Supporting Information

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SI Materials and Methods

CD133$^+$ Cell Transduction. The cord blood (CB) CD133$^+$ cells were infected by retroviral vector and cultured as previously described (1, 2).

For the lentiviral vector production, mouse cDNA *cMyc* was cloned into a lentiviral vector expressing internal ribosomal entry site/enhanced GFP under the control of tetracycline operator and minimal CMV promoter. FU-tet-*o*-hSox2 lentiviral vector was provided by Addgene (no. 19779).

Replication-incompetent lentiviral particles were packaged in 293T cells expressing the lentivirus packaging vectors pMDL, pVSVG, and pREV.

CD133$^+$ cells were infected by spinoculation (1 h; 2,000 × *g* at 32 °C) with concentrated lentivirus particles and Polybrene (8 mg/mL) over RetroNectin-coated plates and in the presence of SCF, Flt3-ligand, TPO, and IL-6 cytokines. After the infection viral medium was removed and replaced with DMEM plus 10% FBS and in the presence of cytokines (SCF, TPO, Flt3, and IL-6). After 24 h, CD133$^+$ cells were transferred to six-well plates in the presence of human foreskin fibroblast (HFF)-1, human ES (hES) medium, 20 ng/mL bFGF, and doxycycline (1 μg/mL).

Purification of Total RNA and Quantitative Real-Time PCR. Isolation of total RNA from CB CD133$^+$ stem cells, CB-induced neuronal-like cells (CB-iNCs), CB-derived mature neurons, and positive controls [hES-derived neural progenitor cells (HUES6-NPCs) and hES-derived neurons (HUES6-derived neurons)] (3) was performed with an RNeasy Mini Kit (Qiagen) or RNAqueous-Micro kit (Ambion) based on the cell number available. To obtain CB-derived mature neurons, CB-iNCs were dissociated by trypsin and plated directly on polyornithine/laminin-coated plates in the presence of DMEM/F12, 1× N2, 1× B27 supplements, retinoic acid (1 μM), 20 ng/mL brain-derived neurotrophic factor (BDNF; Peprotech), 20 ng/mL glial cell line-derived neurotrophic factor (GDNF; Peprotech), 200 nM ascorbic acid (Sigma), 1 mM dibutyryl-cAMP Lam (1 μg/mL), and 0.5% FBS for 4 wk.

All RNA samples were treated with TURBO DNase inhibitor (Ambion) to remove any residual genomic DNA, and 1 μg of RNA was used to synthesize cDNA by using the SuperScript II Reverse Transcriptase kit (Invitrogen). A total of 25 ng of cDNA was used to quantify gene expression by quantitative real-time PCR (qRT-PCR) using the following primers:

Tbr-1, forward, 5′-CAA CTC AGT CAA CAG GAA GGC-3′; reverse, 5′-AAA GAT GAT CTC CAG CAC AGC-3′; Gfap, forward, 5′-CCGACAGCAGGTCCATGT-3′; reverse, 5′-GTTGCTGGACGCCATTG-3′; Map2 , forward, 5′-TTGGTGCCGAGTGAGAAGA-3′; reverse, 5′-GTCTGGCAGTGGTTGGTTAA-3′; Pax6, forward, 5′-AATCAGAGAAGACAGGCCA-3′; reverse, 5′-GTGTAGGTATCATAACTC-3′; Olig2, forward, 5′-CAG AAG CGC TGA TGG TCA T-3′; reverse, 5′-CG GCA GTT TTG GGT TAT TC-3′; Otx2, forward, 5′-GAC CAC TTC GGG TAT GGA CT-3′; reverse, 5′-TGG ACA AGG GAT CTG ACA GT-3′; NeuroD1, forward, 5′-AGCCCCAAGGTCCTCCAA-3′; reverse, 5′-CGTGCTCCTCGTCCTGAGA-3′; doublecortin (Dcx), forward, 5′-GCCAGGGAGAACAAGGACTTT-3′; reverse, 5′-CACCCCACTGCGGATGA-3′; Nestin, forward, 5′-GGGAAGAGGTGATGGAACCA-3′; reverse, 5′-AAGCCCTGAACCCTCTTTGC-3′; Nkx2.2, forward, 5′-GGGACGCCGGCAAGA-3′; reverse, 5′-TAGGTCTGCGCCTTGGAGAA-3′; Sox1, forward, 5′-TCCCCCGCGTGAACTG-3′; reverse, 5′-CAAGGCATTTTGCGTTCACA-3′; Mash1, forward, 5′-CTGGACTTTACCAACTGGTTCTGA-3′; reverse, 5′-CCTGCTTCCAAAGTCCATTCC-3′; Ngn1, forward, 5′-CCAGCCACCACTTCAGTGTGATTT-3′; reverse, 5′-TATTGTCAGCCGGCTCAAACCGAA-3′; Synp1, forward, 5′-GGAAGGGATCACATCATTGAGG-3′; reverse, 5′-TGTTTGTCTTCATCCTGGT-3′; NeuN, forward, 5′-CCAAGCGGCTACACGTCT-3′; reverse, 5′-GCTCGGTCAGCATCTGAG-3′; Musashi1, forward, 5′-GAGACTGACGCGCCCCAGCC-3′; reverse, 5′-CGCCTGGTCCATGAAAGTGACG-3′; Sox10, forward, 5′-AGTACCCGCACCTGCACA-3′; reverse, 5′-GAAGGGGCGCTTGTCACT-3′; Snail, forward, 5′-ATCGGAAGCCTAACTACAGC-3′; reverse, 5′-CAGAGTCCCAGATGAGCATT-3′; Pax3, forward, 5′-GCCAATCAACTGATGGCTTT-3′; reverse, 5′-GGTTGGAAGGAATCGTGCTT-3′; p75, forward, 5′-TCGGAGTCCCCGAAGGA-3′; reverse, 5′-TGACCGAAAGGCACATTCC-3′; Slug, forward, 5′-CTGCGGCAAGGCGTTT-3′; reverse, 5′-CGTGTGAGTTCTAATGTGTCCTTGA-3′; and VGlut1, forward, 5′-AGCCGCCGCATCATGT-3′; reverse, 5′-GCCTCCGCAGTTCATCAACT-3′.

GeneChip Microarray Analysis. The GeneChip microarray processing was performed by the core facility Microarray Analysis Service from Institut de Recerca Hospital del Mar (Barcelona, Spain). Amplification, labeling, and hybridization were performed according to the Ambion and Affymetrix protocols. Briefly, 200 ng of total RNA were amplified by using the Ambion WT Expression Kit (Ambion/Applied Biosystems), labeled by using the WT Terminal Labeling Kit (Affymetrix), and then hybridized to Human Gene 1.0 ST Array in a GeneChip Hybridization Oven 640.

Washing and scanning were performed by using the Hybridization Wash and Stain Kit and the GeneChip System of (Affymetrix; GeneChip Fluidics Station 450 and GeneChip Scanner 3000 7G). The data extraction was done by Command Console software.

The raw CEL files were normalized with the RMA algorithm using ArrayStar4. We considered a probe set as differently expressed between samples if the fold change was at least two with a *P* value lower than 0.05. The hierarchical clustering of samples was performed by using correlation metric and the average linkage method with Cluster software, as visualized by TreeView software.

ChIP Assays. ChIP assays were carried out according to the Millipore protocol. Briefly, 0.5 × 10^6 CB-iNCs were used for each immunoprecipitation. Cells were fixed by using 1% formaldehyde, collected, resuspended in ChIP lysis buffer (1% SDS, 10 mM EDTA, and 50 mM Tris-HCl, pH 8.1), and sonicated by using a Branson Digital Sonifier to generate fragments of 150 to 500 bp.

