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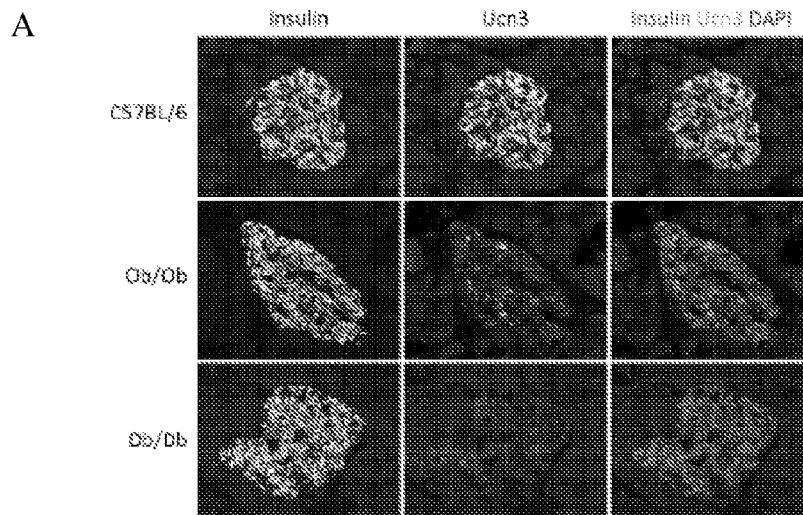
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(54) Title: METHODS FOR DETECTING AND REVERSING BETA CELL DE-DIFFERENTIATION AND USES THEREOF



(57) Abstract: Disclosed herein are methods for detecting, preventing, and/or reversing β cell de-differentiation.

METHODS FOR DETECTING AND REVERSING BETA CELL DE-DIFFERENTIATION AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 61/972,196, filed on March 28, 2014, the contents of which are hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] Dysfunction or death of pancreatic β cells underlies all types of diabetes. In the case of Type 1 diabetes, it is unknown whether the initiating cause of β cell destruction is an immune attack or a β cell pathology that instigates autoimmunity. β cell failure in type 2 diabetes (T2D) is thought to begin as a compensatory response to peripheral insulin resistance and eventually results in the loss of a mature β cell phenotype, without necessarily leading to β cell death (Weir et al., 2013; Weir and Bonner-Weir, 2004). This loss of a mature β cell phenotype, sometimes called de-differentiation, can result from exposure to high levels of glucose, lipids and inflammatory cytokines (Accili et al., 2010). De-differentiation of β -cells in the context of diabetes has been shown *in vivo* with the genetic disruption of key transcription factors, including FoxO1 (Talchai et al., 2012) and NeuroD (Gu et al., 2010), and is also seen in isolated islets cultured *in vitro* on an adherent substrate (Bar-Nur et al., 2011; Bar et al., 2012; Gershengorn et al., 2004; Negi et al., 2012; Russ et al., 2008; Weinberg et al.,

2007). In both the FoxO1 knockout mice and obese diabetic (Db/Db) mice, de-differentiating β -cells gradually lose insulin expression and begin to express progenitor-cell markers including Ngn3 and Sox9 (Talchai et al., 2012). Oxidative stress, also associated with T2D, inactivates the β cell specific transcription factors MafA, Nkx6.1 and Pdx1, again leading to the loss of mature β cell identity (Guo et al., 2013).

[0003] β cell de-differentiation may represent a reversal of the normal ontogeny of β cells, or follow a different pathway, but it is clear that de-differentiation depletes the pool of functionally mature β cells in T2D patients (Weir et al., 2013; Weir and Bonner-Weir, 2004). It is not known whether there are stages of de-differentiation at which the cells can recover or re-differentiate back into fully mature β cells. The commonly used T2D drugs act by suppressing glucose production in the liver (e.g. Metformin), by enhancing peripheral insulin sensitivity (e.g. Rosiglitazone and other thiazolidinediones), or by forcing the secretion of more insulin from the already-stressed β cells (e.g. sulfonylureas such as Glyburide). There is no evidence that any of these drugs reverse β -cell de-differentiation or restore the functionally mature β cell mass after β cell de-differentiation has occurred (Accili et al., 2010; Kahn et al., 2006). Accordingly, there exists a need for a marker for the early detection of β cell de-differentiation, and methods for preventing and/or reversing β cell de-differentiation (e.g., at an early stage).

SUMMARY OF THE INVENTION

[0004] In some aspects, the disclosure provides a method for detecting de-differentiation or re-differentiation of a β cell, the method comprising: a) obtaining a β cell; b) detecting the level of urocortin 3 (Ucn3) expression in the β cell; c) comparing the level of Ucn3 expression detected in the β cell to the level of Ucn3 expression detected in a normal mature β cell; and d) detecting de-differentiation or re-differentiation of the β cell, wherein de-differentiation of the β cell is detected if the level of Ucn3 expression detected in the β cell is decreased relative to the level of Ucn3 expression detected in the normal mature β cell; or wherein re-differentiation of the β cell is detected if the level of Ucn3 expression detected in the β cell is comparable to the level of Ucn3 expression detected in the normal mature β cell.

[0005] In some embodiments, detecting in b) comprises detecting the level of Ucn3 mRNA expression or Ucn3 protein expression. In some embodiments, detecting in b) comprises performing an immunostain using an antibody specific for Ucn3 protein to

detect the level of Ucn3 protein expression in the β cell. In some embodiments, detecting in b) comprises performing an immunostain using a primary antibody specific for Ucn3 protein and a secondary antibody-fluorescent dye conjugate specific for the primary antibody to detect the level of Ucn3 protein expression in the β cell. In some embodiments, the method includes imaging the immunostain using a microscope to obtain a micrograph displaying Ucn3 protein expressed in the β cell in a fluorescent color indicative of the level of Ucn3 protein expressed in the β cell. In some embodiments, comparing in c) comprises displaying a first micrograph showing the results of an immunostain for Ucn3 protein in the β cell and displaying a second micrograph showing the results of an immunostain for Ucn3 protein in the normal mature β cell. In some embodiments, measuring in b) comprises performing a hybridization based assay using one or more nucleic acids which hybridizes to a nucleic acid encoding Ucn3. In some embodiments, measuring in b) comprises performing a polymerase chain reaction based assay using a set of primers and probes specific for Ucn3 cDNA, wherein the level of Ucn3 cDNA detected is indicative of the level of Ucn3 mRNA expressed in the β cell. In some embodiments, measuring in b) comprises: i) isolating total RNA comprising Ucn3 mRNA from the β cell; ii) reverse transcribing the Ucn3 mRNA isolated in step i) to generate Ucn3 cDNA; and iii) performing a quantitative real-time polymerase chain reaction (qRT-PCR) assay using a set of probes and/or primers specific for Ucn3 cDNA to detect the level of Ucn3 mRNA expression in the β cell, wherein the amount of Ucn3 cDNA detected is indicative of the level of Ucn3 mRNA expression in the β cell. In some embodiments, comparing in c) comprises displaying a graph showing the relative expression of Ucn3 mRNA in the β cell compared to the relative expression of Ucn3 mRNA in the normal mature β cell.

[0006] In some embodiments, the method includes detecting the level of insulin expression in the β cell. In some embodiments, detecting comprises detecting the level of expression of insulin mRNA or protein. In some embodiments, detecting the level of expression of insulin protein comprises performing an immunostain using an antibody specific for insulin protein to detect the level of insulin protein expression in the β cell. In some embodiments, detecting the level of expression of insulin protein comprises performing an immunostain using a primary antibody specific for insulin protein and a secondary antibody-fluorescent dye conjugate specific for the primary antibody to detect the level of insulin protein expression in the β cell. In some embodiments, the method

includes imaging the immunostain using a microscope to obtain a micrograph displaying insulin protein expressed in the β cell in a fluorescent color indicative of the level of insulin protein expressed in the β cell. In some embodiments, the method includes comparing the level of insulin expression in the β cell to the level of insulin expression in the normal mature β cell. In some embodiments, comparing comprises displaying a first micrograph showing the results of an immunostain for insulin protein in the β cell and displaying a second micrograph showing the results of an immunostain for insulin protein in the normal mature β cell. In some embodiments, detecting the level of expression of insulin mRNA in the β cell comprises performing a hybridization based assay using one or more nucleic acids which hybridizes to a nucleic acid encoding insulin. In some embodiments, detecting the level of expression of insulin mRNA in the β cell comprises performing a polymerase chain reaction based assay using a set of primers and probes specific for insulin cDNA, wherein the level of insulin cDNA detected is indicative of the level of insulin mRNA in the β cell. In some embodiments, detecting the level of expression of insulin mRNA in the β cell comprises: i) isolating total RNA comprising insulin mRNA from the β cell; ii) reverse transcribing the insulin RNA isolated in step i) to generate insulin cDNA; and iii) performing a quantitative real-time polymerase chain reaction (qRT-PCR) assay using a set of probes and/or primers specific for insulin cDNA to detect the level of insulin mRNA expression in the β cell, wherein the amount of insulin cDNA detected is indicative of the level of insulin mRNA expression in the β cell. In some embodiments, the method includes comparing the level of expression of insulin mRNA in the β cell to the level of expression of insulin mRNA in a normal mature β cell. In some embodiments, comparing comprises displaying a graph showing the relative expression of insulin mRNA in the β cell compared to the relative expression of Ucn3 mRNA in the normal mature β cell.

[0007] In some embodiments, de-differentiation comprises an early stage of de-differentiation in which Ucn3 expression decreases and insulin expression remains the same or increases. In some embodiments, de-differentiation comprises a late stage of de-differentiation in which Ucn3 expression decreases and insulin expression decreases. In some embodiments, de-differentiation is characterized by decreased expression of at least one marker of mature β cells comprising FoxO1, MafA, NeuroD, Nkx6.1, and Pdx1. In some embodiments, re-differentiation is characterized by increased expression of at least one marker of mature β cells comprising FoxO1, MafA, NeuroD, Nkx6.1, and Pdx1. In

some embodiments, de-differentiation is characterized by the absence of an appropriate glucose stimulated insulin secretion (GSIS) response in the β cell. In some embodiments, re-differentiation is characterized by the presence of an appropriate GSIS response in the β cell.

[0008] In some embodiments, the β cell obtained in a) comprises (i) a β cell in or isolated from an islet or a pancreas; or (ii) a β cell differentiated *in vitro*. In some embodiments, the β cell is obtained from a subject who is (i) has diabetes; (ii) is at risk of developing diabetes; (iii) is developing diabetes; or (iv) is suspected of having or developing diabetes. In some embodiments, the β cell obtained from a subject who is (i) non-diabetic; (ii) mildly diabetic, or (iii) severely diabetic.

[0009] In some aspects, the disclosure provides a method of preventing de-differentiation of a β cell, comprising contacting a β cell with an agent that inhibits transforming growth factor- β (TGF β) superfamily signaling.

[0010] In some embodiments, preventing de-differentiation of the β cell causes the β cell to: (i) increase or maintain expression levels of Ucn3; (i) increase or maintain expression levels of at least one marker of mature β cells comprising FoxO1, MafA, NeuroD, Nkx6.1, and Pdx1; and/or (iii) preserve an appropriate GSIS response in the β cell.

[0011] In some aspects, the disclosure provides a method of reversing de-differentiation of a β cell, comprising contacting a de-differentiated β cell with an agent that inhibits transforming growth factor- β (TGF β) superfamily signaling.

[0012] In some embodiments, the de-differentiated β cell comprises: a) a de-differentiated β cell in an early stage of de-differentiation that exhibits at least one of i) decreased Ucn3 expression and increased or unchanged insulin expression; ii) decreased expression of at least one marker of mature β cells comprising FoxO1, MafA, NeuroD, Nkx6.1, and Pdx1; and/or iii) lack of an appropriate GSIS response; or b) a de-differentiated β cell in a late stage of de-differentiation that exhibits at least one of i) decreased Ucn3 expression and decreased insulin expression; ii) decreased expression of at least one marker of mature β cells comprising FoxO1, MafA, NeuroD, Nkx6.1, and Pdx1; and/or iii) lack of an appropriate GSIS response.

[0013] In some embodiments, reversing de-differentiation of the β cell causes the β cell to: (i) increase expression levels of Ucn3; (i) increase expression levels of at least

one marker of mature β cells comprising MafA, Nkx6.1, Pdx1, NeuroD, and FoxO1; and/or (iii) exhibit an appropriate GSIS response in the β cell.

[0014] In some embodiments, TGF β superfamily signaling comprises artemin signaling through receptor tyrosine kinase RET or a GFR α 3 receptor (GRF α 3). In some embodiments, the at least one agent comprises an inhibitor of RET or an inhibitor of GFR α 3. In some embodiments, the at least one agent comprises PHA-739358 or an analog or derivative thereof. In some embodiments, the at least one agent comprises VEGFR inhibitor V or an analog or derivative thereof. In some embodiments, TGF β superfamily signaling comprises TGF β signaling through a receptor serine/threonine kinase. In some embodiments, the at least one agent comprises Alk5 inhibitor II or an analog or derivative thereof. In some embodiments, the at least one agent comprises ALK5 inhibitor I or an analog or derivative thereof. In some embodiments, the at least one agent comprises a SMAD3 inhibitor or an analog or derivative thereof. In some embodiments, the method (e.g., of reversing and/or preventing de-differentiation) includes detecting de-differentiation of the β cell or de-differentiated β cell. In some embodiments, de-differentiation of the β cell or de-differentiated β cell is detected (i) prior to contacting, (ii) contemporaneously with contacting, or (iii) after contacting.

[0015] In some embodiments, contacting occurs *in vitro* or *ex vivo*. In some embodiments, contacting occurs *in vivo*. In some embodiments, the *in vivo* contact occurs in a subject. In some embodiments, the subject (i) has diabetes; (ii) is at risk of developing diabetes; (iii) is developing diabetes; or (iv) is suspected of having or developing diabetes. In some embodiments, the subject is (i) non-diabetic; (ii) mildly diabetic, or (iii) severely diabetic. In some embodiments, the method includes administering to the subject a conventional anti-diabetes therapy.

[0016] In some aspects, the disclosure provides a method of identifying at least one candidate agent for preventing β cell de-differentiation, comprising: a) contacting a β cell with at least one test agent under conditions which cause β cell de-differentiation to occur; and b) assessing the level of Ucn3 expression in β cell in the presence of the at least one test agent, wherein the at least one test agent is identified as at least one candidate agent for preventing β cell de-differentiation if the level of Ucn3 expression in the β cell does not decrease in the presence of the at least one test.

[0017] In some embodiments, the conditions which cause β cell de-differentiation to occur comprise stress induced by a cytokine comprising IL-1 β , TNF α , IFN γ , and combinations thereof.

[0018] In some embodiments, the method includes assessing the level of expression of at least one marker of mature β cells comprising FoxO1, MafA, Nkx6.1, and/or Pdx1, wherein the at least one test agent is identified as at least one candidate agent for preventing β cell de-differentiation if the level of expression of the at least one marker of mature β cells in the β cell does not decrease in the presence of the at least one test agent.

[0019] In some embodiments, the method includes conducting a GSIS assay on the β cell, wherein the at least one test agent is identified as at least one candidate agent for preventing β cell de-differentiation if the β cell maintains its ability to exhibit an appropriate GSIS response in the presence of the at least one test agent.

[0020] In some aspects, the disclosure provides a method of identifying at least one candidate agent for reversing β cell de-differentiation, comprising: a) contacting a de-differentiated β cell with at least one test agent; and b) assessing the level of Ucn3 expression in the de-differentiated β cell in the presence and absence of the at least one test agent, wherein the at least one test agent is identified as at least one candidate agent for reversing β cell de-differentiation if the level of Ucn3 expression in the de-differentiated β cell increases in the presence of the at least one test agent compared to the level of Ucn3 expression in the de-differentiated β cell in the absence of the at least one test agent.

[0021] In some embodiments, the method includes measuring the level of expression of at least one marker of mature β cells comprising FoxO1, MafA, Nkx6.1, and Pdx1, wherein the at least one test agent is identified as a candidate agent for reversing β cell de-differentiation if the level of expression of the at least one marker of mature β cells detected in the de-differentiated β cell increases in the presence of the at least one test agent relative the level of expression of the at least one marker of mature β cells detected in the de-differentiated β cell in the absence of the at least one test agent.

[0022] In some embodiments, the method includes conducting a GSIS assay on the de-differentiated β cell, wherein the at least one test agent is identified as a candidate agent for reversing β cell de-differentiation if the de-differentiated β cell gains the ability to exhibit an appropriate GSIS response after exposure to the at least one test agent.

[0023] In some embodiments, the de-differentiated β cell comprises: (i) a β cell de-differentiated by culturing in adherent conditions, or (ii) a de-differentiated β cell obtained from a diabetic subject.

[0024] In some aspects, the disclosure provides a method of identifying at least one candidate agent for reversing β cell de-differentiation, comprising: a) contacting a β cell with at least one test agent; and b) assessing the ability of the at least one test agent to inhibit Alk5 signaling, wherein at least one test agent that demonstrates the ability to inhibit Alk5 signaling comprises at least one candidate agent for reversing β cell de-differentiation. In some embodiments, the method includes assessing the ability of the at least one candidate agent to reverse β cell de-differentiation, wherein assessing the ability of the at least one candidate agent to reverse β cell de-differentiation comprises: a) contacting a de-differentiated β cell with the at least one candidate agent; and b) detecting the level of Ucn3 expression in the de-differentiated β cell, wherein at least one candidate agent demonstrates the ability to reverse β cell de-differentiation if the level of Ucn3 expression detected in the de-differentiated β cell increases in the presence of the at least one candidate agent relative the level of Ucn3 expression detected in the de-differentiated β cell in the absence of the at least one candidate agent.

[0025] In some embodiments, the method includes measuring the level of expression of at least one marker of mature β cells comprising FoxO1, MafA, Nkx6.1, and Pdx1, wherein the at least one candidate agent demonstrates the ability to reverse β cell de-differentiation if the level of expression of the at least one marker of mature β cells detected in the de-differentiated β cell increases in the presence of the at least one candidate agent relative the level of expression of the at least one marker of mature β cells detected in the de-differentiated β cell in the absence of the at least one candidate agent.

[0026] In some embodiments, the method includes conducting a GSIS assay on the de-differentiated β cell, wherein the at least one candidate agent demonstrates the ability to reverse β cell de-differentiation if the de-differentiated β cell gains the ability to exhibit an appropriate GSIS response after exposure to the at least one candidate agent.

[0027] In some embodiments, the disclosure provides a method of preventing a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of a β cell de-differentiation preventing agent. In some embodiments, the disclosure provides a method of treating a β cell de-

differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of a β cell de-differentiation reversing agent.

[0028] In some embodiments, the β cell de-differentiation preventing agent and/or the β cell de-differentiation reversing agent comprise an inhibitor of TGF β superfamily signaling. In some embodiments, the β cell de-differentiation preventing agent and/or the β cell de-differentiation reversing agent comprise an inhibitor of artemin signaling. In some embodiments, the β cell de-differentiation preventing agent and/or the β cell de-differentiation reversing agent comprises an inhibitor of receptor tyrosine kinase RET or an inhibitor of receptor GRF α 3. In some embodiments, the β cell de-differentiation preventing agent and/or the β cell de-differentiation reversing agent comprises PHA-739358 or an analog or derivative thereof. In some embodiments, the β cell de-differentiation preventing agent and/or the β cell de-differentiation reversing agent comprises VEGFR inhibitor V or an analog or derivative thereof. In some embodiments, the β cell de-differentiation preventing agent and/or the β cell de-differentiation reversing agent comprise an inhibitor of Alk5 signaling. In some embodiments, the β cell de-differentiation preventing agent and/or the β cell de-differentiation reversing agent comprises Alk5 inhibitor II or an analog or derivative thereof. In some embodiments, the β cell de-differentiation preventing agent and/or the β cell de-differentiation reversing agent comprises ALK5 inhibitor I or an analog or derivative thereof. In some embodiments, the β cell de-differentiation preventing agent and/or the β cell de-differentiation reversing agent comprises a SMAD3 inhibitor or an analog or derivative thereof.

[0029] In some embodiments, the subject in need of is a subject who (i) is in need of additional β cell; (ii) has diabetes; (iii) is at risk of developing diabetes; (iv) is developing diabetes; (v) is suspected of having or developing diabetes; (vi) is non-diabetic; (vii) is mildly diabetic; or (viii) severely diabetic. In some embodiments, the subject has, is developing or is at risk of developing, or is suspected of having metabolic syndrome or obesity. In some embodiments, the β cell de-differentiation-related disorder is selected from the group consisting of pre-diabetes, type I diabetes, type II diabetes, type 1.5 diabetes, obesity, metabolic syndrome, or hyperlipidemia. In some embodiments, the method includes selecting a subject in need of treatment for a β cell de-differentiation-related disorder. In some embodiments, the method includes detecting β

cell de-differentiation in the subject. In some embodiments, the method includes administering to the subject an effective amount of an anti-diabetic agent.

[0030] The practice of the present invention will typically employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant nucleic acid (e.g., DNA) technology, immunology, and RNA interference (RNAi) which are within the skill of the art. Non-limiting descriptions of certain of these techniques are found in the following publications: Ausubel, F., et al., (eds.), *Current Protocols in Molecular Biology*, *Current Protocols in Immunology*, *Current Protocols in Protein Science*, and *Current Protocols in Cell Biology*, all John Wiley & Sons, N.Y., edition as of December 2008; Sambrook, Russell, and Sambrook, *Molecular Cloning: A Laboratory Manual*, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 2001; Harlow, E. and Lane, D., *Antibodies – A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1988; Freshney, R.I., “*Culture of Animal Cells, A Manual of Basic Technique*”, 5th ed., John Wiley & Sons, Hoboken, NJ, 2005. Non-limiting information regarding therapeutic agents and human diseases is found in Goodman and Gilman’s *The Pharmacological Basis of Therapeutics*, 11th Ed., McGraw Hill, 2005, Katzung, B. (ed.) *Basic and Clinical Pharmacology*, McGraw-Hill/Appleton & Lange; 10th ed. (2006) or 11th edition (July 2009). Non-limiting information regarding genes and genetic disorders is found in McKusick, V.A.: *Mendelian Inheritance in Man. A Catalog of Human Genes and Genetic Disorders*. Baltimore: Johns Hopkins University Press, 1998 (12th edition) or the more recent online database: *Online Mendelian Inheritance in Man, OMIM™*. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD), as of May 1, 2010, World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/> and in *Online Mendelian Inheritance in Animals (OMIA)*, a database of genes, inherited disorders and traits in animal species (other than human and mouse), at <http://omia.angis.org.au/contact.shtml>. All patents, patent applications, and other publications (e.g., scientific articles, books, websites, and databases) mentioned herein are incorporated by reference in their entirety. In case of a conflict between the specification and any of the incorporated references, the specification (including any amendments thereof, which may be based on an incorporated reference), shall control. Standard art-

accepted meanings of terms are used herein unless indicated otherwise. Standard abbreviations for various terms are used herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

[0032] FIGS. 1A, 1B and 1C demonstrate loss of Ucn3 expression as an early marker for β cell de-differentiation in diabetes. FIG. 1A shows immunostaining with antibodies against insulin (red) and Ucn3 (green) in pancreata from T2D (Ob/Ob and Db/Db) and healthy control (C57BL/6) mice. Ucn3 protein expression is down regulated in diabetic pancreata compared to the healthy control. Nuclei are stained with DAPI (blue). FIG. 1B is a bar graph showing the results of quantitative real-time PCR analysis of Insulin1 and Ucn3 gene expression in islets from non-diabetic control mice ($n=10$; average blood glucose 167 ± 5 mg/dL), mildly diabetic ($n=16$; average blood glucose 381 ± 17 mg/dL) and severely diabetic mice ($n=11$; average blood glucose 588 ± 8 mg/dL). Error bars represent $\pm$ SEM. *** $P<0.005$. FIG. 1C is a bar graph showing that mice treated with S961 for one week have a nearly two-fold increase in insulin mRNA expression (indicating that they are compensating for the insulin resistance by over-producing insulin), and about a twofold decrease in Ucn3 mRNA expression.

[0033] FIGS. 2A, 2B, 2C, 2D, 2E, 2F and 2G demonstrate β cell de-differentiation is reversible. FIG. 2A shows immunostaining with antibodies against insulin (red) and Ucn3 (green) in pancreata from wild-type C57BL/6 mice treated with either vehicle (PBS) or S961 (insulin receptor antagonist) for 7 days (upper and middle panels) or treated with S961 for 7 days followed by a 7 day recovery period in the absence of S961 (lower panel). Ucn3 protein expression is down regulated in β cells following 7-days S961 treatment, but returns to normal expression levels upon remission to normoglycaemia (see text). Nuclei are stained with DAPI (blue). FIG. 2B is a schematic depicting RCU reporter mice made by crossing mice homozygous for the Insulin2-Cre transgene with mice doubly-homozygous for Rosa26-lox-stop-lox-H2BmCherry and Ucn3-GFP. Insulin expression in RCU progeny is permanently marked by red nuclear fluorescence, and Ucn3 expression is marked by green cytoplasmic

fluorescence. FIG. 2C shows confocal imaging of triple hemizygous progeny β cells from RCU mice show red nuclear fluorescence in β cells that is easily distinguished from the cytoplasmic green fluorescence emitted by the Ucn3-GFP reporter. FIG. 2D shows pancreas sections of PBS-treated control and S961-treated diabetic RCU mice. Ucn3-GFP is reduced in diabetic mice, but not in controls, and Ucn3 expression returns after remission from diabetes. All images show live (unstained) reporter fluorescence. FIG. 2E is a bar graph demonstrating that the levels of both insulin1 and Ucn3 in such adherent cultures of islets from wild-type mice were reduced to 4% and 29% of the levels in freshly harvested islets, respectively. FIG. 2F is a bar graph showing that consistent with the loss of the Ucn3 marker, islets completely lose their ability for glucose-stimulated insulin secretion (GSIS). FIG. 2G shows de-differentiation and re-differentiation of RCU islets cultured *in vitro*. Islets from adult RCU mice were isolated and plated on 804G matrix for one week (left and middle panel). Note islet spreading and loss of Ucn3-GFP in the de-differentiated islets (middle panel). After 7-days, the de-differentiated islets were transplanted into euglycemic SCID mice for three weeks (right panel) after which time the transplants show the return of Ucn3 expression in cells.

[0034] FIGS. 3A, 3B, 3C and 3D demonstrate that TGF β pathway inhibitors and inhibitors of Artemin signaling reverse β cell de-differentiation. FIG. 3A is a schematic depicting islets from adult RCU mice are isolated and plated on 804G matrix for one week in a 384-well plate format during which time the β cells de-differentiate. A compound library is added on day 7, and islets are cultured for an additional week in the presence of compounds. Each compound is tested in duplicates of two or three concentrations. Fresh un-manipulated RCU islets are used as a positive control, and DMSO- or untreated islets are used as negative controls. Islets are fixed on day 11 for automated imaging and subsequent analysis. Percentages of mCherry positive cells that co-express GFP are calculated for each well and used to identify conditions that significantly increase the number of GFP positive cells over negative (DMSO- or non-treated) controls. Positive hits are selected according to their statistical significance (P value) over the negative control. FIG. 3B is a graph showing the results of a screen with 114 growth factor proteins. Factors are ordered from left to right based on the P-value of their Ucn3-GFP fluorescence over the negative (non-treated) control. For convenience, values on the Y axis are presented as 1/P-value. Red bar represents the threshold for statistical significance ($P < 0.001$). FIG. 3C is a graph showing the results of a screen with

19 TGF β pathway inhibitors, 18 RET/GFR α 3 inhibitors and 42 known T2D drugs. Factors are ordered from left to right based on the statistical P-value of their Ucn3-GFP fluorescence over the negative (DMSO-treated) control as above. For convenience, values on the Y axis are presented as 1/P-value. Red bar represents the threshold for statistical significance ($P < 0.001$). A full list of the factors tested is presented in Tables 1A, 1B and 2. FIG. 3D is a line graph illustrating results of a dose-response test showing that the effect of Alk5 inhibitor II on Ucn3-GFP expression in de-differentiated RCU β -cells begins at pico-molar concentrations.

