

SUPPLEMENTARY MATERIAL

Figure S1

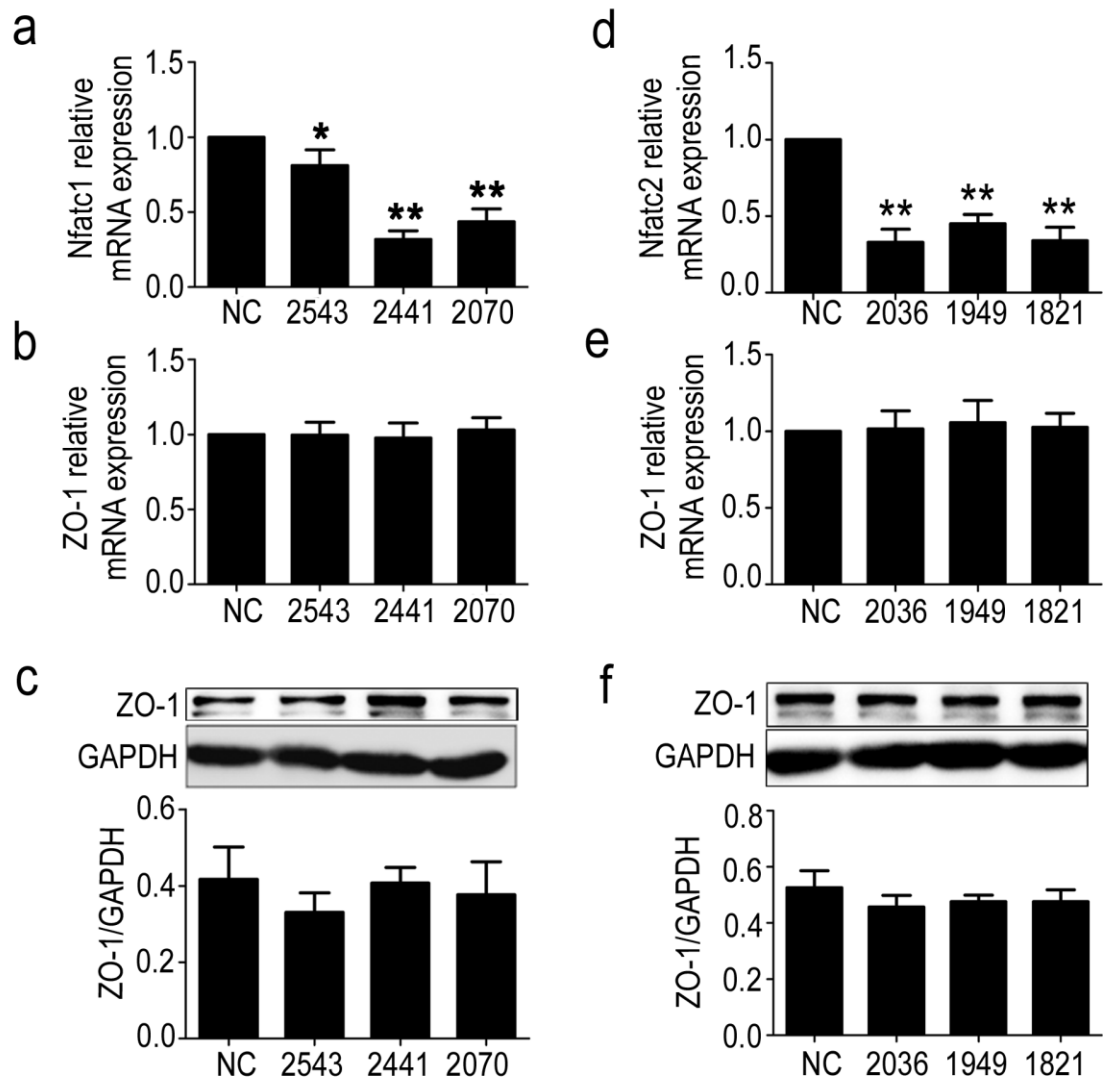


Figure S1. Effect of NFATC1 and NFATC2 interference on zonula occludens-1 (ZO-1)

