YMTHE, Volume *25*

Supplemental Information

Long Noncoding RNA LINC00673 Is Activated by SP1 and Exerts Oncogenic Properties by Interacting with LSD1 and EZH2 in Gastric Cancer

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Supplementary Table1. Primers, siRNAs and shRNAs sequence

Primers for qPCR

GAPDH F	GGGAGCCAAAAGGGTCAT
GAPDH R	GAGTCCTTCCACGATACCAA
LINC00673 F	TACCACACCCTTTCTTGCCC
LINC00673 R	ACACTGGCCTCTTTACACGG
LINC00673 P1 F	CATAGCCTGGGACTTGAA
LINC00673 P1 R	CATGAGAATCGCTTGGAC
LINC00673 P2 F	GGAATGCCCTGTCTATCT
LINC00673 P2 R	GGCATGAGAATCGCTTGG
CADM4 F	CTAGTGGGCATGGTCTGGTG
CADM4 R	TCCCTGTTCATCCAAGCCAC
CLDN6 F	CATGCCATCATCCGGGACTT
CLDN6 R	GCAGGGGCAGATGTTGAGTA
EMP3 F	TCTACACCATGCGACGAGGA
EMP3 R	GGGCGAAGCAGTATCCGAAG
GADD45GIP1 F	GTGGTCCCCGGTTCGTTATG
GADD45GIP1 R	CAGTATCCAGTCCCGCCATC
INHBE F	TCTTGGACACAGCAGGACAC
INHBE R	CAGTATCCAGTCCCGCCATC
KIF14 F	CGGGATTGACGGCAGTAAGA
KIF14 R	ACTGGGTGTGCATTCCTCTG
S100P F	AGTTCATCGTGTTCGTGGCT
S100P R	CACTTTTGGGAAGCCTGGGA
E2F2 F	CCAGCGCATCGCGTCTC
E2F2 R	TAGAGATCGCCGCTTGGAG
CRIP2 F	GTGCGACAAGACCGTGTACT
CRIP2 R	TCGCACTTGAGGCAGAACTT
KLF2 P26-155 F	ACGGGCTTATTGAGGTTGG
KLF2 P26-155 R	GCCTGGGTGACAGAGGAGAC
KLF2 F	AGAGGGTCTCCCTCGATGAC
KLF2 R	TCTCACAAGGCATCACAAGC
LATS2 F	ACCCCAAAGTTCGGACCTTAT
LATS2 R	CATTTGCCGGTTCACTTCTGC

siRNA sequence

si-LINC00673 1#	CAGCCGGAUACAGAGUGAAUAGUUA
si-LINC00673 2#	UGUGCCUUUGUACUCAGCAAUUCUU
si-SP1-1	CAGCGUUUCUGCAGCUACCUUGACU
si-SP1-2	GACAGGUCAGUUGGCAGACUCUACA

si-EZH2-1	AAGACTCTGAATGCAGTTGCT
si-EZH2-2	CGGCUUCCCAAUAACAGUATT
si-LSD1-1	CAACCTCTCAGAAGATGAGTATTAT
si-LSD1-2	CAAAGGAAACTATGTAGCTGATCTT
si-KLF2	CCAAGAGTTCGCATCTGAA
si-LATS2	CAGGTGGACTCACAATTCCAAATAT
shRNA sequence	
sh-LINC00673 1#	CACCGCAGCCGGATACAGAGTGAATAGTTACGAATAACTATTCACTCTGTATCCGGCTG
sh-LINC00673 2#	CACCGTGTGCCTTTGTACTCAGCAATTCTTCGAAAAGAATTGCTGAGTACAAAGGCACA

Supplementary Table 2. Correlation between LINC00673 expression and clinicopathological characteristics of gastric cancer patients (n = 73)

