Supplementary information

Supplementary Tables

Supplementary Table S1. Upregulated genes in AGS cells following galectin-1 silencing Transcriptomic profiling was performed using microarray analysis in AGS cells transfected with galectin-1 siRNA. Genes significantly upregulated compared with control are listed. Differentially expressed genes were defined using a cut-off of |fold change| ≥ 2.0 (linear scale) and adjusted $p < 0.05$.

Gene Symbol	Gene Name	Fold Change (linear)
Response to wounding		
CXCL11	chemokine (C-X-C motif) ligand 11	2
CXCL9	chemokine (C-X-C motif) ligand 9	2
CTGF	connective tissue growth factor	2
ENG	endoglin	2
Biological adhesion		
CDH15	cadherin 15, type 1, M-cadherin	2
TGFBI	transforming growth factor, beta-induced	3.3
BMP1	bone morphogenetic protein 1	2
Cell motility		
CXCL12	chemokine (C-X-C motif) ligand 12	2.5
NRP2	neuropilin 2	2.5
Cell migration		
CDH2	cadherin 2, type 1, N-cadherin	2
DRD2	dopamine receptor D2	2
Immune response		
IL1F10	interleukin 1 family, member 10	2
IL1RN	interleukin 1 receptor antagonist	2
Positive regulation of signal transduction		
LGALS9	lectin, galactoside-binding, soluble, 9	2

Supplementary Table S2. Downregulated genes in AGS cells following galectin-1 silencing Transcriptomic profiling was performed using microarray analysis in AGS cells transfected with galectin-1 siRNA. Genes significantly downregulated compared with control are listed. Differentially expressed genes were defined using a cut-off of |fold change| ≥ 2.0 and adjusted $p < 0.05$.

Gene Symbol	Gene Name	Fold Change (linear)
Response to wounding		
NOX1	NADPH oxidase 1	-2
CXCL1	chemokine (C-X-C motif) ligand 1	-2
CXCL2	chemokine (C-X-C motif) ligand 2	-2
TGFB3	transforming growth factor, beta 3	-2
Blood vessel morphogenesis		
AMOT	angiomotin	-2
PGF	placental growth factor	-2
Glucose metabolic process		
HK2	hexokinase 2 pseudogene; hexokinase 2	-2
LDHA	lactate dehydrogenase A	-2
PDK1	pyruvate dehydrogenase kinase, isozyme 1	-2.5
Cell activation		
NDRG1	N-myc downstream regulated 1	-2.5
Defense response		
CEBPE	CCAAT/enhancer binding protein (C/EBP), epsilon	-2
Regulation of cell proliferation		
SOX2	SRY (sex determining region Y)-box 2	-2
CGREF1	cell growth regulator with EF-hand domain 1	-2.5
DHRS2	dehydrogenase/reductase (SDR family) member 2	-2
FOXO4	forkhead box O4	-2
TGM2	transglutaminase 2	-2
NANOG	Nanog homeobox pseudogene 8; Nanog homeobox	-2.5
Negative regulation of signal transduction		
DDIT4	DNA-damage-inducible transcript 4	-2.5
IGFBP5	insulin-like growth factor binding protein 5	-2.5
KLF9	Kruppel-like factor 9	-2

