Supplementary methods

Immunohistochemistry (IHC)

Brains were fixed for 2 hours in 4% PFA/PBS, incubated overnight at 4°C with 30% (w/v) sucrose in PBS, embedded in OCT compound (Sakura Finetek), and cut with a cryostat to yield 10 or 12 μm-thick sections. Sections were blocked in 0.1% Triton X-100 and 2% donkey serum in Tris-buffered saline (140 mM NaCl, 25 mM Tris-HCl, pH 7.5) for 1 hour at room temperature. Primary antibodies diluted in blocking solution were added before an overnight incubation at 4°C. Then, sections were incubated with secondary antibodies diluted in blocking solution for 40 minutes at room temperature, and mounted in Mowiol (Calbiochem). Images were analyzed using a laser scanning confocal microscope (LSM510, Carl Zeiss; TSC-SP5, Leica). Images were processed with Photoshop CS software (Adobe). Primary antibodies and dilutions used are as follows: rat anti-CTIP2, 1:2000 (Abcam); mouse anti-Ring1B, 1:200 (MBL); rat anti-BrdU, 1:200 (Abcam); mouse anti-BrdU, 1:500 (BD); rabbit anti-Cux1, 1:200 (SantaCruz); rabbit anti-Tbr1, 1:1000 (Abcam); rabbit anti-Sox5, 1:1000 (Abcam); rabbit anti-Pax6, 1:200 (Millipore); rabbit anti-Tbr2, 1:1000 (Abcam); rabbit anti-pH3,

1:200 (Cell Signaling); mouse anti-Satb2, 1:200 (Abcam); mouse anti-βIII-tubulin, 1:1000 (Covance); rabbit anti-Ki67, 1:200 (Novocastra).

RT-qPCR

Total RNA was obtained from NPCs using RNAiso (Takara) following the instructions of the manufacturer. Reverse transcription (RT) was performed with 2-5 μg of total RNA, oligod(T)12-18 (Invitrogen) primers, and ReverTra Ace (TOYOBO). The resulting cDNA was subjected to real-time PCR in a Roche LightCycler with SYBR Premix Ex Taq (Takara; Roche). The amount of target mRNA was normalized relative to that of *Gapdh* mRNA. The primers used are as follows: Fezf2, sense 5'-CTCTACTGACAGCAAACCCA-3' and antisense 5'-CTTTGCACACAAACGGTCT-3'; Gapdh, sense 5'-TGGGTGTGAACCACGA-3' and antisense 5'- AAGTTGTCATGGATGACCTT-3'; βIII-tubulin, sense 5'-ACACAGACGAGACCTACT-3' and antisense 5'-GCAGACACAAGGTGGTT-3'; Ring1B, sense 5'- AGTTACAACGAACACCTCAG-3' and antisense 5'-TCCAAACAAATTGGGCACAT-3'; Pax6, sense

5’-CGGAGGGAGTAAGCCAAGAG-3’ and antisense

5’-TCTGTCTCGGATTTCCCAAG-3’; Ezh2 (SET domain), sense

5’-TTTGCTAATCATTCAGTAAATCCAAAC-3’ and antisense

5’-GCAAAGATGCCTATCCTGTG-3’.

ChIP

For H3K27me3 ChIP, cells were first fixed in 0.5% PFA for 10 minutes at room temperature, put on ice in a Lysis buffer [1% SDS, 10 mM EDTA, 50 mM Tris-HCl (pH 8.1)] for 10 minutes, and then sonicated to shear genomic chromatin into DNA fragments. The lysate was incubated with Dynabeads Protein A (Invitrogen) for 1 hour at 4ºC, after which the beads were removed and the lysate was incubated overnight at 4ºC with antibody and protein A beads. The beads were then isolated and washed 8 times with Wash buffer [50 mM Hepes-KOH (pH7.6), 500 mM LiCl, 1 mM EDTA (pH8.0), 1% NP-40, 0.7% Na-Deoxycholate], and twice with TE buffer [10 mM TrisHCl (pH 8.0), 1 mM EDTA]. To elute immune complexes from the beads, the beads were incubated at 65ºC for 15 minutes in a solution containing 10 mM Tris-HCl (pH

8.0), 5 mM EDTA, 300 mM NaCl, and 0.5% SDS. Then the beads were removed, and the eluate was incubated at 65ºC overnight. The proteins then were eliminated by digestion with proteinase K at 55°C for 1 hour. The DNA was then extracted with PCI (phenol/chlorophorm/isoamylalcohol) and EtOH, after which it was rinsed with 70% EtOH and suspended in water. The eluted DNA was subjected to real-time PCR in a Roche Light- cycler using Thunderbird SYBR qPCR mix (TOYOBO).

