SAFETY SWITCHES FOR REGULATION OF GENE EXPRESSION

CROSS-REFERENCE

[0001] This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application Nos. 62/962,730 filed January 17, 2020, 62/962,739 filed January 17, 2020, and 62/962,764 filed January 17, 2020, the disclosures of which are herein incorporated by reference in their entirety.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled SANA006WO1SeqList.txt, created January 14, 2021, which is 33,948 bytes in size. The information in the electronic format of the Sequence Listing is incorporated by reference in its entirety.

BACKGROUND

[0003] Degenerative diseases pose a disproportionate threat to human health. Often age-related, these diseases result in the progressive deterioration of affected tissues and organs and, ultimately, disability and death of the affected subject. The promise of regenerative medicine is to replace diseased or missing cells with new healthy cells. Over the past five years, a new paradigm for regenerative medicine has emerged — the use of human pluripotent stem cells (hPSCs) to generate any adult cell type for transplantation into patients. In principle, hPSC-based cell therapies have the potential to treat most if not all degenerative illnesses, however the success of such therapies may be limited by a subject's immune response.

[0004] Strategies that have been considered to overcome the immune rejection include HLA-matching (*e.g.*, identical twin or umbilical cord banking), the administration of immunosuppressive drugs to the subject, blocking antibodies, bone marrow suppression/mixed chimerism, HLA-matched stem cell repositories, and autologous stem cell therapy.

[0005] There remains a need for novel approaches, compositions and methods for overcoming immune rejection associated with cell therapies.

SUMMARY

[0006] In one example, provided herein is a method for controlling the immunogenicity of an engineered cell, the method comprising: (a) obtaining an isolated cell; (b) introducing into the

isolated cell (i) a nucleic acid comprising an inducible RNA polymerase promoter operably linked to an shRNA sequence targeting an immunosuppressive factor and (ii) a nucleic acid comprising a promoter operably linked to a transactivator element corresponding to the inducible RNA polymerase promoter to produce an engineered cell; and (c) exposing the engineered cell to an exogenous factor to activate the transactivator element, thereby controlling the immunogenicity of the cell.

[0006a] In another aspect, the present disclosure refers to a method of introducing a construct as described herein into a cell.

[0007] In one example, provided herein is a method for controlling the immunogenicity of an engineered cell, the method comprising: (a) obtaining an isolated cell; (b) introducing into the isolated cell a nucleic acid comprising (i) a sequence encoding an inducible degron element operably linked to an immunosuppressive factor or (ii) a sequence encoding an immunosuppressive factor operably linked to an inducible degron element to produce an engineered cell; and (c) exposing the engineered cell to an exogenous factor to activate the inducible degron element, thereby controlling the immunogenicity of the engineered cell.

[0008] In another example, provided herein is a method for controlling immunogenicity of an engineered cell comprising: (a) obtaining an isolated cell; (b) introducing into the isolated cell: (i) a first construct comprising from 5' end to 3' end: a first promoter and an immunosuppressive factor gene; (ii) a second construct comprising from 5' end to 3' end: a second promoter and a nucleic acid sequence encoding Cas9 or a variant thereof; and (ii) a third construct comprising from 5' end to 3' end: an inducible RNA polymerase promoter, a guide RNA (gRNA) sequence targeting the immunosuppressive factor, a third promoter, and a transactivator element corresponding to the inducible RNA polymerase promoter; and (c) exposing the engineered cell to an exogenous factor to activate the transactivator element, thereby controlling the immunogenicity of the engineered cell.

[0009] In yet another example, provided herein is a method for controlling the immunogenicity of an engineered cell, the method comprising: (a) obtaining an isolated cell; (b) introducing into the isolated cell (i) a nucleic acid comprising an inducible RNA polymerase promoter operably linked to an immune signaling factor gene and (ii) a nucleic acid comprising a promoter operably linked to a transactivator element corresponding to the inducible RNA polymerase promoter to produce an engineered cell; and (c) exposing the engineered cell to an exogenous factor to activate the transactivator element, thereby controlling the immunogenicity of the engineered cell.

[0009a] In another aspect, the present disclosure refers to a method for controlling the immunogenicity of an engineered cell, the method comprising: (1) (a) introducing into an isolated cell (i) a nucleic acid comprising an inducible RNA polymerase promoter operably linked to an shRNA sequence targeting an immunosuppressive factor and (ii) a nucleic acid comprising a promoter operably linked to a transactivator element corresponding to the inducible RNA polymerase promoter to produce an engineered cell; and (b) exposing the engineered cell to an exogenous factor to activate the transactivator element, thereby controlling the immunogenicity of the engineered cell; or (2) (a) introducing into an isolated cell a nucleic acid comprising (i) a sequence encoding an inducible degron element operably linked to an immunosuppressive factor or (ii) a sequence encoding an immunosuppressive factor operably linked to an inducible degron element to produce an engineered cell; and (b) exposing the engineered cell to an exogenous factor to activate the inducible degron element, thereby controlling the immunogenicity of the engineered cell; or (3) (a) introducing into an isolated cell: (i) a first construct comprising from 5' end to 3' end: a first promoter and an immunosuppressive factor gene; (ii) a second construct comprising from 5' end to 3' end: a second promoter and a nucleic acid sequence encoding a Cas nuclease or a variant thereof; optionally wherein the Cas nuclease is Cas9, and (iii) a third construct comprising from 5' end to 3' end: an inducible RNA polymerase promoter, a guide RNA (gRNA) sequence targeting the immunosuppressive factor, a third promoter, and a transactivator element corresponding to the inducible RNA polymerase promoter; and (b) exposing the engineered cell to an exogenous factor to activate the transactivator element, thereby controlling the immunogenicity of the engineered cell; or (4) (a) introducing into the isolated cell (i) a nucleic acid comprising an inducible RNA polymerase promoter operably linked to an immune signaling factor gene and (ii) a nucleic acid comprising a promoter operably linked to a transactivator element corresponding to the inducible RNA polymerase promoter to produce an engineered cell; and (b) exposing the engineered cell to an exogenous factor to activate the transactivator element, thereby controlling the immunogenicity of the engineered cell.

[0010] In some examples, the method further comprises administering the engineered cell to a subject prior to step (c).

[0011] In some examples, the step (b) of any of the methods comprises introducing into the isolated cell a single nucleic acid construct comprising (i) the inducible RNA polymerase promoter operably linked the shRNA sequence targeting the immunosuppressive factor and (ii) the promoter operably linked to the transactivator element. In some examples, construct comprises from 5' end to 3' end: the inducible RNA polymerase promoter; the shRNA sequence; the promoter; and the transactivator element.

[0012] In some examples, the step (b) comprises introducing into the isolated cell a single nucleic acid construct comprising (i) the inducible RNA polymerase promoter operably linked the immune signaling factor gene and (ii) the promoter operably linked to the transactivator element.

[0013] In some examples, the construct comprises from 5' end to 3' end: the inducible RNA polymerase promoter, the immune signaling factor gene, the promoter, and the transactivator element.

[0014] In some examples, the isolated cell is engineered to exogenously express the immunosuppressive factor. In some examples, the isolated cell overexpresses the immunosuppressive factor in the absence of the exogenous factor that activates the transactivator element.

[0015] In some examples, the inducible RNA polymerase promoter is a U6Tet promoter. In some examples, the inducible RNA polymerase promoter is U6Tet promoter, the transactivator element is a Tet Repressor element, and the exogenous factor is tetracycline or a derivative thereof. In some examples, the inducible RNA polymerase promoter is a TRE promoter and the transactivator element is a Tet-On element, and the exogenous factor is tetracycline or a derivative thereof.

[0016] In some examples, a flexible linker connects the inducible degron element to the immunosuppressive factor. In some examples, the flexible linker is selected from the group consisting of $(GSG)_n$, $(GGGS)_n$, and $(GGGSGGGS)_n$, wherein n is 1-10.

[0017] In some examples, the step (b) comprises introducing into the isolated cell a single nucleic acid construct comprising a promoter operably linked to the nucleic acid.

[0018] In some examples, the promoter is a constitutive promoter selected from the group consisting of an EF1A promoter, an EFS promoter, a CMV promoter, a CAGGS promoter, an

SV40 promoter, a COPIA promoter, an ACT5C promoter, a TRE promoter, a CBh promoter, a PGK promoter, and a UBC promoter. In some examples, the first, second and/or third promoters are constitutive promoters, each independently selected from the group consisting of an EF1A promoter, an EFS promoter, a CMV promoter, a CAGGS promoter, a SV40 promoter, a COPIA promoter, an ACT5C promoter, a TRE promoter, a CBh promoter, a PGK promoter, and a UBC promoter.

[0019] In some examples, the immunosuppressive factor is selected from the group consisting of 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.

[0020] In some examples, the construct comprises from 5' end to 3' end: a U6Tet promoter, a shRNA sequence targeting CD47, an EF1a promoter, and a Tet Repressor element, and wherein the exogenous factor is tetracycline or a derivative thereof.

[0021] In some examples, the construct further comprises a vector backbone for lentiviral expression.

[0022] In some examples, the inducible 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 examples, 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 a rapamycin responsive degron element. In some examples, the ligand inducible degron element is a small molecule-assisted shutoff (SMASH) degron element and the exogenous factor is asunaprevir.

[0023] In some examples, the construct further comprises a 5' homology arm and a 3' homology arm for targeted integration to a safe harbor locus selected from the group consisting of an AAVS1 locus, a CLBYL locus, a CXCR4 locus, a Rosa26 locus, and a CCR5 locus.

[0024] In some examples, the isolated cell is an isolated human cell further comprising deletion or reduced expression of MHC class I human leukocyte antigens and/or deletion or reduced expression of MHC class II human leukocyte antigens compared to an unmodified human cell. In some examples, the isolated human cell further comprises deletion or reduced expression of CIITA, B2M, and/or NLRC5.

[0025] In some examples, the isolated human cell is hypoimmunogenic and either a stem cell or a differentiated cell thereof, wherein the stem cell is selected from the group consisting of

an embryonic stem cell, a pluripotent stem cell, an induced pluripotent stem cell, an adult stem cell, and wherein the differentiated cell is selected from the group consisting of a cardiac cell, liver cell, kidney cell, pancreatic cell, neural cell, immune cell, mesenchymal cell, and endothelial cell. In some examples, the isolated human cell is hypoimmunogenic. In some examples, the isolated human cell is a stem cell. In some instances, the stem cell is selected from the group consisting of an embryonic stem cell, a pluripotent stem cell, an induced pluripotent stem cell, and an adult stem cell.

[0026] In another example, provided herein is a construct comprising from 5' end to 3' end: an inducible RNA polymerase promoter; an shRNA sequence targeting an immunosuppressive factor; a constitutive promoter; and a transactivator element corresponding to the inducible RNA polymerase promoter.

[0027] In one example, provided herein is a construct comprising from 5' end to 3' end: an inducible RNA polymerase promoter; an immune signaling factor gene; a promoter; and a transactivator element corresponding to the inducible RNA polymerase promoter.

[0028] In some examples, the inducible RNA polymerase promoter is a U6Tet promoter. In many examples, the inducible RNA polymerase promoter is a TRE promoter.

[0029] In some examples, the immunosuppressive factor of the construct is selected from the group consisting of 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 some examples, the immune signaling factor of the construct is selected from the group consisting of B2M, MIC-A, MIC-B, HLA-A, HLA-B, HLA-C, RFXANK, CTLA-4, PD-1, RAET1E/ULBP4, RAET1G/ULBP5, RAET1H/ULBP2, RAET1/ULBP1, RAET1L/ULBP6, RAET1N/ULBP3, and other ligands of NKG2D.

[0030] In some examples, the promoter of the construct is selected from the group consisting of an EF1A promoter, an EFS promoter, a CMV promoter, a CAGGS promoter, a SV40 promoter, a COPIA promoter, an ACT5C promoter, a TRE promoter, a CBh promoter, a PGK promoter, and a UBC promoter.

[0031] In some examples, the construct comprises from 5' end to 3' end: a U6Tet promoter, a shRNA sequence targeting CD47, an EF1a promoter, and a Tet Repressor element.

[0032] In some examples, the construct comprises from 5' end to 3' end: a TRE promoter, an immune signaling factor gene, an EF1a promoter, and a Tet-On element.

[0033] In some examples, the construct further comprises a vector backbone for lentiviral expression.

[0034] Also provided is a composition comprising an isolated cell comprising any of the constructs described herein.

[0035] Also provided is a composition comprising an isolated cell comprising any of the constructs described herein, wherein the isolated cell is engineered to exogenously express the immunosuppressive factor. In some examples, the isolated cell overexpresses the immunosuppressive factor in the absence of the exogenous factor that activates the transactivator element. In some examples, the isolated cell is exposed to an exogenous factor to activate the transactivator element. In some examples, the isolated cell is a stem cell selected from the group consisting of an embryonic stem cell, a pluripotent stem cell, and an adult stem cell.

[0036] In some examples, the composition comprises isolated differentiated cells prepared by culturing any of the stem cells outlined herein under differentiation conditions appropriate for differentiation of the stem cell into a cell type selected from the group consisting of cardiac cells, liver cells, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells.

[0037] In some examples, provided herein is a method of treating a patient in need of cell therapy comprising: (a) administering any composition outlined herein to a patient; and (b) exposing the composition to an exogenous factor to activate the inducible RNA polymerase promoter, thereby controlling immunogenicity of the cells of the composition.

[0038] Provided is a pluripotent stem cell comprising (i) reduced or silenced expression of MHC class I molecules and/or MHC class II molecules, (ii) overexpression of CD47, and (iii) a factor selected from the group consisting of: an inducible shRNA targeting CD47, an inducible degron element controlling CD47, or a SMASH degron element controlling CD47.

[0039] Also provided herein is a pluripotent stem cell comprising (i) reduced or silenced expression of B2M and CIITA, (ii) overexpression of CD47, and (iii) a factor selected from the group consisting of: an inducible shRNA targeting CD47, an inducible degron element controlling CD47, or a SMASH degron element controlling CD47.

