

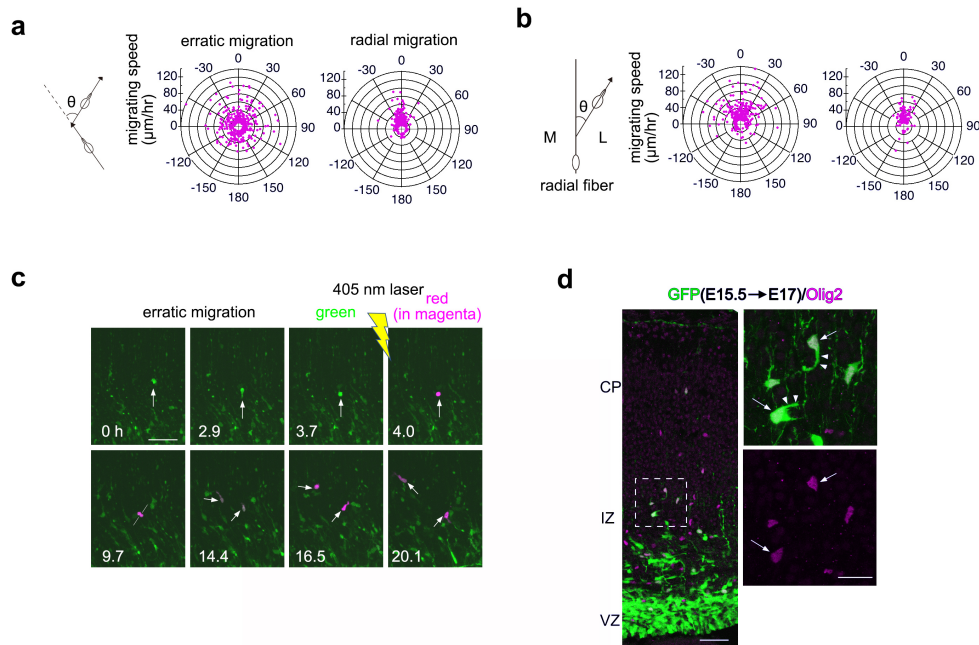
1
2
3
4
5
6
7
8
9
10
11

Supplemental Information

**Erratic and Blood Vessel-Guided Dispersion of Astrocyte Progenitors in the
Cerebral Cortex**

Tabata H. et al.

Supplementary Figure 1

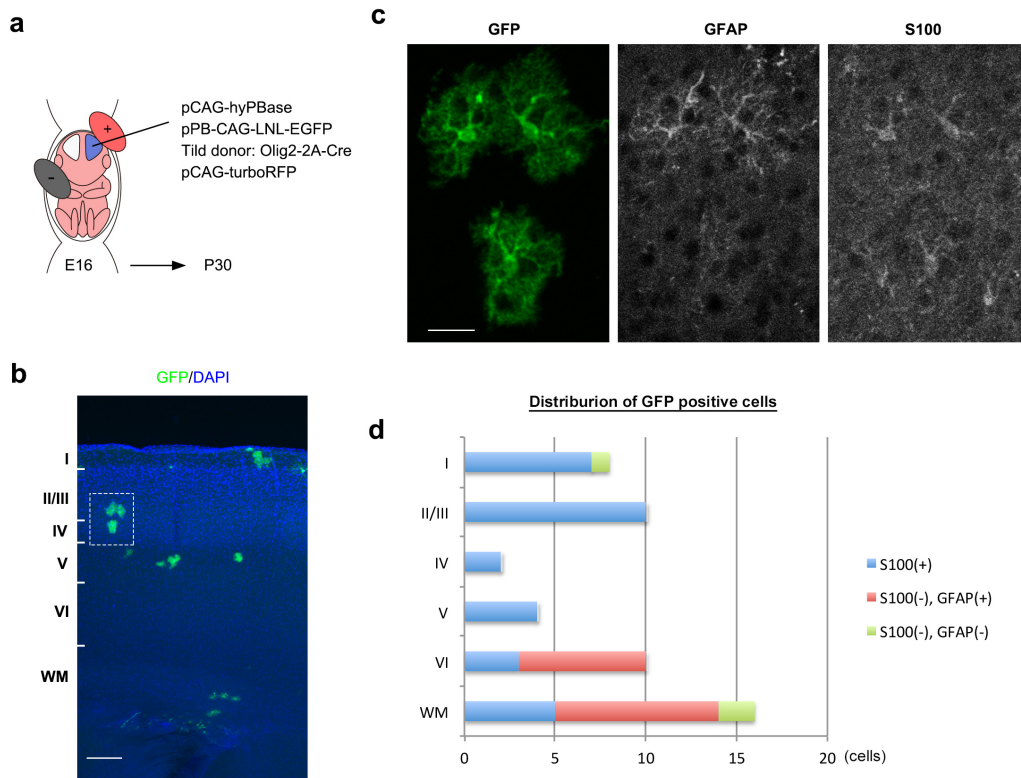


Supplementary Figure 1 Characterization of erratically migration cells.

