Supplemental Figures

Supplementary Figure 1. Developmental regulation of TIMP-1 expression in the central nervous system. qPCR results for TIMP-1 confirmed the previous reports that TIMP-1 levels are elevated in the CNS during early post-natal development and drop throughout adulthood (n=2-6 mice/group). RNA was extracted from the brains of WT mice (P0-P21) using Trizol (Invitrogen, Carlsbad, CA) according to manufacture's protocol. Following DNase treatment, RNA was reversed transcribed using iScript cDNA synthesis kit (BioRad; Hercules, CA) and PCR was performed with iQ SYBR Green Supermix (BioRad; Hercules, CA), using the Eppendorf realplex^2 Mastercycler. Expression of GAPDH was used as an endogenous control reference. All amplifications were performed in triplicate in a 96-well plate. Quantification of gene expression was calculated according to the experimental protocol's $2^{-\Delta\Delta CT}$ method. The following primers were used for detection of TIMP-1:
FWD:CATGGAAAGCCTCTGTGGATATG, REV:AAGCTGCAGGCACTGATGTG
The following primers were used for detection of GAPDH:
FWD:ACCACCATGGAGAAGGC, REV:GGCATGGACTGTGGTCATGA.
Fold changes in expression levels of *TIMP-1* mRNA are relative to P21.

Supplementary Figure 2. A. G ratios of myelinated axons in TIMP-1 KO mice were reduced compared to WT at P7. Analysis of 37500X magnified electron micrographs from the dorsal column of WT and TIMP-1KO mice were used to measure axon diameters and myelin sheath thicknesses using ImageJ software. Error bars represent the mean ± SEM. ***p<0.001. **B. Analysis of axon diameters in dorsal column of spinal cords in P7 TIMP-1KO and age-matched WT mice** revealed no significant differences in the diameters of myelinated axons in TIMP-1KO mice. n=3/group; 21-26 axons/specimen using 17900x magnification electron micrographs. P=0.702.

Supplementary Figure 3. A. Decreased numbers of PDGFRα+ OPCs were observed in the corpus callosum of TIMP-1KO mice relative to WT controls (n=3/group). Oligodendrocyte progenitor cells (OPCs) were identified by immunohistochemistry on coronal brain tissue sections from P7 WT and TIMP-1KO mice using antisera to platelet derived growth factor α receptor (Cell Signaling, Beverly, MA). It was noted in the white matter of TIMP-1KO mice that the OPCs were smaller and exhibited shorter processes indicative of a less developed stage of growth. Scale bar = 200μm. **B. Quantification of PDGFRα+ OPCs in corpus callosum of WT and TIMP-1KO mice during postnatal development** revealed a specific deficit in OPC numbers that coincided with the period of delayed myelination at P7. n=3/genotype/timepoint. *, P<0.05.

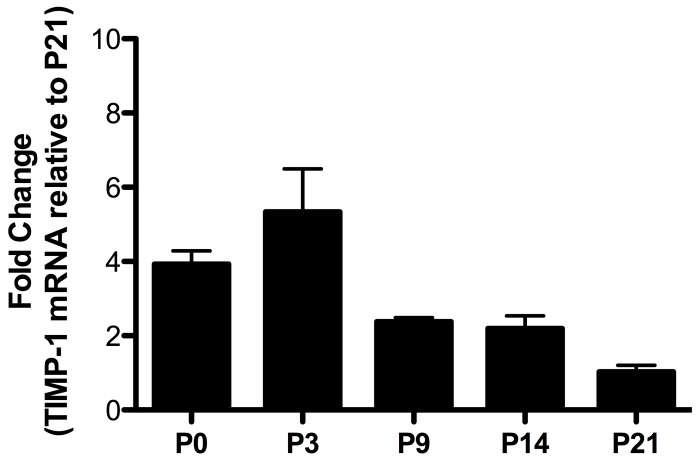
Supplementary Figure 4. Decreased numbers of reactive astrocytes in the developing CNS of TIMP-1 KO mice. Immunohistofluorescent detection of glial fibrillary acidic protein

(GFAP) was used to identify astrocytes in coronal brain tissue sections from P7 WT and TIMP-1KO pups. Upper panel set depicts representative GFAP immunolabeling in cortex and hippocampus with lower images taken from area highlighted by white box. Higher magnification images reveal the paucity of astrocytes in the molecular layer of the hippocampus superior to the granular layer of the dentate gyrus of TIMP-1KO animals. The lower panel set depicts a similar phenomenon in the corpus callosum. Whereas astrocytes clearly identify the corpus callosum in the developing wildtype mouse brain, GFAP+ cells are sparse in the corpus callosum of age-matched TIMP-1KO mice. Schematic brain diagrams to right of upper and lower panels indicate (red box) region shown in low magnification in upper and lower panel sets, respectively. Hence, consistent with observations from spinal cord tissues at the same developmental ages, astrocytic responses during CNS development were globally affected by TIMP-1 deficiency.

