Multimodality analysis confers a prognostic benefit of a T-cell infiltrated tumor microenvironment and peripheral immune status in patients with melanoma

Georgia M. Beasley (1, 2)*, Michael C. Brown (3)*, Norma Farrow (1), Karenia Landa (1), Rami N Al-Rohil (4), Maria Angelica Selim (4), Aaron Therien (1), Sin-Ho Jung, (5), Junheng Gao (5), David Boczkowski (1), Eda Holl, (1), April Salama (2), Darell D. Bigner (2, 3, 4), Matthias Gromeier (2, 3, 6), Smita K. Nair (1, 3, 4).
*co-first authors

1. Duke University, Department of Surgery
2. Duke University, Department of Medicine
3. Duke University, Department of Neurosurgery
4. Duke University, Department of Pathology
5. Duke University, Department of Biostatistics and Bioinformatics
6. Duke University, Department of Molecular Genetics and Microbiology

Corresponding Author (s):
Georgia M. Beasley MD, MHS
Duke University School of Medicine, Box 3118, Durham, NC 27710
Email: georgia.beasley@duke.edu Tel: 919-684-6858

Smita K. Nair, PhD
Duke University School of Medicine, Box 103035, Durham, NC 27710
Email: smita.nair@duke.edu Tel: 919-681-2180

Running Head: Immune status is linked with viral immunotherapy response

Supplementary Table 1

Scan_ID	CD127	CD25	CD27	CD4	CD44	CD45RO	CD8	GZMB	HLA-DR	ICOS	Ki-67	Tim-3
1 Pre	3.96	1.68	6.77	40.77	181.735	12.79	75.685	10.295	73.59	6.735	22.86	4.84
1 Pre-Non	3.43	1.87	1.9	31.4	130.81	4.99	18.87	7.02	65.58	3.76	8.18	3.15
2 Pre	3.795	1.865	2.53	25.5	81.315	4.87	40.71	4.43	90.255	2.16	1.82	3.6
3 Pre	2.915	2.14	9.7	46.58	76.705	10.245	42.885	5.125	38.345	11.6	153.83	3.935
4 Pre	1.845	2.42	3.595	9.69	79.705	3.85	44.05	5.925	16.89	1.465	16.175	1.47
5 Pre	3.935	0.94	3.835	34.745	89.735	6.265	14.55	5.015	46.52	1.485	8.545	2.78
6 Pre	4.225	1.47	3.37	29.59	123.24	8.74	17.485	7.505	27.265	2.12	7.695	1.64
7 Pre	6.66	2	12.02	24.815	188.715	317.225	82.49	14.085	31.26	5.55	66.7	6.465
8 Pre	10.1	4.6	2.5	89.6	248.8	60.3	378.3	23.7	163.5	10.3	110.9	22.2
9 Pre	2.62	1.54	5.955	34.615	113.265	14.225	96.315	9.675	112.1	3.5	97.57	7.705
9 Pre-Non	4.09	1.795	8.725	32.895	175.58	30.26	45.06	8.165	92.88	2.68	46.62	11.215
12 Pre	2.94	2.585	10.255	70.675	228.77	13.65	123.18	11.825	63.825	8.545	119.885	5.065

Supplementary Table 1. Signal to noise ratios for represented proteins in regions of interest chosen by CD3+

Supplementary Table 2

Scan_ID	B7-H3	Bcl-2	CD4	CD44	CD45	CD8	Fibronectin	HLA-DR	Ki-67	MART1	NY-ESO-1	S100B	STING
Patient 1 pre	9.915	8.495	9.045	126.1	37.39	14.7	24.335	35.65	18.46	199.35	4.93	507.115	17.1
Patient 1 pre	24.55	6.2	5.68	202.45	30.68	11.4	81.075	17.765	13.48	183.85	3.95	643.355	29.155
Patient 2 Pre	19.35	9.7975	8.095	171.475	36.6825	14.6	41.79	28.985	15.97	194.6	4.44	609.455	16.7175
Patient 3 Pre	41.965	4.44	4.21	10.6	9.22	2.885	50.735	6.745	33.625	0.775	4.47	21.795	4.26
Patient 4 Pre	21.95	9.7975	7.62	171.475	36.51125	14.55	48.305	25.6525	15.97	192.225	4.44	609.455	16.52625
Patient 5 Pre	17.55	5.37	9.915	249.95	50.59	3.545	47.415	22.895	44.685	41.16	1.585	78.665	11.47
Patient 6 Pre	20.95	13.7	3.825	485.6	10.81	3.025	32.79	6.785	31.015	30.785	3.875	304.28	22.19
Patient 7 Pre	18.2	21.1	8.115	398.9	79.475	12.2	18.78	21.02	28.235	6.84	3.535	45.275	16.045
Patient 8 Pre	9.7097456	16.45	75.25	275.75	594.2	362.05	115.05	406.55	138.8	2.4	2.65	49.4	30.45
Patient 9 Pre	10.75	12.6	26.1	297.25	166.315	71.45	65.555	372.8	122.69	32.345	9.515	218.395	28.49
Patient 9 Pre	10.5	5.83	21.8	627.2	180.5	26.4	242.76	316	31.15	28.99	7.22	152.17	23.85
Patient 12 Pre	8.24	8.035	24.105	124.35	161.515	43.73	181.745	23.605	37.55	6.465	18.505	37.785	32.35

