Supplemental Material

MicroRNA-329 suppresses angiogenesis by targeting CD146

Ping Wang[1,2], Yongting Luo[1], Hongxia Duan[1,2], Shu Xing[1,2], Jianlin Zhang[1,2], Di Lu[1], Jing Feng[1], Dongling Yang[1], Lina Song[1], Xiyun Yan[1,*]

[1] Key Laboratory of Protein and Peptide Pharmaceutical, CAS-University of Tokyo Joint Laboratory of Structural Virology and Immunology, Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Beijing 100101, China

[2] University of Chinese Academy of Sciences, 19A Yuquan Road, Beijing 100049, China

***Corresponding author:**

Xiyun Yan

Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Beijing 100101, China

E-mail: yanxy@ibp.ac.cn

Tel: +86 10 64888583

Fax: +86 10 64888584

18 **Table S1** Primer sequences.

Table S1 Primer sequences

underlined = mutations **bold** = restriction sites

Primers for cloning		Primer sequence (5' to 3')
CD146 for pCR3.1-CD146-3'UTR	Sense	CCC**AAGCTT**ATGGGGCTTCCCAGGCTG
	Antisense	CCG**GAATTC**CTACTTGTCATCGTCATC
CD146 3'UTR for pCR3.1-CD146-3'UTR	Sense	CCG**GAATTC**CCCCGAATCACTTCAGC
	Antisense	TGC**TCTAGA**TATGATTTCTGGGACAATTAAG
CD146 3'UTR for Luc-3'UTR	Sense	CGG**GGTACC**CCCCGAATCACTTCAGCTCCCTT
	Antisense	GA**AGATCT**TATGATTTCTGGGACAATTAAGCTTTATTTTTC
CD146 3'UTR-Mut-N1	Sense	CGGGCCTGGCTAGAGCTTCGCCACACAGTGTCTGTCTGTGTGTATGCATA
	Antisense	TATGCATACACACAGACAGACACTGTGTGGCGAAGCTCTAGCCAGGCCCG
CD146 3'UTR-Mut-N2	Sense	CGGGTGTGTGTGTCTGTCTCACACAATGCATACATATGTGTGTATAT
	Antisense	ATATACACACATATGTATGCATTGTGTGAGACAGACACACACACCCG
CD146 3'UTR-Mut-N3	Sense	GTGTGTATGCATACATATCACACAATATATGGTTTTGTCAGGTG
	Antisense	CACCTGACAAAACCATATATTGTGTGATATGTATGCATACACAC
CD146 3'UTR-Mut-N4	Sense	TGTGTGTATATATGGTTTTGTGTCCACACAAAATTTGCAAATTGTTTCCTT
	Antisense	AAGGAAACAATTTGCAAATTTTGTGTGGACACAAAACCATATATACACACA
CD146 3'UTR-Mut-P4	Sense	TATGCATACATATGTGTGTAAAAGAGGTTAACCAGGTGTGTTAATTTGCAAA
	Antisense	TTTGCAAATTAACACACCTGGTTAACCTCTTTTACACACATATGTATGCATA
Primers for qRT-PCR		**Primer sequence (5' to 3')**
CD146	Sense	TCAACGGCACGGCAAGTG
	Antisense	AGGCCGTGCATTCAACACC
VEGF	Sense	GAGGGCAGAATCATCACGAA
	Antisense	CATGGTGATGTTGGACTCCT
ICAM-1	Sense	CTGCAGACAGTGACCATC
	Antisense	GTCCAGTTTCCCGGACAA
IL-8	Sense	AAGAAACCACCGGAAGGAACC
	Antisense	GTGTTGGCGCAGTGTGGTC
MMP-2	Sense	AGTCTGAAGAGCGTGAAG
	Antisense	CCAGGTAGGAGTGAGAATG
MMP-9	Sense	AACCAATCTCACCGACAG
	Antisense	AAAGGCGTCGTCAATCAC
GAPDH	Sense	TGCACCACCAACTGCTTAGC
	Antisense	GGCATGGACTGTGGTCATGAG
mCD146	Sense	TCAATGGTTCGGCAACTGAATGGA
	Antisense	GGGAGTTGGAGGCTGTACACTCTGCACC
mGAPDH	Sense	CTCACTCAAGATTGTCAGCA
	Antisense	GTCTTCTgGGTGGCAGTGAT
Primers for ChIP		**Primer sequence (5' to 3')**
site 1	Sense	AACTCTCAGTATCCAATCCATCCTC
	Antisense	CAGAGAGTGCATTTAGTGCCCAGAA
site 2	Sense	TCATGGTTGATGTCTGACTTGTGCA
	Antisense	GGTAGCCCATCTCACACAAATCCCT
site 3	Sense	AGGGATTTGTGTGAGATGGGCTACC
	Antisense	AGAGGTGGACATTGGTATAAACAGA
site 4	Sense	CATTGAAGAGTTGCTTTCCTGTGTTGA
	Antisense	AGGAGTTGTTTTGGTCAACCACCTCCCTA
site 5	Sense	GCTTTGTTCATGAAGATGGCATGGTGGTTA
	Antisense	CCCAGATCTTACCTATTAGTGAGCTC
site 6	Sense	GGCACACAGCAGGCACTTAATCAG
	Antisense	CAGGAGTCTCGTCTTCATGGTCGG
site 7	Sense	CCATGACACCCGAAGAGATGATTTT
	Antisense	GCGTAGCGTAGTCAACCACGTCATT
site 8	Sense	AAAAGGGTTCAGCCACACTGCATGTC
	Antisense	TACCCCGAGGCAGTACTTGTACCAGAA
site 9	Sense	GTTGGCCATGCTTCATATGTTTTAC
	Antisense	CAGCACAAAGGAGCCCCTCAAGAAT

