Supporting Information

Nott et al. 10.1073/pnas.1218126110

SI Materials and Methods

Cell Cultures. Embryonic cortical rat and mouse neurons were cultured as described (1). Before treatment, rat neurons were placed in low serum medium (3% FBS) containing DL-2-amino-5-phosphonovaleric acid (50 μ M; Tocris). P19 cells were maintained in MEM Alpha with Glutamax and supplemented with 10% FBS. Cells were transfected in Optimem containing 1 mM siRNA and 1 μ g·mL⁻¹ DNA plasmid. Unless stated otherwise, all tissue culture reagents were obtained from Invitrogen.

Mouse Lines. *nNOS* homozygous null animals or wild-type control animals were of a C57BL/6 background and were intercrossed to generate null or control litters, respectively. Embryos were collected at embryonic day 14.5 (E14.5) for ex vivo electroporations and preparation of primary cultures. *Brm* homozygous null animals or wild-type control animals were of a 129/Sv background and were intercrossed to generate null or control litters, respectively.

Immunostaining of Cryostat Sections. Embryonic brains were fixed in 4% paraformaldehyde (PFA) at room temperature for 1 h and cryoprotected in 30% sucrose overnight at 4 °C. Brains were frozen in optimal cutting temperature (OCT) medium and 10μM sagittal sections were collected using a Leica vibratome. Sections were immediately blocked/permeabilized in PBS (0.5% triton, 3% BSA) at room temperature for 30 min. Sections were incubated in primary antibodies (1:100) overnight at 4 °C in PBS and 1% BSA and secondary antibodies (1:300) with DAPI (1:1,000) at room temperature for 90 min. Sections were washed with PBS and then mounted using Flouromount-G (SouthernBiotech). Primary antibodies used were mouse anti-nNOS (Santa Cruz sc5302), mouse anti-histone deacetylase 2 (HDAC2) (Millipore 05814), rabbit anti-HDAC2 (Santa Cruz sc7899), rabbit anti-Brm (Abcam ab15597), rat anti-Ctip2 (Abcam, ab18465), and rabbit anti-Cux1 (Santa Cruz sc13024). Mouse and rabbit secondary antibodies were AlexaFlour conjugated (Invitrogen). Images were acquired on an SP5 confocal microscope (Leica) using LAS AF software, and images were processed using Fiji software.

5-ethynyl-2'-deoxyuridine (EdU) Migration Assay. E15.5 mice were injected with 5-ethynyl-2'-deoxyuridine (EdU) (5 μg/g of mouse weight) and culled at either E17.5 or E18.5. Embryos were immediately isolated, and brains were fixed with 4% PFA at room temperature for 1 h and cryoprotected with 30% sucrose overnight at 4 °C. Tissues were flash-frozen in OCT with isopentane on dry ice. Ten-micrometer sections were collected using a cryostat (Leica) and stored at -20 °C. Sections were permeabilized in PBS (0.5% triton, 10% donkey serum) for 30 min at room temperature and stained for EdU incorporation using the Click-iT EdU mixture with AlexaFlour 594 (Invitrogen) at room temperature for 60 min followed by incubation with DAPI (1:1,000). Sections were mounted with Flouromount-G (SouthernBiotech) and imaged using an SP5 confocal microscope (Leica). Sagittal sections of the forebrain were matched between control and mutant animals. For quantification of EdU-positive cells, maximum projections of three confocal Z-stacks were used, and quantification was done using Fiji software. EdU-positive cells were identified using find maxima tool.

Ex Vivo Electroporation. The following ex vivo protocol was developed by Franck Polleux (2). DNA ($1 \mu g \cdot \mu L^{-1}$) and siRNA (10

μM) diluted in 0.05% Fast Green Dye (Sigma) were microinjected into the lateral ventricles using a PLI-100 Pico-injector (Harvard Apparatus) set to a pulse length of 3 ms, P_{INJECT} 0.3 and PBALANCE 0.1. Brains were electroporated using an ECM 830 Electro-Square-Porator (Harvard Apparatus) set to three unipolar pulses at 30 V (100-ms interval and length) using goldplated Genepaddles (Fisher Scientific). Brains were embedded in 3% low-melting-point agarose dissolved in HBSS medium. Brains were sliced using a Vibratome 1000 Plus (Leica) set to 250 μm. Slices were cultured on semipermeable organotypic membranes (VWR) in slice culture medium [70% MEM, 30% HBSS, 20 mM glucose, 1 mM glutamine, penicillin (50 UI) and streptomycin (50-µg/mL)] at 37 °C, 5% CO₂ for up to 5 d in vitro. Ex vivo slices were fixed in 4% PFA at room temperature for 1 h and blocked/permeabilized in PBS (0.5% triton, 3% BSA) overnight at 4 °C. Slices were incubated in primary antibodies (1:100) at room temperature for 1 h followed by secondary antibodies (1:300) with DAPI at room temperature for 90 min. Primary antibodies used were chicken anti-GFP (Abcam ab13970) and mouse anti-nestin (Santa Cruz sc33677). Chicken secondary antibodies were Cy2 conjugated (Jackson ImmunoResearch Labs). Anaylsis of radial migration in $nNOS^{-/-}$ mice was performed by electroporating embryos with pCIG-IRES-eGFP and by culturing slices for 3 d in vitro. Analysis of HDAC2 nitrosylation on radial migration was performed using embryos electroporated with pCIG-IRES-eGFP, pCIG-IRES-HDAC2^{WT}, and pCIG-IRES-HDAC2^{C262A/C274A}. Organotypic slices were cultured for 5 d in vitro.

