTCAGGAAC	642	20	SpyCas9
TGFbR2061	GCTGGTGTTATATTCTGATG	643	20	SpyCas9
TGFbR2062	CCGACTTCTGAACGTGCGGT	644	20	SpyCas9
TGFbR2063	TGCTGGCGATACGCGTCCAC	645	20	SpyCas9
TGFbR2064	CCCGACTTCTGAACGTGCGG	646	20	SpyCas9
TGFbR2065	CCACCGCACGTTCAGAAGTC	647	20	SpyCas9

TGFbR2066	TCACCCGACTTCTGAACGTG	648	20	SpyCas9
TGFbR2067	CCCACCGCACGTTCAGAAGT	649	20	SpyCas9
TGFbR2068	CGAGCAGCGGGGTCTGCCAT	650	20	SpyCas9
TGFbR2069	ACGAGCAGCGGGGTCTGCCA	651	20	SpyCas9
TGFbR2070	AGCGGGGTCTGCCATGGGTC	652	20	SpyCas9
TGFbR2071	CCTGAGCAGCCCCCGACCCA	653	20	SpyCas9
TGFbR2072	CCATGGGTCGGGGGCTGCTC	654	20	SpyCas9
TGFbR2073	AACGTGCGGTGGGATCGTGC	655	20	SpyCas9
TGFbR2074	GGACGATGTGCAGCGGCCAC	656	20	SpyCas9
TGFbR2075	GTCCACAGGACGATGTGCAG	657	20	SpyCas9
TGFbR2076	CATGGGTCGGGGGCTGCTCA	658	20	SpyCas9
TGFbR2077	CAGCGGGGTCTGCCATGGGT	659	20	SpyCas9
TGFbR2078	ATGGGTCGGGGGCTGCTCAG	660	20	SpyCas9
TGFbR2079	CGGGGTCTGCCATGGGTCGG	661	20	SpyCas9
TGFbR2080	AGGAAGTCTGTGTGGCTGTA	662	20	SpyCas9
TGFbR2081	CTCCATCTGTGAGAAGCCAC	663	20	SpyCas9
TGFbR2082	ATGATAGTCACTGACAACAA	664	20	SpyCas9
TGFbR2083	GATGCTGCAGTTGCTCATGC	665	20	SpyCas9
TGFbR2084	ACAGCCACACAGACTTCCTG	666	20	SpyCas9
TGFbR2085	GAAGCCACAGGAAGTCTGTG	667	20	SpyCas9
TGFbR2086	TTCCTGTGGCTTCTCACAGA	668	20	SpyCas9
TGFbR2087	CTGTGGCTTCTCACAGATGG	669	20	SpyCas9
TGFbR2088	TCACAAAATTTACACAGTTG	670	20	SpyCas9
TGFbR2089	GACAACATCATCTTCTCAGA	671	20	SpyCas9
TGFbR2090	TCCAGAATAAAGTCATGGTA	672	20	SpyCas9
TGFbR2091	GGTAGGGGAGCTTGGGGTCA	673	20	SpyCas9
TGFbR2092	TTCTCCAAAGTGCATTATGA	674	20	SpyCas9
TGFbR2093	CATCTTCCAGAATAAAGTCA	675	20	SpyCas9
TGFbR2094	CACATGAAGAAAGTCTCACC	676	20	SpyCas9
TGFbR2095	TTCCAGAATAAAGTCATGGT	677	20	SpyCas9
TGFbR2096	TTTTCCTTCATAATGCACTT	678	20	SpyCas9
TGFBR24024	CACAGTTGTGGAAACTTGAC	679	20	AsCpf1
TGFBR24039	CCCAACTCCGTCTTCCGCTC	680	20	AsCpf1
TGFBR24040	GGCTTTCCCTGCGTCTGGAC	681	20	AsCpf1
TGFBR24036	CTGAGGTCTATAAGGCCAAG	682	20	AsCpf1
TGFBR24026	TGATGTGAGATTTTCCACCT	683	20	AsCpf1
TGFBR24038	CCTATGAGGAGTATGCCTCT	684	20	AsCpf1
TGFBR24033	AAGTGACAGGCATCAGCCTC	685	20	AsCpf1
TGFBR24028	CCATGACCCCAAGCTCCCCT	686	20	AsCpf1
TGFBR24031	CTTCATAATGCACTTTGGAG	687	20	AsCpf1
TGFBR24032	TTCATGTGTTCCTGTAGCTC	688	20	AsCpf1
TGFBR24029	TTCTGGAAGATGCTGCTTCT	689	20	AsCpf1
TGFBR24035	CCCACCAGGGTGTCCAGCTC	690	20	AsCpf1
TGFBR24037	AGACAGTGGCAGTCAAGATC	691	20	AsCpf1

TGFBR24041	CCTGCGTCTGGACCCTACTC	692	20	AsCpf1
TGFBR24025	CACAACTGTGTAAATTTTGT	693	20	AsCpf1
TGFBR24030	GAGAAGCAGCATCTTCCAGA	694	20	AsCpf1
TGFBR24027	TGGTTGTCACAGGTGGAAAA	695	20	AsCpf1
TGFBR24034	CCAGGTTGAACTCAGCTTCT	696	20	AsCpf1
TGFBR24043	ATCACAAAATTTACACAGTTG	697	21	SauCas9
TGFBR24065	GGCATCAGCCTCCTGCCACCA	698	21	SauCas9
TGFBR24110	GTTAGCCAGGTCATCCACAGA	699	21	SauCas9
TGFBR24099	GCTGGGCAGCTCCCTCGCCCG	700	21	SauCas9
TGFBR24064	CAGGAGGCTGATGCCTGTCAC	701	21	SauCas9
TGFBR24094	GAGGAGCGGAAGACGGAGTTG	702	21	SauCas9
TGFBR24108	CGTCTGGACCCTACTCTGTCT	703	21	SauCas9
TGFBR24058	TTTTTCCTTCATAATGCACTT	704	21	SauCas9
TGFBR24075	CCATTGAGCTGGACACCCTGG	705	21	SauCas9
TGFBR24057	CTTCTCCAAAGTGCATTATGA	706	21	SauCas9
TGFBR24103	GCCCAAGATGCCCATCGTGCA	707	21	SauCas9
TGFBR24060	TCATGTGTTCCTGTAGCTCTG	708	21	SauCas9
TGFBR24048	GTGATGCTGCAGTTGCTCATG	709	21	SauCas9
TGFBR24087	TCTCATGCTTCAGATTGATGT	710	21	SauCas9
TGFBR24081	TCCCTATGAGGAGTATGCCTC	711	21	SauCas9
TGFBR24044	CATCACAAAATTTACACAGTT	712	21	SauCas9
TGFBR24077	ATTGAGCTGGACACCCTGGTG	713	21	SauCas9
TGFBR24080	CAGTCAAGATCTTTCCCTATG	714	21	SauCas9
TGFBR24046	AGGATTTCTGGTTGTCACAGG	715	21	SauCas9
TGFBR24101	TCCACAGTGATCACACTCCAT	716	21	SauCas9
TGFBR24079	AGCAGAACACTTCAGAGCAGT	717	21	SauCas9
TGFBR24072	CCGGCAAGACGCGGAAGCTCA	718	21	SauCas9
TGFBR24074	GATGTCAGAGCGGTCATCTTC	719	21	SauCas9
TGFBR24062	TCATTGCACTCATCAGAGCTA	720	21	SauCas9
TGFBR24054	CTTCCAGAATAAAGTCATGGT	721	21	SauCas9
TGFBR24045	AGATTTTCCACCTGTGACAAC	722	21	SauCas9
TGFBR24049	ACTGCAGCATCACCTCCATCT	723	21	SauCas9
TGFBR24098	AGCTGGGCAGCTCCCTCGCCC	724	21	SauCas9
TGFBR24090	TGACGGCTGAGGAGCGGAAGA	725	21	SauCas9
TGFBR24076	CATTGAGCTGGACACCCTGGT	726	21	SauCas9
TGFBR24078	AGCAAAGCGACCTTTCCCCAC	727	21	SauCas9
TGFBR24067	CGCGTTAACCGGCAGCAGAAG	728	21	SauCas9
TGFBR24063	GAAATATGACTAGCAACAAGT	729	21	SauCas9
TGFBR24107	AGACAGAGTAGGGTCCAGACG	730	21	SauCas9
TGFBR24047	CAGGATTTCTGGTTGTCACAG	731	21	SauCas9
TGFBR24096	CTCCTGTAGGTTGCCCTTGGC	732	21	SauCas9
TGFBR24105	ACAGAGTAGGGTCCAGACGCA	733	21	SauCas9
TGFBR24056	GCTTCTCCAAAGTGCATTATG	734	21	SauCas9
TGFBR24068	GCAGCAGAAGCTGAGTTCAAC	735	21	SauCas9

TGFBR24093	TGAGGAGCGGAAGACGGAGTT	736	21	SauCas9
TGFBR24055	CTTTGGAGAAGCAGCATCTTC	737	21	SauCas9
TGFBR24053	CTCCCCTACCATGACTTTATT	738	21	SauCas9
TGFBR24106	GACAGAGTAGGGTCCAGACGC	739	21	SauCas9
TGFBR24092	CTGAGGAGCGGAAGACGGAGT	740	21	SauCas9
TGFBR24102	GGGCATCTTGGGCCTCCCACA	741	21	SauCas9
TGFBR24082	CCAAGAGGCATACTCCTCATA	742	21	SauCas9
TGFBR24051	AGAATGACGAGAACATAACAC	743	21	SauCas9
TGFBR24097	CCTGACGCGGCATGTCATCAG	744	21	SauCas9
TGFBR24073	AGCGAGCACTGTGCCATCATC	745	21	SauCas9
TGFBR24104	GCAGGTTAGGTCGTTCTTCAC	746	21	SauCas9
TGFBR24050	ACCTCCATCTGTGAGAAGCCA	747	21	SauCas9
TGFBR24052	TAAAGTCATGGTAGGGGAGCT	748	21	SauCas9
TGFBR24061	TCAGAGCTACAGGAACACATG	749	21	SauCas9
TGFBR24086	TCTCAGACATCAATCTGAAGC	750	21	SauCas9
TGFBR24066	CATCAGCCTCCTGCCACCACT	751	21	SauCas9
TGFBR24089	CGCTCCTCAGCCGTCAGGAAC	752	21	SauCas9
TGFBR24071	AACCTGGGAAACCGGCAAGAC	753	21	SauCas9
TGFBR24095	TCCACGCCAAGGGCAACCTAC	754	21	SauCas9
TGFBR24100	GAGGTGAGCAATCCCCCGGGC	755	21	SauCas9
TGFBR24069	CAGCAGAAGCTGAGTTCAACC	756	21	SauCas9
TGFBR24083	TCCAAGAGGCATACTCCTCAT	757	21	SauCas9
TGFBR24070	AGCAGAAGCTGAGTTCAACCT	758	21	SauCas9
TGFBR24088	CCAGTTCCTGACGGCTGAGGA	759	21	SauCas9
TGFBR24085	AGGAGTATGCCTCTTGGAAGA	760	21	SauCas9
TGFBR24084	TTCCAAGAGGCATACTCCTCA	761	21	SauCas9
TGFBR24042	CAACTGTGTAAATTTTGTGAT	762	21	SauCas9
TGFBR24059	TGAAGGAAAAAAAAAAGCCTG	763	21	SauCas9
TGFBR24091	CGTCTTCCGCTCCTCAGCCGT	764	21	SauCas9
TGFBR24109	CCAGGTCATCCACAGACAGAG	765	21	SauCas9
TGFBR2736	GCCTAGAGTGAAGAGATTCAT	766	21	SpyCas9
TGFBR2737	GTTCTCCAAAGTGCATTATGA	767	21	SpyCas9
TGFBR2738	GCATCTTCCAGAATAAAGTCA	768	21	SpyCas9

[163] 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 7.

Table 7. CISH gRNAs

Name	gRNA Targeting Domain Sequence (DNA)	SEQ ID NO:	Length	Enzyme
CISH0873	CAACCGTCTGGTGGCCGACG	769	20	SpyCas9
CISH0874	CAGGATCGGGGCTGTCGCTT	770	20	SpyCas9

CISH0875	TCGGGCCTCGCTGGCCGTAA	771	20	SpyCas9
CISH0876	GAGGTAGTCGGCCATGCGCC	772	20	SpyCas9
CISH0877	CAGGTGTTGTCGGGCCTCGC	773	20	SpyCas9
CISH0878	GGAGGTAGTCGGCCATGCGC	774	20	SpyCas9
CISH0879	GGCATACTCAATGCGTACAT	775	20	SpyCas9
CISH0880	CCGCCTTGTCATCAACCGTC	776	20	SpyCas9
CISH0881	AGGATCGGGGCTGTCGCTTC	777	20	SpyCas9
CISH0882	CCTTGTCATCAACCGTCTGG	778	20	SpyCas9
CISH0883	TACTCAATGCGTACATTGGT	779	20	SpyCas9
CISH0884	GGGTTCCATTACGGCCAGCG	780	20	SpyCas9
CISH0885	GGCACTGCTTCTGCGTACAA	781	20	SpyCas9
CISH0886	GGTTGATGACAAGGCGGCAC	782	20	SpyCas9
CISH0887	TGCTGGGGCCTTCCTCGAGG	783	20	SpyCas9
CISH0888	TTGCTGGCTGTGGAGCGGAC	784	20	SpyCas9
CISH0889	TTCTCCTACCTTCGGGAATC	785	20	SpyCas9
CISH0890	GACTGGCTTGGGCAGTTCCA	786	20	SpyCas9
CISH0891	CATGCAGCCCTTGCCTGCTG	787	20	SpyCas9
CISH0892	AGCAAAGGACGAGGTCTAGA	788	20	SpyCas9
CISH0893	GCCTGCTGGGGCCTTCCTCG	789	20	SpyCas9
CISH0894	CAGACTCACCAGATTCCCGA	790	20	SpyCas9
CISH0895	ACCTCGTCCTTTGCTGGCTG	791	20	SpyCas9
CISH0896	CTCACCAGATTCCCGAAGGT	792	20	SpyCas9
CISH7048	TACGCAGAAGCAGTGCCCGC	793	20	AsCpf1
CISH7049	AGGTGTACAGCAGTGGCTGG	794	20	AsCpf1
CISH7050	GGTGTACAGCAGTGGCTGGT	795	20	AsCpf1
CISH7051	CGGATGTGGTCAGCCTTGTG	796	20	AsCpf1
CISH7052	CACTGACAGCGTGAACAGGT	797	20	AsCpf1
CISH7053	ACTGACAGCGTGAACAGGTA	798	20	AsCpf1
CISH7054	GCTCACTCTCTGTCTGGGCT	799	20	AsCpf1
CISH7055	CTGGCTGTGGAGCGGACTGG	800	20	AsCpf1
CISH7056	GCTCTGACTGTACGGGGCAA	801	20	AsCpf1 RR
CISH7057	AGCTCTGACTGTACGGGGCA	802	20	AsCpf1 RR
CISH7058	ACAGTACCCCTTCCAGCTCT	803	20	AsCpf1 RR
CISH7059	CGTCGGCCACCAGACGGTTG	804	20	AsCpf1 RR
CISH7060	CCAGCCACTGCTGTACACCT	805	20	AsCpf1 RR
CISH7061	ACCCCGGCCCTGCCTATGCC	806	20	AsCpf1 RR
CISH7062	GGTATCAGCAGTGCAGGAGG	807	20	AsCpf1 RR
CISH7063	GATGTGGTCAGCCTTGTGCA	808	20	AsCpf1 RR
CISH7064	GGATGTGGTCAGCCTTGTGC	809	20	AsCpf1 RR
CISH7065	GGCCACGCATCCTGGCCTTT	810	20	AsCpf1 RR
CISH7066	GAAAGGCCAGGATGCGTGGC	811	20	AsCpf1 RR
CISH7067	ACTGCTTGTCCAGGCCACGC	812	20	AsCpf1 RR
CISH7068	TCTGGACTCCAACTGCTTGT	813	20	AsCpf1 RR
CISH7069	GTCTGGACTCCAACTGCTTG	814	20	AsCpf1 RR

