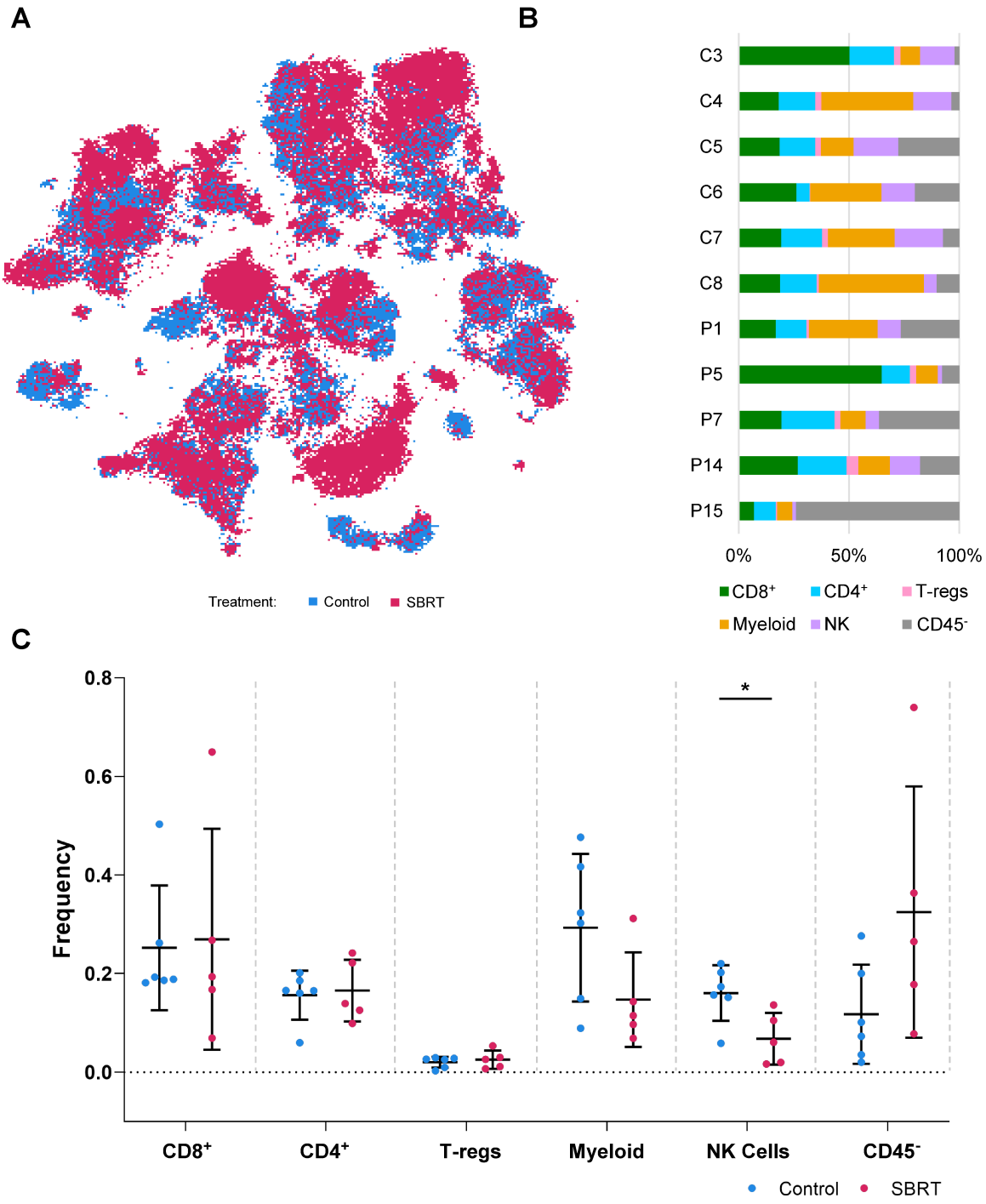
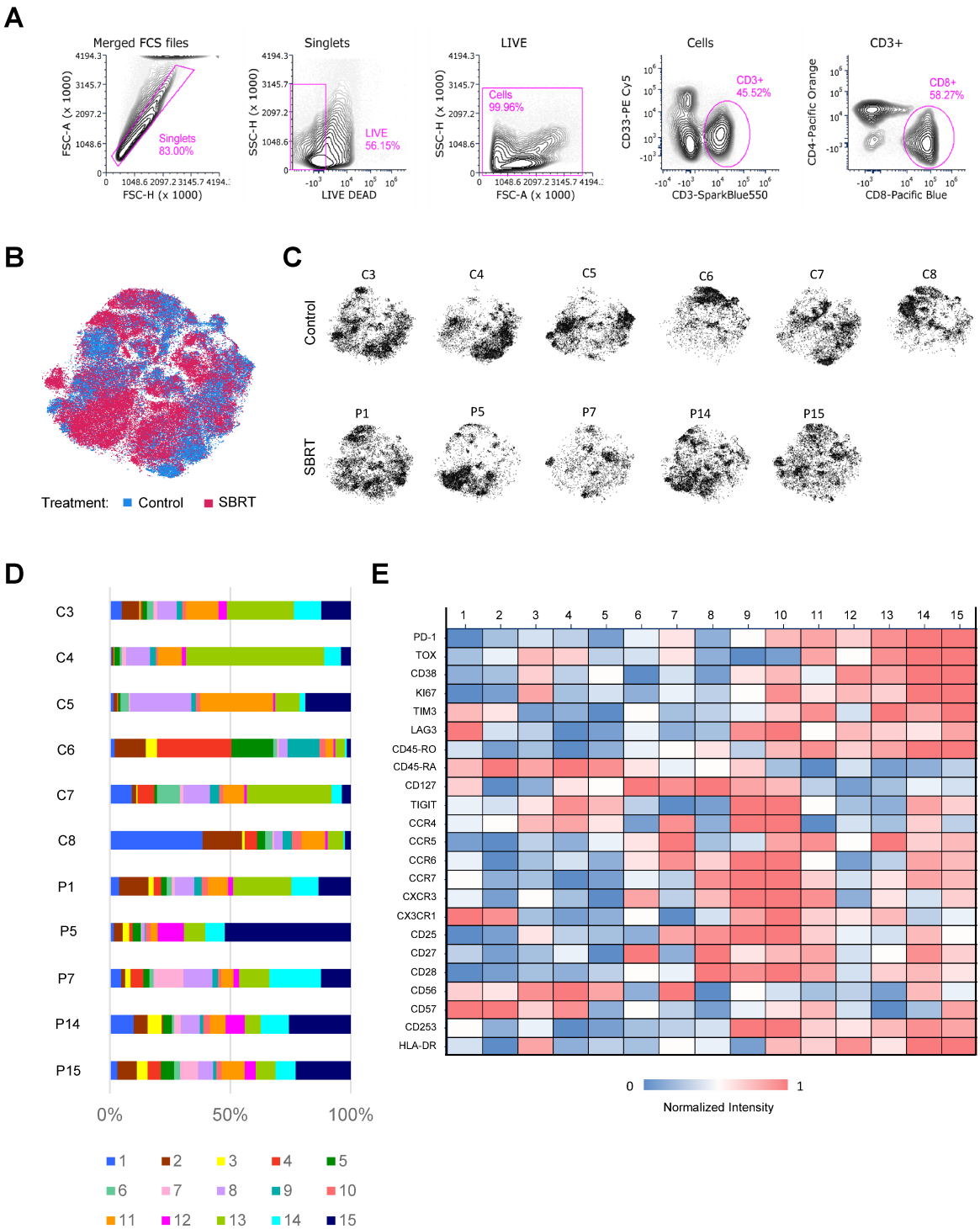
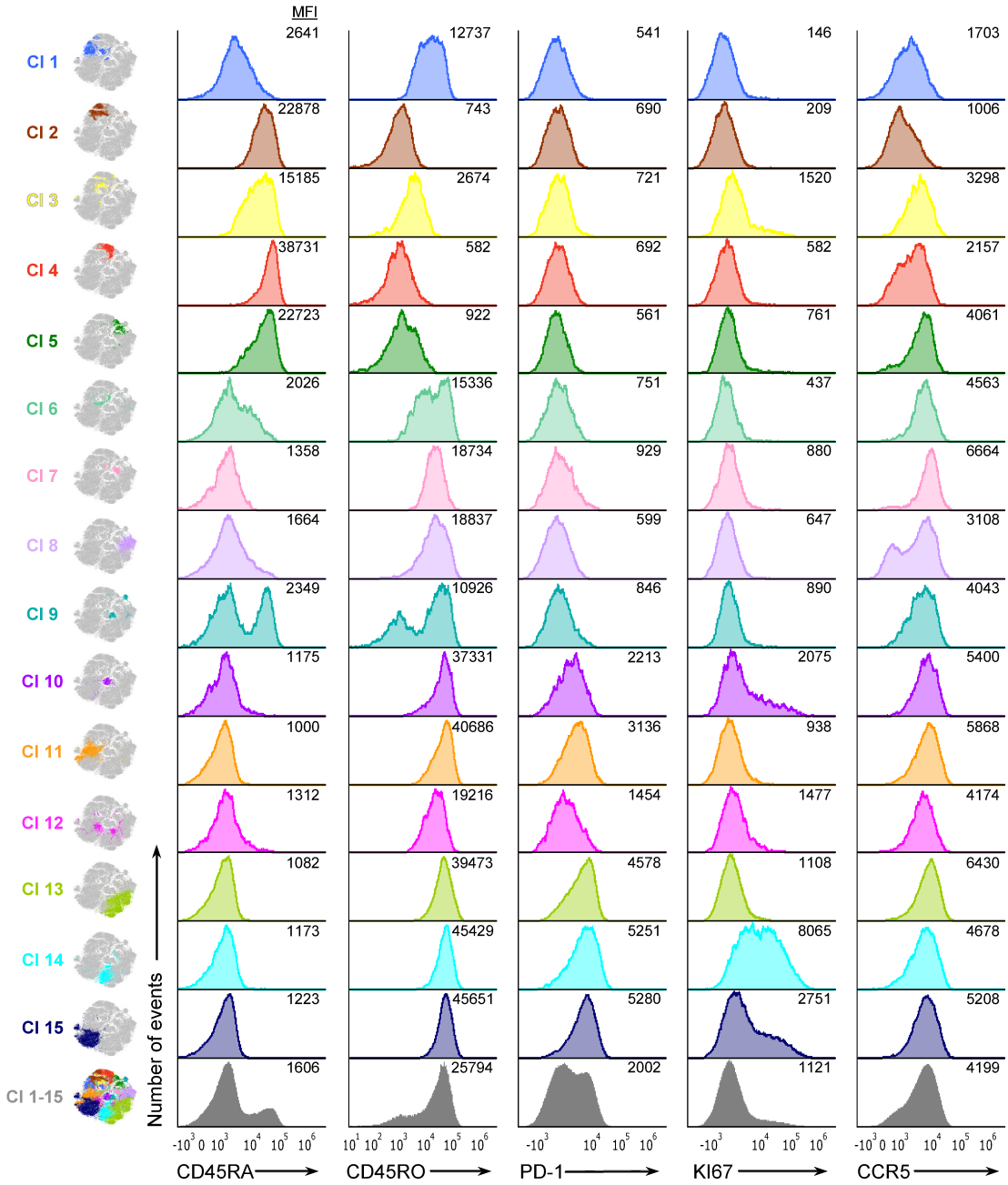