Migration Analysis. Quantitation of radial cell distribution in slice culture was automated using a protocol setup in Excel as previously described (3). Distances migrated were generated as a percentage distance from the ventricular zone (VZ) to the marginal zone. Percentage distances traveled for cells in each slice were binned into thte VZ/subventricular zone (SVZ), intermediate zone (IZ), or cortical plate (CP) using GraphPad Prism software.

Proliferation Assay. Wild-type and $nNOS^{-/-}$ pregnant mice were injected at E13.5 with EdU (5 µg/g of mouse weight) and culled 24 h later. Embryos were immediately isolated and brains were flash-frozen in OCT with isopentane on dry ice. Ten-micrometer sections were collected using a cryostat (Leica) and postfixed on microscope slides with 4% PFA for 20 min at room temperature. Sections were transferred to 100 mM glycine/PBS for 10 min and then permeabilized in PBS (0.1% triton, 1% BSA) for 30 min at 4 °C. Sections were stained for EdU incorporation using the Click-iT EdU mixture with AlexaFlour 594 (Invitrogen) at room temperature for 60 min. Sections were further blocked in 1% BSA for 20 min at room temperature and incubated in rabbit anti-Ki67 (Abcam) primary antibody (1:100) at room temperature for 60 min, followed by donkey anti-rabbit AlexaFlour-488 secondary (Invitrogen) (1:100) and DAPI (1:1,000) for 60 min at room temperature. Sections were mounted in Flouromount-G (SouthernBiotech) and imaged using SP5 confocal microscope (Leica). Images were analyzed by random selection and marking of 50 EdU-positive cells in the 555 channel (20 cells in the SVZ, 20 cells in the IZ, and 10 cells in the CP) using Fiji software. The 488 channel was then overlaid onto the modified 555 channel, and the number of marked cells that were also Ki-67 positive were counted. Cell cycle exit index was calculated as a ratio of cells that costained for Ki-67 and EdU over the

number of EdU-positive cells analyzed. A total of 300 wild-type cells and 250 $nNOS^{-/-}$ cells were analyzed using three different pregnant mice for each strain.

Proliferation Assay in ex Vivo Slices. The proliferation assay for ex vivo slices was modified as follows. Slices were exposed to medium containing 10 μM EdU 16 h post electroporation, removed from EdU medium after 4 h, and incubated in conditioned medium for a further 20 h before fixing in 4% PFA at room temperature for 60 min. Slices were permeabilized and blocked in PBS (0.5% triton, 3% BSA) at room temperature for 60 min and stained for EdU incorporation as described above. Slices were washed with PBS containing DAPI and mounted with Flouromount-G before imaging. Analysis was performed as a percentage of GFP-positive cells that were EdU positive. A total of 205, 185, and 162 cells were analyzed for slices electroporated with pCIG-IRES-eGFP, pCIG-HDAC2^{WT}-IRES-eGFP, and pCIG-HDAC2^{C262A/C274A}-IRES-eGFP, respectively, using five different embryos for each condition.

Morphological Analysis. Ex vivo-electroporated slices were cultured for up to 5 d, and a Z-series of 40 images at 0.5-µm intervals was captured using an SP5 confocal microscope from the IZ and CP of each analyzed slice. Fifty cells were analyzed from both the IZ and the CP of each slice. Cells were chosen with cell bodies that were located midway through the Z-series (when Z = 20/40) to allow analysis of projections both above and below the plane of the cell body. Analyses of slices were performed at 3 d in vitro using four wild-type and four $nNOS^{-/-}$ embryos electroporated with pCIG-IRES-eGFP obtained by two independent ex vivo electroporations for each strain. Analysis of slices were performed at 5 d in vitro using slices from wild-type embryos electroporated with pCIG-empty vector, pCIG-HDAC2^{WT}, and pCIG-HDAC2^{C262A/C274A}. Cells that had either one or two processes projecting from the cell body were recorded as having a monopolar/bipolar morphology. Cells with three or more processes were recorded as being multipolar. The numbers of monopolar/bipolar and multipolar cells were analyzed as a percentage of the number of cells that had migrated to the IZ or CP.

