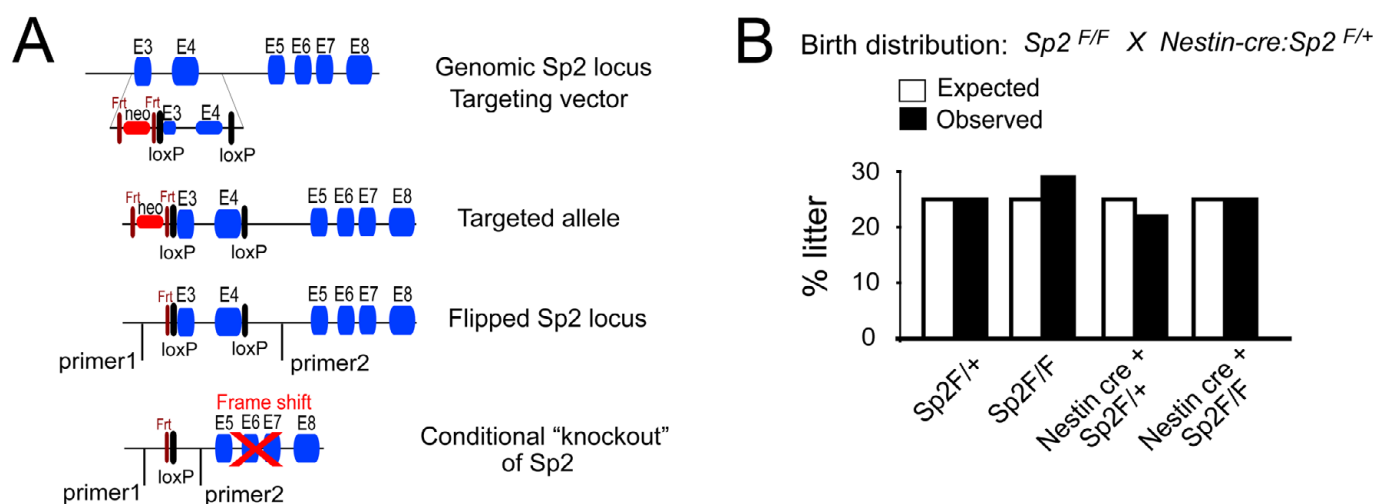
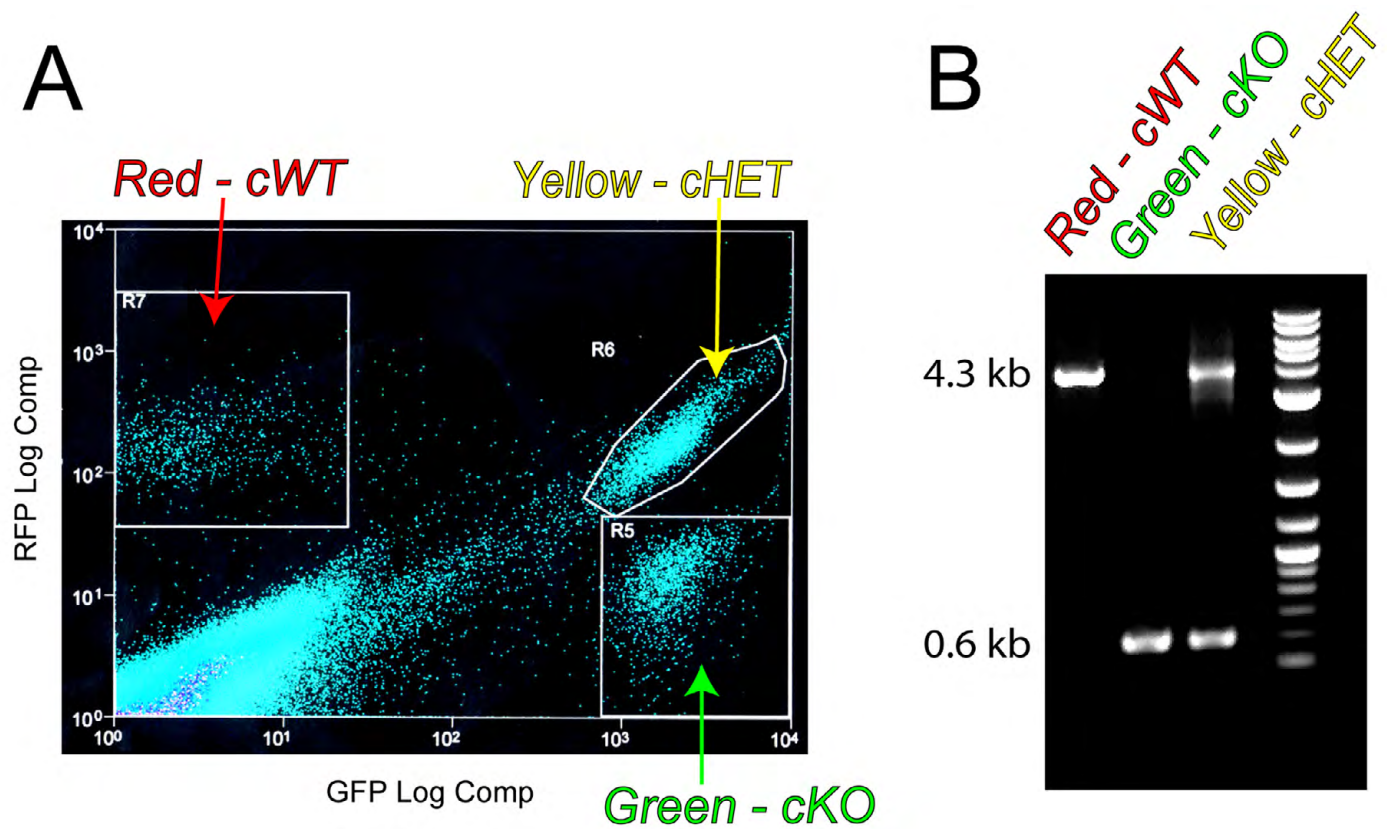


