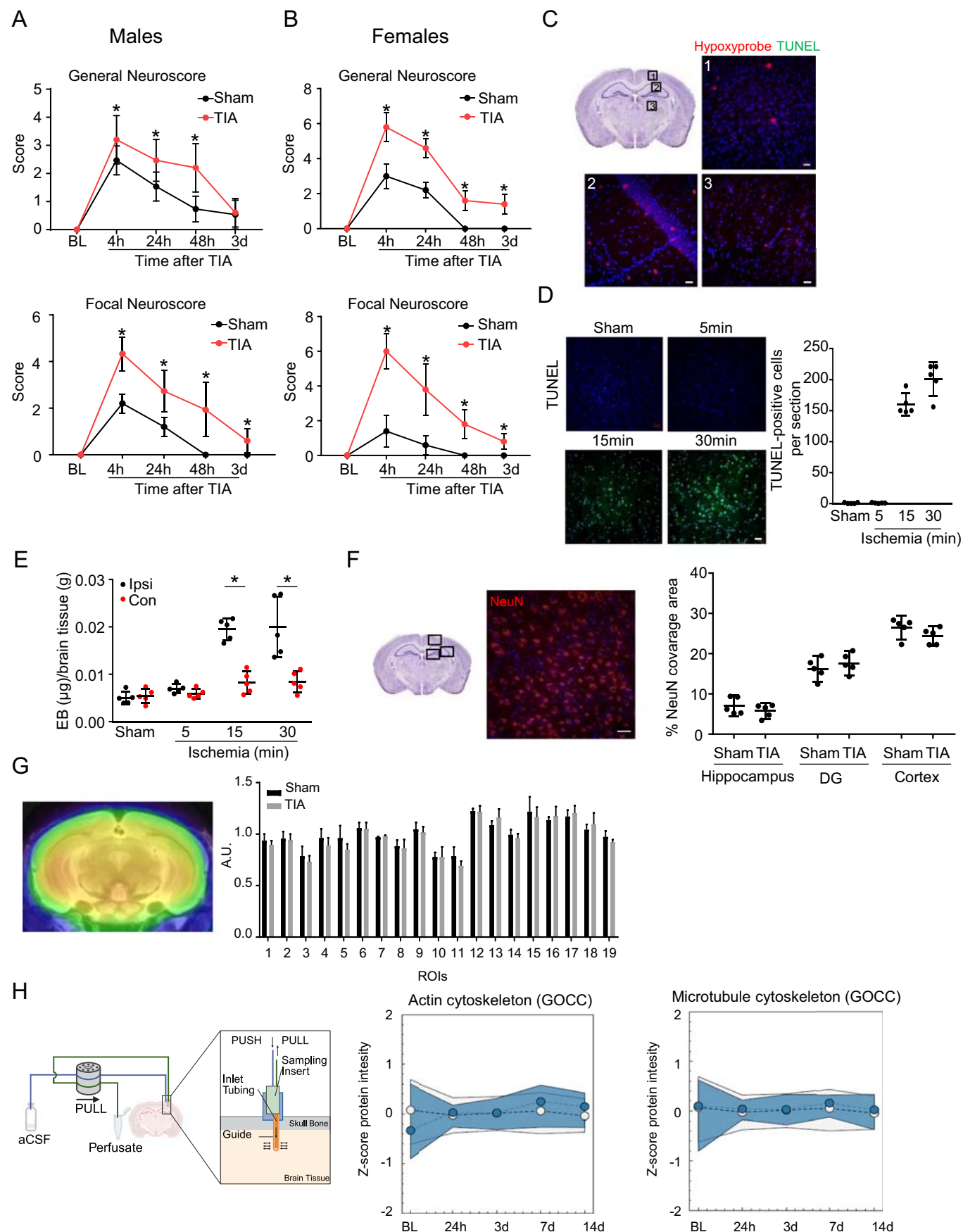


Expanded View Figures

Figure EV1. Characterization of the TIA model.

