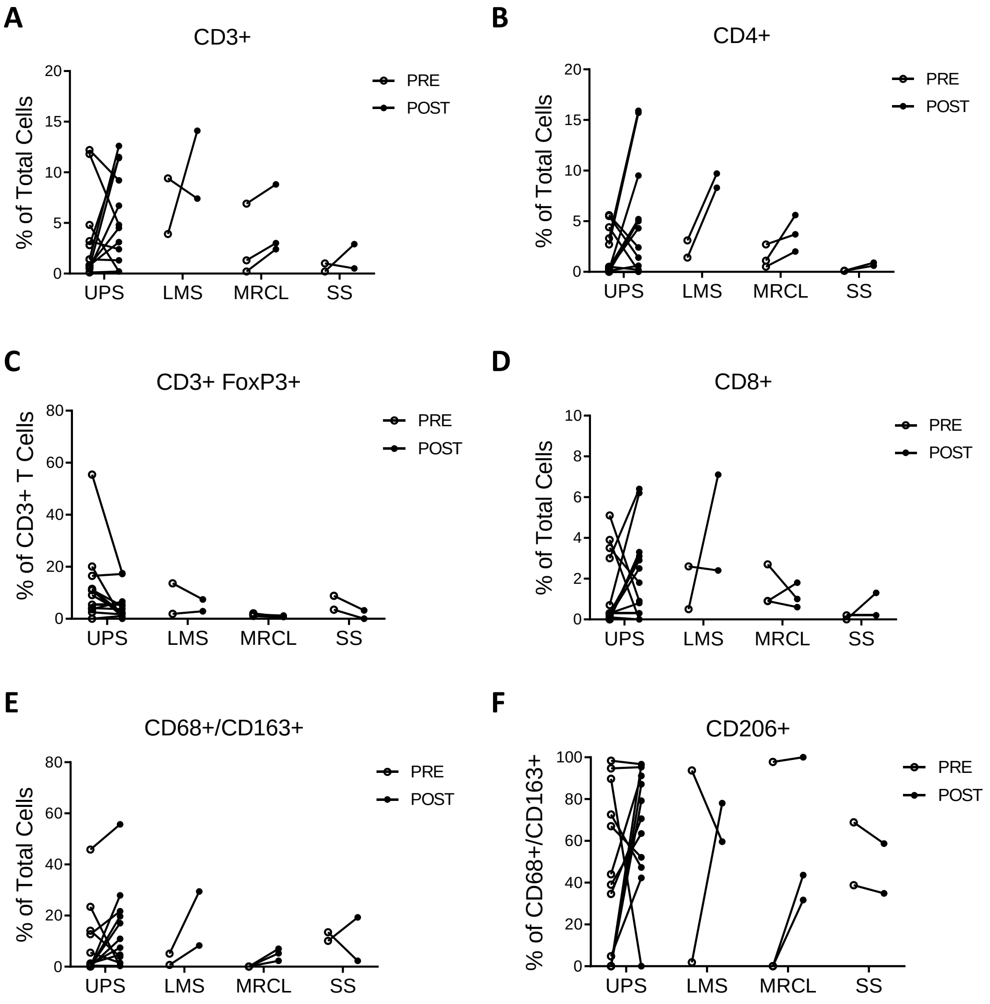
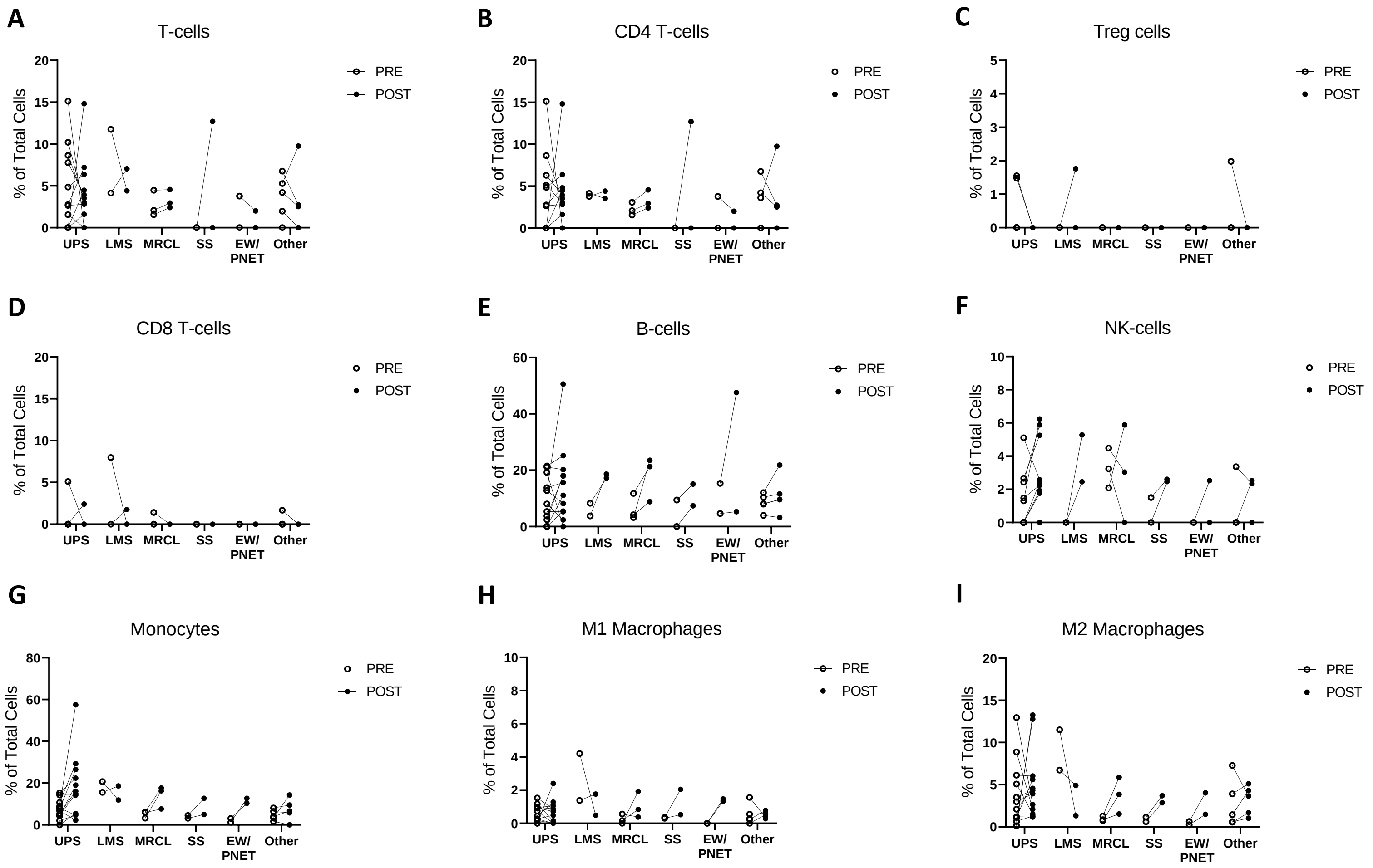
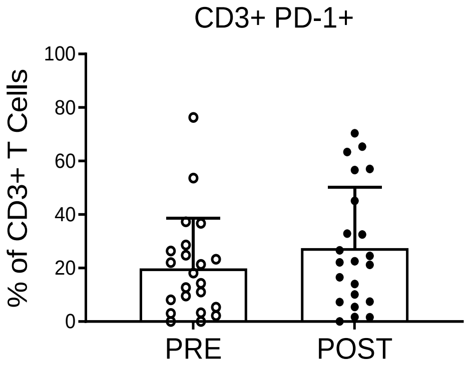


