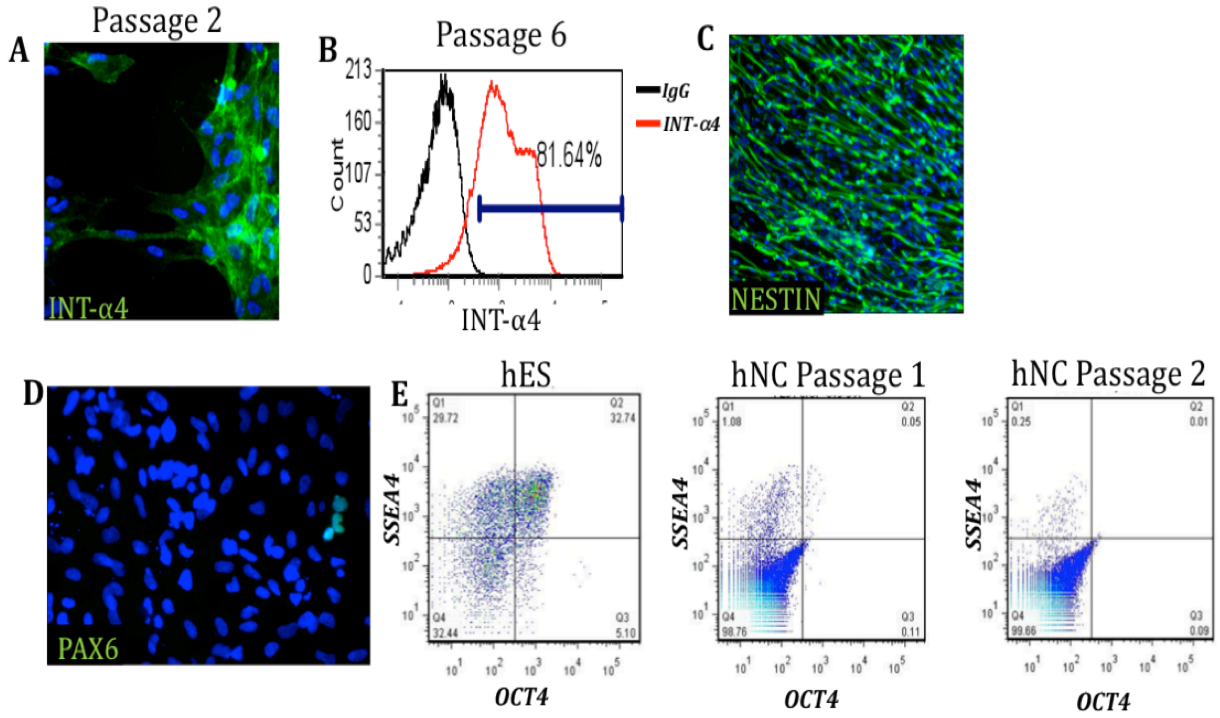
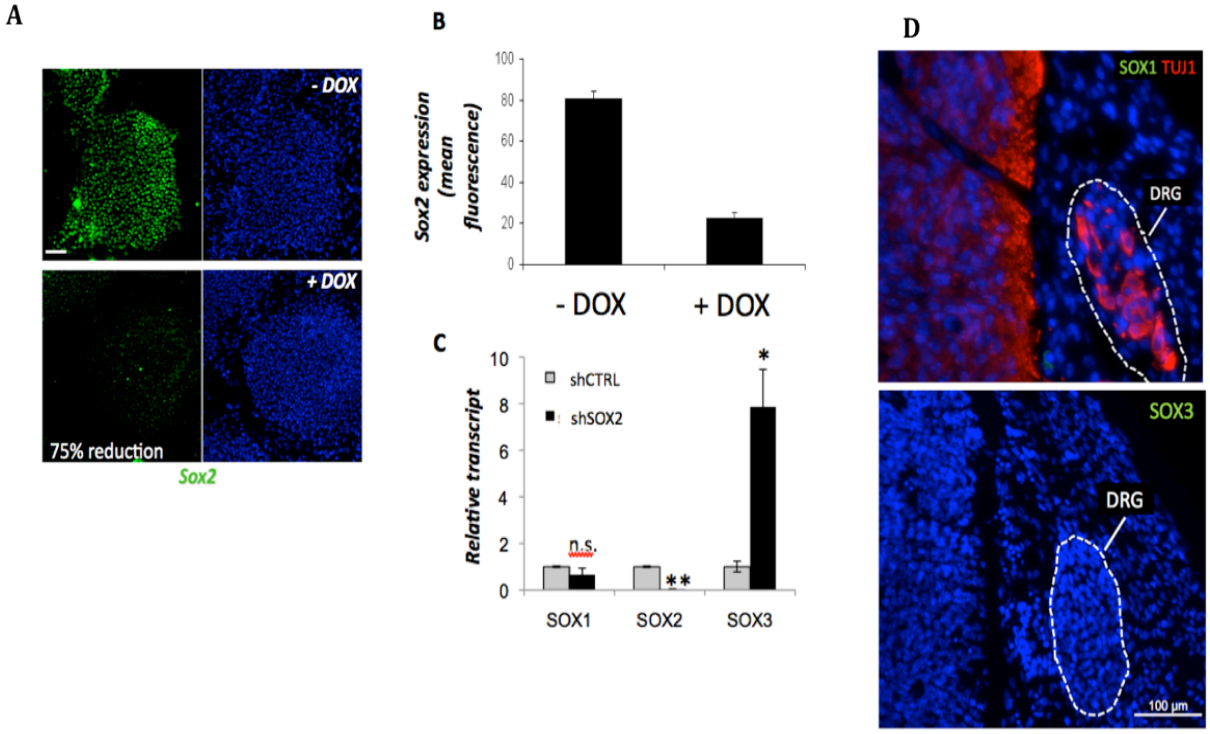
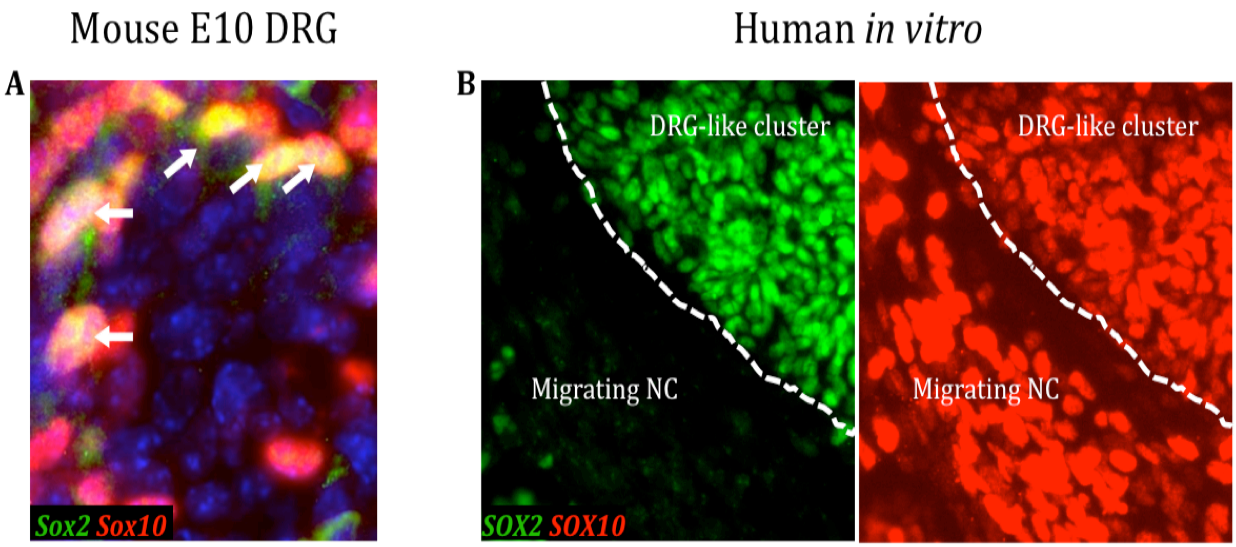


