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Supplemental Information

Combined TCR Repertoire Profiles and Blood Cell Phenotypes Predict Melanoma Patient Response to Personalized Neoantigen Therapy plus Anti-PD-1

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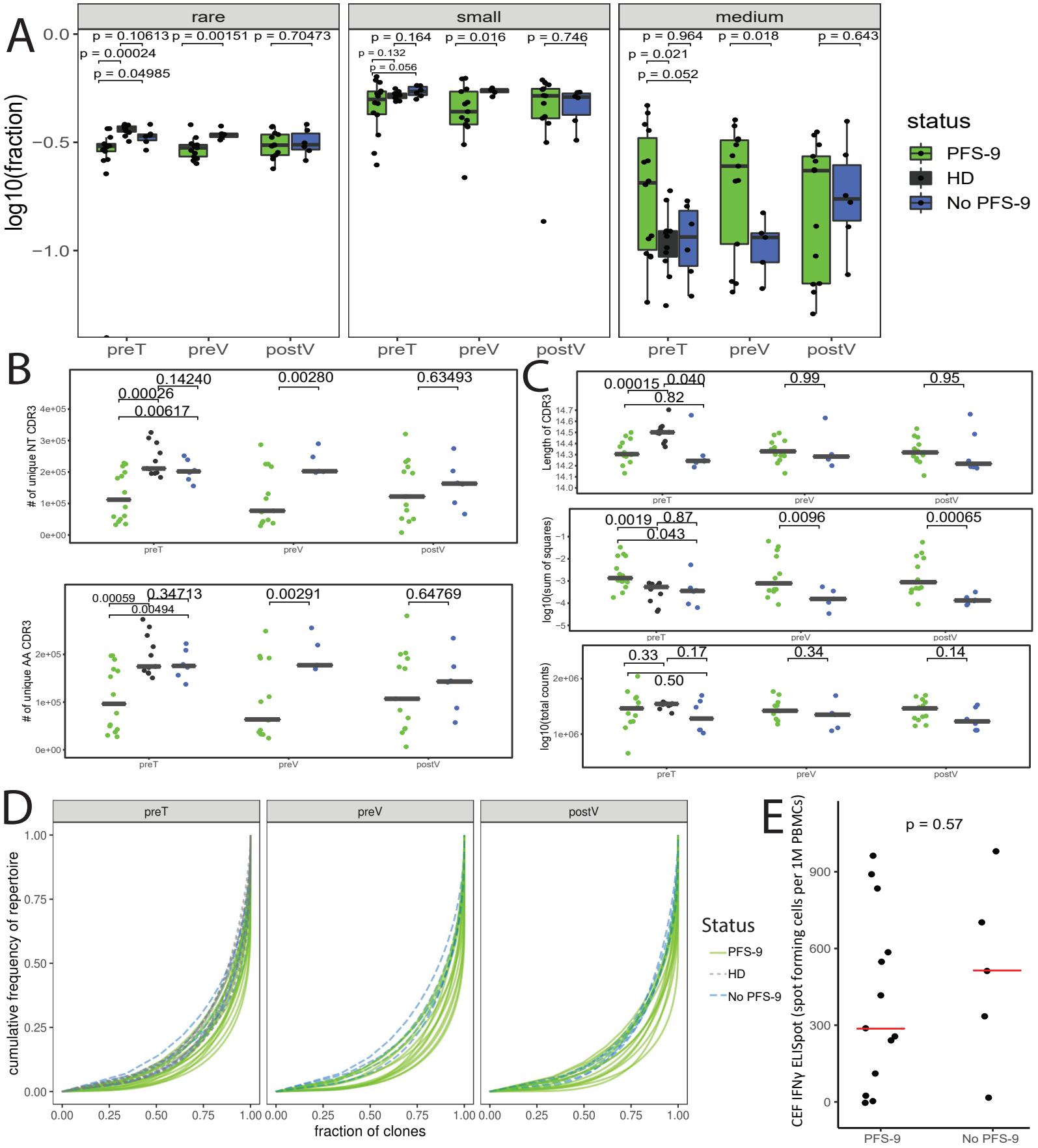

