

COMPOSITIONS AND METHODS FOR GENETICALLY MODIFYING CIITA IN A CELL

[0001] This application claims the benefit under 35 U.S.C. 119(e) of US Provisional Application No. 63/130,098, filed December 23, 2020, US Provisional Application No. 63/251,002, filed September 30, 2021, US Provisional Application No. 63/254,971, filed October 12, 2021, and US Provisional Application No. 63/288,502, filed December 10, 2021; each of which disclosures is herein incorporated by reference in its entirety.

[0002] This application is filed with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled “2021-12-20_01155-0038-00PCT_Seq_List_ST25.txt” created on December 20, 2021, which is 410,044 bytes in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

INTRODUCTION AND SUMMARY

[0003] The ability to downregulate MHC class II is critical for many *in vivo* and *ex vivo* utilities, *e.g.*, when using allogeneic cells (originating from a donor) for transplantation and/or *e.g.*, for creating a cell population *in vitro* that does not activate T cells. In particular, the transfer of allogeneic cells into a subject is of great interest to the field of cell therapy. The use of allogeneic cells has been limited due to the problem of rejection by the recipient subject's immune cells, which recognize the transplanted cells as foreign and mount an attack. To avoid the problem of immune rejection, cell-based therapies have focused on autologous approaches that use a subject's own cells as the cell source for therapy, an approach that is time-consuming and costly.

[0004] Typically, immune rejection of allogeneic cells results from a mismatching of major histocompatibility complex (MHC) molecules between the donor and recipient. Within the human population, MHC molecules exist in various forms, including *e.g.*, numerous genetic variants of any given MHC gene, *i.e.*, alleles, encoding different forms of MHC protein. The primary classes of MHC molecules are referred to as MHC class I and MHC class II. MHC class I molecules (*e.g.*, HLA-A, HLA-B, and HLA-C in humans) are expressed on all nucleated cells and present antigens to activate cytotoxic T cells (CD8⁺ T cells or CTLs). MHC class II molecules (*e.g.*, HLA-DP, HLA-DQ, and HLA-DR in humans) are expressed on only certain cell types (*e.g.*, B cells, dendritic cells, and macrophages) and present antigens to activate helper T cells (CD4⁺ T cells or Th cells), which in turn provide signals to B cells to produce antibodies.

[0005] Slight differences, *e.g.*, in MHC alleles between individuals can cause the T cells in a recipient to become activated. During T cell development, an individual's T cell repertoire is tolerized to one's own MHC molecules, but T cells that recognize another individual's MHC molecules may persist in circulation and are referred to as alloreactive T cells. Alloreactive T cells can become activated *e.g.*, by the presence of another individual's cells expressing MHC molecules in the body, causing *e.g.*, graft versus host disease and transplant rejection.

[0006] Methods and compositions for reducing the susceptibility of an allogeneic cell to rejection are of interest, including *e.g.*, reducing the cell's expression of MHC protein to avoid recipient T cell responses. In practice, the ability to genetically modify an allogeneic cell for transplantation into a subject has been hampered by the requirement for multiple gene edits to reduce all MHC protein expression, while at the same time, avoiding other harmful recipient immune responses. For example, while strategies to deplete MHC class I protein may reduce activation of CTLs, cells that lack MHC class I on their surface are susceptible to lysis by natural killer (NK) cells of the immune system because NK cell activation is regulated by MHC class I-specific inhibitory receptors. Gene editing strategies to deplete MHC class II molecules have also proven difficult particularly in certain cell types for reasons including low editing efficiencies and low cell survival rates, preventing practical application as a cell therapy.

[0007] Thus, there exists a need for improved methods and compositions for modifying allogeneic cells to overcome the problem of recipient immune rejection and the technical difficulties associated with the multiple genetic modifications required to produce a safer cell for transplant.

[0008] The present disclosure provides engineered cells with reduced or eliminated surface expression of MHC class II. The engineered cell comprises a genetic modification in the CIITA gene (class II major histocompatibility complex transactivator), which may be useful in cell therapy. The disclosure further provides compositions and methods to reduce or eliminate surface expression of MHC class II protein in a cell by genetically modifying the CIITA gene. The CIITA protein functions as a transcriptional activator (activating the MHC class II promoter) and is essential for MHC class II protein expression.

[0009] In some embodiments, the disclosure further provides compositions and methods to reduce or eliminate surface expression of MHC class I protein in the cell, *e.g.*, by genetically modifying B2M (β -2-microglobulin) or by genetically modifying the HLA-A gene. The B2M protein forms a heterodimer with MHC class I molecules and is required for MHC class I protein expression on the cell surface. In some embodiments comprising a B2M genetic modification, the disclosure further provides expression of an NK cell inhibitor molecule by

the cell to reduce or eliminate the lytic activity of NK cells. In some embodiments, the disclosure further provides compositions and methods to reduce or eliminate surface expression of HLA-A in cells homozygous for HLA-B and homozygous for HLA-C.

[0010] In some embodiments, the methods and compositions further provide for insertion of an exogenous nucleic acid, *e.g.*, encoding a targeting receptor, other polypeptide expressed on the cell surface, or a polypeptide that is secreted from the cell. In some embodiments, the engineered cell is useful as a “cell factory” for secreting an exogenous protein in a recipient. In some embodiments, the engineered cell is useful as an adoptive cell therapy.

[0011] Provided herein is an engineered cell, which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10902662-chr16:10923285.

[0012] Provided herein is an engineered cell, which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from:

chr16:10902682,	chr16:10902723-10902743,	chr16:10902729-10902749,	chr16:10903747-
10903767,	chr16:10903824-10903844,	chr16:10903824-10903844,	chr16:10903848-
10903868,	chr16:10904761-10904781,	chr16:10904764-10904784,	chr16:10904765-
10904785,	chr16:10904785-10904805,	chr16:10906542-10906562,	chr16:10906556-
10906576,	chr16:10906609-10906629,	chr16:10906610-10906630,	chr16:10906616-
10906636,	chr16:10906682-10906702,	chr16:10906756-10906776,	chr16:10906757-
10906777,	chr16:10906757-10906777,	chr16:10906821-10906841,	chr16:10906823-
10906843,	chr16:10906847-10906867,	chr16:10906848-10906868,	chr16:10906853-
10906873,	chr16:10906904-10906924,	chr16:10906907-10906927,	chr16:10906913-
10906933,	chr16:10906968-10906988,	chr16:10906970-10906990,	chr16:10906985-
10907005,	chr16:10907030-10907050,	chr16:10907058-10907078,	chr16:10907119-
10907139,	chr16:10907139-10907159,	chr16:10907172-10907192,	chr16:10907272-
10907292,	chr16:10907288-10907308,	chr16:10907314-10907334,	chr16:10907315-
10907335,	chr16:10907325-10907345,	chr16:10907363-10907383,	chr16:10907384-
10907404,	chr16:10907385-10907405,	chr16:10907433-10907453,	chr16:10907434-
10907454,	chr16:10907435-10907455,	chr16:10907441-10907461,	chr16:10907454-
10907474,	chr16:10907461-10907481,	chr16:10907476-10907496,	chr16:10907539-
10907559,	chr16:10907586-10907606,	chr16:10907589-10907609,	chr16:10907621-

10907641, chr16:10907622-10907642, chr16:10907623-10907643, chr16:10907730-10907750, chr16:10907731-10907751, chr16:10907757-10907777, chr16:10907781-10907801, chr16:10907787-10907807, chr16:10907790-10907810, chr16:10907810-10907830, chr16:10907820-10907840, chr16:10907870-10907890, chr16:10907886-10907906, chr16:10907924-10907944, chr16:10907928-10907948, chr16:10907932-10907952, chr16:10907935-10907955, chr16:10907978-10907998, chr16:10907979-10907999, chr16:10908069-10908089, chr16:10908073-10908093, chr16:10908101-10908121, chr16:10909056-10909076, chr16:10909138-10909158, chr16:10910195-10910215, chr16:10910196-10910216, chr16:10915592-10915612, chr16:10915626-10915646, chr16:10916375-10916395, chr16:10916382-10916402, chr16:10916426-10916446, chr16:10916432-10916452, chr16:10918486-10918506, chr16:10918492-10918512, chr16:10918493-10918513, chr16:10922435-10922455, chr16:10922441-10922461, chr16:10922441-10922461, chr16:10922444-10922464, chr16:10922460-10922480, chr16:10923257-10923277, and chr16:10923265-10923285.

[0013] Provided herein is a method of making an engineered cell, which has reduced or eliminated surface expression of MHC class II protein relative to an unmodified cell, comprising contacting a cell with a composition comprising: (a) a CIITA guide RNA comprising (i) a guide sequence selected from SEQ ID NOs: 1-117; (ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-117; (iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-117; (iv) a sequence that comprises 10 contiguous nucleotides ± 10 nucleotides of a genomic coordinate listed in Table 2; (v) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (iv); or (vi) a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (v); and (b) optionally an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[0014] Provided herein is a method of reducing or eliminating surface expression of MHC class II protein in an engineered cell relative to an unmodified cell, comprising contacting a cell with a composition comprising: (a) a CIITA guide RNA comprising (i) a guide sequence selected from SEQ ID NOs: 1-117; (ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-117; (iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-117; (iv) a sequence that comprises 10 contiguous nucleotides ± 10 nucleotides of a genomic coordinate listed in Table 2; (v) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (iv); or (vi) a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (v); and (b) optionally an

RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[0015] Further embodiments are provided throughout and described in the claims and Figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] FIGS. 1A-B show results of screening CIITA guides for efficacy in editing T cells with Cas9 in two donors following electroporation with RNP. FIG. 1A shows percent editing following CIITA editing in T cells. FIG. 1B shows percent MHC class II negative cells following CIITA editing in T cells.

[0017] FIGS. 2A-B show dose-response results for editing T cells with Cas9 and three individual CIITA guides (G013674, G013675, G013676) formulated in LNP compositions. FIG. 2A shows percent indel editing in total T cells (n=1). FIG. 2B shows the percentage of MHC class II negative T cells following CIITA editing as compared to untreated T cells.

[0018] FIGS. 3A-B show results of a dose-response screen of four CIITA guides (CR002961, CR009217, CR007982, and CR007994) for editing T cells with Cas9. FIG. 3A shows the percent editing in T cells. FIG. 3B shows the percentage of MHC class II negative T cells following CIITA editing.

[0019] FIGS. 4A-B show results for efficiency of three CIITA guides (G016086, G016092, and G016067) for editing T cells with BC22. FIG. 4A shows the percent C-to-T conversion. FIG. 4B shows the percentage of MHC class II negative T cells.

[0020] FIGS. 5A-B show results for three CIITA guides (G013676, G013675, G015535) with insertion of mCherry at the CIITA locus. FIG. 5A shows the percentage of mCherry positive CD4⁺ and CD8⁺ T cells. FIG. 5B shows the percentage of MHC class II negative T cells with and without insertion of mCherry and as compared to untreated T cells.

[0021] FIGS. 6A-B show results for CIITA guide G016086 with Cas9 or BC22. FIG. 6A shows the percent of total reads for indels, C-to-A/G conversion, and C-to-G conversion with increasing concentration of Cas9 mRNA or BC22 mRNA. FIG. 6B shows the percentage of MHC class II negative T cells with increasing concentration of Cas9 mRNA or BC22 mRNA.

[0022] FIGS. 7A-F show results for sequential editing in CD8⁺ T cells. FIG. 7A shows the percentage of HLA-A positive cells. FIG. 7B shows the percentage of MHC class II positive cells. FIG. 7C shows the percentage of WT1 TCR positive CD3⁺, Vb8⁺ cells. FIG. 7D shows the percentage of CD3⁺ cells displaying mis-paired TCRs. FIG. 7E shows the percentage of

CD3+, Vb8- cells displaying only endogenous TCRs. FIG. 7F shows the percentage of CD3+, Vb8+, positive for the WT1 TCR and negative for HLA-A and MHC class II.

[0023] FIGS. 8A-F show results for sequential editing in CD4+ T cells. FIG. 8A shows the percentage of HLA-A positive cells. FIG. 8B shows the percentage of MHC class II positive cells. FIG. 8C shows the percentage of WT1 TCR positive CD3+, Vb8+ cells. FIG. 8D shows the percentage cells displaying mis-paired TCRs. FIG. 8E shows the percentage of CD3+, Vb8- cells displaying only endogenous TCRs. FIG. 8F shows the percentage of CD3+, Vb8+, positive for the WT1 TCR and negative for HLA-A and MHC class II.

[0024] FIGS. 9A-D show the percent indels following sequential editing of T cells for CIITA (FIG. 9A), HLA-A (FIG. 9B), TRBC1 (FIG. 9C), and TRBC2 (FIG. 9D) in T cells.

[0025] FIG. 10 shows resistance to NK-cell mediated killing of HLA-A knockout (HLA-B/C match) T cells versus B2M knockout T cells, optionally including an exogenous HLA-E construct, as percent T cell lysis. HLA-A knockout, HLA-A, CIITA double knockout, B2M knockout, B2M + HLA-E, and wild type cells are compared.

[0026] FIGS. 11A-B show luciferase expression from B2M, CIITA, HLA-A, or double (HLA-A, CIITA) knockout human T cells administered to mice inoculated human natural killer cells. FIG. 11A shows radiance (photons/s/cm²/sr) from luciferase expressing T cells present at the various time points after injection. FIG. 11B shows radiance (photons/s/cm²/sr) from luciferase expressing T cells present in the various mice groups on Day 27.

[0027] FIGS. 12A-B show luciferase expression from B2M and AlloWT1 knockout human T cells administered to mice inoculated with human natural killer cells. FIG. 12A shows total flux (p/s) from luciferase expressing T cells present at the various time points after injection. FIG. 12B shows total flux (p/s) from luciferase expressing T cells present in the various mice groups after 31 days.

[0028] FIGS. 13A-B show the percent normalized proliferation of host CD4 (FIG. 13A) or host CD8 (FIG. 13B) T cells triggered by HLA class I + HLA class II double knockout or HLA-A and HLA class II double knockout engineered autologous or allogeneic T cells.

[0029] FIGS. 14A-F shows a panel of percent CD8+ (FIG. 14A), endogenous TCR+ (FIG. 14B), WT1 TCR+ (FIG. 14C), HLA-A2 knockout (FIG. 14D), HLA-DRDPDQ knockout (FIG. 14E), and % Allo WT1 (FIG. 14F).

[0030] FIG. 15 shows total flux (p/s) from luciferase expressing T cells present at the various time points after injection out to 18 days.

[0031] FIGS. 16A-16B respectively show release of IFN- γ and IL-2 in supernatants from a killing assay containing a co-culture of engineered T cells from the Allo-WT1, Auto-WT1, TCR KO, and Wildtype (WT) groups with target tumor cells.

[0032] FIGS. 17A-17B show CIITA, HLA-A, TRAC, and TRBC editing and WT1 TCR insertion rates in CD8⁺ T cells in three conditions. The percentage of cells expressing relevant cell surface proteins following sequential T cell engineering are shown in FIG. 17A for CD8⁺ T cells. The percent of T cells with all intended edits (insertion of the WT1-TCR, combined with knockout of HLA-A and CIITA) is shown in FIG 17B.

[0033] FIG. 18 shows mean percent editing at the CIITA locus in T cells treated with sgRNA in the 100-mer or 91-mer formats.

[0034] Fig. 19 shows the mean percentage of CD8⁺ T cells that are negative for HLA-DR, DP, DQ surface receptors following treatment with sgRNAs in the 100-mer or 91-mer formats targeting CIITA.

DETAILED DESCRIPTION

[0035] The present disclosure provides engineered cells, as well as methods and compositions for genetically modifying a cell to make an engineered cell and populations of engineered cells, that are useful, for example, for adoptive cell transfer (ACT) therapies. The disclosure provided herein overcomes certain hurdles of prior methods by providing methods and compositions for genetically modifying CIITA to reduce expression of MHC class II protein on the surface of a cell. In some embodiments, the disclosure provides engineered cells with reduced or eliminated surface expression of MHC class II as a result of a genetic modification in the CIITA gene. In some embodiments, the disclosure provides compositions and methods for reducing or eliminating expression of MHC class II protein and compositions and methods to further reduce the cell's susceptibility to immune rejection. For example, in some embodiments, the methods and compositions comprise reducing or eliminating surface expression of MHC class II protein by genetically modifying CIITA, and reducing or eliminating surface expression of MHC class I protein and/or inserting an exogenous nucleic acid encoding an NK cell inhibitor molecule, or a targeting receptor, or other polypeptide (expressed on the cell surface or secreted) into the cell by genetic modification. The engineered cell compositions produced by the methods disclosed herein have desirable properties, including *e.g.*, reduced expression of MHC molecules, reduced immunogenicity *in vitro* and *in vivo*, increased survival, and increased genetic compatibility with greater subjects for transplant.

[0036] The term “about” or “approximately” means an acceptable error for a particular value as determined by one of ordinary skill in the art, which depends in part on how the value is measured or determined, or a degree of variation that does not substantially affect the properties of the described subject matter, or within the tolerances accepted in the art, *e.g.*, within 10%, 5%, 2%, or 1%. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

I. Definitions

[0037] Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings:

[0038] The term “or combinations thereof” as used herein refers to all permutations and combinations of the listed terms preceding the term. For example, “A, B, C, or combinations thereof” is intended to include at least one of: A, B, C, AB, AC, BC, or ABC, and if order is important in a particular context, also BA, CA, CB, ACB, CBA, BCA, BAC, or CAB. Continuing with this example, expressly included are combinations that contain repeats of one or more item or term, such as BB, AAA, AAB, BBC, CBBA, CABA, and so forth. The skilled artisan will understand that typically there is no limit on the number of items or terms in any combination, unless otherwise apparent from the context.

[0039] As used herein, the term “kit” refers to a packaged set of related components, such as one or more polynucleotides or compositions and one or more related materials such as delivery devices (*e.g.*, syringes), solvents, solutions, buffers, instructions, or desiccants.

[0040] An “allogeneic” cell, as used herein, refers to a cell originating from a donor subject of the same species as a recipient subject, wherein the donor subject and recipient subject have genetic dissimilarity, *e.g.*, genes at one or more loci that are not identical. Thus, *e.g.*, a cell is allogeneic with respect to the subject to be administered the cell. As used herein, a cell that is removed or isolated from a donor, that will not be re-introduced into the original donor, is considered an allogeneic cell.

[0041] An “autologous” cell, as used herein, refers to a cell derived from the same subject to whom the material will later be re-introduced. Thus, *e.g.*, a cell is considered autologous if it is removed from a subject and it will then be re-introduced into the same subject.

[0042] “ β 2M” or “B2M,” as used herein, refers to nucleic acid sequence or protein sequence of “ β -2 microglobulin”; the human gene has accession number NC_000015 (range 44711492..44718877), reference GRCh38.p13. The B2M protein is associated with MHC class I molecules as a heterodimer on the surface of nucleated cells and is required for MHC class I protein expression.

[0043] “*CIITA*” or “CIITA” or “C2TA,” as used herein, refers to the nucleic acid sequence or protein sequence of “class II major histocompatibility complex transactivator;” the human gene has accession number NC_000016.10 (range 10866208..10941562), reference GRCh38.p13. The CIITA protein in the nucleus acts as a positive regulator of MHC class II gene transcription and is required for MHC class II protein expression.

[0044] As used herein, “MHC” or “MHC molecule(s)” or “MHC protein” or “MHC complex(es),” refers to a major histocompatibility complex molecule (or plural), and includes *e.g.*, MHC class I and MHC class II molecules. In humans, MHC molecules are referred to as “human leukocyte antigen” complexes or “HLA molecules” or “HLA protein.” The use of terms “MHC” and “HLA” are not meant to be limiting; as used herein, the term “MHC” may be used to refer to human MHC molecules, *i.e.*, HLA molecules. Therefore, the terms “MHC” and “HLA” are used interchangeably herein.

[0045] The term “HLA-A,” as used herein in the context of HLA-A protein, refers to the MHC class I protein molecule, which is a heterodimer consisting of a heavy chain (encoded by the HLA-A gene) and a light chain (*i.e.*, beta-2 microglobulin). The term “HLA-A” or “HLA-A gene,” as used herein in the context of nucleic acids refers to the gene encoding the heavy chain of the HLA-A protein molecule. The HLA-A gene is also referred to as “HLA class I histocompatibility, A alpha chain;” the human gene has accession number NC_000006.12 (29942532..29945870). The HLA-A gene is known to have thousands of different genotypic versions of the HLA-A gene across the population (and an individual may receive two different alleles of the HLA-A gene). A public database for HLA-A alleles, including sequence information, may be accessed at IPD-IMGT/HLA: <https://www.ebi.ac.uk/ipd/imgt/hla/>. All alleles of HLA-A are encompassed by the terms “HLA-A” and “HLA-A gene.”

[0046] “HLA-B” as used herein in the context of nucleic acids refers to the gene encoding the heavy chain of the HLA-B protein molecule. The HLA-B is also referred to as “HLA class I histocompatibility, B alpha chain;” the human gene has accession number NC_000006.12 (31353875..31357179).

[0047] “HLA-C” as used herein in the context of nucleic acids refers to the gene encoding the heavy chain of the HLA-C protein molecule. The HLA-C is also referred to as “HLA class

I histocompatibility, C alpha chain;” the human gene has accession number NC_000006.12 (31268749..31272092).

[0048] As used herein, the term “within the genomic coordinates” includes the boundaries of the genomic coordinate range given. For example, if chr6:29942854- chr6:29942913 is given, the coordinates chr6:29942854- chr6:29942913 are encompassed. Throughout this application, the referenced genomic coordinates are based on genomic annotations in the GRCh38 (also referred to as hg38) assembly of the human genome from the Genome Reference Consortium, available at the National Center for Biotechnology Information website. Tools and methods for converting genomic coordinates between one assembly and another are known in the art and can be used to convert the genomic coordinates provided herein to the corresponding coordinates in another assembly of the human genome, including conversion to an earlier assembly generated by the same institution or using the same algorithm (e.g., from GRCh38 to GRCh37), and conversion of an assembly generated by a different institution or algorithm (e.g., from GRCh38 to NCBI33, generated by the International Human Genome Sequencing Consortium). Available methods and tools known in the art include, but are not limited to, NCBI Genome Remapping Service, available at the National Center for Biotechnology Information website, UCSC LiftOver, available at the UCSC Genome Browser website, and Assembly Converter, available at the Ensembl.org website.

[0049] An “exon,” as used herein, refers to the nucleic acids within a gene that encode the mature RNA transcript. In the case of the CIITA gene, the genomic coordinates for the start and end of each exon within the gene are known and provided in **Table 1**.

[0050] As used herein, the term “subject” is intended to include living organisms in which an immune response can be elicited, including *e.g.*, mammals, primates, humans.

[0051] “Polynucleotide” and “nucleic acid” are used herein to refer to a multimeric compound comprising nucleosides or nucleoside analogs which have nitrogenous heterocyclic bases or base analogs linked together along a backbone, including conventional RNA, DNA, mixed RNA-DNA, and polymers that are analogs thereof. A nucleic acid “backbone” can be made up of a variety of linkages, including one or more of sugar-phosphodiester linkages, peptide-nucleic acid bonds (“peptide nucleic acids” or PNA; PCT No. WO 95/32305), phosphorothioate linkages, methylphosphonate linkages, or combinations thereof. Sugar moieties of a nucleic acid can be ribose, deoxyribose, or similar compounds with substitutions, *e.g.*, 2’ methoxy or 2’ halide substitutions. Nitrogenous bases can be conventional bases (A, G, C, T, U), analogs thereof (*e.g.*, modified uridines such as 5-methoxyuridine, pseudouridine, or N1-methylpseudouridine, or others); inosine; derivatives of purines or pyrimidines (*e.g.*, N⁴-

methyl deoxyguanosine, deaza- or aza-purines, deaza- or aza-pyrimidines, pyrimidine bases with substituent groups at the 5 or 6 position (e.g., 5-methylcytosine), purine bases with a substituent at the 2, 6, or 8 positions, 2-amino-6-methylaminopurine, O⁶-methylguanine, 4-thio-pyrimidines, 4-amino-pyrimidines, 4-dimethylhydrazine-pyrimidines, and O⁴-alkyl-pyrimidines; US Pat. No. 5,378,825 and PCT No. WO 93/13121). For general discussion see *The Biochemistry of the Nucleic Acids* 5-36, Adams et al., ed., 11th ed., 1992). Nucleic acids can include one or more “abasic” residues where the backbone includes no nitrogenous base for position(s) of the polymer (US Pat. No. 5,585,481). A nucleic acid can comprise only conventional RNA or DNA sugars, bases and linkages, or can include both conventional components and substitutions (e.g., conventional bases with 2' methoxy linkages, or polymers containing both conventional bases and one or more base analogs). Nucleic acid includes “locked nucleic acid” (LNA), an analogue containing one or more LNA nucleotide monomers with a bicyclic furanose unit locked in an RNA mimicking sugar conformation, which enhance hybridization affinity toward complementary RNA and DNA sequences (Vester and Wengel, 2004, *Biochemistry* 43(42):13233-41). RNA and DNA have different sugar moieties and can differ by the presence of uracil or analogs thereof in RNA and thymine or analogs thereof in DNA.

[0052] “Guide RNA”, “gRNA”, and simply “guide” are used herein interchangeably to refer to, for example, the guide that directs an RNA-guided DNA binding agent to a target DNA and can be a single guide RNA, or the combination of a crRNA and a trRNA (also known as tracrRNA). Exemplary gRNAs include Class II Cas nuclease guide RNAs, in modified or unmodified forms. The crRNA and trRNA may be associated as a single RNA molecule (single guide RNA, sgRNA) or in two separate RNA strands (dual guide RNA, dgRNA). “Guide RNA” or “gRNA” refers to each type. The trRNA may be a naturally occurring sequence, or a trRNA sequence with modifications or variations compared to naturally-occurring sequences. As used herein, a “guide sequence” refers to a sequence within a guide RNA that is complementary to a target sequence and functions to direct a guide RNA to a target sequence for binding or modification (e.g., cleavage) by an RNA-guided DNA binding agent. A “guide sequence” may also be referred to as a “targeting sequence,” or a “spacer sequence.” A guide sequence can be 20 base pairs in length, e.g., in the case of *Streptococcus pyogenes* (*i.e.*, Spy Cas9 (SpCas9)) and related Cas9 homologs/orthologs. Shorter or longer sequences can also be used as guides, e.g., 15-, 16-, 17-, 18-, 19-, 21-, 22-, 23-, 24-, or 25-nucleotides in length. In some embodiments, the target sequence is in a gene or on a chromosome, for example, and is complementary to the guide sequence. In some

embodiments, the degree of complementarity or identity between a guide sequence and its corresponding target sequence may be about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%. In some embodiments, the guide sequence and the target region may be 100% complementary or identical. In other embodiments, the guide sequence and the target region may contain at least one mismatch. For example, the guide sequence and the target sequence may contain 1, 2, 3, or 4 mismatches, where the total length of the target sequence is at least 17, 18, 19, 20 or more base pairs. In some embodiments, the guide sequence and the target region may contain 1-4 mismatches where the guide sequence comprises at least 17, 18, 19, 20 or more nucleotides. In some embodiments, the guide sequence and the target region may contain 1, 2, 3, or 4 mismatches where the guide sequence comprises 20 nucleotides.

[0053] Target sequences for RNA-guided DNA binding agents include both the positive and negative strands of genomic DNA (*i.e.*, the sequence given and the sequence's reverse complement), as a nucleic acid substrate for an RNA-guided DNA binding agent is a double stranded nucleic acid. Accordingly, where a guide sequence is said to be "complementary to a target sequence", it is to be understood that the guide sequence may direct a guide RNA to bind to the reverse complement of a target sequence. Thus, in some embodiments, where the guide sequence binds the reverse complement of a target sequence, the guide sequence is identical to certain nucleotides of the target sequence (e.g., the target sequence not including the PAM) except for the substitution of U for T in the guide sequence.

[0054] As used herein, an "RNA-guided DNA binding agent" means a polypeptide or complex of polypeptides having RNA and DNA binding activity, or a DNA-binding subunit of such a complex, wherein the DNA binding activity is sequence-specific and depends on the sequence of the RNA. Exemplary RNA-guided DNA binding agents include Cas cleavases/nickases and inactivated forms thereof ("dCas DNA binding agents"). "Cas nuclease", also called "Cas protein" as used herein, encompasses Cas cleavases, Cas nickases, and dCas DNA binding agents. Cas cleavases/nickases and dCas DNA binding agents include a Csm or Cmr complex of a type III CRISPR system, the Cas10, Csm1, or Cmr2 subunit thereof, a Cascade complex of a type I CRISPR system, the Cas3 subunit thereof, and Class 2 Cas nucleases. As used herein, a "Class 2 Cas nuclease" is a single-chain polypeptide with RNA-guided DNA binding activity. Class 2 Cas nucleases include Class 2 Cas cleavases/nickases (e.g., H840A, D10A, or N863A variants), which further have RNA-guided DNA cleavases or nickase activity, and Class 2 dCas DNA binding agents, in which cleavage/nickase activity is inactivated. Class 2 Cas nucleases include, for example, Cas9, Cpf1, C2c1, C2c2, C2c3, HF Cas9 (e.g., N497A, R661A, Q695A, Q926A variants), HypaCas9

(e.g., N692A, M694A, Q695A, H698A variants), eSPCas9(1.0) (e.g., K810A, K1003A, R1060A variants), and eSPCas9(1.1) (e.g., K848A, K1003A, R1060A variants) proteins and modifications thereof. Cpf1 protein, Zetsche et al., *Cell*, 163: 1-13 (2015), is homologous to Cas9, and contains a RuvC-like nuclease domain. Cpf1 sequences of Zetsche are incorporated by reference in their entirety. See, e.g., Zetsche, Tables S1 and S3. See, e.g., Makarova et al., *Nat Rev Microbiol*, 13(11): 722-36 (2015); Shmakov et al., *Molecular Cell*, 60:385-397 (2015).

[0055] As used herein, the term “editor” refers to an agent comprising a polypeptide that is capable of making a modification within a DNA sequence. In some embodiments, the editor is a cleavase, such as a Cas9 cleavase. In some embodiments, the editor is capable of deaminating a base within a DNA molecule. In some embodiments, the editor is capable of deaminating a cytosine (C) in DNA. In some embodiments, the editor is a fusion protein comprising an RNA-guided nickase fused to a cytidine deaminase. In some embodiments, the editor is a fusion protein comprising an RNA-guided nickase fused to an APOBEC3A deaminase (A3A). In some embodiments, the editor comprises a Cas9 nickase fused to an APOBEC3A deaminase (A3A). In some embodiments, the editor is a fusion protein comprising an RNA-guided nickase fused to a cytidine deaminase and a UGI. In some embodiments, the editor lacks a UGI.

[0056] As used herein, a “cytidine deaminase” means a polypeptide or complex of polypeptides that is capable of cytidine deaminase activity, that is catalyzing the hydrolytic deamination of cytidine or deoxycytidine, typically resulting in uridine or deoxyuridine. Cytidine deaminases encompass enzymes in the cytidine deaminase superfamily, and in particular, enzymes of the APOBEC family (APOBEC1, APOBEC2, APOBEC4, and APOBEC3 subgroups of enzymes), activation-induced cytidine deaminase (AID or AICDA) and CMP deaminases (see, e.g., Conticello et al., *Mol. Biol. Evol.* 22:367-77, 2005; Conticello, *Genome Biol.* 9:229, 2008; Muramatsu et al., *J. Biol. Chem.* 274: 18470-6, 1999); Carrington et al., *Cells* 9:1690 (2020)).

[0057] As used herein, the term “APOBEC3” refers to a APOBEC3 protein, such as an APOBEC3 protein expressed by any of the seven genes (A3A-A3H) of the human APOBEC3 locus. The APOBEC3 may have catalytic DNA or RNA editing activity. An amino acid sequence of APOBEC3A has been described (UniPROT accession ID: p31941) and is included herein as SEQ ID NO: 40. In some embodiments, the APOBEC3 protein is a human APOBEC3 protein and/or a wild-type protein. Variants include proteins having a sequence that differs from wild-type APOBEC3 protein by one or several mutations (i.e. substitutions, deletions, insertions), such as one or several single point substitutions. For instance, a shortened

APOBEC3 sequence could be used, e.g. by deleting several N-term or C-term amino acids, preferably one to four amino acids at the C-terminus of the sequence. As used herein, the term “variant” refers to allelic variants, splicing variants, and natural or artificial mutants, which are homologous to a APOBEC3 reference sequence. The variant is “functional” in that it shows a catalytic activity of DNA or RNA editing. In some embodiments, an APOBEC3 (such as a human APOBEC3A) has a wild-type amino acid position 57 (as numbered in the wild-type sequence). In some embodiments, an APOBEC3 (such as a human APOBEC3A) has an asparagine at amino acid position 57 (as numbered in the wild-type sequence).

[0058] As used herein, a “nickase” is an enzyme that creates a single-strand break (also known as a “nick”) in double strand DNA, i.e., cuts one strand but not the other of the DNA double helix. As used herein, an “RNA-guided DNA nickase” means a polypeptide or complex of polypeptides having DNA nickase activity, wherein the DNA nickase activity is sequence-specific and depends on the sequence of the RNA. Exemplary RNA-guided DNA nickases include Cas nickases. Cas nickases include nickase forms of a Csm or Cmr complex of a type III CRISPR system, the Cas10, Csm1, or Cmr2 subunit thereof, a Cascade complex of a type I CRISPR system, the Cas3 subunit thereof, and Class 2 Cas nucleases. Class 2 Cas nickases include variants in which only one of the two catalytic domains is inactivated, which have RNA-guided DNA nickase activity. Class 2 Cas nickases include, for example, Cas9 (e.g., H840A, D10A, or N863A variants of SpyCas9), Cpf1, C2c1, C2c2, C2c3, HF Cas9 (e.g., N497A, R661A, Q695A, Q926A variants), HypaCas9 (e.g., N692A, M694A, Q695A, H698A variants), eSPCas9(1.0) (e.g., K810A, K1003A, R1060A variants), and eSPCas9(1.1) (e.g., K848A, K1003A, R1060A variants) proteins and modifications thereof. Cpf1 protein, Zetsche et al., *Cell*, 163: 1-13 (2015), is homologous to Cas9, and contains a RuvC-like protein domain. Cpf1 sequences of Zetsche are incorporated by reference in their entirety. See, e.g., Zetsche, Tables S1 and S3. “Cas9” encompasses *S. pyogenes* (Spy) Cas9, the variants of Cas9 listed herein, and equivalents thereof. See, e.g., Makarova et al., *Nat Rev Microbiol*, 13(11): 722-36 (2015); Shmakov et al., *Molecular Cell*, 60:385-397 (2015).

[0059] As used herein, the term “fusion protein” refers to a hybrid polypeptide which comprises protein domains from at least two different proteins. One protein may be located at the amino-terminal (N-terminal) portion of the fusion protein or at the carboxy-terminal (C-terminal) protein thus forming an “amino-terminal fusion protein” or a “carboxy-terminal fusion protein,” respectively. Any of the proteins provided herein may be produced by any method known in the art. For example, the proteins provided herein may be produced via recombinant protein expression and purification, which is especially suited for fusion proteins

comprising a peptide linker. Methods for recombinant protein expression and purification are well known, and include those described by Green and Sambrook, *Molecular Cloning: A Laboratory Manual* (4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012)), the entire contents of which are incorporated herein by reference.

[0060] The term “linker,” as used herein, refers to a chemical group or a molecule linking two adjacent molecules or moieties. Typically, the linker is positioned between, or flanked by, two groups, molecules, or other moieties and connected to each one via a covalent bond. In some embodiments, the linker is an amino acid or a plurality of amino acids (e.g., a peptide or protein) such as a 16-amino acid residue “XTEN” linker, or a variant thereof (See, e.g., the Examples; and Schellenberger et al. A recombinant polypeptide extends the in vivo half-life of peptides and proteins in a tunable manner. *Nat. Biotechnol.* 27, 1186-1190 (2009)). In some embodiments, the XTEN linker comprises the sequence SGSETPGTSESATPES (SEQ ID NO: 900), SGSETPGTSESA (SEQ ID NO: 901), or SGSETPGTSESATPEGGSGGS (SEQ ID NO: 902).

[0061] As used herein, the term “uracil glycosylase inhibitor” or “UGI” refers to a protein that is capable of inhibiting a uracil-DNA glycosylase (UDG) base-excision repair enzyme.

[0062] As used herein, “open reading frame” or “ORF” of a gene refers to a sequence consisting of a series of codons that specify the amino acid sequence of the protein that the gene codes for. The ORF begins with a start codon (e.g., ATG in DNA or AUG in RNA) and ends with a stop codon, e.g., TAA, TAG or TGA in DNA or UAA, UAG, or UGA in RNA.

[0063] As used herein, “ribonucleoprotein” (RNP) or “RNP complex” refers to a guide RNA together with an RNA-guided DNA binding agent, such as a Cas nuclease, e.g., a Cas cleavase, Cas nickase, or dCas DNA binding agent (e.g., Cas9). In some embodiments, the guide RNA guides the RNA-guided DNA binding agent such as Cas9 to a target sequence, and the guide RNA hybridizes with and the agent binds to the target sequence; in cases where the agent is a cleavase or nickase, binding can be followed by cleaving or nicking.

[0064] As used herein, a first sequence is considered to “comprise a sequence with at least X% identity to” a second sequence if an alignment of the first sequence to the second sequence shows that X% or more of the positions of the second sequence in its entirety are matched by the first sequence. For example, the sequence AAGA comprises a sequence with 100% identity to the sequence AAG because an alignment would give 100% identity in that there are matches to all three positions of the second sequence. The differences between RNA and DNA (generally the exchange of uridine for thymidine or vice versa) and the presence of nucleoside analogs such as modified uridines do not contribute to differences in identity or

complementarity among polynucleotides as long as the relevant nucleotides (such as thymidine, uridine, or modified uridine) have the same complement (e.g., adenosine for all of thymidine, uridine, or modified uridine; another example is cytosine and 5-methylcytosine, both of which have guanosine or modified guanosine as a complement). Thus, for example, the sequence 5'-AXG where X is any modified uridine, such as pseudouridine, N1-methyl pseudouridine, or 5-methoxyuridine, is considered 100% identical to AUG in that both are perfectly complementary to the same sequence (5'-CAU). Exemplary alignment algorithms are the Smith-Waterman and Needleman-Wunsch algorithms, which are well-known in the art. One skilled in the art will understand what choice of algorithm and parameter settings are appropriate for a given pair of sequences to be aligned; for sequences of generally similar length and expected identity >50% for amino acids or >75% for nucleotides, the Needleman-Wunsch algorithm with default settings of the Needleman-Wunsch algorithm interface provided by the EBI at the www.ebi.ac.uk web server is generally appropriate.

[0065] "mRNA" is used herein to refer to a polynucleotide and comprises an open reading frame that can be translated into a polypeptide (*i.e.*, can serve as a substrate for translation by a ribosome and amino-acylated tRNAs). mRNA can comprise a phosphate-sugar backbone including ribose residues or analogs thereof, e.g., 2'-methoxy ribose residues. In some embodiments, the sugars of an mRNA phosphate-sugar backbone consist essentially of ribose residues, 2'-methoxy ribose residues, or a combination thereof.

[0066] As used herein, "indels" refer to insertion/deletion mutations consisting of a number of nucleotides that are either inserted or deleted, *e.g.* at the site of double-stranded breaks (DSBs), in a target nucleic acid.

[0067] As used herein, "reduced or eliminated" expression of a protein on a cell refers to a partial or complete loss of expression of the protein relative to an unmodified cell. In some embodiments, the surface expression of a protein on a cell is measured by flow cytometry and has "reduced or eliminated" surface expression relative to an unmodified cell as evidenced by a reduction in fluorescence signal upon staining with the same antibody against the protein. A cell that has "reduced or eliminated" surface expression of a protein by flow cytometry relative to an unmodified cell may be referred to as "negative" for expression of that protein as evidenced by a fluorescence signal similar to a cell stained with an isotype control antibody. The "reduction or elimination" of protein expression can be measured by other known techniques in the field with appropriate controls known to those skilled in the art.

[0068] As used herein, "knockdown" refers to a decrease in expression of a particular gene product (e.g., protein, mRNA, or both), e.g., as compared to expression of an unedited target

sequence. Knockdown of a protein can be measured by detecting total cellular amount of the protein from a sample, such as a tissue, fluid, or cell population of interest. It can also be measured by measuring a surrogate, marker, or activity for the protein. Methods for measuring knockdown of mRNA are known and include analyzing mRNA isolated from a sample of interest. In some embodiments, “knockdown” may refer to some loss of expression of a particular gene product, for example a decrease in the amount of mRNA transcribed or a decrease in the amount of protein expressed by a cell or population of cells (including *in vivo* populations such as those found in tissues).

[0069] As used herein, “knockout” refers to a loss of expression from a particular gene or of a particular protein in a cell. Knockout can result in a decrease in expression below the level of detection of the assay. Knockout can be measured either by detecting total cellular amount of a protein in a cell, a tissue or a population of cells.

[0070] As used herein, a “target sequence” or “genomic target sequence” refers to a sequence of nucleic acid in a target gene that has complementarity to the guide sequence of the gRNA. The interaction of the target sequence and the guide sequence directs an RNA-guided DNA binding agent to bind, and potentially nick or cleave (depending on the activity of the agent), within the target sequence.

[0071] As used herein, “treatment” refers to any administration or application of a therapeutic for disease or disorder in a subject, and includes inhibiting the disease, arresting its development, relieving one or more symptoms of the disease, curing the disease, or preventing one or more symptoms of the disease, including recurrence of the symptom.

[0072] Reference will now be made in detail to certain embodiments of the invention, examples of which are illustrated in the accompanying drawings. While the invention is described in conjunction with the illustrated embodiments, it will be understood that they are not intended to limit the invention to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents, which may be included within the invention as defined by the appended claims and included embodiments.

[0073] Before describing the present teachings in detail, it is to be understood that the disclosure is not limited to specific compositions or process steps, as such may vary. It should be noted that, as used in this specification and the appended claims, the singular form “a”, “an” and “the” include plural references unless the context clearly dictates otherwise. Thus, for example, reference to “a conjugate” includes a plurality of conjugates and reference to “a cell” includes a plurality of cells and the like.

[0074] Numeric ranges are inclusive of the numbers defining the range. Measured and measurable values are understood to be approximate, taking into account significant digits and the error associated with the measurement. Also, the use of “comprise”, “comprises”, “comprising”, “contain”, “contains”, “containing”, “include”, “includes”, and “including” are not intended to be limiting. It is to be understood that both the foregoing general description and detailed description are exemplary and explanatory only and are not restrictive of the teachings.

[0075] Unless specifically noted in the specification, embodiments in the specification that recite “comprising” various components are also contemplated as “consisting of” or “consisting essentially of” the recited components; embodiments in the specification that recite “consisting of” various components are also contemplated as “comprising” or “consisting essentially of” the recited components; and embodiments in the specification that recite “consisting essentially of” various components are also contemplated as “consisting of” or “comprising” the recited components (this interchangeability does not apply to the use of these terms in the claims). The term “or” is used in an inclusive sense, *i.e.*, equivalent to “and/or,” unless the context clearly indicates otherwise.

[0076] The section headings used herein are for organizational purposes only and are not to be construed as limiting the desired subject matter in any way. In the event that any material incorporated by reference contradicts any term defined in this specification or any other express content of this specification, this specification controls. While the present teachings are described in conjunction with various embodiments, it is not intended that the present teachings be limited to such embodiments. On the contrary, the present teachings encompass various alternatives, modifications, and equivalents, as will be appreciated by those of skill in the art.

II. Genetically Modified Cells

A. Engineered Cell Compositions

[0077] The present disclosure provides engineered cell compositions which have reduced or eliminated surface expression of MHC class II relative to an unmodified cell. In some embodiments, the engineered cell composition comprises a genetic modification in the CIITA gene. In some embodiments, the engineered cell is an allogeneic cell. In some embodiments, the engineered cell with reduced MHC class II expression is useful for adoptive cell transfer therapies. In some embodiments, the engineered cell comprises additional genetic modifications in the genome of the cell to yield a cell that is desirable for allogeneic transplant purposes.

[0078] As used herein, the term “within the genomic coordinates” includes the boundaries of the genomic coordinate range given. For example, if chr16:10895702-10895722 is given, the coordinates chr16:10895702 and chr16:10895722 are encompassed.

[0079] In some embodiments, for each given range of genomic coordinates, a range may encompass +/- 10 nucleotides on either end of the specified coordinates. For each given range of genomic coordinates, the range may encompass +/- 5 nucleotides on either end of the range. For example, if chr16:10895702-10895722 is given, in some embodiments the genomic target sequence or genetic modification may fall within chr16:10895692-10895732.

[0080] Genetic modifications in the CIITA gene are described further herein. In some embodiments, a genetic modification in the CIITA gene comprises any one or more of an insertion, deletion, substitution, or deamination of at least one nucleotide in a target sequence.

[0081] In some embodiments, a given range of genomic coordinates may comprise a target sequence on both strands of the DNA (*i.e.*, the plus (+) strand and the minus (-) strand).

[0082] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16: 10902662-chr16:10923285.

[0083] The boundaries of the exons in the CIITA gene are known and provided in **Table 1** below, based on the ENST00000618327 transcript. See https://useast.ensembl.org/Homo_sapiens/Transcript/Exons?db=core;g=ENSG00000179583;r=16:10866222-10943021;t=ENST00000618327.

[0084] **Table 1. CIITA Region Boundaries (hg38 Transcript: CIITA-214 ENST00000618327.4).**

Exon No.	Start (chromosome 6)	End (chromosome 6)
1	10,877,198	10,877,382
2	10,895,282	10,895,428
3	10,895,669	10,895,764
4	10,898,670	10,898,732
5	10,898,922	10,899,002
6	10,901,514	10,901,558
7	10,902,038	10,902,184
8	10,902,658	10,902,801
9	10,903,731	10,903,895
10	10,904,744	10,904,812
11	10,906,499	10,908,149
12	10,909,029	10,909,187
13	10,910,188	10,910,259
14	10,915,570	10,915,650

Exon No.	Start (chromosome 6)	End (chromosome 6)
15	10,916,367	10,916,459
16	10,918,440	10,918,526
17	10,922,167	10,922,250
18	10,922,407	10,922,490
19	10,923,228	10,923,325
20	10,923,878	10,924,983

[0085] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 contiguous nucleotides within the genomic coordinates chr16: 10902662-chr16:10923285.

[0086] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least 5 contiguous nucleotides within the genomic coordinates chr16: 10902662-chr16:10923285.

[0087] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least 6, 7, 8, 9, or 10 contiguous nucleotides within the genomic coordinates chr16: 10902662-chr16:10923285. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least 6 contiguous nucleotides within the genomic coordinates chr16: 10902662- chr16:10923285. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least 7 contiguous nucleotides within the genomic coordinates chr16: 10902662- chr16:10923285. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least 8 contiguous nucleotides within the genomic coordinates chr16: 10902662- chr16:10923285. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least 9 contiguous nucleotides within the genomic

coordinates chr16: 10902662-chr16:10923285. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least 10 contiguous nucleotides within the genomic coordinates chr16: 10902662-chr16:10923285.

[0088] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates chr16: 10902662- chr16:10923285. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one C to T substitution within the genomic coordinates chr16: 10902662-chr16:10923285. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one A to G substitution within the genomic coordinates chr16: 10902662-chr16:10923285.

[0089] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10906542- chr16:10923285.

[0090] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10906542- chr16:10908121.

[0091] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10916432-10916452, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10906985-10907005, chr16:10908073-10908093, chr16:10907433-10907453, chr16:10907979-10907999, chr16:10907139-10907159, chr16:10922435-10922455, chr16:10907384-10907404, chr16:10907434-10907454, chr16:10907119-10907139, chr16:10907539-10907559, chr16:10907810-10907830, chr16:10907315-10907335, chr16:10916426-

10916446, chr16:10909138-10909158, chr16:10908101-10908121, chr16:10907790-10907810, chr16:10907787-10907807, chr16:10907454-10907474, chr16:10895702-10895722, chr16:10902729-10902749, chr16:10918492-10918512, chr16:10907932-10907952, chr16:10907623-10907643, chr16:10907461-10907481, chr16:10902723-10902743, chr16:10907622-10907642, chr16:10922441-10922461, chr16:10902662-10902682, chr16:10915626-10915646, chr16:10915592-10915612, chr16:10907385-10907405, chr16:10907030-10907050, chr16:10907935-10907955, chr16:10906853-10906873, chr16:10906757-10906777, chr16:10907730-10907750, and chr16:10895302-10895322.

[0092] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10907539-10907559, chr16:10916426-10916446, chr16:10906907-10906927, chr16:10895702-10895722, chr16:10907757-10907777, chr16:10907623-10907643, chr16:10915626-10915646, chr16:10906756-10906776, chr16:10907476-10907496, chr16:10907385-10907405, and chr16:10923265-10923285.

[0093] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10907315-10907335, chr16:10916432-10916452, chr16:10907932-10907952, chr16:10915626-10915646, chr16:10907586-10907606, chr16:10916426-10916446, chr16:10907476-10907496, chr16:10907787-10907807, chr16:10907979-10907999, chr16:10906904-10906924, and chr16:10909138-10909158.

[0094] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10895702-10895722, chr16:10916432-10916452, chr16:10907623-10907643, chr16:10907932-10907952, chr16:10906985-10907005, chr16:10915626-10915646, chr16:10907539-10907559, chr16:10916426-10916446, chr16:10907476-10907496, chr16:10907119-10907139, chr16:10907979-10907999, and chr16:10909138-10909158.

[0095] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10906757-10906777, chr16:10895302-10895322, chr16:10907539-10907559, chr16:10907730-10907750, and chr16:10895702-10895722.

[0096] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10922444-10922464, and chr16:10916432-10916452.

[0097] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10906853-10906873. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10922444-10922464. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10916432-10916452. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10906757-10906777. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10895302-10895322. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10907539-10907559. In some

embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10907730-10907750. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10895702-10895722. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10907932-10907952. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10907476-10907496. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10909138-10909158.

[0098] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from:

chr16:10902662-10902682,	chr16:10902723-10902743,	chr16:10902729-10902749,
chr16:10903747-10903767,	chr16:10903824-10903844,	chr16:10903824-10903844,
chr16:10903848-10903868,	chr16:10904761-10904781,	chr16:10904764-10904784,
chr16:10904765-10904785,	chr16:10904785-10904805,	chr16:10906542-10906562,
chr16:10906556-10906576,	chr16:10906609-10906629,	chr16:10906610-10906630,
chr16:10906616-10906636,	chr16:10906682-10906702,	chr16:10906756-10906776,
chr16:10906757-10906777,	chr16:10906757-10906777,	chr16:10906821-10906841,
chr16:10906823-10906843,	chr16:10906847-10906867,	chr16:10906848-10906868,
chr16:10906853-10906873,	chr16:10906853-10906873,	chr16:10906904-10906924,
chr16:10906907-10906927,	chr16:10906913-10906933,	chr16:10906968-10906988,
chr16:10906970-10906990,	chr16:10906985-10907005,	chr16:10907030-10907050,

chr16:10907058-10907078, chr16:10907119-10907139, chr16:10907139-10907159,
 chr16:10907172-10907192, chr16:10907272-10907292, chr16:10907288-10907308,
 chr16:10907314-10907334, chr16:10907315-10907335, chr16:10907325-10907345,
 chr16:10907363-10907383, chr16:10907384-10907404, chr16:10907385-10907405,
 chr16:10907433-10907453, chr16:10907434-10907454, chr16:10907435-10907455,
 chr16:10907441-10907461, chr16:10907454-10907474, chr16:10907461-10907481,
 chr16:10907476-10907496, chr16:10907539-10907559, chr16:10907586-10907606,
 chr16:10907589-10907609, chr16:10907621-10907641, chr16:10907622-10907642,
 chr16:10907623-10907643, chr16:10907730-10907750, chr16:10907731-10907751,
 chr16:10907757-10907777, chr16:10907781-10907801, chr16:10907787-10907807,
 chr16:10907790-10907810, chr16:10907810-10907830, chr16:10907820-10907840,
 chr16:10907870-10907890, chr16:10907886-10907906, chr16:10907924-10907944,
 chr16:10907928-10907948, chr16:10907932-10907952, chr16:10907935-10907955,
 chr16:10907978-10907998, chr16:10907979-10907999, chr16:10908069-10908089,
 chr16:10908073-10908093, chr16:10908101-10908121, chr16:10909056-10909076,
 chr16:10909138-10909158, chr16:10910195-10910215, chr16:10910196-10910216,
 chr16:10915592-10915612, chr16:10915626-10915646, chr16:10916375-10916395,
 chr16:10916382-10916402, chr16:10916426-10916446, chr16:10916432-10916452,
 chr16:10918486-10918506, chr16:10918492-10918512, chr16:10918493-10918513,
 chr16:10922435-10922455, chr16:10922441-10922461, chr16:10922441-10922461,

chr16:10922444-10922464, chr16:10922460-10922480, chr16:10923257-10923277, and chr16:10923265-10923285. In some embodiments, the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the genetic modification comprises at least 5 contiguous nucleotides within the genomic coordinates. In some embodiments, the genetic modification comprises at least 6, 7, 8, 9, or 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates.

[0099] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: chr16:10916432-10916452, chr16:10922444-10922464, chr16:10907924-10907944,

chr16:10906985-10907005, chr16:10908073-10908093, chr16:10907433-10907453,
 chr16:10907979-10907999, chr16:10907139-10907159, chr16:10922435-10922455,
 chr16:10907384-10907404, chr16:10907434-10907454, chr16:10907119-10907139,
 chr16:10907539-10907559, chr16:10907810-10907830, chr16:10907315-10907335,
 chr16:10916426-10916446, chr16:10909138-10909158, chr16:10908101-10908121,
 chr16:10907790-10907810, chr16:10907787-10907807, chr16:10907454-10907474,
 chr16:10895702-10895722, chr16:10902729-10902749, chr16:10918492-10918512,
 chr16:10907932-10907952, chr16:10907623-10907643, chr16:10907461-10907481,
 chr16:10902723-10902743, chr16:10907622-10907642, chr16:10922441-10922461,
 chr16:10902662-10902682, chr16:10915626-10915646, chr16:10915592-10915612,
 chr16:10907385-10907405, chr16:10907030-10907050, chr16:10907935-10907955,
 chr16:10906853-10906873, chr16:10906757-10906777, chr16:10907730-10907750, and
 chr16:10895302-10895322. In some embodiments, the genetic modification comprises at least
 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 contiguous
 nucleotides within the genomic coordinates. In some embodiments, the genetic modification
 comprises at least 5 contiguous nucleotides within the genomic coordinates. In some
 embodiments, the genetic modification comprises at least 6, 7, 8, 9, or 10 contiguous
 nucleotides within the genomic coordinates. In some embodiments, the genetic modification
 comprises at least one C to T substitution or at least one A to G substitution within the genomic
 coordinates.

[00100] In some embodiments, an engineered cell which has reduced or eliminated surface
 expression of MHC class II relative to an unmodified cell is provided, comprising a genetic
 modification in the CIITA gene, wherein the genetic modification comprises an indel, a C to T
 substitution, or an A to G substitution within the genomic coordinates chosen from:
 chr16:10907539-10907559, chr16:10916426-10916446, chr16:10906907-10906927,
 chr16:10895702-10895722, chr16:10907757-10907777, chr16:10907623-10907643,
 chr16:10915626-10915646, chr16:10906756-10906776, chr16:10907476-10907496,
 chr16:10907385-10907405, and chr16:10923265-10923285. In some embodiments, the
 genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at
 least 8, at least 9, or at least 10 contiguous nucleotides within the genomic coordinates. In
 some embodiments, the genetic modification comprises at least 5 contiguous nucleotides
 within the genomic coordinates. In some embodiments, the genetic modification comprises at
 least 6, 7, 8, 9, or 10 contiguous nucleotides within the genomic coordinates. In some

embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates.

[00101] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10907315-10907335, chr16:10916432-10916452, chr16:10907932-10907952, chr16:10915626-10915646, chr16:10907586-10907606, chr16:10916426-10916446, chr16:10907476-10907496, chr16:10907787-10907807, chr16:10907979-10907999, and chr16:10906904-10906924, and chr16:10909138-10909158. In some embodiments, the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the genetic modification comprises at least 5 contiguous nucleotides within the genomic coordinates. In some embodiments, the genetic modification comprises at least 6, 7, 8, 9, or 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates.

[00102] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: chr16:10895702-10895722, chr16:10916432-10916452, chr16:10907623-10907643, chr16:10907932-10907952, chr16:10906985-10907005, chr16:10915626-10915646, chr16:10907539-10907559, chr16:10916426-10916446, chr16:10907476-10907496, chr16:10907119-10907139, chr16:10907979-10907999, and chr16:10909138-10909158. In some embodiments, the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the genetic modification comprises at least 5 contiguous nucleotides within the genomic coordinates. In some embodiments, the genetic modification comprises at least 6, 7, 8, 9, or 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates.

[00103] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10906757-10906777, chr16:10895302-10895322, chr16:10907539-10907559, chr16:10907730-10907750, and chr16:10895702-10895722. In some embodiments, the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the genetic modification comprises at least 5 contiguous nucleotides within the genomic coordinates. In some embodiments, the genetic modification comprises at least 6, 7, 8, 9, or 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates.

[00104] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10922444-10922464, and chr16:10916432-10916452. In some embodiments, the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the genetic modification comprises at least 5 contiguous nucleotides within the genomic coordinates. In some embodiments, the genetic modification comprises at least 6, 7, 8, 9, or 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates.

[00105] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chr16:10906853-10906873. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chr16:10922444-10922464. In some embodiments, an engineered cell which has reduced or eliminated surface

expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chr16:10916432-10916452. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chr16:10906757-10906777. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chr16:10895302-10895322. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chr16:10907539-10907559. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chr16:10907730-10907750. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chr16:10895702-10895722. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chr16:10907932-10907952. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chr16:10907476-10907496. In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises an indel, a C to T

substitution, or an A to G substitution within the genomic coordinates chr16:10909138-10909158. In some embodiments, the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the genetic modification comprises at least 5 contiguous nucleotides within the genomic coordinates. In some embodiments, the genetic modification comprises at least 6, 7, 8, 9, or 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates.

[00106] In some embodiments, an engineered cell is provided wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10902662-10902682, chr16:10902723-10902743, chr16:10902729-10902749, chr16:10903747-10903767, chr16:10903824-10903844, chr16:10903824-10903844, chr16:10903848-10903868, chr16:10904761-10904781, chr16:10904764-10904784, chr16:10904765-10904785, chr16:10904785-10904805, chr16:10906542-10906562, chr16:10906556-10906576, chr16:10906609-10906629, chr16:10906610-10906630, chr16:10906616-10906636, chr16:10906682-10906702, chr16:10906756-10906776, chr16:10906757-10906777, chr16:10906757-10906777, chr16:10906821-10906841, chr16:10906823-10906843, chr16:10906847-10906867, chr16:10906848-10906868, chr16:10906853-10906873, chr16:10906853-10906873, chr16:10906904-10906924, chr16:10906907-10906927, chr16:10906913-10906933, chr16:10906968-10906988, chr16:10906970-10906990, chr16:10906985-10907005, chr16:10907030-10907050, chr16:10907058-10907078, chr16:10907119-10907139, chr16:10907139-10907159, chr16:10907172-10907192, chr16:10907272-10907292, chr16:10907288-10907308, chr16:10907314-10907334, chr16:10907315-10907335, chr16:10907325-10907345, chr16:10907363-10907383, chr16:10907384-10907404, chr16:10907385-10907405, chr16:10907433-10907453, chr16:10907434-10907454, chr16:10907435-10907455, chr16:10907441-10907461, chr16:10907454-10907474, chr16:10907461-10907481, chr16:10907476-10907496, chr16:10907539-10907559, chr16:10907586-10907606, chr16:10907589-10907609, chr16:10907621-10907641, chr16:10907622-10907642, chr16:10907623-10907643, chr16:10907730-10907750, chr16:10907731-10907751, chr16:10907757-10907777, chr16:10907781-10907801, chr16:10907787-10907807, chr16:10907790-10907810, chr16:10907810-10907830, chr16:10907820-10907840, chr16:10907870-10907890, chr16:10907886-10907906, chr16:10907924-

10907944, chr16:10907928-10907948, chr16:10907932-10907952, chr16:10907935-10907955, chr16:10907978-10907998, chr16:10907979-10907999, chr16:10908069-10908089, chr16:10908073-10908093, chr16:10908101-10908121, chr16:10909056-10909076, chr16:10909138-10909158, chr16:10910195-10910215, chr16:10910196-10910216, chr16:10915592-10915612, chr16:10915626-10915646, chr16:10916375-10916395, chr16:10916382-10916402, chr16:10916426-10916446, chr16:10916432-10916452, chr16:10918486-10918506, chr16:10918492-10918512, chr16:10918493-10918513, chr16:10922435-10922455, chr16:10922441-10922461, chr16:10922441-10922461, chr16:10922444-10922464, chr16:10922460-10922480, chr16:10923257-10923277, chr16:10923265-10923285. In some embodiments, the CIITA genomic target sequence comprises at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the CIITA genomic target sequence comprises at least 15 contiguous nucleotides within the genomic coordinates. In some embodiments, the gene editing system comprises an RNA-guided DNA-binding agent. In some embodiments, the RNA-guided DNA-binding agent comprises a Cas9 protein, such as an *S. pyogenes* Cas9. In some embodiments, the RNA-guided DNA binding agent comprises an APOBEC3A deaminase (A3A) and an RNA-guided nickase.

[00107] In some embodiments, an engineered cell is provided wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10906542-10906562, chr16:10906556-10906576, chr16:10906609-10906629, chr16:10906610-10906630, chr16:10906616-10906636, chr16:10906682-10906702, chr16:10906756-10906776, chr16:10906757-10906777, chr16:10906757-10906777, chr16:10906821-10906841, chr16:10906823-10906843, chr16:10906847-10906867, chr16:10906848-10906868, chr16:10906853-10906873, chr16:10906853-10906873, chr16:10906904-10906924, chr16:10906907-10906927, chr16:10906913-10906933, chr16:10906968-10906988, chr16:10906970-10906990, chr16:10906985-10907005, chr16:10907030-10907050, chr16:10907058-10907078, chr16:10907119-10907139, chr16:10907139-10907159, chr16:10907172-10907192, chr16:10907272-10907292, chr16:10907288-10907308, chr16:10907314-10907334, chr16:10907315-10907335, chr16:10907325-10907345, chr16:10907363-10907383, chr16:10907384-10907404, chr16:10907385-10907405, chr16:10907433-10907453, chr16:10907434-10907454, chr16:10907435-10907455, chr16:10907441-10907461, chr16:10907454-10907474, chr16:10907461-10907481, chr16:10907476-10907496, chr16:10907539-

10907559, chr16:10907586-10907606, chr16:10907589-10907609, chr16:10907621-
 10907641, chr16:10907622-10907642, chr16:10907623-10907643, chr16:10907730-
 10907750, chr16:10907731-10907751, chr16:10907757-10907777, chr16:10907781-
 10907801, chr16:10907787-10907807, chr16:10907790-10907810, chr16:10907810-
 10907830, chr16:10907820-10907840, chr16:10907870-10907890, chr16:10907886-
 10907906, chr16:10907924-10907944, chr16:10907928-10907948, chr16:10907932-
 10907952, chr16:10907935-10907955, chr16:10907978-10907998, chr16:10907979-
 10907999, chr16:10908069-10908089, chr16:10908073-10908093, chr16:10908101-
 10908121, chr16:10909056-10909076, chr16:10909138-10909158, chr16:10910195-
 10910215, chr16:10910196-10910216, chr16:10915592-10915612, chr16:10915626-
 10915646, chr16:10916375-10916395, chr16:10916382-10916402, chr16:10916426-
 10916446, chr16:10916432-10916452, chr16:10918486-10918506, chr16:10918492-
 10918512, chr16:10918493-10918513, chr16:10922435-10922455, chr16:10922441-
 10922461, chr16:10922441-10922461, chr16:10922444-10922464, chr16:10922460-
 10922480, chr16:10923257-10923277, chr16:10923265-10923285. In some embodiments, the

CIITA genomic target sequence comprises at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the CIITA genomic target sequence comprises at least 15 contiguous nucleotides within the genomic coordinates. In some embodiments, the gene editing system comprises an RNA-guided DNA-binding agent. In some embodiments, the RNA-guided DNA-binding agent comprises a Cas9 protein, such as an *S. pyogenes* Cas9. In some embodiments, the RNA-guided DNA binding agent comprises an APOBEC3A deaminase (A3A) and an RNA-guided nickase.

[00108] In some embodiments, an engineered cell is provided wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10906542-10906562, chr16:10906556-10906576, chr16:10906609-
 10906629, chr16:10906610-10906630, chr16:10906616-10906636, chr16:10906682-
 10906702, chr16:10906756-10906776, chr16:10906757-10906777, chr16:10906757-
 10906777, chr16:10906821-10906841, chr16:10906823-10906843, chr16:10906847-
 10906867, chr16:10906848-10906868, chr16:10906853-10906873, chr16:10906853-
 10906873, chr16:10906904-10906924, chr16:10906907-10906927, chr16:10906913-
 10906933, chr16:10906968-10906988, chr16:10906970-10906990, chr16:10906985-
 10907005, chr16:10907030-10907050, chr16:10907058-10907078, chr16:10907119-
 10907139, chr16:10907139-10907159, chr16:10907172-10907192, chr16:10907272-

10907292, chr16:10907288-10907308, chr16:10907314-10907334, chr16:10907315-10907335, chr16:10907325-10907345, chr16:10907363-10907383, chr16:10907384-10907404, chr16:10907385-10907405, chr16:10907433-10907453, chr16:10907434-10907454, chr16:10907435-10907455, chr16:10907441-10907461, chr16:10907454-10907474, chr16:10907461-10907481, chr16:10907476-10907496, chr16:10907539-10907559, chr16:10907586-10907606, chr16:10907589-10907609, chr16:10907621-10907641, chr16:10907622-10907642, chr16:10907623-10907643, chr16:10907730-10907750, chr16:10907731-10907751, chr16:10907757-10907777, chr16:10907781-10907801, chr16:10907787-10907807, chr16:10907790-10907810, chr16:10907810-10907830, chr16:10907820-10907840, chr16:10907870-10907890, chr16:10907886-10907906, chr16:10907924-10907944, chr16:10907928-10907948, chr16:10907932-10907952, chr16:10907935-10907955, chr16:10907978-10907998, chr16:10907979-10907999, chr16:10908069-10908089, chr16:10908073-10908093, chr16:10908101-10908121. In some embodiments, the CIITA genomic target sequence comprises at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the CIITA genomic target sequence comprises at least 15 contiguous nucleotides within the genomic coordinates. In some embodiments, the gene editing system comprises an RNA-guided DNA-binding agent. In some embodiments, the RNA-guided DNA-binding agent comprises a Cas9 protein, such as an *S. pyogenes* Cas9. In some embodiments, the RNA-guided DNA binding agent comprises an APOBEC3A deaminase (A3A) and an RNA-guided nickase.

[00109] In some embodiments, an engineered cell is provided wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10916432-10916452, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10906985-10907005, chr16:10908073-10908093, chr16:10907433-10907453, chr16:10907979-10907999, chr16:10907139-10907159, chr16:10922435-10922455, chr16:10907384-10907404, chr16:10907434-10907454, chr16:10907119-10907139, chr16:10907539-10907559, chr16:10907810-10907830, chr16:10907315-10907335, chr16:10916426-10916446, chr16:10909138-10909158, chr16:10908101-10908121, chr16:10907790-10907810, chr16:10907787-10907807, chr16:10907454-10907474, chr16:10895702-10895722, chr16:10902729-10902749, chr16:10918492-10918512, chr16:10907932-10907952, chr16:10907623-10907643, chr16:10907461-10907481, chr16:10902723-10902743, chr16:10907622-10907642, chr16:10922441-10922461, chr16:10902662-10902682, chr16:10915626-10915646, chr16:10915592-

10915612, chr16:10907385-10907405, chr16:10907030-10907050, chr16:10907935-10907955, chr16:10906853-10906873, chr16:10906757-10906777, chr16:10907730-10907750, and chr16:10895302-10895322. In some embodiments, the CIITA genomic target sequence comprises at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the CIITA genomic target sequence comprises at least 15 contiguous nucleotides within the genomic coordinates. In some embodiments, the gene editing system comprises an RNA-guided DNA-binding agent. In some embodiments, the RNA-guided DNA-binding agent comprises a Cas9 protein, such as an *S. pyogenes* Cas9. In some embodiments, the RNA-guided DNA binding agent comprises an APOBEC3A deaminase (A3A) and an RNA-guided nickase.

[00110] In some embodiments, an engineered cell is provided wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10907539-10907559, chr16:10916426-10916446, chr16:10906907-10906927, chr16:10895702-10895722, chr16:10907757-10907777, chr16:10907623-10907643, chr16:10915626-10915646, chr16:10906756-10906776, chr16:10907476-10907496, chr16:10907385-10907405, and chr16:10923265-10923285. In some embodiments, the CIITA genomic target sequence comprises at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the CIITA genomic target sequence comprises at least 15 contiguous nucleotides within the genomic coordinates. In some embodiments, the gene editing system comprises an RNA-guided DNA-binding agent. In some embodiments, the RNA-guided DNA-binding agent comprises a Cas9 protein, such as an *S. pyogenes* Cas9.

[00111] In some embodiments, an engineered cell is provided wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10907539-10907559, chr16:10916426-10916446, chr16:10906907-10906927, chr16:10895702-10895722, chr16:10907757-10907777, chr16:10907623-10907643, chr16:10915626-10915646, chr16:10906756-10906776, chr16:10907476-10907496, chr16:10907385-10907405, and chr16:10923265-10923285. In some embodiments, the CIITA genomic target sequence comprises at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the CIITA genomic target sequence comprises at least 15 contiguous nucleotides within the genomic coordinates. In some embodiments, the gene editing system comprises an RNA-guided DNA-binding agent. In some

embodiments, the RNA-guided DNA binding agent comprises an APOBEC3A deaminase (A3A) and an RNA-guided nickase.

[00112] In some embodiments, an engineered cell is provided wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10907315-10907335, chr16:10916432-10916452, chr16:10907932-10907952, chr16:10915626-10915646, chr16:10907586-10907606, chr16:10916426-10916446, chr16:10907476-10907496, chr16:10907787-10907807, chr16:10907979-10907999, chr16:10906904-10906924, and chr16:10909138-10909158. In some embodiments, the CIITA genomic target sequence comprises at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the CIITA genomic target sequence comprises at least 15 contiguous nucleotides within the genomic coordinates. In some embodiments, the gene editing system comprises an RNA-guided DNA-binding agent. In some embodiments, the RNA-guided DNA-binding agent comprises a Cas9 protein, such as an *S. pyogenes* Cas9.

[00113] In some embodiments, an engineered cell is provided wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10895702-10895722, chr16:10916432-10916452, chr16:10907623-10907643, chr16:10907932-10907952, chr16:10906985-10907005, chr16:10915626-10915646, chr16:10907539-10907559, chr16:10916426-10916446, chr16:10907476-10907496, chr16:10907119-10907139, chr16:10907979-10907999, and chr16:10909138-10909158. In some embodiments, the CIITA genomic target sequence comprises at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the CIITA genomic target sequence comprises at least 15 contiguous nucleotides within the genomic coordinates. In some embodiments, the gene editing system comprises an RNA-guided DNA-binding agent. In some embodiments, the RNA-guided DNA binding agent comprises an APOBEC3A deaminase (A3A) and an RNA-guided nickase.

[00114] In some embodiments, an engineered cell is provided wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10906757-10906777, chr16:10895302-10895322, chr16:10907539-10907559, chr16:10907730-10907750, and chr16:10895702-

10895722. In some embodiments, the CIITA genomic target sequence comprises at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the CIITA genomic target sequence comprises at least 15 contiguous nucleotides within the genomic coordinates. In some embodiments, the gene editing system comprises an RNA-guided DNA-binding agent. In some embodiments, the RNA-guided DNA-binding agent comprises a Cas9 protein, such as an *S. pyogenes* Cas9. In some embodiments, the RNA-guided DNA binding agent comprises an APOBEC3A deaminase (A3A) and an RNA-guided nickase.

[00115] In some embodiments, an engineered cell is provided wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10922444-10922464, and chr16:10916432-10916452. In some embodiments, the CIITA genomic target sequence comprises at least 10 contiguous nucleotides within the genomic coordinates. In some embodiments, the CIITA genomic target sequence comprises at least 15 contiguous nucleotides within the genomic coordinates. In some embodiments, the gene editing system comprises an RNA-guided DNA-binding agent. In some embodiments, the RNA-guided DNA-binding agent comprises a Cas9 protein, such as an *S. pyogenes* Cas9.

[00116] In some embodiments, an engineered cell is provided wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16:10906853-10906873. In some embodiments, an engineered cell is provided wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16:10922444-10922464. In some embodiments, an engineered cell is provided wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16:10906757-10906777. In some embodiments, an engineered cell is provided wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16:10895302-10895322. In some embodiments, an engineered cell is provided wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16:10907539-10907559. In some embodiments, an engineered cell is provided wherein the MHC class II

expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16:10907730-10907750. In some embodiments, an engineered cell is provided wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16:10895702-10895722. In some embodiments, an engineered cell is provided wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16:10907932-10907952. In some embodiments, an engineered cell is provided wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16:10907476-10907496. In some embodiments, an engineered cell is provided wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16:10909138-10909158. In some embodiments, the CIITA genomic target sequence comprises at least 15 contiguous nucleotides within the genomic coordinates. In some embodiments, the gene editing system comprises an RNA-guided DNA-binding agent. In some embodiments, the RNA-guided DNA-binding agent comprises a Cas9 protein, such as an *S. pyogenes* Cas9. In some embodiments, the RNA-guided DNA binding agent comprises an APOBEC3A deaminase (A3A) and an RNA-guided nickase.

[00117] In some embodiments, the engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene as described herein, and wherein the cell further has reduced or eliminated surface expression of HLA-A. In some embodiments, the engineered cell comprises a genetic modification in the HLA-A gene. In some embodiments, the engineered cell comprises a genetic modification in the HLA-A gene and wherein the cell is homozygous for HLA-B and homozygous for HLA-C. In some embodiments, the engineered cell comprises a genetic modification that eliminates expression of MHC class I protein on the surface of the engineered cell.

[00118] The engineered human cells described herein may comprise a genetic modification in any HLA-A allele of the HLA-A gene. The HLA gene is located in chromosome 6 in a genomic region referred to as the HLA superlocus; hundreds of HLA-A alleles have been reported in the art (*see e.g.*, Shiina et al., *Nature* 54:15-39 (2009)). Sequences for HLA-A alleles

are available in the art (*see e.g.*, IPD-IMGT/HLA database for retrieving sequences of specific HLA-A alleles <https://www.ebi.ac.uk/ipd/imgt/hla/allele.html>).

[00119] In any of the embodiments above, the engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the modification comprises at least one nucleotide of an exon within the genomic coordinates chr16: 10902662- chr16:10923285, further comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29942854 to chr6:29942913 and chr6:29943518 to chr6: 29943619. In some embodiments, the cell comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29942864 to chr6: 29942903. In some embodiments, the cell comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29943528 to chr6:29943609. In some embodiments, the cell comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; and chr6:29944026-29944046. In some embodiments, the cell comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; and chr6:29944026-29944046. In some embodiments, the HLA-A expression of the cell is reduced or eliminated by a gene editing system that binds to an HLA-A genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550;

chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; and chr6:29944026-29944046. In some embodiments, the cell is homozygous for HLA-B and homozygous for HLA-C.

[00120] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10906542- chr16:10923285, and wherein the cell further comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29942854 to chr6:29942913 and chr6:29943518 to chr6:29943619. In some embodiments, the cell comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29942864 to chr6:29942903. In some embodiments, the cell comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29943528 to chr6:29943609. In some embodiments, the cell comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; and chr6:29944026-29944046. In some embodiments, the cell comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; and chr6:29944026-29944046. In some embodiments, the HLA-A expression of the cell is reduced or eliminated by a gene editing system that binds to an HLA-A genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146;

chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; and chr6:29944026-29944046. In some embodiments, the cell is homozygous for HLA-B and homozygous for HLA-C.

[00121] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10906542- chr16:10908121, and wherein the cell further comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29942854 to chr6:29942913 and chr6:29943518 to chr6:29943619. In some embodiments, the cell comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29942864 to chr6:29942903. In some embodiments, the cell comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29943528 to chr6:29943609. In some embodiments, the cell comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; and chr6:29944026-29944046. In some embodiments, the cell comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; and chr6:29944026-29944046. In some embodiments, the HLA-A expression of the cell is reduced or eliminated by a gene editing system that binds to an HLA-A genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896;

chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; and chr6:29944026-29944046. In some embodiments, the cell is homozygous for HLA-B and homozygous for HLA-C.

[00122] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10916432-10916452, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10906985-10907005, chr16:10908073-10908093, chr16:10907433-10907453, chr16:10907979-10907999, chr16:10907139-10907159, chr16:10922435-10922455, chr16:10907384-10907404, chr16:10907434-10907454, chr16:10907119-10907139, chr16:10907539-10907559, chr16:10907810-10907830, chr16:10907315-10907335, chr16:10916426-10916446, chr16:10909138-10909158, chr16:10908101-10908121, chr16:10907790-10907810, chr16:10907787-10907807, chr16:10907454-10907474, chr16:10895702-10895722, chr16:10902729-10902749, chr16:10918492-10918512, chr16:10907932-10907952, chr16:10907623-10907643, chr16:10907461-10907481, chr16:10902723-10902743, chr16:10907622-10907642, chr16:10922441-10922461, chr16:10902662-10902682, chr16:10915626-10915646, chr16:10915592-10915612, chr16:10907385-10907405, chr16:10907030-10907050, chr16:10907935-10907955, chr16:10906853-10906873, chr16:10906757-10906777, chr16:10907730-10907750, and chr16:10895302-10895322, and wherein the cell further comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29942854 to chr6:29942913 and chr6:29943518 to chr6:29943619. In some embodiments, the cell comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29942864 to chr6:29942903. In some embodiments, the cell comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29943528 to chr6:29943609. In some embodiments, the cell comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29942864-29942884; chr6:29942868-29942888;

chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903;
 chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549;
 chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569;
 chr6:29943589-29943609; and chr6:29944026-29944046. In some embodiments, the cell
 comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in
 the HLA-A gene comprises an indel, a C to T substitution, or an A to G substitution within the
 genomic coordinates chosen from: chr6:29942864-29942884; chr6:29942868-29942888;
 chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903;
 chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549;
 chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569;
 chr6:29943589-29943609; and chr6:29944026-29944046. In some embodiments, the HLA-A
 expression of the cell is reduced or eliminated by a gene editing system that binds to an HLA-
 A genomic target sequence comprising at least 5 contiguous nucleotides within the genomic
 coordinates chosen from: chr6:29942864-29942884; chr6:29942868-29942888;
 chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903;
 chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549;
 chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569;
 chr6:29943589-29943609; and chr6:29944026-29944046. In some embodiments, the cell is
 homozygous for HLA-B and homozygous for HLA-C.

[00123] In some embodiments, an engineered cell which has reduced or eliminated surface
 expression of MHC class II relative to an unmodified cell is provided, comprising a genetic
 modification in the CIITA gene, wherein the genetic modification comprises at least one
 nucleotide of an exon within the genomic coordinates chosen from: chr16:10907539-
 10907559, chr16:10916426-10916446, chr16:10906907-10906927, chr16:10895702-
 10895722, chr16:10907757-10907777, chr16:10907623-10907643, chr16:10915626-
 10915646, chr16:10906756-10906776, chr16:10907476-10907496, chr16:10907385-
 10907405, and chr16:10923265-10923285, and wherein the cell further comprises a genetic
 modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene
 comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29942854
 to chr6:29942913 and chr6:29943518 to chr6:29943619. In some embodiments, the cell
 comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in
 the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen
 from: chr6:29942864 to chr6:29942903. In some embodiments, the cell comprises a genetic
 modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene

comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29943528 to chr6:29943609. In some embodiments, the cell comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; and chr6:29944026-29944046. In some embodiments, the cell comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; and chr6:29944026-29944046. In some embodiments, the HLA-A expression of the cell is reduced or eliminated by a gene editing system that binds to an HLA-A genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; and chr6:29944026-29944046. In some embodiments, the cell is homozygous for HLA-B and homozygous for HLA-C.

[00124] In some embodiments, an engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10906757-10906777, chr16:10895302-10895322, chr16:10907539-10907559, chr16:10907730-10907750, chr16:10895702-10895722, and wherein the cell further comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29942854 to chr6:29942913 and chr6:29943518 to chr6:29943619. In some embodiments, the cell comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide

within the genomic coordinates chosen from: chr6:29942864 to chr6: 29942903. In some embodiments, the cell comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29943528 to chr6:29943609. In some embodiments, the cell comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; and chr6:29944026-29944046. In some embodiments, the cell comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; and chr6:29944026-29944046. In some embodiments, the HLA-A expression of the cell is reduced or eliminated by a gene editing system that binds to an HLA-A genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; and chr6:29944026-29944046. In some embodiments, the cell is homozygous for HLA-B and homozygous for HLA-C.

[00125] In some embodiments, the engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene as described herein, and wherein the cell further has reduced or eliminated surface expression of MHC class I. In some embodiments, the engineered cell comprises a genetic modification in the beta-2-microglobulin (B2M) gene. In some embodiments, the engineered cell comprises a genetic modification in the beta-2-microglobulin (B2M) gene and insertion of an exogenous nucleic acid encoding an NK cell inhibitor

molecule. In some embodiments, the engineered cell comprises a genetic modification that eliminates expression of MHC class I protein on the surface of the engineered cell.

[00126] In some embodiments, the engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the modification comprises at least one nucleotide of an exon within the genomic coordinates chr16: 10902662-chr16:10923285, and wherein the cell further comprises an exogenous nucleic acid. In some embodiments, the exogenous nucleic acid encodes a targeting receptor that is expressed on the surface of the engineered cell. In some embodiments, the targeting receptor is a chimeric antigen receptor (CAR). In some embodiments, the targeting receptor is a universal CAR (UniCar). In some embodiments, the targeting receptor is a T cell receptor (TCR). In some embodiments, the targeting receptor is a WT1 TCR. In some embodiments, the targeting receptor is a hybrid CAR/TCR. In some embodiments, the targeting receptor comprises an antigen recognition domain (e.g., a cancer antigen recognition domain and a subunit of a TCR). In some embodiments, the targeting receptor is a cytokine receptor. In some embodiments, the targeting receptor is a chemokine receptor. In some embodiments, the targeting receptor is a B cell receptor (BCR). In some embodiments, the exogenous nucleic acid encodes a polypeptide that is secreted by the engineered cell (i.e., a soluble polypeptide). In some embodiments, the exogenous nucleic acid encodes a therapeutic polypeptide. In some embodiments, the exogenous nucleic acid encodes an antibody. In some embodiments, the exogenous nucleic acid encodes an enzyme. In some embodiments, the exogenous nucleic acid encodes a cytokine. In some embodiments, the exogenous nucleic acid encodes a chemokine. In some embodiments, the exogenous nucleic acid encodes a fusion protein.

[00127] In some embodiments, the engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the modification comprises at least one nucleotide of an exon within the genomic coordinates chr16: 10902662-chr16:10923285, wherein the cell further has reduced or eliminated surface expression of MHC class I, and wherein the cell further comprises an exogenous nucleic acid. In some embodiments, the engineered cell comprises a genetic modification in the beta-2-microglobulin (B2M) gene. In some embodiments, the engineered cell comprises a genetic modification that reduces expression of MHC class I protein on the surface of the engineered cell. In some embodiments, the exogenous nucleic acid encodes a targeting receptor that is expressed on the surface of the engineered cell. In some embodiments, the targeting receptor is a chimeric antigen receptor (CAR). In some

embodiments, the targeting receptor is a universal CAR (UniCar). In some embodiments, the targeting receptor is a T cell receptor (TCR). In some embodiments, the targeting receptor is a WT1 TCR. In some embodiments, the targeting receptor is a hybrid CAR/TCR. In some embodiments, the targeting receptor comprises an antigen recognition domain (e.g., a cancer antigen recognition domain and a subunit of a TCR). In some embodiments, the targeting receptor is a cytokine receptor. In some embodiments, the targeting receptor is a chemokine receptor. In some embodiments, the targeting receptor is a B cell receptor (BCR). In some embodiments, the exogenous nucleic acid encodes a polypeptide that is secreted by the engineered cell (i.e., a soluble polypeptide). In some embodiments, the exogenous nucleic acid encodes a therapeutic polypeptide. In some embodiments, the exogenous nucleic acid encodes an antibody. In some embodiments, the exogenous nucleic acid encodes an enzyme. In some embodiments, the exogenous nucleic acid encodes a cytokine. In some embodiments, the exogenous nucleic acid encodes a chemokine. In some embodiments, the exogenous nucleic acid encodes a fusion protein.

[00128] In some embodiments, the engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the modification comprises at least one nucleotide of an exon within the genomic coordinates chr16: 10902662-chr16:10923285, wherein the cell further has reduced or eliminated surface expression of HLA-A, and wherein the cell further comprises an exogenous nucleic acid. In some embodiments, the engineered cell comprises a genetic modification in the HLA-A gene. In some embodiments, the engineered cell comprises a genetic modification that reduces expression of HLA-A protein on the surface of the engineered cell. In some embodiments, the exogenous nucleic acid encodes a targeting receptor that is expressed on the surface of the engineered cell. In some embodiments, the targeting receptor is a chimeric antigen receptor (CAR). In some embodiments, the targeting receptor is a universal CAR (UniCar). In some embodiments, the targeting receptor is a T cell receptor (TCR). In some embodiments, the targeting receptor is a WT1 TCR. In some embodiments, the targeting receptor is a hybrid CAR/TCR. In some embodiments, the targeting receptor comprises an antigen recognition domain (e.g., a cancer antigen recognition domain and a subunit of a TCR). In some embodiments, the targeting receptor is a cytokine receptor. In some embodiments, the targeting receptor is a chemokine receptor. In some embodiments, the targeting receptor is a B cell receptor (BCR). In some embodiments, the exogenous nucleic acid encodes a polypeptide that is secreted by the engineered cell (i.e., a soluble polypeptide). In some embodiments, the exogenous nucleic acid encodes a therapeutic polypeptide. In some

embodiments, the exogenous nucleic acid encodes an antibody. In some embodiments, the exogenous nucleic acid encodes an enzyme. In some embodiments, the exogenous nucleic acid encodes a cytokine. In some embodiments, the exogenous nucleic acid encodes a chemokine. In some embodiments, the exogenous nucleic acid encodes a fusion protein. .

[00129] In some embodiments, the engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the modification comprises at least one nucleotide of an exon within the genomic coordinates chr16: 10902662-chr16:10923285, and wherein the cell further has reduced or eliminated expression of an endogenous TCR protein relative to an unmodified cell. In some embodiments, the engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the modification comprises at least one nucleotide of an exon within the genomic coordinates chr16: 10902662-chr16:10923285, and wherein the cell further comprises an exogenous nucleic acid, and further has reduced or eliminated expression of an endogenous TCR protein relative to an unmodified cell. In some embodiments, the engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the modification comprises at least one nucleotide of an exon within the genomic coordinates chr16: 10902662- chr16:10923285, and wherein the cell further has reduced or eliminated surface expression of MHC class I, and wherein the cell further has reduced or eliminated expression of an endogenous TCR protein relative to an unmodified cell.

[00130] In some embodiments, the engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the modification comprises at least one nucleotide of an exon within the genomic coordinates chr16: 10902662- chr16:10923285, and wherein the cell further comprises an exogenous nucleic acid, and wherein the cell further has reduced or eliminated surface expression of MHC class I, and wherein the cell further has reduced or eliminated expression of an endogenous TCR protein relative to an unmodified cell. In some embodiments, the engineered cell has reduced or eliminated expression of a TRAC protein relative to an unmodified cell. In some embodiments, the engineered cell has reduced or eliminated expression of a TRBC protein relative to an unmodified cell. In some embodiments, the engineered cell comprises a genetic modification in the beta-2-microglobulin (B2M) gene. In some embodiments, the engineered cell comprises a genetic modification that reduces expression of MHC class I protein on the surface of the engineered cell. In some embodiments,

the exogenous nucleic acid encodes a targeting receptor that is expressed on the surface of the engineered cell. In some embodiments, the targeting receptor is a chimeric antigen receptor (CAR). In some embodiments, the targeting receptor is a universal CAR (UniCar). In some embodiments, the targeting receptor is a T cell receptor (TCR). In some embodiments, the targeting receptor is a WT1 TCR. In some embodiments, the targeting receptor is a hybrid CAR/TCR. In some embodiments, the targeting receptor comprises an antigen recognition domain (e.g., a cancer antigen recognition domain and a subunit of a TCR). In some embodiments, the targeting receptor is a cytokine receptor. In some embodiments, the targeting receptor is a chemokine receptor. In some embodiments, the targeting receptor is a B cell receptor (BCR). In some embodiments, the exogenous nucleic acid encodes a polypeptide that is secreted by the engineered cell (i.e., a soluble polypeptide). In some embodiments, the exogenous nucleic acid encodes a therapeutic polypeptide. In some embodiments, the exogenous nucleic acid encodes an antibody. In some embodiments, the exogenous nucleic acid encodes an enzyme. In some embodiments, the exogenous nucleic acid encodes a cytokine. In some embodiments, the exogenous nucleic acid encodes a chemokine. In some embodiments, the exogenous nucleic acid encodes a fusion protein.

[00131] In some embodiments, the engineered cell which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell is provided, comprising a genetic modification in the CIITA gene, wherein the modification comprises at least one nucleotide of an exon within the genomic coordinates chr16: 10902662- chr16:10923285, and wherein the cell further comprises an exogenous nucleic acid, and wherein the cell further has reduced or eliminated surface expression of HLA-A, and wherein the cell further has reduced or eliminated expression of an endogenous TCR protein relative to an unmodified cell. In some embodiments, the engineered cell has reduced or eliminated expression of a TRAC protein relative to an unmodified cell. In some embodiments, the engineered cell has reduced or eliminated expression of a TRBC protein relative to an unmodified cell. In some embodiments, the engineered cell comprises a genetic modification in the HLA-A gene. In some embodiments, the engineered cell comprises a genetic modification that reduces expression of HLA-A protein on the surface of the engineered cell. In some embodiments, the exogenous nucleic acid encodes a targeting receptor that is expressed on the surface of the engineered cell. In some embodiments, the targeting receptor is a chimeric antigen receptor (CAR). In some embodiments, the targeting receptor is a universal CAR (UniCar). In some embodiments, the targeting receptor is a T cell receptor (TCR). In some embodiments, the targeting receptor is a WT1 TCR. In some embodiments, the targeting receptor is a hybrid CAR/TCR. In some

embodiments, the targeting receptor comprises an antigen recognition domain (e.g., a cancer antigen recognition domain and a subunit of a TCR). In some embodiments, the targeting receptor is a cytokine receptor. In some embodiments, the targeting receptor is a chemokine receptor. In some embodiments, the targeting receptor is a B cell receptor (BCR). In some embodiments, the exogenous nucleic acid encodes a polypeptide that is secreted by the engineered cell (i.e., a soluble polypeptide). In some embodiments, the exogenous nucleic acid encodes a therapeutic polypeptide. In some embodiments, the exogenous nucleic acid encodes an antibody. In some embodiments, the exogenous nucleic acid encodes an enzyme. In some embodiments, the exogenous nucleic acid encodes a cytokine. In some embodiments, the exogenous nucleic acid encodes a chemokine. In some embodiments, the exogenous nucleic acid encodes a fusion protein.

[00132] The engineered cell may be any of the exemplary cell types disclosed herein. In some embodiments, the engineered cell is an immune cell. In some embodiments, the engineered cell is a hematopoietic stem cell (HSC). In some embodiments, the engineered cell is an induced pluripotent stem cell (iPSC). In some embodiments, the engineered cell is a monocyte, macrophage, mast cell, dendritic cell, or granulocyte. In some embodiments, the engineered cell is monocyte. In some embodiments, the engineered cell is a macrophage. In some embodiments, the engineered cell is a mast cell. In some embodiments, the engineered cell is a dendritic cell.

[00133] In some embodiments, the engineered cell is a granulocyte. In some embodiments, the engineered cell is a lymphocyte. In some embodiments, the engineered cell is a T cell. In some embodiments, the engineered cell is a CD4⁺ T cell. In some embodiments, the engineered cell is a CD8⁺ T cell. In some embodiments, the engineered cell is a memory T cell. In some embodiments, the engineered cell is a B cell. In some embodiments, the engineered cell is a plasma B cell. In some embodiments, the engineered cell is a memory B cell.

[00134] In some embodiments, the engineered cell is homozygous for HLA-B and homozygous for HLA-C. In some embodiments, the HLA-B allele is selected from any one of the following HLA-B alleles: HLA-B*07:02; HLA-B*08:01; HLA-B*44:02; HLA-B*35:01; HLA-B*40:01; HLA-B*57:01; HLA-B*14:02; HLA-B*15:01; HLA-B*13:02; HLA-B*44:03; HLA-B*38:01; HLA-B*18:01; HLA-B*44:03; HLA-B*51:01; HLA-B*49:01; HLA-B*15:01; HLA-B*18:01; HLA-B*27:05; HLA-B*35:03; HLA-B*18:01; HLA-B*52:01; HLA-B*51:01; HLA-B*37:01; HLA-B*53:01; HLA-B*55:01; HLA-B*44:02; HLA-B*44:03; HLA-B*35:02; HLA-B*15:01; and HLA-B*40:02.

[00135] In some embodiments, the HLA-C allele is selected from any one of the following HLA-C alleles: HLA-C*07:02; HLA-C*07:01; HLA-C*05:01; HLA-C*04:01 HLA-C*03:04; HLA-C*06:02; HLA-C*08:02; HLA-C*03:03; HLA-C*06:02; HLA-C*16:01; HLA-C*12:03; HLA-C*07:01; HLA-C*04:01; HLA-C*15:02; HLA-C*07:01; HLA-C*03:04; HLA-C*12:03; HLA-C*02:02; HLA-C*04:01; HLA-C*05:01; HLA-C*12:02; HLA-C*14:02; HLA-C*06:02; HLA-C*04:01; HLA-C*03:03; HLA-C*07:04; HLA-C*07:01; HLA-C*04:01; HLA-C*04:01; and HLA-C*02:02.

