

Supplemental Figures S1-S6

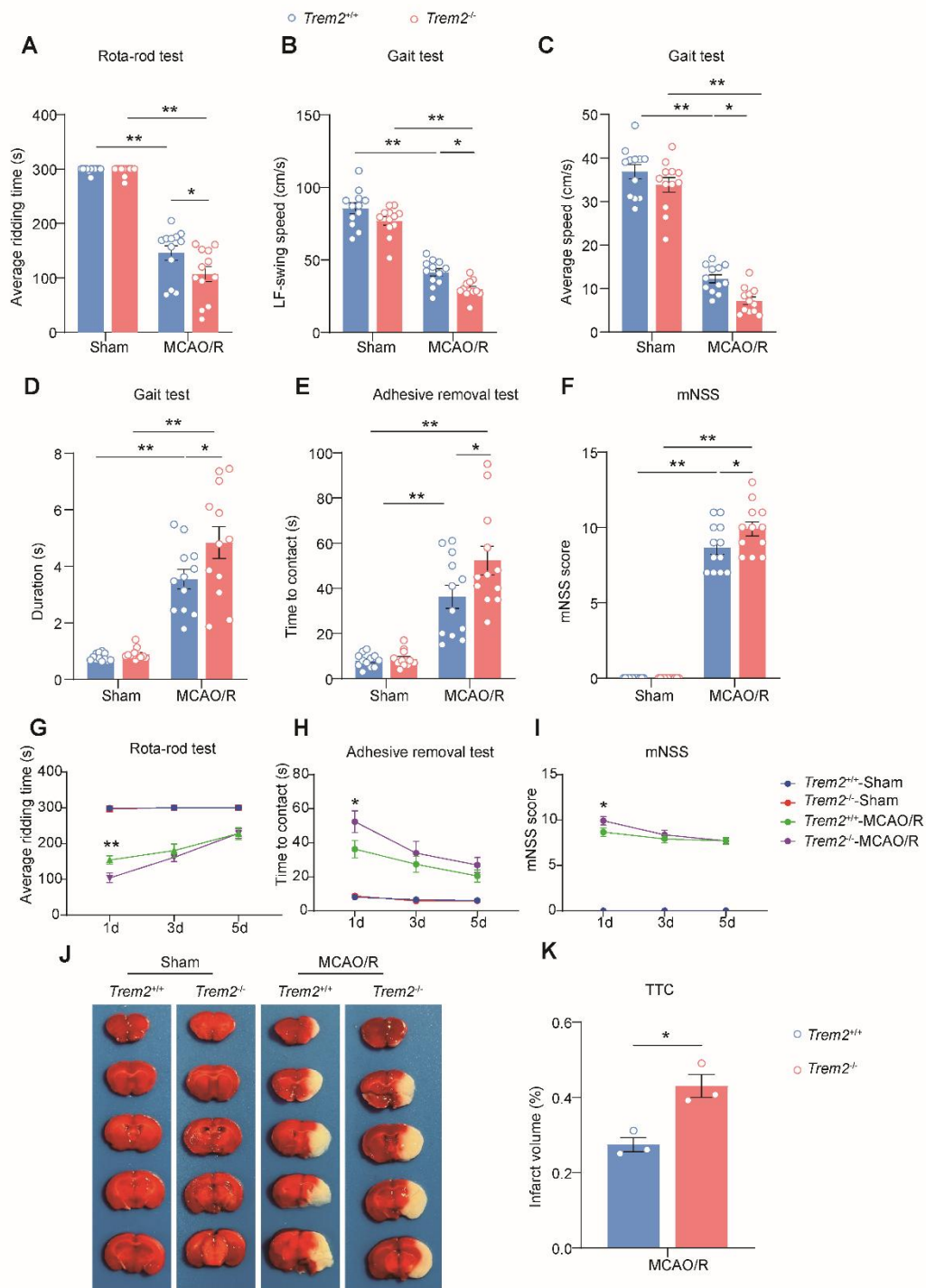


Figure S1. TREM2 deficiency aggravates brain injury in MCAO/R mice, related to Figure 1.