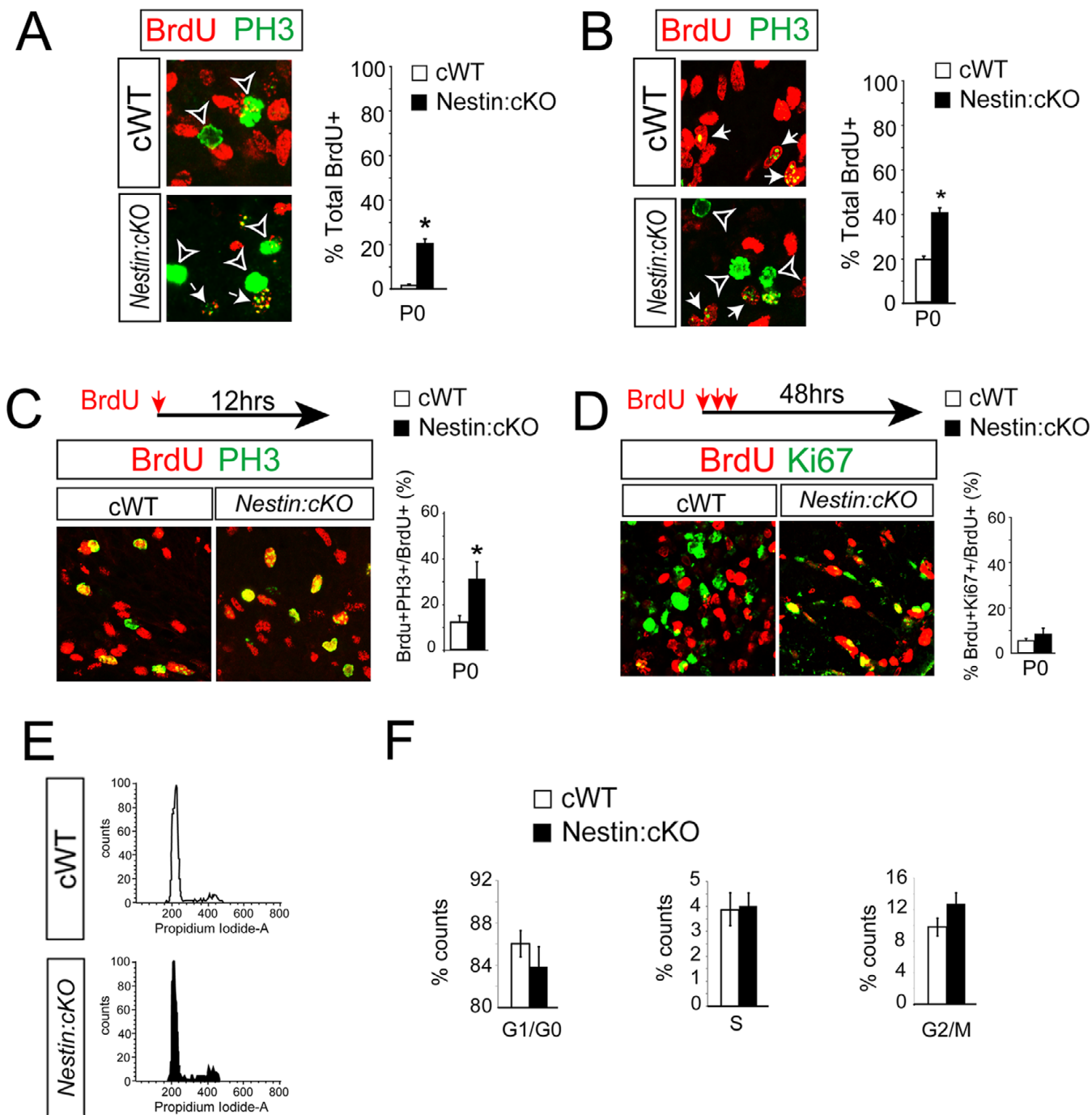
**Fig. S1. *Sp2* expression in the cerebellum and hippocampus of a P56 adult mouse.** (A) *Sp2* is expressed in granular layers of the cerebellum and hippocampus in sagittal sections processed to visualize a DIG-labeled *Sp2* probe (blue). (B) Fluorescence in situ hybridization against the *Sp2* probe (green) co-labeled with NeuN (red) in corresponding granular layer of the cerebellum and the dentate gyrus of the hippocampus.



