

Supplementary Materials and Methods

In situ hybridization (ISH)

Nonradioactive ISH was performed on cryosections of wild-type (WT) mice as described previously (Ernsberger et al., 1997) using a *Tcf3* specific probe. For ISH, Digoxigenin (DIG) labeled probes were diluted 1:1000 in hybridization buffer. Hybridization and subsequent washing was performed at 68°C, washing with maleic acid buffer and 0.1% Tween (MABT) was carried out 2 × 30 min before sections were blocked with lamb serum at RT for 1 h. Anti-DIG alkaline phosphatase conjugate was diluted 1:5000. The color reaction was performed overnight at RT using NBT/BCIP Stock Solution (Roche) diluted 1:100. *In situ* probes were *in vitro* transcribed from linearized plasmids at 37°C for 2 h. The following primers were used:

Tcf3 Fwd: 5`-GTCCTGGGTGGATGATGAACC-3`

Tcf3 Rev: 5`-CAACGAAGAAGCTGTGACG-3`

Immunohistochemistry (IHC)

Primary antibodies used for IHC: rabbit anti-E2A (1:1000, sc-349X, Santa Cruz Biotechnology), goat anti-GFP (1:1000, Abcam), rabbit anti-Ki67 (1:500, Abcam), goat anti-Nestin (1:200, Santa Cruz Biotechnology), rabbit anti-NeuN (1:2000, Abcam), rabbit anti-p57(KIP2) (1:500, Sigma P0357), mouse anti-Pax6 (1:50, Developmental Studies Hybridoma Bank), mouse anti-Satb2 (1:500, Abcam), rabbit anti-Tbr1 (1:500, Abcam), rabbit anti-Tbr2 (1:2000, Millipore), rat anti-Tbr2 (1:500, Invitrogen), guinea pig anti-Vglut1 (1:500, Millipore), mouse anti-Mash1 (1:500, BD Bioscience), rat anti-Ctip2 (1:500, Abcam), mouse anti-Sox2 (1:1000, Abcam), rabbit anti-Ngn2 (1:200, Cell

Signaling), mouse anti-neurofilament (1:100, Developmental Study Hybridoma Bank), mouse anti-Tuj-1 (1:200, Millipore), rabbit anti-cleaved caspase-3 (1:100, Cell Signaling), mouse anti-Gad67 (1:1000, Millipore), rabbit anti-Math2 (1:1000, Abcam), mouse anti-NeuroD1 (1:1000, Abcam). Secondary antibodies used were conjugated with fluorescein isothiocyanate (FITC), Alexa Fluor 488 or 594 (1:200, Jackson ImmunoResearch Laboratories). Sections were coverslipped with 4',6-diamidino-2-phenylindole (DAPI) Fluoromount-G® solution (Southern Biotech).

Immunocytochemistry (ICC)

Primary antibodies used for ICC: rat anti-BrdU (1:300, Abcam), rabbit anti-cleaved caspase-3 (1:100, Cell Signaling), goat anti-GFP (1:1000, Abcam) or mouse anti-Tuj-1 (1:100, Millipore) in 1% BSA/PBS overnight. After three washes in PBS, cells were incubated with an appropriate secondary antibody conjugated with either FITC, Alexa Fluor 488 or 594 (1:200, Jackson ImmunoResearch Laboratories) for 1 h in 1% BSA/PBS at RT, washed in PBS, and mounted with DAPI Fluoromount-G® solution (Southern Biotechnology).

Neurite outgrowth assay

For the neurite outgrowth assays, 125,000 electroporated WT and *E47*^{-/-} cells were cultured in differentiation medium, allowed to extend processes for 48 h (Fig. 7A,B) or for 24 h (Fig 7H,I) and stained for Tuj-1. Neurite outgrowth was determined as the proportion of cells bearing neurites two times longer than the diameter of the cell body, an indication of successful initiation of neurite outgrowth (Schachtrup et al., 2007). The number of neurite-bearing cells was measured from at least 200 GFP⁺ neurons per

condition. Quantification of cell numbers was performed using ImageJ software (National Institutes of Health).

RNA isolation and quantitative PCR

The following primers were used for quantitative PCR:

E12 Fwd: 5'-GGGAGGAGAAAGAGGATGA-3'

E12 Rev: 5'-GCTCCGCCTTCTGCTCTG-3'

E47 Fwd: 5'-GGGAGGAGAAAGAGGATGA-3'

E47 Rev: 5'-CCGGTCCCTCAGGTCCTTC-3'

E-cadherin Fwd: 5'-AATGGCGGCAATGCAATCCCAAGA-3'

E-cadherin Rev: 5'-TGCCACAGACCGATTGTGGAGATA-3'

Gapdh Fwd: 5'-CAAGGCCGAGAATGGGAAG-3'

Gapdh Rev: 5'-GGCCTCACCCCATTTGATGT-3'

Cdkn1a Fwd: 5'- CCT GGTGATGTCCGACCTG-3'

Cdkn1a Rev: 5'- CCATGAGCGCATCGCAATC-3'

Cdkn1b Fwd: 5'- TCAAACGTGAGAGTGTCTAACG-3'

Cdkn1b Rev: 5'- CCGGGCCGAAGAGATTTCTG-3'

Cdkn1c Fwd: 5'-CGAGGAGCAGGACGAGAATC-3'

Cdkn1c Rev: 5'-GAAGAAGTCGTTTCGCATTGGC-3'

Chromatin Immunoprecipitation Sequencing (ChIP-Seq)

Embryonic NSCs (approx. 10×10^6 cells) were fixed with 1.5 mM ethylene glycol bis[succinimidyl succinate] in DMSO for 20 min at RT, followed by adding

formaldehyde to a final concentration of 1%, incubated for 10 min at RT and finally quenched by 0.2 M glycine. Cells were then lysed and sonicated with a microtip for 10 seconds followed by a 1 min break for 10 cycles. After adding 1% Triton-X to the sonicated lysates, ChIP was performed overnight at 4°C with Dynabeads M-280 Sheep anti-Rabbit IgG (Thermo Fisher, 11203D) linked to either rabbit anti-E2A (1.5 µg, sc-349X, Santa Cruz Biotechnology) or control rabbit anti-IgG (1.5 µg, sc-2027, Santa Cruz Biotechnology). 10% of sonicated suspension was kept as input control. After washing and elution of chromatin-antibody complexes from the beads at 65°C overnight, crosslinking was reversed by proteinase K and RNase digestion. Genomic DNA and DNA from the sonicated input were purified using a PCR Purification Kit (Qiagen).

Luciferase reporter transactivation assays

The reporter constructs were co-transfected with the constitutively active *Renilla reniformis* luciferase producing vector pRL-CMV (Promega) into HEK293T cells by calcium phosphate co-precipitation. Cells were lysed 24 h after transfection using passive lysis buffer (Promega). Luciferase activity was determined in duplicates using a 96-well plate reader (PerkinElmer) with automatic injection of 100 µl substrate and measurement for 10 seconds after a 2 second delay. For measuring *Renilla* luciferase activity, the *Renilla Juice Kit* (PJK) was used as a substrate according to the manufacturer's instructions. Firefly luciferase activity was quantified by using firefly substrate solution containing luciferin. For cloning of the reporter construct the following primers were used:

p57-Intron (p57-I) Fwd: 5'-AGATCTCAAGGGCCCAAGAGAGTGC-3'

p57-Intron (p57-I) Rev: 5'- AGATCTGCCAAACTTCTCTGGCCAAT-3'

p57-Downstream (p57-II) Fwd: 5'- AGATCTGTTCT-CCCGCAAGGACCATT-3'

p57-Downstream (p57-II) Rev: 5'- AGATCTGCAGAGGCAGTCCCATGAAA-3'

p57-Distal Intergenic (p57-III) Fwd: 5'-AGATCTAGGTAGGGATGGTCCCAGAC-3'

p57-Distal Intergenic (p57-III) Rev: 5'-AGATCTGGTCATACACCACAAGGGCA-3'

Immunoblots

The following antibodies were used for Western blotting: mouse anti-E12 (1:5000, BD Pharmingen, Cat.no. 6656/A), mouse anti-E47 (1:6000, BD Pharmingen, Cat.no. 554077), mouse anti-E47 (1:6666, BD Pharmingen, Cat.no. 554199), rabbit anti-GAPDH (1:1000, Cell Signaling), rabbit anti-GFP (1:1000, Cell Signaling).

Co-immunoprecipitation

Co-immunoprecipitation was performed as described (Schachtrup et al., 2015). For endogenous co-immunoprecipitation, cell lysates of primary embryonic NSC cultures were incubated with rabbit anti-mouse E2A antibody (5 µg, sc-349X, Santa Cruz Biotechnology) bound to A-agarose beads for 4 h at 4°C. Cell lysates were probed with the following antibodies: rabbit anti-Limk (1:1000, Cell Signaling), mouse anti-NeuroD1 (1:1000, Abcam), rabbit anti-NeuroD2 (1:1000, Abcam), rabbit anti-Math2 (1:500, Abcam), rabbit anti-Ngn2 (1:500, Santa Cruz), and rabbit anti-Mash1(1:500, Abcam).

***In utero* electroporation**

For *in utero* electroporation, DNA constructs were dissolved in sterile PBS (3-4 µg/µl), mixed with a fast green contrast dye and injected into the lateral ventricles of each

embryo at E13.5 of pregnant C57BL/6J mice using a microinjector and pulled Borosilicate glass capillaries. Electroporation was performed by applying ten pulses of 40 V and a pulse length of 50 ms within a 950 ms interval to the head of each embryo with the anode of the electrode oriented toward the injected side. After injection and electroporation, the uterus was replaced in the body cavity, the muscle and the skin sutured and the embryos were allowed to develop until E15.5 before isolation. The following plasmids were used: pCAGGs-IRES-eGFP and hE47^{WT}-IRES-eGFP.

