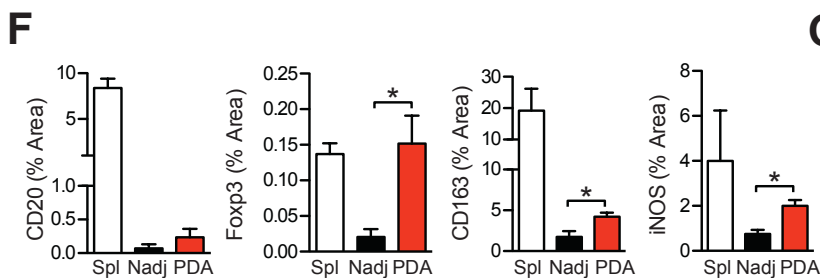
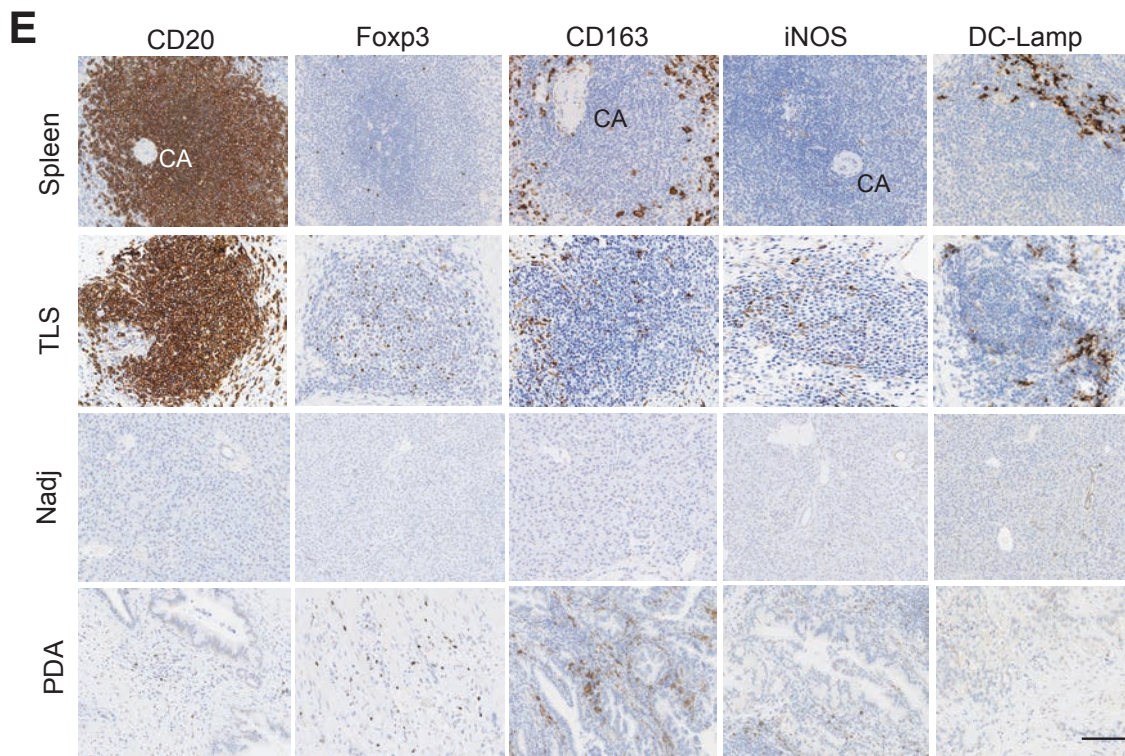
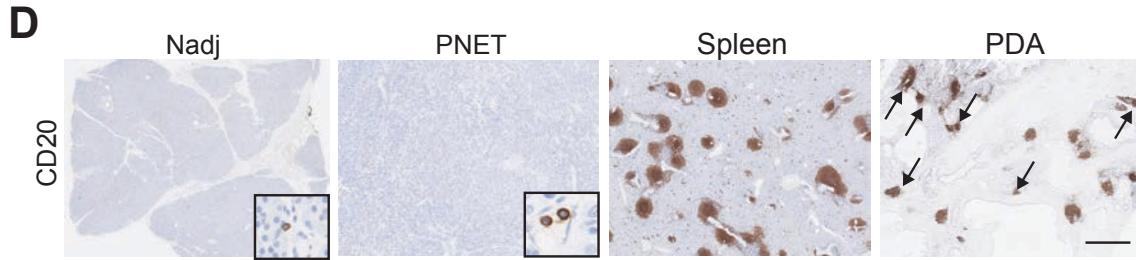
