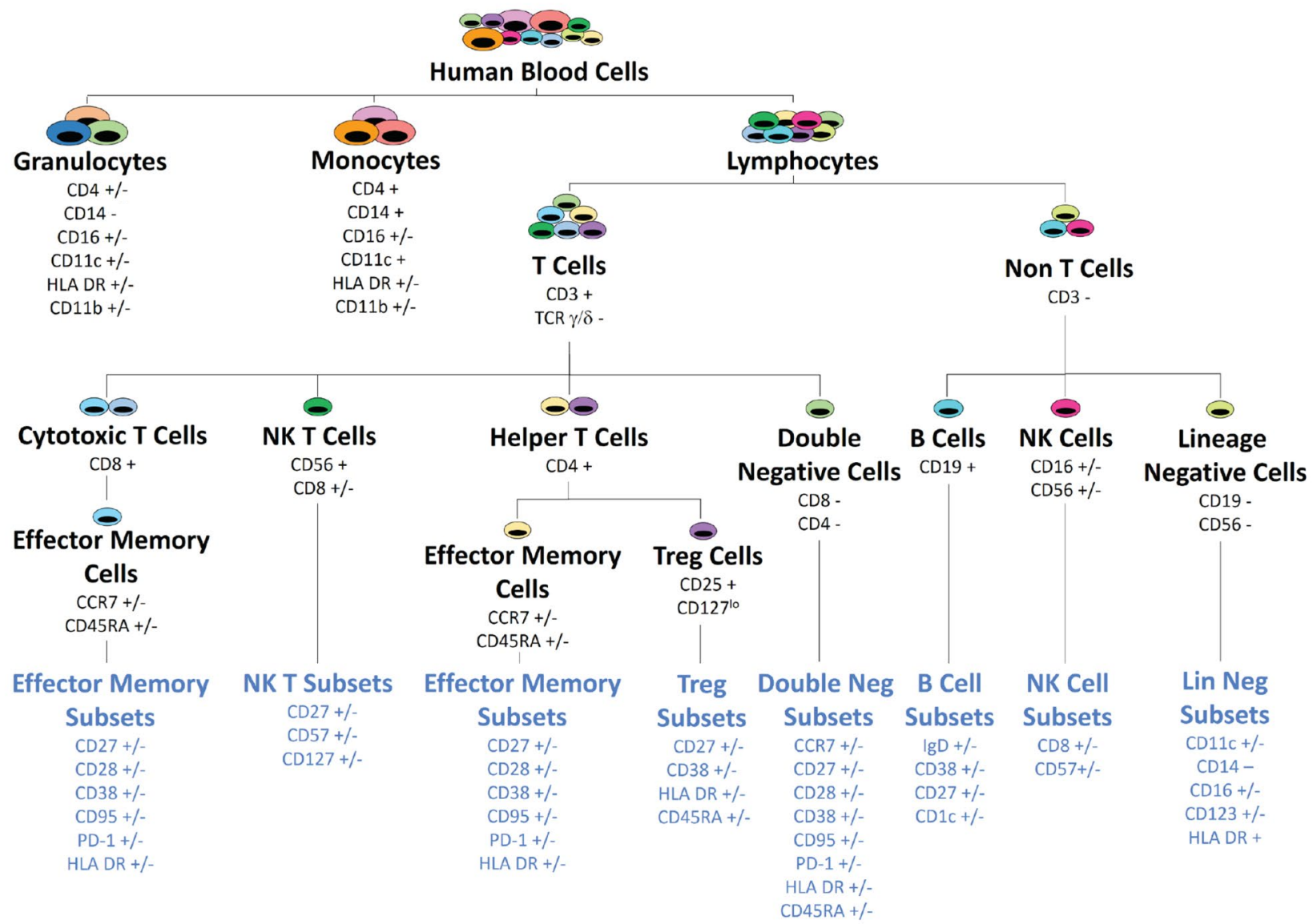


Supplemental Figure S1. Summary of Immune Cell Subtypes by the 24-color spectral flow cytometry panel

