Supporting Information

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SI Materials and Methods

Animal Treatment. Tamoxifen (Sigma) was dissolved in corn oil (Nacalai) at a concentration of 10 mg/mL. Pregnant mice were injected intraperitoneally with 150 μL of tamoxifen solution for inducing ERT2-Cre activity. All mice were maintained according to the protocol approved by the Animal Care and Use Committee of the University of Tokyo.

Mice. For conditional disruption of the Ring1B gene, the *Ring1B*$^{f/f}$ mouse (1) was crossed with a NestinERT2-Cre mouse (ERT2-Cre driven by the nestin enhancer) (2).

Primary Neural Precursor Cell (NPC) Culture. Primary NPCs were prepared from the dorsal cerebral cortex of ICR strain mouse embryos at E11.5 (E1 was defined as 12 h after detection of the vaginal plug). Dissected cortices were transferred to artificial cerebrospinal fluid (aCSF: 124 mM NaCl, 5 mM KCl, 0.1 mM $CaCl_2$, 26 mM $NaHCO_3$, 1.3 mM $MgCl_2$, 10 mM glucose) containing 0.05% trypsin (Sigma) and incubated for 10 min on ice to remove overlying epidermal ectoderm. The cortices were then transferred to aCSF containing 0.1% trypsin, DNase I (0.1 mg/mL) (Roche), and hyaluronidase (0.67 mg/mL) (Sigma) and incubated at 37 °C for 10 min. After the addition of an equal volume of aCSF containing trypsin inhibitor (0.7 mg/mL) (Sigma), the neuroepithelium was transferred to DMEM-F12 (1:1) and mechanically dissociated into single cells. The dissociated cells were cultured in DMEM-F12 (1:1) supplemented with B27 (Invitrogen), FGF2 (20 ng/mL) (R&D), and EGF (20 ng/mL) (Upstate Biotechnology).

ES Cell (ESC) Culture. Mouse Bf1::Venus ESCs were maintained as described previously (3). Differentiation medium was prepared as follows: G-MEM supplemented with 10% (vol/vol) knockout serum replacement (KSR; Invitrogen), 2 mM glutamine, 1 mM pyruvate, 0.1 mM nonessential amino acids, and 0.1 mM 2-ME. To induce NPCs from ESCs by SFEBq (serum-free culture of embryoid body-like quick aggregates), ESCs were dissociated to single cells in 0.25% trypsin-EDTA (Invitrogen) and quickly reaggregated in differentiation medium (3,000 cells/150 μL/well) using 96-well low cell-adhesion plates (Sumitomo Bakelite). The day on which ESCs were seeded to differentiate is defined as differentiation day 0.

qRT-PCR and RT-PCR. Total RNA was obtained from NPCs using TRIzol (Invitrogen) following the instructions of the manufacturer. Reverse transcription (RT) was performed with 1 μg of total RNA, oligo $d(T)_{20}$ primers and ReverTra Ace (Toyobo). The resulting cDNA was subjected to quantitative RT-PCR (qRT-PCR) in a Roche LightCycler with SYBR Premix Ex Taq (Takara) or PCR in a GeneAmp PCR system 9700 (Applied Biosystems). The abundance of target mRNAs was normalized relative to that of *Gapdh* mRNA in qRT-PCR analysis. Relative expression levels were quantified by constructing a standard curve using DNA dilutions derived from BAC clone (RP24-113B18). The sense and antisense primers, respectively, were as follows: For quantitative PCR: *Gapdh*, 5′-TGG GTG TGA ACC ACG AG-3′ and 5′-AAG TTG TCA TGG ATG ACC TT-3′; *Neurog1*, 5′-ATC ACC ACT CTC TGA CCC-3′ and 5′-GAG GAA GAA AGT ATT GAT GTT GCC TTA-3′; *utNgn1*, 5′-CCC CTC CTT TCA GTT TCG CTA GCC TTG T-3′ and 5′-CCT GCT GCG AGA GTT TGA ATA CCG AGA A-3′; *CXCL14*, 5′-TGT TCC CGG AAG GGG CCC AA-3′ and 5′-GGT GCA GGC AGT GCT CCT GG-3′; *H2afy*, 5′-AAG CAG GGA GAA GTG AGC AA-3′ and 5′-GCT CTT GGT GGA GAG GAC AG-3′; *NEAT1*, 5′-TTG GGA CAG TGG ACG TGT GG-3′ and 5′-TCA AGT GCC AGC AGA CAG CA-3′; *Oct3/4*, 5′-GAT GCT GTG AGC CAA GGC AAG-3′ and 5′-GGC TCC TGA TCA ACA GCA TCA C-3′; *Nestin*, 5′-TGA AGC ACT GGG AAG AGT AG-3′ and 5′-TAA CTC ATC TGC CTC ACT GTC-3′; *βIII-tubulin*, 5′-ACA CAG ACG AGA CCT ACT-3′ and 5′-GCA GAC ACA AGG TGG TT-3′, *Hes1*, 5′-CAC TTC GGA CTC CAT GT-3′, 5′-GAG GTG GGC TAG GGA CTT TA-3′; *Neurog1* TSS −1.2 kbp, 5′-CAG AGG GAA GGA GCC AC-3′ and 5′-CCT AGC CTG CTG TCT TCA-3′; *Neurog1* TSS −3.6 kbp, 5′-CAA GAG TGG GTG GAG TAG A-3′ and 5′-CAT GTG CCT ATG GTC CG-3′; and *Neurog1* TSS −4.6 kbp, 5′-GGT TGT GTT TCC ACA GAA GAG CA-3′ and 5′-CCT TGT TTC CCC CCT CCA TTT TA-3′. For PCR: *Neurog1* TSS −6.1 kbp, 5′-AGA AAT GGT TTG AGA GTA GCC CG-3′ and 5′-CTC CTG CTG CGA GAG TTT GAA-3′; *Neurog1* TSS −3.6 kbp, 5′-CAG GTG TTC TGG CTG ACT GA-3′ and 5′-GAG ACT TTG TCC CAC CCT GA-3′; *β-actin*, 5′-AAT AGT CAT TCC AAG TAT CCA TGA AA-3′ and 5′-GCG ACC ATC CTC CTC TTA G-3′; *Tbr2*, 5′-CAT GGA CAT CCA GAA TGA GC-3′ and 5′-CAG GAG GAA CTA ATC TCT TCT TTA AC-3′; *NeuroD1*, 5′-CCA AAG CCA CGG ATC AAT-3′ and 5′-CGA ATG GCT ATC GAA AGA CAT AA-3′; *Trpc7*, 5′-TTC ACC TAC GCC AGG GAT AA-3′ and 5′-TAG GCG ATC CGG GAG AA-3′; *Smad5*, 5′-GAC ATA AGT ATT CCA TAG CAA GAG T-3′ and 5′-AGT AAA GGA ACA TGA ACG GT-3′; *Fbxl21*, 3′-TTG AGT TTG AGC TGA ACC AAT-3′ and 5′-TAC TGA AGA TGT GCA GCG T-3′; *Tgfbi*, 5′-GAC TCT GCC CTT GAA ATC TT-3′ and 5′-CTA ATG CTT CAT CCT CTC CAG TA-3′; *Pcbd2*, 5′-ATC AGG CGT TTG GCT TTA-3′ and 5′-GGG TTA TCT GGA CTT TGT TGT AG-3′; and *Txndc15*, 5′-CAG CAC TGA CAG CCT CAA-3′ and 5′-CAT GAG GTC CTG GGA CAT A-3′.

