

Expanded View Figures

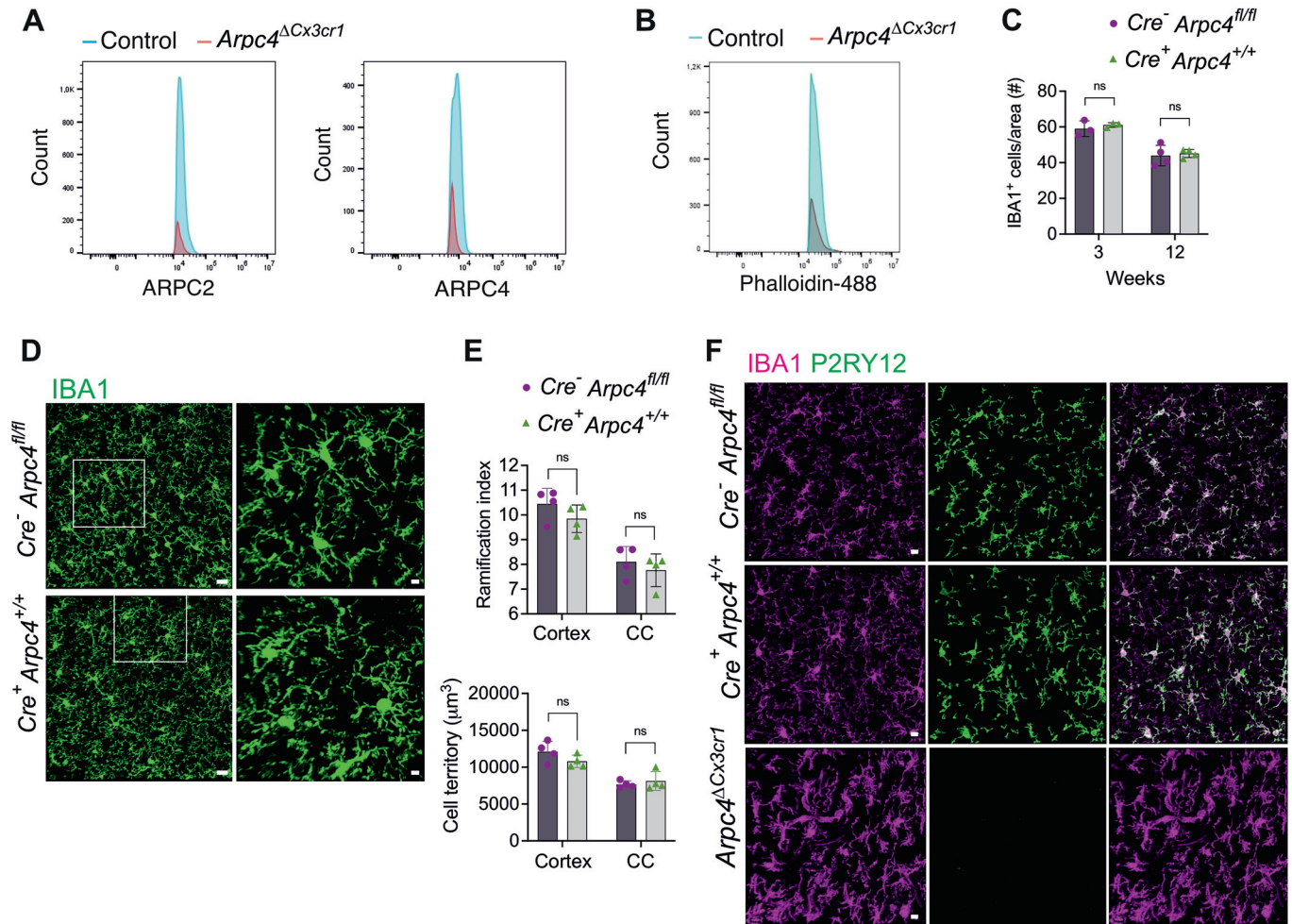


Figure EV1. Microglia characteristics in *Tg(Cx3cr1-Cre)*-expressing and *Tg(Cx3cr1-Cre)*-negative *Arpc4^{+/+}* mice.