Microscopy and imaging analysis

Representative images of *E2A* mRNA expression on sagittal brain sections were acquired with an Axioplan 2 Imaging epifluorescence microscope and dry Plan-NEOFLUAR objectives (10x/0,30 NA; 20x/0,50 NA), an Axiocam HRc CCD camera and the AxioVision image analysis software (Carl Zeiss). For quantification analyses of cell populations or immunoreactivity on sagittal brain sections as well as for *in vitro* overexpression assays, BrdU labeling studies, and apoptosis assays, microscopic images were acquired with an AxioImager.M2 epifluorescence microscope and dry Plan-NEOFLUAR objectives (10x/0,30 NA; 20x/0,50 NA; 40x/0,75 NA), an Axiocam HRc CCD camera and the image analysis software ZEN 2012 blue edition (Carl Zeiss). Representative images and images for quantification analyses were acquired with a TCS SP8 confocal laser scanning microscope using the 40x oil immersion objective and the LAS AF image analysis software (Leica). For quantification analyses of neurosphere diameter, microscopic images were acquired with a Leica DM LED Fluo inverted microscope and dry N PLAN objective (5x/0,12 NA) and HI PLAN objective (10x/0,25), a Leica DFC3000 G camera and the image analysis software LAS AF. For quantification

of the number of Tbr1, Satb2, Ctip2, Pax6, Sox2, NeuN, Ngn2, and cleaved caspase-3 cells, we analyzed an area in the caudal neocortex spanning the cortical wall from the ventricular surface to the pia with a width of 40 μm (Satb2, E14.5), 80 μm (Sox2, E14.5), 100 μm (NeuN, Ngn2, and Tbr2 at E14.5), 120 μm (Satb2 at E18.5), 150 μm (Satb2 at P3), 200 μm (Tbr1, Pax6, and Ctip2 at E14.5), 374 μm (Tbr1 at E18.5), 400 μm (Tbr1 at P3), and 700 μm (cleaved caspase-3). For Tbr1 bin quantification, defined areas as indicated above were subdivided into 10 equal bins along the dorsoventral axis and the number of cells in each bin was determined (Magno et al., 2012). For quantification analysis of p57(KIP2), and neurofilament, we analyzed an area in the caudal neocortex spanning the cortical wall from the ventricular surface to the pia with a width of 145 μm (neurofilament at E14.5), 200 μm (p57(KIP2) at E12.5), and 500 μm (p57(KIP2) at E14.5). For immunoreactivity analysis of E2A in Pax6+ cells (Fig. 1D), we divided Pax6 labeled VZ into apical region (40 μm from the ventricular surface) and basal region (100 μm from the apical region) (Kowalczyk et al., 2009). E2A+ cells colocalizing with Pax6 were analyzed in the apical and basal VZ with a width of 250 μm . For immunoreactivity analysis of E2A in Ki67+ cells (Fig. 1E), E2A+/Ki67+ cells (10 cells per animal) were randomly selected in the total VZ and the number of pixels with an intensity above a predetermined threshold level was determined as E2A^{high} and E2A^{low} (Fig. 1D and Fig. 1E). For quantification of the number of GFP+/p57(KIP2)+ cells after *in utero* electroporation, we analyzed an area in the middle neocortex spanning the cortical wall from the ventricular surface to the pia with a width of 80 μm . For quantification of neurite outgrowth after *in utero* electroporation and after electroporation *in vitro*, GFP+ cells with a neurite length twice that long than the diameter of the cell body of the neuron were counted as neurite bearing cells as previously described

(Schachtrup et al., 2007). For *in vivo* and *in vitro* neurite outgrowth analyses after *in utero electroporation* at least 100 GFP+ neurons per condition were measured. For quantification of the number of GFP+ cells after *in utero electroporation*, GFP+ cells were counted in the cortical plate spanning an area with a width of 80 μm (Fig. 6E, cortical plate. For quantification of the number of Ki67+ cells, EdU+/Ki67+, and PH3+ embryonic NSCs for cell proliferation and cell cycle length analyses, we analyzed an area in the caudal neocortex spanning the cortical wall from the ventricular surface to the pia with a width of 100 μm (Ki67+ and EdU+/Ki67+ cells) and 400 μm (pH3+ cells). At least three brain sections per animal were analyzed for cortical quantifications. For proliferation analyses *in vitro*, the diameter of at least 200 neurospheres and 200 BrdU-labeled embryonic NSCs were assessed. For apoptosis assays *in vitro* at least 200 embryonic NSCs per condition were analyzed.

Supplementary References

- Ernsberger, U., Patzke, H. and Rohrer, H.** (1997). The developmental expression of choline acetyltransferase (ChAT) and the neuropeptide VIP in chick sympathetic neurons: evidence for different regulatory events in cholinergic differentiation. *Mech Dev* **68**, 115-126.
- Kowalczyk, T., Pontious, A., Englund, C., Daza, R. A., Bedogni, F., Hodge, R., Attardo, A., Bell, C., Huttner, W. B. and Hevner, R. F.** (2009). Intermediate neuronal progenitors (basal progenitors) produce pyramidal-projection neurons for all layers of cerebral cortex. *Cereb Cortex* **19**, 2439-2450.
- Magno, L., Oliveira, M. G., Mucha, M., Rubin, A. N. and Kessarlis, N.** (2012). Multiple embryonic origins of nitric oxide synthase-expressing GABAergic neurons of the neocortex. *Front Neural Circuits* **6**, 65.
- Schachtrup, C., Lu, P., Jones, L. L., Lee, J. K., Lu, J., Sachs, B. D., Zheng, B. and Akassoglou, K.** (2007). Fibrinogen inhibits neurite outgrowth via beta 3 integrin-mediated phosphorylation of the EGF receptor. *Proc Natl Acad Sci U S A* **104**, 11814-11819.
- Schachtrup, C., Ryu, J. K., Mammadzada, K., Khan, A. S., Carlton, P. M., Perez, A., Christian, F., Le Moan, N., Vagena, E., Baeza-Raja, B., et al.** (2015). Nuclear pore complex remodeling by p75(NTR) cleavage controls TGF-beta signaling and astrocyte functions. *Nat Neurosci* **18**, 1077-1080.

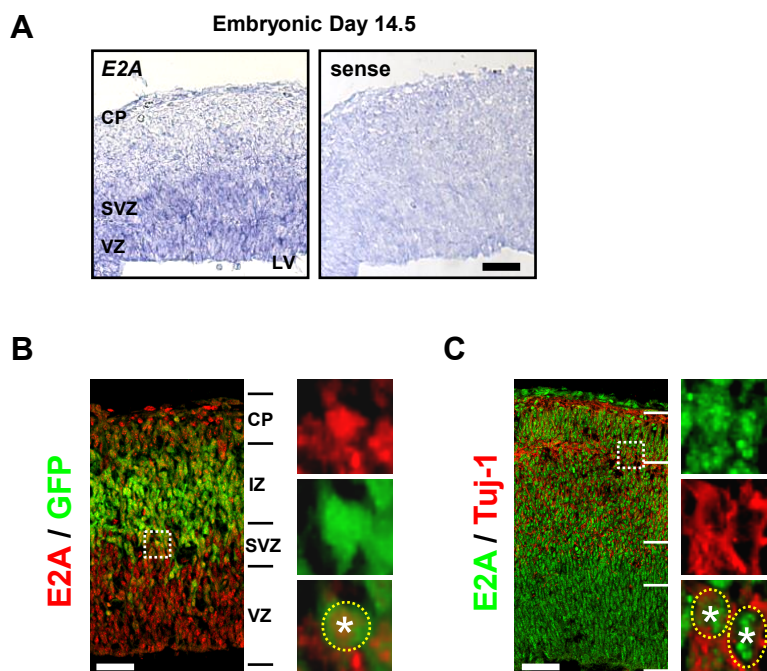


Figure S1. Expression of E2A in intermediate precursor cells and postmitotic neurons during cortical development. (A) *In situ* hybridization of E2A mRNA (blue) using antisense probe (A, left) and a sense control (A, right) of representative sagittal brain sections of C57BL/6 mice at E14.5. Scale bar: 200 μ m. ($n = 3$). (B) Immunolabeling for E2A (red) in the cortex in representative sagittal brain sections of Eomes^{GFP} mice at E14.5. Eomes^{GFP} expressing cells can be identified by green fluorescence. Scale bar: 50 μ m. ($n = 3$ mice). (C) Immunolabeling for E2A (green) in combination with Tuj-1 (red) in the cortex in representative sagittal brain sections of C57BL/6 mice at E14.5. White boxes indicate representative E2A colocalization with GFP+ (A) and Tuj-1+ (B) cells (high magnification images at the right, respectively). Scale bar: 50 μ m. ($n = 3$ mice). Abbreviations: CP, cortical plate; IZ, intermediate zone; SVZ, subventricular zone; VZ, ventricular zone.

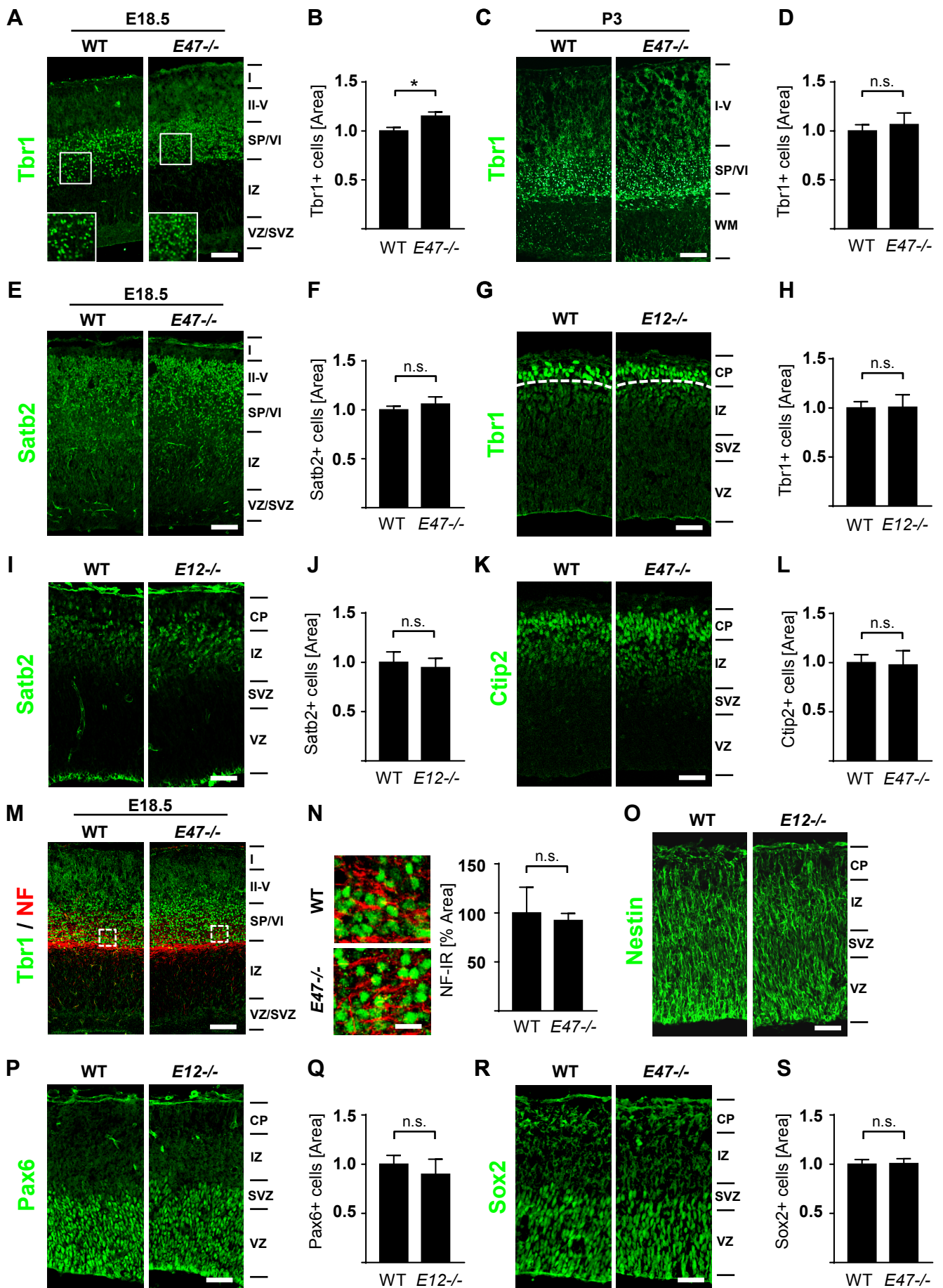


Figure S2. E12 does not regulate deep-layer or upper-layer neurogenesis at E14.5.

