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Supplemental Information

A Chemical Recipe for Generation of Clinical-Grade Striatal Neurons from hESCs

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SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIGURES AND TABLES

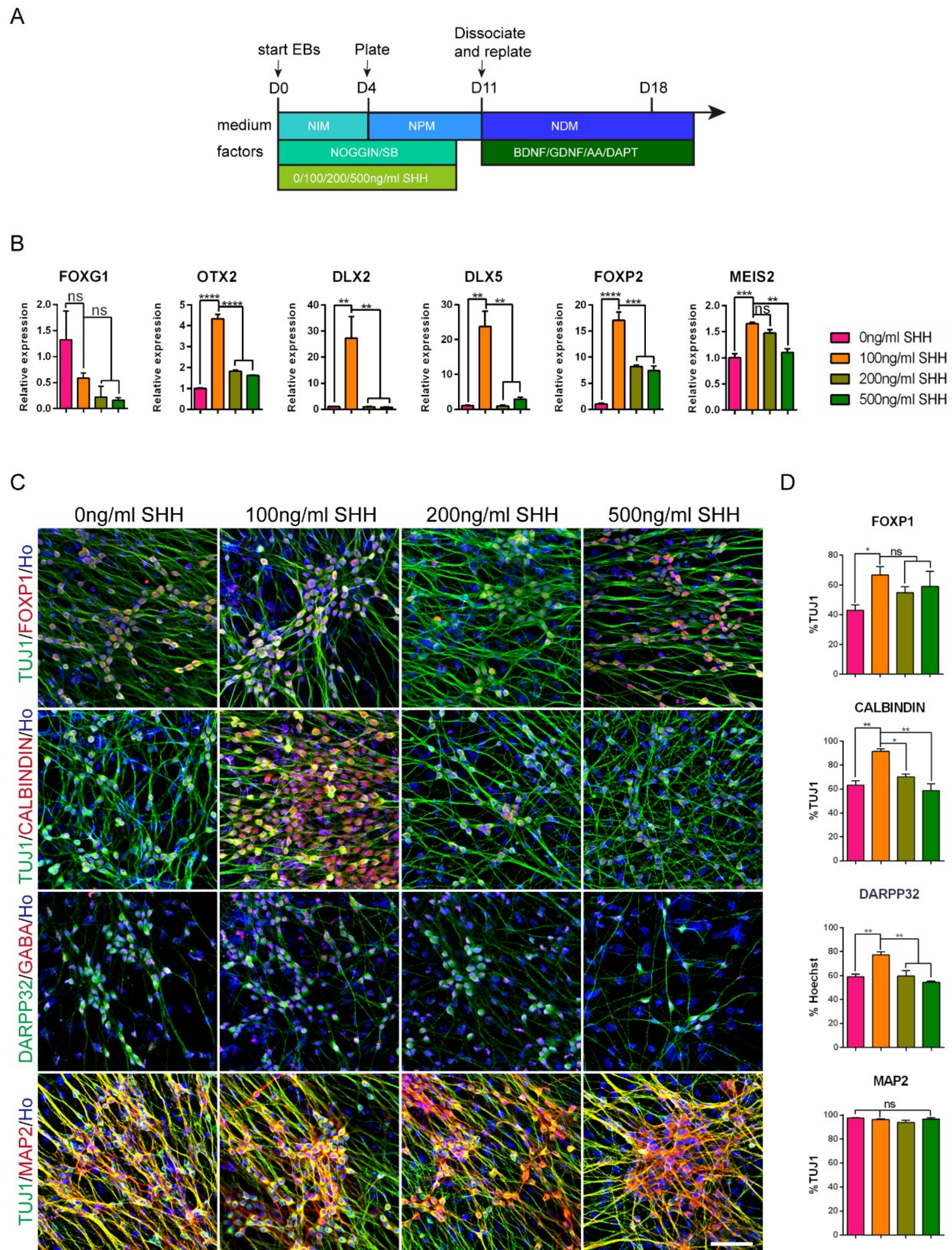


Figure S1. Robust generation of striatal MSNs by optimizing SHH pathway activity.

(A) Overview of differentiation protocol for hESCs. SB represents SB431542; AA represents ascorbic acid.

(B) Gene expression changes of cultures treated with different concentrations of SHH as measured by qRT-PCR on differentiation 14 day. Data are presented as the mean of three independent experiments;

Error bars represent SEM. ns, no significant, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. One-way ANOVA, followed by Tukey's multiple comparisons tests.

(C) Expression of MSN markers after treatment with different concentrations of SHH, imaged by confocal microscopy. Ho represents Hochest33258. Scale bars, 50 μm.

(D) Efficiencies of MSN differentiation, determined by counting FOXP1, CALBINDIN, DARPP32 and MAP2-positive cells. Efficiencies are presented as the percentage of positive cells plus or minus the SEM of all fields counted. ns, no significant, *$P < 0.05$, **$P < 0.01$. One-way ANOVA, followed by Tukey's multiple comparisons tests.

See also Figure 1.

