Supplementary Tables

Supplementary Table 1. The correlation between MMP11 expression and other clinical features in patients with GC

Clinical features	No. Cases	MMP11 expression		P-value
		High (%)	Low (%)	
Gender				
Male	121	82 (67.8)	39 (32.2)	
Female	38	23 (60.5)	15 (39.5)	0.436
Age				
≥60	74	48 (64.9)	26 (35.1)	
<60	85	57 (67.1)	28 (32.9)	0.867
Tumor site				
Cardiac cancer	50	28 (56.0)	22 (44.0)	
Gastric adenocarcinoma	109	77 (70.6)	32 (29.4)	0.075
Survival status				
Live	75	38 (50.7)	37 (49.3)	
Dead	84	67 (79.8)	17 (20.2)	<0.001
Lauren's classification				
Intestinal GC	92	57 (62.0)	35 (38.0)	
Diffused GC	67	48 (71.6)	19 ()28.4	0.237
TNM stage				
I	18	8 (44.4)	10 (55.6)	

II	41	22 (53.7)	19 (46.3)	
III	91	66 (72.5)	25 (27.5)	
IV	9	9 (100.0)	0 (0.0)	0.004
Tumor invasive depth (T)				
1	8	3 (37.5)	5 (62.5)	
2	17	9 (52.9)	8 (47.1)	
3	69	39 (56.5)	30 (43.5)	
4	65	54 (83.1)	11 (16.9)	0.001
Lymph node metastasis (N)				
0	47	23 (48.9)	24 (51.1)	
1	22	18 (81.8)	4 (18.2)	
2	38	25 (65.8)	13 (34.2)	
3	52	39 (75.0)	13 (25.0)	0.017
Distant metastasis (M)				
Yes	9	9 (100.0)	0 (0.0)	
No	150	96 (64.0)	54 (36.0)	0.029
p-STAT3 expression				
High	97	77 (79.4)	20 (20.6)	
Low	62	28 (45.2)	34 (54.8)	<0.001
GAST expression				
High	92	75 (81.5)	17 (18.5)	
Low	67	30 (44.8)	37 (55.2)	<0.001

Supplementary Table 2. The correlation between p-STAT3 expression and other clinical features in patients with GC

Clinical features	No. Cases	p-STAT3 expression		P-value
		High (%)	Low (%)	
Gender				
Male	121	74 (61.2)	47 (38.8)	
Female	38	23 (60.5)	15 (39.5)	>0.999
Age				
≥60	74	43 (58.1)	31 (41.9)	
<60	85	54 (63.5)	31 (36.5)	0.517
Tumor site				
Cardiac cancer	50	31 (62.0)	19 (38.0)	
Gastric adenocarcinoma	109	66 (60.6)	43 (39.4)	>0.999
Survival status				
Live	75	42 (56.0)	33 (44.0)	
Dead	84	55 (65.5)	29 (34.5)	0.256
Lauren's classification				
Intestinal GC	92	56 (60.9)	36 (39.1)	
Diffused GC	67	41 (61.2)	26 (38.8)	>0.999
TNM stage				
I	18	7 (38.9)	11 (61.1)	

II	41	24 (58.5)	17 (41.5)	
III	91	60 (65.9)	31 (34.1)	
IV	9	6 (66.7)	3 (33.3)	0.185
Tumor invasive depth (T)				
1	8	4 (50.0)	4 (50.0)	
2	17	8 (47.1)	9 (52.9)	
3	69	44 (63.8)	25 (36.2)	
4	65	41 (63.1)	24 (36.9)	0.549
Lymph node metastasis (N)				
0	47	21 (44.7)	26 (55.3)	
1	22	17 (77.3)	5 (22.7)	
2	38	23 (60.5)	15 (39.5)	
3	52	36 (69.2)	16 (30.8)	0.027
Distant metastasis (M)				
Yes	9	6 (66.7)	3 (33.3)	
No	150	91 (60.7)	59 (39.3)	>0.999
MMP11 expression				
High	105	77 (73.3)	28 (26.7)	
Low	54	20 (37.0)	34 (63.0)	<0.001
GAST expression				

High	92	64 (69.6)	28 (30.4)	
Low	67	33 (49.3)	34 (50.7)	0.013

Supplementary Table 3. The correlation between GAST expression and other

biomarkers in GC samples and normal tissues.

