

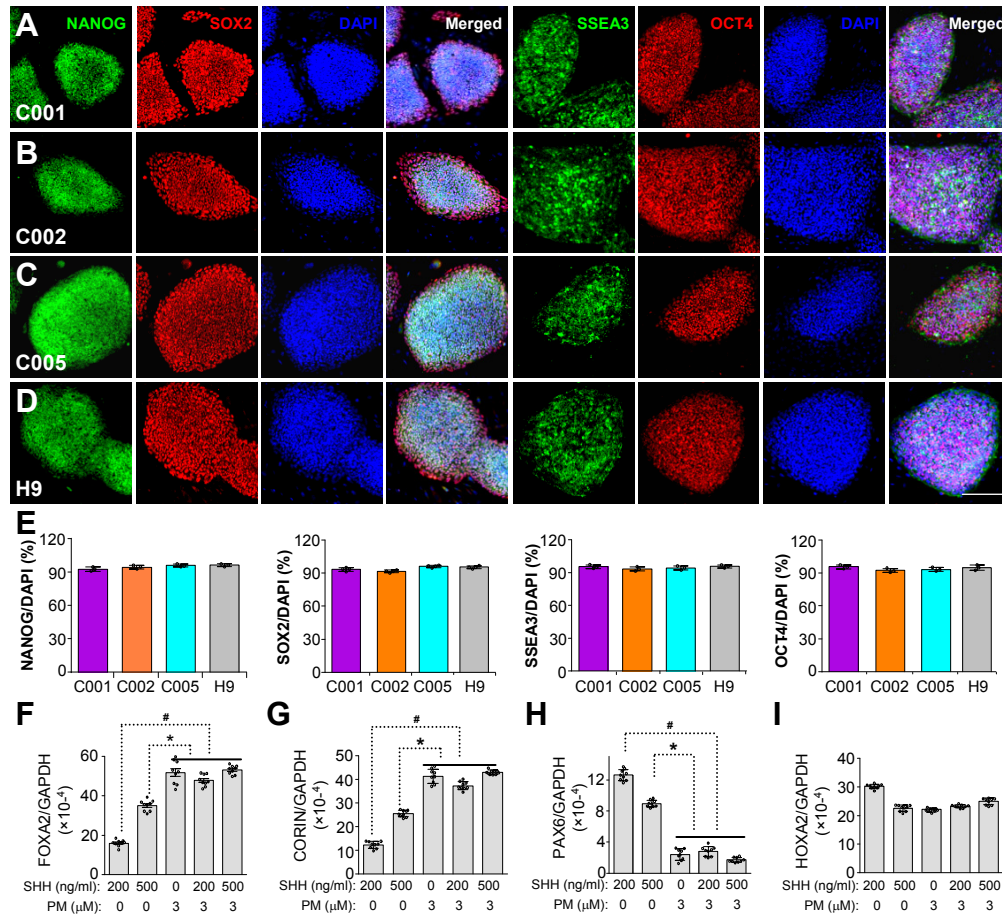


Fig. S1 Pluripotency marker expression and effects of SHH and purmorphamine on differentiation. **A-E**, Immunostaining (A-D) and quantification (E) of the indicated pluripotency markers in C001, C002 and C005 iPSCs and H9 hESCs. Bar, 100 μ m. $n = 3$ independent experiments, each counting cells in at least 20 colonies. **F-I**, C002 iPSCs were differentiated to mFP progenitors in the presence of 0.8 μ M CHIR and SHH and purmorphamine (PM) at the indicated concentrations. At day 14 of differentiation, expression levels of FOXA2 (F), CORIN (G), PAX6 (H) and HOXA2 (I) were analyzed by qRT-PCR. #, $p < 0.01$, unpaired t -test, vs. 200 ng/ml SHH; *, $p < 0.05$, unpaired t -test, vs. 500 ng/ml SHH, $n=9$ from 3 separate experiments.