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Table S2 Screening for miRNAs regulating CD146.

Table S2

Screening for miRNAs regulating CD146

Color	Inhibition
	0
	10%-30%
	30%-50%
	50%-80%

No.	miRNAs	Regulation of CD146 in endothelium tested by western blot	Regulation of CD146 3'UTR tested by luciferase assay
1	miR-17		
2	miR-93		
3	miR-106a		
4	miR-106b		
5	miR-20b		
6	miR-20a		
7	miR-92a		
8	let-7a-2		
9	let-7d		
10	miR-221		
11	miR-122a		
12	miR-34a		
13	miR-96		
14	miR-200b		
15	miR-9		
16	miR-24		
17	miR-34c		
18	miR-199a-5p		
19	miR-107		
20	miR-130a		
21	miR-21		
22	miR-31		
23	miR-185		
24	miR-25		
25	miR-30c		
26	miR-138-2		
27	miR-143		
28	miR-181a		
29	miR-373		
30	miR-342-5P		
31	miR-527		
32	miR-663		
33	miR-632		
34	miR-149		
35	miR-329		

Table S3 Algorithmic prediction of putative NF-κB binding sites within the miR-329 promoter.

Table S3 Putative NF-κB binding sites within the miR-329 promoter

putative binding sites	1	2	3	4	5	6	7	8	9
start position from TSS	-2879	-2800	-2619	-2502	-2354	-2005	-1527	-468	-308
end position from TSS	-2870	-2791	-2609	-2493	-2345	-1996	-1518	-459	-299
sequence	GGCAATTTCT	GGGAGTTTTC	GGGGGACCCAC	AGGGTTTTCC	GGAAATGCCC	GGGACTTTCG	GGGAAATCGC	GGAAAGTCTCC	GGGGCTTTCT

