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Supplemental Information

Enhancing Oligodendrocyte Myelination Rescues Synaptic Loss and Improves Functional Recovery after Chronic Hypoxia

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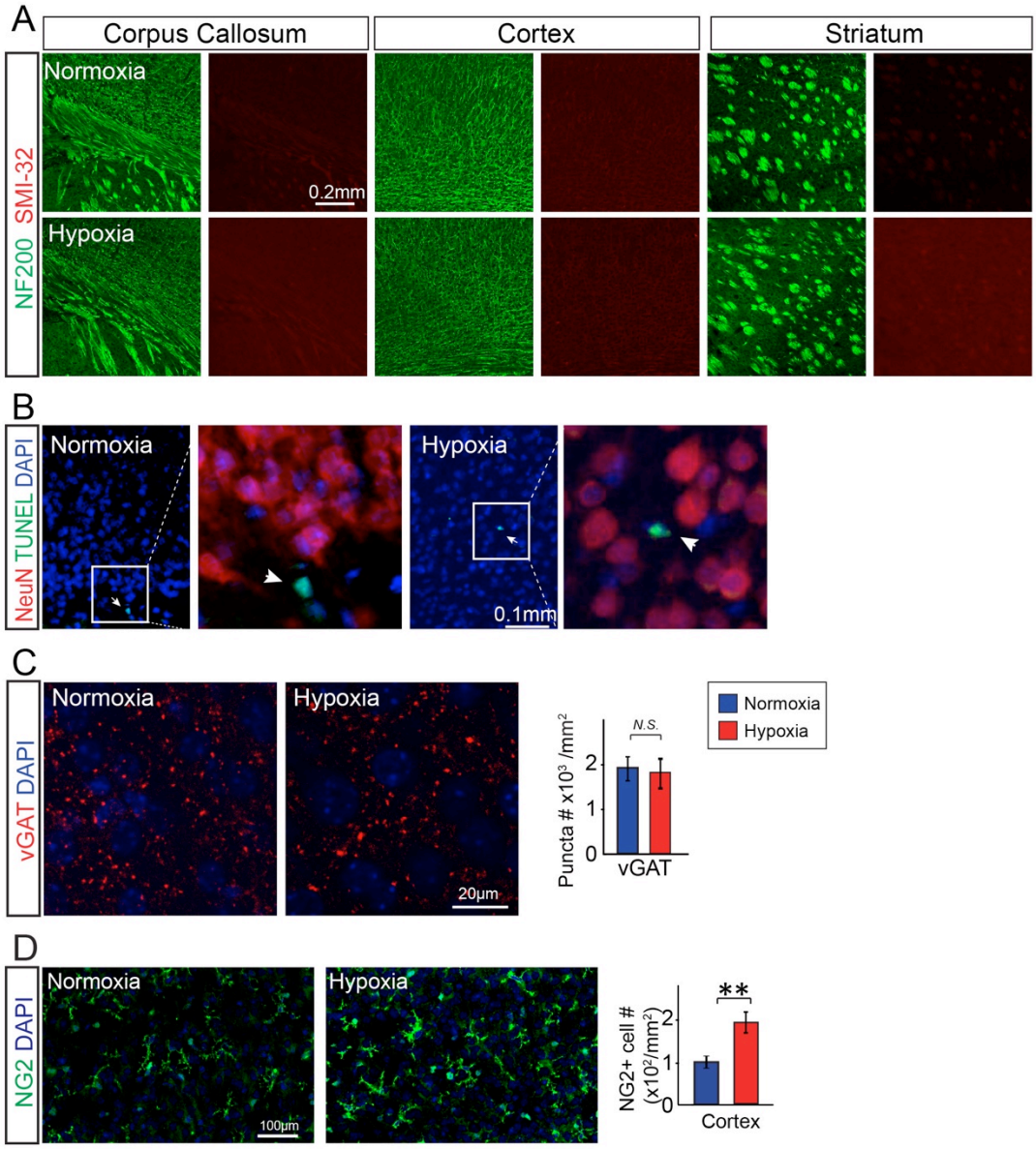
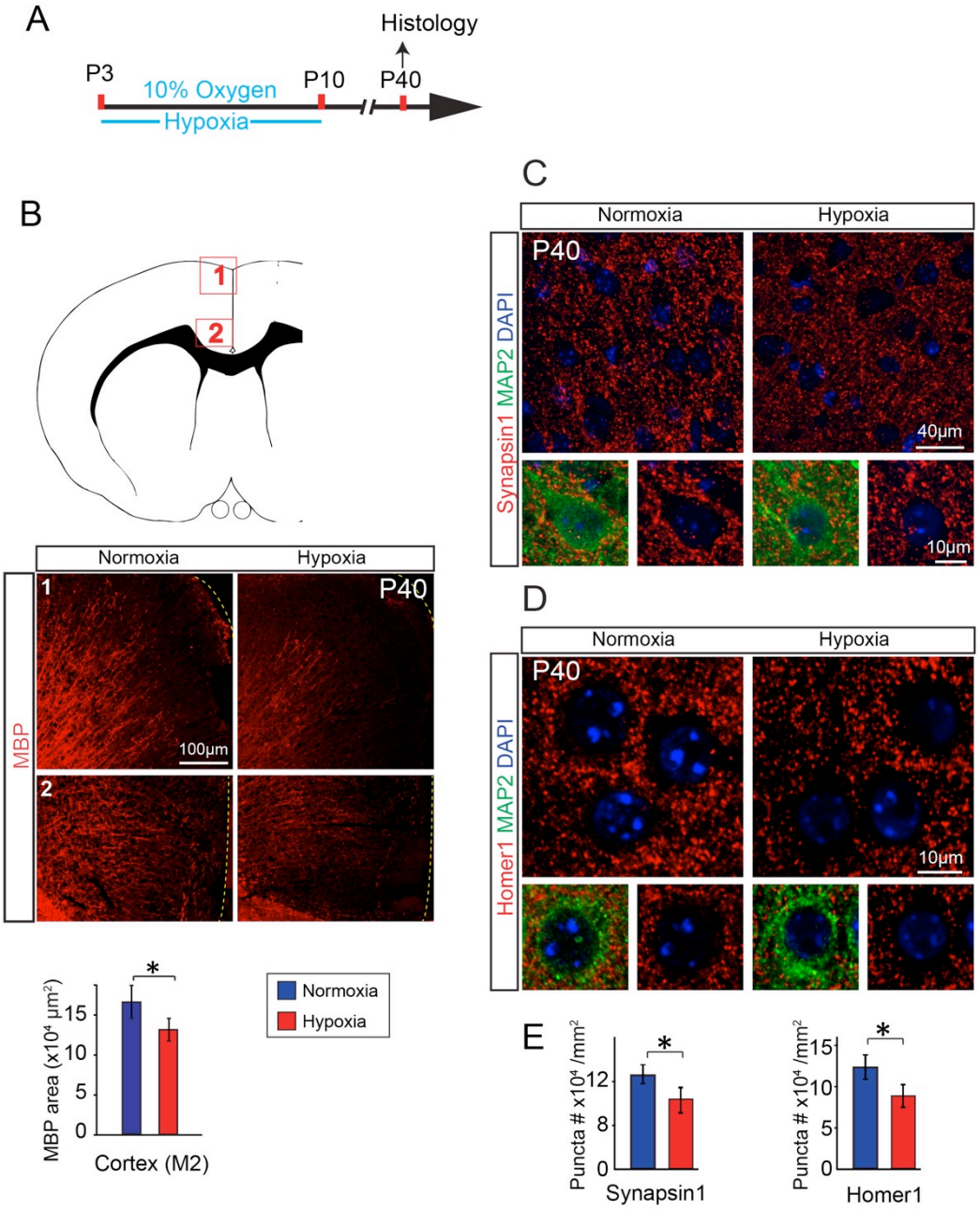
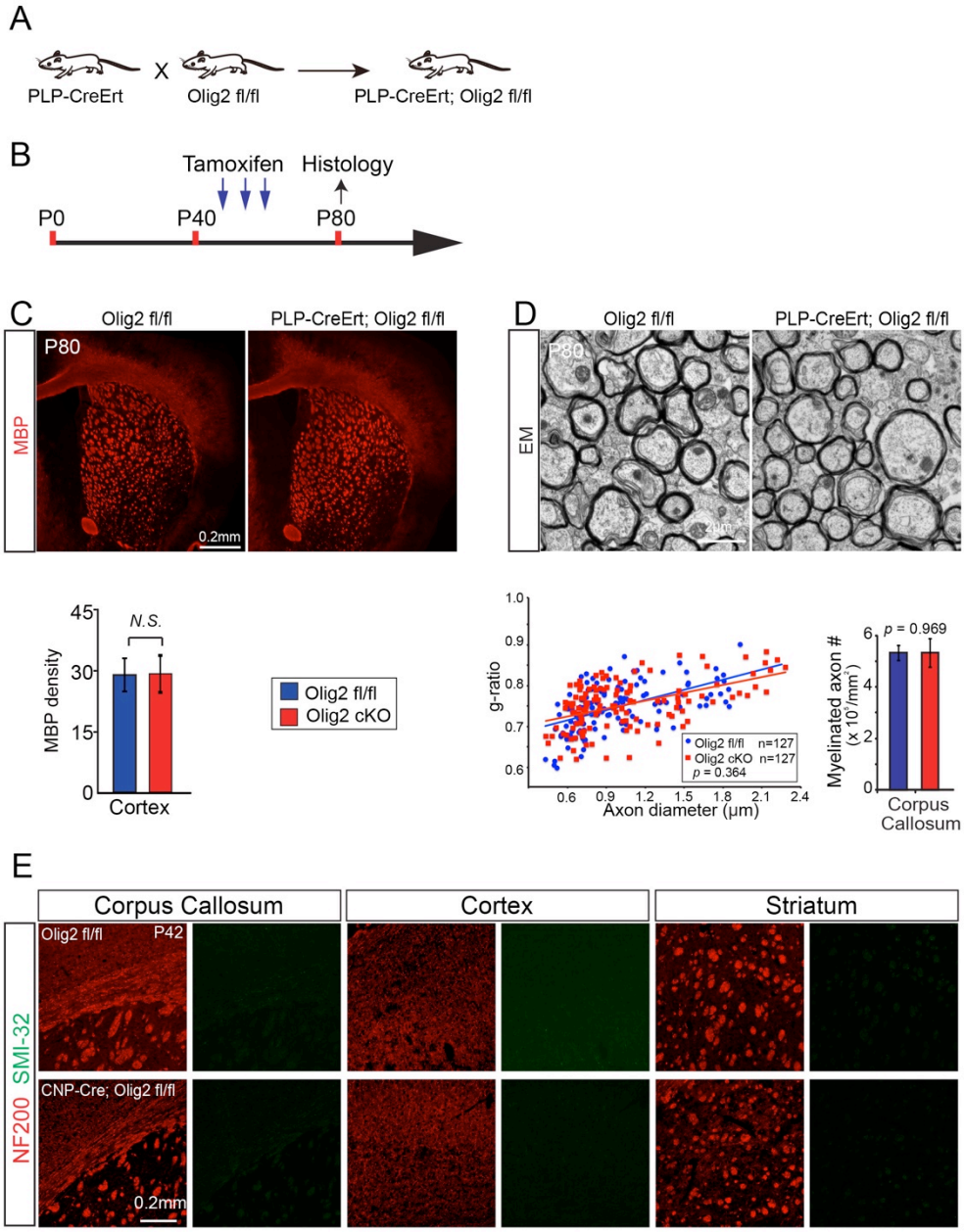


