Supplementary Materials

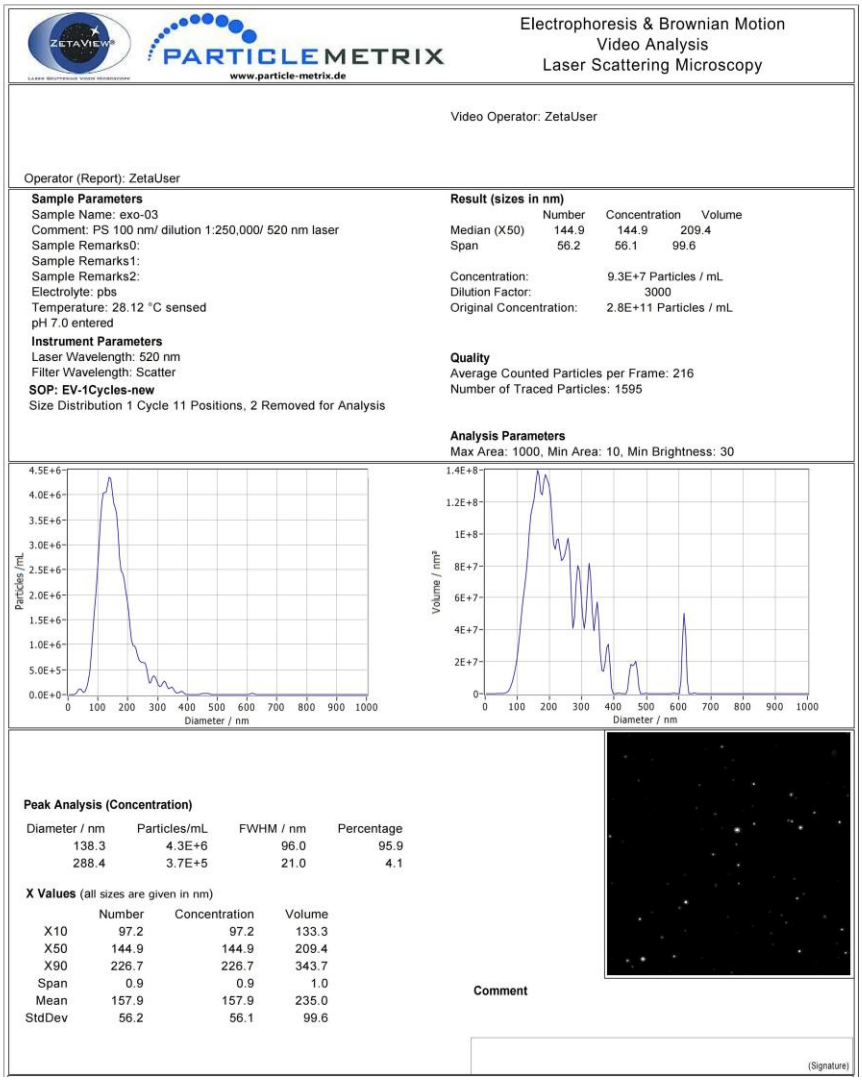
hUMSC-Exosomes Suppress TREM1-p38 MAPK Signaling via HMGB1-Dependent Mechanisms to Reprogram Microglial Function and Promote Neuroprotection in Ischemic Stroke

Zengyu Zhang[1#], Rong Ji[1#], Zhuohang Liu[1#], Zhiwen Jiang[2], Min Chu[3], Yong Wang[4*], Jing Zhao[1,5*]

*Correspondence author:
Jing Zhao, Department of Neurology, Minhang Hospital, Fudan University, Shanghai 201199, China. E-mail address: zhao_jing@fudan.edu.cn;
Yong Wang, Department of Neurology, Zhongshan Hospital, Fudan University, Shanghai 200030, China. E-mail address: yong_wang@fudan.edu.cn

#Zengyu Zhang, Rong Ji and Zhuohang Liu contributed equally to this study.

Fig.S1 Nanoparticle Tracking Analysis (NTA) of hUMSC-Exos showing size distribution, concentration, and median diameter, and original western blots for figure 1.



