Summary of the Invention

[0003a] In a first aspect, the present invention refers to an in vitro cell culture comprising human cells wherein at least 5% of said human cells are endocrine precursor cells that express neurogenin 3 (NEUROG3), paired box 4 (PAX4) and NKX2 transcription factor related locus 2 (NKX2.2), said endocrine precursor cells being multipotent cells that can differentiate into pancreatic islet hormone-expressing cells that express at least one pancreatic hormone selected from the group consisting of insulin, somatostatin, ghrelin, pancreatic polypeptide and glucagon.

[0003b] In a second aspect, the present invention refers to a method of producing endocrine precursor cells, said method comprising the steps of:

(a) obtaining a cell population comprising pluripotent human cells;

(b) providing said cell population with a TGF-β superfamily growth factor, thereby producing definitive endoderm cells;

(c) withdrawing TGF-β superfamily growth factor from said cell population;

(d) providing said definitive endoderm cells with a retinoid, thereby producing human PDX1-positive pancreatic endoderm cells; and

(e) incubating said cell population in a culture medium for a sufficient time to permit formation of human endocrine precursor cells expressing neurogenin 3 (NEUROG3).

[0004] Some ~~embodiments of the present invention~~examples relate to compositions, such as cell cultures, comprising human pancreatic islet hormone-expressing cells. In such ~~embodiments~~examples, the amount of human pancreatic islet hormone-expressing cells can range from about 2% to about 80% of the human cells present in the cell culture. In some ~~embodiments of the present invention~~examples, the pancreatic islet hormone-expressing cells can be either mature pancreatic islet hormone-expressing cells, immature pancreatic islet hormone-expressing cells or combinations of mature and immature pancreatic islet hormone-expressing cells. In certain ~~embodiments~~examples, the human pancreatic islet hormone-expressing cells express one or more hormones selected from the group consisting of ghrelin, insulin, somatostatin and glucagon. In some ~~embodiments~~examples, the islet hormone-expressing cells express insulin in response to glucose stimulation.

[0005] Other ~~embodiments~~examples relate to cell cultures comprising both human pancreatic islet hormone-expressing cells and human endocrine precursor cells. In such ~~embodiments~~examples, the amount of human endocrine precursor cells can range from about 5% to about 80% of the cells present in the cell culture. In some ~~embodiments~~examples, the cell cultures comprise predominately immature pancreatic islet hormone-expressing cells and endocrine precursor cells. In other ~~embodiments~~examples, the cell cultures comprise both mature and immature pancreatic islet hormone-expressing cells as well as endocrine precursor cells.

[0006] Some ~~embodiments~~examples described herein include compositions, such as cell cultures, comprising human endocrine precursor cells but which do not include a substantial amount of human pancreatic islet hormone-expressing cells. In such ~~embodiments~~examples, the amount of human endocrine precursor cells can range from about 5% to about 80% of the human cells present in the cell culture. In certain ~~embodiments~~examples, the human endocrine precursor cells express a marker selected from the group consisting of neurogenin 3 (NEUROG3 or NGN3) paired box 4 (PAX4) and NKX2 transcription factor related locus 2 (NKX2.2).

[0007] Other ~~embodiments~~examples relate to cell cultures comprising both human endocrine precursor cells and human PDX1-positive pancreatic endoderm cells (PDX1-positive foregut endoderm cells), wherein the PDX1-positive pancreatic endoderm cells are PDX1-expressing, multipotent cells that can differentiate into cells, tissues or organs derived from the anterior portion of the gut tube. In such ~~embodiments~~examples, the human endocrine precursor cells can range from about 5% to about 95% of the cells present in said cell culture. In some ~~embodiments~~examples, the amount of human PDX1-positive pancreatic

endoderm cells can range from about 5% to about 95% of the cells present in said cell culture.

[0008] Still further ~~embodiments of the present invention~~examples relate to methods of producing human mature pancreatic islet hormone-expressing cells, human immature pancreatic islet hormone-expressing cells, and human endocrine precursor cells. In some ~~embodiments~~examples, human mature pancreatic islet hormone-expressing cells are produced from human immature pancreatic islet hormone-expressing cells. In some ~~embodiments~~examples, human immature pancreatic islet hormone-expressing cells are produced from human endocrine precursor cells. In some ~~embodiments~~examples, human endocrine precursor cells are produced from human PDX1-positive pancreatic endoderm cells.

[0009] Other ~~embodiments of the present invention~~examples relate to methods for producing human pancreatic islet hormone-expressing cells from human embryonic stem cells (hESCs) or other human pluripotent cells. In such ~~embodiments~~examples, the hESCs or other human pluripotent cells are first differentiated to human definitive endoderm cells. Definitive endoderm cells are multipotent cells that can differentiate into cells of the gut tube or organs derived therefrom. Human definitive endoderm cells and their production have been described in U.S. Patent Application Number 11/021618, filed December 23, 2004, the disclosure of which is incorporated by reference in its entirety. The definitive endoderm cells are then differentiated to foregut endoderm. Human foregut endoderm cells are multipotent cells that can differentiate into cells, tissues or organs derived from the anterior portion of the gut tube. Foregut endoderm cells and their production have been described in U.S. Provisional Patent Application Number 60/730917, filed October 27, 2005, the disclosure of which is incorporated by reference in its entirety. The foregut endoderm cells are then differentiated to PDX1-positive pancreatic endoderm cells (PDX1-positive foregut endoderm). Human PDX1-positive pancreatic endoderm cells are multipotent cells that can differentiate into cells, tissues or organs derived from the anterior portion of the gut tube. PDX1-positive pancreatic endoderm cells and their production have been described in U.S. Patent Application Number 11/115868, filed April 26, 2005 and U.S. Provisional Patent Application Number 60/730917, filed October 27, 2005, the disclosures of which are incorporated herein by reference in their entireties. The PDX1-positive pancreatic endoderm cells are differentiated into endocrine precursor cells, which are differentiated into immature, and then finally mature, pancreatic islet hormone-expressing cells as described in U.S. Provisional Patent Application Number 60/833,633,

filed July 26, 2006, the disclosure of which is incorporated herein by reference in its entirety, as well as the methods described herein.

[0010] Other ~~embodiments~~examples described herein relate to methods of producing cell populations enriched in human endocrine precursor cells and methods of producing cell populations enriched in human immature pancreatic islet hormone-expressing cells. In some ~~embodiments~~examples, methods of producing cell populations enriched in endocrine precursor cells involves providing a cell population that comprises human endocrine precursor cells with a reagent that binds to neural cell adhesion molecule (NCAM), and separating human endocrine precursor cells bound to the reagent from cells that are not bound to the reagent. Similarly, in some ~~embodiments~~examples, methods of producing cell populations enriched in human immature pancreatic islet hormone-expressing cells involves providing a cell population that comprises human immature pancreatic islet hormone-expressing cells with a reagent that binds to NCAM, and separating human immature pancreatic islet hormone-expressing cells bound to the reagent from cells that are not bound to the reagent. In some ~~embodiments~~examples, additional enrichment of immature pancreatic islet hormone-expressing cells can be achieved by contacting the NCAM-positive cell population with a second reagent that binds to CD133, and then removing from the cell population cells that are bound to the second reagent.

[0011] In some ~~embodiments of the present invention~~examples, the cell populations comprising human pancreatic islet hormone-expressing cells produced by the methods described herein can be derived from human endocrine precursor cells. In certain ~~embodiments~~examples of the methods of producing cell populations enriched for human endocrine precursor cells, the endocrine precursor cells can be derived from human PDX1-positive pancreatic endoderm cells. In still further ~~embodiments~~examples, the human PDX1-positive pancreatic endoderm cells are derived from human foregut endoderm cells. In yet further ~~embodiments~~examples, the human foregut endoderm cells are derived from human definitive endoderm cells. In still further ~~embodiments~~examples, the human definitive endoderm cells are derived from human embryonic stem cells.

[0012] Other ~~embodiments of the present invention~~examples relate to cell populations that are enriched for human endocrine precursor cells. In certain ~~embodiments~~examples, the cell populations enriched for human endocrine precursor cells comprise from about 5% human endocrine precursor cells that express Neurogenin 3 (NGN3), but that do not substantially express a marker selected from the group consisting of AFP, SOX7, SOX1, ZIC1, NFM, INS, GCG, SST and GHRL. In some ~~embodiments~~examples, the cell populations that are enriched

for human endocrine precursor cells are obtained using the methods described herein for the production of cell populations enriched for human endocrine precursor cells.

[0013] Still other ~~embodiments of the present invention~~examples relate to cell populations that are enriched for human immature pancreatic islet hormone-expressing cells. The enriched cell populations can be obtained by the methods described herein, comprising providing cell populations comprising immature pancreatic islet hormone-expressing cells with a reagent that binds NCAM, and separating the cells bound to said reagent from cells that are not bound to the reagent. In certain ~~embodiments~~examples, the cell populations comprise at least about 25% to at least about 90% immature pancreatic hormone-expressing cells that express MAFB but do not substantially express MAFA and/or NGN3. In some ~~embodiments~~examples, the enriched cell population comprises at least about 50% immature pancreatic islet hormone-expressing cells that express MAFB but do not substantially express MAFA and/or NGN3.

[0014] Yet other ~~embodiments of the present invention~~examples relate to cell populations that are enriched in human mature pancreatic islet hormone-expressing cells that are derived *in vitro* from human pluripotent cells. The enriched cell populations can be obtained by the methods described herein, such as by providing cell populations comprising pancreatic islet hormone-expressing cells, which are produced *in vitro* from human pluripotent cells, with a reagent that binds NCAM and separating the cells bound to said reagent from cells that are not bound to the reagent. In certain ~~embodiments~~examples, the cell populations comprise at least about 25% to at least about 90% pancreatic hormone-expressing cells that express at least one marker selected from the group consisting of GHRL, IAPP, INS, GCG, NKX6.1, SST and PP but which do not substantially express at least one marker selected from the group consisting of AFP, SOX7, SOX1, ZIC and NFM. In some embodiments, the enriched cell population comprises at least about 50% immature pancreatic islet hormone-expressing cells that express GHRL, IAPP, INS, GCG, NKX6.1, SST and PP but not substantially express at least one marker selected from the group consisting of AFP, SOX7, SOX1, ZIC and NFM.

