

WT GFAP-IL6 GFAP-IFN ---- Microglia ----- Monocytes

Supplementary Figure 1 Surviving microglia repopulate the depleted brain in WT and GFAP-IL6 mice but not in GFAP-IFN mice. (A-C) Representative immunofluorescence images (Iba1<sup>+</sup> microglia/macrophages, green; TMEM119<sup>+</sup> microglia, red; DAPI, blue) from the cerebellum of WT (A), GFAP-IL6 (B) and GFAP-IFN (C) mice given control or PLX5622 diet for 14 days (repopulation day 0) or following 28 days after removal of PLX5622 (repopulation day 28). Scale bars, 20  $\mu$ m. (D-L) Quantification of (D-F) the number of

Iba1<sup>+</sup>TMEM119<sup>+</sup> microglia per mm<sup>2</sup>, (G-I) the number of Iba1<sup>+</sup>TMEM119<sup>-</sup> monocytes/macrophages per mm<sup>2</sup> and (J-L) the percentage of Iba1<sup>+</sup>TMEM119<sup>+</sup> microglia versus Iba1<sup>+</sup>TMEM119<sup>-</sup> monocytes/macrophages in the (D, G, J) cerebellum, (E, H, K) cortex and (F, I, L) hippocampus. In (J-L), solid lines represent Iba1<sup>+</sup>TMEM119<sup>+</sup> microglia and dashed lines represent Iba1<sup>+</sup>TMEM119<sup>-</sup> monocytes/macrophages. n=3-6 mice/group. For (D-I), graphs show individual values per mouse and mean  $\pm$  SEM and for (J-L), graphs show mean  $\pm$  SEM. \*, p<0.05 compared with WT of same diet; ^, p<0.05 compared with GFAP-IL6 of same diet; #, p<0.05 compared with untreated control of same genotype; x, p<0.05 compared with PLX5622 day 0 of same genotype using two-way ANOVA with Tukey's post-test.



**Supplementary Figure 2** Additional images of histopathology in the brain of GFAP-IL6 and GFAP-IFN mice following PLX5622 treatment. (A-F) Representative images of the (A, B, D-F) cerebellum and (C) cortex of mice treated with control or PLX5622 diet for 12 weeks. (A) Iba1, (B, C) GFAP in the (B) cerebellum and (C) cortex, (D) laminin, (E) neurofilament-200 (NF-200) and (F) calbindin (CALB) in the cerebellum. Black arrowheads indicate areas of neuronal cell loss. Scale bars for (A, C, D, F), 20  $\mu$ m. Scale bars for (B, E), 100  $\mu$ m. (G) Quantification of the number of CALB<sup>+</sup> Purkinje neurons per mm<sup>2</sup> in the Purkinje cell layer (PCL) of the cerebellum. Graph shows individual values per mouse and mean ± SEM. n=6-12 mice/group.



**Supplementary Figure 3** Differential expression of *Spp1*, but not *Trem2*, by microglia in GFAP-IL6 versus GFAP-IFN mice. **(A-B)** Quantification of RNA-seq reads (fragments per kilobase of transcript per million mapped reads (FPKM)) for **(A)** *Spp1* and **(B)** *Trem2* in microglia isolated *ex vivo* from the cerebellum of 1-month-old WT, GFAP-IL6 and GFAP-IFN mice [1]. \*, p<0.05 compared with WT; ^, p<0.05 compared with GFAP-IL6 using edgeR. n=3 mice/group.

## References

1. West PK, McCorkindale AN, Guennewig B, Ashhurst TM, Viengkhou B, Hayashida E, et al. The cytokines interleukin-6 and interferon- $\alpha$  induce distinct microglia phenotypes. J Neuroinflammation. 2022;19(1):96.