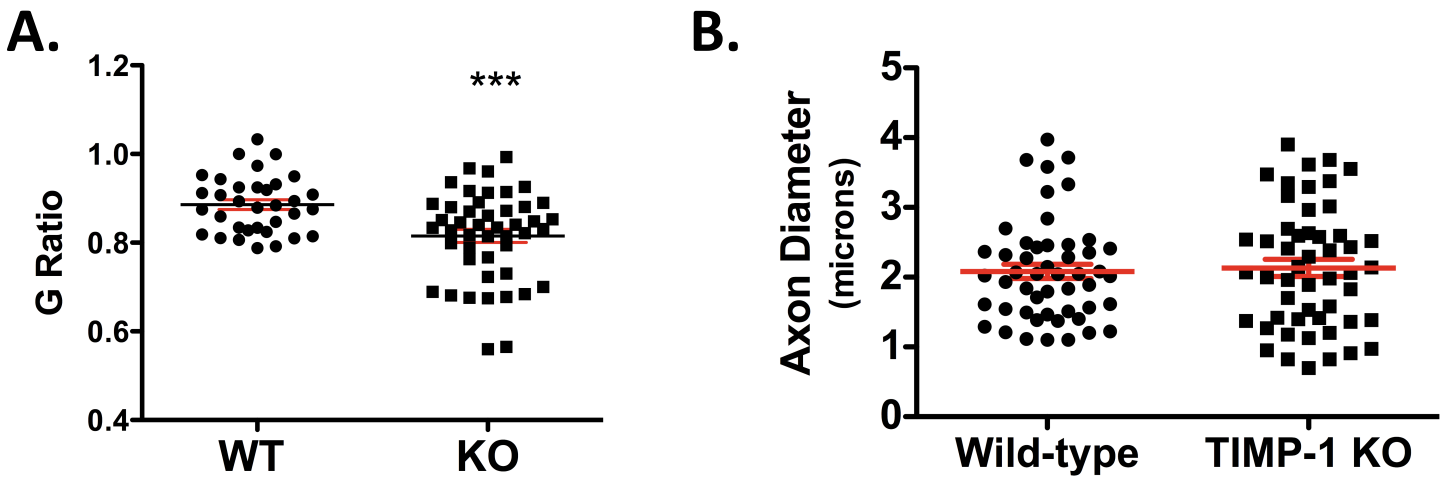
Supplementary Figure 5. TIMP-1 stimulates proliferation of WT astrocytes. Treatment of mixed glial cultures from WT mice were treated with rmTIMP-1 (100ng/mL; R&D Systems) for 48 hours and the number of GFAP+ cells were evaluated by immunocytochemistry. Analysis of rmTIMP-1 treated compared with untreated cultures revealed that addition of rmTIMP-1 stimulated a significant increase (~3-fold) in the number of GFAP+ cells. No effect on astrocyte numbers was observed using either active (A) or inactive (I) forms of the potent pan-MMP inhibitor GM6001 (125μM) (n=3/group, **p<0.01 relative to “No treatment”). Error bars represent the mean ± SEM; scale bar=200μm.

Supplementary Figure 6. TIMP-1 stimulates proliferation of astrocytes *in vitro*. (A) Protein lysates taken from TIMP-1KO primary glial cultures that were treated with rmTIMP-1 (1-100ng/mL) were analyzed by Western blotting for expression of proliferating cell nuclear antigen (PCNA), a marker of cell proliferation, and procaspase-3, a marker for apoptosis. Increased PCNA levels were observed in astrocytes treated with rmTIMP-1 (10ng/mL), a dose that provided the largest increase in GFAP+ cell numbers and in BrdU+ labeled cells, while no differences in procaspase-3 levels were noted. No effects of GM6001 on either PCNA or caspase-3 levels were observed, indicating that MMP inhibition was not a cause of astrocyte proliferation, in addition to providing evidence that this compound was not toxic in this culture system. **(B)** TUNEL assay confirmed that GM6001 did not induce cell death of astrocytes in a mixed glial culture, further supporting that this compound was not cytotoxic. Together, these data indicate that TIMP-1 provided a mitogenic, rather than an anti-apoptotic effect to the astrocytes in culture.