For Ring1B ChIP, cells were first fixed in 1% formaldehyde for 10 minutes at room temperature and then put on ice in a swelling buffer [20 mM Hepes (pH 7.8), 1.5 mM $MgCl_2$, 10 mM KCl, 0.1% NP-40, 1 mM DTT] for 10 minutes. After removing the swelling buffer, they were suspended in RIPA buffer [10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 140 mM NaCl, 1% Triton X-100, 0.1% SDS, 0.1% sodium deoxycholate (DOC)] and sonicated to shear genomic chromatin into DNA fragments. The lysate was incubated with Dynabeads Protein A (Invitrogen) for 1 hour at 4ºC, after which the beads were removed and the lysate was incubated overnight at 4ºC with antibody and protein A beads. The beads were then isolated and rinsed twice with RIPA buffer, then washed 6 times with RIPA buffer, twice with RIPA + 500 mM NaCl buffer, twice with

LiCl wash buffer [10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 250 mM LiCl 0.5% NP-40, 0.5% DOC], and twice with TE buffer [10 mM TrisHCl (pH 8.0), 1 mM EDTA]. To elute immune complexes from the beads, the beads were incubated at 65ºC for 15 minutes in a solution containing 10 mM Tris-HCl (pH8.0), 5 mM EDTA, 300 mM NaCl, and 0.5% SDS. Then the beads were removed, and the eluate was incubated at 65ºC overnight. The proteins then were eliminated by digestion with proteinase K at 55°C for 1 hour. The DNA was then extracted with PCI (phenol/chlorophorm/isoamylalcohol) and EtOH, after which it was rinsed with 70% EtOH and suspended in water. The eluted DNA was subjected to real-time PCR in a Roche Light- cycler using Thunderbird SYBR qPCR mix (TOYOBO).

The sense and antisense primers used are as follows: Fezf2 promoter, sense 5’-ACATCCTAATGAGGTAATTATCATTTG-3’ and antisense 5’-ACCGTGCTAATAAACTGCC-3’; Gapdh promoter, sense 5’-TGCAGTCCGTATTTATAGGAACC-3’ and antisense 5’-CTTGAGCTAGGACTGGATAAGCA-3’; Neurog1 promoter, sense 5’-CATTGTTGCGCGCCGTA-3’ and antisense 5’-GCGATCAGATCAGCTCCT-3’.

siRNA

siRNA oligos used are as fellows: control siRNA oligo, AAA UGC UUA GAU GAU CAC UUA UCC C, Fezf2 siRNA oligo #1, MSS225765 (Invitrogen stealth siRNA), siRNA oligo #2, MSS225766 (Invitrogen stealth siRNA).

Supplementary Figure S1. Ring1B deletion did not change the layer formation of neocortex and excessively-produced-CTIP2^{++} neurons did not express cux1. Ring1B$^{flox/flox}$ (Control; A, C) or Ring1B$^{flox/flox}$;NesCreERT2 (Ring1B KO; B, D) mice were treated with tamoxifen at 13.0 dpc. Then the embryos were fixed at P2.5 and subjected to immunohistochemistry with the antibodies indicated. The primary somatosensory areas are shown. Scale bar: 250 μm. Ring1B$^{flox/flox}$ (Control; E, G, I) or Ring1B$^{flox/flox}$;NesCreERT2 (Ring1B KO; F, H, J) mice were treated with tamoxifen at 13.0 dpc. Then the embryos were fixed at P2.5 and subjected to immunohistochemistry with the antibodies indicated. The primary somatosensory areas are shown. Scale bar: 100 μm.

Supplementary Figure S2. The distribution of Satb2^{+} cells and Cux1^{+} cells in the cortex. Ring1B$^{flox/flox}$ (Control; black bars) or Ring1B$^{flox/flox}$;NesCreERT2 (Ring1B KO; white bars) were treated with tamoxifen at 13.0 dpc. The pups' brains were fixed at P2.5 and subjected to immunohistochemistry with an anti-Satb2 antibody (A) and an anti-Cux1 antibody (B). The pups' brains were fixed at 18.5 dpc and subjected to immunohistochemistry with an anti-CTIP2 antibody (C), an anti-Satb2 antibody (D) and an anti-Cux1 antibody (E). Then cortical plate was equally divided into 10 bins positioned parallel to the brain surface. The number of each type of neurons in each bin was determined. Data are the means±s.d. of values of 16 corresponding areas of three control mice and 31 corresponding areas of five KO mice (A,B) or the means±s.d. of values of five hemispheres of three control mice and seven hemispheres of four KO mice (C-E). *P<0.05, **P<0.01.

