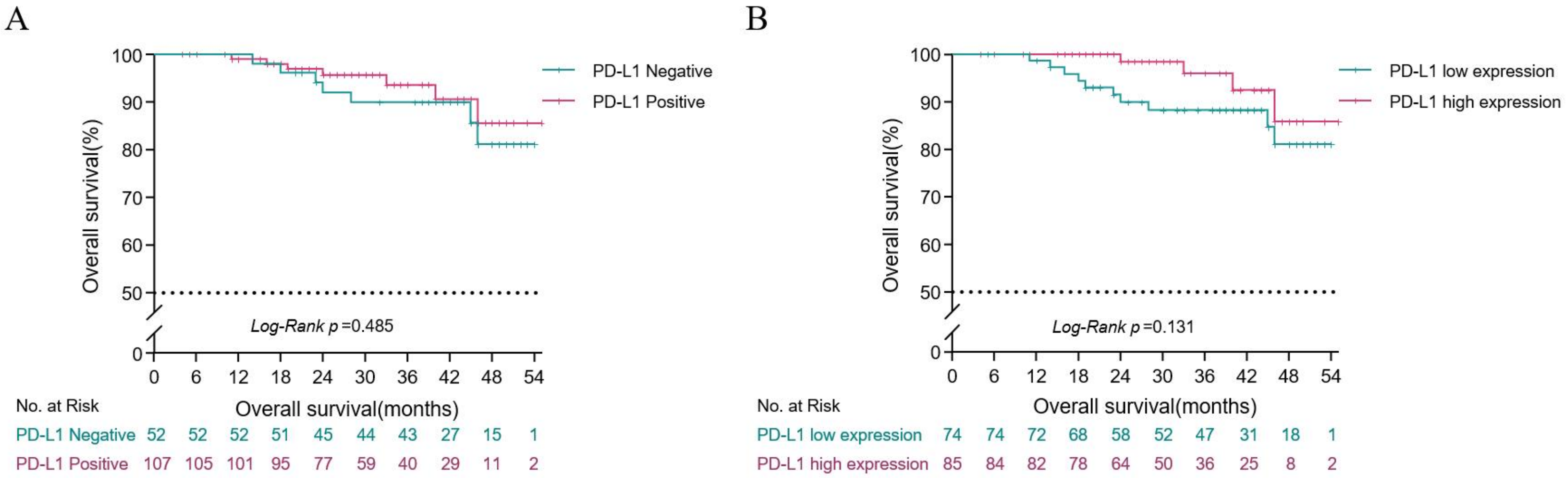
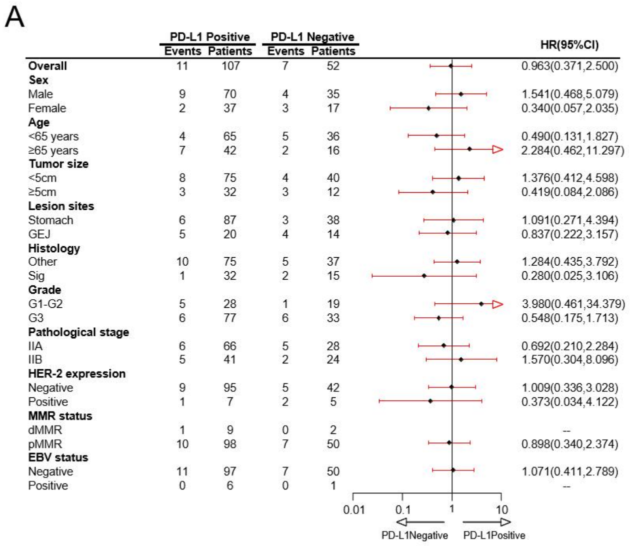
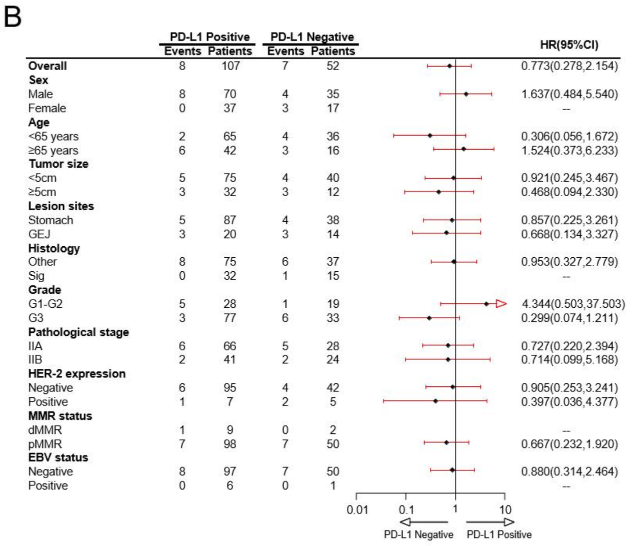


