Supplementary Information

Expression of intestinal stem cell and cancer stem cell markers in submucosal invasion and its prognostic significance in gastric cancers

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Supplementary Table S1 Clinicopathological characteristics of 91 early gastric cancers

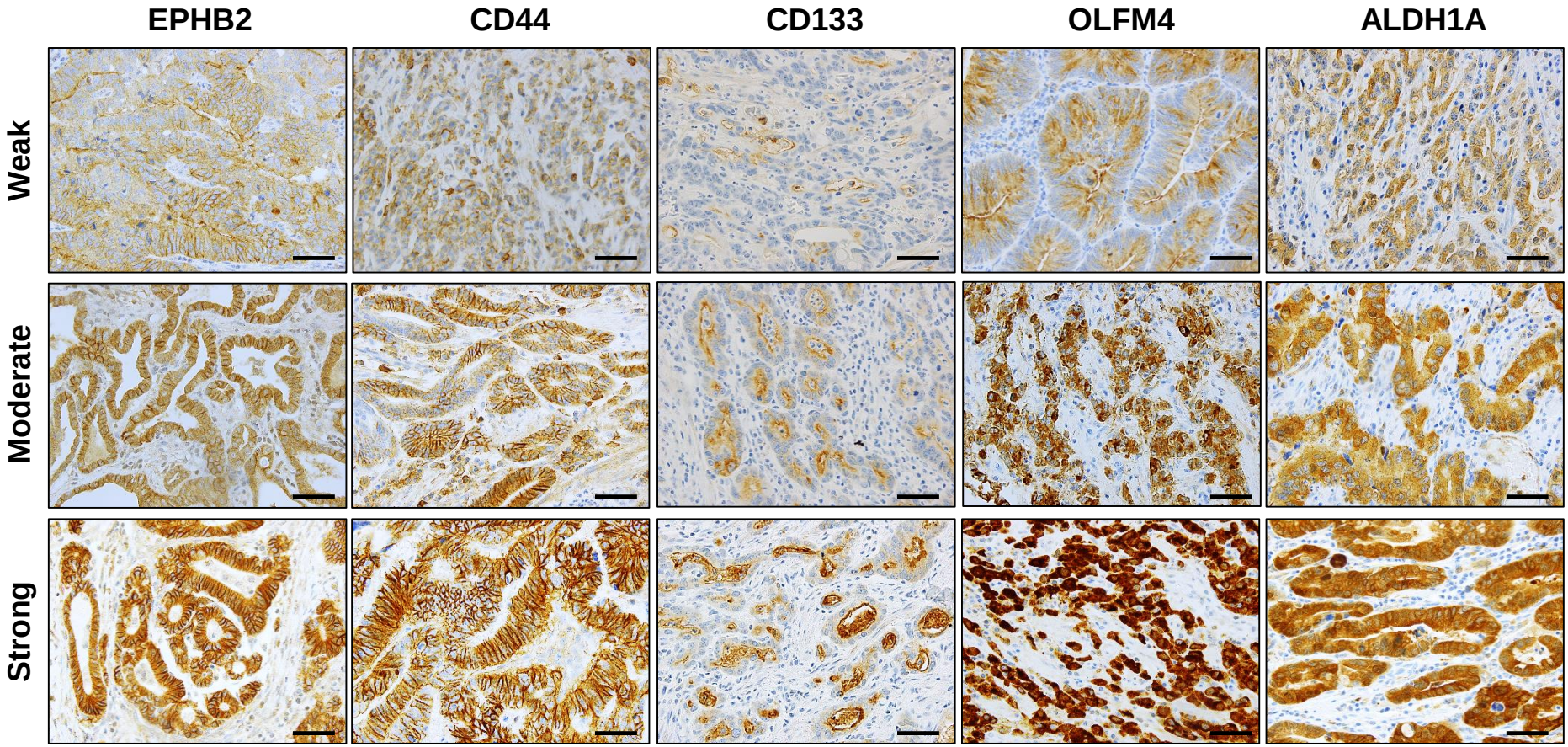
Age	40 - 85 years (median: 67; s.d.: 11.0)
Tumor size	0.4 - 10cm (median: 2.5; s.d.: 1.96)
Sex	
Male	62 (68%)
Female	29 (32%)
Depth	
Lamina propria	14 (15%)
Muscularis mucosa	10 (11%)
Submucosa	67 (74%)
Histology	
WD	23 (25%)
MD	53 (58%)
PD	8 (9%)
PCC	7 (8%)
Lauren	
Intestinal	72 (79%)
Diffuse	11 (12%)
Mixed	8 (9%)
LN invasion	
Absent	77 (85%)
Present	14 (15%)
LN metastasis*	
Absent	59 (80%)
Present	15 (20%)