[0035] FIGS. 4A, 4B and 4C demonstrate that Alk5 inhibitor II induces expression of mature β cell transcription factors and prevents their reduction under cytokine stress induced by IL-1 β (FIG. 4A), TNF α (FIG. 4B) and INF γ (FIG. 4C). Bar graphs show the results of quantitative real-time PCR analysis of gene expression in wild-type islets treated with cytokines as shown. Each bar represents average gene expression in three independent experiments. Expression levels are normalized to the levels of control islets not treated with any cytokine (dashed line). Statistical significance relates to the difference between Alk5i-treated and DMSO-treated islets for each gene. Error bars represent $\pm$ SEM. * $P < 0.05$; *** $P < 0.005$. B.G. = Blood glucose level at time of sacrifice.

[0036] FIGS. 5A, 5B, 5C, 5D, and 5E demonstrate that Alk5 inhibitor II induces expression of mature β cell transcription factors even in β cells that were exposed to extreme diabetic conditions for several months. FIGS. 5A, 5B, 5C and 5D are bar graphs demonstrating that Alk5 inhibitor II (Alk5i) induces expression of specific β cell genes in islets from healthy and severely diabetic mice. Shown are quantitative real-time PCR analysis of gene expression in islets of healthy control (C57BL/6) and diabetic mice (Db/Db, Ob/Ob and Akita). Each bar represents average gene expression in three independent experiments for each group. Statistical significance relates to the difference between Alk5i-treated and DMSO-treated islets for each gene. Expression levels are normalized to the levels of DMSO-treated islets for each gene separately. Error bars represent $\pm$ SEM. * $P < 0.05$; *** $P < 0.005$. B.G. = Blood glucose level at time of sacrifice. FIG. 5E is a bar graph demonstrating that Alk5 inhibitor II (Alk5i) induces expression of specific β -cell transcription factors in human islets. Shown are quantitative Real-Time PCR analyses of gene expression. Error bars represent three technical repeats on islets from a single donor. Error bars represent $\pm$ SEM.

DETAILED DESCRIPTION OF THE INVENTION

[0037] Work described herein demonstrates that Urocortin 3 (Ucn3), a marker for mature β cells, is down-regulated in the early stages of T2D in *in vivo* (e.g., in mice) and when β cells are stressed *in vitro*. Using an insulin expression-coupled lineage tracer, with Ucn3 as a reporter for the mature β cell state, the inventors screened for factors that reverse β cell de-differentiation, and surprisingly found that inhibitors of TGF β receptor I (Alk5) protect cells from the loss of key β cell transcription factors and restore mature β cell identity, even after exposure to prolonged and severe diabetes.

[0038] *Detecting De-Differentiation and/or Redifferentiation of β cells*

[0039] Aspects of the disclosure relate to methods for detecting de-differentiation and/or re-differentiation of β cells. As will be appreciated by those skilled in the art, detecting de-differentiation and/or re-differentiation of β cells finds use in various applications (e.g., diagnostic, prognostic, screening, treatment, etc). Examples of such applications include, without limitation, detecting β cell de-differentiation as an early marker of β cell stress, detecting β cell de-differentiation as an early marker of diabetes (e.g., identifying individuals developing or at increased risk of developing diabetes), identifying disorders associated with β cell de-differentiation, determining the efficacy of a treatment with one or more β cell de-differentiation reversing agents (e.g., by assaying for whether the one or more β cell de-differentiation reversing agents successfully reversed de-differentiation of β cells alone, or in combination with conventional anti-diabetes therapy), and screening for candidate agents that are useful for reversing β cell de-differentiation, etc.

[0040] In some aspects, the disclosure provides a method for detecting de-differentiation of a β cell, the method comprising: a) obtaining a β cell; b) detecting the level of urocortin 3 (Ucn3) expression in the β cell; c) comparing the level of Ucn3 expression detected in the β cell to the level of Ucn3 expression detected in a normal mature β cell; and d) detecting de-differentiation of the β cell, wherein de-differentiation of the β cell is detected if the level of Ucn3 expression detected in the β cell is decreased relative to the level of Ucn3 expression detected in the normal mature β cell.

[0041] In some aspects, the disclosure provides a method for detecting re-differentiation of a β cell, the method comprising: a) obtaining a β cell; b) detecting the level of Ucn3 expression in the β cell; c) comparing the level of Ucn3 expression detected in the β cell to the level of Ucn3 expression detected in a normal mature β cell; and d)

detecting re-differentiation of the β cell, wherein re-differentiation of the β cell is detected if the level of Ucn3 expression detected in the β cell is comparable to the level of Ucn3 expression detected in the normal mature β cell.

[0042] In some aspects, the disclosure provides a method for detecting de-differentiation or re-differentiation of a β cell, the method comprising: a) obtaining a β cell; b) detecting the level of Ucn3 expression in the β cell; c) comparing the level of Ucn3 expression detected in the β cell to the level of Ucn3 expression detected in a normal mature β cell; and d) detecting de-differentiation or re-differentiation of the β cell, wherein de-differentiation of the β cell is detected if the level of Ucn3 expression detected in the β cell is decreased relative to the level of Ucn3 expression detected in the normal mature β cell; or wherein re-differentiation of the β cell is detected if the level of Ucn3 expression detected in the β cell is comparable to the level of Ucn3 expression detected in the normal mature β cell.

[0043] In some aspects, the disclosure provides a method for detecting β cell de-differentiation as an early marker of β cell stress, the method comprising: a) obtaining a β cell; b) detecting the level of urocortin 3 (Ucn3) expression in the β cell; c) comparing the level of Ucn3 expression detected in the β cell to the level of Ucn3 expression detected in a normal mature β cell; and d) detecting β cell de-differentiation as an early marker of β cell stress, wherein β cell de-differentiation is detected as an early marker of β cell stress if the level of Ucn3 expression detected in the β cell is decreased relative to the level of Ucn3 expression detected in the normal mature β cell.

[0044] In some aspects, the disclosure provides a method for detecting β cell de-differentiation as an early marker of diabetes, the method comprising: a) obtaining a β cell; b) detecting the level of urocortin 3 (Ucn3) expression in the β cell; c) comparing the level of Ucn3 expression detected in the β cell to the level of Ucn3 expression detected in a normal mature β cell; and d) detecting β cell de-differentiation as an early marker of diabetes, wherein β cell de-differentiation is detected as an early marker of diabetes if the level of Ucn3 expression detected in the β cell is decreased relative to the level of Ucn3 expression detected in the normal mature β cell.

[0045] As used herein, the phrases “de-differentiation of a β cell” and “ β cell de-differentiation” refer to the loss of a mature β cell phenotype, for example, due to exposure of a β cell to high levels of glucose, lipids, and inflammatory cytokines, diabetes or a pre-diabetic condition, and/or β cell stress (e.g., oxidative stress). A β cell exhibits

loss of a mature β cell phenotype and is thus undergoing de-differentiation if the β cell exhibits a statistically significant and detectable change in any one of the following markers of a mature β cell phenotype: (1) a morphology that resembles the morphology of an endogenous mature β cell (e.g., encapsulation of crystalline insulin into secretory granules); (2) an appropriate GSIS response both *in vitro* and *in vivo*; (3) cytokine-induced apoptosis in response to cytokines; (4) enhanced insulin secretion in response to known antidiabetic drugs (e.g., secretagogues); (5) monohormonal (e.g., lack of expression of hormones other than insulin, such as glucagon, somatostatin or pancreatic polypeptide); (6) a low rate of replication; (7) glucose stimulated calcium flux, i.e., the cells increase intracellular Ca^{2+} in response to glucose; and (8) expression of one or more markers characteristic of a mature β cell. In the context of β cells, the expressions “de-differentiated” or “de-differentiating” are relative terms meaning that the degree of loss of mature β cell phenotype may vary along a de-differentiation continuum from a fully mature β cell (i.e., un-de-differentiated β cell) to a fully de-differentiated β cell (i.e., complete loss of the mature β cell phenotype). Those skilled in the art will appreciate that the degree of loss of mature β cell phenotype (e.g., degree of de-differentiation) in any particular β cell is a function of the amount of markers of the mature β cell phenotype which change compared to the phenotype of a normal mature β cell, as well as the magnitude or extent to which those markers change in the de-differentiating β cell. In other words, a first de-differentiated β cell is considered to be more de-differentiated than a second de-differentiated β cell if the first β cell has undergone more changes in markers of the mature β cell phenotype, or if the magnitude or extent to which the changes in the first de-differentiated β cell exceeds the magnitude or extent of similar changes in the second de-differentiated β cell. For example, if expression of a marker of mature β cells (e.g., Ucn3) is decreased in both a first and second de-differentiated β cell, the first de-differentiated β cell is considered to be more de-differentiated if the magnitude of the decrease in expression of the marker in the first de-differentiated β cell phenotype exceeds the magnitude of the decrease in expression of the marker in the second de-differentiated β cell, e.g., a first β cell in which Ucn3 expression has decreased by 10 fold is considered to be more de-differentiated than a second β cell in which Ucn3 expression has decreased by only 3 fold. As another example, a first β cell in which expression of least three markers of a mature β cell have decreased is considered to be more de-differentiated than a second β cell in which expression of only two of the at least three

markers of mature β cell have decreased. In some contexts, the magnitude or extent of de-differentiation of a β cell can be assessed by the ability of a de-differentiated β cell to function like a normal mature β cell, i.e., the magnitude or extent to which the de-differentiated or de-differentiating β cell is unable to function like a normal mature β cell is indicative of the magnitude or extent to which the de-differentiated or de-differentiating β cell is de-differentiated. Using an appropriate GSIS response as an example, a first β cell exhibiting a higher stimulation index in a GSIS assay is considered to be less de-differentiated than a second β cell exhibiting a lower stimulation index in the GSIS assay, i.e., a lower relative stimulation index is indicative of a weakened GSIS response.

[0046] As used herein, a normal mature β cell refers to a β cell which exhibits markers of a mature β cell phenotype characteristic of endogenous mature β cells in a healthy adult individual (e.g., a normal mature human β cell exhibits markers of the mature β cell phenotype characteristic of endogenous mature β cells in healthy human adults).

[0047] As used herein, the phrases “re-differentiation of a β cell” and “ β cell re-differentiation” refer to the restoration of a mature β cell phenotype, for example, by reversing de-differentiation (e.g., due to exposure of a β cell to high levels of glucose, lipids, and inflammatory cytokines, diabetes or a pre-diabetic condition, and/or β cell stress (e.g., oxidative stress)) according to a method described herein. A β cell exhibits restoration of a mature β cell phenotype and is thus undergoing re-differentiation if the β cell exhibits a statistically significant and detectable change in any one of the following markers of a mature β cell phenotype: (1) a morphology that resembles the morphology of an endogenous mature β cell (e.g., encapsulation of crystalline insulin into secretory granules); (2) an appropriate GSIS response both *in vitro* and *in vivo*; (3) cytokine-induced apoptosis in response to cytokines; (4) enhanced insulin secretion in response to known antidiabetic drugs (e.g., secretagogues); (5) monohormonal (e.g., lack of expression of hormones other than insulin, such as glucagon, somatostatin or pancreatic polypeptide); (6) a low rate of replication; (7) glucose stimulated calcium flux, i.e., the cells increase intracellular Ca^{2+} in response to glucose; and (8) expression of one or more markers characteristic of a mature β cell. In the context of β cells, the expressions “re-differentiated” or “re-differentiating” are relative terms meaning that the degree of restoration of mature β cell phenotype may vary along a re-differentiation continuum

from a fully de-differentiated β cell (i.e., complete loss of the mature β cell phenotype) to a fully mature β cell (i.e., un-de-differentiated β cell). Those skilled in the art will appreciate that the degree of restoration of mature β cell phenotype (e.g., degree of re-differentiation) in any particular β cell is a function of the amount of markers of mature β cell phenotype which changes compared to the phenotype of the de-differentiated β cell (i.e., the markers of mature β cell phenotype return to the original state of the β cell prior to de-differentiation), as well as the magnitude or extent to which those markers change in the re-differentiating β cell. In other words, a first re-differentiated β cell is considered to be more re-differentiated than a second re-differentiated β cell if the first β cell has undergone more changes in markers of the mature β cell phenotype, or if the magnitude or extent to which the changes in the first re-differentiated β cell exceeds the magnitude or extent of similar changes in the second re-differentiated β cell. For example, if expression of a marker of mature β cells (e.g., Ucn3) is increased in both a first and second re-differentiated β cell, the first re-differentiated β cell is considered to be more re-differentiated if the magnitude of the increase in expression of the marker in the first re-differentiated β cell phenotype exceeds the magnitude of the increase in expression of the marker in the second de-differentiated β cell, e.g., a first β cell in which Ucn3 expression has increased by 10 fold is considered to be more re-differentiated than a second β cell in which Ucn3 expression has increased by only 3 fold compared to the de-differentiated β cell prior to reversing de-differentiation. As another example, a first β cell in which expression of least five markers of a mature β cell have increased is considered to be more re-differentiated than a second β cell in which expression of only three of the at least five markers of the mature β cell have increased. In some contexts, the magnitude or extent of re-differentiation of a β cell can be assessed by the ability of a re-differentiated or re-differentiating β cell to function like a normal mature β cell, i.e., the magnitude or extent to which the re-differentiated or re-differentiating β cell is able to function like a normal mature β cell is indicative of the magnitude or extent to which the re-differentiated or re-differentiating β cell has re-differentiated. Using an appropriate GSIS response as an example, a first β cell exhibiting a higher stimulation index in a GSIS assay is considered to be more re-differentiated than a second β cell exhibiting a lower stimulation index in the GSIS assay.

[0048] Those skilled in the art will readily appreciate how to assess the above listed markers of mature β cell phenotype using existing techniques, for example, a

skilled artisan can detect whether a β cell (e.g., de-differentiated, de-differentiating, re-differentiated, or re-differentiating) is monohormonal, e.g., by immunostaining the β cell or a population of cells comprising β cells for expression of hormones such as insulin, glucagon, somatostatin, and pancreatic polypeptide, using antibodies specific for those hormones. β cells expressing insulin and lacking expression of glucagon, somatostatin and pancreatic polypeptide are considered to be monohormonal and indicative of a mature β cell phenotype.

[0049] Aspects of the disclosure involve detecting the levels of expression products (e.g., an expression product of the Ucn3 gene, the insulin gene, and/or at least one marker of mature β cells). Levels of expression products may be assessed using any suitable method. Either mRNA or protein level may be measured. A “polypeptide”, “peptide” or “protein” refers to a molecule comprising at least two covalently attached amino acids. A polypeptide can be made up of naturally occurring amino acids and peptide bonds and/or synthetic peptidomimetic residues and/or bonds. Polypeptides described herein include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells.

[0050] Exemplary methods for measuring mRNA include hybridization based assays, polymerase chain reaction assay, sequencing, in situ hybridization, etc. Exemplary methods for measuring protein levels include ELISA assays, Western blot, mass spectrometry, or immunohistochemistry. It will be understood that suitable controls and normalization procedures can be used to accurately quantify expression. Values can also be normalized to account for the fact that different samples may contain different proportions of a cell type of interest, e.g., mature β cells compared to de-differentiated β cells.

[0051] In some embodiments, detecting in b) comprises detecting the level of Ucn3 mRNA expression or Ucn3 protein expression and/or detecting the level of insulin mRNA expression or insulin protein expression in the β cell. Any suitable method of detecting the level of Ucn3 mRNA expression or Ucn3 protein expression and/or insulin mRNA expression or insulin protein expression can be used.

[0052] In some embodiments, detecting in b) comprises performing an immunostain using an antibody specific for Ucn3 protein to detect the level of Ucn3

protein expression in the β cell. In some embodiments, detecting in b) comprises performing an immunostain using a primary antibody specific for Ucn3 protein and a secondary antibody-fluorescent dye conjugate specific for the primary antibody to detect the level of Ucn3 protein expression in the β cell. In some embodiments, detecting the level of expression of insulin protein comprises performing an immunostain using an antibody specific for insulin protein to detect the level of insulin protein expression in the β cell. In some embodiments, detecting the level of expression of insulin protein comprises performing an immunostain using a primary antibody specific for insulin protein and a secondary antibody-fluorescent dye conjugate specific for the primary antibody to detect the level of insulin protein expression in the β cell.

[0053] The disclosure contemplates the use of any antibody (e.g., primary antibody) specific for Ucn3 protein and/or insulin protein. Suitable antibodies are available from commercial sources. An example of an antibody specific for Ucn3 (e.g., a primary antibody) of use herein comprises rabbit anti-mouse Ucn3 antibody (commercially available from Phoenix Pharmaceuticals). An example of a secondary antibody (e.g., antibody-fluorescent dye conjugate) specific for the primary antibody to detect Ucn3 protein expression in the β cell comprises Alexa Fluor 488 donkey anti-rabbit antibody (commercially available from Invitrogen). An exemplary antibody (e.g., primary antibody) specific for insulin protein comprises guinea pig anti-insulin antibody (commercially available from DAKO). An exemplary secondary antibody (e.g., antibody-fluorescent dye conjugate) specific for the primary antibody comprises DyLight 649 donkey anti-guinea pig antibody (commercially available from Jackson ImmunoResearch).

[0054] The immunostains obtained can be imaged for subsequent analysis (e.g., for comparing expression levels of Ucn3 and/or insulin protein in a β cell or de-differentiated β cell to the expression levels of Ucn3 and/or insulin protein in the β cell or de-differentiated β cell. In some embodiments, the method includes imaging the immunostain using a microscope to obtain a micrograph displaying Ucn3 protein expressed in the β cell in a fluorescent color indicative of the level of Ucn3 protein expressed in the β cell. In some embodiments, the method includes imaging the immunostain using a microscope to obtain a micrograph displaying insulin protein expressed in the β cell in a fluorescent color indicative of the level of insulin protein expressed in the β cell. The disclosure contemplates the use of any fluorescent colors that

are capable of showing a contrast between expression products of interest (e.g., Ucn3 protein and/or insulin protein) in a cell or tissue of interest (e.g., β cell, islet, pancreas, etc). In some embodiments, the fluorescent color indicative of the level of Ucn3 protein expressed in the β cell is green. In some embodiments, the fluorescent color indicative of the level of Ucn3 protein expressed in the β cell is red. In some embodiments, the fluorescent color comprises blue.

[0055] Any suitable microscope can be used. In some embodiments, the microscope comprises a confocal microscope. Examples of suitable microscopes include, without limitation, a Olympus IX51 Microscope and a Zeiss LSC 7000 confocal microscope.

[0056] In some embodiments, comparing in c) comprises displaying a first micrograph showing the results of an immunostain for Ucn3 protein in the β cell and displaying a second micrograph showing the results of an immunostain for Ucn3 protein in the normal mature β cell. In some embodiments, the method further comprises comparing the level of insulin expression in the β cell to the level of insulin expression in the normal mature β cell (e.g., the level of insulin protein). In some embodiments, comparing comprises displaying a first micrograph showing the results of an immunostain for insulin protein in the β cell and displaying a second micrograph showing the results of an immunostain for insulin protein in the normal mature β cell. An exemplary embodiment of comparing the level of expression of Ucn3 protein and/or level of expression of insulin protein to the level of expression of Ucn3 protein and/or level of expression of insulin protein in a β cell or de-differentiated β cell is shown in FIG. 1A. As is shown in FIG. 1A, comparing the level of Ucn3 expression in the cell may be performed at the same time as comparing the level of insulin expression in the cell. In some embodiments, comparing in c) comprises displaying a first micrograph showing the results of an immunostain for Ucn3 protein expression in the β cell and/or de-differentiated β cell compared to the results of an immunostains for Ucn3 protein expression in a normal mature β cell, an displaying a second micrograph showing the results of an immunostains for insulin protein expression in the β cell and/or de-differentiated β cell compared to the results of an immunostains for insulin protein expression in the β cell and/or de-differentiated β cell.

[0057] In some embodiments, measuring in b) comprises performing a hybridization based assay using one or more nucleic acids which hybridizes to a nucleic

acid encoding Ucn3. In some embodiments, measuring in b) comprises performing a polymerase chain reaction based assay using a set of primers and probes specific for Ucn3 cDNA, wherein the level of Ucn3 cDNA detected is indicative of the level of Ucn3 mRNA expressed in the β cell. In some embodiments, measuring in b) comprises: i) isolating total RNA comprising Ucn3 mRNA from the β cell; ii) reverse transcribing the Ucn3 mRNA isolated in step i) to generate Ucn3 cDNA; and iii) performing a quantitative real-time polymerase chain reaction (qRT-PCR) assay using a set of probes and/or primers specific for Ucn3 cDNA to detect the level of Ucn3 mRNA expression in the β cell, wherein the amount of Ucn3 cDNA detected is indicative of the level of Ucn3 mRNA expression in the β cell. In some embodiments, the method further comprises comparing the level of insulin expression in the β cell to the level of insulin expression in the normal mature β cell (e.g., the level of insulin mRNA expression). In some embodiments, detecting the level of expression of insulin mRNA in the β cell comprises performing a hybridization based assay using one or more nucleic acids which hybridizes to a nucleic acid encoding insulin. In some embodiments, detecting the level of expression of insulin mRNA in the β cell comprises performing a polymerase chain reaction based assay using a set of primers and probes specific for insulin cDNA, wherein the level of insulin cDNA detected is indicative of the level of insulin mRNA in the β cell. In some embodiments, detecting the level of expression of insulin mRNA in the β cell comprises: i) isolating total RNA comprising insulin mRNA from the β cell; ii) reverse transcribing the insulin RNA isolated in step i) to generate insulin cDNA; and iii) performing a quantitative real-time polymerase chain reaction (qRT-PCR) assay using a set of probes and/or primers specific for insulin cDNA to detect the level of insulin mRNA expression in the β cell, wherein the amount of insulin cDNA detected is indicative of the level of insulin mRNA expression in the β cell.

[0058] Nucleic acid sequences encoding Ucn3 and/or insulin are available to the skilled artisan utilizing publicly accessible databases (e.g., NCBI GenBank). For example, the human Ucn3 gene (GenBank Gene ID: 114131, also known as SCP, SPC, UCNIH) encodes a 710 bp linear mRNA (GenBank Accession Number NM_053049.2). Similarly, the human insulin (INS) gene (Gene ID: 3630, also known as ILPR, IRDN, IDDM1, IDDM2, and MODY10) encodes at least three insulin isoforms including a 469 bp linear mRNA referred to as transcript variant 1 (GenBank Accession Number NM_000207.2), a 495 bp linear mRNA referred to as transcript variant 2 (GenBank

Accession Number NM_001185097.1), and a 648 bp linear mRNA referred to as transcript variant 3 (GenBank Accession Number NM_001185098.1). Based on the publicly available sequence information, a skilled person can readily produce suitable nucleic acids capable of hybridizing to any of these sequences without routine experimentation. Similarly, suitable probes and/or primers for use in PCR based methods of detecting Ucn3 mRNA and/or insulin mRNA expression can be designed by the skilled artisan. An exemplary set of probes and/or primers for detecting Ucn3 mRNA and/or insulin mRNA expression in a β cell or de-differentiated β cell comprise gene-specific TaqMan probes with TaqMan® Fast Universal PCR Master Mix (Life Technologies) on an ABI 7900 Real-Time PCR machine.

[0059] In some embodiments, comparing in c) comprises displaying a graph showing the relative expression of Ucn3 mRNA in the β cell compared to the relative expression of Ucn3 mRNA in the normal mature β cell. In some embodiments, the method includes comparing the level of expression of insulin mRNA in the β cell to the level of expression of insulin mRNA in a normal mature β cell. In some embodiments, comparing comprises displaying a graph showing the relative expression of insulin mRNA in the β cell compared to the relative expression of Ucn3 mRNA in the normal mature β cell. An exemplary embodiment of comparing in c) is shown in FIG. 1B. As is shown in FIG. 1B, relative mRNA expression of insulin and Ucn3 can be shown in a side-by-side comparison on the same display (e.g., graph).

[0060] In some embodiments, the relative expression of insulin and/or Ucn3 mRNA detected in a sample can be compared to the relative expression of insulin and/or Ucn3 mRNA in a non-diabetic control sample. In some embodiments, the relative expression of insulin and/or Ucn3 mRNA detected in a sample can be compared to the relative expression of insulin and/or Ucn3 mRNA in a mildly diabetic control sample. In some embodiments, the relative expression of insulin and/or Ucn3 mRNA detected in a sample can be compared to the relative expression of insulin and/or Ucn3 mRNA in a severely diabetic control sample.

[0061] Generally, the methods described herein are capable of detecting any amount and/or type of de-differentiation. In some embodiments, de-differentiation comprises an early stage of de-differentiation. As used herein, an “early stage of de-differentiation” comprises de-differentiation of a β cell in which Ucn3 expression decreases and insulin expression remains the same or increases. During the early stage of

β cell de-differentiation, Ucn3 expression in a de-differentiating β cell may decrease as much as 3 fold compared to the level of Ucn3 expression in a normal mature β cell (e.g., a mature β cell obtained from a healthy control individual). In some embodiments, during the early stage of β cell de-differentiation, Ucn3 expression (e.g., mRNA or protein) in a de-differentiating β cell decreases by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 55%, 60%, 66%, or more compared to the level of Ucn3 expression in a normal mature β cell. In some embodiments, during the early stage of β cell de-differentiation, Ucn3 expression (e.g., mRNA or protein) in a de-differentiating β cell decreases by at least 1 fold, 1.1 fold, 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 1.6 fold, 1.7 fold, 1.8 fold, 1.9 fold, 2.0 fold, 2.1 fold, 2.2 fold, 2.3 fold, 2.4 fold, 2.5 fold, 2.6 fold, 2.7 fold, 2.8 fold, or 2.9 fold compared to the level of Ucn3 expression in a normal mature β cell. In some embodiments, levels of expression of Ucn3 mRNA and/or protein decrease to 34% of the level of expression of Ucn3 mRNA or protein in a normal mature β cell. In some embodiments, insulin expression (e.g., mRNA or protein) in a de-differentiating β cell increases slightly compared to the levels of insulin mRNA or protein expression in a normal mature β cell.

[0062] In some embodiments, de-differentiation comprises a late stage of de-differentiation. As used herein, a “late stage of de-differentiation” comprises de-differentiation of a β cell in which Ucn3 expression decreases and insulin expression decreases. During the late stage of β cell de-differentiation, Ucn3 expression in a de-differentiating β cell may decrease as much as 10 fold compared to the level of Ucn3 expression in a normal mature β cell (e.g., a mature β cell obtained from a healthy control individual). In some embodiments, during the late stage of β cell de-differentiation, Ucn3 expression (e.g., mRNA or protein) in a de-differentiating β cell decreases by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 55%, 60%, 66%, 70%, 75%, 80%, 85%, 90% or more compared to the level of Ucn3 expression in a normal mature β cell. In some embodiments, during the early stage of β cell de-differentiation, Ucn3 expression (e.g., mRNA or protein) in a de-differentiating β cell decreases by at least 1 fold, 1.3 fold, 1.5 fold, 1.7 fold, 2.0 fold, 2.3 fold, 2.6 fold, 3.0 fold, 3.4 fold, 3.6 fold, 3.9 fold, 4.1 fold, 4.5 fold, 4.8 fold, 5.2 fold, 5.4 fold, 5.8 fold, 6.1 fold, 6.3 fold, 6.4 fold, 6.6 fold, 6.9 fold, 7.1 fold, 7.5 fold, 7.8 fold, 8.2 fold, 8.4 fold, 8.8 fold, 9.1 fold, 9.3 fold, 9.5 fold, 9.6 fold, 9.7 fold, 9.8 fold, 9.9 fold, or 10 fold or more compared to the level of Ucn3 expression in a normal mature β cell. During the late stage of β cell de-differentiation, insulin

expression (e.g., mRNA or protein) in a de-differentiating β cell may decrease as much as 3.5 fold compared to the level of insulin expression in a normal mature β cell (e.g., a mature β cell obtained from a healthy control individual). In some embodiments, during the late stage of β cell de-differentiation, insulin expression (e.g., mRNA or protein) in a de-differentiating β cell decreases by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 55%, 60%, 70%, 72% or more compared to the level of insulin expression in a normal mature β cell. In some embodiments, during the late stage of β cell de-differentiation, insulin expression (e.g., mRNA or protein) in a de-differentiating β cell decreases by at least 1 fold, 1.1 fold, 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 1.6 fold, 1.7 fold, 1.8 fold, 1.9 fold, 2.0 fold, 2.1 fold, 2.2 fold, 2.3 fold, 2.4 fold, 2.5 fold, 2.6 fold, 2.7 fold, 2.8 fold, 2.9 fold, 3.0 fold, 3.1 fold, 3.2 fold, 3.3 fold, or 3.4 fold compared to the level of insulin expression in a normal mature β cell. In some embodiments, levels of expression of Ucn3 mRNA or protein decrease during the late stage of de-differentiation to 10% of the level of expression of Ucn3 mRNA or protein in a normal mature β cell. In some embodiments, levels of expression of insulin mRNA or protein decrease during the late stage of de-differentiation to 28% of the level of expression of insulin mRNA or protein in a normal mature β cell.