(A-F) Behavioral analyses of *Trem2*^{-/-} and *Trem2*^{+/+} littermate mice starting 24 hours after MCAO/R. The average riding time on the rota-rod was assessed in the rota-rod test (A); the left foot swing (LF-swing) speed (B), the average speed (C), and the duration for completing gait tasks (D) were assessed in the gait test; the time to contact the adhesive was assessed in the adhesive removal test (E); and the modified neurological severity score (mNSS) was evaluated (F). *n* = 12 male mice per group.

(G-I) Treated mice were assessed for neurological functions at 1, 3, and 5 days after MCAO/R using the rota-rod test (G), the adhesive removal test (H), and the mNSS evaluation (I). *n* = 12 male mice per group.

(J) Representative TTC staining images of coronal brain sections of the indicated groups.

(K) Quantitative analysis of the infarct volumes in (J). *n* = 3 male mice per group.

Data are presented as the mean ± SEM. *P* values were determined by two-way ANOVA followed by Sidak's *post hoc* analysis in (A), (B), (C), (D), (E), (F), (G), (H), and (I); and two-tailed unpaired Student's *t*-test in (K). **p* < 0.05; ***p* < 0.01.

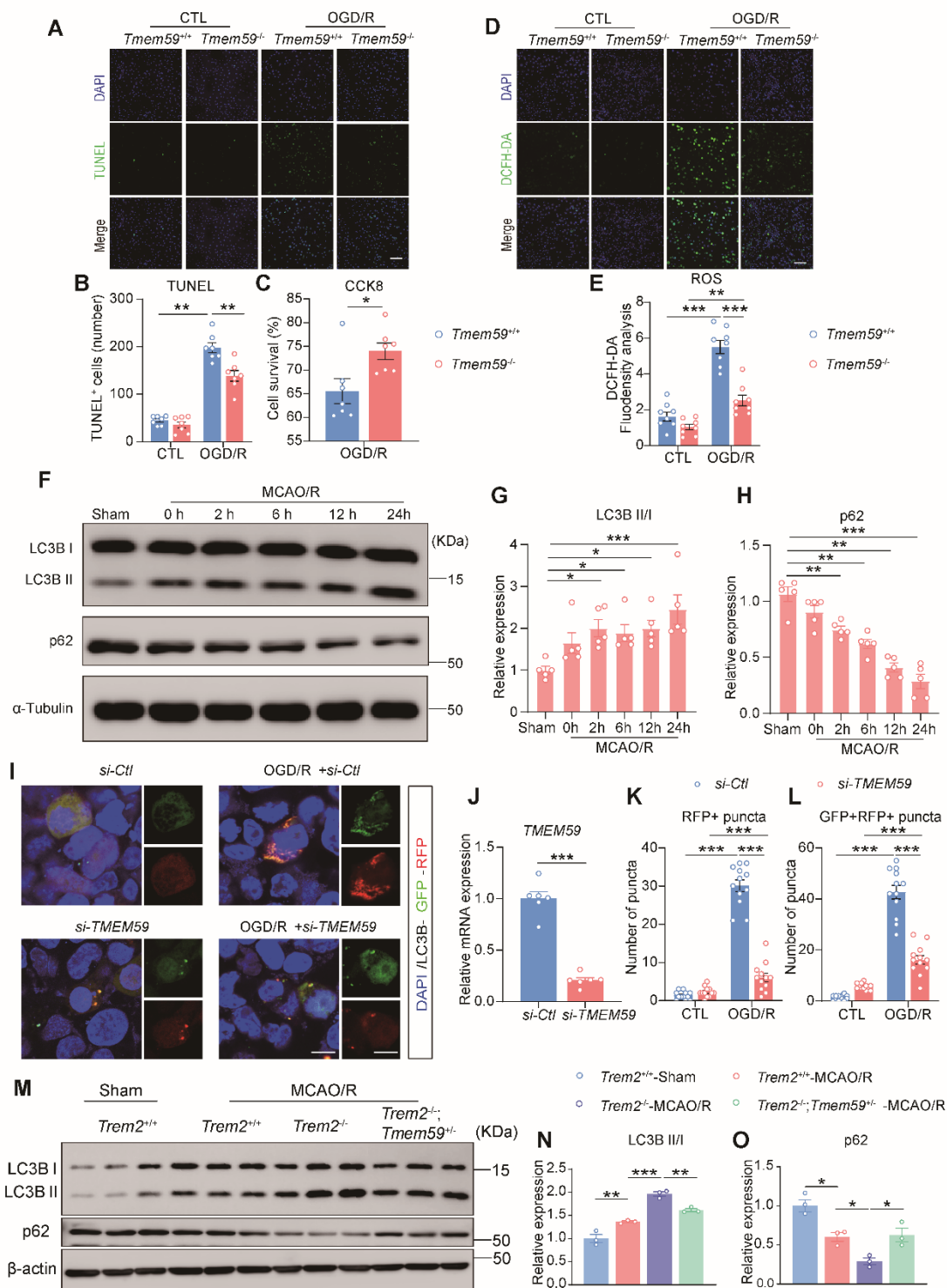


Figure S2. TMEM59 deletion mitigates microglial apoptosis and oxidative stress triggered by OGD/R, related to Figure 4.

(A) Representative images of TUNEL staining (green) in microglia of the indicated groups. DAPI (blue) was co-stained. Scale bar, 100 μ m.

(B) Quantitative analysis of the number of TUNEL-stained cells in (A). $n = 7$ samples per group.

