

Supplemental Information

circ_2858 Helps Blood-Brain Barrier Disruption by Increasing VEGFA via Sponging miR-93-5p during *Escherichia coli* Meningitis

Ruicheng Yang, Jiaqi Chen, Bojie Xu, Bo Yang, Jiyang Fu, Siyu Xiao, Chen Tan, Huanchun Chen, and Xiangru Wang

Supplementary Figures

Figure S1

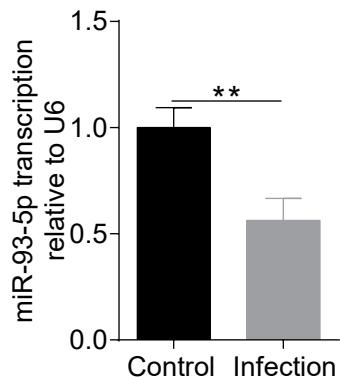


Figure S1. Meningitic *E. coli* infection decreased the transcription of miR-93-5p in hBMECs. U6 was used as the internal reference, and data were presented as mean \pm SD from three independent experiments.

Figure S2

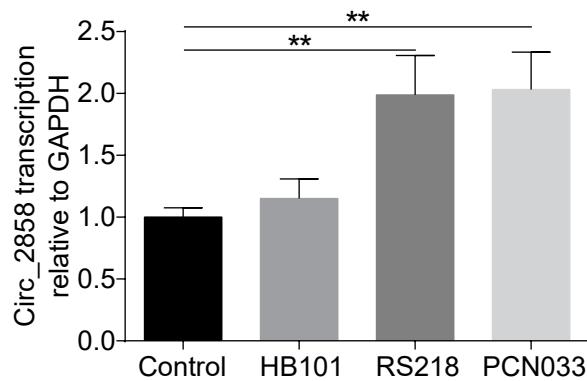


Figure S2. The alteration of circ_2858 in hBMECs by the challenge of meningitic *E. coli* strain RS218 and PCN033, and the non-meningitic *E. coli* strain HB101. GAPDH was used as the internal reference, and data were presented as mean \pm SD from three independent experiments.

Figure S3

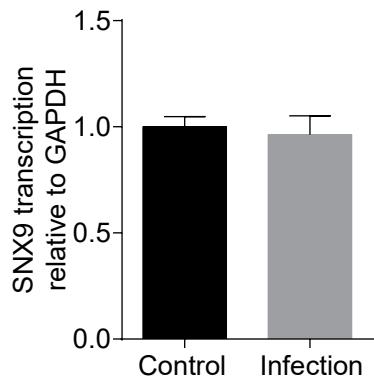


Figure S3. The transcription of SNX9 in hBMECs upon meningitic *E. coli* infection. GAPDH was used as the internal reference, and data were presented as mean \pm SD from three independent experiments.

Figure S4

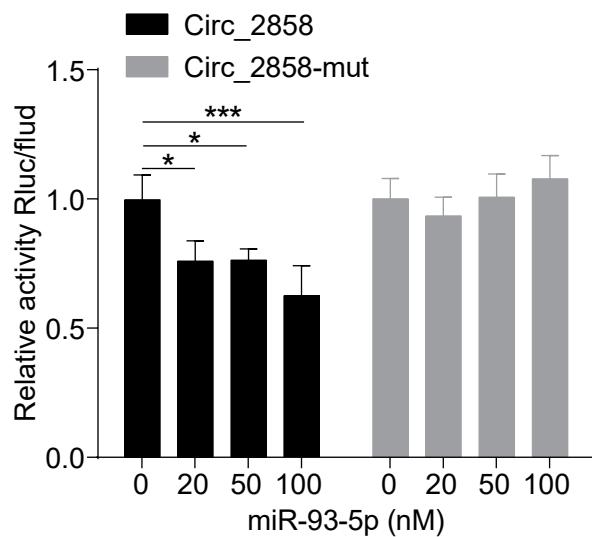


Figure S4. Dual-luciferase reporter assays showing that miR-93-5p significantly and dose-dependently reduced the luciferase activity of the wild-type circ_2858 construct, but not that of the mutant construct. The renilla luciferase activity was normalized to the firefly luciferase activity, and data were presented as mean \pm SD from three independent experiments.

