miRNA-584-3p inhibits gastric cancer progression by repressing Yin Yang 1-facilitated MMP-14 expression

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Supplementary Figure Legends

Supplementary Figure S1 Effects of YY1 on MMP-14 expression. a, over-lapping analysis revealing YY1 as a crucial transcription factor of *MMP-14*. **b**, the correlation between *YY1* and *MMP-14* levels in public gastric cancer datasets derived from Gene Expression Omnibus (GEO). **c**, real-time quantitative RT-PCR showing the effects of YY1 and C646 on cellular *MMP-14* expression. * $P<0.01$ vs. mock.

Supplementary Figure S2 Expression of MMP-14 and VEGF in gastric cancer cells. Real-time quantitative RT-PCR indicating the *MMP-14* and *VEGF* levels in cancer cells with ectopic expression or knockdown of miR-584-3p (**a** and **b**), and those co-transfected with sh-Scb or sh-AGO2 (**c**). RIP and qPCR (**d**), western blot (**e**), and ChIP and qPCR (**f**) assays showing the interaction between miR-548-3p and AGO2, expression of H3K27me3 and H3K9me2, and protein

enrichment on *MMP-14* promoter in gastric cancer cells transfected with mock or miR-584-3p, and those co-transfected with sh-Scb, sh-AGO2, sh-YY1 or treated with GSK343 (1 μmol/L) or A-366 (3 μmol/L). * P<0.01 vs. mock, anti-NC, mock+sh-Scb, IgG, or mock+DMSO.

Supplementary Figure S3 Roles of YY1 and miR-584-3p in cancer cells. Real-time quantitative RT-PCR (**a**), western blot (**b**), dual-luciferase (**c**), ChIP (using YY1 antibody) and qPCR (**d**) assays showing the expression of MMP-14 and YY1, activity of *MMP-14* promoter reporter [pGL3-MMP14 (-1246/+199)], and YY1 binding to *MMP-14* promoter in cancer cells transfected with empty vector (mock) or miR-584-3p precursor, and those co-transfected with *YY1* (mean ± SD, n=4). *P<0.01 vs. mock+mock.

Supplementary Figure S4 Effects of miR-584-3p and YY1 on gastric cancer cells. Western blot (**a**), MTT colorimetric (**b)**, soft agar (**c**), matrigel invasion (**d**), and tube formation (**e**) assays indicating gene expression, viability, growth, invasion, and angiogenesis of cancer cells transfected with sh-Scb, sh-YY1, anti-NC, anti-miR-584-3p inhibitors (100 nmol/L). * P<0.01 vs. sh-Scb+anti-NC.

Supplementary Figure S5 miR-584-3p and YY1 exert functions through regulating MMP-14 expression. Western blot (**a** and **e**), MTT colorimetric (**b** and **f**), matrigel invasion (**c** and **g**), and tube formation (**d** and **h**) assays indicating gene expression, viability, invasion, and angiogenesis of cancer cells transfected as indicated. *P<0.01 vs. mock+mock or mock+sh-Scb.

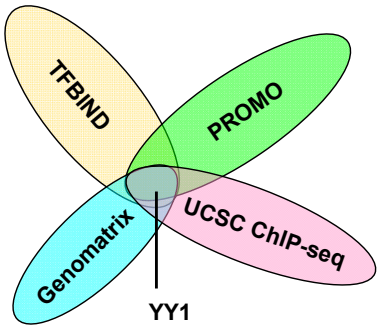
Supplementary Figure S6 Expression of miR-584-3p, SH3TC2, and MMP-14 in publicly available datasets. a and **b**, miR-584-3p and SH3TC2 levels in gastric cancer and adjacent normal

tissues derived from Gene Expression Omnibus (GEO) datasets. **c**, the correlation between SH3TC2 and MMP-14 levels in gastric cancer tissues derived from GEO datasets.

Supplementary Figure S7 Interaction between AGO2 with EZH2, EHMT2, and YY1 in gastric cancer cells. Co-IP and western blot assays showing the interaction of AGO2 with EZH2, EHMT2, and YY1 in MKN-45 cells stably transfected with mock or miR-584-3p precursor.