(C) The viability of OGD/R-treated microglia was assessed using the CCK-8 assay. $n = 7$ samples per group.

(D) Representative images of DCFH-DA staining (green) in microglia of the indicated groups. DAPI (blue) was co-stained. Scale bar, 100 μ m.

(E) Quantitative analysis of the fluorescence intensity of DCFH-DA in (D). $n = 8$ samples per group.

(F) Representative immunoblotting of LC3B (I and II) and p62 in the brains of wild-type (WT) mice subjected to MCAO/R for the indicated time points. α -Tubulin was used as a loading control.

(G) Quantitative analysis of the ratio of LC3B II/I in (F). $n = 5$ male mice per group.

(H) Quantitative analysis of the levels of p62 in (F). $n = 5$ male mice per group.

(I) Representative images of mRFP-GFP-LC3 in HEK293T cell subjected to TMEM59 knockdown (si-*TMEM59*) and OGD/R. DAPI (blue) was co-stained. Scale bar, 10 μ m.

(J) Quantitative real-time PCR (qRT-PCR) analysis of *TMEM59* in HEK293T treated with si-*TMEM59* or si-*Ctl* in (I). $n = 6$ samples per group.

(K) Quantitative comparison of the RFP⁺ puncta in (I). $n = 12$ ROI from 4 slides per group.

(L) Quantitative comparison of the GFP⁺RFP⁺ puncta in (I). $n = 12$ ROI from 4 slides per group.

(M) Representative immunoblotting of LC3B (I and II) and p62 in the brains of the indicated groups. β -actin was used as a loading control.

(N) Quantitative analysis of the ratio of LC3B II/I in (K). $n = 3$ male mice per group.

(O) Quantitative analysis of the levels of p62 in (K). $n = 3$ male mice per group.

