Supplementary Information for

Radiation induces dynamic responses to the T cell repertoire in renal cell carcinoma patients

Jacky Chow, Nicholas C. Hoffend, Scott I. Abrams, Thomas Schwaab, Anurag K. Singh, Jason B. Muhitch

Correspondence: Jason Muhitch
Email: Jason.Muhitch@RoswellPark.org

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Tables S1 and S2
Figures S1 to S7

Table S1. SBRT Patient Samples

(a) All tumor samples presented with clear cell histology

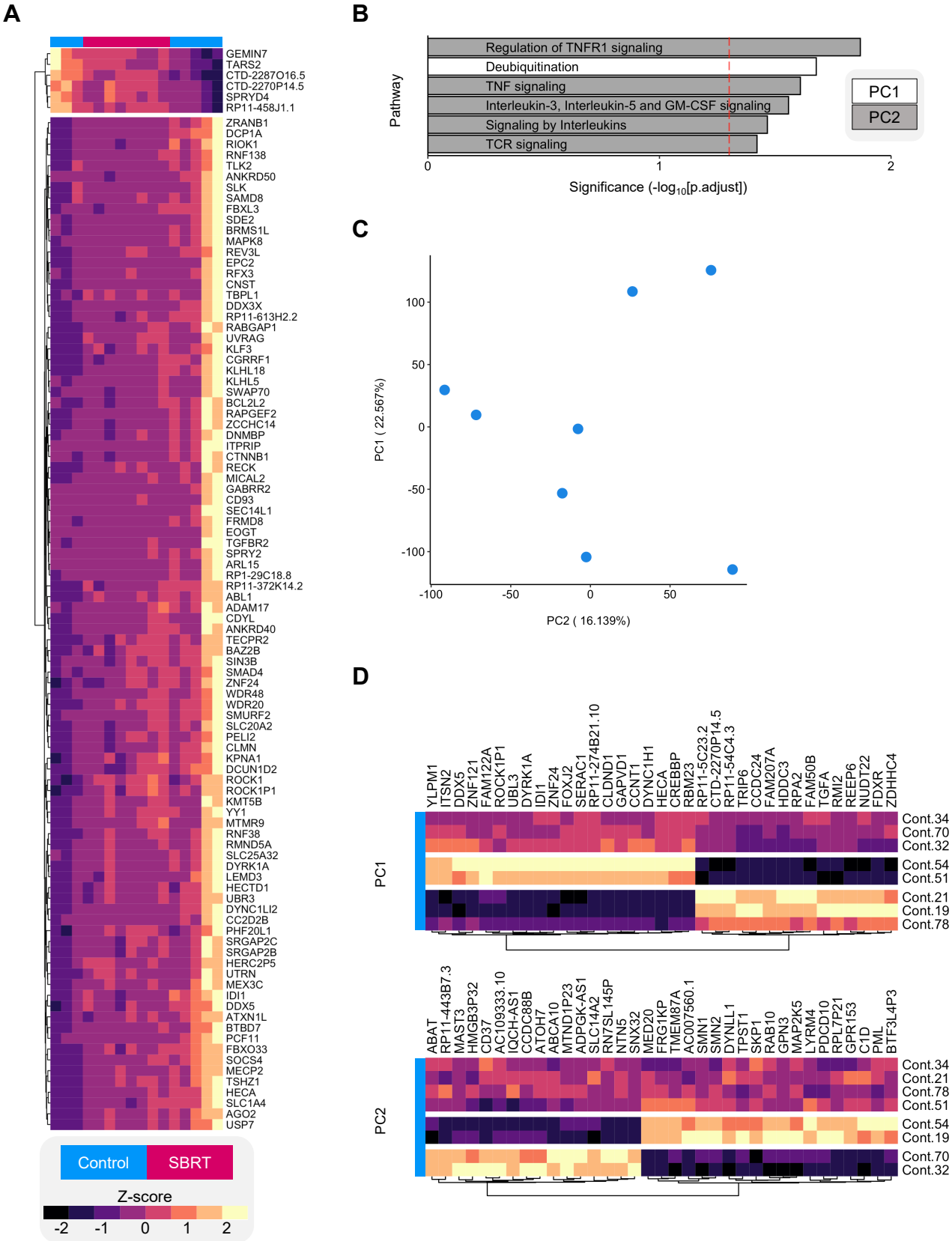
(b) Only patients with both 2 week and 4 week blood sample data had serial blood analysis

