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Supplemental information

GluA2 overexpression in oligodendrocyte progenitors promotes postinjury oligodendrocyte regeneration

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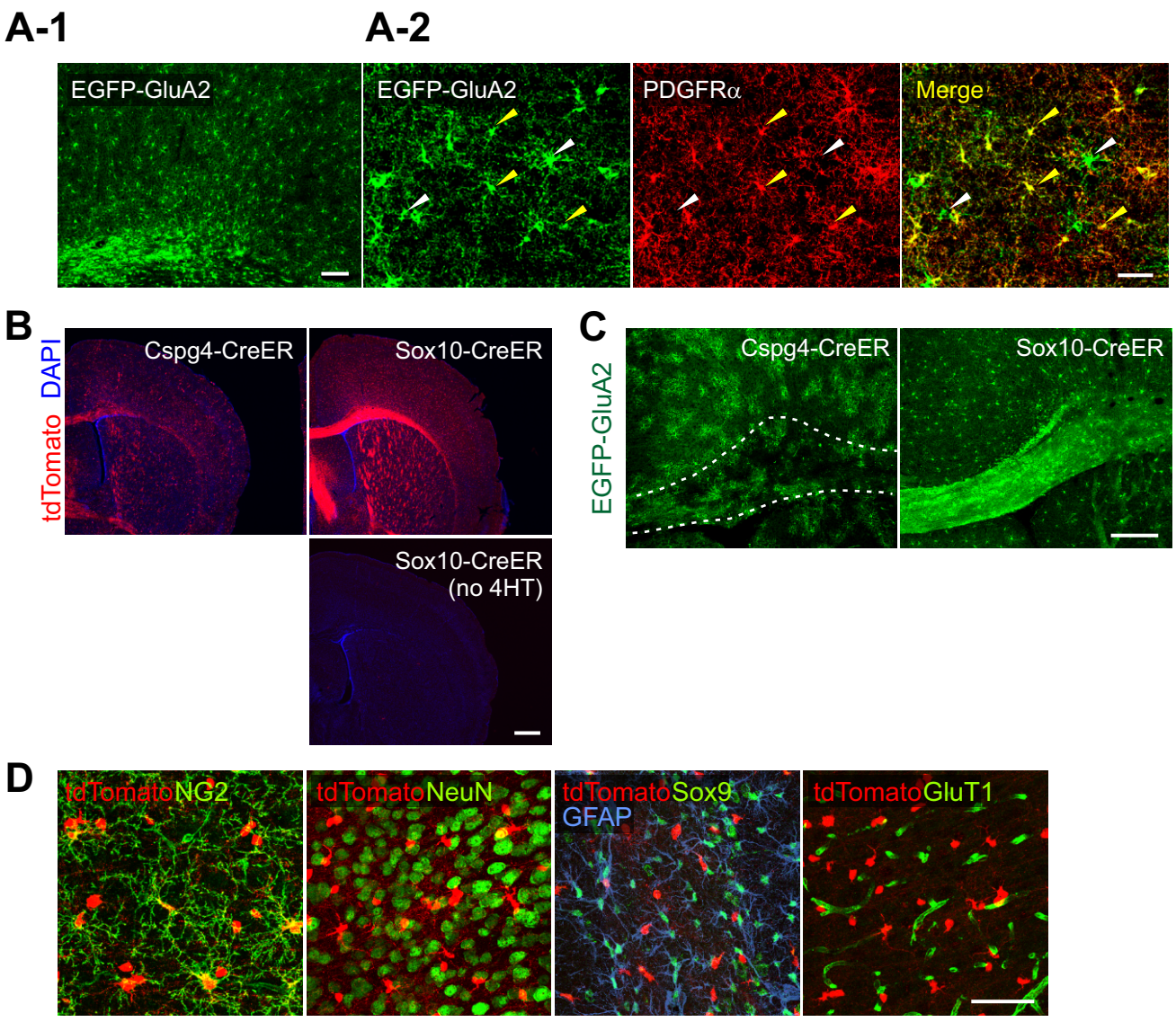

Figure S1. Cre Recombination with *Sox10-CreER* Mice, Related to Figures 1 and 3.

(A) Widespread EGFP expression in the CTX of 4HT-administered *Sox10-CreER; R26-Gria2* mice (P2+8). Fluorescence (A-1) and confocal (A-2) images of EGFP and PDGFRα. Yellow and white arrowheads indicate $EGFP^{+}$ $PDGFR\alpha^{+}$ OPCs and $EGFP^{+}$ $PDGFR\alpha^{-}$ pre-OLs, respectively. Scale bars: 100 μm (A-1) and 25 μm (A-2).

