and (B) culturing the modified PSC under conditions sufficient for differentiation of the modified PSC into the modified SC-beta cell. In one aspect, the disclosure provides a method of generating a modified stem cell derived beta cell (SC-beta cell), the method comprising: (A) providing a modified pluripotent stem cell (PSC) comprising modifications that: (a) inactivate or disrupt one or more alleles of: (i) one or more major histocompatibility complex (MHC) class I molecules or one or more molecules that regulate expression of the one or more MHC class I molecules, and/or (ii) one or more MHC class II molecules or one or more molecules that regulate expression of the one or more MHC class II molecules; and (b) increase expression of one or more tolerogenic factors in the modified PSC, relative to a control or wild-type PSC, wherein the one or more tolerogenic factors is CD47, wherein the modification that increases expression of CD47 comprises an exogenous polynucleotide encoding CD47 that is integrated by targeted insertion into a target genomic locus of the modified PSC, and wherein the target genomic locus is a safe harbor locus, a *B2M* gene locus, a *CIITA* gene locus, or a *CD142* gene locus; and (B) culturing the modified PSC under conditions sufficient for differentiation of the modified PSC into the modified SC-beta cell.

[0005] Provided herein is a method of generating a modified stem cell derived beta cell (SC-beta cell) the method comprising: (A) generating a modified pluripotent stem cell (PSC) comprising: (a) introducing, into a PSC, one or more modifications that inactivate or disrupt one or more alleles of: (i) one or more major histocompatibility complex (MHC) class I molecules or one or more molecules that regulate expression of the one or more MHC class I molecules, and/or (ii) one or more MHC class II molecules or one or more molecules that regulate expression of the one or more MHC class II molecules; and (b) increasing expression of one or more tolerogenic factors in the PSC, relative to a control or wild-type PSC; and (B) culturing the modified PSC under conditions sufficient for differentiation of the modified PSC into a modified SC-beta cell.

[0006] Provided herein is a method of generating a modified stem cell derived beta cell (SC-beta cell), the method comprising (A) providing a modified pluripotent stem cell (PSC) that comprises at least one modification selected from the group consisting of: (a) modifications that inactivate or disrupt one or more alleles of: (i) one or more major histocompatibility complex (MHC) class I molecules or one or more molecules that regulate expression of the one or more MHC class I molecules, and/or (ii) one or more MHC class II molecules or one or more molecules that regulate expression of the one or more MHC class II molecules; and (b) modifications that increase expression of one or more tolerogenic factors in the modified PSC, relative to a control or wild-type cell of the same cell type that does not comprise the modification; (B) culturing the modified PSC under conditions sufficient for differentiation of the modified PSC into a modified SC-beta cell; and (C) introducing one or more additional modifications into the modified SC-beta cell, wherein the one or more additional modifications comprise at least one or more other modifications of (a), (b), or (a) and (b) not present in the modified PSC.

[0007] Provided herein is a method of generating a modified stem cell derived beta cell (SC-beta cell), the method comprising (A) culturing a pluripotent stem cell (PSC) under conditions sufficient for differentiation of the PSC into a SC-beta cell; and (B) generating a modified SC-beta cells comprising: (a) introducing, into the SC-beta cell, one or more modifications that inactivate or disrupt one or more alleles of: (i) one or more major histocompatibility complex (MHC) class I molecules or one or more molecules that regulate expression of the one or more MHC class I molecules, and/or (ii) one or more MHC class II molecules or one or more molecules that regulate expression of the one or more MHC class II molecules; and (b) increasing expression of one or more tolerogenic factors in the SC-beta cell, relative to a control or wild-type SC-beta cell.

[0008] Provided herein is a method of generating a generating a modified stem cell derived beta cell (SC-beta cell), the method comprising (A) providing a modified pluripotent stem cell (PSC) comprising modifications that: (a) reduce expression of one or more major histocompatibility complex (MHC) class I

molecule and/or one or more MHC class II molecule in the modified PSC, relative to a control or wild-type PSC; and (b) increase expression of one or more tolerogenic factors in the modified PSC, relative to the control or wild-type PSC; and (B) culturing the modified PSC under conditions sufficient for differentiation of the modified PSC into the modified SC-beta cell.

[0009] Provided herein is a method of generating a generating a modified stem cell derived beta cell (SC-beta cell), the method comprising (A) generating a modified pluripotent stem cell (PSC) comprising (a) reducing expression of one or more major histocompatibility complex (MHC) class I molecules and/or one or more MHC class II molecules in a PSC, relative to a control or wild-type PSC; and (b) increasing expression of one or more tolerogenic factors in the PSC, relative to the control or wild-type PSC; and (B) culturing the modified PSC under conditions sufficient for differentiation of the modified PSC into a modified SC-beta cell.

[0010] In some of any of the provided examples, in (a) reducing expression of the one or more MHC class I molecules and/or the one or more MHC class II molecules comprises introducing modifications that reduce expression of the one or more MHC class I molecule and/or the one or more MHC class II molecules in the modified PSC, relative to the control or wild-type PSC.

[0011] In some of any of the provided examples, the control or wild-type PSC is an unmodified PSC that does not comprise the modifications.

[0012] In some of any of the provided examples, the PSC does not comprise the modifications.

[0013] In some of any of the provided examples, expression of one or more MHC class I molecules and one or more MHC class II molecules is reduced in the modified PSC.

[0014] In some of any of the provided examples, the modifications in (a) reduce protein expression of the one or more MHC class I molecules. In some of any of the provided examples, the modifications in (a) reduce cell surface expression of the one or more MHC class I molecules. In some of any of the provided examples, the modifications in (a) reduce a function of the one or more MHC class I molecules. In some examples, the function is antigen presentation.

[0015] In some of any of the provided examples, the one or more modifications in (a) reduce cell surface trafficking of the one or more MHC class I molecules.

[0016] In some of any of the provided examples, the one or more MHC class I molecules is one or more human leukocyte antigen (HLA) class I molecules. In some examples, the one or more MHC HLA class I molecules is selected from the group consisting of HLA-A, HLA-B, and HLA-C.

[0017] In some of any of the provided examples, the one or more molecules that regulate cell surface protein expression of the one or more MHC class I molecules are B2M. In some examples, the modification that reduce expression of the one or more MHC class I molecules reduce expression of the B-2 microglobulin (B2M) gene and/or the transporter 1, ATP binding cassette subfamily B member (TAP1) gene. In some examples, the modifications that reduce expression of the one or more MHC

class I molecules reduce expression of the B-2 microglobulin (B2M) gene. In some examples, the modification that reduce expression of the one or more MHC class I molecules reduce expression of the transporter 1, ATP binding cassette subfamily B member (TAP1) gene. In some examples, the modifications reduce expression of the B-2 microglobulin (B2M) gene and the transporter 1, ATP binding cassette subfamily B member (TAP1) gene. In some examples, the modifications reduce expression reduce expression of the B2M gene.

[0018] In some of any of the provided examples, the modification that reduces expression of the one or more MHC class I molecules reduces expression of B2M. In some of any of the provided examples, the modification that reduces expression of the one or more MHC class I molecules reduces mRNA expression of the B2M gene. In some of any of the provided examples, the modification that reduces expression of the one or more MHC class I molecules reduces protein expression of B2M. In some of any of the provided examples, the modification that reduces expression of the one or more MHC class I molecules comprises inactivation or disruption of one allele of the B2M gene. In some of any of the provided examples, the modification that reduces expression of the one or more MHC class I molecules comprises inactivation or disruption of both alleles of the B2M gene. In some of any of the provided examples, the modification that reduces expression of the one or more MHC class I molecules comprises inactivation or disruption of all B2M coding alleles in the cell. In some of any of the provided examples, the inactivation or disruption comprises an indel in the *B2M* gene. In some of any of the provided examples, the inactivation or disruption comprises a frameshift mutation or a deletion of a contiguous stretch of genomic DNA of the B2M gene. In some examples, the B2M gene is knocked out.

[0019] In some of any of the provided examples, the modification that reduces expression of the one or more MHC class I molecules reduces expression of TAP1. In some of any of the provided examples, the modification that reduces expression of the one or more MHC class I molecules reduces mRNA expression of the TAP1 gene. In some examples, the modification that reduces expression of the one or more MHC class I molecules reduce expression reduces protein expression of a protein encoded by the TAP1 gene. In some examples, the modification comprises inactivation or disruption of one allele of the TAP1 gene. In some examples, the modification comprises inactivation or disruption of both alleles of the TAP1 gene. In some examples, the modification comprises inactivation or disruption of all coding sequences of the TAP1 gene in the cell. In some examples, the inactivation or disruption comprises an indel in one allele of the TAP1 gene. In some examples, the inactivation or disruption comprises an indel in both alleles of the TAP1 gene. In some examples, the one or more modifications that reduce expression comprises a frameshift mutation or a deletion of a contiguous stretch of genomic DNA of the TAP1 gene. In some examples, the TAP1 gene is knocked out.

[0020] In some of any of the provided examples, the modifications in (a) reduce protein expression of the one or more MHC class II molecules. In some of any of the provided examples, the modifications in (a) reduce cell surface expression of the one or more MHC class II molecules. In some of any of the provided examples, the modifications in (a) reduce a function of the one or more MHC class II molecules. In some examples, the function is antigen presentation.

[0021] In some of any of the provided examples, the one or more modifications in (a) reduce cell surface trafficking of the one or more MHC class II molecules.

[0022] In some of any of the provided examples, the one or more MHC class II molecules is one or more human leukocyte antigen (HLA) class II molecules. In some examples, the one or more MHC HLA class II molecules is selected from the group consisting of HLA-DP, HLA-DQ, and/or HLA-DR.

[0023] In some of any of the provided examples, the one or more molecules that regulate expression of the one or more MHC class II molecules is/are selected from the group consisting of CIITA and CD74. In some examples, the modification that reduce expression of the one or more MHC class II molecules reduce expression of the CIITA gene and/or CD74 gene. In some examples, the modification is a modification that regulates expression of the one or more MHC class II molecules, and the modification inactivates or disrupts one or more alleles of CIITA. In some examples, the modifications that reduce expression of the one or more MHC class II molecules reduce expression of the CITTA gene. In some examples, the modification that reduce expression of the one or more MHC class II molecules reduce expression of the CD74 gene. In some examples, the modifications reduce expression of the CIITA gene and the CD74 gene.

[0024] In some of any of the provided examples, the modification that reduces expression of the one or more MHC class II molecules reduces expression of CD74. In some of any of the provided examples, the modification that reduces expression of the one or more MHC class II molecules reduces mRNA expression of the CD74 gene. In some examples, the modification that reduces expression of the one or more MHC class II molecules reduce expression reduces protein expression of a protein encoded by the CD74 gene. In some examples, the modification comprises inactivation or disruption of one allele of the CD74 gene. In some examples, the modification comprises inactivation or disruption of both alleles of the CD74 gene. In some examples, the modification comprises inactivation or disruption of all coding sequences of the CD74 gene in the cell. In some examples, the inactivation or disruption comprises an indel in one allele of the CD74 gene. In some examples, the inactivation or disruption comprises an indel in both alleles of the CD74 gene. In some examples, the one or more modifications that reduce expression comprises a frameshift mutation or a deletion of a contiguous stretch of genomic DNA of the CD74 gene. In some examples, the CD74 gene is knocked out.