Supplementary Table S3. Primer sequences used in this study

Gene name	Forward primer (5′→3′)	Reverse primer (5′→3′)
LGALS1	CTCTCGGGTGGAGTCTTCTG	ACGAAGCTCTTAGCGTCAGG
SOX2	AAAACAGCCCGGACCGCGTC	CTCGTCGATGAACGGCCGCT
NANOG	GGCGTCGGGATCGGATAAATA	GAAGAGGAGAGACAGTCTCCGT
CXCL9	GCAAGGAACCCCAGTAGTGA	TTTGGCTGACCTGTTTCTCC
CXCL12	TCAGCCTGAGCTACAGATGC	CTTTAGCTTCGGGTCAATGC
BMP1	GGAGAGACCCTGCAAGACAG	CGATAGGCTCAGGGAGTTTG
CDH2	GACAATGCCCCTCAAGTGTT	CCATTAAGCCGAGTGATGGT
LGALS9	CCTTTGACCTCTGCTTCCTG	AAACAGACAGGCTGGGAGAA
TGFB1	AACCCACAACGAAATCTATG	GTGCTGCTCCACTTTTAACT
NRP2	TCCACTGCTGACAAGGTTTG	ACTGGGGCTCCAGAGGTATT
CXCL1	AGGGAATTCACCCCAAGAAC	CACCAGTGAGCTTCCTCCTC
LDHA	TGGGAGTTCACCCATTAAGC	AGCACTCTCAACCACCTGCT
NDRG1	CGAGAGCTACATGACGTGGA	AAGAGGGGGTTGTAGCAGGT
IGFBP5	AAGAAGCTGACCCAGTCCAA	GAATCCTTTGCGGTCACAAT
NOX1	GGATGATCGTGACTCCCACT	TTTGGATGGGTGCATAACAA
KLF9	TCGCGTTTACAAGTGTCGAG	CTTCAGCTTCGGTTTTCCAG
TGFB3	CTATATCGGTGGCAAGAATC	ATCACCTCGTGAATGTTTTC
HK2	CCACCTTTGTGAGGTCCACT	GTCCTCAGGGATGGCATAGA
TGM2	GGGGTGAGAGAGGAAAGACC	TGCAGTCTAGGGAGCTGGAT

Supplementary Table S4. Association of Galectin-1 and SOX2 expression with clinicopathologic characteristics in gastric cancer

Category	Galectin-1, No. (%)			SOX2, No. (%)		
	Low (*n*=196)	High (*n*=61)	*p* value	Low (*n*=188)	High (*n*=69)	*p* value
Age	55.8 ± 12.3	59.7 ± 10.9	0.029*	56.6 ± 12.4	57.3 ± 11.4	0.688
Gender			0.269			0.365
Female	65 (33.2)	15 (24.6)		62 (33.0)	18 (26.1)	
Male	131 (66.8)	46 (75.4)		126 (67.0)	51 (73.9)	
Differentiation			<0.001*			0.304
Well	27 (13.8)	1 (1.6)		22 (11.7)	6 (8.7)	
Moderate	51 (26.0)	28 (45.9)		61 (32.5)	18 (26.1)	
Poor	78 (39.8)	30 (49.2)		73 (38.8)	35 (50.7)	
Signet-ring cell	35 (17.9)	1 (1.6)		28 (14.9)	8 (11.6)	
Mucinous	5 (2.5)	0		4 (2.1)	1 (1.4)	
Others	0	1 (1.6)		0	1 (1.4)	
pT stage			<0.001*			0.001*
pT1	95 (48.5)	4 (6.6)		85 (45.2)	14 (20.3)	
pT2	19 (9.7)	19 (31.1)		28 (14.9)	10 (14.5)	
pT3	47 (24.0)	26 (42.6)		47 (25.0)	26 (37.7)	
pT4	35 (17.8)	12 (19.7)		28 (14.9)	19 (27.5)	
LN metastasis			0.012*			0.015*
absent	109 (55.6)	22 (36.1)		105 (55.9)	26 (37.7)	
present	87 (44.4)	39 (63.9)		83 (44.1)	43 (62.3)	
LVI			0.016*			0.001*
absent	132 (67.3)	30 (49.2)		130 (69.1)	32 (46.4)	
present	64 (32.7)	31 (50.8)		58 (30.9)	37 (53.6)	
Depth of invasion			<0.001*			<0.001*
EGC	95 (48.5)	4 (6.6)		85 (45.2)	14 (20.3)	
AGC	101 (51.5)	57 (93.4)		103 (54.8)	55 (79.7)	

LN, lymph node; LVI, lymphovascular invasion; EGC, early gastric cancer; AGC, advanced gastric cancer

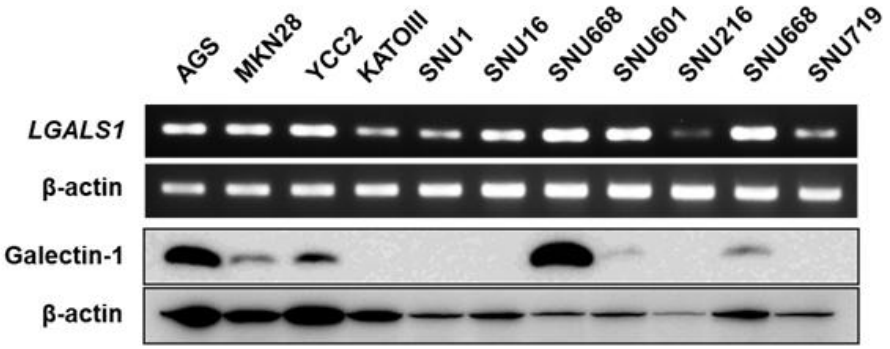
*Statistically significant ($p < 0.05$)

