

miRNA-337-3p inhibits gastric cancer progression through repressing myeloid zinc finger 1-facilitated expression of matrix metalloproteinase 14

Supplementary Materials

Supplementary Table S1: MZF1 and MMP-14 expression in human gastric cancer tissues

Clinicopathologic factor	Total	MZF1 expression		MMP-14 expression	
	<i>n</i> (%)	<i>n</i> (%)	<i>P</i> -value	<i>n</i> (%)	<i>P</i> -value
Age (years)					
≤ 60	26 (52.0)	16 (61.5)	0.817	18 (69.2)	0.902
> 60	24 (48.0)	14 (58.3)		17 (70.8)	
Sex					
Male	35 (70.0)	21 (60.0)	1.000	24 (68.6)	0.736
Female	15 (30.0)	9 (60.0)		11 (73.3)	
Size (diameter)					
≤ 6 cm	33 (66.0)	20 (60.1)	1.000	23 (69.7)	0.948
> 6 cm	17 (34.0)	10 (58.8)		12 (70.6)	
Laurén classification					
Intestinal type	29 (58.0)	17 (58.6)	0.418	20 (68.9)	0.851
Diffuse type	21 (42.0)	13 (61.9)		15 (71.4)	
Gastric wall invasion					
T1/T2	18 (36.0)	2 (11.1)	< 0.001	6 (33.3)	< 0.001
T3/T4	32 (64.0)	28 (87.5)		29 (90.6)	
Lymph node metastasis					
Negative	18 (36.0)	4 (22.2)	< 0.001	5 (27.8)	< 0.001
Positive	32 (64.0)	26 (81.3)		30 (93.8)	
Distant metastasis					
Negative	38 (76.0)	19 (50.0)	0.029	23 (60.5)	0.009
Positive	12 (24.0)	11 (91.7)		12 (100.0)	
TNM stage					
I/II	17 (34.0)	3 (17.6)	< 0.001	5 (29.4)	< 0.001
III/IV	33 (66.0)	27 (81.8)		30 (90.9)	

MZF1, myeloid zinc finger 1; MMP-14, matrix metalloproteinases 14; TNM, tumor-node-metastasis.

Supplementary Table S2: Correlation between the expression of MZF1 and MMP-14

	MZF1 expression		<i>R</i> -value	<i>P</i> -value
	Low	High		
MMP-14 expression				
Low	18	2	0.500	< 0.001
High	12	18		

MZF1, myeloid zinc finger 1; MMP-14, matrix metalloproteinases 14; Pearson's correlation coefficient was applied to determine the expression correlation.

Supplementary Table S3: Comparison of survival curves of gastric cancer patients

Comparison	Chi-square	Bonferroni <i>P</i> -value
MZF1-low v.s. MZF1-high	56.33	< 1.0×10^{-10}
MZF1-low v.s. MMP-14-low	1.49	1
MZF1-low v.s. MMP-14-high	30.61	1.6×10^{-7}
MZF1-low v.s. miR-337-3p-low	46.26	< 1.0×10^{-10}
MZF1-low v.s. miR-337-3p-high	0.02	1
MZF1-high v.s. MMP-14-low	44.17	< 1.0×10^{-10}
MZF1-high v.s. MMP-14-high	5.36	0.1029
MZF1-high v.s. miR-337-3p-low	0.38	1
MZF1-high v.s. miR-337-3p-high	55.72	< 1.0×10^{-10}
MMP-14-low v.s. MMP-14-high	30.41	1.8×10^{-7}
MMP-14-low v.s. miR-337-3p-low	37.09	< 1.0×10^{-10}
MMP-14-low v.s. miR-337-3p-high	1.27	1
MMP-14-high v.s. miR-337-3p-low	2.71	0.4992
MMP-14-high v.s. miR-337-3p-high	31.24	1.1×10^{-7}
miR-337-3p-low v.s. miR-337-3p-high	46.35	< 1.0×10^{-10}

MZF1, myeloid zinc finger 1; MMP-14, matrix metalloproteinases 14; Bonferroni method was applied for the comparison of survival curves.