(A) Flow cytometric analysis of ARPC2 and ARPC4 expression in 12-week-old *Tg(Cx3cr1-Cre) Arpc4^{fl/fl}* (*Arpc4^{ΔCx3cr1}*) and *Arpc4^{fl/fl}* control mice (Biological replicates: $n = 4$ mice per group, Technical replicates: $n = 3$ experiments). (B) Flow cytometric analysis of Alexa Fluor 488-conjugated phalloidin, indicating global levels of F-actin in microglia from 12-week-old *Arpc4^{ΔCx3cr1}* and control mice (Biological replicates: $n = 4$ mice per group, Technical replicates: $n = 2$ experiments). (C) Quantification of microglial cell number in the cortex of 3- and 12-week-old *Cre⁺ Arpc4^{+/+}* and *Cre⁻ Arpc4^{fl/fl}* mice ($n = 3$ –4 mice per group, data were presented as mean ± s.d., two-tailed Student's t -test, ns: $P > 0.9999$). (D, E) Representative images of IBA1⁺ microglia morphology and quantification of microglia ramification and cell territory in 12-week-old *Tg(Cx3cr1-Cre) Arpc4^{+/+}* (*Cre⁺ Arpc4^{+/+}*) and *Cre⁻ Arpc4^{fl/fl}* mice. Scale bar: 20 μm (left) and 5 μm (right) ($n = 4$ mice per group, data are presented as mean ± s.d., two-tailed Student's t -test, ns not significant). (F) Representative images showing IBA1⁺/P2RY12⁺ microglia in 12-week-old *Cre⁺ Arpc4^{+/+}*, *Cre⁻ Arpc4^{fl/fl}* and *Tg(Cx3cr1-Cre) Arpc4^{fl/fl}* (*Arpc4^{ΔCx3cr1}*) mice, scale bar: 20 μm. Source data are available online for this figure.