Data are presented as the mean $\pm$ SEM. P values were determined by two-way ANOVA followed by Sidak's *post hoc* analysis in (B), (E), (K), and (L); two-tailed unpaired Student's *t*-test in (C) and (J); and one-way ANOVA followed by Dunnett's *post hoc* analysis in (G), (H), (N), and (O). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

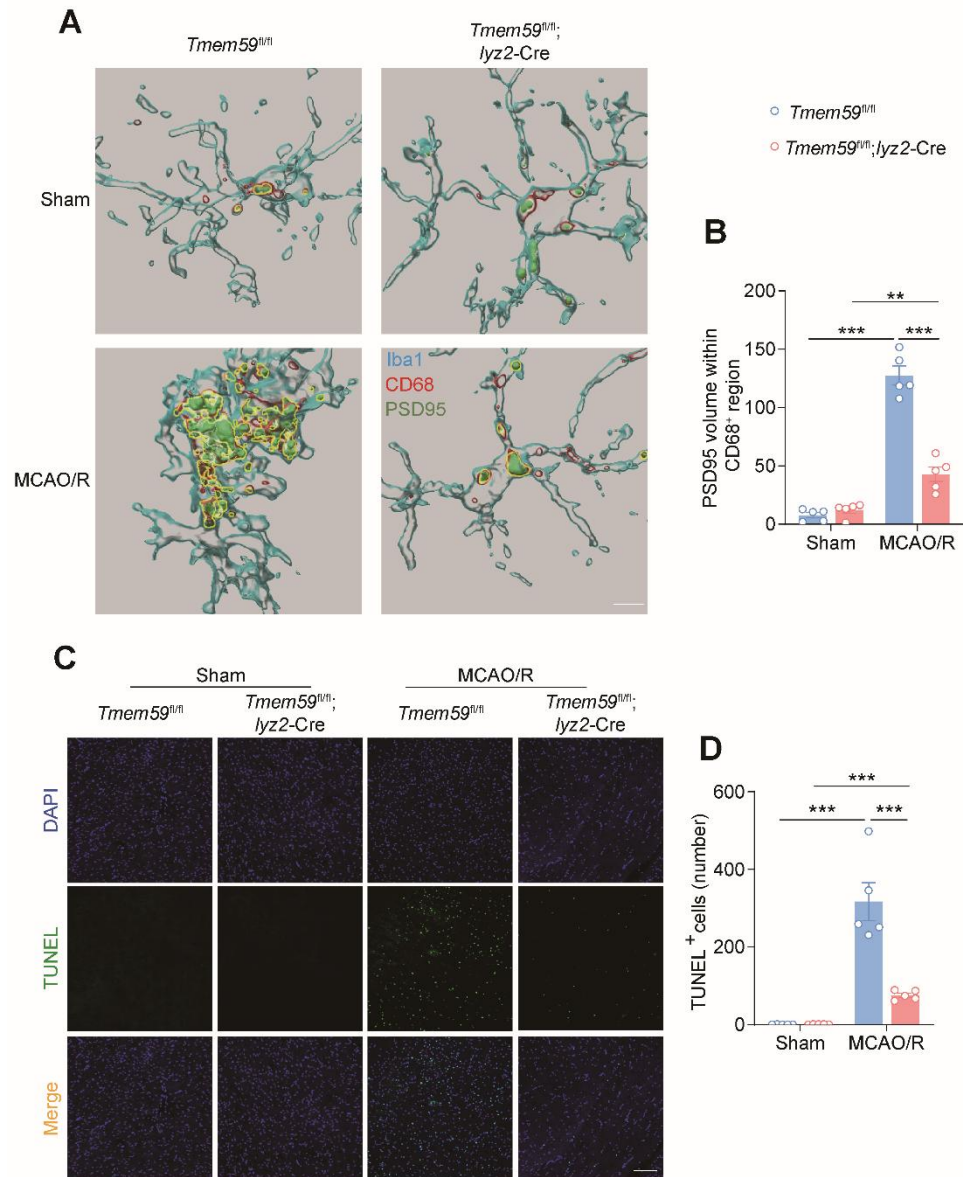


Figure S3. Microglia-specific TMEM59 deletion attenuates apoptosis in MCAO/R mice, related to Figure 5.