Supplemental Figure 1 (Related to Figures 1, 2): Axon degeneration, neuron apoptosis, OPCs proliferation and vGAT expression after hypoxia exposure.
(a) Neonatal mice were exposed to 10% oxygen from P3 to P10. Representative images showing SMI-32 (red) expression in the cortex, corpus callosum and striatum of hypoxic brains as compared to normoxic brains at P10. Axons were revealed by NF200 (green) staining. (b) TUNEL positive apoptotic cells (green) were not NeuN (red) positive. (c) vGAT (red) expression in the M2 cortex. Significance based on Student's t-test with the respective controls, n = 3 for all experiments. (d) Representative and quantification of NG2 positive cells in the hypoxic brain at P10. **p<0.01 or *p<0.05, significance based on Student's t-test with the respective controls, n = 3 for all experiments.



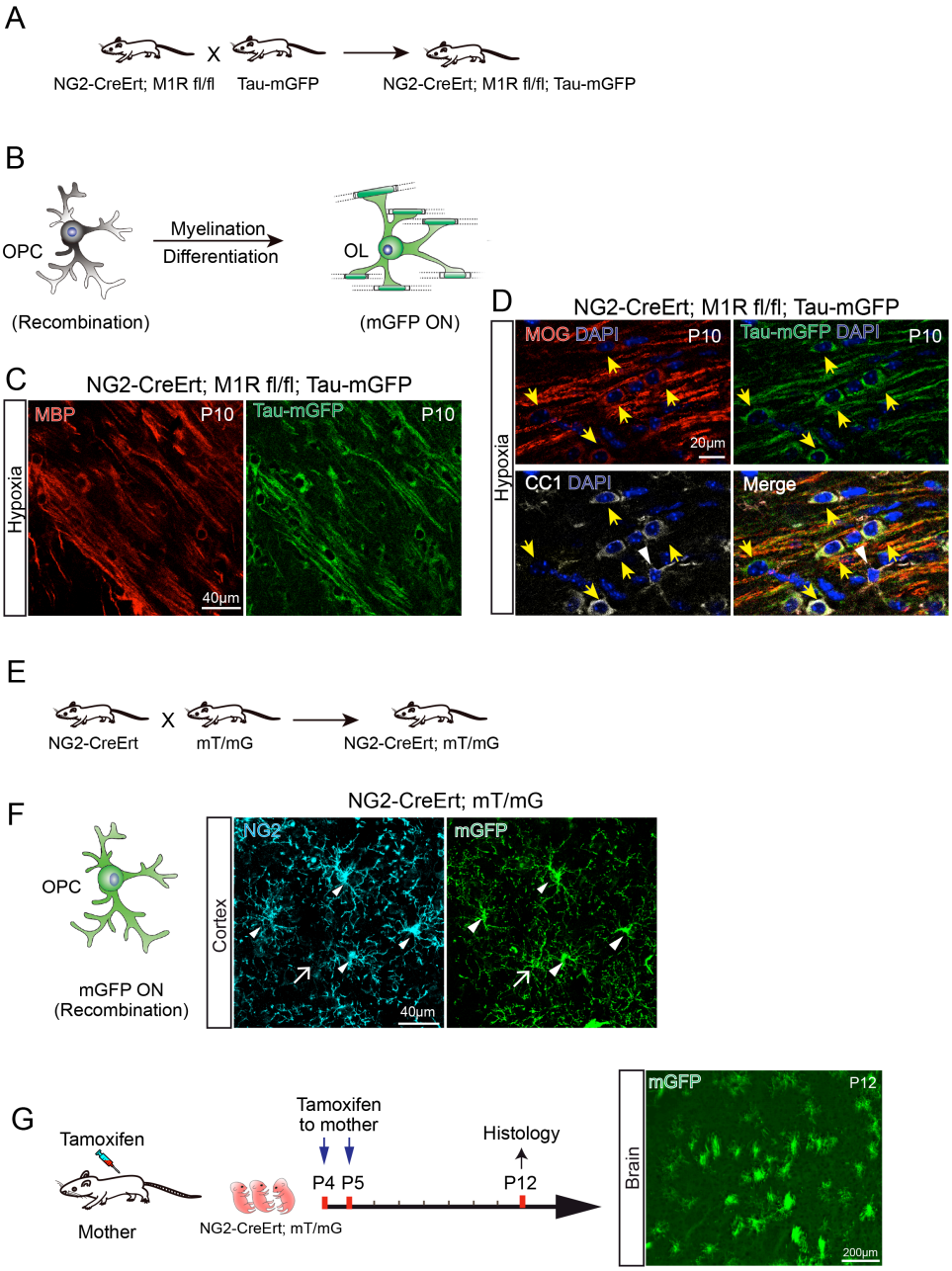
Supplemental Figure 2 (Related to Figure 3): Hypomyelination and synaptic loss in hypoxic brains at P40.

(a) Schematic paradigm showing the time course of hypoxia exposure. (b) Representative images and quantification of MBP positive myelin in the cortex of hypoxia brains at P40; (c-e) Representative images and quantification of synaptic puncta by using synapsin-1 (red, c) and homer1 (red, d) in the M2 cortex of normoxic and hypoxic mice at P40 and neurons were revealed by MAP2 staining (green). The numbers of synaptic puncta were quantified. (e), *p<0.05, significance based on Student's t-test with the respective controls, n = 3 for all experiments.