General and focal Neuroscore at different time points after TIA in (A) males ($n = 15$ per group; General Neuroscore: 4 h: p value = 0.0004, 24 and 48 h: p value <0.0001; Focal Neuroscore: 4, 24, and 48 h: p value <0.0001, 3 d: p value = 0.0012) and (B) females ($n = 5$ per group; General Neuroscore: 4, 24, 48 h and 3 d: p value <0.0001; Focal Neuroscore: 4, 24 h: p value <0.0001, 48 h: p value = 0.0002, 3 d: p value = 0.045). (C) Representative image for brain hypoxia (hypoxyprom in red) and cell death (TUNEL⁺ in green) 24 h after TIA in males in cortex (1), hippocampus (2) and striatum (3), scale bar = 20 μ m. (D) Representative TUNEL⁺ staining and quantification 24 h after different MCA occlusion times in males ($n = 5$ per group), scale bar = 20 μ m. (E) Blood-brain barrier integrity was measured by extravasated Evans Blue per gram of the brain tissue 24 h after different ischemia times in males. Black = ipsilateral hemisphere, red = contralateral hemisphere ($n = 5$ per group; 15 and 30 min ischemia: p value = 0.0079). (F) Representative image and analysis of neural cells (NeuN⁺) in different brain regions in males (DG dentate gyrus), scale bar = 20 μ m. (G) Representative image and analysis of glucose metabolism using in vivo positron-emission tomography 24 h after TIA or Sham males ($n = 6$ per group). 1-Right striatum, 2-Left striatum, 3-Cortex, 4-Right hippocampus, 5-Left hippocampus, 6-Thalamus, 7-Cerebellum, 8-Basal forebrain/septum, 9-Hypothalamus, 10-Right amygdala, 11-Left amygdala, 12-Brainstem, 13-Central gray, 14-Superior colliculi, 15-Olfactory bulb, 16-Midbrain right, 17-Midbrain left, 18-Inferior colliculi left, 19-Inferior colliculi right. (H) Representative scheme of the microperfusion system and protein intensity over time before (BL) and after TIA in males ($n = 5$ per group). Statistical tests: (A, B, E, F) two-way ANOVA, corrected for multiple comparisons using two-stage step-up method of Benjamin Krieger. (H) Two-way ANOVA, Fisher's exact testing. Error bars indicate $\pm$ SD. * $p < 0.05$. Source data are available online for this figure.



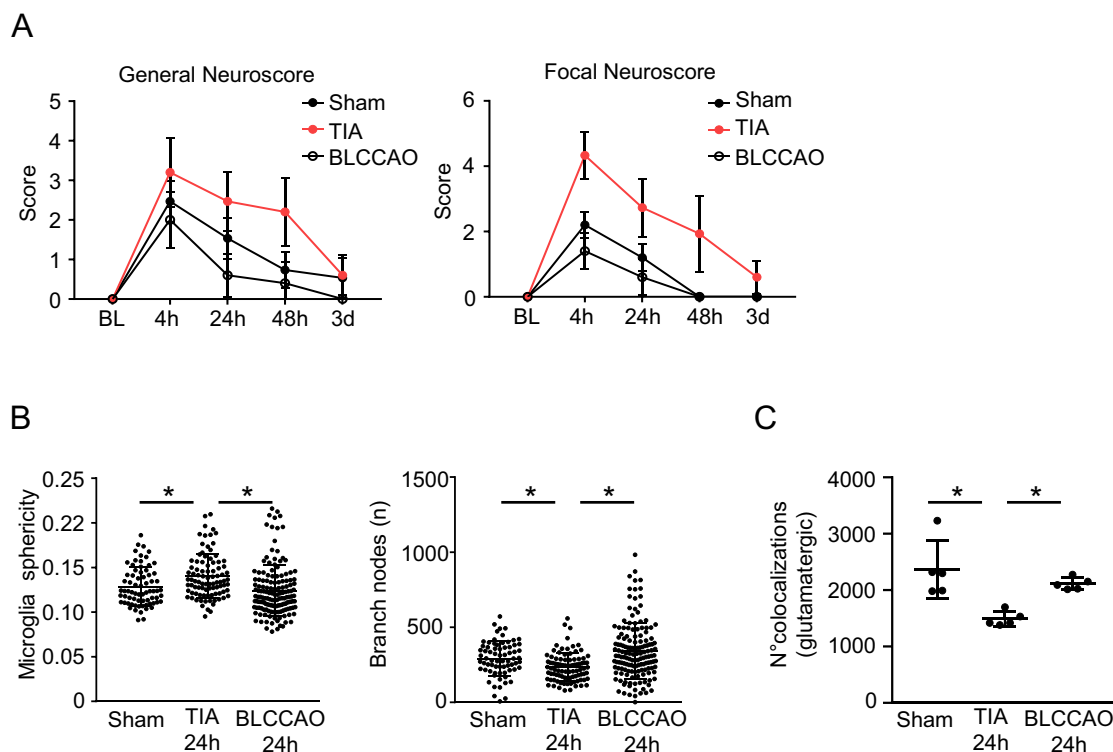


Figure EV2. TIA vs BLCCAO.

