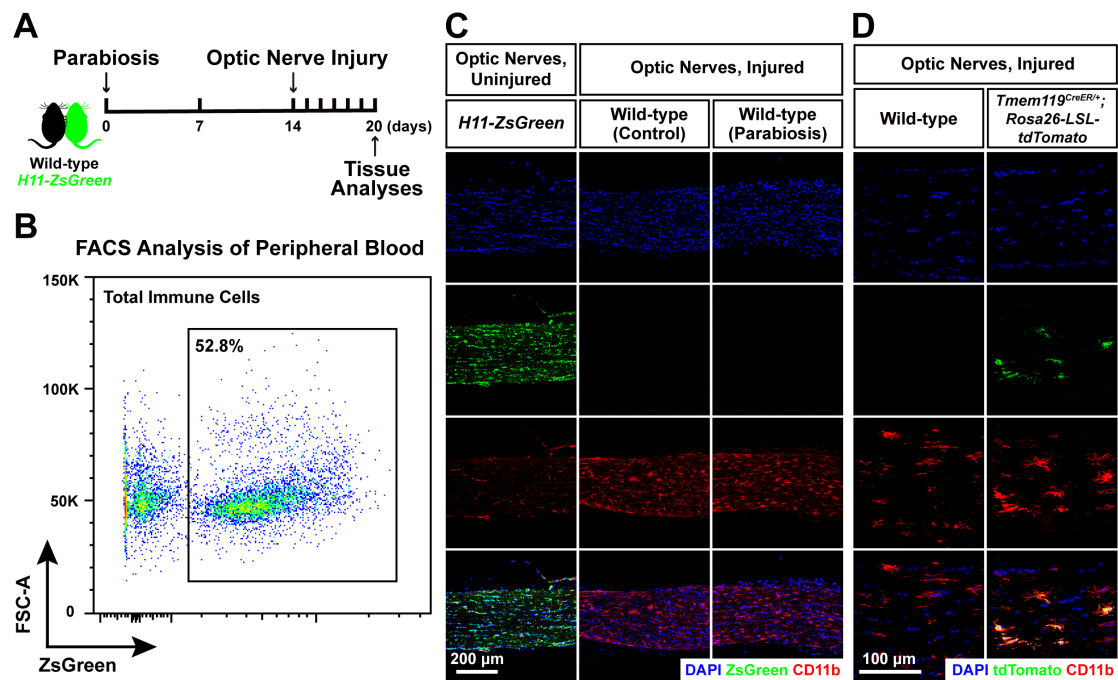


Supplementary Figures

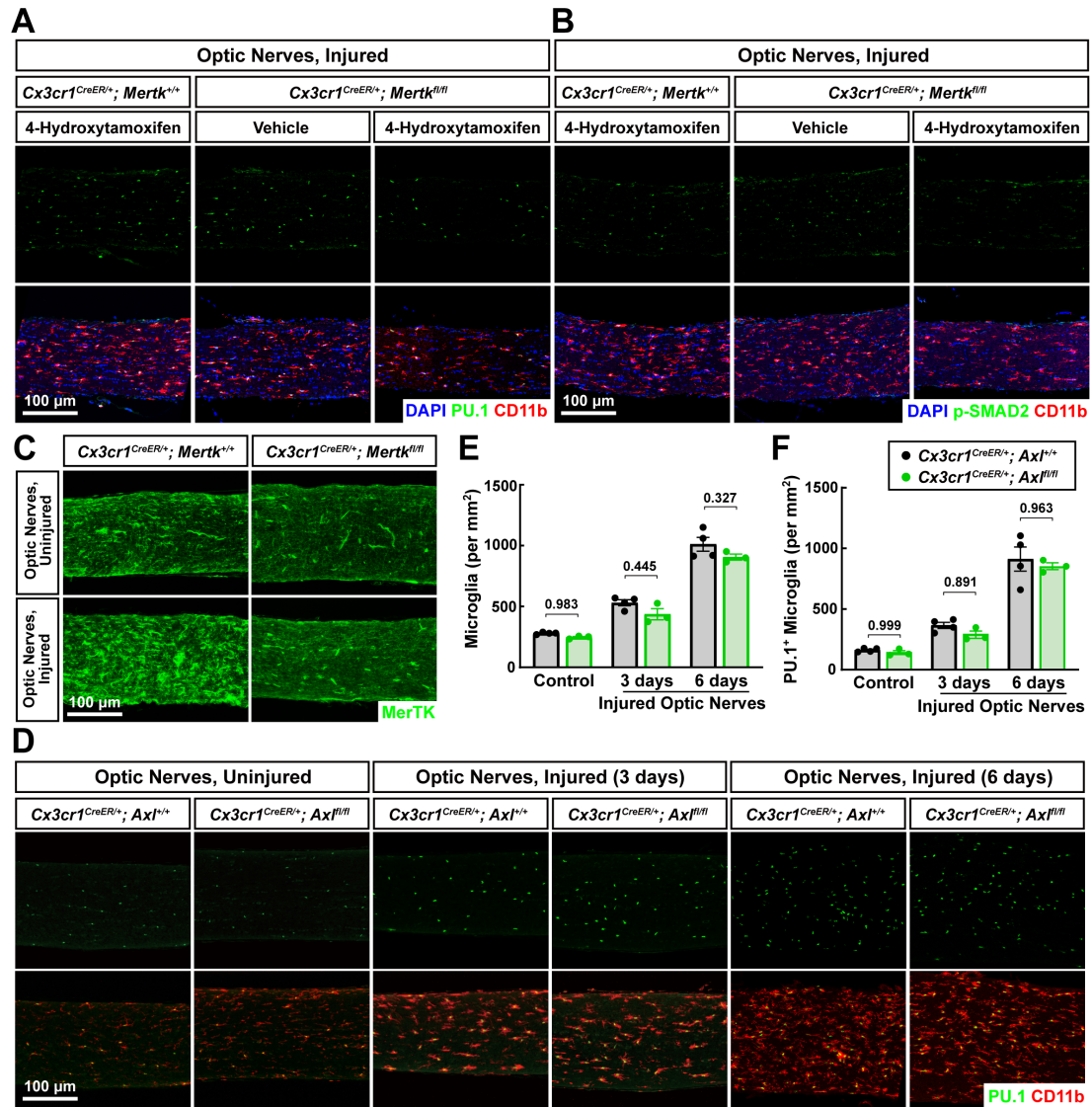


Supplementary Figure 1. A minor involvement of circulating immune cells in the mouse model of optic nerve injury.

(A to C) 8-week-old C57BL/6 wild-type and *H11-ZsGreen* female mice were utilized for parabiosis. (A) Diagram of the experimental procedure. (B) The peripheral blood of the wild-type mice in parabiosis with *H11-ZsGreen* mice was processed for FACS. ZsGreen⁺ cells in CD45⁺ total immune cells were quantified. (C) Control wild-type mice or the wild-type mice in parabiosis with *H11-ZsGreen* mice were subjected to optic nerve injury. Optic