INDUCIBLE SYSTEMS FOR ALTERING GENE EXPRESSION IN HYPOIMMUNOGENIC CELLS

CROSS-REFERNCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 63/232,141, filed August 11, 2021, and U.S. Provisional Application No. 63/270,454, filed October 21, 2021, the disclosure of each of which is herein incorporated in its entirety.

SUMMARY

[0002] Off-the-shelf therapeutic cells can offer advantages over autologous cell-based strategies, including ease of manufacturing, quality control and avoidance of malignant contamination and T cell dysfunction. However, the vigorous host-versus-graft immune response against histoincompatible cells prevents expansion and persistence of allogeneic cells and mitigates the efficacy of this approach.

[0003] There is substantial evidence in both animal models and human patients that hypoimmunogenic cell transplantation is a scientifically feasible and clinically promising approach to the treatment of numerous disorders, conditions, and diseases.

[0004] There remains a need for novel approaches, compositions and methods for producing cell-based therapies that avoid detection by the recipient's immune system.

[0005] In some embodiments, provided herein is an engineered cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

[0006] In some embodiments, provided herein is an engineered cell comprising regulatable modifications that increase expression of CD47, relative to a control.

[0007] In some embodiments, the engineered cell is selected from the group consisting of a stem cell, a pluripotent stem cell (PSC), an induced pluripotent stem cell (iPSC), a mesenchymal stem cell (MSC), a hematopoietic stem cell (HSC), an embryonic stem cell (ESC), pancreatic islet cell, a beta islet cell, an immune cell, a B cell, a T cell, a natural killer (NK) cell, a natural killer T (NKT) cell, a macrophage cell, an immune privileged cell, an optic cell, a retinal

pigmented epithelium cell (RPE), a hepatocyte, a thyroid cell, an endothelial cell, a skin cell, a glial progenitor cell, a neural cell, a muscle cell, a cardiac cell, and a blood cell.

[0008] In some embodiments, provided herein is an engineered pancreatic islet cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

[0009] In some embodiments, the pancreatic islet cell is a beta islet cell.

[0010] In some embodiments, provided herein is an engineered endothelial cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

[0011] In some embodiments, provided herein is an engineered cardiac muscle cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

[0012] In some embodiments, provided herein is an engineered smooth muscle cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

[0013] In some embodiments, provided herein is an engineered skeletal muscle cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

[0014] In some embodiments, provided herein is an engineered hepatocyte comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

[0015] In some embodiments, provided herein is an engineered glial progenitor cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC

class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

[0016] In some embodiments, provided herein is an engineered dopaminergic neuron comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

[0017] In some embodiments, provided herein is an engineered immune privileged cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

[0018] In some embodiments, provided herein is an engineered retinal pigment epithelial cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

[0019] In some embodiments, provided herein is an engineered thyroid cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

[0020] In some embodiments, provided herein is an engineered immune cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

[0021] In some embodiments, the engineered immune cell comprises an exogenous polynucleotide encoding one or more chimeric antigen receptors (CARs).

[0022] In some embodiments, provided herein is an engineered T cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

[0023] In some embodiments, the engineered T cell comprises an exogenous polynucleotide encoding one or more chimeric antigen receptors (CARs).

[0024] In some embodiments, provided herein is an engineered NK cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

[0025] In some embodiments, the engineered T cell comprises an exogenous polynucleotide encoding one or more chimeric antigen receptors (CARs).

[0026] In some embodiments, provided herein is an engineered macrophage cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

[0027] In some embodiments, the cell expresses at least about the same amount of CD47, relative to the control.

[0028] In some embodiments, the cell is an immune privileged cell.

[0029] In some embodiments, the cell expresses at least about a 10% higher amount of CD47, relative to the control.

[0030] In some embodiments, the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, higher amount of CD47, relative to the control.

[0031] In some embodiments, the cell expresses at least about a 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, or 900%, higher amount of CD47, relative to the control.

[0032] In some embodiments, the cell expresses at least about a 1000% higher amount of CD47, relative to the control.

[0033] In some embodiments, the cell expresses at least about 1.1-fold of the level of CD47 expressed in the control.

[0034] In some embodiments, the cell expresses at least about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, or about 5-fold of the level of CD47 expressed in the control.

[0035] In some embodiments, the cell expresses at least about 4-fold, about 4.5-fold, about 5-fold, or about 5.5-fold of the level of CD47 expressed in the control.

[0036] In some embodiments, the cell expresses at least about about 4-fold of the level of CD47 expressed in the control.

[0037] In some embodiments, the cell expresses at least about about 4.5-fold of the level of CD47 expressed in the control.

[0038] In some embodiments, the cell expresses at least about about 5-fold of the level of CD47 expressed in the control.

[0039] In some embodiments, the cell expresses at least about about 5.5-fold of the level of CD47 expressed in the control.

[0040] In some embodiments, the cell expresses at least about about 16-fold, about 17-fold, about 18-fold, about 19-fold, or about 20-fold of the level of CD47 expressed in the control.

[0041] In some embodiments, the control is a wild-type cell, a control cell, or a baseline reference.

[0042] In some embodiments, the control cell is an unmodified or unaltered cell, optionally wherein the unmodified or unaltered cell is of the same cell type as the engineered cell.

[0043] In some embodiments, the control cell is a starting material from a donor or a pool of starting cells from a pool of donors.

[0044] In some embodiments, the baseline reference is an isotype control or a background signal level.

[0045] In some embodiments, the baseline is an isotype control, optionally wherein the CD47 level is determined using an antibody-based assay.

[0046] In some embodiments, the CD47 level is determined using an antibody-based quantitation method, optionally a Quantibrite™ assay.

[0047] In some embodiments, the engineered cell is a beta islet cell that expresses at least about 200,000, 250,000, 300,000, 350,000, or 400,000 CD47 molecules per cell.

[0048] In some embodiments, engineered cell is a retinal pigment epithelial cell that expresses at least about a 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 12-fold, 14-fold, 16-fold, 18-fold, 20-fold, or higher increase in CD47 expression over baseline.

[0049] In some embodiments, the engineered cell is a T cell that expresses at least about 180,000, 190,000, 200,000, 210,000, 220,000, 230,000, 240,000, 250,000, 260,000, 270,000, 280,000, 290,000, 300,000, 350,000, 400,000, 450,000, 500,000, 550,000, 600,000, 650,000, or 700,000 CD47 molecules per cell.

[0050] In some embodiments, provided herein is an engineered cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II human leukocyte antigens, and ii) increase expression of one or more tolerogenic factors, relative to a control, wherein the engineered cell expresses the tolerogenic factor at a threshold level or higher.

[0051] In some embodiments, the engineered cell is selected from the group consisting of a stem cell, a pluripotent stem cell (PSC), an induced pluripotent stem cell (iPSC), a mesenchymal stem cell (MSC), a hematopoietic stem cell (HSC), an embryonic stem cell (ESC), pancreatic islet cell, a beta islet cell, an immune cell, a B cell, a T cell, a natural killer (NK) cell, a natural killer T (NKT) cell, a macrophage cell, an immune privileged cell, an optic cell, a retinal pigmented epithelium cell (RPE), a hepatocyte, a thyroid cell, an endothelial cell, a skin cell, a glial progenitor cell, a neural cell, a muscle cell, a cardiac cell, and a blood cell.

[0052] In some embodiments, the control is a wild-type cell, a control cell, or a baseline reference.

[0053] In some embodiments, the control cell is an unmodified or unaltered cell, optionally wherein the unmodified or unaltered cell is of the same cell type as the engineered cell.

[0054] In some embodiments, the control cell is a starting material from a donor or a pool of starting cells from a pool of donors.

[0055] In some embodiments, the baseline reference is an isotype control or a background signal level.

[0056] In some embodiments, the baseline is an isotype control, optionally wherein the amount of the tolerogenic factor is determined using an antibody-based assay.

[0057] In some embodiments, the amount of the tolerogenic factor is determined using an antibody-based quantitation method, optionally a Quantibrite™ assay.

[0058] In some embodiments, the cell expresses at least about the same amount of tolerogenic factor, relative to the control.

[0059] In some embodiments, the cell is an immune privileged cell.

[0060] In some embodiments, the cell expresses at least about a 10% higher amount of the tolerogenic factor, relative to the control.

[0061] In some embodiments, the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, higher amount of the tolerogenic factor, relative to the control.

[0062] In some embodiments, the cell expresses at least about a 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, or 900%, higher amount of the tolerogenic factor, relative to the control.

[0063] In some embodiments, the cell expresses at least about a 1000% higher amount of the tolerogenic factor, relative to the control.

[0064] In some embodiments, the cell expresses at least about 1.1-fold of the level of the tolerogenic factor expressed in the control.

[0065] In some embodiments, the cell expresses at least about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, or about 5-fold of the level of the tolerogenic factor expressed in the control.

[0066] In some embodiments, the cell expresses at least about 4-fold, about 4.5-fold, about 5-fold, or about 5.5-fold of the level of the tolerogenic factor expressed in the control.

[0067] In some embodiments, the cell expresses at least about about 4-fold of the level of the tolerogenic factor expressed in the control.

[0068] In some embodiments, the cell expresses at least about about 4.5-fold of the level of the tolerogenic factor expressed in the control.

[0069] In some embodiments, the cell expresses at least about about 5-fold of the level of the tolerogenic factor expressed in the control.

[0070] In some embodiments, the cell expresses at least about about 5.5-fold of the level of the tolerogenic factor expressed in the control.

[0071] In some embodiments, the cell expresses at least about about 16-fold, about 17-fold, about 18-fold, about 19-fold, or about 20-fold of the level of the tolerogenic factor expressed in the control.

[0072] In some embodiments, the modifications reduce expression of: (a) MHC class I molecule; (b) MHC class II molecule; or (c) MHC class I molecule and MHC class II molecule.

[0073] In some embodiments, the modifications reduce 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, relative to a control.

[0074] In some embodiments, the cell does not express MHC class I molecule and/or MHC class II molecule.

[0075] In some embodiments, the cell does not express 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, relative to a control.

[0076] In some embodiments, the modifications comprise knock out of one or more targets selected from the group consisting 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.

[0077] In some embodiments, the modifications reduce expression of one or more targets selected from the group consisting of B2M, TAP1, NLRC5 and/or CIITA.

[0078] In some embodiments, the modifications comprise knock out of one or more targets selected from the group consisting of B2M, TAP1, NLRC5 and/or CIITA.

[0079] In some embodiments, the knock out occurs in both alleles.

[0080] In some embodiments, the cell further comprises one or more modifications that reduce expression of CTLA-4, PD-1, IRF1, MIC-A, MIC-B, a protein that is involved in

oxidative or ER stress, TRAC, TRB, CD142, ABO, CD38, PCDH11Y, NLGN4Y and/or RHD, relative to a control.

[0081] In some embodiments, the protein that is involved in oxidative or ER stress is selected from the group consisting of thioredoxin-interacting protein (TXNIP), PKR-like ER kinase (PERK), inositol-requiring enzyme 1α (IRE1α), and DJ-1 (PARK7).

[0082] In some embodiments, the modifications comprise knock out of one or more targets selected from the group consisting of CTLA-4, PD-1, IRF1, MIC-A, MIC-B, a protein that is involved in oxidative or ER stress, TRAC, TRB, CD142, ABO, CD38, PCDH11Y, NLGN4Y and/or RHD.

[0083] In some embodiments, the knock out occurs in both alleles.

[0084] In some embodiments, the modifications reduce expression of B2M.

[0085] In some embodiments, the modifications reduce expression of CIITA.

[0086] In some embodiments, the modifications reduce expression of B2M and CIITA.

[0087] In some embodiments, the modifications comprise knock out of B2M and/or CIITA.

[0088] In some embodiments, the B2M and/or CIITA knock out occurs in both alleles.

[0089] In some embodiments, the modifications reduce expression of a NK cell ligand, optionally MIC-A and/or MIC-B.

[0090] In some embodiments, the modifications comprise knock out of MIC-A and/or MIC-B.

[0091] In some embodiments, the MIC-A and/or MIC-B knock out occurs in both alleles.

[0092] In some embodiments, the cell further comprises a modification that reduces expression of one or more Y chromosome genes, relative to a control.

[0093] In some embodiments, the one or more Y chromosome genes are selected from the group consisting of Protocadherin-11 Y-linked and Neuroligin-4 Y-linked.

[0094] In some embodiments, the modifications reduce expression of TXNIP.

[0095] In some embodiments, the modifications comprise knock out of TXNIP.

[0096] In some embodiments, the TXNIP knock out occurs in both alleles.

[0097] In some embodiments, the cell further comprises modifications that reduce 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, a protein that is involved in oxidative or ER stress, TRAC, TRB, CD142, ABO, CD38, PCDH11Y, NLGN4Y and/or RHD.

[0098] In some embodiments, the cell does not express 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, a protein that is involved in oxidative or ER stress, TRAC, TRB, CD142, ABO, CD38, PCDH11Y, NLGN4Y and/or RHD.

[0099] In some embodiments, the cell further comprises modifications that reduce expression of B2M, CIITA, NLRC5, TRAC, TRB, CD142, ABO, MIC-A/B, CD38, PCDH11Y, NLGN4Y and/or RHD, relative to a control.

[00100] In some embodiments, the cell does not express B2M, CIITA, NLRC5, TRAC, TRB, CD142, ABO, MIC-A/B, CD38, CD52, PCDH11Y, NLGN4Y and/or RHD.

[00101] In some embodiments, the cell comprises further modifications that reduce expression of one or more tolerogenic factors.

[00102] In some embodiments, the one or more tolerogenic factors are selected from the group consisting of 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.

[00103] In some embodiments, the one or more tolerogenic factors are selected from the group consisting of A20/TNFAIP3, C1-Inhibitor, CCL21, CCL22, CD16, CD16 Fc receptor, CD24, CD27, CD35, CD39, CD46, CD47, 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.

[00104] In some embodiments, the one or more tolerogenic factors comprise CD47.

[00105] In some embodiments, the one or more tolerogenic factors comprise HLA-E.

[00106] In some embodiments, the one or more tolerogenic factors comprise CD24.

[00107] In some embodiments, the one or more tolerogenic factors comprise PD-L1.

[00108] In some embodiments, the one or more tolerogenic factors comprise CD46.

[00109] In some embodiments, the one or more tolerogenic factors comprise CD55.

[00110] In some embodiments, the one or more tolerogenic factors comprise CD59.

[00111] In some embodiments, the one or more tolerogenic factors comprise CR1.

[00112] In some embodiments, the one or more tolerogenic factors comprise MANF.

[00113] In some embodiments, the one or more tolerogenic factors comprise A20/TNFAIP3.

[00114] In some embodiments, the one or more tolerogenic factors comprise HLA-E and CD47.

[00115] In some embodiments, the one or more tolerogenic factors comprise one or more of CD24, CD47, and/or PDL1.

[00116] In some embodiments, the one or more tolerogenic factors comprise one or more of HLA-E, CD24, CD47, and/or PDL1.

[00117] In some embodiments, the one or more tolerogenic factors comprise one or more of CD46, CD55, CD59, and/or CR1.

[00118] In some embodiments, the one or more tolerogenic factors comprise one or more of HLA-E, CD46, CD55, CD59, and/or CR1.

[00119] In some embodiments, the one or more tolerogenic factors comprise one or more of HLA-E, CD24, CD47, PDL1, CD46, CD55, CD59, and/or CR1.

[00120] In some embodiments, the one or more tolerogenic factors comprise HLA-E and PDL1.

[00121] In some embodiments, the one or more tolerogenic factors comprise one or more of HLA-E, PDL1, and/or A20/TNFAIP.

[00122] In some embodiments, the one or more tolerogenic factors comprise one or more of HLA-E, PDL1, and/or MANF.

[00123] In some embodiments, the one or more tolerogenic factors comprise one or more of HLA-E, PDL1, A20/TNFAIP, and/or MANF.

[00124] In some embodiments, the modifications: (a) reduce expression of MHC class I and/or MHC class II molecules; (b) reduce expression of MIC-A and/or MIC-B; (c) increase expression of CD47, and optionally CD24 and PD-L1; and (d) increase expression of CD46, CD55, CD59 and CR1.

[00125] In some embodiments, the modification: (a) reduce expression of MHC class I molecule; (b) reduce expression of MIC-A and/or MIC-B; (c) reduce expression of TXNIP; and (d) increase expression of PD-L1 and HLA-E.

[00126] In some embodiments, the modifications further increase expression of A20/TNFAIP3 and MANF.

[00127] In some embodiments, the cell is derived from a human cell or an animal cell.

[00128] In some embodiments, the cell is a differentiated cell derived from a stem cell or a progeny thereof.

[00129] In some embodiments, the stem cell is selected from the group consisting of a pluripotent stem cell, an induced pluripotent stem cell (iPSC), a mesenchymal stem cell (MSC), a hematopoietic stem cell (HSC), and an embryonic stem cell (ESC).

[00130] In some embodiments, the cell is derived from a primary cell or a progeny thereof.

[00131] In some embodiments, the cell evades NK cell mediated cytotoxicity upon administration to a recipient patient.

[00132] In some embodiments, the cell is protected from cell lysis by mature NK cells upon administration to a recipient patient.

[00133] In some embodiments, the cell evades macrophage engulfment upon administration to a recipient patient.

[00134] In some embodiments, the cell does not induce an innate and/or an adaptive immune response to the cell upon administration to a recipient patient.

[00135] In some embodiments, the cell does not induce an antibody-based immune response to the cell upon administration to a recipient patient.

[00136] In some embodiments, one or more of the modifications is a regulatable modification.

[00137] In some embodiments, provided herein is an engineered cell comprising one or more regulatable modifications to alter the expression of one or more targets in the engineered cell, relative to a control, optionally wherein the one or more regulatable modifications increase expression of a CD47, relative to a control.

[00138] In some embodiments, the one or more regulatable modifications comprise a conditional or inducible RNA-based component for i) increasing or ii) reducing or knocking out expression of the one or more targets, relative to a control.

[00139] In some embodiments, the conditional or inducible RNA-based component is selected from the group consisting of conditional or inducible shRNAs, conditional or inducible siRNAs, conditional or inducible miRNAs, and conditional or inducible CRISPR interference (CRISPRi).

[00140] In some embodiments, the conditional RNA-based component is under the control of a conditional promoter selected from the group consisting of a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, and a differentiation-induced promoter.

[00141] In some embodiments, the inducible RNA-based component is under the control of an inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[00142] In some embodiments, the regulatable modifications comprise a conditional or inducible DNA-based component for i) increasing or ii) reducing or knocking out expression of the one or more targets, relative to a control.

[00143] In some embodiments, the conditional or inducible DNA-based component is selected from the group consisting of conditional or inducible CRISPRs, conditional or inducible TALENs, conditional or inducible zinc finger nucleases, conditional or inducible homing endonucleases, conditional or inducible prime editing, conditional or inducible PASTE editing, and conditional or inducible meganucleases.

[00144] In some embodiments, the conditional DNA-based component is under the control of a conditional promoter selected from the group consisting of a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, and a differentiation-induced promoter.

[00145] In some embodiments, the conditional DNA-based component is under the control of an inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[00146] In some embodiments, the regulatable modifications comprise a conditional or inducible protein-based component for i) increasing or ii) reducing or knocking out expression of the one or more targets, relative to a control.

[00147] In some embodiments, the conditional or inducible protein-based component is a conditional or inducible degron component.

[00148] In some embodiments, the conditional or inducible degron component is selected from the group consisting of ligand induced degradation (LID) using a SMASH tag, LID using Shield-1, LID using auxin, LID using rapamycin, conditional or inducible peptidic degrons (*e.g.*, IKZF3 based degrons), and conditional or inducible proteolysis-targeting chimeras (PROTACs).

[00149] In some embodiments, the conditional protein-based component is under the control of a conditional promoter selected the group consisting of from a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, and a differentiation-induced promoter.

[00150] In some embodiments, the protein-based component is under the control of an inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[00151] In some embodiments, the cell comprises a conditional promoter operably linked to an exogenous polynucleotide encoding the one or more tolerogenic factors or the CD47.

[00152] In some embodiments, the cell comprises (i) an exogenous polynucleotide comprising a conditional promoter operably linked to a transposase, and (ii) an exogenous polynucleotide comprising a transposon comprising a cargo polynucleotide encoding the one or more tolerogenic factors or the CD47.

[00153] In some embodiments, the conditional promoter is selected from the group consisting of a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, and a differentiation-induced promoter.

[00154] In some embodiments, the cell comprises an inducible promoter operably linked to an exogenous polynucleotide encoding the one or more tolerogenic factors or the CD47.

[00155] In some embodiments, the cell comprises (i) an exogenous polynucleotide comprising an inducible promoter operably linked to a transposase, and (ii) an exogenous polynucleotide comprising a transposon comprising a cargo polynucleotide encoding the one or more tolerogenic factors or the CD47.

[00156] In some embodiments, the inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[00157] In some embodiments, the cell comprises a CD47 polypeptide having at least 80%, 85%, 90%, 95%, 98%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:129.

[00158] In some embodiments, the cell comprises a CD47 polypeptide having at least 80%, 85%, 90%, 95%, 98%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:130.

[00159] In some embodiments, the cell further comprises regulatable modifications that increase expression of one or more of 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, relative to a control.

[00160] In some embodiments, the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, higher amount of 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, relative to a control.

[00161] In some embodiments, the cell expresses at least about a 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, or 900%, higher amount of 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, relative to a control.

[00162] In some embodiments, the cell expresses at least about a 1000% higher amount of 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, relative to a control.

[00163] In some embodiments, the control is a wild-type cell, a control cell, or a baseline reference.

[00164] In some embodiments, the control cell is an unmodified or unaltered cell, optionally wherein the unmodified or unaltered cell is of the same cell type as the engineered cell.

[00165] In some embodiments, the control cell is a starting material from a donor or a pool of starting cells from a pool of donors.

[00166] In some embodiments, the baseline reference is an isotype control or a background signal level.

[00167] In some embodiments, the one or more tolerogenic factors or the CD47 is encoded by a first exogenous polynucleotide.

[00168] In some embodiments, the cell comprises a second exogenous polynucleotide encoding one or more chimeric antigen receptors (CARs).

[00169] In some embodiments, the first and/or second exogenous polynucleotide is inserted into a first and/or second specific locus of at least one allele of the cell.

[00170] In some embodiments, the first and/or second specific loci are selected from the group consisting of a safe harbor locus, a target locus, an *RHD* locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus, and a *TRB* locus.

[00171] In some embodiments, the safe harbor locus is selected from the group consisting of a *CCR5* locus, a *PPP1R12C* locus, a *Rosa* locus, a *ROSA26* gene locus, and a *CLYBL* locus.

[00172] In some embodiments, the target locus is selected from the group consisting of a *CXCR4* locus, an *ALB* locus, a *SHS231* locus, an *F3* (*CD142*) locus, a *MICA* locus, a *MICB* locus, a *LRP1* (*CD91*) locus, a *HMGB1* locus, an *ABO* locus, a *FUT1* locus, and a *KDM5D* locus.

[00173] In some embodiments, the first and/or second exogenous polynucleotide is introduced into the cell using a lentiviral vector.

[00174] In some embodiments, the first and/or second exogenous polynucleotide is introduced into the cell using fusogen-mediated delivery or a transposase system selected from the group consisting of conditional or inducible transposases, conditional or inducible PiggyBac transposons, conditional or inducible Sleeping Beauty (SB11) transposons, conditional or inducible Mos1 transposons, and conditional or inducible Tol2 transposons.

[00175] In some embodiments, provided herein is a pancreatic islet cell having reduced expression of MHC class I HLA and/or reduced expression of MHC class II HLA and that expresses at least about a 1000% higher amount of CD47, relative to a control.

[00176] In some embodiments, the cell is a primary beta islet cell that expresses at least about 16-fold, about 17-fold, about 18-fold, about 19-fold, or about 20-fold of the level of CD47 expressed in a control.

[00177] In some embodiments, provided herein is an engineered that expresses at least about a 10% higher amount of CD47, relative to a control, or that expresses at least about 1.1-fold of the level of CD47 expressed in a control.

[00178] In some embodiments, provided herein is an engineered cell that expresses at least about a 10% higher amount of CD47, relative to a control, or that expresses at least about 1.1-fold of the level of CD47 expressed in a control.

[00179] In some embodiments, the cell expresses at least about a 20%, about a 30%, about a 40%, about a 50%, about a 60%, about a 70%, about a 80%, about a 90%, about a 100%, about a 200%, about a 300%, about a 400%, about a 500%, about a 600%, about a 700%, about a 800%, about a 900%, or about a 1000% higher amount of CD47, relative to the control.

[00180] In some embodiments, the cell expresses at least about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, or about 5-fold of the level of CD47 expressed in the control.

[00181] In some embodiments, the cell is a primary pancreatic islet cell that expresses at least about a 1000% or at least about a 2000% higher amount of CD47, relative to a control.

[00182] In some embodiments, the control is a wild-type cell, a control cell, or a baseline reference.

[00183] In some embodiments, the control cell is an unmodified or unaltered cell, optionally wherein the unmodified or unaltered cell is of the same cell type as the engineered cell.

[00184] In some embodiments, the control cell is a starting material from a donor or a pool of starting cells from a pool of donors.

[00185] In some embodiments, the baseline reference is an isotype control or a background signal level.

[00186] In some embodiments, the CD47 level is determined using an antibody-based quantitation method, optionally a Quantibrite™ assay.

[00187] In some embodiments, provided herein is an engineered T cell having reduced expression of MHC class I HLA and/or reduced expression of MHC class II HLA and that expresses at least about a 10% higher amount of CD47, relative to a control, that expresses at least about 1.1-fold of the level of CD47 expressed in a control, or that expresses at least about 170,000 CD47 molecules.

[00188] In some embodiments, the cell is a T cell that expresses at least about a 300% or at least about a 400% higher amount of CD47, relative to a control.

[00189] In some embodiments, the cell is a T cell that expresses at least about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, or about 5-fold of the level of CD47 expressed in the control.

[00190] In some embodiments, provided herein is an engineered T cell that expresses at least about 170,000 CD47 molecules.

[00191] In some embodiments, the T cell expresses at least about 180,000 CD47 molecules, at least about 190,000 CD47 molecules, at least about 200,000 CD47 molecules, at least about 210,000 CD47 molecules, at least about 220,000 CD47 molecules, at least about 230,000 CD47 molecules, at least about 240,000 CD47 molecules, at least about 250,000 CD47 molecules, at least about 260,000 CD47 molecules, at least about 270,000 CD47 molecules, at least about 280,000 CD47 molecules, at least about 290,000 CD47 molecules, or at least about 300,000 CD47 molecules.

[00192] In some embodiments, the control is a wild-type cell, a control cell, or a baseline reference.

[00193] In some embodiments, the control cell is an unmodified or unaltered cell, optionally wherein the unmodified or unaltered cell is of the same cell type as the engineered cell.

[00194] In some embodiments, the control cell is a starting material from a donor or a pool of starting cells from a pool of donors.

[00195] In some embodiments, the baseline reference is an isotype control or a background signal level.

[00196] In some embodiments, the CD47 level is determined using an antibody-based quantitation method, optionally a Quantibrite™ assay.

[00197] In some embodiments, the cell comprises 1, 2, 3, 4, or 5 copies of an exogenous polynucleotide encoding CD47.

[00198] In some embodiments, the cell comprises a constitutive promoter operably linked to an exogenous polynucleotide encoding CD47.

[00199] In some embodiments, an exogenous polynucleotide encoding CD47 is delivered to the cell via viral mediated integration.

[00200] In some embodiments, the viral mediated integration is lentivirus mediated.

[00201] In some embodiments, an exogenous polynucleotide encoding CD47 is integrated at a site in the cell genome via HDR.

[00202] In some embodiments, the exogenous polynucleotide encoding CD47 is integrated into a locus in the TRAC gene, a locus in the TRBC gene, or a combination thereof.

[00203] In some embodiments, the exogenous polynucleotide encoding CD47 is integrated into at least one TRAC allele, at least one TRBC allele, or a combination thereof.

[00204] In some embodiments, the exogenous polynucleotide encoding CD47 is integrated into at least two TRAC alleles, at least two TRBC alleles, or a combination thereof.

[00205] In some embodiments, the cell comprises an exogenous polynucleotide comprising a CD47 polypeptide having at least about 80% sequence identity to the amino acid sequence of SEQ ID NO:129, at least about 85% sequence identity to the amino acid sequence of SEQ ID NO:129, at least about 90% sequence identity to the amino acid sequence of SEQ ID NO:129, at least about 95% sequence identity to the amino acid sequence of SEQ ID NO:129, at least about

98% sequence identity to the amino acid sequence of SEQ ID NO:129, at least about 99% sequence identity to the amino acid sequence of SEQ ID NO:129, or having the amino acid sequence of SEQ ID NO:129.

[00206] In some embodiments, the cell comprises an exogenous polynucleotide comprising a CD47 polypeptide having at least about 80% sequence identity to the amino acid sequence of SEQ ID NO:130, at least about 85% sequence identity to the amino acid sequence of SEQ ID NO:130, at least about 90% sequence identity to the amino acid sequence of SEQ ID NO:130, at least about 95% sequence identity to the amino acid sequence of SEQ ID NO:130, at least about 98% sequence identity to the amino acid sequence of SEQ ID NO:130, at least about 99% sequence identity to the amino acid sequence of SEQ ID NO:130, or having the amino acid sequence of SEQ ID NO:130.

[00207] In some embodiments, the cell comprises reduced expression of one or more MHC class I and/or MHC class II molecules, relative to a control.

[00208] In some embodiments, the reduced expression of the one or more MHC class I and/or MHC class II molecules, is caused by constitutve modifications to one or more genes encoding the MHC class I and/or class II HLA.

[00209] In some embodiments, the cell comprises one or more knock outs of targets selected from the group consisting of MHC class I and MHC class II HLA.

[00210] In some embodiments, the one or more knock outs are constitutive knock outs.

[00211] In some embodiments, the cell comprises reduced expression of one or more targets selected from the group consisting of B2M and CIITA, relative to the control.

[00212] In some embodiments, the reduced expression of B2M and/or CIITA is caused by constitutive modifications to the B2M gene and/or the CIITA gene.

[00213] In some embodiments, the cell comprises one or more knock outs of targets selected from the group consisting of B2M and CIITA.

[00214] In some embodiments, the cell comprises knock outs of both alleles of B2M and/or both alleles of CIITA.

[00215] In some embodiments, the one or more knock outs are constitutive knock outs.

[00216] In some embodiments, the cell further comprises an exogenous polynucleotide encoding one or more further tolerogenic factors.

[00217] In some embodiments, the one or more further tolerogenic factors are selected from the group consisting of HLA-C, HLA-E, HLA-F, HLA-G, PD-L1, CTLA-4-Ig, CI-inhibitor, and IL-35.

[00218] In some embodiments, the cell comprises reduced expression of B2M, CIITA, NLRC5, TRAC, TRB, CD142, ABO, MIC-A/B, CD38, CD52, PCDH11Y, NLGN4Y and/or RHD, relative to the control.

[00219] In some embodiments, the cell does not express B2M, CIITA, NLRC5, TRAC, TRB, CD142, ABO, MIC-A/B, CD38, CD52, PCDH11Y, NLGN4Y and/or RHD.

[00220] In some embodiments, the cell is a pluripotent stem cell.

[00221] In some embodiments, the pluripotent stem cell is an induced pluripotent stem cell (iPSC), a mesenchymal stem cell (MSC), a hematopoietic stem cell (HSC), or an embryonic stem cell (ESC).

[00222] In some embodiments, the cell is a differentiated cell derived from a pluripotent stem cell or a progeny thereof.

[00223] In some embodiments, the differentiated cell is selected from the group consisting of a pancreatic islet cell, a T cell, a natural killer (NK) cell, a CAR-M cell, an endothelial cell, a cardiac muscle cell, a smooth muscle cell, a skeletal muscle cell, a hepatocyte, a glial progenitor cell, a dopaminergic neuron, a retinal pigment epithelial cell, and a thyroid cell.

[00224] In some embodiments, the cell is a primary cell or a progeny thereof.

[00225] In some embodiments, the primary cell or a progeny thereof is a T cell or an NK cell.

[00226] In some embodiments, the T cell further comprises reduced expression of T cell receptor (TCR)-alpha and/or TCR-beta.

[00227] In some embodiments, the T cell does not express TCR-alpha and/or TCR-beta.

[00228] In some embodiments, the T cell further comprises a second exogenous polynucleotide encoding one or more chimeric antigen receptors (CARs).

[00229] In some embodiments, the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 expression, relative to a control, and reduced expression of one or more of MHC class I and MHC class II human leukocyte antigens, relative to a control.

[00230] In some embodiments, the cell expresses at least about 2-fold, about 3-fold, about 4-fold, or about 5-fold of the level of CD47 expressed in a wild-type cell or a control cell that has no or low expression of CD47, and reduced expression of one or more of MHC class I and MHC class II human leukocyte antigens, relative to the control cell.

[00231] In some embodiments, the cell expresses at least about 3-fold, about 4-fold, or about 5-fold of the level of CD47 expressed in a wild-type cell or a control cell of the same cell type that has no or low expression of CD47.

[00232] In some embodiments, the control cell is a pancreatic islet cell, a T cell, a natural killer (NK) cell, a CAR-M cell, an endothelial cell, a cardiac muscle cell, a smooth muscle cell, a skeletal muscle cell, a hepatocyte, a glial progenitor cell, a dopaminergic neuron, a retinal pigment epithelial cell, or a thyroid cell.

[00233] In some embodiments, the differentiated cell or the progeny thereof, or the primary immune cell or the progeny thereof evades NK cell mediated cytotoxicity upon administration to a recipient patient, is protected from cell lysis by mature NK cells upon administration to a recipient patient, evades macrophage engulfment upon administration to a recipient patient, does not induce an innate and/or an adaptive immune response to the cell upon administration to a recipient patient, and/or does not induce an antibody-based immune response to the cell upon administration to a recipient patient.

[00234] In some embodiments, the cell is an autologous cell.

[00235] In some embodiments, the cell is an allogeneic cell.

[00236] In some embodiments, provided herein is a pharmaceutical composition comprising a population of the engineered cells disclosed herein, and a pharmaceutically acceptable additive, carrier, diluent or excipient.

[00237] In some embodiments, the engineered cell is a beta islet cell and the pharmaceutical composition further comprises one or more additional pancreatic islet cells.

[00238] In some embodiments, provided herein is a method of treating a patient with a disease or condition who would benefit from a cell-based therapy, comprising administering a clinically effective amount or a therapeutically effective amount of the engineered cells disclosed herein to the patient.

[00239] In some embodiments, provided herein is a method of treating a patient with a disease or condition who would benefit from a cell-based therapy, comprising administering a population of cells comprising the engineered cells disclosed herein to the patient.

[00240] In some embodiments, provided herein is a method of treating a patient with a disease or condition who would benefit from a cell-based therapy, comprising administering a population of cells comprising the differentiated cells disclosed herein to the patient.

[00241] In some embodiments, provided herein is a method of treating a patient with a disease or condition who would benefit from a cell-based therapy, comprising administering a pharmaceutical composition disclosed herein to the patient.

[00242] In some embodiments, disease or condition is selected from the group consisting of a cancer, a genetic disorder, a chronic infectious disease, an autoimmune disorder, a neurological disorder, a cardiac disorder (selected from the group consisting of pediatric cardiomyopathy, age-related cardiomyopathy, dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, chronic ischemic cardiomyopathy, peripartum cardiomyopathy, inflammatory cardiomyopathy, idiopathic cardiomyopathy, other cardiomyopathy, myocardial ischemic reperfusion injury, ventricular dysfunction, heart failure, congestive heart failure, coronary artery disease, end-stage heart disease, atherosclerosis, ischemia, hypertension, restenosis, angina pectoris, rheumatic heart, arterial inflammation, cardiovascular disease, myocardial infarction, myocardial ischemia, myocardial infarction, cardiac ischemia, cardiac injury, myocardial ischemia, vascular disease, acquired heart disease, congenital heart disease, coronary artery disease, dysfunctional conduction systems, dysfunctional coronary arteries, pulmonary hypertension, cardiac arrhythmias, muscular dystrophy, muscle mass abnormality, muscle degeneration, myocarditis, infective myocarditis, drug- or toxin-induced muscle abnormalities, hypersensitivity myocarditis, mitral insufficiency, autoimmune endocarditis, primary arrhythmic diseases, cardiac chanellopathies, long QT syndromes, short QT syndromes, Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia, Jervell and Lange-Nielsen syndrome,

myocardial infarction, heart failure, cardiomyopathy, congenital heart defect, heart valve disease or dysfunction, endocarditis, rheumatic fever, mitral valve prolapse, infective endocarditis, hypertrophic cardiomyopathy, dilated cardiomyopathy, myocarditis, cardiomegaly, mitral insufficiency), a neurological disorder (selected from the group consisting of Alzheimer's disease, Huntington's disease, Parkinson's disease, Pelizaeus-Merzbacher disease, other neurodegenerative disease or condition, attention deficit hyperactivity disorder (ADHD), ischaemia, multiple sclerosis, traumatic brain injury, epilepsy, catalepsy, encephalitis, meningitis, migraine, stroke, transient ischemic attack, subarachnoid hemorrhage, subdural hemorrhage, hematoma, extradural hemorrhage, spinal cord injury, cervical spondylosis, carpal tunnel syndrome, brain or spinal cord tumors, peripheral neuropathy, Guillan-Barre syndrome, neuralgia, amyotrophic lateral sclerosis (ALS), tauopathies, Pick disease, progressive supranuclear palsy, corticobasal degeneration, argyrophilic grain disease, Bell's palsy, cerebral palsy, motor neurone disease, neurofibromatosis, encephalitis, meningitis, Tourette's syndrome, schizophrenia, psychosis, depression, and other neuropsychiatric disorder), vascular dementia, Alzheimer's disease, Parkinson's disease, Huntington disease, multiple sclerosis, other neurodegenerative disease or condition, attention deficit hyperactivity disorder (ADHD), Tourette Syndrome (TS), schizophrenia, psychosis, depression, other neuropsychiatric disorder, HIV-1-associated neurocognitive disorder, traumatic brain injury, stroke, amyotrophic lateral sclerosis (ALS), cerebral hemorrhage, epileptic seizure, spinal cord injury, argyrophilic grain disease (AGD), amyotrophic lateral sclerosis (ALS), cortico-basal degeneration (CBD), Parkinsonism linked to chromosome 17 (FTDP-17), multiple system atrophy (MSA), Parkinson's disease/diffuse Lewy body disease (PD/DLBD), or Alzheimer's disease, atherosclerosis, atherogenesis, arterial thrombosis, venous thrombosis, thrombocytic microangiopathies, vascular leakage, diffuse intravascular coagulation, diabetes, insulin resistance, cardiovascular disease, vascular disease, peripheral vascular disease, ischemic disease, myocardial infarction, congestive heart failure, peripheral vascular obstructive disease, stroke, reperfusion injury, limb ischemia, neuropathy (*e.g.*, peripheral neuropathy or diabetic neuropathy), organ failure (*e.g.*, liver failure, kidney failure, and the like), diabetes, rheumatoid arthritis, osteoporosis, vascular injury, tissue injury, hypertension, angina pectoris and myocardial infarction due to coronary artery disease, renal vascular hypertension, renal failure due to renal artery stenosis, claudication of the lower extremities, transient ischemic attack or

stroke, myocardial infarction, and limb ischemia, repair of ischemic tissues, formation of blood vessels and heart valves, engineering of artificial vessels, repair of damaged vessels, and inducing the formation of blood vessels in engineered tissues (*e.g.*, prior to transplantation), repair or replacement for tissue in need of vascular cells or vascularization a cardiac tissue, liver tissue, pancreatic tissue, renal tissue, muscle tissue, neural tissue, bone tissue, among others, which can be a tissue damaged and characterized by excess cell death, a tissue at risk for damage, or an artificially engineered tissue), coronary artery disease, cerebrovascular disease, aortic stenosis, aortic aneurysm, peripheral artery disease, atherosclerosis, varicose veins, angiopathy, infarcted area of heart lacking coronary perfusion, non-healing wounds, diabetic or non-diabetic ulcers, or any other disease or disorder in which it is desirable to induce formation of blood vessels, improving prosthetic implants (*e.g.*, vessels made of synthetic materials such as Dacron and Gortex.) which are used in vascular reconstructive surgery, a vascular disorder selected from the group consisting of vascular injury, cardiovascular disease, vascular disease, peripheral vascular disease, ischemic disease, myocardial infarction, congestive heart failure, peripheral vascular obstructive disease, hypertension, ischemic tissue injury, reperfusion injury, limb ischemia, stroke, neuropathy (*e.g.*, peripheral neuropathy or diabetic neuropathy), organ failure (*e.g.*, liver failure, kidney failure, and the like), diabetes, rheumatoid arthritis, osteoporosis, cerebrovascular disease, hypertension, angina pectoris and myocardial infarction due to coronary artery disease, renal vascular hypertension, renal failure due to renal artery stenosis, claudication of the lower extremities, other vascular condition or disease, autoimmune thyroiditis, goiter, hyperparathyroidism, hypoparathyroidism (congenital or autoimmune), thyroiditis, Hashimoto's thyroiditis, postpartum thyroiditis, subacute thyroiditis, iatrogenic hypothyroidism, Grave's disease, and thyroid eye disease, infectious hepatitis (A, B, and C), autoimmune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis, non-alcoholic fatty liver disease, cirrhosis, hemochromatosis, hyperoxaluria, alpha-1 antitrypsin deficiency, liver failure, Wilson's disease, hepatic encephalopathy, jaundice, acute hepatic porphyrias, Alagille syndrome, biliary atresia, Budd-Chiari syndrome, hyperbilirubinemias, Crigler-Najjar syndrome, Gilbert-Meulengracht syndrome, Dubin-Johnson syndrome, Rotor syndrome, galactosemia, glycogen storage disease type 1, hepatorenal syndrome, intrahepatic cholestasis of pregnancy, progressive familial intrahepatic cholestasis, Reye's syndrome, lysosomal acid lipase deficiency, alcohol-related pancreatitis, gallstone pancreatitis, diabetes mellitus (type 1 and type

2), prediabetes, gestational diabetes, pancreoprivic diabetes mellitus, pancreatic exocrine insufficiency, acute pancreatitis, chronic pancreatitis, hereditary pancreatitis, hyperinsulinemia, pancreatic cysts, Zollinger-Ellison syndrome, Shwachman-Diamond syndrome, hereditary hemochromatosis, thalassemia, pancreatic iron deposition, cystic fibrosis, pancreas divisum, and pancreatic resection, macular degeneration or a patient having damaged RPE cells, age-related macular degeneration (AMD), early AMD, intermediate AMD, late AMD, non-neovascular age-related macular degeneration, dry macular degeneration (dry age-related macular degeneration), wet macular degeneration (wet age-related macular degeneration), adult-onset vitelliform macular dystrophy (AVMD), Best vitelliform macular dystrophy, Stargardt-like macular dystrophy (STGD3), Sorby's fundus dystrophy (SFD), ABCA4-related disease, Usher type IB, autosomal recessive bestrophinopathy, autosomal dominant vitreoretinochoroidopathy, juvenile macular degeneration (JMD), Leber's Congenital Amaurosis, or retinitis pigmentosa, retinal detachment, retinal tears, severe combined immunodeficiencies (SCID), Omenn syndrome, Cartilage-Hair hypoplasia, reticular dysgenesis, Wiskott-Aldrich syndrome, ataxia telangiectasia, DiGeorge syndrome, immune-osseous dysplasias, dyskeratosis congenita, chronic mucocutaneous candidiasis, hematologic malignancy, follicular lymphoma (FL), myeloid neoplasm, mature T/NK neoplasms, Histiocytic neoplasms, multiple myeloma (MM), myelodysplastic syndromes (MDS), lymphoplasmacytic lymphoma (LPL), Waldenström macroglobulinemia, Burkitt lymphoma (BL), primary mediastinal large B-cell lymphoma (PMBL), Hodgkin lymphoma, Mantle cell lymphoma (MCL), Hairy cell leukemia (HCL), myeloproliferative/myelodysplastic syndromes (MDS) , acute lymphoid leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), Diffuse large B-cell lymphoma (DLBCL), B cell acute lymphoid leukemia (B-ALL), T cell acute lymphoid leukemia (T-ALL), T cell lymphoma, B cell lymphoma, autoimmune disease, including, for example, lupus, systemic lupus erythematosus, rheumatoid arthritis, psoriasis, psoriatic arthritis, multiple sclerosis, Crohn's disease, ulcerative colitis, Addison's disease, Graves' disease, Sjögren's syndrome, Hashimoto's thyroiditis, diabetes mellitus type 1, primary biliary cirrhosis, autoimmune hepatitis, celiac disease, cancers including, but not limited to, 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, bladder cancer, systemic lupus erythematosus (SLE), type 1 diabetes, autoimmune liver disease, Sjögren's syndrome, rheumatoid arthritis, systemic sclerosis (scleroderma), organ-specific autoimmune diseases (autoimmune hepatitis, primary sclerosing chonlangitis), alcohol-related liver disease, multiple sclerosis, NK cell deficiency (NKD) (functional (FNKD) or classical (CNKD)), immunodeficiency-polyendocrinopathy-enteropathy-X-linked (IPEX)-like syndrome, Bloom syndrome, Fanconi's anemia, dyskeratosis congenita, Chediak-Higashi syndrome, familial hematophagocytic lymphohistocytosis (FHL), Griscelli syndrome type 2, Hermansky Pudliak syndrome, Papillon-Lefevre syndrome, Wiskott-Aldrich syndrome, autosomal recessive hyper-IgE syndrome, May Hegglin anomaly, and leucocyte adhesion deficiency type I or type III.

[00243] In some embodiments, the differentiated cells are selected from the group consisting of a mesenchymal stem cell (MSC), a hematopoietic stem cell (HSC), pancreatic islet cell, a beta islet cell, an immune cell, a B cell, a T cell, a natural killer (NK) cell, a natural killer T (NKT) cell, a macrophage cell, an immune privileged cell, an optic cell, a retinal pigmented epithelium cell (RPE), a hepatocyte, a thyroid cell, an endothelial cell, a skin cell, a glial progenitor cell, a neural cell, a muscle cell, a cardiac cell, and a blood cell.

[00244] In some embodiments, an immunosuppressive and/or immunomodulatory agent is not administered to the patient before the administration of the population of cells.

[00245] In some embodiments, the method further comprises administering one or more immunosuppressive agents to the patient.

[00246] In some embodiments, where the patient has been administered one or more immunosuppressive agents.

[00247] In some embodiments, the one or more immunosuppressive agents are a small molecule or an antibody.

[00248] In some embodiments, 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.

[00249] In some embodiments, the one or more immunosuppressive agents comprise cyclosporine.

[00250] In some embodiments, the one or more immunosuppressive agents comprise mycophenolate mofetil.

[00251] In some embodiments, the one or more immunosuppressive agents comprise a corticosteroid.

[00252] In some embodiments, the one or more immunosuppressive agents comprise cyclophosphamide.

[00253] In some embodiments, the one or more immunosuppressive agents comprise rapamycin.

[00254] In some embodiments, the one or more immunosuppressive agents comprise tacrolimus (FK-506).

[00255] In some embodiments, the one or more immunosuppressive agents comprise anti-thymocyte globulin.

[00256] In some embodiments, the one or more immunosuppressive agents are one or more immunomodulatory agents.

[00257] In some embodiments, the one or more immunomodulatory agents are a small molecule or an antibody.

[00258] In some embodiments, 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.

[00259] In some embodiments, the one or more immunosuppressive agents are or have been administered to the patient prior to administration of the engineered cells.

[00260] In some embodiments, 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.

[00261] In some embodiments, 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.

[00262] In some embodiments, 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.

[00263] In some embodiments, 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.

[00264] In some embodiments, 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.

[00265] In some embodiments, the one or more immunosuppressive agents are or have been administered to the patient after administration of the engineered cells.

[00266] In some embodiments, 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.

[00267] In some embodiments, 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.

[00268] In some embodiments, 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.

[00269] In some embodiments, 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.

[00270] In some embodiments, 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.

[00271] In some embodiments, 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.

[00272] In some embodiments, 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.

[00273] In some embodiments, provided herein is a use of a population of the engineered cells disclosed herein for treating a disorder or condition in a recipient patient who would benefit from a cell-based therapy.

[00274] In some embodiments, provided herein is an method for producing the engineered cells disclosed herein or the population of cells comprising the engineered cells disclosed herein, the method comprising: (a) obtaining an isolated cell; and (b) contacting the isolated cell with one or more reagents and/or components to modify gene expression in the isolated cell, thereby producing the engineered cell or the population of cells comprising the engineered cell.

[00275] In some embodiments, the method further comprises determining the CD47 expression levels of the engineered cells or the population of cells.

[00276] In some embodiments, the method further comprises selecting the engineered cell or the population of cells for use in producing a therapeutic product if the engineered cell or the population of cells are determined to express CD47 at a threshold level or higher.

[00277] In some embodiments, the engineered cell or the population of cells express at least about the same amount of CD47, relative to the control.

[00278] In some embodiments, the engineered cell or the population of cells express at least about a 10% higher amount of CD47, relative to the control.

[00279] In some embodiments, the engineered cell or the population of cells express at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, higher amount of CD47, relative to the control.

[00280] In some embodiments, the engineered cell or the population of cells express at least about a 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, or 900%, higher amount of CD47, relative to the control.

[00281] In some embodiments, the engineered cell or the population of cells express at least about a 1000% higher amount of CD47, relative to the control.

[00282] In some embodiments, the engineered cell or the population of cells express at least about 1.1-fold of the level of CD47 expressed in the control.

[00283] In some embodiments, the engineered cell or the population of cells express at least about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, or about 5-fold of the level of CD47 expressed in the control.

[00284] In some embodiments, the engineered cell or the population of cells express at least about 4-fold, about 4.5-fold, about 5-fold, or about 5.5-fold of the level of CD47 expressed in the control.

[00285] In some embodiments, the engineered cell or the population of cells express at least about about 4-fold of the level of CD47 expressed in the control.

[00286] In some embodiments, the engineered cell or the population of cells express at least about about 4.5-fold of the level of CD47 expressed in the control.

[00287] In some embodiments, the engineered cell or the population of cells express at least about about 5-fold of the level of CD47 expressed in the control.

[00288] In some embodiments, the engineered cell or the population of cells express at least about about 5.5-fold of the level of CD47 expressed in the control.

[00289] In some embodiments, the engineered cell or the population of cells express at least about about 16-fold, about 17-fold, about 18-fold, about 19-fold, or about 20-fold of the level of CD47 expressed in the control.

[00290] In some embodiments, the control is a wild-type cell or a population of wild type cells, a control cell or a population of control cells, or a baseline reference.

[00291] In some embodiments, the control cell or the population of control cells comprise an unmodified or unaltered cell, optionally wherein the unmodified or unaltered cell is of the same cell type as the engineered cell.

[00292] In some embodiments, the control cell or the population of control cells is a starting material from a donor or a pool of starting cells from a pool of donors.

[00293] In some embodiments, the baseline reference is an isotype control or a background signal level.

[00294] In some embodiments, the engineered cell is a beta islet cell and the population of cells comprises beta islet cells and additional pancreatic islet cells.

[00295] In some embodiments, the engineered cell comprises regulatable modifications that alter the expression of one or more targets in the engineered cell, relative to a control.

[00296] In some embodiments, the regulatable modifications reduce expression of one or more MHC class I and/or MHC class II molecules, relative to a wild-type cell, a population of wild type cells, a control cell, or a population of control cells.

[00297] In some embodiments, the regulatable modifications increase expression of one or more tolerogenic factors, relative to a wild-type cell, a population of wild type cells, a control cell, or a population of control cells.

[00298] In some embodiments, the one or more reagents to modify gene expression in the isolated cell comprise i) a conditional or inducible RNA-based component for altering expression of the one or more targets, ii) a conditional or inducible DNA-based component for altering expression of the one or more targets, or iii) a conditional or inducible protein-based component for altering expression of the one or more targets.

[00299] In some embodiments, the method further comprises contacting the isolated cell with an exogenous factor or exposing the isolated cell to a condition to activate the conditional or inducible promoter, thereby causing expression of the one or more targets, thereby producing the engineered cell.

[00300] In some embodiments, provided herein is a method for producing an engineered cell comprising regulatable modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of one or more tolerogenic factors, relative to a control, the method comprising: (a) obtaining an isolated cell; (b) introducing into the cell a conditional or inducible RNA-based component for regulatable reduced expression of the MHC class I and/or MHC class II human leukocyte molecules, a conditional or inducible DNA-based component for regulatable reduced expression of the MHC class I and/or MHC class II human leukocyte molecules, or a conditional or inducible protein-based component for regulatable reduced expression of the MHC class I and/or MHC class II human leukocyte molecules; (c) exposing the cell to a condition or an exogenous factor to activate the conditional or inducible component, thereby causing reduced expression of the MHC class I and/or MHC class molecules; (d) introducing into the isolated cell a nucleic acid comprising a conditional or inducible promoter operably linked to an exogenous polynucleotide encoding the one or more tolerogenic factors for regulatable increased expression of the one or more tolerogenic factors; and (e) exposing the engineered cell to a condition or an exogenous factor to activate the conditional or inducible promoter, thereby causing expression of the exogenous one or more tolerogenic factors, and thereby producing the engineered cell.

[00301] In some embodiments, steps (a)-(d) are carried out in any order.

[00302] In some embodiments, one or more of steps (a)-(d) are carried out simultaneously.

[00303] In some embodiments, steps (b) and (c) are carried out before steps (d) and (e).

[00304] In some embodiments, steps (d) and (e) are carried out before steps (b) and (c).

[00305] In some embodiments, steps (c) and (e) are carried out sequentially.

[00306] In some embodiments, steps (c) and (e) are carried out simultaneously.

[00307] In some embodiments, provided herein is a method for identifying a population of cells or a population of cells comprising the engineered cells disclosed herein suitable for use as a therapeutic product, the method comprising: (a) obtaining isolated cells; (b) introducing into the cells one or more modifications that reduce expression of one or more MHC class I and/or MHC class II molecules, relative to a control; (c) introducing into the cells one or more modifications that increase expression of CD47, relative to a control; (d) measuring the CD47 expression levels

of the cells; and (e) selecting a population of cells that express at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47, relative to the control, and identifying the population as suitable for use as a therapeutic product.

[00308] In some embodiments, provided herein is a method for identifying a population of cells or a population of cells comprising the engineered cells disclosed herein suitable for use as a therapeutic product, the method comprising: (a) obtaining isolated cells; (b) introducing into the cells one or more modifications that reduce expression of one or more MHC class I and/or MHC class II molecules, relative to a control; (c) introducing into the cells one or more modifications that increase expression of CD47, relative to a control; (d) measuring the CD47 expression levels of the cells; and (e) selecting a population of cells that express at least about 1.1-fold, about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold, about 10-fold, about 11-fold, about 12-fold, about 13-fold, about 14-fold, about 15-fold, about 16-fold, about 17-fold, about 18-fold, about 19-fold, or about 20-fold of the level of CD47 expressed in the control, and identifying the population as suitable for use as a therapeutic product.

[00309] In some embodiments, step (b) is carried out before step (c).

[00310] In some embodiments, step (c) is carried out before step (b).

[00311] In some embodiments, steps (b) and (c) are carried out simultaneously.

[00312] In some embodiments, provided herein is a method of determining whether a population of cells is suitable for use as a therapeutic product, the method comprising: (a) producing engineered cells comprising a first exogenous polynucleotide encoding CD47, optionally the engineered cells disclosed herein; (b) measuring the CD47 expression levels of the cells; and (c) determining that the population of cells is suitable for use as a therapeutic product if the cells express at about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47, relative to a control.

[00313] In some embodiments, provided herein is a method of determining whether a population of cells is suitable for use as a therapeutic product, the method comprising: (a)

producing engineered cells comprising a first exogenous polynucleotide encoding CD47, optionally the engineered cells disclosed herein; (b) measuring the CD47 expression levels of the cells; and (c) determining that the population of cells is suitable for use as a therapeutic product if the cells express at least about 1.1-fold, about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold, about 10-fold, about 11-fold, about 12-fold, about 13-fold, about 14-fold, about 15-fold, about 16-fold, about 17-fold, about 18-fold, about 19-fold, or about 20-fold of the level of CD47 expressed in a control.

[00314] In some embodiments, the control is a wild-type cell, a control cell, or a baseline reference.

[00315] In some embodiments, the control cell is an unmodified or unaltered cell, optionally wherein the unmodified or unaltered cell is of the same cell type as the engineered cell.

[00316] In some embodiments, the control cell is a starting material from a donor or a pool of starting cells from a pool of donors.

[00317] In some embodiments, the baseline reference is an isotype control or a background signal level.

[00318] In some embodiments, the CD47 level is determined using an antibody-based quantitation method, optionally a Quantibrite™ assay.

[00319] In some embodiments, provided herein is a method of determining a threshold of CD47 expression level required for immune-evasion of hypoimmunogenic cells, the method comprising: (a) producing engineered cells comprising a first exogenous polynucleotide encoding CD47; (b) sorting the engineered cells based on CD47 expression levels, to generate pools of cells having similar CD47 expression levels; (c) assessing the immune response induced by the pools of cells; and (d) determining a threshold of CD47 expression level required for immune-evasion.

[00320] In some embodiments, the CD47 level is determined using an antibody-based quantitation method, optionally a Quantibrite™ assay.

[00321] In some embodiments, step (a) of the method further comprises engineering the cells to comprise reduced expression of one or more Y chromosome genes and major histocompatibility

complex (MHC) class I and/or class II human leukocyte antigens, relative to a wild-type cell or a control cell.

[00322] In some embodiments, the assessing of the immune response is carried out using in vitro assays or in vivo assays.

[00323] In some embodiments, the assessing of the immune response is carried out by measuring NK cell mediated cytotoxicity, lysis by mature NK cells, macrophage engulfment, antibody-based immune response to the cells, or by measuring the percentage of the cells still present in the recipient after a certain period of time upon administration to a recipient patient.

[00324] In some embodiments, provided herein is a method for identifying a population of cells or a population of cells comprising the engineered cells disclosed herein suitable for use as a therapeutic product, the method comprising: (a) introducing into isolated cells one or more modifications that reduce expression of one or more MHC class I and/or MHC class II molecules, relative to a control, and (b) introducing into the cells one or more modifications that increase expression of CD47, relative to a control.

[00325] In some embodiments, the method further comprises step (c) measuring the CD47 expression levels of the cells.

[00326] In some embodiments, the method further comprises step (d) selecting a population of cells that express at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47, relative to the control, and identifying the population as suitable for use as a therapeutic product.

[00327] In some embodiments, the method further comprises step (d) selecting a population of cells that express at least about 1.1-fold, about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold, about 10-fold, about 11-fold, about 12-fold, about 13-fold, about 14-fold, about 15-fold, about 16-fold, about 17-fold, about 18-fold, about 19-fold, or about 20-fold of the level of CD47 expressed in the control, and identifying the population as suitable for use as a therapeutic product.

[00328] In some embodiments, step (a) is carried out before step (b).

[00329] In some embodiments, step (b) is carried out before step (a).

[00330] In some embodiments, steps (a) and (b) are carried out simultaneously.

[00331] Detailed descriptions of hypoimmunogenic cells, methods of producing thereof, and methods of using thereof are found in U.S. Provisional Application No. 63/065,342 filed on August 13, 2020, U.S. Provisional Application No. 63/136,152 filed on December 31, 2020, U.S. Provisional Application No. 63/175,030 filed on April 14, 2021, U.S. Provisional Application No. 63/175,003 filed on April 14, 2021, and U.S. Provisional Application filed on January 11, 2021 (Attorney Docket No. 18615-30046.00), WO2016/183041 filed May 9, 2015, WO2018/132783 filed January 14, 2018, WO2020/018615 filed July 17, 2019, WO2020/018620 filed July 17, 2019, WO2020/168317 filed February 16, 2020, PCT/US2021/029443 filed April 27, 2021, the disclosures of which including the examples, sequence listings and figures are incorporated herein by reference in their entireties.

BRIEF DESCRIPTION OF THE DRAWINGS

[00332] FIGs. 1A, 1C, 1E, 1G, 1I, 1K, and 1M depict flow cytometry data measuring CD47 levels on the cell surface of primary mouse $B2M^{-/-}$; CD47*tg* beta islet cells which were generated from beta islet cells isolated from B2M-knock out C57BL/6 (B6) mice and then transduced with lentiviruses containing CD47 transgenes. Various MOI were evaluated with the $B2M^{-/-}$; CD47*tg* beta islet cells. CD47 levels were compared to an isotype control (left side). FIGs. 1B, 1D, 1F, 1H, 1J, 1L, and 1N depict data of NK cell mediated killing of the $B2M^{-/-}$; CD47*tg* beta islet cells by mouse NK cells.

[00333] FIGs. 2A-2AB depict data from Xelligence assays of NK cell and macrophage mediated killing or lack thereof of $B2M^{-/-}$; CD47*tg* T cells by NK cells and macrophages.

[00334] FIGs. 3A-3L depict data from Xelligence assays of NK cell mediated killing or lack thereof of $B2M^{-/-}$; CD47*tg* T cells by NK cells.

[00335] FIGs. 4A-4C depict flow cytometry data measuring HLA-I, HLA-II, and CD47 levels on the cell surface of unmodified primary RPE cells.

[00336] FIGs. 5A-5D depict cell morphology (5A) and flow cytometry (5B-5D) data measuring HLA-I, HLA-II, and CD47 levels on the cell surface of $B2M^{-/-}$; $CIITA^{-/-}$; CD47*tg* primary RPE cells.

[00337] FIGs. 6A-6I depict flow cytometry data measuring HLA-I, HLA-II, and CD47 levels on the cell surface of unmodified (6A-6C), $B2M^{-/-}$; $CIITA^{-/-}$ (6D-6F), and $B2M^{-/-}$; $CIITA^{-/-}$; CD47*tg* (6G-6I) primary RPE cells.

[00338] FIGS. 7A-7I depict data from Xelligence assays of NK cell and macrophage mediated killing or lack thereof of unmodified (7A-7C), $B2M^{-/-}$; $CIITA^{-/-}$ (7D-7F), and $B2M^{-/-}$; $CIITA^{-/-}$; CD47*tg* (7G-7I) primary RPE cells by NK cells and macrophages.

[00339] Other objects, advantages and embodiments of the present disclosure will be apparent from the detailed description following.

DETAILED DESCRIPTION

I. INTRODUCTION

[00340] Described herein are engineered or modified immune evasive cells based, in part, on the hypoimmune editing platform described in WO2018132783, and PCT/US21/65157 filed 12/23/2021, each of which is incorporated herein by reference in its entirety, including but not limited to human immune evasive cells. To overcome the problem of a subject's immune rejection of these primary and/or stem cell-derived transplants, the inventors have developed and describe herein hypoimmunogenic cells (*e.g.*, hypoimmunogenic pluripotent cells, differentiated cells derived from such, and primary cells) that represent a viable source for any transplantable cell type. Such cells are protected from adaptive and/or innate immune rejection upon administration to a recipient subject. Advantageously, the cells disclosed herein are not rejected by the recipient subject's immune system, regardless of the subject's genetic make-up, as they are protected from adaptive and innate immune rejection upon administration to a recipient subject. In some embodiments, the hypoimmunogenic cells regulatably lack expression of one or more MHC class I and class II antigen molecules and/or T-cell receptors. In certain embodiments, the hypoimmunogenic cells regulatably lack expression of major histocompatibility complex (MHC) I and II antigen molecules and/or T-cell receptors and regulatably overexpress one or more tolerogenic factors. In certain embodiments, the hypoimmunogenic cells such as hypoimmunogenic T cells regulatably lack expression of one or more MHC I and II antigen molecules and/or T-cell receptors, regulatably overexpress CD47 and regulatably express CARs. In some embodiments, the hypoimmunogenic cells regulatably lack expression of one or more MHC I and II antigen molecules and/or T-cell receptors and/or one or more Y chromosome

genes. In certain embodiments, the hypoimmunogenic cells regulatably lack expression of one or more MHC I and II antigen molecules and/or T-cell receptors and/or one or more Y chromosome genes and regulatably overexpress CD47. In certain embodiments, the hypoimmunogenic cells regulatably lack expression of one or more MHC I and II antigen molecules and/or T-cell receptors and/or RHD and regulatably overexpress CD47 proteins. In certain embodiments, the hypoimmunogenic cells regulatably lack expression of one or more MHC I and II antigen molecules and/or T-cell receptors and/or ABO and regulatably overexpress CD47 proteins. In certain embodiments, the hypoimmunogenic cells regulatably lack expression of one or more MHC I and II antigen molecules and/or T-cell receptors and/or MICA and regulatably overexpress CD47 proteins. In certain embodiments, the hypoimmunogenic cells regulatably lack expression of one or more MHC I and II antigen molecules and/or T-cell receptors and/or MICB and regulatably overexpress CD47 proteins. In certain embodiments, the hypoimmunogenic cells such as hypoimmunogenic T cells regulatably lack expression of one or more MHC I and II antigen molecules and/or T-cell receptors and/or one or more Y chromosome genes, regulatably overexpress CD47 and regulatably express CARs.

[00341] In some embodiments, hypoimmunogenic cells outlined herein are not subject to an innate immune cell rejection. In some instances, hypoimmunogenic cells are not susceptible to NK cell-mediated lysis. In some instances, hypoimmunogenic cells are not susceptible to macrophage engulfment. In some embodiments, hypoimmunogenic cells are useful as a source of universally compatible cells or tissues (*e.g.*, universal donor cells or tissues) that are transplanted into a recipient subject with little to no immunosuppressant agent needed. Such hypoimmunogenic cells retain cell-specific characteristics and features upon transplantation, including, *e.g.*, pluripotency, as well as being capable of engraftment and functioning similarly to a corresponding native cell.

[00342] The technology disclosed herein utilizes regulatable expression of tolerogenic factors and regulatable modulation (*e.g.*, reduction or elimination) of MHC I molecules, MHC II molecules, and/or TCR expression in human cells. In some embodiments, regulatable genome editing technologies utilizing regulatable rare-cutting endonucleases (*e.g.*, the CRISPR/Cas, TALEN, zinc finger nuclease, meganuclease, and homing endonuclease systems) are also used to reduce or eliminate expression of genes involved in an innate and/or an adaptive immune response (*e.g.*, by deleting genomic DNA of genes involved in an innate and/or an adaptive

immune response or by insertions of genomic DNA into such genes, such that gene expression is impacted) in the cells. In some embodiments, regulatable genome editing technologies or other gene modulation technologies are used to insert tolerance-inducing (tolerogenic) factors in human cells, rendering the cells and their progeny (include any differentiated cells prepared therefrom) able to evade immune recognition upon engrafting into a recipient subject. As such, the cells described herein exhibit regulatable modulated expression of one or more genes and factors that affect MHC I molecules, MHC II molecules, and/or TCR expression and evade the recipient subject's immune system.

[00343] It has surprisingly been found that some transgenes overexpressing exogenous polynucleotides can become silenced during differentiation of iPSCs and primary cells into, *e.g.*, engineered hypoimmunogenic differentiated cells. Accordingly, the present disclosure provides systems allowing for regulatable expression of exogenous polynucleotides. It has also been found that reduced expression of one or more MHC I molecules, MHC II molecules, and/or TCR is not required prior to the generation of the differentiated cells, *e.g.*, engineered hypoimmunogenic differentiated cells. Accordingly, the present disclosure also provides systems allowing for regulatable knock out or knock down of MHC I molecules, MHC II molecules, and/or TCR.

[00344] The genome editing techniques enable double-strand DNA breaks at desired locus sites. These controlled double-strand breaks promote homologous recombination at the specific locus sites. This process focuses on targeting specific sequences of nucleic acid molecules, such as chromosomes, with endonucleases that recognize and bind to the sequences and induce a double-stranded break in the nucleic acid molecule. The double-strand break is repaired either by an error-prone non-homologous end-joining (NHEJ) or by homologous recombination (HR).

[00345] The practice of the numerous embodiments will employ, unless indicated specifically to the contrary, conventional methods of chemistry, biochemistry, organic chemistry, molecular biology, microbiology, recombinant DNA techniques, genetics, immunology, and cell biology that are within the skill of the art, many of which are described below for the purpose of illustration. Such techniques are explained fully in the literature. See, *e.g.*, Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual (3rd Edition, 2001); Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual (2nd Edition, 1989); Maniatis *et al.*, Molecular Cloning: A Laboratory Manual (1982); Ausubel *et al.*, Current Protocols in Molecular Biology (John Wiley

and Sons, updated July 2008); Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience; Glover, DNA Cloning: A Practical Approach, vol. I & II (IRL Press, Oxford, 1985); Anand, Techniques for the Analysis of Complex Genomes, (Academic Press, New York, 1992); Transcription and Translation (B. Hames & S. Higgins, Eds., 1984); Perbal, A Practical Guide to Molecular Cloning (1984); Harlow and Lane, Antibodies, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1998) Current Protocols in Immunology Q. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach and W. Strober, eds., 1991); Annual Review of Immunology; as well as monographs in journals such as Advances in Immunology.

II. DEFINITIONS

[00346] As described in the present disclosure, the following terms will be employed, and are defined as indicated below.

[00347] The term "antigen", as used herein, refers to a molecule capable of provoking an immune response. Antigens include but are not limited to cells, cell extracts, proteins, polypeptides, peptides, polysaccharides, polysaccharide conjugates, peptide and non-peptide mimics of polysaccharides and other molecules, small molecules, lipids, glycolipids, carbohydrates, viruses and viral extracts and multicellular organisms such as parasites and allergens. The term antigen broadly includes any type of molecule which is recognized by a host immune system as being foreign.

[00348] The terms "autoimmune disease" or “autoimmune disorder” or “inflammatory disease” or “inflammatory disorder” refer to any disease or disorder in which the subject mounts an innate and/or an adaptive immune response against its own tissues and/or cells. Autoimmune disorders can affect almost every organ system in the subject (*e.g.*, human), including, but not limited to, diseases of the nervous, gastrointestinal, and endocrine systems, as well as skin and other connective tissues, eyes, blood and blood vessels. Examples of autoimmune diseases include, but are not limited to Hashimoto's thyroiditis, Systemic lupus erythematosus, Sjogren's syndrome, Graves' disease, Scleroderma, Rheumatoid arthritis, Multiple sclerosis, Myasthenia gravis and Diabetes.

[00349] The term "cancer" as used herein is defined as a hyperproliferation of cells whose unique trait (*e.g.*, loss of normal controls) results in unregulated growth, lack of differentiation, local tissue invasion, and metastasis. With respect to the inventive methods, the cancer can be

any cancer, including any of acute lymphocytic cancer, acute myeloid leukemia, alveolar rhabdomyosarcoma, bladder cancer, bone cancer, brain cancer, breast cancer, cancer of the anus, anal canal, or anorectum, cancer of the eye, cancer of the intrahepatic bile duct, cancer of the joints, cancer of the neck, gallbladder, or pleura, cancer of the nose, nasal cavity, or middle ear, cancer of the oral cavity, cancer of the vulva, chronic lymphocytic leukemia, chronic myeloid cancer, colon cancer, esophageal cancer, cervical cancer, fibrosarcoma, gastrointestinal carcinoid tumor, Hodgkin lymphoma, hypopharynx cancer, kidney cancer, larynx cancer, leukemia, liquid tumors, liver cancer, lung cancer, lymphoma, malignant mesothelioma, mastocytoma, melanoma, multiple myeloma, nasopharynx cancer, non-Hodgkin lymphoma, ovarian cancer, pancreatic cancer, peritoneum, omentum, and mesentery cancer, pharynx cancer, prostate cancer, rectal cancer, renal cancer, skin cancer, small intestine cancer, soft tissue cancer, solid tumors, stomach cancer, testicular cancer, thyroid cancer, ureter cancer, and/or urinary bladder cancer. As used herein, the term "tumor" refers to an abnormal growth of cells or tissues of the malignant type, unless otherwise specifically indicated and does not include a benign type tissue.

[00350] The term "chronic infectious disease" refers to a disease caused by an infectious agent wherein the infection has persisted. Such a disease may include hepatitis (A, B, or C), herpes virus (*e.g.*, VZV, HSV-1, HSV-6, HSV-II, CMV, and EBV), and HIV/AIDS. Non-viral examples may include chronic fungal diseases such Aspergillosis, Candidiasis, Coccidioidomycosis, and diseases associated with Cryptococcus and Histoplasmosis. None limiting examples of chronic bacterial infectious agents may be Chlamydia pneumoniae, Listeria monocytogenes, and Mycobacterium tuberculosis. In some embodiments, the disorder is human immunodeficiency virus (HIV) infection. In some embodiments, the disorder is acquired immunodeficiency syndrome (AIDS).

[00351] [0010] As used herein, “clinically effective amount” refers to an amount sufficient to provide a clinical benefit in the treatment and/or management of a disease, disorder, or condition. In some embodiments, a clinically effective amount is an amount that has been shown to produce at least one improved clinical endpoint to the standard of care for the disease, disorder, or condition. In some embodiments, a clinically effective amount is an amount that has been demonstrated, for example in a clinical trial, to be sufficient to provide statistically significant and meaningful effectiveness for treating the disease, disorder, or condition. In some

embodiments, the clinically effective amount is also a therapeutically effective amount. In other embodiments, the clinically effective amount is not a therapeutically effective amount.

[00352] As used herein, "conditional promoters" are active under certain cellular conditions or under certain cellular stages. As used herein, conditional promoters include, *e.g.*, cell-specific promoters, tissue-specific promoters, lineage-specific promoters, developmentally-specific promoters, cell differentiation-specific promoters, differentiation-induced promoters, cell cycle-specific promoters, and cell phase-specific promoters. "Cell-specific promoters," "tissue-specific promoters," and "lineage-specific promoters" are promoters that cause a nucleotide sequence to be expressed in a specific cell, tissue, or lineage type, such as respiratory, prostatic, pancreatic, mammary, renal, intestinal, neural, skeletal, vascular, hepatic, hematopoietic, muscle, endothelial, epithelial, or cardiac cells. Promoters that cause a nucleotide sequence to be expressed at a specific stage of development or cell differentiation are commonly referred to as "developmentally-specific promoters," "cell differentiation-specific promoters," or "differentiation-induced promoters," and include, *e.g.*, promoters that are activated or inactivated when a cell transitions from one cell type to another cell type, *e.g.*, from an undifferentiated cell to a differentiated cell, *e.g.*, from a stem cell to a multipotential progenitor cell, from a multipotential progenitor cell to a lineage-committed progenitor cell, from a lineage-committed progenitor cell to a precursor cell, or from a precursor cell to a mature cell. Promoters that cause a nucleotide sequence to be expressed during a specific stage of the cell cycle are commonly referred to as "cell cycle-specific promoters" or "cell phase-specific promoters." Numerous standard conditional promoters will be known to one of skill in the art.

[00353] "Constitutive promoters" are typically active, *i.e.*, promote transcription, under most conditions. In some examples, constitutive promoters are capable of directing transcription of an operably linked nucleic acid sequence in the absence of a stimulus (*e.g.*, heat shock, chemicals, etc.). In some examples, constitutive promoters are active in most cell types at most times. Numerous standard conditional promoters will be known to one of skill in the art. Constitutive promoters are included herein as one type of "regulatable promoter".

[00354] In some embodiments, an alteration or modification (including, for example, genetic alterations or modifications) described herein results in reduced expression of a target or selected polynucleotide sequence. In some embodiments, an alteration or modification described herein results in reduced expression of a target or selected polypeptide sequence. In some

embodiments, an alteration or modification described herein results in increased expression of a target or selected polynucleotide sequence. In some embodiments, an alteration or modification described herein results in increased expression of a target or selected polypeptide sequence. The terms "decrease," "reduced," "reduction," and "decrease" are all used herein generally to mean a decrease by a statistically significant amount. However, for avoidance of doubt, decrease," "reduced," "reduction," "decrease" means a decrease by at least 10% as compared to a reference level, for example a decrease by at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% decrease (*i.e.* absent level as compared to a reference sample), or any decrease between 10-100% as compared to a reference level. In some embodiments, the cells are engineered to have reduced expression of one or more targets relative to an unaltered or unmodified wild-type cell.

[00355] In additional or alternative embodiments, the present disclosure contemplates altering target polynucleotide sequences in any manner which is available to the skilled artisan, *e.g.*, utilizing a TALEN system or RNA-guided transposases. It should be understood that although examples of methods utilizing CRISPR/Cas (*e.g.*, Cas9 and Cas12a) and TALEN are described in detail herein, the present disclosure is not limited to the use of these methods/systems. Other methods of targeting, *e.g.*, B2M, to reduce or ablate expression in target cells known to the skilled artisan can be utilized herein.

[00356] "Degron element" as used herein refers to a subunit of a protein that regulates the degradation of the protein. In some instances, a degron comprises a sequence of amino acids, which provides a degradation signal that directs a polypeptide for cellular degradation. The degron may promote degradation of an attached polypeptide through either the proteasome or autophagy-lysosome pathways. In the fusion protein, the degron must be operably linked to the polypeptide of interest, but need not be contiguous with it as long as the degron still functions to direct degradation of the polypeptide of interest. Preferably, the degron induces rapid degradation of the polypeptide of interest. For a discussion of degrons and their function in protein degradation, see, *e.g.*, Kanemaki *et al.* (2013) Pflugers Arch. 465(3):419-425, Erales *et al.* (2014) Biochim Biophys Acta 1843(1):216-221, Schrader *et al.* (2009) Nat. Chem. Biol. 5(11):815-822, Ravid *et al.* (2008) Nat. Rev. Mol. Cell. Biol. 9(9):679-690, Tasaki *et al.* (2007) Trends Biochem Sci. 32(11):520-528, Meinnel *et al.* (2006) Biol. Chem. 387(7):839-851, Kim *et*

al. (2013) Autophagy 9(7):1100-1103, Varshaysky (2012) Methods Mol. Biol. 832:1-11, and Fayadat *et al.* (2003) Mol Biol Cell. 14(3):1268-1278; the contents herein incorporated by reference in their entirety.

[00357] In some embodiments, the engineered and hypoimmunogenic cells described are derived from an iPSC or a progeny thereof. As used herein, the term "derived from an iPSC or a progeny thereof" encompasses the initial iPSC that is generated and any subsequent progeny thereof. As used herein, the term "progeny" encompasses, *e.g.*, a first-generation progeny, *i.e.*, the progeny is directly derived from, obtained from, obtainable from or derivable from the initial iPSC by, *e.g.*, traditional propagation methods. The term "progeny" also encompasses further generations such as second, third, fourth, fifth, sixth, seventh, or more generations, *i.e.*, generations of cells which are derived from, obtained from, obtainable from or derivable from the former generation by, *e.g.*, traditional propagation methods. The term "progeny" also encompasses modified cells that result from the modification or alteration of the initial iPSC or a progeny thereof.

[00358] The term "donor subject" refers to an animal, for example, a human from whom cells can be obtained. The "non-human animals" and "non-human mammals" as used interchangeably herein, includes mammals such as rats, mice, rabbits, sheep, cats, dogs, cows, pigs, and non-human primates. The term "donor subject" also encompasses any vertebrate including but not limited to mammals, reptiles, amphibians and fish. However, advantageously, the donor subject is a mammal such as a human, or other mammals such as a domesticated mammal, *e.g.* dog, cat, horse, and the like, or production mammal, *e.g.* cow, sheep, pig, and the like. A "donor subject" can also refere to more than one donor, for example one or more humans or non-human animals or non-human mammals.

[00359] The term "endogenous" refers to a referenced molecule or polypeptide that is naturally present in the cell. Similarly, the term when used in reference to expression of an encoding nucleic acid refers to expression of an encoding nucleic acid naturally contained within the cell and not exogenously introduced. Similarly, the term when used in reference to a promoter sequence refers to a promoter sequence naturally contained within the cell and not exogenously introduced.

[00360] The term "engineered cell" as used herein refers to a cell that has been altered in at least some way by human intervention, including, for example, by genetic alterations or modifications such that the engineered cell differs from a wild-type cell.

[00361] As used herein, the term "exogenous" in the context of a polynucleotide or polypeptide being expressed is intended to mean that the referenced molecule or the referenced polypeptide is introduced into the cell of interest. The polypeptide can be introduced, for example, by introduction of an encoding nucleic acid into the genetic material of the cells such as by integration into a chromosome or as non-chromosomal genetic material such as a plasmid or expression vector. Therefore, the term as it is used in reference to expression of an encoding nucleic acid refers to introduction of the encoding nucleic acid in an expressible form into the cell.

[00362] An "exogenous" molecule is a molecule, construct, factor and the like that is not normally present in a cell, but can be introduced into a cell by one or more genetic, biochemical or other methods. "Normal presence in the cell" is determined with respect to the particular developmental stage and environmental conditions of the cell. Thus, for example, a molecule that is present only during embryonic development of neurons is an exogenous molecule with respect to an adult neuron cell. An exogenous molecule can comprise, for example, a functioning version of a malfunctioning endogenous molecule or a malfunctioning version of a normally-functioning endogenous molecule.

[00363] An exogenous molecule or factor can be, among other things, a small molecule, such as is generated by a combinatorial chemistry process, or a macromolecule such as a protein, nucleic acid, carbohydrate, lipid, glycoprotein, lipoprotein, polysaccharide, any modified derivative of the above molecules, or any complex comprising one or more of the above molecules. Nucleic acids include DNA and RNA, can be single- or double-stranded; can be linear, branched or circular; and can be of any length. Nucleic acids include those capable of forming duplexes, as well as triplex-forming nucleic acids. See, for example, U.S. Pat. Nos. 5,176,996 and 5,422,251. Proteins include, but are not limited to, DNA-binding proteins, transcription factors, chromatin remodeling factors, methylated DNA binding proteins, polymerases, methylases, demethylases, acetylases, deacetylases, kinases, phosphatases, integrases, recombinases, ligases, topoisomerases, gyrases and helicases.

[00364] An exogenous molecule or construct can be the same type of molecule as an endogenous molecule, *e.g.*, an exogenous protein or nucleic acid. In such instances, the exogenous molecule is introduced into the cell at greater concentrations than that of the endogenous molecule in the cell. In some instances, an exogenous nucleic acid can comprise an infecting viral genome, a plasmid or episome introduced into a cell, or a chromosome that is not normally present in the cell. Methods for the introduction of exogenous molecules into cells are known to those of skill in the art and include, but are not limited to, lipid-mediated transfer (*i.e.*, liposomes, including neutral and cationic lipids), electroporation, direct injection, cell fusion, particle bombardment, calcium phosphate co-precipitation, DEAE-dextran-mediated transfer and viral vector-mediated transfer.

[00365] As used herein, a “fusosome” includes to a gene therapy vector comprising retroviral vector pseudotyped with an engineered fusogen comprising a G protein modified to include a targeting moiety and an F protein blinded to no longer recognize its cognate receptor. In some embodiments, the fusogen protein complex is from a paraymyxovirus, optionally wherein the paraymyxovirus is a Nipah virus. In some embodiments, the retroviral vector is a lentiviral vector.

[00366] A "gene," for the purposes of the present disclosure, includes a DNA region encoding a gene product, as well as all DNA regions which regulate the production of the gene product, whether or not such regulatory sequences are adjacent to coding and/or transcribed sequences. Accordingly, a gene includes, but is not necessarily limited to, promoter sequences, terminators, translational regulatory sequences such as ribosome binding sites and internal ribosome entry sites, enhancers, silencers, insulators, boundary elements, replication origins, matrix attachment sites and/or locus control regions.

[00367] "Gene expression" refers to the conversion of the information, contained in a gene, into a gene product. A gene product can be the direct transcriptional product of a gene (*e.g.*, mRNA, tRNA, rRNA, antisense RNA, ribozyme, structural RNA or any other type of RNA) or a protein produced by translation of an mRNA. Gene products also include RNAs which are modified, by processes such as capping, polyadenylation, methylation, and editing, and proteins modified by, for example, methylation, acetylation, phosphorylation, ubiquitination, ADP-ribosylation, myristoylation, and/or glycosylation.

[00368] The term “genetic modification” and its grammatical equivalents as used herein can refer to one or more alterations of a nucleic acid, *e.g.*, the nucleic acid within an organism's genome. For example, genetic modification can refer to alterations, additions, and/or deletion of genes or portions of genes or other nucleic acid sequences. A genetically modified cell can also refer to a cell with an added, deleted and/or altered gene or portion of a gene. A genetically modified cell can also refer to a cell with an added nucleic acid sequence that is not a gene or gene portion. Genetic modifications include, for example, both transient knock-in or knock-down mechanisms, and mechanisms that result in permanent knock-in, knock-down, or knock-out of target genes or portions of genes or nucleic acid sequences Genetic modifications include, for example, both transient knock-in and mechanisms that result in permanent knock-in of nucleic acids seqeunces Genetic modifications also include, for example, reduced or increased transcription, reduced or increased mRNA stability, reduced or increased translation, and reduced or increased protein stability.

[00369] As used herein, the terms "grafting", "administering," "introducing", "implanting" and "transplanting" as well as grammatical variations thereof are used interchangeably in the context of the placement of cells (*e.g.*, cells described herein) into a subject, by a method or route which results in localization or at least partial localization of the introduced cells at a desired site or systemic introduction (*e.g.* into circulation). The cells can be implanted directly to the desired site, or alternatively be administered by any appropriate route which results in delivery to a desired location in the subject where at least a portion of the implanted cells or components of the cells remain viable. The period of viability of the cells after administration to a subject can be as short as a few hours, e. g. twenty-four hours, to a few days, to as long as several years. In some embodiments, the cells can also be administered (*e.g.*, injected) a location other than the desired site, such as in the brain or subcutaneously, for example, in a capsule to maintain the implanted cells at the implant location and avoid migration of the implanted cells.

[00370] By "HLA" or "human leukocyte antigen" or “HLA molecules” or "human leukocyte antigen molecules” complex is a gene complex encoding the MHC proteins in humans. These cell-surface proteins that make up the HLA complex are responsible for the regulation of the immune response to antigens. In humans, there are two MHCs, class I molecues and class II molecules, "HLA-I" and "HLA-II", or "HLA-I molecules" and "HLA-II molecules". HLA-I includes three proteins, HLA-A, HLA-B and HLA-C, which present peptides from the inside of

the cell, and antigens presented by the HLA-I complex attract killer T-cells (also known as CD8+ T-cells or cytotoxic T cells). The HLA-I proteins are associated with β-2 microglobulin (B2M). HLA-II includes five proteins, HLA-DP, HLA-DM, HLA-DOB, HLA-DQ and HLA-DR, which present antigens from outside the cell to T lymphocytes. This stimulates CD4+ cells (also known as T-helper cells). It should be understood that the use of either "MHC" or "HLA" is not meant to be limiting, as it depends on whether the genes are from humans (HLA) or murine (MHC). Thus, as it relates to mammalian cells, these terms may be used interchangeably herein.

[00371] As used herein to characterize a cell, the terms "immune privileged" and "hypoimmunogenic" are used interchangeably and generally mean that such cell is less prone to innate or adaptive immune rejection by a subject into which such cells are transplanted, *e.g.*, the cell is less prone to allorejection by a subject into which such cells are transplanted. For example, relative to a cell of the same cell type that does not comprise the modifications, such a hypoimmunogenic cell may be about 2.5%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 97.5%, 99% or more less prone to innate or adaptive immune rejection by a subject into which such cells are transplanted. In some embodiments, genome editing technologies are used to modulate the expression of one or more MHC I and MHC II genes, and thus, contribute to generation of a hypoimmunogenic cell. In some embodiments, a hypoimmunogenic cell evades immune rejection in an MHC-mismatched allogeneic recipient. In some instance, differentiated cells produced from the hypoimmunogenic stem cells outlined herein evade immune rejection when administered (*e.g.*, transplanted or grafted) to an MHC-mismatched allogeneic recipient. In some embodiments, a hypoimmunogenic cell is protected from T cell-mediated adaptive immune rejection and/or innate immune cell rejection. Detailed descriptions of hypoimmunogenic cells, methods of producing thereof, and methods of using thereof are found in WO2016183041 filed May 9, 2015; WO2018132783 filed January 14, 2018; WO2018176390 filed March 20, 2018; WO2020018615 filed July 17, 2019; WO2020018620 filed July 17, 2019; PCT/US2020/44635 filed July 31, 2020; WO2021022223 filed July 31, 2020; WO2021041316 filed August 24, 2020; WO2021222285 filed April 27, 2021; and WO2021222285 filed April 27, 2021, the disclosures including the examples, sequence listings and figures are incorporated herein by reference in their entirety.

[00372] Hypoimmunogenicity of a cell can be determined by evaluating the immunogenicity of the cell such as the cell's ability to elicit adaptive and innate immune responses or to avoid

eliciting such adaptive and innate immune responses. Such immune response can be measured using assays recognized by those skilled in the art. In some embodiments, an innate and/or an adaptive immune response assay measures the effect of a hypoimmunogenic cell on T cell proliferation, T cell activation, T cell killing, donor specific antibody generation, NK cell proliferation, NK cell activation, and macrophage activity. In some cases, hypoimmunogenic cells and derivatives thereof undergo decreased killing by T cells and/or NK cells upon administration to a subject. In some instances, the cells and derivatives thereof show decreased macrophage engulfment compared to an unmodified or wild-type cell. In some embodiments, a hypoimmunogenic cell elicits a reduced or diminished immune response in a recipient subject compared to a corresponding unmodified wild-type cell. In some embodiments, a hypoimmunogenic cell is nonimmunogenic or fails to elicit an innate and/or an adaptive immune response in a recipient subject.

[00373] The term percent "identity," in the context of two or more nucleic acid or polypeptide sequences, refers to two or more sequences or subsequences that have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence, as measured using one of the sequence comparison algorithms described below (*e.g.*, BLASTP and BLASTN or other algorithms available to persons of skill) or by visual inspection. Depending on the application, the percent "identity" can exist over a region of the sequence being compared, *e.g.*, over a functional domain, or, alternatively, exist over the full length of the two sequences to be compared. For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[00374] Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in

the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection (see generally Ausubel *et al.,* infra).

[00375] One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul *et al.,* J. Mol. Biol. 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information.

[00376] "Immune signaling factor" as used herein refers to, in some cases, a molecule, protein, peptide and the like that activates immune signaling pathways.

[00377] "Immunosuppressive factor" or "immune regulatory factor" or "tolerogenic factor" as used herein include hypoimmunity factors, complement inhibitors, and other factors that modulate or affect the ability of a cell to be recognized by the immune system of a host or recipient subject upon administration, transplantation, or engraftment. These may be in combination with additional genetic modifications.

[00378] The terms "increased", "increase" or "enhance" or "activate" are all used herein to generally mean an increase by a statically significant amount; for the avoidance of any doubt, the terms "increased", "increase" or "enhance" or "activate" means an increase of at least 10% as compared to a reference level, for example an increase of at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% increase or any increase between 10-100% as compared to a reference level, or at least about a 2-fold, or at least about a 3-fold, or at least about a 4-fold, or at least about a 5-fold or at least about a 10-fold increase, or any increase between 2-fold and 10-fold or greater as compared to a reference level. In some embodiments, the reference level, also referred to as the basal level, is 0.

[00379] In some embodiments, the alteration is an indel. As used herein, "indel" refers to a mutation resulting from an insertion, deletion, or a combination thereof. As will be appreciated by those skilled in the art, an indel in a coding region of a genomic sequence will result in a frameshift mutation, unless the length of the indel is a multiple of three. In some embodiments, the alteration is a point mutation. As used herein, "point mutation" refers to a substitution that replaces one of the nucleotides. A gene editing (*e.g.* CRISPR/Cas) system of the present disclosure can be used to induce an indel of any length or a point mutation in a target polynucleotide sequence.

[00380] "Inducible promoters" are active only under certain conditions, such as but not limited to, in the presence of a given molecule factor (*e.g.*, an agent, biological molecule, chemical, ligand, or the like) or a given environmental condition (*e.g.*, particular CO_2 concentration, nutrient levels, light, heat). In the absence of that condition, inducible promoters typically do not allow significant or measurable levels of transcriptional activity. For example, inducible promoters may be induced according to temperature, pH, a hormone, a metabolite (*e.g.*, lactose, mannitol, an amino acid), light (*e.g.*, wavelength specific), osmotic potential (*e.g.*, salt-induced), heavy metal, or an antibiotic. Numerous standard inducible promoters will be known to one of skill in the art. Indcucible promoters are included herein as one type of "regulatable promoter".

[00381] In some cases, the inducible gene expression system can turn on or turn off transcription in the presence of a ligand, small molecule, peptide, factor, agent, and the like. In some cases, the inducible gene expression system can activate a protein degradation pathway in response to the presence of a ligand, small molecule, peptide, factor, agent, and the like.

[00382] As used herein, "knock down" refers to a reduction in expression of the target mRNA or the corresponding target protein. Knock down is commonly reported relative to levels present following administration or expression of a noncontrol molecule that does not mediate reduction in expression levels of RNA (*e.g.*, a non-targeting control shRNA, siRNA, or miRNA). In some embodiments, knock down of a target gene is achived by way of conditional or inducible shRNAs, conditional or inducible siRNAs, conditional or inducible miRNAs, or conditional or inducible CRISPR interference (CRISPRi). In some embodiments, knock down of a target gene is achieved by way of a protein-based component, such as a conditional or inducible degron method. In some embodiments, knock down of a target gene is achieved by genetic modification, including shRNAs, siRNAs, miRNAs, or use of gene editing systems (*e.g.* CRISPR/Cas).

[00383] Knock down is commonly assessed by measuring the mRNA levels using quantitative polymerase chain reaction (qPCR) amplification or by measuring protein levels by western blot or enzyme-linked immunosorbent assay (ELISA). Analyzing the protein level provides an assessment of both mRNA cleavage as well as translation inhibition. Further techniques for measuring knock down include RNA solution hybridization, nuclease protection, northern hybridization, gene expression monitoring with a microarray, antibody binding, radioimmunoassay, and fluorescence activated cell analysis. Those skilled in the art will readily appreciate how to use the gene editing systems (*e.g.* CRISPR/Cas) of the present disclosure to

knock out a target polynucleotide sequence or a portion thereof based upon the details described herein.

[00384] By "knock in" or “knock-in” herein is meant a genetic modification resulting from the insertion of a DNA sequence into a chromosomal locus in a host cell. This causes initiation of or increased levels of expression of the knocked in gene, portion of gene, or nucleic acid sequence inserted product, *e.g.*, an increase in RNA transcript levels and/or encoded protein levels. As will be appreciated by those in the art, this can be accomplished in several ways, including inserting or adding one or more additional copies of the gene or portion thereof to the host cell or altering a regulatory component of the endogenous gene increasing expression of the protein is made or inserting a specific nucleic acid sequence whose expression is desired. This may be accomplished by modifying a promoter, adding a different promoter, adding an enhancer, adding other regulatory elements, or modifying other gene expression sequences.

[00385] As used herein, "knock out" or “knock-out” includes deleting all or a portion of a target polynucleotide sequence in a way that interferes with the translation or function of the target polynucleotide sequence. For example, a knock out can be achieved by altering a target polynucleotide sequence by inducing an insertion or a deletion (“indel”) in the target polynucleotide sequence, including in a functional domain of the target polynucleotide sequence (*e.g.*, a DNA binding domain). Those skilled in the art will readily appreciate how to use the gene editing systems (*e.g.* CRISPR/Cas)of the present disclosure to knock out a target polynucleotide sequence or a portion thereof based upon the details described herein.

[00386] In some embodiments, a genetic modification or alteration results in a knock out or knock down of the target polynucleotide sequence or a portion thereof. Knocking out a target polynucleotide sequence or a portion thereof using a gene editing system (*e.g.* CRISPR/Cas)of the present disclosure can be useful for a variety of applications. For example, knocking out a target polynucleotide sequence in a cell can be performed in vitro for research purposes. For ex vivo purposes, knocking out a target polynucleotide sequence in a cell can be useful for treating or preventing a disorder associated with expression of the target polynucleotide sequence (*e.g.*, by knocking out a mutant allele in a cell ex vivo and introducing those cells comprising the knocked out mutant allele into a subject) or for changing the genotype or phenotype of a cell.

[00387] "Modulation" of gene expression refers to a change in the expression level of a gene. Modulation of expression can include, but is not limited to, gene activation and gene repression.

Modulation may also be complete, *i.e.* wherein gene expression is totally inactivated or is activated to wild-type levels or beyond; or it may be partial, wherein gene expression is partially reduced, or partially activated to some fraction of wild-type levels. As used herein, the term "modify gene expression" refers to introducing any of the modifications disclosed herein into a cell to make the engineered cells disclosed herein.

[00388] In additional or alternative aspects, the present disclosure contemplates altering target polynucleotide sequences in any manner which is available to the skilled artisan, *e.g.*, utilizing a nuclease system such as a TAL effector nuclease (TALEN) or zinc finger nuclease (ZFN) system. It should be understood that although examples of methods utilizing CRISPR/Cas (*e.g.*, Cas9 and Cas12a) and TALEN are described in detail herein, the disclosure is not limited to the use of these methods/systems. Other methods of targeting to reduce or ablate expression in target cells known to the skilled artisan can be utilized herein. The methods provided herein can be used to alter a target polynucleotide sequence in a cell. The present disclosure contemplates altering target polynucleotide sequences in a cell for any purpose. In some embodiments, the target polynucleotide sequence in a cell is altered to produce a mutant cell. As used herein, a "mutant cell" refers to a cell with a resulting genotype that differs from its original genotype. In some instances, a "mutant cell" exhibits a mutant phenotype, for example when a normally functioning gene is altered using the gene editing systems (*e.g.* CRISPR/Cas)systems of the present disclosure. In other instances, a "mutant cell" exhibits a wild-type phenotype, for example when a gene editing system (*e.g.* CRISPR/Cas)system of the present disclosure is used to correct a mutant genotype. In some embodiments, the target polynucleotide sequence in a cell is altered to correct or repair a genetic mutation (*e.g.*, to restore a normal phenotype to the cell). In some embodiments, the target polynucleotide sequence in a cell is altered to induce a genetic mutation (*e.g.*, to disrupt the function of a gene or genomic element).

[00389] The term "native cell" as used herein refers to a cell that is not otherwise modified (e.g., engineered). In some embodiments, a native cell is a naturally occurring wild-type or a control cell.

[00390] The term "operatively linked" or "operably linked" are used interchangeably with reference to a juxtaposition of two or more components (such as sequence elements), in which the components are arranged such that both components function normally and allow the possibility that at least one of the components can mediate a function that is exerted upon at least

one of the other components. By way of illustration, a transcriptional regulatory sequence, such as a promoter, is operatively linked to a coding sequence if the transcriptional regulatory sequence controls the level of transcription of the coding sequence in response to the presence or absence of one or more transcriptional regulatory factors. A transcriptional regulatory sequence is generally operatively linked in cis with a coding sequence, but need not be directly adjacent to it. For example, an enhancer is a transcriptional regulatory sequence that is operatively linked to a coding sequence, even though they are not contiguous.

[00391] "Pluripotent stem cells" as used herein have the potential to differentiate into any of the three germ layers: endoderm (*e.g.*, the stomach linking, gastrointestinal tract, lungs, etc.), mesoderm (*e.g.*, muscle, bone, blood, urogenital tissue, etc.) or ectoderm (*e.g.*, epidermal tissues and nervous system tissues). The term "pluripotent stem cells," as used herein, also encompasses "induced pluripotent stem cells", or "iPSCs", or a type of pluripotent stem cell derived from a non-pluripotent cell. In some embodiments, a pluripotent stem cell is produced or generated from a cell that is not a pluripotent cell. In other words, pluripotent stem cells can be direct or indirect progeny of a non-pluripotent cell. Examples of parent cells include somatic cells that have been reprogrammed to induce a pluripotent, undifferentiated phenotype by various means. Such " iPS" or "iPSC" cells can be created by inducing the expression of certain regulatory genes or by the exogenous application of certain proteins. Methods for the induction of iPS cells are known in the art and are further described below. (See, *e.g.*, Zhou *et al.*, Stem Cells 27 (11): 2667-74 (2009); Huangfu *et al.*, Nature Biotechnol. 26 (7): 795 (2008); Woltjen *et al.*, Nature 458 (7239): 766-770 (2009); and Zhou *et al.*, Cell Stem Cell 8:381-384 (2009); each of which is incorporated by reference herein in their entirety.) The generation of induced pluripotent stem cells (iPSCs) is outlined below. As used herein, "hiPSCs" are human induced pluripotent stem cells. In some embodiments, "pluripotent stem cells," as used herein, also encompasses mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs), and/or embryonic stem cells (ESCs).

[00392] As used herein, "promoter," "promoter sequence," or "promoter region" refers to a DNA regulatory region/sequence capable of binding RNA polymerase and involved in initiating transcription of a downstream coding or non-coding sequence. In some examples, the promoter sequence includes the transcription initiation site and extends upstream to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above

background. In some embodiments, the promoter sequence includes a transcription initiation site, as well as protein binding domains responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes.

[00393] In some embodiments, the engineered and hypoimmunogenic cells described are propagated from a primary T cell or a progeny thereof. As used herein, the term "propagated from a primary T cell or a progeny thereof" encompasses the initial primary T cell that is isolated from the donor subject and any subsequent progeny thereof. As used herein, the term "progeny" encompasses, *e.g.*, a first-generation progeny, *i.e.*, the progeny is directly derived from, obtained from, obtainable from or derivable from the initial primary T cell by, *e.g.*, traditional propagation methods. The term "progeny" also encompasses further generations such as second, third, fourth, fifth, sixth, seventh, or more generations, *i.e.*, generations of cells which are derived from, obtained from, obtainable from or derivable from the former generation by, *e.g.*, traditional propagation methods. The term "progeny" also encompasses modified cells that result from the modification or alteration of the initial primary T cell or a progeny thereof.

[00394] The term "recipient patient" refers to an animal, for example, a human to whom treatment, including prophylactic treatment, with the cells as described herein, is provided. For treatment of those infections, conditions or disease states, which are specific for a specific animal such as a human patient, the term patient refers to that specific animal. The term "recipient patient" also encompasses any vertebrate including but not limited to mammals, reptiles, amphibians and fish. However, advantageously, the recipient patient is a mammal such as a human, or other mammals such as a domesticated mammal, *e.g.* dog, cat, horse, and the like, or production mammal, *e.g.* cow, sheep, pig, and the like. In some embodiments, the recipient patient has an infection, condition, disease, or disorder. In some embodiments, the recipient patient is suspected of having an infection, condition, disease, or disorder

[00395] "Regulatable modification" as used herein refers to any modification of a cell that is made under certain conditions, such as, but not limited to, cellular conditions or stages, or external conditions. In embodiments, a regulatable modification comprises regulatable knock out of a target gene. In embodiments, a regulatable modification comprises regulatable reduced expression of one or more target genes. In embodiments, a regulatable modification comprises regulatable increased expression of one or endogenous or exogenous genes. In embodiments, regulatable modifications comprise conditional or inducible DNA-based components,

conditional or inducible RNA-based components, or conditional or inducible protein-based components to increase, decrease, or knock out expression of a target gene.

[00396] "Regulatable promoters" as used herein are active only under certain conditions, such as but not limited to, cellular conditions or stages, or external conditions. As used herein, regulatable promoters include conditional promoters and inducible promoters. In some cases, the inducible regulatable gene expression system can turn on or turn off transcription in the presence of a ligand, small molecule, peptide, factor, agent, and the like. In some cases, the regulatable gene expression system can activate a protein degradation pathway in response to the presence of a ligand, small molecule, peptide, factor, agent, and the like.

[00397] As used herein, the terms "regulatory sequences," "regulatory elements," and "control elements" are interchangeable and refer to polynucleotide sequences that are upstream (5' non-coding sequences), within, or downstream (3' non-translated sequences) of a polynucleotide target to be expressed. Regulatory sequences influence, for example but are not limited to, the timing of transcription, amount or level of transcription, RNA processing or stability, and/or translation of the related structural nucleotide sequence. Regulatory sequences may include activator binding sequences, enhancers, introns, polyadenylation recognition sequences, promoters, repressor binding sequences, stem-loop structures, translational initiation sequences, translation leader sequences, transcription termination sequences, translation termination sequences, primer binding sites, and the like. It is recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, nucleotide sequences of different lengths may have identical regulatory or promoter activity.

[00398] "Safe harbor locus" as used herein refers to a gene locus that allows expression of a transgene or an exogenous gene in a manner that enables the newly inserted genetic elements to function predictably and that also may not cause alterations of the host genome in a manner that poses a risk to the host cell. Exemplary "safe harbor" loci include, but are not limited to, a CCR5 gene, a PPP1R12C (also known as AAVS1) gene, a CLYBL gene, and/or a Rosa gene (*e.g.*, ROSA26). "Target locus" as used herein refers to a gene locus that allows expression of a transgene or an exogenous gene. Exemplary "target loci" include, but are not limited to, a CXCR4 gene, an albumin gene, a SHS231 locus, an F3 gene (also known as CD142), a MICA gene, a MICB gene, a LRP1 gene (also known as CD91), a HMGB1 gene, an ABO gene, a RHD gene, a FUT1 gene, and/or a KDM5D gene (also known as HY). The exogenous polynucleotide

encoding the exogenous gene can be inserted in the CDS region for B2M, CIITA, TRAC, TRBC, CCR5, F3 (*i.e.*, CD142), MICA, MICB, LRP1, HMGB1, ABO, RHD, FUT1, KDM5D (*i.e.*, HY), PDGFRa, OLIG2, and/or GFAP. The exogenous polynucleotide encoding the exogenous gene can be inserted in introns 1 or 2 for PPP1R12C (*i.e.*, AAVS1) or CCR5. The exogenous polynucleotide encoding the exogenous gene can be inserted in exons 1 or 2 or 3 for CCR5. The exogenous polynucleotide encoding the exogenous gene can be inserted in intron 2 for CLYBL. The exogenous polynucleotide encoding the exogenous gene can be inserted in a 500 bp window in Ch-4:58,976,613 (*i.e.*, SHS231). The exogenous polynucleotide encoding the exogenous gene can be insert in any suitable region of the aforementioned safe harbor or target loci that allows for expression of the exogenous, including, for example, an intron, an exon or a coding sequence region in a safe harbor or target locus.

[00399] As used herein, a "target" can refer to a gene, a portion of a gene, a portion of the genome, or a protein that is subject to regulatable reduced expression by the methods described herein.

[00400] As used herein, "therapeutically effective amount" refers to an amount sufficient to provide a therapeutic benefit in the treatment and/or management of a disease, disorder, or condition. In some embodiments, a therapeutically effective amount is an amount sufficient to ameliorate, palliate, stabilize, reverse, slow, attenuate or delay the progression of a disease, disorder, or condition, or of a symptom or side effect of the disease, disorder, or condition. In some embodiments, the therapeutically effective amount is also a clinically effective amount. In other embodiments, the therapeutically effective amount is not a clinically effective amount.

[00401] As used herein, the term "treating" and "treatment" includes administering to a subject a therapeutically or clinically effective amount of cells described herein so that the subject has a reduction in at least one symptom of the disease or an improvement in the disease, for example, beneficial or desired therapeutic or clinical results. For purposes of this technology, beneficial or desired therapeutic or clinical results include, but are not limited to, alleviation of one or more symptoms, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. Treating can refer to prolonging survival as compared to expected survival if not receiving treatment. Thus, one of skill in the art realizes that a treatment may improve the disease condition, but may not be a

complete cure for the disease. In some embodiments, one or more symptoms of a condition, disease or disorder are alleviated by at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, or at least 50% upon treatment of the condition, disease or disorder.

[00402] For purposes of this technology, beneficial or desired therapeutic or clinical results of disease treatment include, but are not limited to, alleviation of one or more symptoms, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable.

[00403] A "vector" or "construct" is capable of transferring gene sequences to target cells. Typically, "vector construct," "expression vector," and "gene transfer vector," mean any nucleic acid construct capable of directing the expression of a gene of interest and which can transfer gene sequences to target cells. Thus, the term includes cloning, and expression vehicles, as well as integrating vectors. Methods for the introduction of vectors or constructs into cells are known to those of skill in the art and include, but are not limited to, lipid-mediated transfer (*i.e.*, liposomes, including neutral and cationic lipids), electroporation, direct injection, cell fusion, particle bombardment, calcium phosphate co-precipitation, DEAE-dextran-mediated transfer and/or viral vector-mediated transfer.

[00404] In some embodiments, the cells are engineered to have reduced or increased expression of one or more targets relative to an unaltered or unmodified wild-type cell. In some embodiments, the cells are engineered to have constitutive reduced or increased expression of one or more targets relative to an unaltered or unmodified wild-type cell. In some embodiments, the cells are engineered to have regulatable reduced or increased expression of one or more targets relative to an unaltered or unmodified wild-type cell. In some embodiments, the cells comprise increased expression of CD47 relative to a wild-type cell or a control cell of the same cell type. By “wild-type” or “wt” or “control” in the context of a cell means any cell found in nature. Examples of wild type or control cells include primary cells and T cells found in nature. However, by way of example, in the context of an engineered cell, as used herein, “wild-type” or “control” can also mean an engineered cell that may contain nucleic acid changes resulting in reduced expression of one or more MHC class I molecules and/or class II molecules and/or T-cell receptors, but did not undergo the gene editing procedures to result in overexpression of CD47 proteins. For example, as used herein, “wild-type” or “control” means an engineered cell

that comprises reduced or knocked out expression of B2M, CIITA, and/or TRAC. Also as used herein, "wild-type" or "control" means an engineered cell that comprises reduced or knocked out expression of B2M, CIITA, TRAC, and/or TRBC. As used herein, "wild-type" or "control" also means an engineered cell that may contain nucleic acid changes resulting in overexpression of CD47 proteins, but did not undergo the gene editing procedures to result in reduced expression of one or more MHC class I and/or class II molecules and/or T-cell receptors. In the context of an iPSC or a progeny thereof, "wild-type" or "control" also means an iPSC or progeny thereof that may contain nucleic acid changes resulting in pluripotency but did not undergo the gene editing procedures of the present disclosure to achieve reduced expression of one or more MHC I class and/or class II molecules and/or T-cell receptors, and/or overexpression of CD47 proteins. For example, as used herein, "wild-type" or "control" means an iPSC or progeny thereof that comprises reduced or knocked out expression of B2M, CIITA, and/or TRAC. Also as used herein, "wild-type" or "control" means an iPSC or progeny thereof that comprises reduced or knocked out expression of B2M, CIITA, TRAC, and/or TRBC. In the context of a primary T cell or a progeny thereof, "wild-type" or "control" also means a primary T cell or progeny thereof that may contain nucleic acid changes resulting in reduced expression of one or more MHC class I and/or class II molecules and/or T-cell receptors, but did not undergo the gene editing procedures to result in overexpression of CD47 proteins. For example, as used herein, "wild-type" or "control" means a primary T cell or progeny thereof that comprises reduced or knocked out expression of B2M, CIITA, and/or TRAC. Also as used herein, "wild-type" or "control" means a primary T cell or progeny thereof that comprises reduced or knocked out expression of B2M, CIITA, TRAC, and/or TRBC. Also in the context of a primary T cell or a progeny thereof, "wild-type" or "control" also means a primary T cell or progeny thereof that may contain nucleic acid changes resulting in overexpression of CD47 proteins, but did not undergo the gene editing procedures to result in reduced expression of one or more MHC class I and/or class II molecules and/or T-cell receptors. In some embodiments, the cells are engineered to have regulatable reduced or increased expression of one or more targets relative to a cell of the same cell type that does not comprise the modifications. In some embodiments, the wild-type cell or the control cell is a starting material. In some embodiments, the starting material is otherwise modified or engineered to have altered expression of one or more genes to generate the engineered cell. In some embodiments, the control cell is from the same starting material as the cell described

herein. In some embodiments, the control cell is from a reference starting material. In some embodiments, the starting material is from a single donor. In some embodiments, the starting material is from a pool of donors.

[00405] In some embodiments, the cells are engineered to express a higher amount of a tolerogenic factor relative to control. The term "control" as used herein can be used in the context of a cell, a population of cells, a sample, or a measurement. In some embodiments, the cells are engineered to express a higher amount of a tolerogenic factor relative to a control cell. In some embodiments, the cells are engineered to express a higher amount of a tolerogenic factor relative to a population of control cells. In some embodiments, the cells are engineered to express a higher amount of a tolerogenic factor relative to a control sample. In some embodiments, the cells are engineered to express a higher amount of a tolerogenic factor relative to a control measurement, including, but not limited to, a baseline reference or control signal in an assay or test. As used herein, a "baseline reference" refers to any suitable reference value or signal level known to those skilled in the art in view of the present disclosure, including those used in the examples presented herein. In some embodiments, a baseline reference refers to a control level, and in some levels, a normal level, of expression against which a test level of expression can be compared. In some embodiments, a baseline reference refers to a control or a background level that is appropriate for the particular test or assay used. In some embodiments, a baseline reference refers to a control signal, including, but not limited to, an isotype control value from any suitable test or assay known in the art that can be used to evaluate expression levels. In some embodiments, a baseline reference refers to a background signal from any suitable test or assay known in the art that can be used to evaluate expression levels. In some embodiments, the cells are engineered to expresses a tolerogenic factor at a threshold level or higher. In some embodiments, the cells are engineered to expresses CD47 at a threshold level or higher. A threshold can be determined using any suitable method known to those in the art in view of the specification, including, for example, those disclosed herein. In some embodiments, a baseline reference is specific for an engineered cell or a population of cells comprising the engineered cell.

[00406] It is noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only," and the like in connection with the recitation of claim elements, or use of a

"negative" limitation. As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present disclosure. Any recited method may be carried out in the order of events recited or in any other order that is logically possible. Although any methods and materials similar or equivalent to those described herein may also be used in the practice or testing of the present disclosure, representative illustrative methods and materials are now described.

[00407] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this present disclosure belongs. Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the present disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the present disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the present disclosure. Certain ranges are presented herein with numerical values being preceded by the term "about." The term "about" is used herein to provide literal support for the exact number that it precedes, as well as a number that is near to or approximately the number that the term precedes. In determining whether a number is near to or approximately a specifically recited number, the near or approximating unrecited number may be a number, which, in the context presented, provides the substantial equivalent of the specifically recited number. The term about is used herein to mean plus or minus ten percent (10%) of a value. For example, "about 100" refers to any number between 90 and 110.

[00408] All publications, patents, and patent applications cited in this specification are incorporated herein by reference to the same extent as if each individual publication, patent, or patent application were specifically and individually indicated to be incorporated by reference. Furthermore, each cited publication, patent, or patent application is incorporated herein by reference to disclose and describe the subject matter in connection with which the publications are cited. The citation of any publication is for its disclosure prior to the filing date and should

not be construed as an admission that the technology described herein is not entitled to antedate such publication by virtue of prior technology. Further, the dates of publication provided might be different from the actual publication dates, which may need to be independently confirmed.

[00409] Before the technology is further described, it is to be understood that this technology is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present disclosure will be limited only by the appended claims. It should also be understood that the headers used herein are not limiting and are merely intended to orient the reader, but the subject matter generally applies to the technology disclosed herein.

III. DETAILED DESCRIPTION

A. Hypoimmunogenic Cells

[00410] In some embodiments, the present disclosure provides engineered (*e.g.*, modified and genetically modified) cells that comprise regulatable modifications that i) reduce expression of one or more MHC class I and/or MHC class II human leukocyte antigen molecules relative to a cell of the same cell type that does not comprise the modifications, wherein the regulatable reduced expression is by way of an RNA-based component , a DNA-based component , or a protein-based component , and/or ii) increase expression of a first exogenous polynucleotide encoding one or more tolerogenic factors relative to a cell of the same cell type that does not comprise the modifications, wherein the regulatable overexpression is by way of a conditional or inducible promoter. In some embodiments, the cells are able to evade activating NK cell mediated and/or antibody-based immune responses.

[00411] In some embodiments, the cells are induced pluripotent stem cells, any type of differentiated cells thereof, primary immune cells and other primary cells of any tissue. In some embodiments, the differentiated cells are cardiac cells and subpopulations thereof, neural cells and subpopulations thereof, cerebral endothelial cells and subpopulations thereof, dopaminergic neurons and subpopulations thereof, glial progenitor cells and subpopulations thereof, endothelial cells and subpopulations thereof, thyroid cells and subpopulations thereof, hepatocytes and subpopulations thereof, pancreatic islet cells and subpopulations thereof, or retinal pigmented epithelium cells and subpopulations thereof. In some embodiments, the

differentiated cells are T cells and subpopulations thereof, NK cells and subpopulations thereof. In some embodiments, the primary immune cells are T cells and subpopulations thereof and NK cells and subpopulations thereof. In some embodiments, the primary tissue cells include primary endothelial cells and subpopulations thereof.