Patient	Age	Sex	Pathologic T stage	Histology (a)	RNAseq	Tumor TCRseq	Blood TCRseq (b)
P1	53	M	2A	clear cell	X	X	X
P3	75	F	3A	clear cell	X	X	X
P5	67	M	3A	clear cell		X	X
P6	53	F	4	clear cell	X	X	X
P7	67	M	3A	clear cell		X	X
P9	65	M	1A	clear cell		X	X
P10	75	F	3A	clear cell	X	X	X
P13	60	F	2	clear cell	X	X	
P14	62	M	3A	clear cell	X	X	
P15	57	F	2A	clear cell	X	X	X
P16	63	F	3A	clear cell	X	X	X

Table S2. Control RCC tumor samples

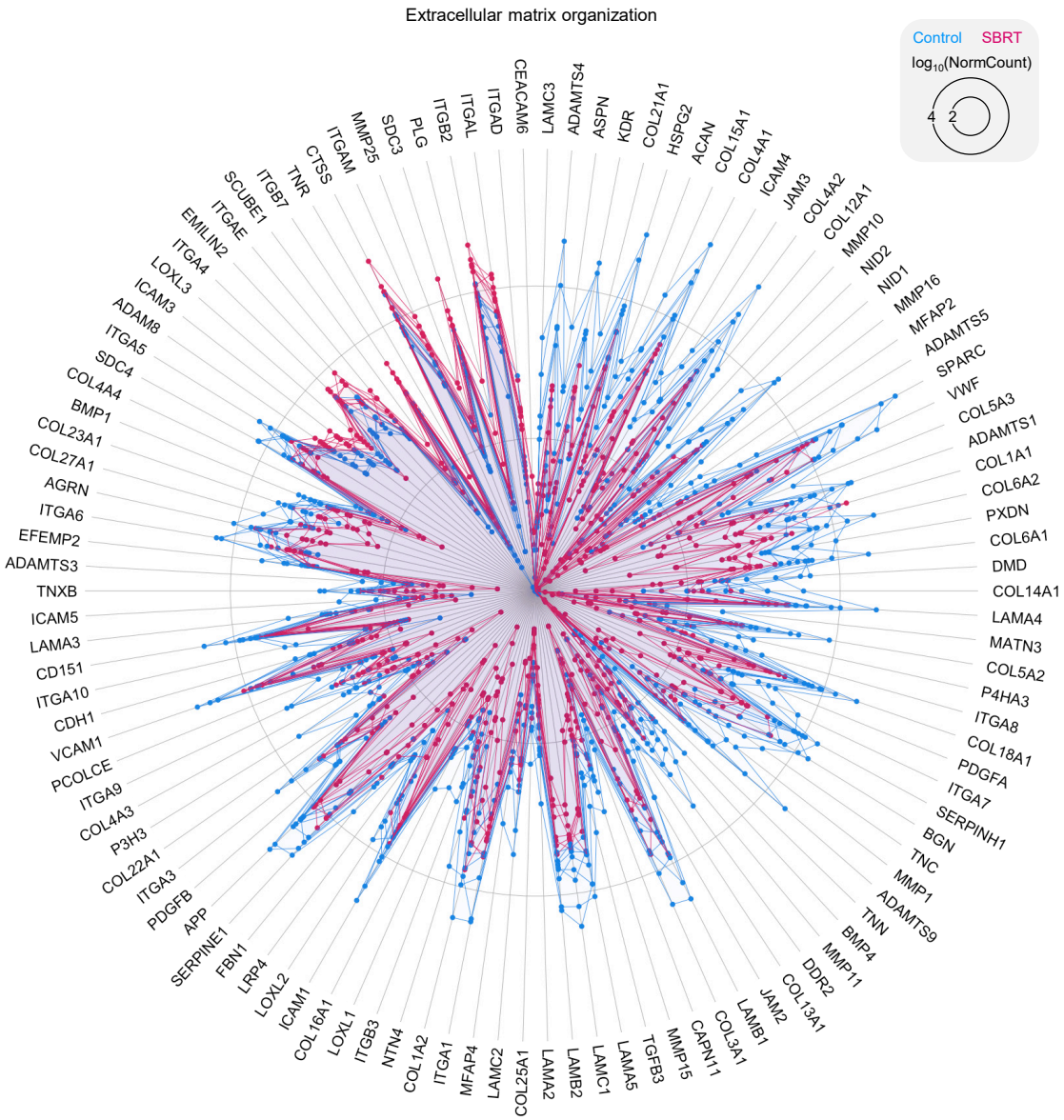
(a) All tumor samples presented with clear cell histology