a Directional changes in the movement (degree; positive values indicate a rightward turn in the right cerebral hemisphere) and migration speed (radius) in each frame. In radial migration, the directional changes between two consequent movements were mostly within 30° ($75.8\% \pm 9.1\%$), whereas those during erratic migration showed a greater divergence ($49.5\% \pm 10.5\%$). **b** Direction of movement relative to the radial fiber (degree; positive values indicate a lateral direction) and the migration speed (radius) in each frame. Although erratically migrating cells moved in various directions, the movement angle relative to the radial fibers and the speed was $-12.8\% \pm 16.5^\circ$ at $8.8 \pm 1.3 \mu\text{m/h}$ (0° is parallel to the radial fibers toward the brain surface; data is for the mean of 11 cells in 4 slices $\pm$ S.E.M.), indicating that they tended to move toward the brain surface. On the other hand, that of radial migration was $-1.2\% \pm 4.2^\circ$ at $16.6 \pm 1.6 \mu\text{m/h}$ (8 cells in 4 slices), representing a slow but steady migration toward the brain surface. **c** (Related to Figure 1d) An example of photoconversion of erratically migrating cells with 405nm laser. The arrows point to a cell

that was migrating in an erratic migration mode; this single cell was irradiated with a 405-nm laser between the “3.7 h” and “4.0 h” time frames, changing in the fluorescence from green to red (the red color is shown in magenta on these images). This cell divided at 9.7 hours, and the daughter cells also adopted an erratic migration mode. **d** GFP⁺/Olig2⁺ astrocyte progenitors represent the erratically migrating cell-like morphology in fixed brains. Embryos were electroporated with pCAG-EGFP (1 µg/µl) at E15.5 and were then fixed 36 hours later. Overall distribution of GFP⁺ cells is shown at left, and high magnification of the cells located in the superficial IZ to bottom of CP is shown at right. GFP⁺/Olig2⁺ astrocyte progenitors begin to enter the superficial IZ and CP earlier than neurons 2 days after in utero electroporation. Their irregular morphology and the short leading process pointing in various directions represented the characteristics of erratically migrating cells. The vast majority of GFP⁺ cells in these regions were Olig2⁺ (right). Scale bars, 100 µm. 50 µm (**d**, left); 20 µm (**d**, right).

Supplementary Figure 2



Supplementary Figure 2 Lineage tracing of Olig2-expressing cells derived from cortical VZ using the Tild-CRISPR method.

