Supplementary Material and Methods

PD-L2 Immunohistochemistry

Rehydrated paraffin embedded tissue sections were boiled in EDTA buffer pH 8 (Life Technologies, Frederick, MD, USA) with a pressure cooker (Biocare Medical, Pacheco, CA, USA) for 30 seconds at 125°C. After cooling down at room temperature (RT), tissue sections were successively incubated with a peroxidase block (Dual Endogenous Enzyme Block, Agilent) and a protein block (Serum Free Block, Agilent) for 10 minutes each. Sections were next incubated for 1 hour at RT with the rabbit anti-PD-L2 antibody (1/50, clone D7U8C, Cell Signaling Technology, Danvers, MA, USA) diluted in Antibody Diluent with Background Reducing Components (Agilent). Tissue sections were then incubated with a mouse monoclonal anti-rabbit antibody (1/100, clone MR12/53, Agilent) for 30 minutes at RT followed by an incubation of 30 minutes at RT with EnVision anti-rabbit horseradish (HRP)-conjugated antibody (Agilent). HRP visualization was performed by applying 3,3-diaminobenzidine substrate (DAB+, Agilent) for 4 minutes. Between each step, tissue sections were washed for 5 minutes in washing buffer (0.1 mM Tris, pH7.4 + 0.05% Tween 20). Nuclei were counterstained with hematoxylin.

Multiplex Immunofluorescence Staining

Rehydrated paraffin embedded tissue sections were boiled in EDTA buffer pH 8 (Life Technologies) with a pressure cooker (Biocare Medical) for 30 seconds at 125°C. After cooling down at RT, tissue sections were successively incubated with a peroxidase block (Dual Endogenous Enzyme Block, Agilent) and a protein block (Serum Free Block, Agilent) for 10 minutes and 15 minutes respectively. Sections were next incubated for 1 hour at RT with the primary antibody diluted in Antibody Diluent with Background Reducing Components (Agilent). Tissue sections were then incubated with EnVision horseradish (HRP)-conjugated antibody (Agilent) for 10 minutes at RT. HRP-mediated visualization was performed by applying tyramide-conjugated-Opal/fluorochrome working solution for 5 minutes (Perkin Elmer,

Waltham, MA, USA). Between each step, tissue sections were washed for 5 minutes in wash buffer (0.1 mM Tris, pH7.4 + 0.05% Tween 20). Primary and secondary antibodies were then stripped, allowing for another round of staining, by boiling the slides in AR6 buffer (Perkin Elmer) using a 1300W microwave oven (Panasonic, Kadoma, Osaka, Japan) for 5 minutes at a power level of 2. After cooling down at RT, tissue sections were incubated with the protein block and another round of staining procedure was repeated. Finally, nuclei were counterstained with DAPI (Perkin Elmer) for 5 min at RT and tissue sections were mounted in Prolong Gold antifade mountant (Life Technologies).

Transcriptome capture

Total RNA was assessed for quality using the Caliper LabChip GX2 (Perkin Elmer). The percentage of fragments with a size greater than 200nt (DV200) was calculated using software. An aliquot of 200ng of RNA was used as the input for first strand cDNA synthesis using TruSeq RNA Access Library Prep Kit (Illumina, San Diego, California, USA). Synthesis of the second strand of cDNA was followed by indexed adapter ligation. Subsequent PCR amplification enriched for adapted fragments. The amplified libraries were quantified using an automated PicoGreen assay (Life Technologies). 200ng of each cDNA library, not including controls, were combined into 4-plex pools. Capture probes that target the exome were added and hybridized for recommended time. Following hybridization, streptavidin magnetic beads were used to capture the library-bound probes from the previous step. Two wash steps effectively remove any nonspecifically bound products. These same hybridization, capture and wash steps are repeated to assure high specificity. A second round of amplification enriches the captured libraries. After enrichment, the libraries were quantified with qPCR using the KAPA Library Quantification Kit for Illumina Sequencing Platforms and then pooled equimolarly. The entire process is in 96-well format and all pipetting is done by either Bravo (Agilent) or Starlet (Hamilton, Reno, Nevada, USA). Pooled libraries were normalized to 2nM and denatured using 0.1 N NaOH prior to sequencing. Flowcell cluster amplification and sequencing were performed according to the manufacturer's protocols using HiSeq 2000 or 2500 (Illumina). Each run was a 76bp paired-end with an eight-base index barcode read. Data was analyzed using the Broad Picard Pipeline which includes de-multiplexing and data aggregation (http://broadinstitute.github.io/picard/). RNA sequencing data was then aligned to the reference human genome using STAR (1). Sample

quality was assessed using RNA-SeQC, and samples with fewer than 10,000 transcripts detected or fewer than 20M total purity filtered reads were excluded from downstream analysis (2). For samples that passed these the quality control thresholds, RSEM was used to quantify the expression of each transcript, expressed as transcripts per million (TPM) (3).

References Supplementary Materials and Methods

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3. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics. 2011 Aug 4;12:323.