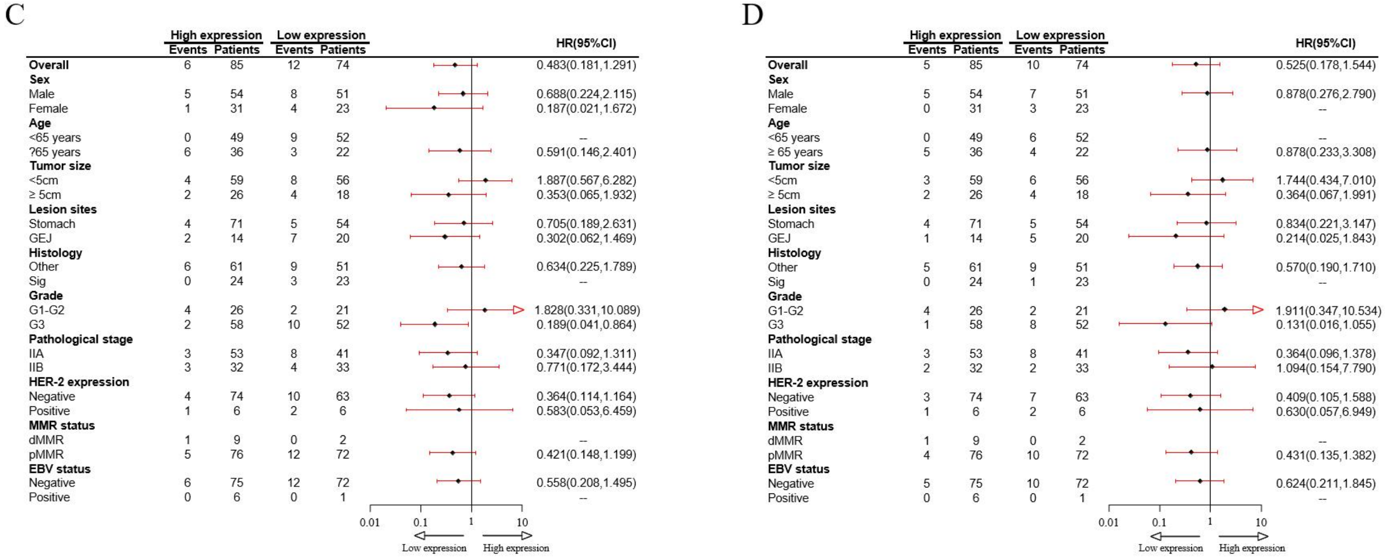
Supplementary Figure 1 Sensitivity analyses of overall survival according to programmed death ligand-1 expression in patients with pathological stage II gastric or gastroesophageal junction cancer. A: Kaplan-Meier curves showing overall survival (OS) between programmed death ligand-1 (PD-L1) positive and PD-L1-negative groups; B: Kaplan-Meier curves showing OS between high and low PD-L1 expression groups. No significant differences in OS were observed between the groups. PD-L1: Programmed death ligand-1.



	PD-L1 Positive		PD-L1 Negative		HR(95%CI)
	Events	Patients	Events	Patients	
Overall	11	107	7	52	0.963(0.371,2.500)
Sex					
Male	9	70	4	35	1.541(0.468,5.079)
Female	2	37	3	17	0.340(0.057,2.035)
Age					
<65 years	4	65	5	36	0.490(0.131,1.827)
≥65 years	7	42	2	16	2.284(0.462,11.297)
Tumor size					
<5cm	8	75	4	40	1.376(0.412,4.598)
≥5cm	3	32	3	12	0.419(0.084,2.086)
Lesion sites					
Stomach	6	87	3	38	1.091(0.271,4.394)
GEJ	5	20	4	14	0.837(0.222,3.157)
Histology					
Other	10	75	5	37	1.284(0.435,3.792)
Sig	1	32	2	15	0.280(0.025,3.106)
Grade					
G1-G2	5	28	1	19	3.980(0.461,34.379)
G3	6	77	6	33	0.548(0.175,1.713)
Pathological stage					
IIA	6	66	5	28	0.692(0.210,2.284)
IIB	5	41	2	24	1.570(0.304,8.096)
HER-2 expression					
Negative	9	95	5	42	1.009(0.336,3.028)
Positive	1	7	2	5	0.373(0.034,4.122)
MMR status					
dMMR	1	9	0	2	--
pMMR	10	98	7	50	0.898(0.340,2.374)
EBV status					
Negative	11	97	7	50	1.071(0.411,2.789)
Positive	0	6	0	1	--