[00412] In some embodiments, cells described herein comprise regulatable reduced expression of one or more MHC class I and/or MHC class II human leukocyte antigen molecules relative to a cell of the same cell type that does not comprise the modifications, wherein the regulatable reduced expression is by way of an RNA-based component . In some embodiments, the RNA-based component is selected from the group consisting of conditional or inducible shRNAs, conditional or inducible siRNAs, conditional or inducible miRNAs, and conditional or inducible CRISPR interference (CRISPRi). In some embodiments, the RNA-based component is under the control of a conditional promoter, wherein the conditional promoter is a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, or a differentiation-induced promoter. In some embodiments, the RNA-based component is under the control of an inducible promoter, wherein the inducible promoter is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[00413] In some embodiments, cells described herein comprise regulatable reduced expression of one or more MHC class I and/or MHC class II human leukocyte antigen molecules relative to a cell of the same cell type that does not comprise the modifications, wherein the regulatable reduced expression is by way of a DNA-based component . In some embodiments, the DNA-based component is a knock out or knock down using a method selected from the group consisting of conditional or inducible CRISPRs, conditional or inducible TALENs, conditional or inducible zinc finger nucleases, conditional or inducible homing endonucleases, and conditional or inducible meganucleases. In some embodiments, the DNA-based component is under the control of a conditional promoter, wherein the conditional promoter is a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, or a differentiation-induced promoter. In some embodiments, the DNA-based component is under the control of an inducible promoter, wherein the inducible promoter is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[00414] In some embodiments, cells described herein comprise regulatable reduced expression of one or more MHC class I and/or MHC class II human leukocyte antigen molecules relative to a cell of the same cell type that does not comprise the modifications, wherein the regulatable reduced expression is by way of a protein-based component . In some embodiments, the protein-based component is a conditional or inducible degron method. In some embodiments, the degron method is selected from the group consisting of ligand induced degradation (LID) using a SMASH tag, LID using Shield-1, LID using auxin, LID using rapamycin, conditional or inducible peptidic degrons (*e.g.*, IKZF3 based degrons), and conditional or inducible proteolysis-targeting chimeras (PROTACs). In some embodiments, the protein-based component is under the control of a conditional promoter, wherein the conditional promoter is a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, or a differentiation-induced promoter. In some embodiments, the protein-based component is under the control of an inducible promoter, wherein the inducible promoter is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[00415] In some embodiments, cells described herein comprise regulatable overexpression of a first exogenous polynucleotide encoding one or more tolerogenic factors, wherein the regulatable overexpression is by way of a conditional or inducible promoter. In some embodiments, the regulatable overexpression is by way of a conditional promoter, wherein the conditional promoter is a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, or a differentiation-induced promoter. In some embodiments, the regulatable overexpression is by way of an inducible promoter that is regulated by a small molecule, a ligand, or a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[00416] In some embodiments, the present disclosure is directed to pluripotent stem cells, (*e.g.*, pluripotent stem cells and induced pluripotent stem cells (iPSCs)), differentiated cells derived from such pluripotent stem cells (such as, but not limited to, T cells, NK cells, cardiac cells, neural cells, cerebral endothelial cells, dopaminergic neurons, glial progenitor cells, endothelial cells, thyroid cells, hepatocytes, pancreatic islet cells, and retinal pigmented epithelium cells), and primary cells (such as, but not limited to, primary T cells and primary NK cells). In some embodiments, the pluripotent stem cells, differentiated cells derived therefrom such as T cells,

NK cells, cardiac cells, neural cells, cerebral endothelial cells, dopaminergic neurons, glial progenitor cells, endothelial cells, thyroid cells, hepatocytes, pancreatic islet cells, and retinal pigmented epithelium cells, and primary cells such as primary T cells and primary NK cells are engineered for regulatable reduced expression or regulatable lack of expression of one or more MHC class I and/or MHC class II human leukocyte antigen molecules, and in some instances, for regulatable reduced expression or regulatable lack of expression of a T-cell receptor (TCR) complex. In some embodiments, the hypoimmune T cells and primary T cells regulatably overexpress CD47 and optionally regulatably overexpress a chimeric antigen receptor (CAR) in addition to (i) regulatable reduced expression or regulatable lack of expression of one or more MHC class I and/or MHC class II human leukocyte antigen molecules, and (ii) regulatable reduced expression or regulatable lack of expression of a T-cell receptor (TCR) complex. In some embodiments, the CAR comprises an antigen binding domain that binds to any one selected from the group consisting of CD19, CD22, CD38, CD123, CD138, and BCMA. In some embodiments, the CAR is a CD19-specific CAR. In some embodiments, the CAR is a CD22-specific CAR. In some instances, the CAR is a CD38-specific CAR. In some embodiments, the CAR is a CD123-specific CAR. In some embodiments, the CAR is a CD138-specific CAR. In some instances, the CAR is a BCMA-specific CAR. In some embodiments, the CAR is a bispecific CAR. In some embodiments, the bispecific CAR is a CD19/CD22-bispecific CAR. In some embodiments, the bispecific CAR is a BCMA/CD38-bispecific CAR. In some embodiments, the cells described express a CD19-specific CAR and a different CAR, such as, but not limited to a CD22-specific CAR, a CD38-specific CAR, a CD123-specific CAR, a CD138-specific CAR, and a BCMA-specific CAR. In some embodiments, the cells described express a CD22-specific CAR and a different CAR, such as, but not limited to a CD19-specific CAR, a CD38-specific CAR, a CD123-specific CAR, a CD138-specific CAR, and a BCMA-specific CAR. In some embodiments, the cells described express a CD38-specific CAR and a different CAR, such as, but not limited to a CD22-specific CAR, a CD18-specific CAR, a CD123-specific CAR, a CD138-specific CAR, and a BCMA-specific CAR. In some embodiments, the cells described express a CD123-specific CAR and a different CAR, such as, but not limited to a CD22-specific CAR, a CD38-specific CAR, a CD19-specific CAR, a CD138-specific CAR, and a BCMA-specific CAR. In some embodiments, the cells described express a CD138-specific CAR and a different CAR, such as, but not limited to a CD22-specific

CAR, a CD38-specific CAR, a CD123-specific CAR, a CD19-specific CAR, and a BCMA-specific CAR. In some embodiments, the cells described express a BCMA-specific CAR and a different CAR, such as, but not limited to a CD22-specific CAR, a CD38-specific CAR, a CD123-specific CAR, a CD138-specific CAR, and a CD19-specific CAR.

[00417] In some embodiments, hypoimmune cells derived from iPSCs, such as, but not limited to, T cells, NK cells, cardiac cells, neural cells, cerebral endothelial cells, dopaminergic neurons, glial progenitor cells, endothelial cells, thyroid cells, hepatocytes, pancreatic islet cells, and retinal pigmented epithelium cells, regulatably overexpress CD47, and include a regulatable genomic modification or regulatable knock out or knock down of the B2M gene. In some embodiments, hypoimmune cells derived from iPSCs, such as, but not limited to, T cells, NK cells, cardiac cells, neural cells, cerebral endothelial cells, dopaminergic neurons, glial progenitor cells, endothelial cells, thyroid cells, hepatocytes, pancreatic islet cells, and retinal pigmented epithelium cells, regulatably overexpress CD47 and include a regulatable genomic modification or regulatable knock out or knock down of the CIITA gene. In some embodiments, the cells are regulatably $B2M^{-/-}$ cells. In some embodiments, the cells are regulatably $CIITA^{-/-}$ cells. In some embodiments, the cells are regulatably *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{indel/indel}$ cells. In some embodiments, the cells are regulatably $CIITA^{indel/inde}$ cells. In some embodiments, the cells are regulatably *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{knock\ down}$ cells. In some embodiments, the cells are regulatably $CIITA^{knock\ down}$ cells. In some embodiments, the cells are regulatably *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{-/-}$, $CIITA^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{indel/indel}$, $CIITA^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{knock\ down}$, $CIITA^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{-/-}$, $CIITA^{-/-}$ cells. In some embodiments, the cells are regulatably $B2M^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably $CIITA^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{indel/indel}$, $CIITA^{indel/indel}$ cells. In some embodiments, the cells are regulatably $B2M^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably $CIITA^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{knock\ down}$, $CIITA^{knock\ down}$ cells. In some embodiments, the cells are regulatably $B2M^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably $CIITA^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{-/-}$, $CIITA^{-/-}$, $TRAC^{-/-}$ cells. In some embodiments, the

cells are regulatably *B2M*$^{-/-}$, *TRAC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRAC*$^{-/-}$ that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRAC*$^{-/-}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$ that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$ that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRBC*$^{-/-}$ cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRBC*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRBC*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRBC*$^{-/-}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRBC*$^{indel/indel}$ cells that also

express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRBC*$^{knock\ down}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$ cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells.

[00418] In some embodiments, hypoimmune cells derived from iPSCs are produced by differentiating induced pluripotent stem cells such as hypoimmunogenic induced pluripotent stem cells.

[00419] In some embodiments, hypoimmune cells derived from ESCs, such as, but not limited to, T cells, NK cells, cardiac cells, neural cells, cerebral endothelial cells, dopaminergic neurons, glial progenitor cells, endothelial cells, thyroid cells, hepatocytes, pancreatic islet cells, and retinal pigmented epithelium cells, regulatably overexpress CD47, and include a regulatable genomic modification or regulatable knock out or knock down of the B2M gene. In some embodiments, hypoimmune cells derived from ESCs, such as, but not limited to, T cells, NK cells, cardiac cells, neural cells, cerebral endothelial cells, dopaminergic neurons, glial progenitor cells, endothelial cells, thyroid cells, hepatocytes, pancreatic islet cells, and retinal pigmented epithelium cells, regulatably overexpress CD47 and include a regulatable genomic modification or regulatable knock out or knock down of the CIITA gene. In some embodiments, the cells are regulatably $B2M^{-/-}$ cells. In some embodiments, the cells are regulatably $CIITA^{-/-}$ cells. In some embodiments, the cells are regulatably *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{indel/indel}$ cells. In some embodiments, the cells are regulatably $CIITA^{indel/inde}$ cells. In some embodiments, the cells are regulatably *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{knock\ down}$ cells. In some embodiments, the cells are regulatably $CIITA^{knock\ down}$ cells. In some embodiments, the cells are regulatably *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{-/-}$, $CIITA^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{indel/indel}$, $CIITA^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{knock\ down}$, $CIITA^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{-/-}$, $CIITA^{-/-}$ cells. In some embodiments, the cells are regulatably $B2M^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably $CIITA^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{indel/indel}$, $CIITA^{indel/indel}$ cells. In some embodiments, the cells are regulatably $B2M^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably $CIITA^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{knock\ down}$, $CIITA^{knock\ down}$ cells. In some embodiments, the cells are regulatably $B2M^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably $CIITA^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{-/-}$, $CIITA^{-/-}$, $TRAC^{-/-}$ cells. In some embodiments, the cells are regulatably $B2M^{-/-}$, $TRAC^{-/-}$, *CD47tg* cells. In some embodiments, the cells are

regulatably *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRAC*$^{-/-}$ that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRAC*$^{-/-}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$ that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$ that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRBC*$^{-/-}$ cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRBC*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRBC*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRBC*$^{-/-}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRBC*$^{indel/indel}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRBC*$^{indel/indel}$,

CD47tg cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRBC*$^{knock\ down}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$ cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells.. In some embodiments, hypoimmune cells derived from iPSCs are produced by differentiating pluripotent stem cells such as hypoimmunogenic embryonic stem cells.

[00420] In some embodiments, hypoimmune T cells derived from iPSCs and primary T cells regulatably overexpress one or more tolerogenic factors and a chimeric antigen receptor (CAR),

and include a regulatable genomic modification or regulatable knock out or knock down of the B2M gene. In some embodiments, hypoimmune T cells derived from iPSCs and primary T cells regulatably overexpress one or more tolerogenic factors and include a regulatable genomic modification or regulatable knock out or knock down of the CIITA gene. In some embodiments, hypoimmune T cells derived from iPSCs and primary T cells regulatably overexpress one or more tolerogenic factors and a CAR, and include a regulatable genomic modification or regulatable knock out or knock down of the TRAC gene. In some embodiments, hypoimmune T cells derived from iPSCs and primary T cells regulatably overexpress one or more tolerogenic factors and a CAR, and include a regulatable genomic modification or regulatable knock out or knock down of the TRB gene. In some embodiments, hypoimmune T cells derived from iPSCs and primary T cells regulatably overexpress one or more tolerogenic factors and a CAR, and include one or more regulatable genomic modifications or regulatable knock outs or knock downs selected from the group consisting of the B2M, CIITA, TRAC, and TRB genes. In some embodiments, hypoimmune T cells derived from iPSCs and primary T cells regulatably overexpress one or more tolerogenic factors and a CAR, and include regulatable genomic modifications or regulatable knock outs or knock downs of the B2M, CIITA, TRAC, and TRB genes. In some embodiments, hypoimmune T cells derived from iPSCs and primary T cells regulatably overexpress CD47 and a chimeric antigen receptor (CAR), and include a regulatable genomic modification or regulatable knock out or knock down of the B2M gene. In some embodiments, hypoimmune T cells derived from iPSCs and primary T cells regulatably overexpress CD47 and include a regulatable genomic modification or regulatable knock out or knock down of the CIITA gene. In some embodiments, hypoimmune T cells derived from iPSCs and primary T cells regulatably overexpress CD47 and a CAR, and include a regulatable genomic modification or regulatable knock out or knock down of the TRAC gene. In some embodiments, hypoimmune T cells derived from iPSCs and primary T cells regulatably overexpress CD47 and a CAR, and include a regulatable genomic modification or regulatable knock out or knock down of the TRB gene. In some embodiments, hypoimmune T cells derived from iPSCs and primary T cells regulatably overexpress CD47 and a CAR, and include one or more regulatable genomic modifications or regulatable knock outs or knock downs selected from the group consisting of the B2M, CIITA, TRAC, and TRB genes. In some embodiments, hypoimmune T cells derived from iPSCs and primary T cells regulatably overexpress CD47 and

a CAR, and include regulatable genomic modifications or regulatable knock outs or knock downs of the B2M, CIITA, TRAC, and TRB genes. In some embodiments, the cells are regulatably *B2M*$^{-/-}$ cells. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$ cells. In some embodiments, the cells are regulatably *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/inde}$ cells. In some embodiments, the cells are regulatably *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$ cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRAC*$^{-/-}$ cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRAC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRAC*$^{-/-}$ that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRAC*$^{-/-}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$ that also express CARs. In some

embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$ that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRBC*$^{-/-}$ cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRBC*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRBC*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRBC*$^{-/-}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRBC*$^{indel/indel}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRBC*$^{knock\ down}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$ cells that also express CARs. In some embodiments, the cells are regulatably

B2M$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$ cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, hypoimmune T cells are produced by differentiating induced pluripotent stem cells such as hypoimmunogenic induced pluripotent stem cells.

[00421] In some embodiments, the hypoimmune T cells derived from iPSCs and primary T cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRAC*$^{-/-}$ that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRAC*$^{-/-}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$ that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$ that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRBC*$^{-/-}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells that also express

CARs. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRBC*$^{indel/indel}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRBC*$^{knock\ down}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells that also express CARs. .

[00422] In some embodiments, the engineered or modified cells described are pluripotent stem cells, induced pluripotent stem cells, NK cells differentiated from such pluriopotent stem cells and induced pluripotent stem cells, T cells differentiated from such pluripotent stem cells and induced pluripotent stem cells, or primary T cells. Non-limiting examples of primary T cells include CD3+ T cells, CD4+ T cells, CD8+ T cells, naïve T cells, regulatory T (Treg) cells, non-regulatory T cells, Th1 cells, Th2 cells, Th9 cells, Th17 cells, T-follicular helper (Tfh) cells, cytotoxic T lymphocytes (CTL), effector T (Teff) cells, central memory T (Tcm) cells, effector memory T (Tem) cells, effector memory T cells express CD45RA (TEMRA cells), tissue-resident memory (Trm) cells, virtual memory T cells, innate memory T cells, memory stem cell (Tsc), γδ T cells, and any other subtype of T cells. In some embodiments, the primary T cells are

selected from a group that includes cytotoxic T-cells, helper T-cells, memory T-cells, regulatory T-cells, tumor infiltrating lymphocytes, and combinations thereof. Non-limiting examples of NK cells and primary NK cells include immature NK cells and mature NK cells. In some embodiments, the cells are modified or engineered as compared to a wild-type or control cell, including an unaltered or unmodified wild-type cell or control cell. In some embodiments, the wild-type cell or the control cell is a starting material. In some embodiments, the starting material is otherwise modified or engineered to have altered expression of one or more genes to generate the engineered cell.

[00423] In some embodiments, the primary T cells are from a pool of primary T cells from one or more donor subjects that are different than the recipient subject (*e.g.,* the patient administered the cells). The primary T cells can be obtained from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 50, 100 or more donor subjects and pooled together. The primary T cells can be obtained from 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10, or more 20 or more, 50 or more, or 100 or more donor subjects and pooled together. In some embodiments, the primary T cells are harvested from one or a plurality of individuals, and in some instances, the primary T cells or the pool of primary T cells are cultured in vitro. In some embodiments, the primary T cells or the pool of primary T cells are engineered to regulatably exogenously express CD47 and cultured in vitro.

[00424] In many embodiments, the primary T cells or the pool of primary T cells are engineered to regulatably express a chimeric antigen receptor (CAR). The CAR can be any known to those skilled in the art. Useful CARs include those that bind an antigen selected from a group that includes CD19, CD20, CD22, CD38, CD123, CD138, and BCMA. In some cases, the CAR is the same or equivalent to those used in FDA-approved CAR-T cell therapies such as, but not limited to, those used in tisagenlecleucel and axicabtagene ciloleucel, or others under investigation in clinical trials.

[00425] In some embodiments, the primary T cells or the pool of primary T cells are engineered to regulatably exhibit reduced expression of an endogenous T cell receptor compared to unmodified primary T cells. In certain embodiments, the primary T cells or the pool of primary T cells are engineered to exhibit reduced expression of CTLA-4, PD-1, or both CTLA-4 and PD-1, as compared to unmodified primary T cells. Methods of genetically modifying a cell including a T cell are described in detail, for example, in WO2020/018620 and WO2016/183041,

the disclosures of which are herein incorporated by reference in their entireties, including the tables, appendices, sequence listing and figures.

[00426] In some embodiments, the CAR-T cells comprise a CAR selected from a group including: (a) a first generation CAR comprising an antigen binding domain, a transmembrane domain, and a signaling domain; (b) a second generation CAR comprising an antigen binding domain, a transmembrane domain, and at least two signaling domains; (c) a third generation CAR comprising an antigen binding domain, a transmembrane domain, and at least three signaling domains; and (d) a fourth generation CAR comprising an antigen binding domain, a transmembrane domain, three or four signaling domains, and a domain which upon successful signaling of the CAR induces expression of a cytokine gene.

[00427] In some embodiments, the CAR-T cells comprise a CAR comprising an antigen binding domain, a transmembrane, and one or more signaling domains. In some embodiments, the CAR also comprises a linker. In some embodiments, the CAR comprises a CD19 antigen binding domain. In some embodiments, the CAR comprises a CD28 or a CD8α transmembrane domain. In some embodiments, the CAR comprises a CD8α signal peptide. In some embodiments, the CAR comprises a Whitlow linker GSTSGSGKPGSGEGSTKG (SEQ ID NO: 15).In some embodiments, the antigen binding domain of the CAR is selected from a group including, but not limited to, (a) an antigen binding domain targets an antigen characteristic of a neoplastic cell; (b) an antigen binding domain that targets an antigen characteristic of a T cell; (c) an antigen binding domain targets an antigen characteristic of an autoimmune or inflammatory disorder; (d) an antigen binding domain that targets an antigen characteristic of senescent cells; (e) an antigen binding domain that targets an antigen characteristic of an infectious disease; and (f) an antigen binding domain that binds to a cell surface antigen of a cell.

[00428] In some embodiments, the CAR further comprises one or more linkers. The format of an scFv is generally two variable domains linked by a flexible peptide sequence, or a "linker," either in the orientation VH-linker-VL or VL-linker-VH. Any suitable linker known to those in the art in view of the specification can be used in the CARs. Examples of suitable linkers include, but are not limited to, a GS based linker sequence, and a Whitlow linker GSTSGSGKPGSGEGSTKG (SEQ ID NO:15). In some embodiments, the linker is a GS or a gly-ser linker. Exemplary gly-ser polypeptide linkers comprise the amino acid sequence $Ser(Gly_4Ser)_n$, as well as $(Gly_4Ser)_n$ and/or $(Gly_4Ser_3)_n$. In some embodiments, n=l. In some

embodiments, n=2. In some embodiments, n=3, *i.e.*, Ser(Gly_4Ser)$_3$. In some embodiments, n=4, i.e., Ser(Gly_4Ser)$_4$. In some embodiments, n=5. In some embodiments, n=6. In some embodiments, n=7. In some embodiments, n=8. In some embodiments, n=9. In some embodiments, n=10. Another exemplary gly-ser polypeptide linker comprises the amino acid sequence Ser(Gly_4Ser)$_n$. In some embodiments, n=l. In some embodiments, n=2. In some embodiments, n=3. In another embodiment, n=4. In some embodiments, n=5. In some embodiments, n=6. Another exemplary gly-ser polypeptide linker comprises (Gly4Ser)n. In some embodiments, n=l. In some embodiments, n=2. In some embodiments, n=3. In some embodiments, n=4. In some embodiments, n=5. In some embodiments, n=6. Another exemplary gly-ser polypeptide linker comprises (Gly_3Ser)$_n$. In some embodiments, n=l. In some embodiments, n=2. In some embodiments, n=3. In some embodiments, n=4. In another embodiment, n=5. In yet another embodiment, n=6. Another exemplary gly-ser polypeptide linker comprises (Gly_4Ser_3)$_n$. In some embodiments, n=l. In some embodiments, n=2. In some embodiments, n=3. In some embodiments, n=4. In some embodiments, n=5. In some embodiments, n=6. Another exemplary gly-ser polypeptide linker comprises (Gly_3Ser)$_n$. In some embodiments, n=l. In some embodiments, n=2. In some embodiments, n=3. In some embodiments, n=4. In another embodiment, n=5. In yet another embodiment, n=6.

[00429] In some embodiments, the antigen binding domain is selected from a group that includes an antibody, an antigen-binding portion or fragment thereof, an scFv, and a Fab. In some embodiments, the antigen binding domain binds to CD19, CD20, CD22, CD38, CD123, CD138, or BCMA. In some embodiments, the antigen binding domain is an anti-CD19 scFv such as but not limited to FMC63.

[00430] In some embodiments, the transmembrane domain comprises one selected from a group that includes a transmembrane region of TCRα, TCRβ, TCRζ, CD3ε, CD3γ, CD3δ, CD3ζ, CD4, CD5, CD8α, CD8β, CD9, CD16, CD28, CD45, CD22, CD33, CD34, CD37, CD40, CD40L/CD154, CD45, CD64, CD80, CD86, OX40/CD134, 4-1BB/CD137, CD154, FcεRIγ, VEGFR2, FAS, FGFR2B, and functional variant thereof.

[00431] In some embodiments, the signaling domain(s) of the CAR comprises a costimulatory domain(s). For instance, a signaling domain can contain a costimulatory domain. Or, a signaling domain can contain one or more costimulatory domains. In certain embodiments, the signaling domain comprises a costimulatory domain. In other embodiments, the signaling

domains comprise costimulatory domains. In some cases, when the CAR comprises two or more costimulatory domains, two costimulatory domains are not the same. In some embodiments, the costimulatory domains comprise two costimulatory domains that are not the same. In some embodiments, the costimulatory domain enhances cytokine production, CAR-T cell proliferation, and/or CAR-T cell persistence during T cell activation. In some embodiments, the costimulatory domains enhance cytokine production, CAR-T cell proliferation, and/or CAR-T cell persistence during T cell activation.

[00432] As described herein, a fourth generation CAR can contain an antigen binding domain, a transmembrane domain, three or four signaling domains, and a domain which upon successful signaling of the CAR induces expression of a cytokine gene. In some instances, the cytokine gene is an endogenous or exogenous cytokine gene of the hypoimmunogenic cells. In some cases, the cytokine gene encodes a pro-inflammatory cytokine. In some embodiments, the pro-inflammatory cytokine is selected from a group that includes IL-1, IL-2, IL-9, IL-12, IL-18, TNF, IFN-gamma, and a functional fragment thereof. In some embodiments, the domain which upon successful signaling of the CAR induces expression of the cytokine gene comprises a transcription factor or functional domain or fragment thereof.

[00433] In some embodiments, the CAR comprises a CD3 zeta (CD3ζ) domain or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof. In some embodiments, the CAR comprises (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; and (ii) a CD28 domain, or a 4-1BB domain, or functional variant thereof. In other embodiments, the CAR comprises (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain or functional variant thereof; and (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof. In certain embodiments, the CAR comprises (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain or functional variant thereof; (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof; and (iv) a cytokine or costimulatory ligand transgene. In some embodiments, the CAR comprises a (i) an anti-CD19 scFv; (ii) a CD8α hinge and transmembrane domain or functional variant thereof; (iii) a 4-1BB costimulatory domain or functional variant thereof; and (iv) a CD3ζ signaling domain or functional variant thereof.

[00434] Methods for introducing a CAR construct or producing a CAR-T cells are well known to those skilled in the art. Detailed descriptions are found, for example, in Vormittag *et al.*, Curr Opin Biotechnol, 2018, 53, 162-181; and Eyquem *et al.*, Nature, 2017, 543, 113-117.

[00435] In some embodiments, the cells derived from primary T cells comprise reduced expression of an endogenous T cell receptor, for example by disruption of an endogenous T cell receptor gene (*e.g.*, T cell receptor alpha constant region (TRAC) or T cell receptor beta constant region (TRB)). In some embodiments, an exogenous nucleic acid encoding a polypeptide as disclosed herein (*e.g.*, a chimeric antigen receptor, CD47, or another tolerogenic factor disclosed herein) is inserted at the disrupted T cell receptor gene. In some embodiments, an exogenous nucleic acid encoding a polypeptide is inserted at a TRAC or a TRB gene locus.

[00436] In some embodiments, the cells derived from primary T cells comprise reduced expression of cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and/or programmed cell death (PD1). Methods of reducing or eliminating expression of CTLA4, PD1 and both CTLA4 and PD1 can include any recognized by those skilled in the art, such as but not limited to, genetic modification technologies that utilize rare-cutting endonucleases and RNA silencing or RNA interference technologies. Non-limiting examples of a rare-cutting endonuclease include any Cas protein, TALEN, zinc finger nuclease, meganuclease, and/or homing endonuclease. In some embodiments, an exogenous nucleic acid encoding a polypeptide as disclosed herein (*e.g.*, a chimeric antigen receptor, CD47, or another tolerogenic factor disclosed herein) is inserted at a CTLA4 and/or PD1 gene locus.

[00437] In some embodiments, a transgene encoding one or more tolerogenic factors with regulatable expression is inserted into a pre-selected locus of the cell. In some embodiments, a transgene encoding a CAR is inserted into a pre-selected locus of the cell. In certain embodiments, a transgene encoding one or more tolerogenic factors with regulatable expression and a transgene encoding a CAR are inserted into a pre-selected locus of the cell. The pre-selected locus can be a safe harbor locus or a target locus. Non-limiting examples of a safe harbor locus include, but are not limited to, a CCR5 gene locus, a PPP1R12C (also known as AAVS1) gene locus, and a CLYBL gene locus, a Rosa gene locus (*e.g.*, ROSA26 gene locus). Non-limiting examples of a target locus include, but are not limited to, a CXCR4 gene locus, an albumin gene locus, a SHS231 gene locus, an F3 gene locus (also known as CD142), a MICA gene locus, a MICB gene locus, a LRP1 gene locus (also known as a CD91 gene locus), a

HMGB1 gene locus, an ABO gene locus, an RHD gene locus, a FUT1 locus, and a KDM5D gene locus. The transgene encoding one or more tolerogenic factors can be inserted in Introns 1 or 2 for PPP1R12C (*i.e.*, AAVS1) or CCR5. The transgene encoding one or more tolerogenic factors can be inserted in Introns 1 or 2 for PPP1R12C (*i.e.*, AAVS1) or CCR5. The transgene encoding one or more tolerogenic factors can be inserted in Exons 1 or 2 or 3 for CCR5. The transgene encoding one or more tolerogenic factors can be inserted in intron 2 for CLYBL. The transgene encoding one or more tolerogenic factors can be inserted in a 500 bp window in Ch-4:58,976,613 (*i.e.*, SHS231). The transgene encoding one or more tolerogenic factors can be insert in any suitable region of the aforementioned safe harbor or target loci that allows for expression of the exogenous, including, for example, an intron, an exon or a coding sequence region in a safe harbor or target locus. In some embodiments, the pre-selected locus is selected from the group consisting of the *B2M* locus, the *CIITA* locus, the *TRAC* locus, and the *TRB* locus. In some embodiments, the pre-selected locus is the *B2M* locus. In some embodiments, the pre-selected locus is the *CIITA* locus. In some embodiments, the pre-selected locus is the *TRAC* locus. In some embodiments, the pre-selected locus is the *TRB* locus.

[00438] In some embodiments, a transgene encoding one or more tolerogenic factors with regulatable expression and a transgene encoding a CAR are inserted into the same locus. In some embodiments, a transgene encoding one or more tolerogenic factors with regulatable expression and a transgene encoding a CAR are inserted into different loci. In many instances, a transgene encoding one or more tolerogenic factors is inserted into a safe harbor or target locus. In many instances, a transgene encoding a CAR is inserted into a safe harbor or target locus. In some instances, a transgene encoding one or more tolerogenic factors is inserted into a *B2M* locus. In some instances, a transgene encoding a CAR is inserted into a *B2M* locus. In certain instances, a transgene encoding one or more tolerogenic factors is inserted into a *CIITA* locus. In certain instances, a transgene encoding a CAR is inserted into a *CIITA* locus. In particular instances, a transgene encoding one or more tolerogenic factors is inserted into a *TRAC* locus. In particular instances, a transgene encoding a CAR is inserted into a *TRAC* locus. In many other instances, a transgene encoding one or more tolerogenic factors is inserted into a *TRB* locus. In many other instances, a transgene encoding a CAR is inserted into a *TRB* locus. In some embodiments, a transgene encoding one or more tolerogenic factors and a transgene encoding a CAR are inserted into a safe harbor or target locus (*e.g.*, a CCR5 gene locus, a CXCR4 gene

locus, a PPP1R12C gene locus, an albumin gene locus, a SHS231 gene locus, a CLYBL gene locus, a Rosa gene locus, an F3 (CD142) gene locus, a MICA gene locus, a MICB gene locus, a LRP1 (CD91) gene locus, a HMGB1 gene locus, an ABO gene locus, an RHD gene locus, a FUT1 locus, and a KDM5D gene locus.

[00439] In many embodiments, a transgene encoding one or more tolerogenic factors with regulatable expression and a transgene encoding a CAR are inserted into a safe harbor or target locus. In certain embodiments, a transgene encoding one or more tolerogenic factors with regulatable expression and a transgene encoding a CAR are controlled by a single promoter and are inserted into a safe harbor or target locus. In certain embodiments, a transgene encoding one or more tolerogenic factors with regulatable expression and a transgene encoding a CAR are controlled by their own promoters and are inserted into a safe harbor or target locus. In certain embodiments, a transgene encoding one or more tolerogenic factors and a transgene encoding a CAR are inserted into a *TRAC* locus. In certain embodiments, a transgene encoding one or more tolerogenic factors and a transgene encoding a CAR are controlled by a single promoter and are inserted into a *TRAC* locus. In certain embodiments, a transgene encoding one or more tolerogenic factors and a transgene encoding a CAR are controlled by their own promoters and are inserted into a *TRAC* locus. In some embodiments, a transgene encoding one or more tolerogenic factors and a transgene encoding a CAR are inserted into a *TRB* locus. In some embodiments, a transgene encoding one or more tolerogenic factors and a transgene encoding a CAR are controlled by a single promoter and are inserted into a *TRB* locus. In some embodiments, a transgene encoding one or more tolerogenic factors and a transgene encoding a CAR are controlled by their own promoters and are inserted into a *TRB* locus. In other embodiments, a transgene encoding one or more tolerogenic factors and a transgene encoding a CAR are inserted into a *B2M* locus. In other embodiments, a transgene encoding one or more tolerogenic factors and a transgene encoding a CAR are controlled by a single promoter and are inserted into a *B2M* locus. In other embodiments, a transgene encoding one or more tolerogenic factors and a transgene encoding a CAR are controlled by their own promoters and are inserted into a *B2M* locus. In various embodiments, a transgene encoding one or more tolerogenic factors and a transgene encoding a CAR are inserted into a *CIITA* locus. In various embodiments, a transgene encoding one or more tolerogenic factors and a transgene encoding a CAR are controlled by a single promoter and are inserted into a *CIITA* locus. In various embodiments, a

transgene encoding one or more tolerogenic factors and a transgene encoding a CAR are controlled by their own promoters and are inserted into a *CIITA* locus.

[00440] In some instances, the promoter controlling expression of any transgene described is a constitutive promoter. In some instances, the promoter controlling expression of any transgene described is a conditional promoter. In other instances, the promoter for any transgene described is an inducible promoter. In some embodiments, the promoter is an EF1α promoter. In some embodiments, the promoter is CAG promoter. In some embodiments, a transgene encoding one or more tolerogenic factors is controlled by a constitutive promoter. In some embodiments, a transgene encoding one or more tolerogenic factors is controlled by a conditional promoter. In some embodiments, the transgene encoding one or more tolerogenic factors is controlled by a cell cycle-specific promoter. In some embodiments, the transgene encoding one or more tolerogenic factors is controlled by a tissue-specific promoter. In some embodiments, the transgene encoding one or more tolerogenic factors is controlled by a lineage-specific promoter. In some embodiments, the transgene encoding one or more tolerogenic factors is controlled by a differentiation-induced promoter. In some embodiments, the transgene encoding one or more tolerogenic factors is controlled by an inducible promoter. In some embodiments, the transgene encoding one or more tolerogenic factors is controlled by an inducible promoter that is regulated by a small molecule. In some embodiments, the transgene encoding one or more tolerogenic factors is controlled by an inducible promoter that is regulated by a ligand. In some embodiments, the transgene encoding one or more tolerogenic factors is controlled by an inducible promoter that is regulated by a biologic agent. In some embodiments, the transgene encoding one or more tolerogenic factors is controlled by an inducible promoter that is regulated by an aptamer-mediated modulator of polyadenylation. In some embodiments, the transgene encoding one or more tolerogenic factors is controlled by an inducible promoter that is regulated by an aptamer-regulated riboswitch. In some embodiments, a CAR transgene is controlled by a constitutive promoter. In some embodiments, a CAR transgene is controlled by a conditional promoter. In some embodiments, the CAR transgene is controlled by a cell cycle-specific promoter. In some embodiments, the CAR transgene is controlled by a tissue-specific promoter. In some embodiments, the CAR transgene is controlled by a lineage-specific promoter. In some embodiments, the CAR transgene is controlled by a differentiation-induced promoter. In some embodiments, the CAR transgene is controlled by an inducible promoter. In some embodiments,

the CAR transgene is controlled by an inducible promoter that is regulated by a small molecule. In some embodiments, the CAR transgene is controlled by an inducible promoter that is regulated by a ligand. In some embodiments, the CAR transgene is controlled by an inducible promoter that is regulated by a biologic agent. In some embodiments, the CAR transgene is controlled by an inducible promoter that is regulated by an aptamer-mediated modulator of polyadenylation. In some embodiments, the CAR transgene is controlled by an inducible promoter that is regulated by an aptamer-regulated riboswitch. In some embodiments, a transgene encoding one or more tolerogenic factors and a transgene encoding a CAR are both controlled by a conditional promoter. In some embodiments, a transgene encoding one or more tolerogenic factors and a transgene encoding a CAR are both controlled by an inducible promoter. In some embodiments, a transgene encoding one or more tolerogenic factors is controlled by a constitutive promoter and a transgene encoding a CAR is controlled by an inducible promoter. In some embodiments, a transgene encoding one or more tolerogenic factors is controlled by a constitutive promoter and a transgene encoding a CAR is controlled by a conditional promoter. In some embodiments, a transgene encoding one or more tolerogenic factors is controlled by a conditional promoter and a transgene encoding a CAR is controlled by an inducible promoter. In some embodiments, a transgene encoding one or more tolerogenic factors is controlled by a conditional promoter and a transgene encoding a CAR is controlled by a constitutive promoter. In some embodiments, a transgene encoding one or more tolerogenic factors is controlled by an inducible promoter and a transgene encoding a CAR is controlled by a conditional promoter. In various embodiments, a transgene encoding one or more tolerogenic factors is controlled by an EF1α promoter and a transgene encoding a CAR is controlled by an EF1α promoter. In some embodiments, a transgene encoding one or more tolerogenic factors is controlled by a CAG promoter and a transgene encoding a CAR is controlled by a CAG promoter. In some embodiments, a transgene encoding one or more tolerogenic factors is controlled by a CAG promoter and a transgene encoding a CAR is controlled by an EF1α promoter. In some embodiments, a transgene encoding one or more tolerogenic factors is controlled by an EF1α promoter and a transgene encoding a CAR is controlled by a CAG promoter. In some embodiments, expression of both a transgene encoding one or more tolerogenic factors and a transgene encoding a CAR is controlled by a single EF1α promoter. In

some embodiments, expression of both a transgene encoding one or more tolerogenic factors and a transgene encoding a CAR is controlled by a single CAG promoter.

[00441] In another embodiment, the present disclosure disclosed herein is directed to pluripotent stem cells, (*e.g.*, pluripotent stem cells and induced pluripotent stem cells (iPSCs)), differentiated cells derived from such pluripotent stem cells (*e.g.*, hypoimmune T cells, cardiac cells, neural cells, cerebral endothelial cells, dopaminergic neurons, glial progenitor cells, endothelial cells, thyroid cells, hepatocytes, pancreatic islet cells, and retinal pigmented epithelium cells), and primary T cells that regulatably overexpress CD47 (such as regulatably exogenously express CD47 proteins), have regulatable reduced expression or lack expression of one or more MHC class I and/or MHC class II human leukocyte antigen molecules, and have regulatable reduced expression or lack expression of a T-cell receptor (TCR) complex. In some embodiments, the hypoimmune T cells and primary T cells regulatably overexpress CD47 (such as regulatably exogenously express CD47 proteins), have regulatable reduced expression or lack expression of one or more MHC class I and/or MHC class II human leukocyte antigen molecules, and have regulatable reduced expression or lack expression of a T-cell receptor (TCR) complex.

[00442] In some embodiments, pluripotent stem cells, (*e.g.*, pluripotent stem cells and induced pluripotent stem cells (iPSCs)), differentiated cells derived from such pluripotent stem cells (*e.g.*, hypoimmune T cells, cardiac cells, neural cells, cerebral endothelial cells, dopaminergic neurons, glial progenitor cells, endothelial cells, thyroid cells, hepatocytes, pancreatic islet cells, and retinal pigmented epithelium cells), and primary T cells regulatably overexpress CD47 and include a regulatable genomic modification of the B2M gene. In some embodiments, pluripotent stem cells, differentiated cell derived from such pluripotent stem cells and primary T cells regulatably overexpress CD47 and include a regulatable genomic modification of the CIITA gene. In some embodiments, the pluripotent stem cells, differentiated cells derived from such pluripotent stem cells, such as, but not limited to, T cells, NK cells, cardiac cells, neural cells, cerebral endothelial cells, dopaminergic neurons, glial progenitor cells, endothelial cells, thyroid cells, hepatocytes, pancreatic islet cells, and retinal pigmented epithelium cells, are regulatably $B2M^{-/-}$ cells. In some embodiments, the cells are regulatably $CIITA^{-/-}$ cells. In some embodiments, the cells are regulatably *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{indel/indel}$ cells. In some embodiments, the cells are regulatably $CIITA^{indel/inde}$ cells.

In some embodiments, the cells are regulatably *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{knock\ down}$ cells. In some embodiments, the cells are regulatably $CIITA^{knock\ down}$ cells. In some embodiments, the cells are regulatably *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{-/-}$, $CIITA^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{indel/indel}$, $CIITA^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{knock\ down}$, $CIITA^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{-/-}$, $CIITA^{-/-}$ cells. In some embodiments, the cells are regulatably $B2M^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably $CIITA^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{indel/indel}$, $CIITA^{indel/indel}$ cells. In some embodiments, the cells are regulatably $B2M^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably $CIITA^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{knock\ down}$, $CIITA^{knock\ down}$ cells. In some embodiments, the cells are regulatably $B2M^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably $CIITA^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{-/-}$, $CIITA^{-/-}$, $TRAC^{-/-}$ cells. In some embodiments, the cells are regulatably $B2M^{-/-}$, $TRAC^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably $CIITA^{-/-}$, $TRAC^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRAC^{indel/indel}$ cells. In some embodiments, the cells are regulatably $B2M^{indel/indel}$, $TRAC^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably $CIITA^{indel/indel}$, $TRAC^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{knock\ down}$, $CIITA^{knock\ down}$, $TRAC^{knock\ down}$ cells. In some embodiments, the cells are regulatably $B2M^{knock\ down}$, $TRAC^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably $CIITA^{knock\ down}$, $TRAC^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{-/-}$, $CIITA^{-/-}$, $TRBC^{-/-}$ cells. In some embodiments, the cells are regulatably $B2M^{-/-}$, $TRBC^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably $CIITA^{-/-}$, $TRBC^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRBC^{indel/indel}$ cells. In some embodiments, the cells are regulatably $B2M^{indel/indel}$, $TRBC^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably $CIITA^{indel/indel}$, $TRBC^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{knock\ down}$, $CIITA^{knock\ down}$, $TRBC^{knock\ down}$ cells. In some embodiments, the cells are regulatably $B2M^{knock\ down}$, $TRBC^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably $CIITA^{knock\ down}$, $TRBC^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{-/-}$, $CIITA^{-/-}$, $TRAC^{-/-}$, $TRBC^{-/-}$ cells. In some

embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, pluripotent stem cells, T cells differentiated from such pluripotent stem cells and primary T cells regulatably overexpress CD47 and include a regulatable genomic modification of the TRAC gene. In some embodiments, pluripotent stem cells, T cells differentiated from such pluripotent stem cells and primary T cells regulatably overexpress CD47 and include a regulatable genomic modification of the TRB gene. In some embodiments, pluripotent stem cells, T cells differentiated from such pluripotent stem cells and primary T cells regulatably overexpress CD47 and include one or more regulatable genomic modifications selected from the group consisting of the B2M, CIITA, TRAC and TRB genes. In some embodiments, pluripotent stem cells, T cells differentiated from such pluripotent stem cells and primary T cells regulatably overexpress CD47 and include regulatable genomic modifications of the B2M, CIITA and TRAC genes. In some embodiments, pluripotent stem cells, T cells differentiated from such pluripotent stem cells and primary T cells regulatably overexpress CD47 and include regulatable genomic modifications of the B2M, CIITA and TRB genes. In some embodiments, pluripotent stem cells, T cells differentiated from such pluripotent stem cells and primary T cells regulatably overexpress CD47 and include regulatable genomic modifications of the B2M, CIITA, TRAC and TRB genes. In certain embodiments, the pluripotent stem cells, differentiated cell derived from such pluripotent stem cells and primary T cells are regulatably *B2M*$^{-/-}$ cells. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$ cells. In some embodiments, the cells are regulatably *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/inde}$ cells. In some embodiments, the cells are regulatably *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$ cells. In some embodiments, the

cells are regulatably *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$ cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRAC*$^{-/-}$ cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRAC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRAC*$^{-/-}$ that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRAC*$^{-/-}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$ that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$ that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRBC*$^{-/-}$ cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells. In some embodiments, the

cells are regulatably *CIITA*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRBC*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRBC*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRBC*$^{-/-}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRBC*$^{indel/indel}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRBC*$^{knock\ down}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$ cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$ cells. In some

embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells.. In some embodiments, the engineered or modified cells described are pluripotent stem cells (*e.g.*, embryonic stem cells or induced pluripotent stem cells), T cells differentiated from such pluripotent stem cells or primary T cells. Non-limiting examples of primary T cells include CD3+ T cells, CD4+ T cells, CD8+ T cells, naïve T cells, regulatory T (Treg) cells, non-regulatory T cells, Th1 cells, Th2 cells, Th9 cells, Th17 cells, T-follicular helper (Tfh) cells, cytotoxic T lymphocytes (CTL), effector T (Teff) cells, central memory T (Tcm) cells, effector memory T (Tem) cells, effector memory T cells express CD45RA (TEMRA cells), tissue-resident memory (Trm) cells, virtual memory T cells, innate memory T cells, memory stem cell (Tsc), γδ T cells, and any other subtype of T cells. In some embodiments, the cells are modified or engineered as compared to a wild-type or control cell, including an unaltered or unmodified wild-type cell or control cell. In some embodiments, the wild-type cell or the control cell is a starting material. In some embodiments, the starting material is otherwise modified or engineered to have altered expression of one or more genes to generate the engineered cell.

[00443] In some embodiments, a transgene encoding one or more tolerogenic factors with regulatable expression is inserted into a pre-selected locus of the cell. The pre-selected locus can be a safe harbor or target locus. Non-limiting examples of a safe harbor locus include a CCR5 gene locus, a PPP1R12C gene locus, and a CLYBL gene locus, a Rosa gene locus. Non-limiting examples of a target locus include a CXCR4 gene locus, an albumin gene locus, a SHS231 gene locus, an F3 (CD142) gene locus, a MICA gene locus, a MICB gene locus, a LRP1 (CD91) gene locus, a HMGB1 gene locus, an ABO gene locus, an RHD gene locus, a FUT1 locus, and a KDM5D gene locus. In some embodiments, the pre-selected locus is the *TRAC* locus. In some embodiments, a transgene encoding one or more tolerogenic factors is inserted into a safe harbor

or target locus (*e.g.*, a CCR5 gene locus, a CXCR4 gene locus, a PPP1R12C gene locus, an albumin gene locus, a SHS231 gene locus, a CLYBL gene locus, a Rosa gene locus, an F3 (CD142) gene locus, a MICA gene locus, a MICB gene locus, a LRP1 (CD91) gene locus, a HMGB1 gene locus, an ABO gene locus, an RHD gene locus, a FUT1 locus, and a KDM5D gene locus. In certain embodiments, a transgene encoding one or more tolerogenic factors is inserted into the *B2M* locus. In certain embodiments, a transgene encoding one or more tolerogenic factors is inserted into the *B2M* locus. In certain embodiments, a transgene encoding one or more tolerogenic factors is inserted into the *TRAC* locus. In certain embodiments, a transgene encoding one or more tolerogenic factors is inserted into the *TRB* locus.

[00444] In some instances, expression of a transgene encoding one or more tolerogenic factors is controlled by a conditional promoter. In other instances, expression of a transgene encoding one or more tolerogenic factors is controlled by an inducible promoter.

[00445] In yet another embodiment, the present disclosure disclosed herein is directed to pluripotent stem cells, (*e.g.*, pluripotent stem cells and induced pluripotent stem cells (iPSCs)), T cells derived from such pluripotent stem cells (*e.g.*, hypoimmune T cells), and primary T cells that have regulatable reduced expression or regulatable lack of expression of one or more MHC class I and/or MHC class II human leukocyte antigen molecules and have regulatable reduced expression or regulatable lack of expression of a T-cell receptor (TCR) complex. In some embodiments, the cells have regulatable reduced or regulatable lack of expression of one or more MHC class I antigen molecules, MHC class II antigen molecules, and TCR complexes.

[00446] In some embodiments, pluripotent stem cells (*e.g.*, iPSCs), differentiated cells derived from such (*e.g.*, T cells, cardiac cells, neural cells, cerebral endothelial cells, dopaminergic neurons, glial progenitor cells, endothelial cells, thyroid cells, hepatocytes, pancreatic islet cells, and retinal pigmented epithelium cells differentiated from such), and primary T cells include a regulatable genomic modification or regulatable knock down of the B2M gene. In some embodiments, pluripotent stem cells (*e.g.*, iPSCs), differentiated cells derived from such (*e.g.*, T cells, cardiac cells, neural cells, cerebral endothelial cells, dopaminergic neurons, glial progenitor cells, endothelial cells, thyroid cells, hepatocytes, pancreatic islet cells, and retinal pigmented epithelium cells differentiated from such), and primary T cells include a regulatable genomic modification or regulatable knock down of the CIITA gene. In some embodiments, the cells,

including iPSCs and differentiated cells derived from such pluripotent stem cells, such as, but not limited to, T cells, NK cells, cardiac cells, neural cells, cerebral endothelial cells, dopaminergic neurons, glial progenitor cells, endothelial cells, thyroid cells, hepatocytes, pancreatic islet cells, and retinal pigmented epithelium cells, are regulatably *B2M*$^{-/-}$ cells. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$ cells. In some embodiments, the cells are regulatably *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/inde}$ cells. In some embodiments, the cells are regulatably *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$ cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRAC*$^{-/-}$ cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRAC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRBC*$^{-/-}$ cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$,

TRBC$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRBC*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$ cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, pluripotent stem cells (*e.g.*, ESCs or iPSCs), T cells differentiated from such, and primary T cells include a regulatable genomic modification or regulatable knock down of the TRAC gene. In some embodiments, pluripotent stem cells (*e.g.*, iPSCs), T cells differentiated from such, and primary T cells include a regulatable genomic modification or regulatable knock down of the TRB gene. In some embodiments, pluripotent stem cells (*e.g.*, iPSCs), T cells differentiated from such, and primary T cells include one or more regulatable genomic modifications or regulatable knock downs selected from the group consisting of the B2M, CIITA and TRAC genes. In some embodiments, pluripotent stem cells (*e.g.*, iPSCs), T cells differentiated from such, and primary T cells include one or more regulatable genomic modifications or regulatable knock downs selected from the group consisting of the B2M, CIITA and TRB genes. In some embodiments, pluripotent stem cells (*e.g.*, iPSCs), T cells differentiated from such, and primary T cells include one or more regulatable genomic modifications or regulatable knock downs selected from the group consisting of the B2M, CIITA, TRAC and TRB genes. In certain embodiments, the cells including iPSCs, T cells differentiated from such, and primary T cells are regulatably *B2M*$^{-/-}$ cells. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$ cells. In some embodiments, the cells are regulatably *CD47tg* cells. In some

embodiments, the cells are regulatably *B2M*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/inde}$ cells. In some embodiments, the cells are regulatably *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$ cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRAC*$^{-/-}$ cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRAC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRAC*$^{-/-}$ that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRAC*$^{-/-}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$ that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$ that also express CARs. In some embodiments, the cells are regulatably *B2M*^{knock}

^{down}, $TRAC^{knock\ down}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably $CIITA^{knock\ down}$, $TRAC^{knock\ down}$, *CD47tg* that also express CARs. In some embodiments, the cells are regulatably $B2M^{-/-}$, $CIITA^{-/-}$, $TRBC^{-/-}$ cells. In some embodiments, the cells are regulatably $B2M^{-/-}$, $TRBC^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably $CIITA^{-/-}$, $TRBC^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRBC^{indel/indel}$ cells. In some embodiments, the cells are regulatably $B2M^{indel/indel}$, $TRBC^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably $CIITA^{indel/indel}$, $TRBC^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{knock\ down}$, $CIITA^{knock\ down}$, $TRBC^{knock\ down}$ cells. In some embodiments, the cells are regulatably $B2M^{knock\ down}$, $TRBC^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably $CIITA^{knock\ down}$, $TRBC^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably $B2M^{-/-}$, $CIITA^{-/-}$, $TRBC^{-/-}$ cells that also express CARs. In some embodiments, the cells are regulatably $B2M^{-/-}$, $TRBC^{-/-}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably $CIITA^{-/-}$, $TRBC^{-/-}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRBC^{indel/indel}$ cells that also express CARs. In some embodiments, the cells are regulatably $B2M^{indel/indel}$, $TRBC^{indel/indel}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably $CIITA^{indel/indel}$, $TRBC^{indel/indel}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably $B2M^{knock\ down}$, $CIITA^{knock\ down}$, $TRBC^{knock\ down}$ cells that also express CARs. In some embodiments, the cells are regulatably $B2M^{knock\ down}$, $TRBC^{knock\ down}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably $CIITA^{knock\ down}$, $TRBC^{knock\ down}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably $B2M^{-/-}$, $CIITA^{-/-}$, $TRAC^{-/-}$, $TRBC^{-/-}$ cells that also express CARs. In some embodiments, the cells are regulatably $B2M^{-/-}$, $TRAC^{-/-}$, $TRBC^{-/-}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably $CIITA^{-/-}$, $TRAC^{-/-}$, $TRBC^{-/-}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRAC^{indel/indel}$, $TRBC^{indel/indel}$ cells that also express CARs. In some embodiments, the cells are regulatably $B2M^{indel/indel}$, $TRAC^{indel/indel}$, $TRBC^{indel/indel}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably $CIITA^{indel/indel}$, $TRAC^{indel/indel}$, $TRBC^{indel/indel}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably $B2M^{knock\ down}$, $CIITA^{knock\ down}$, $TRAC^{knock\ down}$, $TRBC^{knock\ down}$ cells that also express CARs. In some embodiments, the cells

are regulatably *B2M*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells that also express CARs. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$ cells. In some embodiments, the cells are regulatably *B2M*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{-/-}$, *TRAC*$^{-/-}$, *TRBC*$^{-/-}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$ cells. In some embodiments, the cells are regulatably *B2M*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$, *TRBC*$^{indel/indel}$, *CD47tg* cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$ cells. In some embodiments, the cells are regulatably *B2M*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the cells are regulatably *CIITA*$^{knock\ down}$, *TRAC*$^{knock\ down}$, *TRBC*$^{knock\ down}$, *CD47tg* cells. In some embodiments, the modified cells described are pluripotent stem cells, induced pluripotent stem cells, T cells differentiated from such pluripotent stem cells and induced pluripotent stem cells, or primary T cells. Non-limiting examples of primary T cells include CD3+ T cells, CD4+ T cells, CD8+ T cells, naïve T cells, regulatory T (Treg) cells, non-regulatory T cells, Th1 cells, Th2 cells, Th9 cells, Th17 cells, T-follicular helper (Tfh) cells, cytotoxic T lymphocytes (CTL), effector T (Teff) cells, central memory T (Tcm) cells, effector memory T (Tem) cells, effector memory T cells express CD45RA (TEMRA cells), tissue-resident memory (Trm) cells, virtual memory T cells, innate memory T cells, memory stem cell (Tsc), γδ T cells, and any other subtype of T cells. In some embodiments, the cells are modified or engineered as compared to a wild-type or control cell, including an unaltered or unmodified wild-type cell or control cell. In some embodiments, the wild-type cell or the control cell is a starting material. In some embodiments, the starting material is otherwise modified or engineered to have altered expression of one or more genes to generate the engineered cell.

[00447] Cells of the present disclosure exhibit regulatably reduced or regulatable lack of expression of one or more MHC class I antigen molecules, MHC class II antigen molecules, and/or TCR complexes. Reduction of MHC I and/or MHC II expression can be accomplished, for example, by one or more of the following: (1) targeting the polymorphic HLA alleles (HLA-A, HLA-B, HLA-C) and MHC-II genes directly; (2) removal of B2M, which will prevent surface trafficking of all MHC-I molecules; (3) removal of CIITA, which will prevent surface trafficking

of all MHC-II molecules; and/or (4) deletion of components of the MHC enhanceosomes, such as LRC5, RFX5, RFXANK, RFXAP, IRFl, NF-Y (including NFY-A, NFY-B, NFY-C), and CIITA that are critical for HLA expression.

[00448] In some embodiments, HLA expression is interfered with by targeting individual HLAs (*e.g.*, knocking out, knocking down, or reducing expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, and/or HLA-DR), targeting transcriptional regulators of HLA expression (*e.g.*, knocking out or reducing expression of NLRC5, CIITA, RFX5, RFXAP, RFXANK, NFY-A, NFY-B, NFY-C and/or IRF-1), blocking surface trafficking of MHC class I molecules (*e.g.*, knocking out or reducing expression of B2M and/or TAP1), and/or targeting with HLA-Razor (see, *e.g.*, WO2016183041).

[00449] In some embodiments, the cells disclosed herein including, but not limited to, pluripotent stem cells, induced pluripotent stem cells, differentiated cells derived from such stem cells, and primary T cells regulatably do not express one or more human leukocyte antigen molecules (*e.g.*, HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, and/or HLA-DR) corresponding to MHC-I and/or MHC-II and are thus characterized as being hypoimmunogenic. For example, in certain embodiments, the pluripotent stem cells and induced pluripotent stem cells disclosed have been modified such that the stem cell or a differentiated stem cell prepared therefrom regulatably do not express or regulatably exhibit reduced expression of one or more of the following MHC-I molecules: HLA-A, HLA-B and HLA-C. In some embodiments, one or more of HLA-A, HLA-B and HLA-C may be regulatably "knocked-out" of a cell. A cell that has a regulatable knocked-out HLA-A gene, HLA-B gene, and/or HLA-C gene may regulatably exhibit reduced or eliminated expression of each knocked-out gene. In some embodiments, one or more of HLA-A, HLA-B and HLA-C may be regulatably knocked down or knocked out in a cell. A cell that has a knocked-down HLA-A gene, HLA-B gene, and/or HLA-C gene may regulatably exhibit reduced or eliminated expression of each knocked-down gene.

[00450] In some embodiments, guide RNAs, shRNAs, siRNAs, or miRNAs that allow simultaneous deletion of all MHC class I alleles by targeting a conserved region in the HLA genes are identified as HLA Razors. In some embodiments, the gRNAs are part of a CRISPR system, such as a regulatable CRISPR system, such as a conditional or inducible CRISPR system. In alternative embodiments, the gRNAs are part of a TALEN system, such as a regulatable TALEN system, such as a conditional or inducible TALEN system. In some

embodiments, the shRNAs, siRNAs, or mRNAs are part of a regulatable RNAi system, such as a conditional or inducible RNAi system. In some embodiments, an HLA Razor targeting an identified conserved region in HLAs is described in WO2016183041. In some embodiments, multiple HLA Razors targeting identified conserved regions are utilized. It is generally understood that any guide, siRNA, shRNA, or miRNA molecule that targets a conserved region in HLAs can act as an HLA Razor.

[00451] Methods provided are useful for regulatable inactivation or ablation of MHC class I expression and/or MHC class II expression in cells such as but not limited to pluripotent stem cells, differentiated cells, and primary T cells. In some embodiments, regulatable genome editing technologies utilizing rare-cutting endonucleases (*e.g.*, the CRISPR/Cas, TALEN, zinc finger nuclease, meganuclease, and homing endonuclease systems) are also used to reduce or eliminate expression of genes involved in an innate and/or an adaptive immune response (*e.g.*, by deleting genomic DNA of genes involved in an innate and/or an adaptive immune response or by insertions of genomic DNA into such genes, such that gene expression is impacted) in cells. In certain embodiments, regulatable genome editing technologies or other gene modulation technologies are used to insert tolerance-inducing factors in human cells, rendering them and the differentiated cells prepared therefrom hypoimmunogenic cells. As such, the hypoimmunogenic cells have reduced or eliminated expression of one or more MHC I and MHC II expression. In some embodiments, the cells are nonimmunogenic (*e.g.*, do not induce an innate and/or an adaptive immune response) in a recipient subject.

[00452] In some embodiments, the cell includes a modification to regulatably increase expression of CD47 and one or more factors selected from the group consisting of DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9.

[00453] In some embodiments, the cell comprises a regulatable genomic modification or regulatable knock down of one or more target polynucleotide sequences that regulate the expression of either MHC class I molecules, MHC class II molecules, or MHC class I and MHC class II molecules. In some embodiments, a regulatable genetic editing system is used to modify one or more target polynucleotide sequences. In some embodiments, a regulatable RNAi system is used to knock down expression of one or more target polynucleotide sequences. In some

embodiments, the targeted polynucleotide sequence is one or more selected from the group including B2M, CIITA, and NLRC5. In some embodiments, the cell comprises a regulatable regulatable genetic editing modification to the B2M gene. In some embodiments, the cell comprises a regulatable genetic editing modification to the CIITA gene. In some embodiments, the cell comprises a regulatable genetic editing modification to the NLRC5 gene. In some embodiments, the cell comprises regulatable genetic editing modifications to the B2M and CIITA genes. In some embodiments, the cell comprises regulatable genetic editing modifications to the B2M and NLRC5 genes. In some embodiments, the cell comprises regulatable genetic editing modifications to the CIITA and NLRC5 genes. In numerous embodiments, the cell comprises regulatable genetic editing modifications to the B2M, CIITA and NLRC5 genes. In some embodiments, the cell comprises a regulatable RNAi system targeting the B2M gene. In some embodiments, the cell comprises a regulatable RNAi system targeting the CIITA gene. In some embodiments, the cell comprises a regulatable RNAi system targeting the NLRC5 gene. In some embodiments, the cell comprises a regulatable RNAi system targeting the B2M and CIITA genes. In some embodiments, the cell comprises a regulatable RNAi system targeting the B2M and NLRC5 genes. In some embodiments, the cell comprises a regulatable RNAi system targeting the CIITA and NLRC5 genes. In numerous embodiments, the cell comprises a regulatable RNAi system targeting the B2M, CIITA and NLRC5 genes. In certain embodiments, the genome of the cell has been altered to reduce or delete critical components of HLA expression. In certain embodiments, the cell comprises a regulatable RNAi system targeting critical components of HLA expression. In some embodiments, the cells are modified or engineered as compared to a wild-type or control cell, including an unaltered or unmodified wild-type cell or control cell. In some embodiments, the wild-type cell or the control cell is a starting material. In some embodiments, the starting material is otherwise modified or engineered to have altered expression of one or more genes to generate the engineered cell.

[00454] In some embodiments, the present disclosure provides a cell (*e.g.*, stem cell, induced pluripotent stem cell, differentiated cell such as a cardiac cell, neural cell, cerebral endothelial cell, dopaminergic neuron, glial progenitor cell, endothelial cell, thyroid cell, hepatocyte, pancreatic islet cell, or retinal pigmented epithelium cell, hematopoietic stem cell, primary NK cell, CAR-NK cell, primary T cell or CAR-T cell) or population thereof comprising a genome in which a gene has been regulatably edited to delete a contiguous stretch of genomic DNA,

thereby reducing or eliminating surface expression of one or more MHC class I molecules in the cell or population thereof. In certain embodiments, the present disclosure provides a cell (*e.g.*, stem cell, induced pluripotent stem cell, differentiated cell such as a cardiac cell, neural cell, cerebral endothelial cell, dopaminergic neuron, glial progenitor cell, endothelial cell, thyroid cell, hepatocyte, pancreatic islet cell, or retinal pigmented epithelium cell, hematopoietic stem cell, primary NK cell, CAR-NK cell, primary T cell or CAR-T cell) or population thereof comprising a genome in which a gene has been regulatably edited to delete a contiguous stretch of genomic DNA, thereby reducing or eliminating surface expression of one or more MHC class II molecules in the cell or population thereof. In numerous embodiments, the present disclosure provides a cell (*e.g.*, stem cell, induced pluripotent stem cell, differentiated cell such as a cardiac cell, neural cell, cerebral endothelial cell, dopaminergic neuron, glial progenitor cell, endothelial cell, thyroid cell, hepatocyte, pancreatic islet cell, or retinal pigmented epithelium cell, hematopoietic stem cell, primary NK cell, CAR-NK cell, primary T cell or CAR-T cell) or population thereof comprising a genome in which one or more genes has been regulatably edited to delete a contiguous stretch of genomic DNA, thereby reducing or eliminating surface expression of one or more MHC class I and II molecules in the cell or population thereof.

[00455] In many embodiments, the expression of one or more MHC I molecules and/or MHC II molecules is regulatably modulated by targeting and deleting a contiguous stretch of genomic DNA, thereby reducing or eliminating expression of a target gene selected from the group consisting of B2M, CIITA, and NLRC5. In some embodiments, described herein are genetically edited cells (*e.g.*, modified human cells) comprising regulatable exogenous CD47 proteins and regulatably inactivated or modified CIITA gene sequences, and in some instances, additional gene modifications that regulatably inactivate or modify B2M gene sequences. In some embodiments, described herein are genetically edited cells comprising regulatable exogenous CD47 proteins and regulatably inactivated or modified CIITA gene sequences, and in some instances, additional gene modifications that regulatably inactivate or modify NLRC5 gene sequences. In some embodiments, described herein are genetically edited cells comprising regulatable exogenous CD47 proteins and regulatably inactivated or modified B2M gene sequences, and in some instances, additional gene modifications that regulatably inactivate or modify NLRC5 gene sequences. In some embodiments, described herein are genetically edited cells comprising regulatable exogenous CD47 proteins and regulatably inactivated or modified

B2M gene sequences, and in some instances, additional gene modifications that regulatably inactivate or modify CIITA gene sequences and NLRC5 gene sequences.

[00456] Provided herein are cells exhibiting a modification of one or more targeted polynucleotide sequences that regulatably regulates the expression of any one of the following: (a) MHC I antigen molecules, (b) MHC II antigen molecules, (c) TCR complexes, (d) both MHC I and II antigen molecules, and (e) MHC I and II antigen molecules and TCR complexes. In certain embodiments, the modification includes regulatably increasing expression of CD47. In some embodiments, the cells include an exogenous or recombinant CD47 polypeptide. In certain embodiments, the modification includes regulatable expression of a chimeric antigen receptor. In some embodiments, the cells comprise an exogenous or recombinant chimeric antigen receptor polypeptide.

[00457] In some embodiments, the cell includes a genomic modification of one or more targeted polynucleotide sequences that regulatably regulates the expression of one or more MHC I antigen molecules, MHC II antigen molecules and/or TCR complexes. In some embodiments, a genetic editing system is used to regulatably modify one or more targeted polynucleotide sequences. In some embodiments, the polynucleotide sequence targets one or more genes selected from the group consisting of B2M, CIITA, TRAC, and TRB. In certain embodiments, the genome of a T cell (*e.g.*, a T cell differentiated from hypoimmunogenic iPSCs and a primary T cell) has been altered to regulatably reduce or delete critical components of HLA and TCR expression, *e.g.*, HLA-A antigen, HLA-B antigen, HLA-C antigen, HLA-DP antigen, HLA-DQ antigen, HLA-DR antigens, TCR-alpha and TCR-beta.

[00458] In some embodiments, the present disclosure provides a cell or population thereof comprising a genome in which a gene has been regulatably edited to delete a contiguous stretch of genomic DNA, thereby reducing or eliminating surface expression of one or more MHC class I molecules in the cell or population thereof. In certain embodiments, the present disclosure provides a cell or population thereof comprising a genome in which a gene has been regulatably edited to delete a contiguous stretch of genomic DNA, thereby reducing or eliminating surface expression of one or more MHC class II molecules in the cell or population thereof. In certain embodiments, the present disclosure provides a cell or population thereof comprising a genome in which a gene has been regulatably edited to delete a contiguous stretch of genomic DNA, thereby reducing or eliminating surface expression of TCR molecules in the cell or population

thereof. In numerous embodiments, the present disclosure provides a cell or population thereof comprising a genome in which one or more genes has been regulatably edited to delete a contiguous stretch of genomic DNA, thereby reducing or eliminating surface expression of one or more MHC class I and II molecules and TCR complex molecules in the cell or population thereof.

[00459] In some embodiments, the cells and methods described herein include regulatably genomically editing human cells to cleave CIITA gene sequences as well as regulatably editing the genome of such cells to alter one or more additional target polynucleotide sequences such as, but not limited to, B2M TRAC, and TRB. In some embodiments, the cells and methods described herein include regulatably genomically editing human cells to cleave B2M gene sequences as well as regulatably editing the genome of such cells to alter one or more additional target polynucleotide sequences such as, but not limited to, CIITA, TRAC, and TRB. In some embodiments, the cells and methods described herein include regulatably genomically editing human cells to cleave TRAC gene sequences as well as regulatably editing the genome of such cells to alter one or more additional target polynucleotide sequences such as, but not limited to, B2M, CIITA, and TRB. In some embodiments, the cells and methods described herein include regulatably genomically editing human cells to cleave TRB gene sequences as well as regulatably editing the genome of such cells to alter one or more additional target polynucleotide sequences such as, but not limited to, B2M, CIITA, and TRAC.

[00460] Provided herein are hypoimmunogenic stem cells comprising i) regulatable reduced expression of HLA-A, HLA-B, HLA-C, CIITA, TCR-alpha, and TCR-beta relative to a wild-type stem cell, wherein the regulatable reduced expression is by way of an RNA-based component , a DNA-based component , or a protein-based component , and ii) a set of exogenous genes comprising a first regulatable gene encoding one or more tolerogenic factors and a second regulatable gene encoding a chimeric antigen receptor (CAR), wherein the first and/or second regulatable genes are inserted into a specific locus of at least one allele of the cell. Also provided herein are hypoimmunogenic primary T cells including any subtype of primary T cells comprising i) regulatable reduced expression of HLA-A, HLA-B, HLA-C, CIITA, TCR-alpha, and TCR-beta relative to a wild-type primary T cell, wherein the regulatable reduced expression is by way of an RNA-based component , a DNA-based component , or a protein-based component , and ii) a set of exogenous genes comprising a first regulatable gene encoding

one or more tolerogenic factors and a second regulatable gene encoding a chimeric antigen receptor (CAR), wherein the first and/or second regulatable genes are inserted into a specific locus of at least one allele of the cell. Further provided herein are hypoimmunogenic T cells differentiated from hypoimmunogenic induced pluripotent stem cells comprising i) regulatable reduced expression of HLA-A, HLA-B, HLA-C, CIITA, TCR-alpha, and TCR-beta relative to a wild-type primary T cell, wherein the regulatable reduced expression is by way of an RNA-based component , a DNA-based component , or a protein-based component , and ii) a set of exogenous genes comprising a first regulatable gene encoding one or more tolerogenic factors and a second regulatable gene encoding a chimeric antigen receptor (CAR), wherein the first and/or second regulatable genes are inserted into a specific locus of at least one allele of the cell.

[00461] In some embodiments, the population of engineered cells described evades NK cell mediated cytotoxicity upon administration to a recipient patient. In some embodiments, the population of engineered cells evades NK cell mediated cytotoxicity by one or more subpopulations of NK cells. In some embodiments, the population of engineered cells is protected from cell lysis by NK cells, including immature and/or mature NK cells upon administration to a recipient patient. In some embodiments, the population of engineered cells evades macrophage engulfment upon administration to a recipient patient. In some embodiments, the population of engineered cells does not induce an innate and/or an adaptive immune response to the cell upon administration to a recipient patient. In some embodiments, the population of engineered cells evades NK cell mediated cytotoxicity by one or more subpopulations of NK cells, as determined by an in vitro assay or an in vivo assay. In some embodiments, the population of engineered cells is protected from cell lysis by NK cells, including immature and/or mature NK cells upon administration to a recipient patient, as determined by an in vitro assay or an in vivo assay. In some embodiments, the population of engineered cells evades macrophage engulfment upon administration to a recipient patient, as determined by an in vitro assay or an in vivo assay. In some embodiments, the population of engineered cells does not induce an innate and/or an adaptive immune response to the cell upon administration to a recipient patient, as determined by an in vitro assay or an in vivo assay.

[00462] In some embodiments, the cells described herein comprise a safety switch. The term "safety switch" used herein refers to a system for controlling the expression of a gene or protein of interest that, when downregulated or upregulated, leads to clearance or death of the cell, *e.g.*,

through recognition by the host's immune system. A safety switch can be designed to be triggered by an exogenous molecule in case of an adverse clinical event. A safety switch can be engineered by regulating the expression on the DNA, RNA and protein levels. A safety switch includes a protein or molecule that allows for the control of cellular activity in response to an adverse event. In one embodiment, the safety switch is a "kill switch" that is expressed in an inactive state and is fatal to a cell expressing the safety switch upon activation of the switch by a selective, externally provided agent. In one embodiment, the safety switch gene is cis-acting in relation to the gene of interest in a construct. Activation of the safety switch causes the cell to kill solely itself or itself and neighboring cells through apoptosis or necrosis. In some embodiments, the cells described herein, *e.g.,* stem cells, induced pluripotent stem cells, hematopoietic stem cells, primary cells, or differentiated cell, including, but not limited to, cardiac cells, cardiac progenitor cells, neural cells, glial progenitor cells, endothelial cells, T cells, B cells, pancreatic islet cells, retinal pigmented epithelium cells, hepatocytes, thyroid cells, skin cells, blood cells, plasma cells, platelets, renal cells, epithelial cells, CAR-T cells, NK cells, and/or CAR-NK cells, comprise a safety switch.

[00463] In some embodiments, the safety switch comprises a therapeutic agent that inhibits or blocks the interaction of CD47 and SIRPα. In some aspects, the CD47-SIRPα blockade agent is an agent that neutralizes, blocks, antagonizes, or interferes with the cell surface expression of CD47, SIRPα, or both. In some embodiments, the CD47-SIRPα blockade agent inhibits or blocks the interaction of CD47, SIRPα or both. In some embodiments, a CD47-SIRPα blockade agent (e.g., a CD47-SIRPα blocking, inhibiting, reducing, antagonizing, neutralizing, or interfering agent) comprises an agent selected from from a group that includes an antibody or fragment thereof that binds CD47, a bispecific antibody that binds CD47, an immunocytokine fusion protein that bind CD47, a CD47 containing fusion protein, an antibody or fragment thereof that binds SIRPα, a bispecific antibody that binds SIRPα, an immunocytokine fusion protein that bind SIRPα, an SIRPα containing fusion protein, and a combination thereof.

[00464] In some embodiments, the cells described herein comprise a "suicide gene" (or "suicide switch"). The suicide gene can cause the death of the hypoimmunogenic cells should they grow and divide in an undesired manner. The suicide gene ablation approach includes a suicide gene in a gene transfer vector encoding a protein that results in cell killing only when activated by a specific compound. A suicide gene can encode an enzyme that selectively

converts a nontoxic compound into highly toxic metabolites. In some embodiments, the cells described herein, *e.g.,* stem cells, induced pluripotent stem cells, hematopoietic stem cells, primary cells, or differentiated cell, including, but not limited to, cardiac cells, cardiac progenitor cells, neural cells, glial progenitor cells, endothelial cells, T cells, B cells, pancreatic islet cells, retinal pigmented epithelium cells, hepatocytes, thyroid cells, skin cells, blood cells, plasma cells, platelets, renal cells, epithelial cells, CAR-T cells, NK cells, and/or CAR-NK cells, comprise a suicide gene.

[00465] In some embodiments, the population of engineered cells described elicits a reduced level of immune activation or no immune activation upon administration to a recipient subject. In some embodiments, the cells elicit a reduced level of systemic TH1 activation or no systemic TH1 activation in a recipient subject. In some embodiments, the cells elicit a reduced level of immune activation of peripheral blood mononuclear cells (PBMCs) or no immune activation of PBMCs in a recipient subject. In some embodiments, the cells elicit a reduced level of donor-specific IgG antibodies or no donor specific IgG antibodies against the cells upon administration to a recipient subject. In some embodiments, the cells elicit a reduced level of IgM and IgG antibody production or no IgM and IgG antibody production against the cells in a recipient subject. In some embodiments, the cells elicit a reduced level of cytotoxic T cell killing of the cells upon administration to a recipient subject.

B. Conditional HIP Cells and Methods for Conditional Downregulation of Target Genes

[00466] The introduction of regulatable reduced expression of target genes improves the safety of cell therapies developed using hypoimmunogenic cells (HIP cells). In some embodiments, the regulatable reduced expression of target genes makes it possible to avoid potential difficulties when differentiating the cells from pluripotent stem cells. In some embodiments, regulatable reduced expression of a target gene includes regulatable reduced expression, such as regulatable knock out or knock down, of B2M, CIITA, NLRC5, TRAC, TRB, CD142, ABO, MIC-A/B, CD38, CD52, PCDH11Y, NLGN4Y and/or RHD. The regulatable reduced expression of one or more of the target genes functions to control an innate and/or an adaptive immune response by a recipient subject to an engrafted hypoimmunogenic cell.

[00467] Described herein are methods for the reduced expression of a target gene that involves a mechanism to "turn-off" expression of the target gene in a controlled manner. Also described are HIP cells possessing regulatable reduced expression of one or more target genes. In some

cases, the cells can be induced to knock out or knock down expression of the one or more target genes.

[00468] In some embodiments, the hypoimmunity of the cells that are introduced to a recipient subject is achieved through the overexpression of an immunosuppressive molecule including hypoimmunity factors and complement inhibitors accompanied with the repression or genetic disruption of the HLA-I and HLA-II loci. These modifications cloak the cell from the recipient immune system's effector cells that are responsible for the clearance of infected, malignant or non-self cells, such as T cells, B cells, NK cells and macrophages. Cloaking of a cell from the immune system allows for existence and persistence of allogeneic cells within the body. The level of expression of any of the immunosuppressive molecules described can be controlled on the protein level, mRNA level, or DNA level in the cells. Similarly, the level of expression of any of the immune signaling molecules described can be controlled on the protein level, mRNA level, or DNA level in the cells.

[00469] In some embodiments, any of the regulatable reduced expression methods described (*e.g.*, RNA level, DNA level, and protein level methods) are used to decrease the level of a target protein in the cells such that the lower level of the target protein is below a threshold level. In some embodiments, the level of the target protein in the cells is decreased by about 10-fold, 9-fold, 8-fold, 7-fold, 6-fold, 5-fold, 4-fold, 3-fold, 2-fold, 1-fold or 0.5-fold below a threshold level of expression. In some embodiments, the level of the target protein in the cells is decreased by about 10-fold to 5-fold, 10-fold to 3-fold, 9-fold to 1-fold, 8-fold to 1-fold, 7-fold to 0.5-fold, 6-fold, to 1-fold, 5-fold to 0.5-fold, 4-fold to 0.5-fold, 3-fold to 0.5-fold, 2-fold to 0.5-fold, or 1-fold to 0.5-fold below a threshold level of expression. In some embodiments, the threshold level of expression of the target protein is established based on the expression of such factor in an induced pluripotent stem cell. In some embodiments, the threshold level of the target protein expression is established based on the expression level of the target protein in a corresponding hypoimmune cell, such as an MHC I and MHC II knock out cell or an MHC I/MHC II/TCR knock out cell.

1. RNA-Based Components

[00470] Target genes can be targeted by shRNAs, siRNAs, or miRNAs, thereby leading to the degradation of the transcript encoding the factors. A shRNA, siRNA, or miRNA can be exogenously provided or genetically encoded to provide control over transcription of the

inhibitory RNA. The shRNA, siRNA, or miRNA can anneal to the target gene's transcript, resulting in degradation by the RISC complex.

[00471] In some embodiments, methods for inducible RNA regulation to downregulate expression of a target gene include, but are not limited to, conditional or inducible shRNAs, conditional or inducible siRNAs, conditional or inducible miRNAs, conditional or inducible CRISPR interference (CRISPRi), and conditional or inducible RNA targeting nucleases.

[00472] In some embodiments, the method comprises an shRNA, siRNA, or miRNA targeting the RNA of the target gene. In some instances, expression of the shRNA, siRNA, or miRNA is induced by a small molecule or biologic agent. In some instances, expression of the shRNA, siRNA, or miRNA is induced by a cellular condition.

[00473] In some embodiments, provided are methods for controlling the immunogenicity of a mammalian cell (*e.g.*, a human cell) by obtaining an isolated cell and introducing a construct containing a conditional or inducible RNA polymerase promoter operably linked an shRNA, siRNA, or miRNA sequence targeting a target gene that is operably linked to a constitutive promoter that is operably linked to a transactivator element that can control the inducible RNA polymerase promoter. In some embodiments, the construct includes a U6Tet promoter, an shRNA, siRNA, or miRNA targeting a target gene, a constitutive promoter, and a Tet Repressor element that is responsive to tetracycline or a derivative thereof (*e.g.*, doxycycline). In other instances, the shRNA, siRNA, or miRNA eliminates expression of the target gene. In other instances, the shRNA, siRNA, or miRNA decreases expression of the target gene by about 99% or less, *e.g.*, 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 90%, 85% or less. Any of the constitutive promoters, conditional promoters, inducible promoters, target genes, and cells described herein are applicable to the method.

[00474] In many embodiments, the engineered cell expresses an inducible an shRNA, siRNA, or miRNA that targets a target gene. In some embodiments, the expression of the RNA polymerase, shRNA, siRNA, or miRNA is under the control of an inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch. In some embodiments, the cell is contacted by a factor such as, but not limited to, a ligand, molecule, peptide, small molecule, or biologic agent that activates the expression of the shRNA, siRNA, or miRNA to degrade the target gene. In some embodiments, the expression of the RNA polymerase, shRNA, siRNA, or miRNA is

under the control of an aptamer-mediated modulator of polyadenylation or an aptamer-regulated riboswitch. In some embodiments, the expression of the RNA polymerase, shRNA, siRNA, or miRNA is under the control of a conditional promoter, such as, *e.g.*, a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, or a differentiation-induced promoter.

[00475] In some embodiments, provided are methods for controlling the immunogenicity of a mammalian cell (*e.g.*, a human cell) by obtaining an isolated cell and introducing into the cell (i) a first construct comprising a conditional or inducible RNA polymerase promoter operably linked to a shRNA, siRNA, or miRNA targeting a target gene such that the shRNA, siRNA, or miRNA is operably linked to a transactivator element that corresponds to the conditional or inducible RNA polymerase promoter.

[00476] In some embodiments, the method comprises a CRISPR interference system (CRISPRi) for targeting the promoter of a target gene to downregulate its transcription. In some instances, expression of a CRISPRi and/or a gRNA targeting the target gene is induced by a small molecule or biologic agent. In some instances, expression of the CRISPRi and/or a gRNA is induced by a cellular condition. Detailed description of CRISPRi methods are found in, *e.g.*, Engreitz *et al.*, Cold Spring Harb Perspect Biol, 2019, 11:a035386, which is herein incorporated by reference in its entirety. In some embodiments, the CRISPRi system utilizes a dCas9-repressor fusion protein that is controlled by a constitutive promoter and a gRNA specific to the target gene under the control of a conditional or an inducible promoter.

[00477] In some embodiments, the expression of the dCas9-repressor fusion protein and/or the gRNA is under the control of an inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch. In some embodiments, the cell is contacted by a factor such as, but not limited to, a ligand, molecule, peptide, small molecule, or biologic agent that activates the expression of the dCas9-repressor fusion protein and/or the gRNA to degrade the target gene. In some embodiments, the expression of the dCas9-repressor fusion protein and/or the gRNA is under the control of an aptamer-mediated modulator of polyadenylation or an aptamer-regulated riboswitch. In some embodiments, the expression of the dCas9-repressor fusion protein and/or the gRNA is under the control of a conditional promoter, such as, *e.g.*, a cell cycle-specific

promoter, a tissue-specific promoter, a lineage-specific promoter, or a differentiation-induced promoter.

[00478] In some embodiments, the CRISPR based method includes a nuclease for targeting the mRNA sequence corresponding to the target gene such as, but not limited to, Cas13, Cas7, or Csx1. In some instances, expression of a nuclease and/or a gRNA targeting the target gene is induced by a small molecule or biologic agent. In some instances, expression of the nuclease and/or gRNA is induced by a cellular condition.

[00479] In some embodiments, the expression of the nuclease and/or gRNA is under the control of an inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch. In some embodiments, the cell is contacted by a factor such as, but not limited to, a ligand, molecule, peptide, small molecule, or biologic agent that activates the expression of the nuclease and/or gRNA to degrade the target gene. In some embodiments, the expression of the nuclease and/or gRNA is under the control of an aptamer-mediated modulator of polyadenylation or an aptamer-regulated riboswitch. In some embodiments, the expression of the nuclease and/or gRNA is under the control of a conditional promoter, such as, *e.g.*, a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, or a differentiation-induced promoter.

[00480] In some embodiments, provided are methods for controlling the immunogenicity of a mammalian cell (*e.g.*, a human cell) by obtaining an isolated cell and introducing into the cell (i) a first construct comprising a constitutive promoter operably linked to a gene encoding a Cas13a nuclease, a variant thereof, or a fusion protein thereof; and (iii) a second construct comprising a conditional or inducible RNA polymerase promoter operably linked to a gRNA sequence targeting a target gene such that the gRNA sequence is operably linked to a transactivator element that corresponds to the conditional or inducible RNA polymerase promoter.

[00481] In some embodiments, inducible expression systems that are useful for RNA level control of the target gene include, but are not limited to, ligand inducible transcription factor systems, small molecule inducible systems, biologic agent inducible systems, receptor mediated expression control systems, aptamer-mediated modulators of polyadenylation (*see*, *e.g.*, WO 2017/083747 and WO 2021/041924, the contents are herein incorporated by reference in their entirety), and ligand-regulated riboswitches. In some embodiments, the inducible expression system comprises a tetracycline-controlled operator system, a synthetic Notch-based (SynNotch)

system (*see*, *e.g.*, Morsut *et al.*, Cell, 2016, 164:780-791 and Yang *et al.*, Commun Biol, 2020, 3:116), and riboswitch that regulates expression of the target gene by ligand (*e.g.*, aptamer, peptide or small molecule) mediated alternative splicing of the resulting pre-mRNA. Useful riboswitches comprise a sensor region and an effector region that sense the presence of a ligand and alter the splice of the target gene. Detailed descriptions and examples of riboswitch gRNAs are found in *e.g.*, US 9,228,207; US 9,993,491; and US 10,421,989; and Seeliger *et al.*, PLoS One, 2012, 7(1):e29266; the contents are herein incorporated by reference in their entirety.

[00482] In some embodiments, conditional expression systems that are useful for RNA level control of the target gene include, but are not limited to, methods under the control of conditional promoters including, but not limited to, cell cycle-specific promoters, tissue-specific promoters, lineage-specific promoters, and differentiation-induced promoters.

[00483] In some embodiments, the level of a target gene, such as B2M, CIITA, NLRC5, TRAC, TRB, and/or RHD, in the engineered cells is decreased by an RNA-based component by about 10-fold, 9-fold, 8-fold, 7-fold, 6-fold, 5-fold, 4-fold, 3-fold, 2-fold, 1-fold or 0.5-fold below a threshold level of expression. In some embodiments, the level of CD47 in the engineered cells is decreased by about 10-fold to 5-fold, 10-fold to 3-fold, 9-fold to 1-fold, 8-fold to 1-fold, 7-fold to 0.5-fold, 6-fold, to 1-fold, 5-fold to 0.5-fold, 4-fold to 0.5-fold, 3-fold to 0.5-fold, 2-fold to 0.5-fold, or 1-fold to 0.5-fold below a threshold level of expression. In some instances, the threshold level of B2M, CIITA, NLRC5, TRAC, TRB, and/or RHD expression is established based on the endogenous expression of B2M, CIITA, NLRC5, TRAC, TRB, and/or RHD in an induced pluripotent stem cell. In some instances, the threshold level of B2M, CIITA, NLRC5, TRAC, TRB, and/or RHD expression is established based on the endogenous expression of B2M, CIITA, NLRC5, TRAC, TRB, and/or RHD in a wild-type or unmodified cell.

2. DNA-Based Components

[00484] Transcriptional regulation of target genes through employing conditional or inducible promoters provides the ability to turn expression of the gene on or off through the addition or removal of biologic agents or small molecules, such as, but not limited to, doxycycline, or through a change in a cellular condition. Genetic disruption via targeted nuclease activity can eliminate expression of the target genes.

[00485] In some embodiments, methods for conditional or inducible DNA regulation include, but are not limited to, using cell cycle-specific promoters, tissue-specific promoters, lineage-specific promoters, differentiation-induced promoters, inducible promoters, controllable riboswitches, and knock out using a conditional or inducible nuclease (*e.g.*, conditional or inducible CRISPRs, conditional or inducible TALENs, conditional or inducible zinc finger nucleases, conditional or inducible homing endonucleases, conditional or inducible meganucleases, and the like) to target the DNA sequence of one or more target genes. In some embodiments, the conditional or inducible nuclease comprises a nuclease such that its expression is controlled by the presence of a small molecule. In some embodiments, the conditional or inducible nuclease comprises a nuclease such that delivery of the nuclease RNA or protein to a cells is controlled by the presence of a small molecule. In some embodiments, expression of the nuclease is induced by a small molecule or biologic agent. In some embodiments, expression of a Cas nuclease and/or a guide RNA (gRNA) is induced by a small molecule or biologic agent. In some instances, expression of a Cas nuclease and/or a gRNA is induced by a cellular condition.

[00486] In some embodiments, methods for inducible expression include, but are not limited to, ligand inducible transcription factors systems (*e.g.*, a tetracycline-controlled operator system), receptor mediated control of expression system (*e.g.*, a SynNotch system), and a ligand regulated riboswitch system for control of mRNA or gRNA activity. Detailed description of inducible expression methods are found in, *e.g.*, Kallunki *et al.*, Cells, 2019, 796 (doi:10.3390/cells8080796), which is herein incorporated by reference in its entirety.

[00487] Any of the constitutive promoters, conditional promoters, inducible promoters, target genes, and cells described herein are applicable to the method.

[00488] In some embodiments, the present disclosure provides a method of producing a stem cell (*e.g.*, hypoimmunogenic pluripotent stem cell or hypoimmunogenic induced pluripotent stem cell) or a differentiated cell thereof that has been modified to conditionally knock out or knock down any one of the target genes selected from the group consisting of B2M, CIITA, NLRC5, TRAC, TRB, and RHD.

[00489] In some embodiments, inducible expression systems that are useful for DNA level control of the target gene include, but are not limited to, ligand inducible transcription factor systems, small molecule inducible systems, biologic agent inducible systems, receptor mediated expression control systems, aptamer-mediated modulators of polyadenylation (see, *e.g.*, WO

2017/083747 and WO 2021/041924, the contents are herein incorporated by reference in their entirety), and ligand-regulated riboswitches. In some embodiments, the inducible expression system comprises a tetracycline-controlled operator system, a synthetic Notch-based (SynNotch) system (see, *e.g.*, Morsut *et al.*, Cell, 2016, 164:780-791 and Yang *et al.*, Commun Biol, 2020, 3:116), and riboswitch that regulates expression of the target gene by ligand (*e.g.*, aptamer, peptide or small molecule) mediated alternative splicing of the resulting pre-mRNA. Useful riboswitches comprise a sensor region and an effector region that sense the presence of a ligand and alter the splice of the target gene. Detailed descriptions and examples of riboswitch gRNAs are found in *e.g.*, US 9,228,207; US 9,993,491; and US 10,421,989; and Seeliger *et al.*, PLoS One, 2012, 7(1):e29266; the contents are herein incorporated by reference in their entirety.

[00490] In some embodiments, conditional expression systems that are useful for DNA level control of the target gene include, but are not limited to, methods under the control of conditional promoters including, but not limited to, cell cycle-specific promoters, tissue-specific promoters, lineage-specific promoters, and differentiation-induced promoters.

[00491] In some embodiments, the level of a target gene, such as B2M, CIITA, NLRC5, TRAC, TRB, and/or RHD, in the engineered cells is decreased by an DNA-based component by about 10-fold, 9-fold, 8-fold, 7-fold, 6-fold, 5-fold, 4-fold, 3-fold, 2-fold, 1-fold or 0.5-fold below a threshold level of expression. In some embodiments, the level of CD47 in the engineered cells is decreased by about 10-fold to 5-fold, 10-fold to 3-fold, 9-fold to 1-fold, 8-fold to 1-fold, 7-fold to 0.5-fold, 6-fold, to 1-fold, 5-fold to 0.5-fold, 4-fold to 0.5-fold, 3-fold to 0.5-fold, 2-fold to 0.5-fold, or 1-fold to 0.5-fold below a threshold level of expression. In some instances, the threshold level of B2M, CIITA, NLRC5, TRAC, TRB, and/or RHD expression is established based on the endogenous expression of B2M, CIITA, NLRC5, TRAC, TRB, and/or RHD in an induced pluripotent stem cell. In some instances, the threshold level of B2M, CIITA, NLRC5, TRAC, TRB, and/or RHD expression is established based on the endogenous expression of B2M, CIITA, NLRC5, TRAC, TRB, and/or RHD in a wild-type or unmodified cell.

3. Protein-Based Components

[00492] In some embodiments, regulated degradation of a target protein is established by a degron-based method that allows recruitment of the target protein to the endogenous protein turnover machinery. Mechanisms for targeted protein degradation include, but are not limited to,

recruitment to an E3 ligase for ubiquitination and subsequent proteasomal degradation, direct recruitment to the proteasome, and recruitment to the lysosome.

[00493] In some embodiments, methods for inducible protein degradation by a degron includes, but is not limited to, ligand induced degradation (LID) using a SMASH tag, ligand induced degradation using Shield-1, ligand induced degradation using auxin, ligand induced degradation using rapamycin, peptidic degrons (*e.g.*, IKZF3 based degrons), and proteolysis-targeting chimeras (PROTACs). In some embodiments of a ligand induced degradation method, a degron tag that is held in an inactive conformation but is induced to adopt a conformation capable of recognition by the proteasome upon binding of a specific molecule, such as but not limited to, a Shield-1 molecule. See, *e.g.*, Roth *et al.*, Cellular Molecular Life Sciences, 2019, 76(14), 2761-2777, which is herein incorporated by reference in its entirety. Detailed descriptions of SMASH degron technology can be found in Hannah and Zhou, Nat Chem Biol, 2015, 11:637-638 and Chung *et al.*, Nat Chem Biol, 2015, 11:713-720, which are herein incorporated by reference in their entireties. Detailed descriptions of LID degron technologies can be found in Bonger *et al.*, Nat Chem Biol, 2011, 7(8):531-7, which is herein incorporated by reference in its entirety.

[00494] In some embodiments, provided are methods for controlling the immunogenicity of a mammalian cell (*e.g.*, a human cell) by obtaining an isolated cell and introducing a construct containing a conditional or inducible promoter operably linked to peptidic proteolysis targeting chimera (PROTAC) element directed to a target protein, *e.g.*, B2M, CIITA, NLRC5, TRAC, TRB, and/or RHD.

[00495] In some embodiments of a peptidic degron, a peptide tag is used that confers small molecule-mediated recruitment to an E3 ligase. In some embodiments, the peptide tag comprises the lymphoid-restricted transcription factor IKZF3 that is recruited to the E3 ligase receptor (CRBN) in an immunomodulatory drug (IMiD) dependent manner, as described in Koduri *et al.*, Proc Natl Acad Sci, 2019, 116(7), 2539-2544, which is herein incorporated by reference in its entirety. In certain embodiments, the degron is capable of targeting target proteins for degradation (*e.g.*, through a ubiquitination pathway), inducing protein degradation, or degrading proteins.

[00496] In some embodiments of a PROTAC, a bifunctional molecule is used to recruit a target protein to the protein degradation machinery of a cell. In some embodiments, the bi-functional molecule binds to the native or wild-type sequence of the target protein with high affinity. In

some embodiments, the bi-functional molecule comprises a small molecule or a biologic agent (*e.g.*, an antibody or fragment thereof). See, *e.g.*, Burslem *et al.*, Cell Chemical Biology, 2018, 25, 67-77 and Roth *et al.*, Cellular Molecular Life Sciences, 2019, 76(14), 2761-2777, which are herein incorporated by reference in their entirety.

[00497] In some embodiments of a bi-functional antibody, the antibody targets a target protein and a second endogenous receptor which leads to internalization and degradation. Controllable expression of one or more target proteins can be provided by way of a bifunctional antibody (*e.g.*, a chemically reprogrammed bifunctional antibody), inducible protein degradation by a degron, inducible RNA regulation, inducible DNA regulation, and an inducible expression method. See, *e.g.*, Natsume and Kanemaki, Annu Rev Genet, 2017, 51, 82-102; Burslem and Crews, Chem Rev, 2017, 117, 11269-11301; Banik *et al.*, ChemRxiv, 2019; which are herein incorporated by reference in their entirety. In some embodiments, a cell expressing a target protein is contacted by an antibody that binds the cell for degradation.

[00498] In some embodiments, the inducible degron element is selected from the group consisting of a ligand inducible degron element such as a small molecule-assisted shutoff (SMASH) degron element, Shield-1 responsive degron element, auxin responsive degron element, and rapamycin responsive degron element; a peptidic degron element; and a peptidic proteolysis targeting chimera (PROTAC) element. In useful embodiments, the ligand inducible degron element is a small molecule-assisted shutoff (SMASH) degron element and the exogenous factor for controlling immunogenicity is asunaprevir. In some embodiments, the target gene is selected from the group consisting of B2M, CIITA, NLRC5, TRAC, TRB, and RHD.

[00499] In some embodiments, methods for conditional or inducible protein regulation are under the control of cell cycle-specific promoters, tissue-specific promoters, lineage-specific promoters, differentiation-induced promoters, inducible promoters, or controllable riboswitches. In some embodiments, expression of the conditional or inducible degron is controlled by the presence of a small molecule or biologic agent. In some instances, expression of the conditional or inducible degron is controlled by a cellular condition.

[00500] In some embodiments, methods for inducible expression include, but are not limited to, ligand inducible transcription factors systems (*e.g.*, a tetracycline-controlled operator system), receptor mediated control of expression system (*e.g.*, a SynNotch system), and a ligand regulated

riboswitch system for control of mRNA or gRNA activity. Detailed description of inducible expression methods are found in, *e.g.*, Kallunki *et al.*, Cells, 2019, 796 (doi:10.3390/cells8080796), which is herein incorporated by reference in its entirety.

[00501] Any of the constitutive promoters, conditional promoters, inducible promoters, target genes, and cells described herein are applicable to the method.

[00502] In some embodiments, the present disclosure provides a method of producing a stem cell (*e.g.*, hypoimmunogenic pluripotent stem cell or hypoimmunogenic induced pluripotent stem cell) or a differentiated cell thereof that has been modified to conditionally degrade any one of the target proteins selected from the group consisting of B2M, CIITA, NLRC5, TRAC, TRB, and RHD.

[00503] In some embodiments, inducible expression systems that are useful for protein level control of the target gene include, but are not limited to, ligand inducible transcription factor systems, small molecule inducible systems, biologic agent inducible systems, receptor mediated expression control systems, aptamer-mediated modulators of polyadenylation (see, *e.g.*, WO 2017/083747 and WO 2021/041924, the contents are herein incorporated by reference in their entirety), and ligand-regulated riboswitches. In some embodiments, the inducible expression system comprises a tetracycline-controlled operator system, a synthetic Notch-based (SynNotch) system (see, *e.g.*, Morsut *et al.*, Cell, 2016, 164:780-791 and Yang *et al.*, Commun Biol, 2020, 3:116), and riboswitch that regulates expression of the target gene by ligand (*e.g.*, aptamer, peptide or small molecule) mediated alternative splicing of the resulting pre-mRNA. Useful riboswitches comprise a sensor region and an effector region that sense the presence of a ligand and alter the splice of the target gene. Detailed descriptions and examples of riboswitch gRNAs are found in *e.g.*, US 9,228,207; US 9,993,491; and US 10,421,989; and Seeliger *et al.*, PLoS One, 2012, 7(1):e29266; the contents are herein incorporated by reference in their entirety.

[00504] In some embodiments, conditional expression systems that are useful for protein level control of the target gene include, but are not limited to, methods under the control of conditional promoters including, but not limited to, cell cycle-specific promoters, tissue-specific promoters, lineage-specific promoters, and differentiation-induced promoters.

[00505] In some embodiments, the level of a target protein, such as B2M, CIITA, NLRC5, TRAC, TRB, and/or RHD, in the engineered cells is decreased by an protein-based component by about 10-fold, 9-fold, 8-fold, 7-fold, 6-fold, 5-fold, 4-fold, 3-fold, 2-fold, 1-fold or 0.5-fold

below a threshold level of expression. In some embodiments, the level of CD47 in the engineered cells is decreased by about 10-fold to 5-fold, 10-fold to 3-fold, 9-fold to 1-fold, 8-fold to 1-fold, 7-fold to 0.5-fold, 6-fold, to 1-fold, 5-fold to 0.5-fold, 4-fold to 0.5-fold, 3-fold to 0.5-fold, 2-fold to 0.5-fold, or 1-fold to 0.5-fold below a threshold level of expression. In some instances, the threshold level of B2M, CIITA, NLRC5, TRAC, TRB, and/or RHD protein is established based on the endogenous protein of B2M, CIITA, NLRC5, TRAC, TRB, and/or RHD in an induced pluripotent stem cell. In some instances, the threshold level of B2M, CIITA, NLRC5, TRAC, TRB, and/or RHD expression is established based on the endogenous expression of B2M, CIITA, NLRC5, TRAC, TRB, and/or RHD in a wild-type or unmodified cell.

C. Conditional HIP Cells and Methods for Conditional Upregulation of Transgenes

[00506] The introduction of regulatable overexpression of transgenes improves the safety of cell therapies developed using hypoimmunogenic cells (HIP cells). A feature of the HIP cells described herein is the regulatable expression of one or more immune regulatory (immunosuppressive) factors. In some embodiments, an immunosuppressive factor (also referred to herein as "an hypoimmunity factor") includes, but is not limited to, CD47, CD24, CD200, HLA-G, HLA-E, HLA-C, HLA-E heavy chain, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FASL, Serpinb9, CCl21, and Mfge8. In certain embodiments, the immunosuppressive factor is CD47. The regulatable or inducible expression of an immunosuppressive factor functions to control an innate and/or an adaptive immune response by a recipient subject to an engrafted hypoimmunogenic cell.

[00507] Described herein are methods for the expression of an immune signaling factor in a controllable manner as to increase the expression of the factor to alter the hypoimmunogenicity of the cell.

[00508] Controllable expression of one or more immunosuppressive factors can be provided by way of an inducible ligand stabilization system using a degron, an inducible RNA upregulation system (*e.g.*, an inducible CRISPR activation), and an inducible DNA upregulation system. In some embodiments, the inducible DNA upregulation system comprises inducible CRISPR activation (CRISPRa), tissue-specific promoters, inducible promoters, and riboswitches.

[00509] Detailed description of CRISPRa methods are found in, *e.g.*, Engreitz *et al.*, Cold Spring Harb Perspect Biol, 2019, 11:a035386, which is herein incorporated by reference in its

entirety. Detailed descriptions and examples of inducible riboswitches are found in *e.g.*, US 9,228,207; US 9,993,491; and US 10,421,989; and Seeliger *et al.*, PLoS One, 2012, 7(1):e29266; the contents are herein incorporated by reference in their entirety.

[00510] Any of the constitutive promoters, conditional promoters, inducible promoters, target genes, and cells described herein are applicable to the method.

[00511] In some embodiments, the engineered cell comprises an exogenous polynucleotide comprising a conditional or inducible transgene encoding one or more tolerogenic factors. In some embodiments, the expression of the CD47 is under the control of an inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch. In some embodiments, the cell is contacted by a factor such as, but not limited to, a ligand, molecule, peptide, small molecule, or biologic agent that activates the expression of the CD47. In some embodiments, the expression of the CD47 is under the control of an aptamer-mediated modulator of polyadenylation or an aptamer-regulated riboswitch. In some embodiments, the expression of the CD47 is under the control of a conditional promoter, such as, *e.g.*, a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, or a differentiation-induced promoter.

[00512] In some embodiments, provided are methods for controlling the immunogenicity of a mammalian cell (*e.g.*, a human cell) by obtaining an isolated cell and introducing into the cell (i) a first construct comprising a conditional or inducible transgene encoding one or more tolerogenic factors.

D. Regulatory Elements

[00513] Promoters may be derivatives or modified variants of any native or known promoters, including insertions and deletions of native or known promoters and combinations or permutations thereof. Chimeric promoters may also be used comprising sequence elements from two or more different promoters described herein. In any case, any promoter can be tested readily for its effectiveness in the cells described herein.

[00514] Numerous types of appropriate expression vectors and suitable regulatory sequences are known in the art for a variety of host cells. Typically, the one or more regulatory nucleotide sequences may include, but are not limited to, promoter sequences, leader or signal sequences, ribosomal binding sites, transcriptional start and termination sequences, translational start and

termination sequences, polyadenylation sites, Kozak consensus sequences, and enhancer or activator sequences.

1. Constitutive Promoters

[00515] Any constitutive or ubiquitous promoter known in the art can be used in the present disclosure. Examples of constitutive or ubiquitous promoters include, *e.g.*, actin promoter (*e.g.*, ACTB promoter), albumin promoter, baculovirus IE1 promoter, beta-actin promoter, beta-actin promoter linked to the enhancer derived from the cytomegalovirus (CMV) immediate early (IE) promoter (CAG promoter), CaM-kinase promoter, CMV-HSV thymidine kinase promoter, collagen 1A1 promoter (Sokolov *et al.* 1995; Breault *et al.* 1997), collagen 1A2 promoter (Akai *et al.* 1999; Antoniv *et al.* 2001), dihydrofolate reductase promoter, elongation factor 1α (EF1α) promoter, herpes thymidine kinase promoter (Wagner *et al.*, 1981), HPRT promoter, Moloney murine leukemia virus Long Terminal repeat region (MMLV LTR), phosphoglycerate kinase 1 (PGK) promoter, promoters of the E1A or major late promoter (MLP) genes of adenoviruses (Ad), promoters of RNA polymerases pol I, pol II, pol III, U6, or HI, tubulin promoter, ubiquitin (UbC) promoter, vimentin promoter, viral promoters (*e.g.*, avian sarcoma virus, bovine papilloma virus, cytomegalovirus (CMV), (Boshart *et al.,* Cell, 41:521-530 (1985), minimal CMV promoter (Gossen and Bujard, Proc. Natl. Acad. Sci. USA, 1992, 89: 5547-5551), fowlpox virus, hepatitis-B virus, polyoma virus, a retrovirus, retroviral Rous sarcoma virus (RSV) LTR promoter, simian virus 40 (SV40)).

2. Inducible Promoters and Elements

[00516] Any inducible promoter known in the art can be used in the present disclosure. The term "inducible promoter" refers to a promoter that selectively expresses a coding sequence or functional RNA in response to the presence of an endogenous or exogenous stimulus, for example by chemical compounds (chemical inducers) or in response to environmental, hormonal, chemical, and/or developmental signals. Inducible or regulated promoters include, for example, promoters induced or regulated by hormones, steroids, growth factors, cytokines, cytostatics, irradiation, small molecules, metals, heat shock, light, tetracycline, interferon, prodrugs, aptamers, etc. Examples of regulatable promoters are described in Goverdhana et ak, Mol Ther, 12: 189-211, 2005; Agha- Mohammadi and Lotze, J Clin Invest, 105: 1177-1183;

Mullick et ak, BMC Biotech, 6:43, 2006; and US20210155667, each of which is encorporated herein in its entirety.

[00517] Examples of inducible promoters include, *e.g.*, *small molecule or ligand-responsive promoters*, including, *e.g.*, abscisic acid (ABA) system (Liang *et al.*, Sci Signal. 2011 Mar 15;4(164):rs2), coumermycin-responsive promoters (Zhao *et al.*, Hum Gene Ther. 2003 Nov 20;14(17):1619-29), cumate-regulated promoters, dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoters, doxycycline inducible promoters (*e.g.*, tre) (Bohl *et al.*, (1998) Blood 92(5), 1512-7), ecdysone insect promoters (No *et al.*, Proc. Natl. Acad. Sci. USA, 93:3346-3351 (1996)), GAL1-GAL10 promoter, isopropyl-beta-D-thiogalactopyranoside (IPTG)-regulated promoter, lactose induced promoter, mifepristone-responsive promoters (*e.g.*, GAL4-Elb promoter), mouse mammary leukemia virus promoter, pyruvate kinase promoter, rapamycin-inducible promoters (Magari *et al.,* J Clin. Invest., 100:2865-2872 (1997)), RU486-inducible system (Wang *et al.,* Nat. Biotech., 15:239-243 (1997) and Wang *et al.,* Gene Ther., 4:432-441 (1997)), tetracycline-regulated promoters, *e.g.* a tetracycline-repressible system (Gossen *et al.,* Proc. Natl. Acad. Sci. USA, 89:5547-5551 (1992)) or a tetracycline-inducible system [Gossen *et al.,* Science, 268:1766-1769 (1995); see also Harvey *et al.*, Curr. Opin. Chem. Biol, 2:512-518 (1998); see also US20210155667, all incorporated herein by reference in their entirety]; *alcohol-regulated promoters; environment/stress-induced promoters,* including, *e.g.*, heat shock response promoters (*e.g.*, heat shock 70 promoter), hypoxia driven promoters, IL-8 promoters, interferon-responsive promoters, NF-Kb responsive promoter, pH-regulated promoters; *light-responsive promoters*, including, *e.g.*, vivid (VVD) system, photoactivatable (PA)-Tet-OFF/ON system; *metal-responsive promoters*, including, *e.g.*, metallothionein-inducible promoter (*e.g.*, copper inducible ACEl, zinc-inducible sheep metallothionine (MT) promoters); *pathogenesis-regulated promoters*, including, *e.g.*, promoters that are induced by salicylic acid, ethylene or benzothiadiazole (BTH)); *RNA polymerase-inducible promoters,* including, *e.g.*, T3 RNA polymerase promoter, T7 polymerase promoter system (*see*, *e.g.*, WO 98/10088, incorporated herein by reference in its entirety); and *steroid hormone-inducible promoters*, including, *e.g.*, promoters comprising a hormone response element that renders the promoter responsive to a ligand for a hormone receptor, wherein hormone receptors include, *e.g.*, the estrogen, progesterone, and glucocorticoid receptors, and wherein ligands include

physiological ligands, *e.g.*, estrogen, progesterone, or cortisol, and non-physiological ligands, *e.g.*, tamoxifen, dexamethasone.

[00518] In some embodiments, the inducible target gene regulation is by way of inducible CRISPR activation (CRISPRa), including, *e.g.*, dCas9 fused to a scaffold that recruits activator peptides (*e.g.* SunTag), dCas9 fused to a series of activation domains (*e.g.*, dCas9-VPR), dCas9 fused to an activator and a tagged gRNA recruits other activators (*e.g.*, SAM). Detailed description of CRISPRa methods are found in, *e.g.*, Engreitz *et al.*, Cold Spring Harb Perspect Biol, 2019, 11:a035386.

[00519] In some embodiments, the inducible target gene regulation is by way of inducible riboswitches/aptamers. The term "aptamer" as used herein refers to an RNA polynucleotide that specifically binds to a ligand. The term "ligand" refers to a molecule that is specifically bound by the aptamer. The gene regulation cassette refers to a recombinant DNA construct that when incorporated into the DNA of a target gene provides the ability to regulate expression of the target gene by aptamer/ligand mediated alternative splicing of the resulting pre-mRNA. The riboswitch contains a sensor region (*e.g.*, an aptamer) and an effector region that together are responsible for sensing the presence of a small molecule ligand and altering splicing to an alternative exon. In one embodiment, the target gene's expression is increased when the aptamer ligand is present and decreased when the ligand is absent. Detailed descriptions and examples of inducible riboswitches are found in *e.g.*, US 9,228,207; US 9,993,491; and US 10,421,989; and Seeliger *et al.*, PLoS One, 2012, 7(1):e29266.

[00520] In some embodiments, the inducible target gene regulation is by way of regulatory fusion proteins. Gene expression in eukaryotic cells can be tightly regulated using a strong promoter that is controlled by an operator that is in turn regulated by a regulatory fusion protein (RFP). The RFP consists essentially of a transcription blocking domain, and a ligand-binding domain that regulates its activity. In the presence of the cognate ligand for the ligand-binding domain, the RFP binds the operator thereby preventing transcription of the GOI. When the cognate ligand is withdrawn, the RFP is destabilized and transcription of the nucleotide sequence of interest proceeds.

[00521] In some embodiments, the inducible target gene regulation is by way of degrons. In some embodiments, the degron element is selected from the group consisting of a ligand

inducible degron element, a peptidic degron element, and a peptidic proteolysis targeting chimera (PROTAC) element. In some embodiments, the ligand inducible degron element is selected from a small molecule-assisted shutoff (SMASH) degron element, Shield-1 responsive degron element, auxin responsive degron element, and rapamycin responsive degron element. In certain embodiments, the ligand inducible degron element is a small molecule-assisted shutoff (SMASH) degron element and the exogenous factor is asunaprevir. In the case of regulatable up-regulation of a target gene, the degron element can be a degron system as described in Tan *et al.*, Gene Regulation: Methods, vol. 9, suppl. 1, S123, May 01, 2004, in which a degron containing ZFP TF may be combined with a regulatable switch such as the progesterone receptor ligand-binding domain, resulting in mifpristone-dependent up-regulation of the target gene.

3. Cell Cycle-Specific Promoters

[00522] In some embodiments, the conditional target gene regulation is by way of a cell cycle specific promoter. Cell cycle phase specific expression control element may be selected from cell cycle specific promoters and other elements that influence the control of transcription or translation in a cell cycle specific manner. Where the expression control element is a promoter, the choice of promoter will depend on the phase of the cell cycle selected for study.

[00523] Any cell cycle-specific promoter known in the art can be used in the present disclosure. Examples of cell cycle-specific promoters include, *e.g.*, cyclin B1 promoter (Cogswell *et al.,* Mol. Cell Biol., (1995), 15(5), 2782-90, Hwang *et al.,* J. Biol. Chem., (1995), 270(47), 2841 9-24, Piaggio *et al.,* Exp. Cell Res., (1995), 21 6(2), 396-402), Cdc25B promoter (Korner *et al.,* J. Biol. Chem., (2001), 276(1 3), 9662-9); cyclin A2 promoter (Henglein *et al.,* Proc.Nat.Acad.Sci.USA, (1994), 91 (12), 5490-4, Zwicker *et al.,* Embo J., (1995), 14(1 8), 451 4-22), Cdc2 promoter (Tommasi and Pfeifer, Mol. Cell Biol., (1995), 15(12), 6901 -1 3, Zwicker *et al.,* Embo J (1995), 14(1 8), 4514-22), Cdc25C promoter (Korner and Muller, J. Biol. Chem., (2000), 275(25), 1 8676-81 , Korner *et al.,* Nucl. Acids Res., (1997), 25(24), 4933-9), cyclin E promoter (Botz *et al.,* Mol. Cell Biol., (1996), 1 6(7), 3401 -9, Korner and Muller, J. Biol. Chem., (2000), 275(25), 1 8676-81), Cdc6 promoter (Hateboer *et al.,* Mol. Cell Biol., (1998), 1 8(11), 6679-97, Yan *et al.,* Proc.Nat.Acad.Sci.USA, (1998), 95(7), 3603-8), DHFR promoter (Shimada *et al.,* J. Biol. Chem., (1986), 261 (3), 1 445-52, Shimada and Nienhuis, J. Biol.

Chem., (1985), 260(4), 2468-74), histone promoters (van Wijnen *et al.,* Proc.Nat.Acad.Sci.USA, (1994), 91, 1 2882- 1 2886)

[00524] In some embodiments, cell cycle-specific IRES elements are also used in the present disclosure. Examples of cell cycle-specific IRES elements include, *e.g.*, G2-IRES (Cornelis *et al.,* Mol. Cell, (2000), 5(4), 597-605); HCV IRES (Honda *et al.*, Gastroenterology, (2000), 1 1 8, 1 52-1 62); ODC IRES (Pyronet *et al.,* Mol. Cell, (2000), 5, 607-61 6); c-myc IRES (Pyronnet *et al.,* Mol. Cell, (2000), 5(4), 607-1 6) and p58 PITSLRE IRES (Cornelis *et al.*, Mol. Cell, (2000), 5(4), 597-605).

4. Tissue- and Lineage-Specific Promoters

[00525] In some embodiments, the promoter is a spatially restricted promoter (*i.e.*, cell type specific promoter, tissue specific promoter, lineage specific promoter, etc.) such that in a multi-cellular organism, the promoter is active (*i.e.*, "ON") in a subset of specific cells. Spatially restricted promoters may also be referred to as enhancers, transcriptional control elements, control sequences, etc. Any convenient spatially restricted promoter may be used and the choice of suitable promoter will depend on the organism. For example, various spatially restricted promoters are known for plants, flies, worms, mammals, mice, etc. Thus, a spatially restricted promoter can be used to regulate the expression of a nucleic acid encoding a subject site-directed modifying polypeptide in a wide variety of different tissues and cell types, depending on the organism. Some spatially restricted promoters are also temporally restricted such that the promoter is in the "ON" state or "OFF" state during specific stages of embryonic development or during specific stages of a biological process (*e.g.*, hair follicle cycle in mice).