[0040] Additionally, outlined herein is a pluripotent stem cell comprising (i) reduced or silenced expression of MHC class I molecules and/or MHC class II molecules, (ii) overexpression of CD47, (iii) a Cas9 or a variant thereof, and (iv) an inducible guide RNA targeting CD47.

modulating expression of CD47 selected from the group consisting of a tissue specific promoter expression system, an inducible promoter expression system, a molecule regulated riboswitch system, and an inducible nuclease-based genome editing system.

[0048] Provided herein is a pluripotent stem cell comprising (i) reduced or silenced expression of MHC class I molecules and/or MHC class II molecules, (ii) overexpression of CD47, and (iii) an inducible system for modulating expression of CD47. In some examples, the inducible system decreases or reduces expression of CD47 in the cell.

[0049] Also, provided herein is a pluripotent stem cell comprising (i) reduced or silenced expression of B2M and CIITA, (ii) overexpression of CD47, and (iii) an inducible system for modulating expression of CD47. In some examples, the inducible system decreases or reduces expression of CD47.

[0049a] In one aspect, the present disclosure refers to a pluripotent stem cell comprising: (i) reduced or silenced expression of one or more MHC class I molecules and/or one or more MHC class II molecules, or reduced or silenced expression of B2M and CIITA, (ii) overexpression of CD47, and (iii) one of the following: (a) a factor selected from the group consisting of: an inducible shRNA targeting CD47, an inducible degron element controlling CD47, or a SMASH degron element controlling CD47; (b) a Cas nuclease or a variant thereof and an inducible guide RNA targeting CD47, optionally wherein the Cas nuclease is Cas9; (c) an inducible protein degradation system for modulating expression of CD47 selected from the group consisting of a small molecule-assisted shutoff (SMASH) system, a Shield-1-inducible degron, an auxin-inducible degron, an IMid-inducible degron, a peptidic degron, a proteolysis targeting chimera, and an antibody for targeted degradation; (d) an RNA regulation system for modulating expression of CD47 selected from the group consisting of an inducible shRNA, an inducible siRNA, a CRISPR interference (CRISPRi), and an RNA targeting nuclease system; (e) a DNA regulation system for modulating expression of CD47 selected from the group consisting of a tissue specific promoter expression system, an inducible promoter expression system, a molecule regulated riboswitch system, and an inducible nuclease-based genome editing system; or
(f) an inducible system for modulating expression of CD47.

[0049b] In one aspect, the present disclosure refers to a differentiated cell derived from the pluripotent stem cell asd described herein, wherein the differentiated cell is selected from the group consisting of a cardiac cell, liver cell, kidney cell, pancreatic cell, neural cell, immune cell, mesenchymal cell, and endothelial cell.

[0050] In some instances, provided is a differentiated cell derived from any of the pluripotent stem cells outlined, wherein the differentiated cell is selected from the group consisting of a

cardiac cell, liver cell, kidney cell, pancreatic cell, neural cell, immune cell, mesenchymal cell, and endothelial cell.

[0051] In one example, outlined is a construct comprising from 5’ end to 3’ end: a promoter, an inducible degron element, an optional sequence encoding a flexible linker, and an immunosuppressive factor gene.

[0052] In another example, outlined is a construct comprising from 5’ end to 3’ end: a promoter, an immunosuppressive factor gene, an optional sequence encoding a flexible linker, and an inducible degron element.

[0052a] In another aspect, the present disclosure refers to a construct comprising: (1) from 5’ end to 3’ end: an inducible RNA polymerase promoter; an shRNA sequence targeting an immunosuppressive factor; a constitutive promoter; and a transactivator element corresponding to the inducible RNA polymerase promoter; (2) from 5’ end to 3’ end: an inducible RNA polymerase promoter; an immune signaling factor gene; a promoter; and a transactivator element corresponding to the inducible RNA polymerase promoter; (3) from 5’ end to 3’ end: a promoter, an inducible degron element, an optional sequence encoding a flexible linker, and an immunosuppressive factor gene; or (4) from 5' end to 3' end: a promoter, an immunosuppressive factor gene, an optional sequence encoding a flexible linker, and an inducible degron element.

[0052b] In another aspect, the present disclosure refers to an isolated cell comprising a construct as described herein.

[0053] In some examples, the promoter is a constitutive promoter selected from the group consisting of an EF1A promoter, an EFS promoter, a CMV promoter, a CAGGS promoter, a SV40 promoter, a COPIA promoter, an ACT5C promoter, a TRE promoter, a CBh promoter, a PGK promoter, and a UBC promoter.

[0054] In many examples, the flexible linker is selected from the group consisting of consisting of $(GSG)_n$ (SEQ ID NO:3), $(GGGS)_n$ (SEQ ID NO:1), and $(GGGSGGGS)_n$ (SEQ ID NO:2), wherein n is 1-10

[0055] In some examples, the immunosuppressive factor is selected from the group consisting of 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.

[0056] In some examples, the inducible degron element is selected from the group consisting of a ligand inducible degron element, an inducible peptidic degron element, and a peptidic proteolysis targeting chimera (PROTAC) element. In some examples, 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.

[0057] In some examples, the construct further comprises a 5' homology arm and a 3' homology arm for targeted integration to a genomic safe harbor locus selected from the group consisting of an AAVS1 locus, a CLBYL locus, a CXCR4 locus, a Rosa26 locus, and a CCR5 locus.

[0058] Provided is a composition comprising an isolated cell comprising any of the constructs described.

[0059] In some examples, the isolated cell is selected from the group consisting of a stem cell, embryonic stem cell, pluripotent stem cell, and adult stem cell.

[0060] Provided is a composition comprising isolated differentiated cells prepared by culturing any of the stem cells described under differentiation conditions appropriate for differentiation of the stem cell into a cell type selected from the group consisting of cardiac cells, liver cells, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells.

[0061] In some example, provided herein is a method of treating a patient in need of cell therapy comprising:(a) administering the composition described to the patient; and (b) exposing the composition to an exogenous factor to activate the inducible degron element, thereby controlling immunogenicity of the cells of the composition.

[0062] In some example, provided herein is a composition comprising an isolated cell comprising a DNA targeted nuclease system for controlling immunogenicity of the cell comprising: (a) a first element comprising from 5' end to 3' end: a first promoter and an immunosuppressive factor gene; (b) a second element comprising from 5' end to 3' end: a second promoter and a nucleic acid sequence encoding Cas9 or a variant thereof; and (c) a third element comprising from 5' end to 3' end: an inducible RNA polymerase promoter, a guide RNA (gRNA) sequence targeting the immunosuppressive factor, a third promoter, and a transactivator element corresponding to the inducible promoter. In certain examples, immunogenicity of the cell is controllable upon exposing the cell to an exogenous factor to induce activity of the transactivator element. In some

examples, the inducible RNA polymerase promoter is a U6Tet promoter, the transactivator element is a Tet Repressor element, and the exogenous factor is tetracycline or a derivative thereof.

[0062a] In another aspect, the present disclosure refers to an isolated cell comprising a DNA targeted nuclease system for controlling immunogenicity of the cell comprising: (a) a first element comprising from 5' end to 3' end: a first promoter and an immunosuppressive factor gene; (b) a second element comprising from 5' end to 3' end: a second promoter and a nucleic acid sequence encoding a Cas nuclease or a variant thereof, optionally wherein the Cas nuclease is Cas9; and (c) a third element comprising from 5' end to 3' end: an inducible RNA polymerase promoter, a guide RNA (gRNA) sequence targeting the immunosuppressive factor, a third promoter, and a transactivator element corresponding to the inducible promoter, wherein immunogenicity of the cell is controllable upon exposing the cell to an exogenous factor to induce activity of the transactivator element.

[0062b] In another aspect, the present disclosure refers to a composition comprising differentiated cells prepared by culturing the stem cell as described herein under differentiation conditions appropriate for differentiation of the stem cell into a cell type selected from the group consisting of cardiac cells, liver cells, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells.**[0062c]** In another aspect, the present disclosure refers to a composition comprising the primary cell as described herein, wherein the cell type is selected from the group consisting of cardiac cells, liver cells, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells.

[0062d] In another aspect, the present disclosure refers to the use of a cell as described herein in the manufacture of a medicament for cell therapy.

[0062e] In another aspect, the present disclosure refers to a cell as described herein for use in cell therapy.

[0062f] In another aspect, the present disclosure refers to the use of a composition as described herein in the manufacture of a medicament for cell therapy.

[0062g] In another aspect, the present disclosure refers to a composition as described herein for use in cell therapy.

[0062h] In yet another aspect, the present disclosure refers to a composition comprising an isolated mammalian cell comprising a modification comprising a recombinant nucleic acid sequence encoding a system for conditional expression of one or more immunosuppressive factors or one or more immune signaling factors.

[0063] In some examples, the immunosuppressive factor is selected from the group consisting of 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.

[0064] In some examples, the first, second and/or third promoters are constitutive promoters, each independently selected from the group consisting of an EF1A promoter, an EFS promoter, a CMV promoter, a CAGGS promoter, a SV40 promoter, a COPIA promoter, an ACT5C promoter, a TRE promoter, a CBh promoter, a PGK promoter, and a UBC promoter.

[0065] In some examples, the isolated cell is an isolated human cell further comprising deletion or reduced expression of MHC class I human leukocyte antigens and/or deletion or reduced expression of MHC class II human leukocyte antigens compared to an unmodified human cell. In some examples, the isolated human cell further comprises deletion or reduced expression of CIITA, B2M, and/or NLRC5.

[0066] In some examples, the isolated human cell is hypoimmunogenic and a stem cell. In some instances, the stem cell is selected from the group consisting of an embryonic stem cell, a pluripotent stem cell, an induced pluripotent stem cell, and an adult stem cell.

[0067] Provided herein is a composition comprising isolated differentiated cells prepared by culturing the stem cell described under differentiation conditions appropriate for differentiation of the stem cell into a cell type selected from the group consisting of cardiac cells, liver cells, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells.

[0068] In one example, provided is a method of treating a patient in need of cell therapy comprising:(a) administering the composition described above; and (b) exposing the composition to an exogenous factor to activate the inducible RNA polymerase promoter, thereby controlling immunogenicity of the cells of the composition.

[0069] In one example, provided is a composition comprising an isolated mammalian cell comprising a modification comprising a recombinant nucleic acid sequence encoding a system for conditional expression of one or more immunosuppressive factors.

[0070] In one example, provided is a composition comprising an isolated mammalian cell comprising a recombinant nucleic acid sequence encoding a system for conditional expression of one or more immune signaling factors.

[0071] In some examples, the expression of the one or more immunosuppressive factors is controllable by an exogenous factor. In some examples, the expression of the one or more immune signaling factors is controllable by an exogenous factor.

[0072] In some examples, the system comprises an inducible protein degradation system to reduce protein levels of the one or more immunosuppressive factors. In some examples, the inducible protein degradation system is selected from the group consisting of a small molecule-assisted shutoff (SMASH) system, a Shield-1-inducible degron, an auxin-inducible degron, an IMid-inducible degron, a peptidic degron, a proteolysis targeting chimera, and an antibody for targeted degradation. In some examples, the system comprises a RNA regulation system to controllably reduce RNA levels of the one or more immunosuppressive factors. In some examples, the RNA regulation system is selected from the group consisting of an inducible shRNA, an inducible siRNA, a CRISPR interference (CRISPRi), and a RNA targeting nuclease system. In some examples, the RNA regulation system is controllable by a ligand inducible transcription factor, a SynNotch receptor, or a ligand regulated riboswitch.

[0073] In some examples, the system comprises a DNA regulation system to reduce expression levels of the one or more immunosuppressive factors that is selected from the group consisting of a tissue-specific promoter expression system, an inducible promoter expression system, a molecule regulated riboswitch system, and an inducible nuclease-based genome editing system.

[0074] In some examples, the inducible promoter expression system comprises a U6Tet promoter and a Tet Repressor element.

[0075] In some examples, the system comprises an inducible protein stabilization system to increase protein levels of the one or more immune signaling factors.

[0076] In some examples, the inducible protein stabilization system comprises a ligand-inducible protein stabilization system and a small molecule-inducible protein stabilization system.

[0077] In some examples, the system comprises an RNA regulation system to increase RNA levels of the one or more immune signaling factors.

[0078] In some examples, the RNA regulation system comprises a CRISPR activation (CRISPRa) system.

[0079] In some examples, the system comprises a DNA regulation system to increase expression levels of the one or more immune signaling factors.

[0080] In some examples, the DNA regulation system comprises one selected from the group consisting of a CRISPR activation (CRISPRa) system, a tissue-specific promoter, an inducible promoter, and a molecule regulated riboswitch system.

[0081] In some examples, the tissue-specific promoter is selected from the group consisting of a cardiac cell-specific promoter, hepatocyte-specific promoter, kidney cell-specific promoter, pancreatic cell-specific promoter, neural cell-specific promoter, immune cell-specific promoter, mesenchymal cell-specific promoter, and endothelial cell-specific promoter.

[0082] In some examples, the inducible promoter comprises a TetOn system.

[0083] In some examples, the molecule regulated riboswitch system comprises a theophylline regulated riboswitch or a guanine regulated riboswitch.

[0084] In some examples, the inducible nuclease-based genome editing system comprises one selected from the group consisting of CRISPR genome editing comprising an inducible guide RNA targeting the one or more immunosuppressive factors, inducible TALEN genome editing, inducible ZFN genome editing, and small molecule enhanced CRISPR-based genome editing.

[0085] In some examples, the one or more immunosuppressive factors are selected from the group consisting of 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.