TSS, transcription start site

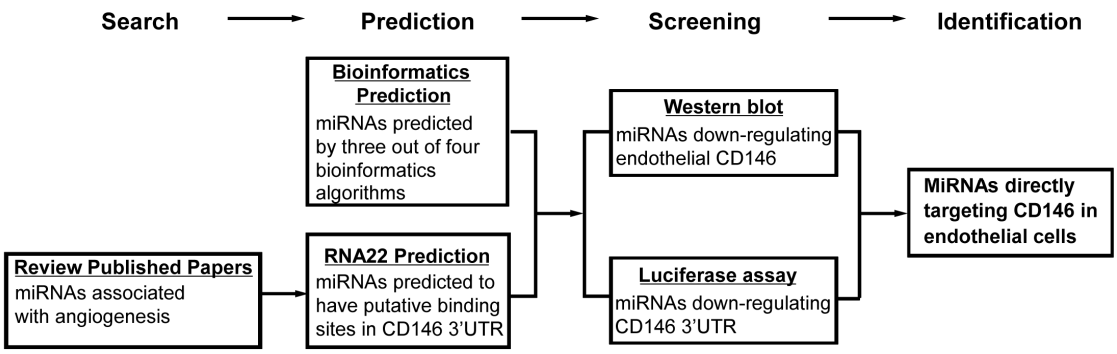


FIG S1. Two screening strategies for miRNAs targeting endothelial CD146.

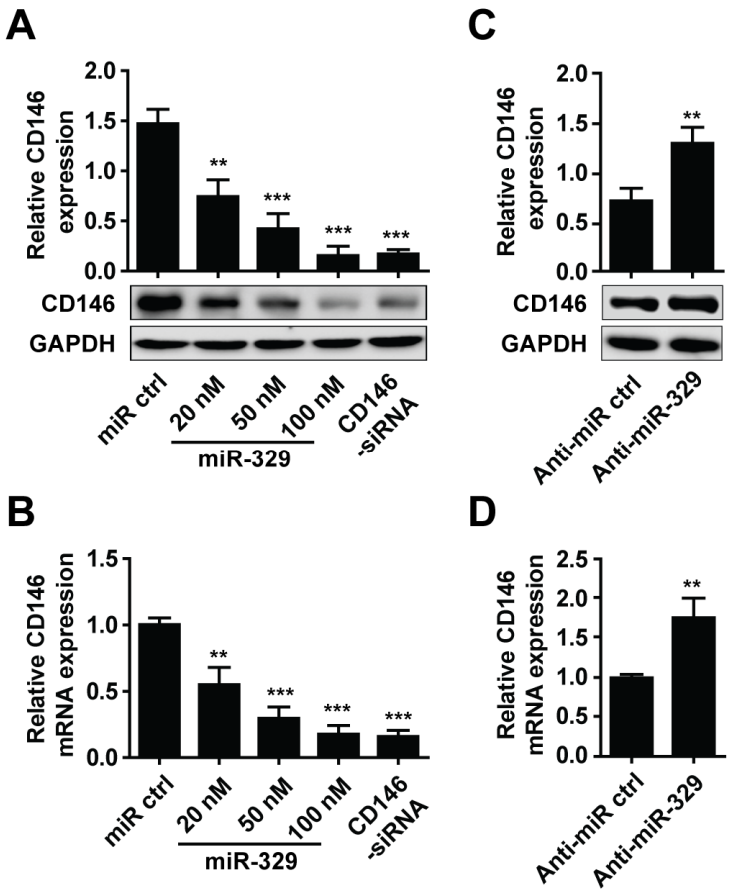


FIG S2. miR-329 down-regulates CD146 in HMECs. (A and B) Different doses of miR-329 mimics or control miRNA (miR ctrl) were transfected into HMECs. The protein and mRNA levels of CD146 were measured by western blot (A) or quantitative real-time PCR (B), respectively. GAPDH was used as an internal control. The mRNA expression was relative to that in HMECs transfected with control miRNA. (C and D) miR-329 inhibitor (anti-miR-329) or control anti-miRNA (anti-miR ctrl) (50 nM) was transfected into HMECs. The protein and mRNA expression of CD146 were analyzed by western blot (C) or quantitative real-time PCR (D), respectively. GAPDH was used as an internal control. The mRNA expression was relative to that in HMECs transfected with control anti-miRNA. ***P*<.01, ****P*<.001. Error bars represent ± s.d. Data are representative of three independent experiments.