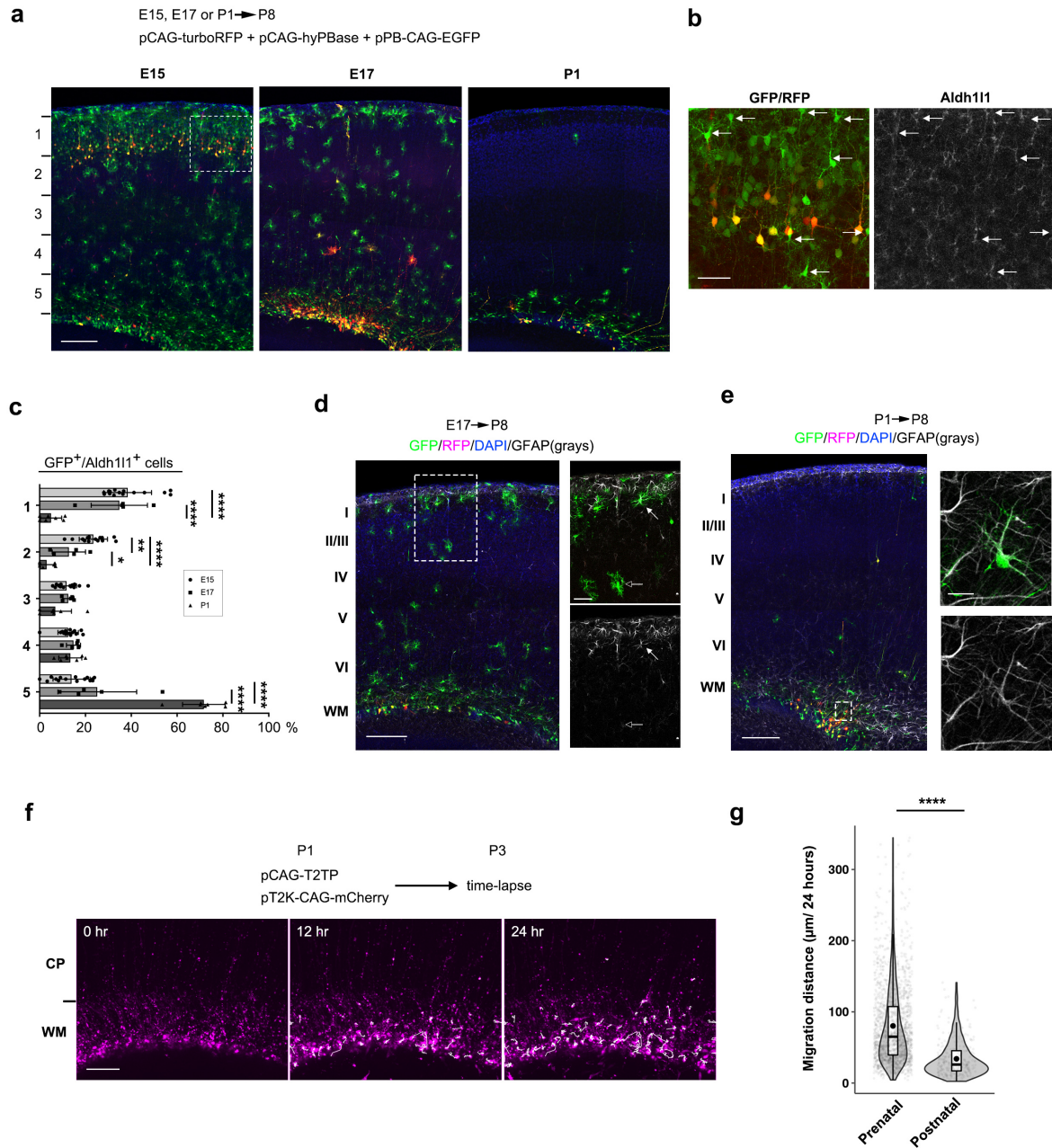
(A) 2A-Cre sequence was inserted into the *Olig2* gene *in utero* at E16 and the GFP-expressing cells were analyzed at P30. (B) One example of the brain containing GFP⁺ cells. (C) High magnification view of the boxed area in (B) and its immunohistochemistry for GFAP and S100 β . (D) Histogram of GFP-positive cells in the CP (layer I~VI) and WM. They were classified into S100 β -positive (protoplasmic astrocyte), S100 β -negative but strongly GFAP-positive (fibrous astrocyte) or double negative cells. The double-positive cells were included in the S100 β -positive cell column. Using the Tild-CRISPR method, fibrous astrocytes in the WM were also labeled, which was not observed when Cre activation was temporally induced with tamoxifen at E17 (Fig. 2H). These fibrous astrocytes were thought to be labeled due to the prolonged expression of Cre in later stages. The cortical VZ-derived

54 Olig2-expressing cells mostly differentiated into either protoplasmic or fibrous astrocytes
55 (94%, n=50). Scale bars, 200 μ m (B); 30 μ m (C).

56

57

Supplementary Figure 3



Supplementary Figure 3 Stage dependent positioning of astrocytes.