Patient	Age	Sex	Pathologic T stage	Histology (a)	Experiment
C70	62	F	1B	clear cell	RNAseq
C34	71	F	1B	clear cell	RNAseq
C32	50	M	2A	clear cell	RNAseq
C78	51	M	2A	clear cell	RNAseq
C21	55	F	1B	clear cell	RNAseq
C19	60	F	3A	clear cell	RNAseq
C54	70	F	1A	clear cell	RNAseq
C51	74	F	1B	clear cell	RNAseq
C12	82	F	3A	clear cell	TCRseq
C13	62	M	3B	clear cell	TCRseq
C14	62	M	2A	clear cell	TCRseq
C16	40	M	3A	clear cell	TCRseq
C27	55	M	2A	clear cell	TCRseq
C30	57	M	3	clear cell	TCRseq
C40	58	M	3A	clear cell	TCRseq
C47	43	M	3A	clear cell	TCRseq
C5	45	M	3A	clear cell	TCRseq
C52	74	F	3A	clear cell	TCRseq
C56	52	F	3A	clear cell	TCRseq
C57	47	M	3A	clear cell	TCRseq
C62	60	F	3A	clear cell	TCRseq
C7	66	M	3A	clear cell	TCRseq
C77	51	M	2A	clear cell	TCRseq

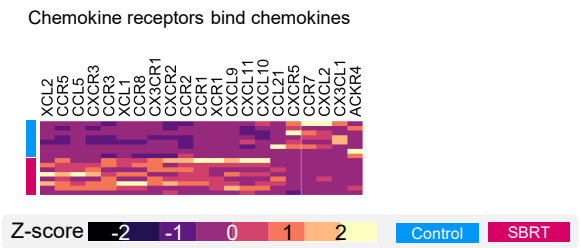


Supplemental Fig 1. Analysis of variance by PC1 and variance among control samples. (A) Heat map of top 100 contributors to PC1. Genes (rows) clustered by normalized expression among patient samples. Samples (columns) arranged according to distribution along PC1. (B) Selected enriched pathways of top 100 contributors to PC1 and PC2. Red line is p.adjust = 0.05. (C) PCA of control samples. (D) Top contributors to PC1 and PC2 for control sample PCA.