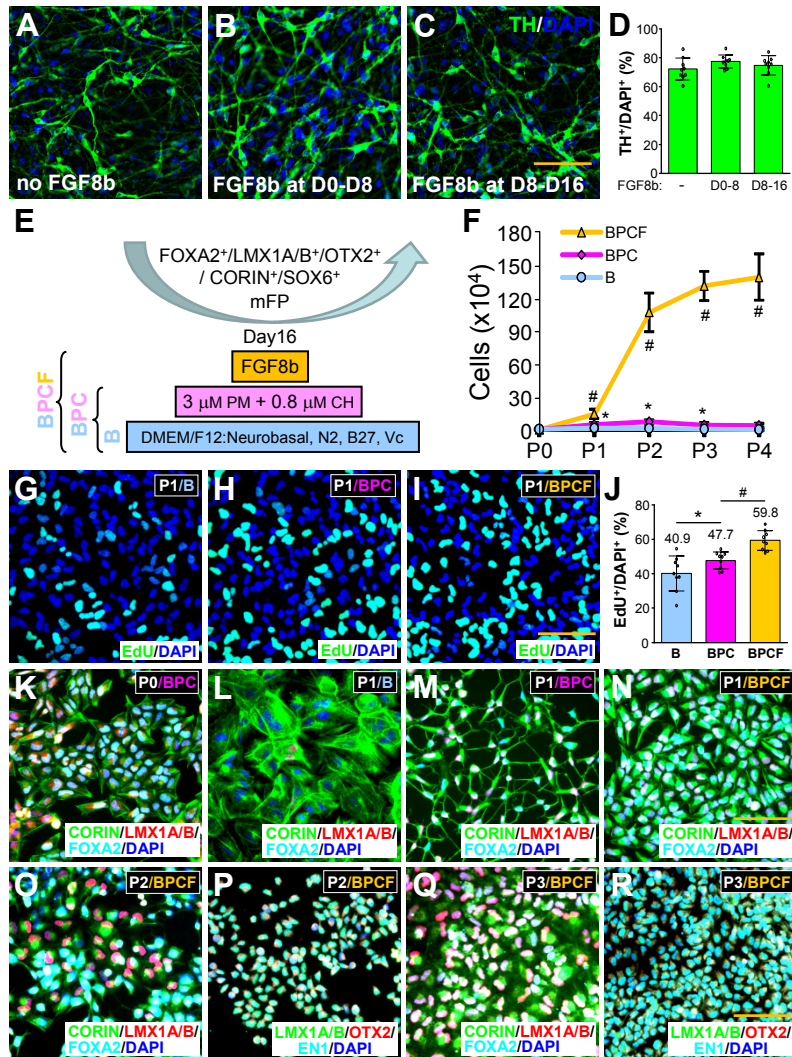


Fig. S2 The roles of FGF8b in the differentiation and proliferation of mFP progenitors. A-D Immunostaining (A-C) and quantification (D) of TH⁺ neurons at day 60. These neurons were differentiated from C002 iPSCs in the absence (A) or presence of FGF8b at days 0-8 (B) or days 8-16 (C). *n* = 9 from three independent experiments with 3 wells in each experiment. **E-F** Grown in different media indicated in (E), the number of midbrain floor plate DA progenitor cells differentiated from C002 iPSCs were counted at different passages (F). B, Basal Medium containing DMEM/F12:Neurobasal (1:1) with N2, B27 (no vitamin A) and 0.2 mM Vitamin C (Vc); BPC, Basal Medium plus 3 μ M purmorphamine and 0.8 μ M CHIR99021; BPCF, BPC plus 100 ng/ml FGF8b. *, *p* < 0.05, unpaired *t*-test, BPC vs. B; #, *p* < 0.01, unpaired *t*-test, BPC vs. BPCF, *n* = 9 from three independent experiments with 3 wells in each experiment. **G-J** Incorporation of EdU in different media (G-I) was quantified (J). *, *p* < 0.05, BPC vs. B, unpaired *t*-test; #, *p* < 0.05, BPC vs. BPCF, unpaired *t*-test, *n* = 9 from three independent experiments with 3 wells in each experiment. **K-N** Midbrain floor plate DA progenitors at passage 0 (P0) in BPC medium (K) were replated to passage 1 (P1) in the indicated media (L-N) and stained as indicated. **O-R** Cells in BPCF medium were replated to passage 2 (P2) and passage 3 (P3), and stained as indicated. Bars, 100 μ m.