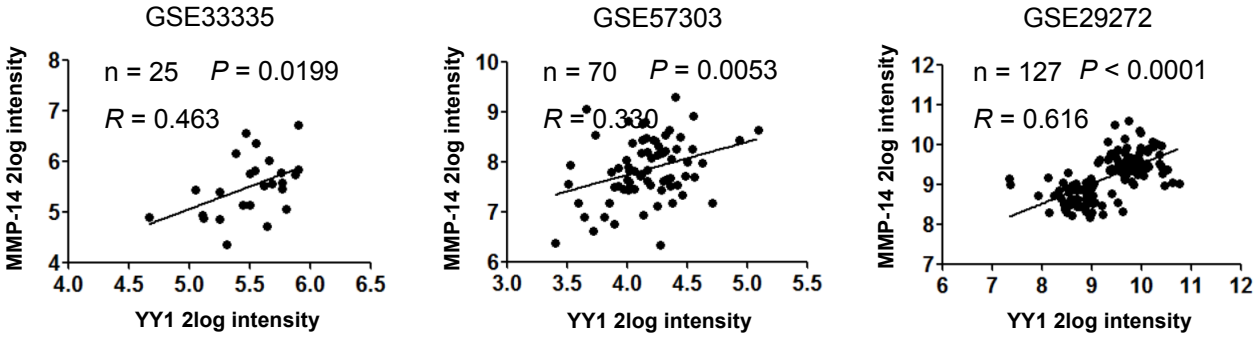
Supplementary Figure S1

a

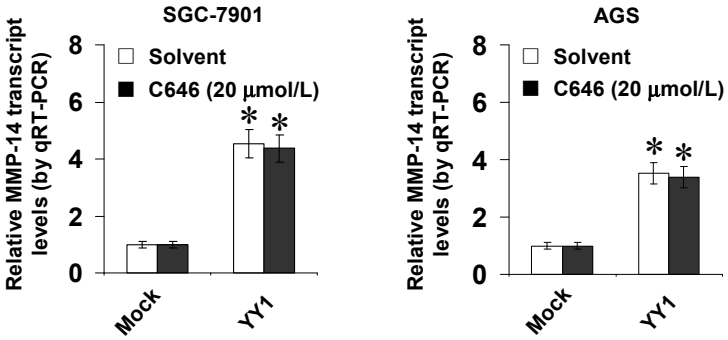


TF name	TF binding site within MMP-14 promoter region (chr14:23304582-23305827)			ChIP-seq peak (UCSC Browser)
	Genomatrix	TFBIND	PROMO	
YY1	Yes	Yes	Yes	Yes
NFY	Yes	Yes	Yes	No
NFE2L2	Yes	Yes	Yes	No