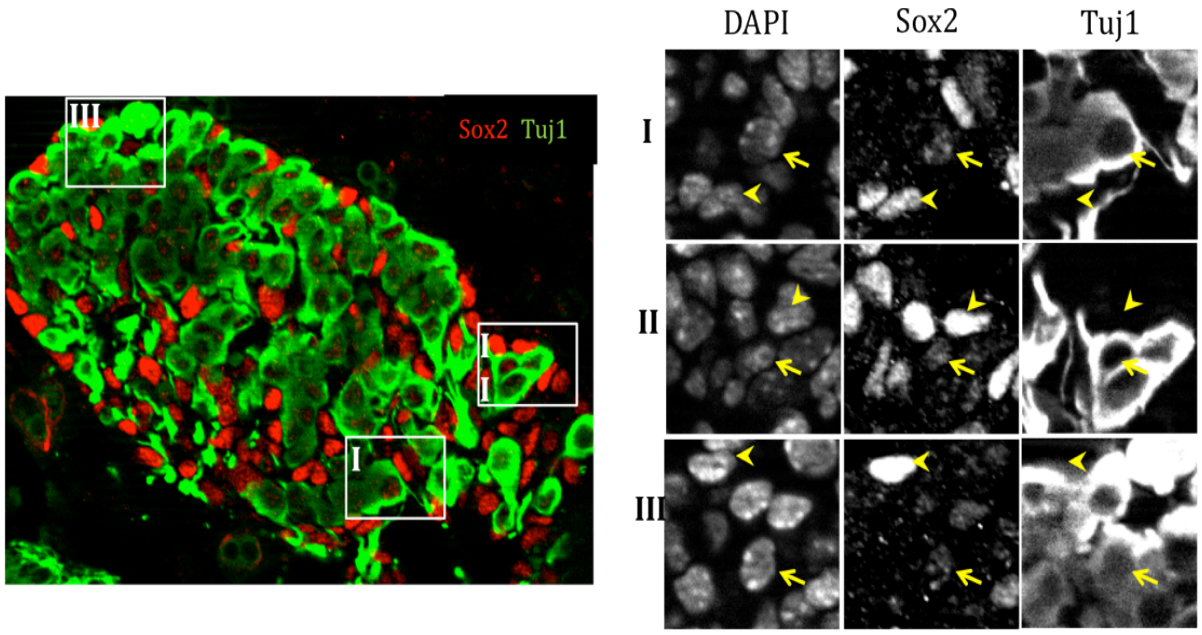
Supplementary Figure S1. INT-α4, NESTIN and PAX6 expression on hES-derived NC cells. (A) immunostaining for INT-α4 on NC cells at passage 2. (B) FACS analysis for INT-α4 on NC cells at passage 6. 81.64% of NC cells were INT- α4 positive. Normal mouse IgG was used as negative control. (C) Immunostaing for the neural marker NESTIN on NC cells at passage 1 witnessing the neural origin of hES-derived NC cells. (D) PAX6 immunostaining on NC cells at passage 1. Only very rare cells could be find positive for such marker. (E) FACS analysis for the hES markers OCT4 and SSE4 in hES cells and hES-derived human NC cultures at different passages. Note that cells already at passage 1 have almost undetectable levels of OCT4. Note also that OCT4/SSE4 double negative cells in hES cultures are MEFs used for co-culture. Such cells are removed enzymatically before differentiation into dorsal neuroepithelial cells.