Northern Blotting. Total RNA was prepared from the dorsal or ventral telencephalon of ICR mouse embryos. A total of 10 μg of total RNA was separated on 1.2% (wt/vol) formaldehyde-agarose gels and transferred to a positively charged nylon membrane (Hybond-N+; Amersham Biosciences). After UV cross-link, the blots were hybridized with RNA probe labeling with digoxigenin (DIG)-UTP (Roche) at 68 °C, using hybridization buffer [50% (vol/vol) formamide deionized, 7% (wt/vol) SDS, 5× saline sodium citrate (SSC), 2% (wt/vol) blocking reagent (Roche), 0.1% *N*-lauroylsarcosine, and 50 mM sodium phosphate]. Blots were stringently washed twice in wash buffer 1 (2× SSC and 0.1% SDS) for 5 min at room temperature, and twice in wash buffer 2 (0.1× SSC and 0.1% SDS) for 15 min at 68 °C, then rinsed in wash buffer 3 [0.1 M maleic acid, 0.15 M NaCl, 0.3% Tween 20 (pH 7.5)] and incubated in blocking reagent (Roche) for 30 min at room temperature. Subsequently, blots were incubated with a 10,000-fold dilution of anti-DIG-AP Fab fragment (Roche) in blocking reagent for 30 min at room temperature, washed twice in wash buffer 3 for 15 min, and immersed in detection buffer [0.1 M Tris·HCl, 0.1 M NaCl (pH 9.5)] for 5 min. Anti–DIG-AP was detected using CDP-star chemiluminescent substrate for alkaline phosphatase (Roche). Signals were detected and analyzed using ImageQuant LAS4000 image analyzer (Fuji Film). The DIG-labeled antisense RNA probe for detecting mouse *Neurog1* corresponds to the *Neurog1* CDS region and that for *utNgn1* correspond to a 460-bp region. Probes were generated by

in vitro transcription with T3 RNA polymerase (Promega) using the DNA templates, which contain a promoter sequence of T3 RNA polymerase (AATTAACCCTCACTAAAGGG) followed by a complimentary sequence of target RNA. DNA templates were amplified by PCR with the following primers: For *utNgn1* probe: (F) ATT AGT ACA CTT ATT GTC CCG TT and (R) AAT TAA CCC TCA CTA AAG GGC ATA AAA GAG ATA GTG AAA TGC TGG; *Neurog1* probe: (F) AAT TAA CCC TCA CTA AAG GGA GGC CTA GTG GTA TGG GAT GAA and (R) CAC ACT GCA AGA TGC CTG C.

