Stem Cell Reports, Volume *8*

Supplemental Information

Direct Conversion of Human Fibroblasts into Neural Progenitors Using Transcription Factors Enriched in Human ESC-Derived Neural Progenitors

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Figure S1. Related to Figure 1

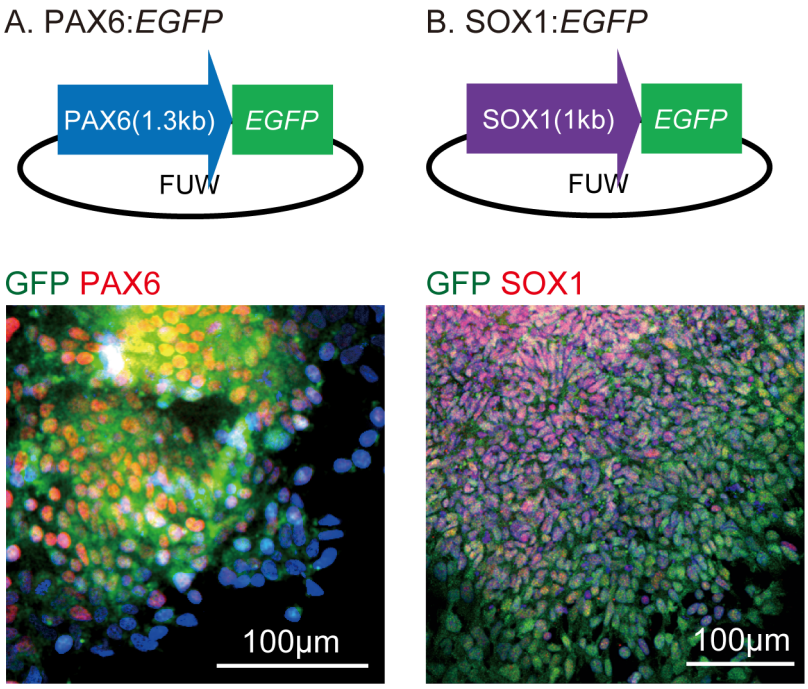


Figure S2. Related to Figure Figures 2 and 3

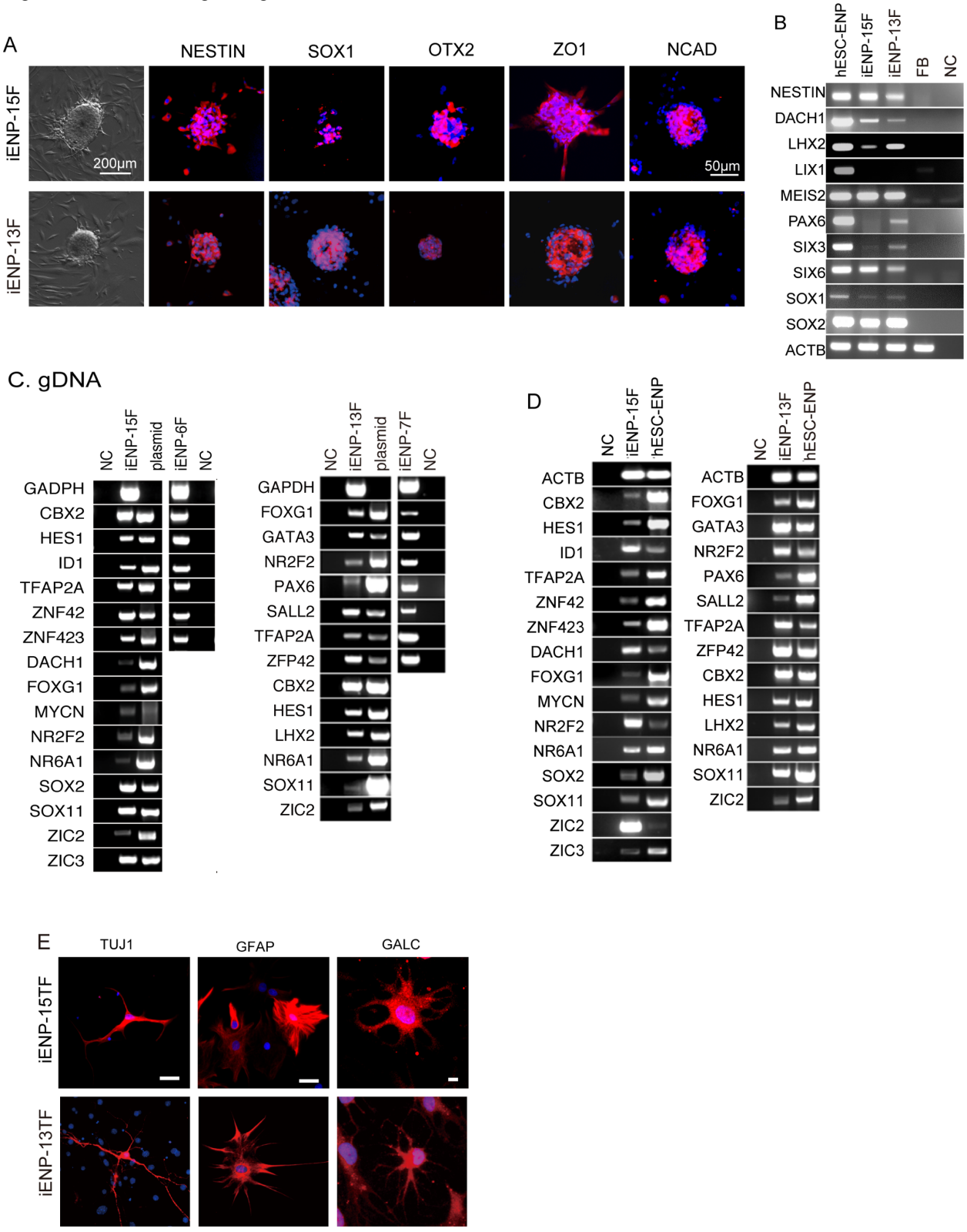


Figure S3: related to Figures 2-5.

