Supplementary Methods:

Patients and tumor samples

In this study, we included 11 SCCOHT cases (Supplementary Table 1, available online), of which 5 (cases 101, 102, 103, 107 and 114) have been previously reviewed and analyzed for *SMARCA4* mutations [1, 2]. Using previously described guidelines [1], specialty gynecologic pathologists reviewed new cases — 115, 116, 117, 118, 120 and 121 — to confirm the diagnosis of SCCOHT. Clinical data collection was limited to only age due to the Health Insurance Portability and Accountability Act regulations. Specific case details were collected directly from involved patients and deemed to be IRB-exempt.

MSK-IMPACT Assay and Sequencing

For the purposes of the MSK-IMPACT assay, DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tumors with at least 50% tumor cell nuclei according to standard protocols (DNeasy Blood&Tissue kit; Qiagen #69506). The assay was performed as previously described [2]. Briefly, barcoded sequence libraries were prepared using 250 ng of genomic DNA (Kapa Biosystems, Wilmington, MA, USA) and combined into a single equimolar pool. The captured pool was subsequently sequenced on an Illumina HiSeq 2500 sequencer (Illumina, San Diego, CA, USA) as paired-end 100-base pair reads, producing at least 250-fold coverage per tumor. A panel of 341 known oncogenes and tumor-suppressor genes frequently altered in cancer were sequenced.

Immunostaining

All assays were performed on formalin-fixed paraffin-embedded slides. Immunohistochemistry staining for SMARCA4 was performed as previously described [1]. Immunofluorescent staining for human CD3 (rabbit polyclonal, DAKO #A0452; 1.2 µg/ml), CD68 (mouse clone KP1, DAKO #M0814; 0.02 µg/ml), and PD-L1 (rabbit clone E1L3N, Cell Signaling Technology #13684; 1.67 µg/ml) were performed with an automated Leica Bond RX processor using a published protocol [3]. Slides were digitized using Pannoramic Flash scanner (3DHistech). Quantification of CD3, CD68, and PD-L1 positive cells was performed by selection of 10 random high power fields (HPFs) in each sample. The random HPFs were converted to 8-bit images and the areas of each signal (DAPI, A488, A594 or A568, and overlapping A488 and A594 or A568) were calculated for each field using ImageJ software. Multiplier constants specific for each stain (CD3, CD68, PD-L1 and double positive CD68 and PD-L1) were used to correct for differences in each signal area compared to that of DAPI for a single cell. These were calculated by dividing the total signal area by the average DAPI area per cell. The estimated cell counts were validated by manual counts by two independent evaluators, including one pathologist, in at least 20 random HPFs from all samples. Percent positive signal was calculated by multiplying the total signal area by the appropriate multiplier constant and then dividing by the total DAPI area within each field. Estimated positive cell counts were calculated by multiplying the total signal area by the appropriate multiplier constant and then dividing by the average DAPI area per cell.

Gene expression profiling

We performed gene expression analysis using the NanoString's nCounter PanCancer Immuno Profiling Panel. RNA was extracted from the FFPE samples using

the RecoverAll Total Nucleic Acid Isolation Kit (Ambion; #AM1975). Quality check (QC) was performed for all RNA samples on an Agilent's 2100 Bioanalyzer. The amount of RNA used for the Immuno Profiling Panel analysis was adjusted according to RNA's QC score. We stratified the SCCOHT cases into two groups; the four highest (101, 102, 117 and 121) versus four lowest (114, 115, 116 and 118) PD-L1-expressing cases. Given the limited number of cases, we excluded the two cases in-between to allow for a more meaningful comparison of the extremes. The cut-offs we set for High-PD-L1 and Low-PD-L1 cases resulted in ≥ 40 and ≤ 20 positive cells per HPF, respectively.

Using the NanoString's nSolver 3.0 software, the data were analyzed following the standard protocol. Prior to normalization, the processed samples were checked for quality using the standard QC protocol. Background subtraction was done following the standard protocol using 8 negative controls. Geometric mean of 40 housekeeping genes was used for normalization. All data, except for volcano plots, were generated by Human PanCancer Immune Profiling Advanced Analysis Software (version 1.0.36). For the statistical analysis, we used pre-defined Benjamini-Yekutieli statistical method (p-value threshold 0.05). The cell type scores are calculated as the average of the log2 expression of all the genes used in a cell type's signature.