[0015] Additional ~~embodiments of the present invention~~examples relate to *ex-vivo* reagent-cell complexes comprising an NCAM binding reagent and a human endocrine precursor cell that expresses NCAM, a human immature pancreatic islet hormone-expressing cell that expresses NCAM or a human mature pancreatic islet hormone-expressing cell that expresses NCAM. In certain ~~embodiments~~examples, the endocrine precursor cells, the immature pancreatic islet hormone-expressing cells and/or the mature pancreatic

islet hormone-expressing cells are derived *in vitro* from human pluripotent cells. The reagent of the reagent-cell complexes can comprise a molecule such as an anti-NCAM antibody, and anti-NCAM antibody fragment, or an NCAM ligand.

[0016] Other ~~aspects of the present invention~~examples relate to *in vitro* cell cultures and *in vitro* cell populations as set forth herein that have not been differentiated in the presence of sodium butyrate or other histone deacetylase inhibitor during any stage of their development. Other ~~aspects~~examples included herein relate to methods of producing endocrine precursor cell cultures or cell populations and/or pancreatic hormone-expressing cell cultures or cell populations in the absence of sodium butyrate or other histone deacetylase inhibitor. In such ~~aspects~~examples, hESCs are differentiated to definitive endoderm cells as well as cell types derived from definitive endoderm, such as endocrine precursor cells and pancreatic hormone-expressing cells, in the absence of sodium butyrate or other histone deacetylase inhibitor.

[0017] Still other ~~aspects of the present invention~~examples relate to cell cultures and cell populations comprising non-recombinant or non-engineered human endocrine precursor cells and/or human pancreatic hormone-expressing cells. In some ~~embodiments~~examples, the non-recombinant human endocrine precursor cells and/or human pancreatic hormone-expressing cells of the cell cultures and/or cell populations are differentiated from non-recombinant hESCs. In some ~~embodiments~~examples, non-recombinant hESCs are differentiated to definitive endoderm cells as well as cell types derived from definitive endoderm, such as endocrine precursor cells and pancreatic hormone-expressing cells.

[0018] In certain jurisdictions, there may not be any generally accepted definition of the term "comprising." As used herein, the term "comprising" is intended to represent "open" language which permits the inclusion of any additional elements. With this in mind, additional ~~embodiments of the present invention~~examples are described with reference to the numbered paragraphs below:

[0019] 1. An *in vitro* cell culture comprising human cells wherein at least about 2% of said human cells are pancreatic islet hormone-expressing cells that express at least one pancreatic hormone selected from the group consisting of ghrelin, insulin, somatostatin and glucagon, said pancreatic islet hormone-expressing cells being derived *in vitro* from human pluripotent cells.

[0020] 2. The *in vitro* cell culture of paragraph 1, wherein at least about 5% of said human cells are pancreatic islet hormone-expressing cells.

WHAT IS CLAIMED IS:

1. An *in vitro* cell culture comprising human cells wherein at least about 2% of said human cells are pancreatic islet hormone-expressing cells that express at least one pancreatic hormone selected from the group consisting of ghrelin, insulin, somatostatin and glucagon, said pancreatic islet hormone-expressing cells being derived *in vitro* from human pluripotent cells.

2. The *in vitro* cell culture of claim 1, wherein at least about 5% of said human cells are pancreatic islet hormone-expressing cells.

3. The *in vitro* cell culture of claim 1, wherein at least about 10% of said human cells are pancreatic islet hormone-expressing cells.

4. The *in vitro* cell culture of any of claims 1 to 3, wherein at least about 10% of said human cells are human endocrine precursor cells that express neurogenin 3 (NEUROG3).

5. The *in vitro* cell culture of claim 4, wherein said human endocrine precursor cells express a marker selected from the group consisting of paired box 4 (PAX4) and NKX2 transcription factor related locus 2 (NKX2.2).

6. The *in vitro* cell culture of any of claims 1 to 3, wherein at least about 50% of said human cells are human endocrine precursor cells that express neurogenin 3 (NEUROG3).

7. The *in vitro* cell culture of claim 6, wherein said human endocrine precursor cells express a marker selected from the group consisting of paired box 4 (PAX4) and NKX2 transcription factor related locus 2 (NKX2.2).

8. The *in vitro* cell culture of claim 1, wherein said pancreatic islet hormone-expressing cells express at least two hormones selected from the group consisting of ghrelin, insulin, somatostatin and glucagon.

9. The *in vitro* cell culture of claim 1, wherein said pancreatic islet hormone-expressing cells express ghrelin, insulin, somatostatin and glucagon.

10. The *in vitro* cell culture of claim 1, wherein at least about 5% of the pancreatic islet hormone-expressing cells express insulin but do not significantly express ghrelin, somatostatin and glucagon.

11. The *in vitro* cell culture of claim 1, wherein at least about 10% of the pancreatic islet hormone-expressing cells express insulin but do not significantly express ghrelin, somatostatin and glucagon.

12. The *in vitro* cell culture of claim 1, wherein at least about 20% of the pancreatic islet hormone-expressing cells express insulin but do not significantly express ghrelin, somatostatin and glucagon.

13. The *in vitro* cell culture of claim 1, wherein at least about 30% of the pancreatic islet hormone-expressing cells express insulin but do not significantly express ghrelin, somatostatin and glucagon.

14. The *in vitro* cell culture of any one of claims 10 to 13, wherein insulin is secreted in response to glucose stimulation.

15. The *in vitro* cell culture of any one of claims 10 to 13, wherein C-peptide is secreted in response to glucose stimulation.

16. The *in vitro* cell culture of claim 1, wherein at said least 10% of said pancreatic islet cells are present in islet cell clusters.

17. The *in vitro* cell culture of claim 1, wherein said pancreatic islet hormone-expressing cells further express a marker selected from the group consisting of pancreatic duodenal homeobox 1 (PDX1), islet amyloid polypeptide (IAPP), pancreatic polypeptide (PP), ISL1 transcription factor (ISL1), NKX6 transcription factor related locus 1 (NKX6.1) and paired box 6 (PAX6).

18. The *in vitro* cell culture of claim 17, wherein said pancreatic islet hormone-expressing cells do not substantially express a marker selected from the group consisting of neurogenin 3 (NEUROG3) and paired box gene 4 (PAX4).

19. The *in vitro* cell culture of claim 1, wherein at least about 1 pancreatic islet hormone-expressing cell is present for about every 10 endocrine precursor cells in said cell culture.

20. The *in vitro* cell culture of claim 1, wherein at least about 1 pancreatic islet hormone-expressing cell is present for about every 5 endocrine precursor cells in said cell culture.

21. The *in vitro* cell culture of claim 1, wherein at least about 1 pancreatic islet hormone-expressing cell is present for about every 2 endocrine precursor cells in said cell culture.

22. The *in vitro* cell culture of claim 1, wherein said pancreatic islet hormone-expressing cells are non-recombinant cells.

23. The *in vitro* cell culture of claim 1 further comprising a medium which comprises a factor selected from the group consisting of nicotinamide (NIC), exendin 4 (Ex4), hepatocyte growth factor (HGF), insulin-like growth factor (IGF) and combinations thereof.

24. The *in vitro* cell culture of claim 1, further comprising a medium which comprises a factor selected from the group consisting of exendin 4 (Ex4), hepatocyte growth factor (HGF), insulin-like growth factor 1 (IGF1) and combinations thereof.

25. The *in vitro* cell culture of claim 1, further comprising a medium which comprises nicotinamide (NIC) at a concentration of about 10 mM.

26. The *in vitro* cell culture of claim 1, further comprising a medium which comprises exendin 4 (Ex4) at a concentration of about 40 ng/ml.

27. The *in vitro* cell culture of claim 1, further comprising a medium which comprises hepatocyte growth factor (HGF) at a concentration of about 25 ng/ml.

28. The *in vitro* cell culture of claim 1, further comprising a medium which comprises insulin-like growth factor 1 (IGF1) at a concentration of about 50 ng/ml.

29. An *in vitro* cell culture comprising human cells wherein at least about 5% of said human cells are endocrine precursor cells that express neurogenin 3 (NEUROG3), said endocrine precursor cells being multipotent cells that can differentiate into pancreatic islet hormone-expressing cells that express at least one pancreatic hormone selected from the group consisting of insulin, somatostatin and glucagon.

30. The *in vitro* cell culture of claim 29, wherein at least about 10% of said human cells are endocrine precursor cells.

31. The *in vitro* cell culture of claim 29, wherein at least about 25% of said human cells are endocrine precursor cells.

32. The *in vitro* cell culture of claim 29, wherein at least about 50% of said human cells are endocrine precursor cells.

33. The *in vitro* cell culture of any of claims 29 to 32, wherein at least about 10% of said human cells are human pancreatic duodenal homeobox 1 (PDX1)-positive pancreatic endoderm cells.

34. The *in vitro* cell culture of any of claims 29 to 32, wherein at least about 25% of said human cells are human pancreatic duodenal homeobox 1 (PDX1)-positive pancreatic endoderm cells.

35. The *in vitro* cell culture of any of claims 29 to 32, wherein at least about 50% of said human cells are human pancreatic duodenal homeobox 1 (PDX1)-positive pancreatic endoderm cells.

36. The *in vitro* cell culture of any of claims 29 to 32, wherein said cell culture is substantially devoid of human pancreatic islet hormone-expressing cells.

37. The *in vitro* cell culture of claim 36, wherein at least about 10% of said human cells are human pancreatic duodenal homeobox 1 (PDX1)-positive pancreatic endoderm cells.

38. The *in vitro* cell culture of claim 36, wherein at least about 25% of said human cells are human pancreatic duodenal homeobox 1 (PDX1)-positive pancreatic endoderm cells.