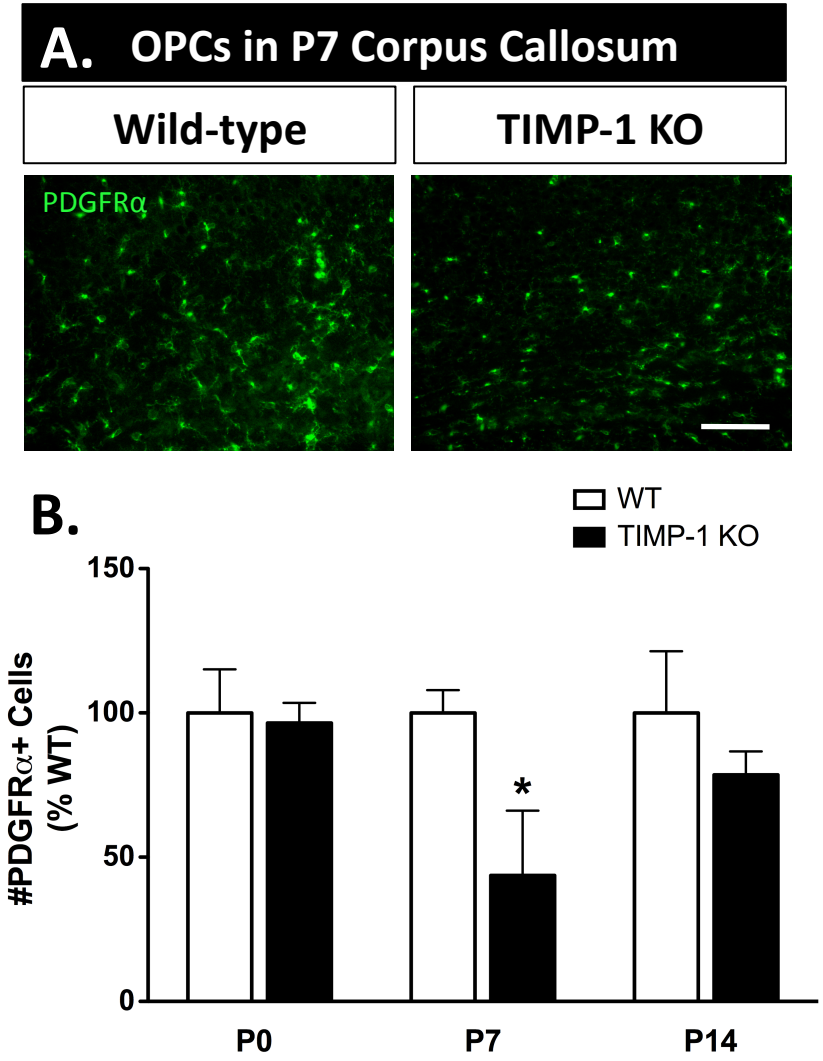
Supplementary Figure 1



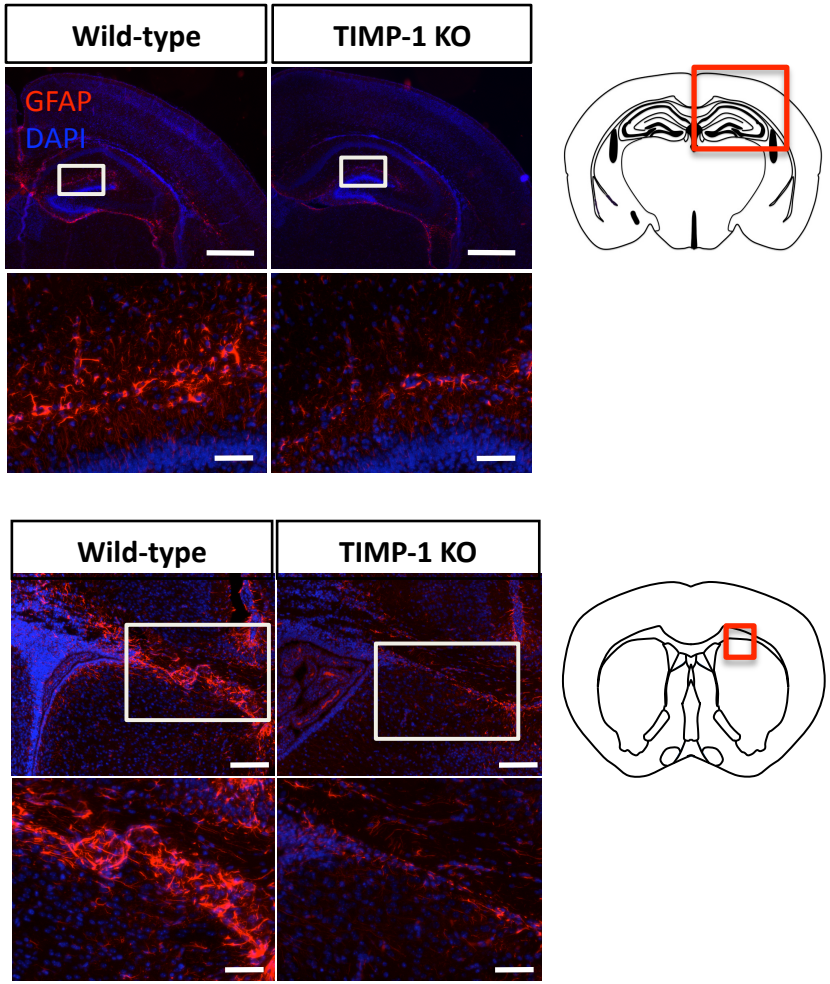