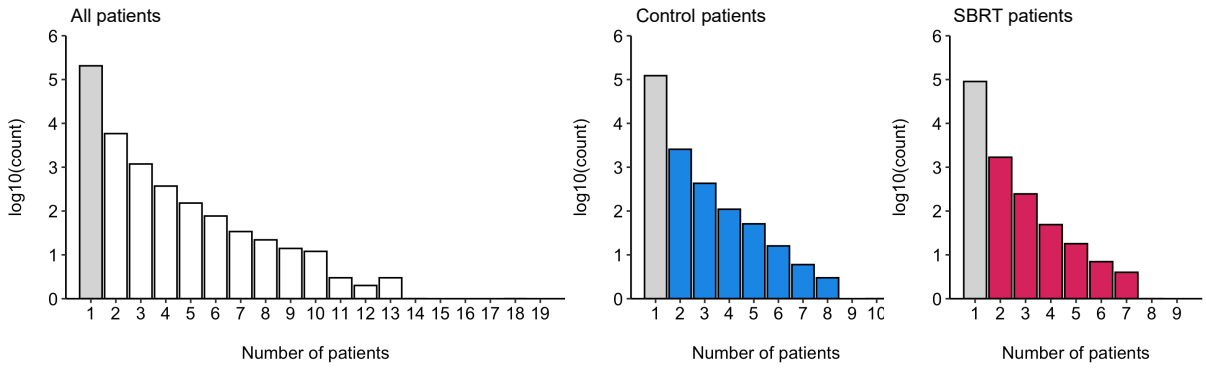
A



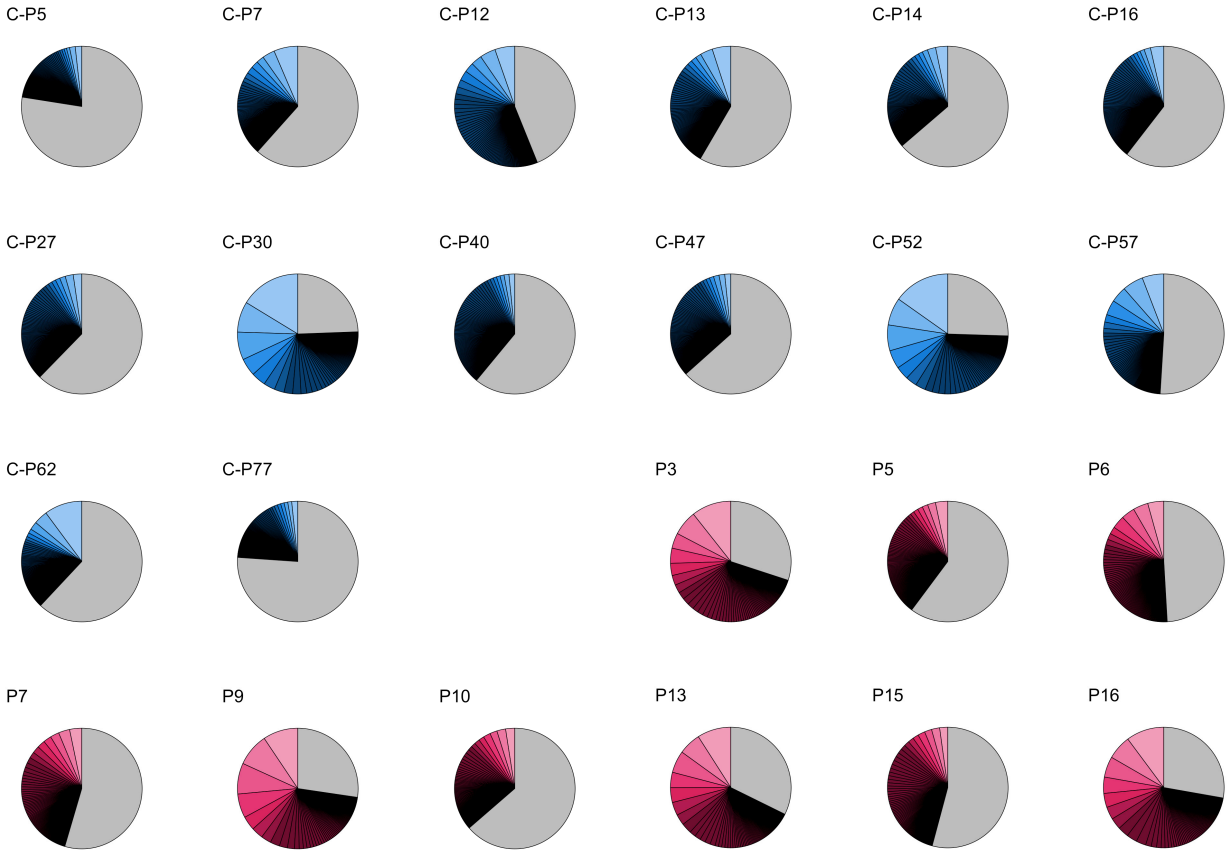
B



Supplemental Fig 2. Differentially expressed genes of (A) Extracellular matrix organization and (B) Chemokine receptors bind chemokines DE gene sets. Lines represent individual patient values for indicated DE genes.



Supplemental Fig 3. Interpatient sharing of CDR3 amino acid sequences. Bar charts showing interpatient sharing of T cell AA sequences among all, control only, and SBRT only tumors; y-axis is number of AA sequences shared among the number of patients in the x-axis. Gray bars are AA sequences specific to one patient.



Supplemental Fig 4. TCR repertoire characteristics. Pie charts reflecting frequency of top 100 clones per patient.