[0086] In some examples, the one or more immune signaling factors are selected from the group consisting of beta-2-microglobulin (B2M), MHC class I chain-related protein A (MIC-A), MHC class I chain-related protein B (MIC-B), HLA-A, HLA-B, HLA-C, RFXANK, CTLA-4, PD-1, RAET1E/ULBP4, RAET1G/ULBP5, RAET1H/ULBP2, RAET1/ULBP1, RAET1L/ULBP6, RAET1N/ULBP3, and other ligands of NKG2D.

[0087] In some examples, the isolated mammalian cell is an isolated human cell further comprising deletion or reduced expression of MHC class I human leukocyte antigens and/or deletion or reduced expression of MHC class II human leukocyte antigens compared to an unmodified human cell.

[0088] In some examples, the isolated human cell further comprises deletion or reduced expression of CIITA, B2M, and/or NLRC5.

[0089] In some examples, the isolated human cell is hypoimmunogenic and a stem cell. In some instances, the stem cell is selected from the group consisting of an embryonic stem cell, a pluripotent stem cell, an induced pluripotent stem cell, and an adult stem cell.

[0090] Provided is a composition comprising an isolated differentiated cell prepared by culturing any of the stem cells described under differentiation conditions appropriate for differentiation of a stem cell into a cell type selected from the group consisting of cardiac cells, liver cells, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells.

[0090a] In another aspect, the present disclosure refers to a composition comprising differentiated cell prepared by culturing the stem cell as described herein under differentiation conditions appropriate for differentiation of the stem cell into a cell type selected from the group consisting of cardiac cells, liver cells, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells.

[0090b] In another aspect, the present disclosure refers to a composition comprising the primary cell as described herein, wherein the cell type is selected from the group consisting of cardiac cells, liver cells, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells.

[0090c] In another aspect, the present disclosure refers to the use of an isolated cell as described herein in the manufacture of a medicament for cell therapy.

[0090d] In another aspect, the present disclosure refers to an isolated cell as described herein for use in cell therapy.

[0090e] In another aspect, the present disclosure refers to the use of a composition as described herein in the manufacture of a medicament for cell therapy.

[0091] In another example, outlined is a method of treating a patient in need of cell therapy comprising: (a) administering the composition outlined; and (b) exposing the composition to an exogenous factor to control expression of the one or more immunosuppressive factors, thereby controlling immunogenicity of the cells of the composition.

[0092] In one example, outlined is a construct comprising from 5' to 3' end: (1) a safety switch transgene; (2) a ribosomal skipping sequence and/or a sequence encoding a linker; (3) a hypoimmunity gene. In another example, outlined is a construct comprising from 5' to 3' end: (1) a hypoimmunity gene; (2) a ribosomal skipping sequence or a linker; (3) a safety switch transgene.

[0092a] In another aspect, the present disclosure refers to a construct comprising from 5' to 3' end: (1) a safety switch transgene; (2) a ribosomal skipping sequence and/or a sequence encoding a linker; (3) a hypoimmunity gene or an essential cell factor gene; or from 5' to 3' end: (1) a hypoimmunity gene or an essential cell factor gene; (2) a ribosomal skipping sequence or a linker; (3) a safety switch transgene.

[0093] I some examples, the safety switch transgene is selected from the group consisting of a HSVtk gene, a cytosine deaminase gene, a nitroreductase gene, a purine nucleoside phosphorylase gene, a horseradish peroxidase gene, iCaspase9 gene, HER1 transgene, RQR8 transgene, CD20 transgene, CCR4 transgene, HER2 transgene, CD19 transgene, MUC1 transgene, EGFR transgene, GD2 transgene, PSMA transgene, CD16 transgene, and CD30 transgene.

[0094] In some examples, the ribosomal skipping sequence comprises a sequence encoding an IRES sequence or a sequence encoding a 2A-coding sequence.

[0095] In some examples, the linker is selected from any one of the linkers provided in Table 3.

[0096] In some examples, the hypoimmunity gene is selected from the group consisting of: CD47, CD24, CD200, HLA-G, HLA-E, HLA-C, HLA-E heavy chain, PD-L1, IDO1, CTLA4-Ig, IL-10, IL-35, FASL, Serpinb9, CCl21, and Mfge8.

[0097] In some examples, the construct further comprises a transcriptional regulatory element operably linked to the safety switch transgene and a polyadenylation sequence at the 3' end of the hypoimmunity gene, or a transcriptional regulatory element operably linked to the hypoimmunity gene and a polyadenylation sequence at the 3' end of the safety switch transgene.

[0098] In some examples, the transcriptional regulatory element of the construct is selected from the group consisting of an EF1A promoter, an EFS promoter, a CMV promoter, a CAGGS promoter, an SV40 promoter, a COPIA promoter, an ACT5C promoter, a TRE promoter, a CBh promoter, a PGK promoter, and a UBC promoter.

[0099] In some examples, the construct further comprises a vector backbone for lentiviral expression.

[00100] In some examples, provided is a method of delivering a construct into an isolated cell comprising transducing an isolated cell with a lentiviral construct comprising any construct outlined and selecting an engineered cell carrying the safety switch transgene and the hypoimmunity gene.

[00101] Provided herein is an isolated cell or a population thereof comprising a construct described. In some examples, the construct has been introduced into a target gene locus. In some examples, the gene locus is either a safe harbor locus selected from the group consisting of an AAVS1 locus, a CLBYL locus, a CXCR4 locus, a Rosa26 locus, and a CCR5 locus, or an immune signaling gene locus selected from the group consisting of B2M, HLA-A, HLA-B, HLA-C, HLA-D, HLA-E, RFXANK, CIITA, CTLA-4, PD-1, RAET1E/ULBP4, RAET1G/ULBP5, RAET1H/ULBP2, RAET1/ULBP1, RAET1L/ULBP6, RAET1N/ULBP3, and other ligands of NKG2D. In some examples, the isolated cell is an isolated engineered human cell further comprising deletion or reduced expression of MHC class I human leukocyte antigens and/or deletion or reduced expression of MHC class II human leukocyte antigens compared to an unmodified human cell. In some examples, the isolated cell further comprises deletion or reduced expression of CIITA, B2M, and/or NLRC5. In some examples, the isolated cell is hypoimmunogenic and is a stem cell.

[00102] Also provided is a differentiated cell or a population thereof prepared by culturing the stem cell under differentiation conditions appropriate for differentiation of the stem cell into a cell type selected from the group consisting of cardiac cells, liver cells, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells.

[00103] Provided is a method of treating a patient in need of cell therapy comprising administering to patient the differentiated cell or the population thereof described. Provided is a method of treating a patient comprising activating a safety switch in a patient previously administered the differentiated cell or the population thereof described.

[00103a] In another aspect, the present disclosure refers to the use of a cell or a population thereof as described herein in the manufacture of a medicament for treatment of a patient in need of cell therapy.

[00103b] In another aspect, the present disclosure refers to a cell or a population as described herein for use in treatment of a patient in need of cell therapy.

[00104] In one example, provided is a construct for homology directed repair into a safe harbor locus comprising from 5' to 3' end: (1) a first homology arm homologous to a first endogenous sequence of a safe harbor locus; (2) a safety switch transgene; (3) a ribosomal skipping sequence and/or a sequence encoding a linker; (4) an hypoimmunity gene; (5) a polyadenylation sequence; and (6) a second homology arm homologous to a second endogenous sequence of the safe harbor locus. In one example, provided is a construct for homology directed repair into a safe harbor locus comprising from 5' to 3' end: (1) a first homology arm homologous to a first endogenous sequence of an immune signaling gene locus; (2) a safety switch transgene; (3) a ribosomal skipping sequence and/or a sequence encoding a linker; (4) an hypoimmunity gene; (5) a polyadenylation sequence; and (6) a second homology arm homologous to a second endogenous sequence of the immune signaling gene locus. In one example, provided is a construct for homology directed repair into a safe harbor locus comprising from 5' to 3' end: (1) a first homology arm homologous to a first endogenous sequence of a safe harbor locus; (2) a safety switch transgene; (3) a ribosomal skipping sequence or a sequence encoding a linker; (4) an essential cell factor gene; (5) a polyadenylation sequence; and (6) a second homology arm homologous to a second endogenous sequence of the safe harbor locus. In another example, provided is a construct for homology directed repair into an immune signaling comprising from 5' to 3' end: (1) a first homology arm homologous to a first endogenous sequence of an immune signaling gene locus; (2) a safety switch transgene; (3) a ribosomal skipping sequence or a sequence encoding a linker; (4) an essential cell factor gene; (5) a polyadenylation sequence; and (6) a second homology arm homologous to a second endogenous sequence of the immune signaling gene locus. In yet another example, provided is a construct for homology directed repair into an essential cell factor gene locus comprising from 5' to 3' end: (1) a first homology arm homologous to a first endogenous sequence of an essential cell factor gene locus; (2) a sequence

encoding a linker; (3) a safety switch transgene; and (4) a second homology arm homologous to a second endogenous sequence of the essential cell factor gene locus.

[00105] In some examples, the hypoimmunity gene is selected from the group consisting of CD47, CD24, CD200, HLA-G, HLA-E, HLA-C, HLA-E heavy chain, PD-L1, IDO1, CTLA4-Ig, IL-10, IL-35, FASL, Serpinb9, CCl21, and Mfge8.

[00106] In some examples, the essential cell factor is selected from the group consisting of RpS2, RpS9, RpS11, RpS13, RpS18, RpL8, RpL11, RpL32, RpL36, Rpn22, Psmd14, PSMA3, a ribosome subunit protein, a proteasome subunit protein, and a spliceosome subunit protein.

[00107] In some examples, the safe harbor locus is selected from the group consisting of an AAVS1 locus, a CLBYL locus, a CXCR4 locus, a Rosa26 locus, and a CCR5 locus.

[00108] In some examples, the immune signaling gene locus is selected from the group consisting of an B2M, HLA-A, HLA-B, HLA-C, HLA-D, HLA-E, RFXANK, CIITA, CTLA-4, PD-1, RAET1E/ULBP4, RAET1G/ULBP5, RAET1H/ULBP2, RAET1/ULBP1, RAE11L/ULBP6, RAET1N/ULBP3, and other ligands of NKG2D.

[00109] In some examples, the immune signaling gene locus is selected from the group consisting of B2M, HLA-A, HLA-B, HLA-C, HLA-D, and HLA-E.

[00110] In some examples, the ribosomal skipping sequence comprises a sequence encoding an IRES sequence or a sequence encoding a 2A-coding sequence.

[00111] In some examples, the 2A-coding sequence is selected from the group consisting of T2A, P2A, E2A, and F2A.

[00112] In some examples, the construct enables a targeting nuclease to cleave the safe harbor locus or the immune signaling gene locus, thereby allowing the construct to recombine into the locus by homology directed repair.

[00113] In some examples, the construct enables a targeting nuclease to cleave the essential cell factor gene locus, thereby allowing the construct to recombine into the locus by homology directed repair.

[00114] In some examples, the construct further comprises a transcriptional regulatory element selected from the group consisting of an EF1A promoter, an EFS promoter, a CMV promoter, a CAGGS promoter, an SV40 promoter, a COPIA promoter, an ACT5C promoter, a TRE promoter, a CBh promoter, a PGK promoter, and a UBC promoter located at the 5’ end of the safety switch transgene.

[00115] In some examples, the safety switch transgene is selected from the group consisting of a HSVtk gene, a cytosine deaminase gene, a nitroreductase gene, a purine nucleoside

phosphorylase gene, a horseradish peroxidase gene, iCaspase9 gene, HER1 transgene, RQR8 transgene, CD20 transgene, CCR4 transgene, HER2 transgene, CD19 transgene, MUC1 transgene, EGFR transgene, GD2 transgene, PSMA transgene, CD16 transgene, and CD30 transgene.

[00116] In some examples, the linker is selected from any one of the linkers provided in Table 3.

[00117] Outlined herein is an isolated cell or a population thereof comprising a safety switch transgene and a hypoimmunity gene integrated into a safe harbor locus or an immune signaling gene locus, wherein any of the constructs above has recombined into the endogenous safe harbor locus of a cell, or wherein any of the constructs above has recombined into the endogenous immune signaling gene locus of a cell. Outlined herein is an isolated cell or a population thereof comprising a safety switch transgene and an essential cell factor gene integrated into a safe harbor locus or an immune signaling gene locus, wherein any of the constructs above has recombined into the endogenous safe harbor locus of a cell, or wherein any of the constructs above has recombined into the endogenous immune signaling gene locus of a cell, and wherein the cell or the population thereof is unable to express the essential cell factor from the endogenous locus.

[00118] In some examples, the isolated cell is an isolated engineered human cell further comprising deletion or reduced expression of MHC class I human leukocyte antigens and/or deletion or reduced expression of MHC class II human leukocyte antigens compared to an unmodified human cell. In some examples, the isolated cell further comprises deletion or reduced expression of CIITA, B2M and/or NLRC5. In some examples, the isolated cell is hypoimmunogenic and a stem cell. In some instances, the stem cell is selected from the group consisting of an embryonic stem cell, a pluripotent stem cell, an induced pluripotent stem cell, and an adult stem cell.

[00119] Provided is a differentiated cell or a population thereof prepared by culturing the any stem cell outlined herein under differentiation conditions appropriate for differentiation of the stem cell into a cell type selected from the group consisting of cardiac cells, liver cell, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells.

[00120] Disclosed is a method of treating a patient in need of cell therapy comprising administering to a patient the differentiated cell or the population thereof outlined. Also disclosed

is a method of treating a patient comprising activating a safety switch in a patient previously administered the differentiated cell or the population thereof outlined.

[00121] Provided is a homology independent donor construct comprising from 5' to 3' end: (1) a 5' long terminal repeats (LTR) comprising a left element (LE); (2) a splice acceptor-viral 2A peptide (SA-2A) element; (3) a safety switch transgene; (4) a ribosomal skipping sequence or sequence encoding a linker; (5) a hypoimmunity gene; (6) a polyadenylation sequence; and (7) 3' LTR comprising a right element (RE).

