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Supplemental Information

Direct Lineage Conversion of Adult Mouse Liver Cells and B Lymphocytes to Neural Stem Cells

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Figure S1

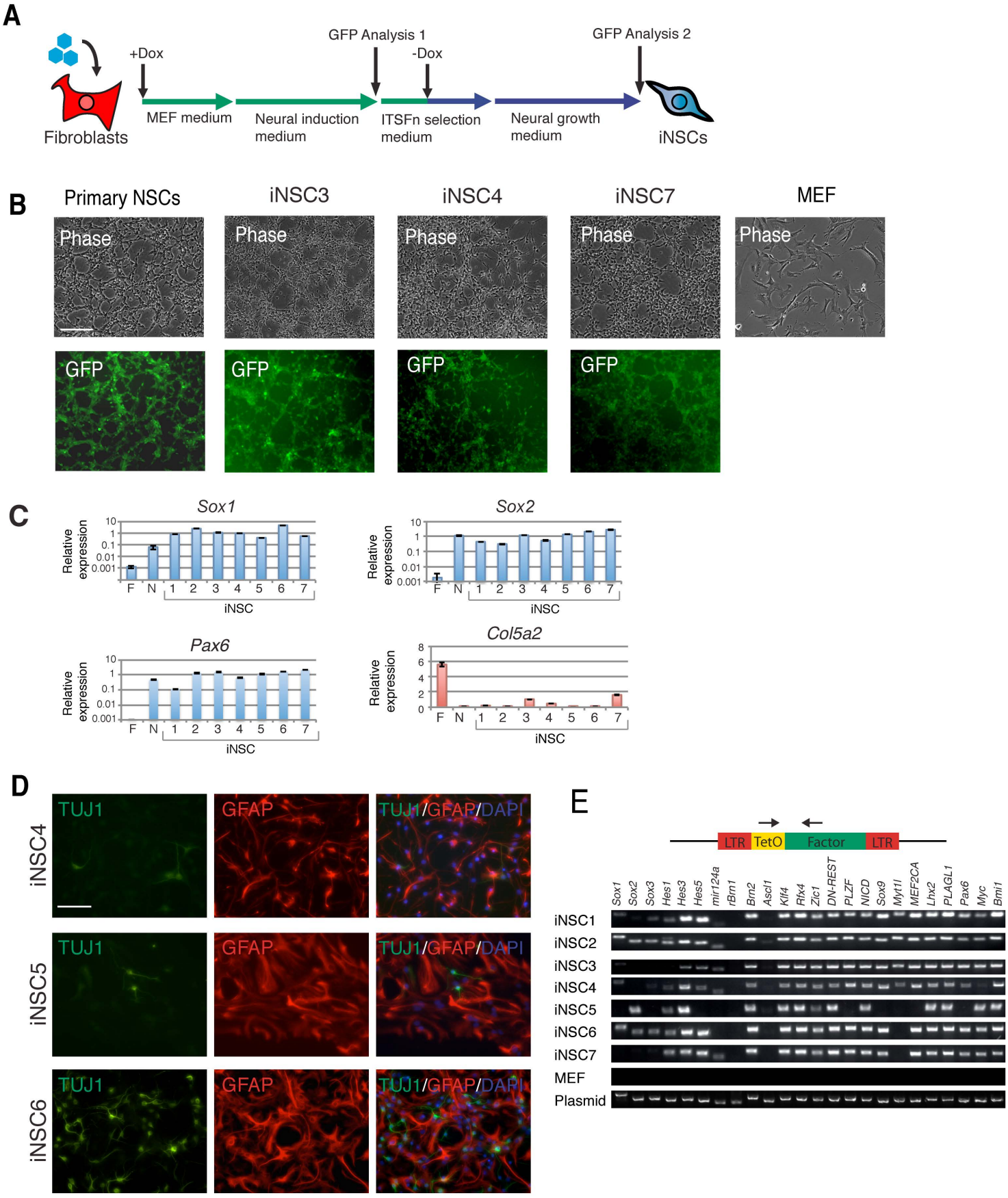

A
+Dox
GFP Analysis 1
-Dox
GFP Analysis 2
Fibroblasts
MEF medium
Neural induction medium
ITSFn selection medium
Neural growth medium
iNSCs
B
Primary NSCs
iNSC3
iNSC4
iNSC7
MEF
Phase
GFP
C
Sox1
Sox2
Pax6
Col5a2
Relative expression
iNSC
F N 1 2 3 4 5 6 7
D
iNSC4
iNSC5
iNSC6
TUJ1
GFAP
TUJ1/GFAP/DAPI
E
LTR
TetO
Factor
LTR
Sox1 Sox2 Sox3 Hes1 Hes3 Hes5 mir124a rBm1 Brn2 Ascl1 Klf4 Rfx4 Zic1 DN-REST PLZF NICD Sox9 Myt1l MEF2CA Lhx2 PLAGL1 Pax6 Myc Bmi1
iNSC1
iNSC2
iNSC3
iNSC4
iNSC5
iNSC6
iNSC7
MEF
Plasmid

Figure S1. Defined growth conditions and factors induce transgene-independent iNSCs

(Related to Figure 1)

(A) Experimental strategy for inducing and selecting transgene-independent NSC-like cells from MEFs. Dox was withdrawn on day 4 of a 10 day growth period in a neural selection medium (ITSFn). See methods section for experimental details.

