

Supporting Information

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SI Materials and Methods

Generation of *Ng2-Cre* Transgenic Mice. To document the *in vivo* fate of NG2⁺ cells in the mammalian central nervous system, we generated a transgenic mouse line that expresses Cre recombinase under *CSPG4* transcriptional regulation, using a BAC transgenic strategy (1). Our *Ng2-Cre* transgenic line specifically represents the NG2⁺ progenitor population or populations *in vivo* throughout the developmental stages and in adult mice. The selected mouse line has been maintained as heterozygous with the wild-type or *Rosa26LacZ* reporter line. For brain tissue collections, mice at various ages of either sex were anesthetized with isoflurane before transcardial perfusion. Mice were perfused with 4% paraformaldehyde in 1× phosphate buffer (PBS). The tissues collected were then postfixed at 4 °C overnight in 4% paraformaldehyde. Later, the tissue was sectioned at 40–50 μm thickness with a vibratome.

BrdU Administration. The generation time of different NG2 progenies during embryonic dates was determined by 1-day BrdU incorporation (BrdU injection every 6 h for 24 h) at embryonic day (E)12.5, E14.5, E 16.5, and E18.5 given intraperitoneally to the pregnant dams. For postnatal birth dating, each animal was injected intraperitoneally with BrdU once a day for 10 d according to three different time frames [postnatal day 0 (P0)–P10, P11–P20, and P21–P30]. Mice were killed at P30, and their brains were sectioned for immunohistochemistry analysis to de-

termine the cell lineage association with birth time. For proliferation analysis, BrdU was injected intraperitoneally at a specific age (e.g., P3) 3 h before tissue collection (for a detailed BrdU injection and experimental timetable, see Fig. S3).

Immunohistochemistry. *Ng2-Cre/Rosa26LacZ* mice were perfused with 4% paraformaldehyde and then sectioned using a vibratome at 40 μm thickness. Floating brain sections were immunostained with various antibodies to β-galactosidase (β-gal; Abcam), oligodendrocyte transcription factor (Olig2) (Millipore), NG2 (Millipore), neuronal lineage marker (Millipore), Tbr1 (Abcam), Ctip (Abcam), GST-π (Cell Signaling), GFAP (Sigma), Cre (Millipore), Calretinin (Millipore), Ki67 (Vector), BrdU (Abcam), and PDGFαR (BD Biosciences). Sections were incubated with primary antibodies at 4 °C overnight, followed by secondary antibody incubation for 2 h at room temperature before PBS washes and mounting.

For BrdU immunostaining, sections were preincubated in 2N HCl at 37 °C for 15 or 30 min, depending on the thickness of the sections, and neutralized with 1× PBS before blocking. For Ki67 immunostaining, sections were preincubated with sodium citrate buffer (10 mM, 0.05 Triton X-100 at pH 6) at 80 °C for 30 min, followed by cooling to room temperature and several PBS washes. After blocking, all of the sections were stained as described in previous studies (2).

1. Yang XW, Model P, Heintz N (1997) Homologous recombination based modification in *Escherichia coli* and germline transmission in transgenic mice of a bacterial artificial chromosome. *Nat Biotechnol* 15(9):859–865.