CISH7070	GCTTCCGTCTGGACTCCAAC	815	20	AsCpf1 RR
CISH7071	GACGGAAGCTGGAGTCGGCA	816	20	AsCpf1 RR
CISH7072	CGCTGTCAGTGAAAACCACT	817	20	AsCpf1 RR
CISH7073	CTGACAGCGTGAACAGGTAG	818	20	AsCpf1 RR
CISH7074	TTACGGCCAGCGAGGCCCGA	819	20	AsCpf1 RR
CISH7075	ATTACGGCCAGCGAGGCCCG	820	20	AsCpf1 RR
CISH7076	GGAATCTGGTGAGTCTGAGG	821	20	AsCpf1 RR
CISH7077	CCCTCAGACTCACCAGATTC	822	20	AsCpf1 RR
CISH7078	CGAAGGTAGGAGAAGGTCTT	823	20	AsCpf1 RR
CISH7079	GAAGGTAGGAGAAGGTCTTG	824	20	AsCpf1 RR
CISH7080	GCACCTTTGGCTCACTCTCT	825	20	AsCpf1 RR
CISH7081	TCGAGGAGGTGGCAGAGGGT	826	20	AsCpf1 RR
CISH7082	TGGAACTGCCCAAGCCAGTC	827	20	AsCpf1 RR
CISH7083	AGGGACGGGGCCCACAGGGG	828	20	AsCpf1 RR
CISH7084	GGGACGGGGCCCACAGGGGC	829	20	AsCpf1 RR
CISH7085	CTCCACAGCCAGCAAAGGAC	830	20	AsCpf1 RR
CISH7086	CAGCCAGCAAAGGACGAGGT	831	20	AsCpf1 RR
CISH7087	CTGCCTTCTAGACCTCGTCC	832	20	AsCpf1 RR
CISH7088	CCTAAGGAGGATGCGCCTAG	833	20	AsCpf1 RVR
CISH7089	TGGCCTCCTGCACTGCTGAT	834	20	AsCpf1 RVR
CISH7090	AGCAGTGCAGGAGGCCACAT	835	20	AsCpf1 RVR
CISH7091	CCGACTCCAGCTTCCGTCTG	836	20	AsCpf1 RVR
CISH7092	GGGGTTCCATTACGGCCAGC	837	20	AsCpf1 RVR
CISH7093	CACAGCAGATCCTCCTCTGG	838	20	AsCpf1 RVR
CISH7094	ATTGCCCCGTACAGTCAGAG	839	21	SauCas9
CISH7095	CCCGTACAGTCAGAGCTGGA	840	21	SauCas9
CISH7096	TGGTGGAGGAGCAGGCAGTG	841	21	SauCas9
CISH7097	TCCTTAGGCATAGGCAGGGC	842	21	SauCas9
CISH7098	CGGCCCTGCCTATGCCTAAG	843	21	SauCas9
CISH7099	TAGGCATAGGCAGGGCCGGG	844	21	SauCas9
CISH7100	AGGCAGGGCCGGGGTGGGAG	845	21	SauCas9
CISH7101	GCAGGATCGGGGCTGTCGCT	846	21	SauCas9
CISH7102	CTGCACAAGGCTGACCACAT	847	21	SauCas9
CISH7103	TGCACAAGGCTGACCACATC	848	21	SauCas9
CISH7104	CTGACCACATCCGGAAAGGC	849	21	SauCas9
CISH7105	GGCCACGCATCCTGGCCTTT	850	21	SauCas9
CISH7106	GCGTGGCCTGGACAAGCAGT	851	21	SauCas9
CISH7107	GACAAGCAGTTGGAGTCCAG	852	21	SauCas9
CISH7108	GTTGGAGTCCAGACGGAAGC	853	21	SauCas9
CISH7109	ATGCGTACATTGGTGGGGCC	854	21	SauCas9
CISH7110	TGGCCCCACCAATGTACGCA	855	21	SauCas9

CISH7111	GCTACCTGTTCACGCTGTCA	856	21	SauCas9
CISH7112	TGACAGCGTGAACAGGTAGC	857	21	SauCas9
CISH7113	GTCGGGCCTCGCTGGCCGTA	858	21	SauCas9
CISH7114	GCACTTGCCTAGGCTGGTAT	859	21	SauCas9
CISH7115	GGGAATCTGGTGAGTCTGAG	860	21	SauCas9
CISH7116	CTCACCAGATTCCCGAAGGT	861	21	SauCas9
CISH7117	CTCCTACCTTCGGGAATCTG	862	21	SauCas9
CISH7118	CAAGACCTTCTCCTACCTTC	863	21	SauCas9
CISH7119	CCAAGACCTTCTCCTACCTT	864	21	SauCas9
CISH7120	GCCAAGACCTTCTCCTACCT	865	21	SauCas9
CISH7121	TATGCACAGCAGATCCTCCT	866	21	SauCas9
CISH7122	CAAAGGTGCTGGACCCAGAG	867	21	SauCas9
CISH7123	GGCTCACTCTCTGTCTGGGC	868	21	SauCas9
CISH7124	AGGGTACCCCAGCCCAGACA	869	21	SauCas9
CISH7125	AGAGGGTACCCCAGCCCAGA	870	21	SauCas9
CISH7126	GTACCCTCTGCCACCTCCTC	871	21	SauCas9
CISH7127	CCTTCCTCGAGGAGGTGGCA	872	21	SauCas9
CISH7128	ATGACTGGCTTGGGCAGTTC	873	21	SauCas9
CISH7129	GGCCCCTGTGGGCCCCGTCC	874	21	SauCas9
CISH7130	AGGACGAGGTCTAGAAGGCA	875	21	SauCas9

[164] 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 8.

Table 8: B2M gRNAs

gRNA name	gRNA Targeting Domain Target sequence (DNA)	SEQ ID NO:	Length	Enzyme
B2M1	TATAAGTGGAGGCGTCGCGC	876	20	SpyCas9
B2M2	GGGCACGCGTTTAATATAAG	877	20	SpyCas9
B2M3	ACTCACGCTGGATAGCCTCC	878	20	SpyCas9
B2M4	GGCCGAGATGTCTCGCTCCG	879	20	SpyCas9
B2M5	CACGCGTTTAATATAAGTGG	880	20	SpyCas9
B2M6	AAGTGGAGGCGTCGCGCTGG	881	20	SpyCas9
B2M7	GAGTAGCGCGAGCACAGCTA	882	20	SpyCas9
B2M8	AGTGGAGGCGTCGCGCTGGC	883	20	SpyCas9
B2M9	GCCCGAATGCTGTCAGCTTC	884	20	SpyCas9
B2M10	CGCGAGCACAGCTAAGGCCA	885	20	SpyCas9
B2M11	CTCGCGCTACTCTCTCTTTC	886	20	SpyCas9
B2M12	GGCCACGGAGCGAGACATCT	887	20	SpyCas9
B2M13	CGTGAGTAAACCTGAATCTT	888	20	SpyCas9
B2M14	AGTCACATGGTTCACACGGC	889	20	SpyCas9

B2M15	AAGTCAACTTCAATGTCGGA	890	20	SpyCas9
B2M16	CAGTAAGTCAACTTCAATGT	891	20	SpyCas9
B2M17	ACCCAGACACATAGCAATTC	892	20	SpyCas9
B2M18	GCATACTCATCTTTTTCAGT	893	20	SpyCas9
B2M19	ACAGCCCAAGATAGTTAAGT	894	20	SpyCas9
B2M20	GGCATACTCATCTTTTTCAG	895	20	SpyCas9
B2M21	TTCCTGAAGCTGACAGCATT	896	20	SpyCas9
B2M22	TCACGTCATCCAGCAGAGAA	897	20	SpyCas9
B2M23	CAGCCCAAGATAGTTAAGTG	898	20	SpyCas9
B2M-c1	AAUUCUCUCUCCAUUCUU	899	18	AsCpf1
B2M-c2	AAUUCUCUCUCCAUUCUUC	900	19	AsCpf1
B2M-c3	AAUUCUCUCUCCAUUCUUCA	901	20	AsCpf1
B2M-c4	AAUUCUCUCUCCAUUCUUCAG	902	21	AsCpf1
B2M-c5	AAUUCUCUCUCCAUUCUUCAGU	903	22	AsCpf1
B2M-c6	AAUUCUCUCUCCAUUCUUCAGUA	904	23	AsCpf1
B2M-c7	AAUUCUCUCUCCAUUCUUCAGUAA	905	24	AsCpf1
B2M-c8	ACUUUCCAUUCUCUGCUG	906	18	AsCpf1
B2M-c9	ACUUUCCAUUCUCUGCUGG	907	19	AsCpf1
B2M-c10	ACUUUCCAUUCUCUGCUGGA	908	20	AsCpf1
B2M-c11	ACUUUCCAUUCUCUGCUGGAU	909	21	AsCpf1
B2M-c12	ACUUUCCAUUCUCUGCUGGAUG	910	22	AsCpf1
B2M-c13	ACUUUCCAUUCUCUGCUGGAUGA	911	23	AsCpf1
B2M-c14	ACUUUCCAUUCUCUGCUGGAUGAC	912	24	AsCpf1
B2M-c15	AGCAAGGACUGGUCUUUC	913	18	AsCpf1
B2M-c16	AGCAAGGACUGGUCUUUCU	914	19	AsCpf1
B2M-c17	AGCAAGGACUGGUCUUUCUA	915	20	AsCpf1
B2M-c18	AGCAAGGACUGGUCUUUCUAU	916	21	AsCpf1
B2M-c19	AGCAAGGACUGGUCUUUCUAUC	917	22	AsCpf1
B2M-c20	AGCAAGGACUGGUCUUUCUAUCU	918	23	AsCpf1
B2M-c21	AGCAAGGACUGGUCUUUCUAUCUC	919	24	AsCpf1
B2M-c22	AGUGGGGGUGAAUUCAGU	920	18	AsCpf1
B2M-c23	AGUGGGGGUGAAUUCAGUG	921	19	AsCpf1
B2M-c24	AGUGGGGGUGAAUUCAGUGU	922	20	AsCpf1
B2M-c25	AGUGGGGGUGAAUUCAGUGUA	923	21	AsCpf1
B2M-c26	AGUGGGGGUGAAUUCAGUGUAG	924	22	AsCpf1
B2M-c27	AGUGGGGGUGAAUUCAGUGUAGU	925	23	AsCpf1
B2M-c28	AGUGGGGGUGAAUUCAGUGUAGUA	926	24	AsCpf1
B2M-c29	AUCCAUCCGACAUUGAAG	927	18	AsCpf1
B2M-c30	AUCCAUCCGACAUUGAAGU	928	19	AsCpf1
B2M-c31	AUCCAUCCGACAUUGAAGUU	929	20	AsCpf1
B2M-c32	AUCCAUCCGACAUUGAAGUUG	930	21	AsCpf1
B2M-c33	AUCCAUCCGACAUUGAAGUUGA	931	22	AsCpf1

