

### Claims

1. A method of differentiating neural cells, the method comprising:
  - (a) performing a first incubation comprising culturing pluripotent stem cells in a non-adherent culture vessel under conditions to produce a cellular spheroid, wherein beginning at the initiation of the first incubation (day 0) the cells are exposed to (i) an inhibitor of TGF- $\beta$ /activating-Nodal signaling; (ii) at least one activator of Sonic Hedgehog (SHH) signaling; (iii) an inhibitor of bone morphogenetic protein (BMP) signaling; and (iv) an inhibitor of glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ) signaling; and
  - (b) performing a second incubation comprising culturing cells of the spheroid in a substrate-coated culture vessel under conditions to neurally differentiate the cells.
2. The method of claim 1, wherein the second incubation begins on about day 7.
3. The method of claim 1 or claim 2, wherein the cells are exposed to the inhibitor of TGF- $\beta$ /activating-Nodal signaling up to a day at or before day 7.
4. The method of any one of claims 1-3, wherein the cells are exposed to the inhibitor of TGF- $\beta$ /activating-Nodal beginning at day 0 and through day 6, inclusive of each day.
5. The method of any one of claims 1-4, wherein the cells are exposed to the at least one activator of SHH signaling up to a day at or before day 7.
6. The method of any one of claims 1-5, wherein the cells are exposed to the at least one activator of SHH signaling beginning at day 0 and through day 6, inclusive of each day.
7. The method of any one of claims 1-6, wherein the cells are exposed to the inhibitor of BMP signaling up to a day at or before day 11.
8. The method of any one of claims 1-7, wherein the cells are exposed to the inhibitor of BMP signaling beginning at day 0 and through day 10, inclusive of each day.

9. The method of any one of claims 1-8, wherein the cells are exposed to the inhibitor of GSK3 $\beta$  signaling up to a day at or before day 13.
10. The method of any one of claims 1-9, wherein the cells are exposed to the inhibitor of GSK3b signaling beginning at day 0 and through day 12, inclusive of each day.
11. The method of any one of claims 1-10, wherein culturing the cells under conditions to neurally differentiate the cells comprises exposing the cells to (i) brain-derived neurotrophic factor (BDNF); (ii) ascorbic acid; (iii) glial cell-derived neurotrophic factor (GDNF); (iv) dibutyryl cyclic AMP (dbcAMP); (v) transforming growth factor beta-3 (TGF $\beta$ 3) (collectively, "BAGCT"); and (vi) an inhibitor of Notch signaling.
12. The method of any one of claims 1-11, wherein the cells are exposed to BAGCT and the inhibitor of Notch signaling beginning on day 11.
13. The method of any one of claims 1-12, wherein the cells are exposed to BAGCT and the inhibitor of Notch signaling beginning at day 11 and until harvest of the neurally differentiated cells, optionally until day 18, optionally until day 25.
14. A method of differentiating neural cells, the method comprising:
- (a) performing a first incubation comprising culturing pluripotent stem cells in a non-adherent culture vessel under conditions to produce a cellular spheroid, wherein beginning at the initiation of the first incubation day (day 0), the cells are exposed to (i) an inhibitor of TGF- $\beta$ /activating-Nodal signaling through day 6, each day inclusive; (ii) at least one activator of Sonic Hedgehog (SHH) signaling through day 6, each day inclusive; (iii) an inhibitor of bone morphogenetic protein (BMP) signaling through day 6, each day inclusive; and (iv) an inhibitor of glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ) signaling through day 6, each day inclusive; and
- (b) beginning on day 7, performing a second incubation to neurally differentiate cells of the spheroid, comprising culturing the cells in a culture vessel coated with a substrate selected from among: laminin, collagen, entactin, heparin sulfate proteoglycans, and combinations thereof, wherein beginning on day 7, the cells are exposed to (i) an inhibitor of BMP signaling and (ii) an inhibitor of GSK3 $\beta$  signaling; and beginning on day 11, the cells are exposed to (i)

brain-derived neurotrophic factor (BDNF); (ii) ascorbic acid; (iii) glial cell-derived neurotrophic factor (GDNF); (iv) dibutyryl cyclic AMP (dbcAMP); (v) transforming growth factor beta-3 (TGFβ3) (collectively, “BAGCT”); and (vi) an inhibitor of Notch signaling.

15. The method of any one of claims 1-14, further comprising harvesting the neurally differentiated cells.

16. The method of claim 15, wherein the harvesting is carried out at about day 16 or later.

17. The method of claim 15 or claim 16, wherein the harvesting is carried out between about day 16 and about day 30.

18. The method of any one of claims 15-17, wherein the harvesting is carried out between day 18 and day 25.

19. The method of any one of claims 15-18, wherein the harvesting is carried out at or about at day 18.

20. The method of any one of claims 15-18, wherein the harvesting is carried out at or about at day 25.

21. The method of any one of claims 1-20, wherein the neurally differentiated cells are determined dopaminergic neuron progenitor cells.

22. The method of claim 21, wherein the dopaminergic neuron progenitor cells are capable of innervating host tissue upon transplantation into a subject.

23. The method of any one of claims 1-22, wherein, prior to performing the second incubation, the spheroid is dissociated to produce a cell suspension, and cells of the cell suspension are cultured in the substrate-coated culture vessel.

