









**Supplemental Figure 1: White matter regions of interest.** 40X image of the WM at P15, showing the two main regions of interest, 1) CG = cingulum and 2) EC = external capsule. The CG region included a large portion of the corpus callosum (CC) and was located directly above the subventricular zone (SVZ). Scale bar = 50  $\mu$ m.

Supplemental Figure 2: Changes in NG2<sup>+</sup> progenitor cell numbers in CNP-EGFP mice after hyperoxia. (A-D) Confocal images of NG2<sup>+</sup> progenitors in P8 and P12 CNP-EGFP mice under control conditions or after hyperoxia. Scale bar = 50  $\mu$ m. (E) The total number of NG2<sup>+</sup> cells is decreased at P8 in the external capsule (EC) and in the cingulum (CG), and increased in the CG after 4 days of recovery at room air at P12. Data are shown as mean  $\pm$  SD (n = 3-5 brains for each group). Unpaired t-test comparing control vs. hyperoxia was used for statistical analysis. \*P < 0.05, \*\*P < 0.025.

Supplemental Figure 3: Hyperoxia does not alter astrocyte proliferation in the developing white matter. (A-F) Immunostaining for glutamine synthetase (GS) and Ki67 in GFAP-EGFP transgenic mice at P8, P10 and P12. No Ki67<sup>+</sup>GFP<sup>+</sup>GS<sup>+</sup> immunostaining was detectable at any time point in either experimental group (hyperoxia vs. control). Scale bar = 50  $\mu$ m.

Supplemental Figure 4: Immunofluorescence staining for cleaved caspase3- $\alpha$  in GFAP-EGFP transgenic mice. Confocal images from GFAP-EGFP transgenic mice following hyperoxia for 6 hours (P6) and 48 hours (P8) vs. litter-matched controls. Staining for cleaved caspase3- $\alpha$  is not detectable in EGFP<sup>+</sup> astrocytes of the white matter at either time point. Scale bar = 50 µm.

**Supplemental Figure 5: Hyperoxia does not cause cell death, but upregulates GFAP levels in cultured astrocytes.** (A) Cell death was assayed by immunocytochemistry for TUNEL in astrocytes following hyperoxia compared to controls. In confluent astrocyte cultures treated with cAMP, TUNEL<sup>+</sup> cells (red) are very rarely detectable under normoxia or hyperoxia, and in sub-confluent cultures no TUNEL<sup>+</sup> cells are visible under both conditions. In the positive control (+ Control = cells treated with DNase), the vast

majority of cells are TUNEL<sup>+</sup>. Scale bar = 50  $\mu$ m. (**B**) Confluent astrocyte cultures were exposed to 72 hours of hyperoxia in the presence (+) cAMP or absence (-) cAMP of 50 uM db-cAMP (cAMP). GFAP protein levels in cultured astrocytes were analyzed by Western blotting. A representative experiment is shown. (**C**) Densitometric analysis reveals an increase in GFAP following hyperoxia. Values represent means of 3 independent experiments <u>+</u> SEM. \**P* < 0.05 (unpaired student's t-test vs. control).