Supplementary Materials for

PD-L2 glycosylation promotes immune evasion and predicts anti-EGFR efficacy

Yiqi Xu, Zhenyue Gao, Ruxin Hu, Yuqing Wang, Yuhong Wang, Zheng Su, Xiaoyue Zhang, Jingxuan Yang, Mei Mei*, Yu Ren*, Min Li*, Xuan Zhou*

*Correspondence to

Professor Xuan Zhou; xuanzhou@tmu.edu.cn

Professor Min Li; min-li@ouhsc.edu

Professor Yu Ren; yuren0925@tmu.edu.cn

Professor Mei Mei; meim@tmu.edu.cn

This PDF file includes:

Supplementary Materials and Methods

Figs. S1 to S9

Table S1 to S4

Xu Y, *et al. J Immunother Cancer* 2022; 9:e002699. doi: 10.1136/jitc-2021-002699

Supplementary Materials and Methods

Cell culture

Human tongue HNSCC cell lines SCC15, SCC25 were purchased from American Type Culture Collection (ATCC). Murine HNSCC cell line SCC7 cells were purchased from Chinese Center for Type Culture Collection. SCC15 and SCC25 cells were cultured in a mixture of Dulbecco's modified Eagle's medium (DMEM) and Ham's F12 medium with 2.5 mM L-glutamine, 1.2 g/L sodium bicarbonate, 15 mM HEPES and 0.5 mM sodium pyruvate and supplemented with 400 ng/mL hydrocortisone and 10% fetal bovine serum (FBS). SCC7 was cultured in Minimum Essential Medium Eagles with Earle’s Balanced Salts (MEM-EBSS) containing 10%FBS. All cell lines were cultured in humidified environment containing 5% CO_2 at 37°C.

Generation of stable cells using lentiviral transfection

To generate human *PD-L2* knockout cells, the SCC15 or SCC25 cells were transfected with lentiviral vectors harboring clustered regularly interspaced short palindromic repeats (CRISPR)/caspase 9 (Cas9) sequences against human *PD-L2*. The target sequences were as follows: 5′-ACTGTCCTTCGTCCCTCACT-3′ (sgPD-L2 #1), 5′-CCGTGAAAGAGCCACTTTGC-3′ (sgPD-L2 #2), and 5′-TTGCAGCTTCACCAGATAGC-3′ (sgPD-L2 #3). To generate mouse *PD-L2* knockout cells, the SCC7 cells were transfected with lentiviral vectors harboring CRISPR/Cas9 sequences against mouse *PD-L2*. The target sequence was 5′-CAGTTTGCAGAAGGTAGAA-3′. The cells were seeded at 50% confluency 24-36 h before lentiviral particle infection. The medium was replaced with a medium containing lentiviral vectors. After transduction for 16 h, the medium was replaced with fresh medium and the transfected cells were selected with 4 μg/mL puromycin (Sigma) for 7 days.

To generate the cells expressing wild-type (WT) or mutant (4NQ or REY) PD-L2 SCC7, SCC15 and SCC25 cells, the lentiviral vectors harboring Flag-tagged WT or mutant PD-L2

sequences, which were purchased from Genechem (China), were transfected into sgPD-L2-infection cells. The HNSCC cells were seeded at 50% confluency 24-36 h before lentiviral particle infection. The medium was replaced with a medium containing lentiviral vectors. After transduction for 16 h, the medium was replaced with fresh medium and the infected cells were selected with 600 μg/mL G418 (Sigma) for 2 weeks.

To establish *FUT8* knockdown stable lines, a lentiviral shRNA system (Genechem) with short hairpin sequences against *FUT8* as follows: 5′-TGGAGTGATCCTGGATATA-3’ (shFUT8#1), 5’-ACAGATGACCCTTCTTTAT-3’ (shFUT8#2) was used. Two sh*STAT3* clones were selected. The target sequences used for shSTAT3 were as follows: 5’-GCAAAGAATCACATGCCACTT-3’ (shSTAT3 #1) and 5’-GCACAATCTACGAAGAATC AA -3’ (shSTAT3 #2). The cells were seeded at 50% confluency 24-36 h before lentiviral particle infection. The medium was replaced with a medium containing lentivirus. After transfection for 16 h, the medium was replaced with fresh medium and the transfected cells were selected with 4 μg/mL puromycin for 7 days.

Antibodies and Reagents.

Cetuximab was purchased from Merck Serono. Cycloheximide and tunicamycin were obtained from Abcam. Stattic (STAT3 inhibitor, S7024), SB203580 (MAPK inhibitor, S1076), GF109203X (PKC inhibitor, S7208), Rapamycin (mTOR inhibitor, S1039), U0126 (MEK inhibitor, S1102), Nutlin-3 (MDM2 inhibitor, S1061) were purchased from Selleck Chemicals. EGF was purchased from Sino Biological (10605-HNAE).

The antibodies used were as follows: Flag (F1804, Sigma), PD-L2 (MAB1224, R&D Systems), Myc (2276S, Cell Signaling Technology), HA (3724, Cell Signaling Technology), FUT8 (ab191571, Abcam), EGFR (ab30, Abcam), phospho-EGFR Tyr1068 (3777, Cell Signaling Technology), STAT3 (ab12640, Abcam), phospho-STAT3 Tyr705 (9145, Cell Signaling Technology), phospho-MDM2 S166 (ab170880, Abcam), phospho-PKCαS657 (ab180848, Abcam), Phospho-MAPK Thr202/Tyr204 (4370, Cell Signaling Technology), Phospho-mTOR Ser2448 (5536, Cell Signaling Technology), Phospho-MEK1/2 Ser221 (2338, Cell Signaling Technology), PD-1 (ab52587, Abcam), LAMP2 (ab125068,

Abcam,1:100), Tubulin (66031-1-Ig, Proteintech).

Tissue sample preparation for western blotting

The tissue sample (30 mg) was lysed with 500 μL of ice-cold lysis buffer (40 mM Tris-HCl, 120 mM NaCl, 1% Triton X-100, and protease inhibitor cocktail) for 30 min at 4°C. Next, the samples were centrifuged at 12,000 rpm for 15 min to remove debris. The supernatant was stored at -80°C. The samples were subjected to western blotting with anti-PD-L2 (MAB1224, R&D Systems) and anti-tubulin (66031-1-Ig, Proteintech) antibodies. The sensitive tissue was obtained from the primary lesion that responded to treatment. Informed consent was obtained from all patients whose tumor tissue were used for this study.

Immunohistochemical (IHC) staining

Paraffin-embedded tissue sections were baked for 3 h at 60°C. The tissue sections were immersed in 10 mM citrate buffer (pH 6.0) and subjected to microwave treatment for antigen retrieval. Next, the samples were incubated with 3% H_2O_2 for 30 min to block endogenous peroxidase and blocked with 3% BSA for 1 h. The samples were then probed with anti-PD-L2 (MAB1224, R&D Systems) antibody in blocking buffer overnight at 4°C and incubated with the secondary antibodies (ZSGB-Bio, China) at room temperature for 30 min. Immunostaining was performed using a 3,3′-diaminobenzidine kit (ZSGB-Bio, China), following the manufacturer's instructions. The samples were counterstained with hematoxylin. The subcellular localization, intensity, and distribution of the target protein in the immuno-positive cells were quantified using a visual grading system based on the extent of staining. The proportion of cancer cells exhibiting positive staining was graded on a scale from 0 to 4 as follows: 0, < 10%; 1, 10%-25%; 2, 26%-50%; 3, 51%-75%; 4,>75%. The intensity of staining was graded on a scale of 0-3 as follows: 0, none; 1, weak staining; 2, moderate staining; and 3, strong staining. The EI value, which varied from 0 to 12, was determined by multiplying extent (E) score and intensity (I) score.

Glycosidase assay

Total proteins were incubated with glycoprotein denaturing buffer (ThermoFisher) at

95°C for 10 min. The samples were treated with endoglycosidase H (Endo H), peptide *N*-glycosidase F (PNGase F), or *O*-glycosidase (Sigma) in the presence of GlycoBuffer 2 (10X) and 10% NP-40 at 37°C for 1 h, following the manufacturer's instructions. Next, the samples were boiled in SDS-PAGE sample buffer at 95°C for 5 min. Glycosylated PD-L2 was stained using the glycoprotein staining kit (ThermoFisher).

Identification of N-glycopeptide

(1) LC MS/MS analysis

The protein was analyzed using LC–MS/MS (Thermo Easy-nLC 1200). Briefly, the peptide solutions were separated by nano liquid chromatography on a capillary column (150μm×150mm) packed with C18 (3 μm, 100 Å) at a 90 min gradient: 6% buffer B (1 min)-36% buffer B (69 min)-60% buffer B (6 min)-100% buffer B (12.5 min)-1% buffer B (1.5 min), and at a flow-rate of 400 nL/min. The mobile phases came with 0.1% Formic in water (A) and 0.1% FA in acetonitrile (B). The mass spectrometer (Thermo Scientific Orbitrap Fusion Lumos) was run under the positive mode. The MS1 was analyzed with a mass range of 350–1600 m/z at a resolution of 120,000 at 200 m/z. The automatic gain control (AGC) was set as $4e^5$ and the maximum injection time (IT) was set as 50 ms. The top 20 precursor ions were selected from each MS full scan with isolation width of m/z^2. The MS2 was analyzed with 110 m/z at a resolution of 30,000 at 200 m/z. The MS2 AGC was set as $5e^4$ at HCD collisional mode and $2e^5$ at ETD collisional mode, respectively. The maximum injection time was 54ms, and the spray voltage was 2.1 kV and the ion transfer tube temperature was 320 °C.

(2) Data Analysis

All spectra were searched with Byonic (Protein Metrics, San Carlos, CA) against the UniProt human haptoglobin database (P00738). Trypsin and GluC were selected as enzymes with a maximum of two missed cleavages allowed. The search was performed using the following parameters: (1) static modification, carbamidomethyl of C; (2) dynamic modifications, oxidation of M, deamindation of N and Q, and glycan modifications; (3) maximum missed cleavages, 2; (4) precursor ion mass tolerance 10 ppm; (5) fragment ion

mass tolerance 0.01 Da. Results were filtered at 1% FDR and confidence threshold of Byonic score >100, and further validation was performed manually. The abundance of a site-specific glycoform was represented by the sum of all glycopeptides containing the same glycan at the glycosite while with different peptides in the case of miscleavage or deamidation. The relative quantitation of each site-specific glycopeptide was achieved at two levels: the total glycopeptide level and its corresponding glycosite level. Each sample was analyzed with two injections, and the average normalized abundance of a given N-glycopeptide from the two replicates was used to measure its relative levels between disease groups.