In Utero Electroporation, FACS Sorting, and mRNA isolation. DNA (2) $\mu g \cdot \mu L^{-1}$) diluted in 10 mM Tris (pH 8.0) with 0.01% Fast Green Dye were microinjected into the lateral ventricles using a PLI-100 Pico-injector (Harvard Apparatus) set at an injection pressure of 17.3 psi, with calibrated injection time equivalent to 1.7 μL. Brains were electroporated using an ECM 830 Electrosquare-porator (Harvard Apparatus) set to five unipolar pulses at 33 V (950-ms interval, 50-ms pulse length) using tweezertrode paddles (Harvard Apparatus). Embryos were electroporated in utero at E14.5 and removed after 18 h. Cortices were dissected out and dissociated using the Papain dissociation system (Worthington), and cells were sorted on a Cytopeia Influx Cell sorter. Live cells were sorted using forward scatter versus side scatter, and clumps were removed by combining the forward scatter versus pulse width. GFP fluorescent cells were sorted on forward scatter versus fluorescence channel 1 (528/38 filter) using a 100μm nozzle at a pressure of 15 psi with an average speed of 7,000 cells/sec⁻¹. Sorted GFP-positive cells were collected directly into RLT buffer, RNA was isolated using the RNeasy micro kit, and DNase was treated on column according to manufacturer's instructions (Qiagen).

Bead-Array Assay. RNA quantity and quality was analyzed using a bioanalyser (Agilent). RNA samples with RNA integrity values (RINs) above 7 were adjusted to 100 pg·mL⁻¹. RNA (500 pg) was amplified and reverse-transcribed using the ovation pico WTA system (Nugene). Mouse whole-genome expression profiling of the samples was performed using mouseWG-6 v2.0

Expression BeadChip (Illumina). Raw data selection and normalization was performed using R package Lumi and subsequent pairwise comparisons of the data was performed by R package Limma (Cambridge Genome Services). Fold changes in relative expression were calculated from the 2logfc [2^{\(\)}(2logfc)]. A 1.6fold cutoff and uncorrected P value ≤ 0.01 was used to identify putative targets that were down-regulated (<1/1.6) and up-regulated (>1.6). In a different statistical approach, sample DM2, which displayed a relatively low degree of HDAC2 overexpression compared with the other samples and clustered away from the other samples based on euclidean distance, was excluded. Subsequently, stringent but nonselective filter criteria were applied: transcripts with fewer than three detected probes were exluded and probes were eliminated if they did not show a relative change of x and an absolute change of y units with (x, y) =(3,100) (4). Heat maps were generated with MeV v4.7.3 using Euclidean distance. To identify processes specifically affected by S-nitrosylation of HDAC2, Gene Ontology (GO) analysis was performed using the BiNGO 2.44 plug-in in Cytoscape 2.8.2 [hypergeometric test; Benjamini–Hochberg FDR correction (P < 0.05); GO Biological Process; whole annotation as a reference set].

RNA Isolation and Reverse Transcription. Cortices were isolated and RNA was extracted using TRIzol according to the manufacturer's protocol (Invitrogen). RNA pellets were resuspended in 50 μL diethylpyrocarbonate (depc)-treated water and immediately DNase-treated using a TURBO DNase kit according to the manufacturer's instructions (Ambion). RNA was tested for genomic contamination by actin PCR. For each sample, 5 μL RNA was reverse-transcribed in a 20- μL reaction volume containing random hexamer mix and SuperScript III reverse transcriptase (50 U; Invitrogen) at 50 °C for 1 h. First-strand cDNAs were diluted, and 3 μL was PCR-amplified in 25 μL PCR containing primers (0.2 μM), dNTPs (200 nM), and GoTaq polymerase (1.25 U; Promega). Primer sequences and PCR conditions are available on request.

Luciferase Assay. E17 rat cortical neurons were cultured in six-well plates and transfected after 4 d in vitro. Neurons were transfected with 1 $\mu g \cdot m L^{-1}$ pME18S-HDAC2, 0.2 $\mu g \cdot m L^{-1}$ Brm-luciferase vector (firefly) (5), and 4 $\mu L \cdot m L^{-1}$ lipofectamine 2000 (Invitrogen). Neurons were incubated with OptiMem mix for 2 h at 37 °C, 10% CO₂, and transfection medium was replaced with original plating medium. Twenty-four hours after transfection, neurons were serum-starved and stimulated 2 h later with 75 ng·mL^{-1} brain-derived neurotrophic factor or 50 mM KCl, and lysates were collected 24 h later in 0.5 mL passive lysis buffer (Promega). Samples were processed using luciferase reporter assay (Promega) according to the manufacturer's instructions.