[0025] In some of any of the provided examples, the modification that reduces expression of the one or more MHC class II molecules comprises reduced expression of CIITA. In some of any of the provided examples, the modification that reduces expression of the one or more MHC class II molecules reduces mRNA expression of the CIITA gene. In some of any of the provided examples, the modification that reduces expression of the one or more MHC class II molecules reduces protein expression of CIITA. In some of any of the provided examples, the modification that reduces expression of the one or more MHC class II molecules comprises inactivation or disruption of one allele of the CIITA gene. In some of any of the provided examples, the modification that reduces expression of the one or more MHC class II molecules comprises inactivation or disruption of both alleles of the CIITA gene. In some of any of the provided examples, the modification that reduces expression of the one or more MHC class II molecules comprises or inactivation or disruption of all CIITA coding alleles in the cell. In some of any of the provided examples, the inactivation or disruption comprises an indel in the CIITA gene. In some of any of the provided examples, the inactivation or disruption is a frameshift mutation or a deletion of a contiguous stretch of genomic DNA of the CIITA gene. In some examples, the CIITA gene is knocked out.

[0026] In some of any of the provided examples, expression of all MHC class I molecules and all MHC class II molecules is reduced in the modified PSC. In some examples, expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, and HLA-DR are reduced in the modified PSC.

[0027] In some of any of the provided examples, the one or more tolerogenic factors is selected from the group consisting of CD47, CD27, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDOl, CTLA4-Ig, Cl-Inhibitor, IL-10, IL-35, FASL, CCL21, MFGE8, and SERPINB9. In some of any of the provided examples, at least one of the one or more tolerogenic factors is CD47. In some of any of the provided examples, the one or more tolerogenic factors is CD47. In some of any of the provided examples, at least one of the one or more tolerogenic factors is PD-L1. In some of any of the provided examples, the one or more tolerogenic factors is PD-L1. In some of any of the provided examples, at least one of the one or more tolerogenic factors is HLA-E. In some of any of the provided examples, the one or more tolerogenic factors is HLA-E. In some of any of the provided examples, at least one of the one or more tolerogenic factors is HLA-G. In some of any of the provided examples, the one or more tolerogenic factors is HLA-G.

[0028] In some of any of the provided examples, increasing expression of the one or more tolerogenic factors comprises introducing a modification that increases expression of the one or more tolerogenic factors in the modified PSC, relative to the control or wild-type PSC. In some of any of the provided examples, the modification to increase expression of the one or more tolerogenic factors comprises an exogenous polynucleotide encoding the one or more tolerogenic factors.

[0029] In some of any of the provided examples, the exogenous polynucleotide encoding the one or more tolerogenic factors is integrated into the genome of the modified PSC. In some of any of the provided examples, the exogenous polynucleotide is integrated by non-targeted insertion into the genome of the modified PSC. In some examples, the non-targeted integration is by introduction of the exogenous polynucleotide into the cell using a lentiviral vector. In some of any of the provided examples, the exogenous polynucleotide is integrated by targeted insertion into a target genomic locus of the cell. In some examples, the targeted insertion is by nuclease-mediated gene editing with homology-directed repair.

[0030] In some of any of the provided examples, increasing expression of the one or more tolerogenic factors comprises introducing a modification that increases expression of the one or more tolerogenic factors in the modified SC-beta cell, relative to the control or wild-type beta cell. In some of any of the provided examples, the modification to increase expression of the one or more tolerogenic factors comprises an exogenous polynucleotide encoding the one or more tolerogenic factors.

[0031] In some of any of the provided examples, the exogenous polynucleotide encoding the one or more tolerogenic factors is integrated into the genome of the modified SC-beta cell. In some of any of the provided examples, the exogenous polynucleotide is integrated by non-targeted insertion into the genome of the modified SC-beta cell. In some examples, the non-targeted integration is by introduction of the exogenous polynucleotide into the cell using a lentiviral vector. In some of any of the provided examples, the exogenous polynucleotide is integrated by targeted insertion into a target genomic locus of the cell. In some examples, the targeted insertion is by nuclease-mediated gene editing with homology-directed repair.

[0032] Also provided herein is a method of generating a modified stem cell derived beta cell (SC-beta cell), the method comprising: (A) providing a modified pluripotent stem cell (PSC) comprising knock out of the B2M gene, knock out of the CIITA gene, and an exogenous polynucleotide encoding CD47 protein, relative to a control or wild-type PSC; and (B) culturing the modified PSC under conditions sufficient for differentiation of the modified PSC into the modified SC-beta cell. In some examples, the modified PSC has the phenotype $B2M^{indel/indel}$; $CIITA^{indel/indel}$; CD47*tg*. In some of any examples, the modified PSC further comprises a modification to increase expression of an exogenous suicide gene. In some of any examples, the modified SC-beta cell further comprises a modification to increase expression of an exogenous suicide gene.

[0033] Also provided herein is a method of generating a modified stem cell derived beta cell (SC-beta cell), the method comprising: (A) providing a pluripotent stem cell (PSC); (B) culturing the PSC under conditions sufficient for differentiation of the PSC into a SC-beta cell; and (C) generating a modified SC-beta cell from the SC-beta cell by introducing modifications, into the SC-beta cell to knock out the B2M gene and to knock out the CIITA gene, and introducing an exogenous polynucleotide

encoding CD47 protein. In some examples, the modified SC-beta cell has the phenotype B2M$^{indel/indel}$; CIITA$^{indel/indel}$; CD47*tg.* In some of any examples, the modified SC-beta cell further comprises a modification to increase expression of an exogenous suicide gene. In some of any examples, the modified SC-beta cell further comprises a modification to increase expression of an exogenous suicide gene.

[0034] Also provided herein is a method of generating a modified stem cell derived beta cell (SC-beta cell), the method comprising: (A) providing a modified pluripotent stem cell (PSC) comprising knock out of the B2M gene, knock out of the CIITA gene, an exogenous polynucleotide encoding CD47 protein, and an exogenous polynucleotide encoding a suicide gene, relative to a control or wild-type PSC; and (B) culturing the modified PSC under conditions sufficient for differentiation of the modified PSC into the modified SC-beta cell. In some examples, the modified PSC has the phenotype B2M$^{indel/indel}$; CIITA$^{indel/indel}$; CD47*tg;* suicide gene*tg.*

[0035] Also provided herein is a method of generating a modified stem cell derived beta cell (SC-beta cell), the method comprising: (A) providing a pluripotent stem cell (PSC); (B) culturing the PSC under conditions sufficient for differentiation of the PSC into a SC-beta cell; and (C) generating a modified SC-beta cell from the SC-beta cell by introducing modifications, into the SC-beta cell to knock out the B2M gene and to knock out the CIITA gene, and introducing an exogenous polynucleotide encoding CD47 protein, and an exogenous polynucleotide encoding a safety switch. In some examples, the modified SC-beta cell has the phenotype B2M$^{indel/indel}$; CIITA$^{indel/indel}$; CD47*tg;* safety switch (e.g., suicide gene) transgene. In some of any examples provided herein, the exogenous polynucleotide encoding CD47 is integrated by non-targeted insertion into the genome of the modified SC-beta cell, optionally by introduction of the exogenous polynucleotide into the cell using a lentiviral vector.

[0036] In some examples of any of the methods of generating a modified SC-beta cell herein, the modified SC-beta cell comprises an exogenous polynucleotide encoding a suicide gene or suicide switch. In some examples, the suicide gene is selected from the group consisting of cytosine deaminase (CyD), herpesvirus thymidine kinase (HSV-Tk), an inducible caspase 9 (iCaspase9), and rapamycin-activated caspase 9 (rapaCasp9). In some examples, the suicide gene or suicide switch and genes associated with the suicide gene or the safety switch are expressed from a bicistronic cassette integrated into the genome of the modified SC-beta cell. In some examples, the suicide gene or suicide switch and the one or more tolerogenic factors are expressed from a bicistronic cassette integrated into the genome of the modified SC-beta cell. In some examples, the bicistronic cassette is integrated by non-targeted insertion into the genome of the modified SC-beta cell. In some examples, the bicistronic cassette is integrated by targeted insertion into a target genomic locus of the modified SC-beta cell. In some examples, the one or more tolerogenic factors is CD47.

[0037] In some of any of the provided examples, suicide gene is selected from the group consisting of cytosine deaminase (CyD), herpesvirus thymidine kinase (HSV-Tk), an inducible caspase 9 (iCaspase9), and rapamycin-activated caspase 9 (rapaCasp9).

[0038] In some of any of the provided examples, the suicide gene and the one or more tolerogenic factors are expressed from a bicistronic cassette integrated into the genome of the modified PSC. In some examples, the one or more tolerogenic factor is or comprises CD47 and the suicide gene and the CD47 are expressed from a bicistronic cassette integrated into the genome of the modified PSC. In some of any of the provided examples, the bicistronic cassette is integrated by non-targeted insertion into the genome of the modified PSC. In some examples, the non-targeted integration is by introduction of the exogenous polynucleotide into the cell using a lentiviral vector. In some of any of the provided examples, the bicistronic cassette is integrated by targeted insertion into a target genomic locus of the cell. In some examples, the targeted insertion is by nuclease-mediated gene editing with homology-directed repair.

[0039] In some of any of the provided examples, the safety switch (e.g., suicide gene) and the one or more tolerogenic factors are expressed from a bicistronic cassette integrated into the genome of the modified cell. In some examples, the safety switch and CD47 are expressed from a bicistronic cassette integrated into the genome of the modified cell. In some of any of the provided examples, the bicistronic cassette is integrated by non-targeted insertion into the genome of the modified SC-beta cell. In some examples, the non-targeted integration is by introduction of the exogenous polynucleotide into the cell using a lentiviral vector. In some of any of the provided examples, the bicistronic cassette is integrated by targeted insertion into a target genomic locus of the cell. In some examples, the targeted insertion is by nuclease-mediated gene editing with homology-directed repair.

[0040] In some of any of the provided examples, the target genomic locus is a safe harbor locus, a B2M gene locus, a CIITA gene locus, or a CD142 gene locus. In some of any of the provided examples, the safe harbor locus is selected from the group consisting of: a CCR5 gene locus, a CXCR4 gene locus, a PPP1R12C (also known as AAVS1) gene, an albumin gene locus, a SHS231 locus, a CLYBL gene locus, and a ROSA26 gene locus.

[0041] In some of any of the provided examples, the modified PSC comprises a modification that reduces expression of CD142, relative to the control or wild-type PSC. In some of any of the provided examples, the modification reduces mRNA expression of the CD142 gene. In some of any of the provided examples, the modification reduces protein expression of CD142. In some of any of the provided examples, the modification comprises inactivation or disruption of one allele of the CD142 gene. In some of any of the provided examples, the modification comprises inactivation or disruption of both alleles of the CD142 gene. In some of any of the provided examples, the modification comprises inactivation or disruption of all CD142 coding alleles in the cell. In some of any

of the provided examples, the inactivation or disruption comprises an indel in the CD142 gene. In some of any of the provided examples, the inactivation or disruption is a frameshift mutation or a deletion of a contiguous stretch of genomic DNA of the CD142 gene.

[0042] In some of any of the provided examples, the modified PSC comprises a modification that increases expression of one or more complement inhibitors selected from the group consisting of CD46, CD59, CD55 and CD35, relative to the control or wild-type PSC. In some of any of the provided examples, the modification to increase expression of the one or more complement inhibitors comprises at least one exogenous polynucleotide selected from the group consisting of an exogenous polynucleotide encoding CD46, an exogenous polynucleotide encoding CD59, an exogenous polynucleotide encoding CD55 and an exogenous polynucleotide encoding CD35. In some of any of the provided examples, the one or more complement inhibitors is CD46 and CD59. In some of any of the provided examples, the one or more complement inhibitor is CD46, CD59 and CD55. In some of any of the provided examples, the at least one exogenous polynucleotide is integrated by non-targeted insertion into the genome of the modified PSC. In some examples the non-targeted insertion is by introduction of the exogenous polynucleotide into the cell using a lentiviral vector. In some of any of the provided examples, the at least one exogenous polynucleotide is integrated by targeted insertion into a target genomic locus of the cell. In some examples, the targeted insertion is by nuclease-mediated gene editing with homology-directed repair. In some of any of the provided examples, the target genomic locus is a safe harbor locus, a B2M gene locus, a CIITA gene locus, or a CD142 gene locus. In some of any of the provided examples, the safe harbor locus is selected from the group consisting of: a CCR5 gene locus, a CXCR4 gene locus, a PPP1R12C (also known as AAVS1) gene, an albumin gene locus, a SHS231 locus, a CLYBL gene locus, and a ROSA26 gene locus.