nerves were collected at 6 days post-injury for the co-immunostaining of ZsGreen and CD11b. n = 3 mice per condition, and representative images are shown.

(D) 8-week-old *Tmem119^{CreER/+}; Rosa26-LSL-tdTomato* and control C57BL/6 wild-

type female mice were subjected to the 4-hydroxytamoxifen-induced Cre-recombinase activity and then utilized for the model of optic nerve injury. Optic nerves were collected at 3 days post-injury for the co-immunostaining of tdTomato and CD11b. n = 5 mice per condition, and representative images are shown.



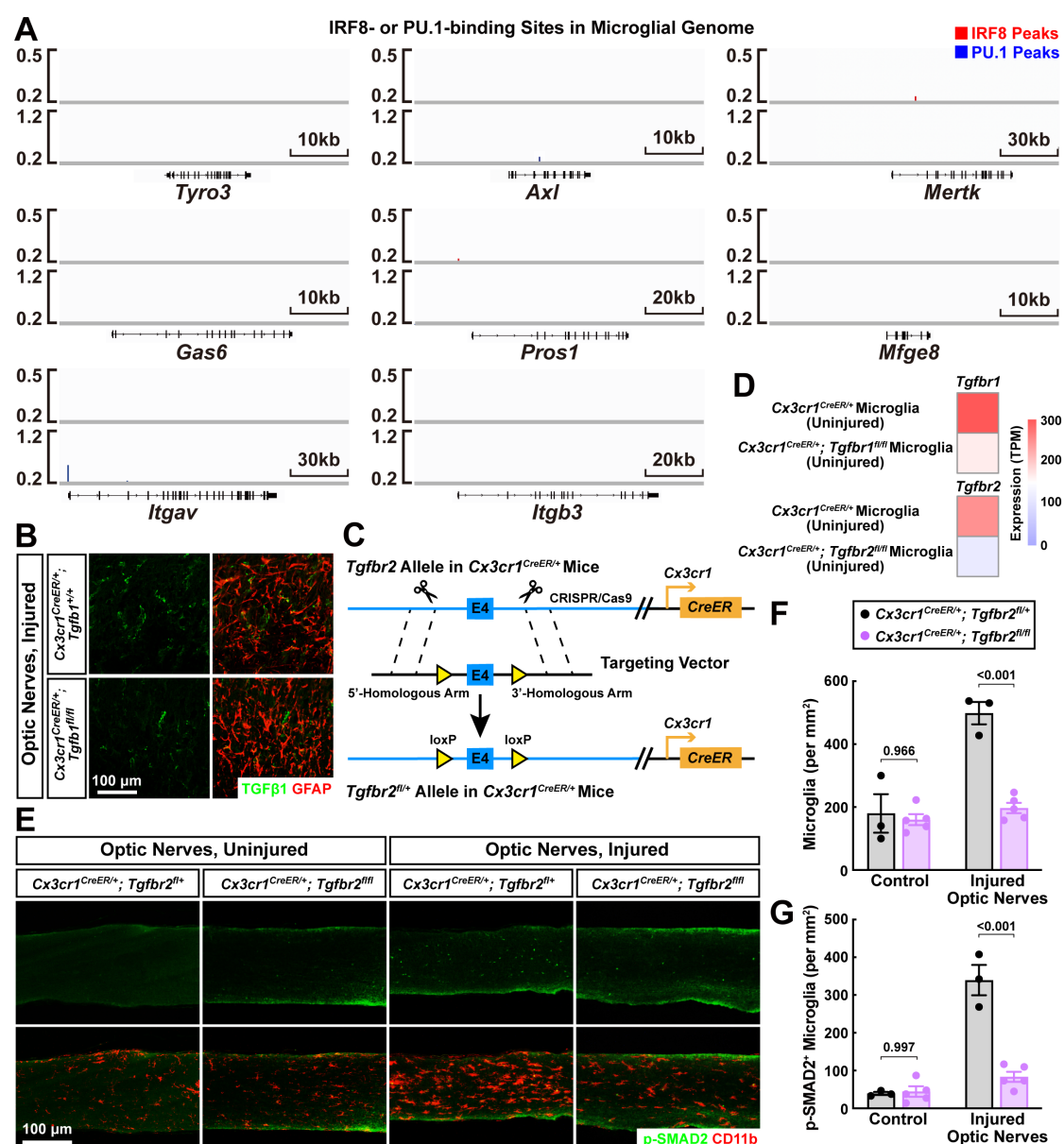
Supplementary Figure 2. Microglial response to pathological axonal