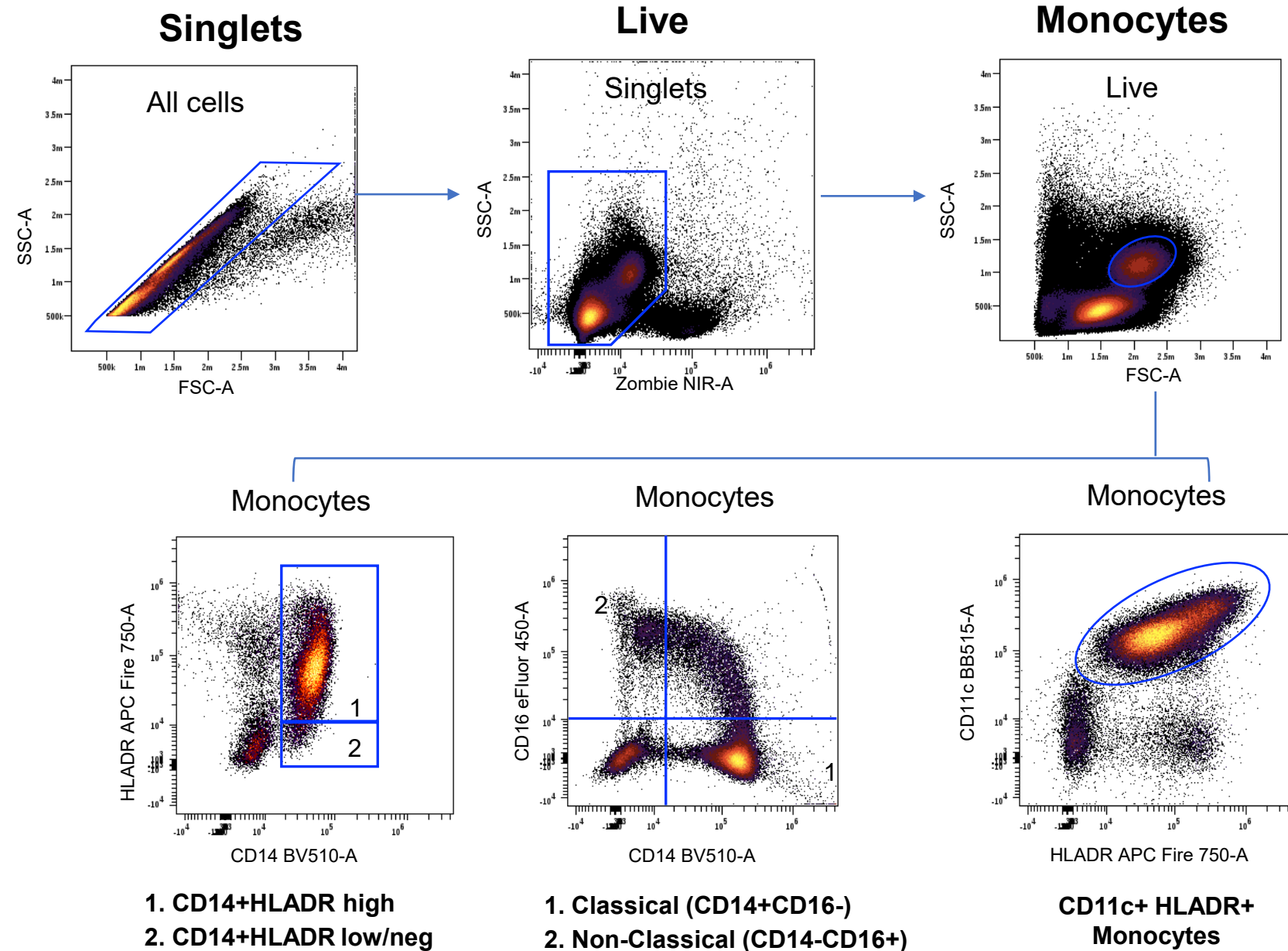


Supplemental Figure S2. Representative data showing the gating strategy for each panel and populations listed in Supplemental Table 1.