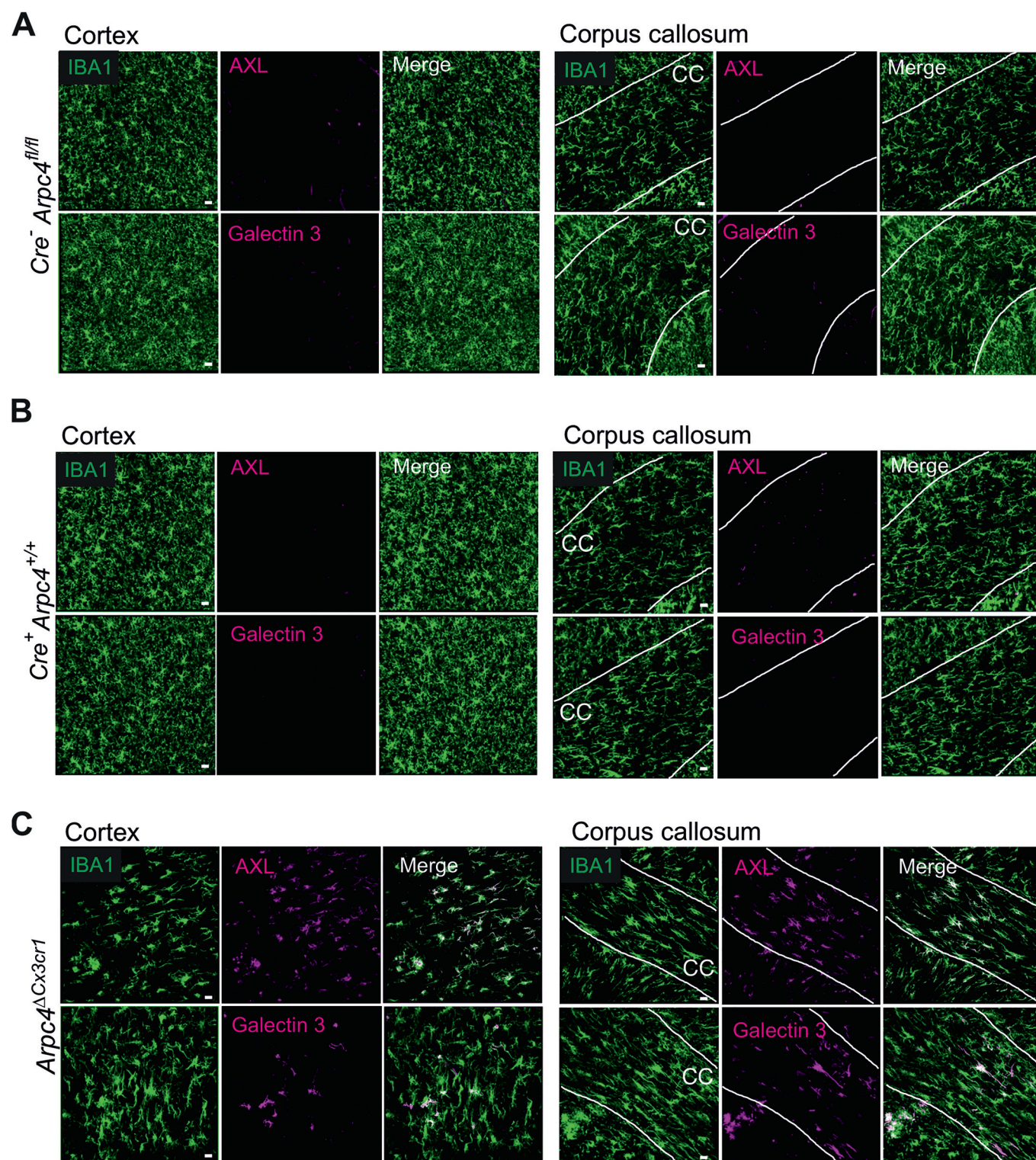
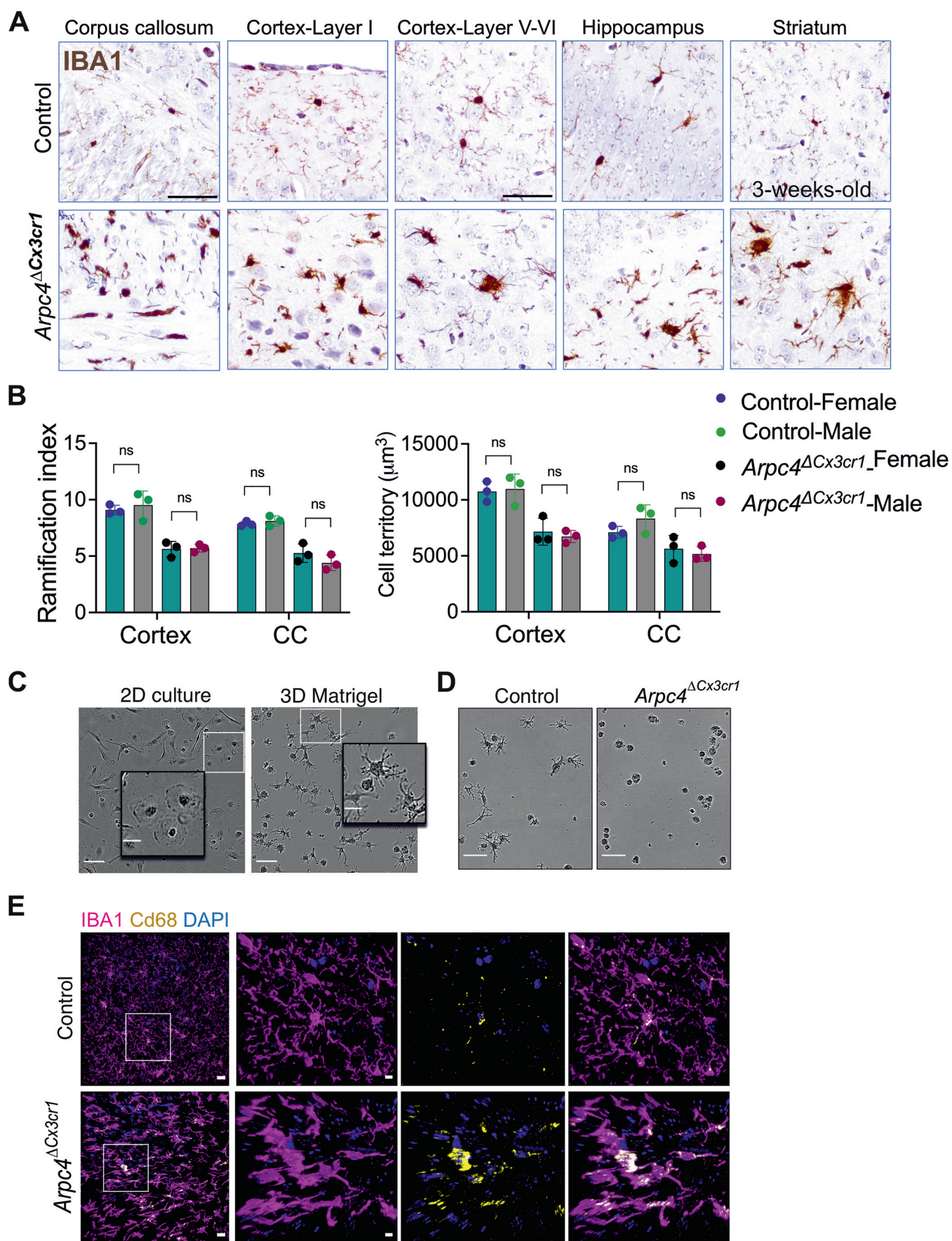