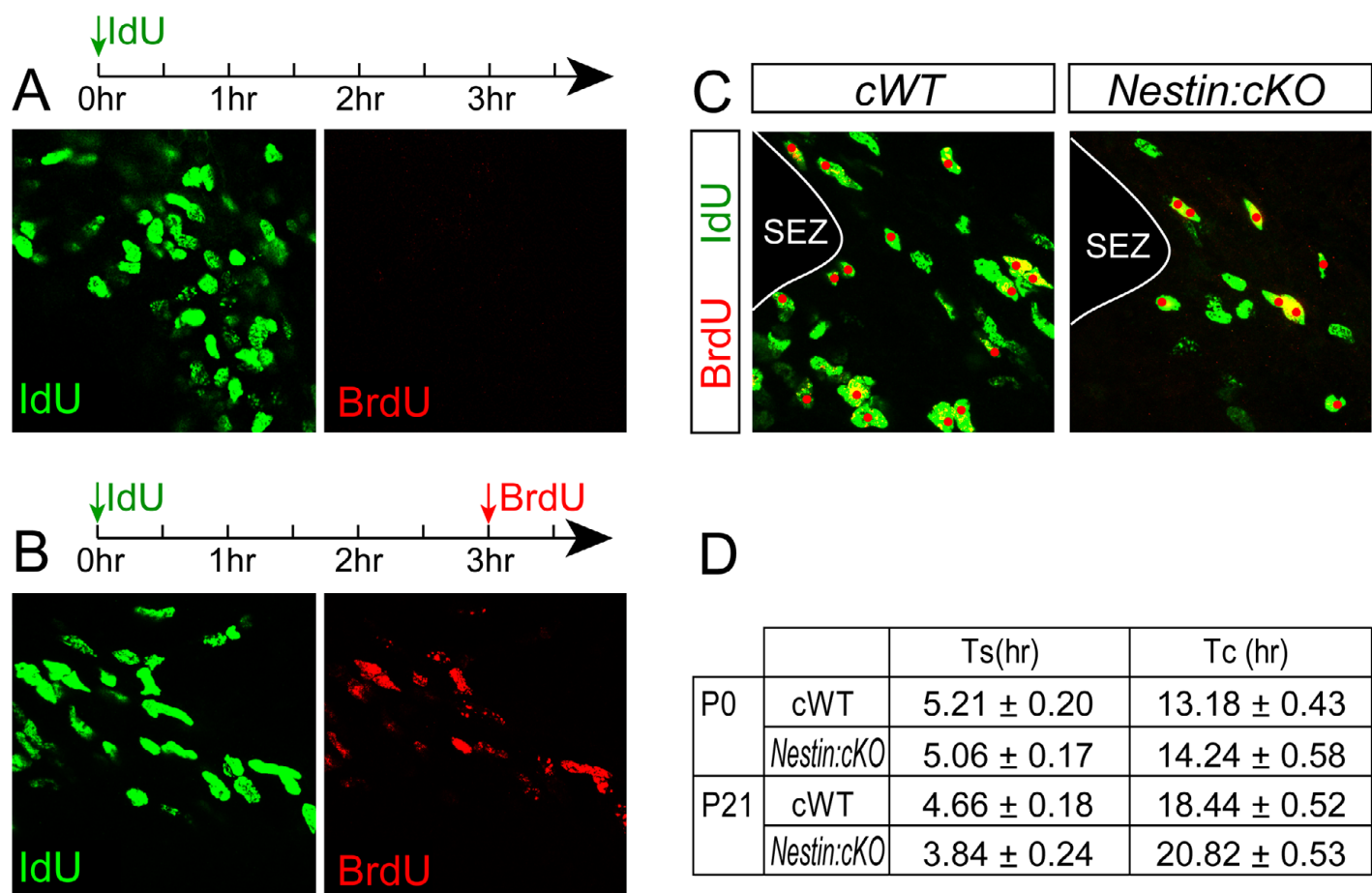
**Fig. S2. Generation and analysis of the *Sp2* floxed mice.** (A) A targeting vector containing an HSVtk-neo cassette (red bar) upstream of *Sp2* exon 3 was generated. LoxP sites (black boxes) were introduced to flank *Sp2* exon 3 and exon 4. Frt sites on both sides of the neo cassette allowed for its removal in targeted ES cells using flip-mediated recombination. Mice carrying the *Sp2* 'floxed' locus were interbred with *Nestin-cre* transgenic mice, which resulted in deletion of exons 3 and 4 in tissue harvested from the postnatal brain. (B) Birth rate of offspring from [*Sp2*<sup>F/F</sup>] crossed to [*Nestin-cre(het):Sp2*<sup>F/+</sup>] mice. The expected genotypes based on Mendelian ratios are 25% for genotypes [*Sp2*<sup>F/+</sup>], [*Sp2*<sup>F/F</sup>], [*Nestin-cre(het):Sp2*<sup>F/+</sup>] and [*Nestin-cre(het):Sp2*<sup>F/F</sup>]. Percentages were calculated for the total number of mice ( $n=203$ , from 22 litters).



