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SUPPLEMENTARY INFORMATION

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Peripheral TREM1 responses to brain and intestinal immunogens amplify stroke severity

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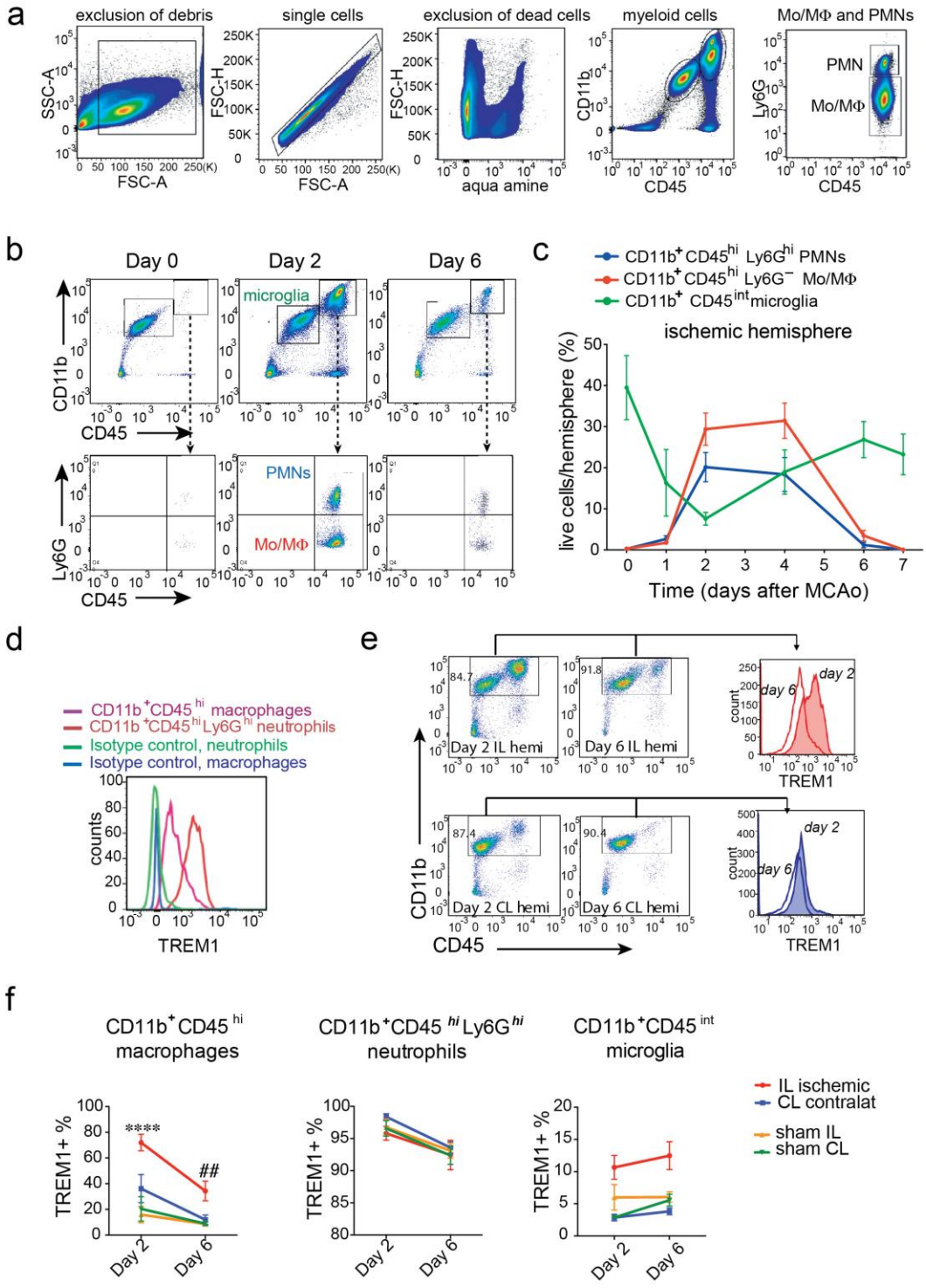
Supplementary Figure 1



Supplementary Figure 1

Temporal dynamics of myeloid cells and TREM1 expression in ipsilateral (IL) hemisphere after MCAo.