Supplementary Table S5. Cox proportional univariate and multivariate analyses of disease-free survival in patients with gastric cancer

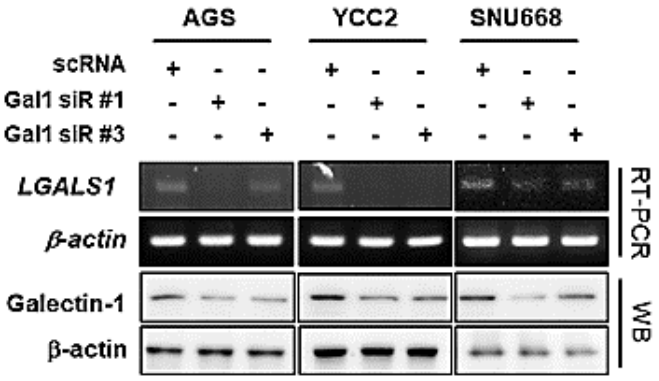
Variables	Univariate analysis		Multivariate analysis		Multivariate analysis (including dual Gal-1/SOX2)	
	HR [95% CI]	*p* value	HR [95% CI]	*p* value	HR [95% CI]	*p* value
Gender (Male)	1.115 [0.665-1.870]	0.681				
Differentiation	1.259 [0.994-1.595]	0.563				
pT	2.910 [2.219-3.817]	<0.001*	2.524 [1.836-3.470]	<0.001*	2.516 [1.833-3.454]	<0.001*
pN	7.858 [4.023-15.348]	<0.001*	3.078 [1.536-6.170]	0.002*	3.082 [1.537-6.179]	0.002*
Gal-1^{high}	2.282 [1.412-3.687]	0.005*	2.086 [1.242-3.502]	0.005*		
SOX2^{high}	1.994 [1.238-3.210]	0.001*	1.356 [0.840-2.190]	0.212		
Gal-1^{high}/SOX2^{high}	1.499 [1.230-1.827]	<0.001*			1.422 [1.143-1.768]	0.002*

HR, Hazard ratio; CI, confidence interval; Gal-1, galectin-1; *Statistically significant (p<0.05)