* Lymph node dissection was performed in 74 cases.
WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated; PCC, poorly cohesive carcinoma; LN, lymph node; s.d., standard deviation.

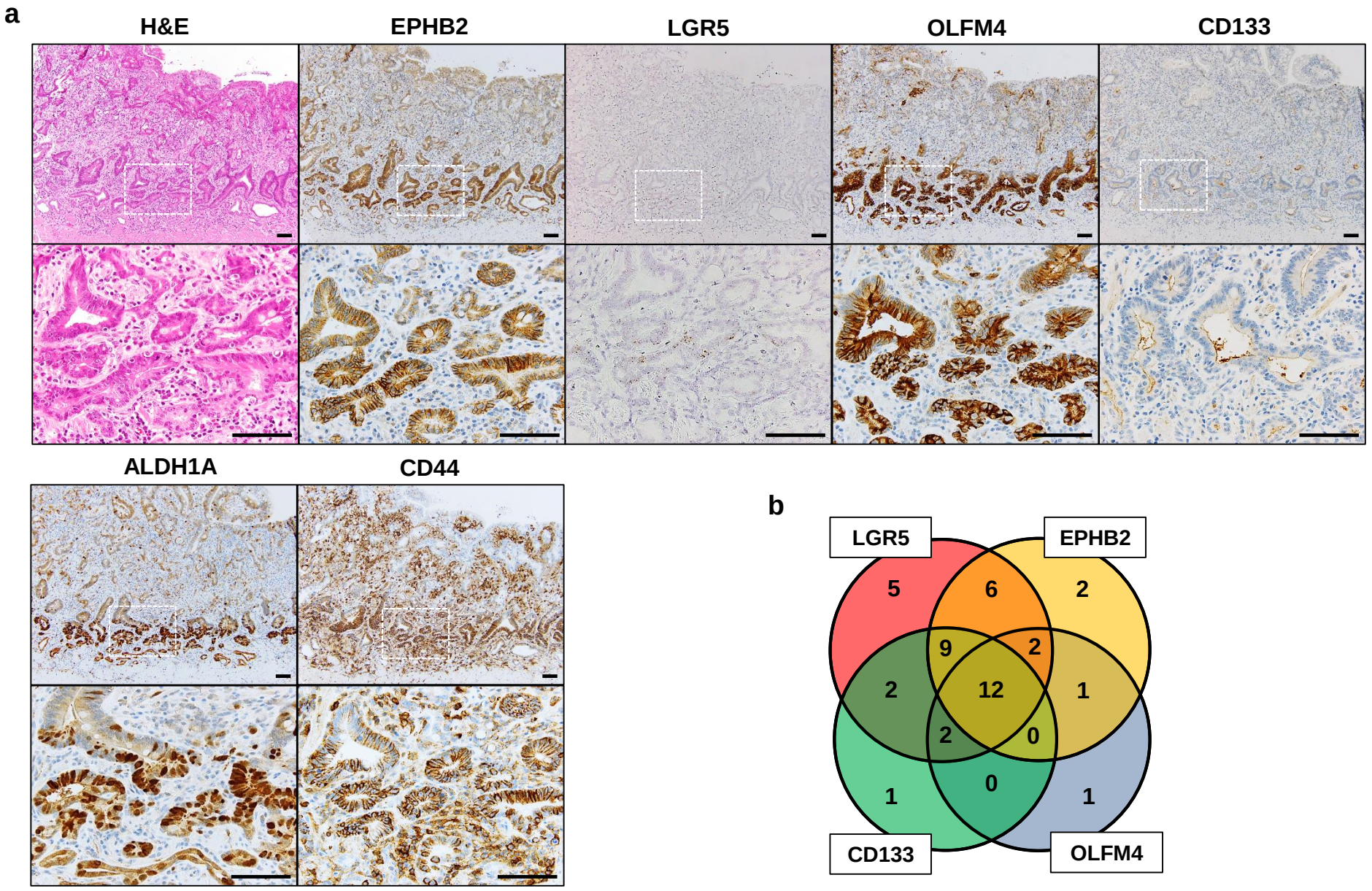
Supplementary Table S2 Association between clinicopathologic parameters and lymph node metastasis in 74 early gastric cancers