degeneration depends on the phagocytic receptor MerTK.

(A to C) 8-week-old *Cx3cr1^{CreER/+}; Mertk^{fl/fl}* and control *Cx3cr1^{CreER/+}; Mertk^{+/+}* female mice were subjected to the 4-hydroxytamoxifen-induced Cre-recombinase activity or vehicle control and then utilized for the model of optic nerve injury. (A and B) Microglial expression of PU.1 (A) and phospho-SMAD2 (p-SMAD2) (B) in the optic nerves at 3 days post-injury was assessed by co-immunostaining with

CD11b. n = 3 mice per condition, and representative images are shown. **(C)** MerTK expression in the optic nerves at 3 days post-injury was assessed by immunostaining. n = 3 mice per condition, and representative images are shown.

(D to F) 8-week-old *Cx3cr1^{CreER/+}; Ax1^{fl/fl}* and control *Cx3cr1^{CreER/+}; Ax1^{+/+}* female mice were subjected to the 4-hydroxytamoxifen-induced Cre-recombinase activity and then utilized for the model of optic nerve injury. **(D)** Microglial expression of PU.1 in the optic nerves at 3 or 6 days post-injury was assessed by co-immunostaining with CD11b, and representative images are shown. **(E and F)** Microglial densities **(E)** and PU.1⁺ microglia **(F)** were quantified. n = 4 mice for *Cx3cr1^{CreER/+}; Ax1^{+/+}* and n = 3 mice for *Cx3cr1^{CreER/+}; Ax1^{fl/fl}*, mean ± SEM, two-way ANOVA test.



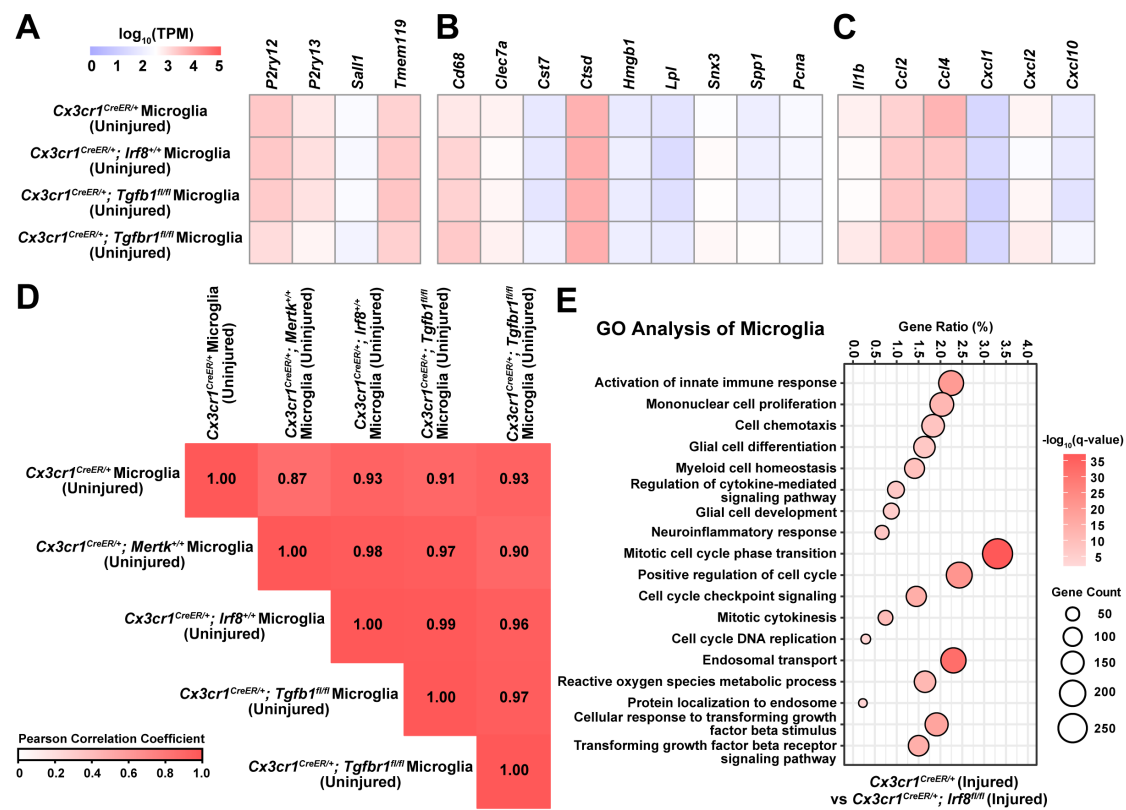
Supplementary Figure 3. Microglial TGFβ1 acts as an autocrine signal in response to pathological axonal degeneration.