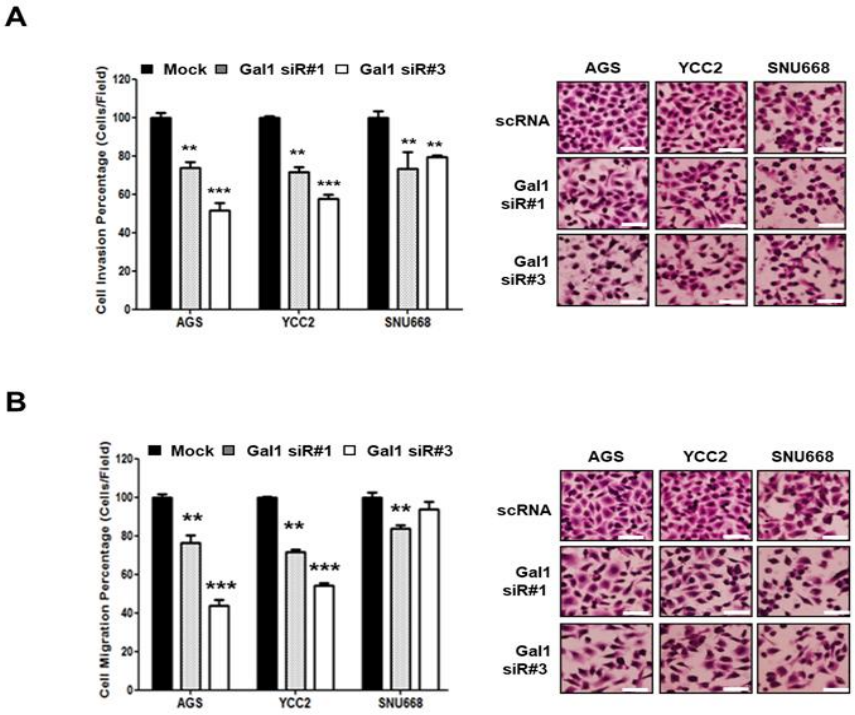
Supplementary Figures



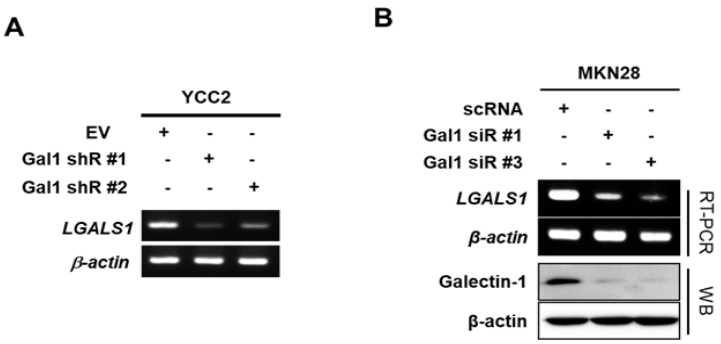
Supplementary Figure S1. Detection of galectin-1 expression in gastric cancer cell lines. mRNA and protein expression levels of galectin-1 were examined in 11 gastric cancer cell lines by RT-PCR and western blotting.



Supplementary Figure S2. Suppression of galectin-1 expression by siRNAs in gastric cancer cell lines. AGS, YCC2, and SNU-668 cells were transfected with scrambled control (scRNA) or two different galectin-1 siRNAs (Gal1 siR#1 and Gal1 siR#3). Cells were harvested 48 h post-transfection, and galectin-1 mRNA and protein levels were analyzed by RT-PCR and western blotting.

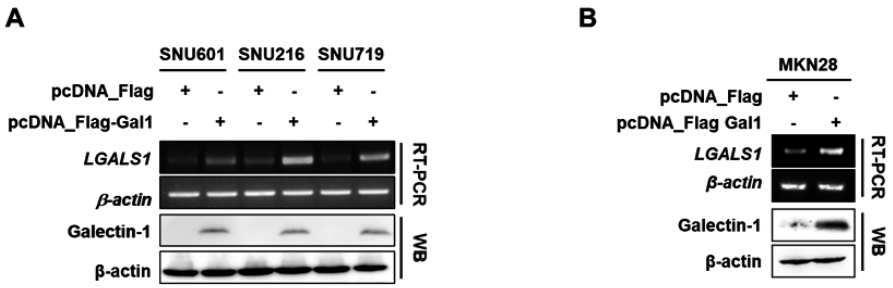


Supplementary Figure S3. Effect of galectin-1 silencing on migration and invasion of gastric cancer cell lines. Transwell assays were performed to evaluate (A) invasion and (B) migration in AGS, YCC-2, and SNU-668 cells transfected with scrambled control (scRNA) or galectin-1 siRNAs. Representative images (right) and quantification (left) are shown. In each group, five random fields were imaged and counted. Scale bar, 5 μm. p-values were calculated using Student's t-test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