24. The method of claim 23, wherein the dissociating is carried out at a time when the spheroid cells express at least one of PAX6 and OTX2.
25. The method of claim 23 or claim 24, wherein the dissociating is carried out on about day 7.
26. The method of any one of claims 1-25, wherein the culture vessel is selected from among the group consisting of: a plate, a dish, a flask, and a bioreactor.
27. The method of any one of claims 1-26, wherein the culture vessel is a plate.
28. The method of claim 27, wherein the plate is a 6-well plate, a 12-well plate, a 24-well plate, or a 96-well plate.
29. The method of claim 27 or claim 28, wherein the plate is a microwell plate.
30. The method of claim 29, wherein the microwell plate is a 6-well plate.
31. The method of claim 29, wherein the microwell plate is a 24-well plate.
32. The method of any one of claims 1-31, wherein the culture vessel is an Aggrewell™ plate.
33. The method of any one of claims 29-32, wherein the culture of pluripotent stem cells on day 0 comprises between about  $0.1 \times 10^6$  cells/cm<sup>2</sup> and about  $2 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.1 \times 10^6$  cells/cm<sup>2</sup> and about  $1 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.1 \times 10^6$  cells/cm<sup>2</sup> and about  $0.8 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.1 \times 10^6$  cells/cm<sup>2</sup> and about  $0.6 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.1 \times 10^6$  cells/cm<sup>2</sup> and about  $0.4 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.1 \times 10^6$  cells/cm<sup>2</sup> and about  $0.2 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.2 \times 10^6$  cells/cm<sup>2</sup> and about  $2 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.2 \times 10^6$  cells/cm<sup>2</sup> and about  $1 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.2 \times 10^6$  cells/cm<sup>2</sup> and about  $0.8 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.2 \times 10^6$  cells/cm<sup>2</sup> and about  $0.6 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.2 \times 10^6$  cells/cm<sup>2</sup> and about  $0.4 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.4 \times 10^6$  cells/cm<sup>2</sup> and about  $2 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.4 \times 10^6$  cells/cm<sup>2</sup> and about  $1 \times$

$10^6$  cells/cm<sup>2</sup>, between about  $0.4 \times 10^6$  cells/cm<sup>2</sup> and about  $0.8 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.4 \times 10^6$  cells/cm<sup>2</sup> and about  $0.6 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.6 \times 10^6$  cells/cm<sup>2</sup> and about  $2 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.6 \times 10^6$  cells/cm<sup>2</sup> and about  $1 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.6 \times 10^6$  cells/cm<sup>2</sup> and about  $0.8 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.8 \times 10^6$  cells/cm<sup>2</sup> and about  $2 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.8 \times 10^6$  cells/cm<sup>2</sup> and about  $1 \times 10^6$  cells/cm<sup>2</sup>, or between about  $1.0 \times 10^6$  cells/cm<sup>2</sup> and about  $2 \times 10^6$  cells/cm<sup>2</sup>.

34. The method of any one of claims 29-33, wherein the culture of pluripotent stem cells on day 0 is carried out in a 6-well plate and comprises between about  $1 \times 10^6$  pluripotent stem cells per well and about  $20 \times 10^6$  pluripotent stem cells per well, between about  $1 \times 10^6$  pluripotent stem cells per well and about  $15 \times 10^6$  pluripotent stem cells per well, between about  $1 \times 10^6$  pluripotent stem cells per well and about  $10 \times 10^6$  pluripotent stem cells per well, between about  $1 \times 10^6$  pluripotent stem cells per well and about  $5 \times 10^6$  pluripotent stem cells per well, between about  $5 \times 10^6$  pluripotent stem cells per well and about  $20 \times 10^6$  pluripotent stem cells per well, between about  $5 \times 10^6$  pluripotent stem cells per well and about  $15 \times 10^6$  pluripotent stem cells per well, between about  $5 \times 10^6$  pluripotent stem cells per well and about  $10 \times 10^6$  pluripotent stem cells per well, between about  $10 \times 10^6$  pluripotent stem cells per well and about  $20 \times 10^6$  pluripotent stem cells per well, between about  $10 \times 10^6$  pluripotent stem cells per well and about  $15 \times 10^6$  pluripotent stem cells per well, or between about  $15 \times 10^6$  pluripotent stem cells per well and about  $20 \times 10^6$  pluripotent stem cells per well.

35. The method of any one of claims 29-34, wherein the culture of pluripotent stem cells on day 0 is carried out in a 24-well plate and comprises between about  $1 \times 10^5$  pluripotent stem cells per well and about  $5 \times 10^6$  pluripotent stem cells per well, between about  $1 \times 10^5$  pluripotent stem cells per well and about  $1 \times 10^6$  pluripotent stem cells per well, between about  $1 \times 10^5$  pluripotent stem cells per well and about  $5 \times 10^5$  pluripotent stem cells per well, between about  $5 \times 10^5$  pluripotent stem cells per well and about  $5 \times 10^6$  pluripotent stem cells per well, between about  $5 \times 10^5$  pluripotent stem cells per well and about  $1 \times 10^6$  pluripotent stem cells per well, or between about  $1 \times 10^6$  pluripotent stem cells per well and about  $5 \times 10^6$  pluripotent stem cells per well.