Supplementary Figure S3. Knockout of the *Ring1B* gene in postmitotic neurons has little effect on the number of CTIP2^{++} cells. The brains of control (A,C,E,G) and Ring1B$^{flox/flox}$;NEX-Cre (B,D,F,H) mice were fixed at P0 (A-F) or P2 (G,H) and subjected to immunohistochemistry using the antibodies indicated. The number of CTIP2^{++} or Cux1^{+} cells was determined (I- K). Data are the means±s.e.m. of values of eight corresponding areas of four control mice and six corresponding areas of three KO mouse (I, J). Data are the means±s.e.m. of values of six corresponding areas of four control mice and five corresponding areas of three KO mouse (K). Scale bar: 50 μm.

Supplementary Figure S4. Ring1B deletion did not significantly affect the number of Pax6-positive neural precursor cells. Ring1B$^{flox/flox}$ (Control, A) or Ring1B$^{flox/flox}$;NesCreERT2 (Ring1B KO NesCreER, B) mice were treated with tamoxifen at 13.0 dpc. The pups' brains were fixed at P2 and subjected to immunohistochemistry with an anti-Pax6 antibody. The number of Pax6^{+} cells was determined (C). Ring1B$^{flox/flox}$ (Control; D, G) or Ring1B$^{flox/flox}$;Emx1-CreERT2 (Ring1B KO Emx1CreER; E, H) mice were treated with tamoxifen at 10 dpc. The pups' brains were fixed at 12 dpc (D,E) or 17 dpc (G,H) and subjected to immunohistochemistry with an anti-Pax6 antibody. The number of Pax6^{+} cells was determined (F,I). Data are the means±s.d. of values of four corresponding areas of two control mice and 4 corresponding areas of three KO mice (C), values of five corresponding areas of one control mice and 22 corresponding areas of three KO mice (F), values of ten corresponding areas of five control mice and six corresponding areas of three KO mice (I). Scale bar: 50 μm.