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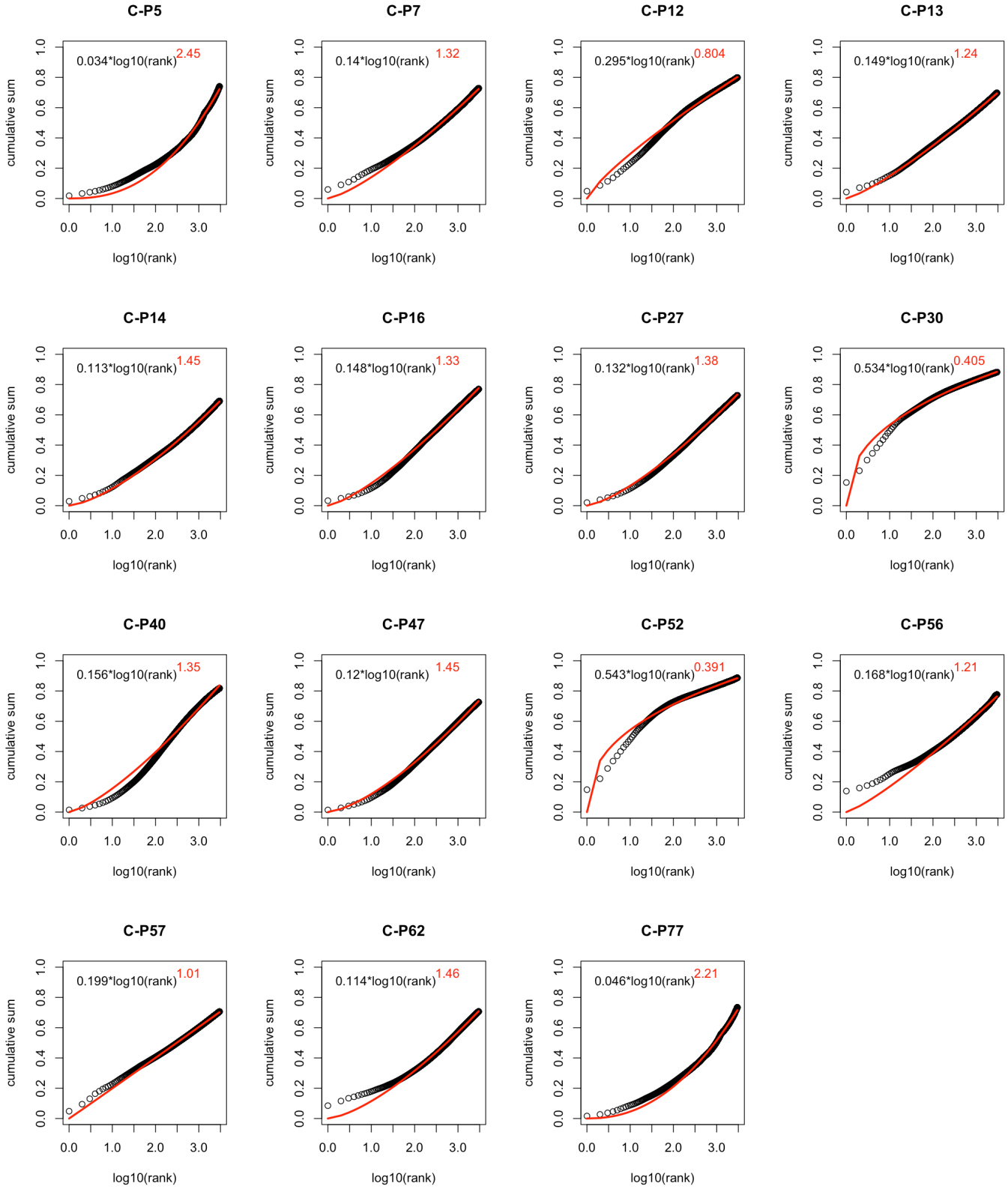
Supplemental Figure 5