Soluble chromatin was diluted 10-fold in ChIP dilution buffer (16.7 mM Tris-HCl, pH 8.1, 167 mM NaCl, 1.2 mM EDTA, 1.1% Triton X-100, 0.01% SDS) and incubated with Dynabeads Protein A (Invitrogen) coupled to the specific antibody. After incubation, the immunocomplexes were washed sequentially with low-salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, and 150 mM NaCl), high-salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, and 500 mM NaCl), LiCl wash buffer (0.25 M LiCl, 1% Nonidet P-40, 1% deoxycholate, 1 mM EDTA, and 10 mM Tris-HCl, pH 8.1), and Tris-EDTA buffer (10 mM Tris-Cl, pH 7.5, and 1 mM EDTA). Immunocomplexes were eluted in ChIP elution buffer

(1% SDS and 0.1 M $NaHCO_3$), and the crosslinking was reverted overnight at 65 °C. Samples were treated with proteinase K and RNase A, extracted with phenol/chloroform, and precipitated with ethanol. Purified chromatin was quantified by quantitative PCR using SYBR green on an ABI Prism 7300 system. Values obtained from immunoprecipitated samples were normalized to the input. The antibodies used were rabbit-IgGs (sc-2027; Santa Cruz Biotechnology) and rabbit anti-SOX2 (ab59776; Abcam). A total of 5 μg of antibody was used for the ChIP experiments. The sequences of oligonucleotides used for ChIP coupled to quantitative PCR are available on request.

Southern Blot. Genomic DNA from each cell line was isolated by using a DNeasy Blood and Tissue Kit (Qiagen) per the manufacturer's guidelines. Each lane of the Southern blot corresponds to 5 μg of genomic DNA digested with 30 U of PstI or HindIII restriction enzyme (New England Biolabs) in the presence of BSA overnight at 37 °C, electrophoresed on a 1% agarose gel, transferred to a neutral nylon membrane (Hybond-N; Amersham), and hybridized with DIG-dUTP–labeled probes generated by PCR using the PCR DIG Probe Synthesis Kit (Roche Diagnostics). Probes were detected by an alkaline phosphatase (ALP)-conjugated DIG antibody (Roche Diagnostics) using CDP-Star (Sigma-Aldrich) as a substrate for chemiluminescence. Conditions were per the instructions of the manufacturer. The probes were generated using hSox2, and h_c-Myc cDNAs as templates with the following primers:

Sox2, forward, 5′-AGTACAACTCCATGACCAGC-3′; reverse, 5′-TCACATGTGTGAGAGGGGC-3′; and c-Myc, forward, 5′-TCCACTCGGAAGGACTATCC-3′; reverse, 5′-TTACGCACAAGAGTTCCGTAG-3.

Immunofluorescence Analysis and Alkaline Phosphatase (AP) Analysis. CB-iNCs were grown on plastic cover-slide chambers in the presence of HFF-1 feeder for 1 wk in the presence of hES medium and fixed with 4% (wt/vol) paraformaldehyde. Direct AP activity was analyzed by using an AP Blue/Red Membrane substrate solution kit (Sigma) according to the manufacturer's guidelines.

The following antibodies were used: TUJ-1 (1:500; Covance), α-fetoprotein (1:400; Dako), α-actin (1:100; Sigma), OCT4 (1:100; Santa Cruz Biotechnology), NANOG (1:100; Everest Biotech), smooth muscle actin (1:400; Sigma), FOXA2 (1:50; R&D Systems), GFAP (1:1,000; Dako), MAP2 (1:100; Sigma), PAX6 (1:100; Dako), neurofilament-200 (1:80; Sigma), NESTIN (1:200; Neuromics), SOX1 (1:200; Chemicon), Ki67 (1:25; Thermo Scientific), SYN1 (1:400; Calbiochem), MUSASHI1 (1:500; Millipore), MASH1 (1:200; Abcam), GABA (1:100; Sigma), A2B5 (1:25; R&D Systems), S100b (1:100; Abcam), p75 (1:100; Alomone), PAX3 (1:500; Calbiochem), SOX10 (1:40; Santa Cruz Biotechnology), and BrdU (1:10; Roche). Images were taken by using a Leica SP5 confocal microscope.

To count the percentage of TUJ-1– and PAX6-positive cells within the CB-iNC colonies, 10 images were taken randomly for each slide by a Leica SPE confocal microscope with a 40× magnification objective. The area of each image was 275 × 275 μm. Manually Count Objects software from MetaMorph (Molecular Devices) was used to count the total number of nuclei (i.e., DAPI), TUJ-1–positive, and PAX6-positive cells. The percentage of positive cells was calculated by dividing the number of positive cells by the number of total cells counted in the investigated area.

Teratoma Assay. SCID beige mice (Charles River Laboratories) were anesthetized, and ~0.5×10^6 CB-iNCs and CB-induced pluripotent stem cell cells, resuspended in 20 to 40 μL of hES media, were injected into the testis. Mice were euthanized 6 to 8 wk after cell injection. Testis and tumors were processed and analyzed following conventional immunohistochemistry protocols (Masson trichromic stain). Anti-human nuclei (1:50; clone 235–1; Millipore) was used to detect human cells into mouse testis and detected by DAB (K4061; Dako).

Karyotyping and DNA Fingerprinting. Standard G-banding chromosome and DNA fingerprinting analysis was performed by Prenatal Genetics and Banc de Sang i Teixits (Barcelona, Spain), respectively.

Calcium Imaging. Neuronal networks derived from CB-iNCs were previously infected with the lentiviral vector carrying the Syn::DsRed reporter construct (obtained by cloning the Synapsin-1 promoter in a lentivirus backbone) (3). Cell cultures were washed twice with sterile Krebs Hepes buffer (KHB; 10 mM Hepes, 4.2 mM $NaHCO_3$, 10 mM dextrose, 1.18 mM $MgSO_4 \cdot 2H_2O$, 1.18 mM KH_2PO_4, 4.69 mM KCl, 118 mM NaCl, 1.29 mM $CaCl_2$; pH 7.3) and incubated with 2 to 5 μM Fluo-4AM (Molecular Probes/Invitrogen) in KHB for 40 min at room temperature. Excess dye was removed by washing twice with KHB, and an additional 20-min incubation was done to equilibrate intracellular dye concentration and allow deesterification. Time-lapse image sequences (100× magnification) of 5,000 frames were acquired at 28 Hz with a region of 336 × 256 pixels by using an ORCA-ER digital camera (Hamamatsu Photonics) with a 488-nm (FITC) filter on an IX81 inverted fluorescence confocal microscope (Olympus). Images were acquired with MetaMorph 7.7 (MDS Analytical Technologies). Images were subsequently processed by using ImageJ (National Institutes of Health) and custom-written routines in Matlab 7.2 (MathWorks).