Supplementary Table S4: Univariate and multivariate analysis of prognostic factors in gastric cancer patients

Variables	<i>n</i>	Univariate analysis		Multivariate analysis		
		Mean ± SEM (months)	<i>P</i> -value	Hazard ratio	95% CI	<i>P</i> -value
Age (years)						
≤ 60	26	35.4 ± 4.9	0.902	1.017	0.482–3.143	0.765
> 60	24	36.7 ± 5.1				
Sex						
Male	35	37.0 ± 4.2	0.872	1.397	0.427–3.465	0.675
Female	15	33.3 ± 6.6				
Size (diameter)						
≤ 6 cm	33	37.2 ± 4.2	0.896	1.807	1.311–5.162	0.467
> 6 cm	17	33.9 ± 6.4				
Laurén classification						
Intestinal type	29	41.2 ± 4.3	0.146	1.596	0.352–4.233	0.545
Diffuse type	21	28.9 ± 5.7				
Gastric wall invasion						
T1/T2	18	64.7 ± 0.2	< 0.001	2.218	1.682–6.924	0.332
T3/T4	32	18.9 ± 2.5				
Lymph node metastasis						
Negative	18	61.5 ± 0.3	< 0.001	2.018	1.339–5.756	0.431
Positive	32	20.7 ± 2.5				
Distant metastasis						
Negative	38	44.8 ± 3.6	< 0.001	2.052	1.976–5.513	0.002
Positive	12	8.1 ± 0.5				
TNM stage						
I/II	17	64.7 ± 0.2	< 0.001	2.722	1.976–6.713	0.005
III/IV	33	20.5 ± 2.8				
MZF1 expression						
Low	30	52.3 ± 3.4	< 0.001	3.516	1.537–8.942	0.018
High	20	11.6 ± 1.3				
miR-337-3p expression						
Low	22	59.3 ± 2.8	< 0.001	0.393	0.128–0.705	0.015
High	28	20.0 ± 3.1				
MMP-14 expression						
Low	20	13.6 ± 2.4	< 0.001	2.319	1.275–6.334	0.028
High	30	53.5 ± 3.3				

MZF1, myeloid zinc finger 1; MMP-14, matrix metalloproteinases 14; TNM, tumor-node-metastasis; Log-rank test and Cox regression model were applied for univariate and multivariate analysis. *n*, number of patients; SEM, standard error of the mean; 95% CI, 95% confidence interval.

Supplementary Table S5: Oligonucleotide sets used for constructs, inhibitors and short hairpin RNAs