39. The *in vitro* cell culture of claim 36, wherein at least about 50% of said human cells are human pancreatic duodenal homeobox 1 (PDX1)-positive pancreatic endoderm cells.

40. The *in vitro* cell culture of claim 29, wherein said endocrine precursor cells express a marker selected from the group consisting of paired box 4 (PAX4) and NKX2 transcription factor related locus 2 (NKX2.2).

41. The *in vitro* cell culture of claim 29, wherein at least about 1 endocrine precursor cell is present for about every 10 PDX1-positive pancreatic endoderm cells in said cell culture.

42. The *in vitro* cell culture of claim 29, wherein at least about 1 endocrine precursor cell is present for about every 5 PDX1-positive pancreatic endoderm cells in said cell culture.

43. The *in vitro* cell culture of claim 29, wherein at least about 1 endocrine precursor cell is present for about every 2 PDX1-positive pancreatic endoderm cells in said cell culture.

44. The *in vitro* cell culture of claim 29, wherein said endocrine precursor cells are non-recombinant cells.

45. The *in vitro* cell culture of claim 29 further comprising a medium which comprises N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT).

46. The *in vitro* cell culture of claim 45, wherein said DAPT concentration is at least about 1 μM.

47. The *in vitro* cell culture of claim 45, wherein said DAPT concentration is about 3 μM.

48. The *in vitro* cell culture of claim 45 further comprising a factor selected from retinoic acid (RA) and exendin 4 (Ex4).

49. The *in vitro* cell culture of claim 45, wherein said medium is CMRL.

50. A method of producing human pancreatic islet hormone-expressing cells, said method comprising the steps of obtaining a cell population comprising human endocrine precursor cells, said human endocrine precursor cells being multipotent cells that can differentiate into human pancreatic islet hormone-expressing cells; and incubating said human endocrine precursor cells in a culture medium for a sufficient time to permit human pancreatic islet hormone-expressing cells to form, wherein said sufficient time for human pancreatic islet hormone-expressing cells to form has been determined by detecting the presence of human pancreatic islet hormone-expressing cells in said cell population.

51. The method of claim 50, wherein at least about 2% of said human cells in said cell population differentiate into human pancreatic islet hormone-expressing cells.

52. The method of claim 50, wherein at least about 5% of said human cells in said cell population differentiate into human pancreatic islet hormone-expressing cells.

53. The method of claim 50, wherein at least about 10% of said human cells in said cell population differentiate into human pancreatic islet hormone-expressing cells.

54. The method of claim 50 further comprising providing said human pancreatic endocrine cells with a factor selected from the group consisting of nicotinamide (NIC), exendin 4 (Ex4), hepatocyte growth factor (HGF), insulin-like growth factor-1 (IGF1) and combinations thereof in an amount sufficient to further promote differentiation of said human endocrine precursor cells to human pancreatic islet hormone-expressing cells, wherein said human pancreatic islet hormone-expressing cells express at least one pancreatic hormone selected from the group consisting of insulin, somatostatin and glucagon.

55. The method of claim 54, wherein said factor is selected from the group consisting of Ex4, HGF and IGF1.

56. The method of claim 54, wherein Ex4 is provided to said cell population of endocrine precursor cells at a concentration ranging from about 10 ng/ml to about 100 ng/ml.

57. The method of claim 54, wherein Ex4 is provided to said cell population of endocrine precursor cells at a concentration of about 40 ng/ml.

58. The method of claim 54, wherein said factor is IGF1.

59. The method of claim 58, wherein IGF1 is provided to said cell population of endocrine precursor cells at a concentration ranging from about 10 ng/ml to about 1000 ng/ml.

60. The method of claim 58, wherein IGF1 is provided to said cell population of endocrine precursor cells at a concentration ranging from about 10 ng/ml to about 100 ng/ml.

61. The method of claim 58, wherein IGF1 is provided to said cell population of endocrine precursor cells at a concentration ranging from about 25 ng/ml to about 75 ng/ml.

62. The method of claim 58, wherein IGF1 is provided to said cell population of endocrine precursor cells at a concentration of about 50 ng/ml.

63. The method of claim 50, wherein detecting the presence of human pancreatic islet hormone-expressing cells in said cell population comprises detecting the expression of at least one marker selected from the group consisting of pancreatic duodenal homeobox 1 (PDX1), ghrelin (GHRL), islet amyloid polypeptide (IAPP), pancreatic polypeptide (PP), ISL1 transcription factor (ISL1), NKX6 transcription factor related locus 1 (NKX6.1) and paired box 6 (PAX6) in cells of said cell population.

64. The method of claim 63, wherein the expression of at least one of said markers is determined by Q-PCR.

65. The method of claim 63, wherein the expression of at least one of said markers is determined by immunocytochemistry.

66. The method of claim 50, wherein the step of obtaining a cell population comprising human endocrine precursor cells comprises the steps of obtaining a population of human PDX1-positive pancreatic endoderm cells, said human PDX1-positive pancreatic endoderm cells being multipotent cells that can differentiate into cells, tissues or organs derived from the anterior portion of the gut tube; and providing said population of human PDX1-positive pancreatic endoderm cells with a gamma secretase inhibitor, thereby producing a population of human endocrine precursor cells.

67. The method of claim 66, wherein said gamma secretase inhibitor comprises N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT).

68. The method of claim 67, wherein DAPT is provided to said population of human PDX1-positive pancreatic endoderm cells at a concentration ranging from about 1 μM to about 10 μM.

69. The method of claim 67, wherein DAPT is provided to said population of human PDX1-positive pancreatic endoderm cells at a concentration of about 3 μM.

70. The method of claim 66 further comprising providing said population of human PDX1-positive pancreatic endoderm cells with exendin 4 (Ex4).

71. The method of claim 70, wherein Ex4 is provided to said population of human PDX1-positive pancreatic endoderm cells at a concentration ranging from about 10 ng/ml to about 100 ng/ml.

72. The method of claim 70, wherein Ex4 is provided to said population of human PDX1-positive pancreatic endoderm cells at a concentration of about 40 ng/ml.

73. The method of claim 70, wherein the step of obtaining a population of human PDX1-positive pancreatic endoderm cells comprises the steps of obtaining a population of human foregut endoderm cells, said human foregut endoderm cells being PDX1-negative multipotent cells that can differentiate into cells, tissues or organs derived from the anterior portion of the gut tube; and providing said population of human foregut endoderm cells with a retinoid, thereby producing a population of human PDX1-positive pancreatic endoderm cells.

74. The method of claim 73, wherein said retinoid is retinoic acid (RA)

75. The method of claim 74, wherein RA is provided to said population of human foregut endoderm cells at a concentration ranging from about 1 nM to about 10 μM

76. The method of claim 73, wherein the step of obtaining a population of human foregut endoderm cells comprises the steps of obtaining a population of human definitive endoderm cells, said human definitive endoderm cells being multipotent cells that can differentiate into cells of the gut tube or organs derived therefrom; and providing said population

of human definitive endoderm cells with fibroblast growth factor 10 (FGF-10) and a hedgehog pathway inhibitor, thereby producing a population of human foregut endoderm cells.

77. The method of claim 76 further comprising withdrawing any growth factor of the TGF-β superfamily that may be present in said population of definitive endoderm cells.

78. The method of claim 77, wherein said growth factor of the TGF-β superfamily is selected from the group consisting of Nodal, activin A, activin B and combinations thereof.

79. The method of claim 77, wherein said growth factor of the TGF-β superfamily is activin A.

80. The method of claim 76, wherein said hedgehog inhibitor comprises KAAD-cyclopamine.

81. The method of claim 80, wherein KAAD-cyclopamine is provided to said population of human definitive endoderm cells at a concentration ranging from about 0.01 μM to about 1 μM.

82. The method of claim 80, wherein KAAD-cyclopamine is provided to said population of human definitive endoderm cells at a concentration of about 0.2 μM.

83. The method of claim 76, wherein FGF-10 is provided to said population of human definitive endoderm cells at a concentration ranging from about 1 ng/ml to about 1000 ng/ml.

84. The method of claim 76, wherein FGF-10 is provided to said population of human definitive endoderm cells at a concentration ranging from about 10 ng/ml to about 100 ng/ml.

85. The method of claim 76, wherein FGF-10 is provided to said population of human definitive endoderm cells at a concentration of about 50 ng/ml.

86. The method of claim 76, wherein the step of obtaining a population of human definitive endoderm cells comprises the steps of obtaining a population of pluripotent human embryonic stem cells; and providing said population of pluripotent human embryonic stem cells with at least one growth factor of the TGF-β superfamily.

87. The method of claim 86, wherein said at least one growth factor is Nodal.

88. The method of claim 86, wherein said at least one growth factor is activin A.

89. The method of claim 86, wherein said at least one growth factor is activin B.

90. The method of claim 86 further comprising providing said population of pluripotent human embryonic stem cells with wingless-type MMTV integration site family member 3A (Wnt3A).

91. The method of claim 86, wherein a plurality of growth factors of the TGFβ superfamily is provided.

92. The method of claim 91, wherein Wnt3A is also provided.

93. The method of claim 86, wherein said at least one growth factor is provided in a concentration of at least about 10 ng/ml.

94. The method of claim 86, wherein said at least one growth factor is provided in a concentration of at least about 100 ng/ml.

95. The method of claim 86, wherein said at least one growth factor is provided in a concentration of at least about 500 ng/ml.

96. The method of claim 86, wherein said at least one growth factor is provided in a concentration of at least about 1000 ng/ml.

97. The method of claim 86, wherein said at least one growth factor is provided in a concentration of at least about 5000 ng/ml.

98. The method of claim 86, wherein said pluripotent human embryonic stem cells are differentiated to human definitive endoderm cells in a medium comprising less than about 2% serum.

99. The method of claim 86, wherein said pluripotent human embryonic stem cells are derived from a tissue selected from the group consisting of the morula, the ICM of an embryo and the gonadal ridges of an embryo.