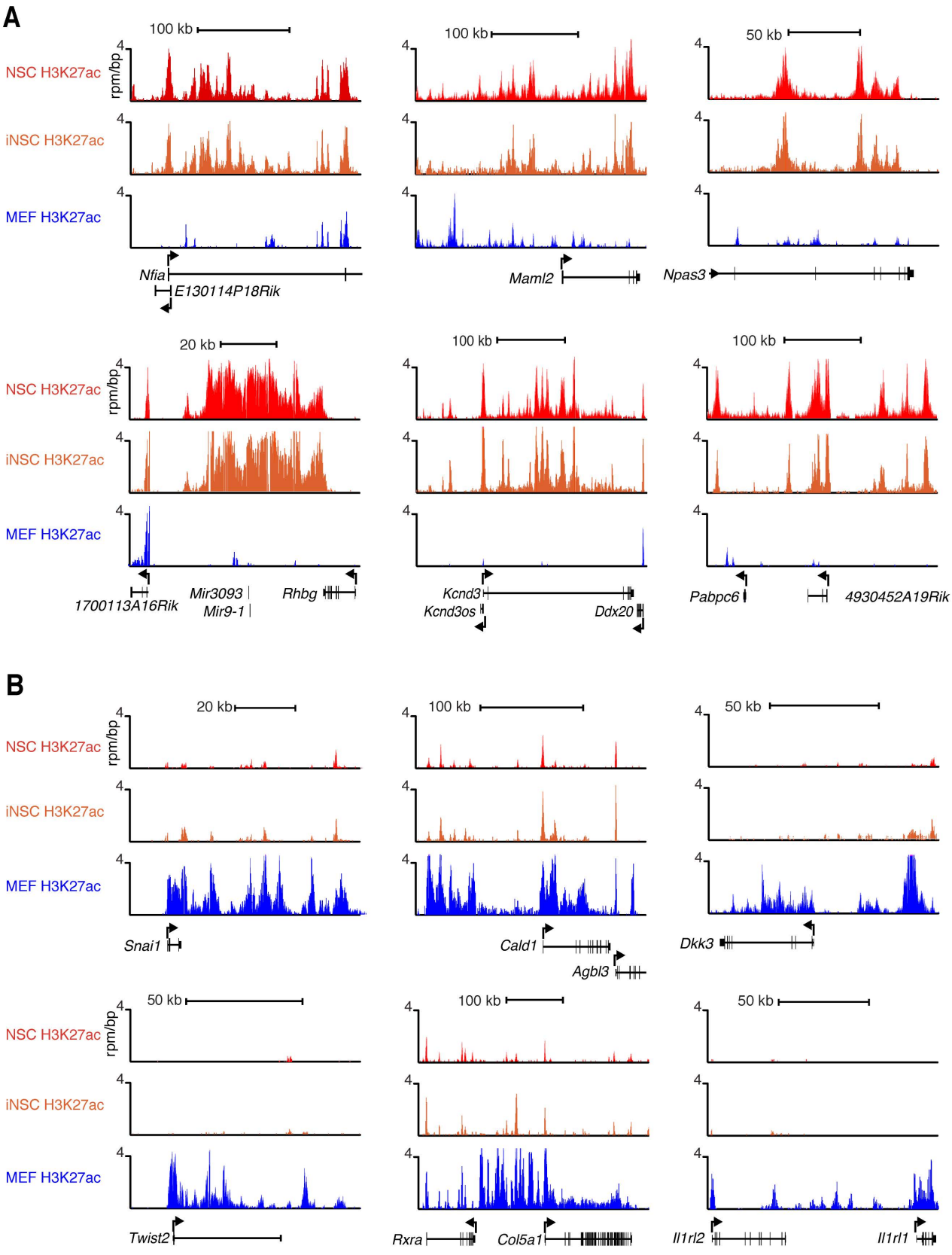
(B) Morphology and GFP fluorescence of iNSCs generated from transduced MEFs using the experimental strategy shown in part (A). Scale bar=200μm.

(C) qPCR analysis of indicated NSC and MEF transcripts in MEFs (F), primary-derived control NSCs (N), and iNSC lines induced from MEFs. Expression values were normalized to *Gapdh* expression for each cell type, and error bars represent standard deviation from the mean (n=3 technical replicates).

(D) Immunostaining of differentiation markers GFAP and TUJ1 after growth factor withdrawal in representative lines. Scale bar=100μm.

(E) Top, schematic showing the PCR strategy to detect factors transduced in the genomic DNA of iNSC lines. Primer sets either included a universal primer recognizing the TetO promoter and a factor-specific reverse primer, or two factor-specific primers separated by at least one intron in the endogenous gene. Bottom, PCR genotyping results for the transduced factors detected in iNSC lines. The asterisk denotes line iNSC5, which contained the fewest number of transduced factors.

Figure S2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100 kb
NSC H3K27ac
iNSC H3K27ac
MEF H3K27ac
rpm/bp
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Npas3
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Rhbg
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Kcnd3
Kcnd3os
Ddx20
100 kb
Pabpc6
4930452A19Rik
B
20 kb
Snai1
100 kb
Cald1
Agbl3
50 kb
Dkk3
50 kb
Twist2
100 kb
Rxra
Col5a1
50 kb
Il1rl2
Il1rl1

Figure S2. H3K27ac profiles at NSC-specific and MEF-specific loci (Related to Figure 2)

(A) H3K27ac ChIP-seq profiles of representative loci with NSC-specific enhancers depicted in Figure 2E. Shown are the profiles for neural-associated transcription factors *Nfia*, *Mastermind-like 2* (*Maml2*), and *Npas3* (top), as well as brain-specific microRNA *miR-9-1*, potassium voltage-gated channel protein gene *Kcnd3*, and the mRNA binding protein *Pacbp6*.

(B) H3K27ac ChIP-seq profiles of representative loci with MEF-specific enhancers depicted in Figure 2E. Shown are profiles for transcription factors *Snai1* and *Twist2* (left), MEF-specific *Caldesmon 1* (*Cald1*) and *Collagen 5a1* (middle), as well as the signaling molecule *Dkk3* and the cytokine receptor gene cluster *Il1lr1/Il1lr2* (right).