Characteristics	Total No.(%)	Lymph node metastasis		*p*-value
		Negative (%)	Positive (%)	
	74 (100)	59 (80)	15 (20)	
Age				
<65	34 (46)	24 (71)	10 (29)	0.071†
≥65	40 (54)	35 (88)	5 (12)	
Sex				
Female	24 (32)	16 (67)	8 (33)	0.053†
Male	50 (68)	43 (86)	7 (14)	
Size				
<2.5	28 (38)	26 (93)	2 (7)	0.028†
≥2.5	46 (62)	33 (56)	13 (28)	
Depth				
Mucosa	20 (27)	19 (95)	1 (5)	0.047†
Submucosa	54 (73)	40 (74)	14 (26)	
Histology				
WD	17 (23)	15 (88)	2 (12)	
MD	42 (57)	33 (79)	9 (21)	0.752†
PD	8 (11)	6 (75)	2 (25)	
PCC	7 (9)	5 (71)	2 (29)	
Lauren				
Intestinal	55 (74)	45 (82)	10 (18)	
Diffuse	11 (15)	8 (73)	3 (27)	0.743†
Mixed	8 (11)	6 (75)	2 (25)	
Lymphatic invasion				
Absent	60 (81)	54 (90)	6 (10)	< 0.001†
Present	14 (19)	5 (36)	9 (64)	

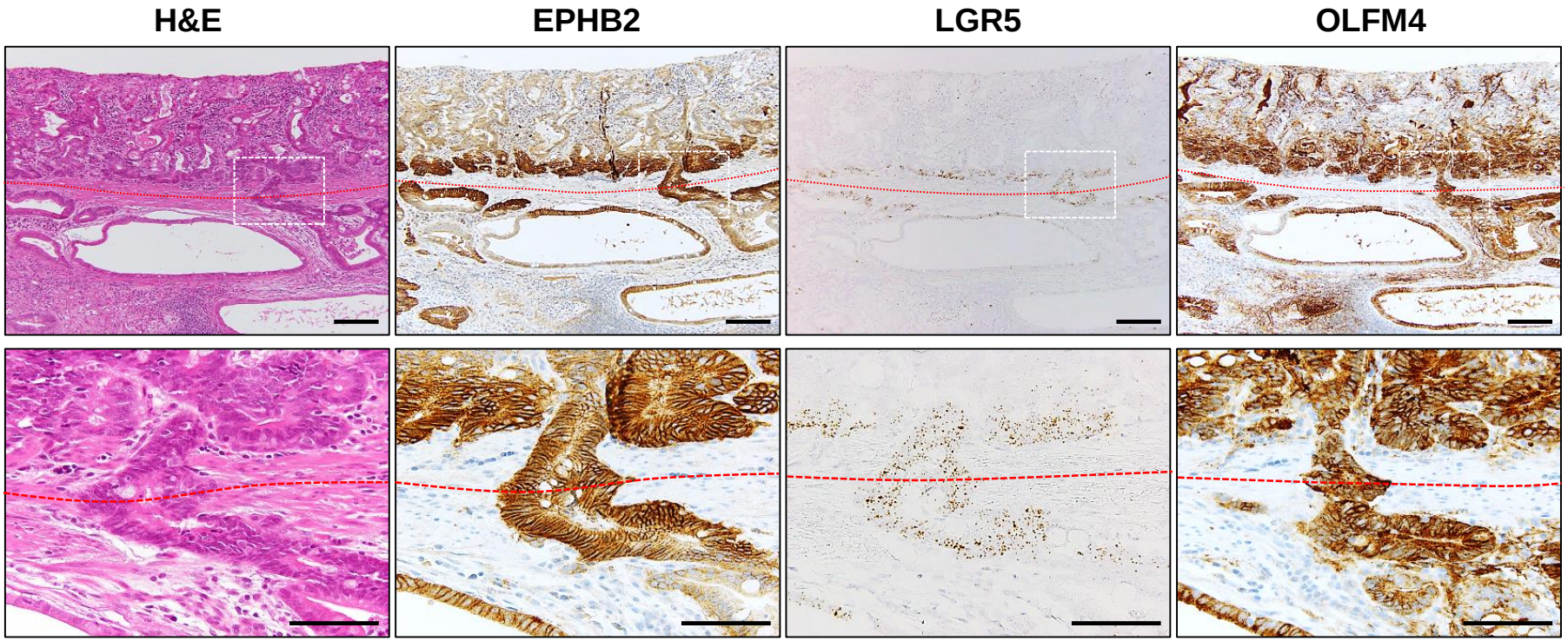
† Pearson Chi-square test.