[0063] De-differentiation may be characterized by decreased expression of at least one marker of mature β cells in the de-differentiated or de-differentiating β cell compared to the level of expression of the at least one marker of mature β cells in a normal mature β cell. Exemplary markers of mature β cells include, without limitation, FoxO1, MafA, NeuroD, Nkx6.1, and/or Pdx1. De-differentiation includes any decrease in expression of the at least one marker of mature β cells in the de-differentiated or de-differentiating β cell relative to the level of expression of the at least one marker of mature β cells in the normal mature β cell.

[0064] In some embodiments, the marker of mature β cells comprises FoxO1, and the level of expression of FoxO1 in the de-differentiated or de-differentiating cells is decreased by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 70%, or more relative to the level of expression of FoxO1 in a normal mature β cell. In some embodiments, the level of expression of FoxO1 in the de-differentiated or de-differentiating cells is decreased by at least 75%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least

98%, or at least 99%, relative to the level of expression of FoxO1 in a normal mature β cell. In some embodiments, the level of expression of FoxO1 in the de-differentiated or de-differentiating cells is decreased by at 100% relative to the level of expression of FoxO1 in a normal mature β cell, i.e., expression of FoxO1 is completely abolished in the de-differentiated or de-differentiating cells.

[0065] In some embodiments, the marker of mature β cells comprises MafA, and the level of expression of MafA in the de-differentiated or de-differentiating cells is decreased by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 70%, or more relative to the level of expression of MafA in a normal mature β cell. In some embodiments, the level of expression of MafA in the de-differentiated or de-differentiating cells is decreased by at least 75%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, relative to the level of expression of MafA in a normal mature β cell. In some embodiments, the level of expression of MafA in the de-differentiated or de-differentiating cells is decreased by at 100% relative to the level of expression of MafA in a normal mature β cell, i.e., expression of MafA is completely abolished in the de-differentiated or de-differentiating cells.

[0066] In some embodiments, the marker of mature β cells comprises NeuroD, and the level of expression of NeuroD in the de-differentiated or de-differentiating cells is decreased by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 70%, or more relative to the level of expression of NeuroD in a normal mature β cell. In some embodiments, the level of expression of NeuroD in the de-differentiated or de-differentiating cells is decreased by at least 75%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, relative to the level of expression of NeuroD in a normal mature β cell. In some embodiments, the level of expression of NeuroD in the de-differentiated or de-differentiating cells is decreased by at 100% relative to the level of expression of NeuroD in a normal mature β cell, i.e., expression of NeuroD is completely abolished in the de-differentiated or de-differentiating cells.

[0067] In some embodiments, the marker of mature β cells comprises Nkx6.1, and the level of expression of Nkx6.1 in the de-differentiated or de-differentiating cells is

decreased by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 70%, or more relative to the level of expression of Nkx6.1 in a normal mature β cell. In some embodiments, the level of expression of Nkx6.1 in the de-differentiated or de-differentiating cells is decreased by at least 75%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, relative to the level of expression of Nkx6.1 in a normal mature β cell. In some embodiments, the level of expression of Nkx6.1 in the de-differentiated or de-differentiating cells is decreased by at 100% relative to the level of expression of Nkx6.1 in a normal mature β cell, i.e., expression of Nkx6.1 is completely abolished in the de-differentiated or de-differentiating cells.

[0068] In some embodiments, the marker of mature β cells comprises Pdx1, and the level of expression of Pdx1 in the de-differentiated or de-differentiating cells is decreased by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 70%, or more relative to the level of expression of Pdx1 in a normal mature β cell. In some embodiments, the level of expression of Pdx1 in the de-differentiated or de-differentiating cells is decreased by at least 75%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, relative to the level of expression of Pdx1 in a normal mature β cell. In some embodiments, the level of expression of Pdx1 in the de-differentiated or de-differentiating cells is decreased by at 100% relative to the level of expression of Pdx1 in a normal mature β cell, i.e., expression of Pdx1 is completely abolished in the de-differentiated or de-differentiating cells.

[0069] In some embodiments, β cell de-differentiation is characterized by decreased expression of at least two markers of mature β cells comprising FoxO1, MafA, NeuroD, Nkx6.1, and/or Pdx1. In some embodiments, β cell de-differentiation is characterized by decreased expression of at least three markers of mature β cells comprising FoxO1, MafA, NeuroD, Nkx6.1, and/or Pdx1. In some embodiments, β cell de-differentiation is characterized by decreased expression of at least four markers of mature β cells comprising FoxO1, MafA, NeuroD, Nkx6.1, and/or Pdx1. In some embodiments, β cell de-differentiation is characterized by decreased expression of at least five markers of mature β cells comprising FoxO1, MafA, NeuroD, Nkx6.1, and/or Pdx1.

Those skilled in the art will appreciate that β cell de-differentiation in certain individuals (e.g., diabetics) reduces the number of functionally mature β cells (e.g., decreasing the percentage of functionally mature β cell mass in those individuals).

[0070] In some embodiments, de-differentiation is characterized by the absence of an appropriate GSIS response in a β cell, a de-differentiated β cell, or a de-differentiating β cell. Aspects of the disclosure involve assaying a β -cell (e.g., a de-differentiating β -cell, de-differentiated β -cell, re-differentiating β -cell, or re-differentiated β -cell, etc.) for the presence or absence of an appropriate glucose stimulated insulin secretion (GSIS) response, for example, to determine whether a β -cell has de-differentiated (i.e., the β -cell exhibits the absence of an appropriate GSIS response) or whether a β -cell has re-differentiated (i.e., the β -cell exhibits the presence of an appropriate GSIS response).

[0071] Assaying a β -cell or population of β -cells for the presence or absence of an appropriate GSIS response comprises assaying a β -cell or population of β -cells for the presence or absence of a GSIS response at low glucose concentrations and/or for the presence or absence of a large fold change in the GSIS response between the low and high glucose concentrations. Such assays are referred to as GSIS assays. Briefly, a GSIS assay involves exposing a β -cell or population of β -cells to varying concentrations of glucose and measuring how much insulin is secreted by the β -cell or population of β -cells in response to the varying glucose concentrations.

[0072] The present disclosure contemplates the use of any method of measuring insulin secretion available to the skilled artisan. An exemplary method of measuring insulin secretion from isolated islets of Langerhans is described by Nolan and O-Dowd (*Methods Mol Biol* 560, 43-51 (2009)). Other suitable methods of measuring insulin secretion are apparent to the skilled artisan.

[0073] As noted above, the presence of a GSIS response at low glucose concentrations is indicative of a de-differentiated β -cell or de-differentiating β -cell. As used herein, "presence of a GSIS response at low glucose concentrations" generally means that a statistically measurable and relevant amount of insulin is secreted by the cells upon exposure to low concentrations of glucose. In some embodiments, the presence of a GSIS response at low glucose concentrations is at least a first phase of insulin secretion in response to the low glucose concentration. In some embodiments, the presence of a GSIS response at low glucose concentrations is a complete GSIS response

comprising a first and second phase of insulin secretion in response to the low glucose concentration.

[0074] The absence of a GSIS response at low glucose concentrations is indicative of a mature β -cell (i.e., a fully re-differentiated β -cell). In some embodiments, the absence of a GSIS response at low glucose concentrations is a lack of insulin secretion in response to the low glucose concentrations.

[0075] As used herein, a “low glucose concentration” refers to concentrations of glucose that are less than or equal to about 5 mM, between about 2.8 mM and about 5 mM, about 2.8 mM, below 2.8 mM, about 0.5 mM. As used herein, a “high glucose concentration” refers to concentrations of glucose that are greater than or equal to about 10 mM, about 16.7 mM, about 20 mM or more.

[0076] In some embodiments, the presence or absence of a large fold change in the GSIS response of a β -cell between exposure to low and high glucose concentrations is a marker for de-differentiation or re-differentiation of β -cells. For example, the absence of a large fold change in the GSIS response between the low and high glucose concentrations is indicative of de-differentiated or de-differentiating β -cells. In contrast, the presence of a large fold change in the GSIS response between the low and high glucose concentrations is indicative of mature β -cells (e.g., fully differentiated β -cells).

[0077] In some embodiments, the large fold change in the GSIS response between the low and high glucose concentrations is at least about 2.5 fold, at least about 3.5 fold, at least about 5 fold, at least about 10 fold, at least about 15, fold, at least about 20 fold, at least about 25, at least about 28 fold, at least about 32 fold, at least about 36 fold, at least about 39 fold, at least about 41 fold, at least about 43 fold, at least about 45 fold, up to at least about 47 fold or more. In some embodiments, the large fold change in the GSIS response between the low and high glucose concentrations is at least about 50 fold. In some embodiments, the large fold change in the GSIS response between the low and high glucose concentrations is at about 50 fold, about 55 fold, about 60 fold, about 70 fold, or up to about 75 fold, or more.

[0078] Re-differentiation may be characterized by increased expression of at least one marker of mature β cells in a re-differentiated or re-differentiating β cell compared to the level of expression of the at least one marker of mature β cells in a de-differentiated β cell from which the re-differentiated β cell was derived, e.g., by reversing de-differentiation in accordance with a method described herein. Re-differentiation includes

any increase in expression of the at least one marker of mature β cells in the re-differentiated or re-differentiating β cell relative to the level of expression of the at least one marker of mature β cells in the de-differentiated β cell from which the re-differentiated β cell was derived.

[0079] In some embodiments, the marker of mature β cells comprises FoxO1, and the level of expression of FoxO1 in the re-differentiated or re-differentiating cells is increased by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 70%, or more relative to the level of expression of FoxO1 in the de-differentiated β cell from which the re-differentiated β cell was derived. In some embodiments, the level of expression of FoxO1 in the re-differentiated or re-differentiating cells is increased by at least 75%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, relative to the level of expression of FoxO1 in the de-differentiated β cell from which the re-differentiated β cell was derived. In some embodiments, the level of expression of FoxO1 in the re-differentiated or re-differentiating cells is increased by at least 1.1 fold, at least 1.2 fold, at least 1.3 fold, at least 1.4 fold, at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2 fold, at least 2.5 fold, at least 3.0 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, at least 6.0 fold, at least 6.5 fold, at least 7.0 fold, at least 7.5 fold, at least 8.0 fold, at least 8.5 fold, at least 9.0 fold, at least 9.5 fold, or at least 10.0 fold relative to the level of expression of FoxO1 in the de-differentiated β cell from which the re-differentiated β cell was derived.

[0080] In some embodiments, the level of expression of FoxO1 in the re-differentiated cells or re-differentiating cells is increased to within at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, at least 50%, at least 45%, or at least 40% of the level of expression of FoxO1 in the β cell prior to de-differentiation of the β cell. In some embodiments, the level of expression of FoxO1 in the re-differentiated cells or re-differentiating cells is increased to within at least 35%, at least 30%, at least 25%, at least 20%, at least 15%, at least 10%, at least 9%, at least 8%, at least 7%, at least 6%, at least 5%, at least 4%, at least 3%, at least 2% or at least 1% of the level of expression of FoxO1 in the β cell prior to de-differentiation of the β cell. In some embodiments, the level of expression of FoxO1 in the re-differentiated cells is increased to the level of expression of FoxO1 in the β cell

prior to de-differentiation of the β cell, i.e., the level of FoxO1 expression in the β cell is comparable to the level of FoxO1 expression in a normal mature β cell.

[0081] In some embodiments, the marker of mature β cells comprises MafA, and the level of expression of MafA in the re-differentiated or re-differentiating cells is increased by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 70%, or more relative to the level of expression of MafA in the de-differentiated β cell from which the re-differentiated β cell was derived. In some embodiments, the level of expression of MafA in the re-differentiated or re-differentiating cells is increased by at least 75%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, relative to the level of expression of MafA in the de-differentiated β cell from which the re-differentiated β cell was derived. In some embodiments, the level of expression of MafA in the re-differentiated or re-differentiating cells is increased by at 1.1 fold, at least 1.2 fold, at least 1.3 fold, at least 1.4 fold, at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2 fold, at least 2.5 fold, at least 3.0 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, at least 6.0 fold, at least 6.5 fold, at least 7.0 fold, at least 7.5 fold, at least 8.0 fold, at least 8.5 fold, at least 9.0 fold, at least 9.5 fold, or at least 10.0 fold relative to the level of expression of MafA in the de-differentiated β cell from which the re-differentiated β cell was derived.

[0082] In some embodiments, the level of expression of MafA in the re-differentiated cells or re-differentiating cells is increased to within at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, at least 50%, at least 45%, or at least 40% of the level of expression of MafA in the β cell prior to de-differentiation of the β cell. In some embodiments, the level of expression of MafA in the re-differentiated cells or re-differentiating cells is increased to within at least 35%, at least 30%, at least 25%, at least 20%, at least 15%, at least 10%, at least 9%, at least 8%, at least 7%, at least 6%, at least 5%, at least 4%, at least 3%, at least 2% or at least 1% of the level of expression of MafA in the β cell prior to de-differentiation of the β cell. In some embodiments, the level of expression of MafA in the re-differentiated cells is increased to the level of expression of MafA in the β cell prior to de-differentiation of the β cell, i.e., the level of MafA expression in the β cell is comparable to the level of MafA expression in a normal mature β cell.

[0083] In some embodiments, the marker of mature β cells comprises NeuroD, and the level of expression of NeuroD in the re-differentiated or re-differentiating cells is increased by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 70%, or more relative to the level of expression of NeuroD in the de-differentiated β cell from which the re-differentiated β cell was derived. In some embodiments, the level of expression of NeuroD in the re-differentiated or re-differentiating cells is increased by at least 75%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, relative to the level of expression of NeuroD in the de-differentiated β cell from which the re-differentiated β cell was derived. In some embodiments, the level of expression of NeuroD in the re-differentiated or re-differentiating cells is increased by at least 1.1 fold, at least 1.2 fold, at least 1.3 fold, at least 1.4 fold, at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2 fold, at least 2.5 fold, at least 3.0 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, at least 6.0 fold, at least 6.5 fold, at least 7.0 fold, at least 7.5 fold, at least 8.0 fold, at least 8.5 fold, at least 9.0 fold, at least 9.5 fold, or at least 10.0 fold relative to the level of expression of NeuroD in the de-differentiated β cell from which the re-differentiated β cell was derived.

[0084] In some embodiments, the level of expression of NeuroD in the re-differentiated cells or re-differentiating cells is increased to within at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, at least 50%, at least 45%, or at least 40% of the level of expression of NeuroD in the β cell prior to de-differentiation of the β cell. In some embodiments, the level of expression of NeuroD in the re-differentiated cells or re-differentiating cells is increased to within at least 35%, at least 30%, at least 25%, at least 20%, at least 15%, at least 10%, at least 9%, at least 8%, at least 7%, at least 6%, at least 5%, at least 4%, at least 3%, at least 2% or at least 1% of the level of expression of NeuroD in the β cell prior to de-differentiation of the β cell. In some embodiments, the level of expression of NeuroD in the re-differentiated cells is increased to the level of expression of NeuroD in the β cell prior to de-differentiation of the β cell, i.e., the level of NeuroD expression in the β cell is comparable to the level of NeuroD expression in a normal mature β cell.

[0085] In some embodiments, the marker of mature β cells comprises Nkx6.1, and the level of expression of Nkx6.1 in the re-differentiated or re-differentiating cells is increased by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 70%, or more relative to the level of expression of Nkx6.1 in the de-differentiated β cell from which the re-differentiated β cell was derived. In some embodiments, the level of expression of Nkx6.1 in the re-differentiated or re-differentiating cells is increased by at least 75%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, relative to the level of expression of Nkx6.1 in the de-differentiated β cell from which the re-differentiated β cell was derived. In some embodiments, the level of expression of Nkx6.1 in the re-differentiated or re-differentiating cells is increased by at 1.1 fold, at least 1.2 fold, at least 1.3 fold, at least 1.4 fold, at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2 fold, at least 2.5 fold, at least 3.0 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, at least 6.0 fold, at least 6.5 fold, at least 7.0 fold, at least 7.5 fold, at least 8.0 fold, at least 8.5 fold, at least 9.0 fold, at least 9.5 fold, or at least 10.0 fold relative to the level of expression of Nkx6.1 in the de-differentiated β cell from which the re-differentiated β cell was derived.

[0086] In some embodiments, the level of expression of Nkx6.1 in the re-differentiated cells or re-differentiating cells is increased to within at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, at least 50%, at least 45%, or at least 40% of the level of expression of Nkx6.1 in the β cell prior to de-differentiation of the β cell. In some embodiments, the level of expression of Nkx6.1 in the re-differentiated cells or re-differentiating cells is increased to within at least 35%, at least 30%, at least 25%, at least 20%, at least 15%, at least 10%, at least 9%, at least 8%, at least 7%, at least 6%, at least 5%, at least 4%, at least 3%, at least 2% or at least 1% of the level of expression of Nkx6.1 in the β cell prior to de-differentiation of the β cell. In some embodiments, the level of expression of Nkx6.1 in the re-differentiated cells is increased to the level of expression of Nkx6.1 in the β cell prior to de-differentiation of the β cell, i.e., the level of Nkx6.1 expression in the β cell is comparable to the level of Nkx6.1 expression in a normal mature β cell.

[0087] In some embodiments, the marker of mature β cells comprises Pdx1, and the level of expression of Pdx1 in the re-differentiated or re-differentiating cells is increased by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 70%, or more relative to the level of expression of Pdx1 in the de-differentiated β cell from which the re-differentiated β cell was derived. In some embodiments, the level of expression of Pdx1 in the re-differentiated or re-differentiating cells is increased by at least 75%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, relative to the level of expression of Pdx1 in the de-differentiated β cell from which the re-differentiated β cell was derived. In some embodiments, the level of expression of Pdx1 in the re-differentiated or re-differentiating cells is increased by at least 1.1 fold, at least 1.2 fold, at least 1.3 fold, at least 1.4 fold, at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2 fold, at least 2.5 fold, at least 3.0 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, at least 6.0 fold, at least 6.5 fold, at least 7.0 fold, at least 7.5 fold, at least 8.0 fold, at least 8.5 fold, at least 9.0 fold, at least 9.5 fold, or at least 10.0 fold relative to the level of expression of Pdx1 in the de-differentiated β cell from which the re-differentiated β cell was derived.

[0088] In some embodiments, the level of expression of Pdx1 in the re-differentiated cells or re-differentiating cells is increased to within at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, at least 50%, at least 45%, or at least 40% of the level of expression of Pdx1 in the β cell prior to de-differentiation of the β cell. In some embodiments, the level of expression of Pdx1 in the re-differentiated cells or re-differentiating cells is increased to within at least 35%, at least 30%, at least 25%, at least 20%, at least 15%, at least 10%, at least 9%, at least 8%, at least 7%, at least 6%, at least 5%, at least 4%, at least 3%, at least 2% or at least 1% of the level of expression of Pdx1 in the β cell prior to de-differentiation of the β cell. In some embodiments, the level of expression of Pdx1 in the re-differentiated cells is increased to the level of expression of Pdx1 in the β cell prior to de-differentiation of the β cell, i.e., the level of Pdx1 expression in the β cell is comparable to the level of Pdx1 expression in a normal mature β cell.

[0089] In some embodiments, β cell re-differentiation is characterized by increased expression of at least two markers of mature β cells comprising FoxO1, MafA,

NeuroD, Nkx6.1, and/or Pdx1. In some embodiments, β cell re-differentiation is characterized by increased expression of at least three markers of mature β cells comprising FoxO1, MafA, NeuroD, Nkx6.1, and/or Pdx1. In some embodiments, β cell re-differentiation is characterized by increased expression of at least four markers of mature β cells comprising FoxO1, MafA, NeuroD, Nkx6.1, and/or Pdx1. In some embodiments, β cell re-differentiation is characterized by increased expression of at least five markers of mature β cells comprising FoxO1, MafA, NeuroD, Nkx6.1, and/or Pdx1. Those skilled in the art will appreciate that β cell re-differentiation in certain individuals (e.g., diabetics) increases the number of functionally mature β cells (e.g., increasing the percentage of functionally mature β cell mass in those individuals).

[0090] In some embodiments, re-differentiation (e.g., of a de-differentiated β cell) is characterized by the presence of an appropriate GSIS response in the β cell (i.e., re-differentiated β cell).

[0091] Aspects of the disclosure involve obtaining a β cell or population of cells comprising β cells. As used herein, β cell refers to an isolated β cell or isolated and/or purified population of β cells, as well as a β cell or population of β cells in islets or pancreata. In some embodiments the β -cell or population of β -cells are obtained from an *in vitro* source. In some embodiments the *in vitro* source is a culture of differentiating stem cells. In some embodiments the stem cells are selected from the group consisting of human embryonic stem cells (hESCs), induced pluripotent stem cells (iPSCs), blood stem cells, and combinations thereof. In some embodiments the *in vitro* source is selected from the group consisting of a cell bank, cell line, cell culture, cell population, and combinations thereof. In some embodiments the *in vitro* source is an ex-planted tissue or organ.

[0092] In some embodiments the β -cell is obtained from an *in vivo* source. In some embodiments the *in vivo* source is an individual who has received an administration of β -cells. In some embodiments the *in vivo* source is an individual suffering from a disorder associated with de-differentiated β -cells (i.e., a β -cells de-differentiation-related disorder). In some embodiments the *in vivo* source is an individual suspected of being in need of functionally mature β -cells.

[0093] Aspects of the disclosure involve obtaining a biological sample comprising β cells from a subject. A biological sample used in the methods described herein will typically comprise or be derived from cells or tissues isolated from a subject.

In some embodiments, biological sample comprises β cells in or isolated from an islet or a pancreas. In some embodiments, the biological sample comprises β cells differentiated *in vitro* according to a directed differentiation protocol.

[0094] Samples can be, e.g., surgical samples, tissue biopsy samples, fine needle aspiration biopsy samples, core needle samples. The sample may be obtained using methods known in the art. A sample can be subjected to one or more processing steps. In some embodiments the sample is frozen and/or fixed. In some embodiments the sample is sectioned and/or embedded, e.g., in paraffin. In some embodiments, β cells, e.g., pancreatic β cells are separated from at least some surrounding tissue (e.g., pancreatic tissue and/or islets of Langerhans). Cells or tissue of interest can be isolated using, e.g., tissue microdissection, e.g., laser capture microdissection. It should be appreciated that a sample can be a sample isolated from any of the subjects described herein. In some embodiments, the sample comprises β cells obtained from a subject who has diabetes. In some embodiments, the sample comprises β cells obtained from a subject who is at risk of developing diabetes. In some embodiments, the sample comprises β cells obtained from a subject who is suspected of having or developing diabetes. In some embodiments, the sample comprises β cells obtained from a subject who is non-diabetic. In some embodiments, the sample comprises β cells obtained from a subject who is mildly diabetic. In some embodiments, the sample comprises β cells obtained from a subject who is severely diabetic. In some embodiments, the sample comprises β cells obtained from a subject who is suffering from metabolic syndrome.

[0095] In some embodiments, cells of the sample are lysed. Nucleic acids or polypeptides may be isolated from the samples (e.g., β cells, islets, or pancreata). In some embodiments DNA (e.g., cDNA), optionally isolated from a sample, is amplified. A wide variety of methods are available for detection of DNA, e.g., Ucn3 cDNA reverse transcribed from Ucn3 mRNA, insulin cDNA reverse transcribed from insulin mRNA, or cDNA encoding at least one marker of mature β cells reverse transcribed from mRNA encoding at least one marker of mature β cells. In some embodiments RNA, optionally isolated from a sample, is reverse transcribed and/or amplified. A wide variety of solution phase or solid phase methods are available for detection of RNA, e.g., Ucn3 mRNA, insulin mRNA, and/or mRNA encoding at least one marker of mature β cells. Suitable methods include e.g., hybridization-based approaches (e.g., nuclease protection assays, Northern blots, microarrays, in situ hybridization), amplification-based

approaches (e.g., reverse transcription polymerase chain reaction (which can be a real-time PCR reaction), or sequencing (e.g., RNA-Seq, which uses high throughput sequencing techniques to quantify RNA transcripts (see, e.g., Wang, Z., et al. Nature Reviews Genetics 10, 57-63, 2009)). In some embodiments of interest a quantitative PCR (qPCR) assay is used. Other methods include electrochemical detection, bioluminescence-based methods, fluorescence-correlation spectroscopy, etc.

[0096] *Preventing and/or Reversing β cell De-differentiation*

[0097] Aspects of the disclosure relate to preventing the de-differentiation of a β cell and/or reversing the de-differentiation of a β cell. As will be appreciated by those skilled in the art, preventing and/or reversing the de-differentiation of a β cell can be useful, e.g., for protecting β cells from β cell stress and the resulting β cell loss during onset of diabetes, e.g., for the treatment and/or prevention of disorders involving β cell de-differentiation, e.g., diabetes, pre-diabetes, metabolic syndrome, obesity, etc.

[0098] The expressions “activate”, “inhibit”, “modulate”, “increase”, “decrease”, “prevent”, “reverse” and “retard” in any grammatical form (e.g., gerund) or the like, e.g., which denote quantitative differences between two states, refer to at least statistically significant differences between the two states. For example, “reversing β cell de-differentiation” means that the rate of de-differentiation of β cells or the fraction of de-differentiated β cells remaining after treatment will be at least statistically significantly different from the de-differentiated β cells in which β cell de-differentiation is not reversed by use of a method, composition, or agent of the disclosure. As another example, “preventing β cell de-differentiation” means that the rate of de-differentiation of β cells or the fraction of de-differentiated β cells remaining after treatment will be at least statistically significantly different from the de-differentiating β cells in which β cell de-differentiation is not prevented by use of a method, composition, or agent of the disclosure. Such terms are applied herein to, for example, rates of cell replication, rates of de-differentiation of β cells, rates of re-differentiation of β cell, levels of expression, levels of transcriptional or translational activity, and levels of enzymatic or protein activity, etc.

[0099] In some aspects, the disclosure provides a method of preventing de-differentiation of a β cell, comprising contacting a β cell with an agent that inhibits transforming growth factor- β (TGF β) superfamily signaling.

[0100] As used herein, the phrase “preventing de-differentiation” refers to retarding the onset of β cell de-differentiation or retarding the rate of β cell de-differentiation in β cells (e.g., in a culture, organ, tissue, or a subject). In some embodiments, preventing β cell de-differentiation comprises retarding the onset of β cell de-differentiation in at least 1%, at least 5%, at least 10%, at least 15%, at least 20% or at least 25% of the β cells in a population (e.g., culture, organ, tissue, or a subject) compared to the onset of β cell de-differentiation in the β cells in the population (e.g., culture, organ, tissue, or a subject) in the absence of treatment. In some embodiments, preventing β cell de-differentiation comprises retarding the onset of β cell de-differentiation in at least 30%, at least 35%, at least 40%, at least 45%, at least 50% or at least 55% of the β cells in a population (e.g., culture, organ, tissue, or a subject) compared to the onset of β cell de-differentiation in the β cells in the population (e.g., culture, organ, tissue, or a subject) in the absence of treatment. In some embodiments, preventing β cell de-differentiation comprises retarding the onset of β cell de-differentiation in at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% of the β cells in a population (e.g., culture, organ, tissue, or a subject) compared to the onset of β cell de-differentiation in the β cells in a population (e.g., culture, organ, tissue, or a subject) in the absence of treatment. In some embodiments, preventing β cell de-differentiation comprises retarding the onset of β cell de-differentiation in at least 96%, at least 97%, at least 98%, or at least 99% of the β cells in a population (e.g., culture, organ, tissue, or a subject) compared to the onset of β cell de-differentiation in the β cells in the population (e.g., culture, organ, tissue, or a subject) in the absence of treatment. In some embodiments, preventing β cell de-differentiation comprises retarding the onset of β cell de-differentiation in all of the β cells in a population (e.g., culture, organ, tissue, or a subject) compared to the onset of β cell de-differentiation in the β cells in the population (e.g., culture, organ, tissue, or a subject) in the absence of treatment.