(A) 8-week-old C57BL/6 wild-type female mice were subjected to the model of optic nerve injury. IRF8- or PU.1-targeted sites on the indicated gene loci in microglia were assessed by ChIP-seq analyses.

(B) 8-week-old *Cx3cr1*^{CreER/+}; *Tgfb1*^{fl/fl} and control *Cx3cr1*^{CreER/+}; *Tgfb1*^{+/+} female

mice were subjected to the 4-hydroxytamoxifen-induced Cre-recombinase activity and then utilized for the model of optic nerve injury. Astrocytic TGFβ1 expression in the optic nerves at 3 days post-injury was assessed by co-immunostaining with GFAP. n = 3 mice for *Cx3cr1*^{CreER/+}; *Tgfb1*^{+/+} and n = 4 mice for *Cx3cr1*^{CreER/+}; *Tgfb1*^{fl/fl}, and representative images are shown.

(C to G) *Cx3cr1*^{CreER/+}; *Tgfb2*^{fl/fl} mice were generated for the model of optic nerve injury. **(C)** Diagram of the second round of gene targeting to obtain the *Cx3cr1*^{CreER/+}; *Tgfb2*^{fl/+} allele. **(D)** 8-week-old *Cx3cr1*^{CreER/+}; *Tgfb1*^{fl/fl}, *Cx3cr1*^{CreER/+}; *Tgfb2*^{fl/fl}, and control *Cx3cr1*^{CreER/+} female mice were subjected to the 4-hydroxytamoxifen-induced Cre-recombinase activity. Microglia were FACS-sorted from the uninjured optic nerves and analyzed by RNA-seq. Pooled RNA-seq data from 4 mice per condition are shown for *Tgfb1* expression in *Cx3cr1*^{CreER/+}; *Tgfb1*^{fl/fl} microglia versus control *Cx3cr1*^{CreER/+} microglia and *Tgfb2* expression in *Cx3cr1*^{CreER/+}; *Tgfb2*^{fl/fl} microglia versus control *Cx3cr1*^{CreER/+} microglia. **(E to G)** 8-week-old *Cx3cr1*^{CreER/+}; *Tgfb2*^{fl/fl} mice and control *Cx3cr1*^{CreER/+}; *Tgfb2*^{fl/+} were subjected to the 4-hydroxytamoxifen-induced Cre-recombinase activity and then utilized for the model of optic nerve injury. Microglial expression of phospho-SMAD2 (p-SMAD2) in the optic nerves at 3 days post-injury was assessed by co-immunostaining with CD11b. **(E)** Representative images are shown. **(F and G)** Microglial densities **(F)** and p-SMAD2⁺ microglia **(G)** were quantified. n = 3 mice for *Cx3cr1*^{CreER/+}; *Tgfb2*^{fl/+} and n = 5 mice for *Cx3cr1*^{CreER/+}; *Tgfb2*^{fl/fl}, mean ± SEM, two-way ANOVA test.

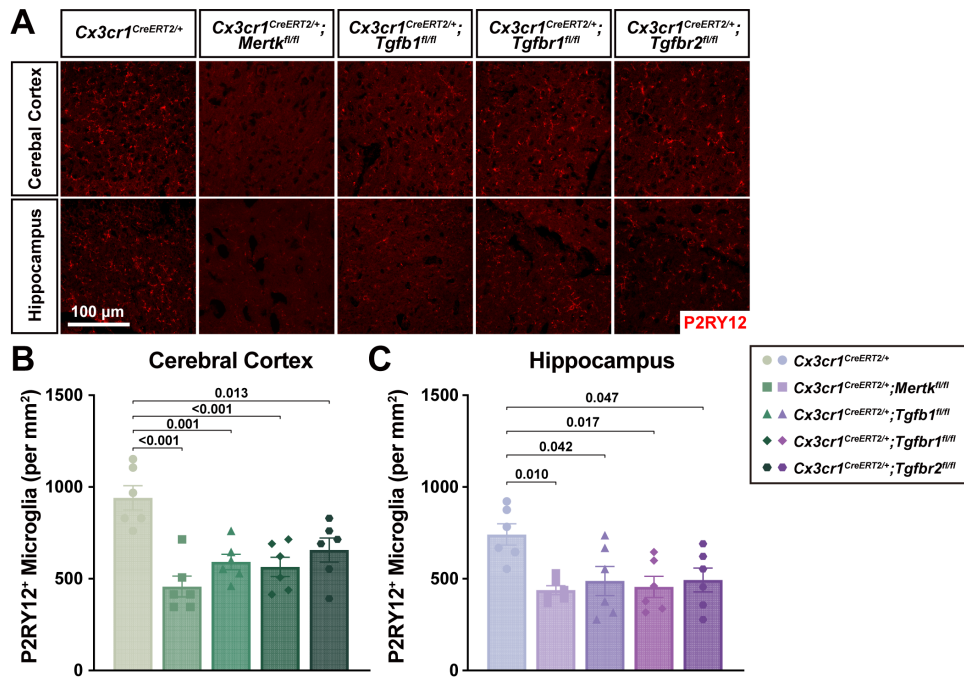


Supplementary Figure 4. An essential role of TGF β 1 autocrine signal in microglial response to pathological axonal degeneration.