**Fig. S3. Distinctly labeled MADM-11 cells faithfully carry allelic deletion of *Sp2* corresponding to expected combinatorial expression of fluorophores.** (A) *Nestin-cre:Sp2<sup>F/+</sup>:MADM-11* cells from P0 mice were harvested and isolated using fluorescence activated cell sorting. (B) PCR analysis of DNA confirmed genotypes of distinctly sorted populations corresponding to red=cWT, green=cKO and yellow=cHet genotypes.

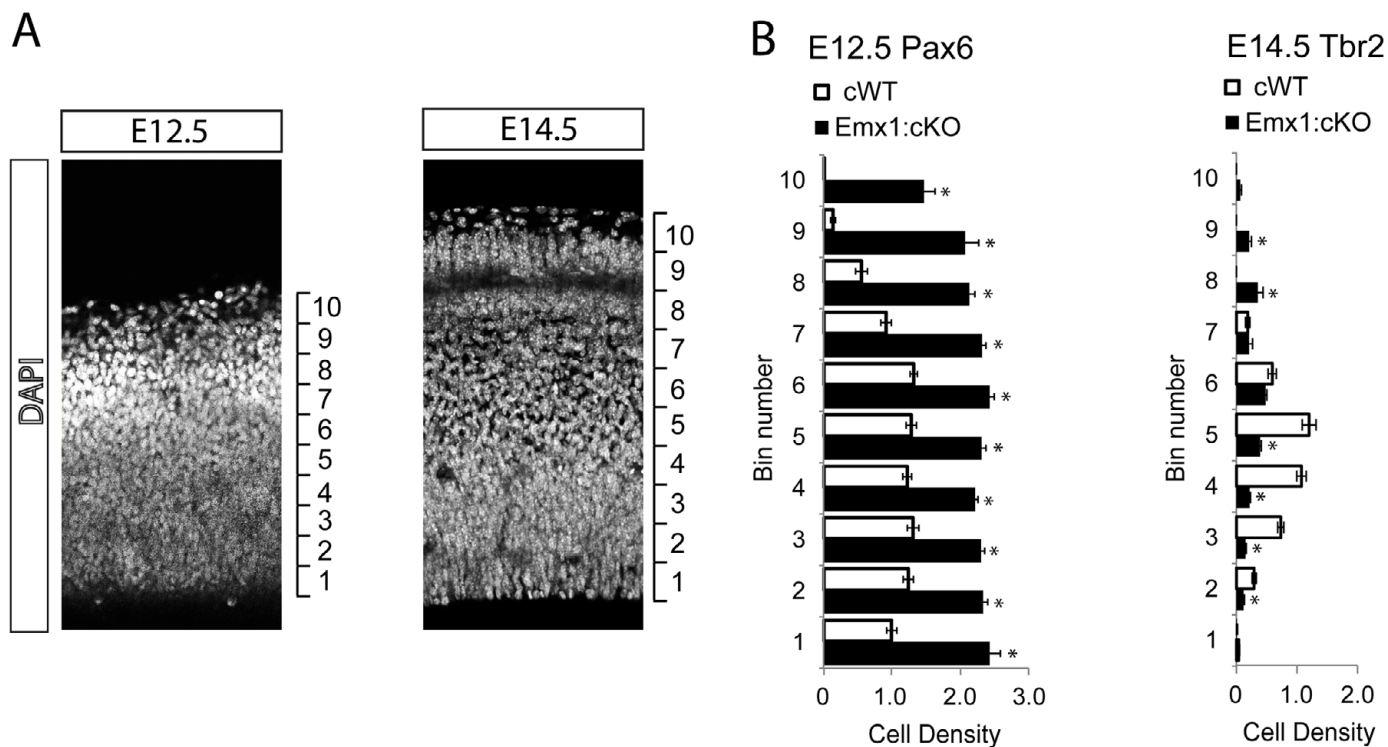


**Fig. S4. Cell cycle progression from S-to-G2/M is disrupted in Nestin:cKO progenitors harvested at P0.** (A) A 1-hour BrdU pulse-chase experiments allowed for identification of proliferating cells that progressed from S to G2/M phases of the cell cycle by co-labeling BrdU (red) with the M-phase marker PH3 (green; arrows). M-phase cells that did not incorporate BrdU during the 1-hour chase period were labeled as PH3+/BrdU negative (arrow heads). The proportion of BrdU+ cells in the SEZ and RMS of Nestin:cKO mice that progressed into G2/M (PH3+) was consistently and significantly higher compared with cWT progenitors at P0. (B) Four-hour BrdU pulse-chase experiments allowed for identification of proliferating Nestin:cKO progenitors that exhibit M phase perdurance. Arrows indicate BrdU+/PH3+ cells; arrowheads