PD-1-binding assay

The cells were fixed with 4% paraformaldehyde at room temperature for 15 min and incubated with recombinant human PD-1 Fc protein (Cohesion, CRP2068) overnight at 4°C. Next, the cells were washed and stained with an anti-human Alexa Fluor 488 dye conjugate (ThermoFisher). Fluorescence intensity was analyzed using a microplate reader (PerkinElmer, Operetta).

T cell-mediated cell killing assay.

T cells were activated by incubation with anti-CD3 antibody (100 ng/mL) and IL-2 (10 ng/mL) for 24h. To analyze the killing of tumor cells by T cell, tumor cells were co-cultured with activated T cells at 1:5 ratios for 96h in the presence of Caspase 3/7 substrate. Washed the cells by PBS to remove the T cells and nuclei of tumor cells were counterstained with Sytox. Representative green fluorescent (nuclear restricted SYTOX), and red fluorescent (NucView 547 caspase-3/7 substrate) merged images was measured and quantified by a using a microplate reader (PerkinElmer, Operetta). Red fluorescent cells were counted as dead cells.

Chromatin immunoprecipitation assay (ChIP)

The ChIP assay was performed using the EZ-ChIP kit (Millipore) as described previously. The cells were fixed with 1% formaldehyde, neutralized with 0.125 M glycine, washed with cold PBS, and lysed with SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM

Tris-HCl (pH 8.0), and protease inhibitor cocktail (Roche, 04693116001). After sonication, 200–1000 bp chromatins fragments were collected and pre-cleared in dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 167 mM Tris-HCl (pH 8.0), and 16.7 mM NaCl). The fragments were incubated with homologous IgG or primary antibody on a rotating platform overnight at 4°C. After washing the immunocomplexes collected using protein G beads, the DNA-bound antibody fragments were eluted for real-time polymerase chain reaction analysis with the following primers: *FUT8* 5′-TGCTAAGCCTTCTGAGTGCC-3′ (forward) and 5′-CACCAGGCCTCATGCAAAAC-3′ (reverse).

Promoter activity assay

Promoter sequence of human FUT8 gene was obtained from USCS genomic browser. The promoter regions of FUT8 wild-type (WT) and 5 mutants were cloned into pGL3-basic plasmid and synthesized by Genechem Company (Shanghai, China). The STAT3 binding sequence were predicted by the JASPAR database. Relative threshold was set to 80%. The FUT8 mutant promoter vector was constructed with STAT3 mutated binding sites at FUT8 promoter regions. The control or STAT3 vector in conjunction with luciferase FUT8 WT or mutant promoter vectors were co-transfected with Renilla luciferase reporter into SCC15 and SCC25 cells, respectively. Firefly and Renilla luciferase activity were measured using the Dual-Luciferase Reporter Assay System (Promega).

Immunofluorescence analysis

Cells were fixed with 4% paraformaldehyde for 15 min at room temperature, permeabilized with 0.25% Triton X-100 for 15 min, and blocked with 3% bovine serum albumin (BSA) for 1 h. Next, the cells were incubated with primary antibodies overnight at 4°C, followed by incubation with anti-mouse Alexa Fluor 488 or 594 dye conjugates and/or anti-rabbit Alexa Fluor 488 dye conjugates (Life Technologies). The nuclei were stained with DAPI (Life Technologies) and the cells were visualized using an FV-1000 laser scanning confocal microscope.

Proximity ligation assays (PLA)

The cells were washed with cold PBS, fixed with 4% formaldehyde for 15 min, and

permeabilized with 0.25% Triton X-100 for 15 min. Next, the cells were incubated with blocking solution for 1 h and incubated with suitable concentrations of primary antibodies at 4°C overnight. The samples were then incubated with PLA probe solution for 1 h at 37 °C, followed by incubation with ligation buffer for 30 min. Further, the samples were incubated with amplification buffer for 100 min at 37°C in the dark. The nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI). The fluorescent signals were detected using an FV-1000 confocal microscope.

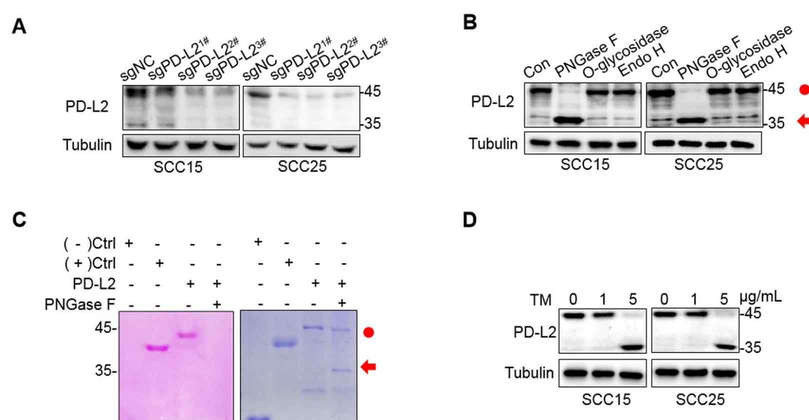
Silver staining and mass spectrometry analysis

The cells were treated with NETN lysis buffer (50 mM Tris, 150 mM NaCl, 0.2 mM EDTA, 1% Triton X-100, 1% NP-40, and protease inhibitor cocktail (Roche, 04693116001) on ice for 30 min. The lysates were centrifuged for 20 min at 4°C. For immunoprecipitation, the cell lysates were incubated with anti-FLAG M2 affinity gel (Sigma) with gentle rocking at 4°C for 3 h. The cell lysates were then incubated with flag-peptides with gentle rocking at 4°C for 3 h. The proteins were denatured by heating at 70°C for 10 min. The eluents were subjected to NuPAGE with a 4 %-12% Bis-Tris gel (Invitrogen). The bands were visualized using silver staining with a silver staining kit (Pierce, Thermo Fisher). Distinct protein bands were retrieved and subjected to liquid chromatography-tandem mass spectrometry (LC-MS/MS).

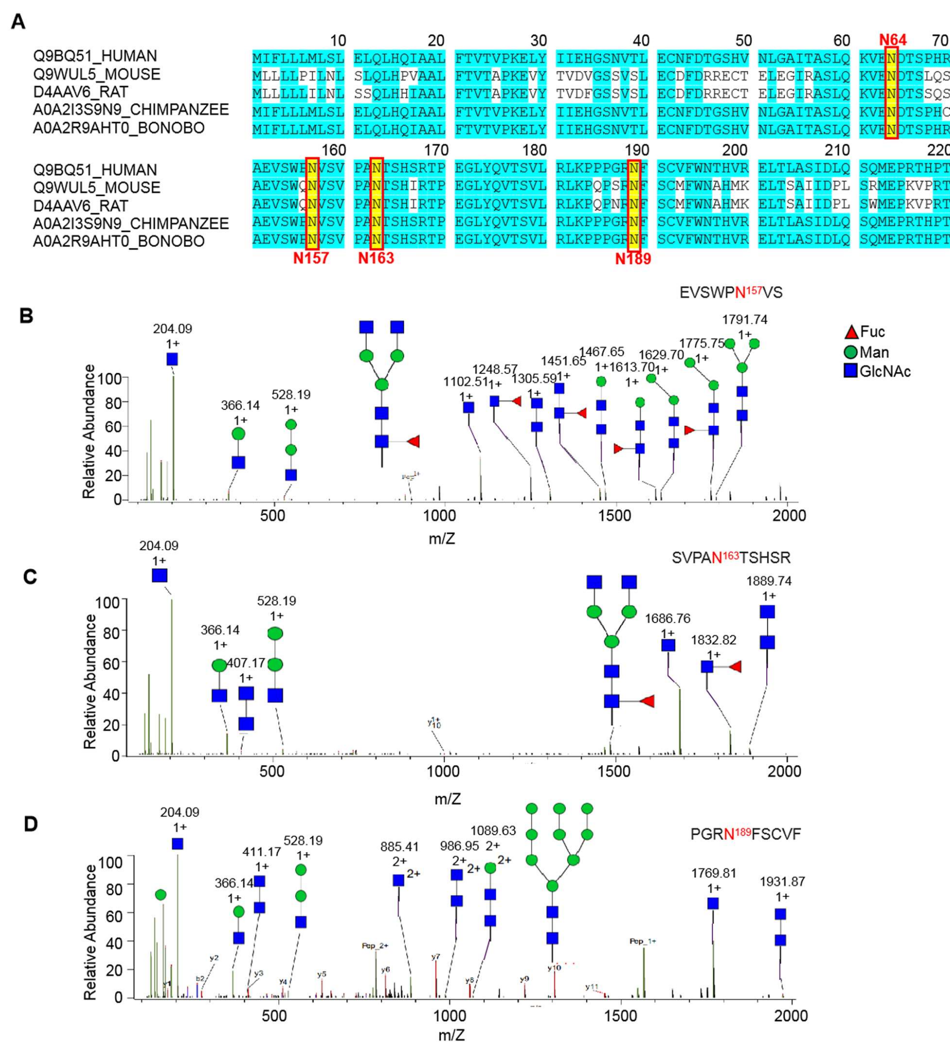
EGF-EGFR binding assay

The 96-wells plates were coated with 5 μg/mL anti-EGFR antibody (100 μL/well) in 0.05 M $NaCO_3$/$NaHCO_3$ (pH 9.6) overnight at 4°C. The plates were washed with PBS containing 0.1% Tween-20 (PBST) and blocked with 1% BSA solution for 1.5 h at 37°C. Then incubated with the cell lysate or negative control overnight. Next, the samples were washed with PBST and incubated with recombinant human biotin-conjugated EGF at 37°C for 1.5 h. The samples were washed with PBST and incubated with streptavidin-conjugated HRP (1:10000 in blocking buffer; 100 μL/well) at room temperature for 30 min. Further, the samples were incubated with 200 μL TMB at room temperature for 30 min. The optical density at 370 nm was determined using a BioTek Cytation 5 microplate reader.

