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Article Title:	Medial ganglionic eminence–like cells derived from human embryonic stem cells correct learning and memory deficits
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Supplementary item and number (e.g., Supplementary Fig. 1)	Title or caption (this should match what is in the PDF)
	Effects of timing and dosage of SHH on progenitor induction.
Supplementary Fig. 1	Effects of timing and dosage of SHH on progenitor induction.
Supplementary Fig. 2	Electrophysiological properties of hESC-derived neurons.
Supplementary Fig. 3	Characteristics of grafted human cells
Supplementary Fig. 4	Subtypes and electrophysiological properties of grafted human neurons.
Supplementary Fig. 5	Transplantation of hESC-derived MGE progenitors contributes to functional recovery.
Supplementary Table 1	
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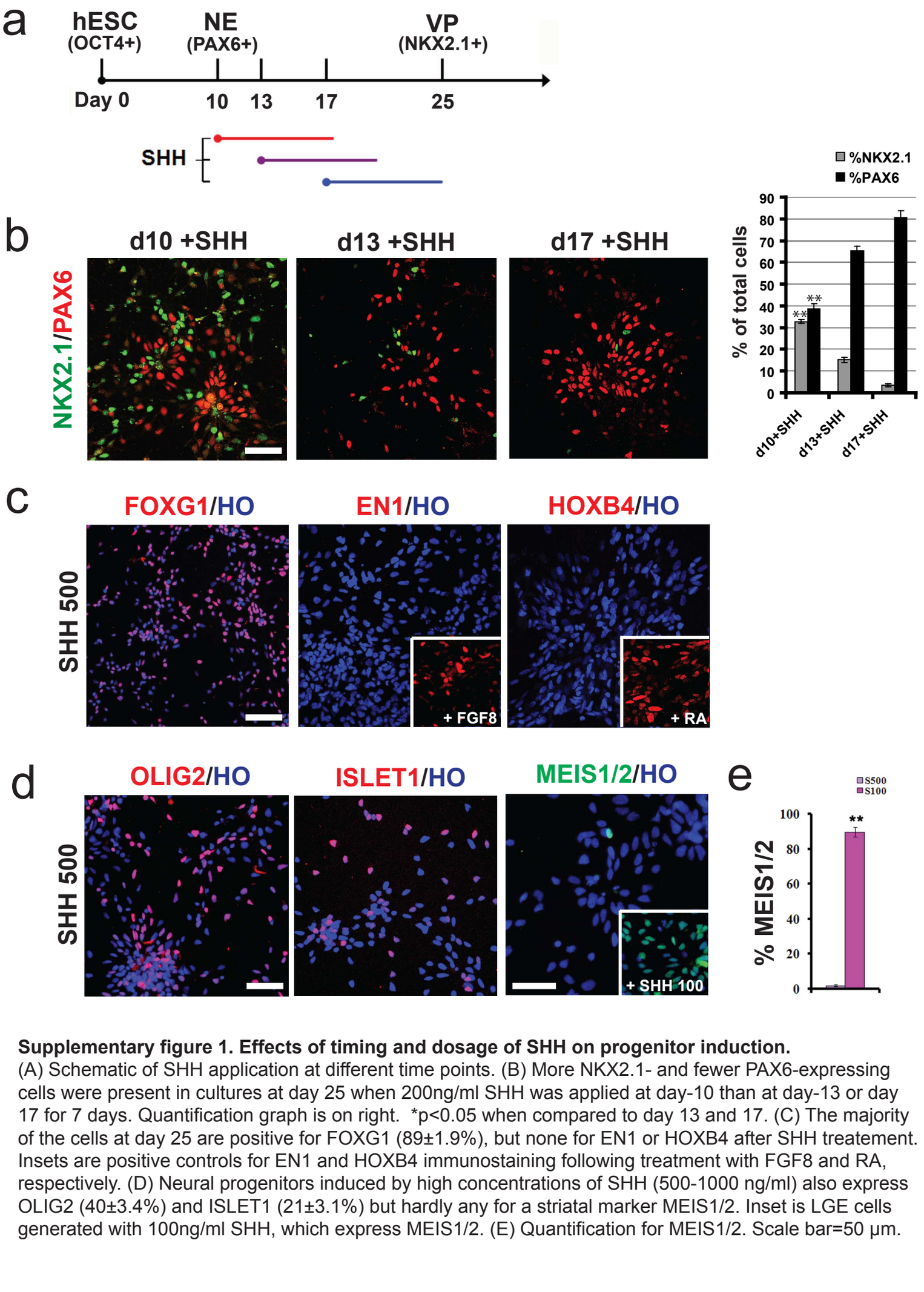
Supplementary Figure 1 (Zhang)