Supplementary Figure 2



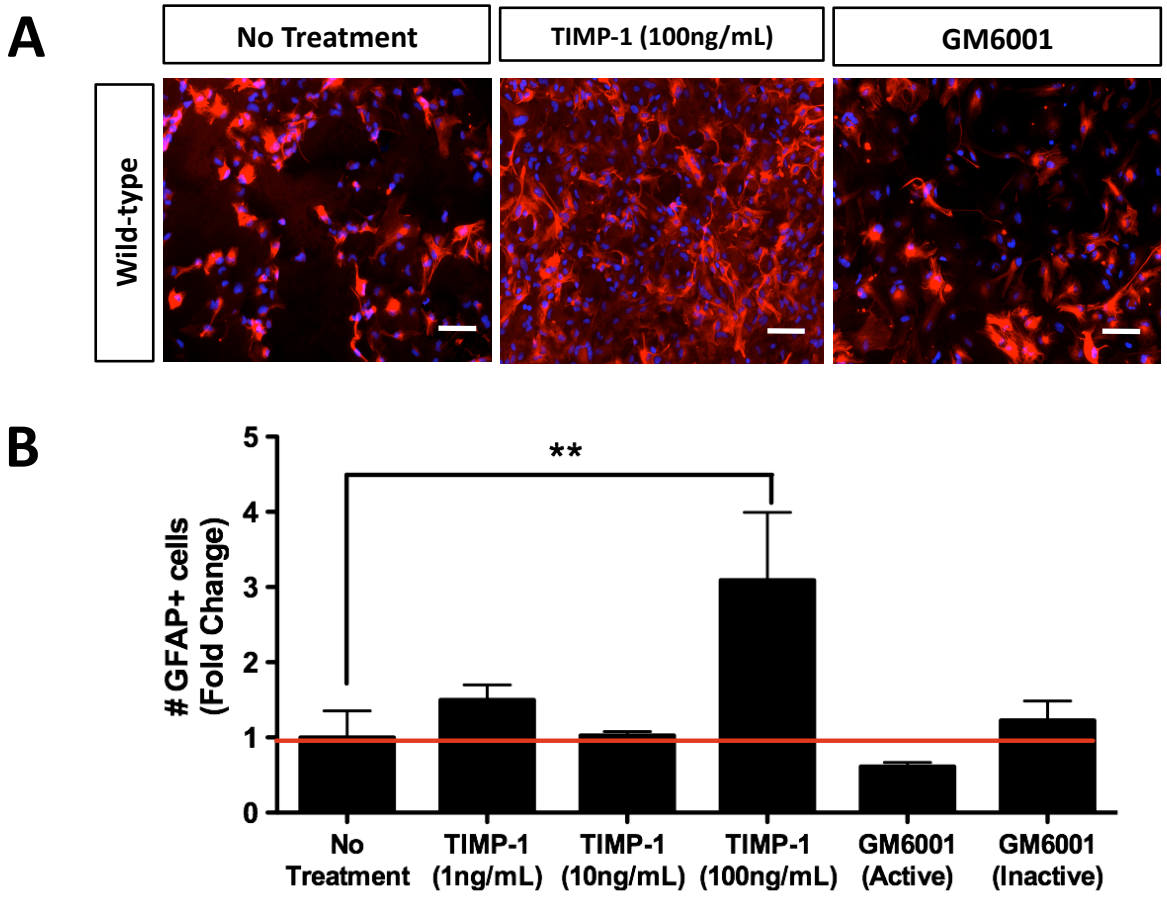
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6