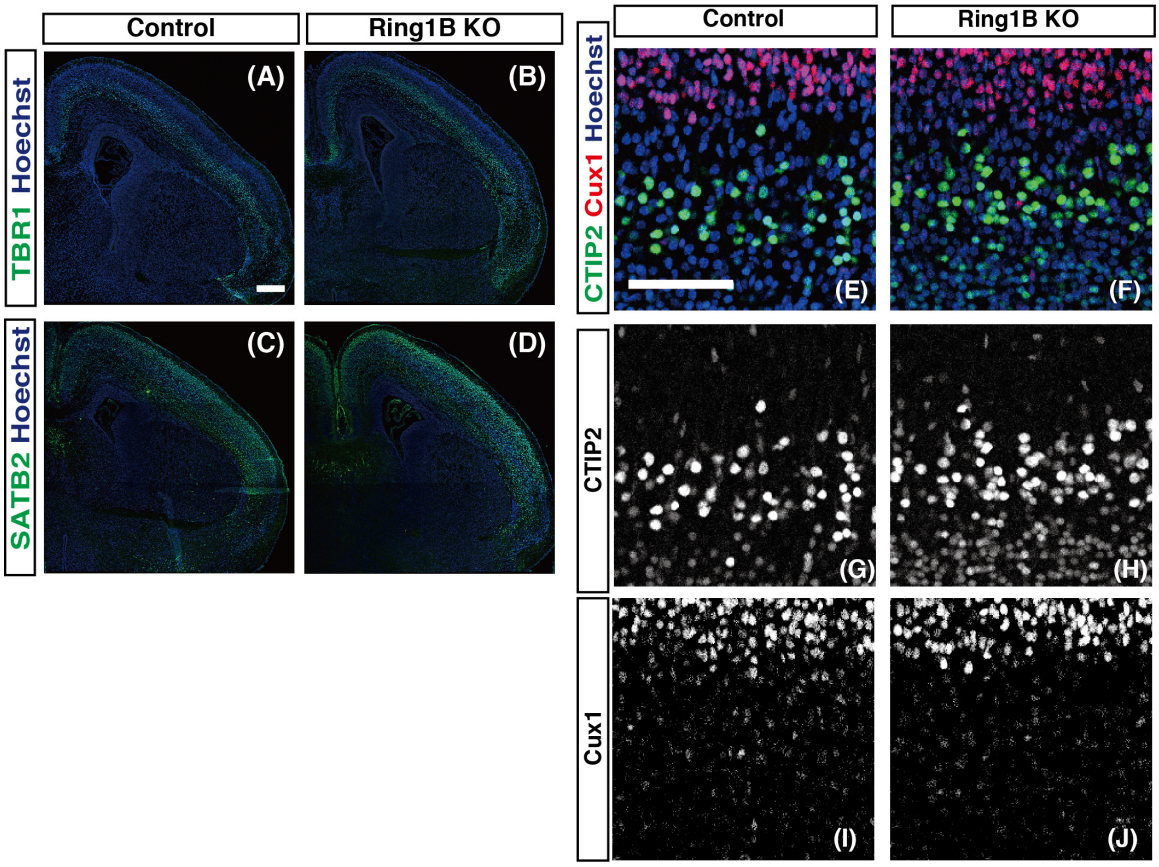
Supplementary Figure S5. FACS gate information. The marked areas in each chart were collected from 14 dpc WT (A), 16 dpc WT (B), and 17 dpc Ring1B;NesCreERTs mice (C). (A,B) Cells were stained with anti-CD133 antibody (red histogram) or not stained (gray histogram) and $CD133^{high}$ and $CD133^{mid}$ (the fractions indicated by the black horizontal lines) were obtained by FACS. (D,E) $CD133^{high}$ and $CD133^{mid}$ cells (each black horizontal line, respectively) were obtained from cells isolated from control (D) and Ring1B KO (E) mouse embryos by FACS at 17 dpc. Cells were stained with anti-CD133 antibody (red histogram) or not stained (gray histogram).

Supplementary Figure S6. The level of Ring1B mRNA did not change between 14 dpc and 16 dpc. The amount of *Ring1B* mRNA in CD133^{high} NPCs of 14 dpc and 16 dpc was assessed using quantitative PCR. Data are means±s.e.m. of values from four experiments.