(A) General and focal Neuroscore at different time points after TIA, Sham and BLCCAO male animals ($n = 5$ per group). (B) Microglia morphology analysis (sphericity and branch nodes) at different time points 24 h after TIA, Sham and BLCCAO male animals ($n = 5$ per group; Microglia sphericity: TIA: p value = 0.0123 and BLCCAO: p value < 0.0001, Branch nodes: TIA: p value = 0.0022 and BLCCAO: p value < 0.0001). (C) Quantification of colocalized glutamatergic presynaptic and postsynaptic particles 24 h after TIA, Sham, and BLCCAO male animals ($n = 5$ per group; TIA: p value = 0.0140 and BLCCAO: p value = 0.0400). Statistical tests: (A–C) two-way ANOVA, corrected for multiple comparisons using two-stage step-up method of Benjamin Krieger. Error bars indicate $\pm$ SD. * $p < 0.05$.

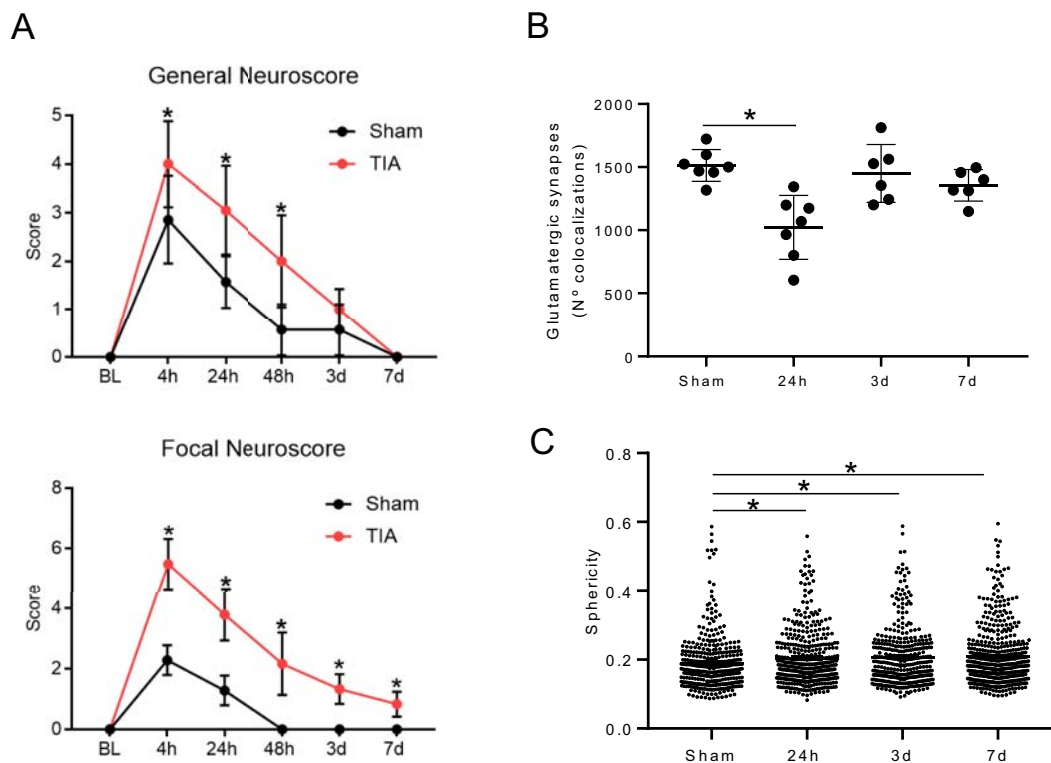


Figure EV3. TIA in aging.

(A) General and focal Neuroscore at different time points after TIA in male aging animals (20 months) (Sham: $n = 7$; TIA: BL-24 h $n = 19$, 48 h-3d $n = 12$, 7 d $n = 6$; General neuroscore: 4 h: p value = 0.0002, 24 and 48 h: p value < 0.0001; Focal Neuroscore: 4, 24, and 48 h: p value < 0.0001, 3 d: p value = 0.0034). (B) Quantification of colocalized glutamatergic presynaptic and postsynaptic particles at different time points after TIA in male aging animals (20 months) (Sham: $n = 7$; TIA: 24 h $n = 7$, 3d-7d $n = 6$; 24 h: p value = 0.0003). (C) Microglia morphology analysis (sphericity) at different time points after TIA in male aging animals (20 months) (Sham: $n = 7$; TIA: 24 h $n = 7$, 3d-7d $n = 6$; 24 h: p value = 0.0083, 3 d: p value = 0.0027, 7 d: p value = 0.0011). Statistical tests: (A-C) two-way ANOVA, corrected for multiple comparisons using two-stage step-up method of Benjamin Krieger. Error bars indicate $\pm$ SD. * p < 0.05.