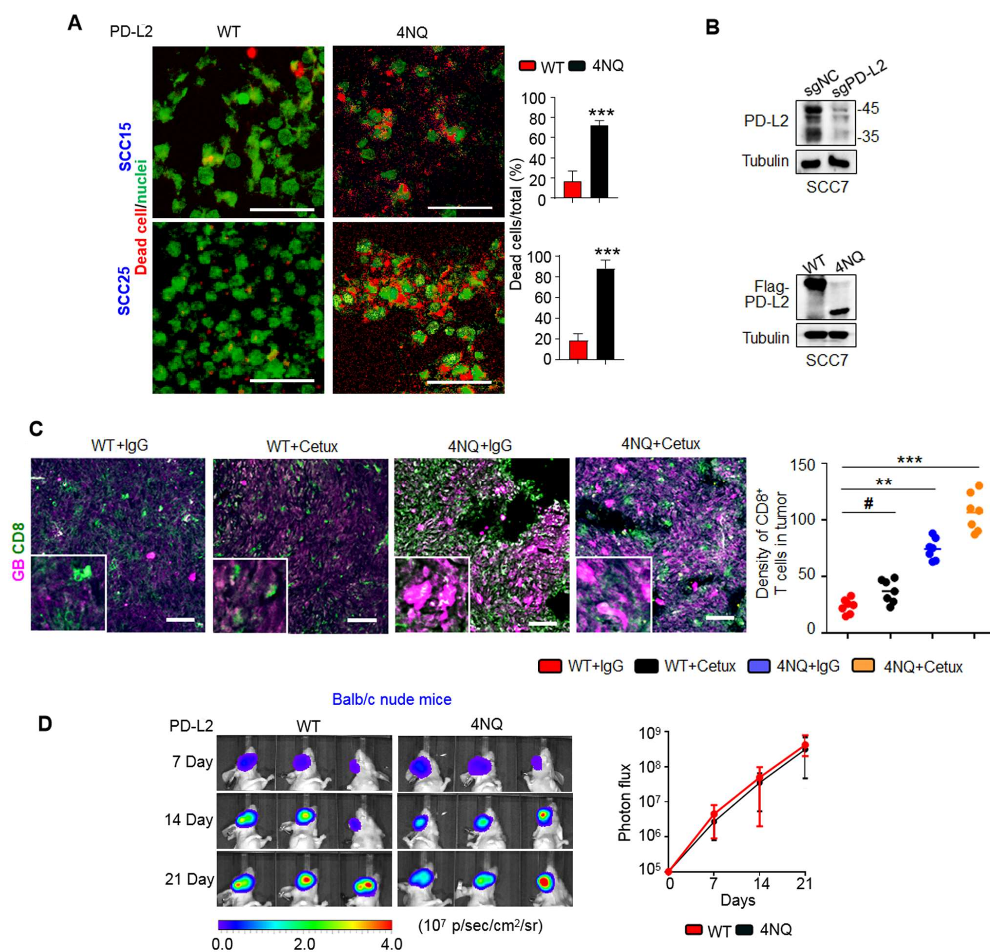
Supplementary Figures



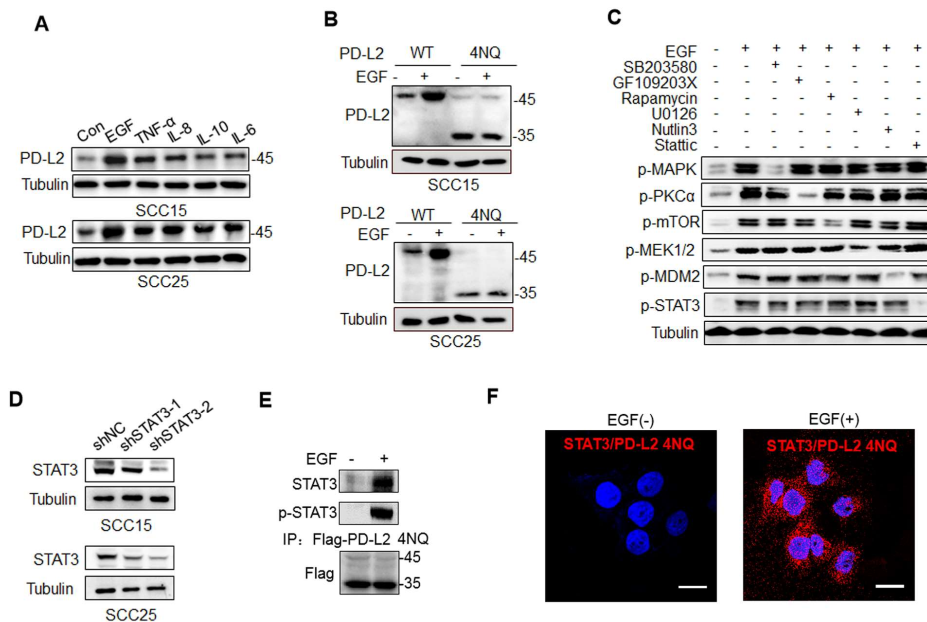
Supplementary Figure 1. PD-L2 is *N*-glycosylated in HNSCC cell lines. (A) PD-L2 protein levels in sgNC and three independent sgPD-L2 stable clones in SCC15 and SCC25 cells. **(B)** The glycosylation patterns of PD-L2 protein in SCC15 and SCC25 cell lysates were incubated with PNGase F, Endo H or *O*-glycosidase, and analyzed by Western blotting. **(C)** Glycoprotein staining of purified PD-L2 protein in the presence or absence PNGase F treatment in SCC15 cells. Coomassie blue staining represents total PD-L2 protein. Circle, glycosylated PD-L2; arrowhead, non-glycosylated PD-L2. (-) Ctrl, a control for non-glycoprotein. (+) Ctrl, a positive control for glycoprotein. **(D)** PD-L2 proteins expression in various dose of Tunicamycin (TM) treated cells.



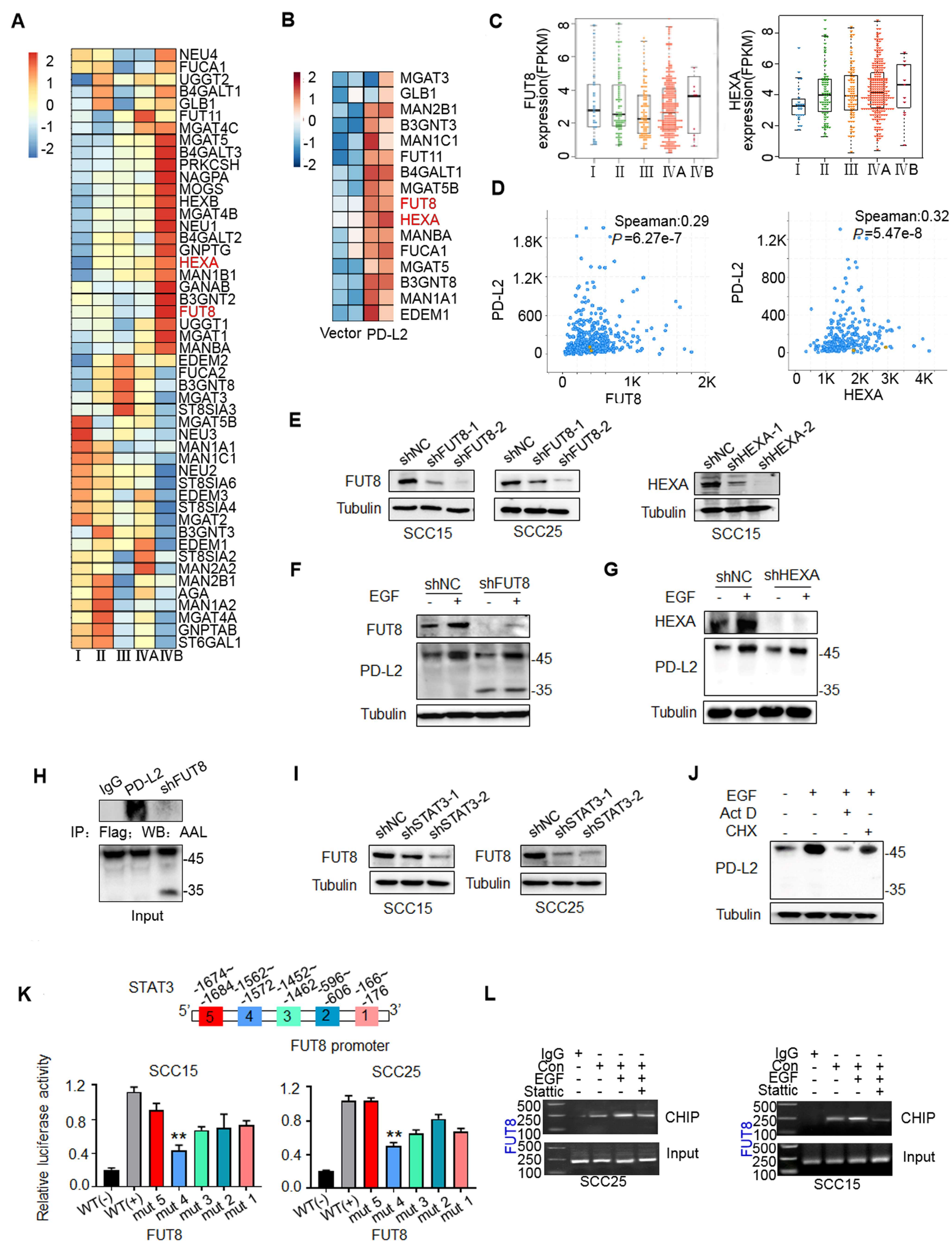
Supplementary Figure 2. Identification of the glycosylation sites of PD-L2. (A) The PD-L2 amino acid sequences alignment from distinct species. Four NXT motifs N64, N157, N163, and N189 are highlighted in yellow. **(B-D)** Identification of *N*-glycopeptides based on LC-MS/MS, corresponding to each of the four *N*-glycosylation sites N157, N163 and N189 of PD-L2 (from SCC15 cells).