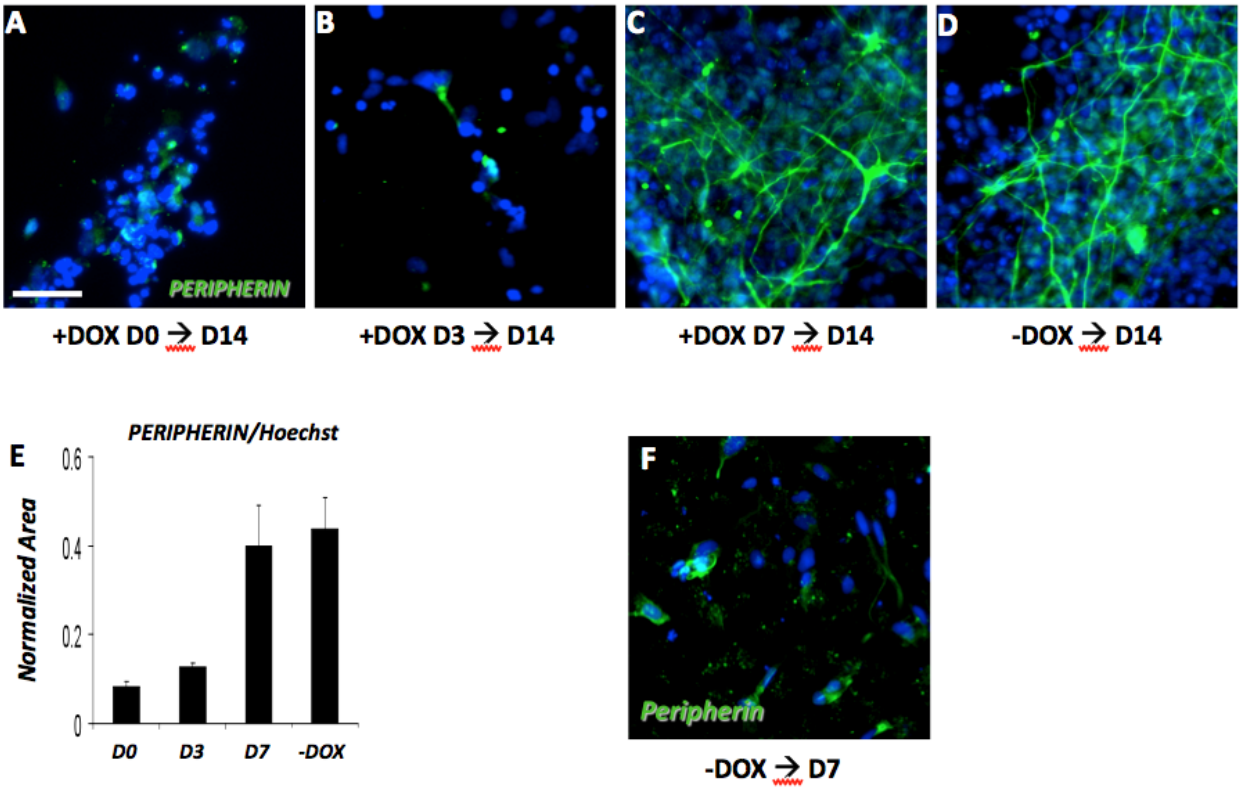
Supplementary Figure S2. Doxycycline Inducible shRNA Targeting SOX2. (A), Immunostaining for SOX2 in hES cells transduced with a lentiviral construct encoding doxycyline inducible SOX2-specific shRNA. Colonies were grown for two days in the absence (-DOX) or in the presence (+DOX) of doxycycline (1 µg/ml). (B), Quantification of SOX2 expression in the conditions presented in (A) reveals a 75% reduction in SOX2 levels. Values are mean fluorescence (arbitrary units) per nucleus in cells within hES colonies. (C), Quantitative PCR analysis of SOXB1 transcripts in ES-derived dNE cells after SOX2 shRNA (shSOX2) induction with DOX (3 days) shows specific reduction of SOX2 transcript. Transcript levels are relative to scrambled shRNA control (shCTRL) and normalized to the expression of 18S housekeeping gene. Note that SOX2 shRNA does not alter SOX1 expression whereas SOX3 levels were found to be increased after SOX2 knockdown, likely because of compensatory mechanisms. Scale bars in pictures are 100 µm. Blue is Hoechst nuclear dye. *P<0.05, **P<0.005. n.s. = not significant. (D) Sox1, Sox3 immunostaining in E11 DRG. Sox1 and Sox3 expression was undetectable in E11 DRG, suggesting that, among SOXB1 members, SOX2 has a specific and not redundant role in sensory neurogenesis. Tuj1 antibody was used to label neurons arising from CNS progenitors and sensory progenitors in DRG.



Supplementary Figure S3. The SOX2/SOX10 co-expression in mouse DRG and hESC-derived peripheral neuronal progenitors. (A) Immunostaining for Sox2 and Sox10 in E10 murine DRG showing expression of Sox2 in Sox10+ neural progenitors (arrows). (B) Similarly, SOX2 and SOX10 tend to be co-expressed in hES-derived NC cells in secondary neurogenic clusters positive for SOX2+ (the dashed line outlines a cluster). Cells SOX10+/SOX2- outside of the clusters are migrating human NC cells.