Supplementary figure 1. Effects of timing and dosage of SHH on progenitor induction.
(A) Schematic of SHH application at different time points. (B) More NKX2.1- and fewer PAX6-expressing cells were present in cultures at day 25 when 200ng/ml SHH was applied at day-10 than at day-13 or day 17 for 7 days. Quantification graph is on right. *p<0.05 when compared to day 13 and 17. (C) The majority of the cells at day 25 are positive for FOXG1 (89±1.9%), but none for EN1 or HOXB4 after SHH treatement. Insets are positive controls for EN1 and HOXB4 immunostaining following treatment with FGF8 and RA, respectively. (D) Neural progenitors induced by high concentrations of SHH (500-1000 ng/ml) also express OLIG2 (40±3.4%) and ISLET1 (21±3.1%) but hardly any for a striatal marker MEIS1/2. Inset is LGE cells generated with 100ng/ml SHH, which express MEIS1/2. (E) Quantification for MEIS1/2. Scale bar=50 μm.

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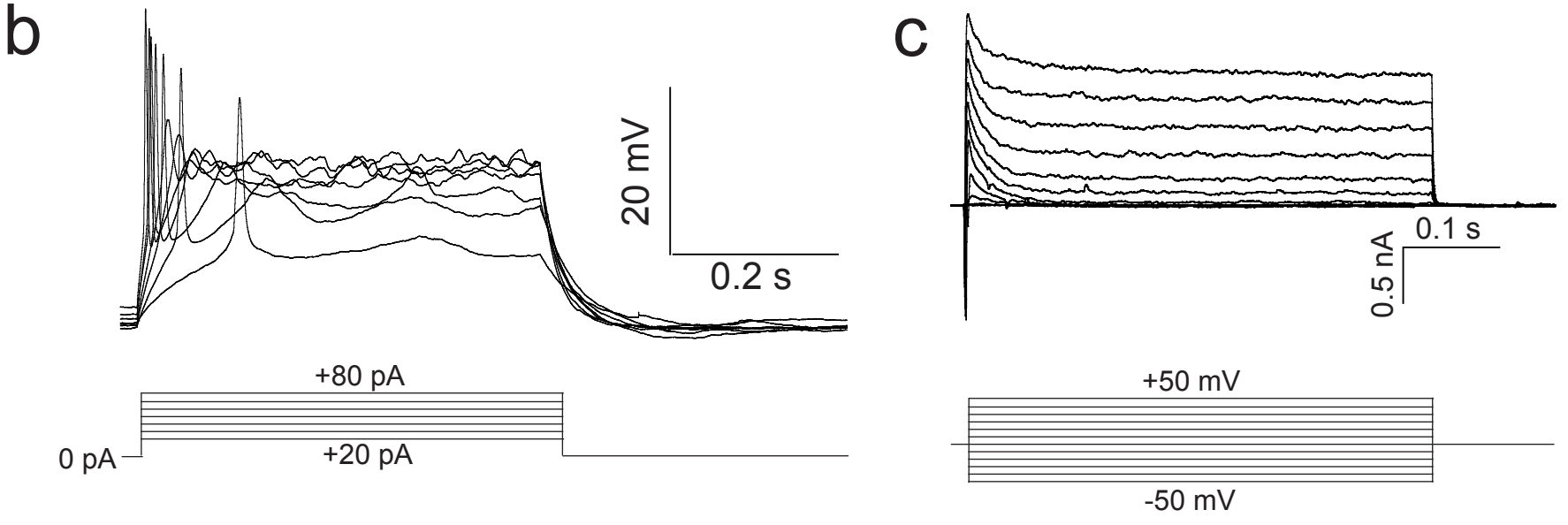
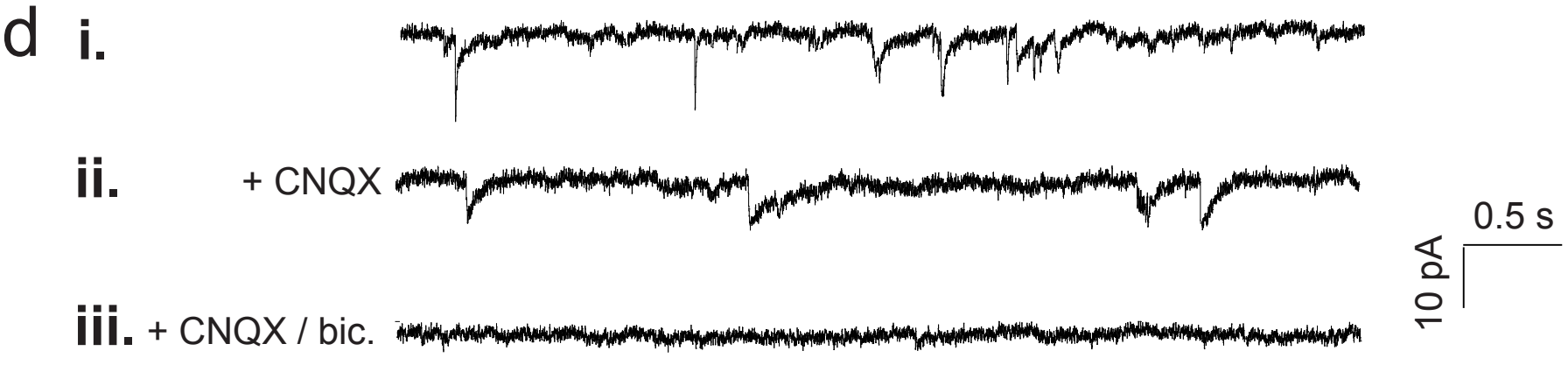
Supplementary Figure 2 (Zhang)