[0043] In some of any of the provided examples, the culturing the PSC under conditions sufficient for differentiation of the PSC into the SC-beta cell comprises one or more of: (i) contacting the PSC with a TGFbeta/Activin agonist and/or, a glycogen synthase kinase 3 (GSK) inhibitor and/or WNT agonist for an amount of time sufficient to form a definitive endoderm cell; (ii) contacting a definitive endoderm cell differentiated from the PSC with a FGFR2b agonist for an amount of time sufficient to form a primitive gut tube cell; (iii) contacting a primitive gut tube cell differentiated from the PSC with a retinoic acid receptor (RAR) agonist, a rho kinase inhibitor, a Smoothened antagonist, a FGFR2b agonist, a protein kinase C activator, and/or a BMP type 1 receptor inhibitor for an amount of time sufficient to form an early pancreas progenitor cell; (iv) incubating an early pancreas progenitor cell differentiated from the PSC for at least about 3 days and contacting the early pancreas progenitor cell with a rho kinase inhibitor, a TGFbeta-/Activin agonist, a Smoothened antagonist, an FGFR2b agonist, a RAR agonist, a protein kinase C activator, and/or a BMP type 1 receptor inhibitor for an amount of time sufficient to form a pancreatic progenitor cell, wherein the RAR agonist concentration is less than the RAR agonist

concentration in step (iii); (v) contacting a pancreatic progenitor cell differentiated from the PSC with an Alk5 inhibitor/TGFbeta receptor inhibitor, a gamma secretase inhibitor, a Smoothened antagonist, an Erbbl (EGFR) or Erbb4 agonist, a thyroid hormone, and/or a RAR agonist for an amount of time sufficient to form an endoderm cell, wherein during at least a portion of the contacting in (v) comprises depolymerizing the actin cytoskeleton at a time and for an amount of time sufficient to increase differentiation efficiency; and/or (vi) incubating an endoderm cell differentiated from the PSC for an amount of time in serum-free media sufficient to form a beta cell.

[0044] In some of any of the provided examples, the culturing the modified PSC under conditions sufficient for differentiation of the modified PSC into the modified SC-beta cell comprises one or more of (i) contacting the modified PSC with a TGF /Activin agonist or a glycogen synthase kinase 3 (GSK) inhibitor or WNT agonist for an amount of time sufficient to form a definitive endoderm cell; (ii) contacting a definitive endoderm cell differentiated from the modified PSC with a FGFR2b agonist for an amount of time sufficient to form a primitive gut tube cell; (iii) contacting a primitive gut tube cell differentiated from the modified PSC with an RAR agonist, and optionally a rho kinase inhibitor, a Smoothened antagonist, a FGFR2b agonist, a protein kinase C activator, or a BMP type 1 receptor inhibitor for an amount of time sufficient to form an early pancreas progenitor cell; (iv) incubating an early pancreas progenitor cell differentiated from the modified PSC for at least about 3 days and optionally contacting the early pancreas progenitor cell with a rho kinase inhibitor, a TGF- /Activin agonist, a Smoothened antagonist, an FGFR2b agonist, or a RAR agonist for an amount of time sufficient to form a pancreatic progenitor cell; (v) contacting a pancreatic progenitor cell differentiated from the modified PSC with an Alk5 inhibitor, a gamma secretase inhibitor, a Smoothened antagonist, an Erbbl (EGFR) or Erbb4 agonist, and/or a RAR agonist for an amount of time sufficient to form an endoderm cell, wherein during at least a portion of the contacting in (v) depolymerizing the actin cytoskeleton at a time and for an amount of time sufficient to increase differentiation efficiency; and/or (vi) incubating an endoderm cell for an amount of time in serum-free media sufficient to form a beta cell, and within about 24 hours of incubation resizing the beta cells that formed into beta cell clusters.

[0045] In some of any of the provided examples, the culturing the modified PSC under conditions sufficient for differentiation of the modified PSC into the modified SC-beta cell comprises (i) contacting the modified PSC with a TGF /Activin agonist or a glycogen synthase kinase 3 (GSK) inhibitor or WNT agonist for an amount of time sufficient to form a definitive endoderm cell; (ii) contacting the definitive endoderm cell with a FGFR2b agonist for an amount of time sufficient to form a primitive gut tube cell; (iii) contacting the primitive gut tube cell with an RAR agonist, and optionally a rho kinase inhibitor, a Smoothened antagonist, a FGFR2b agonist, a protein kinase C activator, or a BMP type 1 receptor inhibitor for an amount of time sufficient to form an early pancreas progenitor cell; (iv) incubating the early pancreas progenitor cell for at least about 3 days and optionally contacting the early

pancreas progenitor cell with a rho kinase inhibitor, a TGF- /Activin agonist, a Smoothened antagonist, an FGFR2b agonist, or a RAR agonist for an amount of time sufficient to form a pancreatic progenitor cell; (v) contacting the pancreatic progenitor cell with an Alk5 inhibitor, a gamma secretase inhibitor, SANT 1 , Erbbl (EGFR) or Erbb4 agonist, or a RAR agonist for an amount of time sufficient to form an endoderm cell, wherein during at least a portion of the contacting in (v) depolymerizing the actin cytoskeleton at a time and for an amount of time sufficient to increase differentiation efficiency; and (vi) incubating the endoderm cell for an amount of time in serum-free media sufficient to form a beta cell, and within about 24 hours of incubation resizing the beta cells that formed into beta cell clusters.

[0046] In some of any of the provided examples, depolymerizing the actin cytoskeleton comprises plating cells on a stiff or soft substrate or introducing a cytoskeletal-modulating agent to cells. In some of any of the provided examples, the cytoskeletal-modulating agent comprises latrunculin A, latrunculin B, nocodazole, cytochalasin D, jasplakinolide, blebbistatin, y-27632, y-15, gdc-0994, or an integrin modulating agent. In some of any of the provided examples, the cytoskeletal-modulating agent is latrunculin A. In some of any of the provided examples, depolymerizing the actin cytoskeleton is initiated at the start of the contacting in (v). In some of any of the provided examples, depolymerizing the actin cytoskeleton comprises adding latrunculin A at the start of the contacting for at least at or about the first 24 hours. In some of any of the provided examples, resizing the beta cell clusters comprises breaking apart clusters and reaggregating.

[0047] In some of any of the provided examples, the TGF /Activin agonist is Activin A. In some of any of the provided examples, the glycogen synthase kinase 3 (GSK) inhibitor or the WNT agonist is CHIR. In some of any of the provided examples, the FGFR2b agonist is KGF. In some of any of the provided examples, the Smoothened antagonist is SANT-1. In some of any of the provided examples, the RAR agonist is retinoic acid (RA). In some of any of the provided examples, the protein kinase C activator is TPPB. In some of any of the provided examples, the BMP type 1 receptor inhibitor is LDN. In some of any of the provided examples, the rho kinase inhibitor is Y27632. In some of any of the provided examples, the Alk5 inhibitor is Alk5i. In some of any of the provided examples, the Erbb4 agonist is betacellulin. In some of any of the provided examples, the thyroid hormone is T3. In some of any of the provided examples, the gamma secretase inhibitor is XXI.

[0048] In some of any of the provided examples, the PSC is an embryonic stem cell. In some of any of the provided examples, the PSC is an induced PSC (iPSC). In some examples, the iPSC is a patient-derived iPSC.

[0049] In some of any of the provided examples, the modified PSC expresses each of the one or more tolerogenic factors at a first level that is greater than at or about 5-fold over a second level expressed by the control or wild-type PSC. In some of any of the provided examples, each of the one

or more tolerogenic factors is expressed at a first level that is greater than at or about 10-fold, greater than at or about 20-fold, greater than at or about 30-fold, greater than at or about 40-fold, greater than at or about 50-fold, greater than at or about 60-fold, or greater than at or about 70-fold over a second level expressed by the control or wild-type PSC.

[0050] In some of any of the provided examples, each of the one or more tolerogenic factors is expressed by the modified PSC at greater than at or about 20,000 molecules per cell. In some of any of the provided examples, each of the one or more tolerogenic factors is expressed by the modified PSC at greater than at or about 30,000 molecules per cell, greater than at or about 50,000 molecules per cell, greater than at or about 100,000 molecules per cell, greater than at or about 200,000 molecules per cell, greater than at or about 300,000 molecules per cell, greater than at or about 400,000 molecules per cell, greater than at or about 500,000 molecules per cell, or greater than at or about 600,000 molecules per cell.

[0051] In some of any of the provided examples, the one or more tolerogenic factors is or comprises CD47 and the modified PSC expresses CD47 at a first level that is greater than at or about 5-fold over a second level expressed by the control or wild-type PSC. In some of any of the provided examples, CD47 is expressed at a first level that is greater than at or about 10-fold, greater than at or about 20-fold, greater than at or about 30-fold, greater than at or about 40-fold, greater than at or about 50-fold, greater than at or about 60-fold, or greater than at or about 70-fold over a second level expressed by the control or wild-type PSC.

[0052] In some of any of the provided examples, the one or more tolerogenic factor is CD47 and CD47 is expressed by the modified PSC at greater than at or about 20,000 molecules per cell. In some of any of the provided examples, CD47 is expressed by the modified PSC at greater than at or about 30,000 molecules per cell, greater than at or about 50,000 molecules per cell, greater than at or about 100,000 molecules per cell, greater than at or about 200,000 molecules per cell, greater than at or about 300,000 molecules per cell, greater than at or about 400,000 molecules per cell, greater than at or about 500,000 molecules per cell, or greater than at or about 600,000 molecules per cell.

[0053] In some of any of the provided examples, the modified SC-beta cell comprises the modifications of the modified PSC.

[0054] In some of any examples of the provided methods, the modified SC-beta cell comprises modifications that (1) reduce expression of one or more MHC class I molecules and/or one or more MHC class II molecules, relative to a control or wild-type beta cell; and (2) increase expression of one or more tolerogenic factors, relative to the control or wild-type beta cell. In some of any of the provided examples, the control or wild-type SC-beta cell is an unmodified SC-beta cell differentiated from an unmodified PSC not comprising modifications that reduce expression of the one or more MHC class I molecules and/or the one or more MHC class II molecules or that increase expression of the one or more

tolerogenic factors. In some of any of the provided examples, the control or wild-type beta cell is a wild-type primary beta cell.

[0055] In some of any of the provided examples, expression of the one or more MHC class I molecules and the one or more MHC class II molecules is reduced in the modified SC-beta cell.

[0056] In some of any of the provided examples, the modifications in (1) reduce protein expression of the one or more MHC class I molecules in the modified SC-beta cell. In some of any of the provided examples, the modifications in (1) reduce cell surface expression of the one or more MHC class I molecules in the modified SC-beta cell. In some of any of the provided examples, the modifications in (1) reduce a function of the one or more MHC class I molecules in the modified SC-beta cell. In some examples, the function is antigen presentation.

[0057] In some of any of the provided examples, the one or more MHC class I molecules is one or more human leukocyte antigen (HLA) class I molecules. In some of any of the provided examples, the one or more MHC HLA class I molecules is selected from the group consisting of HLA-A, HLA-B, and HLA-C.

[0058] In some of any of the provided examples, the modification that reduces expression of the one or more MHC class I molecules in the modified SC-beta cell comprises reduced expression of B2M. In some of any of the provided examples, the modification that reduces expression of the one or more MHC class I molecules in the modified SC-beta cell reduces mRNA expression of the B2M gene. In some of any of the provided examples, the modification that reduces expression of the one or more MHC class I molecules in the modified SC-beta cell reduces protein expression of B2M.