Other biomarkers	No. cases	GAST expression		P-value	Pearson's R
		High (%)	Low (%)		
GC samples	159				
p-STAT3 expression					
High	97	64 (66.0)	33 (34.0)		
Low	62	28 (45.2)	34 (54.8)	0.013	0.206
MMP11 expression					
High	105	75 (71.4)	30 (28.6)		
Low	54	17 (31.5)	37 (68.5)	$<$0.001	0.383
Normal samples	102				
p-STAT3 expression					
High	30	16 (53.3)	14 (46.7)		
Low	72	15 (20.8)	57 (79.2)	0.002	0.322
MMP11 expression					
High	25	15 (60.0)	10 (40.0)		
Low	77	16 (20.8)	61 (79.2)	$<$0.001	0.367

Supplementary Table 4. Primers, probes and nucleotides

| Target Gene	Primer	Sequence (5'-3')
GAST	Forward	CAGGTTATGCGCTGTACGGT
	Reverse	TGATGTAGGCCACGGGATTG
Wt-GAST-Luci	Forward	CCGCTCGAGGAAATGGAAATGCAGCGACTATATG
	Reverse	CGCGCGGCCGCAAGTTCTCATCCTCAGCACTG
Mut-GAST-Luci	Forward	GACTTCGGCCGCCGCGATGCTGGTAGATAGAACTAACA
	Reverse	ACCTACCTACCTGAAGCCGGCGGCGCTACGACCATCTA
miR-30a-3p	Forward	GGCCTCGAGCTAAAGAAGGTATATTGC
	Reverse	GGCGGATCCAAAGTAGCCCCTTGAAGT
miR-30a-3p (RT)		GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGA TACGACGCTGCA
β-actin	Forward	TTAGTTGCGTTACACCCTTTC
	Reverse	ACCTTCACCGTTCCAGTTT
β-actin	Forward	CGGGAAATCGTGCGTGACATT
	Reverse	CTAGAAGCATTTGCGGTGGAC
miR-30a-3p ISH probe		5DigN/GCTGCAAACATCCGACTGAAAG/3Dig_N
U6 ISH probe		CACGAATTTGCGTGTCATCCTT
Scramble ISH probe		GTGTAACACGTCTATACGCCCA
Overexpressed GAST plasmid	Forward	CCGCTCGAGGAAATGGAAATGCAGCGACTATGTG
	Reverse	CGCGGATCCAAGTTCTCATCCTCAGCACTG

Silenced GAST plasmid

shGAST-1	Forward	ATCCGAAGAAGAAGCCTATGGATTTCAAGA	
		GAATCCATAGGCTTCTTCTTCTTTTTTGGAAA	
	Reverse	AGCTTTTCCAAAAAAGAAGAAGAAGCCTAT	
		GGATTCTCTTGAAATCCATAGGCTTCTTCTTC	
shGAST-2	Forward	GATCCGAAGAAGCCTATGGATGGATTCAAGA	
		GATCCATCCATAGGCTTCTTCTTTTTTGGAAA	
	Reverse	AGCTTTTCCAAAAAAGAAGAAGCCTATGGA	
		TGGATCTCTTGAATCCATCCATAGGCTTCTTC	