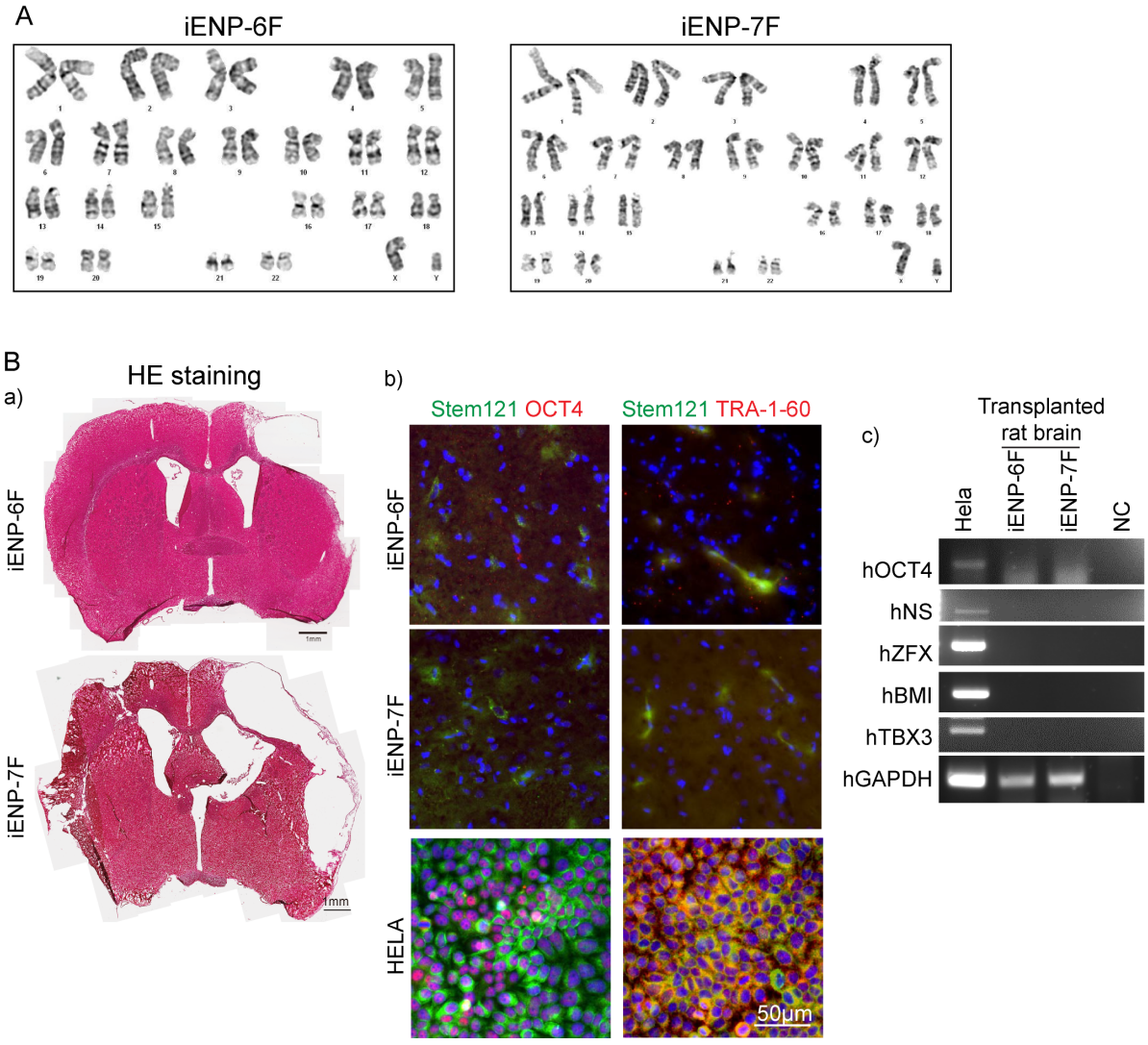


Figure S4: related to Figures 2 and 3.