Supplementary Figure S4. Confocal microscopy analysis of Sox2/Tuj1 co-staining in E11 murine DRG. Left panel, confocal image of E11 DRG section at the level of forelimbs. Right panel, higher magnifications of the respective white boxes in the left image. Individual images for DAPI, Sox2, and TuJ1 channels are shown for each box. Cells with the highest expression of Sox2 do not show Tuj1 expression (arrowheads) while several Tuj1+ cells showed low but detectable levels of Sox2 (arrows), suggesting that at least some of the young ganglionic neurons co-expressed Sox2 and TuJ1.



Supplementary Figure S5. SOX2 Is Required at Early But Not Late Stages of Neuronal Differentiation. (A-D), Doxycycline-dependent SOX2 shRNA expression was induced under neurogenic culture conditions on day 0 - 7 (D0, D3, D7) during neuronal maturation and cells were immunostained for Peripherin at day 14 of differentiation; (D) -DOX = control no DOX added. (E) quantification of Peripherin expression n (A-D). Values represent the area showing immunoreactivity for Peripherin normalized to the total area stained by Hoechst nuclear dye. (F), Human ES cell-derived dNE cells cultured under neurogenic conditions for 7 days show no or little immunostaining for PERIPHERIN and lack of mature neuronal morphology seen at day 14 (D). The downregulation of SOX2 at day 7 do not affect the neuronal maturation (C, E). Scale bars = 50 µm.

Biogroup name	common genes	direction	p-value	
cell cycle	27	down	6.70E-20	
Cell Cycle G1 to S control Reactome	8	down	8.90E-11	
interphase of mitotic cell cycle	5	down	1.20E-06	**Cell cycle and mitosis**
G1/S transition of mitotic cell cycle	3	down	6.30E-05	
Cell Cycle G1/S Check Point	3	down	0.0002	
regulation of progression through mitotic cell cycle	2	down	0.0006	
mitosis	13	down	3.40E-14	
Focal Adhesion	7	up	1.60E-07	
cell-matrix adhesion	3	up	0.0002	
cell-substrate adhesion	3	up	0.0003	
cell-cell adhesion	2	down	0.007	**Adhesion , migration and cell motility**
cell motility	7	up	7.40E-06	
regulation of cell motility	2	up	0.0059	
Leukocyte Transendothelial Migration	5	up	2.50E-06	
cell migration	3	up	0.009	
Striated Muscle Contraction	5	up	2.60E-08	
muscle contraction	6	up	6.40E-07	
structural constituent of muscle	3	up	8.70E-05	
Smooth Muscle Contraction	3	up	0.001	**Muscle differentiation and migration**
smooth muscle contraction	2	up	0.0015	
muscle development	3	up	0.0027	
striated muscle contraction	1	up	0.0094	
neuron projection	3	down	0.0006	
neuron morphogenesis during differentiation	3	down	0.0027	**Neuronal differentiation and maturation**
neuron development	3	down	0.0052	
SRF binding site geneset 4	7	up	1.50E-08	
SRF binding site geneset 2	7	up	2.80E-07	
SRF binding site geneset 3	7	up	4.90E-07	**Serum regulated genes**
SRF binding site geneset 6	4	up	5.00E-06	
SRF binding site geneset 5	6	up	1.30E-05	
SRF binding site geneset 1	3	up	8.20E-05	

Supplementary Figure S6. Nextbio Pathways Meta Analysis Following SOX2 Downregulation Under Neuronal Differentiation Conditions. Genes that passed the Volcano filter built in GeneSpring analysis software (significantly up- or downregulated at least 2 fold) when compared SOX2 shRNA vs scrambled control shRNA at day 3 under neurogenic conditions were identified and further analyzed using the Nextbio search engine / pathway analysis.

Tables S1–S3. NextBio biogroups and pathways potentially regulated by the SOX2 interacting proteins.

Table 1- Gene Ontology	
Biogroup name	**common genes**
regulation of biosynthetic process	64
regulation of nitrogen compound metabolic process	62
transport	59
transcription	57
cytosol	54
cytoskeleton	49
response to stress	42
nucleoplasm	40
RNA binding	39
nucleoside binding	34
nucleolus	33
catabolic process	32
cell death	32
death	32
cell differentiation	28
mitochondrion	26
cell cycle	24
enzyme binding	23
protein transport	23
translation	22
cytoskeleton organization	21
enzyme regulator activity	20
cell proliferation	20

interspecies interaction between organisms	19
envelope	19
positive regulation of biosynthetic process	19
cytoskeletal protein binding	18
negative regulation of biosynthetic process	18
cellular response to stimulus	18
transcription factor binding	17
identical protein binding	17
chromosome	17
response to organic substance	16
endoplasmic reticulum	16
actin binding	15
regulation of cellular component organization	15
insoluble fraction	14
transcription activator activity	13
cellular ketone metabolic process	13
kinase activity	13
cellular response to stress	13
Golgi apparatus	13
receptor binding	13
transporter activity	13
protein dimerization activity	12
cellular component movement	12
intermediate filament	11
perinuclear region of cytoplasm	11
response to external stimulus	11
GTPase regulator activity	11

protein kinase activity	11
heterocycle metabolic process	11
endoplasmic reticulum part	11
kinase binding	10
cellular nitrogen compound biosynthetic process	10
protein domain specific binding	10

Table 2 - Canonical Pathways	
Biogroup name	**common genes**
Cell Communication	11
mRNA Processing Reactome	11
Purine Metabolism	7
Calcineurin Nf At Signaling	6
Pyrimidine Metabolism	5
Ribosome	5
Focal Adhesion	5
MAPK Signaling Pathway	5
MTA3 Pathway	4
Smooth Muscle Contraction	4
WNT Signaling Pathway	4
Regulation of The Actin Cytoskeleton by Rho Gtpases	3
The Role of FYVE-finger Proteins In Vesicle Transport (EEA1)	3
WNT Beta Catenin Pathway	3
P38 MAPK Pathway	3

