CLAIMS

WHAT IS CLAIMED:

1. 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.

2. 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.

3. The method according to claim 1 or the composition according to claim 2, wherein

(a) the one or more modifications comprise a modification that regulates cell surface protein expression of the one or more MHC class I molecules and the modification inactivates or disrupts one or more alleles of B2M; and/or 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; and/or

(b) the modified cell comprises knock out of the B2M gene, knock out of the CIITA gene, and an exogenous polynucleotide encoding CD47 protein; and/or

(c) modified PSC has the phenotype B2M$^{indel/indel}$; CIITA$^{indel/indel}$; CD47*tg*.

4. The method according to claim 1 or claim 3, or the composition according to claim 2 or claim 3, wherein the modified cell further comprises a modification to increase expression of an exogenous safety switch,

optionally wherein

(i) the safety switch is a suicide gene, optionally wherein 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), and/or

(ii) the safety switch and CD47 are expressed from a bicistronic cassette integrated into the genome of the modified cell.

5. The method according to any one of claims 1, 3-4, or the composition according to any one of claims 2-4, wherein 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.

6. The method according to any one of claims 1, 3-5, or the composition according to any one of claims 2-5, wherein the tolerogenic factor is expressed by the modified cell at greater than at or about 20,000 molecules per cell, 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.

7. The method according to any one of claims 1, 3-6, or the composition according to any one of claims 2-6, wherein:

(a) the modified SC-beta cell expresses at least one beta cell marker, optionally wherein 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;

(b) the modified SC-beta cell exhibits one or more functions of a wild-type or control beta cell, optionally wherein 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;

(c) modified SC-beta cell is capable of glucose-stimulated insulin secretion (GSIS), optionally wherein the insulin secretion is in a perfusion GSIS assay;

(d) the level of insulin secretion by the modified SC-beta cells is at least 20% of that observed for primary beta islets, optionally cadaveric islets;

(e) 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, optionally cadaveric islets;

(f) 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;

(g) 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;

(h) the modified SC-beta cell exhibits functionality for 1 or more days following transplantation into a subject;

(i) the modified SC-beta cell exhibits functionality for more than 1 week following transplantation into a subject; and/or

(j) 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.

8. The method according to any of claims 1, 3-7, wherein 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,

optionally

(i) wherein the method comprises aggregating the beta cells formed in step (vi) into clusters;

(ii) wherein depolymerizing the actin cytoskeleton comprises plating cells on a stiff or soft substrate and/or introducing a cytoskeletal-modulating agent to cells, optionally wherein the cytoskeletal-modulating agent comprises latrunculin A, latrunculin B, nocodazole, cytochalasin D, jasplakinolide, blebbistatin, y-27632, y-15, gdc-0994, and/or an integrin modulating agent;

(iii) wherein depolymerizing the actin cytoskeleton is initiated at the start of the contacting in (v);

(iv) wherein depolymerizing the actin cytoskeleton comprises adding latrunculin A at the start of the contacting for at least at or about the first 24 hours;

(v) wherein resizing the beta cell clusters comprises breaking apart clusters and reaggregating; and/or

(vi) wherein:

the TGF /Activin agonist is Activin A;

the glycogen synthase kinase 3 (GSK) inhibitor or the WNT agonist is CHIR99021;

the FGFR2b agonist is KGF;

the smoothened antagonist is SANT-1;

the RAR agonist is retinoic acid (RA);

the protein kinase C activator is TPPB;

the BMP type 1 receptor inhibitor is LDN193189;

the rho kinase inhibitor is Y27632;

the Alk5 inhibitor is Alk5i II;

the Erbb4 agonist is betacellulin;

the thyroid hormone is T3; and/or

the gamma secretase inhibitor is XXI.

9. The method according to any of claims 1, 3-8, wherein the PSC is an embryonic stem cell, an induced PSC (iPSC) or a patient-derived iPSC.

10. The composition according to any one of claims 2-7, wherein at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 99.99% of cells in the population express one or more markers of a beta cell or a marker of an islet, optionally wherein the marker is one or more of INS, CHGA, NKX2-2, PAX6, PDX1, NKX6-1, MAFB, GCK and GLUT1.

11. The composition according to any one of claims 2-7 or 10, wherein the modified SC-beta cell exhibits glucose-stimulated insulin secretion (GSIS).

12. The composition according to any one of claims 2-7, 10 or 11, wherein:

(a) among the cells in the population, the level of expression of MHC class I molecules and/or MHC class II molecules 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;

(b) 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 one or more MHC class I molecules and/or for expression of B2M;

(c) 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 one or more MHC class II molecules and/or for expression of CIITA;

(d) 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 comprise inactivation or disruption of one or more alleles of: one or more MHC class I molecules and/or B2M;

(e) 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 comprise inactivation or disruption of one or more alleles of: one or more MHC class II molecules and/or CIITA; and/or

(f) 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.

13. Use of a composition according to any of claims 2-7 or 10-12 in the manufacture of a medicament for treating diabetes or improving glucose tolerance in a subject.

14. The use according to claim 13, wherein

(a) the diabetes is type I diabetes or type II diabetes; or

(b) the subject is a diabetic patient, optionally wherein the diabetic patient has type I diabetes or type II diabetes.

15. The use according to claim 13 or claim 14, wherein:

(a) an agent that allows for depletion of a modified SC-beta cell of the population of modified SC-beta cells is to be administered, optionally wherein 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; and/or

(b) an agent that recognizes CD47 on the surface of the modified SC-beta cell is to be administered.

16. A composition according to any of claims 2-7 or 10-12 for use as a medicament.