[00136] In some embodiments, the HLA-B allele is selected from any one of the following HLA-B alleles: HLA-B*07:02; HLA-B*08:01; HLA-B*44:02; HLA-B*35:01; HLA-B*40:01; HLA-B*57:01; HLA-B*14:02; HLA-B*15:01; HLA-B*13:02; HLA-B*44:03; HLA-B*38:01; HLA-B*18:01; HLA-B*44:03; HLA-B*51:01; HLA-B*49:01; HLA-B*15:01; HLA-B*18:01; HLA-B*27:05; HLA-B*35:03; HLA-B*18:01; HLA-B*52:01; HLA-B*51:01; HLA-B*37:01; HLA-B*53:01; HLA-B*55:01; HLA-B*44:02; HLA-B*44:03; HLA-B*35:02; HLA-B*15:01; and HLA-B*40:02; and the HLA-C allele is selected from any one of the following HLA-C alleles: HLA-C*07:02; HLA-C*07:01; HLA-C*05:01; HLA-C*04:01 HLA-C*03:04; HLA-C*06:02; HLA-C*08:02; HLA-C*03:03; HLA-C*06:02; HLA-C*16:01; HLA-C*12:03; HLA-C*07:01; HLA-C*04:01; HLA-C*15:02; HLA-C*07:01; HLA-C*03:04; HLA-C*12:03; HLA-C*02:02; HLA-C*04:01; HLA-C*05:01; HLA-C*12:02; HLA-C*14:02; HLA-C*06:02; HLA-C*04:01; HLA-C*03:03; HLA-C*07:04; HLA-C*07:01; HLA-C*04:01; HLA-C*04:01; and HLA-C*02:02.

[00137] In some embodiments, the engineered cell is homozygous for HLA-B and homozygous for HLA-C and the HLA-B and HLA-C alleles are selected from any one of the following HLA-B and HLA-C alleles: HLA-B*07:02 and HLA-C*07:02; HLA-B*08:01 and HLA-C*07:01; HLA-B*44:02 and HLA-C*05:01; HLA-B*35:01 and HLA-C*04:01; HLA-B*40:01 and HLA-C*03:04; HLA-B*57:01 and HLA-C*06:02; HLA-B*14:02 and HLA-C*08:02; HLA-B*15:01 and HLA-C*03:03; HLA-B*13:02 and HLA-C*06:02; HLA-B*44:03 and HLA-C*16:01; HLA-B*38:01 and HLA-C*12:03; HLA-B*18:01 and HLA-C*07:01; HLA-B*44:03 and HLA-C*04:01; HLA-B*51:01 and HLA-C*15:02; HLA-B*49:01 and HLA-C*07:01; HLA-B*15:01 and HLA-C*03:04; HLA-B*18:01 and HLA-C*12:03; HLA-B*27:05 and HLA-C*02:02; HLA-B*35:03 and HLA-C*04:01; HLA-B*18:01 and HLA-C*05:01; HLA-B*52:01 and HLA-C*12:02; HLA-B*51:01 and HLA-C*14:02; HLA-B*37:01 and HLA-C*06:02; HLA-B*53:01 and HLA-C*04:01; HLA-B*55:01 and HLA-C*03:03; HLA-B*44:02 and HLA-C*07:04; HLA-B*44:03 and HLA-C*07:01; HLA-B*35:02 and HLA-C*04:01; HLA-B*15:01 and HLA-C*04:01; and HLA-

B*40:02 and HLA-C*02:02. In some embodiments, the cell is homozygous for HLA-B and homozygous for HLA-C and the HLA-B and HLA-C alleles are HLA-B*07:02 and HLA-C*07:02. In some embodiments, the cell is homozygous for HLA-B and homozygous for HLA-C and the HLA-B and HLA-C alleles are HLA-B*08:01 and HLA-C*07:01. In some embodiments, the cell is homozygous for HLA-B and homozygous for HLA-C and the HLA-B and HLA-C alleles are HLA-B*44:02 and HLA-C*05:01. In some embodiments, the cell is homozygous for HLA-B and homozygous for HLA-C and the HLA-B and HLA-C alleles are HLA-B*35:01 and HLA-C*04:01.

[00138] In some embodiments, the disclosure provides a pharmaceutical composition comprising any one of the engineered cells disclosed herein. In some embodiments, the pharmaceutical composition comprises a population of any one of the engineered cells disclosed herein. In some embodiments, the pharmaceutical composition comprises a population of engineered cells that is at least 65% negative as measured by flow cytometry. In some embodiments, the pharmaceutical composition comprises a population of engineered cells that is at least 70% negative as measured by flow cytometry. In some embodiments, the pharmaceutical composition comprises a population of engineered cells that is at least 80% negative as measured by flow cytometry. In some embodiments, the pharmaceutical composition comprises a population of engineered cells that is at least 90% negative as measured by flow cytometry. In some embodiments, the pharmaceutical composition comprises a population of engineered cells that is at least 91% negative as measured by flow cytometry. In some embodiments, the pharmaceutical composition comprises a population of engineered cells that is at least 92% negative as measured by flow cytometry. In some embodiments, the pharmaceutical composition comprises a population of engineered cells that is at least 93% negative as measured by flow cytometry. In some embodiments, the pharmaceutical composition comprises a population of engineered cells that is at least 94% negative as measured by flow cytometry. In some embodiments, the pharmaceutical composition comprises a population of engineered cells that is at least 95% endogenous TCR protein negative as measured by flow cytometry. In some embodiments, the pharmaceutical composition comprises a population of engineered cells that is at least 97% endogenous TCR protein negative as measured by flow cytometry. In some embodiments, the pharmaceutical composition comprises a population of engineered cells that is at least 98% endogenous TCR protein negative as measured by flow cytometry. In some embodiments, the pharmaceutical composition comprises a population of engineered cells that is at least 99% endogenous TCR protein negative as measured by flow cytometry.

[00139] In some embodiments, methods are provided for administering the engineered cells or pharmaceutical compositions disclosed herein to a subject in need thereof. In some embodiments, methods are provided for administering the engineered cells or pharmaceutical compositions disclosed herein to a subject as an ACT therapy. In some embodiments, methods are provided for administering the engineered cells or pharmaceutical compositions disclosed herein to a subject as a treatment for cancer. In some embodiments, methods are provided for administering the engineered cells or pharmaceutical compositions disclosed herein to a subject as a treatment for an autoimmune disease. In some embodiments, methods are provided for administering the engineered cells or pharmaceutical compositions disclosed herein to a subject as a treatment for an infectious disease.

B. Methods and Compositions for Reducing or Eliminating Surface Expression of MHC Class II

[00140] The present disclosure provides methods and compositions for reducing or eliminating surface expression of MHC class II protein on a cell relative to an unmodified cell by genetically modifying the CIITA gene. The resultant genetically modified cell may also be referred to herein as an engineered cell. In some embodiments, an already-genetically modified (or engineered) cell may be the starting cell for further genetic modification using the methods or compositions provided herein. In some embodiments, the cell is an allogeneic cell. In some embodiments, a cell with reduced MHC class II expression is useful for adoptive cell transfer therapies. In some embodiments, editing of the CIITA gene is combined with additional genetic modifications to yield a cell that is desirable for allogeneic transplant purposes.

[00141] In some embodiments, the methods comprise reducing or eliminating surface expression of MHC class II protein on the surface of a cell comprising contacting a cell with a composition comprising a CIITA guide RNA comprising i) a guide sequence selected from SEQ ID NOs: 1-117; ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-117; ii). a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-117; iv) a sequence that comprises 10 contiguous nucleotides $\pm$ 10 nucleotides of a genomic coordinate listed in **Table 2**; v) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (iv); or vi) a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (v). In some embodiments, the methods further comprise contacting the cell with an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent. In some embodiments, the RNA-guided DNA binding agent is Cas9. In some embodiments, the RNA-guided DNA binding agent is *S.*

pyogenes Cas9. In some embodiments, the CIITA guide RNA is a *S. pyogenes* Cas9 guide RNA. In some embodiments, the RNA-guided DNA binding agent further comprises a deaminase domain. In some embodiments the RNA-guided DNA binding agent comprises an APOBEC3A deaminase (A3A) and an RNA-guided nickase. In some embodiments, the expression of MHC class II protein on the surface of the cell (*i.e.*, engineered cell) is thereby reduced.

[00142] In some embodiments, the methods comprise making an engineered cell, which has reduced or eliminated surface expression of MHC class II protein relative to an unmodified cell, comprising contacting the cell with a composition comprising a CIITA guide RNA comprising i) a guide sequence selected from SEQ ID NOs: 1-117; ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-117; iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-117; iv) a sequence that comprises 10 contiguous nucleotides $\pm$ 10 nucleotides of a genomic coordinate listed in **Table 2**; v) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (iv); or vi) a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (v). In some embodiments, the methods further comprise contacting the cell with an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent. In some embodiments, the RNA-guided DNA binding agent is Cas9. In some embodiments, the RNA-guided DNA binding agent is *S. pyogenes* Cas9. In some embodiments, the CIITA guide RNA is a *S. pyogenes* Cas9 guide RNA. In some embodiments, the RNA-guided DNA binding agent further comprises a deaminase region. In some embodiments the RNA-guided DNA binding agent comprises an APOBEC3A deaminase (A3A) and an RNA-guided nickase. In some embodiments, the expression of MHC class II protein on the surface of the cell (*i.e.*, engineered cell) is thereby reduced.

[00143] In some embodiments, the methods comprise genetically modifying a cell to reduce or eliminate the surface expression of MHC class II protein comprising contacting the cell with a composition comprising a CIITA guide RNA comprising i) a guide sequence selected from SEQ ID NOs: 1-117; ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-117; iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-117; iv) a sequence that comprises 10 contiguous nucleotides $\pm$ 10 nucleotides of a genomic coordinate listed in **Table 2**; v) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (iv); or vi) a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (v). In some embodiments, the methods further comprise contacting the cell with an RNA-guided DNA binding agent or a nucleic acid

encoding an RNA-guided DNA binding agent. In some embodiments, the RNA-guided DNA binding agent is Cas9. In some embodiments, the RNA-guided DNA binding agent is *S. pyogenes* Cas9. In some embodiments, the CIITA guide RNA is a *S. pyogenes* Cas9 guide RNA. In some embodiments, the RNA-guided DNA binding agent further comprises a deaminase region. In some embodiments the RNA-guided DNA binding agent comprises an APOBEC3A deaminase (A3A) and an RNA-guided nickase. In some embodiments, the expression of MHC class II protein on the surface of the cell (*i.e.*, engineered cell) is thereby reduced.

[00144] In some embodiments, the methods of reducing expression of an MHC class II protein on the surface of a cell comprise contacting a cell with any one or more of the CIITA guide RNAs disclosed herein. In some embodiments, the CIITA guide RNA comprises a guide sequence selected from SEQ ID NO: 1-117.

[00145] In some embodiments, compositions are provided comprising a CIITA guide RNA comprising i) a guide sequence selected from SEQ ID NOs: 1-117; ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-117; iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-117; iv) a sequence that comprises 10 contiguous nucleotides ± 10 nucleotides of a genomic coordinate listed in **Table 2**; v) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (iv); or vi) a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (v). In some embodiments, the composition further comprises an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent. In some embodiments, the composition comprises an RNA-guided DNA binding agent that is Cas9. In some embodiments, the RNA-guided DNA binding agent is *S. pyogenes* Cas9. In some embodiments, the CIITA guide RNA is a *S. pyogenes* Cas9 guide RNA. In some embodiments, the RNA-guided DNA binding agent further comprises a deaminase region. In some embodiments the RNA-guided DNA binding agent comprises an APOBEC3A deaminase (A3A) and an RNA-guided nickase.

[00146] In any of the foregoing embodiments, the guide sequence is selected from SEQ ID NO: 32, 64, 67, 68, 74, 76, 84, 86, 90, 91, and 115; ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 32, 64, 67, 68, 74, 76, 84, 86, 90, 91, and 115; iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 32, 64, 67, 68, 74, 76, 84, 86, 90, 91, and 115.

[00147] In some embodiments, the composition further comprises a uracil glycosylase inhibitor (UGI). In some embodiments, the composition comprises an RNA-guided DNA

binding agent that the RNA-guided DNA binding agent generates a cytosine (C) to thymine (T) conversion with the CIITA genomic target sequence. In some embodiments, the composition comprises an RNA-guided DNA binding agent that generates a adenosine (A) to guanine (G) conversion with the CIITA genomic target sequence.

[00148] In some embodiments, an engineered cell produced by the methods described herein is provided. In some embodiments, the engineered cell produced by the methods and compositions described herein is an allogeneic cell. In some embodiments, the methods produce a composition comprising an engineered cell having reduced MHC class II expression. In some embodiments, the methods produce a composition comprising an engineered cell having reduced CIITA protein expression. In some embodiments, the methods produce a composition comprising an engineered cell having reduced CIITA levels in the cell nucleus. In some embodiments, the methods produce a composition comprising an engineered cell that expresses a truncated form of the CIITA protein. In some embodiments, the methods produce a composition comprising an engineered cell that produces no detectable CIITA protein. In some embodiments, the engineered cell has reduced MHC class II expression, reduced CIITA protein, and/or reduced CIITA levels in the cell nucleus as compared to an unmodified cell. In some embodiments, the engineered cell produced by the methods disclosed herein elicits a reduced response from CD4⁺ T cells as compared to an unmodified cell as measured in an *in vitro* cell culture assay containing CD4⁺ T cells.

[00149] In some embodiments, the compositions disclosed herein further comprise a pharmaceutically acceptable carrier. In some embodiments, a cell produced by the compositions disclosed herein comprising a pharmaceutically acceptable carrier is provided. In some embodiments, compositions comprising the cells disclosed herein are provided.

1. CIITA guide RNAs

[00150] The methods and compositions provided herein disclose CIITA guide RNAs useful for reducing the expression of MHC class II protein on the surface of a cell. In some embodiments, such guide RNAs direct an RNA-guided DNA binding agent to a CIITA genomic target sequence and may be referred to herein as “CIITA guide RNAs.” In some embodiments, the CIITA guide RNA directs an RNA-guided DNA binding agent to a human CIITA genomic target sequence. In some embodiments, the CIITA guide RNA comprises a guide sequence selected from SEQ ID NO: 1-117.

[00151] In some embodiments, a composition is provided comprising a CIITA guide RNA described herein and an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[00152] In some embodiments, a CIITA single-guide RNA (sgRNA) comprising a guide sequence selected from SEQ ID NO: 1-117 is provided. In some embodiments, a composition is provided comprising a CIITA single-guide RNA (sgRNA) comprising a guide sequence selected from SEQ ID NO: 1-117. In some embodiments, a composition is provided comprising a CIITA sgRNA described herein and an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[00153] In some embodiments, a CIITA dual-guide RNA (dgRNA) comprising a guide sequence selected from SEQ ID NO: 1-117 is provided. In some embodiments, a composition is provided comprising a CIITA dual-guide RNA (dgRNA) comprising a guide sequence selected from SEQ ID NO: 1-117. In some embodiments, a composition is provided comprising a CIITA dgRNA described herein and an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[00154] Exemplary CIITA guide sequences are shown below in **Table 2** (SEQ ID NOs: 1-117 with corresponding guide RNA sequences SEQ ID NOs: 218-334 and 335-426).

[00155] **Table 2. Exemplary CIITA guide sequences.**

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
CR002961	1	crRNA	CAGCUC ACAGUG UGCCAC CA	CAGCUCACAG UGUGCCACCA GUUUUAGAG CUAUGCUGU UUUG		chr16:10895282-10895302
CR002966	2	crRNA	UUCUAG GGGCCC CAACUC CA	UUCUAGGGG CCCCAACUCC AGUUUUAGA GCUAUGCUG UUUUG		chr16:10895302-10895322
CR002967	3	crRNA	AUGGAG UUGGGG CCCCUA GA	AUGGAGUUG GGGCCCCUAG AGUUUUAGA GCUAUGCUG UUUUG		chr16:10895301-10895321
CR002971	4	crRNA	CUCCAG GUAGCC ACCUUC UA	CUCCAGGUA GCCACCUUCU AGUUUUAGA GCUAUGCUG UUUUG		chr16:10895317-10895337
CR002991	5	crRNA	AGGCUG UUGUGU	AGGCUGUUG UGUGACAUG		chr16:10895706-10895726

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
			GACAUGGA	GAGUUUUAGAGCUAUGCUUUUUG		
CR002995	6	crRNA	GAUAUU GGCAUA AGCCUC CC	GAUAUUGGC AUAAGCCUCC CGUUUUAGA GCUAUGCUG UUUUG		chr16:10895743-10895763
CR003009	7	crRNA	UGAAGU GAUCGG UGAGAG UA	UGAAGUGAU CGGUGAGAG UAGUUUUAG AGCUAUGCU GUUUUG		chr16:10898940-10898960
CR003011	8	crRNA	UGGAGA UGCCAG CAGAAG UU	UGGAGAUGC CAGCAGAAG UUGUUUUAG AGCUAUGCU GUUUUG		chr16:10898960-10898980
CR003014	9	crRNA	GGUCUG CCGGAA GCUCCU CU	GGUCUGCCG GAAGCUCCUC UGUUUUAGA GCUAUGCUG UUUUG		chr16:10901520-10901540
CR007938	10	crRNA	UUUUAC CUUGGG GCUCUG AC	UUUUACCUU GGGGCUCUG ACGUUUUAG AGCUAUGCU GUUUUG		chr16:10877368-10877388
CR007955	11	crRNA	UCCAAG CCCCCU AACAU CU	UCCAAGCCCC CUAACAUAC UGUUUUAGA GCUAUGCUG UUUUG		chr16:10902183-10902203
CR007982	12	crRNA	CCCCCG GACGGU UCAAGC AA	CCCCCGGACG GUUCAAGCA AGUUUUAGA GCUAUGCUG UUUUG		chr16:10906853-10906873
CR007994	13	crRNA	GGACGG UUCAAG CAAUGG CA	GGACGGUUC AAGCAAUGG CAGUUUUAG AGCUAUGCU GUUUUG		chr16:10906848-10906868
CR007997	14	crRNA	CCCGGA UGGCAU CCUAGU GG	CCCGGAUGGC AUCCUAGUG GGUUUUAGA GCUAUGCUG UUUUG		chr16:10906556-10906576
CR009188	15	crRNA	CAGUGG CUGAUG GAGCGA AG	CAGUGGCUG AUGGAGCGA AGGUUUUAG AGCUAUGCU GUUUUG		chr16:10903824-10903844
CR009202	16	crRNA	GAGAAG ACAAAG	GAGAAGACA AAGUCGUAC		chr16:10906821-10906841

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
			UCGUAC UG	UGGUUUUAG AGCUAUGCU GUUUUG		
CR009206	17	crRNA	CGUCUA GGAUGA GCAGAA CG	CGUCUAGGA UGAGCAGAA CGGUUUUAG AGCUAUGCU GUUUUG		chr16:10906970-10906990
CR009208	18	crRNA	GACGGU UCAAGC AAUGGC AG	GACGGUUCA AGCAAUGGC AGGUUUUAG AGCUAUGCU GUUUUG		chr16:10906847-10906867
CR009211	19	crRNA	UGAGAA GAAGUG GCCGGU CC	UGAGAAGAA GUGGCCGGU CCGUUUUAG AGCUAUGCU GUUUUG		chr16:10907288-10907308
CR009217	20	crRNA	UGGUCA GGGCAA GAGCUA UU	UGGUCAGGG CAAGAGCUA UUGUUUUAG AGCUAUGCU GUUUUG		chr16:10906757-10906777
CR009229	21	crRNA	GUUCCU CGGAAG ACACAG CU	GUUCCUCGG AAGACACAG CUGUUUUAG AGCUAUGCU GUUUUG		chr16:10910195-10910215
CR009230	22	crRNA	UUCCUC GGAAGA CACAGC UG	UUCCUCGGA AGACACAGC UGGUUUUAG AGCUAUGCU GUUUUG		chr16:10910196-10910216
CR009234	23	crRNA	GCUGAG UGAGAA CAAGAU CG	GCUGAGUGA GAACAAGAU CGGUUUUAG AGCUAUGCU GUUUUG		chr16:10916375-10916395
CR009235	24	crRNA	GAGAAC AAGAUC GGGGAC GA	GAGAACAAAG AUCGGGGAC GAGUUUUAG AGCUAUGCU GUUUUG		chr16:10916382-10916402
CR009238	25	crRNA	CCACAU GAGGAC ACCUCC GA	CCACAUGAG GACACCUCCG AGUUUUAGA GCUAUGCUG UUUUG		chr16:10922460-10922480
G013674	26	sgRNA	UUCUAG GGGCCC CAACUC CA	UUCUAGGGG CCCCAACUCC AGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU	mU*mU*mC*UAGG GGCCCCAACUCCA GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA	chr16:10895302-10895322

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
				AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	
G013675	27	sgRNA	CCCCCG GACGGU UCAAGC AA	CCCCCGGACG GUUCAAGCA AGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mC*mC*CCGG ACGGUUCAAGCAA GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109068 53-10906873
G013676	28	sgRNA	UGGUCA GGGCAA GAGCUA UU	UGGUCAGGG CAAGAGCUA UUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mU*mG*mG*UCAG GGCAAGAGCUAU UGUUUUAGAmGm CmUmAmGmAmAm AmUmAmGmCAAG UAAAAUAAGGC UAGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109067 57-10906777
G015535	29	sgRNA	UUCUAG GGGCCC CAACUC CA	UUCUAGGGG CCCCAACUCC AGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	UUCUAGGGGCCCC AACUCCAGUUUUA GAGCUAGAAAAU GCAAGUAAAAU AAGGCUAGUCCGU UAUCAACUUGAA AAAGUGGCACCGA GUCGGUGCUUUU	chr16:108953 02-10895322
G016030	30	sgRNA	UCAACU GCGACC AGUUCA GC	UCAACUGCG ACCAGUUCA GCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mU*mC*mA*ACUG CGACCAGUUCAGC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG	chr16:108956 86-10895706

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
					mGmUmGmCmU*mU*mU*mU	
G016031	31	sgRNA	AGCGCA GGCAGU GGCAGG CA	AGCGCAGGC AGUGGCAGG CAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mA*mG*mC*GCAG GCAGUGGCAGGCA GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	chr16:10902105-10902125
G016032	32	sgRNA	ACCUGC AACAAC AGGAUU CA	ACCUGCAACA ACAGGAUUC AGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mA*mC*mC*UGCA ACAACAGGAUUC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	chr16:10923265-10923285
G016033	33	sgRNA	CCAGGA ACACCU GCAACA AC	CCAGGAACAC CUGCAACAAC GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mC*mA*GGAA CACCUGCAACAAC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	chr16:10923257-10923277
G016034	34	sgRNA	CAGCAG CAAGAG CCUGGA GC	CAGCAGCAA GAGCCUGGA GCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mC*mA*mG*CAGC AAGAGCCUGGAGC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	chr16:10906610-10906630

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
G016035	35	sgRNA	GCAGCA GCAAGA GCCUGG AG	GCAGCAGCA AGAGCCUGG AGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mG*mC*mA*GCAG CAAGAGCCUGGAG GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109066 09-10906629
G016036	36	sgRNA	CAAUCU CUUCUU CUCCAG CC	CAAUCUCUUC UUCUCCAGCC GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mA*mA*UCUC UUCUUCUCCAGCC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:108953 96-10895416
G016037	37	sgRNA	AGCAGC UCGCUG CCAGCC UU	AGCAGCUCGC UGCCAGCCUU GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mA*mG*mC*AGCU CGCUGCCAGCCUU GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109224 41-10922461
G016038	38	sgRNA	AGCUCG CUGCCA GCCUUC GG	AGCUCGCUGC CAGCCUUCGG GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mA*mG*mC*UCGC UGCCAGCCUUCGG GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109224 44-10922464
G016039	39	sgRNA	CUCUGG ACCAGG	CUCUGGACCA GGCGGCCCCG GUUUUAGAG	mC*mU*mC*UGGA CCAGGCGGCCCCG GUUUUAGAmGmC	chr16:109071 39-10907159

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
			CGGCCC CG	CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	
G016040	40	sgRNA	GCAGCC CUCGAC AGCCCC CC	GCAGCCUCG ACAGCCCCC GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mC*mA*GCCC UCGACAGCCCCC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109074 33-10907453
G016041	41	sgRNA	CAGCCC UCGACA GCCCCC C	CAGCCUCGA CAGCCCCC GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mA*mG*CCCU CGACAGCCCCC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109074 34-10907454
G016042	42	sgRNA	AGCCAA GUACCC CCUCCC AG	AGCCAAGUA CCCCUCCCA GGUUUAGA GCUAGAAU AGCAAGUUA AAUAAGGCU UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mA*mG*mC*CAAG UACCCCUCCAG GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109037 47-10903767
G016043	43	sgRNA	CAGCCA ACAGCA CCUCAG CC	CAGCCAACAG CACCUCAGCC GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU	mC*mA*mG*CCAA CAGCACCUCAGCC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU	chr16:109066 82-10906702

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
				AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	
G016044	44	sgRNA	UGCCGC GCCCCG AGUGUC CC	UGCCGCGCCC GCAGUGUCCC GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mU*mG*mC*CGCG CCCCGAGUGUCCC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109078 86-10907906
G016045	45	sgRNA	CGACAG CCCCCC GGGGCC C	CGACAGCCCC CCCCGGGGCCC GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mG*mA*CAGC CCCCCGGGGCCC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109074 41-10907461
G016046	46	sgRNA	GGCAGC GAGCUG CUGGGC CC	GGCAGCGAG CUGCUGGGCC CGUUUUAGA GCUAGAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mG*mC*AGCG AGCUGCUGGGCCC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109224 35-10922455
G016047	47	sgRNA	GCCAGC UCUGCC AGGGCC CC	GCCAGCUCUG CCAGGGCCCC GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC	mG*mC*mC*AGCU CUGCCAGGGCCCC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU	chr16:109074 54-10907474

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334) ACCGAGUCG GUGCUUUU	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
					mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	
G016048	48	sgRNA	AGCGAG CGAAGG CAGGGC CU	AGCGAGCGA AGGCAGGGC CUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mA*mG*mC*GAGC GAAGGCAGGGCCU GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109184 86-10918506
G016049	49	sgRNA	AAGGCU GGCAGC GAGCUG CU	AAGGCUGGC AGCGAGCUG CUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mA*mA*mG*GCUG GCAGCGAGCUGCU GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109224 41-10922461
G016050	50	sgRNA	AGCCCU CGACAG CCCCCCC G	AGCCCU CGAC AGCCCCCCCCG GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mA*mG*mC*CCUC GACAGCCCCCG GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109074 35-10907455
G016051	51	sgRNA	GGAUGC AGCGAG CGAAGG CA	GGAUGCAGC GAGCGAAGG CAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mG*mG*mA*UGCA GCGAGCGAAGGCA GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109184 92-10918512

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
					mGmUmGmCmU*mU*mU*mU	
G016052	52	sgRNA	UCCACC GAGGCA GCCGCC GA	UCCACCGAGG CAGCCGCCGA GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mU*mC*mC*ACCG AGGCAGCCGCCGA GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	chr16:109078 20-10907840
G016053	53	sgRNA	UCUCCA ACAAGC UUCCAA AA	UCUCCAACAA GCUUCCAAA AGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mU*mC*mU*CCAA CAAGCUUCCAAAA GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	chr16:109047 85-10904805
G016054	54	sgRNA	AGCAGC CCCCGG AGGGAG CA	AGCAGCCCCC GGAGGGAGC AGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mA*mG*mC*AGCC CCCCGAGGGAGCA GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	chr16:109070 58-10907078
G016055	55	sgRNA	GCCACA GCCCUA CUUUGU GC	GCCACAGCCC UACUUUGUG CGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mC*mC*ACAG CCCUACUUUGUGC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	chr16:109073 14-10907334

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
G016056	56	sgRNA	CUGCGC CCACGA GGCCGA GG	CUGCGCCAC GAGGCCGAG GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mU*mG*CGCC CACGAGGCCGAGG GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109079 24-10907944
G016057	57	sgRNA	CAGCCG CCGAUG GCCCGA GU	CAGCCGCCGA UGGCCCGAG UGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mA*mG*CCGC CGAUGGCCCGAGU GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109078 10-10907830
G016058	58	sgRNA	CGAGGC UCCCCA AUCCAG AG	CGAGGCUCCC CAAUCCAGA GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mG*mA*GGCU CCCCAAUCCAGAG GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109081 01-10908121
G016059	59	sgRNA	CUCAAC GAGGAA CUGGAG AA	CUCAACGAG GAACUGGAG AAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mC*mU*mC*AACG AGGAACUGGAGA AGUUUUAGAmGm CmUmAmGmAmAm AmUmAmGmCAAG UUAAAAUAAGGC UAGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109026 62-10902682
G016060	60	sgRNA	CCACAG CCCUAC	CCACAGCCCU ACUUUGUGC CGUUUUAGA	mC*mC*mA*CAGC CCUACUUUGUGCC GUUUUAGAmGmC	chr16:109073 15-10907335

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
			UUUGUGCC	GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	
G016061	61	sgRNA	CAAGAG CCUGGA GCGGGA AC	CAAGAGCCU GGAGCGGGA ACGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mC*mA*mA*GAGC CUGGAGCGGGAAC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109066 16-10906636
G016062	62	sgRNA	GACUGC CAGUCA CCACAG UG	GACUGCCAG UCACCACAGU GGUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mA*mC*UGCC AGUACCACAGUG GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109020 47-10902067
G016063	63	sgRNA	GCCCAC GAGGCC GAGGAG GC	GCCCACGAGG CCGAGGAGG CGUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mC*mC*CACG AGGCCGAGGAGGC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109079 28-10907948
G016064	64	sgRNA	AUCAGC CCAGCC AGAAAG CG	AUCAGCCCAG CCAGAAAGC GGUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC	mA*mU*mC*AGCC CAGCCAGAAAGCG GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU	chr16:109077 57-10907777

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
					AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	
G016065	65	sgRNA	CAGAGA AGACAA AGUCGU AC	CAGAGAAGA CAAAGUCGU ACGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mC*mA*mG*AGAA GACAAAGUCGUAC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109068 23-10906843
G016066	66	sgRNA	UGGGAG UCCUG CAGCAG CA	UGGGAGUCC CUGCAGCAGC AGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mU*mG*mG*GAGU CCCUGCAGCAGCA GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109090 56-10909076
G016067	67	sgRNA	CAGCAG GCUGUU GUGUGA CA	CAGCAGGCU GUUGUGUGA CAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mC*mA*mG*CAGG CUGUUGUGUGAC AGUUUUAGAmGm CmUmAmGmAmAm AmUmAmGmCAAG UUAAAAUAAGGC UAGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:108957 02-10895722
G016068	68	sgRNA	CUGGUC AGGGCA AGAGCU AU	CUGGUCAGG GCAAGAGCU AUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG	mC*mU*mG*GUCA GGGCAAGAGCUA UGUUUUAGAmGm CmUmAmGmAmAm AmUmAmGmCAAG UUAAAAUAAGGC UAGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU	chr16:109067 56-10906776

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334) GCACCGAGUC GGUGCUUUU	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
					mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	
G016069	69	sgRNA	CAGGUU CAGGCA UGCUGG GC	CAGGUUCAG GCAUGCUGG GCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mC*mA*mG*GUUC AGGCAUGCUGGGC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109038 48-10903868
G016070	70	sgRNA	UUUCCA AGGACU UCAGCU GG	UUUCCAAGG ACUUCAGCU GGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mU*mU*mU*CCAA GGACUUCAGCUGG GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109164 32-10916452
G016071	71	sgRNA	AGGCCG AGGAGG CUGGAA UU	AGGCCGAGG AGGCUGGAA UUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mA*mG*mG*CCGA GGAGGCUGGAAU UGUUUUAGAmGm CmUmAmGmAmAm AmUmAmGmCAAG UUAAAAUAAGGC UAGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109079 35-10907955
G016072	72	sgRNA	CAGUGG CUGAUG GAGCGA AG	CAGUGGCUG AUGGAGCGA AGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mC*mA*mG*UGGC UGAUGGAGCGAA GGUUUUAGAmGm CmUmAmGmAmAm AmUmAmGmCAAG UUAAAAUAAGGC UAGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109038 24-10903844

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
					mGmUmGmCmU*mU*mU*mU	
G016073	73	sgRNA	GGGAUG CAGCGA GCGAAG GC	GGGAUGCAG CGAGCGAAG GCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mG*mG*mG*AUGC AGCGAGCGAAGGC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	chr16:109184 93-10918513
G016074	74	sgRNA	AAGCUG CCCUCC ACGCUC AC	AAGCUGCCCU CCACGCUCAC GUUUUAGAG CUAGAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mA*mA*mG*CUGC CCUCCACGCUCAC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	chr16:109073 85-10907405
G016075	75	sgRNA	UUCUC CUGCAA UGCUUC CU	UUCUCCUGC AAUGCUUCC UGUUUUAGA GCUAGAAAU AGCAAGUUA AAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mU*mU*mC*CUCC UGCAAUGCUUCCU GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	chr16:109076 22-10907642
G016076	76	sgRNA	UCCUCC UGCAAU GCUUCC UG	UCCUCCUGCA AUGCUUCCU GGUUUUAGA GCUAGAAAU AGCAAGUUA AAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mU*mC*mC*UCCU GCAAUGCUUCCUG GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	chr16:109076 23-10907643

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
G016077	77	sgRNA	CAAGCU GCCCUC CACGCU CA	CAAGCUGCCC UCCACGCUCA GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mA*mA*GCUG CCCUCCACGCUCA GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109073 84-10907404
G016078	78	sgRNA	UCCUCG GACCUC CGCAGC AC	UCCUCGGACC UCCGCAGCAC GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mU*mC*mC*CUUG ACCUCCGCAGCAC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109080 69-10908089
G016079	79	sgRNA	UCCAGC CUCCUC GGCCUC GU	UCCAGCCUCC UCGGCCUCGU GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mU*mC*mC*AGCC UCCUCGGCCUCGU GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109079 32-10907952
G016080	80	sgRNA	CUUCUC CAGCCA GGUCCA UC	CUUCUCCAGC CAGGUCCAUC GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mU*mU*CUCC AGCCAGGUCCAUC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:108953 87-10895407
G016081	81	sgRNA	CUUCCU CCUGCA	CUUCCUCCUG CAAUGCUUCC GUUUUAGAG	mC*mU*mU*CCUC CUGCAAUGCUUCC GUUUUAGAmGmC	chr16:109076 21-10907641

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
			AUGCUUCC	CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*mU U*mU*mU	
G016082	82	sgRNA	CGUCCU CCCCAA GCUCCA GC	CGUCCUCCCC AAGCUCCAGC GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mG*mU*CCUC CCCCAAGCUCCAGC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*mU U*mU*mU	chr16:109073 63-10907383
G016083	83	sgRNA	CCAGCU CCUCGA AGCCGU CU	CCAGCUCCUC GAAGCCGUC UGUUUAGA GCUAGAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mC*mA*GCUC CUCGAAGCCGUCU GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*mU U*mU*mU	chr16:109069 85-10907005
G016084	84	sgRNA	CUUUUC CUCCCU GCAGCA UC	CUUUUCCUCC CUGCAGCAUC GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mU*mU*UUC UCCCGCAGCAUC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*mU U*mU*mU	chr16:109156 26-10915646
G016085	85	sgRNA	CGGCCG CCACGA GUGGCU GU	CGGCCGCCAC GAGUGGCUG UGUUUAGA GCUAGAAU AGCAAGUUA AAAUAAGGC	mC*mG*mG*CCGC CACGAGUGGCUGU GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU	chr16:109069 13-10906933

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
				UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	
G016086	86	sgRNA	CGCCCA GGUCCU CACGUC UG	CGCCCAGGUC CUCACGUCUG GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mG*mC*CCAG GUCCUCACGUCUG GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109075 39-10907559
G016087	87	sgRNA	CACGUG CGGACC GGCACC GG	CACGUGCGG ACCGGCACCG GGUUUUAGA GCUAGAAAU AGCAAGUUA AAUAAGGCU UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mA*mC*GUGC GGACCGGCACCGG GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109070 30-10907050
G016088	88	sgRNA	CAGCUU GGCCAG CUCUGC CA	CAGCUUGGCC AGCUCUGCCA GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mA*mG*CUUG GCCAGCUCUGCCA GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109074 61-10907481
G016089	89	sgRNA	UCGGAC UCUGCG GCCCCG GG	UCGGACUCU GCGGCCCGCG GGUUUUAGA GCUAGAAAU AGCAAGUUA AAUAAGGCU UAGUCCGUU AUCAACUUG AAAAAGUGG	mU*mC*mG*GACU CUGCGGCCCGCGG GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU	chr16:109075 86-10907606

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334) CACCGAGUCG GUGCUUUU	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
					mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	
G016090	90	sgRNA	CUUCCC CCAGCU GAAGUC CU	CUUCCCCCAG CUGAAGUCC UGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mU*mU*CCCC CAGCUGAAGUCCU GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109164 26-10916446
G016091	91	sgRNA	GCCCAG CUCCCA GGCCAG CU	GCCCAGCUCC CAGGCCAGCU GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mC*mC*CAGC UCCAGGCCAGCU GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109074 76-10907496
G016092	92	sgRNA	CCCUCC AGCCAG UUGUCA UA	CCCUCCAGCC AGUUGUCAU AGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mC*mC*UCCA GCCAGUUGUCAUA GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109077 30-10907750
G016093	93	sgRNA	GCCCUC CAGCCA GUUGUC AU	GCCCUCAGC CAGUUGUCA UGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mC*mC*CUCC AGCCAGUUGUCAU GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109077 31-10907751

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
					mGmUmGmCmU*mU*mU*mU	
G016094	94	sgRNA	CCCUGA CGCUCC UCCGGG AC	CCCUGACGCU CCUCCGGGAC GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mC*mC*UGAC GCUCCUCCGGGAC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	chr16:109072 72-10907292
G016095	95	sgRNA	CACACU GCCCGG CACAAA GU	CACACUGCCC GGCACAAAG UGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mA*mC*ACUG CCCGGCACAAAGU GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	chr16:109073 25-10907345
G016096	96	sgRNA	CAGCUC ACAGUG UGCCAC CA	CAGCUCACAG UGUGCCACCA GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mA*mG*CUCA CAGUGUGCCACCA GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	chr16:108952 82-10895302
G016097	97	sgRNA	AGCUCG GACUCU GCGGCC CG	AGCUCGGAC UCUGCGGCC GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mA*mG*mC*UCGG ACUCUGCGGCCCG GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	chr16:109075 89-10907609

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
G016098	98	sgRNA	GACGCC CUAUUU GAGCUG UC	GACGCCCUAU UUGAGCUGU CGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGG UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mA*mC*GCCC UAUUUAGCUGU CGUUUUAGAmGm CmUmAmGmAmAm AmUmAmGmCAAG UAAAAUAAGGC UAGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109071 72-10907192
G016099	99	sgRNA	GCCAGU GCUGCG GAGGUC CA	GCCAGUGCU GCGGAGGUC CAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mG*mC*mC*AGUG CUGCGGAGGUCCA GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109080 73-10908093
G016100	100	sgRNA	AGGGCU CCCAGG CAGCGG GC	AGGGCUCCCA GGCAGCGGG CGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGG UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mA*mG*mG*GCUC CCAGGCAGCGGGC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109077 90-10907810
G016101	101	sgRNA	GCCGAG CCCACA GGCCCG GA	GCCGAGCCCG CAGGCCCGGA GUUUUAGAG CUAGAAAU GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mG*mC*mC*GAGC CCGAGGCCCGGA GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109065 42-10906562
G016102	102	sgRNA	GCUCCC AGGCAG	GCUCCCAGGC AGCGGGCGG GGUUUUAGA	mG*mC*mU*CCCA GGCAGCGGGCGG GUUUUAGAmGmC	chr16:109077 87-10907807

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
			CGGGCG GG	GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	
G016103	103	sgRNA	CCUCUC CAGCUG CCGGGC AU	CCUCUCCAGC UGCCGGGCA UGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mC*mU*CUCC AGCUGCCGGGCAU GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109047 65-10904785
G016104	104	sgRNA	AGGCUU UCCCCA AACUGG UG	AGGCUUUCCC CAAACUGGU GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mA*mG*mG*CUUU CCCCAAACUGGUG GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109155 92-10915612
G016105	105	sgRNA	CCAUCU CCACUC UGCCCC AU	CCAUCUCCAC UCUGCCCCAU GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mC*mA*UCUC CACUCUGCCCCAU GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109027 23-10902743
G016106	106	sgRNA	UCCUCC UCACAG CCCGGC CC	UCCUCCUCAC AGCCCGGCC GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU	mU*mC*mC*UCCU CACAGCCCGGCC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU	chr16:109071 19-10907139

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
					AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	
G016107	107	sgRNA	CCACUC UGCCCC AUGGGC UC	CCACUCUGCC CCAUGGGCUC GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mC*mA*CUCU GCCCCAUGGGCUC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109027 29-10902749
G016108	108	sgRNA	CAGCCU CCCGCCC GCUGCC U	CAGCCUCCCG CCCGCUGCCU GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mA*mG*CCUC CCGCCGCUGCCU GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109077 81-10907801
G016109	109	sgRNA	CCCGGC CGCCUC UCUUUU CU	CCCGGCCGCC UCUCUUUUC UGUUUAGA GCUAGAAU AGCAAGUUA AAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mC*mC*GGCC GCCUCUCUUUUCU GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109079 79-10907999
G016110	110	sgRNA	CCUGGG CCCACA GCCACU CG	CCUGGGCCCA CAGCCACUCG GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC	mC*mC*mU*GGGC CCACAGCCACUCG GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU	chr16:109069 04-10906924

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334) ACCGAGUCG GUGCUUUU	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
					mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	
G016111	111	sgRNA	UCCCCG GCUGCA GCCGCU UC	UCCCCGGCUG CAGCCGCUUC GUUUUAGAG CUAGAAUA GCAAGUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mU*mC*mC*CCGG CUGCAGCCGCUUC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109078 70-10907890
G016112	112	sgRNA	CGCGUU CUGCUC AUCCUA GA	CGCGUUCUGC UCAUCCUAG AGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mG*mC*GUUC UGCUCUCCUAGA GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109069 68-10906988
G016113	113	sgRNA	UUCCAC AUCCUU CAGGGA CU	UUCCACAUCC UUCAGGGAC UGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mU*mU*mC*CACA UCCUUCAGGGACU GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109091 38-10909158
G016114	114	sgRNA	CUCUCC AGCUGC CGGGCA UU	CUCUCCAGCU GCCGGGCAU UGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mU*mC*UCCA GCUGCCGGGCAU GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*m U*mU*mU	chr16:109047 64-10904784

Guide ID	SEQ ID NO to the Guide Sequence	Type	Guide Sequence	Exemplary Guide RNA Full Sequence (SEQ ID NOs: 218-334)	Exemplary Guide RNA Modified Sequence (SEQ ID NOs: 335-426)	Genomic Coordinates
					mGmUmGmCmU*mU*mU*mU	
G016115	115	sgRNA	GGGCCC ACAGCC ACUCGU GG	GGGCCCACAG CCACUCGUGG GUUUUAGAG CUAGAAUA GCAAGUAAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mG*mG*mG*CCCA CAGCCACUCGUGG GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	chr16:109069 07-10906927
G016116	116	sgRNA	CCCCGG CCGCCU CUCUUU UC	CCCCGGCCGC CUCUCUUUUC GUUUUAGAG CUAGAAUA GCAAGUAAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mC*mC*CGGC CGCCUCUCUUUUC GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	chr16:109079 78-10907998
G016117	117	sgRNA	UCCAGC UGCCGG GCAUUG GG	UCCAGCUGCC GGGCAUUGG GGUUUAGA GCUAGAAUA AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mU*mC*mC*AGCU GCCGGGCAUUGGG GUUUUAGAmGmC mUmAmGmAmAmA mUmAmGmCAAGU UAAAAUAAGGCU AGUCCGUUAUCA mAmCmUmUmGmA mAmAmAmAmGmU mGmGmCmAmCmC mGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	chr16:109047 61-10904781

[00156] The terms “mA,” “mC,” “mU,” or “mG” may be used to denote a nucleotide that has been modified with 2'-O-Me.

[00157] In some embodiments, the CIITA guide RNA comprises a guide sequence selected from SEQ ID NOs: 1-117. In some embodiments, the CIITA guide RNA comprises a guide sequence that is at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-117. In some embodiments, the CIITA guide RNA comprises a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-117.

In some embodiments, the CIITA guide RNA comprises a guide sequence that is at least 95% identical to a sequence selected from SEQ ID NOs: 1-117. In some embodiments disclosed herein, the guide sequence is (i) a guide sequence of SEQ ID NO: 32, 64, 67, 68, 74, 76, 84, 86, 90, 91, or 115; ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 32, 64, 67, 68, 74, 76, 84, 86, 90, 91, and 115; ii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 32, 64, 67, 68, 74, 76, 84, 86, 90, 91, and 115.

[00158] In some embodiments, the CIITA guide RNA comprises a guide sequence that comprises at least 10 contiguous nucleotides $\pm$ 10 nucleotides of a genomic coordinate listed in **Table 2**. As used herein, at least 10 contiguous nucleotides $\pm$ 10 nucleotides of a genomic coordinate means, for example, at least 10 contiguous nucleotides within the genomic coordinates wherein the genomic coordinates include 10 nucleotides in the 5' direction and 10 nucleotides in the 3' direction from the ranges listed in **Table 2**. For example, a CIITA guide RNA may comprise 10 contiguous nucleotides within the genomic coordinates chr16:10877360-10877380 or within chr16:10877350-10877390, including the boundary nucleotides of these ranges. In some embodiments, the CIITA guide RNA comprises a guide sequence that is at least 17, 18, 19, or 20 contiguous nucleotides of a sequence that comprises 10 contiguous nucleotides $\pm$ 10 nucleotides of a genomic coordinate listed in **Table 2**. In some embodiments, the CIITA guide RNA comprises a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from a sequence that is 17, 18, 19, or 20 contiguous nucleotides of a sequence that comprises 10 contiguous nucleotides $\pm$ 10 nucleotides of a genomic coordinate listed in **Table 2**.

[00159] In some embodiments, the CIITA guide RNA comprises a guide sequence that comprises at least 15 contiguous nucleotides $\pm$ 10 nucleotides of a genomic coordinate listed in **Table 2**. In some embodiments, the CIITA guide RNA comprises a guide sequence that comprises at least 20 contiguous nucleotides $\pm$ 10 nucleotides of a genomic coordinate listed in **Table 2**.

[00160] In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 1. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 2. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 3. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 4. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 5. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 6. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 7. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 8. In some embodiments, the CIITA guide RNA

[illegible]

[illegible]

comprises SEQ ID NO: 94. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 95. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 96. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 97. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 98. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 99. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 100. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 101. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 102. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 103. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 104. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 105. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 106. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 107. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 108. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 109. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 110. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 111. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 112. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 113. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 114. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 115. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 116. In some embodiments, the CIITA guide RNA comprises SEQ ID NO: 117.

[00161] Additional embodiments of CIITA guide RNAs are provided herein, including *e.g.*, exemplary modifications to the guide RNA.

2. Genetic modifications to CIITA

[00162] In some embodiments, the methods and compositions disclosed herein genetically modify at least one nucleotide of an exon in the CIITA gene in a cell. Because CIITA protein regulates expression of MHC class II, in some embodiments, the genetic modification to CIITA alters the production of CIITA protein, and thereby reduces the expression of MHC class II protein on the surface of the genetically modified cell (or engineered cell). Genetic modifications encompass the population of modifications that results from contact with a gene editing system (*e.g.*, the population of edits that result from Cas9 and a CIITA guide RNA, or the population of edits that result from BC22 and a CIITA guide RNA).

[00163] In some embodiments, the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16: 10902662- chr16:10923285. In some embodiments, the genetic modification comprises at least one nucleotide of an exon within the

genomic coordinates chr16:10906542- chr16:10923285. In some embodiments, the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10906542- chr16:10908121. In some embodiments, the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10916432-10916452, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10906985-10907005, chr16:10908073-10908093, chr16:10907433-10907453, chr16:10907979-10907999, chr16:10907139-10907159, chr16:10922435-10922455, chr16:10907384-10907404, chr16:10907434-10907454, chr16:10907119-10907139, chr16:10907539-10907559, chr16:10907810-10907830, chr16:10907315-10907335, chr16:10916426-10916446, chr16:10909138-10909158, chr16:10908101-10908121, chr16:10907790-10907810, chr16:10907787-10907807, chr16:10907454-10907474, chr16:10895702-10895722, chr16:10902729-10902749, chr16:10918492-10918512, chr16:10907932-10907952, chr16:10907623-10907643, chr16:10907461-10907481, chr16:10902723-10902743, chr16:10907622-10907642, chr16:10922441-10922461, chr16:10902662-10902682, chr16:10915626-10915646, chr16:10915592-10915612, chr16:10907385-10907405, chr16:10907030-10907050, chr16:10907935-10907955, chr16:10906853-10906873, chr16:10906757-10906777, chr16:10907730-10907750, and chr16:10895302-10895322. In some embodiments, the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10907539-10907559, chr16:10916426-10916446, chr16:10906907-10906927, chr16:10895702-10895722, chr16:10907757-10907777, chr16:10907623-10907643, chr16:10915626-10915646, chr16:10906756-10906776, chr16:10907476-10907496, chr16:10907385-10907405, and chr16:10923265-10923285. In some embodiments, the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10907315-10907335, chr16:10916432-10916452, chr16:10907932-10907952, chr16:10915626-10915646, chr16:10907586-10907606, chr16:10916426-10916446, chr16:10907476-10907496, chr16:10907787-10907807, chr16:10907979-10907999, chr16:10906904-10906924, and chr16:10909138-10909158. In some embodiments, the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10895702-10895722, chr16:10916432-10916452, chr16:10907623-10907643, chr16:10907932-10907952, chr16:10906985-10907005, chr16:10915626-10915646, chr16:10907539-10907559, chr16:10916426-10916446, chr16:10907476-10907496, chr16:10907119-10907139, chr16:10907979-10907999, and

chr16:10909138-10909158. In some embodiments, the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10906757-10906777, chr16:10895302-10895322, chr16:10907539-10907559, chr16:10907730-10907750, and chr16:10895702-10895722. In some embodiments, the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10922444-10922464, chr16:10916432-10916452. In some embodiments, the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10906853-10906873. In some embodiments, the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10922444-10922464. In some embodiments, the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10906757-10906777. In some embodiments, the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10916432-10916452. In some embodiments, the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10895302-10895322. In some embodiments, the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10907539-10907559. In some embodiments, the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10907730-10907750. In some embodiments, the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10895702-10895722. In some embodiments, the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10907932-10907952. In some embodiments, the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10907476-10907496. In some embodiments, the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10909138-10909158.

[00164] In some embodiments, the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 contiguous nucleotides within the genomic coordinates chosen from: chr16:10902662-10902682, chr16:10902723-10902743, chr16:10902729-10902749, chr16:10903747-10903767, chr16:10903824-10903844, chr16:10903848-10903868, chr16:10904761-10904781, chr16:10904764-10904784, chr16:10904765-10904785, chr16:10904785-10904805, chr16:10906542-10906562, chr16:10906556-10906576, chr16:10906609-10906629, chr16:10906610-10906630, chr16:10906616-10906636, chr16:10906682-10906702, chr16:10906756-10906776, chr16:10906757-10906777, chr16:10906821-10906841, chr16:10906823-

10906843, chr16:10906847-10906867, chr16:10906848-10906868, chr16:10906853-
 10906873, chr16:10906853-10906873, chr16:10906904-10906924, chr16:10906907-
 10906927, chr16:10906913-10906933, chr16:10906968-10906988, chr16:10906970-
 10906990, chr16:10906985-10907005, chr16:10907030-10907050, chr16:10907058-
 10907078, chr16:10907119-10907139, chr16:10907139-10907159, chr16:10907172-
 10907192, chr16:10907272-10907292, chr16:10907288-10907308, chr16:10907314-
 10907334, chr16:10907315-10907335, chr16:10907325-10907345, chr16:10907363-
 10907383, chr16:10907384-10907404, chr16:10907385-10907405, chr16:10907433-
 10907453, chr16:10907434-10907454, chr16:10907435-10907455, chr16:10907441-
 10907461, chr16:10907454-10907474, chr16:10907461-10907481, chr16:10907476-
 10907496, chr16:10907539-10907559, chr16:10907586-10907606, chr16:10907589-
 10907609, chr16:10907621-10907641, chr16:10907622-10907642, chr16:10907623-
 10907643, chr16:10907730-10907750, chr16:10907731-10907751, chr16:10907757-
 10907777, chr16:10907781-10907801, chr16:10907787-10907807, chr16:10907790-
 10907810, chr16:10907810-10907830, chr16:10907820-10907840, chr16:10907870-
 10907890, chr16:10907886-10907906, chr16:10907924-10907944, chr16:10907928-
 10907948, chr16:10907932-10907952, chr16:10907935-10907955, chr16:10907978-
 10907998, chr16:10907979-10907999, chr16:10908069-10908089, chr16:10908073-
 10908093, chr16:10908101-10908121, chr16:10909056-10909076, chr16:10909138-
 10909158, chr16:10910195-10910215, chr16:10910196-10910216, chr16:10915592-
 10915612, chr16:10915626-10915646, chr16:10916375-10916395, chr16:10916382-
 10916402, chr16:10916426-10916446, chr16:10916432-10916452, chr16:10918486-
 10918506, chr16:10918492-10918512, chr16:10918493-10918513, chr16:10922435-
 10922455, chr16:10922441-10922461, chr16:10922441-10922461, chr16:10922444-
 10922464, chr16:10922460-10922480, chr16:10923257-10923277, and chr16:10923265-
 10923285. In some embodiments, the genetic modification comprises at least 2, at least 3, at
 least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 contiguous nucleotides
 within the genomic coordinates chosen from: chr16:10916432-10916452, chr16:10922444-
 10922464, chr16:10907924-10907944, chr16:10906985-10907005, chr16:10908073-
 10908093, chr16:10907433-10907453, chr16:10907979-10907999, chr16:10907139-
 10907159, chr16:10922435-10922455, chr16:10907384-10907404, chr16:10907434-
 10907454, chr16:10907119-10907139, chr16:10907539-10907559, chr16:10907810-
 10907830, chr16:10907315-10907335, chr16:10916426-10916446, chr16:10909138-
 10909158, chr16:10908101-10908121, chr16:10907790-10907810, chr16:10907787-

10907807, chr16:10907454-10907474, chr16:10895702-10895722, chr16:10902729-10902749, chr16:10918492-10918512, chr16:10907932-10907952, chr16:10907623-10907643, chr16:10907461-10907481, chr16:10902723-10902743, chr16:10907622-10907642, chr16:10922441-10922461, chr16:10902662-10902682, chr16:10915626-10915646, chr16:10915592-10915612, chr16:10907385-10907405, chr16:10907030-10907050, chr16:10907935-10907955, chr16:10906853-10906873, chr16:10906757-10906777, chr16:10907730-10907750, and chr16:10895302-10895322. In some embodiments, the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 nucleotides of an exon within the genomic coordinates chosen from: chr16:10907539-10907559, chr16:10916426-10916446, chr16:10906907-10906927, chr16:10895702-10895722, chr16:10907757-10907777, chr16:10907623-10907643, chr16:10915626-10915646, chr16:10906756-10906776, chr16:10907476-10907496, chr16:10907385-10907405, and chr16:10923265-10923285. In some embodiments, the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 nucleotides of an exon within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10907315-10907335, chr16:10916432-10916452, chr16:10907932-10907952, chr16:10915626-10915646, chr16:10907586-10907606, chr16:10916426-10916446, chr16:10907476-10907496, chr16:10907787-10907807, chr16:10907979-10907999, chr16:10906904-10906924, and chr16:10909138-10909158. In some embodiments, the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 nucleotides of an exon within the genomic coordinates chosen from: chr16:10895702-10895722, chr16:10916432-10916452, chr16:10907623-10907643, chr16:10907932-10907952, chr16:10906985-10907005, chr16:10915626-10915646, chr16:10907539-10907559, chr16:10916426-10916446, chr16:10907476-10907496, chr16:10907119-10907139, chr16:10907979-10907999, and chr16:10909138-10909158. In some embodiments, the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 nucleotides of an exon within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10906757-10906777, chr16:10895302-10895322, chr16:10907539-10907559, chr16:10907730-10907750, and chr16:10895702-10895722. In some embodiments, the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 nucleotides of an exon within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10922444-10922464,

chr16:10916432-10916452. In some embodiments, the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 nucleotides of an exon within the genomic coordinates chr16:10906853-10906873. In some embodiments, the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 nucleotide of an exon within the genomic coordinates chr16:10922444-10922464. In some embodiments, the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 nucleotides of an exon within the genomic coordinates chr16:10906757-10906777. In some embodiments, the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 nucleotide of an exon within the genomic coordinates chr16:10916432-10916452. In some embodiments, the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 nucleotides of an exon within the genomic coordinates chr16:10895302-10895322. In some embodiments, the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 nucleotides of an exon within the genomic coordinates chr16:10907539-10907559. In some embodiments, the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 nucleotides of an exon within the genomic coordinates chr16:10907730-10907750. In some embodiments, the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 nucleotides of an exon within the genomic coordinates chr16:10895702-10895722. In some embodiments, the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 nucleotides of an exon within the genomic coordinates chr16:10907932-10907952. In some embodiments, the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 nucleotides of an exon within the genomic coordinates chr16:10907476-10907496. In some embodiments, the genetic modification comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 nucleotides of an exon within the genomic coordinates chr16:10909138-10909158.

[00165] In some embodiments, the genetic modification comprises at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10902662-10902682, chr16:10902723-10902743, chr16:10902729-10902749, chr16:10903747-10903767, chr16:10903824-10903844, chr16:10903848-10903868, chr16:10904761-10904781, chr16:10904764-10904784, chr16:10904765-10904785, chr16:10904785-10904805,

chr16:10906542-10906562, chr16:10906556-10906576, chr16:10906609-10906629,
 chr16:10906610-10906630, chr16:10906616-10906636, chr16:10906682-10906702,
 chr16:10906756-10906776, chr16:10906757-10906777, chr16:10906821-10906841,
 chr16:10906823-10906843, chr16:10906847-10906867, chr16:10906848-10906868,
 chr16:10906853-10906873, chr16:10906853-10906873, chr16:10906904-10906924,
 chr16:10906907-10906927, chr16:10906913-10906933, chr16:10906968-10906988,
 chr16:10906970-10906990, chr16:10906985-10907005, chr16:10907030-10907050,
 chr16:10907058-10907078, chr16:10907119-10907139, chr16:10907139-10907159,
 chr16:10907172-10907192, chr16:10907272-10907292, chr16:10907288-10907308,
 chr16:10907314-10907334, chr16:10907315-10907335, chr16:10907325-10907345,
 chr16:10907363-10907383, chr16:10907384-10907404, chr16:10907385-10907405,
 chr16:10907433-10907453, chr16:10907434-10907454, chr16:10907435-10907455,
 chr16:10907441-10907461, chr16:10907454-10907474, chr16:10907461-10907481,
 chr16:10907476-10907496, chr16:10907539-10907559, chr16:10907586-10907606,
 chr16:10907589-10907609, chr16:10907621-10907641, chr16:10907622-10907642,
 chr16:10907623-10907643, chr16:10907730-10907750, chr16:10907731-10907751,
 chr16:10907757-10907777, chr16:10907781-10907801, chr16:10907787-10907807,
 chr16:10907790-10907810, chr16:10907810-10907830, chr16:10907820-10907840,
 chr16:10907870-10907890, chr16:10907886-10907906, chr16:10907924-10907944,
 chr16:10907928-10907948, chr16:10907932-10907952, chr16:10907935-10907955,
 chr16:10907978-10907998, chr16:10907979-10907999, chr16:10908069-10908089,
 chr16:10908073-10908093, chr16:10908101-10908121, chr16:10909056-10909076,
 chr16:10909138-10909158, chr16:10910195-10910215, chr16:10910196-10910216,
 chr16:10915592-10915612, chr16:10915626-10915646, chr16:10916375-10916395,
 chr16:10916382-10916402, chr16:10916426-10916446, chr16:10916432-10916452,
 chr16:10918486-10918506, chr16:10918492-10918512, chr16:10918493-10918513,
 chr16:10922435-10922455, chr16:10922441-10922461, chr16:10922441-10922461,
 chr16:10922444-10922464, chr16:10922460-10922480, chr16:10923257-10923277, and
 chr16:10923265-10923285. In some embodiments, the genetic modification comprises at least
 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10916432-
 10916452, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10906985-
 10907005, chr16:10908073-10908093, chr16:10907433-10907453, chr16:10907979-
 10907999, chr16:10907139-10907159, chr16:10922435-10922455, chr16:10907384-
 10907404, chr16:10907434-10907454, chr16:10907119-10907139, chr16:10907539-

10907559, chr16:10907810-10907830, chr16:10907315-10907335, chr16:10916426-10916446, chr16:10909138-10909158, chr16:10908101-10908121, chr16:10907790-10907810, chr16:10907787-10907807, chr16:10907454-10907474, chr16:10895702-10895722, chr16:10902729-10902749, chr16:10918492-10918512, chr16:10907932-10907952, chr16:10907623-10907643, chr16:10907461-10907481, chr16:10902723-10902743, chr16:10907622-10907642, chr16:10922441-10922461, chr16:10902662-10902682, chr16:10915626-10915646, chr16:10915592-10915612, chr16:10907385-10907405, chr16:10907030-10907050, chr16:10907935-10907955, chr16:10906853-10906873, chr16:10906757-10906777, chr16:10907730-10907750, and chr16:10895302-10895322. In some embodiments, the genetic modification comprises at least 5 nucleotides of an exon within the genomic coordinates chosen from: chr16:10907539-10907559, chr16:10916426-10916446, chr16:10906907-10906927, chr16:10895702-10895722, chr16:10907757-10907777, chr16:10907623-10907643, chr16:10915626-10915646, chr16:10906756-10906776, chr16:10907476-10907496, chr16:10907385-10907405, and chr16:10923265-10923285. In some embodiments, the genetic modification comprises at least 5 nucleotides of an exon within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10907315-10907335, chr16:10916432-10916452, chr16:10907932-10907952, chr16:10915626-10915646, chr16:10907586-10907606, chr16:10916426-10916446, chr16:10907476-10907496, chr16:10907787-10907807, chr16:10907979-10907999, chr16:10906904-10906924, and chr16:10909138-10909158. In some embodiments, the genetic modification comprises at least 5 nucleotides of an exon within the genomic coordinates chosen from: chr16:10895702-10895722, chr16:10916432-10916452, chr16:10907623-10907643, chr16:10907932-10907952, chr16:10906985-10907005, chr16:10915626-10915646, chr16:10907539-10907559, chr16:10916426-10916446, chr16:10907476-10907496, chr16:10907119-10907139, chr16:10907979-10907999, and chr16:10909138-10909158. In some embodiments, the genetic modification comprises at least 5 nucleotides of an exon within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10906757-10906777, chr16:10895302-10895322, chr16:10907539-10907559, chr16:10907730-10907750, and chr16:10895702-10895722. In some embodiments, the genetic modification comprises at least 5 nucleotides of an exon within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10922444-10922464, chr16:10916432-10916452. In some embodiments, the genetic modification comprises at least 5 nucleotides of an exon within the genomic coordinates chr16:10906853-10906873. In some embodiments, the genetic

modification comprises at least 5 nucleotide of an exon within the genomic coordinates chr16:10922444-10922464. In some embodiments, the genetic modification comprises at least 5 nucleotides of an exon within the genomic coordinates chr16:10906757-10906777. In some embodiments, the genetic modification comprises at least 5 nucleotide of an exon within the genomic coordinates chr16:10916432-10916452. In some embodiments, the genetic modification comprises at least 5 nucleotides of an exon within the genomic coordinates chr16:10895302-10895322. In some embodiments, the genetic modification comprises at least 5 nucleotides of an exon within the genomic coordinates chr16:10907539-10907559. In some embodiments, the genetic modification comprises at least 5 nucleotides of an exon within the genomic coordinates chr16:10907730-10907750. In some embodiments, the genetic modification comprises at least 5 nucleotides of an exon within the genomic coordinates chr16:10895702-10895722. In some embodiments, the genetic modification comprises at least 5 nucleotides of an exon within the genomic coordinates chr16:10907932-10907952. In some embodiments, the genetic modification comprises at least 5 nucleotides of an exon within the genomic coordinates chr16:10907476-10907496. In some embodiments, the genetic modification comprises at least 5 nucleotides of an exon within the genomic coordinates chr16:10909138-10909158.

[00166] In some embodiments, the genetic modification comprises at least 10 contiguous nucleotides within the genomic coordinates chosen from: chr16:10902662-10902682, chr16:10902723-10902743, chr16:10902729-10902749, chr16:10903747-10903767, chr16:10903824-10903844, chr16:10903848-10903868, chr16:10904761-10904781, chr16:10904764-10904784, chr16:10904765-10904785, chr16:10904785-10904805, chr16:10906542-10906562, chr16:10906556-10906576, chr16:10906609-10906629, chr16:10906610-10906630, chr16:10906616-10906636, chr16:10906682-10906702, chr16:10906756-10906776, chr16:10906757-10906777, chr16:10906821-10906841, chr16:10906823-10906843, chr16:10906847-10906867, chr16:10906848-10906868, chr16:10906853-10906873, chr16:10906853-10906873, chr16:10906904-10906924, chr16:10906907-10906927, chr16:10906913-10906933, chr16:10906968-10906988, chr16:10906970-10906990, chr16:10906985-10907005, chr16:10907030-10907050, chr16:10907058-10907078, chr16:10907119-10907139, chr16:10907139-10907159, chr16:10907172-10907192, chr16:10907272-10907292, chr16:10907288-10907308, chr16:10907314-10907334, chr16:10907315-10907335, chr16:10907325-10907345, chr16:10907363-10907383, chr16:10907384-10907404, chr16:10907385-10907405, chr16:10907433-10907453, chr16:10907434-10907454, chr16:10907435-10907455,

chr16:10907441-10907461, chr16:10907454-10907474, chr16:10907461-10907481,
chr16:10907476-10907496, chr16:10907539-10907559, chr16:10907586-10907606,
chr16:10907589-10907609, chr16:10907621-10907641, chr16:10907622-10907642,
chr16:10907623-10907643, chr16:10907730-10907750, chr16:10907731-10907751,
chr16:10907757-10907777, chr16:10907781-10907801, chr16:10907787-10907807,
chr16:10907790-10907810, chr16:10907810-10907830, chr16:10907820-10907840,
chr16:10907870-10907890, chr16:10907886-10907906, chr16:10907924-10907944,
chr16:10907928-10907948, chr16:10907932-10907952, chr16:10907935-10907955,
chr16:10907978-10907998, chr16:10907979-10907999, chr16:10908069-10908089,
chr16:10908073-10908093, chr16:10908101-10908121, chr16:10909056-10909076,
chr16:10909138-10909158, chr16:10910195-10910215, chr16:10910196-10910216,
chr16:10915592-10915612, chr16:10915626-10915646, chr16:10916375-10916395,
chr16:10916382-10916402, chr16:10916426-10916446, chr16:10916432-10916452,
chr16:10918486-10918506, chr16:10918492-10918512, chr16:10918493-10918513,
chr16:10922435-10922455, chr16:10922441-10922461, chr16:10922441-10922461,
chr16:10922444-10922464, chr16:10922460-10922480, chr16:10923257-10923277, and
chr16:10923265-10923285. In some embodiments, the genetic modification comprises at least
10 contiguous nucleotides within the genomic coordinates chosen from: chr16:10916432-
10916452, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10906985-
10907005, chr16:10908073-10908093, chr16:10907433-10907453, chr16:10907979-
10907999, chr16:10907139-10907159, chr16:10922435-10922455, chr16:10907384-
10907404, chr16:10907434-10907454, chr16:10907119-10907139, chr16:10907539-
10907559, chr16:10907810-10907830, chr16:10907315-10907335, chr16:10916426-
10916446, chr16:10909138-10909158, chr16:10908101-10908121, chr16:10907790-
10907810, chr16:10907787-10907807, chr16:10907454-10907474, chr16:10895702-
10895722, chr16:10902729-10902749, chr16:10918492-10918512, chr16:10907932-
10907952, chr16:10907623-10907643, chr16:10907461-10907481, chr16:10902723-
10902743, chr16:10907622-10907642, chr16:10922441-10922461, chr16:10902662-
10902682, chr16:10915626-10915646, chr16:10915592-10915612, chr16:10907385-
10907405, chr16:10907030-10907050, chr16:10907935-10907955, chr16:10906853-
10906873, chr16:10906757-10906777, chr16:10907730-10907750, and chr16:10895302-
10895322. In some embodiments, the genetic modification comprises at least 10 nucleotides
of an exon within the genomic coordinates chosen from: chr16:10907539-10907559,
chr16:10916426-10916446, chr16:10906907-10906927, chr16:10895702-10895722,

chr16:10907757-10907777, chr16:10907623-10907643, chr16:10915626-10915646, chr16:10906756-10906776, chr16:10907476-10907496, chr16:10907385-10907405, and chr16:10923265-10923285. In some embodiments, the genetic modification comprises at least 10 nucleotides of an exon within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10907315-10907335, chr16:10916432-10916452, chr16:10907932-10907952, chr16:10915626-10915646, chr16:10907586-10907606, chr16:10916426-10916446, chr16:10907476-10907496, chr16:10907787-10907807, chr16:10907979-10907999, chr16:10906904-10906924, and chr16:10909138-10909158. In some embodiments, the genetic modification comprises at least 10 nucleotides of an exon within the genomic coordinates chosen from: chr16:10895702-10895722, chr16:10916432-10916452, chr16:10907623-10907643, chr16:10907932-10907952, chr16:10906985-10907005, chr16:10915626-10915646, chr16:10907539-10907559, chr16:10916426-10916446, chr16:10907476-10907496, chr16:10907119-10907139, chr16:10907979-10907999, and chr16:10909138-10909158. In some embodiments, the genetic modification comprises at least 10 nucleotides of an exon within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10906757-10906777, chr16:10895302-10895322, chr16:10907539-10907559, chr16:10907730-10907750, and chr16:10895702-10895722. In some embodiments, the genetic modification comprises at least 10 nucleotides of an exon within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10922444-10922464, and chr16:10916432-10916452. In some embodiments, the genetic modification comprises at least 10 nucleotides of an exon within the genomic coordinates chr16:10906853-10906873. In some embodiments, the genetic modification comprises at least 10 nucleotides of an exon within the genomic coordinates chr16:10922444-10922464. In some embodiments, the genetic modification comprises at least 10 nucleotides of an exon within the genomic coordinates chr16:10906757-10906777. In some embodiments, the genetic modification comprises at least 10 nucleotides of an exon within the genomic coordinates chr16:10916432-10916452. In some embodiments, the genetic modification comprises at least 10 nucleotides of an exon within the genomic coordinates chr16:10895302-10895322. In some embodiments, the genetic modification comprises at least 10 nucleotides of an exon within the genomic coordinates chr16:10907539-10907559. In some embodiments, the genetic modification comprises at least 10 nucleotides of an exon within the genomic coordinates chr16:10907730-10907750. In some embodiments, the genetic modification comprises at least 10 nucleotides of an exon within the genomic coordinates chr16:10895702-10895722. In some embodiments, the genetic modification comprises at least

10 nucleotides of an exon within the genomic coordinates chr16:10907932-10907952. In some embodiments, the genetic modification comprises at least 10 nucleotides of an exon within the genomic coordinates chr16:10907476-10907496. In some embodiments, the genetic modification comprises at least 10 nucleotides of an exon within the genomic coordinates chr16:10909138-10909158.

[00167] In some embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates chosen from:

chr16:10902662-10902682,	chr16:10902723-10902743,	chr16:10902729-10902749,
chr16:10903747-10903767,	chr16:10903824-10903844,	chr16:10903848-10903868,
chr16:10904761-10904781,	chr16:10904764-10904784,	chr16:10904765-10904785,
chr16:10904785-10904805,	chr16:10906542-10906562,	chr16:10906556-10906576,
chr16:10906609-10906629,	chr16:10906610-10906630,	chr16:10906616-10906636,
chr16:10906682-10906702,	chr16:10906756-10906776,	chr16:10906757-10906777,
chr16:10906821-10906841,	chr16:10906823-10906843,	chr16:10906847-10906867,
chr16:10906848-10906868,	chr16:10906853-10906873,	chr16:10906853-10906873,
chr16:10906904-10906924,	chr16:10906907-10906927,	chr16:10906913-10906933,
chr16:10906968-10906988,	chr16:10906970-10906990,	chr16:10906985-10907005,
chr16:10907030-10907050,	chr16:10907058-10907078,	chr16:10907119-10907139,
chr16:10907139-10907159,	chr16:10907172-10907192,	chr16:10907272-10907292,
chr16:10907288-10907308,	chr16:10907314-10907334,	chr16:10907315-10907335,
chr16:10907325-10907345,	chr16:10907363-10907383,	chr16:10907384-10907404,
chr16:10907385-10907405,	chr16:10907433-10907453,	chr16:10907434-10907454,
chr16:10907435-10907455,	chr16:10907441-10907461,	chr16:10907454-10907474,
chr16:10907461-10907481,	chr16:10907476-10907496,	chr16:10907539-10907559,
chr16:10907586-10907606,	chr16:10907589-10907609,	chr16:10907621-10907641,
chr16:10907622-10907642,	chr16:10907623-10907643,	chr16:10907730-10907750,
chr16:10907731-10907751,	chr16:10907757-10907777,	chr16:10907781-10907801,
chr16:10907787-10907807,	chr16:10907790-10907810,	chr16:10907810-10907830,
chr16:10907820-10907840,	chr16:10907870-10907890,	chr16:10907886-10907906,
chr16:10907924-10907944,	chr16:10907928-10907948,	chr16:10907932-10907952,
chr16:10907935-10907955,	chr16:10907978-10907998,	chr16:10907979-10907999,
chr16:10908069-10908089,	chr16:10908073-10908093,	chr16:10908101-10908121,
chr16:10909056-10909076,	chr16:10909138-10909158,	chr16:10910195-10910215,
chr16:10910196-10910216,	chr16:10915592-10915612,	chr16:10915626-10915646,

chr16:10916375-10916395, chr16:10916382-10916402, chr16:10916426-10916446, chr16:10916432-10916452, chr16:10918486-10918506, chr16:10918492-10918512, chr16:10918493-10918513, chr16:10922435-10922455, chr16:10922441-10922461, chr16:10922441-10922461, chr16:10922444-10922464, chr16:10922460-10922480, chr16:10923257-10923277, and chr16:10923265-10923285. In some embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates chosen from: chr16:10916432-10916452, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10906985-10907005, chr16:10908073-10908093, chr16:10907433-10907453, chr16:10907979-10907999, chr16:10907139-10907159, chr16:10922435-10922455, chr16:10907384-10907404, chr16:10907434-10907454, chr16:10907119-10907139, chr16:10907539-10907559, chr16:10907810-10907830, chr16:10907315-10907335, chr16:10916426-10916446, chr16:10909138-10909158, chr16:10908101-10908121, chr16:10907790-10907810, chr16:10907787-10907807, chr16:10907454-10907474, chr16:10895702-10895722, chr16:10902729-10902749, chr16:10918492-10918512, chr16:10907932-10907952, chr16:10907623-10907643, chr16:10907461-10907481, chr16:10902723-10902743, chr16:10907622-10907642, chr16:10922441-10922461, chr16:10902662-10902682, chr16:10915626-10915646, chr16:10915592-10915612, chr16:10907385-10907405, chr16:10907030-10907050, chr16:10907935-10907955, chr16:10906853-10906873, chr16:10906757-10906777, chr16:10907730-10907750, and chr16:10895302-10895322. In some embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates chosen from: chr16:10907539-10907559, chr16:10916426-10916446, chr16:10906907-10906927, chr16:10895702-10895722, chr16:10907757-10907777, chr16:10907623-10907643, chr16:10915626-10915646, chr16:10906756-10906776, chr16:10907476-10907496, chr16:10907385-10907405, and chr16:10923265-10923285. In some embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10907315-10907335, chr16:10916432-10916452, chr16:10907932-10907952, chr16:10915626-10915646, chr16:10907586-10907606, chr16:10916426-10916446, chr16:10907476-10907496, chr16:10907787-10907807, chr16:10907979-10907999, chr16:10906904-10906924, and chr16:10909138-10909158. In some embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates chosen from: chr16:10895702-

10895722, chr16:10916432-10916452, chr16:10907623-10907643, chr16:10907932-10907952, chr16:10906985-10907005, chr16:10915626-10915646, chr16:10907539-10907559, chr16:10916426-10916446, chr16:10907476-10907496, chr16:10907119-10907139, chr16:10907979-10907999, and chr16:10909138-10909158. In some embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10906757-10906777, chr16:10895302-10895322, chr16:10907539-10907559, chr16:10907730-10907750, and chr16:10895702-10895722. In some embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10922444-10922464, chr16:10916432-10916452. In some embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates chr16:10906853-10906873. In some embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates chr16:10922444-10922464. In some embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates chr16:10906757-10906777. In some embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates chr16:10916432-10916452. In some embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates chr16:10895302-10895322. In some embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates chr16:10907539-10907559. In some embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates chr16:10907730-10907750. In some embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates chr16:10895702-10895722. In some embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates chr16:10907932-10907952. In some embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates chr16:10907476-10907496. In some embodiments, the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates chr16:10909138-10909158.

[00168] In some embodiments, the modification to CIITA comprises any one or more of an insertion, deletion, substitution or deamination of at least one nucleotide in a target sequence. In some embodiments, the modification to CIITA comprises an insertion of 1, 2, 3, 4 or 5 or more nucleotides in a target sequence. In some embodiments, the modification to CIITA comprises a deletion of 1, 2, 3, 4 or 5 or more nucleotides in a target sequence. In other embodiments, the modification to CIITA comprises an insertion of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or 25 or more nucleotides in a target sequence. In other embodiments, the modification to CIITA comprises a deletion of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or 25 or more nucleotides in a target sequence. In some embodiments, the modification to CIITA comprises an indel, which is generally defined in the art as an insertion or deletion of less than 1000 base pairs (bp). In some embodiments, the modification to CIITA comprises an indel which results in a frameshift mutation in a target sequence. In some embodiments, the modification to CIITA comprises a substitution of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or 25 or more nucleotides in a target sequence. In some embodiments, the modification to CIITA comprises one or more of an insertion, deletion, or substitution of nucleotides resulting from the incorporation of a template nucleic acid. In some embodiments, the modification to CIITA comprises an insertion of a donor nucleic acid in a target sequence. In some embodiments, the modification to CIITA is not transient.

[00169] In some embodiments, the genetic modification to CIITA results in utilization of an out-of-frame stop codon. In some embodiments, the genetic modification to CIITA results in reduced CIITA protein expression by the cell. In some embodiments, the genetic modification to CIITA results in reduced CIITA in the cell nucleus. In some embodiments, the modification to CIITA results in reduced MHC class II protein expression on the surface of the cell.

[00170] In some embodiments, the genetic modification to CIITA results in a truncated form of the CIITA protein. In some embodiments, the truncated CIITA protein does not bind to GTP. In some embodiments, the truncated CIITA protein does not localize to the nucleus. In some embodiments, the CIITA protein (*e.g.*, a truncated form of the CIITA protein) has impaired activity as compared to the wildtype CIITA protein's activity relating to regulating MHC class II expression. In some embodiments, MHC class II expression on the surface of a cell is reduced as a result of impaired CIITA protein activity. In some embodiments, MHC class II expression on the surface of a cell is absent as a result of impaired CIITA protein activity.

3. Efficacy of CIITA guide RNAs

[00171] The efficacy of a CIITA guide RNA may be determined by techniques available in the art that assess the editing efficiency of a guide RNA, the levels of CIITA protein and/or mRNA, and/or the levels of MHC class II in a target cell.

[00172] In some embodiments, the efficacy of a CIITA guide RNA is determined by measuring levels of CIITA protein in a cell. The levels of CIITA protein may be detected by, *e.g.*, cell lysate and western blot with an anti-CIITA antibody. In some embodiments, the efficacy of a CIITA guide RNA is determined by measuring levels of CIITA protein in the cell nucleus. In some embodiments, the efficacy of a CIITA guide RNA is determined by measuring levels of CIITA mRNA in a cell. The levels of CIITA mRNA may be detected by *e.g.*, RT-PCR. In some embodiments, a decrease in the levels CIITA protein and/or CIITA mRNA in the target cell as compared to an unmodified cell is indicative of an effective CIITA guide RNA.

[00173] An “unmodified cell” (or “unmodified cells”) refers to a control cell (or cells) of the same type of cell in an experiment or test, wherein the “unmodified” control cell has not been contacted with a CIITA guide. Therefore, an unmodified cell (or cells) may be a cell that has not been contacted with a guide RNA, or a cell that has been contacted with a guide RNA that does not target CIITA.

[00174] In some embodiments, the efficacy of a CIITA guide RNA is determined by measuring the reduction or elimination of MHC class II protein expression by the target cells. The CIITA protein functions as a transactivator, activating the MHC class II promoter, and is essential for the expression of MHC class II protein. In some embodiments, MHC class II protein expression may be detected on the surface of the target cells. In some embodiments, MHC class II protein expression is measured by flow cytometry. In some embodiments, an antibody against MHC class II protein (*e.g.*, anti-HLA-DR, -DQ, -DP) may be used to detect MHC class II protein expression *e.g.*, by flow cytometry. In some embodiments, one or more antibodies against MHC class II protein (*e.g.*, anti-HLA-DR, -DQ, -DP) may be used to detect MHC class II protein expression *e.g.*, by flow cytometry. In some embodiments, the one or more antibodies against MHC class II protein comprises one or more of an antibody against HLA-DR, an antibody against HLA-DQ, and an antibody against HLA-DP. In some embodiments, the one or more antibodies against MHC class II protein comprises an antibody against HLA-DR, an antibody against anti-HLA-DQ, and an antibody against HLA-DP. In

some embodiments, the one or more antibodies against MHC class II protein comprises an antibody against HLA-DR, HLA-DQ, and HLA-DP.

[00175] In some embodiments, a reduction or elimination in MHC class II protein on the surface of a cell (or population of cells) as compared to an unmodified cell (or population of unmodified cells) is indicative of an effective CIITA guide RNA. In some embodiments, a cell (or population of cells) that has been contacted with a particular CIITA guide RNA and RNA-guided DNA binding agent that is negative for MHC class II protein by flow cytometry is indicative of an effective CIITA guide RNA.

[00176] In some embodiments, the MHC class II protein expression is reduced or eliminated in a population of cells using the methods and compositions disclosed herein. In some embodiments, the population of cells is enriched (e.g., by FACS or MACS) and is at least 65%, 70%, 80%, 90%, 91%, 92%, 93%, or 94% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is not enriched (e.g., by FACS or MACS) and is at least 65%, 70%, 80%, 90%, 91%, 92%, 93%, or 94% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells.

[00177] In some embodiments, the population of cells is at least 65% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 70% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 80% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 90% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 91% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 92% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 93% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 94% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 95% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 96% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the

population of cells is at least 97% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 98% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 99% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells.

[00178] In some embodiments, the population of cells is at least 65% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using one or more of an antibody against HLA-DR, an antibody against HLA-DQ, and an antibody against HLA-DP. In some embodiments, the population of cells is at least 65% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using an antibody against HLA-DR, an antibody against anti-HLA-DQ, and an antibody against HLA-DP. In some embodiments, the population of cells is at least 65% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using an antibody against HLA-DR, HLA-DQ, and HLA-DP. In some embodiments, the population of cells is at least 70% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using one or more of an antibody against HLA-DR, an antibody against HLA-DQ, and an antibody against HLA-DP. In some embodiments, the population of cells is at least 70% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using an antibody against HLA-DR, an antibody against anti-HLA-DQ, and an antibody against HLA-DP. In some embodiments, the population of cells is at least 70% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using an antibody against HLA-DR, HLA-DQ, and HLA-DP. In some embodiments, the population of cells is at least 80% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using one or more of an antibody against HLA-DR, an antibody against HLA-DQ, and an antibody against HLA-DP. In some embodiments, the population of cells is at least 80% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using an antibody against HLA-DR, an antibody against anti-HLA-DQ, and an antibody against HLA-DP. In some embodiments, the population of cells is at least 80% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using an antibody against HLA-DR, HLA-DQ, and HLA-DP. In some embodiments, the population of cells is at least 90% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using one or more of an antibody against HLA-DR, an antibody against HLA-DQ, and an antibody against HLA-DP. In some embodiments, the population of cells is at least 90% MHC class II negative as measured by

flow cytometry relative to a population of unmodified cells, using an antibody against HLA-DR, an antibody against anti-HLA-DQ, and an antibody against HLA-DP. In some embodiments, the population of cells is at least 90% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using an antibody against HLA-DR, HLA-DQ, and HLA-DP. In some embodiments, the population of cells is at least 92% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using one or more of an antibody against HLA-DR, an antibody against HLA-DQ, and an antibody against HLA-DP. In some embodiments, the population of cells is at least 92% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using an antibody against HLA-DR, an antibody against anti-HLA-DQ, and an antibody against HLA-DP. In some embodiments, the population of cells is at least 92% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using an antibody against HLA-DR, HLA-DQ, and HLA-DP. In some embodiments, the population of cells is at least 93% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using one or more of an antibody against HLA-DR, an antibody against HLA-DQ, and an antibody against HLA-DP. In some embodiments, the population of cells is at least 93% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using an antibody against HLA-DR, an antibody against anti-HLA-DQ, and an antibody against HLA-DP. In some embodiments, the population of cells is at least 93% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using an antibody against HLA-DR, HLA-DQ, and HLA-DP. In some embodiments, the population of cells is at least 94% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using one or more of an antibody against HLA-DR, an antibody against HLA-DQ, and an antibody against HLA-DP. In some embodiments, the population of cells is at least 94% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using an antibody against HLA-DR, an antibody against anti-HLA-DQ, and an antibody against HLA-DP. In some embodiments, the population of cells is at least 94% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using an antibody against HLA-DR, HLA-DQ, and HLA-DP. In some embodiments, the population of cells is at least 95% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using one or more of an antibody against HLA-DR, an antibody against HLA-DQ, and an antibody against HLA-DP. In some embodiments, the population of cells is at least 95% MHC class II negative as measured by flow cytometry relative to a population of unmodified

cells, using an antibody against HLA-DR, an antibody against anti-HLA-DQ, and an antibody against HLA-DP. In some embodiments, the population of cells is at least 95% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using an antibody against HLA-DR, HLA-DQ, and HLA-DP. In some embodiments, the population of cells is at least 96% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using one or more of an antibody against HLA-DR, an antibody against HLA-DQ, and an antibody against HLA-DP. In some embodiments, the population of cells is at least 96% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using an antibody against HLA-DR, an antibody against anti-HLA-DQ, and an antibody against HLA-DP. In some embodiments, the population of cells is at least 96% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using an antibody against HLA-DR, HLA-DQ, and HLA-DP. In some embodiments, the population of cells is at least 97% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using one or more of an antibody against HLA-DR, an antibody against HLA-DQ, and an antibody against HLA-DP. In some embodiments, the population of cells is at least 97% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using an antibody against HLA-DR, an antibody against anti-HLA-DQ, and an antibody against HLA-DP. In some embodiments, the population of cells is at least 97% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using an antibody against HLA-DR, HLA-DQ, and HLA-DP. In some embodiments, the population of cells is at least 98% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using one or more of an antibody against HLA-DR, an antibody against HLA-DQ, and an antibody against HLA-DP. In some embodiments, the population of cells is at least 98% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using an antibody against HLA-DR, an antibody against anti-HLA-DQ, and an antibody against HLA-DP. In some embodiments, the population of cells is at least 98% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using an antibody against HLA-DR, HLA-DQ, and HLA-DP. In some embodiments, the population of cells is at least 99% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using one or more of an antibody against HLA-DR, an antibody against HLA-DQ, and an antibody against HLA-DP. In some embodiments, the population of cells is at least 99% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using an antibody against HLA-DR, an antibody against anti-

HLA-DQ, and an antibody against HLA-DP. In some embodiments, the population of cells is at least 99% MHC class II negative as measured by flow cytometry relative to a population of unmodified cells, using an antibody against HLA-DR, HLA-DQ, and HLA-DP.

[00179] In some embodiments, an effective CIITA guide RNA may be determined by measuring the response of immune cells *in vitro* or *in vivo* (*e.g.*, CD4⁺ T cells) to the genetically modified target cell. A CD4⁺ T cell response may be evaluated by an assay that measures the activation response of CD4⁺ T cells *e.g.*, CD4⁺ T cell proliferation, expression of activation markers, and/or cytokine production (IL-2, IL-12, IFN- γ) (*e.g.*, flow cytometry, ELISA). The response of CD4⁺ T cells may be evaluated in *in vitro* cell culture assays in which the genetically modified cell is co-cultured with cells comprising CD4⁺ T cells. For example, the genetically modified cell may be co-cultured *e.g.*, with PBMCs, purified CD3⁺ T cells comprising CD4⁺ T cells, purified CD4⁺ T cells, or a CD4⁺ T cell line. The CD4⁺ T cell response elicited from the genetically modified cell may be compared to the response elicited from an unmodified cell. A reduced response from CD4⁺ T cells is indicative of an effective CIITA guide RNA.

[00180] The efficacy of a CIITA guide RNA may also be assessed by the survival of the cell post-editing. In some embodiments, the cell survives post editing for at least one week to six weeks. In some embodiments, the cell survives post editing for at least one week to twelve weeks. In some embodiments, the cell survives post editing for at least two weeks. In some embodiments, the cell survives post editing for at least three weeks. In some embodiments, the cell survives post editing for at least four weeks. In some embodiments, the cell survives post editing for at least five weeks. In some embodiments, the cell survives post editing for at least six weeks. The viability of a genetically modified cell may be measured using standard techniques, including *e.g.*, by measures of cell death, by flow cytometry live/dead staining, or cell proliferation.

C. Methods and Compositions for Reducing or Elimination MHC Class II and Additional Modifications

1. MHC class I knock out

[00181] In some embodiments, methods for reducing or eliminating expression of MHC class II protein on the surface of a cell by genetically modifying CIITA as disclosed herein are provided, wherein the methods further provide for reducing or eliminating expression of MHC class I protein on the surface of the cell relative to an unmodified cell. In one approach, MHC class I protein expression is reduced or eliminated by genetically modifying the B2M gene. In

some embodiments, MHC class I protein expression is reduced or eliminated by contacting the cell with a B2M guide RNA. In another approach, expression of a MHC class I protein HLA-A is reduced or eliminated by genetically modifying HLA-A, thereby reducing or eliminating the surface expression of HLA-A in a human cell, wherein the human cell is homozygous for HLA-B and homozygous for HLA-C. Therefore, in some embodiments, or HLA-A protein expression is reduced or eliminated by contacting a human cell with an HLA-A guide RNA, wherein the human cell is homozygous for HLA-B and homozygous for HLA-C. In some embodiments, the resulting cell is an allogeneic cell.

[00182] In some embodiments, the methods comprise reducing or eliminating surface expression of MHC class II protein in an engineered cell relative to an unmodified cell comprising contacting the cell with a composition comprising a CIITA guide RNA disclosed herein, the method further comprising contacting the cell with a B2M guide RNA. In some embodiments, the method further comprises contacting the cell with an RNA-guided DNA binding agent. In some embodiments, the method further comprises inducing a DSB or an SSB in the B2M target sequence. In some embodiments, B2M expression is thereby reduced by the cell. In some embodiments, MHC class I protein expression is thereby reduced or eliminated by the cell.

[00183] In some embodiments, the B2M guide RNA targets the human B2M gene.

[00184] In some embodiments, the B2M guide RNA comprises SEQ ID NO: 701. In some embodiments, the B2M guide RNA comprises a guide sequence that is at least 17, 18, 19, or 20 contiguous nucleotides of SEQ ID NO: 701. In some embodiments, the B2M guide RNA comprises a guide sequence that is at least 95%, 90%, or 85% identical to SEQ ID NO: 701.

[00185] Additional embodiments of B2M guide RNAs are provided herein, including *e.g.*, exemplary modifications to the guide RNA.

[00186] In some embodiments, the efficacy of a B2M guide RNA is determined by measuring levels of B2M protein in a cell relative to an unmodified cell. In some embodiments, the efficacy of a B2M guide RNA is determined by measuring levels of B2M protein expressed by the cell. In some embodiments, an antibody against B2M protein (*e.g.*, anti-B2M) may be used to detect the level of B2M protein by *e.g.*, flow cytometry. In some embodiments, the efficacy of a B2M guide RNA is determined by measuring levels of B2M mRNA in a cell *e.g.*, by RT-PCR. In some embodiments, reduction or elimination in the levels of B2M protein or B2M mRNA is indicative of an effective B2M guide RNA as compared to the levels of B2M protein in an unmodified cell. In some embodiments, a cell (or population of cells) that is negative for B2M protein by flow cytometry as compared to an unmodified cell (or population

of unmodified cells) is indicative of an effective B2M guide RNA. In some embodiments, a cell (or population of cells) that has been contacted with a particular B2M guide RNA and RNA-guided DNA binding agent that is negative for MHC class I protein by flow cytometry is indicative of an effective B2M guide RNA.

[00187] In some embodiments, the efficacy of a B2M guide RNA is determined by measuring levels of MHC class I protein on the surface of a cell. In some embodiments, MHC class I protein levels are measured by flow cytometry (*e.g.*, with an antibody against HLA-A, HLA-B, or HLA-C). In some embodiments, the population of cells is at least 65% MHC class I negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 70% MHC I negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 80% MHC I negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 90% MHC I negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 95% MHC I negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 100% MHC class I negative as measured by flow cytometry relative to a population of unmodified cells.

[00188] In some embodiments, the methods comprise reducing or eliminating surface expression of MHC class II protein in an engineered cell relative to an unmodified cell comprising contacting the cell with a composition comprising a CIITA guide RNA disclosed herein, the method further comprising reducing or eliminating the HLA-A expression of the cell by a gene editing system that binds to an HLA-A genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; and chr6:29944026-29944046. In some embodiments, the methods comprise reducing or eliminating surface expression of MHC class II protein in an engineered cell relative to an unmodified cell comprising contacting the cell with a composition comprising a CIITA guide RNA disclosed herein, the method further comprising reducing or eliminating the HLA-A expression of the cell by a gene editing system that binds to an HLA-A genomic target sequence comprising at least 10 contiguous nucleotides within the genomic coordinates chosen from: chr6:29942864-29942884; chr6:29942868-

29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; and chr6:29944026-29944046. In some embodiments, the HLA-A genomic coordinates are chosen from chr6:29942864-29942884. In some embodiments, the HLA-A genomic coordinates are chosen from chr6:29942868-29942888. In some embodiments, the HLA-A genomic coordinates are chosen from chr6:29942876-29942896. In some embodiments, the HLA-A genomic coordinates are chosen from chr6:29942877-29942897. In some embodiments, the HLA-A genomic coordinates are chosen from chr6:29942883-29942903. In some embodiments, the HLA-A genomic coordinates are chosen from chr6:29943126-29943146. In some embodiments, the HLA-A genomic coordinates are chosen from chr6:29943528-29943548. In some embodiments, the HLA-A genomic coordinates are chosen from chr6:29943529-29943549. In some embodiments, the HLA-A genomic coordinates are chosen from chr6:29943530-29943550. In some embodiments, the HLA-A genomic coordinates are chosen from chr6:29943537-29943557. In some embodiments, the HLA-A genomic coordinates are chosen from chr6:29943549-29943569. In some embodiments, the HLA-A genomic coordinates are chosen from chr6:29943589-29943609. In some embodiments, the HLA-A genomic coordinates are chosen from chr6:29944026-29944046. In some embodiments, the gene editing system comprises an RNA-guided DNA-binding agent. In some embodiments, the RNA-guided DNA-binding agent comprises a Cas9 protein, such as an *S. pyogenes* Cas9. In some embodiments, the cell is homozygous for HLA-B and homozygous for HLA-C.

