CLAIMS

What is claimed is:

1. A stem cell-derived β cell (SC-β).

2. The cell according to claim 1, wherein the cell is mature.

3. The cell according to claims 1 or 2, wherein the cell exhibits an *in vitro* glucose stimulated insulin secretion (GSIS) response.

4. The cell according to any one of claims 1 to 3, wherein the cell exhibits an *in vivo* GSIS response.

5. The cell according to any one of claims 1 to 4, wherein the cell exhibits *in vitro* and *in vivo* glucose stimulated insulin secretion (GSIS) responses.

6. The cell according to any one of claims 1 to 5, wherein the cell exhibits a GSIS response to at least one glucose challenge.

7. The cell according to any one of claims 1 to 6, wherein the cell exhibits a GSIS response to at least two sequential glucose challenges.

8. The cell according to any one of claims 1 to 7, wherein the cell exhibits a GSIS response to at least three sequential glucose challenges.

9. The cell according to any one of claims 1 to 8, wherein the GSIS response is observed immediately upon transplanting the cell into a human or animal.

10. The cell according to any one of claims 1 to 9, wherein the GSIS response is observed within approximately 24 hours of transplanting the cell into a human or animal.

11. The cell according to any one of claims 1 to 10, wherein the GSIS response is observed within approximately two weeks of transplanting the cell into a human or animal.

12. The cell according to any one of claims 1 to 11, wherein the stimulation index of the cell as characterized by the ratio of insulin secreted in response to high glucose concentrations compared to low glucose concentrations is similar to the stimulation index of an endogenous mature pancreatic β cell.

13. The cell according to any one of claims 1 to 12, wherein the stimulation index is greater than or equal to 1, or greater than or equal to 1.1, or greater than or equal to 1.3, or greater than or equal to 2, or greater than or equal to 2.3, or greater than or equal to 2.6.

14. The cell according to any one of claims 1 to 13, wherein the cell exhibits cytokine-induced apoptosis in response to a cytokine.

15. The cell according to any one of claims 1 to 14, wherein the cytokine is selected from the group consisting of interleukin 1β (IL-β), interferon-γ (INF-γ), tumor necrosis factor-α (TNF-α), and combinations thereof.

16. The cell according to any one of claims 1 to 15, wherein insulin secretion from the cell is enhanced in response to an anti-diabetic agent.

17. The cell according to claim 16, wherein the anti-diabetic agent comprises a secretagogue selected from the group consisting of an incretin mimetic, a sulfonylurea, a meglitinide, and combinations thereof.

18. The cell according to any one of claims 1 to 17, wherein the cell is monohormonal.

19. The cell according to any one of claims 1 to 18, wherein the cell exhibits a morphology that resembles the morphology of an endogenous mature pancreatic β cell.

20. The cell according to any one of claims 1 to 19, wherein the cell exhibits encapsulated crystalline insulin granules under electron microscopy that resemble insulin granules of an endogenous mature pancreatic β cell.

21. The cell according to any one of claims 1 to 20, wherein the cell exhibits a low rate of replication.

22. The cell according to any one of claims 1 to 21, wherein the cell exhibits a glucose stimulated Ca^{2+} flux (GSCF) that resembles the GSCF of an endogenous mature pancreatic β cell.

23. The cell according to any one of claims 1 to 22, wherein the cell exhibits a GSCF response to at least one glucose challenge.

24. The cell according to any one of claims 1 to 23, wherein the cell exhibits a GSCF response to at least two glucose challenges.

25. The cell according to any one of claims 1 to 24, wherein the cell exhibits a GSCF response to at least three glucose challenges.

26. The cell according to any one of claims 1 to 25, wherein the cell exhibits an increased calcium flux.

27. The method of claim 26, wherein the increased calcium flux comprises an increased amount of influx or a ratio of influx at low relative to high glucose concentrations.

28. The cell according to any one of claims 1 to 27, wherein the cell expresses at least one marker characteristic of an endogenous mature pancreatic β cell selected from the group consisting of insulin, C-peptide, PDX1, MAFA, NKX6-1, PAX6, NEUROD1, glucokinase (GCK), SLC2A1, PCSK1, KCNJ11, ABCC8, SLC30A8, SNAP25, RAB3A, GAD2, PTPRN, NKX2-2, Pax4.

29. The cell according to any one of claims 1 to 28, wherein the cell does not express at least one marker selected from the group consisting of

a) a hormone selected from the group consisting of

i) glucagon (GCG), and

ii) somatostatin (SST); or

b) an acinar cell marker selected from the group consisting of

i) amylase, and

ii) carboxypeptdase A (CPA1);

c) an α cell marker selected from the group consisting of

i) GCG,

ii) Arx,

iii) Irx1, and

IR; and

d) a ductal cell marker selected from the group consisting of

i) CFTR, and

ii) Sox9.

30. The cell according to any one of claims 1 to 29, wherein the cell is differentiated *in vitro* from an insulin-positive endocrine cell or a precursor thereof selected from the group consisting of a Nkx6-1-positive pancreatic progenitor cell, a Pdx1-positive pancreatic progenitor cell, and a pluripotent stem cell.

31. The cell according to claim 30, wherein the pluripotent stem cell is selected from the group consisting of an embryonic stem cell and induced pluripotent stem cell.

32. The cell according to any one of claims 1 to 31, wherein the cell is human.

33. The cell according to any one of claims 1 to 32, wherein the cell is not genetically modified.

34. The cell according to any one of claims 1 to 33, wherein the cell is genetically modified.

35. The cell according to any one of claims 1 to 34, wherein the insulin produced per cell is between 0.5 and 10 μIU per 1000 cells per 30 minute incubation at a high glucose concentration.

36. The cell according to any one of claims 1 to 35, wherein the insulin produced per cell is approximately 2.5 μIU per 1000 cells per 30 minute incubation at a high glucose concentration.

37. The cell according to claims 35 or 36, wherein the incubation occurs *ex vivo*.

38. A cell line comprising the cell according to any one of claims 1 to 37.

39. The cell line according to claim 38, wherein the cell line stably expresses insulin.

40. The cell line according to claims 38 or 39, wherein the cells can be frozen, thawed, and amplified with a doubling time of between about 24 and 44 hours without significant morphological changes until at least 30 passages.

41. A method of generating a SC-β cell from insulin-positive endocrine cells, the method comprising contacting a population of cells comprising insulin-positive endocrine cells under conditions that promote cell clustering with at least two β cell-maturation factors comprising a) a transforming growth factor β (TGF-β) signaling pathway inhibitor and b) a thyroid hormone signaling pathway activator, to induce the *in vitro* maturation of at least one insulin-positive endocrine cell in the population into a SC-β cell.

