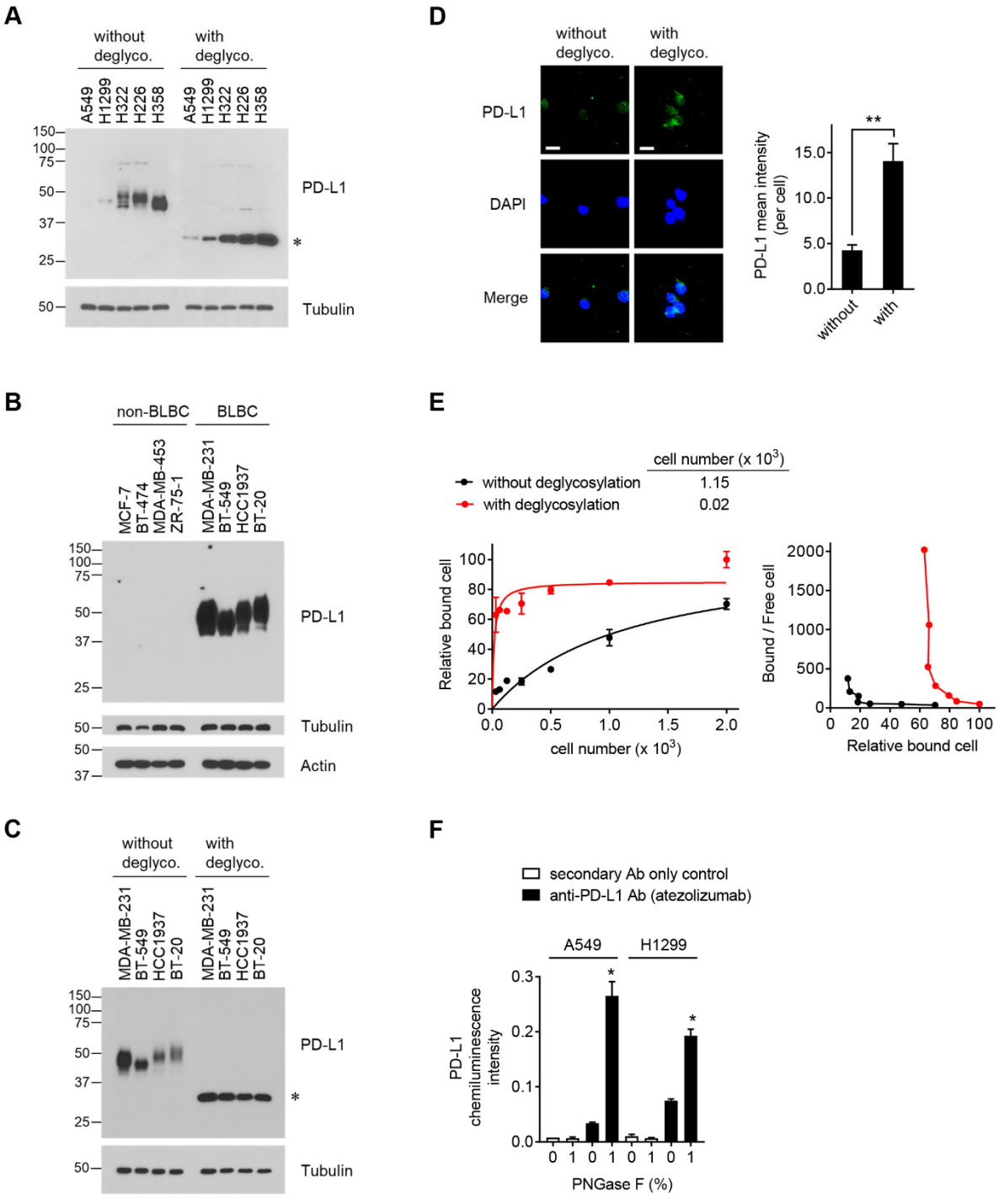

A
without deglyco.
with deglyco.
A549
H1299
H322
H226
H358
150
100
75
50
37
25
PD-L1
*
50
Tubulin
B
non-BLBC
BLBC
MCF-7
BT-474
MDA-MB-453
ZR-75-1
MDA-MB-231
BT-549
HCC1937
BT-20
PD-L1
Tubulin
Actin
C
PD-L1
Tubulin
D
PD-L1
DAPI
Merge
PD-L1 mean intensity (per cell)
**
without
with
E
cell number (x 10^3)
without deglycosylation 1.15
with deglycosylation 0.02
Relative bound cell
cell number (x 10^3)
Bound / Free cell
Relative bound cell
F
secondary Ab only control
anti-PD-L1 Ab (atezolizumab)
A549
H1299
PD-L1 chemiluminescence intensity
PNGase F (%)

Figure S1. Anti-PD-L1 signal is enhanced after deglycosylation in human cancer cells in immunofluorescence and ELISA-based assays. Related to Figure 1.