(A) Three-dimensional rendering of confocal stacks of microglia and engulfed PSD95 puncta (detected within microglial CD68⁺ structures) of treated mice. Scale bar, 5 μ m.

(B) Quantitative analysis of PSD95 engulfment within microglial CD68⁺ structures in

(A). $n = 5$ male mice per group (3 cells were averaged per mouse).

(C) Representative images of TUNEL staining (green) in the cerebral cortex of the indicated groups. DAPI (blue) was co-stained. Scale bar, 100 μm .

(D) Quantitative analysis of the number of TUNEL-positive cells in (A). $n = 5$ male mice per group.

Data are presented as the mean $\pm$ SEM. P values were determined by two-way ANOVA followed by Sidak's *post hoc* analysis. $**p < 0.01$; $***p < 0.001$.

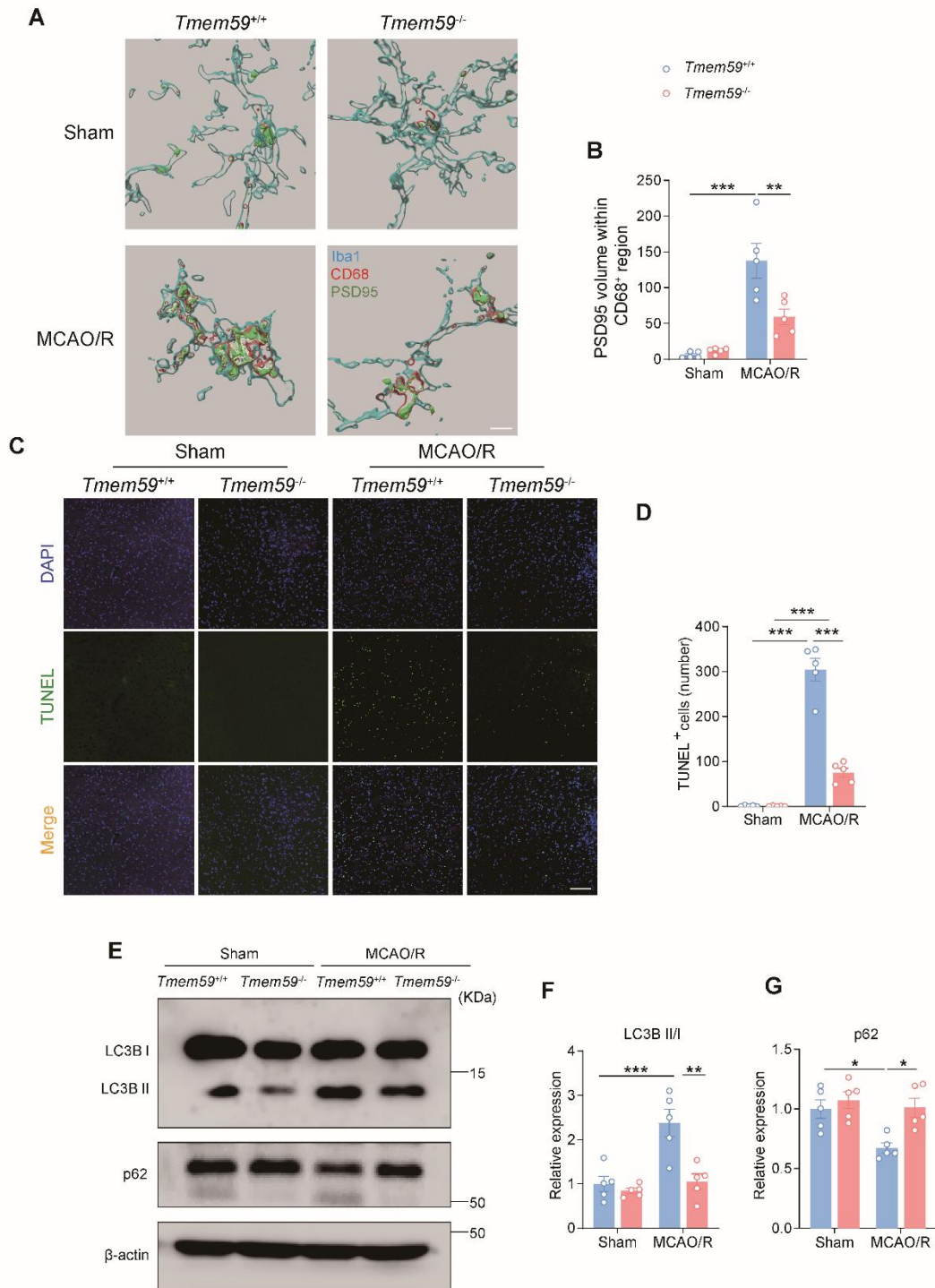


Figure S4. Systemic deletion of TMEM59 attenuates apoptosis and autophagy in MCAO/R mice, related to Figure 6.

(A) Three-dimensional rendering of confocal stacks of microglia and engulfed PSD95

puncta (detected within microglial CD68⁺ structures) of treated mice. Scale bar, 5 μ m.

(B) Quantitative analysis of PSD95 engulfment within microglial CD68⁺ structures in

(A). $n = 5$ male mice per group (3 cells were averaged per mouse).

(C) Representative images of TUNEL staining (green) in the cerebral cortex of the indicated groups. DAPI (blue) was co-stained. Scale bar, 100 μ m.

(D) Quantitative analysis of the number of TUNEL-positive cells in (C). $n = 5$ male mice per group.

(E) Representative immunoblotting of LC3B (I and II) and p62 in the brains of the indicated groups. β -actin was used as a loading control.

(F) Quantitative analysis of the ratio of LC3B II/I in (E). $n = 5$ male mice per group.

(G) Quantitative analysis of the levels of p62 in (E). $n = 5$ male mice per group.

Data are presented as the mean $\pm$ SEM. P values were determined by two-way ANOVA followed by Sidak's *post hoc* analysis. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

