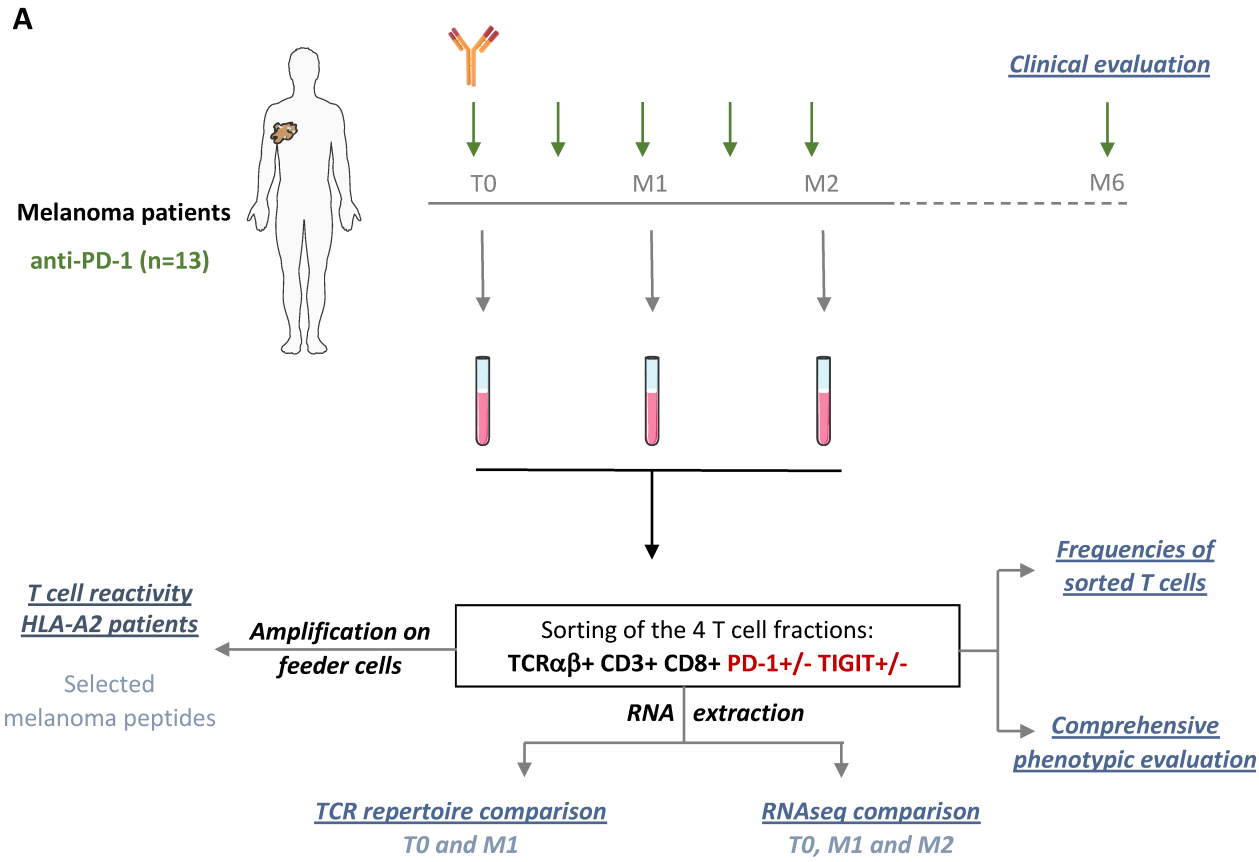

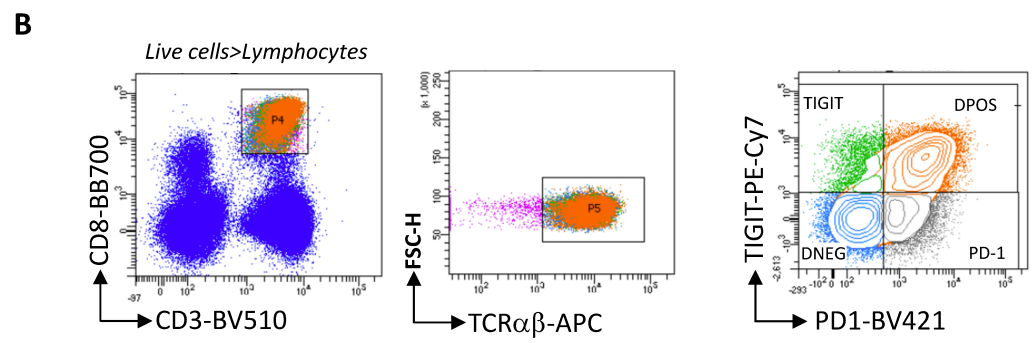

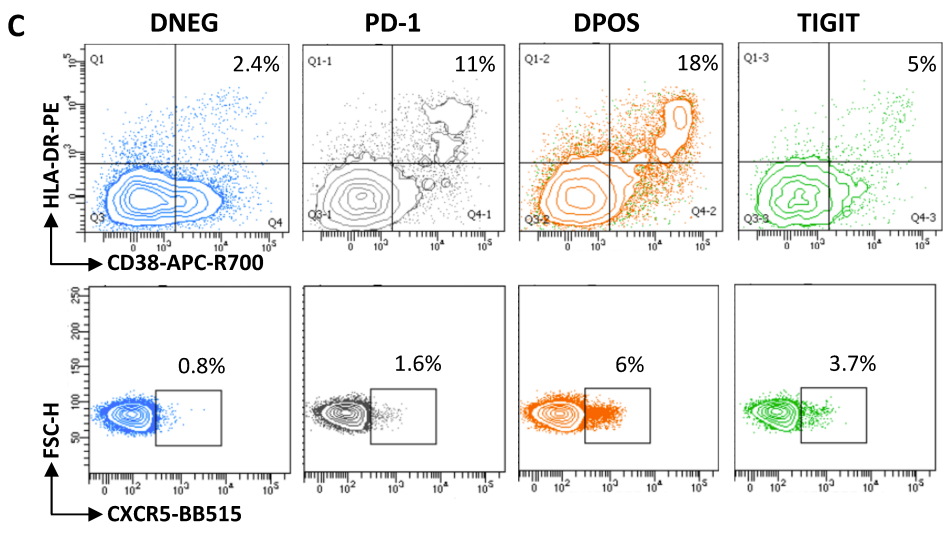

Figure S1: Overall scheme of the study and cell gating strategy for sorting

A) Overall scheme of the study.
B) Gating strategy to sort DNEG, PD-1, DPOS and TIGIT T cell subsets.
C) Representative dot plots of HLA-DR/CD38 expression (upper panel) and CXCR5 expression (lower panel) by the 4 T cell subsets.

