Tsp-1^{+} microglia attenuate retinal neovascularization by maintaining the expression of Smad3 in endothelial cells through exosomes with decreased miR-27a-5p

Supplementary Figures S1-S2 and Tables S1-S3

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Supplementary Figures

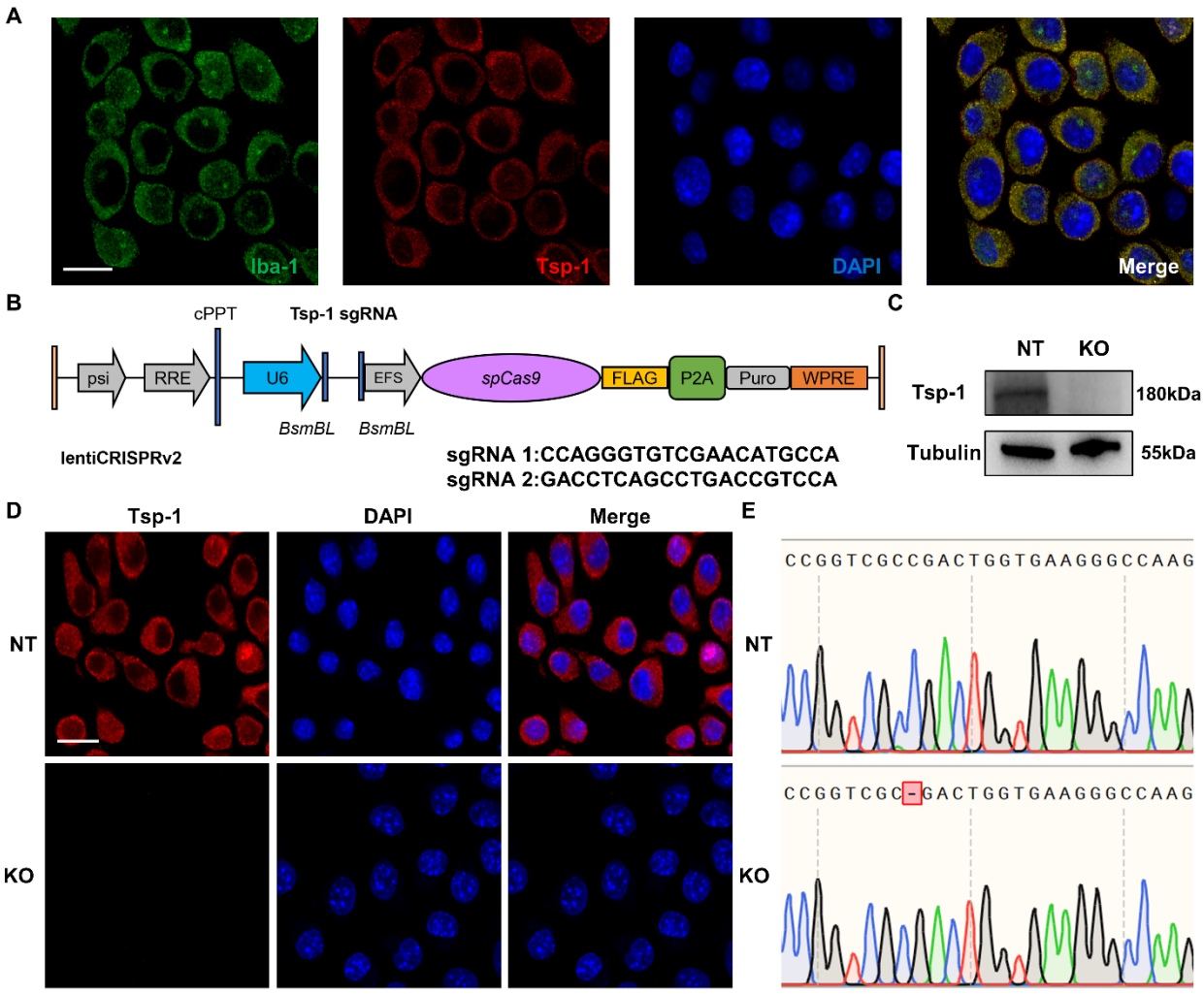

Figure S1. Stable knockout of Tsp-1 in the BV2 microglial cell line. (A) Representative confocal images of double staining for Iba-1 and Tsp-1 in BV2 microglia (Iba-1, green; Tsp-1, red; DAPI, blue). Scale bar: 20 μm. n = 3. **(B)** Schematic diagram of the lentiCRISPRv2 plasmid targeting Tsp-1. Two single-guide RNAs (sgRNAs) were designed to knock out the Tsp-1 gene. Western blot results **(C)** and representative confocal images **(D)** of Tsp-1 expression levels in the negative target (NT) and Tsp-1 knockout (KO) BV2 cells. The BV2-KO cells lacked Tsp-1 expression. (Tsp-1, red; DAPI, blue). Scale bar: 20 μm. n = 3. **(E)** Sanger sequencing of lentivirus-infected microglia, including the BV2-NT and BV2-KO cells. Dashes indicate deleted nucleotides. n = 5.