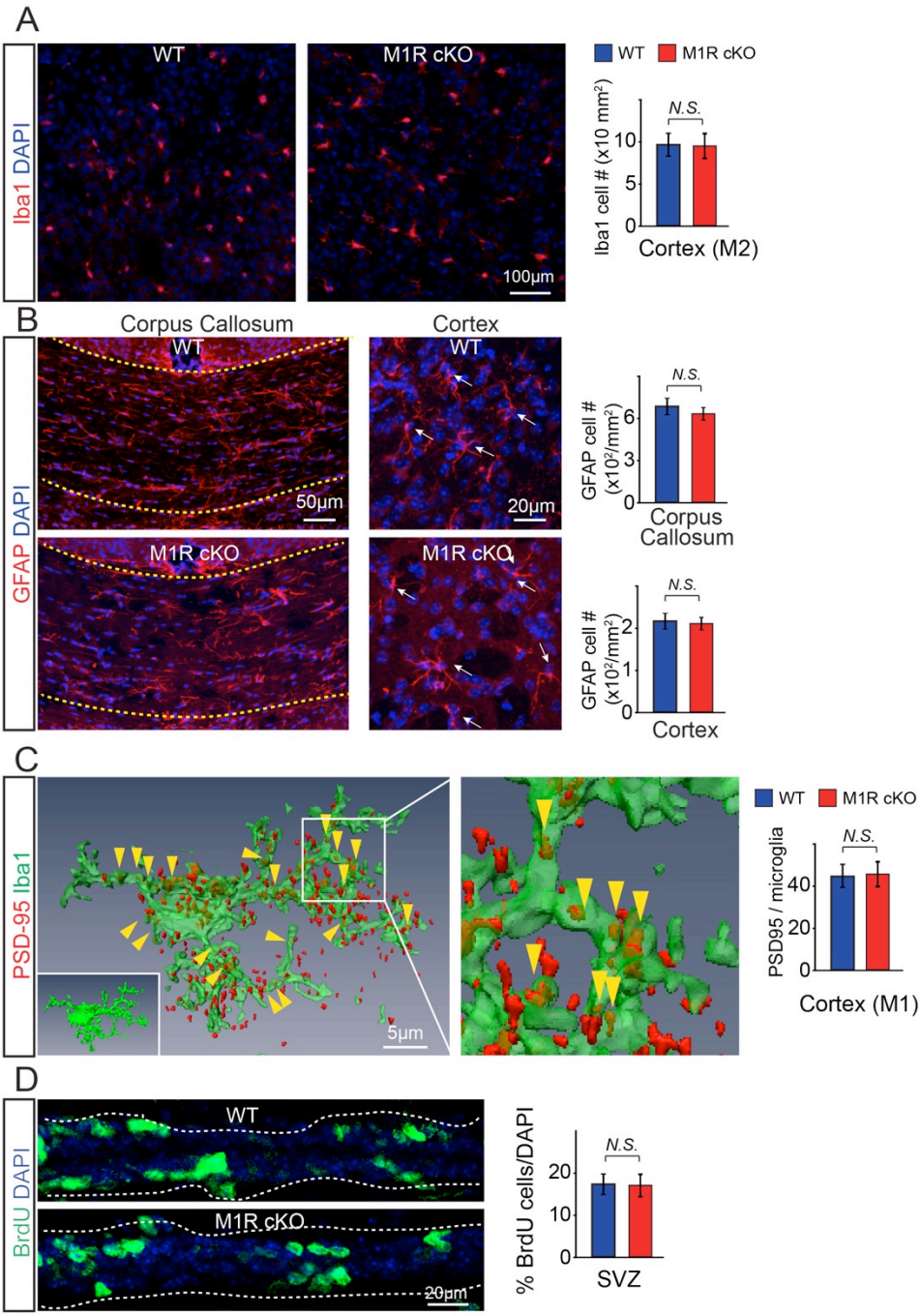
Supplemental Figure 3 (Related to Figure 4): Olig2 deletion in mature OLs does not cause demyelination or axonal degeneration.

(a) The PLP-CreERt line was crossed with the Olig2 fl/fl mouse line; (b) Schematic paradigm showing the time course of tamoxifen induction and histology examination. (c) Representative images and quantification of MBP expression in the Olig2 cKO and wildtype littermate brains. (d) Representative EM images showing corpus callosum in the Olig2 cKO mice and littermates. Myelinated axon numbers in the corpus callosum were quantified. The scatterplot displayed g-ratios of individual axons in Olig2 cKO mice and littermates. Error bars represent mean ± s.e.m. Significance based on Student's t-test with the respective controls. n = 3 for all experiments. (e) Representative images showing SMI-32 (green) and NF200 positive axons (red) in the cortex, corpus callosum and striatum of CNP-Cre; Olig2 fl/fl and Olig2 fl/fl mice at P42.