Characteristics	**Linc00673 Low no. case(%)**	**Linc00673 High no. case(%)**	**P Chi-squared test P-value**
Age(years)			
>65	18(24.7)	16(21.9)	0.352
≤65	25(34.2)	14(19.2)	
Gender			
Male	23(31.5)	19(26.0)	0.474
Female	20(27.4)	11(15.1)	
Histologic			
Well	20(27.4)	4(5.8)	0.026*
Moderately	15(20.5)	15(20.5)	
Poorly	7(9.6)	9(12.3)	
Undifferentiated	1(1.4)	2(2.7)	
TNM Stage			
I	7(9.6)	0(0.0)	0.024*
II	18(24.7)	10(13.7)	
III	16(21.9)	14(19.2)	
IV	2(2.7)	6(8.2)	
Tumor size			
≤5cm	28(38.4)	11(15.1)	0.019*
>5cm	15(20.5)	19(26.0)	
Lymph node metastasis			
Negative	19(26.0)	6(8.2）	0.045*
Positive	24(32.9)	24(32.9)	
Location			
Distal	17(23.3)	14(19.2)	0.741
Middle	15(20.5)	8(11.0)	
Proximal	11(15.1)	8(11.0)	

Supplementary Table 3. Univariate and multivariate analysis of OS in gastric cancer patients (n=73)

Variables	**Univariate analysis**			**Multivariate analysis**		
	HR	**95% CI**	**p value**	**HR**	**95% CI**	**p value**
Age	0.855	0.435-1.683	0.651			
Gender	0.836	0.436-1.605	0.591			
Location	1.232	0.779-1.950	0.372			
Tumor size	1.618	0.846-3.095	0.146			
Histologic	1.742	1.165-2.606	0.007*	1.395	0.903-2.156	0.134
TNM stage	1.695	1.087-2.643	0.020*	1.217	0.725-2.043	0.458
Lymph node metastasis (No vs .Yes)	3.766	1.539-9.217	0.004*	2.556	1.014-6.444	0.047*
LINC00673 expression (High vs. Low)	4.010	2.017-7.969	<0.001*	2.381	1.121-5.056	0.024*

HR, hazard ratio; 95 % CI, 95 % confidence interval, * Overall P < 0.05.