[00526] In certain embodiments, expression of the transgene may be under the control of a promoter that preferentially initiates transcription in certain lineages, such as respiratory, prostatic, pancreatic, mammary, renal, intestinal, neural, skeletal, vascular, hepatic, hematopoietic, muscle or cardiac cell lineages. Examples of lineage-specific promoter include, but not limited to, Sox-2 promoter (neural progenitor cell specific, see US Patent No. 7,781,214), myosin light chain 2 promoter (cardiac-specific, see Huber I *et al.,* FASEB J 2007, 21:2551-63), aMHC promoter (cardiac-specific, see Kita-Matsuo H, PLoS One 2009, 4:e5046; Ritner C *et al.,* PLoS One 2011, 6:el6004), Hb9 promoter (motor neurons specific, see Singh *et al.*, Exp Neurol 2005, 196:224-34), Dazl promoter (germ cell specific, see Nicholas CR *et al.*, Genesis 2009,

47:74- 84), albumin promoter (hepatocyte specific, see Lavon N *et al.*, Differentiation 2004, 72:230- 238), and Pdxl promoter (pancreatic progenitor specific, *see*, Lavon N *et al.*, Stem Cells 2006, 24: 1923-1930).

[00527] In certain embodiments, expression of the transgene may be under the control of a tissue specific promoter, such as a promoter that is specific for: liver, pancreas (exocrine or endocrine portions), spleen, esophagus, stomach, large or small intestine, colon, GI tract, heart, lung, kidney, thymus, parathyroid, pineal gland, pituitary gland, mammary gland, salivary gland, ovary, uterus, cervix (*e.g.*, neck portion), prostate, testis, germ cell, ear, eye, brain, retina, cerebellum, cerebrum, PNS or CNS, placenta, adrenal cortex or medulla, skin, lymph node, muscle, fat, bone, cartilage, synovium, bone marrow, epithelial, endothelial, vascular, nervous tissues, etc. The tissue specific promoter may also be specific for certain disease tissues, such as cancers. *See*, Fukazawa *et al.*, Cancer Research 64: 363-369, 2004 (incorporated herein by reference).

[00528] Any tissue specific promoters known in the art can be used in the instant invention. Merely to illustrate, Chen *et al.* (Nucleic Acid Research, Vol. 34, database issue, pages D104-D107, 2006) described TiProD, the Tissue-specific Promoter Database (incorporated herein by reference). Specifically, TiProD is a database of human promoter sequences for which some functional features are known. It allows a user to query individual promoters and the expression pattern they mediate, gene expression signatures of individual tissues, and to retrieve sets of promoters according to their tissue-specific activity or according to individual Gene Ontology terms the corresponding genes are assigned to. The database has defined a measure for tissue-specificity that allows the user to discriminate between ubiquitously and specifically expressed genes. The database is accessible at tiprod.cbi.pku dot edu.cn:8080/index.html. It covers most (if not all) the tissues described above. Other promoters that can be used include promoters as disclosed online at <biobase/de/pages/ρroducts/transpor html>, which is a database with over 15,000 different promoter sequences classified by genes/activity.

[00529] Examples of cardiac cell-specific promoters include, *e.g.*, α-myosin heavy chain promoter, AE3 promoter, Aplnr promoter, cardiac actin promoter, cardiac troponin C promoter, desmin (DES) promoter, muscle creatine kinase (MCK) promoter, optionally with an MCK or a cardiac troponin-T enhancer, myosin light chain-2 promoter, Nfatc1 promoter, and Npr3

promoter (Franz *et al.* (1997) Cardiovasc. Res. 35:560-566; Robbins *et al.* (1995) Ann. N.Y. Acad. Sci. 752:492-505; Linn *et al.* (1995) Circ. Res. 76:584-591; Parmacek *et al.* (1994) Mol. Cell. Biol. 14:1870-1885; Hunter *et al.* (1993) Hypertension 22:608-617; and Sartorelli *et al.* (1992) Proc. Natl. Acad. Sci. USA 89:4047-4051).

[00530] Examples of muscle cell-specific promoters include, *e.g.*, smooth muscle α-actin (SMA) promoter, SM-myosin heavy chain promoter, calponin-h1 promoter, SM22α promoter, vascular alpha-actin promoter, enteric gamma-actin promoter, skeletal-alpha actin (SkA) promoter, mammalian muscle creatine kinase (MCK) promoter, mammalian desmin (DES) promoter, mammalian troponin I (TNNI2) promoter, and mammalian skeletal alpha-actin (ASKA) promoter.

[00531] Examples of neural cell-specific promoters include, *e.g.*, *astrocytes*: glial fibrillary acidic protein (GFAP) promoter (Smith-Arica *et al.*, 2000; Lee *et al.*, 2008); *GABAergic neuron*: glutamic acid decarboxylase (GAD) promoter (Rasmussen *et al.*, 2007); *glutamatergic neuron*: phosphate-activated glutaminase (PAG) or vesicular glutamate transporter (vGLUT) promoter (Rasmussen *et al.*, 2007); *microglial cells*: F4/80 promoter, CD68 promoter (Rosario *et al.*, 2016); *neurons*: synapsin-1 (Syn1) and neuron-specific enolase (NSE) promoter (Peel *et al.*, 1997; Kugler *et al.*, 2001; Kugler *et al.*, 2003; McLean *et al.*, 2014); *oligodendrocytes*: myelin basic protein (MBP) (von Jonquieres *et al.*, 2013) or human myelin associated glycoprotein (MAG) promoter, the latter in both a full-length and a truncated version (von Jonquieres *et al.*, 2016). Other examples of neural cell-specific promoters include, *e.g.*, aromatic amino acid decarboxylase (AADC) promoter, Ca2+-calmodulin-dependent protein kinase II-alpha (CamKIIα) promoter (*see*, *e.g.*, Mayford *et al.* (1996) Proc. Natl. Acad. Sci. USA 93:13250; and Casanova *et al.* (2001) Genesis 31:37), CMV enhancer/platelet-derived growth factor-β promoter (see, *e.g.*, Liu *et al.* (2004) Gene Therapy 11:52-60), DAT promoter, DNMT promoter (see, *e.g.*, Bartge *et al.* (1988) Proc. Natl. Acad. Sci. USA 85:3648-3652), enkephalin promoter (see, *e.g.*, Comb *et al.* (1988) EMBO J. 17:3793-3805), ENO2 promoter, GnRH promoter (see, *e.g.*, Radovick *et al.* (1991) Proc. Natl. Acad. Sci. USA 88:3402-3406), L7 promoter (see, *e.g.*, Oberdick *et al.* (1990) Science 248:223-226), MAP2 promoter, neurofilament light-chain gene promoter (Piccioli *et al.*, 1991, Proc. Natl. Acad. Sci. USA, 88:5611-5 (1991)), neurofilament promoter, NURR1 promoter, PITX3 promoter, S100 promoter, serotonin receptor promoter (see, *e.g.*, GenBank S62283), Synapsin promoter, Tau promoter, thy-1 promoter (*see*, *e.g.*, Chen *et al.*

(1987) Cell 51:7-19; and Llewellyn, *et al.* (2010) Nat. Med. 16(10):1161-1166), TUBA1A promoter, TUJ1 promoter, tyrosine hydroxylase promoter (TH) (see, *e.g.*, Oh *et al.* (2009) Gene Ther 16:437; Sasaoka *et al.* (1992) Mol. Brain Res. 16:274; Boundy *et al.* (1998) J. Neurosci. 18:9989; and Kaneda *et al.* (1991) Neuron 6:583-594), VGF promoter (Piccioli *et al.*, Neuron 15:373-84 (1995), and VMAT2 promoter.

[00532] Examples of glial progenitor cell-specific promters include, *e.g.*, A2B5 promoter, BLBP promoter, brain derived neurotrophic factor BDNF promoter, CD105 promoter, CD11b promoter, CD11c promoter, CD133 promoter, CD140a promoter, CD45 promoter, CD9 promoter, ciliary neurotrophic factor CNTF promoter, connexin 43 promoter, CX3CR1 promoter, EGFR promoter, epidermal growth factor EGF promoter, FGF8 promoter, FOXG1 promoter, GalC promoter, GAP-43 promoter, GD3 promoter, GLAST, glutamine synthetase promoter, IBA-1 promoter, LNGFR promoter, MBP promoter, Musashi promoter, nerve growth factor NGF promoter, nestin promoter, neutrotrophin-3 NT-3 promoter, NG2 promoter, NKX2.2 promoter, NT-4 promoter, O4 promoter, OLIG1 promoter, OLIG2 promoter, P2RY12 promoter, PAX6 promoter, PDGFαR promoter, S100β promoter, SOX10 promoter, TMEM119 promoter, and vimentin promoter.

[00533] Other examples of neural-specific promoters include, *e.g.*, 2',3'-cyclic-nucleotide 3'-phosphodiesterase CNP promoter, Ach promoter, ASCL1 promoter, beta-tubulin promoter, calbindin promoter, c-fos promoter, ChAT promoter, corin promoter, CRF promoter, CTIP2 promoter, diaminobenzidine (DAB) promoter, DLX1 promoter, DLX2 promoter, DLX5 promoter, DLX6 promoter, dopamine transporter (DAT) promoter, doublecortin promoter, EMX2p75 promoter, Forkhead box protein A2 FOXA2 promoter, Forkhead box protein O1 FOXO1 promoter, Forkhead box protein O4 FOXO4 promoter, FOX3 promoter, FOXG1 promoter, G protein-activated inward rectifier potassium channel 2 (GIRK2) promoter, gamma-aminobutyric acid GABA promoter, glutamate decarboxylase 1 GAD1 promoter, glutamate ionotropic receptor NMDA type subunit 1 GRIN1 promoter, hypocretin promoter, insulin gene enhancer protein (Isl1) promoter, LHX6 promoter, LHX8 promoter, LIM homeobox transcription factor 1-alpha LMX1A promoter, LIM homeobox transcription factor l-beta (LMX1B) promoter, AFB promoter, MAP2 promoter, microtubule-associated protein 2 (MAP-2) promoter, myelin basic protein MBP promoter, NADPH promoter, nestin promoter, NGF promoter, NGFI-B promoter, NKX2.1 promoter, NKX2.2 promoter, NKX6.2 promoter, NPAS1

promoter, Nurr1 promoter, NURR1 promoter, OLIG2 promoter, paired box protein (Pax6) promoter, PAX6 promoter, POMC promoter, PV promoter, RAX promoter, SATB2 promoter, SIX6 promoter, solute carrier family 1 member 6 SLC1A6 promoter, SOX6 promoter, SST promoter, TBR1 promoter, TH promoter, tubulin beta chain 3 NEUN promoter, tubulin beta chain 3 TUB3 promoter, TuJ1 promoter, VAChT promoter, and VGLUT1 promoter.

[00534] Examples of endothelial cell-specific promoters include, *e.g.*, *angiogenic-specific*: Esm1, Apelin; *arterial-specific*: Sox17 promoter, Bmx promoter. Other examples of endothelial cell-specific promoters include, *e.g.*, cadherin 5 (Cdh5, also known as vascular endothelial cadherin) promoter, endothelial cell protein C binding protein (EPCR) promoter, Fabp4 promoter, Kdr (Flk1/VEGFR2) promoter, Platelet-derived growth factor B (PDGFB) promoter, Tek/Tie2 promoter, keratin promoter in the case of keratinocytes, probasin promoter in the case of prostatic epithelium, and VE cadherin promoter.

[00535] Examples of cerebral endothelial cell-specific promoters include, *e.g.*, advanced glycation endproduct-specific receptor AGER promoter, and multidrug resistance-associated protein 5 ABCC5, ATP-ABCC2 binding cassette transporter ABCG2 promoter, ATP-dependent translocase ABCB1 promoter, basal cell adhesion molecule BCAM promoter, canalicular multispecific organic anion transporter 1 ABCC2 promoter, CD117 (c-kit) promoter, CD146 promoter, CD31 promoter, CD34 promoter, CD45 promoter, claudin-5 promoter, CXCR4 promoter, eNOS promoter, excitatory amino acid transporter 3 SLC1A1 promoter, GLUT-1 promoter, insulin receptor INSR promoter, large neutral amino acids transporter small subunit 1 SLC7A5 promoter, leptin receptor LEPR promoter, low density lipoprotein receptor LDLR promoter, low density lipoprotein receptor-related protein 1 LRP1 promoter, multidrug resistance-associated protein 1 ABCC1 promoter, multidrug resistance-associated protein 4 ABCC4 promoter, occludin promoter, PDGF promoter, PECAM-1 promoter, p-glycoprotein promoter, receptor for retinol uptake STRA6 promoter, SDF-1 promoter, sodium-coupled neutral amino acid transporter 5 SLC38A5 promoter, solute carrier family 16 member 1 SLC16A1 promoter, transferrin receptor TFRC promoter, VE cadherin promoter, VE-cadherin promoter, VEGF promoter, von Willebrand factor promoter, and ZO-1 promoter

[00536] Examples of pancreatic islet cell-specific promoters include, *e.g.*, RIP promoter, *islet progenitors*: Pdx1 promoter (NT-009799), Neurogenin 3 promoter (NT—008583), NeuroD1

promoter (NT—005265), Nestin promoter (NT-004858), and Ptf1a-p48 promoter (NT—008705), Pax6, Insm1, Nkx2-2 promoters; *mature islet cells*: insulin promoter (GenBank Accession NT—009308), glucagon promoter (NT-022154), somatostatin promoter (NT—005962), and pancreatic polypeptide promoter (NT-010755), MafB, MafA, Pcsk1, Iapp, G6pc2, and Ins1 promoters.

[00537] Examples of retinal pigmented epithelium cell-specific promoters include, *e.g.*, beta phosphodiesterase gene promoter (Nicoud *et al.*, (2007) J. Gene Med. 9:1015), cone opsin promoter (COP), interphotoreceptor retinoid-binding protein (IRBP) gene enhancer (Nicoud *et al.* (2007)), IRBP gene promoter (Yokoyama *et al.* (1992) Exp Eye Res. 55:225), red/green opsin promoter (COP), retinitis pigmentosa gene promoter (Nicoud *et al.*, (2007) supra), rhodopsin kinase promoter (Young *et al.* (2003) Ophthalmol. Vis. Sci. 44:4076), rhodopsin (ROD) promoter, thymocyte antigen (Thy1.2, 6500 bp) promoter, and vitelliform macular dystrophy (VMD2) promoter.

[00538] Examples of hepatocyte-specific promoters include, *e.g.*, albumin, Miyatake *et al.* J Virol, 71:5124-32 (1997), alpha-fetoprotein (AFP), Arbuthnot *et al.*, Hum. Gene Ther, 7:1503-14 (1996), hepatitis B virus core promoter, Sandig *et al.*, Gene Ther., 3:1002-9 (1996), human alpha-1 anti-trypsin (hAAT) promoter, promoter of the fatty acid binding intestinal protein, thyroxine binding globulin (TBG) promoter, Apo AI and Apo AII promoters, alpha 1-antitrypsin promoter, and transthyretin promoter (Quian *et al.*, (1995) Mol Cell Biol 15, 1364-1376; Bristol J A, Gallo-Penn A, Andrews J, Idamakanti N, Kaleko M, Connelly S. (2001) Hum Gene Ther vol 12(13):1651-61).

[00539] Examples of thyroid cell-specific promoters include, *e.g.*, thyroglobulin (Tg) promoter, thyroperoxidase (TPO) promoter, and TSH receptor (TSHr) promoter.

[00540] Examples of T cell-specific promoters include, *e.g.*, CD2 promoter (Hansal *et al.*, J Immunol, 161:1063-8 (1998), immunoglobulin heavy chain promoter, and T cell receptor a chain promoter.

[00541] Examples of cancer cell-specific promoters include, *e.g.*, tyrosinase promoter or a TRP2 promoter in the case of melanoma cells and melanocytes, MMTV or WAP promoter in the case of breast cells and/or cancers, villin or FABP promoter in the case of intestinal cells and/or

cancers, nestin or GFAP promoter in the case of CNS cells and/or cancers, and Clara cell secretory protein promoter in the case of lung cancer.

5. Differentiation-Specific Promoters

[00542] Any differentiation-specific promoter known in the art can be used in the present disclosure . Examples of differentiation-specific promoters include those described above. Another method of tissue-specific expression includes the "BAC TG-EMBED" method for copy-number dependent, position-independent transgene expression even after induced quiescence and/or cell differentiation into multiple cell types, *e.g.*, using a GAPDH BAC containing ~200 kb of the human GAPDH gene locus and a 1.2 kb human UBC promoter (Chaturvedi, *et al.*, Gene Ther 25, 376–391 (2018)).

6. Transcriptional Regulatory Domains:

[00543] Any transcriptional regulatory domains known in the art can be used in the present disclosure . Common domains include, *e.g.*, transcription factor domains (activators, repressors, co-activators, co-repressors), silencers, oncogenes (*e.g.*, myc, jun, fos, myb, max, mad, rel, ets, bcl, myb, mos family members etc.); DNA repair enzymes and their associated factors and modifiers; DNA rearrangement enzymes and their associated factors and modifiers; chromatin associated proteins and their modifiers (*e.g.* kinases, acetylases and deacetylases); and DNA modifying enzymes (*e.g.*, methyltransferases such as members of the DNMT family (*e.g.*, DNMT1, DNMT3A, DNMT3B, DNMT3L, etc., topoisomerases, helicases, ligases, kinases, phosphatases, polymerases, endonucleases) and their associated factors and modifiers. See, *e.g.*, U.S. Publication No. 2013/0253040, incorporated by reference in its entirety herein.

[00544] Any transcriptional activator known in the art can be used in the present disclosure . *Examples of transcriptional activators include, e.g.,* HSV VP 16 activation domain (*see*, *e.g.*, Hagmann *et al.*, J. Virol. 71, 5952-5962 (1 97)) nuclear hormone receptors (see, *e.g.*, Torchia *et al.*, Curr. Opin. Cell. Biol. 10:373-383 (1998)); the p65 subunit of nuclear factor kappa B (Bitko & Bank, J. Virol. 72:5610-5618 (1998) and Doyle & Hunt, Neuroreport 8:2937-2942 (1997)); Liu *et al.*, Cancer Gene Ther. 5:3-28 (1998)), or artificial chimeric functional domains such as VP64 (Beerli *et al.*, (1998) Proc. Natl. Acad. Sci. USA 95:14623-33), and degron (Molinari *et al.*, (1999) EMBO J. 18, 6439-6447). Additional exemplary activation domains include, Oct 1, Oct-2A, Spl, AP-2, and CTF1 (Seipel *et al.*, EMBOJ. 11, 4961-4968 (1992) as well as p300,

CBP, PCAF, SRC1 PvALF, AtHD2A and ERF-2. See, for example, Robyr *et al.,* (2000) Mol. Endocrinol. 14:329-347; Collingwood *et al.,* (1999) J. Mol. Endocrinol 23:255-275; Leo *et al.,* (2000) Gene 245:1-11; Manteuffel-Cymborowska (1999) Acta Biochim. Pol. 46:77-89; McKenna *et al.,* (1999) J. Steroid Biochem. Mol. Biol. 69:3-12; Malik *et al.,* (2000) Trends Biochem. Sci. 25:277-283; and Lemon *et al.,* (1999) Curr. Opin. Genet. Dev. 9:499-504. Additional exemplary activation domains include, but are not limited to, OsGAI, HALF-1, Cl, AP1, ARF-5, -6,-1, and -8, CPRF1, CPRF4, MYC-RP/GP, and TRAB1 , See, for example, Ogawa *et al.,* (2000) Gene 245:21-29; Okanami *et al.,* (1996) Genes Cells 1 :87-99; Goff *et al.,* (1991) Genes Dev. 5:298-309; Cho *et al.,* (1999) Plant Mol Biol 40:419-429; Ulmason *et al.,* (1999) Proc. Natl. Acad. Sci. USA 96:5844-5849; Sprenger-Haussels *et al.,* (2000) Plant J. 22:1-8; Gong *et al.,* (1999) Plant Mol. Biol. 41:33-44; and Hobo *et al.* , (1999) Proc. Natl. Acad. Sci. USA 96:15,348-15,353.

[00545] Any enhancer known in the art can be used in the present disclosure . Examples of enhancers include, *e.g.*, CMV enhancer (eCMV), RSV enhancer, and SV40 enhancer.

[00546] Any insulator element known in the art can be used in the present disclosure . Examples of insulator elements include, *e.g.*, cHS4 (Chung *et al.*, 1993), and ubiquitous chromatin opening element (UCOE) derived from the human HNRPA2B1-CBX3 locus (A2UCOE).

[00547] Any histone acetyltransferase (HAT) known in the art can be used in the present disclosure . Examples of HATs include, *e.g.*, type- A, nuclear localized such as MYST family members MOZ, Ybf2/Sas3, MOF, and Tip60, GNAT family members Gcn5 or pCAF, the p300 family members CBP, p300 and Rttl09 (Bemdsen and Denu (2008) Curr Opin Struct Biol l8(6):682-689).

[00548] Any histone deacetylase (HDAC) known in the art can be used in the present disclosure . Examples of HDACs include, *e.g.*, class I (HDAC-l, 2, 3, and 8), class II (HDAC IIA (HDAC-4, 5, 7 and 9), HD AC IIB (HDAC 6 and 10)), class IV (HDAC-l 1), and class III (also known as sirtuins (SIRTs); SIRT1-7) (see Mottamal *et al.*, (2015) Molecules 20(3):3898-394l).

[00549] Any histone phosphorylase or kinase known in the art can be used in the present disclosure . Examples of histone phosphorylases or kinases include, *e.g.*, MSK1, MSK2, ATR, ATM, DNA-PK, Bubl, VprBP, IKK-a, PKCpi, Dik/Zip, JAK2, PKC5, WSTF and CK2.

[00550] Any methylation domain known in the art can be used in the present disclosure . Examples of methylation domains include, *e.g.*, Ezh2, PRMT1/6, PRMT5/7, PRMT 2/6, CARM1, set7/9, MLL, ALL-1, Suv 39h, G9a, SETDB1, Ezh2, Set2, Dotl, PRMT 1/6, PRMT 5/7, PR-Set7 and Suv4-20h.

[00551] Any domains involved in sumoylation or biotinylation known in the art can be used in the present disclosure . Examples of domains involved in sumoylation or biotinylation include, *e.g.*, Lys9, 13, 4, 18 and 12 (review see Kousarides (2007) Cell 128:693-705).

[00552] Any post-transcriptional regulatory element known in the art can be used in the present disclosure . Examples of post-transcriptional regulatory elements include, *e.g.*, hepatitis B virus (HBV) post-transcriptional regulatory element (PRE) (HPRE) (Huang, Z. M. and Yen, T. S. (1995) Mol. Cell. Biol. 15: 3864-3869), and Woodchuck hepatitis virus (WHV) PRE (WPRE) (U.S. Pat. Nos. 6,136,597 and 6,287,814).

7. Other Methods to Reverse Transgene Silencing

[00553] Any method known in the art can be used to regulatably overexpress transgenes, *e.g.*, CD47. Examples of methods that can be used to reverse transgene silencing and regulatably overexpress transgenes include, *e.g.*, employment of cytoplasmic-only (non-nuclear) vectors (non-viral mRNA vectors or positive strand RNA-based viral vectors such as Sendai virus based vectors); CpG ablation, CpG depletion and minimized DNA vectors; multiple transgene insertions into random chromosomal sites; site-specific chromosomal integration; episomal localization of a transgene (compact episomal replicons from SV40, polyoma, papilloma viruses; EBNA1-oriP DNA segment of Epstein-Barr Virus (EBV) can be used to support the maintenance of plasmid gene vectors in the nucleoplasm of dividing laboratory cells); employment of the locus control regions within gene therapy vectors (chromatin insulators or other cis-acting locus control regions (LCRs)); repeated vector administration to compensate for silenced transgenes; small molecule enhancers of transgene expression (*e.g.*, substances known to influence chromatin's state, such as histone deacetylase inhibitors Trichostatin A, 4-phenylbutyric acid, butyric acid, valeric acid, caproic acid, valproic acid, and retinoic acid; small molecule enhancers specific for particular vectors for gene transfer, *e.g.*, hydroxyurea is known to boost transgene expression after delivery with AAV vectors); and post-translational regulatory

elements. See, *e.g.*, Tolmachov *et al.* (2013). Silencing of Transgene Expression: A Gene Therapy Perspective. 10.5772/53379.

8. Timing of Regulatable Reduced or Overexpression

[00554] In some embodiments, regulatable reduced expression of a target gene includes regulatable reduced expression, such as regulatable knock out or knock down, of B2M, CIITA, NLRC5, TRAC, TRB, and/or RHD. The regulatable reduced expression can be initiated at any point during the engineering and differentiation of the cell. For example, the regulatable reduced expression can be initiated, introduced, or induced at any point between day 1, *e.g.* the day the primary cell is isolated or the day the cell becomes an iPSC cell, and the day the cell is terminally differentiated.

[00555] In some embodiments, regulatable overexpression of a transgene includes regulatable overexpression of an exogenous polynucleotide, such as regulatable overexpression of an exogenous transgene encoding one or more tolerogenic factors. The regulatable overexpression can be initiated at any point during the engineering and differentiation of the cell. For example, the regulatable overexpression can be initiated, introduced, or induced at any point between day 1, *e.g.* the day the primary cell is isolated or the day the cell becomes an iPSC cell, and the day the cell is terminally differentiated.

E. CIITA

[00556] In some embodiments, the present disclosure regulatably modulates (*e.g.*, reduces or eliminates) the expression of one or more MHC II genes by regulatably targeting and modulating (*e.g.*, reducing or eliminating) Class II transactivator (CIITA) expression. In some embodiments, the modulation occurs using a gene editing system (*e.g.* CRISPR/Cas). In some embodiments, the modulation occurs using an RNA-based component selected from the group consisting of conditional or inducible shRNAs, conditional or inducible siRNAs, conditional or inducible miRNAs, and conditional or inducible CRISPR interference (CRISPRi). In some embodiments, the modulation occurs using a DNA-based component selected from the group consisting of a knock out using a method selected from the group consisting of conditional or inducible CRISPRs, conditional or inducible TALENs, conditional or inducible zinc finger nucleases, conditional or inducible homing endonucleases, and conditional or inducible meganucleases. In

some embodiments, the modulation occurs using a protein-based component that is a conditional or inducible degron method.

[00557] CIITA is a member of the LR or nucleotide binding domain (NBD) leucine-rich repeat (LRR) family of proteins and regulates the transcription of MHC II by associating with the MHC enhanceosome.

[00558] In some embodiments, the target polynucleotide sequence of the present disclosure is a variant of CIITA. In some embodiments, the target polynucleotide sequence is a homolog of CIITA. In some embodiments, the target polynucleotide sequence is an ortholog of CIITA.

[00559] In some embodiments, reduced or eliminated expression of CIITA reduces or eliminates expression of one or more of the following MHC class II are HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, and HLA-DR.

[00560] In some embodiments, the cells described herein comprise regulatable gene modifications at the gene locus encoding the CIITA protein. In other words, the cells comprise a regulatable genetic modification at the CIITA locus. In some instances, the nucleotide sequence encoding the CIITA protein is set forth in RefSeq. No. NM_000246.4 and NCBI Genbank No. U18259. In some instances, the CIITA gene locus is described in NCBI Gene ID No. 4261. In certain cases, the amino acid sequence of CIITA is depicted as NCBI GenBank No. AAA88861.1. Additional descriptions of the CIITA protein and gene locus can be found in Uniprot No. P33076, HGNC Ref. No. 7067, and OMIM Ref. No. 600005.

[00561] In some embodiments, the hypoimmunogenic cells outlined herein comprise a regulatable genetic modification targeting the CIITA gene. In some embodiments, the regulatable genetic modification targeting the CIITA gene is by way of a regulatable rare-cutting endonuclease comprising a regulatable Cas protein or a regulatable polynucleotide encoding a Cas protein, and at least one guide ribonucleic acid sequence for specifically targeting the CIITA gene. In some embodiments, the at least one guide ribonucleic acid sequence for specifically targeting the CIITA gene is selected from the group consisting of SEQ ID NOS:5184-36352 of Table 12 of WO2016183041, which is herein incorporated by reference. In some embodiments, the cell has a reduced ability to induce an innate and/or an adaptive immune response in a recipient subject. In some embodiments, an exogenous nucleic acid encoding a polypeptide as disclosed herein (*e.g.*, a chimeric antigen receptor, CD47, or another tolerogenic factor disclosed herein) is inserted at the CIITA gene.

[00562] In some embodiments, the hypoimmunogenic cells outlined herein comprise regulatable knock out of CIITA expression, such that the cells are regulatably *CIITA*$^{-/-}$. In some embodiments, the hypoimmunogenic cells outlined herein regulatably introduce an indel into the CIITA gene locus, such that the cells are regulatably *CIITA*$^{indel/indel}$. In some embodiments, the hypoimmunogenic cells outlined herein comprise regulatable knock down of CIITA expression, such that the cells are regulatably *CIITA*$^{knock\ down}$.

[00563] Assays to test whether the CIITA gene has been inactivated are known and described herein. In some embodiments, the resulting genetic modification of the CIITA gene by PCR and the reduction of HLA-II expression can be assays by FACS analysis. In another embodiment, CIITA protein expression is detected using a Western blot of cells lysates probed with antibodies to the CIITA protein. In another embodiment, reverse transcriptase polymerase chain reactions (RT-PCR) are used to confirm the presence of the inactivating genetic modification.

F. B2M

[00564] In some embodiments, the technology disclosed herein regulatably modulates (*e.g.*, reduces or eliminates) the expression of one or more MHC-I genes by regulatably targeting and modulating (*e.g.*, reducing or eliminating) expression of the accessory chain B2M. In some embodiments, the modulation occurs using a gene editing system (*e.g.* CRISPR/Cas). In some embodiments, the modulation occurs using an RNA-based component selected from the group consisting of conditional or inducible shRNAs, conditional or inducible siRNAs, conditional or inducible miRNAs, and conditional or inducible CRISPR interference (CRISPRi). In some embodiments, the modulation occurs using a DNA-based component selected from the group consisting of a knock out or knock down using a method selected from the group consisting of conditional or inducible CRISPRs, conditional or inducible TALENs, conditional or inducible zinc finger nucleases, conditional or inducible homing endonucleases, and conditional or inducible meganucleases. In some embodiments, the modulation occurs using a protein-based component that is a conditional or inducible degron method.

[00565] By modulating (*e.g.*, reducing or deleting) expression of B2M, surface trafficking of MHC-I molecules is blocked and the cell rendered hypoimmunogenic. In some embodiments, the cell has a reduced ability to induce an innate and/or an adaptive immune response in a recipient subject.

[00566] In some embodiments, the target polynucleotide sequence of the present disclosure is a variant of B2M. In some embodiments, the target polynucleotide sequence is a homolog of B2M. In some embodiments, the target polynucleotide sequence is an ortholog of B2M.

[00567] In some embodiments, decreased or eliminated expression of B2M reduces or eliminates expression of one or more of the following MHC I molecules: HLA-A, HLA-B, and HLA-C.

[00568] In some embodiments, the cells described herein comprise regulatable gene modifications at the gene locus encoding the B2M protein. In other words, the cells comprise a regulatable genetic modification at the B2M locus. In some instances, the nucleotide sequence encoding the B2M protein is set forth in RefSeq. No. NM_004048.4 and Genbank No. AB021288.1. In some instances, the B2M gene locus is described in NCBI Gene ID No. 567. In certain cases, the amino acid sequence of B2M is depicted as NCBI GenBank No. BAA35182.1. Additional descriptions of the B2M protein and gene locus can be found in Uniprot No. P61769, HGNC Ref. No. 914, and OMIM Ref. No. 109700.

[00569] In some embodiments, the hypoimmunogenic cells outlined herein comprise a regulatable genetic modification targeting the B2M gene. In some embodiments, the regulatable genetic modification targeting the B2M gene is by way of a regulatable rare-cutting endonuclease comprising a regulatable Cas protein or a regulatable polynucleotide encoding a Cas protein, and at least one guide ribonucleic acid sequence for specifically targeting the B2M gene. In some embodiments, the at least one guide ribonucleic acid sequence for specifically targeting the B2M gene is selected from the group consisting of SEQ ID NOS:81240-85644 of Table 15 of WO2016183041, which is herein incorporated by reference. In some embodiments, an exogenous nucleic acid encoding a polypeptide as disclosed herein (*e.g.*, a chimeric antigen receptor, CD47, or another tolerogenic factor disclosed herein) is inserted at the B2M gene.

[00570] Assays to test whether the B2M gene has been inactivated are known and described herein. In some embodiments, the resulting genetic modification of the B2M gene by PCR and the reduction of HLA-I expression can be assays by FACS analysis. In another embodiment, B2M protein expression is detected using a Western blot of cells lysates probed with antibodies to the B2M protein. In another embodiment, reverse transcriptase polymerase chain reactions (RT-PCR) are used to confirm the presence of the inactivating genetic modification.

[00571] In some embodiments, the hypoimmunogenic cells outlined herein comprise regulatable knock out of B2M expression, such that the cells are regulatably $B2M^{-/-}$. In some embodiments, the hypoimmunogenic cells outlined herein regulatably introduce an indel into the B2M gene locus, such that the cells are regulatably $B2M^{indel/indel}$. In some embodiments, the hypoimmunogenic cells outlined herein comprise regulatable knock down of B2M expression, such that the cells are regulatably $B2M^{knock\ down}$.

G. NLRC5

[00572] In certain embodiments, the technology disclosed herein regulatably modulate (*e.g.*, reduces or eliminates) the expression of one or more MHC-I genes by regulatably targeting and modulating (*e.g.*, reducing or eliminating) expression of the NLR family, CARD domain containing 5/NOD27/CLR16.1 (NLRC5). In some embodiments, the modulation occurs using a gene editing system (*e.g.* CRISPR/Cas). In some embodiments, the modulation occurs using an RNA-based component selected from the group consisting of conditional or inducible shRNAs, conditional or inducible siRNAs, conditional or inducible miRNAs, and conditional or inducible CRISPR interference (CRISPRi). In some embodiments, the modulation occurs using a DNA-based component selected from the group consisting of a knock out or knock down using a method selected from the group consisting of conditional or inducible CRISPRs, conditional or inducible TALENs, conditional or inducible zinc finger nucleases, conditional or inducible homing endonucleases, and conditional or inducible meganucleases. In some embodiments, the modulation occurs using a protein-based component that is a conditional or inducible degron method.

[00573] NLRC5 is a critical regulator of MHC-I-mediated immune responses and, similar to CIITA, NLRC5 is highly inducible by IFN-γ and can translocate into the nucleus. NLRC5 activates the promoters of MHC-I genes and induces the transcription of MHC-I as well as related genes involved in MHC-I antigen presentation.

[00574] In some embodiments, the target polynucleotide sequence is a variant of NLRC5. In some embodiments, the target polynucleotide sequence is a homolog of NLRC5. In some embodiments, the target polynucleotide sequence is an ortholog of NLRC5.

[00575] In some embodiments, decreased or eliminated expression of NLRC5 reduces or eliminates expression of one or more of the following MHC I molecules – HLA-A, HLA-B, and HLA-C.

[00576] In some embodiments, the cells outlined herein comprise a regulatable genetic modification targeting the NLRC5 gene. In some embodiments, the regulatable genetic modification targeting the NLRC5 gene is by way of a regulatable rare-cutting endonuclease comprising a regulatable Cas protein or a regulatable polynucleotide encoding a Cas protein, and at least one guide ribonucleic acid sequence for specifically targeting the NLRC5 gene. In some embodiments, the at least one guide ribonucleic acid sequence for specifically targeting the NLRC5 gene is selected from the group consisting of SEQ ID NOS:36353-81239 of Appendix 3 or Table 14 of WO2016183041, the disclosure is incorporated by reference in its entirety.

[00577] Assays to test whether the NLRC5 gene has been inactivated are known and described herein. In some embodiments, the resulting genetic modification of the NLRC5 gene by PCR and the reduction of HLA-I expression can be assays by FACS analysis. In another embodiment, NLRC5 protein expression is detected using a Western blot of cells lysates probed with antibodies to the NLRC5 protein. In another embodiment, reverse transcriptase polymerase chain reactions (RT-PCR) are used to confirm the presence of the inactivating genetic modification.

[00578] In some embodiments, the hypoimmunogenic cells outlined herein comprise regulatable knock out of NLRC5 expression, such that the cells are regulatably *NLRC5*$^{-/-}$. In some embodiments, the hypoimmunogenic cells outlined herein regulatably introduce an indel into the NLRC5 gene locus, such that the cells are regulatably *NLRC5*$^{indel/indel}$. In some embodiments, the hypoimmunogenic cells outlined herein comprise regulatable knock down of NLRC5 expression, such that the cells are regulatably *NLRC5*$^{knock\ down}$.

H. TRAC

[00579] In certain embodiments, the technologies disclosed herein regulatably modulate (*e.g.*, reduce or eliminate) the expression of TCR genes including the TRAC gene by regulatably targeting and modulating (*e.g.*, reducing or eliminating) expression of the constant region of the T cell receptor alpha chain. In some embodiments, the modulation occurs using a gene editing system (*e.g.* CRISPR/Cas). In some embodiments, the modulation occurs using an RNA-based component selected from the group consisting of conditional or inducible shRNAs, conditional or inducible siRNAs, conditional or inducible miRNAs, and conditional or inducible CRISPR interference (CRISPRi). In some embodiments, the modulation occurs using a DNA-based component selected from the group consisting of a knock out or knock down using a method

selected from the group consisting of conditional or inducible CRISPRs, conditional or inducible TALENs, conditional or inducible zinc finger nucleases, conditional or inducible homing endonucleases, and conditional or inducible meganucleases. In some embodiments, the modulation occurs using a protein-based component that is a conditional or inducible degron method.

[00580] By modulating (*e.g.*, reducing or deleting) expression of TRAC, surface trafficking of TCR molecules is blocked. In some embodiments, the cell also has a reduced ability to induce an innate and/or an adaptive immune response in a recipient subject.

[00581] In some embodiments, the target polynucleotide sequence of the present disclosure is a variant of TRAC. In some embodiments, the target polynucleotide sequence is a homolog of TRAC. In some embodiments, the target polynucleotide sequence is an ortholog of TRAC.

[00582] In some embodiments, decreased or eliminated expression of TRAC reduces or eliminates TCR surface expression.

[00583] In some embodiments, the cells, such as, but not limited to, pluripotent stem cells, induced pluripotent stem cells, T cells differentiated from induced pluripotent stem cells, primary T cells, and cells derived from primary T cells comprise regulatable gene modifications at the gene locus encoding the TRAC protein. In other words, the cells comprise a regulatable genetic modification at the TRAC locus. In some instances, the nucleotide sequence encoding the TRAC protein is set forth in Genbank No. X02592.1. In some instances, the TRAC gene locus is described in RefSeq. No. NG_001332.3 and NCBI Gene ID No. 28755. In certain cases, the amino acid sequence of TRAC is depicted as Uniprot No. P01848. Additional descriptions of the TRAC protein and gene locus can be found in Uniprot No. P01848, HGNC Ref. No. 12029, and OMIM Ref. No. 186880.

[00584] In some embodiments, the hypoimmunogenic cells outlined herein comprise a regulatable genetic modification targeting the TRAC gene. In some embodiments, the regulatable genetic modification targeting the TRAC gene is by way of a regulatable rare-cutting endonuclease comprising a regulatable Cas protein or a regulatable polynucleotide encoding a Cas protein, and at least one guide ribonucleic acid sequence for specifically targeting the TRAC gene. In some embodiments, the at least one guide ribonucleic acid sequence for specifically targeting the TRAC gene is selected from the group consisting of SEQ ID NOS:532-609 and 9102-9797 of US20160348073, which is herein incorporated by reference.

[00585] Assays to test whether the TRAC gene has been inactivated are known and described herein. In some embodiments, the resulting genetic modification of the TRAC gene by PCR and the reduction of TCR expression can be assays by FACS analysis. In another embodiment, TRAC protein expression is detected using a Western blot of cells lysates probed with antibodies to the TRAC protein. In another embodiment, reverse transcriptase polymerase chain reactions (RT-PCR) are used to confirm the presence of the inactivating genetic modification.

[00586] In some embodiments, the hypoimmunogenic cells outlined herein comprise regulatable knock out of TRAC expression, such that the cells are regulatably $TRAC^{-/-}$. In some embodiments, the hypoimmunogenic cells outlined herein regulatably introduce an indel into the TRAC gene locus, such that the cells are regulatably $TRAC^{indel/indel}$. In some embodiments, the hypoimmunogenic cells outlined herein comprise regulatable knock down of TRAC expression, such that the cells are regulatably $TRAC^{knock\ down}$.

I. TRB

[00587] In many embodiments, the technologies disclosed herein regulatably modulate (*e.g.*, reduce or eliminate) the expression of TCR genes including the gene encoding T cell antigen receptor, beta chain (*e.g.*, the TRB, TRBC, or TCRB gene) by regulatably targeting and modulating (*e.g.*, reducing or eliminating) expression of the constant region of the T cell receptor beta chain. In some embodiments, the modulation occurs using a gene editing system (*e.g.* CRISPR/Cas). In some embodiments, the modulation occurs using an RNA-based component selected from the group consisting of conditional or inducible shRNAs, conditional or inducible siRNAs, conditional or inducible miRNAs, and conditional or inducible CRISPR interference (CRISPRi). In some embodiments, the modulation occurs using a DNA-based component selected from the group consisting of a knock out or knock down using a method selected from the group consisting of conditional or inducible CRISPRs, conditional or inducible TALENs, conditional or inducible zinc finger nucleases, conditional or inducible homing endonucleases, and conditional or inducible meganucleases. In some embodiments, the modulation occurs using a protein-based component that is a conditional or inducible degron method.

[00588] By modulating (*e.g.*, reducing or deleting) expression of TRB, surface trafficking of TCR molecules is blocked. In some embodiments, the cell also has a reduced ability to induce an innate and/or an adaptive immune response in a recipient subject.

[00589] In some embodiments, the target polynucleotide sequence of the present disclosure is a variant of TRB. In some embodiments, the target polynucleotide sequence is a homolog of TRB. In some embodiments, the target polynucleotide sequence is an ortholog of TRB.

[00590] In some embodiments, decreased or eliminated expression of TRB reduces or eliminates TCR surface expression.

[00591] In some embodiments, the cells, such as, but not limited to, pluripotent stem cells, induced pluripotent stem cells, T cells differentiated from induced pluripotent stem cells, primary T cells, and cells derived from primary T cells comprise regulatable gene modifications at the gene locus encoding the TRB protein. In other words, the cells comprise a regulatable genetic modification at the TRB gene locus. In some instances, the nucleotide sequence encoding the TRB protein is set forth in UniProt No. P0DSE2. In some instances, the TRB gene locus is described in RefSeq. No. NG_001333.2 and NCBI Gene ID No. 6957. In certain cases, the amino acid sequence of TRB is depicted as Uniprot No. P01848. Additional descriptions of the TRB protein and gene locus can be found in GenBank No. L36092.2, Uniprot No. P0DSE2, and HGNC Ref. No. 12155.

[00592] In some embodiments, the hypoimmunogenic cells outlined herein comprise a regulatable genetic modification targeting the TRB gene. In some embodiments, the regulatable genetic modification targeting the TRB gene is by way of a regulatable rare-cutting endonuclease comprising a regulatable Cas protein or a regulatable polynucleotide encoding a Cas protein, and at least one guide ribonucleic acid sequence for specifically targeting the TRB gene. In some embodiments, the at least one guide ribonucleic acid sequence for specifically targeting the TRB gene is selected from the group consisting of SEQ ID NOS:610-765 and 9798-10532 of US20160348073, which is herein incorporated by reference.

[00593] Assays to test whether the TRB gene has been inactivated are known and described herein. In some embodiments, the resulting genetic modification of the TRB gene by PCR and the reduction of TCR expression can be assays by FACS analysis. In another embodiment, TRB protein expression is detected using a Western blot of cells lysates probed with antibodies to the TRB protein. In another embodiment, reverse transcriptase polymerase chain reactions (RT-PCR) are used to confirm the presence of the inactivating genetic modification.

[00594] In some embodiments, the hypoimmunogenic cells outlined herein comprise regulatable knock out of TRB expression, such that the cells are regulatably *TRB*$^{-/-}$. In some

embodiments, the hypoimmunogenic cells outlined herein regulatably introduce an indel into the TRB gene locus, such that the cells are regulatably $TRB^{indel/indel}$. In some embodiments, the hypoimmunogenic cells outlined herein comprise regulatable knock down of TRB expression, such that the cells are regulatably $TRB^{knock\ down}$.

J. CD142

[00595] In certain embodiments, the technology disclosed herein modulate (*e.g.*, reduce or eliminate) the expression of CD142, which is also known as tissue factor, factor III, and F3. In some embodiments, the modulation occurs using a gene editing system (*e.g.* CRISPR/Cas).

[00596] In some embodiments, the target polynucleotide sequence is CD142 or a variant of CD142. In some embodiments, the target polynucleotide sequence is a homolog of CD142. In some embodiments, the target polynucleotide sequence is an ortholog of CD142.

[00597] In some embodiments, the cells outlined herein comprise a genetic modification targeting the CD142 gene. In some embodiments, the genetic modification targeting the CD142 gene by the rare-cutting endonuclease comprises a Cas protein or a polynucleotide encoding a Cas protein, and at least one guide ribonucleic acid (gRNA) sequence for specifically targeting the CD142 gene. Useful methods for identifying gRNA sequences to target CD142 are described below.

[00598] Assays to test whether the CD142 gene has been inactivated are known and described herein. In some embodiments, the resulting genetic modification of the CD142 gene by PCR and the reduction of CD142 expression can be assays by FACS analysis. In another embodiment, CD142 protein expression is detected using a Western blot of cells lysates probed with antibodies to the CD142 protein. In another embodiment, reverse transcriptase polymerase chain reactions (RT-PCR) are used to confirm the presence of the inactivating genetic modification.

[00599] Useful genomic, polynucleotide and polypeptide information about the human CD142 are provided in, for example, the GeneCard Identifier GC01M094530, HGNC No. 3541, NCBI Gene ID 2152, NCBI RefSeq Nos. NM_001178096.1, NM_001993.4, NP_001171567.1, and NP_001984.1, UniProt No. P13726, and the like.

K. RHD

[00600] In some embodiments, the technologies disclosed herein regulatably modulate (*e.g.*, reduce or eliminate) the expression of RhD antigen by regulatably targeting and modulating

(*e.g.*, reducing or eliminating) expression of the RHD gene. In some embodiments, the modulation occurs using a gene editing system (*e.g.* CRISPR/Cas). In some embodiments, the modulation occurs using an RNA-based component selected from the group consisting of conditional or inducible shRNAs, conditional or inducible siRNAs, conditional or inducible miRNAs, and conditional or inducible CRISPR interference (CRISPRi). In some embodiments, the modulation occurs using a DNA-based component selected from the group consisting of a knock out or knock down using a method selected from the group consisting of conditional or inducible CRISPRs, conditional or inducible TALENs, conditional or inducible zinc finger nucleases, conditional or inducible homing endonucleases, and conditional or inducible meganucleases. In some embodiments, the modulation occurs using a protein-based component that is a conditional or inducible degron method.

[00601] In some embodiments, the cell has a reduced ability to induce an innate and/or an adaptive immune response in a recipient subject.

[00602] In some embodiments, the target polynucleotide sequence of the present disclosure is a variant of RHD gene. In some embodiments, the target polynucleotide sequence is a homolog of RHD gene. In some embodiments, the target polynucleotide sequence is an ortholog of RHD gene.

[00603] In some embodiments, the cells described herein comprise gene regulatable modifications at the gene locus encoding the RhD antigen protein. In other words, the cells comprise a regulatable genetic modification at the RHD locus. In some instances, the nucleotide sequence encoding the RhD antigen protein is set forth in RefSeq. Nos. NM_001127691.2, NM_001282868.1, NM_001282869.1, NM_001282871.1, or NM_016124.4, or in Genbank No. L08429. in some instances, the RHD gene locus is described in NCBI Gene ID No.6007. In certain cases, the amino acid sequence of RhD antigen protein is depicted as NCBI GenBank No. AAA02679.1. Additional descriptions of the RhD protein and gene locus can be found in Uniprot No. Q02161, HGNC Ref. No. 10009, and OMIM Ref. No. 111680.

[00604] In some embodiments, the cells outlined herein comprise a regulatable genetic modification targeting the RHD gene. In some embodiments, the regulatable genetic modification targeting the RHD gene is generated by regulatably gene editing the RHD gene using regulatable gene editing tools such as but not limited to regulatable CRISPR/Cas, regulatable TALE- nucleases, regulatable zinc finger nucleases, other regulatable viral based

gene editing system, or regulatable RNA interference. In some embodiments, the gene editing targets the coding sequence of the RHD gene. In some instances, the cells do not generate a functional RHD gene product. In the absence of the RHD gene product, the cells completely lack an Rh blood group antigen.

[00605] In some embodiments, the regulatable genetic modification targeting the RHD gene by the rare-cutting endonuclease comprises a regulatable Cas protein or a polynucleotide encoding a regulatable Cas protein, and at least one guide ribonucleic acid (gRNA) sequence for specifically targeting the RHD gene. Useful methods for identifying gRNA sequences to target RHD are described below.

[00606] Assays to test whether the RHD gene has been inactivated are known and described herein. In some embodiments, the resulting genetic modification of the RHD gene by PCR and the reduction of RHD expression can be assays by FACS analysis. In another embodiment, RhD protein expression is detected using a Western blot of cells lysates probed with antibodies to the RhD protein. In another embodiment, reverse transcriptase polymerase chain reactions (RT-PCR) are used to confirm the presence of the inactivating genetic modification.

L. Protocadherin-11 Y-linked

[00607] In some embodiments, the technologies disclosed herein regulatably modulate (*e.g.*, reduce or eliminate) the expression of one or more Y chromosome genes by regulatably targeting and modulating (*e.g.*, reducing or eliminating) expression of the Y chromosome gene. In some embodiments, the modulation occurs using a gene editing system (*e.g.* CRISPR/Cas). In some embodiments, the modulation occurs using an RNA-based component selected from the group consisting of conditional or inducible shRNAs, conditional or inducible siRNAs, conditional or inducible miRNAs, and conditional or inducible CRISPR interference (CRISPRi). In some embodiments, the modulation occurs using a DNA-based component selected from the group consisting of a knock out or knock down using a method selected from the group consisting of conditional or inducible CRISPRs, conditional or inducible TALENs, conditional or inducible zinc finger nucleases, conditional or inducible homing endonucleases, and conditional or inducible meganucleases. In some embodiments, the modulation occurs using a protein-based component that is a conditional or inducible degron method.

[00608] In some embodiments, the cell has a reduced ability to induce an innate and/or an adaptive immune response in a recipient subject.

[00609] In certain embodiments, the technologies disclosed herein regulatably modulate (*e.g.*, reduce or eliminate) the expression of protocadherin-11 Y-linked antigen by regulatably targeting and modulating (*e.g.*, reducing or eliminating) expression of the protocadherin-11 Y-linked gene, *e.g.*, PCDH11Y. In some embodiments, the modulation occurs using a gene editing system (*e.g.* CRISPR/Cas). In some embodiments, the technologies disclosed herein regulatably modulate (*e.g.*, reduce or eliminate) the expression of one or more Y chromosome genes by regulatably targeting and modulating (*e.g.*, reducing or eliminating) expression of the Y chromosome gene. In some embodiments, the modulation occurs using a CRISPR/Cas system. In some embodiments, the modulation occurs using an RNA-based component selected from the group consisting of conditional or inducible shRNAs, conditional or inducible siRNAs, conditional or inducible miRNAs, and conditional or inducible CRISPR interference (CRISPRi). In some embodiments, the modulation occurs using a DNA-based component selected from the group consisting of a knock out or knock down using a method selected from the group consisting of conditional or inducible CRISPRs, conditional or inducible TALENs, conditional or inducible zinc finger nucleases, conditional or inducible homing endonucleases, and conditional or inducible meganucleases. In some embodiments, the modulation occurs using a protein-based component that is a conditional or inducible degron method.

[00610] In some embodiments, the cell has a reduced ability to induce an innate and/or an adaptive immune response in a recipient subject.

[00611] In some embodiments, the target polynucleotide sequence of the present disclosure is a variant of PCDH11Y gene. In some embodiments, the target polynucleotide sequence is a homolog of PCDH11Y gene. In some embodiments, the target polynucleotide sequence is an ortholog of PCDH11Y gene.

[00612] In some embodiments, the cells described herein comprise regulatable gene modifications at the gene locus encoding the protocadherin-11 Y-linked antigen protein. In other words, the cells comprise a regulatable genetic modification at the PCDH11Y locus. In some instances, the nucleotide sequence encoding the protocadherin-11 Y-linked antigen protein is set forth in RefSeq. Nos. N NM_001278619.1, NM_032971.2, NM_032972.2, NM_032973.2, or

XM_017030082.1, or in Genbank Nos. AJ276803, AF277053, AF332216, AF332217, AJ564958, AJ564959, AJ564960, AJ564961, AJ564962, AJ564963, AJ564966, or AJ56496. In some instances, the PCDH11Y gene locus is described in NCBI Gene ID No. 83259. In certain cases, the amino acid sequence of protocadherin-11 Y-linked antigen is depicted as NCBI GenBank Nos. CAC13122.1, AAL55729.1, AAK13468.1, AAK13469.1, CAD92429.1, CAD92430.1, CAD92431.1, CAD92432.1, CAD92433.1, CAD92434.1, CAD92437.1, or CAD92440.1. Additional descriptions of the protocadherin-11 Y-linked antigen protein and gene locus can be found in Uniprot No. Q9BZA8, HGNC Ref. No. 15813, and OMIM Ref. No. 400022.

[00613] In some embodiments, the hypoimmunogenic cells outlined herein comprise a regulatable genetic modification targeting the PCDH11Y gene. In some embodiments, the regulatable genetic modification targeting the PCDH11Y gene is generated by regulatably gene editing the PCDH11Y gene using regulatable gene editing tools such as but not limited to regulatable CRISPR/Cas, regulatable TALE- nucleases, regulatable zinc finger nucleases, other regulatable viral based gene editing system, or regulatable RNA interference. In some embodiments, the gene editing targets the coding sequence of the PCDH11Y gene. In some instances, the cells do not generate a functional PCDH11Y gene product. In the absence of the PCDH11Y gene product, the cells completely lack a protocadherin-11 Y-linked antigen.

[00614] In some embodiments, the regulatable genetic modification targeting the PCDH11Y gene by the rare-cutting endonuclease comprises a regulatable Cas protein or a polynucleotide encoding a regulatable Cas protein, and at least one guide ribonucleic acid (gRNA) sequence for specifically targeting the PCDH11Y gene. Useful methods for identifying gRNA sequences to target PCDH11Y are described below.

[00615] Assays to test whether the PCDH11Y gene has been inactivated are known and described herein. In one embodiment, the resulting genetic modification of the PCDH11Y gene by PCR and the reduction of protocadherin-11 Y-linked antigen protein expression can be assayed by FACS analysis. In another embodiment, protocadherin-11 Y-linked antigen protein expression is detected using a Western blot of cells lysates probed with antibodies to the protocadherin-11 Y-linked antigen protein. In another embodiment, reverse transcriptase

polymerase chain reactions (RT-PCR) are used to confirm the presence of the inactivating genetic modification.

M. Neuroligin-4 Y-linked

[00616] In some embodiments, the technologies disclosed herein regulatably modulate (*e.g.*, reduce or eliminate) the expression of neuroligin-4 Y-linked antigen by regulatably targeting and modulating (*e.g.*, reducing or eliminating) expression of the neuroligin-4 Y-linked gene, *e.g.*, NLGN4Y. In some embodiments, the modulation occurs using a gene editing system (*e.g.* CRISPR/Cas). In some embodiments, the modulation occurs using an RNA-based component selected from the group consisting of conditional or inducible shRNAs, conditional or inducible siRNAs, conditional or inducible miRNAs, and conditional or inducible CRISPR interference (CRISPRi). In some embodiments, the modulation occurs using a DNA-based component selected from the group consisting of a knock out or knock down using a method selected from the group consisting of conditional or inducible CRISPRs, conditional or inducible TALENs, conditional or inducible zinc finger nucleases, conditional or inducible homing endonucleases, and conditional or inducible meganucleases. In some embodiments, the modulation occurs using a protein-based component that is a conditional or inducible degron method.

[00617] In some embodiments, the cell has a reduced ability to induce an innate and/or an adaptive immune response in a recipient subject.

[00618] In some embodiments, the target polynucleotide sequence of the present disclosure is a variant of NLGN4Y gene. In some embodiments, the target polynucleotide sequence is a homolog of NLGN4Y gene. In some embodiments, the target polynucleotide sequence is an ortholog of NLGN4Y gene.

[00619] In some embodiments, the cells described herein comprise regulatable gene modifications at the gene locus encoding the neuroligin-4 Y-linked antigen protein. In other words, the cells comprise a regulatable genetic modification at the NLGN4Y locus. In some instances, the nucleotide sequence encoding the neuroligin-4 Y-linked antigen protein is set forth in RefSeq. Nos. NM_001164238.1, NM_001206850.1, NM_014893.4, XM_017030034.1, XM_017030035.1, XM_017030036.1, XM_017030037.1, XM_017030038.1, XM_017030040.1, or XM_017030041.1, or in Genbank Nos. AF376804, AB023168, BX537428, AC010726, AC010879, AC010979, AC011903, BC032567, BC113525, or

BC113551. In some instances, the NLGN4Y gene locus is described in NCBI Gene ID No. 22829. In certain cases, the amino acid sequence of neuroligin-4 Y-linked antigen is depicted as NCBI GenBank Nos. AAM46113.1, BAA76795.2, CAD97670.1, AAH32567.1, AAI13526.1, or AAI13552.1. Additional descriptions of the neuroligin-4 Y-linked antigen protein and gene locus can be found in Uniprot No. Q8NFZ3, HGNC Ref. No. 15529, and OMIM Ref. No. 400028.

[00620] In some embodiments, the hypoimmunogenic cells outlined herein comprise a regulatable genetic modification targeting the NLGN4Y gene. In some embodiments, the regulatable genetic modification targeting the NLGN4Y gene is generated by gene editing the NLGN4Y gene using regulatable gene editing tools such as but not limited to regulatable CRISPR/Cas, regulatable TALE- nucleases, regulatable zinc finger nucleases, other regulatable viral based gene editing system, or regulatable RNA interference. In some embodiments, the gene editing targets the coding sequence of the NLGN4Y gene. In some instances, the cells do not generate a functional NLGN4Y gene product. In the absence of the NLGN4Y gene product, the cells completely lack a neuroligin-4 Y-linked antigen.

[00621] In some embodiments, the regulatable genetic modification targeting the NLGN4Y gene by the rare-cutting endonuclease comprises a regulatable Cas protein or a polynucleotide encoding a regulatable Cas protein, and at least one guide ribonucleic acid (gRNA) sequence for specifically targeting the NLGN4Y gene. Useful methods for identifying gRNA sequences to target NLGN4Y are described below.

[00622] Assays to test whether the NLGN4Y gene has been inactivated are known and described herein. In one embodiment, the resulting genetic modification of the NLGN4Y gene by PCR and the reduction of neuroligin-4 Y-linked antigen protein expression can be assayed by FACS analysis. In another embodiment, neuroligin-4 Y-linked antigen protein expression is detected using a Western blot of cells lysates probed with antibodies to the neuroligin-4 Y-linked antigen protein. In another embodiment, reverse transcriptase polymerase chain reactions (RT-PCR) are used to confirm the presence of the inactivating genetic modification.

N. RHD

[00623] In some embodiments, the technologies disclosed herein regulatably modulate (*e.g.*, reduce or eliminate) the expression of RhD antigen by regulatably targeting and modulating (*e.g.*, reducing or eliminating) expression of the RHD gene. In some embodiments, the

modulation occurs using a CRISPR/Cas system. In some embodiments, the modulation occurs using an RNA-based component selected from the group consisting of conditional or inducible shRNAs, conditional or inducible siRNAs, conditional or inducible miRNAs, and conditional or inducible CRISPR interference (CRISPRi). In some embodiments, the modulation occurs using a DNA-based component selected from the group consisting of a knock out or knock down using a method selected from the group consisting of conditional or inducible CRISPRs, conditional or inducible TALENs, conditional or inducible zinc finger nucleases, conditional or inducible homing endonucleases, and conditional or inducible meganucleases. In some embodiments, the modulation occurs using a protein-based component that is a conditional or inducible degron method.

[00624] In some embodiments, the target polynucleotide sequence of the present disclosure is a variant of RHD gene. In some embodiments, the target polynucleotide sequence is a homolog of RHD gene. In some embodiments, the target polynucleotide sequence is an ortholog of RHD gene.

[00625] In some embodiments, the cells described herein comprise regulatable gene modifications at the gene locus encoding the RhD antigen protein. In other words, the cells comprise a regulatable genetic modification at the RHD locus. In some instances, the nucleotide sequence encoding the RhD antigen protein is set forth in RefSeq. Nos. NM_001127691.2, NM_001282868.1, NM_001282869.1, NM_001282871.1, or NM_016124.4, or in Genbank No. L08429. in some instances, the RHD gene locus is described in NCBI Gene ID No.6007. In certain cases, the amino acid sequence of RhD antigen protein is depicted as NCBI GenBank No. AAA02679.1. Additional descriptions of the RhD protein and gene locus can be found in Uniprot No. Q02161, HGNC Ref. No. 10009, and OMIM Ref. No. 111680.

[00626] In some embodiments, the cells outlined herein comprise a regulatable genetic modification targeting the RHD gene. In some embodiments, the genetic modification targeting the RHD gene is generated by gene editing the RHD gene using regulatable gene editing tools such as but not limited to regulatable CRISPR/Cas, regulatable TALE- nucleases, regulatable zinc finger nucleases, other regulatable viral based gene editing system, or regulatable RNA interference. In some embodiments, the gene editing targets the coding sequence of the RHD

gene. In some instances, the cells do not generate a functional RHD gene product. In the absence of the RHD gene product, the cells completely lack an Rh blood group antigen.

[00627] In some embodiments, the regulatable genetic modification targeting the RHD gene by the rare-cutting endonuclease comprises a regulatable Cas protein or a polynucleotide encoding a regulatable Cas protein, and at least one guide ribonucleic acid (gRNA) sequence for specifically targeting the RHD gene. Useful methods for identifying gRNA sequences to target RHD are described below.

[00628] Assays to test whether the RHD gene has been inactivated are known and described herein. In some embodiments, the resulting genetic modification of the RHD gene by PCR and the reduction of RHD expression can be assays by FACS analysis. In another embodiment, RhD protein expression is detected using a Western blot of cells lysates probed with antibodies to the RhD protein. In another embodiment, reverse transcriptase polymerase chain reactions (RT-PCR) are used to confirm the presence of the inactivating genetic modification.

O. ABO

[00629] In some embodiments, the technologies disclosed herein regulatably modulate (*e.g.*, reduce or eliminate) the expression of Histo-blood group ABO system transferase (ABO) by regulatably targeting and modulating (*e.g.*, reducing or eliminating) expression of the ABO gene. In some embodiments, the modulation occurs using a gene editing system (*e.g.* CRISPR/Cas). In some embodiments, the modulation occurs using an RNA-based component selected from the group consisting of conditional or inducible shRNAs, conditional or inducible siRNAs, conditional or inducible miRNAs, and conditional or inducible CRISPR interference (CRISPRi). In some embodiments, the modulation occurs using a DNA-based component selected from the group consisting of a knock out or knock down using a method selected from the group consisting of conditional or inducible CRISPRs, conditional or inducible TALENs, conditional or inducible zinc finger nucleases, conditional or inducible homing endonucleases, and conditional or inducible meganucleases. In some embodiments, the modulation occurs using a protein-based component that is a conditional or inducible degron method.

[00630] In some embodiments, the cell has a reduced ability to induce an innate and/or an adaptive immune response in a recipient subject.

[00631] In some embodiments, the target polynucleotide sequence of the present disclosure is a variant of ABO gene. In some embodiments, the target polynucleotide sequence is a homolog of ABO gene. In some embodiments, the target polynucleotide sequence is an ortholog of ABO gene.

[00632] In some embodiments, the cells described herein comprise regulatable gene modifications at the gene locus encoding the ABO protein. In other words, the cells comprise a regulatable genetic modification at the ABO locus. In some instances, the nucleotide sequence encoding the ABO protein is set forth in RefSeq. No. NM_020469.2, or in Genbank No. AF134412. In some instances, the ABO gene locus is described in NCBI Gene ID No. 28. In certain cases, the amino acid sequence of ABO is depicted as NCBI GenBank No. AAD26572.1. Additional descriptions of the ABO protein and gene locus can be found in Uniprot No. P16442, HGNC Ref. No. 79, and OMIM Ref. No. 110300.

[00633] In some embodiments, the hypoimmunogenic cells outlined herein comprise a regulatable genetic modification targeting the ABO gene. In some embodiments, the regulatable genetic modification targeting the ABO gene is generated by gene editing the ABO gene using regulatable gene editing tools such as but not limited to regulatable CRISPR/Cas, regulatable TALE- nucleases, regulatable zinc finger nucleases, other regulatable viral based gene editing system, or regulatable RNA interference. In some embodiments, the gene editing targets the coding sequence of the ABO gene. In some instances, the cells do not generate a functional ABO gene product. In the absence of the ABO gene product, the cells completely lack an ABO protein.

[00634] In some embodiments, the regulatable genetic modification targeting the ABO gene by the rare-cutting endonuclease comprises a regulatable Cas protein or a polynucleotide encoding a regulatable Cas protein, and at least one guide ribonucleic acid (gRNA) sequence for specifically targeting the ABO gene. Useful methods for identifying gRNA sequences to target ABO are described below.

[00635] Assays to test whether the ABO gene has been inactivated are known and described herein. In one embodiment, the resulting genetic modification of the ABO gene by PCR and the reduction of ABO protein expression can be assayed by FACS analysis. In another embodiment, ABO protein expression is detected using a Western blot of cells lysates probed with antibodies

to the ABO protein. In another embodiment, reverse transcriptase polymerase chain reactions (RT-PCR) are used to confirm the presence of the inactivating genetic modification.

P. MIC-A

[00636] In some embodiments, the technologies disclosed herein regulatably modulate (*e.g.*, reduce or eliminate) the expression of one or more MHC class I polypeptide-related sequence A (MIC-A) by regulatably targeting and modulating (*e.g.*, reducing or eliminating) expression of the MIC-A gene. In some embodiments, the modulation occurs using a gene editing system (*e.g.* CRISPR/Cas). In some embodiments, the modulation occurs using an RNA-based component selected from the group consisting of conditional or inducible shRNAs, conditional or inducible siRNAs, conditional or inducible miRNAs, and conditional or inducible CRISPR interference (CRISPRi). In some embodiments, the modulation occurs using a DNA-based component selected from the group consisting of a knock out or knock down using a method selected from the group consisting of conditional or inducible CRISPRs, conditional or inducible TALENs, conditional or inducible zinc finger nucleases, conditional or inducible homing endonucleases, and conditional or inducible meganucleases. In some embodiments, the modulation occurs using a protein-based component that is a conditional or inducible degron method.

[00637] In some embodiments, the cell has a reduced ability to induce an innate and/or an adaptive immune response in a recipient subject.

[00638] In some embodiments, the target polynucleotide sequence of the present disclosure is a variant of MIC-A gene. In some embodiments, the target polynucleotide sequence is a homolog of MIC-A gene. In some embodiments, the target polynucleotide sequence is an ortholog of MIC-A gene.

[00639] In some embodiments, the cells described herein comprise regulatable gene modifications at the gene locus encoding the MIC-A protein. In other words, the cells comprise a regulatable genetic modification at the MIC-A locus. In some instances, the nucleotide sequence encoding the MIC-A protein is set forth in RefSeq. No. NM_000247.2, or in Genbank No. BC016929. In some instances, the MIC-A gene locus is described in NCBI Gene ID No. 100507436. In certain cases, the amino acid sequence of MIC-A is depicted as NCBI GenBank No. AAH16929.1. Additional descriptions of the MIC-A protein and gene locus can be found in Uniprot No. Q29983, HGNC Ref. No. 7090, and OMIM Ref. No. 600169.

[00640] In some embodiments, the hypoimmunogenic cells outlined herein comprise a regulatable genetic modification targeting the MIC-A gene. In some embodiments, the regulatable genetic modification targeting the MIC-A gene is generated by gene editing the MIC-A gene using regulatable gene editing tools such as but not limited to regulatable CRISPR/Cas, regulatable TALE- nucleases, regulatable zinc finger nucleases, other regulatable viral based gene editing system, or regulatable RNA interference. In some embodiments, the gene editing targets the coding sequence of the MIC-A gene. In some instances, the cells do not generate a functional MIC-A gene product. In the absence of the MIC-A gene product, the cells completely lack a MIC-A protein.

[00641] In some embodiments, the regulatable genetic modification targeting the MIC-A gene by the rare-cutting endonuclease comprises a regulatable Cas protein or a polynucleotide encoding a regulatable Cas protein, and at least one guide ribonucleic acid (gRNA) sequence for specifically targeting the MIC-A gene. Useful methods for identifying gRNA sequences to target MIC-A are described below.

[00642] Assays to test whether the MIC-A gene has been inactivated are known and described herein. In one embodiment, the resulting genetic modification of the MIC-A gene by PCR and the reduction of MIC-A protein expression can be assayed by FACS analysis. In another embodiment, MIC-A protein expression is detected using a Western blot of cells lysates probed with antibodies to the MIC-A protein. In another embodiment, reverse transcriptase polymerase chain reactions (RT-PCR) are used to confirm the presence of the inactivating genetic modification.

Q. MIC-B

[00643] In some embodiments, the technologies disclosed herein regulatably modulate (*e.g.*, reduce or eliminate) the expression of one or more MHC class I polypeptide-related sequence B (MIC-B) by regulatably targeting and modulating (*e.g.*, reducing or eliminating) expression of the MIC-B gene. In some embodiments, the modulation occurs using a gene editing system (*e.g.* CRISPR/Cas). In some embodiments, the modulation occurs using an RNA-based componentselected from the group consisting of conditional or inducible shRNAs, conditional or inducible siRNAs, conditional or inducible miRNAs, and conditional or inducible CRISPR interference (CRISPRi). In some embodiments, the modulation occurs using a DNA-based

componentselected from the group consisting of a knock out or knock down using a method selected from the group consisting of conditional or inducible CRISPRs, conditional or inducible TALENs, conditional or inducible zinc finger nucleases, conditional or inducible homing endonucleases, and conditional or inducible meganucleases. In some embodiments, the modulation occurs using a protein-based component that is a conditional or inducible degron method.

[00644] In some embodiments, the cell has a reduced ability to induce an innate and/or an adaptive immune response in a recipient subject.

[00645] In some embodiments, the target polynucleotide sequence of the present disclosure is a variant of MIC-B gene. In some embodiments, the target polynucleotide sequence is a homolog of MIC-B gene. In some embodiments, the target polynucleotide sequence is an ortholog of MIC-B gene.

[00646] In some embodiments, the cells described herein comprise regulatable gene modifications at the gene locus encoding the MIC-B protein. In other words, the cells comprise a regulatable genetic modification at the MIC-B locus. In some instances, the nucleotide sequence encoding the MIC-B protein is set forth in RefSeq. No. NM_001289160.1, or in Genbank No. AK314228. In some instances, the MIC-B gene locus is described in NCBI Gene ID No. 4277. In certain cases, the amino acid sequence of MIC-B is depicted as NCBI GenBank No. BAG36899.1. Additional descriptions of the MIC-B protein and gene locus can be found in Uniprot No. Q29980, HGNC Ref. No. 7091, and OMIM Ref. No. 602436.

[00647] In some embodiments, the hypoimmunogenic cells outlined herein comprise a regulatable genetic modification targeting the MIC-B gene. In some embodiments, the regulatable genetic modification targeting the MIC-B gene is generated by gene editing the MIC-B gene using regulatable gene editing tools such as but not limited to regulatable CRISPR/Cas, regulatable TALE- nucleases, regulatable zinc finger nucleases, other regulatable viral based gene editing system, or regulatable RNA interference. In some embodiments, the gene editing targets the coding sequence of the MIC-B gene. In some instances, the cells do not generate a functional MIC-B gene product. In the absence of the MIC-B gene product, the cells completely lack a MIC-B protein.

[00648] In some embodiments, the regulatable genetic modification targeting the MIC-B gene by the rare-cutting endonuclease comprises a regulatable Cas protein or a polynucleotide encoding a regulatable Cas protein, and at least one guide ribonucleic acid (gRNA) sequence for specifically targeting the MIC-B gene. Useful methods for identifying gRNA sequences to target MIC-B are described below.

[00649] Assays to test whether the MIC-B gene has been inactivated are known and described herein. In one embodiment, the resulting genetic modification of the MIC-B gene by PCR and the reduction of MIC-B protein expression can be assayed by FACS analysis. In another embodiment, MIC-B protein expression is detected using a Western blot of cells lysates probed with antibodies to the MIC-B protein. In another embodiment, reverse transcriptase polymerase chain reactions (RT-PCR) are used to confirm the presence of the inactivating genetic modification.

R. CTLA-4

[00650] In some embodiments, the target polynucleotide sequence is CTLA-4 or a variant of CTLA-4. In some embodiments, the target polynucleotide sequence is a homolog of CTLA-4. In some embodiments, the target polynucleotide sequence is an ortholog of CTLA-4.

[00651] In some embodiments, the cells outlined herein comprise a genetic modification targeting the CTLA-4 gene. In certain embodiments, primary T cells comprise a genetic modification targeting the CTLA-4 gene. The genetic modification can reduce expression of CTLA-4 polynucleotides and CTLA-4 polypeptides in T cells includes primary T cells and CAR-T cells. In some embodiments, the genetic modification targeting the CTLA-4 gene by the rare-cutting endonuclease comprises a Cas protein or a polynucleotide encoding a Cas protein, and at least one guide ribonucleic acid (gRNA) sequence for specifically targeting the CTLA-4 gene. Useful methods for identifying gRNA sequences to target CTLA-4 are described below.

[00652] Assays to test whether the CTLA-4 gene has been inactivated are known and described herein. In some embodiments, the resulting genetic modification of the CTLA-4 gene by PCR and the reduction of CTLA-4 expression can be assays by FACS analysis. In another embodiment, CTLA-4 protein expression is detected using a Western blot of cells lysates probed with antibodies to the CTLA-4 protein. In another embodiment, reverse transcriptase

polymerase chain reactions (RT-PCR) are used to confirm the presence of the inactivating genetic modification.

[00653] Useful genomic, polynucleotide and polypeptide information about the human CTLA-4 are provided in, for example, the GeneCard Identifier GC02P203867, HGNC No. 2505, NCBI Gene ID 1493, NCBI RefSeq Nos. NM_005214.4, NM_001037631.2, NP_001032720.1 and NP_005205.2, UniProt No. P16410, and the like.

S. PD-1

[00654] In some embodiments, the target polynucleotide sequence is PD-1 or a variant of PD-1. In some embodiments, the target polynucleotide sequence is a homolog of PD-1. In some embodiments, the target polynucleotide sequence is an ortholog of PD-1.

[00655] In some embodiments, the cells outlined herein comprise a genetic modification targeting the gene encoding the programmed cell death protein 1 (PD-1) protein or the PDCD1 gene. In certain embodiments, primary T cells comprise a genetic modification targeting the PDCD1 gene. The genetic modification can reduce expression of PD-1 polynucleotides and PD-1 polypeptides in T cells includes primary T cells and CAR-T cells. In some embodiments, the genetic modification targeting the PDCD1 gene by the rare-cutting endonuclease comprises a Cas protein or a polynucleotide encoding a Cas protein, and at least one guide ribonucleic acid (gRNA) sequence for specifically targeting the PDCD1 gene. Useful methods for identifying gRNA sequences to target PD-1 are described below.

[00656] Assays to test whether the PDCD1 gene has been inactivated are known and described herein. In some embodiments, the resulting genetic modification of the PDCD1 gene by PCR and the reduction of PD-1 expression can be assays by FACS analysis. In another embodiment, PD-1 protein expression is detected using a Western blot of cells lysates probed]with antibodies to the PD-1 protein. In another embodiment, reverse transcriptase polymerase chain reactions (RT-PCR) are used to confirm the presence of the inactivating genetic modification.

[00657] Useful genomic, polynucleotide and polypeptide information about human PD-1 including the PDCD1 gene are provided in, for example, the GeneCard Identifier GC02M241849, HGNC No. 8760, NCBI Gene ID 5133, Uniprot No. Q15116, and NCBI RefSeq Nos. NM_005018.2 and NP_005009.2.

T. CD47

[00658] In some embodiments, the present disclosure provides a cell or population thereof that has been modified to regulatably overexpress the tolerogenic factor (*e.g.*, immunomodulatory polypeptide) CD47. In some embodiments, the present disclosure provides a method for altering a cell genome to regulatably overexpress CD47. In some embodiments, the stem cell regulatably overexpresses exogenous CD47. In some instances, the cell regulatably expresses an expression vector comprising a nucleotide sequence encoding a human CD47 polypeptide. In some embodiments, the cell is genetically modified to comprise an integrated exogenous polynucleotide encoding a regulatable CD47 using homology-directed repair. In some instances, the cell regulatably expresses a nucleotide sequence encoding a human CD47 polypeptide such that the nucleotide sequence is inserted into at least one allele of a safe harbor or target locus. In some instances, the cell regulatably expresses a nucleotide sequence encoding a human CD47 polypeptide wherein the nucleotide sequence is inserted into at least one allele of an *AAVS1* locus. In some instances, the cell regulatably expresses a nucleotide sequence encoding a human CD47 polypeptide wherein the nucleotide sequence is inserted into at least one allele of an *CCR5* locus. In some instances, the cell regulatably expresses a nucleotide sequence encoding a human CD47 polypeptide wherein the nucleotide sequence is inserted into at least one allele of a safe harbor or target gene locus, such as, but not limited to, a CCR5 gene locus, a CXCR4 gene locus, a PPP1R12C gene locus, an albumin gene locus, a SHS231 gene locus, a CLYBL gene locus, a Rosa gene locus, an F3 (CD142) gene locus, a MICA gene locus, a MICB gene locus, a LRP1 (CD91) gene locus, a HMGB1 gene locus, an ABO gene locus, an RHD gene locus, a FUT1 locus, and a KDM5D gene locus. In some instances, the cell regulatably expresses a nucleotide sequence encoding a human CD47 polypeptide wherein the nucleotide sequence is inserted into at least one allele of a *TRAC* locus.

[00659] CD47 is a leukocyte surface antigen and has a role in cell adhesion and modulation of integrins. It is expressed on the surface of a cell and signals to circulating macrophages not to eat the cell.

[00660] In some embodiments, the cell outlined herein comprises a nucleotide sequence encoding a CD47 polypeptide has at least 95% sequence identity (*e.g.*, 95%, 96%, 97%, 98%, 99%, or more) to an amino acid sequence as set forth in NCBI Ref. Sequence Nos. NP_001768.1 and NP_942088.1. In some embodiments, the cell outlined herein comprises a nucleotide

sequence encoding a CD47 polypeptide having an amino acid sequence as set forth in NCBI Ref. Sequence Nos. NP_001768.1 and NP_942088.1. In some embodiments, the cell comprises a nucleotide sequence for CD47 having at least 85% sequence identity (*e.g.*, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) to the sequence set forth in NCBI Ref. Nos. NM_001777.3 and NM_198793.2. In some embodiments, the cell comprises a nucleotide sequence for CD47 as set forth in NCBI Ref. Sequence Nos. NM_001777.3 and NM_198793.2. In some embodiments, the nucleotide sequence encoding a CD47 polynucleotide is a codon optimized sequence. In some embodiments, the nucleotide sequence encoding a CD47 polynucleotide is a human codon optimized sequence.

[00661] In some embodiments, the cell comprises a CD47 polypeptide having at least 95% sequence identity (*e.g.*, 95%, 96%, 97%, 98%, 99%, or more) to an amino acid sequence as set forth in NCBI Ref. Sequence Nos. NP_001768.1 and NP_942088.1. In some embodiments, the cell outlined herein comprises a CD47 polypeptide having an amino acid sequence as set forth in NCBI Ref. Sequence Nos. NP_001768.1 and NP_942088.1.

[00662] Exemplary amino acid sequences of human CD47 with a signal sequence and without a signal sequence are provided in Table 1.

Table 1. Amino acid sequences of human CD47

Protein	SEQ ID NO:	Sequence	Amino acid residues
Human CD47 (without signal sequence)	129	QLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEV YVKWKFKGRDIYTFDGALNKSTVPTDFSSAKIEVS QLLKGDASLKMDKSDAVSHTGNYTCEVTELTREG ETIIELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGI KTLKYRSGGMDEKTIALLVAGLVITVIVIVGAILFVP GEYSLKNATGLGLIVTSTGILILLHYYVFSTAIGLTSF VIAILVIQVIAYILAVVGLSLCIAACIPMHGPLLISGL SILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLN AFKESKGMMNDE	aa 19-323
Human CD47 (with signal sequence)	130	MWPLVAALLLGSACCGSAQLLFNKTKSVEFTFCND TVVIPCFVTNMEAQNTTEVYVKWKFKGRDIYTFDG ALNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDA VSHTGNYTCEVTELTREGETIIELKYRVVSWFSPNE NILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIAL LVAGLVITVIVIVGAILFVPGEYSLKNATGLGLIVTS TGILILLHYYVFSTAIGLTSFVIAILVIQVIAYILAVVG LSLCIAACIPMHGPLLISGLSILALAQLLGLVYMKFV ASNQKTIQPPRKAVEEPLNAFKESKGMMNDE	aa 1-323

[00663] In some embodiments, the cell comprises a CD47 polypeptide having at least 80% sequence identity (*e.g.*, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) to the amino acid sequence of SEQ ID NO:129, wherein the CD47 polypeptide has substantially the same biological function and activity of a CD47 polypeptide having the amino acid sequence of SEQ ID NO:129. In some embodiments, the cell comprises a CD47 polypeptide having the amino acid sequence of SEQ ID NO:129. In some embodiments, the cell comprises a CD47 polypeptide having at least 80% sequence identity (*e.g.*, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more) to the amino acid sequence of SEQ ID NO:14, wherein the CD47 polypeptide has substantially the same biological function and activity of a CD47 polypeptide having the amino acid sequence of SEQ ID NO:130. In some embodiments, the cell comprises a CD47 polypeptide having the amino acid sequence of SEQ ID NO:14.

[00664] In some embodiments, the cell comprises a nucleotide sequence encoding a CD47 polypeptide having at least 95% sequence identity (*e.g.*, 95%, 96%, 97%, 98%, 99%, or more) to the amino acid sequence of SEQ ID NO:129. In some embodiments, the cell comprises a nucleotide sequence encoding a CD47 polypeptide having the amino acid sequence of SEQ ID NO:129. In some embodiments, the cell comprises a nucleotide sequence encoding a CD47 polypeptide having at least 95% sequence identity (*e.g.*, 95%, 96%, 97%, 98%, 99%, or more) to the amino acid sequence of SEQ ID NO:130. In some embodiments, the cell comprises a nucleotide sequence encoding a CD47 polypeptide having the amino acid sequence of SEQ ID NO:130. In some embodiments, the nucleotide sequence is codon optimized for expression in a particular cell.

[00665] In some embodiments, a suitable gene editing system (*e.g.*, CRISPR/Cas system or any of the gene editing systems described herein) is used to facilitate the insertion of a regulatable polynucleotide encoding CD47, into a genomic locus of the hypoimmunogenic cell. In some cases, the regulatable polynucleotide encoding CD47 is inserted into a safe harbor or target locus, such as but not limited to, an AAVS1, CCR5, CLYBL, ROSA26, SHS231, F3 (CD142), MICA, MICB, LRP1 (CD91), HMGB1, ABO, RHD, FUT1, or KDM5D gene locus. In some embodiments, the regulatable polynucleotide encoding CD47 is inserted into a B2M gene locus, a CIITA gene locus, a TRAC gene locus, or a TRB gene locus. In some embodiments, the regulatable polynucleotide encoding CD47 is inserted into any one of the gene loci depicted in

Table 16 provided herein. In certain embodiments, the regulatable polynucleotide encoding CD47 is operably linked to a promoter.

[00666] In some embodiments, the promoter is an endogenous or a constitutive promoter. In some embodiments, the promoter is a conditional or inducible promoter. In some embodiments, the conditional promoter is a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, or a differentiation-induced promoter. In some embodiments, the inducible promoter is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[00667] In some embodiments, the cells are engineered to expresses an increased amount of CD47 relative to a cell of the same cell type that does not comprise the modifications.

[00668] The amount of increased CD47 expression can be measured, for example, as a multiple, a fold, or a percentage of expression relative to the unaltered or unmodified wild-type cell. For example, in some embodiments, the cells described herein express at least about 1x, at least about 1.1x, at least about 1.2x, at least about 1.3x, at least about 1.4x, at least about 1.5x, at least about 1.6x, at least about 1.7x, at least about 1.8x, at least about 1.9x, at least about 2x, at least about 2.1x, at least about 2.2x, at least about 2.3x, at least about 2.4x, at least about 2.5x, at least about 2.6x, at least about 2.7x, at least about 2.8x, at least about 2.9x, at least about 3x, at least about 3.1x, at least about 3.2x, at least about 3.3x, at least about 3.4x, at least about 3.5x, at least about 3.6x, at least about 3.7x, at least about 3.8x, at least about 3.9x, at least about 4x, at least about 4.1x, at least about 4.2x, at least about 4.3x, at least about 4.4x, at least about 4.5x, at least about 4.6x, at least about 4.7x, at least about 4.8x, at least about 4.9x, at least about 5x, at least about 5.1x, at least about 5.2x, at least about 5.3x, at least about 5.4x, at least about 5.5x, at least about 5.6x, at least about 5.7x, at least about 5.8x, at least about 5.9x, at least about 6x, at least about 6.1x, at least about 6.2x, at least about 6.3x, at least about 6.4x, at least about 6.5x, at least about 6.6x, at least about 6.7x, at least about 6.8x, at least about 6.9x, at least about 7x, at least about 7.1x, at least about 7.2x, at least about 7.3x, at least about 7.4x, at least about 7.5x, at least about 7.6x, at least about 7.7x, at least about 7.8x, at least about 7.9x, at least about 8x, at least about 8.1x, at least about 8.2x, at least about 8.3x, at least about 8.4x, at least about 8.5x, at least about 8.6x, at least about 8.7x, at least about 8.8x, at least about 8.9x, at least about 9x, at least about 9.1x, at least about 9.2x, at least about 9.3x, at least about 9.4x, at least about 9.5x, at least about 9.6x, at least about 9.7x, at least about 9.8x, at least about 9.9x, at least about 10x, or

more, of the level of CD47 expressed in an unaltered or unmodified wild-type cell of the same cell type.

[00669] In some embodiments, the cells described herein express at least about 1-fold, at least about 1.1-fold, at least about 1.2-fold, at least about 1.3-fold, at least about 1.4-fold, at least about 1.5-fold, at least about 1.6-fold, at least about 1.7-fold, at least about 1.8-fold, at least about 1.9-fold, at least about 2-fold, at least about 2.1-fold, at least about 2.2-fold, at least about 2.3-fold, at least about 2.4-fold, at least about 2.5-fold, at least about 2.6-fold, at least about 2.7-fold, at least about 2.8-fold, at least about 2.9-fold, at least about 3-fold, at least about 3.1-fold, at least about 3.2-fold, at least about 3.3-fold, at least about 3.4-fold, at least about 3.5-fold, at least about 3.6-fold, at least about 3.7-fold, at least about 3.8-fold, at least about 3.9-fold, at least about 4-fold, at least about 4.1-fold, at least about 4.2-fold, at least about 4.3-fold, at least about 4.4-fold, at least about 4.5-fold, at least about 4.6-fold, at least about 4.7-fold, at least about 4.8-fold, at least about 4.9-fold, at least about 5-fold, at least about 5.1-fold, at least about 5.2-fold, at least about 5.3-fold, at least about 5.4-fold, at least about 5.5-fold, at least about 5.6-fold, at least about 5.7-fold, at least about 5.8-fold, at least about 5.9-fold, at least about 6-fold, at least about 6.1-fold, at least about 6.2-fold, at least about 6.3-fold, at least about 6.4-fold, at least about 6.5-fold, at least about 6.6-fold, at least about 6.7-fold, at least about 6.8-fold, at least about 6.9-fold, at least about 7-fold, at least about 7.1-fold, at least about 7.2-fold, at least about 7.3-fold, at least about 7.4-fold, at least about 7.5-fold, at least about 7.6-fold, at least about 7.7-fold, at least about 7.8-fold, at least about 7.9-fold, at least about 8-fold, at least about 8.1-fold, at least about 8.2-fold, at least about 8.3-fold, at least about 8.4-fold, at least about 8.5-fold, at least about 8.6-fold, at least about 8.7-fold, at least about 8.8-fold, at least about 8.9-fold, at least about 9-fold, at least about 9.1-fold, at least about 9.2-fold, at least about 9.3-fold, at least about 9.4-fold, at least about 9.5-fold, at least about 9.6-fold, at least about 9.7-fold, at least about 9.8-fold, at least about 9.9-fold, at least about 10-fold, or more, of the level of CD47 expressed in an unaltered or unmodified wild-type cell of the same cell type.

[00670] In some embodiments, the cells described herein express at least about 110%, at least about 120%, at least about 130%, at least about 140%, at least about 150%, at least about 160%, at least about 170%, at least about 180%, at least about 190%, at least about 200%, at least about 250%, at least about 300%, at least about 350%, at least about 400%, at least about 450%, at least about 500%, at least about 550%, at least about 600%, at least about 650%, at least about

700%, at least about 750%, at least about 800%, at least about 850%, at least about 900%, at least about 950%, at least about 1000%, at least about 1500%, at least about 2000%, at least about 2500%, at least about 3000%, at least about 3500%, at least about 4000%, at least about 4500%, at least about 5000%, at least about 5500%, at least about 6000%, at least about 6500%, at least about 7000%, at least about 7500%, at least about 8000%, at least about 8500%, at least about 9000%, at least about 10000%, or more, of the level of CD47 expressed in an unaltered or unmodified wild-type cell of the same cell type.

[00671] The amount of increased CD47 expression can also be measured, for example, as a multiple, a fold, or a percentage increase in expression relative to the unaltered or unmodified wild-type cell. For example, in some embodiments, the cells described herein express at least about 0.1x higher, at least about 0.1x higher, at least about 0.2x higher, at least about 0.3x higher, at least about 0.4x higher, at least about 0.5x higher, at least about 0.6x higher, at least about 0.7x higher, at least about 0.8x higher, at least about 0.9x higher, at least about 2x higher, at least about 1x higher, at least about 1.1x higher, at least about 1.2x higher, at least about 1.3x higher, at least about 1.4x higher, at least about 1.5x higher, at least about 1.6x higher, at least about 1.7x higher, at least about 1.8x higher, at least about 1.9x higher, at least about 2x higher, at least about 2.1x higher, at least about 2.2x higher, at least about 2.3x higher, at least about 2.4x higher, at least about 2.5x higher, at least about 2.6x higher, at least about 2.7x higher, at least about 2.8x higher, at least about 2.9x higher, at least about 3x higher, at least about 3.1x higher, at least about 3.2x higher, at least about 3.3x higher, at least about 3.4x higher, at least about 3.5x higher, at least about 3.6x higher, at least about 3.7x higher, at least about 3.8x higher, at least about 3.9x higher, at least about 4x higher, at least about 4.1x higher, at least about 4.2x higher, at least about 4.3x higher, at least about 4.4x higher, at least about 4.5x higher, at least about 4.6x higher, at least about 4.7x higher, at least about 4.8x higher, at least about 4.9x higher, at least about 5x higher, at least about 5.1x higher, at least about 5.2x higher, at least about 5.3x higher, at least about 5.4x higher, at least about 5.5x higher, at least about 5.6x higher, at least about 5.7x higher, at least about 5.8x higher, at least about 5.9x higher, at least about 6x higher, at least about 6.1x higher, at least about 6.2x higher, at least about 6.3x higher, at least about 6.4x higher, at least about 6.5x higher, at least about 6.6x higher, at least about 6.7x higher, at least about 6.8x higher, at least about 6.9x higher, at least about 7x higher, at least about 7.1x higher, at least about 7.2x higher, at least about 7.3x higher, at least about

7.4x higher, at least about 7.5x higher, at least about 7.6x higher, at least about 7.7x higher, at least about 7.8x higher, at least about 7.9x higher, at least about 8x higher, at least about 8.1x higher, at least about 8.2x higher, at least about 8.3x higher, at least about 8.4x higher, at least about 8.5x higher, at least about 8.6x higher, at least about 8.7x higher, at least about 8.8x higher, at least about 8.9x higher, at least about 9x higher, at least about 9.1x higher, at least about 9.2x higher, at least about 9.3x higher, at least about 9.4x higher, at least about 9.5x higher, at least about 9.6x higher, at least about 9.7x higher, at least about 9.8x higher, at least about 9.9x higher, at least about 10x higher, or more, amount of CD47 expression relative to the level of CD47 expressed in an unaltered or unmodified wild-type cell of the same cell type.

[00672] In some embodiments, the cells described herein express at least about 0.1-fold higher, at least about 0.2-fold higher, at least about 0.3-fold higher, at least about 0.4-fold higher, at least about 0.5-fold higher, at least about 0.6-fold higher, at least about 0.7-fold higher, at least about 0.8-fold higher, at least about 0.9-fold higher, at least about 1-fold higher, at least about 1.1-fold higher, at least about 1.2-fold higher, at least about 1.3-fold higher, at least about 1.4-fold higher, at least about 1.5-fold higher, at least about 1.6-fold higher, at least about 1.7-fold higher, at least about 1.8-fold higher, at least about 1.9-fold higher, at least about 2-fold higher, at least about 2.1-fold higher, at least about 2.2-fold higher, at least about 2.3-fold higher, at least about 2.4-fold higher, at least about 2.5-fold higher, at least about 2.6-fold higher, at least about 2.7-fold higher, at least about 2.8-fold higher, at least about 2.9-fold higher, at least about 3-fold higher, at least about 3.1-fold higher, at least about 3.2-fold higher, at least about 3.3-fold higher, at least about 3.4-fold higher, at least about 3.5-fold higher, at least about 3.6-fold higher, at least about 3.7-fold higher, at least about 3.8-fold higher, at least about 3.9-fold higher, at least about 4-fold higher, at least about 4.1-fold higher, at least about 4.2-fold higher, at least about 4.3-fold higher, at least about 4.4-fold higher, at least about 4.5-fold higher, at least about 4.6-fold higher, at least about 4.7-fold higher, at least about 4.8-fold higher, at least about 4.9-fold higher, at least about 5-fold higher, at least about 5.1-fold higher, at least about 5.2-fold higher, at least about 5.3-fold higher, at least about 5.4-fold higher, at least about 5.5-fold higher, at least about 5.6-fold higher, at least about 5.7-fold higher, at least about 5.8-fold higher, at least about 5.9-fold higher, at least about 6-fold higher, at least about 6.1-fold higher, at least about 6.2-fold higher, at least about 6.3-fold higher, at least about 6.4-fold higher, at least about 6.5-fold higher, at least about 6.6-fold higher, at least about 6.7-fold higher, at least about 6.8-fold higher, at least about 6.9-fold

higher, at least about 7-fold higher, at least about 7.1-fold higher, at least about 7.2-fold higher, at least about 7.3-fold higher, at least about 7.4-fold higher, at least about 7.5-fold higher, at least about 7.6-fold higher, at least about 7.7-fold higher, at least about 7.8-fold higher, at least about 7.9-fold higher, at least about 8-fold higher, at least about 8.1-fold higher, at least about 8.2-fold higher, at least about 8.3-fold higher, at least about 8.4-fold higher, at least about 8.5-fold higher, at least about 8.6-fold higher, at least about 8.7-fold higher, at least about 8.8-fold higher, at least about 8.9-fold higher, at least about 9-fold higher, at least about 9.1-fold higher, at least about 9.2-fold higher, at least about 9.3-fold higher, at least about 9.4-fold higher, at least about 9.5-fold higher, at least about 9.6-fold higher, at least about 9.7-fold higher, at least about 9.8-fold higher, at least about 9.9-fold higher, at least about 10-fold higher, or more, amount of CD47 expression relative to the level of CD47 expressed in an unaltered or unmodified wild-type cell of the same cell type.

[00673] In some embodiments, the cells described herein express at least about 10% higher, at least about 20% higher, at least about 30% higher, at least about 40% higher, at least about 50% higher, at least about 60% higher, at least about 70% higher, at least about 80% higher, at least about 90% higher, at least about 100% higher, at least about 125% higher, at least about 150% higher, at least about 200% higher, at least about 250% higher, at least about 300% higher, at least about 350% higher, at least about 400% higher, at least about 450% higher, at least about 500% higher, at least about 550% higher, at least about 600% higher, at least about 650% higher, at least about 700% higher, at least about 750% higher, at least about 800% higher, at least about 850% higher, at least about 900% higher, at least about 950% higher, at least about 1000% higher, at least about 1500% higher, at least about 2000% higher, at least about 2500% higher, at least about 3000% higher, at least about 3500% higher, at least about 4000% higher, at least about 4500% higher, at least about 5000% higher, at least about 5500% higher, at least about 6000% higher, at least about 6500% higher, at least about 7000% higher, at least about 7500% higher, at least about 8000% higher, at least about 8500% higher, at least about 9000% higher, at least about 10000% higher, or more, amount of CD47 expression relative to the level of CD47 expressed in an unaltered or unmodified wild-type cell of the same cell type.

[00674] The amount of CD47 expression can also be measured, for example, as a number of CD47 molecules for cell. For example, in some embodiments, the cells described herein express about 150,000 to about 1,000,000 CD47 molecules per cell. In some embodiments, the cells

described herein express about 150,000 to about 200,000 CD47 molecules per cell. In some embodiments, the cells described herein express about 200,000 to about 250,000 CD47 molecules per cell. In some embodiments, the cells described herein express about 250,000 to about 300,000 CD47 molecules per cell. In some embodiments, the cells described herein express about 300,000 to about 350,000 CD47 molecules per cell. In some embodiments, the cells described herein express about 350,000 to about 400,000 CD47 molecules per cell. In some embodiments, the cells described herein express about 400,000 to about 450,000 CD47 molecules per cell. In some embodiments, the cells described herein express about 450,000 to about 500,000 CD47 molecules per cell. In some embodiments, the cells described herein express about 500,000 to about 550,000 CD47 molecules per cell. In some embodiments, the cells described herein express about 550,000 to about 600,000 CD47 molecules per cell. In some embodiments, the cells described herein express about 600,000 to about 650,000 CD47 molecules per cell. In some embodiments, the cells described herein express about 650,000 to about 700,000 CD47 molecules per cell. In some embodiments, the cells described herein express about 700,000 to about 750,000 CD47 molecules per cell. In some embodiments, the cells described herein express about 750,000 to about 800,000 CD47 molecules per cell. In some embodiments, the cells described herein express about 800,000 to about 850,000 CD47 molecules per cell. In some embodiments, the cells described herein express about 850,000 to about 900,000 CD47 molecules per cell. In some embodiments, the cells described herein express about 900,000 to about 950,000 CD47 molecules per cell. In some embodiments, the cells described herein express about 950,000 to about 1,000,000 CD47 molecules per cell.

[00675] In some embodiments, the cells described herein express at least about 180,000 CD47 molecules, at least about 190,000 CD47 molecules, at least about 200,000 CD47 molecules, at least about 210,000 CD47 molecules, at least about 220,000 CD47 molecules, at least about 230,000 CD47 molecules, at least about 240,000 CD47 molecules, at least about 250,000 CD47 molecules, at least about 260,000 CD47 molecules, at least about 270,000 CD47 molecules, at least about 280,000 CD47 molecules, at least about 290,000 CD47 molecules, at least about 300,000 CD47 molecules, at least about 210,000 CD47 molecules, at least about 220,000 CD47 molecules, at least about 230,000 CD47 molecules, at least about 240,000 CD47 molecules, at least about 250,000 CD47 molecules, at least about 260,000 CD47 molecules, at least about 270,000 CD47 molecules, at least about 280,000 CD47 molecules, at least about 290,000 CD47

molecules, at least about 300,000 CD47 molecules, at least about 210,000 CD47 molecules, at least about 220,000 CD47 molecules, at least about 230,000 CD47 molecules, at least about 240,000 CD47 molecules, at least about 250,000 CD47 molecules, at least about 260,000 CD47 molecules, at least about 270,000 CD47 molecules, at least about 280,000 CD47 molecules, at least about 290,000 CD47 molecules, at least about 300,000 CD47 molecules, at least about 310,000 CD47 molecules, at least about 320,000 CD47 molecules, at least about 330,000 CD47 molecules, at least about 340,000 CD47 molecules, at least about 350,000 CD47 molecules, at least about 360,000 CD47 molecules, at least about 370,000 CD47 molecules, at least about 380,000 CD47 molecules, at least about 390,000 CD47 molecules, at least about 400,000 CD47 molecules, at least about 410,000 CD47 molecules, at least about 420,000 CD47 molecules, at least about 430,000 CD47 molecules, at least about 440,000 CD47 molecules, at least about 450,000 CD47 molecules, at least about 460,000 CD47 molecules, at least about 470,000 CD47 molecules, at least about 480,000 CD47 molecules, at least about 490,000 CD47 molecules, at least about 500,000 CD47 molecules, at least about 510,000 CD47 molecules, at least about 520,000 CD47 molecules, at least about 530,000 CD47 molecules, at least about 540,000 CD47 molecules, at least about 550,000 CD47 molecules, at least about 560,000 CD47 molecules, at least about 570,000 CD47 molecules, at least about 580,000 CD47 molecules, at least about 590,000 CD47 molecules, at least about 600,000 CD47 molecules, at least about 610,000 CD47 molecules, at least about 620,000 CD47 molecules, at least about 630,000 CD47 molecules, at least about 640,000 CD47 molecules, at least about 650,000 CD47 molecules, at least about 660,000 CD47 molecules, at least about 670,000 CD47 molecules, at least about 680,000 CD47 molecules, at least about 690,000 CD47 molecules, at least about 700,000 CD47 molecules, at least about 710,000 CD47 molecules, at least about 720,000 CD47 molecules, at least about 730,000 CD47 molecules, at least about 240,000 CD47 molecules, at least about 750,000 CD47 molecules, at least about 760,000 CD47 molecules, at least about 770,000 CD47 molecules, at least about 780,000 CD47 molecules, at least about 790,000 CD47 molecules, at least about 800,000 CD47 molecules, at least about 810,000 CD47 molecules, at least about 820,000 CD47 molecules, at least about 830,000 CD47 molecules, at least about 840,000 CD47 molecules, at least about 850,000 CD47 molecules, at least about 860,000 CD47 molecules, at least about 870,000 CD47 molecules, at least about 880,000 CD47 molecules, at least about 890,000 CD47 molecules, at least about 900,000 CD47 molecules, at least about 910,000 CD47 molecules, at

least about 920,000 CD47 molecules, at least about 930,000 CD47 molecules, at least about 940,000 CD47 molecules, at least about 950,000 CD47 molecules, at least about 960,000 CD47 molecules, at least about 970,000 CD47 molecules, at least about 980,000 CD47 molecules, at least about 990,000 CD47 molecules, or at least about 1,000,000 CD47 molecules per cell.

[00676] The expression level can be due to a number of factors known to those skilled in the art. For example, expression level of an exogenous polynucleotide encoding CD47 can be affected by, among other factors, the copy number of the exogenous polynucleotide in the cell, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more copies of the exogenous polynucleotide in the cell; the regulatory elements present, such as, *e.g.*, any of the regulatory elements described herein or known in the art, including any of the constitutive, inducible, or conditional promoters described herein or known in the art; the location where the exogenous polynucleotide is inserted into the genome of the cell; the type of vector used to introduce the exogenous polynucleotide into the cell; the ordering of cassettes in the exogenous polynucleotide, *e.g.*, bicistronic, etc.

[00677] The CD47 expression level is measured relative to the level of CD47 expressed in an unaltered or unmodified wild-type cell of the same cell type. For example, the CD47 expression level conferred by the exogenous polynucleotide encoding CD47 in a T cell is conveyed as an expression level relative to that of an unaltered or unmodified wild-type T cell; the CD47 expression level conferred by the exogenous polynucleotide encoding CD47 in an NK cell is conveyed as an expression level relative to that of an unaltered or unmodified wild-type NK cell; the CD47 expression level conferred by the exogenous polynucleotide encoding CD47 in an endothelial cell is conveyed as an expression level relative to that of an unaltered or unmodified wild-type endothelial cell; the CD47 expression level conferred by the exogenous polynucleotide encoding CD47 in a pancreatic islet cell is conveyed as an expression level relative to that of an unaltered or unmodified wild-type pancreatic islet cell; the CD47 expression level conferred by the exogenous polynucleotide encoding CD47 in a cardiac muscle cell is conveyed as an expression level relative to that of an unaltered or unmodified wild-type cardiac muscle cell; the CD47 expression level conferred by the exogenous polynucleotide encoding CD47 in a smooth muscle cell is conveyed as an expression level relative to that of an unaltered or unmodified wild-type smooth muscle cell; the CD47 expression level conferred by the exogenous polynucleotide encoding CD47 in a skeletal muscle cell is conveyed as an expression level relative to that of an unaltered or unmodified wild-type skeletal muscle cell; the CD47

expression level conferred by the exogenous polynucleotide encoding CD47 in a hepatocyte is conveyed as an expression level relative to that of an unaltered or unmodified wild-type hepatocyte; the CD47 expression level conferred by the exogenous polynucleotide encoding CD47 in a glial progenitor cell is conveyed as an expression level relative to that of an unaltered or unmodified wild-type glial progenitor cell; the CD47 expression level conferred by the exogenous polynucleotide encoding CD47 in a dopaminergic neuron is conveyed as an expression level relative to that of an unaltered or unmodified wild-type dopaminergic neuron; the CD47 expression level conferred by the exogenous polynucleotide encoding CD47 in a retinal pigment epithelial cell is conveyed as an expression level relative to that of an unaltered or unmodified wild-type retinal pigment epithelial cell; the CD47 expression level conferred by the exogenous polynucleotide encoding CD47 in a thyroid cell is conveyed as an expression level relative to that of an unaltered or unmodified wild-type thyroid cell.

[00678] In another embodiment, CD47 protein expression is detected using a Western blot of cell lysates probed with antibodies against the CD47 protein. In another embodiment, reverse transcriptase polymerase chain reactions (RT-PCR) are used to confirm the presence of the exogenous CD47 mRNA.

U. CD24

[00679] In some embodiments, the present disclosure provides a cell or population thereof that has been modified to express the tolerogenic factor (*e.g.*, immunomodulatory polypeptide) CD24. In some embodiments, the present disclosure provides a method for altering a cell genome to express CD24. In some embodiments, the stem cell expresses exogenous CD24. In some instances, the cell expresses an expression vector comprising a nucleotide sequence encoding a human CD24 polypeptide.

[00680] CD24 which is also referred to as a heat stable antigen or small-cell lung cancer cluster 4 antigen is a glycosylated glycosylphosphatidylinositol-anchored surface protein (Pirruccello et al., J Immunol, 1986, 136, 3779-3784; Chen et al., Glycobiology, 2017, 57, 800-806). It binds to Siglec-10 on innate immune cells. Recently it has been shown that CD24 via Siglec-10 acts as an innate immune checkpoint (Barkal et al., Nature, 2019, 572, 392-396).

[00681] In some embodiments, the cell outlined herein comprises a nucleotide sequence encoding a CD24 polypeptide has at least 95% sequence identity (*e.g.*, 95%, 96%, 97%, 98%, 99%, or more) to an amino acid sequence set forth in NCBI Ref. Nos. NP_001278666.1,

NP_001278667.1, NP_001278668.1, and NP_037362.1. In some embodiments, the cell outlined herein comprises a nucleotide sequence encoding a CD24 polypeptide having an amino acid sequence set forth in NCBI Ref. Nos. NP_001278666.1, NP_001278667.1, NP_001278668.1, and NP_037362.1.

[00682] In some embodiments, the cell comprises a nucleotide sequence having at least 85% sequence identity (*e.g.*, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) to the sequence set forth in NCBI Ref. Nos. NM_00129737.1, NM_00129738.1, NM_001291739.1, and NM_013230.3. In some embodiments, the cell comprises a nucleotide sequence as set forth in NCBI Ref. Nos. NM_00129737.1, NM_00129738.1, NM_001291739.1, and NM_013230.3.

[00683] In some embodiments, a suitable gene editing system (*e.g.*, CRISPR/Cas system or any of the gene editing systems described herein) is used to facilitate the insertion of a polynucleotide encoding CD24, into a genomic locus of the hypoimmunogenic cell. In some cases, the polynucleotide encoding CD24 is inserted into a safe harbor or target locus, such as but not limited to, an AAVS1, CCR5, CLYBL, ROSA26, SHS231, F3 (CD142), MICA, MICB, LRP1 (CD91), HMGB1, ABO, RHD, FUT1, or KDM5D gene locus. In some embodiments, the polynucleotide encoding CD24 is inserted into a B2M gene locus, a CIITA gene locus, a TRAC gene locus, or a TRB gene locus. In some embodiments, the polynucleotide encoding CD24 is inserted into any one of the gene loci depicted in Table 15 provided herein. In certain embodiments, the polynucleotide encoding CD24 is operably linked to a promoter.

[00684] In another embodiment, CD24 protein expression is detected using a Western blot of cells lysates probed with antibodies against the CD24 protein. In another embodiment, reverse transcriptase polymerase chain reactions (RT-PCR) are used to confirm the presence of the exogenous CD24 mRNA.

[00685] In some embodiments, a suitable gene editing system (*e.g.*, CRISPR/Cas system or any of the gene editing systems described herein) is used to facilitate the insertion of a polynucleotide encoding CD24, into a genomic locus of the hypoimmunogenic cell. In some cases, the polynucleotide encoding CD24 is inserted into a safe harbor or target locus, such as but not limited to, an AAVS1, CCR5, CLYBL, ROSA26, SHS231, F3 (also known as CD142), MICA, MICB, LRP1 (also known as CD91), HMGB1, ABO, RHD, FUT1, or KDM5D gene locus. In some embodiments, the polynucleotide encoding CD24 is inserted into a B2M gene

locus, a CIITA gene locus, a TRAC gene locus, or a TRB gene locus. In some embodiments, the polynucleotide encoding CD24 is inserted into any one of the gene loci depicted in Table 15 provided herein. In certain embodiments, the polynucleotide encoding CD24 is operably linked to a promoter.

V. DUX4

[00686] In some embodiments, the present disclosure provides a cell (*e.g.*, stem cell, induced pluripotent stem cell, differentiated cell, hematopoietic stem cell, primary T cell or CAR-T cell) or population thereof comprising a genome modified to increase expression of a tolerogenic or immunosuppressive factor such as DUX4. In some embodiments, the present disclosure provides a method for altering a cell's genome to provide increased expression of DUX4. In some embodiments, the disclosure provides a cell or population thereof comprising exogenously expressed DUX4 proteins. In some embodiments, increased expression of DUX4 suppresses, reduces or eliminates expression of one or more of the following MHC I molecules: HLA-A, HLA-B, and HLA-C.

[00687] DUX4 is a transcription factor that is active in embryonic tissues and induced pluripotent stem cells, and is silent in normal, healthy somatic tissues (Feng et al., 2015, ELife4; De Iaco et al., 2017, Nat Genet, 49, 941-945; Hendrickson et al., 2017, Nat Genet, 49, 925-934; Snider et al., 2010, PLoS Genet, e1001181; Whiddon et al., 2017, Nat Genet). DUX4 expression acts to block IFN-gamma mediated induction of major histocompatibility complex (MHC) class I gene expression (*e.g.*, expression of *B2M*, *HLA-A*, *HLA-B*, and *HLA-C*). DUX4 expression has been implicated in suppressed antigen presentation by MHC class I (Chew et al., Developmental Cell, 2019, 50, 1-14). DUX4 functions as a transcription factor in the cleavage-stage gene expression (transcriptional) program. Its target genes include, but are not limited to, coding genes, noncoding genes, and repetitive elements.

[00688] There are at least two isoforms of DUX4, with the longest isoform comprising the DUX4 C-terminal transcription activation domain. The isoforms are produced by alternative splicing. See, *e.g.*, Geng et al., 2012, Dev Cell, 22, 38-51; Snider et al., 2010, PLoS Genet, e1001181. Active isoforms for DUX4 comprise its N-terminal DNA-binding domains and its C-terminal activation domain. See, *e.g.*, Choi et al., 2016, Nucleic Acid Res, 44, 5161-5173.

[00689] It has been shown that reducing the number of CpG motifs of DUX4 decreases silencing of a DUX4 transgene (Jagannathan et al., Human Molecular Genetics, 2016,

25(20):4419-4431). The nucleic acid sequence provided in Jagannathan et al., supra represents a codon altered sequence of DUX4 comprising one or more base substitutions to reduce the total number of CpG sites while preserving the DUX4 protein sequence. The nucleic acid sequence is commercially available from Addgene, Catalog No. 99281.

[00690] In many embodiments, at least one or more polynucleotides may be utilized to facilitate the exogenous expression of DUX4 by a cell, *e.g.*, a stem cell, induced pluripotent stem cell, differentiated cell, hematopoietic stem cell, primary T cell or CAR-T cell.

[00691] In some embodiments, a suitable gene editing system (*e.g.*, CRISPR/Cas system or any of the gene editing systems described herein) is used to facilitate the insertion of a polynucleotide encoding DUX4, into a genomic locus of the hypoimmunogenic cell. In some cases, the polynucleotide encoding DUX4 is inserted into a safe harbor or target locus, such as but not limited to, an AAVS1, CCR5, CLYBL, ROSA26, SHS231, F3 (CD142), MICA, MICB, LRP1 (CD91), HMGB1, ABO, RHD, FUT1, or KDM5D gene locus. In some embodiments, the polynucleotide encoding DUX4 is inserted into a B2M gene locus, a CIITA gene locus, a TRAC gene locus, or a TRB gene locus. In some embodiments, the polynucleotide encoding DUX4 is inserted into any one of the gene loci depicted in Table 15 provided herein. In certain embodiments, the polynucleotide encoding DUX4 is operably linked to a promoter.

[00692] In some embodiments, the polynucleotide sequence encoding DUX4 comprises a polynucleotide sequence comprising a codon altered nucleotide sequence of DUX4 comprising one or more base substitutions to reduce the total number of CpG sites while preserving the DUX4 protein sequence. In some embodiments, the polynucleotide sequence encoding DUX4 comprising one or more base substitutions to reduce the total number of CpG sites has at least 85% (*e.g.*, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) sequence identity to SEQ ID NO:1 of PCT/US2020/44635, filed July 31, 2020. In some embodiments, the polynucleotide sequence encoding DUX4 is SEQ ID NO:1 of PCT/US2020/44635.

[00693] In some embodiments, the polynucleotide sequence encoding DUX4 is a nucleotide sequence encoding a polypeptide sequence having at least 95% (*e.g.*, 95%, 96%, 97%, 98%, 99% or 100%) sequence identity to a sequence selected from a group including SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ

ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, and SEQ ID NO:29, as provided in PCT/US2020/44635. In some embodiments, the polynucleotide sequence encoding DUX4 is a nucleotide sequence encoding a polypeptide sequence is selected from a group including SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, and SEQ ID NO:29. Amino acid sequences set forth as SEQ ID NOS:2-29 are shown in Figure 1A-1G of PCT/US2020/44635.