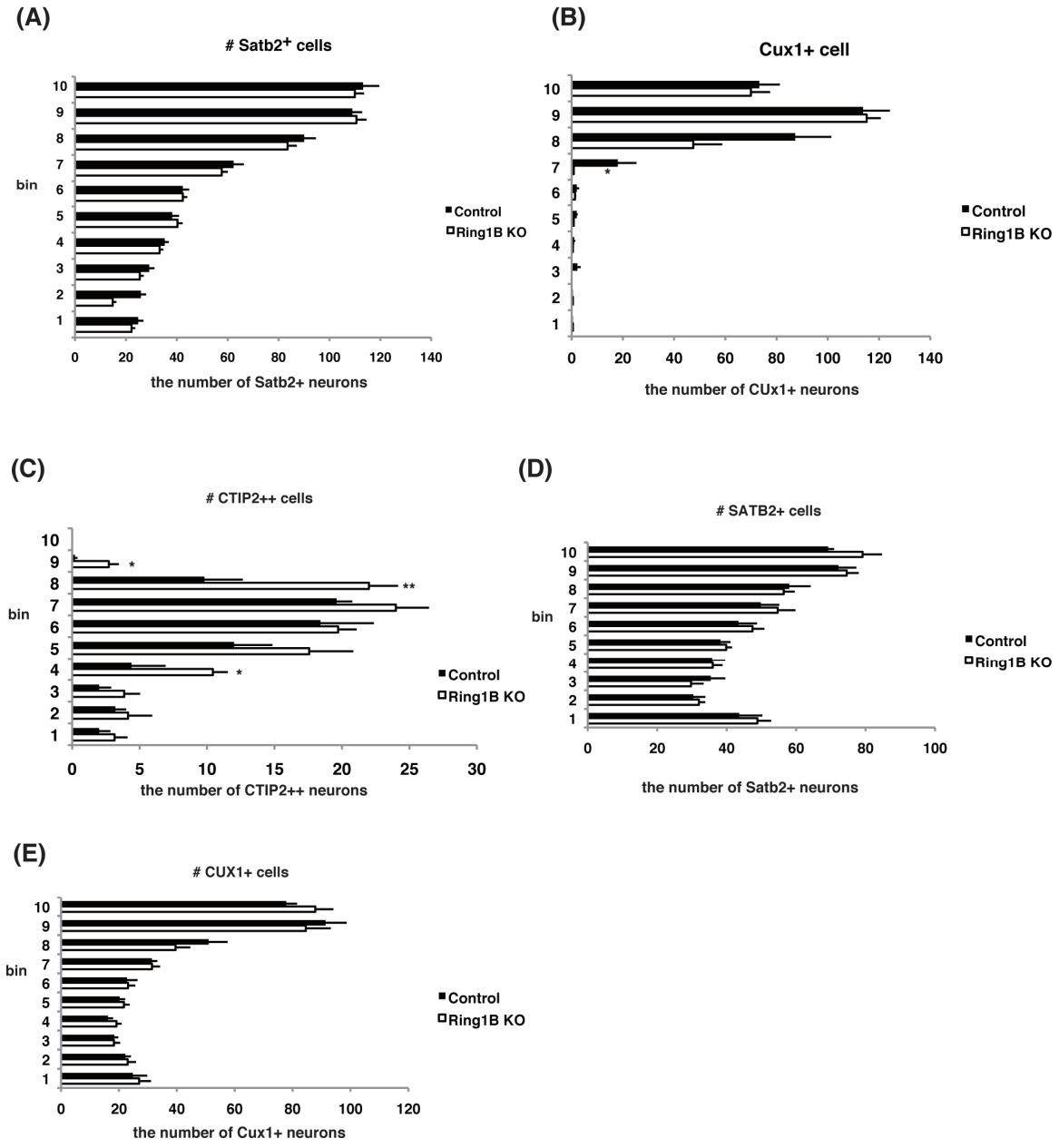
Supplementary Figure S7. The level of H3K27me3 at the *Fezf2* promoter and *Neurog1* promoter increased in the late neurogenic phase. The amount of H3K27me3 at the *Neurog1* promoter was assessed using ChIP and quantitative PCR. The neocortical ventricular zone/subventricular sections of 14 dpc, 16 dpc, or 19 dpc were manually isolated, and the chromatin complex from each stage was immunoprecipitated with anti-trimethylated K27 histoneH3. The immunoprecipitates were subjected to PCR amplification of the promoter region of each gene. The level of H3K27me3 at the *Fezf2* promoter increased between 14 dpc and 16 dpc, whereas the level at the *Gapdh* promoter remained constant. The level *Neurog1* promoter increased gradually during the neurogenic phase. Data are means±s.e.m. of values from three samples. $^{**}P<0.01$.

Supplementary Figure S8. Fezf2 expression was induced in differentiating NPCs in the early stage of neurogenic phase and it was not in the late stage. Proposed model of the role of PcG proteins in terminating SCPN production.