In Situ Hybridization. E13.5 and E18.5 mouse embryos were fixed at 4 °C with 4% (wt/vol) paraformaldehyde in PBS overnight. Samples were cryoprotected overnight with 30% (wt/vol) sucrose in PBS, embedded in OCT (Tissue-Tek), and frozen on dry ice. Frozen embryos were sectioned on a cryostat at 16 μm. Sections were processed for in situ hybridization. Frozen sections were prefixed in 4% paraformaldehyde at room temperature for 10 min and washed three times with PBS. Sections were treated with 0.25 μg/mL proteinase K for 30 min at 37 °C, postfixed in 4% paraformaldehyde at room temperature for 5 min, and washed with PBS. Then the sections were treated sequentially with 0.25% acetic anhydride in 0.1 M triethanolamine for 10 min and 0.3% Triton X-100 in phosphate buffer (pH 7.0) for 20 min at room temperature. Then sections were incubated in a hybridization buffer (50% formamide deionized, 0.1% SDS, 5× SSC, 50 μg/mL heparin, 50 μg/mL yeast tRNA) containing DIG-labeled probes at 65 °C. The RNA probes for *Neurog1* and *utNgn1* are the same as those used in Northern blotting analysis. The DIG-labeled sense RNA probe for *utNgn1* corresponds to the same region as the antisense probe for *utNgn1* in the reverse direction. Hybridized sections were washed three times in 0.2× SSC or 2× SSC, 50% formamide, 0.1% Tween 20 wash buffer at 65 °C for 30 min. After washing, sections were incubated for 1 h in 1% (wt/vol) blocking reagent (Roche) in a buffer containing 0.1 M Tris·HCl, 0.15 M NaCl (pH 7.5), then they were incubated with an alkaline phosphatase (AP)-coupled antibody (Roche) at 4 °C overnight and rinsed, and the signals were visualized with nitro-blue tetrazolium chloride (NBT)/5-Bromo-4-Chloro-3′-Indolylphosphatase p-Toluidine salt (BCIP) (Wako).

5′ and 3′ RACE. 5′ and 3′ RACE were carried out using a SMART RACE cDNA amplification kit (Clontech) or the 5′-RACE system, version 2.0 kit (Invitrogen), following the instructions of the manufacturer. Total RNA was extracted from the dorsal cerebral cortex of ICR mouse embryos at E12.5. With the SMART RACE cDNA amplification kit, total RNA was reverse transcribed using an oligo dT primer containing an anchor sequence (for 3′ RACE) or an oligo dT primer with an anchor-tagged switching template (for 5′ RACE). The cDNA was amplified by PCR using the universal amplification primers (from the kit) and the gene-specific primer R for 3′ RACE or primer F1 for 5′ RACE. With the 5′-RACE system, version 2.0 kit, poly (A) positive RNA or total RNA were reverse transcribed using a gene-specific primer (CGATTAGTACACTTATTGT). Poly (A) selection of total RNA was performed using the oligotex-dT30 kit (Takara). After adding a poly(C) sequence to the 3′ end of the cDNA, it was amplified by two sequential PCRs using the universal amplification primers (included in the kit) and the gene-specific primers: primer F for the first PCR and primer F2 for the second PCR. PCR products were gel purified (QIAquick; Qiagen) and cloned into pCR-Blunt (Invitrogen). The target sequences were determined using the M13 primers. The sequences of the gene-specific primers used in this experiment are the following: primer R, CCT GCT GCG AGA GTT TGA ATA CCG AGA A; primer F, TTG TTA CAG TCC AGG GCC ATT CTC GGT ATT; and primer F2, CCC CTC CTT TCA GTT TCG CTA GCC TTG T.

Quantification and Statistics. Data for mRNA quantification are the mean ± SEM for the three samples unless indicated otherwise in figure legends. These data are representative of results obtained from at least three independent experiments. Statistical significance is determined by Student *t* test.

Cell Fractionation. Cell fractionation of primary neocortical NPCs (prepared from E12.5 mouse cerebral cortex and cultured in suspension for 3 d) into cytoplasmic and nuclear RNA fractions was performed with a nuclear/cytoplasm fractionation kit (PARIS kit; Ambion) following the instructions of the manufacturer. The concentration of total RNA derived from nuclear, cytoplasm, and total fraction were quantified using the Nano drop ND-1000 spectrophotometer (NanoDrop Technologies) and normalized before reverse transcription. Then the amount of cDNA in each fraction was determined by real-time PCR in a Roche LightCycler with SYBR Premix Ex Taq (Takara). PCR primers are described above.