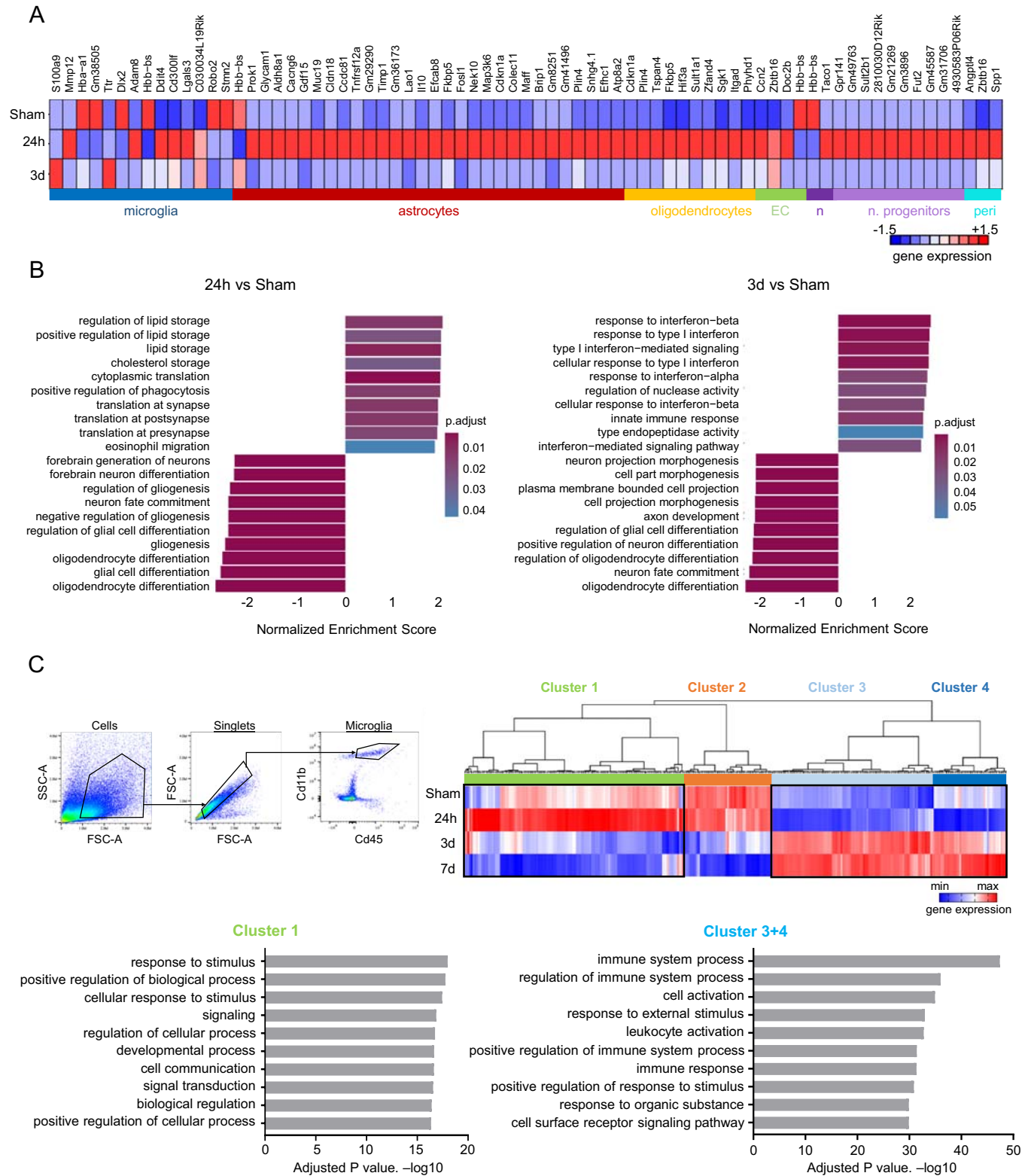


Figure EV4. The impact of TIA on microglia transcriptome.