[00694] In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in GenBank Accession No. ACN62209.1 or an amino acid sequence set forth in GenBank Accession No. ACN62209.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in NCBI RefSeq No. NP_001280727.1 or an amino acid sequence set forth in NCBI RefSeq No. NP_001280727.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in GenBank Accession No. ACP30489.1 or an amino acid sequence set forth in GenBank Accession No. ACP30489.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in UniProt No. P0CJ85.1 or an amino acid sequence set forth in UniProt No. P0CJ85.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in GenBank Accession No. AUA60622.1 or an amino acid sequence set forth in GenBank Accession No. AUA60622.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in GenBank Accession No. ADK24683.1 or an amino acid sequence set forth in GenBank Accession No. ADK24683.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in GenBank Accession No. ACN62210.1 or an amino acid sequence set forth in GenBank Accession No. ACN62210.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence

having at least 95% sequence identity to the sequence set forth in GenBank Accession No. ADK24706.1 or an amino acid sequence set forth in GenBank Accession No. ADK24706.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in GenBank Accession No. ADK24685.1 or an amino acid sequence set forth in GenBank Accession No. ADK24685.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in GenBank Accession No. ACP30488.1 or an amino acid sequence set forth in GenBank Accession No. ACP30488.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in GenBank Accession No. ADK24687.1 or an amino acid sequence set forth in GenBank Accession No. ADK24687.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in GenBank Accession No. ACP30487.1 or an amino acid sequence set forth in GenBank Accession No. ACP30487.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in GenBank Accession No. ADK24717.1 or an amino acid sequence set forth in GenBank Accession No. ADK24717.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in GenBank Accession No. ADK24690.1 or an amino acid sequence set forth in GenBank Accession No. ADK24690.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in GenBank Accession No. ADK24689.1 or an amino acid sequence set forth in GenBank Accession No. ADK24689.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in GenBank Accession No. ADK24692.1 or an amino acid sequence set forth in GenBank Accession No. ADK24692.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in GenBank Accession No. ADK24693.1 or an amino acid sequence of set forth in GenBank Accession No. ADK24693.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in GenBank Accession No. ADK24712.1 or an amino acid sequence set forth in GenBank Accession No. ADK24712.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set

forth in GenBank Accession No. ADK24691.1 or an amino acid sequence set forth in GenBank Accession No. ADK24691.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in UniProt No. P0CJ87.1 or an amino acid sequence of set forth in UniProt No. P0CJ87.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in GenBank Accession No. ADK24714.1 or an amino acid sequence set forth in GenBank Accession No. ADK24714.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in GenBank Accession No. ADK24684.1 or an amino acid sequence of set forth in GenBank Accession No. ADK24684.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in GenBank Accession No. ADK24695.1 or an amino acid sequence set forth in GenBank Accession No. ADK24695.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in GenBank Accession No. ADK24699.1 or an amino acid sequence set forth in GenBank Accession No. ADK24699.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in NCBI RefSeq No. NP_001768.1 or an amino acid sequence set forth in NCBI RefSeq No. NP_001768. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in NCBI RefSeq No. NP_942088.1 or an amino acid sequence set forth in NCBI RefSeq No. NP_942088.1. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO:28 provided in PCT/US2020/44635 or an amino acid sequence of SEQ ID NO:28 provided in PCT/US2020/44635. In some instances, the DUX4 polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO:29 provided in PCT/US2020/44635 or an amino acid sequence of SEQ ID NO:29 provided in PCT/US2020/44635.

[00695] In other embodiments, expression of tolerogenic factors is facilitated using an expression vector. In some embodiments, the expression vector comprises a polynucleotide sequence encoding DUX4 is a codon altered sequence comprising one or more base substitutions to reduce the total number of CpG sites while preserving the DUX4 protein sequence. In some cases, the codon altered sequence of DUX4 comprises SEQ ID NO:1 of PCT/US2020/44635. In

some cases, the codon altered sequence of DUX4 is SEQ ID NO:1 of PCT/US2020/44635. In other embodiments, the expression vector comprises a polynucleotide sequence encoding DUX4 comprising SEQ ID NO:1 of PCT/US2020/44635. In some embodiments, the expression vector comprises a polynucleotide sequence encoding a DUX4 polypeptide sequence having at least 95% sequence identity to a sequence selected from a group including SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, and SEQ ID NO:29 of PCT/US2020/44635. In some embodiments, the expression vector comprises a polynucleotide sequence encoding a DUX4 polypeptide sequence selected from a group including SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, and SEQ ID NO:29 of PCT/US2020/44635.

[00696] An increase of DUX4 expression can be assayed using known techniques, such as Western blots, ELISA assays, FACS assays, immunoassays, and the like.

W. Additional Tolerogenic Factors

[00697] In certain embodiments, one or more tolerogenic factors can be inserted or reinserted into genome-edited cells to create immune-privileged universal donor cells, such as universal donor stem cells, universal donor T cells, or universal donor cells. In certain embodiments, the hypoimmunogenic cells disclosed herein have been further modified to express one or more tolerogenic factors. Exemplary tolerogenic factors include, without limitation, one or more of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and Serpinb9. In some embodiments, the tolerogenic factors are selected from the group consisting of CD200, HLA-G, HLA-E, HLA-C, HLA-E heavy chain, PD-L1, IDO1, CTLA4-Ig, IL-10, IL-35, FasL, Serpinb9, CCL21, CCL22, and Mfge8. In some embodiments, the tolerogenic factors are selected from the

group consisting of DUX4, HLA-C, HLA-E, HLA-F, HLA-G, PD-L1, CTLA-4-Ig, C1-inhibitor, and IL-35. In some embodiments, the tolerogenic factors are selected from the group consisting of HLA-C, HLA-E, HLA-F, HLA-G, PD-L1, CTLA-4-Ig, C1-inhibitor, and IL-35. In some embodiments, the tolerogenic factors are selected from a group including CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and Serpinb9.

[00698] Useful genomic, polynucleotide and polypeptide information about human CD27 (which is also known as CD27L receptor, Tumor Necrosis Factor Receptor Superfamily Member 7, TNFSF7, T Cell Activation Antigen S152, Tp55, and T14) are provided in, for example, the GeneCard Identifier GC12P008144, HGNC No. 11922, NCBI Gene ID 939, Uniprot No. P26842, and NCBI RefSeq Nos. NM_001242.4 and NP_001233.1.

[00699] Useful genomic, polynucleotide and polypeptide information about human CD46 are provided in, for example, the GeneCard Identifier GC01P207752, HGNC No. 6953, NCBI Gene ID 4179, Uniprot No. P15529, and NCBI RefSeq Nos. NM_002389.4, NM_153826.3, NM_172350.2, NM_172351.2, NM_172352.2 NP_758860.1, NM_172353.2, NM_172359.2, NM_172361.2, NP_002380.3, NP_722548.1, NP_758860.1, NP_758861.1, NP_758862.1, NP_758863.1, NP_758869.1, and NP_758871.1.

[00700] Useful genomic, polynucleotide and polypeptide information about human CD55 (also known as complement decay-accelerating factor) are provided in, for example, the GeneCard Identifier GC01P207321, HGNC No. 2665, NCBI Gene ID 1604, Uniprot No. P08174, and NCBI RefSeq Nos. NM_000574.4, NM_001114752.2, NM_001300903.1, NM_001300904.1, NP_000565.1, NP_001108224.1, NP_001287832.1, and NP_001287833.1.

[00701] Useful genomic, polynucleotide and polypeptide information about human CD59 are provided in, for example, the GeneCard Identifier GC11M033704, HGNC No. 1689, NCBI Gene ID 966, Uniprot No. P13987, and NCBI RefSeq Nos. NP_000602.1, NM_000611.5, NP_001120695.1, NM_001127223.1, NP_001120697.1, NM_001127225.1, NP_001120698.1, NM_001127226.1, NP_001120699.1, NM_001127227.1, NP_976074.1, NM_203329.2, NP_976075.1, NM_203330.2, NP_976076.1, and NM_203331.2.

[00702] Useful genomic, polynucleotide and polypeptide information about human CD200 are provided in, for example, the GeneCard Identifier GC03P112332, HGNC No. 7203, NCBI Gene

ID 4345, Uniprot No. P41217, and NCBI RefSeq Nos. NP_001004196.2, NM_001004196.3, NP_001305757.1, NM_001318828.1, NP_005935.4, NM_005944.6, XP_005247539.1, and XM_005247482.2.

[00703] Useful genomic, polynucleotide and polypeptide information about human HLA-C are provided in, for example, the GeneCard Identifier GC06M031272, HGNC No. 4933, NCBI Gene ID 3107, Uniprot No. P10321, and NCBI RefSeq Nos. NP_002108.4 and NM_002117.5.

[00704] Useful genomic, polynucleotide and polypeptide information about human HLA-E are provided in, for example, the GeneCard Identifier GC06P047281, HGNC No. 4962, NCBI Gene ID 3133, Uniprot No. P13747, and NCBI RefSeq Nos. NP_005507.3 and NM_005516.5.

[00705] Useful genomic, polynucleotide and polypeptide information about human HLA-G are provided in, for example, the GeneCard Identifier GC06P047256, HGNC No. 4964, NCBI Gene ID 3135, Uniprot No. P17693, and NCBI RefSeq Nos. NP_002118.1 and NM_002127.5.

[00706] Useful genomic, polynucleotide and polypeptide information about human PD-L1 or CD274 are provided in, for example, the GeneCard Identifier GC09P005450, HGNC No. 17635, NCBI Gene ID 29126, Uniprot No. Q9NZQ7, and NCBI RefSeq Nos. NP_001254635.1, NM_001267706.1, NP_054862.1, and NM_014143.3.

[00707] Useful genomic, polynucleotide and polypeptide information about human IDO1 are provided in, for example, the GeneCard Identifier GC08P039891, HGNC No. 6059, NCBI Gene ID 3620, Uniprot No. P14902, and NCBI RefSeq Nos. NP_002155.1 and NM_002164.5.

[00708] Useful genomic, polynucleotide and polypeptide information about human IL-10 are provided in, for example, the GeneCard Identifier GC01M206767, HGNC No. 5962, NCBI Gene ID 3586, Uniprot No. P22301, and NCBI RefSeq Nos. NP_000563.1 and NM_000572.2.

[00709] Useful genomic, polynucleotide and polypeptide information about human Fas ligand (which is known as FasL, FASLG, CD178, TNFSF6, and the like) are provided in, for example, the GeneCard Identifier GC01P172628, HGNC No. 11936, NCBI Gene ID 356, Uniprot No. P48023, and NCBI RefSeq Nos. NP_000630.1, NM_000639.2, NP_001289675.1, and NM_001302746.1.

[00710] Useful genomic, polynucleotide and polypeptide information about human CCL21 are provided in, for example, the GeneCard Identifier GC09M034709, HGNC No. 10620, NCBI Gene ID 6366, Uniprot No. O00585, and NCBI RefSeq Nos. NP_002980.1 and NM_002989.3.

[00711] Useful genomic, polynucleotide and polypeptide information about human CCL22 are provided in, for example, the GeneCard Identifier GC16P057359, HGNC No. 10621, NCBI Gene ID 6367, Uniprot No. O00626, and NCBI RefSeq Nos. NP_002981.2, NM_002990.4, XP_016879020.1, and XM_017023531.1.

[00712] Useful genomic, polynucleotide and polypeptide information about human Mfge8 are provided in, for example, the GeneCard Identifier GC15M088898, HGNC No. 7036, NCBI Gene ID 4240, Uniprot No. Q08431, and NCBI RefSeq Nos. NP_001108086.1, NM_001114614.2, NP_001297248.1, NM_001310319.1, NP_001297249.1, NM_001310320.1, NP_001297250.1, NM_001310321.1, NP_005919.2, and NM_005928.3.

[00713] Useful genomic, polynucleotide and polypeptide information about human SerpinB9 are provided in, for example, the GeneCard Identifier GC06M002887, HGNC No. 8955, NCBI Gene ID 5272, Uniprot No. P50453, and NCBI RefSeq Nos. NP_004146.1, NM_004155.5, XP_005249241.1, and XM_005249184.4.

[00714] Methods for modulating expression of genes and factors (proteins) include genome editing technologies, and, RNA or protein expression technologies and the like. For all of these technologies, well known recombinant techniques are used, to generate recombinant nucleic acids as outlined herein.

[00715] In some embodiments, the cells (*e.g.*, stem cell, induced pluripotent stem cell, differentiated cell, hematopoietic stem cell, primary T cell or CAR-T cell) possess genetic modifications that inactivate the B2M and CIITA genes and express a plurality of exogenous polypeptides selected from the group including CD47 and DUX4, CD47 and CD24, CD47 and CD27, CD47 and CD46, CD47 and CD55, CD47 and CD59, CD47 and CD200, CD47 and HLA-C, CD47 and HLA-E, CD47 and HLA-E heavy chain, CD47 and HLA-G, CD47 and PD-L1, CD47 and IDO1, CD47 and CTLA4-Ig, CD47 and C1-Inhibitor, CD47 and IL-10, CD47 and IL-35, CD47 and IL-39, CD47 and FasL, CD47 and CCL21, CD47 and CCL22, CD47 and Mfge8, and CD47 and Serpinb9, and any combination thereof. In some instances, such cells also possess a genetic modification that inactivates the CD142 gene.

[00716] In some instances, a gene editing system such as the CRISPR/Cas system is used to facilitate the insertion of tolerogenic factors, such as the tolerogenic factors into a safe harbor or target locus, such as the AAVS1 locus, to actively inhibit immune rejection. In some instances, the tolerogenic factors are inserted into a safe harbor or target locus using an expression vector.

In some embodiments, the safe harbor or target locus is an AAVS1, CCR5, CLYBL, ROSA26, SHS231, F3 (also known as CD142), MICA, MICB, LRP1 (also known as CD91), HMGB1, ABO, RHD, FUT1, or KDM5D gene locus.

[00717] In some embodiments, expression of a target gene (*e.g.*, CD47, or another tolerogenic factor gene) is increased by expression of fusion protein or a protein complex containing (1) a site-specific binding domain specific for the endogenous target gene (*e.g.*, CD47, or another tolerogenic factor gene) and (2) a transcriptional activator.

[00718] In some embodiments, the regulatory factor is comprised of a site specific DNA-binding nucleic acid molecule, such as a guide RNA (gRNA). In some embodiments, the method is achieved by site specific DNA-binding targeted proteins, such as zinc finger proteins (ZFP) or fusion proteins containing ZFP, which are also known as zinc finger nucleases (ZFNs).

[00719] In some embodiments, the regulatory factor comprises a site-specific binding domain, such as using a DNA binding protein or DNA-binding nucleic acid, which specifically binds to or hybridizes to the gene at a targeted region. In some embodiments, the provided polynucleotides or polypeptides are coupled to or complexed with a site-specific nuclease, such as a modified nuclease. For example, in some embodiments, the administration is effected using a fusion comprising a DNA-targeting protein of a modified nuclease, such as a meganuclease or an RNA-guided nuclease such as a clustered regularly interspersed short palindromic nucleic acid (CRISPR)-Cas system, such as CRISPR-Cas9 system. In some embodiments, the nuclease is modified to lack nuclease activity. In some embodiments, the modified nuclease is a catalytically dead dCas9.

[00720] In some embodiments, the site specific binding domain may be derived from a nuclease. For example, the recognition sequences of homing endonucleases and meganucleases such as I-SceI, I-CeuI, PI-PspI, PI-Sce, I-SceIV, I-CsmI, I-PanI, I-SceII, I-PpoI, I-SceIII, I-CreI, I-TevI, I-TevII and I-TevIII. See also U.S. Patent No. 5,420,032; U.S. Patent No. 6,833,252; Belfort *et al.* , (1997) Nucleic Acids Res. 25:3379-3388; Dujon *et al.*, (1989) Gene 82:115-118; Perler *et al.*, (1994) Nucleic Acids Res. 22, 1125-1127; Jasin (1996) Trends Genet. 12:224-228; Gimble *et al.*, (1996) J. Mol. Biol. 263:163-180; Argast *et al.*, (1998) J. Mol. Biol. 280:345-353 and the New England Biolabs catalogue. In addition, the DNA-binding specificity of homing endonucleases and meganucleases can be engineered to bind non-natural target sites. See, for example, Chevalier *et al.*, (2002) Molec. Cell 10:895-905; Epinat *et al.*, (2003) Nucleic Acids

Res. 31 :2952-2962; Ashworth *et al.,* (2006) Nature 441 :656-659; Paques *et al.,* (2007) Current Gene Therapy 7:49-66; U.S. Patent Publication No. 2007/0117128.

[00721] Zinc finger, TALE, and CRISPR system binding domains can be "engineered" to bind to a predetermined nucleotide sequence, for example via engineering (altering one or more amino acids) of the recognition helix region of a naturally occurring zinc finger or TALE protein. Engineered DNA binding proteins (zinc fingers or TALEs) are proteins that are non-naturally occurring. Rational criteria for design include application of substitution rules and computerized algorithms for processing information in a database storing information of existing ZFP and/or TALE designs and binding data. See, for example, U.S. Pat. Nos. 6,140,081; 6,453,242; and 6,534,261; see also WO 98/53058; WO 98/53059; WO 98/53060; WO 02/016536 and WO 03/016496 and U.S. Publication No. 20110301073.

[00722] In some embodiments, the site-specific binding domain comprises one or more zinc-finger proteins (ZFPs) or domains thereof that bind to DNA in a sequence-specific manner. A ZFP or domain thereof is a protein or domain within a larger protein that binds DNA in a sequence-specific manner through one or more zinc fingers, regions of amino acid sequence within the binding domain whose structure is stabilized through coordination of a zinc ion.

[00723] Among the ZFPs are artificial ZFP domains targeting specific DNA sequences, typically 9-18 nucleotides long, generated by assembly of individual fingers. ZFPs include those in which a single finger domain is approximately 30 amino acids in length and contains an alpha helix containing two invariant histidine residues coordinated through zinc with two cysteines of a single beta turn, and having two, three, four, five, or six fingers. Generally, sequence-specificity of a ZFP may be altered by making amino acid substitutions at the four helix positions (−1, 2, 3 and 6) on a zinc finger recognition helix. Thus, in some embodiments, the ZFP or ZFP-containing molecule is non-naturally occurring, *e.g.*, is engineered to bind to a target site of choice. See, for example, Beerli *et al.* (2002) Nature Biotechnol. 20:135-141; Pabo *et al.* (2001) Ann. Rev. Biochem. 70:313-340; Isalan *et al.* (2001) Nature Biotechnol. 19:656-660; Segal *et al.* (2001) Curr. Opin. Biotechnol. 12:632-637; Choo *et al.* (2000) Curr. Opin. Struct. Biol. 10:411-416; U.S. Pat. Nos. 6,453,242; 6,534,261; 6,599,692; 6,503,717; 6,689,558; 7,030,215; 6,794,136; 7,067,317; 7,262,054; 7,070,934; 7,361,635; 7,253,273; and U.S. Patent Publication Nos. 2005/0064474; 2007/0218528; 2005/0267061, all incorporated herein by reference in their entireties.

[00724] Many gene-specific engineered zinc fingers are available commercially. For example, Sangamo Biosciences (Richmond, CA, USA) has developed a platform (CompoZr) for zinc-finger construction in partnership with Sigma–Aldrich (St. Louis, MO, USA), allowing investigators to bypass zinc-finger construction and validation altogether, and provides specifically targeted zinc fingers for thousands of proteins (Gaj *et al.*, Trends in Biotechnology, 2013, 31(7), 397-405). In some embodiments, commercially available zinc fingers are used or are custom designed.

[00725] In some embodiments, the site-specific binding domain comprises a naturally occurring or engineered (non-naturally occurring) transcription activator-like protein (TAL) DNA binding domain, such as in a transcription activator-like protein effector (TALE) protein, See, *e.g.*, U.S. Patent Publication No. 20110301073, incorporated by reference in its entirety herein.

[00726] In some embodiments, the site-specific binding domain is derived from the CRISPR/Cas system. In general, "CRISPR system" refers collectively to transcripts and other elements involved in the expression of or directing the activity of CRISPR-associated ("Cas") genes, including sequences encoding a Cas gene, a tracr (trans-activating CRISPR) sequence (*e.g.* tracrRNA or an active partial tracrRNA), a tracr-mate sequence (encompassing a "direct repeat" and a tracrRNA-processed partial direct repeat in the context of an endogenous CRISPR system), a guide sequence (also referred to as a "spacer" in the context of an endogenous CRISPR system, or a "targeting sequence"), and/or other sequences and transcripts from a CRISPR locus.

[00727] In general, a guide sequence includes a targeting domain comprising a polynucleotide sequence having sufficient complementarity with a target polynucleotide sequence to hybridize with the target sequence and direct sequence-specific binding of the CRISPR complex to the target sequence. In some embodiments, the degree of complementarity between a guide sequence and its corresponding target sequence, when optimally aligned using a suitable alignment algorithm, is about or more than about 50%, 60%, 75%, 80%, 85%, 90%, 95%, 97.5%, 99%, or more. In some examples, the targeting domain of the gRNA is complementary, *e.g.*, at least 80, 85, 90, 95, 98 or 99% complementary, *e.g.*, fully complementary, to the target sequence on the target nucleic acid.

[00728] In some embodiments, the target site is upstream of a transcription initiation site of the target gene. In some embodiments, the target site is adjacent to a transcription initiation site of the gene. In some embodiments, the target site is adjacent to an RNA polymerase pause site downstream of a transcription initiation site of the gene.

[00729] In some embodiments, the targeting domain is configured to target the promoter region of the target gene to promote transcription initiation, binding of one or more transcription enhancers or activators, and/or RNA polymerase. One or more gRNA can be used to target the promoter region of the gene. In some embodiments, one or more regions of the gene can be targeted. In certain aspects, the target sites are within 600 base pairs on either side of a transcription start site (TSS) of the gene.

[00730] It is within the level of a skilled artisan to design or identify a gRNA sequence that is or comprises a sequence targeting a gene, including the exon sequence and sequences of regulatory regions, including promoters and activators. A genome-wide gRNA database for CRISPR genome editing is publicly available, which contains exemplary single guide RNA (sgRNA) target sequences in constitutive exons of genes in the human genome or mouse genome (see *e.g.*, genescript.com/gRNA-database.html; see also, Sanjana *et al.* (2014) Nat. Methods, 11:783-4; www.e-crisp.org/E-CRISP/; crispr.mit.edu/). In some embodiments, the gRNA sequence is or comprises a sequence with minimal off-target binding to a non-target gene.

[00731] In some embodiments, the regulatory factor further comprises a functional domain, *e.g.*, a transcriptional activator.

[00732] In some embodiments, the transcriptional activator is or contains one or more regulatory elements, such as one or more transcriptional control elements of a target gene, whereby a site-specific domain as provided above is recognized to drive expression of such gene. In some embodiments, the transcriptional activator drives expression of the target gene. In some cases, the transcriptional activator, can be or contain all or a portion of an heterologous transactivation domain. For example, in some embodiments, the transcriptional activator is selected from Herpes simplex–derived transactivation domain, Dnmt3a methyltransferase domain, p65, VP16, and VP64.

[00733] In some embodiments, the regulatory factor is a zinc finger transcription factor (ZF-TF). In some embodiments, the regulatory factor is VP64-p65-Rta (VPR).

[00734] In certain embodiments, the regulatory factor further comprises a transcriptional regulatory domain. Common domains include, *e.g.*, transcription factor domains (activators, repressors, co-activators, co-repressors), silencers, oncogenes (*e.g.*, myc, jun, fos, myb, max, mad, rel, ets, bcl, myb, mos family members etc.); DNA repair enzymes and their associated factors and modifiers; DNA rearrangement enzymes and their associated factors and modifiers; chromatin associated proteins and their modifiers (*e.g.* kinases, acetylases and deacetylases); and DNA modifying enzymes (*e.g.*, methyltransferases such as members of the DNMT family (*e.g.*, DNMT1, DNMT3A, DNMT3B, DNMT3L, etc., topoisomerases, helicases, ligases, kinases, phosphatases, polymerases, endonucleases) and their associated factors and modifiers. See, *e.g.*, U.S. Publication No. 2013/0253040, incorporated by reference in its entirety herein.

[00735] Suitable domains for achieving activation include the HSV VP 16 activation domain (see, *e.g.*, Hagmann *et al.*, J. Virol. 71, 5952-5962 (1 97)) nuclear hormone receptors (see, *e.g.*, Torchia *et al.*, Curr. Opin. Cell. Biol. 10:373-383 (1998)); the p65 subunit of nuclear factor kappa B (Bitko & Bank, J. Virol. 72:5610-5618 (1998) and Doyle & Hunt, Neuroreport 8:2937-2942 (1997)); Liu *et al.*, Cancer Gene Ther. 5:3-28 (1998)), or artificial chimeric functional domains such as VP64 (Beerli *et al.*, (1998) Proc. Natl. Acad. Sci. USA 95:14623-33), and degron (Molinari *et al.*, (1999) EMBO J. 18, 6439-6447). Additional exemplary activation domains include, Oct 1, Oct-2A, Spl, AP-2, and CTF1 (Seipel etal, EMBOJ. 11, 4961-4968 (1992) as well as p300, CBP, PCAF, SRC1 PvALF, AtHD2A and ERF-2. See, for example, Robyr *et al.*, (2000) Mol. Endocrinol. 14:329-347; Collingwood *et al.*, (1999) J. Mol. Endocrinol 23:255-275; Leo *et al.*, (2000) Gene 245:1-11; Manteuffel-Cymborowska (1999) Acta Biochim. Pol. 46:77-89; McKenna *et al.*, (1999) J. Steroid Biochem. Mol. Biol. 69:3-12; Malik *et al.*, (2000) Trends Biochem. Sci. 25:277-283; and Lemon *et al.*, (1999) Curr. Opin. Genet. Dev. 9:499-504. Additional exemplary activation domains include, but are not limited to, OsGAI, HALF-1, Cl, AP1, ARF-5, -6,-1, and -8, CPRF1, CPRF4, MYC-RP/GP, and TRAB1 , See, for example, Ogawa *et al.*, (2000) Gene 245:21-29; Okanami *et al.*, (1996) Genes Cells 1 :87-99; Goff *et al.*, (1991) Genes Dev. 5:298-309; Cho *et al.*, (1999) Plant Mol Biol 40:419-429; Ulmason *et al.*, (1999) Proc. Natl. Acad. Sci. USA 96:5844-5849; Sprenger-Haussels *et al.*, (2000) Plant J. 22:1-8; Gong *et al.*, (1999) Plant Mol. Biol. 41:33-44; and Hobo *et al.* , (1999) Proc. Natl. Acad. Sci. USA 96:15,348-15,353.

[00736] Exemplary repression domains that can be used to make genetic repressors include, but are not limited to, KRAB A/B, KOX, TGF-beta-inducible early gene (TIEG), v-erbA, SID, MBD2, MBD3, members of the DNMT family (*e.g.*, DNMT1, DNMT3A, DNMT3B, DNMT3L, etc.), Rb, and MeCP2. See, for example, Bird *et al.,* (1999) Cell 99:451-454; Tyler *et al.,* (1999) Cell 99:443-446; Knoepfler *et al.,* (1999) Cell 99:447-450; and Robertson *et al.,* (2000) Nature Genet. 25:338-342. Additional exemplary repression domains include, but are not limited to, ROM2 and AtHD2A. See, for example, Chem *et al.,* (1996) Plant Cell 8:305-321; and Wu *et al.,* (2000) Plant J. 22:19-27.

[00737] In some instances, the domain is involved in epigenetic regulation of a chromosome. In some embodiments, the domain is a histone acetyltransferase (HAT), *e.g.* type- A, nuclear localized such as MYST family members MOZ, Ybf2/Sas3, MOF, and Tip60, GNAT family members Gcn5 or pCAF, the p300 family members CBP, p300 or Rttl09 (Bemdsen and Denu (2008) Curr Opin Struct Biol 18(6):682-689). In other instances the domain is a histone deacetylase (HD AC) such as the class I (HDAC-l, 2, 3, and 8), class II (HDAC IIA (HDAC-4, 5, 7 and 9), HD AC IIB (HDAC 6 and 10)), class IV (HDAC-l 1), class III (also known as sirtuins (SIRTs); SIRT1-7) (see Mottamal *et al.*, (2015) Molecules 20(3):3898-394l). Another domain that is used in some embodiments is a histone phosphorylase or kinase, where examples include MSK1, MSK2, ATR, ATM, DNA-PK, Bubl, VprBP, IKK-a, PKCpi, Dik/Zip, JAK2, PKC5, WSTF and CK2. In some embodiments, a methylation domain is used and may be chosen from groups such as Ezh2, PRMT1/6, PRMT5/7, PRMT 2/6, CARM1, set7/9, MLL, ALL-1, Suv 39h, G9a, SETDB1, Ezh2, Set2, Dotl, PRMT 1/6, PRMT 5/7, PR-Set7 and Suv4-20h, Domains involved in sumoylation and biotinylation (Lys9, 13, 4, 18 and 12) may also be used in some embodiments (review see Kousarides (2007) Cell 128:693-705).

[00738] Fusion molecules are constructed by methods of cloning and biochemical conjugation that are well known to those of skill in the art. Fusion molecules comprise a DNA-binding domain and a functional domain (*e.g.*, a transcriptional activation or repression domain). Fusion molecules also optionally comprise nuclear localization signals (such as, for example, that from the SV40 medium T-antigen) and epitope tags (such as, for example, FLAG and hemagglutinin). Fusion proteins (and nucleic acids encoding them) are designed such that the translational reading frame is preserved among the components of the fusion.

[00739] Fusions between a polypeptide component of a functional domain (or a functional fragment thereof) on the one hand, and a non-protein DNA-binding domain (*e.g.*, antibiotic, intercalator, minor groove binder, nucleic acid) on the other, are constructed by methods of biochemical conjugation known to those of skill in the art. See, for example, the Pierce Chemical Company (Rockford, IL) Catalogue. Methods and compositions for making fusions between a minor groove binder and a polypeptide have been described. Mapp *et al.,* (2000) Proc. Natl. Acad. Sci. USA 97:3930-3935. Likewise, CRISPR/Cas TFs and nucleases comprising a sgRNA nucleic acid component in association with a polypeptide component function domain are also known to those of skill in the art and detailed herein.

[00740] In some embodiments, the present disclosure provides a cell (*e.g.*, a primary T cell and a hypoimmunogenic stem cell and derivative thereof) or population thereof comprising a genome in which the cell genome has been modified to regulatably express CD47. In some embodiments, the present disclosure provides a method for altering a cell genome to regulatably express CD47. In certain embodiments, at least one ribonucleic acid or at least one pair of ribonucleic acids may be utilized to facilitate the insertion of CD47 into a cell line. In certain embodiments, the at least one ribonucleic acid or the at least one pair of ribonucleic acids is selected from the group consisting of SEQ ID NOS:200784-231885 of Table 29 of WO2016183041, which is herein incorporated by reference.

[00741] In some embodiments, the present disclosure provides a cell (*e.g.*, a primary T cell and a hypoimmunogenic stem cell and derivative thereof) or population thereof comprising a genome in which the cell genome has been modified to express HLA-C. In some embodiments, the present disclosure provides a method for altering a cell genome to express HLA-C. In certain embodiments, at least one ribonucleic acid or at least one pair of ribonucleic acids may be utilized to facilitate the insertion of HLA-C into a cell line. In certain embodiments, the at least one ribonucleic acid or the at least one pair of ribonucleic acids is selected from the group consisting of SEQ ID NOS:3278-5183 of Table 10 of WO2016183041, which is herein incorporated by reference.

[00742] In some embodiments, the present disclosure provides a cell (*e.g.*, a primary T cell and a hypoimmunogenic stem cell and derivative thereof) or population thereof comprising a genome in which the cell genome has been modified to express HLA-E. In some embodiments, the present disclosure provides a method for altering a cell genome to express HLA-E. In certain

embodiments, at least one ribonucleic acid or at least one pair of ribonucleic acids may be utilized to facilitate the insertion of HLA-E into a cell line. In certain embodiments, the at least one ribonucleic acid or the at least one pair of ribonucleic acids is selected from the group consisting of SEQ ID NOS:189859-193183 of Table 19 of WO2016183041, which is herein incorporated by reference.

[00743] In some embodiments, the present disclosure provides a cell (*e.g.*, a primary T cell and a hypoimmunogenic stem cell and derivative thereof) or population thereof comprising a genome in which the cell genome has been modified to express HLA-F. In some embodiments, the present disclosure provides a method for altering a cell genome to express HLA-F. In certain embodiments, at least one ribonucleic acid or at least one pair of ribonucleic acids may be utilized to facilitate the insertion of HLA-F into a cell line. In certain embodiments, the at least one ribonucleic acid or the at least one pair of ribonucleic acids is selected from the group consisting of SEQ ID NOS: 688808-399754 of Table 45 of WO2016183041, which is herein incorporated by reference.

[00744] In some embodiments, the present disclosure provides a cell (*e.g.*, a primary T cell and a hypoimmunogenic stem cell and derivative thereof) or population thereof comprising a genome in which the cell genome has been modified to express HLA-G. In some embodiments, the present disclosure provides a method for altering a cell genome to express HLA-G. In certain embodiments, at least one ribonucleic acid or at least one pair of ribonucleic acids may be utilized to facilitate the insertion of HLA-G into a stem cell line. In certain embodiments, the at least one ribonucleic acid or the at least one pair of ribonucleic acids is selected from the group consisting of SEQ ID NOS:188372-189858 of Table 18 of WO2016183041, which is herein incorporated by reference.

[00745] In some embodiments, the present disclosure provides a cell (*e.g.*, a primary T cell and a hypoimmunogenic stem cell and derivative thereof) or population thereof comprising a genome in which the cell genome has been modified to express PD-L1. In some embodiments, the present disclosure provides a method for altering a cell genome to express PD-L1. In certain embodiments, at least one ribonucleic acid or at least one pair of ribonucleic acids may be utilized to facilitate the insertion of PD-L1 into a stem cell line. In certain embodiments, the at least one ribonucleic acid or the at least one pair of ribonucleic acids is selected from the group

consisting of SEQ ID NOS:193184-200783 of Table 21 of WO2016183041, which is herein incorporated by reference.

[00746] In some embodiments, the present disclosure provides a cell (*e.g.*, a primary T cell and a hypoimmunogenic stem cell and derivative thereof) or population thereof comprising a genome in which the cell genome has been modified to express CTLA4-Ig. In some embodiments, the present disclosure provides a method for altering a cell genome to express CTLA4-Ig. In certain embodiments, at least one ribonucleic acid or at least one pair of ribonucleic acids may be utilized to facilitate the insertion of CTLA4-Ig into a stem cell line. In certain embodiments, the at least one ribonucleic acid or the at least one pair of ribonucleic acids is selected from any one disclosed in WO2016183041, including the sequence listing.

[00747] In some embodiments, the present disclosure provides a cell (*e.g.*, a primary T cell and a hypoimmunogenic stem cell and derivative thereof) or population thereof comprising a genome in which the cell genome has been modified to express CI-inhibitor. In some embodiments, the present disclosure provides a method for altering a cell genome to express CI-inhibitor. In certain embodiments, at least one ribonucleic acid or at least one pair of ribonucleic acids may be utilized to facilitate the insertion of CI-inhibitor into a stem cell line. In certain embodiments, the at least one ribonucleic acid or the at least one pair of ribonucleic acids is selected from any one disclosed in WO2016183041, including the sequence listing.

[00748] In some embodiments, the present disclosure provides a cell (*e.g.*, a primary T cell and a hypoimmunogenic stem cell and derivative thereof) or population thereof comprising a genome in which the cell genome has been modified to express IL-35. In some embodiments, the present disclosure provides a method for altering a cell genome to express IL-35. In certain embodiments, at least one ribonucleic acid or at least one pair of ribonucleic acids may be utilized to facilitate the insertion of IL-35 into a stem cell line. In certain embodiments, the at least one ribonucleic acid or the at least one pair of ribonucleic acids is selected from any one disclosed in WO2016183041, including the sequence listing.

[00749] In some embodiments, the tolerogenic factors are expressed in a cell using an expression vector. For example, the expression vector for expressing CD47 in a cell comprises a polynucleotide sequence encoding CD47. The expression vector can be an inducible expression vector. The expression vector can be a viral vector, such as but not limited to, a lentiviral vector. In some embodiments, the tolerogenic factors are introduced into the cells using fusogen-

mediated delivery or a transposase system selected from the group consisting of conditional or inducible transposases, conditional or inducible PiggyBac transposons, conditional or inducible Sleeping Beauty (SB11) transposons, conditional or inducible Mos1 transposons, and conditional or inducible Tol2 transposons.

[00750] In some embodiments, a suitable gene editing system (*e.g.*, CRISPR/Cas system or any of the gene editing systems described herein) is used to facilitate the insertion of a polynucleotide encoding a tolerogenic factor, into a genomic locus of the hypoimmunogenic cell. In some cases, the polynucleotide encoding the tolerogenic factor is inserted into a safe harbor or target locus, such as but not limited to, an AAVS1, CCR5, CLYBL, ROSA26, SHS231, F3 (CD142), MICA, MICB, LRP1 (CD91), HMGB1, ABO, RHD, FUT1, or KDM5D gene locus. In some embodiments, the polynucleotide encoding the tolerogenic factor is inserted into a B2M gene locus, a CIITA gene locus, a TRAC gene locus, or a TRB gene locus. In some embodiments, the polynucleotide encoding the tolerogenic factor is inserted into any one of the gene loci depicted in Table 16 provided herein. In certain embodiments, the polynucleotide encoding the tolerogenic factor is operably linked to a promoter.

[00751] In some embodiments, the cells are engineered to expresses an increased amount of one or more of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 relative to a cell of the same cell type that does not comprise the modifications.

[00752] The amount of increased CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 expression can be measured, for example, as a multiple, a fold, or a percentage of expression relative to the unaltered or unmodified wild-type cell. For example, in some embodiments, the cells described herein express at least about 1x, at least about 1.1x, at least about 1.2x, at least about 1.3x, at least about 1.4x, at least about 1.5x, at least about 1.6x, at least about 1.7x, at least about 1.8x, at least about 1.9x, at least about 2x, at least about 2.1x, at least about 2.2x, at least about 2.3x, at least about 2.4x, at least about 2.5x, at least about 2.6x, at least about 2.7x, at least about 2.8x, at least about 2.9x, at least about 3x, at least about 3.1x, at least about 3.2x, at least about 3.3x, at least about 3.4x, at least about 3.5x, at least about 3.6x, at least

about 3.7x, at least about 3.8x, at least about 3.9x, at least about 4x, at least about 4.1x, at least about 4.2x, at least about 4.3x, at least about 4.4x, at least about 4.5x, at least about 4.6x, at least about 4.7x, at least about 4.8x, at least about 4.9x, at least about 5x, at least about 5.1x, at least about 5.2x, at least about 5.3x, at least about 5.4x, at least about 5.5x, at least about 5.6x, at least about 5.7x, at least about 5.8x, at least about 5.9x, at least about 6x, at least about 6.1x, at least about 6.2x, at least about 6.3x, at least about 6.4x, at least about 6.5x, at least about 6.6x, at least about 6.7x, at least about 6.8x, at least about 6.9x, at least about 7x, at least about 7.1x, at least about 7.2x, at least about 7.3x, at least about 7.4x, at least about 7.5x, at least about 7.6x, at least about 7.7x, at least about 7.8x, at least about 7.9x, at least about 8x, at least about 8.1x, at least about 8.2x, at least about 8.3x, at least about 8.4x, at least about 8.5x, at least about 8.6x, at least about 8.7x, at least about 8.8x, at least about 8.9x, at least about 9x, at least about 9.1x, at least about 9.2x, at least about 9.3x, at least about 9.4x, at least about 9.5x, at least about 9.6x, at least about 9.7x, at least about 9.8x, at least about 9.9x, at least about 10x, or more, of the level of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 expressed in an unaltered or unmodified wild-type cell of the same cell type.

[00753] In some embodiments, the cells described herein express at least about 1-fold, at least about 1.1-fold, at least about 1.2-fold, at least about 1.3-fold, at least about 1.4-fold, at least about 1.5-fold, at least about 1.6-fold, at least about 1.7-fold, at least about 1.8-fold, at least about 1.9-fold, at least about 2-fold, at least about 2.1-fold, at least about 2.2-fold, at least about 2.3-fold, at least about 2.4-fold, at least about 2.5-fold, at least about 2.6-fold, at least about 2.7-fold, at least about 2.8-fold, at least about 2.9-fold, at least about 3-fold, at least about 3.1-fold, at least about 3.2-fold, at least about 3.3-fold, at least about 3.4-fold, at least about 3.5-fold, at least about 3.6-fold, at least about 3.7-fold, at least about 3.8-fold, at least about 3.9-fold, at least about 4-fold, at least about 4.1-fold, at least about 4.2-fold, at least about 4.3-fold, at least about 4.4-fold, at least about 4.5-fold, at least about 4.6-fold, at least about 4.7-fold, at least about 4.8-fold, at least about 4.9-fold, at least about 5-fold, at least about 5.1-fold, at least about 5.2-fold, at least about 5.3-fold, at least about 5.4-fold, at least about 5.5-fold, at least about 5.6-fold, at least about 5.7-fold, at least about 5.8-fold, at least about 5.9-fold, at least about 6-fold, at least about 6.1-fold, at least about 6.2-fold, at least about 6.3-fold, at least about 6.4-fold, at least about 6.5-

fold, at least about 6.6-fold, at least about 6.7-fold, at least about 6.8-fold, at least about 6.9-fold, at least about 7-fold, at least about 7.1-fold, at least about 7.2-fold, at least about 7.3-fold, at least about 7.4-fold, at least about 7.5-fold, at least about 7.6-fold, at least about 7.7-fold, at least about 7.8-fold, at least about 7.9-fold, at least about 8-fold, at least about 8.1-fold, at least about 8.2-fold, at least about 8.3-fold, at least about 8.4-fold, at least about 8.5-fold, at least about 8.6-fold, at least about 8.7-fold, at least about 8.8-fold, at least about 8.9-fold, at least about 9-fold, at least about 9.1-fold, at least about 9.2-fold, at least about 9.3-fold, at least about 9.4-fold, at least about 9.5-fold, at least about 9.6-fold, at least about 9.7-fold, at least about 9.8-fold, at least about 9.9-fold, at least about 10-fold, or more, of the level of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 expressed in an unaltered or unmodified wild-type cell of the same cell type.

[00754] In some embodiments, the cells described herein express at least about 110%, at least about 120%, at least about 130%, at least about 140%, at least about 150%, at least about 160%, at least about 170%, at least about 180%, at least about 190%, at least about 200%, at least about 250%, at least about 300%, at least about 350%, at least about 400%, at least about 450%, at least about 500%, at least about 550%, at least about 600%, at least about 650%, at least about 700%, at least about 750%, at least about 800%, at least about 850%, at least about 900%, at least about 950%, at least about 1000%, at least about 1500%, at least about 2000%, at least about 2500%, at least about 3000%, at least about 3500%, at least about 4000%, at least about 4500%, at least about 5000%, at least about 5500%, at least about 6000%, at least about 6500%, at least about 7000%, at least about 7500%, at least about 8000%, at least about 8500%, at least about 9000%, at least about 10000%, or more, of the level of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 expressed in an unaltered or unmodified wild-type cell of the same cell type.

[00755] The amount of increased CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF,

and/or Serpinb9 expression can also be measured, for example, as a multiple, a fold, or a percentage increase in expression relative to the unaltered or unmodified wild-type cell. For example, in some embodiments, the cells described herein express at least about 0.1x higher, at least about 0.1x higher, at least about 0.2x higher, at least about 0.3x higher, at least about 0.4x higher, at least about 0.5x higher, at least about 0.6x higher, at least about 0.7x higher, at least about 0.8x higher, at least about 0.9x higher, at least about 2x higher, at least about 1x higher, at least about 1.1x higher, at least about 1.2x higher, at least about 1.3x higher, at least about 1.4x higher, at least about 1.5x higher, at least about 1.6x higher, at least about 1.7x higher, at least about 1.8x higher, at least about 1.9x higher, at least about 2x higher, at least about 2.1x higher, at least about 2.2x higher, at least about 2.3x higher, at least about 2.4x higher, at least about 2.5x higher, at least about 2.6x higher, at least about 2.7x higher, at least about 2.8x higher, at least about 2.9x higher, at least about 3x higher, at least about 3.1x higher, at least about 3.2x higher, at least about 3.3x higher, at least about 3.4x higher, at least about 3.5x higher, at least about 3.6x higher, at least about 3.7x higher, at least about 3.8x higher, at least about 3.9x higher, at least about 4x higher, at least about 4.1x higher, at least about 4.2x higher, at least about 4.3x higher, at least about 4.4x higher, at least about 4.5x higher, at least about 4.6x higher, at least about 4.7x higher, at least about 4.8x higher, at least about 4.9x higher, at least about 5x higher, at least about 5.1x higher, at least about 5.2x higher, at least about 5.3x higher, at least about 5.4x higher, at least about 5.5x higher, at least about 5.6x higher, at least about 5.7x higher, at least about 5.8x higher, at least about 5.9x higher, at least about 6x higher, at least about 6.1x higher, at least about 6.2x higher, at least about 6.3x higher, at least about 6.4x higher, at least about 6.5x higher, at least about 6.6x higher, at least about 6.7x higher, at least about 6.8x higher, at least about 6.9x higher, at least about 7x higher, at least about 7.1x higher, at least about 7.2x higher, at least about 7.3x higher, at least about 7.4x higher, at least about 7.5x higher, at least about 7.6x higher, at least about 7.7x higher, at least about 7.8x higher, at least about 7.9x higher, at least about 8x higher, at least about 8.1x higher, at least about 8.2x higher, at least about 8.3x higher, at least about 8.4x higher, at least about 8.5x higher, at least about 8.6x higher, at least about 8.7x higher, at least about 8.8x higher, at least about 8.9x higher, at least about 9x higher, at least about 9.1x higher, at least about 9.2x higher, at least about 9.3x higher, at least about 9.4x higher, at least about 9.5x higher, at least about 9.6x higher, at least about 9.7x higher, at least about 9.8x higher, at least about 9.9x higher, at least

about 10x higher, or more, amount of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 expression relative to the level of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 expressed in an unaltered or unmodified wild-type cell of the same cell type.

[00756] In some embodiments, the cells described herein express at least about 0.1-fold higher, at least about 0.2-fold higher, at least about 0.3-fold higher, at least about 0.4-fold higher, at least about 0.5-fold higher, at least about 0.6-fold higher, at least about 0.7-fold higher, at least about 0.8-fold higher, at least about 0.9-fold higher, at least about 1-fold higher, at least about 1.1-fold higher, at least about 1.2-fold higher, at least about 1.3-fold higher, at least about 1.4-fold higher, at least about 1.5-fold higher, at least about 1.6-fold higher, at least about 1.7-fold higher, at least about 1.8-fold higher, at least about 1.9-fold higher, at least about 2-fold higher, at least about 2.1-fold higher, at least about 2.2-fold higher, at least about 2.3-fold higher, at least about 2.4-fold higher, at least about 2.5-fold higher, at least about 2.6-fold higher, at least about 2.7-fold higher, at least about 2.8-fold higher, at least about 2.9-fold higher, at least about 3-fold higher, at least about 3.1-fold higher, at least about 3.2-fold higher, at least about 3.3-fold higher, at least about 3.4-fold higher, at least about 3.5-fold higher, at least about 3.6-fold higher, at least about 3.7-fold higher, at least about 3.8-fold higher, at least about 3.9-fold higher, at least about 4-fold higher, at least about 4.1-fold higher, at least about 4.2-fold higher, at least about 4.3-fold higher, at least about 4.4-fold higher, at least about 4.5-fold higher, at least about 4.6-fold higher, at least about 4.7-fold higher, at least about 4.8-fold higher, at least about 4.9-fold higher, at least about 5-fold higher, at least about 5.1-fold higher, at least about 5.2-fold higher, at least about 5.3-fold higher, at least about 5.4-fold higher, at least about 5.5-fold higher, at least about 5.6-fold higher, at least about 5.7-fold higher, at least about 5.8-fold higher, at least about 5.9-fold higher, at least about 6-fold higher, at least about 6.1-fold higher, at least about 6.2-fold higher, at least about 6.3-fold higher, at least about 6.4-fold higher, at least about 6.5-fold higher, at least about 6.6-fold higher, at least about 6.7-fold higher, at least about 6.8-fold higher, at least about 6.9-fold higher, at least about 7-fold higher, at least about 7.1-fold higher, at least about 7.2-fold higher,

at least about 7.3-fold higher, at least about 7.4-fold higher, at least about 7.5-fold higher, at least about 7.6-fold higher, at least about 7.7-fold higher, at least about 7.8-fold higher, at least about 7.9-fold higher, at least about 8-fold higher, at least about 8.1-fold higher, at least about 8.2-fold higher, at least about 8.3-fold higher, at least about 8.4-fold higher, at least about 8.5-fold higher, at least about 8.6-fold higher, at least about 8.7-fold higher, at least about 8.8-fold higher, at least about 8.9-fold higher, at least about 9-fold higher, at least about 9.1-fold higher, at least about 9.2-fold higher, at least about 9.3-fold higher, at least about 9.4-fold higher, at least about 9.5-fold higher, at least about 9.6-fold higher, at least about 9.7-fold higher, at least about 9.8-fold higher, at least about 9.9-fold higher, at least about 10-fold higher, or more, amount of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 expression relative to the level of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 expressed in an unaltered or unmodified wild-type cell of the same cell type.

[00757] In some embodiments, the cells described herein express at least about 10% higher, at least about 20% higher, at least about 30% higher, at least about 40% higher, at least about 50% higher, at least about 60% higher, at least about 70% higher, at least about 80% higher, at least about 90% higher, at least about 100% higher, at least about 125% higher, at least about 150% higher, at least about 200% higher, at least about 250% higher, at least about 300% higher, at least about 350% higher, at least about 400% higher, at least about 450% higher, at least about 500% higher, at least about 550% higher, at least about 600% higher, at least about 650% higher, at least about 700% higher, at least about 750% higher, at least about 800% higher, at least about 850% higher, at least about 900% higher, at least about 950% higher, at least about 1000% higher, at least about 1500% higher, at least about 2000% higher, at least about 2500% higher, at least about 3000% higher, at least about 3500% higher, at least about 4000% higher, at least about 4500% higher, at least about 5000% higher, at least about 5500% higher, at least about 6000% higher, at least about 6500% higher, at least about 7000% higher, at least about 7500% higher, at least about 8000% higher, at least about 8500% higher, at least about 9000% higher, at least about 10000% higher, or more, amount of CD47, DUX4, CD24, CD27, CD35, CD46,

CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 expression relative to the level of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 expressed in an unaltered or unmodified wild-type cell of the same cell type.

[00758] The expression level can be due to a number of factors known to those skilled in the art. For example, expression level of an exogenous polynucleotide encoding CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 can be affected by, among other factors, the copy number of the exogenous polynucleotide in the cell, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more copies of the exogenous polynucleotide in the cell; the regulatory elements present, such as, *e.g.*, any of the regulatory elements described herein or known in the art, including any of the constitutive, inducible, or conditional promoters described herein or known in the art; the location where the exogenous polynucleotide is inserted into the genome of the cell; the type of vector used to introduce the exogenous polynucleotide into the cell; the ordering of cassettes in the exogenous polynucleotide, *e.g.*, bicistronic, etc.

[00759] The CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 expression level is measured relative to the level of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 expressed in an unaltered or unmodified wild-type cell of the same cell type. For example, the CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 expression level conferred by the exogenous polynucleotide encoding CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy

chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 in a T cell is conveyed as an expression level relative to that of an unaltered or unmodified wild-type T cell; the CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 expression level conferred by the exogenous polynucleotide encoding CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 in an NK cell is conveyed as an expression level relative to that of an unaltered or unmodified wild-type NK cell; the CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 expression level conferred by the exogenous polynucleotide encoding CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 in an endothelial cell is conveyed as an expression level relative to that of an unaltered or unmodified wild-type endothelial cell; the CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 expression level conferred by the exogenous polynucleotide encoding CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 in a pancreatic islet cell is conveyed as an expression level relative to that of an unaltered or unmodified wild-type pancreatic islet cell; the CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 expression level conferred by the exogenous polynucleotide encoding CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200,

HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 in a cardiac muscle cell is conveyed as an expression level relative to that of an unaltered or unmodified wild-type cardiac muscle cell; the CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 expression level conferred by the exogenous polynucleotide encoding CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 in a smooth muscle cell is conveyed as an expression level relative to that of an unaltered or unmodified wild-type smooth muscle cell; the CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 expression level conferred by the exogenous polynucleotide encoding CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 in a skeletal muscle cell is conveyed as an expression level relative to that of an unaltered or unmodified wild-type skeletal muscle cell; the CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 expression level conferred by the exogenous polynucleotide encoding CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 in a hepatocyte is conveyed as an expression level relative to that of an unaltered or unmodified wild-type hepatocyte; the CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 expression level conferred by the exogenous polynucleotide encoding CD47,

DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 in a glial progenitor cell is conveyed as an expression level relative to that of an unaltered or unmodified wild-type glial progenitor cell; the CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 expression level conferred by the exogenous polynucleotide encoding CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 in a dopaminergic neuron is conveyed as an expression level relative to that of an unaltered or unmodified wild-type dopaminergic neuron; the CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 expression level conferred by the exogenous polynucleotide encoding CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 in a retinal pigment epithelial cell is conveyed as an expression level relative to that of an unaltered or unmodified wild-type retinal pigment epithelial cell; the CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 expression level conferred by the exogenous polynucleotide encoding CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 in a thyroid cell is conveyed as an expression level relative to that of an unaltered or unmodified wild-type thyroid cell.

[00760] In another embodiment, CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF,

and/or Serpinb9 protein expression is detected using a Western blot of cell lysates probed with antibodies against the CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 protein. In another embodiment, reverse transcriptase polymerase chain reactions (RT-PCR) are used to confirm the presence of the exogenous CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 mRNA.

X. Chimeric Antigen Receptors

[00761] Provided herein are hypoimmunogenic cells comprising a chimeric antigen receptor (CAR). In some embodiments, the CAR is binds to CD19. In some embodiments, the CAR binds to CD22. In some embodiments, the CAR binds to CD19. In some embodiments, the CAR binds to CD19 and CD22. In some embodiments, the CAR is selected from the group consisting of a first generation CAR, a second generation CAR, a third generation CAR, and a fourth generation CAR. In some embodiments, the CAR includes a single binding domain that binds to a single target antigen. In some embodiments, the CAR includes a single binding domain that binds to more than one target antigen, *e.g.*, 2, 3, or more target antigens. In some embodiments, the CAR includes two binding domains such that each binding domain binds to a different target antigens. In some embodiments, the CAR includes two binding domains such that each binding domain binds to the same target antigen. Detailed descriptions of exemplary CARs including CD19-specific, CD22-specific and CD19/CD22-bispecific CARs can be found in WO2012/079000, WO2016/149578 and WO2020/014482, the disclosures including the sequence listings and figures are incorporated herein by reference in their entirety. In some embodiments, the CAR includes two binding domains such that each binding domain binds to the same target antigen. Detailed descriptions of exemplary CARs including CD19-specific, CD22-specific and CD19/CD22-bispecific CARs can be found in WO2012/079000, WO2016/149578 and WO2020/014482, the disclosures including the sequence listings and figures are incorporated herein by reference in their entirety.

[00762] In some embodiments, the CD19 specific CAR includes an anti-CD19 single-chain antibody fragment (scFv), a transmembrane domain such as one derived from human CD8α, a 4-

1BB (CD137) co-stimulatory signaling domain, and a CD3ζ signaling domain. In some embodiments, the CD20 specific CAR includes an anti-CD20 scFv, a transmembrane domain such as one derived from human CD8α, a 4-1BB (CD137) co-stimulatory signaling domain, and a CD3ζ signaling domain. In some embodiments, the CD19/CD20-bispecific CAR includes an anti-CD19 scFv, an anti-CD20 scFv, a transmembrane domain such as one derived from human CD8α, a 4-1BB (CD137) co-stimulatory signaling domain, and a CD3ζ signaling domain. In some embodiments, the CD22 specific CAR includes an anti-CD22 scFv, a transmembrane domain such as one derived from human CD8α, a 4-1BB (CD137) co-stimulatory signaling domain, and a CD3ζ signaling domain. In some embodiments, the CD19/CD22-bispecific CAR includes an anti-CD19 scFv, an anti-CD22 scFv, a transmembrane domain such as one derived from human CD8α, a 4-1BB (CD137) co-stimulatory signaling domain, and a CD3ζ signaling domain.

[00763] In some embodiments, the CAR comprises a commercial CAR construct carried by a T cell. Non-limiting examples of commercial CAR-T cell based therapies include brexucabtagene autoleucel (TECARTUS®), axicabtagene ciloleucel (YESCARTA®), idecabtagene vicleucel (ABECMA®), lisocabtagene maraleucel (BREYANZI®), tisagenlecleucel (KYMRIAH®), Descartes-08 and Descartes-11 from Cartesian Therapeutics, CTL110 from Novartis, P-BMCA-101 from Poseida Therapeutics, AUTO4 from Autolus Limited, UCARTCS from Cellectis, PBCAR19B and PBCAR269A from Precision Biosciences, FT819 from Fate Therapeutics, and CYAD-211 from Clyad Oncology.

[00764] In some embodiments, a hypoimmunogenic cell described herein comprises a polynucleotide encoding a chimeric antigen receptor (CAR) comprising an antigen binding domain. In some embodiments, a hypoimmunogenic cell described herein comprises a chimeric antigen receptor (CAR) comprising an antigen binding domain. In some embodiments, the polynucleotide is or comprises a chimeric antigen receptor (CAR) comprising an antigen binding domain. In some embodiments, the CAR is or comprises a first generation CAR comprising an antigen binding domain, a transmembrane domain, and at least one signaling domain (*e.g.*, one, two or three signaling domains). In some embodiments, the CAR comprises a second generation CAR comprising an antigen binding domain, a transmembrane domain, and at least two signaling domains. In some embodiments, the CAR comprises a third generation CAR comprising an antigen binding domain, a transmembrane domain, and at least three signaling

domains. In some embodiments, a fourth generation CAR comprising an antigen binding domain, a transmembrane domain, three or four signaling domains, and a domain which upon successful signaling of the CAR induces expression of a cytokine gene. In some embodiments, the antigen binding domain is or comprises an antibody, an antibody fragment, an scFv or a Fab.

1. Antigen binding domain (ABD) targets an antigen characteristic of a neoplastic or cancer cell

[00765] In some embodiments, the antigen binding domain (ABD) targets an antigen characteristic of a neoplastic cell. In other words, the antigen binding domain targets an antigen expressed by a neoplastic or cancer cell. In some embodiments, the ABD binds a tumor associated antigen. In some embodiments, the antigen characteristic of a neoplastic cell (*e.g.*, antigen associated with a neoplastic or cancer cell) or a tumor associated antigen is selected from a cell surface receptor, an ion channel-linked receptor, an enzyme-linked receptor, a G protein-coupled receptor, receptor tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, histidine kinase associated receptor, epidermal growth factor receptors (EGFR) (including ErbB1/EGFR, ErbB2/HER2, ErbB3/HER3, and ErbB4/HER4), fibroblast growth factor receptors (FGFR) (including FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, FGF7, FGF18, and FGF21), vascular endothelial growth factor receptors (VEGFR) (including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PIGF), RET Receptor and the Eph Receptor Family (including EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA9, EphA10, EphB1, EphB2. EphB3, EphB4, and EphB6), CXCR1, CXCR2, CXCR3, CXCR4, CXCR6, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR8, CFTR, CIC-1, CIC-2, CIC-4, CIC-5, CIC-7, CIC-Ka, CIC-Kb, Bestrophins, TMEM16A, GABA receptor, glycin receptor, ABC transporters, NAV1.1, NAV1.2, NAV1.3, NAV1.4, NAV1.5, NAV1.6, NAV1.7, NAV1.8, NAV1.9, sphingosin-1-phosphate receptor (S1P1R), NMDA channel, transmembrane protein, multispan transmembrane protein, T-cell receptor motifs, T-cell alpha chains, T-cell β chains, T-cell γ chains, T-cell δ chains, CCR7, CD3, CD4, CD5, CD7, CD8, CD11b, CD11c, CD16, CD19, CD20, CD21, CD22, CD25, CD28, CD34, CD35, CD40, CD45RA, CD45RO, CD52, CD56, CD62L, CD68, CD80, CD95, CD117, CD127, CD133, CD137 (4-1BB), CD163, F4/80, IL-4Ra, Sca-1 , CTLA-4, GITR, GARP, LAP, granzyme B, LFA-1, transferrin receptor, NKp46, perforin, CD4+, Th1, Th2, Th17, Th40, Th22, Th9, Tfh, canonical Treg. FoxP3+, Tr1, Th3, Treg17, TREG; CDCP, NT5E, EpCAM, CEA,

gpA33, mucins, TAG-72, carbonic anhydrase IX, PSMA, folate binding protein, gangliosides (*e.g.*, CD2, CD3, GM2), Lewis-γ^2, VEGF, VEGFR 1/2/3, $\alpha V\beta 3$, $\alpha 5\beta 1$, ErbB1/EGFR, ErbB1/HER2, ErB3, c-MET, IGF1R, EphA3, TRAIL-R1, TRAIL-R2, RANKL, FAP, Tenascin, PDL-1, BAFF, HDAC, ABL, FLT3, KIT, MET, RET, IL-1β, ALK, RANKL, mTOR, CTLA-4, IL-6, IL-6R, JAK3, BRAF, PTCH, Smoothened, PIGF, ANPEP, TIMP1, PLAUR, PTPRJ, LTBR, ANTXR1, folate receptor alpha (FRa), ERBB2 (Her2/neu), EphA2, IL-13Ra2, epidermal growth factor receptor (EGFR), mesothelin, TSHR, CD19, CD123, CD22, CD30, CD171, CS-1, CLL-1, CD33, EGFRvIII, GD2, GD3, BCMA, MUC16 (CA125), L1CAM, LeY, MSLN, IL13Rα1, L1-CAM, Tn Ag, prostate specific membrane antigen (PSMA), ROR1, FLT3, FAP, TAG72, CD38, CD44v6, CEA, EPCAM, B7H3, KIT, interleukin-11 receptor a (IL-11Ra), PSCA, PRSS21, VEGFR2, LewisY, CD24, platelet-derived growth factor receptor-beta (PDGFR-beta), SSEA-4, CD20, MUC1, NCAM, Prostase, PAP, ELF2M, Ephrin B2, IGF-1 receptor, CAIX, LMP2, gpl00, bcr-abl, tyrosinase, Fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, CLDN6, GPRC5D, CXORF61, CD97, CD179a, ALK, Polysialic acid, PLACl, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-A1, legumain, HPV E6, E7, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, major histocompatibility complex class I-related gene protein (MR1), urokinase-type plasminogen activator receptor (uPAR), Fos-related antigen 1, p53, p53 mutant, prostein, survivin, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, androgen receptor, cyclin B1, MYCN, RhoC, TRP-2, CYPIB I, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, CD79a, CD79b, CD72, LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, GPC3, FCRL5, IGLL1, a neoantigen, CD133, CD15, CD184, CD24, CD56, CD26, CD29, CD44, HLA-A, HLA-B, HLA-C, (HLA-A,B,C) CD49f, CD151 CD340, CD200, tkrA, trkB, or trkC, or an antigenic fragment or antigenic portion thereof.

2. ABD targets an antigen characteristic of a T cell

[00766] In some embodiments, the antigen binding domain targets an antigen characteristic of a T cell. In some embodiments, the ABD binds an antigen associated with a T cell. In some

instances, such an antigen is expressed by a T cell or is located on the surface of a T cell. In some embodiments, the antigen characteristic of a T cell or the T cell associated antigen is selected from a cell surface receptor, a membrane transport protein (*e.g.*, an active or passive transport protein such as, for example, an ion channel protein, a pore-forming protein, etc.), a transmembrane receptor, a membrane enzyme, and/or a cell adhesion protein characteristic of a T cell. In some embodiments, an antigen characteristic of a T cell may be a G protein-coupled receptor, receptor tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, histidine kinase associated receptor, AKT1; AKT2; AKT3; ATF2; BCL10; CALM1; CD3D (CD3δ); CD3E (CD3ε); CD3G (CD3γ); CD4; CD8; CD28; CD45; CD80 (B7-1); CD86 (B7-2); CD247 (CD3ζ); CTLA-4 (CD152); ELK1; ERK1 (MAPK3); ERK2; FOS; FYN; GRAP2 (GADS); GRB2; HLA-DRA; HLA-DRB1; HLA-DRB3; HLA-DRB4; HLA-DRB5; HRAS; IKBKA (CHUK); IKBKB; IKBKE; IKBKG (NEMO); IL2; ITPR1; ITK; JUN; KRAS2; LAT; LCK; MAP2K1 (MEK1); MAP2K2 (MEK2); MAP2K3 (MKK3); MAP2K4 (MKK4); MAP2K6 (MKK6); MAP2K7 (MKK7); MAP3K1 (MEKK1); MAP3K3; MAP3K4; MAP3K5; MAP3K8; MAP3K14 (NIK); MAPK8 (JNK1); MAPK9 (JNK2); MAPK10 (JNK3); MAPK11 (p38β); MAPK12 (p38γ); MAPK13 (p38δ); MAPK14 (p38α); NCK; NFAT1; NFAT2; NFKB1; NFKB2; NFKBIA; NRAS; PAK1; PAK2; PAK3; PAK4; PIK3C2B; PIK3C3 (VPS34); PIK3CA; PIK3CB; PIK3CD; PIK3R1; PKCA; PKCB; PKCM; PKCQ; PLCY1; PRF1 (Perforin); PTEN; RAC1; RAF1; RELA; SDF1; SHP2; SLP76; SOS; SRC; TBK1; TCRA; TEC; TRAF6; VAV1; VAV2; or ZAP70.

3. ABD targets an antigen characteristic of an autoimmune or inflammatory disorder

[00767] In some embodiments, the antigen binding domain targets an antigen characteristic of an autoimmune or inflammatory disorder. In some embodiments, the ABD binds an antigen associated with an autoimmune or inflammatory disorder. In some instances, the antigen is expressed by a cell associated with an autoimmune or inflammatory disorder. In some embodiments, the autoimmune or inflammatory disorder is selected from chronic graft-vs-host disease (GVHD), lupus, arthritis, immune complex glomerulonephritis, goodpasture syndrome, uveitis, hepatitis, systemic sclerosis or scleroderma, type I diabetes, multiple sclerosis, cold agglutinin disease, Pemphigus vulgaris, Grave's disease, autoimmune hemolytic anemia, Hemophilia A, Primary Sjogren's Syndrome, thrombotic thrombocytopenia purrpura,

neuromyelits optica, Evan's syndrome, IgM mediated neuropathy, cryoglobulinemia, dermatomyositis, idiopathic thrombocytopenia, ankylosing spondylitis, bullous pemphigoid, acquired angioedema, chronic urticarial, antiphospholipid demyelinating polyneuropathy, and autoimmune thrombocytopenia or neutropenia or pure red cell aplasias, while exemplary non-limiting examples of alloimmune diseases include allosensitization (see, for example, Blazar *et al.*, 2015, Am. J. Transplant, 15(4):931-41) or xenosensitization from hematopoietic or solid organ transplantation, blood transfusions, pregnancy with fetal allosensitization, neonatal alloimmune thrombocytopenia, hemolytic disease of the newborn, sensitization to foreign antigens such as can occur with replacement of inherited or acquired deficiency disorders treated with enzyme or protein replacement therapy, blood products, and gene therapy. In some embodiments, the antigen characteristic of an autoimmune or inflammatory disorder is selected from a cell surface receptor, an ion channel-linked receptor, an enzyme-linked receptor, a G protein-coupled receptor, receptor tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, or histidine kinase associated receptor.

[00768] In some embodiments, an antigen binding domain of a CAR binds to a ligand expressed on B cells, plasma cells, or plasmablasts. In some embodiments, an antigen binding domain of a CAR binds to CD10, CD19, CD20, CD22, CD24, CD27, CD38, CD45R, CD138, CD319, BCMA, CD28, TNF, interferon receptors, GM-CSF, ZAP-70, LFA-1, CD3 gamma, CD5 or CD2. See, *e.g.*, US 2003/0077249; WO 2017/058753; WO 2017/058850, the contents of which are herein incorporated by reference.

4. ABD targets an antigen characteristic of senescent cells

[00769] In some embodiments, the antigen binding domain targets an antigen characteristic of senescent cells, *e.g.*, urokinase-type plasminogen activator receptor (uPAR). In some embodiments, the ABD binds an antigen associated with a senescent cell. In some instances, the antigen is expressed by a senescent cell. In some embodiments, the CAR may be used for treatment or prophylaxis of disorders characterized by the aberrant accumulation of senescent cells, *e.g.*, liver and lung fibrosis, atherosclerosis, diabetes and osteoarthritis.

5. ABD targets an antigen characteristic of an infectious disease

[00770] In some embodiments, the antigen binding domain targets an antigen characteristic of an infectious disease. In some embodiments, the ABD binds an antigen associated with an infectious disease. In some instances, the antigen is expressed by a cell affected by an infectious disease. In some embodiments, wherein the infectious disease is selected from HIV, hepatitis B virus, hepatitis C virus, Human herpes virus, Human herpes virus 8 (HHV-8, Kaposi sarcoma-associated herpes virus (KSHV)), Human T-lymphotrophic virus-1 (HTLV-1), Merkel cell polyomavirus (MCV), Simian virus 40 (SV40), Epstein-Barr virus, CMV, human papillomavirus. In some embodiments, the antigen characteristic of an infectious disease is selected from a cell surface receptor, an ion channel-linked receptor, an enzyme-linked receptor, a G protein-coupled receptor, receptor tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, histidine kinase associated receptor, HIV Env, gpl20, or CD4-induced epitope on HIV-1 Env.

6. ABD binds to a cell surface antigen of a cell

[00771] In some embodiments, an antigen binding domain binds to a cell surface antigen of a cell. In some embodiments, a cell surface antigen is characteristic of (*e.g.*, expressed by) a particular or specific cell type. In some embodiments, a cell surface antigen is characteristic of more than one type of cell.

[00772] In some embodiments, a CAR antigen binding domain binds a cell surface antigen characteristic of a T cell, such as a cell surface antigen on a T cell. In some embodiments, an antigen characteristic of a T cell may be a cell surface receptor, a membrane transport protein (*e.g.*, an active or passive transport protein such as, for example, an ion channel protein, a pore-forming protein, etc.), a transmembrane receptor, a membrane enzyme, and/or a cell adhesion protein characteristic of a T cell. In some embodiments, an antigen characteristic of a T cell may be a G protein-coupled receptor, receptor tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, or histidine kinase associated receptor.

[00773] In some embodiments, an antigen binding domain of a CAR binds a T cell receptor. In some embodiments, a T cell receptor may be AKT1; AKT2; AKT3; ATF2; BCL10; CALM1; CD3D (CD3δ); CD3E (CD3ε); CD3G (CD3γ); CD4; CD8; CD28; CD45; CD80 (B7-1); CD86

(B7-2); CD247 (CD3ζ); CTLA-4 (CD152); ELK1; ERK1 (MAPK3); ERK2; FOS; FYN; GRAP2 (GADS); GRB2; HLA-DRA; HLA-DRB1; HLA-DRB3; HLA-DRB4; HLA-DRB5; HRAS; IKBKA (CHUK); IKBKB; IKBKE; IKBKG (NEMO); IL2; ITPR1; ITK; JUN; KRAS2; LAT; LCK; MAP2K1 (MEK1); MAP2K2 (MEK2); MAP2K3 (MKK3); MAP2K4 (MKK4); MAP2K6 (MKK6); MAP2K7 (MKK7); MAP3K1 (MEKK1); MAP3K3; MAP3K4; MAP3K5; MAP3K8; MAP3K14 (NIK); MAPK8 (JNK1); MAPK9 (JNK2); MAPK10 (JNK3); MAPK11 (p38β); MAPK12 (p38γ); MAPK13 (p38δ); MAPK14 (p38α); NCK; NFAT1; NFAT2; NFKB1; NFKB2; NFKBIA; NRAS; PAK1; PAK2; PAK3; PAK4; PIK3C2B; PIK3C3 (VPS34); PIK3CA; PIK3CB; PIK3CD; PIK3R1; PKCA; PKCB; PKCM; PKCQ; PLCY1; PRF1 (Perforin); PTEN; RAC1; RAF1; RELA; SDF1; SHP2; SLP76; SOS; SRC; TBK1; TCRA; TEC; TRAF6; VAV1; VAV2; or ZAP70.

7. Transmembrane domain

[00774] In some embodiments, the CAR transmembrane domain comprises at least a transmembrane region of the alpha, beta or zeta chain of a T cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, or functional variant thereof. In some embodiments, the transmembrane domain comprises at least a transmembrane region(s) of CD8α, CD8β, 4-1BB/CD137, CD28, CD34, CD4, FcεRIγ, CD16, OX40/CD134, CD3ζ, CD3ε, CD3γ, CD3δ, TCRα, TCRβ, TCRζ, CD32, CD64, CD64, CD45, CD5, CD9, CD22, CD37, CD80, CD86, CD40, CD40L/CD154, VEGFR2, FAS, and FGFR2B, or functional variant thereof. antigen binding domain binds

8. Signaling domain or plurality of signaling domains

[00775] In some embodiments, a CAR described herein comprises one or at least one signaling domain selected from one or more of B7-1/CD80; B7-2/CD86; B7-H1/PD-L1; B7-H2; B7-H3; B7-H4; B7-H6; B7-H7; BTLA/CD272; CD28; CTLA-4; Gi24/VISTA/B7-H5; ICOS/CD278; PD-1; PD-L2/B7-DC; PDCD6); 4-1BB/TNFSF9/CD137; 4-1BB Ligand/TNFSF9; BAFF/BLyS/TNFSF13B; BAFF R/TNFRSF13C; CD27/TNFRSF7; CD27 Ligand/TNFSF7; CD30/TNFRSF8; CD30 Ligand/TNFSF8; CD40/TNFRSF5; CD40/TNFSF5; CD40 Ligand/TNFSF5; DR3/TNFRSF25; GITR/TNFRSF18; GITR Ligand/TNFSF18; HVEM/TNFRSF14; LIGHT/TNFSF14; Lymphotoxin-alpha/TNF-beta; OX40/TNFRSF4; OX40 Ligand/TNFSF4; RELT/TNFRSF19L; TACI/TNFRSF13B; TL1A/TNFSF15; TNF-alpha; TNF

RII/TNFRSF1B); 2B4/CD244/SLAMF4; BLAME/SLAMF8; CD2; CD2F-10/SLAMF9; CD48/SLAMF2; CD58/LFA-3; CD84/SLAMF5; CD229/SLAMF3; CRACC/SLAMF7; NTB-A/SLAMF6; SLAM/CD150); CD2; CD7; CD53; CD82/Kai-1; CD90/Thy1; CD96; CD160; CD200; CD300a/LMIR1; HLA Class I; HLA-DR; Ikaros; Integrin alpha 4/CD49d; Integrin alpha 4 beta 1; Integrin alpha 4 beta 7/LPAM-1; LAG-3; TCL1A; TCL1B; CRTAM; DAP12; Dectin-1/CLEC7A; DPPIV/CD26; EphB6; TIM-1/KIM-1/HAVCR; TIM-4; TSLP; TSLP R; lymphocyte function associated antigen-1 (LFA-1); NKG2C, a CD3 zeta domain, an immunoreceptor tyrosine-based activation motif (ITAM), CD27, CD28, 4-1BB, CD134/OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, or functional fragment thereof.

[00776] In some embodiments, the at least one signaling domain comprises a CD3 zeta domain or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof. In other embodiments, the at least one signaling domain comprises (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; and (ii) a CD28 domain, or a 4-1BB domain, or functional variant thereof. In yet other embodiments, the at least one signaling domain comprises a (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain or functional variant thereof; and (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof. In some embodiments, the at least one signaling domain comprises a (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain or functional variant thereof; (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof; and (iv) a cytokine or costimulatory ligand transgene.

[00777] In some embodiments, the at least two signaling domains comprise a CD3 zeta domain or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof. In other embodiments, the at least two signaling domains comprise (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; and (ii) a CD28 domain, or a 4-1BB domain, or functional variant thereof. In yet other embodiments, the at least one signaling domain comprises a (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain or functional variant thereof; and (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof. In

some embodiments, the at least two signaling domains comprise a (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain or functional variant thereof; (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof; and (iv) a cytokine or costimulatory ligand transgene.

[00778] In some embodiments, the at least three signaling domains comprise a CD3 zeta domain or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof. In other embodiments, the at least three signaling domains comprise (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; and (ii) a CD28 domain, or a 4-1BB domain, or functional variant thereof. In yet other embodiments, the least three signaling domains comprises a (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain or functional variant thereof; and (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof. In some embodiments, the at least three signaling domains comprise a (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain or functional variant thereof; (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof; and (iv) a cytokine or costimulatory ligand transgene.

[00779] In some embodiments, the CAR comprises a CD3 zeta domain or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof. In some embodiments, the CAR comprises (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; and (ii) a CD28 domain, or a 4-1BB domain, or functional variant thereof.

[00780] In some embodiments, the CAR comprises a (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain or functional variant thereof; and (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof.

[00781] In some embodiments, the CAR comprises (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain, or a 4-1BB domain, or functional variant thereof, and/or (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof.

[00782] In some embodiments, the CAR comprises a (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain or functional variant thereof; (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof; and (iv) a cytokine or costimulatory ligand transgene.

9. Domain which upon successful signaling of the CAR induces expression of a cytokine gene

[00783] In some embodiments, a first, second, third, or fourth generation CAR further comprises a domain which upon successful signaling of the CAR induces expression of a cytokine gene. In some embodiments, a cytokine gene is endogenous or exogenous to a target cell comprising a CAR which comprises a domain which upon successful signaling of the CAR induces expression of a cytokine gene. In some embodiments, a cytokine gene encodes a pro-inflammatory cytokine. In some embodiments, a cytokine gene encodes IL-1, IL-2, IL-9, IL-12, IL-18, TNF, IL-4, IL-10, or IFN-gamma, or functional fragment thereof. In some embodiments, a domain which upon successful signaling of the CAR induces expression of a cytokine gene is or comprises a transcription factor or functional domain or fragment thereof. In some embodiments, a domain which upon successful signaling of the CAR induces expression of a cytokine gene is or comprises a transcription factor or functional domain or fragment thereof. In some embodiments, a transcription factor or functional domain or fragment thereof is or comprises a nuclear factor of activated T cells (NFAT), an NF-kB, or functional domain or fragment thereof. See, *e.g.*, Zhang. C. *et al.*, Engineering CAR-T cells. Biomarker Research. 5:22 (2017); WO 2016126608; Sha, H. *et al.* Chimaeric antigen receptor T-cell therapy for tumour immunotherapy. Bioscience Reports Jan 27, 2017, 37 (1).

[00784] In some embodiments, the CAR further comprises one or more spacers, *e.g.*, wherein the spacer is a first spacer between the antigen binding domain and the transmembrane domain. In some embodiments, the first spacer includes at least a portion of an immunoglobulin constant region or variant or modified version thereof. In some embodiments, the spacer is a second spacer between the transmembrane domain and a signaling domain. In some embodiments, the second spacer is an oligopeptide, *e.g.*, wherein the oligopeptide comprises glycine and serine residues such as but not limited to glycine-serine doublets. In some embodiments, the CAR comprises two or more spacers, *e.g.*, a spacer between the antigen binding domain and the

transmembrane domain and a spacer between the transmembrane domain and a signaling domain.

[00785] In some embodiments, any one of the cells described herein comprises a nucleic acid encoding a CAR or a first generation CAR. In some embodiments, a first generation CAR comprises an antigen binding domain, a transmembrane domain, and signaling domain. In some embodiments, a signaling domain mediates downstream signaling during T cell activation.

[00786] In some embodiments, any one of the cells described herein comprises a nucleic acid encoding a CAR or a second generation CAR. In some embodiments, a second generation CAR comprises an antigen binding domain, a transmembrane domain, and two signaling domains. In some embodiments, a signaling domain mediates downstream signaling during T cell activation. In some embodiments, a signaling domain is a costimulatory domain. In some embodiments, a costimulatory domain enhances cytokine production, CAR-T cell proliferation, and/or CAR-T cell persistence during T cell activation.

[00787] In some embodiments, any one of the cells described herein comprises a nucleic acid encoding a CAR or a third generation CAR. In some embodiments, a third generation CAR comprises an antigen binding domain, a transmembrane domain, and at least three signaling domains. In some embodiments, a signaling domain mediates downstream signaling during T cell activation. In some embodiments, a signaling domain is a costimulatory domain. In some embodiments, a costimulatory domain enhances cytokine production, CAR-T cell proliferation, and or CAR-T cell persistence during T cell activation. In some embodiments, a third generation CAR comprises at least two costimulatory domains. In some embodiments, the at least two costimulatory domains are not the same.

[00788] In some embodiments, any one of the cells described herein comprises a nucleic acid encoding a CAR or a fourth generation CAR. In some embodiments, a fourth generation CAR comprises an antigen binding domain, a transmembrane domain, and at least two, three, or four signaling domains. In some embodiments, a signaling domain mediates downstream signaling during T cell activation. In some embodiments, a signaling domain is a costimulatory domain. In some embodiments, a costimulatory domain enhances cytokine production, CAR-T cell proliferation, and or CAR-T cell persistence during T cell activation.

10. ABD comprising an antibody or antigen-binding portion thereof

[00789] In some embodiments, a CAR antigen binding domain is or comprises an antibody or antigen-binding portion thereof. In some embodiments, a CAR antigen binding domain is or comprises an scFv or Fab. In some embodiments, a CAR antigen binding domain comprises an scFv or Fab fragment of a CD19 antibody; CD22 antibody; T-cell alpha chain antibody; T-cell β chain antibody; T-cell γ chain antibody; T-cell δ chain antibody; CCR7 antibody; CD3 antibody; CD4 antibody; CD5 antibody; CD7 antibody; CD8 antibody; CD11b antibody; CD11c antibody; CD16 antibody; CD20 antibody; CD21 antibody; CD25 antibody; CD28 antibody; CD34 antibody; CD35 antibody; CD40 antibody; CD45RA antibody; CD45RO antibody; CD52 antibody; CD56 antibody; CD62L antibody; CD68 antibody; CD80 antibody; CD95 antibody; CD117 antibody; CD127 antibody; CD133 antibody; CD137 (4-1 BB) antibody; CD163 antibody; F4/80 antibody; IL-4Ra antibody; Sca-1 antibody; CTLA-4 antibody; GITR antibody GARP antibody; LAP antibody; granzyme B antibody; LFA-1 antibody; MR1 antibody; uPAR antibody; or transferrin receptor antibody.

[00790] In some embodiments, a CAR comprises a signaling domain which is a costimulatory domain. In some embodiments, a CAR comprises a second costimulatory domain. In some embodiments, a CAR comprises at least two costimulatory domains. In some embodiments, a CAR comprises at least three costimulatory domains. In some embodiments, a CAR comprises a costimulatory domain selected from one or more of CD27, CD28, 4-1BB, CD134/OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83. In some embodiments, if a CAR comprises two or more costimulatory domains, two costimulatory domains are different. In some embodiments, if a CAR comprises two or more costimulatory domains, two costimulatory domains are the same.

[00791] In addition to the CARs described herein, various chimeric antigen receptors and nucleotide sequences encoding the same are known in the art and would be suitable for fusosomal delivery and reprogramming of target cells in vivo and in vitro as described herein. See, *e.g.*, WO2013040557; WO2012079000; WO2016030414; Smith T, *et al.*, Nature Nanotechnology. 2017. DOI: 10.1038/NNANO.2017.57, the disclosures of which are herein incorporated by reference.

11. Additional Descriptions of CARs

[00792] In certain embodiments, the cell may comprise an exogenous polynucleotide encoding a CAR. CARs (also known as chimeric immunoreceptors, chimeric T cell receptors, or artificial T cell receptors) are receptor proteins that have been engineered to give host cells (e.g., T cells) the new ability to target a specific protein. The receptors are chimeric because they combine both antigen-binding and T cell activating functions into a single receptor. The polycistronic vector of the present disclosure may be used to express one or more CARs in a host cell (e.g., a T cell) for use in cell-based therapies against various target antigens. The CARs expressed by the one or more expression cassettes may be the same or different. In these embodiments, the CAR may comprise an extracellular binding domain (also referred to as a "binder") that specifically binds a target antigen, a transmembrane domain, and an intracellular signaling domain. In certain embodiments, the CAR may further comprise one or more additional elements, including one or more signal peptides, one or more extracellular hinge domains, and/or one or more intracellular costimulatory domains. Domains may be directly adjacent to one another, or there may be one or more amino acids linking the domains. The nucleotide sequence encoding a CAR may be derived from a mammalian sequence, for example, a mouse sequence, a primate sequence, a human sequence, or combinations thereof. In the cases where the nucleotide sequence encoding a CAR is non-human, the sequence of the CAR may be humanized. The nucleotide sequence encoding a CAR may also be codon-optimized for expression in a mammalian cell, for example, a human cell. In any of these embodiments, the nucleotide sequence encoding a CAR may be at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to any of the nucleotide sequences disclosed herein. The sequence variations may be due to codon-optimalization, humanization, restriction enzyme-based cloning scars, and/or additional amino acid residues linking the functional domains, etc.

[00793] In certain embodiments, the CAR may comprise a signal peptide at the N-terminus. Non-limiting examples of signal peptides include CD8α signal peptide, IgK signal peptide, and granulocyte-macrophage colony-stimulating factor receptor subunit alpha (GMCSFR-α, also known as colony stimulating factor 2 receptor subunit alpha (CSF2RA)) signal peptide, and variants thereof, the amino acid sequences of which are provided in **Table 2** below.

Table 2. Exemplary sequences of signal peptides

SEQ ID NO:	Sequence	Description
6	MALPVTALLLPLALLLHAARP	CD8α signal peptide
7	METDTLLLWVLLLWVPGSTG	IgK signal peptide
8	MLLLVTSLLLCELPHPAFLLIP	GMCSFR-α (CSF2RA) signal peptide

[00794] In certain embodiments, the extracellular binding domain of the CAR may comprise one or more antibodies specific to one target antigen or multiple target antigens. The antibody may be an antibody fragment, for example, an scFv, or a single-domain antibody fragment, for example, a VHH. In certain embodiments, the scFv may comprise a heavy chain variable region (V_H) and a light chain variable region (V_L) of an antibody connected by a linker. The V_H and the V_L may be connected in either order, i.e., V_H-linker-V_L or V_L-linker-V_H. Non-limiting examples of linkers include Whitlow linker, $(G_4S)_n$ (n can be a positive integer, e.g., 1, 2, 3, 4, 5, 6, etc.) linker, and variants thereof. In certain embodiments, the antigen may be an antigen that is exclusively or preferentially expressed on tumor cells, or an antigen that is characteristic of an autoimmune or inflammatory disease. Exemplary target antigens include, but are not limited to, CD5, CD19, CD20, CD22, CD23, CD30, CD70, Kappa, Lambda, and B cell maturation agent (BCMA), G-protein coupled receptor family C group 5 member D (GPRC5D) (associated with leukemias); CS1/SLAMF7, CD38, CD138, GPRC5D, TACI, and BCMA (associated with myelomas); GD2, HER2, EGFR, EGFRvIII, B7H3, PSMA, PSCA, CAIX, CD171, CEA, CSPG4, EPHA2, FAP, FRα, IL-13Rα, Mesothelin, MUC1, MUC16, and ROR1 (associated with solid tumors). In any of these embodiments, the extracellular binding domain of the CAR can be codon-optimized for expression in a host cell or have variant sequences to increase functions of the extracellular binding domain.

[00795] In certain embodiments, the CAR may comprise a hinge domain, also referred to as a spacer. The terms "hinge" and "spacer" may be used interchangeably in the present disclosure. Non-limiting examples of hinge domains include CD8α hinge domain, CD28 hinge domain, IgG4 hinge domain, IgG4 hinge-CH2-CH3 domain, and variants thereof, the amino acid sequences of which are provided in **Table 3** below.

Table 3. Exemplary sequences of hinge domains

SEQ ID NO:	Sequence	Description
9	TTTPAPRPPTPAPTIASQPLSLRPEACRPAA GGAVHTRGLDFACD	CD8α hinge domain
10	IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSP LFPGPSKP	CD28 hinge domain
113	AAAIEVMYPPPYLDNEKSNGTIIHVKGKHL CPSPLFPGPSKP	CD28 hinge domain
11	ESKYGPPCPPCP	IgG4 hinge domain
12	ESKYGPPCPSCP	IgG4 hinge domain
13	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSQEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKGLPSSIEKTIS KAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSRLTVDKSRWQEGNVFSCS VMHEALHNHYTQKSLSLSLGK	IgG4 hinge-CH2-CH3 domain

[00796] In certain embodiments, the transmembrane domain of the CAR may comprise a transmembrane region of the alpha, beta, or zeta chain of a T cell receptor, CD28, CD3ε, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, or a functional variant thereof, including the human versions of each of these sequences. In other embodiments, the transmembrane domain may comprise a transmembrane region of CD8α, CD8β, 4-1BB/CD137, CD28, CD34, CD4, FcεRIγ, CD16, OX40/CD134, CD3ζ, CD3ε, CD3γ, CD3δ, TCRα, TCRβ, TCRζ, CD32, CD64, CD64, CD45, CD5, CD9, CD22, CD37, CD80, CD86, CD40, CD40L/CD154, VEGFR2, FAS, and FGFR2B, or a functional variant thereof, including the human versions of each of these sequences. **Table 4** provides the amino acid sequences of a few exemplary transmembrane domains.

Table 4. Exemplary sequences of transmembrane domains

SEQ ID NO:	Sequence	Description
14	IYIWAPLAGTCGVLLLSLVITLYC	CD8α transmembrane domain
15	FWVLVVVGGVLACYSLLVTVAFIIF WV	CD28 transmembrane domain
114	MFWVLVVVGGVLACYSLLVTVAFII FWV	CD28 transmembrane domain

[00797] In certain embodiments, the intracellular signaling domain and/or intracellular costimulatory domain of the CAR may comprise one or more signaling domains selected from B7-1/CD80, B7-2/CD86, B7-H1/PD-L1, B7-H2, B7-H3, B7-H4, B7-H6, B7-H7, BTLA/CD272, CD28, CTLA-4, Gi24/VISTA/B7-H5, ICOS/CD278, PD-1, PD-L2/B7-DC, PDCD6, 4-1BB/TNFSF9/CD137, 4-1BB Ligand/TNFSF9, BAFF/BLyS/TNFSF13B, BAFF R/TNFRSF13C, CD27/TNFRSF7, CD27 Ligand/TNFSF7, CD30/TNFRSF8, CD30 Ligand/TNFSF8, CD40/TNFRSF5, CD40/TNFSF5, CD40 Ligand/TNFSF5, DR3/TNFRSF25, GITR/TNFRSF18, GITR Ligand/TNFSF18, HVEM/TNFRSF14, LIGHT/TNFSF14, Lymphotoxin-alpha/TNFβ, OX40/TNFRSF4, OX40 Ligand/TNFSF4, RELT/TNFRSF19L, TACI/TNFRSF13B, TL1A/TNFSF15, TNFα, TNF RII/TNFRSF1B, 2B4/CD244/SLAMF4, BLAME/SLAMF8, CD2, CD2F-10/SLAMF9, CD48/SLAMF2, CD58/LFA-3, CD84/SLAMF5, CD229/SLAMF3, CRACC/SLAMF7, NTB-A/SLAMF6, SLAM/CD150, CD2, CD7, CD53, CD82/Kai-1, CD90/Thy1, CD96, CD160, CD200, CD300a/LMIR1, HLA Class I, HLA-DR, Ikaros, Integrin alpha 4/CD49d, Integrin alpha 4 beta 1, Integrin alpha 4 beta 7/LPAM-1, LAG-3, TCL1A, TCL1B, CRTAM, DAP12, Dectin-1/CLEC7A, DPPIV/CD26, EphB6, TIM-1/KIM-1/HAVCR, TIM-4, TSLP, TSLP R, lymphocyte function associated antigen-1 (LFA-1), NKG2C, CD3ζ, an immunoreceptor tyrosine-based activation motif (ITAM), CD27, CD28, 4-1BB, CD134/OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and a functional variant thereof including the human versions of each of these sequences. In some embodiments, the intracellular signaling domain and/or intracellular costimulatory domain comprises one or more signaling domains selected from a CD3ζ domain, an ITAM, a CD28 domain, 4-1BB domain, or a functional variant thereof. **Table 5** provides the amino acid sequences of a few exemplary intracellular costimulatory and/or signaling domains. In certain embodiments, as in the case of tisagenlecleucel as described below, the CD3ζ signaling domain of SEQ ID NO:18 may have a mutation, e.g., a glutamine (Q) to lysine (K) mutation, at amino acid position 14 (see SEQ ID NO:115).

Table 5. Exemplary sequences of intracellular costimulatory and/or signaling domains

SEQ ID NO:	Sequence	Description
16	KRGRKKLLYIFKQPFMRPVQTTQEEDG CSCRFPEEEEGGCEL	4-1BB costimulatory domain

17	RSKRSRLLHSDYMNMTPRRPGPTRKHY QPYAPPRDFAAYRS	CD28 costimulatory domain
18	RVKFSRSADAPAYQQGQNQLYNELNL GRREEYDVLDKRRGRDPEMGGKPRRK NPQEGLYNELQKDKMAEAYSEIGMKG ERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR	CD3ζ signaling domain
115	RVKFSRSADAPAYKQGQNQLYNELNL GRREEYDVLDKRRGRDPEMGGKPRRK NPQEGLYNELQKDKMAEAYSEIGMKG ERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR	CD3ζ signaling domain (with Q to K mutation at position 14)

[00798] In certain embodiments where the polycistronic vector encodes two or more CARs, the two or more CARs may comprise the same functional domains, or one or more different functional domains, as described. For example, the two or more CARs may comprise different signal peptides, extracellular binding domains, hinge domains, transmembrane domains, costimulatory domains, and/or intracellular signaling domains, in order to minimize the risk of recombination due to sequence similarities. Or, alternatively, the two or more CARs may comprise the same domains. In the cases where the same domain(s) and/or backbone are used, it is optional to introduce codon divergence at the nucleotide sequence level to minimize the risk of recombination.

CD19 CAR

[00799] In some embodiments, the CAR is a CD19 CAR ("CD19-CAR"), and in these embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a CD19 CAR. In some embodiments, the CD19 CAR may comprise a signal peptide, an extracellular binding domain that specifically binds CD19, a hinge domain, a transmembrane domain, an intracellular costimulatory domain, and/or an intracellular signaling domain in tandem.

[00800] In some embodiments, the signal peptide of the CD19 CAR comprises a CD8α signal peptide. In some embodiments, the CD8α signal peptide comprises or consists of an amino acid sequence set forth in SEQ ID NO:6 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:6. In some embodiments, the signal peptide comprises an IgK signal peptide. In some embodiments, the

IgK signal peptide comprises or consists of an amino acid sequence set forth in SEQ ID NO:7 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:7. In some embodiments, the signal peptide comprises a GMCSFR-α or CSF2RA signal peptide. In some embodiments, the GMCSFR-α or CSF2RA signal peptide comprises or consists of an amino acid sequence set forth in SEQ ID NO:8 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:8.

[00801] In some embodiments, the extracellular binding domain of the CD19 CAR is specific to CD19, for example, human CD19. The extracellular binding domain of the CD19 CAR can be codon-optimized for expression in a host cell or to have variant sequences to increase functions of the extracellular binding domain. In some embodiments, the extracellular binding domain comprises an immunogenically active portion of an immunoglobulin molecule, for example, an scFv.

[00802] In some embodiments, the extracellular binding domain of the CD19 CAR comprises an scFv derived from the FMC63 monoclonal antibody (FMC63), which comprises the heavy chain variable region (V_H) and the light chain variable region (V_L) of FMC63 connected by a linker. FMC63 and the derived scFv have been described in Nicholson et al., Mol. Immun. 34(16-17):1157-1165 (1997) and PCT Application Publication No. WO2018/213337, the entire contents of each of which are incorporated by reference herein. In some embodiments, the amino acid sequences of the entire FMC63-derived scFv (also referred to as FMC63 scFv) and its different portions are provided in **Table 6** below. In some embodiments, the CD19-specific scFv comprises or consists of an amino acid sequence set forth in SEQ ID NO:19, 20, or 25, or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:19, 20, or 25. In some embodiments, the CD19-specific scFv may comprise one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 21-23 and 26-28. In some embodiments, the CD19-specific scFv may comprise a light chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 21-23. In some embodiments, the CD19-specific scFv may comprise a heavy chain with one or

more CDRs having amino acid sequences set forth in SEQ ID NOs: 26-28. In any of these embodiments, the CD19-specific scFv may comprise one or more CDRs comprising one or more amino acid substitutions, or comprising a sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical), to any of the sequences identified. In some embodiments, the extracellular binding domain of the CD19 CAR comprises or consists of the one or more CDRs as described herein.

[00803] In some embodiments, the linker linking the V_H and the V_L portions of the scFv is a Whitlow linker having an amino acid sequence set forth in SEQ ID NO:24. In some embodiments, the Whitlow linker may be replaced by a different linker, for example, a $3xG_4S$ linker having an amino acid sequence set forth in SEQ ID NO:30, which gives rise to a different FMC63-derived scFv having an amino acid sequence set forth in SEQ ID NO:29. In certain of these embodiments, the CD19-specific scFv comprises or consists of an amino acid sequence set forth in SEQ ID NO:29 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:29.

Table 6. Exemplary sequences of anti-CD19 scFv and components

SEQ ID NO:	Amino Acid Sequence	Description
19	DIQMTQTTSSLSASLGDRVTISCRAS QDISKYLNWYQQKPDGTVKLLIYHT SRLHSGVPSRFSGSGSGTDYSLTISN LEQEDIATYFCQQGNTLPYTFGGGT KLEITGSTSGSGKPGSGEGSTKGEVK LQESGPGLVAPSQSLSVTCTVSGVSL PDYGVSWIRQPPRKGLEWLGVIWGS ETTYYNSALKSRLTIIKDNSKSQVFL KMNSLQTDDTAIYYCAKHYYYGGS YAMDYWGQGTSVTVSS	Anti-CD19 FMC63 scFv entire sequence, with Whitlow linker
20	DIQMTQTTSSLSASLGDRVTISCRAS QDISKYLNWYQQKPDGTVKLLIYHT SRLHSGVPSRFSGSGSGTDYSLTISN LEQEDIATYFCQQGNTLPYTFGGGT KLEIT	Anti-CD19 FMC63 scFv light chain variable region
21	QDISKY	Anti-CD19 FMC63 scFv light chain CDR1

22	HTS	Anti-CD19 FMC63 scFv light chain CDR2
23	QQGNTLPYT	Anti-CD19 FMC63 scFv light chain CDR3
24	GSTSGSGKPGSGEGSTKG	Whitlow linker
25	EVKLQESGPGLVAPSQSLSVTCTVS GVSLPDYGVSWIRQPPRKGLEWLG VIWGSETTYYNSALKSRLTIIKDNSK SQVFLKMNSLQTDDTAIYYCAKHY YYGGSYAMDYWGQGTSVTVSS	Anti-CD19 FMC63 scFv heavy chain variable region
26	GVSLPDYG	Anti-CD19 FMC63 scFv heavy chain CDR1
27	IWGSETT	Anti-CD19 FMC63 scFv heavy chain CDR2
28	AKHYYYGGSYAMDY	Anti-CD19 FMC63 scFv heavy chain CDR3
29	DIQMTQTTSSLSASLGDRVTISCRAS QDISKYLNWYQQKPDGTVKLLIYHT SRLHSGVPSRFSGSGSGTDYSLTISN LEQEDIATYFCQQGNTLPYTFGGGT KLEITGGGGSGGGGSGGGGSEVKLQ ESGPGLVAPSQSLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVIWGSET TYYNSALKSRLTIIKDNSKSQVFLK MNSLQTDDTAIYYCAKHYYYGGSY AMDYWGQGTSVTVSS	Anti-CD19 FMC63 scFv entire sequence, with 3xG_4S linker
30	GGGGSGGGGSGGGGS	3xG_4S linker

[00804] In some embodiments, the extracellular binding domain of the CD19 CAR is derived from an antibody specific to CD19, including, for example, SJ25C1 (Bejcek et al., Cancer Res. 55:2346-2351 (1995)), HD37 (Pezutto et al., J. Immunol. 138(9):2793-2799 (1987)), 4G7 (Meeker et al., Hybridoma 3:305-320 (1984)), B43 (Bejcek (1995)), BLY3 (Bejcek (1995)), B4 (Freedman et al., 70:418-427 (1987)), B4 HB12b (Kansas & Tedder, J. Immunol. 147:4094-4102 (1991); Yazawa et al., Proc. Natl. Acad. Sci. USA 102:15178-15183 (2005); Herbst et al., J. Pharmacol. Exp. Ther. 335:213-222 (2010)), BU12 (Callard et al., J. Immunology, 148(10): 2983-2987 (1992)), and CLB-CD19 (De Rie Cell. Immunol. 118:368-381(1989)). In any of these embodiments, the extracellular binding domain of the CD19 CAR can comprise or consist of the V_H, the V_L, and/or one or more CDRs of any of the antibodies.

[00805] In some embodiments, the hinge domain of the CD19 CAR comprises a CD8α hinge domain, for example, a human CD8α hinge domain. In some embodiments, the CD8α hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:9 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:9. In some embodiments, the hinge domain comprises a CD28 hinge domain, for example, a human CD28 hinge domain. In some embodiments, the CD28 hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:10 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:10. In some embodiments, the hinge domain comprises an IgG4 hinge domain, for example, a human IgG4 hinge domain. In some embodiments, the IgG4 hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:11 or SEQ ID NO:12, or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:11 or SEQ ID NO:12. In some embodiments, the hinge domain comprises a IgG4 hinge-Ch2-Ch3 domain, for example, a human IgG4 hinge-Ch2-Ch3 domain. In some embodiments, the IgG4 hinge-Ch2-Ch3 domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:13 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:13.

[00806] In some embodiments, the transmembrane domain of the CD19 CAR comprises a CD8α transmembrane domain, for example, a human CD8α transmembrane domain. In some embodiments, the CD8α transmembrane domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:14 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:14. In some embodiments, the transmembrane domain comprises a CD28 transmembrane domain, for example, a human CD28 transmembrane domain. In some embodiments, the CD28 transmembrane domain comprises or consists of an amino acid sequence set forth in SEQ ID

NO:15 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:15.

[00807] In some embodiments, the intracellular costimulatory domain of the CD19 CAR comprises a 4-1BB costimulatory domain. 4-1BB, also known as CD137, transmits a potent costimulatory signal to T cells, promoting differentiation and enhancing long-term survival of T lymphocytes. In some embodiments, the 4-1BB costimulatory domain is human. In some embodiments, the 4-1BB costimulatory domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:16 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:16. In some embodiments, the intracellular costimulatory domain comprises a CD28 costimulatory domain. CD28 is another co-stimulatory molecule on T cells. In some embodiments, the CD28 costimulatory domain is human. In some embodiments, the CD28 costimulatory domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:17 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:17. In some embodiments, the intracellular costimulatory domain of the CD19 CAR comprises a 4-1BB costimulatory domain and a CD28 costimulatory domain as described.

[00808] In some embodiments, the intracellular signaling domain of the CD19 CAR comprises a CD3 zeta (ζ) signaling domain. CD3ζ associates with T cell receptors (TCRs) to produce a signal and contains immunoreceptor tyrosine-based activation motifs (ITAMs). The CD3ζ signaling domain refers to amino acid residues from the cytoplasmic domain of the zeta chain that are sufficient to functionally transmit an initial signal necessary for T cell activation. In some embodiments, the CD3ζ signaling domain is human. In some embodiments, the CD3ζ signaling domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:18 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:18.

[00809] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a CD19 CAR, including, for example, a CD19 CAR comprising the CD19-specific scFv having sequences set forth in SEQ ID NO:19 or SEQ ID NO:29, the CD8α hinge domain of SEQ ID NO:9, the CD8α transmembrane domain of SEQ ID NO:14, the 4-1BB costimulatory domain of SEQ ID NO:16, the CD3ζ signaling domain of SEQ ID NO:18, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof. In any of these embodiments, the CD19 CAR may additionally comprise a signal peptide (e.g., a CD8α signal peptide) as described.

[00810] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a CD19 CAR, including, for example, a CD19 CAR comprising the CD19-specific scFv having sequences set forth in SEQ ID NO:19 or SEQ ID NO:29, the IgG4 hinge domain of SEQ ID NO:11 or SEQ ID NO:12, the CD28 transmembrane domain of SEQ ID NO:15, the 4-1BB costimulatory domain of SEQ ID NO:16, the CD3ζ signaling domain of SEQ ID NO:18, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof. In any of these embodiments, the CD19 CAR may additionally comprise a signal peptide (e.g., a CD8α signal peptide) as described.

[00811] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a CD19 CAR, including, for example, a CD19 CAR comprising the CD19-specific scFv having sequences set forth in SEQ ID NO:19 or SEQ ID NO:29, the CD28 hinge domain of SEQ ID NO:10, the CD28 transmembrane domain of SEQ ID NO:15, the CD28 costimulatory domain of SEQ ID NO:17, the CD3ζ signaling domain of SEQ ID NO:18, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof. In any of these embodiments, the CD19 CAR may additionally comprise a signal peptide (e.g., a CD8α signal peptide) as described.

[00812] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a CD19 CAR as set forth in SEQ ID NO:116 or is at

least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the nucleotide sequence set forth in SEQ ID NO:116 (see **Table 7**). The encoded CD19 CAR has a corresponding amino acid sequence set forth in SEQ ID NO:117 or is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:117, with the following components: CD8α signal peptide, FMC63 scFv (V_L-Whitlow linker-V_H), CD8α hinge domain, CD8α transmembrane domain, 4-1BB costimulatory domain, and CD3ζ signaling domain.

[00813] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a commercially available embodiment of CD19 CAR. Non-limiting examples of commercially available embodiments of CD19 CARs expressed and/or encoded by T cells include tisagenlecleucel, lisocabtagene maraleucel, axicabtagene ciloleucel, and brexucabtagene autoleucel.

[00814] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding tisagenlecleucel or portions thereof. Tisagenlecleucel comprises a CD19 CAR with the following components: CD8α signal peptide, FMC63 scFv (V_L-3xG_4S linker-V_H), CD8α hinge domain, CD8α transmembrane domain, 4-1BB costimulatory domain, and CD3ζ signaling domain. The nucleotide and amino acid sequence of the CD19 CAR in tisagenlecleucel are provided in **Table 7**, with annotations of the sequences provided in **Table 8**.

[00815] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding lisocabtagene maraleucel or portions thereof. Lisocabtagene maraleucel comprises a CD19 CAR with the following components: GMCSFR-α or CSF2RA signal peptide, FMC63 scFv (V_L-Whitlow linker-V_H), IgG4 hinge domain, CD28 transmembrane domain, 4-1BB costimulatory domain, and CD3ζ signaling domain. The nucleotide and amino acid sequence of the CD19 CAR in lisocabtagene maraleucel are provided in **Table 7**, with annotations of the sequences provided in **Table 9**.

[00816] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding axicabtagene ciloleucel or portions thereof. Axicabtagene ciloleucel comprises a CD19 CAR with the following components: GMCSFR-α or

CSF2RA signal peptide, FMC63 scFv (V_L-Whitlow linker-V_H), CD28 hinge domain, CD28 transmembrane domain, CD28 costimulatory domain, and CD3ζ signaling domain. The nucleotide and amino acid sequence of the CD19 CAR in axicabtagene ciloleucel are provided in **Table 7**, with annotations of the sequences provided in **Table 10**.

[00817] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding brexucabtagene autoleucel or portions thereof. Brexucabtagene autoleucel comprises a CD19 CAR with the following components: GMCSFR-α signal peptide, FMC63 scFv, CD28 hinge domain, CD28 transmembrane domain, CD28 costimulatory domain, and CD3ζ signaling domain.

[00818] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a CD19 CAR as set forth in SEQ ID NO: 31, 33, or 35, or is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the nucleotide sequence set forth in SEQ ID NO: 31, 33, or 35. The encoded CD19 CAR has a corresponding amino acid sequence set forth in SEQ ID NO: 32, 34, or 36, respectively, or is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO: 32, 34, or 36, respectively.

Table 7. Exemplary sequences of CD19 CARs

SEQ ID NO:	Sequence	Description
116	atggccttaccagtgaccgccttgctcctgccgctggccttgctgct ccacgccgccaggccggacatccagatgacacagactacatcctc cctgtctgcctctctgggagacagagtcaccatcagttgcagggca agtcaggacattagtaaatatttaaattggtatcagcagaaaccagat ggaactgttaaactcctgatctaccatacatcaagattacactcagg agtcccatcaaggttcagtggcagtgggtctggaacagattattctc tcaccattagcaacctggagcaagaagatattgccacttacttttgcc aacagggtaatacgcttccgtacacgttcggaggggggaccaagc tggagatcacaggctccacctctggatccggcaagcccggatctg gcgagggatccaccaagggcgaggtgaaactgcaggagtcagg acctggcctggtggcgccctcacagagcctgtccgtcacatgcact gtctcaggggtctcattacccgactatggtgtaagctggattcgcca gcctccacgaaagggtctggagtggctgggagtaatatggggtag tgaaaccacatactataattcagctctcaaatccagactgaccatcat caaggacaactccaagagccaagttttcttaaaaatgaacagtctgc	Exemplary CD19 CAR nucleotide sequence

	aaactgatgacacagccatttactactgtgccaaacattattactacg gtggtagctatgctatggactactggggccaaggaacctcagtcac cgtctcctcaaccacgacgccagcgccgcgaccaccaacaccgg cgcccaccatcgcgtcgcagcccctgtccctgcgcccagaggcgt gccggccagcggcgggggggcgcagtgcacacgaggggggctgg acttcgcctgtgatatctacatctgggcgcccttggccgggacttgt ggggtccttctcctgtcactggttatcacccctttactgcaaacggggc agaaagaaactcctgtatatattcaaacaaccatttatgagaccagta caaactactcaagaggaagatggctgtagctgccgatttccagaag aagaagaaggaggatgtgaactgagagtgaagttcagcaggagc gcagacgcccccgcgtaccagcagggccagaaccagctctataa cgagctcaatctaggacgaagagaggagtacgatgttttggacaa gagacgtggccgggaccctgagatgggggggaaagccgagaag gaagaaccctcaggaaggcctgtacaatgaactgcagaaagataa gatggcggaggcctacagtgagattgggatgaaaggcgagcgcc ggaggggcaaggggcacgatggcctttaccagggtctcagtaca gccaccaaggacacctacgacgcccttcacatgcaggccctgccc cctcgc	
117	MALPVTALLLPLALLLHAARPDIQMTQTTS SLSASLGDRVTISCRASQDISKYLNWYQQK PDGTVKLLIYHTSRLHSGVPSRFSGSGSGT DYSLTISNLEQEDIATYFCQQGNTLPYTFG GGTKLEITGSTSGSGKPGSGEGSTKGEVKL QESGPGLVAPSQSLSVTCTVSGVSLPDYGV SWIRQPPRKGLEWLGVIWGSETTYYNSAL KSRLTIIKDNSKSQVFLKMNSLQTDDTAIY YCAKHYYYGGSYAMDYWGQGTSVTVSST TTPAPRPPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACDIYIWAPLAGTCGVLLLS LVITLYCKRGRKKLLYIFKQPFMRPVQTTQ EEDGCSCRFPEEEEGGCELRVKFSRSADAP AYQQGQNQLYNELNLGRREEYDVLDKRR GRDPEMGGKPRRKNPQEGLYNELQKDKM AEAYSEIGMKGERRRGKGHDGLYQGLSTA TKDTYDALHMQALPPR	Exemplary CD19 CAR amino acid sequence
31	atggccttaccagtgaccgccttgctcctgccgctggccttgctgct ccacgccgccaggccggacatccagatgacacagactacatcctc cctgtctgcctctctgggagacagagtcaccatcagttgcagggca agtcaggacattagtaaatatttaaattggtatcagcagaaaccagat ggaactgttaaactcctgatctaccatacatcaagattacactcagg agtcccatcaaggttcagtggcagtgggtctggaacagattattctc tcaccattagcaacctggagcaagaagatattgccacttacttttgcc aacagggtaatacgcttccgtacacgttcggaggggggaccaagc tggagatcacaggtggcggtggctcgggcggtggtgggtcgggt ggcggcggatctgaggtgaaactgcaggagtcaggacctggcct	Tisagenlecleucel CD19 CAR nucleotide sequence

	ggtggcgccctcacagagcctgtccgtcacatgcactgtctcagg ggtctcattacccgactatggtgtaagctggattcgccagcctccac gaaagggtctggagtggctgggagtaatatggggtagtgaaacca catactataattcagctctcaaatccagactgaccatcatcaaggac aactccaagagccaagttttcttaaaaatgaacagtctgcaaactga tgacacagccatttactactgtgccaaacattattactacggtggtag ctatgctatggactactggggccaaggaacctcagtcaccgtctcct caaccacgacgccagcgccgcgaccaccaacaccggcgcccac catcgcgtcgcagcccctgtccctgcgcccagaggcgtgccggc cagcggcgggggcgcagtgcacacgaggggctggacttcgc ctgtgatatctacatctgggcgcccttggccgggacttgtggggtcc ttctcctgtcactggttatcacccttactgcaaacggggcagaaag aaactcctgtatatattcaaacaaccatttatgagaccagtacaaact actcaagaggaagatggctgtagctgccgatttccagaagaagaa gaaggaggatgtgaactgagagtgaagttcagcaggagcgcaga cgcccccgcgtacaagcagggccagaaccagctctataacgagc tcaatctaggacgaagagaggagtacgatgttttggacaagagac gtggccgggaccctgagatgggggaaagccgagaaggaaga accctcaggaaggcctgtacaatgaactgcagaaagataagatgg cggaggcctacagtgagattgggatgaaaggcgagcgccggag gggcaaggggcacgatggcctttaccagggtctcagtacagccac caaggacacctacgacgcccttcacatgcaggccctgcccctcg c	
32	MALPVTALLLPLALLLHAARPDIQMTQTTS SLSASLGDRVTISCRASQDISKYLNWYQQK PDGTVKLLIYHTSRLHSGVPSRFSGSGSGT DYSLTISNLEQEDIATYFCQQGNTLPYTFG GGTKLEITGGGGSGGGGSGGGGSEVKLQE SGPGLVAPSQSLSVTCTVSGVSLPDYGVSW IRQPPRKGLEWLGVIWGSETTYYNSALKSR LTIIKDNSKSQVFLKMNSLQTDDTAIYYCA KHYYYGGSYAMDYWGQGTSVTVSSTTTP APRPPTPAPTIASQPLSLRPEACRPAAGGAV HTRGLDFACDIYIWAPLAGTCGVLLLSLVI TLYCKRGRKKLLYIFKQPFMRPVQTTQEED GCSCRFPEEEEGGCELRVKFSRSADAPAYK QGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEA YSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPR	Tisagenlecleucel CD19 CAR amino acid sequence
33	atgctgctgctggtgaccagcctgctgctgtgcgagctgccccacc ccgcctttctgctgatccccgacatccagatgacccagaccacctc cagcctgagcgccagcctgggcgaccgggtgaccatcagctgcc gggccagccaggacatcagcaagtacctgaactggtatcagcag aagcccgacggcaccgtcaagctgctgatctaccacaccagccg	Lisocabtagene maraleucel CD19 CAR nucleotide sequence

	gctgcacagcggcgtgcccagccggtttagcggcagcggctccg gcaccgactacagcctgaccatctccaacctggaacaggaagata tcgccacctacttttgccagcagggcaacacactgccctacaccttt ggcggcggaacaaagctggaaatcaccggcagcacctccggca gcggcaagcctggcagcggcgagggcagcaccaagggcgagg tgaagctgcaggaaagcggccctggcctggtggcccccagccag agcctgagcgtgacctgcaccgtgagcggcgtgagcctgcccga ctacggcgtgagctggatccggcagccccccaggaagggcctgg aatggctgggcgtgatctggggcagcgagaccacctactacaaca gcgccctgaagagccggctgaccatcatcaaggacaacagcaag agccaggtgttcctgaagatgaacagcctgcagaccgacgacacc gccatctactactgcgccaagcactactactacggcggcagctacg ccatggactactggggccagggcaccagcgtgaccgtgagcagc gaatctaagtacggaccgccctgccccccttgccctatgttctgggt gctggtggtggtcggaggcgtgctggcctgctacagcctgctggt caccgtggccttcatcatcttttgggtgaaacggggcagaaagaaa ctcctgtatatattcaaacaaccatttatgagaccagtacaaactactc aagaggaagatggctgtagctgccgatttccagaagaagaagaag gaggatgtgaactgcgggtgaagttcagcagaagcgccgacgcc cctgcctaccagcagggccagaatcagctgtacaacgagctgaac ctgggcagaagggaagagtacgacgtcctggataagcggagag gccgggaccctgagatgggcggcaagcctcggcggaagaaccc ccaggaaggcctgtataacgaactgcagaaagacaagatggccg aggcctacagcgagatcggcatgaagggcgagcggaggcgggg gcaagggccacgacggcctgtatcagggcctgtccaccgccacc aaggatacctacgacgccctgcacatgcaggccctgcccccaag g	
34	MLLLVTSLLLCELPHPAFLLIPDIQMTQTTS SLSASLGDRVTISCRASQDISKYLNWYQQK PDGTVKLLIYHTSRLHSGVPSRFSGSGSGT DYSLTISNLEQEDIATYFCQQGNTLPYTFG GGTKLEITGSTSGSGKPGSGEGSTKGEVKL QESGPGLVAPSQSLSVTCTVSGVSLPDYGV SWIRQPPRKGLEWLGVIWGSETTYYNSAL KSRLTIIKDNSKSQVFLKMNSLQTDDTAIY YCAKHYYYGGSYAMDYWGQGTSVTVSSE SKYGPPCPPCPMFWVLVVVGGVLACYSLL VTVAFIIFWVKRGRKKLLYIFKQPFMRPVQ TTQEEDGCSCRFPEEEEGGCELRVKFSRSA DAPAYQQGQNQLYNELNLGRREEYDVLD KRRGRDPEMGGKPRRKNPQEGLYNELQK DKMAEAYSEIGMKGERRRGKGHDGLYQG LSTATKDTYDALHMQALPPR	Lisocabtagene maraleucel CD19 CAR amino acid sequence
35	atgcttctcctggtgacaagccttctgctctgtgagttaccacaccca gcattcctcctgatcccagacatccagatgacacagactacatcctc	Axicabtagene ciloleucel CD19

	cctgtctgcctctctgggagacagagtcaccatcagttgcagggca agtcaggacattagtaaatatttaaattggtatcagcagaaaccagat ggaactgttaaactcctgatctaccatacatcaagattacactcagg agtcccatcaaggttcagtggcagtgggtctggaacagattattctc tcaccattagcaacctggagcaagaagatattgccacttacttttgcc aacagggtaatacgcttccgtacacgttcggaggggggactaagtt ggaaataacaggctccacctctggatccggcaagcccggatctgg cgagggatccaccaagggcgaggtgaaactgcaggagtcagga cctggcctggtggcgccctcacagagcctgtccgtcacatgcactg tctcaggggtctcattacccgactatggtgtaagctggattcgccag cctccacgaaagggtctggagtggctgggagtaatatggggtagt gaaaccacatactataattcagctctcaaatccagactgaccatcatc aaggacaactccaagagccaagttttcttaaaaatgaacagtctgca aactgatgacacagccatttactactgtgccaaacattattactacgg tggtagctatgctatggactactggggtcaaggaacctcagtcacc gtctcctcagcggccgcaattgaagttatgtatcctcctccttaccta gacaatgagaagagcaatggaaccattatccatgtgaaagggaaa caccttttgtccaagtcccctatttcccggaccttctaagccctttttggg tgctggtggtggttgggggagtcctggcttgctatagcttgctagta acagtggcctttattattttctgggtgaggagtaagaggagcaggct cctgcacagtgactacatgaacatgactccccgccgccccgggcc cacccgcaagcattaccagccctatgccccaccacgcgacttcgc agcctatcgctccagagtgaagttcagcaggagcgcagacgccc ccgcgtaccagcagggccagaaccagctctataacgagctcaatc taggacgaagagaggagtacgatgttttggacaagagacgtggcc gggaccctgagatgggggggaaagccgagaaggaagaaccctca ggaaggcctgtacaatgaactgcagaaagataagatggcggagg cctacagtgagattgggatgaaaggcgagcgccggaggggcaa ggggcacgatggcctttaccagggtctcagtacagccaccaagga cacctacgacgcccttcacatgcaggccctgcccccctcgc	CAR nucleotide sequence
36	MLLLVTSLLLCELPHPAFLLIPDIQMTQTTS SLSASLGDRVTISCRASQDISKYLNWYQQK PDGTVKLLIYHTSRLHSGVPSRFSGSGSGT DYSLTISNLEQEDIATYFCQQGNTLPYTFG GGTKLEITGSTSGSGKPGSGEGSTKGEVKL QESGPGLVAPSQSLSVTCTVSGVSLPDYGV SWIRQPPRKGLEWLGVIWGSETTYYNSAL KSRLTIIKDNSKSQVFLKMNSLQTDDTAIY YCAKHYYYGGSYAMDYWGQGTSVTVSSA AAIEVMYPPPYLDNEKSNGTIIHVKGKHLC PSPLFPGPSKPFWVLVVVGGVLACYSLLVT VAFIIFWVRSKRSRLLHSDYMNMTPRRPGP TRKHYQPYAPPRDFAAYRSRVKFSRSADA PAYQQGQNQLYNELNLGRREEYDVLDKR RGRDPEMGGKPRRKNPQEGLYNELQKDK	Axicabtagene ciloleucel CD19 CAR amino acid sequence

	MAEAYSEIGMKGERRRGKGHDGLYQGLS TATKDTYDALHMQALPPR	

Table 8. Annotation of tisagenlecleucel CD19 CAR sequences

Feature	Nucleotide Sequence Position	Amino Acid Sequence Position
CD8α signal peptide	1-63	1-21
FMC63 scFv (V_L-3xG_4S linker-V_H)	64-789	22-263
CD8α hinge domain	790-924	264-308
CD8α transmembrane domain	925-996	309-332
4-1BB costimulatory domain	997-1122	333-374
CD3ζ signaling domain	1123-1458	375-486

Table 9. Annotation of lisocabtagene maraleucel CD19 CAR sequences

Feature	Nucleotide Sequence Position	Amino Acid Sequence Position
GMCSFR-α signal peptide	1-66	1-22
FMC63 scFv (V_L-Whitlow linker-V_H)	67-801	23-267
IgG4 hinge domain	802-837	268-279
CD28 transmembrane domain	838-921	280-307
4-1BB costimulatory domain	922-1047	308-349
CD3ζ signaling domain	1048-1383	350-461

Table 10. Annotation of axicabtagene ciloleucel CD19 CAR sequences

Feature	Nucleotide Sequence Position	Amino Acid Sequence Position
CSF2RA signal peptide	1-66	1-22
FMC63 scFv (V_L-Whitlow linker-V_H)	67-801	23-267
CD28 hinge domain	802-927	268-309
CD28 transmembrane domain	928-1008	310-336
CD28 costimulatory domain	1009-1131	337-377

CD3ζ signaling domain	1132-1467	378-489

[00819] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding CD19 CAR as set forth in SEQ ID NO: 31, 33, or 35, or at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the nucleotide sequence set forth in SEQ ID NO: 31, 33, or 35. The encoded CD19 CAR has a corresponding amino acid sequence set forth in SEQ ID NO: 32, 34, or 36, respectively, is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO: 32, 34, or 36, respectively.