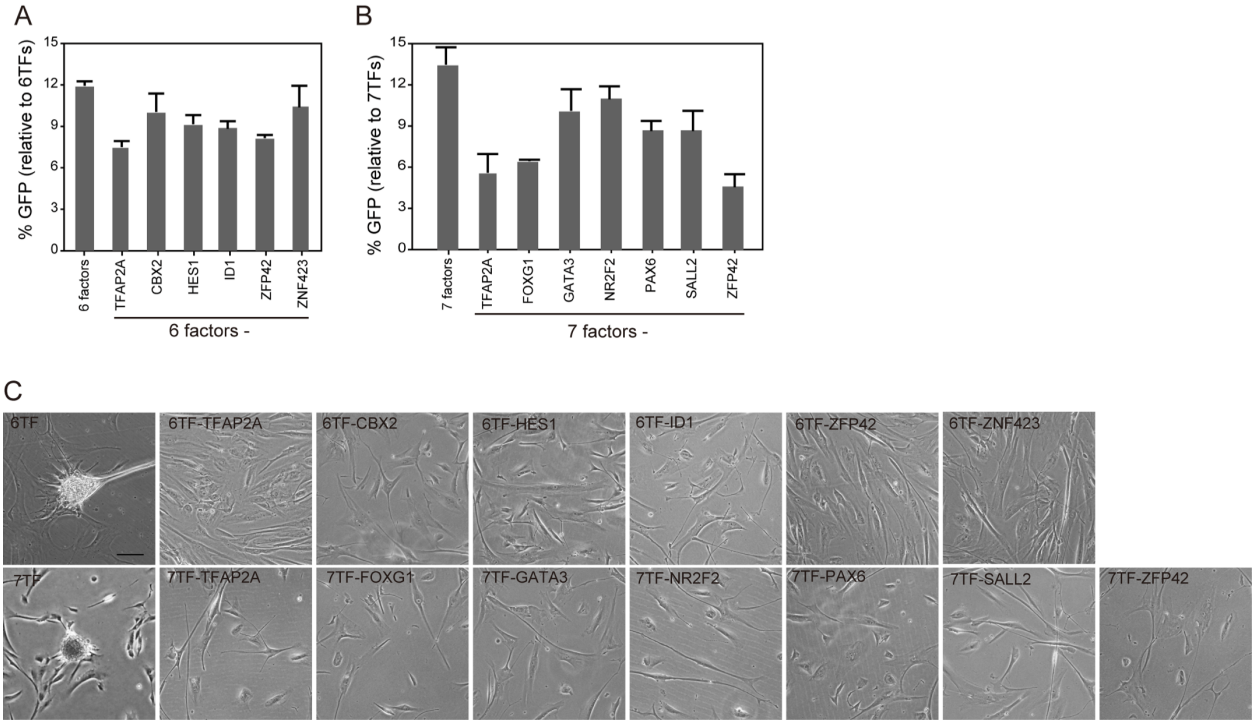
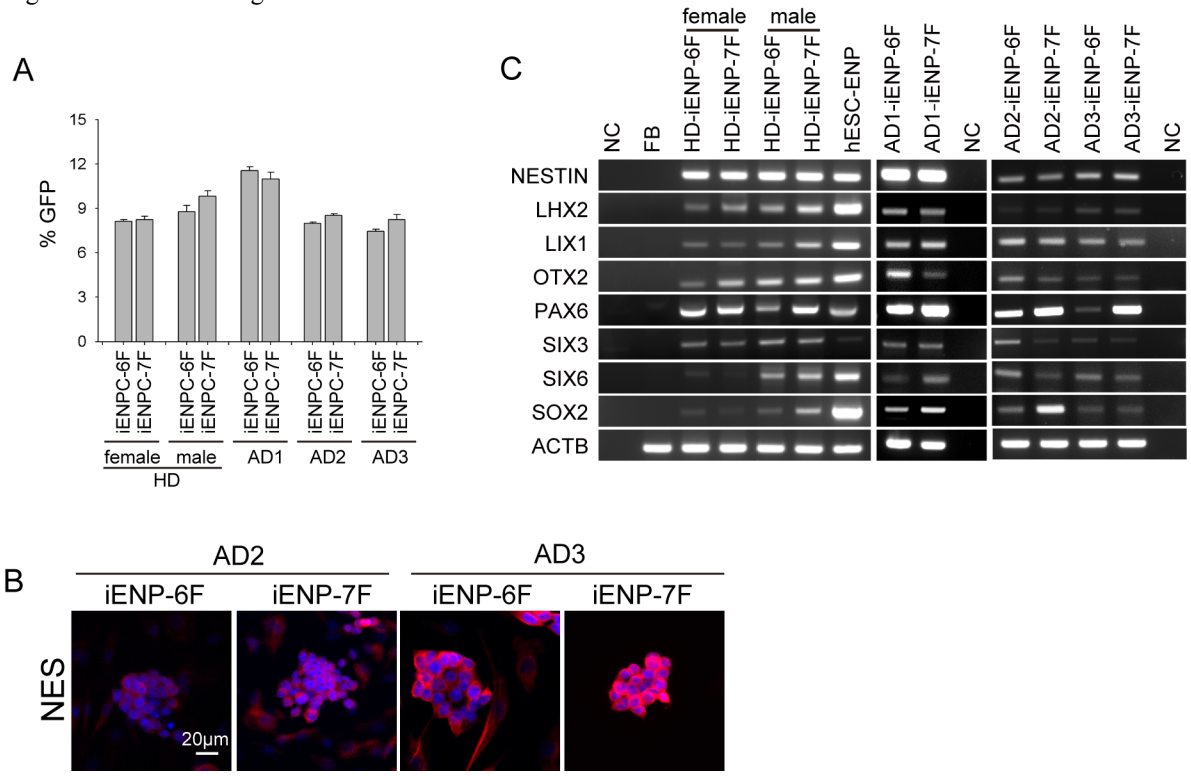


Figure S5: related to Figure 7.