	PD-L1 Positive		PD-L1 Negative		HR(95%CI)
	Events	Patients	Events	Patients	
Overall	8	107	7	52	0.773(0.278,2.154)
Sex					
Male	8	70	4	35	1.637(0.484,5.540)
Female	0	37	3	17	--
Age					
<65 years	2	65	4	36	0.306(0.056,1.672)
≥65 years	6	42	3	16	1.524(0.373,6.233)
Tumor size					
<5cm	5	75	4	40	0.921(0.245,3.467)
≥5cm	3	32	3	12	0.468(0.094,2.330)
Lesion sites					
Stomach	5	87	4	38	0.857(0.225,3.261)
GEJ	3	20	3	14	0.668(0.134,3.327)
Histology					
Other	8	75	6	37	0.953(0.327,2.779)
Sig	0	32	1	15	--
Grade					
G1-G2	5	28	1	19	4.344(0.503,37.503)
G3	3	77	6	33	0.299(0.074,1.211)
Pathological stage					
IIA	6	66	5	28	0.727(0.220,2.394)
IIB	2	41	2	24	0.714(0.099,5.168)
HER-2 expression					
Negative	6	95	4	42	0.905(0.253,3.241)
Positive	1	7	2	5	0.397(0.036,4.377)
MMR status					
dMMR	1	9	0	2	--
pMMR	7	98	7	50	0.667(0.232,1.920)
EBV status					
Negative	8	97	7	50	0.880(0.314,2.464)
Positive	0	6	0	1	--



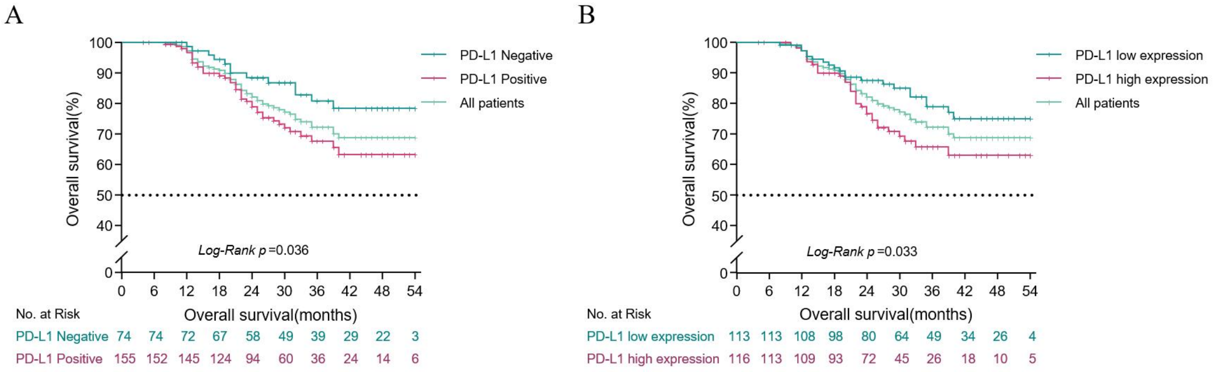
C

	High expression		Low expression		HR(95%CI)
	Events	Patients	Events	Patients	
Overall	6	85	12	74	0.483(0.181,1.291)
Sex					
Male	5	54	8	51	0.688(0.224,2.115)
Female	1	31	4	23	0.187(0.021,1.672)
Age					
<65 years	0	49	9	52	--
?65 years	6	36	3	22	0.591(0.146,2.401)
Tumor size					
<5cm	4	59	8	56	1.887(0.567,6.282)
≥ 5cm	2	26	4	18	0.353(0.065,1.932)
Lesion sites					
Stomach	4	71	5	54	0.705(0.189,2.631)
GEJ	2	14	7	20	0.302(0.062,1.469)
Histology					
Other	6	61	9	51	0.634(0.225,1.789)
Sig	0	24	3	23	--
Grade					
G1-G2	4	26	2	21	1.828(0.331,10.089)
G3	2	58	10	52	0.189(0.041,0.864)
Pathological stage					
IIA	3	53	8	41	0.347(0.092,1.311)
IIB	3	32	4	33	0.771(0.172,3.444)
HER-2 expression					
Negative	4	74	10	63	0.364(0.114,1.164)
Positive	1	6	2	6	0.583(0.053,6.459)
MMR status					
dMMR	1	9	0	2	--
pMMR	5	76	12	72	0.421(0.148,1.199)
EBV status					
Negative	6	75	12	72	0.558(0.208,1.495)
Positive	0	6	0	1	--