Supplementary Figure 3. Glycosylation is required for PD-L2 immunosuppression. (A) T cell-meditated tumor cell-killing effect in PD-L2 WT or 4NQ SCC15 and SCC25 cells. The quantitative ratio of apoptotic cells was shown in bar graph (right). Scale bar, 50 μm. ****P*<0.001. **(B)** Validations of the transfection efficiency in lentivirus infected SCC7 cells by Western blotting. **(C)** Immunofluorescence staining of the protein expression pattern of CD8, and granzyme B (GB) in SCC7 tumor masses. Scale bar, 20 μm. ***P*<0.01; ****P*<0.001; #, not significant. **(D)** Representative BLI of tumors derived from PD-L2 WT and 4NQ mutant expressing SCC7 cells in BALB/c immune deficient nude mice at 21 days (n = 5 mice per group).

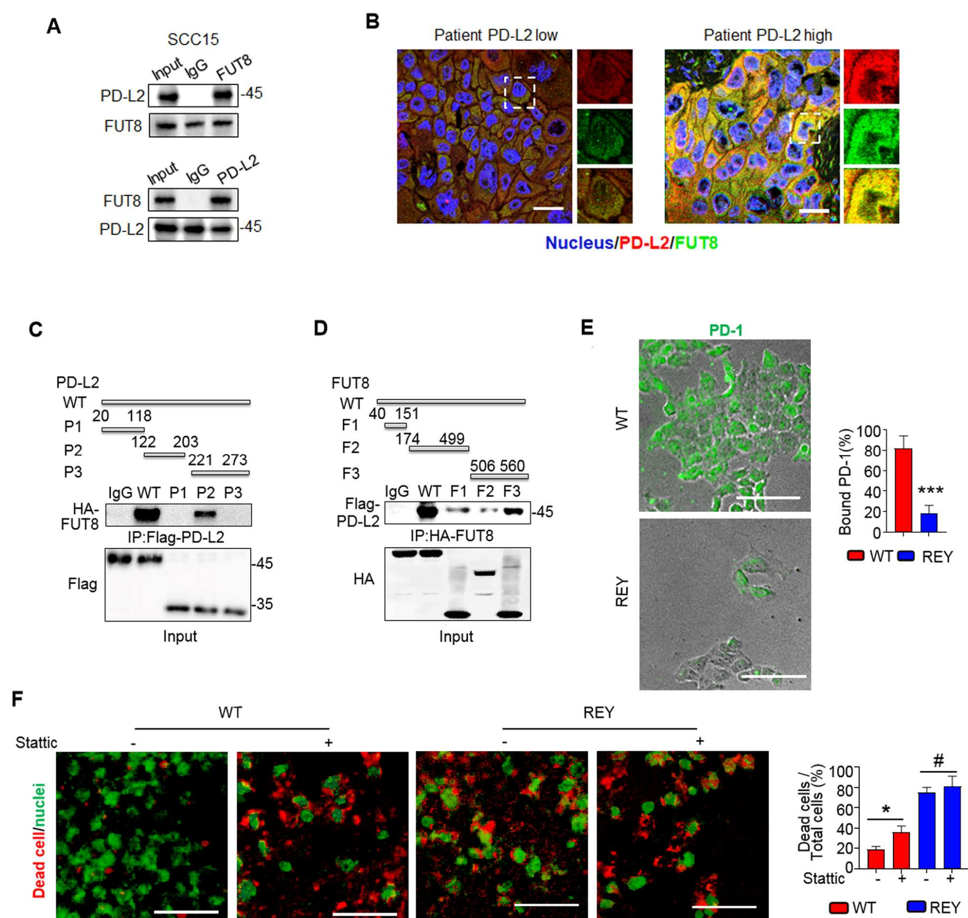


Supplementary Figure 4. EGF/STAT3 signaling regulates PD-L2 glycosylation. (A) PD-L2 protein expression in SCC15 and SCC25 cells treated with multiple cytokines for 15 min. **(B)** WB analysis of exogenous PD-L2 expression in Flag-PD-L2 WT or 4NQ cells with or without exposure to EGF for 15 min. **(C)** SCC15 cells were pretreated with or without 7μM SB203580 (MAPK inhibitor), 7μM GF109203X (PKCα inhibitor), 7μM Rapamycin (mTOR inhibitor), 7μM U0126 (MEK1/2 inhibitor), 7μM Nutlin-3 (MDM2 inhibitor) and 7μM Stattic (STAT3 inhibitor) for 4 h, followed by incubation with EGF for 15 min. Lysates were subjected to immunoblotting analysis. **(D)** Protein levels of STAT3 in shNC or shSTAT3 treated SCC15 and SCC25 cells. **(E)** Co-IP analyzed the (p)STAT3/PD-L2 interaction in SCC15 PD-L2 4NQ cells with or without EGF treatment. **(F)** Immunofluorescence staining of STAT3 and PD-L2 4NQ interaction in SCC15 cells by PLA assay. Scale bar, 20 μm.



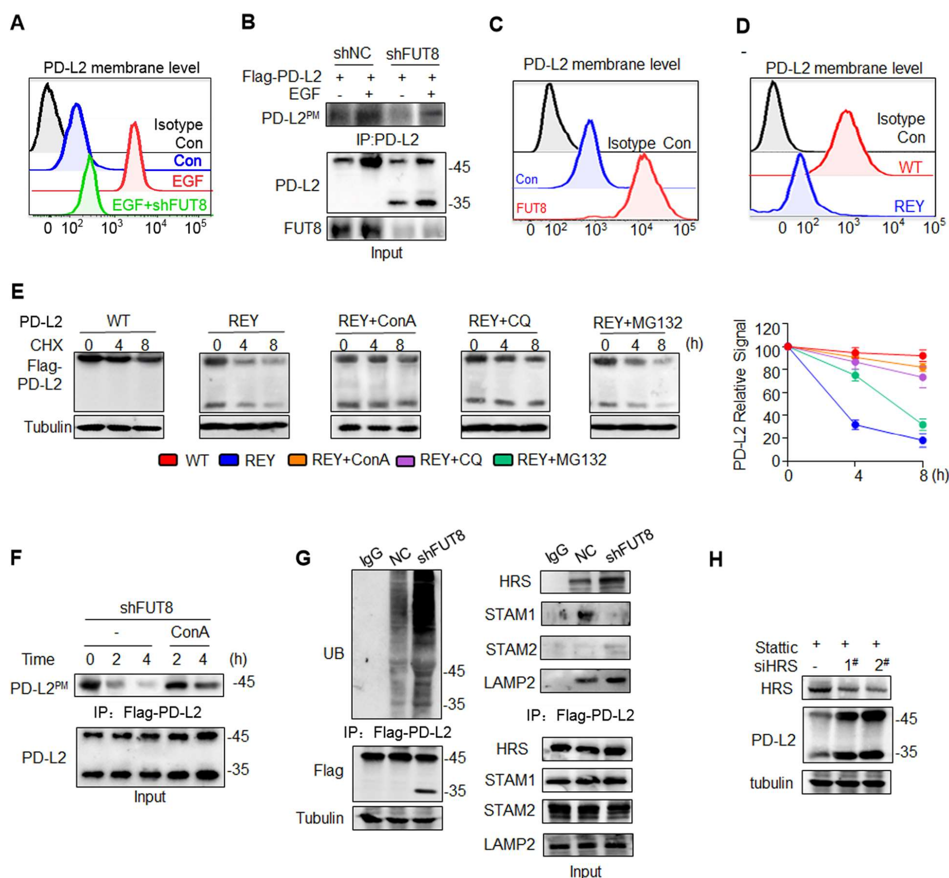
Supplementary Figure 5. Identification of the candidate *N*-glycosyltransferase responsible for PD-L2 glycosylation. (A) Heatmap showed *N*-glycosyltransferase gene expression pattern in HNSCC based on The Cancer Genome Atlas (TCGA) database. **(B)** Transcriptome sequencing showed *N*-glycosyltransferase expression in Vector and PD-L2 overexpressing SCC15 cells. **(C)** *N*-linked glycotransferases, FUT8, HEXA expression strongly correlated tumor stage in HNSCC. **(D)** Correlation analysis between FUT8, HEXA

expression and PD-L2 in HNSCC using TCGA data. **(E)** Validations of the knockdown efficiency in shFUT8-1, shFUT8-2, shHEXA-1 and shHEXA-2 lentivirus infected SCC15 and SCC25 cells by Western blotting. **(F)** Protein levels of PD-L2 and FUT8 in EGF treated shNC or shFUT8 SCC25 cells. **(G)** PD-L2 protein levels by shNC or shRNA for HEXA in EGF pretreated SCC15 cells. **(H)** Proteins in the cell lysates were blotted with biotinylated AAL lectin and subjected to the peroxidase (SK-6604, Vector Laboratories). **(I)** Protein levels of FUT8 in shNC or shSTAT3 treated SCC15 and SCC25 cells. **(J)** PD-L2 protein expression was detected in SCC15/PD-L2 cells treated with actinomycin D (Act D) or CHX for 16 h. **(K)** Luciferase reporter assay revealed that FUT8 was the transcriptional target of STAT3. **$P<0.01$. **(L)** CHIP assay revealed that STAT3 knockdown impaired EGF induced STAT3 binding to the promoter of FUT8 in SCC15 and SCC25 cells.



Supplementary Figure 6. STAT3/FUT8 catalyzes PD-L2 glycosylation contributes to immune surveillance escape in HNSCC. (A) Co-IP analysis of the interaction between PD-L2 and FUT8 in SCC15 cells. **(B)** Immunofluorescence staining of FUT8 and PD-L2 in HNSCC specimens. Scale bar, 20 μm. **(C)** Mapping of the binding site of PD-L2/FUT8 in vitro. Diagrammatic representation of FUT8 and its truncated forms in SCC15 cells. **(D)** Identification of the essential domains required for interactions with FUT8 and PD-L2 in SCC15 cells. **(E)** The bound PD-1/Fc protein in PD-L2 WT, or REY expressing SCC15 cells. Scale bar, 50 μm. Significance was detected by one-way ANOVA. ***P<0.001. **(F)** T-cell-meditated tumor cell-killing assay in PD-L2 WT or REY-expressing SCC15 cells with or without Stattic treatment. Scale bar, 50 μm. *P<0.05; #, not significant.