(B) tdTomato expression in *Cspg4-CreER; Ai14* mice (left) and *Sox10-CreER; Ai14* mice (right) after administration of the same doses of 4HT (0.5 mg per i.p. injection, given at P9, P11, and P13). No tdTomato expression in *Sox10-CreER; Ai14* mice without 4HT (right bottom). Scale bar: 500 μm.

(C) Expression of EGFP-GluA2 in *Cspg4-CreER; R26-Gria2* (left) and *Sox10-CreER; R26-Gria2* (right) mice (P9+26). Scale bar: 200 μm.

(D) Confocal images of tdTomato and other cell-specific markers (green) in the brain of *Sox10-CreER; Ai14* (P9+26) mice. Markers include NG2 (for OPC), NeuN (neuron), Sox9 (astrocyte), GFAP (astrocyte) and glucose transporter T1 (GluT1, endothelial cells). Images collected from the CTX, except for Sox9 and GFAP (collected from the hippocampus). Scale bar: 50 μm.

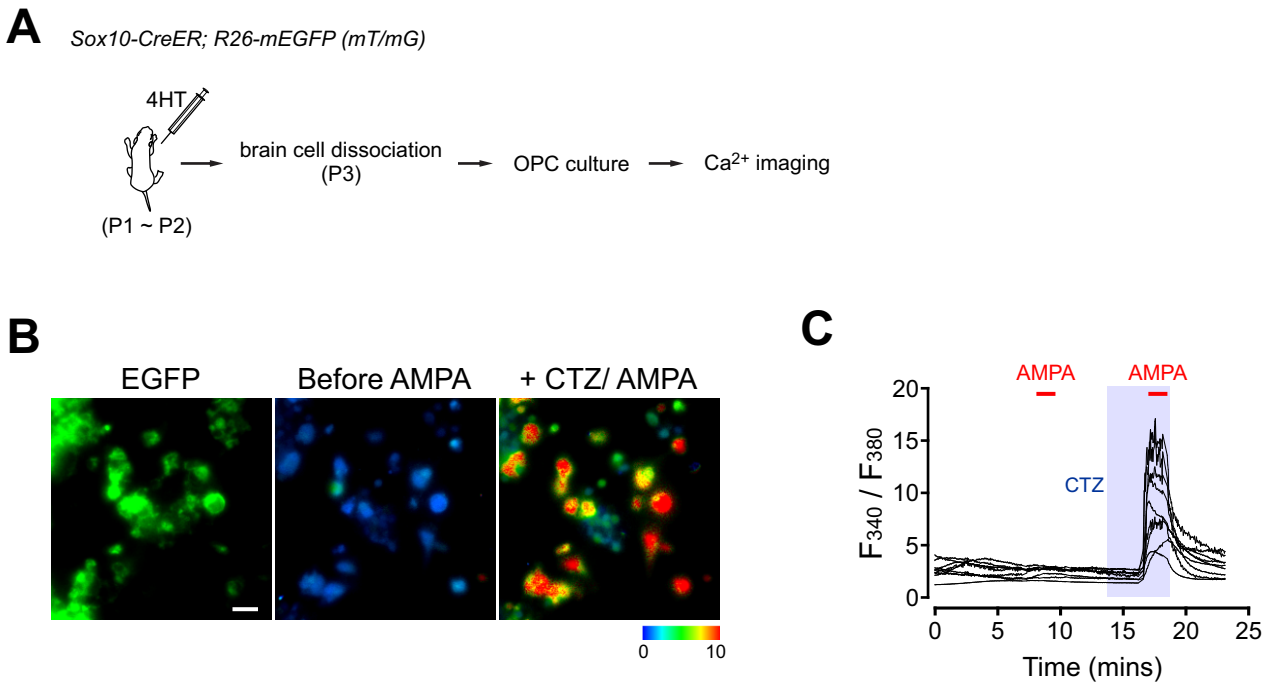

Figure S2. AMPA-Elicited Calcium Responses in Cultured OPCs That Express Membrane-Bound EGFP, Related to Figure 1.

(A) Experimental steps for OPC preparation from *Sox10-CreER; R26-mEGFP (mT/mG)* mice and calcium imaging.

(B) Images of EGFP (left) and calcium responses before (middle) and after (right) the CTZ (100 μM) + AMPA (20 μM) treatment. Scale bar: 25 μm.