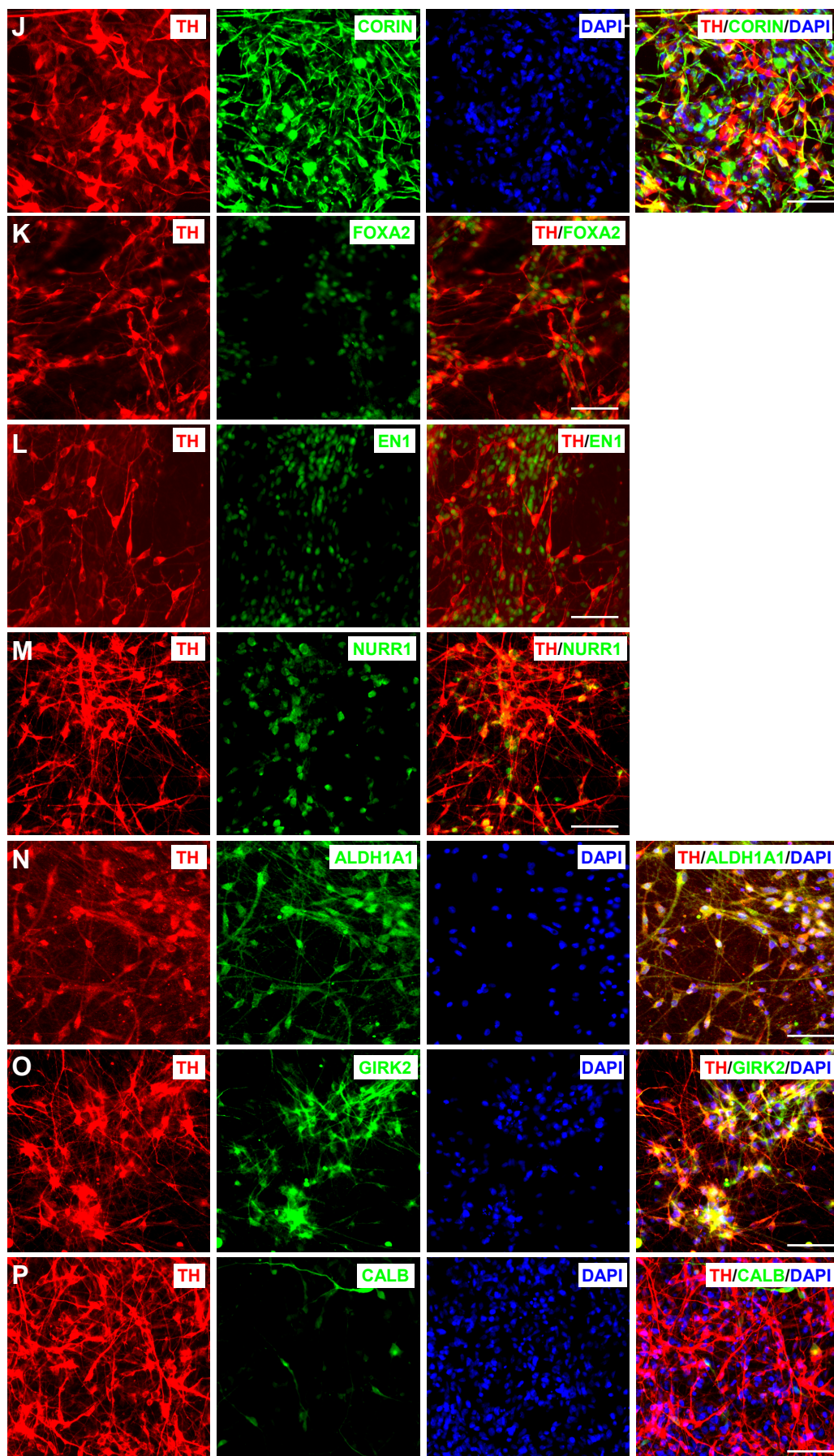


Fig. S3 Separate channels of Fig. 5J-P. Bars, 100 μm .