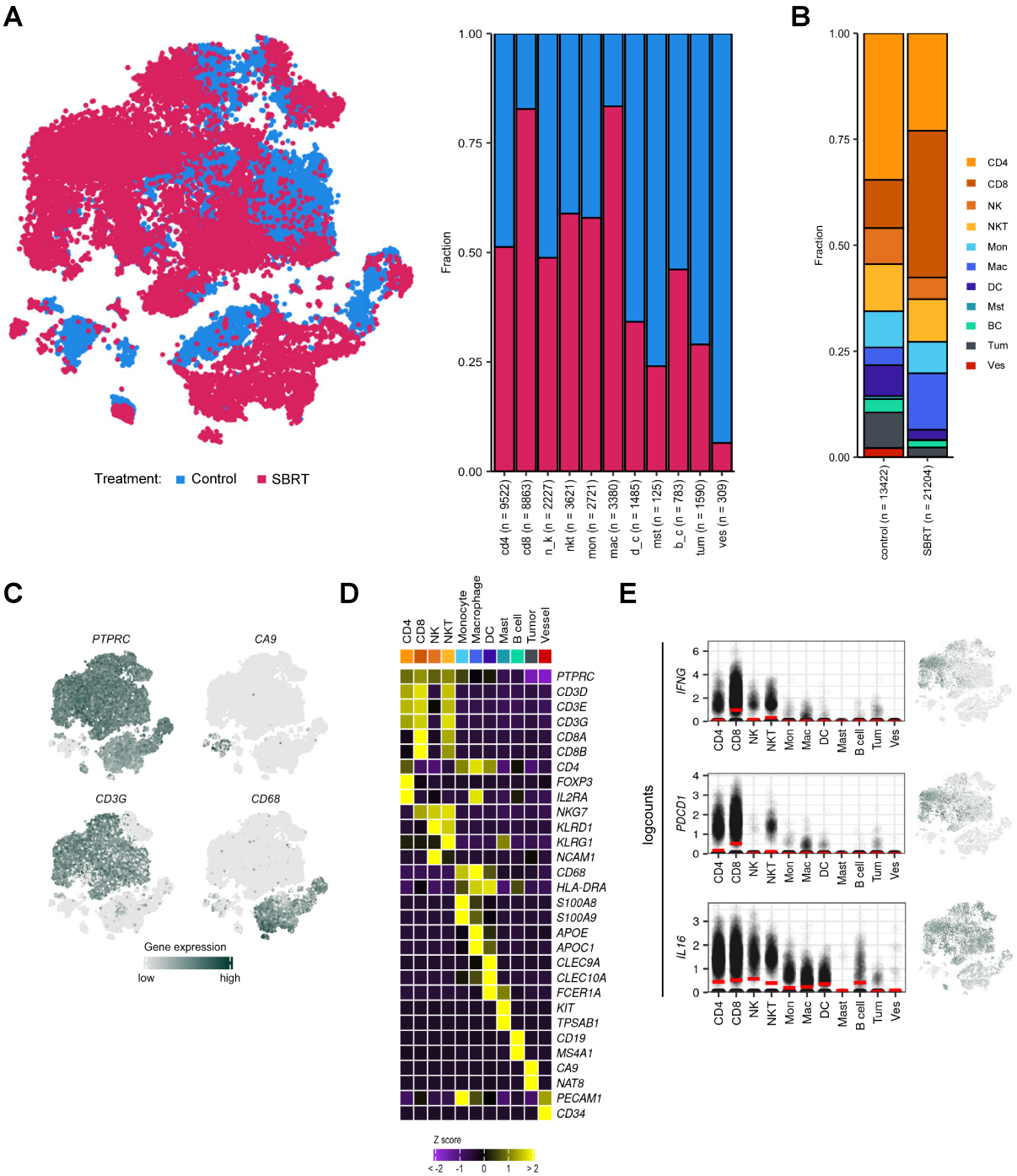
Supplemental Figure 1. Comparison of control vs SBRT treated cells by spectral flow cytometry. (A) t-SNE overlay plot comprising all cell subsets used in the analysis (see also figure 1B-D). (B) Frequency of major cell types within specified patient tumors. Colors and labels denote the major cell subsets. (C) Frequency of cells in each major cell type within control (blue) and SBRT-treated (red) patient samples. Statistical significance was calculated using multiple unpaired t-tests (*p < 0.05). Data presented as mean ± standard deviation.