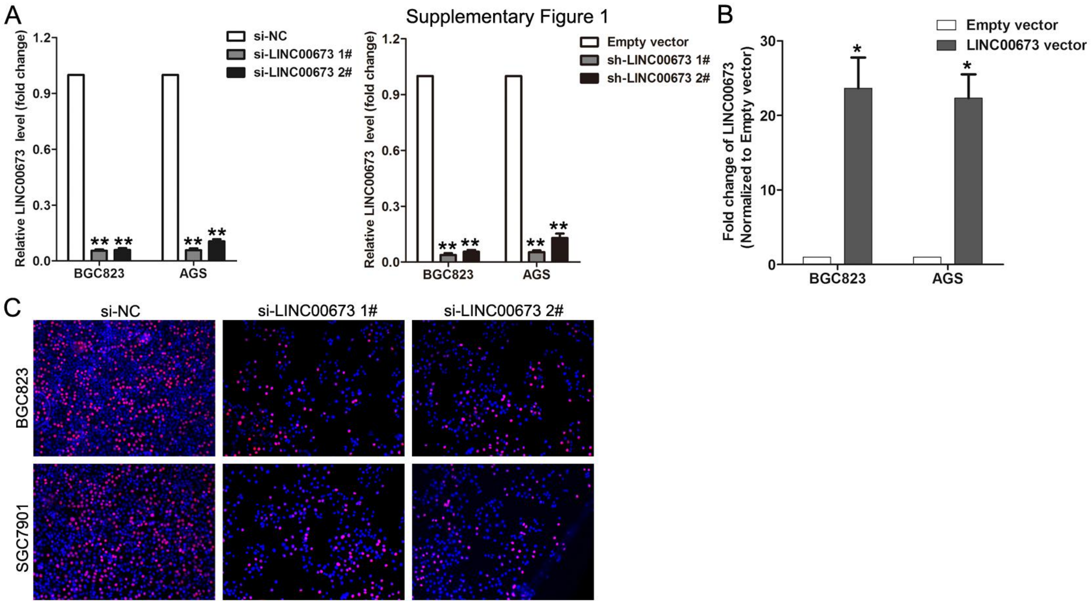

Supplementary Figure 1

Figure S1. (a) qRT-PCR analysis of LINC00673 expression in BGC823 and AGS cells after transfection with si-DUXAP8 or sh-DUXAP8. **(b)** qRT-PCR analysis of LINC00673 expression in BGC823 and AGS cells after transfection with the LINC00673 expression vector or an empty vector. **(c)** Cell proliferation of BGC823 and AGS was evaluated 48 h after transfection with LINC00673 or NC siRNA using EdU-incorporation assays. Red: EdU staining of proliferating cells; blue: DAPI staining of the cell nuclei. The mean values and standard errors were calculated from triplicates of a representative experiment. *P<0.05, **P<0.01.