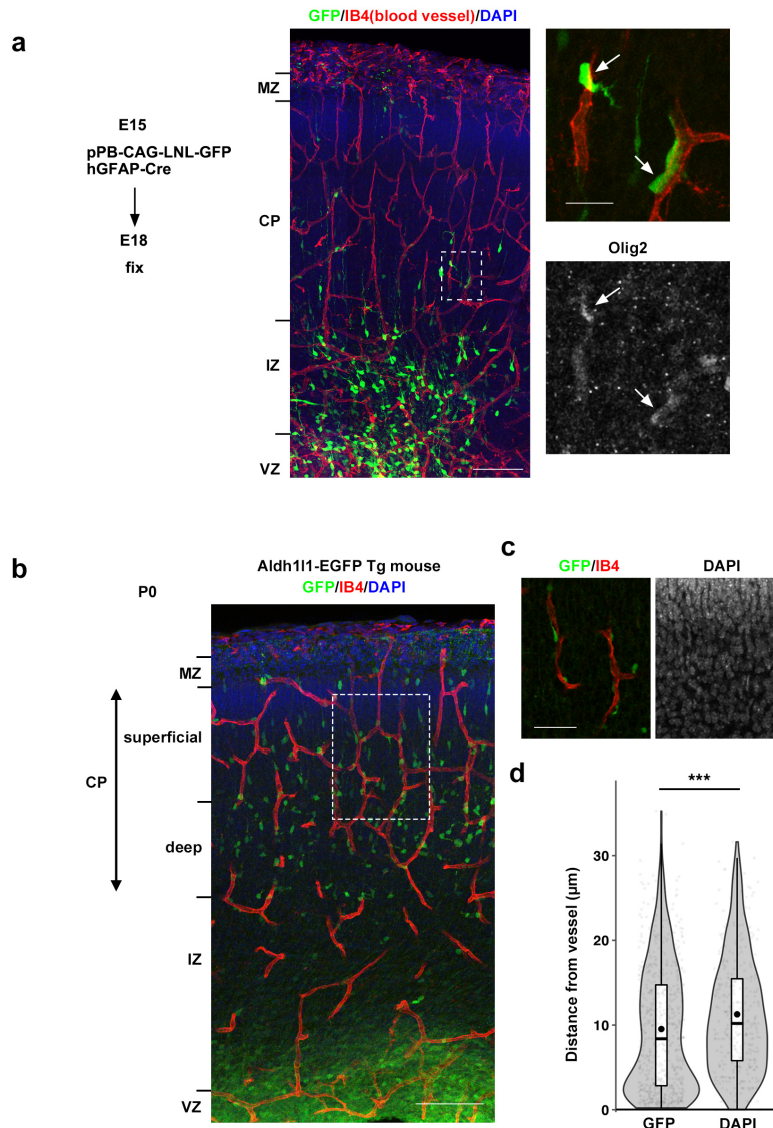
a The final positions of astrocytes electroporated at different timings. We electroporated E15, E17 or P1 embryos/pups with pPB-CAG-EGFP (0.5 $\mu\text{g}/\mu\text{l}$) + pCAG-hyPBBase (0.5 $\mu\text{g}/\mu\text{l}$) + pCAG-turboRFP (0.2 $\mu\text{g}/\mu\text{l}$), which labels astrocytes with GFP and neurons with turboRFP, and fixed the brains at P8. **b** High magnification view of the boxed region in the left panel of

(a). Many GFP⁺/Aldh111⁺ astrocytes (arrows) were found in layers II/III of the brain electroporated at E15. c Histogram of the GFP⁺/Aldh111⁺ cells in the brains electroporated at E15, E17 or P1 among 5 bins shown in the left side of (a). In the brains electroporated at P1, most of the GFP⁺/Aldh111⁺ astrocytes were located in the white matter (WM). In the case of E17, the GFP⁺/Aldh111⁺ astrocytes in the layer I were still produced, but the layer II/III and layer IV astrocytes, which are in bin2, were significantly reduced compared to the brains electroporated at E15 (one-way ANOVA followed by Tukey's multiple comparisons test, E15; 15 brains, E17; 5 brains, P1; 7 brains, average $\pm$ S.D., * $p < 0.05$, ** $P < 0.01$, *** $P < 0.001$). (d and e) GFAP staining of the brains electroporated at E17 or P1. In the brains electroporated at E17, GFP⁺ layer I astrocytes were still labeled (d). The high magnification view of the boxed region in the left panel is shown in the right. The strongly GFAP-positive layer I astrocytes (arrow) and GFAP-negative protoplasmic astrocytes (open arrow) could be observed. Postnatal VZ cells mainly produce fibrous astrocyte in the WM or deep layers of the CP (e). GFP⁺ cells were found mainly in the WM. High magnification view of the boxed region in the left panel is shown in right. The GFP⁺ cells in the WM highly expressed GFAP (arrows), and assumed fibrous astrocyte morphology. f Cells derived from postnatal VZ do not move extensively. P1 mouse pups were electroporated with Tol2 transposon vector system [pCAG-T2TP (1 $\mu\text{g}/\mu\text{l}$) and pT2K-CAG-mCherry (1 $\mu\text{g}/\mu\text{l}$)], and subjected to time-lapse observations 2 days later. The mCherry-labeled cells (magenta) and their trajectories (white lines, cells that can be traced for more than 5 hours) were shown. Scale bar, 100 μm . See also Supplementary Movie 3. g The maximum distance achieved by each cell within 24 hour-observation period was represented in violin and box plots. Migration distances of E15 labeled and E18 observed cells that has been described in Fig.2 (turboRFP⁺) were also analyzed in the same method for P1 labeled cells. The migration distance was significantly decreased in postnatally labeled cells compared to E15 labeled cells (Welch two sample t-

89 test, 1748 E15-labeled cells vs 283 P1-labeled cells, ****P<0.0001) Scale bars, 200 μ m (**a**,
90 **d-left, e-left**), 100 μ m (**f**), 50 μ m (**b, d-right**), 20 μ m (**e-right**).