expression. (a) NFATC1 interference assay. siNfatc1-2543, SiNfatc1-2441 and siNfatc1-2070 reduced Nfatc1 mRNA levels, (b) whereas did not influence levels of ZO-1 mRNA or (c) protein. (d) NFATC2 interference assay. siNfatc2-2036, siNfatc2-1949, and siNfatc2-1821 suppressed Nfatc2 mRNA expression, (e) whereas did not influence levels of ZO-1 mRNA or (f) protein. One-way ANOVA was used for repeated measurements.

Data are shown as the mean \pm SD, n = 3.* p < 0.05 and ** p < 0.01 vs. NC.

Figure S2

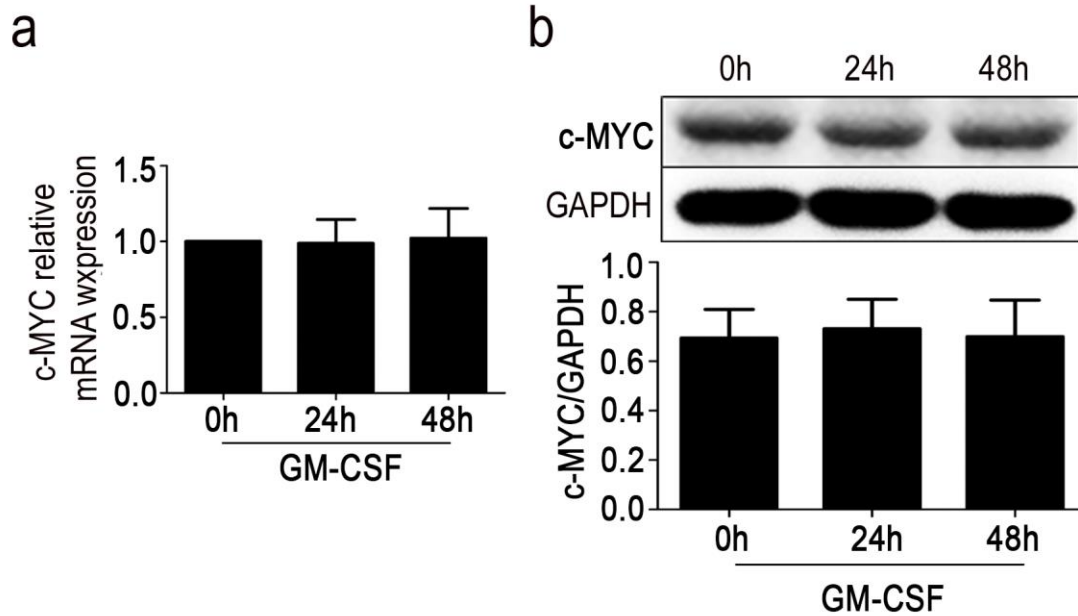


Figure S2. Effect of granulocyte/macrophage colony-stimulating factor (GM-CSF) on c-MYC expression. Levels of c-MYC mRNA (a) and protein (b) were detected in human brain microvascular endothelial cells (HBMECs) stimulated with 20 ng/ml GM-CSF for 24 or 48 h. One-way ANOVA was used for repeated measurements. Data are shown as the mean \pm SD, n = 3.