Tables S1 - S3 are submitted as Excel files and can be downloaded as separate files.

Table S4. Primers used for circRNAs real-time PCR

Primers	Nucleotide sequence (5'-3')	Amplified fragments
P1	TAGTGAGGATGAGGAGGAAGA	
P2	TTAAGGGTGGCTGATGT	hsa_circ_851
P3	AGTCCTCAGTCCTCAGTCATC	
P4	CCTGCTGTTGGGAAGTTCA	hsa_circ_1291
P5	GTTCCCTCTTCCAGACCAT	
P6	CCAACAACAAGTTCATCATCCA	hsa_circ_1507
P7	GTGACTTACCAGGGAAACT	
P8	AGCAAACGTGGGTCTTA	hsa_circ_1647
P9	GCGGAAGTCCTAACAAAGCATAGT	
P10	CTCAAAGCGATTGCCTCCTCTT	hsa_circ_1687
P11	CAACATTGAGCGTGAGTG	
P12	AATAGCAGGTTCTTGATTGG	hsa_circ_1715
P13	TGAGGACTCGCTGATTCTTGAGA	
P14	ACTTCCATTACCACTCCCTCAGA	hsa_circ_1910
P15	GCGGTAGATGATGATGATGAAG	
P16	CAAATCTGCGTGGTTCTCTT	hsa_circ_2146
P17	GCTAACACCTACTAACACTAACATCG	
P18	ACTGCCAACCTGTGACTT	hsa_circ_2858
P19	GAAATGTGAAGCCAGGTTA	
P20	CTTGTCCAGGTTGTAAGC	hsa_circ_3196
P21	GATATGGCTCAGACTCAGT	
P22	TCCGAAGGTCATCAACTAAT	hsa_circ_3779
P23	ACTAATCTCGGGCAGAG	
P24	TCCTGTCATCCTTCTTACG	hsa_circ_4316
P25	CCAGACAAAGTAACAGAGATT	
P26	AAGTTGGAGTAGTGATTGC	hsa_circ_5804
P27	TGCCTCCTGCACCACCAACT	
P28	CGCCTGCTTCACCACCTTC	GAPDH

Table S5. Primers used for hsa_circ_2858 identification

Primers	Nucleotide sequence (5'-3')	Product size	Target product
P1	GCTAACACCTACTAACACTAACATCG	140 bp	Exon 1 and 5 divergent primer
P2	ACTGCCAACCTGTGACTT		
P3	AACTGGTGATGATGATGACT	123 bp	Exon 2 convergent primer
P4	GAGGAACTAGGCACGACTG		
P5	CACAGGTATAAGCACTTTGA	393 bp	Exon 2 and 5 divergent primer
P6	GAGGAACTAGGCACGACTG		
P7	CAGTCGTGCTAGTTCTC	564 bp	Exon 2 divergent primer
P8	AGTCATCATCATCACCAAGTT		
P9	CCATTCCAATCCCTTCTCT	612 bp	Exon 5 divergent primer
P10	GTCAAAGTGCTTATACCTGTG		