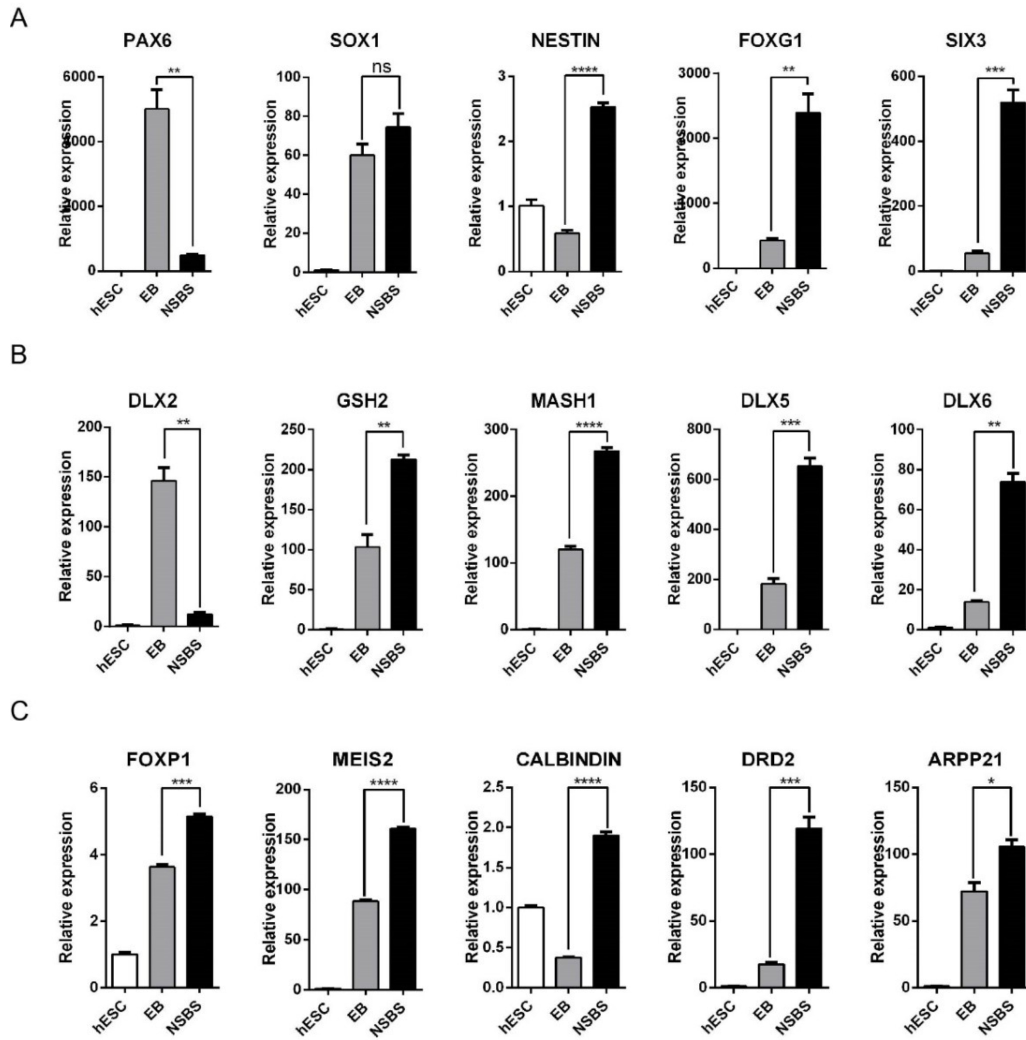


Figure S2. NSBS differentiation protocol is more efficient compared to EB protocol.

(A-C) QPCR analysis of gene expression at different differentiation stages of EB and NSBS protocol. Data are presented as the mean of three independent experiments; error bars represent SEM. ns, no significant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Student's *t*-test.

See also Figure 1.