36. The method of any one of claims 29-35, wherein the culture of pluripotent stem cells on day 0 comprises a number of cells sufficient to produce a spheroid on about day 7

comprising between about 1,000 cells and about 5,000 cells, or between about 2,000 cells and about 3,000 cells.

37. The method of any one of claims 29-36, wherein the culture of pluripotent stem cells on day 0 comprises a number of cells sufficient to produce a spheroid on about day 7 comprising about 2,000 cells.

38. The method of any one of claims 29-36, wherein the culture of pluripotent stem cells on day 0 comprises a number of cells sufficient to produce a spheroid on about day 7 comprising about 3,000 cells.

39. The method of any one of claims 1-38, wherein the culture vessel is treated to reduce or eliminate adherence of cells.

40. The method of 39, wherein the treating the culture vessel comprises incubating the culture vessel with pluronic acid.

41. The method of any one of claims 1-40, wherein, prior to the first incubation, the non-adherent culture vessel is exposed to a surfactant.

42. The method of claim 41, wherein the surfactant is pluronic acid.

43. The method of any one of claims 1-42, wherein the non-adherent culture vessel comprises a low or ultra-low attachment surface.

44. The method of any one of claim 1-43, the non-adherent culture vessel comprises an ultra-low attachment surface.

45. The method of any one of claims 1-44, wherein the substrate is a basement membrane protein.

46. The method of any one of claims 1-45, wherein the substrate is selected from one or more of laminin, collagen, entactin, heparin sulfate proteoglycans, and combinations thereof.

47. The method of any one of claims 1-46, wherein the substrate is a recombinant protein.
48. The method of any one of claims 1-47, wherein the substrate is recombinant laminin.
49. The method of any one of claims 1-48, wherein, the substrate-coated culture vessel is exposed to poly-L-ornithine prior to being used for culturing the cells.
50. The method of any one of claims 1-49, wherein the inhibitor of TGF- $\beta$ /activating-Nodal signaling is SB431542.
51. The method of claim 50, wherein the cells are exposed to SB431542 at a concentration of between about 1  $\mu$ M and about 20  $\mu$ M, between about 5  $\mu$ M and about 15  $\mu$ M, or between about 8  $\mu$ M and about 12  $\mu$ M, optionally about 10  $\mu$ M.
52. The method of any one of claims 1-51, wherein the at least one activator of SHH signaling is SHH or purmorphamine.
53. The method of any one of claims 1-51, wherein the at least one activator of SHH signaling comprises two activators of SHH signaling selected from SHH protein and purmorphamine.
54. The method of claim 52 or claim 53, wherein the cells are exposed to SHH at a concentration of between about 10 ng/mL and 500 ng/mL, between about 20 ng/mL and about 400 ng/mL, between about 50 ng/mL and about 200 ng/mL, or between about 75 ng/mL and about 150 ng/mL, optionally about 100 ng/mL.
55. The method of any one of claims 52-54, wherein the cells are exposed to purmorphamine at a concentration of between about 1  $\mu$ M and about 20  $\mu$ M, between about 5  $\mu$ M and about 15  $\mu$ M, or between about 8  $\mu$ M and about 12  $\mu$ M, optionally about 10  $\mu$ M.

56. The method of any one of claims 1-55, wherein the inhibitor of BMP signaling is LDN193189.

57. The method of claim 56, wherein the cells are exposed to LDN193189 at a concentration of between about 10 nM and 500 nM, between about 20 nM and about 400 nM, between about 50 nM and about 200 nM, or between about 75 nM and about 150 nM, optionally about 100 nM.

58. The method of any one of claims 1-57, wherein the inhibitor of GSK3 $\beta$  signaling is CHIR99021.

59. The method of claim 58, wherein the cells are exposed to CHIR99021 at a concentration of between about 0.1  $\mu$ M and about 5  $\mu$ M, between about 0.5  $\mu$ M and about 4  $\mu$ M, or between about 1  $\mu$ M and about 3  $\mu$ M, optionally about 2  $\mu$ M.

60. The method of any one of claims 11-59, wherein the cells are exposed to GDNF at a concentration of between about 1 ng/mL and about 100 ng/mL, between about 5 ng/mL and about 80 ng/mL, between about 10 ng/mL and about 60 ng/mL, or between about 15 ng/mL and about 30 ng/mL, optionally about 20 ng/mL.

61. The method of any one of claims 11-60, wherein the cells are exposed to BDNF at a concentration of between about 1 ng/mL and about 100 ng/mL, between about 5 ng/mL and about 80 ng/mL, between about 10 ng/mL and about 60 ng/mL, or between about 15 ng/mL and about 30 ng/mL, optionally about 20 ng/mL.

62. The method of any one of claims 11-61, wherein the cells are exposed to dbcAMP at a concentration of between about 0.1 mM and 5 mM, between about 0.2 mM and about 4 mM, between about 0.3 mM and about 3 mM, or between about 0.4 mM and about 2 mM, optionally about 0.5 mM.