[0059] In some of any examples, the modification that reduces expression of the one or more MHC class I molecules in the modified SC-beta cell comprises inactivation or disruption of one allele of the B2M gene. In some of any examples, the modification that reduces expression of the one or more MHC class I molecules in the modified SC-beta cell comprises inactivation or disruption of both alleles of the B2M gene. In some of any examples, the modification that reduces expression of the one or more MHC class I molecules in the modified SC-beta cell comprises inactivation or disruption of all B2M coding alleles in the cell. In some of any examples, the inactivation or disruption comprises an indel in the B2M gene. In some of any examples, the inactivation or disruption comprises a frameshift mutation or a deletion of a contiguous stretch of genomic DNA of the B2M gene.

[0060] In some of any examples, the modifications in (1) reduce protein expression of the one or more MHC class II molecules in the modified SC-beta cell. In some of any examples, the modifications in (1) reduce cell surface expression of the one or more MHC class II molecules in the modified SC-beta cell. In some of any examples, the modifications in (1) reduce a function of the one or more MHC class II molecules in the modified SC-beta cell. In some examples, the function is antigen presentation.

[0061] In some of any examples, the one or more MHC class II molecules is one or more human leukocyte antigen (HLA) class II molecules. In some examples, the one or more MHC class II molecules is selected from the group consisting of HLA-DP, HLA-DQ, and/or HLA-DR.

[0062] In some of any examples, the modification that reduces expression of the one or more MHC class II molecules in the modified SC-beta cell comprises reduced expression of CIITA. In some of any examples, the modification that reduces expression of the one or more MHC class II molecules in the modified SC-beta cell reduces mRNA expression of the CIITA gene. In some of any examples, the modification that reduces expression of the one or more MHC class II molecules in the modified SC-beta cell reduces protein expression of CIITA. In some of any examples, the modification that reduces expression of the one or more MHC class II molecules comprises inactivation or disruption of one allele of the CIITA gene. In some of any examples, the modification that reduces expression of the one or more MHC class II molecules comprises inactivation or disruption of both alleles of the CIITA gene. In some of any examples, the modification that reduces expression of the one or more MHC class II molecules comprises inactivation or disruption of all CIITA coding alleles in the cell. In some of any examples, the inactivation or disruption comprises an indel in the CIITA gene. In some of any examples, the inactivation or disruption is a frameshift mutation or a deletion of a contiguous stretch of genomic DNA of the CIITA gene.

[0063] In some of any of the provided examples, expression of all MHC class I molecules and all MHC class II molecules is reduced in the modified SC-beta cell. In some examples, expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, and HLA-DR are reduced in the modified SC-beta cell.

[0064] In some of any examples, the modified SC-beta cell comprises a modification that reduces expression of CD142. In some of any examples, the modification reduces mRNA expression of the CD142 gene. In some of any examples, the modification reduces protein expression of CD142. In some of any examples, the modification comprises inactivation or disruption of one allele of the CD142 gene. In some of any examples, the modification comprises inactivation or disruption of both alleles of the CD142 gene. In some of any examples, the modification comprises inactivation or disruption of all CD142 coding alleles in the cell. In some of any examples, the inactivation or disruption comprises an indel in the CD142 gene. In some of any examples, the inactivation or disruption is a frameshift mutation or a deletion of a contiguous stretch of genomic DNA of the CD142 gene.

[0065] In some of any examples, the modification to increase expression of the one or more tolerogenic factors in the modified SC-beta cell comprises an exogenous polynucleotide encoding the one or more tolerogenic factors. In some of any examples, the exogenous polynucleotide encoding the one or more tolerogenic factors is integrated into the genome of the modified SC-beta cell. In some of any examples, the exogenous polynucleotide encoding the one or more tolerogenic factors is

integrated into a non-target locus in the genome of the modified SC-beta cell. In some of any examples, the exogenous polynucleotide encoding the one or more tolerogenic factors is integrated into a target genomic locus of the modified SC-beta cell. In some examples, the tolerogenic factor is CD47. In some of any examples, the modified SC-beta cell further comprises a modification for expression of an exogenous suicide gene in the modified SC-beta cell.

[0066] In some examples, the modified SC-beta cell generated by the provided methods comprises knock out of the *B2M* gene, knock out of the CIITA gene, and an exogenous polynucleotide encoding exogenous CD47 protein, relative to a control or wild-type beta cell. In some examples, the modified SC-beta cell has the phenotype $B2M^{indel/indel}$; $CIITA^{indel/indel}$; CD47*tg*.

[0067] In some examples, the modified SC-beta cell generated by the provided methods comprises knock out of the *B2M* gene, knock out of the *CIITA* gene, an exogenous polynucleotide encoding CD47 protein, and an exogenous polynucleotide encoding a suicide gene, relative to a control or wild-type beta cell. In some examples, the modified SC-beta cell has the phenotype $B2M^{indel/indel}$; $CIITA^{indel/indel}$; CD47*tg;* suicide gene*tg*.

[0068] In some of any examples, the exogenous polynucleotide encoding CD47 is integrated into a non-target locus in the genome of the modified SC-beta cell. In some of any examples, the exogenous polynucleotide encoding CD47 is integrated into a target genomic locus of the modified SC-beta cell.

[0069] In some of any examples, the exogenous suicide gene is selected from the group consisting of cytosine deaminase (CyD), herpesvirus thymidine kinase (HSV-Tk), an inducible caspase (iCaspase9), and rapamycin-activated caspase 9 (rapaCasp9). In some of any examples, the suicide gene and the one or more tolerogenic factors are expressed from a bicistronic cassette integrated into the genome of the modified SC-beta cell. In some of any examples, the one or more tolerogenic factors is CD47 and the suicide gene and CD47 are expressed from a bicistronic cassette integrated into the genome of the modified SC-beta cell. In some of any examples, the bicistronic cassette is integrated at a non-target locus in the genome of the modified SC-beta cell. In some of any examples, the bicistronic cassette is integrated into a target genomic locus of the cell. In some of any examples, the target genomic locus is a safe harbor locus, a B2M gene locus, a CIITA gene locus, or a CD142 gene locus. In some of any examples, the safe harbor locus is selected from the group consisting of: a CCR5 gene locus, a CXCR4 gene locus, a PPP1R12C (also known as AAVS1) gene, an albumin gene locus, a SHS231 locus, a CLYBL gene locus, and a ROSA26 gene locus.

[0070] In some of any examples, the methods generate a modified SC-beta cell that comprises a modification that increases expression of one or more complement inhibitors selected from the group consisting of CD46, CD59, CD55 and CD35, relative to the control or wild-type beta cell. In some of any examples, the modification to increase expression of the one or more complement inhibitors in

the modified SC-beta cell comprises at least one exogenous polynucleotide encoding the one or more complement inhibitors selected from the group consisting of an exogenous polynucleotide encoding CD46, an exogenous polynucleotide encoding CD59, an exogenous polynucleotide encoding CD55, and an exogenous polynucleotide encoding CD35. In some of any examples, the one or more complement inhibitors is CD46 and CD59. In some of any examples, the one or more complement inhibitor is CD46, CD59 and CD55.

[0071] In some of any examples, the reduced expression comprises reduced surface expression. In some of any examples, the increased expression comprises increased surface expression. In some of any examples, the level of the reduced expression of (1) and the increased expression of (2) by the modified SC-beta cell is retained or is similar compared to the modified PSC.

[0072] In some of any examples, the methods generate a modified SC-beta cell that expresses the one or more tolerogenic factors at a first level that is greater than at or about 5-fold over a second level expressed by the control or wild-type beta cell. In some of any examples the control or wild-type beta cell is differentiated from an unmodified PSC not comprising modifications that reduce expression of the one or more MHC class I molecules and/or the one or more MHC class II molecules and that increase expression of the one or more tolerogenic factors. In some of any examples, the modified SC-beta cell expresses each of the one or more tolerogenic factors at a first level that is greater than at or about 5-fold over a second level expressed by the control or wild-type beta cell. In some of any examples, each of the one or more tolerogenic factors is expressed at a first level that is greater than at or about 10-fold, greater than at or about 20-fold, greater than at or about 30-fold, greater than at or about 40-fold, greater than at or about 50-fold, greater than at or about 60-fold, or greater than at or about 70-fold over a second level expressed by the control or wild-type beta cell.

[0073] In some of any examples, each of the one or more tolerogenic factors is expressed by the modified SC-beta cell at greater than at or about 20,000 molecules per cell. In some of any examples, each of the one or more tolerogenic factors is expressed by the modified SC-beta cell at greater than at or about 30,000 molecules per cell, greater than at or about 50,000 molecules per cell, greater than at or about 100,000 molecules per cell, greater than at or about 200,000 molecules per cell, greater than at or about 300,000 molecules per cell, greater than at or about 400,000 molecules per cell, greater than at or about 500,000 molecules per cell, or greater than at or about 600,000 molecules per cell.

[0074] In some of any examples, the one or more tolerogenic factors is or comprises CD47 and the modified SC-beta cell expresses CD47 at a first level that is greater than at or about 5-fold over a second level expressed by the control or wild-type beta cell. In some of any examples, the control or wild-type beta cell is differentiated from an unmodified PSC not comprising modifications that reduce expression of the one or more MHC class I molecules and/or the one or more MHC class II molecules and that increase expression of the one or more tolerogenic factors. In some of any examples, the

modified SC-beta cell expresses CD47 at a first level that is greater than at or about 5-fold over a second level expressed by the control or wild-type beta cell. In some of any examples, CD47 is expressed at a first level that is greater than at or about 10-fold, greater than at or about 20-fold, greater than at or about 30-fold, greater than at or about 40-fold, greater than at or about 50-fold, greater than at or about 60-fold, or greater than at or about 70-fold over a second level expressed by the control or wild-type beta cell. In some of any examples, CD47 is expressed by the modified SC-beta cell at greater than at or about 20,000 molecules per cell. In some of any examples, CD47 is expressed by the modified SC-beta cell at greater than at or about 30,000 molecules per cell, greater than at or about 50,000 molecules per cell, greater than at or about 100,000 molecules per cell, greater than at or about 200,000 molecules per cell, greater than at or about 300,000 molecules per cell, greater than at or about 400,000 molecules per cell, greater than at or about 500,000 molecules per cell, or greater than at or about 600,000 molecules per cell.

[0075] In some of any examples, the modified SC-beta cell expresses at least one beta cell marker. In some of any examples, the at least one beta cell marker is selected from the group consisting of INS, CHGA, NKX2-2, PDX1, NKX6-1, MAFB, GCK and GLUT1. In some of any examples, the modified SC-beta cell exhibits one or more functions of a wild-type or control beta cell. In some examples, the one or more functions is selected from the group consisting of in vitro glucose-stimulated insulin secretion (GSIS), glucose metabolism, maintaining fasting blood glucose levels, secreting insulin in response to glucose injections in vivo, and clearing glucose after a glucose injection in vivo.

[0076] In some of any examples, the modified SC-beta cell is capable of glucose-stimulated insulin secretion (GSIS). In some examples, the insulin secretion is in a perfusion GSIS assay. In some of any examples, the GSIS is dynamic GSIS comprising first and second phase dynamic insulin secretion. In some of any examples, the GSIS is static GSIS. In some examples, the static incubation index is greater than at or about 1, greater than at or about 2, greater than at or about 5, greater than at or about 10 or greater than at or about 20. In some of any examples, the level of insulin secretion by the modified SC-beta cells is at least 20% of that observed for primary beta islets, such as observed for cadaveric islets. In some of any examples, the level of insulin secretion by the modified SC-beta cells is at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70% of that observed for primary beta islets, such as observed for cadaveric islets. In some of any examples, the total insulin content of the modified SC-beta cell is greater than at or about 500 µIU Insulin per 5000 cells, greater than at or about 1000 µIU Insulin per 5000 cells, greater than at or about 2000 µIU Insulin per 5000 cells, greater than at or about 3000 µIU Insulin per 5000 cells or greater than at or about 4000 µIU Insulin per 5000 cells. In some of any examples, the proinsulin to insulin ratio of the modified

SC-beta cell is between at or about 0.02 and at or about 0.1, optionally at or about 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and any value between any of the foregoing.

