GENETICALLY MODIFIED PRIMARY CELLS FOR ALLOGENEIC CELL THERAPY

Cross-Reference to Related Applications

[0001] This application claims priority to U.S. Provisional Patent Application No. 63/232,161 filed August 11, 2021, U.S. Provisional Patent Application No. 63/297,694 filed January 7, 2022, U.S. Provisional Patent Application No. 63/344,502 filed May 20, 2022, U.S. Provisional Patent Application No. 63/348,990 filed June 3, 2022, and U.S. Provisional Patent Application No. 63/353,531 filed June 17, 2022, the contents of each of which are herein incoproated by reference in their entireties for all purposes.

Reference to an Electronic Sequence Listing

[0002] The contents of the electronic sequence listing (186152005440SEQLIST.xml; Size: 32,413 bytes; and Date of Creation: August 11, 2022) is herein incorporated by reference in its entirety.

Field

[0003] In certain aspects, the present disclosure is directed to engineered cells, such as engineered primary cells, containing one or more modifications, such as genetic modifications, for use in allogeneic cell therapy. In some embodiments, the engineered primary cells are hyopimmunogenic cells.

Summary

[0004] In some examples, provided herein is an engineered cell, such as an engineered primary cell, comprising modifications that (i) increase expression of one or more tolerogenic factor, and (ii) reduce expression of one or more major histocompatibility complex (MHC) class I molecules and/or one or more MHC class II molecules, wherein the increased expression of (i) and the reduced expression of (ii) is relative to a cell of the same cell type that does not comprise the modifications.

[0005] In some examples, the modification in (ii) reduces expression of one or more MHC class I molecules. In some examples, the modifications in (ii) reduces expression of one or more MHC class I molecules and one or more MHC class II molecules. In some of any of the provided examples, the one or more tolerogenic factors is A20/TNFAIP3, C1-Inhibitor, CCL21, CCL22, CD16, CD16 Fc receptor, CD24, CD27, CD35, CD39, CD46, CD52, CD55, CD59, CD200, CR1, CTLA4-Ig, DUX4, FasL, H2-M3, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, IDO1, IL-10, IL15-RF, IL-35, MANF, Mfge8, PD-1, PD-L1 and/or Serpinb9.In some examples, the one or more tolerogenic factor is selected from the group consisting of CD47, CD27, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDOl, CTLA4-Ig, Cl-Inhibitor, IL-10, IL-35, FASL, CCL21, MFGE8, and SERPINB9, and any combination thereof. In some examples, the one or more tolerogenic factor is selected from the group consisting of CD47, PD-L1, HLA-E, HLA-G, CCL21, FASL, SERPINB9, CD200, MFGE8, and any combination thereof. In some examples, at least one of the one or more tolerogenic factor is CD47.

[0006] In some of any examples, the one or more tolerogenic factors is selected from the group consisting of CD47; HLA-E; CD24; PD-L1; CD46; CD55; CD59; CR1; MANF; A20/TNFAIP3; HLA-E and CD47; CD24, CD47, PD-L1, and any combination thereof; HLA-E, CD24, CD47, and PD-L1, and any combination thereof; CD46, CD55, CD59, and CR1, and any combination thereof; HLA-E, CD46, CD55, CD59, and CR1, and any combination thereof; HLA-E, CD24, CD47, PDL1, CD46, CD55, CD59, and CR1, and any combination thereof; HLA-E and PDL1; HLA-E, PDL1, and A20/TNFAIP, and any combination thereof; HLA-E, PDL1, and MANF, and any combination thereof; HLA-E, PDL1, A20/TNFAIP, and MANF, and any combination thereof; and CD47, PD-L1, HLA-E, HLA-G, CCL21, FASL, SERPINB9, CD200, MFGE8, and any combination thereof.

[0007] In some of any examples, the modifications are selected from modifications that reduce expression of MHC I and/or MHC II; reduce expression of MIC-A and/or MIC-B; increase expression of CD47, and optionally CD24 and PD-L1; and increase expression of CD46, CD55, CD59 and CR1.

[0008] In some of any examples, the modifications are selected from modifications that reducet expression of MHC class I molecule; reduce expression of MIC-A and/or MIC-B; reduce expression of TXNIP; increase expression of PD-L1 and HLA-E; and optionally A20/TNFAIP3 and MANF.

[0009] In some of any examples, the modifications are selected from modifications that increase the expression of CCL21, PD-L1, FASL, SERPINB9, HLA-G, CD47, CD200, and MFGE8; and reduce the expression of a MICA and/ or MICB.

[0010] In some examples, the modifications are selected from modifications that reduce expression of MHC I and/or MHC II; and increase expression of CD47.

[0011] In some examples, any of the above modifications are present in a provided engineered cells along with one or more additional edits that increase or decrease expression of a gene in the cell. In some examples, any one or more of the further modifications can be a modification that that reduces expression, such as disrupts, inactivates or knockout expression, of B2M, TAP I, NLRC5, CIITA, HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, HLA-DR, RFX5, RFXANK, RFXAP, NFY-A, NFY-B, NFY-C, CTLA-4, PD-1, IRF1, MIC-A, MIC-B. In some examples, any one or more of the further modifications can be a modification that reduces expression of a protein that is involved in oxidative or ER stress, TRAC, TRB, CD142, ABO, CD38, PCDH11Y, NLGN4Y and/or RHD. In some examples, proteins that are is involved in oxidative or ER stress include thioredoxin-interacting protein (TXNIP), PKR-like ER kinase (PERK), inositol-requiring enzyme 1α (IRE1α), and DJ-1 (PARK7).

[0012] In some examples, for any of the provided examples in which there is reduced expression of any of the above described target genes (e.g. B2M, TAP I, NLRC5, CIITA, HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, HLA-DR, RFX5, RFXANK, RFXAP, NFY-A, NFY-B, NFY-C, CTLA-4, PD-1, IRF1, MIC-A, MIC-B) the target gene is not expressed by the engineered cell. In some examples, the protein encoded by the target gene is not expressed on the surface of the cell. In some examples, the MHC class I complex and/or MHC class II complex is not expressed on the surface of the cell.

[0013] In some examples, provided herein is an engineered primary cell comprising modifications that (i) increase expression of CD47, and (ii) reduce expression of one or more major histocompatibility complex (MHC) class I molecules and/or one or more MHC class II molecules, wherein the increased expression of (i) and the reduced expression of (ii) is relative to a cell of the same cell type that does not comprise the modifications.

[0014] In some examples, the modification(s) that increase expression comprise increased surface expression, and/or the modifications that reduce expression comprise reduced

surface expression. In some examples, the modification that increases expression of CD47 comprises an exogenous polynucleotide encoding the CD47 protein. In some examples, the exogenous polynucleotide encoding CD47 encodes a sequence of amino acids having at least 85% identity to the amino acid sequence of SEQ ID NO: 2, and reduces innate immune killing of the engineered primary cell. In some examples, the exogenous polynucleotide encoding CD47 encodes a sequence set forth in SEQ ID NO: 2. In some examples, the exogenous polynucleotide encoding CD47 is operably linked to a promoter. In some examples, the promoter is a constitutive promoter. In some examples, the promoter is selected from the group consisting of the CAG promoter, the cytomegalovirus (CMV) promoter, the EF1a promoter, the PGK promoter, adenovirus late promoter, vaccinia virus 7.5K promoter, SV40 promoter, tk promoter of HSV, mouse mammary tumor virus (MMTV) promoter, LTR promoter of HIV, promoter of moloney virus, Epstein barr virus (EBV) promoter, and the Rous sarcoma virus (RSV) promoter. In some examples, the exogenous polynucleotide encoding CD47 is integrated into the genome of the engineered primary cell. In some examples, the exogenous polynucleotide is a multicistronic vector encoding CD47 and an additional transgene encoding a second transgene. In some examples, the integration is by non-targeted insertion into the genome of the engineered primary cell, optionally by introduction of the exogenous polynucleotide into the cell using a lentiviral vector. In some examples, the integration is by targeted insertion into a target genomic locus of the cell. In some examples, the target genomic locus is a safe harbor locus, a *B2M* gene locus, a *CIITA* gene locus, a *TRAC* gene locus, or a *TRBC* gene locus. In some examples, the target genomic locus is selected from the group consisting of: a *CCR5* gene locus, a *CXCR4* gene locus, a *PPP1R12C* (also known as *AAVS1*) gene, an albumin gene locus, a SHS231 locus, a *CLYBL* gene locus, and a *ROSA26* gene locus.