indicate BrdU-/PH3+ cells. Percentage of BrdU+/PH3+ cells among all BrdU incorporated cells is significantly increased in P0 mutant compared with the control. In addition, a significant fraction of PH3+ cells in the Nestin:cKO SEZ and RMS remained BrdU negative. (C) BrdU administration followed by 12 hours of chase allowed for indexing M-phase exiting. The percentage of BrdU+/PH3+ cells in the Nestin:cKO SEZ and RMS was significantly higher than in cWT brains. (D) To quantify cell cycle exiting, three pulses of BrdU were administered every 2 hours followed by a 48-hour survival period. In this regimen, BrdU immunoreactivity was combined with Ki67 staining in order to distinguish progenitors that had remained in, or re-entered, the cell cycle after 48 hours (BrdU+/Ki67+) from cells that had exited the cell cycle (BrdU+/Ki67 negative). Percentage of BrdU+/Ki67+ cells among all BrdU incorporated cells is higher in the Nestin:cKO SEZ/RMS compared with controls. For A-D, data are mean±s.e.m.; \* $P < 0.05$ , Student's  $t$ -test,  $n = 3$ /age group. (E,F) Flow cytometry for cell cycle analysis at P0. Data from cycling cells harvested from P0 SEZ and RMS illustrated higher proportion of cells in the G2/M phases in Nestin:cKOs. \* $P < 0.01$ , Student's  $t$ -test,  $n = 3$ /age group.