CD20 CAR

[00820] In some embodiments, the CAR is a CD20 CAR ("CD20-CAR"), and in these embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a CD20 CAR. CD20 is an antigen found on the surface of B cells as early at the pro-B phase and progressively at increasing levels until B cell maturity, as well as on the cells of most B-cell neoplasms. CD20 positive cells are also sometimes found in cases of Hodgkins disease, myeloma, and thymoma. In some embodiments, the CD20 CAR may comprise a signal peptide, an extracellular binding domain that specifically binds CD20, a hinge domain, a transmembrane domain, an intracellular costimulatory domain, and/or an intracellular signaling domain in tandem.

[00821] In some embodiments, the signal peptide of the CD20 CAR comprises a CD8α signal peptide. In some embodiments, the CD8α signal peptide comprises or consists of an amino acid sequence set forth in SEQ ID NO:6 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:6. In some embodiments, the signal peptide comprises an IgK signal peptide. In some embodiments, the IgK signal peptide comprises or consists of an amino acid sequence set forth in SEQ ID NO:7 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:7. In some embodiments, the signal peptide comprises a GMCSFR-α or CSF2RA signal peptide. In some embodiments, the GMCSFR-α or CSF2RA signal peptide comprises or consists of an amino acid sequence set forth in SEQ ID

NO:8 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:8.

[00822] In some embodiments, the extracellular binding domain of the CD20 CAR is specific to CD20, for example, human CD20. The extracellular binding domain of the CD20 CAR can be codon-optimized for expression in a host cell or to have variant sequences to increase functions of the extracellular binding domain. In some embodiments, the extracellular binding domain comprises an immunogenically active portion of an immunoglobulin molecule, for example, an scFv.

[00823] In some embodiments, the extracellular binding domain of the CD20 CAR is derived from an antibody specific to CD20, including, for example, Leu16, IF5, 1.5.3, rituximab, obinutuzumab, ibritumomab, ofatumumab, tositumumab, odronextamab, veltuzumab, ublituximab, and ocrelizumab. In some embodiments, the CD20 CAR is derived from a CAR specific to CD20, including, for example, MB-106, UCART20, or C-CAR066, as detailed in **Table 11A**. In any of these embodiments, the extracellular binding domain of the CD20 CAR can comprise or consist of the V_H, the V_L, and/or one or more CDRs of any of the antibodies or CARs detailed in **Table 11A**.

Table 11A. Exemplary CD20 -specific CARs

CAR Name	Antigen	Company	Reference
MB-106	CD20	Fred Hutchinson Cancer Research Center	Shadman et al., Blood 134 (Suppl._1):3235 (2019)
UCART20	CD20	Cellectis	www.cellbiomedgroup.com
C-CAR066	CD20	Cellular Biomedicine Group	Liang et al., J Clin Oncol 39(15)_suppl:2508-2508 (2021)

[00824] In some embodiments, the extracellular binding domain of the CD20 CAR comprises an scFv derived from the Leu16 monoclonal antibody, which comprises the heavy chain variable

region (V_H) and the light chain variable region (V_L) of Leu16 connected by a linker. See Wu et al., Protein Engineering. 14(12):1025-1033 (2001). In some embodiments, the linker is a $3xG_4S$ linker. In other embodiments, the linker is a Whitlow linker as described herein. In some embodiments, the amino acid sequences of different portions of the entire Leu16-derived scFv (also referred to as Leu16 scFv) and its different portions are provided in **Table 11B** below. In some embodiments, the CD20-specific scFv comprises or consists of an amino acid sequence set forth in SEQ ID NO:37, 38, or 42, or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:37, 38, or 42. In some embodiments, the CD20-specific scFv may comprise one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 39-41, 43 and 44. In some embodiments, the CD20-specific scFv may comprise a light chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 39-41. In some embodiments, the CD20-specific scFv may comprise a heavy chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 43-44. In any of these embodiments, the CD20-specific scFv may comprise one or more CDRs comprising one or more amino acid substitutions, or comprising a sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical), to any of the sequences identified. In some embodiments, the extracellular binding domain of the CD20 CAR comprises or consists of the one or more CDRs as described herein.

Table 11B. Exemplary sequences of anti-CD20 scFv and components

SEQ ID NO:	Amino Acid Sequence	Description
37	DIVLTQSPAILSASPGEKVTMTCRAS SSVNYMDWYQKKPGSSPKPWIYAT SNLASGVPARFSGSGSGTSYSLTISR VEAEDAATYYCQQWSFNPPTFGGG TKLEIKGSTSGSGKPGSGEGSTKGEV QLQQSGAELVKPGASVKMSCKASG YTFTSYNMHWVKQTPGQGLEWIGA IYPGNGDTSYNQKFKGKATLTADKS SSTAYMQLSSLTSEDSADYYCARSN YYGSSYWFFDVWGAGTTVTVSS	Anti-CD20 Leu16 scFv entire sequence, with Whitlow linker

38	DIVLTQSPAILSASPGEKVTMTCRAS SSVNYMDWYQKKPGSSPKPWIYAT SNLASGVPARFSGSGSGTSYSLTISR VEAEDAATYYCQQWSFNPPTFGGG TKLEIK	Anti-CD20 Leu16 scFv light chain variable region
39	RASSSVNYMD	Anti-CD20 Leu16 scFv light chain CDR1
40	ATSNLAS	Anti-CD20 Leu16 scFv light chain CDR2
41	QQWSFNPPT	Anti-CD20 Leu16 scFv light chain CDR3
42	EVQLQQSGAELVKPGASVKMSCKA SGYTFTSYNMHWVKQTPGQGLEWI GAIYPGNGDTSYNQKFKGKATLTA DKSSSTAYMQLSSLTSEDSADYYCA RSNYYGSSYWFFDVWGAGTTVTVS S	Anti-CD20 Leu16 scFv heavy chain
43	SYNMH	Anti-CD20 Leu16 scFv heavy chain CDR1
44	AIYPGNGDTSYNQKFKG	Anti-CD20 Leu16 scFv heavy chain CDR2

[00825] In some embodiments, the hinge domain of the CD20 CAR comprises a CD8α hinge domain, for example, a human CD8α hinge domain. In some embodiments, the CD8α hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:9 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:9. In some embodiments, the hinge domain comprises a CD28 hinge domain, for example, a human CD28 hinge domain. In some embodiments, the CD28 hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:10 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:10. In some embodiments, the hinge domain comprises an IgG4 hinge domain, for example, a human IgG4 hinge domain. In some embodiments, the IgG4 hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:11 or SEQ ID NO:12, or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least

98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:11 or SEQ ID NO:12. In some embodiments, the hinge domain comprises a IgG4 hinge-Ch2-Ch3 domain, for example, a human IgG4 hinge-Ch2-Ch3 domain. In some embodiments, the IgG4 hinge-Ch2-Ch3 domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:13 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:13.

[00826] In some embodiments, the transmembrane domain of the CD20 CAR comprises a CD8α transmembrane domain, for example, a human CD8α transmembrane domain. In some embodiments, the CD8α transmembrane domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:14 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:14. In some embodiments, the transmembrane domain comprises a CD28 transmembrane domain, for example, a human CD28 transmembrane domain. In some embodiments, the CD28 transmembrane domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:15 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:15.

[00827] In some embodiments, the intracellular costimulatory domain of the CD20 CAR comprises a 4-1BB costimulatory domain, for example, a human 4-1BB costimulatory domain. In some embodiments, the 4-1BB costimulatory domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:16 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:16. In some embodiments, the intracellular costimulatory domain comprises a CD28 costimulatory domain, for example, a human CD28 costimulatory domain. In some embodiments, the CD28 costimulatory domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:17 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:17.

[00828] In some embodiments, the intracellular signaling domain of the CD20 CAR comprises a CD3 zeta (ζ) signaling domain, for example, a human CD3ζ signaling domain. In some embodiments, the CD3ζ signaling domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:18 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:18.

[00829] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a CD20 CAR, including, for example, a CD20 CAR comprising the CD20-specific scFv having sequences set forth in SEQ ID NO:37, the CD8α hinge domain of SEQ ID NO:9, the CD8α transmembrane domain of SEQ ID NO:14, the 4-1BB costimulatory domain of SEQ ID NO:16, the CD3ζ signaling domain of SEQ ID NO:18, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof.

[00830] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a CD20 CAR, including, for example, a CD20 CAR comprising the CD20-specific scFv having sequences set forth in SEQ ID NO:37, the CD28 hinge domain of SEQ ID NO:10, the CD8α transmembrane domain of SEQ ID NO:14, the 4-1BB costimulatory domain of SEQ ID NO:16, the CD3ζ signaling domain of SEQ ID NO:18, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof.

[00831] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a CD20 CAR, including, for example, a CD20 CAR comprising the CD20-specific scFv having sequences set forth in SEQ ID NO:37, the IgG4 hinge domain of SEQ ID NO:11 or SEQ ID NO:12, the CD8α transmembrane domain of SEQ ID NO:14, the 4-1BB costimulatory domain of SEQ ID NO:16, the CD3ζ signaling domain of SEQ ID NO:18, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof.

[00832] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a CD20 CAR, including, for example, a CD20 CAR comprising the CD20-specific scFv having sequences set forth in SEQ ID NO:37, the CD8α hinge domain of SEQ ID NO:9, the CD28 transmembrane domain of SEQ ID NO:15, the 4-1BB costimulatory domain of SEQ ID NO:16, the CD3ζ signaling domain of SEQ ID NO:18, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof.

[00833] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a CD20 CAR, including, for example, a CD20 CAR comprising the CD20-specific scFv having sequences set forth in SEQ ID NO:37, the CD28 hinge domain of SEQ ID NO:10, the CD28 transmembrane domain of SEQ ID NO:15, the 4-1BB costimulatory domain of SEQ ID NO:16, the CD3ζ signaling domain of SEQ ID NO:18, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof.

[00834] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a CD20 CAR, including, for example, a CD20 CAR comprising the CD20-specific scFv having sequences set forth in SEQ ID NO:37, the IgG4 hinge domain of SEQ ID NO:11 or SEQ ID NO:1, the CD28 transmembrane domain of SEQ ID NO:15, the 4-1BB costimulatory domain of SEQ ID NO:16, the CD3ζ signaling domain of SEQ ID NO:18, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof.

CD22 CAR

[00835] In some embodiments, the CAR is a CD22 CAR ("CD22-CAR"), and in these embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a CD22 CAR. CD22, which is a transmembrane protein found mostly on the surface of mature B cells that functions as an inhibitory receptor for B cell receptor (BCR) signaling. CD22 is expressed in 60-70% of B cell lymphomas and leukemias (e.g., B-

chronic lymphocytic leukemia, hairy cell leukemia, acute lymphocytic leukemia (ALL), and Burkitt's lymphoma) and is not present on the cell surface in early stages of B cell development or on stem cells. In some embodiments, the CD22 CAR may comprise a signal peptide, an extracellular binding domain that specifically binds CD22, a hinge domain, a transmembrane domain, an intracellular costimulatory domain, and/or an intracellular signaling domain in tandem.

[00836] In some embodiments, the signal peptide of the CD22 CAR comprises a CD8α signal peptide. In some embodiments, the CD8α signal peptide comprises or consists of an amino acid sequence set forth in SEQ ID NO:6 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:6. In some embodiments, the signal peptide comprises an IgK signal peptide. In some embodiments, the IgK signal peptide comprises or consists of an amino acid sequence set forth in SEQ ID NO:7 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:7. In some embodiments, the signal peptide comprises a GMCSFR-α or CSF2RA signal peptide. In some embodiments, the GMCSFR-α or CSF2RA signal peptide comprises or consists of an amino acid sequence set forth in SEQ ID NO:8 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:8.

[00837] In some embodiments, the extracellular binding domain of the CD22 CAR is specific to CD22, for example, human CD22. The extracellular binding domain of the CD22 CAR can be codon-optimized for expression in a host cell or to have variant sequences to increase functions of the extracellular binding domain. In some embodiments, the extracellular binding domain comprises an immunogenically active portion of an immunoglobulin molecule, for example, an scFv.

[00838] In some embodiments, the extracellular binding domain of the CD22 CAR is derived from an antibody specific to CD22, including, for example, SM03, inotuzumab, epratuzumab, moxetumomab, and pinatuzumab. In any of these embodiments, the extracellular binding

domain of the CD22 CAR can comprise or consist of the V_H, the V_L, and/or one or more CDRs of any of the antibodies.

[00839] In some embodiments, the extracellular binding domain of the CD22 CAR comprises an scFv derived from the m971 monoclonal antibody (m971), which comprises the heavy chain variable region (V_H) and the light chain variable region (V_L) of m971 connected by a linker. In some embodiments, the linker is a 3xG_4S linker. In other embodiments, the Whitlow linker may be used instead. In some embodiments, the amino acid sequences of the entire m971-derived scFv (also referred to as m971 scFv) and its different portions are provided in **Table 12** below. In some embodiments, the CD22-specific scFv comprises or consists of an amino acid sequence set forth in SEQ ID NO:45, 46, or 50, or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:45, 46, or 50. In some embodiments, the CD22-specific scFv may comprise one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 47-49 and 51-53. In some embodiments, the CD22-specific scFv may comprise a heavy chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 47-49. In some embodiments, the CD22-specific scFv may comprise a light chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 51-53. In any of these embodiments, the CD22-specific scFv may comprise one or more CDRs comprising one or more amino acid substitutions, or comprising a sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical), to any of the sequences identified. In some embodiments, the extracellular binding domain of the CD22 CAR comprises or consists of the one or more CDRs as described herein.

[00840] In some embodiments, the extracellular binding domain of the CD22 CAR comprises an scFv derived from m971-L7, which is an affinity matured variant of m971 with significantly improved CD22 binding affinity compared to the parental antibody m971 (improved from about 2 nM to less than 50 pM). In some embodiments, the scFv derived from m971-L7 comprises the V_H and the V_L of m971-L7 connected by a 3xG_4S linker. In other embodiments, the Whitlow linker may be used instead. In some embodiments, the amino acid sequences of the entire m971-L7-derived scFv (also referred to as m971-L7 scFv) and its different portions are provided in **Table 12** below. In some embodiments, the CD22-specific scFv comprises or consists of an

amino acid sequence set forth in SEQ ID NO:54, 55, or 59, or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:54, 55, or 59. In some embodiments, the CD22-specific scFv may comprise one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 56-58 and 60-62. In some embodiments, the CD22-specific scFv may comprise a heavy chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 56-58. In some embodiments, the CD22-specific scFv may comprise a light chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 60-62. In any of these embodiments, the CD22-specific scFv may comprise one or more CDRs comprising one or more amino acid substitutions, or comprising a sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical), to any of the sequences identified. In some embodiments, the extracellular binding domain of the CD22 CAR comprises or consists of the one or more CDRs as described herein.

Table 12. Exemplary sequences of anti-CD22 scFv and components

SEQ ID NO:	Amino Acid Sequence	Description
45	QVQLQQSGPGLVKPSQTLSLTCAISG DSVSSNSAAWNWIRQSPSRGLEWL GRTYYRSKWYNDYAVSVKSRITINP DTSKNQFSLQLNSVTPEDTAVYYCA REVTGDLEDAFDIWGQGTMVTVSS GGGGSGGGGSGGGGSDIQMTQSPSS LSASVGDRVTITCRASQTIWSYLNW YQQRPGKAPNLLIYAASSLQSGVPS RFSGRGSGTDFTLTISSLQAEDFATY YCQQSYSIPQTFGQGTKLEIK	Anti-CD22 m971 scFv entire sequence, with 3xG_4S linker
46	QVQLQQSGPGLVKPSQTLSLTCAISG DSVSSNSAAWNWIRQSPSRGLEWL GRTYYRSKWYNDYAVSVKSRITINP DTSKNQFSLQLNSVTPEDTAVYYCA REVTGDLEDAFDIWGQGTMVTVSS	Anti-CD22 m971 scFv heavy chain variable region
47	GDSVSSNSAA	Anti-CD22 m971 scFv heavy chain CDR1
48	TYYRSKWYN	Anti-CD22 m971 scFv heavy chain CDR2

49	AREVTGDLEDAFDI	Anti-CD22 m971 scFv heavy chain CDR3
50	DIQMTQSPSSLSASVGDRVTITCRAS QTIWSYLNWYQQRPGKAPNLLIYA ASSLQSGVPSRFSGRGSGTDFTLTISS LQAEDFATYYCQQSYSIPQTFGQGT KLEIK	Anti-CD22 m971 scFv light chain
51	QTIWSY	Anti-CD22 m971 scFv light chain CDR1
52	AAS	Anti-CD22 m971 scFv light chain CDR2
53	QQSYSIPQT	Anti-CD22 m971 scFv light chain CDR3
54	QVQLQQSGPGMVKPSQTLSLTCAIS GDSVSSNSVAWNWIRQSPSRGLEW LGRTYYRSTWYNDYAVSMKSRITIN PDTNKNQFSLQLNSVTPEDTAVYYC AREVTGDLEDAFDIWGQGTMVTVS SGGGGSGGGGSGGGGSDIQMIQSPS SLSASVGDRVTITCRASQTIWSYLN WYRQRPGEAPNLLIYAASSLQSGVP SRFSGRGSGTDFTLTISSLQAEDFAT YYCQQSYSIPQTFGQGTKLEIK	Anti-CD22 m971-L7 scFv entire sequence, with 3xG_4S linker
55	QVQLQQSGPGMVKPSQTLSLTCAIS GDSVSSNSVAWNWIRQSPSRGLEW LGRTYYRSTWYNDYAVSMKSRITIN PDTNKNQFSLQLNSVTPEDTAVYYC AREVTGDLEDAFDIWGQGTMVTVS S	Anti-CD22 m971-L7 scFv heavy chain variable region
56	GDSVSSNSVA	Anti-CD22 m971-L7 scFv heavy chain CDR1
57	TYYRSTWYN	Anti-CD22 m971-L7 scFv heavy chain CDR2
58	AREVTGDLEDAFDI	Anti-CD22 m971-L7 scFv heavy chain CDR3
59	DIQMIQSPSSLSASVGDRVTITCRAS QTIWSYLNWYRQRPGEAPNLLIYAA SSLQSGVPSRFSGRGSGTDFTLTISSL QAEDFATYYCQQSYSIPQTFGQGTK LEIK	Anti-CD22 m971-L7 scFv light chain variable region

60	QTIWSY	Anti-CD22 m971-L7 scFv light chain CDR1
61	AAS	Anti-CD22 m971-L7 scFv light chain CDR2
62	QQSYSIPQT	Anti-CD22 m971-L7 scFv light chain CDR3

[00841] In some embodiments, the extracellular binding domain of the CD22 CAR comprises immunotoxins HA22 or BL22. Immunotoxins BL22 and HA22 are therapeutic agents that comprise an scFv specific for CD22 fused to a bacterial toxin, and thus can bind to the surface of the cancer cells that express CD22 and kill the cancer cells. BL22 comprises a dsFv of an anti-CD22 antibody, RFB4, fused to a 38-kDa truncated form of *Pseudomonas* exotoxin A (Bang et al., Clin. Cancer Res., 11:1545-50 (2005)). HA22 (CAT8015, moxetumomab pasudotox) is a mutated, higher affinity version of BL22 (Ho et al., J. Biol. Chem., 280(1): 607-17 (2005)). Suitable sequences of antigen binding domains of HA22 and BL22 specific to CD22 are disclosed in, for example, U.S. Patent Nos. 7,541,034; 7,355,012; and 7,982,011, which are hereby incorporated by reference in their entirety.

[00842] In some embodiments, the hinge domain of the CD22 CAR comprises a CD8α hinge domain, for example, a human CD8α hinge domain. In some embodiments, the CD8α hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:9 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:9. In some embodiments, the hinge domain comprises a CD28 hinge domain, for example, a human CD28 hinge domain. In some embodiments, the CD28 hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:10 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:10. In some embodiments, the hinge domain comprises an IgG4 hinge domain, for example, a human IgG4 hinge domain. In some embodiments, the IgG4 hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:11 or SEQ ID NO:12, or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:11 or

SEQ ID NO:12. In some embodiments, the hinge domain comprises a IgG4 hinge-Ch2-Ch3 domain, for example, a human IgG4 hinge-Ch2-Ch3 domain. In some embodiments, the IgG4 hinge-Ch2-Ch3 domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:13 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:13.

[00843] In some embodiments, the transmembrane domain of the CD22 CAR comprises a CD8α transmembrane domain, for example, a human CD8α transmembrane domain. In some embodiments, the CD8α transmembrane domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:14 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:14. In some embodiments, the transmembrane domain comprises a CD28 transmembrane domain, for example, a human CD28 transmembrane domain. In some embodiments, the CD28 transmembrane domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:15 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:15.

[00844] In some embodiments, the intracellular costimulatory domain of the CD22 CAR comprises a 4-1BB costimulatory domain, for example, a human 4-1BB costimulatory domain. In some embodiments, the 4-1BB costimulatory domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:16 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:16. In some embodiments, the intracellular costimulatory domain comprises a CD28 costimulatory domain, for example, a human CD28 costimulatory domain. In some embodiments, the CD28 costimulatory domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:17 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:17.

[00845] In some embodiments, the intracellular signaling domain of the CD22 CAR comprises a CD3 zeta (ζ) signaling domain, for example, a human CD3ζ signaling domain. In some embodiments, the CD3ζ signaling domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:18 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:18.

[00846] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a CD22 CAR, including, for example, a CD22 CAR comprising the CD22-specific scFv having sequences set forth in SEQ ID NO:45 or SEQ ID NO:54, the CD8α hinge domain of SEQ ID NO:9, the CD8α transmembrane domain of SEQ ID NO:14, the 4-1BB costimulatory domain of SEQ ID NO:16, the CD3ζ signaling domain of SEQ ID NO:18, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof.

[00847] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a CD22 CAR, including, for example, a CD22 CAR comprising the CD22-specific scFv having sequences set forth in SEQ ID NO:45 or SEQ ID NO:54, the CD28 hinge domain of SEQ ID NO:10, the CD8α transmembrane domain of SEQ ID NO:14, the 4-1BB costimulatory domain of SEQ ID NO:16, the CD3ζ signaling domain of SEQ ID NO:18, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof.

[00848] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a CD22 CAR, including, for example, a CD22 CAR comprising the CD22-specific scFv having sequences set forth in SEQ ID NO:45 or SEQ ID NO:54, the IgG4 hinge domain of SEQ ID NO:11 or SEQ ID NO:12, the CD8α transmembrane domain of SEQ ID NO:14, the 4-1BB costimulatory domain of SEQ ID NO:16, the CD3ζ signaling domain of SEQ ID NO:18, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof.

[00849] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a CD22 CAR, including, for example, a CD22 CAR comprising the CD22-specific scFv having sequences set forth in SEQ ID NO:45 or SEQ ID NO:54, the CD8α hinge domain of SEQ ID NO:9, the CD28 transmembrane domain of SEQ ID NO:15, the 4-1BB costimulatory domain of SEQ ID NO:16, the CD3ζ signaling domain of SEQ ID NO:18, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof.

[00850] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a CD22 CAR, including, for example, a CD22 CAR comprising the CD22-specific scFv having sequences set forth in SEQ ID NO:45 or SEQ ID NO:54, the CD28 hinge domain of SEQ ID NO:10, the CD28 transmembrane domain of SEQ ID NO:15, the 4-1BB costimulatory domain of SEQ ID NO:16, the CD3ζ signaling domain of SEQ ID NO:18, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof.

[00851] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a CD22 CAR, including, for example, a CD22 CAR comprising the CD22-specific scFv having sequences set forth in SEQ ID NO:45 or SEQ ID NO:54, the IgG4 hinge domain of SEQ ID NO:11 or SEQ ID NO:12, the CD28 transmembrane domain of SEQ ID NO:15, the 4-1BB costimulatory domain of SEQ ID NO:16, the CD3ζ signaling domain of SEQ ID NO:18, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof.

BCMA CAR

[00852] In some embodiments, the CAR is a BCMA CAR ("BCMA-CAR"), and in these embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a BCMA CAR. BCMA is a tumor necrosis family receptor (TNFR) member expressed on cells of the B cell lineage, with the highest expression on terminally differentiated B cells or mature B lymphocytes. BCMA is involved in mediating the

survival of plasma cells for maintaining long-term humoral immunity. The expression of BCMA has been recently linked to a number of cancers, such as multiple myeloma, Hodgkin's and non-Hodgkin's lymphoma, various leukemias, and glioblastoma. In some embodiments, the BCMA CAR may comprise a signal peptide, an extracellular binding domain that specifically binds BCMA, a hinge domain, a transmembrane domain, an intracellular costimulatory domain, and/or an intracellular signaling domain in tandem.

[00853] In some embodiments, the signal peptide of the BCMA CAR comprises a CD8α signal peptide. In some embodiments, the CD8α signal peptide comprises or consists of an amino acid sequence set forth in SEQ ID NO:6 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:6. In some embodiments, the signal peptide comprises an IgK signal peptide. In some embodiments, the IgK signal peptide comprises or consists of an amino acid sequence set forth in SEQ ID NO:7 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:7. In some embodiments, the signal peptide comprises a GMCSFR-α or CSF2RA signal peptide. In some embodiments, the GMCSFR-α or CSF2RA signal peptide comprises or consists of an amino acid sequence set forth in SEQ ID NO:8 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:8.

[00854] In some embodiments, the extracellular binding domain of the BCMA CAR is specific to BCMA, for example, human BCMA. The extracellular binding domain of the BCMA CAR can be codon-optimized for expression in a host cell or to have variant sequences to increase functions of the extracellular binding domain.

[00855] In some embodiments, the extracellular binding domain comprises an immunogenically active portion of an immunoglobulin molecule, for example, an scFv. In some embodiments, the extracellular binding domain of the BCMA CAR is derived from an antibody specific to BCMA, including, for example, belantamab, erlanatamab, teclistamab, LCAR-B38M, and ciltacabtagene. In any of these embodiments, the extracellular binding domain of the BCMA

CAR can comprise or consist of the V_H, the V_L, and/or one or more CDRs of any of the antibodies.

[00856] In some embodiments, the extracellular binding domain of the BCMA CAR comprises an scFv derived from C11D5.3, a murine monoclonal antibody as described in Carpenter et al., Clin. Cancer Res. 19(8):2048-2060 (2013). See also PCT Application Publication No. WO2010/104949. The C11D5.3-derived scFv may comprise the heavy chain variable region (V_H) and the light chain variable region (V_L) of C11D5.3 connected by the Whitlow linker, the amino acid sequences of which is provided in **Table 13** below. In some embodiments, the BCMA-specific extracellular binding domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:63, 64, or 68, or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:63, 64, or 68. In some embodiments, the BCMA-specific extracellular binding domain may comprise one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 65-67 and 69-71. In some embodiments, the BCMA-specific extracellular binding domain may comprise a light chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 65-67. In some embodiments, the BCMA-specific extracellular binding domain may comprise a heavy chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 69-71. In any of these embodiments, the BCMA-specific scFv may comprise one or more CDRs comprising one or more amino acid substitutions, or comprising a sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical), to any of the sequences identified. In some embodiments, the extracellular binding domain of the BCMA CAR comprises or consists of the one or more CDRs as described herein.

[00857] In some embodiments, the extracellular binding domain of the BCMA CAR comprises an scFv derived from another murine monoclonal antibody, C12A3.2, as described in Carpenter et al., Clin. Cancer Res. 19(8):2048-2060 (2013) and PCT Application Publication No. WO2010/104949, the amino acid sequence of which is also provided in **Table 13** below. In some embodiments, the BCMA-specific extracellular binding domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:72, 73, or 77, or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at

least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:72, 73, or 77. In some embodiments, the BCMA-specific extracellular binding domain may comprise one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 74-76 and 78-80. In some embodiments, the BCMA-specific extracellular binding domain may comprise a light chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 74-76. In some embodiments, the BCMA-specific extracellular binding domain may comprise a heavy chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 78-80. In any of these embodiments, the BCMA-specific scFv may comprise one or more CDRs comprising one or more amino acid substitutions, or comprising a sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical), to any of the sequences identified. In some embodiments, the extracellular binding domain of the BCMA CAR comprises or consists of the one or more CDRs as described herein.

[00858] In some embodiments, the extracellular binding domain of the BCMA CAR comprises a murine monoclonal antibody with high specificity to human BCMA, referred to as BB2121 in Friedman et al., Hum. Gene Ther. 29(5):585-601 (2018)). See also, PCT Application Publication No. WO2012163805.

[00859] In some embodiments, the extracellular binding domain of the BCMA CAR comprises single variable fragments of two heavy chains (VHH) that can bind to two epitopes of BCMA as described in Zhao et al., J. Hematol. Oncol. 11(1):141 (2018), also referred to as LCAR-B38M. See also, PCT Application Publication No. WO2018/028647.

[00860] In some embodiments, the extracellular binding domain of the BCMA CAR comprises a fully human heavy-chain variable domain (FHVH) as described in Lam et al., Nat. Commun. 11(1):283 (2020), also referred to as FHVH33. See also, PCT Application Publication No. WO2019/006072. The amino acid sequences of FHVH33 and its CDRs are provided in **Table 13** below. In some embodiments, the BCMA-specific extracellular binding domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:81 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:81. In some embodiments, the BCMA-specific extracellular binding domain may

comprise one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 82-84. In any of these embodiments, the BCMA-specific extracellular binding domain may comprise one or more CDRs comprising one or more amino acid substitutions, or comprising a sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical), to any of the sequences identified. In some embodiments, the extracellular binding domain of the BCMA CAR comprises or consists of the one or more CDRs as described herein.

[00861] In some embodiments, the extracellular binding domain of the BCMA CAR comprises an scFv derived from CT103A (or CAR0085) as described in U.S. Patent No. 11,026,975 B2, the amino acid sequence of which is provided in **Table 13** below. In some embodiments, the BCMA-specific extracellular binding domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:118, 119, or 123, or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO: 118, 119, or 123. In some embodiments, the BCMA-specific extracellular binding domain may comprise one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 120-122 and 124-126. In some embodiments, the BCMA-specific extracellular binding domain may comprise a light chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 120-122. In some embodiments, the BCMA-specific extracellular binding domain may comprise a heavy chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 124-126. In any of these embodiments, the BCMA-specific scFv may comprise one or more CDRs comprising one or more amino acid substitutions, or comprising a sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical), to any of the sequences identified. In some embodiments, the extracellular binding domain of the BCMA CAR comprises or consists of the one or more CDRs as described herein.

[00862] Additionally, CARs and binders directed to BCMA have been described in U.S. Application Publication Nos. 2020/0246381 A1 and 2020/0339699 A1, the entire contents of each of which are incorporated by reference herein.

Table 13. Exemplary sequences of anti-BCMA binder and components

SEQ ID NO:	Amino Acid Sequence	Description
63	DIVLTQSPASLAMSLGKRATISCRAS ESVSVIGAHLIHWYQQKPGQPPKLLI YLASNLETGVPARFSGSGSGTDFTLT IDPVEEDDVAIYSCLQSRIFPRTFGG GTKLEIKGSTSGSGKPGSGEGSTKG QIQLVQSGPELKKPGETVKISCKASG YTFTDYSINWVKRAPGKGLKWMG WINTETREPAYAYDFRGRFAFSLETS ASTAYLQINNLKYEDTATYFCALDY SYAMDYWGQGTSVTVSS	Anti-BCMA C11D5.3 scFv entire sequence, with Whitlow linker
64	DIVLTQSPASLAMSLGKRATISCRAS ESVSVIGAHLIHWYQQKPGQPPKLLI YLASNLETGVPARFSGSGSGTDFTLT IDPVEEDDVAIYSCLQSRIFPRTFGG GTKLEIK	Anti-BCMA C11D5.3 scFv light chain variable region
65	RASESVSVIGAHLIH	Anti-BCMA C11D5.3 scFv light chain CDR1
66	LASNLET	Anti-BCMA C11D5.3 scFv light chain CDR2
67	LQSRIFPRT	Anti-BCMA C11D5.3 scFv light chain CDR3
68	QIQLVQSGPELKKPGETVKISCKASG YTFTDYSINWVKRAPGKGLKWMG WINTETREPAYAYDFRGRFAFSLETS ASTAYLQINNLKYEDTATYFCALDY SYAMDYWGQGTSVTVSS	Anti-BCMA C11D5.3 scFv heavy chain variable region
69	DYSIN	Anti-BCMA C11D5.3 scFv heavy chain CDR1
70	WINTETREPAYAYDFRG	Anti-BCMA C11D5.3 scFv heavy chain CDR2
71	DYSYAMDY	Anti-BCMA C11D5.3 scFv heavy chain CDR3
72	DIVLTQSPPSLAMSLGKRATISCRAS ESVTILGSHLIYWYQQKPGQPPTLLI QLASNVQTGVPARFSGSGSRTDFTL TIDPVEEDDVAVYYCLQSRTIPRTFG GGTKLEIKGSTSGSGKPGSGEGSTK GQIQLVQSGPELKKPGETVKISCKAS GYTFRHYSMNWVKQAPGKGLKWM GRINTESGVPIYADDFKGRFAFSVET	Anti-BCMA C12A3.2 scFv entire sequence, with Whitlow linker

	SASTAYLVINNLKDEDTASYFCSND YLYSLDFWGQGTALTVSS	
73	DIVLTQSPPSLAMSLGKRATISCRAS ESVTILGSHLIYWYQQKPGQPPTLLI QLASNVQTGVPARFSGSGSRTDFTL TIDPVEEDDVAVYYCLQSRTIPRTFG GGTKLEIK	Anti-BCMA C12A3.2 scFv light chain variable region
74	RASESVTILGSHLIY	Anti-BCMA C12A3.2 scFv light chain CDR1
75	LASNVQT	Anti-BCMA C12A3.2 scFv light chain CDR2
76	LQSRTIPRT	Anti-BCMA C12A3.2 scFv light chain CDR3
77	QIQLVQSGPELKKPGETVKISCKASG YTFRHYSMNWVKQAPGKGLKWMG RINTESGVPIYADDFKGRFAFSVETS ASTAYLVINNLKDEDTASYFCSNDY LYSLDFWGQGTALTVSS	Anti-BCMA C12A3.2 scFv heavy chain variable region
78	HYSMN	Anti-BCMA C12A3.2 scFv heavy chain CDR1
79	RINTESGVPIYADDFKG	Anti-BCMA C12A3.2 scFv heavy chain CDR2
80	DYLYSLDF	Anti-BCMA C12A3.2 scFv heavy chain CDR3
81	EVQLLESGGGLVQPGGSLRLSCAAS GFTFSSYAMSWVRQAPGKGLEWVS SISGSGDYIYYADSVKGRFTISRDISK NTLYLQMNSLRAEDTAVYYCAKEG TGANSSLADYRGQGTLVTVSS	Anti-BCMA FHVH33 entire sequence
82	GFTFSSYA	Anti-BCMA FHVH33 CDR1
83	ISGSGDYI	Anti-BCMA FHVH33 CDR2
84	AKEGTGANSSLADY	Anti-BCMA FHVH33 CDR3
118	DIQMTQSPSSLSASVGDRVTITCRAS QSISSYLNWYQQKPGKAPKLLIYAA SSLQSGVPSRFSGSGSGTDFTLTISSL QPEDFATYYCQQKYDLLTFGGGTK VEIKGSTSGSGKPGSGEGSTKGQLQ	Anti-BCMA CT103A scFv entire sequence, with Whitlow linker

	LQESGPGLVKPSETLSLTCTVSGGSI SSSSYYWGWIRQPPGKGLEWIGSISY SGSTYYNPSLKSRVTISVDTSKNQFS LKLSSVTAADTAVYYCARDRGDTIL DVWGQGTMVTVSS	
119	DIQMTQSPSSLSASVGDRVTITCRAS QSISSYLNWYQQKPGKAPKLLIYAA SSLQSGVPSRFSGSGSGTDFTLTISSL QPEDFATYYCQQKYDLLTFGGGTK VEIK	Anti-BCMA CT103A scFv light chain variable region
120	QSISSY	Anti-BCMA CT103A scFv light chain CDR1
121	AAS	Anti-BCMA CT103A scFv light chain CDR2
122	QQKYDLLT	Anti-BCMA CT103A scFv light chain CDR3
123	QLQLQESGPGLVKPSETLSLTCTVSG GSISSSSYYWGWIRQPPGKGLEWIGS ISYSGSTYYNPSLKSRVTISVDTSKN QFSLKLSSVTAADTAVYYCARDRG DTILDVWGQGTMVTVSS	Anti-BCMA CT103A scFv heavy chain variable region
124	GGSISSSSYY	Anti-BCMA CT103A scFv heavy chain CDR1
125	ISYSGST	Anti-BCMA CT103A scFv heavy chain CDR2
126	ARDRGDTILDV	Anti-BCMA CT103A scFv heavy chain CDR3

[00863] In some embodiments, the hinge domain of the BCMA CAR comprises a CD8α hinge domain, for example, a human CD8α hinge domain. In some embodiments, the CD8α hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:9 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:9. In some embodiments, the hinge domain comprises a CD28 hinge domain, for example, a human CD28 hinge domain. In some embodiments, the CD28 hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:10 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the

amino acid sequence set forth in SEQ ID NO:10. In some embodiments, the hinge domain comprises an IgG4 hinge domain, for example, a human IgG4 hinge domain. In some embodiments, the IgG4 hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:11 or SEQ ID NO:12, or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:11 or SEQ ID NO:12. In some embodiments, the hinge domain comprises a IgG4 hinge-Ch2-Ch3 domain, for example, a human IgG4 hinge-Ch2-Ch3 domain. In some embodiments, the IgG4 hinge-Ch2-Ch3 domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:13 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:13.

[00864] In some embodiments, the transmembrane domain of the BCMA CAR comprises a CD8α transmembrane domain, for example, a human CD8α transmembrane domain. In some embodiments, the CD8α transmembrane domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:14 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:14. In some embodiments, the transmembrane domain comprises a CD28 transmembrane domain, for example, a human CD28 transmembrane domain. In some embodiments, the CD28 transmembrane domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:15 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:15.

[00865] In some embodiments, the intracellular costimulatory domain of the BCMA CAR comprises a 4-1BB costimulatory domain, for example, a human 4-1BB costimulatory domain. In some embodiments, the 4-1BB costimulatory domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:16 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:16. In some embodiments, the intracellular costimulatory domain comprises a CD28 costimulatory

domain, for example, a human CD28 costimulatory domain. In some embodiments, the CD28 costimulatory domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:17 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:17.

[00866] In some embodiments, the intracellular signaling domain of the BCMA CAR comprises a CD3 zeta (ζ) signaling domain, for example, a human CD3ζ signaling domain. In some embodiments, the CD3ζ signaling domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:18 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:18.

[00867] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a BCMA CAR, including, for example, a BCMA CAR comprising any of the BCMA-specific extracellular binding domains as described, the CD8α hinge domain of SEQ ID NO:9, the CD8α transmembrane domain of SEQ ID NO:14, the 4-1BB costimulatory domain of SEQ ID NO:16, the CD3ζ signaling domain of SEQ ID NO:18, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof. In any of these embodiments, the BCMA CAR may additionally comprise a signal peptide (e.g., a CD8α signal peptide) as described.

[00868] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a BCMA CAR, including, for example, a BCMA CAR comprising any of the BCMA-specific extracellular binding domains as described, the CD8α hinge domain of SEQ ID NO:9, the CD8α transmembrane domain of SEQ ID NO:14, the CD28 costimulatory domain of SEQ ID NO:17, the CD3ζ signaling domain of SEQ ID NO:18, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof. In any of these embodiments, the BCMA CAR may additionally comprise a signal peptide as described.

[00869] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a BCMA CAR as set forth in SEQ ID NO:127 or is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the nucleotide sequence set forth in SEQ ID NO:127 (see **Table 14**). The encoded BCMA CAR has a corresponding amino acid sequence set forth in SEQ ID NO:128 or is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:128, with the following components: CD8α signal peptide, CT103A scFv (V_L-Whitlow linker-V_H), CD8α hinge domain, CD8α transmembrane domain, 4-1BB costimulatory domain, and CD3ζ signaling domain.

[00870] In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding a commercially available embodiment of BCMA CAR, including, for example, idecabtagene vicleucel (ide-cel, also called bb2121). In some embodiments, the polycistronic vector comprises an expression cassette that contains a nucleotide sequence encoding idecabtagene vicleucel or portions thereof. Idecabtagene vicleucel comprises a BCMA CAR with the following components: the BB2121 binder, CD8α hinge domain, CD8α transmembrane domain, 4-1BB costimulatory domain, and CD3ζ signaling domain.

Table 14. Exemplary sequences of BCMA CARs

SEQ ID NO:	Sequence	Description
127	atggccttaccagtgaccgccttgctcctgccgctggccttgctgc tccacgccgccaggccggacatccagatgacccagtctccatcct ccctgtctgcatctgtaggagacagagtcaccatcacttgccggg caagtcagagcattagcagctatttaaattggtatcagcagaaacc agggaaagcccctaagctcctgatctatgctgcatccagtttgcaa agtggggtcccatcaaggttcagtggcagtggatctgggacagat ttcactctcaccatcagcagtctgcaacctgaagattttgcaacttac tactgtcagcaaaaatacgacctcctcacttttggcggagggacca aggttgagatcaaaggcagcaccagcggctccggcaagcctgg ctctggcgagggcagcacaaagggacagctgcagctgcagga gtcgggcccaggactggtgaagccttcggagaccctgtccctca cctgcactgtctctggtggctccatcagcagtagtagttactactgg ggctggatccgccagccccccagggaaggggctggagtggattg ggagtatctcctatagtgggagcacctactacaacccgtccctcaa gagtcgagtcaccatatccgtagacacgtccaagaaccagttctc	Exemplary BCMA CAR nucleotide sequence

	cctgaagctgagttctgtgaccgccgcagacacggcggtgtacta ctgcgccagagatcgtggagacaccatactagacgtatggggtc agggtacaatggtcaccgtcagctcattcgtgcccgtgttcctgcc cgccaaacctaccaccacccctgcccctagacctcccaccccag ccccaacaatcgccagccagcctctgtctctgcggcccgaagcct gtagacctgctgccggcggagccgtgcacaccagaggcctgga cttcgcctgcgacatctacatctgggcccctctggccggcacctgt ggcgtgctgctgctgagcctggtgatcaccctgtactgcaaccac cggaacaaacggggcagaaagaaactcctgtatatattcaaacaa ccatttatgagaccagtacaaactactcaagaggaagatggctgta gctgccgatttccagaagaagaagaaggaggatgtgaactgaga gtgaagttcagcagatccgccgacgcccctgcctaccagcaggg acagaaccagctgtacaacgagctgaacctgggcagacgggaa gagtacgacgtgctggacaagcggagaggccgggaccccgag atgggcggaaagcccagacggaagaacccccaggaaggcctg tataacgaactgcagaaagacaagatggccgaggcctacagcg agatcggcatgaagggcgagcggaggcgcggcaagggccac gatggcctgtaccagggcctgagcaccgccaccaaggacacct acgacgccctgcacatgcaggccctgcccccaga	
128	MALPVTALLLPLALLLHAARPDIQMTQSP SSLSASVGDRVTITCRASQSISSYLNWYQQ KPGKAPKLLIYAASSLQSGVPSRFSGSGSG TDFTLTISSLQPEDFATYYCQQKYDLLTFG GGTKVEIKGSTSGSGKPGSGEGSTKGQLQ LQESGPGLVKPSETLSLTCTVSGGSISSSSY YWGWIRQPPGKGLEWIGSISYSGSTYYNP SLKSRVTISVDTSKNQFSLKLSSVTAADTA VYYCARDRGDTILDVWGQGTMVTVSSFV PVFLPAKPTTTPAPRPPTPAPTIASQPLSLR PEACRPAAGGAVHTRGLDFACDIYIWAPL AGTCGVLLLSLVITLYCNHRNKRGRKKLL YIFKQPFMRPVQTTQEEDGCSCRFPEEEEG GCELRVKFSRSADAPAYQQGQNQLYNEL NLGRREEYDVLDKRRGRDPEMGGKPRRK NPQEGLYNELQKDKMAEAYSEIGMKGER RRGKGHDGLYQGLSTATKDTYDALHMQ ALPPR	Exemplary BCMA CAR amino acid sequence

[00871]

Y. Characteristics of Hypoimmunogenic Cells

[00872] In some embodiments, the population of hypoimmunogenic stem cells retains pluripotency as compared to a control stem cell (*e.g.*, a wild-type stem cell or immunogenic stem

cell). In some embodiments, the population of hypoimmunogenic stem cells retains differentiation potential as compared to a control stem cell (*e.g.*, a wild-type stem cell or immunogenic stem cell).

[00873] In some embodiments, the administered population of hypoimmunogenic cells such as hypoimmunogenic differentiated cells and CAR-T cells elicits a decreased or lower level of immune activation in the subject or patient. In some instances, the level of immune activation elicited by the cells is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% lower compared to the level of immune activation produced by the administration of immunogenic cells. In some embodiments, the administered population of hypoimmunogenic cells fails to elicit immune activation in the subject or patient.

[00874] In some embodiments, the administered population of hypoimmunogenic cells such as hypoimmunogenic differentiated cells and CAR-T cells elicits a decreased or lower level of T cell response in the subject or patient. In some instances, the level of T cell response elicited by the cells is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% lower compared to the level of T cell response produced by the administration of immunogenic cells. In some embodiments, the administered population of hypoimmunogenic cells fails to elicit a T cell response to the cells in the subject or patient.

[00875] In some embodiments, the administered population of hypoimmunogenic cells such as hypoimmunogenic differentiated cells and CAR-T cells elicits a decreased or lower level of NK cell response in the subject or patient. In some instances, the level of NK cell response elicited by the cells is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% lower compared to the level of NK cell response produced by the administration of immunogenic cells. In some embodiments, the administered population of hypoimmunogenic cells fails to elicit an NK cell response to the cells in the subject or patient.

[00876] In some embodiments, the administered population of hypoimmunogenic cells such as hypoimmunogenic differentiated cells and CAR-T cells elicits a decreased or lower level of macrophage engulfment in the subject or patient. In some instances, the level of NK cell response elicited by the cells is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%,

55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% lower compared to the level of macrophage engulfment produced by the administration of immunogenic cells. In some embodiments, the administered population of hypoimmunogenic cells fails to elicit macrophage engulfment of the cells in the subject or patient.

[00877] In some embodiments, the administered population of hypoimmunogenic cells such as hypoimmunogenic differentiated cells and CAR-T cells elicits a decreased or lower level of systemic TH1 activation in the subject or patient. In some instances, the level of systemic TH1 activation elicited by the cells is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% lower compared to the level of systemic TH1 activation produced by the administration of immunogenic cells. In some embodiments, the administered population of hypoimmunogenic cells fails to elicit systemic TH1 activation in the subject or patient.

[00878] In some embodiments, the administered population of hypoimmunogenic cells such as hypoimmunogenic differentiated cells and CAR-T cells elicits a decreased or lower level of NK cell killing in the subject or patient. In some instances, the level of NK cell killing elicited by the cells is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% lower compared to the level of NK cell killing produced by the administration of immunogenic cells. In some embodiments, the administered population of hypoimmunogenic cells fails to elicit NK cell killing in the subject or patient.

[00879] In some embodiments, the administered population of hypoimmunogenic cells such as hypoimmunogenic differentiated cells and CAR-T cells elicits a decreased or lower level of immune activation of peripheral blood mononuclear cells (PBMCs) in the subject or patient. In some instances, the level of immune activation of PBMCs elicited by the cells is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% lower compared to the level of immune activation of PBMCs produced by the administration of immunogenic cells. In some embodiments, the administered population of hypoimmunogenic cells fails to elicit immune activation of PBMCs in the subject or patient.

[00880] In some embodiments, the administered population of hypoimmunogenic cells such as hypoimmunogenic differentiated cells and CAR-T cells elicits a decreased or lower level of

donor-specific IgG antibodies in the subject or patient. In some instances, the level of donor-specific IgG antibodies elicited by the cells is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% lower compared to the level of donor-specific IgG antibodies produced by the administration of immunogenic cells. In some embodiments, the administered population of hypoimmunogenic cells fails to elicit donor-specific IgG antibodies in the subject or patient.

[00881] In some embodiments, the administered population of hypoimmunogenic cells such as hypoimmunogenic differentiated cells and CAR-T cells elicits a decreased or lower level of donor-specific IgM antibodies in the subject or patient. In some instances, the level of donor-specific IgM antibodies elicited by the cells is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% lower compared to the level of donor-specific IgM antibodies produced by the administration of immunogenic cells. In some embodiments, the administered population of hypoimmunogenic cells fails to elicit donor-specific IgM antibodies in the subject or patient.

[00882] In some embodiments, the administered population of hypoimmunogenic cells such as hypoimmunogenic differentiated cells and CAR-T cells elicits a decreased or lower level of IgM and IgG antibody production in the subject or patient. In some instances, the level of IgM and IgG antibody production elicited by the cells is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% lower compared to the level of IgM and IgG antibody production produced by the administration of immunogenic cells. In some embodiments, the administered population of hypoimmunogenic cells fails to elicit IgM and IgG antibody production in the subject or patient.

[00883] In some embodiments, the administered population of hypoimmunogenic cells such as hypoimmunogenic differentiated cells and CAR-T cells elicits a decreased or lower level of cytotoxic T cell killing in the subject or patient. In some instances, the level of cytotoxic T cell killing elicited by the cells is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% lower compared to the level of cytotoxic T cell killing produced by the administration of immunogenic cells. In some embodiments, the administered population of hypoimmunogenic cells fails to elicit cytotoxic T cell killing in the subject or patient.

[00884] In some embodiments, the administered population of hypoimmunogenic cells such as hypoimmunogenic differentiated cells and CAR-T cells elicits a decreased or lower level of complement-dependent cytotoxicity (CDC) in the subject or patient. In some instances, the level of CDC elicited by the cells is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% lower compared to the level of CDC produced by the administration of immunogenic cells. In some embodiments, the administered population of hypoimmunogenic cells fails to elicit CDC in the subject or patient.

Z. Therapeutic Cells from Primary T Cells

[00885] Provided herein are hypoimmunogenic cells including, but not limited to, primary T cells that evade immune recognition. In some embodiments, the hypoimmunogenic cells are produced (*e.g.*, generated, cultured, or derived) from T cells such as primary T cells. In some instances, primary T cells are obtained (*e.g.*, harvested, extracted, removed, or taken) from a subject or an individual. In some embodiments, primary T cells are produced from a pool of T cells such that the T cells are from one or more subjects (*e.g.*, one or more human including one or more healthy humans). In some embodiments, the pool of primary T cells is from 1-100, 1-50, 1-20, 1-10, 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 10 or more, 20 or more, 30 or more, 40 or more, 50 or more, or 100 or more subjects. In some embodiments, the donor subject is different from the patient (*e.g.*, the recipient that is administered the therapeutic cells). In some embodiments, the pool of T cells do not include cells from the patient. In some embodiments, one or more of the donor subjects from which the pool of T cells is obtained are different from the patient.

[00886] In some embodiments, the hypoimmunogenic cells do not activate an innate and/or an adaptive immune response in the patient (*e.g.*, recipient upon administration). Provided are methods of treating a disorder by administering a population of hypoimmunogenic cells to a subject (*e.g.*, recipient) or patient in need thereof. In some embodiments, the hypoimmunogenic cells described herein comprise T cells engineered (*e.g.*, are modified) to express a chimeric antigen receptor including but not limited to a chimeric antigen receptor described herein. In some instances, the T cells are populations or subpopulations of primary T cells from one or more individuals. In some embodiments, the T cells described herein such as the engineered or modified T cells comprise reduced expression of an endogenous T cell receptor.

[00887] In some embodiments, the present disclosure is directed to hypoimmunogenic primary T cells that regulatably overexpress CD47 and CARs, and have regulatable reduced expression or lack expression of one or more MHC class I and/or MHC class II human leukocyte antigen molecules and have reduced expression or lack expression of TCR complex molecules. The cells outlined herein regulatably overexpress CD47 and CARs and evade immune recognition. In some embodiments, the primary T cells display regulatable reduced levels or activity of MHC class I antigens/molecules, MHC class II antigens/molecules, and/or TCR complex molecules. In certain embodiments, primary T cells regulatably overexpress CD47 and CARs and harbor a regulatable genomic modification in the B2M gene. In some embodiments, T cells regulatably overexpress CD47 and CARs and harbor a regulatable genomic modification in the CIITA gene. In some embodiments, primary T cells regulatably overexpress CD47 and CARs and harbor a regulatable genomic modification in the TRAC gene. In some embodiments, primary T cells regulatably overexpress CD47 and CARs and harbor a regulatable genomic modification in the TRB gene. In some embodiments, T cells regulatably overexpress CD47 and CARs and harbor regulatable genomic modifications in one or more of the following genes: the B2M, CIITA, TRAC and TRB genes.

[00888] Exemplary T cells of the present disclosure are selected from the group consisting of cytotoxic T cells, helper T cells, memory T cells, central memory T cells, effector memory T cells, effector memory RA T cells, regulatory T cells, tissue infiltrating lymphocytes, and combinations thereof. In certain embodiments, the T cells express CCR7, CD27, CD28, and CD45RA. In some embodiments, the central T cells express CCR7, CD27, CD28, and CD45RO. In other embodiments, the effector memory T cells express PD-1, CD27, CD28, and CD45RO. In other embodiments, the effector memory RA T cells express PD-1, CD57, and CD45RA.

[00889] In some embodiments, the T cell is a modified (*e.g.*, an engineered) T cell. In some cases, the modified T cell comprise a modification causing the cell to express at least one chimeric antigen receptor that specifically binds to an antigen or epitope of interest expressed on the surface of at least one of a damaged cell, a dysplastic cell, an infected cell, an immunogenic cell, an inflamed cell, a malignant cell, a metaplastic cell, a mutant cell, and combinations thereof. In other cases, the modified T cell comprise a modification causing the cell to express at least one protein that modulates a biological effect of interest in an adjacent cell, tissue, or organ when the cell is in proximity to the adjacent cell, tissue, or organ. Useful modifications to

primary T cells are described in detail in US2016/0348073 and WO2020/018620, the disclosures of which are incorporated herein in their entireties.

[00890] In some embodiments, the hypoimmunogenic cells described herein comprise T cells that are engineered (*e.g.*, are modified) to express a chimeric antigen receptor including but not limited to a chimeric antigen receptor described herein. In some instances, the T cells are populations or subpopulations of primary T cells from one or more individuals. In some embodiments, the T cells described herein such as the engineered or modified T cells include reduced expression of an endogenous T cell receptor. In some embodiments, the T cells described herein such as the engineered or modified T cells include reduced expression of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). In other embodiments, the T cells described herein such as the engineered or modified T cells include reduced expression of programmed cell death (PD-1). In certain embodiments, the T cells described herein such as the engineered or modified T cells include reduced expression of CTLA-4 and PD-1. Methods of reducing or eliminating expression of CTLA-4, PD-1 and both CTLA-4 and PD-1 can include any recognized by those skilled in the art, such as but not limited to, genetic modification technologies that utilize rare-cutting endonucleases and RNA silencing or RNA interference technologies. Non-limiting examples of a rare-cutting endonuclease include any Cas protein, TALEN, zinc finger nuclease, meganuclease, and homing endonuclease. In some embodiments, an exogenous nucleic acid encoding a polypeptide as disclosed herein (*e.g.*, a chimeric antigen receptor, CD47, or another tolerogenic factor disclosed herein) is inserted at a CTLA-4 and/or PD-1 gene locus.

[00891] In some embodiments, the T cells described herein such as the engineered or modified T cells include enhanced expression of PD-L1.

[00892] In some embodiments, the hypoimmunogenic T cell includes a polynucleotide encoding a CAR, wherein the polynucleotide is inserted in a genomic locus. In some embodiments, the polynucleotide is inserted into a safe harbor or target locus, such as but not limited to, an AAVS1, CCR5, CLYBL, ROSA26, SHS231, F3 (also known as CD142), MICA, MICB, LRP1 (also known as CD91), HMGB1, ABO, RHD, FUT1, or KDM5D gene locus. In some embodiments, the polynucleotide is inserted in a B2M, CIITA, TRAC, TRB, PD-1 or CTLA-4 gene.

[00893] In some embodiments, the hypoimmunogenic T cell includes a polynucleotide encoding a CAR that is regulatably expressed in a cell using an expression vector. In some embodiments, the CAR is introduced to the cell using a viral expression vector that mediates integration of the CAR sequence using an into the genome of the cell. For example, the expression vector for expressing the CAR in a cell comprises a polynucleotide sequence encoding the CAR. The expression vector can be an inducible expression vector. The expression vector can be a viral vector, such as but not limited to, a lentiviral vector.

[00894] Hypoimmunogenic T cells provided herein are useful for the treatment of suitable cancers including, but not limited to, 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.

AA. Therapeutic Cells Differentiated from Hypoimmunogenic Pluripotent Stem Cells

[00895] Provided herein are hypoimmunogenic cells including, cells derived from pluripotent stem cells, that evade immune recognition. In some embodiments, the cells do not activate an innate and/or an adaptive immune response in the patient or subject (*e.g.*, recipient upon administration). Provided are methods of treating a disorder comprising repeat dosing of a population of hypoimmunogenic cells to a recipient subject in need thereof.

[00896] In an aspect, provided herein are HIP cells that are differentiated into different cell types for subsequent transplantation into recipient subjects. Differentiation can be assayed as is known in the art, generally by evaluating the presence of cell-specific markers. As will be appreciated by those in the art, the differentiated hypoimmunogenic pluripotent cell derivatives can be transplanted using techniques known in the art that depends on both the cell type and the ultimate use of these cells.

[00897] In some embodiments, the pluripotent stem cell and any cell differentiated from such a pluripotent stem cell is modified to exhibit regulatable reduced expression of one or more MHC class I human leukocyte antigen molecules. In other embodiments, the pluripotent stem cell and any cell differentiated from such a pluripotent stem cell is modified to exhibit regulatable reduced expression of one or more MHC class II human leukocyte antigen molecules. In certain

embodiments, the pluripotent stem cell and any cell differentiated from such a pluripotent stem cell is modified to exhibit regulatable reduced expression of TCR complexes. In some embodiments, the pluripotent stem cell and any cell differentiated from such a pluripotent stem cell is modified to exhibit regulatable reduced expression of one or more MHC class I and II human leukocyte antigen molecules. In some embodiments, the pluripotent stem cell and any cell differentiated from such a pluripotent stem cell is modified to exhibit regulatable reduced expression of one or more MHC class I and II human leukocyte antigen molecules and TCR complexes.

[00898] In some embodiments, the pluripotent stem cell and any cell differentiated from such a pluripotent stem cell is modified to exhibit regulatable reduced expression of one or more MHC class I and/or II human leukocyte antigen molecules and exhibit regulatable increased CD47 expression. In some instances, the cell regulatably overexpresses CD47 by harboring one or more transgenes encoding one or more tolerogenic factors . In some embodiments, the pluripotent stem cell and any cell differentiated from such a pluripotent stem cell is modified to regulatably exhibit reduced expression of one or more MHC class I and II human leukocyte antigen molecules and exhibit regulatable increased CD47 expression. In some embodiments, the pluripotent stem cell and any cell differentiated from such a pluripotent stem cell is modified to exhibit regulatable reduced expression of one or more MHC class I and II human leukocyte antigen molecules and TCR complexes and exhibit regulatable increased CD47 expression.

[00899] In some embodiments, the pluripotent stem cell and any cell differentiated from such a pluripotent stem cell is modified to exhibit regulatable reduced expression of one or more MHC class I and/or II human leukocyte antigen molecules, to exhibit regulatable increased CD47 expression, and to regulatably exogenously express a chimeric antigen receptor. In some instances, the cell regulatably overexpresses one or more tolerogenic factors by harboring one or more transgenes encoding one or more tolerogenic factors. In some instances, the cell regulatably overexpresses CARs by harboring one or more CAR transgenes. In some embodiments, the pluripotent stem cell and any cell differentiated from such a pluripotent stem cell is modified to regulatably exhibit reduced expression of one or more MHC class I and II human leukocyte antigen molecules, exhibit increased regulatable expression of one or more tolerogenic factors, and to regulatably exogenously express a chimeric antigen receptor. In some embodiments, the pluripotent stem cell and any cell differentiated from such a pluripotent stem

cell is modified to regulatably exhibit reduced expression of one or more MHC class I and II human leukocyte antigen molecules and TCR complexes, to exhibit regulatable increased expression of one or more tolerogenic factors, and to regulatably exogenously express a chimeric antigen receptor.

[00900] In some embodiments, the pluripotent stem cell and any cell differentiated from such a pluripotent stem cell is modified to exhibit regulatable reduced expression of one or more MHC class I and/or II human leukocyte antigen molecules and exhibit regulatable increased CD47 expression. In some instances, the cell overexpresses CD47 by harboring one or more CD47 transgenes. In some embodiments, the pluripotent stem cell and any cell differentiated from such a pluripotent stem cell is modified to exhibit regulatable reduced expression of one or more MHC class I and II human leukocyte antigen molecules and exhibit regulatable increased CD47 expression. In some embodiments, the pluripotent stem cell and any cell differentiated from such a pluripotent stem cell is modified to exhibit regulatable reduced expression of one or more MHC class I and II human leukocyte antigen molecules and TCR complexes and exhibit regulatable increased CD47 expression.

[00901] In some embodiments, the pluripotent stem cell and any cell differentiated from such a pluripotent stem cell is modified to exhibit regulatable reduced expression of one or more MHC class I and/or II human leukocyte antigen molecules, to exhibit regulatable increased CD47 expression, and to exogenously express a chimeric antigen receptor. In some instances, the cell overexpresses CD47 polypeptides by harboring one or more CD47 transgenes. In some instances, the cell overexpresses CAR polypeptides by harboring one or more CAR transgenes. In some embodiments, the pluripotent stem cell and any cell differentiated from such a pluripotent stem cell is modified to exhibit regulatable reduced expression of one or more MHC class I and II human leukocyte antigen molecules, exhibit regulatable increased CD47 expression, and to exogenously express a chimeric antigen receptor. In some embodiments, the pluripotent stem cell and any cell differentiated from such a pluripotent stem cell is modified to exhibit regulatable reduced expression of one or more MHC class I and II human leukocyte antigen molecules and TCR complexes, to exhibit regulatable increased CD47 expression, and to exogenously express a chimeric antigen receptor.

[00902] Such pluripotent stem cells are hypoimmunogenic stem cells. Such differentiated cells are hypoimmunogenic cells.

[00903] Any of the pluripotent stem cells described herein can be differentiated into any cells of an organism and tissue. In some embodiments, the cells exhibit regulatable reduced expression of one or more MHC class I and/or II human leukocyte antigen molecules and regulatable reduced expression of TCR complexes. In some instances, expression of one or more MHC class I and/or II human leukocyte antigen molecules is regulatably reduced compared to unmodified or wild-type cell of the same cell type. In some instances, expression of TCR complexes is regulatably reduced compared to unmodified or wild-type cell of the same cell type. In some embodiments, the cells exhibit increased CD47 expression. In some instances, expression of CD47 is increased in cells encompassed by the present disclosure as compared to unmodified or wild-type cells of the same cell type. In some embodiments, the cells exhibit exogenous CAR expression. Methods for reducing levels of MHC class I and/or II human leukocyte antigen molecules and TCR complexes and increasing the expression of CD47 and CARs are described herein.

[00904] In some embodiments, the cells used in the methods described herein evade immune recognition and responses when administered to a patient (*e.g.*, recipient subject). The cells can evade killing by immune cells in vitro and in vivo. In some embodiments, the cells evade killing by macrophages and NK cells. In some embodiments, the cells are ignored by immune cells or a subject's immune system. In other words, the cells administered in accordance with the methods described herein are not detectable by immune cells of the immune system. In some embodiments, the cells are cloaked and therefore avoid immune rejection.

[00905] Methods of determining whether a pluripotent stem cell and any cell differentiated from such a pluripotent stem cell evades immune recognition include, but are not limited to, IFN-γ Elispot assays, microglia killing assays, cell engraftment animal models, cytokine release assays, ELISAs, killing assays using bioluminescence imaging or chromium release assay or a real-time, quantitative microelectronic biosensor system for cell analysis (xCELLigence® RTCA system, Agilent), mixed-lymphocyte reactions, immunofluorescence analysis, etc.

[00906] Therapeutic cells outlined herein are useful to treat a disorder such as, but not limited to, a cancer, a genetic disorder, a chronic infectious disease, an autoimmune disorder, a neurological disorder, and the like.

1. Cardiac Cells Differentiated from Hypoimmunogenic Pluripotent Cells

[00907] Provided herein are cardiac cell types differentiated from hypoimmunogenic induced pluripotent (HIP) cells for subsequent transplantation or engraftment into subjects (*e.g.*, recipients). As will be appreciated by those in the art, the methods for differentiation depend on the desired cell type using known techniques. Exemplary cardiac cell types include, but are not limited to, a cardiomyocyte, nodal cardiomyocyte, conducting cardiomyocyte, working cardiomyocyte, cardiomyocyte precursor cell, cardiomyocyte progenitor cell, cardiac stem cell, cardiac muscle cell, atrial cardiac stem cell, ventricular cardiac stem cell, epicardial cell, hematopoietic cell, vascular endothelial cell, endocardial endothelial cell, cardiac valve interstitial cell, cardiac pacemaker cell, and the like.

[00908] In some embodiments, cardiac cells described herein are administered to a recipient subject to treat a cardiac disorder selected from the group consisting of pediatric cardiomyopathy, age-related cardiomyopathy, dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, chronic ischemic cardiomyopathy, peripartum cardiomyopathy, inflammatory cardiomyopathy, idiopathic cardiomyopathy, other cardiomyopathy, myocardial ischemic reperfusion injury, ventricular dysfunction, heart failure, congestive heart failure, coronary artery disease, end-stage heart disease, atherosclerosis, ischemia, hypertension, restenosis, angina pectoris, rheumatic heart, arterial inflammation, cardiovascular disease, myocardial infarction, myocardial ischemia, myocardial infarction, cardiac ischemia, cardiac injury, myocardial ischemia, vascular disease, acquired heart disease, congenital heart disease, coronary artery disease, dysfunctional conduction systems, dysfunctional coronary arteries, pulmonary hypertension, cardiac arrhythmias, muscular dystrophy, muscle mass abnormality, muscle degeneration, myocarditis, infective myocarditis, drug- or toxin-induced muscle abnormalities, hypersensitivity myocarditis, mitral insufficiency, autoimmune endocarditis, primary arrhythmic diseases, cardiac chanellopathies, long QT syndromes, short QT syndromes, Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia, and Jervell and Lange-Nielsen syndrome (see van den Brink *et al., Stem Cells.* 2020 Feb;38(2):174-186).

[00909] Accordingly, provided herein are methods for the treatment and prevention of a cardiac injury or a cardiac disease or disorder in a subject in need thereof. The methods described herein

can be used to treat, ameliorate, prevent or slow the progression of a number of cardiac diseases or their symptoms, such as those resulting in pathological damage to the structure and/or function of the heart. The terms "cardiac disease," "cardiac disorder," and "cardiac injury," are used interchangeably herein and refer to a condition and/or disorder relating to the heart, including the valves, endothelium, infarcted zones, or other components or structures of the heart. Such cardiac diseases or cardiac-related disease include, but are not limited to, myocardial infarction, heart failure, cardiomyopathy, congenital heart defect, heart valve disease or dysfunction, endocarditis, rheumatic fever, mitral valve prolapse, infective endocarditis, hypertrophic cardiomyopathy, dilated cardiomyopathy, myocarditis, cardiomegaly, and/or mitral insufficiency, among others.

[00910] In some embodiments, the cardiomyocyte precursor includes a cell that is capable giving rise to progeny that include mature (end-stage) cardiomyocytes. Cardiomyocyte precursor cells can often be identified using one or more markers selected from GATA-4, Nkx2.5, and the MEF-2 family of transcription factors. In some instances, cardiomyocytes refer to immature cardiomyocytes or mature cardiomyocytes that express one or more markers (sometimes at least 2, 3, 4 or 5 markers) from the following list: cardiac troponin I (cTnl), cardiac troponin T (cTnT), sarcomeric myosin heavy chain (MHC), GATA-4, Nkx2.5, N-cadherin, β2-adrenoceptor, ANF, the MEF-2 family of transcription factors, creatine kinase MB (CK-MB), myoglobin, and atrial natriuretic factor (ANF). In some embodiments, the cardiac cells demonstrate spontaneous periodic contractile activity. In some cases, when that cardiac cells are cultured in a suitable tissue culture environment with an appropriate Ca2+ concentration and electrolyte balance, the cells can be observed to contract in a periodic fashion across one axis of the cell, and then release from contraction, without having to add any additional components to the culture medium. In some embodiments, the cardiac cells are hypoimmunogenic cardiac cells.

[00911] In some embodiments, the method of producing a population of hypoimmunogenic cardiac cells from a population of hypoimmunogenic pluripotent (HIP) cells by in vitro differentiation comprises: (a) culturing a population of hypoimmunogenic induced pluripotent stem cells in a culture medium comprising a GSK inhibitor; (b) culturing the population of HIP cells in a culture medium comprising a WNT antagonist to produce a population of pre-cardiac cells; and (c) culturing the population of pre-cardiac cells in a culture medium comprising insulin to produce a population of hypoimmune cardiac cells. In some embodiments, the GSK inhibitor

is CHIR-99021, a derivative thereof, or a variant thereof. In some instances, the GSK inhibitor is at a concentration ranging from about 2 mM to about 10 mM. In some embodiments, the WNT antagonist is IWR1, a derivative thereof, or a variant thereof. In some instances, the WNT antagonist is at a concentration ranging from about 2 mM to about 10 mM.

[00912] In some embodiments, the population of hypoimmunogenic cardiac cells is isolated from non-cardiac cells. In some embodiments, the isolated population of hypoimmunogenic cardiac cells are expanded prior to administration. In certain embodiments, the isolated population of hypoimmunogenic cardiac cells are expanded and cryopreserved prior to administration.

[00913] Other useful methods for differentiating induced pluripotent stem cells or pluripotent stem cells into cardiac cells are described, for example, in US2017/0152485; US2017/0058263; US2017/0002325; US2016/0362661; US2016/0068814; US9,062,289; US7,897,389; and US7,452,718. Additional methods for producing cardiac cells from induced pluripotent stem cells or pluripotent stem cells are described in, for example, Xu *et al.,* Stem Cells and Development, 2006, 15(5): 631-9, Burridge *et al.,* Cell Stem Cell, 2012, 10: 16-28, and Chen *et al.,* Stem Cell Res, 2015, 15(2):365-375.

[00914] In various embodiments, hypoimmunogenic cardiac cells can be cultured in culture medium comprising a BMP pathway inhibitor, a WNT signaling activator, a WNT signaling inhibitor, a WNT agonist, a WNT antagonist, a Src inhibitor, a EGFR inhibitor, a PCK activator, a cytokine, a growth factor, a cardiotropic agent, a compound, and the like.

[00915] The WNT signaling activator includes, but is not limited to, CHIR99021. The PCK activator includes, but is not limited to, PMA. The WNT signaling inhibitor includes, but is not limited to, a compound selected from KY02111, SO3031 (KY01-I), SO2031 (KY02-I), and SO3042 (KY03-I), and XAV939. The Src inhibitor includes, but is not limited to, A419259. The EGFR inhibitor includes, but is not limited to, AG1478.

[00916] Non-limiting examples of an agent for generating a cardiac cell from an iPSC include activin A, BMP4, Wnt3a, VEGF, soluble frizzled protein, cyclosporin A, angiotensin II, phenylephrine, ascorbic acid, dimethylsulfoxide, 5-aza-2'-deoxycytidine, and the like.

[00917] The cells provided herein can be cultured on a surface, such as a synthetic surface to support and/or promote differentiation of hypoimmunogenic pluripotent cells into cardiac cells. In some embodiments, the surface comprises a polymer material including, but not limited to, a

homopolymer or copolymer of selected one or more acrylate monomers. Non-limiting examples of acrylate monomers and methacrylate monomers include tetra(ethylene glycol) diacrylate, glycerol dimethacrylate, 1,4-butanediol dimethacrylate, poly(ethylene glycol) diacrylate, di(ethylene glycol) dimethacrylate, tetra(ethyiene glycol) dimethacrylate, 1,6-hexanediol propoxylate diacrylate, neopentyl glycol diacrylate, trimethylolpropane benzoate diacrylate, trimethylolpropane eihoxylate (1 EO/QH) methyl, tricyclo[5.2.1.02,6] decane dimethanol diacrylate, neopentyl glycol ethoxylate diacrylate, and trimethylolpropane triacrylate. Acrylate synthesized as known in the art or obtained from a commercial vendor, such as Polysciences, Inc., Sigma Aldrich, Inc. and Sartomer, Inc.

[00918] The polymeric material can be dispersed on the surface of a support material. Useful support materials suitable for culturing cells include a ceramic substance, a glass, a plastic, a polymer or co-polymer, any combinations thereof, or a coating of one material on another. In some instances, a glass includes soda-lime glass, pyrex glass, vycor glass, quartz glass, silicon, or derivatives of these or the like.

[00919] In some instances, plastics or polymers including dendritic polymers include poly(vinyl chloride), poly(vinyl alcohol), poly(methyl methacrylate), poly(vinyl acetate- maleic anhydride), poly(dimethylsiloxane) monomethacrylate, cyclic olefin polymers, fluorocarbon polymers, polystyrenes, polypropylene, polyethyleneimine or derivatives of these or the like. In some instances, copolymers include poly(vinyl acetate-co-maleic anhydride), poly(styrene-co-maleic anhydride), poly(ethylene-co-acrylic acid) or derivatives of these or the like.

[00920] The efficacy of cardiac cells prepared as described herein can be assessed in animal models for cardiac cryoinjury, which causes 55% of the left ventricular wall tissue to become scar tissue without treatment (Li *et al.,* Ann. Thorac. Surg. 62:654, 1996; Sakai *et al.,* Ann. Thorac. Surg. 8:2074, 1999, Sakai *et al.*, Thorac. Cardiovasc. Surg. 118:715, 1999). Successful treatment can reduce the area of the scar, limit scar expansion, and improve heart function as determined by systolic, diastolic, and developed pressure. Cardiac injury can also be modeled using an embolization coil in the distal portion of the left anterior descending artery (Watanabe *et al.*, Cell Transplant. 7:239, 1998), and efficacy of treatment can be evaluated by histology and cardiac function.

[00921] In some embodiments, the administration comprises implantation into the subject's heart tissue, intravenous injection, intraarterial injection, intracoronary injection, intramuscular

injection, intraperitoneal injection, intramyocardial injection, trans-endocardial injection, trans-epicardial injection, or infusion.

[00922] In some embodiments, the patient administered the engineered cardiac cells is also administered a cardiac drug. Illustrative examples of cardiac drugs that are suitable for use in combination therapy include, but are not limited to, growth factors, polynucleotides encoding growth factors, angiogenic agents, calcium channel blockers, antihypertensive agents, antimitotic agents, inotropic agents, anti-atherogenic agents, anti-coagulants, beta- blockers, anti-arhythmic agents, anti-inflammatory agents, vasodilators, thrombolytic agents, cardiac glycosides, antibiotics, antiviral agents, antifungal agents, agents that inhibit protozoans, nitrates, angiotensin converting enzyme (ACE) inhibitors, angiotensin II receptor antagonist, brain natriuretic peptide (BNP); antineoplastic agents, steroids, and the like.

[00923] The effects of therapy according to the methods provided herein can be monitored in a variety of ways. For instance, an electrocardiogram (ECG) or holier monitor can be utilized to determine the efficacy of treatment. An ECG is a measure of the heart rhythms and electrical impulses, and is a very effective and non-invasive way to determine if therapy has improved or maintained, prevented, or slowed degradation of the electrical conduction in a subject's heart. The use of a holier monitor, a portable ECG that can be worn for long periods of time to monitor heart abnormalities, arrhythmia disorders, and the like, is also a reliable method to assess the effectiveness of therapy. An ECG or nuclear study can be used to determine improvement in ventricular function.

2. Neural Cells Differentiated from Hypoimmunogenic Pluripotent Cells

[00924] Provided herein are different neural cell types differentiated from HIP cells that are useful for subsequent transplantation or engraftment into recipient subjects. As will be appreciated by those in the art, the methods for differentiation depend on the desired cell type using known techniques. Exemplary neural cell types include, but are not limited to, cerebral endothelial cells, neurons (*e.g.*, dopaminergic neurons), glial cells, and the like.

[00925] In some embodiments, neural cells described herein are administered to a recipient subject to treat a neurological disorder selected from the group consisting of Alzheimer's disease, Huntington's disease, Parkinson's disease, Pelizaeus-Merzbacher disease, other neurodegenerative disease or condition, attention deficit hyperactivity disorder (ADHD), ischaemia, multiple sclerosis, traumatic brain injury, epilepsy, catalepsy, encephalitis,

meningitis, migraine, stroke, transient ischemic attack, subarachnoid hemorrhage, subdural hemorrhage, hematoma, extradural hemorrhage, spinal cord injury, cervical spondylosis, carpal tunnel syndrome, brain or spinal cord tumors, peripheral neuropathy, Guillan-Barre syndrome, neuralgia, amyotrophic lateral sclerosis (ALS), tauopathies, Pick disease, progressive supranuclear palsy, corticobasal degeneration, argyrophilic grain disease, Bell's palsy, cerebral palsy, motor neurone disease, neurofibromatosis, encephalitis, meningitis, Tourette's syndrome, schizophrenia, psychosis, depression, or other neuropsychiatric disorder (as described in Gorman *et al., J Cell Mol Med.* 2008 Dec;12(6A):2263-2280; Kovacs *et al. Handbook of Clin Neurol.* 2018;145:355-368).

[00926] In some embodiments, differentiation of induced pluripotent stem cells is performed by exposing or contacting cells to specific factors which are known to produce a specific cell lineage(s), so as to target their differentiation to a specific, desired lineage and/or cell type of interest. In some embodiments, terminally differentiated cells display specialized phenotypic characteristics or features. In certain embodiments, the stem cells described herein are differentiated into a neuroectodermal, neuronal, neuroendocrine, dopaminergic, cholinergic, serotonergic (5-HT), glutamatergic, GABAergic, adrenergic, noradrenergic, sympathetic neuronal, parasympathetic neuronal, sympathetic peripheral neuronal, or glial cell population. In some instances, the glial cell population includes a microglial (*e.g.*, amoeboid, ramified, activated phagocytic, and activated non-phagocytic) cell population or a macroglial (central nervous system cell: astrocyte, oligodendrocyte, ependymal cell, and radial glia; and peripheral nervous system cell: Schwann cell and satellite cell) cell population, or the precursors and progenitors of any of the preceding cells.

[00927] Protocols for generating different types of neural cells are described in PCT Application No. WO2010144696, US Patent Nos. 9,057,053; 9,376,664; and 10,233,422. Additional descriptions of methods for differentiating hypoimmunogenic pluripotent cells can be found, for example, in Deuse *et al.*, Nature Biotechnology, 2019, 37, 252-258 and Han *et al.*, Proc Natl Acad Sci USA, 2019, 116(21), 10441-10446. Methods for determining the effect of neural cell transplantation in an animal model of a neurological disorder or condition are described in the following references: for spinal cord injury – Curtis *et al.*, Cell Stem Cell, 2018, 22, 941-950; for Parkinson's disease – Kikuchi *et al.*, Nature, 2017, 548:592-596; for ALS –

Izrael *et al.*, Stem Cell Research, 2018, 9(1):152 and Izrael *et al.*, IntechOpen, DOI: 10.5772/intechopen.72862; for epilepsy – Upadhya *et al.*, PNAS, 2019, 116(1):287-296

3. Cerebral endothelial cells Differentiated from Hypoimmunogenic Pluripotent Cells

[00928] In some embodiments, neural cells are administered to a subject to treat vascular dementia, Alzheimer; disease, Parkinson's disease, Huntington disease, multiple sclerosis, other neurodegenerative disease or condition, attention deficit hyperactivity disorder (ADHD), Tourette Syndrome (TS), schizophrenia, psychosis, depression, other neuropsychiatric disorder, HIV-1-associated neurocognitive disorder, traumatic brain injury (as described in Grammas *et al., Exp Rev Mol Med.* 2011 Mar;13:e19; Wang *et al. Front Aging Neurosci.* 2018 Nov 16;00376). In some embodiments, neural cells described herein are administered to a subject to treat or ameliorate stroke. In some embodiments, the neurons and glial cells are administered to a subject with amyotrophic lateral sclerosis (ALS). In some embodiments, cerebral endothelial cells are administered to alleviate the symptoms or effects of cerebral hemorrhage. In some embodiments, dopaminergic neurons are administered to a patient with Parkinson's disease. In some embodiments, noradrenergic neurons, GABAergic interneurons are administered to a patient who has experienced an epileptic seizure. In some embodiments, motor neurons, interneurons, Schwann cells, oligodendrocytes, and microglia are administered to a patient who has experienced a spinal cord injury.

[00929] In some embodiments, cerebral endothelial cells (ECs), precursors, and progenitors thereof are differentiated from pluripotent stem cells (*e.g.*, induced pluripotent stem cells) on a surface by culturing the cells in a medium comprising one or more factors that promote the generation of cerebral ECs or neural cell. In some instances, the medium includes one or more of the following: CHIR-99021, VEGF, basic FGF (bFGF), and Y-27632. In some embodiments, the medium includes a supplement designed to promote survival and functionality for neural cells.

[00930] In some embodiments, cerebral endothelial cells (ECs), precursors, and progenitors thereof are differentiated from pluripotent stem cells on a surface by culturing the cells in an unconditioned or conditioned medium. In some instances, the medium comprises factors or small molecules that promote or facilitate differentiation. In some embodiments, the medium comprises one or more factors or small molecules selected from the group consisting of VEGR, FGF, SDF-1, CHIR-99021, Y-27632, SB 431542, and any combination thereof. In some

embodiments, the surface for differentiation comprises one or more extracellular matrix proteins. The surface can be coated with the one or more extracellular matrix proteins. The cells can be differentiated in suspension and then put into a gel matrix form, such as matrigel, gelatin, or fibrin/thrombin forms to facilitate cell survival. In some cases, differentiation is assayed as is known in the art, generally by evaluating the presence of cell-specific markers.

[00931] In some embodiments, the cerebral endothelial cells express or secrete a factor selected from the group consisting of CD31, VE cadherin, and a combination thereof. In certain embodiments, the cerebral endothelial cells express or secrete one or more of the factors selected from the group consisting of CD31, CD34, CD45, CD117 (c-kit), CD146, CXCR4, VEGF, SDF-1, PDGF, GLUT-1, PECAM-1, eNOS, claudin-5, occludin, ZO-1, p-glycoprotein, von Willebrand factor, VE-cadherin, low density lipoprotein receptor LDLR, low density lipoprotein receptor-related protein 1 LRP1, insulin receptor INSR, leptin receptor LEPR, basal cell adhesion molecule BCAM, transferrin receptor TFRC, advanced glycation endproduct-specific receptor AGER, receptor for retinol uptake STRA6, large neutral amino acids transporter small subunit 1 SLC7A5, excitatory amino acid transporter 3 SLC1A1, sodium-coupled neutral amino acid transporter 5 SLC38A5, solute carrier family 16 member 1 SLC16A1, ATP-dependent translocase ABCB1, ATP-ABCC2-binding cassette transporter ABCG2, multidrug resistance-associated protein 1 ABCC1, canalicular multispecific organic anion transporter 1 ABCC2, multidrug resistance-associated protein 4 ABCC4, and multidrug resistance-associated protein 5 ABCC5.

[00932] In some embodiments, the cerebral ECs are characterized with one or more of the features selected from the group consisting of high expression of tight junctions, high electrical resistance, low fenestration, small perivascular space, high prevalence of insulin and transferrin receptors, and high number of mitochondria.

[00933] In some embodiments, cerebral ECs are selected or purified using a positive selection strategy. In some instances, the cerebral ECs are sorted against an endothelial cell marker such as, but not limited to, CD31. In other words, CD31 positive cerebral ECs are isolated. In some embodiments, cerebral ECs are selected or purified using a negative selection strategy. In some embodiments, undifferentiated or pluripotent stem cells are removed by selecting for cells that express a pluripotency marker including, but not limited to, TRA-1-60 and SSEA-1.

4. Dopaminergic neurons Differentiated from Hypoimmunogenic Pluripotent Cells

[00934] In some embodiments, HIP cells described herein are differentiated into dopaminergic neurons include neuronal stem cells, neuronal progenitor cells, immature dopaminergic neurons, and mature dopaminergic neurons.

[00935] In some cases, the term "dopaminergic neurons" includes neuronal cells which express tyrosine hydroxylase (TH), the rate-limiting enzyme for dopamine synthesis. In some embodiments, dopaminergic neurons secrete the neurotransmitter dopamine, and have little or no expression of dopamine hydroxylase. A dopaminergic (DA) neuron can express one or more of the following markers: neuron-specific enolase (NSE), 1-aromatic amino acid decarboxylase, vesicular monoamine transporter 2, dopamine transporter, Nurr-l, and dopamine-2 receptor (D2 receptor). In certain cases, the term "neural stem cells" includes a population of pluripotent cells that have partially differentiated along a neural cell pathway and express one or more neural markers including, for example, nestin. Neural stem cells may differentiate into neurons or glial cells (*e.g.*, astrocytes and oligodendrocytes). The term "neural progenitor cells" includes cultured cells which express FOXA2 and low levels of b-tubulin, but not tyrosine hydroxylase. Such neural progenitor cells have the capacity to differentiate into a variety of neuronal subtypes; particularly a variety of dopaminergic neuronal subtypes, upon culturing the appropriate factors, such as those described herein.

[00936] In some embodiments, the DA neurons derived from HIP cells are administered to a patient, *e.g.*, human patient to treat a neurodegenerative disease or condition. In some cases, the neurodegenerative disease or condition is selected from the group consisting of Parkinson's disease, Huntington disease, and multiple sclerosis. In other embodiments, the DA neurons are used to treat or ameliorate one or more symptoms of a neuropsychiatric disorder, such as attention deficit hyperactivity disorder (ADHD), Tourette Syndrome (TS), schizophrenia, psychosis, and depression. In yet other embodiments, the DA neurons are used to treat a patient with impaired DA neurons.

[00937] In some embodiments, the differentiated DA neurons are transplanted either intravenously or by injection at particular locations in the patient. In some embodiments, the DA cells are transplanted into the substantia nigra (particularly in or adjacent of the compact region), the ventral tegmental area (VTA), the caudate, the putamen, the nucleus accumbens, the subthalamic nucleus, or any combination thereof, of the brain to replace the DA neurons whose

degeneration resulted in Parkinson's disease. The DA cells can be injected into the target area as a cell suspension. Alternatively, the DA cells can be embedded in a support matrix or scaffold when contained in such a delivery device. In some embodiments, the scaffold is biodegradable. In other embodiments, the scaffold is not biodegradable. The scaffold can comprise natural or synthetic (artificial) materials.

[00938] In some embodiments, DA neurons, precursors, and progenitors thereof are differentiated from pluripotent stem cells by culturing the stem cells in medium comprising one or more factors or additives. Useful factors and additives that promote differentiation, growth, expansion, maintenance, and/or maturation of DA neurons include, but are not limited to, Wntl, FGF2, FGF8, FGF8a, sonic hedgehog (SHH), brain derived neurotrophic factor (BDNF), transforming growth factor a (TGF-a), TGF-b, interleukin 1 beta, glial cell line-derived neurotrophic factor (GDNF), a GSK-3 inhibitor (*e.g.*, CHIR-99021), a TGF-b inhibitor (*e.g.*, SB-431542), B-27 supplement, dorsomorphin, purmorphamine, noggin, retinoic acid, cAMP, ascorbic acid, neurturin, knock out serum replacement, N-acetyl cysteine, c-kit ligand, modified forms thereof, mimics thereof, analogs thereof, and variants thereof. In some embodiments, the DA neurons are differentiated in the presence of one or more factors that activate or inhibit the WNT pathway, NOTCH pathway, SHH pathway, BMP pathway, FGF pathway, and the like. Differentiation protocols and detailed descriptions thereof are provided in, *e.g.*, US9,968,637, US7,674,620, Kim *et al.,* Nature, 2002, 418,50-56; Bjorklund *et al.,* PNAS, 2002, 99(4), 2344-2349; Grow *et al.*, Stem Cells Transl Med. 2016, 5(9): 1133-44, and Cho *et al.,* PNAS, 2008, 105:3392-3397, the disclosures in their entirety including the detailed description of the examples, methods, figures, and results are herein incorporated by reference.

[00939] In some embodiments, the population of hypoimmunogenic dopaminergic neurons is isolated from non-neuronal cells. In some embodiments, the isolated population of hypoimmunogenic dopaminergic neurons are expanded prior to administration. In certain embodiments, the isolated population of hypoimmunogenic dopaminergic neurons are expanded and cryopreserved prior to administration.

[00940] To characterize and monitor DA differentiation and assess the DA phenotype, expression of any number of molecular and genetic markers can be evaluated. For example, the presence of genetic markers can be determined by various methods known to those skilled in the

art. Expression of molecular markers can be determined by quantifying methods such as, but not limited to, qPCR-based assays, immunoassays, immunocytochemistry assays, immunoblotting assays, and the like. Exemplary markers for DA neurons include, but are not limited to, TH, b-tubulin, paired box protein (Pax6), insulin gene enhancer protein (Isl1), nestin, diaminobenzidine (DAB), G protein-activated inward rectifier potassium channel 2 (GIRK2), microtubule-associated protein 2 (MAP-2), NURR1, dopamine transporter (DAT), forkhead box protein A2 (FOXA2), FOX3, doublecortin, and LIM homeobox transcription factor l-beta (LMX1B), and the like. In some embodiments, the DA neurons express one or more of the markers selected from corin, FOXA2, TuJ1, NURR1, and any combination thereof.

[00941] In some embodiments, DA neurons are assessed according to cell electrophysiological activity. The electrophysiology of the cells can be evaluated by using assays knowns to those skilled in the art. For instance, whole-cell and perforated patch clamp, assays for detecting electrophysiological activity of cells, assays for measuring the magnitude and duration of action potential of cells, and functional assays for detecting dopamine production of DA cells.

[00942] In some embodiments, DA neuron differentiation is characterized by spontaneous rhythmic action potentials, and high-frequency action potentials with spike frequency adaption upon injection of depolarizing current. In other embodiments, DA differentiation is characterized by the production of dopamine. The level of dopamine produced is calculated by measuring the width of an action potential at the point at which it has reached half of its maximum amplitude (spike half-maximal width).

[00943] In some embodiments, the differentiated DA neurons are transplanted either intravenously or by injection at particular locations in the patient. In some embodiments, the differentiated DA cells are transplanted into the substantia nigra (particularly in or adjacent of the compact region), the ventral tegmental area (VTA), the caudate, the putamen, the nucleus accumbens, the subthalamic nucleus, or any combination thereof, of the brain to replace the DA neurons whose degeneration resulted in Parkinson's disease. The differentiated DA cells can be injected into the target area as a cell suspension. Alternatively, the differentiated DA cells can be embedded in a support matrix or scaffold when contained in such a delivery device. In some embodiments, the scaffold is biodegradable. In other embodiments, the scaffold is not biodegradable. The scaffold can comprise natural or synthetic (artificial) materials.

[00944] The delivery of the DA neurons can be achieved by using a suitable vehicle such as, but not limited to, liposomes, microparticles, or microcapsules. In other embodiments, the differentiated DA neurons are administered in a pharmaceutical composition comprising an isotonic excipient. The pharmaceutical composition is prepared under conditions that are sufficiently sterile for human administration. In some embodiments, the DA neurons differentiated from HIP cells are supplied in the form of a pharmaceutical composition. General principles of therapeutic formulations of cell compositions are found in Cell Therapy: Stem Cell Transplantation, Gene Therapy, and Cellular Immunotherapy, G. Morstyn & W. Sheridan eds, Cambridge University Press, 1996, and Hematopoietic Stem Cell Therapy, E. Ball, J. Lister & P. Law, Churchill Livingstone, 2000, the disclosures are incorporated herein by reference.

[00945] Useful descriptions of neurons derived from stem cells and methods of making thereof can be found, for example, in Kirkeby *et al.*, Cell Rep, 2012, 1:703-714; Kriks *et al.*, Nature, 2011, 480:547-551; Wang *et al.*, Stem Cell Reports, 2018, 11(1):171-182; Lorenz Studer, "Chapter 8 - Strategies for Bringing Stem Cell-Derived Dopamine Neurons to the clinic-The NYSTEM Trial" in Progress in Brain Research, 2017, volume 230, pg. 191-212; Liu *et al.*, Nat Protoc, 2013, 8:1670-1679; Upadhya *et al.*, Curr Protoc Stem Cell Biol, 38, 2D.7.1-2D.7.47; US Publication Appl. No. 20160115448, and US8,252,586; US8,273,570; US9,487,752 and US10,093,897, the contents are incorporated herein by reference in their entirety.

[00946] In addition to DA neurons, other neuronal cells, precursors, and progenitors thereof can be differentiated from the HIP cells outlined herein by culturing the cells in medium comprising one or more factors or additive. Non-limiting examples of factors and additives include GDNF, BDNF, GM-CSF, B27, basic FGF, basic EGF, NGF, CNTF, SMAD inhibitor, Wnt antagonist, SHH signaling activator, and any combination thereof. In some embodiments, the SMAD inhibitor is selected from the group consisting of SB431542, LDN-193189, Noggin PD169316, SB203580, LY364947, A77-01, A-83-01, BMP4, GW788388, GW6604, SB-505124, lerdelimumab, metelimumab, GC-I008, AP-12009, AP-110I4, LY550410, LY580276, LY364947, LY2109761, SB-505124, E-616452 (RepSox ALK inhibitor), SD-208, SMI6, NPC-30345, K 26894, SB-203580, SD-093, activin-M108A, P144, soluble TBR2-Fc, DMH-1, dorsomorphin dihydrochloride and derivatives thereof. In some embodiments, the Wnt antagonist is selected from the group consisting of XAV939, DKK1, DKK-2, DKK-3, DKK-4, SFRP-1, SFRP-2, SFRP-3, SFRP-4, SFRP-5, WIF-1, Soggy, IWP-2, IWR1, ICG-001, KY0211,

Wnt-059, LGK974, IWP-L6 and derivatives thereof. In some embodiments, the SHH signaling activator is selected from the group consisting of Smoothened agonist (SAG), SAG analog, SHH, C25-SHH, C24-SHH, purmorphamine, Hg-Ag and/or derivatives thereof.

[00947] In some embodiments, the neurons express one or more of the markers selected from the group consisting of glutamate ionotropic receptor NMDA type subunit 1 GRIN1, glutamate decarboxylase 1 GAD1, gamma-aminobutyric acid GABA, tyrosine hydroxylase TH, LIM homeobox transcription factor 1-alpha LMX1A, Forkhead box protein O1 FOXO1, Forkhead box protein A2 FOXA2, Forkhead box protein O4 FOXO4, FOXG1, 2',3'-cyclic-nucleotide 3'-phosphodiesterase CNP, myelin basic protein MBP, tubulin beta chain 3 TUB3, tubulin beta chain 3 NEUN, solute carrier family 1 member 6 SLC1A6, SST, PV, calbindin, RAX, LHX6, LHX8, DLX1, DLX2, DLX5, DLX6, SOX6, MAFB, NPAS1, ASCL1, SIX6, OLIG2, NKX2.1, NKX2.2, NKX6.2, VGLUT1, MAP2, CTIP2, SATB2, TBR1, DLX2, ASCL1, ChAT, NGFI-B, c-fos, CRF, RAX, POMC, hypocretin, NADPH, NGF, Ach, VAChT, PAX6, EMX2p75, CORIN, TUJ1, NURR1, and/or any combination thereof.

5. Glial cells Differentiated from Hypoimmunogenic Pluripotent Cells

[00948] In some embodiments, the neural cells described include glial cells such as, but not limited to, microglia, astrocytes, oligodendrocytes, ependymal cells and Schwann cells, glial precursors, and glial progenitors thereof are produced by differentiating pluripotent stem cells into therapeutically effective glial cells and the like. Differentiation of hypoimmunogenic pluripotent stem cells produces hypoimmunogenic neural cells, such as hypoimmunogenic glial cells.

[00949] In some embodiments, glial cells, precursors, and progenitors thereof generated by culturing pluripotent stem cells in medium comprising one or more agents selected from the group consisting of retinoic acid, IL-34, M-CSF, FLT3 ligand, GM-CSF, CCL2, a TGFbeta inhibitor, a BMP signaling inhibitor, a SHH signaling activator, FGF, platelet derived growth factor PDGF, PDGFR-alpha, HGF, IGF1, noggin, SHH, dorsomorphin, noggin, and any combination thereof. In certain instances, the BMP signaling inhibitor is LDN193189, SB431542, or a combination thereof. In some embodiments, the glial cells express NKX2.2, PAX6, SOX10, brain derived neurotrophic factor BDNF, neutrotrophin-3 NT-3, NT-4, EGF, ciliary neurotrophic factor CNTF, nerve growth factor NGF, FGF8, EGFR, OLIG1, OLIG2, myelin basic protein MBP, GAP-43, LNGFR, nestin, GFAP, CD11b, CD11c, CX3CR1,

P2RY12, IBA-1, TMEM119, CD45, and any combination thereof. Exemplary differentiation medium can include any specific factors and/or small molecules that may facilitate or enable the generation of a glial cell type as recognized by those skilled in the art.

[00950] To determine if the cells generated according to the in vitro differentiation protocol display glial cell characteristics and features, the cells can be transplanted into an animal model. In some embodiments, the glial cells are injected into an immunocompromised mouse, *e.g.*, an immunocompromised shiverer mouse. The glial cells are administered to the brain of the mouse and after a pre-selected amount of time the engrafted cells are evaluated. In some instances, the engrafted cells in the brain are visualized by using immunostaining and imaging methods. In some embodiments, it is determined that the glial cells express known glial cell biomarkers.

[00951] Useful methods for generating glial cells, precursors, and progenitors thereof from stem cells are found, for example, in US7,579,188; US7,595,194; US8,263,402; US8,206,699; US8,252,586; US9,193,951; US9,862,925; US8,227,247; US9,709,553; US2018/0187148; US2017/0198255; US2017/0183627; US2017/0182097; US2017/253856; US2018/0236004; WO2017/172976; and WO2018/093681. Methods for differentiating pluripotent stem cells are described in, *e.g.*, Kikuchi *et al.*, Nature, 2017, 548, 592-596; Kriks *et al.*, Nature, 2011, 547-551; Doi *et al.*, Stem Cell Reports, 2014, 2, 337-50; Perrier *et al.*, Proc Natl Acad Sci USA, 2004, 101, 12543-12548; Chambers *et al.*, Nat Biotechnol, 2009, 27, 275-280; and Kirkeby *et al.*, Cell Reports, 2012, 1, 703-714.

[00952] The efficacy of neural cell transplants for spinal cord injury can be assessed in, for example, a rat model for acutely injured spinal cord, as described by McDonald, *et al.*, Nat. Med., 1999, 5:1410) and Kim, *et al.*, Nature, 2002, 418:50. For instance, successful transplants may show transplant-derived cells present in the lesion 2-5 weeks later, differentiated into astrocytes, oligodendrocytes, and/or neurons, and migrating along the spinal cord from the lesioned end, and an improvement in gait, coordination, and weight-bearing. Specific animal models are selected based on the neural cell type and neurological disease or condition to be treated.

[00953] In some embodiments, the glial cells differentiated from hypoimmunogenic cells are administered to a subject in need thereof, where the subject suffers a disease or condition, including but not limited to, argyrophilic grain disease (AGD), amyotrophic lateral sclerosis (ALS), cortico-basal degeneration (CBD), Parkinsonism linked to chromosome 17 (FTDP-17),

multiple system atrophy (MSA), Parkinson's disease/diffuse Lewy body disease (PD/DLBD), or Alzheimer's disease (see Miller *et al., Neuron Glia Biol.* 2004 Feb; 1(1): 13–21).

[00954] The neural cells can be administered in a manner that permits them to engraft to the intended tissue site and reconstitute or regenerate the functionally deficient area. For instance, neural cells can be transplanted directly into parenchymal or intrathecal sites of the central nervous system, according to the disease being treated. In some embodiments, any of the neural cells described herein including cerebral endothelial cells, neurons, dopaminergic neurons, ependymal cells, astrocytes, microglial cells, oligodendrocytes, and Schwann cells are injected into a patient by way of intravenous, intraspinal, intracerebroventricular, intrathecal, intra-arterial, intramuscular, intraperitoneal, subcutaneous, intramuscular, intra-abdominal, intraocular, retrobulbar and combinations thereof. In some embodiments, the cells are injected or deposited in the form of a bolus injection or continuous infusion. In certain embodiments, the neural cells are administered by injection into the brain, apposite the brain, and combinations thereof. The injection can be made, for example, through a burr hole made in the subject's skull. Suitable sites for administration of the neural cell to the brain include, but are not limited to, the cerebral ventricle, lateral ventricles, cisterna magna, putamen, nucleus basalis, hippocampus cortex, striatum, caudate regions of the brain and combinations thereof.

[00955] Additional descriptions of neural cells including dopaminergic neurons for use in the present disclosure are found in WO2020/018615, the disclosure is herein incorporated by reference in its entirety.

6. Endothelial Cells Differentiated from Hypoimmunogenic Pluripotent Cells

[00956] Provided herein are hypoimmunogenic pluripotent cells that are differentiated into various endothelial cell types for subsequent transplantation or engraftment into subjects (*e.g.*, recipients). As will be appreciated by those in the art, the methods for differentiation depend on the desired cell type using known techniques.

[00957] In some embodiments, the endothelial cells differentiated from the subject hypoimmunogenic pluripotent cells are administered to a patient, *e.g.*, a human patient in need thereof. The endothelial cells can be administered to a patient suffering from a disease or condition such as, but not limited to, atherosclerosis, atherogenesis, arterial thrombosis, venous thrombosis, thrombocytic microangiopathies, vascular leakage, diffuse intravascular coagulation,

diabetes, insulin resistance (as described in Rajendra *et al., Int J Biol Sci.* 2013 Nov 9;9(10):1057-1069), cardiovascular disease, vascular disease, peripheral vascular disease, ischemic disease, myocardial infarction, congestive heart failure, peripheral vascular obstructive disease, stroke, reperfusion injury, limb ischemia, neuropathy (*e.g.*, peripheral neuropathy or diabetic neuropathy), organ failure (*e.g.*, liver failure, kidney failure, and the like), diabetes, rheumatoid arthritis, osteoporosis, vascular injury, tissue injury, hypertension, angina pectoris and myocardial infarction due to coronary artery disease, renal vascular hypertension, renal failure due to renal artery stenosis, claudication of the lower extremities, and the like. In certain embodiments, the patient has suffered from or is suffering from a transient ischemic attack or stroke, which in some cases, may be due to cerebrovascular disease. In some embodiments, the engineered endothelial cells are administered to treat tissue ischemia *e.g.*, as occurs in atherosclerosis, myocardial infarction, and limb ischemia and to repair of injured blood vessels. In some instances, the cells are used in bioengineering of grafts.

[00958] For instance, the endothelial cells can be used in cell therapy for the repair of ischemic tissues, formation of blood vessels and heart valves, engineering of artificial vessels, repair of damaged vessels, and inducing the formation of blood vessels in engineered tissues (*e.g.*, prior to transplantation). Additionally, the endothelial cells can be further modified to deliver agents to target and treat tumors.

[00959] In certain embodiments, provided herein is a method of repair or replacement for tissue in need of vascular cells or vascularization. The method involves administering to a human patient in need of such treatment, a composition containing the isolated endothelial cells to promote vascularization in such tissue. The tissue in need of vascular cells or vascularization can be a cardiac tissue, liver tissue, pancreatic tissue, renal tissue, muscle tissue, neural tissue, bone tissue, among others, which can be a tissue damaged and characterized by excess cell death, a tissue at risk for damage, or an artificially engineered tissue.

[00960] In some embodiments, vascular diseases, which may be associated with cardiac diseases or disorders can be treated by administering endothelial cells, such as but not limited to, definitive vascular endothelial cells and endocardial endothelial cells derived as described herein. Such vascular diseases include, but are not limited to, coronary artery disease, cerebrovascular disease, aortic stenosis, aortic aneurysm, peripheral artery disease, atherosclerosis, varicose

veins, angiopathy, infarcted area of heart lacking coronary perfusion, non-healing wounds, diabetic or non-diabetic ulcers, or any other disease or disorder in which it is desirable to induce formation of blood vessels.

[00961] In many embodiments, the endothelial cells are used for improving prosthetic implants (*e.g.*, vessels made of synthetic materials such as Dacron and Gortex.) which are used in vascular reconstructive surgery. For example, prosthetic arterial grafts are often used to replace diseased arteries which perfuse vital organs or limbs. In other embodiments, the engineered endothelial cells are used to cover the surface of prosthetic heart valves to decrease the risk of the formation of emboli by making the valve surface less thrombogenic.

[00962] The endothelial cells outlined can be transplanted into the patient using well known surgical techniques for grafting tissue and/or isolated cells into a vessel. In some embodiments, the cells are introduced into the patient's heart tissue by injection (*e.g.*, intramyocardial injection, intracoronary injection, trans-endocardial injection, trans-epicardial injection, percutaneous injection), infusion, grafting, and implantation.

[00963] Administration (delivery) of the endothelial cells includes, but is not limited to, subcutaneous or parenteral including intravenous, intraarterial (*e.g.*, intracoronary), intramuscular, intraperitoneal, intramyocardial, trans-endocardial, trans-epicardial, intranasal administration as well as intrathecal, and infusion techniques.

[00964] As will be appreciated by those in the art, the HIP derivatives are transplanted using techniques known in the art that depend on both the cell type and the ultimate use of these cells. In some embodiments, the cells are transplanted either intravenously or by injection at particular locations in the patient. When transplanted at particular locations, the cells may be suspended in a gel matrix to prevent dispersion while they take hold.

[00965] Exemplary endothelial cell types include, but are not limited to, a capillary endothelial cell, vascular endothelial cell, aortic endothelial cell, arterial endothelial cell, venous endothelial cell, renal endothelial cell, brain endothelial cell, liver endothelial cell, and the like.

[00966] The endothelial cells outlined herein can express one or more endothelial cell markers. Non-limiting examples of such markers include VE-cadherin (CD 144), ACE (angiotensin-converting enzyme) (CD 143), BNH9/BNF13, CD31, CD34, CD54 (ICAM-l), CD62E (E-Selectin), CD105 (Endoglin), CD146, Endocan (ESM-l), Endoglyx-l, Endomucin, Eotaxin-3, EPAS1 (Endothelial PAS domain protein 1), Factor VIII related antigen, FLI-l, Flk-l (KDR,

VEGFR-2), FLT-l (VEGFR-l), GATA2, GBP-l (guanylate- binding protein-l), GRO-alpha, HEX, ICAM-2 (intercellular adhesion molecule 2), LM02, LYVE-l, MRB (magic roundabout), Nucleolin, PAL-E (pathologische anatomie Leiden- endothelium), RTKs, sVCAM-l, TALI, TEM1 (Tumor endothelial marker 1), TEM5 (Tumor endothelial marker 5), TEM7 (Tumor endothelial marker 7), thrombomodulin (TM, CD141), VCAM-l (vascular cell adhesion molecule- 1) (CD106), VEGF, vWF (von Willebrand factor), ZO-l, endothelial cell-selective adhesion molecule (ESAM), CD102, CD93, CD184, CD304, and DLL4.

[00967] In some embodiments, the endothelial cells are genetically modified to express an exogenous polynucleotide encoding a protein of interest such as but not limited to an enzyme, hormone, receptor, ligand, or drug that is useful for treating a disorder/condition or ameliorating symptoms of the disorder/condition. Standard methods for genetically modifying endothelial cells are described, *e.g.*, in US5,674,722.

[00968] Such endothelial cells can be used to provide constitutive synthesis and delivery of polypeptides or proteins, which are useful in prevention or treatment of disease. In this way, the polypeptide is secreted directly into the bloodstream or other area of the body (*e.g.*, central nervous system) of the individual. In some embodiments, the endothelial cells can be modified to secrete insulin, a blood clotting factor (*e.g.*, Factor VIII or von Willebrand Factor), alpha-l antitrypsin, adenosine deaminase, tissue plasminogen activator, interleukins (*e.g.*, IL-l, IL-2, IL-3), and the like.

[00969] In some embodiments, the endothelial cells can be modified in a way that improves their performance in the context of an implanted graft. Non-limiting illustrative examples include secretion or expression of a thrombolytic agent to prevent intraluminal clot formation, secretion of an inhibitor of smooth muscle proliferation to prevent luminal stenosis due to smooth muscle hypertrophy, and expression and/or secretion of an endothelial cell mitogen or autocrine factor to stimulate endothelial cell proliferation and improve the extent or duration of the endothelial cell lining of the graft lumen.

[00970] In some embodiments, the engineered endothelial cells are utilized for delivery of therapeutic levels of a secreted product to a specific organ or limb. For example, a vascular implant lined with endothelial cells engineered (transduced) in vitro can be grafted into a specific organ or limb. The secreted product of the transduced endothelial cells will be delivered in high

concentrations to the perfused tissue, thereby achieving a desired effect to a targeted anatomical location.

[00971] In other embodiments, the endothelial cells are genetically modified to contain a gene that disrupts or inhibits angiogenesis when expressed by endothelial cells in a vascularizing tumor. In some cases, the endothelial cells can also be genetically modified to express any one of the selectable suicide genes described herein which allows for negative selection of grafted endothelial cells upon completion of tumor treatment.