(A) Cell lysates of lung cancer cells processed with or without deglycosylation (deglyco.) by PNGase F (5%) pretreatment and immunoblotting (IB) with the indicated antibodies. Anti-PD-L1 antibody for IB, Cell Signaling (13684). Asterisk indicates non-glycosylated PD-L1.

(B) IB of basal-like breast cancer (BLBC) and non-BLBC cells with the indicated antibodies.

(C) Cell lysates of BLBC cells processed with or without deglycosylation by PNGase F (5%) pretreatment and IB with the indicated antibodies.

(D) Immunofluorescence confocal microscopy of H1299 cells processed with or without deglycosylation by PNGase F (5%) pretreatment stained with DAPI and an anti-PD-L1 antibody (Abcam, ab58810). Bar, 10 μm. Quantification is shown to the right. Data are representative of 3 independent experiments, randomly chosen in 3 different fields.

(E) Left: saturation binding assay of H1299 cell lysates binding to anti-PD-L1 clone 28-8 mAb. Right: scatchard plot of cell number binding to anti-PD-L1 antibody transformed from the left.

(F) ELISA of PD-L1 level by an anti-PD-L1 Ab atezolizmab (MDACC) in A549 and H1299 cells processed with deglycosylation by PNGase F (1%) pretreatment for comparison with cells without deglycosylation (0%). Negative control, secondary Ab only control.

(D–F) Results are presented as mean ± SD. *$p < 0.05$, **$p < 0.01$, Student's t test.