100. A human pancreatic islet hormone-expressing cell produced by the method of claim 86.

101. A method of producing human pancreatic islet hormone-expressing cells, said method comprising the steps of:

(a) obtaining a population of pluripotent human embryonic stem cells;

(b) providing said population of pluripotent human embryonic stem cells with at least one growth factor of the TGF-β superfamily, thereby producing a population of human definitive endoderm cells;

c) providing said population of human definitive endoderm cells with at least one fibroblast growth factor, thereby producing a population of human foregut endoderm cells;

(d) providing said population of human foregut endoderm cells with a retinoid, thereby producing a population of human PDX1-positive pancreatic endoderm cells;

(e) providing said population of human PDX1-positive pancreatic endoderm cells with a gamma secretase inhibitor, thereby producing a population comprising human endocrine precursor cells; and

(f) incubating said population of human endocrine precursor cells in a culture medium for a sufficient time to permit human pancreatic islet hormone-expressing cells to form.

102. The method of claim 101, wherein step (b) further comprises providing a hedgehog pathway inhibitor.

103. The method of claim 101, wherein said fibroblast growth factor is selected from the group consisting of FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, FGF10, FGF11, FGF12, FGF13, FGF14, FGF15, FGF16, FGF17, FGF18, FGF19, FGF20, FGF21, FGF22 and FGF23.

104. The method of claim 101, wherein said fibroblast growth factor comprises FGF10.

105. The method of claim 101, wherein step (d) further comprises providing insulin or an insulin-like growth factor.

106. The method of claim 101 further comprising substantially withdrawing said at least one growth factor of the TGF-β superfamily.

107. The method of claim 101, wherein said retinoid and said gamma secretase inhibitor are provided at about the same time.

108. The method of claim 101, wherein said foregut endoderm cells are competent to further differentiate into pancreatic cells.

109. A method of producing human pancreatic islet hormone-expressing cells, said method comprising the steps of:

(a) obtaining a population of pluripotent human embryonic stem cells;

(b) providing said population of pluripotent human embryonic stem cells with at least one growth factor of the TGF-β superfamily, thereby producing a population of human definitive endoderm cells;

(c) providing said population of human definitive endoderm cells with a retinoid, thereby producing a population of human PDX1-positive pancreatic endoderm cells; and

(d) incubating said population of human PDX1-positive pancreatic endoderm cells in the presence of a retinoid for a sufficient time to permit human pancreatic islet hormone-expressing cells to form.

110. The method of claim 109 further comprising the step of providing said population of human definitive endoderm cells with a fibroblast family growth factor.

111. The method of claim 110, wherein said fibroblast family growth factor comprises FGF10 or FGF7.

112. The method of claim 109, further comprising the step of providing said population of human definitive endoderm cells with a hedgehog pathway inhibitor.

113. The method of claim 112, wherein said hedgehog pathway inhibitor is KAAD-cyclopamine.

114. The method of claim 109, wherein said retinoid is retinoic acid.

115. The method of claim 109, further comprising the step of providing said population of human PDX1-positive pancreatic endoderm cells with a gamma secretase inhibitor.

116. The method of claim 115, gamma secretase inhibitor comprises N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT).

117. A method for producing a cell population enriched in human endocrine precursor cells, said method comprising the steps of providing a cell population comprising human endocrine precursor cells with a reagent that binds to neural cell adhesion molecule (NCAM), and separating human endocrine precursor cells bound to said reagent from cells that are not bound to said reagent, thereby producing a cell population enriched in human endocrine precursor cells.

118. The method of claim 117, wherein said human endocrine precursor cells are derived *in vitro* from human pluripotent cells.

119. The method of claim 117, wherein said human endocrine precursor cells express Neurogenin 3 (NGN3) and do not substantially express at least one marker selected from the group consisting of AFP, SOX7, SOX1, ZIC1, NFM, insulin (INS), glucagon (GCG), somatostatin (SST) and ghrelin (GHRL).

120. The method of claim 119, wherein said human endocrine precursor cells express paired box gene 4 (PAX4).

121. The method of claim 119, wherein said human endocrine precursor cells do not substantially express Paired Box 6 transcription factor (PAX6).

122. The method of claim 117, wherein said reagent comprises a molecule selected from the group consisting of an anti-NCAM antibody, an anti-NCAM antibody fragment and an NCAM ligand.

123. The method of claim 122, wherein said NCAM ligand is NCAM Binding Protein 10 (NBP10).

124. The method of claim 122, wherein said anti-NCAM antibody is labeled.

125. The method of claim 124, wherein said anti-NCAM antibody is fluorescently labeled.

126. The method of claim 117 further comprising providing said cell population and said reagent with a secondary reagent that binds to said reagent.

127. The method of claim 126, wherein said reagent comprises and anti-NCAM antibody and wherein said secondary reagent is fluorescently labeled.

128. The method of claim 125 or claim 127, wherein said separating step comprises using fluorescence activated cell sorting (FACS) to separate said endocrine precursor cells bound to said anti-NCAM antibodies from said cells that are not bound to said anti-NCAM antibodies.

129. The method of claim 117 further comprising the step of disaggregating said cells in said cell population comprising human endocrine precursor cells prior to providing said cell population with said reagent that binds to NCAM.

130. The method of claim 117 further comprising the steps of obtaining a cell population comprising a population of human PDX1-positive pancreatic endoderm cells, said human PDX1-positive pancreatic endoderm cells being multipotent cells that can differentiate into cells, tissues or organs derived from the anterior portion of the gut tube and providing said population of human PDX1-positive pancreatic endoderm cells with a gamma secretase inhibitor, thereby producing a population of human endocrine precursor cells.

131. The method of claim 130, wherein said gamma secretase inhibitor comprises N-[N-(3, 5,-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT).

132. The method of claim 131, wherein said DAPT is provided to said population of human PDX1-positive pancreatic endoderm cells at a concentration ranging from about 1 μM to about 10 μM.

133. The method of claim 131, wherein said DAPT is provided to said population of human PDX1-positive pancreatic endoderm cells at a concentration of about 3 μM.

134. The method of claim 130, further comprising providing said population of human PDX1-positive pancreatic endoderm cells with exendin 4 (Ex4).

135. The method of claim 134, wherein said Ex4 is provided to said population of human PDX1-positive pancreatic endoderm cells at a concentration ranging from about 10 ng/ml to about 100 ng/ml.

136. The method of claim 134, wherein said Ex4 is provided to said population of human PDX1-positive pancreatic endoderm cells at a concentration of about 40 ng/ml.

137. The method of claim 130, wherein the step of obtaining a population of human PDX1-positive pancreatic endoderm cells comprises the steps of obtaining a population of human foregut endoderm cells, said human foregut endoderm cells being PDX1-negative multipotent cells that can differentiate into cells, tissues or organs derived from the anterior portion of the gut tube and providing said population of human foregut endoderm cells with a retinoid, thereby producing a population of human PDX1-positive pancreatic endoderm cells.

138. The method of claim 137, wherein said retinoid is retinoic acid (RA).

139. The method of claim 138, wherein RA is provided to said population of human foregut endoderm cells at a concentration ranging from about 1 nM to about 10 μM.

140. The method of claim 137, wherein the step of obtaining a population of human foregut endoderm cells comprises the steps of obtaining a population of human definitive endoderm cells, said human definitive endoderm cells being multipotent cells that can differentiate into cells of the gut tube or organs derived therefrom and providing said population of human definitive endoderm cells with fibroblast growth factor 10 (FGF-10) and a hedgehog pathway inhibitor, thereby producing a population of human foregut endoderm cells.

141. The method of claim 140, further comprising withdrawing an exogenously added factor of the TGF-β superfamily that may be present in said population of definitive endoderm cells.

142. The method of claim 141, wherein said growth factor of the TGF-β superfamily is selected from the group consisting of Nodal, activin A, activin B and combinations thereof.

143. The method of claim 142, wherein said growth factor of the TGF-β superfamily is activin A.

144. The method of claim 140, wherein said hedgehog inhibitor comprises KAAD-cyclopamine.

145. The method of claim 144, wherein KAAD-cyclopamine is provided to said population of human definitive endoderm cells at a concentration ranging from about 0.01 μM to about 1 μM.

146. The method of claim 145, wherein KAAD-cyclopamine is provided to said population of human definitive endoderm cells at a concentration of about 0.2 μM.

147. The method of claim 140, wherein FGF-10 is provided to said population of human definitive endoderm cells at a concentration ranging from about 1 ng/ml to about 1000 ng/ml.

148. The method of claim 140, wherein FGF-10 is provided to said population of human definitive endoderm cells at a concentration ranging from about 10 ng/ml to about 100 ng/ml.

149. The method of claim 140, wherein FGF-10 is provided to said population of human definitive endoderm cells at a concentration of about 50 ng/ml.

150. The method of claim 140, wherein the step of obtaining a population of human definitive endoderm cells comprises the steps of obtaining a population of human pluripotent cells and providing said population of human pluripotent cells with at least one growth factor of the TGF-β superfamily.

151. The method of claim 150, wherein said at least one growth factor is Nodal.

152. The method of claim 150, wherein said at least one growth factor is activin A.

153. The method of claim 150, wherein said at least one growth factor is activin B.

154. The method of claim 150 further comprising providing said population of human pluripotent cells with wingless-type MMTV integration site family member 3A (Wnt3A).

155. The method of claim 150, wherein a plurality of growth factors of the TGFβ superfamily is provided.

156. The method of claim 155, wherein Wnt3A is also provided.

157. The method of claim 150, wherein said at least one growth factor is provided in a concentration of at least about 10 ng/ml.

158. The method of claim 150, wherein said at least one growth factor is provided in a concentration of at least about 100 ng/ml.

159. The method of claim 150, wherein said at least one growth factor is provided in a concentration of at least about 500 ng/ml.

160. The method of claim 150, wherein said at least one growth factor is provided in a concentration of at least about 1000 ng/ml.

161. The method of claim 150, wherein said at least one growth factor is provided in a concentration of at least about 5000 ng/ml.

162. The method of claim 150, wherein said human pluripotent cells are differentiated to human definitive endoderm cells in a medium comprising less than about 2% serum.