Supplemental Figure legends

Figure S1. Related to Figure 1. Construction and characterization of PAX6:*EGFP* and SOX1:*EGFP* neural reporters.
(A) Schematic drawings of the lentiviral constructs, PAX6:*EGFP* and SOX1:*EGFP*. (B) ICC staining of PAX6:*EGFP*- or SOX1:*EGFP*- transfected hESC-derived neural rosettes (day 20) with antibodies against GFP and PAX6 or SOX1, respectively. Nuclei were counterstained with DAPI (blue).

Figure S2. Related to Figure 2 and 3. Characterization of iENP-15F and iENP-13F.
(A) ICC staining of iENPs induced by the 15 (selected by PAX6:*EGFP*) and 13 (selected by SOX1:*EGFP*) TF combinations, using antibodies against the indicated NP markers. Nuclei were counterstained with DAPI (blue). (B) RT-PCR analysis of the indicated genes using mRNA isolated from undifferentiated iENP-15F and -13F. hESC-ENPs and FBs were used as positive and negative controls, separately. (C) PCR analysis of the integration of the indicated exogenous transgenes using genomic DNA isolated from undifferentiated iENP-15F, iENP-6F, iENP-13F, and iENP-7F. The plasmids of indicated genes were used as positive controls. (D) RT-PCR analysis of the indicated endogenous genes using mRNA isolated from undifferentiated iENP-15F and iENP-13F after doxycycline withdrawal. hESC-ENP was used as a positive control. (E) ICC staining of differentiating iENP-15F and iENP-13F with antibodies against TUJ1, GFAP and GALC. Scale bar = 10μm. NC: negative control (H_2O). Nuclei were counterstained with DAPI (blue).

Figure S3. Related to Figures 2–5. Karyotype of undifferentiated iENPs and absence of tumorigenesis of transplanted iENPs.
(A) Karyotype analysis of undifferentiated iENP-6F at passage 20 and iENP-7F at passage 21. (B)(a) H&E staining showed no obvious nuclear aggregation, and (b) IHC staining using a specific antibody against the tumorigenic marker OCT4 or TRA-1-60 showed no colocalization with the human marker Stem121 at 12 weeks after transplantation of iENP-6F and iENP-7F into the MCAO-injured adult rat brain. Nuclei were counterstained with DAPI (blue). (c) RT-PCR analysis using mRNA isolated from transplanted brain tissue showed no expression of the indicated tumorigenic genes. HELA cells were used as a positive control. NC, negative control (H_2O).

Figure S4. Related to Figure 2 and 3. Essential transcription factor combinations for iENP generation.
(A-B) Effect of single TF dropouts from (A) the 6TF-set on the induction of PAX6:*EGFP*$^+$cells and (B) 7 TF-set on the induction of SOX1:*EGFP*$^+$ cells. (C) Phase contrast image showing iENP-like colony formation during induction with the transfected TF combination. No colony formation was observed after individual TFs were removed from the original 6 TF or 7 TF combinations. All quantitative data were obtained from three independent experiments and are presented as means ± SD.

Figure S5. Related to Figure 7. Generation and characterization of HD-iENP and AD-iENP.
(A) Induction rate of SOX1:*EGFP*$^+$ (putative iENP-6F) and PAX6:*EGFP*$^+$ (putative iENP-7F) from HD and AD FBs. All quantitative data were obtained from three independent experiments and are presented as means ± SD. (B) ICC staining of AD2- and AD3-iENPs using an antibody specifically against neural marker NES. (C) RT-PCR analysis of the indicated genes using mRNA isolated from undifferentiated HD-iENP-6F, HD-iENP-7F, AD-iENP-6F, and AD-iENP-7F. hESC-ENPs and parental FBs were used as positive and negative controls.

Table S1. Primer Sets Used in this Study, Related to Figure 1, 2, 3 and 6.