(A to D) 8-week-old *Cx3cr1^{CreER/+}; Mertk^{fl/fl}*, *Cx3cr1^{CreER/+}; Irf8^{fl/fl}*, *Cx3cr1^{CreER/+}; Tgfb1^{fl/fl}*, *Cx3cr1^{CreER/+}; Tgfb1^{fl/fl}; Tgfb1^{fl/fl}*, and control *Cx3cr1^{CreER/+}* female mice were subjected to the 4-hydroxytamoxifen-induced Cre-recombinase activity. Microglia were FACS-sorted from the uninjured optic nerves and analyzed by RNA-seq. Pooled RNA-seq data from 4 mice per condition are shown. (A to C) Expression levels of homeostatic markers (A), signature genes for microglial response to neurodegenerative insults (B), and proinflammatory cytokines and chemokines (C) in microglia of the indicated conditions. (D) The pairwise comparison of transcriptomic

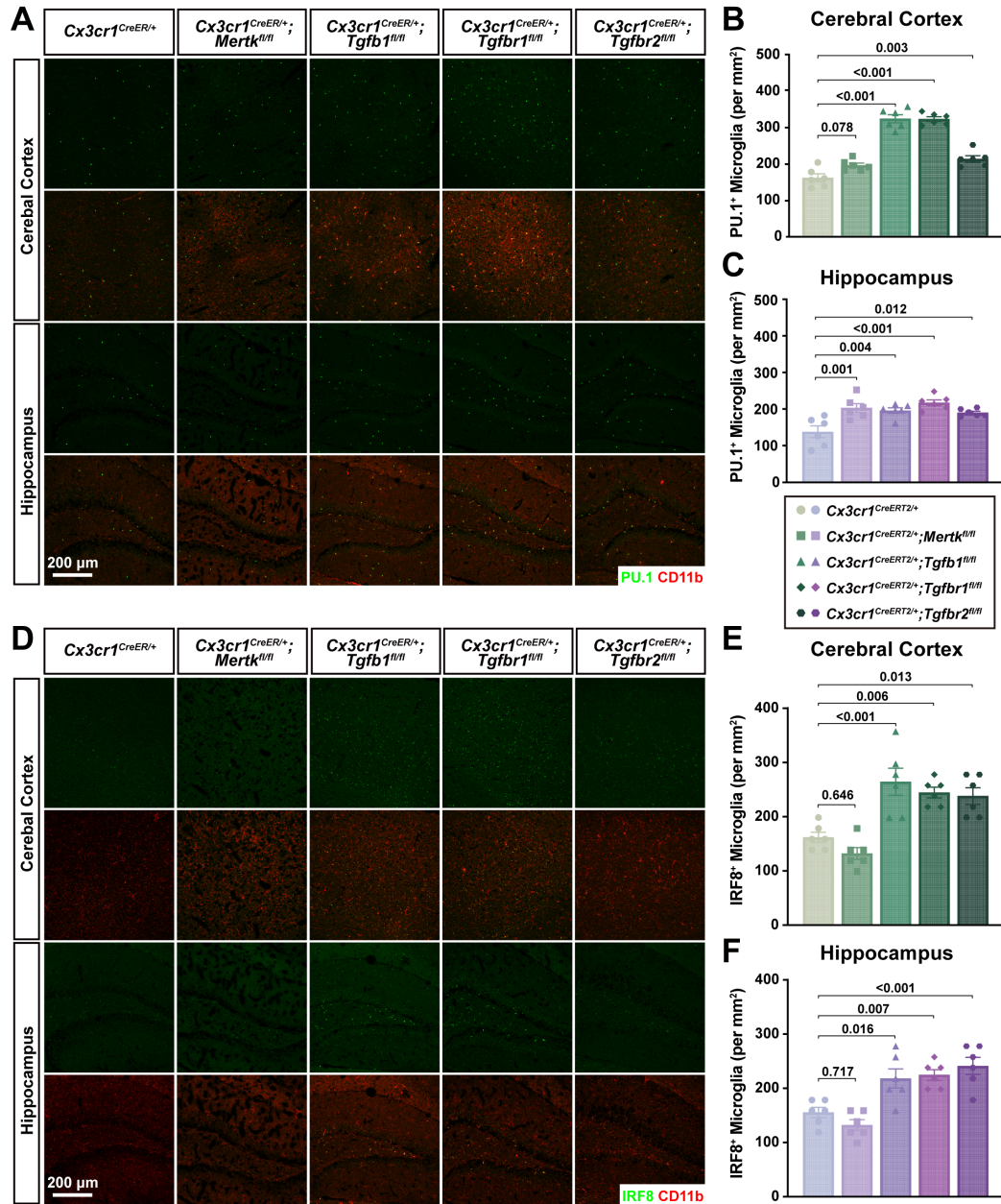
profiles in microglia of the indicated conditions.