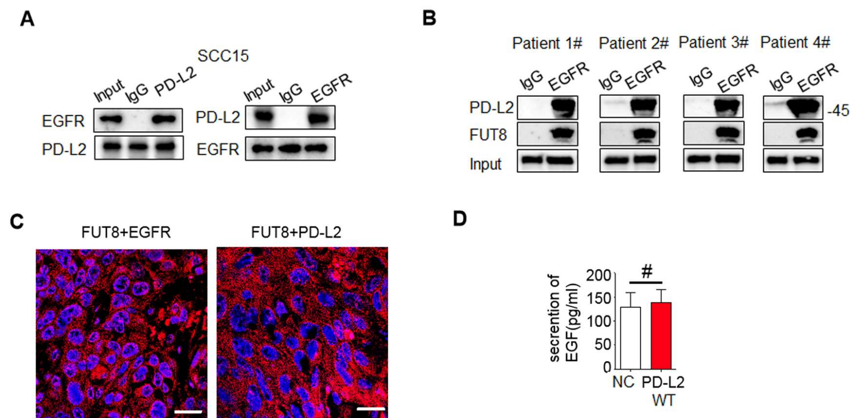
Xu Y, et al. J Immunother Cancer 2022; 9:e002699. doi: 10.1136/jitc-2021-002699



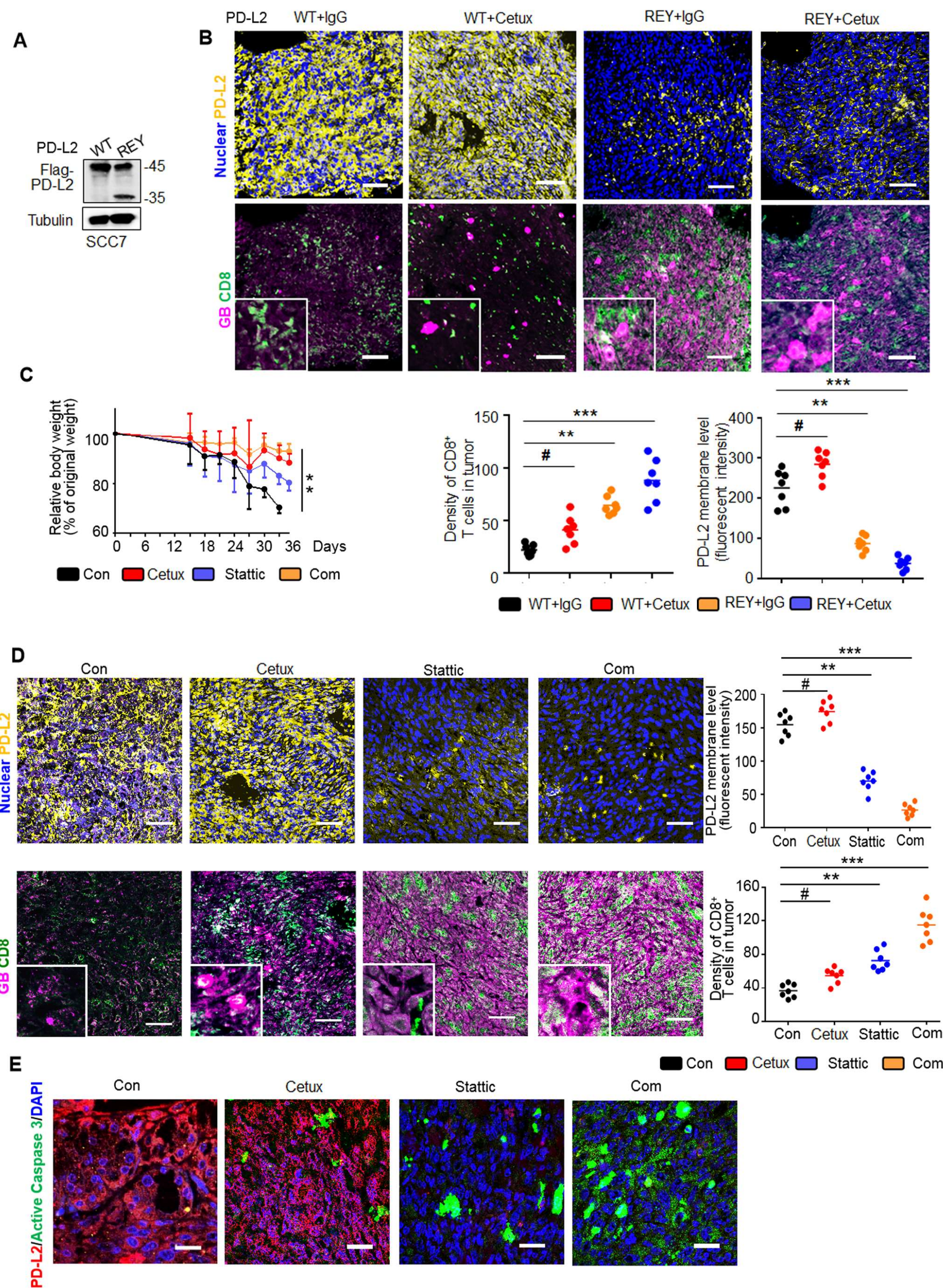
Supplementary Figure 7. PD-L2 glycosylation blocked ubiquitination mediated lysosome degradation and facilitated PD-L2 membrane abundance. (A) Cell membrane PD-L2 level in SCC15 cells with or without EGF treatment and/or shFUT8 infection was analyzed by flow cytometry (n=3). **(B)** Cell surface IP (CS-IP) analyzed membrane PD-L2 in EGF treated SCC15 cells expressing FUT8-shRNAs or control shRNAs. **(C)** Flow cytometry analysis measuring the membrane PD-L2 in vector or FUT8 expressing SCC15 cells (n=3). **(D)** The cell membrane PD-L2 level in SCC15 PD-L2 WT and REY cells analyzed by flow cytometry (n = 3). **(E)** Left, the degradation of PD-L2 WT or REY mutant in SCC15 cells was evaluated by CHX-chase assay in the presence of inhibitors for proteasome (MG132) and lysosome (ConA and CQ). Right, quantification of the PD-L2 intensity. **(F)** Cell membrane PD-L2 was measured in FUT8-knockdown cells by CS-IP. Incubation at 37 °C ± 50 nM ConA for 0, 2, 4h. **(G)** Effect of FUT8 knockdown on PD-L2 ubiquitination. The interaction between HRS, LAMP2, STAM1, STAM2 and PD-L2

was determined by Co-IP in SCC15 expressing FUT8 knockdown cells. **(H)** PD-L2 expression levels in Stattic treated with or without HRS-interfering SCC15 cells.

Xu Y, *et al. J Immunother Cancer* 2022; 9:e002699. doi: 10.1136/jitc-2021-002699



Supplementary Figure 8. PD-L2 interacts with EGFR in HNSCC specimens. (A) Co-IP analysis of the interaction between PD-L2 and EGFR in SCC15 cells. **(B)** Association of PD-L2 and EGFR as well as FUT8 was verified in four HNSCC specimens. **(C)** The direct interaction between PD-L2 and EGFR as well as FUT8 in HNSCC specimens by PLA. Scale bar, 20 μm. **(D)** EGF secretion from control and PD-L2 expressing SCC15 cell culture supernatant was measured by ELISA (n=3). #, not significant.



Supplementary Figure 9. Inhibition of PD-L2 glycosylation improves Cetuximab efficacy. (A) REY mutant PD-L2 transfection efficiency in SCC7 cells was determined by Western blotting. **(B)** Immunofluorescence staining of PD-L2, CD8 and GB in tumors derived from SCC7 cells transfected with PD-L2 WT or REY mutant and treated with Cetux every week. Scale bar, 20 μm. Bottom, Quantification, ***P*<0.01; ****P*<0.001; #, not

significant. **(C)** Survival of C3H mice bearing SCC7 tumors treated with Cetux, Stattic or the combination therapy. ***P*<0.01. **(D)** PD-L2, CD8 and GB protein expression detected by immunofluorescence staining in SCC7 orthotopic tumors. Scale bar, 20 μm. Right, Quantification, ***P*<0.01; ****P*<0.001; #, not significant. **(E)** Immunofluorescence staining of PD-L2 and cleaved-caspase 3 was detected in SCC7 tumor mass. Scale bar, 20 μm.

Xu Y, *et al. J Immunother Cancer* 2022; 9:e002699. doi: 10.1136/jitc-2021-002699

Supplementary Tables.