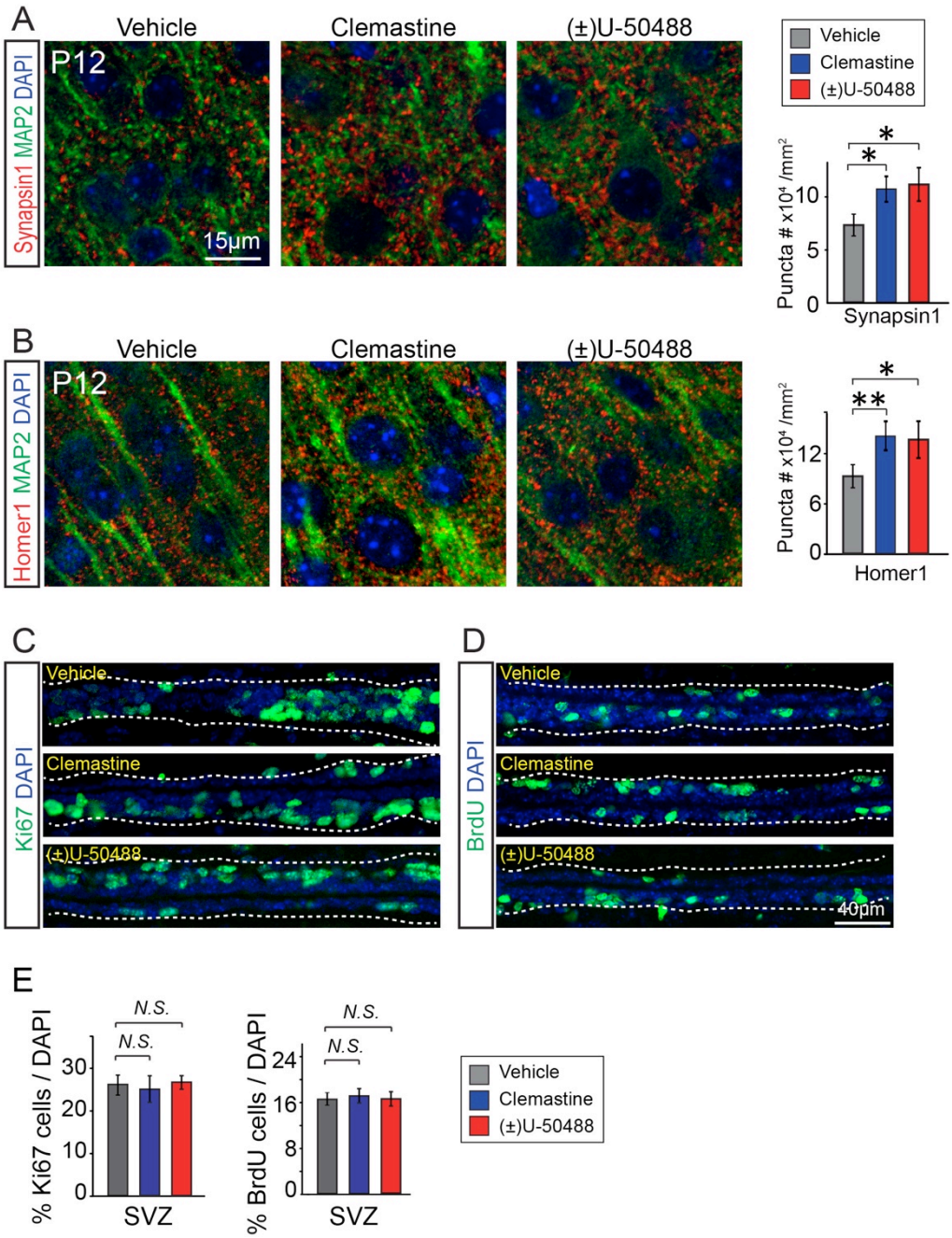
Supplemental Figure 4 (Related to Figure 6): Lineage-specific tracing of M1 null OPCs by newly-formed oligodendrocytes in hypoxic brains and NG2-CreERt induced recombination in OPCs (NG2 positive).

(a) The NG2-CreERt; M1R fl/fl line was crossed with the Tau-mGFP mouse line; (b) Recombination was induced in OPCs and mature OLs were visualized by mGFP expression; (c) Immunostaining showing mGFP positive membranes were also MBP positive. (d) Representative immunofluorescent images showing CC1 positive cells were also MOG and mGFP positive (arrows) and very few mGFP negative and CC1 positive cells (white arrowhead). (e) The NG2-CreERt line was crossed to mT/mG line; (f) Most of the NG2 positive cells express mGFP (arrowheads) and few NG2 weak and mGFP positive cells (arrow) presumably indicating differentiating OPCs. (g) Tamoxifen treatment on mother induce recombination in pups at P12 (green).