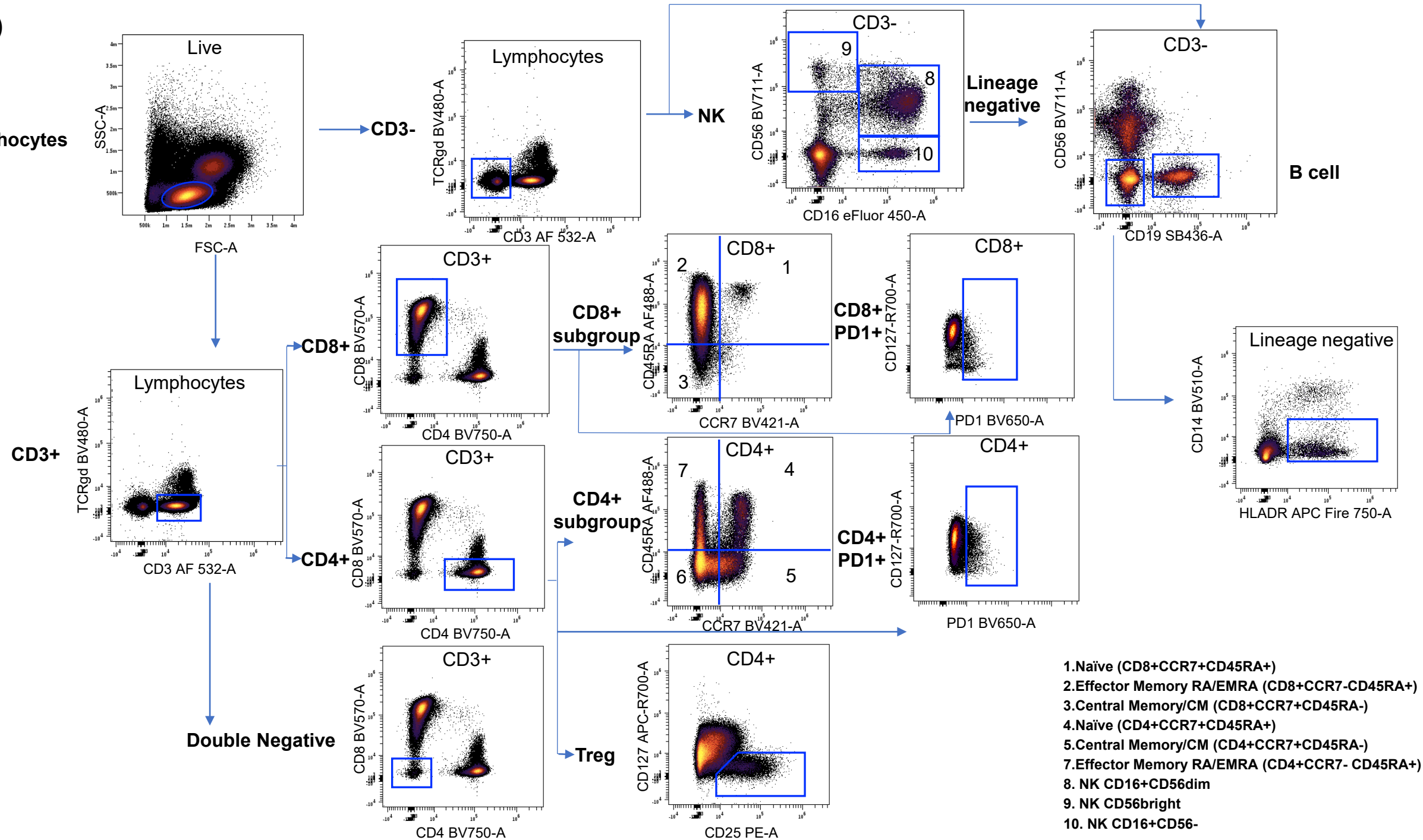
(A)

Ungated
 Singlets
 Live
 Lymphocytes
 CD3-
 B cell
 Lineage negative
 Lineage negative HLADR high
 NK Cell
 CD16+ CD56 dim NK cell
 CD16+ CD56- NK cell
 CD56 bright NK cell
 CD3+
 CD4+
 CD4+Naive
 CD4+CM
 CD4+EM
 CD4+CD28+CD27+
 CD4+CD28+CD27-
 CD4+CD38+HLADR high
 CD4+CD38+HLADR low/neg
 CD4+PD-1
 Treg
 CD8+
 CD8+Naive
 CD8+CM
 CD8+TEMRA
 CD8+CD28+CD27+
 CD8+CD28+CD27-
 CD8+CD38+HLADR high
 CD8+CD38+HLADR low/neg
 CD8+PD-1
 Double negative (DN)
 Monocytes
 CD14+CD16-
 CD14+CD16+
 CD14+CD16+ nonclassical
 CD14+HLADR high
 CD14+HLADR low/neg
 CD11c+HLADR+