Figure S3

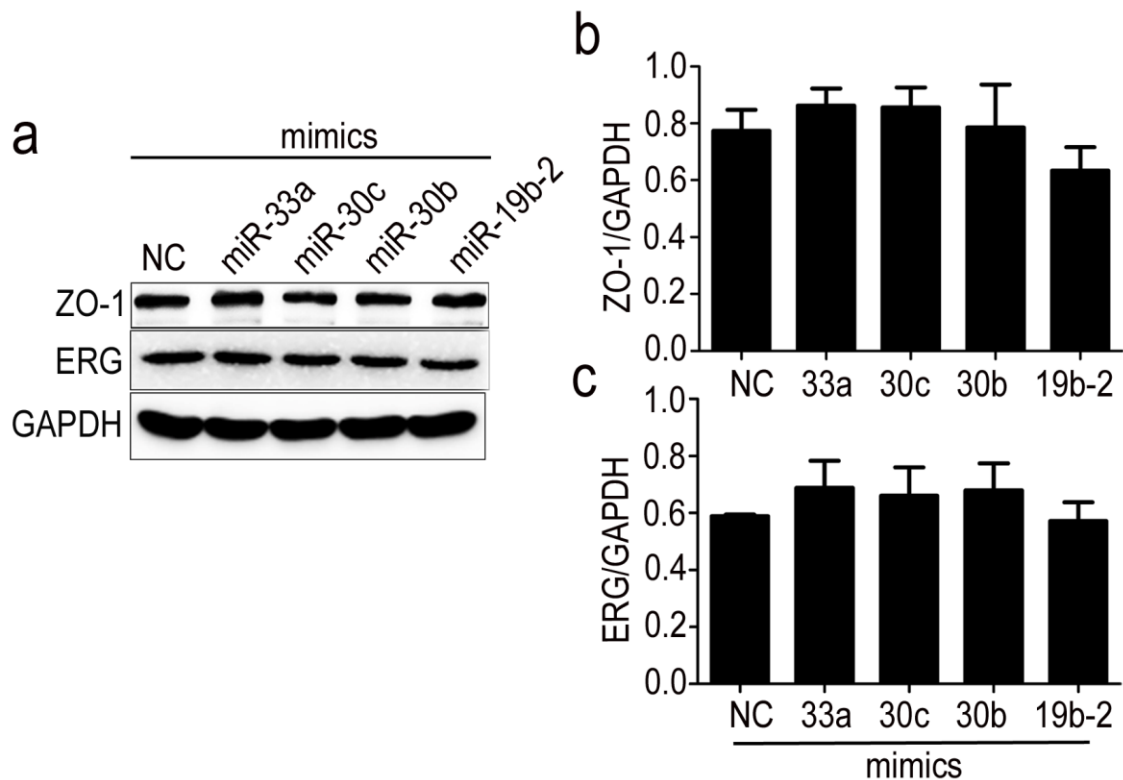


Figure S3. Effects of miR-33a, miR-30c, miR-30b and miR-19b2 on the erythroblast transformation-specific (ETS) transcription factor ERG and zonula occludens-1 (ZO-1) expression. Levels of ZO-1 (a, b) and ERG (a, c) protein were not suppressed by miR-33a, miR-30c, miR-30b or miR-19b2 in human brain microvascular endothelial cells (HBMECs). A two-tailed t-test ($\alpha = 0.05$) was used for repeated measurements. Data are shown as the mean \pm SD, $n = 3$.

Figure S4

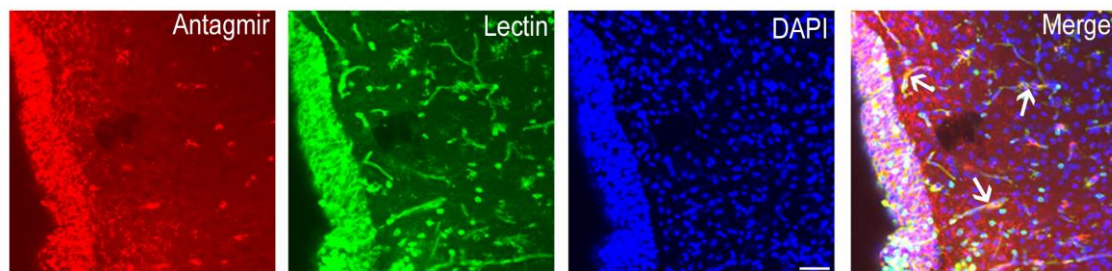


Figure S4. Diffusion of miR-96 antagomir-cy3 in the mouse brain corpus striatum 48 h after an intracerebroventricular injection. Vibratome slices (50 μm thick) of corpus striatum were stained with lectin (green) and 4,6-diamidino-2-phenyl-indole (DAPI) (blue). Diffusion of miR-96 antagomir-cy3 into brain microvessels was indicated by white arrows. Scale bar = 100 μm .