Chromatin Immunoprecipitation Assay. Primary NPCs were cultured in suspension for 0, 3, or 9 d, after which neurospheres were collected and dissociated. The cells were suspended in lysis solution [1% (wt/vol) SDS, 10 mM EDTA, 50 mM Tris·HCl (pH 8.1)] and sonicated to shear genomic chromatin into DNA fragments of ∼0.5–10 kb. The lysate was incubated for 2 h with Dynabeads Protein A (Invitrogen), after which the beads were removed and the lysate was incubated overnight at 4 °C with antibodies. After the addition of protein A beads, the mixture was incubated with rotation for 1 h. The beads were then isolated and washed consecutively with a low-salt solution [0.1% SDS, 1% (vol/vol) Triton X-100, 2 mM EDTA, 20 mM Tris·HCl (pH 8.1), 150 mM NaCl], a high-salt solution [0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris·HCl (pH 8.1), 500 mM NaCl], a LiCl solution [0.25 M LiCl, 1% (vol/vol) Nonidet P-40, 1% (wt/vol) sodium deoxycholate, 1 mM EDTA, 10 mM Tris·HCl (pH 8.1)], and twice with a Tris·EDTA solution [10 mM Tris-HCl (pH 8.0), 1 mM EDTA]. Immune complexes were then eluted from the beads with a solution containing 10 mM DTT, 1% SDS, and 0.1 M $NaHCO_3$, after which NaCl was added to a final concentration of 0.2 M and the eluate was incubated at 65 °C overnight to induce the dissociation of proteins from DNA. The proteins were eliminated by digestion with proteinase K at 45 °C for 1 h and the DNA was purified with a QIAquick spin column (Qiagen). The eluted DNA was subjected to real-time PCR in a Roche LightCycler with SYBR-green Real-Time PCR Master Mix. The abundance of target genome DNA was normalized relative to that of input. The primer sets of 11, 9, 8, and 7 are the same as those of *Neurog1*, TSS −3.6 kbp, TSS −4.6 kbp, and *utNgn1* used in qRT-PCR analysis, respectively. The other sense and antisense primers, respectively, were as follows: primer 10 (*Neurog1* promoter), 5′-CAT TGT TGC GCG CCG TA-3′ and 5′-GCG ATC AGA TCA GCT CCT-3′; primer 6, 5′-CCA TAA AAG AGA TAG TGA AAT GCT G-3′ and 5′-ATT TAT CGC TTT GAA TGT CTT TGG-3′; primer 5, 5′-ACC AGA GAG GCT GAC TAG ATT TAG G-3′ and 5′-CTC CAC CTA CCT TTA CTG TTT TC-3′; primer 4, 5′-GAA GTT TTA ATA TGG GAT AAA TGA CCG-3′ and 5′-CCA GAC TAC GGT GAG AAA AGG AA-3′; primer 3, 5′-CAC GAT AGT CTA GAA CGT GAT TT-3′ and 5′-CAT ATG GCT CAT CTC CAT CTT TG-3′; primer 2, 5′-TCC CAC TGG ATA CAG CC-3′ and 5′-GCA TAG GTA ATA GCT TGC GT-3′; primer 1, 5′-TTT AGT TGA CAG AAT TGG CAC ATC-3′ and 5′-TGA AGC CCA AAT GCT TAG GAA TA-3′; *Gapdh* promoter, 5′-TGC AGT CCG TAT TTA TAG GAA CC-3′ and 5′-CTT GAG CTA GGA CTG GAT AAG CA-3′; *Pax6*, 5′-CGG AGG GAG TAA GCC AAG AG-3′ and 5′-TCT GTC TCG GAT TTC CCA AG-3′; and *Mage-a2*, 5′-GGA CTC TGC GCC ATT TTG TTC TGG-3′ and 5′-GCT AGG CAG GCT AAA GGT TGA CC-3′.

RNA Interference. Primary NPCs were isolated from the E11.5 cerebral cortex and dissociated using Nerve-Cells Dispersion Solutions (Sumitomo Bakelite). Immediately after dissociation of the NPCs, the siRNA (stealth siRNA; Invitrogen) were electroporated three times into them with a pulse voltage of 1,500 V and a pulse width of 10 ms using the Neon transfection system (Invitrogen). The final concentration of siRNA was 160–200 nM. The Stealth RNAi negative controls with low (nos.1 and 2) and middle GC contents (Invitrogen) (indicated in figures as control 1, 3, and 2, respectively) were used as a negative control. The target sequence for the *utNgn1* and *Neurog1* siRNA were the following: *utNgn1* 1, GGG ACA ATA AGT GTA CTA ATC GCT T; *utNgn1* 2, GAG TTG CCT CTG AAG TCG CTT TCT T; *utNgn1* 3, GGG CTA CTC TCA AAC CAT TTC TAA A; and *Neurog1*, CGA CGC CCT GTT TCA TCC CAT ACC A.

Vector Constructs and Luciferase Assay. The *Neurog1* promoter region was digested with BamHI and PstI from mouse genomic DNA (GenBank accession no. AF225969) and cloned into the multicloning site of pBluescript II vector. pGL4.20 (Promega) was digested with BglII and the *Neurog1* promoter region was inserted into this site. *utNgn1* and SV40 poly(A) were amplified from mouse cDNA (derived from E12 neocortex) and pGL4.20 vector, respectively, using the PCR primers indicated below. pGL4.20 with the *Neurog1* promoter region was digested with BamHI and SalI, and *utNgn1* was inserted between these sites. For inserting SV40 poly(A) sequences within the *utNgn1* region, the 5′ and 3′ regions of *utNgn1* were cloned separately with primers containing MluI and BamHI sites, respectively. Two SV40 poly(A) sequences were cloned into these sites. NPCs prepared from E11.5 neocortices were plated (4.0×10^5 cells/mL) on dishes coated with poly-D-lysine and transfected with expression reporter plasmids and the pRL-SV40 plasmid encoding *Renilla* luciferase (Promega) with the use of Lipofectamine 2000 (Invitrogen). The cells were cultured for 12 h with FGF2 and then with or without FGF2 for 20 h. Cell extracts were subsequently prepared and assayed for luciferase activity (Promega). Firefly luciferase activity was normalized relative to the activity of *Renilla* luciferase.

Cloning primers are as follows: for SV40 poly(A), (F) 5′-AAA CGC GTC AGA CAT GAT AAG ATA CAT TGA T-3′ and (R) 5′-AAA CGC GTT ACC ACA TTT GTA GAG GTT TTA C-3′; (F) 5′-AAG GAT CCC AGA CAT GAT AAG ATA CAT TGA T-3′ and (R) 5′-AAG GAT CCT ACC ACA TTT GTA GAG GTT TTA C-3′; for the 5′ region of *utNgn1*: (F) 5′-ATA GAT CTG CAT GCC CCG GAG ACT TTC TTC CCT G-3′ and (R) 5′-ATG GAT CCA TAC GCG TTA CCA TAA CTT TCT GGC ATT TTC TT-3′; and for the 3′ region of *utNgn1*, (F) 5′-GGA TCC AAA CTT GAG TTG CCT CTG AAG TCG CT-3′ and (R) 5′-CTC GAG TTA ATT ATT TTA TTT CAA ACA CTT CTG CC-3′.