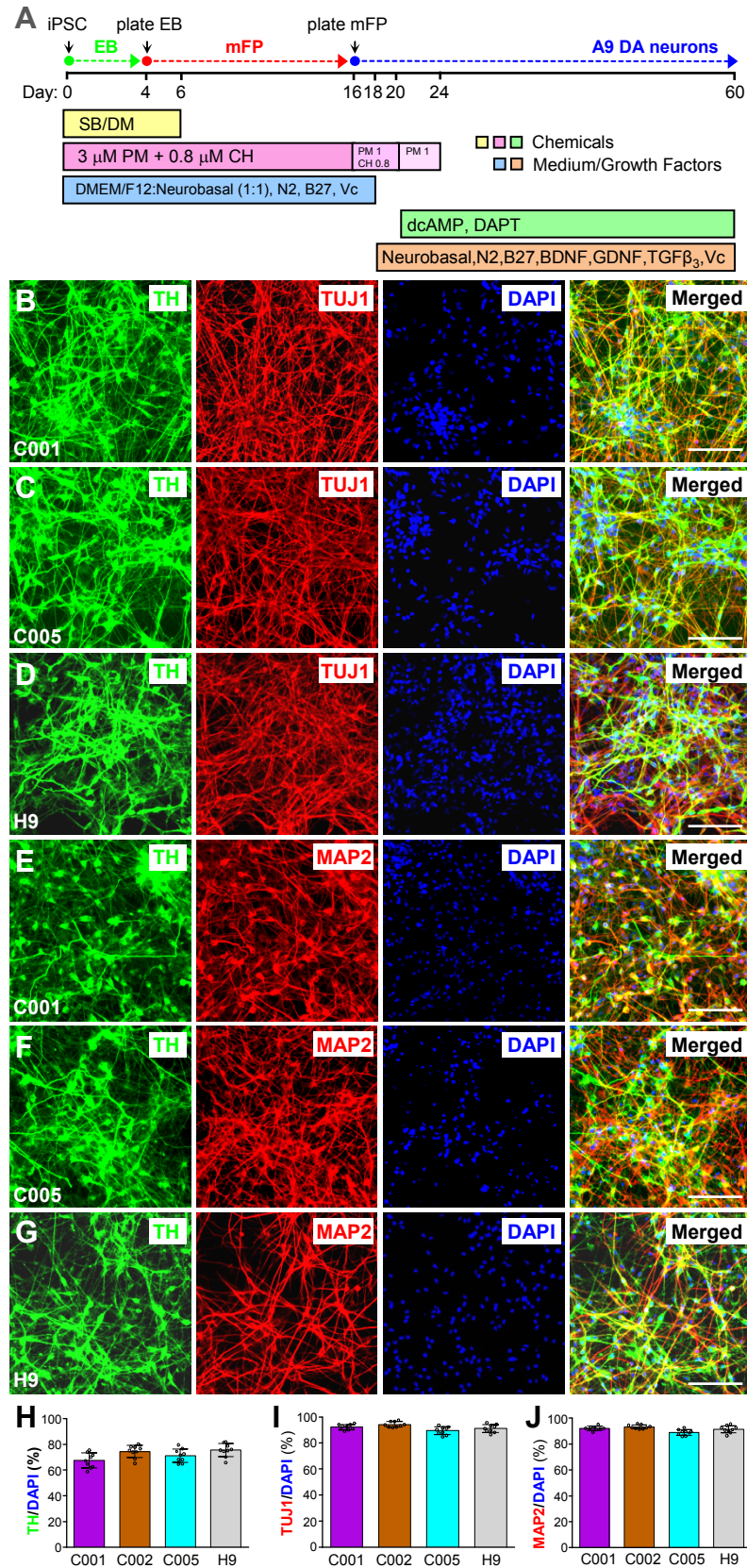


Fig. S4 Differentiation of additional lines of iPSCs. **A** C001, C002, C005 iPSCs and H9 hESCs were differentiated as indicated in the protocol. **B-G** Cells at day 60 were stained for the indicated markers. **H-J** Percentages of TH⁺ (H), Tuj1⁺ (I) or MAP2⁺ (J) neurons in all DAPI⁺ cells were quantified. $n = 9$ from three independent experiments, each counting at least 500 cells. Bars, 100 μ m.