Primers used for amplification of 25 transcription factors

Gene name	Sense strand	Antisense strand
CBX2a	TCTAGAATGGAGGAGCTGAGCAGCG	GAATTCTCAGTAATGCCTCAGGTTGAAGAA
DACH1a	CAATTGATGGCAGTGCCGGCGG	CGACCAATTGTCAGTACATGACA
DEPDC1a	ACTAGTATGGAGAGTCAGGGTGTGCCT	TTCGAATTATCTTAGACTACGGAACTTTGGTTT
FOXG1	GAATTCTGGTCCCAGGGATGTTAATG	GAATTCATGCTGGACATGGGAGATAGG
GATA3a	GAATTCATGGAGGTGACGGCGGAC	GAATTCCTAACCCATGGCGGTGACC
HES1	GAATTCATGCCAGCTGATATAATGGAGAA	GAATTCTCAGTTCCGCCACGGCC
ID1a	TCTAGAATGAAAGTCGCCAGTGGCAG	TTCGAATCAGCGACACAAGATGCGAT
LHX2	CAAGATCTCGGACCGCTACT	CCGTGGTCAGCATCTTGTTA
MBD2a	TCTAGATGGATGCGCGCGCACCC	TTCGAATTAGGCTTCATCTCCACTGTCC
MYCN	GAATTCATGCCGAGCTGCTCCACG	GAATTCCTAGCAAGTCCGAGCGTGTT
MYEF2	TCTAGAATGGCGGACGCCAACAA	TTCGAATTATGCATTACGATCCAAGCG
NR2F2a	GAATTCATGGCAATGGTAGTCAGCACG	GAATTCTTATTGAATTGCCATATACGGC
NR6A1a	GAATTCATGGAGCGGGACGAACCG	GAATTCTCATTCCTTGCCCACACTGG
OTX2b	GAATTCATGATGTCTTATCTTAAGCAACCG	GAATTCTCACAAAACCTGGAATTTCC
PAX6a	CAGCCAGAGCCAGCATGCAG	TTACTGTAATCTTGGCCAGTATTG
SALL2	CAATTGATGTCTCGGCGAAAGCAGC	CAATTGTCATGGGATCGTGGGGTCA
SIX3	GCTAGCATGGTATTCCGCTCCCCCCTAG	GCTAGCTCATACATCACATTCCGAGTCGCTG
SOX1	GCTAGCATGTACAGCATGATGATGGAGACCG	GCTAGCCTAGATGTGCGTCAGGGGCACC
SOX11	CAATTGATGGTGCAGCAGGCGGAG	CAATTGTCAATATGTGAACACCAGGTCG
TFAP2Ab	GAATTCATGTTAGTTCACAGTTTTTCAGCC	GAATTCTCACTTTCTGTGCTTCTCCTCTT
ZFP42	CAATTGATGAGCCAGCAACTGAAGAAAC	CAATTGCTACTTTCCCTCTTGTTCATTCTTG
ZIC2	GAATTCATGCTCCTGGACGCGGG	GAATTCTCACACGTACCATTCATTGAAGTT
ZIC3	TCTAGAATGACGATGCTCCTGGACGG	GAATTCTCAGACGTACCATTCGTTAAAATTAG
ZNF423	GAATTCATGCATAAGAAGAGGGTTGAAGAG	GAATTCTCACTGTGCGTGCTGGCTC

Primers for RT-PCR analysis

Genename	Sense strand	Antisense strand
LHX2	GAAGGGGCGGCCGAGGAAAC	GCTGGTCACGGTCCAGGTGC
PAX6	AACAGACACAGCCCTCACAAACA	CGGGAACTTGAACTGGAACTGAC
SOX1	CAATGCGGGGAGGAGAAGTC	CTCTGGACCAAACTGTGGCG
SOX2	GGCGGCAACCAGAAAAACAG	TAATCCGGGTGCTCCTTCATGT
SIX6	AACAAGAATGAGTCGGTGCT	CAGCGGGAACTTCTTCCTTA
DACH1	CCAACGCAAGTTCTAGACCTGG	GGAAGACAGTCCATCAGGAAACAG
MEIS2	TACCCTTCCGAAGAGCAGAA	TCACTGCTCGATTTGACTGG
LIX1	GGAATTTTGGGAAAGCAAGC	CAGCACTGAAAGTTGCCAAA
SIX3	CCATCAACAAACACGAGTCG	TCCTGTAGGTACCACTCCCG
NESTIN	GCCACCTCACTGCAGTAGTG	AGTCAGCACATGGGAGTGC
OTX2	CGCCTTACGCAGTCAATGGG	CGGGAAGCTGGTGATGCATAG
EMX1	GAGACGCAGGTGAAGGTGT	GTTGATGTGATGGGAGCCCT
EN1	CTGGGTGTACTGCACACGTTAT	TACTCGCTCTCGTCTTTGTCCT
ISL1	ATGACAAAACTAATATCCAGGGG	ATTGACCAGTTGCTGAAAAGC
TH	GTCCCCTGGTTCCCAAGAAAAGT	TCCAGCTGGGGGATATTGTCTTC
AADC	CTCGGACCAAAGTGATCCAT	GGGTGGCAACCATAAAGAAA
ACTB	GAGCACAGAGCCTCGCCTTT	ACATGCCGGAGCCGTTGTC
OCT4	GCCGTGAAGCTGGAGAAGGA	CCCCACATCGGCCTGTGTAT
NS	CAGAGATCCTCTTGGTTGCAG	AATGAGGCACCTGTCTCCTC
ZFX	TGATTCCAGGCAGTACCAAAC	TGACGAAAACCCTTACCACAC
BMI	TACTCCAGTGCAGTCTCCTC	TCCCATCTTTCCTAACACAG
TBX3	GAAGAAGAGGTGGAGGACGA	ATTCAGTTTCGGGGAACAAG
rat GAPDH	GACATGCCGCCTGGAGAAAC	AGCCCAGGATGCCCTTTAGT