163. The method of claim 150, wherein said human pluripotent cells are human embryonic stem cells derived from a tissue selected from the group consisting of the morula, the ICM of an embryo and the gonadal ridges of an embryo.

164. An enriched, *in vitro* human endocrine precursor cell population, wherein said human endocrine precursor cells express NGN3 and do not substantially express at least one marker selected from the group consisting of AFP, SOX7, SOX1, ZIC1, NFM, INS, GCG, SST and GHRL.

165. The enriched, *in vitro* human endocrine precursor cell population of claim 164, wherein said cell population is derived *in vitro* from human pluripotent cells.

166. The enriched, *in vitro* human endocrine precursor cell population of claim 164, wherein said enriched human endocrine precursor cell population is produced by the method of claim 117.

167. The enriched, *in vitro* human endocrine precursor cell population of claim 164, wherein said enriched human endocrine precursor cell population is produced by the method of claim 150.

168. The enriched, *in vitro* human endocrine precursor cell population of claim 164, wherein at least about 5% of said enriched human cell population comprises human endocrine precursor cells that express neurogenin 3 (NGN3) and do not substantially express at least one

marker selected from the group consisting of AFP, SOX7, SOX1, ZIC1 NFM, INS, GCG, SST and GHRL.

169. The enriched, *in vitro* human endocrine precursor cell population of claim 168, wherein said human endocrine precursor cells express PAX4.

170. The enriched, *in vitro* human endocrine precursor cell population of claim 164, wherein said endocrine precursor cells are derived *in vitro* from human PDX1-positive pancreatic endoderm.

171. The enriched, *in vitro* human endocrine precursor cell population of claim 170, wherein said human PDX1-positive pancreatic endoderm cells are derived *in vitro* from human foregut endoderm cells.

172. The enriched, *in vitro* human endocrine precursor cell population of claim 171, wherein said human foregut endoderm cells are derived *in vitro* from definitive endoderm cells.

173. The enriched, *in vitro* human endocrine precursor cell population of claim 172, wherein said definitive endoderm cells are derived *in vitro* from human embryonic stem cells (hESCs).

174. A method for producing a cell population enriched in human immature pancreatic islet hormone-expressing cells, said method comprising the steps of providing a cell population comprising human immature pancreatic islet hormone-expressing cells with a reagent that binds to neural cell adhesion molecule (NCAM) and separating human immature pancreatic islet hormone-expressing cells bound to said reagent from cells that are not bound to said reagent, thereby producing a cell population enriched in human immature pancreatic islet hormone-expressing cells.

175. The method of claim 174, wherein said human immature pancreatic islet hormone-expressing cells are derived *in vitro* from human pluripotent cells.

176. The method of claim 174, wherein said human immature pancreatic islet hormone-expressing cells express MAFB and do not substantially express a marker selected from the group consisting of NGN3 and MAFA.

177. The method of claim 176, wherein said human immature pancreatic islet hormone-expressing cells do not substantially express at least one marker selected from the group consisting of MOX1, CER, POU5F1, AFP, SOX7, SOX1, ZIC1 and NFM.

178. The method of claim 176, wherein said human immature pancreatic islet hormone-expressing cells express at least one marker selected from the group consisting of Synaptophysin (SYP), Chromogranin A (CHGA), NKX2.2, Islet 1 (ISL1), Paired Box Gene 6 (PAX6), and Neurogenic Differentiation 1 (NEUROD), PDX1 and HB9.

179. The method of claim 174, wherein said human immature pancreatic islet hormone-expressing cells process less than about 98% of the insulin produced by said immature pancreatic islet hormone-expressing cells.

180. The method of claim 174, wherein said human immature pancreatic islet hormone-expressing cells process less than about 70% of the insulin produced by said immature pancreatic islet hormone-expressing cells.

181. The method of claim 179 or claim 180, wherein said insulin processing is measured by C-peptide release.

182. The method of claim 174, wherein said reagent comprises a molecule selected from the group consisting of an anti-NCAM antibody, an anti-NCAM antibody fragment and an NCAM ligand.

183. The method of claim 182, wherein said NCAM ligand is NCAM Binding Protein 10 (NBP10).

184. The method of claim 182, wherein said anti-NCAM antibody is labeled.

185. The method of claim 184, wherein said anti-NCAM antibody is fluorescently labeled.

186. The method of claim 174, further comprising providing said cell population with a secondary reagent that binds to said reagent.

187. The method of claim 186 wherein said reagent comprises and anti-NCAM antibody and wherein said secondary reagent that binds to said anti-NCAM antibody is fluorescently labeled.

188. The method of claim 185 or claim 187, wherein said separating step comprises using fluorescence activated cell sorting (FACS) to separate said human immature pancreatic islet hormone-expressing cells bound to said anti-NCAM antibody from said cells that are not bound to said anti-NCAM antibody.

189. The method of claim 174 further comprising the step of providing said human immature pancreatic islet hormone-expressing cells with a second reagent that binds to CD133, and separating said human immature pancreatic islet hormone-expressing cells from cells that are bound to said second reagent.

190. The method of claim 174 further comprising the step of dissociating said cell population prior to providing said cell population with said reagent that binds NCAM.

191. The method of claim 174 further comprising obtaining a cell population comprising human endocrine precursor cells being multipotent cells that can differentiate into human immature pancreatic islet hormone-expressing cells and incubating said human endocrine

precursor cells in a culture medium for a sufficient time to permit human immature pancreatic islet hormone-expressing cells to form.

192. The method of claim 191 further comprising providing said human endocrine precursor cells with a factor selected from the group consisting of nicotinamide (NIC), exendin 4 (Ex4), hepatocyte growth factor (HGF), insulin-like growth factor-1 (IGF1), glucose dependent insulinotropic polypeptide (GIP), nerve growth factor (NGF), vascular endothelial growth factor (VEGF) and combinations thereof in an amount sufficient to further promote differentiation of said human endocrine precursor cells to human immature pancreatic islet hormone-expressing cells.

193. The method of claim 192, wherein said factor is selected from the group consisting of Ex4, HGF and IGF1.

194. The method of claim 193, wherein said factor is Ex4.

195. The method of claim 194, wherein Ex4 is provided to said cell population of endocrine precursor cells at a concentration ranging from about 10 ng/ml to about 100 ng/ml.

196. The method of claim 194, wherein Ex4 is provided to said cell population of endocrine precursor cells at a concentration of about 40 ng/ml.

197. The method of claim 193, wherein said factor is IGF1.

198. The method of claim 197, wherein IGF1 is provided to said cell population of endocrine precursor cells at a concentration ranging from about 10 ng/ml to about 1000 ng/ml.

199. The method of claim 197, wherein IGF1 is provided to said cell population of endocrine precursor cells at a concentration ranging from about 10 ng/ml to about 100 ng/ml.

200. The method of claim 197, wherein IGF1 is provided to said cell population of endocrine precursor cells at a concentration ranging from about 25 ng/ml to about 75 ng/ml.

201. The method of claim 197, wherein IGF1 is provided to said cell population of endocrine precursor cells at a concentration of about 50 ng/ml.

202. The method of claim 191 further comprising the steps of obtaining a cell population comprising a population of human PDX1-positive pancreatic endoderm cells, said human PDX1-positive pancreatic endoderm cells being multipotent cells that can differentiate into cells, tissues or organs derived from the anterior portion of the gut tube and providing said population of human PDX1-positive pancreatic endoderm cells with a gamma secretase inhibitor, thereby producing a population of human endocrine precursor cells.

203. The method of claim 202, wherein said gamma secretase inhibitor comprises N-[N-(3, 5,-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT).

204. The method of claim 203, wherein said DAPT is provided to said population of human PDX1-positive pancreatic endoderm cells at a concentration ranging from about 1 μM to about 10 μM.

205. The method of claim 203, wherein said DAPT is provided to said population of human PDX1-positive pancreatic endoderm cells at a concentration of about 3 μM.

206. The method of claim 202, further comprising providing said population of human PDX1-positive pancreatic endoderm cells with exendin 4 (Ex4).

207. The method of claim 206, wherein said Ex4 is provided to said population of human PDX1-positive pancreatic endoderm cells at a concentration ranging from about 10 ng/ml to about 100 ng/ml.

208. The method of claim 206, wherein said Ex4 is provided to said population of human PDX1-positive pancreatic endoderm cells at a concentration of about 40 ng/ml.

209. The method of claim 202, wherein the step of obtaining a population of human PDX1-positive pancreatic endoderm cells comprises the steps of obtaining a population of human foregut endoderm cells, said human foregut endoderm cells being PDX1-negative multipotent cells that can differentiate into cells, tissues or organs derived from the anterior portion of the gut tube and providing said population of human foregut endoderm cells with a retinoid, thereby producing a population of human PDX1-positive pancreatic endoderm cells.

210. The method of claim 209, wherein said retinoid is retinoic acid (RA).

211. The method of claim 210, wherein RA is provided to said population of human foregut endoderm cells at a concentration ranging from about 1 nM to about 10 μM.

212. The method of claim 209, wherein the step of obtaining a population of human foregut endoderm cells comprises the steps of obtaining a population of human definitive endoderm cells, said human definitive endoderm cells being multipotent cells that can differentiate into cells of the gut tube or organs derived therefrom and providing said population of human definitive endoderm cells with fibroblast growth factor 10 (FGF-10) and a hedgehog pathway inhibitor, thereby producing a population of human foregut endoderm cells.

213. The method of claim 212 further comprising withdrawing an exogenously added factor of the TGF-βsuperfamily that may be present in said population of definitive endoderm cells.

214. The method of claim 213, wherein said growth factor of the TGF-β superfamily is selected from the group consisting of Nodal, activin A, activin B and combinations thereof.

215. The method of claim 214, wherein said growth factor of the TGF-β superfamily is activin A.

216. The method of claim 212, wherein said hedgehog inhibitor comprises KAAD-cyclopamine.

217. The method of claim 216, wherein KAAD-cyclopamine is provided to said population of human definitive endoderm cells at a concentration ranging from about 0.01 μM to about 1 μM.

