Analysis of a novel immune checkpoint, Siglec-15, in pancreatic ductal adenocarcinoma

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Supplementary Material

Supplementary materials and methods

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Multiplexed immunofluorescence (mIF) staining

mIF staining was performed based on the manufacturer's protocol (Opal Multiplex IHC Assay Kit; Akoya Biosciences, MA, USA) to visualize the co-expression of CD68 and Siglec-15. Briefly, TMAs specimens were baked at 65 ℃ for one hour, followed by deparaffinizing with xylene and rehydrating with a graded series of ethanol solutions ethanol. For epitope retrieval, slides were placed in a microwave with AR6 antigen retrieval buffer for 45 s at 100% power and an additional 15 min at 20% power. After blocking, slides were incubated with the first primary antibody (CD68) for one hour at 37℃, followed by incubating the sections with an anti-rabbit horseradish peroxidase-conjugated secondary antibody (Akoya Biosciences) for 10 minutes. The signal was further amplified using the Opal fluorophore working solution containing Opal 540 tyramide signal amplification reagent (Akoya Biosciences). Subsequently, citrate buffer (pH, 6.0) was used to remove the bound antibody. The same procedures were repeated for the second primary antibody [anti-Siglec-15/Opal 650]. Then DAPI were applied to counterstain the slides for 5 minutes at room temperature. At last, the slides were mounted with a hard set medium. Representative images (supplementary material, Figure S1) were acquired by the Vectra Polaris multispectral slide scanner (Akoya Biosciences).

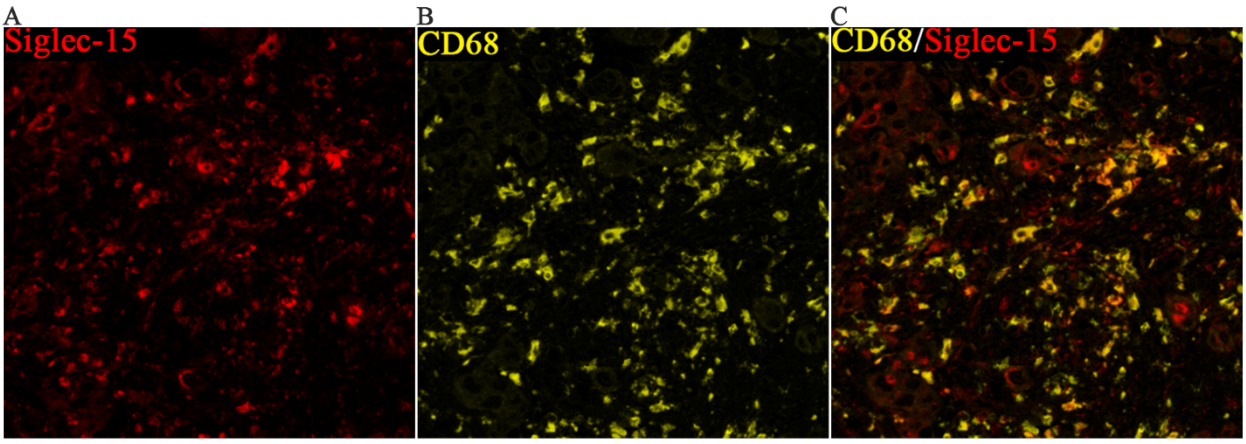

Figure S1. Representative multiplexed immunofluorescence staining of Siglec-15 positivity on macrophages in pancreatic ductal adenocarcinoma. (200×)