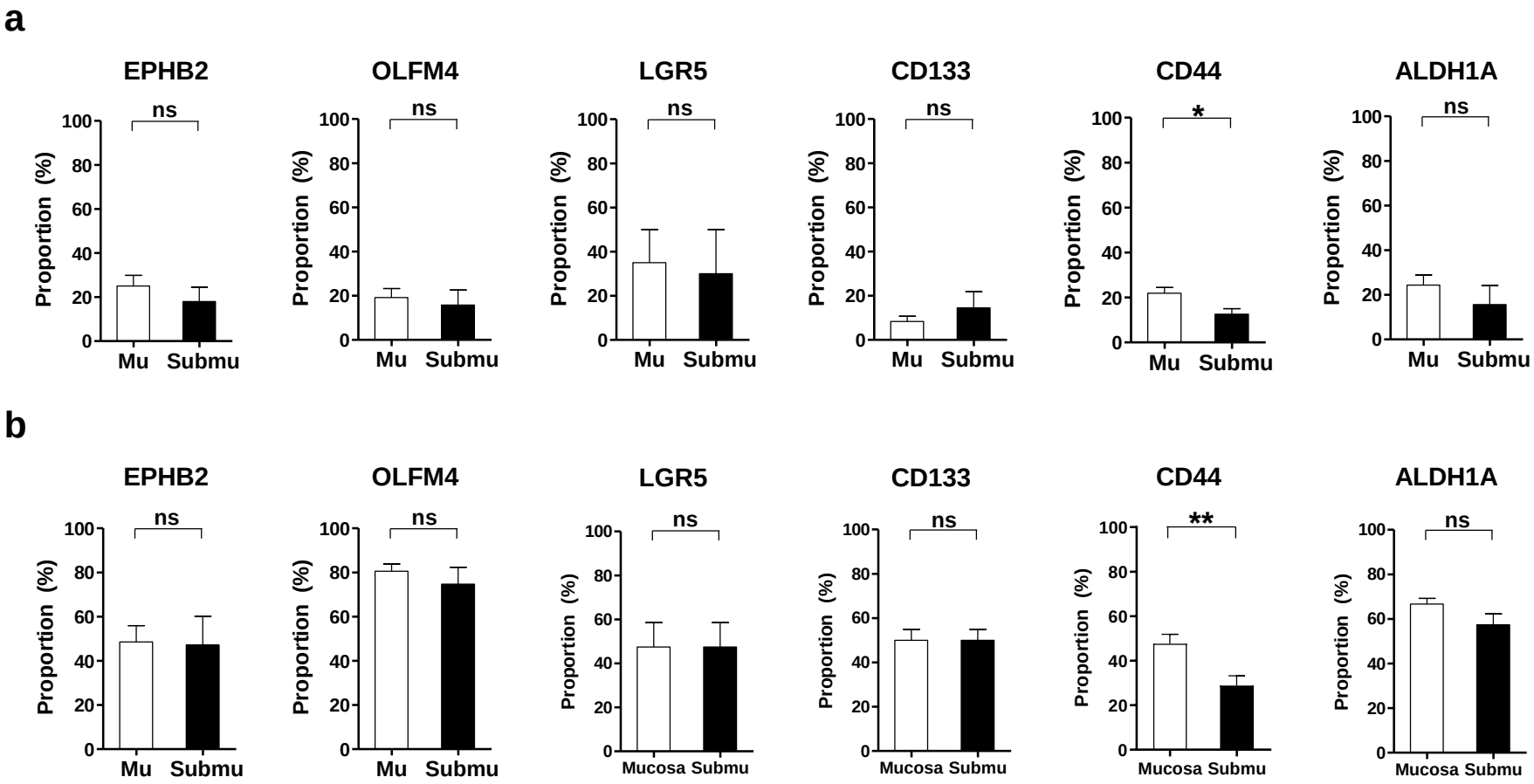
Supplementary Figure S1 Immunohistochemistry for intestinal stem cell markers (EPHB2 and OLFM4) and candidate cancer stem cell markers (CD44, CD133, and ALDHA1). Scale bars: 50μm.