[00122] Provided is a homology independent donor construct comprising from 5' to 3' end: (1) a 5' long terminal repeats (LTR) comprising a left element (LE); (2) a splice acceptor-viral 2A peptide (SA-2A) element; (3) a safety switch transgene; (4) a ribosomal skipping sequence or a sequence encoding a linker; (5) an essential cell factor gene; (6) a polyadenylation sequence; and (7) 3' LTR comprising a right element (RE).

[00123] Provided is a homology independent donor construct comprising from 5' to 3' end: (1) a 5' long terminal repeats (LTR) comprising a left element (LE); (2) a splice acceptor-viral 2A peptide (SA-2A) element; (3) an essential cell factor gene; (4) a ribosomal skipping sequence or a sequence encoding a linker; (5) a safety switch transgene; (6) a polyadenylation sequence; and (7) 3' LTR comprising a right element (RE).

[00124] In some examples, the hypoimmunity gene is selected from the group consisting of CD47, CD24, CD200, HLA-G, HLA-E, HLA-C, HLA-E heavy chain, PD-L1, IDO1, CTLA4-Ig, IL-10, IL-35, FASL, Serpinb9, CCl21, and Mfge8.

[00125] In some examples, the essential cell factor is selected from the group consisting of RpS2, RpS9, RpS11, RpS13, RpS18, RpL8, RpL11, RpL32, RpL36, Rpn22, Psmd14, PSMA3, a ribosome subunit protein, a proteasome subunit protein, and a spliceosome subunit protein.

[00126] In some examples, the construct is configured to integrate into a target gene locus of an isolated cell to disrupt expression of the target gene.

[00127] In some examples, the safety switch transgene is selected from the group consisting of a HSVtk gene, a cytosine deaminase gene, a nitroreductase gene, a purine nucleoside phosphorylase gene, a horseradish peroxidase gene, iCaspase9 gene, HER1 transgene, RQR8 transgene, CD20 transgene, CCR4 transgene, HER2 transgene, CD19 transgene, MUC1 transgene, EGFR transgene, GD2 transgene, PSMA transgene, CD16 transgene, and CD30 transgene.

[00128] In some examples, the target gene locus is an immune signaling gene locus selected from the group consisting of B2M, HLA-A, HLA-B, HLA-C, HLA-D, HLA-E, RFXANK, CIITA, CTLA-4, PD-1, RAET1E/ULBP4, RAET1G/ULBP5, RAET1H/ULBP2, RAET1/ULBP1, RAET1L/ULBP6, RAET1N/ULBP3, and other ligands of NKG2D. In some examples, the target gene locus is immune signaling gene locus selected from the group consisting of B2M, HLA-A, HLA-B, HLA-C, HLA-D, and HLA-E. In some examples, the target gene locus is a safe harbor locus selected from the group consisting of an AAVS1 locus, a CLBYL locus, a CXCR4 locus, a Rosa26 locus, and a CCR5 locus.

[00129] Outlined herein is an isolated cell or a population thereof comprising any of the constructs described, wherein the construct has integrated into an endogenous target gene to disrupt expression target gene expression in the isolated cell. Outlined herein is the isolated cell or the population thereof, wherein the isolated cell is unable to express the essential cell factor from the endogenous loci.

[00130] In some examples, the construct has integrated into the target gene at a nuclease or transposase target site. In some examples, one allele of the target gene are disrupted a nuclease or transposase targeting. In some examples, both alleles of the target gene are disrupted by a nuclease or transposase targeting. In some examples, the isolated cell is an isolated engineered human cell further comprising deletion or reduced expression of MHC class I human leukocyte antigens and/or deletion or reduced expression of MHC class II human leukocyte antigens compared to an unmodified human cell. In some examples, the isolated cell further comprises deletion or reduced expression of CIITA, B2M, and/or NLRC5. In some examples, the isolated cell is hypoimmunogenic and a stem cell. In some instances, the stem cell is selected from the group consisting of an embryonic stem cell, a pluripotent stem cell, an induced pluripotent stem cell, and an adult stem cell.

[00131] In some example, provided herein is a differentiated cell or a population thereof prepared by culturing the stem cell outlined under differentiation conditions appropriate for differentiation of the stem cell into a cell type selected from the group consisting of cardiac cells, liver cells, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells. In some examples, provided is a method of treating a patient in need of cell therapy comprising administering to a patient the differentiated cell or the population thereof. In some

examples, provided is a method of treating a patient comprising activating the safety switch in the patient previously administered the differentiated cell or the population thereof.

[00132] Provided herein is an isolated cell or a population thereof comprising an essential cell factor gene operably linked to a sequence encoding a linker that is operably linked to a safety switch transgene.

[00133] In some examples, the essential cell factor is selected from the group consisting of RpS2, RpS9, RpS11, RpS13, RpS18, RpL8, RpL11, RpL32, RpL36, Rpn22, Psmd14, PSMA3, a ribosome subunit protein, a proteasome subunit protein, and a spliceosome subunit protein. In some examples, the linker is selected from any one of the linkers provided in Table 3.

[00134] In some examples, the safety switch transgene is selected from the group consisting of a HSVtk gene, a cytosine deaminase gene, a nitroreductase gene, a purine nucleoside phosphorylase gene, a horseradish peroxidase gene, iCaspase9 gene, HER1 transgene, RQR8 transgene, CD20 transgene, CCR4 transgene, HER2 transgene, CD19 transgene, MUC1 transgene, EGFR transgene, GD2 transgene, PSMA transgene, CD30 transgene, and CD16 transgene.

[00135] In some example, provided herein is a recombinant peptide epitope fusion protein comprising: (1) a hypoimmunity factor selected from the group consisting of CD47, CD24, CD200, HLA-G, HLA-E, HLA-C, HLA-E heavy chain, PD-L1, IDO1, CTLA4-Ig, IL-10, IL-35, FASL, Serpinb9, CCl21, Mfge8, and membrane-bound forms thereof; and (2) a surface-exposed peptide epitope heterologous to the hypoimmunity factor selected from the group consisting of a CD20 epitope, CCR4 epitope, HER2 epitope, CD19 epitope, MUC1 epitope, EGFR epitope, GD2 epitope, PSMA epitope, CD16 epitope, and CD30 epitope.

[00135a] In another aspect, the present disclosure refers to a recombinant peptide epitope fusion protein comprising: (A) (1) a hypoimmunity factor selected from the group consisting of CD47, CD24, CD200, HLA-G, HLA-E, HLA-C, HLA-E heavy chain, PD-L1, IDO1, CTLA4-Ig, IL-10, IL-35, FASL, Serpinb9, CC121, Mfge8, and membrane-bound forms thereof; and (2) a surface-exposed peptide epitope heterologous to the hypoimmunity factor selected from the group consisting of a CD20 epitope, CCR4 epitope, HER2 epitope, CD19 epitope, MUC1 epitope, EGFR epitope, GD2 epitope, PSMA epitope, CD16 epitope, and CD30 epitope; or (B) (1) an essential cell factor; and (2) a surface-exposed peptide epitope heterologous to the essential cell factor.

[00135b] In another aspect, the present disclosure refers to a construct encoding a recombinant peptide epitope fusion protein as described herein.

[00135c] In another aspect, the present disclosure refers to a method of introducing a construct as described herein into a cell.

[00135d] In yet another aspect, the present disclosure refers to an isolated cell or a population thereof comprising a construct as described herein.

[00136] In some example, provided herein is a construct encoding a recombinant peptide epitope fusion protein comprising: (1) a sequence encoding a hypoimmunity factor selected from the group consisting of CD47, CD24, CD200, HLA-G, HLA-E, HLA-C, HLA-E heavy chain, PD-L1, IDO1, CTLA4-Ig, IL-10, IL-35, FASL, Serpinb9, CCl21, Mfge8, and membrane-bound forms thereof; and (2) a sequence encoding a surface-exposed peptide epitope heterologous to the hypoimmunity factor selected from the group consisting of a CD20 epitope, CCR4 epitope, HER2 epitope, CD19 epitope, MUC1 epitope, EGFR epitope, GD2 epitope, PSMA epitope, CD16 epitope, and CD30 epitope.

[00137] In some examples, the CD20 epitope is recognized by a therapeutic antibody selected from the group consisting of obinutuzumab, ublituximab, ocaratuzumab, rituximab, rituximab-RLIb, and biosimilars thereof; the CCR4 epitope is recognized by a therapeutic antibody selected from the group consisting of mogamulizumab and biosimilars thereof; the HER2 epitope is recognized by a therapeutic antibody selected from the group consisting of margetuximab, trastuzumab, TrasGEX, and biosimilars thereof; the CD19 epitope is recognized by a therapeutic antibody selected from the group consisting of MOR208 and biosimilars thereof; the MUC1 epitope is recognized by a therapeutic antibody selected from the group consisting of gatipotuzumab and biosimilars thereof; the EGFR epitope is recognized by a therapeutic antibody selected from the group consisting of tomuzotuximab, RO5083945 (GA201), cetuximab, and biosimilars thereof; the GD2 epitope is recognized by a therapeutic antibody selected from the group consisting of Hul4.18K322A, Hul4.18-IL2, Hu3F8, dinituximab, c.60C3-RLIc, and biosimilars thereof; the PSMA epitope is recognized by a therapeutic antibody selected from the group consisting of KM2812 and biosimilars thereof; the CD30 or CD16 epitope is recognized by a therapeutic antibody selected from the group consisting of AFM13 and biosimilars thereof, or the CD20 or CD16 epitope is recognized by a therapeutic antibody selected from the group consisting of (CD20)2xCD16 and biosimilars thereof.

[00138] In some examples, the hypoimmunity factor and/or the peptide epitope is at the N-terminus of the fusion protein.

[00139] In some examples, the protein further comprises a linker connecting the hypoimmunity factor and the peptide epitope and/or located at the N-terminus or C-terminus of the fusion protein. In some examples, the linker is selected from any one of the linkers provided in Table 3.

[00140] In some examples, the sequence encoding the hypoimmunity factor of the construct is 5' of the sequence encoding the peptide epitope and/or the sequence encoding the peptide epitope is at the 5' of the sequence encoding the hypoimmunity factor.

[00141] In some examples, the construct further comprises a sequence encoding a linker connecting the sequence encoding the hypoimmunity factor and the sequence encoding the peptide epitope and/or located at the N-terminus or C-terminus of the fusion protein. In some examples, the linker is selected from any one of the linkers provided in Table 3.

[00142] In some examples, the construct further comprises a transcriptional regulatory element selected from the group consisting of an EF1A promoter, an EFS promoter, a CMV promoter, a CAGGS promoter, an SV40 promoter, a COPIA promoter, an ACT5C promoter, a TRE promoter, a CBh promoter, a PGK promoter, and a UBC promoter. In some examples, the construct further comprises a first homology arm and a second homology arm homologous to a target gene locus for CRISPR-based homology directed repair. In some examples, the construct further comprises a vector backbone for lentiviral expression.

[00143] Also provided is a method of delivering a construct into an isolated cell comprising transducing an isolated cell with a lentiviral construct comprising a construct of described herein; and selecting an engineered cell expressing a recombinant peptide epitope fusion protein. In some examples, provided is a method comprising transducing an isolated cell with any of the constructs described and selected the isolated cell that expresses the recombinant peptide epitope fusion protein encoded by the construct.

[00144] Also provided is an isolated cell or a population thereof comprising a construct described herein. In some examples, the isolated cell is an isolated human cell further comprising deletion or reduced expression of MHC class I human leukocyte antigens and/or deletion or reduced expression of MHC class II human leukocyte antigens compared to an unmodified human cell. In some examples, the isolated cell further comprises deletion or reduced expression of CIITA, B2M, and/or NLRC5. In some examples, the isolated cell is hypoimmunogenic and is a stem cell. In some examples, the stem cell is selected from the group consisting of an embryonic stem cell, a pluripotent stem cell, an induced pluripotent stem cell, and an adult stem cell.

[00145] Further, outlined herein is a differentiated cell or a population thereof prepared by culturing the stem cell described under differentiation conditions appropriate for differentiation of the stem cell into a cell type selected from the group consisting of cardiac cells, liver cells, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells.

[00146] In some cases, provided is a method of treating a patient in need of cell therapy comprising administering to patient the differentiated cell or the population thereof described. In some cases, provided is a method of treating a patient comprising administering to a patient previously administered the differentiated cell or the population thereof an antibody that binds the peptide epitope. In some examples, the antibody mediates ADCC or CDC.

[00147] In some examples, outlined is a recombinant CD47-internal-peptide epitope fusion protein comprising from N- to C-terminal: (1) a human CD47 fragment comprising a IgV domain of CD47; (2) a first linker; (3) a heterologous peptide epitope; (4) a second linker; and (5) a human CD47 transmembrane domain.

[00148] In some examples, the human CD47 fragment comprising the IgV domain comprises amino acid residues 1-127 of the human CD47 protein. In some examples, the human CD47 transmembrane domain comprises amino acid residues 128-348 of the human CD47 protein. In some examples, the first and second linkers are selected from any one of the linkers provided in Table 3. In some examples, the peptide epitope is selected from the group consisting of a CD20 epitope, CCR4 epitope, HER2 epitope, CD19 epitope, MUC1 epitope, EGFR epitope, GD2 epitope, PSMA epitope, CD16 epitope, and CD30 epitope. In some examples, the CD20 epitope is recognized by a therapeutic antibody selected from the group consisting of obinutuzumab, ublituximab, ocaratuzumab, rituximab, rituximab-RLIb, and biosimilars thereof; the CCR4 epitope is recognized by a therapeutic antibody selected from the group consisting of mogamulizumab and biosimilars thereof; the HER2 epitope is recognized by a therapeutic antibody selected from the group consisting of margetuximab, trastuzumab, TrasGEX, and biosimilars thereof; the CD19 epitope is recognized by a therapeutic antibody selected from the group consisting of MOR208 and biosimilars thereof; the MUC1 epitope is recognized by a therapeutic antibody selected from the group consisting of gatipotuzumab and biosimilars thereof; the EGFR epitope is recognized by a therapeutic antibody selected from the group consisting of tomuzotuximab, RO5083945 (GA201), cetuximab, and biosimilars thereof; the GD2 epitope is recognized by a therapeutic antibody selected from the group consisting of Hul4.18K322A, Hul4.18-IL2, Hu3F8, dinituximab, c.60C3-RLIc, and biosimilars thereof; the PSMA epitope is recognized by a therapeutic antibody selected from the group consisting of KM2812 and biosimilars thereof; the CD30 or CD16 epitope is recognized by a therapeutic antibody selected from the group consisting of AFM13 and biosimilars thereof, or the CD20 or CD16 epitope is recognized by a therapeutic antibody selected from the group consisting of (CD20)2xCD16 and biosimilars thereof.