91

Supplementary Figure 4

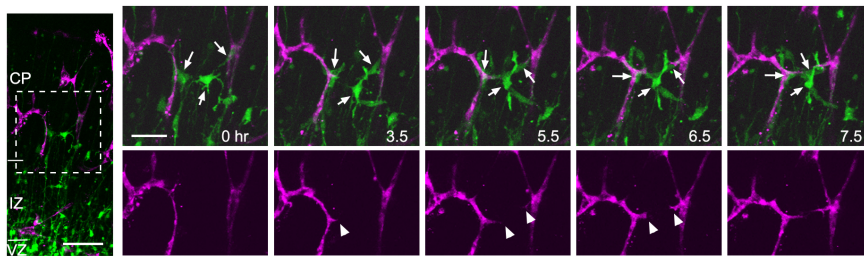


Supplementary Figure 4. Astrocyte progenitors are closely associated with blood vessels *in vivo*.

a Astrocyte progenitors labeled by *in utero* electroporation with a transposon vector were frequently associated with blood vessels. Human GFAP promoter driven Cre expression vector, hGFAP-Cre (1 $\mu\text{g}/\mu\text{l}$), and Cre-dependent GFP-expressing transposon vector, pPB-CAG-LNL-GFP (0.5 $\mu\text{g}/\mu\text{l}$), were electroporated at E15 and the brains were fixed 3 days later. The overview is shown on the left, and the high magnification view of the boxed region

is shown on the right. Double-positive cells for GFP and Olig2, a marker for glial progenitors (pointed by arrows), were frequently associated with blood vessels stained with isolectin B4 (IB4, red). **(b-d)** Genetically labeled astrocyte progenitors were also associated with blood vessels. The fixed P0 Aldh111-GFP mouse brains, in which GFP is expressed in astrocyte progenitors, were stained with IB4 (red), and the relationships with blood vessels were investigated. A single Z plane of the boxed region of **(b)** were shown in **(c)**. GFP (green) and IB4 (red) channels and DAPI (grays) channel were separately presented. The distances between GFP⁺ cells in the superficial CP and blood vessels were measured, and represented in violin and box plots **(d)**. The GFP⁺ cells were significantly closer to blood vessels than all cells stained by DAPI (Welch Two Sample t-test, *** $p < 0.001$, 639 GFP⁺ cells vs 339 DAPI stained cells). Scale bars, 200 μm (**a-left, b**), 20 μm (**a-right**), 50 μm (**c**).