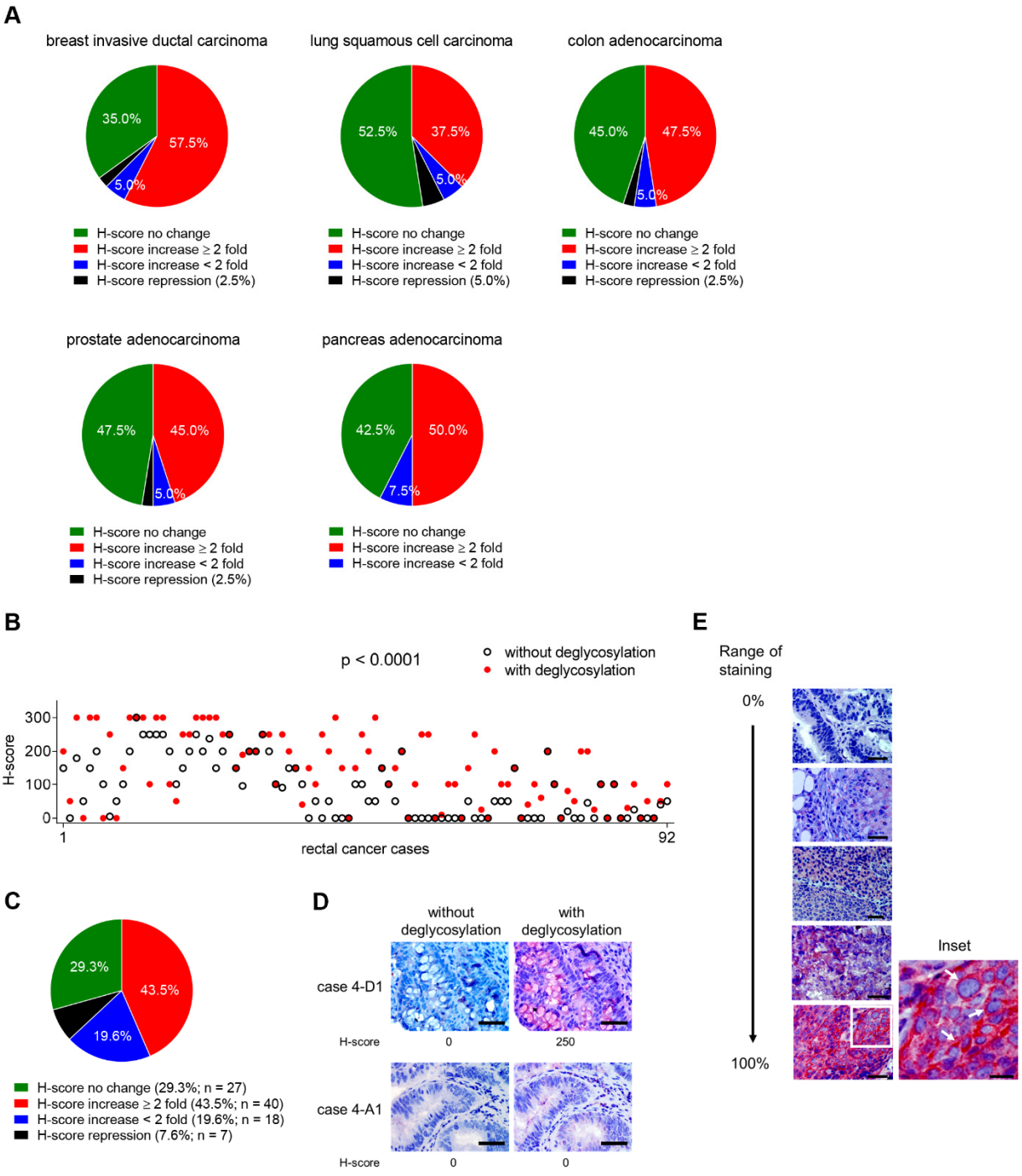


Figure S2. Anti-PD-L1 signal is enhanced after deglycosylation in a major population of patient samples in different cancer types. Related to Figure 2.

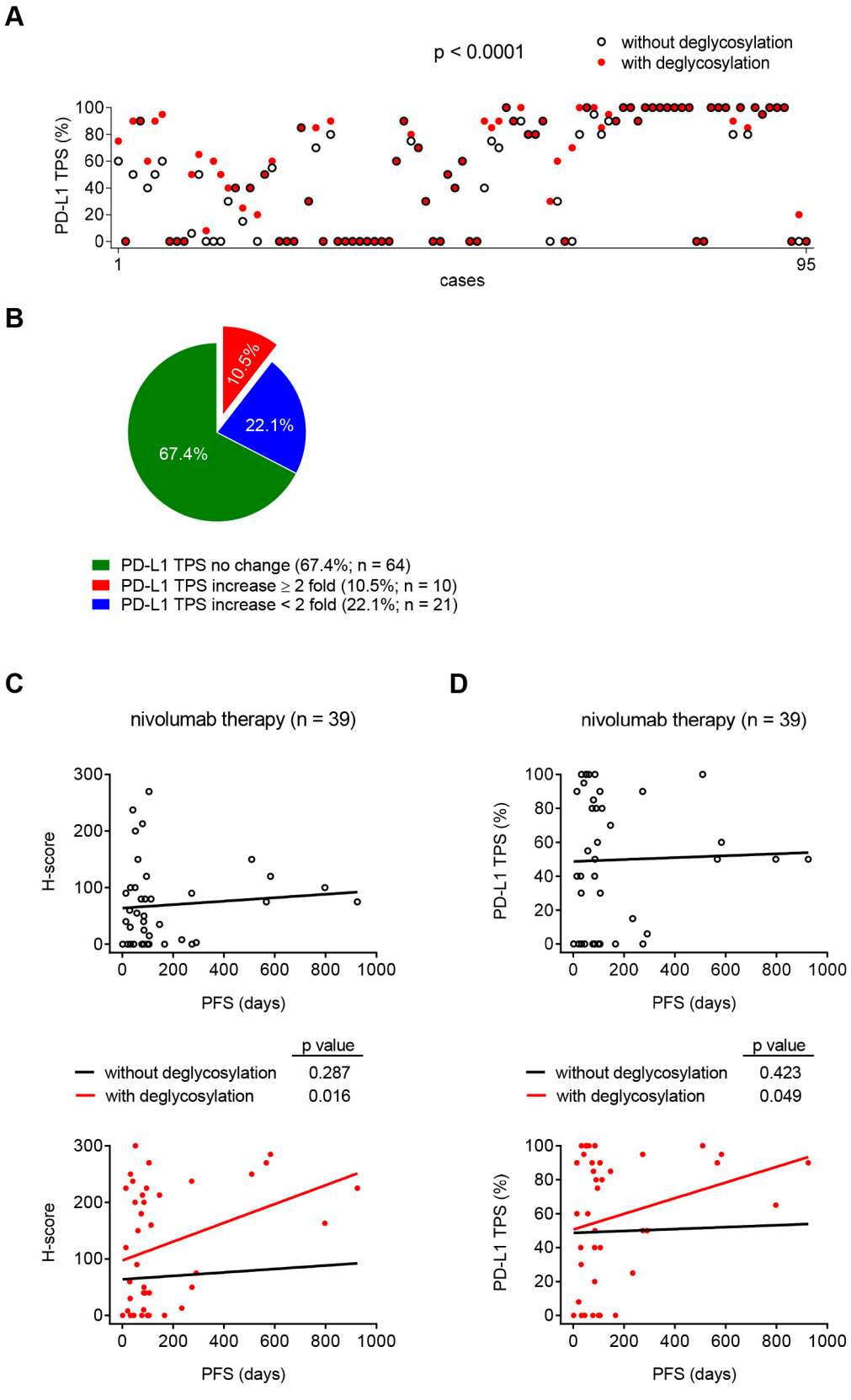
(A) Individual analysis of five cohorts in multi-organ carcinoma TMA from Figure 2A, containing 40 cases each of breast invasive ductal carcinoma, lung squamous cell carcinoma, colon adenocarcinoma, prostate adenocarcinoma, and pancreas adenocarcinoma.