(a) Flow cytometry gating strategy of brain myeloid cells in the ischemic hemisphere 48 h after MCAo.

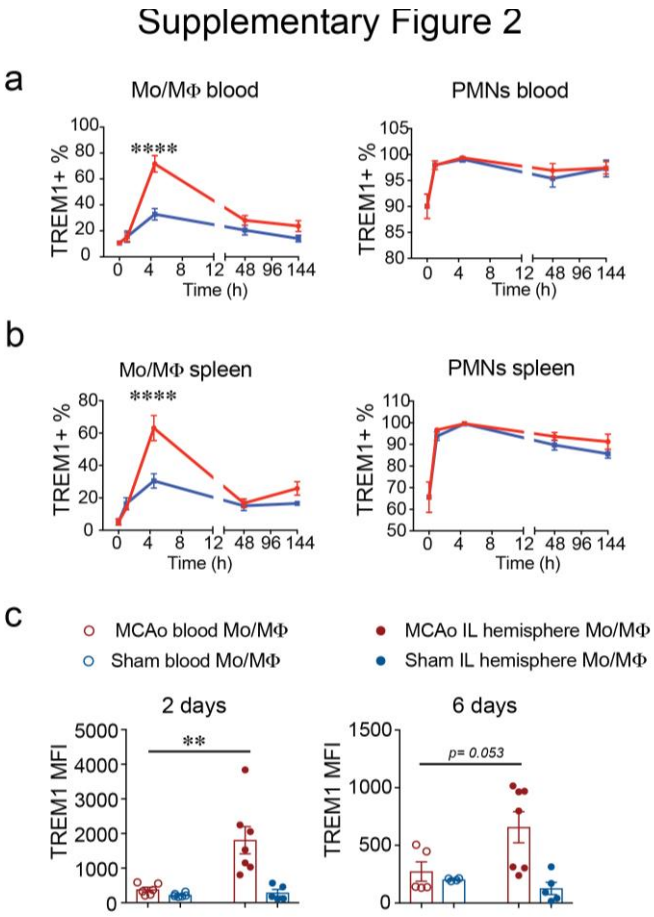
(b) Time courses were carried out over 7 days post MCAo in C56Bl/6J 2-3 mo male mice. Representative plots of $CD11b^{+}CD45^{+}$ myeloid cell populations at day 0 before MCAo, and at 2 and 6 days after MCAo.

(c) Time course of percents of macrophages, neutrophils, and microglia in ischemic hemisphere out to Day 7 post MCAo; n=6 biologically independent samples at days 0 and 7, and n=7 at days 2,4,and 6, mean +/- SEM;1 way ANOVA for each cell type: microglia P=0.015, Mo/MΦ P<0.0001, PMNs P<0.0001.

(d) TREM1 surface expression on $CD11b^{+}CD45^{hi}$ Mo/MΦ and $CD11b^{+}CD45^{hi}Ly6G^{+}$ PMNs 2 days after MCAo compared to isotype control antibody.

(e) Representative plots of $CD11b^{+}CD45^{+}TREM1^{+}$ cells at day 2 and day 6 after MCAo in ischemic ipsilateral (IL) and non-infarcted contralateral (CL) hemispheres.

(f) Percentages of $TREM1^{+}$ macrophages, neutrophils, and microglia in IL and CL hemispheres and in sham IL and CL hemispheres at day 2 and day 6 (n= 6 biologically independent samples for sham, n=7 for MCAo, mean +/- SEM; two-way ANOVA for IL MCAo vs IL sham hemispheres: for macrophages, effect of MCAo P <0.0001, effect of time P <0.01, effect of interaction, P <0.05; Bonferroni post-hoc day 2 IL MCAo vs IL sham **** P <0.0001; post-hoc day 6 IL MCAo vs day 6 IL sham hemisphere ## P <0.01; for microglia, effect of MCAo ** P =0.006).