42. The method of claim 41, wherein the SC-β cell exhibits a response to at least one glucose challenge.

43. The method of claims 41 or 42, wherein the SC-β cell exhibits a response to at least two sequential glucose challenges.

44. The method of any one of claims 41 to 43, wherein the SC-β cell exhibits a response to at least three sequential glucose challenges.

45. The method of any one of claims 41 to 44, wherein the morphology of the SC-β cell resembles the morphology of an endogenous mature β cell.

46. The method of any one of claims 41 to 45, wherein the SC-β cell exhibits *in vitro* and/or *in vivo* glucose stimulated insulin secretion (GSIS) responses.

47. The method of any one of claims 41 to 46, wherein the GSIS response is observed immediately upon transplantation of the SC-β cell into a subject.

48. The method of any one of claims 41 to 47, wherein the GSIS response is observed within approximately 24 hours upon transplantation of the SC-β cell into a subject.

49. The method of any one of claims 41 to 48, wherein the GSIS response is observed within approximately two weeks of transplantation of the SC-β cell into a subject.

50. The method of any one of claims 41 to 49, wherein the population of cells is contacted with the TGF-β signaling pathway inhibitor at a concentration of between 100 nM – 100 μM.

51. The method of any one of claims 41 to 50, wherein the population of cells is contacted with the TGF-β signaling pathway inhibitor at a concentration of 10 μM.

52. The method of any one of claims 41 to 51, wherein the TGF-β signaling pathway comprises TGF-β receptor type I kinase signaling.

53. The method of any one of claims 41 to 52, wherein the TGF-β signaling pathway inhibitor comprises Alk5 inhibitor II.

54. The method of any one of claims 41 to 53, wherein the TGF-β signaling pathway inhibitor comprises an analog or derivative of Alk5 inhibitor II.

55. The method of any one of claims 41 to 54, wherein the population of cells is contacted with the thyroid hormone signaling pathway activator at a concentration of between 0.1 μM – 10 μM.

56. The method of any one of claims 41 to 55, wherein the population of cells is contacted with the thyroid hormone signaling pathway activator at a concentration of 1 μM.

57. The method of any one of claims 41 to 56, wherein the thyroid hormone signaling pathway activator comprises triiodothyronine (T3).

58. The method of any one of claims 41 to 57, wherein the population of cells is optionally contacted with a protein kinase inhibitor.

59. The method of claim 58, wherein the population of cells is not contacted with the protein kinase inhibitor.

60. The method of claim 58, wherein the population of cells is contacted with the protein kinase inhibitor.

61. The method of claim 60, wherein the population of cells is contacted with the protein kinase inhibitor at a concentration of between 10 nM – 1 μM.

62. The method of claims 60 or 61, wherein the population of cells is contacted with the protein kinase inhibitor at a concentration of 100 nM.

63. The method of any one of claims 60 to 62, wherein the protein kinase inhibitor comprises staurosporine.

64. The method of any one of claims 41 to 63, further comprising contacting the population of cells with at least one additional β cell-maturation factor.

65. The method of claim 64, wherein the at least one additional β cell-maturation factor comprises a cystic fibrosis transmembrane conductance regulator (CFTR) inhibitor.

66. The method of claims 64 or 65, wherein the population of cells is contacted with the CFTR inhibitor at a concentration of between 100 nM – 100 μM.

67. The method of any one of claims 64 to 66, wherein the population of cells is contacted with the CFTR inhibitor at a concentration of 10 nm – 10 μM.

68. The method of any one of claims 64 to 67, wherein the CFTR inhibitor comprises Gly-H101.

69. The method of any one of claims 64 to 68, wherein the at least one additional β cell-maturation factor comprises a O-GlcNAcase inhibitor.

70. The method of claim 69, wherein the population of cells is contacted with the O-GlcNAcase inhibitor at a concentration of between 100 nM – 100 μM.

71. The method of claims 69 or 70, wherein the population of cells is contacted with the O-GlcNAcase inhibitor at a concentration of between 10 nM – 10 μM.

72. The method of any one of claims 41 to 71, wherein the inhibitor of O-GlcNAcase comprises Thiamet G.

73. The method of any one of claims 41 to 72, wherein the population of cells is cultured in a suitable culture medium.

74. The method of claim 73, wherein the suitable culture medium comprises Connought Medical Research Laboratories 1066 supplemented islet media (CMRLS) or a component of CMRLS.

75. The method of claim 74, wherein the CMRLS is supplemented with serum.

76. The method of claims 74 or 75, wherein the CMRLS is supplemented with 10% fetal bovine serum.

77. The method of any one of claims 41 to 76, wherein the conditions that promote cell clustering comprise a suspension culture.

78. The method of any one of claim 41 to 77, wherein the population of cells is maintained in a suspension culture for a period of time sufficient to induce the *in vitro* maturation of at least one of the insulin-positive endocrine cells in the population of cells into at least one SC-β cell.

79. The method of claim 78, wherein the period of time comprises at least 7 days.

80. The method of claims 78 or 79, wherein the period of time comprises between 7 days and 21 days.

81. The method of any one of claims 78 to 60, wherein the period of time comprises between 7 and 14 days.

82. The method of any one of claims 78 to 81, wherein the period of time comprises between 10 and 14 days.

83. The method of any one of claims 78 to 82, wherein the period of time comprises 14 days.

84. The method of any one of claims 79 to 83, wherein the β cell-maturation factors are replenished every other day.

85. The method of any one of claims 41 to 84, wherein at least 1% of the insulin-positive endocrine cells in the population of cells are induced to mature into SC-β cells.

86. The method of any one of claims 41 to 85, wherein at least 99% of the insulin-positive endocrine cells in the population are induced to mature into SC-β cells.

87. The method of any one of claims 41 to 86, wherein at least 30% of the resulting cells in the population comprise SC-β cells.

88. The method of any one of claims 41 to 87, wherein the SC-β cells express C-peptide, insulin, NKX6-1, Pdx1, and co-express NKX6-1 and C-peptide.

89. The method of any one of claims 41 to 88, wherein the insulin-positive endocrine cells also express Pdx1 and NKX6-1.

90. The method of any one of claims 41 to 89, wherein the insulin-positive endocrine cells are produced from a population of pluripotent stem cells selected from the group consisting of embryonic stem cells and induced pluripotent stem cells.

91. The method of any one of claims 41 to 90, wherein the SC-β cells comprise human cells.

92. The method of any one of claims 41 to 91, wherein the generation of SC-β cells *in vitro* is scalable.

93. An isolated population of SC-β cells produced according to the methods of any one of claims 41 to 92.

94. A microcapsule comprising the isolated population of SC-β cells according to claim 93 encapsulated therein.

95. A composition comprising a population of SC-β cells produced according to the methods of any one of claims 41 to 92.

96. An assay comprising an isolated population of SC-β cells produced according to the methods of any one of claims 41 to 92.