[0015] In some examples, the modification that reduces expression of one or more MHC class I molecules reduces one or more MHC class I molecules protein expression. In some examples, the modification that reduces expression of one or more MHC class I molecules is a modification that reduces expression of B-2 microglobulin (B2M). In some examples, the modification that reduces expression of one or more MHC class I molecules comprises reduced mRNA expression of B2M. In some examples, the modification that reduces expression of one or more MHC class I molecules comprises reduced protein expression of B2M. In some examples, the modification eliminates *B2M* gene activity. In some examples, the

modification comprises inactivation or disruption of both alleles of the *B2M* gene. In some examples, the modification comprises inactivation or disruption of all *B2M* coding sequences in the cell. In some examples, the inactivation or disruption comprises an indel in the *B2M* gene. In some examples, the modification is a frameshift mutation or a deletion of a contiguous stretch of genomic DNA of the *B2M* gene. In some examples, the *B2M* gene is knocked out. In some examples, the modification is by nuclease-mediated gene editing. In some examples, the nuclease-mediated gene editing is by a zinc finger nuclease (ZFN), a TAL-effector nuclease (TALEN), or a CRISPR-Cas combination that targets the *B2M* gene, optionally wherein the Cas is Cas9. In some examples, the nuclease-mediated gene editing is by a CRISPR-Cas combination and the CRISPR-Cas combination comprises a guide RNA (gRNA) having a targeting domain that is complementary to at least one target site within the *B2M* gene. In some examples, the CRISPR-Cas combination is a ribonucleoprotein (RNP) complex comprising the gRNA and a Cas protein. In some examples, the modification that reduces expression of one or more MHC class I molecules is a modification that reduces expression of an HLA-A protein, an HLA-B protein, or HLA-C protein, optionally wherein a gene encoding said HLA-A protein, an HLA-B protein, or HLA-C protein is knocked out.

[0016] In some examples, the modification that reduces expression of one or more MHC class II molecules reduces one or more MHC class II molecules protein expression. In some examples, the modification that reduces expression of one or more MHC class II molecules is a modification that reduces expression of CIITA. In some examples, the modification that reduces expression of one or more MHC class II molecules comprises reduced mRNA expression of CIITA. In some examples, the modification that reduces expression of one or more MHC class II moelcules comprises reduced protein expression of CIITA. In some examples, the modification eliminates *CIITA* gene activity. In some examples, the modification comprises inactivation or disruption of both alleles of the *CIITA* gene. In some examples, the modification comprises inactivation or disruption of all *CIITA* coding sequences in the cell. In some examples, the inactivation or disruption comprises an indel in the *CIITA* gene. In some examples, the indel is a frameshift mutation or a deletion of a contiguous stretch of genomic DNA of the *CIITA* gene. In some examples, the *CIITA* gene is knocked out. In some examples, the modification is by nuclease-mediated gene editing. In some examples, the nuclease-mediated gene editing is by a zinc finger nuclease (ZFN), a TAL-effector nuclease (TALEN), or a CRISPR-Cas combination that targets the *CIITA* gene,

optionally wherein the Cas is Cas9. In some examples, the nuclease-mediated gene editing is by a CRISPR-Cas combination and the CRISPR-Cas combination comprises a guide RNA (gRNA) having a targeting domain that is complementary to at least one target site within the *CIITA* gene. In some examples, the CRISPR-Cas combination is a ribonucleoprotein (RNP) complex comprising the gRNA and a Cas protein. In some examples, the modification that reduces expression of one or more MHC class II moelcules is a modification that reduces expression of an HLA-DP protein, an HLA-DR protein, or HLA-DQ protein, optionally wherein a gene encoding said HLA-DP protein, an HLA-DR protein, or HLA-DQ protein is knocked out.

[0017] In some examples, the engineered primary cell is a human cell or an animal cell. In some examples, the animal cell is a pig (porcine), cow (bovine) or sheep (ovine) cell. In some examples, the engineered primary cell is a human cell. In some examples, the primary cell is a cell type that is exposed to the blood. In some examples, the engineered primary cell is a primary cell isolated from a donor subject. In some examples, the donor subject is healthy or is not suspected of having a disease or condition at the time the donor sample is obtained from the donor subject. In some examples, the engineered primary cell is selected from an islet cell, a beta islet cell, B cell, T cell, NK cell, retinal pigmented epithelium cell, glial progenitor cell, endothelial cell, hepatocyte, thyroid cell, skin cell, and blood cell. In some examples, the engineered primary cell is an endothelial cell. In some examples, the engineered primary cell is an epithelial cell. In some examples, the engineered primary cell is a T cell. In some examples, the engineered primary cell is an NK cell. In some examples, the engineered primary cell comprises a chimeric antigen receptor (CAR). In some examples, the engineered primary cell is an islet cell. In some examples, the islet cell is a beta islet cell. In some examples, the engineered primary cell is a hepatocyte. In some examples, the engineered primary cell is ABO blood group type O. In some examples, the engineered primary cell is Rhesus factor negative (Rh−).

[0018] In some examples, provided herein is a method of generating an engineered primary cell, the method comprising: a) reducing or eliminating the expression of one or more MHC class I molecules and/or one or more MHC class II molecules in a primary cell; and b) increasing the expression of one or more tolerogenic factors in the primary cell. In some examples, the one or more tolerogenic factor is selected from the group consisting of CD47, CD27, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDOl, CTLA4-Ig, Cl-Inhibitor, IL-10, IL-35, FASL, CCL21, MFGE8, and SERPINB9, and any combination thereof.

In some examples, the one or more tolerogenic factor is selected from the group consisting of CD47, PD-L1, HLA-E, HLA-G, CCL21, FASL, SERPINB9, CD200, MFGE8, and any combination thereof. In some examples, at least one of the one or more tolerogenic factor is CD47. In some examples, the method comprises reducing or eliminating the expression of one or more MHC class I molecules. In some examples, the method comprises reducing or eliminating the expression of one or more MHC class I molecules and one or more MHC class II molecules.

[0019] In some examples, provided herein is a method of generating an engineered primary cell, the method comprising: a. reducing or eliminating the expression of one or more MHC class I and/or one or more MHC class II molecules in the cell; and, b. increasing the expression of CD47 in the cell. In some examples, the method comprises reducing or eliminating the expression of one or more MHC class I molecules. In some examples, the method comprises reducing or eliminating the expression of one or more MHC class I molecules and one or more MHC class II molecules.