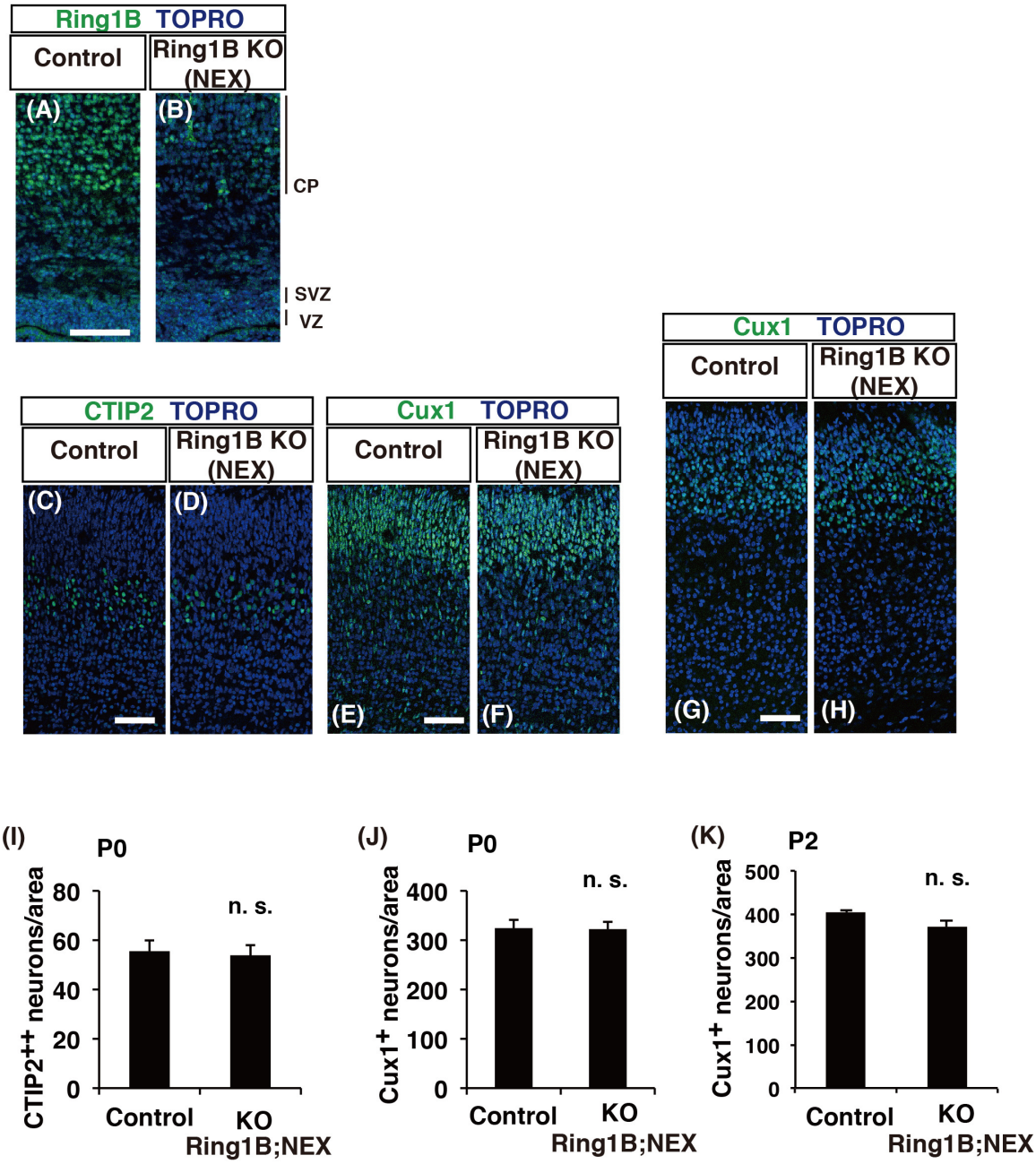
Supplemental Figure 1



Supplemental Figure 2



Supplementary Figure 3



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