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2. Imayoshi I, Ohtsuka T, Metzger D, Chambon P, Kageyama R (2006) Temporal regulation of Cre recombinase activity in neural stem cells. *Genesis* 44:233–238.
3. Eiraku M, et al. (2008) Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. *Cell Stem Cell* 3: 519–532.

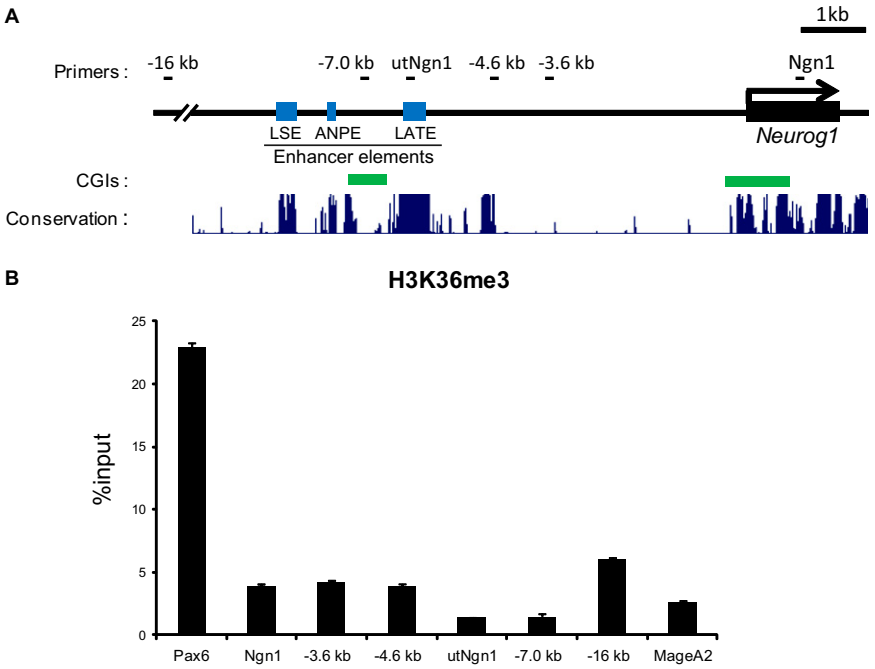

Fig. S1. H3K36me3 at the *Neurog1* locus. (*A*) Positions of PCR primers at the *Neurog1* locus used for ChIP analysis are indicated. (*B*) Primary NPCs isolated from the E11.5 mouse neocortex were harvested and then subjected to ChIP analysis with antibodies to H3K36me3. The *Pax6* and *MageA2* loci were used as positive and negative controls, respectively. Data are expressed as percentage of the input and are means ± SEM ($n = 3$).