(A) Heat map of normalized and scaled (z-score) gene expression of significantly differentially up- and down-regulated genes in each cell type at 24 h and 3 d after TIA compared to Sham in males ($n = 3$ per group). (B) Gene set enrichment analyses (Biological Processes) of differentially regulated genes in microglial cells at 24 h and 3 d compared to Sham in males ($n = 3$ per group). (C) Representative FACS plot sort strategy for microglia-like cells isolation, cluster heat map showing ANOVA+ genes from Nanostring analysis from isolated microglia-like cells at different time points after TIA and pathway analysis from selected cluster 1 ($n = 3$ per group) and cluster 3 + 4 ($n = 3$ per group) in males. Statistical tests: (A) Wilcoxon rank sum test + bonferroni correction. (B) Genes ranked by log2 fold change, permutation-based GSEA, Benjamini-Hochberg correction, (C) Data were analyzed by ROSALIND® (<https://rosalind.bio/>). Significantly regulated genes were identified by ANOVA multiple sample testing ($S0 = 0.1$, permutation-based FDR = 0.05)

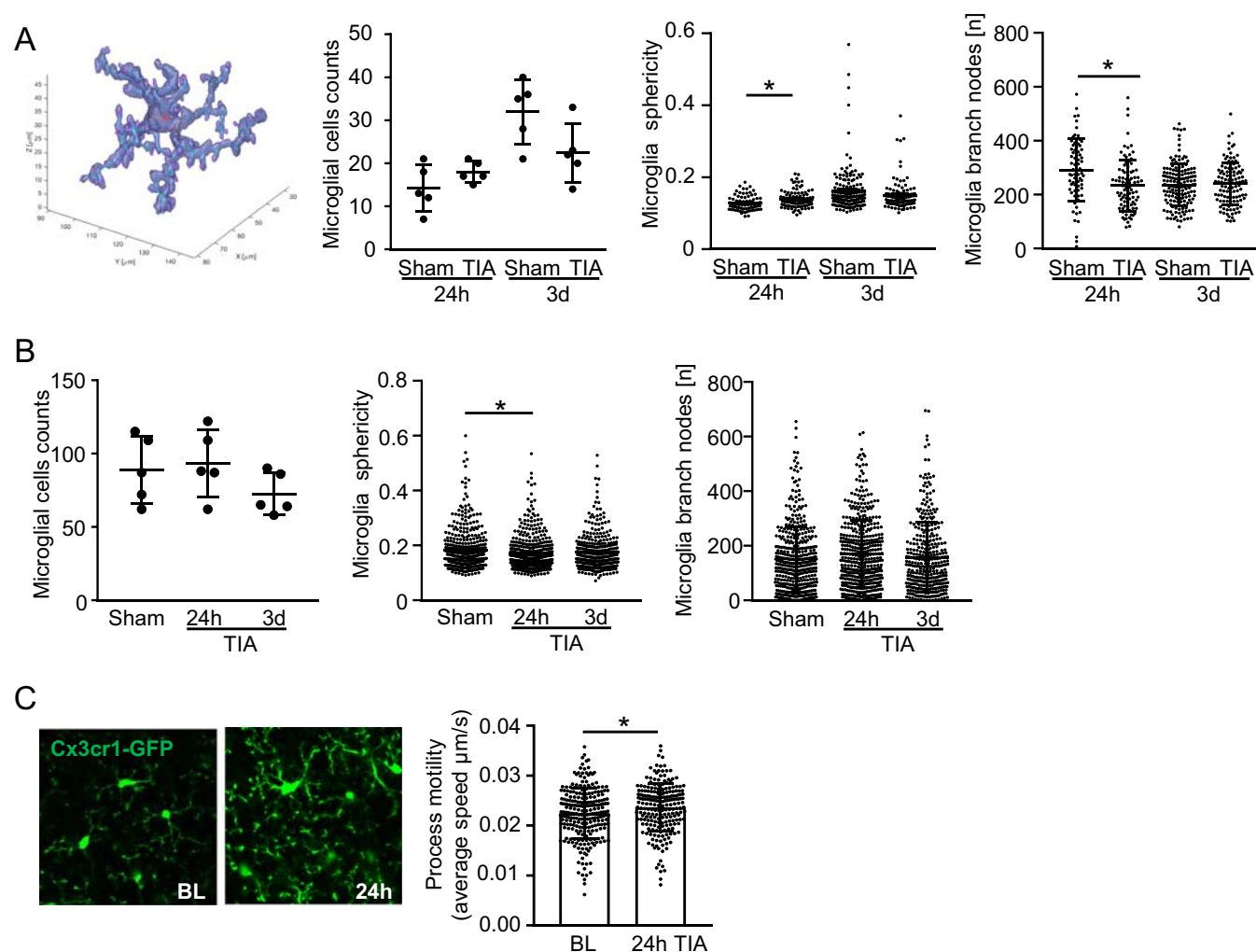


Figure EV5. The impact of TIA on microglia morphology.