a

weeks	Cap (pF)	Rin (GΩ)	RMP (mV)
8-10	30.43 ± 5.32	1.85 ± 0.42	-57.64 ± 4.26





Supplementary figure 2. Electrophysiological properties of hESC-derived neurons.
(A) Electrophysiological characteristics of hESC-derived neurons which were differentiated for 8-10 weeks in vitro. (B) Action potentials were induced by +20 pA to +80 pA current injections. (C) Inward and outward currents were triggered upon -50mV to 50 mV voltage steps. (D) Spontaneous synaptic currents were recorded (i), the AMPA receptor activity was blocked by CNQX (ii), and the GABA receptor activities were further eliminated by application of bicuculline in the presence of CNQX.

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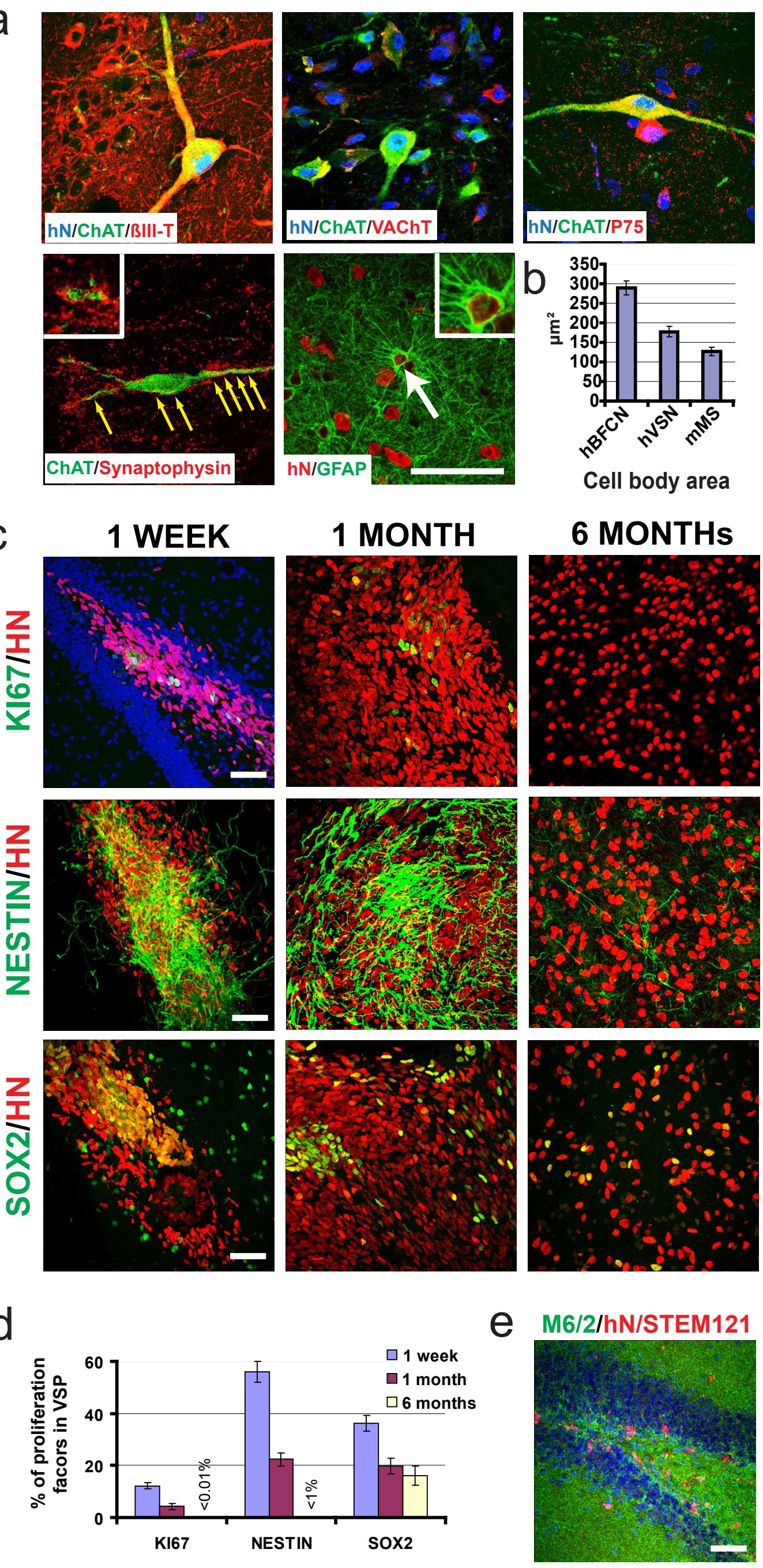
Supplementary Figure 3 (Zhang)



Supplementary figure 3. Characteristics of grafted human cells.
(A) Human ChAT+ co-expressed ßIII-Tubulin, VAChT, P75, and synatophysin. Yellow arrows indicate synapses surrounding CHAT+ neurons, and the inset illustrates higher magnification of the area marked by the white arrow. (B) CHAT+ cell body area in the human MGE graft was larger than that in human VSP grafts and mouse BFCNs in the medial septum (mMS). (C) Cell proliferation and progenitor population at 1 week, 1 month, and 6 months post transplantation. (D) Proportion of KI67-, Nestin-, and SOX2-expressing cells among total human (hN+) cells at 1week, 1 month, and 6 months post-transplantation in VSP. (D) Mouse markers M2/6 and human markers hN and STEM121 are not co-labled. ßIII-T= ßIII-Tubulin; Syn=Synaptophysin; S121=STEM121. All Scale Bars =50 µm.