[0077] In some of any examples, the modified SC-beta cells exhibit functionality for 1 or more days following transplantation into a subject. In some of any examples, the modified SC-beta cells exhibit functionality for more than 1 week following transplantation into a subject. In some of any examples, the functionality is selected from the group consisting of maintaining fasting blood glucose levels, secreting insulin in response to glucose injections in vivo, and clearing glucose after a glucose injection in vivo.

[0078] Provided herein is a composition comprising a population of modified SC-beta cells produced by any of the provided methods. In one aspect, the disclosure provides a composition comprising a population of modified stem-cell derived beta cells (SC-beta cell) comprising one or more modifications that: (a) inactivate or disrupt one or more alleles of: (i) one or more major histocompatibility complex (MHC) class I molecules or one or more molecules that regulate expression of the one or more MHC class I molecules, and/or (ii) one or more MHC class II molecules or one or more molecules that regulate expression of the one or more MHC class II molecules, and/or (b) increase expression of one or more tolerogenic factors, wherein the increased expression is relative to a control or wild-type beta cell that does not comprise the modifications, wherein the tolerogenic factor comprises CD47, wherein the modification that increases expression of CD47 comprises an exogenous polynucleotide encoding CD47 that is integrated by targeted insertion into a target genomic locus of the modified SC-beta cell, wherein the genomic locus is a safe harbor locus, a *B2M* gene locus, a *CIITA* gene locus, or a *CD142* gene locus.

[0079] Provided herein is a modified stem-cell derived beta cell (SC-beta cell) that has been differentiated in vitro from a pluripotent stem cell (PSC), wherein the modified SC-beta cell has (1) reduced expression of one or more major histocompatibility complex (MHC) class I molecules and/or one or more MHC class II molecules, relative to a control or wild-type beta cell; and (2) increased expression of a tolerogenic factor, relative to the control or wild-type beta cell, and wherein the modified SC-beta cell exhibits glucose-stimulated insulin secretion (GSIS).

[0080] In some of any examples, the tolerogenic factor is expressed at a first level that is greater than at or about 10-fold, greater than at or about 20-fold, greater than at or about 30-fold, greater than at or about 40-fold, greater than at or about 50-fold, greater than at or about 60-fold, or greater than at or about 70-fold over a second level expressed by the control or wild-type beta cell.

[0081] Provided herein is a modified stem-cell derived beta cell (SC-beta cell) comprising one or more modifications that: (a) inactivate or disrupt one or more alleles of: (i) one or more major histocompatibility complex (MHC) class I molecules or one or more molecules that regulate expression of the one or more MHC class I molecules, and/or (ii) one or more MHC class II molecules or one or more molecules that regulate expression of the one or more MHC class II molecules, and/or (b) increase expression of one or more tolerogenic factors, wherein the increased expression is relative to a control or wild-type beta cell that does not comprise the modifications. In some examples, the modified SC-beta cell exhibits glucose-stimulated insulin secretion (GSIS).

[0082] Provided herein is a modified stem-cell derived beta cell (SC-beta cell) that has been differentiated in vitro from a pluripotent stem cell (PSC), wherein the modified SC-beta cell has modifications that (a) inactivate or disrupt one or more alleles of: (i) one or more major histocompatibility complex (MHC) class I molecules or one or more molecules that regulate expression of the one or more MHC class I molecules, and/or (ii) one or more MHC class II molecules or one or more molecules that regulate expression of the one or more MHC class II molecules; and (b) increase

expression of one or more tolerogenic factors, relative to a control or wild-type beta cell, and wherein the modified SC-beta cell exhibits glucose-stimulated insulin secretion (GSIS).

[0083] Provided herein is a modified stem cell-derived beta cell (SC-beta cell) that has been differentiated in vitro from a pluripotent stem cell (PSC), wherein the modified SC-beta (1) does not express one or more major histocompatibility complex (MHC) class I molecules and/or one or more MHC class II molecules and (2) overexpresses a tolerogenic factor at a level of greater than at or about 5-fold compared to background, and wherein the modified SC-beta cell exhibits glucose-stimulated insulin secretion (GSIS). In some of any examples, the expression of the tolerogenic factor is by flow cytometry with an antibody directed against the tolerogenic factor and the background is determined by flow cytometry staining with an isotype control of the antibody. In some of any examples, the tolerogenic factor is expressed at a level that is greater than at or about 10-fold, greater than at or about 20-fold, greater than at or about 30-fold, greater than at or about 40-fold, greater than at or about 50-fold, greater than at or about 60-fold, or greater than at or about 70-fold compared to background expression.

[0084] Provided herein is a modified stem cell-derived beta cell (SC-beta cell) that has been differentiated in vitro from a pluripotent stem cell (PSC), wherein the modified SC-beta cell (1) does not express one or more major histocompatibility complex (MHC) class I molecules or one or more MHC class II molecules and (2) overexpresses a tolerogenic factor at a first level of greater than at or about 5-fold over a second level expressed by an unmodified cell, wherein the unmodified cell is an unmodified PSC that does not comprise modifications to reduce the one or more MHC class I molecules and/or the one or more MHC class II molecules and to overexpress the tolerogenic factor or is an unmodified SC-beta cell differentiated from such unmodified PSC; and wherein the modified SC-beta cell exhibits glucose-stimulated insulin secretion (GSIS).

[0085] In some of any examples, the tolerogenic factor is expressed by the modified SC-beta cell at greater than at or about 20,000 molecules per cell.

[0086] Provided herein is a modified stem cell-derived beta cell (SC-beta cell) that has been differentiated in vitro from a pluripotent stem cell (PSC), wherein the modified SC-beta cell (1) does not express one or more major histocompatibility complex (MHC) class I molecules or one or more MHC class II molecules and (2) overexpresses a tolerogenic factor, wherein the tolerogenic factor is expressed at greater than at or about 20,000 molecules per cell, and wherein the modified beta cell exhibits glucose-stimulated insulin secretion (GSIS).

[0087] In some of any examples, the tolerogenic factor is expressed by the modified SC-beta at greater than at or about 30,000 molecules per cell, greater than at or about 50,000 molecules per cell, greater than at or about 100,000 molecules per cell, greater than at or about 200,000 molecules per cell, greater than at or about 300,000 molecules per cell, greater than at or about 400,000 molecules per cell,

greater than at or about 500,000 molecules per cell, or greater than at or about 600,000 molecules per cell.

[0088] In some of any examples, the modified SC-beta cell is differentiated from a PSC in which the PSC is a modified PSC comprising modifications that (a) reduce expression of one or more MHC class I molecules and/or one or more MHC class II molecules, relative to a control or wild-type PSC; and (b) increase expression of a tolerogenic factor, relative to the control or wild-type PSC. In some of any examples, the control or wild-type PSC is an unmodified PSC that does not comprise the modifications. In some of any examples, the modified SC-beta cell expresses the tolerogenic factor at a first level that is greater than at or about 5-fold over a second level expressed by the unmodified PSC or the unmodified SC-beta cell differentiated from the unmodified PSC. In some of any examples, the tolerogenic factor is expressed at a first level that is greater than at or about 10-fold, greater than at or about 20-fold, greater than at or about 30-fold, greater than at or about 40-fold, greater than at or about 50-fold, greater than at or about 60-fold, or greater than at or about 70-fold over a second level expressed by the unmodified PSC or an unmodified SC-beta differentiated from the unmodified PSC.

[0089] Provided herein is a modified stem-cell derived beta cell (SC-beta cell) that has been differentiated in vitro from a modified pluripotent stem cell (PSC), wherein the modified PSC comprises modifications that (a) reduce expression of one or more major histocompatibility complex (MHC) class I molecules or one or more MHC class II molecules, relative to a control or wild-type PSC; and (b) increase expression of a tolerogenic factor, relative to the control or wild-type PSC, and wherein the modified SC-beta cell exhibits glucose-stimulated insulin secretion (GSIS).

[0090] In some of any examples, the control or wild-type PSC is an unmodified PSC that does not comprise the modifications. In some of any examples, the modified PSC expresses the tolerogenic factor at a first level that is greater than at or about 5-fold over a second level expressed by the unmodified PSC that does not comprise the modifications. In some examples, the tolerogenic factor is expressed at a first level that is greater than at or about 10-fold, greater than at or about 20-fold, greater than at or about 30-fold, greater than at or about 40-fold, greater than at or about 50-fold, greater than at or about 60-fold, or greater than at or about 70-fold over the second level expressed by the unmodified PSC.

[0091] In some of any examples, the modified SC-beta cell comprises modifications that (a) reduce expression of the one or more MHC class I molecules and/or or the one or more MHC class II molecule, relative to the unmodified PSC or an unmodified SC-beta differentiated from the unmodified PSC; and (b) increase expression of a tolerogenic factor, compared to the unmodified PSC or the unmodified SC-beta differentiated from the unmodified PSC. In some of any examples, the modified SC-beta expresses the tolerogenic factor at a first level that is greater than at or about 5-fold over a second level expressed by the unmodified PSC or the unmodified SC-beta cell differentiated from an

unmodified PSC. In some of any examples, the tolerogenic factor is expressed at a first level that is greater than at or about 10-fold, greater than at or about 20-fold, greater than at or about 30-fold, greater than at or about 40-fold, greater than at or about 50-fold, greater than at or about 60-fold, or greater than at or about 70-fold over a second level expressed by the unmodified PSC or the unmodified SC-beta cell differentiated from an unmodified PSC. In some of any examples, the tolerogenic factor is expressed by the modified PSC at greater than at or about 20,000 molecules per cell.

[0092] Provided herein is a modified stem cell-derived beta cell (SC-beta cell) that has been differentiated in vitro from a modified pluripotent stem cell (PSC), wherein the modified PSC comprises modifications such that the modified PSC (a) does not express one or more major histocompatibility complex (MHC) class I molecules and/or or one or more MHC class II molecule; and (b) expresses a tolerogenic factor at greater than at or about 20,000 molecules per cell, and wherein the modified SC-beta cell exhibits glucose-stimulated insulin secretion (GSIS).

[0093] In some of any examples, the tolerogenic factor is expressed by the modified PSC at greater than at or about 30,000 molecules per cell, greater than at or about 50,000 molecules per cell, greater than at or about 100,000 molecules per cell, greater than at or about 200,000 molecules per cell, greater than at or about 300,000 molecules per cell, greater than at or about 400,000 molecules per cell, greater than at or about 500,000 molecules per cell, or greater than at or about 600,000 molecules per cell. In some of any examples, the modified SC-beta cell does not express the one or more MHC class I molecule or the one or more MHC class II molecule and expresses the tolerogenic factor at greater than at or about 20,000 molecules per cell. In some of any examples, the tolerogenic factor is expressed by the modified SC-beta cell at greater than at or about 30,000 molecules per cell, greater than at or about 50,000 molecules per cell, greater than at or about 100,000 molecules per cell, greater than at or about 200,000 molecules per cell, greater than at or about 300,000 molecules per cell, greater than at or about 400,000 molecules per cell, greater than at or about 500,000 molecules per cell, or greater than at or about 600,000 molecules per cell.

[0094] In some of any examples, the tolerogenic factor is selected from the group consisting of CD47, CD27, CD200, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDOl, CTLA4-Ig, Cl-Inhibitor, IL-10, IL-35, FASL, CCL21, MFGE8, and SERPINB9, and any combination thereof. In some of any examples, the tolerogenic factor comprises CD47. In some of any examples, the tolerogenic factor comprises PD-L1. In some of any examples, the tolerogenic factor comprises HLA-E. In some of any examples, the tolerogenic factor comprises HLA-G.