97. The assay of claim 96, for use in identifying one or more candidate agents which promote or inhibit a β cell fate selected from the group consisting of β cell proliferation, β cell replication, β cell death, β cell function, β cell susceptibility to immune attack, or β cell susceptibility to dedifferentiation or differentiation.

98. The assay of claim 96, for use in identifying one or more candidate agents which promote the differentiation of at least one insulin-positive endocrine cell or a precursor thereof into at least one SC-β cell.

99. A method for the treatment of a subject in need thereof, the method comprising administering to a subject a composition comprising an isolated population of SC-β cells produced according to the methods of any one of claims 41 to 92.

100. The method of claim 99, wherein the SC-β cells are encapsulated in a microcapsule.

101. The method of claims 99 or 100, wherein the SC-β cells are produced from a population of pluripotent stem cells obtained from the same subject that the SC-β cells are administered to.

102. The method of any one of claims 99 to 101, wherein the SC-β cells are produced from a population of iPS cells, wherein the iPS cells are derived from a cell obtained from the same subject that the SC-β cells are administered to.

103. The method of any one of claims 99 to 102, wherein the subject has, or has an increased risk of developing, diabetes.

104. The method of claim 103, wherein the diabetes is selected from the group of Type I diabetes, Type II diabetes, Type 1.5 diabetes and pre-diabetes.

105. The method of claim any one of claims 99 to 102, wherein the subject has, or has an increased risk of developing a metabolic disorder.

106. Use of an isolated population of SC-β cells produced by the methods according to any one of claims 41 to 102 for administering to a subject in need thereof.

107. The use of claim 106, wherein the isolated population of SC-β cells is administered to the subject encapsulated in microcapsules.

108. The use of claims 106 or 107, wherein the subject has, or has an increased risk of developing diabetes.

109. The use of any one of claims 106 to 108, wherein the diabetes is selected from the group of Type I diabetes, Type II diabetes, Type 1.5 diabetes and pre-diabetes.

110. The use of claims 106 or 107, wherein the subject has, or has an increased risk of developing a metabolic disorder.

111. A culture medium comprising a) Alk5 inhibitor, b) triiodothyronine (T3), optionally c) staurosporine, and optionally d) CMRLS.

112. Use of the culture medium of claim 111 to induce the *in vitro* maturation of insulin-positive endocrine cells into SC-β cells, wherein the SC-β cells exhibit both an *in vitro* and/or *in vivo* GSIS response.

113. A method of producing a NKX6-1-positive pancreatic progenitor cell from a Pdx1-positive pancreatic progenitor cell comprising contacting a population of cells comprising Pdx1-positive pancreatic progenitor cells under conditions that promote cell clustering with at least two β cell-maturation factors comprising a) at least one growth factor from the fibroblast growth factor (FGF) family, b) a sonic

hedgehog pathway inhibitor, and optionally c) a low concentration of a retinoic acid (RA) signaling pathway activator, for a period of at least five days to induce the differentiation of at least one Pdx1-positive pancreatic progenitor cell in the population into NKX6-1-positive pancreatic progenitor cells, wherein the NKX6-1-positive pancreatic progenitor cells express NKX6-1.

114. The method of claim 113, wherein the population of cells is contacted with the at least one growth factor from the FGF family at a concentration of between 1 ng/mL - 100 ng/mL.

115. The method of claims 113 or 114, wherein the population of cells is contacted with the at least one growth factor from the FGF family at a concentration of 50 ng/mL.

116. The method of any one of claims 113 to 115, wherein the at least one growth factor from the FGF family comprises keratinocyte growth factor (KGF).

117. The method of any one of claims 113 to 116, wherein the at least one growth factor from the FGF family is selected from the group consisting of FGF2, FGF8B, FGF10, and FGF21.

118. The method of any one of claims 113 to 117, wherein the population of cells is not contacted with the RA signaling pathway activator.

119. The method of any one of claims 113 to 117, wherein the population of cells is contacted with the RA signaling pathway activator at a concentration of between 0.01 μM – 1.0 μM.

120. The method of any one of claims 113 to 117, wherein the population of cells is contacted with the RA signaling pathway activator at a concentration of 0.1 μM.

121. The method of any one of claims 113 to 120, wherein the RA signaling pathway activator comprises RA.

122. The method of any one of claims 113 to 121, wherein the population of cells is contacted with the SHH pathway inhibitor at a concentration of between 0.1 μM and 0.5 μM.

123. The method of any one of claims 113 to 122, wherein the population of cells is contacted with the SHH pathway inhibitor at a concentration of 0.25 μM.

124. The method of any one of claims 113 to 123, wherein the SHH pathway inhibitor comprises Sant1.

125. The method of any one of claims 113 to 124, further comprising exposing the population of cells to at least one additional β cell-maturation factor.

126. The method of claim 125, wherein the at least one additional β cell-maturation factor comprises at least one growth factor from the EGF family.

127. The method of claim 126, wherein the population of cells is exposed to the at least one growth factor from the EGF family at a concentration of between 2 ng/mL - 200 ng/mL.

128. The method of claims 126 or 127, wherein the population of cells is exposed to the at least one growth factor from the EGF family at a concentration of 20 ng/mL.

129. The method of any one of claims 126 to 128, wherein at least one growth factor from the EGF family is selected from the group consisting of betacellulin and EGF.

130. The method of any one of claims 113 to 129, wherein the population of cells is cultured in a suitable culture medium.

131. The method of any one of claims 113 to 130, wherein the conditions that promote cell clustering comprise a suspension culture.

132. The method of any one of claims 113 to 131, wherein the β cell-maturation factors are replenished every other day.

133. The method of any one of claims 113 to 132, wherein an activator of protein kinase C is not added to the suspension culture during the 5 days.

134. The method of any one of claims 113 to 132, wherein an activator of protein kinase C is removed from the suspension culture prior to the 5 days.

135. The method of claims 113 or 134, wherein the activator of protein kinase C comprises PdbU.

136. The method of any one of claims 113 to 135, wherein a BMP signaling pathway inhibitor is not added to the suspension culture during the 5 days.

137. The method of any one of claims 113 to 135, wherein a BMP signaling pathway inhibitor is removed from the suspension culture prior to the 5 days.

138. The method of claims 136 or 137, wherein the BMP signaling pathway inhibitor comprises LDN193189.

139. The method of any one of claims 113 to 138, wherein at least 10% of the Pdx1-positive pancreatic progenitor cells in the population are induced to differentiate into NKX6-1-positive pancreatic progenitor cells.

140. The method of any one of claims 113 to 139, wherein at least 95% of the Pdx1-positive pancreatic progenitor cells in the population are induced to differentiate into NKX6-1-positive pancreatic progenitor cells.