Supplementary Case Details:

Patient 1

A 29yo was diagnosed with a large ovarian tumor that was completely resected. She received initial treatment with bleomycin/etoposide/cisplatin. She was then disease-free for ~1.5 years and suffered a recurrence in the abdomen and pelvis that was treated with investigational therapy with disease progression in less than 3 months. She was then treated with local radiation and pembrolizumab. She remains on pembrolizumab and continues to have a sustained partial response for 6 months.

Patient 2

A 22yo was diagnosed with an ovarian tumor completely resected. She did not receive and initial adjuvant treatment and remained disease-free for one year when a recurrence was found in the abdomen and pelvis. She was treated with etoposide/cisplatin followed by surgical resection and then platinum/taxane therapy followed by abdominal RT. She remained disease-free for 9 months and suffered an upper abdominal recurrence treated with RT. She then went on an investigational vaccine study and recurred 12 months later. She started nivolumab and remains disease-free for more than 1.5 years.

Patient 3

A 25yo was diagnosed with an ovarian mass that was completely resected. She received initial treatment with doxorubicin / cyclophosphamide /etoposide/cisplatin followed by RT. She was then disease-free for ~1 year and suffered a recurrence in the abdomen. This was surgically resected and taxane/platinum adjuvant therapy was given with concurrent RT. She remained disease-free for six months when she suffered a

recurrence in the same area that was treated with additional RT followed by nivolumab. She remains disease free for ~1.5years.

Patient 4

An 18yo was diagnosed with a large ovarian tumor that was completely resected. She received initial treatment with etoposide/platinum followed by chemoRT to the abdomen. She was then disease-free for ~3 years and suffered a recurrence in the chest that was treated with PARP inhibition followed by retreatment with etoposide/platinum, an investigational agent, local radiation and then two cycles of nivolumab. Nivolumab was stopped due to an exacerbation of rheumatoid arthritis and she remains off treatment for more than 1.5 years without any evidence of disease progression.

Supplementary Figure Legends

Supplementary Figure 1. T cell and macrophage infiltration and PD-L1 expression in SCCOHT. **A)** Two representative SCCOHT cases, 102 and 117, showing H&E staining (left) and PD-L1 IHC single stains (right). Inset shows higher power (400X). **B)** IF staining with PD-L1, CD3 and CD68 antibodies for 8 SCCOHT cases. **Scale bars** = 50μm. IHC = immunohistochemistry; IF = immunofluorescence.

Supplementary Figure 2. PD-L1 expression correlates with T cell infiltration in SCCOHT. **A)** Pearson correlations of PD-L1 and CD3 expression in SCCOHT cases determined by IF. The y-axes and x-axes represent number of CD3- and PD-L1-positive cells per HPF, respectively. Each dot represents 1 HPF. B) Pearson correlation between CD3 and CD68 positive cells in SCCOHT cases. **C)** Pearson correlation between PD-L1 and CD68 expression in SCCOHT cases. **D)** T-cell subtype scores for High and Low PD-L1 subgroups. Boxplot center lines represent tumor medians, box limits are the inter-quartile range from 25% and 75%, whiskers represent the extent of tumors out to 1.5 times the inter-quartile range. Dots represent individual cases. All statistical tests were two-sided. IF = immunofluorescence; HPF=high-power field.

Supplementary Figure 3. The KEGG graph demonstrates that the T-cell receptor signaling pathway activity is elevated in High- versus Low-PD-L1-expressing SCCOHTs. Red boxes indicate over-expressed genes. Grey boxes indicate genes without differential expression. White boxes indicate genes without expression data. KEGG = Kyoto Encyclopedia of Genes and Genomes.

Supplementary Figure 4. T cell-related gene expression in tumor cell- vs. macrophage-predominant PD-L1 expressing tumors. Normalized gene expression obtained from Nanostring for the indicated genes is plotted. The legend indicates whether PD-L1 expression was predominantly seen on tumor cells (tumor) vs. macrophages (Mϕ) in both PD-L1 high and PD-L1- low groups. Statistical comparisons were performed using 2-way ANOVA with multiple comparisons with uncorrected Fisher's LSD test. All statistical tests were two-sided.