(E) 8-week-old *Cx3cr1*^{CreER/+}; *Irf8*^{fl/fl} and control *Cx3cr1*^{CreER/+} female mice were subjected to the 4-hydroxytamoxifen-induced Cre-recombinase activity and then utilized for the model of optic nerve injury. Microglia were FACS-sorted from the injured optic nerves and analyzed by RNA-seq. Pooled RNA-seq data from 4 mice per condition are shown for GO enrichment analysis of microglia from the injured optic nerves of control *Cx3cr1*^{CreER/+} versus *Cx3cr1*^{CreER/+}; *Irf8*^{fl/fl} mice.



Supplementary Figure 5. Blockage of microglial TGF β 1 autocrine signal reduces the homeostatic marker P2RY12.

(A to C) 8-week-old *Cx3cr1^{CreER/+}; Mertk^{fl/fl}*, *Cx3cr1^{CreER/+}; Tgfb1^{fl/fl}*, *Cx3cr1^{CreER/+}; Tgfb1^{fl/fl}*, *Cx3cr1^{CreER/+}; Tgfb2^{fl/fl}*, and control *Cx3cr1^{CreER/+}* female mice were subjected to the 4-hydroxytamoxifen-induced Cre-recombinase activity. Microglial expression of P2RY12 in the cerebral cortex or the hippocampus 4 weeks after the Cre-recombinase induction was examined by immunostaining. (A) Representative images are shown. (B and C) P2RY12⁺ microglia in the cerebral cortex (B) and the hippocampus (C) were quantified. n = 6 mice per condition, mean $\pm$ SEM, one-way ANOVA test.

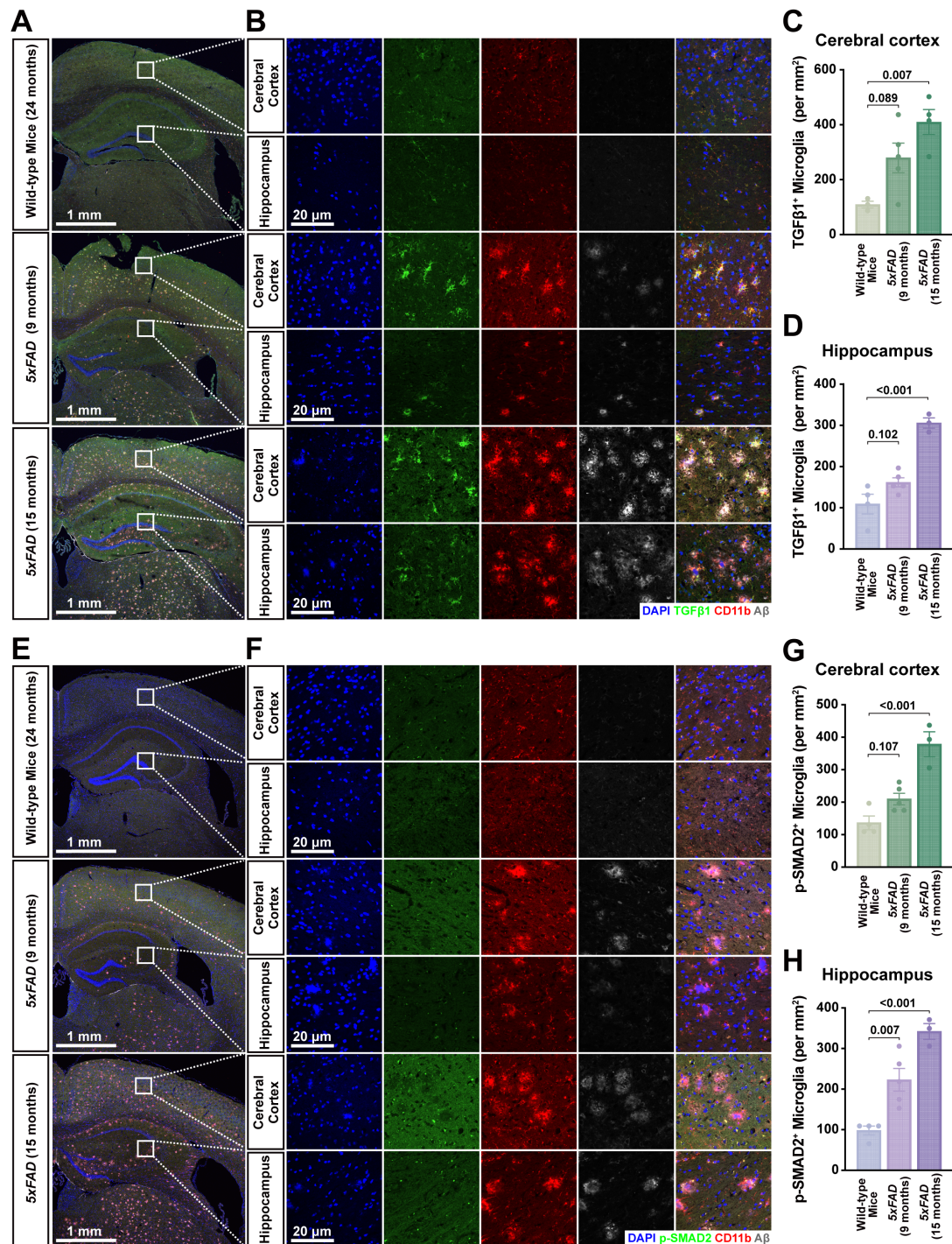


Supplementary Figure 6. Blockage of microglial TGF β 1 autocrine signal elevates the expression of PU.1 and IRF8.