PARTICLEMETRIX
www.particle-metrix.de

Electrophoresis & Brownian Motion
Video Analysis
Laser Scattering Microscopy

Video Operator: ZetaUser

Operator (Report): ZetaUser

Sample Parameters
Sample Name: exo-03
Comment: PS 100 nm/ dilution 1:250,000/ 520 nm laser
Sample Remarks0:
Sample Remarks1:
Sample Remarks2:
Electrolyte: pbs
Temperature: 28.12 °C sensed
pH 7.0 entered

Instrument Parameters
Laser Wavelength: 520 nm
Filter Wavelength: Scatter

SOP: EV-1Cycles-new
Size Distribution 1 Cycle 11 Positions, 2 Removed for Analysis

Result (sizes in nm)

	Number	Concentration	Volume
Median (X50)	144.9	144.9	209.4
Span	56.2	56.1	99.6

Concentration: 9.3E+7 Particles / mL
Dilution Factor: 3000
Original Concentration: 2.8E+11 Particles / mL

Quality
Average Counted Particles per Frame: 216
Number of Traced Particles: 1595

Analysis Parameters
Max Area: 1000, Min Area: 10, Min Brightness: 30

Peak Analysis (Concentration)

Diameter / nm	Particles/mL	FWHM / nm	Percentage
138.3	4.3E+6	96.0	95.9
288.4	3.7E+5	21.0	4.1

X Values (all sizes are given in nm)

	Number	Concentration	Volume
X10	97.2	97.2	133.3
X50	144.9	144.9	209.4
X90	226.7	226.7	343.7
Span	0.9	0.9	1.0
Mean	157.9	157.9	235.0
StdDev	56.2	56.1	99.6

Comment

(Signature)

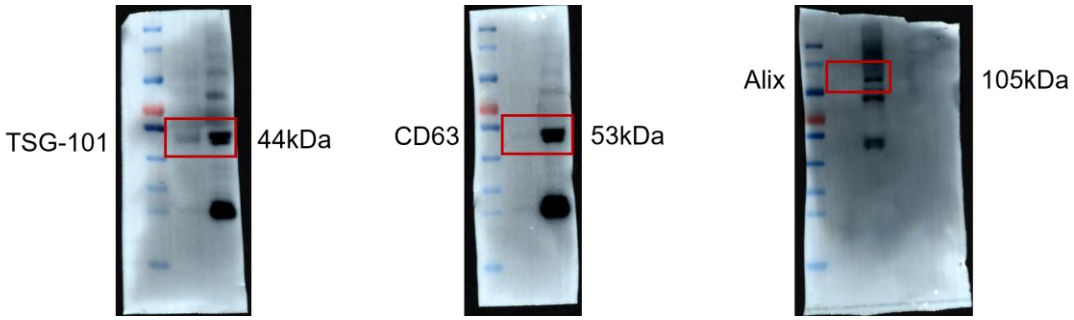


Fig.S2 Accumulation of PKH26-labeled hUMSC-Exo in mouse brain following intranasal administration after tMCAO. Representative fluorescence imaging shows the distribution of PKH26-labeled hUMSC-Exosomes in the brain 12 hours post-administration. Fluorescence signal was detected in both the ischemic and contralateral hemispheres, with a higher intensity observed in the ischemic region. No significant fluorescence was observed in the PBS-treated control group.

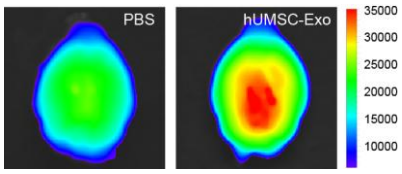


Fig.S3 RT-qPCR analysis of marker gene expression for different neuronal cell populations in the Cd11b (+) group compared to whole brain homogenate. The markers in the figure represent distinct neural cell populations: oligodendrocytes (Mbp), neurons (Grin2b, Rbfox3, Map2), astrocytes (S100β, Gfap, Slc1a3), and microglia (Aif1, Itgam, Cx3cr1, Hexb, P2ry12). Each marker highlights the differential expression patterns in Cd11b (+) microglia versus brain homogenates, reflecting the cellular composition.