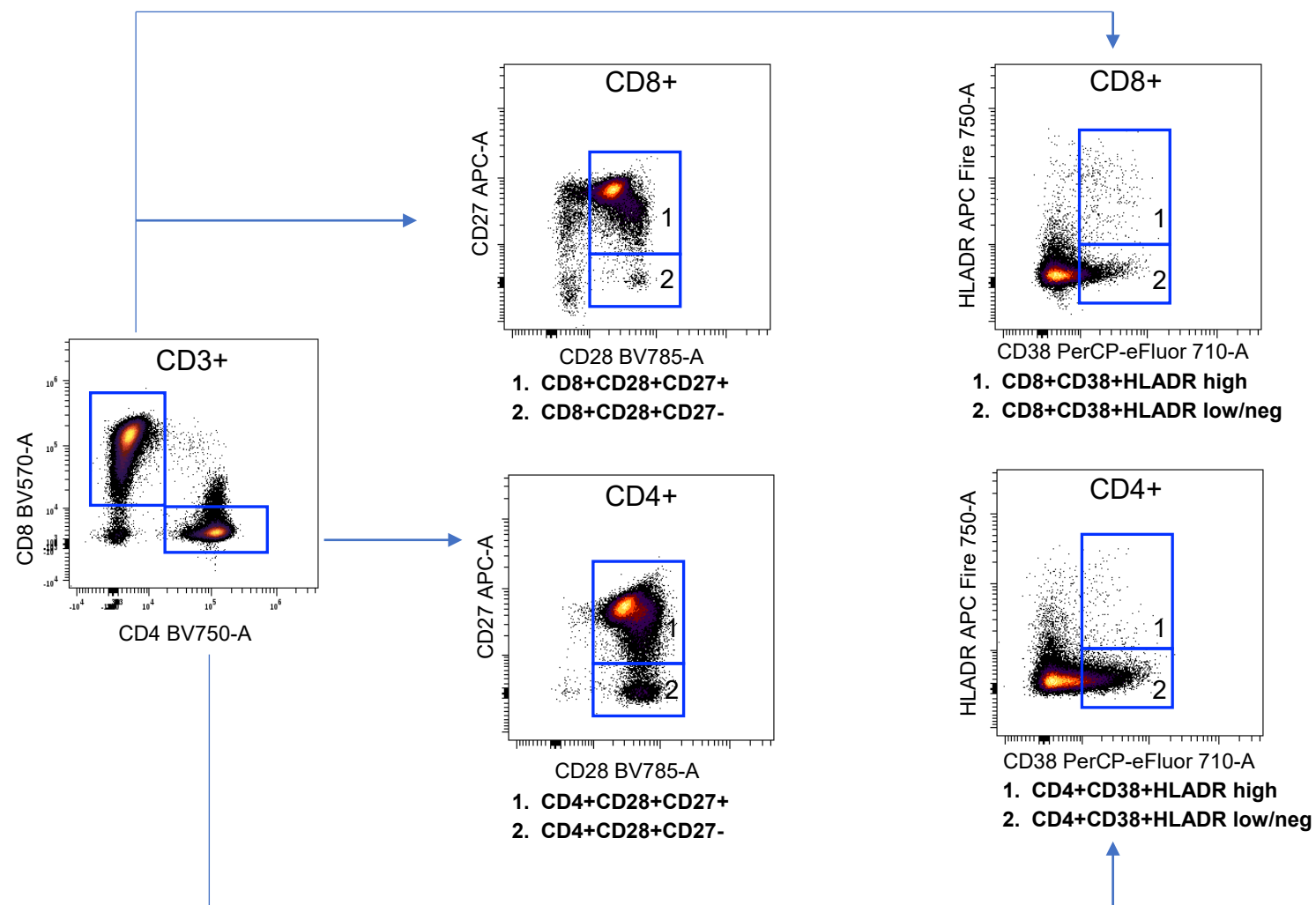
(B)