B2M-c34	AUCCAUCCGACAUUGAAGUUGAC	932	23	AsCpf1
B2M-c35	AUCCAUCCGACAUUGAAGUUGACU	933	24	AsCpf1
B2M-c36	CAAUUCUCUCUCCAUUCU	934	18	AsCpf1
B2M-c37	CAAUUCUCUCUCCAUUCUU	935	19	AsCpf1
B2M-c38	CAAUUCUCUCUCCAUUCUUC	936	20	AsCpf1
B2M-c39	CAAUUCUCUCUCCAUUCUUCA	937	21	AsCpf1
B2M-c40	CAAUUCUCUCUCCAUUCUUCAG	938	22	AsCpf1
B2M-c41	CAAUUCUCUCUCCAUUCUUCAGU	939	23	AsCpf1
B2M-c42	CAAUUCUCUCUCCAUUCUUCAGUA	940	24	AsCpf1
B2M-c43	CAGUGGGGGUGAAUUCAG	941	18	AsCpf1
B2M-c44	CAGUGGGGGUGAAUUCAGU	942	19	AsCpf1
B2M-c45	CAGUGGGGGUGAAUUCAGUG	943	20	AsCpf1
B2M-c46	CAGUGGGGGUGAAUUCAGUGU	944	21	AsCpf1
B2M-c47	CAGUGGGGGUGAAUUCAGUGUA	945	22	AsCpf1
B2M-c48	CAGUGGGGGUGAAUUCAGUGUAG	946	23	AsCpf1
B2M-c49	CAGUGGGGGUGAAUUCAGUGUAGU	947	24	AsCpf1
B2M-c50	CAUUCUCUGCUGGAUGAC	948	18	AsCpf1
B2M-c51	CAUUCUCUGCUGGAUGACG	949	19	AsCpf1
B2M-c52	CAUUCUCUGCUGGAUGACGU	950	20	AsCpf1
B2M-c53	CAUUCUCUGCUGGAUGACGUG	951	21	AsCpf1
B2M-c54	CAUUCUCUGCUGGAUGACGUGA	952	22	AsCpf1
B2M-c55	CAUUCUCUGCUGGAUGACGUGAG	953	23	AsCpf1
B2M-c56	CAUUCUCUGCUGGAUGACGUGAGU	954	24	AsCpf1
B2M-c57	CCCGAUAUUCCUCAGGUA	955	18	AsCpf1
B2M-c58	CCCGAUAUUCCUCAGGUAC	956	19	AsCpf1
B2M-c59	CCCGAUAUUCCUCAGGUACU	957	20	AsCpf1
B2M-c60	CCCGAUAUUCCUCAGGUACUC	958	21	AsCpf1
B2M-c61	CCCGAUAUUCCUCAGGUACUCC	959	22	AsCpf1
B2M-c62	CCCGAUAUUCCUCAGGUACUCCA	960	23	AsCpf1
B2M-c63	CCCGAUAUUCCUCAGGUACUCCAA	961	24	AsCpf1
B2M-c64	CCGAUAUUCCUCAGGUAC	962	18	AsCpf1
B2M-c65	CCGAUAUUCCUCAGGUACU	963	19	AsCpf1
B2M-c66	CCGAUAUUCCUCAGGUACUC	964	20	AsCpf1
B2M-c67	CCGAUAUUCCUCAGGUACUCC	965	21	AsCpf1
B2M-c68	CCGAUAUUCCUCAGGUACUCCA	966	22	AsCpf1
B2M-c69	CCGAUAUUCCUCAGGUACUCCAA	967	23	AsCpf1
B2M-c70	CCGAUAUUCCUCAGGUACUCCAAA	968	24	AsCpf1
B2M-c71	CUCACGUCAUCCAGCAGA	969	18	AsCpf1
B2M-c72	CUCACGUCAUCCAGCAGAG	970	19	AsCpf1
B2M-c73	CUCACGUCAUCCAGCAGAGA	971	20	AsCpf1
B2M-c74	CUCACGUCAUCCAGCAGAGAA	972	21	AsCpf1
B2M-c75	CUCACGUCAUCCAGCAGAGAAU	973	22	AsCpf1

B2M-c76	CUCACGUCAUCCAGCAGAGAAUG	974	23	AsCpf1
B2M-c77	CUCACGUCAUCCAGCAGAGAAUGG	975	24	AsCpf1
B2M-c78	CUGAAUUGCUAUGUGUCU	976	18	AsCpf1
B2M-c79	CUGAAUUGCUAUGUGUCUG	977	19	AsCpf1
B2M-c80	CUGAAUUGCUAUGUGUCUGG	978	20	AsCpf1
B2M-c81	CUGAAUUGCUAUGUGUCUGGG	979	21	AsCpf1
B2M-c82	CUGAAUUGCUAUGUGUCUGGGU	980	22	AsCpf1
B2M-c83	CUGAAUUGCUAUGUGUCUGGGUU	981	23	AsCpf1
B2M-c84	CUGAAUUGCUAUGUGUCUGGGUUU	982	24	AsCpf1
B2M-c85	GAGUACCUGAGGAAUAUC	983	18	AsCpf1
B2M-c86	GAGUACCUGAGGAAUAUCG	984	19	AsCpf1
B2M-c87	GAGUACCUGAGGAAUAUCGG	985	20	AsCpf1
B2M-c88	GAGUACCUGAGGAAUAUCGGG	986	21	AsCpf1
B2M-c89	GAGUACCUGAGGAAUAUCGGGA	987	22	AsCpf1
B2M-c90	GAGUACCUGAGGAAUAUCGGGAA	988	23	AsCpf1
B2M-c91	GAGUACCUGAGGAAUAUCGGGAAA	989	24	AsCpf1
B2M-c92	UAUCUCUUGUACUACACU	990	18	AsCpf1
B2M-c93	UAUCUCUUGUACUACACUG	991	19	AsCpf1
B2M-c94	UAUCUCUUGUACUACACUGA	992	20	AsCpf1
B2M-c95	UAUCUCUUGUACUACACUGAA	993	21	AsCpf1
B2M-c96	UAUCUCUUGUACUACACUGAAU	994	22	AsCpf1
B2M-c97	UAUCUCUUGUACUACACUGAAUU	995	23	AsCpf1
B2M-c98	UAUCUCUUGUACUACACUGAAUUC	996	24	AsCpf1
B2M-c99	UCAAUUCUCUCUCCAUUC	997	18	AsCpf1
B2M-c100	UCAAUUCUCUCUCCAUUCU	998	19	AsCpf1
B2M-c101	UCAAUUCUCUCUCCAUUCUU	999	20	AsCpf1
B2M-c102	UCAAUUCUCUCUCCAUUCUUC	1000	21	AsCpf1
B2M-c103	UCAAUUCUCUCUCCAUUCUUCA	1001	22	AsCpf1
B2M-c104	UCAAUUCUCUCUCCAUUCUUCAG	1002	23	AsCpf1
B2M-c105	UCAAUUCUCUCUCCAUUCUUCAGU	1003	24	AsCpf1
B2M-c106	UCACAGCCCAAGAUAGUU	1004	18	AsCpf1
B2M-c107	UCACAGCCCAAGAUAGUUA	1005	19	AsCpf1
B2M-c108	UCACAGCCCAAGAUAGUUAA	1006	20	AsCpf1
B2M-c109	UCACAGCCCAAGAUAGUUAAG	1007	21	AsCpf1
B2M-c110	UCACAGCCCAAGAUAGUUAAGU	1008	22	AsCpf1
B2M-c111	UCACAGCCCAAGAUAGUUAAGUG	1009	23	AsCpf1
B2M-c112	UCACAGCCCAAGAUAGUUAAGUGG	1010	24	AsCpf1
B2M-c113	UCAGUGGGGGUGAAUUCA	1011	18	AsCpf1
B2M-c114	UCAGUGGGGGUGAAUUCAG	1012	19	AsCpf1
B2M-c115	UCAGUGGGGGUGAAUUCAGU	1013	20	AsCpf1
B2M-c116	UCAGUGGGGGUGAAUUCAGUG	1014	21	AsCpf1
B2M-c117	UCAGUGGGGGUGAAUUCAGUGU	1015	22	AsCpf1

B2M-c118	UCAGUGGGGGUGAAUUCAGUGUA	1016	23	AsCpf1
B2M-c119	UCAGUGGGGGUGAAUUCAGUGUAG	1017	24	AsCpf1
B2M-c120	UGGCCUGGAGGCUAUCCA	1018	18	AsCpf1
B2M-c121	UGGCCUGGAGGCUAUCCAG	1019	19	AsCpf1
B2M-c122	UGGCCUGGAGGCUAUCCAGC	1020	20	AsCpf1
B2M-c123	UGGCCUGGAGGCUAUCCAGCG	1021	21	AsCpf1
B2M-c124	UGGCCUGGAGGCUAUCCAGCGU	1022	22	AsCpf1
B2M-c125	UGGCCUGGAGGCUAUCCAGCGUG	1023	23	AsCpf1
B2M-c126	UGGCCUGGAGGCUAUCCAGCGUGA	1024	24	AsCpf1
B2M-c127	AUAGAUCGAGACAUGUAA	1025	18	AsCpf1
B2M-c128	AUAGAUCGAGACAUGUAAG	1026	19	AsCpf1
B2M-c129	AUAGAUCGAGACAUGUAAGC	1027	20	AsCpf1
B2M-c130	AUAGAUCGAGACAUGUAAGCA	1028	21	AsCpf1
B2M-c131	AUAGAUCGAGACAUGUAAGCAG	1029	22	AsCpf1
B2M-c132	AUAGAUCGAGACAUGUAAGCAGC	1030	23	AsCpf1
B2M-c133	AUAGAUCGAGACAUGUAAGCAGCA	1031	24	AsCpf1
B2M-c134	CAUAGAUCGAGACAUGUA	1032	18	AsCpf1
B2M-c135	CAUAGAUCGAGACAUGUAA	1033	19	AsCpf1
B2M-c136	CAUAGAUCGAGACAUGUAAG	1034	20	AsCpf1
B2M-c137	CAUAGAUCGAGACAUGUAAGC	1035	21	AsCpf1
B2M-c138	CAUAGAUCGAGACAUGUAAGCA	1036	22	AsCpf1
B2M-c139	CAUAGAUCGAGACAUGUAAGCAG	1037	23	AsCpf1
B2M-c140	CAUAGAUCGAGACAUGUAAGCAGC	1038	24	AsCpf1
B2M-c141	CUCCACUGUCUUUUUCAU	1039	18	AsCpf1
B2M-c142	CUCCACUGUCUUUUUCAUA	1040	19	AsCpf1
B2M-c143	CUCCACUGUCUUUUUCAUAG	1041	20	AsCpf1
B2M-c144	CUCCACUGUCUUUUUCAUAGA	1042	21	AsCpf1
B2M-c145	CUCCACUGUCUUUUUCAUAGAU	1043	22	AsCpf1
B2M-c146	CUCCACUGUCUUUUUCAUAGAUC	1044	23	AsCpf1
B2M-c147	CUCCACUGUCUUUUUCAUAGAUCG	1045	24	AsCpf1
B2M-c148	UCAUAGAUCGAGACAUGU	1046	18	AsCpf1
B2M-c149	UCAUAGAUCGAGACAUGUA	1047	19	AsCpf1
B2M-c150	UCAUAGAUCGAGACAUGUAA	1048	20	AsCpf1
B2M-c151	UCAUAGAUCGAGACAUGUAAG	1049	21	AsCpf1
B2M-c152	UCAUAGAUCGAGACAUGUAAGC	1050	22	AsCpf1
B2M-c153	UCAUAGAUCGAGACAUGUAAGCA	1051	23	AsCpf1
B2M-c154	UCAUAGAUCGAGACAUGUAAGCAG	1052	24	AsCpf1
B2M-c155	UCCACUGUCUUUUUCAUA	1053	18	AsCpf1
B2M-c156	UCCACUGUCUUUUUCAUAG	1054	19	AsCpf1
B2M-c157	UCCACUGUCUUUUUCAUAGA	1055	20	AsCpf1
B2M-c158	UCCACUGUCUUUUUCAUAGAU	1056	21	AsCpf1
B2M-c159	UCCACUGUCUUUUUCAUAGAUC	1057	22	AsCpf1

B2M-c160	UCCACUGUCUUUUUCAUAGAUCG	1058	23	AsCpf1
B2M-c161	UCCACUGUCUUUUUCAUAGAUCGA	1059	24	AsCpf1
B2M-c162	UCUCCACUGUCUUUUUCA	1060	18	AsCpf1
B2M-c163	UCUCCACUGUCUUUUUCAU	1061	19	AsCpf1
B2M-c164	UCUCCACUGUCUUUUUCAUA	1062	20	AsCpf1
B2M-c165	UCUCCACUGUCUUUUUCAUAG	1063	21	AsCpf1
B2M-c166	UCUCCACUGUCUUUUUCAUAGA	1064	22	AsCpf1
B2M-c167	UCUCCACUGUCUUUUUCAUAGAU	1065	23	AsCpf1
B2M-c168	UCUCCACUGUCUUUUUCAUAGAUC	1066	24	AsCpf1
B2M-c169	UUCUCCACUGUCUUUUUC	1067	18	AsCpf1
B2M-c170	UUCUCCACUGUCUUUUUCA	1068	19	AsCpf1
B2M-c171	UUCUCCACUGUCUUUUUCAU	1069	20	AsCpf1
B2M-c172	UUCUCCACUGUCUUUUUCAUA	1070	21	AsCpf1
B2M-c173	UUCUCCACUGUCUUUUUCAUAG	1071	22	AsCpf1
B2M-c174	UUCUCCACUGUCUUUUUCAUAGA	1072	23	AsCpf1
B2M-c175	UUCUCCACUGUCUUUUUCAUAGAU	1073	24	AsCpf1
B2M-c176	UUUCUCCACUGUCUUUUU	1074	18	AsCpf1
B2M-c177	UUUCUCCACUGUCUUUUUC	1075	19	AsCpf1
B2M-c178	UUUCUCCACUGUCUUUUUCA	1076	20	AsCpf1
B2M-c179	UUUCUCCACUGUCUUUUUCAU	1077	21	AsCpf1
B2M-c180	UUUCUCCACUGUCUUUUUCAUA	1078	22	AsCpf1
B2M-c181	UUUCUCCACUGUCUUUUUCAUAG	1079	23	AsCpf1
B2M-c182	UUUCUCCACUGUCUUUUUCAUAGA	1080	24	AsCpf1
B2M-c183	UUUUCUCCACUGUCUUUU	1081	18	AsCpf1
B2M-c184	UUUUCUCCACUGUCUUUUU	1082	19	AsCpf1
B2M-c185	UUUUCUCCACUGUCUUUUUC	1083	20	AsCpf1
B2M-c186	UUUUCUCCACUGUCUUUUUCA	1084	21	AsCpf1
B2M-c187	UUUUCUCCACUGUCUUUUUCAU	1085	22	AsCpf1
B2M-c188	UUUUCUCCACUGUCUUUUUCAUA	1086	23	AsCpf1
B2M-c189	UUUUCUCCACUGUCUUUUUCAUAG	1087	24	AsCpf1

[165] 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 9.