Supplementary Table 1. Summary of *SMARCA4* mutation and protein expression in SCCOHTs.

Case number	Age at diagnosis (years)	SMARCA4		
		Gene mutations		IHC
		Protein Change	Domain affected	
101*	40	p.Q1182_splice	Helicase	Loss
102*	22	p.K1390_splice		Loss
103*	19	In-frame Deletion	Helicase	Retained
107*	18	p.E1300_N1303.del		Loss
114*	27	p.X991_splice	SNF2	Loss
115	29	p.L792P and p.X587_splice	SNF2	Equivocal
116	30	p.X813_splice	SNF2	Equivocal
117	32	p.M649Nfs*6 and pE488*		Loss
118	N/A	N/A		Loss
120	31	p.X1072_splice	Helicase	Loss
121	24	p.X1318_splice		Equivocal

IHC (immunohistochemistry), SNF2 (sucrose nonfermenting)
*these data were previously reported [1, 2]

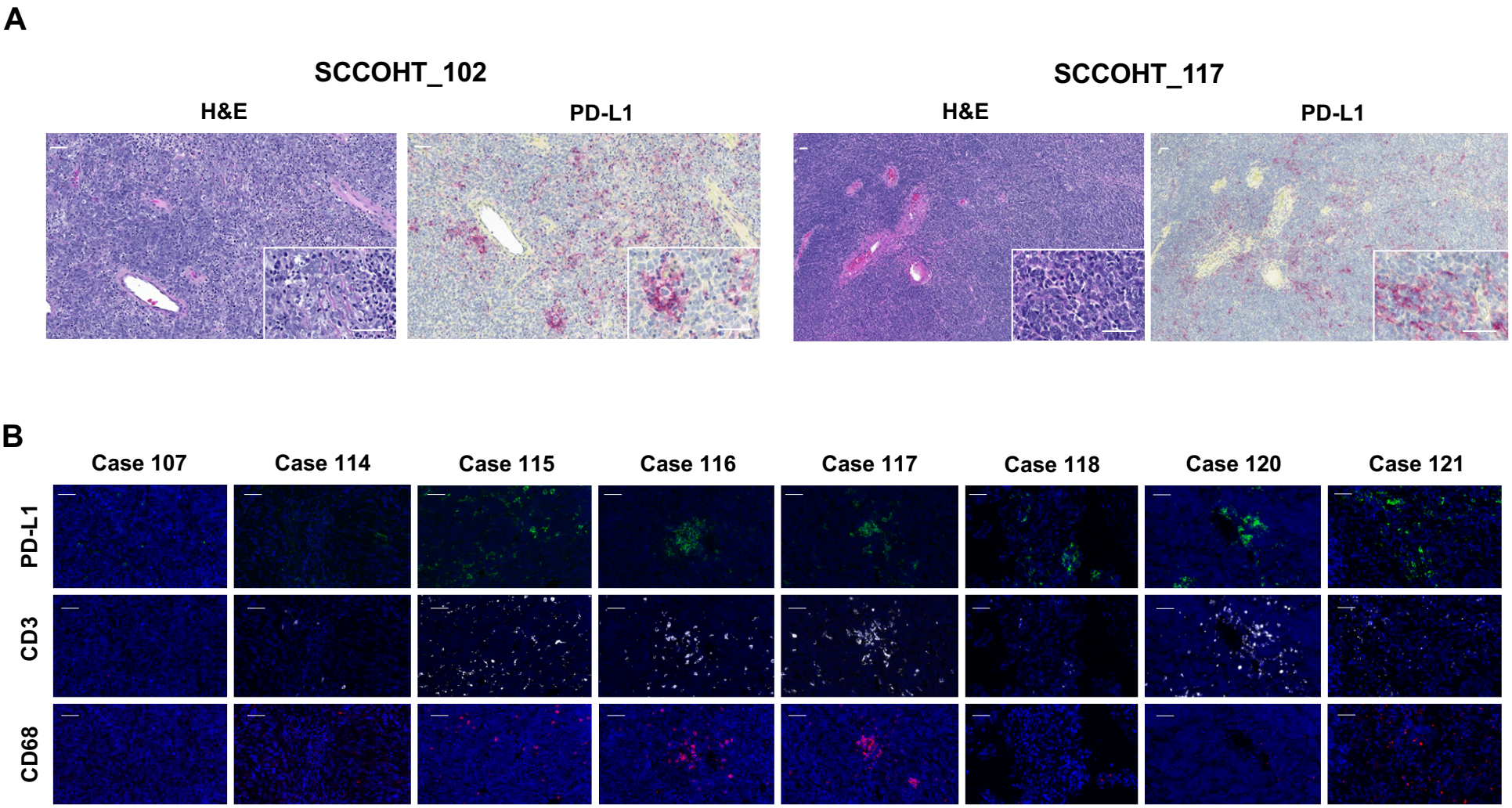
Supplementary Table 2. Differentially expressed genes in High- versus Low-PD-L1 expressing SCCOHTs. Cytotoxicity and T-cell Functions gene sets are in bold.