[00189] In some embodiments, the methods comprise reducing or eliminating surface expression of MHC class II protein in an engineered cell relative to an unmodified cell comprising contacting the cell with a composition comprising a CIITA guide RNA disclosed herein, the method further comprising contacting the cell with an HLA-A guide RNA. In some embodiments the HLA-A guide RNA comprises a guide sequence selected from SEQ ID NOs: 2001-2095 (see **Table 3** below). In some embodiments, the method further comprises contacting the cell with an RNA-guided DNA binding agent. In some embodiments, the RNA-guided DNA-binding agent comprises a Cas9 protein, such as an *S. pyogenes* Cas9. In some embodiments, the cell is homozygous for HLA-B and homozygous for HLA-C.

[00190] In some embodiments, methods are provided for making an engineered cell which has reduced or eliminated surface expression of MHC class II protein relative to an unmodified cell, comprising: a. contacting the cell with a CIITA guide RNA, wherein the guide RNA

comprises a guide sequence selected from SEQ ID NOs: 1-117; and b. contacting the cell with an HLA-A guide RNA, wherein the HLA-A guide RNA comprises a guide sequence selected from any one of SEQ ID NOs: 2001-2095 (see **Table 3** below); and c. optionally contacting the cell with an RNA-guided DNA binding agent or nucleic acid encoding an RNA-guided DNA binding agent; wherein the cell has reduced or eliminated surface expression of HLA-A in the cell relative to an unmodified cell. In some embodiments, the method comprises contacting the cell with an RNA-guided DNA binding agent or nucleic acid encoding an RNA-guided DNA binding agent. In some embodiments, the RNA-guided DNA binding agent comprises an *S. pyogenes* Cas9. In some embodiments, the cell is homozygous for HLA-B and homozygous for HLA-C.

[00191] Exemplary HLA-A guide RNAs are provided in **Table 3** (SEQ ID NOs: 2001-2095 with corresponding guide RNA sequences SEQ ID NOs: 427-521 and 603-697).

[00192] **Table 3. Exemplary HLA-A guide sequences**

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
G018983	2001	UGGAGGGCC UGAUGUGUG UU	UGGAGGGCC UGAUGUGUG UUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mU*mG*mG*AGG GCCUGAUGUGUG UUGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994529 0-29945310 (mismatch to hg38=2)
G018984	2002	GCCUGAUGU GUGUUGGGU GU	GCCUGAUGU GUGUUGGGU GUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mG*mC*mC*UGAU GUGUGUUGGGUG UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU	chr6:2994529 6-29945316 (mismatch to hg38=2)

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
				mGmCmU*mU*mU*mU	
G018985	2003	CCUGAUGUG UGUUGGGUG UU	CCUGAUGUG UGUUGGGUG UUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mC*mC*mU*GAUG UGUGUUGGGUGU UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994529 7-29945317 (mismatch to hg38=1)
G018986	2004	CCCAACACCC AACACACAUC	CCCAACACCC AACACACAUC GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mC*mC*AACA CCCAACACACAUC CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994530 0-29945320 (mismatch to hg38=1)
G018965	2005	UCAGGAAAC AUGAAGAAA GC	UCAGGAAAC AUGAAGAAA GCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mU*mC*mA*GGA AACAUGAAGAAA GCGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2989011 7-29890137
G019018	2006	AGGCGCCUG GGCCUCUCCC G	AGGCGCCUG GGCCUCUCCC GGUUUUAGA	mA*mG*mG*CGCC UGGGCCUCUCCC GGUUUUAGAmGm	chr6:2992705 8-29927078

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
			GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	CmUmAmGmAmA mAmUmAmGmCA AGUUAUUAAUAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018937	2007	CGGGCUGGCC UCCCACAAGG	CGGGCUGGCC UCCCACAAGG GUUUUAGAG CUAGAAAU GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mG*mG*GCUG GCCUCCCACAAG GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAUUAAUAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2993433 0-29934350
G018990	2008	ACGGCCAUC UCGGCGUCU G	ACGGCCAUC UCGGCGUCU GUUUUUAGA GCUAGAAAU AGCAAGUUA AAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mA*mC*mG*GCCA UCCUCGGCGUCU GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAUUAAUAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994254 1-29942561
G018991	2009	GACGGCCAUC CUCGGCGUCU	GACGGCCAUC CUCGGCGUCU GUUUUAGAG CUAGAAAU GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA	mG*mA*mC*GGCC AUCCUCGGCGUC UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAUUAAUAG GCUAGUCCGUUA UCAmAmCmUmU	chr6:2994254 2-29942562

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
			AAAAGUGGC ACCGAGUCG GUGCUUUU	mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018992	2010	GACGCCGAG GAUGGCCGU CA	GACGCCGAG GAUGGCCGU CAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mG*mA*mC*GCCG AGGAUGGCCGUC AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994254 3-29942563
G018993	2011	UGACGGCCA UCCUCGGCGU C	UGACGGCCA UCCUCGGCGU CGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGG UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mU*mG*mA*CGGC CAUCCUCGGCGU CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994254 3-29942563
G018994	2012	GGCGCCAUG ACGGCCAUCC U	GGCGCCAUG ACGGCCAUCC UGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGG UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mG*mC*GCCA UGACGGCCAUCC UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU	chr6:2994255 0-29942570

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
				mGmCmU*mU*mU*mU	
G018995	2013	ACAGCGACGC CGCGAGCCAG	ACAGCGACGC CGCGAGCCAG GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mA*mC*mA*GCGA CGCCGCGAGCCA GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994286 4-29942884
G018996	2014	CGACGCCGCG AGCCAGAGG A	CGACGCCGCG AGCCAGAGG AGUUUUAGA GCUAGAAUA AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mG*mA*CGCC GCGAGCCAGAGG AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994286 8-29942888
G018997	2015	CGAGCCAGA GGAUGGAGC CG	CGAGCCAGA GGAUGGAGC CGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mC*mG*mA*GCCA GAGGAUGGAGCC GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994287 6-29942896
G018998	2016	CGGCUCCAUC CUCUGGCUCG	CGGCUCCAUC CUCUGGCUCG GUUUUAGAG	mC*mG*mG*CUCC AUCCUCUGGCUC GGUUUUAGAmGm	chr6:2994287 6-29942896

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
			CUAGAAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	CmUmAmGmAmA mAmUmAmGmCA AGUUAUUAAUAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018999	2017	GAGCCAGAG GAUGGAGCC GC	GAGCCAGAG GAUGGAGCC GCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mG*mA*mG*CCAG AGGAUGGAGCCG CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAUUAAUAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994287 7-29942897
G019000	2018	GCGCCCGCGG CUCCAUCUC	GCGCCCGCGG CUCCAUCUC GUUUUAGAG CUAGAAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mG*mC*mG*CCCG CGGCUCCAUCUC CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAUUAAUAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994288 3-29942903
G019001	2019	GCCCGUCCGU GGGGGAUGA G	GCCCGUCCGU GGGGGAUGA GGUUUUAGA GCUAGAAAUA AGCAAGUUA AAUAAGGCU UAGUCCGUU AUCAACUUG	mG*mC*mC*CGUC CGUGGGGGAUGA GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAUUAAUAG GCUAGUCCGUUA UCAmAmCmUmU	chr6:2994306 2-29943082

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
			AAAAAGUGG CACCGAGUCG GUGCUUUU	mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G019002	2020	UCAUCCCCCA CGGACGGGCC	UCAUCCCCCA CGGACGGGCC GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mU*mC*mA*UCCC CCACGGACGGGC CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994306 3-29943083
G019003	2021	AUCUCGGACC CGGAGACUG U	AUCUCGGACC CGGAGACUG UGUUUUAGA GCUAGAAUA AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mA*mU*mC*UCGG ACCCGGAGACUG UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994309 2-29943112
G019004	2022	GGGGUCCCGC GGCUUCGGG G	GGGGUCCCGC GGCUUCGGG GUUUUUAGA GCUAGAAUA AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mG*mG*GUCC CGCGGCUUCGGG GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU	chr6:2994311 5-29943135

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
				mGmCmU*mU*mU*mU	
G019005	2023	CUCGGGGUCC CGCGGCUUCG	CUCGGGGUCC CGCGGCUUCG GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mU*mC*GGGG UCCCCGCGCUUC GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994311 8-29943138
G019006	2024	UCUCGGGGU CCCCGCGGCUU C	UCUCGGGGU CCCCGCGGCUU CGUUUUAGA GCUAGAAUA AGCAAGUUA AAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mU*mC*mU*CGGG GUCCCCGCGCUU CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994311 9-29943139
G019007	2025	GUCUCGGGG UCCCCGCGGCU U	GUCUCGGGG UCCCCGCGGCU UGUUUUAGA GCUAGAAUA AGCAAGUUA AAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mU*mC*UCGG GGUCCCCGCGCU UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994312 0-29943140
G019008	2026	GCAAGGGUC UCGGGGUCCC G	GCAAGGGUC UCGGGGUCCC GGUUUUAGA	mG*mC*mA*AGG GUCUCGGGGUCC CGGUUUUAGAmG	chr6:2994312 6-29943146

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
			GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mCmUmAmGmAm AmAmUmAmGmC AAGUUAUUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G019009	2027	GGACCCCGAG ACCCUUGCCC	GGACCCCGAG ACCCUUGCCC GUUUUAGAG CUAGAAAU GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mG*mG*mA*CCCC GAGACCCUUGCC CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994312 8-29943148
G019010	2028	GACCCCGAGA CCCUUGCCCC	GACCCCGAGA CCCUUGCCCC GUUUUAGAG CUAGAAAU GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mG*mA*mC*CCCG AGACCCUUGCCC CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994312 9-29943149
G019011	2029	CGAGACCCUU GCCCCGGGAG	CGAGACCCUU GCCCCGGGAG GUUUUAGAG CUAGAAAU GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA	mC*mG*mA*GACC CUUGCCCCGGGA GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU	chr6:2994313 4-29943154

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
			AAAAGUGGC ACCGAGUCG GUGCUUUU	mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G019012	2030	CUCCCGGGG AAGGGUCUC G	CUCCCGGGG AAGGGUCUC GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mU*mC*CCGG GGCAAGGGUCUC GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994313 4-29943154
G019013	2031	UCUCCCGGG CAAGGGUCU C	UCUCCCGGG CAAGGGUCU CGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mU*mC*mU*CCCG GGGCAAGGGUCU CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994313 5-29943155
G019014	2032	CUCUCCCGG GCAAGGGUC U	CUCUCCCGG GCAAGGGUC UGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mU*mC*UCCC GGGGCAAGGGUC UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU	chr6:2994313 6-29943156

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
				mGmCmU*mU*mU*mU	
G019015	2033	CCUUGCCCCG GGAGAGGCC C	CCUUGCCCCG GGAGAGGCC CGUUUUAGA GCUAGAAAU AGCAAGUUA AAUUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mC*mU*UGCC CCGGGAGAGGCC CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994314 0-29943160
G019016	2034	CUGGGCCUCU CCCCGGGCAA	CUGGGCCUCU CCCCGGGCAA GUUUUAGAG CUAGAAAU GCAAGUUA AAUUAAGGC AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mU*mG*GGCC UCUCCCGGGCA AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994314 2-29943162
G019017	2035	CCUGGGCCUC UCCCGGGGCA	CCUGGGCCUC UCCCGGGGCA GUUUUAGAG CUAGAAAU GCAAGUUA AAUUAAGGC AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mC*mU*GGGC CUCUCCCGGGC AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994314 3-29943163
G019019	2036	UUUAGGCCA AAAAUCCCC C	UUUAGGCCA AAAAUCCCC CGUUUUAGA	mU*mU*mU*AGG CCAAAAUCCCC CCGUUUUAGAmG	chr6:2994318 8-29943208

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
			GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mCmUmAmGmAm AmAmUmAmGmC AAGUUAUUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G021208	2037	CGCUGCAGCG CACGGGUACC	CGCUGCAGCG CACGGGUACC GUUUUAGAG CUAGAAAU GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mG*mC*UGCA GCGCACGGGUAC CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994352 8-29943548
G021209	2038	GCUGCAGCGC ACGGGUACC A	GCUGCAGCGC ACGGGUACC AGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mC*mU*GCAG CGCACGGGUACC AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994352 9-29943549
G021210	2039	CUGCAGCGCA CGGGUACCA G	CUGCAGCGCA CGGGUACCA GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG	mC*mU*mG*CAGC GCACGGGUACCA GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU	chr6:2994353 0-29943550

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
			AAAAAGUGG CACCGAGUCG GUGCUUUU	mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018932	2040	CGCACGGGU ACCAGGGGCC A	CGCACGGGU ACCAGGGGCC AGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mG*mC*ACGG GUACCAGGGGCC AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994353 6-29943556
G018933	2041	GCACGGGUA CCAGGGGCCA C	GCACGGGUA CCAGGGGCCA CGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mC*mA*CGGG UACCAGGGGCCA CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994353 7-29943557
G018934	2042	CACGGGUACC AGGGGCCAC G	CACGGGUACC AGGGGCCAC GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mA*mC*GGGU ACCAGGGGCCAC GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU	chr6:2994353 8-29943558

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
				mGmCmU*mU*mU*mU	
G018935	2043	GGGAGGCGC CCCGUGGCCC C	GGGAGGCGC CCCGUGGCCC CGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mG*mG*AGG CGCCCCGUGGCC CCGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994354 9-29943569
G018936	2044	GCGAUCAGG GAGGCGCCCC G	GCGAUCAGG GAGGCGCCCC GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mC*mG*AUCA GGGAGGCGCCCC GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994355 6-29943576
G021211	2045	UCCUUGUGG GAGGCCAGCC C	UCCUUGUGG GAGGCCAGCC CGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mU*mC*mC*UUGU GGGAGGCCAGCC CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994358 9-29943609
G018938	2046	CUCCUUGUG GGAGGCCAG CC	CUCCUUGUG GGAGGCCAG CCGUUUUAG	mC*mU*mC*CUUG UGGGAGGCCAGC CGUUUUAGAmGm	chr6:2994359 0-29943610

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
			AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018939	2047	GGCUGGCCUC CCACAAGGA G	GGCUGGCCUC CCACAAGGA GGUUUUAGA GCUAGAAAU AGCAAGUUA AAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mG*mC*UGGC CUCCCACAAGGA GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994359 0-29943610
G018940	2048	UUGUCUCCCC UCCUUGUGG G	UUGUCUCCCC UCCUUGUGG GGUUUUAGA GCUAGAAAU AGCAAGUUA AAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mU*mU*mG*UCUC CCCUCCUUGUGG GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994359 9-29943619
G018941	2049	CCACAAGGA GGGGAGACA AU	CCACAAGGA GGGGAGACA AUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU	mC*mC*mA*CAAG GAGGGGAGACAA UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU	chr6:2994360 0-29943620

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
			GAAAAAGUG GCACCGAGUC GGUGCUUUU	mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018942	2050	CACAAGGAG GGGAGACAA UU	CACAAGGAG GGGAGACAA UUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mC*mA*mC*AAGG AGGGGAGACAAU UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994360 1-29943621
G018943	2051	CAAUUGUCU CCCCUCCUUG U	CAAUUGUCU CCCCUCCUUG UGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mA*mA*UUG UCUCCCCUCCUU GUGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994360 2-29943622
G018944	2052	CCAAUUGUC UCCCCUCCUU G	CCAAUUGUC UCCCCUCCUU GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mC*mA*AUUG UCUCCCCUCCUU GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU	chr6:2994360 3-29943623

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
				mGmCmU*mU*mU*mU	
G018945	2053	AUCCUCGAA UACUGAUGA G	AUCCUCGAA UACUGAUGA GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mA*mU*mC*CCUC GAAUACUGAUGA GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994377 4-29943794
G018946	2054	AACCACUCAU CAGUAUUCG A	AACCACUCAU CAGUAUUCG AGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mA*mA*mC*CACU CAUCAGUAUUCG AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994377 9-29943799
G018947	2055	GAACCACUCA UCAGUAUUC G	GAACCACUCA UCAGUAUUC GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mG*mA*mA*CCAC UCAUCAGUAUUC GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994378 0-29943800
G018948	2056	GAGGAAAAG UCACGGGCC A	GAGGAAAAG UCACGGGCC AGUUUUAGA	mG*mA*mG*GAA AAGUCACGGGCC CAGUUUUAGAmG	chr6:2994382 2-29943842

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
			GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mCmUmAmGmAm AmAmUmAmGmC AAGUUAUUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018949	2057	GGCCCGUGAC UUUUCUCUC	GGCCCGUGAC UUUUCUCUC GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mG*mG*mC*CCGU GACUUUUCUCU CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994382 4-29943844
G018950	2058	UGCUUCACAC UCAUGUGU G	UGCUUCACAC UCAUGUGU GUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mU*mG*mC*UUCA CACUCAUGUGU GUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994385 7-29943877
G018951	2059	GCUUCACACU CAAUGUGUG U	GCUUCACACU CAAUGUGUG UGUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG	mG*mC*mU*UCAC ACUCAUGUGUG UGUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU	chr6:2994385 8-29943878

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
			AAAAAGUGG CACCGAGUCG GUGCUUUU	mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018952	2060	CUUCACACUC AAUGUGUGU G	CUUCACACUC AAUGUGUGU GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mU*mU*CACA CUCAAUGUGUGU GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994385 9-29943879
G018953	2061	UUCACACUCA AUGUGUGUG G	UUCACACUCA AUGUGUGUG GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mU*mU*mC*ACAC UCAUGUGUGUG GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994386 0-29943880
G018954	2062	UUGAGAAUG GACAGGACA CC	UUGAGAAUG GACAGGACA CCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mU*mU*mG*AGA AUGGACAGGACA CCGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU	chr6:2994402 6-29944046

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
				mGmCmU*mU*mU*mU	
G021205	2063	AGGCAUUUU GCAUCUGUC AU	AGGCAUUUU GCAUCUGUC AUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mA*mG*mG*CAU UUUGCAUCUGUC AUGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994407 7-29944097
G021206	2064	CAGGCAUUU UGCAUCUGU CA	CAGGCAUUU UGCAUCUGU CAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mC*mA*mG*GCAU UUUGCAUCUGUC AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994407 8-29944098
G018955	2065	AGGGGCCCCU GACCCUGCUA A	AGGGGCCCCU GACCCUGCUA AGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGG UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mA*mG*mG*GGCC CUGACCCUGCUA AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994445 8-29944478
G018956	2066	UGGGAAAAG AGGGGAAGG UG	UGGGAAAAG AGGGGAAGG UGGUUUUAG	mU*mG*mG*GAA AAGAGGGGAAGG UGGUUUUAGAmG	chr6:2994447 8-29944498

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
			AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018957	2067	UGGAGGAGG AAGAGCUCA GG	UGGAGGAGG AAGAGCUCA GGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mU*mG*mG*AGG AGGAAGAGCUCA GGGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994459 7-29944617
G018958	2068	UGAGAUUUC UUGUCUCAC UG	UGAGAUUUC UUGUCUCAC UGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mU*mG*mA*GAU UUCUUGUCUCAC UGGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994464 2-29944662
G018959	2069	GAGAUUUCU UGUCUCACU GA	GAGAUUUCU UGUCUCACU GAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU	mG*mA*mG*AUU UCUUGUCUCACU GAGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU	chr6:2994464 3-29944663

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
			GAAAAAGUG GCACCGAGUC GGUGCUUUU	mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018960	2070	UAAAGCACC UGUUAAAAU GA	UAAAGCACC UGUUAAAAU GAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mU*mA*mA*AGC ACCUGUUAAAAU GAGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994477 2-29944792
G018961	2071	AAUCUGUCC UUCAUUUUA AC	AAUCUGUCC UUCAUUUUA ACGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mA*mA*mU*CUG UCCUUCAUUUUA ACGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994478 2-29944802
G018962	2072	GUCACAGGG GAAGGUCCC UG	GUCACAGGG GAAGGUCCC UGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mG*mU*mC*ACAG GGGAAGGUCCCU GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU	chr6:2994485 0-29944870

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
				mGmCmU*mU*mU*mU	
G018964	2073	AAACAUGAA GAAAGCAGG UG	AAACAUGAA GAAAGCAGG UGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mA*mA*mA*CAU GAAGAAAGCAGG UGGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994490 7-29944927
G018966	2074	UGUCCUGUG AGAUACCAG AA	UGUCCUGUG AGAUACCAG AAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mU*mG*mU*CCUG UGAGAUACCAGA AGUUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994502 4-29945044
G018967	2075	AUGAAGGAG GCUGAUGCC UG	AUGAAGGAG GCUGAUGCC UGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mA*mU*mG*AAG GAGGCUGAUGCC UGGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994509 7-29945117
G018968	2076	AGGCUGAUG CCUGAGGUCC U	AGGCUGAUG CCUGAGGUCC UGUUUUAGA	mA*mG*mG*CUG AUGCCUGAGGUC CUGUUUUAGAmG	chr6:2994510 4-29945124

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
			GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mCmUmAmGmAm AmAmUmAmGmC AAGUUAUUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018969	2077	GGCUGAUGC CUGAGGUCC UU	GGCUGAUGC CUGAGGUCC UUGUUUUAG AGCUAGAAA UAGCAAGUU AAAUAAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mG*mG*mC*UGA UGCCUGAGGUCC UUGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAUUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994510 5-29945125
G018970	2078	CACAAUAUCC CAAGGACCUC	CACAAUAUCC CAAGGACCUC GUUUUAGAG CUAGAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mA*mC*AAUA UCCCAAGGACCU CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAUUAAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994511 6-29945136
G018971	2079	GGUCCUUGG GAUAUUGUG UU	GGUCCUUGG GAUAUUGUG UUGUUUUAG AGCUAGAAA UAGCAAGUU AAAUAAAGG CUAGUCCGU UAUCAACUU	mG*mG*mU*CCUU GGGAUAUUGUGU UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAUUAAAG GCUAGUCCGUUA UCAmAmCmUmU	chr6:2994511 8-29945138

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
			GAAAAAGUG GCACCGAGUC GGUGCUUUU	mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018972	2080	GUCCUUGGG AUAUUGUGU UU	GUCCUUGGG AUAUUGUGU UUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mG*mU*mC*CUUG GGAUAUUGUGUU UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994511 9-29945139
G018973	2081	CUCCCAAACA CAAUAUCCCA	CUCCCAAACA CAAUAUCCCA GUUUUAGAG CUAGAAAUA GCAAGUUAA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mU*mC*CCAA ACACAAUAUCCC AGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994512 4-29945144
G018974	2082	UCCUCUAGCC ACAUCUUCU G	UCCUCUAGCC ACAUCUUCU GGUUUUAGA GCUAGAAAU AGCAAGUUA AAUAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mU*mC*mC*UCUA GCCACAUCUUCU GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU	chr6:2994517 6-29945196

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
				mGmCmU*mU*mU*mU	
G018975	2083	ACAGAAGAU GUGGCUAGA GG	ACAGAAGAU GUGGCUAGA GGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mA*mC*mA*GAA GAUGUGGCUAGA GGGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994517 7-29945197
G018976	2084	CCUCUAGCCA CAUCUUCUG U	CCUCUAGCCA CAUCUUCUG UGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mC*mU*CUAG CCACAUCUUCUG UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994517 7-29945197
G018977	2085	CCCACAGAAG AUGUGGCUA G	CCCACAGAAG AUGUGGCUA GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mC*mC*mC*ACAG AAGAUGUGGCUA GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994518 0-29945200
G018978	2086	GUCAGAUCCC ACAGAAGAU G	GUCAGAUCCC ACAGAAGAU GGUUUUAGA	mG*mU*mC*AGA UCCCACAGAAGA UGGUUUUAGAmG	chr6:2994518 7-29945207

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
			GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG AAAAAGUGG CACCGAGUCG GUGCUUUU	mCmUmAmGmAm AmAmUmAmGmC AAGUUAUUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018979	2087	AUCUUCUGU GGGAUCUGA CC	AUCUUCUGU GGGAUCUGA CCGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mA*mU*mC*UUCU GUGGGAUCUGAC CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994518 8-29945208
G018980	2088	CCCAGGCAGU GACAGUGCCC	CCCAGGCAGU GACAGUGCCC GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mC*mC*AGGC AGUGACAGUGCC CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994522 8-29945248
G018981	2089	CUGGGCACU GUCACUGCCU G	CUGGGCACU GUCACUGCCU GGUUUUAGA GCUAGAAAU AGCAAGUUA AAAUAAAGGC UAGUCCGUU AUCAACUUG	mC*mU*mG*GGCA CUGUCACUGCCU GGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU	chr6:2994523 0-29945250

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
			AAAAAGUGG CACCGAGUCG GUGCUUUU	mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	
G018982	2090	CCUGGGCACU GUCACUGCCU	CCUGGGCACU GUCACUGCCU GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mC*mU*GGGC ACUGUCACUGCC UGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994523 1-29945251
G021207	2091	CCCUGGGCAC UGUCACUGCC	CCCUGGGCAC UGUCACUGCC GUUUUAGAG CUAGAAUA GCAAGUUA AAUAAGGCU AGUCCGUUA UCAACUUGA AAAAGUGGC ACCGAGUCG GUGCUUUU	mC*mC*mC*UGGG CACUGUCACUGC CGUUUUAGAmGm CmUmAmGmAmA mAmUmAmGmCA AGUUAAAAUAAG GCUAGUCCGUUA UCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994523 2-29945252
G018987	2092	UUGGGUGUU GGGCGGAAC AG	UUGGGUGUU GGGCGGAAC AGGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mU*mU*mG*GGU GUUGGGCGGAAC AGGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU	chr6:2994530 8-29945328

Guide ID	SEQ ID NO to the Guide Sequence	Guide Sequence	Exemplary Full Sequence (SEQ ID NOs: 427-521)	Exemplary Mod Sequence (four terminal U residues are optional and may include 0, 1, 2, 3, 4, or more Us) (SEQ ID NOs: 603-697)	Genomic Coordinates
				mGmCmU*mU*mU*mU	
G018988	2093	UGGAUGUAU UGAGCAUGC GA	UGGAUGUAU UGAGCAUGC GAGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mU*mG*mG*AUG UAUUGAGCAUGC GAGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994536 1-29945381
G018989	2094	GGAUGUAUU GAGCAUGCG AU	GGAUGUAUU GAGCAUGCG AUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mG*mG*mA*UGU AUUGAGCAUGCG AUGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:2994536 2-29945382
G018963	2095	AACAUGAAG AAAGCAGGU GU	AACAUGAAG AAAGCAGGU GUGUUUUAG AGCUAGAAA UAGCAAGUU AAAAUAAGG CUAGUCCGU UAUCAACUU GAAAAAGUG GCACCGAGUC GGUGCUUUU	mA*mA*mC*AUG AAGAAAGCAGGU GUGUUUUAGAmG mCmUmAmGmAm AmAmUmAmGmC AAGUUAAAAUAA GGCUAGUCCGUU AUCAmAmCmUmU mGmAmAmAmAm AmGmUmGmGmC mAmCmCmGmAm GmUmCmGmGmU mGmCmU*mU*mU *mU	chr6:3138254 3-31382563

[00193] In some embodiments, the efficacy of an HLA-A guide RNA is determined by measuring levels of HLA-A protein on the surface of a cell. In some embodiments, HLA-A protein levels are measured by flow cytometry (*e.g.*, with an antibody against HLA-A2 and/or HLA-A3). In some embodiments, the population of cells is at least 65% HLA-A negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 70% HLA-A negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 80% HLA-A negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 90% HLA-A negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 95% HLA-A negative as measured by flow cytometry relative to a population of unmodified cells. In some embodiments, the population of cells is at least 100% HLA-A negative as measured by flow cytometry relative to a population of unmodified cells.

[00194] In some embodiments, the efficacy of a B2M guide RNA or an HLA-A guide may be determined by measuring the response of immune cells *in vitro* or *in vivo* (*e.g.*, CD8+ T cells) to the genetically modified target cell as compared to an unmodified cell. For example, a reduced response from CD8+ T cells is indicative of an effective B2M guide RNA or HLA-A guide RNA. A CD8+ T cell response may be evaluated by an assay that measures CD8+ T cell activation responses, *e.g.*, CD8+ T cell proliferation, expression of activation markers, and/or cytokine production (IL-2, IFN- γ , TNF- α) (*e.g.*, flow cytometry, ELISA). The CD8+ T cell response may be assessed *in vitro* or *in vivo*. In some embodiments, the CD8+ T cell response may be evaluated by co-culturing the genetically modified cell with CD8+ T cells *in vitro*. In some embodiments, CD8+ T cell activity may be evaluated in an *in vivo* model, *e.g.*, a rodent model. In an *in vivo* model, *e.g.*, genetically modified cells may be administered with CD8+ T cell; survival of the genetically modified cells is indicative of the ability to avoid CD8+ T cell lysis. In some embodiments, the methods produce a composition comprising a cell that survives *in vivo* in the presence of CD8+ T cells for greater than 1, 2, 3, 4, 5, or 6 weeks or more. In some embodiments, the methods produce a composition comprising a cell that survives *in vivo* in the presence of CD8+ T cells for at least one week to six weeks. In some embodiments, the methods produce a composition comprising a cell that survives *in vivo* in the presence of CD8+ T cells for at least two to four weeks. In some embodiments, the methods produce a composition comprising a cell that survives *in vivo* in the presence of CD8+ T cells

for at least four to six weeks. In some embodiments, the methods produce a composition comprising a cell that survives *in vivo* in the presence of CD8⁺ T cells for more than six weeks.

[00195] In some embodiments, the methods produce a composition comprising a cell having reduced or eliminated MHC class II expression and reduced or eliminated MHC class I expression relative to an unmodified cell. In some embodiments, the methods produce a composition comprising a cell having reduced or eliminated MHC class II protein expression, reduced or eliminated CIITA protein expression, and/or reduced or eliminated CIITA levels in the cell nucleus, and or eliminated reduced MHC class I protein expression. In some embodiments, the methods produce a composition comprising a cell having reduced or eliminated MHC class II protein expression, reduced or eliminated CIITA protein expression, and/or reduced or eliminated CIITA levels in the cell nucleus, and/or eliminated or reduced B2M protein expression. In some embodiments, the methods produce a composition comprising a cell having reduced or eliminated MHC class II protein expression, reduced or eliminated CIITA protein expression, and/or reduced or eliminated CIITA levels in the cell nucleus, and reduced or eliminated B2M mRNA levels. In some embodiments, the cell elicits a reduced or eliminated response from CD8⁺ T cells.

[00196] In some embodiments, the methods produce a composition comprising a cell having reduced or eliminated MHC class II expression and reduced or eliminated HLA-A expression relative to an unmodified cell, wherein the cell is homozygous for HLA-B and homozygous for HLA-C. In some embodiments, the methods produce a composition comprising a cell having reduced or eliminated MHC class II protein expression, reduced or eliminated CIITA protein expression, and/or reduced or eliminated CIITA levels in the cell nucleus, and or eliminated reduced HLA-A protein expression. In some embodiments, the methods produce a composition comprising a cell having reduced or eliminated MHC class II protein expression, reduced or eliminated CIITA protein expression, and/or reduced or eliminated CIITA levels in the cell nucleus, and/or eliminated or reduced HLA-A protein expression. In some embodiments, the cell elicits a reduced or eliminated response from CD8⁺ T cells.

[00197] In some embodiments, an engineered cell is provided wherein the cell has reduced or eliminated expression of MHC class II and MHC class I protein on the cell surface, wherein the cell comprises a genetic modification in CIITA, and wherein the cell comprises a modification in B2M. In some embodiments, the cell elicits a reduced response from CD4⁺ T cells and elicits a reduced response from CD8⁺ T cells.

[00198] In some embodiments, an engineered cell is provided wherein the cell has reduced or eliminated expression of MHC class II and HLA-A protein on the cell surface, wherein the

cell comprises a genetic modification in CIITA, and wherein the cell comprises a genetic modification in the HLA-A gene, wherein the cell is homozygous for HLA-B and homozygous for HLA-C. In some embodiments, an engineered cell is provided wherein the cell has reduced or eliminated expression of MHC class II and HLA-A protein on the cell surface, wherein the cell comprises a genetic modification in CIITA, and wherein the cell comprises a genetic modification in the HLA-A gene. In some embodiments, the cell is homozygous for HLA-B and HLAC. In some embodiments, the cell elicits a reduced response from CD4+ T cells and elicits a reduced response from CD8+ T cells.

2. Exogenous nucleic acids

[00199] In some embodiments, the present disclosure provides methods and compositions for reducing or eliminating expression of MHC class II protein on the surface of a cell by genetically modifying CIITA as disclosed herein, wherein the methods and compositions further provide for expression of an exogenous nucleic acid by the engineered cell.

a) NK cell inhibitor knock-in

[00200] In some embodiments, the present disclosure provides methods for reducing or eliminating expression of MHC class II protein on the surface of a cell by genetically modifying CIITA as disclosed herein, wherein the methods further provide for expression of an exogenous nucleic acid by the cell, wherein the exogenous nucleic acid encodes an NK cell inhibitor molecule. In some embodiments, the NK cell inhibitor molecule is expressed on the surface of the cell, thereby avoiding the activity of NK cells (*e.g.*, lysis of the cell by the NK cell). In some embodiments, the ability of the genetically modified cell to avoid NK cell lysis makes the cell amenable to adoptive cell transfer therapies. In some embodiments, the cell is an allogeneic cell.

[00201] In some embodiments, the methods comprise reducing or eliminating expression of MHC class II protein on the surface of a cell comprising genetically modifying CIITA comprising contacting the cell with a composition comprising a CIITA guide RNA disclosed herein, the method further comprising contacting the cell with a nucleic acid encoding an NK cell inhibitor molecule. In some embodiments, the methods comprise reducing or eliminating expression of MHC class II protein on the surface of a cell comprising modifying CIITA comprising contacting the cell with a composition comprising a CIITA guide RNA disclosed, the method further comprising contacting the cell with a nucleic acid encoding an NK cell inhibitor molecule, and a B2M guide RNA, thereby reducing or eliminating expression of MHC

class I protein on the surface of the cell. In some embodiments, the method further comprises contacting the cell with an RNA-guided DNA binding agent.

[00202] In some embodiments, the methods comprise reducing or eliminating expression of MHC class II protein on the surface of a cell, comprising genetically modifying the cell with one or more compositions comprising a CIITA guide RNA disclosed herein, a B2M guide RNA, a nucleic acid encoding an NK cell inhibitor molecule, and an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[00203] In some embodiments, the methods comprise inducing a DSB or an SSB in CIITA comprising contacting the cell with a composition comprising a CIITA guide RNA disclosed herein, the method further comprising contacting the cell with a nucleic acid encoding an NK cell inhibitor molecule. In some embodiments, the methods comprise inducing a DSB or an SSB in CIITA comprising contacting the cell with a composition comprising a CIITA guide RNA disclosed herein, the method further comprising contacting the cell with a nucleic acid encoding an NK cell inhibitor molecule, and a B2M guide RNA, thereby reducing expression of MHC class I protein on the surface of the cell. In some embodiments, the method further comprises contacting the cell with an RNA-guided DNA binding agent.

[00204] In some embodiments, the methods comprise reducing or eliminating expression of the CIITA protein in a cell comprising delivering a composition comprising a CIITA guide RNA disclosed herein, the method further comprising contacting the cell with a nucleic acid encoding an NK cell inhibitor molecule. In some embodiments, the methods comprise reducing expression of the CIITA protein in a cell comprising delivering a composition comprising a CIITA guide RNA disclosed herein, the method further comprising contacting the cell with a nucleic acid encoding an NK cell inhibitor molecule, and a B2M guide RNA, thereby reducing expression of MHC class I protein on the surface of the cell. In some embodiments, the method further comprises contacting the cell with an RNA-guided DNA binding agent.

[00205] In some embodiments, the NK cell inhibitor molecule binds to an inhibitory receptor on an NK cell. In some embodiments, the NK cell inhibitor molecule binds to an inhibitory receptor specific for MHC class I. In some embodiments, the NK cell inhibitor molecule binds to an inhibitory receptor that is not specific for MHC class I. NK cell inhibitory receptors include *e.g.*, KIR (human), CD94-NKG2A heterodimer (human/mouse), Ly49 (mouse), 2B4, SLAMF6, NKFP-B, TIGIT, KIR2DL4.

[00206] In some embodiments, the NK cell inhibitor molecule binds to NKG2A.

[00207] In some embodiments, the NK cell inhibitor molecule is an MHC class I molecule. In some embodiments, the NK cell inhibitor molecule is a classical MHC class I molecule. In

some embodiments, the NK cell inhibitor molecule is a non-classical MHC class I molecule. In some embodiments, the NK cell inhibitor molecule is an HLA molecule. NK cell inhibitor molecules include *e.g.*, HLA-C, HLA-E, HLA-G, Cd1, CD48, SLAMF6, Clr-b, and CD155.

[00208] In some embodiments, the NK cell inhibitor molecule is HLA-E.

[00209] In some embodiments, the NK cell inhibitor molecule is a fusion protein. In some embodiments, the NK cell inhibitor molecule is a fusion protein comprising HLA-E. In some embodiments, the NK cell inhibitor molecule comprises B2M. In some embodiments, the NK cell inhibitor molecule comprises HLA-E and B2M. In some embodiments, the fusion protein includes a linker. In some embodiments, the HLA-E construct is provided in a vector. In some embodiments, a vector comprising the HLA-E construct is a lentiviral vector. In some embodiments, the HLA-E construct is delivered to the cell via lentiviral transduction.

[00210] In some embodiments, the NK cell inhibitor molecule is inserted into the genome of the target cell. In some embodiments, the NK cell inhibitor molecule is integrated into the genome of the target cell. In some embodiments, the NK cell inhibitor molecule is integrated into the genome of the target cell by homologous recombination (HR). In some embodiments, the NK cell inhibitor molecule is integrated into the genome of the target cell by blunt end insertion. In some embodiments, the NK cell inhibitor molecule is integrated into the genome of the target cell by non-homologous end joining. In some embodiments, the NK cell inhibitor molecule is integrated into a safe harbor locus in the genome of the cell. In some embodiments, the NK cell inhibitor molecule is integrated into one of the TRAC locus, B2M locus, AAVS1 locus, and/or CIITA locus. In some embodiments, the NK cell inhibitor molecule is provided to the cell in a lipid nucleic acid assembly composition. In some embodiments, the lipid nucleic acid assembly composition is a lipid nanoparticle (LNP).

[00211] In some embodiments, the methods produce an engineered cell that elicits a reduced response from NK cells. The NK cell response may be assessed *in vitro* or *in vivo*. In some embodiments, NK cell activity may be evaluated by co-culturing the genetically modified cell with NK cells *in vitro*. In some embodiments, NK cell activity may be evaluated in an *in vivo* model, *e.g.*, a rodent model. In an *in vivo* model, *e.g.*, genetically modified cells may be administered with NK cells; survival of the genetically modified cells is indicative of the ability to avoid NK cell lysis. In some embodiments, the methods produce a composition comprising a cell that survives *in vivo* in the presence of NK cells for greater than 1, 2, 3, 4, 5, or 6 weeks or more. In some embodiments, the methods produce a composition comprising a cell that survives *in vivo* in the presence of NK cells for at least one week to six weeks. In some embodiments, the methods produce a composition comprising a cell that survives *in vivo* in the

presence of NK cells for at least two to four weeks. In some embodiments, the methods produce a composition comprising a cell that survives *in vivo* in the presence of NK cells for at least four to six week. In some embodiments, the methods produce a composition comprising a cell that survives *in vivo* in the presence of NK cells for more than six weeks.

[00212] In some embodiments, the methods produce a composition comprising an engineered cell having reduced or eliminated MHC class II expression and comprising a nucleic acid encoding an NK cell inhibitor molecule. In some embodiments, the methods produce a composition comprising an engineered cell having reduced or eliminated MHC class II expression and expression of an NK cell inhibitor molecule on the cell surface. In some embodiments, the methods produce a composition comprising a cell having reduced or eliminated MHC class II expression and eliciting a reduced response from NK cells. In some embodiments, the methods produce a composition comprising a cell having reduced or eliminated MHC class II protein expression, reduced or eliminated CIITA protein expression, and/or reduced or eliminated CIITA levels in the cell nucleus, and eliciting a reduced response from NK cells, and having reduced or eliminated MHC class I protein expression. In some embodiments, the cell elicits a reduced response from CD4+ T cells, CD8+ T cells, and/or NK cells.

[00213] In some embodiments, an allogeneic cell is provided wherein the cell has reduced or eliminated expression of MHC class II and MHC class I protein on the cell surface, wherein the cell comprises a modification in CIITA as disclosed herein, wherein the cell comprises a genetic modification in B2M, and wherein the cell comprises a nucleic acid encoding an NK cell inhibitor molecule. In some embodiments, the allogeneic cell elicits a reduced response from CD4+ T cells, CD8+ T cells, and/or NK cells.

b) Targeting receptors and other cell-surface expressed polypeptides; secreted polypeptides

[00214] In some embodiments, the present disclosure provides methods for reducing or eliminating expression of MHC class II protein on the surface of a cell by genetically modifying CIITA as disclosed herein, wherein the methods further provide for expression of one or more exogenous nucleic acids (*e.g.*, an antibody, chimeric antigen receptor (CAR), T cell receptor (TCR), cytokine or cytokine receptor, chemokine or chemokine receptor, enzyme, fusion protein, or other type of cell-surface bound or soluble polypeptide). In some embodiments, the exogenous nucleic acid encodes a protein that is expressed on the cell surface. For example, in some embodiments, the exogenous nucleic acid encodes a targeting receptor expressed on the

cell surface (described further herein). In some embodiments, the genetically modified cell may function as a “cell factory” for the expression of a secreted polypeptide encoded by an exogenous nucleic acid, including *e.g.*, as a source for continuous production of a polypeptide *in vivo* (as described further herein). In some embodiments, the cell is an allogeneic cell.

[00215] In some embodiments, the methods comprise reducing or eliminating expression of MHC class II protein on the surface of a cell comprising genetically modifying CIITA comprising contacting the cell with a composition comprising a CIITA guide RNA as disclosed herein, the method further comprising contacting the cell with an exogenous nucleic acid. In some embodiments, the methods comprise reducing or eliminating expression of MHC class II protein on the surface of a cell comprising genetically modifying CIITA comprising contacting the cell with a composition comprising a CIITA guide RNA as disclosed herein, the method further comprising contacting the cell with an exogenous nucleic acid, and a B2M guide RNA, thereby reducing or eliminating expression of MHC class I protein on the surface of the cell. In some embodiments, the methods comprise reducing or eliminating expression of MHC class II protein on the surface of a cell comprising genetically modifying the CIITA gene comprising contacting the cell with a composition comprising a CIITA guide RNA as disclosed herein, the method further comprising contacting the cell with an exogenous nucleic acid, a cell-surface expressed (e.g. targeting receptor) or soluble (e.g. secreted) polypeptide, and a B2M guide RNA, thereby reducing or eliminating expression of MHC class I protein on the surface of the cell. In some embodiments, the methods comprise contacting the cell with more than one exogenous nucleic acid. In some embodiments, the method further comprises contacting the cell with an RNA-guided DNA binding agent.

[00216] In some embodiments, the methods comprise reducing or eliminating expression of MHC class II protein on the surface of a cell comprising genetically modifying CIITA comprising contacting the cell with a composition comprising a CIITA guide RNA as disclosed herein, the method further comprising contacting the cell with an exogenous nucleic acid. In some embodiments, the methods comprise reducing or eliminating expression of MHC class II protein on the surface of a cell comprising genetically modifying CIITA comprising contacting the cell with a composition comprising a CIITA guide RNA as disclosed herein, the method further comprising contacting the cell with an exogenous nucleic acid, and an HLA-A guide RNA, thereby reducing or eliminating expression of HLA-A protein on the surface of the cell. In some embodiments, the methods comprise reducing or eliminating expression of MHC class II protein on the surface of a cell comprising genetically modifying the CIITA gene comprising contacting the cell with a composition comprising a CIITA guide RNA as disclosed herein, the

method further comprising contacting the cell with an exogenous nucleic acid, a cell-surface expressed (e.g. targeting receptor) or soluble (e.g. secreted) polypeptide, and an HLA-A guide RNA, thereby reducing or eliminating expression of HLA-A protein on the surface of the cell. In some embodiments, the methods comprise contacting the cell with more than one exogenous nucleic acid. In some embodiments, the method further comprises contacting the cell with an RNA-guided DNA binding agent.

[00217] In some embodiments, the methods comprise reducing or eliminating expression of MHC class II protein on the surface of a cell, comprising genetically modifying the cell with one or more compositions comprising a CIITA guide RNA as disclosed herein, a B2M guide RNA, an exogenous nucleic acid encoding an NK cell inhibitor molecule, an exogenous nucleic acid encoding a polypeptide (*e.g.*, a targeting receptor), and an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[00218] In some embodiments, the methods comprise reducing or eliminating expression of MHC class II protein and MHC class I protein on the surface of a cell, comprising genetically modifying the cell with one or more compositions comprising a CIITA guide RNA as disclosed herein, a B2M guide RNA, an exogenous nucleic acid encoding an NK cell inhibitor molecule, an exogenous nucleic acid encoding a polypeptide (*e.g.*, a targeting receptor), and an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[00219] In some embodiments, the methods comprise reducing or eliminating expression of MHC class II protein and HLA-A protein on the surface of a cell, comprising genetically modifying the cell with one or more compositions comprising a CIITA guide RNA as disclosed herein, a B2M guide RNA, an exogenous nucleic acid encoding a polypeptide (*e.g.*, a targeting receptor), and an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[00220] In some embodiments, the exogenous nucleic acid encodes a polypeptide that is expressed on the surface of the cell. In some embodiments, the exogenous nucleic acid encodes a soluble polypeptide. As used herein, “soluble” polypeptide refers to a polypeptide that is secreted by the cell. In some embodiments, the soluble polypeptide is a therapeutic polypeptide. In some embodiments, the soluble polypeptide is an antibody. In some embodiments, the soluble polypeptide is an enzyme. In some embodiments, the soluble polypeptide is a cytokine. In some embodiments, the soluble polypeptide is a chemokine. In some embodiments, the soluble polypeptide is a fusion protein.

[00221] In some embodiments, the exogenous nucleic acid encodes an antibody. In some embodiments, the exogenous nucleic acid encodes an antibody fragment (*e.g.*, Fab, Fab2). In

some embodiments, the exogenous nucleic acid encodes is a full-length antibody. In some embodiments, the exogenous nucleic acid encodes is a single-chain antibody (*e.g.*, scFv). In some embodiments, the antibody is an IgG, IgM, IgD, IgA, or IgE. In some embodiments, the antibody is an IgG antibody. In some embodiments, the antibody is an IgG1 antibody. In some embodiments, the antibody is an IgG4 antibody. In some embodiments, the heavy chain constant region contains mutations known to reduce effector functions. In some embodiments, the heavy chain constant region contains mutations known to enhance effector functions. In some embodiments, the antibody is a bispecific antibody. In some embodiments, the antibody is a single-domain antibody (*e.g.*, VH domain-only antibody).

[00222] In some embodiments, the exogenous nucleic acid encodes a neutralizing antibody. A neutralizing antibody neutralizes the activity of its target antigen. In some embodiments, the antibody is a neutralizing antibody against a virus antigen. In some embodiments, the antibody neutralizes a target viral antigen, blocking the ability of the virus to infect a cell. In some embodiments, a cell-based neutralization assay may be used to measure the neutralizing activity of an antibody. The particular cells and readout will depend on the target antigen of the neutralizing antibody. The half maximal effective concentration (EC₅₀) of the antibody can be measured in a cell-based neutralization assay, wherein a lower EC₅₀ is indicative of more potent neutralizing antibody.

[00223] In some embodiments, the exogenous nucleic acid encodes an antibody that binds to an antigen associated with a disease or disorder (*see e.g.*, diseases and disorders described in Section IV).

[00224] In some embodiments, the exogenous nucleic acid encodes a polypeptide that is expressed on the surface of the cell (*i.e.*, a cell-surface bound protein). In some embodiments, the exogenous nucleic acid encodes a targeting receptor. A “targeting receptor” is a receptor present on the surface of a cell, *e.g.*, a T cell, to permit binding of the cell to a target site, *e.g.*, a specific cell or tissue in an organism. In some embodiments, the targeting receptor is a CAR. In some embodiments, the targeting receptor is a universal CAR (UniCAR). In some embodiments, the targeting receptor is a TCR. In some embodiments, the targeting receptor is a TRuC. In some embodiments, the targeting receptor is a B cell receptor (BCR) (*e.g.*, expressed on a B cell). In some embodiments, the targeting receptor is chemokine receptor. In some embodiments, the targeting receptor is a cytokine receptor.

[00225] In some embodiments, targeting receptors include a chimeric antigen receptor (CAR), a T-cell receptor (TCR), and a receptor for a cell surface molecule operably linked through at least a transmembrane domain in an internal signaling domain capable of activating

a T cell upon binding of the extracellular receptor portion. In some embodiments, a CAR refers to an extracellular antigen recognition domain, e.g., an scFv, VHH, nanobody; operably linked to an intracellular signaling domain, which activates the T cell when an antigen is bound. CARs are composed of four regions: an antigen recognition domain, an extracellular hinge region, a transmembrane domain, and an intracellular T-cell signaling domain. Such receptors are well known in the art (see, e.g., WO2020092057, WO2019191114, WO2019147805, WO2018208837). A reversed universal CAR that promotes binding of an immune cell to a target cell through an adaptor molecule (see, e.g., WO2019238722) is also contemplated. CARs can be targeted to any antigen to which an antibody can be developed and are typically directed to molecules displayed on the surface of a cell or tissue to be targeted. In some embodiments, the targeting receptor comprises an antigen recognition domain (e.g., a cancer antigen recognition domain and a subunit of a TCR (e.g., a TRuC). (*See* Baeuerle et al. Nature Communications 2087 (2019).)

[00226] In some embodiments, the exogenous nucleic acid encodes a TCR. In some embodiments, the exogenous nucleic acid encodes a genetically modified TCR. In some embodiments, the exogenous nucleic acid encodes is a genetically modified TCR with specificity for a polypeptide expressed by cancer cells. In some embodiments, the exogenous nucleic acid encodes a targeting receptor specific for Wilms' tumor gene (WT1) antigen. In some embodiments, the exogenous nucleic acid encodes the WT1-specific TCR (*see e.g.*, WO2020/081613A1).

[00227] In some embodiments, an exogenous nucleic acid is inserted into the genome of the target cell. In some embodiments, the exogenous nucleic acid is integrated into the genome of the target cell. In some embodiments, the exogenous nucleic acid is integrated into the genome of the target cell by homologous recombination (HR). In some embodiments, the exogenous nucleic acid is integrated into the genome of the target cell by blunt end insertion. In some embodiments, the exogenous nucleic acid is integrated into the genome of the target cell by non-homologous end joining. In some embodiments, the exogenous nucleic acid is integrated into a safe harbor locus in the genome of the cell. In some embodiments, the exogenous nucleic acid is integrated into one of the TRAC locus, B2M locus, AAVS1 locus, and/or CIITA locus. In some embodiments, the exogenous nucleic acid is provided to the cell in a lipid nucleic acid assembly composition. In some embodiments, the lipid nucleic acid assembly composition is a lipid nanoparticle (LNP).

[00228] In some embodiments, the methods produce a composition comprising an engineered cell having reduced or eliminated MHC class II expression and comprising an

exogenous nucleic acid. In some embodiments, the methods produce a composition comprising an engineered cell having reduced or eliminated MHC class II expression and that secretes and/or expresses a polypeptide encoded by an exogenous nucleic acid integrated into the genome of the cell. In some embodiments, the methods produce a composition comprising an engineered cell having reduced or eliminated MHC class II protein expression, reduced or eliminated CIITA protein expression, and/or reduced or eliminated CIITA levels in the cell nucleus, and eliciting a reduced response from NK cells, and having reduced MHC class I protein expression, and secreting and/or expressing a polypeptide encoded by an exogenous nucleic acid integrated into the genome of the cell. In some embodiments, the engineered cell elicits a reduced response from CD4⁺ T cells, CD8⁺ T cells, and/or NK cells.

[00229] In some embodiments, an allogeneic cell is provided wherein the cell has reduced or eliminated expression of MHC class II and MHC class I protein on the cell surface, wherein the cell comprises a modification in CIITA as disclosed herein, wherein the cell comprises a modification in B2M, wherein the cell comprises an exogenous nucleic acid encoding an NK cell inhibitor molecule, and wherein the cell further comprises an exogenous nucleic acid encoding a polypeptide (e.g., a targeting receptor). In some embodiments, the allogeneic cell elicits a reduced response from CD4⁺ T cells, CD8⁺ T cells, and/or NK cells, and further secretes and/or expresses a therapeutic agent.

[00230] In embodiments, an allogeneic cell is provided wherein the cell has reduced or eliminated expression of MHC class II and HLA-A protein on the cell surface, wherein the cell comprises a modification in CIITA as disclosed herein, wherein the cell comprises a modification in the HLA-A gene, wherein the cell further comprises an exogenous nucleic acid encoding a polypeptide (e.g., a targeting receptor). In some embodiments, the allogeneic cell elicits a reduced response from CD4⁺ T cells, and/or CD8⁺ T cells.

[00231] In some embodiments, the present disclosure provides methods for reducing or eliminating expression of MHC class II protein on the surface of a cell by genetically modifying CIITA as disclosed herein, wherein the methods further provide for reducing expression of one or more additional target genes (e.g., TRAC, TRBC). In some embodiments, the additional genetic modifications provide further advantages for use of the genetically modified cells for adoptive cell transfer applications. In some embodiments, the cell is an allogeneic cell.

[00232] In some embodiments, the methods comprise reducing or eliminating expression of MHC class II protein on the surface of a cell comprising genetically modifying CIITA comprising contacting the cell with a composition comprising a CIITA guide RNA as disclosed herein, the method further comprising contacting the cell with a guide RNA that directs an

RNA-guided DNA binding agent to a target sequence located in an another gene (*e.g.*, a gene other than CIITA or B2M or HLA-A), thereby reducing or eliminating expression of the other gene. In some embodiments, the methods comprise reducing expression of MHC class II protein on the surface of a cell comprising genetically modifying CIITA comprising contacting the cell with a composition comprising a CIITA guide RNA as disclosed herein, the method further comprising contacting the cell with a guide RNA that directs an RNA-guided DNA binding agent to a target sequence located in an another gene, and a B2M guide RNA, thereby reducing or eliminating expression of MHC class I protein on the surface of the cell. In some embodiments, the methods comprise reducing or eliminating expression of MHC class II protein on the surface of a cell comprising genetically modifying CIITA comprising contacting the cell with a composition comprising a CIITA guide RNA as disclosed herein, the method further comprising contacting the cell with a guide RNA that directs an RNA-guided DNA binding agent to a target sequence located in an another gene, thereby reducing or eliminating expression of the other gene, and an exogenous nucleic acid encoding a polypeptide (*e.g.*, a targeting receptor).

[00233] In some embodiments, the methods comprise reducing or eliminating expression of MHC class II protein on the surface of a cell comprising genetically modifying CIITA comprising contacting the cell with a composition comprising a CIITA guide RNA as disclosed herein, the method further comprising contacting the cell with a guide RNA that directs an RNA-guided DNA binding agent to a target sequence located in an another gene (*e.g.*, a gene other than CIITA or B2M or HLA-A), thereby reducing or eliminating expression of the other gene. In some embodiments, the methods comprise reducing expression of MHC class II protein on the surface of a cell comprising genetically modifying CIITA comprising contacting the cell with a composition comprising a CIITA guide RNA as disclosed herein, the method further comprising contacting the cell with a guide RNA that directs an RNA-guided DNA binding agent to a target sequence located in an another gene, and an HLA-A guide RNA, thereby reducing or eliminating expression of HLA-A protein on the surface of the cell. In some embodiments, the methods comprise reducing or eliminating expression of MHC class II protein on the surface of a cell comprising genetically modifying CIITA comprising contacting the cell with a composition comprising a CIITA guide RNA as disclosed herein, the method further comprising contacting the cell with a guide RNA that directs an RNA-guided DNA binding agent to a target sequence located in an another gene, thereby reducing or eliminating expression of the other gene, and an exogenous nucleic acid encoding a polypeptide (*e.g.*, a targeting receptor).

[00234] In some embodiments, the methods comprise reducing or eliminating expression of MHC class II protein on the surface of a cell comprising genetically modifying CIITA comprising contacting the cell with a composition comprising a CIITA guide RNA as disclosed herein, the method further comprising contacting the cell with a guide RNA that directs an RNA-guided DNA binding agent to a target sequence located in an another gene, thereby reducing expression of the other gene, a B2M guide RNA, thereby reducing expression of MHC class I protein on the surface of the cell, and an exogenous nucleic acid encoding an NK cell inhibitor.

[00235] In some embodiments, the methods comprise reducing or eliminating expression of MHC class II protein on the surface of a cell comprising genetically modifying CIITA comprising contacting the cell with a composition comprising a CIITA guide RNA as disclosed herein, the method further comprising contacting the cell with a guide RNA that directs an RNA-guided DNA binding agent to a target sequence located in an another gene, thereby reducing expression of the other gene, and an HLA-A guide RNA, thereby reducing expression of HLA-A protein on the surface of the cell.

[00236] In some embodiments, the methods comprise reducing or eliminating expression of MHC class II protein on the surface of a cell comprising genetically modifying CIITA comprising contacting the cell with a composition comprising a CIITA guide RNA as disclosed herein, the method further comprising contacting the cell with a guide RNA that directs an RNA-guided DNA binding agent to a target sequence located in an another gene, thereby reducing or eliminating expression of the other gene, a B2M guide RNA, thereby reducing or eliminating expression of MHC class I protein on the surface of the cell, and an exogenous nucleic acid encoding a polypeptide (e.g., a targeting receptor).

[00237] In some embodiments, the methods comprise reducing or eliminating expression of MHC class II protein on the surface of a cell comprising genetically modifying CIITA comprising contacting the cell with a composition comprising a CIITA guide RNA as disclosed herein, the method further comprising contacting the cell with a guide RNA that directs an RNA-guided DNA binding agent to a target sequence located in an another gene, an exogenous nucleic acid encoding an NK cell inhibitor molecule, and an exogenous nucleic acid encoding a polypeptide (e.g., a targeting receptor).

[00238] In some embodiments, the methods comprise reducing or eliminating expression of MHC class II protein on the surface of a cell comprising genetically modifying CIITA comprising contacting the cell with a composition comprising a CIITA guide RNA as disclosed herein, the method further comprising contacting the cell with a guide RNA that directs an

RNA-guided DNA binding agent to a target sequence located in an another gene, thereby reducing expression of the other gene, and an HLA-A guide RNA, thereby reducing expression of HLA-A protein on the surface of the cell, and an exogenous nucleic acid encoding a polypeptide (e.g., a targeting receptor).

[00239] In some embodiments, the methods comprise reducing or eliminating expression of MHC class II protein on the surface of a cell comprising genetically modifying CIITA comprising contacting the cell with a composition comprising a CIITA guide RNA as disclosed herein, the method further comprising contacting the cell with a guide RNA that directs an RNA-guided DNA binding agent to a target sequence located in an another gene, thereby reducing or eliminating expression of the additional gene, a B2M guide RNA, thereby reducing or eliminating expression of MHC class I protein on the surface of the cell, an exogenous nucleic acid encoding an NK cell inhibitor molecule, and an exogenous nucleic acid encoding a polypeptide (e.g., a targeting receptor). In some embodiments, the method further comprises contacting the cell with an RNA-guided DNA binding agent.

[00240] In some embodiments, the methods comprise reducing or eliminating expression of MHC class II protein on the surface of a cell, comprising genetically modifying the cell with one or more compositions comprising a CIITA guide RNA as disclosed herein, a B2M guide RNA, an exogenous nucleic acid encoding an NK cell inhibitor molecule, an exogenous nucleic acid encoding polypeptide (e.g., a targeting receptor), a guide RNA that directs an RNA-guided DNA binding agent to a target sequence located in an another gene, thereby reducing or eliminating expression of the other gene, and an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[00241] In some embodiments, the methods comprise reducing or eliminating expression of MHC class II protein on the surface of a cell, comprising genetically modifying the cell with one or more compositions comprising a CIITA guide RNA as disclosed herein, an HLA-A guide RNA, an exogenous nucleic acid encoding polypeptide (e.g., a targeting receptor), a guide RNA that directs an RNA-guided DNA binding agent to a target sequence located in an another gene, thereby reducing or eliminating expression of the other gene, and an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[00242] In some embodiments, the additional target gene is TRAC. In some embodiments, the additional target gene is TRBC.

D. Exemplary Cell Types

[00243] In some embodiments, methods and compositions disclosed herein genetically modify a cell. In some embodiments, the cell is an allogeneic cell. In some embodiments the cell is a human cell. In some embodiments the genetically modified cell is referred to as an engineered cell. An engineered cell refers to a cell (or progeny of a cell) comprising an engineered genetic modification, e.g. that has been contacted with a gene editing system and genetically modified by the gene editing system. The terms “engineered cell” and “genetically modified cell” are used interchangeably throughout. The engineered cell may be any of the exemplary cell types disclosed herein.

[00244] In some embodiments, the cell is an immune cell. As used herein, “immune cell” refers to a cell of the immune system, including *e.g.*, a lymphocyte (*e.g.*, T cell, B cell, natural killer cell (“NK cell”, and NKT cell, or iNKT cell)), monocyte, macrophage, mast cell, dendritic cell, or granulocyte (*e.g.*, neutrophil, eosinophil, and basophil). In some embodiments, the cell is a primary immune cell. In some embodiments, the immune system cell may be selected from CD3⁺, CD4⁺ and CD8⁺ T cells, regulatory T cells (Tregs), B cells, NK cells, and dendritic cells (DC). In some embodiments, the immune cell is allogeneic.

[00245] In some embodiments, the cell is a lymphocyte. In some embodiments, the cell is an adaptive immune cell. In some embodiments, the cell is a T cell. In some embodiments, the cell is a B cell. In some embodiments, the cell is a NK cell. In some embodiments, the lymphocyte is allogeneic.

[00246] As used herein, a T cell can be defined as a cell that expresses a T cell receptor (“TCR” or “αβ TCR” or “γδ TCR”), however in some embodiments, the TCR of a T cell may be genetically modified to reduce its expression (*e.g.*, by genetic modification to the TRAC or TRBC genes), therefore expression of the protein CD3 may be used as a marker to identify a T cell by standard flow cytometry methods. CD3 is a multi-subunit signaling complex that associates with the TCR. Thus, a T cell may be referred to as CD3⁺. In some embodiments, a T cell is a cell that expresses a CD3⁺ marker and either a CD4⁺ or CD8⁺ marker. In some embodiments, the T cell is allogeneic.

[00247] In some embodiments, the T cell expresses the glycoprotein CD8 and therefore is CD8⁺ by standard flow cytometry methods and may be referred to as a “cytotoxic” T cell. In some embodiments, the T cell expresses the glycoprotein CD4 and therefore is CD4⁺ by standard flow cytometry methods and may be referred to as a “helper” T cell. CD4⁺ T cells can differentiate into subsets and may be referred to as a Th1 cell, Th2 cell, Th9 cell, Th17 cell,

Th22 cell, T regulatory (“Treg”) cell, or T follicular helper cells (“Tfh”). Each CD4⁺ subset releases specific cytokines that can have either proinflammatory or anti-inflammatory functions, survival or protective functions. A T cell may be isolated from a subject by CD4⁺ or CD8⁺ selection methods.

[00248] In some embodiments, the T cell is a memory T cell. In the body, a memory T cell has encountered antigen. A memory T cell can be located in the secondary lymphoid organs (central memory T cells) or in recently infected tissue (effector memory T cells). A memory T cell may be a CD8⁺ T cell. A memory T cell may be a CD4⁺ T cell.

[00249] As used herein, a “central memory T cell” can be defined as an antigen-experienced T cell, and for example, may express CD62L and CD45RO. A central memory T cell may be detected as CD62L⁺ and CD45RO⁺ by Central memory T cells also express CCR7, therefore may be detected as CCR7⁺ by standard flow cytometry methods.

[00250] As used herein, an “early stem-cell memory T cell” (or “Tscm”) can be defined as a T cell that expresses CD27 and CD45RA, and therefore is CD27⁺ and CD45RA⁺ by standard flow cytometry methods. A Tscm does not express the CD45 isoform CD45RO, therefore a Tscm will further be CD45RO⁻ if stained for this isoform by standard flow cytometry methods. A CD45RO⁻ CD27⁺ cell is therefore also an early stem-cell memory T cell. Tscm cells further express CD62L and CCR7, therefore may be detected as CD62L⁺ and CCR7⁺ by standard flow cytometry methods. Early stem-cell memory T cells have been shown to correlate with increased persistence and therapeutic efficacy of cell therapy products.

[00251] In some embodiments, the cell is a B cell. As used herein, a “B cell” can be defined as a cell that expresses CD19 and/or CD20, and/or B cell mature antigen (“BCMA”), and therefore a B cell is CD19⁺, and/or CD20⁺, and/or BCMA⁺ by standard flow cytometry methods. A B cell is further negative for CD3 and CD56 by standard flow cytometry methods. The B cell may be a plasma cell. The B cell may be a memory B cell. The B cell may be a naïve B cell. The B cell may be IgM⁺, or has a class-switched B cell receptor (*e.g.*, IgG⁺, or IgA⁺). In some embodiments, the B cell is allogeneic.

[00252] In some embodiments, the cell is a mononuclear cell, such as from bone marrow or peripheral blood. In some embodiments, the cell is a peripheral blood mononuclear cell (“PBMC”). In some embodiments, the cell is a PBMC, *e.g.* a lymphocyte or monocyte. In some embodiments, the cell is a peripheral blood lymphocyte (“PBL”). In some embodiments, the mononuclear cell is allogeneic.

[00253] Cells used in ACT and/or tissue regenerative therapy are included, such as stem cells, progenitor cells, and primary cells. Stem cells, for example, include pluripotent stem

cells (PSCs); induced pluripotent stem cells (iPSCs); embryonic stem cells (ESCs); mesenchymal stem cells (MSCs, *e.g.*, isolated from bone marrow (BM), peripheral blood (PB), placenta, umbilical cord (UC) or adipose); hematopoietic stem cells (HSCs; *e.g.* isolated from BM or UC); neural stem cells (NSCs); tissue specific progenitor stem cells (TSPSCs); and limbal stem cells (LSCs). Progenitor and primary cells include mononuclear cells (MNCs, *e.g.*, isolated from BM or PB); endothelial progenitor cells (EPCs, *e.g.* isolated from BM, PB, and UC); neural progenitor cells (NPCs); and tissue-specific primary cells or cells derived therefrom (TSCs) including chondrocytes, myocytes, and keratinocytes. Cells for organ or tissue transplantations such as islet cells, cardiomyocytes, thyroid cells, thymocytes, neuronal cells, skin cells, and retinal cells are also included.

[00254] In some embodiments, the cell is a human cell, such as a cell isolated from a human subject. In some embodiments, the cell is isolated from human donor PBMCs or leukopaks. In some embodiments, the cell is from a subject with a condition, disorder, or disease. In some embodiments, the cell is from a human donor with Epstein Barr Virus (“EBV”).

[00255] In some embodiments, the methods are carried out *ex vivo*. As used herein, “*ex vivo*” refers to an *in vitro* method wherein the cell is capable of being transferred into a subject, *e.g.* as an ACT therapy. In some embodiments, an *ex vivo* method is an *in vitro* method involving an ACT therapy cell or cell population.

[00256] In some embodiments, the cell is from a cell line. In some embodiments, the cell line is derived from a human subject. In some embodiments, the cell line is a lymphoblastoid cell line (“LCL”). The cell may be cryopreserved and thawed. The cell may not have been previously cryopreserved.

[00257] In some embodiments, the cell is from a cell bank. In some embodiments, the cell is genetically modified and then transferred into a cell bank. In some embodiments the cell is removed from a subject, genetically modified *ex vivo*, and transferred into a cell bank. In some embodiments, a genetically modified population of cells is transferred into a cell bank. In some embodiments, a genetically modified population of immune cells is transferred into a cell bank. In some embodiments, a genetically modified population of immune cells comprising a first and second subpopulations, wherein the first and second sub-populations have at least one common genetic modification and at least one different genetic modification are transferred into a cell bank.

[00258] In some embodiments, when the cell is homozygous for HLA-B the HLA-B allele is selected from any one of the following HLA-B alleles: HLA-B*07:02; HLA-B*08:01; HLA-B*44:02; HLA-B*35:01; HLA-B*40:01; HLA-B*57:01; HLA-B*14:02; HLA-B*15:01;

HLA-B*13:02; HLA-B*44:03; HLA-B*38:01; HLA-B*18:01; HLA-B*44:03; HLA-B*51:01; HLA-B*49:01; HLA-B*15:01; HLA-B*18:01; HLA-B*27:05; HLA-B*35:03; HLA-B*18:01; HLA-B*52:01; HLA-B*51:01; HLA-B*37:01; HLA-B*53:01; HLA-B*55:01; HLA-B*44:02; HLA-B*44:03; HLA-B*35:02; HLA-B*15:01; and HLA-B*40:02.

[00259] In some embodiments, when the cell is homozygous for HLA-C, the HLA-C allele is selected from any one of the following HLA-C alleles: HLA-C*07:02; HLA-C*07:01; HLA-C*05:01; HLA-C*04:01 HLA-C*03:04; HLA-C*06:02; HLA-C*08:02; HLA-C*03:03; HLA-C*06:02; HLA-C*16:01; HLA-C*12:03; HLA-C*07:01; HLA-C*04:01; HLA-C*15:02; HLA-C*07:01; HLA-C*03:04; HLA-C*12:03; HLA-C*02:02; HLA-C*04:01; HLA-C*05:01; HLA-C*12:02; HLA-C*14:02; HLA-C*06:02; HLA-C*04:01; HLA-C*03:03; HLA-C*07:04; HLA-C*07:01; HLA-C*04:01; HLA-C*04:01; and HLA-C*02:02.

[00260] In some embodiments, the cell is homozygous for HLA-B and homozygous for HLA-C and the HLA-B allele is selected from any one of the following HLA-B alleles: HLA-B*07:02; HLA-B*08:01; HLA-B*44:02; HLA-B*35:01; HLA-B*40:01; HLA-B*57:01; HLA-B*14:02; HLA-B*15:01; HLA-B*13:02; HLA-B*44:03; HLA-B*38:01; HLA-B*18:01; HLA-B*44:03; HLA-B*51:01; HLA-B*49:01; HLA-B*15:01; HLA-B*18:01; HLA-B*27:05; HLA-B*35:03; HLA-B*18:01; HLA-B*52:01; HLA-B*51:01; HLA-B*37:01; HLA-B*53:01; HLA-B*55:01; HLA-B*44:02; HLA-B*44:03; HLA-B*35:02; HLA-B*15:01; and HLA-B*40:02; and the HLA-C allele is selected from any one of the following HLA-C alleles: HLA-C*07:02; HLA-C*07:01; HLA-C*05:01; HLA-C*04:01 HLA-C*03:04; HLA-C*06:02; HLA-C*08:02; HLA-C*03:03; HLA-C*06:02; HLA-C*16:01; HLA-C*12:03; HLA-C*07:01; HLA-C*04:01; HLA-C*15:02; HLA-C*07:01; HLA-C*03:04; HLA-C*12:03; HLA-C*02:02; HLA-C*04:01; HLA-C*05:01; HLA-C*12:02; HLA-C*14:02; HLA-C*06:02; HLA-C*04:01; HLA-C*03:03; HLA-C*07:04; HLA-C*07:01; HLA-C*04:01; HLA-C*04:01; and HLA-C*02:02.

[00261] In some embodiments, the cell is homozygous for HLA-B and homozygous for HLA-C and the HLA-B and HLA-C alleles are selected from any one of the following HLA-B and HLA-C alleles: HLA-B*07:02 and HLA-C*07:02; HLA-B*08:01 and HLA-C*07:01; HLA-B*44:02 and HLA-C*05:01; HLA-B*35:01 and HLA-C*04:01; HLA-B*40:01 and HLA-C*03:04; HLA-B*57:01 and HLA-C*06:02; HLA-B*14:02 and HLA-C*08:02; HLA-B*15:01 and HLA-C*03:03; HLA-B*13:02 and HLA-C*06:02; HLA-B*44:03 and HLA-C*16:01; HLA-B*38:01 and HLA-C*12:03; HLA-B*18:01 and HLA-C*07:01; HLA-B*44:03 and HLA-C*04:01; HLA-B*51:01 and HLA-C*15:02; HLA-B*49:01 and HLA-C*07:01; HLA-B*15:01 and HLA-C*03:04; HLA-B*18:01 and HLA-C*12:03; HLA-

B*27:05 and HLA-C*02:02; HLA-B*35:03 and HLA-C*04:01; HLA-B*18:01 and HLA-C*05:01; HLA-B*52:01 and HLA-C*12:02; HLA-B*51:01 and HLA-C*14:02; HLA-B*37:01 and HLA-C*06:02; HLA-B*53:01 and HLA-C*04:01; HLA-B*55:01 and HLA-C*03:03; HLA-B*44:02 and HLA-C*07:04; HLA-B*44:03 and HLA-C*07:01; HLA-B*35:02 and HLA-C*04:01; HLA-B*15:01 and HLA-C*04:01; and HLA-B*40:02 and HLA-C*02:02. In some embodiments, the cell is homozygous for HLA-B and homozygous for HLA-C and the HLA-B and HLA-C alleles are HLA-B*07:02 and HLA-C*07:02. In some embodiments, the cell is homozygous for HLA-B and homozygous for HLA-C and the HLA-B and HLA-C alleles are HLA-B*08:01 and HLA-C*07:01. In some embodiments, the cell is homozygous for HLA-B and homozygous for HLA-C and the HLA-B and HLA-C alleles are HLA-B*44:02 and HLA-C*05:01. In some embodiments, the cell is homozygous for HLA-B and homozygous for HLA-C and the HLA-B and HLA-C alleles are HLA-B*35:01 and HLA-C*04:01.

III. Details of the Gene Editing Systems

[00262] Various suitable gene editing systems may be used to make the engineered cells disclosed herein, including but not limited to the CRISPR/Cas system; zinc finger nuclease (ZFN) system; and the transcription activator-like effector nuclease (TALEN) system. Generally, the gene editing systems involve the use of engineered cleavage systems to induce a double strand break (DSB) or a nick (e.g., a single strand break, or SSB) in a target DNA sequence. Cleavage or nicking can occur through the use of specific nucleases such as engineered ZFN, TALENs, or using the CRISPR/Cas system with an engineered guide RNA to guide specific cleavage or nicking of a target DNA sequence. Further, targeted nucleases are being developed based on the Argonaute system (e.g., from *T. thermophilus*, known as 'TtAgo', see Swarts et al (2014) *Nature* 507(7491): 258-261), which also may have the potential for uses in gene editing and gene therapy.

[00263] In some embodiments, the gene editing system is a TALEN system. Transcription activator-like effector nucleases (TALEN) are restriction enzymes that can be engineered to cut specific sequences of DNA. They are made by fusing a TAL effector DNA-binding domain to a DNA cleavage domain (a nuclease which cuts DNA strands). Transcription activator-like effectors (TALEs) can be engineered to bind to a desired DNA sequence, to promote DNA cleavage at specific locations (see, e.g., Boch, 2011, *Nature Biotech*). The restriction enzymes can be introduced into cells, for use in gene editing or for gene editing in situ, a technique known as gene editing with engineered nucleases. Such methods and compositions for use

therein are known in the art. See, e.g., WO2019147805, WO2014040370, WO2018073393, the contents of which are hereby incorporated in their entireties.

[00264] In some embodiments, the gene editing system is a zinc-finger system. Zinc-finger nucleases (ZFNs) are artificial restriction enzymes generated by fusing a zinc finger DNA-binding domain to a DNA-cleavage domain. Zinc finger domains can be engineered to target specific desired DNA sequences to enable zinc-finger nucleases to target unique sequences within complex genomes. The non-specific cleavage domain from the type II restriction endonuclease FokI is typically used as the cleavage domain in ZFNs. Cleavage is repaired by endogenous DNA repair machinery, allowing ZFN to precisely alter the genomes of higher organisms. Such methods and compositions for use therein are known in the art. See, e.g., WO2011091324, the contents of which are hereby incorporated in their entireties.

[00265] In some embodiments, the gene editing system is a CRISPR/Cas system, including e.g., a CRISPR guide RNA comprising a guide sequence and RNA-guided DNA binding agent, and described further herein.

A. CRISPR Guide RNA

[00266] Provided herein are guide sequences useful for modifying a target sequence, *e.g.*, using a guide RNA comprising a disclosed guide sequence with an RNA-guided DNA binding agent (*e.g.*, a CRISPR/Cas system).

[00267] Each of the guide sequences disclosed herein may further comprise additional nucleotides to form a crRNA, *e.g.*, with the following exemplary nucleotide sequence following the guide sequence at its 3' end: GUUUUAGAGCUAUGCUGUUUUG (SEQ ID NO: 170) in 5' to 3' orientation. In the case of a sgRNA, the above guide sequences may further comprise additional nucleotides (scaffold sequence) to form a sgRNA, *e.g.*, with the following exemplary nucleotide sequence following the 3' end of the guide sequence: GUUUUAGAGCUAGAAUAGCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 171) or GUUUUAGAGCUAGAAUAGCAAGUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC (SEQ ID NO: 172, which is SEQ ID NO: 171 without the four terminal U's) in 5' to 3' orientation. In some embodiments, the four terminal U's of SEQ ID NO: 171 are not present. In some embodiments, only 1, 2, or 3 of the four terminal U's of SEQ ID NO: 171 are present.

[00268] In some embodiments, the sgRNA comprises any one of the guide sequences of SEQ ID Nos: 1-117 and additional nucleotides to form a crRNA, *e.g.*, with the following

exemplary nucleotide sequence following the guide sequence at its 3' end: GUUUUAGAGCUAGAAAUAGCAAGUUAUUAAAGGCUAGUCCGUUAUCAACUU GGCACCGAGUCGGUGC (SEQ ID NO: 173) in 5' to 3' orientation. SEQ ID NO: 173 lacks 8 nucleotides with reference to a wild-type guide RNA conserved sequence: GUUUUAGAGCUAGAAAUAGCAAGUUAUUAAAGGCUAGUCCGUUAUCAACUU GAAAAAGUGGCACCGAGUCGGUGC (SEQ ID NO: 172). Other exemplary scaffold nucleotide sequences are provided in Table 4. In some embodiments, the sgRNA comprises any one of the guide sequences of SEQ ID Nos: 1-117 and additional guide scaffold sequences, in 5' to 3' orientation, in Table 4 including modified versions of the scaffold sequences, as shown.

[00269] In some embodiments, the guide RNA is a sgRNA comprising any one of the sequences shown in Table 2 (SEQ ID NOs: 218-334 and 335-426). In some embodiments, the guide RNA is a chemically modified guide RNA. In some embodiments, the guide RNA is a chemically modified single guide RNA. The chemically modified guide RNAs may comprise one or more of the modifications as shown in Table 2. The chemically modified guide RNAs may comprise one or more of modified nucleotides of any one of SEQ ID NOs: 1006, 1010-1012 and 1014-1017.

[00270] In some embodiments, the guide RNA is a sgRNA comprising any one of SEQ ID NOs: 218-334 with at least one chemical modification disclosed herein. In some embodiments, the guide RNA is a sgRNA comprising a sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any one of SEQ ID NOs: 218-334 with at least one chemical modification disclosed herein.

[00271] In some embodiments, the guide RNA is a sgRNA comprising the modification pattern shown in SEQ ID NO: 1016 or 1017. In some embodiments, the guide RNA is a sgRNA comprising a sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any of the nucleic acids of SEQ ID NOs: 335-426.

[00272] In some embodiments, the guide RNA comprises a sgRNA comprising the modification pattern shown in SEQ ID NO: 1006. In some embodiments, the guide RNA comprises a sgRNA comprising the modified nucleotides of SEQ ID NO: 1006, including a guide sequence comprises a sequence selected from SEQ ID Nos: 1-117. In some embodiments, the guide RNA is a sgRNA comprising a sequence of SEQ ID NO: 1008 or a sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to SEQ ID NO: 1008.

[00273] In some embodiments, the guide RNA is a single guide RNA comprising any one of the sequences of SEQ ID NO: 335-426 and 1008 or a sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any one of the sequences of SEQ ID NO: 335-426 and 1008. In some embodiments, the guide RNA is a single guide RNA comprising any one of sequences SEQ ID NOs: 32, 64, 67, 68, 74, 76, 84, 86, 90, 91, and 115. In some embodiments, the guide RNA is a single guide RNA comprising any one of the sequences SEQ ID NO: 341, 373, 376, 377, 383, 385, 393, 395, 399, 400, and 424, or a sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any one of the sequences SEQ ID NO: 341, 373, 376, 377, 383, 385, 393, 395, 399, 400, and 424.

[00274] The guide RNA may further comprise a trRNA. In each composition and method embodiment described herein, the crRNA and trRNA may be associated as a single RNA (sgRNA) or may be on separate RNAs (dgRNA). In the context of sgRNAs, the crRNA and trRNA components may be covalently linked, e.g., via a phosphodiester bond or other covalent bond. In some embodiments, a crRNA and/or trRNA sequence may be referred to as a “scaffold” or “conserved portion” of a guide RNA.

[00275] In each of the compositions, use, and method embodiments described herein, the guide RNA may comprise two RNA molecules as a “dual guide RNA” or “dgRNA.” The dgRNA comprises a first RNA molecule comprising a crRNA comprising, e.g., a guide sequence shown in **Table 2**, and a second RNA molecule comprising a trRNA. The first and second RNA molecules may not be covalently linked, but may form an RNA duplex via the base pairing between portions of the crRNA and the trRNA.

[00276] In each of the composition, use, and method embodiments described herein, the guide RNA may comprise a single RNA molecule as a “single guide RNA” or “sgRNA”. The sgRNA may comprise a crRNA (or a portion thereof) comprising a guide sequence shown in **Table 2**, covalently linked to a trRNA. The sgRNA may comprise 17, 18, 19, or 20 contiguous nucleotides of a guide sequence shown in **Table 2**. In some embodiments, the crRNA and the trRNA are covalently linked via a linker. In some embodiments, the sgRNA forms a stem-loop structure via the base pairing between portions of the crRNA and the trRNA. In some embodiments, the crRNA and the trRNA are covalently linked via one or more bonds that are not a phosphodiester bond.

[00277] In some embodiments, the trRNA may comprise all or a portion of a trRNA sequence derived from a naturally-occurring CRISPR/Cas system. In some embodiments, the trRNA comprises a truncated or modified wild type trRNA. The length of the trRNA depends

on the CRISPR/Cas system used. In some embodiments, the trRNA comprises or consists of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or more than 100 nucleotides. In some embodiments, the trRNA may comprise certain secondary structures, such as, for example, one or more hairpin or stem-loop structures, or one or more bulge structures.

[00278] In some embodiments, a composition comprising one or more guide RNAs comprising a guide sequence of any one in **Table 2** is provided. In some embodiments, a composition comprising one or more guide RNAs comprising a guide sequence of any one in **Table 2** is provided, wherein the nucleotides of SEQ ID NO: 170, 171, 172, or 173 follow the guide sequence at its 3' end. In some embodiments, the one or more guide RNAs comprising a guide sequence of any one in **Table 2**, wherein the nucleotides of SEQ ID NO: 170, 171, 172, or 173 follow the guide sequence at its 3' end, is modified according to the modification pattern of any one of SEQ ID NOs: 1006, 1010-1012 and 1014-1017.

[00279] In some embodiments, a composition comprising one or more guide RNAs comprising a guide sequence of any one in **Table 2** is provided. In one aspect, a composition comprising one or more gRNAs is provided, comprising a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any of the nucleic acids of SEQ ID NOs: 1-117.

[00280] In other embodiments, a composition is provided that comprises at least one, e.g., at least two gRNA's comprising guide sequences selected from any two or more of the guide sequences shown in **Table 2**. In some embodiments, the composition comprises at least two gRNA's that each comprise a guide sequence at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any of the guide sequences shown in **Table 2**.

[00281] In some embodiments, the guide RNA compositions of the present invention are designed to recognize (*e.g.*, hybridize to) a target sequence in CIITA. For example, the CIITA target sequence may be recognized and cleaved by a provided Cas cleavase comprising a guide RNA. In some embodiments, an RNA-guided DNA binding agent, such as a Cas cleavase, may be directed by a guide RNA to a target sequence in CIITA, where the guide sequence of the guide RNA hybridizes with the target sequence and the RNA-guided DNA binding agent, such as a Cas cleavase, cleaves the target sequence.

[00282] In some embodiments, the selection of the one or more guide RNAs is determined based on target sequences within CIITA. In some embodiments, the compositions comprising one or more guide sequences comprise a guide sequence that is complementary to the corresponding genomic region shown in **Table 2**, according to coordinates from human

reference genome hg38. Guide sequences of further embodiments may be complementary to sequences in the close vicinity of the genomic coordinate listed in any of the **Table 2** within *CIITA*. For example, guide sequences of further embodiments may be complementary to sequences that comprise 10 contiguous nucleotides $\pm$ 10 nucleotides of a genomic coordinate listed in **Table 2**.

[00283] Without being bound by any particular theory, modifications (e.g., frameshift mutations resulting from indels occurring as a result of a nuclease-mediated DSB) in certain regions of the target gene may be less tolerable than mutations in other regions, thus the location of a DSB is an important factor in the amount or type of protein knockdown that may result. In some embodiments, a gRNA complementary or having complementarity to a target sequence within the target gene used to direct an RNA-guided DNA binding agent to a particular location in the target gene.

[00284] In some embodiments, the guide sequence is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 85%, or 80% identical to a target sequence present in the target gene. In some embodiments, the guide sequence is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 85%, or 80% identical to a target sequence present in the human *CIITA* gene.

[00285] In some embodiments, the target sequence may be complementary to the guide sequence of the guide RNA. In some embodiments, the degree of complementarity or identity between a guide sequence of a guide RNA and its corresponding target sequence may be at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%. In some embodiments, the target sequence and the guide sequence of the gRNA may be 100% complementary or identical. In other embodiments, the target sequence and the guide sequence of the gRNA may contain at least one mismatch. For example, the target sequence and the guide sequence of the gRNA may contain 1, 2, 3, or 4 mismatches, where the total length of the guide sequence is 20. In some embodiments, the target sequence and the guide sequence of the gRNA may contain 1-4 mismatches where the guide sequence is 20 nucleotides.

[00286] In some embodiments, a composition or formulation disclosed herein comprises an mRNA comprising an open reading frame (ORF) encoding an RNA-guided DNA binding agent, such as a Cas nuclease as described herein. In some embodiments, an mRNA comprising an ORF encoding an RNA-guided DNA binding agent, such as a Cas nuclease, is provided, used, or administered.

B. Modifications of gRNAs

[00287] In some embodiments, the gRNA (e.g., sgRNA, short-sgRNA, dgRNA, or crRNA) is modified. The term “modified” or “modification” in the context of a gRNA described herein includes, the modifications described above, including, for example, (a) end modifications, e.g., 5' end modifications or 3' end modifications, including 5' or 3' protective end modifications, (b) nucleobase (or “base”) modifications, including replacement or removal of bases, (c) sugar modifications, including modifications at the 2', 3', and/or 4' positions, (d) internucleoside linkage modifications, and (e) backbone modifications, which can include modification or replacement of the phosphodiester linkages and/or the ribose sugar. A modification of a nucleotide at a given position includes a modification or replacement of the phosphodiester linkage immediately 3' of the sugar of the nucleotide. Thus, for example, a nucleic acid comprising a phosphorothioate between the first and second sugars from the 5' end is considered to comprise a modification at position 1. The term “modified gRNA” generally refers to a gRNA having a modification to the chemical structure of one or more of the base, the sugar, and the phosphodiester linkage or backbone portions, including nucleotide phosphates, all as detailed and exemplified herein.

[00288] Further description and exemplary patterns of modifications are provided in Table 1 of WO2019/237069 published December 12, 2019, the entire contents of which are incorporated herein by reference.

[00289] In some embodiments, a gRNA comprises modifications at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or more YA sites. In some embodiments, the pyrimidine of the YA site comprises a modification (which includes a modification altering the internucleoside linkage immediately 3' of the sugar of the pyrimidine). In some embodiments, the adenine of the YA site comprises a modification (which includes a modification altering the internucleoside linkage immediately 3' of the sugar of the adenine). In some embodiments, the pyrimidine and the adenine of the YA site comprise modifications, such as sugar, base, or internucleoside linkage modifications. The YA modifications can be any of the types of modifications set forth herein. In some embodiments, the YA modifications comprise one or more of phosphorothioate, 2'-OMe, or 2'-fluoro. In some embodiments, the YA modifications comprise pyrimidine modifications comprising one or more of phosphorothioate, 2'-OMe, 2'-H, inosine, or 2'-fluoro. In some embodiments, the YA modification comprises a bicyclic ribose analog (e.g., an LNA, BNA, or ENA) within an RNA duplex region that contains one or more YA sites. In some embodiments, the YA modification comprises a bicyclic ribose analog

(e.g., an LNA, BNA, or ENA) within an RNA duplex region that contains a YA site, wherein the YA modification is distal to the YA site.

[00290] In some embodiments, the guide sequence (or guide region) of a gRNA comprises 1, 2, 3, 4, 5, or more YA sites (“guide region YA sites”) that may comprise YA modifications. In some embodiments, one or more YA sites located at 5-end, 6-end, 7-end, 8-end, 9-end, or 10-end from the 5' end of the 5' terminus (where “5-end”, etc., refers to position 5 to the 3' end of the guide region, i.e., the most 3' nucleotide in the guide region) comprise YA modifications. A modified guide region YA site comprises a YA modification.

[00291] In some embodiments, a modified guide region YA site is within 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, or 9 nucleotides of the 3' terminal nucleotide of the guide region. For example, if a modified guide region YA site is within 10 nucleotides of the 3' terminal nucleotide of the guide region and the guide region is 20 nucleotides long, then the modified nucleotide of the modified guide region YA site is located at any of positions 11-20. In some embodiments, a modified guide region YA site is at or after nucleotide 4, 5, 6, 7, 8, 9, 10, or 11 from the 5' end of the 5' terminus.

[00292] In some embodiments, a modified guide region YA site is other than a 5' end modification. For example, a sgRNA can comprise a 5' end modification as described herein and further comprise a modified guide region YA site. Alternatively, a sgRNA can comprise an unmodified 5' end and a modified guide region YA site. Alternatively, a short-sgRNA can comprise a modified 5' end and an unmodified guide region YA site.

[00293] In some embodiments, a modified guide region YA site comprises a modification that at least one nucleotide located 5' of the guide region YA site does not comprise. For example, if nucleotides 1-3 comprise phosphorothioates, nucleotide 4 comprises only a 2'-OMe modification, and nucleotide 5 is the pyrimidine of a YA site and comprises a phosphorothioate, then the modified guide region YA site comprises a modification (phosphorothioate) that at least one nucleotide located 5' of the guide region YA site (nucleotide 4) does not comprise. In another example, if nucleotides 1-3 comprise phosphorothioates, and nucleotide 4 is the pyrimidine of a YA site and comprises a 2'-OMe, then the modified guide region YA site comprises a modification (2'-OMe) that at least one nucleotide located 5' of the guide region YA site (any of nucleotides 1-3) does not comprise. This condition is also always satisfied if an unmodified nucleotide is located 5' of the modified guide region YA site.

[00294] In some embodiments, the modified guide region YA sites comprise modifications as described for YA sites above. The guide region of a gRNA may be modified according to

any embodiment comprising a modified guide region set forth herein. Any embodiments set forth elsewhere in this disclosure may be combined to the extent feasible with any of the foregoing embodiments.

[00295] In some embodiments, the 5' and/or 3' terminus regions of a gRNA are modified.

[00296] In some embodiments, the terminal (i.e., last) 1, 2, 3, 4, 5, 6, or 7 nucleotides in the 3' terminus region are modified. Throughout, this modification may be referred to as a "3' end modification". In some embodiments, the terminal (i.e., last) 1, 2, 3, 4, 5, 6, or 7 nucleotides in the 3' terminus region comprise more than one modification. In some embodiments, the 3' end modification comprises or further comprises any one or more of the following: a modified nucleotide selected from 2'-O-methyl (2'-O-Me) modified nucleotide, 2'-O-(2-methoxyethyl) (2'-O-moe) modified nucleotide, a 2'-fluoro (2'-F) modified nucleotide, a phosphorothioate (PS) linkage between nucleotides, an inverted abasic modified nucleotide, or combinations thereof. In some embodiments, the 3' end modification comprises or further comprises modifications of 1, 2, 3, 4, 5, 6, or 7 nucleotides at the 3' end of the gRNA. In some embodiments, the 3' end modification comprises or further comprises one PS linkage, wherein the linkage is between the last and second to last nucleotide. In some embodiments, the 3' end modification comprises or further comprises two PS linkages between the last three nucleotides. In some embodiments, the 3' end modification comprises or further comprises four PS linkages between the last four nucleotides. In some embodiments, the 3' end modification comprises or further comprises PS linkages between any one or more of the last 2, 3, 4, 5, 6, or 7 nucleotides. In some embodiments, the gRNA comprising a 3' end modification comprises or further comprises a 3' tail, wherein the 3' tail comprises a modification of any one or more of the nucleotides present in the 3' tail. In some embodiments, the 3' tail is fully modified. In some embodiments, the 3' tail comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, or 1-10 nucleotides, optionally where any one or more of these nucleotides are modified. In some embodiments, a gRNA is provided comprising a 3' protective end modification. In some embodiments, the 3' tail comprises between 1 and about 20 nucleotides, between 1 and about 15 nucleotides, between 1 and about 10 nucleotides, between 1 and about 5 nucleotides, between 1 and about 4 nucleotides, between 1 and about 3 nucleotides, and between 1 and about 2 nucleotides. In some embodiments, the gRNA does not comprise a 3' tail.

[00297] In some embodiments, the 5' terminus region is modified, for example, the first 1, 2, 3, 4, 5, 6, or 7 nucleotides of the gRNA are modified. Throughout, this modification may be referred to as a "5' end modification". In some embodiments, the first 1, 2, 3, 4, 5, 6, or 7

nucleotides of the 5' terminus region comprise more than one modification. In some embodiments, at least one of the terminal (i.e., first) 1, 2, 3, 4, 5, 6, or 7 nucleotides at the 5' end are modified. In some embodiments, both the 5' and 3' terminus regions (e.g., ends) of the gRNA are modified. In some embodiments, only the 5' terminus region of the gRNA is modified. In some embodiments, only the 3' terminus region (plus or minus a 3' tail) of the conserved portion of a gRNA is modified. In some embodiments, the gRNA comprises modifications at 1, 2, 3, 4, 5, 6, or 7 of the first 7 nucleotides at a 5' terminus region of the gRNA. In some embodiments, the gRNA comprises modifications at 1, 2, 3, 4, 5, 6, or 7 of the 7 terminal nucleotides at a 3' terminus region. In some embodiments, 2, 3, or 4 of the first 4 nucleotides at the 5' terminus region, and/or 2, 3, or 4 of the terminal 4 nucleotides at the 3' terminus region are modified. In some embodiments, 2, 3, or 4 of the first 4 nucleotides at the 5' terminus region are linked with phosphorothioate (PS) bonds. In some embodiments, the modification to the 5' terminus and/or 3' terminus comprises a 2'-O-methyl (2'-O-Me) or 2'-O-(2-methoxyethyl) (2'-O-moe) modification. In some embodiments, the modification comprises a 2'-fluoro (2'-F) modification to a nucleotide. In some embodiments, the modification comprises a phosphorothioate (PS) linkage between nucleotides. In some embodiments, the modification comprises an inverted abasic nucleotide. In some embodiments, the modification comprises a protective end modification. In some embodiments, the modification comprises a more than one modification selected from protective end modification, 2'-O-Me, 2'-O-moe, 2'-fluoro (2'-F), a phosphorothioate (PS) linkage between nucleotides, and an inverted abasic nucleotide. In some embodiments, an equivalent modification is encompassed.