Table S6. Primers used for miRNAs real-time PCR

Primers	Nucleotide sequence (5'-3')	Amplified fragments
P1	TCAGTGCACTACAGAACTTTGT	hsa-miR-148a-3p
P2	CTAGACTGAAGCTCCTTGAGG	hsa-miR-151a-3p
P3	CAACAAAATCACTAGTCTTCCA	hsa-miR-3529-3p
P4	TGGAAGACTAGTGATTGTTGT	hsa-miR-7-5p
P5	AGGGCTTAGCTGCTGTGA	hsa-miR-27a-5p
P6	CATCTTACCGGACAGTGCT	hsa-miR-200a-5p
P7	AAGTGCTGTTCGTGCAGGTA	hsa-miR-93-5p
P8	CTGAGACCCTTAACCTGTGA	hsa-miR-125a-5p
P9	TAAAGTGCCTATAGTGCAGGTAG	hsa-miR-20a-5p
P10	ACGGAATCCAAAAGCAGCT	hsa-miR-191-5p
P11	CGGATATAATACAACCTGCTAAGTG	hsa-miR-374b-5p
P12	CGGCACTTAGCAGGTTGTATTATAT	hsa-miR-374c-3p
P13	CTAAGGAAGTCCTGTGCTCAG	hsa-miR-4521
P14	CAAAGTGCCTACAGTGCAGGTA	hsa-miR-17-5p
P15	GCAGTGACTGTTCAGACGTC	hsa-miR-2682-5p
P16	TAGCAGCACGTAAATATTGGC	hsa-miR-16-5p
P17	TACCCATTGCATATCGGAGTTG	hsa-miR-660-5p
P18	GGTTATTGCTTAAGAACATCGCGTAG	hsa-miR-137
P19	TGAGAACACCACGTCTGCTCT	hsa-miR-589-5p
P20	AGGCAAGATGCTGGCATA	hsa-miR-31-5p
P21	CAGCATTGTACAGGGCTATCA	hsa-miR-107
P22	TGTCCCTCAGGAGCTCAC	hsa-miR-339-5p
P23	CCAAAACGTGAGTTACTTTGC	hsa-miR-548o-3p
P24	GTGCAAATCCATGCAAAACTG	hsa-miR-19b-3p
P25	TGAAGCGCCTGTGCTCTG	hsa-miR-7706
P26	CCTCCCACACCCAAGGCTTGCA	hsa-miR-532-3p
P27	TAACAGCAACTCCATGTGGA	hsa-miR-194-5p
P28	GCACCTGGCAAGGATTCA	hsa-miR-502-3p
P29	CCGGCCTAATTATGTATAAGC	hsa-miR-590-3p
P30	CGGAGACTTGGCAATTG	hsa-miR-25-5p
P31	AAACGTGAGGCCTGCTAT	hsa-miR-424-3p
P32	AAGGAGCTCACAGTCTATTGAG	hsa-miR-28-5p
P33	AACGCTTCACGAATTGCGT	U6

Table S7. Primers used for mRNAs real-time PCR

Primers	Nucleotide sequence (5'-3')	Amplified fragments
P1	AAGCATGTGTTAACCTCTACC	HILPDA
P2	TGTGTTGGCTAGTGGCTTCT	
P3	GGGTGTGGCGTGTTGAATG	STC2
P4	TTTCCAGCGTTGTGCAGAAAA	
P5	ATGAAGCTGGTTCCGTG	ADM
P6	GACATCCGCAGTCCCTCTT	
P7	TGCCTTGCTGCTCTACCT	VEGFA
P8	GACATCCATGAACCTCACCACTT	
P9	CTCCGGGTGGGTTTTACGAC	SIK1
P10	CTCGGTTTGGTGAATCGATG	
P11	TGTCCGTCAGAACCCATGC	CDKN1A
P12	AAAGTCGAAGTCCATCGCTC	
P13	AAGCCCAGTGCTCTGAGA	RARA
P14	TTCGTAGTGTATTGCCAGC	
P15	TCTGGCATTGAGTCTCTGCG	NFKBIE
P16	AGGAGCCATAGGTGGAATCAG	
P17	CTTGACCCCTGACTATGTTGGC	CSPG4
P18	TGCAGGCGTCCAGAGTAGA	
P19	GCCACAGATTCCCTGTACCTG	ITPRIP
P20	TCCCTGAACGGAATTGATCTT	
P21	GTAGCAGCCGCAGTCATAA	ICAM1
P22	GCCTGTTGAGTCTGTATTCTTG	
P23	CCAACGTGACGGACTTCCC	LIF
P24	TACACGACTATGCGGTACAGC	
P25	TGGAGCCCGTATCCTGGAG	EFNB1
P26	TTGGGGTCGAGAACTGTGCTA	
P27	ATGCCCTGTATCCAAGCCC	NR4A1
P28	GTGTAGCCGTCCATGAAGGT	
P29	TGGGGCTTCCGTATCACAG	PDLIM2
P30	CTCTGGCGGATCTGCTCT	
P31	GGCAGGGGCAGTTACAAAAC	TEX13B
P32	CTGTTCCCTGAGTTCCGTAG	
P33	TGCCTCCTGCACCACCAACT	GAPDH
P34	CGCCTGCTTCACCACCTTC	