Brm Expression in ex Vivo Electroporated Slices. Ex vivo slices were cultured for 5 d before being fixed and stained as previously described. Primary antibodies used were rabbit anti-Brm 1:100 (Abcam ab15597) and chicken anti-GFP 1:1,000 (Abcam ab13970). Secondary antibodies used were anti-rabbit Alexa-Flour-647 (Invitrogen) and anti-chicken-Cy2 (Jackson Laboratories). Slices were imaged in the cortical plate, using an SP5 confocal microscope (Leica), and analyzed for the percentage of GFP-expressing cells positive for Brm staining. Four slices were analyzed (minimum of 20 cells per slice) for each condition taken from two independent ex vivo experiments.

Chromatin Immunoprecipitation on Tissue. Chromatin immunoprecipitation assays were performed on tissue as described (6) with minor modifications. Tissue was dissected and immediately frozen in liquid nitrogen and stored at -80 °C until needed. Samples were thawed at room temperature in 1 mL PBS con-

taining 1% formaldehyde for 15 min. Crosslinking was terminated with glycine (100 µL, 1.25 M) at room temperature for 10 min. Samples were washed once with PBS and homogenized (hand-potter homogenizer; Sigma) in PBS. Cells were collected by centrifugation, and pellets were resuspended in lysis buffer containing 0.1% SDS, 0.5% Triton X-100, 20 mM Tris-HCl (pH 8.1), and 150 mM NaCl. Samples were sonicated using a Bioruptor UCD-200 socinator (Diagenode), set to a 30-s pulse and then to a 30-s interval for 10 min repeated twice for each sample. Cell debris was removed by centrifugation, and supernatants were precleared by incubation with Protein A-Sepharose beads (Amersham Biosciences) for 1 h at 4 °C. Beads were collected by centrifugation, and supernatants were subjected to immunoprecipitation. A fraction of the supernatant was used for immunoprecipitation input control. The volume of each tube was adjusted to 500 µL with lysis buffer, and 5-10 µg of rabbit polyclonal antibody was added overnight at 4 °C. The following antibodies were used: anti-HDAC2 (Santa Cruz H-54), anti-Brm (Abcam ab15597), anti-acetyl histone H3 K9/K14 (Millipore 06– 866), and control rabbit IgG (Santa Cruz). Immune complexes were collected by incubation with Protein A-Sepharose beads for 1 h at 4 °C. Beads were collected and subjected to a series of seven sequential washes, as described (6). After final Tris-EDTA (TE) wash, supernatant was entirely removed, and beads were eluted with 10% Chelex (50 µL), vortexed, and boiled for 10 min.

 Riccio A, et al. (2006) A nitric oxide signaling pathway controls CREB-mediated gene expression in neurons. Mol Cell 21(2):283–294. Beads were spun at 14,000 \times g, and supernatant containing DNA fragments was collected in a fresh tube. Boiling procedure was repeated with 30 μL elution buffer (10 mM Tris-Cl, pH 8.5) and pooled with previous supernatant. DNA fragments were purified using the QIAquick PCR purification kit (Qiagen). PCR conditions and cycle numbers were determined empirically for the different templates and primer pairs. Primers amplified fragments ranging in size from 150 to 250 bp. Primer sequences and PCR conditions are available on request.

Quantitative Real-Time PCR. PCR reactions (20 μ L) contained 10 μ L of DyNAmo Flash Probe qPCR Kit (Thermo Scientific) and 0.5 μ M of primers. All reactions were performed in triplicate with a Mastercycler ep realplex (Eppendorf), and each experiment included a standard curve. Standard curve templates consisted of gel-purified PCR products of the BRM, HDAC2, and rpl11 amplicons of known concentration, and each standard curve consisted of seven serial dilutions of the template. At the end of 46 cycles of amplification, a dissociation curve was performed in which Sybr Green was measured at 1 °C intervals between 50 °C and 100 °C. Results were normalized to rpl11.

Statistical Analysis. Data are expressed as averages and SEM. All analysis was performed using GraphPad Prism version 4.0c for Macintosh (GraphPad Software; www.graphpad.com).