Supplementary Table 1. The clinical characteristics of patients included in single-cell sequencing

Designation	Age	Sex	Primary Site	TNM Stage	Grade
Patient 1	43	male	Tongue	T3N2M0	IVA
Patient 2	47	male	Tongue	T4N1M0	IVA

Supplementary Table 2. The differentially expressed genes in Group1 compared to the other groups. (Related to Figure 1C)

Rank	Gene_ID	Gene_Name	Log2 FC	*P* value
1	ENSG00000118473	SGIP1	1.985817703	5.57E-34
2	ENSG00000111674	ENO2	1.769523712	4.30E-31
3	ENSG00000134243	SORT1	1.645308732	5.48E-15
4	ENSG00000137809	ITGA11	1.581957425	4.56E-33
5	ENSG00000197646	PDCD1LG2	1.5677836	1.64E-07
6	ENSG00000160886	LY6K	1.510625716	1.22E-13
7	ENSG00000164176	EDIL3	1.508244681	6.40E-42
8	ENSG00000117152	RGS4	1.487518878	4.08E-32
9	ENSG00000108691	CCL2	1.365413954	3.60E-42
10	ENSG00000083857	FAT1	1.326366538	1.00E-26
11	ENSG00000144136	SLC20A1	1.273697799	2.94E-22
12	ENSG00000060718	COL11A1	1.260307192	1.59E-25
13	ENSG00000164930	FZD6	1.24709857	2.88E-08
14	ENSG00000106366	SERPINE1	1.204768913	4.43E-34
15	ENSG00000008517	IL32	1.19628867	1.55E-30
16	ENSG00000074416	MGLL	1.178567364	2.94E-26
17	ENSG00000249992	TMEM158	1.137685961	9.60E-16
18	ENSG00000133816	MICAL2	1.084719236	2.68E-19
19	ENSG00000146674	IGFBP3	1.07633818	1.34E-12
20	ENSG00000112715	VEGFA	1.075761367	7.19E-17
21	ENSG00000134013	LOXL2	1.0696223	1.11E-26
22	ENSG00000134363	FST	1.063960056	1.53E-13
23	ENSG00000251493	FOXD1	1.060066217	6.37E-18
24	ENSG00000182667	NTM	1.053483419	4.16E-20
25	ENSG00000175592	FOSL1	1.043497019	2.20E-17
26	ENSG00000185803	SLC52A2	1.039122601	8.81E-10
27	ENSG00000139318	DUSP6	1.034587543	7.37E-19
28	ENSG00000125148	MT2A	1.012969985	7.29E-27
29	ENSG00000149948	HMGA2	1.005930708	1.36E-15

Supplementary Table 3. The Cancer Genome Atlas analyzed 49 *N*-linked glycosyltransferases correlated with the tumor stage in HNSCC (Related to supplemental figure 5A)

Case UUID	Stage	Tissue_of_Origin	Cigarettes_Per_Day	Alcohol_History	MGAT3	B3GNT3	GNPTAB	ST6GAL1	MAN2B1	ST8SIA2	UGGT1	ST8SIA4	B4GALT2	MGAT5	MOGS	MGAT1	MGAT5B	B4GALT1	B3GNT2	MAN1A2	FUT11	B4GALT3	GLB1	MGAT4B	GANAB	PRKCSH	MGAT4C	NEU3	NEU4	MAN1A1	ST8SIA3	NEU2	MGAT2	UGGT2	EDEM2	HEXA	FUCA2	MAN1B1	MAN2A2	ST8SIA6	EDEM1	HEXB	MANBA	NAGPA	AGA	MGAT4A	B3GNT8	EDEM3	NEU1	MAN1C1	FUCA1	GNPTG	FUT8
13f63218-0949-41e6-a661-c844414c45b5	iva	c04.9	.	yes	0.33194	1.59415	5.34451	0.82478	20.2889	0.10485	11.9242	0.6004	18.6849	8.9202	15.5153	20.0335	0.026	66.0233	5.85657	5.4169	5.42627	8.46727	9.20446	9.52136	115.428	58.8496	0.04241	0.87702	0.04829	6.70462	0	0	1.60109	1.04999	11.3352	4.3295	20.5593	18.1356	1.88501	0.20112	6.99587	17.5589	3.70178	1.54695	10.4002	0.70852	5.92852	4.56298	6.19522	0.23247	22.1598	7.74263	2.09755
a2d42990-747c-4618-88dd-e2dece8e566f	ivb	c10.9	0.767123288	yes	0.40826	2.63757	4.68767	1.93297	20.1101	0.70603	17.1565	0.75897	24.5318	21.1605	52.255	27.0573	0.0812	33.9405	65.0557	7.05295	2.57382	20.1588	12.38	17.9267	107.132	65.503	0.00138	2.44013	0.01638	9.71403	0	0	1.61538	2.26902	12.1901	2.63774	11.0436	8.19783	3.27587	0.01462	9.70809	21.1049	3.40775	2.71768	7.39516	1.92258	1.55779	5.55947	20.8389	0.22981	14.6835	8.90999	3.98164
9a76bd98-1d2b-4d0f-9772-883520ff6210	.	c09.9	.	yes	3.33715	1.12971	13.8157	16.4678	42.364	0.02952	13.2234	4.25007	17.8871	17.3122	21.5722	43.1047	0.05458	61.1822	17.1363	5.5613	6.48322	10.1879	17.577	14.3583	90.1576	78.458	0	1.79509	0.04026	14.2412	0.0012	0.01271	2.21048	1.20459	20.3643	5.74622	19.5553	9.1463	2.55975	0.56386	17.7133	23.6562	5.09145	4.71626	6.99779	3.93441	5.9902	7.00615	14.6005	2.19297	89.7367	12.3945	5.26916
70741b73-9683-42bd-87fc-6a021a70103b	ii	c04.9	3.287671233	yes	0.69736	12.535	6.76139	4.3872	13.5642	0.03568	9.15375	1.63774	25.0508	8.2408	19.9868	42.2432	0.07696	98.7325	12.4522	4.61496	3.61786	10.2665	14.1261	22.5778	97.6322	45.1581	0	1.23587	0.03594	13.135	0	0.06143	1.51033	0.9751	14.4742	3.56211	12.6749	13.7019	1.1709	0.43298	12.43	13.8872	1.83083	1.92558	7.45955	1.29488	16.5452	4.4211	8.90247	0.6861	16.3337	6.74735	2.20906
815119e3-13c4-4f63-98c7-10a519034a25	iva	c32.9	2.739726027	no	4.40033	1.09147	5.47305	6.96388	21.6018	1.21516	9.5332	0.9412	20.6165	5.7482	42.8463	30.1012	0.16127	61.5078	54.1856	4.27827	6.0166	15.6483	10.7896	13.0834	93.3996	80.1632	0.02017	1.42518	0.03468	9.52057	0	0.03162	1.439	2.47504	21.7496	5.85128	14.7043	10.9914	7.31796	0.07566	8.69455	18.2607	4.9491	2.02625	6.7342	2.01133	2.86353	4.78562	21.2269	0.96286	18.2624	9.49659	7.56976
fa9f6b24-16b4-4dda-acce-bf2e186d365d	iva	c04.9	.	no	0.09078	3.73158	4.05296	1.18641	7.95423	0.01049	5.44879	0.24629	14.2928	4.48754	15.8727	27.4139	0.00323	54.9383	15.28	3.43969	1.30315	4.97702	9.10462	18.0485	45.8135	32.3269	0	0.44253	0	3.37144	0	0.54174	0.88338	1.69006	6.50723	1.72507	6.52538	7.82404	0.70988	0.08643	7.73018	16.7501	1.70759	1.1103	7.6234	0.80804	9.84654	4.73425	5.75291	0.17837	3.14596	7.59293	0.91495
54dab158-eb39-4334-8b5a-bf290e6b8bb1	iii	c02.9	.	yes	0.4573	4.28304	8.65653	4.46011	18.9092	0.87554	11.3984	3.52524	22.2755	8.99332	26.177	41.0423	0.06546	111.552	12.2645	6.10637	5.14954	12.8238	20.8705	17.3379	87.0191	41.369	0.00228	2.6438	0.04863	25.2818	0	0.02217	1.60288	2.75595	16.8129	5.611	21.9469	10.4156	1.93646	0.5256	10.6299	29.5424	5.48467	2.76032	8.77487	3.73315	3.73804	6.9197	13.868	1.12696	16.2443	11.4878	3.5639
e08e1d99-81e1-454a-9bda-cda92c9e22b4	iii	c02.9	2.246575342	no	0.90858	5.293	5.18569	4.80685	15.0552	0.03015	11.2446	0.67856	19.9527	7.30361	22.8003	34.8633	0.50964	29.3536	6.46988	3.38771	5.01793	11.6216	9.30471	21.3451	85.0088	67.2521	0.26742	1.34265	0.05965	4.56902	0.00421	0.64511	1.60866	2.08195	16.6947	5.20986	17.4151	12.8642	3.45474	0.04839	8.64222	12.8612	4.11968	2.8236	5.57952	0.68912	8.30356	4.18993	13.4303	0.5465	8.73213	13.9776	2.18211
cb78efb3-bc29-4de7-9128-fdc4f9539b48	.	c32.9	2.191780822	yes	0.80186	28.545	4.88491	9.87991	12.2566	0.01827	8.27868	1.00423	27.2776	6.36201	26.0698	20.6651	0.1134	54.3176	13.3934	5.02691	2.03655	9.76335	11.0896	11.4035	91.1392	48.0924	0.00111	1.49466	0.02957	9.60278	0.00128	0.39092	1.47939	1.04068	12.8623	3.04093	23.0312	14.2664	2.05957	0.56304	9.28142	14.0331	5.77621	2.32348	10.7075	1.18716	7.39131	6.38095	11.4358	0.55819	18.3053	11.6965	2.4238
80d51546-c046-4af4-b3b3-b6bf5b1eab02	i	c02.9	.	yes	0.2047	6.49312	10.5994	2.16864	14.9954	1.54641	11.0701	0.35383	43.0197	10.6071	18.9059	28.442	3.92484	70.2222	11.3412	5.37867	6.60126	13.9363	25.7131	21.6024	184.325	85.0775	0.03395	1.76943	0.02233	9.48938	0	0.01527	1.16923	3.38161	40.329	6.92432	42.6634	7.65959	2.53033	0	12.1595	15.184	9.14595	1.97011	6.42701	0.22293	3.60444	7.21512	8.3723	0.29006	11.187	7.05055	7.39322
bfaa4b3f-3e38-478e-99b3-013f2cda80f5	iva	c04.9	1.589041096	yes	0.58958	6.51111	7.18121	5.45635	20.5018	0.57779	17.2078	2.91616	22.6381	11.9443	19.6323	36.8793	0.07568	58.1213	13.374	6.58922	5.21962	13.1055	12.6756	20.1409	106.215	45.2661	0.00149	2.22586	0.02061	11.3986	0.00114	3.63632	1.52627	1.58553	15.2259	5.8054	11.4501	10.4653	3.27607	0.55191	14.6379	21.6677	3.84775	2.66649	8.46053	3.31802	5.86524	10.197	8.38754	1.0816	17.0058	7.37882	4.85354
5d227ea8-bd74-4e8f-9bee-9d981f03d66f	iva	c14.8	1.095890411	yes	0.4299	14.9666	6.09655	3.72587	13.7853	0.47333	11.5165	1.1448	17.8371	8.80222	29.1873	19.9199	0.194	82.7962	23.6043	4.9476	4.46654	9.67627	15.665	13.1771	103.645	57.3511	0.0057	2.30589	0.21643	13.3241	0.00437	0.34592	1.50581	2.39415	17.0874	6.92784	23.0417	8.18677	1.41651	0.04515	6.9642	17.3672	11.0754	2.37683	9.40549	0.91695	5.41528	6.17938	12.6575	1.13163	17.6864	10.4742	5.66129
fc3ea52b-67ac-4876-949d-5464c436f3db	.	c32.9	2.465753425	yes	5.35431	8.69455	10.3098	17.6886	35.0867	0.1641	17.0533	1.35953	21.8443	11.5	35.8701	25.1521	0.81106	31.4383	25.5386	7.67281	3.62363	18.7017	22.7633	15.6964	133.097	83.1129	0.00285	2.69743	0.07589	13.5118	0	0.0173	1.83195	1.91388	38.3326	7.24385	12.7829	10.2487	10.2541	0.64717	17.6335	27.8802	4.55972	3.129	10.5163	3.43852	3.25966	11.0751	27.1567	0.72277	26.2781	7.8507	9.51859

