**Supplementary Methods**

***Patient series***

Tumor microarrays (TMAs) were constructed from 354 resected stage 1-4 esophageal adenocarcinomas. All patients underwent esophagectomy without neoadjuvant treatment. Up to three 0.6 mm cores were obtained from each tumor. The number of cores varied per tumor and was dependent on tumor size; approximately 75% of cases were represented by 3 cores. Detailed clinical annotation was performed by trained data abstractors for relevant demographic, comorbidity, tumor pathology and stage, and recurrence and survival variables as described previously (1) Table 1.

The studies were reviewed and approved by the Institutional Review Boards for both institutions.

***Immunohistochemistry***

Anti-PD-1 (clone EH33), anti-PD-L1 (clone 405.9A11) and anti-PD-L2 (clone 366C.9E5) antibodies are mouse monoclonal antibodies from Dr Gordon Freeman’s lab, Dana-Farber Cancer Institute. 405.9A11 recognizes an epitope in the PD-L1 cytoplasmic domain and reactivity confirms the expression of full-length PD-L1 protein. 366C.9E5 recognizes an epitope in the IgV domain of PD-L2. PD-L2, PD-L1, and PD-L1/CD68 double staining were performed as previously described (9,10). For PD-1 immunohistochemical staining, 4-micrometer-thick sections were deparaffinized, rehydrated, and heated in a steamer for 30 minutes for antigen retrieval in citrate buffer pH 6.0 (Invitrogen, Carlsbad, CA). After cooling for 20 minutes, sections were incubated with peroxidase block (DAKO, Carpinteria, CA) for five minutes and serum-free protein block (DAKO) for 20 minutes. Slides were then incubated at room temperature for one hour with primary antibodies against PD-1 (clone EH33) diluted in Da Vinci green diluent (Biocare Medical, Concord, CA). For the secondary antibody, Envision anti-mouse HRP-labeled polymer (DAKO) was applied for 30 minutes. Immunoreactivity for PD-1 was detected in the tumor infiltrating lymphocytes (TIL)s. Positive TIL cells were counted under 20x middle power field. For the TMA slides, all positive TILs in each core were counted whereas five representative areas in each slide with whole tissue were chosen to count, and the average absolute number was recorded.

PD-L1 (clone 405.9A11) staining was observed in cytoplasm and membranes of tumor cells and immune cells and was considered positive if ≥5% of tumor cells had membranous staining or any positive immune cells. PD-L2 (clone 366C.9E5) staining was observed in cytoplasm and membranes of tumor cells and was scored based on the number of positive cores and staining intensity. A tumor core was considered PD-L2 positive when >50% of tumor cells showed membranous and/or cytoplasmic immunoreactivity. The intensity was scored as follows: 0, negative; 1, weak; 2, moderate; 3, strong. For whole-tissue slides, the percentage of positively stained tumor cells for these two antibodies were assessed. The cutoff of PD-L2 positivity in tumor cells on whole-tissue slides is ≥ 10% and a moderate to strong (2+ and 3+) staining intensity.

*Double staining for PD-L1 and CD163*: After PD-L1 staining and washing, the slides were incubated with mouse monoclonal antibody anti-CD163 (10D6, 1:200, Neomarkers, Fremont, CA) diluted in Da Vinci diluent overnight in cold room. The secondary anti-mouse AP-linked antibody (A3562, DAKO) was used for 30 minutes and the slides were developed by liquid permanent red solution (K0640, DAKO).

All the slides were evaluated and scored by a pathologist (X.L.) blinded to clinical data. The scoring for each marker was performed at least twice with one-week interval.

***Cell lines***

The human esophageal adenocarcinomas cell lines OE19, OACM5.1C, ESO26, KYAE-1, and FLO-1 were purchased from the European Collection of cell cultures (ECACC) and have been authenticated by SNP arrays in 2012. MKN7 cells were purchased from the Broad Institute and characterized within the Cancer Cell Line Encyclopedia (CCLE) project. OE33 was purchased from the Sigma (St. Louis, MO). Only low-passage frozen cell aliquots of original stocks were used for further experiments and therefore cell lines were not revalidated.

Cell lines OE19, OACM5.1C, ESO26, OE33, KYAE-1, and MKN7 were cultured in RPMI-1640 and FLO-1 in DMEM both supplemented with 10% heat-inactivated FBS, 100 U/mL penicillin, 100 mg/mL streptomycin, and 10 mmol/L HEPES.

*FFPE cell pellets* were generated by harvesting 107 cells with PBS/EDTA, washing, and fixing in 2 mL 10% formalin at room temperature for 20 minutes. Cells were then washed, suspended in matrigel, processed, embedded in paraffin as a cell pellet tissue microarray, and cut onto glass slides as per standard histologic procedures.

*IL-4 and IL-13 treatment*: Cells were plated in 6-well plates and stimulated with human recombinant IL-4 (20 ng/ml; BD Pharmingen, #554605) and human recombinant IL-13 (20 ng/ml; Peprotech, #200-13) or with medium for 4, 8, and 24 hours. STAT6 expression and STAT6 phosphorylation were determined by Western blot. Cells were washed twice with PBS and lysed using nuclear and cytoplasmic extraction reagents (NE-PER, Thermo Scientific, #78833). Membranes were incubated with one of the following antibodies: mouse monoclonal anti-pSTAT6 (pY641, 1:500; BD Biosciences; Pharmingen), rabbit polyclonal anti-STAT6 antibody (S-20 1:500; Santa Cruz Biotechnology Inc.), or mouse monoclonal anti–β-actin (1:10,000; Sigma-Aldrich). When necessary, membranes were stripped in 0.2 M glycine/1% w/v sodium dodecyl sulfate, pH 2.5, blocked, and reprobed.

