Supporting Information

Hu et al. 10.1073/pnas.0910012107

SI Text

Two induced pluripotent stem cells (iPSC) lines (iPS-108 and -109) were derived from a female adult (retroviral transduced with OCT4, SOX2, KLF4, and *c-myc*). Both lines were karyotypically normal.

1. Yu J, et al. (2007) Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318:1917–1920.
2. Choi KD, et al. (2009) Hematopoietic and endothelial differentiation of human induced pluripotent stem cells. *Stem Cells* 27:559–567.
3. Yu J, et al. (2009) Human induced pluripotent stem cells free of vector and transgene sequences. *Science* 324:797–801.

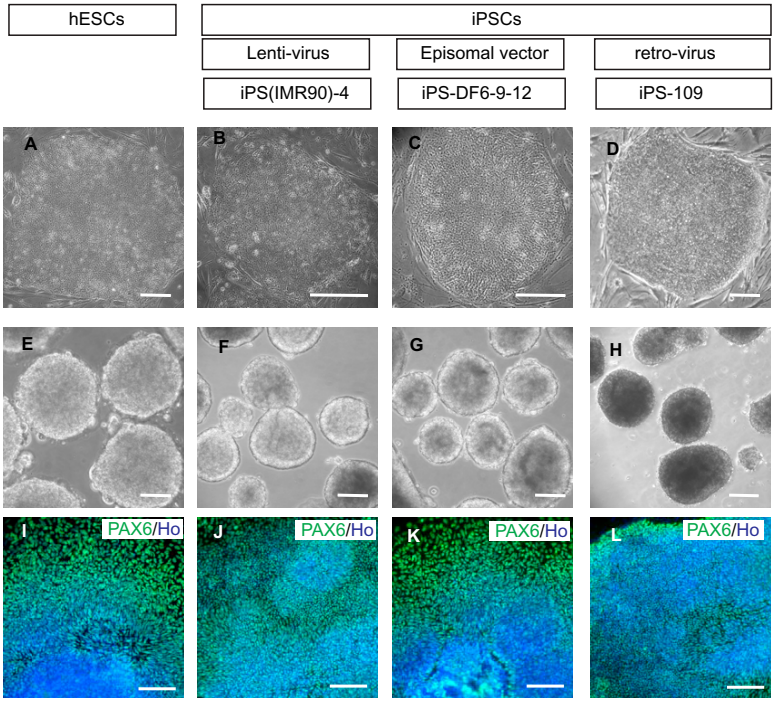

Fig. S1. Human iPSCs are indistinguishable from human embryonic stem cells (hESCs) when growing on MEF and forming aggregates. (*A–D*) Four representative human iPSC lines grow in colonies on mouse embryonic fibroblast (MEF) feeder cells. (*E–H*) After separation from feeder cells, the human iPSCs form aggregates in suspension culture. (*I–L*) hESC- and human iPSC-derived neuroepithelial (NE) cells uniformly express PAX6 at day 10. It should be noted that the iPSC colonies that do not have rosettes do not express PAX6. (Scale bar, 100 μm.)