Quantification of Calcium Transients. For quantification of calcium transients, ImageJ, a National Institutes of Health-funded, open-source, Java-based morphometric application, was used to allow manual selection of individual neurons on the Syn::DsRed image that correspond to each calcium movie using circular regions of interest (ROI) of four pixels (~5μm) in diameter. Each cell was considered as an individual ROI, and the average fluorescence intensity was calculated for each ROI through the entire acquired image sequence. Quantitative signal analysis and processing were done with custom-written Matlab routines. Individual temporal fluorescence intensity signals indicative of intracellular calcium fluctuations were filtered by using power spectrum calculated from Fourier transforms to reduce noise. Amplitude of signals was presented as relative fluorescence changes after background subtraction. A first-derivative filter was used to identify regions of increase in calcium signal, and a calcium event was identified by a positive derivative value of more than two SDs above background and with a rise phase that persisted a minimum of five consecutive frames (~70 ms). To assess changes in calcium signaling in response to perturbation of neuronal activity, 1 μM TTX or the glutamate receptor antagonists 6-cyano-7-nitroquinoxaline-2,3-dione (10 μM) and (2R)-amino-5-phosphonovaleric acid [or (2R)-amino-5-phosphonopentanoate; 20 μM] were applied by bath application.

In Vivo Assay. For the engrafting assay, CB-iNCs were dissociated by trypsin and plated directly on polyornithine/laminin-coated plates in the presence of DMEM/F12 plus N2 and B27 supplements, retinoic acid (1 μM), BDNF, GDNF (both at 20 ng/mL), Lam (1 μg/mL), and 0.5% FBS for 4 wk. Five days before engrafting, the cells were infected with lentiviral vector expressing EGFP. On the day of in vivo injection, CB-derived mature neurons were dissociated with Accutase and resuspended in PBS solution with glucose plus Rho kinase inhibitor, BDNF, and GDNF (50,000 cells/μL). Postnatal day 14 mouse pups were anesthetized with ketamine/xylazine (100 mg/kg and 10 mg/kg, respectively). For transplantation, 1 μL of cell suspension (~50,000 cells) was delivered to the dentate gyrus of the mouse

hippocampus in the right hemisphere through stereotaxic surgery. The injection site was determined based on the difference between bregma and lambda (d), using the position of the bregma as reference: anterior/posterior, $-(1/2) \times d$ mm; lateral, −1.6 mm (if $d < 1.6$) or −1.7 mm; and ventral, −1.9 mm (from dura). CB $CD133^+$ cells infected with Lenti-GFP were engrafted as negative controls.

In Vivo Assay Sample Preparation for Morphological Analysis and Immunohistochemistry. Animals 2 wk, 1 mo, or 3 mo after transplantation were anesthetized with ketamine/xylazine (100 mg/kg and 10 mg/kg, respectively) and perfused transcardially with 0.9% saline solution followed by 4% (wt/vol) paraformaldehyde. The brain samples were postfixed with 4% (wt/vol) paraformaldehyde and equilibrated in 30% (wt/vol) sucrose. Coronal sections of 40 μm were prepared with a sliding microtome. Brain sections of one-in-four series were selected for immunostaining. The following antibodies were used: chicken anti-GFP (gift from Aves Lab), mouse anti–neuronal-specific nuclear protein (NeuN; Chemicon), rabbit anti-Tuj-1 (Chemicon), FITC-conjugated donkey anti-chicken, cyanine 3-conjugated donkey anti-mouse antibodies, and cyanine 5-conjugated donkey anti-rabbit antibodies (Jackson ImmunoResearch). DAPI was used to reveal nuclei.

Ex Vivo Mouse Brain Slice Preparation. Transplanted mice were deeply anesthetized with isoflurane (Butler Schlein) and immediately decapitated. Brains were removed, and coronal slices (200 μm thick) were cut from brain tissue around the transplantation site in the hippocampus. Brain removal and slicing were performed at 4 °C in oxygenated cutting solution adapted to preserve the neural tissue during slicing. The cutting solution contained 110 mM choline chloride, 25 mM $NaHCO_3$, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 25 mM D-glucose, 7 mM $MgSO_4$, 0.5 mM $CaCl_2$, 3.1 mM sodium pyruvate, and 11.6 mM sodium ascorbate bubbled with a mixture of CO_2 (5%) and O_2 (95%). After cutting, the slices were transferred into bubbled artificial cerebrospinal fluid maintained at 35 °C for 20 min, and then kept at 25 °C before recordings were taken. The artificial cerebrospinal fluid contained 124 mM NaCl, 3 mM KCl, 1.3 mM $MgSO_4$, 26 mM $NaHCO_3$, 1.25 mM $NaHPO_4$, 20 mM glucose, and 2 mM $CaCl_2$ (all chemicals from Sigma).

1. Giorgetti A, et al. (2009) Generation of induced pluripotent stem cells from human cord blood using OCT4 and SOX2. *Cell Stem Cell* 5:353–357.
2. Giorgetti A, et al. (2010) Generation of induced pluripotent stem cells from human cord blood cells with only two factors: Oct4 and Sox2. *Nat Protoc* 5:811–820.
3. Marchetto MC, et al. (2010) A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. *Cell* 143:527–539.