Supplementary Figure 2

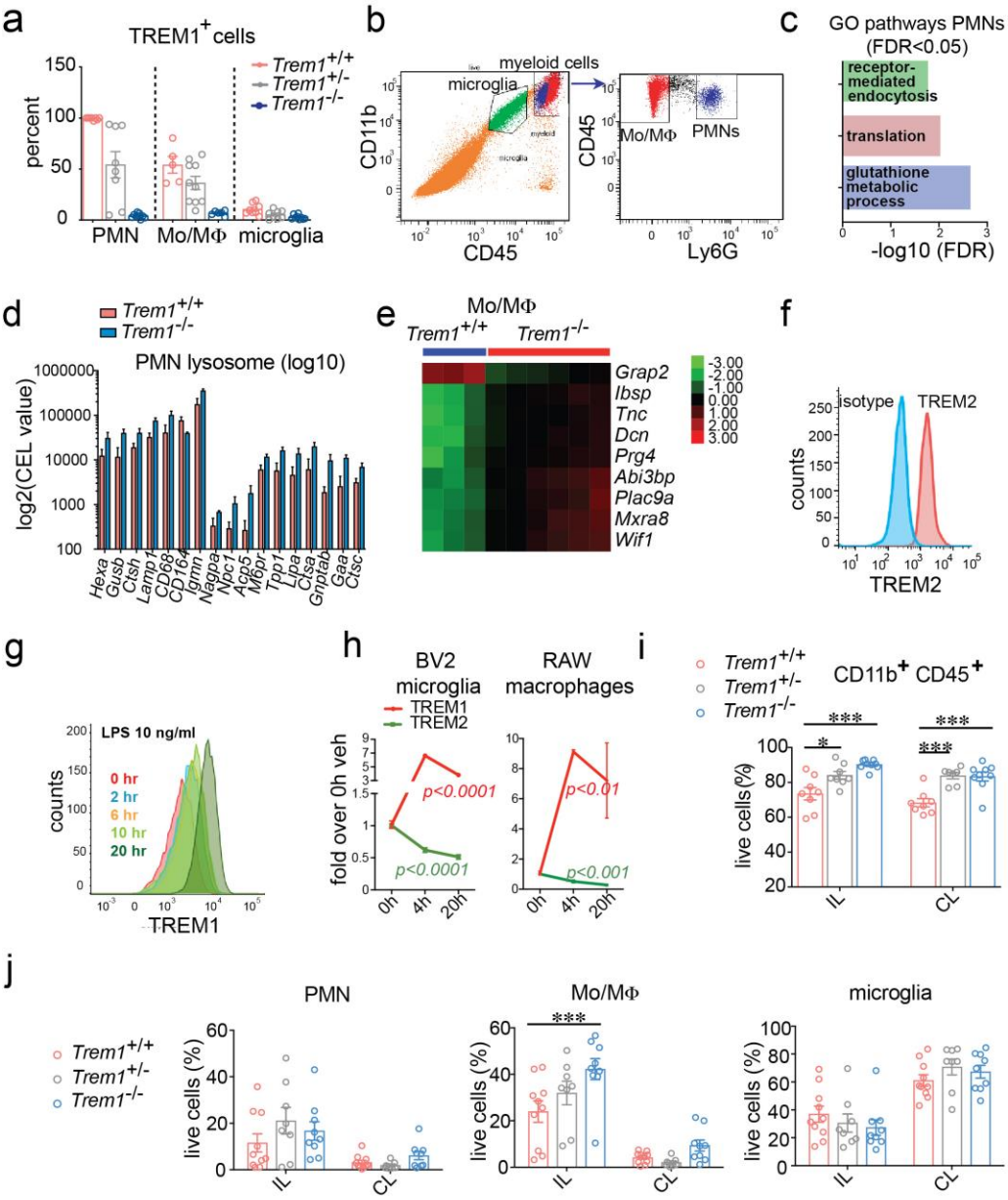
TREM1 surface expression is induced early after MCAo in peripheral Mo/MΦ cells.

(a) TREM1 surface expression was quantified in blood at 0h before sham or MCAo, and 1h, 4.5h, 48h, and 144h (6 days) after sham surgery or MCAo. Percents of TREM1$^+$ Mo/MΦ and PMNs in blood are shown (n=3-12 biologically independent samples per time point, mean +/- SEM; two-way ANOVA for macrophages, effects of MCAo, time, and interaction *P*<0.0001; Bonferroni post hoc **** *P* <0.0001 at 4.5h for MCAo vs sham).