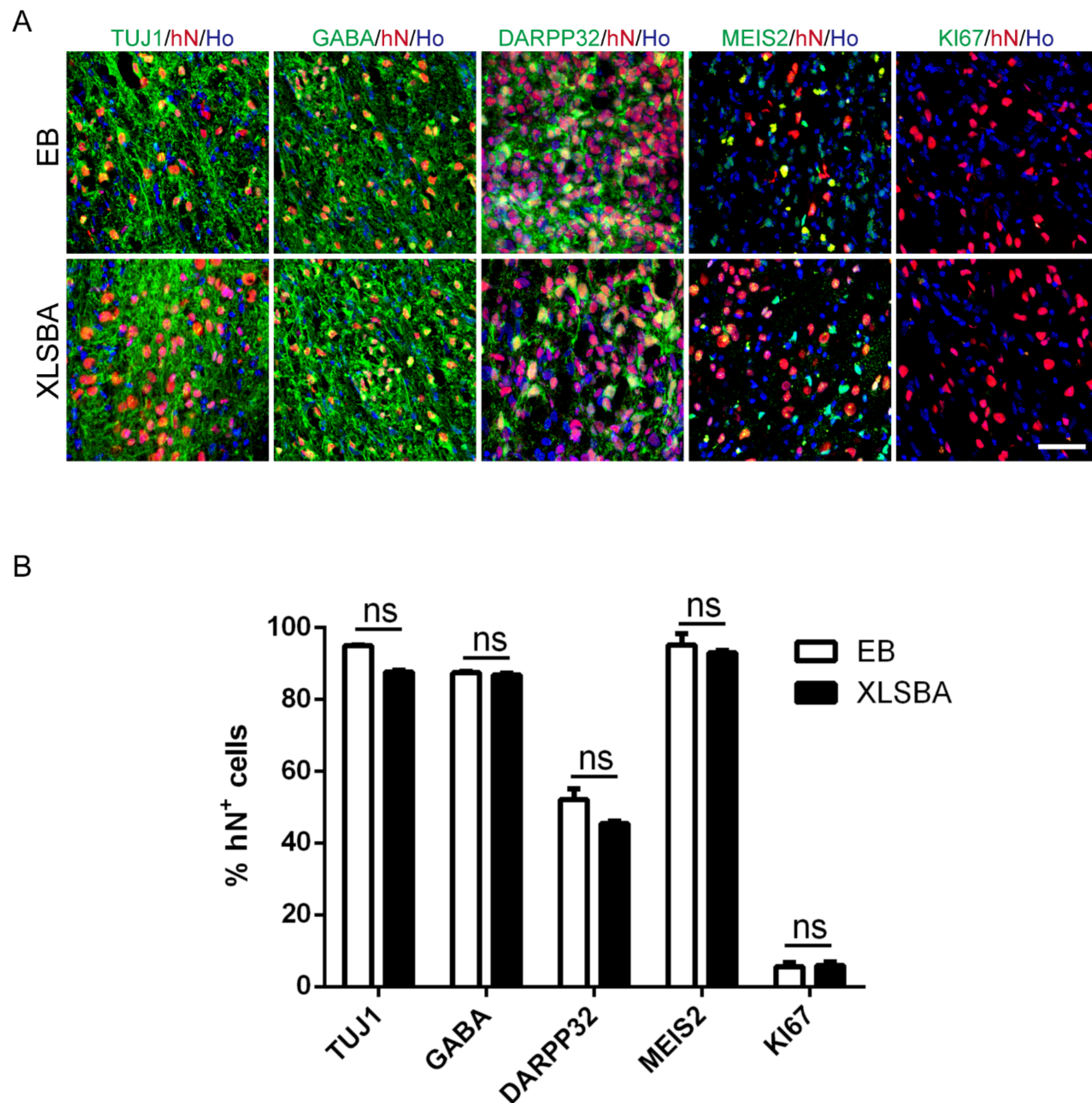
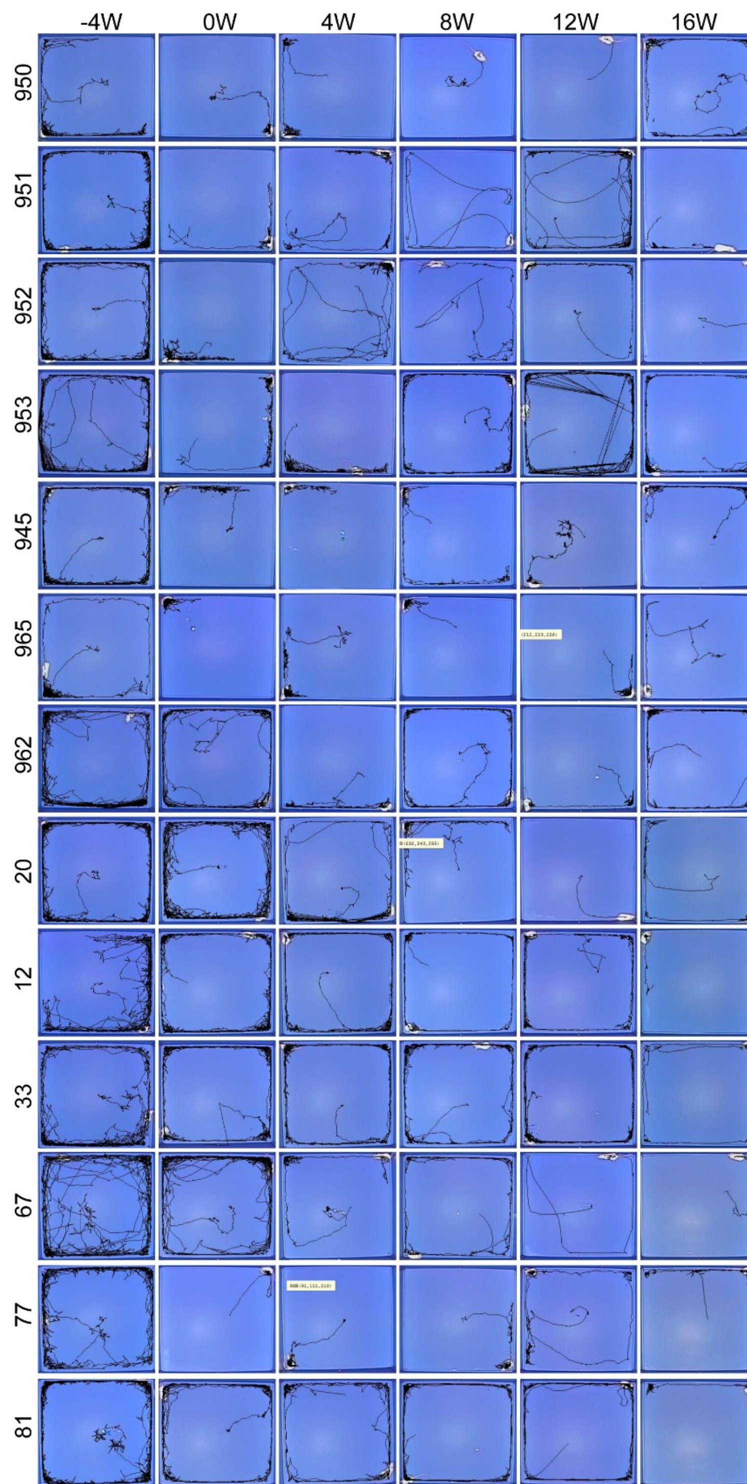