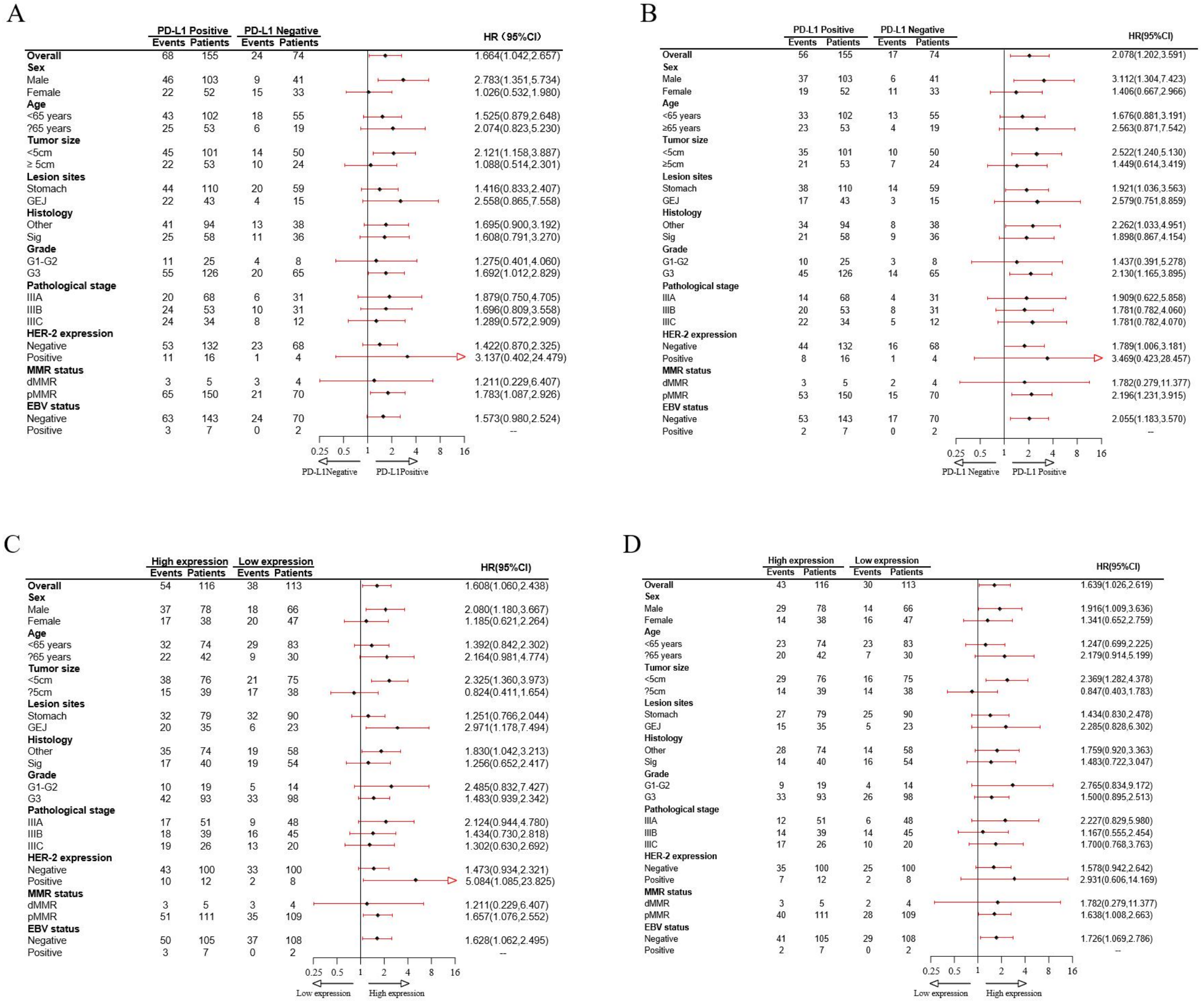
D

	High expression		Low expression		HR(95%CI)
	Events	Patients	Events	Patients	
Overall	5	85	10	74	0.525(0.178,1.544)
Sex					
Male	5	54	7	51	0.878(0.276,2.790)
Female	0	31	3	23	--
Age					
<65 years	0	49	6	52	--
≥ 65 years	5	36	4	22	0.878(0.233,3.308)
Tumor size					
<5cm	3	59	6	56	1.744(0.434,7.010)
≥ 5cm	2	26	4	18	0.364(0.067,1.991)
Lesion sites					
Stomach	4	71	5	54	0.834(0.221,3.147)
GEJ	1	14	5	20	0.214(0.025,1.843)
Histology					
Other	5	61	9	51	0.570(0.190,1.710)
Sig	0	24	1	23	--
Grade					
G1-G2	4	26	2	21	1.911(0.347,10.534)
G3	1	58	8	52	0.131(0.016,1.055)
Pathological stage					
IIA	3	53	8	41	0.364(0.096,1.378)
IIB	2	32	2	33	1.094(0.154,7.790)
HER-2 expression					
Negative	3	74	7	63	0.409(0.105,1.588)
Positive	1	6	2	6	0.630(0.057,6.949)
MMR status					
dMMR	1	9	0	2	--
pMMR	4	76	10	72	0.431(0.135,1.382)
EBV status					
Negative	5	75	10	72	0.624(0.211,1.845)
Positive	0	6	0	1	--

Supplementary Figure 2 Forest plot of disease-free survival and overall survival of different programmed death ligand-1 expression in pathological stage II gastric or gastroesophageal junction cancer. A and B: Disease-free survival (DFS; A) and overall survival (OS; B) in the programmed death ligand-1 (PD-L1) positive and -negative groups; C and D: DFS (C) and OS (D) in the high and low PD-L1 expression groups. For subgroups with zero events in any patients, the HR was not calculated or shown. PD-L1: Programmed death ligand-1; GEJ: Gastroesophageal junction; MMR: Mismatch repair; EBV: Epstein-Barr virus.