141. The method of any one of claims 113 to 140, wherein the NKX6-1-positive pancreatic progenitor cells express Pdx1, NKX6-1, and FoxA2.

142. The method of any one of claims 113 to 141, wherein the Pdx1-positive pancreatic progenitor cells are produced from a population of pluripotent stem cells selected from the group consisting of embryonic stem cells and induced pluripotent stem cells.

143. An isolated population of NKX6-1-positive pancreatic progenitor cells obtained by the method of any one of claims 113 to 142.

144. A microcapsule comprising the isolated population of NKX6-1-positive pancreatic progenitor cells according to claim 143 encapsulated therein.

145. A composition comprising an isolated population of NKX6-1-positive pancreatic progenitor cells produced according to the methods of any one of claims 113 to 142.

146. An assay comprising an isolated population of NKX6-1-positive pancreatic progenitor cells produced according to the methods of any one of claims 113 to 142.

147. The assay of claim 146, for use in identifying one or more candidate agents which promote the differentiation of at least one Pdx1-positive pancreatic progenitor cell or precursor thereof into NKX6-1-positive pancreatic progenitor cells.

148. A method for the treatment of a subject in need thereof, the method comprising administering to a subject a composition comprising an isolated population of NKX6-1-positive pancreatic progenitor cells produced according to the methods of any one of claims 113 to 142.

149. The method of claim 148, wherein the NKX6-1-positive pancreatic progenitor cells are produced from a population of pluripotent stem cells obtained from the same subject as the NKX6-1-positive pancreatic progenitor cells are administered to.

150. The method of claims 148 or 149, wherein the NKX6-1-positive pancreatic progenitor cells are encapsulated in a microcapsule.

151. The method of any one of claims 148 to 150, wherein the subject has, or has an increased risk of developing diabetes.

152. The method of claim 151, wherein the diabetes is selected from the group of Type I diabetes, Type II diabetes, Type 1.5 diabetes and pre-diabetes.

153. The method of any one of claims 148 to 150, wherein the subject has, or has an increased risk of developing a metabolic disorder.

154. Use of an isolated population of NKX6-1-positive pancreatic progenitor cells produced by the methods according to any one of claims 113 to 142 for differentiating into SC-β cells.

155. Use of an isolated population of NKX6-1-positive pancreatic progenitor cells produced by the methods according to any one of claims 113 to 142 for administering to a subject in need thereof.

156. The use of claim 155, wherein the isolated population of NKX6-1-positive pancreatic progenitor cells is administered to the subject encapsulated in microcapsules.

157. The use of claims 155 or 156, wherein the subject has, or has an increased risk of developing diabetes.

158. The use of claim 157, wherein the diabetes is selected from the group of Type I diabetes, Type II diabetes, Type 1.5 diabetes and pre-diabetes.

159. The use of claims 155 or 156, wherein the subject has, or has an increased risk of developing a metabolic disorder.

160. A culture medium comprising a) KGF, b) SANT1), and optionally c) RA, wherein the culture medium is substantially free of PdbU and LDN 193189.

161. Use of the culture medium of claim 160 to induce the *in vitro* differentiation of Pdx1-positive pancreatic progenitor cells into NKX6-1-positive pancreatic progenitor cells.

162. A method of producing an insulin-positive endocrine cell from an NKX6-1-positive pancreatic progenitor cell comprising contacting a population of cells comprising NKX6-1-positive pancreatic progenitor cells under conditions that promote cell clustering with at least two β cell-maturation factors comprising a) a TGF-β signaling pathway inhibitor, and b) thyroid hormone signaling pathway

activator, to induce the differentiation of at least one NKX6-1-positive pancreatic progenitor cell in the population into at least one insulin-positive endocrine cell, wherein the insulin-positive pancreatic progenitor cell expresses insulin.

163. The method of claim 162, wherein the population of cells is contacted with the TGF-β signaling pathway inhibitor at a concentration of between 1 μM – 100 μM.

164. The method of claims 162 or 163, wherein the population of cells is contacted with the TGF-β signaling pathway inhibitor at a concentration of 10 μM.

165. The method of any one of claims 162 to 164, wherein the TGF-β signaling pathway comprises TGF-β receptor type I kinase signaling.

166. The method of any one of claims 162 to 165, wherein the TGF-β signaling pathway inhibitor comprises Alk5 inhibitor II.

167. The method of any one of claims 162 to 166, wherein the population of cells is contacted with the thyroid hormone signaling pathway activator at a concentration of between 0.1 μM – 10 μM.

168. The method of any one of claims 162 to 167, wherein the population of cells is contacted with the thyroid hormone signaling pathway activator at a concentration of 1 μM.

169. The method of any one of claims 162 to 168, wherein the thyroid hormone signaling pathway activator comprises triiodothyronine (T3).

170. The method of any one of claims 162 to 169, further comprising contacting the population of cells with at least one additional β cell-maturation factor.

171. The method of claim 170, wherein the at least one additional β cell-maturation factor comprises a γ-secretase inhibitor.

172. The method of claim 171, wherein the population of cells is contacted with the γ-secretase inhibitor at a concentration of between 0.1 μM - 10 μM.

173. The method of claims 171 or 172, wherein the population of cells is contacted with the γ-secretase inhibitor at a concentration of 1 μM.

174. The method of any one of claims 171 to 173, wherein the γ-secretase inhibitor comprises XXI.

175. The method of any one of claims 171 to 174, wherein the γ-secretase inhibitor comprises DAPT.

176. The method of any one of claims 170 to 175, wherein the at least one additional β cell-maturation factor comprises at least one growth factor from the EGF family.

177. The method of claim 176, wherein the population of cells is contacted with the at least one growth factor from the EGF family at a concentration of between 2 ng/mL - 200 ng/mL.

178. The method of claims 176 or 177, wherein the population of cells is contacted with at least one growth factor from the EGF family at a concentration of 20 ng/mL.

179. The method of any one of claims 176 to 178, wherein the at least one growth factor from the EGF family comprises betacellulin.

180. The method of any one of claims 176 to 178, wherein the at least one growth factor from the EGF family comprises EGF.

181. The method of any one of claims 170 to 180, wherein the at least one additional β cell-maturation factor comprises a low concentration of a retinoic acid (RA) signaling pathway activator.

182. The method of claim 181, wherein the population of cells is contacted with the RA signaling pathway activator at a concentration of between 0.01 μM – 1.0 μM.

183. The method of claims 181 or 182, wherein the population of cells is contacted with the RA signaling pathway activator at a concentration of 0.1 μM.

184. The method of any one of claims 181 to 183, wherein the RA signaling pathway activator comprises RA.

185. The method of any one of claims 170 to 184, wherein the at least one additional β cell-maturation factor comprises a sonic hedgehog (SHH) pathway inhibitor.