[00149] In another example, disclosed is a construct comprising from 5' to 3' end: (1) a transcriptional regulatory element; (2) a sequence encoding a human CD47 fragment comprising a IgV domain of CD47; (3) a first linker; (4) a sequence encoding a peptide epitope; (5) a second

linker; and (6) a sequence encoding a human CD47 fragment comprising a transmembrane domain and C-terminus.

[00150] In some examples, the human CD47 fragment comprising the IgV domain comprises amino acid residues 1-127 of the human CD47 protein. In some examples, the human CD47 fragment comprising the transmembrane domain and C-terminus comprises amino acid residues 128-348 of the human CD47 protein. In some examples, the first and second linkers are selected from any one of the linkers provided in Table 3. In some examples, the peptide epitope encoded by the sequence of (4) of the construct is selected from the group consisting of a CD20 epitope, CCR4 epitope, HER2 epitope, CD19 epitope, MUC1 epitope, EGFR epitope, GD2 epitope, PSMA epitope, CD16 epitope, and CD30 epitope. In some examples, the CD20 epitope is recognized by a therapeutic antibody selected from the group consisting of obinutuzumab, ublituximab, ocaratuzumab, rituximab, rituximab-RLIb, and biosimilars thereof; the CCR4 epitope is recognized by a therapeutic antibody selected from the group consisting of mogamulizumab and biosimilars thereof; the HER2 epitope is recognized by a therapeutic antibody selected from the group consisting of margetuximab, trastuzumab, TrasGEX, and biosimilars thereof; the CD19 epitope is recognized by a therapeutic antibody selected from the group consisting of MOR208 and biosimilars thereof; the MUC1 epitope is recognized by a therapeutic antibody selected from the group consisting of gatipotuzumab and biosimilars thereof; the EGFR epitope is recognized by a therapeutic antibody selected from the group consisting of tomuzotuximab, RO5083945 (GA201), cetuximab, and biosimilars thereof; the GD2 epitope is recognized by a therapeutic antibody selected from the group consisting of Hul4.18K322A, Hul4.18-IL2, Hu3F8, dinituximab, c.60C3-RLIc, and biosimilars thereof; the PSMA epitope is recognized by a therapeutic antibody selected from the group consisting of KM2812 and biosimilars thereof; the CD30 or CD16 epitope is recognized by a therapeutic antibody selected from the group consisting of AFM13 and biosimilars thereof, or the CD20 or CD16 epitope is recognized by a therapeutic antibody selected from the group consisting of (CD20)2xCD16 and biosimilars thereof.

[00151] In some examples, the transcriptional regulatory element is selected from the group consisting of an EF1A promoter, an EFS promoter, a CMV promoter, a CAGGS promoter, an SV40 promoter, a COPIA promoter, an ACT5C promoter, a TRE promoter, a CBh promoter, a PGK promoter, and a UBC promoter.

[00152] In some examples, the construct further comprises a first homology arm and a second homology arm homologous to a target gene locus for CRISPR-based homology directed repair. In some examples, the construct further comprises a vector backbone for lentiviral expression.

[00153] Provided is a method of delivering a construct into an isolated cell comprising transducing an isolated cell with a lentiviral construct comprising a construct; and selecting an engineered cell expressing a CD47-internal-peptide epitope fusion protein. In some examples, the method comprises transducing an isolated cell with any of the constructs described and selected the isolated cell that expresses the CD47-internal-peptide epitope fusion protein encoded by the construct. Also provided is an isolated cell or a population thereof comprising the construct.

[00154] In some examples, the isolated cell is an isolated engineered human cell further comprising deletion or reduced expression of MHC class I human leukocyte antigens and/or deletion or reduced expression of MHC class II human leukocyte antigens compared to an unmodified human cell. In some examples, the isolated cell further comprises deletion or reduced expression of CIITA, B2M, and/or NLRC5. In some examples, the isolated cell is hypoimmunogenic and a stem cell. In some instances, the stem cell is an embryonic stem cell, a pluripotent stem cell, an induced pluripotent stem cell, and an adult stem cell.

[00155] Further, outlined herein is a differentiated cell or a population thereof prepared by culturing the stem cell described under differentiation conditions appropriate for differentiation of the stem cell into a cell type selected from the group consisting of cardiac cells, liver cells, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells.

[00156] In some cases, provided is a method of treating a patient in need of cell therapy comprising administering to patient the differentiated cell or the population thereof described. In some cases, provided is a method of treating a patient previously administered the differentiated cell or the population thereof comprising administering to a patient an antibody that binds the peptide epitope. In some examples, the antibody mediates ADCC or CDC.

[00157] In one example, provided is a construct comprising (1) a transcriptional regulatory element, (2) an essential cell factor gene, (3) a post-transcriptional or post-translational regulatory element, and (4) a polyadenylation sequence.

[00158] In some examples, the essential cell factor is selected from the group consisting of RpS2, RpS9, RpS11, RpS13, RpS18, RpL8, RpL11, RpL32, RpL36, Rpn22, Psmd14, PSMA3, a ribosome subunit protein, a proteasome subunit protein, and a spliceosome subunit protein.

[00159] In some examples, the transcriptional regulatory element is selected from the group consisting of an EF1A promoter, an EFS promoter, a CMV promoter, a CAGGS promoter, an SV40 promoter, a COPIA promoter, an ACT5C promoter, a TRE promoter, a CBh promoter, a PGK promoter, and a UBC promoter. In some examples, the post-transcriptional regulatory element is a RNA regulation system selected from the group consisting of an inducible shRNA, an inducible siRNA, a CRISPR interference (CRISPRi), and a RNA targeting nuclease system. In some examples, the post-translational regulatory element is an inducible protein degradation system is selected from the group consisting of a small molecule-assisted shutoff (SMASH) system, a shield-1-inducible degron, an auxin-inducible degron, an IMid-inducible degron, a peptidic degron, a proteolysis targeting chimera, and an antibody for targeted degradation.

[00160] Also provided is an isolated cell comprising a recombinant essential cell factor under the control of a post-transcriptional or post-translational regulatory element, wherein the endogenous essential cell factor gene is inactivated and expression of the recombinant essential cell factor is controllable by an exogenous factor. In some examples, the essential cell factor is selected from the group consisting of RpS2, RpS9, RpS11, RpS13, RpS18, RpL8, RpL11, RpL32, RpL36, Rpn22, Psmd14, PSMA3, a ribosome subunit protein, a proteasome subunit protein, and a spliceosome subunit protein. In some examples, the post-transcriptional regulatory element is a RNA regulation system selected from the group consisting of an inducible shRNA, an inducible siRNA, a CRISPR interference (CRISPRi), and a RNA targeting nuclease system. In some examples, the post-translational regulatory element is an inducible protein degradation system is selected from the group consisting of a small molecule-assisted shutoff (SMASH) system, a shield-1-inducible degron, an auxin-inducible degron, an IMid-inducible degron, a peptidic degron, a proteolysis targeting chimera, and an antibody for targeted degradation. In some examples, the isolated cell is an autologous human cell or an allogeneic human cell.

[00161] In some examples, the isolated cell is an isolated engineered human cell further comprising deletion or reduced expression of MHC class I human leukocyte antigens and/or deletion or reduced expression of MHC class II human leukocyte antigens compared to an unmodified human cell. In some examples, the isolated cell further comprises deletion or

reduced expression of CIITA, B2M, and/or NLRC5. In some examples, the isolated cell is hypoimmunogenic and selected from the group consisting of a stem cell and a differentiated cell.

[00162] In yet another example, provided herein is a bicistronic construct comprising from 5' to 3' end: (1) a transcriptional regulatory element; (2) a sequence encoding a surface-exposed peptide epitope; (3) a ribosomal skipping sequence; and (4) a sequence encoding a hypoimmunity factor. In some examples, the hypoimmunity factor is selected from the group consisting of CD47, CD24, CD200, HLA-G, HLA-E, HLA-C, HLA-E heavy chain, PD-L1, IDO1, CTLA4-Ig, IL-10, IL-35, FASL, Serpinb9, CCl21, Mfge8, and membrane-bound forms thereof. In some examples, the surface-exposed peptide epitope encoded by the sequence of (2) of the construct is selected from the group consisting of a CD20 epitope, CCR4 epitope, HER2 epitope, CD19 epitope, MUC1 epitope, EGFR epitope, GD2 epitope, PSMA epitope, CD16 epitope, and CD30 epitope. In some examples, the CD20 epitope is recognized by a therapeutic antibody selected from the group consisting of obinutuzumab, ublituximab, ocaratuzumab, rituximab, rituximab-RLIb, and biosimilars thereof; the CCR4 epitope is recognized by a therapeutic antibody selected from the group consisting of mogamulizumab and biosimilars thereof; the HER2 epitope is recognized by a therapeutic antibody selected from the group consisting of margetuximab, trastuzumab, TrasGEX, and biosimilars thereof; the CD19 epitope is recognized by a therapeutic antibody selected from the group consisting of MOR208 and biosimilars thereof; the MUC1 epitope is recognized by a therapeutic antibody selected from the group consisting of gatipotuzumab and biosimilars thereof; the EGFR epitope is recognized by a therapeutic antibody selected from the group consisting of tomuzotuximab, RO5083945 (GA201), cetuximab, and biosimilars thereof; the GD2 epitope is recognized by a therapeutic antibody selected from the group consisting of Hul4.18K322A, Hul4.18-IL2, Hu3F8, dinituximab, c.60C3-RLIc, and biosimilars thereof; the PSMA epitope is recognized by a therapeutic antibody selected from the group consisting of KM2812 and biosimilars thereof; the CD30 or CD16 epitope is recognized by a therapeutic antibody selected from the group consisting of AFM13 and biosimilars thereof, or the CD20 or CD16 epitope is recognized by a therapeutic antibody selected from the group consisting of (CD20)2xCD16 and biosimilars thereof.

[00163] In some examples, the ribosomal skipping sequence comprises a sequence encoding an IRES sequence or a sequence encoding a 2A-coding sequence.

[00164] In some examples, the transcriptional regulatory element is selected from the group consisting of an EF1A promoter, an EFS promoter, a CMV promoter, a CAGGS promoter, an SV40 promoter, a COPIA promoter, an ACT5C promoter, a TRE promoter, a CBh promoter, a PGK promoter, and a UBC promoter.

[00165] In some examples, the construct further comprises a first homology arm and a second homology arm homologous to a target gene locus for CRISPR-based homology directed repair. In some examples, the construct further comprises a vector backbone for lentiviral expression.

[00166] In some examples, outlined is a method of delivering a construct into an isolated cell comprising transducing an isolated cell with a lentiviral construct comprising a construct described; and selecting an engineered cell expressing a hypoimmunity factor and a peptide epitope. In some examples, the method comprises transducing an isolated cell with the construct described; and selecting the isolated cell expressing the hypoimmunity factor and the peptide epitope both encoded by the construct. Also provided is an isolated cell or a population thereof comprising a construct of described.

[00167] In some examples, the isolated cell is an isolated engineered human cell further comprising deletion or reduced expression of MHC class I human leukocyte antigens and/or deletion or reduced expression of MHC class II human leukocyte antigens compared to an unmodified human cell. In some examples, the isolated cell further comprises deletion or reduced expression of CIITA, B2M, and/or NLRC5.

[00168] In some examples, the isolated cell is hypoimmunogenic and a stem cell. In some instances, the stem cell is selected from the group consisting of an embryonic stem cell, a pluripotent stem cell, an induced pluripotent stem cell, and an adult stem cell. In some examples, provided is a differentiated cell or a population thereof prepared by culturing the stem cell described under differentiation conditions appropriate for differentiation of the stem cell into a cell type selected from the group consisting of cardiac cells, liver cells, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells. In some instances, provided is a method of treating a patient in need of cell therapy comprising administering to patient the differentiated cell or the population thereof. In some instances, provided is a method of treating a patient comprising administering to a patient previously

administered the differentiated cell or the population thereof an antibody that binds the peptide epitope. In some cases, the antibody mediates ADCC or CDC.

[00169] In one example, provided is a pluripotent stem cell comprising (i) reduced or silenced expression of MHC class I molecules and/or MHC class II molecules, (ii) a safety switch transgene and (iii) a hypoimmunity gene, wherein expression of the safety switch transgene modulates expression of the hypoimmunity gene.

[00170] In another example, provided is a pluripotent stem cell comprising (i) reduced or silenced expression of B2M and CIITA, (ii) overexpression of CD47, (iii) a safety switch transgene and (iv) a hypoimmunity gene, wherein expression of the safety switch transgene modulates expression of the hypoimmunity gene.

[00171] In yet another example, provided is a pluripotent stem cell comprising (i) reduced or silenced expression of MHC class I molecules and/or MHC class II molecules, (ii) a safety switch and (iii) a hypoimmunity factor, wherein expression of the safety switch modulates expression of the hypoimmunity factor.

[00172] In another example, provided is a pluripotent stem cell comprising (i) reduced or silenced expression of B2M and CIITA, (ii) overexpression of CD47, (iii) a safety switch and (iv) a hypoimmunity factor, wherein expression of the safety switch modulates expression of the hypoimmunity factor.