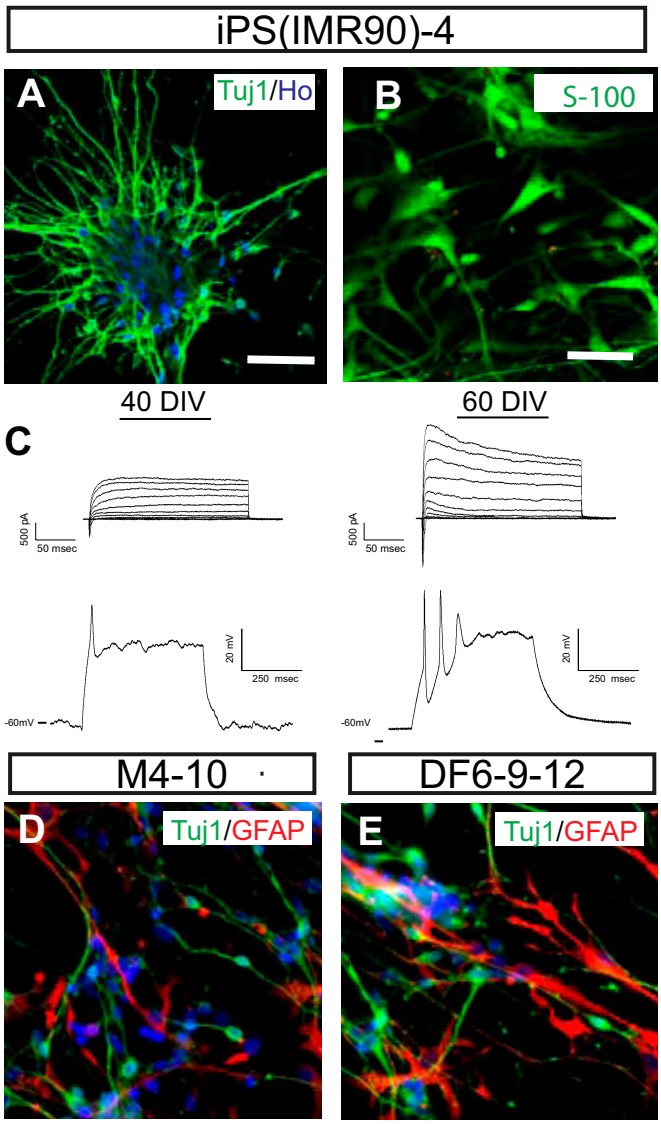

Fig. S2. Sequential generation of neurons and glia from human iPSCs. (*A* and *B*) Human iPS(IMR90)-4 cell derived Tuj1+ neurons and S100β+ astrocyte progenitors are revealed after 6 weeks of differentiation. (*C*) Representative physiological traces from human iPSC-derived neurons at day 40 revealed relatively small inward currents as well as an inability to fire trains of action potential (APs). At day 60, iPSC-derived neurons displayed larger inward and outward currents as well as the ability to fire trains of APs. (*D* and *E*) Human iPSM4-10 and DF6-9–12 derived neurons (Tuj1+) and astrocytes [glial fibrillary acidic protein (GFAP+)] at 3 months of culture. (Scale bar, 50 μm.)

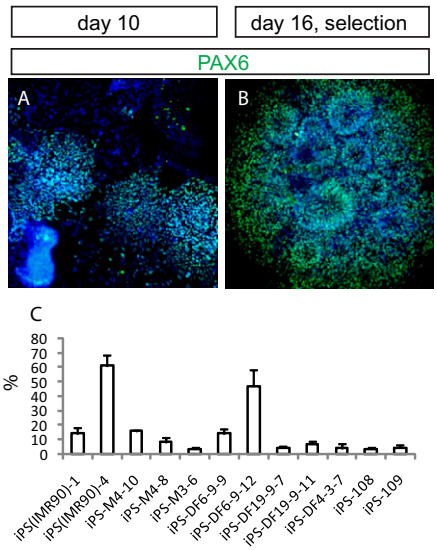

Fig. S3. Manual purification of the iPSC-derived NE cells. (*A*) The heterogeneous culture contained both PAX6+ neuroepithelial cells and PAX6 non-neuroepithelial cells before enrichment. (*B*) Uniform PAX6+ cells in the form of rosettes after enrichment. (*C*) Proportion of colonies that possess typical rosettes among total colonies.