Primers for endogenous gene expression

Gene name	Sense strand	Antisense strand
CBX2a	TGTGTCAAGGGCAGTGCTAC	ACAAGGTGGAGACCAACAAG
DACH1	CTCTCTCTAACTGGGCATGG	TCCCATGACGAATGTCTGAC
FOXG1	TCAATGACTTCGCAGAGCAG	GGTCTGCGTCCACCATATAG
GATA3	ACCCCTGACTATGAAGAAGG	CACAGCACTAGAGACCCTGT
HES1	AAGTGTGCTGGGGAAGTACC	TTCCTGTTTAGAGTCCGGAG
ID1	TTGCCCATTCTGTTTCAGCC	TTCCAACTTCGGATTCCGAG
LHX2	GCTAAGCTGCAACGAAAACG	GGCGAGATCCTAAAATGTGG
MYCN	ATCCTCAAACGATGCCTTCC	ATGTGCAAAGTGGCAGTGAC
NR2F2	TCAAGGCCATAGTCCTGTTC	AGTTGTTCTGACCGACACAG
NR6A1	GCAGGAGCTAATCCTGCTG	TTGGTCTCTCTGGCTTTTCC
PAX6	TTTCAGCACCAGTGTCTACC	CCCCAGTGGTACAATACAGG
SALL2	GGGAAGCAGTGGTGTTTTAG	AAGCTGTTTCTGCTCTGTGG
SOX2	CGGCAGCTACAGCATGATG	TACTCTCCTCTTTTGCACCC
SOX11	GCTACAACGTCGCCAAAGTG	CGAGAGAAAGAGCGAGCAG
TFAP2A	ATGCAAAGAGTTCACCGACC	TCTGTTCTCTTAGGCTCCAC
ZFP42	AGTTGAGGAATAGAGCTGCC	GCACCATGTCCTTAAAACGG
ZIC2	AGCACATGAAGGTCCATGAG	TTAGCAGGGAGGTTTGGTTC
ZIC3	GAAGATCTTTGCCCGTTCTG	GCTACTTTTGGATCTTGCCC
ZNF423	CGAGAACGAGAGAGAGATCC	CACACTAGCTGTAGCAGGAC

Primers used for exogenous gene expression analysis

Gene name	Sense strand	Antisense strand (WPRE)
exo-CBX2a	CAGCAAGAGGGACTGTGTCA	CCACGCCACGTTGCCTGACA
exo-DACH1	ACAGGGGCTGTTGAAAGTTG	CCACGCCACGTTGCCTGACA
exo-FOXG1	AGGGCCGCGTCCTCCTC	CCACGCCACGTTGCCTGACA
exo-GATA3	GTCCTGTGCGAACTGTCAGA	CCACGCCACGTTGCCTGACA
exo-HES1	ATCAATGCCATGACCTACCC	CCACGCCACGTTGCCTGACA
exo-ID1	GTCTGTCTGAGCAGAGCGTG	CCACGCCACGTTGCCTGACA
exo-LHX2	AGTTCAGGCGCAACCTCTTA	CCACGCCACGTTGCCTGACA
exo-MYCN	AAACCACAACATCCTGGAGC	CCACGCCACGTTGCCTGACA
exo-NR2F2	TGCCTGTGGTCTCTCTGATG	CCACGCCACGTTGCCTGACA
exo-NR6A1	GCTAAAGGTCAGCAACGAGG	CCACGCCACGTTGCCTGACA
exo-PAX6	GAACAGACACAGCCCTCACA	CCACGCCACGTTGCCTGACA
exo-SALL2	AGAGAGCAGCAGCAGAAAGG	CCACGCCACGTTGCCTGACA
exo-SOX2	ACCAGCTCGCAGACCTACAT	CCACGCCACGTTGCCTGACA
exo-SOX11	AAGAACATCACCAAGCAGCA	CCACGCCACGTTGCCTGACA
exo-TFAP2A	AGAAGCTGTCCACCTAGCCA	CCACGCCACGTTGCCTGACA
exo-ZFP42	TAGAGCTGCCCTGAGAAAGC	CCACGCCACGTTGCCTGACA
exo-ZIC2	AGCTCCGGCTATGAGTCGT	CCACGCCACGTTGCCTGACA
exo-ZIC3	CCCACACAGGTGAGAAACCT	CCACGCCACGTTGCCTGACA
exo-ZNF423	TTTGAGAGTGCCGAAGACCT	CCACGCCACGTTGCCTGACA

Table S2. Antibodies Used in This Study, Related to Figure 1-7.

Primary Antibody

Name	Company	Catalog	Host	Dilution
BLBP	Millipore	ABN14	Rabbit	1:500
γH2AX	Millipore	05-636	Mouse	1:500
GABA	sigma	A0310	Mouse	1:100
GAL C	chemicon	MAB342	Mouse	1:200
GFAP	Dako	M0761	Mouse	1:100
GFAP	Chemicon	AB5804	Rabbit	1:500
GFP	abcam	ab13970	Chicken	1:500
HB9	Santa cruz	sc-22542	Goat	1:200
HOXB4	DSHB		Rat	1:50
Human nuclei	Chemicon	MAB1281	Mouse	1:200
ISL1	DSHB		Mouse	1:50
LHX/Lim2	chemicon	AB5756	Rabbit	1:200
MAP2	Chemicon	AB5622	Rabbit	1:200
MBP	Chemicon	AB980	Rabbit	1:10000
NCAD	Santa cruz	SC8424	Mouse	1:50
Nestin	chemicon	MAB5326	Mouse	1:200
Nestin	Chemicon	ABD69	Rabbit	1:500
NeuN	Chemicon	MAB377	Mouse	1:10
NFH	Sigma	N4142	Rabbit	1:200
NG2	Chemicon	AB5320	Rabbit	1:200
OCT4	Santa Cruz	SC-9081	Rabbit	1:50
OTX2	R&D	AF1979	Goat	1:100
PAX6	DSHB		Mouse	1:50
AT8	Thermo	MN1020	Mouse	1:40
PRPH	Chemicon	AB1530	Rabbit	1:200
SOX1	chemicon	AB15766	Rabbit	1:100
STEM121	STEM CELLs	AB-121-U-050	Mouse	1:200
SYP	DSHB		Mouse	1:50
TBR1	Millipore	AB10554	Rabbit	1:200
TRA-1-60	Abcam	MAB4360	Mouse	1:100
Tuj1	Chemicon	MAB1637	Mouse	1:200
Tuj1	Covance	MRB-435P	Rabbit	1:10000
ZO1	Santa Cruz	SC-10804	Rabbit	1:200