Supplementary Table 2: Signal to noise ratios for represented proteins in regions of interest chosen by location of tumor
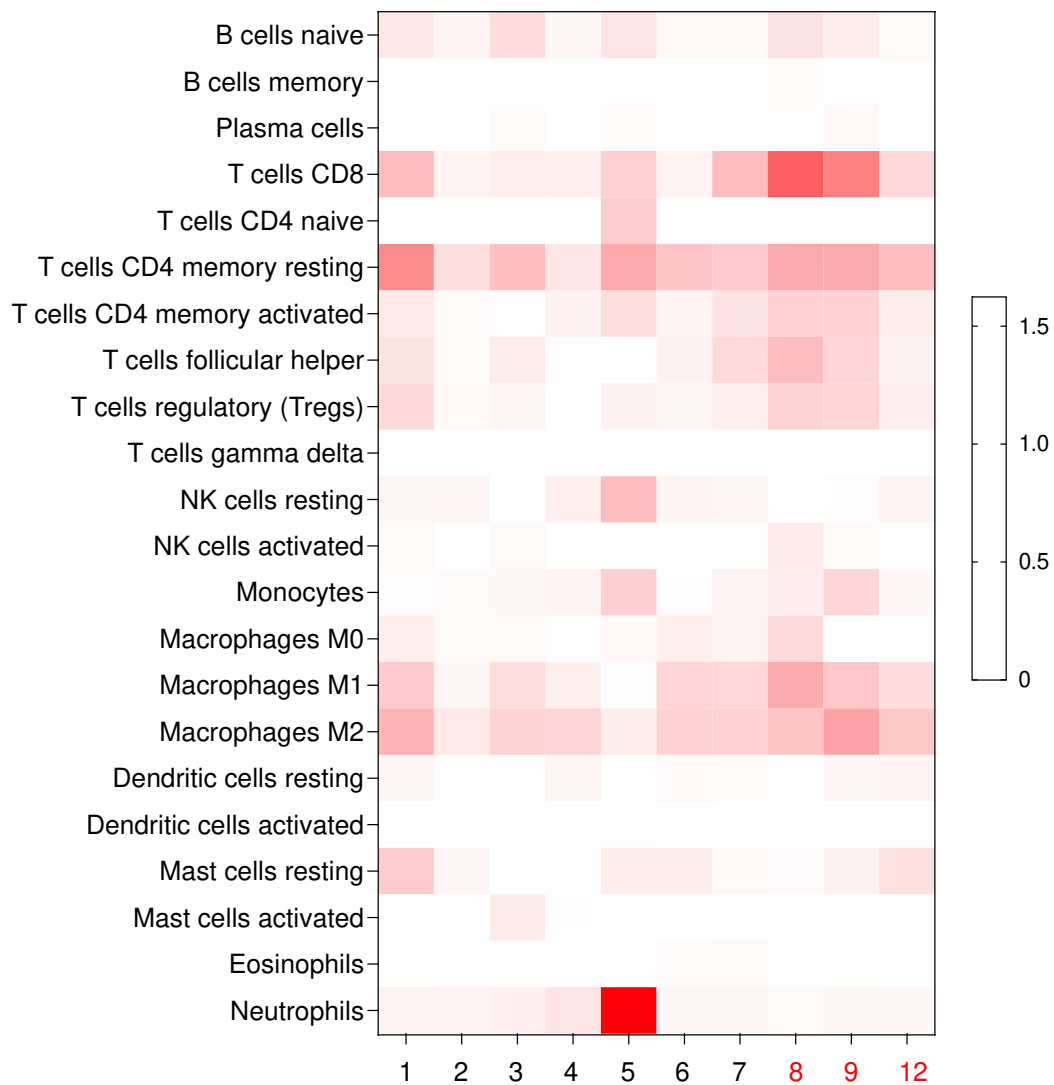
Supplementary Figure 1 (S1)