Table S1. Pluripotent stem cell lines

iPS lines	Source line	Sources	Cat#	Nature	Age	Sex	Reprogramming factors	Methods	Terotoma	Reference
iPS(IMR90)-1	IMR-90	ATCC	CCL-188	Lung fibroblast	16 weeks	F	OCT4, SOX2, NANOG, LIN28	Lentivirus	Y	(1)
iPS(IMR90)-4	IMR-90	ATCC	CCL-188	Lung fibroblast	16 weeks	F	OCT4, SOX2, NANOG, LIN28	Lentivirus	Y	(1)
iPS-M4-10	CCD-1090Sk	ATCC	CRL-2106	Skin fibroblast	46 years	F	OCT4, SOX2, NANOG, LIN28	Lentivirus	Y	(2)
iPS-M4-8	CCD-1090Sk	ATCC	CRL-2106	Skin fibroblast	46 years	F	OCT4, SOX2, NANOG, LIN28	Lentivirus	Y	(2)
iPS-M3-6	CCD-1090Sk	ATCC	CRL-2106	Skin fibroblast	46 years	F	OCT4, SOX2, NANOG	Lentivirus	Y	(2)
iPS-DF6-9–9	CCD-1079Sk	ATCC	CRL-2097	Skin fibroblast	Newborn	M	OCT4, SOX2, NANOG, LIN28, c-Myc, KLF4	Episomal vectors	Y	(3)
iPS-DF6-9–12	CCD-1079Sk	ATCC	CRL-2097	Skin fibroblast	Newborn	M	OCT4, SOX2, NANOG, LIN28, c-Myc, KLF4	Episomal vectors	Y	(3)
iPS-DF19-9–11	CCD-1079Sk	ATCC	CRL-2097	Skin fibroblast	Newborn	M	OCT4, SOX2, NANOG, LIN28, c-Myc, KLF4	Episomal vectors	Y	(3)
iPS-DF19-9–19	CCD-1079Sk	ATCC	CRL-2097	Skin fibroblast	Newborn	M	OCT4, SOX2, NANOG, LIN28, c-Myc, KLF4	Episomal vectors	Y	(3)
iPS-DF4-3–7	CCD-1079Sk	ATCC	CRL-2097	Skin fibroblast	Newborn	M	OCT4, SOX2, NANOG, LIN28, c-Myc, KLF4	Episomal vectors	Y	(3)
iPS-108		Coriell	GM03814	Fibroblast	Adult	F	OCT4, SOX2, c-Myc, KLF4	Retrovirus	NA	
iPS-109		Coriell	GM03814	Fibroblast	Adult	F	OCT4, SOX2, c-Myc, KLF4	Retrovirus	NA	

NA, not analyzed.

Table S2. Primary antibody list

Antibodies	Isotype	Dilution	Source	Cat#
OCT4	Mouse IgG	1:1,000	Santa Cruz Biotechnology, Santa Cruz, CA	SC-5279
SOX2	Mouse IgG	1:1,000	R&D Systems, Minneapolis, MN	MAB2018
PAX6	Mouse IgG	1:5,000	Developmental Studies Hybridoma Bank, Iowa City, IA	
SOX1	Goat IgG	1:1,000	R&D Systems, Minneapolis, MN	AF3366
OTX2	Goat IgG	1:2,000	R&D Systems, Minneapolis, MN	AF1979
HOXB4	Rat IgG	1:50	Developmental Studies Hybridoma Bank, Iowa City, IA	112 anti-Hoxb4
OLIG2	Goat IgG	1:500	Santa Cruz Biotechnology, Santa Cruz, CA)	SC-19969
HB9	Mouse IgG	1:50	Developmental Studies Hybridoma Bank (Iowa City, IA)	81.5C10
Islet1/2	Rabbit IgG	1:4,000	S. Pfaff	
βIII-tubulin	Rabbit IgG	1:5,000	Covance, Emeryville, CA	PRB-435P
MAP2	Mouse IgG	1:2,000	Sigma, Saint Louis, MO	M1406
Synapsin	Rabbit IgG	1:250	CALBIOCHEM, San Diego, CA	574777
S-100	Mouse IgG	1:200	Chemicon, Billerica, MA	MAB079
GFAP	Rabbit IgG	1:5,000	DAKO, Glostrup, Denmark	Z0334
O4	Mouse IgM	1:50	Chemicon, Billerica, MA	MAB344

Table S3. Primers for RT-PCR

Targets	Forward	Reverse
BF1(FOXG1B)	GGACGCAGACCTTGAGAA	CACAAACTGAAGGCAATCGT
OTX2	CAACAGCAGAATGGAGGTCA	CTGGGTGGAAAGAGAAGCTG
LHX2	CAAGATCTCGGACCGCTACT	CCGTGGTCAGCATCTTGTTA
HOXB4	GCAAAGAGCCCGTCGTCTAC	CGTGTCAGGTAGCGGTTGTA
EN1	CCCTGGTTTCTCTGGGACTT	GCAGTCTGTGGGGTCGTATT
GBX2	GCCGTCGCTGATGATGATGC	TCGTCTTTCCCTTGCCCTCG
GAPDH	TCGACAGTCAGCCGCATCTTCTTT	ACCAAATCCGTTGACTCCGACCTT