(A,C) Immunolabeling for Tbr1 (green) in sagittal brain sections of *E47*^{-/-} mice compared to WT littermates at E18.5 (A) and P3 (C). Scale bar: 110 μ m. (B,D) Quantification of the number of Tbr1⁺ neurons ($n = 4$ mice, B; $n = 4$ WT mice, $n = 3$ *E47*^{-/-} mice, D). (E) Immunolabeling for Satb2 (green) in sagittal brain sections of *E47*^{-/-} mice compared to WT littermates at E18.5. Scale bar: 110 μ m. (F) Quantification of the number of Satb2⁺ cells ($n = 4$ mice). (G,I) Immunolabeling for Tbr1 (G, green) and Satb2 (I, green) in sagittal brain sections of *E12*^{-/-} mice compared to WT littermates at E14.5. Scale bar: 40 μ m. (H,J) Quantification of the number of Tbr1⁺ (H) and Satb2⁺ (J) neurons ($n = 5$ WT mice, $n = 3$ *E12*^{-/-} mice, H; $n = 4$ WT mice, $n = 3$ *E12*^{-/-} mice, J). (K) Immunolabeling for Ctip2 (green) in the cortex in sagittal sections of *E47*^{-/-} mice at E14.5. Scale bar: 40 μ m. (L) Quantification of the number of Ctip2⁺ subcortical projection neurons in cortical brain sections of *E47*^{-/-} mice and WT littermates at E14.5 ($n = 4$ WT mice, $n = 5$ *E47*^{-/-} mice). (M) Immunolabeling for Tbr1 (green) and neurofilament (red) in sagittal brain sections of *E47*^{-/-} mice compared to WT littermates at E18.5. Enlargements of regions indicated by rectangles represent area of quantification. Scale bars: 110 μ m (M), 17 μ m (N). (N) Quantification of the immunoreactivity of neurofilament ($n = 3$ mice). (O) Immunolabeling for Nestin (green) in the cortex in representative sagittal sections of *E12*^{-/-} mice at E14.5 ($n = 3$ mice). Scale bar: 40 μ m. (P) Immunolabeling for Pax6 (green) in the cortex in sagittal sections of *E12*^{-/-} mice at E14.5. Scale bar: 40 μ m. (Q) Quantification of the number of Pax6⁺ radial glial cells in cortical brain sections of *E12*^{-/-} mice and WT littermates at E14.5 ($n = 5$ WT mice, $n = 3$ *E12*^{-/-} mice). (R) Immunolabeling for Sox2 (green) in the cortex in sagittal sections of *E47*^{-/-} mice at E14.5. Scale bar: 40 μ m. (S) Quantification of the number of Sox2⁺ radial glial and intermediate progenitor cells in cortical brain sections of *E47*^{-/-}

mice and WT littermates at E14.5 ($n = 7$ WT mice, $n = 6$ *E47*^{-/-} mice). Bar graphs are mean \pm s.e.m. * $P < 0.05$ calculated by Student's *t* test. Abbreviations: CP, cortical plate; SP, subplate; IZ, intermediate zone; NF, neurofilament; SVZ, subventricular zone; VZ, ventricular zone, WM, white matter.

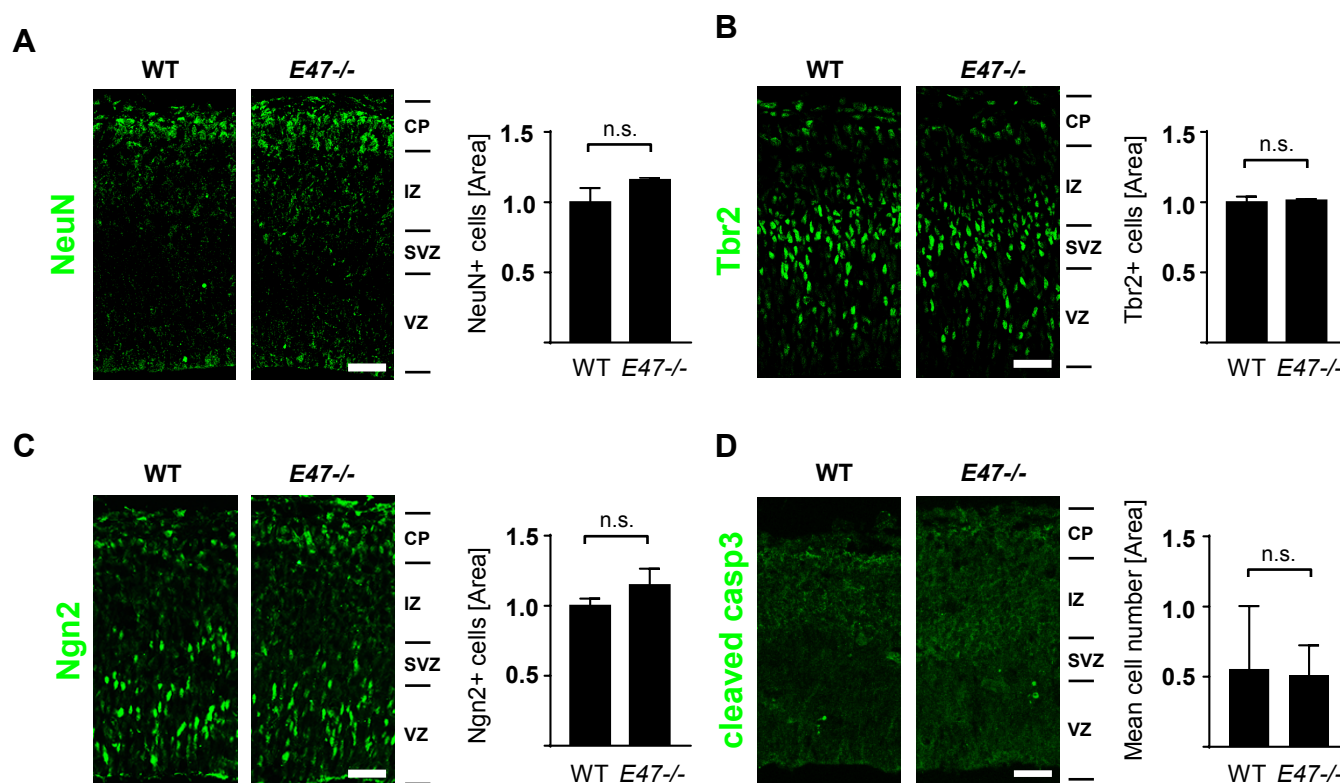


Figure S3. Distribution of intermediate precursor cells and postmitotic neurons in

***E47*^{-/-} cortices at E14.5.** (A) Immunolabeling for NeuN (green) in the cortex in sagittal

brain sections of *E47*^{-/-} mice at E14.5 (left, A) and quantification of NeuN⁺ cells in the cortical area (right, A). Scale bar: 40 μ m. ($n = 3$ mice).

(B) Immunolabeling for Tbr2 (green) in the cortex in sagittal brain sections of *E47*^{-/-} mice and WT littermates at E14.5

(left, B) and quantification of Tbr2⁺ cells in the cortical area (right, B). Scale bar: 40 μ m.

($n = 4$ WT mice, $n = 3$ *E47*^{-/-} mice). (C) Immunolabeling for Ngn2 (green) in the cortex

in sagittal brain sections of *E47*^{-/-} mice and WT littermates at E14.5 (left, C) and

quantification of Ngn2⁺ cells in the cortical area (right, C). Scale bar: 40 μ m. ($n = 6$ WT

mice, $n = 6$ *E47*^{-/-} mice). (D) Immunolabeling for cleaved caspase-3 (green) in the cortex

in sagittal brain sections of *E47*^{-/-} mice and WT littermates at E14.5 (left, D) and

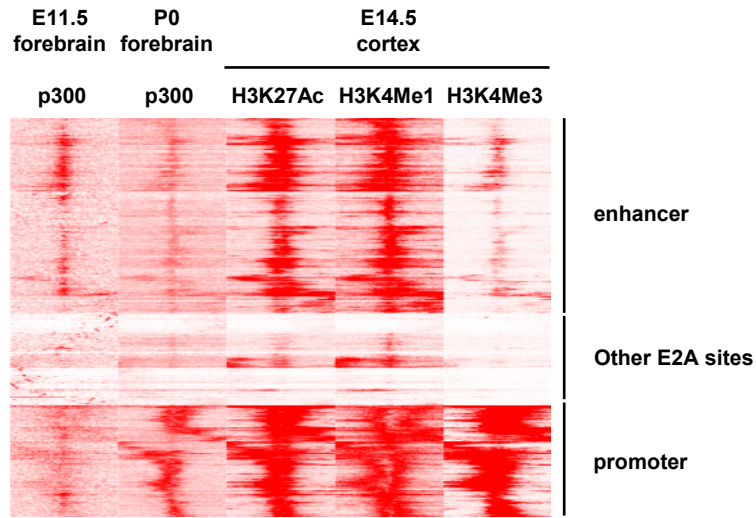
quantification of cleaved caspase-3⁺ cells in the cortical area (right, D). Scale bar: 40 μ m.

($n = 2$ WT mice, $n = 2$ *E47*^{-/-} mice). Bar graphs are mean \pm s.e.m. p -values were

calculated by Student's t test. Abbreviations: CP, cortical plate; IZ, intermediate zone;

SVZ, subventricular zone; VZ, ventricular zone.