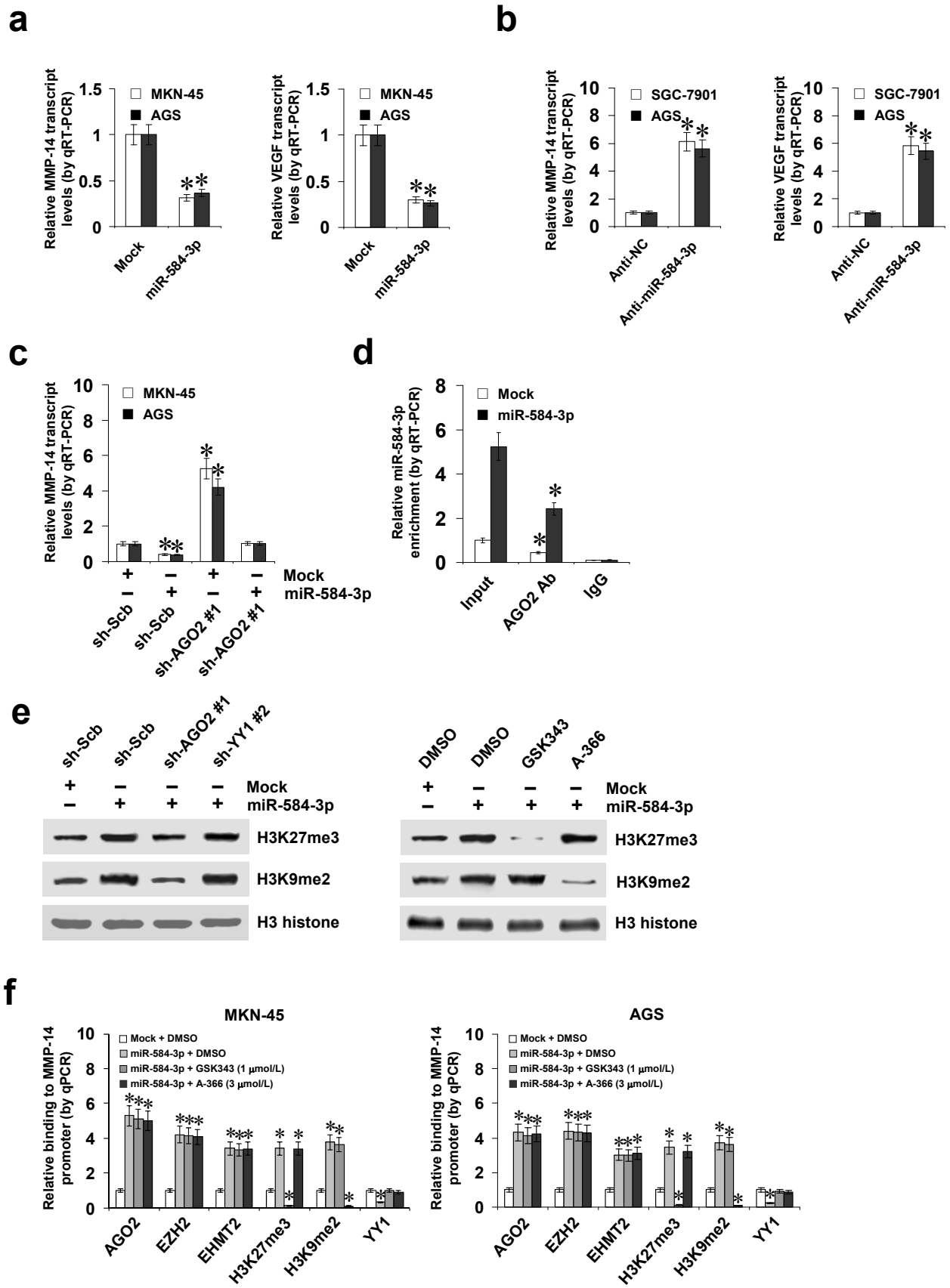
b



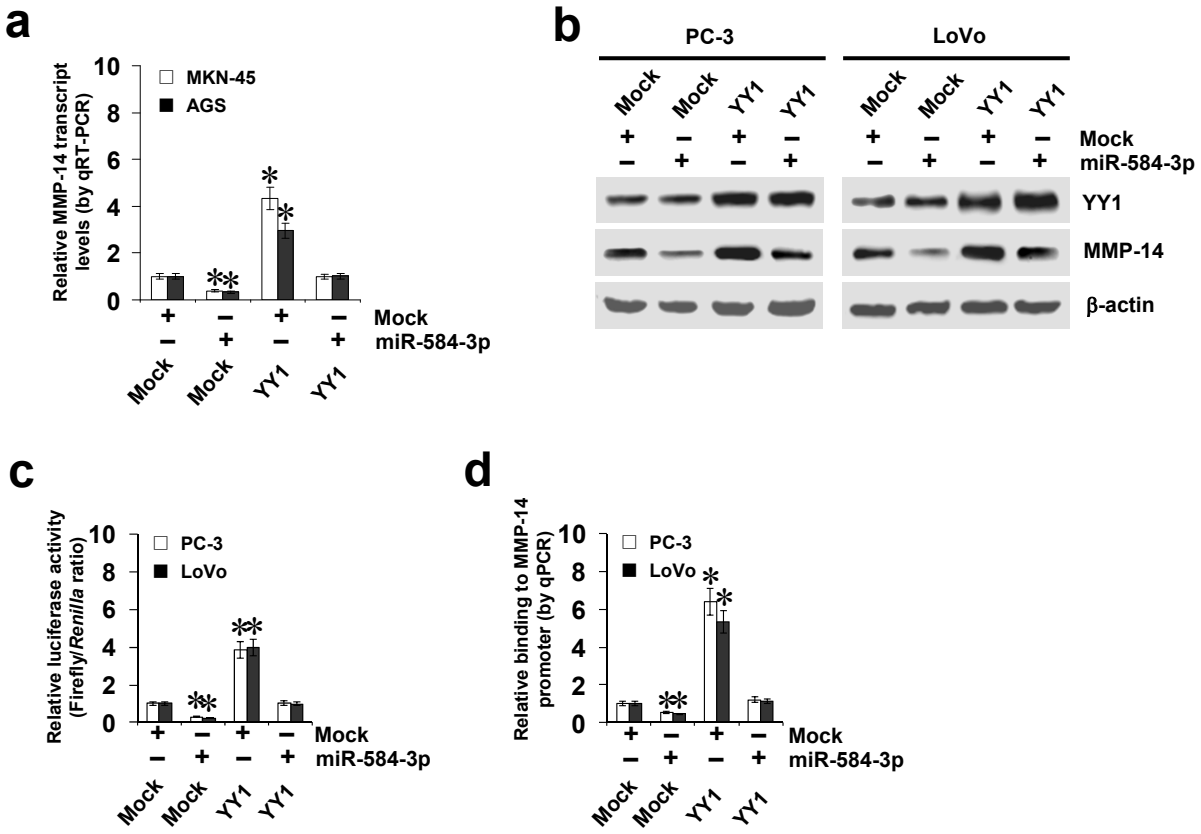
c



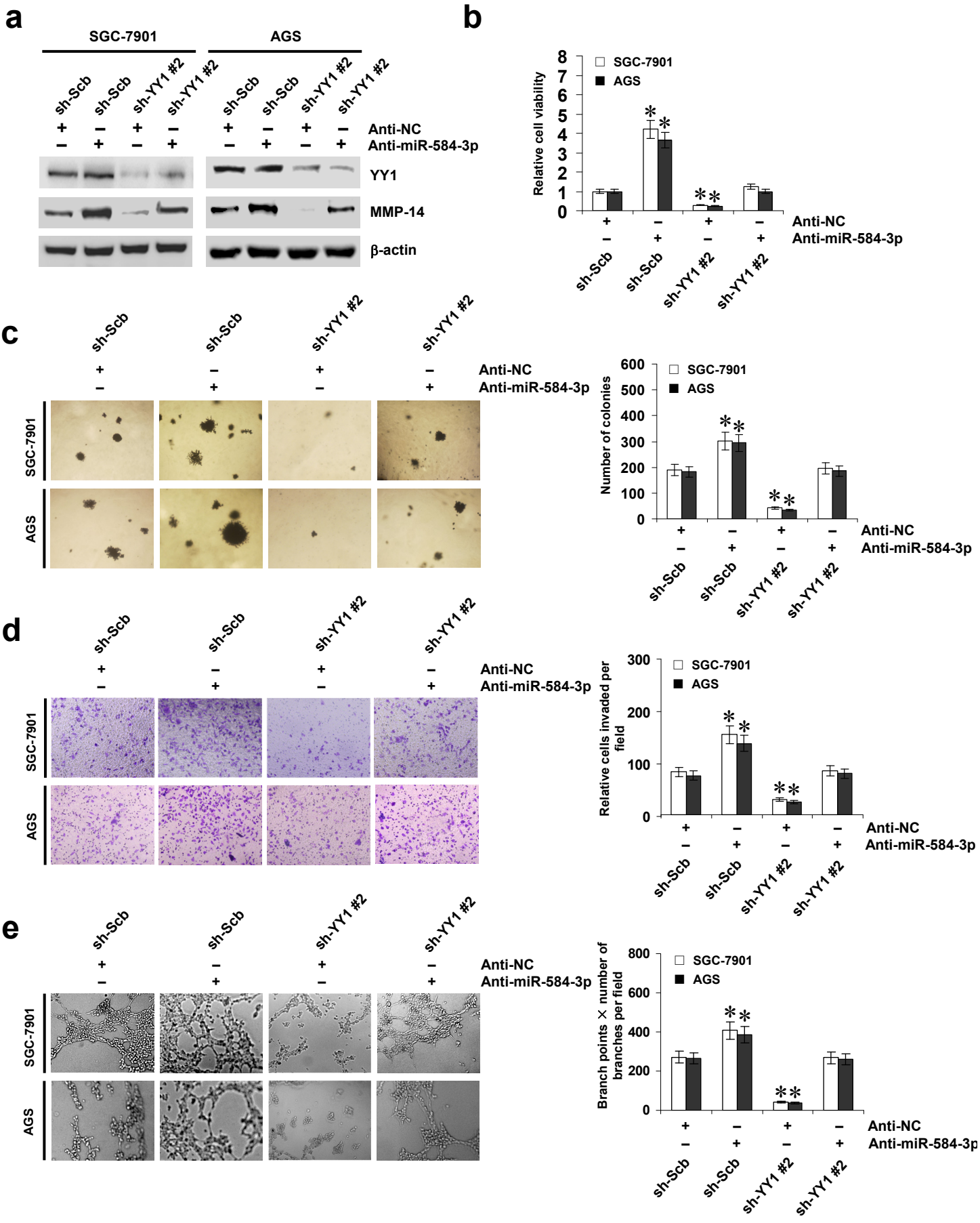
Supplementary Figure S2



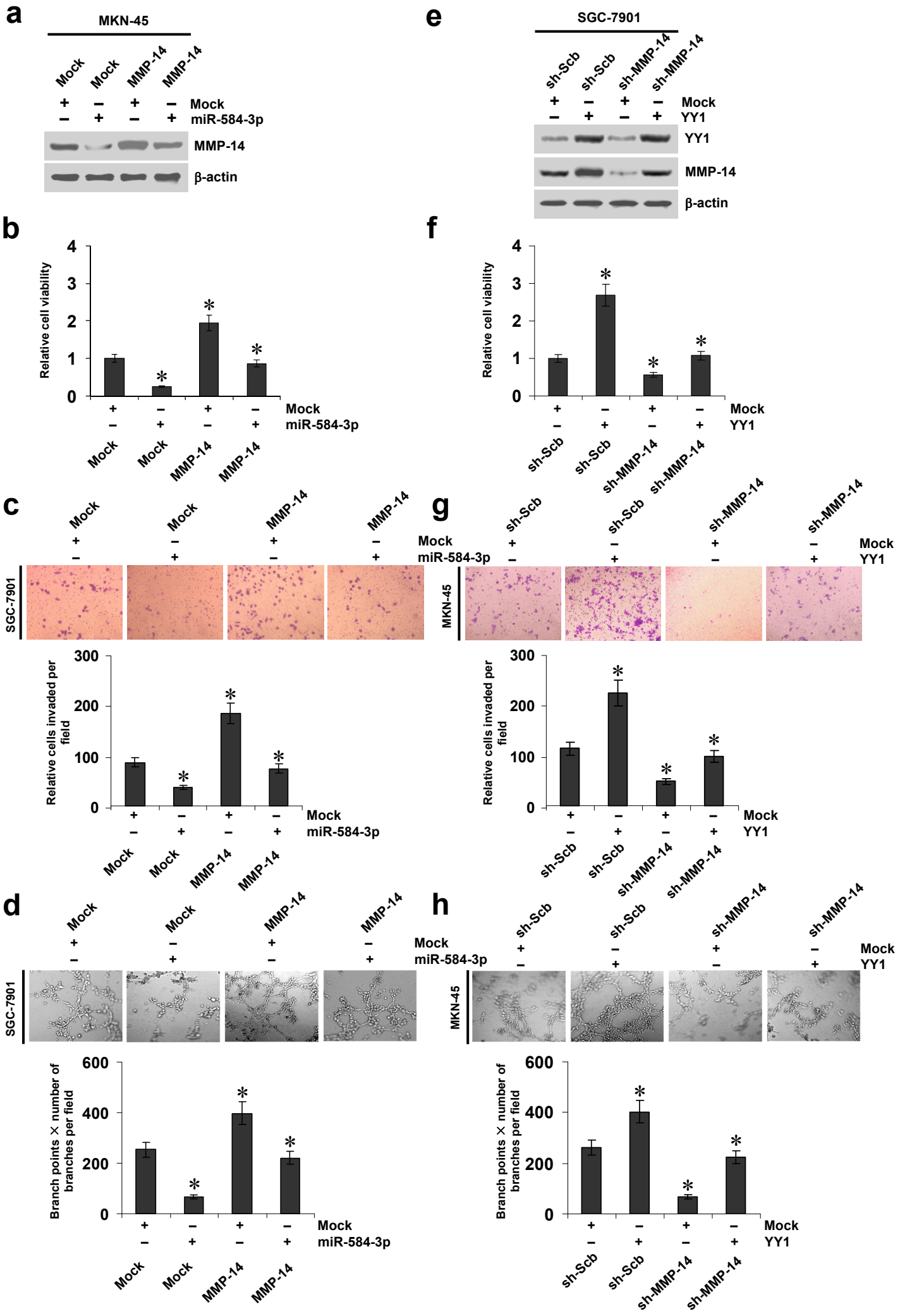
Supplementary Figure S3



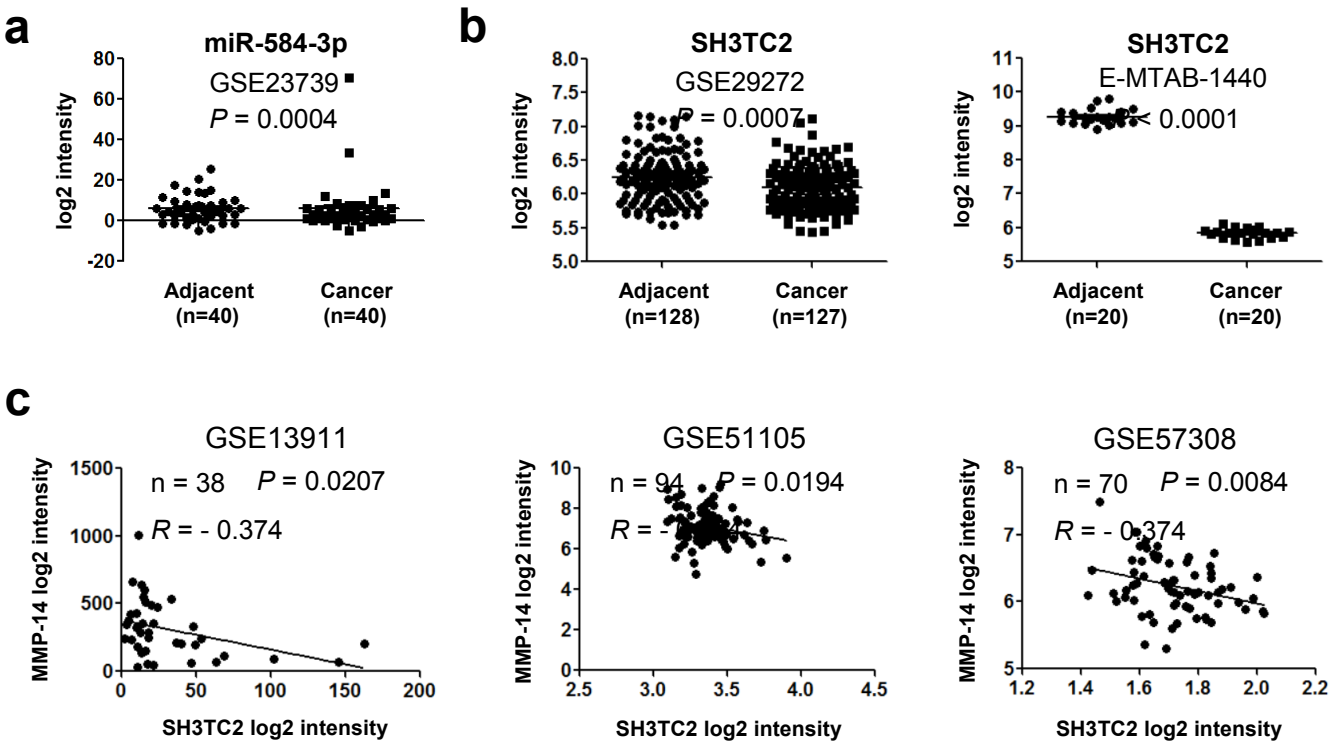
Supplementary Figure S4



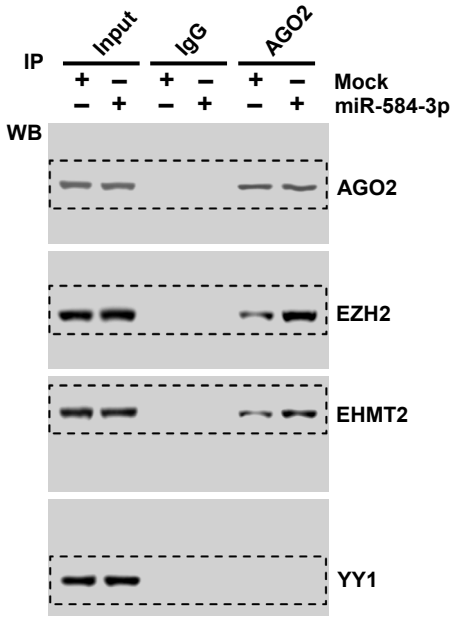
Supplementary Figure S5



Supplementary Figure S6