63. The method of any one of claims 11-62, wherein the cells are exposed to ascorbic at a concentration of between about 0.05 mM and about 5 mM, between about 0.1 mM and about 1 mM, or between about 0.2 mM and about 0.5 mM, optionally about 0.2 mM.

64. The method of any one of claims 11-63, wherein the cells are exposed to TGF $\beta$ 3 at a concentration of between about 0.1 ng/mL and about 5 ng/mL, between about 0.3 ng/mL and about 3 ng/mL, or between about 0.5 ng/mL and about 2 ng/mL, optionally about 1 ng/mL.

65. The method of any one of claims 11-64, wherein the inhibitor of Notch signaling is DAPT.

66. The method of any one of claims 11-65, wherein the cells are exposed to DAPT at a concentration of between about 1  $\mu$ M and about 20  $\mu$ M, between about 5  $\mu$ M and about 15  $\mu$ M, or between about 8  $\mu$ M and about 12  $\mu$ M, optionally about 10  $\mu$ M.

67. The method of any one of claims 1-66, wherein the culturing in the first incubation and/or the second incubation is carried out in media comprising serum or a serum replacement.

68. The method of any one of claims 1-67, wherein the culturing in the first incubation and the second incubation is carried out in media comprising serum or a serum replacement.

69. The method of any one of claims 1-68, wherein the cells are cultured in a media comprising serum or a serum replacement from day about 0 to about day 10.

70. The method of any one of claims 67-69, wherein the serum or serum replacement comprises about 5% of the media (v/v).

71. The method of any one of claims 67-70, wherein the serum or serum replacement comprises about 2% of the media (v/v).

72. The method of any one of claims 67-71, wherein the media comprises about 5% serum or serum replacement (v/v) from about day 0 to about day 1 about 2% serum replacement (v/v) from about day 2 to about day 10.

73. The method of any one of claims 67-72, wherein the media contains a serum replacement.

74. The method of any one of claims 67-73, wherein the serum replacement does not contain fetal bovine serum (FBS).

75. The method of any one of claims 67-74, wherein the serum replacement is KnockOut™ serum replacement.

76. The method of any one of claims 1-66, wherein the cells are cultured in the absence of serum for the duration of culture.

77. The method of any one of claims 1-76, wherein the cells are exposed to an inhibitor of Rho-associated protein kinase (ROCK) signaling on day 0, day 7, day 16, and/or day 20.

78. The method of any one of claims 1-77, wherein the cells are exposed to an inhibitor of Rho-associated kinase protein (ROCK) signaling on days 0, 7, 16, and 20.

79. The method of claim 77 or claim 78, wherein the ROCK inhibitor is Y-27632.

80. The method of any one of claims 77-79, wherein the cells are exposed to the ROCK inhibitor at a concentration of between about 1  $\mu$ M and about 20  $\mu$ M, between about 5  $\mu$ M and about 15  $\mu$ M, or between about 8  $\mu$ M and about 12  $\mu$ M, optionally about 10  $\mu$ M.

81. The method of any one of claims 1-80, wherein at least about 50% of the media is replaced daily or every other day, or every third day.

82. The method of any one of claims 23-81, wherein the spheroid is dissociated by enzymatic dissociation.

83. The method of any one of claims 23-82, wherein the spheroid is dissociated by enzymatic dissociation comprising use of an enzyme selected from among the group consisting of: accutase, dispase, collagenase, and combinations thereof.

84. The method of any one of claims 23-83, wherein the spheroid is dissociated by enzymatic dissociation comprising use of accutase.

85. The method of any one of claims 1-84, wherein the method comprises transferring to the substrate-coated culture vessel between about  $0.1 \times 10^6$  cells/cm<sup>2</sup> and about  $2 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.1 \times 10^6$  cells/cm<sup>2</sup> and about  $1 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.1 \times 10^6$  cells/cm<sup>2</sup> and about  $0.8 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.1 \times 10^6$  cells/cm<sup>2</sup> and about  $0.6 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.1 \times 10^6$  cells/cm<sup>2</sup> and about  $0.4 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.1 \times 10^6$  cells/cm<sup>2</sup> and about  $0.2 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.2 \times 10^6$  cells/cm<sup>2</sup> and about  $2 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.2 \times 10^6$  cells/cm<sup>2</sup> and about  $1 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.2 \times 10^6$  cells/cm<sup>2</sup> and about  $0.8 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.2 \times 10^6$  cells/cm<sup>2</sup> and about  $0.6 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.2 \times 10^6$  cells/cm<sup>2</sup> and about  $0.4 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.4 \times 10^6$  cells/cm<sup>2</sup> and about  $2 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.4 \times 10^6$  cells/cm<sup>2</sup> and about  $1 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.4 \times 10^6$  cells/cm<sup>2</sup> and about  $0.8 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.4 \times 10^6$  cells/cm<sup>2</sup> and about  $0.6 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.6 \times 10^6$  cells/cm<sup>2</sup> and about  $2 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.6 \times 10^6$  cells/cm<sup>2</sup> and about  $1 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.6 \times 10^6$  cells/cm<sup>2</sup> and about  $0.8 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.8 \times 10^6$  cells/cm<sup>2</sup> and about  $2 \times 10^6$  cells/cm<sup>2</sup>, between about  $0.8 \times 10^6$  cells/cm<sup>2</sup> and about  $1 \times 10^6$  cells/cm<sup>2</sup>, or between about  $1.0 \times 10^6$  cells/cm<sup>2</sup> and about  $2 \times 10^6$  cells/cm<sup>2</sup>.