218. The method of claim 216, wherein KAAD-cyclopamine is provided to said population of human definitive endoderm cells at a concentration of about 0.2 μM.

219. The method of claim 212, wherein FGF-10 is provided to said population of human definitive endoderm cells at a concentration ranging from about 1 ng/ml to about 1000 ng/ml.

220. The method of claim 212, wherein FGF-10 is provided to said population of human definitive endoderm cells at a concentration ranging from about 10 ng/ml to about 100 ng/ml.

221. The method of claim 212, wherein FGF-10 is provided to said population of human definitive endoderm cells at a concentration of about 50 ng/ml.

222. The method of claim 212, wherein the step of obtaining a population of human definitive endoderm cells comprises the steps of obtaining a population of human pluripotent cells and providing said population of human pluripotent cells with at least one growth factor of the TGF-β superfamily.

223. The method of claim 222, wherein said at least one growth factor is Nodal.

224. The method of claim 222, wherein said at least one growth factor is activin A.

225. The method of claim 222, wherein said at least one growth factor is activin B.

226. The method of claim 222 further comprising providing said population of human pluripotent cells with wingless-type MMTV integration site family member 3A (Wnt3A).

227. The method of claim 222, wherein a plurality of growth factors of the TGFβ superfamily is provided.

228. The method of claim 227, wherein Wnt3A is also provided.

229. The method of claim 222, wherein said at least one growth factor is provided in a concentration of at least about 10 ng/ml.

230. The method of claim 222, wherein said at least one growth factor is provided in a concentration of at least about 100 ng/ml.

231. The method of claim 222, wherein said at least one growth factor is provided in a concentration of at least about 500 ng/ml.

232. The method of claim 222, wherein said at least one growth factor is provided in a concentration of at least about 1000 ng/ml.

233. The method of claim 222, wherein said at least one growth factor is provided in a concentration of at least about 5000 ng/ml.

234. The method of claim 222, wherein said human pluripotent cells are differentiated to human definitive endoderm cells in a medium comprising less than about 2% serum.

235. The method of claim 222, wherein said human pluripotent cells are human embryonic stem cells derived from a tissue selected from the group consisting of the morula, the ICM of an embryo and the gonadal ridges of an embryo.

236. An enriched, *in vitro* human immature pancreatic islet hormone-expressing cell population, wherein said human immature pancreatic islet hormone-expressing cells express MAFB and do not substantially express NGN3 and MAFA

237. The enriched, *in vitro* human immature pancreatic islet hormone-expressing cell population of claim 236, wherein the enriched cell population is derived *in vitro* from human pluripotent cells.

238. The enriched, *in vitro* human immature pancreatic islet hormone-expressing cell population of claim 236, wherein said enriched cell population is produced by the method of claim 174.

239. The enriched, *in vitro* human immature pancreatic islet hormone-expressing cell population of claim 236, wherein said enriched cell population is produced by the method of claim 222.

240. The enriched, *in vitro* human immature pancreatic islet hormone-expressing cell population of claim 238 or claim 239, wherein at least about 25% of said enriched human cell population comprises human immature pancreatic islet hormone-expressing cells that express MAFB and do not substantially express NGN3 and MAFA.

241. The enriched, *in vitro* human immature pancreatic islet hormone-expressing cell population of claim 238 or claim 239, wherein at least about 50% of said enriched human cell population comprises human immature pancreatic islet hormone-expressing cells that express MAFB and do not substantially express NGN3 and MAFA.

242. The enriched, *in vitro* human immature pancreatic islet hormone-expressing cell population of claim 238 or claim 239, wherein at least about 70% of said enriched human cell population comprises human immature pancreatic islet hormone-expressing cells that express MAFB and do not substantially express NGN3 and MAFA.

243. The enriched, *in vitro* human immature pancreatic islet hormone-expressing cell population of claim 238 or claim 239, wherein at least about 90% of said enriched human cell population comprises human immature pancreatic islet hormone-expressing cells that express MAFB and do not substantially express NGN3 and MAFA.

244. The enriched, *in vitro* human immature pancreatic islet hormone-expressing cell population of claim 236, wherein at least about 25% of said enriched human cell population comprises human immature pancreatic islet hormone-expressing cells that express MAFB and do not substantially express NGN3 and MAFA.

245. The enriched, *in vitro* human immature pancreatic islet hormone-expressing cell population of claim 236, wherein at least about 50% of said enriched human cell population comprises human immature pancreatic islet hormone-expressing cells that express MAFB and do not substantially express NGN3 and MAFA.

246. The enriched, *in vitro* human immature pancreatic islet hormone-expressing cell population of claim 236, wherein at least about 70% of said enriched human cell population comprises human immature pancreatic islet hormone-expressing cells that express MAFB and do not substantially express NGN3 and MAFA.

247. The enriched, *in vitro* human immature pancreatic islet hormone-expressing cell population of claim 236, wherein at least about 90% of said enriched human cell population comprises human immature pancreatic islet hormone-expressing cells that express MAFB and do not substantially express NGN3 and MAFA.

248. The enriched, *in vitro* human immature pancreatic islet hormone-expressing cell population of claim 236, wherein said human immature pancreatic islet hormone-expressing cells do not substantially express at least one marker selected from the group consisting of elected from the group consisting of MOX1, CER, POU5F1, AFP, SOX7, SOX1, ZIC1 and NFM..

249. The enriched, *in vitro* human immature pancreatic islet hormone-expressing cell population of claim 236, wherein said human immature pancreatic islet hormone-expressing cells express at least one marker selected from the group consisting of Synaptophysin (SYP), Chromogranin A (CHGA), NKX2.2, Islet 1 (ISL1), Paired Box Gene 6 (PAX6), Neurogenic Differentiation 1 (NEUROD), PDX1 and HB9.

250. The enriched, *in vitro* human immature pancreatic islet hormone-expressing cell population of claim 236, wherein said human immature pancreatic islet hormone-expressing cells process less than about 98% of the insulin produced by said immature pancreatic islet hormone-expressing cells.

251. The enriched, *in vitro* human immature pancreatic islet hormone-expressing cell population of claim 236, wherein said human immature pancreatic islet hormone-expressing cells process less than about 70% of the insulin produced by said immature pancreatic islet hormone-expressing cells.

252. The enriched, *in vitro* human immature pancreatic islet hormone-expressing cell population of claim 250 or claim 251, wherein said insulin processing is measured by C-peptide release.

253. A method for producing a cell population enriched in human pancreatic islet hormone-expressing cells, said pancreatic hormone-expressing cells being derived *in vitro* from human pluripotent cells, said method comprising the steps of providing a cell population comprising human pancreatic islet hormone-expressing cells with a reagent that binds to neural cell adhesion molecule (NCAM) and separating human endocrine precursor cells bound to said reagent from cells that are not bound to said reagent, thereby producing a cell population enriched in human pancreatic islet hormone-expressing cells.

254. The method of claim 253, wherein said reagent comprises a molecule selected from the group consisting of an anti-NCAM antibody, an anti-NCAM antibody fragment and an NCAM ligand.

255. The method of claim 254, wherein said NCAM ligand is NCAM Binding Protein 10 (NBP10).

256. The method of claim 254, wherein said anti-NCAM antibody is labeled.

257. The method of claim 256, wherein said anti-NCAM antibody is fluorescently labeled.

258. The method of claim 254 further comprising providing said cell population with a secondary reagent that binds to said reagent.

259. The method of claim 258, wherein said reagent comprises and anti-NCAM antibody and wherein said secondary reagent that binds to said anti-NCAM antibody is fluorescently labeled.

260. The method of claim 257 or claim 259, wherein said separating step comprises using fluorescence activated cell sorting (FACS) to separate said pancreatic islet hormone-expressing cells bound to said anti-NCAM antibody from said cells that are not bound to said anti-NCAM antibody.

261. The method of claim 253 further comprising the step of providing said human immature pancreatic islet hormone-expressing cells with a second reagent that binds to CD133, and separating said human immature pancreatic islet hormone-expressing cells from cells that are bound to said second reagent.

262. The method of claim 253 further comprising the step of dissociating said cell population prior to providing said cell population with said reagent that binds NCAM.

263. The method of claim 253 further comprising obtaining a cell population comprising human endocrine precursor cells being multipotent cells that can differentiate into

human pancreatic islet hormone-expressing cells and incubating said human endocrine precursor cells in a culture medium for a sufficient time to permit human pancreatic islet hormone-expressing cells to form.

264. The method of claim 263 further comprising providing said human pancreatic endocrine cells with a factor selected from the group consisting of nicotinamide (NIC), exendin 4 (Ex4), hepatocyte growth factor (HGF), insulin-like growth factor-1 (IGF1), glucose dependent inslulinotropic polypeptide (GIP), nerve growth factor (NGF), vascular endothelial growth factor (VEGF) and combinations thereof in an amount sufficient to further promote differentiation of said human endocrine precursor cells to human pancreatic islet hormone-expressing cells, wherein said human pancreatic islet hormone-expressing cells express at least one pancreatic hormone selected from the group consisting of insulin, somatostatin and glucagon.

265. The method of claim 264, wherein said factor is selected from the group consisting of Ex4, HGF and IGF1.

266. The method of claim 265, wherein said factor is Ex4.

267. The method of claim 266, wherein Ex4 is provided to said cell population of endocrine precursor cells at a concentration ranging from about 10 ng/ml to about 100 ng/ml.

268. The method of claim 266, wherein Ex4 is provided to said cell population of endocrine precursor cells at a concentration of about 40 ng/ml.

269. The method of claim 265, wherein said factor is IGF1.

270. The method of claim 269 wherein IGF1 is provided to said cell population of endocrine precursor cells at a concentration ranging from about 10 ng/ml to about 1000 ng/ml.

271. The method of claim 269, wherein IGF1 is provided to said cell population of endocrine precursor cells at a concentration ranging from about 10 ng/ml to about 100 ng/ml.