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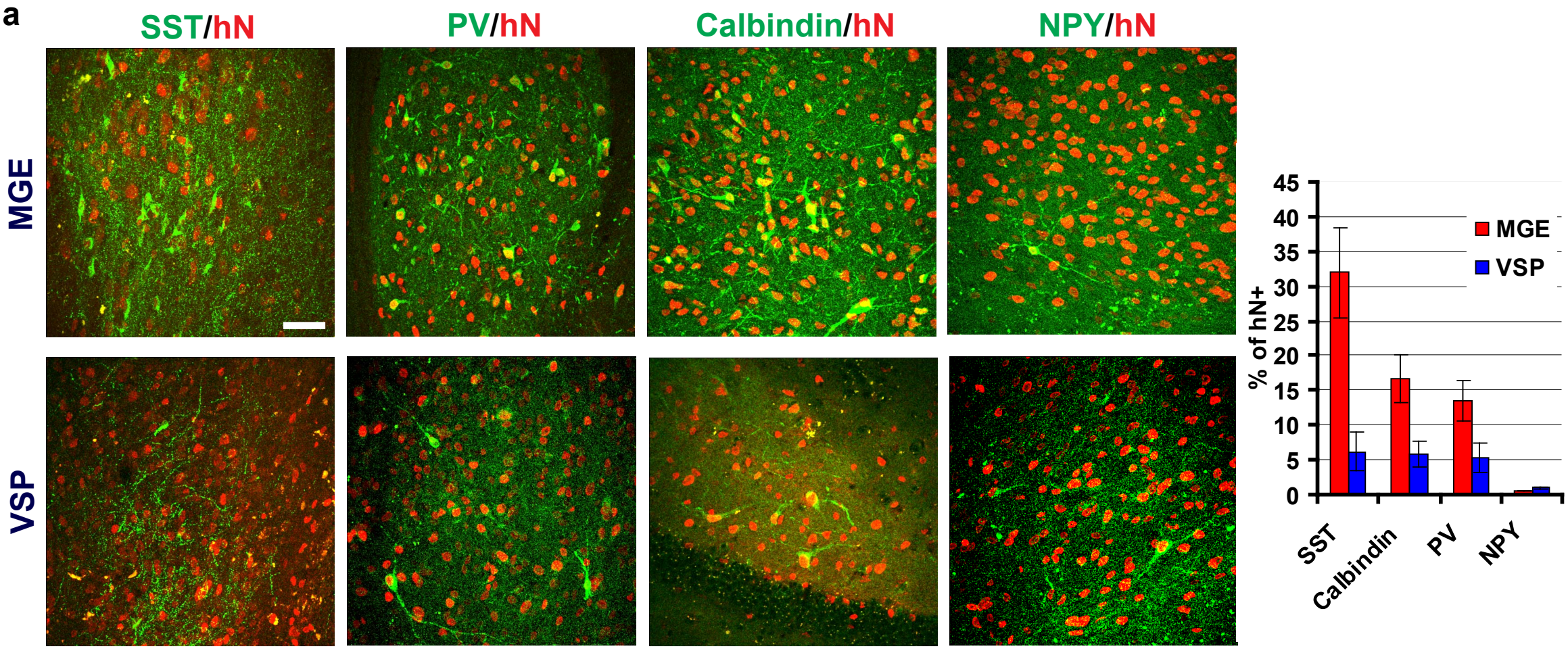
Supplementary Figure 4 (Zhang)



Supplementary figure 4. Subtypes and electrophysiological properties of grafted human neurons.
(A) GABA interneuron subtypes in the MGE and VSP groups. (B) Human cholinergic neurons (hN+/ChAT+) co-expressed muscarinic (m-AChR) and nicotinic (n-AChR) receptors (arrows).(C) (i) Inward and outward currents were triggered in grafted neurons. (ii) Grafted neurons fired action potentials. (iii) Spontaneous synaptic currents were recorded. (iv) m-cherry positive cells are hN positive, arrows point the neurons migrated out of the graft. Inset is showing higher magnification of co-label with hN and M-CHERRY. All Scale Bars =50 μm.