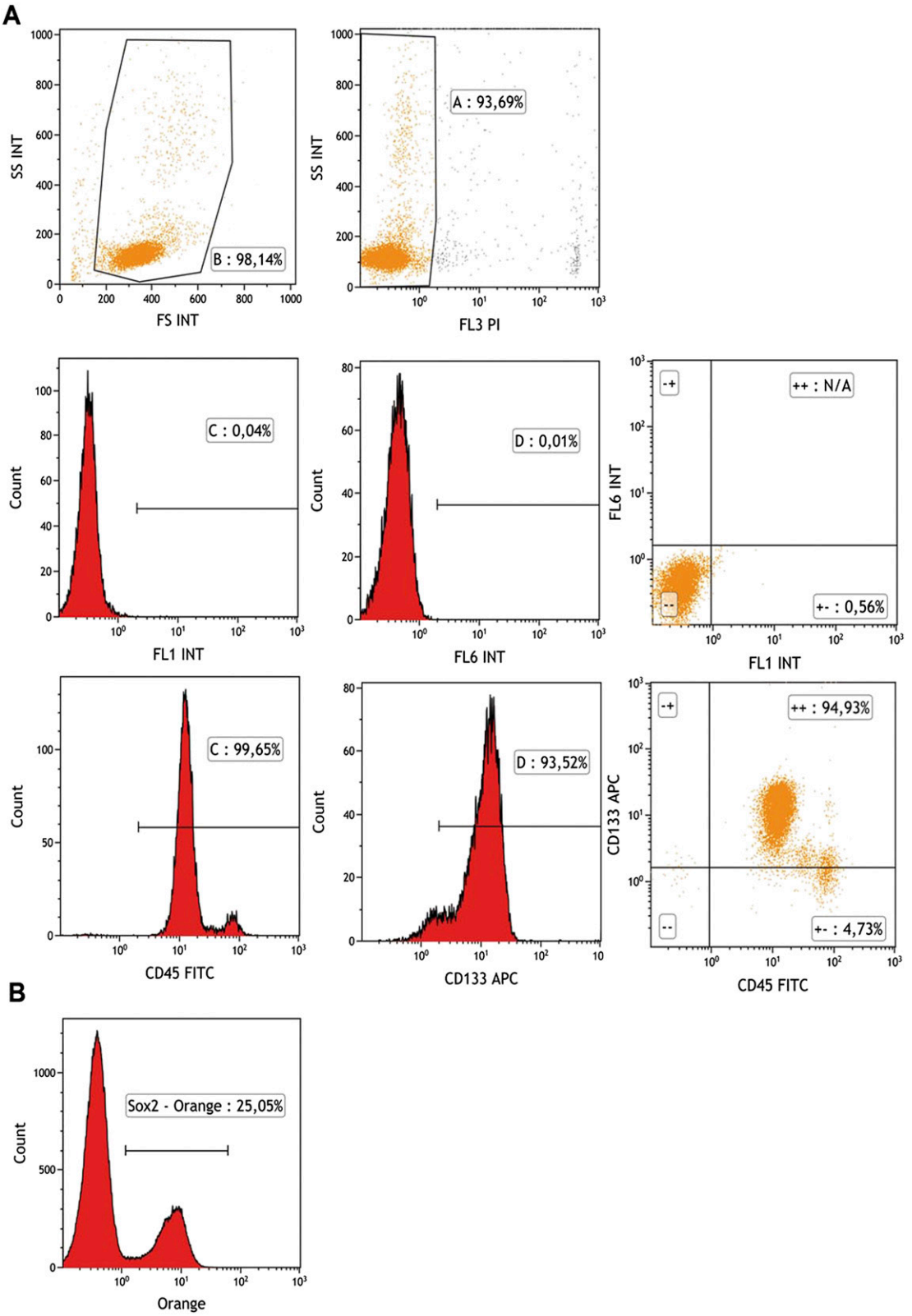

Fig. S1. Representative dot plot for CD133 cell purity. (*A*) Representative quantification of total CD133$^+$ cells and double-positive CD133$^+$/CD45$^+$ cells, after immunoselection, by cytofluorimetric analysis. The cells showed 93.69% viability and were 94.93% double-positive for CD133/CD45. (*B*) Representative histogram for the quantification of CD133-*SOX2*-orange–positive cells after retroviral infection.

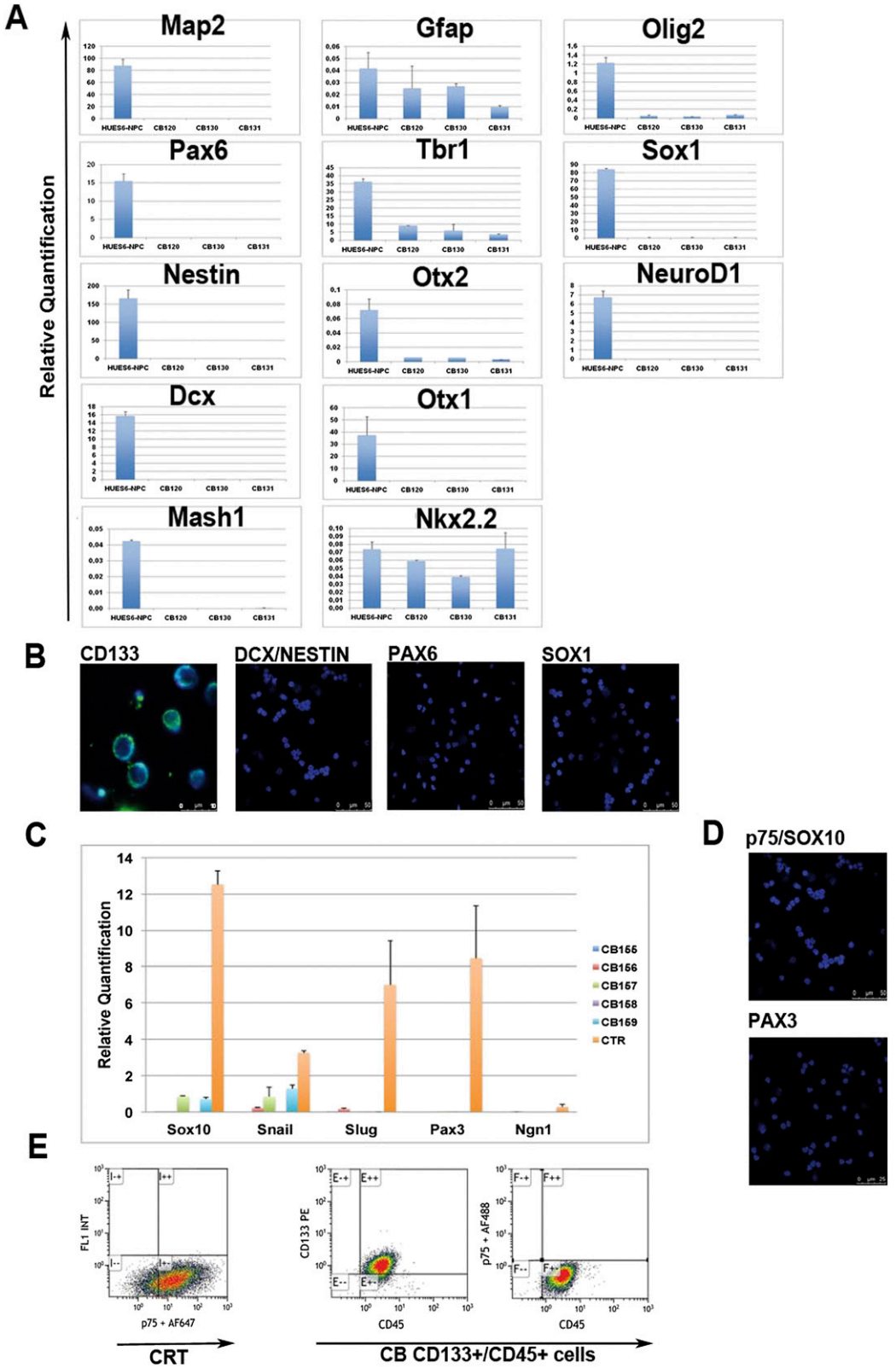

Fig. S2. CB CD133$^+$ cell characterization. (*A*) qRT-PCR analysis for Map2, Pax6, Tbr1, Sox1, Nestin, Otx2, Otx1, NeuroD1, Dcx, Mash-1, Nkx2.2, Olig2, and Gfap in CD133$^+$ cells, isolated from three independent CB units. HUES6-NPCs were analyzed, together with CB CD133$^+$, as positive control. Error bars indicate the SD generated from triplicate experiments. (*B*) Representative images of CB CD133$^+$ cells negative for early neural markers DCX, NESTIN, PAX6, and SOX1 of CD133$^+$ cells at protein level (among approximately 500,000 cells screened): DAPI (blue); CD133 (green) (Scale bar: 10 μm.); DCX, PAX6, and SOX1 (red); and NESTIN (green). (Scale bars, 50 μm.) (*C*) qRT-PCR analysis for neural crest markers Sox10, Snail, Slug, Pax3, and Ngn1. Human total brain RNA (YORBIO) or HUES-NPC RNA were used as positive controls. Error bars indicate the SD generated from triplicate experiments. (*D*) Representative imagine of CD133$^+$ cells negative for NC markers p75 (green), SOX10 (red), and PAX3 (red) at protein level (among 500,000 cells screened). (Scale bar: *Upper*, 50 μm; *Lower*, 25 μm.). (*E*) Cytofluorimetric analysis shows absence of p75-positive cells within CD133$^+$/CD45$^+$ population. Mouse brain (embryonic day 13.5) was used as positive control for p75 antibody.