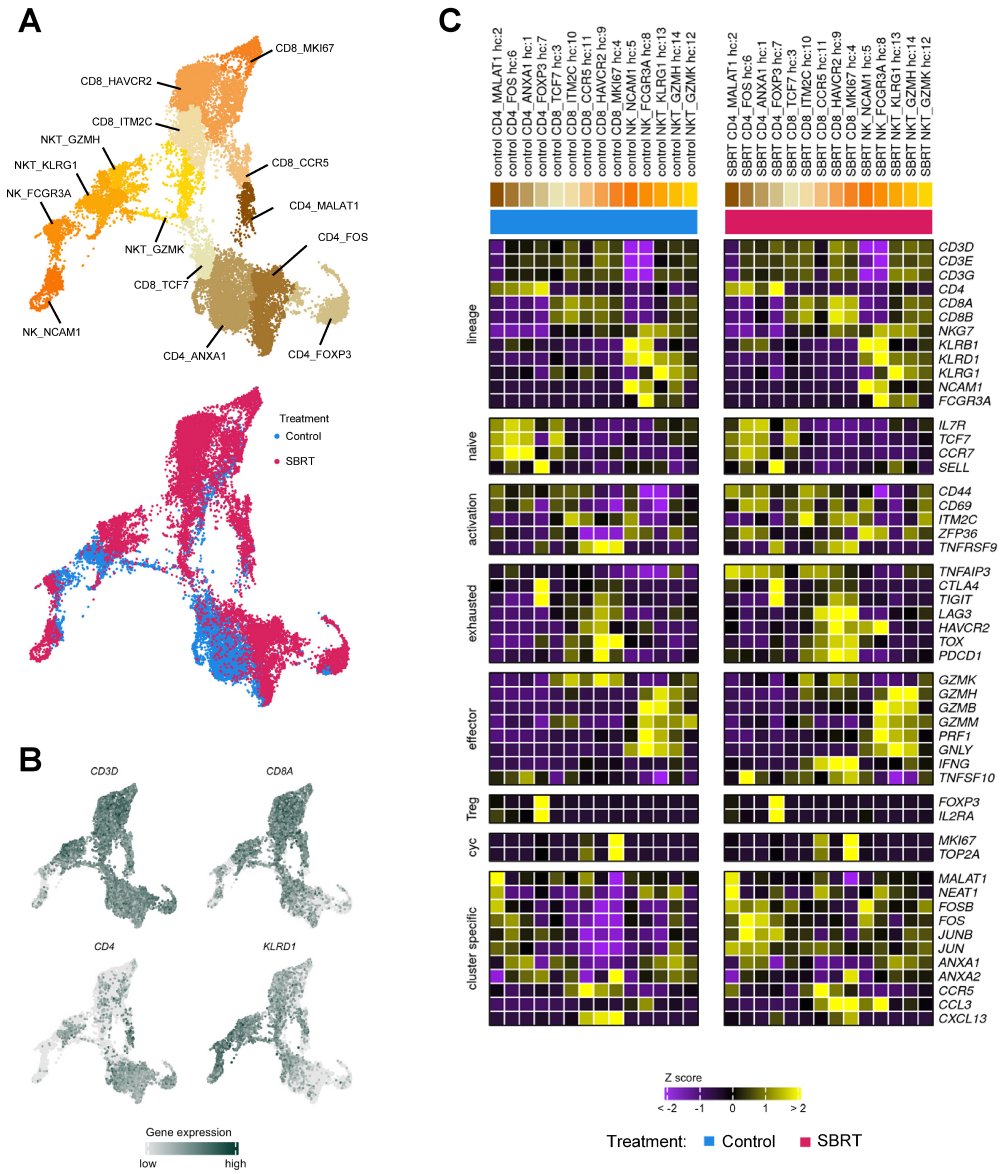
Supplemental Figure 2. Analysis of CD8⁺ T cells by spectral flow cytometry. (A) Gating strategy to export out CD8⁺ T cells from each patient sample for further analysis. (B) t-SNE overlay plot for CD8⁺ T cells used in the analysis. (C) Individual t-SNE plots for indicated samples. (D) Frequency of CD8⁺ T cell clusters within specified patient tumors. Colors and labels indicate the fifteen identified clusters by FlowSOM hierarchical clustering (see also figure 2A). (E) Heatmap showing expression of markers used for generating FlowSOM clusters and t-SNE plots for CD8⁺ T cells in all identified clusters.