86. The method of any one of claims 1-85, wherein the method comprises transferring to the substrate-coated culture vessel between about  $0.4 \times 10^6$  cells/cm<sup>2</sup> and about  $0.8 \times 10^6$  cells/cm<sup>2</sup>.

87. The method of claim 85 or claim 86, wherein the transferring occurs on about day 7.

88. The method of any one of claims 15-87, further comprising formulating the harvested cells with a cryoprotectant.

89. The method of claim 88, wherein the cryoprotectant is selected from among the group consisting of: glycerol, propylene glycol, and dimethyl sulfoxide (DMSO).

90. The method of claim 88 or claim 89, further comprising cryopreserving the harvested cells, and optionally thawing the cells prior to use.

91. The method of any one of claims 1-90, wherein the pluripotent stem cells are embryonic stem (ES) cells, induced pluripotent stem cells (iPSCs), or a combination thereof.

92. The method of any one of claims 1-91, wherein the pluripotent stem cells are embryonic stem (ES) cells, optionally mouse or human embryonic stem cells.

93. The method of any one of claims 1-92, wherein the pluripotent stem cells are human embryonic stem cells.

94. The method of any one of claims 1-91, wherein the pluripotent stem cells are induced pluripotent stem cells, optionally mouse or human induced pluripotent stem cells.

95. The method of any one of claims 1-91 and 94, wherein the pluripotent stem cells are human induced pluripotent stem cells.

96. The method of any one of claims 1-95, wherein the pluripotent stem cells are autologous to a subject to be treated with the neurally differentiated cells.

97. The method of any one of claims 1-95, wherein the pluripotent stem cells are allogeneic to a subject to be treated with the neurally differentiated cells.

98. The method of any one of claims 1-97, wherein the pluripotent stem cells are hypoimmunogenic.

99. The method of claim 98, wherein the pluripotent stem cells are engineered to (a) remove genes encoding one or more of polymorphic HLA-A/-B/-C and HLA class II molecules; and (b) to provide genes encoding one or more of PD-L1, HLA-G, and CD47, optionally into a AAVS1 safe harbor locus.

100. A therapeutic composition produced by the method of any of claims 1-99.

101. The therapeutic composition of claim 100, wherein cells of the composition express EN1 and CORIN and less than 10% of the total cells in the composition express TH.

102. A therapeutic composition comprising determined dopamine neuron progenitor cells (DDPCs) derived from pluripotent stem cells, wherein cells in the composition express EN1 and CORIN and less than 10% of the total cells in the composition express TH.

103. A therapeutic composition comprising determined dopamine neuron progenitor cells (DDPCs) derived from pluripotent stem cells, wherein the therapeutic composition exhibits a ratio of counts per million (CPM) *TH* to CPM *GAPDH* of less than about  $2.5 \times 10^{-2}$ .

104. A therapeutic composition comprising determined dopamine neuron progenitor cells (DDPCs) derived from pluripotent stem cells, wherein the therapeutic composition exhibits one or more of:

(a) a ratio of counts per million (CPM) *NEUROG1* to CPM *GAPDH* of greater than about  $2 \times 10^{-5}$ ;

(b) a ratio of CPM *EDN3* to CPM *GAPDH* of greater than about  $5 \times 10^{-4}$ ;

(c) a ratio of CPM *HES1* to CPM *GAPDH* of greater than about  $4 \times 10^{-3}$ ;

(d) a ratio of CPM *PSRC1* to CPM *GAPDH* of greater than about  $8 \times 10^{-3}$ ;

(e) a ratio of CPM *NEK6* to CPM *GAPDH* of greater than about  $2 \times 10^{-3}$ ;

- (f) a ratio of CPM *IQGAP3* to CPM *GAPDH* of greater than about  $1 \times 10^{-2}$ ;
- (g) a ratio of CPM *USP44* to CPM *GAPDH* of greater than about  $8 \times 10^{-4}$ ;
- (h) a ratio of CPM *CEP55* to CPM *GAPDH* of greater than about  $3 \times 10^{-3}$ ;
- (i) a ratio of CPM *KIF20A* to CPM *GAPDH* of greater than about  $2 \times 10^{-2}$ ;
- (j) a ratio of CPM *AURKA* to CPM *GAPDH* of greater than about  $2 \times 10^{-2}$ ;
- (k) a ratio of CPM *CALCA* to CPM *GAPDH* of less than about  $1 \times 10^{-3}$ ;
- (l) a ratio of CPM *GLRA2* to CPM *GAPDH* of less than about  $1 \times 10^{-3}$ ;
- (m) a ratio of CPM *MAPT* to CPM *GAPDH* of less than about  $1 \times 10^{-1}$ ;
- (n) a ratio of CPM *CAMK2B* to CPM *GAPDH* of less than about  $5 \times 10^{-2}$ ;
- (o) a ratio of CPM *SYT13* to CPM *GAPDH* of less than about  $1 \times 10^{-2}$ ;
- (p) a ratio of CPM *LHFPL4* to CPM *GAPDH* of less than about  $1 \times 10^{-3}$ ;
- (q) a ratio of CPM *RET* to CPM *GAPDH* of less than about  $5 \times 10^{-3}$ ;
- (r) a ratio of CPM *KCND3* to CPM *GAPDH* of less than about  $1 \times 10^{-2}$ ;
- (s) a ratio of CPM *NSG2* to CPM *GAPDH* of less than about  $5 \times 10^{-2}$ ; and
- (t) a ratio of CPM *SNAP25* to CPM *GAPDH* of less than about  $2 \times 10^{-2}$ .