GENE	Fold change	p-value	Gene sets
PRF1	3.25	0.00076	**Cytotoxicity**, Pathogen Defense
NCR1	3.71	0.0032	Cell Functions, NK Cell Functions
TIGIT	2.79	0.0058	**T-Cell Functions**
CLEC4C	4.79	0.0060	
XCR1	2.00	0.0062	Chemokines
TNFRSF9	2.68	0.0065	TNF Superfamily
CXCL13	6.32	0.0097	Chemokines
MEF2C	-2.51	0.012	
CXCL9	3.03	0.015	Chemokines, Regulation, **T-Cell Functions**
CD8A	3.27	0.017	Antigen Processing, Pathogen Defense, **T-Cell Functions**
CXCR3	2.85	0.018	Chemokines, **T-Cell Functions**
IL12RB1	2.16	0.019	**T-Cell Functions**
CD27	4.92	0.019	B-Cell Functions
CD2	3.51	0.019	Leukocyte Functions, **T-Cell Functions**
IL6R	3.29	0.019	Cytokines
CTSW	3.01	0.021	Transporter Functions
GNLY	4.56	0.025	**Cytotoxicity**, Pathogen Defense
PDCD1	2.30	0.025	Regulation, **T-Cell Functions**
NLRC5	3.53	0.027	
GZMB	4.26	0.027	Cell Functions, **Cytotoxicity**
KLRK1	2.60	0.028	NK Cell Functions, Regulation
CCL5	3.41	0.028	Chemokines, Cytokines
ZAP70	2.83	0.029	**T-Cell Functions**
SLAMF7	2.60	0.030	
KLRD1	2.79	0.032	NK Cell Functions, Regulation
IDO1	3.32	0.033	Cytokines, **T-Cell Functions**
CD38	3.43	0.033	B-Cell Functions, Regulation
EWSR1	1.12	0.033	Cell Functions
SH2D1A	3.10	0.036	
GZMH	2.51	0.038	Cell Functions, **Cytotoxicity**
CD9	-2.08	0.039	
BATF	2.53	0.039	Cell Functions
CD3D	3.53	0.040	Regulation, **T-Cell Functions**
LCK	3.29	0.041	Regulation, **T-Cell Functions**
CD5	4.26	0.044	Regulation, **T-Cell Functions**
CD3E	3.12	0.045	**T-Cell Functions**
CD274	1.89	0.092	**T-Cell Functions**
IFNG	1.77	0.090	Cytokines, Interleukins, **T-Cell Functions**