Ceramide Signaling Pathway	3
Stress Pathway	3
Basal Transcription Factors	3
Fas Signaling Pathway	3
GA13 Pathway	3
Chemotaxis	3
Fatty Acid Metabolism	3
Differentiation Pathway In PC12 Cells	3
PIP3 Signaling In Cardiac Myoctes	3
Adipocytokine Signaling Pathway	3
ERBB Signaling Pathway	3
G-Protein Signaling Pathway	3
Regulation of Actin Cytoskeleton	3
HSP27 Pathway	2
Pyruvate Metabolism	2
Lysine Degradation	2
RNA Polymerase Pathway	2
Fatty Acid Elongation In Mitochondria	2
Presenilin-1 (PS1) Pathway	2
RaNK-l Pathway	2
Hypoxia-inducible Factor In The Cardiovascular System (HIF)	2
Role of PI3K Subunit P85 In Regulation of Actin Organization and Cell Migration	2
Glycolysis and Gluconeogenesis	2
Pitx2 Pathway	2
Human Cytomegalovirus and MAP Kinase Pathways (HCMV)	2
AKT Signaling Pathway	2

NDK Dynamin Pathway	2
GPCR Pathway	2
Insulin Receptor Pathway In Cardiac Myocytes	2
PTDINS Pathway	2
Rac CYCD Pathway	2
Inactivation of GSK-3 by AKT Causes Accumulation of beta-Catenin In Alveolar Macrophages	2
PDGF Pathway	2
EGF Signaling Pathway	2
Granule Cell Survival Pathway	2
Tumor Necrosis Factor Pathway	2
TNFR1 Pathway	2
Pyk2 Pathway	2
Citrate Cycle	2
Fas Signaling Pathway (CD95)	2

Table 3 - Regulatory Motifs	
Biogroup name	**common genes**
SP1 binding site geneset 6	75
MAZ binding site geneset 2	56
E12 binding site geneset 2	42
MYC binding site geneset 2	38
LEF1 binding site geneset 4	36
NFAT binding site geneset 3	35

PAX4 binding site geneset 5	29
ELK1 binding site geneset 3	28
NFY binding site geneset 5	27
ERR1 binding site geneset 2	26
FOXO4 binding site geneset 3	26
NRF1 binding site geneset 2	24
AP1 binding site geneset 9	24
E4F1 binding site geneset 2	20
GABP binding site geneset 2	19
LEF1 binding site geneset 3	19
AP4 binding site geneset 5	19
YY1 binding site geneset 4	17
TATA binding site geneset 3	17
MYCMAX binding site geneset 1	14
MAX binding site geneset 1	14
USF binding site geneset 1	14
ETS2 binding site geneset 2	14
PITX2 binding site geneset 2	13
MYOD binding site geneset 4	13
GABP binding site geneset 1	12
USF2 binding site geneset 1	12
NFMUE1 binding site geneset 1	12
ARNT binding site geneset 1	12
AP2 binding site geneset 2	12
SREBP1 binding site geneset 4	12
FREAC2 binding site geneset 2	12

MZF1 binding site geneset 1	11
CACBINDINGPROTEIN binding site geneset 1	11
MAZR binding site geneset 1	11
SP1 binding site geneset 1	11
ARNT binding site geneset 2	11
NRF2 binding site geneset 1	11
AREB6 binding site geneset 5	11
YY1 binding site geneset 2	10
MYCMAX binding site geneset 3	10
USF binding site geneset 5	10
TAXCREB binding site geneset 1	9
PTF1BETA binding site geneset 1	9
AP2GAMMA binding site geneset 1	9
MYCMAX binding site geneset 2	9
E2F1DP1RB binding site geneset 1	9
E2F binding site geneset 10	9
E2F binding site geneset 8	9
HSF1 binding site geneset 2	9
SP1 binding site geneset 2	9
E2F1 binding site geneset 2	9
E2F1 binding site geneset 1	9
AP2 binding site geneset 3	9
COUP binding site geneset 1	9
ELK1 binding site geneset 1	9

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Maintenance of hESCs

H9 hES cells were maintained on MEF feeders and gelatin-coated plates (Sigma, final concentration = 0.1%, 1h coating at room temperature) in knockout DMEM (Gibco), 20% serum replacement (Gibco), 1X non-essential amino acids (Gibco), 2mM L-glutamine (Gibco), 0.1 mM β-mercaptoethanol (Gibco), 1X antibiotics/antimycotics (Omega) and 8 ng/ml bFGF (Sigma). hESCs were routinely passaged every 5-7 days using 1 mg/ml Collagenase type IV (Gibco) diluted in knockout DMEM after manual removal of morphologically identifiable differentiated colonies. Medium was changed daily.