Supplemental Fig. S1. Percentage of immune cell populations detected by mIHC pre- and post-neoadjuvant therapy by sarcoma subtype. Individual samples pre-/post- paired are shown. (A) CD3+ cells, (B) CD4+ cells, (C) FOXP3+ T cells, (D) CD8+ cells, (E) CD68+/CD163+ cells and (F) CD206+ cells. UPS: Undifferentiated pleomorphic sarcoma (n=12); LMS: Leiomyosarcoma (n=2); MRCL: Myxoid/round cell liposarcoma (n=3); SS: Synovial Sarcoma (n=2). Based on the small sample sizes, these represent descriptive comparisons only.



Supplemental Fig. S2. Percentage of immune cell populations analyzed by ImmunoPrism pre- and post- neoadjuvant therapy by sarcoma subtype. Individual samples pre-/post- paired are shown. (A) Total T- cells; (B) CD4 T- cells, (C) T regulatory (Treg) cells; (D) CD8 T cells; (E) B cells; (F) NK cells; (G) Monocytes; (H) M1 Macrophages; and (I) M2 Macrophages. UPS: Undifferentiated pleomorphic sarcoma (n=12); LMS: Leiomyosarcoma (n=2); MRCL: Myxoid/round cell liposarcoma (n=3); SS: Synovial Sarcoma (n=2); EW: Ewing sarcoma (n=2) and other (n=5). Based on the small sample sizes, these represent descriptive comparisons only.

A



B

ImmunoPrism Gene Expression			
Gene ID	**Fold change (post / pre)**	**Paired t-test**	**Significant**
OX40	0.90	**0.0321**	*
TIM-3	3.70	**0.0002**	*
CTLA4	1.70	0.1170	ns
PD-1	3.10	0.3347	ns
PD-L1	1.40	0.7070	ns
BTLA	4.40	0.6345	ns
ICOS	1.90	0.6821	ns
CD47	1.30	0.1127	ns
IDO1	2.30	0.2239	ns
ARG1	1.40	0.9466	ns