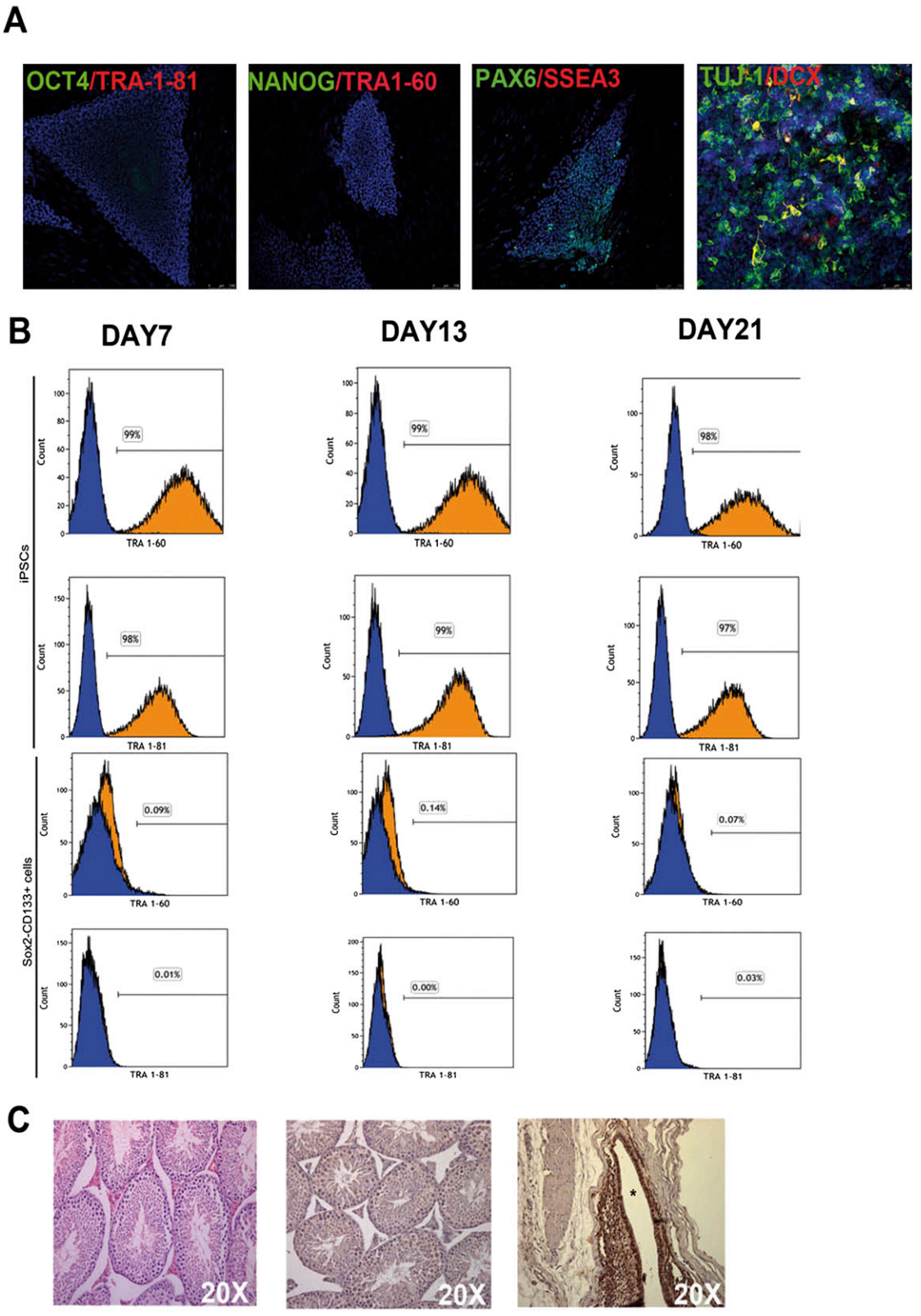

Fig. S3. Sox2 transduced CB cells bypass the pluripotent state. (*A*) CB-iNCs at passage 0 do not express pluripotency markers, whereas the colonies comprise positive cells for PAX6, TUJ-1, and DCX. Neuronal cells at passage 0 show immature morphology. (*B*) Flow cytometry analysis of transduced $CD133^{+}$ stem cells for the embryonic stem cell markers TRA-1–60 (orange) and TRA1-81 (orange), at different time points (days 7, 13, and 17) during the reprogramming process. Matched isotype controls are shown in blue. CB-induced pluripotent stem cell lines were used as technical positive control to test the functionality of antibodies. (*C*) CB-iNCs upon injection into SCID mice did not form teratoma. After 8 wk postinjection, testis tissue showed a normal structure. Any evidence of human cells was found after HuNu antibody staining. As a positive control, a teratoma generated by using human induced pluripotent stem cells is shown (asterisk).

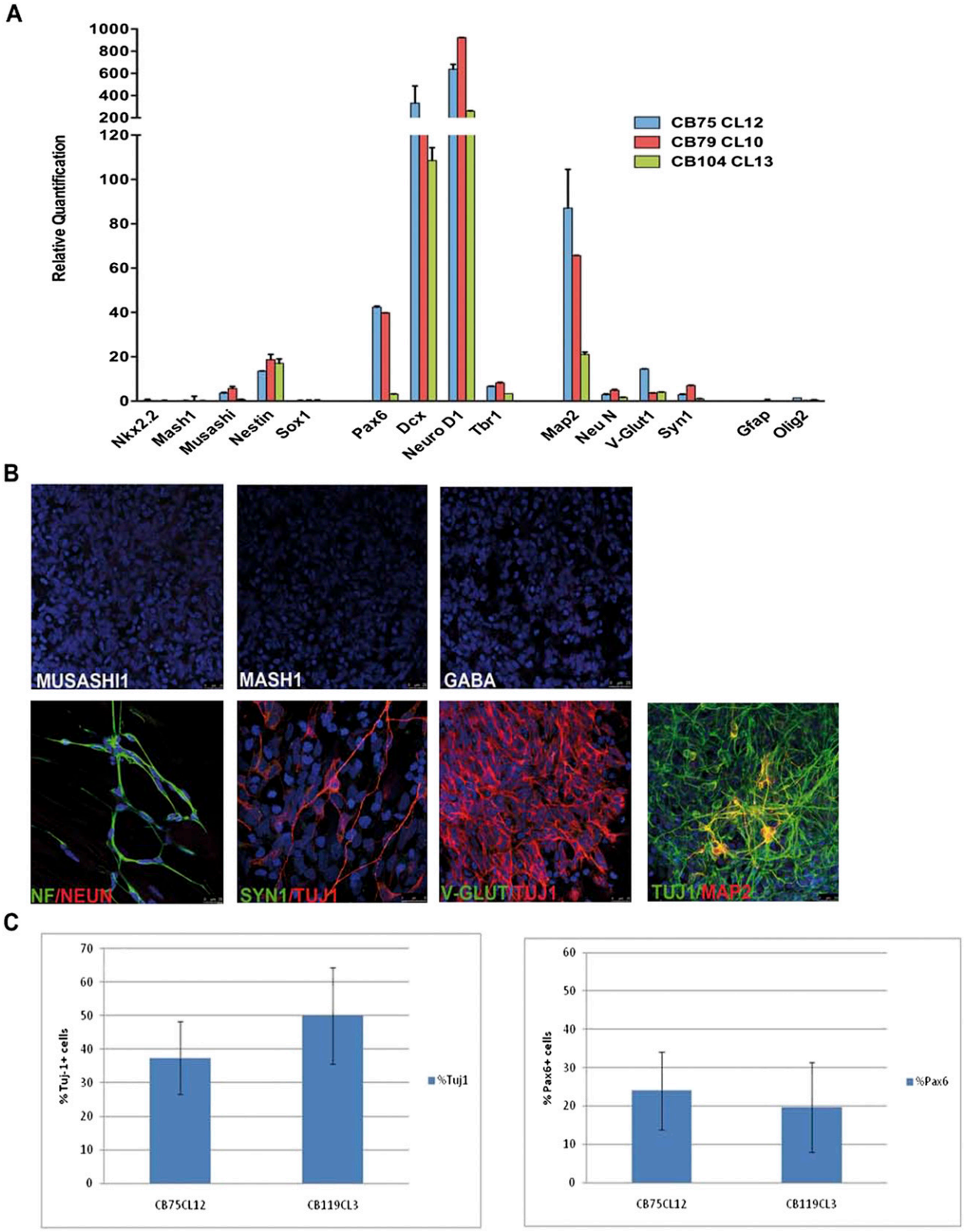

Fig. S4. CB-iNCs comprise a heterogeneous population of cells at different stages of neuronal specification. (*A*) Gene expression characterization of CB-iNCs cultured in the presence of bFGF by qRT-PCR analysis. Human total brain RNA (*YORBIO*) and HUES-NPCs RNA were used as positive control. Error bars indicate the SD generated from triplicate experiments. (*B*) Representative images of immunofluorescence characterization of CB-iNCs cultured in the presence of bFGF. (Scale bar, 25 μm). (*C*) Quantification of percentage of TUJ-1– and PAX6-positive cells within CB-iNC colonies selected randomly.