[0101] In some embodiments, preventing β cell de-differentiation comprises retarding the rate, frequency, magnitude, or extent of β cell de-differentiation by at least 1%, at least 5%, at least 10%, at least 15%, at least 20% or at least 25% in a cell, tissue, organ, or subject compared to the rate, frequency, magnitude, or extent of β cell de-differentiation in a cell, tissue, organ, or subject in the absence of treatment. In some embodiments, preventing β cell de-differentiation comprises retarding the rate, frequency, magnitude, or extent of β cell de-differentiation by at least 30%, at least 35%, at least

40%, at least 45%, at least 50% or at least 55% in a cell, tissue, organ, or subject compared to the rate, frequency, magnitude, or extent of β cell de-differentiation in the cell, tissue, organ, or subject in the absence of treatment. In some embodiments, preventing β cell de-differentiation comprises retarding the rate, frequency, magnitude, or extent of β cell de-differentiation by at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% in a cell, tissue, organ, or subject compared to the rate, frequency, magnitude, or extent of β cell de-differentiation in the cell, tissue, organ, or subject in the absence of treatment. In some embodiments, preventing β cell de-differentiation comprises retarding the rate, frequency, magnitude, or extent of β cell de-differentiation by at least 96%, at least 97%, at least 98%, or at least 99% in a cell, tissue, organ, or subject compared to the rate, frequency, magnitude, or extent of β cell de-differentiation in the cell, tissue, organ, or subject in the absence of treatment. In some embodiments, preventing β cell de-differentiation comprises retarding the rate, frequency, magnitude, or extent of β cell de-differentiation by at least 100% in a cell, tissue, organ, or subject compared to the rate, frequency, magnitude, or extent of β cell de-differentiation in the cell, tissue, organ, or subject in the absence of treatment.

[0102] In some contexts, preventing de-differentiation of the β cell causes the β cell to: (i) increase or maintain expression levels of Ucn3; (i) increase or maintain expression levels of at least one marker of mature β cells comprising FoxO1, MafA, NeuroD, Nkx6.1, and Pdx1; and/or (iii) maintain an appropriate GSIS response in the β cell.

[0103] In some aspects, the disclosure provides a method of reversing de-differentiation of a β cell, comprising contacting a de-differentiated β cell with an agent that inhibits transforming growth factor- β (TGF β) superfamily signaling.

[0104] As used herein, the phrase “reversing de-differentiation” refers to retarding the rate of β cell de-differentiation and/or increasing the rate of β cell re-differentiation. In some embodiments, reversing β cell de-differentiation comprises retarding rate, frequency, magnitude, or extent of β cell de-differentiation by at least 1%, at least 5%, at least 10%, at least 15%, at least 20% or at least 25% in a cell, tissue, organ, or subject compared to the rate, frequency, magnitude, or extent of β cell de-differentiation in the cell, tissue, organ, or subject in the absence of treatment. In some embodiments, reversing β cell de-differentiation comprises retarding the rate, frequency, magnitude, or extent of β cell de-differentiation by at least 30%, at least 35%, at least

40%, at least 45%, at least 50% or at least 55% in a cell, tissue, organ, or subject compared to the rate, frequency, magnitude, or extent of β cell de-differentiation in the cell, tissue, organ, or subject in the absence of treatment. In some embodiments, reversing the β cell de-differentiation comprises retarding the rate, frequency, magnitude, or extent of β cell de-differentiation by at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% in a cell, tissue, organ, or subject compared to the rate, frequency, magnitude, or extent of β cell de-differentiation in the cell, tissue, organ, or subject in the absence of treatment. In some embodiments, reversing β cell de-differentiation comprises retarding the rate, frequency, magnitude, or extent of β cell de-differentiation by at least 96%, at least 97%, at least 98%, or at least 99% in a cell, tissue, organ, or subject compared to the rate, frequency, magnitude, or extent of β cell de-differentiation in the cell, tissue, organ, or subject in the absence of treatment. In some embodiments, reversing β cell de-differentiation comprises retarding the rate, frequency, magnitude, or extent of β cell de-differentiation in a cell, tissue, organ, or subject by 100% compared to the rate, frequency, magnitude, or extent of β cell de-differentiation in the cell, tissue, organ, or subject in the absence of treatment. In some embodiments, reversing β cell de-differentiation comprises retarding the rate, frequency, magnitude, or extent of β cell de-differentiation by at least 1.1 fold, at least 1.2 fold, at least 1.3 fold, at least 1.4 fold, at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2 fold, at least 2.5 fold, at least 3.0 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, at least 6.0 fold, at least 6.5 fold, at least 7.0 fold, at least 7.5 fold, at least 8.0 fold, at least 8.5 fold, at least 9.0 fold, at least 9.5 fold, or at least 10.0 fold in a cell, tissue, organ, or subject compared to the rate, frequency, magnitude, or extent of β cell de-differentiation in the cell, tissue, organ, or subject in the absence of treatment.

[0105] In some embodiments, reversing β cell de-differentiation comprises increasing the rate, frequency, magnitude, or extent of β cell re-differentiation by at least 1%, at least 5%, at least 10%, at least 15%, at least 20% or at least 25% in a cell, tissue, organ, or subject compared to the rate, frequency, magnitude, or extent of β cell re-differentiation in the cell, tissue, organ, or subject in the absence of treatment. In some embodiments, reversing β cell de-differentiation comprises increasing the rate, frequency, magnitude, or extent of β cell re-differentiation by at least 30%, at least 35%, at least 40%, at least 45%, at least 50% or at least 55% in a cell, tissue, organ, or subject

compared to the rate, frequency, magnitude, or extent of β cell re-differentiation in the cell, tissue, organ, or subject in the absence of treatment. In some embodiments, reversing β cell de-differentiation comprises increasing the rate, frequency, magnitude, or extent of β cell re-differentiation by at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% in a cell, tissue, organ, or subject compared to the rate, frequency, magnitude, or extent of β cell re-differentiation in the cell, tissue, organ, or subject in the absence of treatment. In some embodiments, reversing β cell de-differentiation comprises increasing the rate, frequency, magnitude, or extent of β cell re-differentiation by at least 96%, at least 97%, at least 98%, or at least 99% in a cell, tissue, organ, or subject compared to the rate, frequency, magnitude, or extent of β cell re-differentiation in the cell, tissue, organ, or subject in the absence of treatment. In some embodiments, reversing β cell de-differentiation comprises increasing the rate, frequency, magnitude, or extent of β cell re-differentiation in a cell, tissue, organ, or subject by 100% compared to the rate, frequency, magnitude, or extent of β cell re-differentiation in the cell, tissue, organ, or subject in the absence of treatment. In some embodiments, reversing β cell de-differentiation comprises increasing the rate, frequency, magnitude, or extent of β cell re-differentiation by at least 1.1 fold, at least 1.2 fold, at least 1.3 fold, at least 1.4 fold, at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2 fold, at least 2.5 fold, at least 3.0 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, at least 6.0 fold, at least 6.5 fold, at least 7.0 fold, at least 7.5 fold, at least 8.0 fold, at least 8.5 fold, at least 9.0 fold, at least 9.5 fold, or at least 10.0 fold in a cell, tissue, organ, or subject compared to the rate, frequency, magnitude, or extent of β cell re-differentiation in the cell, tissue, organ, or subject in the absence of treatment.

[0106] In some contexts, reversing de-differentiation of the β cell causes the β cell to: (i) increase expression levels of Ucn3; (i) increase expression levels of at least one marker of mature β cells comprising MafA, Nkx6.1, Pdx1, NeuroD, and FoxO1; and/or (iii) exhibit an appropriate GSIS response in the β cell.

[0107] In some embodiments, the de-differentiated β cell comprises a de-differentiated β cell in an early stage of de-differentiation. In some embodiments, the de-differentiated β cell in the early stage of de-differentiation exhibits at least one of (i) decreased Ucn3 expression and increased or unchanged insulin expression; (ii) decreased

expression of at least one marker of mature β cells comprising FoxO1, MafA, NeuroD, Nkx6.1, and Pdx1; and/or (iii) the lack of an appropriate GSIS response.

[0108] In some embodiments, the de-differentiated β cell comprises a de-differentiated β cell in a late stage of de-differentiation. In some embodiments, the de-differentiated β cell in a late stage of de-differentiation exhibits at least one of (i) decreased Ucn3 expression and decreased insulin expression; (ii) decreased expression of at least one marker of mature β cells comprising FoxO1, MafA, NeuroD, Nkx6.1, and Pdx1; and/or (iii) lack of an appropriate GSIS response.

[0109] The work described herein surprisingly and unexpectedly demonstrates that inhibition of artemin signaling through receptor tyrosine kinase RET and/or GFRalpha3 (GFR α 3) receptor and inhibition of Alk5 signaling (e.g., signaling through TGF β receptor type I) not only prevents β cell de-differentiation, for example, due to β cell stress, but also reverses de-differentiation of β cells even after extended exposure to β cell stress and/or diabetic conditions. Moreover, Alk5 inhibition was unexpectedly shown to exhibit such effects using only picomolar concentrations of certain inhibitors (e.g., Alk5 Inhibitor II).

[0110] Accordingly, in some embodiments, TGF β superfamily signaling comprises artemin signaling through receptor tyrosine kinase RET or a GFRalpha3 receptor (GFR α 3).

[0111] Aspects of the disclosure involve the use of agents. The disclosure contemplates the use of any agent that is suitable for a specified purpose (e.g. agents that prevent β cell de-differentiation (de-differentiation preventing agents), agents that reverse β cell de-differentiation (de-differentiation reversing agents or re-differentiating agents), RET inhibitors, GFR α 3 inhibitors, Alk5 inhibitors, anti-diabetic agents, blood glucose lowering agents, agents beneficial for β cells, test agents for preventing and/or reversing β cell de-differentiation etc. Exemplary agents of use herein include, without limitation, small organic or inorganic molecules; saccharides; oligosaccharides; polysaccharides; a biological macromolecule selected from the group consisting of peptides, proteins, peptide analogs and derivatives; peptidomimetics; nucleic acids selected from the group consisting of siRNAs, shRNAs, antisense RNAs, ribozymes, and aptamers; an extract made from biological materials selected from the group consisting of bacteria, plants, fungi, animal cells, and animal tissues; naturally occurring or synthetic compositions; and

any combination thereof. The disclosure contemplates compositions and kits comprising any agent or combination of agents described herein.

[0112] In some embodiments, the at least one agent comprises an inhibitor of RET or an inhibitor of GFR α 3.

[0113] RET (Rearranged ruing Transfection) is a transmembrane tyrosine kinase expressed in the nervous system and neural crest-derived cells, which serves as a co-receptor of GDNF family neurotrophic factor in complex with GRF α family member proteins. RET protein possesses an extracellular portion with four cadherin-like domains, and a cysteine-rich region critical for intermolecular interactions, a hydrophobic transmembrane domain, an intracellular component including a juxtamembrane domain having regulatory function, and a catalytic domain which phosphorylates tyrosine residues of its substrates.

[0114] Exemplary inhibitors of RET include, without limitation, the compounds of Formulas (1A, 1B, and 1C) as disclosed in U.S. Patent Application No. 2011/0201598, such as cyclobenzaprine (SW-01), TG101209, motesanib, diphosphate, sorafenib, VEGFR2 kinase inhibitor III, PP242, RPI-1, PF 477736, VEGFR tyrosine kinase inhibitor V, vatalanib dihydrochloride, and 4,4'-bis(4-aminophenoxy)biphenyl.

[0115] GFR alpha 3 (GFR α 3) is a GPI-anchored receptor that interacts preferentially with GDNF family ligand Artemin. GFR alpha 3 initiates signaling in association with the receptor tyrosine kinase RET and via RET-independent pathways. An exemplary inhibitor of GFR α 3 comprises siRNA targeting GFR α 3 (see, e.g., Jankowski *et al.*, "Enhanced Artemin/GFRalpha3 Levels Regulate Mechanically Insensitive, Heat-Sensitive C-Fiber Recruitment after Axotomy and Regeneration," *J. Neuroscience* 2010; 30(48):16272-16283);

[0116] In some embodiments, the at least one agent comprises PHA-739358 or an analog or derivative thereof.

[0117] In some embodiments, the at least one agent comprises VEGFR inhibitor V or an analog or derivative thereof.

[0118] In some embodiments, TGF β superfamily signaling comprises TGF β signaling through a receptor serine/threonine kinase, i.e., a TGF- β signaling pathway inhibitor.

[0119] In some embodiments, the TGF- β signaling pathway inhibitor comprises ALK5 inhibitor II (CAS 446859-33-2, an ATP-competitive inhibitor of TGF-B RI kinase,

also known as RepSox, IUPAC Name: 2-[5-(6-methylpyridin-2-yl)-1H-pyrazol-4-yl]-1,5-naphthyridine. In some embodiments, the TGF- β signaling pathway inhibitor is an analog or derivative of ALK5 inhibitor II.

[0120] In some embodiments, the analog or derivative of ALK5 inhibitor II is a compound of Formula I as described in U.S. Patent Publication No. 2012/0021519, incorporated by reference herein in its entirety.

[0121] In some embodiments, the TGF- β signaling pathway inhibitor is a TGF- β receptor inhibitor described in U.S. Patent Publication No. 2010/0267731. In some embodiments, the TGF- β signaling pathway inhibitor comprises an ALK5 inhibitor described in U.S. Patent Publication Nos. 2009/0186076 and 2007/0142376.

[0122] In some embodiments, the TGF- β signaling pathway inhibitor is A 83-01. In some embodiments, the TGF- β signaling pathway inhibitor is not A 83-01. In some embodiments, the compositions and methods described herein exclude A 83-01.

[0123] In some embodiments, the TGF- β signaling pathway inhibitor is SB 431542. In some embodiments, the TGF- β signaling pathway inhibitor is not SB 431542. In some embodiments, the compositions and methods described herein exclude SB 431542.

[0124] In some embodiments, the TGF- β signaling pathway inhibitor is D 4476. In some embodiments, the TGF- β signaling pathway inhibitor is not D 4476. In some embodiments, the compositions and methods described herein exclude D 4476.

[0125] In some embodiments, the TGF- β signaling pathway inhibitor is GW 788388. In some embodiments, the TGF- β signaling pathway inhibitor is not GW 788388. In some embodiments, the compositions and methods described herein exclude GW 788388.

[0126] In some embodiments, the TGF- β signaling pathway inhibitor is LY 364947. In some embodiments, the TGF- β signaling pathway inhibitor is not LY 364947. In some embodiments, the compositions and methods described herein exclude LY 364947.

[0127] In some embodiments, the TGF- β signaling pathway inhibitor is LY 580276. In some embodiments, the TGF- β signaling pathway inhibitor is not LY 580276. In some embodiments, the compositions and methods described herein exclude LY 580276.

[0128] In some embodiments, the TGF- β signaling pathway inhibitor is SB 525334. In some embodiments, the TGF- β signaling pathway inhibitor is not SB 525334. In some embodiments, the compositions and methods described herein exclude SB 525334.

[0129] In some embodiments, the TGF- β signaling pathway inhibitor is SB 505124. In some embodiments, the TGF- β signaling pathway inhibitor is not SB 505124. In some embodiments, the compositions and methods described herein exclude SB 505124.

[0130] In some embodiments, the TGF- β signaling pathway inhibitor is SD 208. In some embodiments, the TGF- β signaling pathway inhibitor is not SD 208. In some embodiments, the compositions and methods described herein exclude SD 208.

[0131] In some embodiments, the TGF- β signaling pathway inhibitor is GW 6604. In some embodiments, the TGF- β signaling pathway inhibitor is not GW 6604. In some embodiments, the compositions and methods described herein exclude GW 6604.

[0132] In some embodiments, the TGF- β signaling pathway inhibitor is GW 788388. In some embodiments, the TGF- β signaling pathway inhibitor is not GW 788388. In some embodiments, the compositions and methods described herein exclude GW 788388.

[0133] From the collection of compounds described above, the following can be obtained from various sources: LY-364947, SB-525334, SD-208, and SB-505124 available from Sigma, P.O. Box 14508, St. Louis, Mo., 63178-9916; 616452 and 616453 available from Calbiochem (EMD Chemicals, Inc.), 480 S. Democrat Road, Gibbstown, N.J., 08027; GW788388 and GW6604 available from GlaxoSmithKline, 980 Great West Road, Brentford, Middlesex, TW8 9GS, United Kingdom; LY580276 available from Lilly Research, Indianapolis, Ind. 46285; and SM16 available from Biogen Idec, P.O. Box 14627, 5000 Davis Drive, Research Triangle Park, N.C., 27709-4627.

[0134] In some embodiments, the at least one agent comprises Alk5 inhibitor II or an analog or derivative thereof.

[0135] In some embodiments, the at least one agent comprises ALK5 inhibitor I or an analog or derivative thereof.

[0136] In some embodiments, the at least one agent comprises a SMAD3 inhibitor. Exemplary Smad3 inhibitors include, without limitation, antisense inhibitors of SMAD3 expression, such as those described in U.S. Patent No. 6,013,788, modulators of

SMAD3 expression described in U.S. Publication No. 2011/0213011, and the Smad3 inhibitors described in Published PCT International Application Nos. WO/2001/089556 and WO/2004/064770.

[0137] As used herein, the term “contacting” (e.g., contacting a β cell, de-differentiated or de-differentiating β cell, or a re-differentiating β cell with a de-differentiation preventing agent or re-differentiating agent) is intended to include incubating the agent and the cell together *in vitro* (e.g., adding the de-differentiation preventing agent and/or re-differentiating agent to cells in culture). In some embodiments, the term “contacting” is not intended to include the *in vivo* exposure of cells to the agents as disclosed herein that may occur naturally in a subject (i.e., exposure that may occur as a result of a natural physiological process). The step of contacting a cell (e.g., a β cell, de-differentiated or de-differentiating β cell, or a re-differentiating β cell) with an agent (e.g., de-differentiation preventing agent or re-differentiating agent) as in the embodiments related to preventing and/or reversing β cell de-differentiation can be conducted in any suitable manner. For example, the cells may be treated in adherent culture, or in suspension culture. In some embodiments, the cells are treated in conditions that promote cell clustering. It is understood that the cells contacted with an agent (e.g., de-differentiation preventing agent or re-differentiating agent) can also be simultaneously or subsequently contacted with another agent, such as a growth factor or other anti-diabetic agent or environments to stabilize the cells, or to re-differentiate the cells further.

[0138] In some embodiments, the methods of preventing and/or reversing β cell de-differentiation comprise detecting de-differentiation of the β cell or de-differentiated β cell. In some embodiments, de-differentiation of the β cell or de-differentiated β cell is detected (i) prior to contacting, (ii) contemporaneously with contacting, or (iii) after contacting the β cell or de-differentiated β cell with the at least one agent.

[0139] In some embodiments, contacting occurs *in vitro* or *ex vivo*.

[0140] In some embodiments, contacting occurs *in vivo*. In some embodiments, the *in vivo* contact occurs in a subject, e.g., a subject described herein. Exemplary subjects include humans and animals.

[0141] In some embodiments, the methods of preventing and/or reversing β cell de-differentiation comprise administering to the subject a conventional anti-diabetes therapy. In some embodiments, methods of preventing and/or reversing β cell de-differentiation comprise administering to the subject an anti-diabetic agent. In some

embodiments, the methods of preventing and/or reversing β cell de-differentiation comprise administering to the subject a blood glucose lowering agent. In some embodiments, the methods of preventing and/or reversing β cell de-differentiation comprise administering to the subject an agent that is beneficial to β cells.

[0142] Preventing and/or Treating β Cell De-differentiation-related Disorders

[0143] Aspects of the disclosure relate to preventing and/or treating β cell de-differentiation-related disorders. As used herein, " β cell de-differentiation-related disorder" refers to any disease, condition, or disorder caused by, associated with, correlated to, or otherwise involving β cell de-differentiation. β cell de-differentiation-related disorders include any such disorder characterized by at least a statistically significant amount of β cell de-differentiation, e.g., loss of mature β cells (e.g., as measured by decreased β cell number, function, or mass, etc). Generally, preventing and/or treating β cell de-differentiation-related disorders involves contacting cells with or administering to subjects an effective amount of a β cell de-differentiation preventing agent or β cell re-differentiating agent described herein.

[0144] Aspects of the disclosure involve contacting cells with or administering to subjects an effective amount an agent. As used herein, "an effective amount" refers to an amount of the agents or the compounds mentioned, which result in successful treatment, e.g., preventing β cell de-differentiation, reversing β cell de-differentiation, increasing functional β cell mass, increasing the number of functional β cells, etc., i.e., to effectively inhibit, treat the syndromes of a β cell de-differentiation-related disorder or retard or reverse the rate of β cell de-differentiation or prevent the onset of β cell de-differentiation, for example due to β cell stress. In some embodiments, an "effective amount" is a " β cell de-differentiation-preventing amount". As used herein, " β cell de-differentiation-preventing amount" means a sufficient amount of an agent to provide the desired β cell de-differentiation-preventing effect. For example, in some embodiments, a " β cell de-differentiation-preventing amount" means that dose of agent effective to retard the onset of β cell de-differentiation or retard the rate of β cell de-differentiation or to render the β cells less susceptible to β cell de-differentiation or more susceptible to an antidiabetic therapy, e.g., at least one anti-diabetic agent.

[0145] In some embodiments, an "effective amount" is a " β cell de-differentiation-reversing amount." As used herein, " β cell de-differentiation-reversing amount" means a sufficient amount of an agent to provide the desired β cell de-

differentiation-reversing effect. For example, in some embodiments, “a β cell de-differentiation-preventing amount” means that dose of agent effective to retard or reverse the rate of β cell de-differentiation or to render the β cells more susceptible to β cell re-differentiation or more susceptible to an antidiabetic therapy, e.g., at least one anti-diabetic agent.

[0146] In some aspects, the disclosure provides a method of preventing a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of a β cell de-differentiation preventing agent. In some aspects, the disclosure provides a method of preventing a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of an inhibitor of artemin signaling. In some aspects, the disclosure provides a method of preventing a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of a TGF β pathway inhibitor. In some aspects, the disclosure provides a method of preventing a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of a composition comprising an inhibitor of artemin signaling and a TGF β pathway inhibitor. In some aspects, the disclosure provides a method of preventing a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of a composition comprising an inhibitor of artemin signaling, a TGF β pathway inhibitor, and an anti-diabetic agent (e.g., blood glucose lowering agent). In some aspects, the disclosure provides a method of preventing a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of an Alk5 inhibitor. In some aspects, the disclosure provides a method of preventing a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of Alk5 inhibitor II. In some aspects, the disclosure provides a method of preventing a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of PHA-739358. In some aspects, the disclosure provides a method of preventing a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of VEGFR inhibitor V. In some aspects, the disclosure provides a method of preventing a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of a composition

comprising Alk5 inhibitor II and VEGFR inhibitor V. In some aspects, the disclosure provides a method of preventing a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of a composition comprising Alk5 inhibitor II and PHA-739358. In some aspects, the disclosure provides a method of preventing a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of a composition comprising Alk5 inhibitor II and Alk5 inhibitor I. In some aspects, the disclosure provides a method of preventing a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of a composition comprising Alk5 inhibitor II and Alk5 inhibitor I.

[0147] In some embodiments, preventing the β cell de-differentiation-related disorder comprises retarding the onset of β cell de-differentiation in at least 1%, at least 5%, at least 10%, at least 15%, at least 20% or at least 25% of a subject's β cells compared to the onset of β cell de-differentiation in the absence of treatment. In some embodiments, preventing the β cell de-differentiation-related disorder comprises retarding the onset of β cell de-differentiation in at least 30%, at least 35%, at least 40%, at least 45%, at least 50% or at least 55% of a subject's β cells compared to the onset of β cell de-differentiation in the absence of treatment. In some embodiments, preventing the β cell de-differentiation-related disorder comprises retarding the onset of β cell de-differentiation in at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% of a subject's β cells compared to the onset of β cell de-differentiation in the absence of treatment. In some embodiments, preventing the β cell de-differentiation-related disorder comprises retarding the onset of β cell de-differentiation in at least 96%, at least 97%, at least 98%, or at least 99% of a subject's β cells compared to the onset of β cell de-differentiation in the absence of treatment. In some embodiments, preventing the β cell de-differentiation-related disorder comprises retarding the onset of β cell de-differentiation all of a subject's β cells.

[0148] In some embodiments, preventing the β cell de-differentiation-related disorder comprises retarding the rate, frequency, magnitude, or extent of β cell de-differentiation by at least 1%, at least 5%, at least 10%, at least 15%, at least 20% or at least 25% in a subject compared to the rate, frequency, magnitude, or extent of β cell de-differentiation in the subject in the absence of treatment. In some embodiments, preventing the β cell de-differentiation-related disorder comprises retarding the rate,

frequency, magnitude, or extent of β cell de-differentiation by at least 30%, at least 35%, at least 40%, at least 45%, at least 50% or at least 55% in a subject compared to the rate, frequency, magnitude, or extent of β cell de-differentiation in the subject in the absence of treatment. In some embodiments, preventing the β cell de-differentiation-related disorder comprises retarding the rate, frequency, magnitude, or extent of β cell de-differentiation by at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% in a subject compared to the rate or frequency of β cell de-differentiation in the subject in the absence of treatment. In some embodiments, preventing the β cell de-differentiation-related disorder comprises retarding the rate, frequency, magnitude, or extent of β cell de-differentiation by at least 96%, at least 97%, at least 98%, or at least 99% in a subject compared to the rate, frequency, magnitude, or extent of β cell de-differentiation in the subject in the absence of treatment. In some embodiments, preventing the β cell de-differentiation-related disorder comprises retarding the rate, frequency, magnitude, or extent of β cell de-differentiation by at least 100% in a subject compared to the rate, frequency, magnitude, or extent of β cell de-differentiation in the subject in the absence of treatment, i.e., β cell de-differentiation is completely prevented in the subject.

[0149] In some embodiments, preventing the β cell de-differentiation-related disorder comprises ameliorating insulin resistance in the subject. In some embodiments, preventing the β cell de-differentiation-related disorder comprises preventing onset of diabetes. In some embodiments, preventing the β cell de-differentiation-related disorder comprises preventing progression of diabetes. In some embodiments, preventing the β cell de-differentiation-related disorder comprises preventing β cell loss in diabetic patients.

[0150] In some aspects, the disclosure provides a method of treating a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of a β cell de-differentiation reversing agent. In some aspects, the disclosure provides a method of treating a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of an inhibitor of artemin signaling. In some aspects, the disclosure provides a method of treating a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of a TGF β pathway inhibitor. In some aspects, the disclosure provides a method of treating a β cell de-differentiation-related disorder, the method comprising administering to a subject in need

thereof an effective amount of a composition comprising an inhibitor of artemin signaling and a TGF β pathway inhibitor. In some aspects, the disclosure provides a method of treating a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of a composition comprising an inhibitor of artemin signaling, a TGF β pathway inhibitor, and a anti-diabetic agent (e.g., blood glucose lowering agent). In some aspects, the disclosure provides a method of treating a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of an Alk5 inhibitor. In some aspects, the disclosure provides a method of treating a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of Alk5 inhibitor II. In some aspects, the disclosure provides a method of treating a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of PHA-739358. In some aspects, the disclosure provides a method of treating a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of VEGFR inhibitor V. In some aspects, the disclosure provides a method of treating a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of a composition comprising Alk5 inhibitor II and VEGFR inhibitor V. In some aspects, the disclosure provides a method of treating a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of a composition comprising Alk5 inhibitor II and PHA-739358. In some aspects, the disclosure provides a method of treating a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of a composition comprising Alk5 inhibitor II and Alk5 inhibitor I. In some aspects, the disclosure provides a method of treating a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of a composition comprising Alk5 inhibitor II and Alk5 inhibitor I.