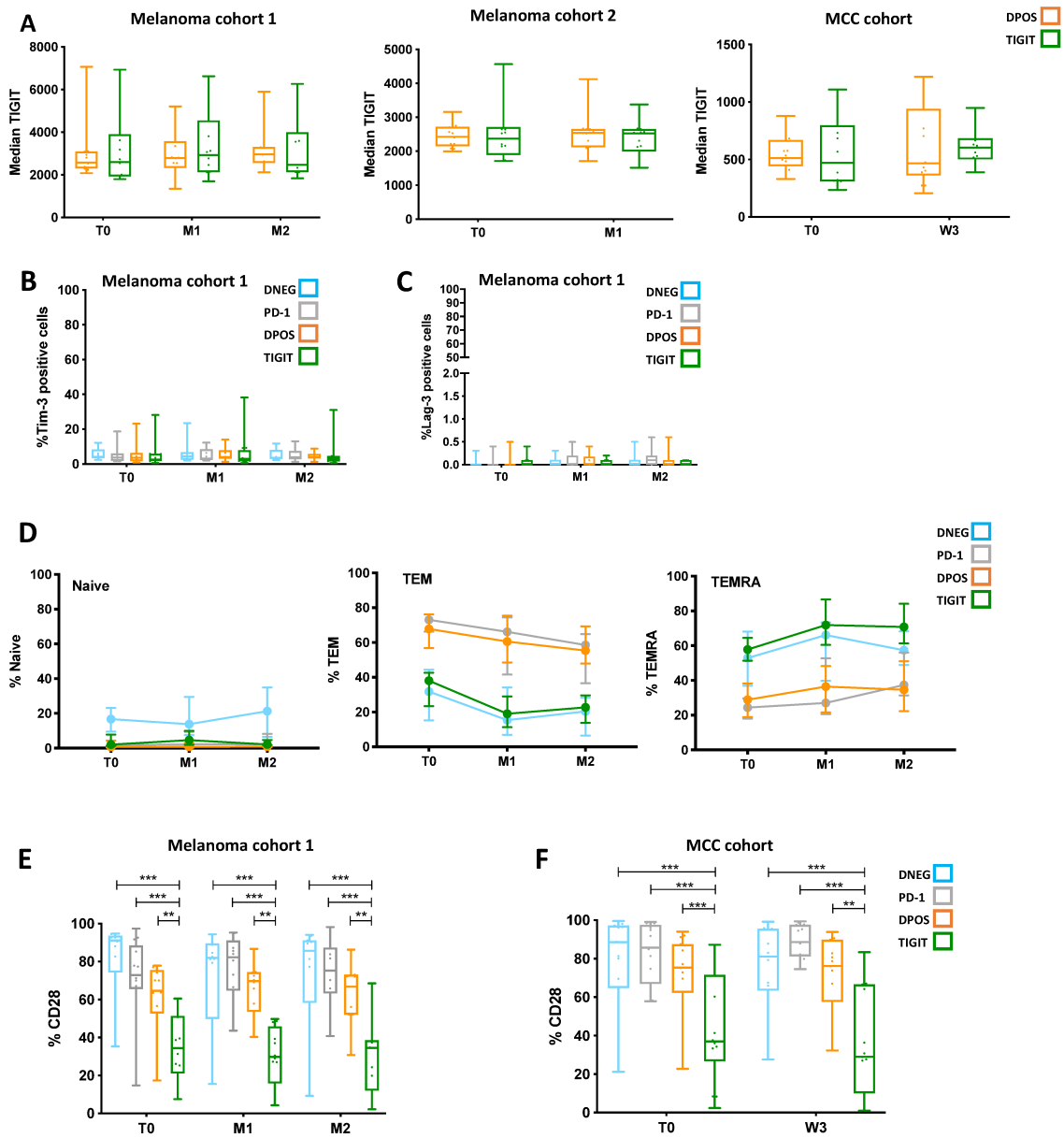

Figure S2: PD-1+TIGIT+ peripheral T cells depict and activated phenotype

A) Median of TIGIT fluorescence in TIGIT and DPOS subsets across time points in the three patient's cohorts. Lines in box-and-whisker-plots indicate median values, boxes indicate IQR values and whiskers minimum and maximum values.

B) Percentage of Tim-3 expression by the 4 T cell subsets across time points in the first cohort of melanoma patients (n=13).

C) Percentage of Lag-3 expression by the 4 T cell subsets across time points in the first cohort of melanoma patients (n=13).

D) Percentage of T naïve, TEM and TEMRA cells in the 4 T cell populations across time points in the first cohort of melanoma patients (n=13) (left).

E and F) CD28 expression by the 4 T cell subsets at baseline, M1 and M2 (Melanoma cohort 1) or at baseline and week 3 (MCC cohort). *** indicates $p < 0.001$ and ** indicates $p < 0.01$ by one-way ANOVA.

