

Supporting Information for

Astrocytic TIMP-1 regulates production of Anastellin, a novel inhibitor of oligodendrocyte differentiation and FTY720 responses

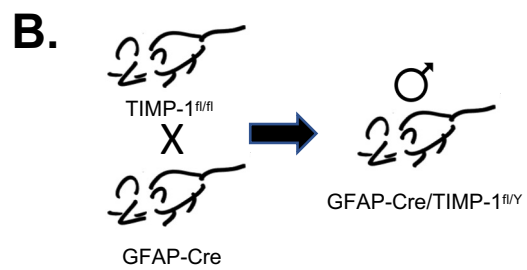
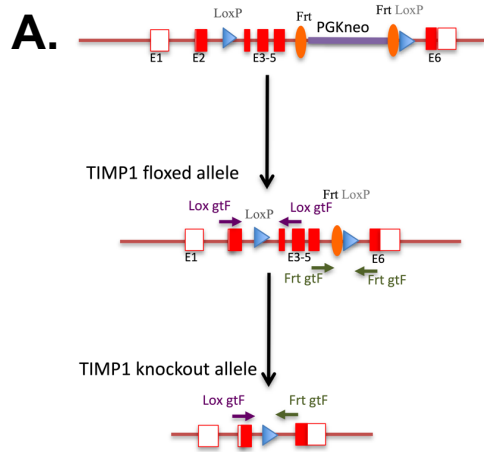
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Supporting text
Figure S1



C. PCR genotyping of TIMP1 cKO mice

Genotyping of TIMP 5'LoxP site:
 Lox gtF: 5'-GTGGTGTGTAGGTGCCTGTG
 Lox gtR: 5'-GCAATCTCAGCACTTGGATG

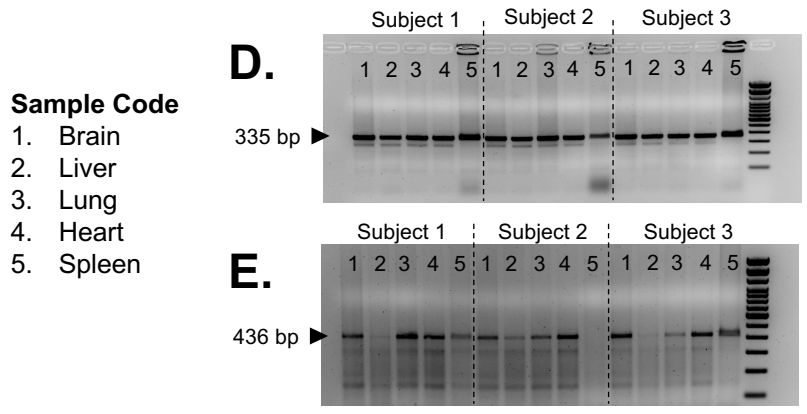
Wt 256bp Floxed 335bp

Genotyping of TIMP 3'LoxP/Frt site:
 Frt gtF: 5'-TCATTGTCCAAGAGCCTTGC
 Frt gtR: 5'-CTTGAAGTCACTCAGACAGG

Wt 328bp Floxed 437bp

Genotyping of TIMP KO/Recombined allele:
 Lox gtF: 5'-GTGGTGTGTAGGTGCCTGTG
 Frt gtR: 5'-CTTGAAGTCACTCAGACAGG

Wt No product. KO/Recomb 436bp



Lox gtF+LoxgtR

Floxed	335 bp
Wt	256 bp

Lox gtF+Frt gtR

KO/Recomb	436 bp
Wt	No product

SI Figure 1. Breeding strategy and genotyping of GFAP-Cre/TIMP-1^{fl/fl} mice. (A) Schematic of targeted TIMP-1 gene locus on X chromosome also indicating the locations of PCR primers for genotyping. (B) Floxed *timp-1* female mice when bred with male GFAP-Cre mice generated F1 generation males which were all Cre⁺/fl⁺. (C) PCR primers designed to identify and distinguish the floxed from Wt and recombined alleles. (D) Analysis of floxed allele in multiple organs from male progeny revealed presence of floxed allele, and not wildtype, in all organs tested, including brain, heart, lung, spleen, and liver. (E) PCR analysis of recombined floxed allele in male progeny identified consistent recombination in brain, but with variable recombination in other organs tested (heart, lung, liver and spleen). Thus, while astrocytic TIMP-1 recombination was consistently demonstrated, possible recombination in other tissues and cell types cannot be excluded in this study.