[00298] In some embodiments, a gRNA is provided comprising a 5' end modification and a 3' end modification. In some embodiments, the gRNA comprises modified nucleotides that are not at the 5' or 3' ends.

[00299] In some embodiments, a sgRNA is provided comprising an upper stem modification, wherein the upper stem modification comprises a modification to any one or more of US1-US12 in the upper stem region. In some embodiments, a sgRNA is provided comprising an upper stem modification, wherein the upper stem modification comprises a modification of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or all 12 nucleotides in the upper stem region. In some embodiments, an sgRNA is provided comprising an upper stem modification, wherein the upper stem modification comprises 1, 2, 3, 4, or 5 YA modifications in a YA site. In some embodiments, the upper stem modification comprises a 2'-OMe modified nucleotide, a 2'-O-moe modified nucleotide, a 2'-F modified nucleotide, and/or combinations thereof.

Other modifications described herein, such as a 5' end modification and/or a 3' end modification may be combined with an upper stem modification.

[00300] In some embodiments, the sgRNA comprises a modification in the hairpin region. In some embodiments, the hairpin region modification comprises at least one modified nucleotide selected from a 2'-O-methyl (2'-OMe) modified nucleotide, a 2'-fluoro (2'-F) modified nucleotide, and/or combinations thereof. In some embodiments, the hairpin region modification is in the hairpin 1 region. In some embodiments, the hairpin region modification is in the hairpin 2 region. In some embodiments, the hairpin modification comprises 1, 2, or 3 YA modifications in a YA site. In some embodiments, the hairpin modification comprises at least 1, 2, 3, 4, 5, or 6 YA modifications. Other modifications described herein, such as an upper stem modification, a 5' end modification, and/or a 3' end modification may be combined with a modification in the hairpin region.

[00301] In some embodiments, a gRNA comprises a substituted and optionally shortened hairpin 1 region, wherein at least one of the following pairs of nucleotides are substituted in the substituted and optionally shortened hairpin 1 with Watson-Crick pairing nucleotides: H1-1 and H1-12, H1-2 and H1-11, H1-3 and H1-10, and/or H1-4 and H1-9. "Watson-Crick pairing nucleotides" include any pair capable of forming a Watson-Crick base pair, including A-T, A-U, T-A, U-A, C-G, and G-C pairs, and pairs including modified versions of any of the foregoing nucleotides that have the same base pairing preference. In some embodiments, the hairpin 1 region lacks any one or two of H1-5 through H1-8. In some embodiments, the hairpin 1 region lacks one, two, or three of the following pairs of nucleotides: H1-1 and H1-12, H1-2 and H1-11, H1-3 and H1-10 and/or H1-4 and H1-9. In some embodiments, the hairpin 1 region lacks 1-8 nucleotides of the hairpin 1 region. In any of the foregoing embodiments, the lacking nucleotides may be such that the one or more nucleotide pairs substituted with Watson-Crick pairing nucleotides (H1-1 and H1-12, H1-2 and H1-11, H1-3 and H1-10, and/or H1-4 and H1-9) form a base pair in the gRNA.

[00302] In some embodiments, the gRNA further comprises an upper stem region lacking at least 1 nucleotide, e.g., any of the shortened upper stem regions indicated in Table 7 of U.S. Application No. 62/946,905, the contents of which are hereby incorporated by reference in its entirety, or described elsewhere herein, which may be combined with any of the shortened or substituted hairpin 1 regions described herein.

[00303] In some embodiments, an sgRNA provided herein is a short-single guide RNAs (short-sgRNAs), e.g., comprising a conserved portion of an sgRNA comprising a hairpin

region, wherein the hairpin region lacks at least 5-10 nucleotides or 6-10 nucleotides. In some embodiments, the 5-10 nucleotides or 6-10 nucleotides are consecutive.

[00304] In some embodiments, a short-sgRNA lacks at least nucleotides 54-58 (AAAAA) of the conserved portion of a spyCas9 sgRNA. In some embodiments, a short-sgRNA is a non-spyCas9 sgRNA that lacks nucleotides corresponding to nucleotides 54-58 (AAAAA) of the conserved portion of a spyCas9 as determined, for example, by pairwise or structural alignment.

[00305] In some embodiments, the short-sgRNA described herein comprises a conserved portion comprising a hairpin region, wherein the hairpin region lacks 5, 6, 7, 8, 9, 10, 11, or 12 nucleotides. In some embodiments, the lacking nucleotides are 5-10 lacking nucleotides or 6-10 lacking nucleotides. In some embodiments, the lacking nucleotides are consecutive. In some embodiments, the lacking nucleotides span at least a portion of hairpin 1 and a portion of hairpin 2. In some embodiments, the 5-10 lacking nucleotides comprise or consist of nucleotides 54-58, 54-61, or 53-60 of SEQ ID NO: 172.

[00306] In some embodiments, the short-sgRNA described herein further comprises a nexus region, wherein the nexus region lacks at least one nucleotide (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in the nexus region). In some embodiments, the short-sgRNA lacks each nucleotide in the nexus region.

[00307] In some embodiments, a SpyCas9 short-sgRNA described herein comprises a sequence of

NNNNNNNNNNNNNNNNNNNNNGUUUAGAGCUAGAAUAGCAAGUUAAAAUAA
GGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGGUGCU (SEQ ID NO: 1005).

[0001] In some embodiments, a short-sgRNA described herein comprises a modification pattern as shown in

mN*mN*mN*NNNNNNNNNNNNNNNNNNNGUUUAGAmGmCmUmAmGmAmAmAmU
mAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCG
GmUmGmC*mU (SEQ ID NO: 1006), where A, C, G, U, and N are adenine, cytosine, guanine, uracil, and any ribonucleotide, respectively, unless otherwise indicated. An m is indicative of a 2'-O-methyl modification, and an * is indicative of a phosphorothioate linkage between the nucleotides.

[0002] In certain embodiments, using SEQ ID NO: 172 ("Exemplary SpyCas9 sgRNA-1") as an example, the Exemplary SpyCas9 sgRNA-1 further includes one or more of:

- A. a shortened hairpin 1 region, or a substituted and optionally shortened hairpin 1 region, wherein
1. at least one of the following pairs of nucleotides are substituted in hairpin 1 with Watson-Crick pairing nucleotides: H1-1 and H1-12, H1-2 and H1-11, H1-3 and H1-10, or H1-4 and H1-9, and the hairpin 1 region optionally lacks
 - a. any one or two of H1-5 through H1-8,
 - b. one, two, or three of the following pairs of nucleotides: H1-1 and H1-12, H1-2 and H1-11, H1-3 and H1-10, and H1-4 and H1-9, or
 - c. 1-8 nucleotides of hairpin 1 region; or
 2. the shortened hairpin 1 region lacks 6-8 nucleotides, preferably 6 nucleotides; and
 - a. one or more of positions H1-1, H1-2, or H1-3 is deleted or substituted relative to Exemplary SpyCas9 sgRNA-1 (SEQ ID NO: 172) or
 - b. one or more of positions H1-6 through H1-10 is substituted relative to Exemplary SpyCas9 sgRNA-1 (SEQ ID NO: 172); or
 3. the shortened hairpin 1 region lacks 5-10 nucleotides, preferably 5-6 nucleotides, and one or more of positions N18, H1-12, or n is substituted relative to Exemplary SpyCas9 sgRNA-1 (SEQ ID NO: 172); or
- B. a shortened upper stem region, wherein the shortened upper stem region lacks 1-6 nucleotides and wherein the 6, 7, 8, 9, 10, or 11 nucleotides of the shortened upper stem region include less than or equal to 4 substitutions relative to Exemplary SpyCas9 sgRNA-1 (SEQ ID NO: 172); or
- C. a substitution relative to Exemplary SpyCas9 sgRNA-1 (SEQ ID NO: 172) at any one or more of LS6, LS7, US3, US10, B3, N7, N15, N17, H2-2 and H2-14, wherein the substituent nucleotide is neither a pyrimidine that is followed by an adenine, nor an adenine that is preceded by a pyrimidine; or
- D. Exemplary SpyCas9 sgRNA-1 (SEQ ID NO: 172) with an upper stem region, wherein the upper stem modification comprises a modification to any one or more of US1-US12 in the upper stem region, wherein

1. the modified nucleotide is optionally selected from a 2'-O-methyl (2'-OMe) modified nucleotide, a 2'-O-(2-methoxyethyl) (2'-O-moe) modified nucleotide, a 2'-fluoro (2'-F) modified nucleotide, a phosphorothioate (PS) linkage between nucleotides, an inverted abasic modified nucleotide, or a combination thereof; or
2. the modified nucleotide optionally includes a 2'-OMe modified nucleotide.

[0003] In certain embodiments, Exemplary SpyCas9 sgRNA-1, or an sgRNA, such as an sgRNA comprising Exemplary SpyCas9 sgRNA-1, further includes a 3' tail, e.g., a 3' tail of 1, 2, 3, 4, or more nucleotides. In certain embodiments, the tail includes one or more modified nucleotides. In certain embodiments, the modified nucleotide is selected from a 2'-O-methyl (2'-OMe) modified nucleotide, a 2'-O-(2-methoxyethyl) (2'-O-moe) modified nucleotide, a 2'-fluoro (2'-F) modified nucleotide, a phosphorothioate (PS) linkage between nucleotides, and an inverted abasic modified nucleotide, or a combination thereof. In certain embodiments, the modified nucleotide includes a 2'-OMe modified nucleotide. In certain embodiments, the modified nucleotide includes a PS linkage between nucleotides. In certain embodiments, the modified nucleotide includes a 2'-OMe modified nucleotide and a PS linkage between nucleotides.

[00308]

[00309] In some embodiments, the gRNA described herein further comprises a nexus region, wherein the nexus region lacks at least one nucleotide.

[00310] In some embodiments, the gRNA is chemically modified. A gRNA comprising one or more modified nucleosides or nucleotides is called a "modified" gRNA or "chemically modified" gRNA, to describe the presence of one or more non-naturally and/or naturally occurring components or configurations that are used instead of or in addition to the canonical A, G, C, and U residues. Modified nucleosides and nucleotides can include one or more of: (i) alteration, e.g., replacement, of one or both of the non-linking phosphate oxygens and/or of one or more of the linking phosphate oxygens in the phosphodiester backbone linkage (an exemplary backbone modification); (ii) alteration, e.g., replacement, of a constituent of the ribose sugar, e.g., of the 2' hydroxyl on the ribose sugar (an exemplary sugar modification); (iii) wholesale replacement of the phosphate moiety with "dephospho" linkers (an exemplary backbone modification); (iv) modification or replacement of a naturally occurring nucleobase, including with a non-canonical nucleobase (an exemplary base modification); (v) replacement or modification of the ribose-phosphate backbone (an exemplary backbone modification); (vi)

modification of the 3' end or 5' end of the oligonucleotide, *e.g.*, removal, modification or replacement of a terminal phosphate group or conjugation of a moiety, cap or linker (such 3' or 5' cap modifications may comprise a sugar and/or backbone modification); and (vii) modification or replacement of the sugar (an exemplary sugar modification).

[00311] Chemical modifications such as those listed above can be combined to provide modified gRNAs comprising nucleosides and nucleotides (collectively “residues”) that can have two, three, four, or more modifications. For example, a modified residue can have a modified sugar and a modified nucleobase. In some embodiments, every base of a gRNA is modified, *e.g.*, all bases have a modified phosphate group, such as a phosphorothioate group. In certain embodiments, all, or substantially all, of the phosphate groups of an gRNA molecule are replaced with phosphorothioate groups. In some embodiments, modified gRNAs comprise at least one modified residue at or near the 5' end of the RNA. In some embodiments, modified gRNAs comprise at least one modified residue at or near the 3' end of the RNA.

[00312] In some embodiments, the gRNA comprises one, two, three or more modified residues. In some embodiments, at least 5% (*e.g.*, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%) of the positions in a modified gRNA are modified nucleosides or nucleotides.

[00313] In some embodiments of a backbone modification, the phosphate group of a modified residue can be modified by replacing one or more of the oxygens with a different substituent. Further, the modified residue, *e.g.*, modified residue present in a modified nucleic acid, can include the wholesale replacement of an unmodified phosphate moiety with a modified phosphate group as described herein. In some embodiments, the backbone modification of the phosphate backbone can include alterations that result in either an uncharged linker or a charged linker with unsymmetrical charge distribution.

[00314] Examples of modified phosphate groups include phosphorothioate, phosphoroselenates, borano phosphates, borano phosphate esters, hydrogen phosphonates, phosphoroamidates, alkyl or aryl phosphonates and phosphotriesters.

[00315] Scaffolds that can mimic nucleic acids can also be constructed wherein the phosphate linker and ribose sugar are replaced by nuclease resistant nucleoside or nucleotide surrogates. Such modifications may comprise backbone and sugar modifications. In some embodiments, the nucleobases can be tethered by a surrogate backbone. Examples can include,

without limitation, the morpholino, cyclobutyl, pyrrolidine and peptide nucleic acid (PNA) nucleoside surrogates.

[00316] The modified nucleosides and modified nucleotides can include one or more modifications to the sugar group, *i.e.* at sugar modification. For example, the 2' hydroxyl group (OH) can be modified, *e.g.* replaced with a number of different “oxy” or “deoxy” substituents. In some embodiments, modifications to the 2' hydroxyl group can enhance the stability of the nucleic acid since the hydroxyl can no longer be deprotonated to form a 2'-alkoxide ion. Examples of 2' hydroxyl group modifications can include alkoxy or aryloxy (OR, wherein “R” can be, *e.g.*, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or a sugar); polyethyleneglycols (PEG), $O(CH_2CH_2O)_nCH_2CH_2OR$ wherein R can be, *e.g.*, H or optionally substituted alkyl, and n can be an integer from 0 to 20. In some embodiments, the 2' hydroxyl group modification can be 2'-O-Me. In some embodiments, the 2' hydroxyl group modification can be a 2'-fluoro modification, which replaces the 2' hydroxyl group with a fluoride. In some embodiments, the 2' hydroxyl group modification can include “locked” nucleic acids (LNA) in which the 2' hydroxyl can be connected, *e.g.*, by a C₁₋₆ alkylene or C₁₋₆ heteroalkylene bridge, to the 4' carbon of the same ribose sugar, where exemplary bridges can include methylene, propylene, ether, or amino bridges. In some embodiments, the 2' hydroxyl group modification can include “unlocked” nucleic acids (UNA) in which the ribose ring lacks the C2'-C3' bond. In some embodiments, the 2' hydroxyl group modification can include the methoxyethyl group (MOE), $(OCH_2CH_2OCH_3)$, *e.g.*, a PEG derivative).

[00317] “Deoxy” 2' modifications can include hydrogen (*i.e.* deoxyribose sugars, *e.g.*, at the overhang portions of partially dsRNA); halo (*e.g.*, bromo, chloro, fluoro, or iodo); amino (wherein amino can be, *e.g.*, NH₂; alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroarylamino, diheteroarylamino, or amino acid); $NH(CH_2CH_2NH)_nCH_2CH_2$ -amino (wherein amino can be, *e.g.*, as described herein), -NHC(O)R (wherein R can be, *e.g.*, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar), cyano; mercapto; alkyl-thio-alkyl; thioalkoxy; and alkyl, cycloalkyl, aryl, alkenyl and alkynyl, which may be optionally substituted with *e.g.*, an amino as described herein.

[00318] The sugar modification can comprise a sugar group which may also contain one or more carbons that possess the opposite stereochemical configuration than that of the corresponding carbon in ribose. Thus, a modified nucleic acid can include nucleotides containing *e.g.*, arabinose, as the sugar. The modified nucleic acids can also include abasic sugars. These abasic sugars can also be further modified at one or more of the constituent sugar

atoms. The modified nucleic acids can also include one or more sugars that are in the L form, *e.g.* L- nucleosides.

[00319] The modified nucleosides and modified nucleotides described herein, which can be incorporated into a modified nucleic acid, can include a modified base, also called a nucleobase. Examples of nucleobases include, but are not limited to, adenine (A), guanine (G), cytosine (C), and uracil (U). These nucleobases can be modified or wholly replaced to provide modified residues that can be incorporated into modified nucleic acids. The nucleobase of the nucleotide can be independently selected from a purine, a pyrimidine, a purine analog, or pyrimidine analog. In some embodiments, the nucleobase can include, for example, naturally-occurring and synthetic derivatives of a base.

[00320] In embodiments employing a dual guide RNA, each of the crRNA and the tracrRNA can contain modifications. Such modifications may be at one or both ends of the crRNA and/or tracrRNA. In embodiments comprising an sgRNA, one or more residues at one or both ends of the sgRNA may be chemically modified, or the entire sgRNA may be chemically modified. Certain embodiments comprise a 5' end modification. Certain embodiments comprise a 3' end modification. In certain embodiments, one or more or all of the nucleotides in single stranded overhang of a gRNA molecule are deoxynucleotides.

[00321] In some embodiments, the gRNAs disclosed herein comprise one of the modification patterns disclosed in WO2018/107028 A1, published June 14, 2018 the contents of which are hereby incorporated by reference in their entirety.

[00322] The terms “mA,” “mC,” “mU,” or “mG” may be used to denote a nucleotide that has been modified with 2'-O-Me. The terms “fA,” “fC,” “fU,” or “fG” may be used to denote a nucleotide that has been substituted with 2'-F. A “*” may be used to depict a PS modification. The terms A*, C*, U*, or G* may be used to denote a nucleotide that is linked to the next (*e.g.*, 3') nucleotide with a PS bond. The terms “mA*,” “mC*,” “mU*,” or “mG*” may be used to denote a nucleotide that has been substituted with 2'-O-Me and that is linked to the next (*e.g.*, 3') nucleotide with a PS bond.

Exemplary spyCas9 sgRNA-1 (SEQ ID NO: 172)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
G	U	U	U	U	A	G	A	G	C	U	A	G	A	A	A	U	A	G	C	A	A	G	U	U	A	A	A	A	U	
LS1-LS6						B1-B2			US1-US12										B2-B6						LS7-LS12					
31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	
A	A	G	G	C	U	A	G	U	C	C	G	U	U	A	U	C	A	A	C	U	U	G	A	A	A	A	A	G	U	
Nexus																		H1-1 through H1-12												
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76															
G	G	C	A	C	C	G	A	G	U	C	G	G	U	G	C															
H2-1 through H2-15																														
N																														

C. Ribonucleoprotein complex

[00323] In some embodiments, the disclosure provides compositions comprising one or more gRNAs comprising one or more guide sequences from **Table 2** and an RNA-guided DNA binding agent, *e.g.*, a nuclease, such as a Cas nuclease, such as Cas9. In some embodiments, the RNA-guided DNA-binding agent has cleavase activity, which can also be referred to as double-strand endonuclease activity. In some embodiments, the RNA-guided DNA-binding agent comprises a Cas nuclease. Examples of Cas9 nucleases include those of the type II CRISPR systems of *S. pyogenes*, *S. aureus*, and other prokaryotes (*see e.g.*, the list in the next paragraph), and modified (*e.g.*, engineered or mutant) versions thereof. *See e.g.*, US2016/0312198 A1; US 2016/0312199 A1. Other examples of Cas nucleases include a Csm or Cmr complex of a type III CRISPR system or the Cas10, Csm1, or Cmr2 subunit thereof; and a Cascade complex of a type I CRISPR system, or the Cas3 subunit thereof. In some embodiments, the Cas nuclease may be from a Type-IIA, Type-IIB, or Type-IIC system. For discussion of various CRISPR systems and Cas nucleases see, *e.g.*, Makarova et al., NAT. REV. MICROBIOL. 9:467-477 (2011); Makarova et al., NAT. REV. MICROBIOL. 13: 722-36 (2015); Shmakov et al., MOLECULAR CELL, 60:385-397 (2015). In some embodiments, the RNA-guided DNA-binding agent comprises a Cas nickase. In some embodiments, the RNA-guided nickase is modified or derived from a Cas protein, such as a Class 2 Cas nuclease (which may be, *e.g.*, a Cas nuclease of Type II, V, or VI). Class 2 Cas nucleases include, for example, Cas9, Cpf1, C2c1, C2c2, and C2c3 proteins and modifications thereof.

[00324] Non-limiting exemplary species that the Cas nuclease or Cas nickase can be derived from include *Streptococcus pyogenes*, *Streptococcus thermophilus*, *Streptococcus sp.*, *Staphylococcus aureus*, *Listeria innocua*, *Lactobacillus gasseri*, *Francisella novicida*, *Wolinella succinogenes*, *Sutterella wadsworthensis*, *Gammaproteobacterium*, *Neisseria meningitidis*, *Campylobacter jejuni*, *Pasteurella multocida*, *Fibrobacter succinogene*, *Rhodospirillum rubrum*, *Nocardiopsis dassonvillei*, *Streptomyces pristinaespiralis*, *Streptomyces viridochromogenes*, *Streptomyces viridochromogenes*, *Streptosporangium roseum*, *Streptosporangium roseum*, *Alicyclobacillus acidocaldarius*, *Bacillus pseudomycooides*, *Bacillus selenitireducens*, *Exiguobacterium sibiricum*, *Lactobacillus delbrueckii*, *Lactobacillus salivarius*, *Lactobacillus buchneri*, *Treponema denticola*, *Microscilla marina*, *Burkholderiales bacterium*, *Polaromonas naphthalenivorans*, *Polaromonas sp.*, *Crocospaera watsonii*, *Cyanothece sp.*, *Microcystis aeruginosa*, *Synechococcus sp.*, *Acetohalobium arabaticum*, *Ammonifex degensii*, *Caldicelulosiruptor*

becscii, *Candidatus Desulforudis*, *Clostridium botulinum*, *Clostridium difficile*, *Finnegoldia magna*, *Natranaerobius thermophilus*, *Pelotomaculum thermopropionicum*, *Acidithiobacillus caldus*, *Acidithiobacillus ferrooxidans*, *Allochromatium vinosum*, *Marinobacter* sp., *Nitrosococcus halophilus*, *Nitrosococcus watsoni*, *Pseudoalteromonas haloplanktis*, *Ktedonobacter racemifer*, *Methanohalobium evestigatum*, *Anabaena variabilis*, *Nodularia spumigena*, *Nostoc* sp., *Arthrospira maxima*, *Arthrospira platensis*, *Arthrospira* sp., *Lyngbya* sp., *Microcoleus chthonoplastes*, *Oscillatoria* sp., *Petrotoga mobilis*, *Thermosiphon africanus*, *Streptococcus pasteurianus*, *Neisseria cinerea*, *Campylobacter lari*, *Parvibaculum lavamentivorans*, *Corynebacterium diphtheria*, *Acidaminococcus* sp., *Lachnospiraceae* bacterium ND2006, and *Acaryochloris marina*.

[00325] In some embodiments, the Cas nuclease is the Cas9 nuclease from *Streptococcus pyogenes*. In some embodiments, the Cas nuclease is the Cas9 nuclease from *Streptococcus thermophilus*. In some embodiments, the Cas nuclease is the Cas9 nuclease from *Neisseria meningitidis*. In some embodiments, the Cas nuclease is the Cas9 nuclease is from *Staphylococcus aureus*. In some embodiments, the Cas nuclease is the Cpf1 nuclease from *Francisella novicida*. In some embodiments, the Cas nuclease is the Cpf1 nuclease from *Acidaminococcus* sp. In some embodiments, the Cas nuclease is the Cpf1 nuclease from *Lachnospiraceae* bacterium ND2006. In further embodiments, the Cas nuclease is the Cpf1 nuclease from *Francisella tularensis*, *Lachnospiraceae* bacterium, *Butyrivibrio proteoclasticus*, *Peregrinibacteria* bacterium, *Parcubacteria* bacterium, *Smithella*, *Acidaminococcus*, *Candidatus Methanoplasma termitum*, *Eubacterium eligens*, *Moraxella bovoculi*, *Leptospira inadai*, *Porphyromonas crevioricanis*, *Prevotella disiens*, or *Porphyromonas macacae*. In certain embodiments, the Cas nuclease is a Cpf1 nuclease from an *Acidaminococcus* or *Lachnospiraceae*.

[00326] In some embodiments, the Cas nickase is derived from the Cas9 nuclease from *Streptococcus pyogenes*. In some embodiments, the Cas nickase is derived from the Cas9 nuclease from *Streptococcus thermophilus*. In some embodiments, the Cas nickase is a nickase form of the Cas9 nuclease from *Neisseria meningitidis*. See e.g., WO/2020081568, describing an Nme2Cas9 D16A nickase fusion protein. In some embodiments, the Cas nickase is derived from the Cas9 nuclease is from *Staphylococcus aureus*. In some embodiments, the Cas nickase is derived from the Cpf1 nuclease from *Francisella novicida*. In some embodiments, the Cas nickase is derived from the Cpf1 nuclease from *Acidaminococcus* sp. In some embodiments, the Cas nickase is derived from the Cpf1 nuclease from *Lachnospiraceae* bacterium ND2006. In further embodiments, the Cas nickase is derived from the Cpf1 nuclease from *Francisella*

ularensis, *Lachnospiraceae* bacterium, *Butyrivibrio proteoclasticus*, *Peregrinibacteria bacterium*, *Parcubacteria bacterium*, *Smithella*, *Acidaminococcus*, *Candidatus Methanoplasma termitum*, *Eubacterium eligens*, *Moraxella bovoculi*, *Leptospira inadai*, *Porphyromonas crevioricanis*, *Prevotella disiens*, or *Porphyromonas macacae*. In certain embodiments, the Cas nickase is derived from a Cpf1 nuclease from an *Acidaminococcus* or *Lachnospiraceae*. As discussed elsewhere, a nickase may be derived from a nuclease by inactivating one of the two catalytic domains, e.g., by mutating an active site residue essential for nucleolysis, such as D10, H840, or N863 in Spy Cas9. One skilled in the art will be familiar with techniques for easily identifying corresponding residues in other Cas proteins, such as sequence alignment and structural alignment, which is discussed in detail below.

[00327] In some embodiments, the gRNA together with an RNA-guided DNA binding agent is called a ribonucleoprotein complex (RNP). In some embodiments, the RNA-guided DNA binding agent is a Cas nuclease. In some embodiments, the gRNA together with a Cas nuclease is called a Cas RNP. In some embodiments, the RNP comprises Type-I, Type-II, or Type-III components. In some embodiments, the Cas nuclease is the Cas9 protein from the Type-II CRISPR/Cas system. In some embodiment, the gRNA together with Cas9 is called a Cas9 RNP.

[00328] Wild type Cas9 has two nuclease domains: RuvC and HNH. The RuvC domain cleaves the non-target DNA strand, and the HNH domain cleaves the target strand of DNA. In some embodiments, the Cas9 protein comprises more than one RuvC domain and/or more than one HNH domain. In some embodiments, the Cas9 protein is a wild type Cas9. In each of the composition, use, and method embodiments, the Cas induces a double strand break in target DNA.

[00329] In some embodiments, chimeric Cas nucleases are used, where one domain or region of the protein is replaced by a portion of a different protein. In some embodiments, a Cas nuclease domain may be replaced with a domain from a different nuclease such as FokI. In some embodiments, a Cas nuclease may be a modified nuclease.

[00330] In other embodiments, the Cas nuclease or Cas nickase may be from a Type-I CRISPR/Cas system. In some embodiments, the Cas nuclease may be a component of the Cascade complex of a Type-I CRISPR/Cas system. In some embodiments, the Cas nuclease may be a Cas3 protein. In some embodiments, the Cas nuclease may be from a Type-III CRISPR/Cas system. In some embodiments, the Cas nuclease may have an RNA cleavage activity.

[00331] In some embodiments, the RNA-guided DNA-binding agent has single-strand nickase activity, i.e., can cut one DNA strand to produce a single-strand break, also known as a “nick.” In some embodiments, the RNA-guided DNA-binding agent comprises a Cas nickase. A nickase is an enzyme that creates a nick in dsDNA, i.e., cuts one strand but not the other of the DNA double helix. In some embodiments, a Cas nickase is a version of a Cas nuclease (e.g., a Cas nuclease discussed above) in which an endonucleolytic active site is inactivated, e.g., by one or more alterations (e.g., point mutations) in a catalytic domain. *See e.g.*, US Pat. No. 8,889,356 for discussion of Cas nickases and exemplary catalytic domain alterations. In some embodiments, a Cas nickase such as a Cas9 nickase has an inactivated RuvC or HNH domain.

[00332] In some embodiments, the RNA-guided DNA-binding agent is modified to contain only one functional nuclease domain. For example, the agent protein may be modified such that one of the nuclease domains is mutated or fully or partially deleted to reduce its nucleic acid cleavage activity. In some embodiments, a nickase is used having a RuvC domain with reduced activity. In some embodiments, a nickase is used having an inactive RuvC domain. In some embodiments, a nickase is used having an HNH domain with reduced activity. In some embodiments, a nickase is used having an inactive HNH domain.

[00333] In some embodiments, a conserved amino acid within a Cas protein nuclease domain is substituted to reduce or alter nuclease activity. In some embodiments, a Cas nuclease may comprise an amino acid substitution in the RuvC or RuvC-like nuclease domain. Exemplary amino acid substitutions in the RuvC or RuvC-like nuclease domain include D10A (based on the *S. pyogenes* Cas9 protein). *See, e.g.*, Zetsche et al. (2015) *Cell* Oct 22:163(3): 759-771. In some embodiments, the Cas nuclease may comprise an amino acid substitution in the HNH or HNH-like nuclease domain. Exemplary amino acid substitutions in the HNH or HNH-like nuclease domain include E762A, H840A, N863A, H983A, and D986A (based on the *S. pyogenes* Cas9 protein). *See, e.g.*, Zetsche et al. (2015). Further exemplary amino acid substitutions include D917A, E1006A, and D1255A (based on the *Francisella novicida* U112 Cpf1 (FnCpf1) sequence (UniProtKB - A0Q7Q2 (CPF1_FRATN)).

[00334] In some embodiments, an mRNA encoding a nickase is provided in combination with a pair of guide RNAs that are complementary to the sense and antisense strands of the target sequence, respectively. In this embodiment, the guide RNAs direct the nickase to a target sequence and introduce a DSB by generating a nick on opposite strands of the target sequence (i.e., double nicking). In some embodiments, use of double nicking may improve specificity and reduce off-target effects. In some embodiments, a nickase is used together with two

separate guide RNAs targeting opposite strands of DNA to produce a double nick in the target DNA. In some embodiments, a nickase is used together with two separate guide RNAs that are selected to be in close proximity to produce a double nick in the target DNA.

[00335] In some embodiments, the RNA-guided DNA-binding agent lacks cleavase and nickase activity. In some embodiments, the RNA-guided DNA-binding agent comprises a dCas DNA-binding polypeptide. A dCas polypeptide has DNA-binding activity while essentially lacking catalytic (cleavase/nickase) activity. In some embodiments, the dCas polypeptide is a dCas9 polypeptide. In some embodiments, the RNA-guided DNA-binding agent lacking cleavase and nickase activity or the dCas DNA-binding polypeptide is a version of a Cas nuclease (*e.g.*, a Cas nuclease discussed above) in which its endonucleolytic active sites are inactivated, *e.g.*, by one or more alterations (*e.g.*, point mutations) in its catalytic domains. See, *e.g.*, US 2014/0186958 A1; US 2015/0166980 A1.

[00336] In some embodiments, the RNA-guided DNA binding agent comprises one or more heterologous functional domains (*e.g.*, is or comprises a fusion polypeptide).

[00337] In some embodiments, the RNA-guided DNA binding agent comprises a APOBEC3 deaminase. In some embodiments, a APOBEC3 deaminase is a APOBEC3A (A3A). In some embodiments, the A3A is a human A3A. In some embodiments, the A3A is a wild-type A3A.

[00338] In some embodiments, the RNA-guided DNA binding agent comprises a deaminase and an RNA-guided nickase. In some embodiments, the mRNA further comprises a linker to link the sequencing encoding A3A to the sequence sequencing encoding RNA-guided nickase. In some embodiments, the linker is an organic molecule, group, polymer, or chemical moiety. In some embodiments, the linker is a peptide linker. In some embodiments, the peptide linker is any stretch of amino acids having at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, or more amino acids. In some embodiments, the peptide linker is the 16 residue "XTEN" linker, or a variant thereof (See, *e.g.*, the Examples; and Schellenberger et al. A recombinant polypeptide extends the in vivo half-life of peptides and proteins in a tunable manner. *Nat. Biotechnol.* 27, 1186-1190 (2009)). In some embodiments, the XTEN linker comprises the sequence SGSETPGTSESATPES (SEQ ID NO: 900), SGSETPGTSESA (SEQ ID NO: 901), or SGSETPGTSESATPEGGSGGS (SEQ ID NO: 902). In some embodiments, the peptide linker comprises one or more sequences selected from SEQ ID NOs: 903-913.

[00339] In some embodiments, the heterologous functional domain may facilitate transport of the RNA-guided DNA-binding agent into the nucleus of a cell. For example, the

heterologous functional domain may be a nuclear localization signal (NLS). In some embodiments, the RNA-guided DNA-binding agent may be fused with 1-10 NLS(s). In some embodiments, the RNA-guided DNA-binding agent may be fused with 1-5 NLS(s). In some embodiments, the RNA-guided DNA-binding agent may be fused with one NLS. Where one NLS is used, the NLS may be fused at the N-terminus or the C-terminus of the RNA-guided DNA-binding agent sequence. It may also be inserted within the RNA-guided DNA binding agent sequence. In other embodiments, the RNA-guided DNA-binding agent may be fused with more than one NLS. In some embodiments, the RNA-guided DNA-binding agent may be fused with 2, 3, 4, or 5 NLSs. In some embodiments, the RNA-guided DNA-binding agent may be fused with two NLSs. In certain circumstances, the two NLSs may be the same (*e.g.*, two SV40 NLSs) or different. In some embodiments, the RNA-guided DNA-binding agent is fused to two NLS sequences (*e.g.*, SV40) fused at the carboxy terminus. In some embodiments, the RNA-guided DNA-binding agent may be fused with two NLSs, one linked at the N-terminus and one at the C-terminus. In some embodiments, the RNA-guided DNA-binding agent may be fused with 3 NLSs. In some embodiments, the RNA-guided DNA-binding agent may be fused with no NLS. In some embodiments, the NLS may be a monopartite sequence, such as, *e.g.*, the SV40 NLS, PKKKRKV (SEQ ID NO: 600) or PKKKRRV (SEQ ID NO: 601). In some embodiments, the NLS may be a bipartite sequence, such as the NLS of nucleoplasmin, KRPAATKKAGQAKKKK (SEQ ID NO: 602). In a specific embodiment, a single PKKKRKV (SEQ ID NO: 600) NLS may be fused at the C-terminus of the RNA-guided DNA-binding agent. One or more linkers are optionally included at the fusion site.

[00340] In some embodiments, the RNA-guided DNA binding agent comprises an editor. An exemplary editor is BC22n which includes a *H. sapiens* APOBEC3A fused to *S. pyogenes*-D10A Cas9 nickase by an XTEN linker, and mRNA encoding BC22n. An mRNA encoding BC22n is provided (SEQ ID NO:804).

[00341] In some embodiments, the heterologous functional domain may be capable of modifying the intracellular half-life of the RNA-guided DNA binding agent. In some embodiments, the half-life of the RNA-guided DNA binding agent may be increased. In some embodiments, the half-life of the RNA-guided DNA-binding agent may be reduced. In some embodiments, the heterologous functional domain may be capable of increasing the stability of the RNA-guided DNA-binding agent. In some embodiments, the heterologous functional domain may be capable of reducing the stability of the RNA-guided DNA-binding agent. In some embodiments, the heterologous functional domain may act as a signal peptide for protein degradation. In some embodiments, the protein degradation may be mediated by proteolytic

enzymes, such as, for example, proteasomes, lysosomal proteases, or calpain proteases. In some embodiments, the heterologous functional domain may comprise a PEST sequence. In some embodiments, the RNA-guided DNA-binding agent may be modified by addition of ubiquitin or a polyubiquitin chain. In some embodiments, the ubiquitin may be a ubiquitin-like protein (UBL). Non-limiting examples of ubiquitin-like proteins include small ubiquitin-like modifier (SUMO), ubiquitin cross-reactive protein (UCRP, also known as interferon-stimulated gene-15 (ISG15)), ubiquitin-related modifier-1 (URM1), neuronal-precursor-cell-expressed developmentally downregulated protein-8 (NEDD8, also called Rub1 in *S. cerevisiae*), human leukocyte antigen F-associated (FAT10), autophagy-8 (ATG8) and -12 (ATG12), Fau ubiquitin-like protein (FUB1), membrane-anchored UBL (MUB), ubiquitin fold-modifier-1 (UFM1), and ubiquitin-like protein-5 (UBL5).

[00342] In some embodiments, the heterologous functional domain may be a marker domain. Non-limiting examples of marker domains include fluorescent proteins, purification tags, epitope tags, and reporter gene sequences. In some embodiments, the marker domain may be a fluorescent protein. Non-limiting examples of suitable fluorescent proteins include green fluorescent proteins (*e.g.*, GFP, GFP-2, tagGFP, turboGFP, sfGFP, EGFP, Emerald, Azami Green, Monomeric Azami Green, CopGFP, AceGFP, ZsGreen1), yellow fluorescent proteins (*e.g.*, YFP, EYFP, Citrine, Venus, YPet, PhiYFP, ZsYellow1), blue fluorescent proteins (*e.g.*, EBFP, EBFP2, Azurite, mKalamal, GFPuv, Sapphire, T-sapphire), cyan fluorescent proteins (*e.g.*, ECFP, Cerulean, CyPet, AmCyan1, Midoriishi-Cyan), red fluorescent proteins (*e.g.*, mKate, mKate2, mPlum, DsRed monomer, mCherry, mRFP1, DsRed-Express, DsRed2, DsRed-Monomer, HcRed-Tandem, HcRed1, AsRed2, eqFP611, mRaspberry, mStrawberry, Jred), and orange fluorescent proteins (mOrange, mKO, Kusabira-Orange, Monomeric Kusabira-Orange, mTangerine, tdTomato) or any other suitable fluorescent protein. In other embodiments, the marker domain may be a purification tag and/or an epitope tag. Non-limiting exemplary tags include glutathione-S-transferase (GST), chitin binding protein (CBP), maltose binding protein (MBP), thioredoxin (TRX), poly(NANP), tandem affinity purification (TAP) tag, myc, AcV5, AU1, AU5, E, ECS, E2, FLAG, HA, nus, Softag 1, Softag 3, Strep, SBP, Glu-Glu, HSV, KT3, S, S1, T7, V5, VSV-G, 6xHis, 8xHis, biotin carboxyl carrier protein (BCCP), poly-His, and calmodulin. Non-limiting exemplary reporter genes include glutathione-S-transferase (GST), horseradish peroxidase (HRP), chloramphenicol acetyltransferase (CAT), beta-galactosidase, beta-glucuronidase, luciferase, or fluorescent proteins.

[00343] In additional embodiments, the heterologous functional domain may target the RNA-guided DNA-binding agent to a specific organelle, cell type, tissue, or organ. In some embodiments, the heterologous functional domain may target the RNA-guided DNA-binding agent to mitochondria.

[00344] In further embodiments, the heterologous functional domain may be an effector domain such as an editor domain. When the RNA-guided DNA-binding agent is directed to its target sequence, *e.g.*, when a Cas nuclease is directed to a target sequence by a gRNA, the effector such as an editor domain may modify or affect the target sequence. In some embodiments, the effector such as an editor domain may be chosen from a nucleic acid binding domain, a nuclease domain (*e.g.*, a non-Cas nuclease domain), an epigenetic modification domain, a transcriptional activation domain, or a transcriptional repressor domain. In some embodiments, the heterologous functional domain is a nuclease, such as a FokI nuclease. See, *e.g.*, US Pat. No. 9,023,649. In some embodiments, the heterologous functional domain is a transcriptional activator or repressor. See, *e.g.*, Qi et al., “Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression,” *Cell* 152:1173-83 (2013); Perez-Pinera et al., “RNA-guided gene activation by CRISPR-Cas9-based transcription factors,” *Nat. Methods* 10:973-6 (2013); Mali et al., “CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering,” *Nat. Biotechnol.* 31:833-8 (2013); Gilbert et al., “CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes,” *Cell* 154:442-51 (2013). As such, the RNA-guided DNA-binding agent essentially becomes a transcription factor that can be directed to bind a desired target sequence using a guide RNA.

D. Determination of Efficacy of Guide RNAs

[00345] In some embodiments, the efficacy of a guide RNA is determined when delivered or expressed together with other components (*e.g.*, an RNA-guided DNA binding agent) forming an RNP. In some embodiments, the guide RNA is expressed together with an RNA-guided DNA binding agent, such as a Cas protein, *e.g.*, Cas9. In some embodiments, the guide RNA is delivered to or expressed in a cell line that already stably expresses an RNA-guided DNA nuclease, such as a Cas nuclease or nickase, *e.g.*, Cas9 nuclease or nickase. In some embodiments the guide RNA is delivered to a cell as part of a RNP. In some embodiments, the guide RNA is delivered to a cell along with a mRNA encoding an RNA-guided DNA nuclease, such as a Cas nuclease or nickase, *e.g.*, Cas9 nuclease or nickase.

[00346] As described herein, use of an RNA-guided DNA nuclease and a guide RNA disclosed herein can lead to DSBs, SSBs, and/or site-specific binding that results in nucleic acid modification in the DNA or pre-mRNA which can produce errors in the form of insertion/deletion (indel) mutations upon repair by cellular machinery. Many mutations due to indels alter the reading frame, introduce premature stop codons, or induce exon skipping and, therefore, produce a non-functional protein.

[00347] In some embodiments, the efficacy of particular guide RNAs is determined based on *in vitro* models. In some embodiments, the *in vitro* model is T cell line. In some embodiments, the *in vitro* model is HEK293 T cells. In some embodiments, the *in vitro* model is HEK293 cells stably expressing Cas9 (HEK293_Cas9). In some embodiments, the *in vitro* model is a lymphoblastoid cell line. In some embodiments, the *in vitro* model is primary human T cells. In some embodiments, the *in vitro* model is primary human B cells. In some embodiments, the *in vitro* model is primary human peripheral blood lymphocytes. In some embodiments, the *in vitro* model is primary human peripheral blood mononuclear cells.

[00348] In some embodiments, the number of off-target sites at which a deletion or insertion occurs in an *in vitro* model is determined, e.g., by analyzing genomic DNA from the cells transfected *in vitro* with Cas9 mRNA and the guide RNA. In some embodiments, such a determination comprises analyzing genomic DNA from cells transfected *in vitro* with Cas9 mRNA, the guide RNA, and a donor oligonucleotide. Exemplary procedures for such determinations are provided in the working examples below.

[00349] In some embodiments, the efficacy of particular gRNAs is determined across multiple *in vitro* cell models for a guide RNA selection process. In some embodiments, a cell line comparison of data with selected guide RNAs is performed. In some embodiments, cross screening in multiple cell models is performed.

[00350] In some embodiments, the efficacy of particular guide RNAs is determined based on *in vivo* models. In some embodiments, the *in vivo* model is a rodent model. In some embodiments, the rodent model is a mouse which expresses the target gene. In some embodiments, the rodent model is a mouse which expresses a *CIITA* gene. In some embodiments, the rodent model is a mouse which expresses a human *CIITA* gene. In some embodiments, the rodent model is a mouse which expresses a *B2M* gene. In some embodiments, the rodent model is a mouse which expresses a human *B2M* gene. In some embodiments, the *in vivo* model is a non-human primate, for example cynomolgus monkey.

[00351] In some embodiments, the efficacy of a guide RNA is evaluated by on target cleavage efficiency. In some embodiments, the efficacy of a guide RNA is measured by percent

editing at the target location, *e.g.*, CIITA, or B2M. In some embodiments, deep sequencing may be utilized to identify the presence of modifications (*e.g.*, insertions, deletions) introduced by gene editing. Indel percentage can be calculated from next generation sequencing “NGS.”

[00352] In some embodiments, the efficacy of a guide RNA is measured by the number and/or frequency of indels at off-target sequences within the genome of the target cell type. In some embodiments, efficacious guide RNAs are provided which produce indels at off target sites at very low frequencies (*e.g.*, <5%) in a cell population and/or relative to the frequency of indel creation at the target site. Thus, the disclosure provides for guide RNAs which do not exhibit off-target indel formation in the target cell type (*e.g.*, T cells or B cells), or which produce a frequency of off-target indel formation of <5% in a cell population and/or relative to the frequency of indel creation at the target site. In some embodiments, the disclosure provides guide RNAs which do not exhibit any off target indel formation in the target cell type (*e.g.*, T cells or B cells). In some embodiments, guide RNAs are provided which produce indels at less than 5 off-target sites, *e.g.*, as evaluated by one or more methods described herein. In some embodiments, guide RNAs are provided which produce indels at less than or equal to 4, 3, 2, or 1 off-target site(s) *e.g.*, as evaluated by one or more methods described herein. In some embodiments, the off-target site(s) does not occur in a protein coding region in the target cell (*e.g.*, T cells or B cells) genome.

[00353] In some embodiments, linear amplification is used to detect gene editing events, such as the formation of insertion/deletion (“indel”) mutations, translocations, and homology directed repair (HDR) events in target DNA. For example, linear amplification with a unique sequence-tagged primer and isolating the tagged amplification products (herein after referred to as “UnIT,” or “Unique Identifier Tagmentation” method) may be used.

[00354] In some embodiments, the efficacy of a guide RNA is measured by the number of chromosomal rearrangements within the target cell type. Kromatid dGH assay may used to detect chromosomal rearrangements, including *e.g.*, translocations, reciprocal translocations, translocations to off-target chromosomes, deletions (*i.e.*, chromosomal rearrangements where fragments were lost during the cell replication cycle due to the editing event). In some embodiments, the target cell type has less than 10, less than 8, less than 5, less than 4, less than 3, less than 2, or less than 1 chromosomal rearrangement. In some embodiments, the target cell type has no chromosomal rearrangements.

E. Delivery of gRNA Compositions

[00355] Lipid nanoparticles (LNP compositions) are a well-known means for delivery of nucleotide and protein cargo and may be used for delivery of the guide RNAs, compositions, or pharmaceutical formulations disclosed herein. In some embodiments, the LNP compositions deliver nucleic acid, protein, or nucleic acid together with protein.

[00356] In some embodiments, the invention comprises a method for delivering any one of the gRNAs disclosed herein to a subject, wherein the gRNA is formulated as an LNP. In some embodiments, the LNP comprises the gRNA and a Cas9 or an mRNA encoding Cas9.

[00357] In some embodiments, the invention comprises a composition comprising any one of the gRNAs disclosed and an LNP. In some embodiments, the composition further comprises a Cas9 or an mRNA encoding Cas9.

[00358] In some embodiments, the LNP compositions comprise cationic lipids. In some embodiments, the LNP compositions comprise (9Z,12Z)-3-((4,4-bis(octyloxy)butanoyl)oxy)-2-(((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl octadeca-9,12-dienoate, also called 3-((4,4-bis(octyloxy)butanoyl)oxy)-2-(((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl (9Z,12Z)-octadeca-9,12-dienoate or another ionizable lipid. See, e.g., lipids of WO/2017/173054 and references described therein. In some embodiments, the LNP compositions comprise molar ratios of a cationic lipid amine to RNA phosphate (N:P) of about 4.5, 5.0, 5.5, 6.0, or 6.5. In some embodiments, the term cationic and ionizable in the context of LNP lipids is interchangeable, e.g., wherein ionizable lipids are cationic depending on the pH.

[00359] In some embodiments, the gRNAs disclosed herein are formulated as LNP compositions for use in preparing a medicament for treating a disease or disorder.

[00360] Electroporation is a well-known means for delivery of cargo, and any electroporation methodology may be used for delivery of any one of the gRNAs disclosed herein. In some embodiments, electroporation may be used to deliver any one of the gRNAs disclosed herein and Cas9 or an mRNA encoding Cas9.

[00361] In some embodiments, the invention comprises a method for delivering any one of the gRNAs disclosed herein to an *ex vivo* cell, wherein the gRNA is formulated as an LNP or not formulated as an LNP. In some embodiments, the LNP comprises the gRNA and a Cas9 or an mRNA encoding Cas9.

[00362] In some embodiments, the guide RNA compositions described herein, alone or encoded on one or more vectors, are formulated in or administered via a lipid nanoparticle; see

e.g., WO/2017/173054 and WO 2019/067992, the contents of which are hereby incorporated by reference in their entirety.

[00363] In certain embodiments, the invention comprises DNA or RNA vectors encoding any of the guide RNAs comprising any one or more of the guide sequences described herein. In some embodiments, in addition to guide RNA sequences, the vectors further comprise nucleic acids that do not encode guide RNAs. Nucleic acids that do not encode guide RNA include, but are not limited to, promoters, enhancers, regulatory sequences, and nucleic acids encoding an RNA-guided DNA nuclease, which can be a nuclease such as Cas9. In some embodiments, the vector comprises one or more nucleotide sequence(s) encoding a crRNA, a trRNA, or a crRNA and trRNA. In some embodiments, the vector comprises one or more nucleotide sequence(s) encoding a sgRNA and an mRNA encoding an RNA-guided DNA nuclease, which can be a Cas nuclease, such as Cas9 or CpfI. In some embodiments, the vector comprises one or more nucleotide sequence(s) encoding a crRNA, a trRNA, and an mRNA encoding an RNA-guided DNA nuclease, which can be a Cas protein, such as, Cas9. In one embodiment, the Cas9 is from *Streptococcus pyogenes* (i.e., Spy Cas9). In some embodiments, the nucleotide sequence encoding the crRNA, trRNA, or crRNA and trRNA (which may be a sgRNA) comprises or consists of a guide sequence flanked by all or a portion of a repeat sequence from a naturally-occurring CRISPR/Cas system. The nucleic acid comprising or consisting of the crRNA, trRNA, or crRNA and trRNA may further comprise a vector sequence wherein the vector sequence comprises or consists of nucleic acids that are not naturally found together with the crRNA, trRNA, or crRNA and trRNA.

IV. Therapeutic Methods and Uses

[00364] Any of the engineered cells and compositions described herein can be used in a method of treating a variety of diseases and disorders, as described herein. In some embodiments, the genetically modified cell (engineered cell) and/or population of genetically modified cells (engineered cells) and compositions may be used in methods of treating a variety of diseases and disorders. In some embodiments, a method of treating any one of the diseases or disorders described herein is encompassed, comprising administering any one or more composition described herein.

[00365] In some embodiments, the methods and compositions described herein may be used to treat diseases or disorders in need of delivery of a therapeutic agent. In some embodiments, the invention provides a method of providing an immunotherapy in a subject, the method including administering to the subject an effective amount of an engineered cell (or population

of engineered cells) as described herein, for example, a cell of any of the aforementioned cell aspects and embodiments.

[00366] In some embodiments, the methods comprise administering to a subject a composition comprising an engineered cell described herein as an adoptive cell transfer therapy. In some embodiments, the engineered cell is an allogeneic cell.

[00367] In some embodiments, the methods comprise administering to a subject a composition comprising an engineered cell described herein, wherein the cell produces, secretes, and/or expresses a polypeptide (e.g., a targeting receptor) useful for treatment of a disease or disorder in a subject. In some embodiments, the cell acts as a cell factory to produce a soluble polypeptide. In some embodiments, the cell acts as a cell factory to produce an antibody. In some embodiments, the cell continuously secretes the polypeptide *in vivo*. In some embodiments, the cell continuously secretes the polypeptide following transplantation *in vivo* for at least 1, 2, 3, 4, 5, or 6 weeks. In some embodiments, the cell continuously secretes the polypeptide following transplantation *in vivo* for more than 6 weeks. In some embodiments, the soluble polypeptide (e.g., an antibody) is produced by the cell at a concentration of at least 10^2 , 10^3 , 10^4 , 10^5 , 10^6 , 10^7 , or 10^8 copies per day. In some embodiments, the polypeptide is an antibody and is produced by the cell at a concentration of at least 10^8 copies per day.

[00368] In some embodiments of the methods, the method includes administering a lymphodepleting agent or immunosuppressant prior to administering to the subject an effective amount of the engineered cell (or engineered cells) as described herein, for example, a cell of any of the aforementioned cell aspects and embodiments. In another aspect, the invention provides a method of preparing engineered cells (e.g., a population of engineered cells).

[00369] Immunotherapy is the treatment of disease by activating or suppressing the immune system. Immunotherapies designed to elicit or amplify an immune response are classified as activation immunotherapies. Cell-based immunotherapies have been demonstrated to be effective in the treatment of some cancers. Immune effector cells such as lymphocytes, macrophages, dendritic cells, natural killer cells, cytotoxic T lymphocytes (CTLs), T helper cells, B cells, or their progenitors such as hematopoietic stem cells (HSC) or induced pluripotent stem cells (iPSC) can be programmed to act in response to abnormal antigens expressed on the surface of tumor cells. Thus, cancer immunotherapy allows components of the immune system to destroy tumors or other cancerous cells. Cell-based immunotherapies have also been demonstrated to be effective in the treatment of autoimmune diseases or transplant rejection. Immune effector cells such as regulatory T cells (Tregs) or mesenchymal

stem cells can be programmed to act in response to autoantigens or transplant antigens expressed on the surface of normal tissues.

[00370] In some embodiments, the invention provides a method of preparing engineered cells (*e.g.*, a population of engineered cells). The population of engineered cells may be used for immunotherapy.

[00371] In some embodiments, the invention provides a method of treating a subject in need thereof that includes administering engineered cells prepared by a method of preparing cells described herein, for example, a method of any of the aforementioned aspects and embodiments of methods of preparing cells.

[00372] In some embodiments, the engineered cells can be used to treat cancer, infectious diseases, inflammatory diseases, autoimmune diseases, cardiovascular diseases, neurological diseases, ophthalmologic diseases, renal diseases, liver diseases, musculoskeletal diseases, red blood cell diseases, or transplant rejections.

[00373] In some embodiments, the engineered cells can be used as a cell therapy comprising an allogeneic stem cell therapy. In some embodiments, the cell therapy comprises induced pluripotent stem cells (iPSCs). iPSCs may be induced to differentiate into other cell types including *e.g.*, beta islet cells, neurons, and blood cells. In some embodiments, the cell therapy comprises hematopoietic stem cells. In some embodiments, the stem cells comprise mesenchymal stem cells that can develop into bone, cartilage, muscle, and fat cells. In some embodiments, the stem cells comprise ocular stem cells. In some embodiments, the allogeneic stem cell transplant comprises allogeneic bone marrow transplant. In some embodiments, the stem cells comprise pluripotent stem cells (PSCs). In some embodiments, the stem cells comprise induced embryonic stem cells (ESCs).

[00374] Engineered cells of the invention are suitable for further engineering, *e.g.*, by introduction of further edited, or modified genes or alleles. In some embodiments, the polypeptide is a wild-type or variant TCR. Cells of the invention may also be suitable for further engineering by introduction of an exogenous nucleic acid encoding *e.g.*, a targeting receptor, *e.g.*, a TCR, CAR, UniCAR. CARs are also known as chimeric immunoreceptors, chimeric T cell receptors or artificial T cell receptors.

[00375] In some embodiments, the cell therapy is a transgenic T cell therapy. In some embodiments, the cell therapy comprises a Wilms' Tumor 1 (WT1) targeting transgenic T cell. In some embodiments, the cell therapy comprises a targeting receptor or a donor nucleic acid encoding a targeting receptor of a commercially available T cell therapy, such as a CAR T cell therapy. There are number of targeting receptors currently approved for cell therapy. The cells

and methods provided herein can be used with these known constructs. Commercially approved cell products that include targeting receptor constructs for use as cell therapies include *e.g.*, Kymriah® (tisagenlecleucel); Yescarta® (axicabtagene ciloleucel); Tecartus™ (brexucabtagene autoleucel); Tabelecleucel (Tab-cel®); Viralym-M (ALVR105); and Viralym-C.

[00376] In some embodiments, the methods provide for administering the engineered cells to a subject, wherein the administration is an injection. In some embodiments, the methods provide for administering the engineered cells to a subject, wherein the administration is an intravascular injection or infusion. In some embodiments, the methods provide for administering the engineered cells to a subject, wherein the administration is a single dose.

[00377] In some embodiments, the methods provide for reducing a sign or symptom associated of a subject's disease treated with a composition disclosed herein. In some embodiments, the subject has a response to treatment with a composition disclosed herein that lasts more than one week. In some embodiments, the subject has a response to treatment with a composition disclosed herein that lasts more than two weeks. In some embodiments, the subject has a response to treatment with a composition disclosed herein that lasts more than three weeks. In some embodiments, the subject has a response to treatment with a composition disclosed herein that lasts more than one month.

[00378] In some embodiments, the methods provide for administering the engineered cells to an subject, and wherein the subject has a response to the administered cell that comprises a reduction in a sign or symptom associated with the disease treated by the cell therapy. In some embodiments, the subject has a response that lasts more than one week. In some embodiments, the subject has a response that lasts more than one month. In some embodiments, the subject has a response that lasts for at least 1-6 weeks.

[00379] **Table 4. ADDITIONAL SEQUENCES**

Description	SEQ ID NO	Sequence
G000644	200	mG*mA*mG*UCCGAGCAGAAGAAGAAGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
G000645	201	mG*mA*mC*CCCCUCCACCCGCCUCGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
G000646	202	mG*mA*mC*UUGUUUUCAUUGUUCUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU

Description	SEQ ID NO	Sequence
G013006	203	mC*mU*mC*UCAGCUGGUACACGGCAGUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
G013009	204	mU*mA*mG*GCAGACAGACUUGUCACGUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
G015964	205	mC*mC*mC*CCCGCCGUGUUUGUGGGGUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
G015991	206	mA*mC*mU*CACGUGGAUAGCCUCCGUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
G015995	207	mU*mU*mA*CCCCACUUAACUAUCUUGUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
G015996	208	mC*mU*mU*ACCCACUUAACUAUCUUGUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
G016016	209	mU*mU*mU*CAAAACCGUCAGUGAUGUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
G016017	210	mU*mU*mC*AAAACCGUCAGUGAUGUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
G016239	211	mG*mG*mC*CUCGGCGCUGACGAUCUGUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
G018081	212	mC*mU*mG*UGUCACCCGUUUCAGGUGUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
G018082	213	mU*mG*mU*GUCACCCGUUUCAGGUGUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
G018995	214	mA*mC*mA*GCGACGCCGAGCCAGGUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
tracr RNA	215	AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAA AAGUGGCACCGAGUCGUGCUUUUUU
G000529	216	mG*mG*mC*CACGGAGCGAGACAUCUGUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
G019000	217	mG*mC*mG*CCCGCGGUCCAUCCUCGUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUm

Description	SEQ ID NO	Sequence
		mGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
Recombinant Cas9-NLS amino acid sequence	800	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSVDKLFQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKD TYDDDLNLLAQIGDQYADLFLAAKNLSDAILSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPIYVGPLARGNSRFAMWTRKSEETITPWNFEVVVDKGASAQSFIERMTNFDKNLPNEKVLPHKSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLEFDREMIEERLKYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDFANRNFQMQLIHDDSLTFKEDIQKAQVSGQGDLSHEHIANLAGSPAIIKKGILQTVKVVDELVKVMGRHKPENIVIAMARENQTTQKGQKNSRERMKRIIEGKELGSQLKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDIRLSDYD VDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKMKMKNYWRQLLNAKLITQRKFDNLTKAERGGSELDKAGFIKRLQVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKS LVSDFRKDFQFYK VREINNYHHAH DAYLNAVVG TALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEI GKATAKYFFYSNIMNFFKTEITLANGEIRKRLIETNGETGEIVWDKGRDFATVRKVLSPQVNVIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKEKVEKSKKLKSVKELLGITIMERSSEFEKNPIDFLEAKGYKEVKD LIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYELKLGSPEDNEQQLFVEQHKHYLDEIIEQISEFSKRVLADANLDKVL SAYN KHRDKPIREQAENIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ SITGLYETRIDLSQLGGDGGGSPKKKRKV
ORF encoding Sp. Cas9	801	ATGGACAAGAAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCTGGGAAACACAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTTCGACAGCGGAGAAACAGCAGAAGCAACAAGACTGAGAGAACAGCAAGAAGATACACAAGAAGAAAGACAGAACTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTGCGACGACAGCTTCTCCACAGACTGGAAGAAAGCTTCTGGTGAAGAAGACAAGAAGCACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTTCGACAGCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCTGATCGAAGGAGACCTGAACCCGGACAACA GCGACGTCGACAAGCTGTTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTGGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCCGGGAGAAAAAGAAACGGACTGTTTCGAAAACCTGATCGC ACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCA GAAGACGCAAGCTGCGAGCTGAGCAAGGACACATACGACGACGACCTGGACAACTGCTGGCACAGATCGGAGACCAGTACGCAGACCTGTTCTCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAAGCATGATCAAGAGATACGACGAACACCACAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCTGCCGGAAGTACAAGGAAATCTTCTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATCAAGCCGATCCTGGAAGAGATGGACGGAACAGAGAACCTTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAGAACAGAACATT CGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAAGACAGGAAGACTTCTACCCGTTCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGACATT CAGAATCCCGTACTACGTCGGACC

Description	SEQ ID NO	Sequence
		GCTGGCAAGAGGAAACAGCAGATTTCGCATGGATGACAAGAAAGAGCGA AGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGC AAGCGCACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTG CCGAACGAAAAGGTCTCTGCCGAAGCACAGCCTGCTGTACGAATACTTCA CAGTCTACAACGAACTGACAAAGGTCAAGTACGTACAGAAAGGAATGA GAAAGCCGGCATTTCTGAGCGGAGAACAGAAGAAGGCAATCTGTCGACC TGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAG ACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGT CGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGCTGAAG ATCATCAAGGACAAGGACTTCTGGACAACGAAGAAAACGAAGACATC CTGGAAGACATCGTCTCTGACACTGACACTGTTCTGAAGACAGAGAAATGA TCGAAGAAAAGACTGAAGACATACGCACACCTGTTCTGACGACAAGGTCAT GAAGCAGCTGAAGAGAAGAAGATACACAGGATGGGGAAGACTGAGCAG AAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCT GGACTTCTGAAGAGCGACGGATTTCGCAACAGAACTTCATGTCAGCTG ATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAG GTCAGCGGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCA GGAAGCCCGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCTGTC GACGAACTGGTCAAGGTCTATGGGAAGACACAAGCCGAAAAACATCGTC ATCGAAATGGCAAGAGAAAAACAGACAACACAGAAGGGACAGAAGAAC AGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCAAGGAACTGGGA AGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAGAAC GAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCTG ACCAGGAACTGGACATCAACAGACTGAGCGACTACGACGTGACCCACAT CGTCCCGCAGAGCTTCTGAAAGGACGACAGCATCGACAACAAGGTCTCTG ACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAA GAAGTCGTCAAGAAGATGAAGAATACTGGAGACAGCTGCTGAACGCA AAGCTGATCACACAGAGAAAAGTTCGACAACCTGACAAAAGGCAGAGAGA GGAGGACTGAGCGAACTGGACAAGGCAGGATTTCATCAAGAGACAGCTG GTCGAAACAAGACAGATCACAAAGCACGTGCGACAGATCCTGGACAGC AGAATGAACACAAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTC AAGGTATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACT TCCAGTTCTACAAGGTCAGAGAAATCAACAATACTACCACCACGCACACGA CGCATACCTGAACGCAGTCTGTCGGAACAGCACTGATCAAGAAGTACCCG AAGCTGGAAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCA GAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAACAGCAA AGTACTTCTTCTACAGCAACATCATGAATCTTCTCAAGACAGAAATCACA CTGGCAAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGA GAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTC AGAAAGGTCCTGAGCATGCCGAGGTCAACATCGTCAAGAAGACAGAA GTCCAGACAGGAGGATTTCAGCAAGGAAAGCATCTGCCGAAGAGAAAC AGCGACAAGCTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTAC GGAGGATTCGACAGCCCCGACAGTCGCATACAGCGTCTGGTCTGTCGAA AGGTGAAAAGGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAAGTGC TGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAAAAGAACCCGATCG ACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCAT CAAGCTGCCGAAGTACAGCCTGTTCTGAACTGGAAAAACGGAAGAAAGAG AATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAACGAACTGGCACT GCCGAGCAAGTACGTCAACTTCTGTACCTGGCAAGCCACTACGAAAAG CTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCTGTCGAA CAGCACAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCA GCAAGAGAGTCATCTGGCAGACGCAAACTGGACAAGGTCTGAGCGC ATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACAT CATCCACCTGTTTCACTGACAAACCTGGGAGCACCGGCAGCATTCAAG TACTTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAA GTCCTGGACGCAACACTGATCCACAGAGCATCACAGGACTGTACGAAA CAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAGGAGGAAGCCCGA AGAAGAAGAGAAAAGGTCTAG

Description	SEQ ID NO	Sequence
ORF encoding Sp. Cas9	802	ATGGACAAGAAGTACTCCATCGGCCTGGACATCGGCACCAACTCCGTGG GCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCTCCAAGAAGTTCAA GGTGTGGGCAACACCGACCGGCACTCCATCAAGAAGAACCTGATCGGC GCCCTGCTGTTCTGACTCCGGCGAGACCGCCGAGGCCACCCGGCTGAAGC GGACCGCCCGCGGCGGTACACCCGGCGGAAGAACCGGATCTGTACTCT GCAGGAGATCTTCTCCAACGAGATGGCCAAGGTGGACGACTCCTTCTTC CACCGGCTGGAGGAGTCTTCTGTTGGAGGAGGACAAGAAGCACGAG CGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGA AGTACCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACTCCACCGA CAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCACATGATCAAG TTCCGGGGCCACTTCTGATCGAGGGCGACCTGAACCCCGACAACCTCCG ACGTGGACAAGCTGTTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTT CGAGGAGAACCCCATCAACGCCTCCGGCGTGGACGCCAAGGCCATCCTG TCCGCCCGGCTGTCCAAGTCCCGGCGGCTGGAGAACCTGATCGCCAGC TGCCCGGCGAGAAGAAGAACGGCCTGTTTCGGCAACCTGATCGCCCTGTC CCTGGGCGCTGACCCCAACTTCAAGTCCAACCTCGACCTGGCCGAGGAC GCCAAGCTGCAGCTGTCCAAGGACACCTACGACGACGACCTGGACAACC TGCTGGCCCAGATCGGCGACCACTACGCCGACCTGTTCTCGCCGCCAA GAACCTGTCCGACGCCATCTGCTGTCCGACATCTGCGGGTGAACACC GAGATACCAAGGCCCCCTGTCCGCTCCATGATCAAGCGGTACGACG AGCACCAACAGGACCTGACCTGCTGAAGGCCCTGGTGGGACGAGCT GCCGAGAAGTACAAGGAGATCTTCTCGACCACTCAAGAACGGCTAC GCCGGCTACATCGACGGCGGCGCCTCCAGGAGGAGTTCTACAAGTTCA TCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAA GCTGAACCGGGAGGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGC TCCATCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCTGCGGC GGCAGGAGGACTTCTACCCCTTCTGAAGGACAACCGGGAGAAGATCGA GAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCTGGCCCGG GCAACTCCCGGTTCCGCTGGATGACCCGGAAGTCCGAGGAGACCATCAC CCCTGGAACTTCGAGGAGGTGGTGGACAAGGGCGCCTCCGCCCACTCC TTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGG TGCTGCCCAAGCACTCCCTGCTGTACGAGTACTTACCGTGTACAACGAG CTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCCGCTTCC TGTCGGGCGAGCAGAAGAAGGCCATCGTGGACCTGCTGTTCAAGAACAA CCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAAGT CGAGTGCTTCGACTCCGTGGAGATCTCCGGCGTGGAGGACCGGTTCAAC GCCTCCCTGGGCACCTACCAAGACCTGCTGAAGATCATCAAGGACAAGG ACTTCTGGACAACGAGGAGAACGAGGACATCTGGAGGACATCGTGCT GACCCTGACCTGTTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAAG ACCTACGCCCACCTGTTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGC GGCGGTACACCGGCTGGGGCCGGCTGTCCCGGAAGCTGATCAACGGCAT CCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCTGAAGTCCGAC GGCTTCGCCAACCGGAACCTTCATGCAGCTGATCCACGACGACTCCCTGA CCTTCAAGGAGGACATCCAGAAGGCCAGGTGTCGGCCAGGGCGACTC CCTGCACGAGCACATCGCCAACCTGGCCGGCTCCCCCGCCATCAAGAAG GGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGG GCCGGACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACC AGACCACCCAGAAGGGCCAGAAGAACTCCGGGAGCGGATGAAGCGGA TCGAGGAGGGCATCAAGGAGCTGGGCTCCAGATCCTGAAGGAGCACCC CGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACTGTACTACCTG CAGAACGGCCGGGACATGTACGTGGACAGGAGCTGGACATCAACCGG CTGTCCGACTACGACGTGGACCATCGTGCCCACTCTTCTGAAGGA CGACTCCATCGACAACAAGGTGCTGACCCGGTCCGACAAGAACCAGGGG AAGTCCGACAACGTGCCCTCCGAGGAGGTGGTGAAGAAGATGAAGAAC TACTGGCGGCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTTCG ACAACCTGACCAAGGCCGAGCGGGGCGGCCTGTCCGAGCTGGACAAGG CCGGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGATCACCAGCA CGTGGCCCAGATCCTGGACTCCCGGATGAACACCAAGTACGACGAGAAC

Description	SEQ ID NO	Sequence
		GACAAGCTGATCCGGGAGGTGAAGGTGATCACCTGAAGTCCAAGCTGG TGTCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAA CACTACCAACACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACC GCCCTGATCAAGAAAGTACCCCAAGCTGGAGTCCGAGTTCGTGTACGGCG ACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGTCCGAGCAGGA GATCGGCAAGGCCACCGCCAAGTACTTCTTACTCCAACATCATGAAGT TCTTCAAGACCGAGATCACCTGGCCAACGGCGAGATCCGGAAGCGGCC CCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGG CCGGGACTTCGCCACCGTGCAGGAAGGTGCTGTCCATGCCCCAGGTGAAC ATCGTGAAGAAGACCGAGGTGCAGACCGGCGGCTTCTCCAAGGAGTCCA TCCTGCCAAGCGGAAGTCCGACAAGCTGATCGCCCGGAAGGAGTGG GGACCCCAAGAAGTACGGCGGCTTCGACTCCCCACCGTGGCCTACTCC GTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGTCCAAGAAGCTGAAG TCCGTGAAGGAGTGTGGGCATCACCATCATGGAGCGGTCTCTCTTCG AGAAGAACCCATCGACTTCTGAGGCAAGGCTCAAGGAGGTGA AGAAGGACCTGATCATCAAGCTGCCAAGTACTCCCTGTTGAGCTGGA GAACGGCCGGAAGCGGATGCTGGCCTCCGCCGCGAGCTGCAGAAGGG CAACGAGCTGGCCCTGCCCTCCAAGTACGTGAAGTCTCTGTACCTGGCCT CCCACTACGAGAAGCTGAAGGGCTCCCCGAGGACAACGAGCAGAAGC AGCTGTCTGTGGAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCA GATCTCCGAGTTCTCCAAGCGGGTGATCCTGGCCGACGCCAACCTGGAC AAGGTGCTGTCCGCCTACAACAAGCACCGGACAAGCCATCCGGGAGC AGGCCGAGAACATCATCCACCTGTTACCCCTGACCAACCTGGGCGCCCC CGCCGCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACC TCCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCACTCATCCCG GCCTGTACGAGACCCGGATCGACCTGTCCAGCTGGGCGGCGACGGCGG CGGCTCCCCCAAGAAGAAGCGGAAGGTGTGA
Open reading frame for Cas9 with Hibt tag	803	AUGGACAAGAAGUACUCCAUCGGCCUGGACAUCGGCACCAACUCCGUG GGCUGGGCCGUGAUCACCGACGAGUACAAGGUGCCCUCCAAGAAGUUC AAGGUGCUUGGCAACACCGACCGGCACUCCAUAAGAAGAACCUGAUC GGCGCCUGCUGUUCGACUCCGGCGAGACCGCCGAGGCCACCCGGCUG AAGCGGACCGCCCGGCGGGCGGUACACCGGGCGGAAGAACCUGAUCUGC UACCUAGCAGGAGAUUCUCCAACGAGAUUGGCCAAGGUGGACGACUCC UUCUCCACCGGCUUGGAGGAGUCCUCCUGGUGGAGGAGGACAAGAA GCACGAGCGGCACCCCAUCUUCGGCAACAUUGGAGCAGAGGUGGCCUA CCACGAGAAGUACCCCAACUACUACCACUGCGGAAGAAGCUGGUGGA CUCCACCGACAAGGCCGACCUUGCGGUGAUCUACCUGGCCUUGGCCCA CAUGAUCAAGUUCGGGGGCCAUUCCUGAUCGAGGGCGACCUGAACCC CGACAACUCCGACGUGGACAAGCUGUUAUCCAGCUGGUGCAGACCUA CAACCAGCUGUUCGAGGAGAAACCCCAUAACGCCUCCGGCGUGGACGC CAAGGCCAUCCUGUCCGCCCGGCUGUCCAAGUCCCGGCGGCGUGGAGAA CCUGAUCGCCCAGCUGCCCAGGCGAGAAGAAGAACGGCCUGUUCGGCAA CCUGAUCGCCCUGUCCUGGGCCUGACCCCAACUUAAGUCCAACUUCU CGACCUGGCCGAGGACGCCAAGCUGCAGCUGUCCAAGGACACCUACGA CGACGACCUUGACAACCUUGCUGGCCAGAUCCGGCAGCAGUACGCCGA CCUGUUCUGGCCGCAAGAACCUGUCCGACGCCAUCCUGCUGUCCGA CAUCCUGCGGGUGAACACCGAGAUACCAAGGCCCCCUUGCCGCCUC CAUGAUCAAGCGGUACGACGAGCACCACAGGACCUAGCCUGCUGAA GGCCUGGUGCGGCAGCAGCUGCCCAGAGAAGUACAAGGAGAUUCUUCU CGACCAGUCCAAGAACGGCUACGCCGGCUACAUCGACGGCGGCGGCCUC CCAGGAGGAGUUCUACAAGUUAUCAAGCCCAUCCUGGAGAAGAUGG ACGGCACCGAGGAGCUGCUGGUGAAGCUGAACCGGGAGGACCUUGCUGC GGAAGCAGCGGACCUUCGACAACGGCUCCAUCCCCACCAGAUCCACC UGGGCGAGCUGCACGCCAUCCUGCGGCGGCAGGAGGACUUCUACCCCU UCCUGAAGGACAACCGGGAGAAGAUUGAGAAGAUCUGACCUUCCGGA UCCCCUACUACGUGGGCCCCUGGCCCGGGGCAACUCCGGUUCGCCU GGAUGACCCGGAAGUCCGAGGAGACCAUACCCCUUGAACUUCGAGG AGGUGGUGGACAAGGGCGCCUCCGCCAGUCCUUAUCGAGCGGAUGA

Description	SEQ ID NO	Sequence
		CCAACUUCGACAAGAACCUGCCCAACGAGAAGGUGCUGCCCAAGCACU CCCUGCUGUACGAGUACUUCACCGUGUACAACGAGCUGACCAAGGUGA AGUACGUGACCGAGGGCAUGCGGAAGCCCGCCUUCUGUCCGGCGAGC AGAAGAAGGCCAUCGUGGACCUGCUGUUAAGACCAACCGGAAGGUG ACCGUGAAGCAGCUGAAGGAGGACUACUUCAGAAGAUUCGAGUGCUU CGACUCCGUGGAGAUUCUCCGGCGUGGAGGACCGGUUCAACGCCUCCU GGGCACCUAACCAGACCUGCUGAAGAUCAUAAGGACAAGGACUCCU GGACAACGAGGAGAACGAGGACAUCCUGGAGGACAUCGUGCUGACCCU GACCCUGUUCGAGGACCGGGAGAUCAUCGAGGAGCGGCUGAAGACCU ACGCCACCUGUUCGACGACAAGGUGAUGAAGCAGCUGAAGCGGGC GGUACACCGGCGUGGGGCCGGCUGUCCCGGAAGCUGAUAACGGCAUCC GGGACAAGCAGUCCGGCAAGACCAUCCUGGACUUCUGAAGUCCGACG GCUUCGCCAACCGGAACUUCAGCAGCUGAUCCACGACGACUCCUGA CCUUAAGGAGGACAUCAGAAAGGCCAGGUGUCCGGCCAGGGCGACU CCCUGCACGAGCACAUCCGCAACCUGGCCGGCUCCCGCCAUCAAGA AGGGCAUCCUGCAGACCGUGAAGGUGGUGGACGAGCUGGUGAAGGUG AUGGGCCGGCACAAGCCCGAGAACAUCGUGAUCGAGAUGGCCCGGGAG AACCAGACCAACCCAGAAGGGCCAGAAGAACUCCCGGGAGCGGAUGAAG CGGAUCGAGGAGGGCAUCAAGGAGCUGGGCUCCAGAUCCUGAAGGA GCACCCCGUGGAGAAACACCCAGCUGCAGAACGAGAAGCUGUACCGUA CUACCUGCAGAACGGCCGGGACAUGUACGUGGACCAGGAGCUGGACAU CAACCGGCUUGCCGACUACGACGUGGACCACAUCGUGCCCCAGUCCU CCUGAAGGACGACUCCAUCGACAACAAGGUGCUGACCCGGUCCGACAA GAACCGGGCAAGUCCGACAACGUGCCCUCCGAGGAGGUGGUGAAGAA GAUGAAGAACUACUGGCGGCAGCUGCUGAAGCACAAGCUGAUCACCCA GCGGAAGUUCGACAACCUGACCAAGGCCGAGCGGGCGGCCUGUCCGA GCUGGACAAGGCCGGCUUCAUCAAGCGGCAGCUGGUGGAGACCCGGCA GAUACCAAGCACGUGGCCCAGAUCCUGGACUCCCGGAUGAACACCAA GUACGACGAGAACGACAAGCUGAUCGGGAGGUGAAGGUGAUCACCC UGAAGUCCAAGCUGGUGUCCGACUUCGGAAGGACUCCAGUUCUACA AGGUGCGGGAGAUCAACAACUACCACCACGCCACGACGCCUACCUGA ACGCCGUGGUGGGACCCGCCUGAUAAGAAGUACCCCAAGCUGGAGU CCGAGUUCGUGUACGGCGACUACAAGGUGUACGACGUGCGGAAGAUG AUCGCCAAGUCCGAGCAGGAGAUCCGCAAGGCCACCGCAAGUACUUC UUCUACUCCAACAUCAUGAACUUCUUAAGACCGAGAUACCCUUGGCC AACGGCGAGAUCCGGAAGCGGCCCUUGAUCGAGACCAACGGCGAGACC GGCGAGAUUCGUGUGGACAAAGGCCGGGACUUCGCCACCGUGCGGAAG GUGCUGUCCAUGCCCCAGGUGAACAUUCGUGAAGAAGACCGAGGUGCAG ACCGGCGGCUUCUCCAAGGAGUCCAUCUGCCCAAGCGGAACUCCGAC AAGCUGAUCGCCCCGAAGAAGGACUGGGACCCCAAGAAGUACGGCGGC UUCGACUCCCCACCGUGGCCUACUCCGUGCUGGUGGUGGCCAAGGUG GAGAAGGGCAAGUCCAAGAAGCUGAAGUCCGUGAAGGAGCUGCUGGG CAUCACCAUCAUGGAGCGGUCCUCCUUCGAGAAGAACCCCAUCGACU CCUGGAGGCCAAGGGCUACAAGGAGGUGAAGAAGGACCUGAUCAUA AGCUGCCCAAGUACUCCUGUUCGAGCUGGAGAACGGCCGGAAGCGGA UGUGGCCUCCGCCGGCGAGCUGCAGAAGGGCAACGAGCUGGCCUGC CCUCCAAGUACGUGAACUUCUGUACCUUGGCCUCCACUACGAGAAGC UGAAGGGCUCCCCGAGGACAACGAGCAGAAGCAGCUGUUCGUGGAGC AGCACAAGCACUACCUGGACGAGAUCAUCGAGCAGAUCCCGAGUUCU CCAAGCGGGUGAUCCUGGCCGACGCCAACCUGGACAAGGUGCUGUCCG CCUACAACAAGCACCGGGACAAGCCAUCCGGGAGCAGGCCGAGAACA UCAUCCACCUGUUCACCCUGACCAACCUGGGCGCCCCCGCCGUUCA AGUACUUCGACACCACCAUCGACCGGAAGCGGUACACCUCCACCAAGG AGGUGCUGGACGCCACCCUGAUCCACAGUCCAUCACCGGCCUGUACG AGACCCGGAUCGACCUGUCCAGCUGGGCGGGCAGCGCGGGCGGCCUCC CCAAGAAGAAGCGGAAGGUGUCCGAGUCCGCCACCCCGAGUCCGUGU CCGGCUUGGCGGCGUGUACAAGAAGAUCCUGA

Description	SEQ ID NO	Sequence
Open Reading frame for BC22n	804	AUGGAGGCCUCCCCGCCUCCGGCCCCGGCACCUGAUGGACCCCCACA UCUUCACCUCCAACUUAACAACGGCAUCGGCCGGCACAAGACCUACC UGUGCUACGAGGUGGAGCGGCUGGACAACGGCACCUCGUGAAGAUG GACCAGCACCGGGGCUUCCUGCACAACCAGGCCAAGAACCUGGUGGC GGCUUCUACGGCCGGCACGCCGAGCUGCGGUUCCUGGACCUGGUGCCC UCCUGCAGCUGGACCCCGCCAGAUUACCGGGUGACCUGGUUAUC UCCUGGUCCCCUGCUUUCUCCUGGGGUGCGCCGGCGAGGUGCGGGCC UUCUGCAGGAGAACACCCACGUGCGGCUGCGGAUCUUCGCCGCCCGG AUCUACGACUACGACCCCCUGUACAAGGAGGCCUGCAGAUGCUGCGG GACGCCGGCGCCAGGUGUCCAUC AUGACCUACGACGAGUUAAGCAC UGCUGGGACACCUUCGUGGACCACAGGGCUGCCCCUCCAGCCUGG GACGGCCUGGACGAGCACUCCAGGCCUGUCCGGCCGGCUGCGGGCC AUCCUGCAGAACCCAGGGCAACUCCGGCUCGAGACCCCGGCACCUCC GAGUCCGCCACCCCGAGUCCGACAAGAAGUACUCCAUCGGCCUGGCC AUCGGCACCAACUCCGUGGGCUGGGCCGUGAUCACCGACGAGUACAAG GUGCCCUCCAAGAAGUUAAGGUGCUGGGCAACACCGACCGGCACUCC AUAAGAAGAACCUGAUCGGCGCCUGCUGUUCGACUCCGGCGAGACC GCCGAGGCCACCCGGCUGAAGCGGACCGCCCGGGCGCGGUACACCCGG CGGAAGAACCGGAUCUGCUACCUAGGAGAUUUUCCAACGAGAUG GCCAAGGUGGACGACUCCUUCUCCACCGGCUGGAGGAGUCCUCCUG GUGGAGGAGGACAAGAAGCACGAGCGGCACCCCAUCUUCGGCAACAUC GUGGACGAGGUGGCCUACCACGAGAAGUACCCACCAUCUACCACUG CGGAAGAAGCUGGUGGACUCCACCGACAAGGCCGACCUGCGGCUGAUC UACCUGGCCUGGGCCACAUGAUCAAGUUCGGGGCCACUCCUGAUC GAGGGCGACCUGAACCCCGACAACUCCGACGUGGACAAGCUGUAUC CAGCUGGUGCAGACCUACAACCAGCUGUUCGAGGAGAACCCCAUAAC GCCUCCGGCGUGGACGCCAAGGCCAUCCUGUCCGCCCGGCUGUCCAAG UCCCGCGCGCUGGAGAACCUGAUCGCCACGUGCCCGCGAGAAGAAG AACGGCCUGUUCGGCAACCUGAUCGCCCGUCCUGGGCCUGACCCCC AACUUAAGUCCAACUUCGACCUUGGCCGAGGACGCCAAGCUGCAGCUG UCCAAGGACACCUACGACGACGACCUGGACAACCUGCUGGGCCAGAU GGCGACCAGUACGCCGACCUGUCCUGGCCGCCAAGAACCUGUCCGAC GCCAUCCUGCUGUCCGACAUCUCCUGCGGGUGAACACCGAGAUACCAAG GCCCCCUGUCCGCCUCCAUGAUCAAGCGGUACGACGAGCACCACAG GACCUGACCCUGCUGAAGGCCUGGUGCGGCAGCAGCUGCCCGAGAAG UACAAGGAGAUUUUUCGACCAAGUCCAAGAAGGCUACGCCGGCUAC AUCGACGGCGCGCCUCCAGGAGGAGUUCUACAAGUUAUCAAGCCC AUCCUGGAGAAGAUGGACGGCACCGAGGAGCUGCUGGUGAAGCUGAA CCGGGAGGACCUGCUGCGGAAGCAGCGGACCUUCGACAACCGGCCAU CCCCACCAAGAUCCACCUGGGCGAGCUGCAGCCAUCCUGCGCGGCA GGAGGACUUCUACCCUUCUGAAGGACAACCGGGAGAAGAUAGAGAA GAUCCUGACCUUCCGGAUCCCUACUACGUGGGCCCCUGGCCCGGGG CAACUCCCGGUUCGCCUGGAUGACCCGGAAGUCCGAGGAGACCAUCAC CCCUUGGAACUUCGAGGAGGUGGUGGACAAGGGCGCCUCCGCCAGUC CUUCAUCGAGCGGAUGACCAACUUCGACAAGAACCUGCCCAACGAGAA GGUGCUGCCCAAGCACUCCUGCUGUACGAGUACUUCACCGUGUACAA CGAGCUGACCAAGGUGAAGUACGUGACCGAGGGCAUGCGGAAGCCCGC CUUCCUGUCCGGCGAGCAGAAGAAGGCCAUCGUGGACCUGCUGUUA GACCAACCGGAAGGUGACCGUGAAGCAGCUGAAGGAGGACUACUUA AGAAGAUCCGAGUGCUUCGACUCCGUGGAGAUUCCGGCGUGGAGGACC GGUUAACGCCUCCUGGGCACCACCACGACCUGUGAAGAUCAUCA AGGACAAGGACUCCUGGACAACGAGGAGAACGAGGACAUCUGGAG GACUUCGUGCUGACCCUGACCCUGUUCGAGGACCGGGAGAUCAUCGAG GAGCGGCUAAGACCUACGCCACCUGUUCGACGACAAGGUGAUGAAG CAGCUGAAGCGGCGCGGUACACCGGCUGGGGCCGGCUGUCCCGGAAG CUGAUCAACGGCAUCCGGGACAAGCAGUCCGGAAGACCAUCCUGGAC UUCUGAAGUCCGACGGCUUCGCCAACCGGAACUUAUGCAGCUGAUC CACGACGACUCCUGACCUUAAGGAGGACAUCAGAAAGGCCAGGUG

Description	SEQ ID NO	Sequence
		UCCGGCCAGGGCGACUCCUGCAGAGCACAU CGCCAACUGGCCGGC UCCCCCGCAUCAAGAAGGGCAUCCUGCAGACCGUGAAGGUGGUGGAC GAGCUGGUGAAGGUGAUGGGCCGGCACAAGCCCGAGAACAUCGUGAU CGAGAUGGCCCGGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAACUC CCGGGAGCGGAUGAAGCGGAUCGAGGAGGGCAUCAAGGAGCUGGGCU CCCAGAUCCUGAAGGAGCACCCCGUGGAGAACACCCAGCUGCAGAACG AGAAGCUGUACCUGUACUACCUGCAGAACGGCCGGGACAUGUACGUGG ACCAGGAGCUGGACAUAACCCGGCUGUCCGACUACGACGUGGACCACA UCGUGCCCCAGUCCUCCUGAAGGACGACUCCAUCGACAACAAGGUGC UGACCCGGUCCGACAAGAACCGGGGCAAGUCCGACAACGUGCCCUCCG AGGAGGUGGUGAAGAAGAUAGAAGACUACUGGCCGGCAGCUGUGAAC GCCAAGCUGAUCACCCAGCGGAAGUUCGACAACCUGACCAAGGCCGAG CGGGGCGGCCUGUCCGAGCUGGACAAGGCCGGCUUCAUAAGCGGCAG CUGGUGGAGACCCGGCAGAUACCAAGCAGUGGGCCAGAUCCUGGAC UCCCGAUGAACAACCAAGUACGACGAGAACGACAAGGAGUCCGGGAG GUGAAGGUGAUCACCCUGAAGUCCAAGCUGGUGUCCGACUUCGGGAAG GACUUCAGUUCUACAAGGUGCGGGAGAUACAACAACUACCACCACGCC CACGACGCCUACUGAACGCCGUGGUGGGCACCGCCUGAUCAGAAG UACCCCAAGCUGGAGUCCGAGUUCGUGUACGGCGACUACAAGGUGUAC GACGUGCGGAAGAUCAUCGCAAGUCCGAGCAGGAGAUCCGCAAGGCC ACCGCAAGUACUUCUUCUACUCCAACAUCAUAGAACUUCUUAAGACC GAGAUACCCUGGCCAACGGCGAGAUCCGGAAGCGGCCCCUGAUCGAG ACCAACGGCGAGACCGGGCGAGAUCCGUGUGGGACAAGGGCCGGGACUUC GCCACCGUGCGGAAGGUGCUGUCCAUGCCCCAGGUGAACAUCCUGAAG AAGACCGAGGUGCAGACCGCGCGCUUCUCCAAGGAGUCCAUCUGGCC AAGCGGAACUCCGACAAGCUGAUCGCCCCGAAGAAGGACUGGGACCCC AAGAAGUACGGCGGCUUCGACUCCCCACCGUGGCCUACUCCGUGCUG GUGGUGGCCAAGGUGGAGAAGGGCAAGUCCAAGAAGCUGAAGUCCGU GAAGGAGCUGCUGGGCAUACCAUCAUGGAGCGGUCCUCCUUCGAGAA GAACCCCAUCGACUUCUGGAGGCCAAGGGCUACAAGGAGGUGAAGAA GGACCUGAUCAUCAAGCUGCCCAAGUACUCCUGUUCGAGCUGGAGAA CGGCCGAAGCGGAUGCUGGCCUCCGCCGGCGAGCUGCAGAAGGGCAA CGAGCUGGCCUGCCCUCCAAGUACGUGAACUUCUGUACCUUGGCCUC CCACUACGAGAAGCUGAAGGGCUCCCCGAGGACAACGAGCAGAAGCA GCUGUUCGUGGAGCAGCACAAAGCACUACCUAGGACGAGAUCAUCGAGCA GAUCUCCGAGUUCUCCAAGCGGGUGAUCCUGGCCGACGCCAACCUUGGA CAAGGUGCUGUCCGCCUACAACAAGCACCGGGACAAGCCCAUCCGGGA GCAGGCCGAGAACAUAUCCACCUGUUCACCCUGACCAACCUGGGCGC CCCCGCCGCCUACAAGUACUUCGACACCACCAUCGACCGGAAGCGGUA CACCUCACCAAGGAGGUGCUGGACGCCACCCUGAUCCACCAGUCCA CACCGGCCUGUACGAGACCCGGAUCGACCUGUCCAGCUGGGCGGCGA CGGCGCGGCUCCCCAAGAAGAAGCGGAAGGUGUGA
Open reading frame for BC22n with Hibit tag	805	AUGGAGGCCUCCCCGCCUCCGGCCCCCGGCACCUGAUGGACCCCCACA UCUUCACCUCCAACUUAACAACGGCAUCGGCCGGCACAAGACCUACC UGUGCUACGAGGUGGAGCGGCUGGACAACGGCACCUCCGUGAAGAUG GACCAGCACCGGGGUUCUCCUGCACAACCAGGCCAAGAACCUGCUGUGC GGCUUCUACGGCCGGCACGCCGAGCUGCGGUUCCUGGACCUGGUGCCC UCCUGCAGCUGGACCCCGCCAGAUUACCGGGUGACCUGGUUCAUC UCCUGUCCCCCUGCUUCUCCUGGGGUGCGCGCGGCGAGGUGCGGGCC UUCUGCAGGAGAACACCCACGUGCGGCGUGCGGAUCUUCGCCGCCCGG AUCUACGACUACGACCCCCUGUACAAGGAGGCCUGCAGAUUGCUGCGG GACGCCGGCGCCAGGUGUCCAUAUGACCUACGACGAGUUAAGCAC UGCUGGGACACCUUCGUGGACCAAGGGCUGCCCCUCCAGCCCUUGG GACGGCCUGGACGAGCACUCCAGGCCUGUCCGGCCGGCUGCGGGCC AUCCUGCAGAACCAGGGCAACUCCGGCUCGAGACCCCGGCACCUCC GAGUCCGCCACCCCGAGUCCGACAAGAAGUACUCCAUCGGCCUGGCC AUCGGCACCAACUCCGUGGGCUGGGCCGUGAUCACCGACGAGUACAAG GUGCCCUCCAAGAAGUUAAGGUGCUGGGCAACACCGACCGGCACUCC