Figure S3

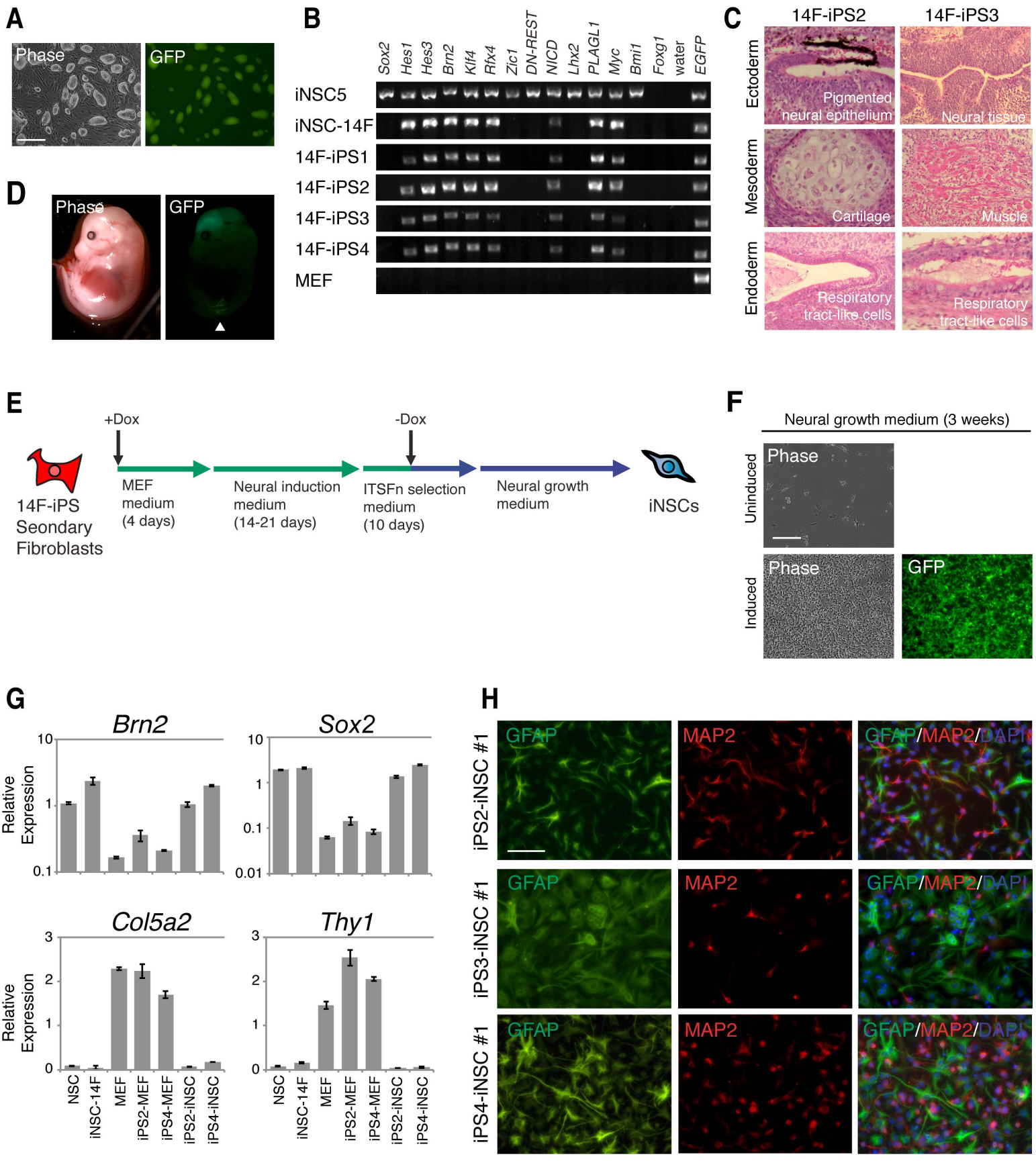
A
Phase
GFP
B
Sox2
Hes1
Hes3
Brn2
Klf4
Rfx4
Zic1
DN-REST
NICD
Lhx2
PLAGL1
Myc
Bmi1
Foxg1
water
EGFP
iNSC5
iNSC-14F
14F-iPS1
14F-iPS2
14F-iPS3
14F-iPS4
MEF
C
14F-iPS2
14F-iPS3
Ectoderm
Mesoderm
Endoderm
Pigmented neural epithelium
Neural tissue
Cartilage
Muscle
Respiratory tract-like cells
Respiratory tract-like cells
D
Phase
GFP
E
+Dox
-Dox
14F-iPS Seondary Fibroblasts
MEF medium (4 days)
Neural induction medium (14-21 days)
ITSFn selection medium (10 days)
Neural growth medium
iNSCs
F
Neural growth medium (3 weeks)
Uninduced
Induced
Phase
Phase
GFP
G
Brn2
Sox2
Col5a2
Thy1
Relative Expression
NSC
iNSC-14F
MEF
iPS2-MEF
iPS4-MEF
iPS2-iNSC
iPS4-iNSC
H
iPS2-iNSC #1
iPS3-iNSC #1
iPS4-iNSC #1
GFAP
MAP2
GFAP/MAP2/DAPI

Figure S3. Genetically homogenous system for iNSC formation

(Related to Figure 3)

(A) ES-like morphology and *Sox2*-GFP expression of iNSC-14F-derived iPS lines. Shown is cell line 14F-iPS1. Scale bar=200μm.

(B) PCR analysis to detect factors integrated in genomic DNA of iNSC-14F and 14F-derived clonal iPS lines (1-4). iNSC5 and untransduced MEFs are shown for reference.

(C) Hematoxylin and eosin staining of teratomas derived from iPS cell lines 14F-iPS2 and 14F-iPS3.

(D) E14.5 chimera derived from blastocyst injection of 14F-iPS4. The GFP fluorescence in the brain and spinal cord (arrowhead) comes from the *Sox2*-GFP reporter of 14F-iPS4 cells.

(E) Schematic for generating secondary iNSCs from 14F-iPS-derived chimera embryonic fibroblasts (Secondary Fibroblasts).

(F) Morphology and *Sox2*-GFP expression at the conclusion of the iNSC induction time course depicted in (E) for both mock-treated cells (Uninduced) and those given dox (Induced). Cells shown in the uninduced condition were non-proliferative and did not express GFP. The cultures were grown in parallel and imaged at the same time. Scale bar=200μm.

(G) qPCR analysis of NSC (*Brn2* and *Sox2*) and fibroblast (*Col5a2* and *Thy1*) marker genes in established secondary iNSC lines (iPS2-iNSC #1 and iPS4-iNSC #1), as well as their starting MEF cultures (iPS2-MEF and iPS4-MEF), a primary-derived control NSC line (NSC), iNSC-14F, and control MEFs. Expression values were normalized to *Actin* expression for each cell type, and error bars represent standard deviation from the mean (n=3 technical replicates).