[0020] In some examples, the modification(s) that increase expression comprise increased surface expression, and/or the modifications that reduce expression comprise reduced surface expression. In some examples, the modification that increases expression of CD47 comprises an exogenous polynucleotide encoding the CD47 protein. In some examples, the exogenous polynucleotide encoding CD47 encodes a sequence of amino acids having at least 85% identity to the amino acid sequence of SEQ ID NO: 2, and reduces innate immune killing of the engineered primary cell. In some examples, the exogenous polynucleotide encoding CD47 encodes a sequence set forth in SEQ ID NO: 2. In some examples, the exogenous polynucleotide encoding CD47 is operably linked to a promoter. In some examples, the exogenous polynucleotide encoding CD47 is integrated into the genome of the engineered primary cell. In some examples, the integration is by non-targeted insertion into the genome of the engineered primary cell, optionally by introduction of the exogenous polynucleotide into the engineered primary cell using a lentiviral vector. In some examples, the integration is by targeted insertion into a target genomic locus of the cell, optionally wherein the targeted insertion is by nuclease-mediated gene editing with homology-directed repair. In some examples, the target genomic locus is a *B2M* gene locus, a *CIITA* gene locus, a *CD142* gene locus, a *TRAC* gene locus, or a *TRBC* gene locus. In some examples, the target genomic locus is selected from the group consisting of: a *CCR5* gene locus, a *CXCR4* gene locus, a

PPP1R12C (also known as *AAVS1*) gene, an albumin gene locus, a SHS231 locus, a *CLYBL* gene locus, and a *ROSA26* gene locus. In some examples, the nuclease-mediated gene editing is by a zinc finger nuclease (ZFN), a TAL-effector nuclease (TALEN), or a CRISPR-Cas combination that targets the target genomic locus, optionally wherein the Cas is Cas9. In some examples, the nuclease-mediated gene editing is by a CRISPR-Cas combination and the CRISPR-Cas combination comprises a guide RNA (gRNA) having a targeting domain that is complementary to a target sequence of the target genomic locus and a homology-directed repair template comprising the exogenous polynucleotide encoding CD47. In some examples, the CRISPR-Cas combination is a ribonucleoprotein (RNP) complex comprising the gRNA and a Cas protein. In some examples, the engineered primary cell is a hypo-immunogenic primary cell.

[0021] In some examples, reducing or eliminating expression of one or more MHC class I molecules comprises introducing a modification that reduces one or more MHC class I molecules protein expression. In some examples, the modification that reduces one or more MHC class I molecules protein expression comprises reduced expression of B2M. In some examples, the modification that reduces one or more MHC class I molecules protein expression comprises reduced protein expression of B2M. In some examples, the modification that reduces one or more MHC class I molecules protein expression eliminates *B2M* gene activity. In some examples, the modification that reduces one or more MHC class I molecules expression comprises inactivation or disruption of both alleles of the *B2M* gene. In some examples, the modification that reduces one or more MHC class I molecules protein expression comprises inactivation or disruption of all B2M coding sequences in the cell. In some examples, the inactivation or disruption comprises an indel in the endogenous *B2M* gene or a deletion of a contiguous stretch of genomic DNA of the endogenous *B2M* gene. In some examples, the indel is a frameshift mutation or a deletion of a contiguous stretch of genomic DNA of the *B2M* gene. In some examples, the endogenous *B2M* gene is knocked out. In some examples, the modification reduces one or more MHC class I molecules protein expression is by nuclease-mediated gene editing. In some examples, the nuclease-mediated gene editing is by a zinc finger nuclease (ZFN), a TAL-effector nuclease (TALEN), or a CRISPR-Cas combination that targets the *B2M* gene, optionally wherein the Cas is Cas9. In some examples, the nuclease-mediated gene editing is by a CRISPR-Cas combination and the CRISPR-Cas combination comprises a guide RNA (gRNA) having a targeting domain that is

complementary to at least one target site within the *B2M* gene. In some examples, the CRISPR-Cas combination is a ribonucleoprotein (RNP) complex comprising the gRNA and a Cas protein. In some examples, the modification that reduces expression of one or more MHC class I molecules reduces HLA-A protein expression, HLA-B protein expression, or HLA-C protein expression, optionally wherein the protein expression is reduced by knocking out a gene encoding said HLA-A protein, HLA-B protein, or HLA-C protein.

[0022] In some examples, reducing or eliminating expression of one or more MHC class II molecules comprises introducing a modification that reduces one or more MHC class II molecules protein expression. In some examples, the genetic modification that reduces one or more MHC class II molecules protein expression comprises reduced expression of CIITA. In some examples, the genetic modification that reduces one or more MHC class II molecules protein expression comprises reduced protein expression of CIITA. In some examples, the modification that reduces one or more MHC class II molecules protein expression eliminates CIITA. In some examples, the modification that reduces one or more MHC class II molecules protein expression comprises inactivation or disruption of both alleles of the *CIITA* gene. In some examples, the modification comprises inactivation or disruption of all *CIITA* coding sequences in the cell. In some examples, the inactivation or disruption comprises an indel in the *CIITA* gene or a deletion of a contiguous stretch of genomic DNA of the *CIITA* gene. In some examples, the indel is a frameshift mutation or a deletion of a contiguous stretch of genomic DNA of the *CIITA* gene. In some examples, the *CIITA* gene is knocked out. In some examples, the genetic modification that reduces expression of one or more MHC class II reduces the expression of a HLA-DP protein, a HLA-DR protein, or a HLA-DQ protein, optionally wherein said HLA-DP protein expression, said HLA-DR protein expression, or said HLA-DQ protein expression is reduced by knocking out a gene encoding said HLA-DP protein, said HLA-DR protein, or said HLA-DQ protein.

[0023] In some examples, the engineered primary cell is a human cell or an animal cell. In some examples, the animal cell is a pig (porcine) cell, cow (bovine) cell, or sheep (ovine) cell. In some examples, the engineered primary cell is a human cell. In some examples, the engineered primary cell is a cell type that is exposed to the blood. In some examples, the engineered primary cell is isolated from a donor subject. In some examples, the engineered primary cell is selected from an islet cell, a beta islet cell, B cell, T cell, NK cell, retinal

pigmented epithelium cell, glial progenitor cell, endothelial cell, hepatocyte, thyroid cell, skin cell, and blood cell. In some examples, the engineered primary cell is an islet cell.

[0024] In some examples, the primary islet cell has been dissociated from a primary islet cluster. In some examples, the primary islet cluster is a human primary cadaveric islet cluster. In some examples, after step a) and/or after step b) the primary islet cell is incubated under conditions for re-clustering into a modified primary islet cluster, wherein at least a portion of the incubating is carried out with motion. In some examples, the incubating further comprises a least a portion of incubating under static conditions. In some examples, the incubating comprises a first incubation under static conditions followed by the incubating with motion. In some examples, the incubating comprises the incubating with motion followed by a second incubation under static conditions. In some examples, prior to the incubating under conditions for reclustering, the method comprises selecting for islet cells that have been modified. In some examples, the selecting is by fluorescence-activated cell sorting (FACS).

[0025] In some examples, the method comprises: i) dissociating a primary islet cluster into a suspension of primary beta islet cells; ii) modifying primary beta islet cells of the suspension to reduce or eliminate the expression of one or more MHC class I and/or one or more MHC class II HLA in primary beta islet cells; iii) incubating the modified primary beta islet cells under conditions for re-clustering into a first modified primary islet cluster, wherein at least a portion of the incubating is carried out with motion; iv) dissociating the modified] primary islet cluster into a suspension of modified primary beta islet cells; v) further modifying the modified primary islet cells of the suspension to increase the expression of one or more tolerogenic factors in the primary cell; and vi) incubating the further modified primary beta islet cells under conditions for re-clustering into a second modified primary islet cluster, wherein at least a portion of the incubating is carried out with motion.

[0025a] In one aspect, the present disclosure refers to a method for modifying primary islet cells, the method comprising: i) dissociating a primary islet cluster into a suspension of primary islet cells; ii) contacting the suspension of primary islet cells with one or more reagents to modify gene expression; and iii) incubating the modified islet cells under conditions for re-clustering the cells into an islet, wherein at least a portion of the incubating is carried out with motion.