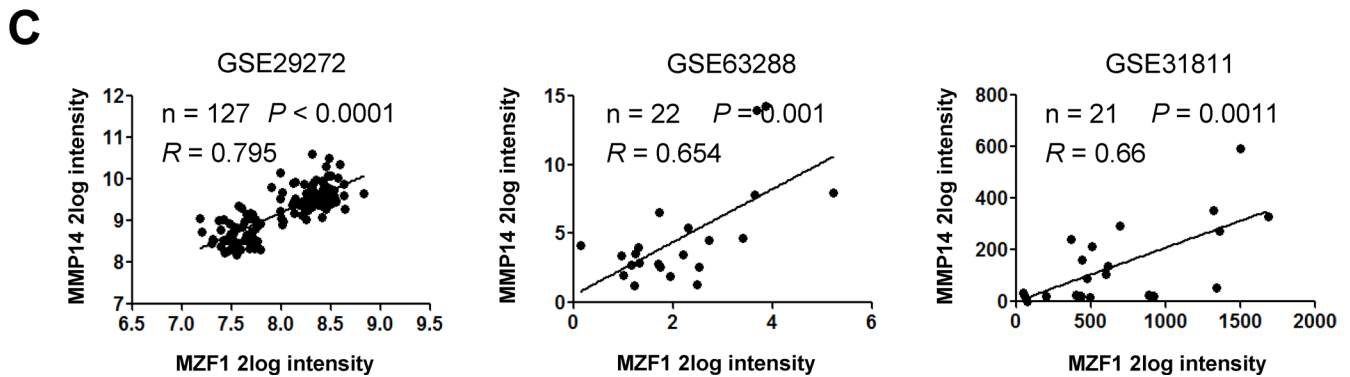
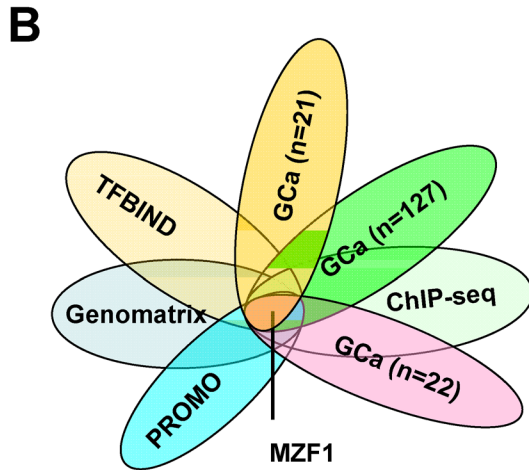
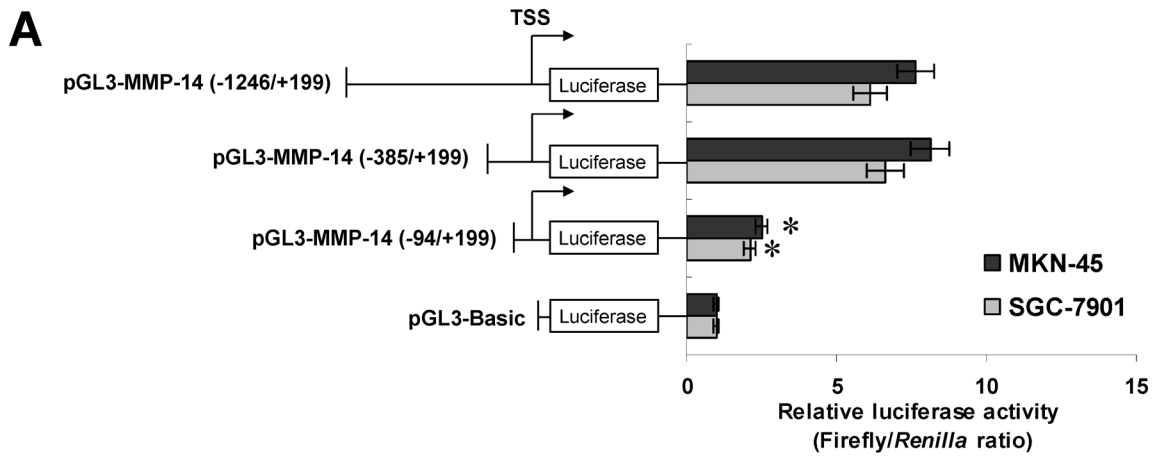
Oligo Set	Sequences
Pre-miR-337-3p	5'-TGCTGCTCCTATATGATGCCTTTCTTCGTTTTGGCCACTGACTGACGAAGAAAGGCATC ATATAGGAG-3'(sense); 5'-CCTGCTCCTATATGATGCCTTTCTTCGTCAGTCAGTGGCCAAAACGAAGAAAGGCATCA TATAGGAGC-3'(antisense)
Pre-miR-NC	5'-TGCTGAAATGTACTGCGCGTGGAGACGTTTTGGCCACTGACTGACGTCTCCACGCAG TACATTT-3'(sense); 5'-CCTGAAATGTACTGCGTGGAGACGTCAGTCAGTGGCCAAAACGTCTCCACGCGCAGT ACATTT-3'(antisense)
pGL3-MMP14 mut (ΔMZF1)	5'-CTGGGGCTTTCACGGAGGAGAGGCTGTGGGAGAAGG-3'(sense); 5'-CTCCTCCGTGAAAGCCCCAGTGCCCTCCTTTCCTGGT-3'(antisense)
pGL3-MMP14 mut (ΔmiR-337-3p)	5'-CTGGGGCGGGGACGGAGGAGCAATTGTGTTCAAAGGGAGGGA-3'(sense); 5'-CTCCTCCGTCCCCGCCCCAGTGCCCTCCTT-3'(antisense)
Anti-NC	RiboBio
Anti-miR-337-3p	RiboBio
sh-Scb	5'-CCGGGCGAACGATCGAGTAAACGGACTCGAGTCCGTTTACTCGATCGTTTCGCTTTTT-3' (sense); 5'-AATTCAAAAAGCGAACGATCGAGTAAACGGACTCGAGTCCGTTTACTCGATCGTTTCGC-3' (antisense)
sh-MZF1-1	5'-CCGGCCACCAGAGCACCAAGCTCATCTCGAGATGAGCTTGGTGCTCTGGTGGTTTTTG-3' (sense); 5'-AATTCAAAAACCACCAGAGCACCAAGCTCATCTCGAGATGAGCTTGGTGCTCTGGTGG-3' (antisense)
sh-MZF1-2	5'-CCGGCCGTTGCGATGTATGTGGCAACTCGAGTTGCCACATACATCGCAACGGTTTTTG-3' (sense); 5'-AATTCAAAAACCGTTGCGATGTATGTGGCAACTCGAGTTGCCACATACATCGCAACGG-3' (antisense)
sh-AGO2-1	5'-CCGGGCACAGCCAGTAATCGAGTTTCTCGAGAAACTCGATTACTGGCTGTGCTTTTTG-3' (sense); 5'-AATTCAAAAAGCACAGCCAGTAATCGAGTTTCTCGAGAAACTCGATTACTGGCTGTGC-3' (antisense)
sh-AGO2-2	5'-CCGGCGTCCGTGAATTTGGAATCATCTCGAGATGATTCCAAATTCACGGACGTTTTTG-3' (sense); 5'-AATTCAAAAACGTCCGTGAATTTGGAATCATCTCGAGATGATTCCAAATTCACGGACG-3' (antisense)