(H) Immunostaining for GFAP and MAP2 expression in differentiation cultures of 3 independent secondary iNSC lines (iPS2-iNSC #1, iPS3-iNSC #1 and iPS4-iNSC #1). Each secondary iNSC line was derived from MEFs generated after chimera formation using different 14F-iPS clones (iPS2-4). The cultures were analyzed 4 days after growth factor withdrawal. Scale bar=100μm.

Figure S4

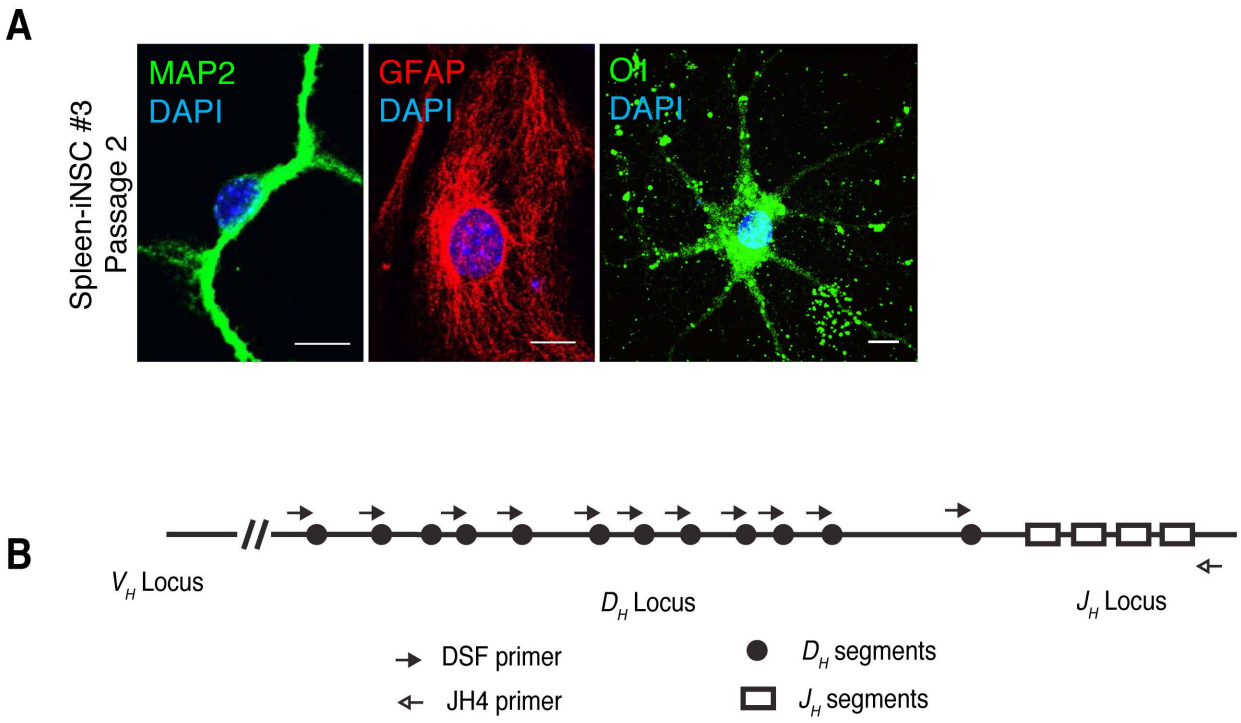
A
Spleen-iNSC #3
Passage 2
MAP2
DAPI
GFAP
DAPI
O4
DAPI
B
V_H Locus
D_H Locus
J_H Locus
DSF primer
JH4 primer
D_H segments
J_H segments
C
D-REGION
P
5'J-REGION
J name
D name
tctatgatggttactac
g
.....ttgactactgg
Musmus IGHJ2*01
Musmus IGHD2-3*01
D2-3
J2

Figure S4. Additional characterization of spleen-derived iNSCs

(Related to Figure 4)

(A) Differentiation analysis of Spleen-iNSC #3 after 10 days of differentiation. Shown are immunostaining for neurons (MAP2), astrocytes (GFAP), and oligodendrocytes (O1). Scale bar=10μm.

(B) PCR strategy for detecting D_H-J_H rearrangements. D_H-J_H rearrangements were amplified using a degenerate PCR primer (DSF) that binds to all D_H-segments and another primer that binds 3' of the J_H4 segment (JH4). The primers only generate a PCR product after genomic rearrangement.

(C) Sequencing analysis of Spleen-iNSC #3 D_H-J_H rearrangement shown in Figure 4F (top panel). The *D* and *J* segments involved in the *IgH* rearrangement are shown (top), as well as a genomic representation of the locus (bottom). Locus not drawn to scale.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Somatic cell isolation and culture

Mouse embryonic fibroblasts (MEFs) were isolated from E14.5 embryos that contained two alleles of the M2 reverse tetracycline trans-activator (M2rtTA) in the constitutively active *Rosa26* locus (*Rosa26*-rtTA +/+) (Beard et al., 2006). The embryos also harbored one allele of *Sox2*-GFP in which the endogenous *Sox2* gene was replaced by a sequence encoding eGFP (Ellis et al., 2004). After removing the head, vertebral column, and internal organs, MEFs were dissociated in 0.25% trypsin (Sigma) for 10 minutes, split onto two 15-cm plates, and grown in MEF medium [DMEM supplemented with 10% FBS (Hyclone), penicillin and streptomycin (100 μg/ml) (Life Technologies), L-glutamine (2 mM) (Life Technologies), and nonessential amino acids (Life Technologies)] until confluent and then frozen. MEFs passaged one to two times were used for transduction experiments. MEFs in the secondary iNSC reprogramming experiments were isolated from E14.5 chimeras using the same protocol as above except that chimera cells were selected in medium containing puromycin (2 μg/ml) for 4-6 days.

