Supplementary Material

This appendix is provided by the authors to give readers additional information about this study.

Change in PD-L1 and CD8 expression after chemoradiotherapy for esophageal squamous cell carcinoma

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Table S1. Clinicopathological characteristics of subgroup ESCC patients

[a]These patients did not provide the tumor grade in pathological reports.

Characteristics	With paired specimens N=23 Mean ±SD or No. (%)	Without paired specimens N= 41 Mean ±SD or No. (%)
Gender		
Male	22 (95.7)	39 (95.1)
Female	1 (4.3)	2 (4.9)
Age (years)	55.97 ±8.62	56.14 ±8.01
Pathologic status		
Stage I	0 (0)	10 (24.4)
Stage II	0 (0)	14 (34.1)
Stage III	22 (95.7)	14 (34.1)
Stage IV	1 (4.3)	3 (7.3)
Tumor differentiation		
Grade 1 (Well)	3 (13.0)	2 (4.9)
Grade 2 (Moderate)	15 (65.2)	34 (82.9)
Grade 3 (Poor)	3 (13.0)	3 (7.3)
Missing[a]	2 (8.7)	2 (4.9)
Treatment		
ESD	0 (0)	5 (12.2)
OP	0 (0)	14 (34.1)
CCRT	7 (30.4)	18 (43.9)
CCRT then OP	16 (69.6)	4 (9.8)
CCRT Response		
Without CCRT	0 (0)	19 (46.3)
Complete response	0 (0)	7 (17.1)
Partial response	22 (95.7)	10 (24.4)
Stable disease	1 (4.3)	4 (9.8)
Progressive disease	0 (0)	1 (2.4)
PD-L1 H-score		
H-score Median < 2	11 (47.8)	18 (43.9)
H-score Median ≥ 2	12 (52.2)	23 (56.1)

Abbreviations: ESD, Endoscopic submucosal dissection; CCRT, Concurrent chemoradiation therapy; CT, chemotherapy; OP, esophageal resection surgery

Table S2. The H-score distribution of 64 patients.

H-score	Numbers (n=64)	Percentage
0	16	25.00%
1	13	20.31%
2 (Median)	6	9.38%
4	1	1.56%
5	2	3.13%
8	1	1.56%
10	2	3.13%
12	1	1.56%
15	2	3.13%
19	1	1.56%
20	1	1.56%
25	2	3.13%
30	2	3.13%
45	1	1.56%
70	1	1.56%
75	2	3.13%
80	1	1.56%
90	1	1.56%
100	1	1.56%
105	1	1.56%
110	1	1.56%
150	1	1.56%
160	1	1.56%
170	1	1.56%
195	1	1.56%
200	1	1.56%

Table S3. Multivariate analysis of factors associated with overall survival in the 23 patients with paired specimens across CCRT by PD-L1 up and down regulation.

Variables		No.	Adjusted HR	95% CI	P value
Post CCRT Minus Pre CCRT	PD-L1 H score				
	Down-regulated	10	1		
	Stable or Up-regulated	13	0.466	0.163-1.133	0.154
	Gender				
	Male	22	1		
	Female	1	0.000	0.00	0.990
	Age	23	1.049	0.983-1.119	0.147

Abbreviations: HR, Hazard Ratio; CI, confidence interval

Table S4. Multiple logistic regression analysis of factors associated with CD8 density in 15 patients before and after CCRT.

Variables	No.	CD8 density (High vs Low)[a] adjusted HR	95% CI	P value
PD-L1 H score (Pre CCRT)				
< 2	10	1		
≥ 2	5	0.36	0.03-3.95	0.40
Gender				
Male	14	1		
Female	1	--		
Age	15	0.97	0.83-1.13	0.72
PD-L1 H score (Post CCRT)				
< 2	10	1		
≥ 2	5	1.32	0.13-13.92	0.82
Gender				
Male	14	1		
Female	1	--		
Age	15	0.98	0.85-1.13	0.81

[a]Median value of CD8 density was used as cutoff. Pre CCRT group: <94.2 vs ≥94.2; Post CCRT group: <149.2 vs ≥149.2

Abbreviations: HR, Hazard Ratio; CI, confidence interval; CCRT, concurrent chemoradiation therapy

Table S5. Literature review of PD-L1 expression rate in ESCC studies.