[00173] In one example, provided is a pluripotent stem cell comprising (i) reduced or silenced expression of MHC class I molecules and/or MHC class II molecules, and (ii) a hypoimmunity factor linked to a surface-exposed peptide epitope; wherein the peptide epitope is selected from the group consisting of a CD20 epitope, CCR4 epitope, HER2 epitope, CD19 epitope, MUC1 epitope, EGFR epitope, GD2 epitope, PSMA epitope, CD16 epitope, and CD30 epitope, and the hypoimmunity factor is selected from the group consisting of CD47, CD24, CD200, HLA-G, HLA-E, HLA-C, HLA-E heavy chain, PD-L1, IDO1, CTLA4-Ig, IL-10, IL-35, FASL, Serpinb9, CCl21, Mfge8, and membrane-bound forms thereof.

[00174] In one example, provided is a pluripotent stem cell comprising (i) reduced or silenced expression of B2M and CIITA, (ii) overexpression of CD47, and (iii) a hypoimmunity factor linked to a surface-exposed peptide epitope; wherein the peptide epitope is selected from the group consisting of a CD20 epitope, CCR4 epitope, HER2 epitope, CD19 epitope, MUC1 epitope, EGFR epitope, GD2 epitope, PSMA epitope, CD16 epitope, and CD30 epitope, and the

hypoimmunity factor is selected from the group consisting of CD47, CD24, CD200, HLA-G, HLA-E, HLA-C, HLA-E heavy chain, PD-L1, IDO1, CTLA4-Ig, IL-10, IL-35, FASL, Serpinb9, CCl21, Mfge8, and membrane-bound forms thereof.

[00175] In one example, provided is a construct comprising from 5' to 3' end: (1) a safety switch transgene; (2) a ribosomal skipping sequence and/or a sequence encoding a linker; and (3) an essential cell factor gene. In some examples, provided is a construct comprising from 5' to 3' end: (1) an essential cell factor gene; (2) a ribosomal skipping sequence or a linker; and (3) a safety switch transgene.

[00176] In some examples, the safety switch transgene is selected from the group consisting of a HSVtk gene, a cytosine deaminase gene, a nitroreductase gene, a purine nucleoside phosphorylase gene, a horseradish peroxidase gene, iCaspase9 gene, HER1 transgene, RQR8 transgene, CD20 transgene, CCR4 transgene, HER2 transgene, CD19 transgene, MUC1 transgene, EGFR transgene, GD2 transgene, PSMA transgene, CD16 transgene, and CD30 transgene.

[00177] In some examples, the ribosomal skipping sequence comprises a sequence encoding an IRES sequence or a sequence encoding a 2A-coding sequence.

[00178] In some examples, the linker is selected from any one of the linkers provided in Table 3.

[00179] In some examples, the hypoimmunity gene is selected from the group consisting of: CD47, CD24, CD200, HLA-G, HLA-E, HLA-C, HLA-E heavy chain, PD-L1, IDO1, CTLA4-Ig, IL-10, IL-35, FASL, Serpinb9, CCl21, and Mfge8.

[00180] In some examples, the construct further comprises a transcriptional regulatory element operably linked to the safety switch transgene and a polyadenylation sequence at the 3' end of the hypoimmunity gene, or a transcriptional regulatory element operably linked to the hypoimmunity gene and a polyadenylation sequence at the 3' end of the safety switch transgene.

[00181] In some examples, the transcriptional regulatory element is selected from the group consisting of an EF1A promoter, an EFS promoter, a CMV promoter, a CAGGS promoter, an SV40 promoter, a COPIA promoter, an ACT5C promoter, a TRE promoter, a CBh promoter, a PGK promoter, and a UBC promoter.

[00182] In some examples, the construct further comprises a vector backbone for lentiviral expression.

[00183] Outlined is a method of delivering a construct into an isolated cell comprising transducing an isolated cell with a lentiviral construct comprising a construct described; and selecting an engineered cell carrying the safety switch transgene and the hypoimmunity gene. In some examples, provided is a method comprising transducing an isolated cell with the construct (e.g., the lentiviral construct) described; and selecting the isolated cell carrying the safety switch transgene and the hypoimmunity gene of the construct. Also provided is an isolated cell or a population thereof comprising any one of the constructs described.

[00184] In some examples, the construct has been introduced into a target gene locus. In some examples, the target gene locus is selected from the group consisting of a safe harbor locus selected from the group consisting of an AAVS1 locus, a CLBYL locus, a CXCR4 locus, a Rosa26 locus, and a CCR5 locus and an immune signaling gene locus selected from the group consisting of B2M, HLA-A, HLA-B, HLA-C, HLA-D, HLA-E, RFXANK, CIITA, CTLA-4, PD-1, RAET1E/ULBP4, RAET1G/ULBP5, RAET1H/ULBP2, RAET1/ULBP1, RAET1L/ULBP6, RAET1N/ULBP3, and other ligands of NKG2D.

[00185] In some examples, the isolated cell is an engineered human cell further comprising deletion or reduced expression of MHC class I human leukocyte antigens and/or deletion or reduced expression of MHC class II human leukocyte antigens compared to an unmodified human cell. In some examples, the isolated cell further comprises deletion or reduced expression of CIITA, B2M, and/or NLRC5. In some examples, the isolated cell is hypoimmunogenic and a stem cell. In some examples, the stem cell is selected from an embryonic stem cell, a pluripotent stem cell, an induced pluripotent stem cell, and an adult stem cell.

[00186] In another example, disclosed is a differentiated cell or a population thereof prepared by culturing any stem cell under differentiation conditions appropriate for differentiation of the stem cell into a cell type selected from the group consisting of cardiac cells, liver cells, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells.

[00186a] In another aspect, the present disclosure refers to the use of a cell or a population thereof as described herein in the manufacture of a medicament for cell therapy.

[00186b] In yet another aspect, the present disclosure refers to the use of an antibody in the manufacture of a medicament for treating a patient who was previously administered the cell or population thereof as described herein, wherein the antibody binds the recombinant peptide epitope fusion protein.

[00186c] In yet another aspect, the present disclosure refers to a cell or a population thereof as described herein for use in cell therapy.

[00187] In some examples, provided is a method of treating a patient in need of cell therapy comprising administering to patient the differentiated cell or the population thereof disclosed. In some examples, provided is a method of treating a patient previously administered the differentiated cell or the population thereof disclosed comprising activating a safety switch in the patient.

[00188] In another example, provided is a recombinant peptide epitope fusion protein comprising: (1) an essential cell factor; and (2) a surface-exposed peptide epitope heterologous to the essential cell factor.

[00189] In some examples, the essential cell factor is selected from the group consisting of RpS2, RpS9, RpS11, RpS13, RpS18, RpL8, RpL11, RpL32, RpL36, Rpn22, Psmd14, PSMA3, a ribosome subunit protein, a proteasome subunit protein, a spliceosome subunit protein, and membrane-bound forms thereof.

[00190] In some examples, the peptide epitope is selected from the group consisting of a CD20 epitope, CCR4 epitope, HER2 epitope, CD19 epitope, MUC1 epitope, EGFR epitope, GD2 epitope, PSMA epitope, CD16 epitope, and CD30 epitope.

[00191] In some examples, the CD20 epitope is recognized by a therapeutic antibody selected from the group consisting of obinutuzumab, ublituximab, ocaratuzumab, rituximab, rituximab-RLIb, and biosimilars thereof; the CCR4 epitope is recognized by a therapeutic antibody selected from the group consisting of mogamulizumab and biosimilars thereof; the HER2 epitope is recognized by a therapeutic antibody selected from the group consisting of margetuximab, trastuzumab, TrasGEX, and biosimilars thereof; the CD19 epitope is recognized by a therapeutic antibody selected from the group consisting of MOR208 and biosimilars thereof; the MUC1 epitope is recognized by a therapeutic antibody selected from the group consisting of gatipotuzumab and biosimilars thereof; the EGFR epitope is recognized by a therapeutic antibody selected from the group consisting of tomuzotuximab, RO5083945 (GA201), cetuximab, and biosimilars thereof; the GD2 epitope is recognized by a therapeutic antibody selected from the group consisting of Hul4.18K322A, Hul4.18-IL2, Hu3F8, dinituximab, c.60C3-RLIc, and biosimilars thereof; the PSMA epitope is recognized by a therapeutic antibody selected from the group consisting of KM2812 and biosimilars thereof; the CD30 or CD16 epitope is recognized by a therapeutic antibody selected from the group consisting of AFM13 and biosimilars thereof, or the CD20 or CD16 epitope is recognized by a therapeutic antibody selected from the group consisting of (CD20)2xCD16 and biosimilars thereof.

[00192] In some examples, the essential cell factor is at the N-terminus of the fusion protein. In some examples, the peptide epitope is at the N-terminus of the fusion protein. In some examples, the protein further comprises a linker connecting the essential cell factor and the peptide epitope. In some examples, the protein further comprises a linker located at the N-

terminus of the peptide epitope. In some examples, the linker is selected from any one of the linkers provided in Table 3.

[00193] In another example, provided is a construct encoding a recombinant peptide epitope fusion protein comprising: (1) a sequence encoding an essential cell factor; and (2) a sequence encoding a surface-exposed peptide epitope heterologous to the essential cell factor.

[00194] In some examples, the essential cell factor is selected from the group consisting of RpS2, RpS9, RpS11, RpS13, RpS18, RpL8, RpL11, RpL32, RpL36, Rpn22, Psmd14, PSMA3, a ribosome subunit protein, a proteasome subunit protein, a spliceosome subunit protein, and membrane-bound forms thereof. In some examples, the peptide epitope is selected from the group consisting of a CD20 epitope, CCR4 epitope, HER2 epitope, CD19 epitope, MUC1 epitope, EGFR epitope, GD2 epitope, PSMA epitope, CD16 epitope, and CD30 epitope. In some examples, the CD20 epitope is recognized by a therapeutic antibody selected from the group consisting of obinutuzumab, ublituximab, ocaratuzumab, rituximab, rituximab-RLIb, and biosimilars thereof; the CCR4 epitope is recognized by a therapeutic antibody selected from the group consisting of mogamulizumab and biosimilars thereof; the HER2 epitope is recognized by a therapeutic antibody selected from the group consisting of margetuximab, trastuzumab, TrasGEX, and biosimilars thereof; the CD19 epitope is recognized by a therapeutic antibody selected from the group consisting of MOR208 and biosimilars thereof; the MUC1 epitope is recognized by a therapeutic antibody selected from the group consisting of gatipotuzumab and biosimilars thereof; the EGFR epitope is recognized by a therapeutic antibody selected from the group consisting of tomuzotuximab, RO5083945 (GA201), cetuximab, and biosimilars thereof; the GD2 epitope is recognized by a therapeutic antibody selected from the group consisting of Hul4.18K322A, Hul4.18-IL2, Hu3F8, dinituximab, c.60C3-RLIc, and biosimilars thereof; the PSMA epitope is recognized by a therapeutic antibody selected from the group consisting of KM2812 and biosimilars thereof; the CD30 or CD16 epitope is recognized by a therapeutic antibody selected from the group consisting of AFM13 and biosimilars thereof, or the CD20 or CD16 epitope is recognized by a therapeutic antibody selected from the group consisting of (CD20)2xCD16 and biosimilars thereof.

[00195] In some examples, the sequence encoding the essential cell factor is 5’ of the sequence encoding the peptide epitope.

[00196] In some examples, the sequence encoding the peptide epitope is at the 5' of the sequence encoding the essential cell factor.

[00197] In some examples, the construct further comprises a sequence encoding a linker connecting the sequence encoding the essential cell factor and the sequence encoding the peptide epitope. In some examples, the construct further comprises a sequence encoding a linker located at the N-terminus or C-terminus of the fusion protein. In some examples, the linker is selected from any one of the linkers provided in Table 3. In some examples, the construct comprises a transcriptional regulatory element selected from the group consisting of an EF1A promoter, an EFS promoter, a CMV promoter, a CAGGS promoter, an SV40 promoter, a COPIA promoter, an ACT5C promoter, a TRE promoter, a CBh promoter, a PGK promoter, and a UBC promoter.

[00198] In some examples, the construct further comprises a first homology arm and a second homology arm homologous to a target gene locus for CRISPR-based homology directed repair. In some examples, the construct further comprises a vector backbone for lentiviral expression.

[00199] In some examples, outlined is a method of delivering a construct into an isolated cell comprising transducing an isolated cell with a lentiviral construct comprising a construct described; and selecting an engineered cell expressing a recombinant peptide epitope fusion protein. In other examples, the method comprises transducing an isolated cell with the lentiviral construct described; and selecting the isolated cell expressing the recombinant peptide epitope fusion protein in the construct. In some examples, an isolated cell or a population thereof comprises a construct mentioned herein.

[00200] In some examples, the isolated cell is an isolated engineered human cell further comprising deletion or reduced expression of MHC class I human leukocyte antigens and/or deletion or reduced expression of MHC class II human leukocyte antigens compared to an unmodified human cell. In some examples, the isolated cell further comprises deletion or reduced expression of CIITA, B2M, and/or NLRC5. In some examples, the isolated cell is hypoimmunogenic and a stem cell. In some instances, the stem cell is selected from the group consisting of an embryonic stem cell, a pluripotent stem cell, an induced pluripotent stem cell, and an adult stem cell.

[00201] In some examples, provided is a differentiated cell or a population thereof prepared by culturing the stem cell described under differentiation conditions appropriate for differentiation of the stem cell into a cell type selected from the group consisting of cardiac cells, liver cells, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells. In some instances, provided is a method of treating a patient in need of cell therapy comprising administering to patient the differentiated cell or the population thereof. In some instances, provided is a method of treating a patient comprising administering to a patient previously administered the differentiated cell or the population thereof an antibody that binds the peptide epitope. In some cases, the antibody mediates ADCC or CDC.