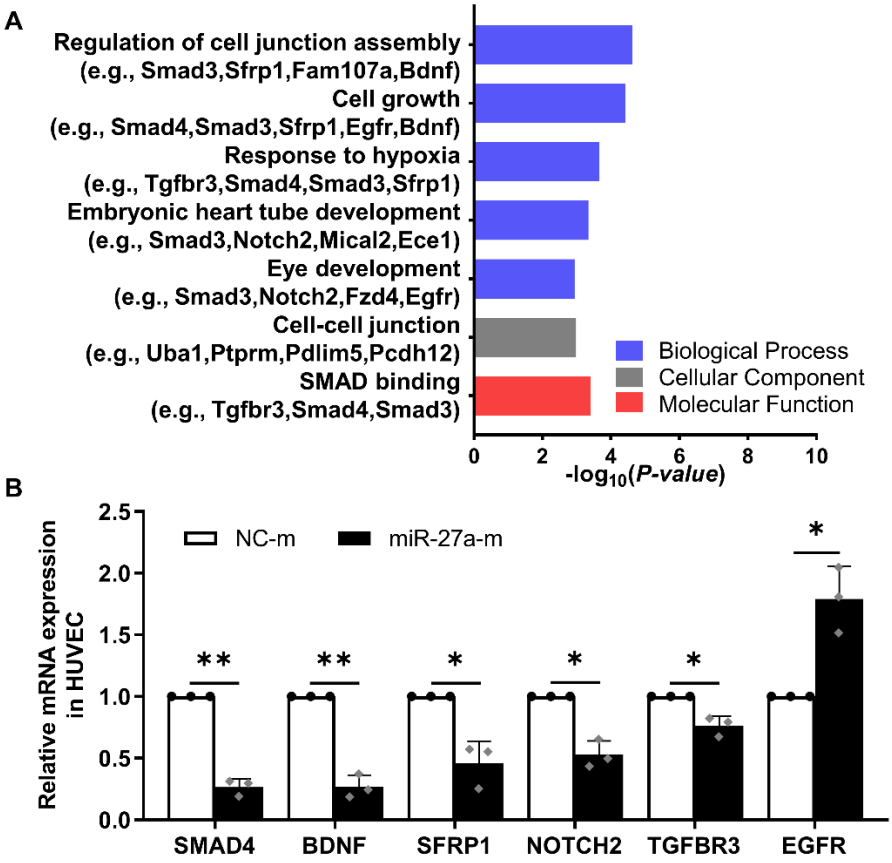

Figure S2. Analysis of miR-27a-5p candidate genes with Gene Ontology enrichment and RT–qPCR. (A) Gene Ontology (GO) analysis of miR-27a-5p candidate target genes. A two-sided statistical test in function enrichGO was used. **(B)** HUVEC were transfected with synthetic mimic control (NC-m) or miR-27a-5p mimic (miR-27a-m), and quantitative real-time PCR revealed the relative mRNA expression levels of SMAD4, BDNF, SFRP1, NOTCH2, TGFBR3, and EGFR. $^{**}P < 0.01$, $^{*}P < 0.05$. n = 3. Results are expressed as mean ± SD.

Supplementary Tables

Table S1. RT–qPCR primer sequences

Primer	Sequence (5′–3′)
F-miR-27a-5p	GGCTTAGCTGCTTGTGAGCA
F-miR-23a-5p	GGTTCCTGGGGATGGGATTT
F-miR-221-5p	CACCTGGCATACAATGTAGATTTCTG
F-miR-193a-5p	GGTCTTTGCGGGCAAGATGA
F- miR-92a-1-5p	GGTTGGGATTTGTCGCAATGC
Universal-R	CAGTGCGTGTCGTGGAGT
F-U6	GCTTCGGCAGCACATATACTAAAAT
R-U6	CGCTTCACGAATTTGCGTGTCAT
F-h-SMAD3	TGAGGCTGTCTACCAGTTGACC
R-h-SMAD3	GTGAGGACCTTGTCAAGCCACT
F-h-SMAD4	CTACCAGCACTGCCAACTTTCC
R-h-SMAD4	CCTGATGCTATCTGCAACAGTCC
F-h-BDNF	CATCCGAGGACAAGGTGGCTTG
R-h-BDNF	GCCGAACTTTCTGGTCCTCATC
F-h-SFRP1	CAATGCCACCGAAGCCTCCAAG
R-h-SFRP1	CAAACTCGCTGGCACAGAGATG

F-h-NOTCH2	GTGCCTATGTCCATCTGGATGG
R-h-NOTCH2	AGACACCTGAGTGCTGGCACAA
F-h-TGFBR3	TGGAGTCTCCTCTGAATGGCTG
R-h-TGFBR3	CCATTATCACCTGACTCCAGATC
F-h-EGFR	AACACCCTGGTCTGGAAGTACG
R-h-EGFR	TCGTTGGACAGCCTTCAAGACC
F-h-ACTIN	CACCACACCTTCTACAATGAG
R-h-ACTIN	TAGCACAGCCTGGATAGCAAC

Table S2. miRNA mimic, inhibitor, and antagomir sequences

Name	Sequence (5′–3′)
miR-27a-5p mimic	AGGGCUUAGCUGCUUGUGAGCA
miRNA mimic-NC	UCACAACCUCCUAGAAAGAGUAGA
miR-27a-5p inhibitor	UGCUCACAAGCAGCUAAGCCCU
miRNA inhibitor-NC	UCUACUCUUUCUAGGAGGUUGUGA
miR-27a-5p antagomir	UGCUCACAAGCAGCUAAGCCCU
miRNA antagomir-NC	CAGUACUUUUGUGUAGUACAAA

Table S3. 3′ UTR target sequences of Smad3 for pMIR-report vector cloning

Name	Sequence (5′–3′)
Luc-Smad3-WT-F	AGCTTGCAAACGGGCTGCCCTAGTCAAGCCCAGTCCCTTCAACAGTATGTCTGAT
Luc-Smad3-WT-R	CTAGATCAGACATACTGTTGAAGGGACTGGGCTTGACTAGGGCAGCCCGTTTGCA
Luc-Smad3-Mut-F	AGCTTGCAAACGGGCTGCCCTAGTCTTCGGGTGTCCCTTCAACAGTATGTCTGAT
Luc-Smad3-Mut-R	CTAGATCAGTCATACTGTTGAAGGGACACCCGAAGACTAGGGCAGCCCGTTTGCA