272. The method of claim 269, wherein IGF1 is provided to said cell population of endocrine precursor cells at a concentration ranging from about 25 ng/ml to about 75 ng/ml.

273. The method of claim 269, wherein IGF1 is provided to said cell population of endocrine precursor cells at a concentration of about 50 ng/ml.

274. The method of claim 263 further comprising the steps of obtaining a cell population comprising a population of human PDX1-positive pancreatic endoderm cells, said human PDX1-positive pancreatic endoderm cells being multipotent cells that can differentiate into cells, tissues or organs derived from the anterior portion of the gut tube and providing said population of human PDX1-positive pancreatic endoderm cells with a gamma secretase inhibitor, thereby producing a population of human endocrine precursor cells.

275. The method of claim 274, wherein said gamma secretase inhibitor comprises N-[N-(3, 5,-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT).

276. The method of claim 275, wherein said DAPT is provided to said population of human PDX1-positive pancreatic endoderm cells at a concentration ranging from about 1 μM to about 10 μM.

277. The method of claim 275, wherein said DAPT is provided to said population of human PDX1-positive pancreatic endoderm cells at a concentration of about 3 μM.

278. The method of claim 274 further comprising providing said population of human PDX1-positive pancreatic endoderm cells with exendin 4 (Ex4).

279. The method of claim 278, wherein said Ex4 is provided to said population of human PDX1-positive pancreatic endoderm cells at a concentration ranging from about 10 ng/ml to about 100 ng/ml.

280. The method of claim 278, wherein said Ex4 is provided to said population of human PDX1-positive pancreatic endoderm cells at a concentration of about 40 ng/ml.

281. The method of claim 274, wherein the step of obtaining a population of human PDX1-positive pancreatic endoderm cells comprises the steps of obtaining a population of human foregut endoderm cells, said human foregut endoderm cells being PDX1-negative multipotent cells that can differentiate into cells, tissues or organs derived from the anterior portion of the gut tube and providing said population of human foregut endoderm cells with a retinoid, thereby producing a population of human PDX1-positive pancreatic endoderm cells.

282. The method of claim 281, wherein said retinoid is retinoic acid (RA).

283. The method of claim 282, wherein RA is provided to said population of human foregut endoderm cells at a concentration ranging from about 1 nM to about 10 μM.

284. The method of claim 281, wherein the step of obtaining a population of human foregut endoderm cells comprises the steps of obtaining a population of human definitive endoderm cells, said human definitive endoderm cells being multipotent cells that can differentiate into cells of the gut tube or organs derived therefrom and providing said population of human definitive endoderm cells with fibroblast growth factor 10 (FGF-10) and a hedgehog pathway inhibitor, thereby producing a population of human foregut endoderm cells.

285. The method of claim 284 further comprising withdrawing an exogenously added factor of the TGF-β superfamily that may be present in said population of definitive endoderm cells.

286. The method of claim 285, wherein said growth factor of the TGF-β superfamily is selected from the group consisting of Nodal, activin A, activin B and combinations thereof.

287. The method of claim 286, wherein said growth factor of the TGF-β superfamily is activin A.

288. The method of claim 284, wherein said hedgehog inhibitor comprises KAAD-cyclopamine.

289. The method of claim 288, wherein KAAD-cyclopamine is provided to said population of human definitive endoderm cells at a concentration ranging from about 0.01 μM to about 1 μM.

290. The method of claim 288, wherein KAAD-cyclopamine is provided to said population of human definitive endoderm cells at a concentration of about 0.2 μM.

291. The method of claim 284, wherein FGF-10 is provided to said population of human definitive endoderm cells at a concentration ranging from about 1 ng/ml to about 1000 ng/ml.

292. The method of claim 284, wherein FGF-10 is provided to said population of human definitive endoderm cells at a concentration ranging from about 10 ng/ml to about 100 ng/ml.

293. The method of claim 284, wherein FGF-10 is provided to said population of human definitive endoderm cells at a concentration of about 50 ng/ml.

294. The method of claim 284, wherein the step of obtaining a population of human definitive endoderm cells comprises the steps of obtaining a population of human pluripotent cells and providing said population of human pluripotent cells with at least one growth factor of the TGF-β superfamily.

295. The method of claim 294, wherein said at least one growth factor is Nodal.

296. The method of claim 294, wherein said at least one growth factor is activin A.

297. The method of claim 294, wherein said at least one growth factor is activin B.

298. The method of claim 294 further comprising providing said population of human pluripotent cells with wingless-type MMTV integration site family member 3A (Wnt3A).

299. The method of claim 294, wherein a plurality of growth factors of the TGFβ superfamily is provided.

300. The method of claim 299, wherein Wnt3A is also provided.

301. The method of claim 294, wherein said at least one growth factor is provided in a concentration of at least about 10 ng/ml.

302. The method of claim 294, wherein said at least one growth factor is provided in a concentration of at least about 100 ng/ml.

303. The method of claim 294, wherein said at least one growth factor is provided in a concentration of at least about 500 ng/ml.

304. The method of claim 294, wherein said at least one growth factor is provided in a concentration of at least about 1000 ng/ml.

305. The method of claim 294, wherein said at least one growth factor is provided in a concentration of at least about 5000 ng/ml.

306. The method of claim 294, wherein said human pluripotent cells are differentiated to human definitive endoderm cells in a medium comprising less than about 2% serum.

307. The method of claim 294, wherein said human pluripotent cells are human embryonic stem cells derived from a tissue selected from the group consisting of the morula, the ICM of an embryo and the gonadal ridges of an embryo.

308. An enriched, *in vitro* human pancreatic islet hormone-expressing cell population derived *in vitro* from human pluripotent cells.

309. The enriched, *in vitro* human pancreatic islet hormone-expressing cell population of claim 308, wherein said enriched cell population is produced by the method of claim 253.

310. The enriched, *in vitro* human pancreatic islet hormone-expressing cell population of claim 308, wherein said enriched cell population is produced by the method of claim 294.

311. The enriched, *in vitro* human pancreatic islet hormone-expressing cell population of claim 309 or 310, wherein at least about 25% of said enriched human cell population comprises human pancreatic islet hormone-expressing cells that express at least one marker selected from the group consisting of ghrelin, islet amyloid polypeptide (IAPP), insulin (INS), glucagon (GCG), NKX6 transcription factor related, locus 1 (NKX6.1), somatostatin (SOM), and pancreatic polypeptide (PP) and do not substantially express at least one marker selected from the group consisting of AFP, SOX7, SOX1, ZIC1 and NFM.

312. The enriched, *in vitro* human pancreatic islet hormone-expressing cell population of claim 311, wherein at least about 50% of said enriched human cell population comprises human pancreatic islet hormone-expressing cells that express at least one marker selected from the group consisting of ghrelin, islet amyloid polypeptide (IAPP), insulin (INS), glucagon (GCG), NKX6 transcription factor related, locus 1 (NKX6.1), somatostatin (SOM), and pancreatic polypeptide (PP) and do not substantially express at least one marker selected from the group consisting of AFP, SOX7, SOX1, ZIC1 and NFM.

313. The enriched, *in vitro* human pancreatic islet hormone-expressing cell population of claim 311, wherein at least about 90% of said enriched human cell population comprises human pancreatic islet hormone-expressing cells that express at least one marker selected from the group consisting of ghrelin, islet amyloid polypeptide (IAPP), insulin (INS), glucagon (GCG), NKX6 transcription factor related, locus 1 (NKX6.1), somatostatin (SOM) and pancreatic polypeptide (PP) and do not substantially express at least one marker selected from the group consisting of AFP, SOX7, SOX1, ZIC1 and NFM..

314. An *ex vivo* reagent-cell complex comprising a human endocrine precursor cell expressing NCAM, said endocrine precursor cell being a multipotent cell that can differentiate into human pancreatic islet hormone-expressing cells, and a reagent bound to said NCAM.

315. The *ex vivo* reagent-cell complex of claim 314, wherein said reagent comprises a molecule selected from the group consisting of an anti-NCAM antibody, an anti-NCAM antibody fragment and an NCAM ligand.

316. The *ex vivo* reagent-cell complex of claim 315, wherein said NCAM ligand is NCAM Binding Protein 10 (NBP10).

317. The *ex-vivo* reagent-cell complex of claim 315, wherein said reagent is an anti-NCAM antibody.

318. The *ex vivo* reagent-cell complex of claim 317, wherein said anti-NCAM antibody is labeled.

319. The *ex vivo* reagent-cell complex of claim 318, wherein said anti-NCAM antibody is fluorescently labeled.

320. The *ex vivo* reagent-cell complex of claim 314 further comprising a secondary reagent that binds to said reagent.

321. The *ex vivo* reagent-cell complex of claim 320, wherein said reagent comprises and anti-NCAM antibody and wherein said secondary reagent that binds to said anti-NCAM antibody is fluorescently labeled.

322. An *ex vivo* reagent-cell complex comprising a human immature islet hormone-expressing cell expressing NCAM, said human immature islet hormone-expressing cell being a multipotent cell that can differentiate into human pancreatic islet hormone-expressing cell, and a reagent bound to said NCAM.

323. The *ex vivo* reagent-cell complex of claim 322, wherein said reagent comprises a molecule selected from the group consisting of an anti-NCAM antibody, an anti-NCAM antibody fragment and an NCAM ligand.

324. The *ex vivo* reagent-cell complex of claim 323, wherein said NCAM ligand is NCAM Binding Protein 10 (NBP10).

325. The *ex-vivo* reagent-cell complex of claim 323, wherein said reagent is an anti-NCAM antibody.

326. The *ex vivo* reagent-cell complex of claim 325, wherein said anti-NCAM antibody is labeled.

327. The *ex vivo* reagent-cell complex of claim 326, wherein said anti-NCAM antibody is fluorescently labeled.

328. The *ex vivo* reagent-cell complex of claim 322 further comprising a secondary reagent that binds to said reagent.

329. The *ex vivo* reagent-cell complex of claim 328, wherein said reagent comprises and anti-NCAM antibody and wherein said secondary reagent that binds to said anti-NCAM antibody is fluorescently labeled.