Adult somatic organs were isolated from 6-week-old chimeric mice as previously described (Carey et al., 2011; Carey et al., 2010). Whole bone marrow was isolated by removing the condyles from the femur and tibia and flushing the bones with DMEM containing 5% FBS (Hyclone). The bone marrow cells were then collected and plated in IMDM with 15% FBS, as well as IL-4, IL-7, SCF (all 10 μg/ml; Peprotech), and

doxycycline (2 μg/ml). Four days later, the medium was changed to neural induction medium for iNSC formation. For splenocytes, the spleen was manually dissociated in RPMI containing 15% FBS and filtered through a 100 μM filter. Cells were collected by centrifugation and plated in neural induction medium for iNSC formation. For the isolation of liver cells, the mice were first perfused with 50 ml HBSS and then with 50 ml HBSS containing collagenase type IV (100 U/ml; Sigma). The liver was dissociated in DMEM with BSA (2 g/L) and filtered two times through a 100 μM filter. Cells were collected by centrifugation at 30g for 3 minutes at 4°C, washed twice, and plated in neural induction medium.

Lentiviral cloning and infections

To create lentiviral vectors, genes of interest were amplified by PCR either from cDNA libraries or from cDNA expression vectors using primers flanked by EcoRI restriction sites (MfeI was used instead of EcoRI for genes that contained internal EcoRI restriction sites). The PCR products were cloned into the TOPO-TA cloning vector (Life Technologies) following the manufacturer's instructions. The genes were then excised from the TOPO-TA vector and ligated into the EcoRI site of the FUW-TetO lentiviral backbone in which transgene expression is controlled by the tetracycline-reponsive operator sequence and a minimal CMV promoter (TetO) (Beard et al., 2006).

Lentivirus was generated in 6-well plates by co-transfecting 293T cells with 2.5 μg of lentiviral vector, 0.625 μg of pMD2.G, and 1.875 μg psPAX2 (packaging vectors from Addgene) using Fugene 6 (Promega) according to the manufacturer's instructions.

MEF medium was replaced 16-24 hours after transfection. Viral supernatants were harvested 48 and 72 hours after transfection and filtered through a 0.45 μm filter. MEFs were transduced by adding a 1:1 mixture of viral supernatants and MEF medium to the cells in the presence of 8 μg/ml polybrene (Sigma). After 24 hours, the MEF medium was replaced and on the following day doxycycline (Sigma) was added (2 ug/ml).

Neural cell culture and differentiation

Primary-derived neural stem cells and established iNSCs were cultured in N2 medium (Okabe et al., 1996) [DMEM/F-12 medium containing insulin (5 μg/ml) (Sigma), transferrin (100 μg/ml) (Sigma), sodium selenite (30 nM) (Sigma), progesterone (20 nM) (Sigma), putrescine (100 nM) (Sigma), and penicillin and streptomycin (100 μg/ml) (Life Technologies)] supplemented with 20 ng/ml epidermal growth factor (EGF) (R & D systems), 20 ng/ml basic fibroblast growth factor (bFGF) (Sigma), and 1 μg/ml laminin (Life Technologies). NSC medium was replenished every 24-48 hours, and cells were passaged every 2-3 days.

For neuronal differentiation, cells were plated on polyornithine- and laminin-coated plates and EGF withdrawn for 2 days followed by bFGF withdrawl and the addition of BDNF (10 μg/ml; Peprotech), ascorbic acid (200 nM; Sigma), and NT3 (10 μg/ml; Peprotech) for an additional 10-14 days (Their et al., 2012). For electrophysiology experiments, the differentiation medium was supplemented with astrocyte conditioned medium (1:4) for 2 more weeks before analysis. Astrocyte differentiation was performed by withdrawing growth factors and supplementing the

medium with 5% FBS for 7-10 days (Conti et al., 2005). For oligodendrocyte differentiation 200,000 cells were plated on polyornithine- and laminin-coated 12-well plates and cultured with bFGF (10 μg/ml), PDGF (10 μg/ml; Peprotech), and forskolin (10 nM; Sigma) for 5 days and then with ascorbic acid (200 nM) and T3 (30 ng/ml; Sigma) for 4 days (Lujan et al., 2012; Glaser et al., 2007).

Control NSC derivation

The forebrain cortex of E12.5 embryos was collected in Hank's buffered saline solution (HBSS). Tissue was dissociated by tritruation and then incubated in HBSS for 10 minutes at room temperature. Cells were collected by centrifugation and plated in N2 containing EGF (20 ng/ml), bFGF (20 ng/ml), and laminin (1 μg/ml) and then cultured for stable cell lines.

iNSC reprogramming

For the generation of iNSCs, transduced MEFs were grown for 4 days in MEF medium supplemented with 2 μg/ml doxycycline (Sigma) before being switched to neural induction medium [N2 medium plus 10 ng/ml EGF, 10 ng/ml FGF (Sigma), 1 μg/ml laminin, and 2 μg/ml doxycycline]. The cells were grown in neural induction medium for 2-3 weeks before the addition of ITSFn selection medium [DMEM/F-12 medium containing insulin (25 μg/ml), transferrin (50 μg/ml), sodium selenite (30 nM), fibronectin (5 μg/ml) (Sigma), and penicillin and streptomycin (100 μg/ml)] (Okabe et al., 1996). The selection medium was supplemented with doxycycline (2 μg/ml) for the first

4 days, and on day 10 the cultures were dissociated by incubating them with 0.25% trypsin (Sigma) for 5-10 minutes. The trypsin was quenched with 10% serum (Hyclone), and the cells were collected by centrifugation and washed once with DMEM/F-12. The cells were then replated to plates coated with polyornithine (15 μg/ml) and laminin (1 μg/ml) (for 24 hours each) and then grown in neural expansion medium [N2 supplemented with 20 ng/ml EGF, 20 ng/ml bFGF, and 1 μg/ml laminin]. Medium was replenished every other day for 2-3 weeks until NSC-like foci became visible, after which cells were fed daily to promote iNSC growth. *Sox2*-GFP+ cells were sorted directly into polyornithine- and laminin-coated plates using the BD FACSAria IIU cell sorter (BD Biosciences). The cells were split at confluence for P1.