Figure EV2. Microglial activation in *Cre⁺ Arpc4^{+/+}* compared to the control (*Cre⁻ Arpc4^{fl/fl}*) and *Arpc4^{ΔCx3cr1}* mice.

Representative images of AXL and Galectin-3 expression in IBA1⁺ microglia in the cortex and corpus callosum in 12-weeks-old *Cre*-negative *Arpc4^{fl/fl}* (*Cre⁻ Arpc4^{fl/fl}*) (A), *Tg(Cx3cr1-Cre) Arpc4^{+/+}* (*Cre⁺ Arpc4^{+/+}*) (B), and *Tg(Cx3cr1-Cre) Arpc4^{+/+}* (*Arpc4^{ΔCx3cr1}*) (C) mice. Scale bar: 20 μ m. Confocal microscopy overview images come from stitched tile scans of immunostained brain tissues. Source data are available online for this figure.



◀ **Figure EV3. Microglial morphology upon *Arpc4* depletion in vivo and in vitro.**

(A) Representative DAB-stained images of microglial morphology in different brain regions in 3-week-old *Arpc4*^{ΔCx3cr1} and control mice. Scale bar: 100 μm. (B) Quantification of microglia ramification and cell territory in 12-week-old female and male *Arpc4*^{fl/fl} (control) and *Tg(Cx3cr1-Cre) Arpc4*^{fl/fl} (*Arpc4*^{ΔCx3cr1}) mice (*n* = 3 mice per group, data are presented as mean ± s.d., two-tailed Student's *t*-test, ns: *P* > 0.9999). (C) Comparison of wild-type microglia morphology in 2D and Matrigel 3D culture systems. Scale bar: 50 μm. (D) *Arpc4*^{ΔCx3cr1} and control microglia in the Matrigel 3D culture after 10 h. Scale bar: 50 μm. (E) Representative images of CD68 immunolabeling of IBA1⁺ microglia in 3-week-old *Arpc4*^{ΔCx3cr1} and control mice. Scale bar: 20 μm (overview), 7 μm (zoom-in). Source data are available online for this figure.