Description	SEQ ID NO	Sequence
		AUCAAGAAGAACCUGAUCGCGCGCCUGCUGUUCGACUCCGGCGAGACC GCCGAGGCCACCCGGCUGAAGCGGACCGCCCGCGCGGCUACACCCGG CGGAAGAACCUGAUCUGCUACCUGCAGGAGAUUUUCCAACGAGAUG GCCAAGGUGGACGACUCCUUCUCCACCGGCUUGGAGGAGUCCUCCUG GUGGAGGAGGACAAGAAGCACGAGCGGCACCCCAUCUUCGGCAACAUC GUGGACGAGGUGGCCUACCACGAGAAGUACCCCAACUUAACCACCU CGGAAGAAGCUGGUGGACUCCACCGACAAGGCCGACCUGCGGCUGAUC UACCGGCCUUGGCCACAUGAUAAGUUCGGGGGCCACUUCUGAUC GAGGGCGACCUGAACCCCGACAACUCCGACGUGGACAAGCUGUUAUC CAGCUGGUGCAGACCUACAACCAGCUGUUCGAGGAGAACCCTCAAC GCCUCCGGCGUGGACGCAAGGCCAUCCUGUCCGGCCGUGUCCAAG UCCCGCGCGCUGGAGAACCUGAUCGCCCAGCUGCCCGCGGAGAAGAAG AACGGCCUGUUCGGCAACCUGAUCGCCCUGUCCUGGGCCUGACCCCC AACUUAAGUCCAACUUCGACCUGGCCGAGGACGCCAAGCUGCAGCUG UCCAAGGACACCUACGACGACGACCUGGACAACCUGGCGCCGUAUC GGCGACCAGUACGCCGACCUGUUCUGGCCGCCAAGAACCUGUCCGAC GCCAUCCUGCUGUCCGACAUCUGCGGGUGAACACCGAGAUACCAAG GCCCCCUUGUCCGCCUCCAUGAUAAGCGGUACGACGAGCACCACAG GACCUGACCCUGCUGAAGGCCCGUGGUGCGGCAGCAGCUGCCCGAGAAG UACAAGGAGAUUUUUCGACCAGUCCAAGAACGGCUACGCCGGCUAC AUCGACGGCGCGCCUCCAGGAGGAGUUCUACAAGUUAUCAAGCCC AUCCUGGAGAAGAUGGACGGCACCGAGGAGCUGCUGGUGAAGCUGAA CCGGGAGGACCUUGCUGCGGAAGCAGCGGACCUUCGACAACGGCUCCAU CCCCACCAGAUCCACCUUGGGCGAGCUGCACGCCAUCCUGCGCGCGGA GGAGGACUUCUACCCCUUCUGAAGGACAACCGGGAGAAGAUCCGAGAA GAUCCUGACCUUCCGGAUCCCUACUACGUGGGCCCCUGGCCCGGGG CAACUCCGGUUCGCCUGGAUGACCCGGAAGUCCGAGGAGACCAUCAC CCCCUGGAACUUCGAGGAGGUGGUGGACAAGGGCGCCUCCGCCAGUC CUUCAUCGAGCGGAUGACCAACUUCGACAAGAACCUGCCCAACGAGAA GGUGCUGCCCAAGCACUCCUGCUGUACGAGUACUUCACCGUGUACAA CGAGCUGACCAAGGUGAAGUACGUGACCGAGGGCAUGCGGAAGCCCGC CUUCCUGUCCGGCGAGCAGAAGAAGGCCAUCCUGGACCUGCUGUUA GACCAACCGGAAGGUGACCGUGAAGCAGCUGAAGGAGGACUACUUA AGAAGAUCCGAGUGCUUCGACUCCGUGGAGAUUCGGCGUGGAGGACC GGUUAACGCCUCCUGGGCACCUACCACGACCUGCUGAAGAUCAUCA AGGACAAGGACUUCUGGACAACGAGGAGAACGAGGACAUCUGGAG GACAUCCUGCUGACCCUGACCCUGUUCGAGGACCGGGAGAUGAUCGAG GAGCGGCUGAAGACCUACGCCCACCUUGUUCGACGACAAGGUGAUGAAG CAGCUGAAGCGGCGCGGUAACCGGCUGGGGCGGGCUGUCCCGGAAG CUGAUCAACGGCAUCCGGGACAAGCAGUCCGGCAAGACCAUCCUGGAC UUCUGAAGUCCGACGGCUUCGCCAACCGGAACUUAUGCAGCUGAUC CACGACGACUCCUGACCUUAAGGAGGACAUCAGAAAGGCCAGGUG UCCGGCCAGGGCGACUCCUGCAGGACACAUCCGCAACCUGGCCGGC UCCCCCGCAUCAAGAAGGGCAUCCUGCAGACCGUGAAGGUGGUGAC GAGCUGGUGAAGGUGAUGGGCCGGCACAAGCCCCGAGAACAUCGUGAU CGAGAUGGCCCGGGAGAACCAGACCCAGAGGGCCAGAAGAACUC CCGGGAGCGGAUGAAGCGGAUCGAGGAGGGCAUCAAGGAGCUGGGCU CCCAGAUCCUGAAGGAGCACCCCGUGGAGAACACCCAGCUGCAGAACG AGAAGCUGUACCUGUACUACCUGCAGAACGGCCGGGACAUGUACGUG ACCAGGAGCUGGACAUAACCGGCUGUCCGACUACGACGUGGACCACA UCGUGCCCCAGUCCUUCUGAAGGACGACUCCAUCGACAACAAGGUGC UGACCCGGUCCGACAAGAACCGGGGCAAGUCCGACAACGUGCCUCCG AGGAGGUGGUGAAGAAGAUGAAGAACUACUGGGCGGAGCUGCUGAAC GCCAAGCUGAUACCCAGCGGAAGUUCGACAACCUGACCAAGGCCGAG CGGGGCGGCCUGUCCGAGCUGGACAAGGCCGGCUUCAUAAGCGGCAG CUGGUGGAGACCCGGCAGAUACCAAGCAGUGGCCAGAUCCUGGAC UCCCGGAUGAACCAAGUACGACGAGAACGACAAGCUGAUCGGGGAG GUGAAGGUGAUCACCCUGAAGUCCAAGCUGGUGUCCGACUUCGGGAAG

Description	SEQ ID NO	Sequence
		GACUUCAGUUCUACAAGGUGCGGGAGAUCAACAACUACCACCACGCC CACGACGCCUACCUGAACGCCGUGGUGGGCACCGCCUGAUCAAGAAG UACCCAAGCUGGAGUCCGAGUUCGUGUACGGCGACUACAAGGUGUAC GACGUGCGGAAGAUGAUCGCCAAGUCCGAGCAGGAGAUCCGCAAGGCC ACCGCAAGUACUUCUUCUACUCCAACAUCAUGAACUUCUUAAGACC GAGAUACCCUGGCCAACGGCGAGAUCCGGAAGCGGCCCCUGAUCGAG ACCAACGGCGAGACCGGCGAGAUCCGUGGGGACAAGGGCCGGGACUUC GCCACCGUGCGGAAGGUGCUGUCCAUGCCCCAGGUGAACAUCCUGAAG AAGACCGAGGUGCAGACCGGCGGCUUCUCCAAGGAGUCCAUCUGCCC AAGCGGAACUCCGACAAGCUGAUCGCCCCGGAAGAAGGACUGGGACCCC AAGAAGUACGGCGGCUUCGACUCCCCACCGUGGCCUACUCCGUGCUG GUGGUGGCCAAGGUGGAGAAGGGCAAGUCCAAGAAGCUGAAGUCCGU GAAGGAGCUGCUGGGCAUACCAUCAUGGAGCGGUCCUCCUUCGAGAA GAACCCAUCCGACUUCUGGAGGCCAAGGGCUACAAGGAGGUGAAGAA GGACCUGAUCAUCAAGCUGCCCAAGUACUCCUGUUCGAGCUGGAGAA CGGCCGGAAGCGGAUGCUGGCCUCCGCCGCGAGCUGCAGAAGGGCAA CGAGCUGGCCUGCCCCUCCAAGUACGUGAACUUCUGUACCUGGCCUC CCACUACGAGAAGCUGAAGGGCUCCCCGAGGACAACGAGCAGAAGCA GCUGUUCGUGGAGCAGCACAAGCACUACCUGGACGAGAUCAUCGAGCA GAUCUCCGAGUUCUCCAAGCGGGUGAUCCUGGCCGACGCCAACCUGGA CAAGGUGCUGUCCGCCUACAACAAGCACCGGGACAAGCCAUCCGGGA GCAGGCCGAGAACAUCAUCCACCUGUUCACCCUGACCAACCUGGGCGC CCCCGCCGCCUUAAGUACUUCGACACCACCAUCGACCGGAAGCGGUA CACCUCACCAAGGAGGUGCUGGACGCCACCCUGAUCCACCAGUCCAU CACCGCCUGUACGAGACCCGGAUCGACCUGUCCAGCUGGGCGGCGA CGGCGGCGGCUCCCCAAGAAGAAGCGGAAGGUGUCCGAGUCCGCCAC CCCGAGUCCGUGUCCGGCUGGGCGGCUUUAAGAAGAUCUCCUGA
Open reading frame for BC22	806	AUGGAAGCAAGCCCGGCAAGCGGACCGAGACACCUGAUGGACCCGCAC AUCUUCACAAGCAACUUCACAACCGAAUCGGAAGACACAAGACAUAC CUGUGCUACGAAAGUCGAAAGACUGGACAACGGAACAAGCGUCAAGAU GGACCAGCACAGAGGAUUCUGCACAACCAGGCAAAGAACCUGCUGUG CGGAUUCUACGGAAGACACGCAGAACUGAGAUUCCUGGACCUGGUCCC GAGCCUGCAGCUGGACCCGGCACAGAUUCACAGAGUCACAUGGUUCAU CAGCUGGAGCCCGUGCUUCAGCUGGGGAUGCGCAGGAGAAGUCAGAGC AUUUCUGCAGGAAAACACACACGUCAGACUGAGAAUUCUCCGACGAAG AAUCUACGACUACGACCCGCUGUACAAGGAAGCACUGCAGAUCCUGAG AGACGCAGGAGCACAGGUCAGCAUCAUGACAUACGACGAUUAAGCA CUGCUGGGGACAUUCGUCGACACCAGGGGAUGCCCGUCCAGCCGUG GGACGGACUGGACGAACACAGCCAGGCACUGAGCGGAAGACUGAGAGC AAUCCUGCAGAAACAGGGAACAGCGGAAGCGAAAACCCGGGAACAAG CGAAAGCGCAACACCGGAAGCGACAAGAAGUACAGCAUCGGACUGGC CAUCGGAACAAACAGCGUCGGAUGGGCAGUCAUCACAGACGAUACAA GGUCCCGAGCAAGAAGUUAAGGUCCUGGGAAACACAGACAGACACAG CAUCAAGAAGAACCUGAUCCGAGCACUGCUGUUCGACAGCGGAGAAAC AGCAGAAGCAACAAGACUGAAGAGAAACAGCAAGAAGAAGAUACACAA GAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUCAGCAACGAA AUGGCAAAGGUCGACGACAGCUUCUCCACAGACUGGAAGAAAGCUUC CUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUUCUGGAAAC AUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCAC CUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACU GAUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUCCU GAUCGAAGGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUU CAUCCAGCUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAU CAACGCAAGCGGAGUCGACGCAAAAGGCAAUCCUGAGCGCAAGACUGAG CAAGAGCAGAAGACUGGAAAACCUGAUCGCACAGCUGCCGGGAGAAA AGAAGAACGGACUGUUCGGAACCUGAUCGCACUGAGCCUGGGACUG ACACCGAACUUAAGAGCAACUUCGACCUGGCAGAAGACGCAAGCUG CAGCUGAGCAAGGACACAUCGACGACGACCUGGACAACCUGCUGGCA

Description	SEQ ID NO	Sequence
		CAGAU CGGAG ACCAGUACGCAG ACCUGUUC CUGGCAGCAAAGA ACCUG AGCGACGCAAUCCUGCUGAGCGCAUCCUGAGAGUCAACACAGAAAUC ACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAGAGAUACGACGAACAC CACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAGCAGCUGCCG GAAAAGUACAAGGAAUUCUUCUGACCAGAGCAAGAACGGAUACGC AGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAGUUC UCAAGCCGAUCCUGGAAAAGAUUGGACGGAACAGAAGAACUGCUGGUC AAGCUGAACAGAGAAGACCUGCUGAGAAAAGCAGAGAACAUUCGACAA CGGAAGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCU GAGAAGACAGGAAGACUUCUACCCGUUCUGAAGGACAACAGAGAAA AGAU CGAAAAGAUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGC UGGCAAGAGGAAACAGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAA GAAACAAUCACCCGUGGAACUUCGAAAGAUUCGUCGACAAGGGAGC AAGCGCACAGAGCUUCAUCGAAAAGAUACAACUUCGACAAGAACC GCCGAACGAAAAGGUCCUGGCCGAAGCACAGCCUGCUGUACGAUACU CACAGUCUACAACGAACUGACAAAAGGUCAAGUACGUCACAGAAGGAA UGAGAAAGCCGGCAUUCUGAGCGGAGAACAGAAGAAGGCAUUCGUC GACCUGCUGUUCAGACAAAACAGAAAGGUACAGUCAAGCAGCUGAA GGAAGACUACUUCAGAAAGAU CGAAUGCUUCGACAGCGUCGAAAUA GCGGAGUCGAAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGAC UGCUGAAGAUCAUCAAGGACAAGGACUUCUGGACAACGAAGAAAAC GAAGACAUCCUGGAAGACAU CGUCCUGACACUGACACUGUUCGAAGAC AGAGAAU GAUUCGAAAGAAAGACUGAAGACAUACGCACACCUGUUCGA CGACAAGGUCAUGAAGCAGCUGAAGAGAAAGAUACACAGGAU GGG GAAGACUGAGCAGAAAAGCUGAUCAACGGAUACAGAGACAAGCAGAGC GGAAAGACA AUCCUGGACUUCUGAAGAGCGACGGAUUCGCAAACAG AAACUUC AUGCAGCUGAUCCACGACGACAGCCUGACAUUCAAGGAAGA CAUCCAGAAAGGCACAGGUCAGCGGACAGGGAGACAGCCUGCACGAACA CAUCGCAAACCUGGCAGGAAGCCCGGCAUUCAGAAAGGGAUUCUGCA GACAGUCAAGGUUCGUGACGGAACUGGUCAAGGUCAUGGGAAAGACACA AGCCGGAAAACAUCGUCAUCGAAAUGGCAAGAGAAAACACAGACAACAC AGAAGGGACAGAAGAACAGCAGAGAAAAGAAUGAAGAGAAUCGAAGAA GGAUUC AAGGAACUGGGAAAGCCAGAUCCUGAAGGAACACCCGGUCGA AAACACACAGCUGCAGAACGAAAAGCUGUACCUUGUACUACCUGCAGAA CGGAAGAGACAU GUACGUGACCGAAGACUGGACAUUAACAGACUGA GCGACUACGACGUCGACCACAU CGUCCCGCAGAGCUUCUGAAGGACG ACAGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGA AAGAGCGACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAU GAAGAA CUACUGGAGACAGCUGCUGAACGCAAGCUGAUACACACAGAGAAAGU UCGACAACCUGACA AAGGCAGAGAGAGAGGAGGACUGAGCGAACUGGAC AAGGCAGGAUUCAUCAAGAGACAGCUGGUUGAAAACAAGACAGAUAC AAAGCAGUCGCA CAGAUCCUGGACAGCAGAAUGAACACAAAGUACGA CGAAAACGACAAGCUGAU CAGAGAAGUCAAGGUCAUCACACUGAAGA GCAAGCUGGUCAGCGACUUCAGAAAAGGACUUCAGUUCUACAAGGUCA GAGAAU CAACAACUACCACCACGCACACGACGCAUACCUGAACGCGAG UCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUGGAAAGCGAA UUCGUCAACGAGACUACAAGGUCAACGACGUCAGAAAAGAU GAUCGC AAAGAGCGAACAGGAAAUCGGAAGGCAACAGCAAAGUACUUCUUCU ACAGCAACAUCAUGAACUUCUUCAGAACAGAAAUCACACUGGCAACG GAGAAU CAGAAAGAGACCGCUGAU CGAAAACAAACGAGAAAACAGGA GAAAU CGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAAGGU CCUGAGCAUGCCCGAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGAC AGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACA AGCUGAU CGCAAGAAAGAAAGGACUGGGACCCGAAGAAAGUACGGAGGA UUCGACAGCCCGACAGUCGCAUACAGCGUCCUGGUCGUCGCAAAGGUC GAAAAGGGAAAGAGCAAGAAGCUGAAGAGCGUCAAGGAACUGCUGGG AAUCACA AUUGGAAAAGAGCAGCUUCGAAAAGAACCCGAUCGACU UCCUGGAAGCAAAGGGAUACAAGGAAGUCAAGAAGGACCUGAUCAUC

Description	SEQ ID NO	Sequence
		AAGCUGCCGAAGUACAGCCUGUUCGAACUGGAAAACGGAAGAAAGAG AAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAACUGGCAC UGCCGAGCAAGUACGUCAACUCCUGUACCUGGCAAGCCACUACGAAA AGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAAGCAGCUGUUCGUC GAACAGCACAAAGCACUACCUGGACGAAAUCAUCGAACAGAUACAGCGAA UUCAGCAAGAGAGUCAUCCUGGCAGACGCAAAACUCCUGGACAAGGUCCUG AGCGCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAA AACAUCAUCCACCUGUUCACACUGACAAACUCCUGGAGCACCAGGCAGCA UUCAAGUACUUCGACACAACAACUUCGACAGAAAAGAGAUACACAAGCACA AAGGAAGUCCUGGACGCAACACUGAUCCACCAGAGCAUCACAGGACUG UACGAAAACAAGAAUCGAUCUGAGCCAGCUGGGAGGAGAGACAGCGGAGG AAGCACAACCCUGAGCGACAUCUAGGAAAAGGAAAACAGGAAAAGCAGC UGGUCUCCAGGAAAAGCAUCCUGAUGCUGCCGGAAGAAGUCGAAGAA GUCAUCGGAACAAGCCGGAAGCGACAUCUCCUGGUCCACACAGCAUAC GACGAAAAGCACAGACGAAAACGUCUAGCUGCUGACAAACGCGACGACCG GAAUACAAGCCGUGGGCACUGGUCAUCCAGGACAGCAACGGAGAAAAC AAGAUCAAGAUUCUGAGCGGAGGAAGCCCGAAGAAGAAGAGAAAAGGU CUAA
Open reading frame for UGI	807	AUGGGACCGAAGAAGAAGAGAAAAGGUCCGAGGAGGAAGCACAAACCU GUCGGACAUAUCGAAAAGGAAACAGGAAAGCAGCUGGUCAUCCAGG AAUCGAUCCUGAUGCUGCCGGAAGAAGUCGAAGAAGUCAUCGGAAC AAGCCGGAUUCGGACAUCUCCUGGUCCACACAGCAUACGACGAAUCGACA GACGAAAACGUCUAGCUGCUGACAUCCGACGACCCGGAUACAAGCCG UGGGCACUGGUCAUCCAGGACUCGAACGGAGAAAACAAGAUCAAGAU GCUGUGA
Open reading frame for UGI	808	AUGACCAACCUGUCCGACAUAUCGAGAAGGAGACCGGAAGCAGCUG GUGAUCCAGGAGUCCAUCUGAUGCUGCCGAGGAGGUGGAGGAGGU GAUCGGCAACAAGCCCGAGUCCGACAUCUCCUGGUGACACCCGCCUACGA CGAGUCCACCGACGAGAACGUGAUGCUGCUGACCUCGACGCCCCCGA GUACAAGCCUUGGGCCCUUGGUGAUCCAGGACUCCAACGGCGAGAACAA GAUCAAGAUGCUGUCCGGCGGCUCCAAGCGGACCGCCGACGGCUCCGA GUUCGAGUCCCCCAAGAAGAAGCGGAAGGUGGAGUGA
Amino acid sequence for Cas9 encoded by SEQ ID Nos. 801-802	809	MDKKYSIGLDIGTNSVGVAVITDEYKVPSSKFKVLGNTDRHSIKKNLIGALL FDSGETAEATRLKRTARRRYTRRKNRICYLQEFSNEMAKVDDSFHRLLES FLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSDDKADLRILIY ALAHMIKFRGHFLIEGDLNPDNSDVKLFIQLVQTYNQLFEENPINASGVDA KAILSARLSKSRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAED AKLQLSKDITYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEIT KAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYID GGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLG ELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVGPLARGNSRFAMWTRKSE ETITPWNFEVVVDKGASAQSFIERMTNFDKNLPNEKVLPHKSLLEYFTVYN ELTKVKYVTEGMRKPAFLSGEQKAIVDLLFKTNRKVTVKQLKEDYFKKIE CFDSVEISGVEDRFNASLGTYHDLKIIKDKDFLDNEENEDILEDIVLTLTLE DREMIEERLKYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDQKSGK TILDFLKSDFANRNFMLIHDDSLTFKEDIQKAQVSGQGDLSLHEHIANLAG SPAIKKGILQTVKVVDELVKVMGRHKPENIVIAMARENQTTQKGQKNSRER MKRIEIGIKELGSQILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDIN RLSDYDVDHIVPQSFLKDDSDNKLVLTRSDKNRGKSDNVPSEEVVKMKMKNY WRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQI LDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVREINNYHHAH DAYLNAVVGTAIIKKYPKLESEFVYGDYKVYDVRKMIKSEQEI GKATAK YFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL MPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV AYSVLVVAKVEKGSKKLKSVKELLGITIMERSSEKPNIDFLEAKGYKEVK KDLIIKLPKYSLEFENGRKRMLASAGELQKGNELALPSKYVNFYLYASHYE KLKGSPEDEQKQLFVEQHKHYLDEIIEQISEFSKRVLADANLDKVL SAYN

Description	SEQ ID NO	Sequence
		KHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLI HQSITGLYETRIDLSQLGGDGGGSPKKKRKV
Amino acid sequence for Cas9 with Hibit tag	810	MDKKYSIGLDIGTNSVGWAVITDEYK VPSKKFKVLGNTDRHSIKKNLIGALL FDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLLES FLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVSTDKADRLRIYL ALAHMIKFRGHFLIEGDLNPDNSVDKLFQQLVQTYNQLFEENPINASGVDA KAILSARLSKSRLENLIALQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAED AKLQLSKD TYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEIT KAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYID GGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLG ELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVGPLARGNSRFAMWTRKSE ETITPWNFEVVDKGASAQSFIERMTNFDKNLPNEKVLPHKSLLEYEFTVYN ELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIE CFDSVEISGVEDRFNASLGTYHDLKIIKDKDFLDNEENEDILEDIVLTLTLFE DREMIEERLKYAHLFDDKVMKQLKRRRYTGWRSLKRLINGIRDKQSGK TILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAG SPAIIKGILQTVKVVDELVKVMGRHKPENIVIAMARENQTTQKGQKNSRER MKRIIEGKELGSQILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDIN RLSDYDVHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKMKNY WRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQI LDSRMNTKYDENDKLIREVKVITLKSLLVSDFRKDFQFYKVR EINNYYHHAH DAYLNAVVGTAIIKKYKLESEFVYGDYKVYDVRKMIKSEQEIGKATAK YFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL MPQVNVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV AYSVLVVAKEKGSKKLSVKELLGITIMERSSEFKNPIDFLEAKGYKEVK KDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFYLASHYE KLKGSPEDEQKQLFVEQHKHYLDEIIEQISEFSKRVLADANLDKVL SAYN KHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLI HQSITGLYETRIDLSQLGGDGGGSPKKKRKVSESATPESVSGWRLFKKIS
Amino acid sequence for BC22n	811	MEASPASGPRHLMDPHIFTSNFNGIGRHKTYLCYEVERLDNGTSVKMDQH RGFLHNQAKNLLCGFYGRHAELRFLDLVPSLQLDPAQIYRVTFWISWSPCFS WGCAGEVRAFLQENTHVRLEIFAARIYDYDPLYKEALQMLRDAGAQVSIM TYDEFKHCWDTFVDHQQCPFPWDGLDEHSQALSGRRLAILQNQNGSGSE TPGTSESATPESDKKYSIGLAIGTNSVGWAVITDEYK VPSKKFKVLGNTDRH SIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKV DDSFHRLLESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVST DKADRLRIYLALAHMIKFRGHFLIEGDLNPDNSVDKLFQQLVQTYNQLFEE NPINASGVDAKAILSARLSKSRLENLIALQLPGEKKNGLFGNLIALSLGLTPN FKSNFDLAEDAKLQLSKD TYDDDLNLLAQIGDQYADLFLAAKNLSDAILL SDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ KNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDN GSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVGPLARGNSR FAWMTRKSEETITPWNFEVVDKGASAQSFIERMTNFDKNLPNEKVLPHK SLLEYEFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQ LKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLKIIKDKDFLDNEENEDIL EDIVLTLTLFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWRSLKRLI NGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDS LHEHIANLAGSPAIIKGILQTVKVVDELVKVMGRHKPENIVIAMARENQTTQ KGQKNSRERMKRIIEGKELGSQILKEHPVENTQLQNEKLYLYLQNGRDM YVDQELDINRLSDYDVHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSE EVVKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVET RQITKHVAQILD SRMNTKYDENDKLIREVKVITLKSLLVSDFRKDFQFYKVR EINNYYHHAH DAYLNAVVGTAIIKKYKLESEFVYGDYKVYDVRKMIKSE QEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRD FATVRKVL SMPQVNVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKK YGGFDSPTVAYSVLVVAKEKGSKKLSVKELLGITIMERSSEFKNPIDFL EAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYV NFLYLASHYEKLKGSPEDEQKQLFVEQHKHYLDEIIEQISEFSKRVLADAN

Description	SEQ ID NO	Sequence
		LDKVL SAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTST KEVL DATLIHQ SITGLYETRIDLSQLGGDGGGSPKKRKRK V*
Amino acid sequence for BC22n with Hibit tag	812	MEAS PASGPRHLM DP HIFTSNFNNGIGRHKTYLCYEVERLDNGTSVKMDQH RGFLHNQAKNLLCGFYGRHAELRFLDLVPSLQLDPAQIYRVTFWISWSPCFS WGCAGEVRAFLQENTH VRLRIFAARIYDYDPLYKEALQMLRDAGA QVSIM TYDEFKHCWDTFVDHQQGCPFPQWDGLDEHSQALSGRRLAILQNQNSGSE TPGTSESATPESDKKYSIGLAIGTNSVGWAVITDEYK VPSKKFKVLGNTDRH SIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKV DDSFHRL EESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLV DST DKADLR LIYLA LAHMIKFRGHFLIEGDLNPDNSVDKLFQIQLVQTYNQLFEE NPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIASLGLTPN FKSNFDLAEDAKLQLSKD TYDDDLNLLAQIGDQYADLFLAAKNLSDAILL SDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQS KNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDN GSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPIYYVGPLARGNSR FAWMTRKSEETITPWNFEVVVDKGASAQSFIERMTNFDKNLPNEK VLPKHS LLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQ LKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDIL EDIVLTLTLFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWRLSRKL I NGIRDKQSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDIQKAQVSGQGDS LHEHIANLAGSPA IKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQ KGQKNSRERMKRIE EGIKELGSQILKEHPVENTQLQNEKLYLYLQNGRDM YVDQELDINRLSDYD VDHVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEE VVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVET RQITKHVAQILD SRMNTKYDENDKLIREVKVITLKS KLVSDFRKDFQFYKVR EINNYHHAHDAYLNAVVG TALIKKYPKLESEFVYGDYKVYDVRKMIKSE QEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRD FATVRKVL SMPQVNI VVKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKK YGGFDSPTVAYSVLVVAKEVGKSKKLKSVKELLGITIMERSSSF EKNPIDFL EAKGYKEVKKDLI IKL PKYSLFELENGRKRMLASAGELQKGNELALPSKYV NFLYLASHYEK LKGS PEDNEQKQLFVEQHKHYLDEIEQISEFSKR VILADAN LDKVL SAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTST KEVL DATLIHQ SITGLYETRIDLSQLGGDGGGSPKKRKRK VSESATPESVSGW RLFKKIS
Amino acid sequence for BC22	813	MEAS PASGPRHLM DP HIFTSNFNNGIGRHKTYLCYEVERLDNGTSVKMDQH RGFLHNQAKNLLCGFYGRHAELRFLDLVPSLQLDPAQIYRVTFWISWSPCFS WGCAGEVRAFLQENTH VRLRIFAARIYDYDPLYKEALQMLRDAGA QVSIM TYDEFKHCWDTFVDHQQGCPFPQWDGLDEHSQALSGRRLAILQNQNSGSE TPGTSESATPESDKKYSIGLAIGTNSVGWAVITDEYK VPSKKFKVLGNTDRH SIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKV DDSFHRL EESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLV DST DKADLR LIYLA LAHMIKFRGHFLIEGDLNPDNSVDKLFQIQLVQTYNQLFEE NPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIASLGLTPN FKSNFDLAEDAKLQLSKD TYDDDLNLLAQIGDQYADLFLAAKNLSDAILL SDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQS KNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDN GSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPIYYVGPLARGNSR FAWMTRKSEETITPWNFEVVVDKGASAQSFIERMTNFDKNLPNEK VLPKHS LLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQ LKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDIL EDIVLTLTLFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWRLSRKL I NGIRDKQSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDIQKAQVSGQGDS LHEHIANLAGSPA IKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQ KGQKNSRERMKRIE EGIKELGSQILKEHPVENTQLQNEKLYLYLQNGRDM YVDQELDINRLSDYD VDHVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEE VVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVET RQITKHVAQILD SRMNTKYDENDKLIREVKVITLKS KLVSDFRKDFQFYKVR EINNYHHAHDAYLNAVVG TALIKKYPKLESEFVYGDYKVYDVRKMIKSE

Description	SEQ ID NO	Sequence
		QEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRLIETNGETGEIVWDKGRD FATVRKVL SMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKK YGGFDSPTVAYSVLVVAKEKGSKKLKSVKELLGITIMERSSEFNPIDFL EAKGYKEVKKDLIIKLPKYSLFELENGRKRLASAGELQKGNELALPSKYV NFLYLASHYEKLKGSPEDEQKQLFVEQHKHYLDEIIEQISEFSKRVLADAN LDKVL SAYNKHHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTST KEVL DATLIHQSI TGLYETRIDLSQLGGDSGGSTNLSDIIEKETGKQLVIQESIL MLPEEV EEVIGNKPESDILVHTAYDESTDENVMLLTSDAPEYKWPALVIQDS NGENKIKMLSGGSPKKKRKV
Amino acid sequence for UGI	814	MTNLSDIIEKETGKQLVIQESILMLPEEV EEVIGNKPESDILVHTAYDESTDEN VMLLTSDAPEYKWPALVIQDSNGENKIKMLSGGSKRTADGSEFESPKKKRKR VE
mRNA sequence encoding UGI	815	GGGAAGCUCAGAAUAAAACGCUCAACUUUGGCCGGAUCUGCCACCAUGA CCAACCUUGUCCGACAUCAUCGAGAAGGAGACCGGCAAGCAGCUGGUGA UCCAGGAGUCCAUCUGAUGCUGCCCGAGGAGGUGGAGGAGGUGAUC GGCAACAAGCCCGAGUCCGACAUCCUGGUGCACACCGCCUACGACGAG UCCACCGACGAGAACGUGAUGCUGCUGACCUCCGACGCCCCCGAGUAC AAGCCUUGGCGCCUUGGUGAUCCAGGACUCCAACGGCGAGAACAAAGAU AAGAUUGCUGUCCGCGCGCUCCAAGCGGACCGCGACGGUCCGAGUUC GAGUCCCCCAAGAAGAAGCGGAAGGUGGAGUGAUAGCUAGCACACGACC UCAAGAACACCCGAAUGGAGUCUCUAAGCUACAUAAUACCAACUUAACA CUUUACAAAUGUUGUCCCCCAAAAUGUAGCCAUUCGUUUCUGCUCCU AAUAAAAAGAAAGUUUCUACAUUCUCUCGAGAAAAAUAUUAUUA GAAAAAUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUA AAAAAUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUA AAAAAUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUA AAAAAUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUA AAAAAUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUA AAAAAUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUA AAUCUAAAAAUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAU ACAAAAAUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAU AAAAAUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUA AAAAAUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUA
	816-899	Not used
Linker	900	SGSETPGTSESATPES
Linker	901	SGSETPGTSESA
Linker	902	SGSETPGTSESATPEGSGGS
	903-971	Not used
mRNA encoding BC22n	972	GGGAAGCUCAGAAUAAAACGCUCAACUUUGGCCGGAUCUGCCACCAUGG AGGCCUCCCCCGCCUCCGGCCCCCGGACCUGAUGGACCCCCACAUCUU CACCUCCAACUUAACAACGGCAUCGGCCGGCACAAGACCUACCUGUG CUACGAGGUGGAGCGGCUUGGACAACGGCACCUCCGUGAAGAUGGACCA GCACCGGGGCUUCCUGCACAACACAGGCCAAGAACCUGCUGUGCGGCUU CUACGCGCGGCACGCGGAGCUGCGGUUCCUGGACCUGGUGCCUCCCU GCAGCUGGACCCCCGCCCAGAUUACCGGGUGACCUGGUUCAUUCUCUG GUCCCCUGCUUUCUUGGGGCUGCGCCGGCGAGGUGCGGGCCUUCU GCAGGAGAACACCCACGUGCGGGCUGCGGAUCUUCGCCGCCCGGAUCUA CGACUACGACCCCCUGUACAAGGAGGCCUGCAGAUUGCUGCGGGACGC CGGCGCCAGGUGUCCAUAUGACCUACGACGAGUUAAGCACUGCUG GGACACCUUCGUGGACACACAGGGCUGCCCCUUCAGCCUUGGGACGG CCUGGACGAGCACUCCAGGCCUGUCCGGCCGGCUGCGGGCCAUCCU GCAGAACCAGGGCAACUCCGGCUCGAGACCCCCGGCACCUCCGAGUC CGCCACCCCGAGUCCGACAAGAAGUACUCCAUCGGCCUGGCCAUCGG CACCACUCCGUGGGCUGGGCCGUGAUCACCGACGAGUACAAGGUGCC CUCCAAGAAGUUAAGGUGCUGGGCAACACCGACCGGCACUCCAUCAA GAAGAACCUGAUCGGCGCCCUUGCUUUCGACUCCGGCGAGACCGCCGA GGCCACCCGGCUGAAGCGGACCGCCGGCGGCGGUACACCCGGCGGAA

Description	SEQ ID NO	Sequence
		GAACCGGAUCUGCUACCUGCAGGAGAUUUUCCAACGAGAUGGCCAA GGUGGACGACUCCUUCUCCACCGGCGUGGAGGAGUCCUCCUGGUGGA GGAGGACAAGAAGCACGAGCGGCACCCCAUCUUCGGCAACAUCGUGGA CGAGGUGGCCUACCCACGAGAAGUACCCACCAUCUACCACCGCGGAA GAAGCUGGUGGACUCCACCGACAAGGCCGACCUGCGGCUGAUCUACC GGCCUUGGCCCAUGAUCAAGUUCGGGGCCACUCCUGAUCGAGGG CGACCUGAACCCCGACAACUCCGACGUGGACAAGCUGUUCAUCCAGCU GGUGCAGACCUACAACCAGCUGUUCGAGGAGAACCCCAUCAACGCCUC CGGCGUGGACGCCAAGGCCAUCCUGUCCGCCCGGCGUCCAAAGUCCCG GCGGCUGGAGAACCUGAUCGCCCAGCUGCCCGGCGAGAAGAAGAACGG CCUGUUCGGCAACCUGAUCGCCUGUCCUGGGGCCUACCCCAACU CAAGUCCAACUUCGACCUGGCCGAGGACGCCAAGCUGCAGCUGUCCAA GGACACCUACGACGACGACCUGGACAACCUGCUGGCCAGAUCCGCCGA CCAGUACGCCGACCUGUCCUGGCCGCCAAGAACCUGUCCGACGCCAU CCUGCUGUCCGACAACCUGCGGGUGAACACCGAGAUCAACCAAGCCCC CCUGUCCGCCUCCAUGAUCAAGCGGUACGACGAGCACCACAGGACCU GACCCUGCUGAAGGCCUGGUGCGGCAGCAGCUGCCCGAGAAGUACAA GGAGAUUUUUCGACCAAGUCCAAAGAACGGCUACGCCGGCUACAUCGA CGGCGGCGCCUCCAGGAGGAGUUCUACAAGUUCAUCAAGCCCAUCCU GGAGAAGAUGGACGGCACCGAGGAGCUGCUGGUGAAGCUGAACCCGG AGGACCUGCUGCGGAAGCAGCGGACCUUCGACAACGGCUCCAUCCCCC ACCAGAUCCACCUGGGCGAGCUGCACGCCAUCCUGCGGCGGCAGGAGG ACUUCUACCCCUUCCUGAAGGACAACCGGGAGAAGAUCCGAGAAGAUC UGACCUUCCGGAUCCCUACUACGUGGGCCCCUGGCCCGGGGCAACU CCCGGUUCGCCUGGAUGACCCGGAAGUCCGAGGAGACCAUACCCCU GGAACUUCGAGGAGGUGGUGGACAAGGGCGCCUCCGCCAGUCCUUA UCGAGCGGAUGACCAACUUCGACAAGAACCUGCCCAACGAGAAGGUGC UGCCCAAGCACUCCUUGCUGUACGAGUACUUCACCGUGUACAACGAGC UGACCAAGGUGAAGUACGUGACCGAGGGCAUGCGGAAGCCCCGCCUUC UGUCCGGCGAGCAGAAGAAGGCCAUCCUGGACCUUGCUGUUAACAGCCA ACCGGAAGGUGACCGUGAAGCAGCUGAAGGAGGACUACUUAAGAAG AUCGAGUGCUUCGACUCCGUGGAGAUUCCCGGCGUGGAGGACCGGUUC AACGCCUCCUGGGCACCUACCACGACCUGCUGAAGAUCAUCAAAGGAC AAGGACUUCUGGACAACGAGGAGAACGAGGACAUCUGGAGGACAUC CGUGCUGACCCUGACCCUGUUCGAGGACCGGGAGAUGAUCGAGGAGCG GCUGAAGACCUACGCCACCUGUUCGACGACAAGGUGAUGAAGCAGCU GAAGCGGCGCGGUACACCGGCUGGGGCCGGCUGUCCCGGAAGCUGAU CAACGGCAUCCGGGACAAGCAGUCCGGCAAGACCAUCCUGGACUUCU GAAGUCCGACGGCUUCGCCAACCAGAACUUAUGCAGCUGAUCCACGA CGACUCCUGACCUUCAAGGAGGACAUCAGAAAGGCCAGGUGUCCGG CCAGGGCGACUCCUGCACGAGCACAUCCGAACCUGGCCGGCUCCCC CGCAUCAAGAAGGGCAUCCUGCAGACCGUGAAGGUGGUGGACGAGCU GGUGAAGGUGAUGGGCCGGCACAAGCCCGAGAACAUCGUGAUCGAGA UGGCCCGGGAGAACCAGACCAACCCAGAAGGGCCAGAAGAACUCCCGG AGCGGAUGAAGCGGAUCGAGGAGGGCAUCAAGGAGCUGGGCUCCAG AUCCUGAAGGAGACCCCGUGGAGAACACCCAGCUGCAGAACGAGAAG CUGUACCUUGAUACCUUGCAGAACGGCCGGGACAUGUACGUGGACGAG GAGCUGGACAUCAACCGGCUUCCGACUACGACGUGGACCAUCUGUG CCCCAGUCCUUCUGAAGGACGACUCCAUCGACAACAAGGUGCUGACC CGGUCCGACAAGAACCAGGGCAAGUCCGACAACGUGCCUCCGAGGAG GUGGUGAAGAAGAUAGAAGACUACUGGCGGCAGCUGCUGAACGCCAA GCUGAUCACCCAGCGGAAGUUCGACAACCUGACCAAGGCCGAGCGGGG CGGCCUGUCCGAGCUGGACAAGGCCGGCUUCAUAAGCGGCAGCUGGU GGAGACCCGGCAGAUACCAAGCACGUGGCCAGAUCCUGGACUCCCG GAUGAACCAAGUACGACGAGAACGACAAGCUGAUCCGGGAGGUGA AGGUGAUCACCUGAAGUCCAAGCUGGUGUCCGACUUCGGGAAGGACU UCCAGUUCUACAAGGUGCGGGAGAUCAACAACUACCACACGCCACG ACGCCUACCUGAACGCCGUGGUGGGCACCGCCUGAUCAAGAAGUACC

Description	SEQ ID NO	Sequence
		CCAAGCUGGAGUCCGAGUUCGUGUACGGCGACUACAAGGUGUACGACG UGCGGAAGAUGAUCGCCAAGUCCGAGCAGGAGAUCCGCAAGGCCACCG CCAAGUACUUCUUCUACUCCAACAUCUAUGAACUUCUUAAGACCGAGA UCACCCUGGGCAACCGGCGAGAUCCGGAAGCGGCCCCUGAUCGAGACCA ACGGCGAGACCGGCGAGAUUCGUGUGGACAAGGGCCGGGACUUCGCCA CCGUGCGGAAGGUGGUGUCCAUGCCCCAGGUGAACAUUCGUGAAGAAGA CCGAGGUGCAGACCGGCGGCUUCUCCAAGGAGUCCAUCUGCCCAAGC GGAACUCCGACAAGCUGAUCGCCCCGGAAGAAGGACUGGGACCCCAAGA AGUACGGCGGCUUCGACUCCCCACCGUGGCCUACUCCGUGCUGGUGG UGGCCAAGGUGGAGAAGGGCAAGUCCAAGAAGCUGAAGUCCGUGAAG GAGCUGCUGGGCAUCACCAUCAUGGAGCGGUCCUUCGAGAAGAAC CCCAUCGACUUCUGGAGGCCAAGGGCUACAAGGAGGUGAAGAAGGAC CUGAUCAUCAAGCUGCCCAAGUACUCCUGUUCGAGCUGGAGAACGGC CGGAAGCGGAUGCUGGCCUCCGCCGCGAGCUGCAGAAGGGCAACGAG CUGGCCUGCCCCCAAGUACGUGAACUUCUGUACUCCUGGCCUCCAC UACGAGAAGCUGAAGGGCUCCCCGAGGACAACGAGCAGAAGCAGCUG UUCGUGGAGCAGCACAAGCACUACCUGGACGAGAUCAUCGAGCAGAUC UCCGAGUUCUCCAAGCGGGUGAUCCUGGCCGACGCCAACCUGGACAAG GUGCUGUCCGCCUACAACAAGCACCGGGACAAGCCCAUCCGGGAGCAG GCCGAGAACAUCAUCCACCUGUUCACCCUGACCAACCUGGGCGCCCC GCCGCCUUAAGUACUUCGACACCACCAUCGACCGGAAGCGGUACACC UCCACCAAGGAGGUGCUGGACGCCACCCUGAUCCACCAGUCCAUCACC GGCCUGUACGAGACCCCGGAUCGACCUGUCCAGCUGGGCGGGCAGCGC GGCGGCUCCCCAAGAAGAAGCGGAAGGUGUGACUAGCACCAGCCUCA AGAACACCCGAAUGGAGUCUCUAAGCUACAUAUACCAACUUAACACU UACAAAUGUUGUCCCCCAAAUGUAGCAUUCGUUACUGCUCUAAU AAAAAGAAAGUUCUUCACAUUCUCUCGAGAAAAAUAUUGGAA AAAAAAAAAACGGAAAAAAGGUAAAAAAAAAAUUAUAAAA AAAAAAAAAACAUAAAAAAAAAAGCAAAAAAAAAAACGUAAAAAA AAAAAACUAAAAAAAAAAGAUAAAAAAAAAACCUGAAAAAAAAA AAUUGUAAAAAAAAAAGGGAAAAAAAAAAACGCAAAAAAAAAA ACACAAAAAAAAAUGCAAAAAAAAAAUCGAAAAAAAAAAAU CUAAAAAAAAAACGAAAAAAAAAACCAAAAAAAAAAAGACA AAAAAAAAAAUAAGAAAAAAAAAAGUAAAAAAAAAACUGAAA AAAAAAAAAAUUAAAAAAAAAAAUUCUAG
mRNA encoding BC22n with HiBit tag	973	GGGAAGCUCAGAAUAAACGCUCAACUUGGCCGGAUCUGCCACCAUGG AGGCCUCCCCGCCUCCGGCCCCCGCACCUGAUGGACCCCCACAUCUU CACCUCCAACUUAACAACGGCAUCGGCCGGCACAAGACCUACCUUG CUACGAGGUGGAGCGGCUUGGACAACGGCACCUCCGUGAAGAUGGACCA GCACCGGGGCUUCCUGCACAACAGGCCAAGAACCUGCUGGCGGCUU CUACGGCCGGCACGCCGAGCUGCGGUUCCUGGACCUGGUGCCUCCCU GCAGCUGGACCCCGCCAGAUUCACCGGGUGACCUGGUUCAUCUCCUG GUCCCCUGCUUCUCCUGGGGCGUGCGCCGGCGAGGUGCGGGCCUUCU GCAGGAGAACACCCACGUGCGGCUGCGGAUCUUCGCCGCCCGGAUCUA CGACUACGACCCCCUGUACAAGGAGGCCUGCAGAUUCGCGGGACGC CGGCGCCAGGUGUCCAUAUGACCUACGACGAGUUAAGCACUGCUG GGACACCUUCGUGGACCAACAGGGCUGCCCCUCCAGCCUUGGACGG CCUGGACGAGCACUCCAGGCCUGUCCGGCCGGCUGCGGGCCAGCU GCAGAACCAAGGCAACUCCGGCUCGAGACCCCGGCACCUCCGAGUC CGCCACCCCGAGUCCGACAAGAAGUACUCCAUCGGCCUGGCCAUCGG CACCAACUCCGUGGGCUGGGCCGUGAUCACCGACGAGUACAAGGUGCC CUCCAAGAAUGUCAAAGGUGCUGGGCAACACCGACCGGCACUCCAUA GAAGAACCUGAUCGGCGCCUGCUGUUCGACUCCGGCGAGACCGCCGA GGCCACCCGGCUGAAGCGGACCGCCGGCGGCGGUACACCCGGCGGAA GAACCGGAUCUGCUACCUAGGAGAUUUCCAACGAGAUGGCCAA GGUGGACGACUCCUUCUCCACCGGCUGGAGGAGUCCUCCUGGUGGA GGAGGACAAGAAGCAGGAGCGGCACCCCAUCUUCGGCAACAUUGGGA CGAGGUGGCCUACCACGAGAAGUACCCCAACUACACCUCCGCGAA

Description	SEQ ID NO	Sequence
		GAAGCUGGUGGACUCCACCGACAAGGCCGACCUGCGGCUGAUCUACCU GGCCCUGGCCCAUGAUAAGUUCGGGGCCACUUCUGAUCGAGGG CGACCUGAACCCCGACAACUCCGACGUGGACAAGCUGUUCUACUCCAGCU GGUGCAGACCUACAACCAGCUGUUCGAGGAGAACCCCAUCAACGCCUC CGGCGUGGACGCCAAGGCCAUCCUGUCCGCGCGGCUGUCCAAGUCCCG GCGGCUGGAGAACCUGAUCGCCCAGCUGCCCGGCGAGAAGAAGAACGG CCUGUUCGGCAACCUGAUCGCCCUGUCCUGGGCCUGACCCCAACUU CAAGUCCAACUUCGACCUGGCCGAGGACGCCAAGCUGCAGCUGUCCAA GGACACCUACGACGACGACCUGGACAACCUGCUGGCCCAGAUCCGGCGA CCAGUACGCCGACCUGUUCUGGCCGCCAAGAACCUGUCCGACGCCAU CCUGCUGUCCGACAUCUCCUGCGGGUGAACACCGAGAUACCAAGGCCCC CCUGUCCGCCUCCAUGAUAAGCGGUACGACGAGCACCACCAGGACCU GACCCUGCUGAAGGCCCUUGGUGCGGCAGCAGCUGCCCCGAGAAGUACAA GGAGAUUUUUUCGACCAGUCCAAGAACGGCUACGCCGGCUACAUCGA CGGCGGCGCCUCCAGGAGGAGUUCUACAAGUUAUCAAGCCCAUCCU GGAGAAGAUUGGACGGCACCGAGGAGCUGCUGGUGAAGCUGAACCGGG AGGACCUGCUGCGGAAGCAGCGGACCUUCGACAACGGCUCCAUCCCCC ACCAGAUCCACCUUGGCGGAGCUGCAGCGCAUCCUGCGGCGGACAGGAG ACUUCUACCCCUUCCUGAAGGACAACCGGGAGAAGAUCCGAGAAGAUCC UGACCUUCCGGAUCCCCUACUACGUGGGCCCCUGGCCCGGGGCAACU CCCGGUUCGCCUGGAUGACCCGGAAGUCCGAGGAGACCAUACCCCCU GGAACUUCGAGGAGGUGGUGGACAAGGGCGCCUCCGCCAGUCCUUA UCGAGCGGAUGACCAACUUCGACAAGAACCUGCCCAACGAGAAGGUGC UGCCCAAGCACUCCUUGCUGUACGAGUACUUCACCGUGUACAACGAGC UGACCAAGGUGAAGUACGUGACCGAGGGCAUGCGGAAGCCCGCCUUC UGUCCGGCGAGCAGAAGAAGGCCAUCCUGGACCUGCUGUUAAGACCA ACCGGAAGGUGACCGUGAAGCAGCUGAAGGAGGACUACUUAAGAAG AUCGAGUGCUUCGACUCCGUGGAGAUUCUCCGGCGUGGAGGACCGGUUC AACGCCUCCUGGGCACCUACCACGACCUGCUGAAGAUCAUCAAGGAC AAGGACUUCUUGGACAACGAGGAGAACGAGGACAUCUUGGAGGACAU CGUGCUGACCCUGACCCUGUUCGAGGACCGGGAGAUGAUCGAGGAGCG GCUGAAGACCUACGCCACCUUGUUCGACGACAAGGUGAUGAAGCAGCU GAAGCGGCGGCGGUACACCGGCGUGGGGCCGGCGUGUCCCGGAAGCUGAU CAACGGCAUCCGGGACAAGCAGUCCGGCAAGACCAUCCUGAUCUCCU GAAGUCCGACGGCUUCGCCAACCGGAACUUAUGCAGCUGAUCCACGA CGACUCCUGACCUUCAAGGAGGACAUCAGAAAGGCCAGGUGUCCGG CCAGGGCGACUCCUGCAGGACACAUCGCCAACUUGGCCGCGUCCCC CGCCAUCAAGAAGGGCAUCCUGCAGACCGUGAAGGUGGUGGACGAGCU GGUGAAGGUGAUGGGCCGGCAACAGCCCGAGAACAUCGUGAUCGAGA UGGCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAUCUCCGGG AGCGGAUGAAGCGGAUCGAGGAGGGCAUCAAGGAGCUGGGCUCCAG AUCCUGAAGGAGACCCCGUGGAGAACACCCAGCUGCAGAACGAGAAG CUGUACCUGUACUACCUGCAGAACGGCCGGGACAUGUACGUGGACCAG GAGCUGGACAUAACCGGCGUGUCCGACUACGACGUGGACCACAUCGUG CCCCAGUCCUCCUGAAGGACGACUCCAUCGACAACAAGGUGCUGACC CGGUCCGACAAGAACCGGGGCAAGUCCGACAACGUGCCCUCCGAGGAG GUGGUGAAGAAGAUGAAGAACUACUGGCGGCAGCUGCUGAACGCCAA GCUGAUCACCCAGCGGAAGUUCGACAACCUGACCAAGGCCGAGCGGGG CGGCCUGUCCGAGCUGGACAAGGCCGGCUUCAUAAGCGGCAGCUGGU GGAGACCCGGCAGAUACCAAGCAGUGGCCCAGAUCCUGGACUCCCG GAUGAACACCAAGUACGACGAGAACGACAAGCUGAUCCGGGAGGUGA AGGUGAUCACCCUGAAGUCCAAGCUGGUGUCCGACUUCGGGAAGGACU UCCAGUUCUACAAGGUGCGGGAGAUCAACAACUACCACCACGCCACG ACGCCUACCUGAACGCCGUGGUGGGCACCGCCUGAUAAGAAGUACC CCAAGCUGGAGUCCGAGUUCGUGUACGGCGACUACAAGGUGUACGACG UGCGGAAGAUUCGCAAGUCCGAGCAGGAGAUCCGCAAGGCCACCG CCAAGUACUUCUUCUACUCCAACAUCAUGAACUUCUUAAGACCGAGA UCACCCUGGCCAACGGCGAGAUCCGGAAGCGGCCCCUGAUCGAGACCA

Description	SEQ ID NO	Sequence
		ACGGCGAGACCGGCGAGAUUCGUGUGGGACAAGGGCCGGGACUUCGCCA CCGUGCGGAAGGUGCUGUCCAUGCCCCAGGUGAACAUUCGUGAAGAAGA CCGAGGUGCAGACCGGCGGCUUCUCCAAGGAGUCCAUCUGCCCAAGC GGAACUCCGACAAGCUGAUUCGCCCCGGAAGAAGGACUGGGACCCCAAGA AGUACGGCGGCUUCGACUCCCCCACCUGGGCCUACUCCGUGCUGGUGG UGGCCAAGGUGGAGAAGGGCAAGUCCAAGAAGCUGAAGUCCGUGAAG GAGCUGCUGGGCAUCACCAUCAUGGAGCGGUCCUCCUUCGAGAAGAAC CCCAUCGACUUCUGGAGGCCAAGGGCUACAAGGAGGUGAAGAAGGAC CUGAUCAUCAAGCUGCCCAAGUACUCCUGUUCGAGCUGGAGAACGGC CGGAAGCGGAUGCUGGCCUCCGCCGGCGAGCUGCAGAAGGGCAACGAG CUGGGCCUGCCCUCCAAGUACGUGAACUCCUGUACCUGGGCCUCCCA UACGAGAAGCUGAAGGGCUCCCCCGAGGACAACGAGCAGAAGCAGCUG UUCGUGGAGCAGCACAAGCACUACCUGGACGAGAUCAUCGAGCAGAUC UCCGAGUUCUCCAAGCGGGUGAUCCUGGCCGACGCCAACCUGGACAAG GUGCUGUCCGCCUACAACAAGCACCGGGACAAGGCCAUCCGGAGCAG GCCGAGAACAUAUCCACCUGUUCACCCUGACCAACCUGGGCGCCCCC GCCGCCUUAAGUACUUCGACACCACCAUCGACCGGAAGCGGUACACC UCCACCAAGGAGGUGCUGGACGCCACCCUGAUCCACCAGUCCAUCACC GGCCUGUACGAGACCCGGAUCGACCUGUCCAGCUGGGCGGGCAGCGC GGCGGCUCCCCAAGAAGAAGCGGAAGGUGUCCGAGUCCGCCACCCCC GAGUCCGUGUCCGGCUGGCGGCGUUAAGAAGAUUCCUGACUAGCA CCAGCCUCAAGAACCCCGAAGGAGUCUCUAAGCUACAUAAUACCAA CUUACACUUUACAAAAUGUUGUCCCCCAAAUAGUAGCCAUUCGUUUCU GCUCCUAAUAAAAAGAAAGUUUCUUCACAUUCUCUCGAGAAAAAAA AAAAUGGAAAAAAAAAAAAACGAAAAAAAAAAAAAGGUAAAAAAAAAA AAUUAUAAAAAAAAAAAAACAUAAAAAAAAAAAAACGAAAAAAAAAAAAAC GUAAAAAAAAAAAAACUCAAAAAAAAAAAAAAGAUAAAAAAAAAAAAACCU AAAAAAAAAAAAAUGUAAAAAAAAAAAAAGGAAAAAAAAAAAAACGCAA AAAAAAAAAACACAAAAAAAAAAAAAUGCAAAAAAAAAAAAAAUCGAAAA AAAAAAAAAUCUAAAAAAAAAAAAACGAAAAAAAAAAAAACCCAAAAAA AAAAAGACAAAAAAAAAAAAAUGAAAAAAAAAAAAAGUUAAAAAAAAAA AAACUGAAAAAAAAAAAAAUUUAAAAAAAAAAAAAUCUAG
mRNA encoding BC22	974	GGGAGACCCAAGCUGGCUAGCGUUUAAACUUAAGCUUUCGCGCAGUCG GCGUCCAGCGGCUUCGCUUGUUCGUGUGUGUGUCGUUGCAGGCCUUAU UCGGAUCCGCCACCAUGGAAGCAAGCCCGGCAAGCGGACCGAGACACC UGAUGGACCCGCACAUUCUACAAGCAACUUAACAACGGAAUCGGAA GACACAAGACAUACCUGUGCUACGAAGUCGAAAGACUGGACAACGGA ACAAGCGUCAAGAUGGACCAGCACAGAGGAUUCUGCACAACCAGGCA AAGAACCUGCUGUGCGGAUUCUACGGAAGACACGCAAGACUGAGAUU CCUGGACCUUGGUCCCGAGCCUGCAGCUGGACCCGGCACAGAUUCACG AGUCACAUGGUUCAUCAGCUGGAGCCCGUGCUUCAGCUGGGGAUGCGC AGGAGAAGUCAGAGCAUUUCUGCAGGAAACACACACGUCAGACUGA GAAUCUUCGAGCAAGAAUUCUACGACUACGACCCGCGUACAAGGAAG CACUGCAGAUUGCUGAGAGACGCAGGAGCACAGGUACAGCAUCAUGACAU ACGACGAUUAAGCACUGCUGGGACACAUUCGUCGACCACCAGGGAU GCCGUUCCAGCCGUGGGACGGACUGGACGAACACAGCCAGGCACUGA GCGGAAGACUGAGAGCAUCCUGCAGAAACAGGGAAACAGCGGAAGC GAAACACCGGGAACAAGCGAAAGCGCAACACCGGAAAGCGACAAGAAG UACAGCAUCGGACUGGCCAUCCGGAACAAACAGCGUCGGAUGGGCAGUC AUCACAGACGAUUAACAAGGUCCCGAGCAAGAAGUUAAGGUCCUGGG AAACACAGACAGACACAGCAUCAAGAAGAACCUGAUUCGGAGCAGUCU GUUCGACAGCGGAGAAACAGCAGAAAGCAACAAGACUGAAGAGAAACAG CAAGAAGAAGAUACACAAGAAAGAAAGAACAGAAUCUGCUACCUGCAG GAAAUUCUACGCAACGAAAUGGCAAGGUUCGACGACAGCUUUCUCCAC AGACUGGAAGAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAG ACACCGAUCUUCGGAACAUCGUCGACGAAGUCGCAUACCACGAAAA GUACCCGACAAUCUACCACUGAGAAAGAAGCUGGUCGACAGCACAGA CAAGGCAGACCUGAGACUGAUUACCUGGCACUGGCACACAUGAUCAA

Description	SEQ ID NO	Sequence
		GUUCAGAGGACACUCCUGAUUCGAAGGAGACCUGAACCCGGACAACAG CGACGUCGACAAGCUGUUAUCCAGCUGGUCCAGACAUACAACCAGCU GUUCGAAGAAAACCCGAUCAACGCAAGCGGAGUCGACGCAAAGGCAU CCUGAGCGCAAGACUGAGCAAGAGCAGAAGACUGGAAAAACUGAUCGC ACAGCUGCCGGGAGAAAAAGAAACGGACUGUUCGGAAACUGAUCG CACUGAGCCUGGGACUGACACCGAACUUAAGAGCAACUUCGACCUGG CAGAAGACGCAAGCUGCAGCUGAGCAAGGACACAUACGACGACGACC UGGACAACCUGCUGGCACAGAUCCGAGACCAGUACGCAGACCUGUUC UGGCAGCAAAGAACUGAGCGACGCAAUCCUGCUGAGCGACAUCUGA GAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUA AGAGAUACGACGAAACACACCAGGACCUGACACUGCUGAAGGCACUGG UCAGACAGCAGCUGCCGAAAAAGUACAAGGAAAAUCUUCUUCGACCAGA GCAAGAACGGAUACGCAGGAUACAUCGACGAGGAGCAAGCCAGGAA GAAUUCUACAAGUUAUCAAGCCGAUCCUGGAAAAAGAUUGGACGGAAC AGAAGAACUGCUGGUCAAGCUGAACAGAGAAGACCUGGAGAAAGC AGAGAACAUCGACAACGGAAGCAUCCCGCACCAGAUCCACCUGGGAG AACUGCACGCAAUCCUGAGAAGACAGGAAGACUUCUACCCGUUCUGA AGGACAACAGAGAAAAGAUUCGAAAAAGAUCCUGACAUUCAGAAUCCCG UACUACGUCGACCGCUGGCAAGAGGAAACAGCAGAUUCGCAUGGAU GACAAGAAAGAGCGAAGAAACAUCACACCGUGGAACUUCGAAGAAG UCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGAAUGACA AACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCACAGC CUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAG UACGUCACAGAAGGAAUGAGAAAAGCCGGCAUCCUGAGCGGAGAAACA GAAGAAGGCAUUCGUCGACCUGCUGUUAAGACAAAACAGAAAGGUC CAGUCAAGCAGCUGAAGGAAGACUACUUAAGAAGAUUGAAUGCUUC GACAGCGUCGAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUG GGAACAUAACACGACCUGCUGAAGAUCAUCAAGGACAAGGACUCCUG GACAACGAAGAAAACGAAGACAUCUUGGAAGACAUCGUCCUGACACUG ACACUGUUCGAAGACAGAGAAAUGAUUCGAAGAAAGACUGAAGACAUA CGCACACCUGUUCGACGACAAGGUCAUGAAGCAGCUGAAGAGAAGAA GAUACACAGGAUGGGGAAGACUGAGCAGAAAAGCUGAUCAACGGAAUC AGAGACAAGCAGAGCGGAAAGACAAUCCUGGACUUCUGAAGAGCGCA CGGAUUCGCAAAACAGAAACUUAUGCAGCUGAUCCACGACGACGCCU GACAUUCAAGGAAGACAUCACAGAAAGGCACAGGUCAGCGGACAGGGAG ACAGCCUGCACGAACACAUCGCAAAACUGGCAGGAAGCCCGGCAAUCA AGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUCAAG GUCAUGGGAAGACACAAGCCGGAAAAACAUCGUAUCGAAAUGGCAAG AGAAAACAGACAACACAGAAGGGACAGAAGAACAGCAGAGAGAAAGAA UGAAGAGAAUCGAAGAAGGAUUAAGGAACUGGGAAGCCAGAUCCUG AAGGAACACCCGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUAC CUGUACUACCUGCAGAACGGAAGAGACAUGUACGUCGACCAGGAACUG GACAUCAACAGACUGAGCGACUACGACGUCGACCACAUUGUCCCGCAG AGCUUCCUGAAGGACGACAGCAUCGACAACAAGGUCCUGACAAGAAGC GACAAGAACAGAGGAAAGAGCGACAACGUCCCGAGCGAAGAAGUCGU CAAGAAGAUAGAACUACUGGAGACAGCUGCUGAACGCAAAGCUGA UCACACAGAGAAAAGUUCGACAACCUGACAAAAGGCAGAGAGAGGAGGA CUGAGCGAACUGGACAAGGCAGGAUUAUCAAGAGAGACGUGGUCGA AACAAGACAGAUCAAAAGCACGUCGACACAGAUCCUGGACAGCAGAAU GAACACAAAGUACGACGAAAAACGACAAGCUGAUCAGAGAAGUCAAGG UCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUC AGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGACG CAUACCUGAACGCAGUCGUCGGAACAGCACUGAUAAGAAGUACCCGA AGCUGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUUCACGACGUC AGAAAGAUCAUCGCAAGAGCGAACAGGAAUUCGAAAGGCAACAGC AAAGUACUUCUUCACAGCAACAUCAGAACUUCUUAAGACAGAAA UCACACUGGCAAAACGGAGAAAUCAGAAAAGAGACCGCUGAUCGAAACA AACGGAGAAACAGGAGAAAUCGUCUGGGACAAGGGAAGAGACUUCGC

Description	SEQ ID NO	Sequence
		AACAGUCAGAAAGGUCCUGAGCAUGCCGCAGGUCAAACUAGCUCAGAA GACAGAAAGUCAGACAGGAGGAUUCAGCAAGGAAAGCAUCCUGCCGA AGAGAAACAGCGCAAGCUGAUUCGCAAGAAAGAAAGCAUGGGGACCCG AAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCAUACAGCGUCCUG GUCGUCGCAAAAGGUCGAAAAGGGAAAGAGCAAGAACGUGAAGAGCGU CAAGGAACUCUGGGAAUACAAUUCAUUGGAAAGAAAGCAGCUCUGGAA AGAAGCCGUAUCGCUCCUGGAAGCAAGGGAUACAAGGAAGUACAAG AAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAA AACGGAAGAAAGAGAAUUCUGGCAAGCGCAGGAGAACUGCAGAAGGG AAACGAACUGGCACUGCCGAGCAAGUACGUAACUUCUGUACCUGGC AAGCCACUACGAAAGCUGAAGGGAAAGCCGGAAGACAACGAACAGA AGCAGUGUUCUGGAACAGCAAGCACAAGCACUACCUGGACGAAAUCAUCG AACAGAUACGCGAAUUCAGCAAGAGAGUCAUCCUGGCAGACGCAAAACC UGGACAAGGUCCUGAGCGCAUACAACAAGCACAGAGACAAGCCGAUCA GAGAACAGGCAGAAAACAUCAUCCACCGUUCACACUGACAAAACCUUG GAGCACCCGGCAGCAUUCAGUACUUCGACACAACAACUACGACAGAAAGA GAUACACAAGCAAAAGGAAGUCCUGGACGCAACACUGAACACCAGAGA GCAUCACAGGACUGUACGAAACAAGAAUUCGAUCUGAGCCAGCUGGGA GGAGACAGCGGAGGAAGCACAAACCUAGAGCGACAUCAUCGAAAAGGA AAGAGAAAGCAGCUGGUCAUCCAGGAAAGCAUCCUGAUGCUGCCGGA AAAGUGUACGAAGAGUACUCGGAACAAGCCGGAAGAGCAGCAUCCUG UCCACACAGCAUACGACGAAAGCACAGACGAAAACGUAUCGUCGUGA CAAGCGACGCACCGGAUACAAGCCGUGGGCAGUGGUCAUCCAGGACA GCAACGGAGAAAACAAGAUCAAGAUGCUGAGCGGAGGAAGCCCGAAG AAGAAGAGAAAGGUCUAAUAGUCUAGACAUCACAUUUAAAAGCAUCU CAGCCUACCAUGAGAAUAAAGAGAAAGAAAUGAAGAUCAAUAGCUUA UUAUCUCUUUUUCUUUUUCGUUGGUGUAAAGCCAACACCCUGUCUAA AAAACAUAAAUUUUUUAAUCAUUUUUGCCUUCUUUUCUCUGUGCUUCA AUUAAUAAAAAUGGAAAGAACCUUGAGAAAAAAGAAAAAAGAAAAA AAAAAAAAAAGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAACC AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAU
mRNA encoding UGI	975	GGGAGACCCAAGCUGGCUAGCUCCCGCAGUCGGCGUCCAGCGGCUCUG CUUGUUCGUGUGUGUGUCGUUGCAGGCCUUAUUCGGAUCCGCCACCAU GGGACCGAAGAAGAAGAGAAAGGUCGGAGGAGGAAGCAACAAACCUGU CGGACAUCUACGAAAAGGAAACAGGAAAGCAGCUGGUCUAUCCAGGAA UCGAUCCUGAUGCUGCCGGAAGAAGUCGAAGAAGUCAUCGGAACAA GCCGGAUUCGGACAUCUCCUGGUCCACACAGCAUACGACGAUUCGACAGA CGAAAACGUAUCGUCGUGACAUCGGACGCACCGGAUACAAGCCGUG GGCACUGGUAUCCAGGACUCGAACGGAGAAAAACAAGAUCAAGAUGA UGUGAUGAUCUAGACAUCACAUUUAAAAGCAUCUACGCCUACCAUGA GAUAAGAGAAAGAAAAUGAAGAUCAAUAGCUUAUUAUCUCUUAUUUU CUUUUUCGUUGGUGUAAAGCCAACACCCUGUCUAAAAAACAUAAAUU UCUUUAUUAUUUUUGCCUUCUUUUCUCUGUGCUUCAUUAUAAAAA UGGAAAGAACCUAGCAGUCUAG
	976-999	Not used
HD1 TCR insertion including ITRs	1000	ttggccactccctctctgcgcgtcgtcgtcactgaggccggggcgaccaaaggtcgcccgacgcccgggctttgcc cggcgcgccctcagtgcgcgagcgcgcgcagaggggagtggtcccaactccatcactaggggttctagatcttggc aacataccataaacctccacttctgctaagtcaggccctaagttggggagaccactccagatgcaagtgtacagtttgc ttgctgggctttttcccatgcctgcctttactctgccagagttatattgctggggttttgaagaagatcctattataaataaaga ataagcagttatttaagtagccctgcatttcaggtttccttgatgtgcagcgccagcgctggccgtgaacgttctactgaaat catggcctctggccaagattgatagcttgctgtcctgtgctccagtcctacagcagcagctggtttctaatagatgctat ttccgtataaagcatgagaccgtgactgtccagccccacagagccccgccctgtccatcattgcatcgtgcatcagtcacg cctgggttggggcaagaggggaatgagatcatgtcctaaccctgatcccttctgccacagatccagatccagcctgacc ctgcggctccgtgtgccgtcagttgggcagagcgcacatcggccacagtcggcaggaagtgtggggggaggggtcggc aattgaaccggtgtcctagagaaggtggcgcggggtaaacctgggaaagtgtatgtcgtgactggctccgctttttcccg gggtggggggagaacggtatataagtgcagtagtcgcgtgaacgttcttttgcgaacgggtttgccggcaaacacag gtaaatcccctgtctgtgttcccgccggcctggcctctttacgggttatggcccttgcctgtcccttgaatttcttccacgccc

209

Description	SEQ ID NO	Sequence
		AGGCTCTGGACCAGGCGCCCCGGGGCCGGGCTGTGAGGAGGAGGGTG CAACCTCGGAGCAGCTTCTTCTGGAAAAGGCCGGCCAGCAGCCCCCGGA GGGAGCAGGGTCCGCCGGTGCCGGTCCGCACGTGCTGTGCAGGAAGCC ATCTTGCGCTTCCAGCTCCTCGAAGCCGTCTAGGATGAGCAGAACGCGG TCAGGTCTCTTCAAGATGTGGCTGAAAACCTCATCGGCCGCCACGAGTG GCTGTGGGCCCCAGGGAGAAGAGCAGATCCTGCAGGCCATAGGCATCCCC CGGACGGTTCAAGGGGGATACCCCTAGAGCCCCAGCTGGTTCTTTCCG CCTCAGAAGCCATAGAGCCCACCGCATCCCCAGCATGCCTGCTATTGTCT TCCCAATCCTCCCCCTTGCTGTCTGCCCCACCCACCCCCAGAATAGA ATGACACCTACTCAGACAATGCGATGCAATTTCTCATTTTATTAGGAAA GGACAGTGGGAGTGGCACCTTCCAGGGTCAAGGAAGGCACGGGGGAGG GGCAAACAACAGATGGCTGGCAACTAGAAGGCACAGTCGAGGACTAGT CTACTTGTACAGCTCGTCCATGCCGCCGGTGGAGTGGCGGCCCTCGGCGC GTTCTGTAAGTGTCCACGATGGTGTAGTCTCGTTGTGGGAGGTGATGTCC AATTGATGTTGACGTTGTAGGCGCCGGGCAGCTGCACGGGCTTCTTGGC CTTGATAGGTGGTCTTGACCTCAGCGTCGTAGTGGCGCCGCTCTTCACT TCAGCCTCTGCTTGATCTCGCCCTTCAGGGCGCCGCTCTCGGGGTACATC CGCTCGGAGGAGGCCTCCAGCCCATGGTCTTCTTCTGCATTACGGGGCC GTCGGAGGGGAAGTTGGTGCCGCGCAGCTTCACTTGTAGATGAACTCG CCGTCTGGAGGGAGGAGTCTGGGTACGGTCACACGCCCGCCGTCTCT CGAAGTTCATCAGCGCTCCCACTTGAAGCCCTCGGGGAAGGACAGCTT CAAGTAGTCGGGGATGTCCGGCGGGGTGCTTACGTAGGCCTTGGAGCCG TACATGAACTGAGGGGACAGGATGTCCAGGCGAAGGGCAGGGGGCCA CCCTTGGTCACCTTCAGCTTGGCGGTCTGGGTGCCCTCGTAGGGGCGGCC CTCGCCCTCGCCCTCGATCTCGAACTCGTGGCCGTTCACGGAGCCCTCCA TGTGCACCTTGAAGCGCATGAACTCCTTGATGATGGCCATGTTATCTCC TCGCCCTTGCTACCATGGTGGCGGCCGCATCAGACACCTGAAATGGA AGAAAAAACTTTGAACCACTGTCTGAGGCTTGAGAATGAACCAAGATC CAAACCTAAAAAGGGCAAATTCGAAGGAGAATTACATCAAGTGCCAAGC TGGCCTAACTTCAGTCTCCACCCACTCAGTGTGGGGAAACTCCATCGCAT AAAACCCCTCCCCCAACCTAAAGACGACGTACTCCAAAAGCTCGAGAA CTAATCGAGGTGCCTGGACGGCGCCCGTACTCCGTGGAGTCACATGAA GCGACGGCTGAGGACGGAAAGGCCCTTTTCTTTGTGTGGGTGACTCAC CCGCCGCTCTCCGAGCGCCGCGTCTCCATTTTGTAGCTCCCTGCAGCA GGGCCGGGAAGCGGCCATCTTTCCGCTCAGCAACTGGTGCCGACCGG CCAGCCTTGCCGCCAGGGCGGGCGATACACGGCGGCGCGAGGCCAG GCACAGAGCAGGCCGGCCAGCTTGAGACTACCCCGTCCGATTCTCGG TGGCCGCGCTCGCAGGCCCGCCTCGCCGAACATGTGCGCTGGGACGCA CGGGCCCCGTGCGCGCCGCGGCCCAAAAACCGAAATACCACTGTGCA CATCTTGGCCCGCATTTACAAGACTATCTTGCCAGAAAAAAGCGTCGC AGCAGGTCAATCAAAAATTTTAAATGGCTAGAGACTTATCGAAAGCAGCG AGACAGGCGCGAAGGTGCCACCAGATTTCGACGCGCGGCCCCAGCGCC CAAGCCAGGCCTCAACTCAAGCACGAGGCGAAGGGGCTCCTTAAGCGCA AGGCTCGAACTCTCCACCCACTTCCAACCCGAAGCTCGGGATCAAGA ATCACGTACTGCAGCCAGGGGCGTGGAAGTAATTCAAGGCACGCAAGGG CCATAACCCGTAAAGAGGCCAGGCCCGCGGGAACCACACACGGCACTTA CCTGTGTTCTGGCGGCAAACCCGTTGCGAAAAAGAACGTTACGGCGAC TACTGCACTTATATACGGTTCTCCCCACCTCGGGAAAAAGGCGGAGC CAGTACACGACATCACTTTCCAGTTTACCCCGCGCCACCTTCTTAGGC ACCGGTTCAATTGCCGACCCCTCCCCCAACTTCTCGGGGACTGTGGGCG ATGTGCGCTCTGCCACTGACGGGCACCGGAGCCCAATGGCAGGGGACA GAGAAGACAAAGTCGTAAGGGGAAGCCGGCCACAAGCCAGGCCCGG CTACTGCCCCAGCCCAATAGCTCTTGCCCTGACCAGCTTTGCCAGCAC AGCAATCACTCGTGTCTACGCGGCCGCGGCTGCTCTTGGCAGCCAAC AGCACCTCAGCCAGGCCTCCTTGGGCCAGCTGCCGTTCTGCCAGTCCGG GGTGGCCAGTTCCCGCTCCAGGCTCTTGCTGCTGCTCTCTCCAGCCTGG CCTGCACCAGATCCACCTCCACTAGGATGCCATCCGGGCCGTGCGGGCTC GGCACCATACGTGTCTGCACTGAGCGGTAGAAGTGTCTCCACCGGCTCT

Description	SEQ ID NO	Sequence
		GCAAAGGCCAGGGGCGTGTACAGGTGGGGGTATGTGAGAGGCAGGGCC AGGGCCAGCCACCACAAGGCCAGCACTGCCACCATCATTTACATCTGTT CCCCACACAGTTTTTTTGTGTTGTTTGTGTTTGTGTTTGTGAGAGATCTAG GAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCT CACTGAGGCCGCCCCGGGCAAAGCCCCGGGCGTCGGGCGACCTTTGGTCGC CCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAA
pAAV_CITA- EF1a-mCherry- G13676	1003	TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACC AAAGGTCGCCCCGACGCCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAG CGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCTAGTA TCTGCATGAGCCCAGGAGGTTGAGGTTGCAGCGAGCTGTGATCACACCA CTGCATTCCAGCCTGGGCAAAAAAGCCAGACCCTGTCTCAAAACAAAA AAAACAAACAAACAAAAAACTGTGTGGGGAACAGATGTAAATGATGG TGGCAGTGCTGGCCTTGTGGTGGCTGGCCCTGGCCCTGCCTCTCACATAC CCCCACCCTGACACGCCCCCTGGCCTTTGCAGAGCCGGTGGAGCAGTTCTA CCGCTCACTGCAGGACACGTATGGTGGCGAGCCCCGAGGCCCGGATGGC ATCCTAGTGGAGGTGGATCTGGTGCAGGCCAGGCTGGAGAGGAGCAGCA GCAAGAGCCTGGAGCGGGAACCTGGCCACCCCGGACTGGGCAGAACGGC AGCTGGCCCAAGGAGGCCTGGCTGAGGTGCTGTTGGCTGCCAAGGAGCA CCGGCGGCCGCGTGAGACACGAGTGATTGCTGTGCTGGGCAAAGCTGGT CAGGGCAAGAGCTGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCG CCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTGCGCAATTGAACCGGTG CCTAGAGAAGGTGGCGCGGGGTAACTGGGAAAGTGATGTCGTGTAAGT GCTCCGCCTTTTTCCCCGAGGGTGGGGGAGAACCGTATATAAGTGACAGTA GTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGT AAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCC CTTGCGTGCCTTGAATTACTTCCACGCCCCCTGGCTGCAGTACGTGATTCT TGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGC GCTTAAGGAGCCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCTTGGGCG CTGGGGCCGCGCGGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTG CTTTCGATAAGTCTCTAGCCATTTAAAAATTTTGTATGACCTGTGCGGACG CTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATGTGCACAC TGGTATTTTCGGTTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTGCCTCC AGCGCACATGTTTCGGCGAGGCGGGGCGCTGCGAGCGCGGCCACCGAGAAT CGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTC GCGCCGCGGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTGCG CACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGG GAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTC ACCCACACAAAGGAAAAAGGGCCTTTCCGTCTCAGCCGTCGCTTCATGT GACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGA GCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATG GAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGC ACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTGGATCTTGGT TCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAAG TGTGCTGATGCGGCCGCCACCATGGTGAGCAAGGGCGAGGAGGATAACA TGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGGCTC CGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCC CTACGAGGGCAGCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCC CTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAA GGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCTT TCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGG CGTGGTGACCGTGACCCAGGACTCCTCCCTCCAGGACGGCGAGTTCATCT ACAAGGTGAAGCTGCGCGGCACCAACTTCCCTCCGACGGCCCCGTAAT GCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCC GAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAG GACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAG AAGCCCGTGCACTGCCCGGCGCCTACAACGTCAACATCAAGTTGGACA TCACCTCCACAAACGAGGACTACACCATCGTGAACAGTACGAACGCGC CGAGGGCCGCCACTCCACGGCGGCATGGACGAGCTGTACAAGTAGACT

Description	SEQ ID NO	Sequence
		AGTCTCTGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCC CCGTGCCTTCTTTGACCCTGGAAGGTGCCACTCCCACTGTCTTTCTTAA TAAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCTATTCT GGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAA TAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGCTTCTGAGGCGGAA AGAACCAGCTGGGGCTCTAGGGGGTATCCCCATTGGGCTGGGGCAGTGA GCCGGGCCTGGGCTTGTGGCCGGCTTCCCCAGTACGACTTTGTCTTCTCT GTCCCTGCCATTGCTTGAACCGTCCGGGGGATGCCTATGGCCTGCAGGA TCTGCTCTTCTCCCTGGGCCCACAGCCACTCGTGGCGGGCGATGAGGTTT TCAGCCACATCTTGAAGAGACCTGACCGCTTCTGCTCATCCTAGACGGC TTCGAGGAGCTGGAAGCGCAAGATGGCTTCTGACAGCAGCAGTGGCGAC CGGCACCGGGCGGAGCCCTGCTCCCTCCGGGGGCTGCTGGCCGGCCTTTTC CAGAAGAAGCTGCTCCGAGGTTGCACCTCCTCCTCACAGCCCGGCCCC GGGGCCGCTGGTCCAGAGCCTGAGCAAGGCCGACGCCCTATTTGAGCT GTCCGGCTTCTCCATGGAGCAGGCCCAGGCATACGTGATGCGTACTTTG AGAGCTCAGGGATGACAGAGCAGCAAGACAGAGCCAGATCTAGGAACC CCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTG AGGCCGCCGGGCAAAGCCCGGGCGTGGGGCGACCTTTGGTCGCCCGGC CTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAA
Lentiviral genome encoding HLA-E expressed by an EF1a promoter	1004	gcgatcgagtaatacaattacggggctcattagttcatagcccatataggagttccggttacataacttacggtaaatggc ccgctggctgaccgcccacgaccccccgccttgacgtcaataatgacgtatgttcccatagtaacccaataggga cttccattgacgtcaatgggtggagtatttacggtaaacgcccacttgccagtcacatcaagtgtatcatatccaagtacg ccccctattgacgtcaatgacggtaaatggccgctggcattatgccagtcacatgacctatgggacttccacttggc agtacatctacgtattagtcacgtattaccatgGTGATGCGGTTTTGGCAGTACATCAATGG GCGTGATAGCGGTTTGACTCACGGGGATTTCGAAGTCTCCACCCCATTTG ACGTCAATGGGAGTTTGTGTTTGGCACCAAAATCAACGGGACTTTCCAAA ATGTCGTAACAACTCCGCCCCATTGACGCAAAATGGGCGGTAGGCGTGTA CGTGTTGGAGGTCTATATAAGCAGAGCTcgttttagtaaccgggtctctctgttagaccagat ctgagcctgggagctctctggctaactagggaaccactgcttaagcccaataaagcttgccttgagtgctcaagtgt gtgtgccgtctgtgtgactctgtaactagagatccctcagacccttttagtcagttgtggaataatctctagcagttggcg cccgaacaggacgtgaaagcgaaagggaaccagagctctctcgacgcaggactcggctgtgtaagcgccacg gcaaggaggcaggggcgccgactggtgagtagcgcacaaatgtttagtagcggaggctagaaggagagagatgggt gcgagagcgtcagttaataagcggggagaattagatcgcgatgggaaaaaattcggttaagggcagggggaaagaaa aaataataaataaacatatagtatggcaagcaggagtagaacgattcgcagttaatcctggcctgttagaacaatca gaaggctgtagacaatactgggacagctacaaccatcccttcagacaggatcagaagaacttagatcattatataatca gtagcaaccctctattgtgtcatcaaaagatagagataaaagacaccaaggaaagctttagacaagatagaggaaagc aaaacaaaagtaagaccaccgacagcaagcgccgctgacttccagacctggaggaggagatagagggaacttg gagaagtgtaattatataataaagtagtaaaattgaaccattagagtagcaccaccaaggcgaaggaagagatg gtgcagagagaaaaagagcagtggaataggagctttgtccttgggttcttgggagcagcaggaaagcactatgggc gcagcctcaatgacgctgacggtacaggccagacaattattgtctgttatagtcagcagcagacaataattgctgagggc tattgaggcgcaacagcatctgttgcaactcacagctctgggcatcaagcagctccaggcaagaatcctgctgtggaa agatacctaaagatcaacagctcctggggatttggggttgccttggaaaactcatttgcaccactgctgtgccttgaatg ctagttggagtaataaattcttggacaagatttggaaacacagacctggatggagtgggacagagaaatfaacaattaca caagcttaatacactccttaattgaagaatcgcaaaaccagcaagaaaaaatgaacaagaatttggaaatagataaat gggcaagtttggaaattggttaacatacaaatggctgtgtatataaaattattcataatagtagtaggagcgttggtag gttaagaatagttttgctgtacttctatagtaaatagagtaggcagggaattaccattatcgtttcagaccacacccc aaccggaggggagcccgacaggcccggaaggaatagaagaagaaaggtagagagagagacagagacatccattcg attagtagacgagatcgacggatcggtaacttttaaaagaaagggggattgggggtacagtgccaggggaaaga atagtagacataatagcaacagacatacaactaaaattacaaaaaacaattacaaaaattcaaaatttggctcccgat cgttgcgttacacacacaattactgctgacgagtgtagccttcccacagtccccgagaagttggggggagggtcgcgc aattgaaccgggtcctagagaagggtggcggggttaactgggaagtgtatgctgtgactggctccgcttttccga gggtgggggagaaaccgtatataagtcagtagtcgctggaacgttcttttcgaacgggttggccgacagaacacag gtaagtgcctgtgtgttcccgccgctggttctttacgggttatggccttgcgtgccttgaattactccacgcccc tggctgcagtacgtgattcttgatcccgagcttcgggttgaagtgggtgggagagtgtaggccttgcgtcttaaggagc cccttcgctcgtgtgtgagttgagccgtggttggcgcttggggcgccgctgtagcaatctgtgtgacaccttgcgcct gtctcgtcgtttcgataagtcctagccatttaaaattttgatgacctgctgcgacgttttttgcgaagatagcttgaat atcggggccaagatgtgcactgtatttctgttttggggccggggcgacggggccgctgcttccacgca catgttcggcgaggcgggcctgcgagcgcggccaccgagaatcgacgggggtagctcaagctggcgccgtgc

Description	SEQ ID NO	Sequence
		tctggtgcctggcctcgcgcgcgtgtatcggccgcccggcgcccaaggctggccggctggcaccagttgcgtg agcggaaagatggccgcttcccgccctgctgcaggagctcaaaatggagacgcggcgctcggagagcgggc gggtgagtcaccacacaaaggaaaaggcccttccgtcctcagccgtcgtctcatgtactccaggagtagccggc gccgtccaggcacctcgattagttctcagcttttgagtagctgtcttttaggtggggaggggtttatgcgatggag ttccccacactgagtggtggagactgaagtaggcccagcttggcactgatgtaattccttgaatttgccttttgag tttgatcttggttctcctcaagcctcagacagtggtcctcaagtttttttCTTCCATTTCAGGTGTCTGTGAi ctagacgccaccATGTCTCGCTCCGTGGCCTTAGCTGTGCTCGCGCTACTCTCTC TTTCTGGCCTAGAGGCTGTTATGGCTCCGCGGACTTAAATTTTAGTGGT GGCGGATCCGGTGGAGGCGGTTCTGGTGGAGGCGGCTCCATCCAGCGTA CGCCAAAGATTTCAGGTTTACTACGTCATCCAGCAGAGAATGGAAGT AAATTTCTGAATTGCTATGTGTCTGGGTTTCATCCATCCGACATTGAAG TTGACTTACTGAAGAATGGAGAGAGAATTGAAAAAGTGGAGCATTGAGA CTGTCTTTTACGAAGGACTGGTCTTTCTATCTCTTGTACTACACTGAATT CACCCCACTGAAAAAGATGAGTATGCCGTGCCGTGTGAACCATGTGACT TTGTCACAGCCCAAGATAGTTAAGTGGGATCGCGACATGGTGGTGGCG GTTCTGGTGGTGGCGGTAGTGGCGCGCGGAGGAAGCGGTGGTGGCGGTT CGGATCTCACTCCTTGAAGTATTTCCACACTTCCGTGTCCCGGCCCGGCC GCGGGGAGCCCGCTTCATCTGTGGGCTACGTGGACGACACCCAGTT CGTGCGCTTCGACAACGACGCCGCGAGTCCGAGGATGGTGCCGCGGGCG CCGTGGATGGAGCAGGAGGGGTACAGATATTGGGACCGGGAGACACGG AGCGCCAGGGACACCGCACAGATTTTCCGAGTGAACCTGCGGACGCTGC GCGGCTACTACAATCAGAGCGAGGCGGGTCTCACACCCTGCAGTGGAT GCATGGCTGCGAGCTGGGGCCCGACAGGCGCTTCCTCCGCGGGTATGAA CAGTTCGCTACGACGGCAAGGATTATCTCACCTGAATTGAGGACCTGC GCTCTGGACCGCGGTGGACACGGCGGCTCAGATCTCCGACAAAAGTC AAATGATGCCTCTGAGGCGGAGCACCAGAGAGCCTACCTGGAAGACACA TGCGTGGAGTGGCTCCACAAATACCTGGAGAAGGGGAAGGAGACGCTG CTTCACCTGGAGCCCCCAAAGACACACGTGACTACCAACCCATCTCTGA CCATGAGGCCACCCTGAGGTGCTGGGCTCTGGGCTTCTACCTGCGGAG ATCACACTGACCTGGCAGCAGGATGGGGAGGGCCATACCCAGGACACG GAGCTCGTGGAGACCAGGCCTGCTGGGGATGGAACCTTCCAGAAGTGGG CAGCTGTGGTGGTGCCTTCTGGAGAGGAGCAGAGATACACGTGCCATGT GCAGCATGAGGGGCTACCCGAGCCCGTCACCCTGAGATGGAAGCCGGCT TCCCAGCCCACCATCCCCATCGTGGGCATCATTGCTGGCCTGGTTCTCT TGGATCTGTGGTCTCTGGAGCTGTGGTTGCTGTGTGATATGGGAAGA AGAGCTCAGGTGGAAGAGGAGGAGCTACTATAAGGCTGAGTGGAGCG ACAGTGCCAGGGGTCTGAGTCTACAGCTTGTAaagtagaagttgtctcctgca ctgactgactgataaatcatttctgtagccgagccctctgtagaagttgtctcctctgactgactgataaat cgatttctgtagccgagccctctgtagctttagtactgactgactgacAAATCAACCTCTGGATTACAA AATTTGTGAAAGATTGACTGGTATTCTTAACTATGTTGCTCCTTTGACGT ATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTAT GGCTTTCATTTCTCCTCCTTGATAAATCCTGGTTGCTGTCTCTTTATGA GGAGTTGTGGCCCGTTGTGAGCAACGTGGCGTGGTGTGCACTGTGTTT CTGACGCAACCCCACTGGTTGGGGCATTGCCACCACCTGTGAGCTCCTT TCCGGGACTTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACCTATCGC CGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGGCACTGAC AATTCCGTGGTGTGTGCGGGGAAGCTGACGTCTTTCCATGGCTGCTCGC CTGTGTTGCCACCTGGATTCTGCGCGGGACGTCTCTGTGCTACGCTCCCTT CGGCCCTCAATCCAGCGGACCTTCTTCCCGCGGCTGCTGCGCGCTCTG CGGCCTCTTCCGCGTCTTTCGCTTTCGCCCTCAGACGAGTCGGATCTCCCT TTGGGCcgccctcccccgctggaattcagctcggtagctttaaagaccaatgacttacaaggcagctgtagatcttag ccactttttaaagaaaaggggggactggaagggttaattcactccaacgaagacaagatctgttttctgtactgg gtctctgtggttagaccagatctgagccctgggagctctctggtactaggaaccactgcttaagcctcaataaagctt gccttgagtgctcaagtagtgtgtgcccgtctgtgtgtgactctggtactagagatccctcagacccttttagtcagtgtg gaaaatcttagcagctctgccaacgtgagcaccgtgctgacctcaaatatcgttaagctggagcctgggagccggc ctggccctccgcccccccccccccgagccacccctggctttgaataaagctgagtgagtgccgacagtgcccg tgaggtctctgactgaggtgagggccggcgttagggacacgtccgtgacgtgcccagggccctgtgacgtg caaggagacaggccttagccctgagggcctaactccgccatcccgccctaactccggccagtccgccattctcggcc

Description	SEQ ID NO	Sequence
		tcatggctgactaatTTTTTtattatgcagaggccgaggccgctcggcctctgagctattccagaagtagtgaggacgct ttttggaggccgaggcttttgcacaaagatcgaacaagagacaggacctgcagggttaataaattaaatcatgtgagcaaa agggccagcaaaaggccaggaaccgtaaaaaggccgctgtgctggcgtttttccatagctccgccccctgacgagca tcacaaaaatcgacgctcaagtcagagggtggcgaaccgacaggactataaagataaccaggcgtttccccctggaag ctccctcgtgcgtctcctgttccgacctgccgttaccggataacctgtccgctttcccttcgggaagcgtgcgcttt ctcatagctcacgctgtaggtatctcagttcgggtgtaggtcgttcgctcaagctgggctgtgtgcacgaacccccgttca gcccagccgtcgccttatccggtaactatcgtctgagtcacccggtaagacacgacttatccactggcagcag ccactggtaacaggattagcagagcaggtatgtagcgggtgctacagagttctgaagtgtggtccctaactacggctac actagaagaacagtafttggatctcgcgtctgctgaagccagttaccttcgaaaaagagttgtagctcttgatccggca acaaaaccaccgctggtagcgggtgttttttttgcgaagcagcagattacgcgcagaaaaaaagatctcaagaagat cctttgatcttttctacggggtcgtgacgctcagtggaacgaaaactcacgttaagggttttggatgagattcaaaaag gatcttcacatagatccttttaataaaaaatgaagtttaaatcaatcaaaagtatatagtaaaactgtgctgacagtacc aatgcttaatcagtgaggcacctatctcagcgtatctgtctatctgttcacatagttgcatftaaatggccgctggcgc gccgtttaaacctagatattgatagctgacggtcaacgtataatcagagtcctagtttgcacacatctatcaagagacag gatcagcagagggttccgcatgagttatcaacattccgtcgtcccttattccctttttggcgttttgccttctgtttttg ctacccagaaacgctggtgaaagttaaagatgctgaagatcagttgggtgcgcgagtggttcatcgaactgcatct caacagcggtaagatccttgagagtttgcctccgaagaacgcttccaatgatgagcattttaaagttctgctatgtggc gcggtattatccgtattgacgcggcgcaagagcaactcggcgcgcatacactattctcagaatgactgtggtgagtatt caccagtcacagaaaagcatcttacgcatggcatgacagtaagagaattatgcagtgtgccataacctgagtataac actgcggccaacttactctgacaacgattggaggaccgaaggagctaaccgctttttgcacaacatgggggatcatgta actcgccttgatcgttgggaaccggagctgaatgaagccatacacaacgacgagcgtgacaccagatgcctgtagca atggcaacaaccttgcgtaaactattaactggcgaactacttactctagcttccggcaacagttgatagactggatggag gcggataaagttgagggaccacttctcgcctcggcccttccggctgggtgtttattgctgataaatctggagccggtag cgtggtctcgcggatcattgcagcactggggccagatggtaagccctcccgtatcgtagttatctacacgacggggag tcaggcaactatggatgaacgaatagacagatcgtgagataggtgctcactgattagcattgtaaccgatttagg tgcatggcgcaaaaaaatgacctgatgcgacgtcgcgtcttatactccacatatgccagattcagcaacggatag ggcttcccaacttgccttccatcagtgctccttaccagaaattatccttaagatcccgaatcgttttaaac
Exemplary shortened SpyCas9 guide RNA	1005	NNNNNNNNNNNNNNNNNNNNNNGUUUUAGAGCUAGAAAUAGCAAGUUA AAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGGUGCU
Exemplary shortened SpyCas9 modified guide RNA	1006	mN*mN*mN*NNNNNNNNNNNNNNNNNNNGUUUUAGAmGmCmUmAmGmAm AmAmUmAmGmCAAGUUA AAAUAAGGCUAGUCCGUUAUCACGAAAGGG CACCGAGUCGGmUmGmC*mU
G023521 Exemplary 91-mer full sequence	1007	CGCCCAGGUCCUCACGUCUGGUUUUAGAGCUAGAAAUAGCAAGUUA AAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGGUGCU
G023521 Exemplary 91-mer modified sequence	1008	mC*mG*mC*CCAGGUCCUCACGUCUGGUUUUAGAmGmCmUmAmGmAmA mAmUmAmGmCAAGUUA AAAUAAGGCUAGUCCGUUAUCACGAAAGGGC ACCGAGUCGGmUmGmC*mU
Guide scaffold 90-mer	1009	GUUUUAGAGCUAGAAAUAGCAAGUUA AAAUAAGGCUAGUCCGUUAUC ACGAAAGGGCACCGAGUCGGUGC
Guide scaffold 90-mer with modification	1010	GUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUUA AAAUAAGG CUAGUCCGUUAUCACGAAAGGGCACCGAGUCGG*mU*mG*mC
Guide scaffold 90-mer with modification	1011	GUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUUA AAAUAAGG CUAGUCCGUUAUCAmCmGmAmAmAmGmGmCmAmCmCmGmAmGmU mCmGmG*mU*mG*mC
Guide scaffold 88-mer with modification	1012	GUUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUUA AAAUAAGG CUAGUCCGUUAUCAACUUGGCACCGAGUCGG*mU*mG*mC
Guide scaffold 88-mer	1013	GUUUUAGAGCUAGAAAUAGCAAGUUA AAAUAAGGCUAGUCCGUUAUC AAAAUGGCACCGAGUCGGUGC

Description	SEQ ID NO	Sequence
Guide scaffold 88-mer with modification	1014	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAAAUGGCACCGAGUCGG*mU*mG*mC
Guide scaffold 88-mer with modification	1015	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmAmAmUmGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC
Guide scaffold	1016	GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmA mCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU
Guide scaffold	1017	mN*mN*mN*NNNNNNNNNNNNNNNNNNNGUUUAGAmGmCmUmAmGmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU

(In each of the sequences in the Table above or described herein, a modified sequence can be unmodified or modified in an alternative way.)