Fig. S1 – **Comparison of additional TCR repertoire features and clonality measurements between patients with and without PFS-9 (Related to Figure 1). A)** The (log-)fraction of clones belonging to the "rare" (left), "small" (center), or "medium" (right), of patients with or without PFS-9 at each time-point, in addition to 11 healthy donors. Boxplots indicate 25%, 50% and 75% percentiles and whiskers extend to the smallest/largest value within 1.5 times the interquartile range. Related to Fig. 1C. **B)** The number of unique amino acid (AA, bottom) and nucleotide (NT, top) sequences of each patient or HD across time-points. Black line indicates median. Related to Fig. 1D. **C)** Average length of the CDR3 (top), log10(sum of squares) (middle), and the total counts of TCRs (bottom) of each patient or HD across time-points. Black line indicates median. Related to Fig. 1D. **D)** Lorentz curves of the TCR repertoires of each patient or HD across time-points. Lines represent cumulative frequency of clones, sorted from least frequent to most frequent. Dotted lines indicate PFS-9, solid lines indicate no PFS-9. Related to Fig. 1D, "DE50". **E)** Ex vivo IFN-g ELISpot assay spot counts comparing reactivity to CMV, EBV, or influenza between patients with and without PFS-9. Red bar represents median.

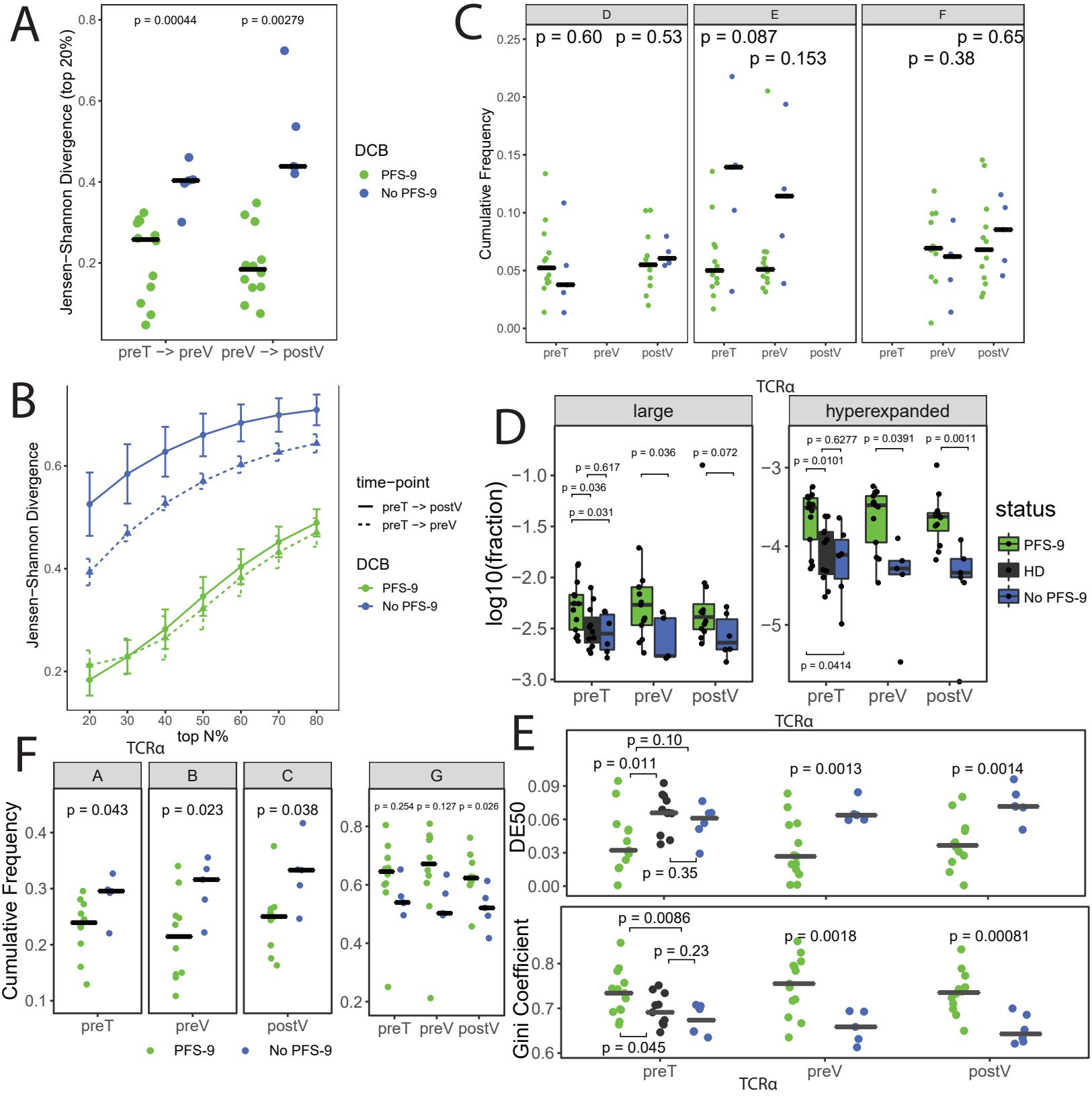

Fig. S2 – **Expanded TCRβ repertoire stability measurements and TCRα repertoire measurements (Related to Figure 1)**