Table 9: NKG2A gRNAs

Name	gRNA Targeting Domain Sequence (DNA)	SEQ ID NO:	Length	Enzyme
NKG2A55	GAGGTAAAGCGTTTGCATTTG	1088	21	AsCpf1
NKG2A56	CCTCTAAAGCTTATGCTTACA	1089	21	AsCpf1
NKG2A57	AGTCGATTTACTTGTAGCACT	1090	21	AsCpf1

NKG2A58	CTTGTAGCACTGCACAGTTAA	1091	21	AsCpf1
NKG2A59	TCCATTACAGGATAAAAGACT	1092	21	AsCpf1
NKG2A60	CTCCATTACAGGATAAAAGAC	1093	21	AsCpf1
NKG2A61	TCTCCATTACAGGATAAAAGA	1094	21	AsCpf1
NKG2A62	ATCCTGTAATGGAGAAAAATC	1095	21	AsCpf1
NKG2A63	TCCTGTAATGGAGAAAAATCC	1096	21	AsCpf1
NKG2A136	AAACATGAGTAAGTTGTTTTG	1097	21	AsCpf1
NKG2A137	GCTTTCAAACATGAGTAAGTT	1098	21	AsCpf1
NKG2A138	AAAGCCAAACCATTCATTGTC	1099	21	AsCpf1
NKG2A139	GTAACAGCAGTCATCATCCAT	1100	21	AsCpf1
NKG2A140	ACCATCCTCATGGATTGGTGT	1101	21	AsCpf1
NKG2A141	TGTCCATCATTTCACCATCCT	1102	21	AsCpf1
NKG2A142	GAAATTTCTGTCCATCATTTC	1103	21	AsCpf1
NKG2A143	AGAAATTTCTGTCCATCATTT	1104	21	AsCpf1
NKG2A144	TTTTAGAAATTTCTGTCCATC	1105	21	AsCpf1
NKG2A145	CTTTTAGAAATTTCTGTCCAT	1106	21	AsCpf1
NKG2A146	TTTTCTTTTAGAAATTTCTGT	1107	21	AsCpf1
NKG2A147	TAAAAGAAAAGAAAGAATTTT	1108	21	AsCpf1
NKG2A270	AAACATTTACATCTTACCATT	1109	21	AsCpf1
NKG2A271	CATCTTACCATTTCTTCTTCA	1110	21	AsCpf1
NKG2A272	TATAGATAATGAAGAAGAAAT	1111	21	AsCpf1
NKG2A273	TTCTTCATTATCTATAGAAAG	1112	21	AsCpf1
NKG2A274	CTGGCCTGTACTTCGAAGAAC	1113	21	AsCpf1
NKG2A275	CTTACCAATGTAGTAACAACT	1114	21	AsCpf1
NKG2A276	GCACGTCATTGTGGCCATTGT	1115	21	AsCpf1
NKG2A277	TTTAGCACGTCATTGTGGCCA	1116	21	AsCpf1
NKG2A414	CCATCAGCTCCAGAGAAGCTC	1117	21	AsCpf1
NKG2A415	TCTCCCTGCAGATTTACCATC	1118	21	AsCpf1
NKG2A437	AAATGCTTTACCTTTGCAGTG	1119	21	AsCpf1
NKG2A438	AATGCTTTACCTTTGCAGTGA	1120	21	AsCpf1
NKG2A439	CCTTTGCAGTGATAGGTTTTG	1121	21	AsCpf1
NKG2A440	CAGTGATAGGTTTTGTCATTC	1122	21	AsCpf1
NKG2A441	AAGGGAATGACAAAACCTATC	1123	21	AsCpf1
NKG2A442	CAAGGGAATGACAAAACCTAT	1124	21	AsCpf1
NKG2A443	GTCATTCCCTTGAAAATCCTG	1125	21	AsCpf1
NKG2A444	TCATTCCCTTGAAAATCCTGA	1126	21	AsCpf1
NKG2A445	TGAAGGTTTAATTCCGCATAG	1127	21	AsCpf1
NKG2A446	GAAGGTTTAATTCCGCATAGG	1128	21	AsCpf1
NKG2A447	AAGGTTTAATTCCGCATAGGT	1129	21	AsCpf1
NKG2A448	ATTCCGCATAGGTTATTTCCT	1130	21	AsCpf1
NKG2A449	GCAACTGAACAGGAAATAACC	1131	21	AsCpf1
NKG2A450	AGCAACTGAACAGGAAATAAC	1132	21	AsCpf1

NKG2A451	CTGTTCAGTTGCTAAAATGGA	1133	21	AsCpf1
NKG2A452	TATTGCCTTTAGGTTTTCGTT	1134	21	AsCpf1
NKG2A453	ATTGCCTTTAGGTTTTCGTTG	1135	21	AsCpf1
NKG2A454	TTGCCTTTAGGTTTTCGTTGC	1136	21	AsCpf1
NKG2A455	GGTTTTCGTTGCTGCCTCTTT	1137	21	AsCpf1
NKG2A456	CGTTGCTGCCTCTTTGGGTTT	1138	21	AsCpf1
NKG2A457	GTTGCTGCCTCTTTGGGTTTG	1139	21	AsCpf1
NKG2A458	GGTTTGGGGGCAGATTCAGGT	1140	21	AsCpf1
NKG2A459	GGGGCAGATTCAGGTCTGAGT	1141	21	AsCpf1

[166] In some embodiments the gRNA for use in the disclosure is a gRNA targeting PD1. In some embodiments the gRNA for use in the disclosure is a gRNA targeting PD1. The gRNAs garneting B2M and PD1 for use in the disclosure are further described in WO2015161276 and WO2017152015 by Welstead et al. ("Welstead"); both incorporated in their entirety herein by reference.

RNA-guided nucleases

[167] RNA-guided nucleases according to the present disclosure include, but are not limited to, naturally-occurring Class 2 CRISPR nucleases such as Cas9, and Cpf1, as well as other nucleases derived or obtained therefrom. In functional terms, RNA-guided 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, RNA-guided nucleases can be defined, in broad terms, by their PAM specificity and cleavage activity, even though variations may exist between individual RNA-guided nucleases that share the same PAM specificity or cleavage activity. Skilled artisans will appreciate that some aspects of the present disclosure relate to systems, methods and compositions that can be implemented using any suitable RNA-guided nuclease having a certain PAM specificity and/or cleavage activity. For this reason, unless otherwise specified, the term RNA-guided 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 RNA-guided nuclease.

[168] 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 RNA-guided nuclease / gRNA combinations.

terminal nuclear localization signals. Nuclear localization sequences are known in the art and are described in Maeder and elsewhere.

[185] 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 RNA-guided nucleases, but it should be understood that the RNA-guided nucleases used may be modified in ways that do not alter their operating principles. Such modifications are within the scope of the present disclosure.

[186] Exemplary suitable nuclease variants include, but are not limited to , AsCpf1 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). 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.

[187] His-AsCpf1-sNLS-sNLS H800A amino acid sequence (SEQ ID NO:1142)

[188] MGHHHHHHGSTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKAR
NDHYKELKPIIDRIYKTYADQCLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNA
IHDYFIGRTDNLTDAINKRHAEIYKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKFTT
YFSGFYENRKNVFSAEDISTAIPHRIVQDNFPKFKENCHIFTRLITAVPSLREHFENVKKAI
GIFVSTSIEEVFSFPFYNQLLTQTQIDLYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDET
AHIIASLPHRFIPLFKQILSDRNTLSFILEEFKSDEEVIQSFCKYKTLLRNENVLETAEALF
NELNSIDLTHIFISHKKLETISSALCDHWDTLRNALYERRISELTGKITKSAKEKVQRSLKH
EDINLQEIISAAGKELSEAFKQKTSEILSHAHAALDQPLPTTLKKQEEKEILKSQLDSLLGL
YHLLDWFAVDESNEVDPEFSARLTGIKLEMEPSLSFYNKARNYATKKPYSVEKFKLNFQMPT
LASGWDVNKEKNNGAILFVKNGLYYLGIMPKQKGRYKALSFEPTEKTSEGFDKMYYDYFPDA
AKMIPKCSTQLKAVTAHFQTHTTPILLSNNFIEPLEITKEIYDLNNPEKEPKKFQTAYAKKT
GDQKGYREALCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQYKDLGEYYAELNPLLYHISFQR
IAEKEIMDAVETGKLYLFQIYNKDFAKGHHGKPNLHTLYWTGLFSPENLAKTSIKLNGQAEL
FYRPKSRMKRMAARLGEKMLNKKLKDQKTPIPDTLYQELYDYVNHRLSHDLSDEARALLPNV
ITKEVSHEIIKDRRFTSDKFFFHVPITLNYQAANSPSKFNQRVNAYLKEHPETPIIGIDRGE
RNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSVVGTIKDLKQGYLS
QVIHEIVDLMIHYQAVVVLENLNFGFKSKRTGIAEKAVYQQFEKMLIDKLNCLVLKDYPAEK
VGGVLNPYQLTDQFTSFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTIKNHESRKHF
LEGFDFLHYDVKTGDFILHFKMNRNLSFQRGLPGFMPAWDIVFEKNETQFDAKGTPFIAGKR
IVPVIENHRFTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTMVALIRS
VLQMRNSNAATGEDYINSPVRDLNGVCFDSRFQNPEWPMDADANGAYHIALKGQLLLNHLKE
SKDLKLQNGISNQDWLAYIQELRNGSPKKKRKVGSPKKKRKV

[189] Cpf1 variant 1 amino acid sequence (SEQ ID NO:1143)

[190] MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKPIIDRI
YKTYADQCLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIGRTDNLTD
AINKRHAEIYKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYENRKNVFS
AEDISTAIPHRIVQDNFPKFKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIEEVFSF
PFYNQLLTQTQIDLYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPHRFIPL
FKQILSDRNTLSFILEEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNELNSIDLTHIFIS
HKKLETISSALCDHWDTLRNALYERRISELTGKITKSAKEKVQRSLKHEDINLQEIISAAGK
ELSEAFKQKTSEILSHAHAALDQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAVDESNE
VDPEFSARLTGIKLEMEPSLSFYNKARNYATKKPYSVEKFKLNFQRPTLASGWDVNKEKNNG
AILFVKNGLYYLGIMPKQKGRYKALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCSTQLKAV
TAHFQTHTTPILLSNNFIEPLEITKEIYDLNNPEKEPKKFQTAYAKKTGDQKGYREALCKWI
DFTRDFLSKYTKTTSIDLSSLRPSSQYKDLGEYYAELNPLLYHISFQRIAEKEIMDAVETGK
LYLFQIYNKDFAKGHHGKPNLHTLYWTGLFSPENLAKTSIKLNGQAELFYRPKSRMKRMAHR
LGEKMLNKKLKDQKTPIPDTLYQELYDYVNHRLSHDLSDEARALLPNVITKEVSHEIIKDRR
FTSDKFLFHVPITLNYQAANSPSKFNQRVNAYLKEHPETPIIGIDRGERNLIYITVIDSTGK
ILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSVVGTIKDLKQGYLSQVIHEIVDLMIHYQ
AVVVLENLNFGFKSKRTGIAEKAVYQQFEKMLIDKLNCLVLKDYPAEKVGGVLNPYQLTDQF
TSFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTIKNHESRKHFLEGFDFLHYDVKTG
DFILHFKMNRNLSFQRGLPGFMPAWDIVFEKNETQFDAKGTPFIAGKRIVPVIENHRFTGRY
RDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTMVALIRSVLQMRNSNAATGED
YINSPVRDLNGVCFDSRFQNPEWPMDADANGAYHIALKGQLLLNHLKESKDLKLQNGISNQD
WLAYIQELRNGRSSDDEATADSQHAAPPKKKRKVGGSGGSGGSGGSGGSGGSGGSGGSLEHH
HHHH

[191] Cpf1 variant 2 amino acid sequence (SEQ ID NO:1144)

[192] MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKP
IIDRIYKTYADQCLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIGRT
DNLTDAINKRHAEIYKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYENR
KNVFSAEDISTAIPHRIVQDNFPKFKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIE
EVFSFPFYNQLLTQTQIDLYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPH
RFIPLFKQILSDRNTLSFILEEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNELNSIDLT
HIFISHKKLETISSALCDHWDTLRNALYERRISELTGKITKSAKEKVQRSLKHEDINLQEII
SAAGKELSEAFKQKTSEILSHAHAALDQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAV
DESNEVDPEFSARLTGIKLEMEPSLSFYNKARNYATKKPYSVEKFKLNFQMPTLASGWDVNK
EKNNGAILFVKNGLYYLGIMPKQKGRYKALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCST
QLKAVTAHFQTHTTPILLSNNFIEPLEITKEIYDLNNPEKEPKKFQTAYAKKTGDQKGYREA
LCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQYKDLGEYYAELNPLLYHISFQRIAEKEIMDA
VETGKLYLFQIYNKDFAKGHHGKPNLHTLYWTGLFSPENLAKTSIKLNGQAELFYRPKSRMK
RMAHRLGEKMLNKKLKDQKTPIPDTLYQELYDYVNHRLSHDLSDEARALLPNVITKEVSHEI
IKDRRFTSDKFFFHVPITLNYQAANSPSKFNQRVNAYLKEHPETPIIGIDRGERNLIYITVI
DSTGKILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSVVGTIKDLKQGYLSQVIHEIVDL
MIHYQAVVVLENLNFGFKSKRTGIAEKAVYQQFEKMLIDKLNCLVLKDYPAEKVGGVLNPYQ
LTDQFTSFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTIKNHESRKHFLEGFDFLHY
DVKTGDFILHFKMNRNLSFQRGLPGFMPAWDIVFEKNETQFDAKGTPFIAGKRIVPVIENHR
FTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTMVALIRSVLQMRNSNA
ATGEDYINSPVRDLNGVCFDSRFQNPEWPMDADANGAYHIALKGQLLLNHLKESKDLKLQNG
ISNQDWLAYIQELRNGRSSDDEATADSQHAAPPKKKRKVGGSGGSGGSGGSGGSGGSGGSGG
SLEHHHHHH

[193] Cpf1 variant 3 amino acid sequence (SEQ ID NO:1145)