186. The method of claim 185, wherein the population of cells is contacted with the SHH pathway inhibitor at a concentration of between 0.1 μM and 0.5 μM.

187. The method of claims 185 or 186, wherein the population of cells is contacted with the SHH pathway inhibitor at a concentration of 0.25 μM.

188. The method of any one of claims 185 to 187, wherein the SHH pathway inhibitor comprises Sant1.

189. The method of any one of claims 162 to 188, wherein the population of cells is optionally contacted with a protein kinase inhibitor.

190. The method of claim 189, wherein the population of cells is not contacted with the protein kinase inhibitor.

191. The method of claim 189, wherein the population of cells is contacted with the protein kinase inhibitor.

192. The method of claim 191, wherein the population of cells is contacted with the protein kinase inhibitor at a concentration of between 10 nM – 1 μM.

193. The method of claims 191 or 192, wherein the population of cells is contacted with the protein kinase inhibitor at a concentration of 100 nM.

194. The method of any one of claims 191 to 193, wherein the protein kinase inhibitor comprises staurosporine.

195. The method of any one of claims 162 to 194, further comprising exposing the population of cells to glucose.

196. The method of claim 195, wherein the population of cells is exposed to glucose at a concentration of between 1 mM – 50 mM.

197. The method of claims 195 or 196, wherein the population of cells is exposed to glucose at a concentration of 25 mM.

198. The method of any one of claims 192 to 197, wherein the conditions that promote cell clustering comprise a suspension culture.

199. The method of any one of claims 192 to 198, wherein the population of cells is maintained in suspension culture for a period of time sufficient to induce the differentiation of at least one of the NKX6-1-positive pancreatic progenitor cells in the population into an insulin-positive endocrine cell.

200. The method of claim 199, wherein the period of time is at least 7 days.

201. The method of claims 199 or 200, wherein the β cell-maturation factors are replenished in the suspension culture every other day.

202. The method of any one of claims 162 to 201, wherein at least 15% of the NKX6-1-positive pancreatic progenitor cells in the population are induced to differentiate into insulin-positive endocrine cells.

203. The method of any one of claims 162 to 202, wherein at least 99% of the NKX6-1-positive pancreatic progenitor cells in the population are induced to differentiate into insulin-positive endocrine cells.

204. The method of any one of claims 162 to 203, wherein the insulin-positive endocrine cells express Pdx1, NKX6-1, NKX2-2, Mafb, glis3, Sur1, Kir6.2, Znt8, SLC2A1, SLC2A3 and/or insulin.

205. The method of any one of claims 162 to 204, wherein the NKX6-1-positive pancreatic progenitor cells are produced from a population of pluripotent stem cells selected from the group consisting of embryonic stem cells and induced pluripotent stem cells.

206. An isolated population of insulin-positive endocrine cells produced according to a method of any one of claims 162 to 205.

207. A microcapsule comprising the isolated population of insulin-positive endocrine cells according to claim 206 encapsulated therein.

208. A composition comprising a population of insulin-positive endocrine cells produced according to the methods of any one of claims 162 to 205.

209. A method for the treatment of a subject in need thereof, the method comprising administering to a subject a composition comprising an isolated population of insulin-positive endocrine cells produced according to the methods of any one of claims 162 to 205.

210. The method of claim 209, wherein the insulin-positive endocrine cells are produced from a population of pluripotent stem cells obtained from the same subject as the insulin-positive endocrine cells are administered to.

211. The method of claims 209 or 210, wherein the insulin-positive endocrine cells are encapsulated in a microcapsule.

212. The method of any one of claims 209 to 211, wherein the subject has, or has an increased risk of developing diabetes.

213. The method of claim 212, wherein the diabetes is selected from the group of Type I diabetes, Type II diabetes, Type 1.5 diabetes and pre-diabetes.

214. The method of any one of claims 209 to 211, wherein the subject has, or has an increased risk of developing a metabolic disorder.

215. Use of an isolated population of insulin-positive endocrine cells produced by the methods according to any one of claims 162 to 205 for differentiating into SC-β cells.

216. Use of an isolated population of insulin-positive endocrine cells produced by the methods according to any one of claims 162 to 205 for administering to a subject in need thereof.

217. The use of claim 216, wherein the isolated population of insulin-positive endocrine cells is administered to the subject encapsulated in microcapsules.

218. The use of claims 216 or 217, wherein the subject has, or has an increased risk of developing diabetes.

219. The use of claim 218, wherein the diabetes is selected from the group of Type I diabetes, Type II diabetes, Type 1.5 diabetes and pre-diabetes.

220. The use of claims 216 or 217, wherein the subject has, or has an increased risk of developing a metabolic disorder.

221. A culture medium comprising a) TGF-β signaling pathway inhibitor, b) a TH pathway activator, and at least one additional β cell-maturation factor selected from the group consisting of i) XXI, ii) Betacellulin, iii) a low concentration of a RA signaling pathway activator, and iv) a SHH pathway inhibitor.

222. Use of the culture medium of claim 221 to induce the *in vitro* differentiation of NKX6-1-positive pancreatic progenitor cells into insulin-positive endocrine cells.

223. A method of generating SC-β cells, the method comprising: contacting Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells under conditions that promote cell clustering with i) a transforming growth factor β (TGF-β) signaling pathway inhibitor, ii) a thyroid hormone signaling pathway activator, and optionally iii) a protein kinase inhibitor, to induce the *in vitro* maturation of at least some of the Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells into SC-β cells, wherein the SC-β cells exhibit a GSIS response *in vitro* and/or *in vivo*.

224. The method of claim 223, wherein the GSIS response is observed (i) immediately upon transplantation of the SC-β cell into a subject; (ii) within approximately 24

hours of transplantation into a subject; or (iii) within approximately two weeks of transplantation into a subject.

225. The method of claims 223 or 224, wherein the SC-β cells exhibit a response to (i) at least one glucose challenge; (ii) at least two sequential glucose challenges; or (iii) at least three sequential glucose challenges.

226. The method of any one of claims 223 to 225, wherein the morphology of the SC-β cells resembles the morphology of endogenous β cells.

227. The method of any one of claims 223 to 226, wherein the Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells are contacted with the TGF-β signaling pathway inhibitor at a concentration of between 100 nM – 100 μM.

228. The method of any one of claims 223 to 227, wherein the Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells are contacted with the TGF-β signaling pathway inhibitor at a concentration of 10 μM.

229. The method of any one of claims 223 to 228, wherein the TGF-β signaling pathway comprises TGF-β receptor type I kinase signaling.

230. The method of any one of claims 223 to 229, wherein the TGF-β signaling pathway inhibitor comprises Alk5 inhibitor II.

231. The method of any one of claims 223 to 230, wherein the Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells are contacted with the thyroid hormone signaling pathway activator at a concentration of between 0.1 μM – 10 μM.