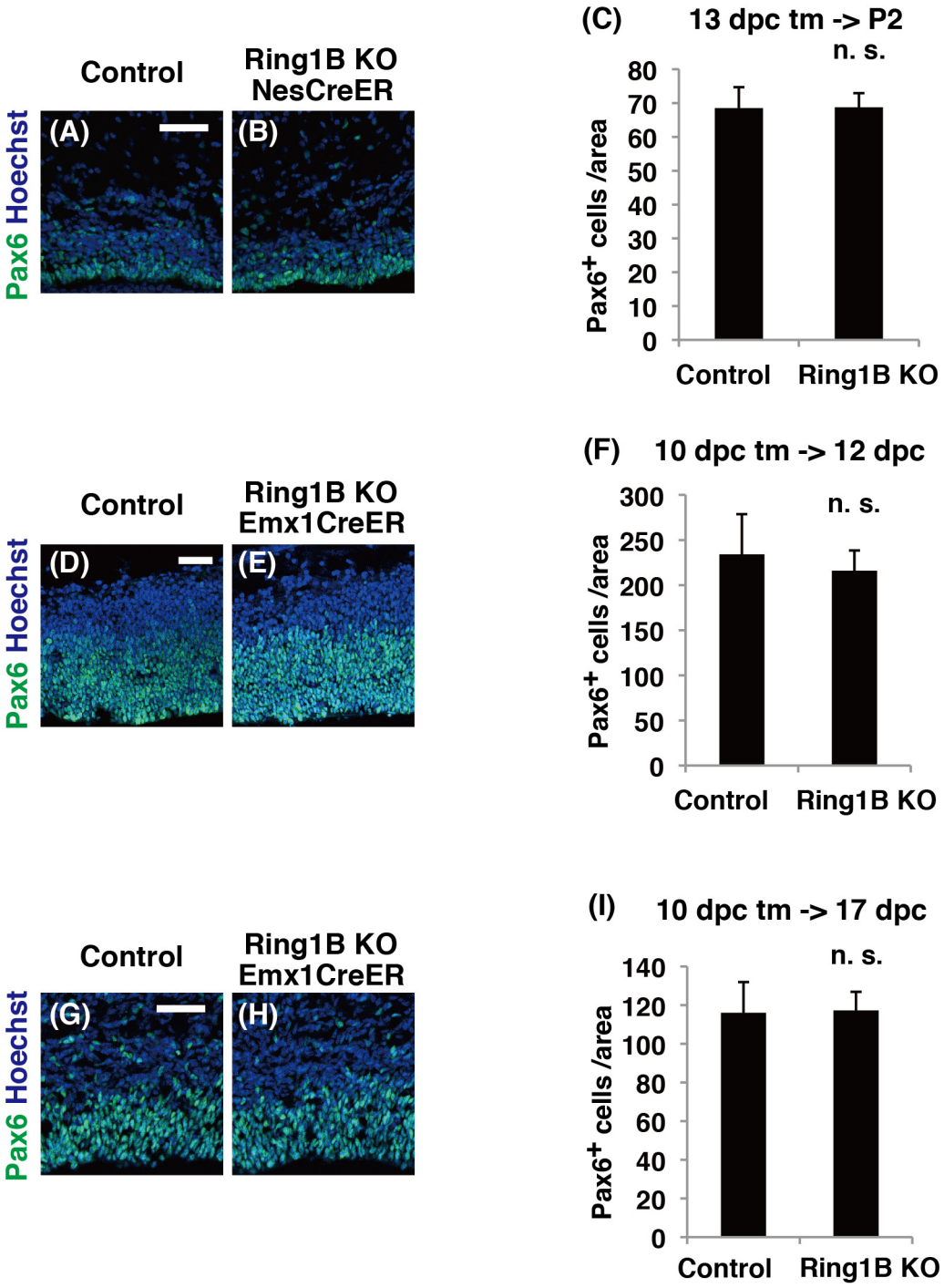

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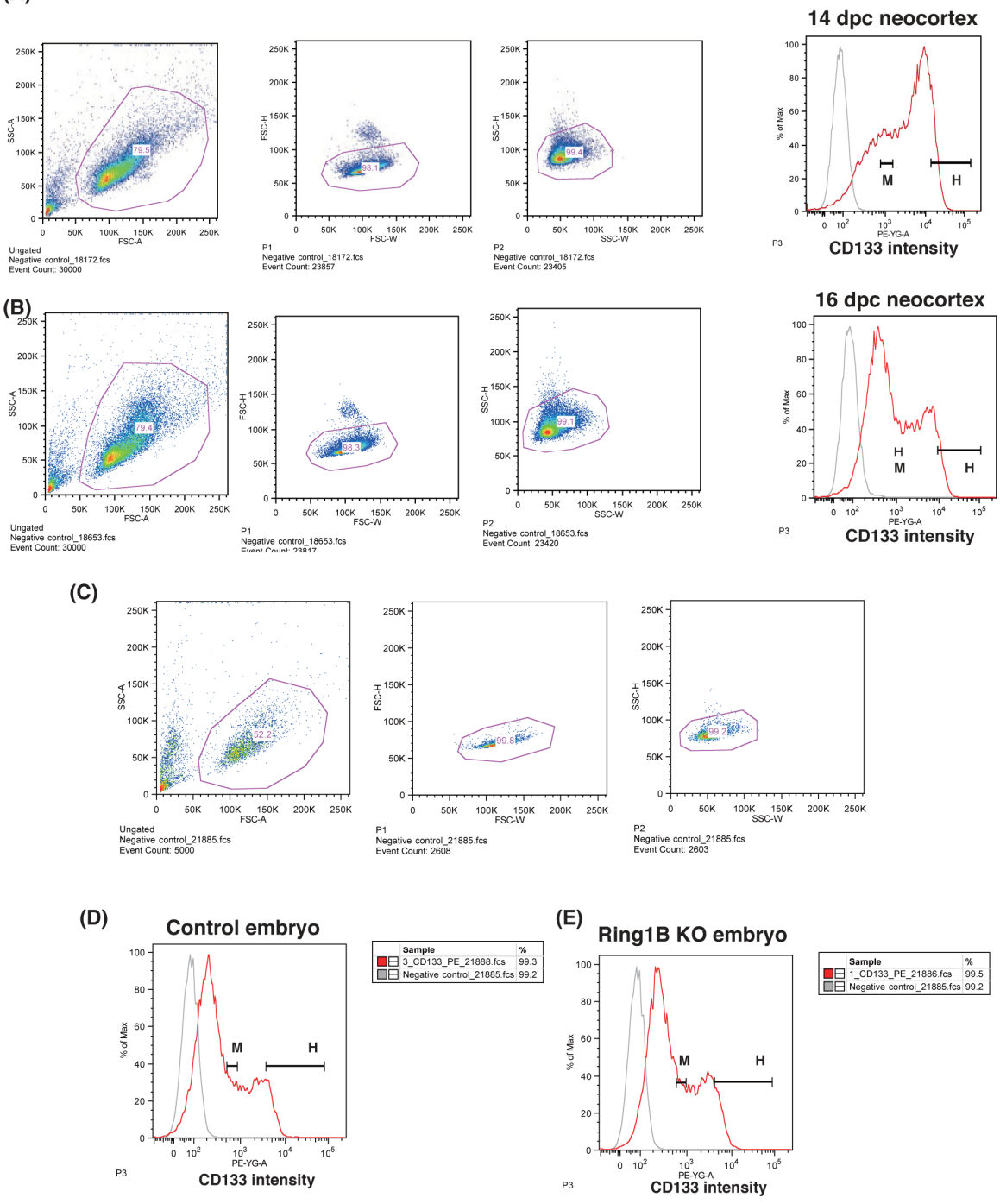