Patient numbers	Ethnicity	PD-L1 expression rate	PDL1_Overall Survival (OS)	Publications
536	Chinese	117/349 (33.5%)	Survival analysis (poor prognosis, P = 0.047)	(1)
162	Chinese	74/162 (45%)	Survival analysis (poor prognosis, P = 0.000); Multivariate analysis (poor prognosis, OR=0.380, 95%CI=0.200-0.648, P = 0.001)	(2)
233	Chinese	129/233 (55.4%)	Survival analysis (good prognosis, P = 0.023); Multivariate analysis (good prognosis, HR=0.697, 95%CI=0.498-0.976, P = 0.035)	(3)
246	Chinese	60/246 (24.4%)	Survival analysis in OP only (not significant, P = 0.706); Survival analysis in post chemotherapy (poor prognosis, P = 0.765); Survival analysis in post radiotherapy (poor prognosis, P = 0.047); Survival analysis in post CCRT (poor prognosis, P = 0.061)	(4)
106	Chinese	57/106 (46.2%)	Survival analysis (poor prognosis, P = 0.027)	(5)
338	Chinese	113/378 (29.9%)	Survival analysis (not significant, P = 0.140); Multivariate analysis (not significant, HR= 0.882, 95%CI = 0.648–1.200, P = 0.423)	(6)
146	Chinese	90/146(61.7%)	Survival analysis (poor prognosis, P = 0.010); Multivariate analysis (poor prognosis, HR= 1.643, 95%CI = 1.038–	(7)

			2.601, P = 0.034)	
133	Chinese	56/133 (42.1%)	Survival analysis (poor prognosis, P = 0.01); Multivariate analysis (poor prognosis, HR=1.957, 95%CI: 1.303–2.939, P = 0.001)	(8)
279	Chinese	Intraepithelial: 74.28%; Tumor: 32.59%	Survival analysis (poor prognosis, P = 0.046)	(9)
138 (82 had paired pre- and post-CRT)	Chinese	57/138 (41.3%)	Survival analysis (not significant, P = 0.133); Univariate analysis (not significant, HR=0.642, 95%CI: 0.360–1.145, P = 0.133)	(10)
41	Japanese	qPCR: 15/31 (48.4%); IHC: 13/31 (41.9%)	Survival analysis (poor prognosis, P = 0.025); Multivariate analysis (poor prognosis, P = 0.0001 without detailing data)	(11)
90	Japanese	17/91(18.9%)	Survival analysis (poor prognosis, P = 0.027); Multivariate analysis with HLA class I (poor prognosis, HR=2.95, 95% C=1.03-7.50, P = 0.0447)	(12)
111 with chemotherapy 69 without chemotherapy	Japanese	53/180 (29.4%)	Survival analysis (poor prognosis, P =0.0010); Survival analysis with chemotherapy (poor prognosis, P = 0.0064); Multivariate analysis (poor prognosis, HR= 1.7480, 95%CI = 1.1373–2.6578, P = 0.0114);	(13)
90	Japanese	57/90 (63.3%)	Survival analysis (poor prognosis, P = 0.0112);	(14)