232. The method of any one of claims 223 to 231, wherein the Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells are contacted with the thyroid hormone signaling pathway activator at a concentration of 1 μM.

233. The method of any one of claims 223 to 232, wherein the thyroid hormone signaling pathway activator comprises triiodothyronine (T3).

234. The method of any one of claims 223 to 233, wherein the Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells are not contacted with the protein kinase inhibitor.

235. The method of any one of claims 223 to 233, wherein the Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells are contacted with the protein kinase inhibitor.

236. The method of claim 235, wherein the Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells are contacted with the protein kinase inhibitor at a concentration of between 10 nM – 1 μM.

237. The method of claims 235 or 236, wherein the Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells are contacted with the protein kinase inhibitor at a concentration of 100 nM.

238. The method of any one of claims 235 to 237, wherein the protein kinase inhibitor comprises staurosporine.

239. The method of any one of claims 223 to 238, further comprising contacting the Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells with a cystic fibrosis transmembrane conductance regulator (CFTR) inhibitor.

240. The method of claim 239, wherein the Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells are contacted with the CFTR inhibitor at a concentration of between 100 nM and 100 μM.

241. The method of claims 239 or 240, wherein the Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells are contacted with the CFTR inhibitor at a concentration of 10 nM and 10 uM.

242. The method of any one of claims 239 to 221, wherein the CFTR inhibitor comprises Gly-H101.

243. The method of any one of claims 223 to 242, further comprising contacting the Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells with a O-GlcNAcase inhibitor.

244. The method of claim 243, wherein the Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells are contacted with the O-GlcNAcase inhibitor at a concentration of between 100 nM and 100 μM.

245. The method of claims 243 or 244, wherein the Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells are contacted with the O-GlcNAcase inhibitor at a concentration of between 10 nM and 10 uM.

246. The method of claims 244 to 245, wherein the inhibitor of O-GlcNAcase comprises Thiamet G.

247. The method of any one of claims 223 to 246, wherein the Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells are cultured in a suitable culture medium.

248. The method of claim 247, wherein the suitable culture medium comprises Connought Medical Research Laboratories 1066 supplemented islet media (CMRLS) or a component of CMRLS.

249. The method of claim 248, wherein the CMRLS is supplemented with serum.

250. The method of claims 228 or 229, wherein the CMRLS is supplemented with 10% fetal bovine serum.

251. The method of any one of claims 74-76 and 248-250, further comprising Sant1.

252. The method of any one of claims 74 – 76 and 248-251, further comprising XXI.

253. The method of any one of claims 74 – 76 and 248-252, further comprising SSP.

254. The method of any one of claims 223 to 253, wherein the conditions that promote cell clustering comprise suspension culture.

255. The method of any one of claim 223 to 254, wherein the Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells are maintained in a suspension culture for a period of time sufficient to induce the *in vitro* maturation of at least some of the Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells into SC-β cells.

256. The method of claim 255, wherein the period of time comprises at least 7 days.

257. The method of claims 255 or 256, wherein the period of time comprises between 7 days and 21 days.

258. The method of any one of claims 255 to 257, wherein the period of time comprises between 7 and 14 days.

259. The method of any one of claims 255 to 258, wherein the period of time comprises 14 days.

260. The method of any one of claims 255 to 259, wherein the suspension culture is replenished every other day.

261. The method of any one of claims 223 to 260, wherein at least 30% of the cells generated comprise SC-β cells.

262. The method of any one of claims 203 to 261, wherein the SC-β cells express C-peptide, insulin, NKX6-1, Pdx1, and co-express NKX6-1 and C-peptide.

263. The method of any one of claims 223 to 262, wherein the SC-β cells comprise human cells.

264. The method of any one of claims 223 to 263, wherein the generation of the SC-β cells *in vitro* is scalable.

265. The method of any one of claims 223 to 264, wherein the insulin-positive, endocrine cells are obtained by contacting Pdx1-positive, NKX6-1-positive pancreatic progenitor cells under conditions that promote cell clustering with i) a TGF-β signaling pathway inhibitor, and ii) a thyroid hormone signaling pathway activator, to induce the differentiation of at least some of the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells into Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells, wherein the Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells express Pdx1, NKX6-1, NKX2-2, Mafb, glis3, Sur1, Kir6.2, Znt8, SLC2A1, SLC2A3 and/or insulin.

266. The method of claim 265, wherein the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells are contacted with the TGF-β signaling pathway inhibitor at a concentration of between 100 nM – 100 μM.

267. The method of claims 265 or 266, wherein the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells are contacted with the TGF-β signaling pathway inhibitor at a concentration of 10 μM.

268. The method of any one of claims 265 to 267, wherein the TGF-β signaling pathway comprises TGF-β receptor type I kinase signaling.

269. The method of any one of claims 265 to 268, wherein the TGF-β signaling pathway inhibitor comprises Alk5 inhibitor II.

270. The method of any one of claims 265 to 269, wherein the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells are contacted with the thyroid hormone signaling pathway activator at a concentration of between 0.1 μM – 10 μM.

271. The method of any one of claims 265 to 270, wherein the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells are contacted with the thyroid hormone signaling pathway activator at a concentration of 1 μM.

272. The method of any one of claims 265 to 271, wherein the thyroid hormone signaling pathway activator comprises triiodothyronine (T3).

273. The method of any one of claims 265 to 272, further comprising contacting the Pdx1-positive NKX6-1-positive pancreatic progenitor cells with at least one of i) a SHH pathway inhibitor, ii) a RA signaling pathway activator, iii) a γ-secretase inhibitor, iv) at least one growth factor from the epidermal growth factor (EGF) family, and optionally v) a protein kinase inhibitor.

274. The method of claim 273, wherein the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells are contacted with the SHH pathway inhibitor at a concentration of between 0.1 μM and 0.5 μM.

275. The method of claims 273 or 274, wherein the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells are contacted with a SHH pathway inhibitor at a concentration of 0.25 μM.

276. The method of any one of claims 273 to 275, wherein the SHH pathway inhibitor comprises Sant1.

277. The method of any one of claims 273 to 276, wherein the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells are contacted with the RA signaling pathway activator at a concentration of between 0.01 μM – 1.0 μM.

278. The method of any one of claims 273 to 277, wherein the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells are contacted with the RA signaling pathway activator at a concentration of 0.1 μM.

279. The method of any one of claims 273 to 278, wherein the RA signaling pathway activator comprises RA.

280. The method of any one of claims 273 to 279, wherein the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells are contacted with the γ-secretase inhibitor at a concentration of between 0.1 μM – 10 μM.

281. The method of any one of claims 273 to 280, wherein the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells are contacted with the γ-secretase inhibitor at a concentration of 1 μM.