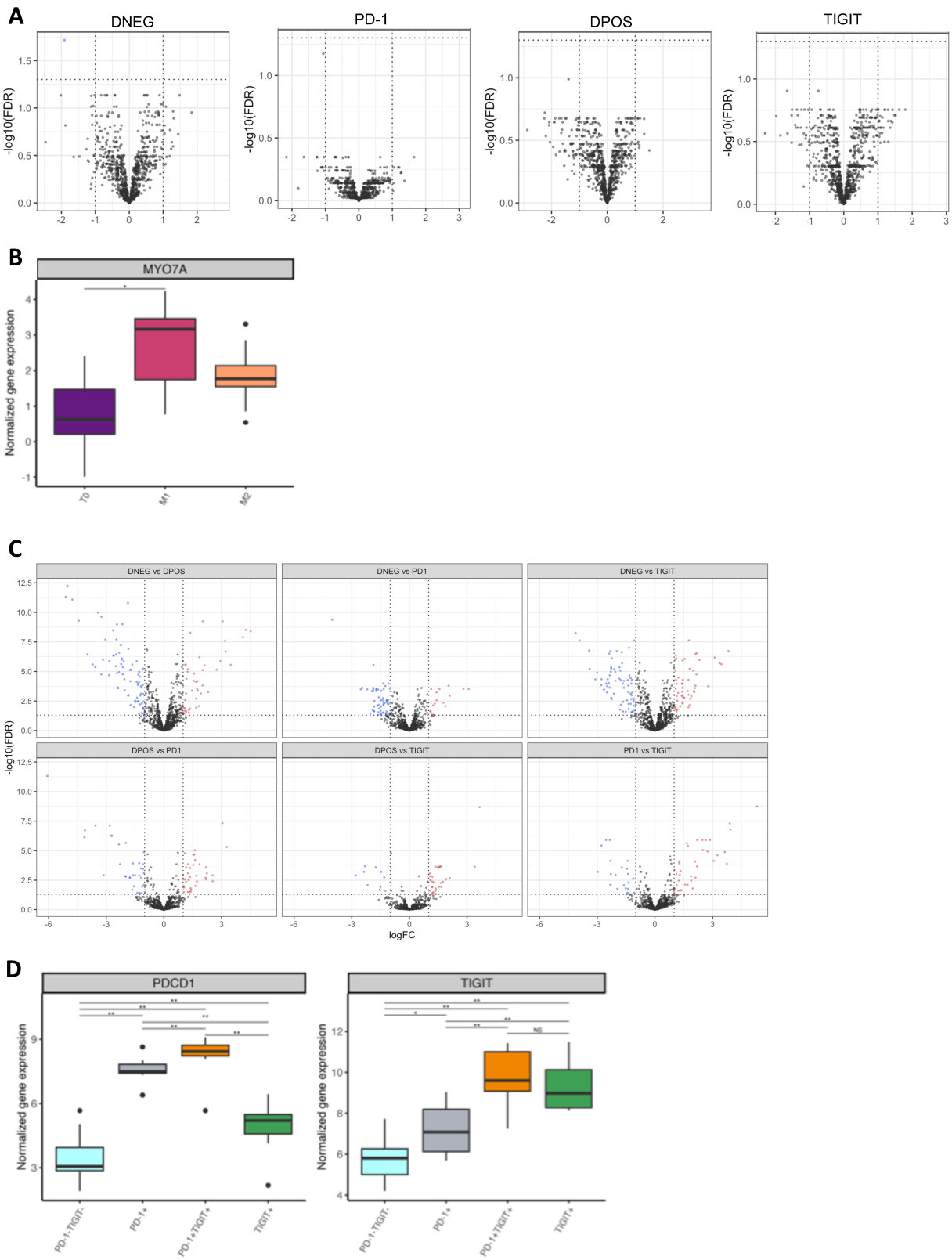

Figure S3: Volcano plots for DEG identified between T0 and M1 or between the different fractions at M1, MYO7A expression in the DNEG fraction upon time and PDCD1 and TIGIT expression on the 4 T cell subsets at M1

A) Volcano plots depicting differentially expressed genes identified between T0 and M1 for the DNEG, PD-1, DPOS and TIGIT populations.

B) Box plot of MYO7A expression by the DNEG population at T0, M1 and M2. * indicates $p < 0.05$ by one-way ANOVA. Lines in box-and-whisker-plots indicate median values, boxes indicate IQR values and whiskers minimum and maximum values.

C) Volcano plots depicting differentially expressed genes identified comparing the different fractions at M1.

D) Box plots of PD-1 and TIGIT expression by the DNEG, PD-1, DPOS and TIGIT populations at M1 (n=11). ** indicates $p < 0.01$ and * indicates $p < 0.05$ by one-way ANOVA.