(C) Traces of the changes in intracellular calcium levels of individual $EGFP^+$ OPCs in response to CTZ and AMPA. Red bars and shaded blue regions represent the duration of AMPA and CTZ applications, respectively. Responses obtained from 9 EGFP-expressing cells.

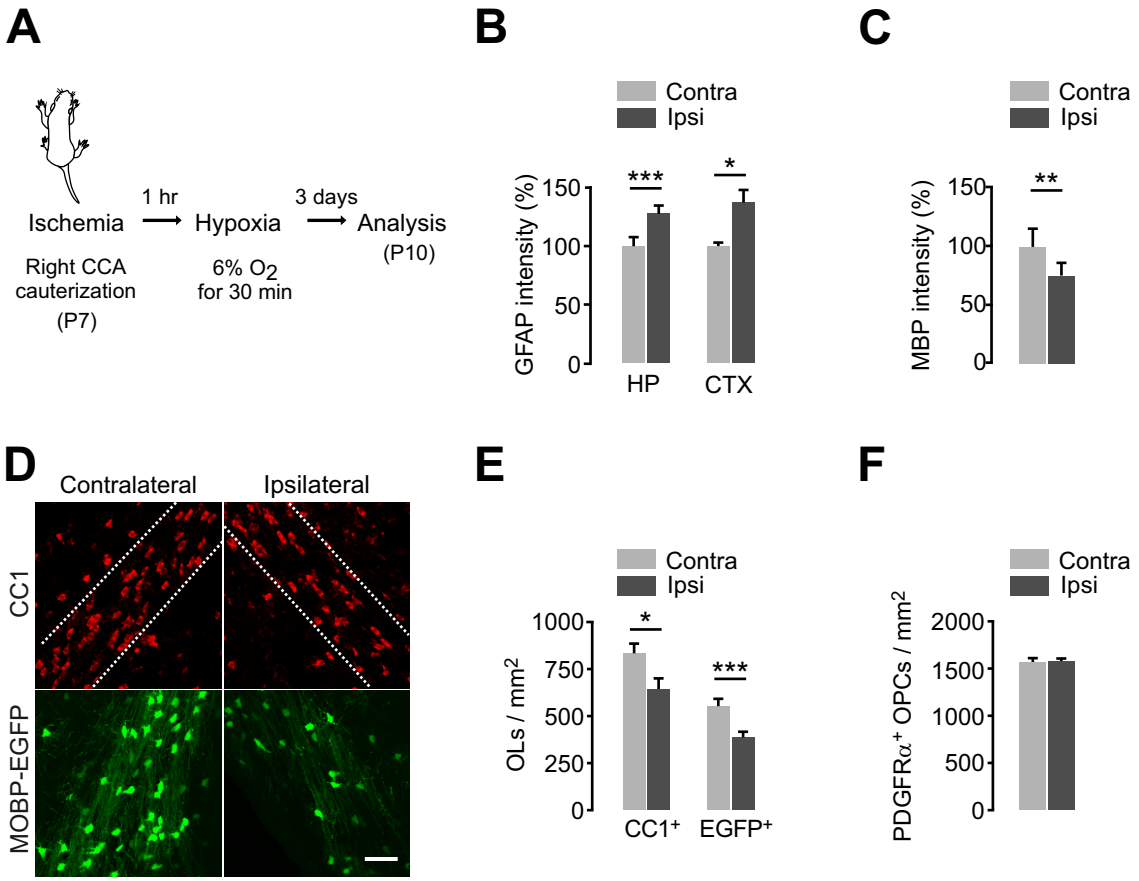

Figure S3. Astrogliosis and Oligodendrocyte Loss After Neonatal Hypoxic-ischemic (H/I) Brain Injury, Related to Figure 5.

(A) Illustration of the procedure for H/I brain injury induction.

(B) Quantification of GFAP-immunopositive pixels in the hippocampus (HP) and CTX after H/I injury (contralateral vs. ipsilateral side, HP: P = 0.0004; CTX: P = 0.038). n = 5 mice.

(C) Quantification of MBP-immunopositive pixels in the EC (contralateral vs. ipsilateral side, P = 0.008). n = 6 mice.

(D) Confocal images of OLs in the EC of a P10 *Mobp-EGFP* mouse that received H/I. OLs were identified with anti-APC (CC1) staining or EGFP. Scale bar: 50 μm.

(E) Densities of OLs (OLs) in EC (contralateral vs. ipsilateral side, $CC1^+$ OLs: P = 0.011, n = 3 mice; $EGFP^+$ OLs: P = 0.00004, n = 9 mice)

(F) Densities of $PDGFR\alpha^+$ OPCs in EC. (contralateral vs. ipsilateral side, P = 0.942, n = 9 mice).