Figure S3. Comparisons of the survival and differentiation of grafted cells between EB and XLSBA group.

(A) Immunostaining images of 16-week-old grafts for TUJ1, GABA, DARPP32, MEIS2 and KI67. Grafted human cells were revealed by human nuclei (hN) staining. Scale bars, 50 μm. (B) Quantification for the proportion of indicated markers in (A). ns, no significant, Student's t-test. Data are presented as mean ±SEM.

See also Figure 6.

A Control group:



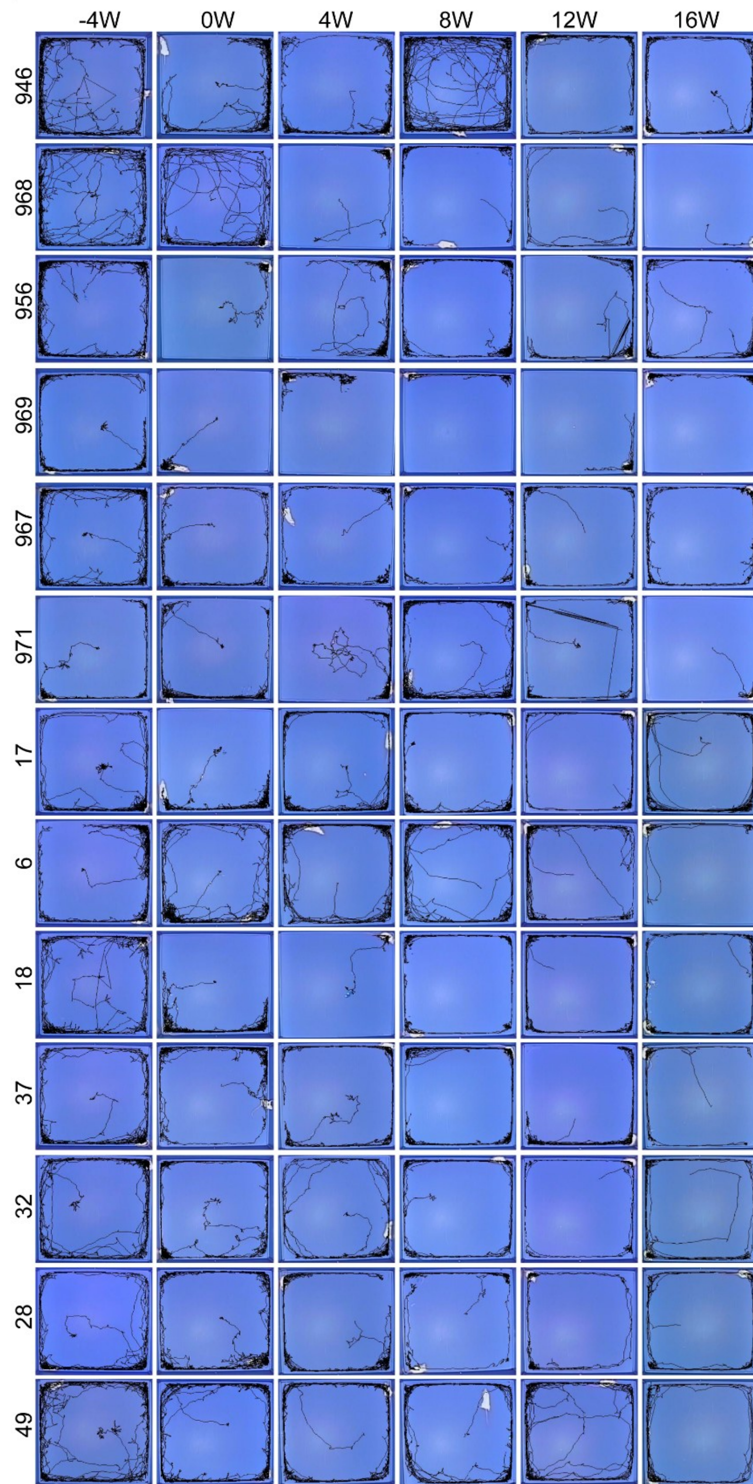


Figure S4. Functional recovery is evaluated by behavior tests after transplantation of hESC-derived LGE progenitors.