Supplementary Figure 3 Sensitivity analyses of overall survival according to programmed death ligand-1 expression in patients with pathological stage III gastric or gastroesophageal junction cancer. A: Kaplan-Meier curves showing overall survival (OS) between programmed death ligand-1 (PD-L1) positive and PD-L1 negative groups; B: Kaplan-Meier curves showing OS between high and low PD-L1 expression groups. Patients with PD-L1-positive or high expression had significantly shorter OS than those with PD-L1-negative or low expression. PD-L1: Programmed death ligand-1.



A

	PD-L1 Positive		PD-L1 Negative		HR（95%CI）
	Events	Patients	Events	Patients	
Overall	68	155	24	74	1.664(1.042,2.657)
Sex					
Male	46	103	9	41	2.783(1.351,5.734)
Female	22	52	15	33	1.026(0.532,1.980)
Age					
<65 years	43	102	18	55	1.525(0.879,2.648)
?65 years	25	53	6	19	2.074(0.823,5.230)
Tumor size					
<5cm	45	101	14	50	2.121(1.158,3.887)
≥ 5cm	22	53	10	24	1.088(0.514,2.301)
Lesion sites					
Stomach	44	110	20	59	1.416(0.833,2.407)
GEJ	22	43	4	15	2.558(0.865,7.558)
Histology					
Other	41	94	13	38	1.695(0.900,3.192)
Sig	25	58	11	36	1.608(0.791,3.270)
Grade					
G1-G2	11	25	4	8	1.275(0.401,4.060)
G3	55	126	20	65	1.692(1.012,2.829)
Pathological stage					
IIIA	20	68	6	31	1.879(0.750,4.705)
IIIB	24	53	10	31	1.696(0.809,3.558)
IIIC	24	34	8	12	1.289(0.572,2.909)
HER-2 expression					
Negative	53	132	23	68	1.422(0.870,2.325)
Positive	11	16	1	4	3.137(0.402,24.479)
MMR status					
dMMR	3	5	3	4	1.211(0.229,6.407)
pMMR	65	150	21	70	1.783(1.087,2.926)
EBV status					
Negative	63	143	24	70	1.573(0.980,2.524)
Positive	3	7	0	2	--