Supplementary Figure S7



Supplementary Table S1 YY1 and MMP-14 expression in human gastric cancer tissues

Clinicopathologic factor	Total	YY1 expression		MMP-14 expression	
	n (%)	n (%)	*P*-value	n (%)	*P*-value
Age (years)					
≤60	31 (51.7)	19 (61.3)	0.833	22 (70.9)	0.866
>60	29 (48.3)	17 (58.6)		20 (68.9)	
Sex					
Male	42 (70.0)	25 (59.5)	0.908	29 (69.0)	0.806
Female	18 (30.0)	11 (61.1)		13 (72.2)	
Size (diameter)					
≤6 cm	40 (66.7)	24 (60.0)	1.000	28 (70.0)	1.000
>6 cm	20 (33.3)	12 (60.0)		14 (70.0)	
Laurén classification					
Intestinal type	35 (58.3)	20 (57.1)	0.593	24 (68.6)	0.775
Diffuse type	25 (41.7)	16 (64.0)		18 (72.0)	
Gastric wall invasion					
T1/T2	22 (36.7)	3 (13.6)	<0.001	8 (36.4)	<0.001
T3/T4	38 (63.3)	33 (86.8)		34 (89.5)	
Lymph node metastasis					
Negative	21 (35.0)	5 (23.8)	<0.001	6 (28.6)	<0.001
Positive	39 (65.0)	31 (79.5)		36 (92.3)	
Distant metastasis					
Negative	46 (76.7)	24 (52.2)	0.025	28 (60.9)	0.005
Positive	14 (23.3)	12 (85.7)		14 (100.0)	
TNM stage					
I/II	20 (33.3)	5 (25.0)	<0.001	6 (30.0)	<0.001
III/IV	40 (66.7)	31 (77.5)		36 (90.0)	

YY1, Yin Yang 1; MMP-14, matrix metalloproteinases 14; TNM, tumor-node-metastasis.