[00972] In some embodiments, endothelial cells described herein are administered to a recipient subject to treat a vascular disorder selected from the group consisting of vascular injury, cardiovascular disease, vascular disease, peripheral vascular disease, ischemic disease, myocardial infarction, congestive heart failure, peripheral vascular obstructive disease, hypertension, ischemic tissue injury, reperfusion injury, limb ischemia, stroke, neuropathy (*e.g.*, peripheral neuropathy or diabetic neuropathy), organ failure (*e.g.*, liver failure, kidney failure, and the like), diabetes, rheumatoid arthritis, osteoporosis, cerebrovascular disease, hypertension, angina pectoris and myocardial infarction due to coronary artery disease, renal vascular hypertension, renal failure due to renal artery stenosis, claudication of the lower extremities, and/or other vascular condition or disease.

[00973] In some embodiments, the hypoimmunogenic pluripotent cells are differentiated into endothelial colony forming cells (ECFCs) to form new blood vessels to address peripheral arterial disease. Techniques to differentiate endothelial cells are known. See, *e.g.*, Prasain *et al.*, doi: 10.1038/nbt.3048, incorporated herein by reference in its entirety and specifically for the methods and reagents for the generation of endothelial cells from human pluripotent stem cells, and also for transplantation techniques. Differentiation can be assayed as is known in the art, generally by evaluating the presence of endothelial cell associated or specific markers or by measuring functionally.

[00974] In some embodiments, the method of producing a population of hypoimmunogenic endothelial cells from a population of hypoimmunogenic pluripotent cells by in vitro differentiation comprises: (a) culturing a population of HIP cells in a first culture medium comprising a GSK inhibitor; (b) culturing the population of HIP cells in a second culture medium comprising VEGF and bFGF to produce a population of pre-endothelial cells; and (c)

culturing the population of pre-endothelial cells in a third culture medium comprising a ROCK inhibitor and an ALK inhibitor to produce a population of hypoimmunogenic endothelial cells.

[00975] In some embodiments, the GSK inhibitor is CHIR-99021, a derivative thereof, or a variant thereof. In some instances, the GSK inhibitor is at a concentration ranging from about 1 mM to about 10 mM. In some embodiments, the ROCK inhibitor is Y-27632, a derivative thereof, or a variant thereof. In some instances, the ROCK inhibitor is at a concentration ranging from about 1 pM to about 20 pM. In some embodiments, the ALK inhibitor is SB-431542, a derivative thereof, or a variant thereof. In some instances, the ALK inhibitor is at a concentration ranging from about 0.5 pM to about 10 pM.

[00976] In some embodiments, the first culture medium comprises from 2 pM to about 10 pM of CHIR-99021. In some embodiments, the second culture medium comprises 50 ng/ml VEGF and 10 ng/ml bFGF. In other embodiments, the second culture medium further comprises Y-27632 and SB-431542. In various embodiments, the third culture medium comprises 10 pM Y-27632 and 1 pM SB-431542. In certain embodiments, the third culture medium further comprises VEGF and bFGF. In particular instances, the first culture medium and/or the second medium is absent of insulin.

[00977] The cells provided herein can be cultured on a surface, such as a synthetic surface to support and/or promote differentiation of hypoimmunogenic pluripotent cells into cardiac cells. In some embodiments, the surface comprises a polymer material including, but not limited to, a homopolymer or copolymer of selected one or more acrylate monomers. Non-limiting examples of acrylate monomers and methacrylate monomers include tetra(ethylene glycol) diacrylate, glycerol dimethacrylate, 1,4-butanediol dimethacrylate, poly(ethylene glycol) diacrylate, di(ethylene glycol) dimethacrylate, tetra(ethyiene glycol) dimethacrylate, 1,6-hexanediol propoxylate diacrylate, neopentyl glycol diacrylate, trimethylolpropane benzoate diacrylate, trimethylolpropane eihoxylate (1 EO/QH) methyl, tricyclo[5.2.1.02,6] decane dimethanol diacrylate, neopentyl glycol exhoxylate diacrylate, and trimethylolpropane triacrylate. Acrylate synthesized as known in the art or obtained from a commercial vendor, such as Polysciences, Inc., Sigma Aldrich, Inc. and Sartomer, Inc.

[00978] In some embodiments, the endothelial cells may be seeded onto a polymer matrix. In some cases, the polymer matrix is biodegradable. Suitable biodegradable matrices are well known in the art and include collagen-GAG, collagen, fibrin, PLA, PGA, and PLA/PGA co-

polymers. Additional biodegradable materials include poly(anhydrides), poly(hydroxy acids), poly(ortho esters), poly(propylfumerates), poly(caprolactones), polyamides, polyamino acids, polyacetals, biodegradable polycyanoacrylates, biodegradable polyurethanes and polysaccharides.

[00979] Non-biodegradable polymers may also be used as well. Other non- biodegradable, yet biocompatible polymers include polypyrrole, polyanibnes, polythiophene, polystyrene, polyesters, non-biodegradable polyurethanes, polyureas, poly(ethylene vinyl acetate), polypropylene, polymethacrylate, polyethylene, polycarbonates, and poly(ethylene oxide). The polymer matrix may be formed in any shape, for example, as particles, a sponge, a tube, a sphere, a strand, a coiled strand, a capillary network, a film, a fiber, a mesh, or a sheet. The polymer matrix can be modified to include natural or synthetic extracellular matrix materials and factors.

[00980] The polymeric material can be dispersed on the surface of a support material. Useful support materials suitable for culturing cells include a ceramic substance, a glass, a plastic, a polymer or co-polymer, any combinations thereof, or a coating of one material on another. In some instances, a glass includes soda-lime glass, pyrex glass, vycor glass, quartz glass, silicon, or derivatives of these or the like.

[00981] In some instances, plastics or polymers including dendritic polymers include poly(vinyl chloride), poly(vinyl alcohol), poly(methyl methacrylate), poly(vinyl acetate- maleic anhydride), poly(dimethylsiloxane) monomethacrylate, cyclic olefin polymers, fluorocarbon polymers, polystyrenes, polypropylene, polyethyleneimine or derivatives of these or the like. In some instances, copolymers include poly(vinyl acetate-co-maleic anhydride), poly(styrene-co-maleic anhydride), poly(ethylene-co-acrylic acid) or derivatives of these or the like.

[00982] In some embodiments, the population of hypoimmunogenic endothelial cells is isolated from non-endothelial cells. In some embodiments, the isolated population of hypoimmunogenic endothelial cells are expanded prior to administration. In certain embodiments, the isolated population of hypoimmunogenic endothelial cells are expanded and cryopreserved prior to administration.

[00983] Additional descriptions of endothelial cells for use in the methods provided herein are found in WO2020/018615, the disclosure is herein incorporated by reference in its entirety.

7. Thyroid Cells Differentiated from Hypoimmunogenic Pluripotent Cells

[00984] In some embodiments, the hypoimmunogenic pluripotent cells are differentiated into thyroid progenitor cells and thyroid follicular organoids that can secrete thyroid hormones to address autoimmune thyroiditis. Techniques to differentiate thyroid cells are known the art. See, *e.g.,* Kurmann *et al.*, Cell Stem Cell, 2015 Nov 5;17(5):527-42, incorporated herein by reference in its entirety and specifically for the methods and reagents for the generation of thyroid cells from human pluripotent stem cells, and also for transplantation techniques. Differentiation can be assayed as is known in the art, generally by evaluating the presence of thyroid cell associated or specific markers or by measuring functionally.

[00985] In some embodiments, the thyroid cells differentiated from the subject hypoimmunogenic pluripotent cells are administered to a patient, *e.g.*, a human patient suffering from a disease or condition such as, but not limited to, goiter, hyperparathyroidism, hypoparathyroidism (congenital or autoimmune), thyroiditis, Hashimoto's thyroiditis, postpartum thyroiditis, subacute thyroiditis, iatrogenic hypothyroidism, Grave's disease, and thyroid eye disease (see Lassen *et al., Ann Endocrinol (Paris).* 2019 Sep;80(4):240-249; Bilezikian *et al., Lancet.* 2018 Jan 13;391(10116):168-178; Weetman, *J Endocrinol Invest.* 2021 May;44(5):883-890; Weiler, *Clin Exp Optom.* 2017 Jan;100(1):20-25).

8. Hepatocytes Differentiated from Hypoimmunogenic Pluripotent Cells

[00986] In some embodiments, the hypoimmunogenic induced pluripotent stem (HIP) cells are differentiated into hepatocytes to address loss of the hepatocyte functioning or cirrhosis of the liver. There are a number of techniques that can be used to differentiate HIP cells into hepatocytes; see for example, Pettinato *et al.*, doi: 10.1038/spre32888, Snykers *et al.*, Methods Mol Biol, 2011 698:305-314, Si-Tayeb *et al.*, Hepatology, 2010, 51:297-305 and Asgari *et al.,* Stem Cell Rev, 2013, 9(4):493- 504, all of which are incorporated herein by reference in their entirety and specifically for the methodologies and reagents for differentiation. Differentiation can be assayed as is known in the art, generally by evaluating the presence of hepatocyte associated and/or specific markers, including, but not limited to, albumin, alpha fetoprotein, and fibrinogen. Differentiation can also be measured functionally, such as the metabolization of ammonia, LDL storage and uptake, ICG uptake and release, and glycogen storage.

[00987] In some embodiments, the hepatocytes differentiated from the subject hypoimmunogenic pluripotent cells are administered to a patient, *e.g.*, a human patient suffering from a disease or condition such as, but not limited to, infectious hepatitis (A, B, and C; Bianco *et al., Dig Liver Dis.* 2004 Dec;36(12):834-842), autoimmune hepatitis (Sirbe *et al., Int J Mol Sci.* 2021 Dec;22(24):13578), primary biliary cholangitis, primary sclerosing cholangitis (both described in Park *et al. Biomedicines.* 2022 Jun;10(6):1288), non-alcoholic fatty liver disease (Francque *et al., JHEP Rep.* 2021 Oct;3(5):100322), cirrhosis (Yoshiji *et al., J Gastroenterol.* 2021;56(7):593-619), hemochromatosis (Brissot *et al., Nat Rev Dis Primers.* 2018 Apr 5;4:18016), hyperoxaluria (Hoppe and Martin-Higueras, *Drugs.* 2022;82(10):1077-1094), alpha-1 antitrypsin deficiency (Chapman *et al., Int J Chron Obstruct Pulmon Dis.* 2018;13:419-432), liver failure (Zaccherini *et al., JHEP Rep.* 2021 Feb:3(1):100176), Wilson's disease (Yuan *et al., Curr Neuropharmacol.* 2021 Apr;19(4):465-485), hepatic encephalopathy (Goh *et al., Cochrane Database Syst Rev.* 2018 May;2018(5):CD012410), jaundice (Chee *et al., Hong Kong Med J.* 2018 Jun;24(3):285-292), acute hepatic porphyrias (Wang *et al., Hepatol. Commun.* 2018 Dec 20;3(2):193-206), Alagille syndrome (Kohut *et al., Semin Liver Dis.* 2021 Nov;41(4):525-537), biliary atresia (Lakshminarayann and Davenport, *J Autoimmun.* 2016 Sep;73:1-9), Budd-Chiari syndrome (Iliescu *et al., Med Ultrason.* 2019 Aug 31;21(3):344-348), hyperbilirubinemias, Crigler-Najjar syndrome, Gilbert-Meulengracht syndrome, Dubin-Johnson syndrome, Rotor syndrome (all described in Strassburg, *Best Pract Res Clin Gastroenterol.* 2010 Oct;24(5):555-571), galactosemia (Coelho *et al., J Inherit Metab Dis.* 2017 May;40(3):325-342), glycogen storage disease type 1 (Kishnani *et al., Genet Med.* 2014 Nov;16(11):e1), hepatorenal syndrome (Ojeda-Yuren *et al., Ann Hepatol.* 2021 May-Jun;22:100236), intrahepatic cholestasis of pregnancy (Smith and Rood, *Clin Obstet Gynecol.* 2020 Mar;63(1):134-151), progressive familial intrahepatic cholestasis (Baker *et al., Clin Res Hepaol Gastroenterol.* 2019 Feb;43(1):20-36), Reye's syndrome (Maheady, *J Pediatr Health Care.* 1989 Sep-Oct;3(5):246-250), or lysosomal acid lipase deficiency (Pastores and Hughes, *Drug Des Devel Ther.* 2020 Feb 11;14:591-601).

9. Pancreatic Islet Cells Differentiated from Hypoimmunogenic Pluripotent Cells

[00988] In some embodiments, pancreatic islet cells (also referred to as beta cells) are derived from the HIP cells described herein. In some instances, hypoimmunogenic pluripotent cells that are differentiated into various pancreatic islet cell types are transplanted or engrafted into

subjects (*e.g.*, recipients). As will be appreciated by those in the art, the methods for differentiation depend on the desired cell type using known techniques. Exemplary pancreatic islet cell types include, but are not limited to, pancreatic islet progenitor cell, immature pancreatic islet cell, mature pancreatic islet cell, and the like. In some embodiments, pancreatic cells described herein are administered to a subject to treat diabetes.

[00989] In some embodiments, the pancreatic islet cells differentiated from the subject hypoimmunogenic pluripotent cells are administered to a patient, e.g., a human patient suffering from a disease or condition such as, but not limited to, alcohol-related pancreatitis, gallstone pancreatitis, diabetes mellitus (type 1 and type 2), prediabetes, gestational diabetes, pancreoprivic diabetes mellitus, pancreatic exocrine insufficiency, acute pancreatitis, chronic pancreatitis, hereditary pancreatitis, hyperinsulinemia, pancreatic cysts, Zollinger-Ellison syndrome, Shwachman-Diamond syndrome, hereditary hemochromatosis, thalassemia, pancreatic iron deposition, cystic fibrosis, pancreas divisum, and pancreatic resection (see Ciochina *et al., Biomolecules* 2022 May;12(5):618).

[00990] In some embodiments, pancreatic islet cells are derived from the hypoimmunogenic pluripotent cells described herein. Useful method for differentiating pluripotent stem cells into pancreatic islet cells are described, for example, in US9,683,215; US9,157,062; and US8,927,280. In some emboriments, the pancreatic islet cells comprise alpha, beta, delta, PP (pancreatic polypeptide-producing), and/or ε-cells (ghrelin-producing) islet cells. In some embodiments, the pancreatic islet cells comprise iPSC-derived beta cells.

[00991] In some embodiments, the pancreatic islet cells produced by the methods as disclosed herein secretes insulin. In some embodiments, a pancreatic islet cell exhibits at least two characteristics of an endogenous pancreatic islet cell, for example, but not limited to, secretion of insulin in response to glucose, and expression of beta cell markers.

[00992] Exemplary beta cell markers or beta cell progenitor markers include, but are not limited to, c-peptide, Pdxl, glucose transporter 2 (Glut2), HNF6, VEGF, glucokinase (GCK), prohormone convertase (PC 1/3), Cdcpl, NeuroD, Ngn3, Nkx2.2, Nkx6.l, Nkx6.2, Pax4, Pax6, Ptfla, Isll, Sox9, Soxl7, and FoxA2.

[00993] In some embodiments, the isolated pancreatic islet cells produce insulin in response to an increase in glucose. In various embodiments, the isolated pancreatic islet cells secrete insulin

in response to an increase in glucose. In some embodiments, the cells have a distinct morphology such as a cobblestone cell morphology and/or a diameter of about 17 pm to about 25 pm.

[00994] In some embodiments, the hypoimmunogenic pluripotent cells are differentiated into beta-like cells or islet organoids for transplantation to address type I diabetes mellitus (T1DM). Cell systems are a promising way to address T1DM, see, *e.g.*, Ellis *et al.,* Nat Rev Gastroenterol Hepatol. 2017 Oct;14(10):612-628, incorporated herein by reference. Additionally, Pagliuca *et al.* (Cell, 2014, 159(2):428-39) reports on the successful differentiation of β-cells from hiPSCs, the contents incorporated herein by reference in its entirety and in particular for the methods and reagents outlined there for the large-scale production of functional human β cells from human pluripotent stem cells). Furthermore, Vegas *et al.* shows the production of human β cells from human pluripotent stem cells followed by encapsulation to avoid immune rejection by the host; Vegas *et al.*, Nat Med, 2016, 22(3):306-11, incorporated herein by reference in its entirety and in particular for the methods and reagents outlined there for the large-scale production of functional human β cells from human pluripotent stem cells.

[00995] In some embodiments, the method of producing a population of hypoimmunogenic pancreatic islet cells from a population of hypoimmunogenic pluripotent cells by in vitro differentiation comprises: (a) culturing the population of HIP cells in a first culture medium comprising one or more factors selected from the group consisting insulin-like growth factor, transforming growth factor, FGF, EGF, HGF, SHH, VEGF, transforming growth factor-b superfamily, BMP2, BMP7, a GSK inhibitor, an ALK inhibitor, a BMP type 1 receptor inhibitor, and retinoic acid to produce a population of immature pancreatic islet cells; and (b) culturing the population of immature pancreatic islet cells in a second culture medium that is different than the first culture medium to produce a population of hypoimmune pancreatic islet cells. In some embodiments, the GSK inhibitor is CHIR-99021, a derivative thereof, or a variant thereof. In some instances, the GSK inhibitor is at a concentration ranging from about 2 mM to about 10 mM. In some embodiments, the ALK inhibitor is SB-431542, a derivative thereof, or a variant thereof. In some instances, the ALK inhibitor is at a concentration ranging from about 1 pM to about 10 pM. In some embodiments, the first culture medium and/or second culture medium are absent of animal serum.

[00996] In some embodiments, the population of hypoimmunogenic pancreatic islet cells is isolated from non-pancreatic islet cells. In some embodiments, the isolated population of

hypoimmunogenic pancreatic islet cells are expanded prior to administration. In certain embodiments, the isolated population of hypoimmunogenic pancreatic islet cells are expanded and cryopreserved prior to administration.

[00997] Differentiation is assayed as is known in the art, generally by evaluating the presence of β cell associated or specific markers, including but not limited to, insulin. Differentiation can also be measured functionally, such as measuring glucose metabolism, see generally Muraro *et al.*, Cell Syst. 2016 Oct 26; 3(4): 385–394.e3, hereby incorporated by reference in its entirety, and specifically for the biomarkers outlined there. Once the beta cells are generated, they can be transplanted (either as a cell suspension or within a gel matrix as discussed herein) into the portal vein/liver, the omentum, the gastrointestinal mucosa, the bone marrow, a muscle, or subcutaneous pouches.

[00998] Additional descriptions of pancreatic islet cells including dopaminergic neurons for use in the present disclosure are found in WO2020/018615, the disclosure is herein incorporated by reference in its entirety.

10. Retinal Pigmented Epithelium (RPE) Cells Differentiated from Hypoimmunogenic Pluripotent Cells

[00999] Provided herein are retinal pigmented epithelium (RPE) cells derived from the HIP cells described above. For instance, human RPE cells can be produced by differentiating human HIP cells. In some embodiments, hypoimmunogenic pluripotent cells that are differentiated into various RPE cell types are transplanted or engrafted into subjects (*e.g.*, recipients). As will be appreciated by those in the art, the methods for differentiation depend on the desired cell type using known techniques.

[001000] The term "RPE" cells refers to pigmented retinal epithelial cells having a genetic expression profile similar or substantially similar to that of native RPE cells. Such RPE cells derived from pluripotent stem cells may possess the polygonal, planar sheet morphology of native RPE cells when grown to confluence on a planar substrate.

[001001] The RPE cells can be implanted into a patient suffering from macular degeneration or a patient having damaged RPE cells. In some embodiments, the patient has age-related macular degeneration (AMD), early AMD, intermediate AMD, late AMD, non-neovascular age-related macular degeneration, dry macular degeneration (dry age-related macular degeneration), wet macular degeneration (wet age-related macular degeneration), adult-onset vitelliform macular

dystrophy (AVMD), Best vitelliform macular dystrophy, Stargardt-like macular dystrophy (STGD3), Sorby's fundus dystrophy (SFD), ABCA4-related disease, Usher type IB, autosomal recessive bestrophinopathy, autosomal dominant vitreoretinochoroidopathy, juvenile macular degeneration (JMD) (*e.g.*, Stargardt disease, Best disease, and juvenile retinoschisis), Leber's Congenital Amaurosis, or retinitis pigmentosa (all described in Yang *et al., Front Pharmacol.* 2021 Jul 28;12:727870; Sparrow *et al., Curr Mol Med.* 2010 Dec;10(9):802-823). In other embodiments, the patient suffers from retinal detachment or retinal tears.

[001002] Exemplary RPE cell types include, but are not limited to, retinal pigmented epithelium (RPE) cell, RPE progenitor cell, immature RPE cell, mature RPE cell, functional RPE cell, and the like.

[001003] Useful methods for differentiating pluripotent stem cells into RPE cells are described in, for example, US9,458,428 and US9,850,463, the disclosures are herein incorporated by reference in their entirety, including the specifications. Additional methods for producing RPE cells from human induced pluripotent stem cells can be found in, for example, Lamba *et al.*, PNAS, 2006, 103(34): 12769-12774; Mellough *et al.,* Stem Cells, 2012, 30(4):673-686; Idelson *et al.,* Cell Stem Cell, 2009, 5(4): 396-408; Rowland *et al.,* Journal of Cellular Physiology, 2012, 227(2):457-466, Buchholz *et al.,* Stem Cells Trans Med, 2013, 2(5): 384-393, and da Cruz *et al.,* Nat Biotech, 2018, 36:328-337.

[001004] Human pluripotent stem cells have been differentiated into RPE cells using the techniques outlined in Kamao et al , Stem Cell Reports 2014:2:205-18, hereby incorporated by reference in its entirety and in particular for the methods and reagents outlined there for the differentiation techniques and reagents; see also Mandai *et al.*, N Engl J Med, 2017, 376:1038-1046, the contents herein incorporated in its entirety for techniques for generating sheets of RPE cells and transplantation into patients. Differentiation can be assayed as is known in the art, generally by evaluating the presence of RPE associated and/or specific markers or by measuring functionally. See for example Kamao *et al.*, Stem Cell Reports, 2014, 2(2):205-18, the contents incorporated herein by reference in its entirety and specifically for the markers outlined in the first paragraph of the results section.

[001005] In some embodiments, the method of producing a population of hypoimmunogenic retinal pigmented epithelium (RPE) cells from a population of hypoimmunogenic pluripotent

cells by in vitro differentiation comprises: (a) culturing the population of hypoimmunogenic pluripotent cells in a first culture medium comprising any one of the factors selected from the group consisting of activin A, bFGF, BMP4/7, DKK1, IGF1, noggin, a BMP inhibitor, an ALK inhibitor, a ROCK inhibitor, and a VEGFR inhibitor to produce a population of pre-RPE cells; and (b) culturing the population of pre-RPE cells in a second culture medium that is different than the first culture medium to produce a population of hypoimmunogenic RPE cells. In some embodiments, the ALK inhibitor is SB-431542, a derivative thereof, or a variant thereof. In some instances, the ALK inhibitor is at a concentration ranging from about 2 mM to about 10 pM. In some embodiments, the ROCK inhibitor is Y-27632, a derivative thereof, or a variant thereof. In some instances, the ROCK inhibitor is at a concentration ranging from about 1 pM to about 10 pM. In some embodiments, the first culture medium and/or second culture medium are absent of animal serum.

[001006] Differentiation can be assayed as is known in the art, generally by evaluating the presence of RPE associated and/or specific markers or by measuring functionally. See for example Kamao *et al.*, Stem Cell Reports, 2014, 2(2):205-18, the contents are herein incorporated by reference in its entirety and specifically for the results section.

[001007] Additional descriptions of RPE cells for use in the present disclosure are found in WO2020/018615, the disclosure is herein incorporated by reference in its entirety.

[001008] For therapeutic application, cells prepared according to the disclosed methods can typically be supplied in the form of a pharmaceutical composition comprising an isotonic excipient, and are prepared under conditions that are sufficiently sterile for human administration. For general principles in medicinal formulation of cell compositions, see "Cell Therapy: Stem Cell Transplantation, Gene Therapy, and Cellular Immunotherapy," by Morstyn & Sheridan eds, Cambridge University Press, 1996; and "Hematopoietic Stem Cell Therapy," E. D. Ball, J. Lister & P. Law, Churchill Livingstone, 2000. The cells can be packaged in a device or container suitable for distribution or clinical use.

11. T Lymphocytes Differentiated from Hypoimmunogenic Pluripotent Cells

[001009] Provided herein, T lymphocytes (T cells, including primary T cells) are derived from the HIP cells described herein (*e.g.*, hypoimmunogenic iPSCs). Methods for generating T cells, including CAR-T cells, from pluripotent stem cells (*e.g.*, iPSCs) are described, for example, in

Iriguchi *et al.*, Nature Communications 12, 430 (2021); Themeli *et al.*, Cell Stem Cell, 16(4):357-366 (2015); Themeli *et al.*, Nature Biotechnology 31:928-933 (2013).

[001010] T lymphocyte derived hypoimmunogenic cells include, but are not limited to, primary T cells that evade immune recognition. In some embodiments, the hypoimmunogenic cells are produced (*e.g.*, generated, cultured, or derived) from T cells such as primary T cells. In some instances, primary T cells are obtained (*e.g.*, harvested, extracted, removed, or taken) from a subject or an individual. In some embodiments, primary T cells are produced from a pool of T cells such that the T cells are from one or more subjects (*e.g.*, one or more human including one or more healthy humans). In some embodiments, the pool of primary T cells is from 1-100, 1-50, 1-20, 1-10, 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 10 or more, 20 or more, 30 or more, 40 or more, 50 or more, or 100 or more subjects. In some embodiments, the donor subject is different from the patient (*e.g.*, the recipient that is administered the therapeutic cells). In some embodiments, the pool of T cells does not include cells from the patient. In some embodiments, one or more of the donor subjects from which the pool of T cells is obtained are different from the patient.

[001011] In some embodiments, the T lymphocytes differentiated from the subject hypoimmunogenic pluripotent cells are administered to a patient, e.g., a human patient suffering from a disease or condition such as, but not limited to, severe combined immunodeficiencies (SCID), Omenn syndrome, Cartilage-Hair hypoplasia, reticular dysgenesis (all described in Shearer et al., J Allergy Clin Immunol. 2014 Apr;133(4):1092-8), Wiskott-Aldrich syndrome (Massaad *et al., Ann N Y Acad Sci.* 2013 May;1285:26-4), ataxia telangiectasia (Rothblum-Oviatt et al., Orphnet J Rare Dis. 2016, Nov 25;11(1):159), DiGeorge syndrome (22q11.2 deletion syndrome, see McDonald-McGinn et al., Nat Rev Dis Primers. 2015 Nov 19;1:15071), immune-osseous dysplasias (Saraiva et al., J Med Genet. 1999 Oct;36(10):786-789), dyskeratosis congenita (Stoopler and Shanti, Mayo Clin Proc. 2019 Sep;94(9):1668-1669), or chronic mucocutaneous candidiasis (Kirkpatrick, J Am Acad Dermatol. 1994 Sep;31(3 Pt 2):S14-17). In other embodiments, T cells provided herein are useful for the treatment of suitable cancers including, but not limited to, 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 (see Mohanti et al., Oncol Rep 42: 2183-2195, 2019).

[001012] In some embodiments, the T lymphocytes differentiated from the subject hypoimmunogenic pluripotent cells are administered to a patient, e.g., a human patient suffering from a disease or condition such as, but not limited to, severe combined immunodeficiencies (SCID), Omenn syndrome, Cartilage-Hair hypoplasia, reticular dysgenesis (all described in Shearer et al., J Allergy Clin Immunol. 2014 Apr;133(4):1092-8), Wiskott-Aldrich syndrome (Massaad *et al., Ann N Y Acad Sci.* 2013 May;1285:26-4), ataxia telangiectasia (Rothblum-Oviatt et al., Orphnet J Rare Dis. 2016, Nov 25;11(1):159), DiGeorge syndrome (22q11.2 deletion syndrome, see McDonald-McGinn et al., Nat Rev Dis Primers. 2015 Nov 19;1:15071), immune-osseous dysplasias (Saraiva et al., J Med Genet. 1999 Oct;36(10):786-789), dyskeratosis congenita (Stoopler and Shanti, Mayo Clin Proc. 2019 Sep;94(9):1668-1669), or chronic mucocutaneous candidiasis (Kirkpatrick, J Am Acad Dermatol. 1994 Sep;31(3 Pt 2):S14-17). In other embodiments, T cells provided herein are useful for the treatment of suitable cancers including, but not limited to, 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 (see Mohanti et al., Oncol Rep 42: 2183-2195, 2019).

[001013] In some embodiments, the cancer is a hematologic malignancy. Non-limiting examples of hematologic malignancies include follicular lymphoma (FL), myeloid neoplasm, mature T/NK neoplasms, Histiocytic neoplasms, multiple myeloma (MM), myelodysplastic syndromes (MDS), lymphoplasmacytic lymphoma (LPL), Waldenström macroglobulinemia, Burkitt lymphoma (BL), primary mediastinal large B-cell lymphoma (PMBL), Hodgkin lymphoma, Mantle cell lymphoma (MCL), Hairy cell leukemia (HCL), myeloproliferative/myelodysplastic syndromes (MDS) , acute lymphoid leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), Diffuse large B-cell lymphoma (DLBCL), B cell acute lymphoid leukemia (B-

ALL), T cell acute lymphoid leukemia (T-ALL), T cell lymphoma, and B cell lymphoma (see Taylor et al., Blood 2017 Jul 27; 130(4): 410–423).

[001014] In some embodiments, the disease is an autoimmune disease, including, for example, lupus, systemic lupus erythematosus, rheumatoid arthritis, psoriasis, psoriatic arthritis, multiple sclerosis, Crohn's disease, ulcerative colitis, Addison's disease, Graves' disease, Sjögren's syndrome, Hashimoto's thyroiditis, diabetes mellitus type 1, primary biliary cirrhosis, autoimmune hepatitis, and celiac disease (described in *Wang et al., J Intern Med.* 2015;278(4):369-395).

[001015] In some embodiments, the hypoimmunogenic cells do not activate an innate and/or an adaptive immune response in the patient (*e.g.*, recipient upon administration). Provided are methods of treating a disorder by administering a population of hypoimmunogenic cells to a subject (*e.g.*, recipient) or patient in need thereof. In some embodiments, the hypoimmunogenic cells described herein comprise T cells engineered (*e.g.*, are modified) to express a chimeric antigen receptor including but not limited to a chimeric antigen receptor described herein. In some instances, the T cells are populations or subpopulations of primary T cells from one or more individuals. In some embodiments, the T cells described herein such as the engineered or modified T cells comprise reduced expression of an endogenous T cell receptor.

[001016] In some embodiments, the HIP--derived T cell includes a chimeric antigen receptor (CAR). Any suitable CAR can be included in the HIP-derived T cell, including the CARs described herein. In some embodiments, the HIP-derived T cell includes a polynucleotide encoding a CAR, wherein the polynucleotide is inserted in a genomic locus. In some embodiments, the polynucleotide is inserted into a safe harbor or a target locus. In some embodiments, the polynucleotide is inserted in a B2M, CIITA, TRAC, TRB, PD-1 or CTLA-4 gene. Any suitable method can be used to insert the CAR into the genomic locus of the hypoimmunogenic cell including the gene editing methods described herein (*e.g.*, a CRISPR/Cas system).

[001017] HIP-derived T cells provided herein are useful for the treatment of suitable cancers including, but not limited to, 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.

12. NK Cells Derived from Hypoimmunogenic Pluripotent Cells

[001018] Provided herein, natural killer (NK) cells are derived from the HIP cells described herein (*e.g.*, hypoimmunogenic iPSCs).

[001019] NK cells (also defined as 'large granular lymphocytes') represent a cell lineage differentiated from the common lymphoid progenitor (which also gives rise to B lymphocytes and T lymphocytes). Unlike T-cells, NK cells do not naturally comprise CD3 at the plasma membrane. Importantly, NK cells do not express a TCR and typically also lack other antigen-specific cell surface receptors (as well as TCRs and CD3, they also do not express immunoglobulin B-cell receptors, and instead typically express CD16 and CD56). NK cell cytotoxic activity does not require sensitization but is enhanced by activation with a variety of cytokines including IL-2. NK cells are generally thought to lack appropriate or complete signaling pathways necessary for antigen-receptor-mediated signaling, and thus are not thought to be capable of antigen receptor-dependent signaling, activation and expansion. NK cells are cytotoxic, and balance activating and inhibitory receptor signaling to modulate their cytotoxic activity. For instance, NK cells expressing CD16 may bind to the Fc domain of antibodies bound to an infected cell, resulting in NK cell activation. By contrast, activity is reduced against cells expressing high levels of MHC class I proteins/molecules. On contact with a target cell NK cells release proteins such as perforin, and enzymes such as proteases (granzymes). Perforin can form pores in the cell membrane of a target cell, inducing apoptosis or cell lysis.

[001020] There are a number of techniques that can be used to generate NK cells, including CAR-NK-cells, from pluripotent stem cells (*e.g.*, iPSC); see, for example, Zhu *et al.*, *Methods Mol Biol.* 2019; 2048:107-119; Knorr *et al.*, *Stem Cells Transl Med.* 2013 2(4):274-83. doi: 10.5966/sctm.2012-0084; Zeng *et al.*, *Stem Cell Reports.* 2017 Dec 12;9(6):1796-1812; Ni *et al.*, *Methods Mol Biol.* 2013;1029:33-41; Bernareggi *et al.*, *Exp Hematol.* 2019 71:13-23; Shankar *et al.*, *Stem Cell Res Ther.* 2020;11(1):234, all of which are incorporated herein by reference in their entirety and specifically for the methodologies and reagents for differentiation. Differentiation can be assayed as is known in the art, generally by evaluating the presence of NK cell associated and/or specific markers, including, but not limited to, CD56, KIRs, CD16,

NKp44, NKp46, NKG2D, TRAIL, CD122, CD27, CD244, NK1.1, NKG2A/C, NCR1, Ly49, CD49b, CD11b, KLRG1, CD43, CD62L, and/or CD226.

[001021] In some embodiments, the NK cells differentiated from the subject hypoimmunogenic pluripotent cells are administered to a patient, e.g., a human patient suffering from a disease or condition such as, but not limited to, systemic lupus erythematosus (SLE), type 1 diabetes, autoimmune liver disease, Sjögren's syndrome, rheumatoid arthritis, systemic sclerosis (scleroderma), organ-specific autoimmune diseases (autoimmune hepatitis, primary sclerosing chonlangitis), alcohol-related liver disease, multiple sclerosis (all described in Liu *et al., Front Immunol.* 2021; 12: 624687), NK cell deficiency (NKD) (functional (FNKD) or classical (CNKD)), immunodeficiency-polyendocrinopathy-enteropathy-X-linked (IPEX)-like syndrome, Bloom syndrome, Fanconi's anemia, dyskeratosis congenita, Chediak-Higashi syndrome, familial hematophagocytic lymphohistocytosis (FHL), Griscelli syndrome type 2, Hermansky Pudliak syndrome, Papillon-Lefevre syndrome, Wiskott-Aldrich syndrome, autosomal recessive hyper-IgE syndrome, May Hegglin anomaly, or leucocyte adhesion deficiency type I or type III (all described in Orange, J. S, *J Allergy Clin Immunol.* 2013 Sep; 132(3): 515–526). In other embodiments, NK cells provided herein are useful for the treatment of suitable cancers including, but not limited to, 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 (see Mohanti *et al., Oncol Rep* 42: 2183-2195, 2019).

[001022] In some embodiments, the NK cells do not activate an innate and/or an adaptive immune response in the patient (*e.g.*, recipient upon administration). Provided are methods of treating a disorder by administering a population of NK cells to a subject (*e.g.*, recipient) or patient in need thereof. In some embodiments, the NK cells described herein comprise NK cells engineered (*e.g.*, are modified) to express a chimeric antigen receptor including but not limited to a chimeric antigen receptor described herein. Any suitable CAR can be included in the NK cells, including the CARs described herein. In some embodiments, the NK cell includes a polynucleotide encoding a CAR, wherein the polynucleotide is inserted in a genomic locus. In

some embodiments, the polynucleotide is inserted into a safe harbor or a target locus. In some embodiments, the polynucleotide is inserted in a B2M, CIITA, TRAC, TRB, PD1 or CTLA4 gene. Any suitable method can be used to insert the CAR into the genomic locus of the NK cell including the gene editing methods described herein (*e.g.*, a CRISPR/Cas system).

[001023]

BB. Methods of Genetic Modifications

[001024] In some embodiments, a vector herein is a nucleic acid molecule capable transferring or transporting another nucleic acid molecule, including into the cell or into genome of a cell. The transferred nucleic acid is generally linked to, e.g., inserted into, the vector nucleic acid molecule. A vector may include sequences that direct autonomous replication in a cell or may include sequences sufficient to allow integration into host cell DNA. Useful vectors include, for example, plasmids (e.g., DNA plasmids or RNA plasmids), transposons, cosmids, bacterial artificial chromosomes, and viral vectors. Useful viral vectors include, e.g., replication defective retroviruses and lentiviruses. Non-viral vectors may require a delivery vehicle to facilitate entry of the nucleic acid molecule into a cell.

[001025] A viral vector can comprise a nucleic acid molecule that includes virus-derived nucleic acid elements that typically facilitate transfer of the nucleic acid molecule or integration into the genome of a cell or to a viral particle that mediates nucleic acid transfer. Viral particles will typically include various viral components and sometimes also host cell components in addition to nucleic acid(s). A viral vector can comprise, e.g., a virus or viral particle capable of transferring a nucleic acid into a cell, or to the transferred nucleic acid (e.g., as naked DNA). Viral vectors and transfer plasmids can comprise structural and/or functional genetic elements that are primarily derived from a virus. A retroviral vector can comprise a viral vector or plasmid containing structural and functional genetic elements, or portions thereof, that are primarily derived from a retrovirus.

[001026] In some vectors described herein, at least part of one or more protein coding regions that contribute to or are essential for replication may be absent compared to the corresponding wild-type virus. This makes the viral vector replication-defective. In some embodiments, the vector is capable of transducing a target non-dividing host cell and/or integrating its genome into a host genome.

[001027] In some embodiments, the retroviral nucleic acid comprises one or more of (e.g., all of): a 5' promoter (e.g., to control expression of the entire packaged RNA), a 5' LTR (e.g., that includes R (polyadenylation tail signal) and/or U5 which includes a primer activation signal), a primer binding site, a psi packaging signal, a RRE element for nuclear export, a promoter directly upstream of the transgene to control transgene expression, a transgene (or other exogenous agent element), a polypurine tract, and a 3' LTR (e.g., that includes a mutated U3, a R, and U5). In some embodiments, the retroviral nucleic acid further comprises one or more of a cPPT, a WPRE, and/or an insulator element.

[001028] A retrovirus typically replicates by reverse transcription of its genomic RNA into a linear double-stranded DNA copy and subsequently covalently integrates its genomic DNA into a host genome. The structure of a wild-type retrovirus genome often comprises a 5' long terminal repeat (LTR) and a 3' LTR, between or within which are located a packaging signal to enable the genome to be packaged, a primer binding site, integration sites to enable integration into a host cell genome and gag, pol and env genes encoding the packaging components which promote the assembly of viral particles. More complex retroviruses have additional features, such as rev and RRE sequences in HIV, which enable the efficient export of RNA transcripts of the integrated provirus from the nucleus to the cytoplasm of an infected target cell. In the provirus, the viral genes are flanked at both ends by regions called long terminal repeats (LTRs). The LTRs are involved in proviral integration and transcription. LTRs also serve as enhancer-promoter sequences and can control the expression of the viral genes. Encapsidation of the retroviral RNAs occurs by virtue of a psi sequence located at the 5' end of the viral genome.

[001029] The LTRs themselves are typically similar (e.g., identical) sequences that can be divided into three elements, which are called U3, R and U5. U3 is derived from the sequence unique to the 3' end of the RNA. R is derived from a sequence repeated at both ends of the RNA and U5 is derived from the sequence unique to the 5' end of the RNA. The sizes of the three elements can vary considerably among different retroviruses.

[001030] For the viral genome, the site of transcription initiation is typically at the boundary between U3 and R in one LTR and the site of poly (A) addition (termination) is at the boundary between R and U5 in the other LTR. U3 contains most of the transcriptional control

elements of the provirus, which include the promoter and multiple enhancer sequences responsive to cellular and in some cases, viral transcriptional activator proteins. Some retroviruses comprise any one or more of the following genes that code for proteins that are involved in the regulation of gene expression: tot, rev, tax and rex.

[001031] With regard to the structural genes gag, pol and env themselves, gag encodes the internal structural protein of the virus. Gag protein is proteolytically processed into the mature proteins MA (matrix), CA (capsid) and NC (nucleocapsid). The pol gene encodes the reverse transcriptase (RT), which contains DNA polymerase, associated RNase H and integrase (IN), which mediate replication of the genome. The env gene encodes the surface (SU) glycoprotein and the transmembrane (TM) protein of the virion, which form a complex that interacts specifically with cellular receptor proteins. This interaction promotes infection, e.g., by fusion of the viral membrane with the cell membrane.

[001032] In a replication-defective retroviral vector genome gag, pol and env may be absent or not functional. The R regions at both ends of the RNA are typically repeated sequences. U5 and U3 represent unique sequences at the 5' and 3' ends of the RNA genome respectively. Retroviruses may also contain additional genes which code for proteins other than gag, pol and env. Examples of additional genes include (in HIV), one or more of vif, vpr, vpx, vpu, tat, rev and nef. EIAV has (amongst others) the additional gene S2.

[001033] Illustrative retroviruses suitable for use in particular embodiments, include, but are not limited to: Moloney murine leukemia virus (M-MuLV), Moloney murine sarcoma virus(MoMSV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), gibbon ape leukemia virus (GaLV), feline leukemia virus (FLV), spumavirus, Friend murine leukemia virus, Murine Stem Cell Virus (MSCV) and Rous Sarcoma Virus (RSV)) and lentivirus.

[001034] In some embodiments the retrovirus is a Gammretrovirus. In some embodiments the retrovirus is an Epsilonretrovirus. In some embodiments the retrovirus is an Alpharetrovirus. In some embodiments the retrovirus is a Betaretro virus. In some embodiments the retrovirus is a Deltaretro virus. In some embodiments the retrovirus is a Spumaretrovirus. In some embodiments the retrovirus is an endogenous retrovirus. In some embodiments the retrovirus is a lentivirus.

[001035] In some embodiments, a retroviral or lentivirus vector further comprises one or more insulator elements, e.g., an insulator element described herein. In various embodiments, the vectors comprise a promoter operably linked to a polynucleotide encoding an exogenous agent. The vectors may have one or more LTRs, wherein either LTR comprises one or more modifications, such as one or more nucleotide substitutions, additions, or deletions. The vectors may further comprise one of more accessory elements to increase transduction efficiency (e.g., a cPPT/FLAP), viral packaging (e.g., a Psi (Y) packaging signal, RRE), and/or other elements that increase exogenous gene expression (e.g., poly (A) sequences), and may optionally comprise a WPRE or HPRE. In some embodiments, a lentiviral nucleic acid comprises one or more of, e.g., all of, e.g., from 5' to 3', a promoter (e.g., CMV), an R sequence (e.g., comprising TAR), a U5 sequence (e.g., for integration), a PBS sequence (e.g., for reverse transcription), a DIS sequence (e.g., for genome dimerization), a psi packaging signal, a partial gag sequence, an RRE sequence (e.g., for nuclear export), a cPPT sequence (e.g., for nuclear import), a promoter to drive expression of the exogenous agent, a gene encoding the exogenous agent, a WPRE sequence (e.g., for efficient transgene expression), a PPT sequence (e.g., for reverse transcription), an R sequence (e.g., for polyadenylation and termination), and a U5 signal (e.g., for integration).

[001036] Illustrative lentiviruses include, but are not limited to: HIV (human immunodeficiency virus; including HIV type 1, and HIV type 2); visna-maedi virus (VMV) virus; the caprine arthritis-encephalitis virus (CAEV); equine infectious anemia virus (EIAV); feline immunodeficiency virus (FIV); bovine immune deficiency virus (BIV); and simian immunodeficiency virus (SIV). In some embodiments, HIV based vector backbones (i.e., HIV cis-acting sequence elements) are used.A lentivirus vector can comprise a viral vector or plasmid containing structural and functional genetic elements, or portions thereof, including LTRs that are primarily derived from a lentivirus.

[001037] In embodiments, a lentivirus vector (e.g., lentiviral expression vector) may comprise a lentiviral transfer plasmid (e.g., as naked DNA) or an infectious lentiviral particle. With respect to elements such as cloning sites, promoters, regulatory elements, heterologous nucleic acids,etc., it is to be understood that the sequences of these elements can be present in RNA form in lentiviral particles and can be present in DNA form in DNA plasmids.

[001038] In embodiments, a lentivirus vector is a vector with sufficient retroviral genetic information to allow packaging of an RNA genome, in the presence of packaging components, into a viral particle capable of infecting a target cell. Infection of the target cell can comprise reverse transcription and integration into the target cell genome. The RLV typically carries non-viral coding sequences which are to be delivered by the vector to the target cell. In embodiments, an RLV is incapable of independent replication to produce infectious retroviral particles within the target cell. Usually the RLV lacks a functional gag-pol and/or env gene and/or other genes involved in replication. The vector may be configured as a split-intron vector, e.g., as described in PCT patent application WO 99/15683, which is herein incorporated by reference in its entirety.

[001039] In some embodiments, the lentivirus vector comprises a minimal viral genome, e.g., the viral vector has been manipulated so as to remove the non-essential elements and to retain the essential elements in order to provide the required functionality to infect, transduce and deliver a nucleotide sequence of interest to a target host cell, e.g., as described in WO 98/17815, which is herein incorporated by reference in its entirety.

[001040] A minimal lentiviral genome may comprise, e.g., (5')R-U5-one or more first nucleotide sequences-U3-R(3')· However, the plasmid vector used to produce the lentiviral genome within a source cell can also include transcriptional regulatory control sequences operably linked to the lentiviral genome to direct transcription of the genome in a source cell. These regulatory sequences may comprise the natural sequences associated with the transcribed retroviral sequence, e.g., the 5' U3 region, or they may comprise a heterologous promoter such as another viral promoter, for example the CMV promoter. Some lentiviral genomes comprise additional sequences to promote efficient virus production. For example, in the case of HIV, rev and RRE sequences may be included.

[001041] In some embodiments, the rare-cutting endonuclease is introduced into a cell containing the target polynucleotide sequence in the form of a nucleic acid encoding a rare-cutting endonuclease. The process of introducing the nucleic acids into cells can be achieved by any suitable technique. Suitable techniques include calcium phosphate or lipid-mediated transfection, electroporation, and transduction or infection using a viral vector. In some embodiments, the nucleic acid comprises DNA. In some embodiments, the nucleic acid

comprises a modified DNA, as described herein. In some embodiments, the nucleic acid comprises mRNA. In some embodiments, the nucleic acid comprises a modified mRNA, as described herein (*e.g.*, a synthetic, modified mRNA).

[001042] The present disclosure contemplates altering target polynucleotide sequences in any manner which is available to the skilled artisan utilizing a gene editing system (*e.g.* CRISPR/Cas)of the present disclosure. Any CRISPR/Cas system that is capable of altering a target polynucleotide sequence in a cell can be used. Such CRISPR-Cas systems can employ a variety of Cas proteins (Haft *et al.* PLoS Comput Biol. 2005; 1(6)e60). The molecular machinery of such Cas proteins that allows the CRISPR/Cas system to alter target polynucleotide sequences in cells include RNA binding proteins, endo- and exo-nucleases, helicases, and polymerases. In some embodiments, the CRISPR/Cas system is a CRISPR Type I system. In some embodiments, the CRISPR/Cas system is a CRISPR Type II system. In some embodiments, the CRISPR/Cas system is a CRISPR Type V system.

[001043] The CRISPR/Cas systems of the present disclosure can be used to alter any target polynucleotide sequence in a cell. Those skilled in the art will readily appreciate that desirable target polynucleotide sequences to be altered in any particular cell may correspond to any genomic sequence for which expression of the genomic sequence is associated with a disorder or otherwise facilitates entry of a pathogen into the cell. For example, a desirable target polynucleotide sequence to alter in a cell may be a polynucleotide sequence corresponding to a genomic sequence which contains a disease associated single polynucleotide polymorphism. In such example, the CRISPR/Cas systems of the present disclosure can be used to correct the disease associated SNP in a cell by replacing it with a wild-type allele. As another example, a polynucleotide sequence of a target gene which is responsible for entry or proliferation of a pathogen into a cell may be a suitable target for deletion or insertion to disrupt the function of the target gene to prevent the pathogen from entering the cell or proliferating inside the cell.

[001044] In some embodiments, the target polynucleotide sequence is a genomic sequence. In some embodiments, the target polynucleotide sequence is a human genomic sequence. In some embodiments, the target polynucleotide sequence is a mammalian genomic sequence. In some embodiments, the target polynucleotide sequence is a vertebrate genomic sequence.

[001045] In some embodiments, a CRISPR/Cas system of the present disclosure includes a Cas protein and at least one to two ribonucleic acids that are capable of directing the Cas protein to

and hybridizing to a target motif of a target polynucleotide sequence. As used herein, "protein" and "polypeptide" are used interchangeably to refer to a series of amino acid residues joined by peptide bonds (*i.e.*, a polymer of amino acids) and include modified amino acids (*e.g.*, phosphorylated, glycated, glycosylated, etc.) and amino acid analogs. Exemplary polypeptides or proteins include gene products, naturally occurring proteins, homologs, paralogs, fragments and other equivalents, variants, and analogs of the above.

[001046] In some embodiments, a Cas protein comprises one or more amino acid substitutions or modifications. In some embodiments, the one or more amino acid substitutions comprises a conservative amino acid substitution. In some instances, substitutions and/or modifications can prevent or reduce proteolytic degradation and/or extend the half-life of the polypeptide in a cell. In some embodiments, the Cas protein can comprise a peptide bond replacement (*e.g.*, urea, thiourea, carbamate, sulfonyl urea, etc.). In some embodiments, the Cas protein can comprise a naturally occurring amino acid. In some embodiments, the Cas protein can comprise an alternative amino acid (*e.g.*, D-amino acids, beta-amino acids, homocysteine, phosphoserine, etc.). In some embodiments, a Cas protein can comprise a modification to include a moiety (*e.g.*, PEGylation, glycosylation, lipidation, acetylation, end-capping, etc.).

[001047] In some embodiments, a Cas protein comprises a core Cas protein, isoform thereof, or any Cas-like protein with similar function or activity of any Cas protein or isoform thereof. In some embodiments, a Cas protein comprises a core Cas protein. Exemplary Cas core proteins include, but are not limited to Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 and Cas9. In some embodiments, a Cas protein comprises type V Cas protein. In some embodiments, a Cas protein comprises a Cas protein of an E. coli subtype (also known as CASS2). Exemplary Cas proteins of the E. Coli subtype include, but are not limited to Cse1, Cse2, Cse3, Cse4, and Cas5e. In some embodiments, a Cas protein comprises a Cas protein of the Ypest subtype (also known as CASS3). Exemplary Cas proteins of the Ypest subtype include, but are not limited to Csy1, Csy2, Csy3, and Csy4. In some embodiments, a Cas protein comprises a Cas protein of the Nmeni subtype (also known as CASS4). Exemplary Cas proteins of the Nmeni subtype include, but are not limited to Csn1 and Csn2. In some embodiments, a Cas protein comprises a Cas protein of the Dvulg subtype (also known as CASS1). Exemplary Cas proteins of the Dvulg subtype include Csd1, Csd2, and Cas5d. In some embodiments, a Cas protein comprises a Cas protein of the Tneap subtype (also known as CASS7). Exemplary Cas proteins of the Tneap

subtype include, but are not limited to, Cst1, Cst2, Cas5t. In some embodiments, a Cas protein comprises a Cas protein of the Hmari subtype. Exemplary Cas proteins of the Hmari subtype include, but are not limited to Csh1, Csh2, and Cas5h. In some embodiments, a Cas protein comprises a Cas protein of the Apern subtype (also known as CASS5). Exemplary Cas proteins of the Apern subtype include, but are not limited to Csa1, Csa2, Csa3, Csa4, Csa5, and Cas5a. In some embodiments, a Cas protein comprises a Cas protein of the Mtube subtype (also known as CASS6). Exemplary Cas proteins of the Mtube subtype include, but are not limited to Csm1, Csm2, Csm3, Csm4, and Csm5. In some embodiments, a Cas protein comprises a RAMP module Cas protein. Exemplary RAMP module Cas proteins include, but are not limited to, Cmr1, Cmr2, Cmr3, Cmr4, Cmr5, and Cmr6. See, *e.g.*, Klompe *et al.*, Nature 571, 219–225 (2019); Strecker *et al.*, Science 365, 48–53 (2019). Examples of Cas proteins include, but are not limited to: Cas3, Cas8a, Cas5, Cas8b, Cas8c, Cas10d, Cse1, Cse2, Csy1, Csy2, Csy3, and/or GSU0054. In some embodiments, a Cas protein comprises Cas3, Cas8a, Cas5, Cas8b, Cas8c, Cas10d, Cse1, Cse2, Csy1, Csy2, Csy3, and/or GSU0054. In some embodiments, examples of Cas proteins include, but are not limited to: Cas9, Csn2, and/or Cas4. In some embodiments, a Cas protein comprises Cas9, Csn2, and/or Cas4. In some embodiments, examples of Cas proteins include, but are not limited to: Cas10, Csm2, Cmr5, Cas10, Csx11, and/or Csx10. In some embodiments, a Cas protein comprises a Cas10, Csm2, Cmr5, Cas10, Csx11, and/or Csx10. In some embodiments, examples of Cas proteins include, but are not limited to: Csf1. In some embodiments, a Cas protein comprises Csf1.In some embodiments, examples of Cas proteins include, but are not limited to: Cas12a, Cas12b, Cas12c, C2c4, C2c8, C2c5, C2c10, and C2c9; as well as CasX (Cas12e) and CasY (Cas12d). Also see, *e.g.*, Koonin *et al.*, *Curr Opin Microbiol.* 2017; 37:67-78: "Diversity, classification and evolution of CRISPR-Cas systems." In some embodiments, a Cas protein comprises Cas12a, Cas12b, Cas12c, Cas12d, Cas12e, Cas12d, and/or Cas12e. In some embodiments, examples of Cas proteins include, but are not limited to: Cas13, Cas13a, C2c2, Cas13b, Cas13c, and/or Cas13d. In some embodiments, a Cas protein comprises Cas13, Cas13a, C2c2, Cas13b, Cas13c, and/or Cas13d.

[001048] In some embodiments, a Cas protein comprises any one of the Cas proteins described herein or a functional portion thereof. As used herein, "functional portion" refers to a portion of a peptide which retains its ability to complex with at least one ribonucleic acid (*e.g.*, guide RNA (gRNA)) and cleave a target polynucleotide sequence. In some embodiments, the functional

portion comprises a combination of operably linked Cas9 protein functional domains selected from the group consisting of a DNA binding domain, at least one RNA binding domain, a helicase domain, and an endonuclease domain. In some embodiments, the functional portion comprises a combination of operably linked Cas12a (also known as Cpf1) protein functional domains selected from the group consisting of a DNA binding domain, at least one RNA binding domain, a helicase domain, and an endonuclease domain. In some embodiments, the functional domains form a complex. In some embodiments, a functional portion of the Cas9 protein comprises a functional portion of a RuvC-like domain. In some embodiments, a functional portion of the Cas9 protein comprises a functional portion of the HNH nuclease domain. In some embodiments, a functional portion of the Cas12a protein comprises a functional portion of a RuvC-like domain.

[001049] In some embodiments, exogenous Cas protein can be introduced into the cell in polypeptide form. In certain embodiments, Cas proteins can be conjugated to or fused to a cell-penetrating polypeptide or cell-penetrating peptide. As used herein, "cell-penetrating polypeptide" and "cell-penetrating peptide" refers to a polypeptide or peptide, respectively, which facilitates the uptake of molecule into a cell. The cell-penetrating polypeptides can contain a detectable label.

[001050] In many embodiments, Cas proteins can be conjugated to or fused to a charged protein (*e.g.*, that carries a positive, negative or overall neutral electric charge). Such linkage may be covalent. In some embodiments, the Cas protein can be fused to a superpositively charged GFP to significantly increase the ability of the Cas protein to penetrate a cell (Cronican *et al.* ACS Chem Biol. 2010; 5(8):747-52). In certain embodiments, the Cas protein can be fused to a protein transduction domain (PTD) to facilitate its entry into a cell. Exemplary PTDs include Tat, oligoarginine, and penetratin. In some embodiments, the Cas9 protein comprises a Cas9 polypeptide fused to a cell-penetrating peptide. In some embodiments, the Cas9 protein comprises a Cas9 polypeptide fused to a PTD. In some embodiments, the Cas9 protein comprises a Cas9 polypeptide fused to a tat domain. In some embodiments, the Cas9 protein comprises a Cas9 polypeptide fused to an oligoarginine domain. In some embodiments, the Cas9 protein comprises a Cas9 polypeptide fused to a penetratin domain. In some embodiments, the Cas9 protein comprises a Cas9 polypeptide fused to a superpositively charged GFP. In some embodiments, the Cas12a protein comprises a Cas12a polypeptide fused to a cell-penetrating

peptide. In some embodiments, the Cas12a protein comprises a Cas12a polypeptide fused to a PTD. In some embodiments, the Cas12a protein comprises a Cas12a polypeptide fused to a tat domain. In some embodiments, the Cas12a protein comprises a Cas12a polypeptide fused to an oligoarginine domain. In some embodiments, the Cas12a protein comprises a Cas12a polypeptide fused to a penetratin domain. In some embodiments, the Cas12a protein comprises a Cas12a polypeptide fused to a superpositively charged GFP.

[001051] In some embodiments, the Cas protein can be introduced into a cell containing the target polynucleotide sequence in the form of a nucleic acid encoding the Cas protein. The process of introducing the nucleic acids into cells can be achieved by any suitable technique. Suitable techniques include calcium phosphate or lipid-mediated transfection, electroporation, and transduction or infection using a viral vector. In some embodiments, the nucleic acid comprises DNA. In some embodiments, the nucleic acid comprises a modified DNA, as described herein. In some embodiments, the nucleic acid comprises mRNA. In some embodiments, the nucleic acid comprises a modified mRNA, as described herein (*e.g.*, a synthetic, modified mRNA).

[001052] In some embodiments, the Cas protein is complexed with one to two ribonucleic acids. In some embodiments, the Cas protein is complexed with two ribonucleic acids. In some embodiments, the Cas protein is complexed with one ribonucleic acid. In some embodiments, the Cas protein is encoded by a modified nucleic acid, as described herein (*e.g.*, a synthetic, modified mRNA).

[001053] The methods of the present disclosure contemplate the use of any ribonucleic acid that is capable of directing a Cas protein to and hybridizing to a target motif of a target polynucleotide sequence. In some embodiments, at least one of the ribonucleic acids comprises tracrRNA. In some embodiments, at least one of the ribonucleic acids comprises CRISPR RNA (crRNA). In some embodiments, a single ribonucleic acid comprises a guide RNA that directs the Cas protein to and hybridizes to a target motif of the target polynucleotide sequence in a cell. In some embodiments, at least one of the ribonucleic acids comprises a guide RNA that directs the Cas protein to and hybridizes to a target motif of the target polynucleotide sequence in a cell. In some embodiments, both of the one to two ribonucleic acids comprise a guide RNA that directs the Cas protein to and hybridizes to a target motif of the target polynucleotide sequence in a cell. The ribonucleic acids of the present disclosure can be selected to hybridize to a variety of

different target motifs, depending on the particular CRISPR/Cas system employed, and the sequence of the target polynucleotide, as will be appreciated by those skilled in the art. The one to two ribonucleic acids can also be selected to minimize hybridization with nucleic acid sequences other than the target polynucleotide sequence. In some embodiments, the one to two ribonucleic acids hybridize to a target motif that contains at least two mismatches when compared with all other genomic nucleotide sequences in the cell. In some embodiments, the one to two ribonucleic acids hybridize to a target motif that contains at least one mismatch when compared with all other genomic nucleotide sequences in the cell. In some embodiments, the one to two ribonucleic acids are designed to hybridize to a target motif immediately adjacent to a deoxyribonucleic acid motif recognized by the Cas protein. In some embodiments, each of the one to two ribonucleic acids are designed to hybridize to target motifs immediately adjacent to deoxyribonucleic acid motifs recognized by the Cas protein which flank a mutant allele located between the target motifs.

[001054] In some embodiments, each of the one to two ribonucleic acids comprises guide RNAs that directs the Cas protein to and hybridizes to a target motif of the target polynucleotide sequence in a cell.

[001055] In some embodiments, one or two ribonucleic acids (*e.g.*, guide RNAs) are complementary to and/or hybridize to sequences on the same strand of a target polynucleotide sequence. In some embodiments, one or two ribonucleic acids (*e.g.*, guide RNAs) are complementary to and/or hybridize to sequences on the opposite strands of a target polynucleotide sequence. In some embodiments, the one or two ribonucleic acids (*e.g.*, guide RNAs) are not complementary to and/or do not hybridize to sequences on the opposite strands of a target polynucleotide sequence. In some embodiments, the one or two ribonucleic acids (*e.g.*, guide RNAs) are complementary to and/or hybridize to overlapping target motifs of a target polynucleotide sequence. In some embodiments, the one or two ribonucleic acids (*e.g.*, guide RNAs) are complementary to and/or hybridize to offset target motifs of a target polynucleotide sequence.

[001056] In some embodiments, nucleic acids encoding Cas protein and nucleic acids encoding the at least one to two ribonucleic acids are introduced into a cell via viral transduction (*e.g.*, lentiviral transduction). In some embodiments, the Cas protein is complexed with 1-2 ribonucleic acids. In some embodiments, the Cas protein is complexed with two ribonucleic

acids. In some embodiments, the Cas protein is complexed with one ribonucleic acid. In some embodiments, the Cas protein is encoded by a modified nucleic acid, as described herein (*e.g.*, a synthetic, modified mRNA).

[001057] Exemplary gRNA sequences useful for CRISPR/Cas-based targeting of genes described herein are provided in Table 15. The sequences can be found in WO2016183041 filed May 9, 2016, the disclosure including the Tables, Appendices, and Sequence Listing is incorporated herein by reference in its entirety.

Table 15. Exemplary gRNA sequences useful for targeting genes

Gene Name	SEQ ID NO:	WO2016183041
HLA-A	SEQ ID NOs: 2-1418	Table 8, Appendix 1
HLA-B	SEQ ID NOs: 1419-3277	Table 9, Appendix 2
HLA-C	SEQ ID NOS:3278-5183	Table 10, Appendix 3
RFX-ANK	SEQ ID NOs: 95636-102318	Table 11, Appendix 4
NFY-A	SEQ ID NOs: 102319-121796	Table 13, Appendix 6
RFX5	SEQ ID NOs: 85645-90115	Table 16, Appendix 9
RFX-AP	SEQ ID NOs: 90116-95635	Table 17, Appendix 10
NFY-B	SEQ ID NOs: 121797-135112	Table 20, Appendix 13
NFY-C	SEQ ID NOs: 135113-176601	Table 22, Appendix 15
IRF1	SEQ ID NOs: 176602-182813	Table 23, Appendix 16
TAP1	SEQ ID NOs: 182814-188371	Table 24, Appendix 17
CIITA	SEQ ID NOS:5184-36352	Table 12, Appendix 5
B2M	SEQ ID NOS:81240-85644	Table 15, Appendix 8
NLRC5	SEQ ID NOS:36353-81239	Table 14, Appendix 7
CD47	SEQ ID NOS:200784-231885	Table 29, Appendix 22
HLA-E	SEQ ID NOS:189859-193183	Table 19, Appendix 12
HLA-F	SEQ ID NOS:688808-699754	Table 45, Appendix 38
HLA-G	SEQ ID NOS:188372-189858	Table 18, Appendix 11
PD-L1	SEQ ID NOS:193184-200783	Table 21, Appendix 14
Gene Name	SEQ ID NO:	US20160348073
TRAC	SEQ ID NOS: 532-609 and 9102-9797	
TRB (also TCRB and TRBC)	SEQ ID NOS:610-765 and 9798-10532	

[001058] Other exemplary gRNA sequences useful for CRISPR/Cas-based targeting of genes described herein are provided in U.S. Provisional Patent Application Number 63/190,685, filed May 19, 2021, and in U.S. Provisional Patent Application No. 63/221,887, filed July 14, 2021,

the disclosures of which, including the Tables, Appendices, and Sequence Listings, are incorporated herein by reference in their entireties.

[001059] In some embodiments, the cells of the technology are made using Transcription Activator-Like Effector Nucleases (TALEN) methodologies.

[001060] By a "TALE-nuclease" (TALEN) is intended a fusion protein consisting of a nucleic acid-binding domain typically derived from a Transcription Activator Like Effector (TALE) and one nuclease catalytic domain to cleave a nucleic acid target sequence. The catalytic domain is preferably a nuclease domain and more preferably a domain having endonuclease activity, like for instance I-TevI, ColE7, NucA and Fok-I. In numerous embodiments, the TALE domain can be fused to a meganuclease like for instance I-CreI and I-OnuI or functional variant thereof. In a more preferred embodiment, said nuclease is a monomeric TALE-Nuclease. A monomeric TALE-Nuclease is a TALE-Nuclease that does not require dimerization for specific recognition and cleavage, such as the fusions of engineered TAL repeats with the catalytic domain of I-TevI described in WO2012138927. Transcription Activator like Effector (TALE) are proteins from the bacterial species Xanthomonas comprise a plurality of repeated sequences, each repeat comprising di-residues in position 12 and 13 (RVD) that are specific to each nucleotide base of the nucleic acid targeted sequence. Binding domains with similar modular base-per-base nucleic acid binding properties (MBBBD) can also be derived from new modular proteins recently discovered by the applicant in a different bacterial species. The new modular proteins have the advantage of displaying more sequence variability than TAL repeats. Preferably, RVDs associated with recognition of the different nucleotides are HD for recognizing C, NG for recognizing T, NI for recognizing A, NN for recognizing G or A, NS for recognizing A, C, G or T, HG for recognizing T, IG for recognizing T, NK for recognizing G, HA for recognizing C, ND for recognizing C, HI for recognizing C, HN for recognizing G, NA for recognizing G, SN for recognizing G or A and YG for recognizing T, TL for recognizing A, VT for recognizing A or G and SW for recognizing A. In another embodiment, critical amino acids 12 and 13 can be mutated towards other amino acid residues in order to modulate their specificity towards nucleotides A, T, C and G and in particular to enhance this specificity. TALEN kits are sold commercially.

[001061] In some embodiments, the cells are manipulated using zinc finger nuclease (ZFN). A "zinc finger binding protein" is a protein or polypeptide that binds DNA, RNA and/or protein,

preferably in a sequence-specific manner, as a result of stabilization of protein structure through coordination of a zinc ion. The term zinc finger binding protein is often abbreviated as zinc finger protein or ZFP. The individual DNA binding domains are typically referred to as "fingers." A ZFP has least one finger, typically two fingers, three fingers, or six fingers. Each finger binds from two to four base pairs of DNA, typically three or four base pairs of DNA. A ZFP binds to a nucleic acid sequence called a target site or target segment. Each finger typically comprises an approximately 30 amino acid, zinc-chelating, DNA-binding subdomain. Studies have demonstrated that a single zinc finger of this class consists of an alpha helix containing the two invariant histidine residues co-ordinated with zinc along with the two cysteine residues of a single beta turn (see, *e.g.*, Berg & Shi, Science 271:1081-1085 (1996)).

[001062] In some embodiments, the cells of the present disclosure are made using a homing endonuclease. Such homing endonucleases are well-known to the art (Stoddard 2005). Homing endonucleases recognize a DNA target sequence and generate a single- or double-strand break. Homing endonucleases are highly specific, recognizing DNA target sites ranging from 12 to 45 base pairs (bp) in length, usually ranging from 14 to 40 bp in length. The homing endonuclease according to the technology may for example correspond to a LAGLIDADG endonuclease, to a HNH endonuclease, or to a GIY-YIG endonuclease. Preferred homing endonuclease according to the present disclosure can be an I-CreI variant.

[001063] In some embodiments, the cells of the technology are made using a meganuclease. Meganucleases are by definition sequence-specific endonucleases recognizing large sequences (Chevalier, B. S. and B. L. Stoddard, Nucleic Acids Res., 2001, 29, 3757-3774). They can cleave unique sites in living cells, thereby enhancing gene targeting by 1000-fold or more in the vicinity of the cleavage site (Puchta *et al.*, Nucleic Acids Res., 1993, 21, 5034-5040; Rouet *et al.*, Mol. Cell. Biol., 1994, 14, 8096-8106; Choulika *et al.*, Mol. Cell. Biol., 1995, 15, 1968-1973; Puchta *et al.*, Proc. Natl. Acad. Sci. USA, 1996, 93, 5055-5060; Sargent *et al.*, Mol. Cell. Biol., 1997, 17, 267-77; Donoho *et al.*, Mol. Cell. Biol, 1998, 18, 4070-4078; Elliott *et al.*, Mol. Cell. Biol., 1998, 18, 93-101; Cohen-Tannoudji *et al.*, Mol. Cell. Biol., 1998, 18, 1444-1448).

[001064] In some embodiments, the cells of the technology are made using RNA silencing or RNA interference (RNAi) to knock down (*e.g.*, decrease, eliminate, or inhibit) the expression of a polypeptide such as a tolerogenic factor. Useful RNAi methods include those that utilize synthetic RNAi molecules, short interfering RNAs (siRNAs), PIWI-interacting NRAs (piRNAs),

short hairpin RNAs (shRNAs), microRNAs (miRNAs), and other transient knock down methods recognized by those skilled in the art. Reagents for RNAi including sequence specific shRNAs, siRNA, miRNAs and the like are commercially available. For instance, CIITA can be knocked down in a pluripotent stem cell by introducing a CIITA siRNA or transducing a CIITA shRNA-expressing virus into the cell. In some embodiments, RNA interference is employed to reduce or inhibit the expression of at least one selected from the group consisting of CIITA, B2M, NLRC5, TCR-alpha, and TCR-beta.

[001065] In some embodiments, the cells provided herein are genetically modified to reduce expression of one or more immune factors (including target polypeptides) to create immune-privileged or hypoimmunogenic cells. In certain embodiments, the cells (*e.g.*, stem cells, induced pluripotent stem cells, differentiated cells, hematopoietic stem cells, primary T cells and CAR-T cells) disclosed herein comprise one or more genetic modifications to reduce expression of one or more target polynucleotides. Non-limiting examples of such target polynucleotides and polypeptides include CIITA, B2M, NLRC5, CTLA-4, PD-1, HLA-A, HLA-BM, HLA-C, RFX-ANK, NFY-A, RFX5, RFX-AP, NFY-B, NFY-C, IRF1, and TAP1.

[001066] In some embodiments, the genetic modification occurs using a CRISPR/Cas system. By modulating (*e.g.*, reducing or deleting) expression of one or a plurality of the target polynucleotides, such cells exhibit decreased immune activation when engrafted into a recipient subject. In some embodiments, the cell is considered hypoimmunogenic, *e.g.*, in a recipient subject or patient upon administration.

A. Gene editing systems

[001067] In some embodiments, the methods for genetically modifying cells to knock out, knock down, or otherwise modify one or more genes comprise using a site-directed nuclease, including, for example, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), meganucleases, transposases, and clustered regularly interspaced short palindromic repeat (CRISPR)/Cas systems, as well as nickase systems, base editing systems, prime editing systems, and gene writing systems known in the art.

1. ZFNs

[001068] ZFNs are fusion proteins comprising an array of site-specific DNA binding domains adapted from zinc finger-containing transcription factors attached to the endonuclease domain of the bacterial FokI restriction enzyme. A ZFN may have one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9,

10, or more) of the DNA binding domains or zinc finger domains. See, *e.g.*, Carroll *et al.*, *Genetics Society of America* (2011) 188:773-782; Kim *et al.*, *Proc. Natl. Acad. Sci. USA* (1996) 93:1156-1160. Each zinc finger domain is a small protein structural motif stabilized by one or more zinc ions and usually recognizes a 3- to 4-bp DNA sequence. Tandem domains can thus potentially bind to an extended nucleotide sequence that is unique within a cell's genome.

[001069] Various zinc fingers of known specificity can be combined to produce multi-finger polypeptides which recognize about 6, 9, 12, 15, or 18-bp sequences. Various selection and modular assembly techniques are available to generate zinc fingers (and combinations thereof) recognizing specific sequences, including phage display, yeast one-hybrid systems, bacterial one-hybrid and two-hybrid systems, and mammalian cells. Zinc fingers can be engineered to bind a predetermined nucleic acid sequence. Criteria to engineer a zinc finger to bind to a predetermined nucleic acid sequence are known in the art. See, *e.g.*, Sera *et al.*, *Biochemistry* (2002) 41:7074-7081; Liu *et al.*, *Bioinformatics* (2008) 24:1850-1857.

[001070] ZFNs containing FokI nuclease domains or other dimeric nuclease domains function as a dimer. Thus, a pair of ZFNs are required to target non-palindromic DNA sites. The two individual ZFNs must bind opposite strands of the DNA with their nucleases properly spaced apart. See Bitinaite *et al.*, *Proc. Natl. Acad. Sci. USA* (1998) 95:10570-10575. To cleave a specific site in the genome, a pair of ZFNs are designed to recognize two sequences flanking the site, one on the forward strand and the other on the reverse strand. Upon binding of the ZFNs on either side of the site, the nuclease domains dimerize and cleave the DNA at the site, generating a DSB with 5′ overhangs. HDR can then be utilized to introduce a specific mutation, with the help of a repair template containing the desired mutation flanked by homology arms. The repair template is usually an exogenous double-stranded DNA vector introduced to the cell. See Miller *et al.*, *Nat. Biotechnol.* (2011) 29:143-148; Hockemeyer *et al.*, *Nat. Biotechnol.* (2011) 29:731-734.

2. TALENs

[001071] TALENs are another example of an artificial nuclease which can be used to edit a target gene. TALENs are derived from DNA binding domains termed TALE repeats, which usually comprise tandem arrays with 10 to 30 repeats that bind and recognize extended DNA sequences. Each repeat is 33 to 35 amino acids in length, with two adjacent amino acids (termed

the repeat-variable di-residue, or RVD) conferring specificity for one of the four DNA base pairs. Thus, there is a one-to-one correspondence between the repeats and the base pairs in the target DNA sequences.

[001072] TALENs are produced artificially by fusing one or more TALE DNA binding domains (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) to a nuclease domain, for example, a FokI endonuclease domain. See Zhang, *Nature Biotech.* (2011) 29:149-153. Several mutations to FokI have been made for its use in TALENs; these, for example, improve cleavage specificity or activity. See Cermak *et al.*, *Nucl. Acids Res.* (2011) 39:e82; Miller *et al.*, *Nature Biotech.* (2011) 29:143-148; Hockemeyer *et al.*, *Nature Biotech.* (2011) 29:731-734; Wood *et al.*, *Science* (2011) 333:307; Doyon *et al.*, *Nature Methods* (2010) 8:74-79; Szczepek *et al.*, *Nature Biotech* (2007) 25:786-793; Guo *et al.*, *J. Mol. Biol.* (2010) 200:96. The FokI domain functions as a dimer, requiring two constructs with unique DNA binding domains for sites in the target genome with proper orientation and spacing. Both the number of amino acid residues between the TALE DNA binding domain and the FokI nuclease domain and the number of bases between the two individual TALEN binding sites appear to be important parameters for achieving high levels of activity. Miller *et al.*, *Nature Biotech.* (2011) 29:143-148.

[001073] By combining engineered TALE repeats with a nuclease domain, a site-specific nuclease can be produced specific to any desired DNA sequence. Similar to ZFNs, TALENs can be introduced into a cell to generate DSBs at a desired target site in the genome, and so can be used to knock out genes or knock in mutations in similar, HDR-mediated pathways. See Boch, *Nature Biotech.* (2011) 29:135-136; Boch *et al.*, *Science* (2009) 326:1509-1512; Moscou *et al.*, *Science* (2009) 326:3501.

3. Meganucleases

[001074] Meganucleases are enzymes in the endonuclease family which are characterized by their capacity to recognize and cut large DNA sequences (from 14 to 40 base pairs). Meganucleases are grouped into families based on their structural motifs which affect nuclease activity and/or DNA recognition. The most widespread and best known meganucleases are the proteins in the LAGLIDADG family, which owe their name to a conserved amino acid sequence. See Chevalier *et al.*, *Nucleic Acids Res.* (2001) 29(18): 3757-3774. On the other hand, the GIY-YIG family members have a GIY-YIG module, which is 70-100 residues long and includes four

or five conserved sequence motifs with four invariant residues, two of which are required for activity. See Van Roey *et al.*, *Nature Struct. Biol.* (2002) 9:806-811. The His-Cys family meganucleases are characterized by a highly conserved series of histidines and cysteines over a region encompassing several hundred amino acid residues. See Chevalier *et al.*, *Nucleic Acids Res.* (2001) 29(18):3757-3774. Members of the NHN family are defined by motifs containing two pairs of conserved histidines surrounded by asparagine residues. See Chevalier *et al.*, *Nucleic Acids Res.* (2001) 29(18):3757-3774.

[001075] Because the chance of identifying a natural meganuclease for a particular target DNA sequence is low due to the high specificity requirement, various methods including mutagenesis and high throughput screening methods have been used to create meganuclease variants that recognize unique sequences. Strategies for engineering a meganuclease with altered DNA-binding specificity, *e.g.*, to bind to a predetermined nucleic acid sequence are known in the art. See, *e.g.*, Chevalier *et al.*, *Mol. Cell.* (2002) 10:895-905; Epinat *et al.*, *Nucleic Acids Res* (2003) 31:2952-2962; Silva *et al.*, *J Mol. Biol.* (2006) 361:744-754; Seligman *et al.*, *Nucleic Acids Res* (2002) 30:3870-3879; Sussman *et al.*, *J Mol Biol* (2004) 342:31-41; Doyon *et al.*, *J Am Chem Soc* (2006) 128:2477-2484; Chen *et al.*, *Protein Eng Des Sel* (2009) 22:249-256; Arnould *et al.*, *J Mol Biol.* (2006) 355:443-458; Smith *et al.*, *Nucleic Acids Res.* (2006) 363(2):283-294.

[001076] Like ZFNs and TALENs, Meganucleases can create DSBs in the genomic DNA, which can create a frame-shift mutation if improperly repaired, *e.g.*, via NHEJ, leading to a decrease in the expression of a target gene in a cell. Alternatively, foreign DNA can be introduced into the cell along with the meganuclease. Depending on the sequences of the foreign DNA and chromosomal sequence, this process can be used to modify the target gene. See Silva *et al.*, *Current Gene Therapy* (2011) 11:11-27.

4. Transposases

[001077] Transposases are enzymes that bind to the end of a transposon and catalyze its movement to another part of the genome by a cut and paste mechanism or a replicative transposition mechanism. By linking transposases to other systems such as the CRISPER/Cas system, new gene editing tools can be developed to enable site specific insertions or manipulations of the genomic DNA. There are two known DNA integration methods using transposons which use a catalytically inactive Cas effector protein and Tn7-like transposons.

The transposase-dependent DNA integration does not provoke DSBs in the genome, which may guarantee safer and more specific DNA integration.

5. CRISPR/Cas systems

[001078] The CRISPR system was originally discovered in prokaryotic organisms (*e.g.*, bacteria and archaea) as a system involved in defense against invading phages and plasmids that provides a form of acquired immunity. Now it has been adapted and used as a popular gene editing tool in research and clinical applications.

[001079] CRISPR/Cas systems generally comprise at least two components: one or more guide RNAs (gRNAs) and a Cas protein. The Cas protein is a nuclease that introduces a DSB into the target site. CRISPR-Cas systems fall into two major classes: class 1 systems use a complex of multiple Cas proteins to degrade nucleic acids; class 2 systems use a single large Cas protein for the same purpose. Class 1 is divided into types I, III, and IV; class 2 is divided into types II, V, and VI. Different Cas proteins adapted for gene editing applications include, but are not limited to, 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. The most widely used Cas9 is described herein as illustrative. These Cas proteins may be originated from different source species. For example, Cas9 can be derived from *S. pyogenes* or *S. aureus*.

[001080] In the original microbial genome, the type II CRISPR system incorporates sequences from invading DNA between CRISPR repeat sequences encoded as arrays within the host genome. Transcripts from the CRISPR repeat arrays are processed into CRISPR RNAs (crRNAs) each harboring a variable sequence transcribed from the invading DNA, known as the "protospacer" sequence, as well as part of the CRISPR repeat. Each crRNA hybridizes with a second transactivating CRISPR RNA (tracrRNA), and these two RNAs form a complex with the Cas9 nuclease. The protospacer-encoded portion of the crRNA directs the Cas9 complex to cleave complementary target DNA sequences, provided that they are adjacent to short sequences known as "protospacer adjacent motifs" (PAMs).

[001081] Since its discovery, the CRISPR system has been adapted for inducing sequence specific DSBs and targeted genome editing in a wide range of cells and organisms spanning from

bacteria to eukaryotic cells including human cells. In its use in gene editing applications, artificially designed, synthetic gRNAs have replaced the original crRNA:tracrRNA complex. For example, the gRNAs can be single guide RNAs (sgRNAs) composed of a crRNA, a tetraloop, and a tracrRNA. The crRNA usually comprises a complementary region (also called a spacer, usually about 20 nucleotides in length) that is user-designed to recognize a target DNA of interest. The tracrRNA sequence comprises a scaffold region for Cas nuclease binding. The crRNA sequence and the tracrRNA sequence are linked by the tetraloop and each have a short repeat sequence for hybridization with each other, thus generating a chimeric sgRNA. One can change the genomic target of the Cas nuclease by simply changing the spacer or complementary region sequence present in the gRNA. The complementary region will direct the Cas nuclease to the target DNA site through standard RNA-DNA complementary base pairing rules.

[001082] In order for the Cas nuclease to function, there must be a PAM immediately downstream of the target sequence in the genomic DNA. Recognition of the PAM by the Cas protein is thought to destabilize the adjacent genomic sequence, allowing interrogation of the sequence by the gRNA and resulting in gRNA-DNA pairing when a matching sequence is present. The specific sequence of PAM varies depending on the species of the Cas gene. For example, the most commonly used Cas9 nuclease derived from *S. pyogenes* recognizes a PAM sequence of 5'-NGG-3' or, at less efficient rates, 5'-NAG-3', where "N" can be any nucleotide. Other Cas nuclease variants with alternative PAMs have also been characterized and successfully used for genome editing, which are summarized in **Table 19** below.

Table 19. Exemplary Cas nuclease variants and their PAM sequences

CRISPR Nuclease	Source Organism	PAM Sequence (5'→3')
SpCas9	*Streptococcus pyogenes*	NGG or NAG
SaCas9	*Staphylococcus aureus*	NGRRT or NGRRN
NmeCas9	*Neisseria meningitidis*	NNNNGATT
CjCas9	*Campylobacter jejuni*	NNNNRYAC
StCas9	*Streptococcus thermophilus*	NNAGAAW
TdCas9	*Treponema denticola*	NAAAAC
LbCas12a (Cpf1)	*Lachnospiraceae bacterium*	TTTV
AsCas12a (Cpf1)	*Acidaminococcus sp.*	TTTV

AacCas12b	*Alicyclobacillus acidiphilus*	TTN
BhCas12b v4	*Bacillus hisashii*	ATTN, TTTN, or GTTN

R = A or G; Y = C or T; W = A or T; V = A or C or G; N = any base

In some embodiments, Cas nucleases may comprise one or more mutations to alter their activity, specificity, recognition, and/or other characteristics. For example, the Cas nuclease may have one or more mutations that alter its fidelity to mitigate off-target effects (*e.g.*, eSpCas9, SpCas9-HF1, HypaSpCas9, HeFSpCas9, and evoSpCas9 high-fidelity variants of SpCas9). For another example the Cas nuclease may have one or more mutations that alter its PAM specificity.

6. Nickases

[001083] Nuclease domains of the Cas, in particular the Cas9, nuclease can be mutated independently to generate enzymes referered to as DNA "nickases". Nickases are capable of introducing a single-strand cut with the same specificity as a regular CRISPR/Cas nucleas system, including for example CRISPR/Cas9. Nickases can be employed to generate double-strand breaks which can find use in gene editing systems (Mali *et al.*, *Nat Biotech*, 31(9):833-838 (2013); Mali *et al. Nature Methods,* 10:957–963 (2013); Mali *et al.*, *Science*, 339(6121):823-826 (2013)). In some instances, when two Cas nickases are used, long overhangs are produced on each of the cleaved ends instead of blunt ends which allows for additional control over precise gene integration and insertion (Mali *et al.*, *Nat Biotech*, 31(9):833-838 (2013); Mali *et al. Nature Methods,* 10:957–963 (2013); Mali *et al.*, *Science*, 339(6121):823-826 (2013)). As both nicking Cas enzymes must effectively nick their target DNA, paired nickases can have lower off-target effects compared to the double-strand-cleaving Cas-based systems (Ran *et al.*, *Cell*, 155(2):479-480(2013); Mali *et al.*, *Nat Biotech*, 31(9):833-838 (2013); Mali *et al. Nature Methods,* 10:957–963 (2013); Mali *et al.*, *Science*, 339(6121):823-826 (2013)).

[001084] Prime editing is a versatile and precise genome editing method that directly writes new genetic information into a specified DNA site using a catalytically impaired Cas9 endonuclease fused to an engineered reverse transcriptase, programmed with a prime editing guide RNA (pegRNA) that both specifies the target site and encodes the desired edit. See, e.g., Anzalone et al., Nature, 576:149-157 (2019); WO2021072328; WO2022067130, all of which are incorporated herein by reference in their entirety. Cas9 and a reverse transcriptase can also be used to insert an integrase site into the genome for insertion of a nucleic acid of interest in a

process called Programmable Addition via Site-specific Targeting Elements (PASTE) editing. See, e.g., Ioannidi et al., bioRxiv 2021.11.01.466786; doi.org/10.1101/2021.11.01.466786.

CC. Methods of Recombinant Expression of Tolerogenic Factors and/or Chimeric Antigen Receptors

[001085] For all of these technologies, well-known recombinant techniques are used, to generate recombinant nucleic acids as outlined herein. In certain embodiments, the recombinant nucleic acids encoding a tolerogenic factor or a chimeric antigen receptor may be operably linked to one or more regulatory nucleotide sequences in an expression construct. Regulatory nucleotide sequences will generally be appropriate for the host cell and recipient subject to be treated. Numerous types of appropriate expression vectors and suitable regulatory sequences are known in the art for a variety of host cells. Typically, the one or more regulatory nucleotide sequences may include, but are not limited to, promoter sequences, leader or signal sequences, ribosomal binding sites, transcriptional start and termination sequences, translational start and termination sequences, and enhancer or activator sequences. Constitutive or inducible promoters as known in the art are also contemplated. The promoters may be either naturally occurring promoters, hybrid promoters that combine elements of more than one promoter, or synthetic promoters. An expression construct may be present in a cell on an episome, such as a plasmid, or the expression construct may be inserted in a chromosome such as in a gene locus. In some embodiment, the expression vector includes a selectable marker gene to allow the selection of transformed host cells. Some embodiments, include an expression vector comprising a nucleotide sequence encoding a variant polypeptide operably linked to at least one regulatory sequence. Regulatory sequence for use herein include promoters, enhancers, and other expression control elements. In some embodiments, an expression vector is designed for the choice of the host cell to be transformed, the particular variant polypeptide desired to be expressed, the vector's copy number, the ability to control that copy number, and/or the expression of any other protein encoded by the vector, such as antibiotic markers.

[001086] Examples of suitable mammalian promoters include, for example, promoters from the following genes: elongation factor 1 alpha (EF1α) promoter, CAG promoter, ubiquitin/S27a promoter of the hamster (WO 97/15664), Simian vacuolating virus 40 (SV40) early promoter, adenovirus major late promoter, mouse metallothionein-I promoter, the long terminal repeat region of Rous Sarcoma Virus (RSV), mouse mammary tumor virus promoter (MMTV),

Moloney murine leukemia virus Long Terminal repeat region, and the early promoter of human Cytomegalovirus (CMV). Examples of other heterologous mammalian promoters are the actin, immunoglobulin or heat shock promoter(s). In additional embodiments, promoters for use in mammalian host cells can be obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 Jul. 1989), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40). In further embodiments, heterologous mammalian promoters are used. Examples include the actin promoter, an immunoglobulin promoter, and heat-shock promoters. The early and late promoters of SV40 are conveniently obtained as an SV40 restriction fragment which also contains the SV40 viral origin of replication (Fiers *et al.*, Nature 273: 113-120 (1978)). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII restriction enzyme fragment (Greenaway *et al.*, Gene 18: 355-360 (1982)). The foregoing references are incorporated by reference in their entirety.

[001087] In some embodiments, the expression vector is a bicistronic or multicistronic expression vector. Bicistronic or multicistronic expression vectors may include (1) multiple promoters fused to each of the open reading frames; (2) insertion of splicing signals between genes; (3) fusion of genes whose expressions are driven by a single promoter; and (4) insertion of proteolytic cleavage sites between genes (self-cleavage peptide) or insertion of internal ribosomal entry sites (IRESs) between genes.

[001088] The process of introducing the polynucleotides described herein into cells can be achieved by any suitable technique. Suitable techniques include calcium phosphate or lipid-mediated transfection, electroporation, fusogens, and transduction or infection using a viral vector. In some embodiments, the polynucleotides are introduced into a cell via viral transduction (*e.g.*, AAV transduction, lentiviral transduction) or otherwise delivered on a viral vector (*e.g.*, fusogen-mediated delivery). In some embodiments, the polynucleotides are introduced into a cell via a fusogen-mediated delivery or a transposase system selected from the group consisting of conditional or inducible transposases, conditional or inducible PiggyBac transposons, conditional or inducible Sleeping Beauty (SB11) transposons, conditional or inducible Mos1 transposons, and conditional or inducible Tol2 transposons.

[001089] In some embodiments, the cells provided herein are genetically modified to include one or more exogenous polynucleotides inserted into one or more genomic loci of the

hypoimmunogenic cell. In some embodiments, the exogenous polynucleotide encodes a protein of interest, *e.g.*, a chimeric antigen receptor. Any suitable method can be used to insert the exogenous polynucleotide into the genomic locus of the hypoimmunogenic cell including the gene editing methods described herein (*e.g.*, a CRISPR/Cas system). In some embodiments, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction, for example, with a vector. In some embodiments, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the exogenous polynucleotide. In some embodiments, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the exogenous polynucleotide. In some embodiments, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction. In some embodiments, the exogenous polynucleotide is inserted into at least one allele of the cell using a lentivirus based viral vector.

[001090] Unlike certain methods of introducing the polynucleotides described herein into cells which generally involve activating cells, such as activating T cells (*e.g.*, $CD8^+$ T cells), suitable techniques can be utilized to introduce polynucleotides into non-activated T cells. Suitable techniques include, but are not limited to, activation of T cells, such as $CD8^+$ T cells, with one or more antibodies which bind to CD3, CD8, and/or CD28, or fragments or portions thereof (*e.g.*, scFv and VHH) that may or may not be bound to beads. Surprisingly, fusogen-mediated introduction of polynucleotides into T cells is performed in non-activated T cells (*e.g.*, $CD8^+$ T cells) that have not been previously contacted with one or more activating antibodies or fragments or portions thereof (*e.g.*, CD3, CD8, and/or CD28). In some embodiments, fusogen-mediated introduction of polynucleotides into T cells is performed in vivo (*e.g.*, after the T cells have been administered to a subject). In other embodiments, fusogen-mediated introduction of polynucleotides into T cells is performed in vitro (*e.g.*, before the T cells are been administered to a subject).

[001091] Provided herein are non-activated T cells comprising regulatable reduced expression of HLA-A, HLA-B, HLA-C, CIITA, TCR-alpha, and/or TCR-beta relative to a wild-type T cell, wherein the non-activated T cell further comprises a first exogenous polynucleotide encoding a regulatable one or more tolerogenic factors.

[001092] In some embodiments, the non-activated T cell has not been treated with an anti-CD3 antibody, an anti-CD28 antibody, a T cell activating cytokine, or a soluble T cell costimulatory

molecule. In some embodiments, the non-activated T cell does not express activation markers. In some embodiments, the non-activated T cell expresses CD3 and CD28, and wherein the CD3 and/or CD28 are inactive.

[001093] In some embodiments, the anti-CD3 antibody is OKT3. In some embodiments, the anti-CD28 antibody is CD28.2. In some embodiments, the T cell activating cytokine is selected from the group of T cell activating cytokines consisting of IL-2, IL-7, IL-15, and IL-21. In some embodiments, the soluble T cell costimulatory molecule is selected from the group of soluble T cell costimulatory molecules consisting of an anti-CD28 antibody, an anti-CD80 antibody, an anti-CD86 antibody, an anti-CD137L antibody, and an anti-ICOS-L antibody.

[001094] In some embodiments, the non-activated T cell is a primary T cell. In other embodiments, the non-activated T cell is differentiated from the hypoimmunogenic cells of the present disclosure. In some embodiments, the T cell is a $CD8^+$ T cell.

[001095] In some embodiments, the non-activated T cell further comprises a second exogenous polynucleotide encoding a regulatable chimeric antigen receptor (CAR). In some embodiments, the CAR is selected from the group consisting of a CD19-specific CAR and a CD22-specific CAR.

[001096] In some embodiments, the first and/or second exogenous polynucleotide is carried by a viral vector, including a lentiviral vector. In some embodiments, the first and/or second exogenous polynucleotide is carried by a lentiviral vector that comprises a CD8 binding agent. In some embodiments, the first and/or second exogenous polynucleotide is introduced into the cells using fusogen-mediated delivery or a transposase system selected from the group consisting of conditional or inducible transposases, conditional or inducible PiggyBac transposons, conditional or inducible Sleeping Beauty (SB11) transposons, conditional or inducible Mos1 transposons, and conditional or inducible Tol2 transposons.

[001097] In some embodiments, the non-activated T cell further comprises a second exogenous polynucleotide encoding CD47. In some embodiments, the first and/or second exogenous polynucleotides are inserted into a specific locus of at least one allele of the T cell. In some embodiments, the specific locus is selected from the group consisting of a safe harbor locus or a target locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus, and a *TRB* locus. In some embodiments, the second exogenous polynucleotide encoding CD47 is inserted into the specific locus selected from the group consisting of a safe harbor or target locus, a target locus, a B2M

locus, a CIITA locus, a TRAC locus and a TRB locus. In some embodiments, the first exogenous polynucleotide encoding the CAR is inserted into the specific locus selected from the group consisting of a safe harbor locus, a target locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus and a *TRB* locus. In some embodiments, the first exogenous polynucleotide encoding CD47 is inserted into the specific locus selected from the group consisting of a safe harbor locus, a target locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus and a *TRB* locus. In some embodiments, the second exogenous polynucleotide encoding the CAR and the first exogenous polynucleotide encoding CD47 are inserted into different loci. In some embodiments, the second exogenous polynucleotide encoding the CAR and the first exogenous polynucleotide encoding one or more tolerogenic factors are inserted into the same locus. In some embodiments, the second exogenous polynucleotide encoding the CAR and the first exogenous polynucleotide encoding one or more tolerogenic factors are inserted into the *B2M* locus. In some embodiments, the second exogenous polynucleotide encoding the CAR and the first exogenous polynucleotide encoding one or more tolerogenic factors are inserted into the *CIITA* locus. In some embodiments, the second exogenous polynucleotide encoding the CAR and the first exogenous polynucleotide encoding one or more tolerogenic factors are inserted into the *TRAC* locus. In some embodiments, the second exogenous polynucleotide encoding the CAR and the first exogenous polynucleotide encoding one or more tolerogenic factors are inserted into the *TRB* locus. In some embodiments, the second exogenous polynucleotide encoding the CAR and the first exogenous polynucleotide encoding one or more tolerogenic factors are inserted into the safe harbor or target locus. In some embodiments, the safe harbor or target locus is selected from the group consisting of a CCR5 gene locus, a CXCR4 gene locus, a PPP1R12C gene locus, an albumin gene locus, a SHS231 gene locus, a CLYBL gene locus, a Rosa gene locus, an F3 (CD142) gene locus, a MICA gene locus, a MICB gene locus, a LRP1 (CD91) gene locus, a HMGB1 gene locus, an ABO gene locus, an RHD gene locus, a FUT1 locus, and a KDM5D gene locus.

[001098] In some embodiments, the non-activated T cell does not express HLA-A, HLA-B, and/or HLA-C antigens. In some embodiments, the non-activated T cell does not express B2M. In some embodiments, the non-activated T cell does not express HLA-DP, HLA-DQ, and/or HLA-DR antigens. In some embodiments, the non-activated T cell does not express CIITA. In some embodiments, the non-activated T cell does not express TCR-alpha. In some embodiments,

the non-activated T cell does not express TCR-beta. In some embodiments, the non-activated T cell does not express TCR-alpha and TCR-beta.

[001099] In some embodiments, the non-activated T cell is a *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$ cell comprising the first regulatable gene encoding one or more tolerogenic factors and/or the second regulatable gene encoding CAR inserted into the *TRAC* locus. In some embodiments, the first and/or second exogenous polynucleotides are inserted into at least one allele of the cell using viral transduction. In some embodiments, the first and/or second exogenous polynucleotides are inserted into at least one allele of the cell using a lentivirus based viral vector. In some embodiments, the non-activated T cell is a *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$ cell comprising the first regulatable gene encoding one or more tolerogenic factors and the second regulatable gene encoding CAR inserted into the *TRAC* locus. In some embodiments, the non-activated T cell is a *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$ cell comprising the first regulatable gene encoding one or more tolerogenic factors and/or the second regulatable gene encoding CAR inserted into the *TRB* locus. In some embodiments, the non-activated T cell is a *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$ cell comprising the first regulatable gene encoding one or more tolerogenic factors and the second regulatable gene encoding CAR inserted into the *TRB* locus. In some embodiments, the non-activated T cell is a *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$ cell comprising the first regulatable gene encoding one or more tolerogenic factors and/or the second regulatable gene encoding CAR inserted into the *B2M* locus. In some embodiments, the non-activated T cell is a *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$ cell comprising the first regulatable gene encoding one or more tolerogenic factors and the second regulatable gene encoding CAR inserted into a *B2M* locus. In some embodiments, the non-activated T cell is a *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$ cell comprising the first regulatable gene encoding one or more tolerogenic factors and/or the second regulatable gene encoding CAR inserted into the *CIITA* locus. In some embodiments, the non-activated T cell is a *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRAC*$^{indel/indel}$ cell comprising the first regulatable gene encoding one or more tolerogenic factors and the second regulatable gene encoding CAR inserted into a *CIITA* locus.

[001100] In some embodiments, the non-activated T cell is a *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRB*$^{indel/indel}$ cell comprising the first regulatable gene encoding one or more tolerogenic factors and/or the second regulatable gene encoding CAR inserted into the *TRAC* locus. In some

embodiments, the non-activated T cell is a *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRB*$^{indel/indel}$ cell comprising the first regulatable gene encoding one or more tolerogenic factors and the second regulatable gene encoding CAR inserted into the *TRAC* locus. In some embodiments, the non-activated T cell is a *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRB*$^{indel/indel}$ cell comprising the first regulatable gene encoding one or more tolerogenic factors and/or the second regulatable gene encoding CAR inserted into the *TRB* locus. In some embodiments, the non-activated T cell is a *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRB*$^{indel/indel}$ cell comprising the first regulatable gene encoding one or more tolerogenic factors and the second regulatable gene encoding CAR inserted into the *TRB* locus. In some embodiments, the non-activated T cell is a *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRB*$^{indel/indel}$ cell comprising the first regulatable gene encoding one or more tolerogenic factors and/or the second regulatable gene encoding CAR inserted into the *B2M* locus. In some embodiments, the non-activated T cell is a *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRB*$^{indel/indel}$ cell comprising the first regulatable gene encoding one or more tolerogenic factors and the second regulatable gene encoding CAR inserted into a *B2M* locus. In some embodiments, the non-activated T cell is a *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRB*$^{indel/indel}$ cell comprising the first regulatable gene encoding one or more tolerogenic factors and/or the second regulatable gene encoding CAR inserted into the *CIITA* locus. In some embodiments, the non-activated T cell is a *B2M*$^{indel/indel}$, *CIITA*$^{indel/indel}$, *TRB*$^{indel/indel}$ cell comprising the first regulatable gene encoding one or more tolerogenic factors and the second regulatable gene encoding CAR inserted into a *CIITA* locus.

[001101] Provided herein are engineered T cells comprising regulatable reduced expression of HLA-A, HLA-B, HLA-C, CIITA, TCR-alpha, and/or TCR-beta relative to a wild-type T cell, wherein the engineered T cell further comprises a first exogenous polynucleotide encoding a regulatable one or more tolerogenic factors carried by a lentiviral vector that comprises a CD8 binding agent.

[001102] In some embodiments, the engineered T cell is a primary T cell. In other embodiments, the engineered T cell is differentiated from the hypoimmunogenic cell of the present disclosure. In some embodiments, the T cell is a $CD8^+$ T cell. In some embodiments, the T cell is a $CD4^+$ T cell.

[001103] In some embodiments, the engineered T cell does not express activation markers. In some embodiments, the engineered T cell expresses CD3 and CD28, and wherein the CD3 and/or CD28 are inactive.

[001104] In some embodiments, the engineered T cell has not been treated with an anti-CD3 antibody, an anti-CD28 antibody, a T cell activating cytokine, or a soluble T cell costimulatory molecule. In some embodiments, the anti-CD3 antibody is OKT3, wherein the anti-CD28 antibody is CD28.2, wherein the T cell activating cytokine is selected from the group of T cell activating cytokines consisting of IL-2, IL-7, IL-15, and IL-21, and wherein soluble T cell costimulatory molecule is selected from the group of soluble T cell costimulatory molecules consisting of an anti-CD28 antibody, an anti-CD80 antibody, an anti-CD86 antibody, an anti-CD137L antibody, and an anti-ICOS-L antibody. In some embodiments, the engineered T cell has not been treated with one or more T cell activating cytokines selected from the group consisting of IL-2, IL-7, IL-15, and IL-21. In some instances, the cytokine is IL-2. In some embodiments, the one or more cytokines is IL-2 and another selected from the group consisting of IL-7, IL-15, and IL-21.

[001105] In some embodiments, the non-activated T cell further comprises a second exogenous polynucleotide encoding a regulatable chimeric antigen receptor (CAR). In some embodiments, the CAR is selected from the group consisting of a CD19-specific CAR and a CD22-specific CAR.

[001106] In some embodiments, the engineered T cell further comprises a second exogenous polynucleotide encoding a regulatable chimeric antigen receptor (CAR).. In some embodiments, the first and/or second exogenous polynucleotides are inserted into a specific locus of at least one allele of the T cell. In some embodiments, the specific locus is selected from the group consisting of a safe harbor locus, a target locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus, and a *TRB* locus. In some embodiments, the first regulatable gene encoding one or more tolerogenic factors is inserted into the specific locus selected from the group consisting of a safe harbor locus, a target locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus and a *TRB* locus. In some embodiments, the second regulatable gene encoding the CAR is inserted into the specific locus selected from the group consisting of a safe harbor locus, a target locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus and a *TRB* locus. In some embodiments, the first regulatable gene encoding one or more tolerogenic factors and the second regulatable gene encoding the CAR are inserted into different loci. In some embodiments, the first regulatable gene encoding one or more tolerogenic factors and the second regulatable gene encoding the CAR are inserted into the same locus. In some embodiments, the first regulatable gene encoding one or more tolerogenic factors and the second

regulatable gene encoding the CAR are inserted into the *B2M* locus, the *CIITA* locus, the *TRAC* locus, the *TRB* locus, or the safe harbor or target locus. In some embodiments, the safe harbor or target locus is selected from the group consisting of a CCR5 gene locus, a CXCR4 gene locus, a PPP1R12C gene locus, an albumin gene locus, a SHS231 gene locus, a CLYBL gene locus, a Rosa gene locus, an F3 (CD142) gene locus, a MICA gene locus, a MICB gene locus, a LRP1 (CD91) gene locus, a HMGB1 gene locus, an ABO gene locus, an RHD gene locus, a FUT1 locus, and a KDM5D gene locus.

[001107] In some embodiments, the CAR is selected from the group consisting of a CD19-specific CAR and a CD22-specific CAR. In some embodiments, the CAR is a CD19-specific CAR. In some embodiments, the CAR is a CD22-specific CAR. In some embodiments, the CAR comprises an antigen binding domain that binds to any one selected from the group consisting of CD19, CD22, CD38, CD123, CD138, and BCMA.

[001108] In some embodiments, the engineered T cell does not express HLA-A, HLA-B, and/or HLA-C antigens, wherein the engineered T cell does not express B2M, wherein the engineered T cell does not express HLA-DP, HLA-DQ, and/or HLA-DR antigens, wherein the engineered T cell does not express CIITA, and/or wherein the engineered T cell does not express TCR-alpha and TCR-beta.

[001109] In some embodiments, the engineered T cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRAC^{indel/indel}$ cell comprising the first regulatable gene encoding one or more tolerogenic factors and/or the second regulatable gene encoding CAR inserted into the *TRAC* locus, into the *TRB* locus, into the *B2M* locus, or into the *CIITA* locus. In some embodiments, the engineered T cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRB^{indel/indel}$ cell comprising the first regulatable gene encoding one or more tolerogenic factors and/or the second regulatable gene encoding CAR inserted into the *TRAC* locus, into the *TRB* locus, into the *B2M* locus, or into the *CIITA* locus.

[001110] In some embodiments, the non-activated T cell and/or the engineered T cell of the present disclosure are in a subject. In other embodiments, the non-activated T cell and/or the engineered T cell of the present disclosure are in vitro.

[001111] In some embodiments, the non-activated T cell and/or the engineered T cell of the present disclosure express a CD8 binding agent. In some embodiments, the CD8 binding agent is an anti-CD8 antibody. In some embodiments, the anti-CD8 antibody is selected from the group consisting of a mouse anti-CD8 antibody, a rabbit anti-CD8 antibody, a human anti-CD8

antibody, a humanized anti-CD8 antibody, a camelid (*e.g.*, llama, alpaca, camel) anti-CD8 antibody, and a fragment thereof. In some embodiments, the fragment thereof is an scFv or a VHH. In some embodiments, the CD8 binding agent binds to a CD8 alpha chain and/or a CD8 beta chain.

[001112] In some embodiments, the CD8 binding agent is fused to a transmembrane domain incorporated in the viral envelope. In some embodiments, the lentivirus vector is pseudotyped with a viral fusion protein. In some embodiments, the viral fusion protein comprises one or more modifications to reduce binding to its native receptor.

[001113] In some embodiments, the viral fusion protein is fused to the CD8 binding agent. In some embodiments, the viral fusion protein comprises Nipah virus F glycoprotein and Nipah virus G glycoprotein fused to the CD8 binding agent. In some embodiments, the lentivirus vector does not comprise a T cell activating molecule or a T cell costimulatory molecule. In some embodiments, the lentivirus vector encodes the first exogenous polynucleotide and/or the second exogenous polynucleotide.

[001114] In some embodiments, following transfer into a first subject, the non-activated T cell or the engineered T cell exhibits one or more responses selected from the group consisting of (a) a T cell response, (b) an NK cell response, and (c) a macrophage response, that are reduced as compared to a wild-type cell following transfer into a second subject. In some embodiments, the first subject and the second subject are different subjects. In some embodiments, the macrophage response is engulfment.

[001115] In some embodiments, following transfer into a subject, the non-activated T cell or the engineered T cell exhibits one or more selected from the group consisting of (a) reduced TH1 activation in the subject, (b) reduced NK cell killing in the subject, and (c) reduced killing by whole PBMCs in the subject, as compared to a wild-type cell following transfer into the subject.

[001116] In some embodiments, following transfer into a subject, the non-activated T cell or the engineered T cell elicits one or more selected from the group consisting of (a) reduced donor specific antibodies in the subject, (b) reduced IgM or IgG antibodies in the subject, and (c) reduced complement-dependent cytotoxicity (CDC) in a subject, as compared to a wild-type cell following transfer into the subject.

[001117] In some embodiments, the non-activated T cell or the engineered T cell is transduced with a lentivirus vector comprising a CD8 binding agent within the subject. In some embodiments, the lentivirus vector carries a gene encoding the CAR and/or CD47.

[001118] In some embodiments, following transfer into a first subject, the cell described herein exhibits one or more responses selected from the group consisting of (a) a T cell response, (b) an NK cell response, and (c) a macrophage response, that are reduced as compared to a wild-type cell following transfer into a second subject. In some embodiments, the first subject and the second subject are different subjects. In some embodiments, the macrophage response is engulfment.

[001119] In some embodiments, following transfer into a subject, the cell described herein exhibits one or more selected from the group consisting of (a) reduced TH1 activation in the subject, (b) reduced NK cell killing in the subject, and (c) reduced killing by whole PBMCs in the subject, as compared to a wild-type cell following transfer into the subject.

[001120] In some embodiments, following transfer into a subject, the cell described herein elicits one or more selected from the group consisting of (a) reduced donor specific antibodies in the subject, (b) reduced IgM or IgG antibodies in the subject, and (c) reduced complement-dependent cytotoxicity (CDC) in a subject, as compared to a wild-type cell following transfer into the subject.

[001121] In some embodiments, the cell described herein is transduced with a lentivirus vector comprising a CD8 binding agent within the subject. In some embodiments, the lentivirus vector carries a gene encoding the CAR and/or CD47.

[001122] In some embodiments, the gene encoding the CAR and/or CD47 is introduced into the cells using a gene therapy vector or a transposase system selected from the group consisting of conditional or inducible transposases, conditional or inducible PiggyBac transposons, conditional or inducible Sleeping Beauty (SB11) transposons, conditional or inducible Mos1 transposons, and conditional or inducible Tol2 transposons. In some embodiments, the gene therapy vector is a retrovirus or a fusosome.

[001123] Provided herein are pharmaceutical compositions comprising a population of the non-activated T cells and/or the engineered T cells of the present disclosure and a pharmaceutically acceptable additive, carrier, diluent or excipient.

[001124] Provided herein are methods comprising administering to a subject a composition comprising a population of the non-activated T cells and/or the engineered T cells of the present disclosure, or one or more the pharmaceutical compositions of the present disclosure.

[001125] In some embodiments, the subject is not administered a T cell activating treatment before, after, and/or concurrently with administration of the composition. In some embodiments, the T cell activating treatment comprises lymphodepletion.

[001126] Provided herein are methods of treating a subject suffering from cancer, comprising administering to a subject a composition comprising a population of the non-activated T cells and/or the engineered T cells of the present disclosure, or one or more the pharmaceutical compositions of the present disclosure, wherein the subject is not administered a T cell activating treatment before, after, and/or concurrently with administration of the composition. In some embodiments, the T cell activating treatment comprises lymphodepletion.

[001127] Provided herein are methods for expanding T cells capable of recognizing and killing tumor cells in a subject in need thereof within the subject, comprising administering to a subject a composition comprising a population of the non-activated T cells and/or the engineered T cells of the present disclosure, or one or more the pharmaceutical compositions of the present disclosure, wherein the subject is not administered a T cell activating treatment before, after, and/or concurrently with administration of the composition. In some embodiments, the T cell activating treatment comprises lymphodepletion.

[001128] Provided herein are dosage regimens for treating a condition, disease or disorder in a subject comprising administration of a pharmaceutical composition comprising a population of the non-activated T cells and/or the engineered T cells of the present disclosure, or one or more the pharmaceutical compositions of the present disclosure, and a pharmaceutically acceptable additive, carrier, diluent or excipient, wherein the pharmaceutical composition is administered in about 1-3 therapeutically effective doses. Provided herein are dosage regimens for treating a condition, disease or disorder in a subject comprising administration of a pharmaceutical composition comprising a population of the cells of the present disclosure, or one or more the pharmaceutical compositions of the present disclosure, and a pharmaceutically acceptable additive, carrier, diluent or excipient, wherein the pharmaceutical composition is administered in about 1-3 clinically effective doses.

[001129] Once altered, the presence of expression of any of the molecule described herein can be assayed using known techniques, such as Western blots, ELISA assays, FACS assays, other immunoassays, reverse transcriptase polymerase chain reactions (RT-PCR), and the like.

DD. Generation of Induced Pluripotent Stem Cells

[001130] The technology provides methods of producing hypoimmunogenic pluripotent cells. In some embodiments, the method comprises generating pluripotent stem cells. The generation of mouse and human pluripotent stem cells (generally referred to as iPSCs; miPSCs for murine cells or hiPSCs for human cells) is generally known in the art. As will be appreciated by those in the art, there are a variety of different methods for the generation of iPCSs. The original induction was done from mouse embryonic or adult fibroblasts using the viral introduction of four transcription factors, Oct3/4, Sox2, c-Myc and Klf4; see Takahashi and Yamanaka Cell 126:663-676 (2006), hereby incorporated by reference in its entirety and specifically for the techniques outlined therein. Since then, a number of methods have been developed; see Seki *et al.,* World J. Stem Cells 7(1): 116-125 (2015) for a review, and Lakshmipathy and Vermuri, editors, Methods in Molecular Biology: Pluripotent Stem Cells, Methods and Protocols, Springer 2013, both of which are hereby expressly incorporated by reference in their entirety, and in particular for the methods for generating hiPSCs (see for example Chapter 3 of the latter reference).

[001131] Generally, iPSCs are generated by the transient expression of one or more reprogramming factors" in the host cell, usually introduced using episomal vectors. Under these conditions, small amounts of the cells are induced to become iPSCs (in general, the efficiency of this step is low, as no selection markers are used). Once the cells are "reprogrammed", and become pluripotent, they lose the episomal vector(s) and produce the factors using the endogenous genes.

[001132] As is also appreciated by those of skill in the art, the number of reprogramming factors that can be used or are used can vary. Commonly, when fewer reprogramming factors are used, the efficiency of the transformation of the cells to a pluripotent state goes down, as well as the "pluripotency", *e.g.,* fewer reprogramming factors may result in cells that are not fully pluripotent but may only be able to differentiate into fewer cell types.

[001133] In some embodiments, a single reprogramming factor, OCT4, is used. In other embodiments, two reprogramming factors, OCT4 and KLF4, are used. In other embodiments,

three reprogramming factors, OCT4, KLF4 and SOX2, are used. In other embodiments, four reprogramming factors, OCT4, KLF4, SOX2 and c-Myc, are used. In other embodiments, 5, 6 or 7 reprogramming factors can be used selected from SOKMNLT; SOX2, OCT4 (POU5F1), KLF4, MYC, NANOG, LIN28, and SV40L T antigen. In general, these reprogramming factor genes are provided on episomal vectors such as are known in the art and commercially available.

[001134] In general, as is known in the art, iPSCs are made from non-pluripotent cells such as, but not limited to, blood cells, fibroblasts, etc., by transiently expressing the reprogramming factors as described herein.

EE. Assays for Hypoimmunogenicity Phenotypes and Retention of Pluripotency

[001135] Once the hypoimmunogenic cells have been generated, they may be assayed for their hypoimmunogenicity and/or retention of pluripotency as is described in WO2016183041 and WO2018132783.

[001136] In some embodiments, hypoimmunogenicity is assayed using a number of techniques as exemplified in Figure 13 and Figure 15 of WO2018132783. These techniques include transplantation into allogeneic hosts and monitoring for hypoimmunogenic pluripotent cell growth (*e.g.,* teratomas) that escape the host immune system. In some instances, hypoimmunogenic pluripotent cell derivatives are transduced to express luciferase and can then followed using bioluminescence imaging. Similarly, the T cell and/or B cell response of the host animal to such cells are tested to confirm that the cells do not cause an immune reaction in the host animal. T cell responses can be assessed by Elispot, ELISA, FACS, PCR, or mass cytometry (CYTOF). B cell responses or antibody responses are assessed using FACS or Luminex. Additionally or alternatively, the cells may be assayed for their ability to avoid innate immune responses, *e.g.*, NK cell killing, as is generally shown in Figures 14 and 15 of WO2018132783.

[001137] In some embodiments, the immunogenicity of the cells is evaluated using T cell immunoassays such as T cell proliferation assays, T cell activation assays, and T cell killing assays recognized by those skilled in the art. In some cases, the T cell proliferation assay includes pretreating the cells with interferon-gamma and coculturing the cells with labelled T cells and assaying the presence of the T cell population (or the proliferating T cell population) after a preselected amount of time. In some cases, the T cell activation assay includes

coculturing T cells with the cells outlined herein and determining the expression levels of T cell activation markers in the T cells.