B

	PD-L1 Positive		PD-L1 Negative		HR(95%CI)
	Events	Patients	Events	Patients	
Overall	56	155	17	74	2.078(1.202,3.591)
Sex					
Male	37	103	6	41	3.112(1.304,7.423)
Female	19	52	11	33	1.406(0.667,2.966)
Age					
<65 years	33	102	13	55	1.676(0.881,3.191)
≥65 years	23	53	4	19	2.563(0.871,7.542)
Tumor size					
<5cm	35	101	10	50	2.522(1.240,5.130)
≥5cm	21	53	7	24	1.449(0.614,3.419)
Lesion sites					
Stomach	38	110	14	59	1.921(1.036,3.563)
GEJ	17	43	3	15	2.579(0.751,8.859)
Histology					
Other	34	94	8	38	2.262(1.033,4.951)
Sig	21	58	9	36	1.898(0.867,4.154)
Grade					
G1-G2	10	25	3	8	1.437(0.391,5.278)
G3	45	126	14	65	2.130(1.165,3.895)
Pathological stage					
IIIA	14	68	4	31	1.909(0.622,5.858)
IIIB	20	53	8	31	1.781(0.782,4.060)
IIIC	22	34	5	12	1.781(0.782,4.070)
HER-2 expression					
Negative	44	132	16	68	1.789(1.006,3.181)
Positive	8	16	1	4	3.469(0.423,28.457)
MMR status					
dMMR	3	5	2	4	1.782(0.279,11.377)
pMMR	53	150	15	70	2.196(1.231,3.915)
EBV status					
Negative	53	143	17	70	2.055(1.183,3.570)
Positive	2	7	0	2	--

C

	High expression		Low expression		HR(95%CI)
	Events	Patients	Events	Patients	
Overall	54	116	38	113	1.608(1.060,2.438)
Sex					
Male	37	78	18	66	2.080(1.180,3.667)
Female	17	38	20	47	1.185(0.621,2.264)
Age					
<65 years	32	74	29	83	1.392(0.842,2.302)
?65 years	22	42	9	30	2.164(0.981,4.774)
Tumor size					
<5cm	38	76	21	75	2.325(1.360,3.973)
?5cm	15	39	17	38	0.824(0.411,1.654)
Lesion sites					
Stomach	32	79	32	90	1.251(0.766,2.044)
GEJ	20	35	6	23	2.971(1.178,7.494)
Histology					
Other	35	74	19	58	1.830(1.042,3.213)
Sig	17	40	19	54	1.256(0.652,2.417)
Grade					
G1-G2	10	19	5	14	2.485(0.832,7.427)
G3	42	93	33	98	1.483(0.939,2.342)
Pathological stage					
IIIA	17	51	9	48	2.124(0.944,4.780)
IIIB	18	39	16	45	1.434(0.730,2.818)
IIIC	19	26	13	20	1.302(0.630,2.692)
HER-2 expression					
Negative	43	100	33	100	1.473(0.934,2.321)
Positive	10	12	2	8	5.084(1.085,23.825)
MMR status					
dMMR	3	5	3	4	1.211(0.229,6.407)
pMMR	51	111	35	109	1.657(1.076,2.552)
EBV status					
Negative	50	105	37	108	1.628(1.062,2.495)
Positive	3	7	0	2	--

D

	High expression		Low expression		HR(95%CI)
	Events	Patients	Events	Patients	
Overall	43	116	30	113	1.639(1.026,2.619)
Sex					
Male	29	78	14	66	1.916(1.009,3.636)
Female	14	38	16	47	1.341(0.652,2.759)
Age					
<65 years	23	74	23	83	1.247(0.699,2.225)
?65 years	20	42	7	30	2.179(0.914,5.199)
Tumor size					
<5cm	29	76	16	75	2.369(1.282,4.378)
?5cm	14	39	14	38	0.847(0.403,1.783)
Lesion sites					
Stomach	27	79	25	90	1.434(0.830,2.478)
GEJ	15	35	5	23	2.285(0.828,6.302)
Histology					
Other	28	74	14	58	1.759(0.920,3.363)
Sig	14	40	16	54	1.483(0.722,3.047)
Grade					
G1-G2	9	19	4	14	2.765(0.834,9.172)
G3	33	93	26	98	1.500(0.895,2.513)
Pathological stage					
IIIA	12	51	6	48	2.227(0.829,5.980)
IIIB	14	39	14	45	1.167(0.555,2.454)
IIIC	17	26	10	20	1.700(0.768,3.763)
HER-2 expression					
Negative	35	100	25	100	1.578(0.942,2.642)
Positive	7	12	2	8	2.931(0.606,14.169)
MMR status					
dMMR	3	5	2	4	1.782(0.279,11.377)
pMMR	40	111	28	109	1.638(1.008,2.663)
EBV status					
Negative	41	105	29	108	1.726(1.069,2.786)
Positive	2	7	0	2	--