2. Wojtowicz JM, Kee N (2006) BrdU assay for neurogenesis in rodents. *Nat Protoc* 1(3): 1399–1405.

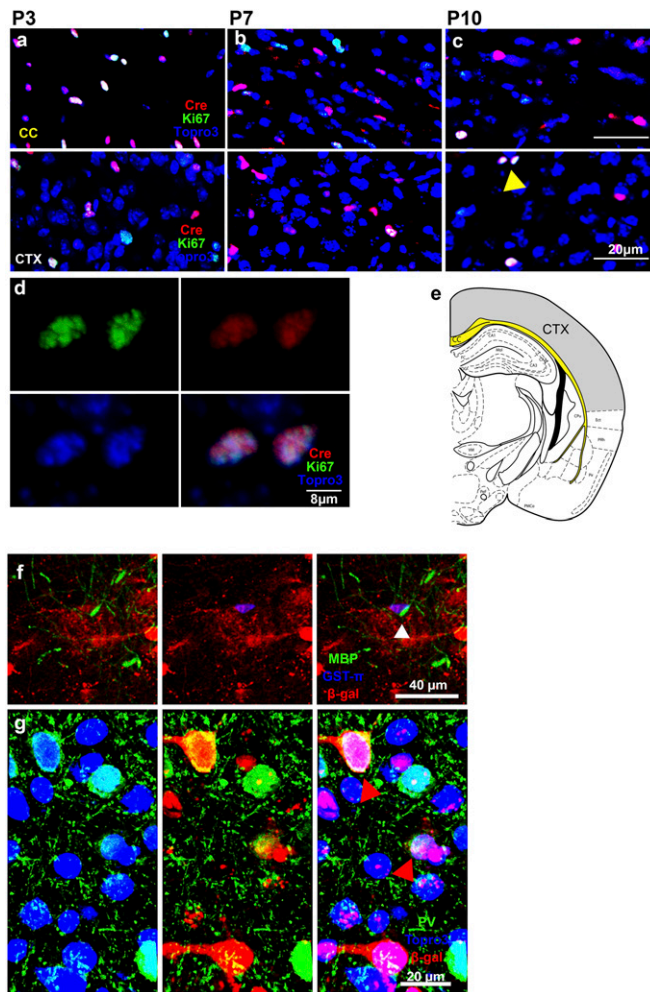


Fig. S1. The NG2⁺ proliferative activity and differentiation rates suggested the heterogeneous nature of NG2 cells in corpus callosum (CC) and cortex (CTX). (A–C) The tissue sections were immunolabeled with Ki67 and Cre at the age of P3, P7, and P10. The Ki67 labeling indicated the Ng2/Cre⁺ cells are in mitotic state at early postnatal stages but gradually decline as CC reaches maturation. (D) Enlarged image of Ki67 and Cre staining in C. (E) The diagram that depicts the areas of the CC and dorsal CTX that were analyzed. (F) Immunostaining with mature oligodendrocyte (OL) markers (myelin basic protein and GST-π) showed that NG2 progenies differentiate into mature OLs. (G) Fluorescent immunostainings of the CTX with the interneuronal marker Parvalbumin.