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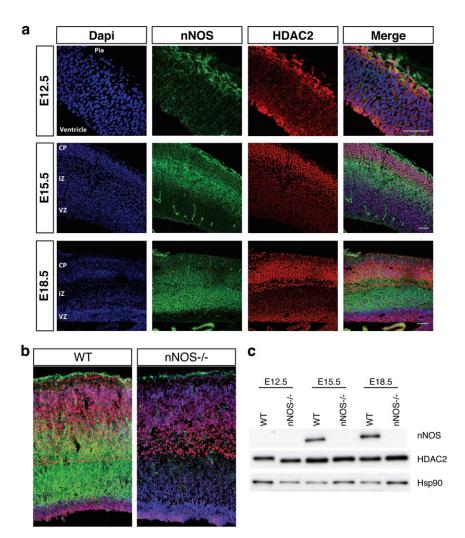


Fig. S1. HDAC2 and neuronal nitric oxide synthase (nNOS) expression during cortical development. (A) Sagittal sections of E12.5 (*Top*), E15.5 (*Middle*), and E18.5 embryos (*Bottom*) were stained using DAPI (blue), anti-nNOS (green), or anti-HDAC2 (red), and images were captured using a Leica SP5 confocal microscope. [Scale bars, 50 μ m (*Top* and *Middle*) and 100 μ m (*Bottom*).] n=3. (B) Confocal images of cortex from E18.5 coronal sections taken from wild-type (*Left*; WT) and nNOS^{-/-} (*Right*) embryos stained with DAPI (blue), HDAC2 (red), and nNOS (green). n=2. (C) Western blot analysis of HDAC2, nNOS, and Hsp90 in cortical lysates obtained from either WT or nNOS^{-/-} embryos dissected at the indicated stages. n=3.

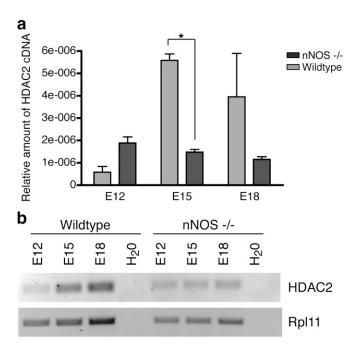


Fig. S2. HDAC2 expression increases during cortical development. (A) Relative mRNA quantitation of HDAC2 normalized to Rpl11 expression was assessed by quantitative RT-PCR using cDNA synthesized from cortical RNA of E12.5, E15.5, and E18.5 embryos from wild-type and nNOS^{-/-} mice. *P < 0.01; two-way ANOVA, Bonferroni post hoc test; n = 4. (B) HDAC2 and Rpl11 mRNA levels assessed by RT-PCR using cDNA synthesized from cortical RNA of E12.5, E15.5, and E18.5 embryos from wild-type and nNOS^{-/-} mice. n = 3.

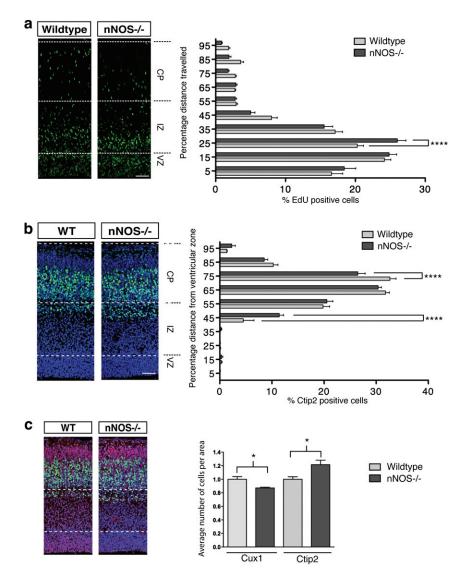


Fig. S3. Lack of nitric oxide signaling impairs cortical development. (A) (Left) Cortex of WT and nNOS $^{-/-}$ embryos from mice injected with EdU at E15.5 and killed 48 h later. (Right) Quantitative analysis of distance traveled by EdU-positive neurons. Values are percentage of distance from VZ. Shown are averages and SEM; **** ^+P < 0.0001, two-way ANOVA, Bonferroni post hoc test. (Scale bar, 50 μ m.) WT embyos, n = 14; nNOS $^{-/-}$ embryos, n = 16. (B) (Left) Cortex of WT and nNOS $^{-/-}$ E17.5 embryos stained with DAPI (blue) and anti-Ctip2 (green). (Right) Quantitative analysis of the distribution of Ctip2-positive cells. Values are percentage distance from VZ. Shown are averages and SEM; *** ^+P < 0.0001, two-way ANOVA, Bonferroni post hoc test. (Scale bar, 50 μ m.) WT embyos, n = 6. (C) (Left) Confocal images of WT and nNOS $^{-/-}$ E17.5 embryos stained with DAPI (blue), anti-Cux1 (red), and anti-Ctip2 (green). (Right) Quantitative analysis of the average number of Cux1- or Ctip2-positive cells per area. Shown are averages and SEM; * ^+P < 0.05, unpaired ^+t test; WT embyos, ^-t embryos, $^-$