[0025b] In one aspect, the present disclosure refers to a method for modifying primary islet cells, the method comprising: i) dissociating a primary islet cluster into a suspension of primary islet cells; ii) contacting the suspension of primary islet cells with one or more first reagents to modify gene expression to produce modified islet cells, wherein the one or more first reagents comprise (1) a gene editing system comprising a genome-modifying protein for gene editing of a target gene encoding an endogenous protein and/or (2) an agent comprising an exogenous polynucleotide encoding an exogenous protein; iii) after the contacting, incubating the modified islet cells under conditions for re-clustering the cells into a first modified primary islet cluster, wherein at least a portion of the incubating is carried out with shaking comprising orbital motion; iv) dissociating the first modified primary islet cluster into a suspension of modified primary islet cells; v) further contacting the modified primary islet cells of the suspension with one or more second reagents, wherein the one or more second reagents comprise (i) a gene editing system comprising a genome-modifying protein for gene editing a target gene encoding an endogenous protein and/or (ii) an agent comprising an exogenous polynucleotide encoding an exogenous protein; and vi) incubating the further modified primary islet cells after the further contacting under conditions for re-clustering into a second modified primary islet cluster, wherein at least a portion of the incubating is carried out with shaking comprising orbital motion.

[0026] In some examples, the one or more MHC class I HLA is an HLA-A protein, an HLA-B protein, or HLA-C protein. In some examples, the one or more MHC class II HLA is an HLA-DP protein, an HLA-DR protein, or an HLA-DQ protein. In some examples, the modifying is by genetic engineering. In some examples, the motion is shaking. In some examples, the shaking comprises orbital motion. In some examples, the shaking comprises bidirectional linear movement. In some examples, the shaking is with an orbital shaker. In some examples, the incubating in (iii) and/or the incubating in vi) further comprises a least a portion of incubating under static conditions. In some examples, the

incubating in iii) and/or the incubating in vi) comprises a first incubation under static conditions followed by the incubating with motion. In some examples, the incubating comprises the incubating with motion followed by a second incubation under static conditions.

[0027] In some examples, prior to v), the method comprises selecting, from the dissociated islet cells in iv), beta islet cells that have been modified, and optionally repeating steps iii) and iv) on the selected islet cells. In some examples, after the incubating in vi), the method comprises dissociating the second modified primary islet cluster into a suspension of modified primary beta islet cells and selecting for islet cells that have been modified. In some examples, incubating the selected modified primary beta islet cells under conditions for re-clustering into a modified primary islet cluster, wherein at least a portion of the incubating is carried out with motion.

[0028] In some examples, provided herein is use of motion to promote modification of a population of cells, wherein the population of cells has been contacted with one or more reagents to modify gene expression in cells of the population before subjecting to the motion.

[0029] In some examples, provided herein is a method of enhancing modification of a population of cells, in which the method includes: i) contacting a population of cells with one or more reagents to modify gene expression in cells of the population; and ii) subjecting the population of cells to motion after contact with the one or more reagents.

[0030] In some examples, provided herein is a method of enhancing viability of a population of cells, in which the method includes: i) contacting a population of cells with one or more reagents to modify gene expression in cells of the population; and ii) subjecting the population of cells to motion after contact with the one or more reagents.

[0031] In some examples, provided herein is a method of modification of a population of cells, in which the method includes i) contacting a population of cells with one or more reagents to modify gene expression in cells of the population; and ii) subjecting the population of cells to motion after contact with the one or more reagents.

[0031a] In one aspect, the present disclosure refers to a method of modification of a population of cells, the method comprising: i) contacting a population of cells with one or more reagents to modify gene expression in cells of the population; and ii) subjecting the population of cells to motion after contact with the one or more reagents.

[0032] In some of any examples of a provided use or method, the population of cells are primary cells. In some f any examples of a provided use or methods, the population of cells are cells derived from stem cells. In some examples, the stem cells are selected from the group consisting of a pluripotent stem cell (PSC), an induced pluripotent stem cell, an embryonic stem cell, a hematopoietic stem cell, a mesenchymal stem cell, an endothelial stem cell, an epithelial stem cell, an adipose stem cell, a germline stem cell, a lung stem cell, a cord

blood stem cell, and a multipotent stem cell. In some examples, the stem cells are pluripotent stem cell. In some examples, the stem cells are induced pluripotent stem cells, mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs), or embryonic stem cells (ESCs). In some examples, the population of cells are selected from the group consisting of islet cells, immune cells, B cells, T cells, natural killer (NK) cells, natural killer T (NKT) cells, macrophages, endothelial cells, muscle cells, cardiac muscle cells, smooth muscle cells, skeletal muscle cells, hepatocytes, a glial progenitor cells, dopaminergic neurons, retinal pigment epithelial cells, thyroid cells, skin cells, glial progenitor cells, neural cells, cardiac cells, and blood cells.

[0033] In some of any examples of a provided use or method, the population of cells are naturally present in a 3D network.

[0034] In some of any examples of a provided use or method, the population of cells are in suspension. In some examples, the population of cells are in a vessel that has a low-attachment surface. In some examples, the population of cells are maintained in a vessel in a minimum volume of media sufficient to cover the cells. In some examples, the population of cells in suspension are produced by dissociating cells from an adherent culture or a cell cluster prior to the contacting.

[0034a] In one aspect, the present disclosure refers to a population of engineered cells produced by the method disclosed herein.

[0035] In some of any examples of a provided use or method, the population of cells are islet cells. In some examples, the population of cells comprise beta islet cells. In some examples, the population of cells comprising beta islet cells are produced by dissociating a primary islet cluster into a suspension of cells comprising primary beta islet cells.

[0036] In some of any examples of a provided use or method, the contacting is carried out for less than two days prior to subjecting the cells to motion. In some examples, the contacting is carried out for 30 seconds to 24 hours prior to subjecting the cells to motion. In some examples, the contacting is carried out for 1 minute to 60 minutes, 2 minutes to 30 minutes, 5 minutes to 15 minutes prior to subjecting the cells to motion.

[0037] In some of any examples of a provided use or method, subjecting the population of cells to motion promotes the formation of cell aggregates. In some examples, subjecting the population of cells to motion forms cell clusters.

[0038] In some of any examples of a provided use or method, the method or use further includes incubating the cells under static conditions after subjecting the cells to motion. In some

of any examples of a provided use or method, the methor or use further includes incubating the cells under static conditions after the contacting and before subjecting the cells to motion.

[0039] In some of any examples of a provided use or method, the one or more reagents comprise at least two different reagents. In some examples, each of the at least two different reagents is for modulating expression of a different gene.

[0040] In some of any examples of a provided use or method, the steps of the method are repeated. In some emobdiments, the one or more reagents in the first iteration of the method are different from the the one or more reagents in the repeated iteration of the method. In some examples, the or more reagents in the first iteration of the method are different from the one or more reagents in the second iteration of the method.

[0041] In some of any examples of a provided use or method, such may further comprise before subjecting the cells to motion after the contacting with the one or more reagents, selecting for cells that have modified gene expression. In some of any examples, selecting cells that have modified gene expression may be relative to the cells before the contacting. In some examples, the modified gene expression is increased, such as relative to the expression of the gene in the cell before the contacting. In some examples, the modified gene expression is decreased, such as relative to the expression of the gene in the cell before the contacting.

[0042] Provided herein is A method for modifying primary islet cells, the method comprising: i) dissociating a primary islet cluster into a suspension of primary islet cells; ii) contacting the suspension of primary islet cells with one or more reagents to modify gene expression; and iii) after the contacting incubating the modified islet cells under conditions for re-clustering the cellsinto an islet, wherein at least a portion of the incubating is carried out with motion.

[0043] In some examples, provided herein is a method for gene editing primary islet cells, the method comprising: i) dissociating a primary islet cluster into a suspension of primary beta islet cells; ii) modifying primary beta islet cells of the suspension; and iii) incubating the modified primarybeta islet cells under conditions for re-clustering the modified primarybeta islet cells into an islet, wherein at least a portion of the incubating is carried out with shaking. In some examples, the primary islet cluster is a human primary cadaveric islet cluster. In some examples, the modifying comprises introducing one more modifications into the cell to reduce expression of one or more genes encoding an endogenous protein in the cell or to increase expression of one or more heterologous proteins in the cell.