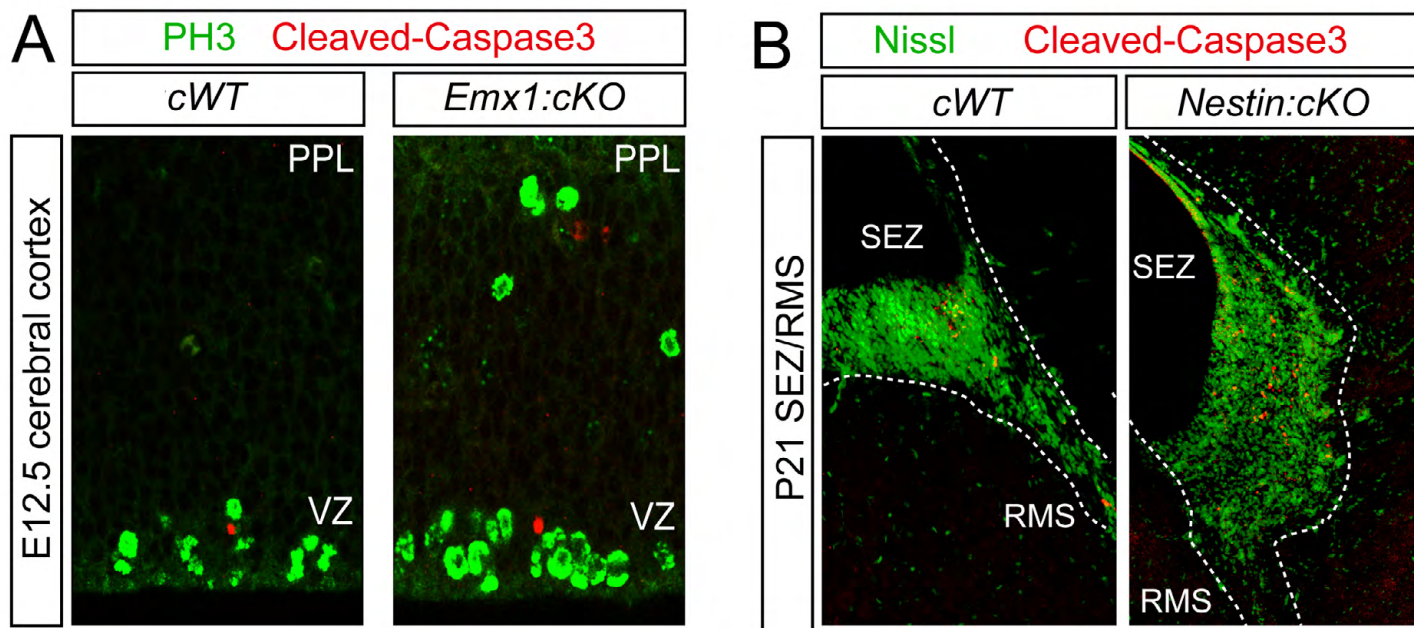


**Fig. S5. IdU/BrdU dual labeling for estimating the lengths of the S-phase and total cell cycle.** (A) An antibody specific to BrdU failed to label IdU incorporated cells (green) in the absence of BrdU injection. (B) A pulse of IdU was followed by a pulse of BrdU 3 hours later that could be distinguished using an antibody that recognizes both BrdU and IdU (green), and one that is specific to BrdU (red). (C) Representative confocal micrographs of IdU (green) and BrdU (red) labeled nuclei in P21 cWT and Nestin:cKO SEZs. Red dots highlight IdU+/BrdU+ cells. (D) Estimated lengths of S (Ts) and total cell cycle (Tc) in the P0 and P21 wild-type and mutant SEZ progenitors. Data are mean±s.e.m.,  $n=3$ /genotype/age.