[0151] In some embodiments, treating the β cell de-differentiation-related disorder comprises retarding rate, frequency, magnitude, or extent of β cell de-differentiation by at least 1%, at least 5%, at least 10%, at least 15%, at least 20% or at least 25% in a subject compared to the rate, frequency, magnitude, or extent of β cell de-differentiation in the subject in the absence of treatment. In some embodiments, treating the β cell de-differentiation-related disorder comprises retarding the rate, frequency,

magnitude, or extent of β cell de-differentiation by at least 30%, at least 35%, at least 40%, at least 45%, at least 50% or at least 55% in a subject compared to the rate, frequency, magnitude, or extent of β cell de-differentiation in the subject in the absence of treatment. In some embodiments, treating the β cell de-differentiation-related disorder comprises retarding the rate, frequency, magnitude, or extent of β cell de-differentiation by at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% in a subject compared to the rate, frequency, magnitude, or extent of β cell de-differentiation in the subject in the absence of treatment. In some embodiments, treating the β cell de-differentiation-related disorder comprises retarding the rate, frequency, magnitude, or extent of β cell de-differentiation by at least 96%, at least 97%, at least 98%, or at least 99% in a subject compared to the rate, frequency, magnitude, or extent of β cell de-differentiation in the subject in the absence of treatment. In some embodiments, treating the β cell de-differentiation-related disorder comprises retarding the rate, frequency, magnitude, or extent of β cell de-differentiation in a subject by 100% compared to the rate, frequency, magnitude, or extent of β cell de-differentiation in a subject in the absence of treatment. In some embodiments, treating the β cell de-differentiation-related disorder comprises retarding the rate, frequency, magnitude, or extent of β cell de-differentiation by at least 1.1 fold, at least 1.2 fold, at least 1.3 fold, at least 1.4 fold, at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2 fold, at least 2.5 fold, at least 3.0 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, at least 6.0 fold, at least 6.5 fold, at least 7.0 fold, at least 7.5 fold, at least 8.0 fold, at least 8.5 fold, at least 9.0 fold, at least 9.5 fold, or at least 10.0 fold in a subject compared to the rate, frequency, magnitude, or extent of β cell de-differentiation in the subject in the absence of treatment.

[0152] In some embodiments, treating the β cell de-differentiation-related disorder comprises increasing the rate, frequency, magnitude, or extent of β cell re-differentiation by at least 1%, at least 5%, at least 10%, at least 15%, at least 20% or at least 25% in a subject compared to the rate, frequency, magnitude, or extent of β cell re-differentiation in the subject in the absence of treatment. In some embodiments, treating the β cell de-differentiation-related disorder comprises increasing the rate, frequency, magnitude, or extent of β cell re-differentiation by at least 30%, at least 35%, at least 40%, at least 45%, at least 50% or at least 55% in a subject compared to the rate, frequency, magnitude, or extent of β cell re-differentiation in the subject in the absence of

treatment. In some embodiments, treating the β cell de-differentiation-related disorder comprises increasing the rate, frequency, magnitude, or extent of β cell re-differentiation by at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% in a subject compared to the rate, frequency, magnitude, or extent of β cell re-differentiation in the subject in the absence of treatment. In some embodiments, treating the β cell de-differentiation-related disorder comprises increasing the rate, frequency, magnitude, or extent of β cell re-differentiation by at least 96%, at least 97%, at least 98%, or at least 99% in a subject compared to the rate, frequency, magnitude, or extent of β cell re-differentiation in the subject in the absence of treatment. In some embodiments, treating the β cell de-differentiation-related disorder comprises increasing the rate, frequency, magnitude, or extent of β cell re-differentiation in a subject by 100% compared to the rate, frequency, magnitude, or extent of β cell re-differentiation in a subject in the absence of treatment. In some embodiments, treating the β cell de-differentiation-related disorder comprises increasing the rate, frequency, magnitude, or extent of β cell re-differentiation by at least 1.1 fold, at least 1.2 fold, at least 1.3 fold, at least 1.4 fold, at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2 fold, at least 2.5 fold, at least 3.0 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, at least 6.0 fold, at least 6.5 fold, at least 7.0 fold, at least 7.5 fold, at least 8.0 fold, at least 8.5 fold, at least 9.0 fold, at least 9.5 fold, or at least 10.0 fold in a subject compared to the rate, frequency, magnitude, or extent of β cell re-differentiation in the subject in the absence of treatment.

[0153] In some embodiments, treating the β cell de-differentiation-related disorder comprises ameliorating insulin resistance in the subject. In some embodiments, treating the β cell de-differentiation-related disorder comprises preventing onset of diabetes. In some embodiments, preventing the β cell de-differentiation-related disorder comprises preventing progression of diabetes. In some embodiments, treating the β cell de-differentiation-related disorder comprises preventing β cell loss in diabetic patients. In some embodiments, treating the β cell de-differentiation-related disorder comprises treating diabetes (e.g., pre-diabetes, type 1 diabetes, type 1.5 diabetes, type 2 diabetes).

[0154] The disclosure contemplates the use of any β cell de-differentiation preventing agent and/or the β cell de-differentiation reversing agent individually, or in combination with each other or a conventional anti-diabetic therapy (e.g., formulated as a pharmaceutical composition), as long as the β cell de-differentiation preventing agent and

the β cell de-differentiation reversing agent exhibit their intended effect. In this regard, the disclosure provides guidance for the skilled person to identify suitable such β cell de-differentiation preventing agents and β cell de-differentiation reversing agents, and the methods described herein contemplate the use of those agents identified.

[0155] In some embodiments, the β cell de-differentiation preventing agent and/or the β cell de-differentiation reversing agent comprise an inhibitor of TGF β superfamily signaling. In some embodiments, the β cell de-differentiation preventing agent and the β cell de-differentiation reversing agent comprise a TGF β pathway inhibitor.

[0156] In some embodiments, the β cell de-differentiation preventing agent and/or the β cell de-differentiation reversing agent comprise an inhibitor of artemin signaling. In some embodiments, the β cell de-differentiation preventing agent and/or the β cell de-differentiation reversing agent comprises an inhibitor of receptor tyrosine kinase RET or an inhibitor of receptor GRF α 3. In some embodiments, the β cell de-differentiation preventing agent and/or the β cell de-differentiation reversing agent comprises PHA-739358 or an analog or derivative thereof. In some embodiments, the β cell de-differentiation preventing agent and/or the β cell de-differentiation reversing agent comprises VEGFR inhibitor V or an analog or derivative thereof.

[0157] In some embodiments, the β cell de-differentiation preventing agent and/or the β cell de-differentiation reversing agent comprise an inhibitor of Alk5 signaling. In some embodiments, the at least one agent comprises Alk5 inhibitor II or an analog or derivative thereof. In some embodiments, the β cell de-differentiation preventing agent and/or the β cell de-differentiation reversing agent comprises ALK5 inhibitor I or an analog or derivative thereof. In some embodiments, the β cell de-differentiation preventing agent and/or the β cell de-differentiation reversing agent comprises a SMAD3 inhibitor or an analog or derivative thereof.

[0158] In some embodiments, the subject in need of is a subject who (i) is in need of additional β cell; (ii) has diabetes; (iii) is at risk of developing diabetes; (iv) is developing diabetes; (v) is suspected of having or developing diabetes; (vi) is non-diabetic; (vii) is mildly diabetic; or (viii) severely diabetic. In some embodiments, the subject has, is developing or is at risk of developing, or is suspected of having metabolic syndrome or obesity. In some embodiments, the β cell de-differentiation-related disorder

is selected from the group consisting of pre-diabetes, type I diabetes, type II diabetes, type 1.5 diabetes, obesity, metabolic syndrome, or hyperlipidemia.

[0159] In some embodiments, the methods include selecting a subject in need of treatment for a β cell de-differentiation-related disorder. For example, a subject can be selected as a subject in need of treatment for β cell de-differentiation-related disorder if β cell de-differentiation is detected in the subject, e.g., in accordance with a method described herein. In some embodiments, the methods include detecting β cell de-differentiation in the subject. In some embodiments, a subject can be selected as a subject in need of treatment for β cell de-differentiation-related disorder if the subject exhibits a decrease in β cell dysfunction, number, or mass.

[0160] In some embodiments, the methods include administering to the subject an effective amount of an anti-diabetic agent (e.g., a secretagogue).

[0161] β cell de-differentiation preventing agents and/or β cell de-differentiation reversing agents may be administered either as a monotherapy or as a combination therapy with each other and/or other pharmaceutical agents. For example, they may be administered together with other pharmaceutical agents suitable for the treatment or prevention of diabetes and/or obesity and/or metabolic syndrome. In some embodiments, a combination therapy includes co-administration of a β cell de-differentiation preventing agent and/or a β cell de-differentiation reversing agent, and an additional agent. As used herein, the term "co-administration" refers to administration of two or more biologically active substances to a subject. Co-administration can be simultaneous or sequential. The two or more biologically active substances can be part of a single composition or separate compositions. In some embodiments, a combination therapy of the present invention comprises co-administration of a β cell de-differentiation preventing agent and/or a β cell de-differentiation reversing agent with one or more blood glucose lowering agents or agents that are beneficial to β cells. These agents include, but are not limited to, Metformin or other Biguanides, DPP4 inhibitors, Sulfonylureas or Metiglitinides, SGLT2 inhibitors, Glucokinase activators, Thiazolidinediones, PPARdelta agonists, non-activating PPARGgamma modulators, Glp-1 analogs, GIP analogs, Glp-1-receptor agonists, combined Glp-1/GIP receptor agonists, FGF21, agonistic FGFR monoclonal antibodies, Oxyntomodulin analogs, IAPP analogs, Leptin or Leptin analogs, Adiponectin or Adiponectin analogs, Insulin or Insulin analogs, proton pump inhibitors or gastrin receptor agonists, Reg family proteins/Reg family protein derived peptides or alpha-

glucosidase inhibitors. Further, they may be administered together with pharmaceutical agents which have an immunosuppressive or immunomodulatory activity, e.g., antibodies, polypeptides and/or peptidic or non-peptidic low molecular weight substances.

[0162] *Identifying Candidate Agents*

[0163] Aspects of the disclosure relate to identifying candidate agents for preventing and/or reversing β cell de-differentiation. In some aspects, the disclosure provides a method of identifying at least one candidate agent for preventing β cell de-differentiation, comprising: a) contacting a β cell with at least one test agent under conditions which cause β cell de-differentiation to occur; and b) assessing the level of Ucn3 expression in β cell in the presence of the at least one test agent, wherein the at least one test agent is identified as at least one candidate agent for preventing β cell de-differentiation if the level of Ucn3 expression in the β cell does not decrease in the presence of the at least one test.

[0164] It is contemplated that any condition which causes β cell de-differentiation to occur can be used in the methods of identifying candidate agents useful for preventing β cell de-differentiation. In some embodiments, the conditions which cause β cell de-differentiation to occur comprise stress induced by a cytokine comprising IL-1 β , TNF α , IFN γ , and combinations thereof. In some embodiments, the conditions which cause β cell de-differentiation to occur comprise oxidative stress. In some embodiments, the conditions which cause β cell de-differentiation to occur comprise diabetic conditions. In some embodiments, the conditions which cause β cell de-differentiation to occur comprise high levels of glucose (e.g., hyperglycemia), and/or lipids (e.g., hyperlipidemia). In some embodiments, the conditions which cause β cell de-differentiation to occur comprise insulin resistance.

[0165] In some embodiments, the methods of identifying candidate agents useful for preventing β cell de-differentiation comprise assessing the level of expression of at least one marker of mature β cells. In some embodiments, the methods comprise assessing the level of expression of at least one marker of mature β cells comprising FoxO1, MafA, Nkx6.1, and/or Pdx1, wherein the at least one test agent is identified as at least one candidate agent for preventing β cell de-differentiation if the level of expression of the at least one marker of mature β cells in the β cell does not decrease in the presence of the at least one test agent.

[0166] In some embodiments, the methods of identifying candidate agents useful for preventing β cell de-differentiation comprise conducting a GSIS assay on the β cell. In some embodiments, the methods comprise conducting a GSIS assay on the β cell, wherein the at least one test agent is identified as at least one candidate agent for preventing β cell de-differentiation if the β cell maintains its ability to exhibit an appropriate GSIS response in the presence of the at least one test agent.

[0167] In some aspects, the disclosure provides a method of identifying at least one candidate agent for reversing β cell de-differentiation, comprising: a) contacting a de-differentiated β cell with at least one test agent; and b) assessing the level of Ucn3 expression in the de-differentiated β cell in the presence and absence of the at least one test agent, wherein the at least one test agent is identified as at least one candidate agent for reversing β cell de-differentiation if the level of Ucn3 expression in the de-differentiated β cell increases in the presence of the at least one test agent compared to the level of Ucn3 expression in the de-differentiated β cell in the absence of the at least one test agent.

[0168] In some embodiments, the methods of identifying candidate agents for reversing β cell de-differentiation comprise measuring the level of expression of at least one marker of mature β cells. In some embodiments, the methods comprise measuring the level of expression of at least one marker of mature β cells comprising FoxO1, MafA, Nkx6.1, and Pdx1, wherein the at least one test agent is identified as a candidate agent for reversing β cell de-differentiation if the level of expression of the at least one marker of mature β cells detected in the de-differentiated β cell increases in the presence of the at least one test agent relative the level of expression of the at least one marker of mature β cells detected in the de-differentiated β cell in the absence of the at least one test agent.

[0169] In some embodiments, the methods of identifying candidate agents for reversing β cell de-differentiation comprise conducting a GSIS assay on the de-differentiated β cell. In some embodiments, the methods comprise conducting a GSIS assay on the de-differentiated β cell, wherein the at least one test agent is identified as a candidate agent for reversing β cell de-differentiation if the de-differentiated β cell gains the ability to exhibit an appropriate GSIS response after exposure to the at least one test agent.

[0170] The disclosure contemplates the use of a de-differentiated β cell which has been de-differentiated according to any suitable method. In some embodiments, the

de-differentiated β cell comprises a β cell de-differentiated by culturing in adherent conditions. In some embodiments, the de-differentiated β cell comprises β cells de-differentiated by culturing islets in adherent conditions. In some embodiments, the de-differentiated β cell is obtained directly from a subject, e.g., a subject described herein, e.g., a diabetic subject.

[0171] In some embodiments, the disclosure provides a method of identifying at least one candidate agent for reversing β cell de-differentiation, comprising: a) contacting a β cell with at least one test agent; and b) assessing the ability of the at least one test agent to inhibit Alk5 signaling, wherein at least one test agent that demonstrates the ability to inhibit Alk5 signaling comprises at least one candidate agent for reversing β cell de-differentiation.

[0172] A variety of assays for assessing the ability of a test agent to inhibit Alk5 signaling are known in the art. Exemplary such assays include the ALK5 Enzyme Assay 1, ALK5 Enzyme Assay 2, and Cell Assay as described in Published PCT International Application WO/2009/022171. Briefly, the ALK5 Enzyme Assay 1 measures the ability of a compound to bind to and inhibit the activity of a tagged (e.g., 6His) recombinant ALK5 protein *in vitro*. Similarly, the ALK5 Enzyme Assay 2 measures the ability of a compound to bind and inhibit ALK5 by its capacity to displace a probe molecule from recombinant ALK5 in an *in vitro* assay, using a probe molecule derivatized with a fluorescent probe or probe molecule that is fluorescent itself. Binding affinity can be measured using Fluorescence Polarization. The exemplary Cell Assay relies on ligand mediated translocation of R-Smads (Smads 1,2,3,5,8), which are a well-documented phenomenon in a variety of cell types. (Derynck, R. and Zhang, Y., 2003, Nature, 425, 577-584, Shi, Y. and Massague, J., 2003, Cell, 113, 685-700). Specifically TGF β 1 and TGF β 3 cause phosphorylation and nuclear translocation of Smad2 and Smad3 transcription factors. Thus compound inhibition of TGF β signaling can be estimated by measuring cellular distribution of Smad2 or 3 under activated TGF β pathway. The Smad2 RedistributionTM Assay (Fisher Biolmage ApS) can be used to assess *in vitro* cellular activity of agents of interest. IC₅₀ values for test agents when tested in one or more of the above assays are expected to be typically less than 10 μ M.

[0173] In some embodiments, the methods of identifying candidates for reversing β cell de-differentiation comprise assessing the ability of the at least one candidate agent to reverse β cell de-differentiation, wherein assessing the ability of the at least one

candidate agent to reverse β cell de-differentiation comprises: a) contacting a de-differentiated β cell with the at least one candidate agent; and b) detecting the level of Ucn3 expression in the de-differentiated β cell, wherein at least one candidate agent demonstrates the ability to reverse β cell de-differentiation if the level of Ucn3 expression detected in the de-differentiated β cell increases in the presence of the at least one candidate agent relative the level of Ucn3 expression detected in the de-differentiated β cell in the absence of the at least one candidate agent.

[0174] In some embodiments, the methods of identifying candidate agents for reversing β cell de-differentiation comprise measuring the level of expression of at least one marker of mature β cells comprising FoxO1, MafA, Nkx6.1, and Pdx1, wherein the at least one candidate agent demonstrates the ability to reverse β cell de-differentiation if the level of expression of the at least one marker of mature β cells detected in the de-differentiated β cell increases in the presence of the at least one candidate agent relative the level of expression of the at least one marker of mature β cells detected in the de-differentiated β cell in the absence of the at least one candidate agent.

[0175] In some embodiments, the methods of identifying candidate agents for reversing β cell de-differentiation comprise conducting a GSIS assay on the de-differentiated β cell, wherein the at least one candidate agent demonstrates the ability to reverse β cell de-differentiation if the de-differentiated β cell gains the ability to exhibit an appropriate GSIS response after exposure to the at least one candidate agent.

* * *

[0176] One skilled in the art readily appreciates that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The details of the description and the examples herein are representative of certain embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Modifications therein and other uses will occur to those skilled in the art. These modifications are encompassed within the spirit of the invention. It will be readily apparent to a person skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

[0177] The articles “a” and “an” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to include the plural referents. Claims or descriptions that include “or” between one or more members of a

group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention also includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process. Furthermore, it is to be understood that the invention provides all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, etc., from one or more of the listed claims is introduced into another claim dependent on the same base claim (or, as relevant, any other claim) unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise. It is contemplated that all embodiments described herein are applicable to all different aspects of the invention where appropriate. It is also contemplated that any of the embodiments or aspects can be freely combined with one or more other such embodiments or aspects whenever appropriate. Where elements are presented as lists, e.g., in Markush group or similar format, it is to be understood that each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements, features, etc., certain embodiments of the invention or aspects of the invention consist, or consist essentially of, such elements, features, etc. For purposes of simplicity those embodiments have not in every case been specifically set forth in so many words herein. It should also be understood that any embodiment or aspect of the invention can be explicitly excluded from the claims, regardless of whether the specific exclusion is recited in the specification. For example, any one or more nucleic acids, polypeptides, cells, species or types of organism, disorders, subjects, or combinations thereof, can be excluded.

[0178] Where the claims or description relate to a composition of matter, e.g., a nucleic acid, polypeptide, cell, or non-human transgenic animal, it is to be understood that methods of making or using the composition of matter according to any of the methods disclosed herein, and methods of using the composition of matter for any of the purposes disclosed herein are aspects of the invention, unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would

arise. Where the claims or description relate to a method, e.g., it is to be understood that methods of making compositions useful for performing the method, and products produced according to the method, are aspects of the invention, unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise.

[0179] Where ranges are given herein, the invention includes embodiments in which the endpoints are included, embodiments in which both endpoints are excluded, and embodiments in which one endpoint is included and the other is excluded. It should be assumed that both endpoints are included unless indicated otherwise. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise. It is also understood that where a series of numerical values is stated herein, the invention includes embodiments that relate analogously to any intervening value or range defined by any two values in the series, and that the lowest value may be taken as a minimum and the greatest value may be taken as a maximum. Numerical values, as used herein, include values expressed as percentages. For any embodiment of the invention in which a numerical value is prefaced by “about” or “approximately”, the invention includes an embodiment in which the exact value is recited. For any embodiment of the invention in which a numerical value is not prefaced by “about” or “approximately”, the invention includes an embodiment in which the value is prefaced by “about” or “approximately”. “Approximately” or “about” generally includes numbers that fall within a range of 1% or in some embodiments within a range of 5% of a number or in some embodiments within a range of 10% of a number in either direction (greater than or less than the number) unless otherwise stated or otherwise evident from the context (except where such number would impermissibly exceed 100% of a possible value). It should be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one act, the order of the acts of the method is not necessarily limited to the order in which the acts of the method are recited, but the invention includes embodiments in which the order is so limited. It should also be understood that unless otherwise indicated or evident from the context, any product or composition described herein may be considered “isolated”.

* * *

[0180] EXAMPLES

[0181] Example 1: Reversal of β cell de-differentiation by a small molecule inhibitor of the TGF β pathway

[0182] *Introduction*

[0183] The gene Urocortin 3 (Ucn3) is a marker for functionally mature β cells, cells capable of glucose stimulated insulin secretion (Blum et al., 2012). Ucn3 expression appears relatively late in postnatal mouse development and its expression levels correlates with functional β cell maturation in mice, and with the maturation of human pluripotent stem cell-derived β -cells after transplantation (Blum et al., 2012; Hua et al., 2013; van der Meulen et al., 2012). The inventors hypothesized that Ucn3 expression may be lost or reduced early during β cell de-differentiation in T2D and if so, could be used to investigate the first steps of stress-induced β cell de-differentiation.

[0184] *Results*

[0185] *Loss of Ucn3 expression is an early event in β cell de-differentiation in diabetes*

[0186] Ucn3 and insulin expression in β cells of T2D mice was examined by immunostaining on pancreata of obese diabetic (Ob/Ob and Db/Db) mice and compared to pancreata of age matched non-diabetic (C57BL/6) mice. The intensity of insulin staining in diabetic mice is indistinguishable from non-diabetic controls, but the immunoreactivity of Ucn3 is almost completely abolished in islets of diabetic mice (FIG. 1A). The disappearance of Ucn3 protein from β cells that still express high levels of insulin suggests that the loss Ucn3 is an early marker of β -cell stress in diabetes, occurring before reduction in insulin expression (Guo et al., 2013; Talchai et al., 2012).

[0187] Insulin expression has been previously reported to be diminished in β cells of severely diabetic mice, those with blood glucose levels exceeding 500mg/dl (Guo et al., 2013). To confirm that loss of Ucn3 is an early marker of diabetes, the inventors divided diabetic mice (Ob/Ob, Db/Db and Akita) into groups of mildly diabetic (blood glucose levels between 200-500mg/dL) and severely diabetic (blood glucose levels >500mg/dL) and compared the expression of insulin1 and Ucn3 mRNA to age-matched non-diabetic controls (C57BL/6, blood glucose levels <200mg/dL). The average (non-fasting) blood glucose level was 381 $\pm$ 18mg/dL in mildly diabetic mice, 588 $\pm$ 8mg/dL in

the severely diabetic mice, and 167 ± 5 mg/dl in the non-diabetic control mice. The expression level of insulin1 mRNA was slightly, but not significantly, higher in islets of mildly diabetic mice as compared to non-diabetic controls, but was reduced to 28% of control levels in islets of the severely diabetic group ($P < 0.001$). In contrast to the late reduction in insulin expression, the levels of Ucn3 mRNA in the mildly diabetic group were already reduced 3 fold, to 34% of the level in the healthy control group ($P < 0.001$), and by 10 fold, to approximately 10% of the control levels, in the severely diabetic group ($P < 0.001$) (FIG. 1B). We conclude that loss of Ucn3 mRNA is an early event in β cell de-differentiation.

[0188] *Using Ucn3 as a marker for the mature β cell state reveals reversibility of β cell de-differentiation*

[0189] Because Ucn3 expression is reduced early during β cell de-differentiation, its expression could be used to test whether β -cells at early or late stages of de-differentiation are able to regain a fully mature state. The hypothesis is that while late-stage de-differentiated β cells (negative for both insulin and Ucn3) may not be able to re-differentiate into fully mature β cells, cells at an earlier stage (negative for Ucn3, but still expressing insulin) may be able to recover from their de-differentiation if the stress inducing factor (i.e. the diabetes) is removed.

[0190] To test this hypothesis, the inventors induced transient insulin resistance in healthy, lean wild-type mice with the insulin-receptor antagonist S961 (Vikram and Jena, 2010; Yi et al., 2013). Mice treated with S961 develop acute insulin resistance and severe diabetes within one week, with non-fasting glucose levels of ≥ 500 mg/dL. Removal of S961 relieves the diabetes, and the mice restore their glucose control within one week.

[0191] Mice treated with S961 for one week have a nearly two-fold increase in insulin mRNA expression (indicating that they are compensating for the insulin resistance by over-producing insulin), and about a twofold decrease in Ucn3 mRNA expression (FIG. 1C). We induced transient hyperglycemia in wild type mice with S961 for one week; control animals were similarly treated with PBS. At the end of the first week, half of the animals were sacrificed for analysis, and half were taken off S961 treatment and allowed to recover from diabetes for another week by which time their blood glucose levels returned to normal (≤ 200 mg/dL). Immunostaining of pancreata from all groups shows the levels of Ucn3 and insulin proteins (FIG. 2A). As expected, animals treated with S961 developed diabetes (reaching blood glucose levels ≥ 460 mg/dL) and show an

increase in insulin, while Ucn3 staining was almost completely abolished. In the diabetic animals that recovered and showed normoglycemia following withdrawal of S961 for one week, there was a complete recovery of Ucn3 staining, with a staining intensity comparable to that of the PBS-treated controls (FIG. 2A).

[0192] The inventors next tested whether more severely de-differentiated β cells can also return to a mature state after removal of the de-differentiation inducing stress. It has previously been reported that substantial β cell de-differentiation occurs when islets are cultured *in vitro* on an adherent substrate (Gershengorn et al., 2004; Negi et al., 2012; Russ et al., 2008; Weinberg et al., 2007). Cells de-differentiated using this method can be analyzed for the loss of their functional character, and can be transplanted back into non-diabetic mice to test their differentiation state after being returned to a healthy environment (Bar-Nur et al., 2011; Bar et al., 2012).

[0193] In order to follow de-differentiated β cells, even after they cease to express insulin, the inventors developed a lineage tracing system that marks cells that have expressed insulin (transcribed the insulin gene) in the past. Insulin2-Cre transgenic mice were crossed with mice carrying a floxed reporter of histone H2B fused to mCherry (R26H2BCherry), such that cells that had expressed insulin are marked with nuclear mCherry. These mice also contained a transgene driving cytoplasmic EGFP protein under the control of the Ucn3 promoter (FIG. 2B). The consequence of this genetic system is that cells with nuclear mCherry have, at some time, transcribed the insulin gene, but need not be actively producing insulin protein, and the (reversible) expression of cytoplasmic GFP indicates whether the β -cell is fully mature (GFP positive) or de-differentiated (GFP negative). The inventors labeled this genetic system "RCU", for R26H2BmCherry; Ins2-Cre; Ucn3-GFP (FIG. 2B).

[0194] Triple hemizygous RCU progeny are healthy and euglycemic (data not shown). The frequency of cytoplasmic Ucn3-derived GFP staining in all Ins2-Cre derived H2BCherry labeled cells was determined by FACS to be $57 \pm 16\%$ in both male and female mice, between one month to four months of age (data not shown). Confocal imaging of triple hemizygous progeny β cells from RCU mice show red nuclear fluorescence in β cells that is easily distinguished from the cytoplasmic green fluorescence emitted by the Ucn3-GFP reporter (FIG. 2C).

[0195] T2D-like symptoms were induced in RCU mice using the insulin antagonist S961 as described above. Ucn3-GFP levels are down-regulated in diabetic

mice, treated with S961 for 6 days, but not in PBS-infused controls (FIG. 2C, left and middle panels). After removal of S961, the expression level of Ucn3-GFP was up-regulated, returning to levels comparable to control animals (FIG. 2C, right panel), corresponding to the remission of hyperglycemia (FIG. 2C, right panel). These data show that loss of Ucn3 expression is not permanent and that β cells can return to a mature Ucn3-positive state after a 7 day period of hyperglycemia.

[0196] When RCU islets are plated on an adherent matrix and cultured for 7 days, the islets flatten, cells spread out, and β cells lose Ucn3-GFP expression (FIG. 2D, left and middle panels). The levels of both insulin1 and Ucn3 in such adherent cultures of islets from wild-type mice were reduced to 4% and 29% of the levels in freshly harvested islets, respectively (FIG. 2E). Consistent with the loss of the Ucn3 marker, these islets completely lose their ability for glucose-stimulated insulin secretion (GSIS, FIG. 2F). Most notably, the β cells re-express Ucn3-GFP three weeks after transplantation into the kidney capsule of euglycemic SCID mice (FIG. 2D, right panel). These data suggest that the β cell de-differentiation caused by culturing cells *ex vivo* on adherent culture is reversible.