105. The therapeutic composition of claim 104, wherein:

- (a) the ratio of CPM *NEUROG1* to CPM *GAPDH* is between about  $5 \times 10^{-5}$  and about  $5 \times 10^{-3}$ ;
- (b) the ratio of CPM *EDN3* to CPM *GAPDH* is between about  $1 \times 10^{-3}$  and about  $5 \times 10^{-2}$ ;
- (c) the ratio of CPM *HES1* to CPM *GAPDH* is between about  $5 \times 10^{-3}$  and about  $5 \times 10^{-2}$ ;
- (d) the ratio of CPM *PSRC1* to CPM *GAPDH* is between about  $1 \times 10^{-2}$  and about  $5 \times 10^{-2}$ ;
- (e) the ratio of CPM *NEK6* to CPM *GAPDH* is between about  $2 \times 10^{-2}$  and about  $5 \times 10^{-1}$ ;
- (f) the ratio of CPM *IQGAP3* to CPM *GAPDH* is between about  $1.5 \times 10^{-2}$  and about  $1 \times 10^{-1}$ ;
- (g) the ratio of CPM *USP44* to CPM *GAPDH* is between about  $5 \times 10^{-3}$  and about  $5 \times 10^{-2}$ ;
- (h) the ratio of CPM *CEP55* to CPM *GAPDH* is between about  $4 \times 10^{-3}$  and about  $5 \times 10^{-2}$ ;

- (i) the ratio of CPM *KIF20A* to CPM *GAPDH* is between about  $2.5 \times 10^{-2}$  and about  $2 \times 10^{-1}$ ;
- (j) the ratio of CPM *AURKA* to CPM *GAPDH* is between about  $2.6 \times 10^{-2}$  and about  $2 \times 10^{-1}$ ;
- (k) the ratio of CPM *CALCA* to CPM *GAPDH* is between about  $1 \times 10^{-5}$  and about  $1 \times 10^{-3}$ ;
- (l) the ratio of CPM *GLRA2* to CPM *GAPDH* is between about  $1 \times 10^{-6}$  and about  $1 \times 10^{-3}$ ;
- (m) the ratio of CPM *MAPT* to CPM *GAPDH* is between about  $5 \times 10^{-3}$  and about  $1 \times 10^{-1}$ ;
- (n) the ratio of CPM *CAMK2B* to CPM *GAPDH* is between about  $1 \times 10^{-3}$  and about  $5 \times 10^{-2}$ ;
- (o) the ratio of CPM *SYT13* to CPM *GAPDH* is between about  $1 \times 10^{-3}$  and about  $1 \times 10^{-2}$ ;
- (p) the ratio of CPM *LHFPL4* to CPM *GAPDH* is between about  $1 \times 10^{-4}$  and about  $1 \times 10^{-3}$ ;
- (q) the ratio of CPM *RET* to CPM *GAPDH* is between about  $1 \times 10^{-4}$  and about  $4 \times 10^{-3}$ ;
- (r) the ratio of CPM *KCND3* to CPM *GAPDH* is between about  $1 \times 10^{-3}$  and about  $1 \times 10^{-2}$ ;
- (s) the ratio of CPM *NSG2* to CPM *GAPDH* is between about  $1 \times 10^{-3}$  and about  $2.4 \times 10^{-2}$ ; and/or
- (t) the ratio of CPM *SNAP25* to CPM *GAPDH* is between about  $1 \times 10^{-3}$  and about  $1 \times 10^{-2}$ .

106. The therapeutic composition of claim 104 or claim 105, wherein the therapeutic composition exhibits between two and twenty of (a)-(t).

107. The therapeutic composition of any one of claims 100 and 103-106, wherein cells in the therapeutic composition express EN1 and CORIN.

108. The therapeutic composition of any one of claims 100-107, wherein the therapeutic composition exhibits:

(a) a ratio of counts per million (CPM) *ENI* to CPM *GAPDH* of greater than about  $1 \times 10^{-4}$ ; and/or

(b) a ratio of CPM *CORIN* to CPM *GAPDH* of greater than about  $2 \times 10^{-2}$ .

109. The therapeutic composition of any one of claims 100-108, wherein the cells of the composition exhibit:

a ratio of counts per million (CPM) *ENI* to CPM *GAPDH* of between about  $1.5 \times 10^{-3}$  and  $1 \times 10^{-2}$ ; and/or

a ratio of CPM *CORIN* to CPM *GAPDH* of between about  $5 \times 10^{-2}$  and  $5 \times 10^{-1}$ .