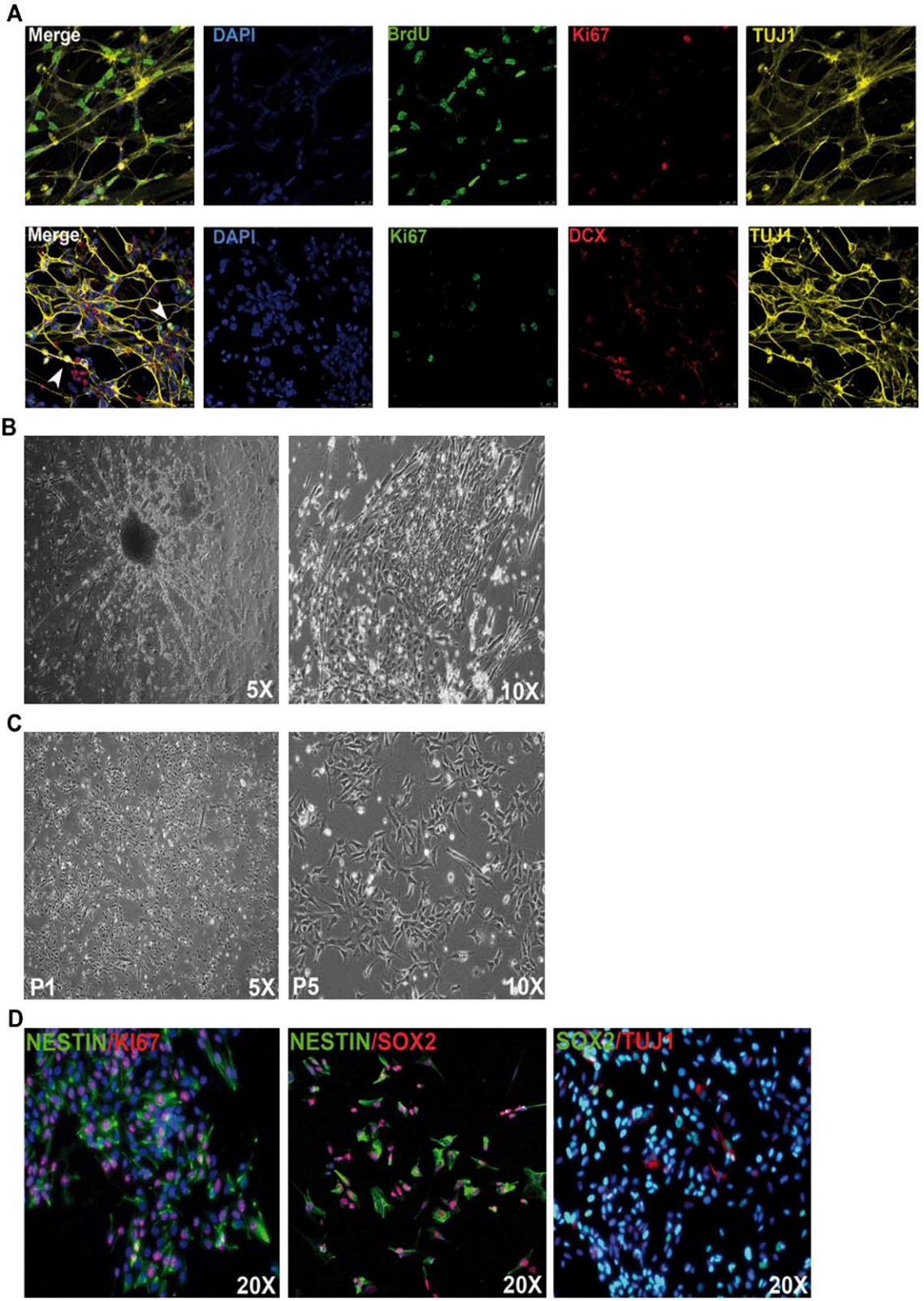

Fig. S5. CB-iNCs containing a small proliferative neuronal progenitor-like population. (*A*) Representative images show the presence of BrdU/TUJ-1- and Ki67/TUJ-1-positive cells within CB-iNCs when cultured in the presence of bFGF. (Scale bar, 25 μm.) White arrows indicate cells in mitosis. (Scale bar: 25 μm.) (*B*) Representative images of clonal 3D colony derived from CB-iNCs. CB-iNCs were dissociated into single cells and seeded on HFFs in the presence of bFGF. Adherent neuroepithelium-like colonies were also detected. (*C*) Phase-contrast images of clonal neuronal-like progenitor cells at passages 1 and 5 cultured on Matrigel and in the presence of bFGF. Culture conditions are described in the text. (*D*) Immunostaining of proliferating clonal population of neuronal-like cells at passage 5. The cells express homogenously neural progenitor markers such as NESTIN and SOX2 and were negative for TUJ-1.