			Multivariate analysis (poor prognosis, HR=1.957, 95%CI: 1.303–2.939, P = 0.001)	
76	Japanese	Tumor 30/76(39.5%); Stroma 39/76(51.3%)	Survival analysis in tumor (not significant, P = 0.84); Survival analysis in stroma (good prognosis, P = 0.04); Multivariate analysis in stroma (good prognosis, HR= 0.45, 95%CI = 0.23-0.85, P <0.05)	(15)
19 with pre- and post-CRT paired tissue 9 with Chemotherapy alone 45 with only Post-CRT tissue	Korean	41/73(56.2%)	Survival analysis (poor prognosis, P = 0.020); Multivariate analysis (poor prognosis, HR= 2.29, 95%CI = 1.12–4.69, P = 0.023)	(16)
200	Korean	67/200(33.5%)	Survival analysis (not significant, P =0.656)	(17)
58	Korean	CPS>=1 29/51 (56.9%); CPS>=10, 14/51 (27.5%); CPS>=20, 9/51 (17.6%)	Better OS trend in CPS >=10 (Not significant)	(18)

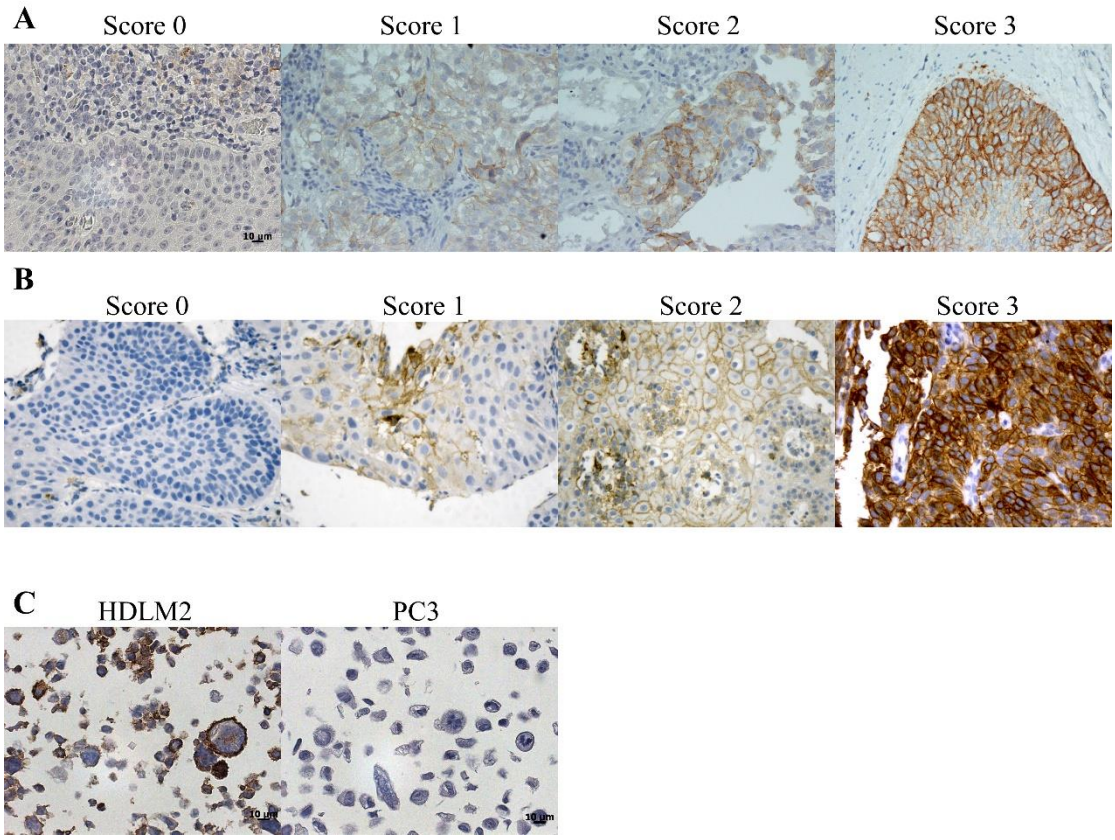

Figure S1. Scoring criteria of PD-L1 immunohistochemistry staining and antibody specificity validation. All images were taken under 200 × magnification. A) Representative images of membrane intensity score by E1L3N clone.; B) Representative images of membrane intensity score by 22C3 clone.; C) The presence and absence of anti-PD-L1 antibody E1L3N clone signals in positive control HDLM2 cells, and in negative control PC3 cells