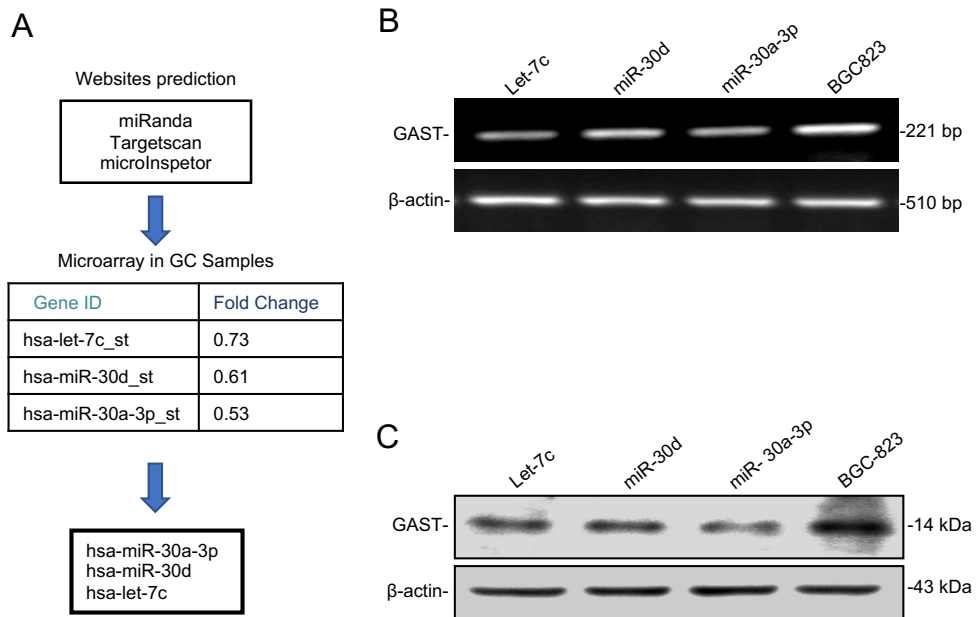**Supplementary Figures and Figure Legends**
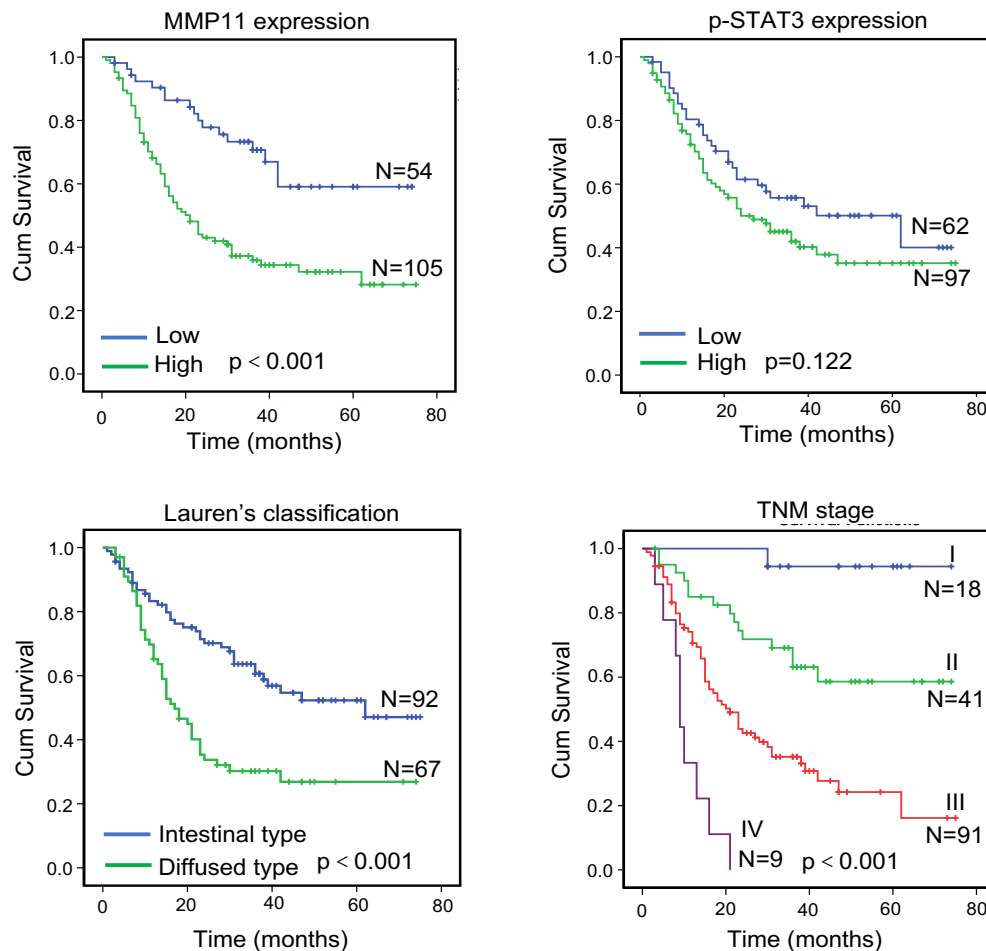
Supplementary Figure 1. The prognostic values of clinical features in GC