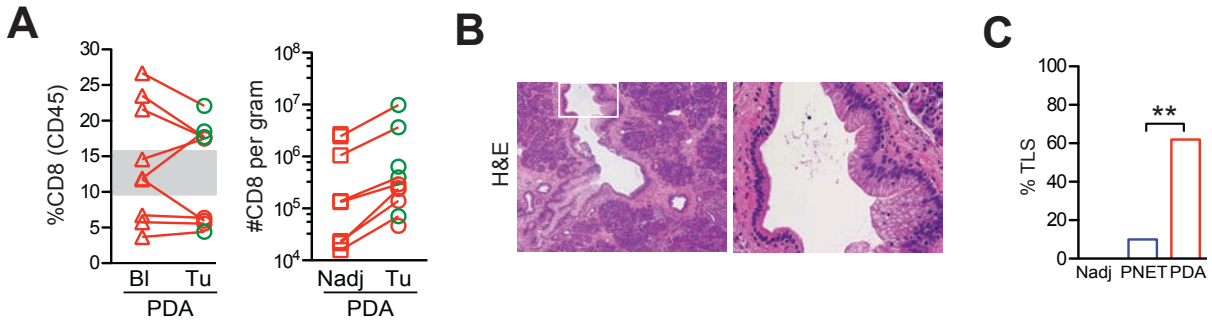
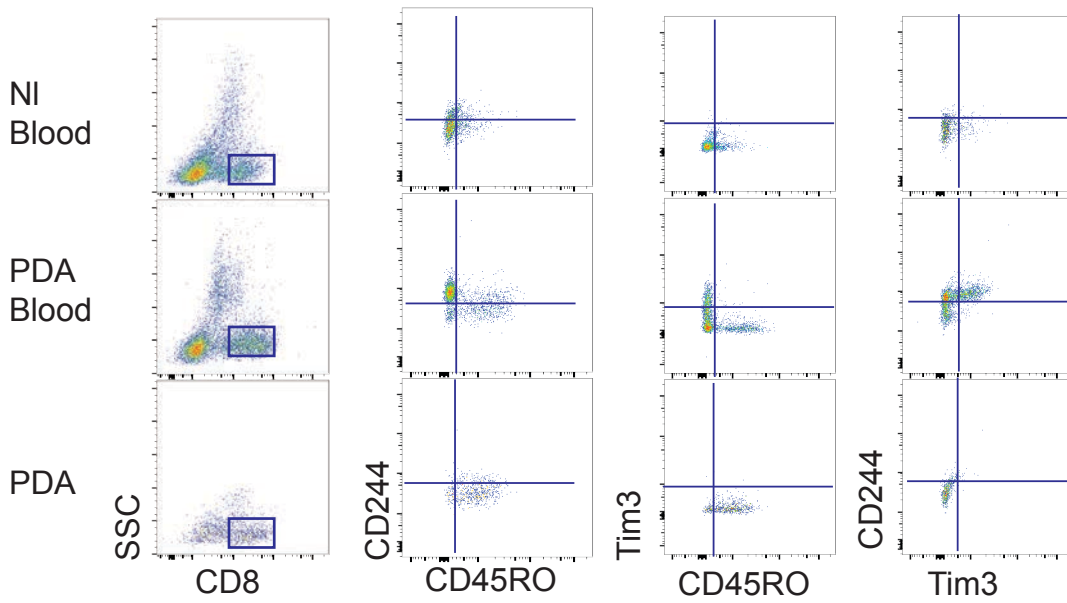