Derivation, Maintenance and Differentiation of Human dNE Cells

Dorsal neuroepithelium (dNE) was generated from hES cells as described (Cimadamore et al., 2009). Monolayer cultures of hES-derived neuroepithelial cells were obtained by enzymatic dissociation of neurospheres (Accutase [Chemicon], 10 min at 37ºC). Dissociated cells were plated under different conditions according to experimental needs. For propagation, cells were seeded onto Matrigel-coated plates (BD Biosciences, final concentration = 1:30, 2h coating at room temperature) using base medium (1:1 ratio of DMEM/F12 Glutamax-neurobasal medium [Gibco], 2% B27 supplement without vitamin A [Gibco], 10% BIT 9500 [StemCell Technologies], and 1 mM glutamine [Gibco]) supplemented with 20ng/ml EGF (Chemicon), 20ng/ml bFGF, 5 µg/ml insulin (Sigma) and 5 mM nicotinamide (Sigma)). For segregation between epithelial and mesenchymal cells, accutase dissociated dNE cells were seeded onto FN-coated plates (1µg/ml, overnight coating) at a density of 45000 cells/cm^2 in the presence of EGF 100 ng/ml for 12 days. For differentiation into smooth muscle cells, dNE cells were seeded onto FN-coated plates (1 µg/ml, overnight coating) at a density of 45,000 cells/cm^2 in base medium supplemented with 40 ng/ml EGF. Cells were allowed to differentiate for 7 days. Gliogenic conditions were obtained by seeding dNE cells at 20000 cells/cm^2 in FN-coated plates in base medium supplemented with 1% horse serum. Cells were allowed to differentiate for 12 days. For neuronal differentiation (neurogenic conditions), dNE cells were seeded onto FN-coated plates at 45,000 cells/cm^2 in base medium supplemented with 40 ng/ml bFGF and 40ng/ml BDNF and allowed to differentiate for 14 days. Other conditions were as described in the text. For neurosphere-based migration assays, hES-derived neurospheres were plated on different substrates as described and cells allowed to migrate for a minimum of 1 day to a maximum of 4 days on laminin/polyornithine coated plates in base media supplemented with 20ng/ml EGF, 20ng/ml bFGF, 5 µg/ml insulin and 5 mM nicotinamide.

Gel Invasion / 3D Migration Assay

The assay was adapted from (Voroteliak et al., 2002). Single dNE cells were obtained by enzymatic digestion with Accutase (10 min at 37º) and seeded on the top of a 1% collagen gel of 0.4 cm thickness. Gels were prepared as described elsewhere (Voroteliak et al., 2002). 4 days after the initial seeding, gel and cells that failed to migrate were manually removed while cells populating the bottom of the well were immediately processed for immunocytochemistry.

Immunostaining

Cells were rinsed with PBS and fixed in 4% paraformaldehyde/PBS for 15 min at room temperature. After blocking with PBSAT (3% BSA and 0.5% Triton X-100 diluted in PBS) for 1h at room temperature, cells were incubated overnight at 4°C with the primary antibodies (see supplementary experimental procedures for the antibody list) diluted in PBSAT. To detect membrane proteins, Triton was omitted from the PBSAT buffer. The appropriate fluorochrome-conjugated secondary antibody was used with every primary antibody at a 1:1000 dilution. Nuclear co-staining was performed by incubating cells with Hoechst nuclear dye (1:1,000). Immunocytochemistry, with the exception of N-CAD staining, was performed as follows: PPFA-fixed mouse sections (12 µm thickness) were warmed in a microwave in low pH Antigen Unmasking Solution (Vector laboratories) until boiling and then cooled to room temperature. The process was repeated 3-4 times, and sections were washed with PBS and blocked with PBSGT (5% BSA, 5% goat serum, 0.5% Triton diluted in PBS) for 1h at room temperature. Sections were incubated with primary antibody overnight at 4°C, washed with PBSGT, and incubated 1h at room temperature with the appropriate fluorochrome-conjugated secondary antibody. For immunodetection of N-CAD *in vivo*, the antigen retrieval step was omitted and the Triton concentration in the PBSGT buffer was reduced to 0.1%. For chicken experiments, embryos were dissected into Ringers solution, fixed 1-2h in 4% paraformaldehyde (in PBS) and were dehydrated in PBS-sucrose before being transferred to gelatin and embedded for cryo-sectioning. Embryos were sectioned at 12⌠m thickness and processed for immunohistochemistry.

RT-PCR, qPCR and Microarray Analysis

For RT-PCR and qPCR, Total RNA was extracted using an RNeasy kit (Qiagen) and 1 µg total RNA was reverse transcribed using the Quantitect kit (Qiagen), according to the manufacturer's instructions. 2-4

µl of purified cDNA served as template for RT-PCR to amplify genes of interest (see supplementary experimental procedures for a primer list). For quantitative qPCR, 2 µl of purified cDNA diluted 1:8 was used as a template. qPCR was performed with SYBRGreen master mix (Invitrogen) according to the manufacturer's recommendations. 18S rRNA was used for normalization and the data were analyzed using the ⊗(⊗CT) method. For microarray analysis, total RNA was extracted with the RNeasy kit. RNA purity was assessed by A260/A280 absorption and RNA integrity was verified by agarose gel electrophoresis. Labeled cRNA was prepared from 500 ng RNA using the Illumina® RNA Amplification Kit from Ambion (Austin, TX, USA). Labeled cRNA (1500 ng) was hybridized overnight at 55°C to the Sentrix ® HumanRef-6 Expression BeadChip (>46,000 gene transcripts; Illumina, San Diego, CA, USA) according to the manufacturer's instructions. BeadChips were subsequently washed and developed with fluorolink streptavidin-Cy3 (GE Healthcare). BeadChips were scanned with an Illumina BeadArray Reader, and hybridization efficiency was monitored using BeadStudio software (Illumina) to measure internal controls built into the Illumina system. Data were analyzed using the Nextbio search engine (www.nextbio.com).