757c56d0-8a49-464f-866a-ae98816de60b	iva	c02.9	.	no	0.49694	8.00338	4.71021	3.74469
9180e195-dae9-4af9-90cf-71778bc9aefc	iii	c02.9	.	yes	0.65637	0.61758	8.3451	1.21041
89f91e36-fed8-4da3-8a35-c761d6f65285	iii	c09.9	0.821917808	yes	25.1505	25.1669	14.3826	9.8249
6f8b9c64-16f1-4cd0-9cd6-a3c27d311cf8	iii	c02.9	.	no	0.46448	7.00267	3.4459	0.93778
20ad4ac0-6a87-461f-83a5-fded51bf821a	iva	c32.9	3.287671233	yes	1.08954	8.90495	8.50285	2.51477
27707915-d36e-4da7-9b39-2fdd9ce0bdc5	iii	c06.0	1.150684932	yes	0.47505	0.4194	6.3828	3.63101
ebe99910-e9f2-43dc-ad57-ac2f610b60c8	iii	c32.9	1.643835616	yes	0.46964	8.96776	5.73188	4.28176
91a712b5-a724-48d0-ba06-4f6857683463	.	c02.9	0.273972603	yes	0.26531	3.06828	5.45315	2.8419
3c5bc678-41e9-46b3-9686-5cd95659ab71	i	c00.9	1.534246575	no	10.701	1.38862	7.07317	12.1589
8fc1f1be-d2d5-4b3a-9973-f4d964018beb	iii	c02.9	.	no	0.04544	10.4714	3.19846	0.28453
8f6c64c9-7799-436d-bff0-b166e56532a8	iva	c02.9	1.095890411	yes	0.05099	6.63232	5.67136	0.48651
67560840-70fb-45fd-8572-648f74811aec	iva	c09.9	.	no	1.11626	3.0503	8.34137	26.7609
731f6967-5a01-40b7-9f88-64d980214bb2	iii	c03.9	1.369863014	yes	0.0884	14.7535	3.20294	0.76764
abbcc1d8-ab74-4459-bf28-cc627bef440e	iva	c09.9	3.287671233	yes	3.30844	5.49926	5.89987	1.99992
78e67b26-c473-449f-95e7-c4d11f066d5b	iii	c14.8	2.356164384	no	0.36971	16.1915	5.52729	1.57513
20ece2ca-8601-4cca-aec5-0a5c0b92594d	ii	c02.9	.	no	0.10701	9.77104	7.46498	1.13805
38cfe5af-02e4-4271-8978-8e2566d3dca7	iva	c02.9	.	no	1.3964	10.2331	9.69915	15.4125
765cc306-bdae-4caf-ad1e-35b8fe95c220	iva	c06.0	.	no	0.26856	13.1953	7.1424	2.13325
bf6b931e-cb2e-4cc0-b603-e9fc71fdc509	iva	c02.9	0.054794521	yes	0.14063	1.52957	7.782	2.68646
241d9310-9137-42dd-b28d-0dc50c44cb43	ii	c32.9	.	yes	3.47597	37.7008	5.81835	2.50714

65ba24fb-07df-47ce-91c4-677684b64cf4	iva	c14.8	.	no	0.26678	14.5389	9.0207	4.37905
b47fb978-2dfb-4abc-a5af-6e14dd684182	iva	c32.9	.	no	0.08147	0.43727	1.28912	0.22501
9e178d80-fe90-4998-9dae-726c4beceef7	.	c01.9	1.643835616	yes	50.2938	5.57355	9.8595	6.04525
16779eed-f386-4e8f-8475-04d33b9dd160	i	c09.9	6.02739726	yes	1.144	1.25422	4.33099	3.49232
d189a6c6-210f-4957-b500-457d5ce3f867	.	c09.9	.	yes	1.47442	4.08389	8.75194	8.6464
ce603d46-299a-4ffb-84ab-b3b914a561b9	iva	c06.2	2.191780822	yes	0.19584	3.41376	5.0353	2.09925
3cf77e08-ea34-4091-b242-75c7e35ff699	.	c32.9	.	no	0.47614	7.6328	6.22438	14.2524
a9017cd4-df30-46eb-b08f-39540b15243f	.	c09.9	1.917808219	yes	6.21703	0.83893	13.1359	11.486
08a45833-16a3-4a57-a310-307ec086e558	i	c02.9	.	no	0.42863	6.37081	7.22194	7.95695
76214b90-c323-4fdc-92f4-1180abdb1380	iii	c32.9	.	no	1.29759	23.3714	6.41427	2.66584
cac30b32-03ef-4ecb-88d8-d5577ab6b2a6	iii	c01.9	5.616438356	yes	31.9069	3.6893	6.8277	1.65486
7e3b90c1-8772-4e12-8050-a156558beb82	iva	c32.9	4.520547945	yes	0.93832	17.1083	6.73558	21.7933
c7353d6a-82a2-4a0d-8cd4-8c8957943edb	.	c32.9	4.109589041	no	4.85535	0.91473	13.7711	3.05405
3ee863da-7991-414f-82d8-2b6502d4c061	iva	c01.9	.	yes	0.14766	0.4796	4.65602	1.26147
a40bb4bc-9ee6-4a8b-8a33-3a4ed78f69a7	iva	c02.9	.	yes	0.50147	2.23864	4.89233	2.94864
a71b425b-ccd3-42f0-8163-3721cb1794ef	iva	c03.9	1.643835616	yes	0.69581	1.02425	5.4442	3.91468
63f6a127-e4e8-4e1c-8236-a2adb9594c8f	iva	c32.9	2.191780822	yes	3.73171	35.3724	11.581	30.1749

c539274c-e2bf-4b5f-ab56-35eff1569bac	ii	c32.9	.	no	0.52134	50.2081	5.30244	1.64664	6.05215	0.00727	6.24938	0.79975	11.757	6.92652	12.1524	27.5064	0.06716	72.2723
9ae5101b-6031-4ab1-bfca-198c41af3184	i	c09.9	0.602739726	yes	0.99941	0.12173	11.4943	16.8746	41.1274	0.02791	13.9418	4.17943	20.5915	6.93412	35.9543	50.7189	0.29671	41.8692
7de19081-d5fd-468c-ad0d-f6e3e8b2ad70	.	c32.9	1.643835616	yes	0.13675	7.63504	5.44984	2.58616	20.0789	0.08307	12.4328	0.42401	24.1468	3.1889	18.5122	34.97	0.24038	21.3685
b3342767-62bc-407f-8a83-5be50e3bdc3e	ii	c14.8	.	no	0.13213	2.42197	5.23407	2.8792	14.004	0.32572	9.07188	0.64565	20.1905	4.49872	21.2687	28.403	0.04469	31.7561
30eb06a5-8e88-4c46-bebb-fb93ca07f108	iva	c32.9	4.383561644	yes	0.14417	6.49611	8.57551	2.14184	14.6367	0.48024	13.5401	0.90496	14.9125	6.72192	34.6902	21.0444	0.6542	50.3731
31e58fac-3ac3-4eeb-82d6-3f2b5cb8a60d	iva	c04.9	1.97260274	yes	0.1809	8.07326	4.14193	0.76656	8.00452	0.12416	8.81288	0.54693	29.9417	5.52561	19.4062	49.9903	0.30916	50.3093
116ad004-929d-4c8d-8eee-883d132e0fe5	ii	c02.9	3.287671233	yes	0.17455	27.1677	5.76314	0.74805	25.5181	0.06291	12.1346	0.3915	33.8477	6.72106	28.4307	20.716	0.09354	15.1994
f22eac21-8a74-433a-b65c-3540d14a45cb	iva	c32.9	.	no	0.14252	7.23075	6.98015	3.74103	13.2937	0.32938	6.92509	0.73414	21.8042	3.45017	39.2208	33.7378	0.13484	30.7828
3bd2fcee-5b16-49f3-a717-f1e106bbc12f	iva	c32.9	2.301369863	yes	0.40661	16.2739	2.03438	2.80612	14.2515	0.05068	4.00687	3.84168	26.6758	2.7785	29.7876	32.0326	0.02342	18.4706
b8ac8500-abcd-40c4-9c24-7af557fd260a	iva	c13.9	1.369863014	yes	0.0742	11.6144	4.67216	0.79881	14.2566	0.00722	10.1535	2.19296	22.8258	5.28939	18.7943	30.8335	0.15018	50.5634
9283709d-d019-4291-a097-611a5c05244b	iii	c04.9	.	yes	0.29238	5.80437	4.46692	2.25879	14.1777	0.42518	10.702	0.81195	28.0554	8.95112	23.7368	31.0962	0.04211	39.7832
396ccdcb-40eb-4299-8575-b26753054434	iva	c04.9	.	no	0.08339	10.9647	4.70041	1.54151	8.78427	0.17701	9.23534	1.13092	22.3358	3.55224	35.5883	36.0451	0.00792	30.0137
90a8d2cb-1f31-45f9-8575-eed3cb9a7798	iii	c14.8	.	no	0.13456	9.30263	5.10475	6.38275	9.71354	0.049	17.0494	2.22966	22.8821	7.49391	31.3604	45.3854	0.08014	45.2917
df37eed0-ea63-485d-a831-28d3061852bd	ii	c02.2	4.383561644	yes	0.44064	1.54778	4.7326	2.17895	11.7896	0.48804	5.85249	1.18748	37.4966	2.80051	28.2187	33.3968	0.06394	45.0031
10593e5b-7acb-498b-bdf7-705e3e2bc443	.	c06.9	2.95890411	yes	0.14434	1.42056	1.50855	0.39593	6.82994	0.04003	4.37578	0.33349	34.0991	3.17471	21.7429	31.5354	0.05242	33.4318