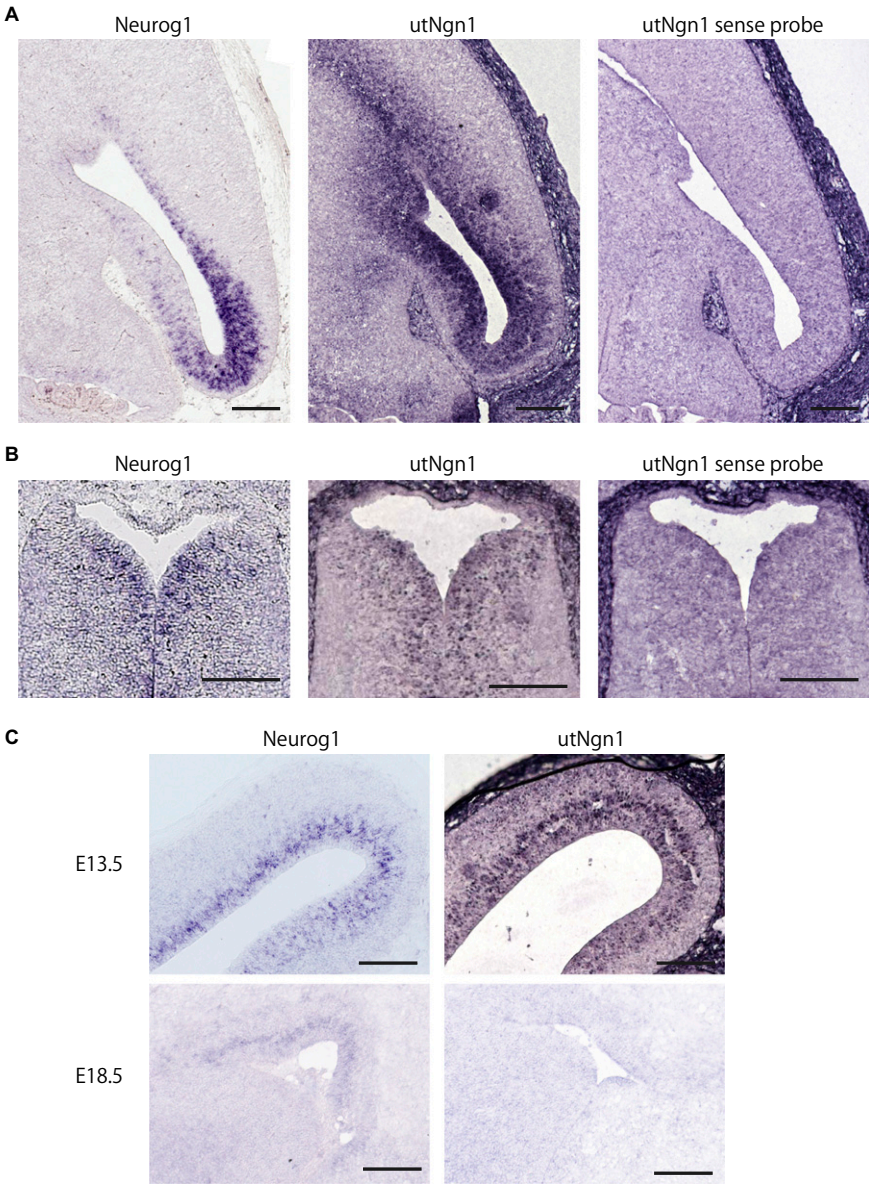

Fig. S2. In situ hybridization of *Neurog1* and *utNgn1*. (*A*) Sagittal sections of the midbrain of an E13.5 embryo. *Neurog1* and *utNgn1* RNA were detected by DIG-labeled RNA probes. Sense probe for *utNgn1* was used as a control. (*B*) Coronal sections of the dorsal diencephalon of an E13.5 embryo. (*C*) Coronal sections of the neocortex of E13.5 and E18.5 embryos. (Scale bar, 100 μm.)

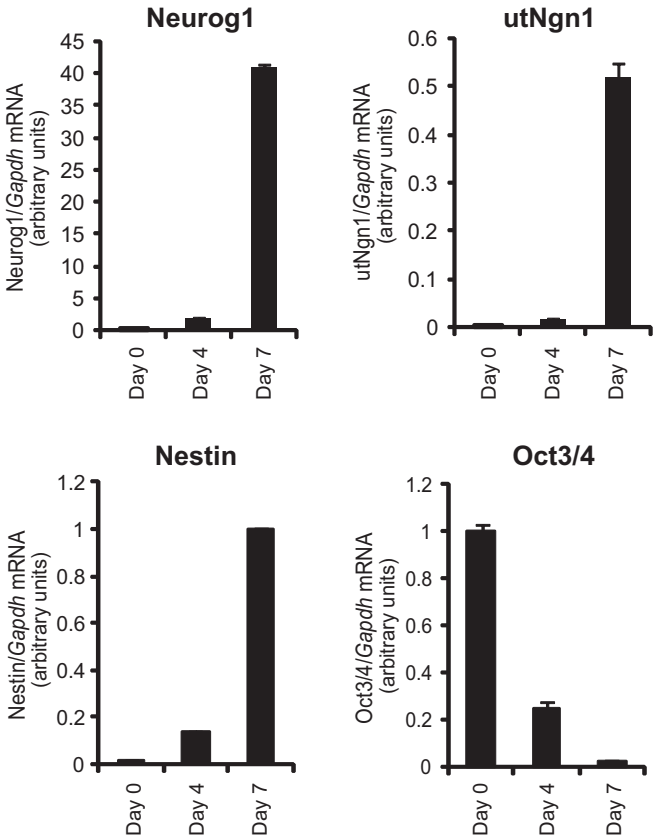

Fig. S3. Expression pattern of *utNgn1* and *Neurog1* during ES cell differentiation into NPCs. Dissociated ES cells were cultured in the wells of a low-adhesion plate and allowed to reaggregate into cell masses of uniform size. They were then induced to differentiate selectively into neural cells (by the SFEBq method) (3) and were harvested after 0, 4, or 7 d in culture. Total RNA was isolated from the cells and subjected to RT-PCR analysis of *utNgn1* as well as *Neurog1*, *Oct3/4*, and *Nestin* mRNAs. Relative amounts of *Neurog1* and *utNgn1* were determined using a standard curve derived from a BAC clone containing both *Neurog1* and *utNgn1* loci, making the values of *Neurog1* and *utNgn1* transcripts comparable. Data are normalized by the amount of *Gapdh* mRNA and are means ± SEM (n = 3).

