

Efficient Generation of Corticofugal Projection Neurons from Human Embryonic Stem Cells

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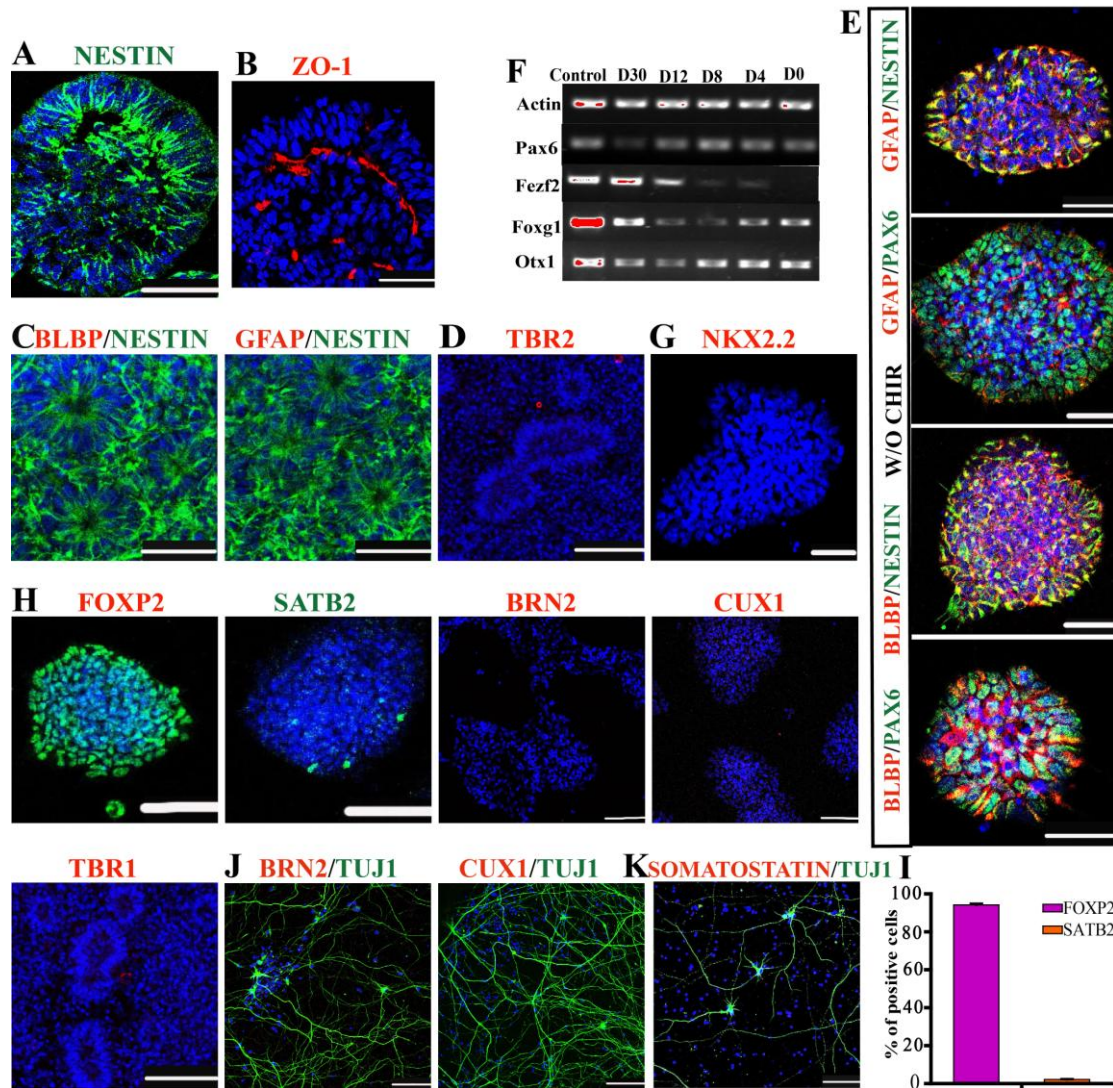


Fig.S1. Neuroepithelial stem cells (NESCs) display the telecephalic identity. (A-B)