110. The therapeutic composition of any one of claims 100 and 104-109, wherein cells in the composition express TH.

111. The therapeutic composition of any one of claims 100 and 103-110, wherein less than 10% of the total cells in the composition express TH.

112. The therapeutic composition of any one of claims 100-111, wherein the therapeutic composition exhibits a ratio of counts per million (CPM) *TH* to CPM *GAPDH* of less than about  $3 \times 10^{-2}$ .

113. The therapeutic composition of any one of claims 100-112, wherein between about 2% and about 10%, between about 2% and about 8%, between about 2% and about 6%, between about 2% and about 4%, between about 4% and about 10%, between about 4% and about 8%, between about 4% and about 6%, between about 6% and about 10%, between about 6% and about 8%, or between about 8% and 10% of the total cells in the composition express TH.

114. The therapeutic composition of any one of claims 100-113, wherein less than 5% of the total cells in the composition express TH.

115. The therapeutic composition of any one of claims 100-114, wherein between about 1% and about 5% or between about 2% and about 4% of the total cells in the composition express TH.

116. The therapeutic composition of any one of claims 100-115, wherein the determined cells are capable of engrafting and innervating other cells in vivo.

117. The therapeutic composition of any one of claims 100-116, wherein the determined cells are capable of producing dopamine, optionally wherein the determined cells do not produce or do not substantially produce norepinephrine.

118. The therapeutic composition of any one of claims 100-117, wherein the therapeutic composition comprises at least 5 million total cells, at least 10 million total cells, at least 15 million total cells, at least 20 million total cells, at least 30 million total cells, at least 40 million total cells, at least 50 million total cells, at least 100 million total cells, at least 150 million total cells, or at least 200 million total cells.

119. The therapeutic composition of any one of claims 100-118, wherein the therapeutic composition comprises between at or about 5 million total cells and at or about 200 million total cells, between at or about 5 million total cells and at or about 150 million total cells, between at or about 5 million total cells and at or about 100 million total cells, between at or about 5 million total cells and at or about 50 million total cells, between at or about 5 million total cells and at or about 25 million total cells, between at or about 5 million total cells and at or about 10 million total cells, between at or about 10 million total cells and at or about 200 million total cells, between at or about 10 million total cells and at or about 150 million total cells, between at or about 10 million total cells and at or about 100 million total cells, between at or about 10 million total cells and at or about 50 million total cells, between at or about 10 million total cells and at or about 25 million total cells, between at or about 25 million total cells and at or about 200 million total cells, between at or about 25 million total cells and at or about 150 million total cells, between at or about 25 million total cells and at or about 100 million total cells, between at or about 25 million total cells and at or about 50 million total cells, between at or about 50 million total cells and at or about 200 million total cells, between at or about 50 million total cells and at or about 150 million total cells, between at or about 50 million total cells and at or about 100 million total cells, between at or about 100 million total cells and at or about 200 million total cells, between at or about 100 million total cells and at or about 150

million total cells, or between at or about 150 million total cells and at or about 200 million total cells.

120. The therapeutic composition of any one of claims 100-119, wherein the therapeutic composition comprises at least 10 million total cells.

121. The therapeutic composition of any one of claims 100-120, wherein the therapeutic composition comprises at least 20 million total cells.

122. The therapeutic composition of any one of claims 100-121, wherein the therapeutic composition comprises at least 30 million total cells.

123. The therapeutic composition of any one of claims 100-122, wherein the therapeutic composition comprises at least 40 million total cells.

124. The therapeutic composition of any one of claims 100-123, wherein at least about 70%, 75%, 80%, 85%, 90%, or 95% of the total cells in the therapeutic composition are viable.

125. The therapeutic composition of any one of claims 100-124, wherein the therapeutic composition comprises a cryoprotectant.

126. The therapeutic composition of claim 125, wherein the cryoprotectant is selected from among the group consisting of: glycerol, propylene glycol, and dimethyl sulfoxide (DMSO).

127. The therapeutic composition of any one of claims 100-126, wherein the composition is for use in treatment of a neurodegenerative disease or condition in a subject.

128. The therapeutic composition of any one of claims 100-127, wherein the neurodegenerative disease or condition comprises a loss of dopaminergic neurons, optionally in the SN, optionally in the SNc.

129. The therapeutic composition of claim 128, wherein the neurodegenerative disease or condition is Parkinson's disease.

130. The therapeutic composition of claim 128, wherein the neurodegenerative disease or condition is Parkinsonism.

131. A method of treatment, comprising administering to a subject in need thereof a therapeutically effective amount of the therapeutic composition of any one of claims 100-130.