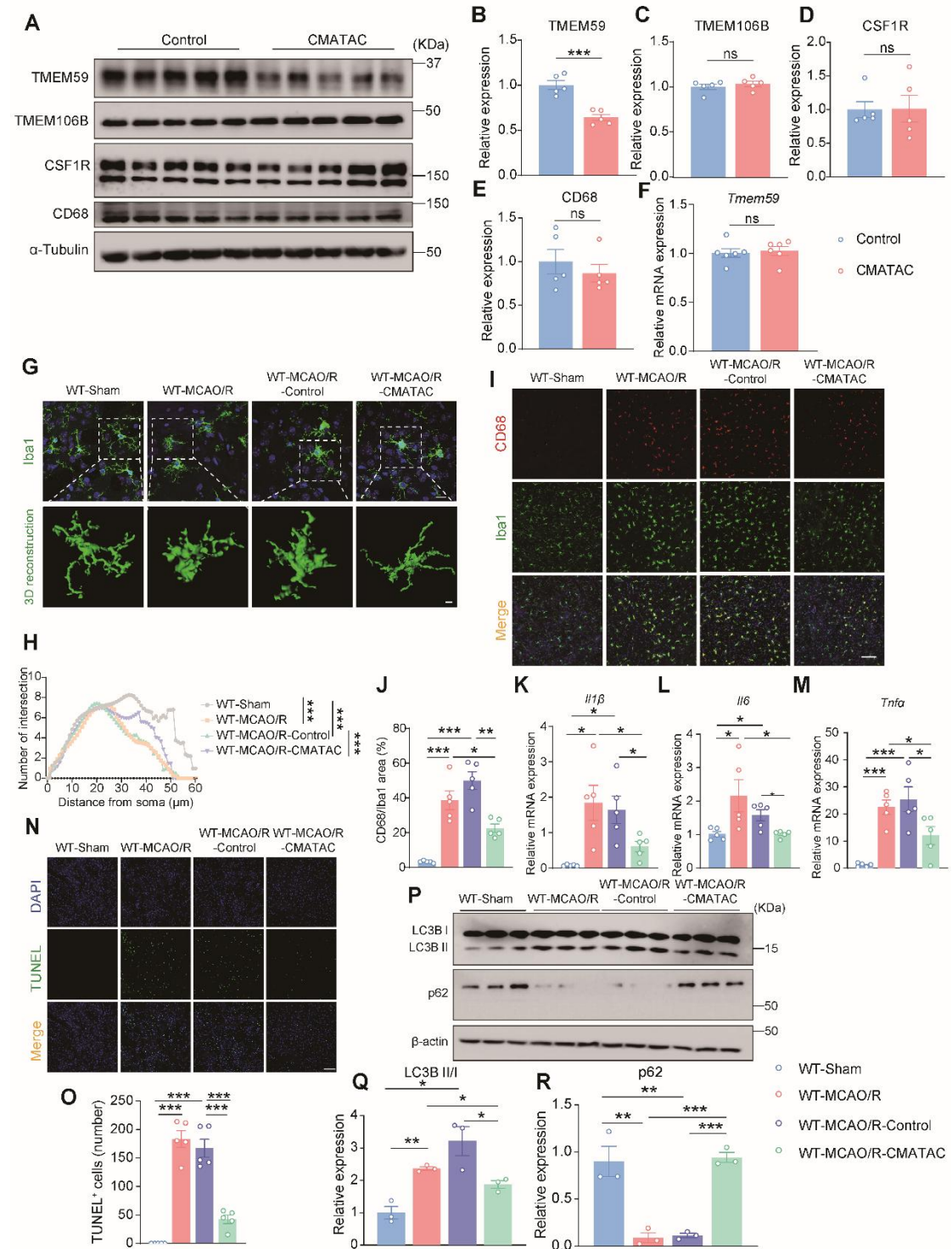


Figure S5. TMEM59-targeting CMATAC peptide attenuates apoptosis and autophagy in MCAO/R mice, related to Figure 7.

(A) Representative immunoblotting of indicated proteins in wild-type (WT) mouse

primary microglia following CMATAC or control peptide treatment. α -Tubulin was used as a loading control.

(B-E) Quantitative analysis of the levels of proteins in (A). $n = 5$ samples per group.

(F) Quantitative real-time PCR (qRT-PCR) analysis of *Tmem59* mRNA expression in WT mouse primary following CMATAC or control peptide treatment. $n = 6$ samples per group.

(G) Representative immunofluorescence images of Iba1 (green) in the cerebral cortex of the indicated groups. DAPI (blue) was co-stained. Scale bar, 20 μ m. Three-dimensional visualization was performed using Imaris software. Scale bar, 5 μ m.

(H) Sholl analysis of microglia in (G). $n = 10$ samples from 5 male mice per group.

(I) Representative immunofluorescence images of CD68 (red) and Iba1 (green) in the cerebral cortex of the indicated groups. DAPI (blue) was co-stained. Scale bar, 100 μ m.

(J) Quantitative analysis of the percentage CD68 area/Iba1 area in (I). $n = 5$ male mice per group.

(K-M) qRT-PCR analysis of pro-inflammatory cytokines *Il1 β* (K), *Il6* (L), and *Tnf α* (M) in the cerebral cortex of mice at 24 h after MCAO/R. $n = 5$ male mice per group.

(N) Representative images of TUNEL staining (green) in the cerebral cortex of the indicated groups. DAPI (blue) was co-stained. Scale bar, 100 μ m.