Pre-miR-NC, negative control pre-miRNA; MMP-14, matrix metalloproteinases 14; MZF1, myeloid zinc finger 1; Anti-NC, negative control inhibitor; sh-Scb, scramble short hairpin RNA; AGO2, argonaute 2.

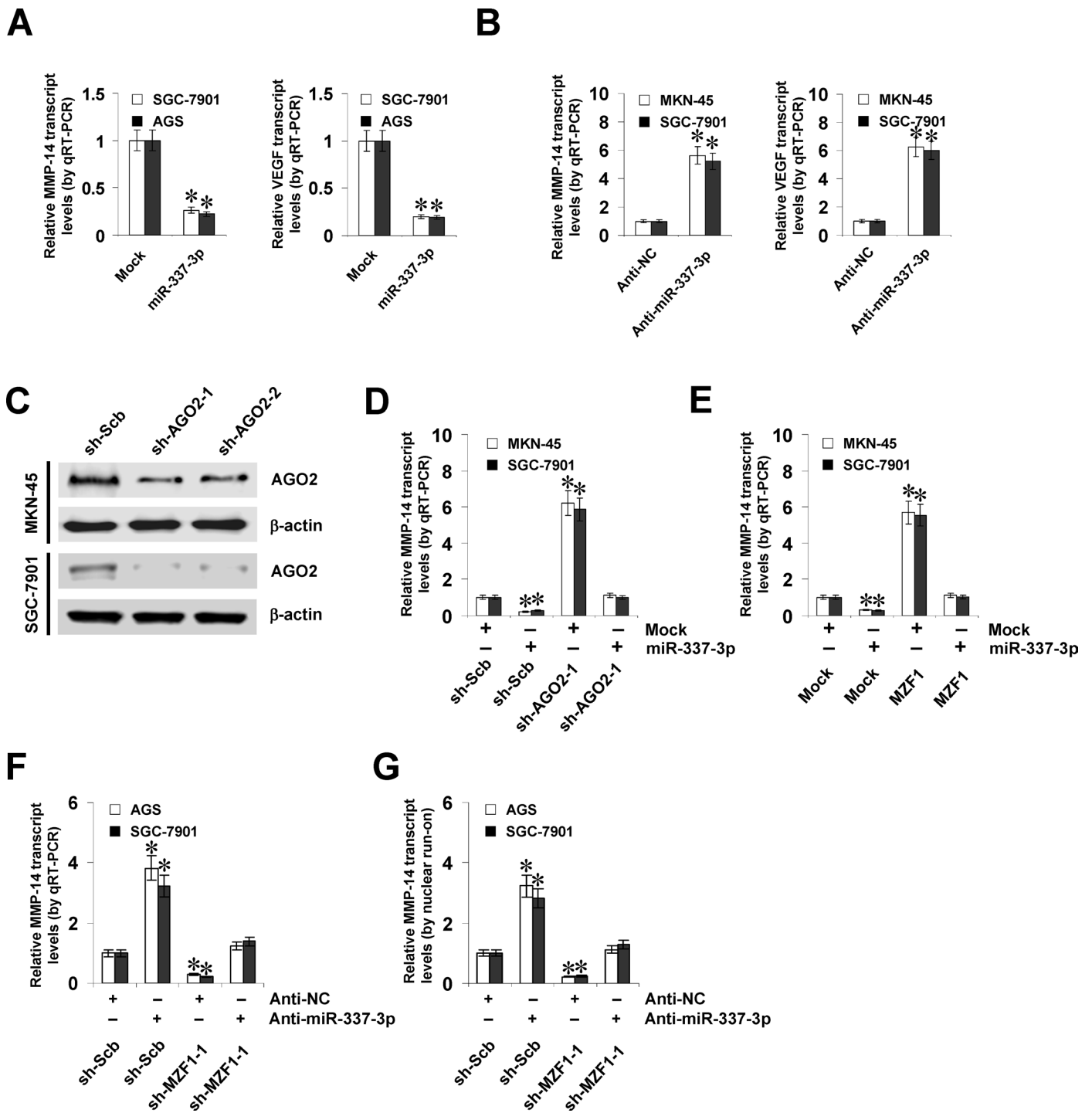
Supplementary Table S6: Primer sets used for qRT-PCR, nuclear run-on, and ChIP