A Coverage at E2A sites in NSCs

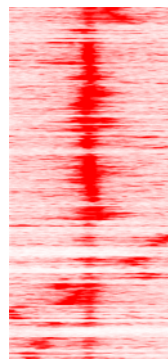


B



C

NeuroD2 coverage around E2A peaks



D

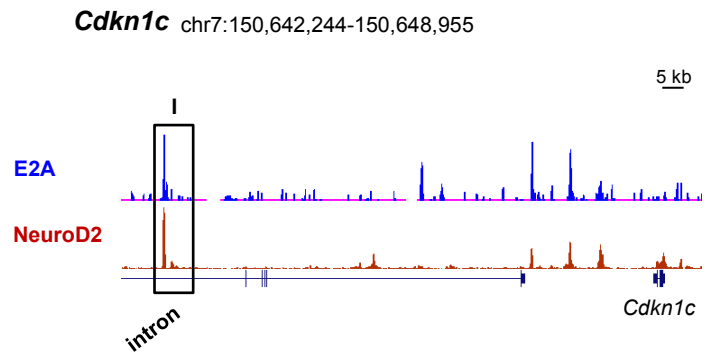


Figure S4. E2A binds predominantly to active enhancer and promoter in neuronal tissue, partially sharing binding sites with NeuroD2.

(A) Comparative analysis of genome-wide enrichment of p300 (Visel et al., 2013) and H3K4Me1, H3K4Me3 and H3K27Ac histone marks (Shen et al., 2012) around E2A binding sites in embryonic NSCs (our study). Epigenetic signatures distinguish active enhancers (H3K27Ac/H3K4Me1^{high}/H3K4Me3^{low}) from promoter regions (H3K27Ac/H3K4Me1^{low}/H3K4Me3^{high}). Note that, at promoter regions H3K4Me1 was low and H3K4Me3 was high around the peak center of E2A binding, while H3K4Me1 was preferentially increased in the surrounding regions. (B) E2A ChIP-Seq reproducibility in WT embryonic NSCs. The Venn diagram depicts the E2A binding sites identified in 3 independent ChIP-Seq experiments. Binding sites are called "reproducible" when they were identified in 2 out of 3 experiments (FDR<0.01). The ChIP-Seq tracks for the *Cdkn1c* locus show 4 reproducibly identified E2A binding sites (black arrow heads). One of those being the functional E47 enhancer in the intron of *Kcnq1* (black box I). (C,D) Genome-wide coverage of NeuroD2 binding events (Bayam et al., 2015) around E2A binding sites in embryonic NSCs (C) including the identified poised enhancer p-57 I located in the *Cdkn1c* locus (D).

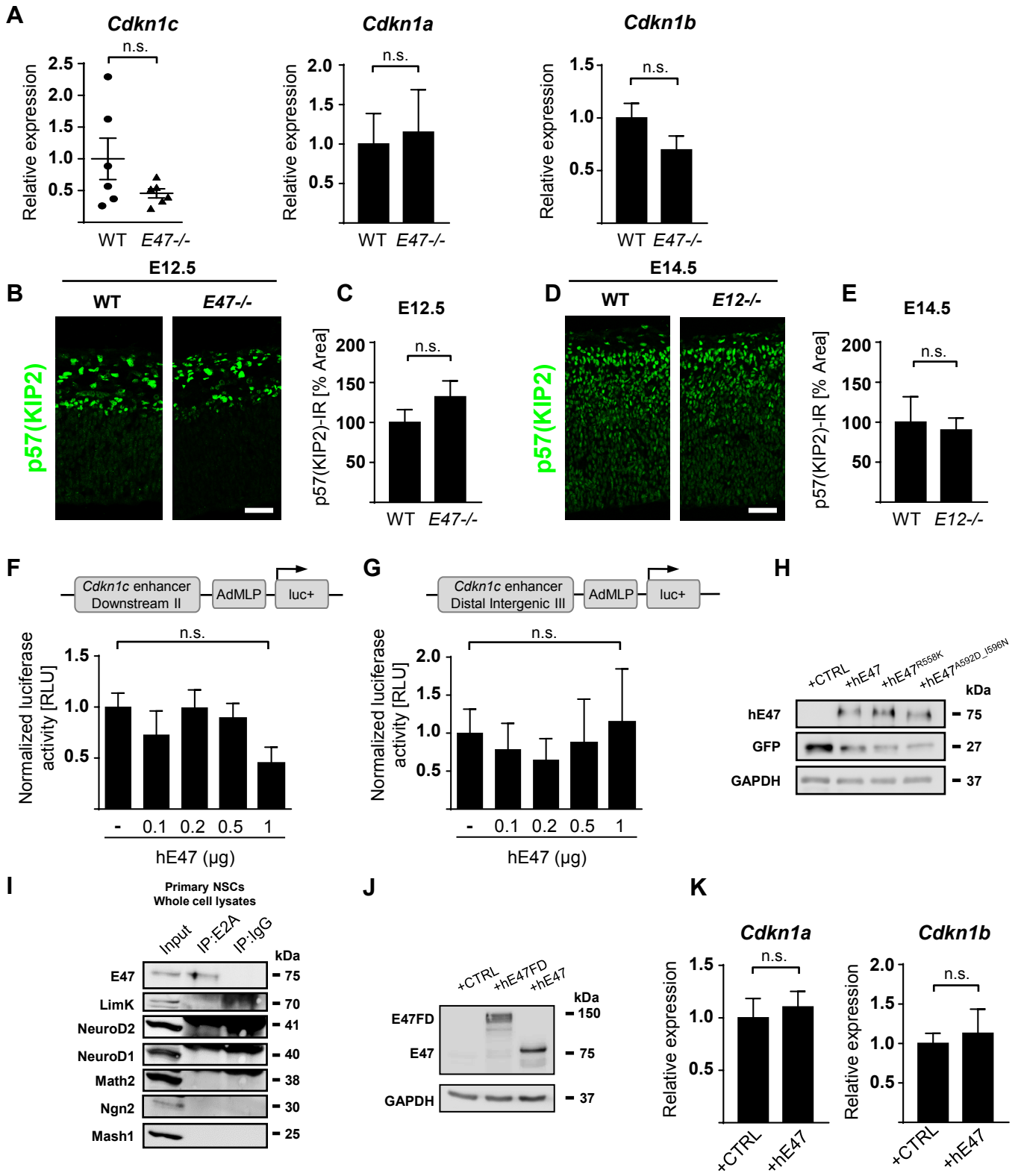


Figure S5. E47 does not regulate mRNA expression of *Cdkn1a* and *Cdkn1b* in embryonic NSCs. (A) Expression of *Cdkn1c*, *Cdkn1a*, and *Cdkn1b* mRNA in embryonic WT and *E47*^{-/-} NSCs under proliferation conditions. Quantitative PCR results are of four independent experiments performed in duplicate. (B) Immunolabeling for p57(KIP2) (green) in sagittal brain sections of *E47*^{-/-} mice compared to WT littermates at E12.5. Scale bar: 40 μ m. (C) Quantification of the p57(KIP2) immunoreactivity ($n = 3$ WT mice, $n = 4$ *E47*^{-/-} mice). (D) Immunolabeling for p57(KIP2) (green) in sagittal brain sections of *E12*^{-/-} mice compared to WT littermates at E14.5. Scale bar: 40 μ m. (E) Quantification of the p57(KIP2) immunoreactivity ($n = 3$ WT mice, $n = 3$ *E12*^{-/-} mice). (F,G) Luciferase reporter assay in HEK293T cells using the indicated p57(KIP2) luciferase reporter construct (F, *Cdkn1c* enhancer II termed as downstream) and (G, *Cdkn1c* enhancer III termed as distal intergenic). Luciferase reporter assay results are of three independent experiments performed in duplicate. (H) Immunoblot protein expression analysis for E47 in FACS sorted mouse embryonic NSCs 24 h after electroporation with WT hE47^{WT}-IRES-eGFP, mutant hE47^{R558K}-IRES-eGFP and mutant hE47^{A592D_I596N}-IRES-eGFP plasmid. Representative Western blots are shown from two independent experiments. (I) Endogenous coimmunoprecipitation of E47 with potential interaction partners in whole embryonic NSC lysates. Representative immunoblots are shown from two independent experiments. (J) Immunoblot protein expression analysis for hE47 forced homodimer (hE47^{FD}) in HEK293T cells 24 h after transfection with hE47^{FD}-pMIG plasmid. Representative Western blots are shown from two independent experiments. (K) Expression of *Cdkn1a* mRNA (left) and *Cdkn1b* mRNA (right) in embryonic NSCs 24 h after electroporation with hE47^{WT}-IRES-eGFP plasmid in comparison to cells electroporated with pCAGGs-IRES-eGFP control plasmid determined by quantitative PCR and normalized to *GAPDH*. Quantitative PCR results are of six independent experiments performed in duplicate. Bar graphs are mean \pm s.e.m. p -values were calculated by Student's t test (A,C,E,K) or by one-way ANOVA (F,G)]. Abbreviations: CDKI, cyclin-dependent kinase inhibitor protein; RLU, relative light unit.

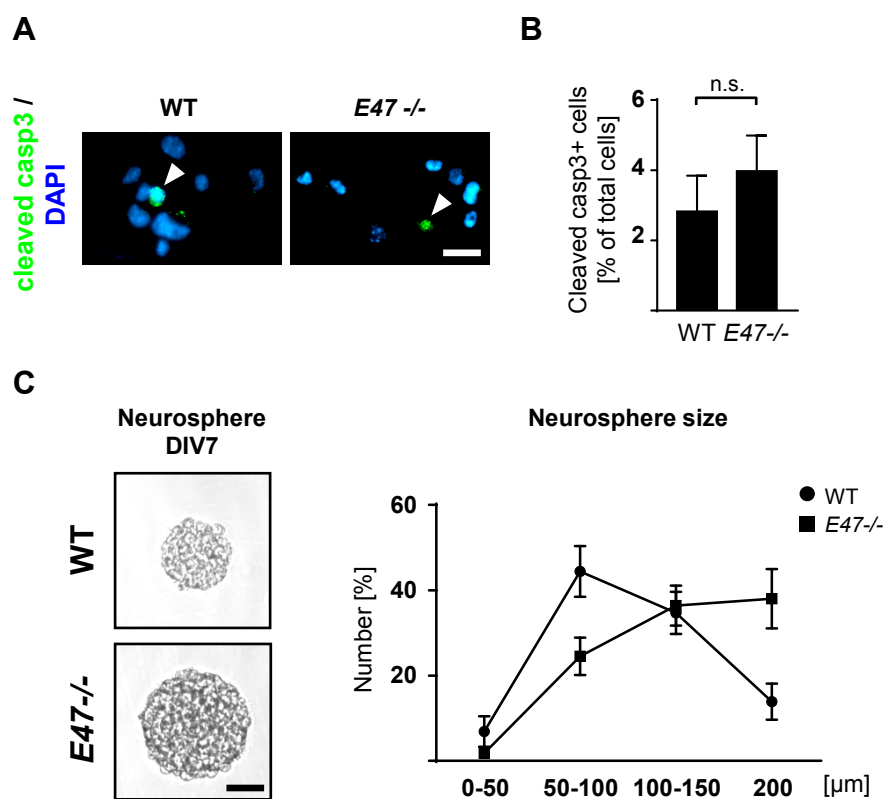


Figure S6. E47-deficiency does not affect apoptotic cell death but neurosphere size of embryonic NSCs *in vitro*. (A,B) Immunolabeling for cleaved caspase-3 (green) of *E47*^{-/-} and WT embryonic NSCs (A) and quantification of cleaved caspase-3+ cells (B). Scale bar: 20 μm. Apoptotic cell death results are of three independent experiments. (C) Distribution of the diameter of individual formed neurospheres from *E47*^{-/-} and WT dissociated primary NSCs after 7 DIV in proliferation medium. *E47*^{-/-} embryonic NSCs formed increased numbers of bigger size neurospheres (200 μm), but less medium size neurospheres (50-100 μm) compared to WT NSCs. Scale bar: 40 μm. Neurosphere size results are of three independent experiments. Values are mean ± s.e.m. *p*-values were calculated by Student's *t* test (B).