(A) Traces of open field test in animals receiving sham operation. (B) Traces of open field test in animals receiving cell grafts. The numbers represent the identifier of each mouse.

See also Figure 6.

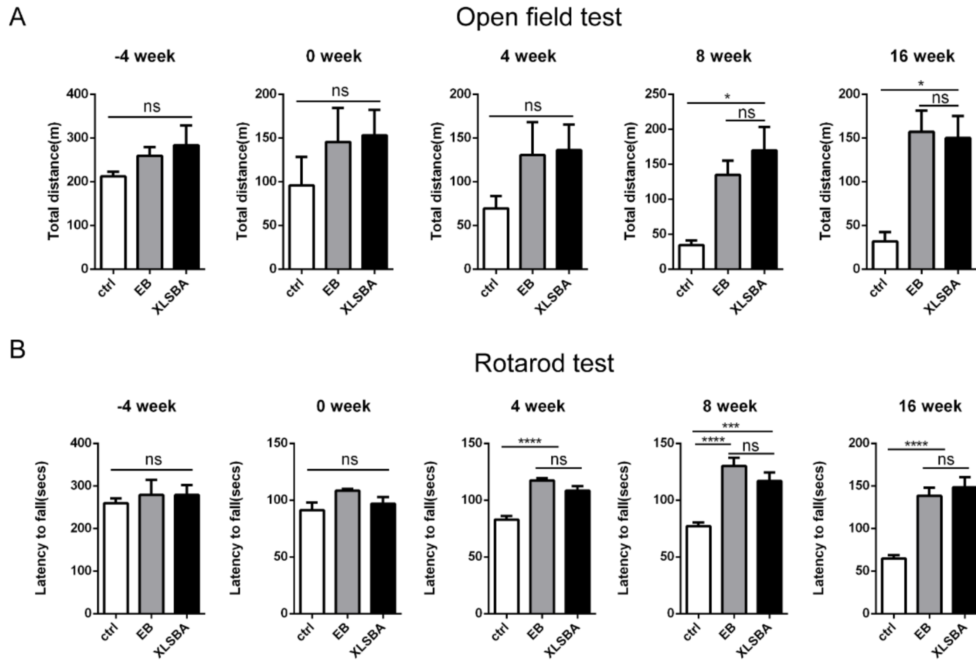


Figure S5. Comparisons of behavior performances between EB and XLSBA group.

(A) Open field tests indicated increased total distance in mice grafted with cells generated by EB and XLSBA protocol but not control group after 8 week and 16 week post-transplantation. The total distance showed no change in mice between EB and XLSBA group. (B) The rotarod test showed increased latency in mice transplanted with cells generated by EB and XLSBA protocol but not control group starting from 4 week post-transplantation. The performance showed no difference between EB and XLSBA group. The tests were analyzed by one-way ANOVA (ns, no significant, *p< 0.05, ***p< 0.001, ****p< 0.0001, control group: n =13, EB group: n=13, XLSBA group: n=13). Data are presented as mean ±SEM.

See also Figure 6.

Table S1. Electrophysiological properties of neurons during differentiation.

Group	D20 (n=39)	D22 (n=54)	D24 (n=16)	Co-culture (n=20)
RMP (mV)	-40.5179±2.1788	-42.5796±1.8247	-39.2188±3.8054	-54.36±2.6579
R_{in} (GΩ)	4.9938±0.4654	4.0574±0.4345	3.3242±0.4852	2.0702±0.568
C_m (pF)	20.8231±0.7453	25.0344±0.7008	23.7881±1.9527	25.467±1.3996

RMP: Resting Membrane Potential; R_{in}: Input Resistance; C_m: Capacitance.

Tables S2. Primary antibodies used for immunofluorescence staining.