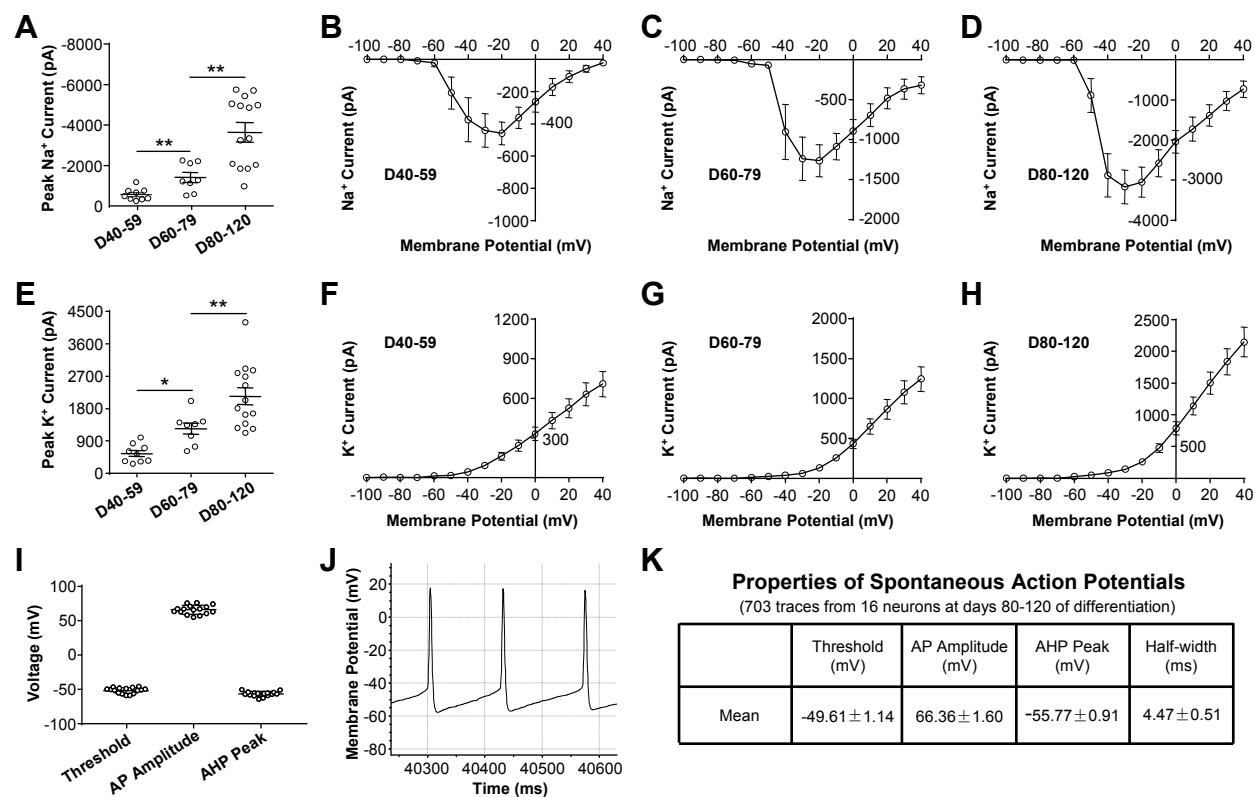


Fig. S5 Electrophysiological Properties of C002 iPSC-derived Neurons. A-H Voltage-gated Na⁺ currents (A-D) and K⁺ currents (E-H) were recorded under a voltage clamp protocol, from -100 to 40 mV at 10 mV steps. Peak Na⁺ currents (A) and I-V curves for voltage-gated Na⁺ currents (B-D), as well as peak K⁺ currents and I-V curves of voltage-gated K⁺ currents at days 40-59 (n=9) (B,F), 60-79 (n=8) (C,G), and 80-120 (n=14) (D,H) showed a significant increase of these currents with time. *, $p < 0.05$; **, $p < 0.01$; unpaired t test. I-K Threshold, Action Potential (AP) Amplitude, Afterhyperpolarization (AHP) peak, and half-width of spontaneous APs were calculated (I) using 703 traces from 16 neurons, each with 20-60 traces, with representative APs (J) and a numerical summary (K).