Supplementary Figure 4 Forest plot of disease-free survival and overall survival of different programmed death ligand-1 expression in pathological stage III gastric or gastroesophageal junction cancer. A and B disease-free survival (DFS; A) and overall survival (OS; B) in the programmed death ligand-1 (PD-L1) positive and -negative groups; C and D: DFS (C) and OS (D) in the high and low PD-L1 expression groups. For subgroups with zero events in any patients, the HR was not calculated or shown. PD-L1: Programmed death ligand-1; GEJ: Gastroesophageal junction; MMR: Mismatch repair; EBV: Epstein-Barr virus.

Supplementary Table 1 Tumor microenvironment analysis of programmed death ligand-1 high expression in patients with locally advanced gastric or gastroesophageal junction cancer

Cell type or other indicators	Marker	Area	Cell number, cells/mm^2 (positive rate, %)							
			Pathological stage II				Pathological stage III			
			Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Total T cells	CD3+	Tumor	2414 (19.42)	836 (9.52)	349.35 (6.92)	426.86 (4.93)	-	-	156 (1.93)	2425 (21.48)
		Stroma	2704 (21.86)	133 (1.86)	1310.70 (14.11)	193.33 (2.31)	-	-	1079 (11.77)	472 (3.47)
Cytotoxic T cells	CD8+	Tumor	10 (0.08)	223 (2.77)	316.51 (5.19)	192.42 (1.89)	388 (6.13)	95 (1.28)	13 (0.13)	890 (7.97)
		Stroma	44 (0.27)	16 (0.21)	780.68 (7.72)	137.63 (1.27)	68 (0.97)	1159 (4.16)	4 (0.04)	32 (0.25)
PD-1 positive effector T	PD-1+CD8+	Tumor	0 (0.00)	0 (0.00)	32.04 (0.52)	28.86 (0.28)	-	-	0 (0.00)	31 (0.27)
		Stroma	4	0	148.48	4.37	-	-	0	1

cells			(0.02)	(0.00)	(1.47)	(0.04)			(0.00)	(0.01)
Helper T Cells	CD3+CD4+	Tumor	471 (3.79)	10 (0.12)	48.19 (0.95)	124.50 (1.44)	-	-	32 (0.39)	1271 (11.26)
		Stroma	384 (3.10)	3 (0.04)	303.58 (3.27)	57.69 (0.69)	-	-	398 (4.34)	258 (1.90)
Regulatory T cells	CD3+CD4+FoxP3+	Tumor	88 (0.71)	0 (0.00)	11.41 (0.23)	26.68 (0.31)	-	-	14 (0.17)	347 (3.08)
		Stroma	13 (0.11)	0 (0.00)	49.79 (0.54)	18.71 (0.22)	-	-	39 (0.42)	40 (0.29)
PD-L1 positive macrophages	PD-L1+CD68+	Tumor	4 (0.03)	2 (0.02)	107.64 (1.76)	3.21 (0.03)	16 (0.26)	9 (0.12)	0 (0.00)	246 (2.20)
		Stroma	25 (0.16)	0 (0.00)	68.69 (0.68)	0.00 (0.00)	8 (0.11)	28 (0.10)	14 (0.12)	365 (2.93)
M1 macrophages	CD68+CD163-	Tumor	232 (1.78)	54 (0.67)	130.07 (2.13)	93.00 (0.91)	265 (4.19)	57 (0.76)	136 (1.33)	181 (1.62)
		Stroma	208 (1.28)	234 (3.07)	167.44 (1.65)	69.91 (0.65)	230 (3.28)	187 (0.67)	465 (3.89)	412 (3.31)