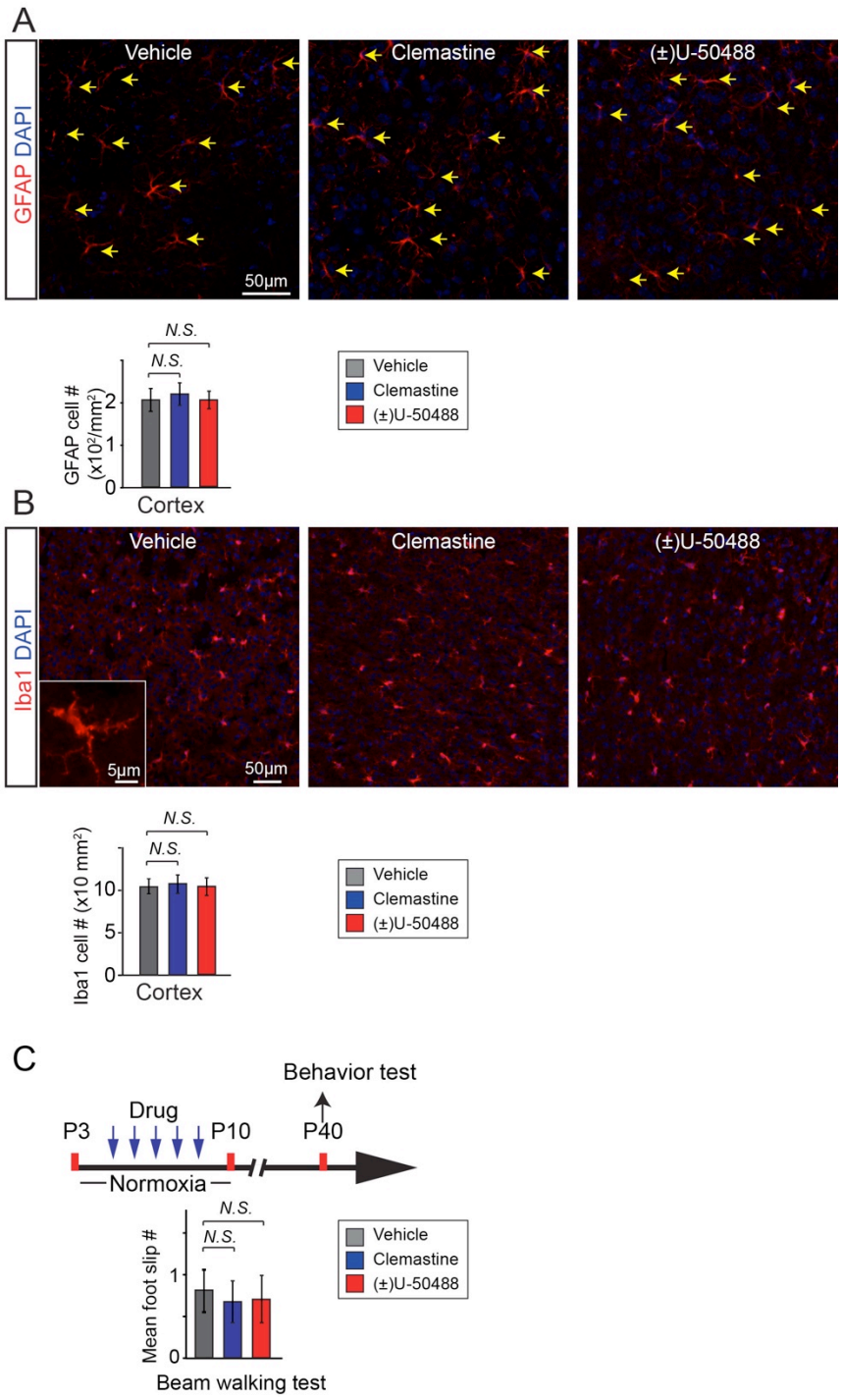


Supplemental Figure 5 (Related to Figure 7): Synapse engulfment by microglia and SVZ proliferation in the M1R cKO mice after hypoxia exposure.

(a, b) Representative images and quantification of Iba1 positive (a) and GFAP positive cells (b) in the cortex of hypoxic M1R cKO mice and wildtype littermates at P10; (c) Rendering of microglial/PSD-95 co-localization (yellow arrowheads) in the cortex of hypoxic M1R cKO and littermate wildtype mice at P10; (d) Representative images of BrdU positive cells in the SVZ of hypoxic M1R cKO mice and wildtype littermates at P10. Error bars represent mean ± s.e.m. Significance based on Student's t-test with the respective controls. n = 3 for all experiments.