(O) Quantitative analysis of the number of TUNEL-positive cells in (N). $n = 5$ male

mice per group.

(P) Representative immunoblotting of LC3B (I and II) and p62 in the cerebral cortex of the indicated groups. β -actin was used as a loading control.

(Q) Quantitative analysis of the ratios of LC3B II/I in (P). $n = 3$ male mice per group.

(R) Quantitative analysis of the levels of p62 in (P). $n = 3$ male mice per group.

Data are presented as the mean $\pm$ SEM. P values were determined by two-tailed unpaired Student's t -test in (B), (C), (D), (E), and (F); and repeated-measures ANOVA followed by Bonferroni-adjusted *post hoc* analysis in (H); and one-way ANOVA followed by Dunnett's *post hoc* analysis in (J), (K), (L), (M), (O), (Q), and (R). ns, not significant; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

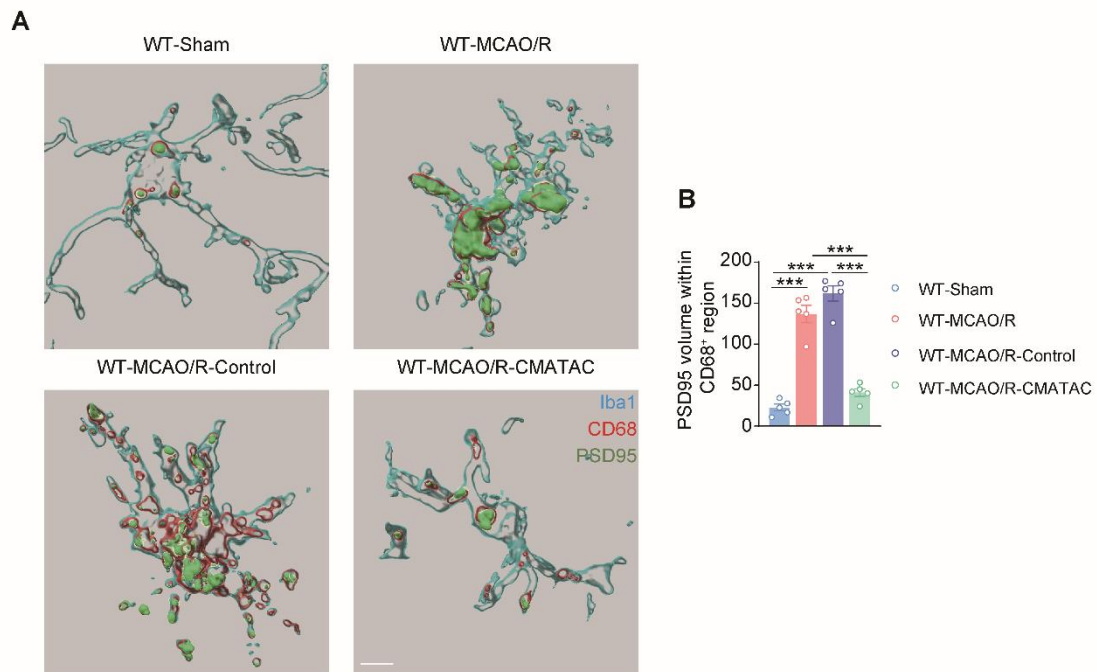


Figure S6. TMEM59-targeting CMATAC peptide attenuates aberrant microglial synaptic engulfment in the MCAO/R mice, related to Figure 7.

(A) Three-dimensional rendering of confocal stacks of microglia and engulfed PSD95 puncta (detected within microglial CD68⁺ structures) of treated mice. Scale bar, 5 μ m.

(B) Quantitative analysis of PSD95 engulfment within microglial CD68⁺ structures in (A). $n = 5$ male mice per group (3 cells were averaged per mouse).

Data are presented as the mean $\pm$ SEM. P values were determined by one-way ANOVA followed by Dunnett's *post hoc* analysis. *** $p < 0.001$.