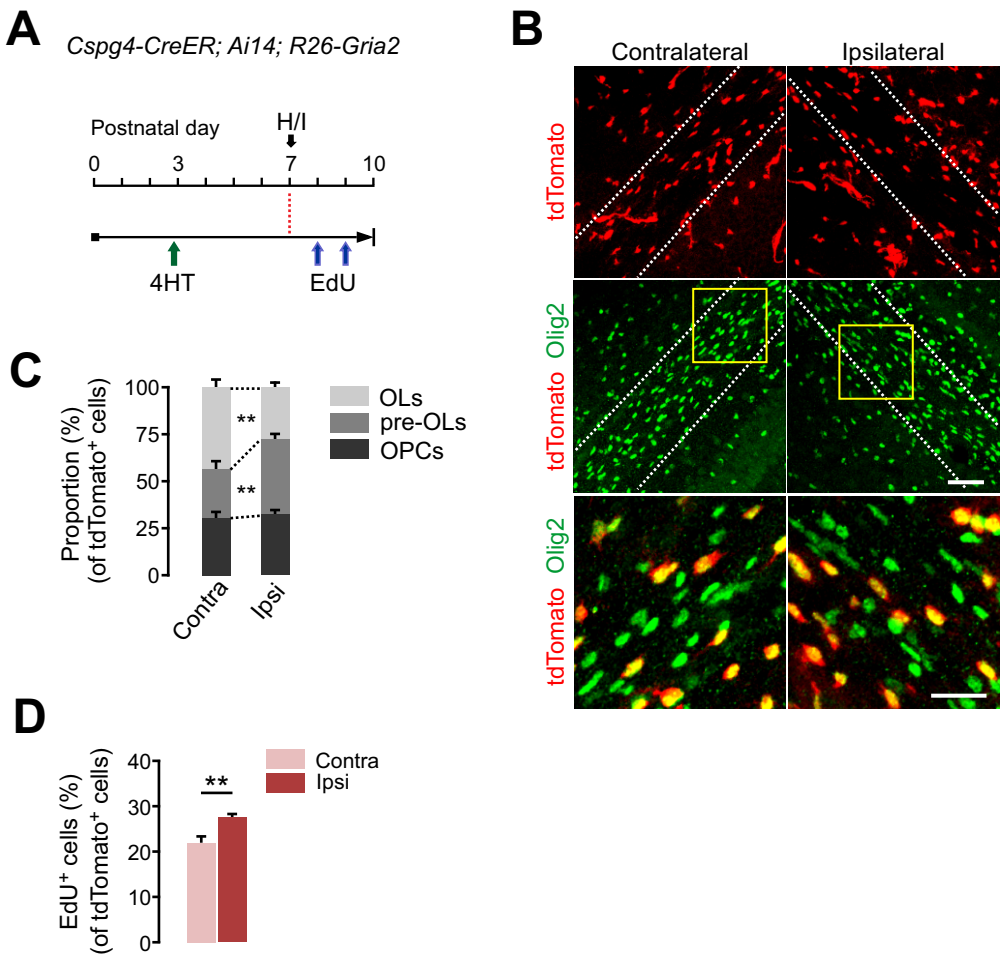

Figure S4. Differential Responses of Oligodendrocyte Lineage Cells to H/I Injury According to Cell Maturation Stage, Related to Figure 5.

(A) Timeline for 4HT injection, H/I induction, and sampling of *Cspg4-CreER; Ai14; R26-Gria2* mice.

(B) Confocal images of tdTomato and Olig2 in the injured brain (at 3 dpi). Dotted lines indicate boundaries of the EC. tdTomato$^+$ pericytes were identified based on their morphology and excluded from the analysis. Bottom panels: enlarged images of the boxed areas of upper panels. Scale bars: 50 μm (middle) or 25 μm (bottom).

(C) The proportion of OLs, pre-OLs and OPCs among tdTomato$^+$ EGFP$^-$ cells in EC (contralateral vs. ipsilateral side, OLs: P = 0.002; pre-OLs: P = 0.002; OPCs: P = 0.235). n = 8 mice.

(D) Percentage of EdU$^+$ cells among the total tdTomato$^+$ EGFP$^-$ cells (contralateral vs. ipsilateral side, P = 0.0082). n = 5 mice. Paired Student's t-test (C,D).

Data are represented as mean + SEM. ** P < 0.01.