(B) H-score values representing PD-L1 protein expression from IHC staining of a human rectal cancer TMA (n = 92) processed with or without deglycosylation by PNGase F (5%) pretreatment. Results were analyzed by the Wilcoxon signed-rank test.

(C) A pie chart highlighting the fold change of H-score after N-linked glycosylation removal through PNGase F treatment from (B).

(D) Two representative cases of IHC staining from (B). Bar, 50 μm.

(E) Representative images of PD-L1 IHC staining in the lung cancer tumor microarray (Biomax, #NSC151). Samples displayed varying percentages of the stained cells spanning a wide range from negative (0%) to strongly positive (100%) staining of the tumor cells. Bar, 50 μm. Inset: PD-L1 membrane staining (arrows); bar, 20 μm.


A
p < 0.0001
without deglycosylation
with deglycosylation
PD-L1 TPS (%)
cases
B
10.5%
22.1%
67.4%
PD-L1 TPS no change (67.4%; n = 64)
PD-L1 TPS increase ≥ 2 fold (10.5%; n = 10)
PD-L1 TPS increase < 2 fold (22.1%; n = 21)
C
nivolumab therapy (n = 39)
H-score
PFS (days)
p value
without deglycosylation 0.287
with deglycosylation 0.016
D
nivolumab therapy (n = 39)
PD-L1 TPS (%)
PFS (days)
p value
without deglycosylation 0.423
with deglycosylation 0.049