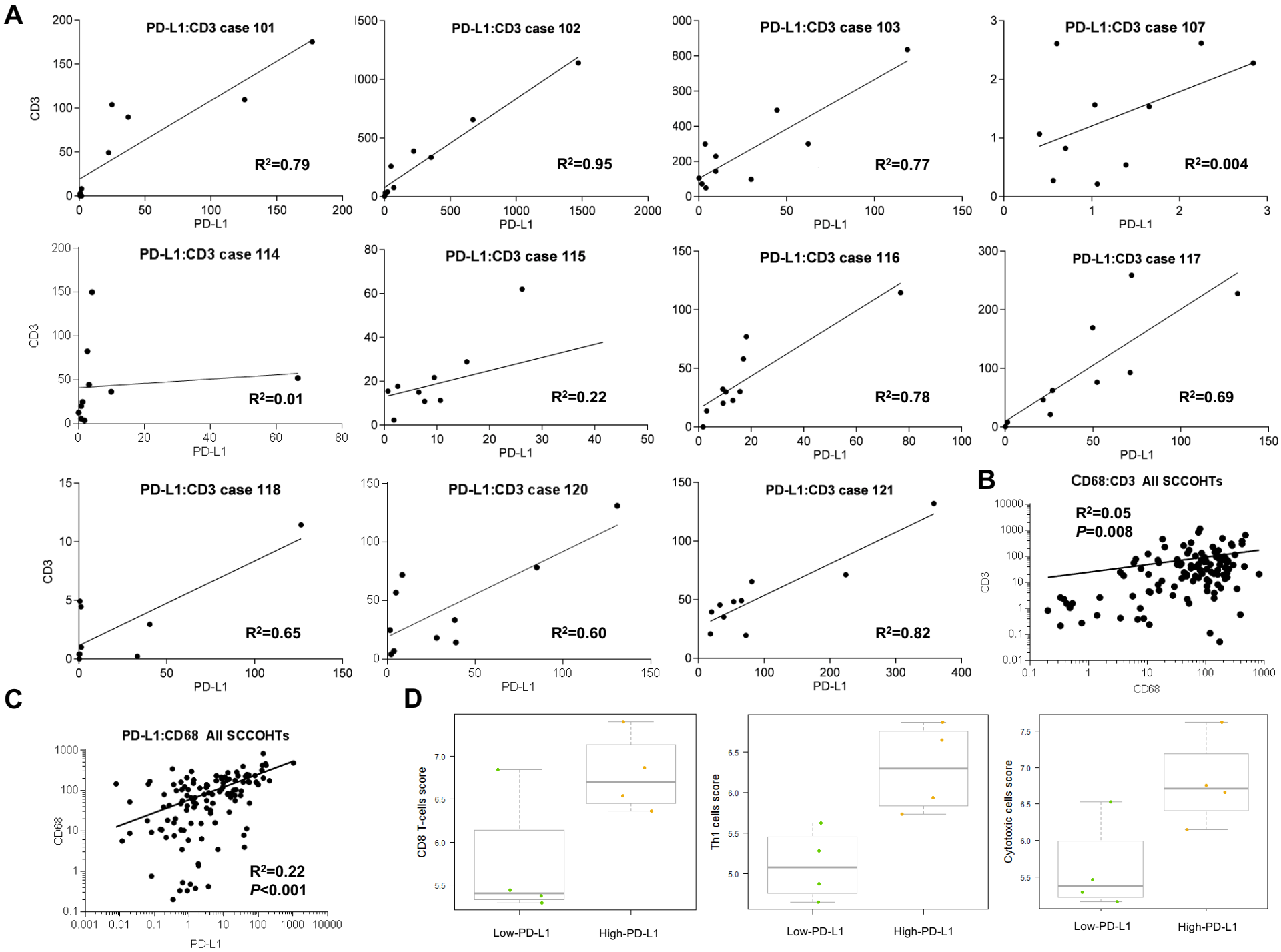
Supplementary References

1. Jelinic P, Mueller JJ, Olvera N*, et al.* Recurrent SMARCA4 mutations in small cell carcinoma of the ovary. Nat Genet 2014;46(5):424-6.
2. Jelinic P, Schlappe BA, Conlon N*, et al.* Concomitant loss of SMARCA2 and SMARCA4 expression in small cell carcinoma of the ovary, hypercalcemic type. Mod Pathol 2016;29(1):60-6.
3. Ricca J, Turkekul M, Barlas A*, et al.* Validation of Anti-Mouse PDL-1 Goat Polyclonal Antibody Staining with Mouse PDL-1 In Situ Hybridization on Adjacent Sections of Cell Pellets and Mouse Tumors. Methods Mol Biol 2017;1554:253-262.