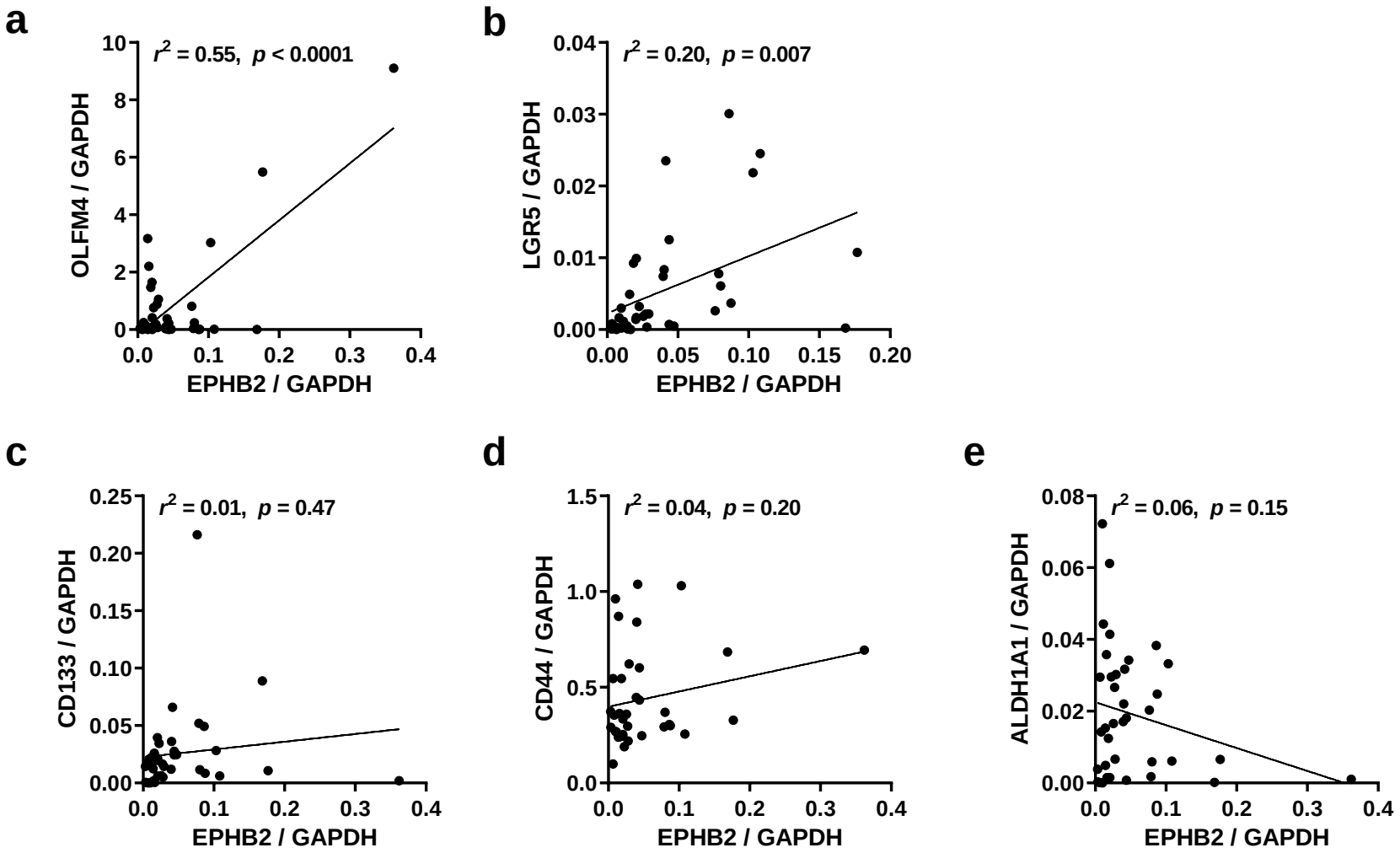
Supplementary Figure S2 Gastric cancers with basal expression of multiple intestinal stem cell and cancer stem cell markers. (**a**) Representative case of gastric cancer with basal EPHB2, LGR5, OLFM4, CD133, ALDH1A, and CD44. (**b**) A diagram showing the numbers of gastric cancer cases with basal expression of EPHB2, LGR5, OLFM4, and CD133 (n = 43). Higher magnification views of the white boxed areas are shown in the lower pictures. Scale bars: 50μm.