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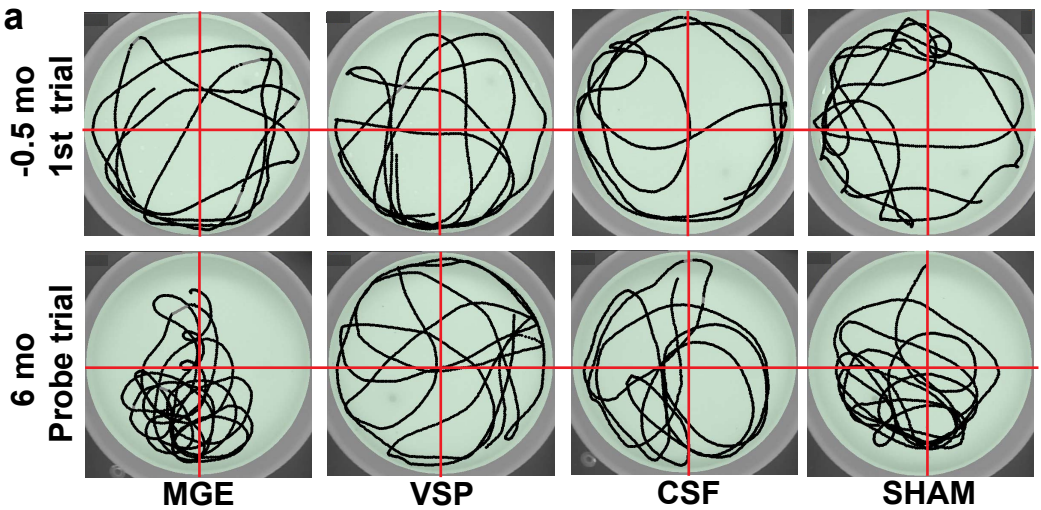
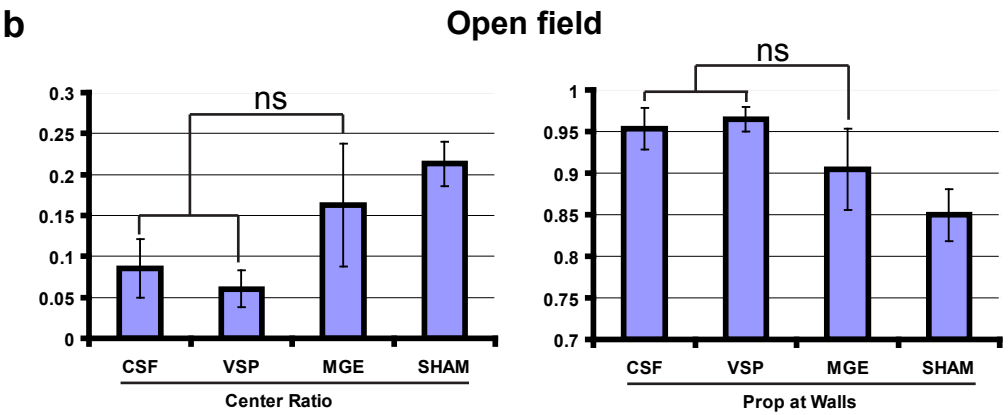
Supplementary Figure 5 (Zhang)





Supplementary figure 5. Transplantation of hESC-derived MGE progenitors contributes to functional recovery.
(A) (i) Swimming traces from the first trials before transplantation for each group. Each groups are similar, which all are tend to close to the wall. (ii) Swimming traces from the probe trials for each group. Similar with SHAM animals, MGE transplanted group had significant more crossings and spent more time in the platform quadrant than other 3 quadrants. (B) There are no significant differences among the three lesioned groups in open field tests.

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Supplementary Table 1. Behavioral tests subgroups

Group name	Animal number	Lesion (P75-SAP injection)	Transplantation
MGE	13	Yes	MGE progenitors
VSP	9	Yes	VSP progenitors
CSF	7	Yes	Injected CSF
SHAM	10	No (injected CSF instead)	No surgery

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Supplementary Table 2. Side-by-side comparison with Bissonnette's method

	Liu et al	Bissonnette et al
Differentiation method	Chemically defined	Matrigel + suspension
Retinoic acid (RA)	None	10μM
bFGF	None	4ng/ml
SHH	Day 10-25 1,000 ng/ml	Day 15-17 200 ng/ml Day18 100 ng/ml
FGF8	None	100 ng/ml
BMP9	None	10 ng/ml, 3 days (day 18-21)
Ara C	None	2.66 μM
Cellular identity	38% BFCN	85% BFCN
MGE progenitors	Yes (93%)	Yes (85%, but only some are FoxG1+ in cytoplasm)
ChAT	Yes	Yes
Co-expression w/ FOXG1	Yes	None
Co-expression w/ NKX2.1	Yes	None
Co-expression w/VAChT	Yes	Yes, also in MAP2- cells
MGE-derived GABA neurons	Yes	None
electrophysiological property	Yes	Yes when co-cultured w/brain slices
Transplantation	Yes	No.