132. The method of claim 131, wherein the number of cells administered to the subject is between about  $0.25 \times 10^6$  cells and about  $20 \times 10^6$  cells, between about  $0.25 \times 10^6$  cells and about  $15 \times 10^6$  cells, between about  $0.25 \times 10^6$  cells and about  $10 \times 10^6$  cells, between about  $0.25 \times 10^6$  cells and about  $5 \times 10^6$  cells, between about  $0.25 \times 10^6$  cells and about  $1 \times 10^6$  cells, between about  $0.25 \times 10^6$  cells and about  $0.75 \times 10^6$  cells, between about  $0.25 \times 10^6$  cells and about  $0.5 \times 10^6$  cells, between about  $0.5 \times 10^6$  cells and about  $20 \times 10^6$  cells, between about  $0.5 \times 10^6$  cells and about  $15 \times 10^6$  cells, between about  $0.5 \times 10^6$  cells and about  $10 \times 10^6$  cells, between about  $0.5 \times 10^6$  cells and about  $5 \times 10^6$  cells, between about  $0.5 \times 10^6$  cells and about  $1 \times 10^6$  cells, between about  $0.5 \times 10^6$  cells and about  $0.75 \times 10^6$  cells, between about  $0.75 \times 10^6$  cells and about  $20 \times 10^6$  cells, between about  $0.75 \times 10^6$  cells and about  $15 \times 10^6$  cells, between about  $0.75 \times 10^6$  cells and about  $10 \times 10^6$  cells, between about  $0.75 \times 10^6$  cells and about  $5 \times 10^6$  cells, between about  $0.75 \times 10^6$  cells and about  $1 \times 10^6$  cells, between about  $1 \times 10^6$  cells and about  $20 \times 10^6$  cells, between about  $1 \times 10^6$  cells and about  $15 \times 10^6$  cells, between about  $1 \times 10^6$  cells and about  $10 \times 10^6$  cells, between about  $1 \times 10^6$  cells and about  $5 \times 10^6$  cells, between about  $5 \times 10^6$  cells and about  $20 \times 10^6$  cells, between about  $5 \times 10^6$  cells and about  $15 \times 10^6$  cells, between about  $5 \times 10^6$  cells and about  $10 \times 10^6$  cells, between about  $10 \times 10^6$  cells and about  $20 \times 10^6$  cells, between about  $10 \times 10^6$  cells and about  $15 \times 10^6$  cells, or between about  $15 \times 10^6$  cells and about  $20 \times 10^6$  cells.

133. The method of claim 131 or claim 132, wherein the subject has a neurodegenerative disease or condition.

134. The method of any one of claims 131-133, wherein the neurodegenerative disease or condition comprises the loss of dopaminergic neurons.

135. The method of any one of claims 131-134, wherein the subject has lost at least 50%, at least 60%, at least 70%, or at least 80% of dopaminergic neurons, optionally in the substantia nigra (SN), optionally in the SN pars compacta (SNc).

136. The method of any one of 131-135, wherein the neurodegenerative disease or condition is Parkinsonism.

137. The method of any one of claims 131-135, wherein the neurodegenerative disease or condition is Parkinson's disease, optionally idiopathic Parkinson's disease.

138. The method of any one of claims 131-137, wherein the administering comprises delivering cells of a composition by stereotactic injection.

139. The method of any one of claims 131-138, wherein the administering comprises delivering cells of a composition through a catheter.

140. The method of claim 138 or claim 139, wherein the cells are delivered to the striatum of the subject.

141. The method of any one of claims 131-140, wherein the cells of the therapeutic composition are autologous to the subject.

142. The method of any one of claims 131-141, wherein the cells of the therapeutic composition are allogeneic to the subject.

143. The method of any one of claims 131-142, wherein the cells of the therapeutic composition are hypoinmunogenic.

144. The method of claim 143, wherein the cells of the therapeutic composition are engineered to (a) remove genes encoding one or more of polymorphic HLA-A/-B/-C and HLA class II molecules; and (b) to provide genes encoding one or more of PD-L1, HLA-G, and CD47, optionally into a AAVS1 safe harbor locus.

145. Use of a composition of any one of claims 100-130 for formulation of a medicament for treating a subject having a neurodegenerative disease or condition.

146. A pharmaceutical composition of any one of claims 100-115 for use in treating a subject having a neurodegenerative disease or condition.

147. The use of claim 145 or the pharmaceutical composition for use of claim 146, wherein the neurodegenerative disease or condition comprises the loss of dopaminergic neurons.

148. The use or the pharmaceutical composition for use of any one of claims 145-147, wherein the subject has lost at least 50%, at least 60%, at least 70%, or at least 80% of dopaminergic neurons, optionally in the substantia nigra (SN), optionally in the SN pars compacta (SNc).

149. The use or the pharmaceutical composition for use of any one of claims 145-148, wherein the neurodegenerative disease or condition is Parkinsonism.

150. The use or the pharmaceutical composition for use of any one of claims 145-148, wherein the neurodegenerative disease or condition is Parkinson's disease, optionally idiopathic Parkinson's disease.

151. The use or the pharmaceutical composition for use of any one of claims 145-150, wherein the cells of the composition are autologous to the subject.

152. The use or the pharmaceutical composition for use of any one of claims 145-150, wherein the cells of the composition are allogeneic to the subject.

153. The use or the pharmaceutical composition for use of any one of claims 145-152, wherein the cells of the therapeutic composition are hypoimmunogenic.

154. The use or the pharmaceutical composition for use of claim 153, wherein the cells of the therapeutic composition are engineered to (a) remove genes encoding one or more of polymorphic HLA-A/-B/-C and HLA class II molecules; and (b) to provide genes encoding one or more of PD-L1, HLA-G, and CD47, optionally into a AAVS1 safe harbor locus.