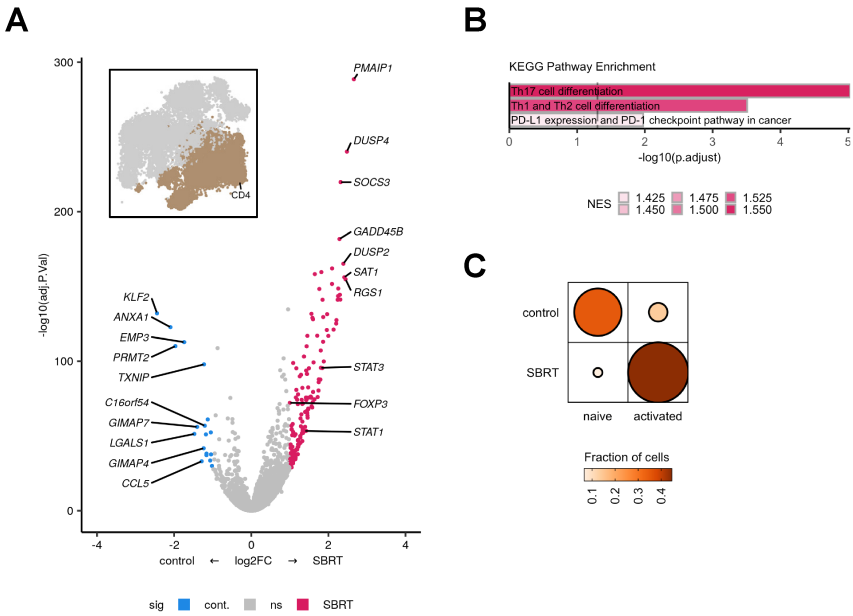
Supplemental Figure 3. Histogram analysis of all CD8⁺ T cell clusters identified by FlowSOM. Single parameter histograms are shown for comparison across 15 clusters identified by FlowSOM analysis. Numbers indicate median fluorescent intensity (MFI) value. Histogram for cumulative expression of listed markers in all CD8⁺ T cells is also included (Cl 1-15).



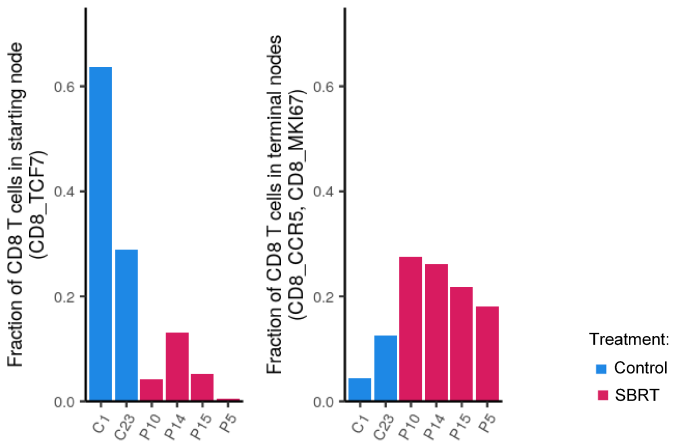
Supplemental Figure 4. Distribution of cells within cell classes and treatment groups. (A) Left, scRNAseq data of all cells plotted by treatment group (see also figure 3A). Right, overall number and distribution by treatment group of cells within broad cell classes. (B) Overall number and distribution by broad cell class within treatment groups. (C) Localized gene expression for indicated genes within the t-SNE. (D) Heatmap showing expression of established lineage markers by broad cell class. (E) Left: Expression of indicated genes by main class. Y axis is log counts. Red dash is mean expression. For each plot, p < 2.2e-16 by ANOVA. Right: Gene expression plotted on parent t-SNE.



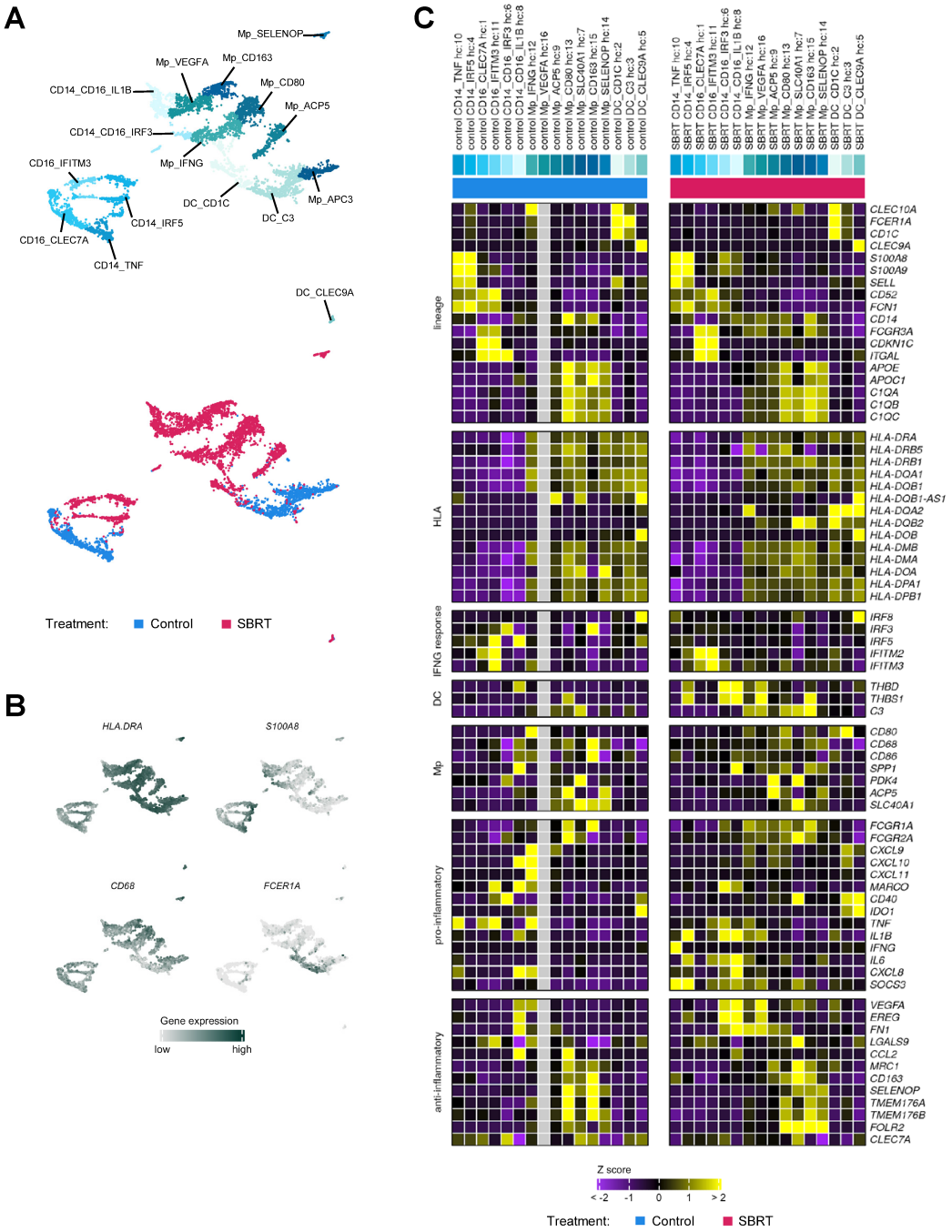
Supplemental Figure 5. Subclassification of lymphocytes. (A) UMAP recalculated for lymphocytes subset from the parent data. Top, lymphocyte reclustering into 14 subclusters. Bottom, distribution of cells by treatment group. (B) Localized gene expression for indicated genes within the lymphocyte UMAP. (C) Heatmaps showing expression of genes used for lymphocyte subclassification, divided by treatment group.