EXAMPLES

[00380] The following examples are provided to illustrate certain disclosed embodiments and are not to be construed as limiting the scope of this disclosure in any way.

Example 1. General Methods

1.1. Preparation of lipid nanoparticles

[00381] In general, the lipid components were dissolved in 100% ethanol at various molar ratios. The RNA cargos (*e.g.*, Cas9 mRNA and sgRNA) were dissolved in 25 mM citrate buffer, 100 mM NaCl, pH 5.0, resulting in a concentration of RNA cargo of approximately 0.45 mg/mL.

[00382] The lipid nucleic acid assemblies contained ionizable Lipid A ((9Z,12Z)-3-((4,4-bis(octyloxy)butanoyl)oxy)-2-(((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl octadeca-9,12-dienoate, also called 3-((4,4-bis(octyloxy)butanoyl)oxy)-2-(((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl (9Z,12Z)-octadeca-9,12-dienoate), cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:3 molar ratio, respectively. The lipid nucleic acid assemblies were formulated with a lipid amine to RNA phosphate (N:P) molar ratio of about 6, and a ratio of gRNA to mRNA of 1:1 or 1:2 by weight.

[00383] LNP compositions were prepared using a cross-flow technique utilizing impinging jet mixing of the lipid in ethanol with two volumes of RNA solutions and one volume of water. The lipids in ethanol were mixed through a mixing cross with the two volumes of RNA solution. A fourth stream of water was mixed with the outlet stream of the cross through an inline tee (*See* WO2016010840 Fig. 2). The LNP compositions were held for 1 hour at room temperature, and further diluted with water (approximately 1:1 v/v). LNP compositions were

concentrated using tangential flow filtration on a flat sheet cartridge (Sartorius, 100kD MWCO) and buffer exchanged using PD-10 desalting columns (GE) into 50 mM Tris, 45 mM NaCl, 5% (w/v) sucrose, pH 7.5 (TSS). Alternatively, the LNP's were optionally concentrated using 100 kDa Amicon spin filter and buffer exchanged using PD-10 desalting columns (GE) into TSS. The resulting mixture was then filtered using a 0.2 μ m sterile filter. The final LNP was stored at 4°C or -80°C until further use.

1.2. In vitro transcription (“IVT”) of mRNA

[00384] Capped and polyadenylated mRNA containing N1-methyl pseudo-U was generated by in vitro transcription using a linearized plasmid DNA template and T7 RNA polymerase. Plasmid DNA containing a T7 promoter, a sequence for transcription, and a polyadenylation sequence was linearized by incubating at 37°C for 2 hours with XbaI with the following conditions: 200 ng/ μ L plasmid, 2 U/ μ L XbaI (NEB), and 1x reaction buffer. The XbaI was inactivated by heating the reaction at 65°C for 20 min. The linearized plasmid was purified from enzyme and buffer salts. The IVT reaction to generate modified mRNA was performed by incubating at 37°C for 1.5-4 hours in the following conditions: 50 ng/ μ L linearized plasmid; 2-5 mM each of GTP, ATP, CTP, and N1-methyl pseudo-UTP (Trilink); 10-25 mM ARCA (Trilink); 5 U/ μ L T7 RNA polymerase (NEB); 1 U/ μ L Murine RNase inhibitor (NEB); 0.004 U/ μ L Inorganic E. coli pyrophosphatase (NEB); and 1x reaction buffer. TURBO DNase (ThermoFisher) was added to a final concentration of 0.01 U/ μ L, and the reaction was incubated for an additional 30 minutes to remove the DNA template. The mRNA was purified using a MegaClear Transcription Clean-up kit (ThermoFisher) or a RNeasy Maxi kit (Qiagen) per the manufacturers' protocols. Alternatively, the mRNA was purified through a precipitation protocol, which in some cases was followed by HPLC-based purification. Briefly, after the DNase digestion, mRNA is purified using LiCl precipitation, ammonium acetate precipitation and sodium acetate precipitation. For HPLC purified mRNA, after the LiCl precipitation and reconstitution, the mRNA was purified by RP-IP HPLC (see, *e.g.*, Kariko, et al. Nucleic Acids Research, 2011, Vol. 39, No. 21 e142). The fractions chosen for pooling were combined and desalted by sodium acetate/ethanol precipitation as described above. In a further alternative method, mRNA was purified with a LiCl precipitation method followed by further purification by tangential flow filtration. RNA concentrations were determined by measuring the light absorbance at 260 nm (Nanodrop), and transcripts were analyzed by capillary electrophoresis by Bioanalyzer (Agilent).

[00385] *Streptococcus pyogenes* (“Spy”) Cas9 mRNA was generated from plasmid DNA encoding an open reading frame according to SEQ ID NOs: 801-803 (see sequences in **Table 4**). BC22n mRNA was generated from plasmid DNA encoding an open reading frame according to SEQ ID NOs: 804-805. BC22 mRNA was generated from plasmid DNA encoding an open reading frame according to SEQ ID NO: 806. UGI mRNA was generated from plasmid DNA encoding an open reading frame according to SEQ ID NOs: 807-808. When SEQ ID NOs: 801-808 are referred to below with respect to RNAs, it is understood that Ts should be replaced with Us (which were N1-methyl pseudouridines as described above). Messenger RNAs used in the Examples include a 5’ cap and a 3’ polyadenylation region, *e.g.*, up to 100 nts, and are identified by the SEQ ID NOs: 801-808 in **Table 4**.

1.3. Next-generation sequencing (“NGS”) and analysis for on-target editing efficiency

[00386] Genomic DNA was extracted using QuickExtract™ DNA Extraction Solution (Lucigen, Cat. QE09050) according to the manufacturer's protocol.

[00387] To quantitatively determine the efficiency of editing at the target location in the genome, deep sequencing was utilized to identify the presence of insertions and deletions introduced by gene editing. PCR primers were designed around the target site within the gene of interest (*e.g.*, TRAC) and the genomic area of interest was amplified. Primer sequence design was done as is standard in the field.

[00388] Additional PCR was performed according to the manufacturer's protocols (Illumina) to add chemistry for sequencing. The amplicons were sequenced on an Illumina MiSeq instrument. The reads were aligned to the human reference genome (*e.g.*, hg38) after eliminating those having low quality scores. Reads that overlapped the target region of interest were re-aligned to the local genome sequence to improve the alignment. Then the number of wild type reads versus the number of reads which contain C-to-T mutations, C-to-A/G mutations or indels was calculated. Insertions and deletions were scored in a 20 bp region centered on the predicted Cas9 cleavage site. Indel percentage is defined as the total number of sequencing reads with one or more base inserted or deleted within the 20 bp scoring region divided by the total number of sequencing reads, including wild type. C-to-T mutations or C-to-A/G mutations were scored in a 40 bp region including 10 bp upstream and 10 bp downstream of the 20 bp sgRNA target sequence. The C-to-T editing percentage is defined as the total number of sequencing reads with either one or more C-to-T mutations within the 40 bp region divided by the total number of sequencing reads, including wild type. The percentage of C-to-A/G mutations are calculated similarly.

Example 2. Screen 1 of CIITA Guide RNAs

[00389] CIITA guide RNAs were screened for efficacy in T cells by assessing loss of MHC class II cell surface expression. The percentage of T cells negative for MHC class II protein (“% MHC class II negative”) was assayed following CIITA editing.

2.1. T cells editing with ribonucleoprotein

[00390] Cas9 editing activity was assessed using electroporation of Cas9 ribonucleoprotein (RNP). Upon thaw, Pan CD3⁺ T cells were plated at a density of 0.5×10^6 cells/mL in T cell RPMI media composed of RPMI 1640 (Invitrogen, Cat. 22400-089) containing 5% (v/v) of fetal bovine serum, 1x Glutamax (Gibco, Cat. 35050-061), 50 μ M of 2-Mercaptoethanol, 100 μ M non-essential amino acids (Invitrogen, Cat. 11140-050), 1 mM sodium pyruvate, 10 mM HEPES buffer, 1% of Penicillin-Streptomycin, and 100 U/mL of recombinant human interleukin-2 (Peprotech, Cat. 200-02). T cells were activated with Dynabeads™ Human T-Expander CD3/CD28 (3:1, Invitrogen). Cells were expanded in T cell RPMI media for 72 hours prior to RNP transfection.

[00391] RNP was generated by pre-annealing individual CIITA targeting crRNA and trRNA (SEQ ID NO: 215) by mixing equivalent amounts of reagent and incubating at 95°C for 2 min and cooling to room temperature. The dual guide (dgRNA) consisting of pre-annealed crRNA and trRNA, was incubated with recombinant Spy Cas9 protein (SEQ ID NO: 800) to form a ribonucleoprotein (RNP) complex. RNP mixture of 50 μ M dgRNA and 50 μ M Cas9-NLS protein was prepared and incubated at 25°C for 10 minutes. Five μ L of RNP mixture was combined with 100,000 cells in 20 μ L P3 electroporation Buffer (Lonza). 22 μ L of RNP/cell mix was transferred to the corresponding wells of a Lonza shuttle 96-well electroporation plate. Cells were electroporated in triplicate with the manufacturer’s pulse code. T cell RPMI media was added to the cells immediately post electroporation. Electroporated T cells were subsequently cultured. Two days post edit, a portion of electroporated T cells as collected for NGS sequencing.

2.2. Flow cytometry

[00392] On day 7 post-edit, T cells were phenotyped by flow cytometry to determine MHC class II protein expression. Briefly, T cells were incubated in antibody targeting HLA-DR (BioLegend® Cat. No. 307622) and Isotype Control-AF647 (BioLegend® Cat. No. 400234). Cells were subsequently washed, processed on a Cytotflex flow cytometer (Beckman Coulter) and analyzed using the FlowJo software package. T cells were gated based on size, shape,

viability, and MHC class II expression. DNA samples were subjected to PCR and subsequent NGS analysis. **Table 5** and **Fig. 1A** show results for percent editing following *CITTA* editing with various guides in CD3⁺ T cells. **Table 5** and **Fig. 1A** show results for percent of MHC-II negative cells, using HLA-DR as a marker, following *CITTA* editing with various guides in T cells.

[00393] **Table 5 - Percent editing and percent of HLA-DR⁺ cells following *CITTA* editing**

Guide	Editing				MHC Class II Expression, HLA-DR			
	Donor B		Donor 26		Donor B		Donor 26	
	% Edit	SD	% Edit	SD	% neg	SD	% neg	SD
CR002966	47.1	3.9	50.9	1.7	42.9	2.0	38.0	3.9
CR002959	37.1	1.7	42.2	3.8	15.8	3.2	3.9	5.2
CR002961	16.4	1.1	18.4	1.8	3.4	2.9	3.7	3.2
CR002967	23.9	1.0	29.8	0.3	23.7	5.0	19.7	6.5
CR002971	13.3	0.4	13.8	1.1	6.9	3.6	10.5	4.0
CR002991	13.6	0.5	15.9	1.0	10.4	1.0	14.4	3.4
CR002995	No data	No Data	8.5	0.6	7.6	4.3	-6.6	6.3
CR003009	7.9	0.3	7.2	0.8	11.6	3.0	0.0	8.4
CR003011	9.9	1.2	13.0	0.3	13.1	1.9	5.4	4.0
CR003014	17.9	1.0	17.1	1.6	13.4	0.6	5.4	2.3
CR007938	58.1	2.2	57.5	2.8	22.2	2.3	11.6	3.3
CR007955	11.6	1.0	13.7	1.2	6.3	2.7	-1.8	5.5
CR007982	24.2	1.1	29.9	4.9	38.2	1.9	35.6	1.5
CR007994	12.6	0.8	12.6	0.8	20.7	2.5	5.5	4.4
CR007997	11.4	1.6	9.2	2.0	11.7	1.2	4.9	5.5
CR009188	5.5	1.0	6.0	0.2	-0.7	1.6	-8.5	6.2
CR009202	8.2	0.1	9.4	0.8	6.8	5.0	5.4	3.3
CR009206	8.3	0.9	9.3	0.6	15.8	4.1	13.0	4.4
CR009208	7.9	1.0	6.8	0.5	8.3	2.2	12.4	1.4
CR009211	4.4	0.8	4.7	0.2	0.9	1.7	-3.1	4.6
CR009217	23.2	1.8	29.0	3.2	29.0	3.3	29.6	0.6
CR009229	17.4	1.1	19.6	0.5	18.7	3.0	19.0	3.4
CR009230	5.2	0.5	6.2	0.9	6.3	4.3	28.1	19.7
CR009234	19.9	0.6	23.7	1.4	17.7	0.4	12.0	5.1
CR009235	11.9	0.5	10.9	0.8	13.8	1.2	1.2	5.6
CR009238	9.0	1.0	13.4	1.7	15.4	1.7	11.4	1.8

Example 3 – sgRNA Dose Response Editing

3.1 T cell preparation

[00394] Healthy human donor apheresis was obtained commercially (Hemacare), and cells were washed and re-suspended in CliniMACS® PBS/EDTA buffer (Miltenyi Biotec Cat. No. 130-070-525) on the LOVO device. T cells were isolated via positive selection using CD4 and CD8 magnetic beads (Miltenyi Biotec Cat. No. 130-030-401/130-030-801) using the CliniMACS® Plus and CliniMACS® LS disposable kit. T cells were aliquoted into vials and cryopreserved in a 1:1 formulation of Cryostor® CS10 (StemCell Technologies Cat. No. 07930) and Plasmalyte A (Baxter Cat. No. 2B2522X) for future use.

[00395] Upon thaw, T cells were plated at a density of 1.5×10^6 cells/mL in OpTmizer-based media containing CTS OpTmizer T Cell Expansion SFM (Gibco, Cat. A3705001), 5% human AB serum (Gemini, Cat. 100-512) 1% of Penicillin-Streptomycin, 1X Glutamax, 10 mM HEPES, 200 U/mL recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/ml recombinant human interleukin 7 (Peprotech, Cat. 200-07), and 5 ng/ml recombinant human interleukin 15 (Peprotech, Cat. 200-15). T-cells were activated with TransAct™ (1:100 dilution, Miltenyi Biotec) in this media for 48 hours.

3.2 T cell editing

[00396] LNP compositions containing mRNA encoding Cas9 (SEQ ID NO: 802) and a sgRNA targeting CIITA were formulated as described in **Example 1**. Each LNP preparation was incubated in OpTmizer-based media with cytokines as described above supplemented with 10 ug/ml recombinant human ApoE3 (Peprotech, Cat. 350-02) for 5 minutes at 37°C. Forty-eight hours post activation, T cells were washed and suspended in OpTmizer media with cytokines as described but without human serum. Pre-incubated LNP mix was added to the each well to yield a final concentration of as described in **Table 6**. A control group including unedited T cells (no LNP) was also included. After 24 hours, T cells were collected, washed, and cultured for 7 days in OpTmizer-based media before being evaluated harvested for evaluation by NGS and flow cytometry. All groups were done with replicate wells (n=2). Expanded T cells were cryopreserved for functional assays. NGS analysis performed as described in **Example 1** for a single set of replicate samples. **Table 6** and **Fig. 2A** show results for percent editing following *CIITA* editing with various guides in T cells.

[00397] **Table 6 – Percent indel editing following CIITA editing in total T cells (n=1)**

LNP Dose (ug/ml)	G013674	G013675	G013676
5	99.5	99.6	99.6
2.5	99.4	99.5	98.8
1.25	99.2	98.6	96.8
0.63	94.6	80	72.8
0.31	67.5	34.2	29.2
0.16	40	11.8	11
0.08	14.6	3.8	3.8
0.04	5.2	1.6	1.5

3.3 Flow cytometry

[00398] On day 7 post-edit, T cells were phenotyped by flow cytometry to determine MHC class II protein expression. Briefly, T cells were incubated with antibody targeting HLA-DR DP-DQ (Biolegend, Cat. 361706) before being washed and analyzed on a Cytotflex flow cytometer (Beckman Coulter). Data analysis was performed using the FlowJo software package. T cells were gated based on size, shape, viability, and MHC class II (HLA-DRDP-DQ) expression. **Table 7** and **Fig. 2B** show results for percent of MHC-II negative cells (HLA-DR-DP-DQ-) following CIITA editing with various guides in CD4+, CD8+, or total T cells.

[00399] **Table 7 – Mean percentage of MHC Class II negative cells following CIITA editing**

Cell type	LNP Dose (ug/ml)	G013674		G013675		G013676		Untreated	
		% neg	SD	% neg	SD	% neg	SD	% neg	SD
Total T cells	0.04	21.8	0.1	20.8	0.4	19.5	0.5	17.2	0.3
	0.08	23.3	1.4	22.8	1.0	21.4	1.1	18.7	1.5
	0.16	29.5	2.2	24.7	0.1	23.7	0.8	19.5	1.3
	0.31	44.7	1.6	37.8	1.9	30.4	3.8	19.4	0.7
	0.63	63.8	0.1	76.2	0.9	61.6	6.4	20.4	1.2
	1.25	67.1	0.6	93.4	0.7	91.8	0.1	20.6	1.2
	2.50	65.6	0.1	94.4	0.8	94.7	0.1	19.0	1.7
	5.00	63.3	1.0	92.6	0.6	94.0	0.4	19.4	0.5
CD4+	0.04	31.5	0.4	30.5	1.1	30.0	0.9	27.6	2.1
	0.08	33.9	0.8	32.9	0.0	31.4	1.1	28.1	2.5
	0.16	39.1	2.4	34.6	0.8	33.8	0.4	29.4	2.4
	0.31	52.2	1.2	47.7	1.1	40.7	3.5	29.9	0.0
	0.63	70.5	0.6	80.1	0.1	68.5	5.0	31.1	1.9
	1.25	73.1	1.3	95.2	0.7	94.1	0.1	30.3	2.3
	2.50	72.4	0.7	96.1	0.6	96.3	0.4	29.5	3.2
	5.00	69.4	0.1	94.9	0.0	96.5	0.6	30.5	1.4

Cell type	LNP Dose (ug/ml)	G013674		G013675		G013676		Untreated	
		% neg	SD	% neg	SD	% neg	SD	% neg	SD
CD8+	0.04	17.4	0.1	16.4	0.1	14.7	0.2	14.9	0.3
	0.08	17.7	1.6	17.5	1.6	16.2	1.2	14.4	1.3
	0.16	24.7	2.3	19.8	0.4	18.6	0.8	16.2	0.7
	0.31	41.1	2.0	32.8	2.5	25.2	4.0	15.4	1.1
	0.63	61.2	0.1	75.1	1.1	58.7	7.2	14.6	1.1
	1.25	64.8	0.4	93.2	0.6	91.4	0.3	15.0	0.8
	2.5	63.1	0.1	94.2	0.6	94.8	0.1	14.3	1.0
	5	61.8	1.1	92.4	0.8	93.7	0.4	13.2	0.4

Example 4 –CIITA Guide RNAs

4.1 T cell preparation

[00400] Healthy human donor apheresis was obtained commercially (Hemacare), and cells were washed and re-suspended in 2% PBS/EDTA buffer. T cells were isolated on the MultiMACS (Miltenyi Biotec Cat. No. 130-098-637) via positive selection using StraightFrom® Leukopak® CD4/CD8 MicroBead Kit (Miltenyi Biotec Cat. No. 130-122-352). T cells were aliquoted into vials and cryopreserved in Cryostor® CS10 (StemCell Technologies Cat. No. 07930).

[00401] Upon thaw, T cells were plated at a density of 1.0×10^6 cells/mL in T cell basal media composed of X-VIVO 15™ serum-free hematopoietic cell medium (Lonza Bioscience) containing 5% (v/v) of fetal bovine serum, 55 μ M of 2-Mercaptoethanol, 10 mM of N-Acetyl-L-(+)-cysteine, 10 U/mL of Penicillin-Streptomycin, in addition to 1X cytokines (200 U/mL of recombinant human interleukin-2, 5 ng/mL of recombinant human interleukin-7 and 5 ng/mL of recombinant human interleukin-15). T-cells were activated with TransAct™ (1:100 dilution, Miltenyi Biotec). Cells were expanded in T cell basal media containing TransAct™ for 48 hours prior to electroporation.

4.2 T cells editing with ribonucleoprotein

[00402] RNP was generated by pre-annealing individual crRNA and trRNA by mixing equivalent amounts of reagent and incubating at 95°C for 2 min and snap cooled. The dual guide (dgRNA) consisting of pre-annealed crRNA and trRNA, was incubated with Spy Cas9 protein (SEQ ID NO: 800) at a 2:1 dgRNA/protein molar ratio to form a ribonucleoprotein (RNP) complex. CD3⁺ T cells were transfected in duplicate with an RNP at the concentrations indicated in **Table 8** using the P3 Primary Cell 96-well Nucleofector™ Kit (Lonza, Cat. V4SP-

3960) and the manufacturer's pulse code. T cell media was added to cells immediately post-nucleofection and cultured for 2 days or more.

[00403] Four days post nucleofection, genomic DNA was prepared as described in Example 1 and NGS analysis performed. **Table 8** and **Fig. 3A** show results for percent editing following *CITA* editing with various guides in CD3⁺ T cells.

4.3. Flow cytometry

[00404] On day 10 post-edit, T cells were phenotyped by flow cytometry to determine MHC class II protein expression. Briefly, T cells were incubated in cocktails of antibodies targeting HLA-DR-DP-DQ (Biolegend, Cat. 361704) and CD3 (BioLegend, Cat. 300322). Cells were subsequently washed, processed on a Cytoflex flow cytometer (Beckman Coulter) and analyzed using the FlowJo software package. T cells were gated based on size, shape, viability, and MHC class II expression. **Table 8** and **Fig. 3B** show results for percent of MHC-II negative cells following *CITA* editing with various guides in CD3⁺ T cells.

[00405] **Table 8 - Percent editing and percent of MHC-II negative cells following *CITA* editing**

Guide	RNP (uM)	% Edit	SD	% MHCII neg	SD
CR002961	0	0.2	0.1	6.7	0.7
	0.0625	5.0	0.2	13.2	0.9
	0.125	11.6	0.5	15.4	0.4
	0.25	23.3	2.1	14.6	0.6
	0.5	49.2	0.8	16.1	0.8
	0.75	65.9	1.9	21.2	0.4
	1	69.2	1.4	22.9	0.1
	1.5	81.9	0.3	25.4	0.1
CR009217	0	0.3	0.1	8.6	0.2
	0.0625	9.6	0.4	16.4	0.1
	0.125	19.2	0.4	20.6	0.6
	0.25	37.9	0.8	28.0	2.2
	0.5	65.2	1.8	48.1	0.7
	0.75	80.3	2.0	58.4	1.6
	1	82.8	3.3	65.8	0.4
	1.5	91.8	1.3	73.7	0.9
CR007982	0	0.1	0.0	7.4	0.0
	0.0625	8.9	0.1	15.0	2.8
	0.125	21.3	1.2	15.2	7.1
	0.25	39.3	3.0	25.5	0.2
	0.5	65.9	3.1	48.8	2.3

Guide	RNP (uM)	% Edit	SD	% MHCII neg	SD
	0.75	80.0	1.8	59.3	1.3
	1	83.9	0.4	68.2	2.8
	1.5	92.3	0.5	71.3	5.2
CR007994	0	0.2	0.1	5.6	1.1
	0.0625	5.1	1.0	13.8	0.8
	0.125	9.7	0.2	14.7	0.5
	0.25	20.7	0.6	12.2	1.3
	0.5	46.7	2.4	30.8	1.5
	0.75	61.8	1.1	41.6	1.5
	1	70.2	3.5	50.5	2.1
	1.5	83.4	1.2	56.4	2.3

Example 5 – T Cell Editing, CIITA Guide RNAs with Cas9 and BC22

5.1 T cell Preparation

[00406] T cells were edited at the CIITA locus with UGI in trans and either BC22 or Cas9 to assess the impact on editing type on MHC class II antigens.

[00407] T cells were prepared from a leukopak using the EasySep Human T cell Isolation Kit (Stem Cell Technology, Cat. 17951) following the manufacturers protocol. T cells were cryopreserved in Cryostor CS10 freezing media (Cat. 07930) for future use. Upon thaw, T cells were plated at a density of 1.0×10^6 cells/mL in T cell R10 media composed of RPMI 1640 (Corning, Cat. 10-040-CV) containing 10% (v/v) of fetal bovine serum, 2 mM Glutamax (Gibco, Cat. 35050-061), 22 μ M of 2-Mercaptoethanol, 100 uM non-essential amino acids (Corning, Cat. 25-025-CI), 1 mM sodium pyruvate, 10 mM HEPES buffer, 1% of Penicillin-Streptomycin, plus 100 U/mL of recombinant human interleukin-2 (Peprotech, Cat. 200-02). T cells were activated with Dynabeads® Human T-Activator CD3/CD28 (Gibco, Cat. 11141D). Cells were expanded in T cell media for 72 hours prior to mRNA transfection.

5.2 T cell editing with RNA electroporation

[00408] Solutions containing mRNA encoding Cas9 protein (SEQ ID NO: 801), BC22 (SEQ ID NO: 806) or UGI (SEQ ID NO: 807) were prepared in sterile water. 50 μ M CIITA targeting sgRNAs were removed from their storage plates and denatured for 2 minutes at 95°C before cooling on ice. Seventy-two hours post activation, T cells were harvested, centrifuged, and resuspended at a concentration of 12.5×10^6 T cells/mL in P3 electroporation buffer (Lonza). For each well to be electroporated, 1×10^5 T cells were mixed with 200 ng of editor mRNA,

200 ng of UGI mRNA and 20 pmols of sgRNA as described in **Table 9** in a final volume of 20 μ L of P3 electroporation buffer. This mix was transferred in duplicate to a 96-well Nucleofector™ plate and electroporated using the manufacturer's pulse code. Electroporated T cells were rested in 180 μ L of R10 media plus 100 U/mL of recombinant human interleukin-2 before being transferred to a new flat-bottom 96-well plate. The resulting plate was incubated at 37°C for 4 days. On day 10 post-editing cells were collected for flow cytometry analysis and NGS sequencing.

5.3 Flow cytometry and NGS sequencing

[00409] On day10 post-editing, T cells were phenotyped by flow cytometry to determine MHC class II protein expression as described in **Example 4** using antibodies targeting HLA-DR, DQ, DP-PE (BioLegend® Cat. No. 361704) and Isotype Control-PE (BioLegend® Cat. No. 400234). DNA samples were subjected to PCR and subsequent NGS analysis, as described in **Example 1**. **Table 9** shows CIITA gene editing and MHC class II negative results for cells edited with BC22. **Table 10** shows CIITA gene editing and MHC class II negative results for cells edited with Cas9.

[00410] **Table 9 – Percent editing and percent of MHC-II negative cells following CIITA editing with BC22**

Guide	% C to T			% A to G			% Indel			% MHC Class II negative		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
G016030	40.2	14.2	2	3.5	1.1	2	2.4	1.1	2	39.7	5.6	2
G016031	58.8	0.0	1	3.4	3.2	2	0.6	0.8	2	41.5	3.0	2
G016032	1.8	0.6	2	18.2	1.3	2	75.2	0.6	2	45.9	4.2	2
G016033	1.6	0.1	2	30.7	2.2	2	18.0	5.2	2	38.5	1.5	2
G016034	52.8	14.8	2	1.5	0.5	2	2.0	0.3	2	41.8	2.2	2
G016035	50.3	14.5	2	1.6	0.6	2	1.4	0.4	2	40.8	2.5	2
G016036	14.2	4.9	2	2.2	0.3	2	2.4	0.3	2	40.1	2.4	2
G016037	10.1	4.5	2	1.3	0.4	2	0.3	0.1	2	38.2	0.8	2
G016038	71.3	6.5	2	3.2	0.1	2	3.0	0.6	2	45.6	2.6	2
G016039	66.0	5.9	2	5.0	0.1	2	10.5	2.9	2	38.6	0.5	2
G016040	No data			0.0	0.0	1	0.0	0.0	1	38.4	0.7	2
G016041	No data			3.1	4.3	2	3.3	1.2	2	40.4	2.1	2
G016042	21.5	7.9	2	3.2	0.1	2	1.6	0.9	2	44.4	3.7	2
G016043	44.7	11.5	2	2.3	0.1	2	3.2	0.5	2	40.5	1.6	2
G016044	93.4	2.3	2	1.8	0.4	2	4.9	0.7	2	39.9	0.9	2
G016045	7.1	3.0	2	1.2	0.1	2	2.2	0.2	2	39.8	0.7	2
G016046	63.7	11.5	2	2.9	0.1	2	3.3	0.1	2	46.4	1.4	2

Guide	% C to T			% A to G			% Indel			% MHC Class II negative		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
G016047	72.7	3.0	2	2.9	0.2	2	4.6	0.9	2	45.4	2.0	2
G016048	6.2	2.4	2	2.4	0.1	2	0.2	0.1	2	38.4	0.5	2
G016049	66.8	9.0	2	5.4	0.1	2	2.8	0.1	2	42.4	2.5	2
G016050	45.4	9.0	2	2.3	0.3	2	3.0	0.6	2	39.1	2.8	2
G016051	86.2	5.9	2	3.7	0.1	2	1.3	0.3	2	39.5	0.6	2
G016052	53.7	13.7	2	4.2	0.9	2	7.4	0.4	2	42.1	1.8	2
G016053	38.6	18.7	2	1.3	0.3	2	3.1	0.8	2	40.4	1.7	2
G016054	42.0	10.7	2	1.9	0.1	2	2.1	0.4	2	42.1	4.2	2
G016055	36.6	13.1	2	3.5	0.9	2	8.2	2.3	2	41.3	1.5	2
G016056	78.0	9.1	2	3.4	0.1	2	2.0	0.1	2	39.8	3.4	2
G016057	73.3	9.1	2	3.4	0.6	2	5.3	0.0	2	39.7	1.3	2
G016058	75.0	9.1	2	1.7	0.1	2	4.2	0.0	2	46.0	2.3	2
G016059	66.5	12.0	2	4.3	0.2	2	4.7	0.7	2	41.0	0.8	2
G016060	55.6	5.2	2	3.5	0.4	2	10.7	1.0	2	44.2	1.6	2
G016061	65.5	9.3	2	2.5	0.4	2	1.2	0.0	2	38.6	2.0	2
G016062	65.8	9.1	2	3.0	0.2	2	4.3	0.3	2	39.8	0.1	2
G016063	10.2	4.2	2	0.9	0.2	2	0.3	0.1	2	39.9	2.5	2
G016064	66.8	12.2	2	3.5	0.0	2	4.4	1.1	2	59.2	2.2	2
G016065	13.5	5.5	2	0.6	0.2	2	1.1	0.4	2	40.4	2.5	2
G016066	0.1	0.0	2	0.7	0.1	2	0.3	0.0	2	35.4	1.5	2
G016067	82.7	6.4	2	2.6	0.6	2	4.6	0.2	2	59.8	1.2	2
G016068	60.1	8.9	2	2.5	0.8	2	1.1	0.1	2	54.0	0.7	2
G016069	55.8	11.6	2	2.6	0.6	2	2.6	1.1	2	34.3	4.0	2
G016070	76.5	9.6	2	3.5	1.0	2	4.4	0.3	2	53.3	1.7	2
G016071	54.3	6.8	2	4.1	0.5	2	1.4	0.6	2	45.8	0.1	2
G016072	82.0	0.0	1	4.1	0.0	1	5.1	0.0	1	25.1	1.1	2
G016073	63.4	11.1	2	3.5	0.5	2	0.9	0.0	2	38.7	1.3	2
G016074	62.7	13.0	2	3.9	0.2	2	4.2	0.7	2	53.3	2.0	2
G016075	41.2	17.2	2	1.0	0.4	2	8.0	1.7	2	48.1	0.7	2
G016076	42.8	14.3	2	0.9	0.1	2	10.2	2.8	2	55.6	2.9	2
G016077	65.1	10.6	2	5.4	0.2	2	2.4	0.1	2	46.0	0.2	2
G016078	44.1	15.1	2	2.0	0.6	2	6.6	2.0	2	41.6	0.1	2
G016079	79.8	4.9	2	5.6	0.5	2	4.6	0.9	2	53.4	2.8	2
G016080	39.0	12.2	2	4.3	0.6	2	13.1	3.1	2	49.5	3.1	2
G016081	9.6	4.9	2	0.5	0.3	2	2.5	0.8	2	39.0	0.3	2
G016082	20.3	8.6	2	2.0	0.3	2	7.0	3.3	2	40.1	4.1	2
G016083	74.5	9.8	2	3.6	0.1	2	8.0	0.6	2	48.6	2.3	2
G016084	46.0	8.5	2	3.9	0.0	2	23.5	3.2	2	54.5	0.7	2
G016085	35.6	6.7	2	1.4	0.1	2	1.6	0.1	2	41.9	3.0	2
G016086	75.0	10.3	2	3.9	0.4	2	3.5	0.2	2	63.1	1.3	2

Guide	% C to T			% A to G			% Indel			% MHC Class II negative		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
G016087	45.3	9.9	2	1.2	0.1	2	3.4	0.5	2	40.3	3.7	2
G016088	67.8	10.5	2	5.6	1.4	2	6.9	1.4	2	44.1	3.3	2
G016089	64.4	10.5	2	4.5	0.1	2	1.5	0.3	2	38.3	1.8	2
G016090	67.1	7.1	2	1.7	0.1	2	16.9	2.2	2	61.3	2.3	2
G016091	47.4	12.0	2	2.1	0.9	2	2.9	2.2	2	54.0	3.9	2
G016092	71.4	11.5	2	3.3	0.1	2	6.5	0.5	2	54.9	0.9	2
G016093	76.6	8.3	2	3.3	0.2	2	3.7	0.1	2	52.2	2.5	2
G016094	75.5	7.5	2	3.6	0.4	2	1.7	0.2	2	44.0	2.5	2
G016095	76.1	7.2	2	7.6	0.4	2	2.8	0.4	2	44.1	0.4	2
G016096	77.6	8.5	2	2.1	0.2	2	4.6	0.4	2	41.2	3.0	2
G016097	44.7	15.0	2	2.2	0.1	2	0.7	0.1	2	38.6	3.2	2
G016098	28.9	8.8	2	1.6	0.4	2	1.6	0.2	2	40.1	4.7	2
G016099	68.8	11.9	2	2.2	0.1	2	3.9	0.7	2	44.8	1.0	2
G016100	85.4	6.9	2	2.4	0.6	2	3.3	0.4	2	43.5	1.9	2
G016101	4.8	1.1	2	0.8	0.1	2	0.2	0.0	2	38.0	3.3	2
G016102	57.5	14.4	2	1.9	0.1	2	2.3	0.4	2	42.6	3.3	2
G016103	69.4	12.8	2	2.4	0.0	2	5.6	0.5	2	39.1	2.7	2
G016104	66.5	12.2	2	1.6	0.7	2	11.1	0.4	2	49.0	3.3	2
G016105	58.4	14.3	2	4.4	0.6	2	8.3	0.7	2	38.4	3.0	2
G016106	74.8	5.7	2	3.3	0.3	2	7.3	0.3	2	51.8	0.1	2
G016107	45.2	12.2	2	5.8	1.1	2	5.4	0.8	2	39.6	1.0	2
G016108	15.3	3.5	2	1.2	0.1	2	1.2	0.4	2	41.5	0.1	2
G016109	77.5	3.7	2	4.5	0.4	2	3.4	0.6	2	48.3	2.5	2
G016110	43.1	15.3	2	1.7	0.2	2	6.9	1.6	2	45.4	0.6	2
G016111	89.2	1.3	2	4.2	0.1	2	5.6	0.8	2	40.6	4.4	2
G016112	68.8	13.2	2	3.8	0.4	2	1.4	0.1	2	43.4	2.7	2
G016113	65.2	9.8	2	3.6	0.2	2	6.9	1.1	2	58.3	2.5	2
G016114	75.5	11.5	2	2.7	0.1	2	5.1	0.6	2	38.9	4.0	2
G016115	72.1	8.6	2	1.2	0.1	2	7.9	0.3	2	60.2	0.9	2
G016116	36.3	7.5	2	2.3	0.8	2	3.0	0.4	2	41.1	0.4	2
G016117	75.4	7.7	2	3.5	0.4	2	5.9	0.4	2	37.1	2.8	2

[00411] Table 10 – Percent editing and percent of MHC-II negative cells following CHITA editing with Cas9

Guide	% C to T			% A to G			% Indel			% MHC Class II negative		
	Mean	SD	n	Mean	SD	N	Mean	SD	n	Mean	SD	n
G016030	0.0	0.0	2	0.4	0.0	2	30.1	8.3	2	42.7	0.4	2
G016031	0.0	0.0	1	0.1	0.0	1	79.5	0.0	1	45.9	1.0	2

	% C to T			% A to G			% Indel			% MHC Class II negative		
Guide	Mean	SD	n	Mean	SD	N	Mean	SD	n	Mean	SD	n
G016032	0.1	0.1	2	14.8	2.6	2	77.7	4.8	2	43.6	0.1	2
G016033	0.1	0.1	2	16.1	2.4	2	61.5	5.3	2	44.1	1.8	2
G016034	0.0	0.0	2	0.1	0.0	2	49.7	6.2	2	46.1	2.7	2
G016035	0.0	0.0	2	0.1	0.0	2	44.7	6.4	2	46.2	0.6	2
G016036	0.0	0.0	2	1.8	0.3	2	5.6	0.1	2	38.3	1.8	2
G016037	0.1	0.1	2	1.2	0.1	2	3.4	0.4	2	35.9	1.1	2
G016038	0.0	0.0	2	0.6	0.1	2	88.3	3.5	2	65.7	2.9	2
G016039	0.0	0.0	2	0.1	0.0	2	91.9	2.6	2	62.9	2.5	2
G016040	No data									63.2	0.6	2
G016041	No data									62.3	0.6	2
G016042	0.0	0.0	2	1.5	0.3	2	40.6	10.8	2	43.9	0.6	2
G016043	0.0	0.0	2	1.3	0.1	2	26.7	4.9	2	42.9	0.4	2
G016044	No data			0.2	0.0	1	74.4	0.0	1	54.9	0.8	2
G016045	0.1	0.1	2	0.8	0.0	2	13.9	4.3	2	40.6	0.8	2
G016046	0.0	0.0	2	0.2	0.1	2	92.8	2.4	2	62.5	0.6	2
G016047	0.1	0.1	2	0.2	0.0	2	80.0	2.0	2	59.8	0.9	2
G016048	0.0	0.0	2	2.1	0.0	2	9.3	2.5	2	41.0	0.8	2
G016049	0.0	0.0	2	0.1	0.0	2	85.8	3.0	2	56.9	0.3	2
G016050	0.1	0.1	2	0.6	0.1	2	38.4	1.6	2	45.0	0.4	2
G016051	0.0	0.0	2	0.4	0.0	2	82.6	3.6	2	58.8	1.4	2
G016052	0.1	0.1	2	0.6	0.1	2	69.8	7.0	2	53.8	0.4	2
G016053	No data									43.4	1.2	2
G016054	0.0	0.0	2	1.1	0.1	2	28.1	7.0	2	44.9	3.2	2
G016055	0.0	0.0	2	0.6	0.1	2	37.1	7.7	2	47.5	0.4	2
G016056	0.0	0.0	2	0.1	0.0	2	89.5	5.3	2	63.8	0.6	2
G016057	0.0	0.0	2	0.1	0.0	2	84.7	4.0	2	61.6	3.1	2
G016058	0.0	0.0	2	0.2	0.1	2	82.3	5.9	2	60.4	2.2	2
G016059	0.0	0.0	2	0.1	0.0	2	75.1	3.7	2	56.6	1.6	2
G016060	0.0	0.0	2	0.2	0.0	2	84.3	3.8	2	61.5	1.8	2
G016061	0.0	0.0	2	0.1	0.0	2	55.2	2.9	2	47.4	0.6	2
G016062	0.0	0.0	2	0.5	0.1	2	71.1	5.9	2	46.4	0.7	2
G016063	0.0	0.0	2	0.6	0.0	2	5.1	1.4	2	36.1	1.4	2
G016064	0.0	0.0	2	1.3	0.1	2	42.7	5.7	2	49.1	0.7	2
G016065	0.0	0.0	2	0.1	0.0	2	11.0	2.0	2	40.0	0.9	2
G016066	0.0	0.0	2	0.6	0.0	2	0.4	0.1	2	36.8	0.6	2
G016067	0.1	0.0	2	0.1	0.0	2	85.4	3.3	2	59.7	3.3	2
G016068	0.0	0.0	2	0.1	0.0	2	59.2	6.9	2	54.0	0.2	2
G016069	0.0	0.0	2	0.4	0.0	2	39.5	3.7	2	40.7	0.0	2
G016070	0.1	0.1	2	0.1	0.1	2	92.3	2.3	2	66.4	3.1	2
G016071	0.0	0.0	2	0.2	0.0	2	73.1	2.2	2	55.6	3.0	2

	% C to T			% A to G			% Indel			% MHC Class II negative		
Guide	Mean	SD	n	Mean	SD	N	Mean	SD	n	Mean	SD	n
G016072	0.0	0.0	2	0.1	0.1	2	91.1	1.4	2	51.6	1.1	2
G016073	0.0	0.0	2	1.5	0.1	2	38.3	4.2	2	43.5	0.8	2
G016074	0.2	0.1	2	0.6	0.0	2	72.4	7.5	2	56.1	1.4	2
G016075	0.1	0.1	2	0.3	0.1	2	72.9	10.3	2	57.8	4.4	2
G016076	0.0	0.0	2	0.3	0.1	2	66.7	13.4	2	58.4	4.6	2
G016077	0.0	0.0	2	0.5	0.1	2	80.0	5.2	2	62.5	1.5	2
G016078	0.1	0.1	2	0.3	0.2	2	59.3	10.5	2	51.6	4.5	2
G016079	0.0	0.0	2	0.7	0.1	2	81.9	4.0	2	58.4	1.0	2
G016080	0.0	0.0	2	0.5	0.1	2	71.8	6.2	2	44.9	1.4	2
G016081	0.0	0.0	2	0.4	0.0	2	8.1	1.3	2	39.1	0.7	2
G016082	0.1	0.1	2	2.1	0.0	2	10.0	2.5	2	39.0	0.8	2
G016083	0.1	0.1	2	0.2	0.1	2	92.2	1.5	2	63.6	0.7	2
G016084	0.0	0.0	2	0.4	0.0	2	70.7	6.4	2	56.1	2.6	2
G016085	0.0	0.0	2	0.3	0.1	2	17.5	0.7	2	42.3	0.4	2
G016086	0.1	0.1	2	0.2	0.1	2	85.8	6.1	2	62.2	3.0	2
G016087	0.0	0.0	2	0.2	0.0	2	89.5	2.1	2	56.1	0.1	2
G016088	0.8	0.0	2	0.3	0.1	2	76.8	4.6	2	58.1	0.5	2
G016089	0.1	0.1	2	0.3	0.1	2	73.3	6.4	2	54.2	0.0	2
G016090	0.2	0.0	2	0.3	0.0	2	88.3	5.1	2	61.2	2.3	2
G016091	0.0	0.0	1	0.7	0.0	1	42.0	0.0	1	49.5	3.8	2
G016092	0.1	0.1	2	0.5	0.1	2	60.9	10.0	2	52.0	2.9	2
G016093	0.0	0.0	2	0.5	0.1	2	68.8	8.1	2	50.6	2.5	2
G016094	0.1	0.1	2	0.1	0.0	2	71.3	6.5	2	50.5	3.3	2
G016095	0.0	0.0	2	0.6	0.1	2	70.5	5.4	2	51.6	5.0	2
G016096	0.2	0.1	2	0.1	0.0	2	94.9	2.0	2	51.2	0.1	2
G016097	0.1	0.1	2	0.3	0.0	2	39.7	12.4	2	50.9	4.5	2
G016098	0.1	0.1	2	0.2	0.0	2	23.4	7.5	2	47.2	0.5	2
G016099	0.1	0.0	2	0.2	0.0	2	84.7	5.8	2	63.2	2.5	2
G016100	0.0	0.0	2	0.3	0.1	2	79.8	7.1	2	60.3	0.6	2
G016101	0.1	0.1	2	0.6	0.1	2	2.3	0.8	2	38.8	1.5	2
G016102	0.0	0.0	2	0.4	0.1	2	75.7	8.9	2	59.9	7.1	2
G016103	0.2	0.1	2	0.6	0.1	2	76.8	4.7	2	46.9	3.4	2
G016104	1.4	0.0	1	1.1	0.0	1	66.8	0.0	1	56.1	3.1	2
G016105	0.1	0.1	2	0.7	0.3	2	90.7	5.1	2	58.0	3.0	2
G016106	0.0	0.0	2	0.2	0.0	2	95.1	2.1	2	62.2	3.0	2
G016107	0.1	0.1	2	0.2	0.0	2	84.9	2.6	2	59.5	1.1	2
G016108	0.0	0.0	2	0.6	0.1	2	19.1	4.8	2	43.3	0.8	2
G016109	0.0	0.0	2	0.1	0.0	2	86.5	3.3	2	62.9	3.5	2
G016110	0.0	0.0	2	0.6	0.1	2	34.9	10.0	2	48.0	4.2	2
G016111	65.6*	3.7	2	1.4	0.1	2	32.9	3.7	2	44.5	1.3	2

	% C to T			% A to G			% Indel			% MHC Class II negative		
Guide	Mean	SD	n	Mean	SD	N	Mean	SD	n	Mean	SD	n
G016112	0.0	0.0	2	0.8	0.1	2	60.7	6.7	2	54.1	5.3	2
G016113	1.1	0.4	2	0.2	0.0	2	84.2	6.7	2	60.5	5.2	2
G016114	0.1	0.1	2	0.4	0.0	2	87.6	3.5	2	50.2	2.5	2
G016115	0.0	0.0	2	0.3	0.1	2	69.3	7.4	2	52.8	4.8	2
G016116	0.0	0.0	2	0.4	0.0	2	16.6	5.0	2	38.7	0.4	2
G016117	0.0	0.0	2	0.4	0.1	2	85.6	4.9	2	49.0	3.0	2

*There is a naturally occurring C/T single nucleotide polymorphism for G016111 target sequence.

Example 6 – Dose Response and Multiplexed Editing

[00412] Three guides from **Table 9**, G016086, G016092, and G016067, were further characterized for editing efficacy with increasing amounts of guide and in combination with guides targeting TRAC (G013009, G016016, or G016017) and B2M (G015991, G015995, or G015996). Generally, unless otherwise indicated, guide RNAs used throughout the Examples identified as “GXXXXXX” refer to 100-nt modified sgRNA format, unless indicated otherwise, such as those shown in the Tables provided herein.

[00413] Cell preparation, activation, and electroporation were performed as described in **Example 5** with the following deviations. Editing was performed using two mRNA species encoding BC22 (SEQ ID NO: 806) and UGI (SEQ ID NO: 807) respectively. Editing was assessed at multiple concentrations of sgRNA, as indicated in **Table 11** and **Table 12**. When multiple guides were used in a single reaction, each guide represented one quarter of the total guide concentration.

[00414] On day 10 post-editing, T cells were phenotyped by flow cytometry to determine MHC class II protein expression as described in Example 6. In addition, B2M detection was performed with B2M-FITC antibody (BioLegend, Cat. 316304) and CD3 expression was assayed using CD3-BV605 antibody (BioLegend, Cat. 317322). DNA samples were subjected to PCR and subsequent NGS analysis, as described in **Example 1**. **Table 11** provides MHC Class II negative flow cytometry results and NGS editing for cells edited with BC22 and individual guides targeting CIITA, with **FIG. 4A** graphing the percent C-to-T conversion and **Fig. 4B** graphing the percent MHC class II negative. **Table 12** shows MHC Class II negative results for cells edited simultaneously with CIITA, B2M, TRAC and TRBC guides.

[00415] **Table 11 – Percent MHC-II negative cells and NGS outcomes following *CIITA* editing (n=2)**

Guide Concentration		2 uM		1 uM		0.5 uM		0.25 uM	
Assay	Guide	Mean	SD	Mean	SD	Mean	SD	Mean	SD
MHC-II neg	G016086	80.2	12.0	72.2	19.7	60.5	14.3	49.7	16.1
	G016092	64.5	11.4	59.3	11.8	49.0	5.7	42.6	15.1
	G016067	77.3	4.4	76.8	2.1	64.5	6.3		
C-to-T	G016086	75.4	12.7	66.1	25.0	53.9	20.1	38.3	28.3
	G016092	71.5	18.5	62.5	25.5	45.2	13.0	36.0	28.1
	G016067	83.1	6.8	82.9	5.7	66.9	8.8	50.1	28.1
C-to-A/G	G016086	1.8	0.1	1.6	0.1	1.7	0.1	1.1	0.6
	G016092	1.6	0.1	1.3	0.2	1.2	0.0	1.2	0.5
	G016067	1.0	0.3	1.2	0.1	1.5	0.5	0.9	0.5
Indel	G016086	2.5	1.4	1.4	0.5	1.5	0.3	1.2	0.5
	G016092	2.9	0.3	3.0	0.6	3.1	0.5	2.5	1.7
	G016067	3.3	0.1	2.4	0.0	3.4	1.4	2.1	1.2

[00416] **Table 12 - Percent antigen negative cells following *CIITA*, *TRAC*, *TRBC*, and *B2M* editing**

Assay	Concentration per guide:	0.5 uM		0.25 uM		0.125 uM	
	Guide	Mean	SD	Mean	SD	Mean	SD
Triple Neg	G015995 G016086 G016017	62.1	9.3	51.9	9.4	26.1	13.6
	G015991 G016092 G016016	43.8	15.6	20.2	7.4	8.2	7.6
	G015996 G016067 G013009	35.3	17.7	15.4	12.3	6.8	7.3
MHC Class II Neg	G015995 G016086 G016017	67.0	8.0	57.6	7.8	38.6	5.4
	G015991 G016092 G016016	57.2	8.2	45.2	3.2	36.8	5.3
	G015996 G016067 G013009	53.1	7.9	69.1	43.1	41.3	7.1
CD3 Neg	G015995 G016086 G016017	92.5	2.3	90.5	3.2	77.3	15.1
	G015991 G016092 G016016	88.1	6.2	87.6	3.2	74.2	14.0
	G015996 G016067 G013009	92.8	2.0	89.5	4.8	79.3	14.4
B2M Neg	G015995 G016086 G016017	94.9	2.5	90.0	6.4	63.2	28.0
	G015991 G016092 G016016	73.4	19.2	29.1	13.1	14.3	15.2
	G015996 G016067 G013009	60.8	25.7	29.1	21.4	14.3	15.0

Example 7 – sgRNA Comparison in T Cells

[00417] T cells were edited at the *CIITA* locus Cas9 to assess the impact on editing type on MHC class II antigens.

7.1 T cell preparation

[00418] Healthy human donor apheresis was obtained (Hemacare), and cells were washed and re-suspended in CliniMACS® PBS/EDTA buffer (Miltenyi Biotec Cat. No. 130-070-525) on the LOVO device. T cells were isolated via positive selection using CD4 and CD8 magnetic beads (Miltenyi Biotec Cat. No. 130-030-401/130-030-801) using the CliniMACS® Plus and CliniMACS® LS disposable kit. T cells were aliquoted into vials and cryopreserved in a 1:1 formulation of Cryostor® CS10 (StemCell Technologies Cat. No. 07930) and Plasmalyte A (Baxter Cat. No. 2B2522X) for future use. Upon thaw, T cells were plated at a density of 1.0×10^6 cells/mL in T cell basal media composed of X-VIVO 15™ serum-free hematopoietic cell medium (Lonza Bioscience) containing 5% (v/v) of fetal bovine serum, 50 μ M of 2-Mercaptoethanol, 10 mM of N-Acetyl-L-(+)-cysteine, 10 U/mL of Penicillin-Streptomycin, in addition to 1X cytokines (200 U/mL of recombinant human interleukin-2, 5 μ g/mL of recombinant human interleukin-7 and 5 μ g/mL of recombinant human interleukin-15). T-cells were activated with TransAct™ (1:100 dilution, Miltenyi Biotec). Cells were expanded in T cell basal media containing TransAct™ for 72 hours prior to electroporation.

7.2 T cell editing with RNA electroporation

[00419] A solution containing mRNA encoding Cas9 (SEQ ID NO: 802) and = mRNA encoding UGI (SEQ ID NO: 807) was prepared in sterile water. Guide RNAs were denatured for 2 minutes at 95°C before cooling on ice. Seventy-two hours post activation, T cells were harvested, and resuspended at a concentration of 12.5×10^6 T cells/mL in P3 electroporation buffer (Lonza). For each well to be electroporated, 1×10^5 T cells were mixed with 200 ng of editor mRNA, 200 ng of UGI mRNA and 40 pmols of sgRNA as described in **Table 13** in a final volume of 20 μ L of P3 electroporation buffer. This mix was transferred in duplicate to a 96-well Nucleofector™ plate and electroporated using the manufacturer's pulse code. Electroporated T cells were immediately rested in cytokine free Optimizer-based media. Cells were incubated at 37°C for 4 days in Optimizer-based media with cytokines. After 96 hours, some cells were harvested for NGS analysis and remaining T cells were diluted 1:3 into fresh Optimizer-based media with cytokines. Electroporated T cells were subsequently cultured for 11 additional days and were collected for flow cytometry analysis.

7.3 Flow cytometry

[00420] On day 11 post-editing, T cells were phenotyped by flow cytometry to determine MHC class II protein expression as described in **Example 4** using antibodies

targeting HLA-DR, DQ, DP-FITC (BioLegend® Cat. No. 361706). **Table 13** shows MHC class II protein expression following electroporation with UGI mRNA combined with Cas9.

[00421] **Table 13 - Percent of MHC-II negative cells following *CIITA* editing**

Guide	% MHC Class II neg	SD
G013675	93.1	4.2
G013676	79.3	6.4
G015964	49.6	27.4
G016030	62.5	2.5
G016031	36.5	0.5
G016032	94.3	8.1
G016033	69.7	2.1
G016034	79.0	1.7
G016035	86.3	3.0
G016037	33.2	4.2
G016038	93.1	7.1
G016039	89.2	0.4
G016040	80.1	1.6
G016041	80.1	9.3
G016042	62.2	4.2
G016043	68.7	6.0
G016044	88.3	11.1
G016045	69.5	5.1
G016046	88.2	12.9
G016047	85.2	8.1
G016048	46.5	0.1
G016049	90.8	4.6
G016050	84.3	0.4
G016051	87.4	9.3
G016052	67.7	0.4
G016053	57.6	5.2
G016054	75.8	4.2
G016055	80.0	1.2
G016056	92.8	2.1
G016057	88.3	2.2
G016058	87.1	11.6
G016059	72.1	2.4
G016060	93.1	2.0
G016061	70.6	2.0
G016062	58.9	25.1
G016063	53.5	8.2
G016064	82.8	1.6

Guide	% MHC Class II neg	SD
G016065	61.3	1.3
G016066	52.1	11.8
G016067*	72.4	0.2
G016068	84.8	3.7
G016069	54.0	5.7
G016070	96.0	1.1
G016071	85.4	15.6
G016072	77.9	4.7
G016073	78.3	3.0
G016074	86.4	12.9
G016075	78.7	2.1
G016076	89.6	3.1
G016077	81.1	7.7
G016078	89.6	10.3
G016079	97.1	0.2
G016080	59.0	7.8
G016081	64.7	9.1
G016082	58.4	5.5
G016083	34.7	4.2
G016084	92.9	6.8
G016085	66.8	0.6
G016086*	51.2	1.8
G016087	77.4	2.2
G016088	88.1	10.5
G016089	91.8	2.9
G016090	92.1	2.6
G016091	95.9	0.6
G016092*	81.2	9.3
G016093	85.7	2.9
G016094	87.6	6.2
G016095	83.3	12.7
G016096	48.5	0.4
G016097	74.1	7.7
G016098	79.7	1.9
G016099	86.2	17.2
G016100	88.6	0.3
G016101	38.5	3.3
G016102	93.4	0.0
G016103	60.8	9.2
G016104	91.8	5.3
G016105	71.2	3.0

Guide	% MHC Class II neg	SD
G016106	78.2	12.1
G016107	62.6	5.8
G016108	64.8	4.6
G016109	93.1	1.0
G016110	90.3	1.9
G016111	87.0	15.4
G016112	51.3	32.7
G016113	98.0	1.1
G016114	44.9	9.7
G016115	80.2	11.4
G016116	80.1	12.9
G016117	58.8	16.1
G018081	94.5	0.8
G018082	94.9	1.7

*Concentration may have technical issue

Example 8 – CIITA Insertion

8.1 T cell preparation

[00422] Healthy human donor apheresis was obtained commercially (Hemacare), and cells were washed and re-suspended in 2% PBS/EDTA buffer. T cells were isolated on the MultiMACS (Miltenyi Biotec Cat. No. 130-098-637) via positive selection using StraightFrom® Leukopak® CD4/CD8 MicroBead Kit (Miltenyi Biotec Cat. No. 130-122-352). T cells were aliquoted into vials and cryopreserved in Cryostor® CS10 (StemCell Technologies Cat. No. 07930).

[00423] Upon thaw, T cells were plated at a density of 1.0×10^6 cells/mL in T cell basal media composed of X-VIVO 15™ serum-free hematopoietic cell medium (Lonza Bioscience) containing 5% (v/v) of fetal bovine serum, 55 μ M of 2-Mercaptoethanol, 10 mM of N-Acetyl-L-(+)-cysteine, 10 U/mL of Penicillin-Streptomycin, in addition to 1X cytokines (200 U/mL of recombinant human interleukin-2, 5 ng/mL of recombinant human interleukin-7 and 5 ng/mL of recombinant human interleukin-15). The next day, the T-cells were activated with TransAct™ (1:100 dilution, Miltenyi Biotec). Cells were expanded in T cell basal media containing TransAct™ for 48 hours prior to electroporation.

8.2 T cell editing with ribonucleoprotein and AAV

[00424] Select sgRNAs were incubated with recombinant Sp. Cas9-NLS protein (SEQ ID NO: 800) to form ribonucleoprotein (RNP) complexes. CIITA targeting sgRNAs were

denatured for 2 minutes at 95°C before cooling at room temperature. RNP mixture of 40 uM sgRNA and 20 uM Cas9-NLS protein was prepared and incubated at 25°C for 10 minutes. 2.5 µL of RNP mixture was combined with 1,000,000 CD3+ T cells in 20 µL P3 electroporation Buffer (Lonza). 25 µL of RNP/cell mix was transferred to the corresponding wells of a Lonza shuttle 96-well electroporation plate. Cells were electroporated in duplicate with the manufacturer's pulse code. T cell basal media was added to cells immediately post-nucleofection and the cells were transferred to a 24 well plate containing T cells media containing cytokines. AAV constructs were designed encoding an mCherry reporter gene flanked by homology arms immediately 5' and 3' to each guide's cut site (SEQ ID NOs. 1001-1003). AAV was added at MOI 3×10^5 to the respective wells. The cells were transferred to a 24-well Grex plate (Wilson Wolf, Cat. 80192) the next day and expanded for 10 days with media changes according to the manufacturer's protocol.

8.3 Flow cytometry

[00425] Day 10 post-edit, T cells were phenotyped by flow cytometry to determine MHC class II protein expression and expression of the mCherry reporter. Briefly, T cells were incubated in cocktails of antibodies consisting of CD4-BV605 (BioLegend® Cat. No. 317438), CD8-AF700 (BioLegend® Cat. No. 344724) and HLA-DR, DQ, DP-FITC (BioLegend® Cat. No. 361706). Cells were subsequently washed, processed on a Cytotflex flow cytometer (Beckman Coulter) and analyzed using the FlowJo software package. T cells were gated based on size, shape, followed by the CD4 and CD8 gating. Insertion was then quantified using mCherry expression as shown in **Table 14** and **Fig. 5A**. MHC class II expression was also assayed to quantify editing frequency, as shown in **Table 15** and **Fig. 5B**.

[00426] **Table 14 – Mean percentage of cells positive for mCherry following editing.**

Insertion	Guide	CD4		CD8		N
		% mCherry+	SD	% mCherry+	SD	
With AAV	G013676	12.9	0.8	17.2	3.2	2
	G013675	24.9	0.1	27.8	0.7	2
	G015535	13.7	0.1	17.4	1.8	2
No AAV	G013676	0.0	NA	0.0	NA	1
	G013675	0.0	NA	0.0	NA	1
	G015535	0.1	NA	0.0	NA	1

[00427] **Table 15 – Mean percentage of MHC Class II negative cells following editing**

Insertion	Guide	% MHC Class II neg	SD	n
With AAV	G013676	86.9	1.1	2
	G013675	89.6	0.2	2
	G015535	57.1	0.7	2
No AAV	G013676	87.5	n/a	1
	G013675	86.3	n/a	1
	G015535	51.5	n/a	1
untreated		34	n/a	1

Example 9 - LNP titration in T cells with fixed ratio of BC22n:UGI

[00428] Using LNP delivery to activated human T cells, the potency of single-target editing was assessed with either Cas9 or BC22n.

9.1. T cell preparation.

[00429] Healthy human donor apheresis was obtained commercially (Hemacare), and cells were washed and re-suspended in CliniMACS® PBS/EDTA buffer (Miltenyi Biotec Cat. No. 130-070-525) on the LOVO device. T cells were isolated via positive selection using CD4 and CD8 magnetic beads (Miltenyi Biotec Cat. No. 130-030-401/130-030-801) using the CliniMACS® Plus and CliniMACS® LS disposable kit. T cells were aliquoted into vials and cryopreserved in a 1:1 formulation of Cryostor® CS10 (StemCell Technologies Cat. No. 07930) and Plasmalyte A (Baxter Cat. No. 2B2522X) for future use. Upon thaw, T cells were plated at a density of 1.0×10^6 cells/mL in T cell basal media composed of X-VIVO 15™ serum-free hematopoietic cell medium (Lonza Bioscience) containing 5% (v/v) of fetal bovine serum, 50 μ M of 2-Mercaptoethanol, 10 mM of N-Acetyl-L-(+)-cysteine, 10 U/mL of Penicillin-Streptomycin, in addition to 1X cytokines (200 U/mL of recombinant human interleukin-2, 5 ng/mL of recombinant human interleukin-7 and 5 ng/mL of recombinant human interleukin-15). T cells were activated with TransAct™ (1:100 dilution, Miltenyi Biotec). Cells were expanded in T cell basal media for 72 hours prior to LNP transfection.

9.2 T cell editing

[00430] Each RNA species, i.e. UGI mRNA, sgRNA or editor mRNA, was formulated separately in an LNP as described in **Example 1**. Editor mRNAs encoded either BC22n (SEQ ID NO: 805) or Cas9 (SEQ ID NO: 803). A sgRNA targeting CIITA (G016086) (SEQ ID NO: 395) was used. UGI mRNA (SEQ ID NO: 807) is delivered in both Cas9 and BC22n arms of

the experiment to normalize lipid amounts. Previous experiments have established UGI mRNA does not impact total editing or editing profile when used with Cas9 mRNA. LNP compositions were mixed to fixed total mRNA weight ratios of 6:3:2 for editor mRNA, guide RNA, and UGI mRNA respectively as described in **Table 16**. LNP mixtures were incubated for 5 minutes at 37°C in T cell basal media substituting 6% cynomolgus monkey serum (Bioreclamation IVT, Cat. CYN220760) for fetal bovine serum.

[00431] Seventy-two hours post activation, T cells were washed and suspended in basal T cell media. Pre-incubated LNP mix was added to the each well with 1×10^5 cells/well. T cells were incubated at 37°C with 5% CO₂ for the duration of the experiment. T cell media was changed 6 days and 8 days after activation and on tenth day post activation, cells were harvested for analysis by NGS and flow cytometry. NGS analysis was performed as described in **Example 1**. **Table 16** and **Fig. 6A** describe editing of T cells. Total editing and C to T editing showed direct, dose responsive relationships to increasing amounts of BC22n mRNA, UGI mRNA and guide across all guides tested. Indel and C conversions to A or G are in an inverse relationship with dose where lower doses resulted in a higher percentage of these mutations. In samples edited with Cas9, total editing and indel activity increase with the total RNA dose.

[00432] **Table 16 - Editing as a percent of total reads – single guide delivery (n=2)**

Guide	Editor	Total RNA (ng)	% C-to-T		% C-to-A/G		% Indel	
			mean	SD	mean	SD	mean	SD
G016086	BC22n	0.0	0.2	0.0	1.0	0.1	0.1	0.0
		8.6	23.5	1.8	3.2	0.1	3.7	0.1
		17.2	40.9	1.1	4.4	0.7	4.6	1.0
		34.4	58.0	0.5	4.6	0.3	3.8	0.6
		68.8	73.5	0.7	3.7	0.0	2.8	0.5
		137.5	83.8	1.1	3.7	0.5	2.0	0.7
		275.0	90.1	2.4	3.1	0.1	1.9	0.8
		550.0	93.4	0.9	3.0	0.2	1.2	0.3
	Cas9	0.0	0.2	0.0	1.0	0.1	0.1	0.0
		8.6	0.2	0.0	1.1	0.2	7.4	0.7
		17.2	0.2	0.0	1.1	0.3	17.7	1.0
		34.4	0.2	0.0	0.8	0.1	32.1	0.1
		68.8	0.2	0.0	0.7	0.2	51.5	0.8
		137.5	0.2	0.0	0.4	0.0	69.3	0.1
		275.0	0.3	0.1	0.3	0.1	84.2	0.1
		550.0	0.3	0.0	0.1	0.1	90.0	0.7

[00433] On day 10 post-activation, T cells were phenotyped by flow cytometry to measure loss of cell surface proteins using antibodies targeting HLA DR DQ DP-PE (BioLegend, Cat 361704) and DAPI (BioLegend, Cat 422801) as described in **Example 5**. A subset of unedited cells was incubated with Isotype Control-PE (BioLegend® Cat. No. 400234).

[00434] **Table 17** and **Fig. 6B** report phenotyping results as percent of cells negative for antibody binding. The percentage of antigen negative cells increased in a dose responsive manner with increasing total RNA for both BC22n and Cas9 samples. Cells edited with BC22n showed comparable or higher protein knockout compared to cells edited with Cas9 for all guides tested.

[00435] **Table 17 – Flow cytometry data – percent cells MHC class II negative (n=2)**

Guide(s)	Phenotype	Total RNA (ng)	BC22n		Cas9	
			Mean %	SD	Mean %	SD
G016086 CIITA	HLA DR DP DQ neg	550.0	96.0	0.1	90.9	0.7
		275.0	93.7	0.1	87.4	0.3
		137.5	88.4	0.5	76.3	0.6
		68.8	80.0	0.7	66.1	1.8
		34.4	69.2	1.5	53.4	1.1
		17.2	56.4	0.4	41.9	0.8
		8.6	45.2	2.9	37.3	0.1
		0.0	30.1	0.9	36.8	0.4

Example 10 – Off-Target Analysis

10.1 Biochemical Off-Target Analysis

[00436] A biochemical method (See, e.g., Cameron et al., *Nature Methods*. 6, 600-606; 2017) was used to determine potential off-target genomic sites cleaved by Cas9 using specific guides targeting *CIITA*. In this experiment, two sgRNAs targeting human *CIITA* were screened using genomic DNA purified from lymphoblast cell line NA24385 (Coriell Institute) alongside three control guides with known off-target profiles. The number of potential off-target sites detected using a guide concentration of 192 nM and 64 nM Cas9 protein in the biochemical assay are shown in **Table 18**.

[00437] **Table 18: Biochemical Off-Target Analysis**

SEQ ID NO:	Guide ID	Target	Number of Sites
27	G013675	CIITA	16
28	G013676	CIITA	124
200	G000644	EMX1	276

SEQ ID NO:	Guide ID	Target	Number of Sites
201	G000645	VEGFA	3259
202	G000646	RAG1B	32

10.2 Targeted sequencing for validating potential off-target sites

[00438] Potential off-target sites predicted by detection assays such as the biochemical method used above, may be assessed using targeted sequencing of the identified potential off-target sites to determine whether off-target cleavage at that site is detected.

[00439] In one approach, Cas9 and a sgRNA of interest (e.g., a sgRNA having potential off-target sites for evaluation) are introduced to primary T cells. The T cells are then lysed and primers flanking the potential off-target site(s) are used to generate an amplicon for NGS analysis. Identification of indels at a certain level may validate a potential off-target site, whereas the lack of indels found at the potential off-target site may indicate a false positive from the off-target predictive assay that was utilized.

Example 11 - Multi-editing T Cells with Sequential LNP Delivery

[00440] T cells were engineered with a series of gene disruptions and insertions. Healthy donor cells were treated sequentially with four LNP compositions, each LNP co-formulated with mRNA encoding Cas9 (SEQ ID NO. 802) and a sgRNA targeting either TRAC (G013006), TRBC (G016239), CIITA (G013676), or HLA-A (G018995). LNP compositions were formulated with lipid A, cholesterol, DSPC, and PEG2k-DMG in a 50:38.5:10:1.5 molar ratio, respectively. The lipid nucleic acid assemblies were formulated with a lipid amine to RNA phosphate (N:P) molar ratio of about 6, and a ratio of gRNA to mRNA of 1:2 by weight. A transgenic T cell receptor targeting Wilm's tumor antigen (WT1 TCR) (SEQ ID NO: 1000) was integrated into the TRAC cut site by delivering a homology directed repair template using AAV.

11.1. T cell Preparation

[00441] T cells were isolated from the leukapheresis products of three healthy HLA-A2+ donors (STEMCELL Technologies). T cells were isolated using EasySep Human T cell Isolation kit (STEMCELL Technologies, Cat. 17951) following manufacturers protocol and cryopreserved using Cryostor CS10 (STEMCELL Technologies, Cat. 07930). The day before initiating T cell editing, cells were thawed and rested overnight in T cell activation media (TCAM): CTS OpTmizer (Thermofisher, Cat. A3705001) supplemented with 2.5% human AB

serum (Gemini, Cat. 100-512), 1X GlutaMAX (Thermofisher, Cat.35050061), 10 mM HEPES (Thermofisher, Cat. 15630080), 200 U/mL IL-2 (Peprotech, Cat. 200-02), IL-7 (Peprotech, Cat. 200-07), IL-15 (Peprotech, Cat. 200-15).

11.2. LNP Treatment and Expansion of T cells

[00442] LNP compositions were prepared each day in ApoE containing media and delivered to T cells as described in **Table 19** and below.

[00443] **Table 19: – Order of editing for T cell engineering**

Group	Day 1	Day 2	Day 3	Day 4
1	Unedited	Unedited	Unedited	Unedited
2	TRBC	CIITA	TRAC	HLA-A
3	TRBC	HLA-A	TRAC	CIITA
4	TRBC		TRAC	

[00444] On day 1, LNP compositions as indicated in **Table 19** were incubated at a concentration of 5 ug/mL in TCAM containing 5 ug/mL rhApoE3 (Peprotech, Cat. 350-02). Meanwhile, T cells were harvested, washed, and resuspended at a density of 2×10^6 cells/mL in TCAM with a 1:50 dilution of T Cell TransAct, human reagent (Miltenyi, Cat. 130-111-160). T cells and LNP-ApoE media were mixed at a 1:1 ratio and T cells plated in culture flasks overnight.

[00445] On day 2, LNP compositions as indicated in **Table 19** were incubated at a concentration of 25 ug/mL in TCAM containing 20 ug/mL rhApoE3 (Peprotech, Cat. 350-02). LNP-ApoE solution was then added to the appropriate culture at a 1:10 ratio.

[00446] On day 3, TRAC-LNP compositions was incubated at a concentration of 5 ug/mL in TCAM containing 10 ug/mL rhApoE3 (Peprotech, Cat. 350-02). T cells were harvested, washed, and resuspended at a density of 1×10^6 cells/mL in TCAM. T cells and LNP-ApoE media were mixed at a 1:1 ratio and T cells plated in culture flasks. WT1 AAV (SEQ ID NO: 1000) was then added to each group at a MOI of 3×10^5 genome copies/cell.

[00447] On day 4, LNP compositions as indicated in **Table 19** were incubated at a concentration of 5 ug/mL in TCAM containing 5 ug/mL rhApoE3 (Peprotech, Cat. 350-02). LNP-ApoE solution was then added to the appropriate culture at a 1:1 ratio.

[00448] On days 5-11, T cells were transferred to a 24-well GREX plate (Wilson Wolf, Cat. 80192) in T cell expansion media (TCEM): CTS OpTmizer (Thermofisher, Cat. A3705001) supplemented with 5% CTS Immune Cell Serum Replacement (Thermofisher, Cat.

A2596101), 1X GlutaMAX (Thermofisher, Cat. 35050061), 10 mM HEPES (Thermofisher, Cat. 15630080), 200 U/mL IL-2 (Peprotech, Cat. 200-02), IL-7 (Peprotech, Cat. 200-07), and IL-15 (Peprotech, Cat. 200-15). Cells were expanded per manufacturers protocols. T-cells were expanded for 6-days, with media exchanges every other day. Cells were counted using a Vi-CELL cell counter (Beckman Coulter) and fold expansion was calculated by dividing cell yield by the starting material as shown in **Table 20**.

[00449] **Table 20 – Fold expansion following multi-edit T cell engineering**

Group	Donor A	Donor B	Donor C	Mean	SD
1	331.40	362.24	533.18	408.94	108.69
2	61.82	72.15	116.13	83.37	28.84
3	64.08	76.29	157.75	99.37	50.92
4	No data	146.78	331.67	239.22	130.74

11.3. Quantification of T cell editing by flow cytometry and NGS

[00450] Post expansion, edited T cells were assayed by flow cytometry to determine HLA-A2 expression (HLA-A⁺), HLA-DR-DP-DQ expression (MHC II⁺) following knockdown CIITA, WT1-TCR expression (CD3⁺ Vb8⁺), and the expression of residual endogenous TCRs (CD3⁺ Vb8⁻) or mispaired TCRs (CD3⁺ Vb8^{low}). T cells were incubated with an antibody cocktail targeting the following molecules: CD4 (Biolegend, Cat. 300524), CD8 (Biolegend, Cat. 301045), Vb8 (Biolegend, Cat. 348106), CD3 (Biolegend, Cat. 300327), HLA-A2 (Biolegend, Cat. 343306), HLA-DRDPDQ (Biolegend, Cat 361706), CD62L (Biolegend, Cat. 304844), CD45RO (Biolegend, Cat. 304230). Cells were subsequently washed, analyzed on a Cytotflex LX instrument (Beckman Coulter) using the FlowJo software package. T cells were gated on size and CD4/CD8 status, before expression of editing and insertion markers was determined. The percentage of cells expressing relevant cell surface proteins following sequential T cell engineering are shown in **Table 21** and **Figs. 7A-F** for CD8⁺ T cells and **Table 22** and **Figs. 8A-F** for CD4⁺ T cells. The percent of fully edited CD4⁺ or CD8⁺ T cells was gated as % CD3⁺ Vb8⁺ HLA-A⁻ MHC II⁻. High levels of HLA-A and MHC II knockdown, as well as WT1-TCR insertion and endogenous TCR KO are observed in edited samples. In addition to flow cytometry analysis, genomic DNA was prepared and NGS analysis performed as described in **Example 1** to determine editing rates at each target site. **Table 23** and **Figs. 9A-D** show results for percent editing at the CIITA, HLA-A, and TRBC1/2 loci, with patterns

across the groups consistent with what was identified by flow cytometry. TRBC1/2 loci were edited to >90-95% in all groups.

[00451] Table 21: Percentage of CD8⁺ cell with cell surface phenotype following sequential T cell engineering

Donor	Group	% HLA-A + HLA-A2 ⁺	% MHC II ⁺ HLA-DR- DP-DQ ⁺	% WT1 TCR CD3 ⁺ Vb8 ⁺	% Mispaired TCR CD3 ⁺ Vb8 ^{low}	% Residual endogenous TCR CD3 ⁺ Vb8 ⁺	% Fully edited CD3 ⁺ Vb8 ⁺ HLA-A2 ⁻ HLA-DR-DP-DQ ⁻
A	1 Unedited	100.0	60.9	6.7	0.8	93.2	0.0
B		99.7	71.0	3.4	0.6	96.1	0.2
C		99.7	52.2	5.7	0.8	94.0	0.0
A	2	2.7	1.2	68.9	1.3	0.4	66.7
B		1.3	21.0	50.4	3.1	4.5	43.3
C		1.8	2.9	62.2	2.6	2.7	60.3
A	3	1.3	0.8	66.0	1.4	0.3	64.4
B		1.4	2.2	56.8	2.2	2.0	55.1
C		1.2	5.7	63.3	1.0	0.9	60.6
B	4	99.8	64.8	62.3	2.0	2.5	0.1
C		99.0	51.5	71.0	1.0	0.5	0.4

[00452] Table 22: Percentage of CD4+ cells with cell surface phenotype following sequential T cell engineering

Donor	Group	% HLA-A +	% MHC II ⁺	% WT1 TCR	% Mispaired TCR	% Residual endogenous TCR	% Fully edited
		HLA-A2 ⁺	HLA-DR- DP-DQ ⁺	CD3 ⁺ Vb8 ⁺	CD3 ⁺ Vb8 ^{low}	CD3 ⁺ Vb8 ⁻	CD3 ⁺ Vb8 ⁺ HLA-A2 ⁻ HLA-DR-DP-DQ ⁻
A	1 Unedited	100.0	36.3	5.4	0.4	94.5	0.0
B		98.7	27.6	5.6	0.4	94.3	0.0
C		99.3	32.3	6.2	0.3	93.6	0.1
A	2	2.6	0.7	62.4	2.4	1.1	60.9
B		1.8	0.5	59.7	2.2	1.0	58.5
C		1.7	3.2	58.6	1.6	1.8	55.8
A	3	1.3	0.8	63.0	3.4	0.8	61.7
B		1.1	1.1	61.8	2.6	0.9	60.6
C		1.1	0.4	60.9	1.7	1.0	59.9
B	4	99.5	25.1	61.9	1.9	5.2	0.1
C		97.9	40.1	69.5	4.7	1.9	0.8

[00453] **Table 23: Percent indels at CIITA, HLA-A, TRBC1 and TRBC2 following sequential T cell editing**

	CIITA (G013676)			HLA-A (G018995)			TRBC1 (G016239)			TRBC2 (G016239)		
Gro up	Don or A	Don or B	Don or C	Don or A	Don or B	Don or C	Don or A	Don or B	Don or C	Don or A	Don or B	Don or C
1	0.2	0.2	0.2	6.9	3.3	2.3	0.1	0.3	0.2	0.3	0.3	0.3
2	98.2	81.8	93.8	94.1	90.2	90.6	97.6	89.9	91.4	98.7	86.8	94.9
3	98.9	98.1	98.9	97.2	86.4	93.1	98.6	94.4	94.7	98.6	94.2	96.6
4	0.1	0.2	0.6	7.6	2.7	3.2	98.9	94	95	98.6	93.2	97.4

Example 12. NK cell functional killing assays

[00454] T cells edited in various combinations to disrupt CIITA, HLA-A, or B2M or to overexpress HLA-E were tested for their ability to resist natural killer (NK) cell mediated killing.

12.1. Engineering T cells and purification

[00455] Upon thaw, Pan CD3+ T cells (StemCell, HLA-A*02.01/ A*03.01) were plated at a density of 0.5×10^6 cells/mL in T cell RPMI media composed of RPMI 1640 (Invitrogen, Cat. 22400-089) containing 5% (v/v) of fetal bovine serum, 1x Glutamax (Gibco, Cat. 35050-061), 50 μ M of 2-Mercaptoethanol, 100 μ M non-essential amino acids (Invitrogen, Cat. 11140-050), 1 mM sodium pyruvate, 10 mM HEPES buffer, 1% of Penicillin-Streptomycin, and 100 U/mL of recombinant human interleukin-2 (Peprotech, Cat. 200-02). T cells were activated with TransAct™ (1:100 dilution, Miltenyi Biotec).

[00456] As described in **Table 24**, one day following activation, T cells were edited with to disrupt the B2M gene. Briefly, LNP compositions containing Cas9 mRNA and sgRNA G000529 (SEQ ID NO: 216) targeting B2M were formulated as described in **Example 1**. LNP compositions were incubated in RPMI-based media with cytokines as described above supplemented with 1 μ g/ml recombinant human ApoE3 (Peprotech, Cat. 350-02) for 15 minutes at 37°C. LNP mix was added to two million activated T cells to yield a final concentration of 2.5 μ g total LNP/mL.

[00457] **Table 24 – Order of sequential editing and viral transduction**

Condition	Day 1	Day2	Day 3
Unedited			
B2M ⁻	B2M LNP		
B2M ⁻ + HLA-E	B2M LNP		HLA-E lentivirus
HLA-A ⁻ MHC II ⁻		CIITA LNP	HLA-A LNP
HLA-A ⁻			HLA-A LNP

[00458] Two days post activation, additional T cells were edited with LNP compositions to disrupt the CIITA gene. This was performed as described for B2M editing using LNP compositions containing Cas9 mRNA and sgRNA G013675 (sgRNA comprising SEQ ID NO: 27, as shown in Table 2) targeting CIITA. LNP compositions used in this step were formulated with lipid A, cholesterol, DSPC, and PEG2k-DMG in a 50:38.5:10:1.5 molar ratio, respectively. The lipid nucleic acid assemblies were formulated with a lipid amine to RNA phosphate (N:P) molar ratio of about 6, and a ratio of gRNA to mRNA of 1:2 by weight.

[00459] Three days post activation, all edited and unedited cells were resuspended in fresh media without TransAct. A B2M-edited T cell sample was transduced by centrifugation at 1000g at 37C for 1 hour with lentivirus expressing HLA-E from an EF1a promoter (SEQ ID No. 1004) at an MOI of 10. A CIITA-edited T cell sample was further edited with LNP compositions to disrupt the HLA-A gene. Editing was performed as described for B2M editing above using LNP compositions containing Cas9 mRNA and sgRNA G019000 targeting HLA-A formulated with lipid A, cholesterol, DSPC, and PEG2k-DMG in a 50:38.5:10:1.5 molar ratio, respectively. The lipid nucleic acid assemblies were formulated with a lipid amine to RNA phosphate (N:P) molar ratio of about 6, and a ratio of gRNA to mRNA of 1:2 by weight.. Four days post activation, all cells were transferred to GREX plate (Wilson Wolf, Cat. 80240M) for expansion.

[00460] Seven days post activation, HLA-E infected T cells were selected for HLA-E expression using Biotinylated Anti-HLA-E Antibody (Biolegend). and Anti-Biotin microbeads (Miltenyi Biotec, Cat#130-090-485) and a magnetic LS Column (Miltenyi Biotec, Cat# 130-042-401) according to manufacturer's protocols.

[00461] Similarly, nine days post activation CIITA edited T cells were negatively selected for lack of MHC II expression. using Biotinylated Anti-HLA-Class II Antibody (Miltenyi, Cat. 130-104-823), Anti-Biotin microbeads (Miltenyi Biotec, Cat. 130-090-485) and a magnetic LS Column (Miltenyi Biotec, Cat. 130-042-401) according to manufacturer's protocols.

12.2 Flow cytometry

[00462] NK cell mediated cytotoxicity towards engineered T cells was assayed. For this the T cells were co-cultured with the HLA-B/C matched CTV labelled NK cells at effector to target ratios (E:T) of 10:1, 5:1, 2.5:1, 1.25:1 and 0.625:1 for 21 hours. The cells were stained with 7AAD (BD Pharmingen, Cat. 559925), processed on a Cytotflex flow cytometer (Beckman Coulter) and analyzed using the FlowJo software package. T cells were gated based on CTV negativity, size, and shape and viability. **Table 25** and **Fig. 10** show the percentage of T cell lysis following NK cell challenge.

[00463] **Table 25 – Percentage T cell lysis following NK cell challenge to engineered T cells**

Log(E:T)	Unedited		HLA-A ⁻		HLA-A ⁻ MHC II ⁻		B2M ⁻		B2M ⁻ + HLA-E		n
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Basal	12.0	1.9	15.5	0.2	8.2	0.4	11.1	0.1	18.1	2.5	2
-0.20	15.1	0.0	16.0	0.5	11.2	0.8	32.6	1.6	25.0	0.9	2
0.10	14.5	0.2	15.6	0.4	10.6	0.1	44.7	2.3	29.4	0.1	2
0.40	12.8	0.6	13.6	0.4	9.3	0.1	66.0	1.8	39.3	0.1	2
0.70	10.4	0.4	11.9	0.2	9.2	0.4	71.2	1.3	51.9	1.6	2
1.00	8.4	0.1	9.4	0.6	7.6	0.1	62.8	0.6	51.7	2.8	2

Example 13: HLA-A and CIITA Partial-Matching in an NK Cell *In Vivo* Killing Mouse Model

[00464] Female NOG-hIL-15 mice were engrafted with 1.5×10^6 primary NK cells followed by the injection of engineered T cells containing luciferase +/- HLA-A, CIITA, or HLA-A/CIITA KO 4 weeks later in order to determine 1) whether engrafted NK cells can readily lyse control T cells (B2M^{-/-}), and 2) whether the addition of a partial-matching edit (HLA-A or CIITA) provides a protective effect for T cells from NK cell lysis in vivo.

13.1. Preparation of T cells containing luciferase +/- HLA-A, CIITA, or HLA-A/CIITA KO

[00465] T cells were isolated from peripheral blood of a healthy human donor with the following MHC I phenotype: HLA-A*02:01:01G, 03:01:01G, HLA-B*07:02:01G, HLA-C*07:02:01G. Briefly, a leukapheresis pack (Stemcell Technologies) was treated in ammonium chloride RBC lysis buffer (Stemcell Technologies; Cat. 07800) for 15 minutes to lyse red blood cells. Peripheral blood mononuclear cell (PBMC) count was determined post lysis and T cell isolation was performed using EasySep Human T cell isolation kit (Stemcell Technologies,

Cat. 17951) according to manufacturer's protocol. Isolated CD3⁺ T cells were re-suspended in Cryostor CS10 media (Stemcell Technologies, Cat. 07930) and frozen down in liquid nitrogen until further use.

[00466] Frozen T cells were thawed at a cell concentration of 1×10^6 cells/ml into T cell growth media (TCGM) composed of OpTmizer TCGM as described in Example 3 further supplemented with 100 U/mL of recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/ml IL-7 (Peprotech, Cat. 200-07), 5ng/ml IL-15 (Peprotech, Cat. 200-15). Cells were activated using T cell TransAct™ (Miltenyi Biotec, Cat. 130-111-160) at 1:100 dilution at 37°C for 24 hours.

[00467] Twenty-four hours post activation, 1×10^6 T cells in 500 ul fresh TCGM without cytokines were transduced by centrifugation 1000xG for 60 minutes at 37°C with 150 ul of Luciferase lentivirus (Imanix Life Sciences, Cat# LV050L). Transduced cells were expanded in 24-well G-Rex plate (Wilson Wolf, Cat. 80192M) in TCGM with cytokines at 37°C for 24 hours.

[00468] Forty-eight hours post activation, luciferase LV infected T cells were edited to disrupt the B2M or HLA-A genes. Briefly, LNP compositions containing mRNA encoding cas9 (SEQ ID NO:802) and sgRNA G019000 (SEQ ID NO: 217) targeting HLA-A were formulated with lipid A, cholesterol, DSPC, and PEG2k-DMG in a 50:38.5:10:1.5 molar ratio, respectively. The lipid nucleic acid assemblies were formulated with a lipid amine to RNA phosphate (N:P) molar ratio of about 6, and a ratio of gRNA to mRNA of 1:2 by weight. LNP compositions containing the Cas9 mRNA and sgRNA G000529 (SEQ ID NO: 216) targeting B2M were formulated as described in **Example 1**. LNP compositions were incubated in Optimizer TCGM without serum or cytokines further supplemented with 1 ug/ml recombinant human ApoE3 (Peprotech, Cat. 350-02) for 15 minutes at 37°C. T cells were washed and suspended in TCGM with cytokines. Pre-incubated LNP and T cells were mixed to yield final concentrations of 0.5×10^6 T cells/ml and 2.5 µg total RNA/mL of LNP in TCGM with 5% human AB serum, 100 U/mL of recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/ml IL-7 (Peprotech, Cat. 200-07), 5ng/ml IL-15 (Peprotech, Cat. 200-15). An additional group of cells were mock edited with media containing ApoE3 but no LNP compositions. All cells were incubated at 37°C for 24 hours.

[00469] Seventy-two hours post activation, the cells were edited to disrupt CIITA, and LNP were administered either on luciferase and HLA-A edited cells or luciferase cells alone. Briefly, cells were transduced with LNP compositions containing the Cas9 mRNA and sgRNA G013675 (sgRNA comprising SEQ ID NO: 27, as shown in Table 2) as described for HLA-A

editing. Ninety-six hours post activation, cells were washed and transferred to a 24-well G-Rex. Media with fresh cytokines was replaced every 2 days. On day 15 post activation, edited T cells were sorted on GFP⁺ cells using BD FACS Aria Flow Sorter to enrich for luciferase-expressing cells. For B2M KO luciferase group, cells were sorted on GFP⁺ and MHC-I⁻. Sorted cells were rested overnight in TCGM media with cytokines in a 37°C incubator. The next day, T cells were re-stimulated with T-cell TransAct™ at 1:100 dilution for 24 hours. Twenty-four hours after restimulation, TransAct was washed out and T cells were cultured and maintained in G-Rex plate for 15 days with regular changes in media and cytokines.

[00470] Fifteen days after restimulation, NK cell mediated cytotoxicity towards engineered T cells was assayed *in vitro* as in **Example 12** with the following exceptions. Assays were performed using OpTmizer TCGM with 100 µl/ml IL-2. T cells were co-cultured overnight with the HLA-B/C matched CTV labelled NK cells at effector to target ratios (E:T) of 10:1, 5:1, 2.5:1, 1.25:1 and 0.625:1. The cells were incubated with BrightGlo Luciferase reagents (Promega, Cat. E2620) and processed on the CellTiter Glo Program in ClarioStar to determine lysis of T cells by NK cells based on luciferase signal. **Table 26** shows the percentage of T cell lysis following NK cell challenge. In vitro, B2M edited cells showed sensitivity to NK killing, while HLA-A edited, CIITA edited and HLA-A, CIITA double edited cells showed protection from NK mediated lysis.

[00471] **Table 26 – Percentage of lysis of luciferase transduced T cell following NK cell challenge**

	No edit		HLA-A KO		CIITA KO		HLA-A KO, CIITA KO		B2M KO		
E:T	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	n
10	19.22	3.16	28.55	1.02	22.96	3.59	22.22	3.15	68.09	0.11	2
5	13.04	1.71	27.18	4.35	22.85	6.93	13.78	4.55	53.87	3.30	2
2.5	1.56	1.35	26.56	3.75	26.59	2.44	21.32	0.72	39.46	7.05	2
1.25	-0.26	1.94	19.78	3.24	19.91	5.38	12.86	0.54	25.79	7.96	2
0.625	8.67	6.81	25.44	0.23	18.32	4.28	19.80	7.20	29.31	2.67	2
0.3125	2.96	7.66	22.40	0.83	19.13	1.34	13.34	2.48	9.32	0.84	2

13.2. HLA-A and CIITA double knockout T cells are protected from NK killing

[00472] For the *in vivo* study, NK cells isolated from a leukopak by methods known in the art were washed with HBSS (Gibco, Cat. No. 14025-092) and resuspended at 10×10^6 cells/mL for injection in 150 µL HBSS. Twenty-two female NOG-hIL-15 mice (Taconic) were dosed by tail vein injection with 1.5×10^6 isolated NK cells. An addition 27 female NOG-hIL-15 served NK-non-injected controls.

[00473] Twenty-eight days after NK cell injection, mice were injected with unedited or engineered T cells as described in **Table 26**. Briefly, engineered T cells were injected 16 days post second activation after washing in PBS and resuspending in HBSS solution at a concentration of 6×10^6 cells/150 μ L.

[00474] IVIS imaging of live mice was performed to identify luciferase-positive T cells by IVIS spectrum. IVIS imaging was done at 6 hours, 24 hours, 48 hours, 8 days, 13 days, 18 days, and 27 days after T cell injection. Mice were prepared for imaging with an injection of D-luciferin i.p. at 10 μ L/g body weight per the manufacturer's recommendation, about 150 μ L per animal. Animals were anesthetized and then placed in the IVIS imaging unit. The visualization was performed with the exposure time set to auto, field of view D, medium binning, and F/stop set to 1. **Table 27** and **Fig. 11A** shows radiance (photons/s/cm²/sr) from luciferase expressing T cells present at the various time points after injection. **Fig. 11B** shows radiance (photons/s/cm²/sr) from luciferase expressing T cells present in the various mice groups after 27 days. In vivo, B2M edited cells showed sensitivity to NK killing, while HLA-A edited, CIITA edited and HLA-A, CIITA double edited cells showed protection from NK mediated lysis. Unexpectedly, even after a reduction in one of the three highly polymorphic MHC class I proteins (HLA-A) the cells are protected against NK-mediated rejection.