330. The method of any one of claims 86, 150, 222 or 294, wherein said human pluripotent cells are human embryonic stem cells derived from a preimplantation embryo.

331. A method of producing human pancreatic hormone-expressing cells, said method comprising the steps of:

(a) providing a population of pluripotent human embryonic stem cells (hESCs) with at least one growth factor of the TGF-β superfamily, thereby producing a population of human definitive endoderm cells;

(b) providing said population of human definitive endoderm cells with at least one fibroblast growth factor, thereby producing a population of human foregut endoderm cells;

(c) providing said population of human foregut endoderm cells with noggin, thereby producing a population comprising human endocrine precursor cells; and

(d) incubating said population of human endocrine precursor cells in a culture medium for a sufficient time to permit human pancreatic islet hormone-expressing cells to form, wherein said sufficient time for human pancreatic hormone-expressing cells to form has been determined by detecting the presence of human pancreatic hormone-expressing cells in said cell population.

332. The method of claim 331, wherein at least about 2% of said human cells in said cell population differentiate into human pancreatic hormone-expressing cells.

333. The method of claim 331, wherein at least about 5% of said human cells in said cell population differentiate into human pancreatic hormone-expressing cells.

334. The method of claim 331, wherein at least about 10% of said human cells in said cell population differentiate into human pancreatic hormone-expressing cells.

335. The method of claim 331, wherein at least about 20% of said human cells in said cell population differentiate into human pancreatic hormone-expressing cells.

336. The method of claim 331, wherein at least about 40% of said human cells in said cell population differentiate into human pancreatic hormone-expressing cells.

337. The method of claim 331, wherein at least about 50% of said human cells in said cell population differentiate into human pancreatic hormone-expressing cells.

338. The method of claim 331, wherein at least about 70% of said human cells in said cell population differentiate into human pancreatic hormone-expressing cells.

339. The method of claim 331, wherein at least about 90% of said human cells in said cell population differentiate into human pancreatic hormone-expressing cells.

340. The method of claim 331, wherein detecting the presence of human pancreatic islet hormone-expressing cells in said cell population comprises detecting the expression of at least one marker selected from the group consisting of pancreatic duodenal homeobox 1 (PDX1), ghrelin (GHRL), islet amyloid polypeptide (IAPP), pancreatic polypeptide (PP), ISL1 transcription factor (ISL1), NKX6 transcription factor related locus 1 (NKX6.1) and paired box 6 (PAX6) in cells of said cell population.

341. The method of claim 340, wherein the expression of at least one of said markers is determined by Q-PCR.

342. The method of claim 340, wherein the expression of at least one of said markers is determined by immunocytochemistry.

343. The method of claim 331 further comprising providing the cell population with a gamma secretase inhibitor.

344. The method of claim 343, wherein said gamma secretase inhibitor comprises N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT).

345. The method of claim 343, wherein said gamma secretase inhibitor is provided to the cell population at about the same time as providing noggin or after providing noggin.

346. The method of claim 344, wherein said gamma secretase inhibitor is provided to the cell population at a concentration ranging from about 0.1 μM to about 10 μM.

347. The method of claim 331, wherein said at least one fibroblast growth factor is fibroblast growth factor 7 (FGF-7).

348. The method of claim 347, wherein said FGF-7 is provided to the cell culture at a concentration ranging from about 1 ng/ml to about 1000 ng/ml.

349. The method of claim 331 further comprising providing the cell population with a hedgehog inhibitor at about the same time as adding the at least one fibroblast growth factor.

350. The method of claim 349, wherein said hedgehog inhibitor comprises KAAD-cyclopamine.

351. The method of claim 350, wherein said KAAD-cyclopamine is provided to the cell population at a concentration ranging from about 0.01 μM to about 10 μM.

352. The method of claim 331 further comprising withdrawing any growth factor of the TGF-β superfamily that may be present in said population of definitive endoderm cells.

353. The method of claim 331, wherein said growth factor of the TGF-β superfamily is selected from the group consisting of Nodal, activin A, activin B and combinations thereof.

354. The method of claim 353, wherein said growth factor of the TGF-β superfamily comprises activin A.

355. The method of claim 354, wherein said activin A is provided to said hESCs at a concentration ranging from about 10 ng/ml to about 1000 ng/ml.

356. The method of claim 331 further comprising providing the hESCs with wingless-type MMTV integration site family member 3A (Wnt3A).

357. The method of claim 356, wherein said Wnt3A is provided at a concentration ranging from about 1 ng/ml to about 1000 ng/ml.

358. The method of claim 331, wherein said hESCs are differentiated to human definitive endoderm cells in a medium comprising less than about 2% serum.

359. The method of claim 331, wherein said hESCs are derived from a tissue selected from the group consisting of the morula, the ICM of an embryo and the gonadal ridges of an embryo.

360. The method of claim 331 further comprising providing a retinoid to the cell population at about the same time as providing noggin.

361. The method of claim 331 further comprising providing a retinoid to the cell population at about the same time or after adding at least one fibroblast growth factor.

362. The method of claim 360 or 361, wherein the retinoid is retinol.

363. The method of claim 360 or 361, wherein the retinoid is retinoic acid.

364. The method of claim 363, wherein the retinoic acid is provided at a concentration ranging from about 0.01 μM to about 10 μM.

365. The cell culture or cell population of any one of claims 1-49, 164-173, 236-252 or 308-313, wherein at least some of the cells are non-recombinant cells.

366. The cell culture or cell population of any one of claims 1-49, 164-173, 236-252 or 308-313, wherein the cells are non-recombinant cells.

367. The cell culture or cell population of any one of claims 1-49, 164-173, 236-252 or 308-313, wherein the cells have not been cultured in the presence of a histone deacetylase inhibitor.

368. The cell culture or cell population of claim 367, wherein said histone deacetylase inhibitor comprises sodium butyrate.

369. The method of any one of claims 50-163, 174-235, 253-307 or 331-364, wherein at least some of the cells are non-recombinant cells.

370. The method of any one of claims 50-163, 174-235, 253-307 or 331-364, wherein the cells are non-recombinant cells.

371. The method of any one of claims 50-163, 174-235, 253-307 or 331-364, wherein the cells have not been cultured in the presence of a histone deacetylase inhibitor.

372. The method of claim 371, wherein said histone deacetylase inhibitor comprises sodium butyrate.

WHAT IS CLAIMED IS:

1. An in vitro cell culture comprising human cells wherein at least 5% of said human cells are endocrine precursor cells that express neurogenin 3 (NEUROG3), paired box 4 (PAX4) and NKX2 transcription factor related locus 2 (NKX2.2), said endocrine precursor cells being multipotent cells that can differentiate into pancreatic islet hormone-expressing cells that express at least one pancreatic hormone selected from the group consisting of insulin, somatostatin, ghrelin, pancreatic polypeptide and glucagon.

2. The in vitro cell culture of claim 1, wherein at least 10% of said human cells are endocrine precursor cells.

3. The in vitro cell culture of claim 1, wherein at least 50% of said human cells are endocrine precursor cells.

4. The in vitro cell culture of any one of claims 1 to 3, wherein at least 10% of said human cells are human pancreatic duodenal homeobox 1 (PDX1)-positive pancreatic endoderm cells.

5. The in vitro cell culture of any one of claims 1 to 3, wherein at least 50% of said human cells are human pancreatic duodenal homeobox 1 (PDX1)-positive pancreatic endoderm cells.

6. The in vitro cell culture of any one of claims 1 to 5, wherein said endocrine precursor cells are multipotent cells that can differentiate into pancreatic islet hormone-expressing cells that express at least one pancreatic hormone selected from the group consisting of somatostatin, ghrelin, pancreatic polypeptide and glucagon.

7. The in vitro cell culture of any one of claims 1 to 6, wherein said endocrine precursor cells do not substantially express a pancreatic hormone selected from the group consisting of ghrelin, insulin, somatostatin and glucagon.

8. The in vitro cell culture of any one of claims 1 to 7, wherein said endocrine precursor cells are non-recombinant cells.

9. The in vitro cell culture of any one of claims 1 to 8, further comprising a retinoid.

10. The in vitro cell culture of any one of claims 1 to 9, wherein said endocrine precursor cells express NCAM.

11. The in vitro cell culture of any one of claims 1 to 10, wherein said endocrine precursor cells are multipotent cells that can differentiate into at least two types of pancreatic islet hormone-expressing cells selected from the group consisting of pancreatic hormone-expressing cells expressing somatostatin, pancreatic hormone-expressing cells expressing ghrelin, pancreatic hormone-expressing cells expressing pancreatic polypeptide and pancreatic hormone-expressing cells expressing glucagon.

12. A method of producing endocrine precursor cells, said method comprising the steps of:

(a) obtaining a cell population comprising pluripotent human cells;

(b) providing said cell population with a TGF-β superfamily growth factor, thereby producing definitive endoderm cells;

(c) withdrawing TGF-β superfamily growth factor from said cell population;

(d) providing said definitive endoderm cells with a retinoid, thereby producing human PDX1-positive pancreatic endoderm cells; and

(e) incubating said cell population in a culture medium for a sufficient time to permit formation of human endocrine precursor cells expressing neurogenin 3 (NEUROG3).

13. The method of claim 12, wherein said endocrine precursor cells further express paired box 4 (PAX4) and NKX2 transcription factor related locus 2 (NKX2.2).

14. The method of claim 12 or 13, wherein step (c) further comprises providing said cell population with a FGF-family growth factor.

15. The method of any one of claims 12 to 14, wherein said retinoid comprises retinoic acid.

16. The method of any one of claims 12 to 15, wherein said pluripotent human cells are non-recombinant cells.

17. The method of any one of claims 12 to 16, wherein step (e) further comprises providing said cell population with a gamma secretase inhibitor.

18. The method of any one of claims 12 to 17, wherein step (b) further comprises providing said cell population with wingless-type MMTV integration site family member 3A (Wnt3A).

19. An endocrine cell population produced by the method of any one of claims 12 to 18.