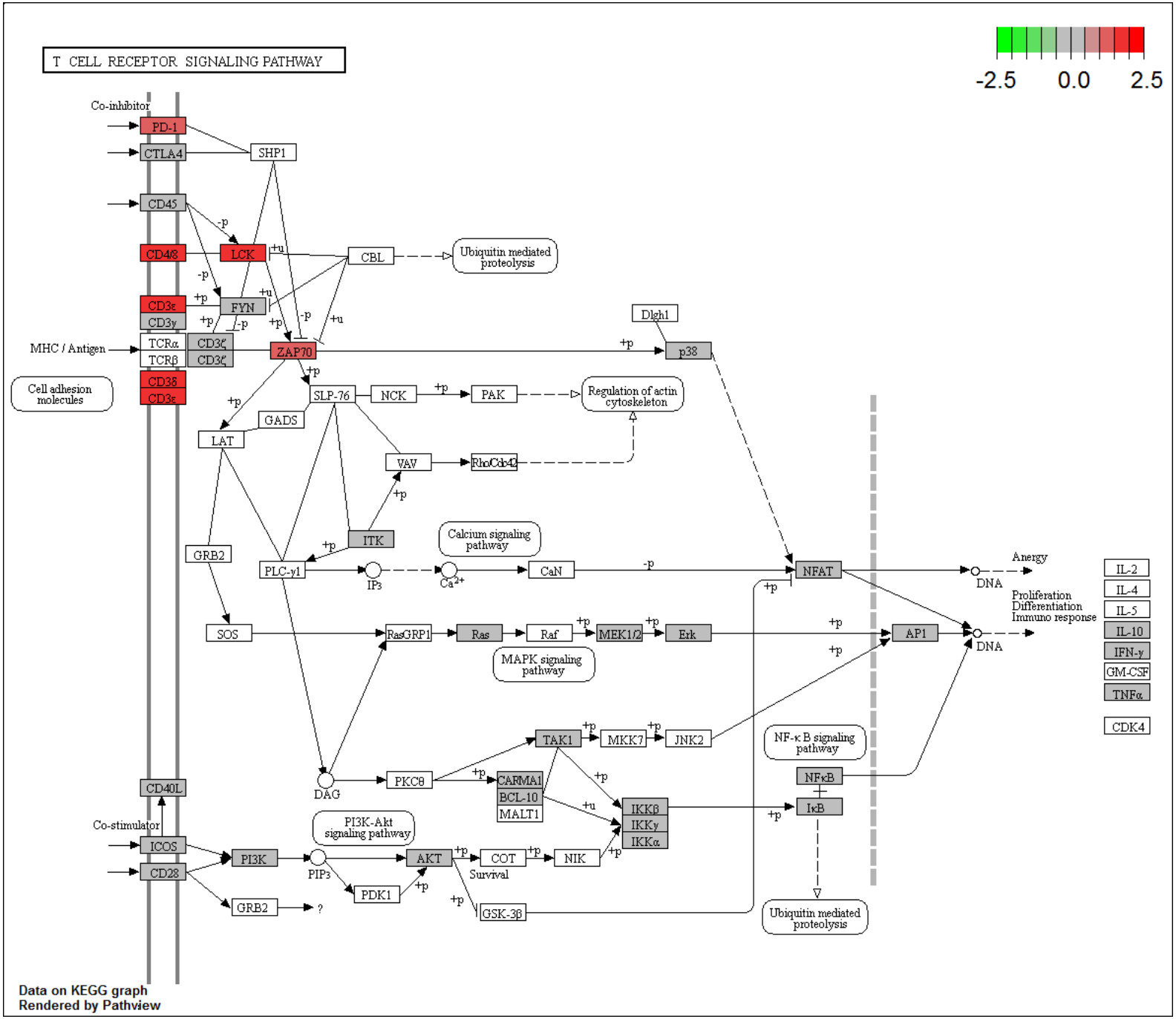
Supplementary Figure 1. T cell and macrophage infiltration and PD-L1 expression in SCCOHT



Supplementary Figure 2. PD-L1 expression correlates with T cell infiltration in SCCOHT.



Supplementary Figure 3. T-cell receptor signaling pathway activity is increased in high- versus low-PD-L1-expressing SCCOHTs.



Supplementary Figure 4. T cell-related gene expression in tumor cell- vs. macrophage-predominant PD-L1 expressing tumors.