Immunofluorescence of NESTIN (A) and ZO-1(B) showed the neural body formed a typical two-layer structure. (C-D) NESCs were negative for GFAP and BLBP (two radial glial progenitor cell makers), and Tbr2 (an intermediate cortical progenitor marker). (E) Removal of CHIR99021 in CHbFSB+LIF media resulted in the loss of NT formation and subsequent transition of radial glial progenitor cell (RGPC) from NESCs within 14 days. Transited cells express RGPC markers PAX6 and BLBP, NESTIN and BLBP, PAX6 and GFAP, NESTIN and GFAP. (F) RT-PCR data show that NESCs and its differentiated cells express the transcription factors involved in telecephalic development. (G) NESCs were negative for dorsal marker NKX2.2 in telecephalon. (H) NESCs were positive for FOXP2 (a cortical deep layer marker) along with few SATB2⁺ (a marker of callosal neurons from layers V or upper layer neurons) cells, but not for TBR1 (a marker of cortical deep layer projection neurons) and either BRN2 or Cux1 (two cortical upper

layer markers). (I) Quantification of FOXP2 and SATB2 positive NESCs. Data expressed as mean \pm s.d (n=3). (J) The neurons derived from NESCs at pdD70 were negative for upper-layer markers BRN2/CUX1. (K) Somatostatin positive neurons were not detected at pdD29. Scale bars: A-B, D, G, J, K, H ,100 μ m ;C, E, 50 μ m. Blue: DAPI, nuclear staining.