Figure S1. CIBERSORT results of bulk RNA sequencing data from pre-treatment tumor tissue.
Patient number is indicated on X-axis. Patients in red have median PFS of 2.3 years after lerapolturev and patients in black have median PFS 1.6 months after lerapolturev.

Supplemental material

BMJ Publishing Group Limited (BMJ) disclaims all liability and responsibility arising from any reliance placed on this supplemental material which has been supplied by the author(s)
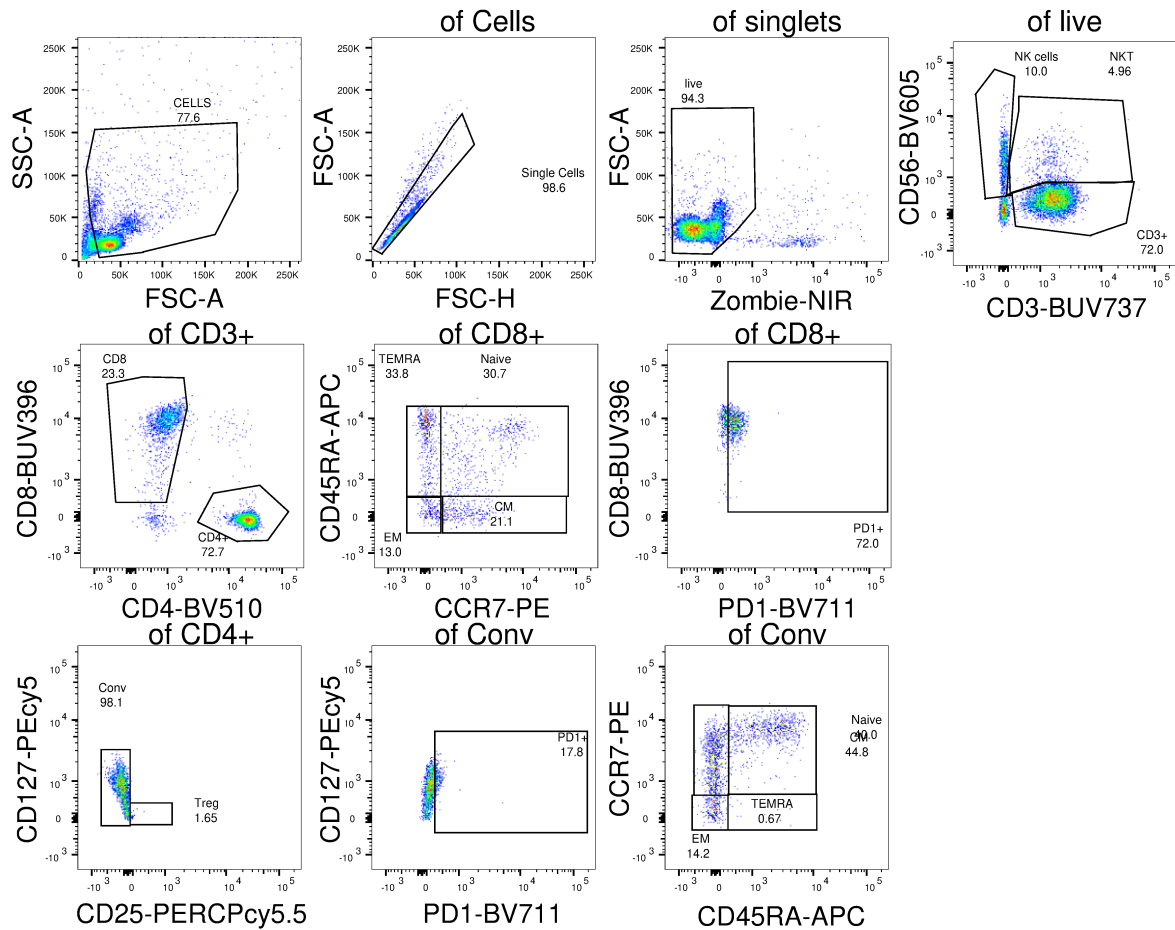
J Immunother Cancer