Antibody	Species	Catalog Number	Company	Dilution
CALBINDIN	rabbit	AB1778	Millipore	1/1000
ChAT	goat	AB144P	Millipore	1/500
CTIP2	rat	ab18465	Abcam	1/500
DARPP32	rabbit	ab40801	Abcam	1/500
DLX2	rabbit	ab5726	Millipore	1/500
Met-Enkephalin	rabbit	ab5026	Millipore	1/1000
FOXG1	rabbit	ab18259	Abcam	1/500
FOXP1	rabbit	ab16645	Abcam	1/500
GABA	rabbit	A2052	Sigma	1/500
GABA	mouse	A0310	Sigma	1/200
GFAP	rabbit	180063	Invitrogen	1/200
GSH2	rabbit	ABN162	Millipore	1/500
hNuclei	mouse	MAB1281	Millipore	1/200
5-HT	rabbit	20080	Immunostar	1/500
IBA1	rabbit	019-19741	Wako	1/200
KI67	rabbit	AB9260	Millipore	1/500
MAP2	mouse	ab11267	Abcam	1/1000
NESTIN	mouse	MAB5326	Millipore	1/500
NESTIN	rabbit	ABD69	Millipore	1/1000
NEUN	mouse	MAB377	Millipore	1/500
OLIG2	goat	SC-19969	Santa Cruz	1/200
PAX6	rabbit	ab5790	Abcam	1/500
PAX6	mouse	MAB5552	Millipore	1/500
PSD95	goat	ab12093	Abcam	1/1000
PV	mouse	MAB1572	Millipore	1/2000
SOX1	goat	AF3369	R&D	1/2000
SOMATOSTATIN	rabbit	ab5494	Millipore	1/1000
Substance P	rabbit	AB1566	Millipore	1/1000
Synapsin1	mouse	106011	Synaptic systems	1/500
Synaptotagmin1	mouse	105011	Synaptic systems	1/500
TH	rabbit	AB152	AB152	1/200
TUJ1	mouse	T8660	Sigma	1/1000
TUJ1	rabbit	T2200	Sigma	1/1000
VGLUT1	rabbit	135302	Synaptic systems	1/2000

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Table S3. Sequence of qRT-PCR primers used in this study.

Target gene	Primer forward	Primer reverse
ASCL1	GTCCTGTCGCCCACCATCTC	CCCTCCCAACGCCACTGAC
ARPP21	GTGCAAAGCGTGATGGTTTCC	CCTTGACCTGCCTGGTTAGG
CALBINDIN1	ATCAGGACGGCAATGGATAC	TAAGAGCAAGATCCGTTCGG
CTIP2	ATCCTCAGCCCCTTTTGTTT	GCCGTTGTTCCTGAATTGTT
DARPP32	CCTGAAGGTCATCAGGCAGT	GGTCTTCCACTTGGTCCTCA
DCX	CAAGTCTAAGCAGTCTCCCATC	ATAGCCCTGTTGGACACTTG
DLX2	ACCAGACCTCGGGATCCGCC	CTGCGGGGTCTGAGTGGGGT
DLX5	TTCAGAAGACTCAGTACCTCGC	GAGTTACACGCCATTGGGTC
DLX6	TACCTCCAGTCCTACCACAAC	AATAAATGGTCCGAGGCTTCCG
DRD2	CTGAGGGCTCCACTAAAGGAG	CATTCTTCTCTGGTTTGGCG
EAR	GAGGCTGAGGCAGGAGAATCG	GTCGCCCAGGCTGGAGTG
EMX2	GGGATCCGTCCACCTTCTAC	CTCAAAGGCGTGTTCCAGCC
EN1	CGTGGCTTACTCCCCATTTA	TCTCGCTGTCTCTCCCTCTC
FOXG1	TGTTGACTCAGAACTCGCTGG	CTGCTCTGCGAAGTCATTGAC
FOXP1	CTACCGCTTCCATGGGAAATC	CTGTTGTCACTAAGGACAGGG
FOXP2	AATGTGGGAGCCATACGAAG	GCCTGCCTTATGAGAGTTGC
GAPDH	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTC
GBX2	CTCGCTGCTCGCCTTCTC	GCCAGTCAGTCAGATTGTCATCCG
GSH2	TATGTCGACTCGCTCATCATC	CAAGCGGGATGAAGAAATCC
HOXA4	ACGCTCTGTTTGTCTGAGCGCC	AGAGGCCGAGGCCGAATTGGA
LMX1A	CGCATCGTTTCTTCTCCTCT	CAGACAGACTTGGGGCTCAC
MAP2	AAAGCTGATGAGGGCAAGAA	GGCCCCTGAATAAATTCCAT
MEIS2	GATGAAAGAGACGGCAGCTCC	GGGTTGAGGTTGCATCATCG
NESTIN	GGAAGAGAACCTGGGAAAGG	CTTGGTCCTTCTCCACCGTA
NKX2.1	ACCGGGTTCAGACTCAGTTC	ATCGACATGATTCGGCGTCGG
NOLZ1	ACATTTTGCACCCCGAGTAC	GGAGTACGGCTTGAAACTCG
OTX2	TCAACTTGCCCGAGTCGAGG	CAATGGTCGGGACTGAGGTG
PAX6	TCCATCAGTTCCAACGGAGAA	GTGGAATTGGTTGGTAGACAC
SIX3	CCGGAAGAGTTGTCCATGTT	CGACTCGTGTTTGTTGATGG
SOX1	ATGCACCGCTACGACATGG	CTCATGTAGCCCTGCGAGTTG
SOX2	CAAGATGCACAACTCGGAGA	CGGGGCCGGTATTTATAATC
TBR2	CACCGCCACCAAACTGAGAT	CGAACACATTGTAGTGGGCAG
TUJ1	AACGAGGCCTCTTCTCACAA	GGCCTGAAGAGATGTCCAAA