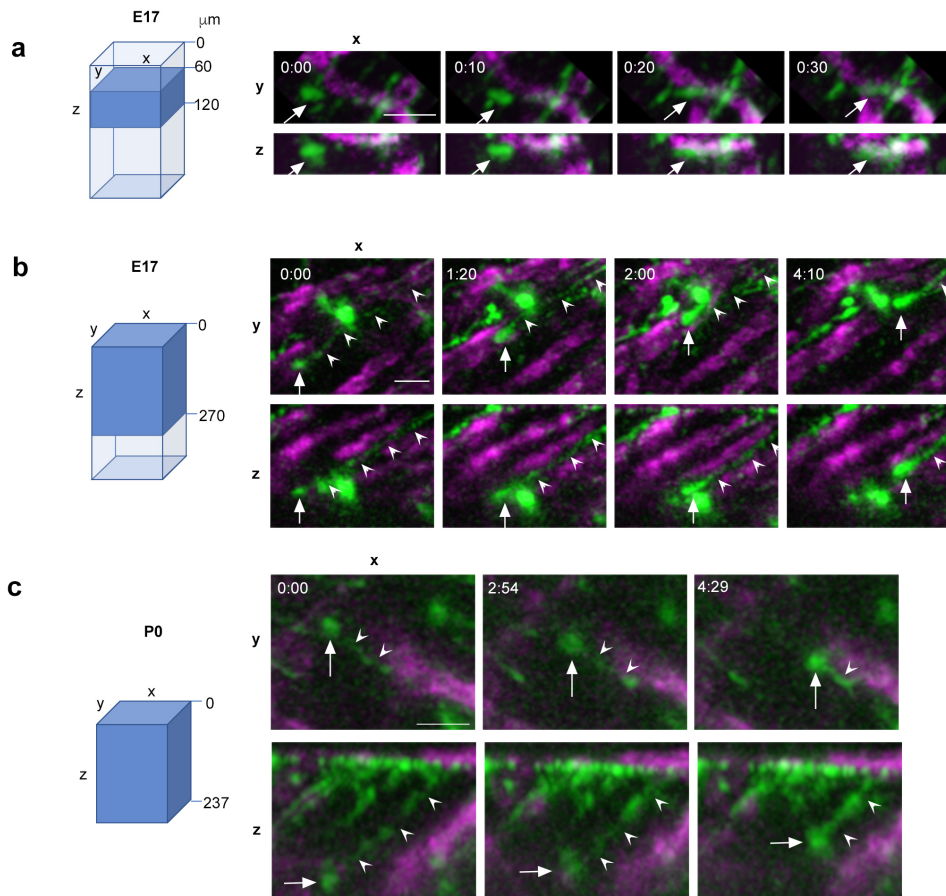
Supplementary Figure 5



Supplementary Figure 5 Astrocyte progenitors support blood vessel branch formation.

An example of blood vessel branch formation supported by astrocyte progenitors other than Figure 3F in slice culture. E15 *flt1*-DsRed mouse embryos were electroporated with pCAG-LNL-EGFP (0.5 $\mu\text{g}/\mu\text{l}$) + pCAG-LNL-LynEGFP (1 $\mu\text{g}/\mu\text{l}$) + Nestin-Cre (0.3 $\mu\text{g}/\mu\text{l}$) and subjected to time-lapse observations next day. The time-lapse images in the boxed regions of the left panels are shown on the right. Three astrocyte progenitors (arrows) formed a bridge between two adjacent blood vessels, and then both blood vessels extended a process (arrowhead) along the astrocyte progenitors. Scale bars, 100 μm (left), 50 μm (right). See also Supplementary Movie 7.