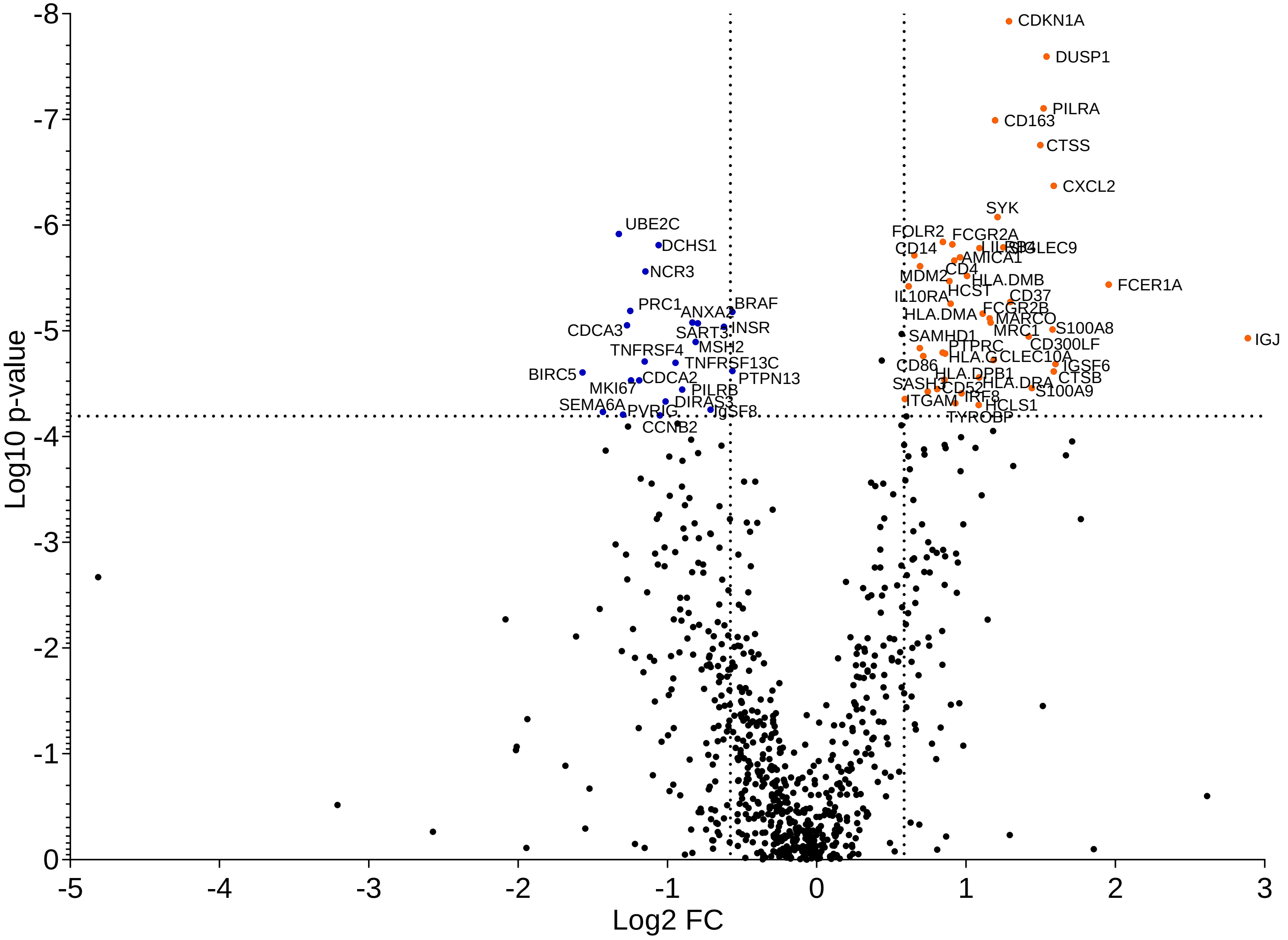
C

NanoString Immune Inhibitory Molecules			
Gene ID	**Fold change (post / pre)**	**q-value**	**Significant**
CD276 (B7-H3)	0.58	**0.0013**	*
PDCD1LG2 (PD-L2)	1.43	**0.0027**	*
ADORA2A	0.66	**0.0132**	*
HAVCR2 (TIM3)	1.34	**0.0047**	*
CD96	1.60	0.0528	ns
CTLA4	0.62	0.4929	ns
CD274 (PD-L1)	0.96	0.6662	ns
LAG3	0.97	0.9218	ns
TIGIT	0.98	0.8343	ns
PDCD1 (PD1)	0.89	0.9855	ns

D

NanoString Immune Stimulating Molecules			
Gene ID	**Fold change (post / pre)**	**q-value**	**Significant**
TNFRSF4 (OX40)	0.45	**0.0003**	*
TNFSF18	1.37	**0.0527**	*
CD86 (B7-2)	1.64	**0.0003**	*
CD28	1.37	0.2657	ns
CD226	0.89	0.2906	ns
TNFSF4 (OX-40L)	0.87	0.3957	ns
ICOS	0.74	0.4032	ns
TNFRSF9 (CD137)	0.66	0.4555	ns
CD40	0.89	0.7071	ns
CD27	0.67	0.7870	ns
TNFRSF18	0.98	0.8110	ns
TNFSF9 (CD137L)	1.08	0.8276	ns
CD80 (B7-1)	0.92	0.8472	ns
CD40LG	1.07	0.9539	ns

Supplemental Fig. S3. Changes in immune modulatory gene expression in the sarcoma tumor immune microenvironment from pre- to post-neoadjuvant therapy as determined by (A) multiplex IHC assaying the percentage of CD3+ cells that are positive for PD-1, and transcriptional profiling with (B) ImmunoPrism, and (C-D) NanoString. Results are reported fold-change (post / pre neoadjuvant therapy) for ImmunoPrism and NanoString data with statistical analyses utilizing paired t-test and the Benjamini-Hochberg q-values, respectively.



Supplemental Fig. S4. Volcano plot showing genes significantly up-regulated (≥1.5, n=43, orange) or down-regulated (≤0.67, n=22, blue) pre- versus post- neoadjuvant therapy. Symbols for the genes with p-value <0.0000643 (Bonferroni cut-off 0.05/778) are indicated.