A) Jensen-Shannon Divergence (JSD) of clones accounting for the top 20% of the repertoire between preV and pre T (left) or postV and preV (right) of patients with and without PFS-9. Low JSD values represent repertoire stability. Related to Fig. 2A. Black line indicates median.

B) Jensen-Shannon Divergence (JSD) of clones accounting for the top 20 - 80% of the repertoire between preV (dotted line) or postV (solid line) and baseline of patients with and without PFS-9. Low JSD values represent repertoire stability. Related to Fig. 2A.

C) Comparison of the cumulative frequencies of clones in either one the segments: D, E, and F between patients with and without PFS-9, at each time-point. High cumulative frequency of segment represents repertoire stability. Black line indicates median. Related to Fig. 2C.

D) The (log-)fraction of clones belonging to the "large" (left) or "hyperexpanded" (right), for patients with and without PFS-9 at each time-point or HDs. Boxplots indicate 25%, 50% and 75% percentiles and whiskers extend to the smallest/largest value within 1.5 times the interquartile range.

E) The skewedness of the TCRα repertoire frequency distribution measured by the Gini Coefficient (bottom) and DE50 (top) of each HD and patient across time-points. Black line indicates median.

F) Comparison of the cumulative frequencies of TCRα CDR3 sequences in either of the segments: A, B, C, and G between patients with and without PFS-9, at each time-point High cumulative frequency of G represents repertoire stability. Black line indicates median.