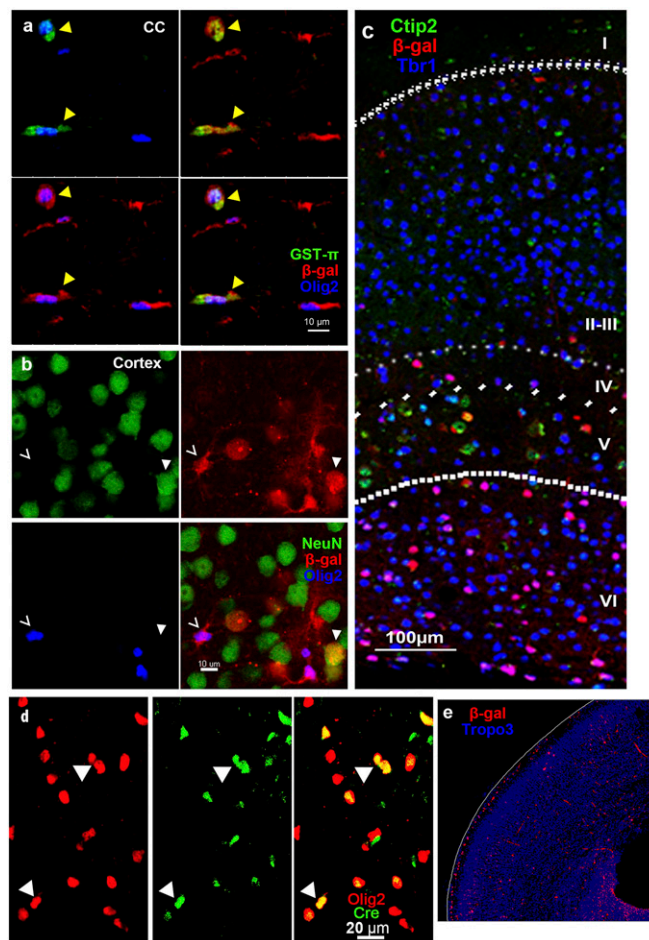


Fig. S2. Tangential migration of NG2⁺ progenitor cells during early embryonic development hinted their cortical interneuronal fate. (A and B) Brain sections of P30 *Ng2-Cre/Rosa26LacZ* were immunolabeled for Olig2, β -gal with neuronal lineage marker, or GST- π . (A) In CC, all matured OLs (yellow arrowheads) and β -gal⁺ NG2 progenies were stained with Olig2. (B) In dorsal CTX, some β -gal⁺ NG2-derived progenies were Olig2-negative but were positive with neuronal lineage marker (NeuN) staining (white arrowheads). (C) Layer-specific markers staining in P30 tissue showing the layer bias distribution of NG2 progenitor-derived neurons in dorsal CTX. (D) The enlarged confocal image of the region indicated by the yellow arrowhead in Fig. 3D, showing the coexpression of Cre and Olig2 in E14.5 *Ng2-Cre/Rosa26LacZ* brain sections. (E) The migrating NG2-derived progenies at neonatal stage are found in cortical layer 1 at P0.

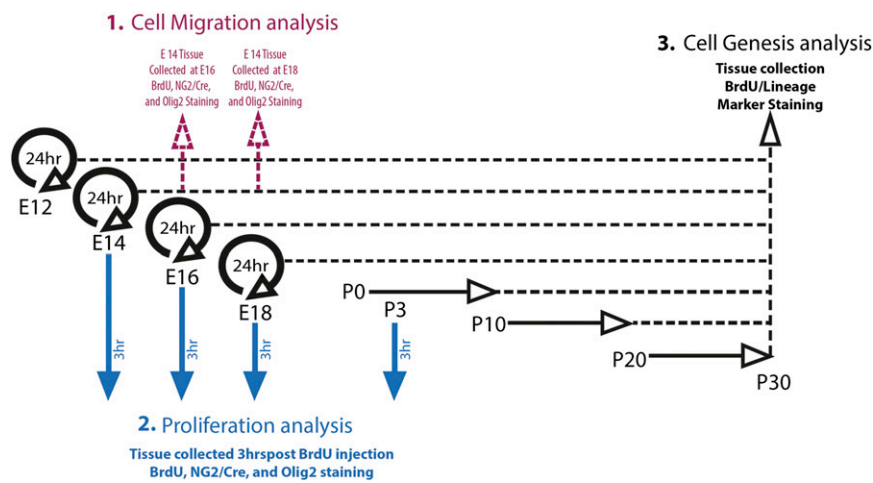


Fig. S3. Schematic illustration for BrdU pulse-chase experiments. (1) For NG2⁺ cell migration analysis, BrdU was injected into E14 time-pregnant mice for a 24-h cycle, and then the tissue was collected on E16 or E18 to trace the migration patterns of NG2 cells/progenies in these times. (2) To check the proliferating activities of NG2 cells, BrdU was injected into time-pregnant mice at embryonic dates E14.5, E16.5, and E18.5, plus one postnatal time, P3. Three hours after injection, the tissues were collected for BrdU staining analysis. (3) Cell genesis analysis was conducted by BrdU injection into time-pregnant mice at E12, E14, E16, or E18 for a 24-h cycle. For a postnatal cell genesis study, BrdU was injected once a day for three different times (P0–P10, P11–P20, and P21–30). After BrdU injection, all the samples were collected at P30 for BrdU and lineage-specific marker staining.