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Supplementary Table 3. Antibodies

Antibody	Isotype	Dilution	Source
MAP2	Mouse IgG	1:1,000	Chemicon & Millipore
BMPI (VGLUT1)	Goat IgG	1:100	Santa Cruz
Calbindin	Rabbit IgG	1:1,000	Chemicon & Millipore
Calretinin	Rabbit IgG	1:200	Epitomics
ChAT	Goat IgG	1:300	Chemicon & Millipore
DARPP32	Rabbit IgG	1:1,000	Chemicon & Millipore
EN1	Mouse IgG	1:1,000	DSHB, Iowa City, IA
FOXG1	Rabbit IgG	1:100	Abcam
GABA	Rabbit IgG	1:10,000	Sigma
GABA	Mouse IgG	1:100	Sigma
GAD65	Rabbit IgG	1:5,000	Chemicon & Millipore
GFAP	Rabbit IgG	1:5,000	DAKO
HOXB4	Rat IgG	1:50	DSHB, Iowa City, IA
Human Nuleui	Mouse IgG	1:200	Chemicon & Millipore
Human Tau	Mouse IgG	1:200	ZYMED
ISLET1	Mouse IgG	1:500	DSHB, Iowa City, IA
KI67	Rabbit IgG	1:200	ZYMED
mAChR	Rat IgG	1:500	Synaptic system
MEIS 1/2	Goat IgG	1:500	Santa Cruz
nAChR	Rat IgG	1:100	Sigma
Nestin	Goat IgG	1:2,000	Santa Cruz
Neuropeptide Y	Rabbit IgG	1:200	ImmunoStar
NKX2.1	Mouse IgG	1:500	Chemicon & Millipore
P75	Rabbit IgG	1:500	Chemicon & Millipore
Parvabumin	Rabbit IgG	1:2,000	Abcam
Parvabumin	Mouse IgG	1:1,000	Sigma
PAX6	Rabbit IgG	1:1,000	Covance

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Somatostatin and receptor	Rat IgG	1:500	Chemicon & Millipore
SOX2	Goat IgG	1:1,000	R&D
ßIII-tubulin	Rabbit IgG	1:10,000	Covance Research Products
ßIII-tubulin	Chick IgG	1:1000	Chemicon & Millipore
STEM121	Mouse IgG	1:500	StemCells
Synapsin	Rabbit IgG	1:1,000	CALBIOCHEM
Synaptophysin	Mouse IgG1	1:1000	Chemicon & Millipore
VAChT	Rabbit IgG	1:1,000	Sigma
VGLU	Rabbit IgG	1:1,000	Synaptic System

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Supplementary Table 4. Primers (5' to 3') for quantitative reverse-transcription PCR

Human gene	Forward	Reverse
Emx1	TTCAATGGGAGAGGGAGAGTG CTT	CCG TCAGCCTTTGTGAATGGT GTT
Lhx6	ACAGATCTACGCCAGCGACT	CATGGTGTCGTAGTGGATGC
Mash1	GTCTCCCGGGGATTTTGTAT	TCTCCATCTTGGCAGAGCTT
Lhx8	CCAAAACCAGCAAAAAGAGC	TGGCGTGCTCTACAATTCTG
Islet1	GTTTGAAATGTGCGGAGTGTAAT	TTCTTGCTGAAGCCGATGC
Nkx2.1	CGCATCCAATCTCAAGGAAT	CAGAGTGTGCCCAGAGTGAA
Pax6	ACAGATCTACGCCAGCGACT	CATGGTGTCGTAGTGGATGC
Olig2	GGTAAGTGCGCAATGCTAAGCTGT	TACAAAGCCCAGTTTGCAACGCAG
Gapdh	TCGACAGTCAGCCGCATCTTCTTT	ACCAAATCCGTTGACTCCGACCTT

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