[0044] In some examples, the incubating in (iii) and/or the incubating in vi) further comprises a least a portion of incubating under static conditions. In some examples, the incubating comprises a first incubation under static conditions followed by the incubating with motion. In some examples, the incubating comprises the incubating with motion followed by a second incubation under static conditions. In some examples, steps i)-iii) are repeated. In some examples, the modifying in the first iteration of the method is different from the modifying in the repeated iteration of the method. In some examples the one or more reagents in the first iteration of the method are different from the one or more reagents in the repeated iteration of the method.

[0045] In some examples, the re-clustered islet cells are a first modified primary islet cluster and wherein the method further comprises: iv) dissociating the first modified primary islet cluster into a suspension of modified primary beta islet cells; v) further modifying the modified primary islet cells of the suspension; and vi) incubating the further modified primary beta islet cells under conditions for re-clustering into a second modified primary islet cluster, wherein at least a portion of the incubating is carried out with motion.

[0046] In some examples, the one or more reagents are one or more first reagents and the re-clustered islet cells are a first modified primary islet cluster and wherein the method further comprises: iv) dissociating the first modified primary islet cluster into a suspension of modified primary islet cells; v) further contacting the modified primary islet cells of the suspension with one or more further reagents to modify gene expression; and vi) incubating the further modified primary islet cells after the further contacting under conditions for re-clustering into a second modified primary islet cluster, wherein at least a portion of the incubating is carried out with motion.

[0047] In some examples, prior to the incubating in iii), the method comprises selecting for islet cells that have been modified. In some examples, prior to v), the method comprises selecting, from the dissociated islet cells in iv), beta islet cells that have been modified, and optionally repeating steps iii) and iv) on the selected islet cells. In some examples, after the incubating in vi), the method comprises dissociating the second modified primary islet cluster into a suspension of modified primary islet cells and selecting for islet cells that have been modified. In some examples, cells that have been modified have modified gene expression, such as modified relative to the primary islet cells before the contacting.

[0048] In some examples, incubating the selected modified primary beta islet cells under conditions for re-clustering into a modified primary islet cluster, wherein at least a portion of the incubating is carried out with motion. In some examples, the selecting comprises fluorescence-activated cell sorting (FACS).

[0049] In some examples, the suspension is a single cell suspension.

[0050] In some examples, the suspension of primary islet cells is present in a vessel that has a low-attachment surface. In som examples, the vessel has a minimum volume of media sufficient to cover the cells.

[0051] In some of any examples, the motion is at a speed of between about 20 rpm and about180 rpm, between about 40 rpm and about 125 rpm or between about 60 rpm and about 100 rpm, each inclusive. In some examples, the motion is at a speed of between about 85 rpm and about 95 rpm, inclusive.

[0052] In some examples, the motion is shaking. In some examples, the shaking comprises orbital motion. In some examples, the motion is an undulating motion. In some examples, the undulating motion is with a shaker device that combines orbital movement and rocking movement. In some examples, the shaking comprises bidirectional linear movement. In some examples, the shaking is with an orbital shaker. In some examples, the motion is with a tilt angle. In some examples, the tilt angle is between 1° and 8°.

[0053] In some examples, one of the first modifying or further modifying comprises reducing expression of one or more genes encoding an endogenous protein in the cell and the other of the first modifying or the further modifying comprises increasing expression of one or more exogenous proteins in the cell. In some examples, the first modifying comprises reducing expression of one or more genes encoding an endogenous protein in the cell and the further modifying comprises increasing expression of one or more exogenous proteins in the cell.

[0054] In some examples, the one or more reagents reduce expression of one or more genes encoding an endogenous protein in the cell or increase expression of one or more heterologous proteins in the cell. In some examples, at least one of the first one or more reagents is for reducing expression of one or more genes encoding an endogenous protein in the cell and at least one of the further one or more reagents is forincreasing expression of one or more exogenous proteins in the cell. In some examples, at least one of the first one or more reagents is for increasing expression of one or more exogenous proteins in the cell and at least

one of the further one or more reagents is for reducing expression of one or more genes encoding an endogenous protein in the cell. In some examples, the first one or more reagents are for reducing expression of one or more genes encoding an endogenous protein in the cell and the further one or more reagents are for increasing expression of one or more exogenous proteins in the cell. In some examples, the one or more reagents comprise a genome-modifying protein for gene editing a target gene encoding an endogenous protein and/or an agent comprising an exogenous polynucleotide encoding an exogenous protein.

[0055] In some examples, reducing expression of one or more genes encoding an endogenous protein in the cell is by introducing a gene-editing system into the cell. In some examples, the gene-editing system comprises a sequence specific nuclease. In some of any examples, the one or more reagents for reducing expression of one or more genes encoding an endogenous protein in the cell comprise a genome-modifying protein. the genome-modifying protein is associated with gene editing by a sequenc-specific nuclease, a CRISPR-associated transposase (CAST), prime editing, or Programmable Addition via Site-specific Targeting Elements (PASTE). In some examples, the genome-modifying protein is a sequence specific nuclease. In some examples, the sequence specific nuclease is an RNA-guided nuclease.

[0056] In some examples, the sequence specific nuclease is selected from the group consisting of a RNA-guided DNA endonuclease, a meganuclease, a transcription activator-like effector nuclease (TALEN), and a zinc-finger nuclease (ZFN). In some examples, the gene-editing system comprises an RNA-guided nuclease. In some examples, the RNA-guided nuclease comprises a Cas nuclease and a guide RNA. In some examples, the RNA-guided-nuclease is a Type II or Type V Cas protein. In some examples, the RNA-guided-nuclease is a Cas9 homologue or a Cpf1 homologue. In some examples, the genome-modifying protein is selected from the group consisting of Cas3, Cas4, Cas5, Cas8a, Cas8b, Cas8c, Cas9, Cas10, Cas12, Cas12a (Cpf1), Cas12b (C2c1), Cas12c (C2c3), Cas12d (CasY), Cas12e (CasX), Cas12f (C2c10), Cas12g, Cas12h, Cas12i, Cas12k (C2c5), Cas13, Cas13a (C2c2), Cas13b, Cas13c, Cas13d, C2c4, C2c8, C2c9, Cmr5, Cse1, Cse2, Csf1, Csm2, Csn2, Csx10, Csx11, Csy1, Csy2, Csy3, and Mad7. In some examples, the Cas is a Cas9. In some examples, the Cas is a Cas12.

[0057] In some examples, the one or more reagents are for reducing expression of one or more major histocompatibility complex (MHC) class I molecules and/or for reducing expression

of one or more MHC class II molecules. In some examples, the first modifying comprises reducing expression of one or more major histocompatibility complex (MHC) class I molecules and/or one or more MHC class II molecules. In some examples, the modifying is genetic engineering. In some examples, the one or more MHC class I HLA is an HLA-A protein, an HLA-B protein, or HLA-C protein. In some examples, the one or more MHC class II HLA is an HLA-DP protein, an HLA-DR protein, or an HLA-DQ protein.

[0058] In some examples, the one or more reagents for reducing expression of one or more MHC class I molecule or MHC class II molecule reduces expression of one or more of B2M, TAP I, NLRC5, CIITA, HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, HLA-DR, RFX5, RFXANK, RFXAP, NFY-A, NFY-B and/or NFY-C. In some examples, reducing expression of one or more MHC class I is by reducing expression of B-2 microglobulin (B2M). In some examples, reducing expression of one or more MHC class II is by reducing expression of CIITA.

[0059] In some examples, the further modifying comprises increasing expression of one or more tolerogenic factor in the cell. In some examples, the one or more exogenous proteins is one or more tolerogenic factors. In some examples, the one or more reagents comprise an agent for increasing expression of one or more tolerogenic factors. In some examples, the agent is a lipid particle or a a viral vector. In some examples, the viral vector is a lentiviral vector.