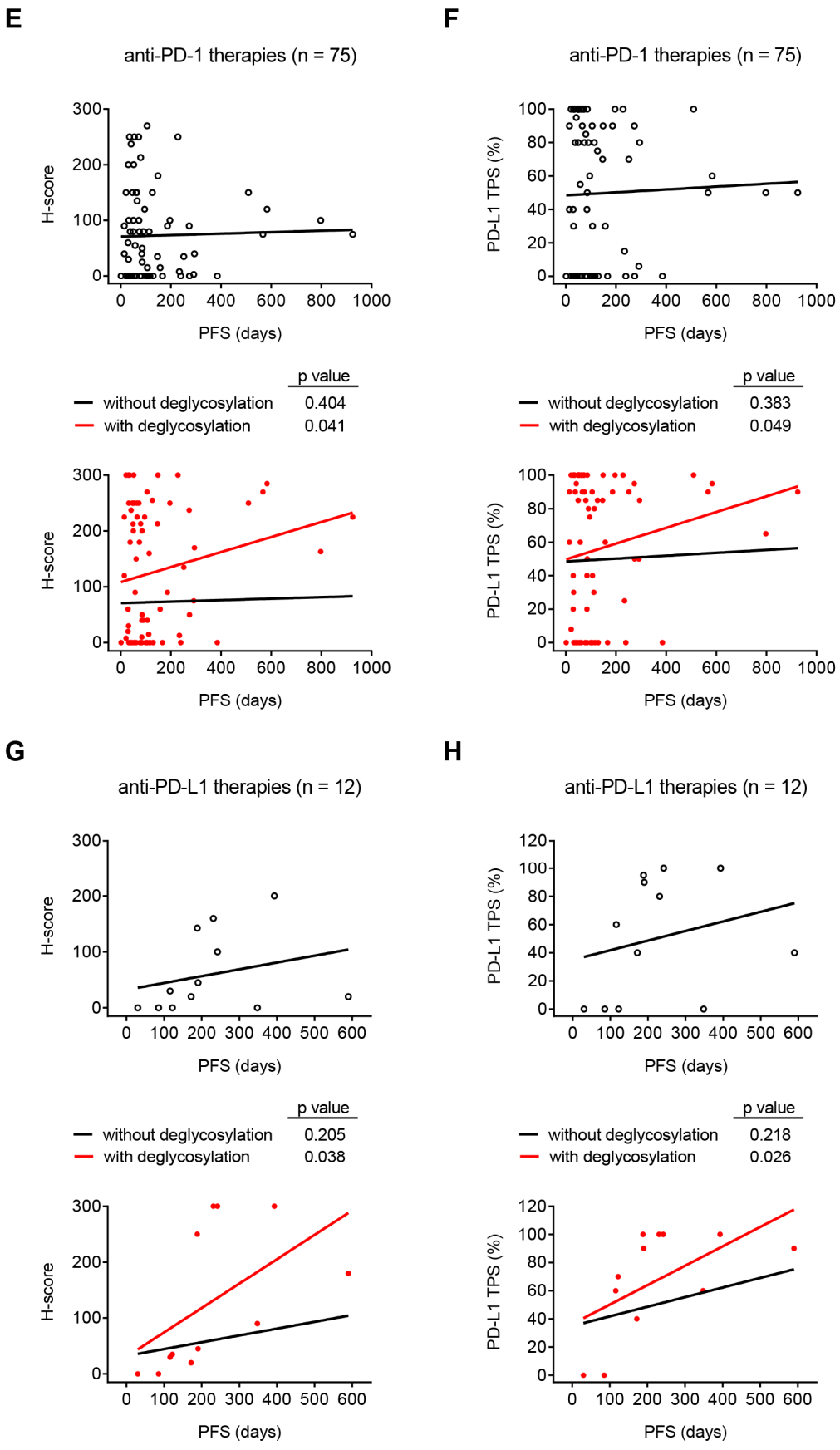

E
anti-PD-1 therapies (n = 75)
H-score
PFS (days)
p value
without deglycosylation 0.404
with deglycosylation 0.041
F
anti-PD-1 therapies (n = 75)
PD-L1 TPS (%)
PFS (days)
p value
without deglycosylation 0.383
with deglycosylation 0.049
G
anti-PD-L1 therapies (n = 12)
H-score
PFS (days)
p value
without deglycosylation 0.205
with deglycosylation 0.038
H
anti-PD-L1 therapies (n = 12)
PD-L1 TPS (%)
PFS (days)
p value
without deglycosylation 0.218
with deglycosylation 0.026

Figure S3. Deglycosylation improves PD-L1 detection in clinical samples and correlation with patient responses to anti-PD-1/PL1 therapy. Related to Figure 3.

(A) The percentage of PD-L1 positive signals in tumor cells (TPS; tumor proportion score; % positive cells) from IHC staining of patient tissue slides processed with or without deglycosylation from Figure 3A (n = 95). Results were analyzed by the Wilcoxon signed-rank test.

(B) A pie chart highlighting the fold change in PD-L1 TPS after N-linked glycosylation removal through PNGase F treatment from (A).

(C and D) Correlation between PD-L1 H-score (C) or PD-L1 TPS (D) in patient tissue slides processed with or without deglycosylation and the corresponding progression-free survival (PFS) from nivolumab therapy (n = 39) from Figure 3A.

(E and F) Correlation between PD-L1 H-score (E) or PD-L1 TPS (F) in patient tissue slides processed with or without deglycosylation and the corresponding PFS from anti-PD-1 therapy (nivolumab, pembrolizumab, and camrelizumab; n = 75) from Figure 3A.

(G and H) Correlation between PD-L1 H-score (G) or PD-L1 TPS (H) in patient tissue slides processed with or without deglycosylation and the corresponding PFS from anti-PD-L1 therapy (atezolizumab and durvalumab; n = 12) from Figure 3A.

(C–H) One-tailed p values are shown, Pearson correction test.