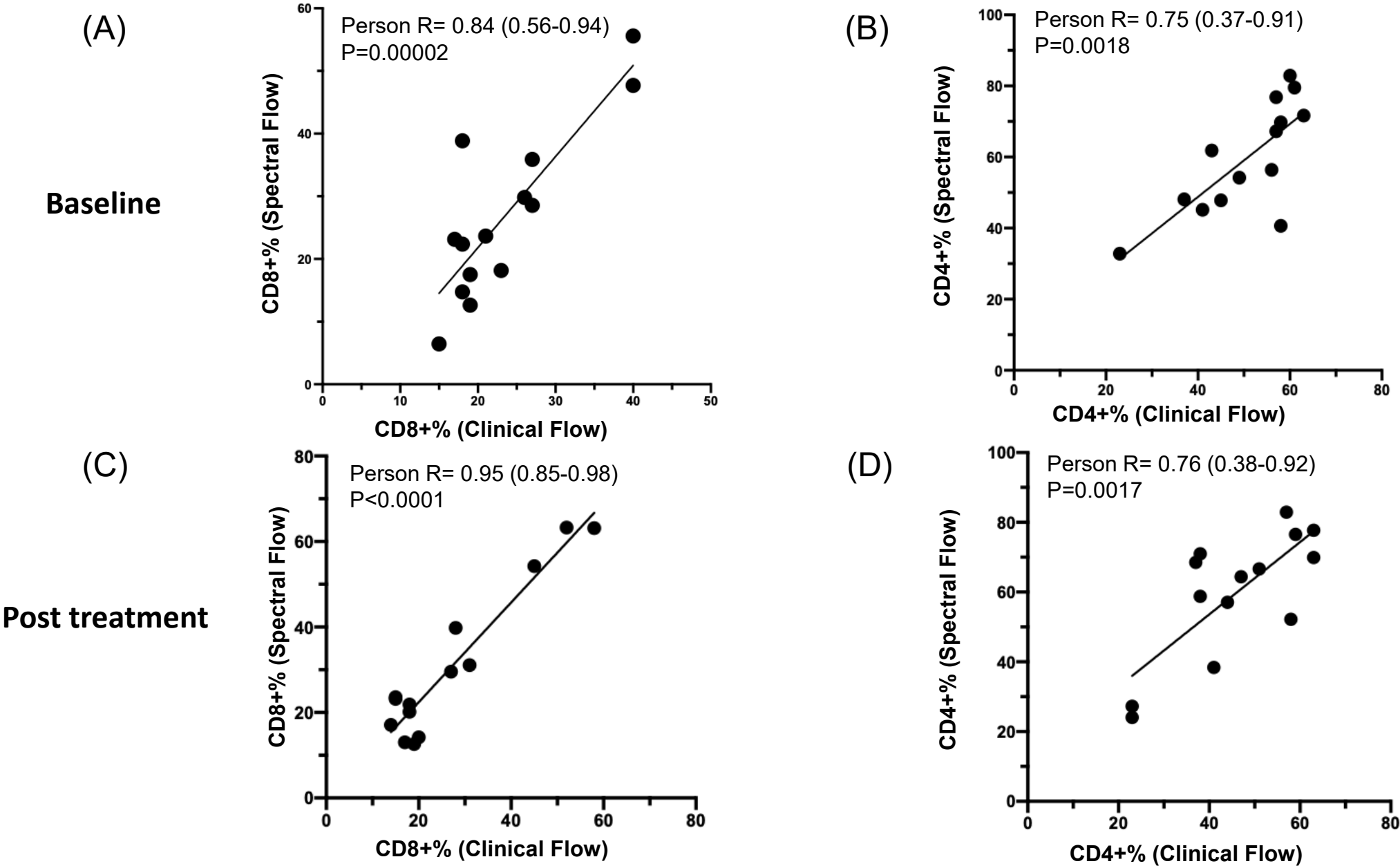
Lymphocytes



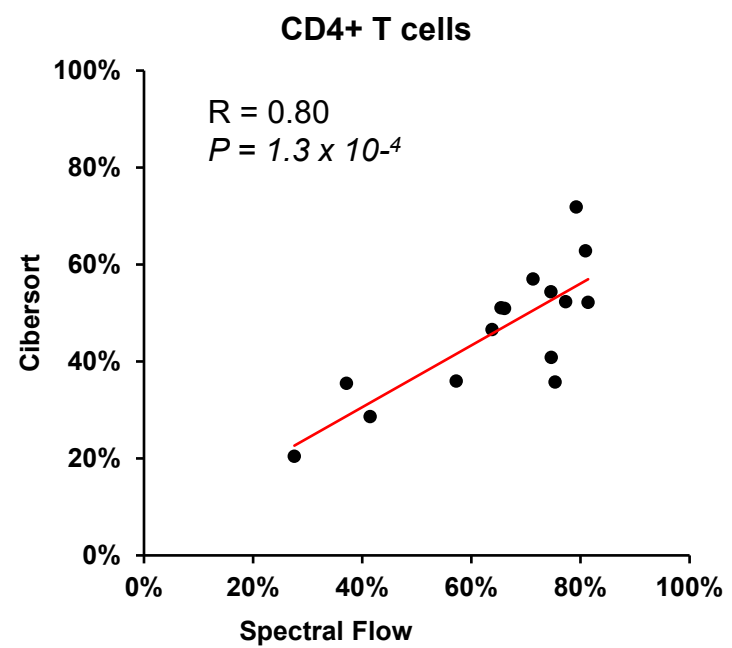
(D)