[001138] In vivo assays can be performed to assess the immunogenicity of the cells outlined herein. In some embodiments, the survival and immunogenicity of hypoimmunogenic cells is determined using an allogenic humanized immunodeficient mouse model. In some instances, the hypoimmunogenic pluripotent stem cells are transplanted into an allogenic humanized NSG-SGM3 mouse and assayed for cell rejection, cell survival, and teratoma formation. In some instances, grafted hypoimmunogenic pluripotent stem cells or differentiated cells thereof display long-term survival in the mouse model.

[001139] Additional techniques for determining immunogenicity including hypoimmunogenicity of the cells are described in, for example, Deuse *et al.*, Nature Biotechnology, 2019, 37, 252-258 and Han *et al.*, Proc Natl Acad Sci USA, 2019, 116(21), 10441-10446, the disclosures including the figures, figure legends, and description of methods are incorporated herein by reference in their entirety.

[001140] Similarly, the retention of pluripotency is tested in a number of ways. In some embodiments, pluripotency is assayed by the expression of certain pluripotency-specific factors as generally described herein and shown in Figure 29 of WO2018132783. Additionally or alternatively, the pluripotent cells are differentiated into one or more cell types as an indication of pluripotency.

[001141] As will be appreciated by those in the art, the successful reduction of the MHC I function (HLA I when the cells are derived from human cells) in the pluripotent cells can be measured using techniques known in the art and as described below; for example, FACS techniques using labeled antibodies that bind the HLA complex; for example, using commercially available HLA-A, HLA-B, and HLA-C antibodies that bind to the alpha chain of the human major histocompatibility HLA Class I antigen molecules.

[001142] In addition, the cells can be tested to confirm that the HLA I complex is not expressed on the cell surface. This may be assayed by FACS analysis using antibodies to one or more HLA cell surface components as discussed above.

[001143] The successful reduction of the MHC II function (HLA II when the cells are derived from human cells) in the pluripotent cells or their derivatives can be measured using techniques

known in the art such as Western blotting using antibodies to the protein, FACS techniques, RT-PCR techniques, etc.

[001144] In addition, the cells can be tested to confirm that the HLA II complex is not expressed on the cell surface. Again, this assay is done as is known in the art (See Figure 21 of WO2018132783, for example) and generally is done using either Western Blots or FACS analysis based on commercial antibodies that bind to human HLA Class II HLA-DR, DP and most DQ antigens.

[001145] In addition to the reduction ofone or more HLA I and II (or MHC I and II) molecules, the hypoimmunogenic cells of the technology have a reduced susceptibility to macrophage phagocytosis and NK cell killing. The resulting hypoimmunogenic cells "escape" the immune macrophage and innate pathways due to reduction or lack of the TCR complex and the expression of one or more tolerogenic factor transgenes.

FF. Exogenous Polynucleotides

[001146] In some embodiments, the hypoimmunogenic cells provided herein are genetically modified to include one or more exogenous polynucleotides inserted into one or more genomic loci of the hypoimmunogenic cell. In some embodiments, the exogenous polynucleotide encodes a protein of interest, *e.g.*, a chimeric antigen receptor. Any suitable method can be used to insert the exogenous polynucleotide into the genomic locus of the hypoimmunogenic cell including the gene editing methods described herein (*e.g.*, a CRISPR/Cas system).

[001147] The exogenous polynucleotide can be inserted into any suitable genomic loci of the hypoimmunogenic cell. In some embodiments, the exogenous polynucleotide is inserted into a safe harbor or target locus as described herein. Suitable safe harbor and target loci include, but are not limited to, a CCR5 gene, a CXCR4 gene, a PPP1R12C (also known as AAVS1) gene, an albumin gene, a SHS231 locus, a CLYBL gene, a Rosa gene (*e.g.*, ROSA26), an F3 gene (also known as CD142), a MICA gene, a MICB gene, a LRP1 gene (also known as CD91), a HMGB1 gene, .an ABO gene, a RHD gene, a FUT1 gene, a PDGFRa gene, an OLIG2 gene, a GFAP gene, and a KDM5D gene (also known as HY). In some embodiments, the exogenous polynucleotide is interested into an intron, exon, or coding sequence region of the safe harbor or target gene locus. In some embodiments, the exogenous polynucleotide is inserted into an endogenous gene wherein the insertion causes silencing or reduced expression of the endogenous gene. In some embodiments, the polynucleotide is inserted in a B2M, CIITA, TRAC, TRB, PD-1

or CTLA-4 gene locus. Exemplary genomic loci for insertion of an exogenous polynucleotide are depicted in Tables 16 and 17.

Table 16: Exemplary genomic loci for insertion of exogenous polynucleotides

Number	species	name	Ensembl ID	Target region for cleavage	Also known as
1	human	B2M	ENSG00000166710	CDS	
2	human	CIITA	ENSG00000179583	CDS	
3	human	TRAC	ENSG00000277734	CDS	
4	human	PPP1R12C	ENSG00000125503	Intron 1 and 2	AAVS1
5	human	CLYBL	ENSG00000125246	Intron 2	
6	human	CCR5	ENSG00000160791	Exons 1-3, introns 1-2, and CDS	
7	human	THUMPD3-AS1	ENSG00000206573	Intron 1	ROSA26
8	human	Ch-4:58,976,613		500 bp window	SHS231
9	human	F3	ENSG00000117525	CDS	CD142
10	human	MICA	ENSG00000204520	CDS	
11	human	MICB	ENSG00000204516	CDS	
12	human	LRP1	ENSG00000123384	CDS	
13	human	HMGB1	ENSG00000189403	CDS	
14	human	ABO	ENSG00000175164	CDS	
15	human	RHD	ENSG00000187010	CDS	
16	human	FUT1	ENSG00000174951	CDS	
17	human	KDM5D	ENSG00000012817	CDS	HY

Table 17: Non-limiting examples of Cas9 guide RNAs

Gene	SEQ ID NO:	guide sequence	PAM	Target site	gRNA cut location
ABO	1	UCUCUCCAUGUGCAGUAGGA	AGG	Exon 7	chr9:133,257,541
FUT1	2	CUGGAUGUCGGAGGAGUACG	CGG	Exon 4	chr19:48,750,822
RH	3	GUCUCCGGAAACUCGAGGUG	AGG	Exon 2	chr1:25,284,622
F3 (CD142)	4	ACAGUGUAGACUUGAUUGAC	GGG	Exon 2	chr1:94,540,281
B2M	5	CGUGAGUAAACCUGAAUCUU	TGG	Exon 2	chr15:44,715,434
CIITA	6	GAUAUUGGCAUAAGCCUCCC	TGG	Exon 3	chr16:10,895,747
TRAC	7	AGAGUCUCUCAGCUGGUACA	CGG	Exon 1	chr14:22,5547,533

[001148] For the Cas9 guides, the spacer sequence for all Cas9 guides is provided in Table 18. with description that the 20nt guide sequence corresponds to a unique guide sequence and can be any of those described herein, including for example those listed in Table 18.

Table 18: Cas9 guide RNAs

Description	SEQ ID NO:	Sequence
20 nt guide sequence*	8	NNNNNNNNNNNNNNNNNNN
12 nt crRNA repeat sequence	9	GUUUUAGAGCUA
4 nt tetraloop sequence	10	GAAA
64 nt tracrRNA sequence	11	UAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACU UGAAAAAGUGGCACCGAGUCGGUGCUUU
Exemplary full sequence	12	NNNNNNNNNNNNNNNNNNNGUUUUAGAGCUAGAA AUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAC UUGAAAAAGUGGCACCGAGUCGGUGCUUU

[001149] In some embodiments, the hypoimmunogenic cell that includes the exogenous polynucleotide is derived from a hypoimmunogenic induced pluripotent cell (HIP), for example, as described herein. Such hypoimmunogenic cells include, for example, cardiac cells, neural cells, cerebral endothelial cells, dopaminergic neurons, glial progenitor cells, endothelial cells, thyroid cells, pancreatic islet cells (beta cells), retinal pigmented epithelium cells, NK cells, and T cells. In some embodiments, the hypoimmunogenic cell that includes the exogenous polynucleotide is a beta cell, a T cell (*e.g.*, a primary T cell), or a glial cell.

[001150] In some embodiments, the exogenous polynucleotide encodes an exogenous CD47 polypeptide (*e.g.*, a human CD47 polypeptide) and the exogenous polypeptide is inserted into a safe harbor or target gene loci or a safe harbor or target site as disclosed herein or a genomic locus that causes silencing or reduced expression of the endogenous gene. In some embodiments, the polynucleotide is inserted in a B2M, CIITA, TRAC, TRB, PD1 or CTLA4 gene locus.

[001151] In some embodiments, the hypoimmunogenic cell that includes the exogenous polynucleotide is a primary T cell or a T cell derived from a hypoimmunogenic pluripotent cell (*e.g.*, a hypoimmunogenic iPSC). In some embodiments, the exogenous polynucleotide is a chimeric antigen receptor (*e.g.*, any of the CARs described herein). In some embodiments, the

exogenous polynucleotide is operably linked to a promoter for expression of the exogenous polynucleotide in the hypoimmunogenic cell.

GG. Pharmaceutically Acceptable Carriers

[001152] In some embodiments, the pharmaceutical composition provided herein further include a pharmaceutically acceptable carrier. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.*, Zn-protein complexes); salts such as sodium chloride; and/or non-ionic surfactants such as polysorbates (TWEEN™), poloxamers (PLURONICS™) or polyethylene glycol (PEG). In some embodiments, the pharmaceutical composition includes a pharmaceutically acceptable buffer (*e.g.*, neutral buffer saline or phosphate buffered saline).

[001153] In some embodiments, the pharmaceutical composition includes one or more electrolyte base solutions selected from the group consisting of lactated CryoStor®, Ringer's solution, PlasmaLyte-A™, Iscove's Modified Dulbecco's Medium, Normosol-R™, Veen-D™, Polysal® and Hank's Balanced Salt Solution (containing no phenol red). These base solutions closely approximate the composition of extracellular mammalian physiological fluids.

[001154] In some embodiments, the pharmaceutical composition includes one or more cryoprotective agents selected from the group consisting of arabinogalactan, glycerol, polyvinylpyrrolidone (PVP), dextrose, dextran, trehalose, sucrose, raffinose, hydroxyethyl starch (HES), propylene glycol, human serum albumin (HSA), and dimethylsulfoxide (DMSO). In some embodiments, the pharmaceutically acceptable buffer is neutral buffer saline or phosphate

buffered saline. In some embodiments, pharmaceutical compositions provided herein include one or more of CryoStor® CSB, Plasma-Lyte-A™, HSA, DMSO, and trehalose.

[001155] CryoStor® is an intracellular-like optimized solution containing osmotic/oncotic agents, free radical scavengers, and energy sources to minimize apoptosis, minimize ischemia/reperfusion injury and maximize the post-thaw recovery of the greatest numbers of viable, functional cells. CryoStor® is serum- and protein-free, and non-immunogenic. CryoStor® is cGMP-manufactured from raw materials of USPgrade or higher. CryoStor® is a family of solutions pre-formulated with 0%, 2%, 5% or 10% DMSO. CryoStor® CSB is a DMSO-free version of CryoStor®. In some embodiments, the pharmaceutical composition includes a base solution of CryoStor® CSB at a concentration of about 0-100%, 5-95%, 10-90%, 15-85%, 20-80%, 30-80%, 40-80%, 50-80%, 60-80%, 70-80%, 25-75%, 30-70%, 35-65%, 40-60%, or 45-55% w/w. In some embodiments, the pharmaceutical composition includes a base solution of CryoStor® CSB at a concentration of about 0%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% w/w.

[001156] PlasmaLyte-A™ is a non-polymeric plasma expander and contains essential salts and nutrients similar to those found in culture medium but does not contain additional constituents found in tissue culture medium which are not approved for human infusion, e.g., phenol red, or are unavailable in U.S.P. grade. PlasmaLyte-A™ contains about 140 mEq/liter of sodium (Na), about 5 mEq/liter of potassium (K), about 3 mEq/liter of magnesium (Mg), about 98 mEq/liter of chloride (Cl), about 27 mEq/liter of acetate, and about 23 mEq/liter of gluconate. (PlasmaLyte-A™ is commercially available from Baxter, Hyland Division, Glendale Calif., product No. 2B2543). In some embodiments, the pharmaceutical composition includes a base solution of PlasmaLyte-A™ at a concentration of about 0-100%, 5-95%, 10-90%, 15-85%, 15-80%, 15-75%, 15-70%, 15-65%, 15-60%, 15-55%, 15-50%, 15-45%, 15-40%, 15-35%, 15-30%, 15-25%, 20-80%, 20-75%, 20-70%, 20-65%, 20-60%, 20-55%, 20-50%, 20-45%, 20-40%, 20-35%, 20-30%, 25-75%, 30-70%, 35-65%, 40-60%, or 45-55% w/w. In some embodiments, the pharmaceutical composition includes a base solution of PlasmaLyte-A™ at a concentration of about 0%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% w/w.

[001157] In some embodiments, the pharmaceutical composition includes human serum albumin (HSA) at a concentration of about 0-10%, 0.3-9.3%, 0.3-8.3%, 0.3-7.3%, 0.3-6.3%, 0.3-5.3%, 0.3-4.3%, 0.3-3.3%, 0.3-2.3%, 0.3-1.3%, 0.6-8.3%, 0.9-7.3%, 1.2-6.3%, 1.5-5.3%, 1.8-4.3%, or 2.1-3.3% w/v. In some embodiments, the pharmaceutical composition includes HSA at a concentration of about 0%, 0.3%, 0.6%, 0.9%, 1.2%, 1.5%, 1.8%, 2.1%, 2.4%, 2.7%, 3.0%, 3.3%, 3.6%, 3.9%, 4.3%, 4.6%, 4.9%, 5.3%, 5.6%, 5.9%, 6.3%, 6.6%, 6.9%, 7.3%, 7.6%, 7.9%, 8.3%, 8.6%, 8.9%, 9.3%, 9.6%, 9.9%, or 10% w/v.

[001158] In some embodiments, the pharmaceutical composition includes dimethylsulfoxide (DMSO) at a concentration of about 0-10%, 0.5-9.5%, 1-9%, 1.5-8.5%, 2-8%, 3-8%, 4-8%, 5-8%, 6-8%, 7-8%, 2.5-7.5%, 3-7%, 3.5-6.5%, 4-6%, or 4.5-5.5% v/v. In some embodiments, the pharmaceutical composition includes HSA at a concentration of about 0%, 0.25%, 0.5%, 0.75%, 1.0%,1.25%, 1.5%, 1.75%, 2.0%, 2.25%, 2.5%, 2.75%, 3.0%, 3.25%, 3.5%, 3.75%, 4.0%, 4.25%, 4.5%, 4.75%, 5.0%, 5.25%, 5.5%, 5.75%, 6.0%, 6.25%, 6.5%, 6.75%, 7.0%, 7.25%, 7.5%, 7.75%, 8.0%, 8.25%, 8.5%, 8.75%, 9.0%, 9.25%, 9.5%, 9.75%, or 10.0% v/v.

[001159] In some embodiments, the pharmaceutical composition includes trehalose at a concentration of about 0-500 mM, 50-450 mM, 100-400 mM, 150-350 mM, or 200-300 mM. In some embodiments, the pharmaceutical composition includes trehalose at a concentration of about 0 mM, 10 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, 100 mM, 125 mM, 150 mM, 175 mM, 200 mM, 225 mM, 250 mM, 275 mM, 300 mM, 325 mM, 350 mM, 375 mM, 400 mM, 425 mM, 450 mM, 475 mM, or 500 mM.

[001160] Exemplary pharmaceutical composition components are shown in Table 34.

Table 34. Exemplary pharmaceutical composition components.

Formulation	Base Solution	c[DMSO]	Additional c[HSA]*	c[trehalose]
A	75% CroStor® CSB + 25% PlasmaLyte A™ + 1.2% HSA	7.5%	0.3%	
B		3.75%	0.3%	
C			5.3%	
D			0.3%	250 mM

E	100% PlasmaLyte A™ + 1.2% HSA	7.5%	0.3%	
F		7.5%	5.3%	
G		7.5%	5.3%	250 mM

* Additional HSA in addition to PlasmaLyte.

[001161] In some embodiments, the pharmaceutical composition comprises hypoimmunogenic cells described herein and a pharmaceutically acceptable carrier comprising 31.25 % (v/v) Plasma-Lyte A, 31.25 % (v/v) of 5% dextrose/0.45% sodium chloride, 10% dextran 40 (LMD)/5% dextrose, 20% (v/v) of 25% human serum albumin (HSA), and 7.5% (v/v) dimethylsulfoxide (DMSO).

HH. Formulations and Dosage Regimens

[001162] Any therapeutically effective amount of cells described herein can be included in the pharmaceutical composition, depending on the indication being treated. Non-limiting examples of the cells include primary T cells, T cells differentiated from hypoimmunogenic induced pluripotent stem cells, and other cells differentiated from hypoimmunogenic induced pluripotent stem cells described herein. In some embodiments, the pharmaceutical composition includes at least about 1×10^2, 5×10^2, 1×10^3, 5×10^3, 1×10^4, 5×10^4, 1×10^5, 5×10^5, 1×10^6, 5×10^6, 1×10^7, 5×10^7, 1×10^8, 5×10^8, 1×10^9, 5×10^9, 1×10^{10}, or 5×10^{10} cells. In some embodiments, the pharmaceutical composition includes up to about 1×10^2, 5×10^2, 1×10^3, 5×10^3, 1×10^4, 5×10^4, 1×10^5, 5×10^5, 1×10^6, 5×10^6, 1×10^7, 5×10^7, 1×10^8, 5×10^8, 1×10^9, 5×10^9, 1×10^{10}, or 5×10^{10} cells. In some embodiments, the pharmaceutical composition includes up to about 6.0×10^8 cells. In some embodiments, the pharmaceutical composition includes up to about 8.0×10^8 cells. In some embodiments, the pharmaceutical composition includes at least about 1×10^2-5×10^2, 5×10^2-1×10^3, 1×10^3-5×10^3, 5×10^3-1×10^4, 1×10^4-5×10^4, 5×10^4-1×10^5, 1×10^5-5×10^5, 5×10^5-1×10^6, 1×10^6-5×10^6, 5×10^6-1×10^7, 1×10^7-5×10^7, 5×10^7-1×10^8, 1×10^8-5×10^8, 5×10^8-1×10^9, 1×10^9-5×10^9, 5×10^9-1×10^{10}, or 1×10^{10} - 5×10^{10} cells. In some embodiments, the pharmaceutical composition includes from about 1.0×10^6 to about 2.5×10^8 cells. In certain embodiments, the pharmaceutical composition includes from about 2.0×10^6 to about 2.0×10^8 cells, such as but not limited to, primary T cells, T cells differentiated from hypoimmunogenic induced pluripotent stem cells.

[001163] In some embodiments, the pharmaceutical composition has a volume of at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, or 500 ml. In some embodiments, the pharmaceutical composition has a volume of up to about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, or 500 ml. In some embodiments, the pharmaceutical composition has a volume of about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, or 500 ml. In some embodiments, the pharmaceutical composition has a volume of from about 1-50 ml, 50-100 ml, 100-150 ml, 150-200 ml, 200-250 ml, 250-300 ml, 300-350 ml, 350-400 ml, 400-450 ml, or 450-500 ml. In some embodiments, the pharmaceutical composition has a volume of from about 1-50 ml, 50-100 ml, 100-150 ml, 150-200 ml, 200-250 ml, 250-300 ml, 300-350 ml, 350-400 ml, 400-450 ml, or 450-500 ml. In some embodiments, the pharmaceutical composition has a volume of from about 1-10 ml, 10-20 ml, 20-30 ml, 30-40 ml, 40-50 ml, 50-60 ml, 60-70 ml, 70-80 ml, 70-80 ml, 80-90 ml, or 90-100 ml. In some embodiments, the pharmaceutical composition has a volume that ranges from about 5 ml to about 80 ml. In some embodiments, the pharmaceutical composition has a volume that ranges from about 10 ml to about 70 ml. In certain embodiments, the pharmaceutical composition has a volume that ranges from about 10 ml to about 50 ml.

[001164] The specific amount/dosage regimen will vary depending on the weight, gender, age and health of the individual, the formulation, the biochemical nature, bioactivity, bioavailability and the side effects of the cells and the number and identity of the cells in the complete therapeutic regimen.

[001165] In some embodiments, a therapeutically effective dose or a clinically effective dose of the pharmaceutical composition includes about 1.0×10^5 to about 2.5×10^8 cells at a volume of about 10 ml to 50 ml and the pharmaceutical composition is administered as a single therapeutically effective dose or clinically effective dose. In some cases, the therapeutically effective dose or clinically effective dose includes about 1.0×10^5 to about 2.5×10^8 primary T cells described herein at a volume of about 10 ml to 50 ml. In some cases, the therapeutically effective dose or clinically effective dose includes about 1.0×10^5 to about 2.5×10^8 primary T cells that have been described above at a volume of about 10 ml to 50 ml. In various cases, the therapeutically effective dose or clinically effective dose includes about 1.0×10^5 to about $2.5 \times$

10^8 T cells differentiated from hypoimmunogenic induced pluripotent stem cells described herein at a volume of about 10 ml to 50 ml. In some embodiments, the therapeutically effective dose or clinically effective dose is 1.0 x 105, 1.1 x 105, 1.2 x 105, 1.3 x 105, 1.4 x 105, 1.5 x 105, 1.6 x 105, 1.7 x 105, 1.8 x 105, 1.9 x 105, 2.0 x 105, 2.1 x 105, 2.2 x 105, 2.3 x 105, 2.4 x 105, 2.5 x 105, 1.0 x 106, 1.1 x 106, 1.2 x 106, 1.3 x 106, 1.4 x 106, 1.5 x 106, 1.6 x 106, 1.7 x 106, 1.8 x 106, 1.9 x 106, 2.0 x 106, 2.1 x 106, 2.2 x 106, 2.3 x 106, 2.4 x 106, 2.5 x 106, 1.0 x 107, 1.1 x 107, 1.2 x 107, 1.3 x 107, 1.4 x 107, 1.5 x 107, 1.6 x 107, 1.7 x 107, 1.8 x 107, 1.9 x 107, 2.0 x 107, 2.1 x 107, 2.2 x 107, 2.3 x 107, 2.4 x 107, 2.5 x 107, 1.0 x 108, 1.1 x 108, 1.2 x 108, 1.3 x 108, 1.4 x 108, 1.5 x 108, 1.6 x 108, 1.7 x 108, 1.8 x 108, 1.9 x 108, 2.0 x 108, 2.1 x 108, 2.2 x 108, 2.3 x 108, 2.4 x 108, or 2.5 x 108 T cells differentiated from hypoimmunogenic induced pluripotent stem cells described herein at a volume of about 10 ml to 50 ml. In other cases, the therapeutically effective dose or clinically effective dose is at a range that is lower than about 1.0 x 10^5 to about 2.5 x 10^8 T cells, including primary T cells or T cells differentiated from hypoimmunogenic induced pluripotent stem cells. In yet other cases, the therapeutically effective dose or clinically effective dose is at a range that is higher than about 1.0 x 10^5 to about 2.5 x 10^8 T cells, including primary T cells and T cells differentiated from hypoimmunogenic induced pluripotent stem cells.

[001166] In some embodiments, the pharmaceutical composition is administered as a single therapeutically effective dose or clinically effective dose of from about 1.0 x 10^5 to about 1.0 x 10^7 cells (such as primary T cells and T cells differentiated from hypoimmunogenic induced pluripotent stem cells) per kg body weight for subjects 50 kg or less. In some embodiments, the pharmaceutical composition is administered as a single therapeutically effective dose or clinically effective dose of from about 0.5 x 10^5 to about 1.0 x 10^7, about 1.0 x 10^5 to about 1.0 x 10^7, about 1.0 x 10^5 to about 1.0 x 10^7, about 5.0 x 10^5 to about 1 x 10^7, about 1.0 x 10^6 to about 1 x 10^7, about 5.0 x 10^6 to about 1.0 x 10^7, about 1.0 x 10^5 to about 5.0 x 10^6, about 1.0 x 10^5 to about 1.0 x 10^6, about 1.0 x 10^5 to about 5.0 x 10^5, about 1.0 x 10^5 to about 5.0 x 10^6, about 2.0 x 10^5 to about 5.0 x 10^6, about 3.0 x 10^5 to about 5.0 x 10^6, about 4.0 x 10^5 to about 5.0 x 10^6, about 5.0 x 10^5 to about 5.0 x 10^6, about 6.0 x 10^5 to about 5.0 x 10^6, about 7.0 x 10^5 to about 5.0 x 10^6, about 8.0 x 10^5 to about 5.0 x 10^6, or about 9.0 x 10^5 to about 5.0 x 10^6 cells per kg body weight for subjects 50 kg or less. In some embodiments, the therapeutically effective dose or clinically effective dose is from about 0.2 x 10^6 to about 5.0 x 10^6 cells per kg body weight for

subjects 50 kg or less. In certain embodiments, the therapeutically effective dose or clinically effective dose is at a range that is lower than from about 0.2×10^6 to about 5.0×10^6 cells per kg body weight for subjects 50 kg or less. In some embodiments, the therapeutically effective dose or clinically effective dose is 0.5×10^5, 0.6×10^5, 0.7×10^5, 0.8×10^5, 0.9×10^5, 1.0×10^5, 1.1×10^5, 1.2×10^5, 1.3×10^5, 1.4×10^5, 1.5×10^5, 1.6×10^5, 1.7×10^5, 1.8×10^5, 1.9×10^5, 2.0×10^5, 2.1×10^5, 2.2×10^5, 2.3×10^5, 2.4×10^5, 2.5×10^5, 2.6×10^5, 2.7×10^5, 2.8×10^5, 2.9×10^5, 3.0×10^5, 3.1×10^5, 3.2×10^5, 3.3×10^5, 3.4×10^5, 3.5×10^5, 3.6×10^5, 3.7×10^5, 3.8×10^5, 3.9×10^5, 4.0×10^5, 4.1×10^5, 4.2×10^5, 4.3×10^5, 4.4×10^5, 4.5×10^5, 4.6×10^5, 4.7×10^5, 4.8×10^5, 4.9×10^5, 5.0×10^5, 0.5×10^6, 0.6×10^6, 0.7×10^6, 0.8×10^6, 0.9×10^6, 1.0×10^6, 1.1×10^6, 1.2×10^6, 1.3×10^6, 1.4×10^6, 1.5×10^6, 1.6×10^6, 1.7×10^6, 1.8×10^6, 1.9×10^6, 2.0×10^6, 2.1×10^6, 2.2×10^6, 2.3×10^6, 2.4×10^6, 2.5×10^6, 2.6×10^6, 2.7×10^6, 2.8×10^6, 2.9×10^6, 3.0×10^6, 3.1×10^6, 3.2×10^6, 3.3×10^6, 3.4×10^6, 3.5×10^6, 3.6×10^6, 3.7×10^6, 3.8×10^6, 3.9×10^6, 4.0×10^6, 4.1×10^6, 4.2×10^6, 4.3×10^6, 4.4×10^6, 4.5×10^6, 4.6×10^6, 4.7×10^6, 4.8×10^6, 4.9×10^6, 5.0×10^6, 5.1×10^6, 5.2×10^6, 5.3×10^6, 5.4×10^6, 5.5×10^6, 5.6×10^6, 5.7×10^6, 5.8×10^6, 5.9×10^6, 6.0×10^6, 6.1×10^6, 6.2×10^6, 6.3×10^6, 6.4×10^6, 6.5×10^6, 6.6×10^6, 6.7×10^6, 6.8×10^6, 6.9×10^6, 7.0×10^6, 7.1×10^6, 7.2×10^6, 7.3×10^6, 7.4×10^6, 7.5×10^6, 7.6×10^6, 7.7×10^6, 7.8×10^6, 7.9×10^6, 8.0×10^6, 8.1×10^6, 8.2×10^6, 8.3×10^6, 8.4×10^6, 8.5×10^6, 8.6×10^6, 8.7×10^6, 8.8×10^6, 8.9×10^6, 9.0×10^6, 9.1×10^6, 9.2×10^6, 9.3×10^6, 9.4×10^6, 9.5×10^6, 9.6×10^6, 9.7×10^6, 9.8×10^6, 9.9×10^6, 0.5×10^7, 0.6×10^7, 0.7×10^7, 0.8×10^7, 0.9×10^7, or 1.0×10^7 cells per kg body weight for subjects 50 kg or less. In some embodiments, the therapeutically effective dose or clinically effective dose is from about 0.2×10^6 to about 5.0×10^6 cells per kg body weight for subjects 50 kg or less. In certain embodiments, the therapeutically effective dose or clinically effective dose is at a range that is higher than from about 0.2×10^6 to about 5.0×10^6 cells per kg body weight for subjects 50 kg or less. In some embodiments, the single therapeutically effective dose or clinically effective dose is at a volume of about 10 ml to 50 ml. In some embodiments, the therapeutically effective dose or clinically effective dose is administered intravenously.

[001167] In some embodiments, the cells are administered in a single therapeutically effective dose or clinically effective dose of from about 1.0×10^6 to about 5.0×10^8 cells (such as primary T cells and T cells differentiated from hypoimmunogenic induced pluripotent stem cells) for subjects above 50 kg. In some embodiments, the pharmaceutical composition is administered as

a single therapeutically effective dose or clinically effective dose of from about 0.5×10^6 to about 1.0×10^9, about 1.0×10^6 to about 1.0×10^9, about 1.0×10^6 to about 1.0×10^9, about 5.0×10^6 to about 1.0×10^9, about 1.0×10^7 to about 1.0×10^9, about 5.0×10^7 to about 1.0×10^9, about 1.0×10^6 to about 5.0×10^7, about 1.0×10^6 to about 1.0×10^7, about 1.0×10^6 to about 5.0×10^7, about 1.0×10^7 to about 5.0×10^8, about 2.0×10^7 to about 5.0×10^8, about 3.0×10^7 to about 5.0×10^8, about 4.0×10^7 to about 5.0×10^8, about 5.0×10^7 to about 5.0×10^8, about 6.0×10^7 to about 5.0×10^8, about 7.0×10^7 to about 5.0×10^8, about 8.0×10^7 to about 5.0×10^8, or about 9.0×10^7 to about 5.0×10^8 cells per kg body weight for subjects 50 kg or less. In some embodiments, the therapeutically effective dose or clinically effective dose is 1.0×10^6, 1.1×10^6, 1.2×10^6, 1.3×10^6, 1.4×10^6, 1.5×10^6, 1.6×10^6, 1.7×10^6, 1.8×10^6, 1.9×10^6, 2.0×10^6, 2.1×10^6, 2.2×10^6, 2.3×10^6, 2.4×10^6, 2.5×10^6, 2.6×10^6, 2.7×10^6, 2.8×10^6, 2.9×10^6, 3.0×10^6, 3.1×10^6, 3.2×10^6, 3.3×10^6, 3.4×10^6, 3.5×10^6, 3.6×10^6, 3.7×10^6, 3.8×10^6, 3.9×10^6, 4.0×10^6, 4.1×10^6, 4.2×10^6, 4.3×10^6, 4.4×10^6, 4.5×10^6, 4.6×10^6, 4.7×10^6, 4.8×10^6, 4.9×10^6, 5.0×10^6, 5.1×10^6, 5.2×10^6, 5.3×10^6, 5.4×10^6, 5.5×10^6, 5.6×10^6, 5.7×10^6, 5.8×10^6, 5.9×10^6, 6.0×10^6, 6.1×10^6, 6.2×10^6, 6.3×10^6, 6.4×10^6, 6.5×10^6, 6.6×10^6, 6.7×10^6, 6.8×10^6, 6.9×10^6, 7.0×10^6, 7.1×10^6, 7.2×10^6, 7.3×10^6, 7.4×10^6, 7.5×10^6, 7.6×10^6, 7.7×10^6, 7.8×10^6, 7.9×10^6, 8.0×10^6, 8.1×10^6, 8.2×10^6, 8.3×10^6, 8.4×10^6, 8.5×10^6, 8.6×10^6, 8.7×10^6, 8.8×10^6, 8.9×10^6, 9.0×10^6, 9.1×10^6, 9.2×10^6, 9.3×10^6, 9.4×10^6, 9.5×10^6, 9.6×10^6, 9.7×10^6, 9.8×10^6, 9.9×10^6, 1.0×10^7, 1.1×10^7, 1.2×10^7, 1.3×10^7, 1.4×10^7, 1.5×10^7, 1.6×10^7, 1.7×10^7, 1.8×10^7, 1.9×10^7, 2.0×10^7, 2.1×10^7, 2.2×10^7, 2.3×10^7, 2.4×10^7, 2.5×10^7, 2.6×10^7, 2.7×10^7, 2.8×10^7, 2.9×10^7, 3.0×10^7, 3.1×10^7, 3.2×10^7, 3.3×10^7, 3.4×10^7, 3.5×10^7, 3.6×10^7, 3.7×10^7, 3.8×10^7, 3.9×10^7, 4.0×10^7, 4.1×10^7, 4.2×10^7, 4.3×10^7, 4.4×10^7, 4.5×10^7, 4.6×10^7, 4.7×10^7, 4.8×10^7, 4.9×10^7, 5.0×10^7, 5.1×10^7, 5.2×10^7, 5.3×10^7, 5.4×10^7, 5.5×10^7, 5.6×10^7, 5.7×10^7, 5.8×10^7, 5.9×10^7, 6.0×10^7, 6.1×10^7, 6.2×10^7, 6.3×10^7, 6.4×10^7, 6.5×10^7, 6.6×10^7, 6.7×10^7, 6.8×10^7, 6.9×10^7, 7.0×10^7, 7.1×10^7, 7.2×10^7, 7.3×10^7, 7.4×10^7, 7.5×10^7, 7.6×10^7, 7.7×10^7, 7.8×10^7, 7.9×10^7, 8.0×10^7, 8.1×10^7, 8.2×10^7, 8.3×10^7, 8.4×10^7, 8.5×10^7, 8.6×10^7, 8.7×10^7, 8.8×10^7, 8.9×10^7, 9.0×10^7, 9.1×10^7, 9.2×10^7, 9.3×10^7, 9.4×10^7, 9.5×10^7, 9.6×10^7, 9.7×10^7, 9.8×10^7, 9.9×10^7, 1.0×10^8, 1.1×10^8, 1.2×10^8, 1.3×10^8, 1.4×10^8, 1.5×10^8, 1.6×10^8, 1.7×10^8, 1.8×10^8, 1.9×10^8, 2.0×10^8, 2.1×10^8, 2.2×10^8, 2.3×10^8, 2.4×10^8, 2.5×10^8, 2.6×10^8, 2.7×10^8, 2.8×10^8, 2.9×10^8, 3.0×10^8, 3.1×10^8, 3.2×10^8, 3.3×10^8, 3.4×10^8, 3.5×10^8, 3.6×10^8, 3.7×10^8, 3.8×10^8, 3.9×10^8, $4.0 \times$

10^8, 4.1 x 10^8, 4.2 x 10^8, 4.3 x 10^8, 4.4 x 10^8, 4.5 x 10^8, 4.6 x 10^8, 4.7 x 10^8, 4.8 x 10^8, 4.9 x 10^8, or 5.0 x 10^8 cells per kg body weight for subjects 50 kg or less. In certain embodiments, the cells are administered in a single therapeutically effective dose or clinically effective dose of about 1.0 x 10^7 to about 2.5 x 10^8 cells for subjects above 50 kg. In some embodiments, the cells are administered in a single therapeutically effective dose or clinically effective dose of a range that is less than about 1.0 x 10^7 to about 2.5 x 10^8 cells for subjects above 50 kg. In some embodiments, the cells are administered in a single therapeutically effective dose or clinically effective dose of a range that is higher than about 1.0 x 10^7 to about 2.5 x 10^8 cells for subjects above 50 kg. In some embodiments, the dose is administered intravenously. In some embodiments, the single therapeutically effective dose or clinically effective dose is at a volume of about 10 ml to 50 ml. In some embodiments, the therapeutically effective dose or clinically effective dose is administered intravenously.

[001168] In some embodiments, the therapeutically effective dose or clinically effective dose is administered intravenously at a rate of about 1 to 50 ml per minute, 1 to 40 ml per minute, 1 to 30 ml per minute, 1 to 20 ml per minute, 10 to 20 ml per minute, 10 to 30 ml per minute, 10 to 40 ml per minute, 10 to 50 ml per minute, 20 to 50 ml per minute, 30 to 50 ml per minute, 40 to 50 ml per minute. In numerous embodiments, the pharmaceutical composition is stored in one or more infusion bags for intravenous administration. In some embodiments, the dose is administered completely at no more than 10 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes, 35 minutes, 40 minutes, 45 minutes, 50 minutes, 55 minutes, 60 minutes, 70 minutes, 80 minutes, 90 minutes, 120 minutes, 150 minutes, 180 minutes, 240 minutes, or 300 minutes.

[001169] In some embodiments, a single therapeutically effective dose or clinically effective dose of the pharmaceutical composition is present in a single infusion bag. In other embodiments, a single therapeutically effective dose or clinically effective dose of the pharmaceutical composition is divided into 2, 3, 4 or 5 separate infusion bags.

[001170] In some embodiments, the cells described herein are administered in a plurality of doses such as 2, 3, 4, 5, 6 or more doses, wherein the plurality of doses together constitute a therapeutically effective dose or clinically effective dose regimen. In some embodiments, each dose of the plurality of doses is administered to the subject ranging from 1 to 24 hours apart. In some instances, a subsequent dose is administered from about 1 hour to about 24 hours (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or about 24

hours) after an initial or preceding dose. In some embodiments, each dose of the plurality of doses is administered to the subject ranging from about 1 day to 28 days apart. In some instances, a subsequent dose is administered from about 1 day to about 28 days (*e.g.*, about 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, or about 28 days) after an initial or preceding dose. In certain embodiments, each dose of the plurality of doses is administered to the subject ranging from 1 week to about 6 weeks apart. In certain instances, a subsequent dose is administered from about 1 week to about 6 weeks (*e.g.*, about 1, 2, 3, 4, 5, or 6 weeks) after an initial or preceding dose. In several embodiments, each dose of the plurality of doses is administered to the subject ranging from about 1 month to about 12 months apart. In several instances, a subsequent dose is administered from about 1 month to about 12 months (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months) after an initial or preceding dose.

[001171] In some embodiments, a subject is administered a first dosage regimen at a first timepoint, and then subsequently administered a second dosage regimen at a second timepoint. In some embodiments, the first dosage regimen is the same as the second dosage regimen. In other embodiments, the first dosage regimen is different than the second dosage regimen. In some instances, the number of cells in the first dosage regimen and the second dosage regimen are the same. In some instances, the number of cells in the first dosage regimen and the second dosage regimen are different. In some cases, the number of doses of the first dosage regimen and the second dosage regimen are the same. In some cases, the number of doses of the first dosage regimen and the second dosage regimen are different.

[001172] In some embodiments, the first dosage regimen includes hypoimmune (HIP) T cells or primary T cells expressing a first CAR and the second dosage regimen includes hypoimmune (HIP) T cells or primary T cells expressing a second CAR such that the first CAR and the second CAR are different. For instance, the first CAR and second CAR bind different target antigens. In some cases, the first CAR includes an scFv that binds an antigen and the second CAR includes an scFv that binds a different antigen. In some embodiments, the first dosage regimen includes hypoimmune (HIP) T cell or primary T cells expressing a first CAR and the second dosage regimen includes hypoimmune (HIP) T cell or primary T cells expressing a second CAR such that the first CAR and the second CAR are the same. The first dosage regimen can be administered to the subject at least 1 month, 2 months, 3 months, 4 months, 5 months, 6 months,

7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 1-3 months, 1-6 months, 4-6 months, 3-9 months, 3-12 months, or more months apart from the second dosage regimen. In some embodiments, a subject is administered a plurality of dosage regimens during the course of a disease (*e.g.*, cancer) and at least two of the dosage regimens comprise the same type of hypoimmune (HIP) T cells or primary T cells described herein. In other embodiments, at least two of the plurality of dosage regimens comprise different types of hypoimmune (HIP) T cells or primary T cells described herein.

[001173] In some embodiments, the CD19 specific (CD19) CAR-T cells described herein are administered to a subject at a dose of about 50 x 10^6 to about 110 x 10^6 (e.g., 50 x 10^6, 51 x 10^6, 52 x 10^6, 53 x 10^6, 54 x 10^6, 55 x 10^6, 56 x 10^6, 57 x 10^6, 58 x 10^6, 59 x 10^6, 60 x 10^6, 61 x 10^6, 62 x 10^6, 63 x 10^6, 64 x 10^6, 65 x 10^6, 66 x 10^6, 67 x 10^6, 68 x 10^6, 69 x 10^6, 70 x 10^6, 71 x 10^6, 72 x 10^6, 73 x 10^6, 74 x 10^6, 75 x 10^6, 76 x 10^6, 77 x 10^6, 78 x 10^6, 79 x 10^6, 80 x 10^6, 81 x 10^6, 82 x 10^6, 83 x 10^6, 84 x 10^6, 85 x 10^6, 86 x 10^6, 87 x 10^6, 88 x 10^6, 89 x 10^6, 90 x 10^6, 91 x 10^6, 92 x 10^6, 93 x 10^6, 94 x 10^6, 95 x 10^6, 96 x 10^6, 97 x 10^6, 98 x 10^6, 99 x 10^6, 100 x 10^6, 101 x 10^6, 102 x 10^6, 103 x 10^6, 104 x 10^6, 105 x 10^6, 106 x 10^6, 107 x 10^6, 108 x 10^6, 109 x 10^6, or 110 x 10^6) viable CD19 specific CAR-T cells. In some embodiments, the dose is a therapeutically effective amount of viable CD19 specific CAR-T cells. In other embodiments, the dose is a clinically effective amount of viable CD19 specific CAR-T cells. In some embodiments, the viable CD19 specific CAR-T cells include CD19 specific CAR expressing CD4+ T cells and CD19 specific CAR expressing CD8+ T cells at a ratio of about 1:1. In some embodiments, the CD19 specific CAR of the cells is lisocabtagene maraleucel (BREYANZI®), a structural equivalent thereof, or a functional equivalent thereof.

[001174] In some embodiments, a subject is administered about 50 x 10^6 to about 110 x 10^6 (e.g., 50 x 10^6, 51 x 10^6, 52 x 10^6, 53 x 10^6, 54 x 10^6, 55 x 10^6, 56 x 10^6, 57 x 10^6, 58 x 10^6, 59 x 10^6, 60 x 10^6, 61 x 10^6, 62 x 10^6, 63 x 10^6, 64 x 10^6, 65 x 10^6, 66 x 10^6, 67 x 10^6, 68 x 10^6, 69 x 10^6, 70 x 10^6, 71 x 10^6, 72 x 10^6, 73 x 10^6, 74 x 10^6, 75 x 10^6, 76 x 10^6, 77 x 10^6, 78 x 10^6, 79 x 10^6, 80 x 10^6, 81 x 10^6, 82 x 10^6, 83 x 10^6, 84 x 10^6, 85 x 10^6, 86 x 10^6, 87 x 10^6, 88 x 10^6, 89 x 10^6, 90 x 10^6, 91 x 10^6, 92 x 10^6, 93 x 10^6, 94 x 10^6, 95 x 10^6, 96 x 10^6, 97 x 10^6, 98 x 10^6, 99 x 10^6, 100 x 10^6, 101 x 10^6, 102 x 10^6, 103 x 10^6, 104 x 10^6, 105 x 10^6, 106 x 10^6, 107 x 10^6, 108 x 10^6, 109 x 10^6, or 110 x 10^6) viable CD19 specific CAR-T cells described herein. In some embodiments, the dose is a therapeutically effective amount of viable CD19 specific CAR-T

cells. In other embodiments, the dose is a clinically effective amount of viable CD19 specific CAR-T cells. In some instances, 50% of the viable CD19 specific CAR-T cells are CD19 specific CAR expressing CD4+ T cells and 50% of the viable CD19 specific CAR-T cells are CD19 specific CAR expressing CD8+ T cells. In some embodiments, the CD19 specific CAR of the cells is lisocabtagene maraleucel (BREYANZI®), a structural equivalent thereof, or a functional equivalent thereof.

[001175] In some embodiments, the CD19 specific CAR-T cells described herein are administered to a subject at a dose of about 2×10^6 per kg of body weight. In some embodiments, a maximum dose administered is about 2×10^8 viable CD19 specific CAR-T cells. In some embodiments, the dose is a therapeutically effective amount of viable CD19 specific CAR-T cells. In other embodiments, the dose is a clinically effective amount of viable CD19 specific CAR-T cells. In some embodiments, the CD19 specific CAR of the cells is the same CD19 specific CAR as axicabtagene ciloleucel (YESCARTA®), a structural equivalent thereof, or a functional equivalent thereof.

[001176] In some embodiments, the CD19 specific CAR-T cells described herein are administered to a subject at a dose of about 2×10^6 per kg of body weight. In some embodiments, a maximum dose of about 2×10^8 viable CD19 specific CAR-T cells is administered to a patient of about 100 kg of body weight and above. In some embodiments, the dose is a therapeutically effective amount of viable CD19 specific CAR-T cells. In other embodiments, the dose is a clinically effective amount of viable CD19 specific CAR-T cells. In some embodiments, the CD19 specific CAR of the cells is the same CD19 specific CAR as brexucabtagene autoleucel (TECARTUS®), a structural equivalent thereof, or a functional equivalent thereof.

[001177] In some embodiments, the CD19 specific CAR-T cells described herein are administered to a subject at a dose of up to about 2×10^8 viable CD19 specific CAR-T cells. In some embodiments, a subject is administered from about 0.2×10^6 to about 5.0×10^6 (e.g., about 0.2×10^6, 0.4×10^6, 0.5×10^6, 0.6×10^6, 0.8×10^6, 0.9×10^6, 1.0×10^6, 1.2×10^6, 1.4×10^6, 1.5×10^6, 1.6×10^6, 1.8×10^6, 1.9×10^6, 2.0×10^6, 2.2×10^6, 2.4×10^6, 2.5×10^6, 2.6×10^6, 2.8×10^6, 2.9×10^6, 3.0×10^6, 3.2×10^6, 3.4×10^6, 3.5×10^6, 3.6×10^6, 3.8×10^6, 3.9×10^6, 4.0×10^6, 4.2×10^6, 4.4×10^6, 4.5×10^6, 4.6×10^6, 4.8×10^6, 4.9×10^6, or 5.0×10^6) viable CD19 specific CAR-

T cells per kg of body weight for a subject with a body weight of about 50 kg or less. In some embodiments, a subject is administered from about 0.1 x 10^8 to about 2.5 x 10^8 (e.g., about 0.1 x 10^6, 0.2 x 10^6, 0.4 x 10^6, 0.5 x 10^6, 0.6 x 10^6, 0.8 x 10^6, 0.9 x 10^6, 1.0 x 10^6, 1.2 x 10^6, 1.4 x 10^6, 1.5 x 10^6, 1.6 x 10^6, 1.8 x 10^6, 1.9 x 10^6, 2.0 x 10^6, 2.2 x 10^6, 2.4 x 10^6, or 2.5 x 10^6) viable CD19 specific CAR-T cells for a subject with a body weight of greater than about 50 kg. In some embodiments, a subject is administered from about 0.6 x 10^8 to about 6.0 x 10^8 (e.g., about 0.6 x 10^8, 0.8 x 10^8, 0.9 x 10^8, 1.0 x 10^8, 1.2 x 10^8, 1.4 x 10^8, 1.5 x 10^8, 1.6 x 10^8, 1.8 x 10^8, 1.9 x 10^8, 2.0 x 10^8, 2.2 x 10^8, 2.4 x 10^8, 2.5 x 10^8, 2.6 x 10^8, 2.8 x 10^8, 2.9 x 10^8, 3.0 x 10^8, 3.2 x 10^8, 3.4 x 10^8, 3.5 x 10^8, 3.6 x 10^8, 3.8 x 10^8, 3.9 x 10^8, 4.0 x 10^8, 4.2 x 10^8, 4.4 x 10^8, 4.5 x 10^8, 4.6 x 10^8, 4.8 x 10^8, 4.9 x 10^8, 5.0 x 10^8, 5.2 x 10^8, 5.4 x 10^8, 5.5 x 10^8, 5.6 x 10^8, 5.8 x 10^8, 5.9 x 10^8, or 6.0 x 10^8) viable CD19 specific CAR-T cells. In some embodiments, the dose is a therapeutically effective amount of viable CD19 specific CAR-T cells. In other embodiments, the dose is a clinically effective amount of viable CD19 specific CAR-T cells. In some embodiments, the CD19 specific CAR of the cells is the same CD19 specific CAR as tisagenlecleucel (KYMRIAH®), a structural equivalent thereof, or a functional equivalent thereof.

[001178] In some embodiments, a single dose of any of the CD19 specific CAR-T cells described herein includes about 50 x 10^6 to about 110 x 10^6 (e.g., 50 x 10^6, 51 x 10^6, 52 x 10^6, 53 x 10^6, 54 x 10^6, 55 x 10^6, 56 x 10^6, 57 x 10^6, 58 x 10^6, 59 x 10^6, 60 x 10^6, 61 x 10^6, 62 x 10^6, 63 x 10^6, 64 x 10^6, 65 x 10^6, 66 x 10^6, 67 x 10^6, 68 x 10^6, 69 x 10^6, 70 x 10^6, 71 x 10^6, 72 x 10^6, 73 x 10^6, 74 x 10^6, 75 x 10^6, 76 x 10^6, 77 x 10^6, 78 x 10^6, 79 x 10^6, 80 x 10^6, 81 x 10^6, 82 x 10^6, 83 x 10^6, 84 x 10^6, 85 x 10^6, 86 x 10^6, 87 x 10^6, 88 x 10^6, 89 x 10^6, 90 x 10^6, 91 x 10^6, 92 x 10^6, 93 x 10^6, 94 x 10^6, 95 x 10^6, 96 x 10^6, 97 x 10^6, 98 x 10^6, 99 x 10^6, 100 x 10^6, 101 x 10^6, 102 x 10^6, 103 x 10^6, 104 x 10^6, 105 x 10^6, 106 x 10^6, 107 x 10^6, 108 x 10^6, 109 x 10^6, or 110 x 10^6) viable CD19 specific CAR-T cells. In some embodiments, the dose is a therapeutically effective amount of viable CD19 specific CAR-T cells. In other embodiments, the dose is a clinically effective amount of viable CD19 specific CAR-T cells. In some embodiments, the viable CD19 specific CAR-T cells include CD19 specific CAR expressing CD4+ T cells and CD19 specific CAR expressing CD8+ T cells at a ratio of about 1:1. In some embodiments, the CD19 specific CAR is the same CD19 specific CAR as lisocabtagene maraleucel (BREYANZI®), a structural equivalent thereof, or a functional equivalent thereof.

[001179] In some embodiments, a single dose of any of the CD19 specific CAR-T cells described herein includes about 2 x 10^8 viable CD19 specific CAR-T cells. In some embodiments, a single infusion bag of any of the CD19 specific CAR-T cells described herein includes about 2 x 10^8 viable CD19 specific CAR-T cells in a cell suspension of about 68 mL. In some embodiments, the CD19 specific CAR is the same CD19 specific CAR as axicabtagene ciloleucel (YESCARTA®), a structural equivalent thereof, or a functional equivalent thereof.

[001180] In some embodiments, a single dose of any of the CD19 specific CAR-T cells described herein includes about 2 x 10^8 viable CD19 specific CAR-T cells. In some embodiments, a single infusion bag of any of the CD19 specific CAR-T cells described herein includes about 2 x 10^8 viable CD19 specific CAR-T cells in a cell suspension of about 68 mL. In some embodiments, the CD19 specific CAR is the same CD19 specific CAR as brexucabtagene autoleucel (TECARTUS®), a structural equivalent thereof, or a functional equivalent thereof.

[001181] In some embodiments, a single dose of any of the CD19 specific CAR-T cells described herein includes about 0.2 x 10^6 to about 5.0 x 10^6 (e.g., about 0.2 x 10^6, 0.3 x 10^6, 0.4 x 10^6, 0.5 x 10^6, 0.6 x 10^6, 0.7 x 10^6, 0.8 x 10^6, 0.9 x 10^6, 1.0 x 10^6, 1.1 x 10^6, 1.2 x 10^6, 1.3 x 10^6, 1.4 x 10^6, 1.5 x 10^6, 1.6 x 10^6, 1.7 x 10^6, 1.8 x 10^6, 1.9 x 10^6, 2.0 x 10^6, 2.1 x 10^6,2.2 x 10^6, 2.3 x 10^6, 2.4 x 10^6, 2.5 x 10^6, 2.6 x 10^6, 2.7 x 10^6, 2.8 x 10^6, 2.9 x 10^6, 3.0 x 10^6, 3.1 x 10^6, 3.2 x 10^6, 3.3 x 10^6, 3.4 x 10^6, 3.5 x 10^6, 3.6 x 10^6, 3.7 x 10^6, 3.8 x 10^6, 3.9 x 10^6, 4.0 x 10^6, 4.1 x 10^6, 4.2 x 10^6, 4.3 x 10^6, 4.4 x 10^6, 4.5 x 10^6, 4.6 x 10^6, 4.7 x 10^6, 4.8 x 10^6, 4.9 x 10^6, or 5.0 x 10^6) viable CD19 specific CAR-T cells per kg of body weight for a subject with a body weight of 50 kg or less. In some embodiments, a single dose of any of the CD19 specific CAR-T cells described herein includes about 0.1 x 10^8 to about 2.5 x 10^8 (e.g., about 0.1 x 10^6, 0.2 x 10^6, 0.3 x 10^6, 0.4 x 10^6, 0.5 x 10^6, 0.6 x 10^6, 0.7 x 10^6, 0.8 x 10^6, 0.9 x 10^6, 1.0 x 10^6, 1.1 x 10^6, 1.2 x 10^6, 1.3 x 10^6, 1.4 x 10^6, 1.5 x 10^6, 1.6 x 10^6, 1.7 x 10^6, 1.8 x 10^6, 1.9 x 10^6, 2.0 x 10^6, 2.1 x 10^6, 2.2 x 10^6, 2.3 x 10^6, 2.4 x 10^6, or 2.5 x 10^6) viable CD19 specific CAR-T cells per kg of body weight for a subject with a body weight of more than 50 kg. In some embodiments, a single dose of any of the CD19 specific CAR-T cells described herein includes about 0.6 x 10^8 to about 6.0 x 10^8 (e.g., about 0.6 x 10^8, 0.7 x 10^8, 0.8 x 10^8, 0.9 x 10^8, 1.0 x 10^8, 1.1 x 10^8,1.2 x 10^8, 1.3 x 10^8, 1.4 x 10^8, 1.5 x 10^8, 1.6 x 10^8, 1.7 x 10^8, 1.8 x 10^8, 1.9 x 10^8, 2.0 x 10^8, 2.1 x 10^8, 2.2 x 10^8, 2.3 x 10^8, 2.4 x 10^8, 2.5 x 10^8, 2.6 x 10^8, 2.7 x 10^8, 2.8 x 10^8, 2.9 x 10^8, 3.0 x 10^8, 3.1 x 10^8, 3.2 x 10^8, 3.3 x 10^8, 3.4 x 10^8, 3.5 x 10^8, 3.6 x 10^8, 3.7 x 10^8, 3.8 x 10^8, 3.9 x 10^8, 4.0 x 10^8, 4.1 x 10^8, 4.2 x 10^8, 4.3 x

10^8, 4.4 x 10^8, 4.5 x 10^8, 4.6 x 10^8, 4.7 x 10^8, 4.8 x 10^8, 4.9 x 10^8, 5.0 x 10^8, 5.1 x 10^8, 5.2 x 10^8, 5.3 x 10^8, 5.4 x 10^8, 5.5 x 10^8, 5.6 x 10^8, 5.7 x 10^8, 5.8 x 10^8, 5.9 x 10^8, or 6.0 x 10^8) viable CD19 specific CAR-T cells. In some embodiments, a single infusion bag of any of the CD19 specific CAR-T cells described herein includes about 0.6 x 10^8 to about 6.0 x 10^8 (e.g., about 0.6 x 10^8, 0.7 x 10^8, 0.8 x 10^8, 0.9 x 10^8, 1.0 x 10^8, 1.1 x 10^8, 1.2 x 10^8, 1.3 x 10^8, 1.4 x 10^8, 1.5 x 10^8, 1.6 x 10^8, 1.7 x 10^8, 1.8 x 10^8, 1.9 x 10^8, 2.0 x 10^8, 2.1 x 10^8, 2.2 x 10^8, 2.3 x 10^8, 2.4 x 10^8, 2.5 x 10^8, 2.6 x 10^8, 2.7 x 10^8, 2.8 x 10^8, 2.9 x 10^8, 3.0 x 10^8, 3.1 x 10^8, 3.2 x 10^8, 3.3 x 10^8, 3.4 x 10^8, 3.5 x 10^8, 3.6 x 10^8, 3.7 x 10^8, 3.8 x 10^8, 3.9 x 10^8, 4.0 x 10^8, 4.1 x 10^8, 4.2 x 10^8, 4.3 x 10^8, 4.4 x 10^8, 4.5 x 10^8, 4.6 x 10^8, 4.7 x 10^8, 4.8 x 10^8, 4.9 x 10^8, 5.0 x 10^8, 5.1 x 10^8, 5.2 x 10^8, 5.3 x 10^8, 5.4 x 10^8, 5.5 x 10^8, 5.6 x 10^8, 5.7 x 10^8, 5.8 x 10^8, 5.9 x 10^8, or 6.0 x 10^8) viable CD19 specific CAR-T cells in a cell suspension of from about 10 mL to about 50 mL. In some embodiments, the dose is a therapeutically effective amount of viable CD19 specific CAR-T cells. In other embodiments, the dose is a clinically effective amount of viable CD19 specific CAR-T cells. In some embodiments, the CD19 specific CAR of the cells is the same CD19 specific CAR as tisagenlecleucel (KYMRIAH®), a structural equivalent thereof, or a functional equivalent thereof.

[001182] In some embodiments, the BCMA specific (BCMA) CAR-T cells described herein are administered to a subject at a dose of about 250 x 10^6 to about 500 x 10^6 (e.g., 250 x 10^6, 255 x 10^6, 260 x 10^6, 265 x 10^6, 270 x 10^6, 275 x 10^6, 280 x 10^6, 285 x 10^6, 290 x 10^6, 295 x 10^6, 300 x 10^6, 305 x 10^6, 310 x 10^6, 315 x 10^6, 320 x 10^6, 325 x 10^6, 330 x 10^6, 335 x 10^6, 340 x 10^6, 345 x 10^6, 350 x 10^6, 355 x 10^6, 360 x 10^6, 365 x 10^6, 370 x 10^6, 375 x 10^6, 380 x 10^6, 385 x 10^6, 390 x 10^6, 395 x 10^6, 400 x 10^6, 405 x 10^6, 410 x 10^6, 415 x 10^6, 420 x 10^6, 425 x 10^6, 430 x 10^6, 435 x 10^6, 440 x 10^6, 445 x 10^6, 450 x 10^6, 455 x 10^6, 460 x 10^6, 465 x 10^6, 470 x 10^6, 475 x 10^6, 480 x 10^6, 485 x 10^6, 490 x 10^6, 495 x 10^6, or 500 x 10^6) viable BCMA specific CAR-T cells. In some embodiments, the dose is a therapeutically effective amount of viable BCMA specific CAR-T cells. In other embodiments, the dose is a clinically effective amount of viable BCMA specific CAR-T cells. In some embodiments, the viable BCMA specific CAR-T cells include BCMA specific CAR expressing CD4+ T cells and BCMA specific CAR expressing CD8+ T cells at a ratio of about 1:1. In some embodiments, the BCMA specific CAR of the cells is idecabtagene vicleucel (ABECEMA®), a structural equivalent thereof, or a functional equivalent thereof.

[001183] In some embodiments, a subject is administered about 250 x 10^6 to about 500 x 10^6 (e.g., 250 x 10^6, 255 x 10^6, 260 x 10^6, 265 x 10^6, 270 x 10^6, 275 x 10^6, 280 x 10^6, 285 x 10^6, 290 x 10^6, 295 x 10^6, 300 x 10^6, 305 x 10^6, 310 x 10^6, 315 x 10^6, 320 x 10^6, 325 x 10^6, 330 x 10^6, 335 x 10^6, 340 x 10^6, 345 x 10^6, 350 x 10^6, 355 x 10^6, 360 x 10^6, 365 x 10^6, 370 x 10^6, 375 x 10^6, 380 x 10^6, 385 x 10^6, 390 x 10^6, 395 x 10^6, 400 x 10^6, 405 x 10^6, 410 x 10^6, 415 x 10^6, 420 x 10^6, 425 x 10^6, 430 x 10^6, 435 x 10^6, 440 x 10^6, 445 x 10^6, 450 x 10^6, 455 x 10^6, 460 x 10^6, 465 x 10^6, 470 x 10^6, 475 x 10^6, 480 x 10^6, 485 x 10^6, 490 x 10^6, 495 x 10^6, or 500 x 10^6) viable BCMA specific CAR-T cells described herein. In some embodiments, the dose is a therapeutically effective amount of viable BCMA specific CAR-T cells. In other embodiments, the dose is a clinically effective amount of viable BCMA specific CAR-T cells. In some instances, 50% of the viable BCMA specific CAR-T cells are BCMA specific CAR expressing CD4+ T cells and 50% of the viable BCMA specific CAR-T cells are BCMA specific CAR expressing CD8+ T cells. In some embodiments, the BCMA specific CAR of the cells is idecabtagene vicleucel (ABECEMA®), a structural equivalent thereof, or a functional equivalent thereof.

[001184] In some embodiments, the BCMA specific CAR-T cells described herein are administered to a subject at a dose of up to about 5 x 10^8 viable BCMA specific CAR-T cells. In some embodiments, a subject is administered from about 2.5 x 10^8 to about 5.0 x 10^8 (e.g., about 0.2 x 10^8, 0.4 x 10^8, 0.5 x 10^8, 0.6 x 10^8, 0.8 x 10^8, 0.9 x 10^8, 1.0 x 10^8, 1.2 x 10^8, 1.4 x 10^8, 1.5 x 10^8, 1.6 x 10^8, 1.8 x 10^8, 1.9 x 10^8, 2.0 x 10^8, 2.2 x 10^8, 2.4 x 10^8, 2.5 x 10^8, 2.6 x 10^8, 2.8 x 10^8, 2.9 x 10^8, 3.0 x 10^8, 3.2 x 10^8, 3.4 x 10^8, 3.5 x 10^8, 3.6 x 10^8, 3.8 x 10^8, 3.9 x 10^8, 4.0 x 10^8, 4.2 x 10^8, 4.4 x 10^8, 4.5 x 10^8, 4.6 x 10^8, 4.8 x 10^8, 4.9 x 10^8, or 5.0 x 10^8) viable BCMA specific CAR-T cells per kg of body weight. In some embodiments, the dose is a therapeutically effective amount of viable BCMA specific CAR-T cells. In other embodiments, the dose is a clinically effective amount of viable BCMA specific CAR-T cells. In some embodiments, the BCMA specific CAR of the cells is the same BCMA specific CAR as idecabtagene vicleucel (ABECEMA®), a structural equivalent thereof, or a functional equivalent thereof.

[001185] In some embodiments, a single dose of any of the BCMA specific CAR-T cells described herein includes about 250 x 10^6 to about 500 x 10^6 (e.g., 250 x 10^6, 255 x 10^6, 260 x 10^6, 265 x 10^6, 270 x 10^6, 275 x 10^6, 280 x 10^6, 285 x 10^6, 290 x 10^6, 295 x 10^6, 300 x 10^6, 305 x 10^6, 310 x 10^6, 315 x 10^6, 320 x 10^6, 325 x 10^6, 330 x 10^6, 335 x 10^6, 340 x 10^6, 345 x 10^6, 350 x 10^6, 355 x 10^6, 360 x 10^6, 365 x 10^6, 370 x 10^6, 375 x 10^6, 380 x 10^6, 385 x 10^6, 390 x 10^6, 395 x

10^6, 400 x 10^6, 405 x 10^6, 410 x 10^6, 415 x 10^6, 420 x 10^6, 425 x 10^6, 430 x 10^6, 435 x 10^6, 440 x 10^6, 445 x 10^6, 450 x 10^6, 455 x 10^6, 460 x 10^6, 465 x 10^6, 470 x 10^6, 475 x 10^6, 480 x 10^6, 485 x 10^6, 490 x 10^6, 495 x 10^6, or 500 x 10^6) viable BCMA specific CAR-T cells. In some embodiments, the dose is a therapeutically effective amount of viable BCMA specific CAR-T cells. In other embodiments, the dose is a clinically effective amount of viable BCMA specific CAR-T cells. In some embodiments, the viable BCMA specific CAR-T cells include BCMA specific CAR expressing CD4+ T cells and BCMA specific CAR expressing CD8+ T cells at a ratio of about 1:1. In some embodiments, the BCMA specific CAR is the same BCMA specific CAR as idecabtagene vicleucel (ABECEMA®), a structural equivalent thereof, or a functional equivalent thereof.

[001186] In some embodiments, a single dose of any of the BCMA specific CAR-T cells described herein includes about 250 x 10^6 to about 500 x 10^6 (e.g., 250 x 10^6, 255 x 10^6, 260 x 10^6, 265 x 10^6, 270 x 10^6, 275 x 10^6, 280 x 10^6, 285 x 10^6, 290 x 10^6, 295 x 10^6, 300 x 10^6, 305 x 10^6, 310 x 10^6, 315 x 10^6, 320 x 10^6, 325 x 10^6, 330 x 10^6, 335 x 10^6, 340 x 10^6, 345 x 10^6, 350 x 10^6, 355 x 10^6, 360 x 10^6, 365 x 10^6, 370 x 10^6, 375 x 10^6, 380 x 10^6, 385 x 10^6, 390 x 10^6, 395 x 10^6, 400 x 10^6, 405 x 10^6, 410 x 10^6, 415 x 10^6, 420 x 10^6, 425 x 10^6, 430 x 10^6, 435 x 10^6, 440 x 10^6, 445 x 10^6, 450 x 10^6, 455 x 10^6, 460 x 10^6, 465 x 10^6, 470 x 10^6, 475 x 10^6, 480 x 10^6, 485 x 10^6, 490 x 10^6, 495 x 10^6, or 500 x 10^6) viable BCMA specific CAR-T cells per kg of body weight. In some embodiments, a single dose of any of the BCMA specific CAR-T cells described herein includes about 2.5 x 10^8 to about 5.0 x 10^8 (e.g., about 0.2 x 10^8, 0.4 x 10^8, 0.5 x 10^8, 0.6 x 10^8, 0.8 x 10^8, 0.9 x 10^8, 1.0 x 10^8, 1.2 x 10^8, 1.4 x 10^8, 1.5 x 10^8, 1.6 x 10^8, 1.8 x 10^8, 1.9 x 10^8, 2.0 x 10^8, 2.2 x 10^8, 2.4 x 10^8, 2.5 x 10^8, 2.6 x 10^8, 2.8 x 10^8, 2.9 x 10^8, 3.0 x 10^8, 3.2 x 10^8, 3.4 x 10^8, 3.5 x 10^8, 3.6 x 10^8, 3.8 x 10^8, 3.9 x 10^8, 4.0 x 10^8, 4.2 x 10^8, 4.4 x 10^8, 4.5 x 10^8, 4.6 x 10^8, 4.8 x 10^8, 4.9 x 10^8, or 5.0 x 10^8) viable BCMA specific CAR-T cells per kg of body weight. In some embodiments, a single dose of any of the BCMA specific CAR-T cells described herein includes about 2.5 x 10^8 to about 5.0 x 10^8 (e.g., about 0.2 x 10^8, 0.4 x 10^8, 0.5 x 10^8, 0.6 x 10^8, 0.8 x 10^8, 0.9 x 10^8, 1.0 x 10^8, 1.2 x 10^8, 1.4 x 10^8, 1.5 x 10^8, 1.6 x 10^8, 1.8 x 10^8, 1.9 x 10^8, 2.0 x 10^8, 2.2 x 10^8, 2.4 x 10^8, 2.5 x 10^8, 2.6 x 10^8, 2.8 x 10^8, 2.9 x 10^8, 3.0 x 10^8, 3.2 x 10^8, 3.4 x 10^8, 3.5 x 10^8, 3.6 x 10^8, 3.8 x 10^8, 3.9 x 10^8, 4.0 x 10^8, 4.2 x 10^8, 4.4 x 10^8, 4.5 x 10^8, 4.6 x 10^8, 4.8 x 10^8, 4.9 x 10^8, or 5.0 x 10^8) viable BCMA specific CAR-T cells. In some embodiments, a single infusion bag of any of the BCMA specific CAR-T cells described herein

includes about 2.5 x 10^8 to about 5.0 x 10^8 (e.g., about 0.2 x 10^8, 0.4 x 10^8, 0.5 x 10^8, 0.6 x 10^8, 0.8 x 10^8, 0.9 x 10^8, 1.0 x 10^8, 1.2 x 10^8, 1.4 x 10^8, 1.5 x 10^8, 1.6 x 10^8, 1.8 x 10^8, 1.9 x 10^8, 2.0 x 10^8, 2.2 x 10^8, 2.4 x 10^8, 2.5 x 10^8, 2.6 x 10^8, 2.8 x 10^8, 2.9 x 10^8, 3.0 x 10^8, 3.2 x 10^8, 3.4 x 10^8, 3.5 x 10^8, 3.6 x 10^8, 3.8 x 10^8, 3.9 x 10^8, 4.0 x 10^8, 4.2 x 10^8, 4.4 x 10^8, 4.5 x 10^8, 4.6 x 10^8, 4.8 x 10^8, 4.9 x 10^8, or 5.0 x 10^8) viable BCMA specific CAR-T cells in a cell suspension of from about 10 mL to about 500 mL. In some embodiments, the cell suspension is about 50 mL, 250 mL, or about 500 mL. In some embodiments, the dose is a therapeutically effective amount of viable BCMA specific CAR-T cells. In other embodiments, the dose is a clinically effective amount of viable BCMA specific CAR-T cells. In some embodiments, the BCMA specific CAR of the cells is the same BCMA specific CAR as idecabtagene vicleucel (ABECEMA®), a structural equivalent thereof, or a functional equivalent thereof.

II. Methods for Administering Hypoimmunogenic Cells Including T Cells

[001187] As is described in further detail herein, provided herein are methods for treating a patient with a condition, disorder, or disorder through administration of hypoimmunogenic cells, particularly hypoimmunogenic T cells. As will be appreciated, for all the multiple embodiments described herein related to the timing and/or combinations of therapies, the administration of the cells is accomplished by a method or route which results in at least partial localization of the introduced cells at a desired site. The cells can be infused, implanted, or transplanted directly to the desired site, or alternatively be administered by any appropriate route which results in delivery to a desired location in the subject where at least a portion of the implanted cells or components of the cells remain viable.

[001188] Provided herein are methods for treating a patient with a condition, disorder, or disorder includes administration of a population of hypoimmunogenic cells (*e.g.*, primary T cells, T cells differentiated from hypoimmunogenic induced pluripotent stem cells, or other cells differentiated from hypoimmunogenic induced pluripotent stem cells described herein) to a subject, *e.g.*, a human patient. For instance, a population of hypoimmunogenic primary T cells such as, but limited to, CD3+ T cells, CD4+ T cells, CD8+ T cells, naïve T cells, regulatory T (Treg) cells, non-regulatory T cells, Th1 cells, Th2 cells, Th9 cells, Th17 cells, T-follicular helper (Tfh) cells, cytotoxic T lymphocytes (CTL), effector T (Teff) cells, central memory T (Tcm) cells, effector memory T (Tem) cells, effector memory T cells that express CD45RA (TEMRA cells), tissue-resident memory (Trm) cells, virtual memory T cells, innate memory T

cells, memory stem cell (Tsc), γδ T cells, and any other subtype of T cell is administered to a patient to treat a condition, disorder, or disorder. In some embodiments, an immunosuppressive and/or immunomodulatory agent (such as, but not limited to a lymphodepletion agent) is not administered to the patient before the administration of the population of hypoimmunogenic cells. In some embodiments, an immunosuppressive and/or immunomodulatory agent is administered at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more before the administration of the cells. In some embodiments, an immunosuppressive and/or immunomodulatory agent is administered at least 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks or more before the administration of the cells. In numerous embodiments, an immunosuppressive and/or immunomodulatory agent is not administered to the patient after the administration of the cells, or is administered at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more after the administration of the cells. In some embodiments, an immunosuppressive and/or immunomodulatory agent is administered 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 the administration of the cells. In some embodiments where an immunosuppressive and/or immunomodulatory agent is administered to the patient before or after the administration of the cells, the administration is at a lower dosage than would be required for cells with one or more MHC I and/or MHC II molecule expression and without exogenous expression of CD47.

[001189] Non-limiting examples of an immunosuppressive and/or immunomodulatory agent (such as, but not limited to a lymphodepletion agent) include cyclosporine, azathioprine, mycophenolic acid, mycophenolate mofetil, corticosteroids such as 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 similar agents. In some embodiments, the immunosuppressive and/or immunomodulatory agent is selected from a group of immunosuppressive antibodies consisting of antibodies binding to p75 of the IL-2 receptor, antibodies binding to, for instance, 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, or CD58, and antibodies binding to any of their ligands. In some embodiments, such an immunosuppressive and/or immunomodulatory agent may be selected from soluble IL-15R, IL-10, B7 molecules (*e.g.*, B7-1, B7-2, variants thereof, and fragments thereof), ICOS, and OX40,

an inhibitor of a negative T cell regulator (such as an antibody against CTLA-4) and similar agents.

[001190] In some embodiments, where an immunosuppressive and/or immunomodulatory agent is administered to the patient before or after the administration of the cells, the administration is at a lower dosage than would be required for cells with one or more MHC I and/or MHC II molecule expression, TCR expression and without exogenous expression of CD47. In some embodiments, where an immunosuppressive and/or immunomodulatory agent is administered to the patient before or after the first administration of the cells, the administration is at a lower dosage than would be required for cells with one or more MHC I and MHC II expression, TCR expression and without exogenous expression of CD47.

[001191] In some embodiments, the cells described are co-administered with a therapeutic agent that that binds to and/or interacts with one or more receptors selected from the group consisting of CD94, KIR2DL4, PD-1, an inhibitory NK cell receptor, and an activating NK receptor. In some instances, the therapeutic agent binds to a receptor on the surface of an NK cell, including one or more subpopulations of NK cells. In some embodiments, the therapeutic agent is selected from the group consisting of an antibody and fragments and variants thereof, an antibody mimetic, a small molecule, a blocking peptide, and a receptor antagonist.

[001192] For therapeutic application, cells prepared according to the disclosed methods can typically be supplied in the form of a pharmaceutical composition comprising an isotonic excipient, and are prepared under conditions that are sufficiently sterile for human administration. For general principles in medicinal formulation of cell compositions, see "Cell Therapy: Stem Cell Transplantation, Gene Therapy, and Cellular Immunotherapy," by Morstyn & Sheridan eds, Cambridge University Press, 1996; and "Hematopoietic Stem Cell Therapy," E. D. Ball, J. Lister & P. Law, Churchill Livingstone, 2000. The cells can be packaged in a device or container suitable for distribution or clinical use.

[001193] In some embodiments, the cells described herein are contraindicated in patients with known Type I hypersensitivity or anaphylactic reactions to murine proteins, Chinese Hamster Ovary (CHO) cell proteins, or to any component of the compositions described herein. In some embodiments, the cells described herein are contraindicated in patients who have or have had progressive multifocal leukoencephalopathy (PML). In some embodiments, the cells described herein are not recommended for use in patients with severe, active infections.

[001194] In some embodiments, the cells described herein are administered to a subject with an autoimmune disease/disorder and/or inflammatory disease/disorder who has been previously treated with rituximab (RITUXAN®). In some embodiments, the cells described herein are administered to a subject with an autoimmune disease/disorder and/or inflammatory disease/disorder who has been previously treated with rituximab (RITUXAN®) and has failed and/or not responded to the rituximab treatment. In some embodiments, the patent has rheumatoid arthritis (RA). In some embodiments, the patient has RA and the rituximab treatment is in combination with methotrexate. In some embodiments, the patient is an adult patient that has moderately-to severely-active RA. In some embodiments, the patient is an adult patient that has moderately-to severely-active RA and the rituximab treatment is in combination with methotrexate. In some embodiments, the patient is an adult patient that has moderately-to severely-active RA who has inadequate response to one or more TNF antagonist therapies and the rituximab treatment is in combination with methotrexate. In some embodiments, the rituximab dose for RA in combination with methotrexate is two-1000 mg intravenous infusions separated by 2 weeks (one course) every 24 weeks and/or based on clinical evaluation, but not sooner than every 16 weeks. In some embodiments, the Methylprednisolone 100 mg intravenous or equivalent glucocorticoid is recommended 30 minutes prior to each infusion.

[001195] In some embodiments, the cells described herein are administered to a subject with an autoimmune disease/disorder and/or inflammatory disease/disorder who has been previously treated with rituximab (RITUXAN®). In some embodiments, the cells described herein are administered to a subject with an autoimmune disease/disorder and/or inflammatory disease/disorder who has been previously treated with rituximab (RITUXAN®) and has failed and/or not responded to the rituximab treatment. In some embodiments, the patent has granulomatosis with polyangiitis (GPA) (Wegener's Granulomatosis). In some embodiments, the patent has Microscopic polyangiitis (MPA) in adult patients in combination with glucocorticoids. In some embodiments, the rituximab dose for GPA and MPA in combination with glucocorticoids is 375 mg/m^2 once weekly for 4 weeks. In some embodiments, the rituximab is administered as a 100 mg/10 mL solution in a single-use vial. In some embodiments, the rituximab is administered as a 500 mg/50 mL solution in a single-use vial.

[001196] *Immunosuppressive Agent*

[001197] In some embodiments, an immunosuppressive and/or immunomodulatory agent is not administered to the patient before the first administration of the population of engineered primary cells, or a composition containing the same.

[001198] In some embodiments, an immunosuppressive and/or immunomodulatory agent may be administered to a patient received administration of engineered primary cells. In some embodiments, the immunosuppressive and/or immunomodulatory agent is administered prior to administration of the engineered primary cells. In some embodiments, the immunosuppressive and/or immunomodulatory agent is administered prior to administration of a first and/or second administration of engineered primary cells.

[001199] Non-limiting examples of an immunosuppressive and/or immunomodulatory agent include cyclosporine, azathioprine, mycophenolic acid, mycophenolate mofetil, corticosteroids such as 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 similar agents. In some embodiments, the immunosuppressive and/or immunomodulatory agent is selected from a group of immunosuppressive antibodies consisting of antibodies binding to p75 of the IL-2 receptor, antibodies binding to, for instance, 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, or CD58, and antibodies binding to any of their ligands. In some embodiments where an immunosuppressive and/or immunomodulatory agent is administered to the patient before or after the first administration of the cells, the administration is at a lower dosage than would be required for cells with one or more MHC class I molecules and/or one or more MHC class II molecules expression and without exogenous expression of CD47.

[001200] In one embodiment, such an immunosuppressive and/or immunomodulatory agent may be selected from soluble IL-15R, IL-10, B7 molecules (e.g., B7-1, B7-2, variants thereof, and fragments thereof), ICOS, and OX40, an inhibitor of a negative T cell regulator (such as an antibody against CTLA-4) and similar agents.

[001201] In some embodiments, an immunosuppressive and/or immunomodulatory agent can be administered to the patient before the first administration of the population of engineered primary cells. In some embodiments, an immunosuppressive and/or immunomodulatory agent is

administered at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more before the first administration of the cells. In some embodiments, an immunosuppressive and/or immunomodulatory agent is administered at least 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks or more before the first administration of the cells.

[001202] In particular embodiments, an immunosuppressive and/or immunomodulatory agent is not administered to the patient after the first administration of the cells, or is administered at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more after the first administration of the cells. In some embodiments, an immunosuppressive and/or immunomodulatory agent is administered 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 the first administration of the cells.

[001203] In some embodiments, an immunosuppressive and/or immunomodulatory agent is not administered to the patient before the administration of the population of enginered cells. In many embodiments, an immunosuppressive and/or immunomodulatory agent is administered to the patient before the first and/or second administration of the population of engineered primary cells. In some embodiments, an immunosuppressive and/or immunomodulatory agent is administered at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more before the administration of the cells. In some embodiments, an immunosuppressive and/or immunomodulatory agent is administered at least 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks or more before the first and/or second administration of the cells. In particular embodiments, an immunosuppressive and/or immunomodulatory agent is administered at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more after the administration of the cells. In some embodiments, an immunosuppressive and/or immunomodulatory agent is administered 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 the first and/or second administration of the cells.

[001204] In some embodiments where an immunosuppressive and/or immunomodulatory agent is administered to the patient before or after the administration of the cells, the administration is at a lower dosage than would be required for immunogenic cells (e.g. a population of cells of the same or similar cell type or phenotype but that do not contain the modifications, e.g. genetic modifications, of the engineered primary cells, e.g. with one or more MHC class I molecules

and/or one or more MHC class II molecules expression and without exogenous expression of CD47.

ADDITIONAL EMBODIMENTS

[001205] In some embodiments, provided herein is an engineered cell comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of one or more tolerogenic factors, relative to a cell of the same cell type that does not comprise the modifications.

[001206] In some embodiments, provided herein is a hypoimmunogenic cell comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of one or more tolerogenic factors, relative to a cell of the same cell type that does not comprise the modifications.

[001207] In some embodiments, the regulatable modifications comprise regulatable knock out of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules.

[001208] In some embodiments, the regulatable modifications comprise regulatable reduced expression of one or more targets selected from B2M and CIITA relative to a cell of the same cell type that does not comprise the modifications.

[001209] In some embodiments, the regulatable modifications comprise regulatable knock out of one or more targets selected from B2M and CIITA.

[001210] In some embodiments, provided herein is an engineered cell comprising regulatable modifications that i) reduce expression of one or more targets selected from beta-2-microglobulin (B2M) and MHC class II transactivator (CIITA), and ii) increase expression of one or more tolerogenic factors, relative to a cell of the same cell type that does not comprise the modifications.

[001211] In some embodiments, provided herein is ahypoimmunogenic cell comprising regulatable modifications that i) reduce expression of one or more targets selected from beta-2-microglobulin (B2M) and MHC class II transactivator (CIITA), and ii) increase expression of

one or more tolerogenic factors, relative to a cell of the same cell type that does not comprise the modifications.

[001212] In some embodiments, the cell further comprises regulatable modifications that reduce or knock out expression of one or more Y chromosome genes.

[001213] In some embodiments, the cell comprises regulatable modifications that reduce or knock out expression of one or more targets selected from Protocadherin-11 Y-linked and Neuroligin-4 Y-linked relative to a cell of the same cell type that does not comprise the modifications.

[001214] In some embodiments, the regulatable modifications comprise a conditional or inducible RNA-based component for reducing or knocking out expression of the one or more targets relative to a cell of the same cell type that does not comprise the modifications.

[001215] In some embodiments, the conditional or inducible RNA-based component is selected from the group consisting of conditional or inducible shRNAs, conditional or inducible siRNAs, conditional or inducible miRNAs, and conditional or inducible CRISPR interference (CRISPRi).

[001216] In some embodiments, the conditional RNA-based component is under the control of a conditional promoter selected from the group consisting of a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, and a differentiation-induced promoter.

[001217] In some embodiments, the inducible RNA-based component is under the control of an inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[001218] In some embodiments, the regulatable modifications comprise a conditional or inducible DNA-based component for reducing or knocking out expression of the one or more targets relative to a cell of the same cell type that does not comprise the modifications.

[001219] In some embodiments, the conditional or inducible DNA-based component is a knock out using a method selected from the group consisting of conditional or inducible CRISPRs, conditional or inducible TALENs, conditional or inducible zinc finger nucleases, conditional or inducible homing endonucleases, and conditional or inducible meganucleases.

[001220] In some embodiments, the conditional DNA-based component is under the control of a conditional promoter selected from the group consisting of a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, and a differentiation-induced promoter.

[001221] In some embodiments, the conditional DNA-based component is under the control of an inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[001222] In some embodiments, the regulatable modifications comprise a conditional or inducible protein-based component for reducing or knocking out expression of the one or more targets relative to a cell of the same cell type that does not comprise the modifications.

[001223] In some embodiments, the conditional or inducible protein-based component is a conditional or inducible degron method.

[001224] In some embodiments, the conditional or inducible degron method is selected from the group consisting of ligand induced degradation (LID) using a SMASH tag, LID using Shield-1, LID using auxin, LID using rapamycin, conditional or inducible peptidic degrons (*e.g.*, IKZF3 based degrons), and conditional or inducible proteolysis-targeting chimeras (PROTACs).

[001225] In some embodiments, the conditional protein-based component is under the control of a conditional promoter selected from the group consisting of a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, and a differentiation-induced promoter.

[001226] In some embodiments, the protein-based component is under the control of an inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[001227] In some embodiments, the cell comprises a conditional promoter operably linked to an exogenous polynucleotide encoding one or more tolerogenic factors.

[001228] In some embodiments, the cell comprises (i) an exogenous polynucleotide comprising a conditional promoter operably linked to a transposase, and (ii) an exogenous polynucleotide comprising a transposon comprising a cargo polynucleotide encoding one or more tolerogenic factors.

[001229] In some embodiments, the conditional promoter is a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, or a differentiation-induced promoter.

[001230] In some embodiments, the cell comprises an inducible promoter operably linked to an exogenous polynucleotide encoding one or more tolerogenic factors.

[001231] In some embodiments, the cell comprises (i) an exogenous polynucleotide comprising an inducible promoter operably linked to a transposase, and (ii) an exogenous polynucleotide comprising a transposon comprising a cargo polynucleotide encoding one or more tolerogenic factors.

[001232] In some embodiments, the inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[001233] In some embodiments, the one or more tolerogenic factors are selected from the group consisting of HLA-C, HLA-E, HLA-F, HLA-G, PD-L1, CTLA-4-Ig, CD47, CI-inhibitor, and IL-35.

[001234] In some embodiments, the cell comprises a conditional or an inducible promoter operably linked to an exogenous polynucleotide encoding CD47.

[001235] In some embodiments, the cell comprises a CD47 polypeptide having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:13.

[001236] In some embodiments, the cell comprises a CD47 polypeptide having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:14.

[001237] In some embodiments, the cell expresses an increased amount of CD47 relative to a cell of the same cell type that does not comprise the modifications.

[001238] In some embodiments, the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications.

[001239] In some embodiments, the cell expresses at least about a 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, or 900%, higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications.

[001240] In some embodiments, the cell expresses at least about a 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications.

[001241] In some embodiments, the cell further comprises regulatable modifications that reduce expression of B2M, CIITA, NLRC5, TRAC, TRB, CD142, ABO, MIC-A/B, CD38, CD52, PCDH11Y, NLGN4Y and/or RHD relative to a cell of the same cell type that does not comprise the modifications.

[001242] In some embodiments, the cell does not express B2M, CIITA, NLRC5, TRAC, TRB, CD142, ABO, MIC-A/B, CD38, CD52, PCDH11Y, NLGN4Y and/or RHD.

[001243] In some embodiments, the cell further comprises regulatable modifications that increase expression of one or more of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 relative to a cell of the same cell type that does not comprise the modifications.

[001244] In some embodiments, the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, higher amount of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 relative to a cell of the same cell type that does not comprise the modifications.

[001245] In some embodiments, the cell expresses at least about a 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, or 900%, higher amount of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 relative to a cell of the same cell type that does not comprise the modifications.

[001246] In some embodiments, the cell expresses at least about a 1000% higher amount of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 relative to a cell of the same cell type that does not comprise the modifications.

[001247] In some embodiments, the cell is derived from a human cell or an animal cell.

[001248] In some embodiments, the human cell or the animal cell is a pluripotent stem cell.

[001249] In some embodiments, the pluripotent stem cell is an induced pluripotent stem cell (iPSC), a mesenchymal stem cell (MSC), or an embryonic stem cell (ESC).

[001250] In some embodiments, the engineered cell or the hypoimmunogenic cell is a differentiated cell derived from a pluripotent stem cell or a progeny thereof.

[001251] In some embodiments, the pluripotent stem cell is an induced pluripotent stem cell (iPSC), a mesenchymal stem cell (MSC), or an embryonic stem cell (ESC).

[001252] In some embodiments, the differentiated cell is selected from the group consisting of a T cell, a natural killer (NK) cell, an endothelial cell, a pancreatic islet cell, a cardiac muscle cell, a smooth muscle cell, a skeletal muscle cell, a hepatocyte, a glial progenitor cell, a dopaminergic neuron, a retinal pigment epithelial cell, and a thyroid cell.

[001253] In some embodiments, the engineered cell or the hypoimmunogenic cell is a primary immune cell or a progeny thereof.

[001254] In some embodiments, the primary immune cell or a progeny thereof is a T cell or an NK cell.

[001255] In some embodiments, the T cell further comprises reduced expression of T cell receptor (TCR)-alpha and/or TCR-beta.

[001256] In some embodiments, the T cell does not express TCR-alpha and/or TCR-beta.

[001257] In some embodiments, the T cell further comprises a second exogenous polynucleotide encoding one or more chimeric antigen receptors (CARs).

[001258] In some embodiments, the first and/or second exogenous polynucleotide is inserted into a first and/or second specific locus of at least one allele of the cell.

[001259] In some embodiments, the first and/or second specific loci are selected from the group consisting of a safe harbor locus, a target locus, an *RHD* locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus, and a *TRB* locus.

[001260] In some embodiments, the safe harbor locus is selected from the group consisting of a *CCR5* locus, a *PPP1R12C* locus, a *Rosa* locus, and a *CLYBL* locus.

[001261] In some embodiments, the target locus is selected from the group consisting of a *CXCR4* locus, an *ALB* locus, a *SHS231* locus, an *F3* (*CD142*) locus, a *MICA* locus, a *MICB* locus, a *LRP1* (*CD91*) locus, a *HMGB1* locus, an *ABO* locus, a *FUT1* locus, and a *KDM5D* locus.

[001262] In some embodiments, the first and/or second exogenous polynucleotide is introduced into the engineered cell or the hypoimmunogenic cell using a lentiviral vector.

[001263] In some embodiments, the first and/or second exogenous polynucleotide is introduced into the engineered cell or the hypoimmunogenic cell using fusogen-mediated delivery or a transposase system selected from the group consisting of conditional or inducible transposases, conditional or inducible PiggyBac transposons, conditional or inducible Sleeping Beauty (SB11) transposons, conditional or inducible Mos1 transposons, and conditional or inducible Tol2 transposons.

[001264] In some embodiments, the differentiated cell or the progeny thereof, or the primary immune cell or the progeny thereof evades NK cell mediated cytotoxicity upon administration to a recipient patient.

[001265] In some embodiments, the differentiated cell or the progeny thereof, or the primary immune cell or the progeny thereof is protected from cell lysis by mature NK cells upon administration to a recipient patient.

[001266] In some embodiments, the differentiated cell or the progeny thereof, or the primary immune cell or the progeny thereof evades macrophage engulfment upon administration to a recipient patient.

[001267] In some embodiments, the differentiated cell or the progeny thereof, or the primary immune cell or the progeny thereof does not induce an innate and/or an adaptive immune response to the cell upon administration to a recipient patient.

[001268] In some embodiments, the differentiated cell or the progeny thereof, or the primary immune cell or the progeny thereof does not induce an antibody-based immune response to the cell upon administration to a recipient patient.

[001269] In some embodiments, provided herein is an engineered cell comprising regulatable modifications that increase expression of CD47, relative to a cell of the same cell type that does not comprise the modifications.

[001270] In some embodiments, provided herein is a hypoimmunogenic cell comprising regulatable modifications that increase expression of CD47, relative to a cell of the same cell type that does not comprise the modifications.

[001271] In some embodiments, the cell comprises a conditional promoter operably linked to an exogenous polynucleotide encoding CD47.

[001272] In some embodiments, the cell comprises (i) an exogenous polynucleotide comprising a conditional promoter operably linked to a transposase, and (ii) an exogenous polynucleotide comprising a transposon comprising a cargo polynucleotide encoding CD47.

[001273] In some embodiments, the conditional promoter is a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, or a differentiation-induced promoter.

[001274] In some embodiments, the cell comprises an inducible promoter operably linked to an exogenous polynucleotide encoding CD47.

[001275] In some embodiments, the cell comprises (i) an exogenous polynucleotide comprising an inducible promoter operably linked to a transposase, and (ii) an exogenous polynucleotide comprising a transposon comprising a cargo polynucleotide encoding CD47.

[001276] In some embodiments, the inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[001277] In some embodiments, the exogenous polynucleotide cell comprises a CD47 polypeptide having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:13.

[001278] In some embodiments, the cell comprises a CD47 polypeptide having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:14.

[001279] In some embodiments, the cell expresses an increased amount of CD47 relative to a cell of the same cell type that does not comprise the modifications.

[001280] In some embodiments, the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications.

[001281] In some embodiments, the cell expresses at least about a 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, or 900%, higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications.

[001282] In some embodiments, the cell expresses at least about a 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications.

[001283] In some embodiments, the cell further comprises regulatable modifications that reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, relative to a cell of the same cell type that does not comprise the modifications.

[001284] In some embodiments, the cell comprises regulatable knock out of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules.

[001285] In some embodiments, the regulatable modifications comprise regulatable reduced expression of one or more targets selected from B2M and CIITA relative to a cell of the same cell type that does not comprise the modifications.

[001286] In some embodiments, the regulatable modifications comprise regulatable knock out of one or more targets selected from B2M and CIITA.

[001287] In some embodiments, the cell further comprises regulatable modifications that reduce or knock out expression of one or more Y chromosome genes.

[001288] In some embodiments, the cell comprises regulatable modifications that reduce or knock out expression of one or more targets selected from Protocadherin-11 Y-linked and Neuroligin-4 Y-linked relative to a cell of the same cell type that does not comprise the modifications.

[001289] In some embodiments, the regulatable modifications comprise a conditional or inducible RNA-based component for reducing or knocking out expression of the one or more targets relative to a cell of the same cell type that does not comprise the modifications.

[001290] In some embodiments, the conditional or inducible RNA-based component is selected from the group consisting of conditional or inducible shRNAs, conditional or inducible siRNAs, conditional or inducible miRNAs, and conditional or inducible CRISPR interference (CRISPRi).

[001291] In some embodiments, the conditional RNA-based component is under the control of a conditional promoter selected from the group consisting of a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, and a differentiation-induced promoter.

[001292] In some embodiments, the inducible RNA-based component is under the control of an inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[001293] In some embodiments, the regulatable modifications comprise a conditional or inducible DNA-based component for reducing or knocking out expression of the one or more targets relative to a cell of the same cell type that does not comprise the modifications.

[001294] In some embodiments, the conditional or inducible DNA-based component is a knock out using a method selected from the group consisting of conditional or inducible CRISPRs, conditional or inducible TALENs, conditional or inducible zinc finger nucleases, conditional or inducible homing endonucleases, and conditional or inducible meganucleases.

[001295] In some embodiments, the conditional DNA-based component is under the control of a conditional promoter selected from the group consisting of a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, and a differentiation-induced promoter.

[001296] In some embodiments, the conditional DNA-based component is under the control of an inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[001297] In some embodiments, the regulatable modifications comprise a conditional or inducible protein-based component for reducing or knocking out expression of the one or more targets relative to a cell of the same cell type that does not comprise the modifications.

[001298] In some embodiments, the conditional or inducible protein-based component is a conditional or inducible degron method.

[001299] In some embodiments, the conditional or inducible degron method is selected from the group consisting of ligand induced degradation (LID) using a SMASH tag, LID using Shield-1,

LID using auxin, LID using rapamycin, conditional or inducible peptidic degrons (*e.g.*, IKZF3 based degrons), and conditional or inducible proteolysis-targeting chimeras (PROTACs).

[001300] In some embodiments, the conditional protein-based component is under the control of a conditional promoter selected from the group consisting of a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, and a differentiation-induced promoter.

[001301] In some embodiments, the protein-based component is under the control of an inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[001302] In some embodiments, the cell further comprises a conditional promoter operably linked to an exogenous polynucleotide encoding one or more further tolerogenic factors.

[001303] In some embodiments, the cell comprises (i) an exogenous polynucleotide comprising a conditional promoter operably linked to a transposase, and (ii) an exogenous polynucleotide comprising a transposon comprising a cargo polynucleotide encoding one or more further tolerogenic factors.

[001304] In some embodiments, the conditional promoter is a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, or a differentiation-induced promoter.

[001305] In some embodiments, the cell comprises an inducible promoter operably linked to an exogenous polynucleotide encoding one or more further tolerogenic factors.

[001306] In some embodiments, the cell comprises (i) an exogenous polynucleotide comprising an inducible promoter operably linked to a transposase, and (ii) an exogenous polynucleotide comprising a transposon comprising a cargo polynucleotide encoding one or more further tolerogenic factors.

[001307] In some embodiments, the inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[001308] In some embodiments, the one or more further tolerogenic factors are selected from the group consisting of HLA-C, HLA-E, HLA-F, HLA-G, PD-L1, CTLA-4-Ig, CI-inhibitor, and IL-35.

[001309] In some embodiments, the cell further comprises regulatable modifications that reduce expression of B2M, CIITA, NLRC5, TRAC, TRB, CD142, ABO, MIC-A/B, CD38, CD52, PCDH11Y, NLGN4Y and/or RHD relative to a cell of the same cell type that does not comprise the modifications.

[001310] In some embodiments, the cell does not express B2M, CIITA, NLRC5, TRAC, TRB, CD142, ABO, MIC-A/B, CD38, CD52, PCDH11Y, NLGN4Y and/or RHD.

[001311] In some embodiments, the cell further comprises regulatable modifications that increase expression of one or more of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 relative to a cell of the same cell type that does not comprise the modifications.

[001312] In some embodiments, the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, higher amount of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 relative to a cell of the same cell type that does not comprise the modifications.

[001313] In some embodiments, the cell expresses at least about a 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, or 900%, higher amount of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 relative to a cell of the same cell type that does not comprise the modifications.

[001314] In some embodiments, the cell expresses at least about a 1000% higher amount of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 relative to a cell of the same cell type that does not comprise the modifications.

[001315] In some embodiments, the cell is derived from a human cell or an animal cell.

[001316] In some embodiments, the human cell or the animal cell is a pluripotent stem cell.

[001317] In some embodiments, the pluripotent stem cell is an induced pluripotent stem cell (iPSC), a mesenchymal stem cell (MSC), or an embryonic stem cell (ESC).

[001318] In some embodiments, the engineered cell or the hypoimmunogenic cell is a differentiated cell derived from a pluripotent stem cell or a progeny thereof.

[001319] In some embodiments, the pluripotent stem cell is an induced pluripotent stem cell (iPSC), a mesenchymal stem cell (MSC), or an embryonic stem cell (ESC).

[001320] In some embodiments, the differentiated cell is selected from the group consisting of a T cell, a natural killer (NK) cell, an endothelial cell, a pancreatic islet cell, a cardiac muscle cell, a smooth muscle cell, a skeletal muscle cell, a hepatocyte, a glial progenitor cell, a dopaminergic neuron, a retinal pigment epithelial cell, and a thyroid cell.

[001321] In some embodiments, the engineered cell or the hypoimmunogenic cell is a primary immune cell or a progeny thereof.

[001322] In some embodiments, the primary immune cell or a progeny thereof is a T cell or an NK cell.

[001323] In some embodiments, the T cell further comprises reduced expression of T cell receptor (TCR)-alpha and/or TCR-beta.

[001324] In some embodiments, the T cell does not express TCR-alpha and/or TCR-beta.

[001325] In some embodiments, the T cell further comprises a second exogenous polynucleotide encoding one or more chimeric antigen receptors (CARs).

[001326] In some embodiments, the first and/or second exogenous polynucleotide is inserted into a first and/or second specific locus of at least one allele of the cell.

[001327] In some embodiments, the first and/or second specific loci are selected from the group consisting of a safe harbor locus, a target locus, an *RHD* locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus, and a *TRB* locus.

[001328] In some embodiments, the safe harbor locus is selected from the group consisting of a *CCR5* locus, a *PPP1R12C* locus, a *Rosa* locus, and a *CLYBL* locus.

[001329] In some embodiments, the target locus is selected from the group consisting of a *CXCR4* locus, an *ALB* locus, a *SHS231* locus, an *F3* (*CD142*) locus, a *MICA* locus, a *MICB* locus, a *LRP1* (*CD91*) locus, a *HMGB1* locus, an *ABO* locus, a *FUT1* locus, and a *KDM5D* locus.

[001330] In some embodiments, the first and/or second exogenous polynucleotide is introduced into the engineered cell or the hypoimmunogenic cell using a lentiviral vector.

[001331] In some embodiments, the first and/or second exogenous polynucleotide is introduced into the engineered cell or the hypoimmunogenic cell using fusogen-mediated delivery or a transposase system selected from the group consisting of conditional or inducible transposases, conditional or inducible PiggyBac transposons, conditional or inducible Sleeping Beauty (SB11) transposons, conditional or inducible Mos1 transposons, and conditional or inducible Tol2 transposons.

[001332] In some embodiments, the differentiated cell or the progeny thereof, or the primary immune cell or the progeny thereof evades NK cell mediated cytotoxicity upon administration to a recipient patient.

[001333] In some embodiments, the differentiated cell or the progeny thereof, or the primary immune cell or the progeny thereof is protected from cell lysis by mature NK cells upon administration to a recipient patient.

[001334] In some embodiments, the differentiated cell or the progeny thereof, or the primary immune cell or the progeny thereof evades macrophage engulfment upon administration to a recipient patient.

[001335] In some embodiments, the differentiated cell or the progeny thereof, or the primary immune cell or the progeny thereof does not induce an innate and/or an adaptive immune response to the cell upon administration to a recipient patient.

[001336] In some embodiments, the differentiated cell or the progeny thereof, or the primary immune cell or the progeny thereof does not induce an antibody-based immune response to the cell upon administration to a recipient patient.

[001337] In some embodiments, provided herein is an engineered endothelial cell comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC

class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of CD47, relative to an endothelial cell that does not comprise the modifications, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications, and wherein the endothelial cell is derived from a pluripotent stem cell or a progeny thereof.

[001338] In some embodiments, provided herein is an engineered pancreatic islet cell comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of CD47, relative to a pancreatic islet cell cell that does not comprise the modifications, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications, and wherein the pancreatic islet cell cell is derived from a pluripotent stem cell or a progeny thereof.

[001339] In some embodiments, provided herein is an engineered cardiac muscle cell comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of CD47, relative to a cardiac muscle cell that does not comprise the modifications, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications, and wherein the cardiac muscle cell is derived from a pluripotent stem cell or a progeny thereof.

[001340] In some embodiments, provided herein is an engineered smooth muscle cell comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of CD47, relative to a smooth muscle cell that does not comprise the modifications, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of

CD47 relative to a cell of the same cell type that does not comprise the modifications, and wherein the smooth muscle cell is derived from a pluripotent stem cell or a progeny thereof.

[001341] In some embodiments, provided herein is an engineered skeletal muscle cell comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of CD47, relative to a skeletal muscle cell that does not comprise the modifications, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications, and wherein the skeletal muscle cell is derived from a pluripotent stem cell or a progeny thereof.

[001342] In some embodiments, provided herein is an engineered hepatocyte comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of CD47, relative to a hepatocyte that does not comprise the modifications, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications, and wherein the hepatocyte is derived from a pluripotent stem cell or a progeny thereof.

[001343] In some embodiments, provided herein is an engineered glial progenitor cell comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of CD47, relative to a glial progenitor cell that does not comprise the modifications, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications, and wherein the glial progenitor cell is derived from a pluripotent stem cell or a progeny thereof.

[001344] In some embodiments, provided herein is an engineered dopaminergic neuron comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of CD47, relative to a dopaminergic neuron that does not comprise the modifications,

wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications, and wherein the dopaminergic neuron is derived from a pluripotent stem cell or a progeny thereof.

[001345] In some embodiments, provided herein is an engineered retinal pigment epithelial cell comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of CD47, relative to a retinal pigment epithelial cell that does not comprise the modifications, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications, and wherein the retinal pigment epithelial cell is derived from a pluripotent stem cell or a progeny thereof.

[001346] In some embodiments, provided herein is an engineered thyroid cell comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of CD47, relative to a thyroid cell that does not comprise the modifications, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications, and wherein the thyroid cell is derived from a pluripotent stem cell or a progeny thereof.

[001347] In some embodiments, provided herein is an engineered T cell comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of CD47, relative to a T cell that does not comprise the modifications, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications, wherein the T cell optionally further comprises an exogenous polynucleotide encoding one or more chimeric antigen receptors (CARs), and

wherein the T cell is derived from a pluripotent stem cell or a progeny thereof, or the T cell is a primary immune cell or a progeny thereof.

[001348] In some embodiments, provided herein is an engineered NK cell comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of CD47, relative to a NK cell that does not comprise the modifications, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications, wherein the NK cell optionally further comprises an exogenous polynucleotide encoding one or more chimeric antigen receptors (CARs), and wherein the NK cell is derived from a pluripotent stem cell or a progeny thereof, or the NK cell is a primary immune cell or a progeny thereof.

[001349] In some embodiments, provided herein is a cell that expresses at least about a 10% higher amount of CD47 relative to a wild-type cell or a control cell of the same cell type, or that expresses at least about 1.1-fold of the level of CD47 expressed in a wild-type cell or a control cell of the same cell type.

[001350] In some embodiments, provided herein is a cell that expresses at least about a 10% higher amount of CD47 relative to a starting cell from a donor or a pool of starting cells from a pool of donors, or that expresses at least about 1.1-fold of the level of CD47 expressed in a starting cell from a donor or a pool of starting cells from a pool of donors.

[001351] In some embodiments, the cell expresses at least about a 20%, about a 30%, about a 40%, about a 50%, about a 60%, about a 70%, about a 80%, about a 90%, about a 100%, about a 200%, about a 300%, about a 400%, about a 500%, about a 600%, about a 700%, about a 800%, about a 900%, or about a 1000% higher amount of CD47 relative to the wild-type cell or the control cell.

[001352] In some embodiments, the cell expresses at least about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, or about 5-fold of the level of CD47 expressed in the wild-type cell of the same cell type.

[001353] In some embodiments, provided herein is a T cell having reduced expression of one or more MHC class I HLA and/or reduced expression of one or more MHC class II HLA and that expresses at least about a 10% higher amount of CD47 relative to a wild-type T cell or a control T cell, that expresses at least about 1.1-fold of the level of CD47 expressed in a wild-type T cell or a control T cell, or that expresses at least about 170,000 CD47 molecules.

[001354] In some embodiments, the cell is a T cell that expresses at least about a 300% or at least about a 400% higher amount of CD47 relative to a wild-type T cell or a control T cell.

[001355] In some embodiments, the cell is a T cell that expresses at least about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, or about 5-fold of the level of CD47 expressed in a wild-type T cell or a control T cell.

[001356] In some embodiments, provided herein is a T cell that expresses at least about 170,000 CD47 molecules.

[001357] In some embodiments, the T cell expresses at least about 180,000 CD47 molecules, at least about 190,000 CD47 molecules, at least about 200,000 CD47 molecules, at least about 210,000 CD47 molecules, at least about 220,000 CD47 molecules, at least about 230,000 CD47 molecules, at least about 240,000 CD47 molecules, at least about 250,000 CD47 molecules, at least about 260,000 CD47 molecules, at least about 270,000 CD47 molecules, at least about 280,000 CD47 molecules, at least about 290,000 CD47 molecules, or at least about 300,000 CD47 molecules.

[001358] In some embodiments, provided herein is a T cell that expresses at least about a 10% higher amount of CD47 relative to a starting T cell from a donor or a pool of starting T cells from a pool of donors, or that expresses at least about 1.1-fold of the level of CD47 expressed in a starting cell from a donor or a pool of starting cells from a pool of donors.

[001359] In some embodiments, the cell is a primary pancreatic islet cell that expresses at least about a 1000% or at least about a 2000% higher amount of CD47 relative to a wild-type pancreatic islet cell or a control pancreatic islet cell.

[001360] In some embodiments, provided herein is a pancreatic islet cell having reduced expression of one or more MHC class I HLA and/or reduced expression of one or more MHC

class II HLA and that expresses at least about a 1000% higher amount of CD47 relative to a wild-type pancreatic islet cell or a control pancreatic islet cell.

[001361] In some embodiments, the cell is a primary beta islet cell that expresses at least about 16-fold, about 17-fold, about 18-fold, about 19-fold, or about 20-fold of the level of CD47 expressed in a wild-type beta islet cell or a control beta islet cell.

[001362] In some embodiments, the cell comprises 1, 2, 3, 4, or 5 copies of an exogenous polynucleotide encoding CD47.

[001363] In some embodiments, the cell comprises a constitutive promoter operably linked to an exogenous polynucleotide encoding CD47.

[001364] In some embodiments, the exogenous polynucleotide encoding CD47 is delivered to the cell via viral mediated integration.

[001365] In some embodiments, the viral mediated integration is lentivirus mediated.

[001366] In some embodiments, the exogenous polynucleotide encoding CD47 is integrated at a site in the cell genome via HDR.

[001367] In some embodiments, the exogenous polynucleotide encoding CD47 is integrated into a locus in the TRAC gene, a locus in the TRBC gene, or a combination thereof.

[001368] In some embodiments, the exogenous polynucleotide encoding CD47 is integrated into at least one TRAC allele, at least one TRBC allele, or a combination thereof.

[001369] In some embodiments, the exogenous polynucleotide encoding CD47 is integrated into at least two TRAC alleles, at least two TRBC alleles, or a combination thereof.

[001370] In some embodiments, the cell comprises an exogenous polynucleotide comprising a CD47 polypeptide having at least about 95% sequence identity to the amino acid sequence of SEQ ID NO:13, at least about 98% sequence identity to the amino acid sequence of SEQ ID NO:13, at least about 99% sequence identity to the amino acid sequence of SEQ ID NO:13, or having the amino acid sequence of SEQ ID NO:13.

[001371] In some embodiments, the cell comprises an exogenous polynucleotide comprising a CD47 polypeptide having at least about 95% sequence identity to the amino acid sequence of SEQ ID NO:14, at least about 98% sequence identity to the amino acid sequence of SEQ ID

NO:14, at least about 99% sequence identity to the amino acid sequence of SEQ ID NO:14, or having the amino acid sequence of SEQ ID NO:14.

[001372] In some embodiments, the cell comprises reduced expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules (HLA), relative to the wild-type cell or the control cell of the same cell type that does not comprise the modifications.

[001373] In some embodiments, the reduced expression of the one or more of MHC class I and MHC class II HLA, is caused by constitutve modifications to one or more genes encoding the MHC class I and/or class II HLA.

[001374] In some embodiments, the cell comprises one or more knock outs of targets selected from MHC class I and MHC class II HLA.

[001375] In some embodiments, the one or more knock outs are constitutive knock outs.

[001376] In some embodiments, the cell comprises reduced expression of one or more targets selected from B2M and CIITA, relative to the wild-type cell or the control cell of the same cell type that does not comprise the modifications.

[001377] In some embodiments, the reduced expression of B2M and/or CIITA is caused by constitutive modifications to the B2M gene and/or the CIITA gene.

[001378] In some embodiments, wherein the cell comprises one or more knock outs of targets selected from B2M and CIITA.

[001379] In some embodiments, the cell comprises knock outs of both alleles of B2M and/or both alleles of CIITA.

[001380] In some embodiments, the one or more knock outs are constitutive knock outs.

[001381] In some embodiments, the cell further comprises an exogenous polynucleotide encoding one or more further tolerogenic factors.

[001382] In some embodiments, the one or more further tolerogenic factors are selected from the group consisting of HLA-C, HLA-E, HLA-F, HLA-G, PD-L1, CTLA-4-Ig, CI-inhibitor, and IL-35.

[001383] In some embodiments, the cell comprises reduced expression of B2M, CIITA, NLRC5, TRAC, TRB, CD142, ABO, MIC-A/B, CD38, CD52, PCDH11Y, NLGN4Y and/or RHD, relative to the wild-type cell or the control cell of the same cell type.

[001384] In some embodiments, the cell does not express B2M, CIITA, NLRC5, TRAC, TRB, CD142, ABO, MIC-A/B, CD38, CD52, PCDH11Y, NLGN4Y and/or RHD.

[001385] In some embodiments, the cell is a pluripotent stem cell.

[001386] In some embodiments, the pluripotent stem cell is an induced pluripotent stem cell (iPSC), a mesenchymal stem cell (MSC), or an embryonic stem cell (ESC).

[001387] In some embodiments, the cell is a differentiated cell derived from a pluripotent stem cell or a progeny thereof.

[001388] In some embodiments, the differentiated cell is selected from the group consisting of a T cell, a natural killer (NK) cell, an endothelial cell, a pancreatic islet cell, a cardiac muscle cell, a smooth muscle cell, a skeletal muscle cell, a hepatocyte, a glial progenitor cell, a dopaminergic neuron, a retinal pigment epithelial cell, and a thyroid cell.

[001389] In some embodiments, the cell is a primary cell or a progeny thereof.

[001390] In some embodiments, the primary cell or a progeny thereof is a T cell or an NK cell.

[001391] In some embodiments, the T cell further comprises reduced expression of T cell receptor (TCR)-alpha and/or TCR-beta.

[001392] In some embodiments, the T cell does not express TCR-alpha and/or TCR-beta.

[001393] In some embodiments, the T cell further comprises a second exogenous polynucleotide encoding one or more chimeric antigen receptors (CARs).

[001394] In some embodiments, the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 expression relative to a control cell of the same or a different cell type that has no or low expression of CD47, and reduced expression of one or more of MHC class I and MHC class II human leukocyte antigen molecules, relative to the control cell.

[001395] In some embodiments, the cell expresses at least about 2-fold, about 3-fold, about 4-fold, or about 5-fold of the level of CD47 expressed in a control cell of the same or a different

cell type that has no or low expression of CD47, and reduced expression of one or more of MHC class I and MHC class II human leukocyte antigen molecules, relative to the control cell.

[001396] In some embodiments, the cell expresses at least about 3-fold, about 4-fold, or about 5-fold of the level of CD47 expressed in a control cell of the same cell type that has no or low expression of CD47.

[001397] In some embodiments, the control cell is a T cell, a natural killer (NK) cell, an endothelial cell, a pancreatic islet cell, a cardiac muscle cell, a smooth muscle cell, a skeletal muscle cell, a hepatocyte, a glial progenitor cell, a dopaminergic neuron, a retinal pigment epithelial cell, or a thyroid cell.

[001398] In some embodiments, the differentiated cell or the progeny thereof, or the primary immune cell or the progeny thereof evades NK cell mediated cytotoxicity upon administration to a recipient patient, is protected from cell lysis by mature NK cells upon administration to a recipient patient, evades macrophage engulfment upon administration to a recipient patient, does not induce an innate and/or an adaptive immune response to the cell upon administration to a recipient patient, and/or does not induce an antibody-based immune response to the cell upon administration to a recipient patient.

[001399] In some embodiments, provided herein is a pharmaceutical composition comprising a population of the engineered cells or of the hypoimmunogenic cells described herein, and a pharmaceutically acceptable additive, carrier, diluent or excipient.

[001400] In some embodiments, provided herein is a method of treating a patient with a disease or condition who would benefit from a cell-based therapy, comprising administering a population of the engineered cells or of the hypoimmunogenic cells described herein to the patient.

[001401] In some embodiments, provided herein is a method of treating a patient with a disease or condition who would benefit from a cell-based therapy, comprising administering a population of the differentiated cells described herein to the patient.

[001402] In some embodiments, the differentiated cells are selected from the group consisting of T cells, natural killer (NK) cells, endothelial cells, pancreatic islet cells, cardiac muscle cells,

smooth muscle cells, skeletal muscle cells, hepatocytes, glial progenitor cells, dopaminergic neurons, retinal pigment epithelial cells, and thyroid cells.

[001403] In some embodiments, provided herein is a use of a population of the engineered cells or of the hypoimmunogenic cells described herein for treating a disorder or condition in a recipient patient who would benefit from a cell-based therapy.