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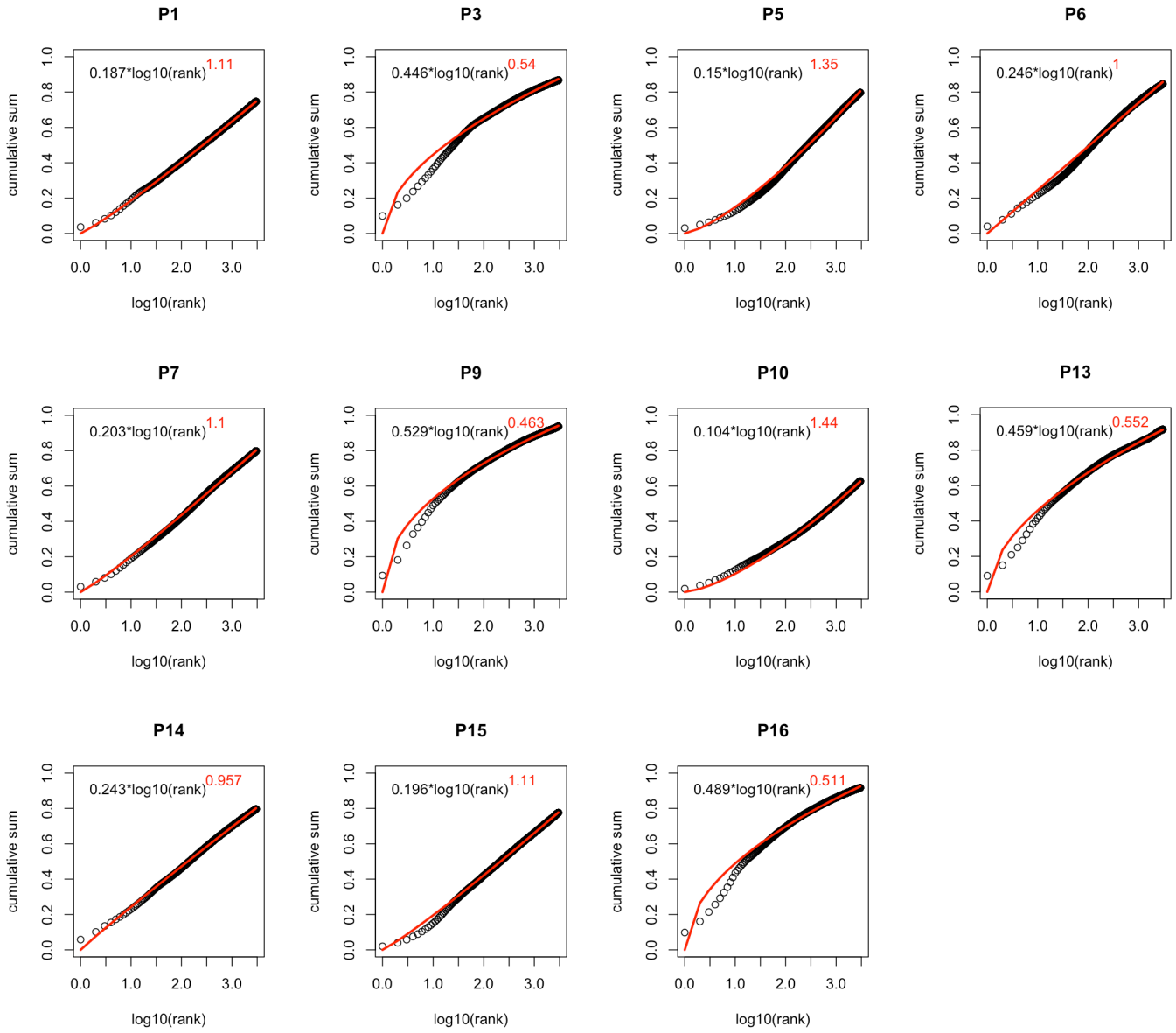
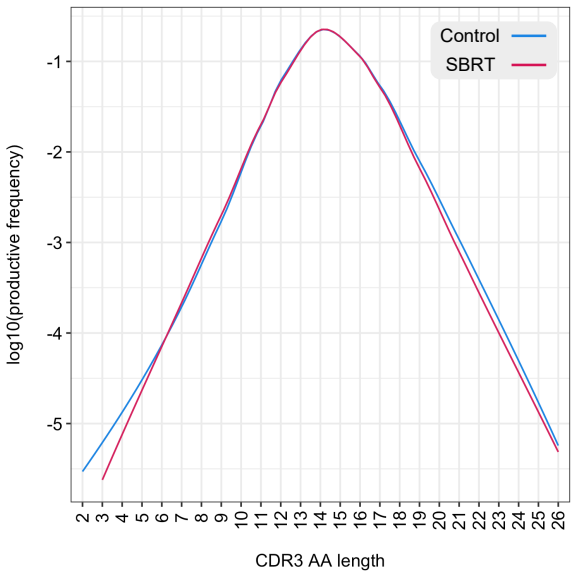
Supplemental Figure 5 (cont.)



Supplemental Fig 5. Non-linear least squares regression of cumulative sum of TCR clonotypes. Dot plot of cumulative sum for top 10,000 clonotypes by patient, red line is regression. Regression equation at top, red is power coefficient, all coefficients are significant $P < 0.001$.



Supplemental Fig 6. Frequency of CDR3 AA length. Linear model showing total frequency of T cell AA residue of a given length in control and patient tumors.