[0095] In some of any examples, the modified SC-beta cell is differentiated from a modified PSC and expression of the one or more MHC class I molecules and the one or more MHC class II molecules is reduced in the modified PSC.

[0096] In some of any examples, the modifications in (a) reduce protein expression of the one or more MHC class I molecules. In some of any examples, the modifications in (a) reduce cell surface expression of one or more MHC class I molecules. In some of any examples, the one or more modifications in (a) reduce cell surface trafficking of the one or more MHC class I molecules. In some of any examples, the modifications in (a) reduce a function of one or more MHC class I molecules. In some examples, the function is antigen presentation.

[0097] In some of any examples, the modified SC-beta cell is differentiated from a modified PSC in which the modification that reduces expression of the one or more MHC class I reduces expression of B2M. In some of any examples, the modification that reduces expression of the one or more MHC class I molecules reduces mRNA expression of the B2M gene. In some of any examples, the modification that reduces expression of the one or more MHC class I molecules reduces protein expression of B2M. In some of any examples, the modification that reduces expression of the one or more MHC class I molecules in the modified PSC comprises inactivation or disruption of one allele of the B2M gene. In some of any examples, the modification that reduces expression of the one or more MHC class I molecules in the modified PSC comprises inactivation or disruption of both alleles of the B2M gene. In some of any examples, the modification that reduces expression of the one or more MHC class I molecules in the modified PSC comprises inactivation or disruption of all B2M coding alleles in the cell. In some of any examples, the inactivation or disruption comprises an indel in the *B2M* gene. In some of any examples, the inactivation or disruption is a frameshift mutation or a deletion of a contiguous stretch of genomic DNA of the *B2M* gene.

[0098] In some of any examples, the modifications in (a) reduce protein expression of the one or more MHC class II molecules. In some of any examples, the modifications in (a) reduce cell surface expression of the one or more MHC class II molecules. In some of any examples, the one or more modifications in (a) reduce cell surface trafficking of the one or more MHC class II molecules. In some of any examples, the modifications in (a) reduce a function of the one or more MHC class II molecules. In some examples, the function is antigen presentation.

[0099] In some of any examples, the modified SC-beta cell is differentiated from a modified PSC in which the modification that reduces expression of the one or more MHC class II comprises reduced expression of CIITA. In some of any examples, the modification that reduces expression of the one or more MHC class II molecules in the modified PSC reduces mRNA expression of the CIITA gene. In some of any examples, the modification that reduces expression of the one or more MHC class II molecules in the modified PSC reduces protein expression of CIITA. In some of any examples, the modification that reduces expression of the one or more MHC class II molecules in the modified PSC comprises inactivation or disruption of one allele of the CIITA gene. In some of any examples, the modification that reduces expression of the one or more MHC class II molecules in the

modified PSC comprises inactivation or disruption of both alleles of the CIITA gene. In some of any examples, the modification that reduces expression of the one or more MHC class II molecules in the modified PSC comprises inactivation or disruption of all CIITA coding alleles in the cell. In some of any examples, the inactivation or disruption comprises an indel in the CIITA gene. In some of any examples, the inactivation or disruption is a frameshift mutation or a deletion of a contiguous stretch of genomic DNA of the CIITA gene.

[0100] In some of any of the provided examples, expression of all MHC class I molecules and all MHC class II molecules is reduced in the modified PSC. In some examples, expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, and HLA-DR are reduced in the modified PSC.

[0101] In some of any examples, the modified SC-beta cell is differentiated from a modified PSC and the modified PSC comprises a modification that reduces expression of CD142. In some of any examples, the modification reduces mRNA expression of the CD142 gene. In some of any examples, the modification reduces protein expression of CD142. In some of any examples, the modification that reduces expression of CD142 in the modified PSC comprises inactivation or disruption of one allele of the CD142 gene. In some of any examples, the modification that reduces expression of CD142 in the modified PSC comprises inactivation or disruption of both alleles of the CD142 gene. In some of any examples, the modification that reduces expression of CD142 in the modified PSC comprises inactivation or disruption of all CD142 coding alleles in the cell. In some of any examples, the inactivation or disruption comprises an indel in the CD142 gene. In some of any examples, the inactivation or disruption is a frameshift mutation or a deletion of a contiguous stretch of genomic DNA of the CD142 gene.

[0102] In some of any examples, the modification to increase expression of the tolerogenic factor in the modified PSC comprises an exogenous polynucleotide encoding the tolerogenic factor. In some of any examples, the exogenous polynucleotide encoding the tolerogenic factor is integrated into the genome of the modified PSC. In some of any examples, the exogenous polynucleotide is integrated by non-targeted insertion into the genome of the modified PSC. In some of any examples, the exogenous polynucleotide is integrated by targeted insertion into a target genomic locus of the modified PSC.

[0103] In some of any examples, the modified SC-beta cell is differentiated from a modified PSC in which the modified PSC further comprises an exogenous polynucleotide encoding a suicide gene. In some of any examples, the suicide gene is selected from the group consisting of cytosine deaminase (CyD), herpesvirus thymidine kinase (HSV-Tk), an inducible caspase (iCaspase9), and rapamycin-activated caspase 9 (rapaCasp9).

[0104] In some of any examples, the suicide gene and the tolerogenic factor are expressed from a bicistronic cassette integrated into the genome of the modified PSC. In some examples, the

tolerogenic factor is CD47 and the suicide gene and CD47 are expressed from a bicistronic cassette integrated into the genome of the modified PSC. In some of any examples, the bicistronic cassette is integrated by non-targeted insertion into the genome of the modified PSC. In some of any examples, the bicistronic cassette is integrated by targeted insertion into a target genomic locus of the modified PSC. In some of any examples, the target genomic locus is a safe harbor locus, a B2M gene locus, a CIITA gene locus, or a CD142 gene locus. In some of any examples, the safe harbor locus is selected from the group consisting of: a CCR5 gene locus, a CXCR4 gene locus, a PPP1R12C (also known as AAVS1) gene, an albumin gene locus, a SHS231 locus, a CLYBL gene locus, and a ROSA26 gene locus.

[0105] In some of any examples, the modified SC-beta cell is differentiated from a modified PSC and the modified PSC comprises a modification that increases expression of one or more complement inhibitors selected from the group consisting of CD46, CD59, CD55 and CD35, relative to the control or wild-type PSC. In some of any examples, the modification to increase expression of one or more complement inhibitors comprises at least one exogenous polynucleotide encoding one or more complement inhibitors selected from the group consisting of an exogenous polynucleotide encoding CD46, an exogenous polynucleotide encoding CD59, an exogenous polynucleotide encoding CD55, and an exogenous polynucleotide encoding CD35. In some of any examples, the one or more complement inhibitors is CD46 and CD59. In some of any examples, the one or more complement inhibitor is CD46, CD59 and CD55. In some of any examples, the at least one exogenous polynucleotide is integrated by non-targeted insertion into the genome of the modified PSC. In some of any examples, the at least one exogenous polynucleotide is integrated by targeted insertion into a target genomic locus of the cell. In some of any examples, the target genomic locus is a safe harbor locus, a B2M gene locus, a CIITA gene locus, or a CD142 gene locus. In some of any examples, the safe harbor locus is selected from the group consisting of: a CCR5 gene locus, a CXCR4 gene locus, a PPP1R12C (also known as AAVS1) gene, an albumin gene locus, a SHS231 locus, a CLYBL gene locus, and a ROSA26 gene locus.

[0106] In some of any examples, the expression of one or more MHC class I molecules and one or more MHC class II molecules is reduced in the modified SC-beta cell.

[0107] In some of any examples, the modifications in (1) reduce protein expression of one or MHC class I molecules in the modified SC-beta cell. In some of any examples, the modifications in (1) reduce cell surface expression of one or more MHC class I molecules in the modified SC-beta cell. In some of any examples, the modifications in (1) reduce a function of MHC class I molecules in the modified SC-beta cell. In some examples, the function is antigen presentation. In some of any examples, the modification that reduces expression of one or more MHC class I molecules in the modified SC-beta cell comprises reduced expression of B2M. In some of any examples, the modification that reduces expression of one or more MHC class I in the modified SC-beta cell reduces

mRNA expression of the B2M gene. In some of any examples, the modification that reduces expression of one or more MHC class I molecules in the modified SC-beta cell reduces protein expression of B2M. In some of any examples, the modification that reduces expression of one or more MHC class I molecules in the modified SC-beta cell comprises inactivation or disruption of one allele of the B2M gene. In some of any examples, the modification that reduces expression of one or more MHC class I molecules in the modified SC-beta cell comprises inactivation or disruption of both alleles of the B2M gene. In some of any examples, the modification that reduces expression of one or more MHC class I molecules in the modified SC-beta cell comprises inactivation or disruption of all B2M coding alleles in the cell. In some of any examples, the inactivation or disruption comprises an indel in the B2M gene. In some of any examples, the inactivation or disruption is a frameshift mutation or a deletion of a contiguous stretch of genomic DNA of the B2M gene.

[0108] In some of any examples, the modifications in (1) reduce protein expression of one or more MHC class II molecules in the modified SC-beta cell. In some of any examples, the modifications in (1) reduce cell surface expression of one or more MHC class II molecules in the modified SC-beta cell. In some of any examples, the modifications in (1) reduce a function of one or more MHC class II molecules in the SC-beta cell. In some examples, the function is antigen presentation. In some of any examples, the modification that reduces expression of one or more MHC class II molecules in the modified SC-beta cell comprises reduced expression of CIITA. In some of any examples, the modification that reduces expression of one or more MHC class II molecules in the modified SC-beta cell reduces mRNA expression of the CIITA gene. In some of any examples, the modification that reduces expression of one or more MHC class II molecules in the modified SC-beta cell reduces protein expression of CIITA. In some of any examples, the modification that reduces expression of one or more MHC class II molecules in the modified SC-beta cell comprises inactivation or disruption of one allele of the CIITA gene. In some of any examples, the modification that reduces expression of one or more MHC class II molecules in the modified SC-beta cell comprises inactivation or disruption of both alleles of the CIITA gene. In some of any examples, the modification that reduces expression of one or more MHC class II molecules in the modified SC-beta cell comprises inactivation or disruption of all CIITA coding alleles in the cell. In some of any examples, the inactivation or disruption comprises an indel in the CIITA gene. In some of any examples, the inactivation or disruption is a frameshift mutation or a deletion of a contiguous stretch of genomic DNA of the CIITA gene.

[0109] In some of any of the provided examples, expression of all MHC class I molecules and all MHC class II molecules is reduced in the modified SC-beta cell. In some examples, expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, and HLA-DR are reduced in the modified SC-beta cell.

[0110] In some of any examples, the modified SC-beta cell comprises a modification that reduces expression of CD142, relative to a control or wild-type beta cell. In some of any examples, the modification reduces mRNA expression of the CD142 gene. In some of any examples, the modification reduces protein expression of CD142. In some of any examples, the modifications that reduce expression of CD142 in the modified SC-beta cell comprises inactivation or disruption of one allele of the CD142 gene. In some of any examples, the modifications that reduce expression of CD142 in the modified SC-beta cell comprises inactivation or disruption of both alleles of the CD142 gene. In some of any examples, the modifications that reduce expression of CD142 in the modified SC-beta cell comprises inactivation or disruption of all CD142 coding alleles in the cell. In some of any examples, the inactivation or disruption comprises an indel in the CD142 gene. In some of any examples, the inactivation or disruption comprises a frameshift mutation or a deletion of a contiguous stretch of genomic DNA of the CD142 gene.