High level of MMP11 expression was significantly correlated with the poor survival of GC using Kaplan-Meier analysis ($p < 0.001$). High level of p-STAT3 expression was correlated with the poor survival of GC, however, this difference was not statistically significant difference using Kaplan-Meier analysis ($p = 0.122$). Lauren's classification and TNM stage is the important prognostic biomarkers in GC using Kaplan-Meier analyses ($p < 0.001$, respectively).
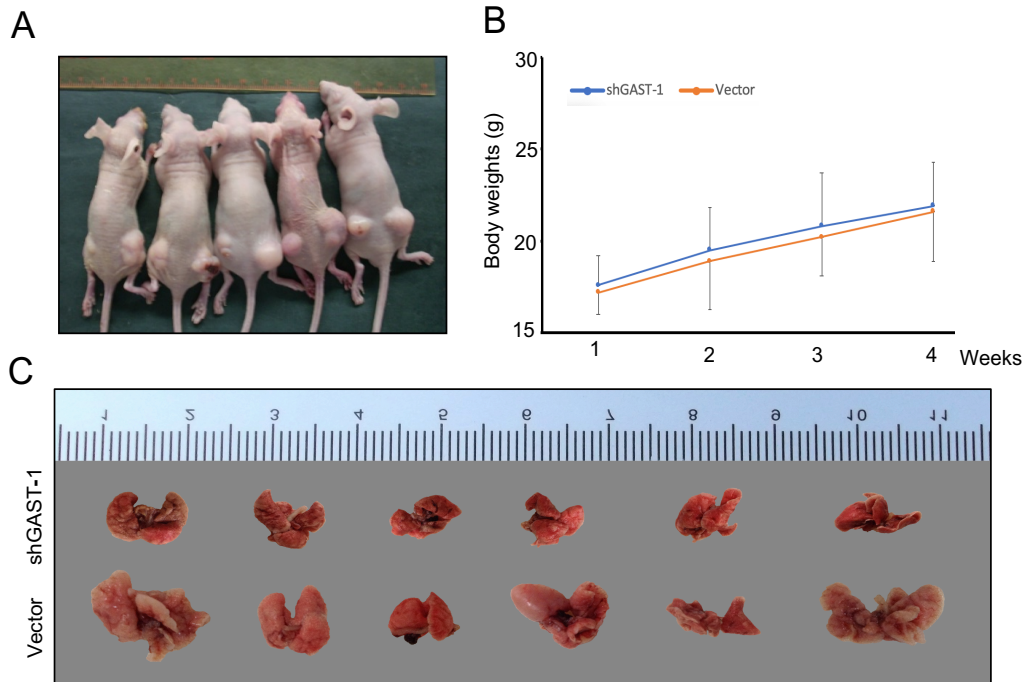
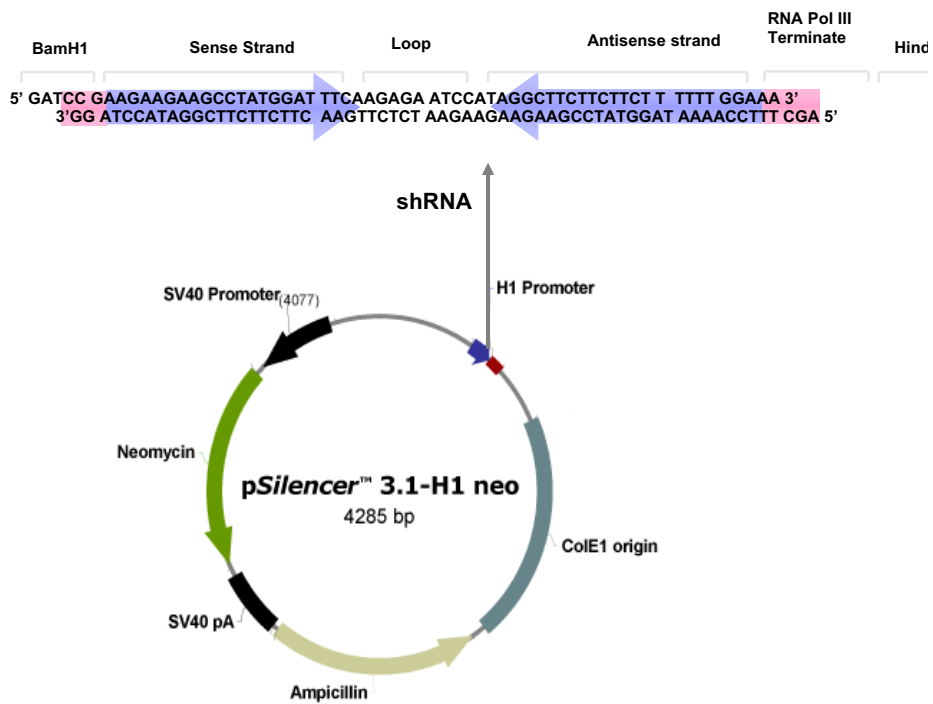**Supplementary Figure 2.** Silenced GAST suppressed tumor malignancy *in vivo*