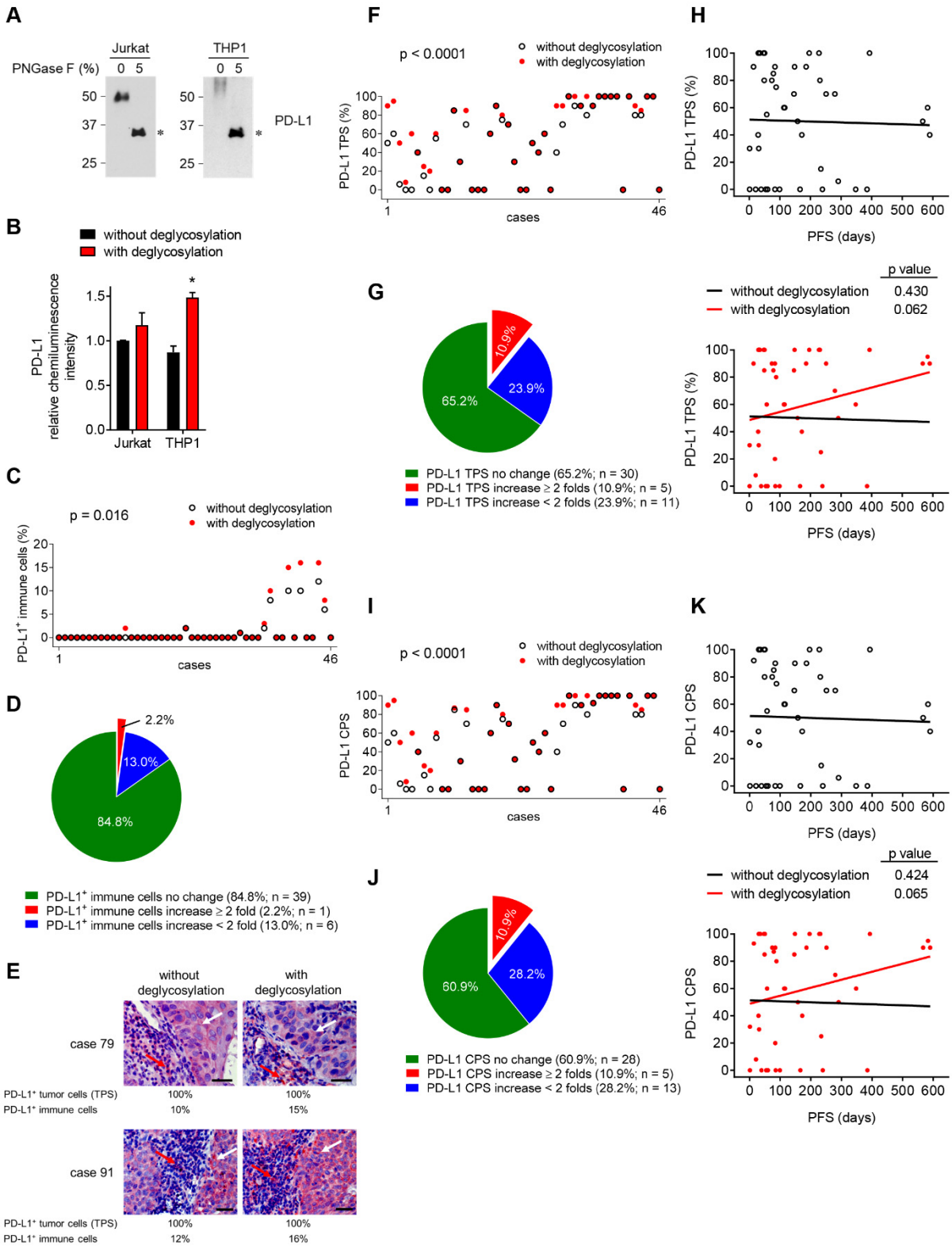

A
Jurkat
THP1
PNGase F (%)
0 5
0 5
50
37
25
PD-L1
B
without deglycosylation
with deglycosylation
PD-L1 relative chemiluminescence intensity
Jurkat
THP1
C
p = 0.016
without deglycosylation
with deglycosylation
PD-L1+ immune cells (%)
cases
D
2.2%
13.0%
84.8%
PD-L1+ immune cells no change (84.8%; n = 39)
PD-L1+ immune cells increase ≥ 2 fold (2.2%; n = 1)
PD-L1+ immune cells increase < 2 fold (13.0%; n = 6)
E
without deglycosylation
with deglycosylation
case 79
PD-L1+ tumor cells (TPS)
100%
100%
PD-L1+ immune cells
10%
15%
case 91
PD-L1+ tumor cells (TPS)
100%
100%
PD-L1+ immune cells
12%
16%
F
p < 0.0001
without deglycosylation
with deglycosylation
PD-L1 TPS (%)
cases
G
10.9%
23.9%
65.2%
PD-L1 TPS no change (65.2%; n = 30)
PD-L1 TPS increase ≥ 2 folds (10.9%; n = 5)
PD-L1 TPS increase < 2 folds (23.9%; n = 11)
H
PD-L1 TPS (%)
PFS (days)
p value
without deglycosylation 0.430
with deglycosylation 0.062
I
p < 0.0001
without deglycosylation
with deglycosylation
PD-L1 CPS
cases
J
10.9%
28.2%
60.9%
PD-L1 CPS no change (60.9%; n = 28)
PD-L1 CPS increase ≥ 2 folds (10.9%; n = 5)
PD-L1 CPS increase < 2 folds (28.2%; n = 13)
K
PD-L1 CPS
PFS (days)
p value
without deglycosylation 0.424
with deglycosylation 0.065

Figure S4. Sample deglycosylation enhances PD-L1 detection in a small fraction of tumor-associated lymphocytes. Related to Figure 3.

(A) Cell lysates of human immune cells, including Jurkat (T lymphocytes) and THP1 (monocytes), processed with deglycosylation by PNGase F (5%) pretreatment for comparison with cell lysates without deglycosylation (0%) and IB with anti-PD-L1 Ab (Cell Signaling, 13684). Asterisk indicates non-glycosylated PD-L1.

(B) ELISA of PD-L1 levels (clone 28-8 mAb) in Jurkat and THP1 cells processed with or without deglycosylation by PNGase F (1%) pretreatment. Results are presented as mean ± SD. *$p < 0.05$, Student's t test.