[0111] In some of any examples, the modification to increase expression of the tolerogenic factor in the modified SC-beta cell comprises an exogenous polynucleotide encoding the tolerogenic factor. In some of any examples, the exogenous polynucleotide encoding the tolerogenic factor is integrated into the genome of the modified SC-beta cell. In some of any examples, the exogenous polynucleotide is integrated into a non-target locus in the genome of the modified SC-beta cell. In some of any examples, the exogenous polynucleotide is integrated into a target genomic locus of the modified SC-beta cell.

[0112] In some of any examples, the tolerogenic factor is CD47 and the modification to increase expression of CD47 in the modified SC-beta cell comprises an exogenous polynucleotide encoding CD47. In some of any examples, the exogenous polynucleotide encoding CD47 is integrated into the genome of the modified SC-beta cell. In some of any examples, the exogenous polynucleotide is integrated into a non-target locus in the genome of the modified SC-beta cell. In some of any examples, the exogenous polynucleotide is integrated into a target genomic locus of the modified SC-beta cell.

[0113] Also provided herein is a modified stem cell derived beta cell (SC-beta cell) that has been differentiated in vitro from a modified pluripotent stem cell (PSC), wherein the modified SC-beta cell comprises knock out of the B2M gene, knock out of the CIITA gene, and an exogenous polynucleotide encoding exogenous CD47 protein, relative to a control or wild-type beta cell. In some examples, the modified SC-beta cell has the phenotype $B2M^{indel/indel}$; $CIITA^{indel/indel}$; CD47*tg*. In some examples, the modified SC-beta cell further comprises an exogenous polynucleotide encoding a suicide gene.

[0114] Also provided herein is a modified stem cell derived beta cell (SC-beta cell) that has been differentiated in vitro from a modified pluripotent stem cell (PSC), wherein the modified SC-beta cell comprises knock out of the B2M gene, knock out of the CIITA gene, an exogenous polynucleotide

encoding CD47 protein, and an exogenous polynucleotide encoding a suicide gene, relative to a control or wild-type beta cell. In some examples, the modified SC-beta cell has the phenotype B2M$^{indel/indel}$; CIITA$^{indel/indel}$; CD47*tg;* suicide gene*tg.*

[0115] In some of any examples, the suicide gene is selected from the group consisting of cytosine deaminase (CyD), herpesvirus thymidine kinase (HSV-Tk), an inducible caspase (iCaspase9), and rapamycin-activated caspase 9 (rapaCasp9). In some of any examples, the suicide gene and the tolerogenic factor are expressed from a bicistronic cassette integrated into the genome of the modified SC-beta cell. In some of any examples, the tolerogenic factor is CD47 and the suicide gene and CD47 are expressed from a bicistronic cassette integrated into the genome of the modified SC-beta cell. In some of any examples, the bicistronic cassette is integrated at a non-target locus in the genome of the modified SC-beta cell. In some of any examples, the bicistronic cassette is integrated into a target genomic locus of the cell. In some of any examples, the target genomic locus is a safe harbor locus, a *B2M* gene locus, a *CIITA* gene locus, or a *CD142* gene locus. In some of any examples, the safe harbor locus is selected from the group consisting of: a CCR5 gene locus, a CXCR4 gene locus, a PPP1R12C (also known as AAVS1) gene, an albumin gene locus, a SHS231 locus, a CLYBL gene locus, and a ROSA26 gene locus.

[0116] In some of any examples, the modified SC-beta cell comprises a modification that increases expression of one or more complement inhibitors selected from the group consisting of CD46, CD59, CD55 and CD35 relative to the control or wild-type beta cell. In some of any examples the modification to increase expression of the one or more complement inhibitors in the modified SC-beta cell comprises at least one exogenous polynucleotide encoding one or more complement inhibitors selected from the group consisting of an exogenous polynucleotide encoding CD46, an exogenous polynucleotide encoding CD59, an exogenous polynucleotide encoding CD55, and an exogenous polynucleotide encoding CD35. In some of any examples, the one or more complement inhibitors is CD46 and CD59. In some of any examples, the one or more complement inhibitor is CD46, CD59 and CD55.

[0117] In some of any examples, the modified SC-beta cell expresses CD47 at a first level that is greater than at or about 5-fold over a second level expressed by the control or wild-type beta cell. In some examples, the control or wild-type beta cell is differentiated from an unmodified PSC not comprising modifications that reduce expression of one or more MHC class I molecules and/or one or more MHC class II molecules and that increase expression of the one or more tolerogenic factors. In some of any examples, the modified SC-beta cell expresses CD47 at a first level that is greater than at or about 5-fold over a second level expressed by the control or wild-type beta cell. In some of any examples, wherein CD47 is expressed at a first level that is greater than at or about 10-fold, greater than at or about 20-fold, greater than at or about 30-fold, greater than at or about 40-fold, greater than at

or about 50-fold, greater than at or about 60-fold, or greater than at or about 70-fold over a second level expressed by the control or wild-type beta cell.

[0118] In some of any examples, CD47 is expressed by the modified SC-beta cell at greater than at or about 20,000 molecules per cell. In some of any examples, CD47 is expressed by the modified SC-beta cell at greater than at or about 30,000 molecules per cell, greater than at or about 50,000 molecules per cell, greater than at or about 100,000 molecules per cell, greater than at or about 200,000 molecules per cell, greater than at or about 300,000 molecules per cell, greater than at or about 400,000 molecules per cell, greater than at or about 500,000 molecules per cell, or greater than at or about 600,000 molecules per cell.

[0119] In some examples of any of the modified SC-beta cells disclosed herein, the modified SC-beta cell comprises an exogenous polynucleotide encoding a suicide gene or a suicide switch. In some examples, the suicide gene or suicide switch is selected from the group consisting of cytosine deaminase (CyD), herpesvirus thymidine kinase (HSV-Tk), an inducible caspase 9 (iCaspase9), and rapamycin-activated caspase 9 (rapaCasp9). In some examples, the suicide gene or suicide switch and genes associated with the suicide gene or the safety switch are expressed from a bicistronic cassette integrated into the genome of the modified SC-beta cell. In some examples, the suicide gene or suicide switch and the one or more tolerogenic factors are expressed from a bicistronic cassette integrated into the genome of the modified SC-beta cell. In some examples, the bicistronic cassette is integrated by non-targeted insertion into the genome of the modified SC-beta cell, optionally by introduction of the exogenous polynucleotide into the cell using a lentiviral vector. In some examples, the bicistronic cassette is integrated by targeted insertion into a target genomic locus of the cell, optionally wherein the targeted insertion is by nuclease-mediated gene editing with homology-directed repair. In some examples, the one or more tolerogenic factors is CD47.

[0120] In some of any examples, the modified SC-beta cell expresses at least one beta cell marker. In some examples, the at least one beta cell marker is selected from the group consisting of INS, CHGA, NKX2-2, PDX1, NKX6-1, MAFB, GCK and GLUT1. In some of any examples, the modified SC-beta cell exhibits one or more functions of a wild-type or control beta cell. In some examples, the one or more functions is selected from the group consisting of in vitro glucose-stimulated insulin secretion (GSIS), glucose metabolism, maintaining fasting blood glucose levels, secreting insulin in response to glucose injections in vivo, and clearing glucose after a glucose injection in vivo. In some of any examples, the GSIS is measured in a perfusion GSIS assay. In some of any examples, the GSIS is dynamic GSIS comprising first and second phase dynamic insulin secretion. In some of any examples, the GSIS is static GSIS. In some examples, the static stimulation index is greater than at or about 1, greater than at or about 1.5, greater than at or about 2, greater than at or about 5, greater than at or about 10, greater than at or about 15, or greater than at or about 20.

[0121] In some of any examples, the level of insulin secretion by the modified SC-beta cell is at least 20% of that observed for primary beta islets, such as cadaveric islets. In some of any examples, the level of insulin secretion by the modified SC-beta cells is at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70% of that observed for primary beta islets, such as cadaveric islets. In some of any examples, the total insulin content of the modified SC-beta is greater than at or about 500 µIU Insulin per 5000 cells, greater than at or about 1000 µIU Insulin per 5000 cells, greater than at or about 2000 µIU Insulin per 5000 cells, greater than at or about 3000 µIU Insulin per 5000 cells or greater than at or about 4000 µIU Insulin per 5000 cells. In some of any examples, the proinsulin to insulin ratio of the modified SC-beta is between at or about 0.02 and at or about 0.1, optionally at or about 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, and any value between any of the foregoing.

[0122] In some of any examples, the modified SC-beta cell exhibits functionality for 1 or more days following transplantation into a subject. In some of any examples, the modified SC-beta cell exhibits functionality for more than 1 week following transplantation into a subject. In some of any examples, the functionality is selected from the group consisting of maintaining fasting blood glucose levels, secreting insulin in response to glucose injections in vivo, and clearing glucose after a glucose injection in vivo.

[0123] Provided herein is a composition comprising any of the provided SC-beta cells. Also provided herein is a composition comprising a population of any of the provided modified SC-beta cells.

[0124] In some of any examples of a provided composition comprising a population of modified SC-beta cells, among the cells in the population, the level of the reduced expression of MHC HLA class I and/or MHC HLA class II and/or the level of the increased expression of the tolerogenic factor is retained or is similar compared to the modified PSC in at least at or about 50%, at least at or about 60%, at least at or about 70%, at least at or about 80%, or at least at or about 90% of the cells in the population. In some of any examples, at least at or about 50%, at least at or about 60%, at least at or about 70%, at least at or about 80%, or at least at or about 90% of the cells in the population are reduced for expression of MHC HLA class I or for B2M. In some of any examples, at least at or about 50%, at least at or about 60%, at least at or about 70%, at least at or about 80%, or at least at or about 90% of the cells in the population are reduced for expression of MHC HLA class II or for CIITA.

[0125] In some of any examples of a provided composition comprising a population of modified SC-beta cells, at least at or about 50%, at least at or about 60%, at least at or about 70%, at least at or about 80%, or at least at or about 90% of the cells in the population express the tolerogenic factor at a first level that is greater than at or about 5-fold, greater than at or about 10-fold, greater than at or about 20-fold, greater than at or about 30-fold, greater than at or about 40-fold, greater than at or about 50-fold, greater than at or about 60-fold, or greater than at or about 70-fold over a second level expressed by the control or wild-type beta cell. In some examples, the control or wild-type beta cell is a wild-type

primary beta cell. In some of any examples, at least at or about 50%, at least at or about 60%, at least at or about 70%, at least at or about 80%, or at least at or about 90% of the cells in the population express the tolerogenic factor at a first level that is greater than at or about 5-fold, greater than at or about 10-fold, greater than at or about 20-fold, greater than at or about 30-fold, greater than at or about 40-fold, greater than at or about 50-fold, greater than at or about 60-fold, or greater than at or about 70-fold over a second level expressed by an unmodified PSC not comprising the modifications or an unmodified SC-beta cell differentiated from the unmodified PSC.

[0126] In some of any examples of a provided composition comprising a population of modified SC-beta cells, at least at or about 50%, at least at or about 60%, at least at or about 70%, at least at or about 80%, or at least at or about 90% of the cells in the population expresses the tolerogenic factor at greater than at or about 20,000 molecules per cell, at greater than at or about 30,000 molecules per cell, greater than at or about 50,000 molecules per cell, greater than at or about 100,000 molecules per cell, greater than at or about 200,000 molecules per cell, greater than at or about 300,000 molecules per cell, greater than at or about 400,000 molecules per cell, greater than at or about 500,000 molecules per cell, or greater than at or about 600,000 molecules per cell.

[0127] In some of any examples of a provided composition comprising a population of modified SC-beta cells, at least at or about 50%, at least at or about 60%, at least at or about 70%, at least at or about 80%, or at least at or about 90% of the cells in the population are reduced for expression of CD142.

[0128] In some of any examples, the provided composition comprises a pharmaceutically acceptable excipient. In some of any examples, the provided composition comprises a cryoprotectant.