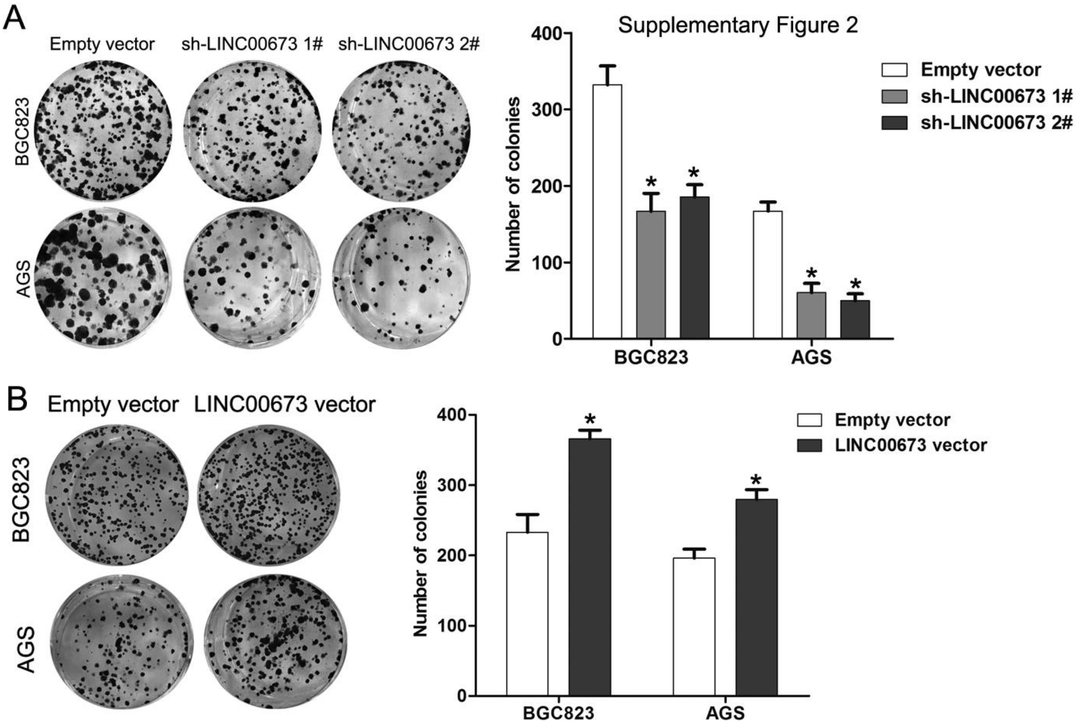
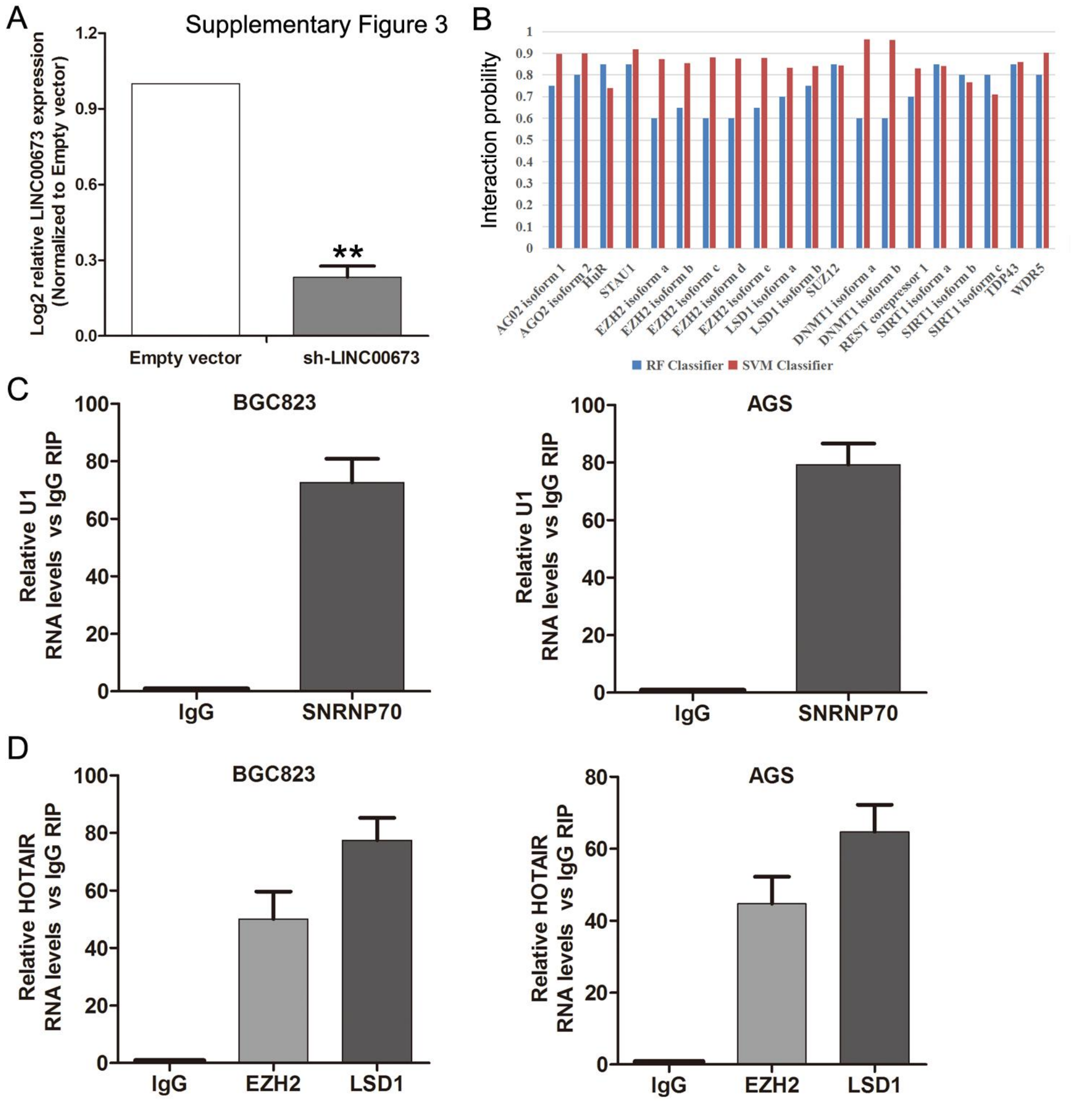
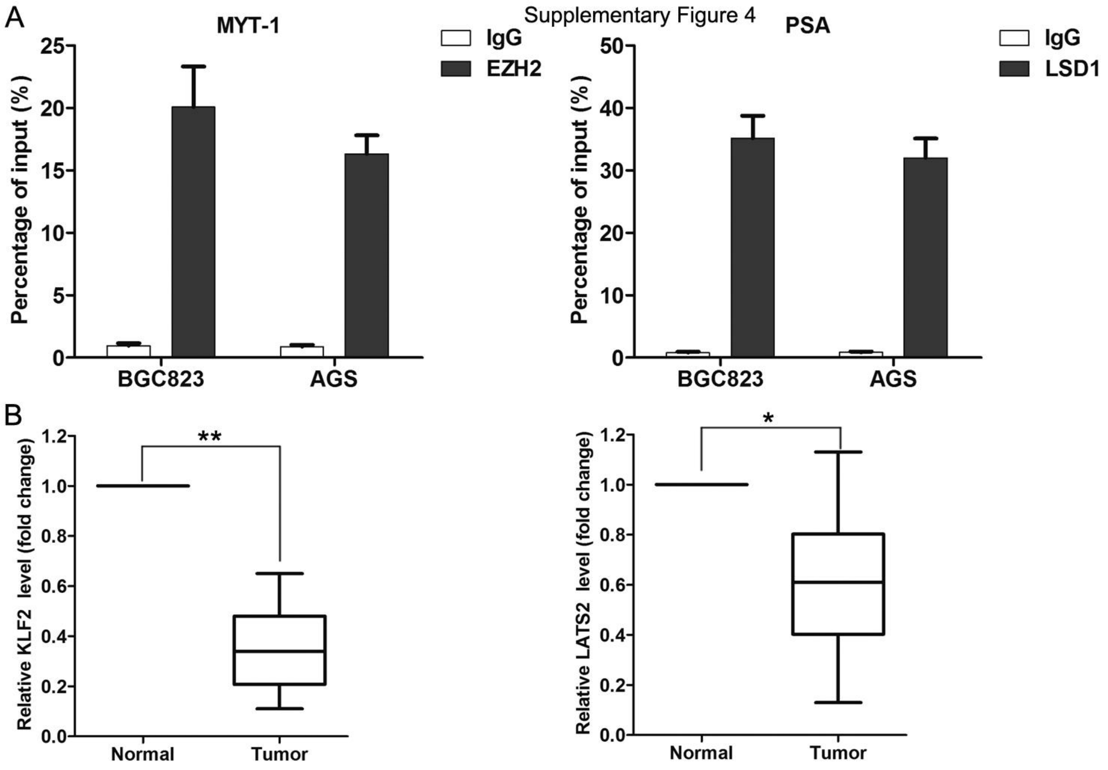