*STAT6 knockdown*: Transfection of siRNA against STAT6 was performed using siRNA for the STAT6 gene (ON-TARGETplus SMARTPOOL, L-006690-00-0005). Non-targeting control siRNA was similarly obtained from Dharmacon (ON-TARGETplus Non-Targeting Pool D-001810-10-05). Cells were plated in a 6-well plate and transfected at a density of 2x 105 per well and transfected on day 2 with 40 nM siRNA (OE33) or 20 nM siRNA (MKN7) following complexation with 7.5 μl Lipofectamine RNAiMAX (Invitrogen, #13778150), according to the manufacturer's instructions. Knockdown efficiency was assessed at the protein level by Western blot analysis. Cells were lysed in ice-cold RIPA buffer containing phosphatase and protease inhibitors. PD-L2 expression was assessed by qRT-PCR and flow cytometry.

***Real-Time PCR***

Total RNA was extracted using the RNeasy mini kit (QIAGEN; #) following the manufacturer’s instructions. Possible genomic DNA contaminants were removed by DNase treatment with the RNase-free DNase set (Qiagen). Complementary DNA synthesis was performed using the

iScript complementary DNA synthesis kit (Bio-Rad, Richmond, CA). Quantitative reverse transcription–PCR was performed using SYBR Green PCR master mix and the following primers: PD-L2\_forward: 5’-GGCAGAAACTTCAGCTGTGTG-3’, PD-L2\_reverse: 5’-GGTCCTGGGTTCCATCTGAC-3’, STAT6\_forward: 5’-GGCATCTTCTGGGTGACTGG-3’, STAT6\_reverse: 5’-GGCATCTTCTGGGTGACTGG-3’, CYCLOPHILIN\_forward: 5’-ctcgaataagtttgacttgtgttt-3’, CYCLOPHILIN\_reverse: 5’-Ctaggcatgggagggaaca-3’. Triplicates were run for each sample. CYCLOPHILIN was used as internal control, and the ΔΔCT method was used to calculate relative mRNA levels.

***Flow cytometry***

*Cell lines*: Adherent cells were treated for 4-20 minutes with EDTA and harvested for flow cytometry. For PD-L2 expression analysis, cells were stained with anti-human PD-L2 antibody (clone 24F.10C12 PE, Biolegend, #329605) and isotype control mouse IgG2a (clone MOPC-173, PE, BioLegend, #400213).

*Xenografts*. Freshly biopsied EAC were implanted into the flank of a nude mouse and subsequently propagated. PD-L2 expression was assessed in the primary tumor and in the tumor grown in the xenograft by IHC and western blot (anti PD-L2, clone 366C.9E5, 1:2500 dilution). Two out of 4 xenografts had PD-L2 expression in more than 20% of cells in both primary cancer tissues and the xenograft and used for further analyses. At the time of xenograft passage mice were sacrificed, and blood was collected through cardiac puncture. Tumors were cut into pieces and incubated in collagenase-containing buffer: 100 U/mL of collagenase type IV (Invitrogen), 50 μg/mL of DNase I (Roche), and 10% FBS in RPMI-1640 medium for 45 minutes. After incubation, cells were treated with red blood cell (RBC) lysis buffer and passed through a cell strainer to remove debris. The cell pellet was resuspended in 2% fetal calf serum in PBS and used for flow cytometry analysis. Isolated cells were stained with the LIVE/DEAD Fixable Dead Cell Stain Kit (Invitrogen). Cells were subsequently stained with anti–PD-L2 antibody, PE isotype control, and anti-EpCAM (clone 9C4, Pacific Blue, Biolegend, #324218).

Flow cytometry was performed with a BD FACSCanto II flow cytometer equipped with Diva software (BD Biosciences). The final analysis and graphical output were performed using FlowJo (TreeStar).

***Statistical analyses***

All continuous data are presented as mean + SEM and categorical data as proportions. Data were stratified by expression status for PD-1, PD-L1, PD-L2 and PD-1 co-expression with PD-L1 and PD-L2, and associations with patient demographics, comorbidity and tumor pathology were analyzed using two-tailed Student t-test and Pearson’s chi-2 or Fisher’s Exact test, where appropriate. Overall survival curves were estimated by the Kaplan-Meier method, stratified by PD-1, PD-L1, PD-L2 and PD-1 coexpression with PD-L1 and PD-L2 and compared by means of the log-rank test. Multivariate Cox proportional hazards analysis was used to control for other significant clinical prognostic factors. All *p-*values are two sided, and a *p*-value <0.05 was considered statistically significant. Analysis was performed using SPSS (version 20) and STATA 13.

Reference

25. Davison JM, Ellis ST, Foxwell TJ, Luketich JD, Gibson MK, Kuan SF, et al. MUC2 expression is an adverse prognostic factor in superficial gastroesophageal adenocarcinomas. Human pathology. 2014;45:540-8.