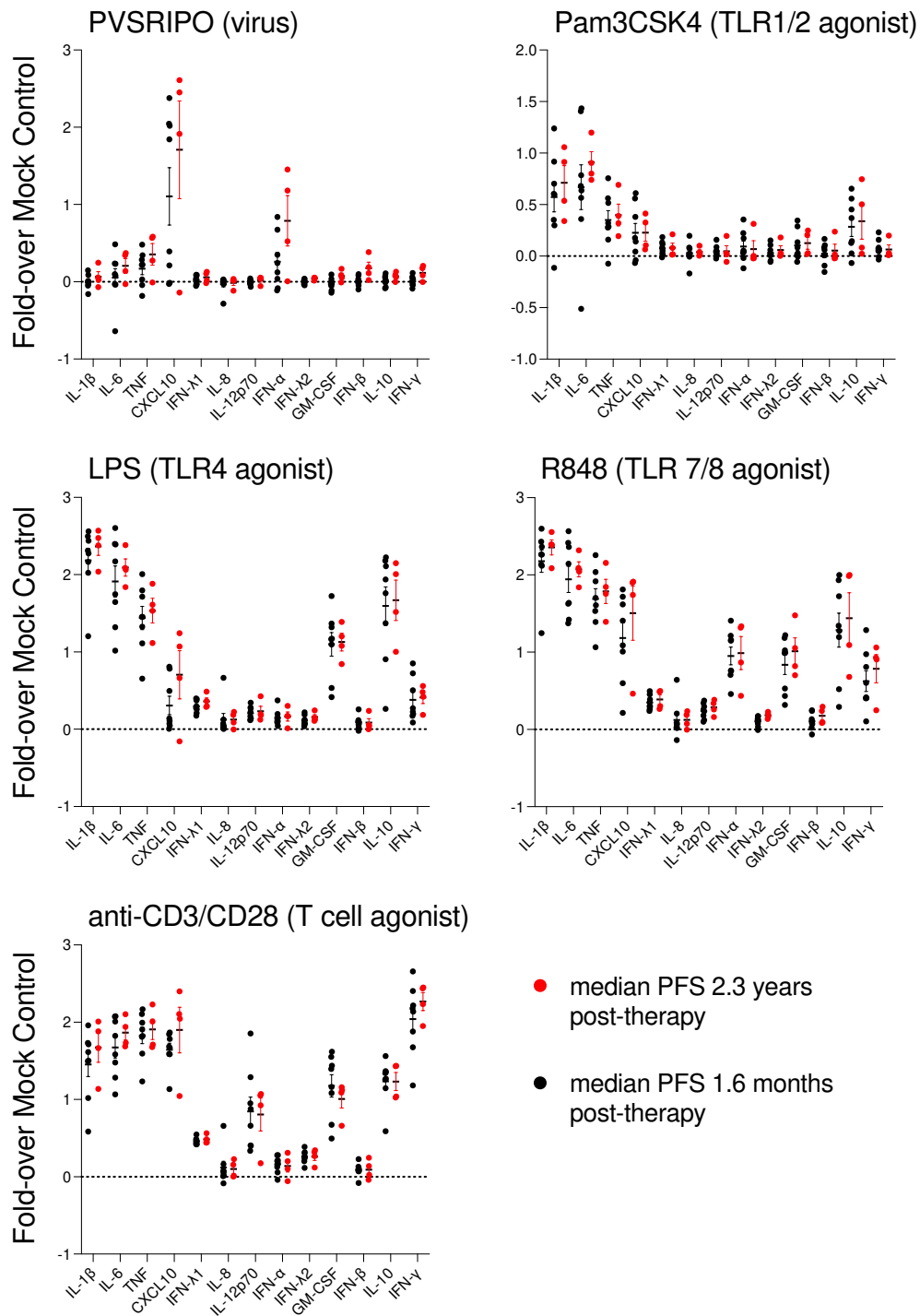