Secondary antibody

Host	Target	Fluorescence	Company	Catalog	Dilution
Donkey	Mouse	488	Thermo	R37114	1:500
Donkey	Mouse	594	Thermo	R37115	1:500
Goat	Mouse-IgM	Cy3	Jackson	115-165-105	1:500
Donkey	Rabbit	488	Thermo	R37118	1:500
Donkey	Rabbit	594	Thermo	R37119	1:500
Donkey	Goat	488	Thermo	A-11055	1:500
Goat	Chicken	488	Thermo	A-11039	1:500
Donkey	Rat	594	Thermo	A-21209	1:500

Supplemental Experimental Procedure

Construct generation

Constructs carrying candidate neural transcription factors were generated from the coding sequences of *FOXG1, GATA3, MBD2, MYCN* (all from transOMIC technologies), *SOX2* (FUW-teto-*SOX2*; Addgene), *CBX2, DACH1, DEPDC1, HES1, ID1, LHX2, MYEF2, NR2F2, NR6A1, OTX2a, PAX6a, SALL2, SIX3, SOX1, SOX11, TFAP2A, ZFP42, ZIC2, ZIC3*, and *ZNF423* (all from cDNA of hESC H9-derived neural progenitors). The coding sequences were cloned into FUW or FUW-teto vector for further experiments. Reporter constructs were generated by cloning 1.3kb PAX6 P1 promoter (Plaza et al., 1995) and 1kb SOX1 promoter into FUW vector to generate PAX6:*EGFP* and SOX1:*EGFP*, respectively, and UbC:*EGFP* was used as a control.

Generation of Induced Embryonic Neural Progenitors (iENP)

The iENPs were generated by producing lentiviral particles carrying candidate transcription factors with the use of 293FT cells in accordance with standard procedures. CCD112SK foreskin fibroblasts (FBs), HD FBs isolated from one female patient and one male patient (both *HTT* with 431 CAG repeats), and AD FBs (AD1 with a *APOE4/E4* mutation, AD2 with the *PSEN1* E184D mutation, and AD3 with *PSEN1* P264L from Coriell Cell Repository) were infected with lentiviruses carrying the candidate transcription factor or reporter, and then cultured in FB media [DMEM, 10%FBS]. At one day after infection, media were replaced with neural induction media [DMEM/F12, N2 supplement, 20μg/ml bFGF, 1% NEAA, 2mM glutamine, 1mM sodium pyruvate (Invitrogen), 2μg/ml doxycycline (Sigma), 10ng/ml LIF (Invitrogen), 3μM CHIR99021 (Sigma) and 2μM SB431542 (R&D) (Li et al., 2011)]; media were subsequently replaced every 2 days. After a week of induction, GFP positive cells were purified on a BD FASCAriaII sorter and planted on matrigel-coated dishes with iENP media containing 2μg/ml doxycycline [N2B27: 50% DMEM/F12, 50% Neurobasal, 0.5X N2 supplement, 0.5X B27 supplement, 10μg/ml bFGF, 1% NEAA, 2mM glutamine, 1mM sodium pyruvate, 10ng/ml LIF (Invitrogen), 3μM CHIR99021 (Sigma), and 2μM SB431542 (R&D)] (Figure 1B). Cells spontaneously formed neural sphere-like structures after 2 or 3 days. The neural sphere-like structures were collected and trypsinized into single cells and then plated on ornithine-laminin coated dishes with iENP media containing 2μg/ml doxycycline. The efficiency of iENP generation was measured by combining two parameters: the percentage of GFP positive cells driven by either PAX6*:EGFP* or SOX1*:EGFP* at day 6 post lentiviral infection, and the percentage of neurosphere formation at day 2 post purification. After 2 to 3 passages, doxycycline was removed from culture media and the cells were maintained and subcultivated every 7 days. After 2 passages without doxycycline, iENPs were examined for expression of neural genes, endogenous neural genes, and exogenous genes by RT-PCR analysis, exogenous gene insertion by PCR analysis, and neural gene expression by ICC analysis.

Chromosome karyotyping

After cell cultures were reach 80% confluence, colcemid solution (KaryoMAX® Colcemid® Solution, 10 μg/ml, Invitrogen) were added at a final concentration of 0.05 μg/ml for 1 hr to arrest cells in metaphase. Cells were then harvest by trypsin and treated with hypotonic solution (0.075 M potassium chloride solution, SIGMA) for 15 minutes, and subsequently with fresh Carnoy's fixative solution (3:1 ratio of methanol:glacial acetic acid). Standard cytogenetic G-banding procedures was applied for chromosome karyotype analysis.