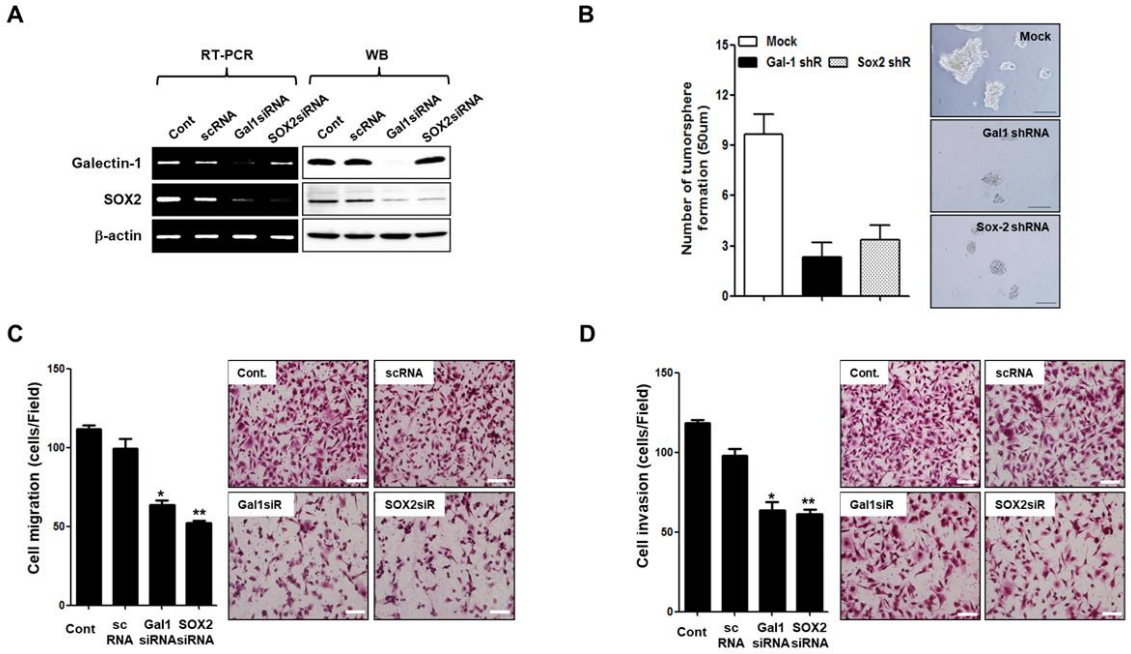


Supplementary Figure S4. Suppression of galectin-1 expression by shRNA or siRNA in gastric cancer cell lines. (A) YCC-2 cells were transfected with empty vector (EV) or two independent galectin-1 shRNAs. mRNA expression levels of galectin-1 were measured by RT-PCR after 48 h.

(B) MKN28 cells were transfected with scrambled control (scRNA) or two independent galectin-1 siRNAs. Galectin-1 mRNA and protein levels were assessed by RT-PCR and western blotting after 48 h.

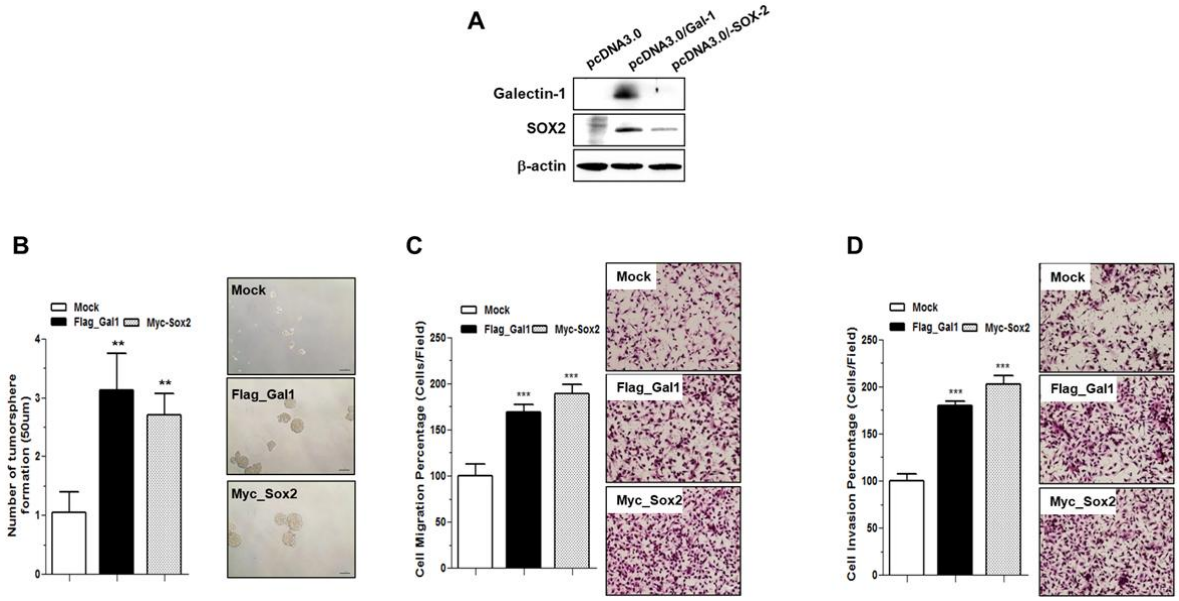


Supplementary Figure S5. Overexpression of galectin-1 in gastric cancer cells. (A) SNU601, SNU216, and SNU719 cells were transfected with empty vector (pcDNA_Flag) or galectin-1 overexpression vector (pcDNA_Flag-Gal1). mRNA and protein levels of galectin-1 were measured by RT-PCR and western blotting after 48 h. (B) MKN28 cells were transfected with empty vector (pLECE3) or galectin-1 overexpression vector (pLECE3-Gal1). Galectin-1 expression was confirmed by RT-PCR and western blotting after 48 h.