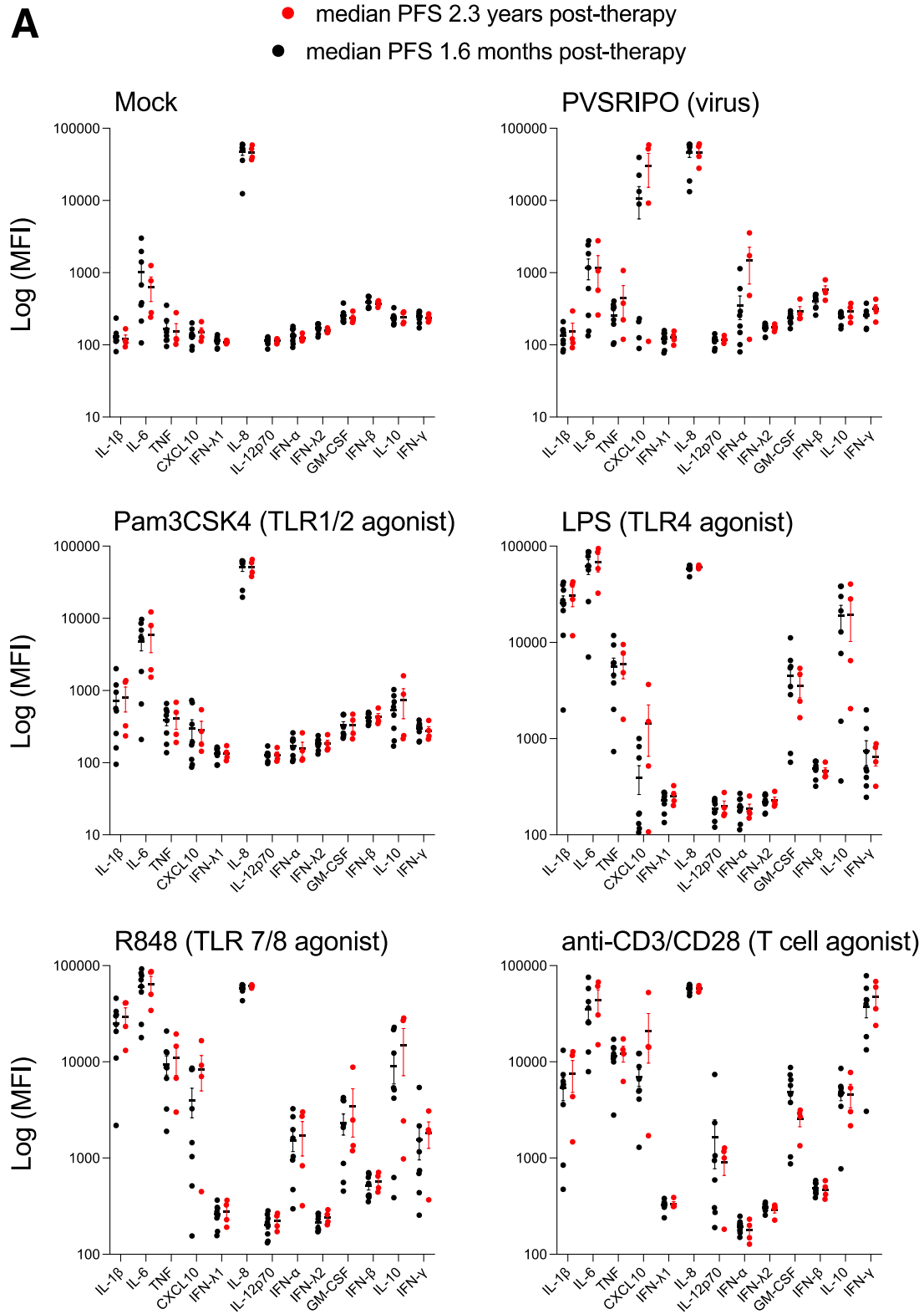Supplementary Figure 2 (S2)