[0060] In some examples, the one or more tolerogenic factors is CD47, A20/TNFAIP3, C1-Inhibitor, CCL21, CCL22, CD16, CD16 Fc receptor, CD24, CD27, CD35, CD39, CD46, CD52, CD55, CD59, CD200, CR1, CTLA4-Ig, DUX4, FasL, H2-M3, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, IL-10, IL15-RF, IL-35, MANF, Mfge8, PD-1, PD-L1, or Serpinb9. In some examples, the one or more tolerogenic factor is selected from the group consisting of CD47, CD27, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDOl, CTLA4-Ig, Cl-Inhibitor, IL-10, IL-35, FASL, CCL21, MFGE8, and SERPINB9, and any combination thereof. In some examples, the one or more tolerogenic factor is selected from the group consisting of CD47, PD-L1, HLA-E, HLA-G, CCL21, FASL, SERPINB9, CD200, MFGE8, and any combination thereof. In some examples, at least one of the one or more tolerogenic factor is CD47.

[0061] In some examples, increasing expression of one or more exogenous proteins in the cell is by introducing an exogenous polynucleotide. In some examples, the exogenous

polynucleotide is operably linked to a promoter. In some examples, the promoter is a constitutive promoter. In some examples, the promoter is selected from the group consisting of the CAG promoter, the cytomegalovirus (CMV) promoter, the EF1a promoter, the PGK promoter, adenovirus late promoter, vaccinia virus 7.5K promoter, SV40 promoter, tk promoter of HSV, mouse mammary tumor virus (MMTV) promoter, LTR promoter of HIV, promoter of moloney virus, Epstein barr virus (EBV) promoter, the Rous sarcoma virus (RSV) promoter and the UBC promoter. In some examples, the exogenous polynucleotide is integrated into the genome of the cell. In some examples, the exogenous polynucleotide is a multicistronic vector. In some examples, the integration is by non-targeted insertion into the genome of the cell, optionally by introduction of the exogenous polynucleotide into the cell using a lentiviral vector. In some examples, the integration is by targeted insertion into a target genomic locus of the cell. In some examples, the islet cell is a beta islet cell.

[0062] In some examples, the engineered primary cell is a hepatocyte. In some examples, the engineered primary cell is a T cell. In some examples, the engineered primary cell is an endothelial cell. In some examples, the engineered primary cell is a thyroid cell. In some examples, the engineered primary cell is a skin cell. In some examples, the engineered primary cell is a retinal pigmented epithelium cell.

[0063] In some examples, provided herein is an engineered cell produced according to the methods described herein. In some examples, the engineered cell is any cell as described herein. In some examples, the engineered cell is a primary cell. In some of any examples, the engineered primary cell is selected from an islet cell, a beta islet cell, B cell, T cell, NK cell, retinal pigmented epithelium cell, glial progenitor cell, endothelial cell, hepatocyte, thyroid cell, skin cell, and blood cell. In some examples, the primary cell is an islet cell. In some examples, the islet cell is a beta islet cell.

[0064] In some examples, the viability of the cells produced by the method is greater than about 30%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% or more. In some examples, the percentage of cells of the population modified by the method is greater than about 30%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% or more.

[0065] In some of any examples, the modifications as described in any of the provided engineered cells reduces innate immune killing of the engineered cell.

[0066] In some examples, the engineered primary cell is capable of evading NK cell mediated cytotoxicity upon administration to a recipient patient. In some examples, the

engineered primary cell is protected from cell lysis by mature NK cells upon administration to a recipient patient. In some examples, the engineered primary cell does not induce an immune response to the cell upon administration to a recipient patient. In some examples, the engineered primary cell does not induce a systemic inflammatory response to the cell upon administration to a recipient patient. In some examples, the engineered primary cell does not induce a local inflammatory response to the cell upon administration to a recipient patient.

[0067] In some examples, provided herein is a population of engineered primary cells comprising a plurality of any of the engineered primary cells described herein. In some examples, the plurality of the engineered primary cells are derived from cells pooled from more than one donor subject. In some examples, each of the more than one donor subjects are healthy subjects or are not suspected of having a disease or condition at the time the donor sample is obtained from the donor subject. In some examples, at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 99.99% of cells in the population comprise the modifications. In some examples, at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 99.99% of cells in the population comprise an exogenous polynucleotide encoding CD47. In some examples, at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 99.99% of cells in the population comprise reduced expression of one or more MHC class I molecules and/or one or more MHC class II molecules relative to a cell of the same cell type that does not comprises the modification(s). In some examples, at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 99.99% of cells in the population comprise reduced expression of B2M and/or CIITA relative to a cell of the same cell type that does not comprises the modification(s). In some examples, at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 99.99% of cells in the population comprise reduced expression of B2M relative to a cell of the same cell type that does not comprises the modification(s). In some examples, at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 99.99% of cells in the population comprise reduced expression of B2M and CIITA relative to a cell of the same cell type that does not comprises the modification(s). In some examples, at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 99.99% of cells in the population comprise one or more alterations that inactivate both alleles of an endogenous *B2M* gene. In some examples, at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 99.99% of cells in the population comprise one or more alterations that inactivate both alleles of an endogenous *CIITA* gene.

[0068] In some examples, provided herein is a composition comprising any one of the populations described herein.

[0069] In some examples, provided herein is a composition comprising an engineered primary islet cluster produd by any one of the methods described herein.

[0070] In some examples, provided herein is a composition comprising a population of engineered primary islet cells, wherein the engineered primary islet cells comprise: (i) a transgene comprising an exogenous polynucleotide encoding CD47 and (ii) inactivation or disruption of both alleles of a *B2M* gene. In some examples, the population of engineered primary islet cells is a cluster of primary islet cells. In some examples, the population of engineered primary islet cells is a population of engineered primary beta islet cells.

[0070a] In one aspect, the present disclosure refers to a population of engineered primary islet cells comprising a plurality of engineered primary islet cells, wherein the engineered primary islet cell comprises modifications comprising: (i) a transgene comprising an exogenous polynucleotide encoding CD47, (ii) inactivation or disruption of both alleles of a *B2M* gene, and (iii) inactivation or disruption of both alleles of a *CIITA* gene, wherein: at least 90% of cells in the population comprise reduced expression of one or more MHC class I molecules relative to a primary islet cell that does not comprise the modifications and at least 90% of cells in the population comprise the exogenous polynucleotide encoding CD47.

[0071] In some examples, provided herein is a composition comprising a population of engineered primary T cells, wherein the engineered primary T cells comprise: (i) a transgene comprising an exogenous polynucleotide encoding CD47 and (ii) inactivation or disruption of both alleles of a *B2M* gene.

[0071a] In one aspect, the present disclosure refers to a composition comprising the population disclosed herein.

[0071b] In one aspect, the presemt disclosure refers to a composition comprising a population of engineered primary cells, wherein the engineered primary cells comprise: (i) a transgene comprising an exogenous polynucleotide encoding CD47 and (ii) inactivation or disruption of both alleles of a *B2M* gene, and wherein the primary cells comprise primary islet cells, T cells, thyroid cells, skin cells, endothelial cells, or retinal pigmented cells.

[0072] In some examples, provided herein is a composition comprising a population of engineered primary thyroid cells, wherein the engineered primary thyroid cells comprise: (i) a transgene comprising an exogenous polynucleotide encoding CD47 and (ii) inactivation or disruption of both alleles of a *B2M* gene.

[0073] In some examples, provided herein is a composition comprising a population of engineered primary skin cells, wherein the engineered primary skin cells comprise: (i) a transgene comprising an exogenous polynucleotide encoding CD47 and (ii) inactivation or disruption of both alleles of a *B2M* gene.

[0074] In some examples, provided herein is a composition comprising a population of engineered primary endothelial cells, wherein the engineered primary endothelial cells comprise: (i) a transgene comprising an exogenous polynucleotide encoding CD47 and (ii) inactivation or disruption of both alleles of a *B2M* gene.

[0075] In some examples, provided herein is a composition comprising a population of engineered primary retinal pigmented epithelium cells, wherein the engineered primary retinal pigmented epithelium cells comprise: (i) a transgene comprising an exogenous polynucleotide encoding CD47 and (ii) inactivation or disruption of both alleles of a *B2M* gene.