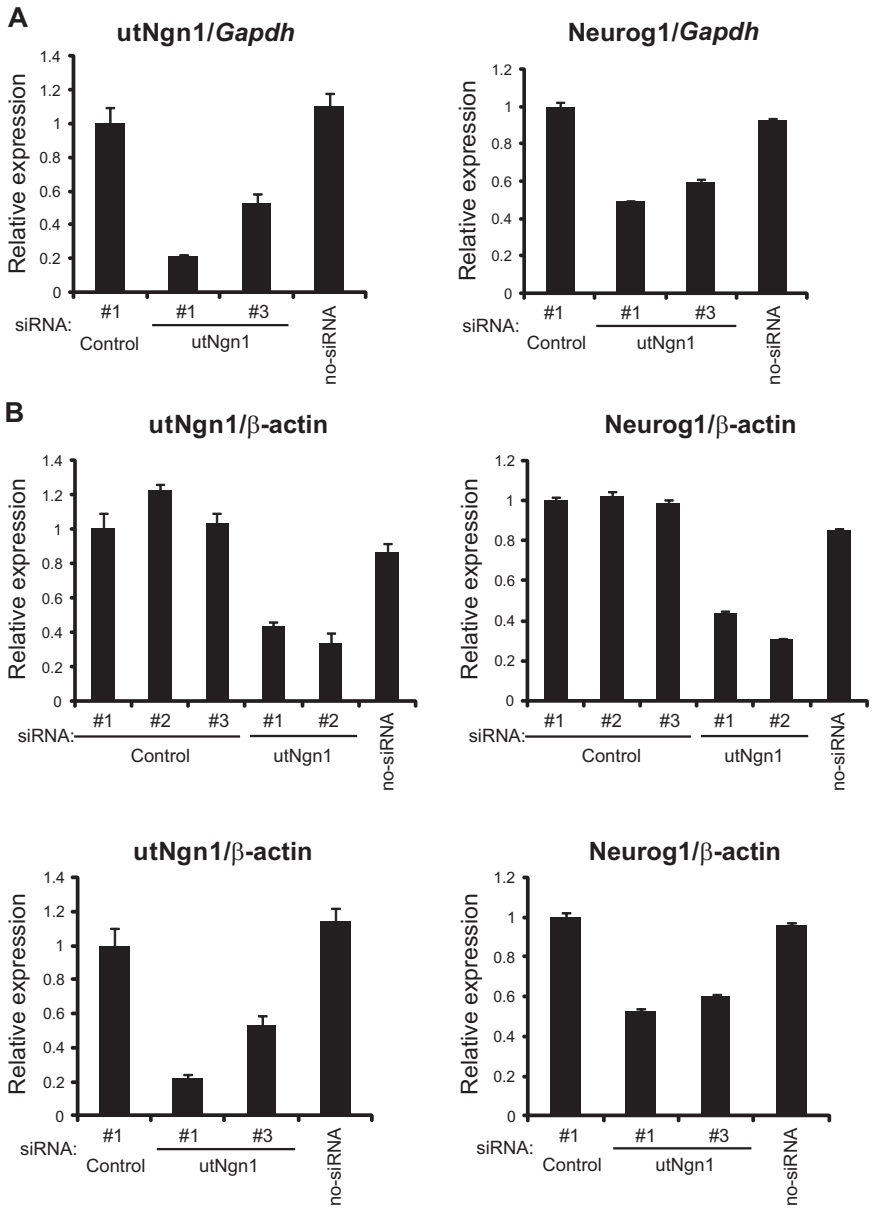

Fig. S4. Validation of *utNgn1* knockdown effects on *Neurog1* expression. (*A* and *B*) Primary NPCs prepared from the E11.5 neocortex were transfected with control siRNA, with *utNgn1* siRNAs or without siRNAs. Cells were cultured for 2 d with FGF2 and then for 10 h without FGF2. Total RNA was then extracted from the cells and subjected to qRT-PCR analysis of *utNgn1* and *Neurog1* mRNA. Data are normalized by the amount of *Gapdh* mRNA (*A*) or *β-actin* mRNA (*B*) and are expressed relative to the corresponding value for cells transfected with the control siRNA 1. (*A*) Cells were transfected with either control siRNA 1, *utNgn1* siRNA 1, 3, or without siRNAs (no-siRNA). Data are means ± SEM (n = 3). (*B*) Cells were transfected with either of the three controls (siRNAs 1, 2, and 3), three *utNgn1* siRNAs 1, 2, and 3, or without siRNAs (no-siRNA). Data are means ± SEM (n = 3).