(A to F) 8-week-old *Cx3cr1^{CreER/+}; Mertk^{fl/fl}*, *Cx3cr1^{CreER/+}; Tgfb1^{fl/fl}*, *Cx3cr1^{CreER/+}; Tgfb1^{fl/fl}*, *Cx3cr1^{CreER/+}; Tgfb1^{fl/fl}*, *Cx3cr1^{CreER/+}; Tgfb2^{fl/fl}*, and control *Cx3cr1^{CreER/+}* female mice were subjected to the 4-hydroxytamoxifen-induced Cre-recombinase activity. 4 weeks after the Cre-recombinase induction, microglial expression of PU.1 (A to C) and IRF8 (D

to F) in the cerebral cortex or the hippocampus were examined by co-immunostaining with CD11b. **(A)** Representative images of microglial PU.1 expression are shown. **(B and C)** Densities of PU.1⁺ microglia in the cerebral cortex **(B)** and the hippocampus **(C)** were quantified. n = 6 mice per condition, mean ± SEM, one-way ANOVA test.

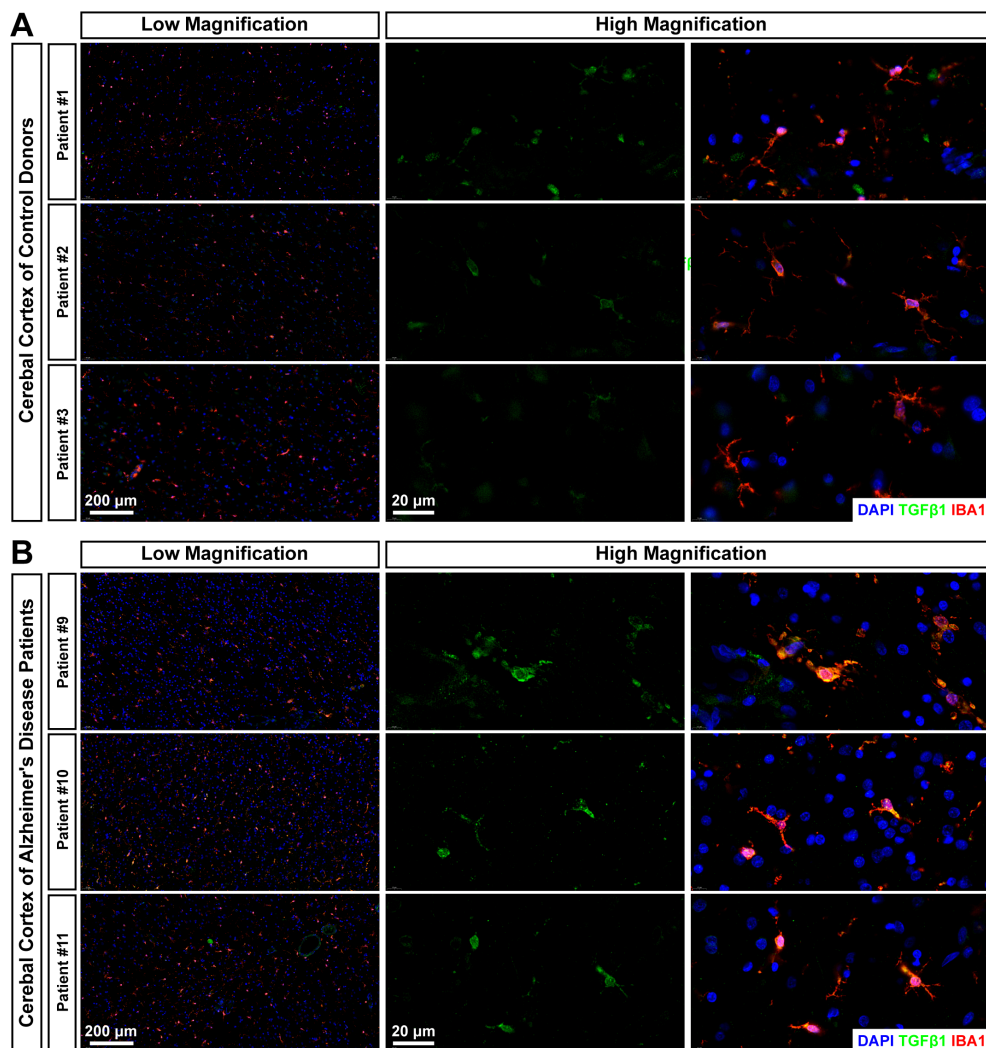
(D) Representative images of microglial IRF8 expression are shown. **(E and F)** Densities of IRF8⁺ microglia in the cerebral cortex **(E)** and the hippocampus **(F)** were quantified. n = 6 mice per condition, mean ± SEM, one-way ANOVA test.