Electrophysiology

Artificial cerebro-spinal fluid (ACSF) [125 mM NaCl, 3 mM KCl, 1.25 mM NaH_2PO_4, 25 mM $NaHCO_3$, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, and 20 mM Dextrose, with osmolality adjust to 312 ± 3mOsm] was continuously bubbled with 95% O_2 and 5% CO_2 and perfused over individual coverslips during the recordings. Putative neurons within a mixed culture were visually identified for whole-cell patch clamp using differential interference contrast (DIC) optics on an Olympus BX61 microscope (Olympus Corp., USA). Patch pipettes, pulled from borosilicate capillaries (Sutter Instruments, Novato, CA, USA), were filled with a solution containing 120 mM K-Gluconate, 10 mM KCl, 10 mM HEPES, 4 mM Mg-ATP, 0.3 mM Na-GTP, 10 mM Na_2-Creatinine Phosphate, with pH 7.3 and osmolality adjusted to 300 ± 3 mOsm with Sucrose. Data was acquired

using a Multiclamp 700B (Molecular Devices, Sunnyvale, CA, USA) amplifier, low pass filtered at 10 kHz (current clamp) or 2 kHz (voltage clamp), and digitized at 10 kHz with a Digidata 1440 system (Molecular Devices). During current clamp recordings, a constant hyperpolarizing current injection was applied to hold the membrane potential at approximately -65mV, and 500ms long depolarizing current steps of varying amplitude were delivered to evoke action potentials. To detect voltage-gated ion currents, cells were continuously held at -70mV in voltage clamp mode while briefly delivering voltage pulses from -100mV to 50mV, in 10mV increments. Leak current and capacitive transients during voltage steps were subtracted from raw traces. All data were acquired and analyzed using pClamp10 software (Molecular Devices).

Immunostaining

For NSC and iNSC immunostaining, cells were washed twice with HBS, fixed in 4% paraformaldehyde for 20 minutes at room temperature, and then washed twice in PBS containing magnesium and calcium ions (PBS+). Cells were blocked by incubating them in PBS+ containing 5% normal donkey serum (Jackson Immunoresearch) and 0.3% Triton X-100 (Sigma) for 60 minutes at room temperature. Antibodies were diluted in a solution of PBS+ with 1% BSA (Sigma) and 0.3% Triton X-100. Primary antibodies were incubated overnight at 4°C, and secondary antibodies were incubated for 60 minutes at room temperature in the dark. Cells were washed 3 times with PBS+ after the primary antibody incubation and twice after secondary antibody incubation. To stain nuclei, cells were incubated with 4',6-diamidino-2-phenylindole (DAPI) for 5-10 minutes

and subsequently washed with PBS+. The following primary antibodies were used: rabbit anti-GFAP (DAKO, 1:2000), chicken anti-GFP (Aves Labs, 1:1000), mouse anti-MAP2 (Sigma, 1:1000), rat anti-MBP (Abcam, 1:500), mouse anti-NESTIN (Developmental Studies Hybridoma Bank, 1:500), mouse anti-O1 (R&D systems, 1:100), mouse anti-O4 (R&D systems, 1:100), rabbit anti-PAX6 (Covance, 1:250), mouse anti-TUJ1 (Covance, 1:1000).

iPS reprogramming

The Moloney viral vectors pMXs-*Oct4*, *Klf4*, *Myc*, *Nanog*, and *Sall4* were purchased from Addgene (Takahashi and Yamanaka, 2006). pMXs-*Esrrb* was generated by digesting TetO-*Esrrb* (Buganim et al., 2012b) with EcoRI and then ligating it into the EcoRI site of pMXs. For transductions, equal amounts of pCLeco and either *Oct4*, *Klf4*, *Myc*, *Nanog*, *Sall4*, or *Esrrb* retroviral vectors were co-transfected into 293T cells using the Fugene 6 transfection reagent (Promega). One day after transfection, the 293T culture medium was exchanged for N2 medium supplemented with 20 ng/ml EGF, 20 ng/ml bFGF, 1 μg/ml laminin, and 5 μg/ml fibronectin. Viral supernatants were collected 48 and 72 hours after transfection and pooled for infection.

The infected cells were grown in NSC medium for 4 days and then grown in ES medium [DMEM supplemented with 10% FBS (Hyclone), penicillin and streptomycin (100 μg/ml) (Life Technologies), L-glutamine (2mM) (Life Technologies), nonessential amino acids (Life Technologies), 0.1 mM β-mercaptoethanol, and leukemia inhibitory factor (Lif)]. When pre-iPS colonies began to emerge, the culture was switched to

serum-free 2i/Lif medium [1:1 mixture of DMEM/F-12 (Life Technologies) and Neurobasal (Life Technologies) base mediums plus N2 supplement (Life Technologies), B27 supplement (Life Technologies), recombinant human LIF, 2 mM L-glutamine (Life Technologies), 1% nonessential amino acids (Life Technologies), 0.1 mM β-mercaptoethanol (Sigma), penicillin and streptomycin (100 μg/ml) (Life Technologies), 5 μg/mL BSA (Sigma), 1 μM PD0325901 (Stemgent), and 3 μM CHIR99021 (Stemgent)] (Hanna et al., 2010). iPS colonies were manually picked and after 1 passage in 2i/lif medium, they were cultured in ES medium.

Teratomas and blastocyst injections

For teratoma analysis, cells were dissociated in 0.25% trypsin and then collected in ES medium. 5×10^5 cells were injected subcutaneously into both flanks of recipient immunocomprimised SCID mice (Brambrink et al., 2008). Tumors approximately 1 cm in diameter were harvested 3-4 weeks after injection for paraffin sectioning and stained with hematoxylin and eosin. Blastocyst injections were performed as described (Wernig et al., 2008b) except that E14.5 embryos were extracted from pregnant females for the isolation of MEFs.

VDJ rearrangement analysis

IgH, *Igκ*, and *Igλ* rearrangements were PCR amplified as previously described (Hanna et al., 2008; Chang et al., 1992; Cobaleda et al., 2007). To characterize individual VDJ rearrangements, PCR fragments were cloned into the TOPO vector, and

at least 8 clones of an individual PCR band were sequenced. Sequences were analyzed using the IMGT database (www.imgt.org) (Brochet et al., 2008). For D_H-J_H rearrangements, the sequencing result was appended to a known V_H segment for IMGT analysis.