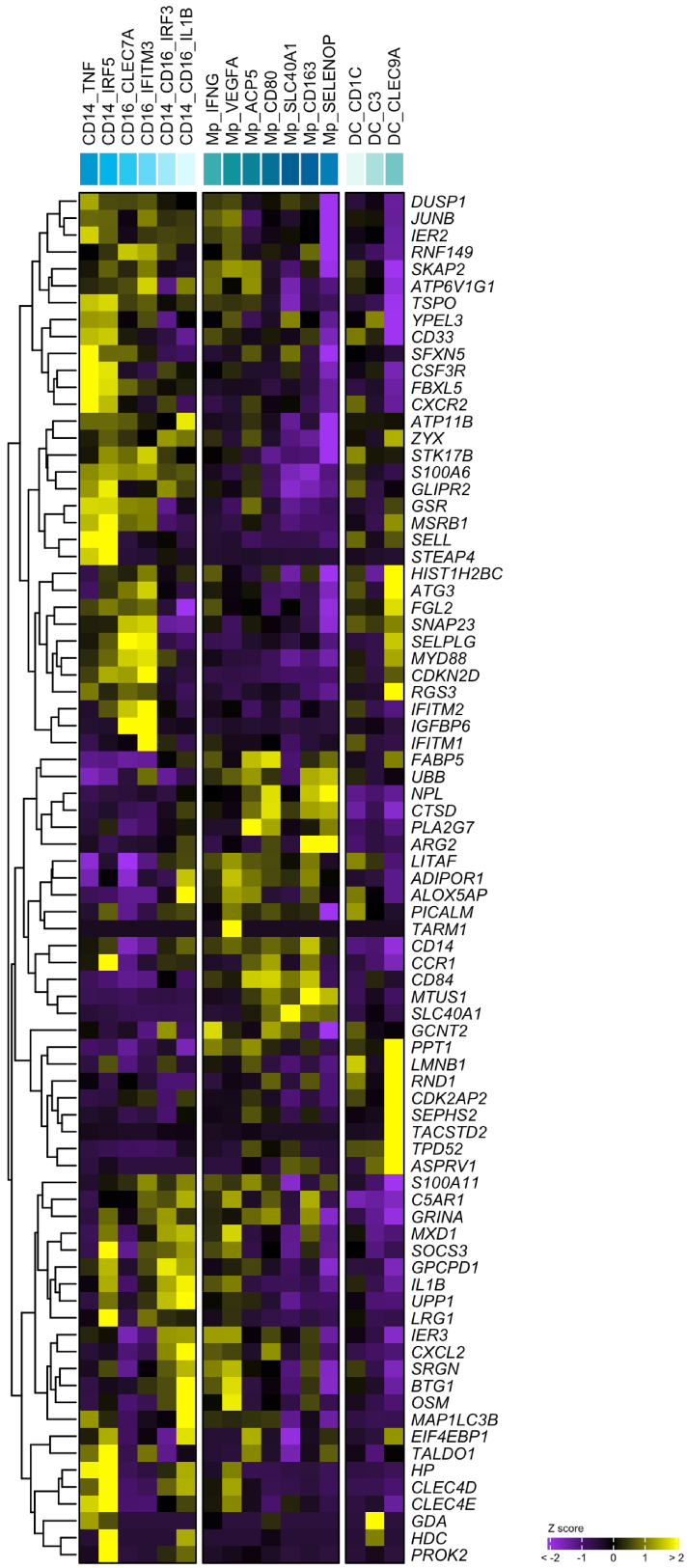
Supplemental Figure 6. Analysis of CD4⁺ T cells. (A) Volcano plot showing DGE within CD4⁺ T cells between treatment groups. Color is differential gene expression, abs(log2FC) > 1; adj.p < 0.05. Inset, CD4⁺ T cells subset from lymphocyte t-SNE. (B) GSEA of KEGG pathways. Opacity is NES. All shown pathways are significant, p.adj < 0.05. (C) Visualized chi square of naïve CD4⁺ T cells versus treatment group. Size and color show relative abundance after controlling for different total CD4⁺ T cell numbers between treatment groups.