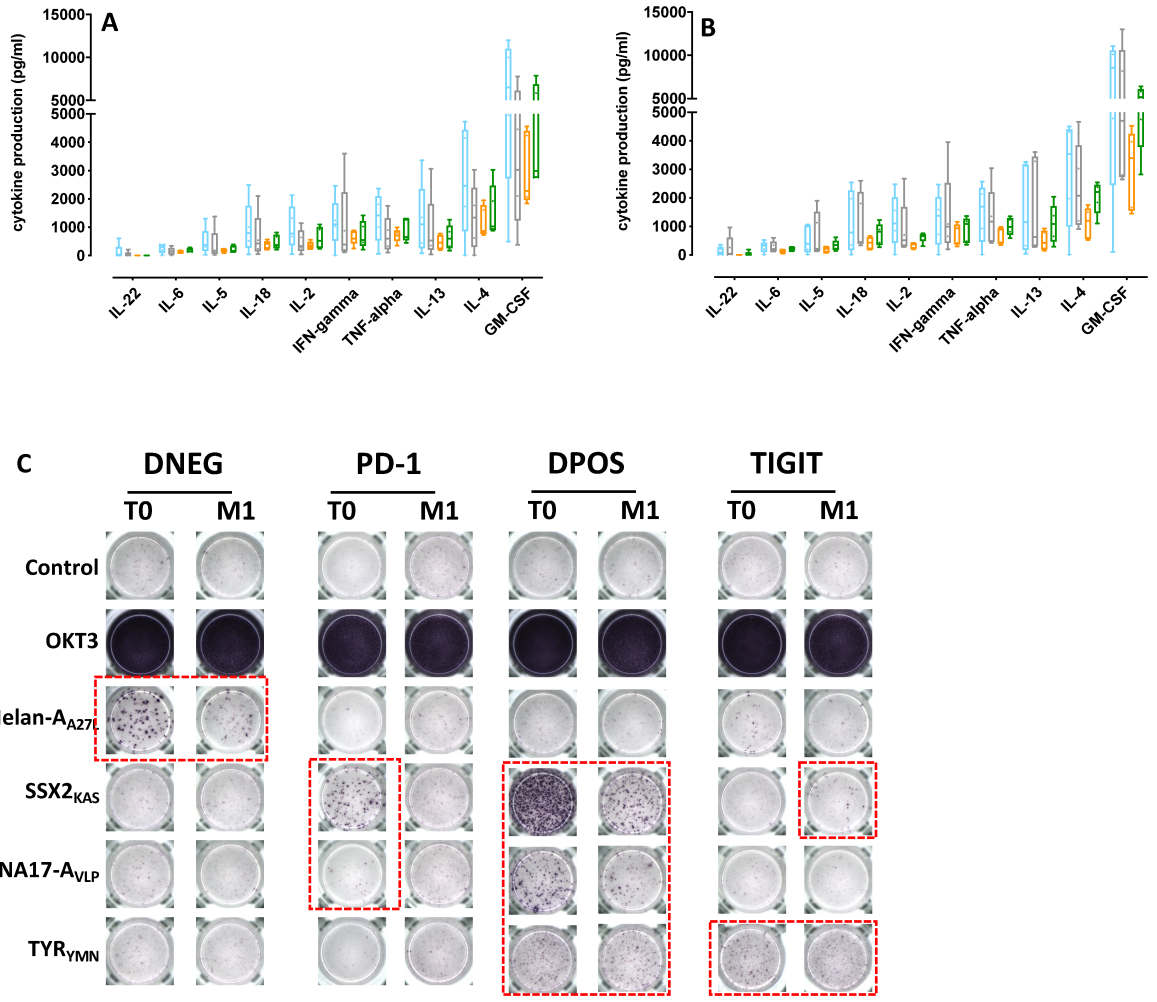

Figure S4: DPOS T cells are polyfunctional and enriched in antigen-specific T cells

Cytokine production assessed by Luminex analysis of culture supernatants for the 4 T cell subset from 5 patients at baseline **(A)** and M1 **(B)** previously expanded on feeder cells and activated with anti-CD3 (500 ng/mL, overnight).

C) Example of ELISPOT responses from P19 patient (one of the three replicates is shown). Positive responses after background deduction are framed in red.