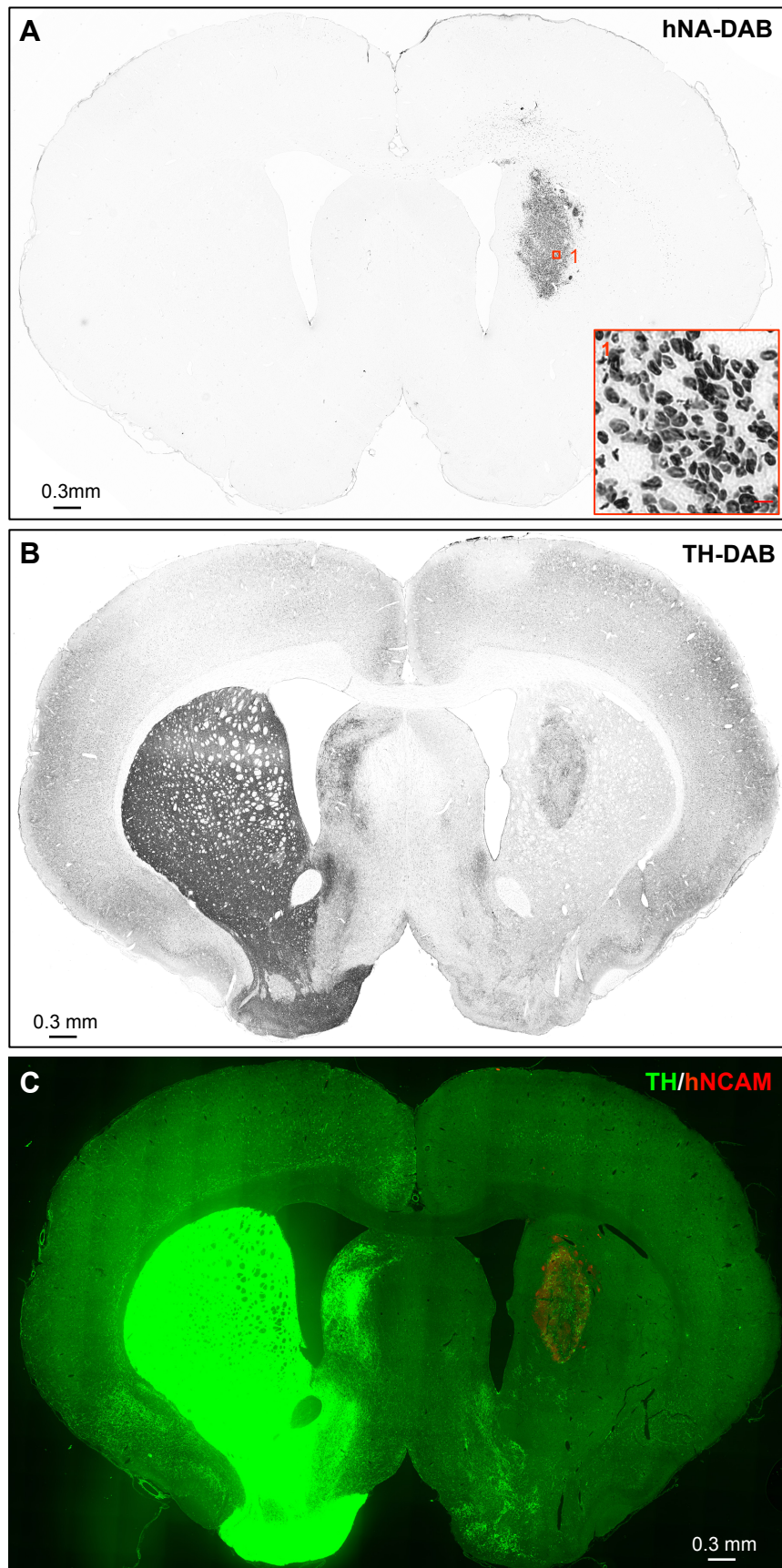


Fig. S6 Transplantation of iPSC-derived Neurons. A-C C002 iPSC-derived A9 DA neurons at day 37 of differentiation were transplanted to the striatum of 6-OHDA-lesioned nude rats. Brain sections were DAB-stained for human Nuclear Antigen (hNA). Inset, enlargement of boxed area 1 (A) or DAB-stained for TH (B), or costained for TH and hNCAM (C). Bars, 0.3 mm.