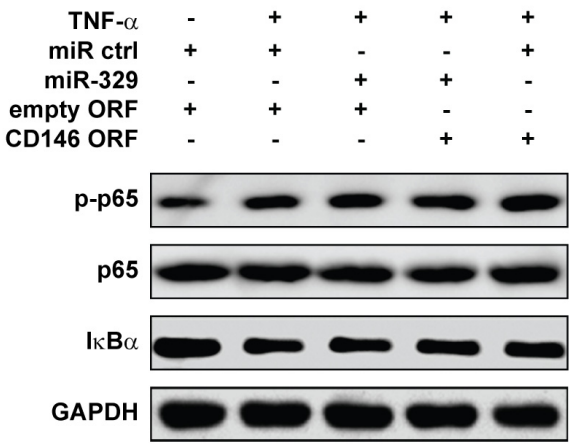
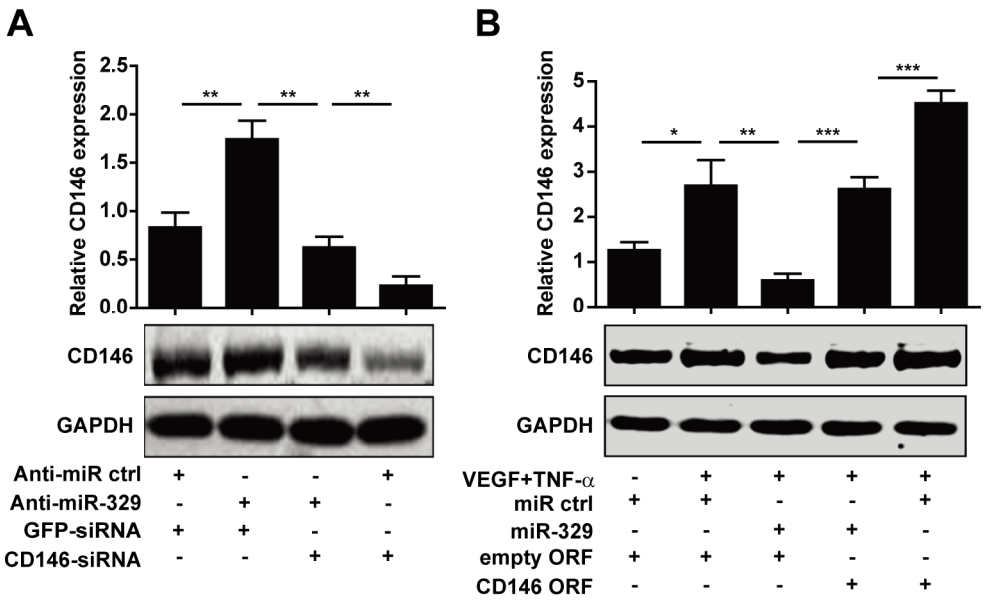


FIG S3. miR-329 does not impair TNF-α-induced NF-κB activation. NF-κB activation (NF-κB p65 phosphorylation and IκBα degradation) induced by TNF-α was determined by western blot after HUVECs were transfected with miR-329 or co-transfected with CD146 ORF (open reading frame without a 3'UTR). GAPDH was used as an internal control for IκBα. Total p65 served as an internal control for p-p65.



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54 **FIG S4.** miR-329 regulates CD146 expression in HUVECs. (A) HUVECs were
55 transfected with anti-miR-329 or co-transfected with CD146 siRNA as indicated. The
56 expression of CD146 was measured by western blot. GAPDH served as an internal
57 control. (B) HUVECs were transfected with miR-329 or co-transfected with CD146
58 ORF (open reading frame without 3'UTR) as indicated. Protein levels of CD146 were
59 determined by western blot. GAPDH was used as an internal control. **P*<.05, ***P*<.01,
60 ****P*<.001. Results are presented as the mean ± s.d. of three independent assays.