ChIP and qPCR

ChIP was performed using the Ez-ChIP kit (Millipore) according to the manufacturer's recommendations with the following modifications: 2⊕10^6 cells were used for each immunoprecipitation, cells were sonicated in order to yield chromatin fragment of 200-500 bp, 5 µg of immunoprecipitating antibodies were employed in each ChIP. Antibodies were rabbit anti-SOX2 (Millipore AB5603) and normal rabbit IgG (Millipore PP64) as a non-specific control. To evaluate SOX2 enrichment at NGN1 promoter sites, qPCR was performed using the purified chromatin as a template. Amplification was performed with site specific primers designed to flank the putative SOX binding sites. For a primer list see supplementary experimental procedures. qPCR values were analyzed with the Δ(ΔCT) method and normalized to the values obtained with the non-specific antibody.

Identification of SOX2-Interacting Proteins

Briefly, hES cell-derived NC cells were cultured as described above. IP was performed using the Pierce® Crosslink Immunoprecipitation Kit with anti-SOX2 (Millipore AB5603) or normal rabbit control (PP64) antibodies. Samples were reduced, alkylated, and digested with sequencing grade, modified Trypsin (Promega) using standard procedures. The resulting peptides were desalted with a

peptide microtrap (Michrom Bioresources), dried in a speed vac and resuspend in 0.1% formic acid /5.0% acetonitrile. Each sample was run in triplicate LC-MS/MS analyses using a HTC-PAL autosampler/ Paradigm MS2 HPLC connected to a 0.2 X 150 mm Magic C18 column/captive spray source (Michrom) coupled to an LTQ Orbitrap Velos mass spectrometer equipped with ETD (Thermo Fisher), using a decision tree (Swaney et al., 2008) top-20 data-dependent method and a 15 min HPLC gradient. Spectra were searched against an ipi.v.3.73 human protein database using a Sorcerer™-SEQUEST® Enterprise (SageN Research), and results filtered to a false discovery rate of 0.005-0.008 using ProteinProphet (Trans-Proteomic Pipeline).

Flow Cytometry

Cells were dissociated with Accutase (10 min at 37°C) and washed with PBS. Cells were blocked with 2% BSA in PBS (PBS/BSA) for 30 min, spun down and resuspended in PBS/BSA at a density of 106 cells/100 µl with the primary antibody (incubation on ice for 1h). Cells were washed with PBS/BSA and subsequently incubated with secondary antibody on ice for 30 min. Cells were washed with PBS/BSA and analyzed with a BD biosciences FACSCanto flow cytometer.

Sox2 Manipulation in Chick Embryo

Fertilized chicken embryos (Gallus gallus domesticus) were obtained from McIntryre Farms, Lakeside CA and incubated in a 38°C humidified incubator until HH10 according to the staging of Hamburger (Hamburger, 1988). miR30 plasmid (4µg/µl concentration) was injected by air pressure into the neural tube of the embryo in ovo using a pulled glass needle. Platinum electrodes were placed across the neural tube and a current of 3 x 21V of 50ms in 100ms intervals was used to electroporate the cells on one half of the neural tube. Embryos were resealed and re-incubated a further 24 hours.

Transgenic Mice

Mice carrying $Sox2^{loxP}$ alleles (Favaro et al., 2009) were crossed with the mice expressing Cre recombinase under the control Wnt1 promoter (wnt1:Cre) (Danielian et al., 1998), resulting in *Wnt1*:*Cre*/*Sox2*$^{LoxP/LoxP}$ mice. Only mice carrying 2 copies of the $Sox2^{LoxP}$ allele were further analyzed and littermates carrying 2 copies of wt Sox2 allele were used as a control. *Wnt1*:*Cre*/*Sox2*$^{LoxP/LoxP}$ mice were not found indicating likely in utero lethality. The phenotype in embryonic DRG was investigated by means of immunocytochemistry at different developmental stages. Serial sections from different animals used for quantification started at the level of the forelimbs. Total 23 sections were used for Tuj1 quantification at E11, 32 sections for

Hu/CD quantification E11 and 50 sections for Tuj1 quantification at E14.5. Sections covered the space of ~4 DRG. Immunocytochemistry was performed as indicated above. *Wnt1*:*Cre*/*Sox2*$^{LoxP/LoxP}$ mice were crossed to Z/EG mice (Jackson Laboratories) which activates GFP upon Cre recombination to monitor Wnt1:Cre activity in DRG.

Lentivirus-Mediated shRNA and Overexpression

For shRNA experiments, vectors expressing doxycyclin-inducible SOX2 shRNA or scrambled (SCR) shRNA were purchased from Cellecta (www.cellecta.com) and used to generate lentiviruses. H9 hES cells were infected with lentiviral particles to obtain lines stably carrying either SOX2 or SCR shRNA. For SOX2 overexpression, human SOX2 coding sequence CDS was amplified from cDNA obtained from hES-derived dNE cells and cloned into a lentivector expressing GFP under control of the PGK promoter. SOX2 CDS was inserted upstream of GFP. Reverse primers for SOX2 amplification were designed to remove the SOX2 stop codon, resulting in a SOX2-GFP fusion protein. Primers, vectors and details about the cloning strategy are available upon request. All lentivectors were packaged into lentiviral particles at the Viral Vectors Facility at the Sanford-Burnham Medical Research Institute (La Jolla, CA).

Digital Image Analysis

Some cell types such as neurons could be seen only in highly confluent cell cultures, making it difficult to manually count the proportion of cells positive for a given marker. We therefore took a semi-automated approach to evaluate marker expression using immunocytochemistry. Pictures were taken with the same exposure time and contrast/brightness parameters. The total area showing immunoreactivity for a particular marker was determined using ImageJ software (http://rsb.info.nih.gov/ij/) and normalized to the total area positive for Hoechst nuclear staining to estimate the total number of cells in a field. A minimum of 5 pictures containing at least 100 cells was analyzed for each condition, as reported in the text.