Supplementary Figure 1



Supplementary Figure 1. TLS composition and immune features of human PDA. **A**, CD8 T cell frequency and number was determined by flow cytometry. Green circles indicate TLS⁺ tumors. Grey box (left panel) demarcates T cell frequency range in blood that does not directly correlate with T cell frequency in tumors. **B**, Example of a preinvasive intraepithelial lesion in Nadj pancreas obtained from a resected patient sample. **C**, Frequency of TLS in Nadj pancreas, PNET and PDA specimens. **D**, CD20 staining of Nadj pancreas, PNET, spleen and PDA demarcate TLS. Inset, rare CD20⁺ B cells in Nadj pancreas and spleen. Arrows, TLS. Scale bar represents 100 μm. **E**, Cellular composition of lymphoid follicles in spleen and tumoral TLS. CA, central arteriole. Scale bar represents 25 μm. **F**, Quantification of IHC staining from Supplementary Figure 1E and was determined by digital analysis of scanned slides. Spl, spleen. **G**, Example of a TLS associated with cytokeratin (CK)⁺ tumor cells and surrounded by αSMA⁺ fibroblasts. Scale bar represents 25 μm.

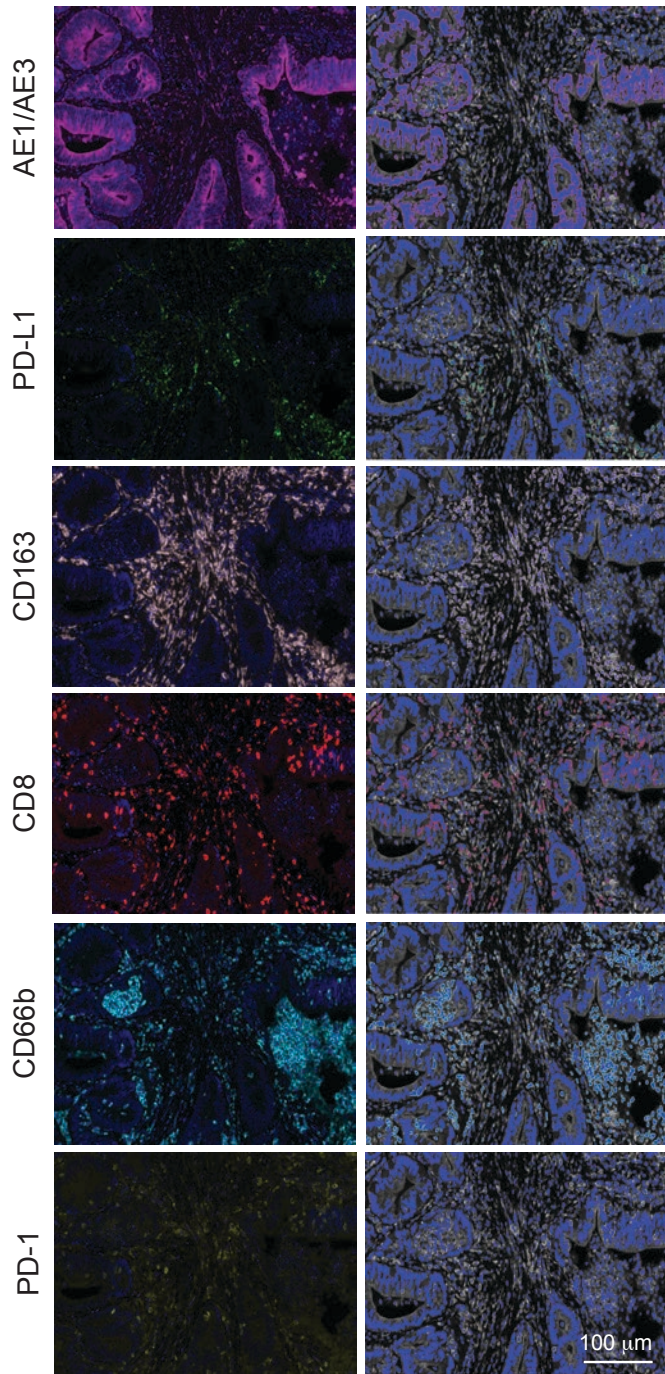
Supplementary Figure 2



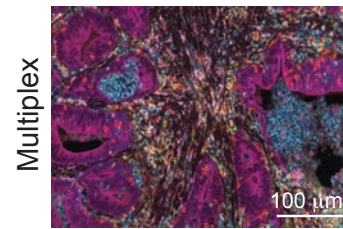
Supplementary Figure 2. Gating strategy for analyzing CD244 (2B4) and Tim3 expression on CD8 T cells in blood of normal individuals (NI), in blood of PDA patients (PDA blood) and in tumors. The far left panel is first gated on CD45⁺ cells. The right three panels are gated on the CD8⁺ T cell gate as shown.