(b) TREM1 surface expression was quantified in spleen at 0h before sham or MCAo, and 1h, 4.5h, 48h, and 144h (6 days) after sham surgery or after MCAo. Percents TREM1$^+$ Mo/MΦ and PMNs in spleen are shown (n=3-12 biologically independent samples per time point, represented as mean +/- SEM; two-way ANOVA for macrophages, effect of MCAo and time, *P*<0.0001; effect of interaction *P*<0.001, Bonferroni post hoc **** *P* <0.0001 at 4.5h for MCAo vs sham).

(c) TREM1 MFI in blood and IL hemisphere Mo/MΦ subsets 2 days (n=5-7 biologically independent samples per group, mean +/- SEM) and 6 days (n=4-7 biologically independent samples per group, mean +/- SEM) after MCAo (Student's two tailed t-test, ** *P* <0.01).

Supplementary Figure 3



Supplementary Figure 3

Genetic ablation of *Trem1* improves outcome after MCAo

(a) Percent TREM1 expression in $CD11b^{+}CD45^{hi}Ly6G^{hi}$ PMNs, $CD11b^{+}CD45^{hi}$ Mo/MΦ, and $CD11b^{+}CD45^{int}$ microglia 2 days after MCAo from *Trem1*$^{+/+}$, *Trem1*$^{+/-}$, and *Trem1*$^{-/-}$ ischemic hemispheres (n=5-10 biologically independent samples per cell type per genotype, mean +/- SEM).

(b) Representative plots of $CD11b^{+}CD45^{hi}Ly6G^{hi}$ PMNs, $CD11b^{+}CD45^{hi}$ Mo/MΦ, and $CD11b^{+}CD45^{int}$ microglia that were sorted and isolated for transcriptomic analysis at 2 days after MCAo.

(c) Top three Gene Ontology pathways (FDR<0.05, absolute fold ≥2).

(d) KEGG lysosomal genes are induced in *Trem1*$^{-/-}$ vs *Trem1*$^{+/+}$ PMNs (note log10 scale).

(e) Heat map of macrophage genes differentially regulated and FDR corrected (P<0.05) in *Trem1*$^{+/+}$ vs *Trem1*$^{-/-}$ ischemic hemispheres at 2 days after MCAo (absolute fold ≥2).

(f) Histogram of TREM2 antibody vs isotype control.

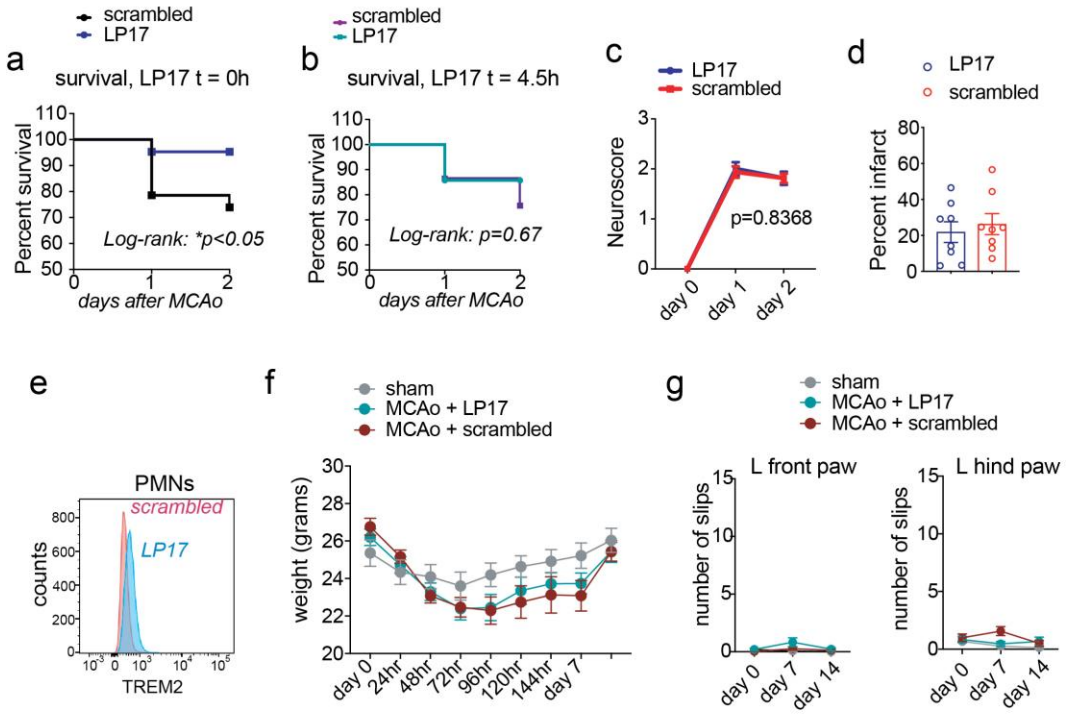
(g) BV2 microglia were stimulated with LPS 10 ng/ml and surface expression of TREM1 determined at 2, 6, 10, and 20h after stimulation.