[00475] **Table 27 – Radiance (photons/s/cm²/sr) from luciferase expressing T cells in treated mice at intervals after T cell injection.**

T cell injection	Timepoint (days)	No NK cell injection			NK cell injection		
		Mean	SD	n	Mean	SD	n
No T cells	0.25	5,065	474	2	6,010	651	2
	1	5,225	431	2	5,150	467	2
	4	4,715	403	2	4,860	57	2
	6	5,145	884	2	5,110	226	2
	11	5,230	382	2	4,700	99	2
	13	6,920	948	2	6,735	35	2
	18	5,055	148	2	5,570	28	2
	27	4,740	311	2	5,185	290	2
No edit	0.25	477,200	51,237	5	464,000	112,493	4
	1	547,600	59,315	5	517,500	95,710	4
	4	285,600	43,328	5	219,750	77,298	4
	6	249,400	58,748	5	137,000	69,190	4
	11	131,500	28,671	5	111,150	36,287	4
	13	147,000	15,732	5	43,168	52,128	4
	18	112,100	20,768	5	55,825	47,391	4
	27	53,960	13,546	5	59,700	31,479	4

T cell injection	Timepoint (days)	No NK cell injection			NK cell injection		
		Mean	SD	n	Mean	SD	n
B2M KO	0.25	662,600	193,865	5	261,850	135,636	4
	1	555,200	122,508	5	89,400	41,151	4
	4	266,200	68,845	5	25,175	11,072	4
	6	202,600	41,825	5	18,500	7,048	4
	11	106,320	14,377	5	17,100	9,440	4
	13	57,714	45,535	5	7,048	2,735	4
	18	77,080	7,792	5	9,453	4,592	4
	27	55,240	12,780	5	6,860	1,207	4
HLA-A KO	0.25	160,000	30,315	5	111,500	30,533	4
	1	206,800	38,493	5	153,000	24,427	4
	4	120,200	23,488	5	91,025	69,091	4
	6	81,100	16,903	5	91,408	106,141	4
	11	55,520	6,843	5	53,367	21,985	3
	13	30,716	23,658	5	33,233	13,615	3
	18	21,802	10,911	5	35,667	5,601	3
	27	20,600	808	4	46,900	4,937	3
CIITA KO	0.25	121,400	19,680	5	116,350	82,606	4
	1	168,200	32,760	5	120,225	43,535	4
	4	93,600	23,187	5	76,450	31,056	4
	6	71,298	40,161	5	52,500	35,590	4
	11	59,100	13,805	5	73,500	77,242	4
	13	43,870	22,810	5	31,760	30,831	4
	18	28,422	14,019	5	35,000	7,902	3
	27	18,780	3,505	5	69,067	31,194	3
HLA-A KO CIITA KO	0.25	259,250	59,824	4	363,000	113,731	4
	1	456,750	69,188	4	481,500	142,778	4
	4	170,500	26,665	4	200,750	70,415	4
	6	108,950	11,046	4	98,633	27,450	3
	11	97,350	19,982	4	93,867	32,173	3
	13	85,708	58,720	4	68,357	54,428	3
	18	20,923	22,172	4	98,633	27,450	3
	27	37,375	10,602	4	31,733	2,593	3

Example 14: HLA-A and CIITA Partial-Matching in an NK Cell In Vivo Killing Mouse Model

[00476] Female NOG-hIL-15 mice were engrafted with 1.5×10^6 primary NK cells followed by the injection of engineered T cells containing luciferase +/- HLA-A/CIITA KO with HD1 TCR 4 weeks later in order to determine 1) whether engrafted NK cells can readily lyse control T cells (B2M^{-/-}), and 2) whether the addition of a partial-matching edit (HLA-A & CIITA)

provides a protective effect for T cells with the exogenous HD1 TCR from NK cell lysis *in vivo*.

14.1. Preparation of T cells containing luciferase +/-HLA-A/CIITA KO and HD1 TCR

[00477] T cells were isolated from peripheral blood of a healthy human donor with the following MHC I phenotype: HLA-A*02:01:01G, 03:01:01G, HLA-B*07:02:01G, HLA-C*07:02:01G. Briefly, a leukapheresis pack (Stemcell Technologies) was treated in ammonium chloride red blood cell lysis buffer (Stemcell Technologies; Cat. 07800) for 15 minutes to lyse red blood cells. Peripheral blood mononuclear cell (PBMC) count was determined post lysis, and T cell isolation was performed using EasySep Human T cell isolation kit (Stemcell Technologies, Cat. 17951) according to manufacturer's protocol. Isolated CD3+ T cells were re-suspended in Cryostor CS10 media (Stemcell Technologies, Cat. 07930) and frozen down in liquid nitrogen until further use.

[00478] Frozen T cells were thawed at a cell concentration of 1.5×10^6 cells/ml into T cell activation media (TCAM) composed of OpTmizer TCGM as described in **Example 3** and further supplemented with 100 U/mL of recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/ml IL-7 (Peprotech, Cat. 200-07), 5 ng/ml IL-15 (Peprotech, Cat. 200-15). Cells were rested at 37 °C for 24 hours.

[00479] Twenty-four hours post thawing, T cells were counted and resuspended at 2×10^6 cells/ml in TCAM media and 1:50 of Transact was added. Cells were mixed and incubated for 20-30 mins at 37°C. LNP compositions containing mRNA encoding Cas9 (SEQ ID NO:802) and sgRNA G013675 (sgRNA comprising SEQ ID NO: 27, as shown in Table 2, targeting CIITA) were formulated with lipid A, cholesterol, DSPC, and PEG2k-DMG in a 50:38.5:10:1.5 molar ratio, respectively. The lipid nucleic acid assemblies were formulated with a lipid amine to RNA phosphate (N:P) molar ratio of about 6, and a ratio of gRNA to mRNA of 1:2 by weight. LNP compositions at 5 ug/ml were incubated in OpTmizer TCAM and further supplemented with 5 ug/ml recombinant human ApoE3 (Peprotech, Cat. 350-02) for 15 minutes at 37 °C. Pre-incubated LNP compositions and T cells with Transact were mixed to yield final concentrations of 1×10^6 T cells/ml and 2.5 µg total RNA/mL of LNP in TCAM media with 2.5% human AB serum, 100 U/mL of recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/ml IL-7 (Peprotech, Cat. 200-07), and 5 ng/ml IL-15 (Peprotech, Cat. 200-15). An additional group of cells were mock-edited with media containing ApoE3 but no LNP compositions. All cells were incubated at 37 °C for 24 hours.

[00480] After 48 hours post activation, all groups were transduced with EF1 α -GFP-Luc lentivirus. Lentivirus was removed from -80 °C and thawed on ice. Cells were collected as per groups and centrifuged at 500Xg for 5 mins to wash off the LNP compositions and media. Cells were resuspended, individually according to their groups, at 2x10⁶ cells/ml in TCAM media. 500 ul of the cell suspension was then transferred to a sterile Eppendorf tube (total 1x10⁶ cells), and 100 ul of lentivirus was added. Cells were centrifuged at 1000XG for 60 minutes at 37 °C. After centrifugation, the cells were combined according to their groups and resuspended at 1x10⁶ cells/ml of TCAM media containing final concentration of 2.5% human AB serum, 100 U/mL of recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/ml IL-7 (Peprotech, Cat. 200-07), and 5 ng/ml IL-15 (Peprotech, Cat. 200-15) followed by incubating at 37 °C for 24 hours.

[00481] Seventy-two hours post activation, luciferase-transduced T cells were treated with LNP compositions to disrupt TRAC genes and further treated with HD1 AAV to insert the HD1 TCR at the TRAC locus. Cells were collected as per groups and centrifuged at 500Xg for 5 mins to wash off the lentivirus and media. The cells were then resuspended in TCAM media at 1x10⁶ cells/ml in TCAM media. LNP compositions containing mRNA encoding Cas9 (SEQ ID NO:802) and sgRNA G013006 (SEQ ID NO: 203, targeting TRAC) were formulated with lipid A, cholesterol, DSPC, and PEG2k-DMG in a 50:38.5:10:1.5 molar ratio, respectively. The lipid nucleic acid assemblies were formulated with a lipid amine to RNA phosphate (N:P) molar ratio of about 6, and a ratio of gRNA to mRNA of 1:2 by weight. LNP compositions at 5 ug/ml were incubated in OpTmizer TCAM and further supplemented with 5 ug/ml recombinant human ApoE3 (Peprotech, Cat. 350-02) for 15 minutes at 37 °C. Pre-incubated LNP compositions and T cells with Transact were mixed to yield final concentrations of 1x10⁶ T cells/ml and 2.5 μ g total RNA/mL of LNP in TCAM with 2.5% human AB serum, 100 U/mL of recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/ml IL-7 (Peprotech, Cat. 200-07), and 5 ng/ml IL-15 (Peprotech, Cat. 200-15). A vial of EF1 α -HD1 AAV was thawed on benchtop and added to the TRAC LNP treated cells at 3x10⁵ GC/cell. Cells were then incubated at 37 °C for 24hours.

[00482] Ninety-six hours post activation cells were then treated for a final round of editing either with TRBC LNP alone or in combination with HLA-A LNP. The B2M KO group was treated with B2M LNP. Cells were collected as per groups and centrifuged at 500Xg for 5 mins to wash off the LNP compositions and media. The cells were then resuspended in TCAM media at 1x10⁶ cells/ml in TCAM media. Briefly, LNP compositions containing mRNA encoding Cas9 (SEQ ID NO:802) and sgRNA G018995 (SEQ ID NO: 214 targeting HLA-A

were formulated as described in **Example 1**). LNP compositions containing the Cas9 mRNA and sgRNA G000529 (SEQ ID NO: 216) targeting B2M and LNP compositions containing the Cas9 mRNA and sgRNA G016239 (SEQ ID NO: 211) targeting TRBC were formulated with lipid A, cholesterol, DSPC, and PEG2k-DMG in a 50:38.5:10:1.5 molar ratio, respectively. The lipid nucleic acid assemblies were formulated with a lipid amine to RNA phosphate (N:P) molar ratio of about 6, and a ratio of gRNA to mRNA of 1:2 by weight. LNP compositions at 5 ug/ml were incubated in OpTmizer TCAM and further supplemented with 5 ug/ml recombinant human ApoE3 (Peprtech, Cat. 350-02) for 15 minutes at 37 °C. Pre-incubated LNP compositions and T cells with Transact were mixed to yield final concentrations of 1×10^6 T cells/ml and 2.5 µg total RNA/mL of LNP in TCAM with 2.5% human AB serum, 100 U/mL of recombinant human interleukin-2 (Peprtech, Cat. 200-02), 5 ng/ml IL-7 (Peprtech, Cat. 200-07), and 5ng/ml IL-15 (Peprtech, Cat. 200-15). For simultaneous TRBC and HLA-A editing, LNP and ApoE3 were formulated at 4X the final concentration followed by adding TRBC LNP first to the T cells and incubating at 37 °C for 15 mins. After incubation preformulated HLA-A LNP compositions were added, the cells were incubated for 24 hours.

[00483] After the final round of editing, the cells were washed by spinning at 500XG for 5 mins and resuspended in TCGM media containing with 5% human AB serum, 100 U/mL of recombinant human interleukin-2 (Peprtech, Cat. 200-02), 5 ng/ml IL-7 (Peprtech, Cat. 200-07), and 5 ng/ml IL-15 (Peprtech, Cat. 200-15).

[00484] On day 5 post activation, edited T cells were sorted on GFP⁺ cells using a BD FACS Aria Flow Sorter to enrich for luciferase-expressing cells. Sorted cells were rested overnight in TCGM media with cytokines in a 37 °C incubator. The next day, T cells were re-stimulated with T-cell TransAct™ at 1:100 dilution for 24 hours. Twenty-four hours after restimulation, TransAct™ was washed out and T cells were cultured and maintained in G-Rex plate for 15 days with regular changes in media and cytokines.

[00485] Fifteen days after first restimulation, editing levels were confirmed via flow cytometry, and cells were washed and resuspend in HBSS buffer for injections.

14.2. HLA-A and CIITA double knockout T cells show protection from NK killing

[00486] For the *in vivo* study, NK cells isolated from a leukopak by methods known in the art were washed with HBSS (Gibco, Cat. No. 14025-092) and resuspended at 10×10^6 cells/mL for injection in 150 µL HBSS. Thirty female NOG-hIL-15 mice (Taconic) were dosed by tail vein injection with 1.5×10^6 isolated NK cells. An addition 25 female NOG-hIL-15 served as NK-non-injected controls.

[00487] Twenty-eight days after NK cell injection, mice were injected with unedited or engineered T cells as described in **Table 28**. Briefly, 0.2×10^6 engineered T cells were injected 16 days post second activation after washing in PBS and resuspending in HBSS solution at a concentration of 6.0×10^6 cells/150 μ L.

[00488] IVIS imaging of live mice was performed to identify luciferase-positive T cells by IVIS spectrum. IVIS imaging was done at 24 hours, 48 hours, 72 hours, 6 days, 10 days, 13 days, 17 days, 20 days, 24 days, 27 days, 31 days, 34 days, 38 days, 42 days, 44 days, 48 days, 55 days, 63 days, 72 days, 77 days, 85 days, and 91 days after T cell injection. Mice were prepared for imaging with an injection of D-luciferin i.p. at 10 μ L/g body weight per the manufacturer's recommendation, about 150 μ L per animal. Animals were anesthetized and then placed in the IVIS imaging unit. The visualization was performed with the exposure time set to auto, field of view D, medium binning, and F/stop set to 1. **Table 29** and **FIG. 12A** shows radiance (photons/s/cm²/sr) from luciferase expressing T cells present at the various time points after injection out to 91 days. **FIG. 12B** shows radiance (photons/s/cm²/sr) from luciferase expressing T cells present in the various mice groups after 31 days. *In vivo*, B2M edited cells showed sensitivity to NK killing, while the HLA-A, CIITA double edited cells showed protection from NK mediated lysis.

[00489] **Table 28 - T-Cell Engineering**

Group	Day 0	Day 1	Day 2	Day 3	Day 4	Day 6	Day 7	Day 8	Day 16
HLA-A CIITA KO	Thaw	CIITA	GFP-Luc LV	TRAC+AAV	TRBC, HLA-A	Flow & Sort	Re-stim	Expand in G-Rex	Wash & Inject
B2M Control	Thaw	B2M	GFP-Luc LV	TRAC+AAV	TRBC	Flow & Sort	Re-stim	Expand in G-Rex	Wash & Inject
No Edit	Thaw	-	GFP-Luc LV	-	-	Flow & Sort	Re-stim	Expand in G-Rex	Wash & Inject

[00490] **Table 29 –Total Flux (photons/s) from luciferase expressing T cells in treated mice at intervals after T cell injection.**

T cell injection	Timepoint (days)	No NK cell injection			NK cell injection		
		Mean	SD	n	Mean	SD	n
No T cells	1	1170000	0	1	1060000	0	1
	2	884000	0	1	728000	0	1
	3	1090000	0	1	771000	0	1
	6	1040000	0	1	888000	0	1

T cell injection	Timepoint (days)	No NK cell injection			NK cell injection		
		Mean	SD	n	Mean	SD	n
	10	741000	0	1	799000	0	1
	13	1350000	0	1	751000	0	1
	17	1210000	0	1	709000	0	1
	20	1530000	0	1	1190000	0	1
	24	1280000	0	1	823000	0	1
	27	1430000	0	1	577000	0	1
	31	1310000	0	1	970000	0	1
	34	1840000	0	1	800000	0	1
	38	937000	0	1	750000	0	1
	42	1450000	0	1	757000	0	1
	44	1770000	0	1	797000	0	1
	48	1850000	0	1	666000	0	1
	55	1170000	0	1	723000	0	1
	63	1680000	0	1	799000	0	1
	72	1400000	0	1	840000	0	1
	77	1570000	0	1	801000	0	1
	85	1220000	0	1	770000	0	1
	91	1580000	0	1	905000	0	1
No edit	1	37560000	34014482.9	5	27882000	27141262.31	5
	2	40698000	22307084.5	5	28640000	14568047.23	5
	3	34210000	18847559.5	5	25692000	14362636.25	5
	6	51440000	10855551.6	5	37700000	34510288.32	5
	10	29460000	5028220.36	5	34060000	24420544.63	5
	13	17350000	8731122.49	5	42864000	47552123.82	5
	17	17380000	4065956.22	5	124180000	217126534.5	5
	20	35860000	9912012.91	5	329720000	644006666.9	5
	24	41400000	6393355.93	5	1784780000	3583692731	5
	27	70500000	28116809.9	5	9112600000	19172106869	5
	31	124260000	57196923	5	14383000000	27254468202	5
	34	313000000	256943574	5	17450000000	24859612829	5
	38	667800000	614512978	5	25316000000	26111305597	5
	42	1727400000	1703225998	5	21084000000	16956611690	5
	44	2101400000	2213844349	5	16975000000	13721121188	4
	48	5068000000	4995313854	5	15106666667	11613532337	3
	55	6386750000	5350377767	4	16303333333	11913187371	3
	63	8105750000	6722716632	4			
	72						

T cell injection	Timepoint (days)	No NK cell injection			NK cell injection		
		Mean	SD	n	Mean	SD	n
	77						
	85						
	91						
B2M KO	1	96334000	62882587.3	5	7192000	6901425.215	5
	2	138300000	57619007.3	5	7296000	2213194.524	5
	3	117980000	43943736.8	5	7342000	2837475.991	5
	6	104240000	34772230.3	5	7276000	2743998.907	5
	10	81120000	19876921.3	5	6124000	1967035.841	5
	13	45386000	24729233.3	5	5748000	3248448.861	5
	17	50600000	19718899.6	5	4390000	902607.3343	5
	20	38200000	12211470	5	2772000	947507.2559	5
	24	32180000	17561520.4	5	4566000	1182742.576	5
	27	35840000	15497354.6	5	3626000	1995903.304	5
	31	41380000	12243243	5	3344000	1295812.486	5
	34	40740000	13481394.6	5	3864000	506635.964	5
	38	33980000	15116117.2	5	3468000	1330139.09	5
	42	38840000	15452605	5	3504000	688534.676	5
	44	35280000	19116929.7	5	3266000	910291.1622	5
	48	31600000	17624982.3	5	3196000	726691.1311	5
	55	38920000	30824779	5	2654000	475794.0731	5
	63	29300000	22330584.4	5	2530000	274135.0032	5
	72	19070000	13309188.6	5	2522000	437344.258	5
	77	30680000	24960508.8	5	2650000	531554.3246	5
	85	24738000	22937833.8	5	1816000	410524.0553	5
	91	18234000	10913394.5	5	1736000	297707.9105	5
HLA-A KO CIITA KO	1	63960000	33085918.5	5	59320000	32265414.92	5
	2	55412000	31461432.3	5	49560000	9862707.539	5
	3	64686000	39918742.2	5	41264000	22521777.9	5
	6	88440000	22053865.9	5	33442000	18099663.53	5
	10	68320000	18250397.3	5	42040000	4585084.514	5
	13	57880000	8452041.17	5	37028000	20443236.53	5
	17	39320000	11283040.4	5	41400000	10968135.67	5
	20	40480000	12259363.8	5	37540000	8371260.359	5
	24	39900000	18287017.3	5	37740000	9070446.516	5
	27	37800000	14406422.2	5	31840000	11387185.78	5
	31	46160000	13751836.2	5	25020000	11377477.75	5
	34	39820000	8990383.75	5	28980000	5348551.206	5
	38	42620000	8249363.61	5	31000000	7146677.55	5
	42	30740000	10083798.9	5	16928000	9138868.639	5
	44	31740000	9619667.35	5	26580000	7343500.528	5
	48	30740000	9147021.37	5	28620000	3141178.123	5
	55	27600000	5482244.07	5	21340000	3673281.911	5
	63	24820000	6599015.08	5	12428000	3646082.83	5
	72	10918000	3813609.84	5	13094000	3349355.162	5
	77	24840000	4728953.37	5	14200000	3801973.172	5
	85	15520000	4283923.44	5	14580000	2920102.738	5

T cell injection	Timepoint (days)	No NK cell injection			NK cell injection		
		Mean	SD	n	Mean	SD	n
	91	17260000	5452797.45	5	11256000	2456141.283	5

Example 15: MHCI and MHCII KO in-vivo efficacy of HD1 T cells

[00491] Female NOG-hIL-15 mice were engrafted with 0.2×10^6 human acute lymphoblastic leukemia cell line 697-Luc2, followed by the injection of 10×10^6 engineered T cells with various edits in order to determine whether the edits provide a specific anti-tumor effect. Groups of T cells studied include: a control group of T cells with no edits (697 only); T cells with edits in TRAC and TRBC (TCR KO); T cells with edits in TRAC and TRBC and insertion of HD1 (TCR KO/WT1 insert); T cells with edits in TRAC and TRBC, insertion of HD1, and disruption in HLA-A (HLA-A KO); T cells with edits in TRAC and TRBC, insertion of HD1, and edits in HLA-A and in CIITA (AlloWT1); and T cells with edits in TRAC and TRBC and insertion of HD1 in the presence of a DNA PKi compound, and edits in HLA-A and in CIITA (AlloWT1+PKi Compound 1).

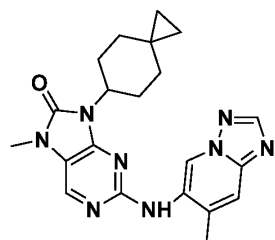
15.1. T cell Preparation

[00492] T cells from HLA-A2+ donor (110046967) were isolated from the leukopheresis products of healthy donor (STEMCELL Technologies). T cells were isolated using EasySep Human T cell isolation kit (STEMCELL Technologies, Cat#17951) following manufacturer's protocol and cryopreserved using Cryostor CS10 (STEMCELL Technologies, Cat# 07930). The day before initiating T cell editing, cells were thawed and rested overnight in T cell activation media TCAM: CTS OpTmizer (Thermofisher #A3705001) supplemented with 2.5% human AB serum (Gemini #100-512), 1X GlutaMAX (Thermofisher #35050061), 10mM HEPES (Thermofisher #15630080), 200 U/mL IL-2 (Peprotech #200-02), IL-7 (Peprotech #200-07), IL-15 (Peprotech #200-15).

15.2. Multi-editing T cells with sequential LNP delivery

[00493] T cells were prepared by treating healthy donor cells sequentially with four LNP compositions co-formulated with Cas9 mRNA and sgRNA targeting either TRAC, TRBC, CIITA, and HLA-A. The lipid portion of the LNP compositions included Lipid A, cholesterol, DSPC, and PEG2k-DMG in a 50:38.5:10:1.5 molar ratio, respectively. The lipid nucleic acid assemblies were formulated with a lipid amine to RNA phosphate (N:P) molar ratio of about 6, and a ratio of gRNA to mRNA of 1:2 by weight. A transgenic WT1-targeting TCR was site-specifically integrated into

the TRAC cut site by delivering a homology-directed repair template using AAV indicated in **Table 30**, in combination with the small molecule inhibitor of DNA-dependent protein kinase to boost the tgTCR insertion rate. The inhibitor, referred to hereinafter as “DNAPKI Compound 1” is 9-(4,4-difluorocyclohexyl)-7-methyl-2-((7-methyl-[1,2,4]triazolo[1,5-a]pyridin-6-yl)amino)-7,9-dihydro-8H-purin-8-one, also depicted as:



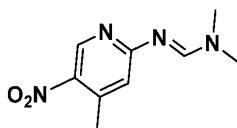
[00494] DNAPKI Compound 1 was prepared as follows:

General Information

[00495] All reagents and solvents were purchased and used as received from commercial vendors or synthesized according to cited procedures. All intermediates and final compounds were purified using flash column chromatography on silica gel. NMR spectra were recorded on a Bruker or Varian 400 MHz spectrometer, and NMR data were collected in CDCl₃ at ambient temperature. Chemical shifts are reported in parts per million (ppm) relative to CDCl₃ (7.26). Data for ¹H NMR are reported as follows: chemical shift, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets m = multiplet), coupling constant, and integration. MS data were recorded on a Waters SQD2 mass spectrometer with an electrospray ionization (ESI) source. Purity of the final compounds was determined by UPLC-MS-ELS using a Waters Acquity H-Class liquid chromatography instrument equipped with SQD2 mass spectrometer with photodiode array (PDA) and evaporative light scattering (ELS) detectors.

[00496] Example 1 - Compound 1

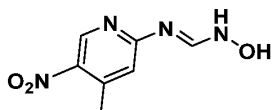
Intermediate 1a: (E)-N,N-dimethyl-N'-((4-methyl-5-nitropyridin-2-yl)formimidamide



[00497] To a solution of 4-methyl-5-nitro-pyridin-2-amine (5 g, 1.0 equiv.) in toluene (0.3 M) was added DMF-DMA (3.0 equiv.). The mixture was stirred at 110 °C for 2 h. The reaction mixture was concentrated under reduced pressure to give a residue and purified by column

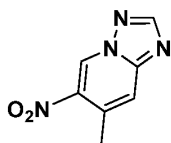
chromatography to afford product as a yellow solid (59%). ¹H NMR (400 MHz, (CD₃)₂SO) δ 8.82 (s, 1H), 8.63 (s, 1H), 6.74 (s, 1H), 3.21 (m, 6H).

Intermediate 1b: (E)-N-hydroxy-N'-(4-methyl-5-nitropyridin-2-yl)formimidamide



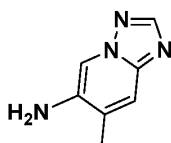
[00498] To a solution of Intermediate 1a (4 g, 1.0 equiv.) in MeOH (0.2 M) was added NH₂OH·HCl (2.0 equiv.). The reaction mixture was stirred at 80 °C for 1 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue. The residue was partitioned between H₂O and EtOAc, followed by 2x extraction with EtOAc. The organic phases were concentrated under reduced pressure to give a residue and purified by column chromatography to afford product as a white solid (66%). ¹H NMR (400 MHz, (CD₃)₂SO) δ 10.52 (d, J = 3.8 Hz, 1H), 10.08 (dd, J = 9.9, 3.7 Hz, 1H), 8.84 (d, J = 3.8 Hz, 1H), 7.85 (dd, J = 9.7, 3.8 Hz, 1H), 7.01 (d, J = 3.9 Hz, 1H), 3.36 (s, 3 H).

Intermediate 1c: 7-methyl-6-nitro-[1,2,4]triazolo[1,5-a]pyridine



[00499] To a solution of Intermediate 1b (2.5 g, 1.0 equiv.) in THF (0.4 M) was added trifluoroacetic anhydride (1.0 equiv.) at 0 °C. The mixture was stirred at 25 °C for 18 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography to afford product as a white solid (44%). ¹H NMR (400 MHz, CDCl₃) δ 9.53 (s, 1H), 8.49 (s, 1H), 7.69 (s, 1H), 2.78 (d, J = 1.0 Hz, 3H).

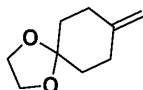
Intermediate 1d: 7-methyl-[1,2,4]triazolo[1,5-a]pyridin-6-amine



[00500] To a mixture of Pd/C (10% w/w, 0.2 equiv.) in EtOH (0.1 M) was added Intermediate 1c (1.0 equiv. and ammonium formate (5.0 equiv.). The mixture was heated at 105 °C for 2 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography to

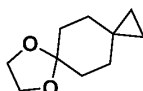
afford product as a pale brown solid. ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{SO}$) δ 8.41 (s, 2H), 8.07 (d, J = 9.0 Hz, 2H), 7.43 (s, 1H), 2.22 (s, 3H).

Intermediate 1e: 8-methylene-1,4-dioxaspiro[4.5]decane



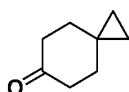
[00501] To a solution of methyl(triphenyl)phosphonium bromide (1.15 equiv.) in THF (0.6 M) was added *n*-BuLi (1.1 equiv.) at -78°C dropwise, and the mixture was stirred at 0°C for 1 h. Then, 1,4-dioxaspiro[4.5]decan-8-one (50 g, 1.0 equiv.) was added to the reaction mixture. The mixture was stirred at 25°C for 12 h. The reaction mixture was poured into aq. NH_4Cl at 0°C , diluted with H_2O , and extracted 3x with EtOAc. The combined organic layers were concentrated under reduced pressure to give a residue and purified by column chromatography to afford product as a colorless oil (51%). ^1H NMR (400 MHz, CDCl_3) δ 4.67 (s, 1H), 3.96 (s, 4 H), 2.82 (t, J = 6.4 Hz, 4 H), 1.70 (t, J = 6.4 Hz, 4 H).

Intermediate 1f: 7,10-dioxadispiro[2.2.4⁶.2³]dodecane



[00502] To a solution of Intermediate 4a (5 g, 1.0 equiv.) in toluene (3 M) was added ZnEt_2 (2.57 equiv.) dropwise at -40°C and the mixture was stirred at -40°C for 1 h. Then diiodomethane (6.0 equiv.) was added dropwise to the mixture at -40°C under N_2 . The mixture was then stirred at 20°C for 17 h under N_2 atmosphere. The reaction mixture was poured into aq. NH_4Cl at 0°C and extracted 2x with EtOAc. The combined organic phases were washed with brine (20 mL), dried with anhydrous Na_2SO_4 , filtered, and the filtrate was concentrated in vacuum. The residue was purified by column chromatography to afford product as a pale yellow oil (73%).

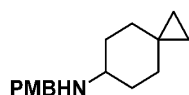
Intermediate 1g: spiro[2.5]octan-6-one



[00503] To a solution of Intermediate 4b (4 g, 1.0 equiv.) in 1:1 THF/ H_2O (1.0 M) was added TFA (3.0 equiv.). The mixture was stirred at 20°C for 2 h under N_2 atmosphere. The reaction mixture was concentrated under reduced pressure to remove THF, and the residue adjusted pH to 7 with 2 M NaOH (aq.). The mixture was poured into water and 3x extracted with EtOAc. The combined organic phase was washed with brine, dried with anhydrous Na_2SO_4 , filtered, and the filtrate was concentrated in vacuum. The residue was purified by

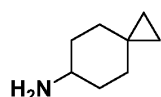
column chromatography to afford product as a pale yellow oil (68%). ^1H NMR (400 MHz, CDCl_3) δ 2.35 (t, J = 6.6 Hz, 4H), 1.62 (t, J = 6.6 Hz, 4H), 0.42 (s, 4H).

Intermediate 1h: N-(4-methoxybenzyl)spiro[2.5]octan-6-amine



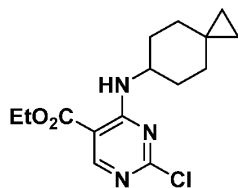
[00504] To a mixture of Intermediate 4c (2 g, 1.0 equiv.) and (4-methoxyphenyl)methanamine (1.1 equiv.) in DCM (0.3 M) was added AcOH (1.3 equiv.). The mixture was stirred at 20 °C for 1 h under N_2 atmosphere. Then, $\text{NaBH}(\text{OAc})_3$ (3.3 equiv.) was added to the mixture at 0 °C, and the mixture was stirred at 20 °C for 17 h under N_2 atmosphere. The reaction mixture was concentrated under reduced pressure to remove DCM, and the resulting residue was diluted with H_2O and extracted 3x with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 , filtered, and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography to afford product as a gray solid (51%). ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{SO}$) δ 7.15 – 7.07 (m, 2H), 6.77 – 6.68 (m, 2H), 3.58 (s, 3H), 3.54 (s, 2H), 2.30 (ddt, J = 10.1, 7.3, 3.7 Hz, 1H), 1.69 – 1.62 (m, 2H), 1.37 (td, J = 12.6, 3.5 Hz, 2H), 1.12 – 1.02 (m, 2H), 0.87 – 0.78 (m, 2H), 0.13 – 0.04 (m, 2H).

Intermediate 1i: spiro[2.5]octan-6-amine



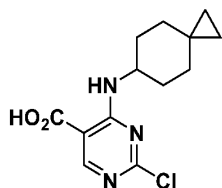
[00505] To a suspension of Pd/C (10% w/w, 1.0 equiv.) in MeOH (0.25 M) was added Intermediate 4d (2 g, 1.0 equiv.) and the mixture was stirred at 80 °C at 50 Psi for 24 h under H_2 atmosphere. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue that was purified by column chromatography to afford product as a white solid. ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{SO}$) δ 2.61 (tt, J = 10.8, 3.9 Hz, 1H), 1.63 (ddd, J = 9.6, 5.1, 2.2 Hz, 2H), 1.47 (td, J = 12.8, 3.5 Hz, 2H), 1.21 – 1.06 (m, 2H), 0.82 – 0.72 (m, 2H), 0.14 – 0.05 (m, 2H).

Intermediate 1j: ethyl 2-chloro-4-(spiro[2.5]octan-6-ylamino)pyrimidine-5-carboxylate



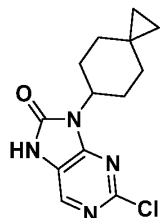
[00506] To a mixture of ethyl 2,4-dichloropyrimidine-5-carboxylate (2.7 g, 1.0 equiv.) and Intermediate 1i (1.0 equiv.) in ACN (0.5 – 0.6 M) was added K_2CO_3 (2.5 equiv.) in one portion under N_2 . The mixture was stirred at 20 °C for 12 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography to afford product as a white solid (54%). ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 8.64 (s, 1H), 8.41 (d, J = 7.9 Hz, 1H), 4.33 (q, J = 7.1 Hz, 2H), 4.08 (d, J = 9.8 Hz, 1H), 1.90 (dd, J = 12.7, 4.8 Hz, 2H), 1.64 (t, J = 12.3 Hz, 2H), 1.52 (q, J = 10.7, 9.1 Hz, 2H), 1.33 (t, J = 7.1 Hz, 3H), 1.12 (d, J = 13.0 Hz, 2H), 0.40 – 0.21 (m, 4H).

Intermediate 1k: 2-chloro-4-((spiro[2.5]octan-6-ylamino)pyrimidine-5-carboxylic acid



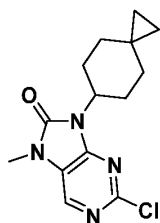
[00507] To a solution of Intermediate 1j (2 g, 1.0 equiv.) in 1:1 THF/ H_2O (0.3 M) was added LiOH (2.0 equiv.). The mixture was stirred at 20 °C for 12 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue. The residue was adjusted to pH 2 with 2 M HCl, and the precipitate was collected by filtration, washed with water, and dried under vacuum. Product was used directly in the next step without additional purification (82%). ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 13.54 (s, 1H), 8.38 (d, J = 8.0 Hz, 1H), 8.35 (s, 1H), 3.82 (qt, J = 8.2, 3.7 Hz, 1H), 1.66 (dq, J = 12.8, 4.1 Hz, 2H), 1.47 – 1.34 (m, 2H), 1.33 – 1.20 (m, 2H), 0.86 (dt, J = 13.6, 4.2 Hz, 2H), 0.08 (dd, J = 8.3, 4.8 Hz, 4H).

Intermediate 1l: 2-chloro-9-((spiro[2.5]octan-6-yl)-7,9-dihydro-8H-purin-8-one



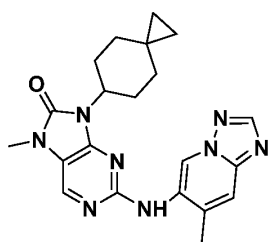
[00508] To a mixture of Intermediate 1k (1.5 g, 1.0 equiv.) and Et₃N (1.0 equiv.) in DMF (0.3 M) was added DPPA (1.0 equiv.). The mixture was stirred at 120 °C for 8 h under N₂ atmosphere. The reaction mixture was poured into water. The precipitate was collected by filtration, washed with water, and dried under vacuum to give a residue that was used directly in the next step without additional purification (67%). ¹H NMR (400 MHz, (CD₃)₂SO) δ 11.68 (s, 1H), 8.18 (s, 1H), 4.26 (ddt, J = 12.3, 7.5, 3.7 Hz, 1H), 2.42 (qd, J = 12.6, 3.7 Hz, 2H), 1.95 (td, J = 13.3, 3.5 Hz, 2H), 1.82 – 1.69 (m, 2H), 1.08 – 0.95 (m, 2H), 0.39 (tdq, J = 11.6, 8.7, 4.2, 3.5 Hz, 4H).

Intermediate 1m: 2-chloro-7-methyl-9-(spiro[2.5]octan-6-yl)-7,9-dihydro-8H-purin-8-one



[00509] To a mixture of Intermediate 1l (1.0 g, 1.0 equiv.) and NaOH (5.0 equiv.) in 1:1 THF/H₂O (0.3-0.5 M) was added MeI (2.0 equiv.). The mixture was stirred at 20 °C for 12 h under N₂ atmosphere. The reaction mixture was concentrated under reduced pressure to afford a residue that was purified by column chromatography to afford product as a pale yellow solid (67%). ¹H NMR (400 MHz, CDCl₃) δ 7.57 (s, 1H), 4.03 (tt, J = 12.5, 3.9 Hz, 1H), 3.03 (s, 3H), 2.17 (qd, J = 12.6, 3.8 Hz, 2H), 1.60 (td, J = 13.4, 3.6 Hz, 2H), 1.47 – 1.34 (m, 2H), 1.07 (s, 1H), 0.63 (dp, J = 14.0, 2.5 Hz, 2H), -0.05 (s, 4H).

Compound 1: 7-methyl-2-((7-methyl-[1,2,4]triazolo[1,5-a]pyridin-6-yl)amino)-9-(spiro[2.5]octan-6-yl)-7,9-dihydro-8H-purin-8-one



[00510] To a mixture of Intermediate 1m (1.0 equiv.) and Intermediate 1d (1.0 equiv.), Pd(dppf)Cl₂ (0.2 equiv.), XantPhos (0.4 equiv.), and Cs₂CO₃ (2.0 equiv.) in DMF (0.2 – 0.3

M) was degassed and purged 3x with N₂, and the mixture was stirred at 130 °C for 12 h under N₂ atmosphere. The mixture was then poured into water and extracted 3x with DCM. The combined organic phase was washed with brine, dried over Na₂SO₄, filtered, and the filtrate was concentrated in vacuum. The residue was purified by column chromatography to afford product as an off-white solid. ¹H NMR (400 MHz, (CD₃)₂SO) δ 9.09 (s, 1H), 8.73 (s, 1H), 8.44 (s, 1H), 8.16 (s, 1H), 7.78 (s, 1H), 4.21 (t, J = 12.5 Hz, 1H), 3.36 (s, 3H), 2.43 (s, 3H), 2.34 (dt, J = 13.0, 6.5 Hz, 2H), 1.93 – 1.77 (m, 2H), 1.77 – 1.62 (m, 2H), 0.91 (d, J = 13.2 Hz, 2H), 0.31 (t, J = 7.1 Hz, 2H). MS: 405.5 m/z [M+H].

[00511] The sequential edits occurred for each group as illustrated in **Table 30**.

[00512] Table 30 T cell engineering

Group Name	Day 1	Day 2	Day 3	Day 4
TCR KO	TRBC		TRAC	
TCR KO/WT1 Insert	TRBC		TRAC/AAV	
WT1/HLA-A		HLA-A	TRAC/AAV	TRBC
AlloWT1	CIITA	HLA-A	TRAC/AAV	TRBC
AlloWT1+DNA PKi Compound 1	CIITA	HLA-A	TRAC/AAV +Compound 1 (0.25uM)	TRBC

15.3. LNP Treatment and Expansion of T cells

[00513] LNP compositions were formulated in ApoE-containing media and delivered to T cells as follows: on day 1, LNP compositions as indicated in **Table 30** were incubated at a concentration of 5 ug/mL in TCAM containing 5 ug/mL rhApoE3 (Peprtech 350-02). Meanwhile, T cells were harvested, washed, and resuspended at a density of 2×10^6 cells/mL in TCAM with a 1:50 dilution of T Cell TransAct, human reagent (Miltenyi, 130-111-160). T cells and LNP-ApoE media were mixed at a 1:1 ratio and T cells plated in culture flasks overnight.

[00514] On day 2, LNP compositions as indicated in **Table 30** were incubated at a concentration of 25 ug/mL in TCAM containing 20 ug/mL rhApoE3 (Peprtech 350-02). LNP-ApoE solution was then added to the appropriate culture at a 1:10 ratio.

[00515] On day 3, TRAC-LNP compositions (**Table 30**) were incubated at a concentration of 5 ug/mL in TCAM containing 10 ug/mL rhApoE3 (Peprtech 350-02). Meanwhile, T cells were harvested, washed, and resuspended at a density of 1×10^6 cells/mL in TCAM. T cells and LNP-ApoE media were mixed at a 1:1 ratio, and T cells were plated in culture flasks. WT1

AAV was then added to the relevant groups at an MOI of 3×10^5 GC/cell. Compound 1 was added to the relevant groups at a final concentration of 0.25 μ M.

[00516] On day 4, LNP compositions as indicated in **Table 30** were incubated at a concentration of 5 μ g/mL in TCAM containing 5 μ g/mL rhApoE3 (Peprotech 350-02). T cells were washed by centrifugation and resuspended at a density of 1×10^6 cells/mL LNP-ApoE solution was then added to the appropriate cultures at a 1:1 ratio.

[00517] On days 5 through 11, T cells were transferred to a GREX plate (Wilson Wolf) in T cell expansion media (TCM: CTS OpTmizer (Thermofisher #A3705001) supplemented with 5% CTS Immune Cell Serum Replacement (Thermofisher #A2596101), 1X GlutaMAX (Thermofisher #35050061), 10 mM HEPES (Thermofisher #15630080), 200 U/mL IL-2 (Peprotech #200-02), IL-7 (Peprotech #200-07), IL-15 (Peprotech #200-15) and expanded. Briefly, T-cells were expanded for 6-days, with fresh cytokine supplementation every other day. Cells were counted using a Vi-CELL cell counter (Beckman Coulter) and fold expansion was calculated by dividing cell yield by the starting material.

15.4. Quantification of T cell editing by flow cytometry and NGS

[00518] Post expansion, edited T cells were stained in an antibody cocktail to determine HLA-A2 knockout (HLA-A2⁻), HLA-DR-DP-DQ knockdown via CIITA knockout (HLA-DRDPDQ⁻), WT1-TCR insertion (CD3⁺Vb8⁺), and the percentage of cells expressing residual endogenous (CD3⁺Vb8⁻). Cells were subsequently washed, analyzed on a Cytotflex LX instrument (Beckman Coulter) using the FlowJo software package. T cells were gated on size and CD8⁺ status, before editing and insertion rates were determined. Editing and insertion rates can be found in **Table 31** and **Figures 14A-14F**. The percent of fully edited AlloWT1-T cells expressing the WT1-TCR with knockout of HLA-A and CIITA was gated as % CD3⁺Vb8⁺HLA-A⁻HLA-DRDPDQ⁻. High levels of HLA-A and CIITA knockout, as well as WT1-TCR insertion and endogenous TCR KO were observed in edited samples. Notably, T cells receiving DNA PK inhibitor Compound 1 showed improved editing efficiencies.

[00519] IVIS imaging of live mice was performed to identify luciferase-positive tumor cells by IVIS spectrum. IVIS imaging was done at 2 days, 6 days, 9 days, 13 days, 16 days, and 18 days after T cell injection. Mice were prepared for imaging with an injection of D-luciferin i.p. at 10 μ L/g body weight per the manufacturer's recommendation, about 150 μ L per animal. Animals were anesthetized and then placed in the IVIS imaging unit. The visualization was performed with the exposure time set to auto, field of view D, medium binning, and F/stop set

to 1. **Table 32** and **Figure 15** show radiance (photons/s/cm²/sr) from luciferase expressing T cells present at the various time points after injection out to 18 days.

[00520] **Table 31 -T cell editing efficiency**

	CD8+	Endogenous TCR+	WT1 TCR+	HLA-A2-	HLA-DRDPDQ-	AlloWT1+
Unedited	26.9	95.4	4.39	0.66	35.7	0.00292
TCR KO	31.1	5.12	0.5	0.62	30.8	0.23
WT1	34.2	1.2	78.5	0.47	49.7	0.03
WT1/HLA-A	24.8	0.93	63.3	99.1	56.4	40.5
AlloWT1	28.8	0.51	69.3	98.7	96.2	66.1
AlloWT1 + Compound 1	29.2	0.23	89.8	99	96.5	86

[00521] **Table 32 – Total Flux (photons/s) from luciferase-expressing target cells in treated mice at intervals after T cell injection.**

		Mean	SD	n
IR Control	2	668000	0	1
	6	662000	0	1
	9	802000	0	1
	13	834000	0	1
	16	799000	0	1
	18	727000	0	1
697 Only	2	11695000	6766940.65	8
	6	11756250	6759771.63	8
	9	6542375000	4097940177	8
	13	34156125000	19588932739	8
	16	56000000000	14890936841	8
	18			
TCR KO	2	8696250	3615004.20	8
	6	8755000	3659211.47	8
	9	1985750000	1311102671	8
	13	39295000000	18556359711	8
	16	50442857143	12082474518	7
	18	35000000000	0	1
TCR KO/WT1 Insert	2	1395750	651356.99	8
	6	1418625	660585.66	8
	9	13293750	10040193.42	8
	13	416762500	340405656.90	8
	16	987625000	637380114.80	8
	18	2523750000	1518542699	8
HLA-A KO	2	1306375	514478.92	8
	6	1323750	504219.55	8
	9	1785000	691416.77	8

		Mean	SD	n
	13	9851428.57	13794971.82	7
	16	35832857.14	53937852.11	7
	18	53608571.43	65167479.22	7
AlloWT1	2	1085625	137185.94	8
	6	1100250	136031.25	8
	9	12085000	20455051.77	8
	13	43676250	87426018.67	8
	16	146917500	310795920.60	8
	18	31418750	33596200.65	8
AlloWT1 + DNAPki	2	1138000	429877.06	8
	6	1152750	420860.26	8
	9	1720000	654391.77	8
	13	3976250	5828721.83	8
	16	39420000	97704137.36	8
	18	80597500	162813409.10	8

15.5. Engineered T Cell Cytokine Release

[00522] Engineered T cells prepared as described in Examples 10.1 and 10.2 were assayed for their cytokine release profiles. In vitro OCI-AML3 tumor cell killing assays were separately performed (data not shown) using the engineered T cells. The supernatants from the tumor cell killing assays were used to evaluate each engineered T cell's cytokine release profile.

[00523] Briefly, TCR KO T cells, Autologous WT1 T cells (TCR KO + WT1 TCR insertion), and Allogeneic WT1 T cells (as indicated in **Table 33**) were thawed and rested overnight in TCGM supplemented with IL-2, IL-7, and IL-15. The following day, a coculture assay was set up where each group of engineered T cells was co-cultured with OCI-AML3 target tumor. First, OCI-AML3 target tumor cells were pulsed with VLD peptide at different concentrations (500, 50, 5, 0.5, 0.05, and 0.005 nM) for 1 hr. Next, T cells from each group were counted and resuspended in TCGM media without cytokines and co-cultured with pulsed OCI-AML3 at 1:1 E:T ratio. The T cell numbers in the co-culture were normalized to the insertion rates to keep the E:T consistent among different groups. After 24 hours of co-culture, the supernatant from each co-culture sample was diluted 5x in Diluent 2 from the U-PLEX Immuno-Oncology Group 1 (hu) Assays kit (MSD, Cat No. K151AEL-2). 50 µL of diluted samples from each group were loaded onto the meso scale discovery (MSD) plate and incubated for 1 hour.

[00524] **Table 33 – T cell engineering.**

	CD8+	Endogenous TCR+	WT1 TCR+	HLA- A2-	HLA- DRDPDQ-	AlloWT1+
Unedited	26.9	95.4	4.39	0.66	35.7	0.00292
TCR KO	31.1	5.12	0.5	0.62	30.8	0.23
WT1	34.2	1.2	78.5	0.47	49.7	0.03
WT1/HLA-A	24.8	0.93	63.3	99.1	56.4	40.5
AlloWT1	28.8	0.51	69.3	98.7	96.2	66.1
AlloWT1 + Compound 1	29.2	0.23	89.8	99	96.5	86

[00525] For each of the cytokines measured, biotinylated capture antibody from the U-
PLEX Immuno-Oncology Group 1 (hu) Assays (MSD, Cat No. K151AEL-2) was added to
the assigned linker according to the kit's protocol. The antibody-linker mixtures were
vortexed and incubated at room temperature for 30 minutes. Post incubation, the plate was
washed, sealed, and stored overnight.

[00526] The following day, calibrators containing standards for each of the cytokines (IL-2
and IFN- γ) to be assayed were reconstituted as per the manufacturer's instructions and
diluted to create a 4-fold standard curve.

[00527] The plates were washed, and 50 μ L of the detection antibody solution (prepared
according to kit instructions) was added to each well of the MSD plate. The plate was
incubated for 1 hour.

[00528] After incubation, the plate was washed and read immediately on the MSD
instrument. Cytokine release is shown in **Tables 34-35** and **Figs. 16A-16B**.

[00529] **Table 34: IFN- γ**

IFN- γ						
Log[peptide (nM)]	TCR KO		AutoWT1		AlloWT1	
2.70	122.55	25.96	93417.51	7094.06	147620.65	9709.50
1.70	134.20	16.97	60680.24	2770.37	104018.15	10358.48
0.70	144.94	24.90	41863.52	1759.74	99896.25	7700.60
-0.30	146.14	58.09	4812.67	175.51	31820.97	1331.50
-1.30	155.20	11.49	77.72	23.65	1592.76	131.04
-2.30	110.63	22.03	69.41	3.27	351.29	23.17

[00530] **Table 35: IL-2**

IL-2						
Log[peptide (nM)]	TCR KO		AutoWT1		AlloWT1	
2.70	4.21	0.63	6031.67	373.56	7525.26	1116.85
1.70	4.17	0.76	3419.94	97.86	4450.71	861.82
0.70	5.28	0.25	1882.55	204.86	3780.66	381.75
-0.30	6.62	2.96	69.51	6.86	452.94	20.13
-1.30	5.87	1.47	4.88	1.07	10.91	2.80
-2.30	6.55	2.18	5.19	1.32	4.94	2.17

Example 16: HLA-A + CIITA DKO T Cells Do Not Elicit Host CD4 or CD8 Proliferation in a Mixed Lymphocyte Reaction Assay

[00531] T cells were isolated from peripheral blood of a healthy human donor with the following MHC I phenotype: HLA-A*02:01:01G, 03:01:01G, HLA-B*07:02:01G, HLA-C*07:02:01G. Briefly, a leukapheresis pack (Stemcell Technologies) was treated in ammonium chloride RBC lysis buffer (Stemcell Technologies; Cat. 07800) for 15 minutes to lyse red blood cells. Peripheral blood mononuclear cell (PBMC) count was determined post lysis and T cell isolation was performed using EasySep Human T cell isolation kit (Stemcell Technologies, Cat. 17951) according to manufacturer's protocol. Isolated CD3+ T cells were re-suspended in Cryostor CS10 media (Stemcell Technologies, Cat. 07930) and frozen down in liquid nitrogen until further use.

[00532] Frozen T cells were thawed at a cell concentration of 1.5×10^6 cells/ml into T cell activation media (TCAM) composed of OpTmizer TCGM as described in Example 3 further supplemented with 100 U/mL of recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/ml IL-7 (Peprotech, Cat. 200-07), 5 ng/ml IL-15 (Peprotech, Cat. 200-15). Cells were rested at 37°C for 24 hours.

[00533] Twenty-four hours post thawing T cells were counted and resuspended at 2×10^6 cells/ml in TCAM media and 1:50 v/v of TransAct (Miltenyi Biotec Cat. 30-111-160) was added. 1×10^6 cells were added to each well of a 24-well tissue culture plate, keeping 2 wells for each group to be engineered and 2 wells as unedited controls (Groups engineered: Unedited or WT, B2M KO (also indicated as HLA-I or HLA class I), CIITA (also indicated as HLA class II or HLA-II) KO, B2M + CIITA DKO, HLA-A KO, HLA-A + CIITA DKO). The plate was transferred to a 37 °C incubator. LNP compositions containing mRNA encoding cas9

(SEQ ID NO:802) and sgRNA G013675 (SEQ ID NO: 27), targeting CIITA were formulated with lipid A, cholesterol, DSPC, and PEG2k-DMG in a 50:38.5:10:1.5 molar ratio, respectively. The lipid nucleic acid assemblies were formulated with a lipid amine to RNA phosphate (N:P) molar ratio of about 6, and a ratio of gRNA to mRNA of 1:2 by weight. LNP compositions at 5ug/ml were incubated in OpTmizer TCAM, further supplemented with 5 ug/ml recombinant human ApoE3 (Peprtech, Cat. 350-02) for 15 minutes at 37 °C. In 6 out of the 12 wells, pre-incubated LNP and T cells with Transact were mixed to yield final concentrations of 1×10^6 T cells/ml and 2.5 µg total RNA/mL of LNP in TCAM media with 2.5% human AB serum, 100 U/mL of recombinant human interleukin-2 (Peprtech, Cat. 200-02), 5 ng/ml IL-7 (Peprtech, Cat. 200-07), 5ng/ml IL-15 (Peprtech, Cat. 200-15) (2 wells for the CIITA KO group, 2 wells for HLA-A + CIITA DKO group and 2 wells for the B2M + CIITA DKO group). All the additional wells were mock edited with media containing ApoE3 but no LNP compositions. All cells were incubated at 37 °C for 24 hours.

[00534] 24 hours post activation, 2 previously untreated wells and 2 CIITA LNP containing wells were treated with LNP compositions for B2M (for B2M KO and B2M + CIITA DKO groups); and 2 previously untreated wells and 2 CIITA LNP containing wells were treated with LNP compositions for HLA-A (for HLA-A KO and HLA-A + CIITA DKO groups). LNP compositions containing the Cas9 mRNA and sgRNA G000529 (SEQ ID NO: 216) targeting B2M, and LNP compositions containing mRNA encoding cas9 (SEQ ID NO:802) and sgRNA G018995 (sgRNA comprising SEQ ID NO: 214, as shown in Table 4) targeting HLA-A were formulated lipid A, cholesterol I, DSPC, and PEG2k-DMG in a 50:38.5:10:1.5 molar ratio, respectively. The lipid nucleic acid assemblies were formulated with a lipid amine to RNA phosphate (N:P) molar ratio of about 6, and a ratio of gRNA to mRNA of 1:2 by weight. LNP compositions at 25ug/ml were incubated in OpTmizer TCAM, further supplemented with 20ug/ml recombinant human ApoE3 (Peprtech, Cat. 350-02) for 15 minutes at 37°C. The B2M and HLA-A LNP compositions, were added to the appropriate wells of the 24 well plate, as mentioned above, to yield final concentrations of 2.5 µg total RNA/mL of LNP in TCAM media with 2.5% human AB serum, 100 U/mL of recombinant human interleukin-2 (Peprtech, Cat. 200-02), 5 ng/ml IL-7 (Peprtech, Cat. 200-07), 5 ng/ml IL-15 (Peprtech, Cat. 200-15). An additional group of cells were mock edited with media containing ApoE3 but no LNP compositions, to serve as the unedited or WT control. All cells were incubated at 37°C for 24 hours.

[00535] 24 hours post the second round of editing, cells were washed by spinning at 500XG for 5mins and resuspended in TCEM media containing with 5% CTS™ Immune Cell SR

(Gibco Cat. A2596101), 100 U/mL of recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/ml IL-7 (Peprotech, Cat. 200-07), 5ng/ml IL-15 (Peprotech, Cat. 200-15. The cells were cultured and maintained in G-Rex plate for 7 days with regular changes in media and cytokines, after which they were re-suspended in Cryostor CS10 media (Stemcell Technologies, Cat. 07930) and frozen down in liquid nitrogen until further use.

[00536] For the MLR assay, six groups of donor T cells (wildtype unedited, B2M KO, HLA-A KO, CIITA KO, HLA-A + CIITA DKO, B2M + CIITA DKO) were thawed and resuspended in TCGM at 1×10^6 /mL + 100 U/ml IL-2, 0.5 ng/mL IL-7 & IL-15 (Donor and Host HLA-genotypes are shown below in **Table 36**). Peripheral blood mononuclear cells (PBMCs) from 3 hosts (Autologous host, Allogeneic host (HLA-B and C matched host), and Positive control host (HLA-A, HLA-B and HLA-C mismatched) were thawed, resuspended in TCGM at 1×10^6 /mL + 100 U/ml IL-2, 0.5 ng/mL IL-7 & IL-15. Donor and host cells were rested overnight in a 37 °C incubator. The following day, donor cell flasks were irradiated at 4000 rad and spun down, and each group was resuspended at 1×10^6 /mL in TCGM without cytokines. Host PBMCs from the two hosts were depleted of CD56⁺ cells using the CD56 MicroBeads (Miltenyi Biotec, Cat. No. 130-050-401). About 1×10^6 cells from each host were saved in 15 mL tubes for unlabeled flow controls. To label 18×10^6 cells of each host, a vial of Cell Trace Violet (Thermo Fisher, Cat. No. C34571) was brought to room temperature and reconstituted using 20 μ L DMSO to generate a stock of 5 mM CTV. Host cells were resuspended at $\sim 1 \times 10^6$ /mL in phosphate buffered saline (Corning, Cat. No. 21-040-CV) and transferred to another 50 mL conical tube. After adding 18 μ L CTV into the tubes to stain host cells, the tubes were transferred to a 37 °C incubator for 15 minutes. Following that, the tubes were topped up to 40 mL with TCGM without cytokines to absorb any unbound dye. The labelled host cells were then spun down at 500xg for 5 minutes and resuspended in TCGM without cytokines at 1×10^6 /mL. 50,000 cells per 50 μ L per well of host PBMCs were plated per well from appropriate hosts. In the wells requiring 4x host cells (control samples to normalize the data), 200,000 host cells were plated per 200 μ L per well. In the host cells labelled “host + TransAct” (proliferation positive control), 50,000 cells per 50 μ L per well of host PBMCs were seeded followed by the addition of 1 μ L of T Cell TransAct™, human (Miltenyi Biotec, Cat. No. 130-111-160), and the volume of these wells was made up to 200 μ L with cytokine free TCGM. The irradiated donor cells were plated according to the plate layout at 150,000 cells per 150 μ L per well. For flow controls, 50,000 cells from one donor and host each were plated together. The volume in all wells was filled to 200 μ L with TCGM without cytokines.

[00537] On day 5 post co-culture, half the media (~100 μ L) from each well was replaced with fresh media (TCGM without cytokines).

[00538] On day 8 post co-culture, the assay plate was stained and analyzed by flow cytometry. For the purpose of staining, the plate was spun at 600xg for 3 minutes, flicked to remove media, and 100 μ L of a 1:100 v/v solution of Fc blocker (Biolegend, Cat # 422302) in FACS buffer was added to each well. Cells were resuspended in the Fc blocker, and the plate was incubated at room temperature for 5 minutes. An antibody cocktail was prepared such that each antibody was present at a 1:100 v/v dilution, and 100 μ L of this antibody mixture was added to each sample well. The plate was protected from light by covering with an aluminum foil and incubated at 2-8 °C for 20-30 minutes. After staining, the plate was spun at 600xg for 3 minutes, flicked to remove media and washed with 200 μ L of FACS buffer. The plate was washed again, and the cell pellets were resuspended in 70 μ L of a 1:200 v/v solution of the viability dye 7-AAD (BD Pharmingen, Cat# 51-68981E). Unstained wells were resuspended in 70 μ L of FACS buffer. The plate was run on fast mode (60 seconds per well) on Cytoflex flow cytometer. The results, shown in **Tables 37A and 37B** and **Figures 13A and 13B** (figures show a subset of data for Wildtype, B2M KO, and HLA-A + CIITA DKO), demonstrate that the HLA-A + CIITA DKO cells elicit minimal CD4 and CD8 responses in the allogeneic host (HLA-B and C matched), which were comparable to the response elicited by B2M + CIITA DKO cells. Results for each group have been normalized to that of the proliferation of the 4x host group, for the respective host.

[00539] **Table 36 – Genotypes of T cell donor and PBMC Hosts**

	HLA-A	HLA-B	HLA-C	HLA-DR	HLA-DQ	HLA-DP
T cell Donor and Autologous Host	A*02:01:01G, 03:01:01G	B*07:02:01G	C*07:02:01G	DRB1*15:01:01G, DRB5*01:01:01G	DQA1*01:02:01G, DQB1*06:02:01G	DPA1*01:03:01G, 02:07:01G, DPB1*04:01:01G, 19:01:01G
B, C matched Host	A*02:01:01G	B*07:02:01G, 44:02:01G	C*05:01:01G, 07:02:01G	DRB1*13:01:01G, 15:01:01G, DRB3*01:01:02G, DRB5*01:01:01G	DQB1*06:02:01G, 06:03:01G, DQA1*01:02:01G, 01:03:01G	DPB1*02:01:02G, 04:02:01G, DPA1*01:03:01G

	HLA-A	HLA-B	HLA-C	HLA-DR	HLA-DQ	HLA-DP
HLA mismatched Host	A*11:01:01G, 24:02:01G	B*40:01:01G	C*03:04:01G	DRB1*08:01:01G, 13:02:01G, DRB3*03:01:01G	DQB1*04:02:01G, 06:04:01G	DPB1*03:01:01G, 05:01:01G

[00540] **Table 37A – Proliferation of Host CD4+ T Cells**

Group	Autologous Host		Allogeneic Host		Positive Control Host	
	Average % Normalized Proliferation	SD % Normalized Proliferation	Average % Normalized Proliferation	SD % Normalized Proliferation	Average % Normalized Proliferation	SD % Normalized Proliferation
WT	-13.76	3.05	5.93	1.72	39.07	3.68
B2M KO	-13.50	2.66	-3.22	5.10	42.47	3.20
CIITA KO	-12.62	4.27	-7.00	5.54	-8.83	14.93
B2M + CIITA KO	-11.98	2.76	-5.15	5.21	-14.20	4.64
HLA-A KO	-9.14	7.96	7.67	12.41	41.83	5.01
HLA-A + CIITA KO	-11.33	2.03	-3.00	4.47	-3.97	6.57

[00541] **Table 37B - Proliferation of Host CD8+ T Cells**

Group	Autologous Host		Allogeneic Host		Positive Control Host	
	Average % Normalized Proliferation	SD % Normalized Proliferation	Average % Normalized Proliferation	SD % Normalized Proliferation	Average % Normalized Proliferation	SD % Normalized Proliferation
WT	7.53	6.95	35.71	12.28	74.00	1.42
B2M KO	-8.87	3.75	20.41	0.95	31.97	11.70
CIITA KO	1.43	5.24	6.17	4.89	56.07	8.53
B2M + CIITA KO	9.63	14.50	-0.05	4.59	0.47	5.23
HLA-A KO	22.40	23.65	25.31	16.59	71.83	2.25

Group	Autologous Host		Allogeneic Host		Positive Control Host	
	Average % Normalized Proliferation	SD % Normalized Proliferation	Average % Normalized Proliferation	SD % Normalized Proliferation	Average % Normalized Proliferation	SD % Normalized Proliferation
HLA-A + CIITA KO	17.57	12.00	5.14	2.88	58.13	7.02

Example 17: Sequential Delivery of Multiple LNP Compositions for Multiple Gene Disruptions and Insertions

[00542] T cells were engineered with a series of gene disruptions and insertions. Healthy donor cells were treated sequentially with four LNP compositions, each LNP composition co-formulated with mRNA encoding Cas9 (SEQ ID NO: 802) and sgRNA targeting either TRAC (G013006) (SEQ ID NO: 203), TRBC (G016239) (SEQ ID NO: 211), CIITA (G013675) (SEQ ID NO: 27), or HLA-A (G018995) (sgRNA comprising SEQ ID NO: 214, as shown in Table 4). LNP compositions were formulated according to the Groups indicated in **Table 38** with either lipid A, cholesterol, DSPC, and PEG2k-DMG in a 35:47.5:15:2.5 molar ratio (Groups 1 and 2), respectively or lipid A, cholesterol, DSPC, and PEG2k-DMG in a 50:35.5:10:1.5 molar ratio (Group 3), respectively at the indicated doses. Groups 1 and 2 differ in LNP concentration. The lipid nucleic acid assemblies were formulated with a lipid amine to RNA phosphate (N:P) molar ratio of about 6, and a ratio of gRNA to mRNA of 1:2 by weight. A transgenic WT1 targeting TCR was site-specifically integrated into the TRAC cut site by delivering a homology directed repair template using AAV. LNP compositions were prepared each day and delivered to T cells as described in **Table 38**.

17.1. T cell Preparation

[00543] T cells from three HLA-A*02:01+ serotypes were isolated from the leukopheresis products of two healthy donors (STEMCELL Technologies). T cells were isolated using EasySep Human T cell isolation kit (STEMCELL Technologies, Cat#17951) following manufacturer's protocol and cryopreserved using Cryostor CS10 (STEMCELL Technologies, Cat# 07930). The day before initiating T cell editing, cells were thawed and rested overnight in T cell activation media (TCAM: CTS OpTmizer, Thermofisher #A3705001) supplemented with 2.5% human AB serum (Gemini #100-512), 1X GlutaMAX (Thermofisher #35050061),

10 mM HEPES (Thermofisher #15630080), 200 U/mL IL-2 (Peprotech #200-02), IL-7 (Peprotech #200-07), and IL-15 (Peprotech #200-15).

17.2. LNP Treatment and Expansion of T cells

[00544] LNP compositions were thawed and diluted on each day in ApoE containing media and delivered to T cells as follows.

[00545] **Table 38 – Order of Editing for T Cell Engineering**

Group	Day 1 Edit (LNP formulation & final concentration)	Day 2 Edit (LNP formulation & final concentration)	Day 3 Edit (LNP formulation & final concentration)	Day 4 Edit (LNP formulation & final concentration)
Group 1	CIITA KO (Lipid A: 35:47.5:15:2.5, 0.65 µg/mL)	HLA-A KO (Lipid A: 35:47.5:15:2.5, 0.65 µg/mL)	TRAC KI (Lipid A: 35:47.5:15:2.5, 0.65 µg/mL)	TRBC KO (Lipid A: 35:47.5:15:2.5, 0.65 µg/mL)
Group 2	CIITA KO (Lipid A: 35:47.5:15:2.5, 2.5 µg/mL)	HLA-A KO (Lipid A: 35:47.5:15:2.5, 2.5 µg/mL)	TRAC KI (Lipid A: 35:47.5:15:2.5, 2.5 µg/mL)	TRBC KO (Lipid A: 35:47.5:15:2.5, 2.5 µg/mL)
Group 3	CIITA KO (Lipid A: 50:35.5:10:1.5, 2.5 µg/mL)	HLA-A KO (Lipid A: 50:35.5:10:1.5, 2.5 µg/mL)	TRAC KI (Lipid A: 50:35.5:10:1.5, 2.5 µg/mL)	TRBC KO (Lipid A: 50:35.5:10:1.5, 2.5 µg/mL)
Unedited	None	None	None	None

[00546] On day 1, LNP compositions as indicated in **Table 38** were incubated in TCAM containing 5 µg/mL rhApoE3 (Peprotech 350-02). Meanwhile, T cells were harvested, washed, and resuspended at a density of 2×10^6 cells/mL in TCAM with a 1:50 dilution of T Cell TransAct, human reagent (Miltenyi, 130-111-160). T cells and LNP-ApoE media were mixed at a 1:1 ratio and T cells plated in culture flasks overnight.

[00547] On day 2, LNP compositions as indicated in **Table 38** were incubated at a concentration of 25 µg/mL in TCAM containing 20 µg/mL rhApoE3 (Peprotech 350-02). LNP-ApoE solution was then added to the appropriate culture at a 10:1 ratio.

[00548] On day 3, as indicated in **Table 38** TRAC-LNP compositions were incubated in TCAM containing 5 µg/mL rhApoE3 (Peprotech 350-02). Meanwhile, T cells were harvested, washed, and resuspended at a density of 1×10^6 cells/mL in TCAM. T cells and LNP-ApoE media were mixed at a 1:1 ratio, and T cells were plated in culture flasks. WT1 AAV was then

added to each group at a MOI of 3×10^5 GC/cell. The DNA-PK inhibitor “Compound 1” was added to each group at a concentration of 0.25 μ M

[00549] On day 4, LNP compositions as indicated in **Table 38** were incubated in TCAM containing 5 μ g/mL rhApoE3 (Peprtech 350-02). Meanwhile, T cells were harvested, washed, and resuspended at a density of 1×10^6 cells/mL in TCAM. T cells and LNP-ApoE media were mixed at a 1:1 ratio and T cells plated in culture flasks.

[00550] On days 5-13, T cells were transferred to a 24-well GREX plate (Wilson Wolf, 80192) in T cell expansion media (TCM: CTS OpTmizer, Thermofisher #A3705001) supplemented with 5% human AB serum (Gemini #100-512), 1X GlutaMAX (Thermofisher #35050061), 10 mM HEPES (Thermofisher #15630080), 200 U/mL IL-2 (Peprtech #200-02), IL-7 (Peprtech #200-07), IL-15 (Peprtech #200-15) and expanded per manufacturers' protocols. Briefly, T-cells were expanded for 8-days, with media exchanges every 2-3 days.

[00551] Post expansion, edited T cells were assayed by flow cytometry to determine HLA-A*02:01 knockout, HLA-DR-DP-DQ knockdown via CIITA knockout, WT1-TCR insertion ($CD3^+Vb8^+$), and the percentage of cells expressing residual endogenous ($CD3^+Vb8^-$). T Cells were incubated with an antibody cocktail targeting the following molecules: Vb8 (Biolegend, Cat. 348104), HLA-A2 (Biolegend, Cat. 343320), HLA-DRDPDQ (Biolegend, Cat. 361712), CD4 (Biolegend, Cat. 300538), CD8 (Biolegend, Cat. 301046), CD3 (Biolegend, Cat. 317336), CCR7 (Biolegend, Cat. 353214), CD62L (Biolegend, Cat. 304820), CD45RA (Biolegend, Cat. 304134), CD45RO (Biolegend, Cat. 304230), CD56 (Biolegend, Cat. 318328), Viakrome (Beckman Coulter, Cat. C36628). Cells were subsequently washed, processed on a Cytotflex LX instrument (Beckman Coulter) and analyzed using the FlowJo software package. T cells were gated on size and CD4/CD8 status, before editing and insertion rates were determined. The percentage of cells expressing relevant cell surface proteins following sequential T cell engineering are shown in **Table 39** and **Figure 17A** for $CD8^+$ T cells respectively. The percent of T cells with all intended edits (insertion of the WT1-TCR, combined with knockout of HLA-A and CIITA) was gated as % $CD3^+Vb8^+ HLA-A^-HLA-DRDPDQ^-$ and is shown in **Figure 17B**. High levels of HLA-A and CIITA knockout, as well as WT1-TCR insertion were observed in edited samples from all groups yielding >75% of fully edited $CD8^+$ T cells. The lower dosage (0.65 μ g/mL) used with Lipid A 35:15:47.5:2.5 composition showed similar potency in editing T cells across all targets as the Lipid A 50:10:35.5:1.5 formulation at a higher dose (2.5 μ g/mL).

[00552] **Table 39. Editing rates in $CD8^+$ T cells**

	Group 1			Group 2			Group 3			Unedited		
Edit	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Fully Edited (Vb8+,CD3+,HLA-DRPDPDQ-,HLA-A*02:01-)	79.6	4.7	3.0	80.5	4.2	3.0	76.8	1.9	3.0	0.2	0.2	3.0
HLA-A KO (HLA-A*02:01-)	97.1	3.6	3.0	96.4	4.7	3.0	96.4	4.4	3.0	3.6	3.8	3.0
CIITA KO (HLA-DRPDPDQ-)	99.3	0.4	3.0	97.7	2.1	3.0	98.7	0.9	3.0	na	na	na
TCR KO (CD3-)	99.3	0.1	3.0	99.7	0.1	3.0	98.7	1.1	3.0	1.8	1.4	3.0
WT1 TCR Insertion (Vb8+)	82.6	2.0	3.0	85.6	0.8	3.0	81.1	2.1	3.0	0.2	0.2	3.0

Example 18: CIITA Guide RNA screening in T cells with BC22n

[00553] Different sgRNAs were screened for their potency in knocking out the CIITA gene in human T cells using C to T base editing. The percentage of T cells negative for MHC class II and/or CD74 protein expression was assayed following CIITA editing following electroporation with mRNA and different sgRNAs.

18.1 T cell Preparation

[00554] Healthy human donor apheresis was obtained commercially (Hemacare), and cells were washed and resuspended in CliniMACS® PBS/EDTA buffer (Miltenyi Biotec Cat. 130-070-525) and processed in a MultiMACS™ Cell 24 Separator Plus device (Miltenyi Biotec). T cells were isolated via positive selection using a Straight from Leukopak® CD4/CD8 MicroBead kit, human (Miltenyi Biotec Cat. 130-122-352). T cells were aliquoted and cryopreserved for future use in Cryostor® CS10 (StemCell Technologies Cat. 07930).

[00555] Upon thaw, T cells were plated at a density of 1.0×10^6 cells/mL in T cell growth media (TCGM) composed of CTS OpTimizer T Cell Expansion SFM and T Cell Expansion Supplement (ThermoFisher Cat. A1048501), 5% human AB serum (GeminiBio, Cat. 100-512) 1X Penicillin-Streptomycin, 1X Glutamax, 10 mM HEPES, 200 U/mL recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/mL recombinant human interleukin 7 (Peprotech, Cat. 200-07), and 5 ng/mL recombinant human interleukin 15 (Peprotech, Cat. 200-15). T cells were rested in this media for 24 hours, at which time they were activated with T Cell TransAct™, human reagent (Miltenyi, Cat. 130-111-160) added at a 1:100 ratio by volume. T cells were activated for 48 hours prior to electroporation.

18.2 T cell editing with RNA electroporation

[00556] Solutions containing mRNA encoding BC22n (SEQ ID NO: 972) and UGI (SEQ ID NO: 815) were prepared in P3 buffer. One hundred μM of CIITA-targeting sgRNAs were removed from their storage plates and denatured for 2 minutes at 95 °C and incubated at room temperature for 5 minutes. Forty-eight hours post activation, T cells were harvested, centrifuged, and resuspended at a concentration of 12.5×10^6 T cells/mL in P3 electroporation buffer (Lonza). For electroporation, 1×10^5 T cells were mixed with 20 ng/ μL of BC22n mRNAs, 20 ng/ μL of UGI mRNA, and 20 pmols of sgRNA in a final volume of 20 μL of P3 electroporation buffer. This mix was transferred in duplicate to a 96-well Nucleofector™ plate and electroporated using the manufacturer's pulse code. Electroporated T cells were immediately rested in 80 μL of CTS Optimizer T cell growth media without cytokines for 15 minutes before being transferred to new flat-bottom 96-well plates containing an additional 80 μL of CTS Optimizer T cell growth media supplemented with 2X cytokines. The resulting plates were incubated at 37 °C for 10 days. On day 4 post-electroporation, cells were split 1:2 in 2 U-bottom plates. One plate was collected for NGS sequencing, while the other plate was replenished with CTS Optimizer fresh media with 1X cytokines. This plate was used for flow cytometry on Day 7.

18.3 Flow cytometry and NGS sequencing

[00557] On day 7 post-editing, T cells were assayed by flow cytometry to determine the surface expression of CD74 and HLA-DR, DP, DQ. The results are shown in **Table 40**. Briefly, T cells were incubated for 30 minutes at 4 °C with a mixture of antibodies diluted in cell staining buffer (BioLegend, Cat. No. 420201). Antibodies against CD3 (BioLegend, Cat. No. 317336), CD4 (BioLegend, Cat. No. 317434), CD8 (BioLegend, Cat. No. 301046), and Viakrome (Beckman Coulter, Cat. No. C36628) were diluted at 1:100, and antibodies against HLA II-DR (BioLegend, Cat. No. 327018), HLA II-DP (BD Biosciences Cat No. 750872), HLA II-DQ (BioLegend, Cat. No. 561504), and CD74 (BioLegend, Cat. No. 326808) were diluted at 1:50. Cells were subsequently washed, resuspended in 100 μL of cell staining buffer and processed on a Cytotflex flow cytometer (Beckman Coulter). Flow cytometry data was analyzed using the FlowJo software package. T cells were gated based on size, shape, viability, CD8, HLA II-DP, HLA II-DQ, HLA II-DR, and CD74 expression.

[00558] **Table 40. Percentage of cells negative for surface protein following genomic editing of *CIITA* with BC22n. (n=2)**

Guide ID	%HLA II-DP-		%HLA II-DQ-		%HLA II-DR-		%CD74-	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
G000502	68.70	3.54	76.30	4.24	76.70	3.96	66.25	5.87
G016788	62.95	1.91	73.40	3.11	73.50	4.38	61.35	4.60
G016053	70.25	6.72	71.50	4.95	73.90	4.10	60.20	6.08
G016103	63.05	6.29	73.95	1.20	75.30	0.71	63.25	1.63
G016114	65.80	1.98	74.70	4.38	75.85	5.30	65.25	5.87
G016117	63.70	0.57	74.60	3.25	76.00	3.11	63.45	5.30
G016034	85.55	2.19	86.30	1.13	87.95	0.07	80.30	0.14
G016035	85.35	3.75	83.55	1.06	84.05	0.35	75.25	1.20
G016039	74.95	0.35	78.20	0.71	78.50	0.28	68.90	1.27
G016040	61.90	0.85	76.30	2.26	77.80	1.56	64.10	2.55
G016041	68.60	1.84	77.30	0.85	76.75	0.35	64.20	0.00
G016043	79.95	1.48	82.55	0.35	82.50	0.71	73.80	0.00
G016044	87.84	3.17	87.80	1.56	88.65	1.34	83.75	1.91
G016045	82.25	2.90	88.70	0.99	88.35	0.78	83.40	0.99
G016047	76.85	0.21	85.40	0.28	85.05	0.21	79.20	0.00
G016050	59.05	1.91	86.80	0.71	83.40	0.57	79.00	0.99
G016052	76.85	0.21	79.25	0.78	80.55	0.92	70.85	1.34
G016054	70.30	1.70	79.85	1.20	79.30	0.42	70.35	0.35
G016055	73.35	1.34	82.15	2.19	82.15	1.77	73.10	1.56
G016056	75.80	1.41	86.05	1.20	86.35	1.34	79.05	1.91
G016057	77.90	0.71	83.95	0.35	84.45	0.21	75.30	0.99
G016058	83.65	2.19	87.25	1.06	88.20	0.99	81.20	1.41
G016060	72.55	0.78	82.70	1.84	83.05	2.47	73.55	2.76
G016061	73.15	6.29	83.10	0.57	82.55	0.21	74.50	0.42
G016063	74.60	7.35	83.75	0.64	83.50	0.71	75.45	0.35
G016064	97.98	0.17	97.80	0.18	96.83	0.13	98.58	0.04
G016065	77.80	0.28	77.70	1.98	80.00	1.56	69.35	3.18
G016068	97.73	0.26	98.07	0.81	97.59	0.66	98.55	0.66
G016071	87.05	4.31	88.55	0.49	89.45	0.49	84.80	0.85
G016074	96.88	0.19	96.34	0.04	95.85	0.57	96.56	0.23
G016075	86.05	0.92	88.20	0.85	88.50	0.14	83.50	1.27
G016076	96.69	0.64	96.67	0.01	96.38	0.02	96.34	0.18
G016077	92.27	2.50	91.20	1.22	91.40	1.44	89.39	1.68
G016078	71.20	0.28	79.55	1.48	80.65	1.48	70.40	0.85
G016079	89.31	1.57	91.24	0.55	90.24	0.00	88.40	0.14
G016081	74.35	0.07	83.05	1.06	83.00	0.42	73.20	1.41
G016082	82.30	5.09	87.50	0.71	88.80	0.14	82.25	0.07
G016083	74.95	4.88	82.90	0.57	83.95	0.21	74.65	1.06
G016085	79.90	2.97	85.40	0.71	87.45	0.07	79.45	0.49
G016086	97.71	0.33	98.06	0.44	96.63	0.08	98.89	0.18

Guide ID	%HLA II-DP-		%HLA II-DQ-		%HLA II-DR-		%CD74-	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
G016087	70.25	9.12	78.55	3.61	78.30	3.39	69.30	4.24
G016088	82.25	5.16	87.40	0.57	88.30	0.42	81.75	1.06
G016089	69.00	1.27	76.65	1.34	79.00	1.70	67.35	1.06
G016091	95.35	0.95	96.81	0.24	96.64	0.25	97.15	0.28
G016092	94.89	0.61	94.87	0.45	95.35	0.83	94.65	0.37
G016093	91.33	0.31	92.35	0.26	93.94	0.23	90.51	0.37
G016094	79.70	4.24	84.85	2.05	85.70	2.69	78.75	2.90
G016095	83.00	2.12	90.55	0.23	90.54	0.24	85.25	0.49
G016097	71.85	6.86	82.80	1.56	82.40	1.27	73.00	1.70
G016098	74.40	5.66	82.35	3.32	83.50	2.69	74.55	4.45
G016099	86.30	2.69	89.71	1.00	90.57	0.35	86.25	1.06
G016100	76.65	1.34	83.90	2.69	86.25	2.19	77.25	3.04
G016101	69.30	0.71	77.55	1.48	78.55	1.77	68.65	1.77
G016102	71.00	1.84	80.70	0.99	80.60	1.56	70.10	0.99
G016106	82.00	1.27	87.55	0.64	88.80	0.71	81.30	1.41
G016108	88.24	3.73	91.49	0.02	91.42	0.66	87.00	0.85
G016109	88.05	0.92	90.20	1.27	90.41	1.85	86.85	2.05
G016110	88.50	3.25	91.12	0.99	90.14	1.05	87.35	1.20
G016111	78.15	0.92	84.45	0.78	85.80	0.85	78.45	1.20
G016112	72.20	3.82	79.85	2.33	81.70	2.26	73.05	2.47
G016115	95.58	1.10	98.36	0.73	97.69	0.48	98.54	0.40
G016116	88.95	0.35	91.15	2.47	92.29	1.79	88.08	2.94
G016066	68.90	0.57	73.20	1.70	74.20	0.99	62.25	2.76
G016113	93.60	1.15	93.02	0.98	93.75	0.66	92.13	1.20
G016084	96.42	0.83	98.38	0.33	97.32	0.69	98.77	0.17
G016104	84.95	1.77	89.69	1.53	91.20	0.97	86.00	1.41
G016070	90.52	0.16	92.10	0.50	92.49	0.64	89.75	0.91
G016090	96.41	1.27	98.06	0.08	97.43	0.15	98.66	0.10
G016048	76.80	1.56	80.60	0.99	81.70	1.41	72.25	2.19
G016051	65.85	20.44	83.15	1.63	84.45	1.34	75.20	2.40
G016073	82.00	1.98	82.65	1.20	83.15	2.76	75.90	0.14
G016037	80.55	1.91	78.05	0.35	80.15	0.07	70.20	0.57
G016038	85.45	0.49	82.45	0.35	84.90	0.28	76.10	1.13
G016046	90.10	0.05	90.75	0.47	91.09	0.86	87.45	0.78
G016049	84.50	0.00	84.90	0.42	86.75	0.07	78.75	0.21
G016036	85.05	1.06	83.15	1.48	85.00	0.99	76.45	2.47
G016080	91.13	2.02	92.11	1.07	92.99	0.97	89.72	1.16
G016096	75.00	4.38	82.90	3.82	83.45	3.61	75.65	4.31
G016032	97.50	0.09	97.42	0.65	96.30	0.33	98.19	0.36
G016033	91.32	0.88	87.75	0.49	88.20	0.28	84.10	0.14

Guide ID	%HLA II-DP-		%HLA II-DQ-		%HLA II-DR-		%CD74-	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
G016030	93.42	0.08	88.10	0.71	88.40	0.42	85.10	0.28
G016067	96.69	0.25	95.82	0.69	95.14	0.64	96.30	0.48
G016031	80.45	1.20	82.10	0.99	83.95	0.07	74.80	0.57
G016062	74.80	11.17	86.60	0.28	86.75	0.35	80.15	0.35
G016059	77.05	3.75	83.05	0.92	83.85	0.92	75.00	1.41
G016105	66.55	5.87	74.90	3.68	76.50	3.68	65.25	3.75
G016107	71.80	4.10	80.70	0.28	80.75	0.49	71.00	0.57
G016042	95.06	0.02	95.90	0.30	94.17	0.35	94.89	1.02
G016069	52.65	3.18	76.35	0.21	75.90	0.28	62.35	0.21
G016072	46.45	4.45	73.50	0.57	71.35	0.92	56.55	0.64

[00559] On day 4 post-editing, DNA samples were subjected to PCR and subsequent NGS analysis, as described in Example 1. **Table 41** shows CIITA editing outcomes in T cells edited with BC22n.

[00560] **Table 41. Mean percent editing at CIITA locus with BC22n. (n=2)**

Guide ID	%C to T		%C to A/G		% Indels	
	Mean	SD	Mean	SD	Mean	SD
G000502	84.63	2.39	0.82	0.19	1.26	0.37
G016788	95.63	0.06	0.99	0.39	0.72	0.15
G016053	94.77	1.07	1.66	0.43	0.71	0.02
G016103	95.60	0.97	0.63	0.23	0.75	0.29
G016114	96.42	0.23	0.66	0.59	0.65	0.09
G016117	96.23	0.70	0.82	0.28	0.90	0.17
G016034	93.11	1.07	0.61	0.21	1.49	0.52
G016035	95.41	0.59	0.52	0.31	0.62	0.24
G016039	88.20	1.33	1.51	0.42	7.99	1.32
G016043	88.51	2.91	1.27	0.11	2.23	0.46
G016044	0.13	0.03	90.04	0.38	9.83	0.41
G016045	61.66	0.51	1.73	0.90	7.12	0.22
G016047	92.74	0.48	0.80	0.06	3.00	0.13
G016050	90.63	0.04	1.35	0.38	1.87	0.56
G016052	92.68	1.74	0.83	0.44	3.26	1.66
G016054	90.82	2.94	1.03	0.89	4.38	4.28
G016055	83.61	1.98	0.46	0.21	10.63	1.04
G016056	96.69	0.65	0.30	0.03	0.64	0.35
G016057	93.83	0.35	0.98	0.18	0.99	0.47
G016058	95.49	0.06	0.35	0.14	0.41	0.31
G016060	85.95	0.54	0.49	0.16	7.63	1.63
G016061	92.81	2.51	0.59	0.34	0.75	0.19

Guide ID	%C to T		%C to A/G		% Indels	
	Mean	SD	Mean	SD	Mean	SD
G016063	70.60	1.87	0.31	0.15	0.25	0.07
G016064	94.14	2.28	0.81	0.41	0.97	0.33
G016065	57.50	1.68	0.61	0.37	1.15	0.32
G016068	94.26	0.97	0.34	0.16	0.52	0.21
G016071	93.78	0.73	0.84	0.14	2.17	0.40
G016074	93.88	1.20	0.92	0.19	2.41	0.28
G016075	91.72	0.73	0.58	0.15	2.89	0.52
G016076	91.24	1.10	0.28	0.20	3.02	1.02
G016077	94.94	1.07	1.08	0.53	1.50	0.46
G016078	93.52	1.78	0.41	0.15	3.30	1.54
G016079	96.29	0.05	0.51	0.08	0.81	0.13
G016081	75.32	7.28	1.33	1.44	7.61	7.04
G016082	31.35	5.20	0.07	0.14	34.39	6.24
G016083	0.24	0.08	99.63	0.11	0.08	0.06
G016085	80.16	3.07	0.74	0.37	0.36	0.12
G016086	96.02	1.68	1.45	0.46	0.84	0.38
G016087	90.30	1.43	0.32	0.20	5.11	0.54
G016088	92.14	0.51	1.05	0.33	2.24	0.86
G016089	94.49	0.39	0.61	0.30	1.14	0.30
G016091	95.93	0.99	1.03	0.23	0.37	0.09
G016092	95.62	0.79	1.17	0.40	0.71	0.28
G016093	95.74	0.81	0.94	0.43	0.43	0.18
G016094	95.85	1.03	0.58	0.39	0.99	0.48
G016095	94.77	0.52	1.32	0.31	0.72	0.37
G016097	94.90	1.64	0.55	0.19	1.40	0.51
G016098	91.71	1.11	0.48	0.18	0.50	0.36
G016099	93.42	1.55	0.56	0.05	3.64	1.06
G016100	96.54	0.93	0.46	0.22	0.46	0.36
G016101	41.21	1.15	0.77	0.07	0.22	0.04
G016102	96.06	1.23	0.46	0.05	0.69	0.25
G016106	90.41	1.16	3.33	0.54	2.66	0.49
G016108	76.19	0.74	0.42	0.28	0.69	0.38
G016109	94.93	0.35	0.46	0.21	1.86	0.59
G016110	87.64	1.01	0.55	0.20	7.63	0.72
G016111	92.93	1.09	1.17	0.58	2.11	1.08
G016112	1.56	0.46	1.79	0.90	0.03	0.05
G016115	89.86	1.01	0.67	0.29	7.30	0.50
G016116	91.37	0.33	0.54	0.27	0.59	0.09
G016066	1.02	0.16	0.32	0.07	0.40	0.21
G016113	93.23	0.98	1.10	0.16	2.40	0.57

Guide ID	%C to T		%C to A/G		% Indels	
	Mean	SD	Mean	SD	Mean	SD
G016084	74.10	1.35	0.87	0.16	21.41	0.94
G016104	0.00	0.00	0.00	0.00	0.00	0.00
G016070	94.80	0.30	0.54	0.04	1.66	0.17
G016090	84.09	0.00	0.51	0.00	12.29	0.00
G016048	48.81	1.82	0.78	0.07	0.54	0.18
G016051	95.69	0.45	1.06	0.09	0.91	0.25
G016073	94.11	0.83	0.76	0.18	1.37	0.29
G016037	64.35	3.31	1.61	0.81	3.46	4.94
G016038	94.99	2.01	1.80	0.64	1.25	0.88
G016046	82.29	2.89	0.88	0.54	14.28	2.86
G016049	95.33	0.15	1.41	0.87	0.46	0.66
G016036	71.71	3.82	0.63	0.14	1.94	0.40
G016080	81.09	1.58	1.33	0.47	5.64	0.94
G016096	94.79	0.33	0.45	0.10	1.81	0.37
G016032	90.27	1.53	2.25	2.37	2.97	0.38
G016033	83.90	0.89	1.79	1.63	5.03	0.94
G016030	96.66	1.35	0.65	0.39	0.31	0.14
G016067	95.79	0.26	0.61	0.17	1.37	0.26
G016031	94.51	0.24	0.75	0.07	2.35	0.68
G016062	93.37	0.45	1.17	0.13	1.91	0.33
G016059	90.26	0.93	0.80	0.30	5.62	0.33
G016105	95.81	1.17	0.53	0.24	0.47	0.45
G016107	91.94	2.74	0.66	0.36	3.18	2.72
G016042	90.07	0.00	0.97	0.00	2.51	0.00
G016069	93.31	0.96	0.96	0.31	1.03	0.33
G016072	84.94	7.39	0.39	0.08	11.91	8.00

Example 19: Screening CIITA sgRNAs in dose-response with BC22n in T cells

[00561] Highly efficient CIITA sgRNAs identified in Example 18 were further assayed for base editing efficacy at multiple guide concentrations in T cells. The potency of each was assayed for genome editing efficacy by NGS or by disruption of surface protein expression of HLA-DR, DP, DQ by flow cytometry.

19.1 T cell Preparation

[00562] Healthy human donor apheresis was obtained commercially (Hemacare), and cells were washed and resuspended in in ClinMACS® PBS/EDTA buffer (Miltenyi Biotec Cat. 130-070-525) and processed in a MultiMACS™ Cell 24 Separator Plus device

(Miltenyi Biotec). T cells were isolated via positive selection using a Straight from Leukopak® CD4/CD8 MicroBead kit, human (Miltenyi Biotec Cat. 130-122-352). T cells were aliquoted and cryopreserved for future use in Cryostor® CS10 (StemCell Technologies Cat. 07930).

[00563] Upon thaw, T cells were plated at a density of 1.0×10^6 cells/mL in T cell growth media (TCGM) composed of CTS OpTmizer T Cell Expansion SFM and T Cell Expansion Supplement (ThermoFisher Cat. A1048501), 5% human AB serum (GeminiBio, Cat. 100-512), 1X Penicillin-Streptomycin, 1X Glutamax, 10 mM HEPES, 200 U/mL recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/mL recombinant human interleukin 7 (Peprotech, Cat. 200-07), and 5 ng/mL recombinant human interleukin 15 (Peprotech, Cat. 200-15). T cells were rested in this media for 24 hours, at which time they were activated with T Cell TransAct™ human reagent (Miltenyi, Cat. 130-111-160) added at a 1:100 ratio by volume. T cells were activated for 48 hours prior to electroporation.

19.2 T cell editing with RNA electroporation

[00564] Solutions containing mRNAs encoding BC22n (SEQ ID NO: 972) and UGI (SEQ ID NO: 815) were prepared in P3 buffer. 100 μ M CIITA targeting sgRNAs were removed from their storage plates and denatured for 2 minutes at 95 °C and incubated at room temperature for 5 minutes. Forty-eight hours post activation, T cells were harvested, centrifuged, and resuspended at a concentration of 12.5×10^6 T cells/mL in P3 electroporation buffer (Lonza). Each sgRNA was serially diluted in ratio of 1:2 in P3 electroporation buffer starting from 60 pmols in a 96-well PCR plate in duplicate. Following dilution, 1×10^5 T cells, 20 ng/ μ L of BC22n mRNAs, and 20 ng/ μ L of UGI mRNA were mixed with sgRNA plate to make the final volume of 20 μ L of P3 electroporation buffer. This mix was transferred to 4 corresponding 96-well Nucleofector™ plates and electroporated using the manufacturer's pulse code. Electroporated T cells were immediately rested in 80 μ L of CTS Optimizer T cell growth media without cytokines for 15 minutes before being transferred to new flat-bottom 96-well plates containing an additional 80 μ L of CTS OpTmizer T cell growth media supplemented with 2X cytokines. The resulting plates were incubated at 37 °C for 7 days. On day 4 post-electroporation, cells were split 1:2 in two U-bottom plates, and one plate was collected for NGS sequencing, while the other plate was replenished with CTS Optimizer fresh media with 1X cytokines. This plate was used for flow cytometry on Day 7.

19.3 Flow cytometry and NGS sequencing

[00565] On day 7 post-editing, T cells were assayed by flow cytometry to determine surface expression of HLA-DR, DP, DQ. Briefly, T cells were incubated for 30 minutes at 4 °C with a mixture of antibodies diluted in cell staining buffer (BioLegend, Cat. No. 420201). Antibodies against CD3 (BioLegend, Cat. No. 317336), CD4 (BioLegend, Cat. No. 317434), CD8 (BioLegend, Cat. No. 301046), and Viakrome (Beckman Coulter, Cat. No. C36628) were diluted at 1:100, and antibodies against HLA II-DR, DP, DQ (BioLegend, Cat. No. 361714) were diluted at 1:50. Cells were subsequently washed, resuspended in 100 µL of cell staining buffer and processed on a Cytoflex flow cytometer (Beckman Coulter). Flow cytometry data was analyzed using the FlowJo software package. T cells were gated based on size, shape, viability, CD8, and HLA-DR, DP, DQ.

[00566] **Table 42** shows CIITA editing outcomes and the percentage of T cells negative for HLA-DR, DP, DQ in T cells following base editing with BC22n.