Table S1. siRNA sequences

Target gene	siRNA name	5'–3' siRNA sequence
Human ERG	605-sense	GAACAUCGAUGGGAAGGAATT
	605-antisense	UCCUUCUCCAUUGAUGUUCTT
	518-sense	GCUAUGGAGUACAGACCAUTT
	518-antisense	AUGGUCUGUACUCCAUAAGCTT
	165-sense	GACCAGUCGUUGUUUGAGUTT
	165-antisense	ACUCAACAACGACUGGUUCTT
Human NFATC1	2543-sense	GGUAACGCCAUCUUCUAATT
	2543-antisense	UUAGAAAGAUGGCGUACCTT
	2441-sense	CGGAAUCAGAGGAUAACCATT
	2441-antisense	UGGUUAUCCUCUGAUUCCGTT
	2070-sense	GAAACUCCGACAUUGAACUTT
	2070-antisense	AGUUCAAUGUCGGAGUUUCTT
Human NFATC2	2036-sense	GUCCAAAGUUGUGUUUACUTT
	2036-antisense	AGUAAACACAACUUUGGACTT
	1949-sense	GCCCAUGGUUGAAAGACAATT
	1949-antisense	UUGUCUUUCAACCAUGGGCTT
	1812-sense	GAGACGGACAUUGGAAGAATT
	1812-antisense	UUCUUCCAAUGUCCGUCUCTT
	1475-sense	UCUCCACACAUCAGCACAATT
	1475-antisense	UUGUGCUGAUGUGUGGAGATT

Human c-MYC	1094-sense	GCUUGUACCUGCAGGAUCUTT
	1094-antisense	AGAUCCUGCAGGUACAAGCTT
	587-sense	GCUUCACCAACAGGAACUATT
	587-antisense	UAGUUCCUGUUGGUGAAGCTT
Negative control	Sense	UUCUCCGAACGUGUCACGUTT
	antisense	ACGUGACACGUUCGGAGAATT

Table S2. Primers of real-time RT-PCR

Target	Oligonucleotide sequence	5'–3'	Nucleotide	GenBank
gene	forward, reverse	position		accession
ZO-1	TCAAAGGGAAAGCCTCCTGA	4673–4692		NM_001330239.1
	ATACTGCGAGGGCAATGGAG	4780–4761		
ERG	CGCCGACATCCTTCTCTCAC	676–695		NM_001331025.1
	CCGTGGAGAGTTTTGTAAGGCT	775–754		
c-MYC	GCGAACACACAACGTCTTGG	1638–1657		NM_002467.4
	ACTACCTTGGGGGCCTTTTC	1751–1732		
NFATC1	GTGGTTGAGATCCCGCCATT	2048–2067		NM_001278675.1
	GTGAAACGCTGGTACTGGCT	2154–2135		
NFATC2	GGTCAGTCAAGGTCAGAGGC	2250–2269		NM_001258297.1
	CTTTGGCTCGTGGCATTCTG	2324–2305		
GAPDH	AGGCTGGGGCTCATTTGCAG	542–561		NM_001256799.2
	TGGTGGTGCAGGAGGCATTG	685–666		

Table S3. Primers for CHIP and re-CHIP

Name	Target	Oligonucleotide sequence	5'–3'	PCR
	gene	forward, reverse	Nucleotide position	products
ChIP	ZO-1	GCAGCTCTGGCGGACAT	-2804– –2787	
		CTCGCTCTCGGGAGATGTTTA	-2701– –2681	124bp
Re-ChI	ZO-1	GCCTAGAACTAGGTGTGGTGTC	-107– –86	
P		CTAGCATTAAATTACACATTGGGC	0 – +22	130bp