Supplementary Figure 6

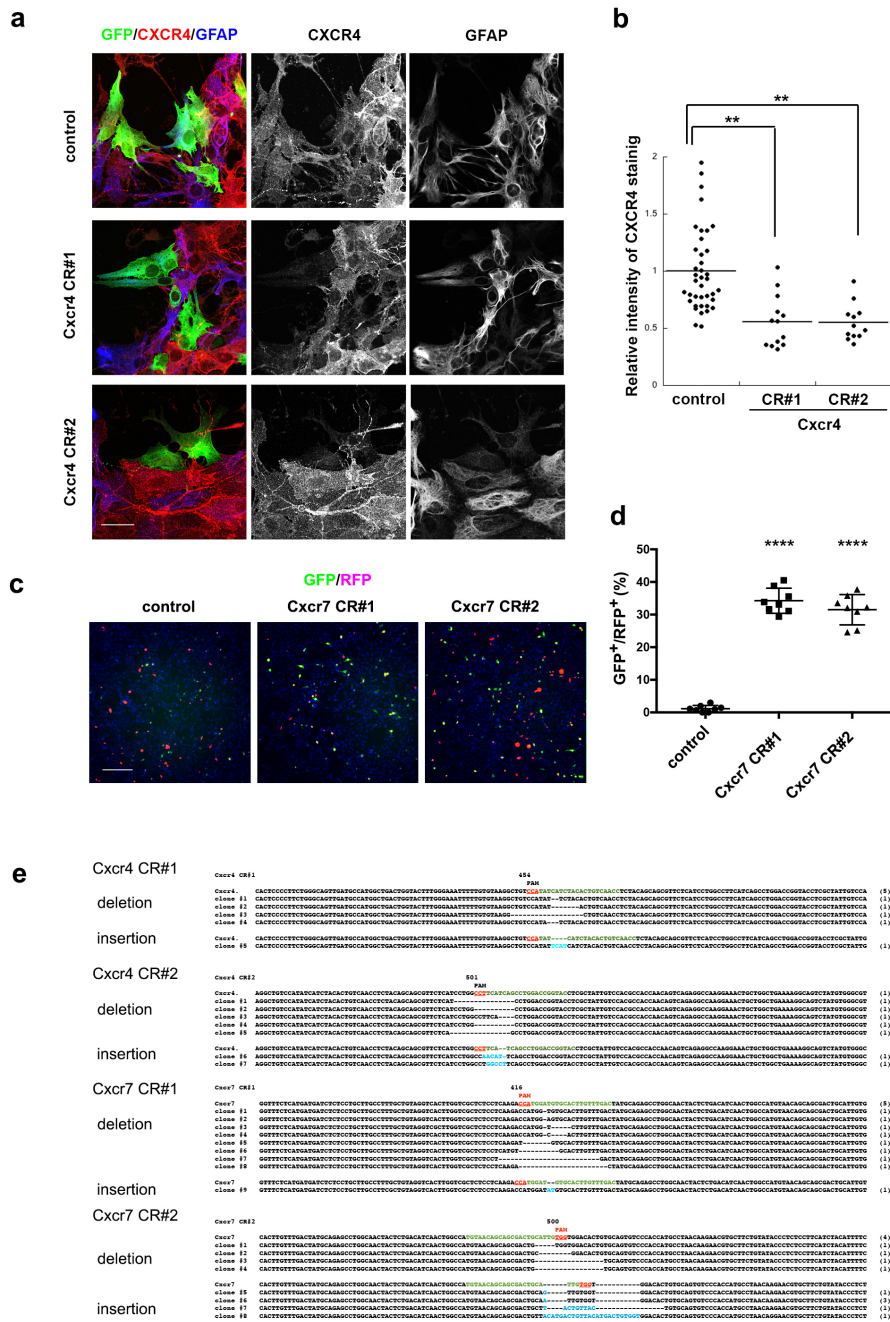


Supplementary Figure 6 Two-photon live imaging of astrocyte progenitors.

Embryos of *flt1*-DsRed mice were electroporated with the rDIO system and employed to *in vivo* imaging at E15 (**a**, **b**) or P0 (**c**). **a** One GFP positive cell (arrow) moved in the vicinity of a blood vessel, attached it, and migrated along it. See also Supplementary Movie 10. **b** Somal translocation mode observed in a living embryo. A GFP-positive cell (arrow) extending a long ascending process (arrowheads) to the brain surface retracted the process to elevate the cell body. See also Supplementary Movie 13. **c** Somal translocation of a GFP-positive cell (arrow) extending a long ascending process (arrowhead) observed in the intact P0 brain. See also Supplementary Movie 14. Scale bars: 20 μ m.

DEGs, and performed GO (biological processes, BP) enrichment analysis for each gene set (**b** and **c**). We selected cell adhesion molecules (BP=cell adhesion), and classified them into gene families based on molecular structures (D-G). (**b** and **c**) GO (BP) enrichment analysis of astrocyte progenitor-enriched DEGs **b** and migrating neuron-enriched DEGs (C) (top-30 GO in P value for enrichment). (D-G) DEGs annotated as “cell adhesion” were selected and were subjected into “protein domain” enrichment analysis. Genes including domains whose false discovery rate is less than 0.05 were taken into consideration. The Y-axis indicates the fold change (FC) of expression (astrocyte progenitors per migrating neurons).