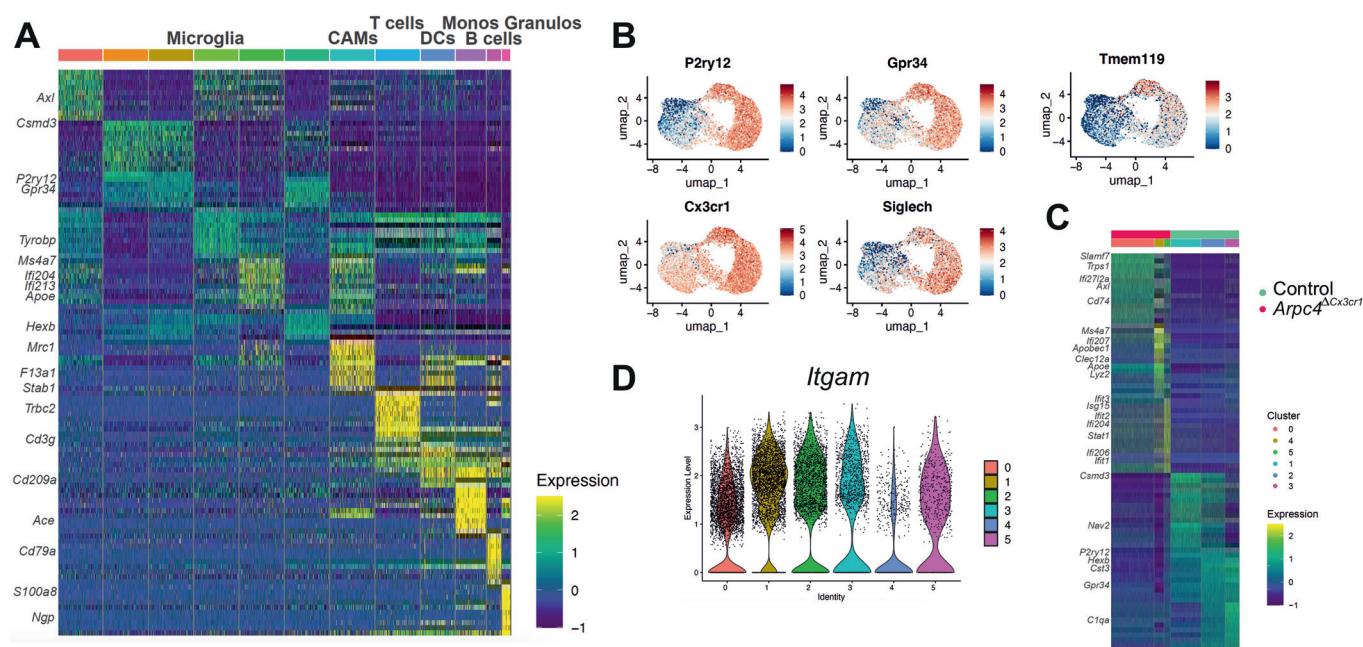


Figure EV4. Downregulation of TGF β -dependent microglial homeostatic signature, their reactive phenotype in *Arpc4* Δ *Cx3cr1* mice.

(A) Heat map of genes (rows) of all the cell types that are highlighted in Fig. 3A. B. Key genes are highlighted. Colors in the heat map correspond to normalized, scaled expression. (B) Expression levels of microglial homeostatic genes, *P2ry12*, *Gpr34*, *Cx3cr1*, *Siglech*, and *Tmem119* in microglia clusters. (C) Heat map of genes (rows) of microglia clusters that are highlighted in Fig. 3C. Key genes are highlighted. Colors in the heat map correspond to normalized, scaled expression. (D) Violin plots showing the expression level of *Itgam* in the microglial clusters in *Arpc4* Δ *Cx3cr1* and control mice ($n = 4$ control and 3 *Arpc4* Δ *Cx3cr1* mice).