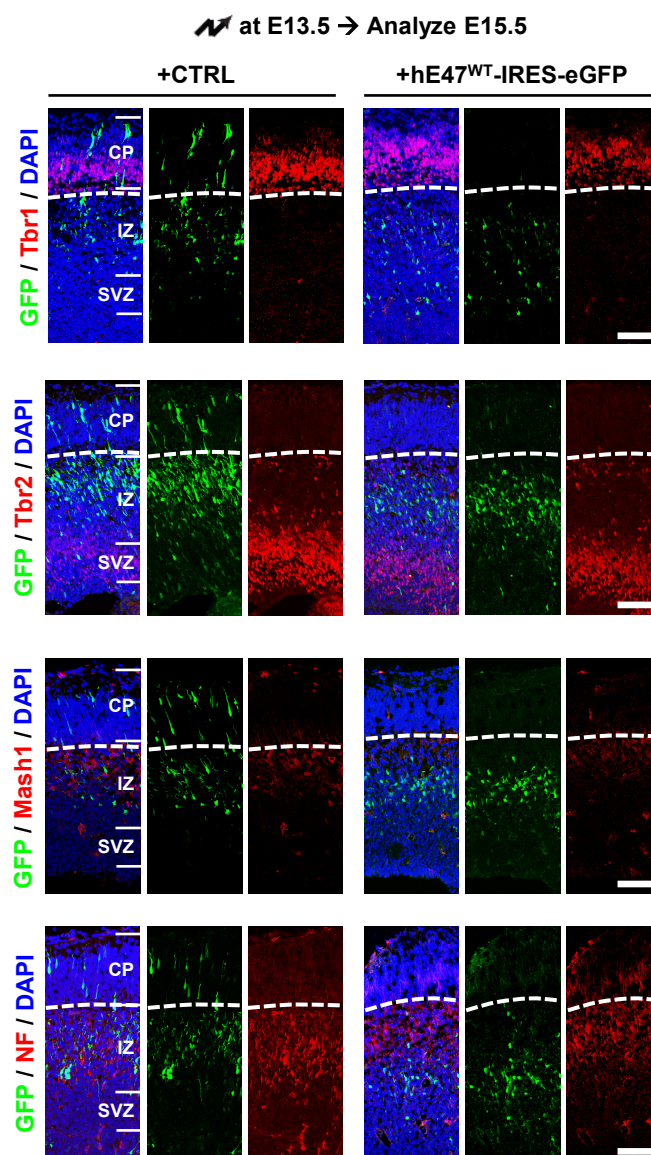


Figure S7. E47 overexpressing cells show a failure in lower layer neuron differentiation. *In utero* electroporation of WT mice at E13.5 with either pCAGGs-IRES-eGFP or hE47^{WT}-IRES-eGFP. Immunolabeling was performed for Tbr1 (red, deep-layer neuron marker, top row), Tbr2 (red, intermediate progenitor cells, second top row), Mash1 (red, ventral telencephalon neuron, second bottom row), neurofilament (red, projecting axons, bottom) in combination with GFP (green, electroporated cells) in sagittal brain sections at E15.5. Scale bars: 80 μ m (left), Nuclei are stained with DAPI (blue). Abbreviations: CP, cortical plate; IZ, intermediate zone; NF, neurofilament; SVZ, subventricular zone.

Table S1: Overlapping differentially expressed genes in *E12*^{-/-} and *E47*^{-/-} primary embryonic NSCs having an E2A binding site in WT cells. Depicted are the results from RNA-Seq indicating the fold-change in gene expression as log₂ (log₂FC) in *E47*^{-/-} or *E12*^{-/-} NSCs, variance between triplicates as P.

Symbol	log ₂ FC <i>E47</i> ^{-/-} -P <i>E47</i> ^{-/-}	log ₂ FC <i>E12</i> ^{-/-} -P <i>E12</i> ^{-/-}	
Tdg 120	-1.75	5.15E-45	-1.64 1.39E-
Csmd3 01	-1.23	7.20E-03	-0.60 2.03E-
Ckmt1 02	-1.14	5.40E-03	-0.72 6.99E-
1190007I07Rik 04	-1.07	3.65E-05	-1.00 3.40E-
Adams3 12	-1.06	4.05E-04	-0.84 9.06E-
Coll1a1 13	-1.05	1.87E-01	-1.85 6.19E-
Plppr5 04	-0.97	3.13E-03	-0.86 4.49E-
Pcdh15 03	-0.96	3.15E-03	-1.32 7.25E-
Cntn1 02	-0.90	2.87E-05	-0.96 1.98E-
Plppr1 04	-0.90	3.37E-04	-0.97 1.57E-
Moxd1 03	-0.86	9.05E-02	-1.45 5.06E-
Brinp1 02	-0.86	3.70E-02	-1.18 1.10E-
Unc5c 02	-0.84	1.87E-02	-0.80 1.48E-
Dpp10 01	-0.83	2.82E-03	-1.20 1.43E-
Clic5 03	-0.81	2.06E-02	0.84 2.29E-
Ptpn5 03	-0.80	3.94E-03	-0.97 2.86E-
Grik3 02	-0.78	2.06E-05	-0.95 1.39E-
Shisa7 01	-0.73	8.15E-03	-0.99 1.27E-

Hmcn1 04	-0.72	5.81E-02	0.69	2.92E-
Nkd2 03	-0.72	7.53E-02	-0.63	6.61E-
Kcnq2 02	-0.71	9.02E-02	-1.13	2.10E-
Gabrb2 03	-0.70	2.23E-02	-0.85	4.36E-
Cobl 02	-0.70	1.82E-01	-0.67	3.18E-
Spo11 06	-0.69	1.60E-01	-1.43	6.96E-
Vat1l 02	-0.67	9.92E-02	-1.12	9.67E-
Cntnap5b 03	-0.66	2.16E-02	-0.74	9.49E-
B3gat2 03	-0.66	2.07E-03	-0.74	6.41E-
Atp10b 02	-0.66	1.24E-01	-0.88	2.40E-
Ppfia2 02	-0.65	9.62E-02	-0.71	1.45E-
3110035E14Rik 03	-0.65	9.81E-02	-1.37	3.46E-
Gm3739 02	-0.65	1.34E-01	-0.65	6.95E-
Stk32a 02	-0.63	5.19E-02	-1.81	1.66E-
AW551984 02	-0.63	6.08E-02	-0.59	9.32E-
Ugt8a 02	-0.63	4.09E-02	-0.71	1.14E-
Hrk 01	-0.61	3.65E-01	-0.60	1.16E-
Clgn 03	-0.61	1.62E-01	-0.71	4.93E-
Slc1a1 02	-0.61	1.18E-01	-1.04	2.88E-
Rasgrp3 02	-0.60	3.99E-01	0.61	4.45E-
Tenm2 03	-0.59	6.81E-02	-1.07	1.06E-
Chrm1 02	-0.58	1.44E-01	-0.69	2.00E-
Dmbx1 02	-0.58	3.05E-01	0.87	1.20E-

Nefl 02	-0.58	3.33E-01	-0.90	1.76E-
Kif5c 03	-0.58	5.28E-02	-0.63	4.11E-
Cdo1 04	0.63	2.51E-01	-0.94	9.17E-
Tnfaip2 02	0.67	2.87E-01	-0.59	8.87E-
Dscaml1 03	0.68	2.90E-01	-0.91	1.83E-
Plp1 03	0.71	2.04E-01	-0.70	4.63E-
Junb 02	0.78	3.92E-02	0.86	3.27E-
Ky 02	1.08	3.30E-02	0.68	6.99E-
Thy1 02	1.14	3.93E-02	0.67	3.19E-
Selenbp1 02	1.14	5.20E-02	-0.61	5.45E-
Agt 04	1.16	1.56E-01	1.11	1.61E-
Cacng5 02	1.19	1.06E-02	0.72	1.25E-
Als2cl 04	1.21	2.22E-02	0.69	5.20E-
Sox10 01	1.25	5.10E-02	-1.29	1.01E-
Six3os1 01	1.47	3.79E-01	-1.07	6.03E-
Slc30a10 02	1.57	4.68E-02	-0.62	3.63E-
Ndr2 02	1.67	1.42E-01	-1.05	1.17E-
Emp1 02	2.04	9.43E-02	-0.71	1.02E-
Gm9855 214	6.76	7.57E-111	6.92	7.96E-

Table S2: Differentially expressed genes in *E47*^{-/-} primary embryonic NSCs having an E2A binding site in WT cells. Depicted are the results from RNA-Seq indicating the fold-change in gene expression as log₂ (log₂FC), variance between triplicates as P or Benjamin-Hochberg adjusted P value (adjP).