[194] MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKP
IIDRIYKTYADQCLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIGRT
DNLTDAINKRHAEIYKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYENR
KNVFSAEDISTAIPHRIVQDNFPKFKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIE
EVFSFPFYNQLLTQTQIDLYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPH
RFIPLFKQILSDRNTLSFILEEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNELNSIDLT
HIFISHKKLETISSALCDHWDTLRNALYERRISELTGKITKSAKEKVQRSLKHEDINLQEII
SAAGKELSEAFKQKTSEILSHAHAALDQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAV
DESNEVDPEFSARLTGIKLEMEPSLSFYNKARNYATKKPYSVEKFKLNFQRPTLASGWDVNK
EKNNGAILFVKNGLYYLGIMPKQKGRYKALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCST
QLKAVTAHFQTHTTPILLSNNFIEPLEITKEIYDLNNPEKEPKKFQTAYAKKTGDQKGYREA
LCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQYKDLGEYYAELNPLLYHISFQRIAEKEIMDA
VETGKLYLFQIYNKDFAKGHHGKPNLHTLYWTGLFSPENLAKTSIKLNGQAELFYRPKSRMK
RMAARLGEKMLNKKLKDQKTPIPDTLYQELYDYVNHRLSHDLSDEARALLPNVITKEVSHEI
IKDRRFTSDKFLFHVPITLNYQAANSPSKFNQRVNAYLKEHPETPIIGIDRGERNLIYITVI
DSTGKILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSVVGTIKDLKQGYLSQVIHEIVDL
MIHYQAVVVLENLNFGFKSKRTGIAEKAVYQQFEKMLIDKLNCLVLKDYPAEKVGGVLNPYQ
LTDQFTSFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTIKNHESRKHFLEGFDFLHY
DVKTGDFILHFKMNRNLSFQRGLPGFMPAWDIVFEKNETQFDAKGTPFIAGKRIVPVIENHR
FTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTMVALIRSVLQMRNSNA
ATGEDYINSPVRDLNGVCFDSRFQNPEWPMDADANGAYHIALKGQLLLNHLKESKDLKLQNG
ISNQDWLAYIQELRNGRSSDDEATADSQHAAPPKKKRKVGGSGGSGGSGGSGGSGGSGGSGG
SLEHHHHHH

[195] Cpf1 variant 4 amino acid sequence (SEQ ID NO:1146)

[196] MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKP
IIDRIYKTYADQCLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIGRT
DNLTDAINKRHAEIYKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYENR
KNVFSAEDISTAIPHRIVQDNFPKFKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIE
EVFSFPFYNQLLTQTQIDLYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPH
RFIPLFKQILSDRNTLSFILEEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNELNSIDLT
HIFISHKKLETISSALCDHWDTLRNALYERRISELTGKITKSAKEKVQRSLKHEDINLQEII
SAAGKELSEAFKQKTSEILSHAHAALDQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAV
DESNEVDPEFSARLTGIKLEMEPSLSFYNKARNYATKKPYSVEKFKLNFQRPTLASGWDVNK
EKNNGAILFVKNGLYYLGIMPKQKGRYKALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCST
QLKAVTAHFQTHTTPILLSNNFIEPLEITKEIYDLNNPEKEPKKFQTAYAKKTGDQKGYREA
LCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQYKDLGEYYAELNPLLYHISFQRIAEKEIMDA
VETGKLYLFQIYNKDFAKGHHGKPNLHTLYWTGLFSPENLAKTSIKLNGQAELFYRPKSRMK
RMAARLGEKMLNKKLKDQKTPIPDTLYQELYDYVNHRLSHDLSDEARALLPNVITKEVSHEI
IKDRRFTSDKFLFHVPITLNYQAANSPSKFNQRVNAYLKEHPETPIIGIDRGERNLIYITVI
DSTGKILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSVVGTIKDLKQGYLSQVIHEIVDL
MIHYQAVVVLENLNFGFKSKRTGIAEKAVYQQFEKMLIDKLNCLVLKDYPAEKVGGVLNPYQ
LTDQFTSFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTIKNHESRKHFLEGFDFLHY
DVKTGDFILHFKMNRNLSFQRGLPGFMPAWDIVFEKNETQFDAKGTPFIAGKRIVPVIENHR
FTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTMVALIRSVLQMRNSNA
ATGEDYINSPVRDLNGVCFDSRFQNPEWPMDADANGAYHIALKGQLLLNHLKESKDLKLQNG
ISNQDWLAYIQELRNGRSSDDEATADSQHAAPPKKKRKV

[197] Cpf1 variant 5 amino acid sequence (SEQ ID NO:1147)

[198] MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKP
IIDRIYKTYADQCLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIGRT
DNLTDAINKRHAEIYKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYENR
KNVFSAEDISTAIPHRIVQDNFPKFKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIE
EVFSFPFYNQLLTQTQIDLYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPH
RFIPLFKQILSDRNTLSFILEEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNELNSIDLT
HIFISHKKLETISSALCDHWDTLRNALYERRISELTGKITKSAKEKVQRSLKHEDINLQEII
SAAGKELSEAFKQKTSEILSHAHAALDQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAV
DESNEVDPEFSARLTGIKLEMEPSLSFYNKARNYATKKPYSVEKFKLNFQRPTLASGWDVNK
EKNNGAILFVKNGLYYLGIMPKQKGRYKALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCST
QLKAVTAHFQTHTTPILLSNNFIEPLEITKEIYDLNNPEKEPKKFQTAYAKKTGDQKGYREA
LCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQYKDLGEYYAELNPLLYHISFQRIAEKEIMDA
VETGKLYLFQIYNKDFAKGHHGKPNLHTLYWTGLFSPENLAKTSIKLNGQAELFYRPKSRMK
RMAHRLGEKMLNKKLKDQKTPIPDTLYQELYDYVNHRLSHDLSDEARALLPNVITKEVSHEI
IKDRRFTSDKFLFHVPITLNYQAANSPSKFNQRVNAYLKEHPETPIIGIDRGERNLIYITVI
DSTGKILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSVVGTIKDLKQGYLSQVIHEIVDL
MIHYQAVVVLENLNFGFKSKRTGIAEKAVYQQFEKMLIDKLNCLVLKDYPAEKVGGVLNPYQ
LTDQFTSFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTIKNHESRKHFLEGFDFLHY
DVKTGDFILHFKMNRNLSFQRGLPGFMPAWDIVFEKNETQFDAKGTPFIAGKRIVPVIENHR
FTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTMVALIRSVLQMRNSNA
ATGEDYINSPVRDLNGVCFDSRFQNPEWPMDADANGAYHIALKGQLLLNHLKESKDLKLQNG
ISNQDWLAYIQELRNGRSSDDEATADSQHAAPPKKKRKV

[199] Cpf1 variant 6 amino acid sequence (SEQ ID NO:1148)

[200] MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKP
IIDRIYKTYADQCLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIGRT
DNLTDAINKRHAEIYKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYENR
KNVFSAEDISTAIPHRIVQDNFPKFKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIE
EVFSFPFYNQLLTQTQIDLYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPH
RFIPLFKQILSDRNTLSFILEEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNELNSIDLT
HIFISHKKLETISSALCDHWDTLRNALYERRISELTGKITKSAKEKVQRSLKHEDINLQEII
SAAGKELSEAFKQKTSEILSHAHAALDQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAV
DESNEVDPEFSARLTGIKLEMEPSLSFYNKARNYATKKPYSVEKFKLNFQRPTLASGWDVNK
EKNNGAILFVKNGLYYLGIMPKQKGRYKALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCST
QLKAVTAHFQTHTTPILLSNNFIEPLEITKEIYDLNNPEKEPKKFQTAYAKKTGDQKGYREA
LCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQYKDLGEYYAELNPLLYHISFQRIAEKEIMDA
VETGKLYLFQIYNKDFAKGHHGKPNLHTLYWTGLFSPENLAKTSIKLNGQAELFYRPKSRMK
RMAHRLGEKMLNKKLKDQKTPIPDTLYQELYDYVNHRLSHDLSDEARALLPNVITKEVSHEI
IKDRRFTSDKFLFHVPITLNYQAANSPSKFNQRVNAYLKEHPETPIIGIDRGERNLIYITVI
DSTGKILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSVVGTIKDLKQGYLSQVIHEIVDL
MIHYQAVVVLENLNFGFKSKRTGIAEKAVYQQFEKMLIDKLNCLVLKDYPAEKVGGVLNPYQ
LTDQFTSFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTIKNHESRKHFLEGFDFLHY
DVKTGDFILHFKMNRNLSFQRGLPGFMPAWDIVFEKNETQFDAKGTPFIAGKRIVPVIENHR
FTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTMVALIRSVLQMRNSNA
ATGEDYINSPVRDLNGVCFDSRFQNPEWPMDADANGAYHIALKGQLLLNHLKESKDLKLQNG
ISNQDWLAYIQELRNGRSSDDEATADSQHAAPPKKKRKVGGSGGSGGSGGSGGSGGSGGSGG
SLEHHHHHH

[201] Cpf1 variant 7 amino acid sequence (SEQ ID NO:1149)

[202] MGRDPGKPIPNPLLGLDSTAPKKKRKVGIHGVPAATQFEGFTNLYQVSKTLR
FELIPQGKTLKHIQEQGFIEEDKARNDHYKELKPIIDRIYKTYADQCLQLVQLDWENLSAAI
DSYRKEKTEETRNALIEEQATYRNAIHDYFIGRTDNLTDAINKRHAEIYKGLFKAELFNGKV
LKQLGTVTTTEHENALLRSFDKFTTYFSGFYENRKNVFSAEDISTAIPHRIVQDNFPKFKEN
CHIFTRLITAVPSLREHFENVKKAIGIFVSTSIEEVFSFPFYNQLLTQTQIDLYNQLLGGIS
REAGTEKIKGLNEVLNLAIQKNDETAHIIASLPHRFIPLFKQILSDRNTLSFILEEFKSDEE
VIQSFCKYKTLLRNENVLETAEALFNELNSIDLTHIFISHKKLETISSALCDHWDTLRNALY
ERRISELTGKITKSAKEKVQRSLKHEDINLQEIISAAGKELSEAFKQKTSEILSHAHAALDQ
PLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAVDESNEVDPEFSARLTGIKLEMEPSLSFY
NKARNYATKKPYSVEKFKLNFQMPTLASGWDVNKEKNNGAILFVKNGLYYLGIMPKQKGRYK
ALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCSTQLKAVTAHFQTHTTPILLSNNFIEPLEI
TKEIYDLNNPEKEPKKFQTAYAKKTGDQKGYREALCKWIDFTRDFLSKYTKTTSIDLSSLRP
SSQYKDLGEYYAELNPLLYHISFQRIAEKEIMDAVETGKLYLFQIYNKDFAKGHHGKPNLHT
LYWTGLFSPENLAKTSIKLNGQAELFYRPKSRMKRMAHRLGEKMLNKKLKDQKTPIPDTLYQ
ELYDYVNHRLSHDLSDEARALLPNVITKEVSHEIIKDRRFTSDKFFFHVPITLNYQAANSPS
KFNQRVNAYLKEHPETPIIGIDRGERNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLDNRE
KERVAARQAWSVVGTIKDLKQGYLSQVIHEIVDLMIHYQAVVVLENLNFGFKSKRTGIAEKA
VYQQFEKMLIDKLNCLVLKDYPAEKVGGVLNPYQLTDQFTSFAKMGTQSGFLFYVPAPYTSK
IDPLTGFVDPFVWKTIKNHESRKHFLEGFDFLHYDVKTGDFILHFKMNRNLSFQRGLPGFMP
AWDIVFEKNETQFDAKGTPFIAGKRIVPVIENHRFTGRYRDLYPANELIALLEEKGIVFRDG
SNILPKLLENDDSHAIDTMVALIRSVLQMRNSNAATGEDYINSPVRDLNGVCFDSRFQNPEW
PMDADANGAYHIALKGQLLLNHLKESKDLKLQNGISNQDWLAYIQELRNPKKKRKVKLAAAL
EHHHHHH

[203] Exemplary AsCpf1 wild-type amino acid sequence (SEQ ID NO: 1150):

[204] MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKP
IIDRIYKTYADQCLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIGRT
DNLTDAINKRHAEIYKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYENR
KNVFSAEDISTAIPHRIVQDNFPKFKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIE
EVFSFPFYNQLLTQTQIDLYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPH
RFIPLFKQILSDRNTLSFILEEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNELNSIDLT
HIFISHKKLETISSALCDHWDTLRNALYERRISELTGKITKSAKEKVQRSLKHEDINLQEII
SAAGKELSEAFKQKTSEILSHAHAALDQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAV
DESNEVDPEFSARLTGIKLEMEPSLSFYNKARNYATKKPYSVEKFKLNFQMPTLASGWDVNK
EKNNGAILFVKNGLYYLGIMPKQKGRYKALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCST
QLKAVTAHFQTHTTPILLSNNFIEPLEITKEIYDLNNPEKEPKKFQTAYAKKTGDQKGYREA
LCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQYKDLGEYYAELNPLLYHISFQRIAEKEIMDA
VETGKLYLFQIYNKDFAKGHHGKPNLHTLYWTGLFSPENLAKTSIKLNGQAELFYRPKSRMK
RMAHRLGEKMLNKKLKDQKTPIPDTLYQELYDYVNHRLSHDLSDEARALLPNVITKEVSHEI
IKDRRFTSDKFFFHVPITLNYQAANSPSKFNQRVNAYLKEHPETPIIGIDRGERNLIYITVI
DSTGKILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSVVGTIKDLKQGYLSQVIHEIVDL
MIHYQAVVVLENLNFGFKSKRTGIAEKAVYQQFEKMLIDKLNCLVLKDYPAEKVGGVLNPYQ
LTDQFTSFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTIKNHESRKHFLEGFDFLHY
DVKTGDFILHFKMNRNLSFQRGLPGFMPAWDIVFEKNETQFDAKGTPFIAGKRIVPVIENHR
FTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTMVALIRSVLQMRNSNA
ATGEDYINSPVRDLNGVCFDSRFQNPEWPMDADANGAYHIALKGQLLLNHLKESKDLKLQNG
ISNQDWLAYIQELRN

Example 10: Gene edited NK cells, exhibiting loss-of-function of CISH and/or TGFBR2, demonstrate improved effector function in response to tumor cells

[451] A next-generation allogeneic NK cell therapy was developed using CRISPR-Cpf1 gene editing to enhance NK cell function through knockout of the CISH and TGFBR2 genes.