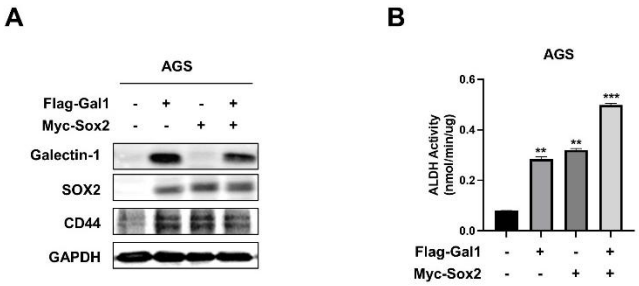


Supplementary Figure S6. Effects of galectin-1 and SOX2 knockdown on stemness and motility in AGS gastric cancer cells. (A) RT-PCR and western blot analysis of galectin-1 and SOX2 expression in AGS cells transfected with scRNA, galectin-1 siRNA, or SOX2 siRNA for 48 h.

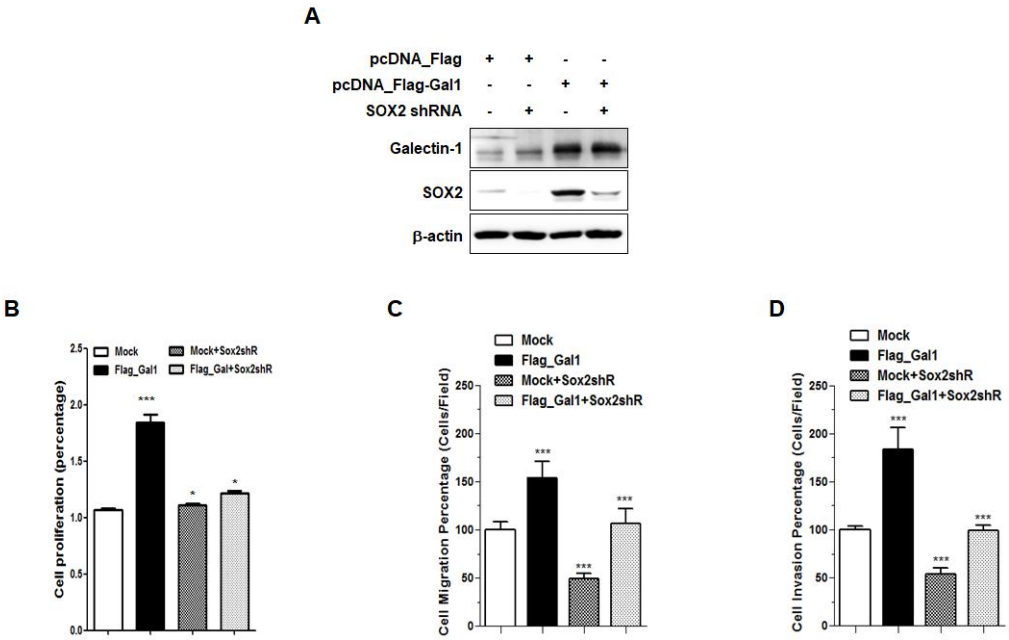
(B) Tumorsphere formation assay of AGS cells transfected with galectin-1 or SOX2 siRNA. Representative images (left) and quantification of sphere number (right) are shown (magnification, ×100; mean ± SD, n = 3). Scale bar = 50 μm. (C–D) Transwell migration (C) and invasion (D) assays of AGS cells transfected with galectin-1 or SOX2 siRNA. Representative images (right) and quantification (left) are shown. Scale bar = 50 μm. Statistical significance was determined using Student's t-test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).