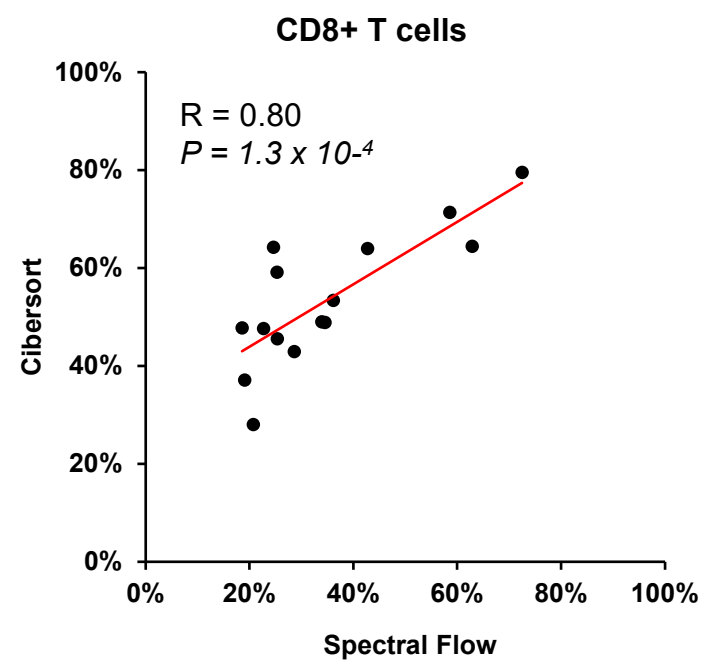
Supplemental Figure S3
Validation of CD4+ and CD8+ T cell proportions by multicolor spectral flow cytometry with clinical flow cytometry and RNA sequencing.



(E)



(F)



Supplemental Figure S4. ROC analysis for pertinent immune cell subtypes

(A) CM (CCR7+CD45RA-) CD4+ T cells (baseline) (B) DN (CD4-CD8-CD3+) T cells (baseline) (C) CD16+CD56dim NK difference (post-treatment) (D) CD14+HLADRhigh difference (post-treatment)

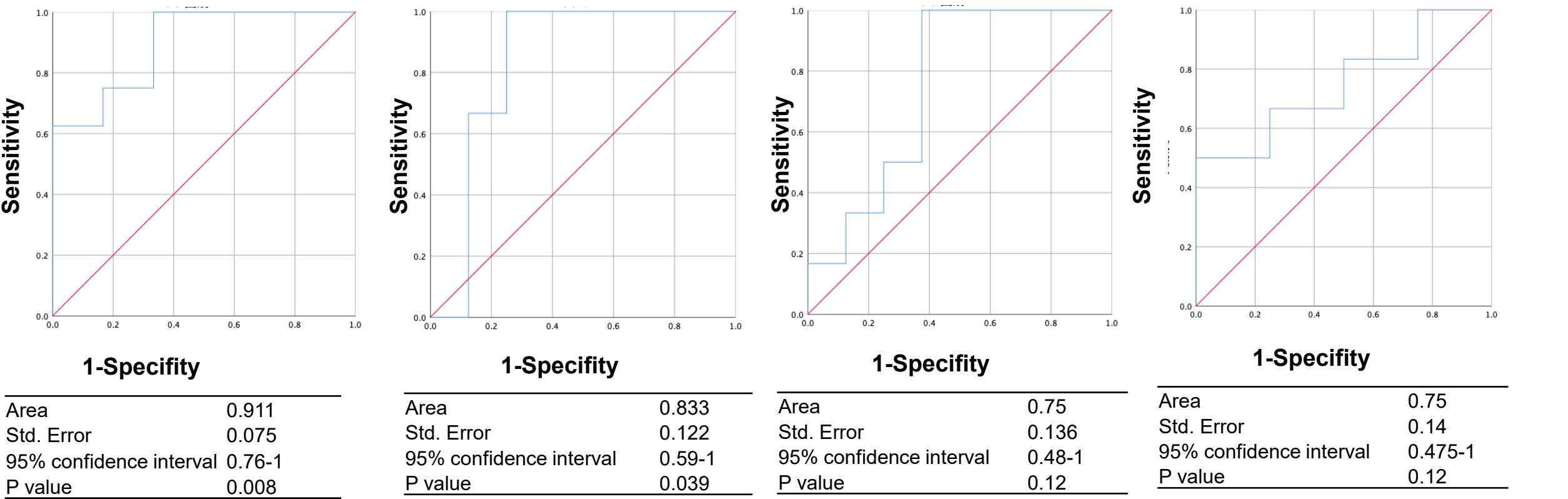


Table S1. Summary of antibodies and dyes used for multi-color spectral flow cytometry