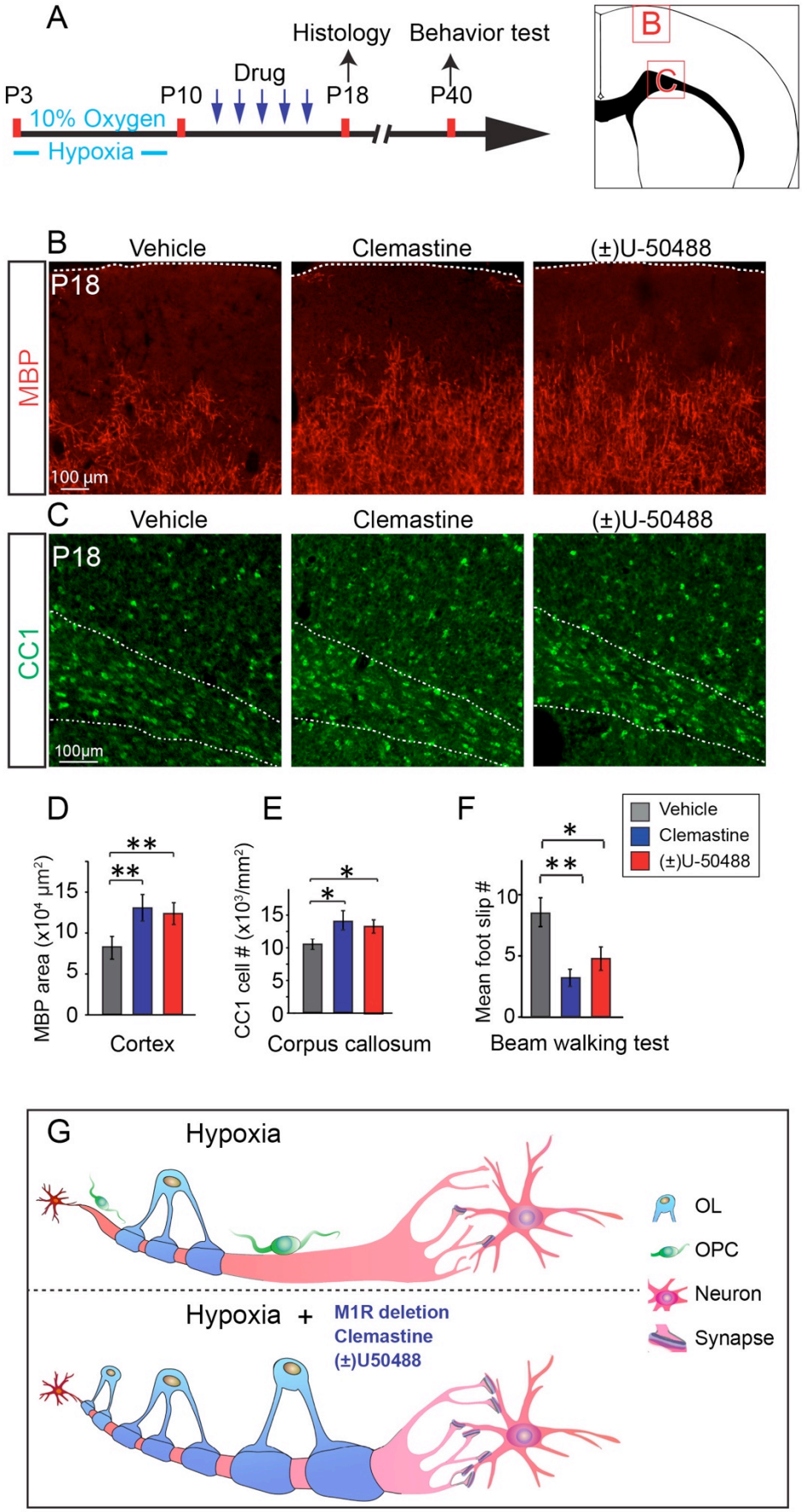


Supplemental Figure 6 (Related to Figure 8): Myelin-enhancing drug treatment rescues synaptic loss caused by hypoxia without changing SVZ proliferation. (a, b) Representative images and quantification of synaptic puncta (red) by labeling synapsin-1 (a) or homer1 (b) in the M2 cortex of hypoxia brains treated with vehicle, clemastine or (±)U50488 at P12 and neurons were revealed by MAP2 staining (green). n = 3 for all experiments; (c-e) Representative images and quantification of Ki67 (c) or BrdU (d) positive cells in the SVZ of hypoxic brains treated with vehicle, clemastine or (±)U50488 at P12. **p<0.01 or *p<0.05, significance based on Student's t-test by comparing clemastine or (±)U50488 to vehicle, n = 3 for all experiments.



Supplemental Figure 7 (Related to Figure 8): Microglia and astrocytes in the drug treated hypoxic brains.

(a, b) Representative images and quantification of Iba1 positive (a) and GFAP positive cells in the cortex of hypoxia brains treated with vehicle, clemastine or (±)U50488. Significance based on Student's t-test, n = 3 for all experiments. (c) The beam-walking test showing the frequency of foot slips of the clemastine, (±)U50488 or vehicle treated normoxic mice at P40 (n = 5). Significance based on non-parametric Mann-Whitney test.


A
Histology
Behavior test
Drug
P3
P10
P18
P40
10% Oxygen
Hypoxia
B
C
B
Vehicle
Clemastine
(±)U-50488
P18
MBP
100 μm
C
Vehicle
Clemastine
(±)U-50488
P18
CC1
100μm
D
MBP area (x10⁴ μm²)
Cortex
E
CC1 cell # (x10³/mm²)
Corpus callosum
F
Vehicle
Clemastine
(±)U-50488
Mean foot slip #
Beam walking test
G
Hypoxia
Hypoxia +
M1R deletion
Clemastine
(±)U50488
OL
OPC
Neuron
Synapse

Supplemental Figure 8 (Related to Figure 8): Post-hypoxia treatment of myelin-enhancing drugs promotes myelination and improve functional recovery. (a) Experimental paradigm showing the time course of drug treatment and hypoxia exposure; (b, c) Representative images showing MBP expression (b) and CC1 positive OLs (c) in the hypoxic brains treated with clemastine, (±)U50488 or vehicle. (d, e) MBP positive areas (d) and CC1 (e) positive cell numbers were quantified. Error bars represent mean ± s.e.m. *p<0.05 or **p<0.01, significance based on Student's t-test with the respective controls, n = 3 for all experiments. (f) The beam-walking test showing the frequency of foot slips of the clemastine, (±)U50488 or vehicle treated mice at P40 (n = 7). **p<0.01 or *p<0.05, significance based on non-parametric Mann-Whitney test.