[00567] Table 42. Percent editing and percent of HLA II-DP, DQ, DR negative cells following CIITA editing with BC22n base editor

	sgRNA (pmols)	C>T			% HLA II-DR, DP, DQ			% CD74		
		Ave	SD	N	Ave	SD	N	Ave	SD	N
G016064	60	96.20%	0.69%	2	99.65	0.07	2	96.2	0.14	2
	30	94.85%	0.69%	2	99.15	0.35	2	92.65	0.21	2
	15	90.26%	0.03%	2	96.70	0.28	2	90.15	2.19	2
	7.5	72.95%	1.76%	2	87.45	1.20	2	80.95	3.32	2
	3.75	49.55%	2.09%	2	73.05	0.64	2	66.35	4.31	2
	1.88	28.63%	1.34%	2	59.40	1.70	2	55.2	5.8	2
	0.94	14.98%	0.01%	2	54.60	2.40	2	46.9	1.84	2
G016068	0	0.26%	0.02%	2	46.70	5.52	2	45.15	0.49	2
	60	97.40%	0.87%	2	99.70	0.00	2	94.25	1.2	2
	30	90.31%	0.83%	2	93.15	0.07	2	85.15	1.2	2
	15	75.08%	0.17%	2	82.45	0.21	2	74.65	1.48	2
	7.5	49.64%	2.08%	2	68.65	1.91	2	57	1.27	2
	3.75	28.95%	1.51%	2	57.85	1.63	2	43.9	1.27	2
	1.88	14.90%	0.58%	2	51.10	2.26	2	35.85	3.75	2
G016074	0.94	7.11%	0.22%	2	49.50	4.67	2	38.2	4.1	2
	0	0.21%	0.02%	2	51.50	1.13	2	44.8	1.56	2
	60	96.62%		2	96.95	0.21	2	90.6	0.28	2
	30	94.89%	0.58%	2	93.50	1.27	2	85.45	1.06	2
	15	89.79%	3.18%	2	88.50	0.28	2	80.35	0.64	2
	7.5	72.00%	1.61%	2	77.75	0.35	2	68.35	1.77	2

		C>T		% HLA II-DR, DP, DQ		% CD74	
	3.75	47.99%	0.39%	65.30	1.84	53.05	1.91
	1.88	27.92%	0.44%	57.60	0.14	45	0.42
	0.94	13.11%	1.79%	54.30	0.71	37.9	0
	0	0.41%	0.13%	49.15	2.05	44.05	1.91
G016076	60	96.03%	0.07%	94.30	0.14	90	0.71
	30	93.29%	ND	89.10	0.99	81.65	1.48
	15	80.74%	0.41%	83.55	0.35	74.1	0.85
	7.5	51.33%	1.82%	69.05	1.91	57.7	2.97
	3.75	28.40%	2.41%	58.90	2.26	43.15	0.64
	1.88	15.12%	1.91%	56.05	0.78	38.45	1.77
	0.94	7.23%	0.26%	52.80	0.71	38.2	0.28
	0	0.27%	0.00%	47.70	1.70	43.5	3.68
G016086	60	97.81%	0.16%	99.25	0.35	94.35	0.92
	30	ND	ND	98.65	0.07	90.25	1.2
	15	ND	ND	98.20	0.28	88.8	1.98
	7.5	97.19%	1.91%	95.85	0.07	87.5	0.14
	3.75	ND	ND	87.10	0.28	74.95	2.47
	1.88	ND	ND	72.45	0.07	60.75	2.76
	0.94	ND	ND	59.10	0.85	48.05	3.89
	0	ND	ND	50.65	2.76	46.15	2.19
G016091	60	ND	ND	98.05	0.07	89.85	0.21
	30	ND	ND	96.95	0.07	87.9	0.85
	15	ND	ND	91.40	1.84	83.65	2.76
	7.5	ND	ND	82.00	1.27	74	3.11

		C>T		% HLA II-DR, DP, DQ			% CD74				
	3.75		ND	ND	2	69.70	0.99	2	62.75	2.33	2
	1.88		ND	ND	2	59.40	1.41	2	54.4	3.54	2
	0.94		ND	ND	2	54.25	2.05	2	46.3	5.09	2
	0		ND	ND	2	46.30	2.26	2	48.3	4.67	2
G016115	60		93.33%	0.70%	2	96.80	0.14	2	92.55	0.07	2
	30		94.46%	0.23%	2	92.70	1.41	2	87.8	1.98	2
	15		93.51%	0.70%	2	89.85	0.35	2	82.75	3.75	2
	7.5		90.13%	0.37%	2	83.35	0.35	2	78.35	2.47	2
	3.75		75.18%	1.51%	2	71.40	1.27	2	65.5	2.4	2
	1.88		51.65%	1.64%	2	64.20	4.95	2	56.1	1.84	2
	0.94		30.19%	2.14%	2	55.25	3.04	2	49.15	4.88	2
	0		0.26%	0.03%	2	46.85	7.57	2	47.1	3.25	2
G016084	60		73.43%	0.13%	2	97.00	0.14	2	91.35	1.06	2
	30		73.70%	0.38%	2	94.80	0.42	2	84.9	0.42	2
	15		67.30%	1.19%	2	91.60	0.71	2	80.6	0.57	2
	7.5		48.91%	0.27%	2	80.20	0.00	2	67.5	0.42	2
	3.75		28.24%	0.35%	2	67.50	0.57	2	51.85	3.18	2
	1.88		16.89%	0.30%	2	59.05	2.76	2	42.7	0.14	2
	0.94		7.42%	0.06%	2	54.65	0.92	2	40.05	0.78	2
	0		1.06%	0.06%	2	49.25	2.47	2	46.9	1.98	2
G016090	60		87.05%	2.52%	2	98.70	1.27	2	93.25	2.19	2
	30		88.93%	0.06%	2	98.90	0.14	2	91	0.99	2
	15		89.51%	0.24%	2	98.70	0.28	2	90.45	0.21	2
	7.5		83.78%	0.02%	2	93.80	0.57	2	85.05	0.07	2

		C>T			% HLA II-DR, DP, DQ			% CD74			
	3.75		66.23%	0.40%	2	83.35	1.06	2	72.35	0.35	2
	1.88		44.49%	0.27%	2	69.50	0.57	2	57.75	1.34	2
	0.94		22.90%	1.49%	2	57.25	1.77	2	45.15	2.47	2
	0		0.61%	0.14%	2	49.55	1.63	2	47.5	4.1	2
G016032	60		91.66%	0.76%	2	99.35	0.07	2	93.65	2.05	2
	30		86.77%	2.13%	2	94.25	1.06	2	87.45	1.63	2
	15		75.23%	1.22%	2	86.10	2.26	2	74.65	0.78	2
	7.5		54.08%	0.63%	2	74.10	0.85	2	60.65	1.34	2
	3.75		33.34%	2.39%	2	63.20	3.82	2	45.95	3.75	2
	1.88		16.30%	1.21%	2	57.20	0.57	2	39.7	0.28	2
	0.94		7.33%	0.38%	2	53.10	0.85	2	37.6	1.13	2
	0		0.33%	0.00%	2	52.65	4.31	2	47.75	2.62	2
G016067	60		97.37%	0.56%	2	93.10	0.14	2	85.95	2.19	2
	30		97.47%	0.36%	2	93.00	0.28	2	81.8	3.54	2
	15		95.78%	0.13%	2	89.30	1.41	2	79.45	0.49	2
	7.5		86.53%	0.24%	2	81.05	2.19	2	71	2.26	2
	3.75		68.12%	1.27%	2	69.35	1.20	2	58.3	2.4	2
	1.88		43.82%	0.48%	2	57.70	1.98	2	48.2	0.99	2
	0.94		22.80%	1.26%	2	54.95	0.35	2	42.55	1.34	2
	0		0.14%	0.06%	2	49.55	1.63	2	43.7	1.98	2

Example 20: Editing human T cells with BC22n, UGI and 91-mer sgRNAs

[00568] The base editing efficacy of 91-mer sgRNA as assessed by NGS and receptor knockout was compared to that of a 100-mer sgRNA format with the same guide sequence.

[00569] The tested 91-mer sgRNA include a 20-nucleotide guide sequence (as represented by N) and a guide scaffold as follows:

mN*mN*mN*NNNNNNNNNNNNNNNNNNNGUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUA AAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCG GmUmGmC*mU (SEQ ID NO: 1006), where A, C, G, U, and N are adenine, cytosine, guanine, uracil, and any ribonucleotide, respectively, unless otherwise indicated. An m is indicative of a 2'-O-methyl modification, and an * is indicative of a phosphorothioate linkage between the nucleotides. Unmodified and modified versions of the guide is provided in Table 4 (Sequence Table).

Example 20.1. T cell preparation

[00570] Healthy human donor apheresis was obtained commercially (Hemacare), and cells were washed, re-suspended in CliniMACS® PBS/EDTA buffer (Miltenyi Biotec Cat. 130-070-525) and processed in a MultiMACS™ Cell 24 Separator Plus device (Miltenyi Biotec). T cells were isolated via positive selection using a Straight from Leukopak® CD4/CD8 MicroBead kit, human (Miltenyi Biotec Cat. 130-122-352). T cells were aliquoted and cryopreserved for future use in Cryostor® CS10 (StemCell Technologies Cat. 07930).

[00571] Upon thaw, T cells were plated at a density of 1.0×10^6 cells/mL in T cell growth media (TCGM) composed of CTS OpTmizer T Cell Expansion SFM and T Cell Expansion Supplement (ThermoFisher Cat. A1048501), 5% human AB serum (GeminiBio, Cat. 100-512) 1X Penicillin-Streptomycin, 1X Glutamax, 10 mM HEPES, 200 U/mL recombinant human interleukin-2 (Peprotech, Cat. 200-02), 5 ng/ml recombinant human interleukin 7 (Peprotech, Cat. 200-07), and 5 ng/ml recombinant human interleukin 15 (Peprotech, Cat. 200-15). T cells were rested in this media for 24 hours, at which time they were activated with T Cell TransAct™, human reagent (Miltenyi, Cat. 130-111-160) added at a 1:100 ratio by volume. T cells were activated for 48 hours prior to LNP treatments.

Example 20.2. T cell LNP treatment and expansion

[00572] Forty-eight hours post-activation, T cells were harvested, centrifuged at 500 g for 5 min, and resuspended at a concentration of 1×10^6 T cells/mL in T cell plating media

(TCPM): a serum-free version of TCGM containing 400 U/mL recombinant human interleukin-2 (Peprotech, Cat. 200-02), 10 ng/ml recombinant human interleukin 7 (Peprotech, Cat. 200-07), and 10 ng/ml recombinant human interleukin 15 (Peprotech, Cat. 200-15). 50 μ L of T cells in TCPM (5×10^4 T cells) were added per well to be treated in flat-bottom 96-well plates.

[00573] LNPs were prepared as described in Example 1 at a ratio of 35:47.5:15:2.5 (Lipid A/ cholesterol/DSPC/PEG2k-DMG). The LNPs were formulated with a lipid amine to RNA phosphate (N:P) molar ratio of about 6. LNPs encapsulated a single RNA species, either a sgRNA as described in **Table 43**, BC22n mRNA (SEQ ID No: 972), or UGI mRNA (SEQ ID No: 815).

Table 43 - 100-mer and 91-mer sgRNAs.

Gene target	100-mer	91-mer
CIITA	G016086 (SEQ ID NO: 395)	G023521 (SEQ ID NO: 1008)

[00574] Prior to T cell treatment, LNPs encapsulating a sgRNA were diluted to 6.64 μ g/mL in T cell treatment media (TCTM): a version of TCGM containing 20 μ g/mL rhApoE3 in the absence of interleukins 2, 5 or 7. These LNPs were incubated at 37°C for 15 minutes and serially diluted 1:4 using TCTM, which resulted in an 8-point dilution series ranging from 6.64 μ g/mL to zero. Similarly, single-cargo LNPs with BC22n mRNA (SEQ ID NO: 972) or UGI mRNA (SEQ ID NO: 815) were diluted in TCTM to 3.32 and 1.67 μ g/mL, respectively, incubated at 37°C for 15 minutes, and mixed 1:1 by volume with sgRNA LNPs serially diluted in the previous step. Last, 50 μ L from the resulting mix was added to T cells in 96-well plates at a 1:1 ratio by volume. T cells were incubated at 37 °C for 24 hours, at which time they were harvested, centrifuged at 500 g for 5 min, resuspended in 200 μ L of TCGM and returned to the incubator.

Example 20.3. Evaluation of editing outcomes by next generation sequencing (NGS)

[00575] Four days post-LNP treatment, T cells were subjected to lysis, PCR amplification of each targeted locus and subsequent NGS analysis, as described in Example 1. **Table 44** and **Fig. 18** shows editing levels and the C to T editing purity in T cells treated with a decreasing mass of 100-mer or 91-mer sgRNA targeting CIITA.

[00576] When compared to the 100-mer version, 91-mer sgRNA resulted in higher editing frequencies when delivered at the same concentration. No differences in C to T editing purity were observed between 100-mer and 91-mer sgRNAs.

Table 44 - Mean percent editing at the CIITA locus in T cells treated with sgRNAs in the 100-mer (G016086) or 91-mer format (G023521).

sgRNA (ng)	CIITA											
	100-mer						91-mer					
	C to T		C to A/G		Indels		C to T		C to A/G		Indels	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
166.00	98.1	0.2	0.7	0.1	0.6	0.1	96.9	0.3	1.3	0.0	1.4	0.2
41.50	97.3	0.2	0.4	0.1	0.3	0.1	98.1	0.2	0.6	0.1	0.8	0.1
10.38	82.9	0.3	0.3	0.0	0.2	0.1	97.5	0.4	0.5	0.2	0.4	0.1
2.59	43.3	0.5	0.3	0.1	0.2	0.1	88.0	1.1	0.4	0.1	0.3	0.1
0.65	14.5	0.9	0.3	0.1	0.1	0.1	50.4	1.9	0.3	0.1	0.1	0.1
0.16	4.3	0.2	0.2	0.1	0.0	0.0	17.7	0.5	0.3	0.0	0.1	0.1
0.04	1.8	0.2	0.3	0.1	0.1	0.0	5.7	0.1	0.2	0.1	0.1	0.0
0.00	0.6	0.1	0.2	0.1	0.1	0.0	0.8	0.0	0.2	0.1	0.1	0.0

Example 20.4. Evaluation of receptor knockout by flow cytometry

[00577] Seven days post LNP treatment, T cells were assayed by flow cytometry to evaluate receptor knockout. T cells were incubated with a fixable viability dye (Beckman Coulter, Cat. C36628) and an antibody cocktail targeting HLA-DR, DP, DQ (Biolegend, Cat. 361714). Cells were subsequently washed, analyzed on a Cytotflex LX instrument (Beckman Coulter) using the FlowJo software package. T cells were gated on size, viability and CD8 positivity before expression of any markers was determined. The resulting data was plotted on GraphPad Prism v. 9.0.2 and analyzed using a variable slope (four parameter) non-linear regression.

[00578] As shown in **Tables 45-46** and **Fig. 19**, the 91-mer sgRNA tested outperformed the 100-mer version. Targets with a lower potency (i.e., higher EC50) in the 100-mer format (CIITA) seem to benefit the most from usage of 91-mer sgRNAs.

Table 45 – Mean percentage of CD8+ T cells that are negative for HLA-DR, DP, DQ surface receptors following treatment sgRNA targeting CIITA, respectively, in the 100-mer or 91-mer formats.

sgRA (ng)	CIITA (HLA-DR, DP, DQ-)			
	100-mer		91-mer	
	Mean	SD	Mean	SD
166.00	98.3	0.2	98.7	0.2
41.50	96.9	0.7	98.4	0.3
10.38	85.2	0.7	97.7	0.3
2.59	58.7	0.2	89.4	1.3
0.65	44.8	1.1	63.4	0.5
0.16	38.2	1.6	45.2	2.6

sgRA (ng)	CIITA (HLA-DR, DP, DQ-)			
	100-mer		91-mer	
	Mean	SD	Mean	SD
0.04	37.8	1.0	38.2	0.5
0.00	35.1	2.5	37.6	1.7

Table 46 – Amount (pmol) of sgRNA that lead to a 50% loss of receptor expression in the surface of CD8+ T cells (EC50s). The far right column shows the fold-increase in potency achieved by 91-mer sgRNA when compared to the 100-mer with the same guide sequence.

Gene target	100-mer		91-mer		EC50 shift (100-mer/91-mer)
	sgRNA ID	EC50 (pmols)	sgRNA ID	EC50 (pmols)	
CIITA	G016086	0.123	G023521	0.027	4.60

Example 21. Additional Embodiments

[00579] The disclosure further includes the following embodiments.

[00580] Embodiment 1 is an engineered cell, which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10902662- chr16:10923285.

[00581] Embodiment 2 is the engineered cell of embodiment 1, wherein the genetic modification comprises at least 5 contiguous nucleotides within the genomic coordinates chr16:10902662- chr16:10923285.

[00582] Embodiment 3 is the engineered cell of any one of the preceding embodiments, wherein the genetic modification comprises at least 6, 7, 8, 9, or 10 contiguous nucleotides within the genomic coordinates chr16:10902662- chr16:10923285.

[00583] Embodiment 4 is the engineered cell of any one of the preceding embodiments, wherein the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates chr16:10902662- chr16:10923285.

[00584] Embodiment 5 is the engineered cell of any one of the preceding embodiments, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10906542- chr16:10923285.

[00585] Embodiment 6 is the engineered cell of any one of the preceding embodiments, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10906542- chr16:10908121.

[00586] Embodiment 7 is the engineered cell of any one of the preceding embodiments, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10907539-10907559, chr16:10916426-10916446, chr16:10906907-10906927, chr16:10895702-10895722, chr16:10907757-10907777, chr16:10907623-10907643, chr16:10915626-10915646, chr16:10906756-10906776, chr16:10907476-10907496, chr16:10907385-10907405, and chr16:10923265-10923285.

[00587] Embodiment 8 is the engineered cell of any one of the preceding embodiments, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10916432-10916452, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10906985-10907005, chr16:10908073-10908093, chr16:10907433-10907453, chr16:10907979-10907999, chr16:10907139-10907159, chr16:10922435-10922455, chr16:10907384-10907404, chr16:10907434-10907454, chr16:10907119-10907139, chr16:10907539-10907559, chr16:10907810-10907830, chr16:10907315-10907335, chr16:10916426-10916446, chr16:10909138-10909158, chr16:10908101-10908121, chr16:10907790-10907810, chr16:10907787-10907807, chr16:10907454-10907474, chr16:10895702-10895722, chr16:10902729-10902749, chr16:10918492-10918512, chr16:10907932-10907952, chr16:10907623-10907643, chr16:10907461-10907481, chr16:10902723-10902743, chr16:10907622-10907642, chr16:10922441-10922461, chr16:10902662-10902682, chr16:10915626-10915646, chr16:10915592-10915612, chr16:10907385-10907405, chr16:10907030-10907050, chr16:10907935-10907955, chr16:10906853-10906873, chr16:10906757-10906777, chr16:10907730-10907750, and chr16:10895302-10895322.

[00588] Embodiment 9 is the engineered cell of any one of the preceding embodiments, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10907315-10907335, chr16:10916432-10916452, chr16:10907932-10907952, chr16:10915626-10915646, chr16:10907586-10907606, chr16:10916426-10916446, chr16:10907476-10907496, chr16:10907787-10907807, chr16:10907979-10907999, chr16:10906904-10906924, and chr16:10909138-10909158.

[00589] Embodiment 10 is the engineered cell of any one of the preceding embodiments, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10895702-10895722, chr16:10916432-10916452, chr16:10907623-10907643, chr16:10907932-10907952, chr16:10906985-10907005, chr16:10915626-10915646, chr16:10907539-10907559, chr16:10916426-10916446,

chr16:10907476-10907496, chr16:10907119-10907139, chr16:10907979-10907999, and chr16:10909138-10909158.

[00590] Embodiment 11 is the engineered cell of any one of the preceding embodiments, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10906757-10906777, chr16:10895302-10895322, chr16:10907539-10907559, chr16:10907730-10907750, chr16:10895702-10895722.

[00591] Embodiment 12 is the engineered cell of any one of the preceding embodiments, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10922444-10922464, and chr16:10916432-10916452.

[00592] Embodiment 13 is the engineered cell of any one of the preceding embodiments, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10906853-10906873.

[00593] Embodiment 14 is the engineered cell of any one of the preceding embodiments, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10922444-10922464.

[00594] Embodiment 15 is the engineered cell of any one of the preceding embodiments, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10916432-10916452.

[00595] Embodiment 16 is the engineered cell of any one of the preceding embodiments, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10906757-10906777.

[00596] Embodiment 17 is the engineered cell of any one of the preceding embodiments, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10895302-10895322.

[00597] Embodiment 18 is the engineered cell of any one of the preceding embodiments, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10907539-10907559.

[00598] Embodiment 19 is the engineered cell of any one of the preceding embodiments, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10907730-10907750.

[00599] Embodiment 20 is the engineered cell of any one of the preceding embodiments, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10895702-10895722.

[00600] Embodiment 21 is the engineered cell of any one of the preceding embodiments, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10907932-10907952.

[00601] Embodiment 22 is the engineered cell of any one of the preceding embodiments, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10907476-10907496.

[00602] Embodiment 23 is the engineered cell of any one of the preceding embodiments, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10909138-10909158.

[00603] Embodiment 24 is an engineered cell, which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises an indel, a C to T

substitution, or an A to G substitution within the genomic coordinates chosen from:
chr16:10902662-10902682, chr16:10902723-10902743, chr16:10902729-10902749,
chr16:10903747-10903767, chr16:10903824-10903844, chr16:10903824-10903844,
chr16:10903848-10903868, chr16:10904761-10904781, chr16:10904764-10904784,
chr16:10904765-10904785, chr16:10904785-10904805, chr16:10906542-10906562,
chr16:10906556-10906576, chr16:10906609-10906629, chr16:10906610-10906630,
chr16:10906616-10906636, chr16:10906682-10906702, chr16:10906756-10906776,
chr16:10906757-10906777, chr16:10906757-10906777, chr16:10906821-10906841,
chr16:10906823-10906843, chr16:10906847-10906867, chr16:10906848-10906868,
chr16:10906853-10906873, chr16:10906904-10906924, chr16:10906907-10906927,
chr16:10906913-10906933, chr16:10906968-10906988, chr16:10906970-10906990,
chr16:10906985-10907005, chr16:10907030-10907050, chr16:10907058-10907078,
chr16:10907119-10907139, chr16:10907139-10907159, chr16:10907172-10907192,
chr16:10907272-10907292, chr16:10907288-10907308, chr16:10907314-10907334,
chr16:10907315-10907335, chr16:10907325-10907345, chr16:10907363-10907383,
chr16:10907384-10907404, chr16:10907385-10907405, chr16:10907433-10907453,
chr16:10907434-10907454, chr16:10907435-10907455, chr16:10907441-10907461,
chr16:10907454-10907474, chr16:10907461-10907481, chr16:10907476-10907496,
chr16:10907539-10907559, chr16:10907586-10907606, chr16:10907589-10907609,

chr16:10907621-10907641, chr16:10907622-10907642, chr16:10907623-10907643, chr16:10907730-10907750, chr16:10907731-10907751, chr16:10907757-10907777, chr16:10907781-10907801, chr16:10907787-10907807, chr16:10907790-10907810, chr16:10907810-10907830, chr16:10907820-10907840, chr16:10907870-10907890, chr16:10907886-10907906, chr16:10907924-10907944, chr16:10907928-10907948, chr16:10907932-10907952, chr16:10907935-10907955, chr16:10907978-10907998, chr16:10907979-10907999, chr16:10908069-10908089, chr16:10908073-10908093, chr16:10908101-10908121, chr16:10909056-10909076, chr16:10909138-10909158, chr16:10910195-10910215, chr16:10910196-10910216, chr16:10915592-10915612, chr16:10915626-10915646, chr16:10916375-10916395, chr16:10916382-10916402, chr16:10916426-10916446, chr16:10916432-10916452, chr16:10918486-10918506, chr16:10918492-10918512, chr16:10918493-10918513, chr16:10922435-10922455, chr16:10922441-10922461, chr16:10922441-10922461, chr16:10922444-10922464, chr16:10922460-10922480, chr16:10923257-10923277, and chr16:10923265-10923285.

[00604] Embodiment 25 is the engineered cell of embodiment 24, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10916432-10916452, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10906985-10907005, chr16:10908073-10908093, chr16:10907433-10907453, chr16:10907979-10907999, chr16:10907139-10907159, chr16:10922435-10922455, chr16:10907384-10907404, chr16:10907434-10907454, chr16:10907119-10907139, chr16:10907539-10907559, chr16:10907810-10907830, chr16:10907315-10907335, chr16:10916426-10916446, chr16:10909138-10909158, chr16:10908101-10908121, chr16:10907790-10907810, chr16:10907787-10907807, chr16:10907454-10907474, chr16:10895702-10895722, chr16:10902729-10902749, chr16:10918492-10918512, chr16:10907932-10907952, chr16:10907623-10907643, chr16:10907461-10907481, chr16:10902723-10902743, chr16:10907622-10907642, chr16:10922441-10922461, chr16:10902662-10902682, chr16:10915626-10915646, chr16:10915592-10915612, chr16:10907385-10907405, chr16:10907030-10907050, chr16:10907935-10907955, chr16:10906853-10906873, chr16:10906757-10906777, chr16:10907730-10907750, chr16:10907586-10907606, chr16:10907476-10907496, chr16:10906904-10906924, and chr16:10895302-10895322.

[00605] Embodiment 26 is the engineered cell of embodiments 24 or 25, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10907539-10907559, chr16:10916426-10916446,

chr16:10906907-10906927, chr16:10895702-10895722, chr16:10907757-10907777, chr16:10907623-10907643, chr16:10915626-10915646, chr16:10906756-10906776, chr16:10907476-10907496, chr16:10907385-10907405, and chr16:10923265-10923285.

[00606] Embodiment 27 is the engineered cell of embodiments 24 or 25, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10907315-10907335, chr16:10916432-10916452, chr16:10907932-10907952, chr16:10915626-10915646, chr16:10907586-10907606, chr16:10916426-10916446, chr16:10907476-10907496, chr16:10907787-10907807, chr16:10907979-10907999, chr16:10906904-10906924, and chr16:10909138-10909158.

[00607] Embodiment 28 is the engineered cell of embodiments 24 or 25, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10895702-10895722, chr16:10916432-10916452, chr16:10907623-10907643, chr16:10907932-10907952, chr16:10906985-10907005, chr16:10915626-10915646, chr16:10907539-10907559, chr16:10916426-10916446, chr16:10907476-10907496, chr16:10907119-10907139, chr16:10907979-10907999, and chr16:10909138-10909158.

[00608] Embodiment 29 is the engineered cell of embodiments 24 or 25, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10906757-10906777, chr16:10895302-10895322, chr16:10907539-10907559, chr16:10907730-10907750, chr16:10895702-10895722.

[00609] Embodiment 30 is the engineered cell of any one of embodiments 24 or 25, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10922444-10922464, and chr16:10916432-10916452.

[00610] Embodiment 31 is the engineered cell of any one of embodiments 24-30, wherein the genetic modification comprises at least 5 contiguous nucleotides within the genomic coordinates.

[00611] Embodiment 32 is the engineered cell of any one of embodiments 24-31, wherein the genetic modification comprises at least 6, 7, 8, 9, or 10 contiguous nucleotides within the genomic coordinates.

[00612] Embodiment 33 is the engineered cell of any one of embodiments 24-32, wherein the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates.

[00613] Embodiment 34 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10902662-10902682, chr16:10902723-10902743, chr16:10902729-10902749, chr16:10903747-10903767, chr16:10903824-10903844, chr16:10903824-10903844, chr16:10903848-10903868, chr16:10904761-10904781, chr16:10904764-10904784, chr16:10904765-10904785, chr16:10904785-10904805, chr16:10906542-10906562, chr16:10906556-10906576, chr16:10906609-10906629, chr16:10906610-10906630, chr16:10906616-10906636, chr16:10906682-10906702, chr16:10906756-10906776, chr16:10906757-10906777, chr16:10906757-10906777, chr16:10906821-10906841, chr16:10906823-10906843, chr16:10906847-10906867, chr16:10906848-10906868, chr16:10906853-10906873, chr16:10906853-10906873, chr16:10906904-10906924, chr16:10906907-10906927, chr16:10906913-10906933, chr16:10906968-10906988, chr16:10906970-10906990, chr16:10906985-10907005, chr16:10907030-10907050, chr16:10907058-10907078, chr16:10907119-10907139, chr16:10907139-10907159, chr16:10907172-10907192, chr16:10907272-10907292, chr16:10907288-10907308, chr16:10907314-10907334, chr16:10907315-10907335, chr16:10907325-10907345, chr16:10907363-10907383, chr16:10907384-10907404, chr16:10907385-10907405, chr16:10907433-10907453, chr16:10907434-10907454, chr16:10907435-10907455, chr16:10907441-10907461, chr16:10907454-10907474, chr16:10907461-10907481, chr16:10907476-10907496, chr16:10907539-10907559, chr16:10907586-10907606, chr16:10907589-10907609, chr16:10907621-10907641, chr16:10907622-10907642, chr16:10907623-10907643, chr16:10907730-10907750, chr16:10907731-10907751, chr16:10907757-10907777, chr16:10907781-10907801, chr16:10907787-10907807, chr16:10907790-10907810, chr16:10907810-10907830, chr16:10907820-10907840, chr16:10907870-10907890, chr16:10907886-10907906, chr16:10907924-10907944, chr16:10907928-10907948, chr16:10907932-10907952, chr16:10907935-10907955, chr16:10907978-10907998, chr16:10907979-10907999, chr16:10908069-10908089, chr16:10908073-10908093, chr16:10908101-10908121, chr16:10909056-10909076, chr16:10909138-10909158, chr16:10910195-10910215, chr16:10910196-10910216, chr16:10915592-10915612, chr16:10915626-

10915646, chr16:10916375-10916395, chr16:10916382-10916402, chr16:10916426-10916446, chr16:10916432-10916452, chr16:10918486-10918506, chr16:10918492-10918512, chr16:10918493-10918513, chr16:10922435-10922455, chr16:10922441-10922461, chr16:10922441-10922461, chr16:10922444-10922464, chr16:10922460-10922480, chr16:10923257-10923277, and chr16:10923265-10923285.

[00614] Embodiment 35 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10906542-10906562, chr16:10906556-10906576, chr16:10906609-10906629, chr16:10906610-10906630, chr16:10906616-10906636, chr16:10906682-10906702, chr16:10906756-10906776, chr16:10906757-10906777, chr16:10906757-10906777, chr16:10906821-10906841, chr16:10906823-10906843, chr16:10906847-10906867, chr16:10906848-10906868, chr16:10906853-10906873, chr16:10906853-10906873, chr16:10906904-10906924, chr16:10906907-10906927, chr16:10906913-10906933, chr16:10906968-10906988, chr16:10906970-10906990, chr16:10906985-10907005, chr16:10907030-10907050, chr16:10907058-10907078, chr16:10907119-10907139, chr16:10907139-10907159, chr16:10907172-10907192, chr16:10907272-10907292, chr16:10907288-10907308, chr16:10907314-10907334, chr16:10907315-10907335, chr16:10907325-10907345, chr16:10907363-10907383, chr16:10907384-10907404, chr16:10907385-10907405, chr16:10907433-10907453, chr16:10907434-10907454, chr16:10907435-10907455, chr16:10907441-10907461, chr16:10907454-10907474, chr16:10907461-10907481, chr16:10907476-10907496, chr16:10907539-10907559, chr16:10907586-10907606, chr16:10907589-10907609, chr16:10907621-10907641, chr16:10907622-10907642, chr16:10907623-10907643, chr16:10907730-10907750, chr16:10907731-10907751, chr16:10907757-10907777, chr16:10907781-10907801, chr16:10907787-10907807, chr16:10907790-10907810, chr16:10907810-10907830, chr16:10907820-10907840, chr16:10907870-10907890, chr16:10907886-10907906, chr16:10907924-10907944, chr16:10907928-10907948, chr16:10907932-10907952, chr16:10907935-10907955, chr16:10907978-10907998, chr16:10907979-10907999, chr16:10908069-10908089, chr16:10908073-10908093, chr16:10908101-10908121, chr16:10909056-10909076, chr16:10909138-10909158, chr16:10910195-10910215, chr16:10910196-10910216, chr16:10915592-10915612, chr16:10915626-10915646, chr16:10916375-10916395, chr16:10916382-10916402, chr16:10916426-10916446, chr16:10916432-10916452, chr16:10918486-

10918506, chr16:10918492-10918512, chr16:10918493-10918513, chr16:10922435-10922455, chr16:10922441-10922461, chr16:10922441-10922461, chr16:10922444-10922464, chr16:10922460-10922480, chr16:10923257-10923277, and chr16:10923265-10923285.

[00615] Embodiment 36 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10906542-10906562, chr16:10906556-10906576, chr16:10906609-10906629, chr16:10906610-10906630, chr16:10906616-10906636, chr16:10906682-10906702, chr16:10906756-10906776, chr16:10906757-10906777, chr16:10906757-10906777, chr16:10906821-10906841, chr16:10906823-10906843, chr16:10906847-10906867, chr16:10906848-10906868, chr16:10906853-10906873, chr16:10906853-10906873, chr16:10906904-10906924, chr16:10906907-10906927, chr16:10906913-10906933, chr16:10906968-10906988, chr16:10906970-10906990, chr16:10906985-10907005, chr16:10907030-10907050, chr16:10907058-10907078, chr16:10907119-10907139, chr16:10907139-10907159, chr16:10907172-10907192, chr16:10907272-10907292, chr16:10907288-10907308, chr16:10907314-10907334, chr16:10907315-10907335, chr16:10907325-10907345, chr16:10907363-10907383, chr16:10907384-10907404, chr16:10907385-10907405, chr16:10907433-10907453, chr16:10907434-10907454, chr16:10907435-10907455, chr16:10907441-10907461, chr16:10907454-10907474, chr16:10907461-10907481, chr16:10907476-10907496, chr16:10907539-10907559, chr16:10907586-10907606, chr16:10907589-10907609, chr16:10907621-10907641, chr16:10907622-10907642, chr16:10907623-10907643, chr16:10907730-10907750, chr16:10907731-10907751, chr16:10907757-10907777, chr16:10907781-10907801, chr16:10907787-10907807, chr16:10907790-10907810, chr16:10907810-10907830, chr16:10907820-10907840, chr16:10907870-10907890, chr16:10907886-10907906, chr16:10907924-10907944, chr16:10907928-10907948, chr16:10907932-10907952, chr16:10907935-10907955, chr16:10907978-10907998, chr16:10907979-10907999, chr16:10908069-10908089, chr16:10908073-10908093, and chr16:10908101-10908121.

[00616] Embodiment 37 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10916432-10916452, chr16:10922444-

10922464, chr16:10907924-10907944, chr16:10906985-10907005, chr16:10908073-10908093, chr16:10907433-10907453, chr16:10907979-10907999, chr16:10907139-10907159, chr16:10922435-10922455, chr16:10907384-10907404, chr16:10907434-10907454, chr16:10907119-10907139, chr16:10907539-10907559, chr16:10907810-10907830, chr16:10907315-10907335, chr16:10916426-10916446, chr16:10909138-10909158, chr16:10908101-10908121, chr16:10907790-10907810, chr16:10907787-10907807, chr16:10907454-10907474, chr16:10895702-10895722, chr16:10902729-10902749, chr16:10918492-10918512, chr16:10907932-10907952, chr16:10907623-10907643, chr16:10907461-10907481, chr16:10902723-10902743, chr16:10907622-10907642, chr16:10922441-10922461, chr16:10902662-10902682, chr16:10915626-10915646, chr16:10915592-10915612, chr16:10907385-10907405, chr16:10907030-10907050, chr16:10907935-10907955, chr16:10906853-10906873, chr16:10906757-10906777, chr16:10907730-10907750, and chr16:10895302-10895322.

[00617] Embodiment 38 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10907539-10907559, chr16:10916426-10916446, chr16:10906907-10906927, chr16:10895702-10895722, chr16:10907757-10907777, chr16:10907623-10907643, chr16:10915626-10915646, chr16:10906756-10906776, chr16:10907476-10907496, chr16:10907385-10907405, and chr16:10923265-10923285.

[00618] Embodiment 39 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10907315-10907335, chr16:10916432-10916452, chr16:10907932-10907952, chr16:10915626-10915646, chr16:10907586-10907606, chr16:10916426-10916446, chr16:10907476-10907496, chr16:10907787-10907807, chr16:10907979-10907999, chr16:10906904-10906924, and chr16:10909138-10909158.

[00619] Embodiment 40 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10895702-10895722, chr16:10916432-

10916452, chr16:10907623-10907643, chr16:10907932-10907952, chr16:10906985-10907005, chr16:10915626-10915646, chr16:10907539-10907559, chr16:10916426-10916446, chr16:10907476-10907496, chr16:10907119-10907139, chr16:10907979-10907999, and chr16:10909138-10909158.

[00620] Embodiment 41 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10906757-10906777, chr16:10895302-10895322, chr16:10907539-10907559, chr16:10907730-10907750, and chr16:10895702-10895722.

[00621] Embodiment 42 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10906853-10906873, chr16:10922444-10922464, and chr16:10916432-10916452.

[00622] Embodiment 43 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16: 10916426-10916446.

[00623] Embodiment 44 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16: 10906907-10906927.

[00624] Embodiment 45 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16: 10907757-10907777.

[00625] Embodiment 46 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16:10907623-10907643.

[00626] Embodiment 47 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that

binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16: 10915626-10915646.

[00627] Embodiment 48 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16: 10906756-10906776.

[00628] Embodiment 49 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16:10907385-10907405.

[00629] Embodiment 50 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16: 10923265-10923285.

[00630] Embodiment 51 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16:10906853-10906873.

[00631] Embodiment 52 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinateschr16:10922444-10922464.

[00632] Embodiment 53 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16:10916432-10916452.

[00633] Embodiment 54 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16:10906757-10906777.

[00634] Embodiment 55 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16:10895302-10895322.

[00635] Embodiment 56 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16:10907539-10907559.

[00636] Embodiment 57 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16:10907730-10907750.

[00637] Embodiment 58 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16:10895702-10895722.

[00638] Embodiment 59 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16:10907932-10907952.

[00639] Embodiment 60 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16:10907476-10907496.

[00640] Embodiment 61 is the engineered cell of any one of the preceding embodiments, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chr16:10909138-10909158.

[00641] Embodiment 62 is the engineered cell of any one of embodiments 34-61, wherein the CIITA genomic target sequence comprises at least 10 contiguous nucleotides within the genomic coordinates.

[00642] Embodiment 63 is the engineered cell of any one of embodiments 34-62, wherein the CIITA genomic target sequence comprises at least 15 contiguous nucleotides within the genomic coordinates.

[00643] Embodiment 64 is the engineered cell of any one of embodiments 34-63, wherein the gene editing system comprises an RNA-guided DNA-binding agent.

[00644] Embodiment 65 is the engineered cell of embodiment 64, wherein the RNA-guided DNA-binding agent comprises a Cas9 protein, such as an *S. pyogenes* Cas9.

[00645] Embodiment 66 is the engineered cell of any one of the preceding embodiments, wherein the engineered cell further has reduced or eliminated surface expression of MHC class I.

[00646] Embodiment 67 is the engineered cell of embodiment 66, wherein the engineered cell comprises a genetic modification in the beta-2-microglobulin (B2M) gene.

[00647] Embodiment 68 is the engineered cell of embodiment 66, wherein the engineered cell comprises a genetic modification in an HLA-A gene.

[00648] Embodiment 69 is the engineered cell of any one of the preceding embodiments, wherein the engineered cell further comprises an exogenous nucleic acid.

[00649] Embodiment 70 is the engineered cell of any one of the preceding embodiments, wherein the engineered cell comprises an exogenous nucleic acid encoding a targeting receptor that is expressed on the surface of the engineered cell.

[00650] Embodiment 71 is the engineered cell of embodiment 70, wherein the targeting receptor is a CAR.

[00651] Embodiment 72 is the engineered cell of embodiment 70, wherein the targeting receptor is a TCR.

[00652] Embodiment 73 is the engineered cell of embodiment 70, wherein the targeting receptor is a WT1 TCR.

[00653] Embodiment 74 is the engineered cell of any one of the preceding embodiments, wherein the engineered cell further comprises an exogenous nucleic acid encoding a polypeptide that is secreted by the engineered cell.

[00654] Embodiment 75 is the engineered cell of any one of the preceding embodiments, wherein the engineered cell is an immune cell.

[00655] Embodiment 76 is the engineered cell of any one of the preceding embodiments, wherein the engineered cell is a monocyte, macrophage, mast cell, dendritic cell, or granulocyte.

[00656] Embodiment 77 is the engineered cell of any one of the preceding embodiments, wherein the engineered cell is a lymphocyte.

[00657] Embodiment 78 is the engineered cell of embodiment 77, wherein the engineered cell is a T cell.

[00658] Embodiment 79 is the engineered cell of embodiment 78, wherein the engineered cell further has reduced or eliminated expression of an endogenous T-cell receptor (TCR) protein relative to an unmodified cell.

[00659] Embodiment 80 is the engineered cell of any one of embodiments 78-79, wherein the cell has reduced or eliminated expression of a TRAC protein relative to an unmodified cell.

[00660] Embodiment 81 is the engineered cell of any one of embodiments 78-80, wherein the cell has reduced expression of a TRBC protein relative to an unmodified cell.

[00661] Embodiment 82 is a pharmaceutical composition comprising the engineered cell of any one of the preceding embodiments.

[00662] Embodiment 83 is a population of cells comprising the engineered cell of any one of the preceding embodiments.

[00663] Embodiment 84 is a pharmaceutical composition comprising a population of cells, wherein the population of cells comprises engineered cell of any one of the preceding embodiments.

[00664] Embodiment 85 is the population of cells of embodiment 83 or pharmaceutical composition of embodiment 84, wherein the population of cells is at least 65% MHC class II negative as measured by flow cytometry.

[00665] Embodiment 86 is the population of cells of embodiment 83 or pharmaceutical composition of embodiment 84, wherein the population of cells is at least 70% MHC class II negative as measured by flow cytometry.

[00666] Embodiment 87 is the population of cells of embodiment 83 or pharmaceutical composition of embodiment 84, wherein the population of cells is at least 80% MHC class II negative as measured by flow cytometry.

[00667] Embodiment 88 is the population of cells of embodiment 83 or pharmaceutical composition of embodiment 84, wherein the population of cells is at least 90% MHC class II negative as measured by flow cytometry.

[00668] Embodiment 89 is the population of cells of embodiment 83 or pharmaceutical composition of embodiment 84, wherein the population of cells is at least 92% MHC class II negative as measured by flow cytometry.

[00669] Embodiment 90 is the population of cells of embodiment 83 or pharmaceutical composition of embodiment 84, wherein the population of cells is at least 93% MHC class II negative as measured by flow cytometry.

[00670] Embodiment 91 is the population of cells of embodiment 83 or pharmaceutical composition of embodiment 84, wherein the population of cells is at least 94% MHC class II negative as measured by flow cytometry.

[00671] Embodiment 92 is the population of cells of embodiment 83 or pharmaceutical composition of embodiment 84, wherein the population of cells is at least 95% MHC class II negative as measured by flow cytometry.

[00672] Embodiment 93 is the population of cells of embodiment 83 or pharmaceutical composition of embodiment 84, wherein the population of cells is at least 96% MHC class II negative as measured by flow cytometry.

[00673] Embodiment 94 is the population of cells of embodiment 83 or pharmaceutical composition of embodiment 84, wherein the population of cells is at least 97% MHC class II negative as measured by flow cytometry.

[00674] Embodiment 95 is the population of cells of embodiment 83 or pharmaceutical composition of embodiment 84, wherein the population of cells is at least 98% MHC class II negative as measured by flow cytometry.

[00675] Embodiment 96 is the population of cells of embodiment 83 or pharmaceutical composition of embodiment 84, wherein the population of cells is at least 99% MHC class II negative as measured by flow cytometry.

[00676] Embodiment 97 is the population of cells or pharmaceutical composition of any one of embodiments 83-96, wherein the population of cells is at least 95% endogenous TCR protein negative as measured by flow cytometry.

[00677] Embodiment 98 is the population of cells or pharmaceutical composition of any one of embodiments 83-96, wherein the population of cells is at least 97% endogenous TCR protein negative as measured by flow cytometry.

[00678] Embodiment 99 is the population of cells or pharmaceutical composition of any one of embodiments 83-96, wherein the population of cells is at least 98% endogenous TCR protein negative as measured by flow cytometry.

[00679] Embodiment 100 is the population of cells or pharmaceutical composition of any one of embodiments 83-96, wherein the population of cells is at least 99% endogenous TCR protein negative as measured by flow cytometry.

[00680] Embodiment 101 is a method of administering the engineered cell, population of cells, or pharmaceutical composition of any one of the preceding embodiments to a subject in need thereof.

[00681] Embodiment 102 is a method of administering the engineered cell, population of cells, or pharmaceutical composition of any one of the preceding embodiments to a subject as an adoptive cell transfer (ACT) therapy.

[00682] Embodiment 103 is a method of making an engineered cell, which has reduced or eliminated surface expression of MHC class II protein relative to an unmodified cell, comprising contacting a cell with a composition comprising: (a) a CIITA guide RNA comprising (i) a guide sequence selected from SEQ ID NOs: 1-117; (ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-117; (iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-117; (iv) a sequence that comprises 10 contiguous nucleotides ± 10 nucleotides of a genomic coordinate listed in Table 2; (v) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (iv); or (vi) a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (v); and (b) optionally an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[00683] Embodiment 104 is a method of reducing or eliminating surface expression of MHC class II protein in an engineered cell relative to an unmodified cell, comprising contacting a cell with a composition comprising: (a) a CIITA guide RNA comprising (i) a guide sequence selected from SEQ ID NOs: 1-117; (ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-117; (iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-117; (iv) a sequence that comprises 10 contiguous nucleotides ± 10 nucleotides of a genomic coordinate listed in Table 2; (v) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (iv); or (vi) a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (v); and (b) optionally an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

[00684] Embodiment 105 is the method of embodiment 103 or 104, wherein the CIITA guide RNA comprises (i) a guide sequence selected from SEQ ID NOs: 32, 64, 67, 68, 74, 76, 84, 86, 90, 91, and 115; (ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NO: 32, 64, 67, 68, 74, 76, 84, 86, 90, 91, and 115; or (iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NO: 32, 64, 67, 68, 74, 76, 84, 86, 90, 91, and 115.

[00685] Embodiment 106 is the method of any one of embodiments 103-105, further comprising reducing or eliminating the surface expression of MHC class I protein in the cell relative to an unmodified cell.

[00686] Embodiment 107 is the method of any one of embodiments 103-106, further comprising reducing or eliminating the surface expression of B2M protein in the cell relative to an unmodified cell.

[00687] Embodiment 108 is the method of any one of embodiments 103-107, further comprising reducing or eliminating the surface expression of HLA-A protein in the cell relative to an unmodified cell.

[00688] Embodiment 109 is the method of any one of embodiments 103-108, further comprising reducing or eliminating the surface expression of a TCR protein in the cell relative to an unmodified cell.

[00689] Embodiment 110 is the method of any one of embodiments 103-109, further comprising contacting the cell with an exogenous nucleic acid.

[00690] Embodiment 111 is the method of any one of embodiments 103-110, further comprising contacting the cell with a DNA-dependent protein kinase inhibitor (DNAPKi).

[00691] Embodiment 112 is the method of embodiment 111, wherein the DNAPKi is Compound 1.

[00692] Embodiment 113 is the method of embodiment 110, further comprising contacting the cell with an exogenous nucleic acid encoding a targeting receptor.

[00693] Embodiment 114 is the method of embodiment 110, further comprising contacting the cell with an exogenous nucleic acid encoding a polypeptide that is secreted by the cell.

[00694] Embodiment 115 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the cell is an allogeneic cell.

[00695] Embodiment 116 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the cell is a human cell.

[00696] Embodiment 117 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the cell is a primary cell.

[00697] Embodiment 118 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the cell is a CD4⁺ T cell.

[00698] Embodiment 119 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the cell is a CD8⁺ T cell.

[00699] Embodiment 120 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the cell is a memory T cell.

[00700] Embodiment 121 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the cell is a B cell.

[00701] Embodiment 122 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the cell is a plasma B cell.

[00702] Embodiment 123 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the cell is memory B cell.

[00703] Embodiment 124 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the cell is a hematopoietic stem cell (HSC).

[00704] Embodiment 125 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the cell is an activated cell.

[00705] Embodiment 126 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the cell is a non-activated cell.

[00706] Embodiment 127 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, comprising an exogenous nucleic acid or contacting the cell with an exogenous nucleic acid, wherein the exogenous nucleic acid encodes an NK cell inhibitor molecule.

[00707] Embodiment 128 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, comprising an exogenous nucleic acid or contacting the cell with an exogenous nucleic acid, wherein the exogenous nucleic acid encodes an NK cell inhibitor molecule, wherein the NK cell inhibitor molecule binds to an inhibitory receptor on an NK cell.

[00708] Embodiment 129 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, comprising an exogenous nucleic acid or contacting the cell with an exogenous nucleic acid, wherein the exogenous nucleic acid encodes an NK cell inhibitor molecule, wherein the NK cell inhibitor molecule binds to NKG2A on an NK cell.

[00709] Embodiment 130 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, comprising an exogenous

nucleic acid or contacting the cell with an exogenous nucleic acid, wherein the exogenous nucleic acid encodes an NK cell inhibitor molecule, wherein the NK cell inhibitor molecule is a non-classical MHC class I molecule.

[00710] Embodiment 131 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, comprising an exogenous nucleic acid or contacting the cell with an exogenous nucleic acid, wherein the exogenous nucleic acid encodes an NK cell inhibitor molecule, wherein the NK cell inhibitor molecule is HLA-E.

[00711] Embodiment 132 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, comprising an exogenous nucleic acid or contacting the cell with an exogenous nucleic acid, wherein the exogenous nucleic acid encodes an NK cell inhibitor molecule, wherein the NK cell inhibitor molecule is a fusion protein.

[00712] Embodiment 133 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, comprising an exogenous nucleic acid or contacting the cell with an exogenous nucleic acid, wherein the exogenous nucleic acid encodes an NK cell inhibitor molecule, wherein the NK cell inhibitor molecule is a fusion protein comprising HLA-E and B2M.

[00713] Embodiment 134 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, comprising an exogenous nucleic acid encoding a polypeptide that is secreted by the cell or contacting the cell with said exogenous nucleic acid, wherein the secreted polypeptide is an antibody or antibody fragment.

[00714] Embodiment 135 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, comprising an exogenous nucleic acid encoding a polypeptide that is secreted by the cell or contacting the cell with said exogenous nucleic acid, wherein the secreted polypeptide is a full-length IgG antibody.

[00715] Embodiment 136 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, comprising an exogenous nucleic acid encoding a polypeptide that is secreted by the cell or contacting the cell with said exogenous nucleic acid, wherein the secreted polypeptide is a single chain antibody.

[00716] Embodiment 137 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, comprising an exogenous

nucleic acid encoding a polypeptide that is secreted by the cell or contacting the cell with said exogenous nucleic acid, wherein the secreted polypeptide is a neutralizing antibody.

[00717] Embodiment 138 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, comprising an exogenous nucleic acid encoding a polypeptide that is secreted by the cell or contacting the cell with said exogenous nucleic acid, wherein the secreted polypeptide is an enzyme.

[00718] Embodiment 139 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, comprising an exogenous nucleic acid encoding a polypeptide that is secreted by the cell or contacting the cell with said exogenous nucleic acid, wherein the secreted polypeptide is a cytokine.

[00719] Embodiment 140 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, comprising an exogenous nucleic acid encoding a polypeptide that is secreted by the cell or contacting the cell with said exogenous nucleic acid, wherein the secreted polypeptide is a fusion protein.

[00720] Embodiment 141 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, comprising an exogenous nucleic acid encoding a polypeptide that is secreted by the cell or contacting the cell with said exogenous nucleic acid, wherein the secreted polypeptide comprises a soluble receptor. The engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, comprising an exogenous nucleic acid encoding a targeting receptor or contacting the cell with an exogenous nucleic acid encoding a targeting receptor, wherein the targeting receptor is a T cell receptor (TCR).

[00721] Embodiment 142 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, comprising an exogenous nucleic acid encoding a targeting receptor or contacting the cell with an exogenous nucleic acid encoding a targeting receptor, wherein the targeting receptor is a genetically modified TCR.

[00722] Embodiment 143 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, comprising an exogenous nucleic acid encoding a targeting receptor or contacting the cell with an exogenous nucleic acid encoding a targeting receptor, wherein the targeting receptor is the WT1 TCR.

[00723] Embodiment 144 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, comprising an exogenous

nucleic acid encoding a targeting receptor or contacting the cell with an exogenous nucleic acid encoding a targeting receptor, wherein the targeting receptor is a CAR.

[00724] Embodiment 145 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA is provided to the cell in a vector.

[00725] Embodiment 146 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA RNA-guided DNA binding agent is provided to the cell in a vector, optionally in the same vector as the CIITA guide RNA.

[00726] Embodiment 147 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the exogenous nucleic acid is provided to the cell in a vector.

[00727] Embodiment 148 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the vector is a viral vector.

[00728] Embodiment 149 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the vector is a lentiviral vector.

[00729] Embodiment 150 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the vector is an AAV.

[00730] Embodiment 151 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the vector is a non-viral vector.

[00731] Embodiment 152 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein a gene editing system component is provided to the cell in a lipid nucleic acid assembly composition.

[00732] Embodiment 153 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the guide RNA is provided to the cell in a lipid nucleic acid assembly composition, optionally in the same lipid nucleic acid assembly composition as an RNA-guided DNA binding agent.

[00733] Embodiment 154 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the exogenous nucleic acid is provided to the cell in a lipid nucleic acid assembly composition.

[00734] Embodiment 155 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the lipid nucleic acid assembly composition is a lipid nanoparticle (LNP).

[00735] Embodiment 156 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the exogenous nucleic acid is integrated into the genome of the cell.

[00736] Embodiment 157 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the exogenous nucleic acid is integrated into the genome of the cell by homologous recombination (HR).

[00737] Embodiment 158 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the exogenous nucleic acid is integrated into a safe harbor locus in the genome of the cell.

[00738] Embodiment 159 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 1.

[00739] Embodiment 160 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 2.

[00740] Embodiment 161 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 3.

[00741] Embodiment 162 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 4.

[00742] Embodiment 163 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 5.

[00743] Embodiment 164 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 6.

[00744] Embodiment 165 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 7.

[00745] Embodiment 166 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 8.

[00746] Embodiment 167 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 9.

[00747] Embodiment 168 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 10.

[00748] Embodiment 169 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 11.

[00749] Embodiment 170 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 12.

[00750] Embodiment 171 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 13.

[00751] Embodiment 172 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 14.

[00752] Embodiment 173 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 15.

[00753] Embodiment 174 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 16.

[00754] Embodiment 175 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 17.

[00755] Embodiment 176 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 18.

[00756] Embodiment 177 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 19.

[00757] Embodiment 178 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 20.

[00758] Embodiment 179 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 21.

[00759] Embodiment 180 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 22.

[00760] Embodiment 181 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 23.

[00761] Embodiment 182 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 24.

[00762] Embodiment 183 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 25.

[00763] Embodiment 184 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 26.

[00764] Embodiment 185 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 27.

[00765] Embodiment 186 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 28.

[00766] Embodiment 187 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 29.

[00767] Embodiment 188 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 30.

[00768] Embodiment 189 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 31.

[00769] Embodiment 190 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 32.

[00770] Embodiment 191 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 33.

[00771] Embodiment 192 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 34.

[00772] Embodiment 193 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 35.

[00773] Embodiment 194 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 36.

[00774] Embodiment 195 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 37.

[00775] Embodiment 196 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 38.

[00776] Embodiment 197 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 39.

[00777] Embodiment 198 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 40.

[00778] Embodiment 199 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 41.

[00779] Embodiment 200 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 42.

[00780] Embodiment 201 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 43.

[00781] Embodiment 202 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 44.

[00782] Embodiment 203 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 45.

[00783] Embodiment 204 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 46.

[00784] Embodiment 205 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 47.

[00785] Embodiment 206 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 48.

[00786] Embodiment 207 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 49.

[00787] Embodiment 208 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 50.

[00788] Embodiment 209 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 51.

[00789] Embodiment 210 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 52.

[00790] Embodiment 211 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 53.

[00791] Embodiment 212 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 54.

[00792] Embodiment 213 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 55.

[00793] Embodiment 214 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 56.

[00794] Embodiment 215 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 57.

[00795] Embodiment 216 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 58.

[00796] Embodiment 217 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 59.

[00797] Embodiment 218 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 60.

[00798] Embodiment 219 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 61.

[00799] Embodiment 220 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 62.

[00800] Embodiment 221 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 63.

[00801] Embodiment 222 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 64.

[00802] Embodiment 223 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 65.

[00803] Embodiment 224 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 66.

[00804] Embodiment 225 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 67.

[00805] Embodiment 226 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 68.

[00806] Embodiment 227 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 69.

[00807] Embodiment 228 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 70.

[00808] Embodiment 229 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 71.

[00809] Embodiment 230 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 72.

[00810] Embodiment 231 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 73.

[00811] Embodiment 232 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 74.

[00812] Embodiment 233 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 75.

[00813] Embodiment 234 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 76.

[00814] Embodiment 235 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 77.

[00815] Embodiment 236 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 78.

[00816] Embodiment 237 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 79.

[00817] Embodiment 238 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 80.

[00818] Embodiment 239 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 81.

[00819] Embodiment 240 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 82.

[00820] Embodiment 241 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 83.

[00821] Embodiment 242 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 84.

[00822] Embodiment 243 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 85.

[00823] Embodiment 244 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 86.

[00824] Embodiment 245 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 87.

[00825] Embodiment 246 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 88.

[00826] Embodiment 247 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 89.

[00827] Embodiment 248 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 90.

[00828] Embodiment 249 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 91.

[00829] Embodiment 250 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 92.

[00830] Embodiment 251 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 93.

[00831] Embodiment 252 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 94.

[00832] Embodiment 253 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 95.

[00833] Embodiment 254 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 96.

[00834] Embodiment 255 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 97.

[00835] Embodiment 256 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 98.

[00836] Embodiment 257 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 99.

[00837] Embodiment 258 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 100.

[00838] Embodiment 259 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 101.

[00839] Embodiment 260 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 102.

[00840] Embodiment 261 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 103.

[00841] Embodiment 262 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 104.

[00842] Embodiment 263 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 105.

[00843] Embodiment 264 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 106.

[00844] Embodiment 265 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 107.

[00845] Embodiment 266 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 108.

[00846] Embodiment 267 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 109.

[00847] Embodiment 268 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 110.

[00848] Embodiment 269 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 111.

[00849] Embodiment 270 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 112.

[00850] Embodiment 271 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 113.

[00851] Embodiment 272 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 114.

[00852] Embodiment 273 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 115.

[00853] Embodiment 274 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 116.

[00854] Embodiment 275 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises SEQ ID NO: 117.

[00855] Embodiment 276 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises at least one modification.

[00856] Embodiment 277 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises at least one modification, wherein the at least one modification includes a 2'-O-methyl (2'-O-Me) modified nucleotide.

[00857] Embodiment 278 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises at least one modification, comprising a phosphorothioate (PS) bond between nucleotides.

[00858] Embodiment 279 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises at least one modification, comprising a 2'-fluoro (2'-F) modified nucleotide.

[00859] Embodiment 280 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises at least one modification, comprising a modification at one or more of the first five nucleotides at the 5' end of the guide RNA.

[00860] Embodiment 281 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises at least one modification, comprising a modification at one or more of the last five nucleotides at the 3' end of the guide RNA.

[00861] Embodiment 282 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises at least one modification, comprising a PS bond between the first four nucleotides of the guide RNA.

[00862] Embodiment 283 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises at least one modification, comprising a PS bond between the last four nucleotides of the guide RNA.

[00863] Embodiment 284 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises at least one modification, comprising a 2'-O-Me modified nucleotide at the first three nucleotides at the 5' end of the guide RNA.

[00864] Embodiment 285 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the CIITA guide RNA comprises at least one modification, comprising a 2'-O-Me modified nucleotide at the last three nucleotides at the 3' end of the guide RNA.

[00865] Embodiment 286 is an engineered cell or population of cells comprising a genetic modification that includes an indel within the genomic region targeted by the CIITA guide RNA of any of the preceding embodiments.

[00866] Embodiment 287 is an engineered cell or population of cells comprising a genetic modification that includes a C to T substitution or an A to G substitution within the genomic region targeted by the CIITA guide RNA of any of the preceding embodiments.

[00867] Embodiment 288 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, for use to express a TCR with specificity for a polypeptide expressed by cancer cells.

[00868] Embodiment 289 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, for use in administering to a subject as an adoptive cell transfer (ACT) therapy.

[00869] Embodiment 290 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, for use in treating a subject with cancer.

[00870] Embodiment 291 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, for use in treating a subject with an infectious disease.

[00871] Embodiment 292 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, for use in treating a subject with an autoimmune disease.

[00872] Embodiment 293 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the genetic modification comprises an indel.

[00873] Embodiment 294 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the genetic modification comprises a C to T substitution.

[00874] Embodiment 295 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the genetic modification comprises an A to G substitution.

[00875] Embodiment 296 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the cell is homozygous for HLA-B and homozygous for HLA-C.

[00876] Embodiment 297 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the cell further comprises a genetic modification in an HLA-A gene, wherein the cell is homozygous for HLA-B and homozygous for HLA-C, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: (a) chr6:29942854 to chr6:29942913 and (b) chr6:29943518 to chr6: 29943619.

[00877] Embodiment 298 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the cell further comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29942864 to chr6: 29942903.

[00878] Embodiment 299 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the cell further comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29943528 to chr6:29943609.

[00879] Embodiment 300 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the cell further comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; and chr6:29944026-29944046.

[00880] Embodiment 301 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the cell further comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903;

chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549;
chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569;
chr6:29943589-29943609; and chr6:29944026-29944046.

[00881] Embodiment 302 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the HLA-A expression of the cell is reduced or eliminated by a gene editing system that binds to an HLA-A genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr6:29942864-29942884; chr6:29942868-29942888; chr6:29942876-29942896; chr6:29942877-29942897; chr6:29942883-29942903; chr6:29943126-29943146; chr6:29943528-29943548; chr6:29943529-29943549; chr6:29943530-29943550; chr6:29943537-29943557; chr6:29943549-29943569; chr6:29943589-29943609; and chr6:29944026-29944046.

[00882] Embodiment 303 is a method of making an engineered cell, which has reduced or eliminated surface expression of MHC class II protein and HLA-A protein relative to an unmodified cell, comprising: (a) contacting the cell with a CIITA guide RNA, wherein the guide RNA comprises a guide sequence selected from SEQ ID NOs: 1-117; (b) contacting the cell with an HLA-A guide RNA, wherein the HLA-A guide RNA comprises a guide sequence selected from any one of SEQ ID NOs: 2001-2095; and (c) optionally contacting the cell with an RNA-guided DNA binding agent or nucleic acid encoding an RNA-guided DNA binding agent; thereby reducing or eliminating the surface expression of MHC class II protein and HLA-A protein in the cell relative to an unmodified cell.

[00883] Embodiment 304 is the method of embodiment 303, wherein the CIITA guide RNA comprises a sequence selected from SEQ ID NO: 32, 64, 67, 68, 74, 76, 84, 86, 90, 91, and 115.

[00884] Embodiment 305 is the method of embodiment 303 or 304, comprising contacting the cell with an RNA-guided DNA binding agent or nucleic acid encoding an RNA-guided DNA binding agent, optionally wherein the RNA-guided DNA binding agent comprises an *S. pyogenes* Cas9.

[00885] Embodiment 306 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the HLA-B is selected from any one of the following HLA-B alleles: HLA-B*07:02; HLA-B*08:01; HLA-B*44:02; HLA-B*35:01; HLA-B*40:01; HLA-B*57:01; HLA-B*14:02; HLA-B*15:01; HLA-B*13:02; HLA-B*44:03; HLA-B*38:01; HLA-B*18:01; HLA-B*44:03; HLA-B*51:01; HLA-B*49:01; HLA-B*15:01; HLA-B*18:01; HLA-B*27:05; HLA-B*35:03;

HLA-B*18:01; HLA-B*52:01; HLA-B*51:01; HLA-B*37:01; HLA-B*53:01; HLA-B*55:01; HLA-B*44:02; HLA-B*44:03; HLA-B*35:02; HLA-B*15:01; and HLA-B*40:02.

[00886] Embodiment 307 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the HLA-C is selected from any one of the following HLA-C alleles: HLA-C*07:02; HLA-C*07:01; HLA-C*05:01; HLA-C*04:01 HLA-C*03:04; HLA-C*06:02; HLA-C*08:02; HLA-C*03:03; HLA-C*06:02; HLA-C*16:01; HLA-C*12:03; HLA-C*07:01; HLA-C*04:01; HLA-C*15:02; HLA-C*07:01; HLA-C*03:04; HLA-C*12:03; HLA-C*02:02; HLA-C*04:01; HLA-C*05:01; HLA-C*12:02; HLA-C*14:02; HLA-C*06:02; HLA-C*04:01; HLA-C*03:03; HLA-C*07:04; HLA-C*07:01; HLA-C*04:01; HLA-C*04:01; and HLA-C*02:02.

[00887] Embodiment 308 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the HLA-B allele is selected from any one of the following HLA-B alleles: HLA-B*07:02; HLA-B*08:01; HLA-B*44:02; HLA-B*35:01; HLA-B*40:01; HLA-B*57:01; HLA-B*14:02; HLA-B*15:01; HLA-B*13:02; HLA-B*44:03; HLA-B*38:01; HLA-B*18:01; HLA-B*44:03; HLA-B*51:01; HLA-B*49:01; HLA-B*15:01; HLA-B*18:01; HLA-B*27:05; HLA-B*35:03; HLA-B*18:01; HLA-B*52:01; HLA-B*51:01; HLA-B*37:01; HLA-B*53:01; HLA-B*55:01; HLA-B*44:02; HLA-B*44:03; HLA-B*35:02; HLA-B*15:01; and HLA-B*40:02; and the HLA-C allele is selected from any one of the following HLA-C alleles: HLA-C*07:02; HLA-C*07:01; HLA-C*05:01; HLA-C*04:01 HLA-C*03:04; HLA-C*06:02; HLA-C*08:02; HLA-C*03:03; HLA-C*06:02; HLA-C*16:01; HLA-C*12:03; HLA-C*07:01; HLA-C*04:01; HLA-C*15:02; HLA-C*07:01; HLA-C*03:04; HLA-C*12:03; HLA-C*02:02; HLA-C*04:01; HLA-C*05:01; HLA-C*12:02; HLA-C*14:02; HLA-C*06:02; HLA-C*04:01; HLA-C*03:03; HLA-C*07:04; HLA-C*07:01; HLA-C*04:01; HLA-C*04:01; and HLA-C*02:02.

[00888] Embodiment 309 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the HLA-B and HLA-C alleles are selected from any one of the following HLA-B and HLA-C alleles: HLA-B*07:02 and HLA-C*07:02; HLA-B*08:01 and HLA-C*07:01; HLA-B*44:02 and HLA-C*05:01; HLA-B*35:01 and HLA-C*04:01; HLA-B*40:01 and HLA-C*03:04; HLA-B*57:01 and HLA-C*06:02; HLA-B*14:02 and HLA-C*08:02; HLA-B*15:01 and HLA-C*03:03; HLA-B*13:02 and HLA-C*06:02; HLA-B*44:03 and HLA-C*16:01; HLA-B*38:01 and HLA-C*12:03; HLA-B*18:01 and HLA-C*07:01; HLA-B*44:03 and HLA-C*04:01; HLA-B*51:01 and HLA-C*15:02; HLA-B*49:01 and HLA-C*07:01; HLA-

B*15:01 and HLA-C*03:04; HLA-B*18:01 and HLA-C*12:03; HLA-B*27:05 and HLA-C*02:02; HLA-B*35:03 and HLA-C*04:01; HLA-B*18:01 and HLA-C*05:01; HLA-B*52:01 and HLA-C*12:02; HLA-B*51:01 and HLA-C*14:02; HLA-B*37:01 and HLA-C*06:02; HLA-B*53:01 and HLA-C*04:01; HLA-B*55:01 and HLA-C*03:03; HLA-B*44:02 and HLA-C*07:04; HLA-B*44:03 and HLA-C*07:01; HLA-B*35:02 and HLA-C*04:01; HLA-B*15:01 and HLA-C*04:01; and HLA-B*40:02 and HLA-C*02:02.

[00889] Embodiment 310 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the HLA-B and HLA-C alleles are HLA-B*07:02 and HLA-C*07:02.

[00890] Embodiment 311 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the HLA-B and HLA-C alleles are HLA-B*08:01 and HLA-C*07:01.

[00891] Embodiment 312 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the HLA-B and HLA-C alleles are HLA-B*44:02 and HLA-C*05:01.

[00892] Embodiment 313 is the engineered cell, population of cells, pharmaceutical composition, or method of any one of the preceding embodiments, wherein the HLA-B and HLA-C alleles are HLA-B*35:01 and HLA-C*04:01.

What is claimed is:

1. An engineered cell, which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10902662- chr16:10923285.
2. The engineered cell of claim 1, wherein the genetic modification comprises at least 5, 6, 7, 8, 9, or 10 contiguous nucleotides within the genomic coordinates chr16:10902662- chr16:10923285.
3. The engineered cell of claim 1 or 2, wherein the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates chr16:10902662- chr16:10923285.
4. The engineered cell of any one of claims 1-3, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10906542- chr16:10923285.
5. The engineered cell of any one of claims 1-4, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10906542- chr16:10908121.
6. The engineered cell of any one of claims 1-5, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10907539-10907559, chr16:10916426-10916446, chr16:10906907-10906927, chr16:10895702-10895722, chr16:10907757-10907777, chr16:10907623-10907643, chr16:10915626-10915646, chr16:10906756-10906776, chr16:10907476-10907496, chr16:10907385-10907405, and chr16:10923265-10923285.
7. The engineered cell of any one of claims 1-6, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10916432-10916452, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10906985-10907005, chr16:10908073-10908093, chr16:10907433-10907453, chr16:10907979-10907999, chr16:10907139-10907159, chr16:10922435-10922455, chr16:10907384-10907404, chr16:10907434-10907454, chr16:10907119-10907139, chr16:10907539-10907559, chr16:10907810-10907830, chr16:10907315-10907335, chr16:10916426-10916446, chr16:10909138-10909158, chr16:10908101-10908121, chr16:10907790-10907810, chr16:10907787-10907807, chr16:10907454-10907474, chr16:10895702-10895722, chr16:10902729-10902749, chr16:10918492-10918512,

chr16:10907932-10907952, chr16:10907623-10907643, chr16:10907461-10907481,
 chr16:10902723-10902743, chr16:10907622-10907642, chr16:10922441-10922461,
 chr16:10902662-10902682, chr16:10915626-10915646, chr16:10915592-10915612,
 chr16:10907385-10907405, chr16:10907030-10907050, chr16:10907935-10907955,
 chr16:10906853-10906873, chr16:10906757-10906777, chr16:10907730-10907750, and
 chr16:10895302-10895322.

8. An engineered cell, which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: chr16:10902662-10902682, chr16:10902723-10902743, chr16:10902729-10902749, chr16:10903747-10903767, chr16:10903824-10903844, chr16:10903824-10903844, chr16:10903848-10903868, chr16:10904761-10904781, chr16:10904764-10904784, chr16:10904765-10904785, chr16:10904785-10904805, chr16:10906542-10906562, chr16:10906556-10906576, chr16:10906609-10906629, chr16:10906610-10906630, chr16:10906616-10906636, chr16:10906682-10906702, chr16:10906756-10906776, chr16:10906757-10906777, chr16:10906757-10906777, chr16:10906821-10906841, chr16:10906823-10906843, chr16:10906847-10906867, chr16:10906848-10906868, chr16:10906853-10906873, chr16:10906904-10906924, chr16:10906907-10906927, chr16:10906913-10906933, chr16:10906968-10906988, chr16:10906970-10906990, chr16:10906985-10907005, chr16:10907030-10907050, chr16:10907058-10907078, chr16:10907119-10907139, chr16:10907139-10907159, chr16:10907172-10907192, chr16:10907272-10907292, chr16:10907288-10907308, chr16:10907314-10907334, chr16:10907315-10907335, chr16:10907325-10907345, chr16:10907363-10907383, chr16:10907384-10907404, chr16:10907385-10907405, chr16:10907433-10907453, chr16:10907434-10907454, chr16:10907435-10907455, chr16:10907441-10907461, chr16:10907454-10907474, chr16:10907461-10907481, chr16:10907476-10907496, chr16:10907539-10907559, chr16:10907586-10907606, chr16:10907589-10907609, chr16:10907621-10907641, chr16:10907622-10907642, chr16:10907623-10907643, chr16:10907730-10907750, chr16:10907731-10907751, chr16:10907757-10907777, chr16:10907781-10907801, chr16:10907787-10907807, chr16:10907790-10907810, chr16:10907810-10907830, chr16:10907820-10907840, chr16:10907870-10907890, chr16:10907886-10907906, chr16:10907924-10907944, chr16:10907928-10907948, chr16:10907932-10907952, chr16:10907935-10907955, chr16:10907978-10907998, chr16:10907979-10907999, chr16:10908069-

10908089, chr16:10908073-10908093, chr16:10908101-10908121, chr16:10909056-10909076, chr16:10909138-10909158, chr16:10910195-10910215, chr16:10910196-10910216, chr16:10915592-10915612, chr16:10915626-10915646, chr16:10916375-10916395, chr16:10916382-10916402, chr16:10916426-10916446, chr16:10916432-10916452, chr16:10918486-10918506, chr16:10918492-10918512, chr16:10918493-10918513, chr16:10922435-10922455, chr16:10922441-10922461, chr16:10922441-10922461, chr16:10922444-10922464, chr16:10922460-10922480, chr16:10923257-10923277, and chr16:10923265-10923285.

9. The engineered cell of claim 8, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10916432-10916452, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10906985-10907005, chr16:10908073-10908093, chr16:10907433-10907453, chr16:10907979-10907999, chr16:10907139-10907159, chr16:10922435-10922455, chr16:10907384-10907404, chr16:10907434-10907454, chr16:10907119-10907139, chr16:10907539-10907559, chr16:10907810-10907830, chr16:10907315-10907335, chr16:10916426-10916446, chr16:10909138-10909158, chr16:10908101-10908121, chr16:10907790-10907810, chr16:10907787-10907807, chr16:10907454-10907474, chr16:10895702-10895722, chr16:10902729-10902749, chr16:10918492-10918512, chr16:10907932-10907952, chr16:10907623-10907643, chr16:10907461-10907481, chr16:10902723-10902743, chr16:10907622-10907642, chr16:10922441-10922461, chr16:10902662-10902682, chr16:10915626-10915646, chr16:10915592-10915612, chr16:10907385-10907405, chr16:10907030-10907050, chr16:10907935-10907955, chr16:10906853-10906873, chr16:10906757-10906777, chr16:10907730-10907750, chr16:10907586-10907606, chr16:10907476-10907496, chr16:10906904-10906924, and chr16:10895302-10895322.

10. The engineered cell of claim 8 or 9, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10907539-10907559, chr16:10916426-10916446, chr16:10906907-10906927, chr16:10895702-10895722, chr16:10907757-10907777, chr16:10907623-10907643, chr16:10915626-10915646, chr16:10906756-10906776, chr16:10907476-10907496, chr16:10907385-10907405, and chr16:10923265-10923285.

11. The engineered cell of any one of claims 8-10, wherein the genetic modification comprises at least 5, 6, 7, 8, 9, or 10 contiguous nucleotides within the genomic coordinates.

12. The engineered cell of any one of claims 8-11, wherein the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates.

13. The engineered cell of any one of claims 1-12, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from:

chr16:10902662-10902682,	chr16:10902723-10902743,	chr16:10902729-10902749,
chr16:10903747-10903767,	chr16:10903824-10903844,	chr16:10903824-10903844,
chr16:10903848-10903868,	chr16:10904761-10904781,	chr16:10904764-10904784,
chr16:10904765-10904785,	chr16:10904785-10904805,	chr16:10906542-10906562,
chr16:10906556-10906576,	chr16:10906609-10906629,	chr16:10906610-10906630,
chr16:10906616-10906636,	chr16:10906682-10906702,	chr16:10906756-10906776,
chr16:10906757-10906777,	chr16:10906757-10906777,	chr16:10906821-10906841,
chr16:10906823-10906843,	chr16:10906847-10906867,	chr16:10906848-10906868,
chr16:10906853-10906873,	chr16:10906853-10906873,	chr16:10906904-10906924,
chr16:10906907-10906927,	chr16:10906913-10906933,	chr16:10906968-10906988,
chr16:10906970-10906990,	chr16:10906985-10907005,	chr16:10907030-10907050,
chr16:10907058-10907078,	chr16:10907119-10907139,	chr16:10907139-10907159,
chr16:10907172-10907192,	chr16:10907272-10907292,	chr16:10907288-10907308,
chr16:10907314-10907334,	chr16:10907315-10907335,	chr16:10907325-10907345,
chr16:10907363-10907383,	chr16:10907384-10907404,	chr16:10907385-10907405,
chr16:10907433-10907453,	chr16:10907434-10907454,	chr16:10907435-10907455,
chr16:10907441-10907461,	chr16:10907454-10907474,	chr16:10907461-10907481,
chr16:10907476-10907496,	chr16:10907539-10907559,	chr16:10907586-10907606,
chr16:10907589-10907609,	chr16:10907621-10907641,	chr16:10907622-10907642,
chr16:10907623-10907643,	chr16:10907730-10907750,	chr16:10907731-10907751,
chr16:10907757-10907777,	chr16:10907781-10907801,	chr16:10907787-10907807,
chr16:10907790-10907810,	chr16:10907810-10907830,	chr16:10907820-10907840,
chr16:10907870-10907890,	chr16:10907886-10907906,	chr16:10907924-10907944,
chr16:10907928-10907948,	chr16:10907932-10907952,	chr16:10907935-10907955,
chr16:10907978-10907998,	chr16:10907979-10907999,	chr16:10908069-10908089,
chr16:10908073-10908093,	chr16:10908101-10908121,	chr16:10909056-10909076,
chr16:10909138-10909158,	chr16:10910195-10910215,	chr16:10910196-10910216,
chr16:10915592-10915612,	chr16:10915626-10915646,	chr16:10916375-10916395,

chr16:10916382-10916402, chr16:10916426-10916446, chr16:10916432-10916452,
 chr16:10918486-10918506, chr16:10918492-10918512, chr16:10918493-10918513,
 chr16:10922435-10922455, chr16:10922441-10922461, chr16:10922441-10922461,
 chr16:10922444-10922464, chr16:10922460-10922480, chr16:10923257-10923277, and
 chr16:10923265-10923285.

14. The engineered cell of any one of claims 1-13, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from:

chr16:10906542-10906562, chr16:10906556-10906576, chr16:10906609-10906629,
 chr16:10906610-10906630, chr16:10906616-10906636, chr16:10906682-10906702,
 chr16:10906756-10906776, chr16:10906757-10906777, chr16:10906757-10906777,
 chr16:10906821-10906841, chr16:10906823-10906843, chr16:10906847-10906867,
 chr16:10906848-10906868, chr16:10906853-10906873, chr16:10906853-10906873,
 chr16:10906904-10906924, chr16:10906907-10906927, chr16:10906913-10906933,
 chr16:10906968-10906988, chr16:10906970-10906990, chr16:10906985-10907005,
 chr16:10907030-10907050, chr16:10907058-10907078, chr16:10907119-10907139,
 chr16:10907139-10907159, chr16:10907172-10907192, chr16:10907272-10907292,
 chr16:10907288-10907308, chr16:10907314-10907334, chr16:10907315-10907335,
 chr16:10907325-10907345, chr16:10907363-10907383, chr16:10907384-10907404,
 chr16:10907385-10907405, chr16:10907433-10907453, chr16:10907434-10907454,
 chr16:10907435-10907455, chr16:10907441-10907461, chr16:10907454-10907474,
 chr16:10907461-10907481, chr16:10907476-10907496, chr16:10907539-10907559,
 chr16:10907586-10907606, chr16:10907589-10907609, chr16:10907621-10907641,
 chr16:10907622-10907642, chr16:10907623-10907643, chr16:10907730-10907750,
 chr16:10907731-10907751, chr16:10907757-10907777, chr16:10907781-10907801,
 chr16:10907787-10907807, chr16:10907790-10907810, chr16:10907810-10907830,
 chr16:10907820-10907840, chr16:10907870-10907890, chr16:10907886-10907906,
 chr16:10907924-10907944, chr16:10907928-10907948, chr16:10907932-10907952,
 chr16:10907935-10907955, chr16:10907978-10907998, chr16:10907979-10907999,
 chr16:10908069-10908089, chr16:10908073-10908093, chr16:10908101-10908121,
 chr16:10909056-10909076, chr16:10909138-10909158, chr16:10910195-10910215,
 chr16:10910196-10910216, chr16:10915592-10915612, chr16:10915626-10915646,
 chr16:10916375-10916395, chr16:10916382-10916402, chr16:10916426-10916446,
 chr16:10916432-10916452, chr16:10918486-10918506, chr16:10918492-10918512,

chr16:10918493-10918513, chr16:10922435-10922455, chr16:10922441-10922461,
chr16:10922441-10922461, chr16:10922444-10922464, chr16:10922460-10922480,
chr16:10923257-10923277, and chr16:10923265-10923285.

15. The engineered cell of any one of claims 1-14, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from:

chr16:10906542-10906562, chr16:10906556-10906576, chr16:10906609-10906629,
chr16:10906610-10906630, chr16:10906616-10906636, chr16:10906682-10906702,
chr16:10906756-10906776, chr16:10906757-10906777, chr16:10906757-10906777,
chr16:10906821-10906841, chr16:10906823-10906843, chr16:10906847-10906867,
chr16:10906848-10906868, chr16:10906853-10906873, chr16:10906853-10906873,
chr16:10906904-10906924, chr16:10906907-10906927, chr16:10906913-10906933,
chr16:10906968-10906988, chr16:10906970-10906990, chr16:10906985-10907005,
chr16:10907030-10907050, chr16:10907058-10907078, chr16:10907119-10907139,
chr16:10907139-10907159, chr16:10907172-10907192, chr16:10907272-10907292,
chr16:10907288-10907308, chr16:10907314-10907334, chr16:10907315-10907335,
chr16:10907325-10907345, chr16:10907363-10907383, chr16:10907384-10907404,
chr16:10907385-10907405, chr16:10907433-10907453, chr16:10907434-10907454,
chr16:10907435-10907455, chr16:10907441-10907461, chr16:10907454-10907474,
chr16:10907461-10907481, chr16:10907476-10907496, chr16:10907539-10907559,
chr16:10907586-10907606, chr16:10907589-10907609, chr16:10907621-10907641,
chr16:10907622-10907642, chr16:10907623-10907643, chr16:10907730-10907750,
chr16:10907731-10907751, chr16:10907757-10907777, chr16:10907781-10907801,
chr16:10907787-10907807, chr16:10907790-10907810, chr16:10907810-10907830,
chr16:10907820-10907840, chr16:10907870-10907890, chr16:10907886-10907906,
chr16:10907924-10907944, chr16:10907928-10907948, chr16:10907932-10907952,
chr16:10907935-10907955, chr16:10907978-10907998, chr16:10907979-10907999,
chr16:10908069-10908089, chr16:10908073-10908093, and chr16:10908101-10908121.

16. The engineered cell of any one of claims 1-15, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from:

chr16:10916432-10916452, chr16:10922444-10922464, chr16:10907924-10907944,
chr16:10906985-10907005, chr16:10908073-10908093, chr16:10907433-10907453,
chr16:10907979-10907999, chr16:10907139-10907159, chr16:10922435-10922455,

chr16:10907384-10907404, chr16:10907434-10907454, chr16:10907119-10907139,
 chr16:10907539-10907559, chr16:10907810-10907830, chr16:10907315-10907335,
 chr16:10916426-10916446, chr16:10909138-10909158, chr16:10908101-10908121,
 chr16:10907790-10907810, chr16:10907787-10907807, chr16:10907454-10907474,
 chr16:10895702-10895722, chr16:10902729-10902749, chr16:10918492-10918512,
 chr16:10907932-10907952, chr16:10907623-10907643, chr16:10907461-10907481,
 chr16:10902723-10902743, chr16:10907622-10907642, chr16:10922441-10922461,
 chr16:10902662-10902682, chr16:10915626-10915646, chr16:10915592-10915612,
 chr16:10907385-10907405, chr16:10907030-10907050, chr16:10907935-10907955,
 chr16:10906853-10906873, chr16:10906757-10906777, chr16:10907730-10907750, and
 chr16:10895302-10895322.

17. The engineered cell of any one of claims 1-16, wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from:
 chr16:10907539-10907559, chr16:10916426-10916446, chr16:10906907-10906927,
 chr16:10895702-10895722, chr16:10907757-10907777, chr16:10907623-10907643,
 chr16:10915626-10915646, chr16:10906756-10906776, chr16:10907476-10907496,
 chr16:10907385-10907405, and chr16:10923265-10923285.

18. The engineered cell of any one of claims 13-17, wherein the CIITA genomic target sequence comprises at least 10 or at least 15 contiguous nucleotides within the genomic coordinates.

19. The engineered cell of any one of claims 13-18, wherein the gene editing system comprises an RNA-guided DNA-binding agent, optionally wherein the RNA-guided DNA-binding agent comprises a Cas9 protein, such as an *S. pyogenes* Cas9.

20. The engineered cell of any one of claims 1-19, wherein the engineered cell further has reduced or eliminated surface expression of MHC class I.

21. The engineered cell of claim 20, wherein the engineered cell comprises a genetic modification in the beta-2-microglobulin (B2M) gene.

22. The engineered cell of claim 20, wherein the engineered cell comprises a genetic modification in an HLA-A gene.

23. The engineered cell of any one of claims 1-22, wherein the engineered cell comprises an exogenous nucleic acid encoding a targeting receptor that is expressed on the surface of the engineered cell.

24. The engineered cell of claim 23, wherein the targeting receptor is a CAR, a T-cell receptor (TCR), or a WT1 TCR.
25. The engineered cell of any one of claims 1-24, wherein the engineered cell further comprises an exogenous nucleic acid encoding a polypeptide that is secreted by the engineered cell.
26. The engineered cell of any one of claims 1-25, wherein the engineered cell is a T cell and further has reduced or eliminated expression of an endogenous T-cell receptor (TCR) protein relative to an unmodified cell.
27. The engineered cell of claim 26, wherein the cell has reduced or eliminated expression of a TRAC protein or a TRBC protein relative to an unmodified cell.
28. A pharmaceutical composition comprising the engineered cell of any one of claims 1-27.
29. A population of cells comprising the engineered cell of any one of claims 1-27.
30. A pharmaceutical composition comprising a population of cells, wherein the population of cells comprises the engineered cell of any one of claims 1-27.
31. The population of cells of claim 29 or pharmaceutical composition of claim 30, wherein the population of cells is at least 65%, at least 70%, at least 80%, at least 90%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% MHC class II negative as measured by flow cytometry.
32. The population of cells or pharmaceutical composition of any one of claims 29-31, wherein the population of cells is at least 95%, at least 97%, at least 98%, or at least 99% endogenous TCR protein negative as measured by flow cytometry.
33. A method of administering the engineered cell, population of cells, or pharmaceutical composition of any one of claims 1-32 to a subject in need thereof.
34. A method of administering the engineered cell, population of cells, or pharmaceutical composition of any one of claims 1-33 to a subject as an adoptive cell transfer (ACT) therapy.
35. A method of making an engineered cell, which has reduced or eliminated surface expression of MHC class II protein relative to an unmodified cell, comprising contacting a cell with a composition comprising:
- a. a CIITA guide RNA comprising
 - i) a guide sequence selected from SEQ ID NOs: 1-117;
 - ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-117;

- iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-117;
 - iv) a sequence that comprises 10 contiguous nucleotides ± 10 nucleotides of a genomic coordinate listed in Table 2;
 - v) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (iv); or
 - vi) a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (v); and
 - b. optionally an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.
36. A method of reducing or eliminating surface expression of MHC class II protein in an engineered cell relative to an unmodified cell, comprising contacting a cell with a composition comprising:
- a. a CIITA guide RNA comprising
 - i) a guide sequence selected from SEQ ID NOs: 1-117;
 - ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-117;
 - iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-117;
 - iv) a sequence that comprises 10 contiguous nucleotides ± 10 nucleotides of a genomic coordinate listed in Table 2;
 - v) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (iv); or
 - vi) a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (v); and
 - b. optionally an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.
37. The method of claim 35 or 36, wherein the CIITA guide RNA comprises
- i) a guide sequence selected from SEQ ID NOs: 32, 64, 67, 68, 74, 76, 84, 86, 90, 91, and 115;
 - ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NO: 32, 64, 67, 68, 74, 76, 84, 86, 90, 91, and 115; or
 - iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NO: 32, 64, 67, 68, 74, 76, 84, 86, 90, 91, and 115.

38. The method of any one of claims 35-37, further comprising reducing or eliminating the surface expression of MHC class I protein in the cell relative to an unmodified cell.
39. The method of any one of claims 35-38, further comprising reducing or eliminating the surface expression of B2M protein in the cell relative to an unmodified cell.
40. The method of any one of claims 35-39, further comprising reducing or eliminating the surface expression of HLA-A protein in the cell relative to an unmodified cell.
41. The method of any one of claims 35-40, further comprising reducing or eliminating the surface expression of a TCR protein in the cell relative to an unmodified cell.
42. The method of any one of claims 35-41, further comprising contacting the cell with an exogenous nucleic acid.
43. The method of any one of claims 35-42, further comprising contacting the cell with a DNA-dependent protein kinase inhibitor (DNAPKi).
44. The method of claim 43, wherein the DNAPKi is Compound 1.
45. The method of claim 42, further comprising contacting the cell with an exogenous nucleic acid encoding a targeting receptor or a polypeptide that is secreted by the cell.
46. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-45, comprising an exogenous nucleic acid or contacting the cell with an exogenous nucleic acid, wherein the exogenous nucleic acid encodes an NK cell inhibitor molecule.
47. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-46, comprising an exogenous nucleic acid or contacting the cell with an exogenous nucleic acid, wherein the exogenous nucleic acid encodes an NK cell inhibitor molecule, wherein the NK cell inhibitor molecule binds to an inhibitory receptor on an NK cell; the NK cell inhibitor molecule binds to NKG2A on an NK cell; the NK cell inhibitor molecule is a non-classical MHC class I molecule; the NK cell inhibitor molecule is HLA-E; the NK cell inhibitor molecule is a fusion protein; or the NK cell inhibitor molecule is a fusion protein comprising HLA-E and B2M.
48. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-47, comprising an exogenous nucleic acid encoding a polypeptide that is secreted by the cell or contacting the cell with said exogenous nucleic acid, wherein the secreted polypeptide is an antibody or antibody fragment.
49. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-48, comprising an exogenous nucleic acid encoding a polypeptide that is

secreted by the cell or contacting the cell with said exogenous nucleic acid, wherein the secreted polypeptide is a full-length IgG antibody, a single chain antibody, or a neutralizing antibody.

50. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-49, comprising an exogenous nucleic acid encoding a polypeptide that is secreted by the cell or contacting the cell with said exogenous nucleic acid, wherein the secreted polypeptide is an enzyme, a cytokine, or a fusion protein.

51. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-50, comprising an exogenous nucleic acid encoding a polypeptide that is secreted by the cell or contacting the cell with said exogenous nucleic acid, wherein the secreted polypeptide comprises a soluble receptor.

52. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-51, comprising an exogenous nucleic acid encoding a targeting receptor or contacting the cell with an exogenous nucleic acid encoding a targeting receptor, wherein the targeting receptor is a T cell receptor (TCR), a genetically modified TCR, a WT1 TCR, or a CAR.

53. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 23-52, wherein the CIITA guide RNA, the RNA-guided DNA binding agent, and/or the exogenous nucleic acid is provided to the cell in a vector, optionally wherein the CIITA guide RNA and the RNA-guided DNA binding agent are provided in the same vector.

54. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-53, wherein the exogenous nucleic acid is provided to the cell in a vector, optionally wherein the vector is a viral vector or a non-viral vector.

55. The engineered cell, population of cells, pharmaceutical composition, or method of claim 54, wherein the vector is a lentiviral vector or an AAV.

56. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-55, wherein a gene editing system component is provided to the cell in a lipid nucleic acid assembly composition.

57. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-56, wherein the guide RNA or the exogenous nucleic acid is provided to the cell in a lipid nucleic acid assembly composition, optionally in the same lipid nucleic acid assembly composition as an RNA-guided DNA binding agent.

58. The engineered cell, population of cells, pharmaceutical composition, or method of claim 56 or 57, wherein the lipid nucleic acid assembly composition is a lipid nanoparticle (LNP).

59. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 35-58, wherein
- (i) wherein the CIITA guide RNA is a single guide RNA comprising any one of the sequences of SEQ ID NO: 335-426 and 1008 or a sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any one of the sequences of SEQ ID NO: 335-426 and 1008;
 - (ii) the CIITA guide RNA comprises any one of sequences SEQ ID NOs: 32, 64, 67, 68, 74, 76, 84, 86, 90, 91, and 115;
 - (iii) wherein the CIITA guide RNA is a single guide RNA comprising any one of the sequences SEQ ID NO: 341, 373, 376, 377, 383, 385, 393, 395, 399, 400, and 424, or a sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any one of the sequences SEQ ID NO: 341, 373, 376, 377, 383, 385, 393, 395, 399, 400, and 424.
60. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 35-59, wherein the CIITA guide RNA comprises at least one modification, wherein the at least one modification includes (i) a 2'-O-methyl (2'-O-Me) modified nucleotide, (ii) a phosphorothioate (PS) bond between nucleotides, (iii) a 2'-fluoro (2'-F) modified nucleotide, (iv) a modification at one or more of the first five nucleotides at the 5' end of the guide RNA, (v) a modification at one or more of the last five nucleotides at the 3' end of the guide RNA, (vi) a PS bond between the first four nucleotides of the guide RNA, (vii) a PS bond between the last four nucleotides of the guide RNA, (viii) a 2'-O-Me modified nucleotide at the first three nucleotides at the 5' end of the guide RNA, (ix) a 2'-O-Me modified nucleotide at the last three nucleotides at the 3' end of the guide RNA, or combinations of one or more of (i)-(ix).
61. An engineered cell or population of cells comprising a genetic modification that includes an indel within the genomic region targeted by the CIITA guide RNA of any one of claims 35-60.
62. An engineered cell or population of cells comprising a genetic modification that includes a C to T substitution or an A to G substitution within the genomic region targeted by the CIITA guide RNA of any one of claims 35-61.
63. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-62, for use to express a TCR with specificity for a polypeptide expressed by cancer cells.

64. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-63, for use in administering to a subject as an adoptive cell transfer (ACT) therapy.
65. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-64, for use in treating a subject with a cancer, an infectious disease, or an autoimmune disease.
66. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-65, wherein the genetic modification comprises an indel.
67. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-66, wherein the genetic modification comprises a C to T substitution.
68. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-67, wherein the genetic modification comprises an A to G substitution.
69. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-68, wherein the cell is homozygous for HLA-B and homozygous for HLA-C.
70. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-69, wherein the cell further comprises a genetic modification in an HLA-A gene, wherein the cell is homozygous for HLA-B and homozygous for HLA-C, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from:
- a. chr6:29942854 to chr6:29942913 and
 - b. chr6:29943518 to chr6: 29943619.
71. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-70, wherein the cell further comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from: chr6:29942864 to chr6: 29942903 and chr6:29943528 to chr6:29943609.
72. A method of making an engineered cell, which has reduced or eliminated surface expression of MHC class II protein and HLA-A protein relative to an unmodified cell, comprising:
- a. contacting the cell with a CIITA guide RNA, wherein the guide RNA comprises a guide sequence selected from SEQ ID NOs: 1-117;
 - b. contacting the cell with an HLA-A guide RNA, wherein the HLA-A guide RNA comprises a guide sequence selected from any one of SEQ ID NOs: 2001-2095;
- and

- c. optionally contacting the cell with an RNA-guided DNA binding agent or nucleic acid encoding an RNA-guided DNA binding agent;
- thereby reducing or eliminating the surface expression of MHC class II protein and HLA-A protein in the cell relative to an unmodified cell.
73. The method of claim 72, wherein the CIITA guide RNA comprises a sequence selected from SEQ ID NO: 32, 64, 67, 68, 74, 76, 84, 86, 90, 91, and 115.