M2 macrophages	CD68+CD163+	Tumor	18 (0.14)	26 (0.32)	192.22 (3.15)	19.24 (0.19)	142 (2.24)	3 (0.04)	7 (0.06)	198 (1.77)
		Stroma	18 (0.11)	163 (2.15)	409.30 (4.05)	15.29 (0.14)	61 (0.86)	14 (0.05)	13 (0.11)	173 (1.39)
Natural killer (NK) cells	CD56bright	Tumor	1 (0.01)	0 (0.00)	0.00 (0.00)	0.00 (0.00)	0 (0.00)	6 (0.08)	0 (0.00)	13 (0.12)
		Stroma	4 (0.03)	0 (0.00)	0.00 (0.00)	0.00 (0.00)	0 (0.00)	28 (0.10)	0 (0.00)	33 (0.25)
	CD56dim	Tumor	54 (0.43)	0 (0.00)	62.77 (1.24)	0.00 (0.00)	24 (0.38)	72 (0.96)	0 (0.00)	7 (0.06)
		Stroma	194 (1.57)	0 (0.00)	201.18 (2.17)	0.00 (0.00)	8 (0.11)	354 (1.27)	23 (0.25)	22 (0.17)
B cells	CD20+	Tumor	101 (0.81)	3 (0.03)	0.63 (0.01)	31.12 (0.36)	-	-	3 (0.04)	395 (3.50)
		Stroma	647 (5.23)	9 (0.12)	3.61 (0.04)	9.35 (0.11)	-	-	62 (0.67)	817 (6.01)
PD-1	PD-1+	Tumor	75	18	171.71	378.43	-	-	38	132

positive cell			(0.57)	(0.22)	(2.81)	(3.71)			(0.37)	(1.18)
		Stroma	185 (1.14)	93 (1.22)	804.29 (7.95)	327.69 (3.03)	-	-	160 (1.33)	260 (2.09)
TPS	PD-L1+ (E1 L3N antibody)		<1%	<1%	0.16	<1%	<1%	<1%	<1%	0.03
CPS	PD-L1+ (E1 L3N antibody)		<1	<1	16	<1	<1	<1	<1	18
Tertiary lymphoid structure[1]	CD3+、CD20+	/	0.15 4829.10	0 0	0 0	0 0	- -	- -	0.47 29136.70	0.36 42672.07

[1] The count and density of tertiary lymphatic structures are filled in the table. The unit is number/mm^2 (μm^2/mm^2).

TME: Tumor microenvironment; G/GEJC: Gastric or gastroesophageal junction cancer.

Supplementary Table 2 Clinicopathological features associated with Programmed death ligand-1 positive group in pathological stage III gastric or gastroesophageal junction cancer

Variables	PD-L1 positive (*n* = 155)	PD-L1 negative (*n* = 74)	Statistic	*P* value
Sex [n(%)]			2.618	0.106

Male	103 (66.5%)	41 (55.4%)		
Female	52 (33.5%)	33 (44.6%)		
Age (mean ± SD)	58.57 ± 12.47	55.22 ± 13.34	-1.859	0.064
Tumor size [n(%)]			0.088	0.767
< 5cm	101 (65.6%)	50 (67.6%)		
≥ 5cm	53 (34.4%)	24 (32.4%)		
Lesion sites [n(%)]			1.609	0.205
Stomach	110 (71.9%)	59 (79.7%)		
GEJ	43 (28.1%)	15 (20.3%)		
Histology [n(%)]			2.255	0.133
Sig	58 (38.2%)	36 (48.6%)		
Other	94 (61.8%)	38 (51.4%)		
Grade [n(%)]			1.227	0.268
G1-G2	25 (16.6%)	8 (11.0%)		
G3	126 (83.4%)	65 (89.0%)		
Pathological stage [n(%)]			1.670	0.434
pIIIA	68 (43.9%)	31 (41.9%)		

pIIIB	53 (34.2%)	31 (41.9%)		
pIIIC	34 (21.9%)	12 (16.2%)		
HER-2 expression [n(%)]			1.619	0.203
Negative	132 (89.2%)	68 (94.4%)		
Positive	16 (10.8%)	4 (5.6%)		
MMR status [n(%)]			0.185	0.667
dMMR	5 (3.2%)	4 (5.4%)		
pMMR	150 (96.8%)	70 (94.6%)		
EBV status [n(%)]			0.093	0.761
Negative	143 (95.3%)	70 (97.2%)		
Positive	7 (4.7%)	2 (2.8%)		
Ki-67 index (mean ± SD)	62.85 ± 21.90	52.85 ± 23.97	-3.125	0.002
Postoperative adjuvant chemotherapy [n(%)]			0.064	0.801
Yes	100 (64.5%)	49 (66.2%)		
No	55 (35.5%)	25 (33.8%)		

GEJ: Gastroesophageal junction; Sig: Signet ring cell carcinoma; pIII: Pathological stage III; MMR: Mismatch repair; dMMR: Deficient mismatch repair; pMMR: Proficient mismatch repair; EBV: Epstein-Barr virus; PD-L1: Programmed death ligand-1.