Supplementary Figure 3

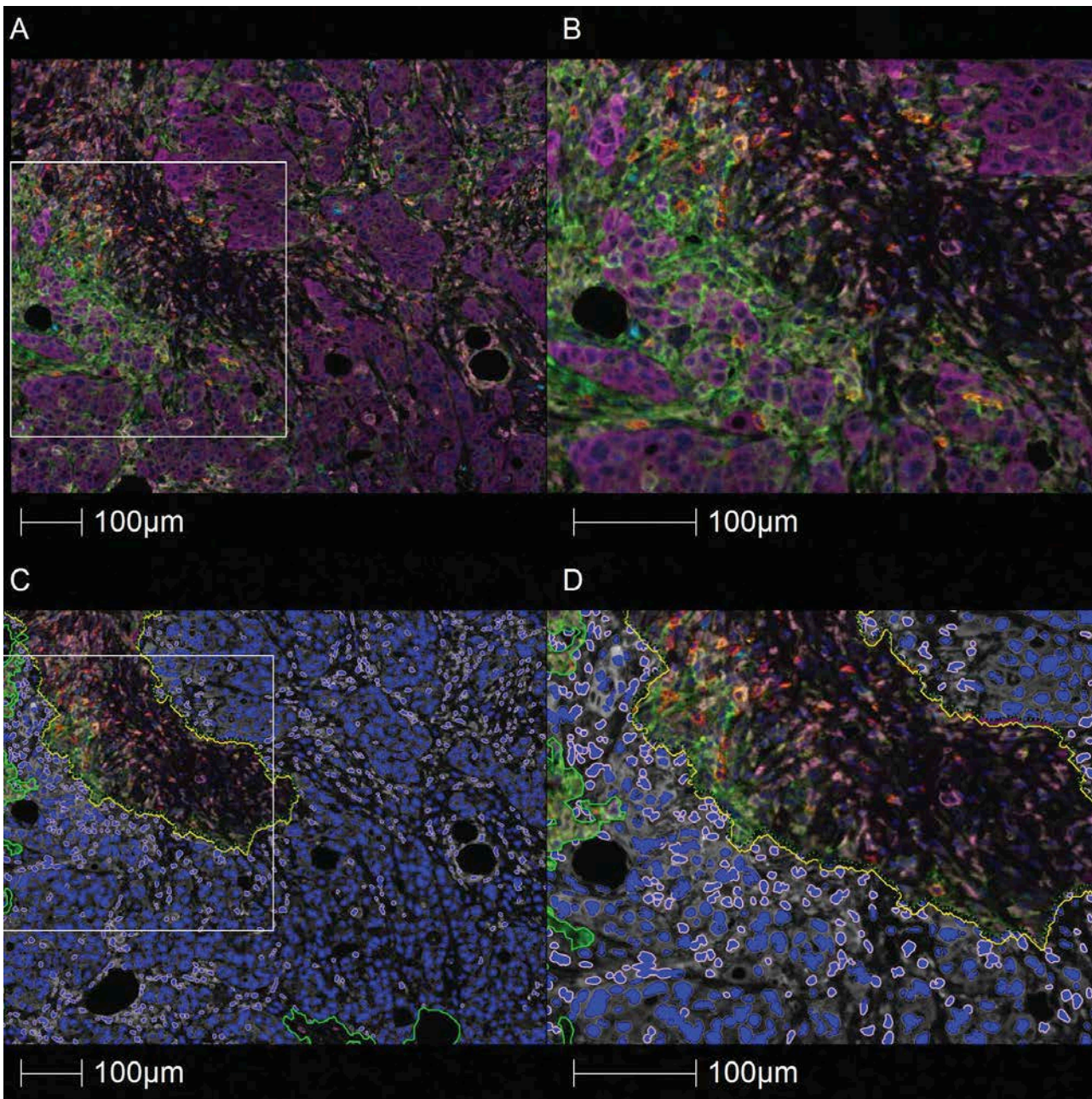
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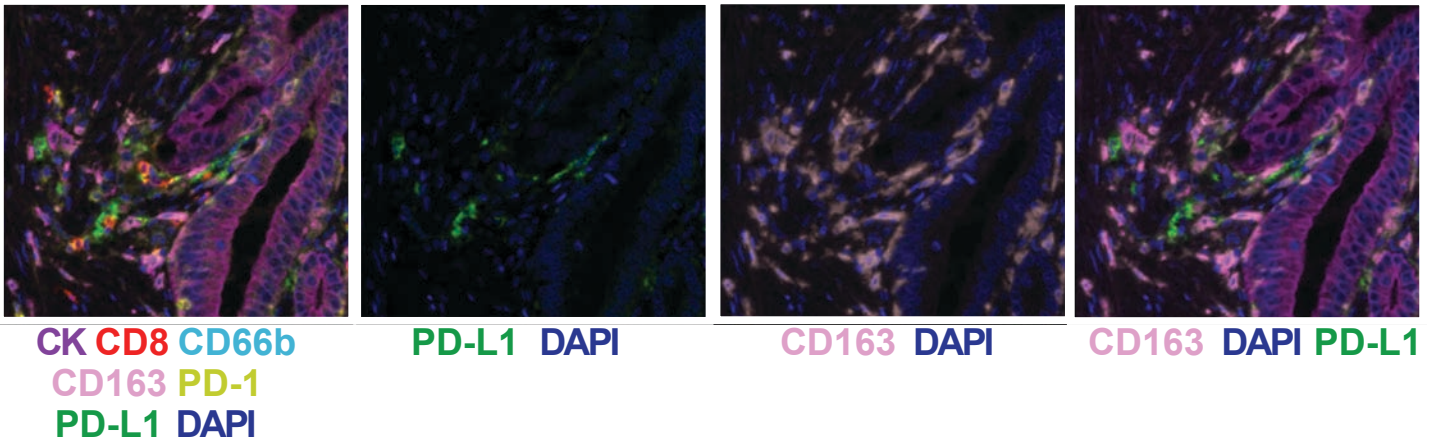
B