Supplementary Figure 7. Microglial TGFβ1 signal in the mouse model of Alzheimer's disease.

(A to H) Brain tissues were collected from 9-month-old or 15-month-old 5×FAD and 24-month-old C57BL/6 wild-type male mice. Microglial expression of TGFβ1 (A to

D) and phospho-SMAD2 (p-SMAD2) (**E to H**) in the cerebral cortex or the hippocampus was examined by co-immunostaining with CD11b and A β . (**A and B**) Representative images of microglial TGF β 1 expression at low (**A**) and high magnification (**B**) are shown. (**C and D**) Densities of TGF β 1⁺ microglia in the cerebral cortex (**C**) and the hippocampus (**D**) were quantified. n = 4 mice for 24-month-old wild-type, n = 5 mice for 9-month-old *5 $\times$ FAD*, and n = 3 mice for 15-month-old *5 $\times$ FAD*, mean $\pm$ SEM, one-way ANOVA test. (**E and F**) Representative images of microglial p-SMAD2 expression at low (**E**) and high magnification (**F**) are shown. (**G and H**) Densities of p-SMAD2⁺ microglia in the cerebral cortex (**G**) and the hippocampus (**H**) were quantified. n = 4 mice for 24-month-old wild-type, n = 5 mice for 9-month-old *5 $\times$ FAD*, and n = 3 mice for 15-month-old *5 $\times$ FAD*, mean $\pm$ SEM, one-way ANOVA test.



Supplementary Figure 8. Microglial TGFβ1 signal in Alzheimer's disease patients.

(A and B) Microglial expression of TGFβ1 in the cerebral cortex of control donors or Alzheimer's disease patients (information in Supplementary Table 3) was examined by co-immunostaining with IBA1. $n = 8$ patients per condition. Representative images of control donors **(A)** or Alzheimer's disease patients **(B)** at low and high magnification are shown.

Supplementary Table 1. Expression of the components of phagocytosis pathways in mouse or human microglia.

Gene Name	Mouse Gene ID	Expression (FPKM, this study)	Human Gene ID	Expression (nTPM, Human Protein)
<i>Tyro3 / TYRO3</i>	22174	0.12	7301	0.9
<i>Axl / AXL</i>	26362	8.13	558	29.5
<i>Mertk / MERTK</i>	17289	135.32	10461	119.0
<i>Gas6 / GAS6</i>	14456	116.41	2621	39.4
<i>Pros1 / PROS1</i>	19128	121.6	5627	25.9
<i>Mfge8 / MFGE8</i>	17304	24.03	4204	14.3
<i>Itgav / ITGAV</i>	16410	11.02	3685	72.5
<i>Itgb3 / ITGB3</i>	16416	11.27	3690	0.9
<i>Adgrb1 / ADGRB1</i>	107831	0.25	575	8.9
<i>Timd4 / TIMD4</i>	276891	3.54	91937	2.7
<i>Stab2 / STAB2</i>	192188	0.00	55576	0.9

Supplementary Table 2. Information of Alzheimer's disease patients in the analyses of published snRNA-seq datasets.

Patient Group	Patient ID	Gender	Age	Final Consensus Cognitive Score
Mathys et al., Nature 2019 (syn18681734)	R9426782	Male	89	4
	R8608442	Male	88	4
	R7944883	Female	75	4
	R9094222	Female	90+	4
	R2895885	Male	86	4
	R4567280	Female	90+	4
	R8629052	Female	90+	4
	R4379962	Male	90	4
	R2830542	Female	81	4
	R4482444	Female	84	6
	R7770387	Female	83	4
	R4042599	Female	90+	4
	R1538032	Female	84	4
	R5885245	Female	90+	4
	R6176158	Female	88	5
	R9551808	Male	80	4
	R7160627	Female	88	4
	R2081705	Male	86	4
	R8451530	Male	87	4
	R4262244	Male	83	4
	R3884524	Female	90+	4
	R2294544	Male	87	4
	R3900996	Male	86	4
Zhou et al., Nature Medicine 2020 (syn21670836)	R2008064	Female	90+	4
	R4235867	Female	90	5
	R4388056	Male	90+	4
	R5138383	Female	90+	4
	R5394614	Female	90+	4
	R5525186	Male	90+	4
	R5691943	Female	87	4

	R6411801	Male	86	4
	R6528749	Female	86	4
	R8399817	Female	89	4
	R8608442	Male	88	4
	R9255163	Female	83	4
	R9453274	Male	84	4
	R9679238	Male	82	4

Supplementary Table 3. Information of human brain tissues for immunostaining analyses.

Patient Group	Patient ID	Cause of Death
Control Donor	#1	Pneumonia
	#2	Primary nephropathy
	#3	Pneumonia
	#4	Intestinal cancer
	#5	Breast cancer
	#6	Breast cancer
	#7	Cardiac arrest
	#8	Lung cancer
Alzheimer's Disease	#9	Pneumonia
	#10	Pneumonia
	#11	Acute coronary syndrome
	#12	Sepsis
	#13	Pneumonia
	#14	Pneumonia
	#15	Multiple organ failure
	#16	Multiple organ failure