Specificity	Fluorochrome	Catalog #	Vendor	Clone
CCR7	BV421	353208	BioLegend	G043H7
CD19	SB436	62-0199-42	Thermo Fisher	HIB19
CD16	eFluor 450	48-0168-42	Thermo Fisher	CB16
TCR $\gamma\delta$	BV480	566076	BD	B1
CD14	BV510	301842	BioLegend	M5E2
CD8	BV570	301038	BioLegend	RPA-T8
CD1c	BV605	331538	BioLegend	L161
PD-1	BV650	329950	BioLegend	EH12.2H7
CD56	BV711	318336	BioLegend	HCD56
CD4	BV750	566355	BD	SK3
CD28	BV785	302950	BioLegend	CD28.2
CD11c	BB515	564490	BD	B-ly6
CD45RA	Alexa 488	304114	BioLegend	HI100
CD3	Alexa 532	58-0038-42	Thermo Fisher	UCHT1
CD25	PE	356104	BioLegend	M-A251
IgD	PE-Dazzle 594	348240	BioLegend	IA6-2
CD95	PE-Cy5	305610	BioLegend	DX2
CD11b	PerCP-Cy5.5	301328	BioLegend	ICRF44
CD38	PerCP-eFluor 710	46-0388-42	Thermo Fisher	HB7
CD57	PE-Cy7	359624	BioLegend	HNK-1
CD27	APC	337169	BD	L128
CD123	Alexa 647	306024	BioLegend	6H6
CD127	APCR-700	565185	BD	HIL-7R-M21
HLA-DR	APC-Fire 750	307658	BioLegend	L243

Table S2. Baseline level of immune cell subgroups

Cell Marker	Patients (N=14)		Normal control (N=4)		P value
	Percent (%)	SD (%)	Percent (%)	SD (%)	
Live	88.3	18.7	99.0	0.2	0.24
Monocytes	26.8	18.6	20.4	2.4	0.57
CD14+CD16-	62.7	25.6	24.0	10.5	0.02
CD14+HLADRhigh	56.5	32.5	80.8	4.1	0.19
CD14+HLADRlow	5.1	5.6	1.0	0.6	0.17
Lymphocytes	45.7	20.2	58.9	2.5	0.23
CD3+	48.1	24.6	71.8	8.3	0.06
CD4+	59.6	15.6	46.9	11.5	0.19
CD4 EM	19.1	15.7	15.7	11.2	0.69
CD4 CM	30.7	11.8	28.9	5.9	0.77
Tregs	11.0	6.3	6.9	1.2	0.22
CD8+	26.8	13.8	36.1	9.6	0.13
CD8 naive	9.5	8.3	56.2	23.2	0.001
CD8 CM	31.7	14.4	16.3	6.1	0.03
CD8 TEMRA	27.3	15.5	14.1	14.9	0.16
CD3-	48.3	26.0	25.6	7.6	0.13
NK CD16+CD56dim	19.8	14.8	35.1	10.7	0.13
NK CD56bright	1.4	1.6	1.3	0.3	0.45
NK CD16+CD56-	3.9	3.3	2.9	0.9	0.65
LINEAGE NEGATIVE	48.0	26.8	20.6	3.2	0.03
Lineage Negative HLADR High	15.6	14.9	11.9	4.1	0.99
DN	6.5	7.8	13.3	1.6	0.048
CD11c HLADR+ Mono	77.9	27.7	89.7	3.1	0.95
NK CD16+CD56+ PD1+ CD27-	25.1	14.9	11.4	4.2	0.13
CD8+ PD1+	16.0	10.8	12.4	4.8	0.96
CD4+ PD1+	15.8	10.3	11.3	6.5	0.28
CD8+CD38+	9.1	7.8	8.2	4.4	0.97
CD4+CD38+	11.8	7.6	20.3	8.2	0.08
CD8+CD28+	50.9	26.9	77.6	21.4	0.06
CD8+CD27+CD28+	42.4	26.6	75.4	22.4	0.08
CD8+CD27-CD28+	8.5	5.8	2.3	1.2	0.06
CD4+CD27+CD28+	75.3	20.9	88.1	10.8	0.23
CD4+CD27-CD28+	18.2	16.4	9.4	6.8	0.38
CD4+CD28+	93.5	7.5	97.6	4.0	0.19
B cells	21.9	14.1	40.6	10.1	0.03

Table S3. Summary of all flow cytometry data with paired samples.