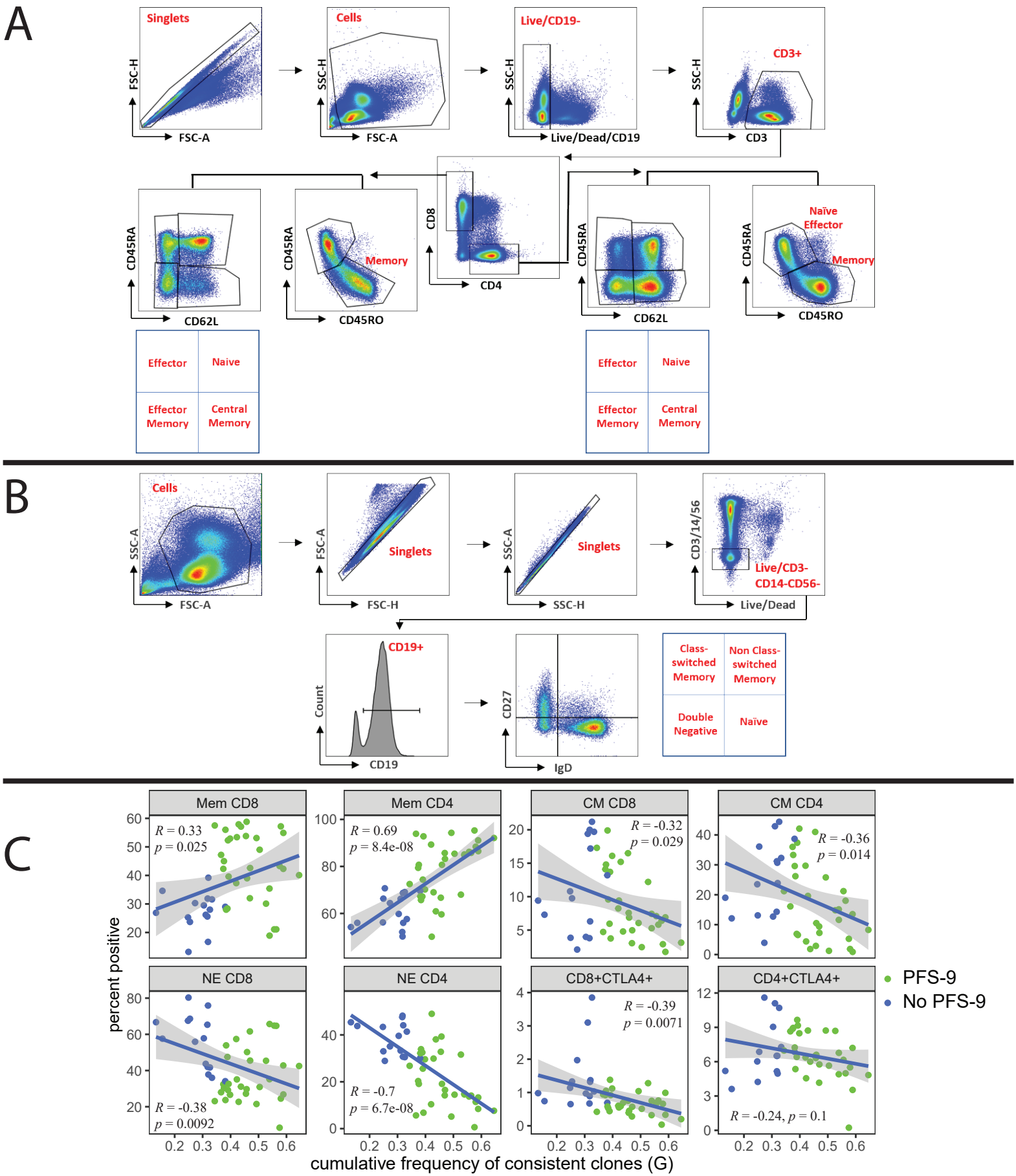

Fig. S3 – **Flow-cytometry gating strategy and additional correlations between TCRβ repertoire and phenotyping (Related to Figure 3)**
A) Gating strategy for CD4+ and CD8+ T cell subpopulations using the FlowJo software. Gating was performed in the sequence displayed, starting with singlets and cells, followed by gating on live, CD19- cells, then CD3+, CD4+ vs. CD8+, and finally CD62L+ vs CD45RA+ or CD45RO vs CD45RA.
B) Gating strategy for B cell subpopulations using the FlowJo software. Gating was performed in the sequence displayed, starting with cells and singlets, followed by gating on live, CD3/CD14/CD56- cells, then CD19+, and finally CD27+ vs IgD.
C) The percent positive of memory(Mem) CD8+, memory (Mem) CD4+, central memory (CM) CD8+, central memory (CM) CD4+, naïve-effector (NE) CD8+, naïve-effector (NE) CD4+, CD8+CTLA4+, CD4+CTLA4+ cell populations as a function of the cumulative frequency of the G segment (the persistent TCRβ clones). Color indicates patients with and without PFS-9. Related to Fig. 3B.