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Generation of striatal MSNs from hESCs with chemical cocktails

Differentiation media were described as previously (Kirkeby et al., 2012a; Kirkeby et al., 2012b): neural induction medium (NIM) consisted of DMEM/F12: Neurobasal (1:1), N2 supplement (1:100), B27 supplement (1:50) and GlutaMAX (1:100); neural proliferation medium (NPM) consisted of DMEM/F12: Neurobasal (1:1), N2 supplement (1:200), B27 supplement (1:100) and GlutaMAX (1:100); and neural differentiation medium (NDM) consisted of Neurobasal, B27 supplement (1:50) and GlutaMAX (1:100). For differentiation, hESCs were detached with dispase to form EBs in NIM. Then the EBs were plated in NPM onto plastic plate coated with Matrigel on day 4. From D0 to D9, SB431542 and LDN-193189, Dorsomorphin or NOGGIN was present in the medium. Patterning factors SHH-C24II, SAG or Purmorphamine were also in present in the medium from d0-d9 or d0-d5. On D11 of differentiation, the cells were dissociated to small clusters with Accutase and replated onto the matrigel-coated plates in NDM. Brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), ascorbic acid (AA) and DAPT were added for terminal differentiation. For clinical-grade hESC differentiation, all Clinical Therapy Systems (CTS) reagents were purchased from Life Technologies, and cells were replated on the PO/FN/lam-coated plates (Fibronectin, Gibco; Laminin, Sigma; Polyornithine, Sigma). Small-molecule compounds were as follows: XAV939 (0-4μM, Selleck), Dorsomorphin (100 nM, Selleck), LDN-193189 (100 nM, Selleck), SB431542 (10 μM, Merck), SAG (0-100nM, Stemgent), Purmorphamine (0-1μM, Stemgent), ascorbic acid (200 mM, sigma) and DAPT (1μM, Tocris). Recombinant growth factors were as follows: SHH (C25II, 0–500 ng/ml), NOGGIN (200 ng/ml), BDNF (20 ng/ml) and GDNF (10 ng/ml), all from Peprotech.

1. **Day 0**: Check that the colonies appear pluripotent by visual criteria, and if needed, remove differentiated colonies from the culture. Aspirate E8 medium and add appropriate volume of dispase (2U/ml) to the cells (i.e., 1 ml for a 6-well) and incubate at 37°C for 8-12 min. When the edges of hPSC colonies begin to curl, aspirate off the dispase.
2. Add 2 ml of fresh E8 medium to each well. Gently pipette colonies off the dish. Collect hESC colonies into a 15-ml tube, and centrifuge at 600rpm for 2 min. Alternatively, let the hESC colonies settle down by letting the tube rest for 3–5 min. Aspirate off the supernatant without disturbing the cells.
3. To start differentiation, resuspend the cells in differentiation medium: NIM + LDN193189 (100nM) + SB431542 (10 μM) +XAV939 (4 μM) +SAG (100 nM). Plate cell suspension in non-treated cell culture dishes for EB formation.
4. **Day 1**: Transfer EB suspensions to a tube and centrifuge at 600rpm for 2 min. Alternatively, let the EBs settle down by letting the tube rest for 3–5 min. Aspirate the medium and re-suspend the EBs in new NIM medium + LDN193189 (100nM) + SB431542 (10 μM) +XAV939(4 μM)+SAG (100 nM). Transfer EBs into the same dishes.
5. **Day 2-3**: Change the medium as step 4 every day.
6. **Day 4**: Transfer EB suspensions to a tube and spin down gently at 600rpm for 2 min. Resuspend EBs in NPM medium+ LDN193189 (100 nM) + SB431542 (10 μM) +XAV939(4 μM)+SAG (100 nM). Replate the EBs into wells coated with PO/lam/FN or Matrigel.
7. **Day 5**: Change the medium with NPM medium+ LDN193189 (100 nM) + SB431542 (10 μM).
8. **Day 7**: Change the medium with NPM medium+ LDN193189 (100 nM) + SB431542 (10 μM).

9. **Day 9**: Change the medium with NPM medium without any small molecules.
10. **Day 11-16**: Change the medium with NDM medium+BDNF (20 ng/ml) +GDNF (10 ng/ml) +AA (0.2 mM) +DAPT (1μM). Or passage cells: wash cells with PBS, and add Accutase to the wells. Incubate the cells at 37℃ for 3-5 min. Dissociate cells with a pipette and spin down at 600rpm for 3 min (before spinning, take an aliquot out for cell counting). Resuspend cells in NDM medium + BDNF (20 ng/ml) +GDNF (10 ng/ml) +AA (0.2 mM) +DAPT (1μM). Then replate the cells into wells coated with PO/lam/FN or Matrigel in a density of 2.5–5x10^6 cells for one well of a 6-well plate.
11. **Day 17-24**: Change the medium with NDM medium+BDNF (20 ng/ml) +GDNF (10 ng/ml) +AA (0.2 mM) +DAPT (1μM) every other day.