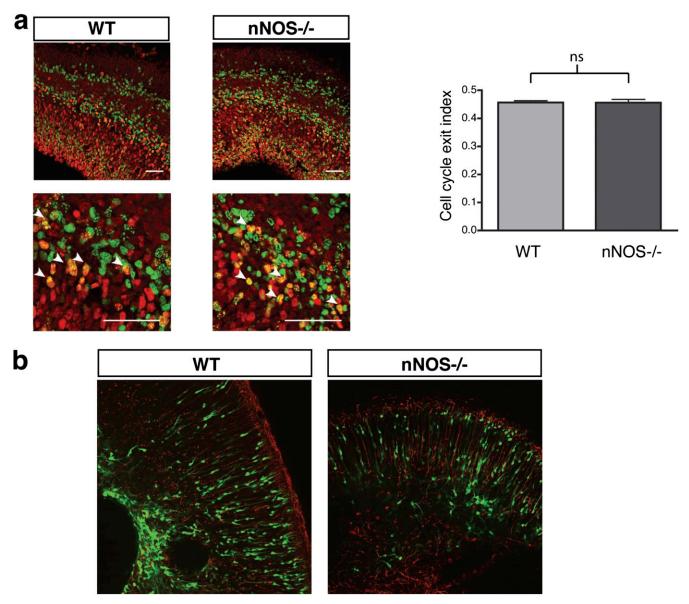


Fig. S4. Lack of nitric oxide does not influence cell proliferation and radial glia formation. (A) (Left) Cortex of either WT or nNOS^{-/-} E13.5 embryos obtained from pregnant mice injected with EdU and killed 24 h after treatment. Sections were stained with EdU (green) and Ki-67 (red). (Scale bar, 50 μ m.) (Right) Ratio of EdU-positive to Ki-67–expressing cells is shown (cell cycle exit index). Shown are averages and SEM; n=3; ns, nonsignificant, unpaired t test. (B) Confocal images of ex vivo organotypic slices from wild-type (Left) and nNOS^{-/-} (Right) embryos electroporated with pCIG-IRES-eGFP at E14.5 and cultured for 3 d in vitro. Green indicates GFP-transfected cells, and red indicates anti-nestin staining. n=2.

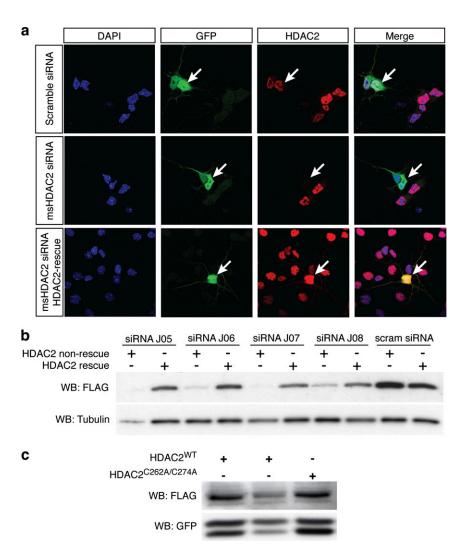


Fig. 55. HDAC2 protein levels were reduced following introduction of msHDAC2 siRNA. (A) Confocal images of dissociated cells from E14.5 embryos electroporated with pCIG-IRES-eGFP and scramble siRNA (*Top*), pCIG-IRES-eGFP and msHDAC2 J06 siRNA (*Middle*) or pCIG-HDAC2WT-IRES-eGFP and msHDAC2 J06 siRNA (*Bottom*) and cultured for 5 d in vitro. Blue denotes DAPI, green GFP, and red anti-HDAC2 stain; n = 3. (B) P19 cells were cotransfected with one of four msHDAC2 siRNA oligomers (1 mM; Dharmacon) and either wild-type FLAG-HDAC2 (HDAC2 nonrescue) or a mutated FLAG-HDAC2 with six to seven synonymous mutations within the siRNA target regions (HDAC2 rescue). Cells were cultured for 72 h and subjected to Western blot analysis for FLAG and tubulin; n = 3. (C) Organotypic slices from E14.5 embryos electroporated with FLAG-tagged pCIG-HDAC2WT-IRES-eGFP or pCIG-HDAC2C262A/C274A-IRES-eGFP were cultured for 5 d and then lysed and subjected to Western blot analysis for FLAG and GFP. n = 2.