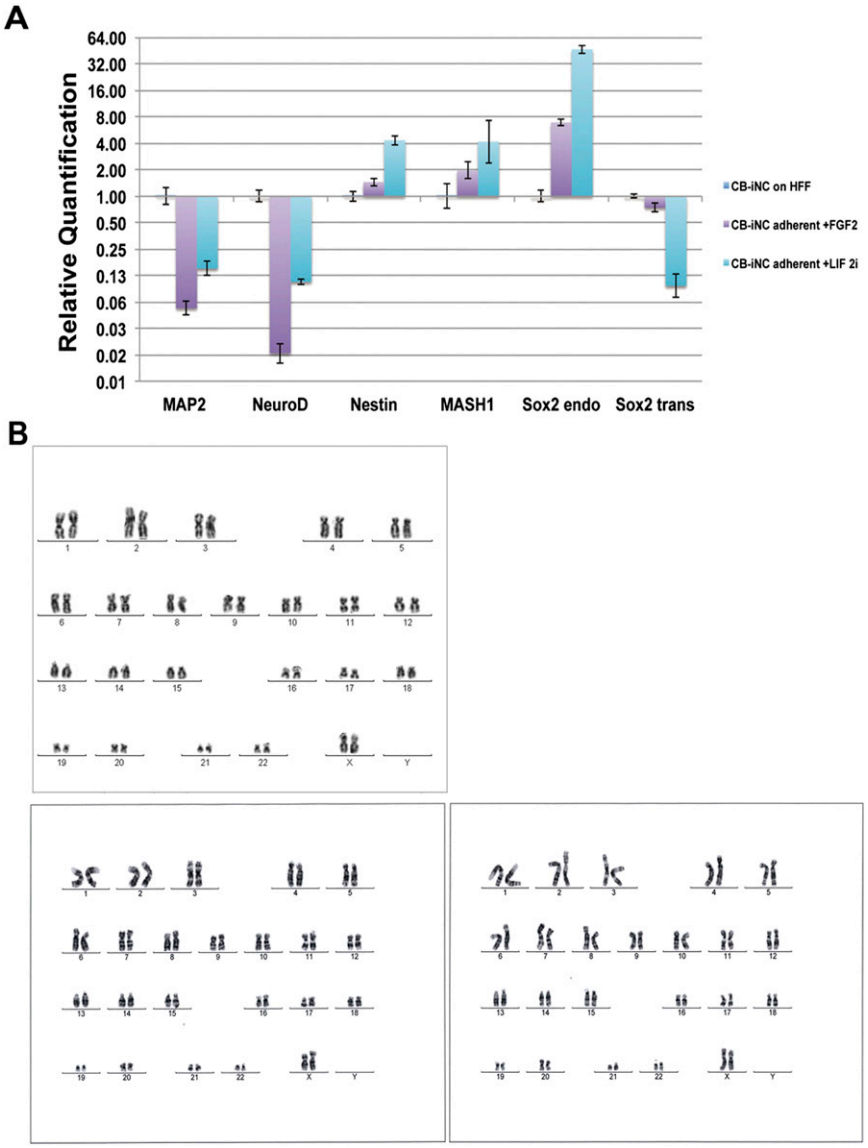

Fig. S6. CB-iNCs contain neuronal progenitor cells and are genetically stable. (*A*) qRT-PCR analysis of indicated markers in non clonal CB-iNCs and clonal neural-like progenitor cells. Purple bars represent culture condition with bFGF2, and blue bars represent culture condition without bFGF2 but with leukemia inhibitory factor and small-molecule inhibitors to GSK3, TGF-β, and Notch signaling pathways (1). All values are relative to expression in CB-iNC culture on HFF. Error bars indicate SD generated from triplicate experiments. (*B*) Representative high-resolution, G-banded karyotype showing a normal, diploid, female chromosomal content in CB-iNCs cells analyzed after passage 15. A minimum of 20 metaphases were analyzed.

1. Li W, 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 USA* 108: 8299–8304.

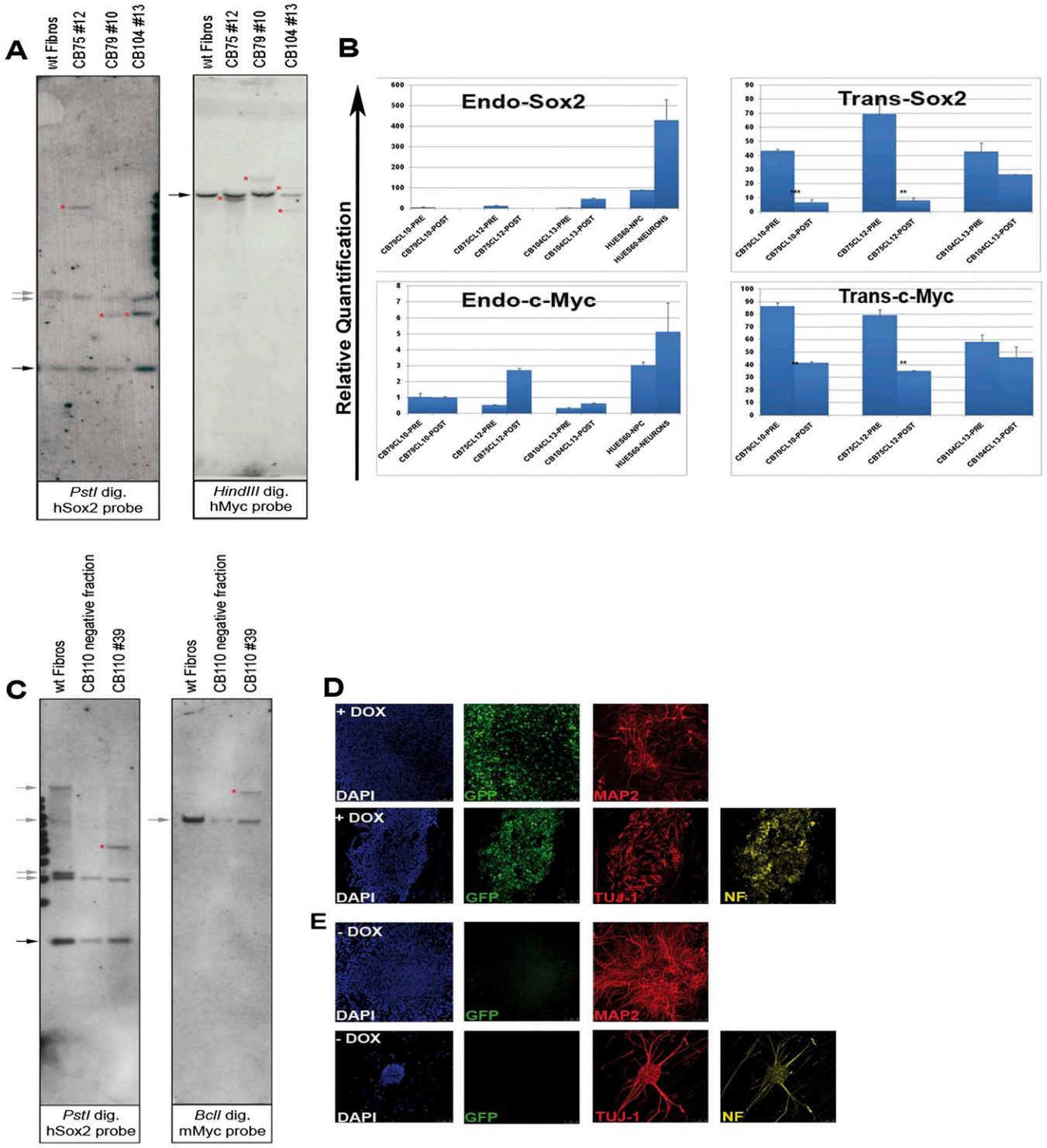

Fig. S7. CB-iNCs have a neuronal phenotype independent of viral transgene expression. (*A*) Southern blotting to confirm number of retroviral integrations. *1*, Genomic DNA digested with *PstI* hybridized with Sox2 probe. Endogenous band: 0.9 kb (black arrowhead). Additional bands in the different CB-iNCs (CB75CL12, CB79CL10, and CB104CL13) clones correspond each to single transgene insertions (red asterisks). Endogenous unspecific bands are marked with gray arrowheads. *2*, Genomic DNA digested with *HindIII* hybridized with Myc probe. Endogenous band: 11 kb (black arrowhead). Additional bands in the different CB-iNCs clones each correspond to single transgene insertions (red asterisks). (*B*) qRT-PCR analysis for endogenous and exogenous Sox2 and c-Myc genes. Error bars indicate SD generated from triplicate experiments (***$P < 0.001$ and **$P < 0.01$, ANOVA). (*C*) Southern blotting to confirm number of lentiviral integrations. *1*, Genomic DNA digested with *PstI* hybridized with Sox2 probe. Endogenous band: 0.9 kb (black arrowhead). Additional bands in representative CB-iNC clones each correspond to single transgene insertions (red asterisks). Endogenous unspecific bands are marked with gray arrowheads. *2*, Genomic DNA digested with *BclI* hybridized with mouse Myc probe. Endogenous unspecific bands are marked with gray arrowheads. Additional bands in the different CB-iNCs clones each correspond to single transgene insertions (red asterisks). (*D*) Representative images of CB-iNCs generated by doxycycline inducible system showing the expression of neural markers MAP2, TUJ-1, and neurofilament in the presence of doxycycline. (Scale bar, 50 μm.) GFP staining (green) represents the transgene activity. (*E*) Representative images of CB-iNCs generated by a doxycycline-inducible system after doxycycline withdrawal. (Scale bar, 50 μm.) The absence of GFP-positive cells indicates down-regulation of transgene.