Supplementary Figure 8

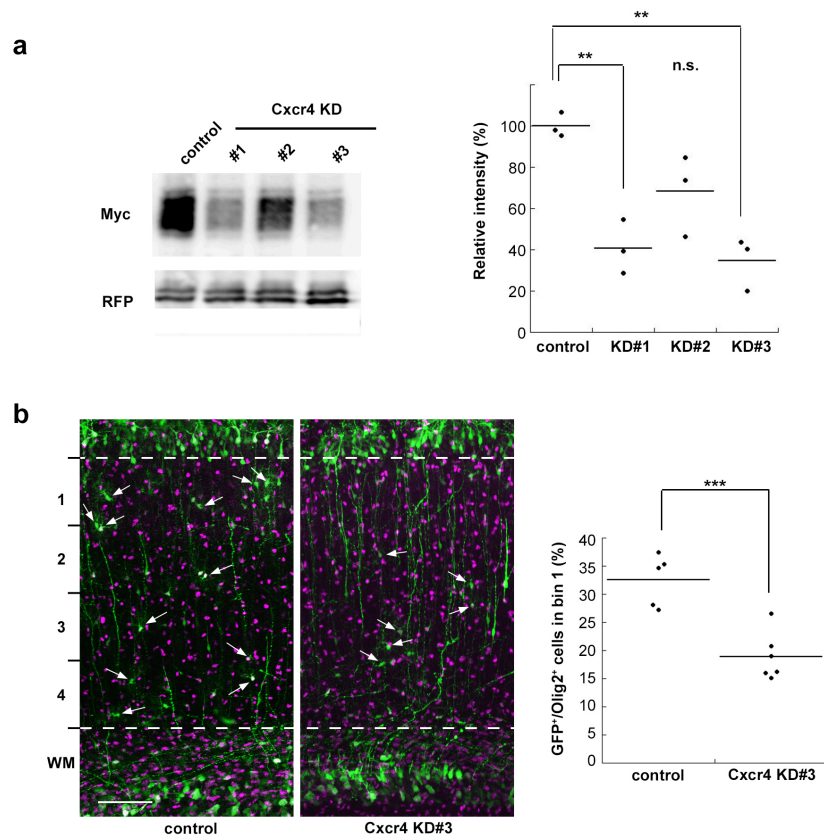


Supplementary Figure 8 Validation of CRISPR vectors for Cxcr4 and Cxcr7.

a Immunohistochemistry for endogenous Cxcr4 and GFAP of primary astrocytes transfected with two independent CRISPR vectors for Cxcr4. E16 mouse embryos were electroporated with CRISPR vectors [Cxcr4 CR#1 or CR#2 (1 µg/µl)] together with transposon EGFP

expression vectors [pT2K-CAG-EGFP (0.5 $\mu\text{g}/\mu\text{l}$) + pCAG-T2TP (1 $\mu\text{g}/\mu\text{l}$)]. On the next day, the transfected brains were taken out and cultivated *in vitro* for 4 days before the immunostaining. **b** Relative intensities of Cxcr4 staining in GFP⁺/GFAP⁺ cells against controls were evaluated (Dunnett's multiple comparisons test, **P < 0.01). **c** Validation of CRISPR vectors for Cxcr7 by using a pCAG-EGxxFP vector. Two CRISPR vectors against distinct target sequences of Cxcr7 were constructed and their efficiencies were evaluated by EGFP fluorescence using the pCAG-EGxxFP vector system (see Methods). COS7 cells were co-transfected with CRISPR vectors, corresponding pCAG-EGxxFP-Cxcr7, and pCAG-mCherry (RFP) vectors. GFP and mCherry expressions were observed two days later. **d** GFP⁺ rate in mCherry⁺ cells in randomly selected objective fields were calculated (Dunnett's multiple comparisons test, ***P < 0.001, 8 objective fields/2 dishes/group). **e** CRISPR vector-mediated mutations in the *Cxcr4* and *Cxcr7* locus. Genomic sequences of *Cxcr4* and *Cxcr7* loci after transfection of two independent CRISPR vectors are shown. The target loci were amplified by PCR and cloned into a plasmid vector, and the amplicons were sequenced. The wild-type sequence of mouse *Cxcr4* and *Cxcr7* genes are shown in the first row, and the mutated sequences of deletion and insertion we observed are shown below. The observed numbers of each mutation or non-mutation are shown on the right end. The PAM sequence and the target sequence are highlighted in red and green, respectively. The insertions are highlighted in blue. The indel ratios of Cxcr4 CR#1 and CR#2, Cxcr7 CR#1 and CR#2 were 50% (5/10), 88% (7/8), 64% (9/14) and 71% (10/14), respectively. Scale bar, (A) 30 μm , (C) 200 μm .

Supplementary Figure 9



Supplementary Figure 9 Knockdown of Cxcr4 disrupt the positioning of protoplasmic astrocytes in the CP.

a Three independent miRNA expressing transposon vectors against mouse Cxcr4 were constructed (Cxcr4 KD#1~3). COS7 cells were transfected with these miRNA vectors together with Myc-tagged mouse Cxcr4 expression vector (pCAG-Myc-Cxcr4) and mCherry expression vector (pCAG-mCherry). After 48h, cells were harvested and subjected to Western blotting with anti-Myc. Anti-RFP was used as a loading control (left). The relative intensities of Myc to control (miRNA vector for lacZ) are shown on the right (averages of three independent experiments). miR-CXCR4 #1 and #3 were effective (Dunnett's multiple comparisons test, $**p < 0.01$). **b** E16 brains were electroporated with Cxcr4 KD#3 together with transposase expression vectors (pT2TP). The brains were fixed at P4 and the

187 GFP⁺/Olig2⁺ cells in the 4 bins in the CP were counted. The percentage of the cells found in
188 bin 1 was significantly reduced (Student's t-test, control vs. Cxcr4 KD#3, ***p<0.001). Scale
189 bar, 100 μ m.

190