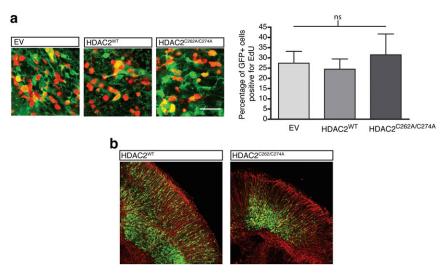


Fig. S6. S-nitrosylation of HDAC2 does not influence cell proliferation and radial glia formation. (A) (Left) Cortices from embryos ex vivo electroporated with siHDAC2 and pCIG-IRES-eGFP, pCIG-HDAC2WT, or pCIG-HDAC2C262A/C274A. Organotypic slices were cultured in vitro for 16 h and then treated with EdU for 4 h and fixed 20 h later. Sections were stained for GFP (green) and EdU (red). (Scale bar, 50 μ m.) (Right) Quantitative analysis was performed by calculating the percentage of GFP-expressing cells staining positive for EdU. Averages and SEM; ns, nonsignificant, unpaired t test; n=5. (B) Confocal images of ex vivo organotypic slices from wild-type embryos electroporated with pCIG-HDAC2WT (Left) and pCIG-HDAC2C262A/C274A (Right) at E14.5 and cultured for 5 d in vitro. Green indicates GFP signal, and red indicates anti-nestin staining. n=2.

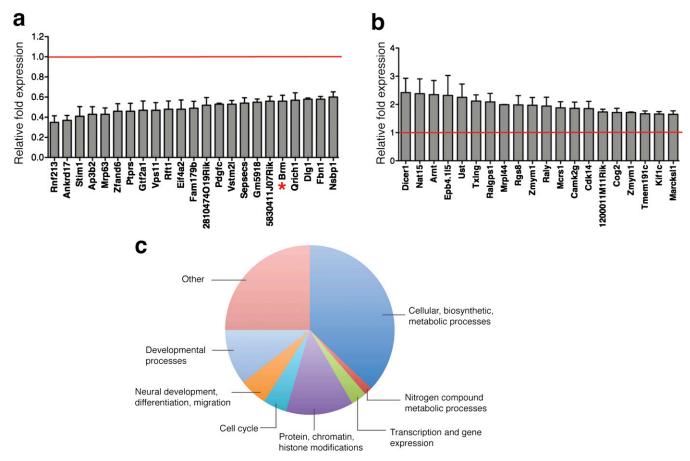


Fig. S7. Characterization of the transcriptional program regulated by HDAC2 S-nitrosylation. (A) Identification of 23 transcripts with >1.6-fold decrease in expression and an uncorrected P value ≤ 0.01 in HDAC2C262A/C274A samples versus HDAC2WT samples. (B) Identification of 20 transcripts with >1.6-fold increased expression and an uncorrected P value ≤ 0.01 in HDAC2C262A/C274A versus HDAC2WT samples. (C) GO analysis performed using targets from the microarray screen with a fold change >1.3 and an uncorrected P value ≤ 0.05 were broadly classified according to biological function.

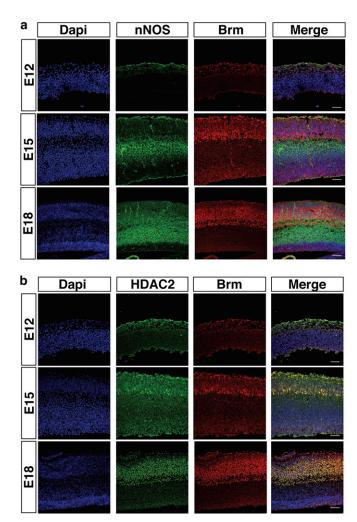


Fig. S8. Brm expression is both temporal and spatially regulated during cortical development similarly to HDAC2. (A) Sagittal sections of E12.5 (*Top*), E15.5 (*Middle*), and E18.5 embryos (*Bottom*) were stained using DAPI (blue), anti-nNOS (green), and anti-Brm (red), and images were captured using a Leica SP5 confocal microscope. [Scale bars, 50 μm (*Top* and *Middle*) and 100 μm (*Bottom*).] n = 3. (B) Sagittal sections of E12.5 (*Top*), E15.5 (*Middle*), and E18.5 embryos (*Bottom*) were stained using DAPI (blue), anti-HDAC2 (green), and anti-Brm (red), and images were captured using a Leica SP5 confocal microscope. [Scale bars, 50 μm (*Top* and *Middle*) and 100 μm (*Bottom*).] n = 3.