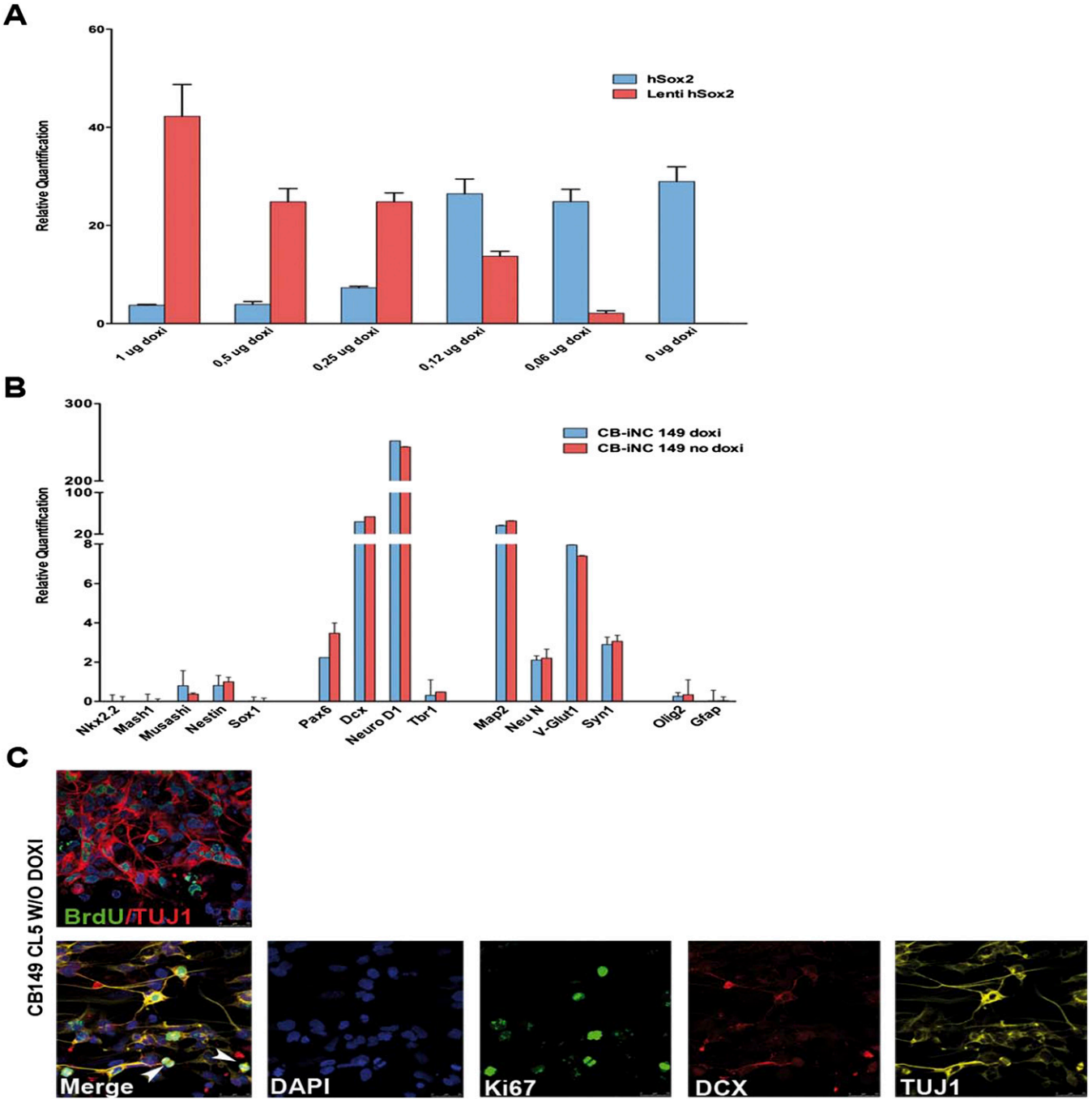

Fig. S8. CB-iNCs can be maintained for several passages after withdrawal of doxycycline. (*A*) qRT-PCR analysis shows down-regulation of the transgene Sox2 and the concomitant activation of endogenous Sox2 after reducing doxycycline concentration. Error bars indicate the SD generated from triplicate experiments. (*B*) qRT-PCR analysis shows that CB-iNCs maintained a stable neural phenotype for as many as 15 passages in the absence of doxycycline. Error bars indicate SD generated from triplicate experiments. (*C*) CB-iNCs maintained the capability to proliferate after withdrawal of doxycycline. $BrdU^+/TUJ\text{-}1^+$ cells were found in absence of doxycycline (at approximately 20 passages). (Scale bar: 50 μm.) Cells in mitosis are highlighted by white arrows (Scale bars, 25 μm.)

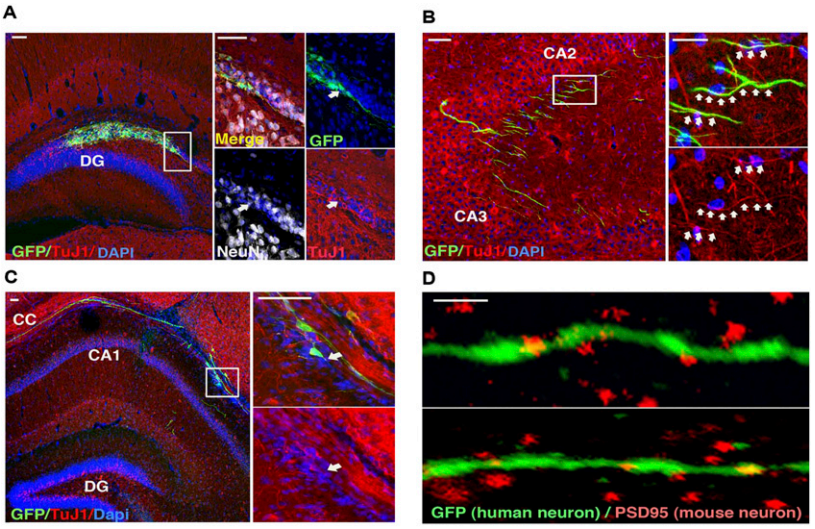

Fig. S9. Transplantation and engraftment of CB-derived neurons. (*A*) Representative example of transplanted neurons integrating in the dorsal blade of the dentate gyrus (DG) of the hippocampus. Grafted neurons were positive for NEUN and extended TUJ-1$^+$ processes around the granule neurons of the host tissue (arrow). (*B*) Grafted neurons also extended TUJ-1 processes along the mossy fiber track to contact endogenous pyramidal neurons in the CA2/CA3 regions (arrows). (*C*) Interestingly, neurons not grafted to the hippocampus also showed the ability to integrate into the corpus callosum (CC), sending TUJ-1$^+$ processes contralaterally (arrow). (Scale bars, 50 μm.) (*D*) Transplanted CB-derived human neurons (green) have postsynaptic (PSD95) puncta (red) adjacent to their processes, suggesting that the human neurons are integrated on the mouse network 3 mo after transplantation. (Scale bar, 2 μm.)