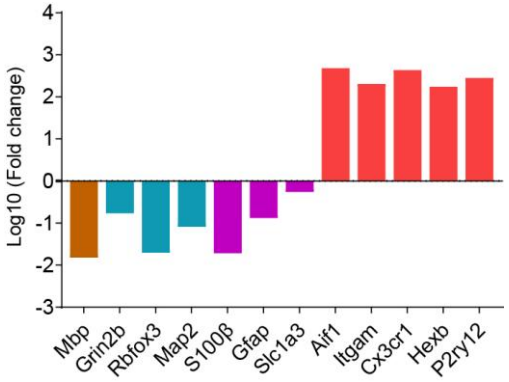


Fig.S4 Quality control and coverage analysis of RNA-seq data across Sham, tMCAO, and tMCAO+Exo Groups. **A** The distribution of RNA-seq reads aligned to genomic regions, including introns, exons, and intergenic regions, across all samples. **B** Gene coverage distribution for representative samples (Sham_1, tMCAO_1, tMCAO+Exo_1). Most genes exhibit high coverage (80-100%), indicating consistent transcriptome profiling across conditions. **C** Saturation curves for representative samples. **D** Transcripts per million (TPM) distribution for all samples. **E** Violin plots of sample expression distribution (log10 TPM). **F** Sample correlation heatmap.