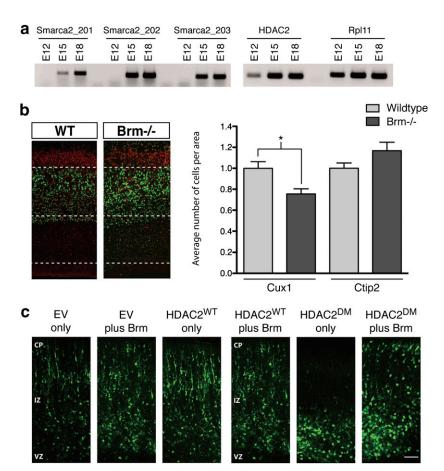


Fig. 59. Brm expression during cortical development and its regulation by HDAC2 nitrosylation. (A) Representative RT-PCR of three splice variants of Brm (Smarca2), HDAC2, and the ribosomal protein Rpl11. mRNA was obtained from cortices of embryos dissected at the indicated embryonic stages. n = 3. (B) (Left) Cortex of WT and Brm^{-/-} E17.5 embryos stained with anti-Cux1 (red) and anti-Ctip2 (green). (C) Images of cortex from E14.5 embryos ex vivo electroporated with siHDAC2 and pCIG-IRES-eGFP, pCIG-HDAC2WT, or pCIG-HDAC2C262A/C274A. Brains were coelectroporated with either pDEST26-hBrm78 (Brm) or empty vector (EV). Organotypic slices were cultured for 5 d in vitro before imaging.

Table S1. FDR-corrected values for putative target genes with an uncorrected P value ≤ 0.01 between HDAC2^{WT} (n=3) and HDAC2^{C262A/C274A} (n=2)

Gene	Fold change (including DM2)	P value (including DM2)	Fold change (excluding DM2)	P value (excluding DM2)	FDR-corrected (excluding DM2)
1200011A11Rik	0.5	0.01	NA	NA	NA
Rnf213	0.4	0.00	0.4	0.000	0.2
Ankrd17	0.4	0.00	0.3	0.000	0.2
Stim1	0.4	0.01	0.6	0.010	0.3
Ap3b2	0.4	0.01	0.4	0.003	0.2
Mrp63	0.4	0.00	0.4	0.000	0.2
Zfand6	0.5	0.01	0.5	0.003	0.2
Ptprs	0.5	0.01	0.6	0.016	0.3
Gtf2a1	0.5	0.01	0.5	0.003	0.2
Rft1	0.5	0.01	0.6	0.011	0.3
Eif4a2	0.5	0.01	0.5	0.002	0.2
Fam179b	0.5	0.01	0.5	0.004	0.2
2810474O19Rik	0.5	0.01	0.5	0.006	0.2
Pdgfc	0.5	0.01	0.5	0.002	0.2
LOC100045403	0.5	0.01	0.4	0.002	0.2
Sepsecs	0.5	0.01	0.5	0.003	0.2
Gm5918	0.5	0.01	0.5	0.008	0.3
5830411J07Rik	0.6	0.01	0.5	0.004	0.2
Brm	0.6	0.01	0.6	0.005	0.2
Qrich1	0.6	0.01	NA	NA	NA
LOC100047603	0.6	0.01	0.5	0.006	0.2
Fbn1	0.6	0.01	0.6	0.005	0.2
Nsbp1	0.6	0.01	NA.	NA	NA
Marcksl1	1.7	0.01	NA	NA	NA
Kif1c	1.7	0.01	NA	NA	NA
Tmem191c	1.7	0.01	1.8	0.002	0.2
Zmym1	1.7	0.01	1.9	0.006	0.2
Cog2	1.7	0.01	1.7	0.004	0.2
1200011M11Rik	1.7	0.01	1.8	0.002	0.2
Cdk14	1.8	0.01	NA	NA	NA
Camk2g	1.9	0.01	1.8	0.005	0.2
Mcrs1	1.9	0.01	1.9	0.003	0.2
Raly	1.9	0.01	2.3	0.003	0.2
Zmym1	2.0	0.01	1.9	0.005	0.2
Rgs8	2.0	0.01	1.9	0.005	0.2
Mrpl44	2.0	0.00	NA	NA	NA
Ralgps1	2.1	0.01	2.0	0.002	0.2
TxIng	2.1	0.01	2.7	0.002	0.2
Ust	2.2	0.01	2.1	0.001	0.2
A430088H15Rik	2.3	0.01	NA	NA	NA
Arnt	2.4	0.01	2.2	0.001	0.2
Nat15	2.4	0.01	2.2	0.010	0.3
Dicer1	2.4	0.01	3.6	0.000	0.2

NA, not applicable (see SI Materials and Methods for criteria).