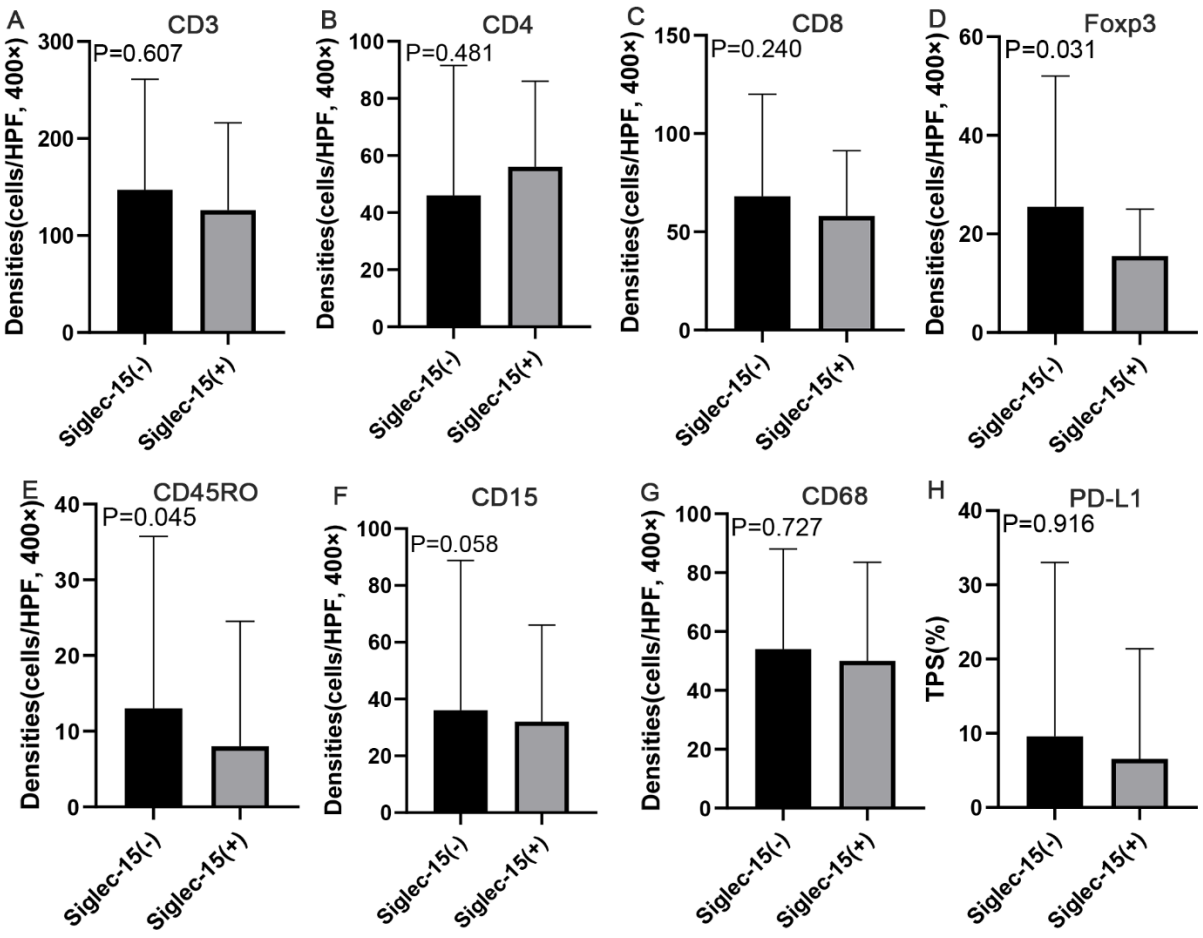

Figure S2. Comparison between Siglec-15 and stromal densities of (A) CD3+ T cells, (B) CD4+ T cells, (C) CD8+ T cells, (D) FOXP3+ T cells, (E) CD45RO+ T cells, (F) CD15+neutrpophils, (G) CD68+macrophages, and (H) TPS of PD-L1 in PDAC using t tests.