ensembl	log ₂ FC	P	adjP	Symbol
ENSMUSG00000034674	-1.75	5.15E-45	3.31E-41	Tdg
ENSMUSG00000063320	-1.07	3.65E-05	4.26E-02	1190007I07Rik
ENSMUSG00000043635	-1.06	4.05E-04	2.89E-01	Adamts3
ENSMUSG00000001506	-1.05	1.87E-01	9.99E-01	Coll1a1
ENSMUSG00000022206	-0.97	1.15E-01	9.99E-01	Npr3
ENSMUSG00000052613	-0.96	3.15E-03	9.20E-01	Pcdh15
ENSMUSG00000063446	-0.90	3.37E-04	2.57E-01	Plppr1
ENSMUSG00000030222	-0.90	3.71E-02	9.99E-01	Rerg
ENSMUSG00000068373	-0.88	7.73E-02	9.99E-01	D430041D05Rik
ENSMUSG00000020000	-0.86	9.05E-02	9.99E-01	Moxd1
ENSMUSG00000029334	-0.85	3.56E-03	9.58E-01	Prkg2
ENSMUSG00000036815	-0.83	2.82E-03	9.20E-01	Dpp10
ENSMUSG00000049252	-0.82	1.26E-03	6.72E-01	Lrp1b
ENSMUSG00000030854	-0.80	3.94E-03	9.68E-01	Ptpn5
ENSMUSG00000041670	-0.76	3.05E-03	9.20E-01	Rims1
ENSMUSG00000066842	-0.72	5.81E-02	9.99E-01	Hmcn1
ENSMUSG00000046500	-0.71	6.18E-02	9.99E-01	Fam19a4
ENSMUSG00000033147	-0.71	1.97E-02	9.99E-01	Slc22a15
ENSMUSG00000020173	-0.70	1.82E-01	9.99E-01	Cobl
ENSMUSG00000005883	-0.69	1.60E-01	9.99E-01	Spo11
ENSMUSG00000026748	-0.68	1.07E-06	2.75E-03	Plxdc2
ENSMUSG00000071424	-0.67	1.08E-01	9.99E-01	Grid2
ENSMUSG00000063434	-0.67	8.20E-03	9.99E-01	Sorcs3
ENSMUSG00000064293	-0.67	2.14E-02	9.99E-01	Cntn4
ENSMUSG00000020181	-0.67	1.41E-02	9.99E-01	Nav3
ENSMUSG00000067028	-0.66	2.16E-02	9.99E-01	Cntnap5b
ENSMUSG00000047261	-0.66	1.43E-01	9.99E-01	Gap43
ENSMUSG00000053825	-0.65	9.62E-02	9.99E-01	Ppfi2
ENSMUSG00000008658	-0.65	2.75E-01	9.99E-01	Rbfox1
ENSMUSG00000073805	-0.65	2.57E-02	9.99E-01	Fam196a
ENSMUSG00000030077	-0.64	2.46E-01	9.99E-01	Chl1
ENSMUSG00000056758	-0.64	9.09E-02	9.99E-01	Hmga2
ENSMUSG00000022021	-0.63	3.53E-06	7.56E-03	Diaph3
ENSMUSG00000039954	-0.63	5.19E-02	9.99E-01	Stk32a
ENSMUSG00000000296	-0.62	5.13E-02	9.99E-01	Tpd52l1
ENSMUSG00000037855	-0.62	2.31E-02	9.99E-01	Zfp365
ENSMUSG00000044770	-0.61	2.91E-01	9.99E-01	Scml4

ENSMUSG00000022894	-0.61	5.00E-02	9.99E-01	Adamts5
ENSMUSG00000009628	-0.61	2.01E-01	9.99E-01	Tex15
ENSMUSG00000038168	-0.61	2.26E-01	9.99E-01	P3h2
ENSMUSG00000024935	-0.61	1.18E-01	9.99E-01	Slc1a1
ENSMUSG00000046159	-0.60	1.34E-01	9.99E-01	Chrm3
ENSMUSG00000035150	-0.60	4.34E-03	9.96E-01	Eif2s3x
ENSMUSG00000071042	-0.60	3.99E-01	9.99E-01	Rasgrp3
ENSMUSG00000032024	-0.60	6.52E-02	9.99E-01	Clmp
ENSMUSG00000049336	-0.59	6.81E-02	9.99E-01	Tenm2
ENSMUSG00000030022	-0.59	1.11E-01	9.99E-01	Adamts9
ENSMUSG00000026514	-0.59	4.56E-02	9.99E-01	Cnih3
ENSMUSG00000079410	-0.59	1.96E-01	9.99E-01	Gm2897
ENSMUSG00000026764	-0.58	5.28E-02	9.99E-01	Kif5c
ENSMUSG00000035342	0.58	2.35E-02	9.99E-01	Lzts2
ENSMUSG00000031661	0.58	2.37E-01	9.99E-01	Nkd1
ENSMUSG00000035547	0.58	1.16E-01	9.99E-01	Capn5
ENSMUSG00000044164	0.59	2.09E-01	9.99E-01	Rnf182
ENSMUSG00000035891	0.59	1.63E-01	9.99E-01	Cerk
ENSMUSG00000020263	0.59	1.69E-01	9.99E-01	Appl2
ENSMUSG00000026923	0.60	5.18E-02	9.99E-01	Notch1
ENSMUSG00000044197	0.60	2.55E-01	9.99E-01	Gpr146
ENSMUSG00000020893	0.60	1.34E-01	9.99E-01	Per1
ENSMUSG00000022425	0.60	2.70E-01	9.99E-01	Enpp2
ENSMUSG00000047085	0.60	4.94E-02	9.99E-01	Lrrc4b
ENSMUSG00000018451	0.61	1.49E-01	9.99E-01	6330403K07Rik
ENSMUSG00000046961	0.61	3.23E-01	9.99E-01	Gpr156
ENSMUSG00000037003	0.61	1.17E-01	9.99E-01	Tns2
ENSMUSG00000039095	0.61	1.25E-01	9.99E-01	En2
ENSMUSG00000022122	0.61	1.92E-01	9.99E-01	Ednrb
ENSMUSG00000055652	0.61	6.88E-02	9.99E-01	Klhl25
ENSMUSG00000030986	0.61	9.15E-02	9.99E-01	Dhx32
ENSMUSG00000068099	0.61	2.87E-01	9.99E-01	1500009C09Rik
ENSMUSG00000029723	0.61	1.40E-01	9.99E-01	Tsc22d4
ENSMUSG00000032204	0.61	2.11E-01	9.99E-01	Aqp9
ENSMUSG00000048001	0.62	6.11E-02	9.99E-01	Hes5
ENSMUSG00000021250	0.62	1.23E-01	9.99E-01	Fos
ENSMUSG00000044786	0.62	1.31E-01	9.99E-01	Zfp36
ENSMUSG00000021806	0.62	2.21E-02	9.99E-01	Nid2
ENSMUSG00000028756	0.62	1.40E-01	9.99E-01	Pink1
ENSMUSG00000021453	0.62	8.16E-02	9.99E-01	Gadd45g
ENSMUSG00000029049	0.62	8.53E-02	9.99E-01	Morn1
ENSMUSG00000036306	0.62	1.66E-01	9.99E-01	Lzts1
ENSMUSG00000015090	0.62	6.85E-02	9.99E-01	Ptgds
ENSMUSG00000040260	0.63	2.14E-01	9.99E-01	Daam2
ENSMUSG00000030790	0.63	1.41E-01	9.99E-01	Adm
ENSMUSG00000000794	0.63	3.09E-01	9.99E-01	Kcnn3
ENSMUSG00000026227	0.63	1.47E-01	9.99E-01	2810459M11Rik

ENSMUSG00000002908	0.63	1.77E-01	9.99E-01	Kcnn1
ENSMUSG00000056204	0.63	1.18E-01	9.99E-01	Pgpep1
ENSMUSG00000022565	0.63	1.24E-01	9.99E-01	Plec
ENSMUSG00000032269	0.63	3.09E-01	9.99E-01	Htr3a
ENSMUSG00000034472	0.63	3.61E-01	9.99E-01	Rasd2
ENSMUSG00000032298	0.64	3.12E-02	9.99E-01	Neil1
ENSMUSG00000027333	0.64	1.02E-01	9.99E-01	Smox
ENSMUSG00000024206	0.64	1.07E-02	9.99E-01	Rfx2
ENSMUSG00000037705	0.64	2.24E-01	9.99E-01	Tecta
ENSMUSG00000056938	0.65	5.67E-02	9.99E-01	Acbd4
ENSMUSG00000051703	0.65	1.09E-01	9.99E-01	Tmem198
ENSMUSG00000030337	0.65	1.12E-01	9.99E-01	Vamp1
ENSMUSG00000033083	0.65	1.16E-01	9.99E-01	Tbc1d4
ENSMUSG00000042647	0.66	1.58E-01	9.99E-01	Acad12
ENSMUSG00000021379	0.66	1.47E-01	9.99E-01	Id4
ENSMUSG00000033059	0.66	1.31E-01	9.99E-01	Pygb
ENSMUSG00000023800	0.66	1.63E-01	9.99E-01	Tiam2
ENSMUSG00000027684	0.66	2.05E-01	9.99E-01	Mecom
ENSMUSG00000004415	0.67	1.68E-01	9.99E-01	Col26a1
ENSMUSG00000023990	0.67	4.72E-02	9.99E-01	Tfeb
ENSMUSG00000002409	0.67	1.85E-01	9.99E-01	Dyrk1b
ENSMUSG00000040841	0.67	2.59E-02	9.99E-01	Six5
ENSMUSG00000020297	0.67	1.64E-01	9.99E-01	Nsg2
ENSMUSG00000006205	0.67	1.06E-01	9.99E-01	Htra1
ENSMUSG00000055799	0.67	6.79E-02	9.99E-01	Tcf7l1
ENSMUSG00000058586	0.67	7.10E-02	9.99E-01	Serhl
ENSMUSG00000041351	0.67	5.43E-02	9.99E-01	Rap1gap
ENSMUSG00000032656	0.68	1.96E-01	9.99E-01	March3
ENSMUSG00000069633	0.68	1.63E-01	9.99E-01	Pex11g
ENSMUSG00000042804	0.68	1.86E-01	9.99E-01	Gpr153
ENSMUSG00000032087	0.68	2.90E-01	9.99E-01	Dscaml1
ENSMUSG00000032741	0.69	9.26E-02	9.99E-01	Tpcn1
ENSMUSG00000003436	0.69	1.03E-01	9.99E-01	Dll3
ENSMUSG00000050953	0.69	8.04E-03	9.99E-01	Gja1
ENSMUSG00000020785	0.70	1.42E-01	9.99E-01	Camkk1
ENSMUSG00000005686	0.70	6.05E-02	9.99E-01	Ampd3
ENSMUSG00000044337	0.71	3.65E-01	9.99E-01	Ackr3
ENSMUSG00000021127	0.71	6.95E-02	9.99E-01	Zfp361l
ENSMUSG00000034227	0.71	3.92E-02	9.99E-01	Foxj1
ENSMUSG00000074170	0.71	8.35E-02	9.99E-01	Plekhf1
ENSMUSG00000030621	0.71	1.91E-01	9.99E-01	Me3
ENSMUSG00000031765	0.72	5.48E-02	9.99E-01	Mt1
ENSMUSG00000058056	0.72	4.01E-02	9.99E-01	Palld
ENSMUSG00000001911	0.73	2.66E-02	9.99E-01	Nfix
ENSMUSG00000043557	0.73	8.60E-02	9.99E-01	Mdgal
ENSMUSG00000046618	0.74	1.58E-01	9.99E-01	Olfml2a
ENSMUSG00000010660	0.74	1.71E-01	9.99E-01	Plcd1