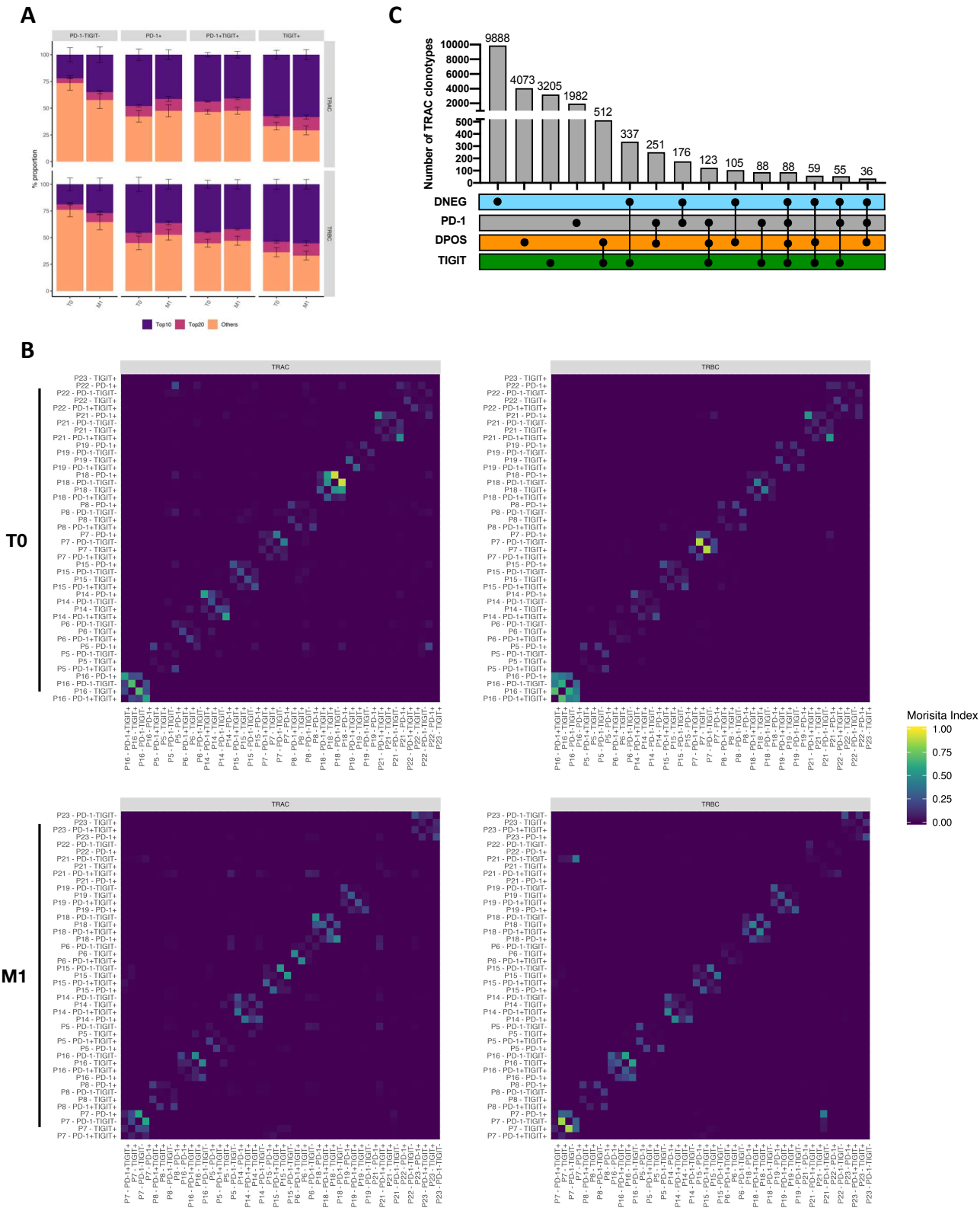

Figure S5: Dominant clonotypes and overlap between patients and fractions

A) Proportions of the top 10, top 20 and others clonotypes (TRAC and TRBC) in all four fractions at T0 and M1 (n=11).

B) Morisita's overlap index between individual fractions from 12 patients

C) Upset diagram showing private and shared TRAC sequences across the 4 fractions at M1. Individual Black circles indicate TRAC repertoires private to a given populations. Black circles connected with solid black lines indicate TRAC repertoires shared between the given fractions.