A. Downregulation of GAST expression suppressed tumorigenicity in nude mice. Mice injected with silenced GAST cells on the left flank and control cells on the right flank. The mouse on the right formed a bigger tumor from the control cells than from the silenced GAST cells. C. Silencing of GAST in BGC823 cells significantly reduced the number of metastatic lesions in the lungs (n = 6/group). Representative macroscopic appearances of lung metastasis.
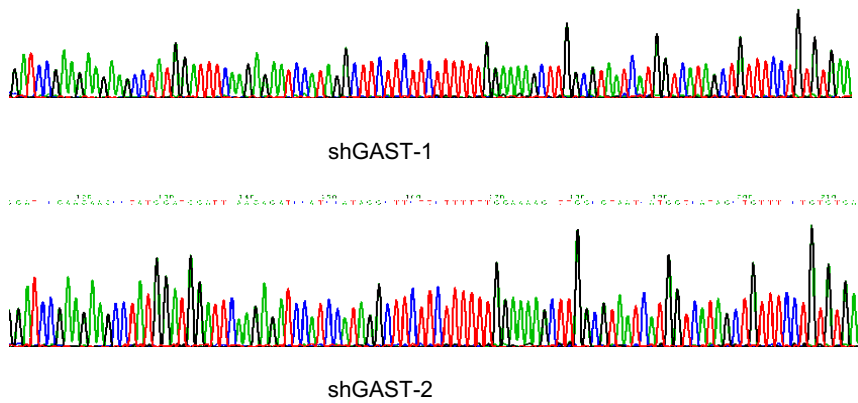
Supplementary Figure 3. Bioinformatical identification of miRNAs downregulating

the level of GAST expression

A. Based our previous miRNA expression profiling within 10 pairs of tumor and their

adjacent normal tissue samples, and we integrated this miRNA expression profiling

data with TargetScan website prediction, and identified 3 down-regulated miRNAs that

might affect the expression of GAST, miR-30a-3p, miR-30d and let-7c. B, C.

Subsequent RT-PCR and Western blot analyses were shown that the mRNA and

protein levels of GAST were both suppressed by these three miRNAs when compared

with negative control BGC823 cells, indicating that these three miRNAs were negative

regulators for GAST. And the most decrease of GAST was correlated with miR-30a-

3p.

A

BamH1	Sense Strand	Loop	Antisense strand	RNA Pol III Terminate	Hind

5' GATCC GAAGAAGAAGCCTATGGAT TTCAAGAGA ATCCATAGGCTTCTTCTTCT T TTTT GGAAA 3'
3'GG ATCCATAGGCTTCTTCTTC AAGTTCTCT AAGAAGAAGAAGCCTATGGAT AAAACCTTT CGA 5'

shRNA

H1 Promoter

SV40 Promoter (4077)

Neomycin

pSilencer™ 3.1-H1 neo
4285 bp

ColE1 origin

SV40 pA

Ampicillin

B

shGAST-1

shGAST-2

Supplementary Figure 4. The silenced GAST plasmids were designed and

identified by sequencing

A. The knockdown of GAST plasmids based on the pSilencer 3.1H1 construct. B.

The correct shGAST plasmids identified by sequencing.