282. The method of any one of claims 273 to 281, wherein the γ-secretase inhibitor comprises XXI.

283. The method of any one of claims 273 to 282, wherein the γ-secretase inhibitor comprises DAPT.

284. The method of any one of claims 273 to 283, wherein the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells are contacted with the at least one growth factor from the EGF family at a concentration of between 2 ng/mL – 200 ng/mL.

285. The method of any one of claims 273 to 284, wherein the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells are contacted with the at least one growth factor from the EGF family at a concentration of 20 ng/mL.

286. The method of any one of claims 273 to 285, wherein the at least one growth factor from the EGF family comprises betacellulin.

287. The method of any one of claims 273 to 286, wherein the at least one growth factor from the EGF family comprises EGF.

288. The method of any one of claims 273 to 287, wherein the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells are not contacted with the protein kinase inhibitor.

289. The method of any one of claims 273 to 288, wherein the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells are contacted with the protein kinase inhibitor.

290. The method of claim 289, wherein the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells are contacted with the protein kinase inhibitor at a concentration of between 10 nM – 1 μM.

291. The method of claims 289 or 290, wherein the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells are contacted with the protein kinase inhibitor at a concentration of 100 nM.

292. The method of any one of claims 289 to 291, wherein the protein kinase inhibitor comprises staurosporine.

293. The method of any one of claims 265 to 292, further comprising exposing the population of cells to glucose.

294. The method of claim 293, wherein the population of cells is exposed to glucose at a concentration of between 1 mM – 50 mM.

295. The method of claims 293 or 294, wherein the population of cells is exposed to glucose at a concentration of 25 mM.

296. The method of any one of claims 265 to 296, wherein the conditions that promote cell clustering comprise suspension culture.

297. The method of any one of claims 265 to 296, wherein the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells are maintained in suspension culture for a period of time sufficient to induce the differentiation of at least some of the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells into Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells.

298. The method of claim 297, wherein the period of time is at least 7 days.

299. The method of claims 297 or 298, wherein the suspension culture is replenished every other day.

300. The method of any one of claims 265 to 299, wherein at least 15% of the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells are induced to differentiate into Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells.

301. The method of any one of claims 265 to 300, wherein at least 99% of the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells are induced to differentiate into Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells.

302. The method of any one of claims 265 to 301, wherein the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells are obtained by contacting Pdx1-positive pancreatic progenitor cells under conditions that promote cell clustering with i) at least one growth factor from the FGF family, ii) at least one SHH pathway inhibitor, and optionally iii) low concentrations of a RA signaling pathway activator, for a period of five days to induce the differentiation of at least some of the Pdx1-positive pancreatic progenitor cells into Pdx1-positive, NKX6-1-positive pancreatic progenitor cells, wherein the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells expresses Pdx1 and NKX6-1.

303. The method of claim 302, wherein the Pdx1-positive pancreatic progenitor cells are contacted with the at least one growth factor from the FGF family at a concentration of between 1 ng/mL - 100 ng/mL.

304. The method of claims 302 or 303, wherein the Pdx1-positive pancreatic progenitor cells are contacted with the at least one growth factor from the FGF family at a concentration of 50 ng/mL.

305. The method of any one of claims 302 to 304, wherein the at least one growth factor from the FGF family comprises keratinocyte growth factor (KGF).

306. The method of any one of claims 302 to 305, wherein the at least one growth factor from the FGF family is selected from the group consisting of FGF2, FGF8B, FGF10, and FGF21.

307. The method of any one of claims 302 to 306, wherein the Pdx1-positive pancreatic progenitor cells are contacted with the at least one SHH pathway inhibitor at a concentration of between 0.1 μM and 0.5 μM.

308. The method of any one of claims 302 to 307, wherein the Pdx1-positive pancreatic progenitor cells are contacted with the at least one SHH pathway inhibitor at a concentration of 0.25 μM.

309. The method of any one of claims 302 to 308, wherein the at least one SHH pathway inhibitor comprises Sant1.

310. The method of any one of claims 302 to 309, wherein the Pdx1-positive pancreatic progenitor cells are contacted with the RA signaling pathway activator at a concentration of between 0.01 μM – 1.0 μM.

311. The method of any one of claims 302 to 310, wherein the Pdx1-positive pancreatic progenitor cells are contacted with the RA signaling pathway activator at a concentration of 0.1 μM.

312. The method of any one of claims 302 to 311, wherein the RA signaling pathway activator comprises RA.

313. The method of any one of claims 302 to 312, further comprising contacting the Pdx1-positive pancreatic progenitor cells with at least one growth factor from the EGF family.

314. The method of claim 313, wherein the Pdx1-positive pancreatic progenitor cells are contacted with the at least one growth factor from the EGF family at a concentration of between 2 ng/mL – 200 ng/mL.

315. The method of claims 313 or 314, wherein the Pdx1-positive pancreatic progenitor cells are contacted with the at least one growth factor from the EGF family at a concentration of 20 ng/mL.

316. The method of any one of claims 313 to 315, wherein the at least one growth factor from the EGF family comprises betacellulin.

317. The method of any one of claims 313 to 316, wherein the at least one growth factor from the EGF family comprises EGF.

318. The method of any one of claims 302 to 317, wherein the Pdx1-positive pancreatic progenitor cells are cultured in a suitable culture medium.

319. The method of any one of claims 302 to 318, wherein the conditions that promote cell clustering comprise suspension culture.

320. The method of claim 319, wherein the suspension culture is replenished every other day.

321. The method of any one of claims 302 to 320, wherein an activator of protein kinase C is not added to the suspension culture during the 5 days.

322. The method of any one of claims 302 to 321, wherein an activator of protein kinase C is removed from the suspension culture prior to the 5 days.

323. The method of claims 321 or 322, wherein the activator of protein kinase C comprises PdbU.

324. The method of any one of claims 302 to 323, wherein a BMP signaling pathway inhibitor is not added to the suspension culture during the 5 days.

325. The method of any one of claims 302 to 324, wherein a BMP signaling pathway inhibitor is removed from the suspension culture prior to the 5 days.

326. The method of claims 324 or 325, wherein the BMP signaling pathway inhibitor comprises LDN193189.

327. The method of any one of claims 302 to 325, wherein at least 10% of the Pdx1-positive pancreatic progenitor cells in the population are induced to differentiate into Pdx1-positive, NKX6-1-positive pancreatic progenitor cells.

328. The method of any one of claims 302 to 327, wherein at least 95% of the Pdx1-positive pancreatic progenitor cells are induced to differentiate into Pdx1-positive, NKX6-1-positive pancreatic progenitor cells.