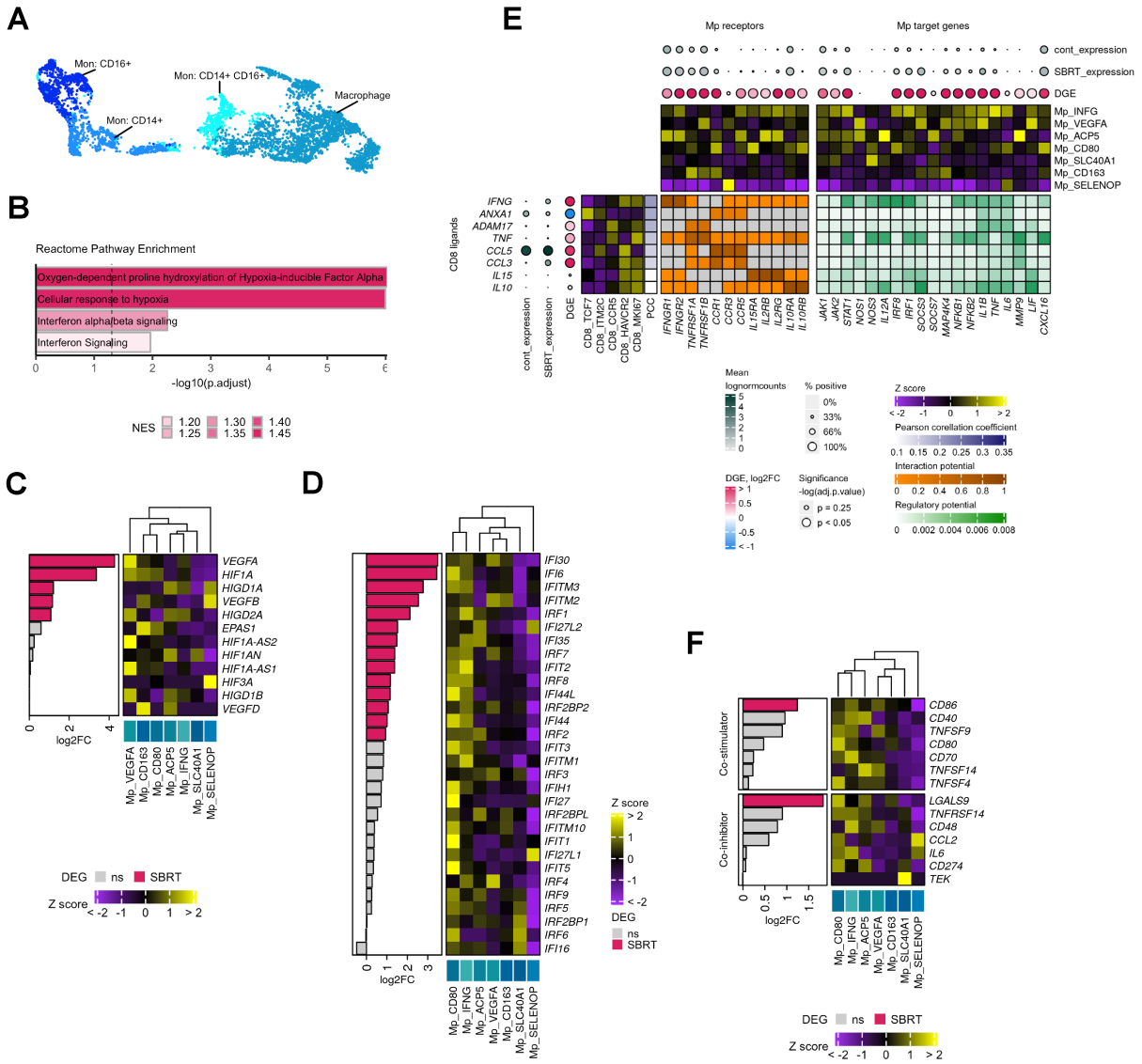
Supplemental Figure 7. Analysis of CD8⁺ T cell maturation across individual patient samples. Bar chart showing the fraction of CD8⁺ T cells in the pseudotime starting node (CD8_TCF7 dense) or pooled terminal nodes (CD8_MKI67, CD8_CCR5) for each patient. Color is treatment group for the sample.



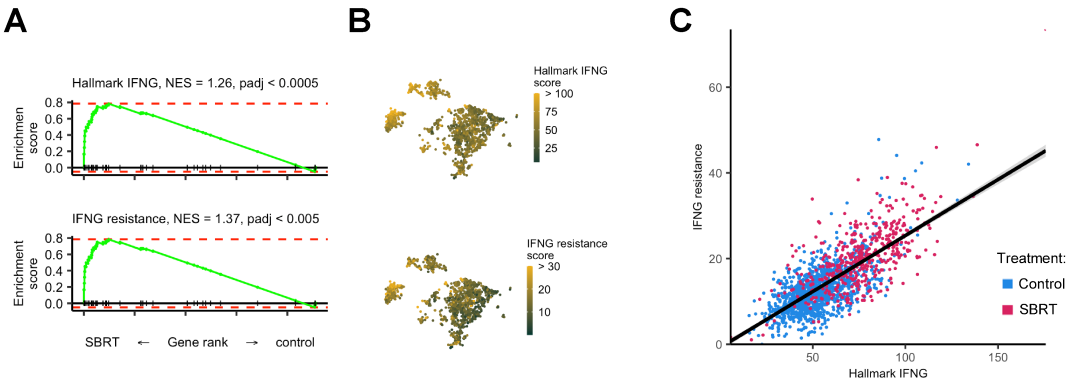
Supplemental Figure 8. Subclassification of myeloid cells. (A) UMAP recalculated for myeloid cells subset from the parent data. Top, myeloid cells reclustering into 16 subclusters. Bottom, distribution of cells by treatment group. (B) Localized gene expression for indicated genes within the myeloid UMAP. (C) Heatmaps showing expression of genes used for myeloid subclassification, divided by treatment group. Grey is no cells in the indicated subclass.