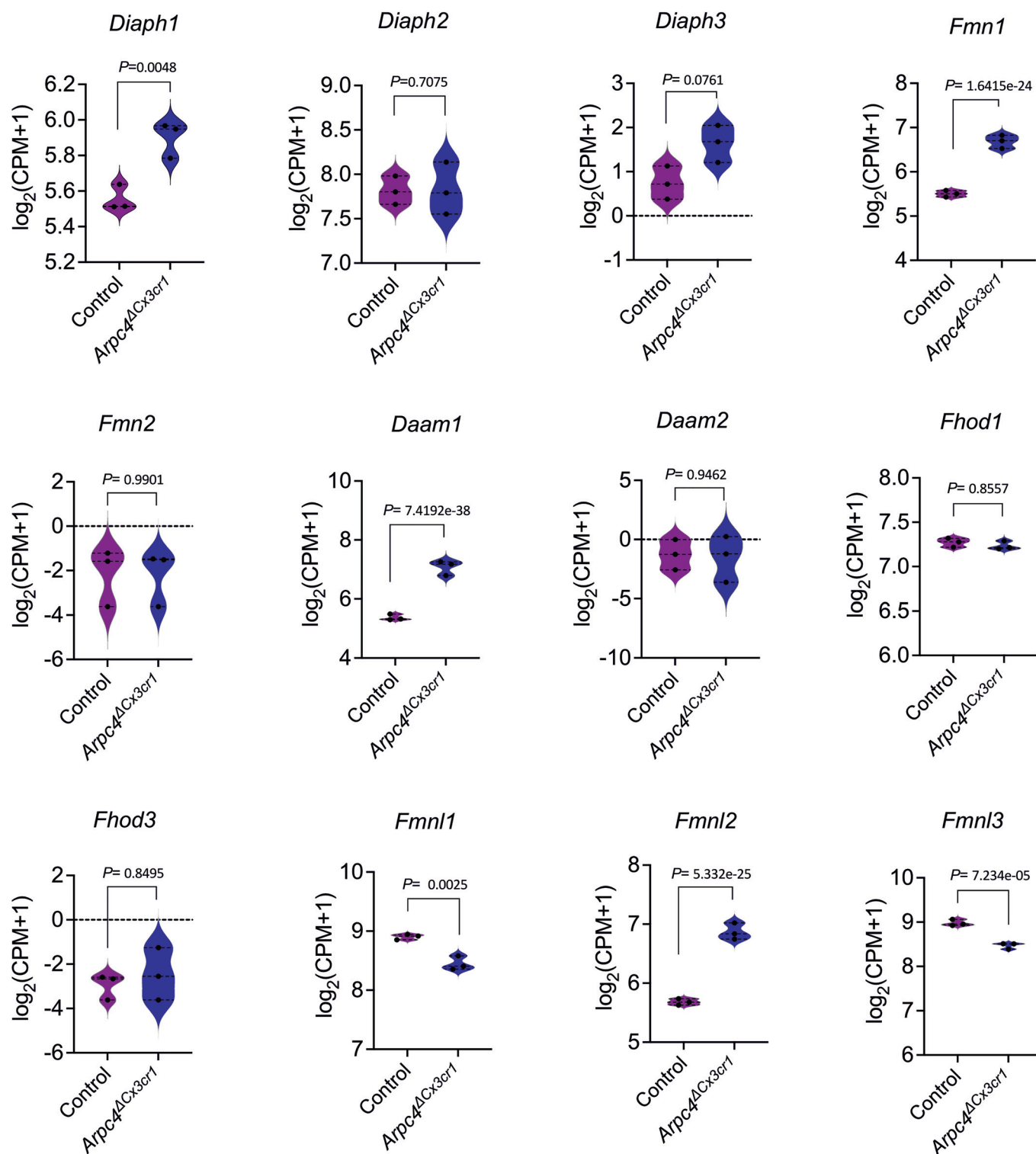


Figure EV5. Expression levels of formin/diaphanous family of proteins.

Violin plots showing gene expression levels of formin/diaphanous family of proteins in microglia isolated from *Arpc4*^{ΔCx3cr1} and control mice (n = 4 control and 3 *Arpc4*^{ΔCx3cr1} mice). Data were retrieved from bulk RNA-seq data sets from CNS-isolated microglia, analyzed in Fig. 3C-F. CPM counts per million.