Supplementary Figure 3. Representative human PDA section stained with multiplex immunohistochemical panel. **A**, Individual antibody stains (left) and positive cell subsets outlined (right). **B**, Same image stamp from (A) with combined antibodies.



Supplementary Figure 4. Example of multiplex IHC analyses of human PDA using HALO software. Top row (**A, B**) shows representative staining of tumor cells (magenta), immune cells and PD-L1 (green). Bottom row (**C, D**) stromal and tumor regions outlined and cell numbers quantified. Tumor cell nuclei are shown in blue. Light pink outlines PD-L1⁺ cells.



Supplementary Figure 5. PD-L1⁺ stromal cells in PDA include CD163⁺ macrophages (Figure 6A) as well as an additional CD163⁻ cell subset shown here.

Supplementary Table 1. Antibodies used for tissue multiplex in Figure 6.

Position	Antibody	Clone/Host	Company	Concentration	OPAL Fluor
1	CD8	144B/Mouse	DAKO	0.2 µg/ml	540
2	CD66b	G10F5/Mouse	BD Biosciences	1.25 µg/ml	520
3	PD-1	D4W2J/Rabbit	Cell Signaling	0.06 µg/ml	570
4	PD-L1	E1L3N/Rabbit	Cell Signaling	0.5 µg/ml	620
5	CD163	EP324/Rabbit	Bio SB	0.25 µg/ml	650
6	AE1/AE3	AE1/AE3/Mouse	DAKO	0.34 µg/ml	690

Supplementary Table 2. Patient characteristics.