ENSMUSG00000063160	0.74	8.40E-02	9.99E-01	Numbl
ENSMUSG00000049556	0.74	9.13E-02	9.99E-01	Lingo1
ENSMUSG00000055782	0.74	1.21E-01	9.99E-01	Abcd2
ENSMUSG00000001750	0.75	4.08E-02	9.99E-01	Tcirg1
ENSMUSG00000001552	0.75	5.94E-02	9.99E-01	Jup
ENSMUSG00000049608	0.75	8.31E-02	9.99E-01	Gpr55
ENSMUSG00000035357	0.75	6.39E-02	9.99E-01	Pdzrn3
ENSMUSG00000022790	0.75	1.50E-01	9.99E-01	Igsf11
ENSMUSG00000049265	0.76	2.90E-01	9.99E-01	Kcnk3
ENSMUSG00000092274	0.76	1.14E-01	9.99E-01	Neat1
ENSMUSG00000032368	0.76	1.86E-02	9.99E-01	Zic1
ENSMUSG00000027298	0.76	4.17E-02	9.99E-01	Tyro3
ENSMUSG00000016028	0.77	9.62E-02	9.99E-01	Celsr1
ENSMUSG00000022382	0.77	3.07E-01	9.99E-01	Wnt7b
ENSMUSG00000000325	0.79	8.53E-02	9.99E-01	Arvcf
ENSMUSG00000032744	0.79	2.51E-01	9.99E-01	Heyl
ENSMUSG00000006958	0.80	2.65E-02	9.99E-01	Chrd
ENSMUSG00000031983	0.81	9.49E-02	9.99E-01	2310022B05Rik
ENSMUSG00000071604	0.81	1.43E-01	9.99E-01	Fam189a2
ENSMUSG00000036528	0.81	3.22E-01	9.99E-01	Ppfibp2
ENSMUSG00000023913	0.81	3.57E-01	9.99E-01	Pla2g7
ENSMUSG00000004105	0.82	1.24E-01	9.99E-01	Angptl2
ENSMUSG00000020108	0.83	2.67E-01	9.99E-01	Ddit4
ENSMUSG00000030087	0.84	3.11E-01	9.99E-01	Klf15
ENSMUSG00000028927	0.84	2.53E-01	9.99E-01	Padi2
ENSMUSG00000030428	0.84	3.02E-01	9.99E-01	Ttyh1
ENSMUSG00000052135	0.84	1.52E-02	9.99E-01	Foxo6
ENSMUSG00000068299	0.85	1.24E-01	9.99E-01	Nat8f4
ENSMUSG00000060376	0.85	5.58E-02	9.99E-01	Bckdha
ENSMUSG00000041544	0.85	2.57E-02	9.99E-01	Disp3
ENSMUSG00000023885	0.86	2.24E-01	9.99E-01	Thbs2
ENSMUSG00000040659	0.86	2.93E-02	9.99E-01	Efhd2
ENSMUSG00000034614	0.86	1.28E-01	9.99E-01	Pik3ip1
ENSMUSG00000019960	0.87	2.74E-01	9.99E-01	Dusp6
ENSMUSG00000029413	0.88	1.97E-01	9.99E-01	Naaa
ENSMUSG00000024076	0.88	6.08E-02	9.99E-01	Vit
ENSMUSG00000028558	0.88	2.97E-01	9.99E-01	Calr4
ENSMUSG00000047786	0.89	9.87E-05	9.75E-02	Lix1
ENSMUSG00000020591	0.89	1.01E-01	9.99E-01	Ntsr2
ENSMUSG00000057060	0.89	1.77E-01	9.99E-01	Slc35f3
ENSMUSG00000040093	0.90	3.65E-01	9.99E-01	Bmf
ENSMUSG00000027447	0.92	6.95E-02	9.99E-01	Cst3
ENSMUSG00000002346	0.93	9.56E-02	9.99E-01	Slc25a42
ENSMUSG00000032394	0.93	9.59E-02	9.99E-01	Igdcc3
ENSMUSG00000053113	0.93	2.11E-01	9.99E-01	Socs3
ENSMUSG00000047361	0.93	1.33E-01	9.99E-01	Gm973
ENSMUSG00000032268	0.94	1.10E-01	9.99E-01	Tmprss5

ENSMUSG00000030605	0.95	1.93E-01	9.99E-01	Mfge8
ENSMUSG00000036995	0.96	8.24E-02	9.99E-01	Asap3
ENSMUSG00000070509	0.96	2.22E-01	9.99E-01	Rgma
ENSMUSG00000069805	0.96	5.21E-02	9.99E-01	Fbp1
ENSMUSG00000022358	0.97	2.99E-01	9.99E-01	Fbxo32
ENSMUSG00000036611	0.99	7.63E-02	9.99E-01	Eepd1
ENSMUSG00000022438	1.00	4.86E-02	9.99E-01	Parvb
ENSMUSG00000025911	1.00	6.89E-02	9.99E-01	Adhfe1
ENSMUSG00000028919	1.01	5.78E-02	9.99E-01	Arhgef19
ENSMUSG00000034714	1.02	2.17E-01	9.99E-01	Ttyh2
ENSMUSG00000063171	1.03	4.01E-03	9.68E-01	Rps4l
ENSMUSG00000039533	1.03	3.00E-01	9.99E-01	Mmd2
ENSMUSG00000024552	1.04	3.16E-02	9.99E-01	Slc14a2
ENSMUSG00000019359	1.05	6.38E-02	9.99E-01	Gdpd2
ENSMUSG00000018169	1.06	1.76E-02	9.99E-01	Mfng
ENSMUSG00000032556	1.08	1.58E-01	9.99E-01	Bfsp2
ENSMUSG00000035606	1.08	3.30E-02	9.99E-01	Ky
ENSMUSG00000047793	1.09	9.86E-02	9.99E-01	Sned1
ENSMUSG00000019577	1.10	1.84E-01	9.99E-01	Pdk4
ENSMUSG00000071550	1.11	1.77E-01	9.99E-01	Cfap44
ENSMUSG00000026475	1.12	5.18E-03	9.99E-01	Rgs16
ENSMUSG00000057880	1.12	2.40E-01	9.99E-01	Abat
ENSMUSG00000059742	1.13	1.11E-01	9.99E-01	Kcnh7
ENSMUSG00000041771	1.15	8.22E-02	9.99E-01	Slc24a4
ENSMUSG00000028167	1.16	1.06E-01	9.99E-01	Bdh2
ENSMUSG00000030088	1.17	9.51E-03	9.99E-01	Aldh11l
ENSMUSG00000014846	1.19	2.84E-02	9.99E-01	Tppp3
ENSMUSG00000040373	1.19	1.06E-02	9.99E-01	Cacng5
ENSMUSG00000024227	1.21	1.35E-01	9.99E-01	Pdzph1
ENSMUSG00000040495	1.21	9.03E-02	9.99E-01	Chrm4
ENSMUSG00000046182	1.22	1.86E-01	9.99E-01	Gsg1l
ENSMUSG00000025780	1.26	2.11E-01	9.99E-01	Itih5
ENSMUSG00000034706	1.29	9.17E-02	9.99E-01	Dnaic2
ENSMUSG00000039410	1.31	4.33E-02	9.99E-01	Prdm16
ENSMUSG00000041556	1.32	2.92E-02	9.99E-01	Fbxo2
ENSMUSG00000047935	1.32	7.87E-03	9.99E-01	Gm5607
ENSMUSG00000071847	1.33	1.54E-01	9.99E-01	Apcdd1
ENSMUSG00000017692	1.33	1.03E-01	9.99E-01	Rhbdl3
ENSMUSG00000051041	1.34	2.06E-01	9.99E-01	Olfml1
ENSMUSG00000022494	1.40	8.11E-02	9.99E-01	Shisa9
ENSMUSG00000049555	1.40	2.28E-02	9.99E-01	Tmie
ENSMUSG00000029005	1.42	1.05E-01	9.99E-01	Draxin
ENSMUSG00000027215	1.42	1.67E-01	9.99E-01	Cd82
ENSMUSG00000053846	1.47	4.45E-02	9.99E-01	Lipg
ENSMUSG00000090122	1.48	3.25E-03	9.28E-01	Kcne1l
ENSMUSG00000054477	1.48	2.30E-01	9.99E-01	Kcnn2
ENSMUSG00000021933	1.49	5.24E-02	9.99E-01	Gucy1b2

ENSMUSG00000024197	1.54	7.38E-03	9.99E-01	Plin3
ENSMUSG00000026614	1.57	4.68E-02	9.99E-01	Slc30a10
ENSMUSG00000007279	1.61	6.41E-02	9.99E-01	Scube2
ENSMUSG00000042607	1.81	8.73E-03	9.99E-01	Asb4
ENSMUSG00000030208	2.04	9.43E-02	9.99E-01	Emp1
ENSMUSG00000021848	2.73	9.92E-02	9.99E-01	Otx2

Table S3: Differentially expressed genes in *E12*^{-/-} primary embryonic NSCs having an E2A binding site in WT cells. Depicted are the results from RNA-Seq indicating the fold-change in gene expression as log₂ (log₂FC), variance between triplicates as P or Benjamin-Hochberg adjusted P value (adjP).