Figure S2. (a) Colony formation analysis of BGC823 and AGS cell proliferation after transfection with sh-LIN00673 or an empty vector. **(b)** Colony formation analysis of BGC823 and AGS cell proliferation after transfection with the LINC0673 expression vector or an empty vector. The mean values and standard errors were calculated from triplicates of a representative experiment. *P<0.05.



Supplementary Figure 3. (a) qRT-PCR analysis of LINC00673 expression in mouse tumor tissues from sh-LINC00673 or the control group. **(b)** Prediction of the interaction probability between LINC00673 and RNA binding proteins using the online database at http://pridb.gdcb.iastate.edu/RPISeq/. **(c)** U1 RNA levels in SNRNP70 immunoprecipitates were determined by qRT-PCR. The expression levels for U1 RNA are presented as fold enrichment values relative to the IgG immunoprecipitates. **(d)** HOTAIR RNA levels in EZH2 and LSD1 immunoprecipitates were determined by qRT-PCR. Expression levels for HOTAIR are presented as fold enrichment values relative to the IgG immunoprecipitates. The mean values and standard errors were calculated from triplicates of a representative experiment. **P<0.01.



Supplementary Figure 4. (a) ChIP-qPCR analysis of EZH2 or LSD1 occupancy in the MYT-1 and PSA promoters in BGC823 and AGS cells, with IgG used as the negative control. **(b)** qRT-PCR analysis of KLF2 and LATS2 expression in 20 paired GC tumor tissues and their adjacent non-tumor tissues. The mean values and standard errors were calculated from triplicates of a representative experiment. *P<0.05, **P<0.01.