(h) Raw macrophages and BV2 microglia were stimulated with LPS 10 ng/ml and qRT-PCR performed for TREM1 and TREM2 expression at 0, 4, and 20 h (n=3 biologically independent samples per time point per group, mean +/- SEM; 1-way ANOVA, P values for TREM1 in red and TREM2 in blue).

(i) Percent CD11b^{+}CD45^{+} myeloid cells in *Trem1*$^{+/+}$, *Trem1*$^{+/-}$, and *Trem1*$^{-/-}$ IL and CL hemispheres 2 days after MCAo (n=8 biologically independent samples per genotype for *Trem1*$^{+/+}$ and *Trem1*$^{+/-}$ mice and n=9 for *Trem1*$^{-/-}$ mice, mean +/- SEM; two-way ANOVA, effect of genotype P <0.0001, effect of hemisphere P <0.05; post-hoc * P <0.05 and *** P <0.001).

(j) Percent CD11b^{+}CD45^{hi}Ly6G^{hi} PMNs, CD11b^{+}CD45^{hi} Mo/MΦ, and CD11b^{+}CD45^{int} microglia in *Trem1*$^{+/+}$, *Trem1*$^{+/-}$, and *Trem1*$^{-/-}$ IL and CL hemispheres 2 days after MCAo (n=8 biologically independent samples for *Trem1*$^{+/+}$ and *Trem1*$^{+/-}$ mice and n=9 samples for *Trem1*$^{-/-}$ mice, mean +/- SEM; two-way ANOVA, effect of hemisphere P <0.0001 all three cell types, effect of genotype P <0.01 for Mo/MΦ only; post-hoc *** P <0.001).

Supplementary Figure 4



Supplementary Figure 4

Administration of LP17 to *Trem1*$^{-/-}$ mice.

(a) LP17 reduces mortality post-stroke (n=26 mice per group; Log-rank test * *P* =0.011).

(b) LP17 administered at 4.5h after MCAo does not affect survival (n=28-36 mice per group).

(c) Neuroscores of *Trem1*$^{-/-}$ mice that underwent MCAo and received LP17 or scrambled peptide at the time of reperfusion (n=8 mice per group; mean +/- SEM).