Cell Marker	Before treatment				P value	After treatment				P value
	Good response (N=11)		Poor response (N=3)			Good response (N=11)		Poor response (N=3)		
	Percent (%)	SD (%)	Percent (%)	SD (%)		Percent (%)	SD (%)	Percent (%)	SD (%)	
Live	86.8	20.9	93.9	5.1	0.99	86.0	22.4	72.5	34.8	0.52
Monocytes	25.6	20.6	31.2	8.6	0.88	29.3	19.1	20.8	1.9	0.37
CD14+CD16-	57.1	25.8	83.1	10.9	0.09	64.5	17.9	66.3	41.2	0.91
Lymphocytes	45.2	21.4	47.6	18.7	0.99	41.4	15.4	34.7	17.9	0.66
CD3+	45.7	23.2	57.1	33.1	0.49	46.3	18.9	57.9	20.0	0.37
CD4+	58.3	15.7	64.6	17.0	0.55	57.0	19.2	69.4	12.3	0.32
CD4 EM	20.1	17.1	15.2	10.4	0.65	17.9	15.0	18.2	13.6	0.97
CD4 CM	30.1	12.7	34.9	5.9	0.37	23.7	10.0	30.5	17.0	0.38
Tregs	9.7	4.3	15.9	11.0	0.14	9.6	5.7	15.2	5.2	0.15
CD8+	28.4	15.2	21.0	3.1	0.43	33.7	18.9	18.8	5.6	0.21
CD8 naive	10.1	9.3	7.4	3.9	0.63	8.5	8.2	8.7	9.0	0.96
CD8 CM	28.2	13.1	44.4	13.6	0.09	27.4	16.0	32.9	6.0	0.58
CD8 TEMRA	29.1	15.1	21.1	18.7	0.45	33.4	20.3	26.0	21.1	0.59
CD3-	51.6	23.9	36.3	35.5	0.39	48.3	21.2	38.5	16.7	0.47
NK CD16+CD56dim	18.0	15.2	31.8	4.3	0.37	32.0	13.4	23.7	3.6	0.29
NK CD56+ Bright	1.4	1..69	1.5	1.5	0.66	2.3	3.0	0.8	0.7	0.23
NK CD56- CD16+	4.0	3.7	3.6	0.6	0.88	5.8	4.7	1.2	0.7	0.13
LINEAGE NEGATIVE	49.8	29.9	41.2	11.2	0.64	31.9	17.5	34.6	22.8	0.83
Lineage Negative HLADR high	15.4	16.8	16.0	4.9	0.95	22.1	18.1	29.9	27.1	0.56
DN T cells	5.8	5.8	9.4	14.6	0.5	5.1	4.8	23.0	31.3	0.07
CD11c HLADR+ Mono	74.2	30.2	91.7	5.7	0.35	79.1	25.8	60.3	17.7	0.26
CD8+ PD1+	13.6	9.8	25.0	11.0	0.13	20.9	22.5	9.3	8.0	0.41
CD4+ PD1+	14.3	10.7	21.3	7.8	0.32	16.7	15.6	16.6	17.7	0.99
B cells	20.3	14.4	28.1	13.2	0.42	22.3	15.8	15.6	8.8	0.5
CD14+HLADR-	5.7	6.2	2.8	0.6	0.74	6.8	13.1	7.1	11.3	0.75
CD14+HLADR+	50.5	34.2	78.3	10.3	0.29	56.9	33.6	43.0	37.1	0.37
CD8+CD38+	10.4	8.4	4.4	0.8	0.55	9.2	10.2	8.4	4.9	0.66
CD8+CD38+HLADR-	4.0	3.0	2.1	0.9	0.29	4.5	5.5	3.7	1.4	0.55
CD8+CD38+HLADR+	6.4	8.2	2.3	1.5	0.25	4.7	5.2	4.7	3.5	0.87
CD4+CD38+	10.4	6.3	17.1	11.3	0.23	9.7	7.6	10.3	7.3	0.99
CD4+CD38+HLADR+	2.7	1.9	1.8	0.8	0.55	2.7	2.3	2.3	2.4	0.99
CD4+CD38+HLADR-	7.7	5.9	15.3	12.0	0.36	7.0	6.2	8.0	6.1	0.88
CD8+CD28+	46.4	27.8	67.5	18.0	0.29	45.3	27.5	54.3	26.8	0.66
CD8+CD27+CD28+	37.0	26.4	62.0	19.7	0.23	34.2	24.3	43.9	31.4	0.66
CD8+CD27-CD28+	9.4	6.3	5.4	2.2	0.36	11.1	14.9	10.4	4.8	0.37
CD4+CD27+CD28+	70.5	21.2	93.0	1.7	0.09	74.5	17.7	79.8	12.7	0.99
CD4+CD27-CD28+	21.5	17.1	5.9	0.7	0.06	15.3	13.0	16.6	10.8	0.77
CD4+CD28+	92.0	7.9	98.9	1.1	0.12	89.8	10.5	96.4	2.3	0.77