[00202] Provided is a construct for homology directed repair into a safe harbor locus comprising from 5' to 3' end: (1) a first homology arm homologous to a first endogenous sequence of a safe harbor locus; (2) a transcriptional regulatory element; (3) an HSVtk safety switch transgene; (4) a ribosomal skipping sequence and/or a sequence encoding a linker; (5) a CD47 hypoimmunity gene; (6) a polyadenylation sequence; and (7) a second homology arm homologous to a second endogenous sequence of the safe harbor locus. Provided is a construct for homology directed repair into a safe harbor locus comprising from 5' to 3' end: (1) a first homology arm homologous to a first endogenous sequence of an immune signaling gene locus; (2) a transcriptional regulatory element; (3) an HSVtk safety switch transgene; (4) a ribosomal skipping sequence and/or a sequence encoding a linker; (5) an CD47 hypoimmunity gene; (6) a polyadenylation sequence; and (7) a second homology arm homologous to a second endogenous sequence of the immune signaling gene locus.

[00203] In some examples, the transcriptional regulatory element is selected from the group consisting of an EF1A promoter, an EFS promoter, a CMV promoter, a CAGGS promoter, an SV40 promoter, a COPIA promoter, an ACT5C promoter, a TRE promoter, a CBh promoter, a PGK promoter, and a UBC promoter. In some examples, the construct further comprises a vector backbone for lentiviral expression.

[00204] Provided is an isolated cell or a population thereof comprising a safety switch transgene and a hypoimmunity gene integrated into a safe harbor locus or an immune signaling gene locus, wherein the construct of described herein has recombined into the endogenous safe harbor locus of the isolated cell or into the endogenous targeted gene locus of the isolated cell.

[00205] In some examples, the isolated cell further comprises deletion or reduced expression of MHC class I human leukocyte antigens and/or deletion or reduced expression of MHC class II human leukocyte antigens compared to an unmodified human cell. In some examples, the isolated cell further comprises deletion or reduced expression of CIITA, B2M, and/or NLRC5. In some examples, the isolated cell is hypoimmunogenic and a stem cell. In some instances, the stem cell is selected from the group consisting of an embryonic stem cell, a pluripotent stem cell, an induced pluripotent stem cell, and an adult stem cell.

[00206] Provided herein is a differentiated cell or a population thereof prepared by culturing the stem cell described under differentiation conditions appropriate for differentiation of any of the stem cells into pancreatic cells.

[00206a] In another aspect, the present disclosure refers to a differentiated cell or a population thereof prepared by culturing the stem cell as described herein under differentiation conditions appropriate for differentiation of the stem cell into a cell type selected from the group consisting of cardiac cells, liver cells, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells. In some examples, the pancreatic cells are beta-islet cells.

[00207] Outlined is a method of treating a patient in need of cell therapy comprising administering to a patient the differentiated cell or the population thereof disclosed, and activating the safety switch in a patient previously administered the differentiated cell or the population thereof as described.

[00208] Detailed descriptions of hypoimmunogenic cells, methods of producing thereof, and methods of using thereof are found in WO2016183041 filed May 9, 2015 and WO2018132783 filed January 14, 2018, the disclosures including the sequence listings and Figures are incorporated herein by reference in their entirety.

[00209] Other objects, advantages and examples of the present technology will be apparent from the detailed description following.

BRIEF DESCRIPTION OF THE DRAWINGS

[00210] Figure 1 depicts data in a HEK293 cell line engineered to express mouse CD47, showing various knockdown efficiencies of different shRNA constructs. Downregulation of exogenous CD47 was by way of an inducible shRNA controlled by a TetO system.

[00211] Figure 2 provides a schematic diagram of the small-molecule assisted shutoff (SMASH) system (see Hannah et al., Nature Chemical Biology 11637-638 (2015)) for inducing CD47 degradation. SMASH is a system using the hepatitis C virus (HCV) nonstructural protein 3 (NS3) protease and elements in the NS4A protein to effectively shut off expression of CD47 protein fused to a SMASH-tag with clinically tested HCV protease inhibitors. In the absence of protease

inhibitor (asunaprevir), a cryptic degron sequence is excised, leading to an unmodified gene

WHAT IS CLAIMED IS:

1. A construct comprising:

from 5' to 3' end: (1) a safety switch transgene; (2) a ribosomal skipping sequence and/or a sequence encoding a linker; (3) a hypoimmunity gene or an essential cell factor gene; or

from 5' to 3' end: (1) a hypoimmunity gene or an essential cell factor gene; (2) a ribosomal skipping sequence or a linker; (3) a safety switch transgene.

2. The construct of claim 1, wherein:

the construct is configured to integrate into a target gene locus of an isolated cell to disrupt expression of the target gene; and/or

the construct further comprises a first homology arm and a second homology arm homologous to a target gene locus for CRISPR-based homology directed repair.

3. The construct of claim 1 or claim 2, wherein the construct further comprises: (a) a first homology arm homologous to a first endogenous sequence of a safe harbor locus and a second homology arm homologous to a second endogenous sequence of the safe harbor locus; or (b) a first homology arm homologous to a first endogenous sequence of an immune signaling gene locus and a second homology arm homologous to a second endogenous sequence of the immune signaling gene locus.

4. The construct of claim 3, wherein:

the safe harbor locus is selected from the group consisting of an AAVS1 locus, a CLBYL locus, a CXCR4 locus, a Rosa26 locus, and a CCR5 locus; or

the immune signaling gene locus is selected from the group consisting of an B2M, HLA-A, HLA-B, HLA-C, HLA-D, HLA-E, RFXANK, CIITA, CTLA-4, PD-1, RAET1E/ULBP4, RAET1G/ULBP5, RAET1H/ULBP2, RAET1/ULBP1, RAET1L/ULBP6, RAET1N/ULBP3, and other ligands of NKG2D.

5. The construct of claim 1, wherein the construct is a homology independent donor construct and comprises:

(A) from 5' to 3' end: (1) a 5' long terminal repeats (LTR) comprising a left element (LE); (2) a splice acceptor-viral 2A peptide (SA-2A) element; (3) a safety switch transgene; (4)

a ribosomal skipping sequence or sequence encoding a linker; (5) a hypoimmunity gene; (6) a polyadenylation sequence; and (7) 3' LTR comprising a right element (RE);

(B) from 5' to 3' end: (1) a 5' long terminal repeats (LTR) comprising a left element (LE); (2) a splice acceptor-viral 2A peptide (SA-2A) element; (3) a hypoimmunity gene; (4) a ribosomal skipping sequence or sequence encoding a linker; (5) a safety switch transgene; (6) a polyadenylation sequence; and (7) 3' LTR comprising a right element (RE);

(C) from 5' to 3' end: (1) a 5' long terminal repeats (LTR) comprising a left element (LE); (2) a splice acceptor-viral 2A peptide (SA-2A) element; (3) a safety switch transgene; (4) a ribosomal skipping sequence or a sequence encoding a linker; (5) an essential cell factor gene; (6) a polyadenylation sequence; and (7) 3' LTR comprising a right element (RE); or

(D) from 5' to 3' end: (1) a 5' long terminal repeats (LTR) comprising a left element (LE); (2) a splice acceptor-viral 2A peptide (SA-2A) element; (3) an essential cell factor gene; (4) a ribosomal skipping sequence or a sequence encoding a linker; (5) a safety switch transgene; (6) a polyadenylation sequence; and (7) 3' LTR comprising a right element (RE).

6. The construct of any one of claims 1-5, wherein the construct is configured to integrate into a target gene locus of an isolated cell to disrupt expression of the target gene, wherein:

the target gene locus is an immune signaling gene locus selected from the group consisting of B2M, HLA-A, HLA-B, HLA-C, HLA-D, HLA-E, RFXANK, CIITA, CTLA-4, PD-1, RAET1E/ULBP4, RAET1G/ULBP5, RAET1H/ULBP2, RAET1/ULBP1, RAET1L/ULBP6, RAET1N/ULBP3, and other ligands of NKG2D; or

the target gene locus is a safe harbor locus selected from the group consisting of an AAVS1 locus, a CLBYL locus, a CXCR4 locus, a Rosa26 locus, and a CCR5 locus.

7. The construct of claim 1 or claim 5, wherein:

the safety switch transgene is selected from the group consisting of a HSVtk transgene, a cytosine deaminase transgene, a nitroreductase transgene, a purine nucleoside phosphorylase transgene, a horseradish peroxidase transgene, iCaspase9 transgene, HER1 transgene, RQR8 transgene, CD20 transgene, CCR4 transgene, HER2 transgene, CD19 transgene, MUC1 transgene, EGFR transgene, GD2 transgene, PSMA transgene, CD16 transgene, and CD30 transgene.

8. The construct of any one of claims 1-7, wherein the hypoimmunity gene is selected from the group consisting of: CD47, CD24, CD200, HLA-G, HLA-E, HLA-C, HLA-E heavy chain, PD-L1, IDO1, CTLA4-Ig, IL-10, IL-35, FASL, Serpinb9, CC121, and Mfge8.

9. The construct of any one of claims 1-7, wherein the essential cell factor is selected from the group consisting of RpS2, RpS9, RpS11, RpS13, RpS18, RpL8, RpL11, RpL32, RpL36, Rpn22, Psmdi4, PSMA3, a ribosome subunit protein, a proteasome subunit protein, and a spliceosome subunit protein.

10. The construct of any one of claims 1-9, wherein the ribosomal skipping sequence comprises a sequence encoding an IRES sequence or a sequence encoding a 2A-coding sequence.

11. The construct of claim 1, further comprising a transcriptional regulatory element operably linked to the safety switch transgene and a polyadenylation sequence at the 3' end of the hypoimmunity gene, or a transcriptional regulatory element operably linked to the hypoimmunity gene and a polyadenylation sequence at the 3' end of the safety switch transgene.

12. The construct of claim 1 comprising:

(A) from 5' to 3' end: (1) a first homology arm homologous to a first endogenous sequence of a safe harbor locus; (2) a transcriptional regulatory element; (3) a cytosine deaminase safety switch transgene; (4) a ribosomal skipping sequence and/or a sequence encoding a linker; (5) a CD47 hypoimmunity gene; (6) a polyadenylation sequence; and (7) a second homology arm homologous to a second endogenous sequence of the safe harbor locus; or

(B) from 5' to 3' end: (1) a first homology arm homologous to a first endogenous sequence of an immune signaling gene locus; (2) a transcriptional regulatory element; (3) a cytosine deaminase safety switch transgene; (4) a ribosomal skipping sequence and/or a sequence encoding a linker; (5) an CD47 hypoimmunity gene; (6) a polyadenylation sequence; and (7) a second homology arm homologous to a second endogenous sequence of the immune signaling gene locus;

(C) from 5' to 3' end: (1) a first homology arm homologous to a first endogenous sequence of a safe harbor locus; (2) a transcriptional regulatory element; (3) a CD47 hypoimmunity gene; (4) a ribosomal skipping sequence and/or a sequence encoding a linker; (5)

a cytosine deaminase safety switch transgene; (6) a polyadenylation sequence; and (7) a second homology arm homologous to a second endogenous sequence of the safe harbor locus; or

(D) from 5' to 3' end: (1) a first homology arm homologous to a first endogenous sequence of an immune signaling gene locus; (2) a transcriptional regulatory element; (3) an CD47 hypoimmunity gene; (4) a ribosomal skipping sequence and/or a sequence encoding a linker; (5) a cytosine deaminase safety switch transgene; (6) a polyadenylation sequence; and (7) a second homology arm homologous to a second endogenous sequence of the immune signaling gene locus.

13. The construct of claim 1 or claim 12 comprising:

(A) from 5' to 3' end: (1) a first homology arm homologous to a first endogenous sequence of a safe harbor locus; (2) a transcriptional regulatory element; (3) an HSVtk safety switch transgene; (4) a ribosomal skipping sequence and/or a sequence encoding a linker; (5) a CD47 hypoimmunity gene; (6) a polyadenylation sequence; and (7) a second homology arm homologous to a second endogenous sequence of the safe harbor locus;

(B) from 5' to 3' end: (1) a first homology arm homologous to a first endogenous sequence of an immune signaling gene locus; (2) a transcriptional regulatory element; (3) an HSVtk safety switch transgene; (4) a ribosomal skipping sequence and/or a sequence encoding a linker; (5) an CD47 hypoimmunity gene; (6) a polyadenylation sequence; and (7) a second homology arm homologous to a second endogenous sequence of the immune signaling gene locus;

(C) from 5' to 3' end: (1) a first homology arm homologous to a first endogenous sequence of a safe harbor locus; (2) a transcriptional regulatory element; (3) a CD47 hypoimmunity gene; (4) a ribosomal skipping sequence and/or a sequence encoding a linker; (5) an HSVtk safety switch transgene; (6) a polyadenylation sequence; and (7) a second homology arm homologous to a second endogenous sequence of the safe harbor locus; or

(D) from 5' to 3' end: (1) a first homology arm homologous to a first endogenous sequence of an immune signaling gene locus; (2) a transcriptional regulatory element; (3) an CD47 hypoimmunity gene; (4) a ribosomal skipping sequence and/or a sequence encoding a linker; (5) an HSVtk safety switch transgene; (6) a polyadenylation sequence; and (7) a second homology arm homologous to a second endogenous sequence of the immune signaling gene locus.

14. The construct of any one of claims 11-13, wherein the transcriptional regulatory element is selected from the group consisting of an EF1A promoter, an EFS promoter, a CMV promoter, a CAGGS promoter, an SV4O promoter, a COPIA promoter, an ACT5C promoter, a TRE promoter, a CBh promoter, a PGK promoter, and a UBC promoter.

15. A method of introducing a construct of any one of claims 1-14 into a cell.

16. The method of claim 15, wherein the construct is introduced by targeted integration into a target gene locus by CRISPR-based methods or wherein the construct is introduced by transducing a cell with a lentiviral vector comprising the construct.