[0197] *Screen using de-differentiated RCU islets identifies roles for TGF β pathway inhibitors and Artemin signaling in reversing β cell de-differentiation*

[0198] The reversion of de-differentiated β cells to a mature state after transplantation to a healthy *in vivo* environment prompted us to look for factors that can recapitulate this phenomenon, as these factors could be candidates for drug development aimed at reversing β cell de-differentiation in T2D. The inventors used the RCU platform to screen an array of 114 growth factors representing most major signaling pathways (Tables 1A and 1B). The experimental design, outlined in FIG. 3A, employs healthy islets from adult RCU mice, isolated on day 1 and plated on an adherent matrix in a 384-well plate format. The islets were first cultured for one week to achieve adequate de-differentiation (see FIGS. 2D and 2E). Test compounds were then added on day 7 for another week. Each compound was tested in duplicate at two or three concentrations (listed in Tables 1A, 1B and 2). Fresh un-manipulated RCU islets were used as a positive control, and DMSO- or non-treated cultures were used as a negative control. The islets were fixed on day 11 for automated imaging and subsequent analysis. Percentages of mCherry positive cells that co-express GFP were calculated for each well and used to identify conditions that significantly increased the number of GFP positive cells over

negative (DMSO- or non-treated) controls (FIG. 3A). Positive hits were selected according to their statistical significance (P value) over the negative control. Of the 114 tested factors, three growth factors restored Ucn3-GFP expression with a high statistical significance ($P < 0.01$, FIG. 3B). These factors are BMP9, soluble TGF β receptor 3 (TGF β sRIII, also known as β glycan), and the GDNF-family member Artemin.

[0199] Both BMP9 and TGF β sRIII signal through receptors of the TGF β receptor family (David et al., 2007; Massague and Chen, 2000), whereas Artemin signals through RET and GFR α 3 receptors (Airaksinen and Saarna, 2002). To delve deeper into the effects of BMP9, TGF β sRIII and Artemin on β -cell re-differentiation, a second screen was performed using a library of 19 small molecule kinase inhibitors of TGF β signaling and 18 small molecules inhibitors of RET/GFR α 3 signaling (FIG. 3C and Table 2). In addition, we included a library of 42 known drugs for T2D (FIG. 3C and Table 2).

[0200] Among the 19 small molecules tested in the TGF β receptor inhibitors group, Alk5 inhibitor I, Alk5 inhibitor II and a SMAD3 inhibitor, restored Ucn3-GFP expression in de-differentiated β -cells ($P > 0.01$; FIG. 2C). Of the 18 RET/GFR α 3 inhibitors two molecules with relatively low specificity to the RET kinase, namely PHA-739358 and VEGFR inhibitor V, induced Ucn3-GFP in the cells with P-values below 0.01, and of the 42 known T2D drugs, only the two potassium-channels blockers, Repaglinide and Tolbutamide, gave marginal results. Alk5 inhibitor II showed the strongest effect among all molecules tested, both on the levels of Ucn3-GFP and by its reproducibility (as measured by its statistical P value over DMSO treated controls). A dose-response test showed that its effect on Ucn3-GFP expression in de-differentiated RCU β -cells begins at pico-molar concentrations (FIG. 3D).

[0201] *Alk5 inhibitor II up regulates expression of β cell transcription factors and prevents their loss under cytokine stress*

[0202] It has recently been shown that under diabetes-related stress, the expression and activity of key β -cell transcription factors, including MafA, Nkx6.1 and Pdx1, are compromised (Guo et al., 2013). We tested whether the most potent small molecule, Alk5 inhibitor II, is capable of preventing the down regulation in expression of these transcription factors. Islets harvested from lean, non-diabetic mice, were exposed to a diabetes-related cytokine challenge for 24 hours, with or without the presence of Alk5 inhibitor II, and the expression of several β cell genes was measured by qRT-PCR and compared to islets not treated with cytokines (FIG. 4).

[0203] Islets exposed to 10ng/ml of either IL-1 β , TNF α or IFN γ showed reduced expression of Ucn3, MafA, Nkx6.1 and Pdx1 mRNAs, whereas expression of Insulin1 and FoxO1 was less affected (FIG. 4). Addition of 1 μ M Alk5 inhibitor II with any of the cytokines prevented the diminution of expression levels for Ucn3, MafA, Nkx6.1 and Pdx1. In fact, the expression levels of the latter three genes remained at levels comparable to, and in some cases higher than, that found in control islets (those not exposed to cytokines) (FIG. 4).

[0204] *Alk5 inhibitor II can restore expression of β -cell transcription factors even in β cells that were exposed to extreme diabetic conditions for several months*

[0205] The inventors explored whether Alk5 inhibitor II could restore the expression levels of specific β cell genes in β cells from severely diabetic mice, β cells that were exposed to an extreme diabetic environment for several months. To answer this question, we performed gene-expression analyses on islets from lean non-diabetic C57BL/6 mice and from mice with advanced to severe diabetes (Db/Db, Ob/Ob and Akita; blood glucose levels of 406 $\pm$ 39mg/dL, 527 $\pm$ 48mg/dL and >600mg/dL, respectively). The islets isolated from these diabetic animals, and controls, were cultured *in vitro* for 24 hours with or without Alk5 inhibitor II (FIG. 5). Culturing control healthy islets for 24 hours with Alk5 inhibitor II results in a 1.5-2.5 fold higher expression of Ucn3, MafA, Nkx6.1, Pdx1 and FoxO1 compared to DMSO treated controls (FIG. 5A). The inventors also observed an unexplained 2 fold decrease in insulin1 expression (FIG. 5). Similarly, islets from mice with advanced diabetes (FIG. 5B), and islets from mice with severe diabetes (FIGS. 5C and 5D) responded to the Alk5 inhibitor II. The increase in Ucn3, Nkx6.1 and Pdx1 gene expression caused by Alk5 inhibitor II in severely diabetic mice was 1.5-2.5 fold, similar to the effect on non-diabetic islets. The induction of MafA expression by Alk5 inhibitor II in the severely diabetic islets increased to 5-6 fold over DMSO-treated controls (FIGS. 5C and 5D). This may reflect the early role of MafA disappearance in β cell stress (Guo et al., 2013). The work described herein demonstrates that Alk5 inhibitor II can induce mature gene expression in β cells that have been exposed to extreme diabetic conditions for several months. The inventors also tested whether the Alk5 inhibitor II is effective in restoring specific β cell gene expression in human islets. Primary human islets were treated with Alk5 inhibitor II for 24 hours, and subjected to gene transcript analyses (FIG. 5E). Similar to results with mouse islets,

human islets treated with Alk5 inhibitor II show an increase in mRNA expression for Insulin, MafA, Nkx6.1 and Pdx1 mRNAs, but not for FoxO1 (FIG. 5E).

[0206] *Discussion*

[0207] The response of β cells to the progression of T2D begins with an adaptive stage, in which the cells compensate for insulin resistance by over-production and over-secretion of insulin, as well as increasing β cell replication (Guo et al., 2013; Weir and Bonner-Weir, 2004; Yi et al., 2013). This adaptation is reversible, as can be seen when β -cell function returns with the remission from T2D after bariatric surgery (Bradley et al., 2012). However, if the metabolic stress persists, β cells surrender to the metabolic overload and de-differentiation occurs. This de-differentiation begins with translocation of the transcription factor FoxO1 to the nucleus, and continues with an inactivation of β cell-specific transcription factors including MafA, Nkx6.1 and Pdx1 and consequently, a reduction in insulin production and secretion. All together, these changes result in the escalation of the disease and eventually to a non-recoverable loss of a functionally mature β cell mass (Guo et al., 2013; Talchai et al., 2012; Weir and Bonner-Weir, 2004).

[0208] The results described herein put the loss of Ucn3 expression as an early event in β cell stress, occurring at the compensation stage, before reduction in insulin expression and deterioration to frank diabetes. Ucn3 protein and mRNA were dramatically down regulated even in mildly diabetic mice, some of which had blood glucose levels that were just slightly above normal. This is further demonstrated by the loss of Ucn3 in S961-treated mice, exposed to insulin resistance and hyperglycemia for only a week. In severely diabetic mice, we observed a dramatic reduction in insulin expression indicative of advanced β cell de-differentiation and the expression of Ucn3 mRNA was almost completely abolished. It is noteworthy, that loss of expression of Ucn3 *per se* is not a driver of diabetes, but is rather caused by it, as mice homozygous for a Ucn3-null allele are not diabetic, and even show slightly better glucose tolerance under high-fat feeding and aging (Li et al., 2007).

[0209] To utilize the finding that Ucn3 is an early marker of β cell de-differentiation, we developed triple-transgenic mice, in which a sensitive Ucn3-regulated GFP reporter is combined with β -cell lineage tracing. This genetic system allows one to trace β cells even after profound de-differentiation. Using this system, we show that β cell de-differentiation can be reversed after one week of S961 treatment *in vivo* or after one week of adherent culture *in vitro*. A screen for pathways that can rescue β cells from de-

differentiation identified three growth factors that restored Ucn3-GFP expression, namely BMP9, TGF β sRIII and Artemin, all belonging to the TGF β superfamily. Of those, only BMP9 had previously been identified as having an active role in glucose homeostasis (Chen et al., 2003). The gene encoding TGF β receptor III has been shown to be up regulated in pancreata from obese human patients compared to lean subjects (Muharram et al., 2005), while Artemin and its receptor GFR α 3 have, to the best of our knowledge, not been described in pancreatic islet function.

[0210] Tests on small molecule mediators of BMP/TGF β and Artemin signaling identified Alk5 inhibitor II as a potent compound able to restore mature β -cell identity even in islets from severely diabetic mice. This inhibitor also blocked the loss of specific β cell gene expression under cytokine-induced stress. Alk5 inhibitor II, which we identified using mouse β cells, can induce the expression of key β cell transcription factors in human islets. While human UCN3 is a marker of the functional maturation for both β and α cells (van der Meulen et al., 2012), and despite evidence that UCN3 is not a faithful marker for functional β -cell maturation in human islets during human pancreas development (Hrvatin et al., 2014), the signals that reverse β cell de-differentiation (i.e. inhibition of Alk5 signaling) may be conserved between mouse and human.

[0211] Alk5 inhibitor II has been previously identified by Rezanian and colleagues in an independent screen aimed at inducing functionally mature endocrine cells from human embryonic stem cells (Rezanian et al., 2011). Ichida and colleagues showed that this inhibitor can replace Sox2 in cell reprogramming (Ichida et al., 2009). Interestingly, it was recently reported that β cells of mice carrying a conditional deletion of both Alk5 (referred to as TGF β receptor I) and TGF β receptor II do not proliferate in response to inflammatory cytokines (Xiao et al., 2013). The results described herein demonstrate that Alk5 inhibitor II restores specific β -cell gene expression in de-differentiated β cells, blocks cytokine-induced β cell stress, and stimulates over-expression of these genes in β cells from healthy, non-diabetic mice and humans. Taken together, these results suggest that Alk5 signaling may be constitutively active in β cells, that sustaining mature β -cell phenotype depends on constant inhibition of this signal, and that the inhibition of Alk5 signaling may confer its effect by inducing expression of β cell transcription factors including MafA, Nkx6.1 and Pdx1. This postulated inhibition of Alk5 signaling in mature β cells develops during the first postnatal weeks, when the cells reach their fully mature state (Blum et al., 2012), and is reduced under diabetic stress or when the cells are taken

out of their niche and grown *in vitro*. If inhibition of Alk5 signaling is not restored, the β cells will evidently de-differentiate and disappear. The work described herein indicates that screening for compounds that inhibit Alk5 signaling specifically in β cells may yield compounds that, in combination with traditional blood-glucose lowering medicines, will delay, prevent or even restore the loss of healthy, mature β cell function in T2D patients.

[0212] Experimental Procedures

[0213] *Animals*

[0214] Animal experiments were performed in compliance with the Harvard University International Animal Care and Use Committee (IACUC) guidelines. Mouse strains used were C57BL/6, Ob/Ob (Zhang et al., 1994), Db/Db (Chen et al., 1996), Insulin2Akita (Wang et al., 1999), Insulin2-Cre transgenic mice (Postic et al., 1999), Ucn3-GFP transgenic mice (Gong et al., 2003), SCID-beige mice, R26H2BCherry mice and RCU mice. R26H2BCherry mice (carrying a floxed nuclear-labeling reporter composed of histone H2B fused mCherry) were generated by genetic targeting of the Rosa26 locus of V6.5 mouse ES cells with the construct Rosa26-Puro-p (A)-CAGS-lox-PGK:neo-p (A)-lox-H2BCherry-p (A). Targeted ES cells were injected into BDF1xB6 blastocysts, and germline transmission was detected through breeding of chimeras with C57BL/6 females. To generate RCU mice, mice homozygous for both R26H2BCherry and Ucn3-GFP were crossed with homozygous Insulin2-Cre mice. All RCU progeny are triple hemizygous at all three alleles. Induction of transient insulin resistance by S961 was done with an osmotic pump as previously described (Yi et al., 2013). Blood glucose levels were measured in non-fasted animals using OneTouch Ultra2 glucometer (LifeScan). For islet isolation, adult pancreata were perfused through the common bile duct with 0.8mM Collagenase P (Roche) and fetal and neonatal pancreata were dissected wholly without perfusion. Pancreata were digested with 0.8mM Collagenase P (Roche) and purified by centrifugation in Histopaque gradient (Sigma).

[0215] *Immunostaining*

[0216] Pancreata were fixed by immersion in 4% paraformaldehyde overnight at 4 °C. Samples were washed with PBS, incubated in 30% sucrose solution overnight and embedded with optimal cutting temperature compound (Tissue-Tek). 10 μ m sections were blocked with 10% donkey serum (Jackson ImmunoResearch) in PBS/0.1% Triton X and incubated with primary antibodies overnight at 4°C. Secondary antibodies were incubated for 1hr at room temperature. Antibodies and dilutions used include rabbit anti-mouse

Ucn3 (1:600-1:800, Phoenix Pharmaceuticals), Guinea Pig anti-insulin (1:800, DAKO), Alexa Fluor 488 donkey anti-rabbit (1:400, Invitrogen) and DyLight 649 donkey anti-guinea pig (1:400, Jackson Immunoresearch). Nuclei were visualized with DAPI. Images were taken using an Olympus IX51 Microscope or Zeiss LSC 700 confocal microscope.

[0217] *Automated screen*

[0218] Islets from adult RCU mice were isolated and plated on 804G matrix (Lefebvre et al., 1998) for one week in a 384-well plate format. Compound libraries were added on day 7, and islets were cultured for an additional week in the presence of compounds. Each compound was tested in duplicates of two or three concentrations. A list of all compounds and concentrations appears in Tables 1A, 1B and 2. Fresh un-manipulated RCU islets were used as a positive control, and DMSO- or untreated islets were used as a negative control. The islets were fixed on day 11 for automated image acquisition and analysis using a Cellomics ArrayScanVTI. Cell nuclei of target cells were identified by nuclear mCherry expression and a 2 pixel cytoplasmic mask was drawn around each nucleus. The GFP fluorescence in the cytoplasmic mask of freshly isolated islets was used as a control to identify fluorescence intensity thresholds that enabled automated calls on each individual cell. Cells that displayed GFP fluorescence equal or greater than found in control cells were identified as being positive for the Ucn3-GFP reporter. Percentages of mCherry positive cells that co-express GFP were calculated for each well and used to identify conditions that significantly increased the number of GFP positive cells over negative controls. Positive hits are selected according to their statistical significance (P value by t.test) over the negative control.

[0219] Table 1A: Growth Factors, Set#1

Drug Name	Conc (ng/ml)	Average % Ucn3High	T-Test (Vs.No Factors)
Fresh Islets		22.99	7.08
No Factors		5.96	1.000
Recombinant Human BMP9	10, 1	23.54	5.46
Recombinant Human Arterin	10, 1	28.48	5.08
Recombinant Human FGF-8a	10, 1, 0.1	24.54	8.06
Recombinant Rat FGF basic (FGF-2)	10, 1, 0.1	0.49	0.013
Reg3a	200, 20	19.62	0.017
Recombinant Human FGF4	10, 1	18.43	0.019
Recombinant Mouse Wnt5a	10, 1, 0.1	0.34	0.026
Recombinant Human PDGFBB	10, 1	20.75	0.029
Recombinant Human TGF-β1.2	10, 1, 0.1	0.57	0.032
Recombinant Human SCF	10, 1	19.93	0.047
Recombinant Human Leptin	10, 1	0.13	0.058
Recombinant Human FGF basic (145 aa)	10, 1, 0.1	1.24	0.059
Recombinant Human IGFII	10, 1	0.35	0.062
Recombinant Human BMP5	10, 1, 0.1	19.66	0.071
Recombinant Human FGF	10, 1, 0.1	19.62	0.076

Recombinant Mouse IGF1	10, 1	0.76	0.089
Recombinant Human IL15	10, 1, 0.1	1.97	0.113
Recombinant Human FGF6	10, 1, 0.1	1.87	0.122
Recombinant Human FGF basic 157 aa	10, 1	13.40	0.123
Recombinant Mouse GDF8/Myostatin	10, 1	1.45	0.134
Neurturin	100, 10	13.59	0.145
Recombinant Human IL3	10, 1, 0.1	11.28	0.195
Recombinant Human GM-CSF	10, 1, 0.1	12.78	0.198
Recombinant Mouse FGF basic (FGF2)	10, 1	19.01	0.197
Human PDGF	10, 1	17.28	0.203
Recombinant Human IL2	10, 1, 0.1	2.68	0.222
Recombinant Human IL9	10, 1	2.30	0.223
Recombinant Human IL5	10, 1, 0.1	2.63	0.238
Recombinant Human TGF- β 1	10, 1, 0.1	10.35	0.243
Recombinant Human BMP4	10, 1	10.24	0.256
Recombinant Human IL31	10, 1	2.54	0.278
Recombinant Human FGF9	10, 1	14.38	0.281
PBS + 0.1% BSA	1:10, 1:100	12.35	0.282
Recombinant Human FGF acidic (FGF1)	10, 1, 0.1	13.63	0.336
Recombinant Bovine FGF basic	10, 1	3.09	0.347
Recombinant Human BDNF	10, 1	3.24	0.365
Recombinant Human Cardiotrophin-1	10, 1	3.22	0.368
Recombinant Mouse TGF β RII Fc Chimera	10, 1	8.99	0.370
Recombinant Mouse GM-CSF	10, 1, 0.1	11.26	0.388
Recombinant Amphibian TGF- β 5	10, 1, 0.1	9.05	0.412
Recombinant Human MCSF	10, 1, 0.1	3.74	0.428
Recombinant Human TGF β 2	10, 1, 0.1	9.05	0.438
Recombinant Human NT4	10, 1	3.56	0.462
Recombinant Mouse TGF β RII/Mouse Fc	10, 1	3.53	0.471
Recombinant Feline Flt-3 Ligand	10, 1	10.56	0.473
Recombinant Human Thrombopoietin	10, 1	3.67	0.475
Recombinant Human IL11	10, 1, 0.1	10.41	0.493
Recombinant Human PDGFDD	10, 1	8.93	0.513
Recombinant Human FGF-2e	10, 1	4.17	0.547
4mM HCl 0.1%B5A	1:50	9.14	0.549
Recombinant Human PDGFAA	10, 1	4.14	0.577
Recombinant Mouse Wnt3a	10, 1	4.28	0.586
Recombinant Rat EGF	10, 1, 0.1	7.25	0.598
Recombinant Human Oncostatin M/OSM	10, 1, 0.1	4.50	0.657
Recombinant Human TGF β 3	10, 1, 0.1	7.98	0.672
Recombinant Human Flt3Ligand	10, 1, 0.1	4.79	0.716
Recombinant Human IL6	10, 1, 0.1	5.01	0.727
Bovine FGF acidic (FGF1)	10, 1, 0.1	4.77	0.746
Porcine TGF β 1	10, 1, 0.1	7.32	0.757
Recombinant Human GM-CSF	10, 1, 0.1	6.96	0.765
Recombinant Human GDNF	10, 1	6.98	0.789
Recombinant Human FGF20	10, 1	4.97	0.800
Recombinant Human IL7	10, 1, 0.1	6.50	0.853
Recombinant Human/Mouse/Rat Activin A	10, 1	6.71	0.870
Recombinant Human IL4	10, 1, 0.1	5.46	0.875
Recombinant Human Amphiregulin	10, 1	5.61	0.924
BMP2/7	200, 20	6.17	0.943
Recombinant Human PDGFAB	10, 1	5.78	0.960
Recombinant Human IGF1	10, 1	5.79	0.964

[0220] Table 1B: Growth Factors, Set#2

Drug Name	Conc (ng/ml)	Average % Ucn3High	T-Test (Vs.No Factors)
Fresh Islets		85.35	1.390E-16

neg control		8.20	1.000
Recombinant Human Artemin	10	30.53	1.512E-03
Recombinant Human BMP9	10	33.94	2.349E-03
Recombinant Human TGF- β soluble Receptor III	100, 20	28.51	6.251E-03
Recombinant Human FGF8f	500, 100, 20	30.50	0.013
Recombinant Human FGF10	500, 100, 20	28.80	0.015
Recombinant Human PDGFCC	10, 1, 0.1	25.05	0.037
Recombinant Human BMP6	10, 1, 0.1	33.19	0.043
Recombinant Human BMP7	10, 1, 0.1	26.73	0.048
Recombinant Mouse Lefty-1	500, 100, 20	21.18	0.052
Recombinant Human FGF-22	500, 100, 20	21.42	0.062
Recombinant Canine KGF/FGF7	500, 100, 20	21.64	0.068
Recombinant Human FGF-8a	50	20.30	0.073
Recombinant Mouse TGF β RI/ALK5 Fc Chimera	10, 1, 0.1	20.73	0.085
Recombinant Mouse BMPRII/ALK-6/Fc Chimera	10, 1, 0.1	19.32	0.125
Recombinant Human TGF β RII Fc Chimera	10, 1	22.72	0.129
Recombinant Human FGF-17	10, 1, 0.1	19.34	0.146
Recombinant Human Klotho (Klotho-a)	10, 1, 0.1	17.47	0.147
PBS + 0.1% BSA	1:10, 1:50, 1:100	17.80	0.153
Recombinant Human Sonic Hedgehog Nterminus	500, 100, 20	17.63	0.155
Recombinant Human BMPRII Fc Chimera	10, 1, 0.1	17.20	0.164
Recombinant Human FGF21	500, 100, 20	17.47	0.178
Recombinant Human FGF-16	10, 1	19.08	0.189
Recombinant Human CNIF	500, 100, 20	16.05	0.224
Recombinant Zebrafish BMP4	500, 100, 20	16.20	0.229
Recombinant Mouse FGF8b Isoform	500, 100, 20	17.97	0.235
Recombinant Mouse FGF-8c	10, 1, 0.1	16.72	0.245
Recombinant Human Follistatin	500, 100, 20	18.03	0.263
Recombinant Human FGF19	10, 1, 0.1	15.19	0.278
Recombinant Human FGF23	10, 1, 0.1	15.52	0.285
Recombinant Human BMP-3b/GDF-10	500, 100, 20	15.54	0.313
Recombinant Human FGF acidic (FGF-1)/BECGF	10, 1, 0.1	15.03	0.345
Recombinant Human FGF-12	500, 100, 20	13.35	0.411
Recombinant Human FGF basic 145 aa	500, 100, 20	13.74	0.436
Recombinant Rat FGFBP	10, 1	12.84	0.558
Recombinant Human FGF basic 146 aa	10, 1, 0.1	12.06	0.599
Recombinant Mouse DLL1 Fc Chimera	10, 1, 0.1	11.47	0.602
Recombinant Human FGF-5	10, 1	11.91	0.645
Reg3A	10	3.85	0.685
PDGFBB	10	12.65	0.687
Recombinant Mouse Cerberus 1 (DAND4)	10, 1, 0.1	6.34	0.766
Recombinant Human Endoglin/CD105	10, 1, 0.1	6.47	0.789
Recombinant Human FGF3	500, 100, 20	9.64	0.817
Recombinant Mouse Cripto	10, 1, 0.1	6.81	0.822
Recombinant Human HGF	10, 1, 0.1	6.89	0.830
Recombinant Mouse FGF acidic	10, 1, 0.1	9.32	0.860
Bovine FGF basic	10, 1, 0.1	9.12	0.884
Recombinant Mouse PDGFCC	10, 1, 0.1	7.38	0.892
Recombinant Human BMP10	10, 1, 0.1	7.57	0.921
Recombinant Human BMP2	10, 1, 0.1	7.64	0.930
Fgf4 human	100	8.92	0.946
Fgf4 mouse		7.58	0.954
Recombinant Human BMPRIA/ALK-3 Fc Chimera	10, 1, 0.1	8.22	0.997

[0221] Table 2: Focused Small Molecules Screen

Drug Name	List	CAS No.	Conc (μ M)	Average % Ucn3 ^{High}	T-Test (Vs. DMSO only)
Fresh islets				42.83	9.20E-21
DMSO only				2.31	1.00

ALK5 Inhibitor II (RepSox)	BMP/TGFβ	446859-33-2	10, 1	42.40	1.16E-14
ALK5 Inhibitor I (LY 364947)	BMP/TGFβ	396129-53-6	10, 1	7.76	1.57E-03
Smad3 inhibitor, SIS3	BMP/TGFβ	1009104-85-1	10, 1	6.88	0.01
SB-431542	BMP/TGFβ	301836-41-9	10, 1	8.29	0.01
BMP inhibitor LDN-193189	BMP/TGFβ	1062368-24-4	10, 1	9.06	0.03
TGF-β RI Kinase Inhibitor VI (ALK5 Inhibitor VII)	BMP/TGFβ	396129-53-6	10, 1	5.10	0.10
SB-525334 (TGF-β RI Kinase Inhibitor VIII)	BMP/TGFβ	356559-20-1	10, 1	0.00	0.11
SB-505124 (TGF-beta RI Inhibitor III)	BMP/TGFβ	694433-59-5	10, 1	0.00	0.11
A-83-01 (TGF-beta RI Inhibitor IV)	BMP/TGFβ	909910-43-6	10, 1	0.00	0.11
Casein Kinase I Inhibitor, D4476	BMP/TGFβ	301836-43-1	10, 1	0.00	0.11
NVP-TAE684 - TAE 684	BMP/TGFβ	761439-42-3	10, 1	0.00	0.11
AP26113	BMP/TGFβ	1197958-12-5	10, 1	5.56	0.13
TGF-β RI Kinase Inhibitor V (SD-209)	BMP/TGFβ	627536-09-8	10, 1	0.65	0.25
AMPK Inhibitor, Compound C, Dorsomorphin	BMP/TGFβ	866405-64-3	10, 1	3.10	0.58
PF-2331066 (Ciclotinib)	BMP/TGFβ	877399-52-5	10, 1	1.52	0.59
ALK5 inhibitor	BMP/TGFβ	259870-33-2	10, 1	1.65	0.64
GSK1838705A	BMP/TGFβ	1116235-97-2	10, 1	2.94	0.70
Pirfenidone (S-7701)	BMP/TGFβ	53179-13-8	10, 1	2.15	0.91
FR 180204	BMP/TGFβ	865362-74-9	10, 1	2.21	0.94
Danuseritin (PHA-739558)	RET/GFRα3	827318-97-8	10, 1	25.20	5.24E-11
VEGFR Tyrosine Kinase Inhibitor V	RET/GFRα3	396129-53-6	10, 1	10.79	1.55E-04
S6K1 Inhibitor II, DG2	RET/GFRα3	871340-88-4	10, 1	6.67	0.01
AMG-706 (Motesanib)	RET/GFRα3	453562-69-1	10, 1	6.27	0.03
ELLAGIC ACID	RET/GFRα3	1953-04-4	10, 1	7.10	0.04
ZD6474 (Vandetanib)	RET/GFRα3	443913-73-3	10, 1	5.08	0.07
Ponatinib (AP24834)	RET/GFRα3	943319-70-8	10, 1	0.00	0.11
Lestaurtinib (CEP-701)	RET/GFRα3	111358-88-4	10, 1	0.00	0.11
Amuvatinib (MP-470, HPK 56)	RET/GFRα3	850679-09-3	10, 1	0.00	0.11
SU11248 (Sunitinib)	RET/GFRα3	341031-54-7	10, 1	4.84	0.12
BAY 73-4506	RET/GFRα3	755037-03-7	10, 1	0.64	0.35
Mer RTK inhibitor, UNC569	RET/GFRα3	1350547-65-7	10, 1	1.19	0.44
TG101209	RET/GFRα3	936091-14-4	10, 1	1.67	0.66
BAY 43-9006 (Sorafenib)	RET/GFRα3	284461-73-0	10, 1	1.84	0.74
PP-1	RET/GFRα3	172889-26-8	10, 1	2.00	0.83
XL-184 (Cabozantinib)	RET/GFRα3	849217-68-1	10, 1	2.10	0.88
PP242	RET/GFRα3	1092351-67-1	10, 1	2.09	0.88
RPI-1	RET/GFRα3	269730-03-2	10, 1	2.22	0.94
Repaglinide	Diabetes	135062-02-1	10, 1	8.24	1.97E-03
Tolbutamide	Diabetes	64-77-7	10, 1	7.02	3.67E-03
KR-62436	Diabetes	761414-79-3	10, 1	9.98	0.01
Glipizide	Diabetes	29094-61-9	10, 1	6.02	0.02
Troglitazone	Diabetes	97322-87-7	10, 1	5.65	0.05
Chlorpropamide	Diabetes	94-20-2	10, 1	5.23	0.06
GW 9508	Diabetes	885101-89-3	10, 1	5.36	0.07
Gliquidone	Diabetes	33342-05-1	10, 1	5.11	0.07
Saxagliptin (BMS-447118)	Diabetes	361442-04-8	10, 1	4.94	0.11
SR11720	Diabetes	1001645-58-4	10, 1	0.00	0.11
JNK Inhibitor X, BI-78D3	Diabetes	883065-90-5	10, 1	0.00	0.11
GSK1292263	Diabetes	1032823-75-8	10, 1	0.00	0.11
Acetohexamide	Diabetes	968-81-0	10, 1	4.58	0.12
CAY10591 (Sirtuin 1 Activator 3)	Diabetes	839699-72-8	10, 1	0.14	0.14
Vildagliptin (LAF237 - Galvus)	Diabetes	274901-16-5	10, 1	5.26	0.15
PIOGUTAZONE HYDROCHLORIDE	Diabetes	111025-46-8	10, 1	4.26	0.18
ACARBOSE	Diabetes	56180-94-0	10, 1	0.47	0.20
NVP-DPP-728 dihydrochloride	Diabetes	247016-68-9	10, 1	0.50	0.21
Harpagoside	Diabetes	19210-12-9	10, 1	0.69	0.26
Phenformin hydrochloride (PhenF)	Diabetes	834-28-6	10, 1	0.70	0.26
JNK inhibitor XIII (SU 3327)	Diabetes	40045-50-9	10, 1	0.71	0.27
S26948	Diabetes	353280-43-0	10, 1	0.71	0.27
METFORMIN HYDROCHLORIDE	Diabetes	1115-70-4	10, 1	3.93	0.29
SR 202	Diabetes	76541-72-5	10, 1	1.10	0.39
Alogliptin (SYR 322)	Diabetes	850649-61-5	10, 1	3.53	0.41
Ile-Pro-Ile (Diprotin A)	Diabetes	90614-48-5	10, 1	3.64	0.44
Sitagliptin Phosphate	Diabetes	654671-78-0	10, 1	1.20	0.44