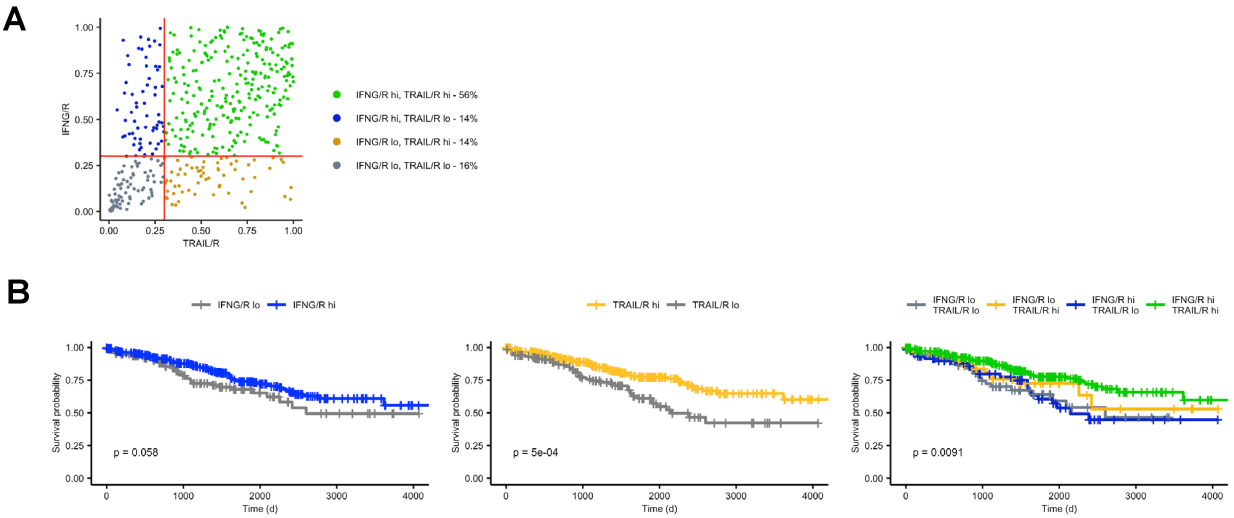
Supplemental Figure 9. MDSC gene signature in myeloid subclasses. Heatmap showing expression of MDSC signature genes across all identified myeloid subclasses.



Supplemental Figure 10. Analysis of macrophage subclasses. (A) Macrophages subset from monocyte and macrophage parent data. (B) GSEA of Reactome pathways. Opacity is NES. All shown pathways are significant, p.adj < 0.05. (C) Heatmap showing expression of indicated hypoxia associated genes by macrophage subclasses. Columns are clustered by gene expression. Bar graph shows log2FC by treatment group. Color is differential gene expression, log2FC > 1; p.adj < 0.05. (D) Heatmap showing expression of indicated interferon response genes by macrophage subclasses. Columns are clustered by gene expression. Bar graph shows log2FC by treatment group. Color is differential gene expression, log2FC > 1; p.adj < 0.05. (E) Predicted interactions from CD8 ligands to macrophage receptors and target genes. CD8 ligands are ordered by decreasing correlation to target gene expression, PCC. Z score and DGE are calculated separately for either the CD8$^+$ T cells or macrophage subsets. (F) Heatmap showing expression of indicated co-stimulatory and co-inhibitory genes by macrophage subclasses. Columns are clustered by gene expression. Bar graph shows log2FC by treatment group. Color is differential gene expression, log2FC > 1; p.adj < 0.05.



Supplemental Figure 11. Analysis of hallmark IFNG and IFNG resistance gene signatures in tumor cells. (A) GSEA of hallmark IFNG response and IFNG resistance pathways.[36] (B) Localization of hallmark IFNG and resistance signal by tumor cell t-SNE coordinates. (C) Dot plot showing hallmark IFNG response score versus IFNG resistance score with linear regression. Color is treatment group.



Supplemental Figure 12. Analysis of potential IFNG and TRAIL ligand to receptor interactions for RCC patient survival and ICB treatment response. Figure S12A-B analyze TCGA-KIRC primary tumors for stages I-III. (A) Dot plot showing distribution of TCGA patient tumors by TRAIL/TRAIL receptor and IFNG/IFNG receptor. Red lines show thresholds from hi and lo designations. Color is classification. (B) Kaplan-Meier curves comparing cross sections of samples from Figure S12A. Left: IFNG/R hi vs. low (right vs. left of vertical line); hazard ratio of IFNG/R hi = 0.68. Middle: TRAIL/R hi vs. low (above vs. below horizontal line); hazard ratio of TRAIL/R hi = 0.54. Right: Mixed effect model of IFNG/R score + TRAIL/R score; hazard ratio of IFNG/R hi = 0.8; hazard ratio of TRAIL/R hi = 0.57.

Patient	Treatment	Sex	Age	Path (T) Stage	Histology	scRNA Seq	Spectral Flow Cytometry
C1	Control	F	79	1B	Clear Cell	x	
C3	Control	F	53	1B	Clear Cell		x
C4	Control	M	63	3A	Clear Cell		x
C5	Control	F	61	1B	Clear Cell		x
C6	Control	M	55	2B	Clear Cell		x
C7	Control	M	53	3	Clear Cell		x
C8	Control	M	70	1B	Clear Cell		x
C23	Control	M	67	1B	Clear Cell	x	
P1	SBRT	M	53	2A	Clear Cell		x
P5	SBRT	M	67	3A	Clear Cell	x	x
P7	SBRT	M	67	3A	Clear Cell		x
P10	SBRT	F	75	3A	Clear Cell	x	
P14	SBRT	M	62	3A	Clear Cell	x	x
P15	SBRT	F	57	2A	Clear Cell	x	x

Supplemental Table 1. Patient demographic and clinical characteristics. List of patients, their treatment method, demographics (sex and age), pathology, histology, and the method of analysis.

Sl. No.	Marker	Clone	Fluorochrome	Dilution	Supplier	Catalog#
1	CCR5	2D7	BUV395	1 in 50	BD	565224
2	Live/Dead		LIVE/DEAD™ Blue	1 in 500	ThermoFisher	L34962
3	CD31	L1331.1	BUV496	1 in 200	BD	749833
4	CD14	MφP9	BUV563	1 in 100	BD	741441
5	CD261	S35-934	BUV615	1 in 50	BD	752308
6	CD11c	B-ly6	BUV661	1 in 200	BD	612967
7	CD56	NCAM16.2	BUV737	1 in 200	BD	612766
8	CD45RO	UCHL1	BUV805	1 in 100	BD	748367
9	CD68	Y1/82A	BV421	1 in 50	BD	564943
10	CD27	O323	SB436	1 in 200	ThermoFisher	62-0279-42
11	CD8	SK1	Pacific Blue	1 in 200	Biolegend	980906
12	KI67	B56	BV480	1 in 50	BD	566109
13	CD204	U23-56	BV510	1 in 50	BD	742439
14	CD4	RPA-T4	Pacific Orange	1 in 100	ThermoFisher	79-0049-42
15	CD28	CD28.2	BV605	1 in 100	Biolegend	302967
16	CXCR3	G025H7	BV650	1 in 50	Biolegend	353729
17	CCR6	G034E3	BV711	1 in 100	Biolegend	353435
18	CCR4	1G1	BV750	1 in 50	BD	746980
19	CCR7	G043H7	BV785	1 in 50	Biolegend	353229
20	CD57	NK-1	FITC	1 in 200	BD	555619
21	CD3	SK-7	Spark Blue 550	1 in 100	Biolegend	344852
22	CD45	2D1	Nova Blue 610	1 in 100	ThermoFisher	H005T03B05
23	PD-1	EH12.1	PerCP-Cy5.5	1 in 50	BD	561273
24	LAG3	3DS223H	PerCP-eFluor710	1 in 50	ThermoFisher	46-2239-42
25	TOX	REA473	PE	1 in 50	Miltenyi	130-120-716
26	TIGIT	A15153G	PE/Dazzle 594	1 in 50	Biolegend	372716
27	CD25	M-A251	PE-Fire/640	1 in 50	Biolegend	356148
28	CD33	HIM3-4	PE-Cy5	1 in 100	ThermoFisher	15-0339-42
29	CD127	A019D5	PE-Fire 700	1 in 100	Biolegend	351366
30	CD253	RIK-2	PE Cy7	1 in 50	Biolegend	308216
31	TIM3	F38-2E2	APC	1 in 50	ThermoFisher	62-3109-42
32	CD45RA	HI100	Spark NIR 685	1 in 200	Biolegend	304168
33	CX3CR1	G025H7	R718	1 in 50	Biolegend	353730
34	CD38	HIT2	APC-eFluor780	1 in 200	ThermoFisher	47-0389-42
35	HLA-DR	L243	APC/Fire 810	1 in 100	Biolegend	307674