Supplementary Table S2 Correlation among the expression of YY1 and MMP-14

	YY1 expression			
	Low	High	*R*-value	*P*-value
MMP-14 expression				
Low	28	2	0.802	<0.001
High	4	26		

YY1, Yin Yang 1; MMP-14, matrix metalloproteinases 14; Pearson's correlation coefficient was applied to determine the expression correlation.

Supplementary Table S3 Univariate and multivariate analysis of prognostic factors in gastric cancer patients

Clinicopathologic factor	n	Univariate analysis		Multivariate analysis		
		Mean ± SEM (months)	*P*-value	Hazard ratio	95% CI	*P*-value
Age (years)						
≤60	31	33.2 ± 5.2	0.865	1.121	0.421-3.012	0.712
>60	29	35.1 ± 4.3				
Sex						
Male	42	34.4 ± 4.5	0.826	1.285	0.412-3.223	0.716
Female	18	33.5 ± 5.3				
Size (diameter)						
≤6 cm	40	34.1 ± 4.6	0.804	1.657	1.242-5.021	0.424
>6 cm	20	33.5 ± 6.2				
Laurén classification						
Intestinal type	35	39.3 ± 4.1	0.142	1.612	0.376-4.021	0.514
Diffuse type	25	30.1 ± 5.2				
Gastric wall invasion						
T1/T2	22	61.5 ± 0.6	<0.001	2.134	1.455-6.252	0.446
T3/T4	38	19.5 ± 2.4				
Lymph node metastasis						
Negative	21	60.2 ± 0.8	<0.001	2.018	1.212-5.323	0.523
Positive	39	21.5 ± 2.2				
Distant metastasis						
Negative	46	42.9 ± 3.2	<0.001	2.126	1.854-5.421	0.003
Positive	14	9.8 ± 0.7				
TNM stage						
I/II	20	62.1 ± 0.9	<0.001	2.352	1.698-6.124	0.001
III/IV	40	21.4 ± 2.7				
miR-584-3p expression						
Low	31	18.5 ± 2.7	<0.001	0.312	0.131-0.765	0.018
High	29	55.7 ± 2.5				
YY1 expression						
Low	32	54.5 ± 3.1	<0.001	2.743	1.215-5.236	0.011
High	28	18.1 ± 2.3				

YY1, Yin Yang 1; 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 S4 Oligonucleotide sets used for constructs, inhibitors and short hairpin RNAs