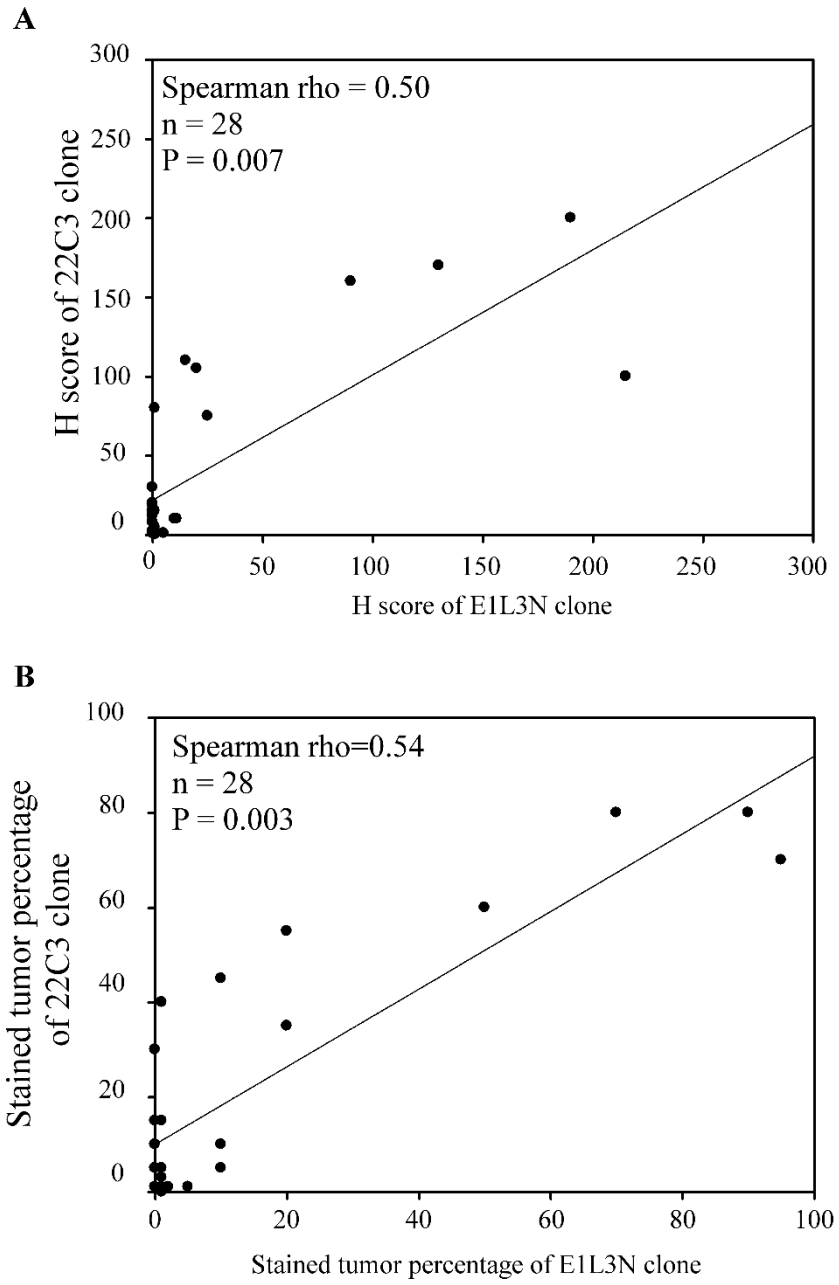

Figure S2. Analysis of PD-L1 antibody sensitivity from 28 specimens. A) Scatter plot comparing the H scores between E1L3N and 22C3 clone;
B) Scatter plot comparing the stained tumor proportion between E1L3N and 22C3 clone

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