[452] NK cells were expanded from $CD3^-$ PBMC cultures in 20ng/mL IL-15. Gene editing was performed at different NK cell expansion stages (between day 8-21). For editing of CISH and TGFBR2, guides for either targets were complexed with Cpf1 nuclease at a 2:1 ratio to form ribonucleoproteins (RNPs). Where cells were edited with both targets, RNP complexation for each target was done separately and then mixed at a 1:1 ratio prior to electroporation.

[453] For electroporation, NK cells were suspended in HyClone buffer at a density of $80x10^6$ cells/mL. Ninety microliters of NK cells were mixed with 10 microliters of the appropriate RNPs. The cell and RNP mixtures were then transferred to a MaxCyte OC-100 or OC-400 cassette for electroporation. Immediately post-electroporation, NK cells were recovered in 100microliters of culture media for 10 minutes at 37°C, before transferring to a 24-well Grex plate for post editing recovery and functional analyses.

[454] The following guide RNA sequences were used for editing of CISH and TGFBR2: Both guides were generated with a targeting domain consisting of RNA, an AsCpf1 scaffold of the sequence UAAUUUCUACUCUUGUAGAU (SEQ ID NO: 1151) 5' of the targeting domain, and a 25-mer DNA extension of the sequence ATGTGTTTTTGTCAAAAGACCTTTT (SEQ ID NO: 1152) at the 5' terminus of the scaffold sequence.

Table 12:

Target	**gRNA targeting domain Sequence (DNA)**	**gRNA sequence**
CISH 7050	GGTGTACAGCAGTGGCTGGT (SEQ ID NO: 1153)	ATGTGTTTTTGTCAAAAGACCTTTTrUrArArUrUrUrCrUrArCrUrCrUrUrGrUrArGrArUrGrGrUrGrUrArCrArGrCrArGrUrGrGrCrUrGrGrU(SEQ ID NO: 1154)
TGFBR2 24026	TGATGTGAGATTTTCCACCT (SEQ ID NO: 1155)	ATGTGTTTTTGTCAAAAGACCTTTTrUrArArUrUrUrCrUrArCrUrCrUrUrGrUrArGrArUrGrArUrGrUrGrArGrArUrUrUrUrCrCrArCrCrU (SEQ ID NO: 1156)

[455] As demonstrated in **Figs. 1A-1B**, robust single and double-gene editing of TGFBR2 and CISH was achieved in NK cells. Both single and simultaneous targeting of TGFBR2 and CISH in NK cells using CRISPR-Cpf1 produced indels at both targets in greater than 80% of NK cells, with greater than 90% of edited NK cells viable at 72 hours post-editing.

[456] The efficacy of the effector cells was assessed *in vitro* by 3D tumor spheroid assays

[457] To form spheroids, 5,000 NucLight Red labeled PC-3 or SK-OV-3 tumor cells were plated in a single well of ultra-low attachment 96 well plates, centrifuged at 1,000rpm for 10 minutes, and incubated for 96 hours at 37° C. At 96 hours, effector cells (primary human NK cells treated with different RNPs) were added to spheroids at multiple effector to target cell ratios with or without 10ng/mL of TGF-beta. Red object intensity was measured every two hours for 6 days on an Incucyte imaging system. 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 (**Figs. 2A-2B**).

[458] Moreover, CISH KO NK cells reduced the growth of SK-OV-3 ovarian (**Figs. 3A-3B** and **Fig. 5A**) and PC-3 prostate tumor spheroids (**Figs. 4A-4B** and **Fig. 5B**) by an average of 23% and 12% ($p<0.0001$ in both cases), respectively, in comparison to unedited controls. However, the activity of CISH KO NK cells were dampened by addition of exogenous TGF-β.

[459] Given this observation, a knockout of the TGF-β receptor gene, TGFBR2, with the CISH KO was generated. Single knockout of TGFBR2 rendered NK cells resistant to TGFβ inhibition ($p<0.0001$). Importantly, across 4 unique donors and 7 independent experiments, TGFBR2/CISH double knockout (DKO) NK cells demonstrated superior effector function and attenuated SK-OV-3 and PC-3 tumor spheroid growth by greater than 60% for both tumor types, with supplement of exogenous TGF-β (**Figs. 3A-3B** and **Figs. 4A-4B**) and without supplement of exogenous TGF-β (**Fig. 5A-5B**). These effector functions were statistically greater than control NK cells or TGFBR2 and CISH single knockout NK cells ($p<0.0001$ in all cases). In addition, TGFBR2/CISH DKO NK cells produced higher concentrations of TNF-α (**Fig. 6A**) and IFN-γ (**Fig. 6B**) $p<0.01$ in both cases as assessed by ELISA.

[460] Double KO NK cells expressed significantly higher levels of activation markers CD25 and CD69 as compared to control NK cells (**Fig. 6C**).

[461] Anti-tumor activity of edited NK cells was measured in an in vivo model. NSG mice received an intraperitoneal injection of 500,000 SKOV3 tumor cells labeled with luciferase.

Seven days post-tumor implantation, 10 million edited (CISH/TGFBR2 double-knockout) or unedited (control) NK cells were injected into the peritoneal cavity of the tumor-bearing mice. Tumor burden was monitored weekly by IP administration of luciferin and IVIS imaging. Two-way ANOVA analysis was performed at day 34 to determine statistical significance between control and DKO NK cell groups (****, P<0.0001) (**Fig 6.D**).

[462] These results demonstrate efficient editing of primary human NK cells at two unique targets simultaneously with CRISPR-Cpf1. Together, the increased effector function of CISH/TGFBR2 DKO primary human NK cells *in vitro* and *in vivo* relative to either single knockout or unedited NK cells indicates an enhanced and synergistic effect of the CISH/TGFBR2 DKO.

Example 11: Gene edited NK cells, exhibiting loss-of-function of TIGIT, NKG2A or ADORA2A, demonstrate improved effector function in response to tumor cells

[463] A next-generation allogeneic NK cell therapy was developed using CRISPR-Cpf1 gene editing to enhance NK cell function through knockout of the TIGIT, NKG2A, or ADORA2A genes.

[464] NK cells were expanded as previously described in Example 10. Briefly, NK cells were expanded *ex-vivo* for 14 days in IL15, and then edited with the respective targeting RNP complex. Gene editing was performed at different NK cell expansion stages (between day 8-21). For editing of TIGIT, NKG2A, or ADORA2A, guides for the corresponding targets were complexed with Cpf1 nuclease at a 2:1 ratio to form ribonucleoproteins (RNPs). Where cells were edited with both targets, RNP complexation for each target was done separately and then mixed at a 1:1 ratio prior to electroporation. NK cells were electroporated as previously described in Example 10.

[465] The following guide RNA sequences were used for editing of TIGIT, NKG2A, or ADORA2A:

Table 13:

Target	**gRNA targeting domain Sequence (DNA)**	**gRNA sequence**
TIGIT	TGCAGAGAAAGGTGGCTCT A (SEQ ID NO: 1157)	ATGTGTTTTTGTCAAAAGACCT TTTrUrArArUrUrUrCrUrArCrUrCr UrUrGrUrArGrArUrUrGrCrArGrAr GrArArArGrGrUrGrGrCrUrCrUrA (SEQ ID NO: 1158)
NKG2A	GCAACTGAACAGGAAATAA CC (SEQ ID NO: 1159)	UAAUUUCUACUCUUGUAGAUG CAACUGAACAGGAAAUAACC

		(SEQ ID NO: 1160)
ADORA2A	CCTGTGTGCTGGTGCCCCTG (SEQ ID NO: 1161)	ATGTGTTTTTGTCAAAAGACCT TTTrUrArArUrUrUrCrUrArCrUrCr UrUrGrUrArGrArUrCrCrUrGrUrGr UrGrCrUrGrGrUrGrCrCrCrCrUrG (SEQ ID NO: 1162)

[466] As demonstrated in **Figs. 7A-7C**, robust single gene editing of TIGIT, NKG2A, and ADORA2A was achieved in NK cells.

[467] The efficacy of the effector cells (primary human NK cells treated with different RNPs), was assessed *in vitro* to determine the function of TIGIT single KO **(Figs. 8A-8B)**, NKG2A single KO **(Figs. 9A-9B)** and ADORA2A single KO **(Figs. 10A-10B)** by 3D tumor spheroid assays

[468] To form spheroids, 5,000 NucLight Red labeled PC-3 or SK-OV-3 tumor cells were plated in a single well of ultra-low attachment 96 well plates, centrifuged at 1,000rpm for 10 minutes, and incubated for 96 hours at 37° C. At 96 hours, effector cells (primary human NK cells treated with different RNPs) were added to spheroids at multiple effector to target cell ratios with or without 10ng/mL of TGF-beta. Red object intensity was measured every two hours for 6 days on an Incucyte imaging system. Data shown are normalized to the red object intensity at time of effector addition.

[469] Across 2 unique donors and 2 independent experiments, TIGIT single KO **(Figs. 8A-8B)**, NKG2A single KO **(Figs. 9A-9B)** and ADORA2A single KO **(Figs. 10A-10B)** NK cells demonstrated superior effector function and attenuated SK-OV-3 and PC-3 tumor spheroid growth. These data demonstrate efficient editing of primary human NK cells at three independent unique targets with CRISPR-Cpf1, resulting in increased effector function of TIGIT single KO, NKG2A single KO and ADORA2A single KO primary human NK cells *in vitro* relative to unedited NK cells.

Example 12: Gene edited NK cells, exhibiting loss-of-function of CISH, TGFBR2 and TIGIT, demonstrate improved effector function in response to tumor cells

[470] A next-generation allogeneic NK cell therapy was developed using CRISPR-Cpf1 gene editing to enhance NK cell function through knockout of the CISH, TGFBR2 and TIGIT genes.

[471] NK cells were expanded as previously described in Example 10. Briefly, NK cells were expanded *ex-vivo* for 14 days in IL15, and then edited with the respective targeting RNP complex. Gene editing was performed at different NK cell expansion stages (between day 8-21). For editing of CISH, TGFBR2 and TIGIT, guides for the targets were complexed with Cpf1 nuclease to form ribonucleoproteins (RNPs), RNP complexation for each target was done separately and then mixed at

a 1:1 ratio prior to electroporation. NK cells were electroporated as previously described in Example 10.

[472] The guide RNA sequences that were used for editing of CISH, TGFBR2 and TIGIT are indicated in Table 14 below:

Table 14

Target	**gRNA targeting domain Sequence (DNA)**	**gRNA sequence**
CISH	ACTGACAGCGTGAACAGGTAG (SEQ ID NO: 1163)	ATGTGTTTTTGTCAAAAGACCTTTTrUrArArUrUrUrCrUrArCrUrCrUrUrGrUrArGrArUrArCrUrGrArCrArGrCrGrUrGrArArCrArGrGrUrArG(SEQ ID NO: 1164)
TGFBR2	TGATGTGAGATTTTCCACCT (SEQ ID NO: 1165)	ATGTGTTTTTGTCAAAAGACCTTTTrUrArArUrUrUrCrUrArCrUrCrUrUrGrUrArGrArUrUrGrArUrGrUrGrArGrArUrUrUrUrCrCrArCrCrU (SEQ ID NO: 1166)
TIGIT	TGCAGAGAAAGGTGGCTCTA (SEQ ID NO: 1167)	ATGTGTTTTTGTCAAAAGACCTTTTrUrArArUrUrUrCrUrArCrUrCrUrUrGrUrArGrArUrUrGrCrArGrArGrArArArGrGrUrGrGrCrUrCrUrA (SEQ ID NO: 1168)

[473] As demonstrated in **Fig. 11**, robust triple-gene editing of TGFBR2, CISH and TIGIT was achieved in NK cells.

[474] The efficacy of the effector cells was assessed *in vitro* by 3D tumor spheroid assays.

[475] To form spheroids, 5,000 NucLight Red labeled PC-3 or SK-OV-3 tumor cells were plated in a single well of ultra-low attachment 96 well plates, centrifuged at 1,000rpm for 10 minutes, and incubated for 96 hours at 37° C. At 96 hours, effector cells (primary human NK cells treated with different RNPs) were added to spheroids at multiple effector to target cell ratios with or without 10ng/mL of TGF-beta. Red object intensity was measured every two hours for 6 days on an Incucyte imaging system. 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.

[476] TGFBR2/CISH/TIGIT triple knockout (TKO) NK cells demonstrated superior effector function and attenuated SK-OV-3 and PC-3 tumor spheroid growth (**Figs. 12A-12B**). These effector functions were statistically greater than control NK cells. These results demonstrate efficient editing of primary human NK cells at three unique targets simultaneously with CRISPR-Cpf1. Together, the increased effector function of CISH/TGFBR2/TIGIT TKO primary human NK cells *in vitro* and relative to unedited NK cells indicates an enhanced effect of the CISH/TGFBR2/TIGIT TKO.

CLAIMS

1. A modified lymphocyte, wherein the modified lymphocyte:

(a) does not express endogenous CD3, CD4, and/or CD8; and

(b) expresses at least one endogenous gene encoding:

(i) CD56 (NCAM), CD49, and/or CD45;

(ii) NK cell receptor immunoglobulin gamma Fc region receptor III (FcγRIII, cluster of differentiation 16 (CD16));

(iii) natural killer group-2 member D (NKG2D);

(iv) CD69;

(v) a natural cytotoxicity receptor;

or any combination of two or more thereof;

wherein the modified lymphocyte further:

(1) comprises at least one exogenous nucleic acid expression construct comprising a nucleic acid sequence encoding:

(i) a chimeric antigen receptor (CAR);

(ii) a non-naturally occurring variant of FcγRIII (CD16);

(iii) interleukin 15 (IL-15);

(iv) IL-15 receptor (IL-15R), or a variant thereof;

(v) interleukin 12 (IL-12);

(vi) IL-12 receptor (IL-12R), or a variant thereof;

(vii) human leukocyte antigen G (HLA-G);

(viii) human leukocyte antigen E (HLA-E);

(ix) leukocyte surface antigen cluster of differentiation CD47 (CD47);

or any combination of two or more thereof;

and/or

(2) exhibits a loss of function of at least one of:

(i) transforming growth factor beta receptor 2 (TGFβR2);

(ii) adenosine A2a receptor (ADORA2A);

(iii) T cell immunoreceptor with Ig and ITIM domains (TIGIT);

(iv) β-2 microgobulin (B2M);

(v) programmed cell death protein 1 (PD-1);

(vi) cytokine inducible SH2 containing protein (CISH);

(vii) class II, major histocompatibility complex, transactivator (CIITA);

(viii) natural killer cell receptor NKG2A (natural killer group 2A);

(ix) two or more HLA class II histocompatibility antigen alpha chain genes, and/or two or more HLA class II histocompatibility antigen beta chain genes;

(x) cluster of differentiation 32B (CD32B, FCGR2B);

(xi) T cell receptor alpha constant (TRAC);

or any combination of two or more thereof.