329. A method of generating SC-β cells from pluripotent cells, the method comprising:

a) differentiating pluripotent stem cells in a population into Pdx1-positive pancreatic progenitor cells;

b) differentiating at least some of the Pdx1-positive pancreatic progenitor cells into Pdx1-positive, NKX6-1-positive pancreatic progenitor cells by a process of contacting the Pdx1-positive pancreatic progenitor cells under conditions that promote cell clustering with i) at least one growth factor from the FGF family, ii)

at least one SHH pathway inhibitor, and optionally iii) a RA signaling pathway activator, every other day for a period of five days to induce the differentiation of at least some of the Pdx1-positive pancreatic progenitor cells in the population into NKX6-1-positive pancreatic progenitor cells, wherein the NKX6-1-positive pancreatic progenitor cells expresses Pdx1 and NKX6-1;

c) differentiating at least some of the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells into Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells by a process of contacting the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells under conditions that promote cell clustering with i) a TGF-β signaling pathway inhibitor, b) a TH signaling pathway activator, and optionally c) at least one SHH pathway inhibitor, ii) a RA signaling pathway activator, iii) a γ-secretase inhibitor, and vi) at least one growth factor from the epidermal growth factor (EGF) family, every other day for a period of between five and seven days to induce the differentiation of at least some of the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells into Pdx1-positive, NKX6-1, insulin-positive endocrine cells, wherein the Pdx1-positive, NKX6-1, insulin-positive endocrine cells express Pdx1, NKX6-1, NKX2-2, Mafb, glis3, Sur1, Kir6.2, Znt8, SLC2A1, SLC2A3 and/or insulin; and

d) differentiating at least some of the Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells into SC-β cells by a process of contacting the Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells under conditions that promote cell clustering with i) a transforming growth factor β (TGF-β) signaling pathway inhibitor, ii) a thyroid hormone signaling pathway activator, and optionally iii) a protein kinase inhibitor, every other day for a period of between seven and 14 days to induce the *in vitro* maturation of at least some of the Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells into SC-β cells, wherein the SC-β cells exhibit a GSIS response *in vitro* and/or *in vivo*.

330. A method of generating SC-β cells from pluripotent cells, the method comprising:

a) differentiating at least some pluripotent cells in a population into Pdx1-positive pancreatic progenitor cells;

b) differentiating at least some of the Pdx1-positive pancreatic progenitor cells into Pdx1-positive, NKX6-1-positive pancreatic progenitor cells by a process of contacting the Pdx1-positive pancreatic progenitor cells under conditions that

promote cell clustering with i) KGF, ii) Sant1, and optionally iii) low concentrations of RA, every other day for a period of five days to induce the differentiation of at least one Pdx1-positive pancreatic progenitor cell in the population into NKX6-1-positive pancreatic progenitor cells, wherein the NKX6-1-positive pancreatic progenitor cells expresses Pdx1 and NKX6-1;

c) differentiating at least some of the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells into Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells by a process of contacting the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells with i) Alk5 Inhibitor II, ii) T3, and optionally iii) Sant1, iv) RA, v) XXI, and vi) betacellulin, every other day for a period of between five and seven days to induce the differentiation of at least some of the Pdx1-positive, NKX6-1-positive pancreatic progenitor cells into Pdx1-positive, NKX6-1, insulin-positive endocrine cells, wherein the Pdx1-positive, NKX6-1, insulin-positive endocrine cells express Pdx1, NKX6-1, NKX2-2, Mafb, glis3, Sur1, Kir6.2, Znt8, SLC2A1, SLC2A3 and/or insulin; and

d) differentiating at least some of the Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells into SC-β cells by a process of contacting the Pdx1-positive, NKX6-1-positive, insulin-positive endocrine cells under conditions that promote cell clustering with i) Alk5 inhibitor II, ii) T3, and optionally iii) staurosporine, every other day for a period of between seven and 14 days to induce the *in vitro* maturation of at least some of the Pdx1-positive, NKX6-1-positive, insulin-producing endocrine cells into SC-β cells, wherein the SC-β cells exhibit a GSIS response *in vitro* and/or *in vivo*.

331. A method of any one of claims 329 to 330, further comprising culturing a stem cell in the presence of a Rock inhibitor wherein said culturing improves survival rate and/or differentiation efficiency of the cell.

332. A method of any one of claims 329 to 331, further comprising culturing a stem cell in the presence of Activin A wherein said culturing improves survival rate and/or differentiation efficiency of the cell.

333. A method of any one of claims 329 to 333, further comprising culturing a stem cell in the presence of Nicotinamide wherein said culturing improves survival rate, differentiation efficiency of the cell, and/or downregulates SOX2 expression.

334. A method of any one of claims 329 to 334, further comprising culturing a stem cell in the presence of staurosporine wherein said culturing generates a near pure endocrine population and/or increases NKX6-1/C-peptide+ cells.

335. A method of any one of claims 329 to 334, further comprising culturing a stem cell in the presence of XXI in combination with Alk5i and T3 wherein said culturing increases NKX6-1+ endocrine cells.

336. A method of any one of claims 329 to 335, further comprising culturing a stem cell in the presence of a gamma-secretase inhibitor in combination with Alk5i and T3 wherein said culturing increases NKX6-1+ endocrine cells.

337. An artificial islet comprising SC-β cells differentiated *in vitro* from pluripotent stem cells.

338. An artificial pancreas comprising SC-β cells differentiated *in vitro* from pluripotent stem cells.

CLAIMS

1. A method for differentiating PDX1-positive pancreatic progenitor cells into NKX6.1 positive pancreatic progenitor cells with a culture medium comprising a) KGF and b) SANT1, wherein the culture medium is substantially free of a Protein Kinase C (PKC) Activator.

2. The method of claim 1, wherein the PKC Activator is Phorbol 12,13-dibutyrate (PdBu) or TPB.

3. The method of claim 1, wherein the PKC Activator is PdBu.

4. The method of claim 1, wherein the PKC Activator is TPB.

5. The method of claim 1, wherein the culture medium further comprises retinoic acid (RA).

6. A method for differentiating PDX1-positive pancreatic progenitor cells into NKX6.1 positive pancreatic progenitor cells with a culture medium comprising a) KGF and b) SANT1, wherein the culture medium is substantially free of a BMP signaling pathway inhibitor.

7. The method of claim 6, wherein the BMP signaling pathway inhibitor is selected from the group consisting of noggin, chordin, gremlin, follistatin and LDN 193189

8. The method of claim 6, wherein the BMP signaling pathway inhibitor is noggin.

9. The method of claim 6, wherein the BMP signaling pathway inhibitor is chordin.

10. The method of claim 6, wherein the BMP signaling pathway inhibitor is gremlin.

11. The method of claim 6, wherein the BMP signaling pathway inhibitor is follistatin.

12. The method of claim 6, wherein the BMP signaling pathway inhibitor is LDN 193189.