Statistical Analysis

For quantifications, experiments were performed at least in triplicate. Statistical significance was assessed using Student t-test. A P value < 0.05 was considered significant. In all graphs, error bars represent standard error values, with the exception of Figure 6A where standard deviations are shown.

ANTIBODIES

Name	specie	Company	Dilution	application
Active Caspase 3	Rb	Promega	1:125	ICC
BRN3a	Rb	Millipore	1:200	ICC
GFP	Rb	Invitrogen	1:100	IHC
HNF3β	ms	developmental hybridoma Bank	1:50	ICC
HUC/D	Ms	Molecular Probes	1:100	IHC
INTEGRINα4	Ms	R&D	1:100	ICC,FACS
KI67	Rb	Novocastra labs	1:200	ICC
MAP2	ms	Sigma	1:500	ICC
MUSASHI1	ms	Milllipore	1:500	ICC
N-CAD	ms	Sigma	1:100	IHC,ICC
NESTIN	ms	Millipore	1:500	ICC
NKX2.2	ms	developmental hybridoma Bank	1:500	ICC
P75NTR	Rb	Alomone	1:200	ICC
PAX3	Rb	Zymed	1:200	ICC
PAX6	Rb	Covance	1:500	ICC
PERIPHERIN	Rb	Chemicon	1:1000	ICC
SMA	ms	Sigma	1:500	ICC
SOX10	ms	Wegner	1:100	ICC
SOX2	Rb	Millipore	1:500	ICC
SOX2	ms	R&D	1:100	IHC,ICC
SOX9	Rb	Chemicon	1:200	ICC
TUJ1	Ms	Covance	1:500	ICC

ZO-1	ms	BD transduction laboratories	1:100	ICC

PRIMERS

qPCR and RT primers:

Gene	Sequence F	Sequence R	application
18S	**ATCAACTTTCGATGGTAGTCG**	**TCCTTGGATGTGGTAGCCG**	**qPCR**
ADAM10	**CGGAAGATGGTGTTGCTGAG**	**CCCTGAGGACCGTATTTATGG**	**RT-PCR**
DLL1	**AAGTGAGATGGCAAGACTCCCGTT**	**TGAACTCGGTTTCTCAGCAGCAGT**	**qPCR**
GAPDH	**CCCCTTCATTGACCTCAACTACA**	**TTGCTGATGATCTTGAGGCTGT**	**RT-PCR**
HES1	**TGTCAACACGACACCGGATAAACC**	**TGGAATGCCGCGAGCTATCTTTCT**	**qPCR**
HES5	**TACCTGAAGCACAGCAAAGCCTTC**	**TGGAAGTGGTACAGCAGCTTCATC**	**qPCR**
JAG1	**TGCTACAACCGTGCCAGTGACTAT**	**AGTGGTCTTTCAGGTGTGAGCAGT**	**qPCR**
MASH1	**CTGGTGCGAATGGACTTTGG**	**ACAGTGTGAGGGAAGCCATG**	**RT-PCR**
MASH1	**AAGAGCAACTGGGACCTGAGTCAA**	**AGCAAGAACTTTCAGCTGTGCGTG**	**qPCR**
NGN1	**GACGACACCAAGCTCACCAA**	**AACAAGCGGCTCAGGTATCC**	**RT-PCR**
NGN1	**CCAGCCACCACTTCAGTGTGATTT**	**TATTGTCAGCCGGCTCAAACCGAA**	**qPCR**
NOTCH1	**TCAGGGTGTGCACTGTGAGATCAA**	**AGGTGCCGTTGTTAAAGCACTTGG**	**qPCR**
NOTCH2	**ACAGTTGTGTCTGCTCACCAGGAT**	**GCGGAAACCATTCACACCGTTGAT**	**qPCR**
RBPJ	**ATCCTTCGAGCCAATTCAAGCCAG**	**TGTGCTGGCGTTTGTGTAACTTCC**	**qPCR**
SLUG	**AGCGAACTGGACACACATAC**	**TCTAGACTGGGCATCGCAG**	**RT-PCR**
SOX1	**TGTTGGCATCTAGGTCTTGGCTCA**	**TGTGCACGAAGCACCTGCAATAAG**	**qPCR**

SOX2	**TTTGTCGGAGACGGAGAAGC**	**ACTTGACCACCGAACCCATG**	**RT-PCR**
SOX2	**CACAACTCGGAGATCAGCAA**	**CGGGGCCGGTATTTATAATC**	**qPCR**
SOX3	**GGGACGCCTTGTTTAGCTTTGCTT**	**TAACACAGCGATTCCCAGCCTACA**	**qPCR**

ChIP-qPCR primers:

Promoter	**Sequence F**	**Sequence R**	**Site**
MASH1	**CCTCCCATCTTTTGCTTCAC**	**CTTGGGGATTCTACCAAGAGAG**	**-2889**
MASH1	**ATGTTCTGGCGGTTTTGG**	**GAAATGGGTGCCCAAATG**	**-2524**
MASH1	**TGGGAGGAAGAGGTAAGAGG**	**TGGCCAGAAGTGAGAGAGTG**	**-30**
NGN1	**GGAGGCAGAGATTGGAGTGA**	**GCTTCTAGTGGTGGCTCCTG**	**-2021**
NGN1	**GGCAGGCACTTTGCTGAA**	**GTCACCTACACTCACCCAGGA**	**-1286**
NGN1	**CCTCCCGCGAGCATAAAT**	**CCTCAGGACCCCTTAAGTACCC**	**-58**