2. The modified lymphocyte of claim 1, wherein the lymphocyte exhibits a loss of function of :

(i) TGFβR2, CISH, TIGIT, ADORA2A, or NKG2A;

(ii) TGFβR2 and CISH, TGFβR2 and TIGIT, TGFβR2 and ADORA2A, TGFβR2 and NKG2A, CISH and TIGIT, CISH and ADORA2A, CISH and NKG2A, TIGIT and ADORA2A, TIGIT and NKG2A, or ADORA2A and NKG2A; or

(iii) TGFβR2, CISH and TIGIT; TGFβR2, CISH and ADORA2A; TGFβR2, CISH and NKG2A; TGFβR2, TIGIT and ADORA2A; TGFβR2, TIGIT and NKG2A; TGFβR2, ADORA2A and NKG2A; CISH, TIGIT and ADORA2A; CISH, TIGIT and NKG2A; CISH, ADORA2A and NKG2A; or TIGIT, ADORA2A and NKG2A.

3. The modified lymphocyte of claim 1 or 2, wherein the lymphocyte comprises a rearranged endogenous T-cell receptor (TCR) locus.

4. The modified lymphocyte of claim 3, wherein the rearranged TCR comprises TCRα VJ and/or TCRβ V(D)J section rearrangements and complete V-domain exons.

5. The modified lymphocyte of claim 1, 2, 3 or 4 wherein the natural cytotoxicity receptor is NKp30, NKp44, NKp46, and/or CD158b.

6. The modified lymphocyte of any of claims 1-5, wherein the IL-15R variant is a constitutively active IL-15R variant and/or wherein the IL12-R variant is a constitutively active IL12-R variant.

7. The modified lymphocyte of claim 6, wherein the constitutively active IL-15R variant is a fusion between IL-15R and an IL-15R agonist (IL-15RA), and/or wherein the constitutively active IL-12R variant is a fusion between IL-12R and an IL-12R agonist (IL-12RA).

8. The modified lymphocyte of claim 7, wherein the IL-15R agonist is IL-15, or an IL-15R-binding variant thereof; and/or wherein the IL-12R agonist is IL-12, or an IL-12R-binding variant thereof.

9. The modified lymphocyte of any one of claims 1-8, wherein the loss of TGFβR2 is associated with exogenous expression of a dominant-negative variant of TGFβ receptor II (DN-TGFβR2).

10. The modified lymphocyte of claim 1, wherein the CAR is capable of binding mesothelin, EGFR, HER2, MICA/B, BCMA, CD19, CD22, CD20, CD33, CD123, androgen receptor, PSMA, PSCA, Muc1, HPV viral peptides (ie. E7), EBV viral peptides, CD70, WT1, CEA, EGFRvIII, IL13Rα2, and GD2, CA125, CD7, EpCAM, Muc16, or CD30,

11. The modified lymphocyte of any one of claims 1-10, wherein the lymphocyte is derived from a pluripotent or multipotent stem cell.

12. The modified lymphocyte of claim 11, wherein the multipotent stem cell is a hematopoietic stem cell (HSC).

13. The modified lymphocyte of claim 11, wherein the pluripotent stem cell is an induced pluripotent stem cell (iPSC).

14. The modified lymphocyte of claim 11, wherein the pluripotent stem cell is an embryonic stem cell (ESC).

15. The modified lymphocyte of any one of claims 1-11, wherein the lymphocyte is derived from a pluripotent or multipotent stem cell that comprises at least one or more

exogenous nucleic acid constructs encoding any of (1)(i)-(1)(xi), or any combination thereof; and/or at least one genomic alteration that effects the loss-of-function of any of (2)(i)-(2)(xi), or any combination thereof, in the lymphocyte.

16. The modified lymphocyte of claim 15, wherein the lymphocyte is derived from a pluripotent or multipotent stem cell that comprises at least one genomic alteration that effects the loss-of-function of any of (2)(i)-(2)(xi), or any combination thereof, in the lymphocyte.

17. The modified lymphocyte of claim 15 or claim 16, wherein the at least one genomic alteration that effects the loss-of-function of one or more (2)(i)-(2)(xi) in the lymphocyte comprises an insertion of an exogenous nucleic acid construct.

18. The modified lymphocyte of claim 17, wherein the exogenous nucleic acid construct encodes any of (1)(i)-(1)(ix), or any combination thereof.

19. The modified lymphocyte of any one of claims 1-18, wherein the lymphocyte exhibits a loss-of-function in two or more of the genes/proteins listed under (2).

20. The modified lymphocyte of any one of claims 1-19, wherein the lymphocyte comprises an indel or an insertion of an exogenous nucleotide construct into a genomic locus harboring a gene or encoding a protein under (2).

21. The modified lymphocyte of any one of claims 1-20, wherein the lymphocyte comprises an indel or an insertion of an exogenous nucleotide construct into two or more genomic loci harboring a gene or encoding a protein under (2).

22. The modified lymphocyte of any one of claims 1-21, wherein the lymphocyte is obtained by editing a genomic locus with an RNA-guided nuclease.

23. The modified lymphocyte of claim 22, wherein the RNA-guided nuclease is a CRISPR/Cas nuclease.

24. The modified lymphocyte of claim 22, wherein the RNA-guided nuclease is selected from the group consisting of SpCas9, SaCas9, (KKH) SaCas9, AsCpf1 (AsCas12a), LbCpf1,

(LbCas12a), CasX, CasY, Cas12h1, Cas12i1, Cas12c1, Cas12c2, eSpCas9, Cas9-HF1, HypaCas9, dCas9-Fokl, Sniper-Cas9, xCas9, AaCas12b, evoCas9, SpCas9-NG, VRQR, VRER, NmeCas9, CjCas9, BhCas12b, and BhCas12b V4.

25. The modified lymphocyte of any one of claims 1-24, wherein the lymphocyte is obtained by editing two or more genomic loci harboring genes encoding any of the proteins under (2).

26. The modified lymphocyte of claim 25, wherein at least two of the two or more genomic loci harboring genes encoding any of the proteins under (2) have been edited by a different RNA-guided nuclease.

27. The modified lymphocyte of claim 25, wherein at least one of the two or more genomic loci harboring genes encoding any of the proteins under (2) has been edited by Cas9, and wherein at least one of the loci has been edited by Cpf1.

28. The modified lymphocyte of any one of claims 1-27, wherein the modified lymphocyte expresses endogenous CD56, CD49, and CD45.

29. The modified lymphocyte of any one of claims 1-28, wherein the lymphocyte is a natural killer (NK) cell.

30. A modified cell, wherein the modified cell

(1) comprises at least one exogenous nucleic acid expression construct comprising a nucleic acid sequence encoding:

(i) a chimeric antigen receptor (CAR);

(ii) a non-naturally occurring variant of FcγRIII (CD16);

(iii) interleukin 15 (IL-15);

(iv) IL-15 receptor (IL-15R), or a variant thereof;

(v) interleukin 12 (IL-12);

(vi) IL-12 receptor (IL-12R), or a variant thereof;

(vii) human leukocyte antigen G (HLA-G);

(viii)human leukocyte antigen E (HLA-E);

(ix) leukocyte surface antigen cluster of differentiation CD47 (CD47);

or any combination of two or more thereof;

and/or

(2) exhibits a loss of function of at least one of:

(i) transforming growth factor beta receptor 2 (TGFβR2);

(ii) adenosine A2a receptor (ADORA2A);

(iii) T cell immunoreceptor with Ig and ITIM domains (TIGIT);

(iv) β-2 microgobulin (B2M);

(v) programmed cell death protein 1 (PD-1);

(vi) cytokine inducible SH2 containing protein (CISH);

(vii) class II, major histocompatibility complex, transactivator (CIITA);

(viii) natural killer cell receptor NKG2A (natural killer group 2A);

(ix) two or more HLA class II histocompatibility antigen alpha chain genes, and/or two or more HLA class II histocompatibility antigen beta chain genes;

(x) cluster of differentiation 32B (CD32B, FCGR2B);

(xi) T cell receptor alpha constant (TRAC);

or any combination of two or more thereof.

31. The modified cell of claim 30, wherein the modified cell exhibits a loss of function of:

(i) TGFβR2, CISH, TIGIT, ADORA2A, or NKG2A;

(ii) TGFβR2 and CISH, TGFβR2 and TIGIT, TGFβR2 and ADORA2A, TGFβR2 and NKG2A, CISH and TIGIT, CISH and ADORA2A, CISH and NKG2A, TIGIT and ADORA2A, TIGIT and NKG2A, or ADORA2A and NKG2A; or

(iii) TGFβR2, CISH and TIGIT; TGFβR2, CISH and ADORA2A; TGFβR2, CISH and NKG2A; TGFβR2, TIGIT and ADORA2A; TGFβR2, TIGIT and NKG2A; TGFβR2, ADORA2A and NKG2A; CISH, TIGIT and ADORA2A; CISH, TIGIT and NKG2A; CISH, ADORA2A and NKG2A; or TIGIT, ADORA2A and NKG2A.

32. The modified cell of claim 30 or 31, wherein the modified cell is an immune cell.

33. The modified cell of claim 32, wherein the immune cell is a lymphocyte.

34. The modified cell of claim 33, wherein the lymphocyte is an NK cell.

35. The modified cell of claim 30, wherein the cell is a pluripotent stem cell, or a differentiated daughter cell derived therefrom.

36. The modified cell of claim 34 or claim 35, wherein the modified cell does not express an endogenous T-cell co-receptor.

37. The modified cell of claim 33, wherein the lymphocyte is a T cell.

38. The modified cell of claim 30, wherein the cell comprises a rearranged endogenous TCR locus, wherein the rearranged TCR comprises TCRα VJ and/or TCRβ V(D)J section rearrangements and complete V-domain exons.

39. The modified cell of any one of claims 30-38, wherein the modified cell expresses at least one endogenous gene encoding:

(i) CD56 (NCAM), CD49, and/or CD45;

(ii) FcγRIII (CD16);

(iii) natural killer group-2 member D (NKG2D);

(iv) CD69;

(v) a natural cytotoxicity receptor;

or any combination of two or more thereof.

40. The modified cell of claim 39, wherein the natural cytotoxicity receptor is NKp30, NKp44, NKp46, and/or CD158b.

41. The modified cell of any one of claims 30-40, wherein the cell expresses at least one NK cell biomarker.

42. The modified cell of claim 41, wherein the NK cell biomarker is CD56, CD49, and/or CD45.

43. A population of cells comprising the modified lymphocyte of any one of claims 1-28, or the modified cell of any one of claims 30-42.

44. A pharmaceutical composition comprising the population of cells of claim 43.

45. An isolated population of lymphocytes, wherein the population comprises at least $1x10^3$, at least $1x10^4$, at least $1x10^5$, at least $2x10^5$, at least $3x10^5$, at least $4x10^5$, at least $5x10^5$,at least $1x10^6$, at least $2x10^6$, at least $3x10^6$, at least $4x10^6$, at least $5x10^6$,at least $1x10^7$, at least $1x10^7$, at least $2x10^7$, at least $3x10^7$, at least $4x10^7$, at least $5x10^7$,at least $1x10^8$, at least $2x10^8$, at least $3x10^8$, at least $4x10^8$, at least $5x10^8$,at least $1x10^9$, at least $1x10^9$, at least $2x10^9$, at least $3x10^9$, at least $4x10^9$, at least $5x10^9$,at least $1x10^{10}$, at least $2x10^{10}$, at least $3x10^{10}$, at least $4x10^{10}$, at least $5x10^{10}$, at least $1x10^{11}$, or at least $1x10^{12}$ cells, and wherein at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.9%, at least 99.99%, at least 99.999%, or virtually 100% of the lymphocytes in the population:

(a) comprise a rearranged T-cell receptor (TCR) locus;

(b) do not express endogenous CD3;

(c) express endogenous CD56 (NCAM), CD49, and/or CD45; and

(d) expresses at least one endogenous gene encoding:

(i) FcγRIII (CD16);

(ii) natural killer group-2 member D (NKG2D);

(iii) CD69;

(iv) a natural cytotoxicity receptor;

or any combination of two or more thereof; and

wherein the modified lymphocyte further:

(1) comprises at least one exogenous nucleic acid expression construct comprising a nucleic acid sequence encoding:

(i) chimeric antigen receptor (CAR);

(ii) non-naturally occurring variant of immunoglobulin gamma Fc region receptor III (FcγRIII, CD16);

(iii) interleukin 15 (IL-15);

(iv) IL-15 receptor (IL-15R), or a variant thereof;

(v) interleukin 12 (IL-12);

(vi) IL-12 receptor (IL-12R), or a variant thereof;

(vii) human leukocyte antigen G (HLA-G);

(viii) human leukocyte antigen E (HLA-E);

(ix) leukocyte surface antigen cluster of differentiation CD47 (CD47);

or any combination of two or more thereof;

and/or

(2) exhibits a loss of function of at least one of:

(i) transforming growth factor beta receptor 2 (TGFβR2);

(ii) adenosine A2a receptor (ADORA2A);

(iii) T cell immunoreceptor with Ig and ITIM domains (TIGIT);

(iv) β-2 microgobulin (B2M);

(v) programmed cell death protein 1 (PD-1);

(vi) cytokine inducible SH2 containing protein (CISH);

(vii) class II, major histocompatibility complex, transactivator (CIITA);

(viii) natural killer cell receptor NKG2A (natural killer group 2A);

(ix) two or more HLA class II histocompatibility antigen alpha chain genes, and/or two or more HLA class II histocompatibility antigen beta chain genes;

(x) cluster of differentiation 32B (CD32B, FCGR2B);

(xi) T cell receptor alpha constant (TRAC);

or any combination of two or more thereof.