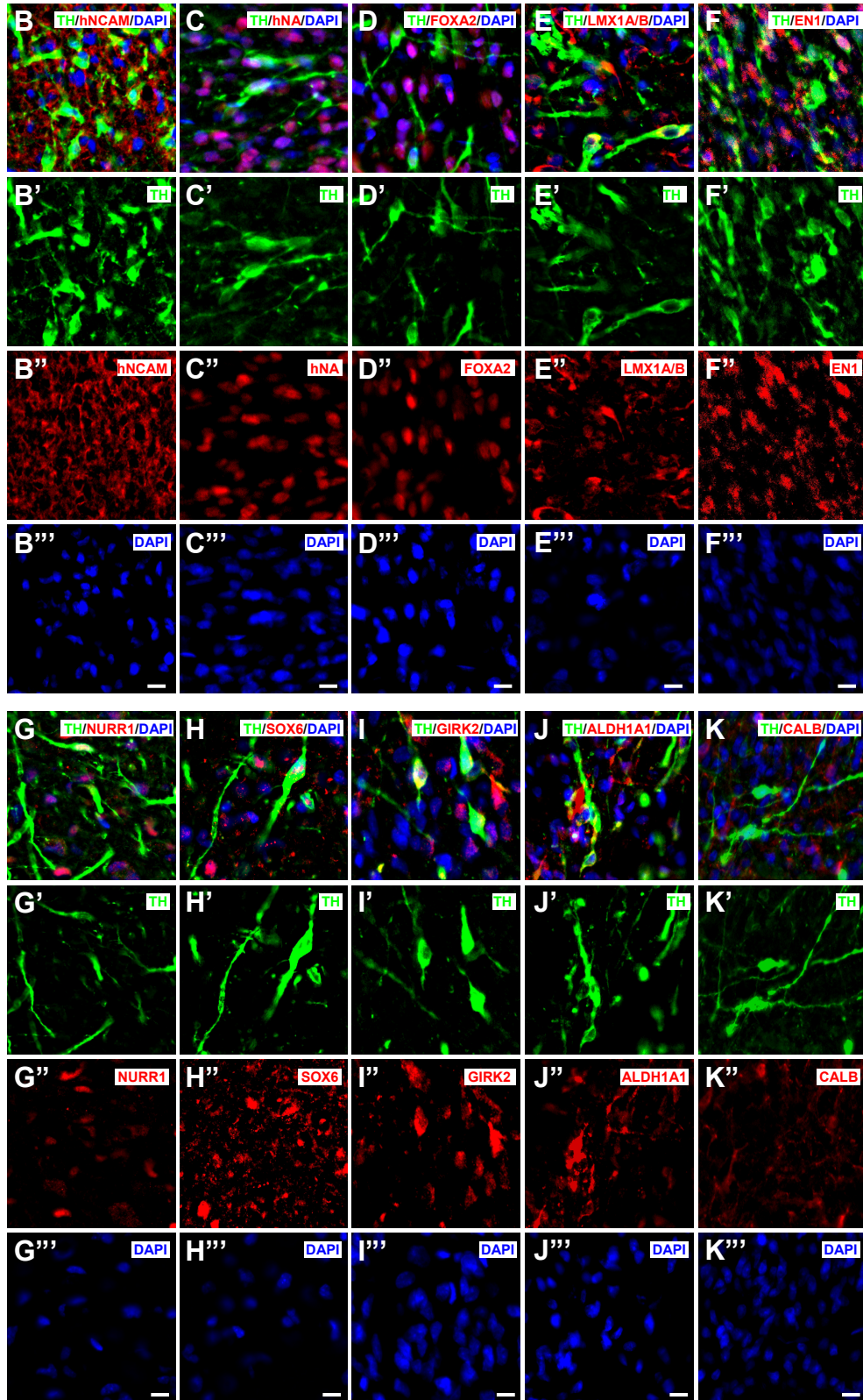


Fig. S7 Immunostaining of transplanted neurons. B-K''', Rat brain sections containing transplanted human iPSC-derived neurons were costained as indicated. Merged images and separate channels corresponding to Fig. 7B-K are shown. Bars, 10 μ m.