Primer set	Primers	Sequence	Product size (bp)	Application
MMP-14	Forward	5'-GCCTTCTGTTCCCTGATAA-3'	225	qRT-PCR
	Reverse	5'-CCATCCTTCCTCTCGTAG-3'		nuclear run-on
VEGF	Forward	5'-ATGACGAGGGCCTGGAGTGT-3'	226	qRT-PCR
	Reverse	5'-CATTTACACGTCTGCGGATCT-3'		
MZF1	Forward	5'-CTGAAACTGAGCCTCCAACCTCC-3'	232	qRT-PCR
	Reverse	5'-CCAGTCTTGCTGTGGGGAAA-3'		
β-actin	Forward	5'-ATCTACGAGGGGTATGCC-3'	227	qRT-PCR
	Reverse	5'-TAGCTCTTCTCCAGGGAG-3'		
miR-337-3p	Forward	RiboBio		qRT-PCR
	Reverse	RiboBio		
U6	Forward	RiboBio		qRT-PCR
	Reverse	RiboBio		
MMP-14 set 1 (-326/-157)	Forward	5'-TCAAGCCACTCAGAATATGC-3'	170	ChIP
	Reverse	5'-ACCAAGAACTGGAAGGAAAA-3'		
MMP-14 set 2 (-122/+69)	Forward	5'-CAACCAGGAAAGGAGGGC-3'	191	ChIP
	Reverse	5'-TCGGCTTGAGTTAAAGG-3'		