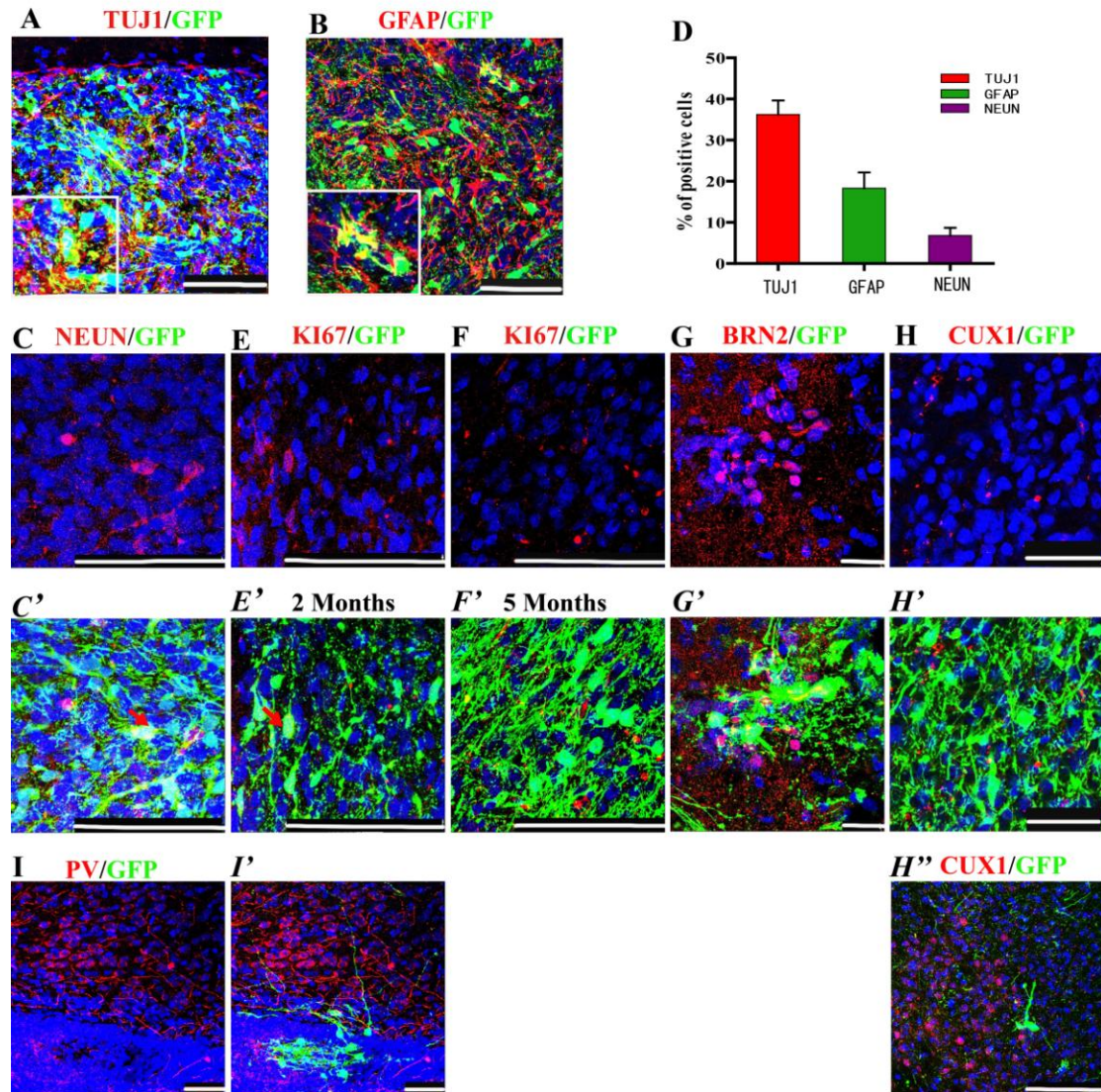


Fig.S2. relative to Figure 5 and 6. Grafted neuroepithelial stem cells (NESCs) differentiated into CfupNs in mouse brain. (A-B) Immunofluorescence showing TUJ1 positive neurons and GFAP positive astrocytes derived from grafted NESCs. (C) NeuN staining of grafted cells two months after transplantation. Red arrows indicate positive cells. (D) Quantification of TUJ1, GFAP and NeuN positive cells in vivo at two months after transplantation. Data were expressed as mean \pm s.d (n=3). (E) A few grafted cells expressed proliferation marker Ki67 two months after transplantation. red arrows indicated positive cells. (F) No any Ki67 cell was detected at the fifth

month post graft. (G-H) No any BRN2 or CUX1 positive upper layer projection neuron was found in grafted three months after transplantation. (I) No any PV (Parvalbumin) positive cell was found in grafted cell. Scale bars: A, B, C-F, I, H, 50µm; G, 25µm, H, 100µm. Blue: DAPI, nuclear staining.

A			B			
Gene	Forward	Reverse	Antibodies	Company	Cat No.	Dilution
Pax6	CCCGTCCATCTTTGCTTG	TCATAACTCCGCCAATCA	Calretinin	Millpore	AB5054	1:1000
CTIP2	TCACCCACGAAAGGCATCTGT	TGAAGGGCTGCTGCATGTTG	Tbr2	Millpore	AB2283	1:300
Otx1	TCTTCGCCAAGACTCGCTAC	CTGCATACACGAGGTGTTGCT	NeuN	Millpore	ABN78	1:400
Ngn2	CCGAGGAATCAGAAAGGCTACA	CTCCCGACAAGCACCGCTAT	Tuj1	Millpore	MAB1637	1:1000
Foxg1	ACCTCGCTGACACTCCACA	GCACCCGTCATGACTTCG	TH	Millpore	AB152	1:400
Reelin	CAACAGCGTAGGAGAAAG	GCCTTCTTCTCGCCTTCTCT	CHAT	Millpore	AP144P	1:600
Fezf2	AGCCAGACCGTTCGT	CGGATATGCGTGTGA	Sox2	Millpore	MAB5603	1:400
CUX1	ACCATCGGCTTCTTCTACAC	TGGTCAGCGAACTTCTTGG	Nestin	Millpore	AB5922	1:400
BRN2	CGGCGGTTTGCTCTATTTC	ATGGTGTGGCTCATCGTG	MAP-2	Millpore	AB5622	1:600
TBR1	TCCCAGTGCCATGTCCC	AACCCATTGCTCCTTGA	Tbr1	Millpore	AB10554	1:200
SOX5	CCTTCCCATCAAGCACCT	CTCAAAGCCTCTGTCCCA	Synapsin I	Sigma	S193	1:500
SATB2	GTGGGCTCAGATTAGCAG	TGATGTGGCAATGGAAGAA	Parvalbumin	Sigma	P3088	1:1000
β-actin	ACTGGAACGGTGAAGGTGAC	TTTTAGGAGGGCAAGGGAC	GFAP	Sigma	G6926	1:2000
			Brdu	Sigma	B8434	1:300
			Brn2	Sigma	SAB2501452	1:400
			GABA	Sigma	A2052	1:600
			Vglut1	Sigma	V0389	1: 1000
			Serotonin	Sigma	S5545	1: 1500
			P-Vimentin	MBLInternational	D076-3S	1:200
			Satb2	ABCAM	ab 51502	1:200
			Foxp2	ABCAM	ab16046	1:250
			PSD95	ABCAM	ab2723	1:300
			CUX1	SANTACRUZE	SC-7815	1:200
			BLBP	ABCAM	ab27171	1:200
			S100	ABCAM	ab52642	1:200

Fig.S3. relative to materials and methods. (A)The primers used for semi-quantitative PCR and RT-PCR. (B) The primary antibody list.

Supplemental Videos

Video S1, relative to Figure 5G-b. The 3D reconstruction of SMI312 (a marker of axon) stain demonstrating that the orientations of axon outgrowth were same with that of endogenous neuron axons.

Video S2, relative to Figure 6A. The 3D reconstruction of a representative GFP+ neuron exhibiting a pyramidal morphology in the cortex three months after graft.