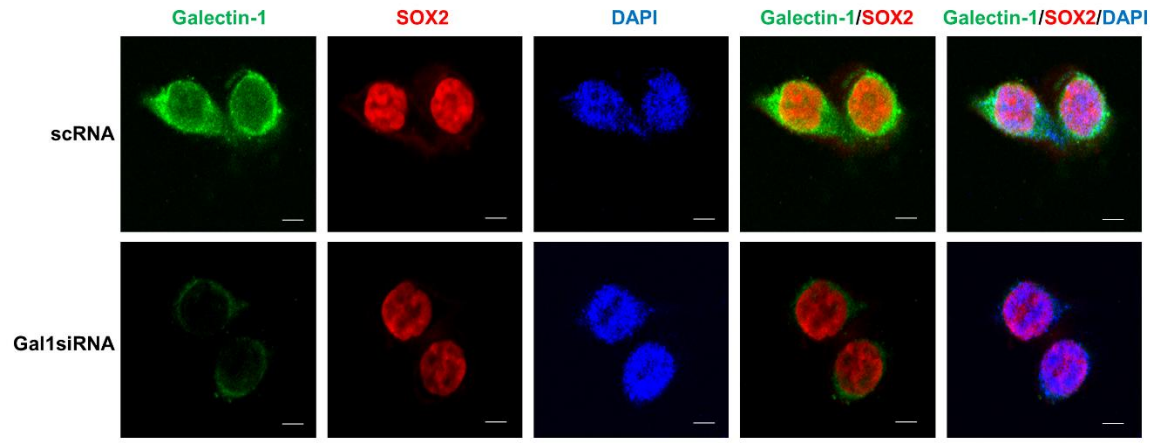
Supplementary Figure S7. Effects of galectin-1 and SOX2 overexpression on stemness and motility in AGS gastric cancer cells. (A) Western blot analysis of galectin-1 and SOX2 protein expression in AGS cells transfected with empty vector, pcDNA3.0-Flag-Galectin-1, or pcDNA3.0-Myc-SOX2. (B) Tumorsphere formation assay of AGS cells following overexpression of galectin-1 or SOX2. Representative images (left) and quantification of sphere number (right) are shown (magnification, ×100; mean ± SD, n = 3). Scale bar = 50 μm. (C–D) Transwell migration (C) and invasion (D) assays of AGS cells overexpressing galectin-1 or SOX2. Representative images (right) and quantification (left) are shown. Scale bar = 50 μm. Statistical significance was determined using Student's t-test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).



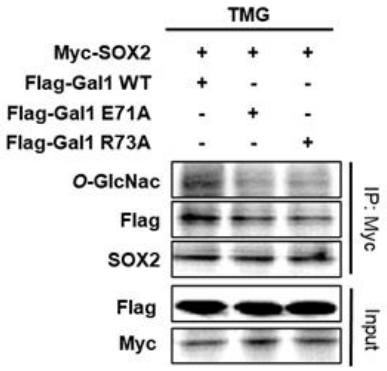
Supplementary Figure S8. Effects of galectin-1 and SOX2 overexpression on stemness in AGS gastric cancer cells. (A) Western blot analysis of galectin-1, SOX2 and CD44 protein expression in AGS cells transfected with empty vector, pcDNA3.0-Flag-Galectin-1, or pcDNA3.0-Myc-SOX2. (B) Quantification of ALDH activity in AGS cells overexpressing vector, pcDNA3.0-Flag-Galectin-1, or pcDNA3.0-Myc-SOX2. Statistical significance was determined using Student's t-test (**$p < 0.01$; ***$p < 0.001$).