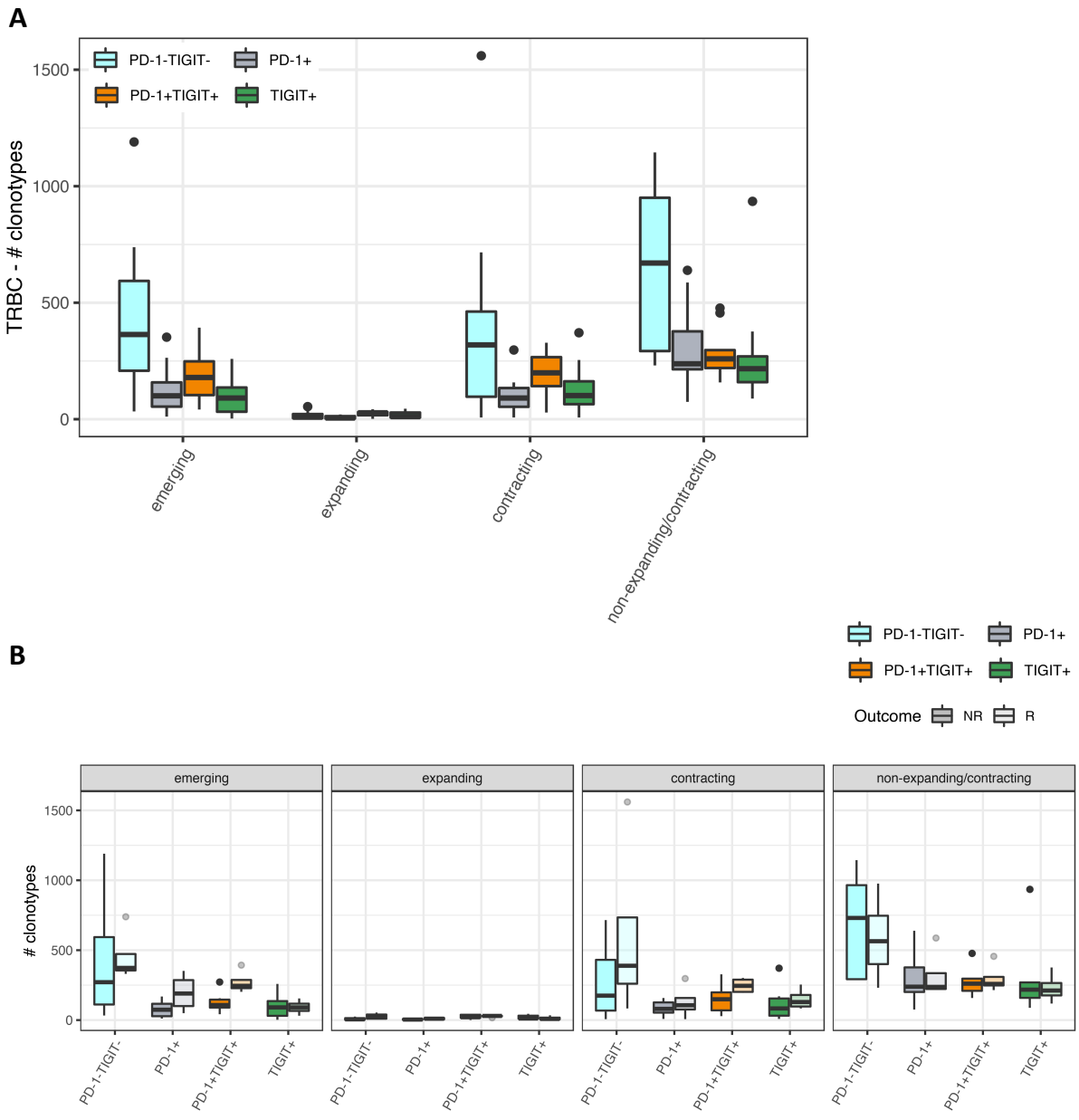

Figure S6: Absolute numbers of TRBC clonotypes across clusters and fractions and clinical outcome
A) Absolute numbers of TRBC clonotypes across clusters and fractions. Lines in box-and-whisker-plots indicate median values, boxes indicate IQR values and whiskers minimum and maximum values.
B) Absolute number of TRBC clonotypes across subsets, clusters and outcome at M1.