NATEGUNIOP	Diabetes	105816-04-4	10	1.40	0.46
SALSALATE	Diabetes	552-94-3	10	1.40	0.53
Tolazamide	Diabetes	1156-19-0	10	3.18	0.54
Voglibose	Diabetes	83480-29-9	10	1.53	0.58
Rosiglitazone hydrochloride	Diabetes	122320-73-4	10	1.55	0.60
Glinapiptide	Diabetes	93479-97-1	10	1.59	0.61
Ciglitazone	Diabetes	74772-77-3	10	1.61	0.62
Glibenclamide	Diabetes	10238-21-8	10	3.00	0.63
CRYPTOTANSHINONE	Diabetes	35825-57-1	10	1.66	0.64
Linagliptin (BI-1356)	Diabetes	668270-12-0	10	2.98	0.65
MIGLITOL	Diabetes	72432-03-2	10	1.96	0.80
DPPI 1c hydrochloride	Diabetes	866396-34-1	10	2.68	0.82
Lisinopril	Diabetes	1953-04-4	10	2.21	0.94
SIRT1 Inhibitor IV, (S)-35, sc 204279, CHC-35	Diabetes	848193-72-6	10	2.40	0.95
GLICLAZIDE	Diabetes	21187-98-4	10	2.35	0.98

[0222] *Quantitative real-time PCR and cytokine treatment*

[0223] Total RNA from fresh or cytokine treated whole islets was isolated using RNeasy Plus Mini Kit (Qiagen). cDNA was prepared with random primers using SuperScript III reverse transcriptase (Life Technologies). For cytokine treatment, isolated islets were recovered overnight in islet media (DMEM containing 1gr/L glucose, 10% v/v FBS, 0.1% v/v Penicillin/Streptomycin), followed by 24 hours incubation with 10ng/ml of either mouse IL-1 β , mouse TNF α or mouse INF γ (R&D Systems), with the addition of Alk5 inhibitor II (1 μ M, Axxora) or vehicle (DMSO) at the same dilution. Relative expression of Ucn3, Ins1, Nkx6.1, Pdx1 and FoxO1 were determined using gene-specific TaqMan probes with TaqMan® Fast Universal PCR Master Mix (Life Technologies) on an ABI 7900 Real-Time PCR machine. Relative expression of mouse MafA was determined using Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix (Agilent) on the same machine. Primers for mouse MafA were 5'-AGCGGCACATTCTGGAGAG-3' (SEQ ID NO: 1) forward and 5'-TTGTACAGGTCCCGCTCCTT-3' (SEQ ID NO: 2) reverse. Levels of gene expression were normalized to the expression of Ubc or Eif2A genes.

[0224] *Human Islets*

[0225] Human islets were obtained from NDRI (The National Disease Research Interchange) and were grown in CMRL 1066 Supplemental medium (Mediatech), 10% v/v HyClone FBS (Thermo Scientific), 1% v/v Penicillin/Streptomycin (Corning Cellgro) for 4 days before treatment for 24h with Alk5 inhibitor II (Axxora).

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CLAIMS

What is claimed is:

1. A method for detecting de-differentiation or re-differentiation of a β cell, the method comprising:
 - a) obtaining a β cell;
 - b) detecting the level of urocortin 3 (Ucn3) expression in the β cell;
 - c) comparing the level of Ucn3 expression detected in the β cell to the level of Ucn3 expression detected in a normal mature β cell;
 - d) detecting de-differentiation or re-differentiation of the β cell,
wherein de-differentiation of the β cell is detected if the level of Ucn3 expression detected in the β cell is decreased relative to the level of Ucn3 expression detected in the normal mature β cell; or
wherein re-differentiation of the β cell is detected if the level of Ucn3 expression detected in the β cell is comparable to the level of Ucn3 expression detected in the normal mature β cell.
2. The method of claim 1, wherein detecting in b) comprises detecting the level of Ucn3 mRNA expression or Ucn3 protein expression.
3. The method of claims 1 or 2, wherein detecting in b) comprises performing an immunostain using an antibody specific for Ucn3 protein to detect the level of Ucn3 protein expression in the β cell.
4. The method of any one of claims 1 to 3, wherein detecting in b) comprises performing an immunostain using a primary antibody specific for Ucn3 protein and a secondary antibody-fluorescent dye conjugate specific for the primary antibody to detect the level of Ucn3 protein expression in the β cell.
5. The method of claims 3 or 4, further comprising imaging the immunostain using a microscope to obtain a micrograph displaying Ucn3 protein expressed in the β cell in a fluorescent color indicative of the level of Ucn3 protein expressed in the β cell.

6. The method of any one of claims 1 to 5, wherein comparing in c) comprises displaying a first micrograph showing the results of an immunostain for Ucn3 protein in the β cell and displaying a second micrograph showing the results of an immunostain for Ucn3 protein in the normal mature β cell.
7. The method of any one of claims 1 to 6, wherein measuring in b) comprises performing a hybridization based assay using one or more nucleic acids which hybridizes to a nucleic acid encoding Ucn3.
8. The method of any one of claims 1 to 7, wherein measuring in b) comprises performing a polymerase chain reaction based assay using a set of primers and probes specific for Ucn3 cDNA, wherein the level of Ucn3 cDNA detected is indicative of the level of Ucn3 mRNA expressed in the β cell.
9. The method of any one of claims 1 to 8, wherein measuring in b) comprises:
 - i) isolating total RNA comprising Ucn3 mRNA from the β cell;
 - ii) reverse transcribing the Ucn3 mRNA isolated in step i) to generate Ucn3 cDNA; and
 - iii) performing a quantitative real-time polymerase chain reaction (qRT-PCR) assay using a set of probes and/or primers specific for Ucn3 cDNA to detect the level of Ucn3 mRNA expression in the β cell, wherein the amount of Ucn3 cDNA detected is indicative of the level of Ucn3 mRNA expression in the β cell.
10. The method of any one of claims 7 to 9, wherein comparing in c) comprises displaying a graph showing the relative expression of Ucn3 mRNA in the β cell compared to the relative expression of Ucn3 mRNA in the normal mature β cell.
11. The method of any one of claims 1 to 10, further comprising detecting the level of insulin expression in the β cell.
12. The method of claim 11, wherein detecting comprises detecting the level of expression of insulin mRNA or protein.

13. The method of claim 11 or 12, wherein detecting the level of expression of insulin protein comprises performing an immunostain using an antibody specific for insulin protein to detect the level of insulin protein expression in the β cell.

14. The method of any one of claims 11 to 13, wherein detecting the level of expression of insulin protein comprises performing an immunostain using a primary antibody specific for insulin protein and a secondary antibody-fluorescent dye conjugate specific for the primary antibody to detect the level of insulin protein expression in the β cell.

15. The method of claims 13 or 14, further comprising imaging the immunostain using a microscope to obtain a micrograph displaying insulin protein expressed in the β cell in a fluorescent color indicative of the level of insulin protein expressed in the β cell.

16. The method of any one of claims 13 to 15, further comprising comparing the level of insulin expression in the β cell to the level of insulin expression in the normal mature β cell.

17. The method of claim 16, wherein comparing comprises displaying a first micrograph showing the results of an immunostain for insulin protein in the β cell and displaying a second micrograph showing the results of an immunostain for insulin protein in the normal mature β cell.

18. The method of any one of claims 11 to 17, wherein detecting the level of expression of insulin mRNA in the β cell comprises performing a hybridization based assay using one or more nucleic acids which hybridizes to a nucleic acid encoding insulin.

19. The method of any one of claims 11 to 18, wherein detecting the level of expression of insulin mRNA in the β cell comprises performing a polymerase chain reaction based assay using a set of primers and probes specific for insulin cDNA, wherein the level of insulin cDNA detected is indicative of the level of insulin mRNA in the β cell.

20. The method of any one of claims 11 to 19, wherein detecting the level of expression of insulin mRNA in the β cell comprises:
- iv) isolating total RNA comprising insulin mRNA from the β cell;
 - v) reverse transcribing the insulin RNA isolated in step i) to generate insulin cDNA; and
 - vi) performing a quantitative real-time polymerase chain reaction (qRT-PCR) assay using a set of probes and/or primers specific for insulin cDNA to detect the level of insulin mRNA expression in the β cell, wherein the amount of insulin cDNA detected is indicative of the level of insulin mRNA expression in the β cell.
21. The method of any one of claims 18 to 20, further comprising comparing the level of expression of insulin mRNA in the β cell to the level of expression of insulin mRNA in a normal mature β cell.
22. The method of claim 21, wherein comparing comprises displaying a graph showing the relative expression of insulin mRNA in the β cell compared to the relative expression of Ucn3 mRNA in the normal mature β cell.
23. The method of any one of claims 1 to 22, wherein de-differentiation comprises an early stage of de-differentiation in which Ucn3 expression decreases and insulin expression remains the same or increases.
24. The method of any one of claim 1 to 22, wherein de-differentiation comprises a late stage of de-differentiation in which Ucn3 expression decreases and insulin expression decreases.
25. The method of any one of claims 1 to 24, wherein de-differentiation is characterized by decreased expression of at least one marker of mature β cells comprising FoxO1, MafA, NeuroD, Nkx6.1, and Pdx1.
26. The method of any one of claims 1 to 25, wherein re-differentiation is characterized by increased expression of at least one marker of mature β cells comprising FoxO1, MafA, NeuroD, Nkx6.1, and Pdx1.

27. The method of any one of claim 1 to 26, wherein de-differentiation is characterized by the absence of an appropriate glucose stimulated insulin secretion (GSIS) response in the β cell.
28. The method of any one of claims 1 to 26, wherein re-differentiation is characterized by the presence of an appropriate GSIS response in the β cell.
29. The method of any one of claims 1 to 28, wherein the β cell obtained in a) comprises (i) a β cell in or isolated from an islet or a pancreas; or (ii) a β cell differentiated *in vitro*.
30. The method of any one of claims 1 to 29, wherein the β cell is obtained from a subject who is (i) has diabetes; (ii) is at risk of developing diabetes; (iii) is developing diabetes; or (iv) is suspected of having or developing diabetes.
31. The method of any one of claim 1 to 30, wherein the β cell obtained from a subject who is (i) non-diabetic; (ii) mildly diabetic, or (iii) severely diabetic.
32. A method of preventing de-differentiation of a β cell, comprising contacting a β cell with an agent that inhibits transforming growth factor- β (TGF β) superfamily signaling.
33. The method of claim 32, wherein preventing de-differentiation of the β cell causes the β cell to: (i) increase or maintain expression levels of Ucn3; (i) increase or maintain expression levels of at least one marker of mature β cells comprising FoxO1, MafA, NeuroD, Nkx6.1, and Pdx1; and/or (iii) preserve an appropriate GSIS response in the β cell.
34. A method of reversing de-differentiation of a β cell, comprising contacting a de-differentiated β cell with an agent that inhibits transforming growth factor- β (TGF β) superfamily signaling.
35. The method of claim 34, wherein the de-differentiated β cell comprises:
(a) a de-differentiated β cell in an early stage of de-differentiation that exhibits at least one of

- (i) decreased Ucn3 expression and increased or unchanged insulin expression;
 - (ii) decreased expression of at least one marker of mature β cells comprising FoxO1, MafA, NeuroD, Nkx6.1, and Pdx1; and/or
 - (iii) lack of an appropriate GSIS response; or
- (b) a de-differentiated β cell in a late stage of de-differentiation that exhibits at least one of
- (i) decreased Ucn3 expression and decreased insulin expression;
 - (ii) decreased expression of at least one marker of mature β cells comprising FoxO1, MafA, NeuroD, Nkx6.1, and Pdx1; and/or
 - (iii) lack of an appropriate GSIS response.

36. The method of claim 34, wherein reversing de-differentiation of the β cell causes the β cell to: (i) increase expression levels of Ucn3; (i) increase expression levels of at least one marker of mature β cells comprising MafA, Nkx6.1, Pdx1, NeuroD, and FoxO1; and/or (iii) exhibit an appropriate GSIS response in the β cell.

37. The method of any one of claims 32 to 36, wherein TGF β superfamily signaling comprises artemin signaling through receptor tyrosine kinase RET or a GFR α 3 receptor (GRF α 3).

38. The method of any one of claims 32 to 37, wherein the at least one agent comprises an inhibitor of RET or an inhibitor of GFR α 3.

39. The method of any one of claims 32 to 38, wherein the at least one agent comprises PHA-739358 or an analog or derivative thereof.

40. The method of any one of claims 32 to 38, wherein the at least one agent comprises VEGFR inhibitor V or an analog or derivative thereof.

41. The method of any one of claims 32 to 40, wherein TGF β superfamily signaling comprises TGF β signaling through a receptor serine/threonine kinase.

42. The method of any one of claims 32 to 41, wherein the at least one agent comprises Alk5 inhibitor II or an analog or derivative thereof.

43. The method of any one of claims 32 to 42, wherein the at least one agent comprises ALK5 inhibitor I or an analog or derivative thereof.
44. The method of any one of claims 32 to 43, wherein the at least one agent comprises a SMAD3 inhibitor or an analog or derivative thereof.
45. The method of any one of claims 32 to 44, further comprising detecting de-differentiation of the β cell or de-differentiated β cell.
46. The method of claim 45, wherein de-differentiation of the β cell or de-differentiated β cell is detected (i) prior to contacting, (ii) contemporaneously with contacting, or (iii) after contacting.
47. The method of any one of claims 32 to 46, wherein contacting occurs *in vitro* or *ex vivo*.
48. The method of any one of claims 32 to 47, wherein contacting occurs *in vivo*.
49. The method of claim 48, wherein the *in vivo* contact occurs in a subject.
50. The method of claim 49, wherein the subject (i) has diabetes; (ii) is at risk of developing diabetes; (iii) is developing diabetes; or (iv) is suspected of having or developing diabetes.
51. The method of claims 49 or 50, wherein the subject is (i) non-diabetic; (ii) mildly diabetic, or (iii) severely diabetic.
52. The method of claims 49 to 51, further comprising administering to the subject a conventional anti-diabetes therapy.
53. A method of identifying at least one candidate agent for preventing β cell de-differentiation, comprising:
- a) contacting a β cell with at least one test agent under conditions which cause β cell de-differentiation to occur; and
 - b) assessing the level of Ucn3 expression in β cell in the presence of the at least one test agent, wherein the at least one test agent is identified as at least one candidate agent

for preventing β cell de-differentiation if the level of Ucn3 expression in the β cell does not decrease in the presence of the at least one test.

54. The method of claim 53, wherein the conditions which cause β cell de-differentiation to occur comprise stress induced by a cytokine comprising IL-1 β , TNF α , IFN γ , and combinations thereof.

55. The method of claims 53 or 54, further comprising assessing the level of expression of at least one marker of mature β cells comprising FoxO1, MafA, Nkx6.1, and/or Pdx1, wherein the at least one test agent is identified as at least one candidate agent for preventing β cell de-differentiation if the level of expression of the at least one marker of mature β cells in the β cell does not decrease in the presence of the at least one test agent.

56. The method of any one of claims 53 to 55, further comprising conducting a GSIS assay on the β cell, wherein the at least one test agent is identified as at least one candidate agent for preventing β cell de-differentiation if the β cell maintains its ability to exhibit an appropriate GSIS response in the presence of the at least one test agent.

57. A method of identifying at least one candidate agent for reversing β cell de-differentiation, comprising:

- a) contacting a de-differentiated β cell with at least one test agent; and
- b) assessing the level of Ucn3 expression in the de-differentiated β cell in the presence and absence of the at least one test agent, wherein the at least one test agent is identified as at least one candidate agent for reversing β cell de-differentiation if the level of Ucn3 expression in the de-differentiated β cell increases in the presence of the at least one test agent compared to the level of Ucn3 expression in the de-differentiated β cell in the absence of the at least one test agent.

58. The method of claims 57, further comprising measuring the level of expression of at least one marker of mature β cells comprising FoxO1, MafA, Nkx6.1, and Pdx1, wherein the at least one test agent is identified as a candidate agent for reversing β cell de-differentiation if the level of expression of the at least one marker of mature β cells detected in the de-differentiated β cell increases in the presence of the at least one test

agent relative the level of expression of the at least one marker of mature β cells detected in the de-differentiated β cell in the absence of the at least one test agent.

59. The method of claims 57 or 58, further comprising conducting a GSIS assay on the de-differentiated β cell, wherein the at least one test agent is identified as a candidate agent for reversing β cell de-differentiation if the de-differentiated β cell gains the ability to exhibit an appropriate GSIS response after exposure to the at least one test agent.

60. The method of any one of claims 57 to 59, wherein the de-differentiated β cell comprises: (i) a β cell de-differentiated by culturing in adherent conditions, or (ii) a de-differentiated β cell obtained from a diabetic subject.

61. A method of identifying at least one candidate agent for reversing β cell de-differentiation, comprising:

- a) contacting a β cell with at least one test agent; and
- b) assessing the ability of the at least one test agent to inhibit Alk5 signaling, wherein at least one test agent that demonstrates the ability to inhibit Alk5 signaling comprises at least one candidate agent for reversing β cell de-differentiation.

62. The method of claim 61, further comprising assessing the ability of the at least one candidate agent to reverse β cell de-differentiation, wherein assessing the ability of the at least one candidate agent to reverse β cell de-differentiation comprises:

- a) contacting a de-differentiated β cell with the at least one candidate agent; and
- b) detecting the level of Ucn3 expression in the de-differentiated β cell, wherein at least one candidate agent demonstrates the ability to reverse β cell de-differentiation if the level of Ucn3 expression detected in the de-differentiated β cell increases in the presence of the at least one candidate agent relative the level of Ucn3 expression detected in the de-differentiated β cell in the absence of the at least one candidate agent.

63. The method of claims 61 or 62, further comprising measuring the level of expression of at least one marker of mature β cells comprising FoxO1, MafA, Nkx6.1, and Pdx1, wherein the at least one candidate agent demonstrates the ability to reverse β cell de-differentiation if the level of expression of the at least one marker of mature β

cells detected in the de-differentiated β cell increases in the presence of the at least one candidate agent relative the level of expression of the at least one marker of mature β cells detected in the de-differentiated β cell in the absence of the at least one candidate agent.

64. The method of any one of claims 61 to 63, further comprising conducting a GSIS assay on the de-differentiated β cell, wherein the at least one candidate agent demonstrates the ability to reverse β cell de-differentiation if the de-differentiated β cell gains the ability to exhibit an appropriate GSIS response after exposure to the at least one candidate agent.

65. A method of preventing a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of a β cell de-differentiation preventing agent.

66. A method of treating a β cell de-differentiation-related disorder, the method comprising administering to a subject in need thereof an effective amount of a β cell de-differentiation reversing agent.

67. The method of claims 65 or 66, wherein the β cell de-differentiation preventing agent and the β cell de-differentiation reversing agent comprise an inhibitor of TGF β superfamily signaling.

68. The method of any one of claims 65 to 67, wherein the β cell de-differentiation preventing agent and the β cell de-differentiation reversing agent comprise an inhibitor of artemin signaling.

69. The method of any one of claims 65 to 68, wherein the β cell de-differentiation preventing agent and the β cell de-differentiation reversing agent comprises an inhibitor of receptor tyrosine kinase RET or an inhibitor of receptor GRF α 3.

70. The method of any one of claims 65 to 69, wherein the β cell de-differentiation preventing agent and the β cell de-differentiation reversing agent comprises PHA-739358 or an analog or derivative thereof.

71. The method of any one of claims 65 to 70, wherein the β cell de-differentiation preventing agent and the β cell de-differentiation reversing agent comprises VEGFR inhibitor V or an analog or derivative thereof.

72. The method of any one of claims 65 to 71, wherein the β cell de-differentiation preventing agent and the β cell de-differentiation reversing agent comprise an inhibitor of Alk5 signaling.

73. The method of any one of claims 65 to 72, wherein the β cell de-differentiation preventing agent and the β cell de-differentiation reversing agent comprises Alk5 inhibitor II or an analog or derivative thereof.

74. The method of any one of claims 65 to 73, wherein the β cell de-differentiation preventing agent and the β cell de-differentiation reversing agent comprises ALK5 inhibitor I or an analog or derivative thereof.

75. The method of any one of claims 65 to 74, wherein the β cell de-differentiation preventing agent and the β cell de-differentiation reversing agent comprises a SMAD3 inhibitor or an analog or derivative thereof.

76. The method of any one of claims 65 to 75, wherein the subject in need of is a subject who (i) is in need of additional β cell; (ii) has diabetes; (iii) is at risk of developing diabetes; (iv) is developing diabetes; (v) is suspected of having or developing diabetes; (vi) is non-diabetic; (vii) is mildly diabetic; or (viii) severely diabetic.

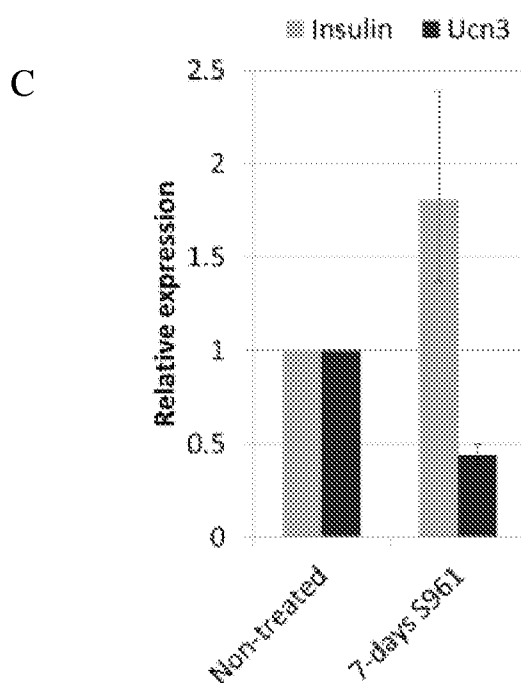
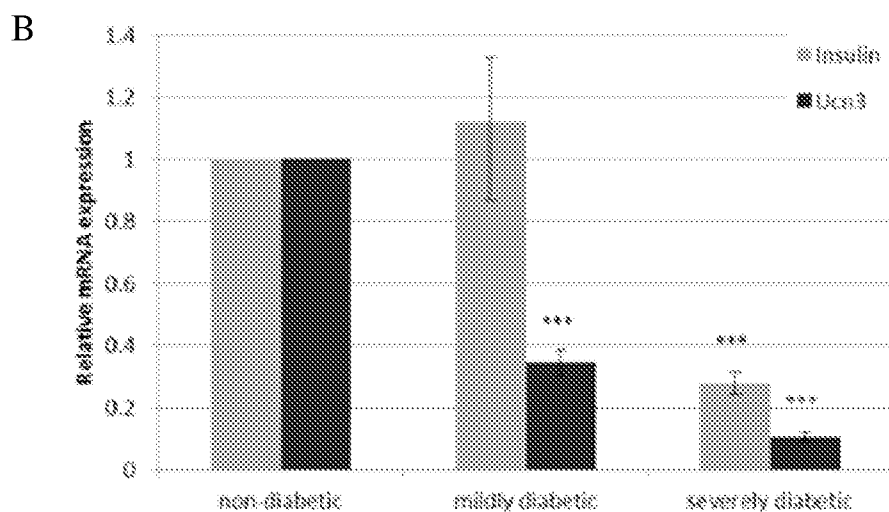
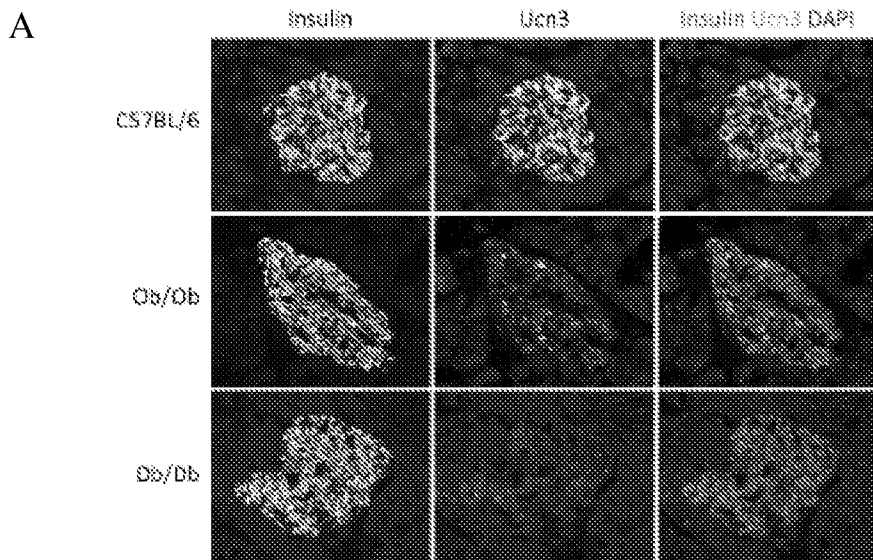
77. The method of any one of claims 65 to 75, wherein the subject has, is developing or is at risk of developing, or is suspected of having metabolic syndrome or obesity.

78. The method of any one of claims 65 to 77, wherein the β cell de-differentiation-related disorder is selected from the group consisting of pre-diabetes, type I diabetes, type II diabetes, type 1.5 diabetes, obesity, metabolic syndrome, or hyperlipidemia.