# ¹	Diagnosis	Differentiation Status/Grade	Stage	Neoadjuvant	Survival (Days)	Cause of death	TLS
PC-1	PDA	Moderate	T3N1	N	Alive	Alive	Y
PC-2	PDA	Poor	T1N1	N	864	PDA	Y
PC-3	PDA	Well	T3N0	N	ND ²	-	Y
PC-4	PDA	Poor	T3N1	N	478	Unknown	N
PC-5	PDA	Well	T3N0	N	ND	-	Y
PC-6	PDA	Moderate	T3N1	N	343	Metastatic angiosarcoma	N
PC-7	PDA	Well	T3N0	N	ND	-	N
PC-8	PDA	Well	T2N1	N	ND	-	N
PC-9	PDA	Poor	T3N0	N	702	Unknown	N
PC-10	PDA	Moderate	T3N1	N	696	PDA	Y
PC-11	PDA	Moderate	T3N1	N	1033	Unknown	Y
PC-12	PDA	Moderate	T3N1	N	626	Unknown	Y
PC-13	PDA	Moderate	T3N1	N	755	Unknown	Y
PC-14	PDA	Moderate	T3N0	N	ND	-	Y
PC-15	PDA	Moderate	T3N0	Y (Chemorads ³)	983	Unknown	N
PC-16	PDA	Moderate	T3N0	Y (Chemo ⁴)	ND	-	Y
PC-17	PDA	No PDA ⁵	NA ⁶	Y (Chemorads)	624	PDA	N
PC-18	PDA	Moderate	T0N1	Y (Folfinrox)	961	PDA	N
PC-19	PDA	Poor	T3N0	Y (Folfinrox)	ND	-	N
PC-20	PDA	Moderate	T2N0	Y (Chemorads ⁷)	ND	-	N
PC-21	PNET	High	T2N1	N	ND	-	N
PC-22	PNET	Low	T2N0	Y (Chemo ⁸)	Alive	Alive	N
PC-23	PNET	Low	T2N1	N	Alive	Alive	N
PC-24	PNET	Intermediate	T2N0	N	ND	-	N
PC-25	PNET	Low	T3N0	N	ND	-	N
PC-26	PNET	High ⁹	T3N1	N	ND	-	Y
PC-27	PNET	Low	T3N1	Y (Chemorads ¹⁰)	ND	-	N

¹Sample #PC1-PC9 correspond to the same tumors #PC1-PC9 as described in Supplementary Table 3

²ND, not determined

³Gemcitabine/Taxotere/Oxaliplatin/Capecitabine/XRT

⁴Gemcitabine/Taxotere/Capecitabine

⁵Pancreas with atrophy, fibrosis, dilated ducts, and residual islet cell islands

⁶NA, not applicable

⁷Folfinrox/Capecitabine/XRT

⁸Capecitabine /Temozolomide

⁹Mixed neuroendocrine and acinar features

¹⁰Capecitabine/XRT

Supplementary Table 3. TCR β CDR3 sequence data in blood and tumors from PDA patients.

Sample	Tissue	# Seq	# Unique	# Prod	# Unique	% Prod	Entropy	Clonality	Max Freq (%)
PC-1	Blood	22683	18915	17596	14718	77.6	13.56	0.02	0.69
PC-2	Blood	22616	16801	18317	13573	81.0	12.92	0.06	1.19
PC-3	Blood	34536	24286	28189	20166	81.6	12.64	0.12	7.91
PC-4	Blood	40341	19349	32245	15554	79.9	11.00	0.21	7.13
PC-5	Blood	55151	45949	45332	37807	82.2	14.84	0.02	0.42
PC-6	Blood	41850	35797	34637	29492	82.8	14.69	0.01	0.20
PC-7	Blood	257543	211787	205772	167410	79.9	16.23	0.06	3.13
PC-1	Tumor	9369	4572	7069	3402	75.5	10.49	0.11	2.31
PC-2	Tumor	8782	4069	7126	3264	81.1	10.31	0.12	2.74
PC-3	Tumor	1149	922	873	708	76.0	9.13	0.04	4.26
PC-4	Tumor	11053	8100	8705	6443	78.8	12.06	0.05	1.44
PC-5	Tumor	13490	7696	11145	6252	82.6	11.66	0.08	1.16
PC-6	Tumor	23728	11148	20217	9237	85.2	11.73	0.11	3.22
PC-7	Tumor	8095	5418	6226	4152	76.9	11.28	0.06	1.54
PC-8	Tumor	86957	14772	69736	11968	80.2	9.44	0.30	8.53
PC-9	Tumor	22738	7735	17322	5756	76.2	10.57	0.15	1.83

TCR β CDR3 sequence data is located at Adaptive Biotechnologies immunoSEQ ANALYZER:

<https://clients.adaptivebiotech.com/pub/stromnes-2017-cancerimmunologyresearch>