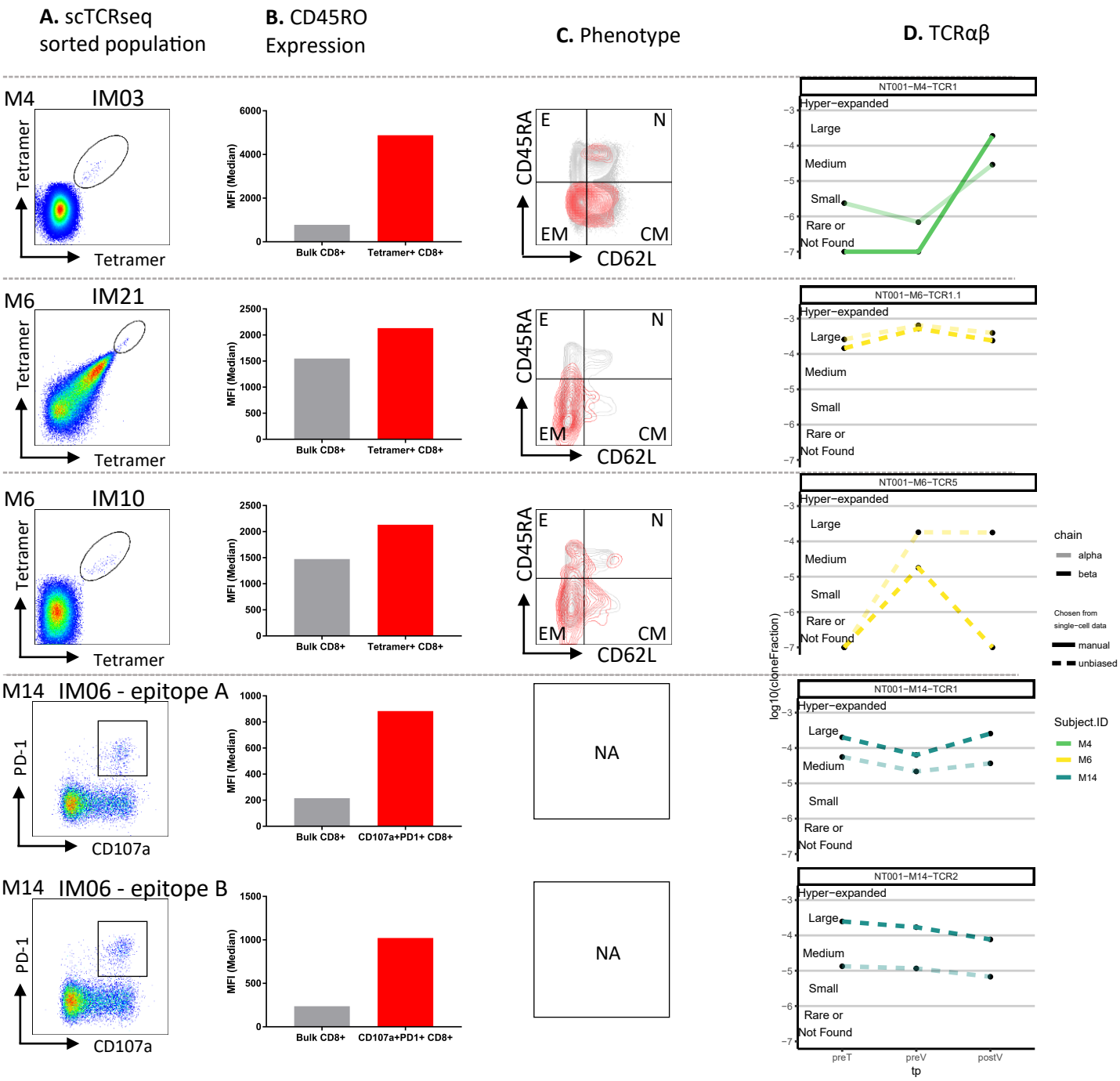

Fig. S4 – **Single-cell neoantigen-specific T cell: Frequency and the phenotype (Related to Figure 3)**
Phenotypic analysis of neoantigen-specific T cells sorted for single-cell TCR sequencing were evaluated in a parallel experiment. Each column depicts the population sorted for single cell TCR sequencing (scTCRseq sorted population), and subsequent phenotype, and TCRα/β expression in the periphery for that specific T cell population. Immunizing peptide (IM) number is indicated.
A) Neoantigen-specific T cells were sorted for single cell TCR sequencing using either MHC class I tetramers (M4, M6) or activation markers (M14). Tetramer-positive populations were pre-gated on singlet, live, CD19-, CD3+, CD8+ T cells. M14 neoantigen-specific T cells were pre-gated on singlet, live, CD19-CD3+CD8+CD69+CD26+ T cells. The immunizing peptide (IM) containing the neoantigen is indicated; each TCR clone from M14 is specific to a different epitope contained within IM06.
B) The expression of CD45RO was evaluated on bulk CD8+ T cells (gray bars) and neoantigen-specific T cells (red bars).
C) Tetramer-positive neoantigen-specific T cells were also evaluated for CD45RA and CD62L (red) compared to bulk CD8+ T cells (gray). E= Effector, N=Naïve, EM=Effector Memory, CM=Central Memory; NA=Not available.
D) the frequency of the TCRα and TCRβ of 5/8 validated TCRs in the peripheral blood. Color indicates patient ID, line-type indicates whether the TCRαβ was chosen for validation based on or regardless of its peripheral frequency, and transparency indicates TCRα vs TCRβ chains.