[0076] In some examples, engineered primary cells of the population of engineered primary cells comprise an indel in both alleles of the *B2M* gene. In some examples, the

engineered primary cells of the population of engineered primary cells further comprise inactivation or disruption of both alleles of a *CIITA* gene. In some examples, engineered primary cells of the population of engineered primary cells comprise an indel in both alleles of the *CIITA* gene. In some examples, the engineered primary cells of the population of engineered primary cells have the phenotype $B2M^{indel/indel}$; $CIITA^{indel/indel}$; CD47*tg*.

[0077] In some examples, the composition is a pharmaceutical composition. In some examples, the composition comprises a pharmaceutically acceptable excipient. In some examples, the pharmaceutically acceptable excipient is a buffered solution, such as saline. In some examples, the composition is formulated in a serum-free cryopreservation medium comprising a cryoprotectant. In some examples, the cryoprotectant is DMSO and the cryopreservation medium is 5% to 10% DMSO (v/v). In some examples, the cryoprotectant is or is about 10% DMSO (v/v). In some examples, the composition is sterile. In some examples, provided herein is a container, comprising any of the compositions described herein. In some examples, the container is a sterile bag. In some examples, the bag is a cryopreservation-compatible bag.

[0078] In some examples, provided herein is a method of treating a disease, condition, or cellular deficiency in a patient in need thereof comprising administering to the patient an effective amount of any of the populations, compositions, or the pharmaceutical compositions described herein. In some examples, the population is formulated as a pharmaceutical composition comprising a pharmaceutically acceptable excipient. In some examples, the population of cells comprises islet cells, including beta islet cells. In some examples, the population of islet cells is administered as a cluster of islet cells. In some examples, the population of islet cells is administered as a cluster of beta islet cells. In some examples, the population of cells are hepatocytes. In some examples, the population of cells comprises T cells. In some examples, the population of cells comprises thyroid cells. In some examples, the population of cells comprises skin cells. In some examples, the population of cells comprises endothelial cells. In some examples, the population of cells comprises retinal pigmented epithelium cells.

[0079] In some examples, the condition or disease is selected from the group consisting of diabetes, cancer, vascularization disorders, ocular disease, thyroid disease, skin diseases, and liver diseases. In some examples, the cellular deficiency is associated with diabetes or the

cellular therapy is for the treatment of diabetes, optionally wherein the diabetes is Type I diabetes.

[0080] In some examples, the population of cells is a population of islet cells, including beta islet cells. In some examples, the population of cells is administered as a cluster of islet cells.

[0081] In some examples provided herein is a method of treating diabetes in a patient in need thereof, the method comprising administering to the patient an effective amount of any of the populations of islet cells, compositions, or the pharmaceutical compositions described herein. In some examples, the cluster of islet cells is a cluster of beta islet cells. In some examples, the cellular deficiency is associated with a vascular condition or disease or the cellular therapy is for the treatment of a vascular condition or disease. In some examples, the population of cells is a population of endothelial cells. In some examples, the cellular deficiency is associated with autoimmune thyroiditis or the cellular therapy is for the treatment of autoimmune thyroiditis. In some examples, the cellular deficiency is associated with a liver disease or the cellular therapy is for the treatment of liver disease. In some examples, the liver disease comprises cirrhosis of the liver. In some examples, the population of cells is a population of hepatocytes. In some examples, the cellular deficiency is associated with a corneal disease or the cellular therapy is for the treatment of corneal disease. In some examples, the corneal disease is Fuchs dystrophy or congenital hereditary endothelial dystrophy. In some examples, the population of cells is a population of corneal endothelial cells. In some examples, the cellular deficiency is associated with a kidney disease or the cellular therapy is for the treatment of a kidney disease. In some examples, the population of cells is a population of renal cells. In some examples, the cellular therapy is for the treatment of a cancer. In some examples, the cancer is selected from the group consisting of B cell acute lymphoblastic leukemia (B-ALL), diffuse large B-cell lymphoma, liver cancer, pancreatic cancer, breast cancer, ovarian cancer, colorectal cancer, lung cancer, non-small cell lung cancer, acute myeloid lymphoid leukemia, multiple myeloma, gastric cancer, gastric adenocarcinoma, pancreatic adenocarcinoma, glioblastoma, neuroblastoma, lung squamous cell carcinoma, hepatocellular carcinoma, and bladder cancer. In some examples, the population of cells is a population of T cells or NK cells. In some examples, the cells are expanded and cryopreserved prior to administration.

[0081a] In one aspect, the present disclosure refers to use of the population disclosed herein, the composition disclosed herein, or the pharmaceutical composition disclosed herein, in the manufacture of a medicament for treating a disease, condition, or cellular deficiency in a patient in need thereof.

[0081b] In one aspect, the present disclosure refers to use of a population of islet cells disclosed herein, the composition disclosed herein, or the pharmaceutical composition disclosed herein, in the manufacture of a medicament for treating diabetes in a patient in need thereof, optionally wherein the population of cells is to be administered as a cluster of islet cells.

[0082] In some examples, administering the population comprises intravenous injection, intramuscular injection, intravascular injection, or transplantation of the population. In some examples, the population is transplanted via kidney capsule transplant or intramuscular injection. In some examples, the population is derived from a donor subject, wherein the HLA type of the donor does not match the HLA type of the patient. In some examples, the population is a human cell population and the patient is a human patient. In some examples, the beta islet cells improve glucose tolerance in the subject. In some examples, the subject is a diabetic patient. In some examples, the diabetic patient has type I diabetes or type II diabetes. In some examples, glucose tolerance is improved relative to the subject's glucose tolerance prior to administration of the islet cells. In some examples, the beta islet cells reduce exogenous insulin usage in the subject. In some examples, glucose tolerance is improved as measured by HbA1c levels. In some examples, the subject is fasting. In some examples, the islet cells improve insulin secretion in the subject. In some examples, insulin secretion is improved relative to the subject's insulin secretion prior to administration of the islet cells.

[0083] In some examples of the method of treating a disease, the method further comprises administering one or more immunosuppressive agents to the patient. In some examples, the patient has been administered one or more immunosuppressive agents. In some examples, the one or more immunosuppressive agents are a small molecule or an antibody. In some examples, the one or more immunosuppressive agents are selected from the group consisting of cyclosporine, azathioprine, mycophenolic acid, mycophenolate mofetil, a corticosteroids, prednisone, methotrexate, gold salts, sulfasalazine, antimalarials, brequinar, leflunomide, mizoribine, 15-deoxyspergualine, 6-mercaptopurine, cyclophosphamide, rapamycin, tacrolimus (FK-506), OKT3, anti-thymocyte globulin, thymopentin (thymosin-α), and an immunosuppressive antibody. In some examples, the one or more immunosuppressive agents comprise cyclosporine. In some examples, the one or more immunosuppressive agents comprise mycophenolate mofetil. In some examples, the one or more immunosuppressive agents comprise a corticosteroid. In some examples, the one or more immunosuppressive agents comprise cyclophosphamide. In some examples, the one or more immunosuppressive agents comprise rapamycin. In some examples, the one or more immunosuppressive agents comprise tacrolimus (FK-506). In some examples, the one or

more immunosuppressive agents comprise anti-thymocyte globulin. In some examples, the one or more immunosuppressive agents are one or more immunomodulatory agents.

[0084] In some examples of the method of treating a disease, the one or more immunomodulatory agents are a small molecule or an antibody. In some examples, the antibody binds to one or more of receptors or ligands selected from the group consisting of p75 of the IL-2 receptor, MHC, CD2, CD3, CD4, CD7, CD28, B7, CD40, CD45, IFN-gamma, TNF-alpha, IL-4, IL-5, IL-6R, IL-6, IGF, IGFR1, IL-7, IL-8, IL-10, CD11a, CD58, and antibodies binding to any of their ligands.