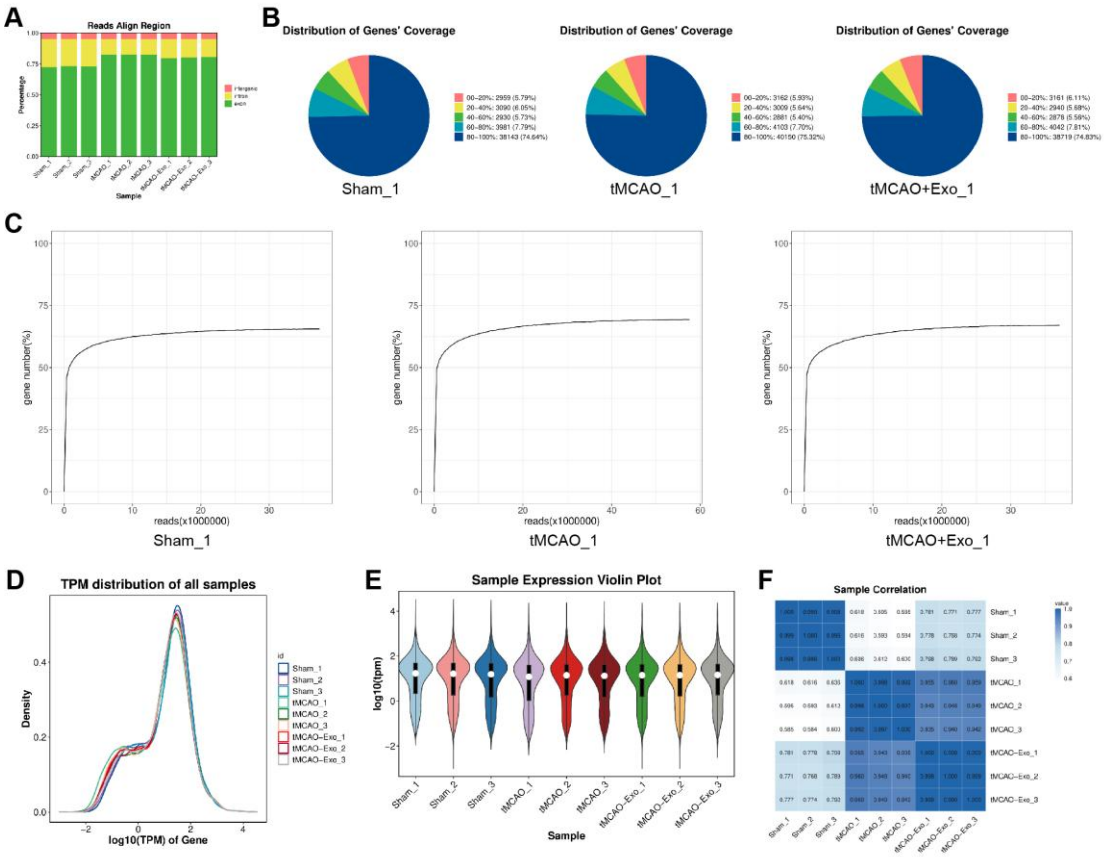


Fig.S5 hUMSC-Exos suppress pro-inflammatory gene expression in microglia in vitro and in vivo following ischemic injury. **A** Quantification of Trem1 fluorescence intensity in Iba1$^+$ primary microglia stimulated with LPS and IFN-γ, with or without hUMSC-Exo treatment. Exosome treatment significantly reduced Trem1 expression. N = 23 cells per group. **B** RT-qPCR analysis of Ccl6, Vegfa, and Mmp9 mRNA levels in isolated microglia from mice 3 days after tMCAO. **C** RT-qPCR analysis of additional inflammation-related genes (Trem1, Cxcl2, Cxcl3, Ccl6, and Mmp9) in brain tissue from mice at day 3 post-tMCAO. N = 5 per group. Data are presented as mean ± SEM. One-way ANOVA with Tukey's post hoc test. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

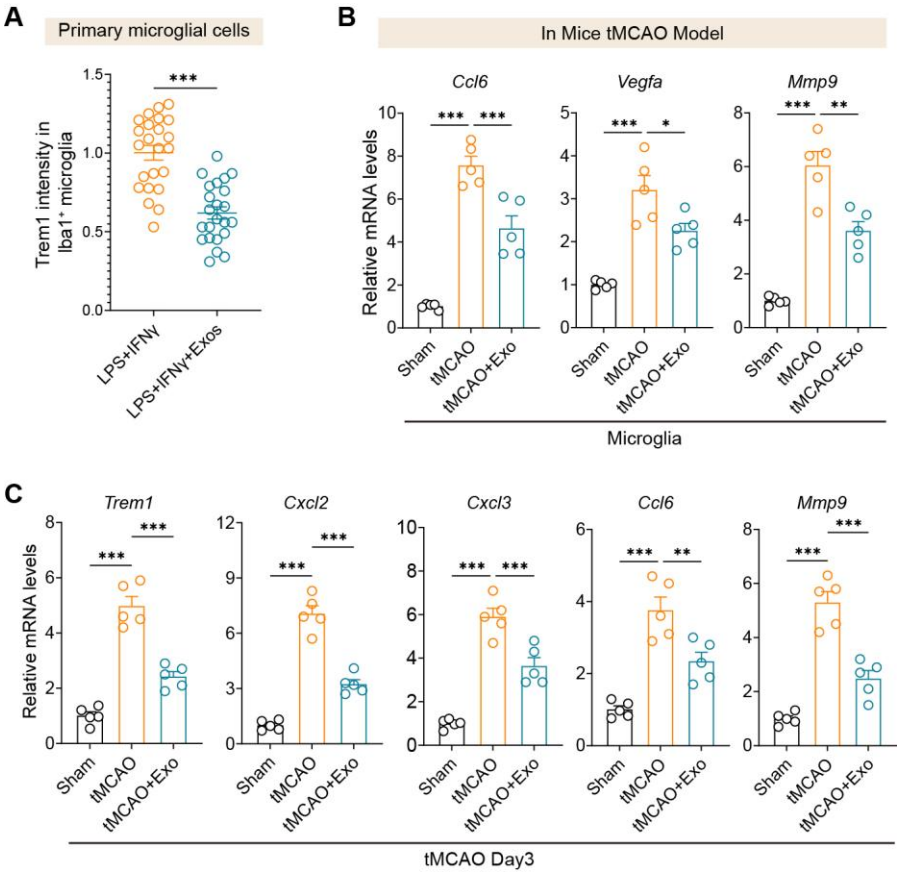


Fig.S6 The effects of LPS+IFN-γ, Exos, and BAY11-7082 on the expression of inflammatory and anti-inflammatory markers in treated BV2 cells. The bar graphs illustrate the relative mRNA levels of *Trem1*, *TNFα*, *IL1β*, *Il6*, *Arg1*, and *Il10* across different treatment groups. Each group consists of n = 5 biological replicates. Data are presented as mean ± SEM. One-way ANOVA with Tukey's post hoc test. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. "ns" indicates no significant difference.