Differentiation

General neural differentiation was examined using differentiation media [Neurobasal, B27 supplement, 1% NEAA, 2mM glutamine, 1mM sodium pyruvate (Invitrogen), 300μg/ml dbcAMP, 50μM ascorbic acid (Sigma), 20ng/ml BDNF, 20ng/ml GDNF, 50ng/ml NGF (Peprotech)], while specific neuronal differentiation were examined using cortical neuronal differentiation media (Maroof et al., 2003), dopaminergic neuronal differentiation media (Nguyen et al., 2011), and PNS neuronal differentiation media (Chambers et al., 2012) [cortical neuronal differentiation media: Neurobasal, N2 supplement, B27 supplement (Invitrogen), 100ng/ml SHH, 125ng/ml Noggin, 250ng/ml DKK1, 10ng/ml BDNF, 10ng/ml bFGF (R&D), 2μM XAV939, 100nM LDN93189, 10μM SB431542, 200μM ascorbic acid, 200μM dbcAMP (Sigma); dopaminergic neuronal differentiation media: DMEM/F12, N2 supplement (Invitrogen), 20ng/ml BDNF, 200ng/ml SHH, 100ng/ml FGF8β (R&D), 200μM ascorbic acid (Sigma); PNS neuronal differentiation media: DMEM/F12, N2 supplement (Invitrogen), 3μM CHIR99021, 10μM SU5402, 10μM DAPT, 200μM dbcAMP].

Aβ measurement

AD-iENP and CCD1112sk (CTL)-iENP were plated in 24 well plates ($8x10^5$ cells/well) and then induced to differentiate into cortical neurons. Media were harvested at 20 days after plating and stored at -80 degree till

analysis. Secreted Aβ42 and 40 were measured using Aβ42 and 40 human ELISA kits (KHB3544 and KHB3482, Thermo Fisher Scientific), and detected using a Benchmark plus microplate spectrophotometer (BIO-RAD). Each experiment was performed in biological triplicates.

Electrophysiology

For electrophysiological recording, iENP-derived neurons were further co-cultured with mouse glial cells in neuronal maturation media [B27: Neurobasal, B27 supplement, 1% NEAA, 2mM glutamine, 1mM sodium pyruvate (Invitrogen), 20ng/ml BDNF, 20ng/ml GDNF, 50ng/ml NGF (Peprotech)] for 2 weeks. Mouse glial cells were isolated from the brains of P1 ICR mice and subcultivated for more than 3 passages to eliminate neuron contamination, which was confirmed by examination of mouse Tuj1 mRNA and protein expression using RT-PCR analysis and ICC analysis, respectively. Electrophysiological properties were determined by whole cell patch clamp recording at room temperature with external solution [115mM NaCl, 2mM KCl, 10mM HEPES, 1.5mM $MgCl_2$, 3mM $CaCl_2$, 10mM Glucose. pH 7.4, 300mOsm]; the patch pipettes were 5-10 MΩ filled with internal solution [130 mM K-gluconate, 10mM NaCl, 2mM MgCl2, 10mM HEPES, 0.5mM EGTA, 3mM ATP]. TTX (1μM) in external solution was used to block TTX-sensitive sodium channels. Seal resistance in the whole cell mode was over 1GΩ. Cells were visualized under a 20X Olympus BX51WI water-immersion lens with Sony CCD; action potentials were recorded in whole cell current-clamp mode, and sodium current was recorded in voltage-clamp mode using a Multiclamp 700B (Molecular Devices) controlled by Signal software and Power 1401 (CED). Results were analyzed using Microsoft Excel 2010.

Reverse transcription-polymerase chain reaction, RT-PCR

RNA was extracted with TRIzol reagent following the standard extraction protocol (Molecular Research Center). Extracted RNA was reverse-transcribed into cDNA with SuperScript III Reverse Transcriptase (Invitrogen). Each PCR used 25 ng of cDNA. GoTaq Green Master Mix (Promega) was used for RT-PCR analysis. For quantitative PCR (qPCR), SYBR® FAST 2X qRT-PCR Master Mix (KAPA) and a 7900HT Fast Real-Time PCR System (Applied Biosystems) were used. Primers are listed in Table S1.

Flow analysis

To evaluate the proportion of GFP-positive cells, cells were dissociated and then resuspended in PBS. The results were recorded and analyzed with a BD FACSCalibur flow cytometer. The effect of withdrawing an individual factor was determined by normalizing the proportion of each set to the control.

Cell proliferation and death analysis

iENP-6F, iENP-7F, NP, and GFP control cells were seeded in 24 wells in iENP medium without doxycycline. Cell number was counted at day 1, 2, 3, 4, and 5. Results are shown relative to those of day 1. BrdU (93-3943, Thermo Fisher Scientific) incorporation and TUNEL (G3250, Promega) assays were performed following standard protocols. Images were detected using a Zeiss microscope and Spot software, and analyzed by Metamorph software.

Supplemental References

Li, W., Sun, W., Zhang, Y., Wei, W., Ambasudhan, R., Xia, P., Talantova, M., Lin, T., Kim, J., Wang, X.*, et al.* (2011). Rapid induction and long-term self-renewal of primitive neural precursors from human embryonic stem cells by small molecule inhibitors. Proc Natl Acad Sci U S A *108*, 8299-8304.