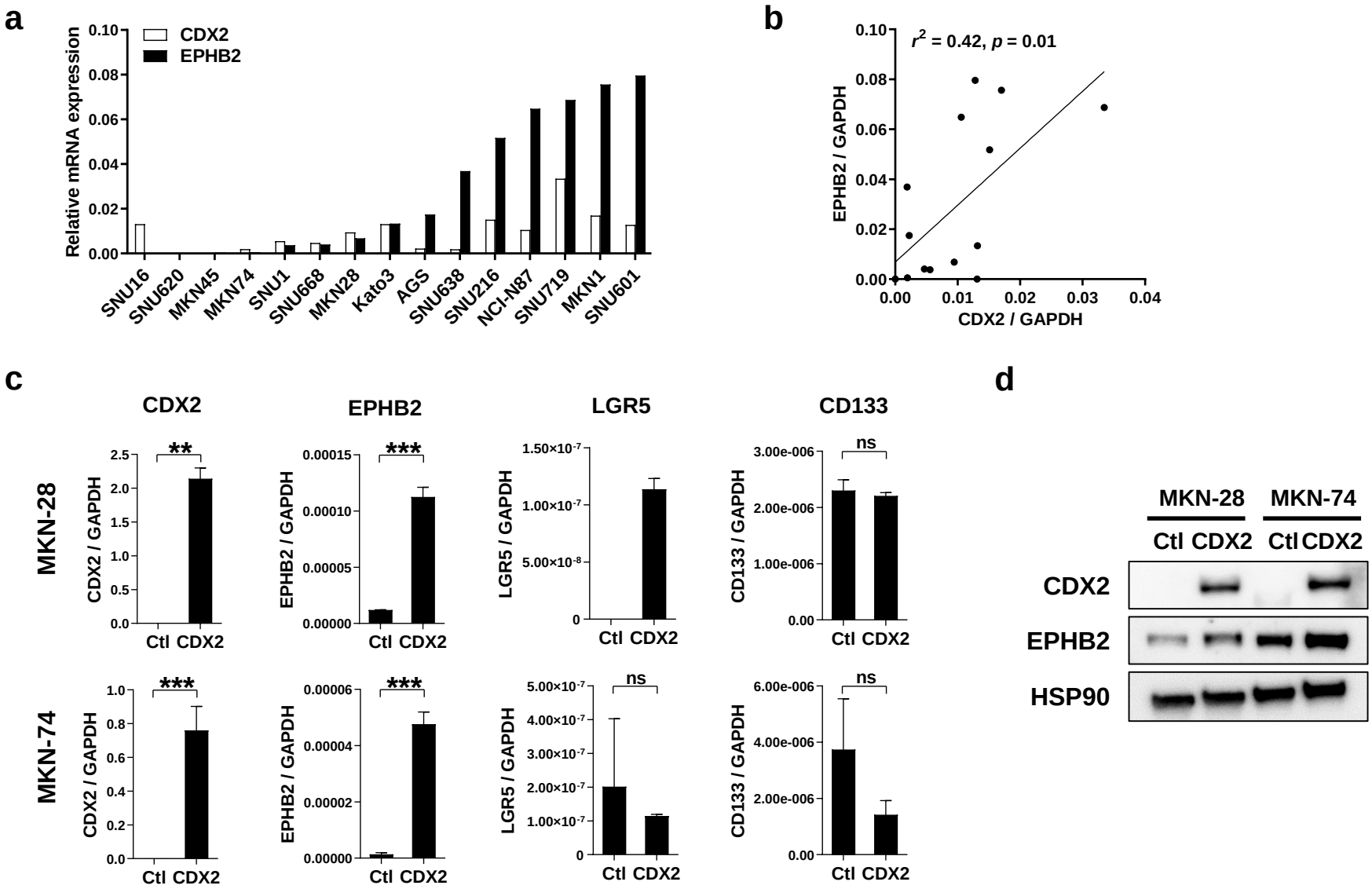
Supplementary Figure S3 Symmetrical distribution of multiple intestinal stem cell markers in the gastric cancer with submucosal invasion. Sequential immunostaining of EPHB2 and OLFM4, and RNA in situ hybridization of LGR5. The basally located cancer cells right above muscularis mucosa (indicated by red dotted line) and cancer cells invading into submucosa are positive for EPHB2, LGR5, and OLFM4. White boxed areas are shown at higher magnification in lower panel. Scale bars: 50μm.