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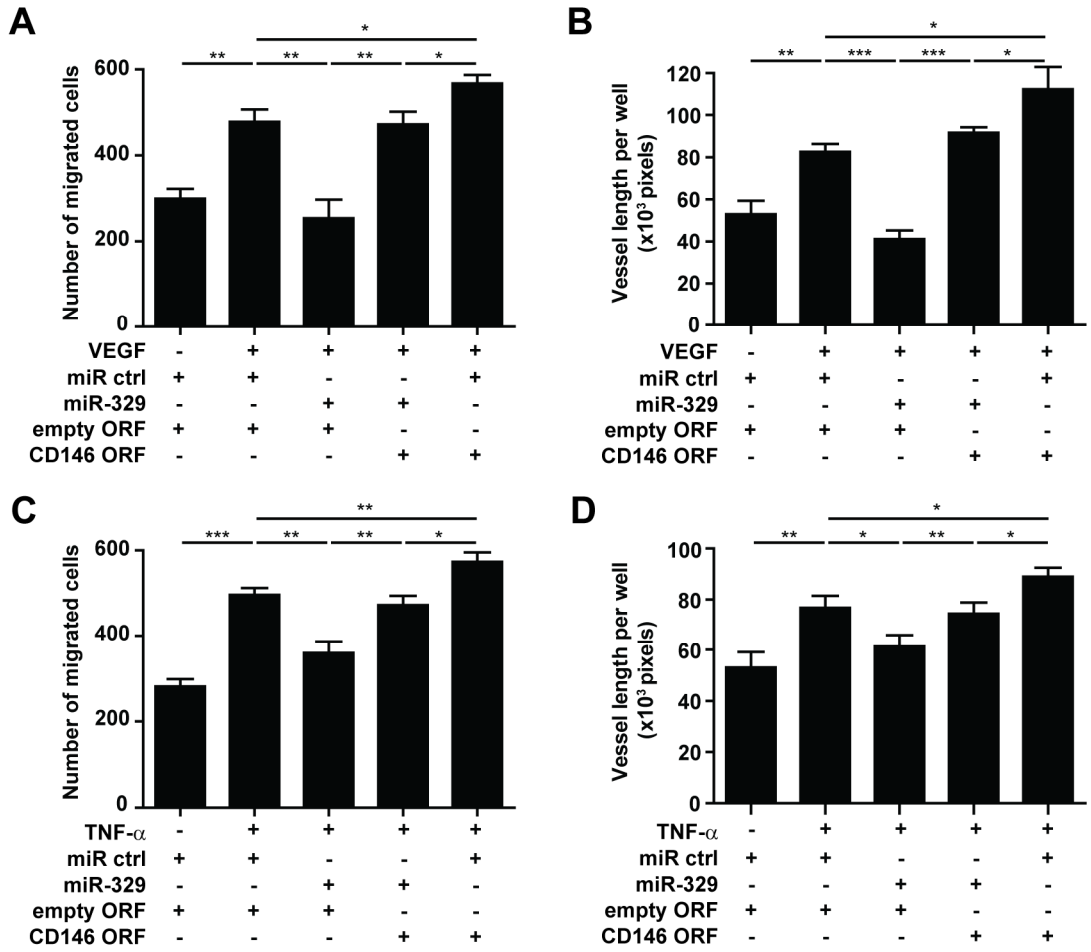

FIG S5. miR-329 inhibits VEGF- and TNF-α-induced endothelial cell tube formation and migration. VEGF-induced cell migration (A) or tube formation (B) was measured after HUVECs were transfected with miR-329 or co-transfected with CD146 ORF. The number of migrated cells or the length of the vessels was measured from 3 independent assays. TNF-α-induced cell migration (C) or tube formation (D) was determined in HUVECs with transfections as indicated. The number of migrated cells or the length of the vessels was quantified and presented in the histogram. **P*<.05, ***P*<.01, ****P*<.001. Results are presented as the mean ± s.d. of three independent assays.