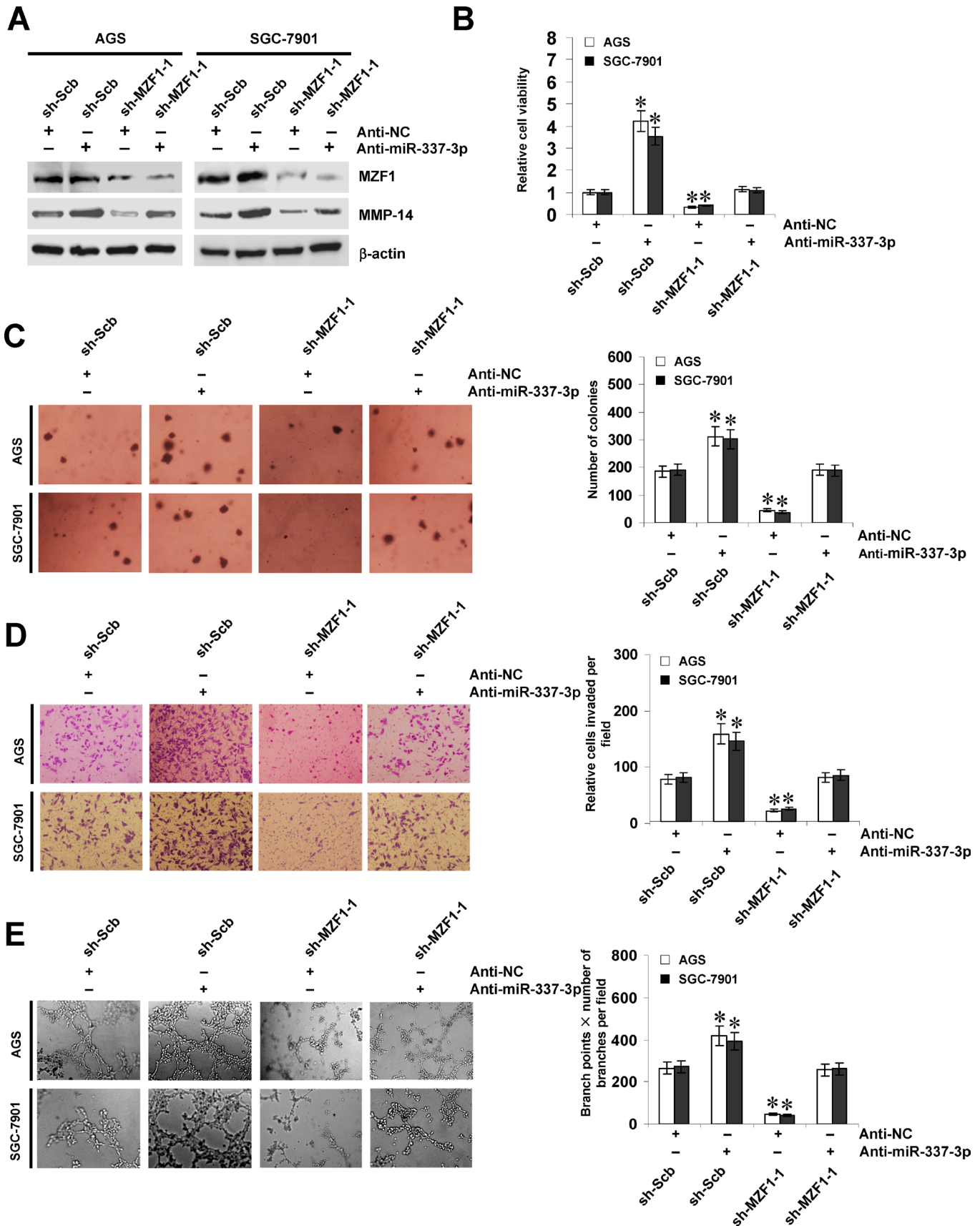
MMP-14, matrix metalloproteinase 14; VEGF, vascular endothelial growth factor; MZF1, myeloid zinc finger 1.