c539274c-e2bf-4b5f-ab56-35eff1569bac	16.2552	5.54285	2.12262	6.47563	5.74606	13.0736	61.4656	31.5704	0.00103	0.73412	0.00915	7.00562	0	0.06254	0.44161	0.803	8.02355	1.8399	7.0842	8.19411
9ae5101b-6031-4ab1-bfca-198c41af3184	15.9872	12.9298	4.91617	16.4852	11.9498	15.5892	155.94	101.389	0	2.17189	0.07731	14.9651	0	0	4.47023	1.21052	21.8774	5.49136	25.6973	13.3064
7de19081-d5fd-468c-ad0d-f6e3e8b2ad70	14.7627	2.11949	7.46006	13.4337	8.07586	17.8636	70.6377	47.1605	0.00307	0.64899	0	4.0056	0	0.70882	1.99956	2.88267	14.1434	5.91724	83.1147	11.8977
b3342767-62bc-407f-8a83-5be50e3bdc3e	5.90671	5.65614	4.3504	9.20979	10.8943	19.86	80.2497	57.0848	0.01353	0.88864	0.01281	31.0405	0	0.47312	0.85533	1.78	17.9725	9.24083	18.1031	10.9104
30eb06a5-8e88-4c46-bebb-fb93ca07f108	8.5433	4.9376	5.77645	9.33442	9.98964	11.5078	124.926	57.6668	0.0127	0.94244	0.00579	11.7894	0	0.53402	1.9132	1.38228	19.5862	6.00276	11.7186	19.4351
31e58fac-3ac3-4eeb-82d6-3f2b5cb8a60d	7.37655	3.08052	3.85957	9.52787	7.49905	15.4252	111.539	57.9518	0.00108	1.30448	0.02233	8.00052	0	0	1.5252	1.94251	8.95548	3.81189	22.6363	22.7829
116ad004-929d-4c8d-8eee-883d132e0fe5	15.2137	5.06541	1.51255	12.3224	8.36099	20.1201	93.3523	49.7628	0	1.42945	0.17904	3.20707	0	1.243	1.74247	2.80655	11.2231	4.93525	21.8559	14.734
f22eac21-8a74-433a-b65c-3540d14a45cb	7.4125	4.93203	5.86073	10.1608	5.94796	12.0557	119.473	70.2847	0.03252	0.52016	0.00592	7.78887	0	0	1.99509	0.97261	11.0584	5.85876	9.38803	25.5474
3bd2fcee-5b16-49f3-a717-f1e106bbc12f	4.85373	3.06898	3.75233	12.4398	8.80923	22.8484	57.2018	65.7151	0.01347	0.78198	0.29775	1.14859	0.0062	0.04362	0.51031	1.50076	20.5787	4.83357	11.3988	16.468
b8ac8500-abcd-40c4-9c24-7af557fd260a	8.9835	7.73584	5.60352	17.7724	8.09684	15.5173	109.284	62.7137	0.6901	0.62333	0.00455	4.08465	0	0	1.85594	1.42683	13.571	7.83616	47.4151	16.0726
9283709d-d019-4291-a097-611a5c05244b	10.0759	6.37758	3.44672	14.6091	9.15262	17.8001	174.063	66.254	0.00065	4.15678	0.10323	5.83156	0	0.04705	1.03414	1.08554	12.1446	2.99949	15.1159	8.31702
396ccdcb-40eb-4299-8575-b26753054434	8.09392	3.68033	5.48854	11.7368	7.92084	16.0009	64.3732	40.3199	0	0.74498	0.01438	3.94279	0	0.14745	0.62722	0.83079	13.2966	5.98446	13.5588	16.8171
90a8d2cb-1f31-45f9-8575-eed3cb9a7798	9.41054	7.70851	9.136	15.2553	8.58117	22.2306	195.38	88.2971	0.02244	0.88638	0.01898	8.7113	0	0	1.48229	1.74284	13.543	8.14448	35.5871	22.1093
df37eed0-ea63-485d-a831-28d3061852bd	5.30439	2.03918	4.15804	8.11157	12.3246	16.3641	73.8879	45.6015	0.0049	1.63318	0.42089	8.81769	0.00376	0	0.9793	1.37359	20.4502	7.72395	18.4935	11.8282
10593e5b-7acb-498b-bdf7-705e3e2bc443	7.04087	1.14883	2.17363	9.93157	4.37228	16.4588	45.4026	51.8538	0.00142	0.75574	0.0042	1.66121	0.00326	0.22396	0.27115	0.62533	15.3916	4.29858	15.2071	24.6309

c539274c-e2bf-4b5f-ab56-35eff1569bac	1.31929	0.31564	8.7094	9.17258	1.84021	1.29303	12.4739	1.33462	20.3689	5.27412	5.70135	0.48943	11.6285	5.99806	0.86205
9ae5101b-6031-4ab1-bfca-198c41af3184	10.0504	0.20071	10.2522	21.7381	3.15786	3.85478	4.60116	5.09187	1.31884	7.67041	15.7523	2.14858	87.213	9.38313	4.78316
7de19081-d5fd-468c-ad0d-f6e3e8b2ad70	1.55397	0.13797	12.7173	21.4469	4.45518	1.86614	23.385	0.79925	13.2088	4.45865	19.7024	0.11811	6.30142	10.7643	8.53781
b3342767-62bc-407f-8a83-5be50e3bdc3e	2.99617	0.21729	5.81696	26.429	2.47835	3.3664	9.91132	0.62706	3.48683	5.36836	13.9498	0.71562	9.15081	15.1459	5.73504
30eb06a5-8e88-4c46-bebb-fb93ca07f108	1.79281	0.01549	9.50766	16.0107	5.71049	3.83374	8.29053	0.78595	3.82891	6.81851	11.2096	0.29004	8.81297	16.3582	10.1953
31e58fac-3ac3-4eeb-82d6-3f2b5cb8a60d	0.71929	0.02277	6.23908	32.9455	3.15201	2.06463	6.82709	0.42867	1.99533	3.35593	9.45745	0.15411	8.6147	9.64437	1.69885
116ad004-929d-4c8d-8eee-883d132e0fe5	1.59641	0.01229	9.3577	9.77549	4.69018	2.28159	9.75933	0.62869	3.56668	7.79314	14.6951	0.127	27.5259	8.8714	0.75664
f22eac21-8a74-433a-b65c-3540d14a45cb	2.10779	0.0793	3.45662	21.4831	3.80084	3.13268	14.7292	0.77589	5.29883	4.96869	18.0384	0.31697	7.11707	22.4268	4.64526
3bd2fcee-5b16-49f3-a717-f1e106bbc12f	1.66256	0.17082	5.63325	13.5012	3.22976	2.37307	10.3545	0.63169	12.798	1.70274	12.7498	0.29278	19.0483	13.2616	0.75909
b8ac8500-abcd-40c4-9c24-7af557fd260a	2.09238	0.00811	7.67886	14.376	5.19844	1.58555	11.5839	0.85423	26.8271	12.024	18.262	0.0732	15.4085	11.0974	4.78398
9283709d-d019-4291-a097-611a5c05244b	2.33546	0.68925	14.0012	20.1846	2.78142	2.43215	8.48448	0.86751	23.2653	7.96438	17.5741	0.40518	10.2379	11.8481	1.96933
396ccdcb-40eb-4299-8575-b26753054434	2.12143	1.45632	10.7211	12.7856	1.27045	2.64604	4.31576	0.93456	9.06528	3.44888	14.309	0.42199	7.31937	10.7604	1.81364
90a8d2cb-1f31-45f9-8575-eed3cb9a7798	2.93208	0.12705	9.92367	17.8288	2.48961	3.07552	7.76909	0.87571	1.60292	7.36754	14.6324	0.32048	10.9787	11.4726	5.64574
df37eed0-ea63-485d-a831-28d3061852bd	1.68511	0.02591	10.5131	19.4073	2.12943	3.49532	16.4769	0.94224	6.88763	2.17426	19.2973	1.58082	10.9488	9.93203	1.84522
10593e5b-7acb-498b-bdf7-705e3e2bc443	0.24609	0.0075	2.19113	14.3709	2.90103	2.75402	6.93419	0.29445	8.23787	1.41047	6.84408	0.17016	6.31998	11.7849	0.58485

Supplementary Table 4. RNA sequencing revealed the expression of 16 *N*-linked glycosyltransferase in PD-L2 overexpression cells compared to the control cells (Related to supplemental figure 5B)

Gene_ID	Gene_name	Log2FC	*P* value
ENSG00000213614	HEXA	0.719369396	1.73E-08
ENSG00000086062	B4GALT1	0.603475124	0.002689879
ENSG00000104774	MAN2B1	0.586699342	0.178050062
ENSG00000177191	B3GNT8	0.552771466	0.21114345
ENSG00000179163	FUCA1	0.506717284	0.080456431
ENSG00000196968	FUT11	0.319348886	0.045714148
ENSG00000167889	MGAT5B	0.353358629	0.012103315
ENSG00000033170	FUT8	0.394192744	6.37E-07
ENSG00000109323	MANBA	0.413757055	0.040504746
ENSG00000167889	MGAT5	0.394192744	0.012103315
ENSG00000128268	MGAT3	0.2224182	0.068201423
ENSG00000111885	MAN1A1	0.183144943	0.005996254
ENSG00000134109	EDEM1	0.138106781	0.057009002
ENSG00000163521	GLB1	0.130131352	0.001719166
ENSG00000179913	B3GNT3	0.092059765	0.008080418
ENSG00000117643	MAN1C1	0.003980993	0.039809928

Xu Y, *et al. J Immunother Cancer* 2022; 9:e002699. doi: 10.1136/jitc-2021-002699