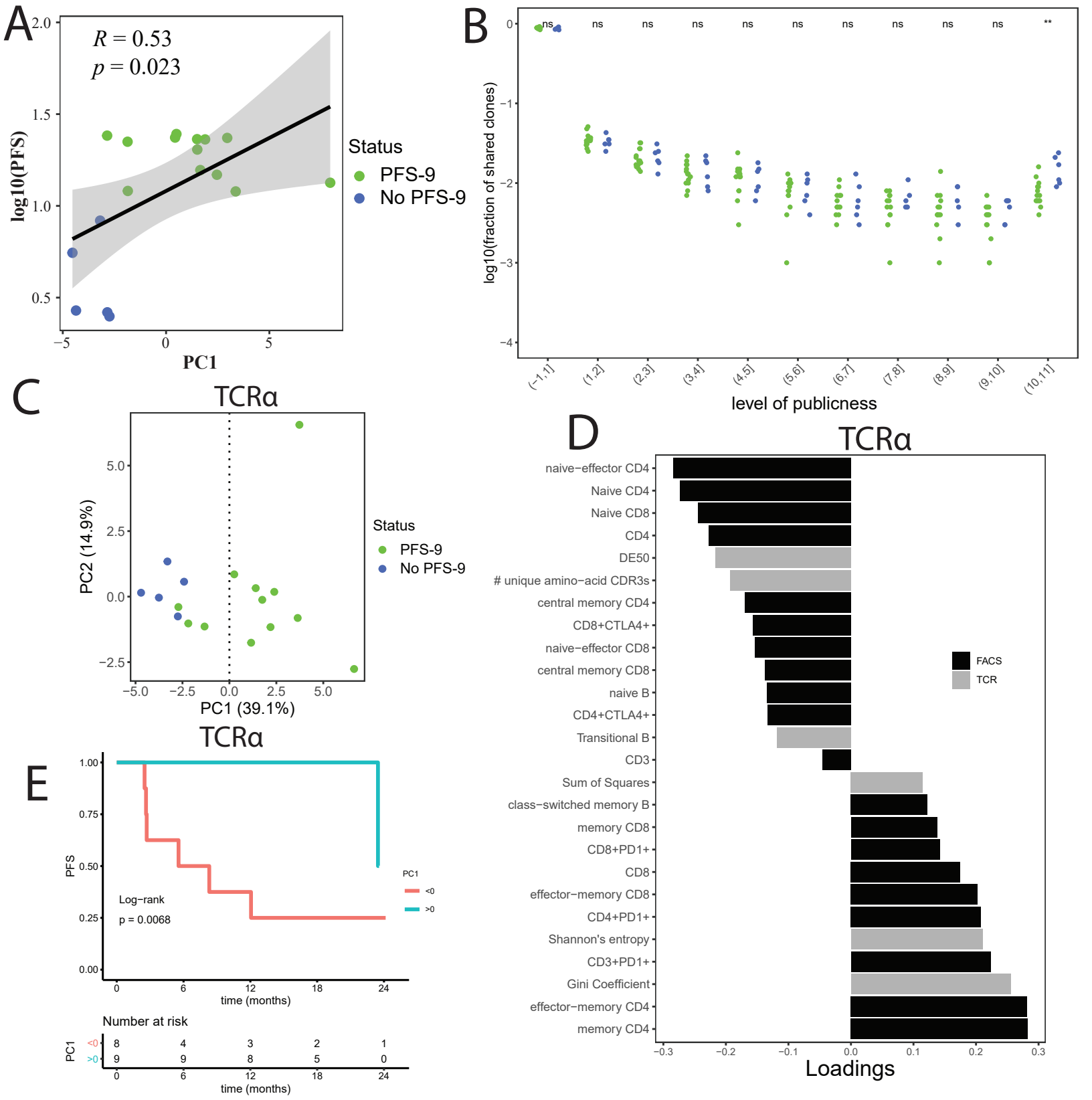

Fig. S5 – **Expanded PCA-related analyses, publicness and concordance with TCRα measurements (Related to Figure 4)**
A) log10(PFS) as a function of each patient's PC1 score. Related to Fig. 4A and 4C. Correlation and its statistical significance are indicated.
B) Fraction of public clones in patients' repertoires as a function of the level of publicness (see Methods). Related to Fig. 4E. ns indicates no significance. ** indicates P-value ≤ 0.01.
C) First 2 components from a Principal Component Analysis of the aggregate peripheral measurements from the TCRα repertoire and immunophenotyping (related to Fig. 4A)
D) The contributions (loadings) of the measured features to PC1, where TCR repertoire features were obtained from the TCRα CDR3 sequences. Color indicates source of data. (related to Fig. 4A)
E) Kaplan-Meier curves for progression-free survival (PFS) of patients with PC1>0 (teal) versus patients with PC1<0 (red), based on the PCA analysis in Fig. S4C. This result demonstrates that the pre-treatment TCRα repertoires are as predictive for PFS-9 status as the TCRβ repertoires. Median PFS was 6.93 months for PC1<0 while at 20.3 months the median has not yet been reached for PC1>0, hazard ratio=0.093, p=0.0068.

Supplementary Table 1 (related to Figure 1)

Table providing the age, sex, background-subtracted CEF values, PFS-9 status and the sample availability for TCRα and TCRβ sequencing at each time-point.