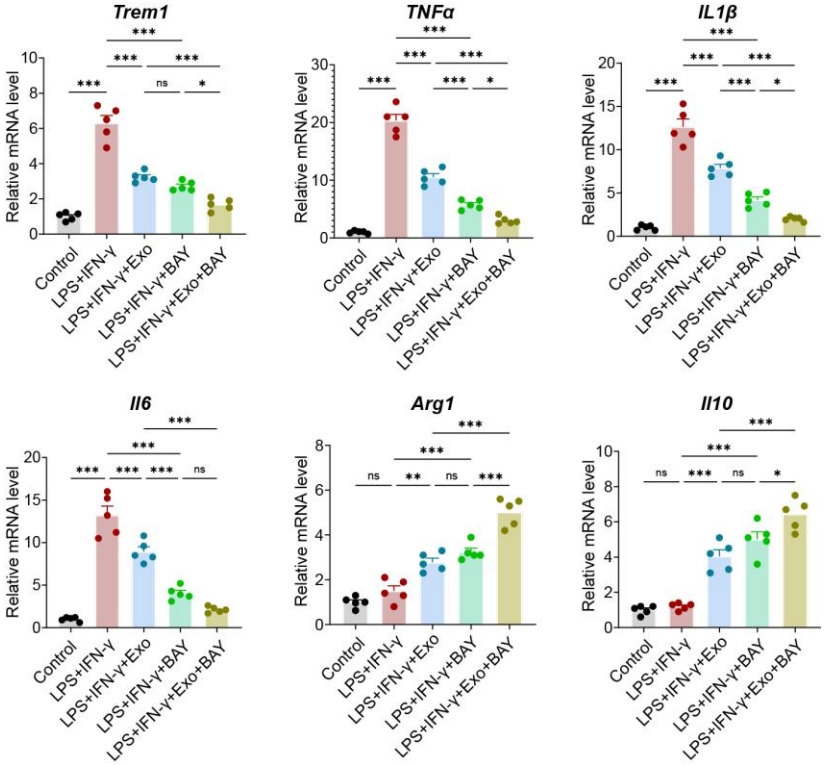


Fig.S7 hUMSC-Exos inhibit microglial migration via TREM1-p38 MAPK signaling. **A** Representative images of Transwell migration assays in BV2 microglia under various conditions. Scale bar, 100 μm. **B** Quantification of migrated cells per field. hUMSC-Exos significantly decreased microglial migration compared to LPS + IFN-γ alone. This inhibitory effect was enhanced by LP17 and SB203580, indicating that hUMSC-Exos act via the TREM1-p38 MAPK axis. N = 9 per group. Data are presented as mean ± SEM. One-way ANOVA with Tukey's post hoc test. *$p < 0.05$ and ***$p < 0.001$. "ns" indicates no significant difference.

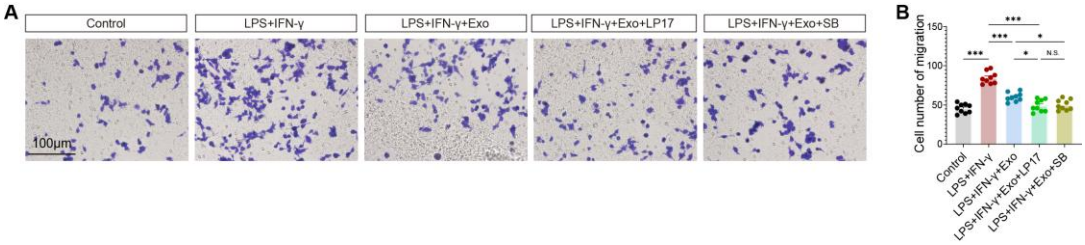


Fig. S8 Validation of Trem1 overexpression and knockdown in BV2 microglial cells. **A** Representative Western blot and densitometric analysis of Trem1 protein expression in BV2 cells transfected with an OE-Trem1 plasmid. Trem1 levels were significantly increased compared to control cells. **B** Representative Western blot and quantification of Trem1 protein levels in BV2 cells transfected with siRNA targeting Trem1 (si-Trem1), showing effective knockdown relative to controls. N = 3 per group. Data are presented as mean ± SEM. ***$p <$ 0.001, unpaired two-tailed *t*-test.