ensembl	log ₂ FC	P	adjP	Symbol
ENSMUSG00000001506	-1.85	6.19E-13	1.91E-09	Col1a1
ENSMUSG000000039954	-1.81	1.66E-02	3.60E-01	Stk32a
ENSMUSG000000034674	-1.64	1.39E-120	8.59E-117	Tdg
ENSMUSG000000020000	-1.45	5.06E-03	2.35E-01	Moxd1
ENSMUSG00000005883	-1.43	6.96E-06	4.77E-03	Spo11
ENSMUSG000000023236	-1.40	2.50E-02	4.15E-01	Scg5
ENSMUSG000000029231	-1.35	7.45E-03	2.77E-01	Pdgfra
ENSMUSG000000052613	-1.32	7.25E-03	2.75E-01	Pcdh15
ENSMUSG000000030302	-1.31	2.77E-02	4.30E-01	Atp2b2
ENSMUSG000000043753	-1.27	5.40E-03	2.40E-01	Dmrta1
ENSMUSG000000036815	-1.20	1.43E-01	6.85E-01	Dpp10
ENSMUSG000000062760	-1.15	1.46E-03	1.32E-01	1810041L15Rik
ENSMUSG000000027584	-1.13	2.56E-03	1.73E-01	Oprl1
ENSMUSG000000049336	-1.07	1.06E-03	1.08E-01	Tenm2
ENSMUSG000000069763	-1.07	1.26E-02	3.34E-01	Tmem100
ENSMUSG000000044519	-1.06	8.86E-06	5.21E-03	Zfp488
ENSMUSG000000024935	-1.04	2.88E-02	4.34E-01	Slc1a1
ENSMUSG000000023011	-1.04	4.30E-02	5.04E-01	Faim2
ENSMUSG000000005672	-1.01	1.67E-02	3.60E-01	Kit
ENSMUSG000000063320	-1.00	3.40E-04	5.86E-02	1190007I07Rik
ENSMUSG000000018537	-0.99	5.53E-02	5.49E-01	Pcgf2
ENSMUSG000000028655	-0.99	7.73E-02	5.93E-01	Mfsd2a
ENSMUSG000000063446	-0.97	1.57E-04	3.65E-02	Plppr1
ENSMUSG000000022367	-0.97	1.88E-04	3.99E-02	Has2
ENSMUSG000000030854	-0.97	2.86E-03	1.80E-01	Ptpn5
ENSMUSG000000031285	-0.92	6.14E-02	5.58E-01	Dcx
ENSMUSG000000032087	-0.91	1.83E-03	1.43E-01	Dscaml1
ENSMUSG000000024347	-0.87	1.28E-02	3.36E-01	Psd2
ENSMUSG000000025429	-0.84	1.55E-03	1.35E-01	Pstpip2
ENSMUSG000000050908	-0.84	9.49E-02	6.28E-01	Tvp23a
ENSMUSG000000043635	-0.84	9.06E-12	2.24E-08	Adamts3
ENSMUSG000000005125	-0.83	8.56E-02	6.10E-01	Ndrgr1
ENSMUSG000000028626	-0.83	1.08E-02	3.17E-01	Col9a2
ENSMUSG000000026547	-0.78	4.11E-02	4.96E-01	Tagln2
ENSMUSG000000035105	-0.77	1.64E-01	7.09E-01	Egln3
ENSMUSG000000041073	-0.76	1.53E-02	3.51E-01	Nacad
ENSMUSG000000010505	-0.76	6.15E-02	5.58E-01	Myt1

ENSMUSG00000067028	-0.74	9.49E-03	3.11E-01	Cntnap5b
ENSMUSG00000028358	-0.74	6.49E-04	8.47E-02	Zfp618
ENSMUSG00000074607	-0.74	1.30E-03	1.23E-01	Tox2
ENSMUSG00000018865	-0.74	5.21E-02	5.37E-01	Sult4a1
ENSMUSG00000048978	-0.74	1.43E-02	3.46E-01	Nrsn1
ENSMUSG00000022044	-0.74	1.43E-01	6.85E-01	Stmn4
ENSMUSG00000062591	-0.73	7.49E-03	2.78E-01	Tubb4a
ENSMUSG00000037625	-0.73	1.22E-02	3.31E-01	Cldn11
ENSMUSG00000027419	-0.72	9.94E-02	6.34E-01	Pcsk2
ENSMUSG00000040606	-0.72	9.31E-03	3.08E-01	Kazn
ENSMUSG00000038457	-0.72	1.92E-02	3.80E-01	Tmem255b
ENSMUSG00000020034	-0.72	3.57E-04	5.86E-02	Tcp11l2
ENSMUSG00000022346	-0.72	2.63E-01	7.81E-01	Myc
ENSMUSG00000053825	-0.71	1.45E-02	3.46E-01	Ppfi2
ENSMUSG00000030208	-0.71	1.02E-02	3.16E-01	Emp1
ENSMUSG00000021451	-0.70	6.32E-02	5.59E-01	Sema4d
ENSMUSG00000028137	-0.70	2.57E-02	4.24E-01	Celf3
ENSMUSG00000035778	-0.69	1.01E-01	6.34E-01	Ggta1
ENSMUSG00000026778	-0.68	7.45E-06	4.84E-03	Prkcq
ENSMUSG00000024500	-0.67	3.55E-04	5.86E-02	Ppp2r2b
ENSMUSG00000020173	-0.67	3.18E-02	4.50E-01	Cobl
ENSMUSG00000028128	-0.67	3.16E-06	2.44E-03	F3
ENSMUSG00000037754	-0.67	2.68E-04	5.11E-02	Ppp1r16b
ENSMUSG00000041261	-0.67	1.02E-06	1.05E-03	Car8
ENSMUSG00000049612	-0.67	6.93E-05	2.19E-02	Omg
ENSMUSG00000062110	-0.66	3.78E-05	1.41E-02	Scfd2
ENSMUSG00000015829	-0.66	3.32E-03	1.94E-01	Tnr
ENSMUSG00000032425	-0.65	1.03E-02	3.16E-01	Zfp949
ENSMUSG00000037664	-0.65	9.26E-05	2.60E-02	Cdkn1c
ENSMUSG00000038068	-0.64	2.68E-02	4.26E-01	Rnf144b
ENSMUSG00000023341	-0.64	1.39E-02	3.42E-01	Mx2
ENSMUSG00000021696	-0.63	3.31E-02	4.52E-01	Elovl7
ENSMUSG00000026764	-0.63	4.11E-03	2.12E-01	Kif5c
ENSMUSG00000026442	-0.63	3.08E-02	4.47E-01	Nfasc
ENSMUSG00000031673	-0.62	1.75E-03	1.42E-01	Cdh11
ENSMUSG00000042751	-0.62	7.94E-05	2.39E-02	Nmnat2
ENSMUSG00000026614	-0.62	3.63E-02	4.72E-01	Slc30a10
ENSMUSG00000056966	-0.61	7.53E-02	5.92E-01	Gjc3
ENSMUSG00000041817	-0.61	2.13E-02	3.97E-01	Fam169a
ENSMUSG00000058966	-0.61	1.55E-02	3.54E-01	Fam57b
ENSMUSG00000021895	-0.61	5.08E-05	1.69E-02	Arhgef3
ENSMUSG00000029053	-0.60	2.78E-02	4.30E-01	Prkcz
ENSMUSG00000031028	-0.60	9.40E-02	6.27E-01	Tub
ENSMUSG00000041607	-0.60	9.96E-02	6.34E-01	Mbp
ENSMUSG00000036800	-0.60	1.58E-01	7.01E-01	Fam135b
ENSMUSG00000060988	-0.59	7.76E-02	5.93E-01	Galnt13
ENSMUSG00000030096	-0.58	9.19E-04	1.03E-01	Slc6a6

ENSMUSG00000032017	-0.58	4.55E-02	5.11E-01	Grik4
ENSMUSG00000087579	0.58	4.52E-02	5.10E-01	1500017E21Rik
ENSMUSG00000083022	0.58	6.86E-02	5.75E-01	Rps15a-ps6
ENSMUSG00000039115	0.59	6.86E-03	2.66E-01	Itga9
ENSMUSG00000029408	0.59	6.08E-02	5.58E-01	Abcb9
ENSMUSG00000078234	0.59	1.17E-01	6.58E-01	Klhdc7a
ENSMUSG00000026131	0.60	1.12E-03	1.10E-01	Dst
ENSMUSG00000071042	0.61	4.45E-02	5.07E-01	Rasgrp3
ENSMUSG00000032193	0.61	1.99E-05	9.43E-03	Ldlr
ENSMUSG00000070047	0.61	4.32E-02	5.04E-01	Fat1
ENSMUSG00000046186	0.61	2.58E-06	2.12E-03	Cd109
ENSMUSG00000031465	0.61	8.55E-04	1.00E-01	Angpt2
ENSMUSG00000029378	0.63	5.93E-02	5.58E-01	Areg
ENSMUSG00000025475	0.63	2.77E-02	4.30E-01	Adgra1
ENSMUSG00000037010	0.63	9.98E-02	6.34E-01	Apln
ENSMUSG00000003032	0.65	3.68E-03	2.04E-01	Klf4
ENSMUSG00000092035	0.65	1.01E-01	6.35E-01	Peg10
ENSMUSG00000064373	0.65	2.40E-01	7.68E-01	Selenop
ENSMUSG00000028780	0.65	7.80E-04	9.39E-02	Sema3c
ENSMUSG00000046743	0.66	2.45E-04	4.87E-02	Fat4
ENSMUSG00000021991	0.67	1.95E-05	9.43E-03	Cacna2d3
ENSMUSG00000035606	0.68	6.99E-02	5.79E-01	Ky
ENSMUSG00000066842	0.69	2.92E-04	5.29E-02	Hmcn1
ENSMUSG00000026628	0.69	2.14E-01	7.45E-01	Atf3
ENSMUSG00000032332	0.69	1.61E-03	1.37E-01	Col12a1
ENSMUSG00000054612	0.69	6.09E-03	2.54E-01	Mgmt
ENSMUSG00000040373	0.72	1.25E-02	3.34E-01	Cacng5
ENSMUSG00000068794	0.74	6.57E-04	8.47E-02	Col28a1
ENSMUSG00000063558	0.75	8.94E-03	3.07E-01	Aox1
ENSMUSG00000022594	0.76	6.04E-11	1.24E-07	Lynx1
ENSMUSG00000032440	0.77	9.77E-04	1.05E-01	Tgfbr2
ENSMUSG00000061808	0.78	5.47E-01	8.99E-01	Ttr
ENSMUSG00000003617	0.80	7.85E-02	5.93E-01	Cp
ENSMUSG00000052854	0.82	1.35E-02	3.38E-01	Nrk
ENSMUSG00000059003	0.84	3.20E-02	4.50E-01	Grin2a
ENSMUSG00000040605	0.87	1.05E-03	1.08E-01	Bace2
ENSMUSG00000040998	0.89	7.89E-06	4.87E-03	Npnt
ENSMUSG00000025582	0.93	2.84E-08	3.90E-05	Nptx1
ENSMUSG00000028214	0.95	2.44E-05	1.01E-02	Gem
ENSMUSG00000027861	0.95	6.64E-03	2.61E-01	Casq2
ENSMUSG00000022330	1.09	3.56E-02	4.67E-01	Osr2
ENSMUSG00000035686	1.12	1.00E-05	5.63E-03	Thrsp
ENSMUSG00000003545	1.14	4.97E-09	7.67E-06	Fosb