[001404] In some embodiments, provided herein is a method for producing an engineered cell comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and/or MHC class II human leukocyte antigen molecules, and ii) increase expression of one or more tolerogenic factors, relative to a cell of the same cell type that does not comprise the modifications, the method comprising:

(a) obtaining an isolated cell;

(b) introducing into the cell a conditional or inducible RNA-based component for regulatable reduced expression of the one or more targets, a conditional or inducible DNA-based component for regulatable reduced expression of the one or more targets, or a conditional or inducible protein-based component for regulatable reduced expression of the one or more targets;

(c) exposing the cell to a condition or an exogenous factor to activate the conditional or inducible method, thereby causing reduced expression of the MHC class I and/or MHC class II human leukocyte antigen molecules;

(d) introducing into the isolated cell a nucleic acid comprising a conditional or inducible promoter operably linked to an exogenous polynucleotide encoding the one or more tolerogenic factors; and

(e) exposing the engineered cell to a condition or an exogenous factor to activate the conditional or inducible promoter, thereby causing expression of the exogenous one or more tolerogenic factors, and thereby producing the engineered cell.

[001405] In some embodiments, steps (a)-(d) are carried out in any order.

[001406] In some embodiments, one or more of steps (a)-(d) are carried out simultaneously.

[001407] In some embodiments, steps (b) and (c) are carried out before steps (d) and (e).

[001408] In some embodiments, steps (d) and (e) are carried out before steps (b) and (c).

[001409] In some embodiments, steps (c) and (e) are carried out sequentially.

[001410] In some embodiments, steps (c) and (e) are carried out simultaneously.

[001411] In some embodiments, provided herein is a method of determining a threshold of CD47 expression level required for immune-evasion of hypoimmunogenic cells, the method comprising:

(a) producing engineered cells comprising a first exogenous polynucleotide encoding CD47;

(b) sorting the engineered cells based on CD47 expression levels, to generate pools of cells having similar CD47 expression levels;

(c) assessing the immune response induced by the pools of cells; and

(d) determining a threshold of CD47 expression level required for immune-evasion.

[001412] In some embodiments, step (a) of the method further comprises engineering the cells to comprise reduced expression of one or more Y chromosome genes and major histocompatibility complex (MHC) class I and/or class II human leukocyte antigen molecules relative to a cell of the same cell type that does not comprise the modifications.

[001413] In some embodiments, the assessing of the immune response is carried out using in vitro assays or in vivo assays.

[001414] In some embodiments, the assessing of the immune response is carried out by measuring NK cell mediated cytotoxicity, lysis by mature NK cells, macrophage engulfment, antibody-based immune response to the cells, or by measuring the percentage of the cells still present in the recipient after a certain period of time upon administration to a recipient patient.

[001415]

IV. EXAMPLES

Example 1: Generation of B2M and CIITA Double Knock Out Glial Progenitor Cells Having Regulatable Expression of Exogenous CD47

[001416] Described herein is an exemplary method for producing B2M and CIITA double knock out glial progenitor cells having regulatable expression of an exogenous CD47 transgene. An

exogenous polynucleotide encoding CD47 operably linked to conditional promoter is introduced into iPSCs using lentiviral expression technology, and the B2M and CIITA genes are inactivated in the iPSCs using CRISPR/Cas9 technology.

[001417] A. Double knock out of B2M/CIITA:

[001418] iPSC cells are edited using CRISPR/Cas9 technology by any method recognized by those in the art. In one example, guide RNAs targeting each of B2M and CIITA are complexed with sp Cas9 to form Cas9 ribonucleoproteins (RNPs). The RNPs for each of B2M and CIITA are complexed separately at a particular sgRNA:Cas9 ratio, followed by mixture of the two RNPs. The RNP mixture is then mixed with the iPSCs, and nucleofected under specific conditions. Nucleofected cells are seeded and cultured.

[001419] B. Introduction of CD47 transgene:

[001420] Polynucleotides encoding CD47 operably linked to different conditional promoters that are cell-cycle specific promoters and tissue/differentiation-specific promoters are generated using any method recognized by those in the art. Promoters that are used include, but are not limited to:

- *Cell cycle-specific promoters*: cyclin B1 promoter, Cdc25B promoter, cyclin A2 promoter, Cdc2 promoter, Cdc25C promoter, cyclin E promoter, Cdc6 promoter, DHFR promoter, and histone promoters. Without being bound by theory, use of a cell cycle-specific promoter causes expression of the operably linked CD47 transgene in the iPSCs at various stages of the cell cycle.
- *Tissue/differentiation-specific promoters*: Sox-2 promoter (neural progenitor cell specific), glial fibrillary acidic protein (GFAP) promoter (astrocyte specific), myelin basic protein (MBP) promoter (oligodendrocyte specific), human myelin associated glycoprotein (MAG) promoter (oligodendrocyte specific), aromatic amino acid decarboxylase (AADC) promoter, Ca2+-calmodulin-dependent protein kinase II-alpha (CamKIIα) promoter, CMV enhancer/platelet-derived growth factor-β promoter, DAT promoter, DNMT promoter, enkephalin promoter, ENO2 promoter, GnRH promoter, L7 promoter, MAP2 promoter, neurofilament light-chain gene promoter, neurofilament promoter, NURR1 promoter, PITX3 promoter, S100 promoter, serotonin receptor promoter, Synapsin promoter, Tau promoter, thy-1 promoter, TUBA1A promoter, TUJ1 promoter, tyrosine hydroxylase (TH) promoter, VGF promoter, VMAT2 promoter, A2B5

promoter, BLBP promoter, brain derived neurotrophic factor BDNF promoter, CD105 promoter, CD11b promoter, CD11c promoter, CD133 promoter, CD140a promoter, CD45 promoter, CD9 promoter, ciliary neurotrophic factor CNTF promoter, connexin 43 promoter, CX3CR1 promoter, EGFR promoter, epidermal growth factor EGF promoter, FGF8 promoter, FOXG1 promoter, GalC promoter, GAP-43 promoter, GD3 promoter, GLAST, glutamine synthetase promoter, IBA-1 promoter, LNGFR promoter, MBP promoter, Musashi promoter, nerve growth factor NGF promoter, nestin promoter, neutrotrophin-3 NT-3 promoter, NG2 promoter, NKX2.2 promoter, NT-4 promoter, O4 promoter, OLIG1 promoter, OLIG2 promoter, P2RY12 promoter, PAX6 promoter, PDGFαR promoter, S100β promoter, SOX10 promoter, TMEM119 promoter, and vimentin promoter. Without being bound by theory, use of a tissue/differentiation-specific promoter causes expression of the operably linked CD47 transgene in the iPSCs at various stages as they differentiate into GPCs.

[001421] Lentiviral vectors are produced by any method recognized by those in the art. For example, cell lines are transfected using standard chemical transfection complexes containing viral expression and transfer vectors harboring the polynucleotides encoding CD47 operably linked to a conditional promoter, *e.g.* a cell cycle-specific or a tissue/differentiation-specific promoter. Cell cultures are harvested and clarified post transfection, followed by centrifugation for concentration. Lentiviral pellets are resuspended to a final concentrate.

[001422] The lentivirus concentrate is mixed with iPSCs in standard culture plates, and the iPSCs are spinfected and transduced with the constructs containing the polynucleotides encoding CD47 operably linked to a conditional promoter.

[001423] Note that step A can occur before, simultaneously with, or after step B to generate B2M/CIITA double knock out iPSCs having a regulatable exogenous CD47 transgene.

[001424] Differentiation into GPCs:

[001425] Glial progenitor cells are differentiated from iPSCs by any method recognized by those in the art.

[001426] In some cases, glial cells, precursors, and progenitors thereof are generated by culturing the B2M/CIITA double knock out iPSCs having a regulatable exogenous CD47 transgene in medium comprising one or more agents selected from the group consisting of retinoic acid, IL-34, M-CSF, FLT3 ligand, GM-CSF, CCL2, a TGFbeta inhibitor, a BMP

signaling inhibitor (such as LDN193189, SB431542, or a combination thereof), a SHH signaling activator, FGF, platelet derived growth factor PDGF, PDGFR-alpha, HGF, IGF-1, noggin, sonic hedgehog (SHH), dorsomorphin, noggin, and any combination thereof. The differentiation medium includes any specific factors and/or small molecules that may facilitate or enable the generation of a glial cell type as recognized by those skilled in the art.

[001427] Separately or additionally, differentiation of pluripotent stem cells is performed by exposing or contacting cells to specific factors which are known to produce a glial cell such as a microglial cell (such as a amoeboid, ramified, activated phagocytic, and activated non-phagocytic cell), a macroglial cell (such as a astrocyte, oligodendrocyte, ependymal cell, radial glia, Schwann cell and satellite cell, a precursor thereof, and a progenitor thereof. Useful methods for generating glial cells, precursors, and progenitors thereof from stem cells are found, for example, in US Patent Nos. 7,579,188; 7,595,194; 8,263,402; 8,206,699; 8,227,247; 8,252,586; 9,193,951; 9,709,553; and 9,862,925; and US Publ. Application Nos. 2018/0187148; 2017/0198255; 2017/0183627; 2017/0182097; 2017/253856; 2018/0236004; and PCT Publ. Application Nos. WO2017/172976 and WO2018/093681, each of which is incorporated herein by reference in its entirety.

[001428] The glial cells are selected or purified using a positive selection strategy, a negative selection strategy, or both.

[001429] Characterization of GPCs:

[001430] In some cases, to monitor glial cell differentiation as well as to assess the phenotype of a glial cell, the expression of any number of molecular and genetic markers specific to glial cells and progenitors thereof are evaluated. For example, the presence of genetic markers is determined by various methods known to those skilled in the art. Expression of molecular markers is determined by quantifying methods such as, but not limited to, qPCR-based assays, RNA-seq assays, proteomic assays, immunoassays, immunocytochemistry assays, immunoblotting assays, and the like.

[001431] In some cases, the glial cells express NKX2.2, PAX6, SOX10, brain derived neurotrophic factor BDNF, neutrotrophin-3 NT-3, NT-4, epidermal growth factor EGF, ciliary neurotrophic factor CNTF, nerve growth factor NGF, FGF8, EGFR, OLIG1, OLIG2, myelin basic protein MBP, GAP-43, LNGFR, nestin, GFAP, CD11b, CD11c, CD105, CX3CR1, P2RY12, IBA-1, TMEM119, CD45, and any combination thereof. In some cases, the glial cells

including oligodendrocytes, astrocytes, and progenitors thereof express one or more of the markers selected from A2B5, CD9, CD133, CD140a, FOXG1, GalC, GD3, GFAP, nestin, NG2, MBP, Musashi, O4, Olig1, Olig2, PDGFαR, S100β, glutamine synthetase, connexin 43, vimentin, BLBP, GLAST, and the like. In some cases, the glial cells including oligodendrocytes, astrocytes, and progenitors thereof do not express one or more of the markers selected from PSA-NCAM, CD9, CD11, CD32, CD36, CD105, CD140a, nestin, PDGFαR, and the like. Any of these exemplary markers are used to characterize glial cells described herein.

[001432] Separately or additionally, the glial cells are characterized according to morphology as determined by immunocytochemistry and immunohistochemistry. In some cases, glial cells are assessed according to functional characterization assays such as, but not limited to, a neuronal co-culture assay, stimulation assay with lipopolysaccharides (LPS), in vitro myelination assay, ATP influx with calcium wave oscillation assay, and the like.

[001433] Separately or additionally, to determine that the glial cells display cell-specific characteristics and features, the cells are transplanted into an animal model. In some cases, the glial cells are injected into an immunocompromised mouse, *e. g.,* an immunocompromised shiverer mouse. The glial cells are administered to the brain of the mouse and after a pre-selected amount of time, the engrafted cells are evaluated. In some instances, the engrafted cells in the brain are visualized using immunostaining and imaging methods. In some cases, expression of known glial cell biomarkers can be determined in the engrafted cells.

[001434] Additional methods for determining the effect of neural cell transplantation in an animal model of a neurological disorder or condition are described in the following references: for spinal cord injury – Curtis *et al.*, Cell Stem Cell, 2018, 22, 941-950; for Parkinson's disease – Kikuchi *et al.*, Nature, 2017, 548:592-596; for ALS – Izrael *et al.*, Stem Cell Research, 2018, 9(1):152 and Izrael *et al.*, IntechOpen, DOI: 10. 5772/intechopen. 72862; for epilepsy – Upadhya *et al.*, PNAS, 2019, 116(1):287-296, each of which is incorporated herein by reference in its entirety.

[001435] The efficacy of neural cell transplants for spinal cord injury are assessed in, for example, a rat model for acutely injured spinal cord, as described by McDonald *et al.*, Nat. Med., 1999, 5:1410 and Kim *et al.*, Nature, 2002, 418:50. For instance, successful transplants may show transplant-derived cells present in the lesion 2-5 weeks later, differentiated into astrocytes, oligodendrocytes, and/or neurons, and migrating along the spinal cord from the lesioned end, and

an improvement in gait, coordination, and weight-bearing. Specific animal models are selected based on the neural cell type and neurological disease or condition to be treated.

Example 2: Exemplary Embodiments

[001436] Cells

[001437] An engineered cell is generated comprising regulatable modifications that i) regulatable reduced expression of one or more targets selected from beta-2-microglobulin (B2M) and MHC class II transactivator (CIITA) relative to an unaltered or unmodified wild-type cell , and ii) regulatable overexpressionincrease expression of one or more tolerogenic factors, relative to a cell of the same cell type that does not comprise the modifications.

[001438] A hypoimmunogenic cell is generated comprising regulatable modifications that i) regulatable reduced expression of one or more targets selected from beta-2-microglobulin (B2M) and MHC class II transactivator (CIITA), and ii) regulatable overexpressionincrease expression of one or more tolerogenic factors, relative to a cell of the same cell type that does not comprise the modifications.

[001439] An engineered cell is generated comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of one or more tolerogenic factors, relative to a cell of the same cell type that does not comprise the modifications.

[001440] A hypoimmunogenic cell is generated comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of one or more tolerogenic factors, relative to a cell of the same cell type that does not comprise the modifications.

[001441] Genetic modifications

[001442] The engineered cells or the hypoimmunogenic cells can be generated to comprise regulatable modifications that comprise regulatable knock out of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules. Optionally, the engineered cells or the hypoimmunogenic cells can be generated to comprise regulatable modifications that comprise regulatable reduced expression of one or more targets selected from B2M and CIITA relative to a cell of the same cell type that does not comprise the modifications. Optionally, the engineered cells or the hypoimmunogenic cells can be generated to comprise regulatable modifications that comprise regulatable knock out of one or more targets selected

from B2M and CIITA. Optionally, the engineered cells or the hypoimmunogenic cells can be generated to further comprise regulatable reduced expression or regulatable knock out of one or more Y chromosome genes. Optionally, the engineered cells or the hypoimmunogenic cells can be generated to comprise reduced expression or regulatable knock out Protocadherin-11 Y-linked and/or Neuroligin-4 Y-linked.

[001443] Conditional or inducible expression

[001444] The engineered cells or the hypoimmunogenic cells can be generated to comprise a conditional or inducible RNA-based component for regulatable reduced expression or knock out of the one or more targets relative to a cell of the same cell type that does not comprise the modifications. Optionally, the conditional or inducible RNA-based component is selected from the group consisting of conditional or inducible shRNAs, conditional or inducible siRNAs, conditional or inducible miRNAs, and conditional or inducible CRISPR interference (CRISPRi). Optionally, the conditional RNA-based component is under the control of a conditional promoter selected from the group consisting of a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, and a differentiation-induced promoter. Optionally, the inducible RNA-based component is under the control of an inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[001445] The engineered cells or the hypoimmunogenic cells can be generated to comprise a conditional or inducible DNA-based component for regulatable reduced expression knock out of the one or more targets relative to a cell of the same cell type that does not comprise the modifications. Optionally, the conditional or inducible DNA-based component is a knock out using a method selected from the group consisting of conditional or inducible CRISPRs, conditional or inducible TALENs, conditional or inducible zinc finger nucleases, conditional or inducible homing endonucleases, and conditional or inducible meganucleases. Optionally, the conditional DNA-based component is under the control of a conditional promoter selected from the group consisting of a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, and a differentiation-induced promoter. Optionally, the conditional DNA-based component is under the control of an inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[001446] The engineered cells or the hypoimmunogenic cells can be generated to comprise a conditional or inducible protein-based component for regulatable reduced expression or knock out of the one or more targets a cell of the same cell type that does not comprise the modifications. Optionally, the conditional or inducible protein-based component is a conditional or inducible degron method. Optionally, the conditional or inducible degron method is selected from the group consisting of ligand induced degradation (LID) using a SMASH tag, LID using Shield-1, LID using auxin, LID using rapamycin, conditional or inducible peptidic degrons (*e.g.*, IKZF3 based degrons), and conditional or inducible proteolysis-targeting chimeras (PROTACs). Optionally, the conditional protein-based component is under the control of a conditional promoter selected from the group consisting of a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, and a differentiation-induced promoter. Optionally, the protein-based component is under the control of an inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[001447] Optionally, the cell comprises a conditional promoter operably linked to an exogenous polynucleotide encoding one or more tolerogenic factors. Optionally, the cell comprises (i) an exogenous polynucleotide comprising a conditional promoter operably linked to a transposase, and (ii) an exogenous polynucleotide comprising a transposon comprising a cargo polynucleotide encoding one or more tolerogenic factors. Optionally, the conditional promoter is a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, or a differentiation-induced promoter.

[001448] Optionally, the cell comprises an inducible promoter operably linked to an exogenous polynucleotide encoding one or more tolerogenic factors. Optionally, the cell comprises (i) an exogenous polynucleotide comprising an inducible promoter operably linked to a transposase, and (ii) an exogenous polynucleotide comprising a transposon comprising a cargo polynucleotide encoding one or more tolerogenic factors. Optionally, the inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[001449] Optionally, the one or more tolerogenic factors are selected from the group consisting of HLA-C, HLA-E, HLA-F, HLA-G, PD-L1, CTLA-4-Ig, CD47, CI-inhibitor, and IL-35. Optionally, engineered cell or the hypoimmunogenic cell is engineered to comprise an inducible

promoter operably linked to an exogenous polynucleotide encoding CD47. Optionally, the cell comprises a CD47 polypeptide having at least 80% sequence identity to the amino acid sequence of SEQ ID NO:129. Optionally, the cell comprises a CD47 polypeptide having at least 80% sequence identity to the amino acid sequence of SEQ ID NO:130 . Optionally, the cell expresses an increased amount of CD47 relative to a cell of the same cell type that does not comprise the modifications. Optionally, the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications. Optionally, the cell expresses at least about a 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, or 900%, higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications. Optionally, the cell expresses at least about a 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications. Optionally, the cell expresses an increased amount of CD47 relative to a baseline reference. In some embodiments, the baseline reference is a background signal. In some embodiments, the baseline reference is a control signal. In some embodiments, the baseline reference is an isotype control signal. In some embodiments, the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, higher amount of CD47 relative to a control. In some embodiments, the cell expresses at least about a 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, or 900%, higher amount of CD47 relative to a control. In some embodiments, the cell expresses at least about a 1000% higher amount of CD47 relative to a control. In some embodiments, the cell expresses at least about 1.1-fold, about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold, about 10-fold, about 11-fold, about 12-fold, about 13-fold, about 14-fold, about 15-fold, about 16-fold, about 17-fold, about 18-fold, about 19-fold, or about 20-fold of the level of CD47 expressed in a control. In some embodimens, the cell expresses at least about 180,000 CD47 molecules, at least about 190,000 CD47 molecules, at least about 200,000 CD47 molecules, at least about 210,000 CD47 molecules, at least about 220,000 CD47 molecules, at least about 230,000 CD47 molecules, at least about 240,000 CD47 molecules, at least about 250,000 CD47 molecules, at least about 260,000 CD47 molecules, at least about 270,000 CD47 molecules, at least about 280,000 CD47 molecules, at least about 290,000 CD47 molecules, at least about 300,000 CD47 molecules, at least about 350,000, at least about 400,000 CD47 molecules per cell.

[001450] Any suitable method for molecule quantitation known to those skilled in the art in view of the present disclosure can be used in the disclosure. Examples of methods that can be used to determine expression level include, but are not limited to, flow cytometry-based methods, cell surface biotinylation methods, chymotrypsin methods, imaging methods, antibody laebling methods, surface plasmon resonance methods, and those described in Prasad et al., Methods Enzymol. 2010;484:179-95, and Drescher et al., Methods Mol Biol. 2009;493:323-43, both of which are incorporated herein by reference in their entireties. In some embodiments, the level of tolerogenic factor or CD47 expression is determined using an antibody-based quantitation method, optionally a Quantibrite™ assay.

[001451] Additional modifications

[001452] Optionally, the cell comprises reduced expression of B2M, CIITA, NLRC5, TRAC, TRB, CD142, ABO, MIC-A/B, CD38, CD52 PCDH11Y, NLGN4Y and/or RHD relative to a cell of the same cell type that does not comprise the modifications. Optionally, the cell does not express B2M, CIITA, NLRC5, TRAC, TRB, CD142, ABO, MIC-A/B, CD38, CD52, PCDH11Y, NLGN4Y and/or RHD. Optionally, the cell further comprises regulatable modifications that increase expression of one or more exogenous polynucleotides encoding one or more of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9. Optionally, the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, higher amount of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 relative to a cell of the same cell type that does not comprise the modifications. Optionally, the cell expresses at least about a 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, or 900%, higher amount of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 relative to a cell of the same cell type that does not comprise the modifications. Optionally, the cell expresses at least about a 1000% higher amount of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-

L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 relative to a cell of the same cell type that does not comprise the modifications.

[001453] Cell types

[001454] Optionally, the cell is derived from a human cell or an animal cell. Optionally, the human cell or the animal cell is a pluripotent stem cell. Optionally, the pluripotent stem cell is an induced pluripotent stem cell (iPSC), a mesenchymal stem cell (MSC), or an embryonic stem cell (ESC). Optionally, the cell is a differentiated cell derived from a pluripotent stem cell or a progeny thereof. Optionally, the pluripotent stem cell is an induced pluripotent stem cell (iPSC) , a mesenchymal stem cell (MSC), or an embryonic stem cell (ESC). Optionally, the differentiated cell is selected from the group consisting of a T cell, a natural killer (NK) cell, an endothelial cell, a pancreatic islet cell, a cardiac muscle cell, a smooth muscle cell, a skeletal muscle cell, a hepatocyte, a glial progenitor cell, a dopaminergic neuron, a retinal pigment epithelial cell, and a thyroid cell. Optionally, the cell is a primary immune cell or a progeny thereof.

[001455] Optionally, the primary immune cell or a progeny thereof is a T cell or an NK cell. Optionally, the T cell further comprises reduced expression of T cell receptor (TCR)-alpha and/or TCR-beta. Optionally, the T cell does not express TCR-alpha and/or TCR-beta. Optionally, the T cell further comprises a second exogenous polynucleotide encoding one or more chimeric antigen receptors (CARs).

[001456] Integration sites

[001457] Optionally, the first and/or second exogenous polynucleotide is inserted into a first and/or second specific locus of at least one allele of the cell. Optionally, the first and/or second specific loci are selected from the group consisting of a safe harbor locus, a target locus, an RHD locus, a B2M locus, a CIITA locus, a TRAC locus, and a TRB locus. Optionally, the safe harbor locus is selected from the group consisting of a CCR5 locus, a PPP1R12C locus, a Rosa locus, and a CLYBL locus. Optionally, the target locus is selected from the group consisting of a CXCR4 locus, an ALB locus, a SHS231 locus, an F3 (CD142) locus, a MICA locus, a MICB locus, a LRP1 (CD91) locus, a HMGB1 locus, an ABO locus, a FUT1 locus, and a KDM5D locus.

[001458] Optionally, the first and/or second exogenous polynucleotide is introduced into the engineered cell or the hypoimmunogenic cell using a lentiviral vector. Optionally, the first and/or second exogenous polynucleotide is introduced into the engineered cell or the hypoimmunogenic cell using fusogen-mediated delivery or a transposase system selected from the group consisting of conditional or inducible transposases, conditional or inducible PiggyBac transposons, conditional or inducible Sleeping Beauty (SB11) transposons, conditional or inducible Mos1 transposons, and conditional or inducible Tol2 transposons.

[001459] Patient response

[001460] Optionally, the cells evade NK cell mediated cytotoxicity upon administration to a recipient patient. Optionally, the cells are protected from cell lysis by mature NK cells upon administration to a recipient patient. Optionally, the cells evade macrophage engulfment upon administration to a recipient patient. Optionally, the cells do not induce an innate and/or an adaptive immune response to the cell upon administration to a recipient patient. Optionally, the cells do not induce an antibody-based immune response to the cell upon administration to a recipient patient.

[001461] Additional cells

[001462] An engineered cell is generated comprising regulatable modifications that increase expression of CD47, relative to a cell of the same cell type that does not comprise the modifications

[001463] A hypoimmunogenic cell is generated comprising regulatable modifications that increase expression of CD47, relative to a cell of the same cell type that does not comprise the modifications

[001464] Optionally, the engineered or hypoimmunogenic cell comprises a conditional promoter operably linked to an exogenous polynucleotide encoding CD47. Optionally, the cell comprises (i) an exogenous polynucleotide comprising a conditional promoter operably linked to a transposase, and (ii) an exogenous polynucleotide comprising a transposon comprising a cargo polynucleotide encoding CD47. Optionally, the conditional promoter is a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, or a differentiation-induced promoter. Optionally, the cell comprises an inducible promoter operably linked to an exogenous polynucleotide encoding CD47. Optionally, the cell comprises (i) an exogenous polynucleotide comprising an inducible promoter operably linked to a transposase, and (ii) an exogenous

polynucleotide comprising a transposon comprising a cargo polynucleotide encoding CD47. Optionally, the inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch. Optionally, the exogenous polynucleotide cell comprises a CD47 polypeptide having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:13. Optionally, the cell comprises a CD47 polypeptide having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:14.

[001465] Optionally, the cell expresses an increased amount of CD47 relative to a cell of the same cell type that does not comprise the modifications. Optionally, the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications. Optionally, the cell expresses at least about a 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, or 900%, higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications. Optionally, the cell expresses at least about a 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications.

[001466] Optionally, the cell further comprises regulatable modifications that reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, relative to a cell of the same cell type that does not comprise the modifications. Optionally, the cell comprises regulatable knock out of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules. Optionally, the regulatable modifications comprise regulatable reduced expression of one or more targets selected from B2M and CIITA relative to a cell of the same cell type that does not comprise the modifications. Optionally, the regulatable modifications comprise regulatable knock out of one or more targets selected from B2M and CIITA. Optionally,the cell further comprises regulatable modifications that reduce or knock out expression of one or more Y chromosome genes. Optionally, the cell comprises regulatable modifications that reduce or knock out expression of one or more targets selected from Protocadherin-11 Y-linked and Neuroligin-4 Y-linked relative to a cell of the same cell type that does not comprise the modifications.

[001467] Optionally, the regulatable modifications comprise a conditional or inducible RNA-based component for reducing or knocking out expression of the one or more targets relative to a cell of the same cell type that does not comprise the modifications. Optionally, the conditional or

inducible RNA-based component is selected from the group consisting of conditional or inducible shRNAs, conditional or inducible siRNAs, conditional or inducible miRNAs, and conditional or inducible CRISPR interference (CRISPRi). Optionally, the conditional RNA-based component is under the control of a conditional promoter selected from the group consisting of a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, and a differentiation-induced promoter. Optionally, the inducible RNA-based component is under the control of an inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[001468] Optionally, the regulatable modifications comprise a conditional or inducible DNA-based component for reducing or knocking out expression of the one or more targets relative to a cell of the same cell type that does not comprise the modifications. Optionally, the conditional or inducible DNA-based component is a knock out using a method selected from the group consisting of conditional or inducible CRISPRs, conditional or inducible TALENs, conditional or inducible zinc finger nucleases, conditional or inducible homing endonucleases, and conditional or inducible meganucleases. Optionally, the conditional DNA-based component is under the control of a conditional promoter selected from the group consisting of a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, and a differentiation-induced promoter. Optionally, the conditional DNA-based component is under the control of an inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[001469] Optionally, the regulatable modifications comprise a conditional or inducible protein-based component for reducing or knocking out expression of the one or more targets relative to a cell of the same cell type that does not comprise the modifications. Optionally, the conditional or inducible protein-based component is a conditional or inducible degron method. Optionally, the conditional or inducible degron method is selected from the group consisting of ligand induced degradation (LID) using a SMASH tag, LID using Shield-1, LID using auxin, LID using rapamycin, conditional or inducible peptidic degrons (*e.g.*, IKZF3 based degrons), and conditional or inducible proteolysis-targeting chimeras (PROTACs). Optionally, the conditional protein-based component is under the control of a conditional promoter selected from the group consisting of a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific

promoter, and a differentiation-induced promoter. Optionally, the protein-based component is under the control of an inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

[001470] Optionally, the cell further comprises a conditional promoter operably linked to an exogenous polynucleotide encoding one or more further tolerogenic factors. Optionally, the cell comprises (i) an exogenous polynucleotide comprising a conditional promoter operably linked to a transposase, and (ii) an exogenous polynucleotide comprising a transposon comprising a cargo polynucleotide encoding one or more further tolerogenic factors. Optionally, the conditional promoter is a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, or a differentiation-induced promoter. Optionally, the cell comprises an inducible promoter operably linked to an exogenous polynucleotide encoding one or more further tolerogenic factors. Optionally, the cell comprises (i) an exogenous polynucleotide comprising an inducible promoter operably linked to a transposase, and (ii) an exogenous polynucleotide comprising a transposon comprising a cargo polynucleotide encoding one or more further tolerogenic factors. Optionally, the inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch. Optionally, the one or more further tolerogenic factors are selected from the group consisting of HLA-C, HLA-E, HLA-F, HLA-G, PD-L1, CTLA-4-Ig, CI-inhibitor, and IL-35.

[001471] Optionally, the cell further comprises regulatable modifications that reduce expression of B2M, CIITA, NLRC5, TRAC, TRB, CD142, ABO, MIC-A/B, CD38, CD52, PCDH11Y, NLGN4Y and/or RHD relative to a cell of the same cell type that does not comprise the modifications. Optionally, the cell does not express B2M, CIITA, NLRC5, TRAC, TRB, CD142, ABO, MIC-A/B, CD38, CD52, PCDH11Y, NLGN4Y and/or RHD.

[001472] Optionally, the cell further comprises regulatable modifications that increase expression of one or more of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 relative to a cell of the same cell type that does not comprise the modifications. Optionally, the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or

90%, higher amount of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 relative to a cell of the same cell type that does not comprise the modifications. Optionally, the cell expresses at least about a 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, or 900%, higher amount of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 relative to a cell of the same cell type that does not comprise the modifications. Optionally, the cell expresses at least about a 1000% higher amount of CD47, DUX4, CD24, CD27, CD35, CD46, CD55, CD59, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, CTLA4-Ig, C1-Inhibitor, IL-10, IL-35, FasL, CCL21, CCL22, Mfge8, CD16, CD52, H2-M3, CD16 Fc receptor, IL15-RF, and/or Serpinb9 relative to a cell of the same cell type that does not comprise the modifications.

[001473] Optionally, the cell is derived from a human cell or an animal cell. Optionally, the human cell or the animal cell is a pluripotent stem cell. Optionally, the pluripotent stem cell is an induced pluripotent stem cell (iPSC), a mesenchymal stem cell (MSC), or an embryonic stem cell (ESC). Optionally, the engineered cell or the hypoimmunogenic cell is a differentiated cell derived from a pluripotent stem cell or a progeny thereof. Optionally, the pluripotent stem cell is an induced pluripotent stem cell (iPSC), a mesenchymal stem cell (MSC), or an embryonic stem cell (ESC). Optionally, the differentiated cell is selected from the group consisting of a T cell, a natural killer (NK) cell, an endothelial cell, a pancreatic islet cell, a cardiac muscle cell, a smooth muscle cell, a skeletal muscle cell, a hepatocyte, a glial progenitor cell, a dopaminergic neuron, a retinal pigment epithelial cell, and a thyroid cell. Optionally, the engineered cell or the hypoimmunogenic cell is a primary immune cell or a progeny thereof. Optionally, the primary immune cell or a progeny thereof is a T cell or an NK cell. Optionally, the T cell further comprises reduced expression of T cell receptor (TCR)-alpha and/or TCR-beta. Optionally, the T cell does not express TCR-alpha and/or TCR-beta. Optionally, the T cell further comprises a second exogenous polynucleotide encoding one or more chimeric antigen receptors (CARs). Optionally, the first and/or second exogenous polynucleotide is inserted into a first and/or second specific locus of at least one allele of the cell.

[001474] Optionally, the first and/or second specific loci are selected from the group consisting of a safe harbor locus, a target locus, an *RHD* locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus, and a *TRB* locus. Optionally, the safe harbor locus is selected from the group consisting of a *CCR5* locus, a *PPP1R12C* locus, a *Rosa* locus, and a *CLYBL* locus. Optionally, the target locus is selected from the group consisting of a *CXCR4* locus, an *ALB* locus, a *SHS231* locus, an *F3* (*CD142*) locus, a *MICA* locus, a *MICB* locus, a *LRP1* (*CD91*) locus, a *HMGB1* locus, an *ABO* locus, a *FUT1* locus, and a *KDM5D* locus. Optionally, the first and/or second exogenous polynucleotide is introduced into the engineered cell or the hypoimmunogenic cell using a lentiviral vector. Optionally, the first and/or second exogenous polynucleotide is introduced into the engineered cell or the hypoimmunogenic cell using fusogen-mediated delivery or a transposase system selected from the group consisting of conditional or inducible transposases, conditional or inducible PiggyBac transposons, conditional or inducible Sleeping Beauty (SB11) transposons, conditional or inducible Mos1 transposons, and conditional or inducible Tol2 transposons.

[001475] Optionally, the differentiated cell or the progeny thereof, or the primary immune cell or the progeny thereof evades NK cell mediated cytotoxicity upon administration to a recipient patient. Optionally, the differentiated cell or the progeny thereof, or the primary immune cell or the progeny thereof is protected from cell lysis by mature NK cells upon administration to a recipient patient. Optionally, the differentiated cell or the progeny thereof, or the primary immune cell or the progeny thereof evades macrophage engulfment upon administration to a recipient patient. Optionally, the differentiated cell or the progeny thereof, or the primary immune cell or the progeny thereof does not induce an innate and/or an adaptive immune response to the cell upon administration to a recipient patient. Optionally, the differentiated cell or the progeny thereof, or the primary immune cell or the progeny thereof does not induce an antibody-based immune response to the cell upon administration to a recipient patient.

[001476] An engineered endothelial cell is generated comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of CD47, relative to an endothelial cell that does not comprise the modifications, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 relative to a cell of the same cell type that does

not comprise the modifications, and wherein the endothelial cell is derived from a pluripotent stem cell or a progeny thereof.

[001477] An engineered pancreatic islet cell is generated comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of CD47, relative to a pancreatic islet cell cell that does not comprise the modifications, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications, and wherein the pancreatic islet cell cell is derived from a pluripotent stem cell or a progeny thereof.

[001478] An engineered cardiac muscle cell is generated comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of CD47, relative to a cardiac muscle cell that does not comprise the modifications, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications, and wherein the cardiac muscle cell is derived from a pluripotent stem cell or a progeny thereof.

[001479] An engineered smooth muscle cell is generated comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of CD47, relative to a smooth muscle cell that does not comprise the modifications, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications, and wherein the smooth muscle cell is derived from a pluripotent stem cell or a progeny thereof.

[001480] An engineered skeletal muscle cell is generated comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of CD47, relative to a skeletal muscle cell that does not comprise the modifications, wherein the cell expresses at least about a

10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications, and wherein the skeletal muscle cell is derived from a pluripotent stem cell or a progeny thereof.

[001481] An engineered hepatocyte is generated comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of CD47, relative to a hepatocyte that does not comprise the modifications, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications, and wherein the hepatocyte is derived from a pluripotent stem cell or a progeny thereof.

[001482] An engineered glial progenitor cell is generated comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of CD47, relative to a glial progenitor cell that does not comprise the modifications, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications, and wherein the glial progenitor cell is derived from a pluripotent stem cell or a progeny thereof.

[001483] An engineered dopaminergic neuron is generated comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of CD47, relative to a dopaminergic neuron that does not comprise the modifications, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications, and wherein the dopaminergic neuron is derived from a pluripotent stem cell or a progeny thereof.

[001484] An engineered retinal pigment epithelial cell is generated comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and

MHC class II human leukocyte antigen molecules, and/or ii) increase expression of CD47, relative to a retinal pigment epithelial cell that does not comprise the modifications, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications, and wherein the retinal pigment epithelial cell is derived from a pluripotent stem cell or a progeny thereof.

[001485] An engineered thyroid cell is generated comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of CD47, relative to a thyroid cell that does not comprise the modifications, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications, and wherein the thyroid cell is derived from a pluripotent stem cell or a progeny thereof.

[001486] An engineered T cell is generated comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of CD47, relative to a T cell that does not comprise the modifications, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the modifications, wherein the T cell optionally further comprises an exogenous polynucleotide encoding one or more chimeric antigen receptors (CARs), and wherein the T cell is derived from a pluripotent stem cell or a progeny thereof, or the T cell is a primary immune cell or a progeny thereof.

[001487] An engineered NK cell comprising regulatable modifications that i) reduce expression of one or more targets selected from MHC class I and MHC class II human leukocyte antigen molecules, and/or ii) increase expression of CD47, relative to a NK cell that does not comprise the modifications, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 relative to a cell of the same cell type that does not comprise the

modifications, wherein the NK cell optionally further comprises an exogenous polynucleotide encoding one or more chimeric antigen receptors (CARs), and wherein the NK cell is derived from a pluripotent stem cell or a progeny thereof, or the NK cell is a primary immune cell or a progeny thereof.

[001488] Pharmaceutical compositions

[001489] The cells can be formulated into a pharmaceutical composition. Optionally, the pharmaceutical composition comprises a population of the engineered cells or of the hypoimmunogenic cells described herein, and a pharmaceutically acceptable additive, carrier, diluent or excipient.

[001490] Methods of treatment

[001491] The cells can be used in methods of treating a patient with a disease or condition who would benefit from a cell-based therapy. Optionally, the method comprises administering a population of the engineered cells or of the hypoimmunogenic cells described herein to the patient. Optionally, the method comprises administering a population of the differentiated cells described herein to the patient. Optionally, the differentiated cells are selected from the group consisting of T cells, natural killer (NK) cells, endothelial cells, pancreatic islet cells, cardiac muscle cells, smooth muscle cells, skeletal muscle cells, hepatocytes, glial progenitor cells, dopaminergic neurons, retinal pigment epithelial cells, and thyroid cells.

[001492] Methods for producing cells

[001493] An engineered cell comprising regulatable modifictions that i) reduce expression of one or more targets selected from MHC class I and/or MHC class II human leukocyte antigen molecules, and ii) increase expression of one or more tolerogenic factors, relative to a cell of the same cell type that does not comprise the modifications, is generated by:

(a) obtaining an isolated cell;

(b) introducing into the cell a conditional or inducible RNA-based component for regulatable reduced expression of the one or more targets, a conditional or inducible DNA-based component for regulatable reduced expression of the one or more targets, or a conditional or inducible protein-based component for regulatable reduced expression of the one or more targets;

(c) exposing the cell to a condition or an exogenous factor to activate the conditional or inducible method, thereby causing reduced expression of the MHC class I and/or MHC class II human leukocyte antigen molecules;

(d) introducing into the isolated cell a nucleic acid comprising a conditional or inducible promoter operably linked to an exogenous polynucleotide encoding the one or more tolerogenic factors; and

(e) exposing the engineered cell to a condition or an exogenous factor to activate the conditional or inducible promoter, thereby causing expression of the exogenous one or more tolerogenic factors, and thereby producing the engineered cell.

[001494] Optionally, steps (a)-(d) are carried out in any order. Optionally, one or more of steps (a)-(d) are carried out simultaneously. Optionally, steps (b) and (c) are carried out before steps (d) and (e). Optionally, steps (d) and (e) are carried out before steps (b) and (c). Optionally, steps (c) and (e) are carried out sequentially. Optionally, steps (c) and (e) are carried out simultaneously.

[001495] Methods of determining CD47 threshold levels

[001496] A method of determining a threshold of CD47 expression level required for immune-evasion of hypoimmunogenic cells is carried out, the method comprising:

(a) producing engineered cells comprising a first exogenous polynucleotide encoding CD47;

(b) sorting the engineered cells based on CD47 expression levels, to generate pools of cells having similar CD47 expression levels;

(c) assessing the immune response induced by the pools of cells; and

(d) determining a threshold of CD47 expression level required for immune-evasion.

[001497] Optionally, step (a) of the method further comprises engineering the cells to comprise regulatable reduced expression of one or more Y chromosome genes and MHC class I and/or class II human leukocyte antigen molecules relative to a cell of the same cell type that does not comprise the modifications. Optionally, the assessing of the immune response is carried out using in vitro assays or in vivo assays., including, but not limited to, flow cytometry methods, antibody methods (donor specific antibody detection, ELISA, Western blot analysis, standard antibody detection methods), cell killing assays, immune cell activation assays. Optionally, the assessing of the immune response is carried out by measuring NK cell mediated cytotoxicity, lysis by mature NK cells, macrophage engulfment, antibody-based immune response to the cells, or by measuring the percentage of the cells still present in the recipient after a certain period of time upon administration to a recipient patient.

Example 3: Reducing MHC-II expression and Increasing CD47 Expression of Primary Pancreatic Islet Cells

[001498] Primary beta islet cells from B2M-knock out C57BL/6 (B6) mice (MHC haplotype $H2^b$) were transduced with a lentiviral vector containing CD47 transgene to generate $B2M^{-/-}$; CD47*tg* primary beta islets. A range of MOIs was tested for lentiviral particles carrying for the CD47 cDNA transgenes. After 24 hours, virus was removed and complete media change was performed. Cells underwent sorting using a fluorescence-labeled anti-CD47 using a standard FACS system.

[001499] $B2M^{-/-}$ primary islet cells isolated from C57BL/6 (B6) mice do not naturally express MHC-II molecules, nor are MHC-II molecules upregulated following stimulation. Surface expression of one or more MHC-I, MHC-II, and CD47 on the $B2M^{-/-}$; CD47*tg* primary beta islets were assessed by flow cytometry using antibody-specific reagents. Isotype antibodies were used as a control. The effect of varying CD47 protein levels in the $B2M^{-/-}$; CD47*tg* beta islet cells and the cells' ability to evade immune responses was evaluated.

[001500] In order to determine CD47 threshold levels required for primary beta islet cells to evade NK cell mediated cell killing, expression levels of CD47 in the $B2M^{-/-}$; CD47*tg* beta islet cells compared to isotype control were analyzed in parallel with killing by mouse NK cells. The $B2M^{-/-}$; CD47*tg* beta islet cells were analyzed by flow cytometry (using standard methods). Cells were blocked with anti-Fc receptor antibodies and stained with an anti-CD47 antibody that was concentration matched to an isotype control. As shown in FIGs. 1A-1N, the $B2M^{-/-}$; CD47*tg* beta islet cells expressed varying levels of CD47 relative to baseline. NK cell killing assays with mouse NK cells were performed on the XCelligence MP platform (ACEA BioSciences, San Diego, CA). As shown in FIGs. 1A-1N, NK cell killing was observed for the $B2M^{-/-}$; CD47*tg* beta islet cells expressing a 6.2-fold, an 8.9-fold, a 11.7-fold or a 15.3-fold increase in CD47 above baseline. In contrast, no NK cell killing was observed for the $B2M^{-/-}$; CD47*tg* beta islet cells expressing a 19.1-fold, a 38.4-fold or a 72.5-fold increase in CD47 over baseline. The data shows that mouse $B2M^{-/-}$; CD47*tg* primary beta islet cells that express CD47 at or above a threshold level evaded immune response by NK cells. Such primary mouse beta islets having 15.3-fold or less expression of CD47 relative to baseline were killed by mouse NK cells, while the primary mouse islet cells having 19.1-fold or greater expression of CD47 relative to baseline were not killed by mouse NK cells.

Example 4: Reducing MHC-I/II expression and Increasing CD47 Expression in T Cells

[001501] BD Quantibrite™ Beads were used to assess the total CD47 molecule number in B2M$^{-/-}$; CIITA$^{-/-}$; CD47*tg* primary human T cells. The effect of varying CD47 protein levels in the B2M$^{-/-}$; CIITA$^{-/-}$; CD47*tg* primary human T cells on the cells' ability to evade immune responses was evaluated.

[001502] In order to determine CD47 threshold levels required for T cells to evade NK cell mediated cell killing, NK cell and macrophage killing assays were performed on the XCelligence MP platform (ACEA BioSciences, San Diego, CA) with the modified primary T cells comprising varying numbers of CD47 molecules per cell. As shown in FIGs. 2A-2AB, NK cell and macrophage killing was observed for the B2M$^{-/-}$; CIITA$^{-/-}$ T cells expressing endogenous CD47 levels of 53,118 or 64,778 molecules per cell, as well as for the B2M$^{-/-}$; CIITA$^{-/-}$; CD47*tg* T cells expressing CD47 levels of 131,534 molecules per cell (about 2 to about 2.5-fold of the endogenous level of CD47 expressed in the control cells, or about 100% to about 150% higher amount of CD47 relative to the control cells), 95,893 molecules per cell (about 1.5 to about 1.8-fold of the endogenous level of CD47 expressed in the control cells, or about 50% to about 80% higher amount of CD47 relative to the control cells),135,284 molecules per cell (about 2.1 to about 2.5-fold of the endogenous level of CD47 expressed in the control cells, or about 110% to about 150% higher amount of CD47 relative to the control cells), 168,751 molecules per cell (about 2.6 to about 3.2-fold of the endogenous level of CD47 expressed in the control cells, or about 160% to about 220% higher amount of CD47 relative to the control cells), or 179,236 molecules per cell (about 2.8 to about 3.4-fold of the endogenous level of CD47 expressed in the control cells, or about 180% to about 240% higher amount of CD47 relative to the control cells). In contrast, no NK cell killing was observed for the B2M$^{-/-}$; CIITA$^{-/-}$;CD47*tg* T cells expressing CD47 levels of 222,777 molecules per cell (about 3.4 to about 4.2-fold of the endogenous level of CD47 expressed in the control cells, or about 240% to about 320% higher amount of CD47 relative to the control cells), 290,942 molecules per cell (about 4.5 to about 5.5-fold of the endogenous level of CD47 expressed in the control cells, or about 350% to about 450% higher amount of CD47 relative to the control cells), 369,671 molecules per cell (about 5.7 to about 7-fold of the endogenous level of CD47 expressed in the control cells, or about 470% to about 600% higher amount of CD47 relative to the control cells), 415,996 molecules per cell (about 6.4 to about 7.8-fold of the endogenous level of CD47 expressed in the control cells, or about 540%

to about 680% higher amount of CD47 relative to the control cells), 439,189 molecules per cell (about 6.8 to about 8.3-fold of the endogenous level of CD47 expressed in the control cells, or about 580% to about 730% higher amount of CD47 relative to the control cells), 585,377 molecules per cell (about 9 to about 11-fold of the endogenous level of CD47 expressed in the control cells, or about 800% to about 1000% higher amount of CD47 relative to the control cells), or 689,168 molecules per cell (about 10.6 to about 13-fold of the endogenous level of CD47 expressed in the control cells, or about 960% to about 1200% higher amount of CD47 relative to the control cells).

[001503] The data shows that $B2M^{-/-}$; $CIITA^{-/-}$;CD47*tg* primary T cells that express CD47 at or above a threshold level evaded immune response by NK cells. Such T cells having 179,236 or fewer CD47 molecules per cell were killed by NK cells and macrophages, while the T cells having 222,777 or more CD47 molecules cell were not killed by NK cells or macrophages.

Example 5: Reducing MHC-II expression and Increasing CD47 Expression of Primary Human Pancreatic Islet Cells

[001504] BD Quantibrite™ Beads were used to assess the total CD47 molecule number in $B2M^{-/-}$; CD47*tg* primary human beta islets. The effect of varying CD47 protein levels in the $B2M^{-/-}$; CD47*tg* primary beta islet cells' ability to evade immune responses was evaluated.

[001505] In order to determine CD47 threshold levels required for islet cells to evade NK cell mediated cell killing, NK cell killing assays were performed on the XCelligence MP platform (ACEA BioSciences, San Diego, CA) with the modified islet cells comprising varying numbers of CD47 molecules per cell.

[001506] As shown in FIGs. 3A-3L, NK cell killing was observed for the $B2M^{-/-}$; CD47*tg* primary beta islet cells expressing endogenous CD47 levels of 53,174 or 60,410 molecules per cell, as well as for the $B2M^{-/-}$; CD47*tg* primary beta islet cells expressing 91,558 molecules per cell (about 1.7-fold or 1.5-fold of the endogenous level of CD47 expressed in the control cells, or about 70% or 50% higher amount of CD47 relative to the control cells), 109,366 molecules per cell (about 2-fold or 1.8-fold of the endogenous level of CD47 expressed in the control cells, or about 100% or 80% higher amount of CD47 relative to the control cells), 152,475 molecules per cell (about 3-fold or 2.5-fold of the endogenous level of CD47 expressed in the control cells, or about 200% or 150% higher amount of CD47 relative to the control cells), and 184,773 molecules per cell (about 3.5-fold or 3-fold of the endogenous level of CD47 expressed in the

control cells, or about 250% or 200% higher amount of CD47 relative to the control cells). In contrast, no NK cell killing was observed for the B2M$^{-/-}$; CD47*tg* primary beta islet cells expressing CD47 levels of 203,853 molecules per cell (about 3.8-fold of the endogenous level of CD47 expressed in the control cells, or about 280% higher amount of CD47 relative to the control cells), 226,566 molecules per cell (about 4.3-fold of the endogenous level of CD47 expressed in the control cells, or about 330% higher amount of CD47 relative to the control cells), 271,191 molecules per cell (about 5-fold of the endogenous level of CD47 expressed in the control cells, or about 400% higher amount of CD47 relative to the control cells), 297,154 molecules per cell (about 5.6-fold of the endogenous level of CD47 expressed in the control cells, or about 460% higher amount of CD47 relative to the control cells), 572,920 molecules per cell (about 10.8-fold of the endogenous level of CD47 expressed in the control cells, or about 980% higher amount of CD47 relative to the control cells), or 802,429 molecules per cell (about 15.1-fold of the endogenous level of CD47 expressed in the control cells, or about 1410% higher amount of CD47 relative to the control cells).

[001507] The data shows that B2M$^{-/-}$; CD47*tg* primary human beta islet cells that express CD47 at or above a threshold level evaded immune response by NK cells. Such islet cells having 184,773 or fewer CD47 molecules per cell were killed by NK cells, while the islet cells having 203,853 or more CD47 molecules cell were not killed by NK cells.

Example 6: Editing of Primary Human RPE cells

[001508] Unmodified primary human retinal pigment epithelium (RPE) cells express HLA-I, no HLA-II, and low CD47 (see, e.g., FIGs. 4A-4C).

[001509] Primary human RPE cells were transduced with a lentiviral vector containing CD47 transgene to generate B2M$^{-/-}$; CIITA$^{-/-}$; CD47*tg* primary RPE cells. A range of MOIs was tested for lentiviral particles carrying for the CD47 cDNA transgenes. After 24 hours, virus was removed and complete media change was performed. Cells underwent sorting using a fluorescence-labeled anti-CD47 using a standard FACS system.

[001510] Surface expression of one or more HLA-I, HLA-II, and CD47 on the B2M$^{-/-}$; CIITA$^{-/-}$; CD47*tg* primary RPEs were assessed by flow cytometry using antibody-specific reagents. Isotype antibodies were used as a control. See FIGs. 5A-5D.

[001511] The effect of varying CD47 protein levels in the B2M$^{-/-}$; CIITA$^{-/-}$; CD47*tg* RPE cells and the cells' ability to evade immune responses was evaluated.

[001512] Expression levels of CD47 in the unmodified, B2M$^{-/-}$; CIITA$^{-/-}$ (dKO) and B2M$^{-/-}$; CIITA$^{-/-}$; CD47*tg* (HIP) primary RPE cells compared to isotype control were analyzed in parallel with assessment of killing by NK cells and macrophages. The RPE cells were analyzed by flow cytometry (using standard methods). Cells were blocked with anti-Fc receptor antibodies and stained with an anti-CD47 antibody that was concentration matched to an isotype control. As shown in FIGs. 6A-6I, the B2M$^{-/-}$; CIITA$^{-/-}$ (dKO) RPE cells expressed about 1.8-fold levels of CD47 relative to baseline, and the B2M$^{-/-}$; CIITA$^{-/-}$; CD47*tg* (HIP) RPE cells expressed about 20-fold levels of CD47 relative to baseline. Killing assays with NK cells and macrophages were performed on the XCelligence MP platform (ACEA BioSciences, San Diego, CA). As shown in FIGs. 7A-7I, NK and macrophage cell killing was observed for the dKO RPEs, but not for the HIP RPEs.

WHAT IS CLAIMED IS:

1. An engineered cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

2. An engineered cell comprising regulatable modifications that increase expression of CD47, relative to a control.

3. The engineered cell of claim 1 or 2, wherein the engineered cell is selected from the group consisting of a stem cell, a pluripotent stem cell (PSC), an induced pluripotent stem cell (iPSC), a mesenchymal stem cell (MSC), a hematopoietic stem cell (HSC), an embryonic stem cell (ESC), pancreatic islet cell, a beta islet cell, an immune cell, a B cell, a T cell, a natural killer (NK) cell, a natural killer T (NKT) cell, a macrophage cell, an immune privileged cell, an optic cell, a retinal pigmented epithelium cell (RPE), a hepatocyte, a thyroid cell, an endothelial cell, a skin cell, a glial progenitor cell, a neural cell, a muscle cell, a cardiac cell, and a blood cell.

4. An engineered pancreatic islet cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

5. The engineered pancreatic islet cell of claim 4, wherein the pancreatic islet cell is a beta islet cell.

6. An engineered endothelial cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

7. An engineered cardiac muscle cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

8. An engineered smooth muscle cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

9. An engineered skeletal muscle cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

10. An engineered hepatocyte comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

11. An engineered glial progenitor cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

12. An engineered dopaminergic neuron comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

13. An engineered immune privileged cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

14. An engineered retinal pigment epithelial cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

15. An engineered thyroid cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

16. An engineered immune cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

17. The engineered immune cell of claim 16, wherein the engineered immune cell comprises an exogenous polynucleotide encoding one or more chimeric antigen receptors (CARs).

18. An engineered T cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

19. The engineered T cell of claim 18, wherein the engineered T cell comprises an exogenous polynucleotide encoding one or more chimeric antigen receptors (CARs).

20. An engineered NK cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

21. The engineered T cell of claim 20, wherein the engineered T cell comprises an exogenous polynucleotide encoding one or more chimeric antigen receptors (CARs).

22. An engineered macrophage cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of CD47, relative to a control, wherein the engineered cell expresses CD47 at a threshold level or higher.

23. The engineered cell of any one of claims 1-22, wherein the cell expresses at least about the same amount of CD47, relative to the control.

24. The engineered cell of claim 23, wherein the cell is an immune privileged cell.

25. The engineered cell of any one of claims 1-24, wherein the cell expresses at least about a 10% higher amount of CD47, relative to the control.

26. The engineered cell of any one of claims 1-25, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, higher amount of CD47, relative to the control.

27. The engineered cell of any one of claims 1-26, wherein the cell expresses at least about a 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, or 900%, higher amount of CD47, relative to the control.

28. The engineered cell of any one of claims 1-27, wherein the cell expresses at least about a 1000% higher amount of CD47, relative to the control.

29. The engineered cell of any one of claims 1-24, wherein the cell expresses at least about 1.1-fold of the level of CD47 expressed in the control.

30. The engineered cell of any one of claims 1-24 or 29, wherein the cell expresses at least about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, or about 5-fold of the level of CD47 expressed in the control.

31. The engineered cell of any one of claims 1-24, 29, or 30, wherein the cell expresses at least about 4-fold, about 4.5-fold, about 5-fold, or about 5.5-fold of the level of CD47 expressed in the control.

32. The engineered cell of any one of claims 1-24 or 29-31, wherein the cell expresses at least about about 4-fold of the level of CD47 expressed in the control.

33. The engineered cell of any one of claims 1-24 or 29-32, wherein the cell expresses at least about about 4.5-fold of the level of CD47 expressed in the control.

34. The engineered cell of any one of claims 1-24 or 29-33, wherein the cell expresses at least about about 5-fold of the level of CD47 expressed in the control.

35. The engineered cell of any one of claims 1-24 or 29-34, wherein the cell expresses at least about about 5.5-fold of the level of CD47 expressed in the control.

36. The engineered cell of any one of claims 1-24 or 29-35, wherein the cell expresses at least about about 16-fold, about 17-fold, about 18-fold, about 19-fold, or about 20-fold of the level of CD47 expressed in the control.

37. The engineered cell of any one of claims 1-36, wherein the control is a wild-type cell, a control cell, or a baseline reference.

38. The engineered cell of claim 37, wherein the control cell is an unmodified or unaltered cell, optionally wherein the unmodified or unaltered cell is of the same cell type as the engineered cell.

39. The engineered cell of claim 37 or 38, wherein the control cell is a starting material from a donor or a pool of starting cells from a pool of donors.

40. The engineered cell of claim 37, wherein the baseline reference is an isotype control or a background signal level.

41. The engineered cell of claim 37 or 40, wherein the baseline is an isotype control, optionally wherein the CD47 level is determined using an antibody-based assay.

42. The engineered cell of claim 41, wherein the CD47 level is determined using an antibody-based quantitation method, optionally a Quantibrite™ assay.

43. The engineered cell of any one of claims 40-42, wherein the engineered cell is a beta islet cell that expresses at least about 200,000, 250,000, 300,000, 350,000, or 400,000 CD47 molecules per cell.

44. The engineered cell of any one of claims 40-42, wherein engineered cell is a retinal pigment epithelial cell that expresses at least about a 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 12-fold, 14-fold, 16-fold, 18-fold, 20-fold, or higher increase in CD47 expression over baseline.

45. The engineered cell of any one of claims 40-42, wherein the engineered cell is a T cell that expresses at least about 180,000, 190,000, 200,000, 210,000, 220,000, 230,000, 240,000, 250,000, 260,000, 270,000, 280,000, 290,000, 300,000, 350,000, 400,000, 450,000, 500,000, 550,000, 600,000, 650,000, or 700,000 CD47 molecules per cell.

46. An engineered cell comprising modifications that i) reduce expression of one or more MHC class I and/or MHC class II human leukocyte antigens, and ii) increase expression of one

or more tolerogenic factors, relative to a control, wherein the engineered cell expresses the tolerogenic factor at a threshold level or higher.

47. The engineered cell of claim 46, wherein the engineered cell is selected from the group consisting of a stem cell, a pluripotent stem cell (PSC), an induced pluripotent stem cell (iPSC), a mesenchymal stem cell (MSC), a hematopoietic stem cell (HSC), an embryonic stem cell (ESC), pancreatic islet cell, a beta islet cell, an immune cell, a B cell, a T cell, a natural killer (NK) cell, a natural killer T (NKT) cell, a macrophage cell, an immune privileged cell, an optic cell, a retinal pigmented epithelium cell (RPE), a hepatocyte, a thyroid cell, an endothelial cell, a skin cell, a glial progenitor cell, a neural cell, a muscle cell, a cardiac cell, and a blood cell.

48. The engineered cell of claim 46 or 47, wherein the control is a wild-type cell, a control cell, or a baseline reference.

49. The engineered cell of claim 48, wherein the control cell is an unmodified or unaltered cell, optionally wherein the unmodified or unaltered cell is of the same cell type as the engineered cell.

50. The engineered cell of claim 48 or 49, wherein the control cell is a starting material from a donor or a pool of starting cells from a pool of donors.

51. The engineered cell of claim 48, wherein the baseline reference is an isotype control or a background signal level.

52. The engineered cell of claim 48 or 51, wherein the baseline is an isotype control, optionally wherein the amount of the tolerogenic factor is determined using an antibody-based assay.

53. The engineered cell of claim 52, wherein the amount of the tolerogenic factor is determined using an antibody-based quantitation method, optionally a Quantibrite™ assay.

54. The engineered cell of any one of claims 46-53, wherein the cell expresses at least about the same amount of tolerogenic factor, relative to the control.

55. The engineered cell of claim 54, wherein the cell is an immune privileged cell.

56. The engineered cell of any one of claims 46-55, wherein the cell expresses at least about a 10% higher amount of the tolerogenic factor, relative to the control.

57. The engineered cell of any one of claims 46-56, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, higher amount of the tolerogenic factor, relative to the control.

58. The engineered cell of any one of claims 46-57, wherein the cell expresses at least about a 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, or 900%, higher amount of the tolerogenic factor, relative to the control.

59. The engineered cell of any one of claims 46-58, wherein the cell expresses at least about a 1000% higher amount of the tolerogenic factor, relative to the control.

60. The engineered cell of any one of claims 46-55, wherein the cell expresses at least about 1.1-fold of the level of the tolerogenic factor expressed in the control.

61. The engineered cell of any one of claims 46-55 or 60, wherein the cell expresses at least about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, or about 5-fold of the level of the tolerogenic factor expressed in the control.

62. The engineered cell of any one of claims 46-55, 60, or 61, wherein the cell expresses at least about 4-fold, about 4.5-fold, about 5-fold, or about 5.5-fold of the level of the tolerogenic factor expressed in the control.

63. The engineered cell of any one of claims 46-55or 60-62, wherein the cell expresses at least about about 4-fold of the level of the tolerogenic factor expressed in the control.

64. The engineered cell of any one of claims 46-55or 60-63, wherein the cell expresses at least about about 4.5-fold of the level of the tolerogenic factor expressed in the control.

65. The engineered cell of any one of claims 46-55or 60-64, wherein the cell expresses at least about about 5-fold of the level of the tolerogenic factor expressed in the control.

66. The engineered cell of any one of claims 46-55or 60-65, wherein the cell expresses at least about about 5.5-fold of the level of the tolerogenic factor expressed in the control.

67. The engineered cell of any one of claims 46-55or 60-66, wherein the cell expresses at least about about 16-fold, about 17-fold, about 18-fold, about 19-fold, or about 20-fold of the level of the tolerogenic factor expressed in the control.

68. The engineered cell of any one of claims 1-67, wherein the modifications reduce expression of:

a. MHC class I molecule;
b. MHC class II molecule; or
c. MHC class I molecule and MHC class II molecule.

69. The engineered cell of any one of claims 1-68, wherein the modifications reduce 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, relative to a control.

70. The engineered cell of any one of claims 1-66, wherein the cell does not express MHC class I molecule and/or MHC class II molecule.

71. The engineered cell of any one of claims 1-70, wherein the cell does not express 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, relative to a control.

72. The engineered cell of claim 71, wherein the modifications comprise knock out of one or more targets selected from the group consisting 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.

73. The engineered cell of any one of claims 1-72, wherein the modifications reduce expression of one or more targets selected from the group consisting of B2M, TAP1, NLRC5 and/or CIITA.

74. The engineered cell of claim 73, wherein the modifications comprise knock out of one or more targets selected from the group consisting of B2M, TAP1, NLRC5 and/or CIITA.

75. The engineered cell of claim 72 or 74, wherein the knock out occurs in both alleles.

76. The engineered cell of any one of claims 1-75, wherein the cell further comprises one or more modifications that reduce expression of CTLA-4, PD-1, IRF1, MIC-A, MIC-B, a protein that is involved in oxidative or ER stress, TRAC, TRB, CD142, ABO, CD38, PCDH11Y, NLGN4Y and/or RHD, relative to a control.

77. The engineered cell of claim 76, wherein the protein that is involved in oxidative or ER stress is selected from the group consisting of thioredoxin-interacting protein (TXNIP), PKR-like ER kinase (PERK), inositol-requiring enzyme 1α (IRE1α), and DJ-1 (PARK7).

78. The engineered cell of claim 76 or 77, wherein the modifications comprise knock out of one or more targets selected from the group consisting of CTLA-4, PD-1, IRF1, MIC-A, MIC-B, a protein that is involved in oxidative or ER stress, TRAC, TRB, CD142, ABO, CD38, PCDH11Y, NLGN4Y and/or RHD.

79. The engineered cell of claim 78, wherein the knock out occurs in both alleles.

80. The engineered cell of any one of claims 1-79, wherein the modifications reduce expression of B2M.

81. The engineered cell of any one of claims 1-79, wherein the modifications reduce expression of CIITA.

82. The engineered cell of any one of claims 1-79, wherein the modifications reduce expression of B2M and CIITA.

83. The engineered cell of any one of claims 80-82, wherein the modifications comprise knock out of B2M and/or CIITA.

84. The engineered cell of claim 83, wherein the B2M and/or CIITA knock out occurs in both alleles.

85. The engineered cell of any one of claims 1-84, wherein the modifications reduce expression of a NK cell ligand, optionally MIC-A and/or MIC-B.

86. The engineered cell of claim 85, wherein the modifications comprise knock out of MIC-A and/or MIC-B.

87. The engineered cell of claim 86, wherein the MIC-A and/or MIC-B knock out occurs in both alleles.

88. The engineered cell of any one of claims 1-87, wherein the cell further comprises a modification that reduces expression of one or more Y chromosome genes, relative to a control.

89. The engineered cell of claim 88, wherein the one or more Y chromosome genes are selected from the group consisting of Protocadherin-11 Y-linked and Neuroligin-4 Y-linked.

90. The engineered cell of any one of claims 1-89, wherein the modifications reduce expression of TXNIP.

91. The engineered cell of claim 90, wherein the modifications comprise knock out of TXNIP.

92. The engineered cell of claim 91, wherein the TXNIP knock out occurs in both alleles.

93. The engineered cell of any one of claims 1-92, wherein the cell further comprises modifications that reduce 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, a protein that is involved in oxidative or ER stress, TRAC, TRB, CD142, ABO, CD38, PCDH11Y, NLGN4Y and/or RHD.

94. The engineered cell of claim 92, wherein the cell does not express 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, a protein that is involved in oxidative or ER stress, TRAC, TRB, CD142, ABO, CD38, PCDH11Y, NLGN4Y and/or RHD.

95. The engineered cell of any one of claims 1-92, wherein the cell further comprises modifications that reduce expression of B2M, CIITA, NLRC5, TRAC, TRB, CD142, ABO, MIC-A/B, CD38, PCDH11Y, NLGN4Y and/or RHD, relative to a control.

96. The engineered cell of claim 95, wherein the cell does not express B2M, CIITA, NLRC5, TRAC, TRB, CD142, ABO, MIC-A/B, CD38, CD52, PCDH11Y, NLGN4Y and/or RHD.

97. The engineered cell of any one of claims 1-45, wherein the cell comprises further modifications that reduce expression of one or more tolerogenic factors.

98. The engineered cell of claim 97, wherein the one or more tolerogenic factors are selected from the group consisting of 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.

99. The engineered cell of any one of claims 46-67, wherein the one or more tolerogenic factors are selected from the group consisting of A20/TNFAIP3, C1-Inhibitor, CCL21, CCL22, CD16, CD16 Fc receptor, CD24, CD27, CD35, CD39, CD46, CD47, 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.

100. The engineered cell of any one of claims 46-67 and 99, wherein the one or more tolerogenic factors comprise CD47.

101. The engineered cell of any one of claims 46-67 and 97-100, wherein the one or more tolerogenic factors comprise HLA-E.

102. The engineered cell of any one of claims 46-67 and 97-101, wherein the one or more tolerogenic factors comprise CD24.

103. The engineered cell of any one of claims 46-67 and 97-102, wherein the one or more tolerogenic factors comprise PD-L1.

104. The engineered cell of any one of claims 46-67 and 97-103, wherein the one or more tolerogenic factors comprise CD46.

105. The engineered cell of any one of claims 46-67 and 97-104, wherein the one or more tolerogenic factors comprise CD55.

106. The engineered cell of any one of claims 46-67 and 97-105, wherein the one or more tolerogenic factors comprise CD59.

107. The engineered cell of any one of claims 46-67 and 97-106, wherein the one or more tolerogenic factors comprise CR1.

108. The engineered cell of any one of claims 46-67 and 97-107, wherein the one or more tolerogenic factors comprise MANF.

109. The engineered cell of any one of claims 46-67 and 97-108, wherein the one or more tolerogenic factors comprise A20/TNFAIP3.

110. The engineered cell of any one of claims 46-67 and 97-109, wherein the one or more tolerogenic factors comprise HLA-E and CD47.

111. The engineered cell of any one of claims 46-67 and 97-110, wherein the one or more tolerogenic factors comprise one or more of CD24, CD47, and/or PDL1.

112. The engineered cell of any one of claims 46-67 and 97-111, wherein the one or more tolerogenic factors comprise one or more of HLA-E, CD24, CD47, and/or PDL1.

113. The engineered cell of any one of claims 46-67 and 97-112, wherein the one or more tolerogenic factors comprise one or more of CD46, CD55, CD59, and/or CR1.

114. The engineered cell of any one of claims 46-67 and 97-113, wherein the one or more tolerogenic factors comprise one or more of HLA-E, CD46, CD55, CD59, and/or CR1.

115. The engineered cell of any one of claims 46-67 and 97-114, wherein the one or more tolerogenic factors comprise one or more of HLA-E, CD24, CD47, PDL1, CD46, CD55, CD59, and/or CR1.

116. The engineered cell of any one of claims 46-67 and 97-115, wherein the one or more tolerogenic factors comprise HLA-E and PDL1.

117. The engineered cell of any one of claims 46-67 and 97-116, wherein the one or more tolerogenic factors comprise one or more of HLA-E, PDL1, and/or A20/TNFAIP.

118. The engineered cell of any one of claims 46-67 and 97-117, wherein the one or more tolerogenic factors comprise one or more of HLA-E, PDL1, and/or MANF.

119. The engineered cell of any one of claims 46-67 and 97-118, wherein the one or more tolerogenic factors comprise one or more of HLA-E, PDL1, A20/TNFAIP, and/or MANF.

120. The engineered cell of any one of claims 1-99, wherein the modifications:

a. reduce expression of MHC class I and/or MHC class II molecules;

b. reduce expression of MIC-A and/or MIC-B;

c. increase expression of CD47, and optionally CD24 and PD-L1; and

d. increase expression of CD46, CD55, CD59 and CR1.

121. The engineered cell of any one of claims 1-99, wherein the modification:

a. reduce expression of MHC class I molecule;

b. reduce expression of MIC-A and/or MIC-B;

c. reduce expression of TXNIP; and

d. increase expression of PD-L1 and HLA-E.

122. The engineered cell of claim 121, wherein the modifications further increase expression of A20/TNFAIP3 and MANF.

123. The engineered cell of any one of claims 1-122, wherein the cell is derived from a human cell or an animal cell.

124. The engineered cell of any one of claims 1-123, wherein the cell is a differentiated cell derived from a stem cell or a progeny thereof.

125. The engineered cell of claim 124, wherein the stem cell is selected from the group consisting of a pluripotent stem cell, an induced pluripotent stem cell (iPSC), a mesenchymal stem cell (MSC), a hematopoietic stem cell (HSC), and an embryonic stem cell (ESC).

126. The engineered cell of any one of claims 1-125, wherein the cell is derived from a primary cell or a progeny thereof.

127. The engineered cell of any one of claims 1-126, wherein the cell evades NK cell mediated cytotoxicity upon administration to a recipient patient.

128. The engineered cell of any one of claims 1-127, wherein the cell is protected from cell lysis by mature NK cells upon administration to a recipient patient.

129. The engineered cell of any one of claims 1-128, wherein the cell evades macrophage engulfment upon administration to a recipient patient.

130. The engineered cell of any one of claims 1-129, wherein the cell does not induce an innate and/or an adaptive immune response to the cell upon administration to a recipient patient.

131. The engineered cell of any one of claims 1-130, wherein the cell does not induce an antibody-based immune response to the cell upon administration to a recipient patient.

132. The engineered cell of any one of claims 1-131, wherein one or more of the modifications is a regulatable modification.

133. An engineered cell comprising one or more regulatable modifications to alter the expression of one or more targets in the engineered cell, relative to a control, optionally wherein the one or more regulatable modifications increase expression of a CD47, relative to a control.

134. The engineered cell of claim 132 or 133, wherein the one or more regulatable modifications comprise a conditional or inducible RNA-based component for i) increasing or ii) reducing or knocking out expression of the one or more targets, relative to a control.

135. The engineered cell of claim 134, wherein the conditional or inducible RNA-based component is selected from the group consisting of conditional or inducible shRNAs, conditional or inducible siRNAs, conditional or inducible miRNAs, and conditional or inducible CRISPR interference (CRISPRi).

136. The engineered cell of claim 134 or 135, wherein the conditional RNA-based component is under the control of a conditional promoter selected from the group consisting of a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, and a differentiation-induced promoter.

137. The engineered cell of claim 134 or 135, wherein the inducible RNA-based component is under the control of an inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

138. The engineered cell of claim 132 or 133, wherein the regulatable modifications comprise a conditional or inducible DNA-based component for i) increasing or ii) reducing or knocking out expression of the one or more targets, relative to a control.

139. The engineered cell of claim 138, wherein the conditional or inducible DNA-based component is selected from the group consisting of conditional or inducible CRISPRs, conditional or inducible TALENs, conditional or inducible zinc finger nucleases, conditional or inducible homing endonucleases, conditional or inducible prime editing, conditional or inducible PASTE editing, and conditional or inducible meganucleases.

140. The engineered cell of claim 138 or 139, wherein the conditional DNA-based component is under the control of a conditional promoter selected from the group consisting of a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, and a differentiation-induced promoter.

141. The engineered cell of claim 138 or 139, wherein the conditional DNA-based component is under the control of an inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

142. The engineered cell of claim 138 or 139, wherein the regulatable modifications comprise a conditional or inducible protein-based component for i) increasing or ii) reducing or knocking out expression of the one or more targets, relative to a control.

143. The engineered cell of claim 142, wherein the conditional or inducible protein-based component is a conditional or inducible degron component.

144. The engineered cell cell of claim 143, wherein the conditional or inducible degron component is selected from the group consisting of ligand induced degradation (LID) using a SMASH tag, LID using Shield-1, LID using auxin, LID using rapamycin, conditional or inducible peptidic degrons (*e.g.*, IKZF3 based degrons), and conditional or inducible proteolysis-targeting chimeras (PROTACs).

145. The engineered cell of any one of claims 142-144, wherein the conditional protein-based component is under the control of a conditional promoter selected the group consisting of from a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, and a differentiation-induced promoter.

146. The engineered cell of any one of claims 142-144, wherein the protein-based component is under the control of an inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

147. The engineered cell of any one of claims 142-146, wherein the cell comprises a conditional promoter operably linked to an exogenous polynucleotide encoding the one or more tolerogenic factors or the CD47.

148. The engineered cell of any one of claims 142-146, wherein the cell comprises (i) an exogenous polynucleotide comprising a conditional promoter operably linked to a transposase, and (ii) an exogenous polynucleotide comprising a transposon comprising a cargo polynucleotide encoding the one or more tolerogenic factors or the CD47.

149. The engineered cell of claim 147 or 148, wherein the conditional promoter is selected from the group consisting of a cell cycle-specific promoter, a tissue-specific promoter, a lineage-specific promoter, and a differentiation-induced promoter.

150. The engineered cell of any one of claims 132-146, wherein the cell comprises an inducible promoter operably linked to an exogenous polynucleotide encoding the one or more tolerogenic factors or the CD47.

151. The engineered cell of any one of claims 132-146, wherein the cell comprises (i) an exogenous polynucleotide comprising an inducible promoter operably linked to a transposase, and (ii) an exogenous polynucleotide comprising a transposon comprising a cargo polynucleotide encoding the one or more tolerogenic factors or the CD47.

152. The engineered cell of claim 150 or 151, wherein the inducible promoter that is regulated by a small molecule, a ligand, a biologic agent, an aptamer-mediated modulator of polyadenylation, or an aptamer-regulated riboswitch.

153. The engineered cell of any one of claims 1-45 or 99-152, wherein the cell comprises a CD47 polypeptide having at least 80%, 85%, 90%, 95%, 98%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:129.

154. The engineered cell of any one of claims 1-45 or 99-152, wherein the cell comprises a CD47 polypeptide having at least 80%, 85%, 90%, 95%, 98%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:130.

155. The engineered cell of any one of claims 132-154, wherein the cell further comprises regulatable modifications that increase expression of one or more of 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, relative to a control.

156. The engineered cell of claim 155, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, higher amount of 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, relative to a control.

157. The engineered cell of claim 155 or 156, wherein the cell expresses at least about a 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, or 900%, higher amount of 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, relative to a control.

158. The engineered cell of any one of claims 155-157, wherein the cell expresses at least about a 1000% higher amount of 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, relative to a control.

159. The engineered cell of any one of claims 155-158, wherein the control is a wild-type cell, a control cell, or a baseline reference.

160. The engineered cell of claim 159, wherein the control cell is an unmodified or unaltered cell, optionally wherein the unmodified or unaltered cell is of the same cell type as the engineered cell.

161. The engineered cell of claim 159 or 160 wherein the control cell is a starting material from a donor or a pool of starting cells from a pool of donors.

162. The engineered cell of claim 159, wherein the baseline reference is an isotype control or a background signal level.

163. The engineered cell of any one of claims 1-162, wherein the one or more tolerogenic factors or the CD47 is encoded by a first exogenous polynucleotide.

164. The engineered cell of any one of claims 1-163, wherein the cell comprises a second exogenous polynucleotide encoding one or more chimeric antigen receptors (CARs).

165. The engineered cell of claim 163 or 164, wherein the first and/or second exogenous polynucleotide is inserted into a first and/or second specific locus of at least one allele of the cell.

166. The engineered cell of claim 165, wherein the first and/or second specific loci are selected from the group consisting of a safe harbor locus, a target locus, an *RHD* locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus, and a *TRB* locus.

167. The engineered cell of claim 166, wherein the safe harbor locus is selected from the group consisting of a *CCR5* locus, a *PPP1R12C* locus, a *Rosa* locus, a *ROSA26* gene locus, and a *CLYBL* locus.

168. The engineered cell of claim 166, wherein the target locus is selected from the group consisting of a *CXCR4* locus, an *ALB* locus, a *SHS231* locus, an *F3* (*CD142*) locus, a *MICA* locus, a *MICB* locus, a *LRP1* (*CD91*) locus, a *HMGB1* locus, an *ABO* locus, a *FUT1* locus, and a *KDM5D* locus.

169. The engineered cell of any one of claims 163-168, wherein the first and/or second exogenous polynucleotide is introduced into the cell using a lentiviral vector.

170. The engineered cell of any one of claims 163-169, wherein the first and/or second exogenous polynucleotide is introduced into the cell using fusogen-mediated delivery or a transposase system selected from the group consisting of conditional or inducible transposases, conditional or inducible PiggyBac transposons, conditional or inducible Sleeping Beauty (SB11) transposons, conditional or inducible Mos1 transposons, and conditional or inducible Tol2 transposons.

171. A pancreatic islet cell having reduced expression of MHC class I HLA and/or reduced expression of MHC class II HLA and that expresses at least about a 1000% higher amount of CD47, relative to a control.

172. The cell of claim 171, wherein the cell is a primary beta islet cell that expresses at least about 16-fold, about 17-fold, about 18-fold, about 19-fold, or about 20-fold of the level of CD47 expressed in a control.

173. An engineered that expresses at least about a 10% higher amount of CD47, relative to a control, or that expresses at least about 1.1-fold of the level of CD47 expressed in a control.

174. An engineered cell that expresses at least about a 10% higher amount of CD47, relative to a control, or that expresses at least about 1.1-fold of the level of CD47 expressed in a control.

175. The engineered cell of claim 173 or 174, wherein the cell expresses at least about a 20%, about a 30%, about a 40%, about a 50%, about a 60%, about a 70%, about a 80%, about a 90%, about a 100%, about a 200%, about a 300%, about a 400%, about a 500%, about a 600%, about a 700%, about a 800%, about a 900%, or about a 1000% higher amount of CD47, relative to the control.

176. The engineered cell of any one of claims 173-175, wherein the cell expresses at least about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, or about 5-fold of the level of CD47 expressed in the control.

177. The engineered cell of any one of claims 173-176, wherein the cell is a primary pancreatic islet cell that expresses at least about a 1000% or at least about a 2000% higher amount of CD47, relative to a control.

178. The engineered cell of any one of claims 171-177, wherein the control is a wild-type cell, a control cell, or a baseline reference.

179. The engineered cell of claim 178, wherein the control cell is an unmodified or unaltered cell, optionally wherein the unmodified or unaltered cell is of the same cell type as the engineered cell.

180. The engineered cell of claim 178 or 179, wherein the control cell is a starting material from a donor or a pool of starting cells from a pool of donors.

181. The engineered cell of claim 178, wherein the baseline reference is an isotype control or a background signal level.

182. The engineered cell of any one of claims 171-181, wherein the CD47 level is determined using an antibody-based quantitation method, optionally a Quantibrite™ assay.

183. An engineered T cell having reduced expression of MHC class I HLA and/or reduced expression of MHC class II HLA and that expresses at least about a 10% higher amount of CD47, relative to a control, that expresses at least about 1.1-fold of the level of CD47 expressed in a control, or that expresses at least about 170,000 CD47 molecules.

184. The engineered cell of claim 221, wherein the cell is a T cell that expresses at least about a 300% or at least about a 400% higher amount of CD47, relative to a control.

185. The engineered cell of claim 183, wherein the cell is a T cell that expresses at least about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, or about 5-fold of the level of CD47 expressed in the control.

186. An engineered T cell that expresses at least about 170,000 CD47 molecules.

187. The engineered T cell of claim 186, wherein the T cell expresses at least about 180,000 CD47 molecules, at least about 190,000 CD47 molecules, at least about 200,000 CD47 molecules, at least about 210,000 CD47 molecules, at least about 220,000 CD47 molecules, at least about 230,000 CD47 molecules, at least about 240,000 CD47 molecules, at least about 250,000 CD47 molecules, at least about 260,000 CD47 molecules, at least about 270,000 CD47 molecules, at least about 280,000 CD47 molecules, at least about 290,000 CD47 molecules, or at least about 300,000 CD47 molecules.

188. The engineered cell of any one of claims 183-187, wherein the control is a wild-type cell, a control cell, or a baseline reference.

189. The engineered cell of claim 188, wherein the control cell is an unmodified or unaltered cell, optionally wherein the unmodified or unaltered cell is of the same cell type as the engineered cell.

190. The engineered cell of claim 188 or 189, wherein the control cell is a starting material from a donor or a pool of starting cells from a pool of donors.

191. The engineered cell of claim 190, wherein the baseline reference is an isotype control or a background signal level.

192. The engineered cell of any one of claims 183-191, wherein the CD47 level is determined using an antibody-based quantitation method, optionally a Quantibrite™ assay.

193. The engineered cell of any one of claims 1-192, wherein the cell comprises 1, 2, 3, 4, or 5 copies of an exogenous polynucleotide encoding CD47.

194. The engineered cell of claim 193, wherein the cell comprises a constitutive promoter operably linked to an exogenous polynucleotide encoding CD47.

195. The engineered cell of any one of claims 1-194, wherein an exogenous polynucleotide encoding CD47 is delivered to the cell via viral mediated integration.

196. The engineered cell of claim 195, wherein the viral mediated integration is lentivirus mediated.

197. The engineered cell of any one of claims 1-196, wherein an exogenous polynucleotide encoding CD47 is integrated at a site in the cell genome via HDR.

198. The engineered cell of claim 197, wherein the exogenous polynucleotide encoding CD47 is integrated into a locus in the TRAC gene, a locus in the TRBC gene, or a combination thereof.

199. The T cell of claim 198, wherein the exogenous polynucleotide encoding CD47 is integrated into at least one TRAC allele, at least one TRBC allele, or a combination thereof.

200. The T cell of claim 198 or 199, wherein the exogenous polynucleotide encoding CD47 is integrated into at least two TRAC alleles, at least two TRBC alleles, or a combination thereof.

201. The cell of any one of claims 1-200, wherein the cell comprises an exogenous polynucleotide comprising a CD47 polypeptide having at least about 80% sequence identity to the amino acid sequence of SEQ ID NO:129, at least about 85% sequence identity to the amino

acid sequence of SEQ ID NO:129, at least about 90% sequence identity to the amino acid sequence of SEQ ID NO:129, at least about 95% sequence identity to the amino acid sequence of SEQ ID NO:129, at least about 98% sequence identity to the amino acid sequence of SEQ ID NO:129, at least about 99% sequence identity to the amino acid sequence of SEQ ID NO:129, or having the amino acid sequence of SEQ ID NO:129.

202. The cell of any one of claims 1-200, wherein the cell comprises an exogenous polynucleotide comprising a CD47 polypeptide having at least about 80% sequence identity to the amino acid sequence of SEQ ID NO:130, at least about 85% sequence identity to the amino acid sequence of SEQ ID NO:130, at least about 90% sequence identity to the amino acid sequence of SEQ ID NO:130, at least about 95% sequence identity to the amino acid sequence of SEQ ID NO:130, at least about 98% sequence identity to the amino acid sequence of SEQ ID NO:130, at least about 99% sequence identity to the amino acid sequence of SEQ ID NO:130, or having the amino acid sequence of SEQ ID NO:130.

203. The cell of any one of claims 171-202, wherein the cell comprises reduced expression of one or more MHC class I and/or MHC class II molecules, relative to a control.

204. The cell of claim 203, wherein the reduced expression of the one or more MHC class I and/or MHC class II molecules, is caused by constitutve modifications to one or more genes encoding the MHC class I and/or class II HLA.

205. The cell of any one of claims 204 or 205, wherein the cell comprises one or more knock outs of targets selected from the group consisting of MHC class I and MHC class II HLA.

206. The cell of claim 205, wherein the one or more knock outs are constitutive knock outs.

207. The cell of any one of claims 204-206, wherein the cell comprises reduced expression of one or more targets selected from the group consisting of B2M and CIITA, relative to the control.

208. The cell of claim 207, wherein the reduced expression of B2M and/or CIITA is caused by constitutive modifications to the B2M gene and/or the CIITA gene.

209. The cell of any one of claims 204-208, wherein the cell comprises one or more knock outs of targets selected from the group consisting of B2M and CIITA.

210. The cell of claim 209, wherein the cell comprises knock outs of both alleles of B2M and/or both alleles of CIITA.

211. The cell of claim 209 or 210, wherein the one or more knock outs are constitutive knock outs.

212. The cell of any one of claims 204-211, wherein the cell further comprises an exogenous polynucleotide encoding one or more further tolerogenic factors.

213. The cell of claim 212, wherein the one or more further tolerogenic factors are selected from the group consisting of HLA-C, HLA-E, HLA-F, HLA-G, PD-L1, CTLA-4-Ig, CI-inhibitor, and IL-35.

214. The cell of any one of claims 204-213, wherein the cell comprises reduced expression of B2M, CIITA, NLRC5, TRAC, TRB, CD142, ABO, MIC-A/B, CD38, CD52, PCDH11Y, NLGN4Y and/or RHD, relative to the control.

215. The cell of claim 214, wherein the cell does not express B2M, CIITA, NLRC5, TRAC, TRB, CD142, ABO, MIC-A/B, CD38, CD52, PCDH11Y, NLGN4Y and/or RHD.

216. The cell of any one of claims 204-215, wherein the cell is a pluripotent stem cell.

217. The cell of claim 216, wherein the pluripotent stem cell is an induced pluripotent stem cell (iPSC), a mesenchymal stem cell (MSC), a hematopoietic stem cell (HSC), or an embryonic stem cell (ESC).

218. The cell of any one of claims 204-217 wherein the cell is a differentiated cell derived from a pluripotent stem cell or a progeny thereof.

219. The cell of claim 218, wherein the differentiated cell is selected from the group consisting of a pancreatic islet cell, a T cell, a natural killer (NK) cell, a CAR-M cell, an endothelial cell, a cardiac muscle cell, a smooth muscle cell, a skeletal muscle cell, a hepatocyte, a glial progenitor cell, a dopaminergic neuron, a retinal pigment epithelial cell, and a thyroid cell.

220. The cell of any one of claims 204-219, wherein the cell is a primary cell or a progeny thereof.

221. The cell of claim 220, wherein the primary cell or a progeny thereof is a T cell or an NK cell.

222. The cell of any one of claims 219 or 221, wherein the T cell further comprises reduced expression of T cell receptor (TCR)-alpha and/or TCR-beta.

223. The cell of claim 222, wherein the T cell does not express TCR-alpha and/or TCR-beta.

224. The cell of any one of claims 219 or 221-223, wherein the T cell further comprises a second exogenous polynucleotide encoding one or more chimeric antigen receptors (CARs).

225. The cell of any one of claims 204-224, wherein the cell expresses at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47 expression, relative to a control, and reduced expression of one or more of MHC class I and MHC class II human leukocyte antigens, relative to a control.

226. The cell of any one of claims 204-225, wherein the cell expresses at least about 2-fold, about 3-fold, about 4-fold, or about 5-fold of the level of CD47 expressed in a wild-type cell or a control cell that has no or low expression of CD47, and reduced expression of one or more of MHC class I and MHC class II human leukocyte antigens, relative to the control cell.

227. The cell of claim 226, wherein the cell expresses at least about 3-fold, about 4-fold, or about 5-fold of the level of CD47 expressed in a wild-type cell or a control cell of the same cell type that has no or low expression of CD47.

228. The cell of any one of claims 225-227, wherein the control cell is a pancreatic islet cell, a T cell, a natural killer (NK) cell, a CAR-M cell, an endothelial cell, a cardiac muscle cell, a smooth muscle cell, a skeletal muscle cell, a hepatocyte, a glial progenitor cell, a dopaminergic neuron, a retinal pigment epithelial cell, or a thyroid cell.

229. The cell of any one of claims 219-228, wherein the differentiated cell or the progeny thereof, or the primary immune cell or the progeny thereof evades NK cell mediated cytotoxicity upon administration to a recipient patient, is protected from cell lysis by mature NK cells upon administration to a recipient patient, evades macrophage engulfment upon administration to a recipient patient, does not induce an innate and/or an adaptive immune response to the cell upon administration to a recipient patient, and/or does not induce an antibody-based immune response to the cell upon administration to a recipient patient.

230. The engineered cell of any one of claims 1-229, wherein the cell is an autologous cell.

231. The engineered cell of any one of claims 1-229, wherein the cell is an allogeneic cell.

232. A pharmaceutical composition comprising a population of the engineered cells of any one of claims 1-231, and a pharmaceutically acceptable additive, carrier, diluent or excipient.

233. The pharmaceutical composition of claim 232, wherein the engineered cell is a beta islet cell and the pharmaceutical composition further comprises one or more additional pancreatic islet cells.

234. A method of treating a patient with a disease or condition who would benefit from a cell-based therapy, comprising administering a clinically effective amount or a therapeutically effective amount of the engineered cells of any one of claims 1-231 to the patient.

235. A method of treating a patient with a disease or condition who would benefit from a cell-based therapy, comprising administering a population of cells comprising the engineered cells of any one of claims 1-231 to the patient.

236. A method of treating a patient with a disease or condition who would benefit from a cell-based therapy, comprising administering a population of cells comprising the differentiated cells of any one of claims 1-231 to the patient.

237. A method of treating a patient with a disease or condition who would benefit from a cell-based therapy, comprising administering the pharmaceutical composition of claim 302 or 303 to the patient.

238. The method of claim 234-237, wherein the disease or condition is selected from the group consisting of a cancer, a genetic disorder, a chronic infectious disease, an autoimmune disorder, a neurological disorder, a cardiac disorder (selected from the group consisting of pediatric cardiomyopathy, age-related cardiomyopathy, dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, chronic ischemic cardiomyopathy, peripartum cardiomyopathy, inflammatory cardiomyopathy, idiopathic cardiomyopathy, other cardiomyopathy, myocardial ischemic reperfusion injury, ventricular dysfunction, heart failure, congestive heart failure, coronary artery disease, end-stage heart disease, atherosclerosis, ischemia, hypertension, restenosis, angina pectoris, rheumatic heart, arterial inflammation, cardiovascular disease, myocardial infarction, myocardial ischemia, myocardial infarction, cardiac ischemia, cardiac injury, myocardial ischemia, vascular disease, acquired heart disease, congenital heart disease, coronary artery disease, dysfunctional conduction systems, dysfunctional coronary arteries, pulmonary hypertension, cardiac arrhythmias, muscular dystrophy, muscle mass abnormality, muscle degeneration, myocarditis, infective myocarditis, drug- or toxin-induced muscle abnormalities, hypersensitivity myocarditis, mitral insufficiency, autoimmune endocarditis, primary arrhythmic diseases, cardiac chanellopathies, long QT syndromes, short QT syndromes, Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia, Jervell and Lange-Nielsen syndrome, myocardial infarction, heart failure, cardiomyopathy, congenital heart defect, heart valve disease or dysfunction, endocarditis, rheumatic fever, mitral valve prolapse, infective endocarditis, hypertrophic cardiomyopathy, dilated cardiomyopathy, myocarditis, cardiomegaly, mitral insufficiency), a neurological disorder (selected from the group consisting of Alzheimer's disease, Huntington's disease, Parkinson's disease, Pelizaeus-Merzbacher disease, other neurodegenerative disease or condition, attention deficit hyperactivity disorder (ADHD), ischaemia, multiple sclerosis, traumatic brain injury, epilepsy, catalepsy, encephalitis, meningitis, migraine, stroke, transient ischemic attack, subarachnoid hemorrhage, subdural hemorrhage, hematoma, extradural hemorrhage, spinal cord injury, cervical spondylosis, carpal tunnel syndrome, brain or spinal cord tumors, peripheral neuropathy, Guillan-Barre syndrome, neuralgia, amyotrophic lateral sclerosis (ALS), tauopathies, Pick disease, progressive supranuclear palsy, corticobasal degeneration, argyrophilic grain disease, Bell's palsy, cerebral palsy, motor neurone disease, neurofibromatosis, encephalitis, meningitis, Tourette's syndrome, schizophrenia, psychosis,

depression, and other neuropsychiatric disorder), vascular dementia, Alzheimer's disease, Parkinson's disease, Huntington disease, multiple sclerosis, other neurodegenerative disease or condition, attention deficit hyperactivity disorder (ADHD), Tourette Syndrome (TS), schizophrenia, psychosis, depression, other neuropsychiatric disorder, HIV-1-associated neurocognitive disorder, traumatic brain injury, stroke, amyotrophic lateral sclerosis (ALS), cerebral hemorrhage, epileptic seizure, spinal cord injury, argyrophilic grain disease (AGD), amyotrophic lateral sclerosis (ALS), cortico-basal degeneration (CBD), Parkinsonism linked to chromosome 17 (FTDP-17), multiple system atrophy (MSA), Parkinson's disease/diffuse Lewy body disease (PD/DLBD), or Alzheimer's disease, atherosclerosis, atherogenesis, arterial thrombosis, venous thrombosis, thrombocytic microangiopathies, vascular leakage, diffuse intravascular coagulation, diabetes, insulin resistance, cardiovascular disease, vascular disease, peripheral vascular disease, ischemic disease, myocardial infarction, congestive heart failure, peripheral vascular obstructive disease, stroke, reperfusion injury, limb ischemia, neuropathy (*e.g.*, peripheral neuropathy or diabetic neuropathy), organ failure (*e.g.*, liver failure, kidney failure, and the like), diabetes, rheumatoid arthritis, osteoporosis, vascular injury, tissue injury, hypertension, angina pectoris and myocardial infarction due to coronary artery disease, renal vascular hypertension, renal failure due to renal artery stenosis, claudication of the lower extremities, transient ischemic attack or stroke, myocardial infarction, and limb ischemia, repair of ischemic tissues, formation of blood vessels and heart valves, engineering of artificial vessels, repair of damaged vessels, and inducing the formation of blood vessels in engineered tissues (*e.g.*, prior to transplantation), repair or replacement for tissue in need of vascular cells or vascularization a cardiac tissue, liver tissue, pancreatic tissue, renal tissue, muscle tissue, neural tissue, bone tissue, among others, which can be a tissue damaged and characterized by excess cell death, a tissue at risk for damage, or an artificially engineered tissue), coronary artery disease, cerebrovascular disease, aortic stenosis, aortic aneurysm, peripheral artery disease, atherosclerosis, varicose veins, angiopathy, infarcted area of heart lacking coronary perfusion, non-healing wounds, diabetic or non-diabetic ulcers, or any other disease or disorder in which it is desirable to induce formation of blood vessels, improving prosthetic implants (*e.g.*, vessels made of synthetic materials such as Dacron and Gortex.) which are used in vascular reconstructive surgery, a vascular disorder selected from the group consisting of vascular injury, cardiovascular disease, vascular disease, peripheral vascular disease, ischemic disease,

myocardial infarction, congestive heart failure, peripheral vascular obstructive disease, hypertension, ischemic tissue injury, reperfusion injury, limb ischemia, stroke, neuropathy (*e.g.*, peripheral neuropathy or diabetic neuropathy), organ failure (*e.g.*, liver failure, kidney failure, and the like), diabetes, rheumatoid arthritis, osteoporosis, cerebrovascular disease, hypertension, angina pectoris and myocardial infarction due to coronary artery disease, renal vascular hypertension, renal failure due to renal artery stenosis, claudication of the lower extremities, other vascular condition or disease, autoimmune thyroiditis, goiter, hyperparathyroidism, hypoparathyroidism (congenital or autoimmune), thyroiditis, Hashimoto's thyroiditis, postpartum thyroiditis, subacute thyroiditis, iatrogenic hypothyroidism, Grave's disease, and thyroid eye disease, infectious hepatitis (A, B, and C), autoimmune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis, non-alcoholic fatty liver disease, cirrhosis, hemochromatosis, hyperoxaluria, alpha-1 antitrypsin deficiency, liver failure, Wilson's disease, hepatic encephalopathy, jaundice, acute hepatic porphyrias, Alagille syndrome, biliary atresia, Budd-Chiari syndrome, hyperbilirubinemias, Crigler-Najjar syndrome, Gilbert-Meulengracht syndrome, Dubin-Johnson syndrome, Rotor syndrome, galactosemia, glycogen storage disease type 1, hepatorenal syndrome, intrahepatic cholestasis of pregnancy, progressive familial intrahepatic cholestasis, Reye's syndrome, lysosomal acid lipase deficiency, alcohol-related pancreatitis, gallstone pancreatitis, diabetes mellitus (type 1 and type 2), prediabetes, gestational diabetes, pancreoprivic diabetes mellitus, pancreatic exocrine insufficiency, acute pancreatitis, chronic pancreatitis, hereditary pancreatitis, hyperinsulinemia, pancreatic cysts, Zollinger-Ellison syndrome, Shwachman-Diamond syndrome, hereditary hemochromatosis, thalassemia, pancreatic iron deposition, cystic fibrosis, pancreas divisum, and pancreatic resection, macular degeneration or a patient having damaged RPE cells, age-related macular degeneration (AMD), early AMD, intermediate AMD, late AMD, non-neovascular age-related macular degeneration, dry macular degeneration (dry age-related macular degeneration), wet macular degeneration (wet age-related macular degeneration), adult-onset vitelliform macular dystrophy (AVMD), Best vitelliform macular dystrophy, Stargardt-like macular dystrophy (STGD3), Sorby's fundus dystrophy (SFD), ABCA4-related disease, Usher type IB, autosomal recessive bestrophinopathy, autosomal dominant vitreoretinochoroidopathy, juvenile macular degeneration (JMD), Leber's Congenital Amaurosis, or retinitis pigmentosa, retinal detachment, retinal tears, severe combined immunodeficiencies (SCID), Omenn syndrome, Cartilage-Hair hypoplasia, reticular dysgenesis,

Wiskott-Aldrich syndrome, ataxia telangiectasia, DiGeorge syndrome, immune-osseous dysplasias, dyskeratosis congenita, chronic mucocutaneous candidiasis, hematologic malignancy, follicular lymphoma (FL), myeloid neoplasm, mature T/NK neoplasms, Histiocytic neoplasms, multiple myeloma (MM), myelodysplastic syndromes (MDS), lymphoplasmacytic lymphoma (LPL), Waldenström macroglobulinemia, Burkitt lymphoma (BL), primary mediastinal large B-cell lymphoma (PMBL), Hodgkin lymphoma, Mantle cell lymphoma (MCL), Hairy cell leukemia (HCL), myeloproliferative/myelodysplastic syndromes (MDS) , acute lymphoid leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), Diffuse large B-cell lymphoma (DLBCL), B cell acute lymphoid leukemia (B-ALL), T cell acute lymphoid leukemia (T-ALL), T cell lymphoma, B cell lymphoma, autoimmune disease, including, for example, lupus, systemic lupus erythematosus, rheumatoid arthritis, psoriasis, psoriatic arthritis, multiple sclerosis, Crohn's disease, ulcerative colitis, Addison's disease, Graves' disease, Sjögren's syndrome, Hashimoto's thyroiditis, diabetes mellitus type 1, primary biliary cirrhosis, autoimmune hepatitis, celiac disease, cancers including, but not limited to, 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, bladder cancer, systemic lupus erythematosus (SLE), type 1 diabetes, autoimmune liver disease, Sjögren's syndrome, rheumatoid arthritis, systemic sclerosis (scleroderma), organ-specific autoimmune diseases (autoimmune hepatitis, primary sclerosing chonlangitis), alcohol-related liver disease, multiple sclerosis, NK cell deficiency (NKD) (functional (FNKD) or classical (CNKD)), immunodeficiency-polyendocrinopathy-enteropathy-X-linked (IPEX)-like syndrome, Bloom syndrome, Fanconi's anemia, dyskeratosis congenita, Chediak-Higashi syndrome, familial hematophagocytic lymphohistocytosis (FHL), Griscelli syndrome type 2, Hermansky Pudliak syndrome, Papillon-Lefevre syndrome, Wiskott-Aldrich syndrome, autosomal recessive hyper-IgE syndrome, May Hegglin anomaly, and leucocyte adhesion deficiency type I or type III.

239. The method of claim 234-238, wherein the differentiated cells are selected from the group consisting of a mesenchymal stem cell (MSC), a hematopoietic stem cell (HSC), pancreatic islet cell, a beta islet cell, an immune cell, a B cell, a T cell, a natural killer (NK) cell, a natural killer T (NKT) cell, a macrophage cell, an immune privileged cell, an optic cell, a retinal pigmented epithelium cell (RPE), a hepatocyte, a thyroid cell, an endothelial cell, a skin cell, a glial progenitor cell, a neural cell, a muscle cell, a cardiac cell, and a blood cell.

240. The method of any one of claims 234-239, wherein an immunosuppressive and/or immunomodulatory agent is not administered to the patient before the administration of the population of cells.

241. The method of any of claims 234-240, further comprising administering one or more immunosuppressive agents to the patient.

242. The method of any of claims 234-241, where the patient has been administered one or more immunosuppressive agents.

243. The method of claim 241 or 242, wherein the one or more immunosuppressive agents are a small molecule or an antibody.

244. The method of any of claims 241-243, wherein 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.

245. The method of any of claims 241-244, wherein the one or more immunosuppressive agents comprise cyclosporine.

246. The method of any of claims 241-245, wherein the one or more immunosuppressive agents comprise mycophenolate mofetil.

247. The method of any of claims 241-246, wherein the one or more immunosuppressive agents comprise a corticosteroid.

248. The method of any of claims 241-247, wherein the one or more immunosuppressive agents comprise cyclophosphamide.

249. The method of any of claims 241-248, wherein the one or more immunosuppressive agents comprise rapamycin.

250. The method of any of claims 241-249, wherein the one or more immunosuppressive agents comprise tacrolimus (FK-506).

251. The method of any of claims 241-250, wherein the one or more immunosuppressive agents comprise anti-thymocyte globulin.

252. The method of any of claims 241-251, wherein the one or more immunosuppressive agents are one or more immunomodulatory agents.

253. The method of claim 252, wherein the one or more immunomodulatory agents are a small molecule or an antibody.

254. The method of claim 243 or claim 253, wherein 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.

255. The method of any of claims 241-254, wherein the one or more immunosuppressive agents are or have been administered to the patient prior to administration of the engineered cells.

256. The method of any of claims 241-255, wherein 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.

257. The method of any of claims 241-256, wherein 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.

258. The method of any of claims 241-257, wherein 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.

259. The method of any of claims 241-258, wherein 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.

260. The method of any of claims 241-259, wherein 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.

261. The method of any of claims 241-260, wherein the one or more immunosuppressive agents are or have been administered to the patient after administration of the engineered cells.

262. The method of any of claims 241-261, wherein 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.

263. The method of any of claims 241-262, wherein 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.

264. The method of any of claims 241-263, wherein 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.

265. The method of any of claims 241-264, wherein 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.

266. The method of any of claims 241-265, wherein 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.

267. The method of any of claims 241-266, wherein 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.

268. The method of any of claims 241-267, wherein 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.

269. Use of a population of the engineered cells of any one of claims 1-231 for treating a disorder or condition in a recipient patient who would benefit from a cell-based therapy.

270. A method for producing the engineered cell of any one of claims 1-231 or the population of cells comprising the engineered cell of any one of claims 1-231, the method comprising

a. obtaining an isolated cell; and

b. contacting the isolated cell with one or more reagents and/or components to modify gene expression in the isolated cell, thereby producing the engineered cell or the population of cells comprising the engineered cell.

271. The method of claim 270, wherein the method further comprises determining the CD47 expression levels of the engineered cells or the population of cells.

272. The method of claim 270 or 271, wherein the method further comprises selecting the engineered cell or the population of cells for use in producing a therapeutic product if the engineered cell or the population of cells are determined to express CD47 at a threshold level or higher.

273. The method of any one of claims 27-272, wherein the engineered cell or the population of cells express at least about the same amount of CD47, relative to the control.

274. The method of any one of claims 270-273, wherein the engineered cell or the population of cells express at least about a 10% higher amount of CD47, relative to the control.

275. The method of any one of claims 270-274, wherein the engineered cell or the population of cells express at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, higher amount of CD47, relative to the control.

276. The method of any one of claims 270-275, wherein the engineered cell or the population of cells express at least about a 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, or 900%, higher amount of CD47, relative to the control.

277. The method of any one of claims 270-276, wherein the engineered cell or the population of cells express at least about a 1000% higher amount of CD47, relative to the control.

278. The method of any one of claims 270-277, wherein the engineered cell or the population of cells express at least about 1.1-fold of the level of CD47 expressed in the control.

279. The method of any one of claims 270-273 and 278, wherein the engineered cell or the population of cells express at least about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, or about 5-fold of the level of CD47 expressed in the control.

280. The method of any one of claims 270-273, 278, and 279, wherein the engineered cell or the population of cells express at least about 4-fold, about 4.5-fold, about 5-fold, or about 5.5-fold of the level of CD47 expressed in the control.

281. The method of any one of claims 270-273 and 278-280, wherein the engineered cell or the population of cells express at least about about 4-fold of the level of CD47 expressed in the control.

282. The method of any one of claims 270-273 and 278-281, wherein the engineered cell or the population of cells express at least about about 4.5-fold of the level of CD47 expressed in the control.

283. The method of any one of claims 270-273 and 278-282, wherein the engineered cell or the population of cells express at least about about 5-fold of the level of CD47 expressed in the control.

284. The method of any one of claims 270-273 and 278-283, wherein the engineered cell or the population of cells express at least about about 5.5-fold of the level of CD47 expressed in the control.

285. The method of any one of claims 270-273 and 278-284, wherein the engineered cell or the population of cells express at least about about 16-fold, about 17-fold, about 18-fold, about 19-fold, or about 20-fold of the level of CD47 expressed in the control.

286. The method of any one of claims 270-285, wherein the control is a wild-type cell or a population of wild type cells, a control cell or a population of control cells, or a baseline reference.

287. The method of claim 286, wherein the control cell or the population of control cells comprise an unmodified or unaltered cell, optionally wherein the unmodified or unaltered cell is of the same cell type as the engineered cell.

288. The method of claim 286 or 287, wherein the control cell or the population of control cells is a starting material from a donor or a pool of starting cells from a pool of donors.

289. The method of claim 286, wherein the baseline reference is an isotype control or a background signal level.

290. The method of any one of claims 270-289, wherein the engineered cell is a beta islet cell and the population of cells comprises beta islet cells and additional pancreatic islet cells.

291. The method of any one of claims 270-289, wherein the engineered cell comprises regulatable modifications that alter the expression of one or more targets in the engineered cell, relative to a control.

292. The method of claim 291, wherein the regulatable modifications reduce expression of one or more MHC class I and/or MHC class II molecules, relative to a wild-type cell, a population of wild type cells, a control cell, or a population of control cells.

293. The method of claim 291 or 292, wherein the regulatable modifications increase expression of one or more tolerogenic factors, relative to a wild-type cell, a population of wild type cells, a control cell, or a population of control cells.

294. The method of any one of claims 293-294, wherein the one or more reagents to modify gene expression in the isolated cell comprise i) a conditional or inducible RNA-based component for altering expression of the one or more targets, ii) a conditional or inducible DNA-based component for altering expression of the one or more targets, or iii) a conditional or inducible protein-based component for altering expression of the one or more targets.

295. The method of claim 294, wherein the method further comprises contacting the isolated cell with an exogenous factor or exposing the isolated cell to a condition to activate the conditional or inducible promoter, thereby causing expression of the one or more targets, thereby producing the engineered cell.

296. A method for producing an engineered cell comprising regulatable modifications that i) reduce expression of one or more MHC class I and/or MHC class II molecules, and ii) increase expression of one or more tolerogenic factors, relative to a control, the method comprising:

(a) obtaining an isolated cell;

(b) introducing into the cell a conditional or inducible RNA-based component for regulatable reduced expression of the MHC class I and/or MHC class II human leukocyte molecules, a conditional or inducible DNA-based component for regulatable reduced

expression of the MHC class I and/or MHC class II human leukocyte molecules, or a conditional or inducible protein-based component for regulatable reduced expression of the MHC class I and/or MHC class II human leukocyte molecules;

(c) exposing the cell to a condition or an exogenous factor to activate the conditional or inducible component, thereby causing reduced expression of the MHC class I and/or MHC class molecules;

(d) introducing into the isolated cell a nucleic acid comprising a conditional or inducible promoter operably linked to an exogenous polynucleotide encoding the one or more tolerogenic factors for regulatable increased expression of the one or more tolerogenic factors; and

(e) exposing the engineered cell to a condition or an exogenous factor to activate the conditional or inducible promoter, thereby causing expression of the exogenous one or more tolerogenic factors, and thereby producing the engineered cell.

297. The method of claim 296, wherein steps (a)-(d) are carried out in any order.

298. The method of claim 296, wherein one or more of steps (a)-(d) are carried out simultaneously.

299. The method of claim 296, wherein steps (b) and (c) are carried out before steps (d) and (e).

300. The method of claim 296, wherein steps (d) and (e) are carried out before steps (b) and (c).

301. The method of claim 296, wherein steps (c) and (e) are carried out sequentially.

302. The method of claim 296, wherein steps (c) and (e) are carried out simultaneously.

303. A method for identifying a population of cells or a population of cells comprising the engineered cells of any one of claims 1-231 suitable for use as a therapeutic product, the method comprising:

(a) obtaining isolated cells;

(b) introducing into the cells one or more modifications that reduce expression of one or more MHC class I and/or MHC class II molecules, relative to a control;

(c) introducing into the cells one or more modifications that increase expression of CD47, relative to a control;

(d) measuring the CD47 expression levels of the cells; and

(e) selecting a population of cells that express at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47, relative to the control, and identifying the population as suitable for use as a therapeutic product.

304. A method for identifying a population of cells or a population of cells comprising the engineered cells of any one of claims 1-231 suitable for use as a therapeutic product, the method comprising:

(a) obtaining isolated cells;

(b) introducing into the cells one or more modifications that reduce expression of one or more MHC class I and/or MHC class II molecules, relative to a control;

(c) introducing into the cells one or more modifications that increase expression of CD47, relative to a control;

(d) measuring the CD47 expression levels of the cells; and

(e) selecting a population of cells that express at least about 1.1-fold, about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold, about 10-fold, about 11-fold, about 12-fold, about 13-fold, about 14-fold, about 15-fold, about 16-fold, about 17-fold, about 18-fold, about 19-fold, or about 20-fold of the level of CD47 expressed in the control, and identifying the population as suitable for use as a therapeutic product.

305. The method of claim 303 or 304, wherein step (b) is carried out before step (c).

306. The method of claim 303 or 304, wherein step (c) is carried out before step (b).

307. The method of claim 303 or 304, wherein steps (b) and (c) are carried out simultaneously.

308. A method of determining whether a population of cells is suitable for use as a therapeutic product, the method comprising:

(a) producing engineered cells comprising a first exogenous polynucleotide encoding CD47, optionally the engineered cells of any one of claims 1-231;

(b) measuring the CD47 expression levels of the cells; and

(c) determining that the population of cells is suitable for use as a therapeutic product if the cells express at about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47, relative to a control.

309. A method of determining whether a population of cells is suitable for use as a therapeutic product, the method comprising:

(a) producing engineered cells comprising a first exogenous polynucleotide encoding CD47, optionally the engineered cells of any one of claims 1-231;

(b) measuring the CD47 expression levels of the cells; and

(c) determining that the population of cells is suitable for use as a therapeutic product if the cells express at least about 1.1-fold, about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold, about 10-fold, about 11-fold, about 12-fold, about 13-fold, about 14-fold, about 15-fold, about 16-fold, about 17-fold, about 18-fold, about 19-fold, or about 20-fold of the level of CD47 expressed in a control.

310. The method of any one of claims 296-309, wherein the control is a wild-type cell, a control cell, or a baseline reference.

311. The method of claim 310, wherein the control cell is an unmodified or unaltered cell, optionally wherein the unmodified or unaltered cell is of the same cell type as the engineered cell.

312. The method of claim 310 or 311, wherein the control cell is a starting material from a donor or a pool of starting cells from a pool of donors.

313. The method of claim 310, wherein the baseline reference is an isotype control or a background signal level.

314. The method of any one of claims 296-313, wherein the CD47 level is determined using an antibody-based quantitation method, optionally a Quantibrite™ assay.

315. A method of determining a threshold of CD47 expression level required for immune-evasion of hypoimmunogenic cells, the method comprising:

(a) producing engineered cells comprising a first exogenous polynucleotide encoding CD47;

(b) sorting the engineered cells based on CD47 expression levels, to generate pools of cells having similar CD47 expression levels;

(c) assessing the immune response induced by the pools of cells; and

(d) determining a threshold of CD47 expression level required for immune-evasion.

316. The method of claim 315, wherein the CD47 level is determined using an antibody-based quantitation method, optionally a Quantibrite™ assay.

317. The method of claim 315, wherein step (a) of the method further comprises engineering the cells to comprise reduced expression of one or more Y chromosome genes and major histocompatibility complex (MHC) class I and/or class II human leukocyte antigens, relative to a wild-type cell or a control cell.

318. The method of claim 315 or 317, wherein the assessing of the immune response is carried out using in vitro assays or in vivo assays.

319. The method of claim 318, wherein the assessing of the immune response is carried out by measuring NK cell mediated cytotoxicity, lysis by mature NK cells, macrophage engulfment, antibody-based immune response to the cells, or by measuring the percentage of the cells still present in the recipient after a certain period of time upon administration to a recipient patient.

320. A method for identifying a population of cells or a population of cells comprising the engineered cells of any one of claims 1-231 suitable for use as a therapeutic product, the method comprising:

(a) introducing into isolated cells one or more modifications that reduce expression of one or more MHC class I and/or MHC class II molecules, relative to a control, and

(b) introducing into the cells one or more modifications that increase expression of CD47, relative to a control.

321. The method of claim 320, further comprising step (c) measuring the CD47 expression levels of the cells.

322. The method of claim 321, further comprising step (d) selecting a population of cells that express at least about a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% higher amount of CD47, relative to the control, and identifying the population as suitable for use as a therapeutic product.

323. The method of claim 321, further comprising step (d) selecting a population of cells that express at least about 1.1-fold, about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 3.5-fold, about 4-fold, about 4.5-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold, about 10-fold, about 11-fold, about 12-fold, about 13-fold, about 14-fold, about 15-fold, about 16-fold, about 17-fold, about 18-fold, about 19-fold, or about 20-fold of the level of CD47 expressed in the control, and identifying the population as suitable for use as a therapeutic product.

324. The method of any one of claims 320-323, wherein step (a) is carried out before step (b).

325. The method of any one of claims 320-323, wherein step (b) is carried out before step (a).

326. The method of any one of claims 320-323, wherein steps (a) and (b) are carried out simultaneously.