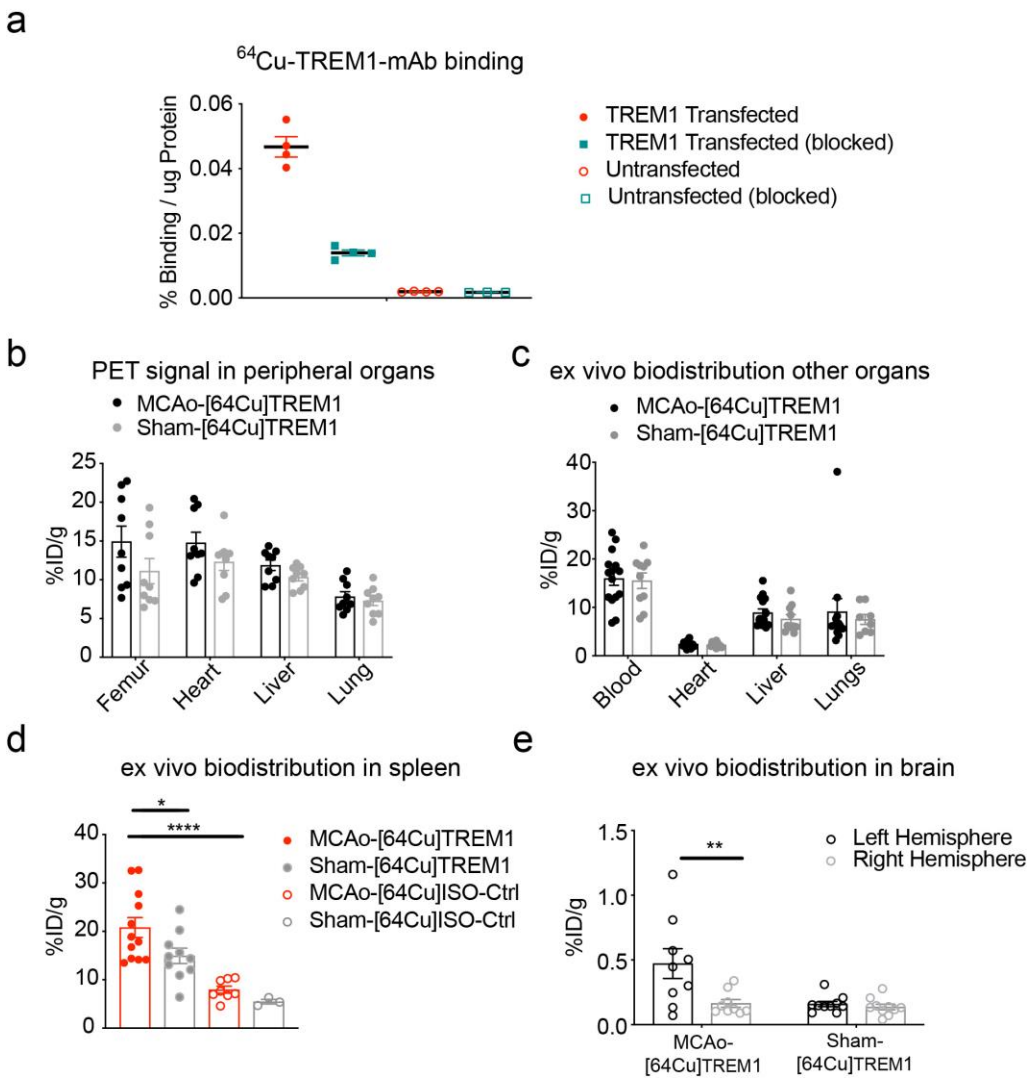
(d) Percent infarct volume in *Trem1*$^{-/-}$ mice treated with LP17 or scrambled peptide (n= 8 mice per group, mean +/- SEM).

(e) Representative histogram of TREM2 expression on CD11b^{+}CD45^{+}Ly6G^{+} PMNs 2 days after MCAo +/- LP17 or scrambled peptide treatment at time of reperfusion.

(f) Body weights of sham and MCAo mice from **Fig. 4m-n**.

(g) Number of left front and left hind paw slips in beam-tested mice (n=5 sham, n=8 scrambled, n=11 LP17).

Supplementary Figure 5



Supplementary Figure 5

TREM1 signal is increased in peripheral myeloid tissues after MCAo.

(a) ^{64}Cu-labeled anti-TREM1-mAb (i.e., [^{64}Cu]TREM1-mAb) was generated with high specific radioactivity (>0.400 MBq/μg), radiochemical purity (>99%), and labeling efficiency (70-95%), and formulated in phosphate-buffered saline [0.1 mol/L NaCl, 0.05 mol/L sodium phosphate (pH 7.4)] (see Methods). HEK293 cells transiently expressing murine *Trem1* cDNA and control empty vector transfected cells were assayed for binding of [^{64}Cu]TREM1-mAb at 1 hour (n=3-4 biologically independent samples per group, mean +/- SEM). Unlabeled TREM1 antibody was used to block binding.