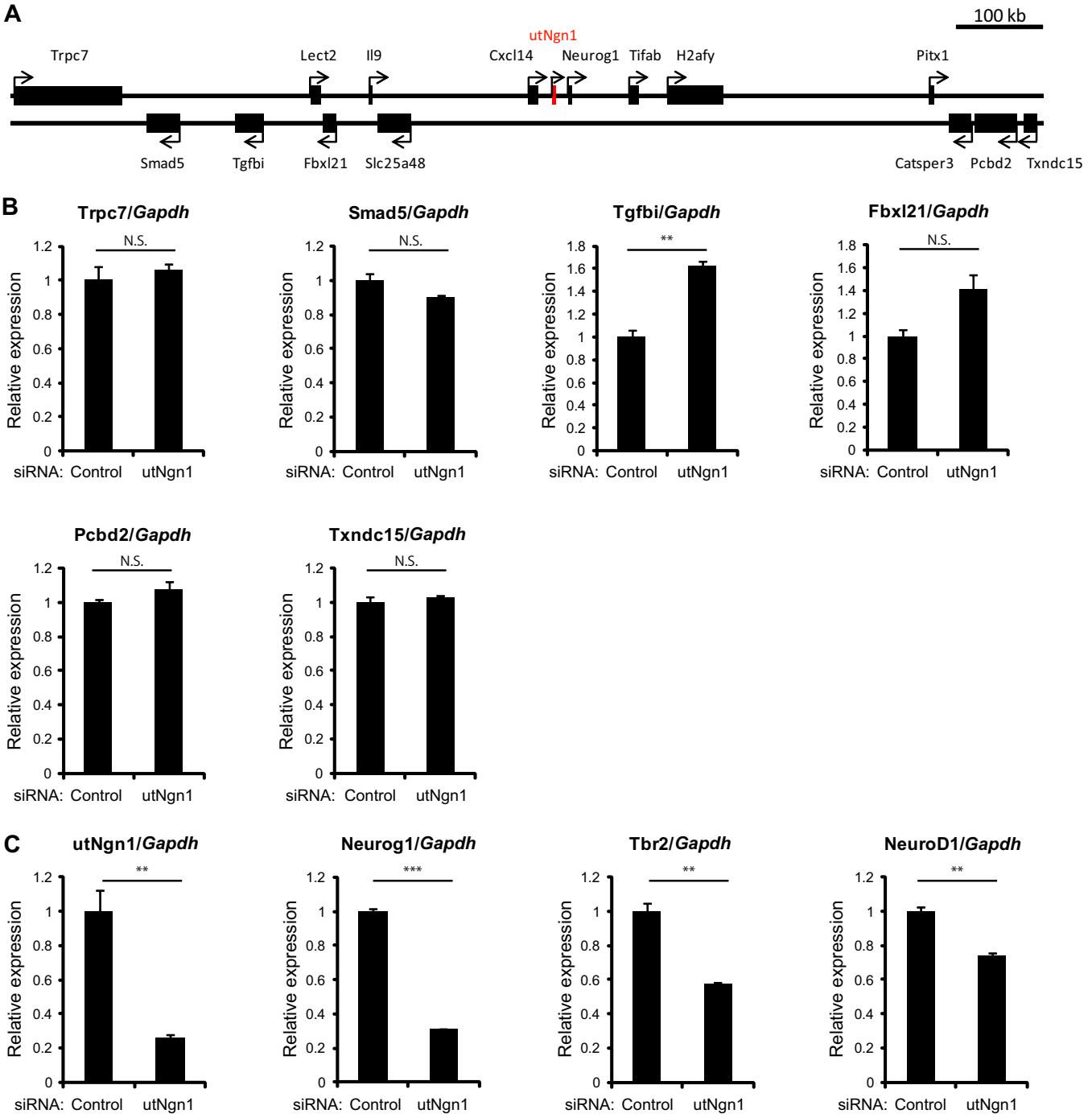

Fig. S5. Effects of *utNgn1* knockdown on gene expression. (*A*) Mapping of the genes located in the 1-Mb region neighboring the *Neurog1* locus. The black rectangles represent the genomic region, including introns, of each gene. Arrows indicate the transcriptional direction of each gene. (*B* and *C*) Primary NPCs prepared from the E11.5 neocortex were transfected with control siRNA (control siRNA 1) or with siRNA against *utNgn1* (*utNgn1* siRNA 2). The cells were cultured for 2 d with FGF2 and then for 8 h without FGF2. Total RNA was then extracted from the cells and analyzed by qRT-PCR. Data are normalized by the amount of *Gapdh* mRNA, are expressed relative to the corresponding value for cells transfected with the control siRNA, and are means ± SEM ($n = 3$). $^{**}P < 0.01$, $^{***}P < 0.001$ (Student *t* test). Genes indicated in *A*, but not in *B* or Fig. 3*C*, were expressed at levels undetectable by qRT-PCR in NPCs.

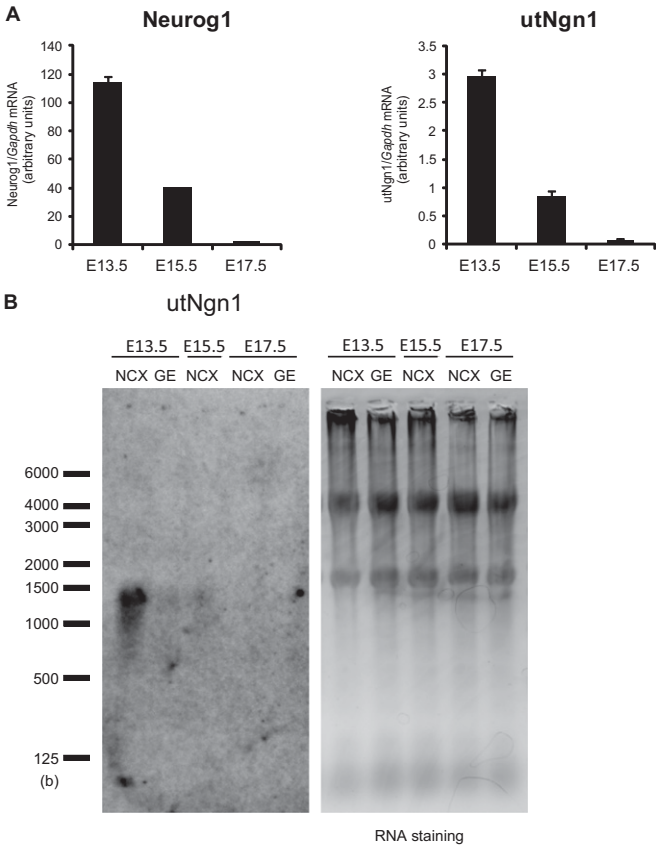

Fig. S6. Expression of *Neurog1* and *utNgn1* is dramatically reduced during development. (*A*) Total RNA isolated from the neocortex of E13.5, E15.5, and E17.5 mouse embryos was analyzed by qRT-PCR with primers for *Neurog1* and *utNgn1*. Relative amounts of *Neurog1* and *utNgn1* were determined using a standard curve derived from a BAC clone containing both *Neurog1* and *utNgn1* loci, making the values of *Neurog1* and *utNgn1* transcripts comparable. Data are normalized by the amount of *Gapdh* mRNA and are means ± SEM ($n = 3$). (*B*) Northern blotting analysis of *utNgn1* expression in E13.5, E15.5, and E17.5 brains. The neocortex (NCX) or ganglionic eminences (GE) were dissected from embryos at each stage and 10 μg of the total RNA extracted from these samples was used for Northern blotting.