MiR-338-3p inhibits epithelial-mesenchymal transition in gastric cancer cells by targeting ZEB2 and MACC1/Met/Akt signaling

Supplementary Material

Supplementary Table 1

The sequences of primer used for dual-luciferase reporter plasmid.

Primer	Forward (5'-3')	Reverse (5'-3')
ZEB2	TAAACTACTGCATTTTAAGC	ACTGAAAAACTGTCTTAGAAAATCA
	TTCCTATTT	CG
Mut ZEB2	AATTTCTTTTGAAAATGACC	AGTTTGGCTACATTTTTATT <u>GCTCGT</u>
	<u>TACGAGC</u> AATAAAAATGTA	<u>A</u> GGTCATTTTCAAAAGAAATT
	GCCAAACT	
MACC1	CCGCTCGAGGGAGCTGAAT	ATAAGAATGCGGCCGCCTGCCTCAG
	AGGTGCAGAT	CCTCCTGAGTA
Mut MACC1	CCACCCTGGGATATTTGGTA	AATTTGAATTACCATTG <u>GTG</u> GCATAC
	TGC <u>CAC</u> CAATGGTAATTCAA	CAAATATCCCAGGGTGG
	ATT	

Supplementary Table 2 $\label{eq:Table 2}$ The sequences of primer used for qRT-PCR.

Primer	Forward (5' -3')	Reverse (5' -3')		
E-cadherin	CTGCTGCAGGTCTCCTCTTG	TGTCGACCGGTGCAATCTTC		
N-cadherin	ACAGTGGCCACCTACAAAGG	CCGAGATGGGGTTGATAATG		
Fibronectin	GACCACATCGAGCGGATCTG	GTCTCTTGGCAGCTGACTCC		
Vimentin	AAGGCGAGGAGAGCAGGATT	GGTCATCGTGATGCTGAGAAG		
ZEB2	CTCTTCCCACACGCTTAGTT	GGCCTAAGCTTACAGTGTCATG		
β-actin	TGGATCAGCAAGCAGGAGTA	TCGGCCACATTGTGAACTTT		

Supplementary Results

Supplementary Table 3

Clinicopathological characteristics of GC patients

Case	Age	Gender	Histological differentiation	Clinical stage	T classification	N classification	Metastasis
1	74	Male	Moderately	IV	T4b	N1	Yes
2	53	Male	Poorly	IIIC	T4b	N3	No
3	71	Male	Moderately	IIB	T4a	N0	No
4	55	Male	Poorly	IV	T4a	N1	Yes
5	69	Male	Poorly	IIA	T2	N1	No
6	44	Male	Poorly	IIIB	T4a	N2	No
7	62	Female	Well	IA	T1b	N0	No
8	46	Male	Poorly	IIA	T1b	N2	No
9	70	Male	Poorly	IIB	T2	N2	No

10	79	Male	Poorly	IB	T2	N0	No
11	41	Male	Poorly	IA	T1b	N0	No
12	56	Male	Well	IB	T2	N0	No
13	77	Male	Poorly	IIIC	T4a	N3	No
14	57	Male	Poorly	IIIB	T4b	N1	No
15	73	Male	Moderately	IIIA	T4a	N1	No
16	51	Female	Poorly	IIIA	T4a	N1	No
17	61	Male	Moderately	IIA	T2	N1	No
18	58	Female	Poorly	IIIB	T4a	N2	No
19	78	Male	Moderately	IIIC	T4b	N2	No
20	48	Male	Poorly	IIIA	T4a	N1	No

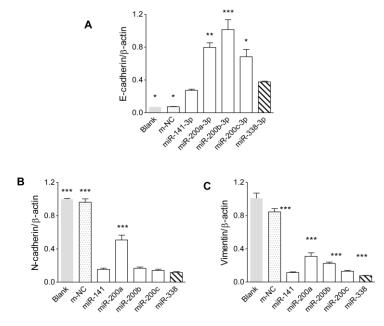


Figure S1. E-cadherin, N-cadherin, and vimentin mRNA expression levels in MKN-28 cells transfected with miR-200 family (miR-200a-3p, miR-200b-3p, miR-200c-3p and miR-141-3p) mimics or miR-338-3p mimics by qRT-PCR.

(A) Compared with miR-338-3p, the expression of E-cadherin mRNA was significantly increased in MKN-28 cells transfected with miR-200b-3p, miR-200a-3p, or miR-200c-3p. **(B** and **C)** N-cadherin and vimentin mRNA levels in miR-338-3p-transfected cells were lower than the levels in miR-200a-3p-transfected cells.

*P < 0.05, **P < 0.01 and ***P < 0.001.

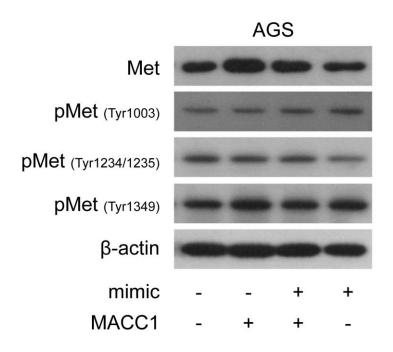


Figure S2. Western blot analysis of MACC1, Met and p-Met protein expression in AGS cells.

MiR-338-3p decreased Met and pMet (Tyr1234/1235) expression. Restoring MACC1 expression increased the protein expression of Met and pMet (Tyr1234/1235) in mimic transfected cells.

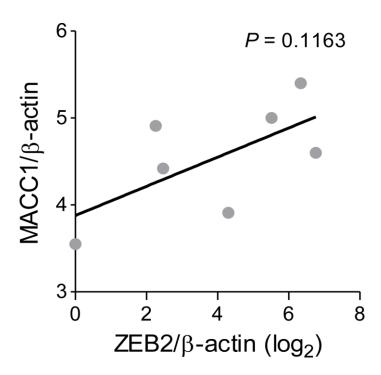


Figure S3. No siginificance correlation was found between MACC1 and ZEB2.