17. The method of claim 16, wherein the construct is introduced into the cell with calcium phosphate-mediated transfection, lipid-mediated transfection, or electroporation.

18. An isolated cell or a population thereof comprising a construct of any one of claims 1-14.

19. The cell or the population thereof of claim 18, wherein the cell is an engineered cell further comprising one or more modifications selected from deletion or reduced expression of one or more MHC class I human leukocyte antigens, deletion or reduced expression of one or more MHC class II human leukocyte antigens, or deletion or reduced expression of CIITA, B2M, and/or NLRC5, wherein the reduced expression is compared to an unmodified human cell.

20. The cell or the population thereof of claim 18 or claim 19, wherein the cell is a stem cell.

21. The cell or the population thereof of claim 20, wherein the stem cell is selected from the group consisting of an embryonic stem cell, a pluripotent stem cell, and an adult stem cell.

22. A differentiated cell or a population thereof prepared by culturing the stem cell of claim 20 or claim 21 under differentiation conditions appropriate for differentiation of the stem cell into a cell type selected from the group consisting of cardiac cells, liver cells, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells.

23. The differentiated cell or the population thereof of claim 22, wherein the pancreatic cells are beta-islet cells.

24. The cell or the population thereof of claim 18 or claim 19, wherein the cell is a primary cell.

25. The cell or the population thereof of claim 24, wherein the cell type is selected from the group consisting of cardiac cells, liver cells, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells.

26. Use of a cell or a population thereof of any one of claims 18-25 in the manufacture of a medicament for treatment of a patient in need of cell therapy.

27. A cell or a population thereof of any one of claims 18-25 for use in treatment of a patient in need of cell therapy.

28. The use of claim 26 or the cell or the population thereof for use of claim 27, wherein the treatment comprises activating a safety switch transgene in a patient previously administered the cell or population thereof.

29. The use or the cell or the population thereof for use of claim 28, wherein the safety switch transgene is a thymidylate synthase gene or a mutant thereof or an HSVtk gene that is controlled by a ganciclovir prodrug, the safety switch transgene is a CD20 transgene that is controlled by anti-CD20 antibody, the safety switch transgene is a cytosine deaminase gene that is controlled by an exogenous factor 5-fluorocytosine, the safety switch transgene is a nirtroreductase transgene that is controlled by the exogenous factor CB 1954, the safety switch transgene is a purine nucleoside phosphorylase gene that is controlled by the exogenous factor 6-methylpurine deoxyriboside or fludarabine, the safety switch transgene is a horseradish peroxidase gene that is controlled by the exogenous factor indole 3-acetic acid, the safety switch transgene is iCaspase9 transgene that is controlled by the exogenous factor rimiducid (AP1903), AP20187 or rapamycin, the safety switch transgene is a human truncated EGFR transgene that is controlled by the exogenous antibody cetuximab or a variant thereof that recognizes the same or similar epitope, the safety switch transgene is human HER1 that is controlled by the exogenous

antibody cetuximab or a variant thereof that recognizes the same or similar epitope, or the safety switch transgene is the human RQR8 transgene that is controlled by the exogenous antibody rituximab or a variant thereof that recognizes the same or similar epitope.

30. A recombinant peptide epitope fusion protein comprising:

(A) (1) a hypoimmunity factor selected from the group consisting of CD47, CD24, CD200, HLA-G, HLA-E, HLA-C, HLA-E heavy chain, PD-L1, IDO1, CTLA4-Ig, IL-10, IL-35, FASL, Serpinb9, CC121, Mfge8, and membrane-bound forms thereof; and (2) a surface-exposed peptide epitope heterologous to the hypoimmunity factor selected from the group consisting of a CD20 epitope, CCR4 epitope, HER2 epitope, CD19 epitope, MUC1 epitope, EGFR epitope, GD2 epitope, PSMA epitope, CD16 epitope, and CD30 epitope; or

(B) (1) an essential cell factor; and (2) a surface-exposed peptide epitope heterologous to the essential cell factor.

31. The recombinant peptide epitope fusion protein of claim 30 that is a recombinant CD47-internal-peptide epitope fusion protein comprising from N- to C-terminal: (1) a human CD47 fragment comprising a IgV domain of CD47; (2) a first linker; (3) a heterologous peptide epitope; (4) a second linker; and (5) a human CD47 transmembrane domain.

32. A construct encoding a recombinant peptide epitope fusion protein of claim 30 or claim 31.

33. The construct of claim 32 that is a bicistronic construct comprising from 5' to 3' end: (1) a transcriptional regulatory element; (2) a sequence encoding the peptide epitope fusion protein; (3) a ribosomal skipping sequence; and (4) a sequence encoding a hypoimmunity factor.

34. A method of introducing a construct of claim 32 or claim 33 into a cell.

35. An isolated cell or a population thereof comprising a construct of claim 32 or claim 33.

36. The cell or the population thereof of claim 35, wherein the cell is a stem cell.

37. A differentiated cell or a population thereof prepared by culturing the stem cell of claim 36 under differentiation conditions appropriate for differentiation of a stem cell into a cell type selected from the group consisting of cardiac cells, liver cells, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells.

38. The cell or the population thereof of claim 35, wherein the cell is a primary cell.

39. The cell or the population thereof of claim 38, wherein the cell type is selected from the group consisting of cardiac cells, liver cells, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells.

40. Use of a cell or a population thereof of any one of claims 35-39 in the manufacture of a medicament for cell therapy.

41. Use of an antibody in the manufacture of a medicament for treating a patient who was previously administered the cell or population thereof of any one of claims 35-39, wherein the antibody binds the recombinant peptide epitope fusion protein.

42. A cell or a population thereof of any one of claims 35-39 for use in cell therapy.

43. A construct comprising (1) a transcriptional regulatory element, (2) an essential cell factor gene, (3) a post-transcriptional or post-translational regulatory element, and (4) a polyadenylation sequence.

44. A method for controlling the immunogenicity of an engineered cell, the method comprising:

(1) (a) introducing into an isolated cell (i) a nucleic acid comprising an inducible RNA polymerase promoter operably linked to an shRNA sequence targeting an immunosuppressive factor and (ii) a nucleic acid comprising a promoter operably linked to a transactivator element corresponding to the inducible RNA polymerase promoter to produce an engineered cell; and (b) exposing the engineered cell to an exogenous factor to activate the transactivator element, thereby controlling the immunogenicity of the engineered cell; or

(2) (a) introducing into an isolated cell a nucleic acid comprising (i) a sequence encoding an inducible degron element operably linked to an immunosuppressive factor or (ii) a sequence encoding an immunosuppressive factor operably linked to an inducible degron element to produce an engineered cell; and (b) exposing the engineered cell to an exogenous factor to activate the inducible degron element, thereby controlling the immunogenicity of the engineered cell; or

(3) (a) introducing into an isolated cell: (i) a first construct comprising from 5' end to 3' end: a first promoter and an immunosuppressive factor gene; (ii) a second construct comprising from 5' end to 3' end: a second promoter and a nucleic acid sequence encoding a Cas nuclease or a variant thereof; optionally wherein the Cas nuclease is Cas9, and (iii) a third construct comprising from 5' end to 3' end: an inducible RNA polymerase promoter, a guide RNA (gRNA) sequence targeting the immunosuppressive factor, a third promoter, and a transactivator element corresponding to the inducible RNA polymerase promoter; and (b) exposing the engineered cell to an exogenous factor to activate the transactivator element, thereby controlling the immunogenicity of the engineered cell; or

(4) (a) introducing into the isolated cell (i) a nucleic acid comprising an inducible RNA polymerase promoter operably linked to an immune signaling factor gene and (ii) a nucleic acid comprising a promoter operably linked to a transactivator element corresponding to the inducible RNA polymerase promoter to produce an engineered cell; and (b) exposing the engineered cell to an exogenous factor to activate the transactivator element, thereby controlling the immunogenicity of the engineered cell.

45. A construct comprising:

(1) from 5' end to 3' end: an inducible RNA polymerase promoter; an shRNA sequence targeting an immunosuppressive factor; a constitutive promoter; and a transactivator element corresponding to the inducible RNA polymerase promoter;

(2) from 5' end to 3' end: an inducible RNA polymerase promoter; an immune signaling factor gene; a promoter; and a transactivator element corresponding to the inducible RNA polymerase promoter;

(3) from 5' end to 3' end: a promoter, an inducible degron element, an optional sequence encoding a flexible linker, and an immunosuppressive factor gene; or

(4) from 5' end to 3' end: a promoter, an immunosuppressive factor gene, an optional sequence encoding a flexible linker, and an inducible degron element.

46. An isolated cell comprising a construct of claim 43 or claim 45.

47. The isolated cell of claim 46, wherein the cell is a stem cell.

48. A composition comprising differentiated cell prepared by culturing the stem cell of claim 43 under differentiation conditions appropriate for differentiation of the stem cell into a cell type selected from the group consisting of cardiac cells, liver cells, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells.

49. The isolated cell of claim 46, wherein the cell is a primary cell.

50. A composition comprising the primary cell of claim 49, wherein the cell type is selected from the group consisting of cardiac cells, liver cells, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells.

51. Use of an isolated cell of any one of claims 46-47 and 49 in the manufacture of a medicament for cell therapy.

52. An isolated cell of any one of claims 46-47 and 49 for use in cell therapy.

53. The use of claim 51 or the isolated cell for use of claim 52, wherein it is comprised that the cell is to be administered to the patient, and the cell is exposed to an exogenous factor to activate the inducible RNA polymerase promoter, thereby controlling immunogenicity of the cell in the patient.

54. Use of a composition of claim 48 or claim 50 in the manufacture of a medicament for cell therapy.

55. A composition of claim 48 or claim 50 for use in cell therapy.

56. The use of claim 48 or the composition for use of claim 55, wherein it is comprised that the composition is to be administered to the patient, and the composition is

exposed to an exogenous factor to activate the inducible RNA polymerase promoter, thereby controlling immunogenicity of the cells of the composition in the patient.

57. A pluripotent stem cell comprising:

(i) reduced or silenced expression of one or more MHC class I molecules and/or one or more MHC class II molecules, or reduced or silenced expression of B2M and CIITA,

(ii) overexpression of CD47, and

(iii) one of the following:

(a) a factor selected from the group consisting of: an inducible shRNA targeting CD47, an inducible degron element controlling CD47, or a SMASH degron element controlling CD47;

(b) a Cas nuclease or a variant thereof and an inducible guide RNA targeting CD47, optionally wherein the Cas nuclease is Cas9;

(c) an inducible protein degradation system for modulating expression of CD47 selected from the group consisting of a small molecule-assisted shutoff (SMASH) system, a Shield-1-inducible degron, an auxin-inducible degron, an IMid-inducible degron, a peptidic degron, a proteolysis targeting chimera, and an antibody for targeted degradation;

(d) an RNA regulation system for modulating expression of CD47 selected from the group consisting of an inducible shRNA, an inducible siRNA, a CRISPR interference (CRISPRi), and an RNA targeting nuclease system;

(e) a DNA regulation system for modulating expression of CD47 selected from the group consisting of a tissue specific promoter expression system, an inducible promoter expression system, a molecule regulated riboswitch system, and an inducible nuclease-based genome editing system; or

(f) an inducible system for modulating expression of CD47.

58. A differentiated cell derived from the pluripotent stem cell of claim 57, wherein the differentiated cell is selected from the group consisting of a cardiac cell, liver cell, kidney cell, pancreatic cell, neural cell, immune cell, mesenchymal cell, and endothelial cell.

59. An isolated cell comprising a DNA targeted nuclease system for controlling immunogenicity of the cell comprising:

(a) a first element comprising from 5’ end to 3’ end: a first promoter and an immunosuppressive factor gene;

(b) a second element comprising from 5’ end to 3’ end: a second promoter and a nucleic acid sequence encoding a Cas nuclease or a variant thereof, optionally wherein the Cas nuclease is Cas9; and

(c) a third element comprising from 5’ end to 3’ end: an inducible RNA polymerase promoter, a guide RNA (gRNA) sequence targeting the immunosuppressive factor, a third promoter, and a transactivator element corresponding to the inducible promoter, wherein immunogenicity of the cell is controllable upon exposing the cell to an exogenous factor to induce activity of the transactivator element.

60. The isolated cell of claim 59, wherein the cell is a stem cell.

61. A composition comprising differentiated cells prepared by culturing the stem cell of claim 60 under differentiation conditions appropriate for differentiation of the stem cell into a cell type selected from the group consisting of cardiac cells, liver cells, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells.

62. The isolated cell of claim 59, wherein the cell is a primary cell.

63. A composition comprising the primary cell of claim 62, wherein the cell type is selected from the group consisting of cardiac cells, liver cells, kidney cells, pancreatic cells, neural cells, immune cells, mesenchymal cells, and endothelial cells.

64. Use of a cell of claim 60 or claim 62 in the manufacture of a medicament for cell therapy.

65. A cell of claim 60 or claim 62 for use in cell therapy.

66. The use of claim 64 or the cell for use of claim 65, wherein it is comprised that the cell is to be administered to the patient, and the cell is exposed to an exogenous factor to activate the inducible RNA polymerase promoter, thereby controlling immunogenicity of the cell in the patient.

67. Use of a composition of claim 61 or claim 63 in the manufacture of a medicament for cell therapy.

68. A composition of claim 61 or claim 63 for use in cell therapy.

69. The use of claim 67 or the composition of claim 68, wherein it is comprised that the composition is to be administered to the patient, and the composition is exposed to an exogenous factor to activate the inducible RNA polymerase promoter, thereby controlling immunogenicity of the cells of the composition in the patient.

70. A composition comprising an isolated mammalian cell comprising a modification comprising a recombinant nucleic acid sequence encoding a system for conditional expression of one or more immunosuppressive factors or one or more immune signaling factors.