Patient	Age	Sex	CEF [spot forming cells per 1M PBMCs]	PFS-9	Pre-Treatment Sample	Pre-Vaccine Sample	Post-Vaccine Sample
M1	55	F	549	Yes	1	1	1
M10	63	M	24	Yes	1	1	1
M12	63	M	239	Yes	1	1	1
M13	60	M	0	Yes	1	1	1
M14	77	M	419	Yes	1	1	1
M15	80	F	514	No	1	1	1
M16	25	M	700	No	0	0	1
M17	37	M	16	No	1	1	1
M18	71	M	2	Yes	1	1	1
M2	65	M	109	Yes	1	1	1
M20	59	M	980	No	1	1	1
M22	47	F	892	Yes	1	0	1
M23	67	M	583	Yes	1	1	1
M3	62	M	333	No	1	1	1
M4	52	F	N/A	No	1	1	1
M5	57	M	256	Yes	1*	1	1
M6	54	F	N/A	Yes	1	1	1
M7	84	M	286	Yes	1	1	1
M8	59	M	836	Yes	1	1	1*
M9	50	F	963	Yes	1	1	0
NV10	59	M	N/A	No	1	0	0
HD1	50	M					
HD2	44	F					
HD3	51	F					
HD4	40	M					
HD5	31	M					
HD6	49	M					
HD7	21	F					
HD8	32	F					
HD9	29	M					
HD10	25	M					
HD11	34	M					

* Only TCRβ sample available.

Supplementary Table 2 (related to Fig. 3)

Table summarizing the eight validated TCRs following the single-cell TCR sequencing.

Patient	PFS9	Co-Receptor	CDR3α	CDR3β	Selection Method
M1**	Yes	CD8	AGSSASKII	ASSEAFSNYGYT	Manual*
M4	No	CD8	VAYNAGNMLT	ASYQTSGH	Manual*
M6	Yes	CD8	AVRPGSQGNLI	ASSVGGGTVEQF	unbiased
M6	Yes	CD8	LVGDIGGATNKLI	ASRPGQGLEKLF	unbiased
M13**	Yes	CD4	AGNNARLM	ASSPIRGAQH	unbiased
M13**	Yes	CD4	AGSNARLM	ASSLIRGTQY	unbiased
M14	Yes	CD8	VVIDNKLI	ASSLNRESQPQH	unbiased
M14	Yes	CD8	ASVGDTGGFKTI	ASSLSETYEQY	unbiased

*Manual means these clones were selected for validation due to being undetected at the pre-treatment time-point and significantly expanded at the post-vaccine time-point.

**Previously reported in Ott, et al, Cell 2020[7].