(C) The percentage of PD-L1 positive signals in immune cells (% PD-L1$^+$ immune cells) from IHC staining of patient tissue slides processed with or without deglycosylation from Figure 3A (n = 46 containing tumor-associated immune cells). Results were analyzed by the Wilcoxon signed-rank test.

(D) A pie chart highlighting the fold change in the percentage of PD-L1$^+$ immune cells after N-linked glycosylation removal through PNGase F treatment from (C).

(E) Two representative cases of IHC staining from (C). PD-L1$^+$ tumor cells (TPS), white arrows; PD-L1$^+$ immune cells, red arrows. Bar, 50 μm.

(F) The percentage of PD-L1 TPS from IHC staining of patient tissue slides processed with or without deglycosylation from (C). Results were analyzed by the Wilcoxon signed-rank test.

(G) A pie chart highlighting the fold change in PD-L1 TPS after N-linked glycosylation removal through PNGase F treatment from (F).

(H) Correlation between PD-L1 TPS in patient tissue slides processed with or without deglycosylation and the corresponding PFS from anti-PD-1/PD-L1 therapy from (F). Pearson correction test; one-tailed.

(I) PD-L1 positive signals in both tumor and immune cells (CPS; combined positive score) from IHC staining of patient tissue slides processed with or without deglycosylation from (C). Results were analyzed by the Wilcoxon signed-rank test.

(J) A pie chart highlighting the fold change in PD-L1 CPS after N-linked glycosylation removal through PNGase F treatment from (I).

(K) Correlation between PD-L1 CPS in patient tissue slides processed with or without deglycosylation and the corresponding PFS from anti-PD-1/PD-L1 therapy from (I). Pearson correction test; one-tailed.