**Fig. S6. Distribution of Pax6 and Tbr2 progenitors in the E12.5 and E14.5 cerebral cortex.** (A) The thickness of E12.5 and E14.5 cerebral cortices were visualized by DAPI nuclear staining and divided into 10 equidistant bins to quantify distribution of distinct makers. (B) Quantified densities of Pax6+ or Tbr2+ cells in each bin of *Emx1:cWT* and *Emx1:cKO* cerebral cortices at each age. Data are mean $\pm$ s.e.m.; \* $P < 0.01$ , Student's *t*-test,  $n = 3$ /age group/genotype.



**Fig. S7. Analysis of caspase-dependent apoptosis in conditionally *Sp2*-deleted embryonic cerebral cortex and postnatal SEZ/RMS.** (A) Confocal images of tissue stained for cleaved caspase 3 (red) and PH3 (green) in the E12.5 cerebral cortex. The density and distribution of cleaved caspase 3-labeled apoptotic cells were indistinguishable in *Emx1:cWT* and *Emx1:cKO* cortices. (B) At P21, an increase in immunoreactivity for cleaved-caspase 3 (red) emerged within the cellular bulge revealed by Nissl stain (green) in *Nestin:cKO* compared with cWT SEZ and RMS.

**Table S1.** Mice purchased from the Jackson laboratory

<b>Name</b>	<b>Jackson Laboratory ID</b>	<b>Catalogue #</b>
<i>Nestin-cre</i>	B6.Cg-Tg ( <i>Nes-cre</i> )1Kln/J	003771
<i>tdTomato</i>	B6.129S6-Gt ( <i>ROSA</i> ) 26Sor <sup><i>tm14</i></sup> ( <i>CAG-tdTomato</i> ) Hze/J	007908
<i>Emx1<sup>cre</sup></i>	B6.129S2- <i>Emx1</i> <sup><i>tm1(cre)</i></sup> Krf/J	005628

<b>Antibody</b>	<b>Source</b>	<b>Dilution</b>
rabbit anti-Ki67	Vision Biosystems	1:500
rabbit anti-PH3	Millipore	1:500
mouse anti- PH3	Abcam	1:500
mouse anti-BrdU	BD Bioscience	13:1000
guinea pig anti-Dcx	Millipore	1:1000
mouse anti-GFAP	Millipore	1:1000
mouse anti-NeuN	Millipore	1:1000
rabbit anti-S100_	Sigma	1:1000
rabbit anti-Dlx2	Gift of Dr. David Eisenstat	1:1000
rabbit anti-Gsx2	Gift of Dr. Kenny Campbell	1:4000
rabbit anti-Pax6	Millipore	1:500
rabbit anti-cleaved caspase3	Cell Signaling	1:1000
rabbit anti-BLBP	Millipore	1:500
rabbit anti-Tbr2	Abcam	1:500
Rabbit anti-NG2	Millipore	1:1000
Mouse anti-Tuj1	Covance	1:1000
Rabbit anti-Cux1	Santa Cruz	1:500
Rabbit anti-Ctip2	Abcam	1:500
Rat anti-BrdU	Abcam	1:200
chicken anti-GFP	Abcam	1:1000
rabbit anti-RFP	Abcam	1:1000
Goat anti-rabbit Alexa488	Invitrogen	1:1000
Goat anti-mouse Alexa488	Invitrogen	1:1000
Goat anti-chicken Alexa488	Invitrogen	1:1000
Goat anti-rabbit Cy3	Millipore	1:1000
Goat anti-mouse Cy3	Millipore	1:1000
Goat anti-rabbit Alexa647	Invitrogen	1:500
Goat anti-mouse Alexa647	Invitrogen	1:500
Goat anti-guinea pig Cy3	Millipore	1:1000

**Table S2.** Primary and secondary antibodies used in the study.