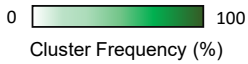
Supplemental Table 2. Antibody panel details. List of antibodies, clones, fluorochromes, dilutions, manufacturer, and catalog numbers used in spectral flow cytometry analysis.

Patients	Cluster frequencies of major cell subsets in each patient					
	CD8$^+$	CD4$^+$	T-regs	Myeloid	NK	CD45$^-$
C3	50.3%	20.2%	2.9%	8.9%	15.7%	2.0%
C4	18.1%	16.5%	2.8%	41.7%	17.3%	3.5%
C5	18.6%	16.0%	2.6%	14.9%	20.2%	27.6%
C6	26.2%	6.0%	0.3%	32.3%	15.2%	20.0%
C7	19.3%	18.5%	2.6%	30.2%	22.0%	7.3%
C8	18.8%	16.5%	1.0%	47.7%	5.9%	10.1%
P1	16.8%	13.9%	1.1%	31.2%	10.5%	26.5%
P5	65.0%	12.6%	3.0%	9.7%	2.0%	7.8%
P7	19.4%	24.2%	2.6%	11.5%	6.1%	36.4%
P14	26.8%	22.2%	5.3%	14.3%	13.6%	17.8%
P15	6.9%	9.9%	0.7%	6.9%	1.7%	74.0%

Cluster Frequency (%)

Supplemental Table 3. Cluster frequencies from spectral flow cytometry analysis. Cluster frequencies for major cell subsets identified by spectral flow cytometry analysis in six control (C3, C4, C5, C6, C7, and C8) and five SBRT treated (P1, P5, P7, P14, and P15) patient samples.

Patient	Cluster frequencies for CD8⁺ T cells in each cluster identified by FlowSOM														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
C3	5.09%	7.16%	0.48%	0.79%	1.85%	2.71%	1.66%	7.99%	2.33%	1.50%	13.63%	3.37%	27.94%	11.32%	12.17%
C4	0.90%	0.82%	0.16%	0.08%	2.19%	0.89%	1.71%	9.99%	2.38%	0.93%	9.90%	1.60%	57.43%	6.95%	4.05%
C5	1.52%	1.55%	0.10%	0.34%	0.97%	3.36%	0.66%	25.36%	1.82%	1.91%	30.30%	0.79%	10.17%	2.29%	18.84%
C6	2.02%	13.00%	4.57%	30.94%	17.37%	1.49%	0.84%	3.64%	13.17%	2.63%	3.24%	0.79%	3.87%	0.86%	1.58%
C7	9.16%	1.81%	0.49%	7.05%	1.15%	9.44%	1.37%	11.10%	3.80%	1.75%	8.69%	1.02%	35.17%	4.34%	3.66%
C8	38.54%	16.42%	1.12%	5.10%	3.30%	3.01%	0.67%	3.61%	3.80%	4.04%	9.81%	1.13%	6.41%	0.66%	2.37%
P1	3.85%	12.28%	2.09%	3.14%	2.27%	2.15%	1.15%	8.18%	3.01%	2.62%	8.39%	2.06%	24.30%	11.20%	13.32%
P5	1.59%	3.79%	2.69%	1.38%	3.38%	0.23%	0.32%	1.21%	0.33%	2.10%	2.91%	10.85%	8.85%	8.14%	52.21%
P7	4.71%	1.63%	2.33%	5.28%	2.44%	1.85%	12.39%	12.08%	2.27%	1.34%	5.06%	2.31%	12.63%	21.45%	12.23%
P14	9.97%	5.77%	5.73%	0.51%	3.80%	0.89%	2.77%	7.97%	1.43%	2.72%	6.55%	7.90%	6.68%	11.68%	25.62%
P15	3.16%	8.13%	4.41%	5.52%	5.42%	2.54%	7.43%	6.26%	1.29%	2.30%	9.60%	4.46%	8.25%	8.44%	22.79%



Supplemental Table 4. Cluster frequencies of CD8⁺ T cells from spectral flow cytometry analysis. Cluster frequencies for CD8⁺ T cell clusters identified by spectral flow cytometry analysis in six control (C3, C4, C5, C6, C7, and C8) and five SBRT treated (P1, P5, P7, P14, and P15) samples.

Sub class	Raw cell numbers					
	C1	C23	P10	P14	P15	P5
CD4_ANXA1	3170	165	188	37	233	177
CD4_FOS	157	654	430	576	746	704
CD4_FOXP3	241	88	328	384	160	462
CD4_MALAT1	161	6	179	91	96	89
CD8_CCR5	25	6	84	112	67	338
CD8_HAVCR2	202	178	252	463	150	3315
CD8_ITM2C	164	46	142	179	182	958
CD8_MKI67	26	42	76	165	32	604
CD8_TCF7	728	111	25	140	24	27
NK_FCGR3A	202	545	77	158	47	37
NK_NCAM1	302	92	235	377	62	93
NKT_GZMH	5	10	74	107	197	450
NKT_GZMK	49	145	101	434	56	65
NKT_KLRG1	58	1223	58	396	164	29

Supplemental Table 5. Single-cell RNA sample composition. Raw cell numbers for distinct T and NK cell subclasses identified in control (C1 and C23) and SBRT treated (P10, P14, P15, and P5) patients by single-cell RNA seq analysis.