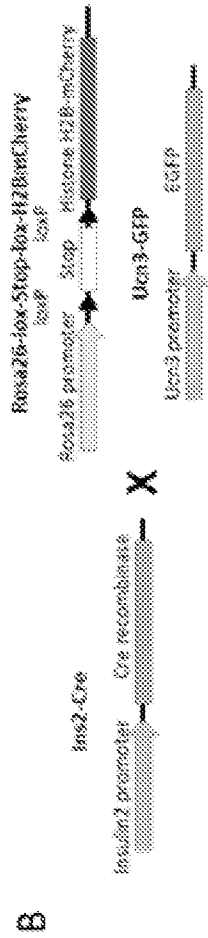
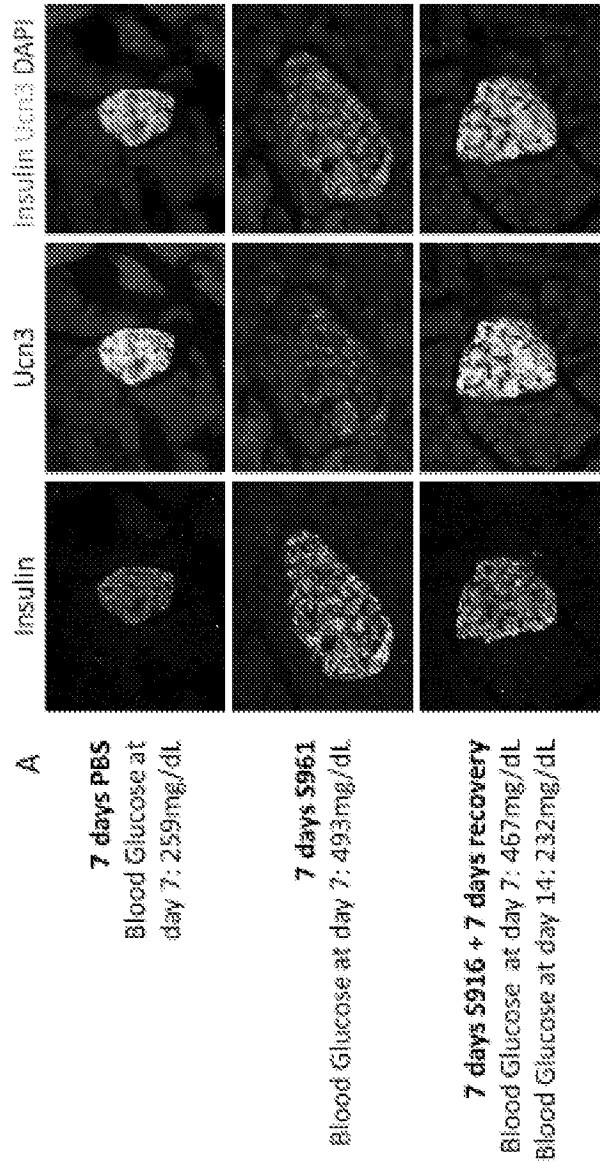
79. The method of any one of claims 65 to 78, further comprising selecting a subject in need of treatment for a β cell de-differentiation-related disorder.

80. The method of any one of claims 65 to 79, further comprising detecting β cell de-differentiation in the subject.

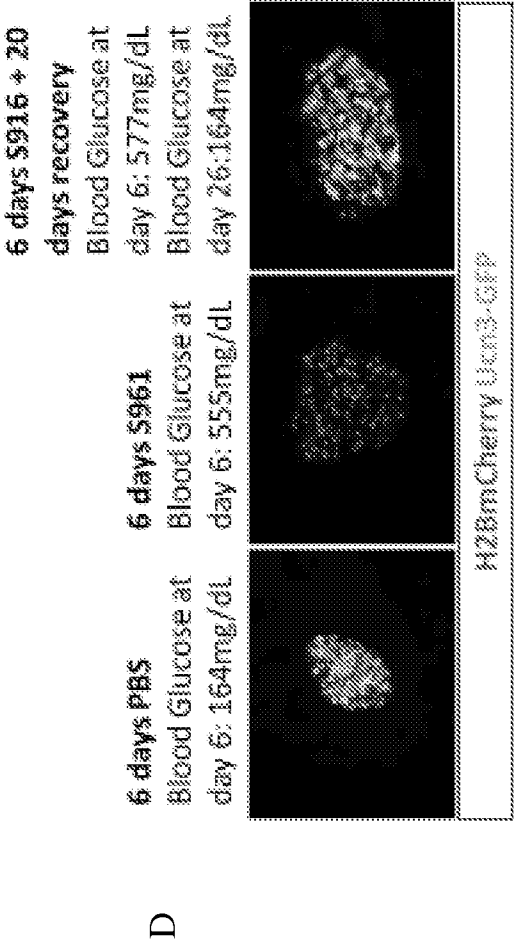
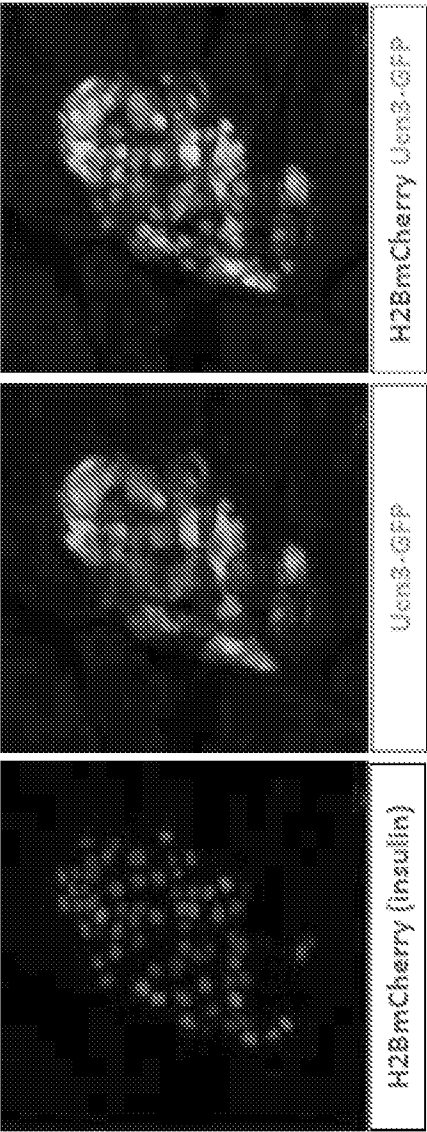
81. The method of any one of claims 65 to 80, further comprising administering to the subject an effective amount of an anti-diabetic agent.



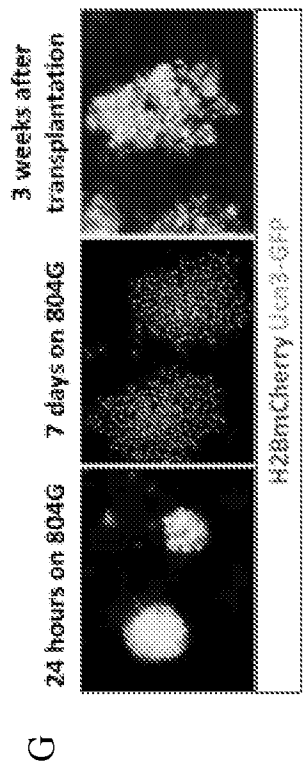
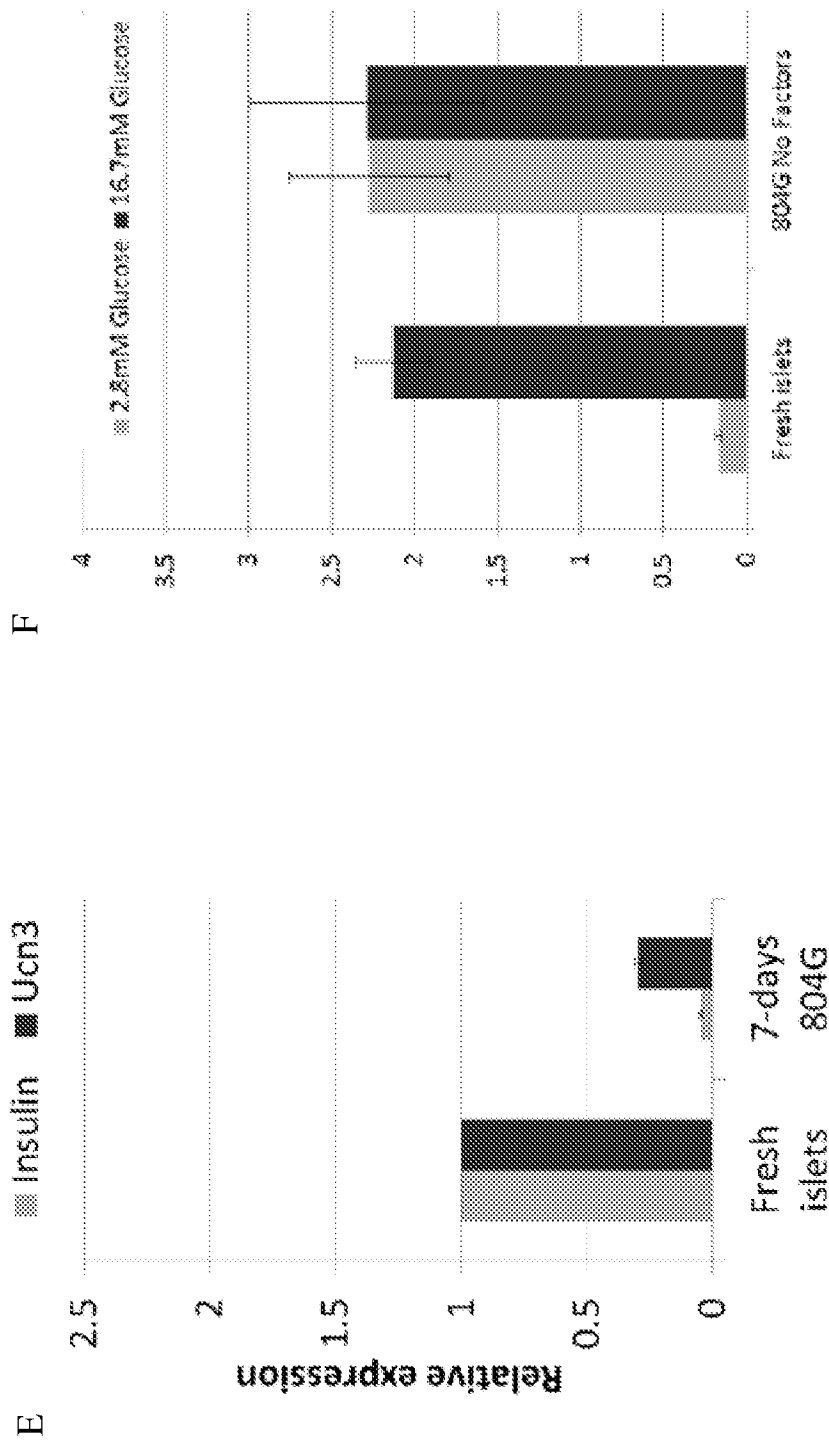
FIGS. 1A-1C



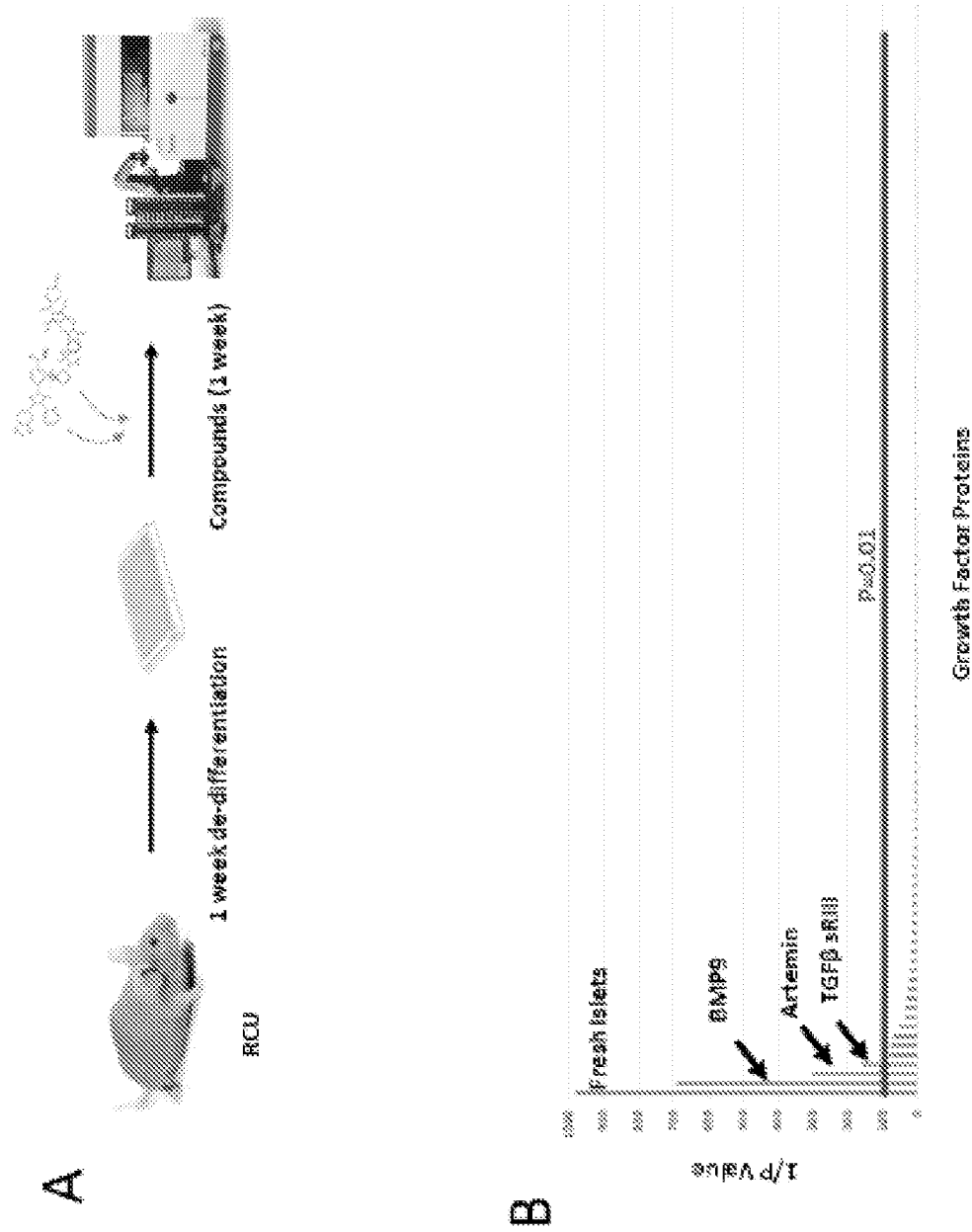
FIGS. 2A-2B



FIGS. 2C-2D

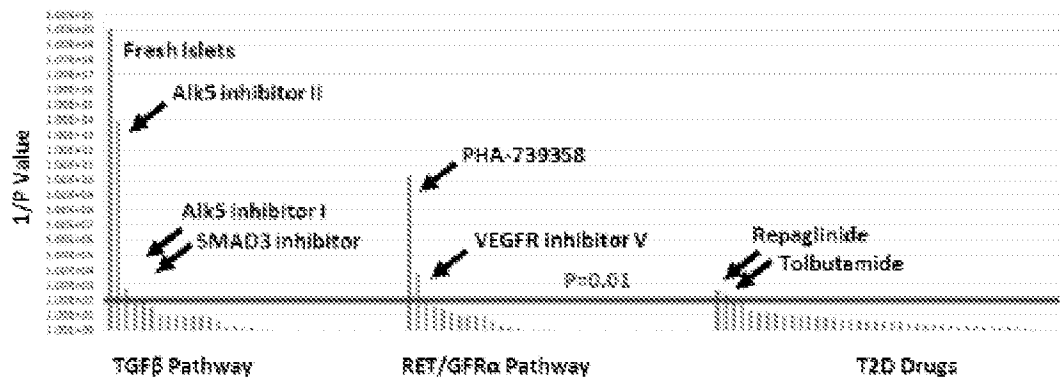


FIGS. 2E-2G

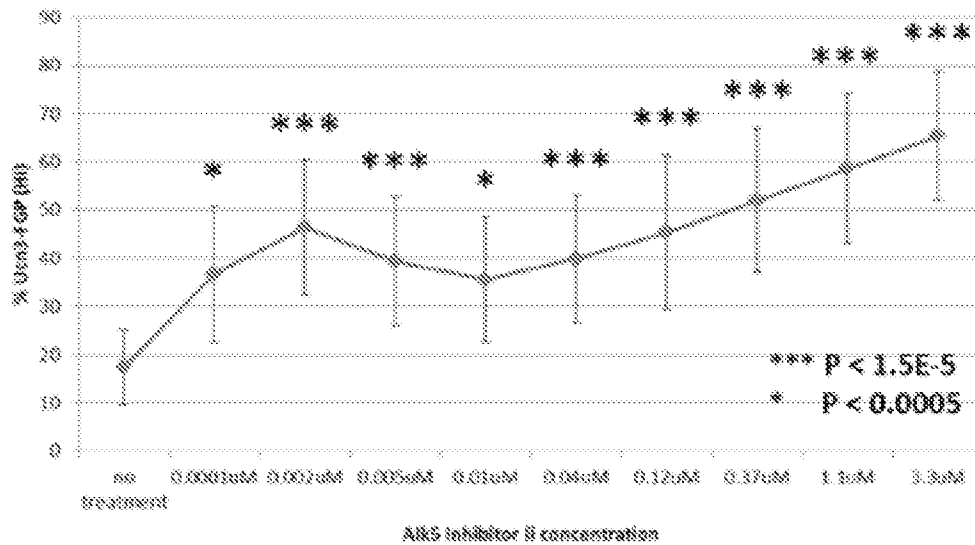


FIGS. 3A-3B

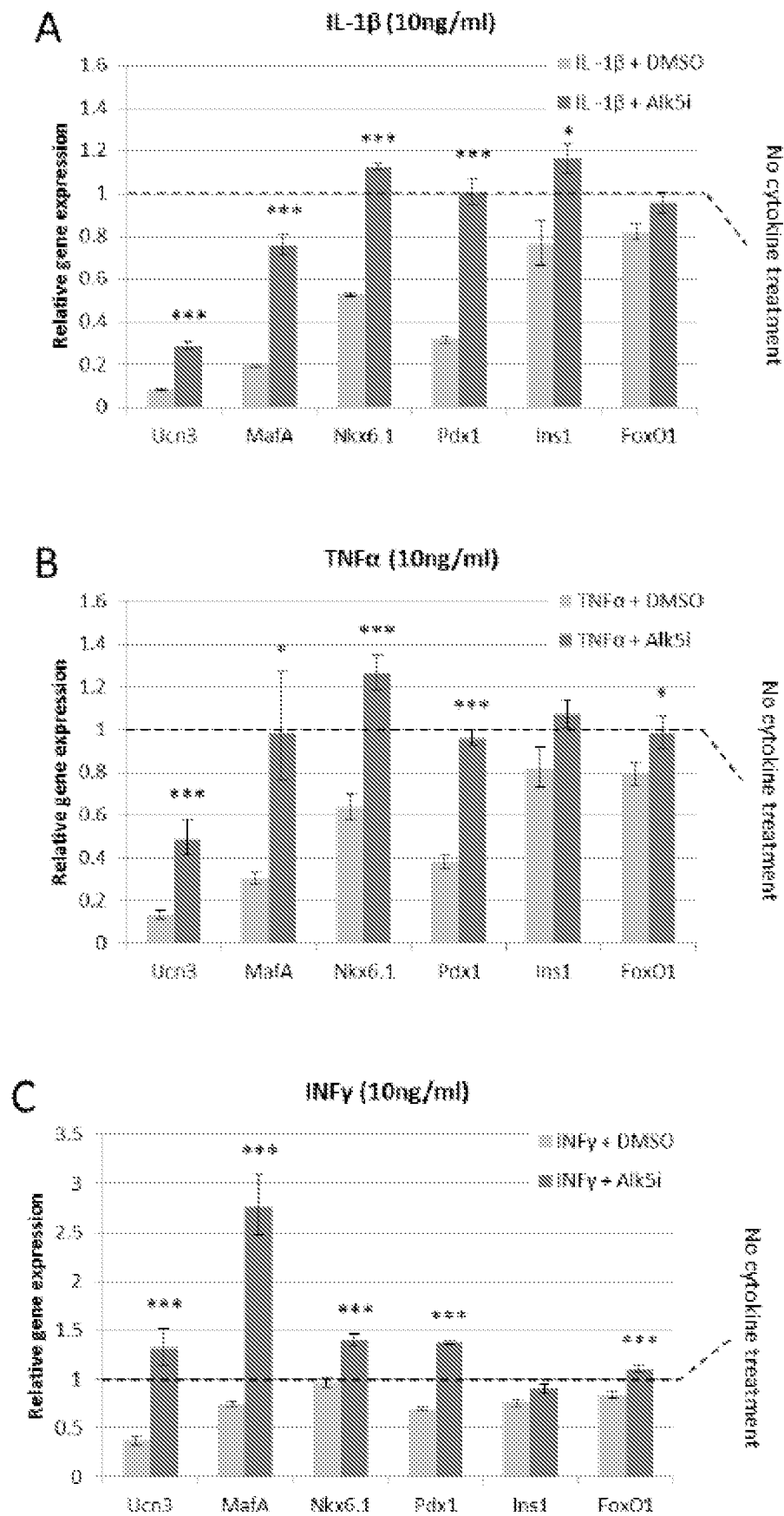
C



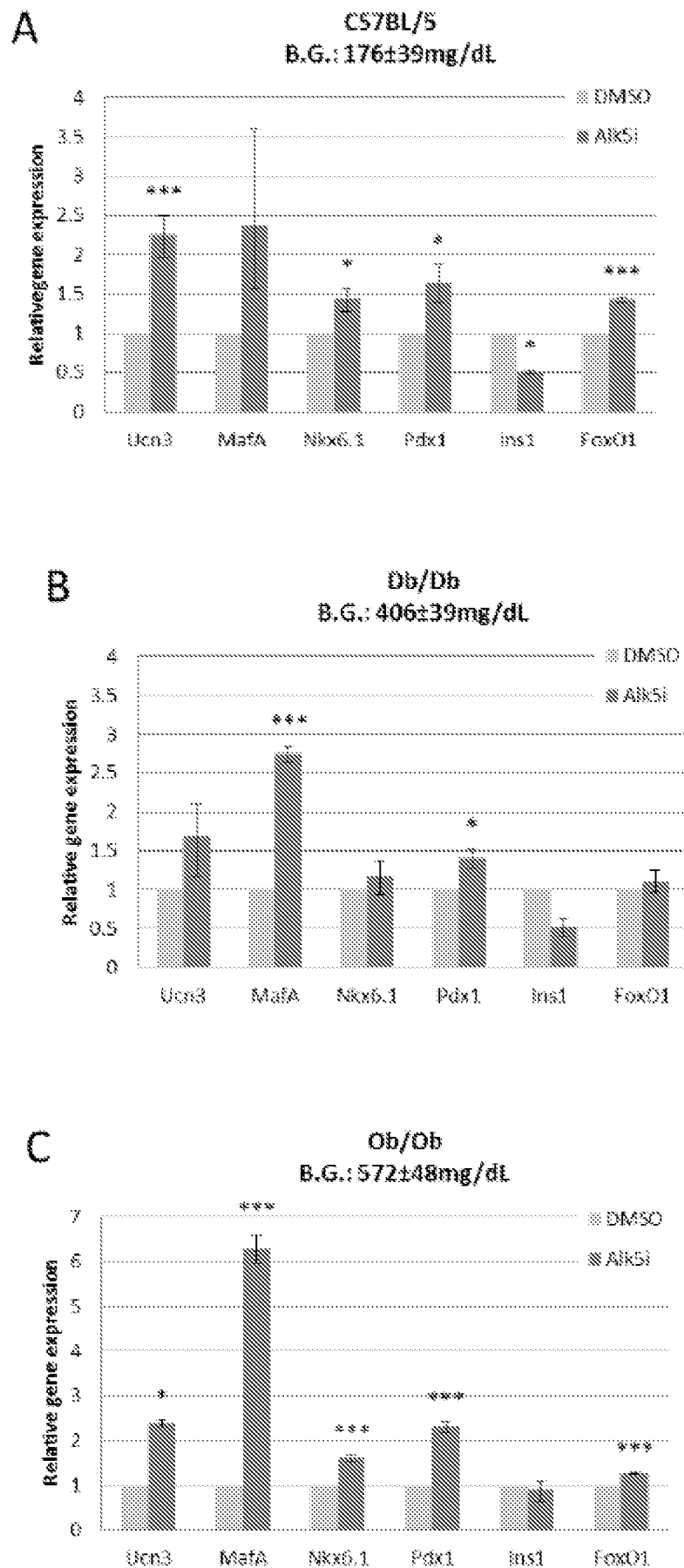
D



FIGS. 3C-3D

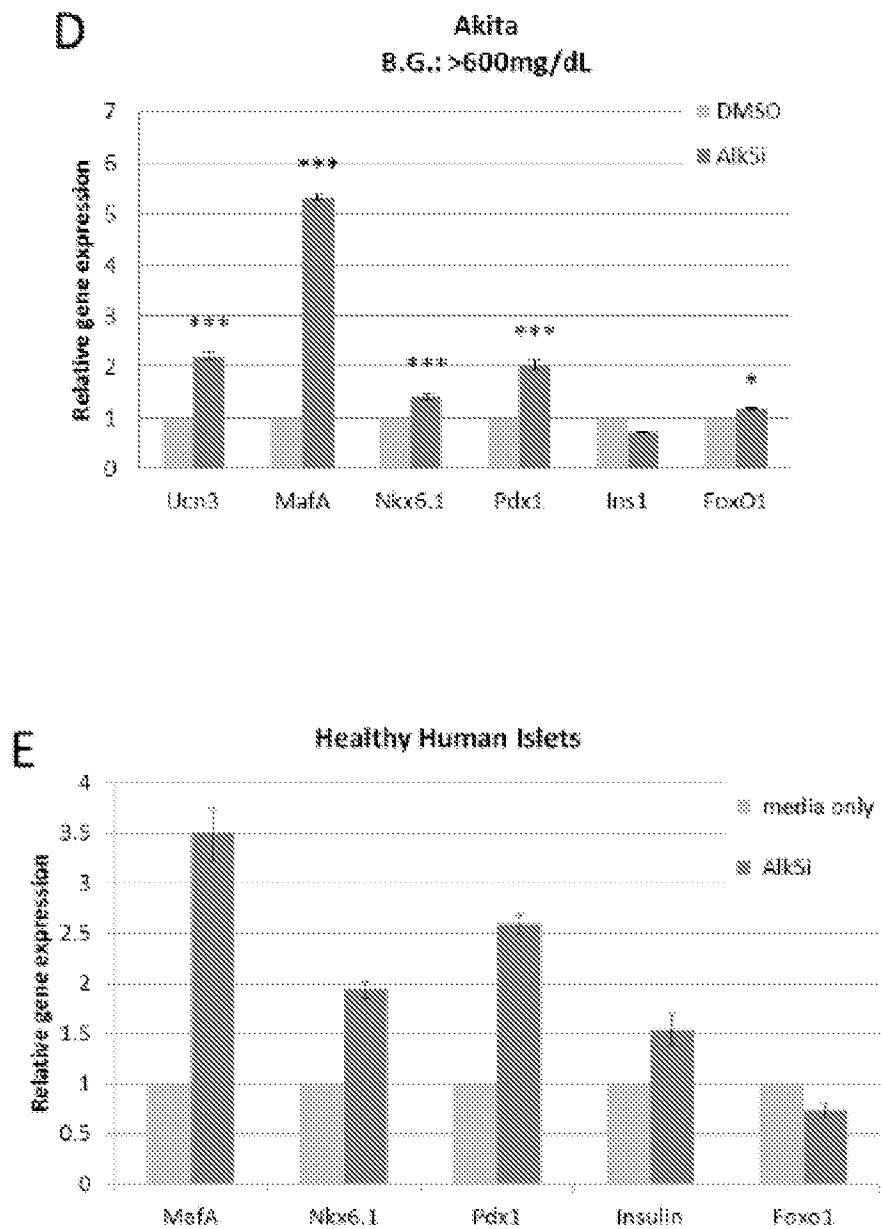


FIGS. 4A-4C



FIGS. 5A-5C

SUBSTITUTE SHEET (RULE 26)



FIGS. 5D-5E