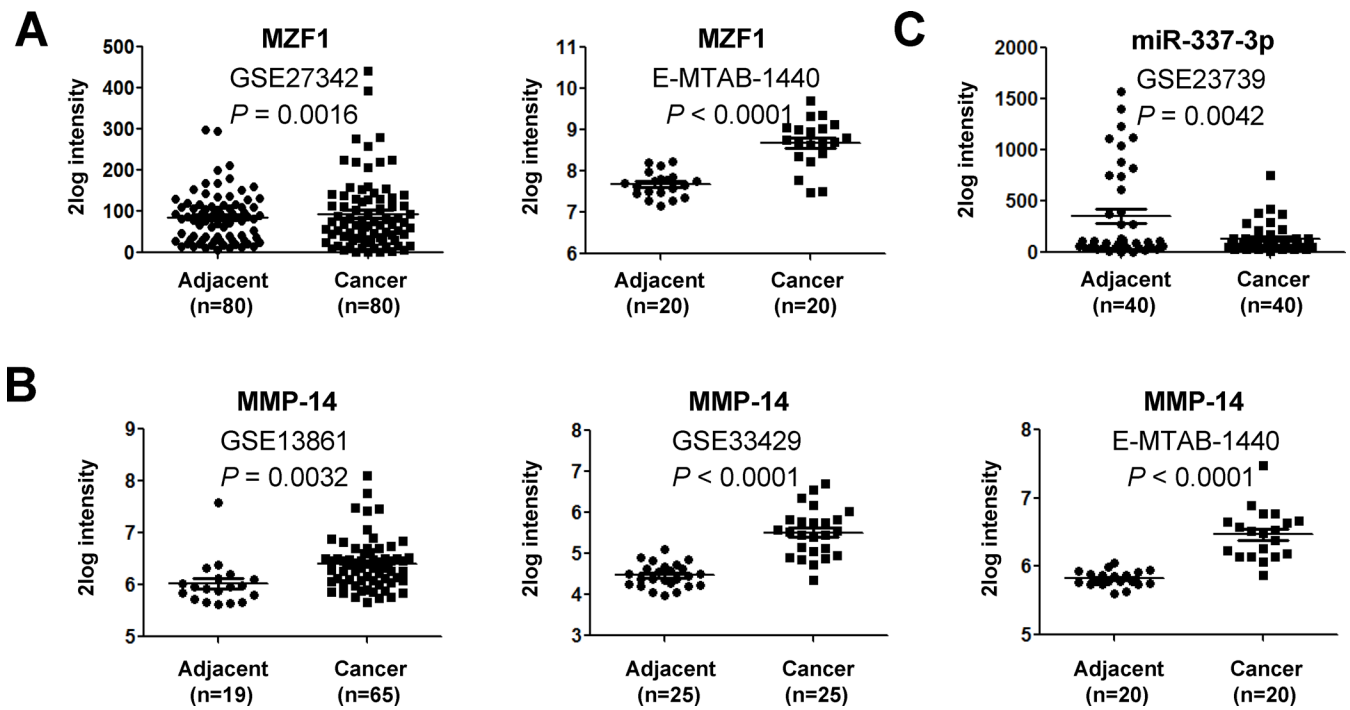
Supplementary Figure S1: Determining the transcription factor crucial for MMP-14 expression. (A), dual-luciferase assay showing the activity of different *MMP-14* promoter fragments in gastric cancer cells. (B), over-lapping analysis revealing MZF1 as a crucial transcription factor binding to the *MMP-14* promoter region (chr14:23305444-23305733). (C), the correlation between MZF1 and MMP-14 levels in gastric cancer cohorts derived from Gene Expression Omnibus (GEO) datasets. * $P < 0.01$ vs. pGL3-MMP-14 (-1246/+199).



Supplementary Figure S2: Expression of MMP-14 and VEGF in gastric cancer cells. (A and B), real-time quantitative RT-PCR showing the transcript levels of *MMP-14* and *VEGF* in gastric cancer cells transfected with empty vector (mock), miR-337-3p precursor, anti-NC (100 nmol/L), or anti-miR-337-3p inhibitor (100 nmol/L). (C), western blot indicating the AGO2 expression in gastric cancer cells transfected with scramble shRNA (sh-Scb) or AGO2 shRNA (sh-AGO2). (D and E), real-time quantitative RT-PCR showing the transcript levels of *MMP-14* in gastric cancer cells stably transfected with mock or miR-337-3p precursor, and those co-transfected with sh-Scb, sh-AGO2, mock, or MZF1. (F and G), real-time quantitative RT-PCR and nuclear run-on assays indicating the transcript levels and nascent transcription of *MMP-14* in gastric cancer cells transfected with anti-NC (100 nmol/L) or anti-miR-337-3p inhibitor (100 nmol/L), and co-transfected with sh-Scb or sh-MZF1. * $P < 0.01$ vs. mock, anti-NC, mock+sh-Scb, or anti-NC+sh-Scb.



Supplementary Figure S3: Effects of miR-337-3p and MZF1 on the growth, invasion, and angiogenesis of gastric cancer cells. Gastric cancer cells were transfected with anti-NC (100 nmol/L) or anti-miR-337-3p inhibitor (100 nmol/L), and co-transfected with sh-Scb or sh-MZF1. Western blot assay (A) showing the expression of MZF1 and MMP-14. MTT colorimetric (B), soft agar (C), matrigel invasion (D), tube formation (E) assays indicating the cellular viability, growth, invasion, angiogenesis capability, respectively. * $P < 0.01$ vs. anti-NC+sh-Scb.



Supplementary Figure S4: Expression of MZF1, MMP-14, and miR-337-3p in public databases. (A, B, and C), the MZF1, MMP-14, and miR-337-3p expression in gastric cancer and adjacent normal tissues derived from Gene Expression Omnibus (GEO) and ArrayExpress (<http://www.ebi.ac.uk/arrayexpress>) datasets.