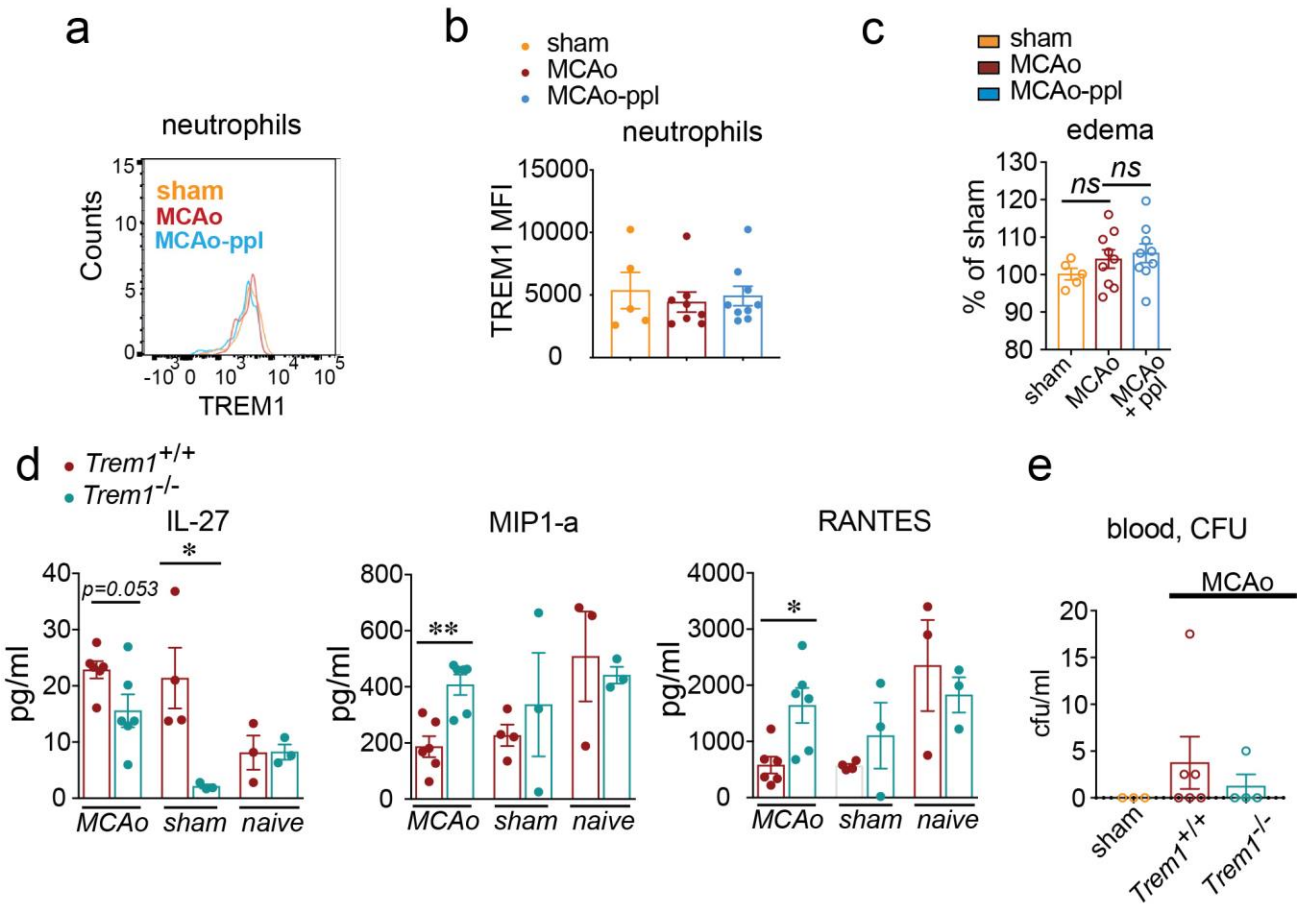
(b) Quantification of PET signal in peripheral organs from MCAo and sham mice (36 h post-MCAo; n=9 biologically independent samples per group, mean +/- SEM)

(c) Quantification of TREM1 signal from *ex vivo* biodistribution studies of blood, heart, liver and lungs (n=8-15 biologically independent samples per group, mean +/- SEM).

(d) Quantification of spleen TREM1 signal from *ex vivo* biodistribution studies shows higher uptake of [^{64}Cu]TREM1-mAb in MCAo mice compared to shams and compared to MCAo and sham mice injected with [^{64}Cu]Isotype control (n=12, MCAo-[64Cu]TREM1; n=10, sham-[64Cu]TREM1; n=8, MCAo-[64Cu]ISO-Ctrl; n=3, Sham-[64Cu]ISO-Ctrl) mice, mean +/- SEM).