Chromatin Immunoprecipitation (ChIP)

ChIPs were performed as previously described (Lee et al., 2006). Briefly, MEF, NSC, and iNSC-13F cells were grown to a final count of 20 million cells and crosslinked for 12 minutes at room temperature by the addition of one-tenth volume of 11% formaldehyde solution (11% formaldehyde, 50mM HEPES pH 7.3, 100 mM NaCl, 1 mM EDTA pH 8.0, 0.5 mM EGTA pH8.0). Cells were washed twice with PBS, scraped, and frozen in liquid nitrogen. 20 µl of Dynal magnetic beads (Sigma) were blocked with 0.5% BSA (w/v) in PBS and then bound with 5 µg of H3K27ac antibody (Abcam, ab4729) overnight at 4°C with rotation. Nuclear extracts were prepared by resuspending crosslinked cells with 50 mM HEPES pH 7.3, 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% NP-40, and 0.25% Triton X-100 and rotating them for 10 minutes at 4°C, followed by centrifugation at 1350g for 5 min. Nuclei were resuspended in sonication buffer (50 mM Tris-HCl pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% Na-deoxycholate, 0.1% SDS) and sonicated using Bioruptor® Standard (Diagenode) for 20 x 30 second pulses (30 second pause between pulses). Sonicated lysates were cleared and incubated overnight at 4°C with magnetic beads bound with antibody to enrich for DNA fragments bound by the H3K27ac mark. Beads were washed two times with sonication buffer, one time with sonication buffer with 500 mM NaCl, one time with

LiCl wash buffer (20 mM Tris pH 8.0, 1 mM EDTA, 250 mM LiCl, 0.5% NP-40, 0.5% Na-deoxycholate), and one time with TE. DNA was eluted in elution buffer. Crosslinks were reversed overnight at 65°C. RNA and protein were digested using RNase A and Proteinase K, respectively, and DNA was purified with phenol:chloroform extraction and ethanol precipitation.

Identifying ChIP-Seq enriched regions

All ChIP-seq datasets were aligned using Bowtie (version 0.12.2, Langmead et al., 2009) to the build version NCBI37/MM9 of the mouse genome with -k 1 -m 1 -n 2 setting. The MACS version 1.4.1 (Model-based analysis of ChIP-Seq) (Zhang et al., 2008) peak finding algorithm was used to identify regions of ChIP-Seq enrichment over background. A p-value threshold of enrichment of 1×10^{-9} was used for all data sets.

Defining active enhancers

Active enhancers were defined as regions of enrichment for H3K27ac outside of promoters (greater than 5 kb away from any transcriptional start site (TSS)). H3K27ac is a histone modification associated with active enhancers (Creyghton et al., 2010; Rada-Iglesias et al., 2011).

ChIP-Seq density heatmaps and composite ChIP-Seq density profiles

In order to display ChIP-Seq levels at enhancers, a heatmap representation was used. The enhancer regions of NSCs and MEFs were aligned at the center in the

composite view of signal density profile. The average ChIP-Seq read density (rpm/bp) around +/- 5 kb centered on the centers in 50 bp bin was calculated and displayed.

Microarray analysis

All expression profiles including the previous published expression datasets were processed together to generate Affymetrix MAS5-normalized probe set values. We processed all CEL files by using the probe definition ("mouse4302 cdf") and the standard MAS5 normalization technique within the affy package in R to get probe set expression values.

The expression profiles were compared and clustered by hierarchical clustering using average linkage. The distance matrix was calculated using Pearson correlation coefficients of the top 50 percent of probe sets with the largest coefficients of variation across expression profiles.

The differentially expressed probe sets between the published datasets of mouse embryonic fibroblasts (MEFs) and mouse neural precursor cells (NPCs) were determined using a linear model within the limma package in R. The empirical Bayes approach was used to estimate variances. The differentially expressed probe sets were required to have absolute value of log2-fold change greater than 2 and FDR-adjusted p-value less than 0.01.

Previously published gene expression datasets

Two previously published datasets using the Affymetrix Mouse Genome 430 2.0

Array (platform ID GPL1261) microarray platform were obtained from the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) database (see below). For Mikkelsen et al. (2007), data were obtained from the GEO database accession GSE8024. For Cahoy et al. (2008), data were obtained from the GEO database accession GSE15148.

Previously Published Datasets

Cell Type	GEO ID	Name	Reference
Neural precursor cells	GSM198065	ES derived NPC Replicate 1	Mikkelsen et al., 2007
Neural precursor cells	GSM198066	ES derived NPC Replicate 2	Mikkelsen et al., 2007
Neural precursor cells	GSM198067	ES derived NPC Replicate 3	Mikkelsen et al., 2007
Mouse Embryonic Stem Cells	GSM198062	Mouse Embryonic Stem Cells Replicate 1	Mikkelsen et al., 2007
Mouse Embryonic Stem Cells	GSM198063	Mouse Embryonic Stem Cells Replicate 2	Mikkelsen et al., 2007
Mouse Embryonic Stem Cells	GSM198064	Mouse Embryonic Stem Cells Replicate 3	Mikkelsen et al., 2007
Mouse embryonic fibroblasts	GSM198070	Mouse embryonic fibroblasts Replicate 1	Mikkelsen et al., 2007
Mouse embryonic fibroblasts	GSM198072	Mouse embryonic fibroblasts Replicate 2	Mikkelsen et al., 2007
Oligodendrocytes	GSM241889	Oligodendrocytes	Cahoy et al., 2008
Oligodendrocytes	GSM241891	Oligodendrocytes	Cahoy et al., 2008
Oligodendrocytes	GSM241892	Oligodendrocytes	Cahoy et al., 2008
Oligodendrocytes	GSM241917	Oligodendrocytes	Cahoy et al., 2008
Astrocytes	GSM241912	Astrocytes	Cahoy et al., 2008
Astrocytes	GSM241914	Astrocytes	Cahoy et al., 2008
Astrocytes	GSM241926	Astrocytes	Cahoy et al., 2008
Neurons	GSM241904	Neurons	Cahoy et al., 2008
Neurons	GSM241896	Neurons	Cahoy et al., 2008