Reagent setup:

Neural induction medium (NIM):

H9/H1 hESC	Q-CTS-hESC-2	Volume
DMEM/F12	CTS™ KnockOut™ DMEM/F-12	240 ml
Neurobasal	CTS™ Neurobasal® Medium	240 ml
N2 (100X)	CTS™ N-2 Supplement	5 ml
B27 (50X)	CTS™ B-27® Supplement	10 ml
GlutaMAX (100X)	CTS™ GlutaMAX™-I	5 ml
Total		500 ml

Neural proliferation medium (NPM):

H9/H1 hESC	Q-CTS-hESC-2	Volume
DMEM/F12	CTS™ KnockOut™ DMEM/F-12	243.75 ml
Neurobasal	CTS™ Neurobasal® Medium	243.75 ml
N2 (100X)	CTS™ N-2 Supplement	2.5 ml
B27 (50X)	CTS™ B-27® Supplement	5 ml
GlutaMAX (100X)	CTS™ GlutaMAX™-I	5 ml
Total		500 ml

Neural differentiation medium (NDM):

H9/H1 hESC	Q-CTS-hESC-2	Volume
Neurobasal	CTS™ Neurobasal® Medium	485 ml
B27 (50X)	CTS™ B-27® Supplement	10 ml
GlutaMAX (100X)	CTS™ GlutaMAX™-I	5 ml
Total		500 ml

Electrophysiological assessment. Whole-cell current and clamp recordings were performed at 22 °C in artificial cerebral spinal fluid, bubbled with 95% O_2 and 5% CO_2. The extracellular fluid consisted of (in mM) 124 NaCl, 3.3 KCl, 2.4 $MgSO_4$, 1.2 KH_2PO_4, 26 $NaHCO_3$, 2.5 $CaCl_2$ and 10 Glucose (at pH 7.4). TTX (100 nM), bicuculline (30 μM), or GABA (10 μM) were used in the bath solution. Borosilicate glass electrodes (resistance 6–10 MΩ) were filled with an intracellular solution containing 135 mM potassium gluconate, 7 mM NaCl, 10 mM HEPES, 2 mM MgATP, 0.3 mM Na_2GTP and 2 mM $MgCl_2$,

adjusted to pH 7.4 with KOH. Cell visualization and patch pipette micromanipulation were performed by video-microscopy, employing a 40× water-immersion objective mounted on an upright microscope equipped with infrared differential interference contrast (Nikon, Eclipse fn1, Japan). Intracellular membrane electrical potentials were recorded in current-clamp mode, using a Multi-clamp 700B amplifier (Molecular Devices, Palo Alto, CA, USA). For voltage clamp recordings, cells were held at -70 mV. (-)-Bicuculline methochloride and tetrodotoxin (TTX) were purchased from Shanghai yuanye Bio-Technology Co., Ltd; GABA were purchased from Solarbio (Beijing, China). All other compounds were obtained from Sigma (St. Louis, MO). Data were digitized at 10 kHz with a 2 kHz low-pass filter. Data processing and analysis were performed using Clampfit 10.6 (Axon Instruments).

Neural transplantation and quinolinic acid striatal lesions. All animal experiments were carried out in accordance with the instructions for the Care and Use of Animals in Research published by the Institute of Zoology of the Chinese Academy of Sciences. Transplantation into the neonatal NOD-SCID mice was conducted as described previously (Danjo et al., 2011). In brief, transplantation was performed by anesthetizing day 1 postnatal NOD-SCID mice on ice for 3 min, and injecting the cell suspension (~$5x10^4$ cells per μl) into the striatum (coordinated 1 mm anterior, 1 mm lateral, and 2.5 mm deep from the bregma). The establishment of quinolinic acid (QA)-lesioned mouse model of HD and cell transplantation were performed as previously described (Ma et al., 2012). To establish unilateral lesion of mouse model of Hunting's disease, adult male SCID mice (8 weeks of age) were anesthetized and received a stereotaxic injection of 1ul of 60nM quinolinic acid (QA, P63204; Sigma, in saline with 0.2 mg/ml ascorbic acid) into the right striatum on a coordinator (anterior-posterior [AP] = +0.7 mm, lateral [L] = +1.7 mm, vertical [V] =-3.5 mm). Differentiated LGE-like progenitors (12-14 days of differentiation from hESCs) were dissociated with Accutase and prepared at approximately $5x10^4$ cells/ul in PBS containing B27, 0.2 mM AA, 20 ng/ml BDNF, and 10 ng/ml GDNF. Cell suspension (2 μl) was injected into the lesioned striatum (AP = +0.8 mm, L = +1.8 mm, V = -3.5 mm) of anesthetized animals 2 weeks after QA lesion with a glass pipette (0.3–0.5 mm in diameter) over a period of 5 min. The QA-lesioned animals receiving the same surgery and injection of 2 μl of PBS solution (without cells) served as controls. For analysis, mice were perfused transcardially with 4% paraformaldehyde, and 20μm-thick brain sections were taken for immunostaining.