[0129] In some examples of any of the compositions disclosed herein, modified SC-beta cells of the population of modified SC-beta cells comprise an exogenous polynucleotide encoding a suicide gene or a suicide switch. In some examples, the suicide gene or suicide switch is selected from the group consisting of cytosine deaminase (CyD), herpesvirus thymidine kinase (HSV-Tk), an inducible caspase 9 (iCaspase9), and rapamycin-activated caspase 9 (rapaCasp9). In some examples, the suicide gene and genes associated with the suicide gene or the safety switch are expressed from a bicistronic cassette integrated into the genome of modified SC-beta cells of the population of modified SC-beta cells. In some examples, the suicide gene or suicide switch and the exogenous CD47 are expressed from a bicistronic cassette integrated into the genome of the modified SC-beta cell. In some examples, the bicistronic cassette is integrated by non-targeted insertion into the genome, optionally by introduction of the exogenous polynucleotide into modified SC-beta cells of the population of modified SC-beta cells using a lentiviral vector. In some examples, the bicistronic cassette is integrated by targeted insertion into a target genomic locus of modified SC-beta cells of the population of modified SC-beta cells, optionally wherein the targeted insertion is by nuclease-mediated gene editing with homology-directed repair.

[0130] Provided herein is a method of treating diabetes in a subject, the method comprising administering any of the modified SC-beta cells or any of the provided compositions to a subject in need of treatment thereof. In one aspect, the disclosure provides use of a composition as disclosed herein in the manufacture of a medicament for treating diabetes or improving glucose tolerance in a subject. In one aspect, the disclosure provides a composition as disclosed herein for use as a medicament.

[0131] In some of any examples, the diabetes is type I diabetes. In some of any examples, the diabetes is type II diabetes. In some of any examples, the modified SC-beta cells improve glucose tolerance in the subject.

[0132] Provided herein is a method for improving glucose tolerance in a subject, the method comprising administering any of the provided modified SC-beta cells or any of the provided compositions to a subject in need of treatment thereof. In some of any examples, the subject is a diabetic patient. In some of any examples, the diabetic patient has type I diabetes or type II diabetes.

[0133] In some of any examples, glucose tolerance is improved relative to the subject's glucose tolerance prior to administration of the modified SC-beta cells. In some of any examples, administration of the modified SC-beta cells reduces exogenous insulin usage in the subject. In some of any examples, glucose tolerance is improved as measured by HbA1c levels. In some of any examples, the subject is fasting. In some of any examples, administration of the modified SC-beta cells improves insulin secretion in the subject. In some of any examples, insulin secretion is improved relative to the subject's insulin secretion prior to administration of the modified SC-beta cells.

[0134] In some examples, the method further comprises administering one or more immunosuppressive agents to the subject. In some examples, the subject has been administered one or more immunosuppressive agents. In some examples, the one or more immunosuppressive agents are a small molecule or an antibody. In some examples, the one or more immunosuppressive agents are selected from the group consisting of cyclosporine, azathioprine, mycophenolic acid, mycophenolate mofetil, a corticosteroids, prednisone, methotrexate, gold salts, sulfasalazine, antimalarials, brequinar, leflunomide, mizoribine, 15-deoxyspergualine, 6-mercaptopurine, cyclophosphamide, rapamycin, tacrolimus (FK-506), OKT3, anti-thymocyte globulin, thymopentin (thymosin-α), and an immunosuppressive antibody. In some examples, the one or more immunosuppressive agents comprise cyclosporine. In some examples, the one or more immunosuppressive agents comprise mycophenolate mofetil. In some examples, the one or more immunosuppressive agents comprise a corticosteroid. In some examples, the one or more immunosuppressive agents comprise cyclophosphamide. In some examples, the one or more immunosuppressive agents comprise rapamycin. In some examples, the one or more immunosuppressive agents comprise tacrolimus (FK-506). In some examples, the one or more immunosuppressive agents comprise anti-thymocyte globulin. In some examples, the one or more immunosuppressive agents are one or more immunomodulatory agents. In some examples, the one or more immunomodulatory agents are a small molecule or an antibody. In some examples, the antibody binds to one or more of receptors or

ligands selected from the group consisting of p75 of the IL-2 receptor, MHC, CD2, CD3, CD4, CD7, CD28, B7, CD40, CD45, IFN-gamma, TNF-alpha, IL-4, IL-5, IL-6R, IL-6, IGF, IGFR1, IL-7, IL-8, IL-10, CD11a, CD58, and antibodies binding to any of their ligands.

[0135] In some examples, the one or more immunosuppressive agents are or have been administered to the subject prior to administration of the modified SC-beta cells. In some examples, the one or more immunosuppressive agents are or have been administered to the subject at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days prior to administration of the modified SC-beta cells. In some examples, the one or more immunosuppressive agents are or have been administered to the subject at least 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks or more prior to administration of the SC-beta cells. In some examples, the one or more immunosuppressive agents are or have been administered to the subject at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days after administration of the modified SC-beta cells. In some examples, the one or more immunosuppressive agents are or have been administered to the subject at least 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, or more, after administration of the modified SC-beta cells. In some examples, the one or more immunosuppressive agents are or have been administered to the subject on the same day as the first administration of the modified SC-beta cells.

[0136] In some examples, the one or more immunosuppressive agents are or have been administered to the subject after administration of the modified SC-beta cells. In some examples, the one or more immunosuppressive agents are or have been administered to the subject after administration of a first and/or second administration of the modified SC-beta cells. In some examples, the one or more immunosuppressive agents are or have been administered to the subject prior to administration of a first and/or second administration of the modified SC-beta cells. In some examples, the one or more immunosuppressive agents are or have been administered to the subject at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days prior to administration of a first and/or second administration of the modified SC-beta cells. In some examples, the one or more immunosuppressive agents are or have been administered to the subject at least 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks or more prior to administration of a first and/or second administration of the modified SC-beta cells. In some examples, the one or more immunosuppressive agents are or have been administered to the subject at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days after administration of a first and/or second administration of the modified SC-beta cells. In some examples, the one or more immunosuppressive agents are or have been administered to the subject at least 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, or more, after administration of a first and/or second administration of the modified SC-beta cells. In some examples, the one or more immunosuppressive agents are administered at a lower dosage compared

to the dosage of one or more immunosuppressive agents administered to reduce immune rejection of immunogenic cells that do not comprise the modifications of the modified SC-beta cells.

[0137] In some examples, the modified SC-beta cell is capable of controlled killing of the modified SC-beta cell. In some examples, the modified SC-beta cell comprises a suicide gene or a suicide switch. In some examples, the suicide gene or the suicide switch induces controlled cell death in the presence of a drug or prodrug, or upon activation by a selective exogenous compound. In some examples, the suicide gene or the suicide switch is an inducible protein capable of inducing apoptosis of the modified SC-beta cell. In some examples, the inducible protein capable of inducing apoptosis of the modified SC-beta cell is a caspase protein. In some examples, the caspase protein is caspase 9. In some examples, the suicide gene or suicide switch is selected from the group consisting of cytosine deaminase (CyD), herpesvirus thymidine kinase (HSV-Tk), an inducible caspase 9 (iCaspase9), and rapamycin-activated caspase 9 (rapaCasp9). In some examples, the suicide gene or the suicide switch is activated to induce controlled cell death after the administration of the one or more immunosuppressive agents to the subject. In some examples, the suicide gene or the suicide switch is activated to induce controlled cell death prior to the administration of the one or more immunosuppressive agents to the subject. In some examples, the suicide gene or the suicide switch is activated to induce controlled cell death after the administration of the modified SC-beta cell to the subject. In some examples, the suicide gene or the suicide switch is activated to induce controlled cell death in the event of cytotoxicity or other negative consequences to the subject.

[0138] In some examples, the method comprises administering an agent that allows for depletion of a modified SC-beta cell of the population of modified SC-beta cells. In some examples, the agent that allows for depletion of the modified SC-beta cell is an antibody that recognizes a protein expressed on the surface of the modified SC-beta cell. In some examples, the antibody is selected from the group consisting of an antibody that recognizes CCR4, CD16, CD19, CD20, CD30, EGFR, GD2, HER1, HER2, MUC1, PSMA, and RQR8. In some examples, the antibody is selected from the group consisting of mogamulizumab, AFM13, MOR208, obinutuzumab, ublituximab, ocaratuzumab, rituximab, rituximab-Rllb, tomuzotuximab, RO5083945 (GA201), cetuximab, Hul4.18K322A, Hul4.18-IL2, Hu3F8, dinituximab, c.60C3-Rllc, and biosimilars thereof. In some examples, the method comprises administering an agent that recognizes the one or more tolerogenic factors on the surface of the modified SC-beta cell. In some examples, the modified SC-beta cell is engineered to express the one or more tolerogenic factors. In some examples, the one or more tolerogenic factors is CD47.

[0139] In some examples, the method comprises administering one or more additional therapeutic agents to the subject. In some examples, the subject has been administered one or more additional therapeutic agents.

[0140] In some examples, the method comprises monitoring the therapeutic efficacy of the method. In some examples, the method comprises monitoring the prophylactic efficacy of the method. In some examples, the method is repeated until a desired suppression of one or more disease symptoms occurs.

Brief Description of the Drawings

[0141] FIG. 1A shows glucose levels measured over time in humanized NSG diabetic mice transplanted with wild-type (WT), B2M$^{indel/indel}$, CIITA$^{indel/indel}$, or B2M$^{indel/indel}$, CIITA$^{indel/indel}$, CD47*tg* modified SC-beta cells.

[0142] FIG. 1B shows serum levels of human c-peptide in humanized NSG diabetic mice transplanted with wild-type (WT), B2M$^{indel/indel}$, CIITA$^{indel/indel}$, or B2M$^{indel/indel}$, CIITA$^{indel/indel}$, CD47*tg* modified SC-beta cells and subjected to a glucose challenge on da 29 after transplant, one hour prior to sacrifice.

[0143] FIG. 2A shows IFN-γ spot frequencies enumerated using an Elispot plate reader as an assay for TH1 T cell response in mice administered wild-type, B2M$^{indel/indel}$, CIITA$^{indel/indel}$, or B2M$^{indel/indel}$, CIITA$^{indel/indel}$, CD47*tg* human SC-beta cells.

[0144] FIG. 2B shows the mean fluorescence intensity (MFI) of cells labelled with FITC-conjugated goat anti-IgM and analyzed by flow cytometry for cell suspensions of sera from recipient wild-type, B2M$^{indel/indel}$, CIITA$^{indel/indel}$, and B2M$^{indel/indel}$, CIITA$^{indel/indel}$, CD47*tg* human SC-beta mice incubated with wild-type, B2M$^{indel/indel}$, CIITA$^{indel/indel}$, and B2M$^{indel/indel}$, CIITA$^{indel/indel}$, CD47*tg* human cells.

[0145] FIG. 2C shows a comparison of NK cell killing using IL-2 stimulated human NK cells as effector and wild-type, B2M$^{indel/indel}$, CIITA$^{indel/indel}$, or B2M$^{indel/indel}$, CIITA$^{indel/indel}$, CD47*tg* human SC-beta cells as target cells.

Detailed Description

[0146] Provided herein are beta cells differentiated from pluripotent stem cells (PSCs) in which the resulting beta cells contain one or more modifications that make the resulting differentiated beta cells hypoimmune to reduce or evade immune rejection. The modified beta cells that have been differentiated in vitro from PSCs and that contain the one more more modifications are called modified stem cell-derived beta cell (also called "modified SC-beta cell" or "modified SC-β cell"). In some cases, at least one or all of the modifications to the differentiated beta-cell are introduced to the beta-cells after the diffierentiation from the PSCs. In some cases, the PSCs are first modified with the one or more modifications, or in some embodiments each of the hypoimmune modifications, and then are