46. The isolated population of lymphocytes of claim 45, wherein the modified lymphocyte exhibits a loss of function of :

(i) TGFβR2, CISH, TIGIT, ADORA2A, or NKG2A;

(ii) TGFβR2 and CISH, TGFβR2 and TIGIT, TGFβR2 and ADORA2A, TGFβR2 and NKG2A, CISH and TIGIT, CISH and ADORA2A, CISH and NKG2A, TIGIT and ADORA2A, TIGIT and NKG2A, or ADORA2A and NKG2A; or

(iii) TGFβR2, CISH and TIGIT; TGFβR2, CISH and ADORA2A; TGFβR2, CISH and NKG2A; TGFβR2, TIGIT and ADORA2A; TGFβR2, TIGIT and NKG2A; TGFβR2, ADORA2A and NKG2A; CISH, TIGIT and ADORA2A; CISH, TIGIT and NKG2A; CISH, ADORA2A and NKG2A; or TIGIT, ADORA2A and NKG2A.

47. The isolated population of lymphocytes of claim 45 or 46, wherein the rearranged TCR locus comprises of TCRα VJ and/or TCRβ V(D)J section rearrangements and complete V-domain exons.

48. The isolated *in vitro* population of lymphocytes of claim 47, wherein the rearranged endogenous TCR locus consists of no more than two rearranged alleles.

49. The isolated *in vitro* population of lymphocytes of claim 45, wherein the natural cytotoxicity receptor is NKp30, NKp44, NKp46, and/or CD158b.

50. The isolated *in vitro* population of lymphocytes of any one of claims 45-49, wherein the population does not comprise more than 1%, more than 0.1%, more than 0.001%, more than 0.0001%, more than 0.00001%, more than 0.000001%, more than 0.0000001%, more than 0.00000001%, more than 0.000000001%, more than 0.0000000001%, or more than more than 0.00000000001% of cells expressing a reprogramming factor from an exogenous nucleic acid construct.

51. The isolated *in vitro* population of lymphocytes of claim 50, wherein the population does not comprise a cell expressing a reprogramming factor from an exogenous nucleic acid construct.

52. The isolated *in vitro* population of lymphocytes of claim 50 or claim 51, wherein the reprogramming factor is Oct-4 and/or Sox-2.

53. The isolated *in vitro* population of lymphocytes of claim 45, wherein the population does not comprise cells harboring episomal expression constructs encoding a reprogramming factor.

54. The isolated *in vitro* population of lymphocytes of any one of claims 45-53, wherein each cell in the population of cells comprises the same combination of (1) and (2).

55. The isolated *in vitro* population of lymphocytes of any one of claims 45-54, wherein the cell population comprises less than 0.001%, less than 0.002%, less than 0.003%, less than

0.004%, less than 0.005%, less than 0.006%, less than 0.007%, less than 0.008%, less than 0.009%, less than 0.01%, less than 0.02%, less than 0.03%, less than 0.04%, less than 0.05%, less than 0.06%, less than 0.07%, less than 0.08%, less than 0.09%, less than 0.1%, less than 0.2%, less than 0.3%, less than 0.4%, less than 0.5%, less than 0.6%, less than 0.7%, less than 0.8%, less than 0.9%, less than 1%, less than 2%, less than 3%, less than 4%, less than 5%, less than 6%, less than 7%, less than 8%, less than 9%, or less than 10% cell that harbor a chromosomal translocation.

56. The lymphocyte of any one of claims 1-29, the modified cell of any one of claims 30-42, the population of cells of claim 41, the pharmaceutical composition of claim 44, or the isolated *in vitro* population of lymphocytes of any one of claims 45-55, for use in therapy.

57. Use of the lymphocyte of any one of claims 1-29, the modified cell of any one of claims 30-42, the population of cells of claim 41, the pharmaceutical composition of claim 44, or the isolated *in vitro* population of lymphocytes of any one of claims 45-55, in the manufacture of a medicament for treating a proliferative disease.

58. The use of claim 57, wherein the proliferative disease is cancer.

59. The use of claim 58, wherein the cancer is breast cancer, colorectal cancer, gastric cancer, renal cell carcinoma (RCC), or non-small cell lung cancer (NSCLC), solid tumors, 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, soft tissue sarcomas, and hematological cancers like ALL, CLL, NHL, DLBCL, AML, CML, multiple myeloma (MM).

60. A method of generating the lymphocyte of any one of claims 1-29, the modified cell of any one of claims 30-42, the population of cells of claim 43, or the isolated *in vitro* population of lymphocytes of any one of claims 44-54, the method comprising:

(a) obtaining an induced pluripotent stem cell (iPSC);

(b) modifying the iPSC, or an undifferentiated or differentiated daughter cell thereof, to comprise express at least one exogenous nucleic acid expression construct of (1) and/or to comprise a loss of function in at least one gene of (2);

(c) directing differentiation of the iPSC to hematopoietic lineage cells,

wherein the hematopoietic lineage cells retain the edited genetic loci comprised in the iPSCs.

61. The method of claim 60, wherein directing differentiation comprises:

(i) contacting iPSCs with a composition comprising a BMP pathway activator, and optionally bFGF, to obtain mesodermal cells; and

(ii) contacting the mesodermal cells with a composition comprising a BMP pathway activator, bFGF, and a WNT pathway activator, to obtain mesodermal cells having definitive hemogenic endothelium (HE) potential, wherein the mesodermal cells having definitive hemogenic endothelium (HE) potential are capable of providing hematopoietic lineage cells;

wherein mesodermal cells and mesodermal cells having definitive HE potential are obtained in steps (i) and (ii) without the step of forming embryoid bodies;

wherein the hematopoietic lineage cells comprise definitive hemogenic endothelium cells, hematopoietic stem and progenitor cells (HSC), hematopoietic multipotent progenitor cell (MPP), pre-T cell progenitor cells, pre-NK cell progenitor cells, T cell progenitor cells, NK cell progenitor cells, T cells, NK cells, NKT cells, or B cells.

62. The method of claim 61, wherein directing differentiation of iPSCs to hematopoietic lineage cells further comprises: contacting the mesodermal cells having definitive HE potential with a composition comprising bFGF and a ROCK inhibitor to obtain definitive HE cells.

63. The method of claim 60, claim 61, or claim 62, further comprising: contacting the definitive HE cells with a composition comprising a BMP activator, and optionally a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6, Flt3L and IL11 to obtain hematopoietic multipotent progenitor cells (MPP).

64. The method of any one of claims 60-63, further comprising: contacting the definitive HE cells with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, and IL7; and optionally one or more of a BMP activator, a ROCK inhibitor, TPO, VEGF and bFGF to obtain pre-T cell progenitors, T cell progenitors, and/or T cells.

65. The method of any one of claims 60-63, further comprising: contacting the definitive HE cells with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, TPO, IL7 and IL15, and optionally one or more of a BMP activator, a ROCK inhibitor, VEGF and bFGF to obtain pre-NK cell progenitors, NK cell progenitors, and/or NK cells.

66. The method of any one of claims 60-65, further comprising: prior to step c), contacting the pluripotent stem cells with a composition comprising a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, to seed and expand the cells.

67. The method of any one of claims 60-65, further comprising detecting a rearranged T-cell receptor (TCR) locus in the hematopoietic lineage cells.

68. The method of claim 67, further comprising selecting the hematopoietic lineage cells comprising the rearranged TCR locus based on the TCR encoded by the rearranged TCR locus binding an antigen of interest.

69. The method of claim 68, wherein the antigen of interest is a tumor antigen.

70. A method, the method comprising:
reprogramming a donor cell to a pluripotent state;
editing a target locus in the donor cell genome; and
differentiating the reprogrammed donor cell into a lymphocyte.

71. The method of claim 70, wherein the editing is performed before or during the step of reprogramming of the donor cell to a pluripotent state.

72. The method of claim 70 or 71, wherein the donor cell is a fibroblast, a peripheral blood cell, a lymphocyte, or a T cell.

73. A method, the method comprising:
differentiating a genetically modified pluripotent stem cell into a lymphocyte, wherein the genetically modified pluripotent stem cell comprises:

(1) an exogenous nucleic acid expression construct comprising:

(i) a nucleic acid sequence encoding a chimeric antigen receptor (CAR);

(ii) a nucleic acid sequence encoding a non-naturally occurring variant of FcγRIII (CD16);

(iii) a nucleic acid sequence encoding interleukin 15 (IL-15);

(iv) a nucleic acid sequence encoding interleukin 15 receptor (IL-15R) or a variant thereof;

(v) a nucleic acid sequence encoding interleukin 12 (IL12);

(vi) a nucleic acid sequence encoding interleukin-12 receptor (IL-12R) or a variant thereof;

(vii) a nucleic acid sequence encoding human leukocyte antigen G (HLA-G);

(viii) a nucleic acid sequence encoding human leukocyte antigen E (HLA-E);

(ix) a nucleic acid sequence encoding leukocyte surface antigen cluster of differentiation CD47 (CD47);

or any combination of two or more thereof; and

(2) an indel, or an insertion of an exogenous nucleic acid in one or more of the following genetic loci:

(i) transforming growth factor beta receptor 2 (TGFβR2);

(ii) adenosine A2a receptor (ADORA2A);

(iii) T cell immunoreceptor with Ig and ITIM domains (TIGIT);

(iv) β-2 microgobulin (B2M);

(v) programmed cell death protein 1 (PD-1, CD279);

(vi) cytokine inducible SH2 containing protein (CISH);

(vii) class II, major histocompatibility complex, transactivator (CIITA);

(viii) natural killer cell receptor NKG2A (natural killer group 2A);

(ix) two or more HLA class II histocompatibility antigen alpha chain genes, and/or two or more HLA class II histocompatibility antigen beta chain genes;

(x) cluster of differentiation 32B (CD32B, FCGR2B);

(xi) T cell receptor alpha constant (TRAC);

or any combination of two or more thereof,

wherein the indel or insertion results in a loss-of-function of a gene product encoded by the respective genetic locus or loci.

74. The method of claim 73, wherein the indel, or the insertion of an exogenous nucleic acid is in the following genetic loci :

(i) TGFβR2, CISH, TIGIT, ADORA2A, or NKG2A;

(ii) TGFβR2 and CISH, TGFβR2 and TIGIT, TGFβR2 and ADORA2A, TGFβR2 and NKG2A, CISH and TIGIT, CISH and ADORA2A, CISH and NKG2A, TIGIT and ADORA2A, TIGIT and NKG2A, or ADORA2A and NKG2A; or

(iii) TGFβR2, CISH and TIGIT; TGFβR2, CISH and ADORA2A; TGFβR2, CISH and NKG2A; TGFβR2, TIGIT and ADORA2A; TGFβR2, TIGIT and NKG2A; TGFβR2, ADORA2A and NKG2A; CISH, TIGIT and ADORA2A; CISH, TIGIT and NKG2A; CISH, ADORA2A and NKG2A; or TIGIT, ADORA2A and NKG2A,

wherein the indel or insertion results in a loss-of-function of a gene product encoded by the respective genetic locus or loci.

75. The method of claim 73 or claim 74, wherein the exogenous nucleic acid of (2) is the exogenous nucleic acid of (1).

76. The method of claim 73 or claim 75, wherein the pluripotent stem cell is an iPS cell.

77. The method of claim 73 or claim 75, wherein the differentiating comprises contacting the pluripotent stem cell with a differentiation medium or a sequence of differentiation media.

78. The modified lymphocyte of any preceding claim, wherein the two or more HLA class II histocompatibility antigen alpha chain genes are selected from HLA-DQA1, HLA-DRA, HLA-DPA1, HLA-DMA, HLA-DQA2, and HLA-DOA.

79. The modified lymphocyte of any preceding claim, wherein the two or more HLA class II histocompatibility antigen beta chain genes are selected from HLA-DMB, HLA-DOB, HLA-DPB1, HLA-DQB1, HLA-DQB3, HLA-DQB2, HLA-DRB1, HLA-DRB3, HLA-DRB4, and HLA-DRB5.

80. The modified lymphocyte of any one of claims 1-29 and 78-79, the modified cell of any one of claims 30-42, the population of cells of claim 43, the pharmaceutical composition of claim 44, the isolated *in vitro* population of lymphocytes of any one of claims 45-55, or the method of any one of claims 56-77, wherein the exogenous nucleic acid expression construct

comprises the encoding nucleic acid sequence listed under (1) under the control of a heterologous promoter.

81. The modified lymphocyte, modified cell, population of cells, pharmaceutical composition, isolated *in vitro* population of lymphocytes, or method of claim 80, wherein the heterologous promoter is an NK-cell specific promoter.

82. The modified lymphocyte, modified cell, population of cells, pharmaceutical composition, isolated *in vitro* population of lymphocytes, or method of claim 80, wherein the NK-cell specific promoter is a promoter of a gene known to be expressed specifically in NK cells, or a variant thereof.

83. The modified lymphocyte, modified cell, population of cells, pharmaceutical composition, isolated *in vitro* population of lymphocytes, or method of claim 80, wherein the NK-cell specific promoter is a CD56 (NCAM), CD49, or CD45 promoter, or a variant thereof.

84. The modified lymphocyte, modified cell, population of cells, pharmaceutical composition, isolated in vitro population of lymphocytes, or method of claim 80, wherein the NK-cell specific promoter is an FcγRIII promoter, an NKG2D promoter, a CD69 promoter, or a natural cytotoxicity receptor promoter, or a variant thereof.

85. The modified lymphocyte of any one of the claims 78-80, the modified cell of any one of the claims 80-84, or the population of cells of any one of the claims 80-84 for use in therapy.

86. Use of the modified lymphocyte of any one of the claims 78-80, the modified cell of any one of the claims 80-84, or the population of cells of any one of the claims 80-84 in the manufacture of a medicament for treating a proliferative disease.

87. The use of claim 86, wherein the proliferative disease is cancer.

88. The use of claim 87, wherein the cancer is breast cancer, colorectal cancer, gastric cancer, renal cell carcinoma (RCC), or non-small cell lung cancer (NSCLC), solid tumors, 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, soft tissue sarcomas, and hematological cancers like ALL, CLL, NHL, DLBCL, AML, CML, multiple myeloma (MM).