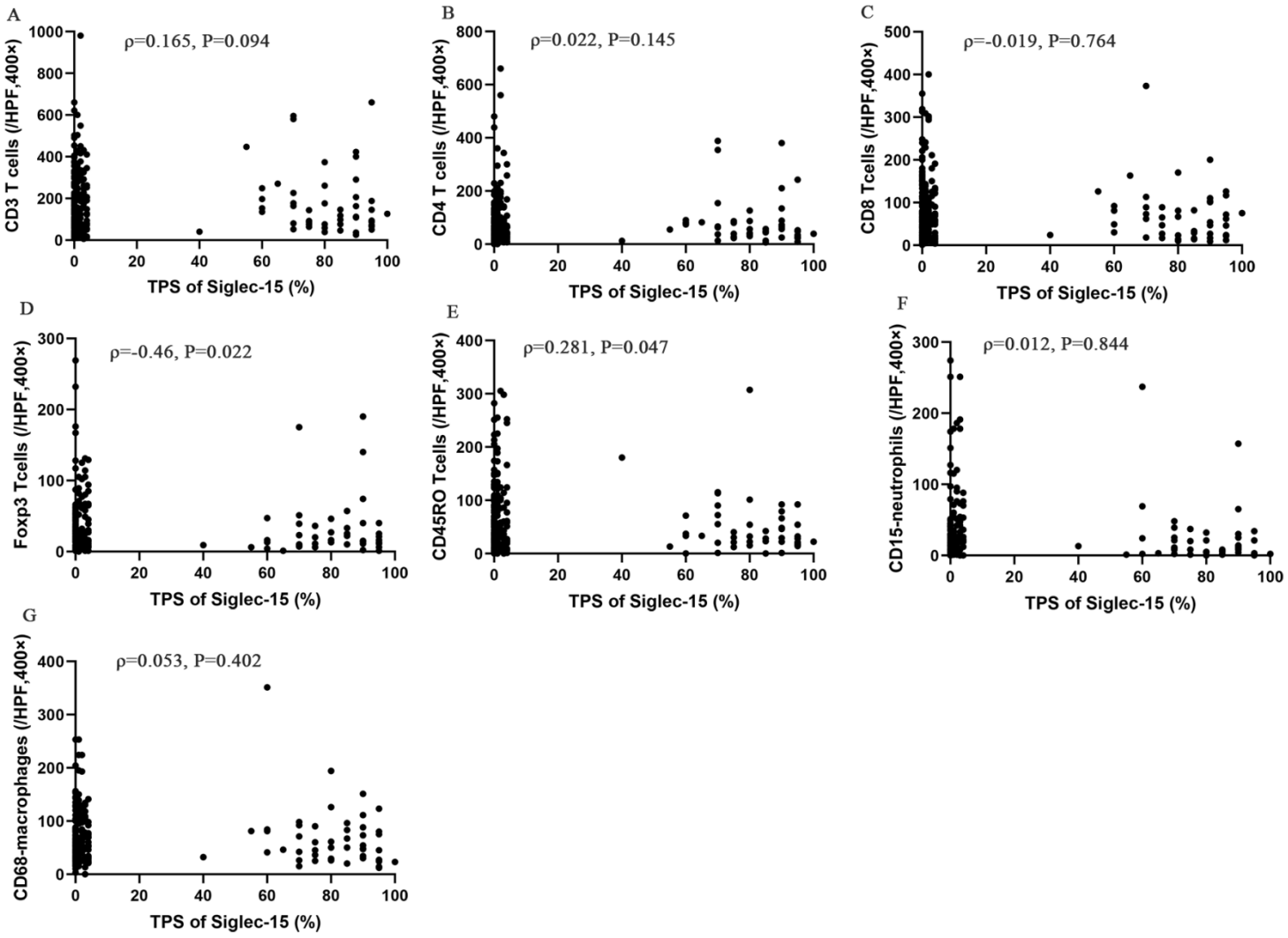

Figure S3. Associations of TPS of Siglec-15 with (A) CD3+ T cells, (B) CD4+ T cells, (C) CD8+ T cells, (D) FOXP3+ T cells, (E) CD45RO+ T cells, (F) CD15+neutrpophils, and (G) CD68+macrophages using Spearman's correlation.

Table S1. Association between clinicopathological features with Siglec-15 and PD-L1 expression.

Variables	N	Siglec-15		
		Negative	Positive	P value
Sex				0.435
Female	121	96(79.3)	25(20.7)	
Male	142	118(83.1)	24(16.9)	
Age, years				0.550
<60	112	93(83.0)	19(17.0)	
≧60	151	121(80.1)	30(19.9)	
Location				0.680
Head & neck	165	133(80.6)	32(19.4)	
Body & tail	98	81(82.7)	17(17.3)	
Tumour size, cm				0.051
<3	102	77(75.5)	25(24.5)	
≧3	161	137(85.1)	24(14.9)	
PNI				0.042
Absent	86	76(88.4)	10(11.6)	
Present	177	138(78.0)	39(22.0)	
LVI				0.568
Absent	165	136(82.4)	29(17.6)	
Present	98	78(79.6)	20(20.4)	
Lymph node status				0.058
Negative	101	88(87.0)	13(12.9)	
Positive	164	126(77.8)	36(22.2)	
Differentiation (grade)				0.018
Well & moderate	171	132(77.2)	39(22.8)	
Poor	92	82(89.1)	10(10.9)	
AJCC				0.230
I-II	209	167(79.9)	42(20.1)	
III-IV	54	47(87.0)	7(13.0)	
PD-L1				0.706
Negative	183	150(82.0)	33(18.0)	
Positive	å80	64(80.0)	16(20.0)	

AJCC, American Joint Committee on Cancer; LVI, lymphovascular invasion; PNI, perineural invasion.

Table S2. Association between DNA damage repair molecules with Siglec-15 and PD-L1 expression.

Variables	N	Siglec-15		
		Negative	Positive	P value
p53 status (N=253)				0.379
Wild type	75	63(84.0)	12(116.0)	
Mutant	178	141(79.2)	37(20.8)	
BRCA1 (N=260)				0.001
Low	234	196(83.8)	38(16.2)	
High	26	15(57.7)	11(42.3)	
BRCA2 (N=260)				0.252
Low	172	143(83.1)	29(16.9)	
High	88	68(77.3)	20(22.3)	

Table S3. Association of Siglec-15 with immune cells.

Stromal immune cells	Spearman's ρ	P-value
CD3+ T cells	0.165	0.094
CD4+ T cells	0.022	0.145
CD8+ T cells	-0.019	0.764
Foxp3+ T cells	-0.46	0.022
CD45RO+ T cells	0.281	0.047
CD15+ neutrophils	0.012	0.844
CD68+ macrophages	0.053	0.402