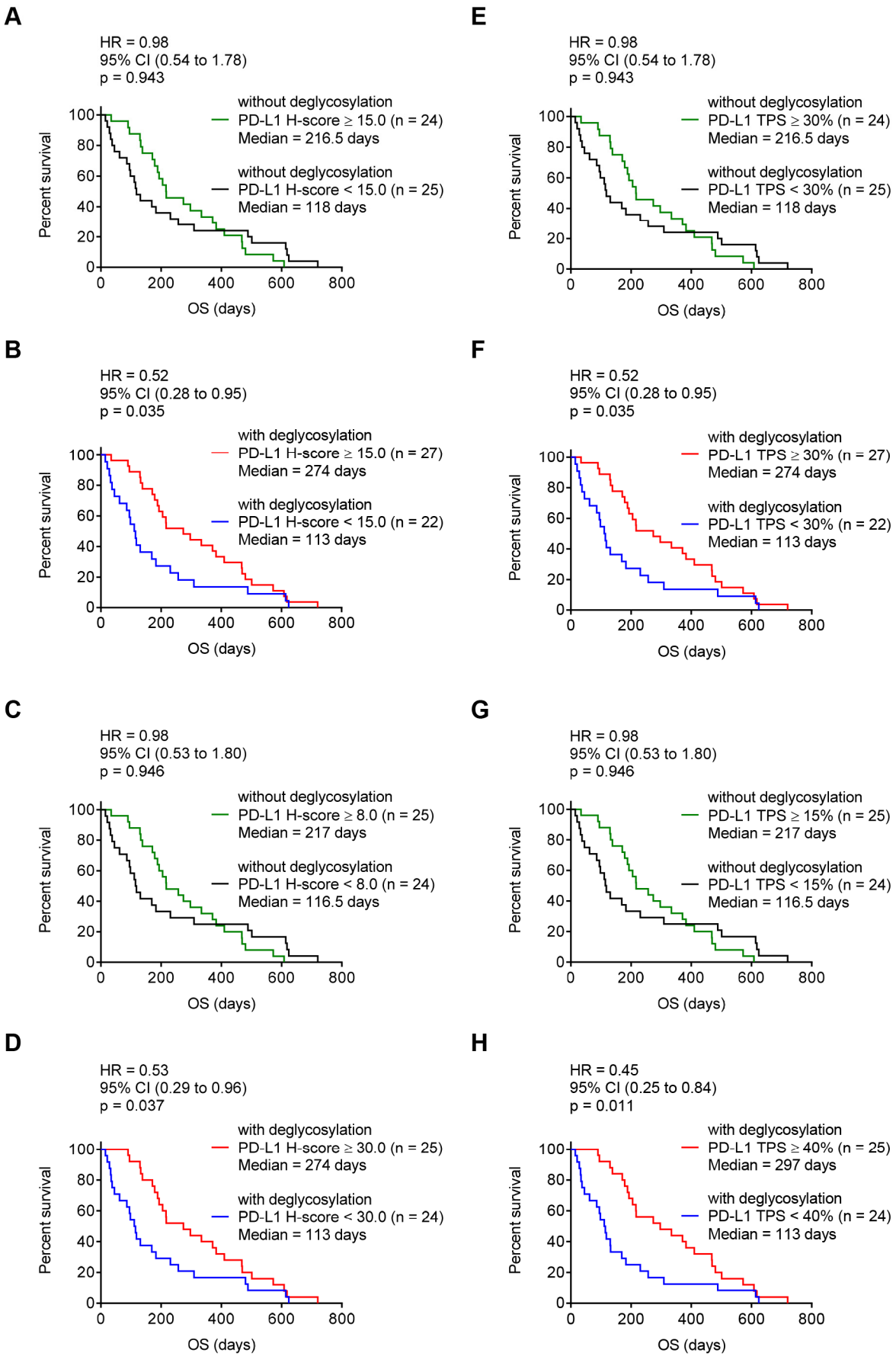

A
HR = 0.98
95% CI (0.54 to 1.78)
p = 0.943
without deglycosylation
PD-L1 H-score ≥ 15.0 (n = 24)
Median = 216.5 days
without deglycosylation
PD-L1 H-score < 15.0 (n = 25)
Median = 118 days
Percent survival
OS (days)
B
HR = 0.52
95% CI (0.28 to 0.95)
p = 0.035
with deglycosylation
PD-L1 H-score ≥ 15.0 (n = 27)
Median = 274 days
with deglycosylation
PD-L1 H-score < 15.0 (n = 22)
Median = 113 days
C
HR = 0.98
95% CI (0.53 to 1.80)
p = 0.946
without deglycosylation
PD-L1 H-score ≥ 8.0 (n = 25)
Median = 217 days
without deglycosylation
PD-L1 H-score < 8.0 (n = 24)
Median = 116.5 days
D
HR = 0.53
95% CI (0.29 to 0.96)
p = 0.037
with deglycosylation
PD-L1 H-score ≥ 30.0 (n = 25)
Median = 274 days
with deglycosylation
PD-L1 H-score < 30.0 (n = 24)
Median = 113 days
E
HR = 0.98
95% CI (0.54 to 1.78)
p = 0.943
without deglycosylation
PD-L1 TPS ≥ 30% (n = 24)
Median = 216.5 days
without deglycosylation
PD-L1 TPS < 30% (n = 25)
Median = 118 days
F
HR = 0.52
95% CI (0.28 to 0.95)
p = 0.035
with deglycosylation
PD-L1 TPS ≥ 30% (n = 27)
Median = 274 days
with deglycosylation
PD-L1 TPS < 30% (n = 22)
Median = 113 days
G
HR = 0.98
95% CI (0.53 to 1.80)
p = 0.946
without deglycosylation
PD-L1 TPS ≥ 15% (n = 25)
Median = 217 days
without deglycosylation
PD-L1 TPS < 15% (n = 24)
Median = 116.5 days
H
HR = 0.45
95% CI (0.25 to 0.84)
p = 0.011
with deglycosylation
PD-L1 TPS ≥ 40% (n = 25)
Median = 297 days
with deglycosylation
PD-L1 TPS < 40% (n = 24)
Median = 113 days

Figure S5. Antigen retrieval by protein deglycosylation improves the utility of PD-L1 as a predictive biomarker for immunotherapy. Related to Figure 5.

(A and B) The overall survival (OS) of cancer patient samples processed without (A) or with (B) deglycosylation by PNGase F (5%) pretreatment. Cases with H-score equal to or higher than the median value of total 49 cases (H-score = 15.0) were considered as high expression and those with H-score less than the median value as low expression.

(C and D) The OS of cancer patient samples processed without (C) or with (D) deglycosylation by PNGase F (5%) pretreatment. Cases with H-score equal to or higher than the median value of individual group [H-score = 8.0 in the group of without glycosylation (C) and H-score = 30.0 in the group of with glycosylation (D), respectively] were considered as high expression and those with H-score less than the respective median value as low expression.

(E and F) The OS of cancer patient samples processed without (E) or with (F) deglycosylation by PNGase F (5%) pretreatment. Cases with PD-L1 TPS equal to or higher than the median value of total 49 cases (PD-L1 TPS = 30%) were considered as high expression and those with PD-L1 TPS less than the median value as low expression.

(G and H) The OS of cancer patient samples processed without (G) or with (H) deglycosylation by PNGase F (5%) pretreatment. Cases with PD-L1 TPS equal to or higher than the median value of individual group [PD-L1 TPS = 15% in the group of without glycosylation (G) and PD-L1 TPS = 40% in the group of with glycosylation (H), respectively] were considered as high expression and those with PD-L1 TPS less than the respective median value as low expression.

(A–H) Cohort size for each group is indicated. p values were determined by Log-rank (Mantel-Cox) test. Hazard Ratio (HR) and 95% confidence interval (CI) were determined by Mantel-Haenszel method.