[0085] In some examples of the method of treating a disease, the one or more immunosuppressive agents are or have been administered to the patient prior to administration of the engineered cells. In some examples, the one or more immunosuppressive agents are or have been administered to the patient at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days prior to administration of the engineered cells. In some examples, the one or more immunosuppressive agents are or have been administered to the patient at least 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks or more prior to administration of the engineered cells. In some examples, the one or more immunosuppressive agents are or have been administered to the patient at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days after administration of the engineered cells. In some examples, the one or more immunosuppressive agents are or have been administered to the patient at least 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, or more, after administration of the engineered cells. In some examples, the one or more immunosuppressive agents are or have been administered to the patient on the same day as the first administration of the engineered cells. In some examples, the one or more immunosuppressive agents are or have been administered to the patient after administration of the engineered cells. In some examples, the one or more immunosuppressive agents are or have been administered to the patient after administration of a first and/or second administration of the engineered cells. In some examples, the one or more immunosuppressive agents are or have been administered to the patient prior to administration of a first and/or second administration of the engineered cells. In some examples, the one or more immunosuppressive agents are or have been administered to the patient at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days prior to administration of a first and/or second administration of the engineered cells. In some examples, the one or more

immunosuppressive agents are or have been administered to the patient at least 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks or more prior to administration of a first and/or second administration of the engineered cells. In some examples, the one or more immunosuppressive agents are or have been administered to the patient at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days after administration of a first and/or second administration of the engineered cells. In some examples, the one or more immunosuppressive agents are or have been administered to the patient at least 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, or more, after administration of a first and/or second administration of the engineered cells. In some examples, the one or more immunosuppressive agents are administered at a lower dosage compared to the dosage of one or more immunosuppressive agents administered to reduce immune rejection of immunogenic cells that do not comprise the modifications of the engineered cells.

[0086] In some examples of the method of treating a disease, the engineered cell is capable of controlled killing of the engineered cell. In some examples, the engineered cell comprises a suicide gene or a suicide switch. In some examples, the suicide gene or the suicide switch induces controlled cell death in the presence of a drug or prodrug, or upon activation by a selective exogenous compound. In some examples, the suicide gene or the suicide switch is an inducible protein capable of inducing apoptosis of the engineered cell. In some examples, the inducible protein capable of inducing apoptosis of the engineered cell is a caspase protein. In some examples, the caspase protein is caspase 9. In some examples, the suicide gene or suicide switch is selected from the group consisting of cytosine deaminase (CyD), herpesvirus thymidine kinase (HSV-Tk), an inducible caspase 9 (iCaspase9), and rapamycin-activated caspase 9 (rapaCasp9). In some examples, the suicide gene or the suicide switch is activated to induce controlled cell death after the administration of the one or more immunosuppressive agents to the patient. In some examples, the suicide gene or the suicide switch is activated to induce controlled cell death prior to the administration of the one or more immunosuppressive agents to the patient. In some examples, the suicide gene or the suicide switch is activated to induce controlled cell death after the administration of the engineered cell to the patient. In some examples, the suicide gene or the suicide switch is activated to induce controlled cell death in the event of cytotoxicity or other negative consequences to the patient.

[0087] In some examples of the method of treating a disease, the method comprises administering an agent that allows for depletion of an engineered cell of the population of engineered cells. In some examples, the agent that allows for depletion of the engineered cell is an antibody that recognizes a protein expressed on the surface of the engineered cell. In some examples, the antibody is selected from the group consisting of an antibody that recognizes CCR4, CD16, CD19, CD20, CD30, EGFR, GD2, HER1, HER2, MUC1, PSMA, and RQR8. In some examples, the antibody is selected from the group consisting of mogamulizumab, AFM13, MOR208, obinutuzumab, ublituximab, ocaratuzumab, rituximab, rituximab-Rllb, tomuzotuximab, RO5083945 (GA201), cetuximab, Hul4.18K322A, Hul4.18-IL2, Hu3F8, dinituximab, c.60C3-Rllc, and biosimilars thereof.

[0088] In some examples of the method of treating a disease, the method comprises administering an agent that recognizes the one or more tolerogenic factors on the surface of the engineered cell. In some examples, the engineered cell is engineered to express the one or more tolerogenic factors. In some examples, the one or more tolerogenic factors is CD47.

[0089] In some examples of the method of treating a disease, the method further comprises administering one or more additional therapeutic agents to the patient. In some examples, the patient has been administered one or more additional therapeutic agents.

[0090] In some examples of the method of treating a disease, the method further comprises monitoring the therapeutic efficacy of the method. In some examples, the method further comprises monitoring the prophylactic efficacy of the method. In some examples, the method is repeated until a desired suppression of one or more disease symptoms occurs.

[0091] In some examples of the engineered cell, the engineered cell comprises an exogenous polynucleotide encoding a suicide gene or a suicide switch. In some examples, the suicide gene or suicide switch is selected from the group consisting of cytosine deaminase (CyD), herpesvirus thymidine kinase (HSV-Tk), an inducible caspase 9 (iCaspase9), and rapamycin-activated caspase 9 (rapaCasp9). In some examples, the suicide gene or suicide switch and genes associated with the suicide gene or the safety switch are expressed from a bicistronic cassette integrated into the genome of the engineered cell. In some examples, the suicide gene or suicide switch and the one or more tolerogenic factors are expressed from a bicistronic cassette integrated into the genome of the engineered cell. In some examples, the bicistronic cassette is integrated by non-targeted insertion into the genome of the engineered cell, optionally by introduction of the exogenous polynucleotide into the cell using a lentiviral

vector. In some examples, the bicistronic cassette is integrated by targeted insertion into a target genomic locus of the engineered cell, optionally wherein the targeted insertion is by nuclease-mediated gene editing with homology-directed repair. In some examples, the one or more tolerogenic factors is CD47.

[0092] In some examples of the method of generating an engineered cell, the engineered cell comprises an exogenous polynucleotide encoding a suicide gene or suicide switch. In some examples, the suicide gene is selected from the group consisting of cytosine deaminase (CyD), herpesvirus thymidine kinase (HSV-Tk), an inducible caspase 9 (iCaspase9), and rapamycin-activated caspase 9 (rapaCasp9). In some examples, the suicide gene or suicide switch and genes associated with the suicide gene or the safety switch are expressed from a bicistronic cassette integrated into the genome of the engineered cell. In some examples, the suicide gene or suicide switch and the one or more tolerogenic factors are expressed from a bicistronic cassette integrated into the genome of the engineered cell. In some examples, the bicistronic cassette is integrated by non-targeted insertion into the genome of the engineered cell. In some examples, the bicistronic cassette is integrated by targeted insertion into a target genomic locus of the engineered cell. In some examples, the one or more tolerogenic factors is CD47.

[0093] In some examples of the composition of engineered cells, engineered cells of the population of engineered cells comprise an exogenous polynucleotide encoding a suicide gene or a suicide switch. In some examples, the suicide gene or suicide switch is selected from the group consisting of cytosine deaminase (CyD), herpesvirus thymidine kinase (HSV-Tk), an inducible caspase 9 (iCaspase9), and rapamycin-activated caspase 9 (rapaCasp9). In some examples, the suicide gene and genes associated with the suicide gene or the safety switch are expressed from a bicistronic cassette integrated into the genome of engineered cells of the population of engineered cells. In some examples, the suicide gene or suicide switch and the exogenous CD47 are expressed from a bicistronic cassette integrated into the genome of the engineered cell. In some examples, the bicistronic cassette is integrated by non-targeted insertion into the genome, optionally by introduction of the exogenous polynucleotide into engineered cells of the population of engineered cells using a lentiviral vector. In some examples, the bicistronic cassette is integrated by targeted insertion into a target genomic locus of engineered cells of the population of engineered cells, optionally wherein the targeted insertion is by nuclease-mediated gene editing with homology-directed repair.