Defined factors screened for iNSC formation in MEFs

Gene Name	Species	Genbank Accession
Ascl1	Mouse	NM_008553
Bmi1	Mouse	NM_007552
Brn2	Mouse	NM_008899
DN-REST (P73 REST)*	Human	NM_005612 (REST)
Hes1	Mouse	NM_008235
Hes3	Mouse	NM_008237
Hes5	Mouse	NM_010419
Klf4	Mouse	NM_010637
Lhx2	Mouse	NM_010710
MEF2CA**	Human	NM_002397 (MEF2C)
mir124a	Human	NR_029668
c-Myc	Mouse	NM_010849
Myt1l	Mouse	NM_001093775
Ngn2	Mouse	NM_009718
NOTCH- intracellular domain (ICD)	Human	NM_017617 (NOTCH1)
Olig1	Mouse	NM_016968
Olig2	Mouse	NM_016967
Pax6	Mouse	NM_001244198
PLAGL1	Human	NM_002656
PLZF	Human	NM_006006
Rfx4	Mouse	NM_001024918
Sox1	Mouse	NM_009233
Sox2	Mouse	NM_011443
SOX3	Human	NM_005634
Sox9	Mouse	NM_011448
Zic1	Mouse	NM_009573

* Dominant-Negative REST. Chong el al. 1995

** Constitutively active MEF2. Li et al. 2008

Primers for PCR genotyping the transduced factors

Gene Name	Forward Primer	Reverse Primer
Ascl1	ATCCACGCTGTTTTGACCTC	GTTTGCAGCGCATCAGTTC
Bmi1	GTGTACGGTGGGAGGCCTAT	TGCAACTTCTCCTCGGTCTT
Brn2	GTGTACGGTGGGAGGCCTAT	CACCCTGCTGTACCACCAC
DN-REST	GAATCTGGCTCTTCCACTGC	GAGGTTTAGGCCCATTGTGA
Foxg1	GTGTACGGTGGGAGGCCTAT	CGCCCCTTTCTTCTCCTC
Hes1	ATCCACGCTGTTTTGACCTC	GTCACCTCGTTCATGCACTC
Hes3	GTGTACGGTGGGAGGCCTAT	AGGCAAGGGTTGAGAACAGA
Hes5	GTGTACGGTGGGAGGCCTAT	CAGGAGTAGCCCTCGCTGTA
Klf4	GTGTACGGTGGGAGGCCTAT	CTAGGTCCAGGAGGTCGTTG
Lhx2	GCGAATACCCAGCACACTTT	TAAAAGGTTGCGCCTGAACT
MEF2CA	CTTATGAGCTGAGCGTGCTG	GTGAGCCAGTGGCAATAGGT
mir124a	ATCCACGCTGTTTTGACCTC	AATCAAGGTCCGCTGTGAAC
c-Myc	GTGTACGGTGGGAGGCCTAT	ACCGCAACATAGGATGGAGA
Myt1l	GTGTACGGTGGGAGGCCTAT	CCCCTTGCTCATCATTGTCT
Ngn2	ATCCACGCTGTTTTGACCTC	GTCTTCTTGATGCGCTGCAC
NOTCH ICD	CATGGACGACAACCAGAATG	CATGTTGTCCTGGATGTTGG
Olig1	ATCCACGCTGTTTTGACCTC	GTGGCAATCTTGGAGAGCTT
Olig2	ATCCACGCTGTTTTGACCTC	GGGCTCAGTCATCTGCTTCT
PLAGL1	CAAGTGTGTGCAGCCTGACT	ATCTCTGGGCACAGAACTGG
PLZF	GTGTACGGTGGGAGGCCTAT	CTGGATGGTCTCCAGCATCT
Rfx4	GTTACTGGAGGAACCCGACA	GAATATGCCACCGTCTGCTT
Sox1	GTGTACGGTGGGAGGCCTAT	GTCCTTCTTGAGCAGCGTCT
Sox2	GTGTACGGTGGGAGGCCTAT	CTCCGGGAAGCGTGTACTTA
SOX3	GTGTACGGTGGGAGGCCTAT	CTGCGTTCGCACTACTCTTG
Sox9	AGGAAGCTGGCAGACCAGTA	CCCTCTCGCTTCAGATCAAC
Zic1	TCTGCTTCTGGGAGGAGTGT	CTGTTGTGGGAGACACGATG

Primers used for qPCR analysis

Gene	Forward Primer	Reverse Primer
AAT	GGCTGACCTCTCCGGAAT	GTCAGCACGGCCTTATGC
Actin	CTTCTTTGCAGCTCCTTCGTT	TTCTGACCCATTCCCACCA
Albumin	AGTGTTGTGCAGAGGCTGAC	TTCTCCTTCACACCATCAAGC
Brn2	AGCAGTTCGCCAAGCAATTC	CGAGAACACGTTGCCGTACA
Col5a2	TAGAGGAAGAAAGGGACAAAAAGG	GTTACAACAGGCACTAATCCTGGTT
Gapdh	TTCACCACCATGGAGAAGGC	CCCTTTTGGCTCCACCCT
Ncan	GGACAGACAACACAGGACTGC	CCCACCTGCGAAGAAATTAT
Nestin	GATCGCTCAGATCCTGGAAG	CCAAGAGAAGCCTGGGAACT
Olig2	AGACCGAGCCAACACCAG	AAGCTCTCGAATGATCCTTCTTT
Pax5	CGCGTGTTTGAGAGACAGCACTACT	GTCTCGGCCTGTGACAATAGGGTAG
Pax6	GAAGGAGGGGGAGAGAACAC	CTCCAGAGCCTCAATCTGCT
Pu.1	CGGATGACTTGGTTACTTACG	GTAGGAAACCTGGTGACTGAG
Sox1	TGTAATCCGGGTGTTCCTTC	AACCCCAAGATGCACAACTC
Sox2	ACTTTTGTCCGAGACCGAGA	CTCCGGGAAGCGTGTACTTA
Sox3	GCCTTGTACCGAAGATGAGG	ACAAAACCCCGACAGTTACG
Thy1	CGAATCCCATGAGCTCCAAT	CCAGCTTGTCTCTATACACACTGATA

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