Figure S2. Flow cytometry gating strategy for detection of memory T cell populations and PD1 expression on CD8+ and CD4+ conventional T cells in Figure 5.

Supplemental material

BMJ Publishing Group Limited (BMJ) disclaims all liability and responsibility arising from any reliance placed on this supplemental material which has been supplied by the author(s)

J Immunother Cancer

Supplementary Figure 3 (S3)

Figure S3. Assessment of baseline peripheral immune cell function in response to lerapolturev.
PBMCs from the pre-treatment time point were challenged with laboratory grade PVSRIPO in vitro for 24 hours. Supernatant was tested for pro-inflammatory cytokines. Patients in red have median PFS of 2.3 years after lerapolturev and patients in black have median PFS 1.6 months after lerapolturev.

Supplemental material

BMJ Publishing Group Limited (BMJ) disclaims all liability and responsibility arising from any reliance placed on this supplemental material which has been supplied by the author(s)

J Immunother Cancer

Supplementary Figure 4A (S4A)

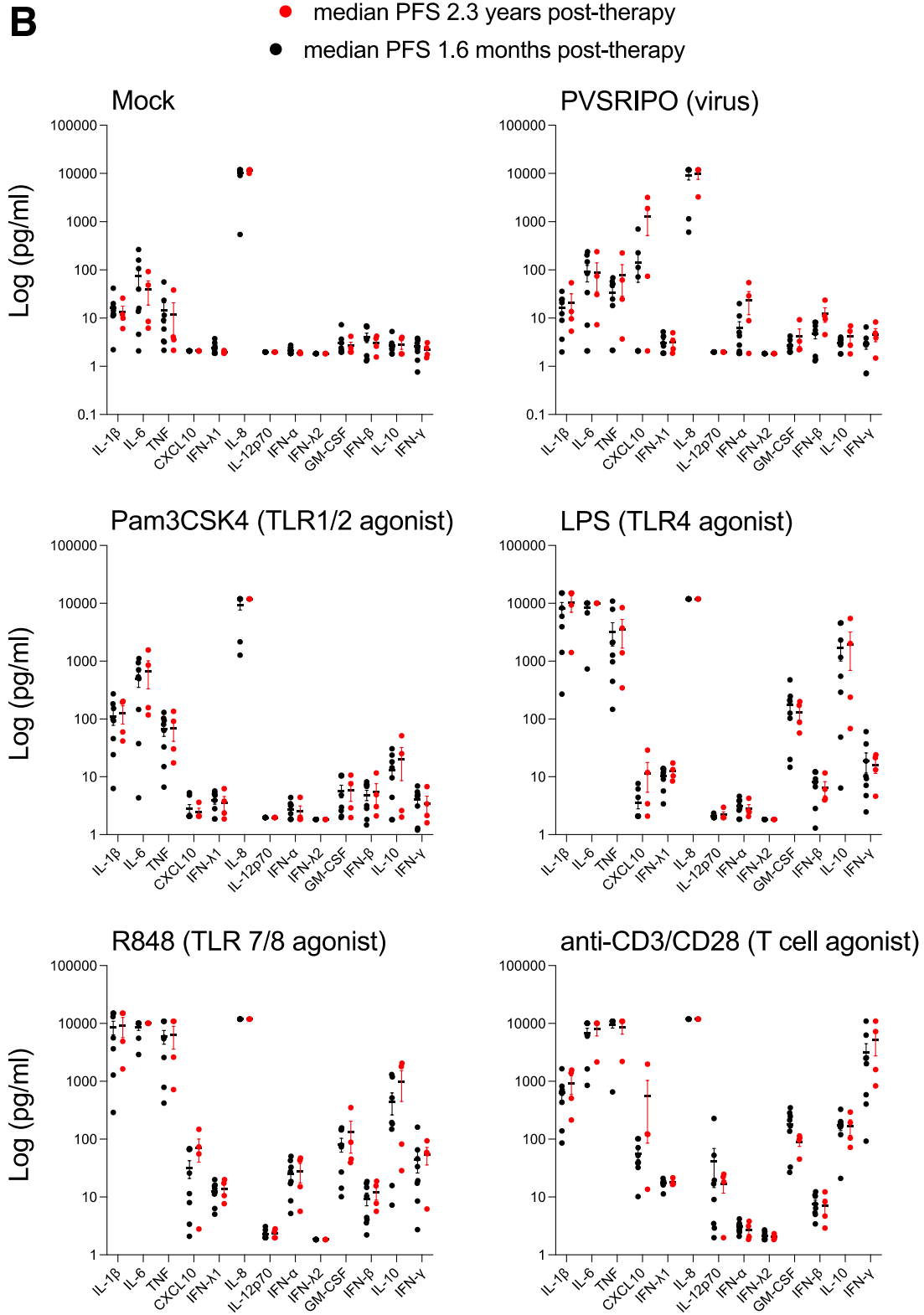Supplementary Figure 4B (S4B)

Figure S4. Peripheral immune cell function at baseline.
Raw MFI (A) or concentration (pg/ml, B) of analytes after each treatment corresponding to data presented in Figure 6. (B) For values below or above the maximum standard concentration threshold, values were set to the minimum or maximum threshold, respectively. Patients in red have median PFS of 2.3 years after lerapolturev and patients in black have median PFS 1.6 months after lerapolturev.