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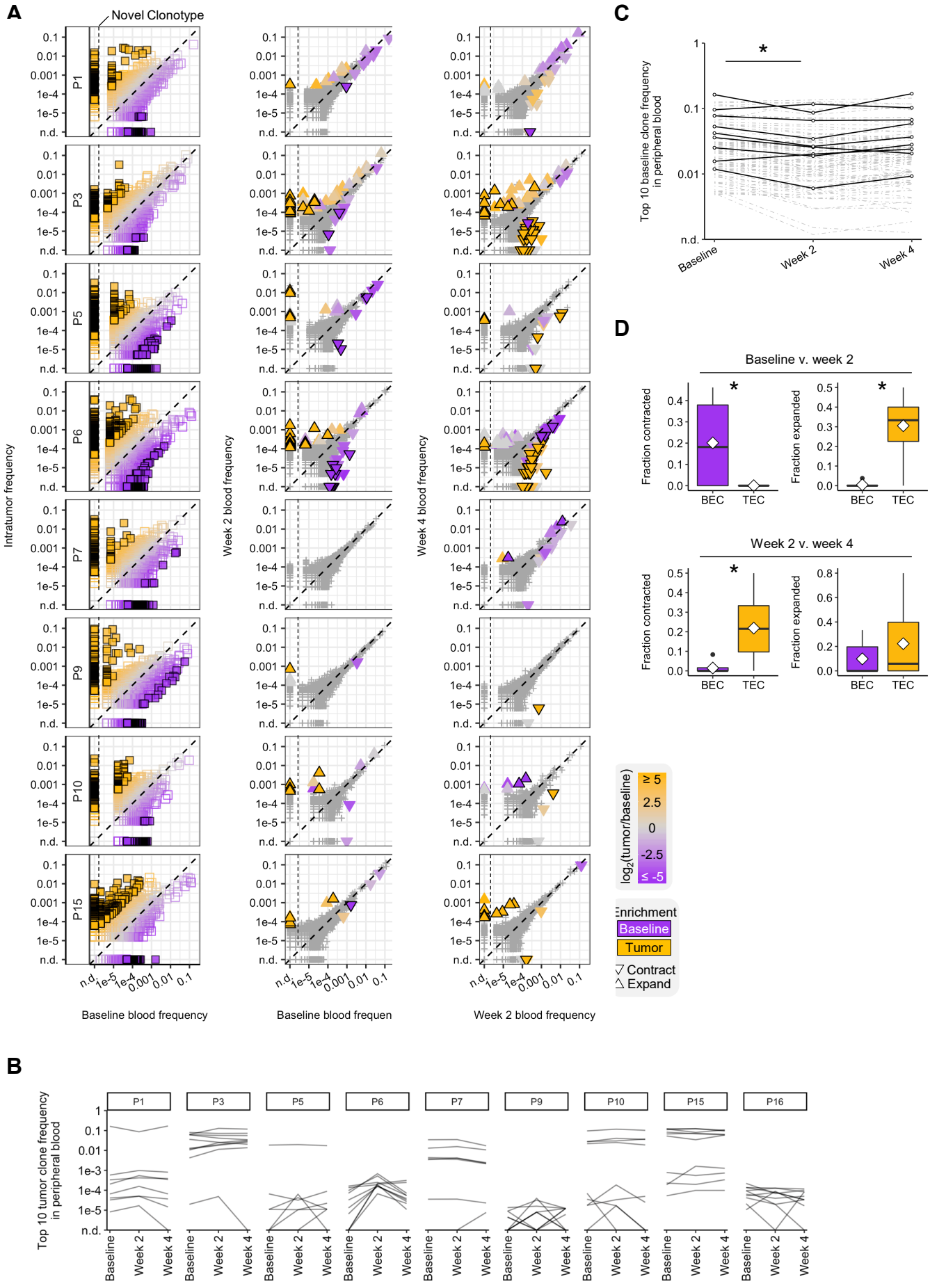
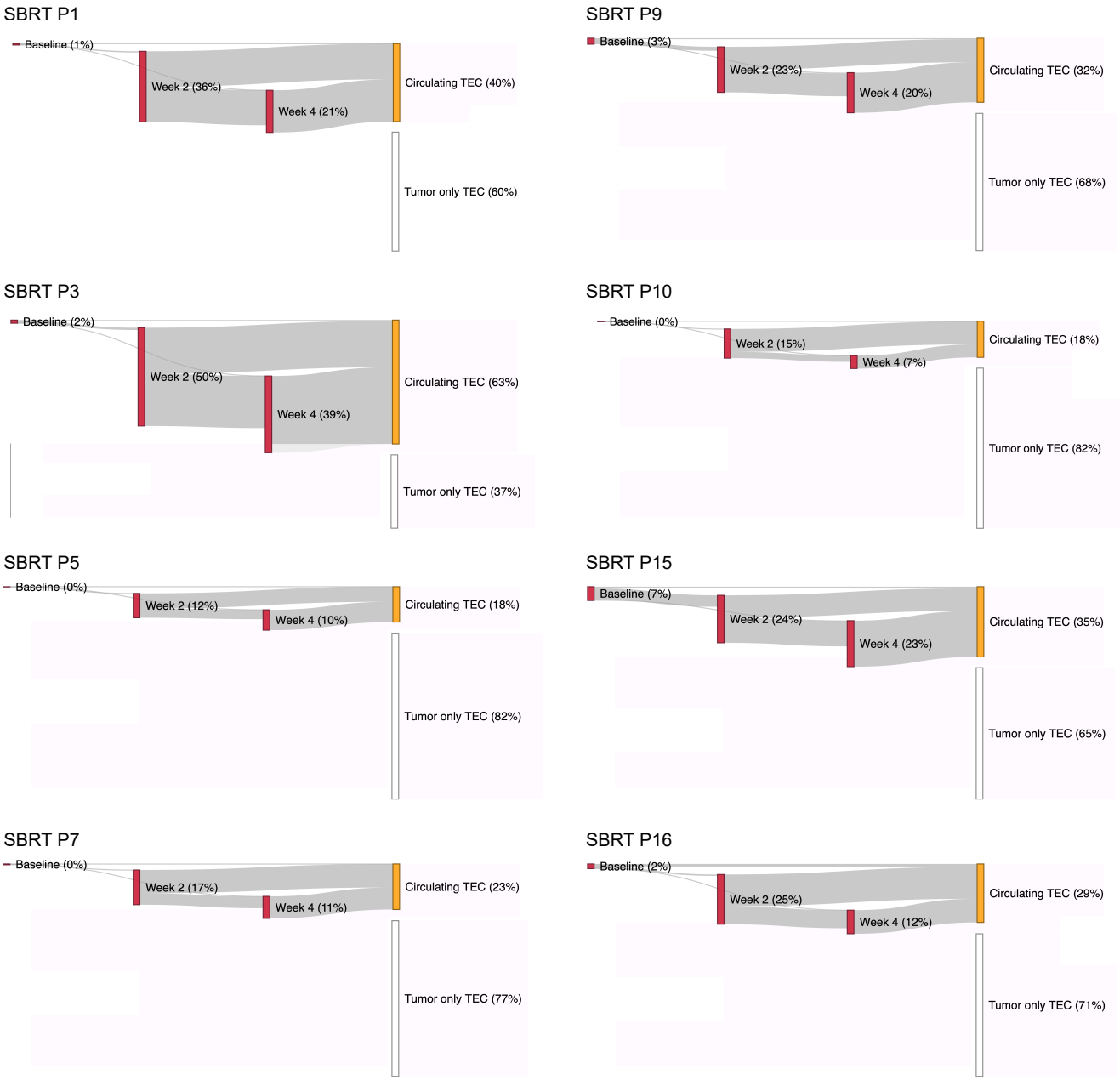
Supplemental Figure 7



Supplemental Fig 7. Dynamic peripheral changes in T cell clonotype frequencies. (A) Scatter plots showing distribution of clonotypes among intrapatient samples. Baseline peripheral blood v. the tumor. Color shows relative frequency in the tumor (orange) or baseline blood (purple), sample enriched clonotypes are filled squares, left of vertical line are novel tumor clonotypes. Comparisons between longitudinal peripheral blood samples. Up triangle indicates expansion, down triangle indicates contraction, color is sample enrichment. (B) From Fig. 5C, frequency of top 10 most abundant tumor clones in peripheral blood divided by patient. (C) Frequency of top 10 most abundant baseline peripheral blood clones in pateint blood. Black lines are representative clones per patient. (D) Box plots showing fraction of expanded or contracted clonotypes being baseline blood or tumor enriched. Tumor only TEC is fraction of TECs that were not detected in any blood sample. All * $P < 0.05$; Mann-Whitney-Wilcoxon paired two-tail test.



Supplemental Fig 8. Emergence and detection of TEC in peripheral blood after SBRT. Sankey plots tracking TEC in serial peripheral blood samples after therapeutic radiation for indicated patients.