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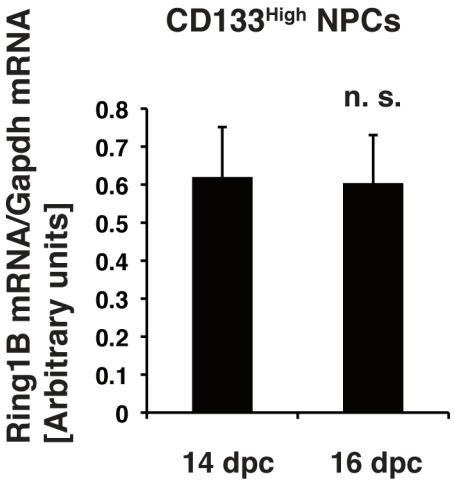
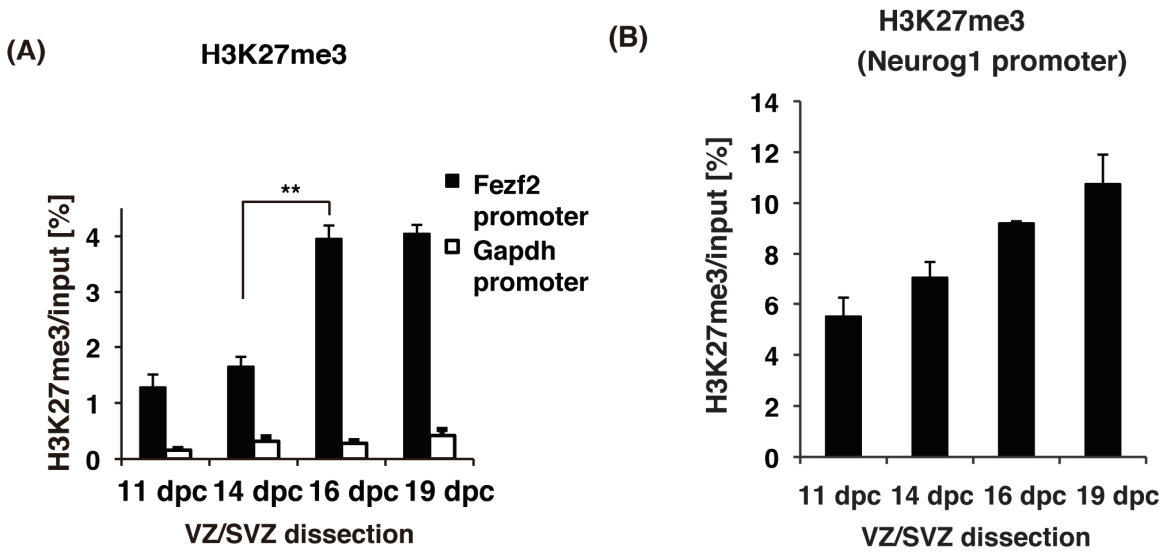


Supplemental Figure 7



Supplementary Figure 8