(e) *Ex vivo* biodistribution of brain hemispheres corroborates brain PET imaging findings (*n*=10 MCAo-[^{64}Cu]TREM1, *n*=9 sham-[^{64}Cu]TREM1 biologically independent samples, mean +/- SEM). All comparisons are two-tailed unpaired Student's t-test **** P <0.001, ** P <0.01, * P <0.05.

Supplementary Figure 6



Supplementary Figure 6

TREM1 is induced in the intestinal inflammatory Mo/MΦ subset after MCAo.

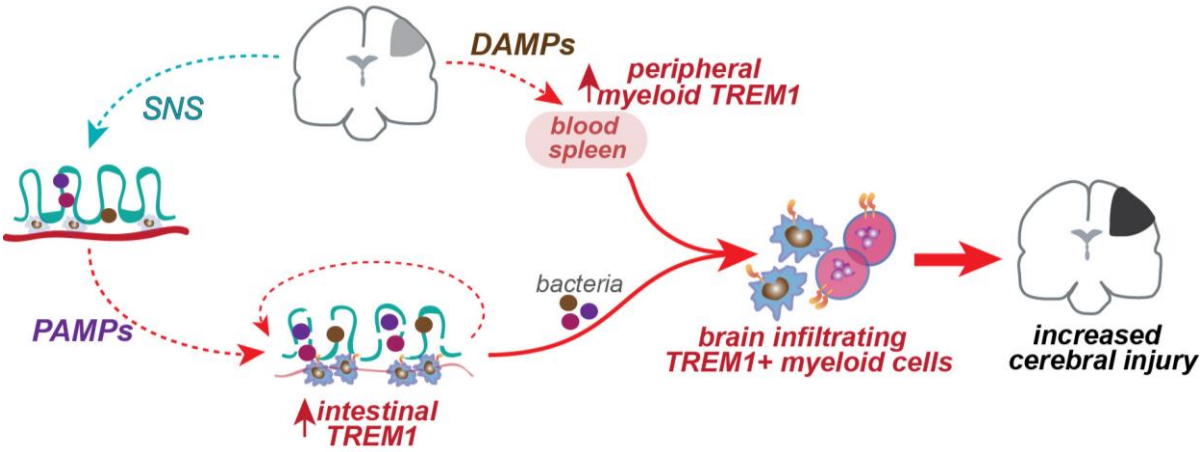
(a) ß-adrenergic inhibition with propranolol (ppl) has no effect on neutrophil TREM1 expression after MCAo

(b) TREM1 MFI in neutrophils in sham, MCAo, and MCAo treated with propranolol (n=5 sham, n=8 MCAo and n=9 MCAo+ppl mice, mean +/- SEM).

(c) Percent change in volume of IL hemisphere in sham and MCAo mice +/- ppl 4.5h after MCAo (n=5 sham, n=9 MCAo and n=9 MCAo+ppl, mean +/- SEM).

(d) Immune factor changes at 4.5h in small intestine lamina propria (n=3-6 biologically independent samples per group, mean +/- SEM.; ** *P* <0.01 Student's two tailed t-test).

(e) Quantification of bacterial colonies that grew out of blood collected from *Trem1*$^{+/+}$ and *Trem1*-/- mice at 4.5h after MCAo (n=3 sham *Trem1*$^{+/+}$, n=6 MCAo *Trem1*$^{+/+}$ and n=4 MCAo *Trem1*$^{-/-}$ mice, mean +/- SEM).



Supplementary Figure 7

Model of dual TREM1 amplification of immune responses after cerebral ischemia.

(**Left side**) Cerebral injury activates the sympathetic nervous system (SNS) within hours of MCAo, leading to early disruption of the gut barrier and translocation of bacterial PAMPs across the epithelial barrier. There, PAMPs induce and activate TREM1 signaling in lamina propria Mo/MΦ subsets, amplifying the innate immune response and further disrupting gut barrier integrity and facilitating translocation of bacteria to the periphery. (**Right side**): Cerebral infarction induces the release of sterile DAMPs that activate TREM1 on circulating peripheral and splenic myeloid cells. Thus, TREM1 is induced in myeloid cells in two spatially distinct processes after MCAo. Dual brain-derived and intestinal-derived TREM1 responses converge and amplify the post-stroke innate immune response, increasing cerebral injury.