(A) Representative image of 3D reconstructed microglia for microglia cells counts and microglia morphology analysis for two representative features: sphericity and branches nodes at 24 h after TIA ($n = 5$ per group; sphericity 24 h:p value = 0.019; branch nodes 24 h:p value = 0.022) in males and (B) females ($n = 5$ per group; sphericity 24 h:p value = 0.0032). (C) Representative images of in vivo two-photon imaging of the microglia process motility and quantification at baseline (BL) and 24 h after TIA in males ($n = 8$ per group; 24 h:p value = 0.025). Statistical tests: (A, C) two-way ANOVA, corrected for multiple comparisons using two-stage step-up method of Benjamin Kriegl. (B) two-way Student's *t*-test. Error bars are mean $\pm$ SD. * $P < 0.05$. Source data are available online for this figure.

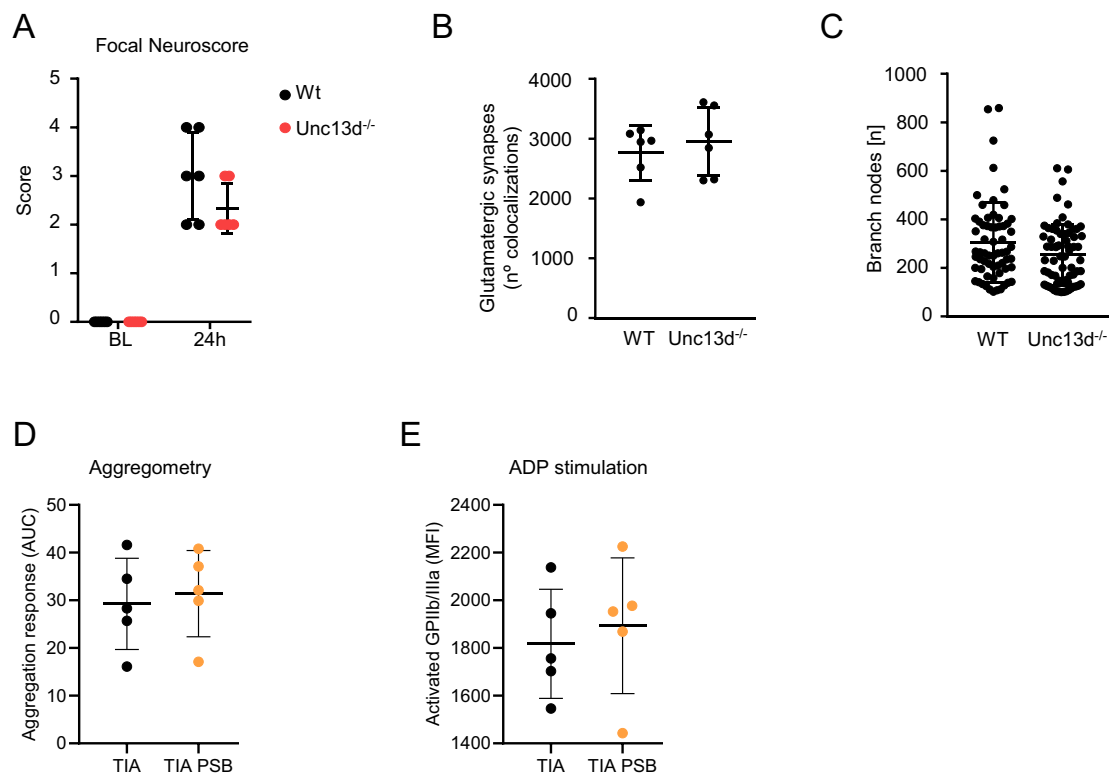


Figure EV6. Platelets are not critical after a TIA.

(A) Focal neuroscore, (B) glutamatergic synapses, and (C) microglia branch nodes analysis of Unc13KO and littermates wild-type (WT) animals 24 h after TIA in males. (D) Platelet aggregation and (E) activation in TIA in vehicle (Veh) or P2Y₁₂Ri (-inhibitor) treated male animals ($n = 5$ per group). Statistical tests: (A–E) two-way Student's t -test. Error bars are mean $\pm$ SD.