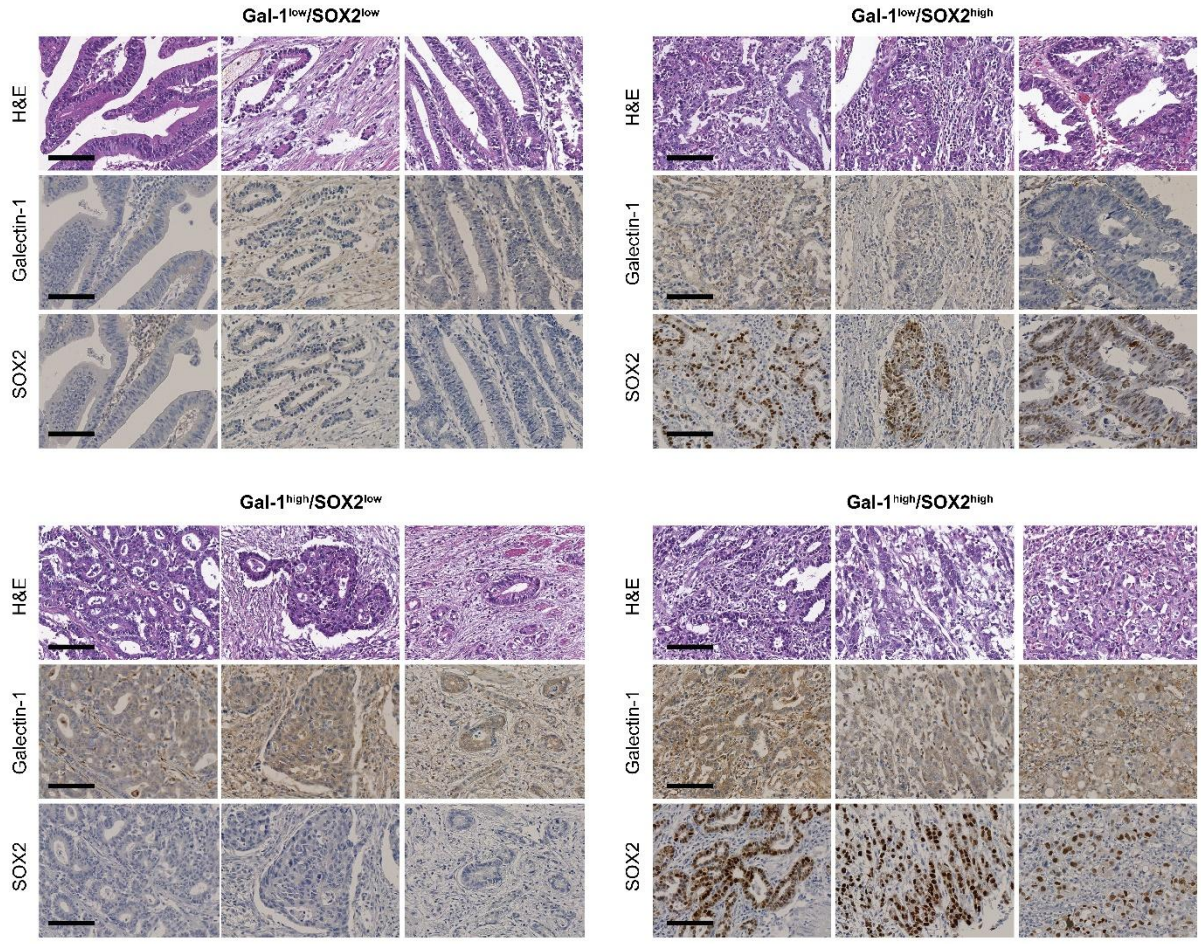
Supplementary Figure S9. SOX2 silencing abrogates the proliferative and motility effects induced by galectin-1 overexpression in AGS gastric cancer cells. (A) Western blot analysis of SOX2 and galectin-1 expression in AGS cells co-transfected with empty vector, galectin-1 overexpression vector, and/or SOX2 shRNA. (B) Cell proliferation was assessed using the WST assay in the indicated AGS cell groups. Data are presented as mean ± SD (n = 3). (C–D) Transwell migration (C) and invasion (D) assays were performed in AGS cells co-transfected with galectin-1 overexpression vector and SOX2 shRNA. Representative images and quantification are shown. Scale bar = 50 μm. Statistical significance was determined using Student's t-test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).



Supplementary Figure S10. Nuclear co-localization of galectin-1 and SOX2 in gastric cancer cells. AGS cells were transfected with scRNA or galectin-1 siRNA #1. AGS cells were transfected with scRNA or galectin-1 siRNA #1. IF for Galectin-1 (green) and SOX2 (red) was performed and visualized using confocal microscopy. Nuclei were stained with 4′,6-diamidino-2-phenylindole (blue). Galectin-1/SOX2, merged image of Galectin-1 and SOX2; Galectin-1/SOX2/DAPI, merged image of Galectin-1, SOX2, and DAPI. Image magnification, × 400; scale bar, 20 μm.



Supplementary Figure S11. Analysis of SOX2 O-GlcNAcylation under wild-type or mutant galectin-1 expression. Gastric cancer cells were transfected with wild-type galectin-1 or mutants (E71A or R73A). Cell lysates were subjected to immunoprecipitation using an anti-Myc antibody, followed by immunoblotting with an anti–O-GlcNAc antibody to assess O-GlcNAcylation levels of SOX2.



Supplementary Figure S12. Representative hematoxylin and eosin (H&E) staining and immunohistochemical detection of Galectin-1 and SOX2 illustrating four distinct expression patterns. Three formalin-fixed, paraffin-embedded gastric cancer cases per group are shown at high magnification. Scale bar, 100 μm.