Oligo Set	Sequences
Pre-miR-584-3p	5'-TGCTGTTATGGTTTGCCTGGGACTGAGGTTTTGGCCACTGACTGACCTCAGTCCCAG GCAAACCATAA-3' (sense);
	5'-CCTGTTATGGTTTGCCTGGGACTGAGGTCAGTCAGTGGCCAAAACCTCAGTCCCAGG CAAACCATAAC-3' (antisense)
Pre-miR-NC	5'-TGCTGAAATGTACTGCGCGTGGAGACGTTTTGGCCACTGACTGACGTCTCCACGCAG TACATTT-3' (sense);
	5'-CCTGAAATGTACTGCGTGGAGACGTCAGTCAGTGGCCAAAACGTCTCCACGCGCAGT ACATTTC-3' (antisense)
pGL3-MMP14 mut	5'-CAACCCCTGCGTGCAATAGCCTGCACCACAAAAAGGCAAC TTAGAGGT-3' (sense);
(ΔYY1)	5'-TGGTGCAGGCTATTGCACGCAGGGGTTGAGGCCGCGATGGACAGCAGG-3' (antisense)
pGL3-MMP14 mut (ΔmiR-584-3p)	5'-CATCCCGTCCAAGTTAGCCAACTACGGCCCCCTGCTGTCCATCGCGGCCTCAACCCC-3' (sense);
	5'-CAGCAGGGGGCCGTAGTTGGCTAACTTGGACGGGATGTGGGAGACTTTGTCCCTATA-3' (antisense)
Anti-NC	RiboBio
Anti-miR-584-3p	RiboBio
sh-Scb	5'-CCGGGCGAACGATCGAGTAAACGGACTCGAGTCCGTTTACTCGATCGTTCGCTTTTT-3' (sense);
	5'-AATTCAAAAAGCGAACGATCGAGTAAACGGACTCGAGTCCGTTTACTCGATCGTTCGC-3' (antisense)
sh-YY1 #1	5'-CCGGCGATGGTTGTAATAAGAAGTTCTCGAGAACTTCTTATTACAACCATCGTTTTTG-3' (sense);
	5'-AATTCAAAAACGATGGTTGTAATAAGAAGTTCTCGAGAACTTCTTATTACAACCATCG-3' (antisense)
sh-YY1 #2	5'-CCGGCCCAAACAACTGGCAGAATTTCTCGAGAAATTCTGCCAGTTGTTTGGGTTTTTG-3' (sense);
	5'-AATTCAAAAACCCAAACAACTGGCAGAATTTCTCGAGAAATTCTGCCAGTTGTTTGGG-3' (antisense)
sh-p65 #1	5'-CCGGGCCTTAATAGTAGGGTAAGTTCTCGAGAACTTACCCTACTATTAAGGCTTTTTG-3' (sense);
	5'-AATTCAAAAAGCCTTAATAGTAGGGTAAGTTCTCGAGAACTTACCCTACTATTAAGGC-3' (antisense)
sh-p65 #2	5'-CCGGCGGATTGAGGAGAAACGTAAACTCGAGTTTACGTTTCTCCTCAATCCGTTTTTG-3' (sense);
	5'-AATTCAAAAACGGATTGAGGAGAAACGTAAACTCGAGTTTACGTTTCTCCTCAATCCG-3' (antisense)
sh-AGO2 #1	5'-CCGGGCACAGCCAGTAATCGAGTTTCTCGAGAAACTCGATTACTGGCTGTGCTTTTTG-3' (sense);
	5'-AATTCAAAAAGCACAGCCAGTAATCGAGTTTCTCGAGAAACTCGATTACTGGCTGTGC-3' (antisense)
sh-AGO2 #2	5'-CCGGCGTCCGTGAATTTGGAATCATCTCGAGATGATTCCAAATTCACGGACGTTTTTG-3' (sense);
	5'-AATTCAAAAACGTCCGTGAATTTGGAATCATCTCGAGATGATTCCAAATTCACGGACG-3' (antisense)
sh-MMP-14	5'-CCGGCGCCAGAAGCTGAAGGTAGAACTCGAGTTCTACCTTCAGCTTCTGGCGTTTTTG-3' (sense);
	5'-AATTCAAAAACGCCAGAAGCTGAAGGTAGAACTCGAGTTCTACCTTCAGCTTCTGGCG-3' (antisense)

Pre-miR-NC, negative control pre-miRNA; MMP-14, matrix metalloproteinases 14; Anti-NC, negative control inhibitor; sh-Scb, scramble short hairpin RNA; YY1, Yin Yang 1; AGO1, argonaute 1; AGO2, argonaute 2.

Supplementary Table S5 Primer sets used for qRT-PCR, nuclear run-on, RIP and ChIP

Primer set	Primers	Sequence	Product size (bp)	Application
MMP-14	Forward	5'-GCCTTCTGTTCCTGATAA-3'	225	qRT-PCR
	Reverse	5'-CCATCCTTCCTCTCGTAG-3‘		nuclear run-on
VEGF	Forward	5'-ATGACGAGGGCCTGGAGTGT-3'	226	qRT-PCR
	Reverse	5'-CATTTACACGTCTGCGGATCT-3'		
YY1	Forward	5'-GTGCAGAATGTGGCAAAGC-3'	168	qRT-PCR
	Reverse	5'-AGGGCCTGTCTCCGGTATG-3'		
β-actin	Forward	5'-ATCTACGAGGGGTATGCC-3'	227	qRT-PCR
	Reverse	5'-TAGCTCTTCTCCAGGGAG-3'		
miR-584-3p	Forward	RiboBio		qRT-PCR, RIP
	Reverse	RiboBio		
U6	Forward	RiboBio		qRT-PCR
	Reverse	RiboBio		
MMP-14 set 1	Forward	5'-TCAAGCCACTCAGAATATGC-3'	197	ChIP
(-326/-130)	Reverse	5'-GGTTGTTTTAGCCTGAATCC-3'		
MMP-14 set 2	Forward	5'-CAACCAGGAAAGGAGGGC-3'	191	ChIP
(-122/+69)	Reverse	5'-TCGGCTTGGAGTTAAAGG-3'		

MMP-14, matrix metalloproteinases 14; VEGF, vascular endothelial growth factor; YY1, Yin Yang 1.