Supplementary Figure S4 Proportion of stem cell (SC) marker-positive cancer cells in the submucosal invading cells. Proportion of SC marker-expressing cells among submucosal cancer cells in the gastric cancers (GCs) with focal distribution pattern (**a**) and in GCs with diffuse pattern (**b**) for each marker. The proportion of CD44-positive gastric cancer cells significantly decreased in the submucosa than in the mucosa of gastric cancers with both focal and diffuse patterns for CD44. ns, not significant. *$p < 0.05$, **$p < 0.005$



Supplementary Figure S5 Association of EPHB2 expression with stem cell markers in gastric cancers (n = 37). Real-time PCR was performed to measure the expression of intestinal and cancer stem cell markers, and association of EPHB2 with OLFM4 **(a)**, LGR5**(b)**, CD133 **(c)**, CD44 **(d)**, and ALDH1A1 **(e)** was evaluated.



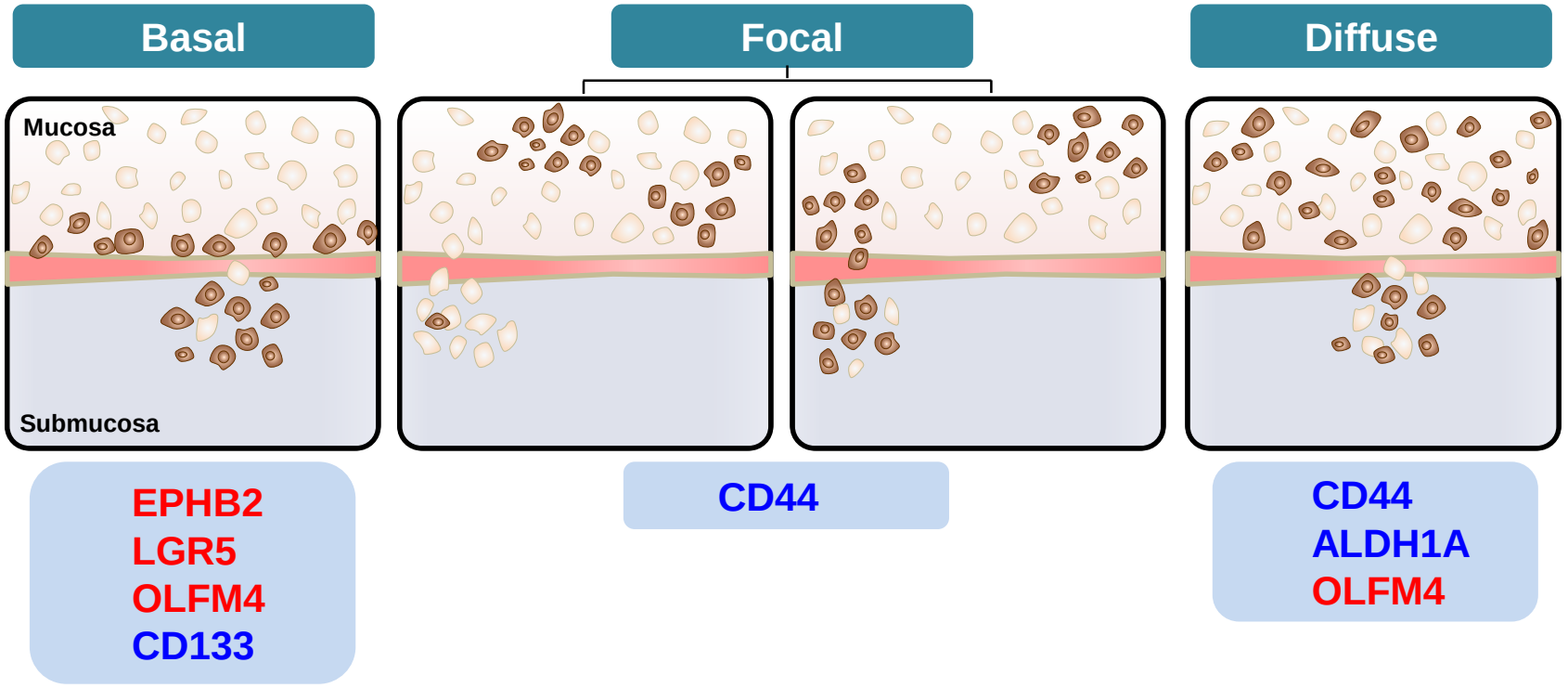
Supplementary Figure S6 Effect of CDX2 on the expression of EPHB2 in gastric cancer (GC) cells. (**a**) CDX2 and EPHB2 expression in 15 GC cell lines. (**b**) Correlation of CDX2 with EPHB2 expression in GC cell lines. (**c**) Influence of CDX2 overexpression in MKN-28 and MKN-74 cells on the expression of EPHB2, LGR5, and CD133. (**d**) Western blot for CDX2 and EPHB2 after transfection of CDX2-expressing plasmid and control vector. ns, not significant. Ctl, control. ***, $p < 0.001$

Supplementary Table S3 The results of multivariate analysis for survival rate for all cases

Variables	HR	95% CI	*p* value[a]
Age	1.428	1.089 - 1.872	0.010
Histology	1.026	0.920 - 1.145	0.642
Lymphatic invasion	1.496	1.017 - 2.200	0.041
Venous invasion	1.374	1.032 - 1.830	0.029
TNM_7th	3.605	2.954- 4.399	< 0.001
CDX2	0.711	0.458 - 1.102	0.127
CD133	1.111	0.815 - 1.614	0.507
OLFM4	0.832	0.624 - 1.110	0.211
EPHB2	0.861	0.640 - 1.158	0.321

HR, Hazard ratio; CI, confidence interval

[a] Cox proportional hazard model



Supplementary Figure S7 Schematic diagram showing distribution of intestinal stem cell (ISC) markers and candidate cancer stem cell (CSC) markers in gastric cancers with submucosal invasion. All ISC markers and CD133 predominantly exhibited a basal distribution, resulting in the increased proportion of stem cell (SC) marker-positive cancer cells in the submucosal invading cells, whereas CD44 and ALDH1A mainly displayed focal or diffuse patterns, which showed a decline or no difference in the proportion of SC marker-positive cells.