Supplementary Table 1 Antibodies, chemicals and growth factors used in the study

Antibody	Source	Catalog Number	Dilution
SSEA-3	Millipore	MAB4303	1:1000
Oct4	Millipore	MAB4401	1:1000
Nanog	Millipore	MABD24	1:1000
SOX2	Millipore	MAB4343	1:1000
TH	Thermo Fisher	OPA1-04050	1:1000
TH	Immunostar	22941	1:1000
MAP2	Santa Cruz Biotechnology	SC-74421	1:1000
β 3-tubulin (TuJ1)	BioLegend	801201	1:2000
β 3-tubulin (TuJ1)	Abcam	Ab18207	1:2000
FOXA2	Santa Cruz Biotechnology	SC101060	1:1000
CORIN	R & D	MAB2209	1:1000
LMX1	Millipore	AB10533	1:500
OTX2	R & D	AF1979	1:1000
SOX1	R & D	AF3369	1:1000
NESTIN	Millipore	ABD69	1:2000
SOX6	abcam	Ab30455	1:1000
EN1	Developmental Studies Hybridoma Bank (DSHB)	4G11	1:200
NURR1	Santa Cruz Biotechnology	SC990	1:1000
ALDH1A1	Abcam	Ab23375	1:1000
GIRK2	Abcam	Ab30738	1:1000
CALBINDIN	Abcam	Ab11426	1:1000
HNA	Millipore	MAB1281	1:1000
NCAM	Santa Cruz Biotechnology	SC-106	1:1000
Donkey anti-Rabbit IgG(H+L), Alexa Fluor 488	Thermo Fisher	A21206	1:2000
Donkey anti-Mouse IgG(H+L), Alexa Fluor 546	Thermo Fisher	A10036	1:2000
Donkey anti-Goat IgG(H+L), Alexa Fluor 647	Thermo Fisher	A21447	1:2000
Donkey anti-Rabbit IgG(H+L), Alexa Fluor 546	Thermo Fisher	A11030	1:2000
Donkey anti-Mouse IgG(H+L), Alexa Fluor 488	Thermo Fisher	A32723	1:2000
Donkey anti-Mouse IgG(H+L), Alexa Fluor 647	Thermo Fisher	A32728	1:2000
Donkey anti-Rabbit IgG(H+L), Alexa Fluor 647	Thermo Fisher	A32733	1:2000
SB431542	Tocris	1614	10 μ M
Dorsomorphin dihydrochloride	Stemgent	04-0024	5 μ M
Purmorphamine	Stemgent	04-0009	3 μ M
CHIR99021	Stemgent	04-0004	0.8 μ M
Dibutyryladenosine 3',5'-cyclic monophosphate	Millipore-Sigma	D0260	0.5 mM
DAPT	Millipore-Sigma	D5942	1 μ M
L-Ascorbic acid	Millipore-Sigma	A5960	0.2 mM
Recombinant Human GDNF	PeproTech	450-10	20 ng/ml
Recombinant Human BDNF	PeproTech	450-02	20 ng/ml
Recombinant Human TGF- β 3	PeproTech	100-36E	1 ng/ml
Recombinant Human FGF2	PeproTech	100-18B	8 ng/ml

Supplementary Table S2**Sequences of qRT-PCR primers**

Gene	Forward	Reverse
FOXA2	CTGGGAGCGGTGAAGATGGA	ACGTACGACGACATGTTTCATGGAG
CORIN	CATATCTCCATCGCCTCAGTTG	GGCAGGAGTCCATGACTGT
LMX1A	AAACCCCATTGACCATCTGTACTC	GGAAATGCTGAGCTACACCATATC
LMX1B	AACTGTACTGCAAACAAGACTACC	TTCATGTCCCATCTTCATCCTC
PAX6	TAAGGATGTTGAACGGGCAG	TGGTATTCTCTCCCCTCCT
HOXA2	CGTCGCTCGCTGAGTGCCTG	TGTCGAGTGTGAAAGCGTCGAGG
OTX2	ACAAGTGCCAATTCATCC	GAGGTGGACAAGGGATCTGA
SOX6	GATGCCATCAACTCCACAGC	GCTGCAGAGCCATTCAATTGC
OTC4	CCTCACTTCACTGCACTGTA	CAGGTTTTCTTTCCCTAGCT
NANOG	CCAAATTCTCTGCCAGTGAC	CACGTGGTTTCCAAACAAGAAA
SOX1	TACAGCCCCATCTCCAATC	GCTCCGACTTCACCAGAGAG
Nestin	GCGTTGGAAACAGAGGTTGGA	TGGGAGCAAAGATCCAAGAC
PITX3	GTGGGTGGAGAGGAGAACAA	TTCCTCCCTCAGGAACAATG
ASCL1	TACTCGTCGGACGAGGGCTCTTA	GCACTAAAGATGCAGGTTGTGCGA
MSX1	AGAAGATGCGCTCGTCAAAG	ATCTTCAGCTTCTCCAGCTC
MSX2	AGATGGAGCGGCGTGGATGC	ACTCTGCACGCTCTGCAATGG
EN1	CCCTGGTTTCTCTGGGACTT	GCAGTCTGTGGGGTCGTATT
TH	TCATCACCTGGTCACCAAGTT	GGTCGCCGTGCCTGTACT
GIRK2	ATCCAGAGGTACGTGAGGAAAGA	CACTGTGTAAACCATGACAAAAATC
ALDH1A1	GAATTTCCCGTTGGTTATGCT	TGTAGGCCCATACCAGGAA
NURR1	TGTGTTCAAGGCGCAGTATGG	TCCCGAAGAGTGGTAACTGTAGC
SHH	CCAATTACAACCCGACATC	AGTTTCACTCCTGGCCACTG
GAPDH	GACAACAGCCTCAAGATCATCAG	ATGGCATGGACTGTGGTCATGAG