

Figure S2

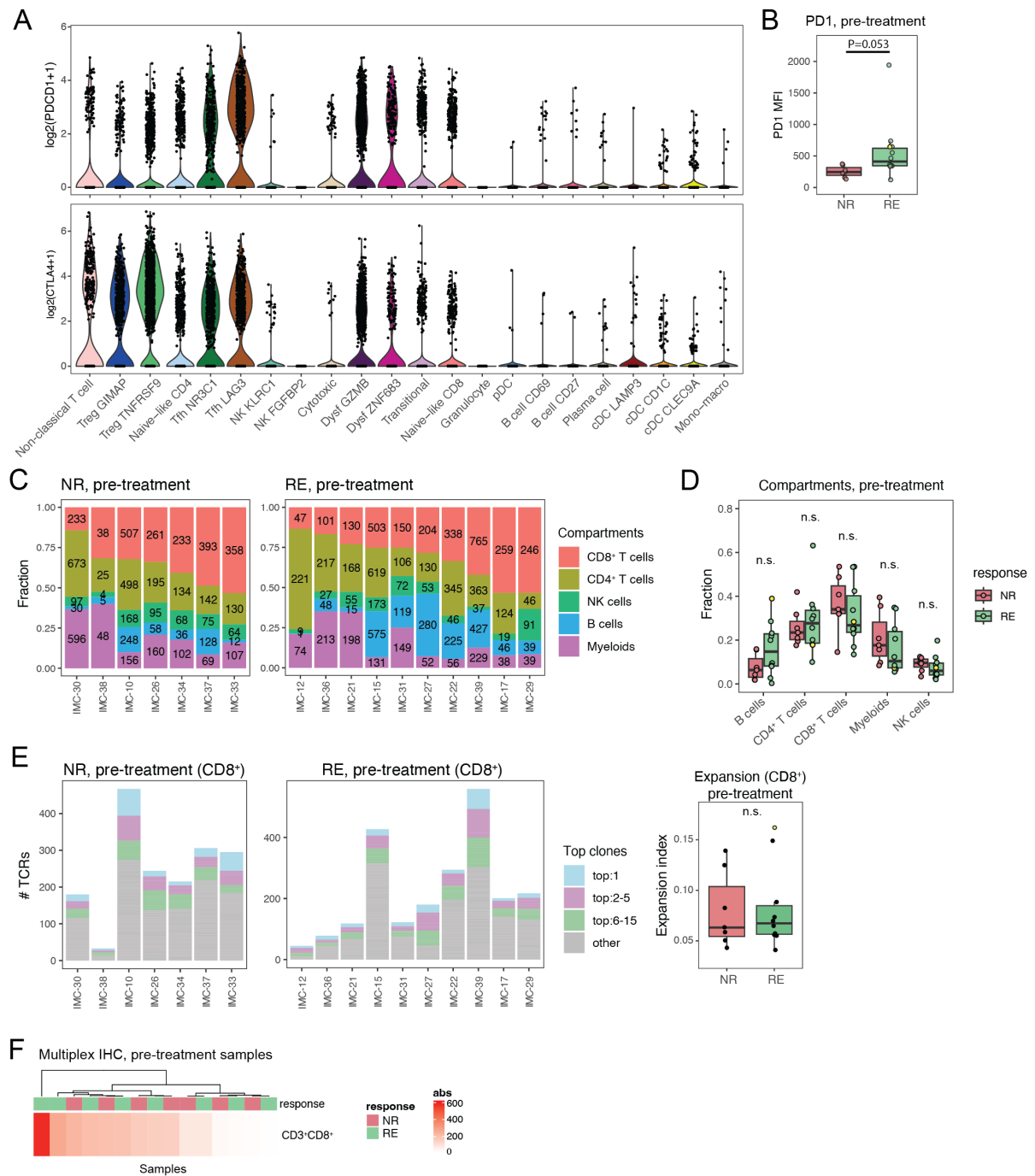


Figure S2. Cell state fractions and transcriptional differences at baseline

A) Pooled analysis of expression of PD-1 and CTLA4 in the indicated cell subsets at baseline in responding and non-responding patients (n=17, excluding nivolumab monotherapy-treated patient ICM-04). Violinplots and datapoints represent log normalized expression of the genes in individual cells. B) Median fluorescence intensity of the PD-1 signal in CD3⁺ cells at baseline in responding and non-responding patients, as measured by flow cytometry. Boxes show the median and 25th and 75th

percentiles. Whiskers depict $1.5 \times \text{IQR}$ and data points represent individual biopsies. Wilcoxon Signed Rank test was performed. C) Cell type composition of the immune infiltrates of tumors from non-responding (left) and responding (right) patients at baseline. D) Abundance of CD4⁺ and CD8⁺ T cell, B cell, NK cell, and myeloid cell subsets in biopsies from non-responding (red) and responding (green) patients at baseline. Data points from the one patient with a partial pathological response (IMC-27) are marked in yellow. Boxes show the median and 25th and 75th percentiles. Boxes, whiskers and individual data points as in B. E) TCR clonality within the CD8⁺ T cell population of pre-treatment biopsies across all patients. Left panel shows the number of T cells harboring a specific TCR per patient (top 1, 2-5, and 6-15 expanded clones are highlighted). Right panel shows the STARTRAC expansion index across non-responding and responding patients³⁷. Data point from IMC-27 is marked in yellow. A two-tailed Mann–Whitney U test was performed between responders and non-responders. F) Quantification of intratumoral CD3⁺CD8⁺ densities at baseline of CD3-CD8-stained slides from all patients of the scRNAseq cohort. Top annotation bar represents responders in green and non-responders in red.