Figure S4

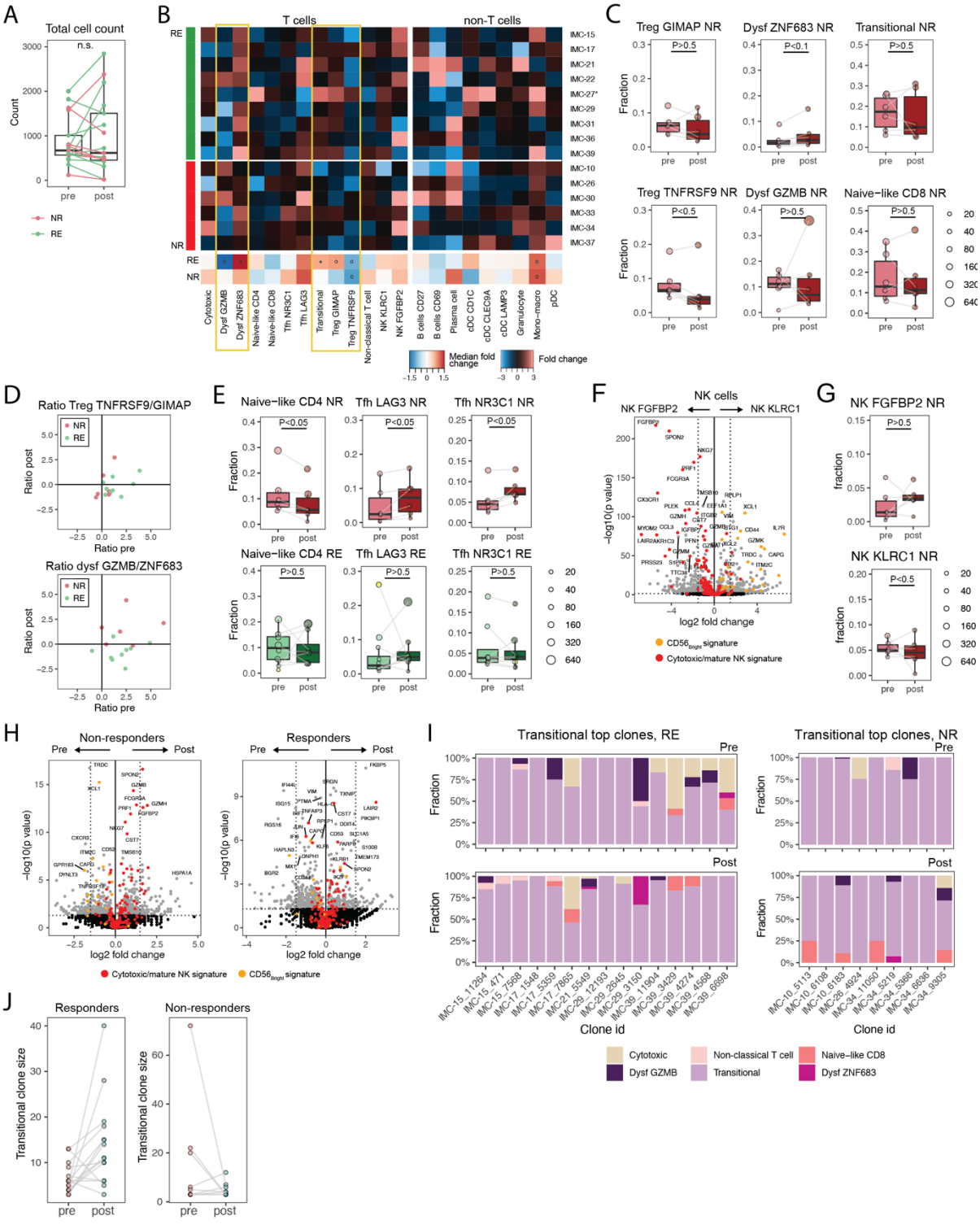

Figure S4. ICB-induced changes in immune cell state abundance and gene expression profiles

A) Quantification of the number of total immune cells in pre- and on-treatment biopsies of non-responding patients, depicted as absolute cell counts. Boxes show the median and 25th and 75th percentiles. Whiskers depict 1.5 × IQR, data points represent individual patients and are colored by

clinical response. A Paired Wilcoxon signed ranks test was performed (RE&NR: *P*=0.65, RE: *P*=0.76, NR: *P*=0.3). B) Top: heatmap depicting changes in abundance of T cell states upon therapy in responding (green) and non-responding (red) patients. Log fold change of the fractions of each cell state in on-treatment versus pre-treatment biopsies, either in T cell or non-T cell compartment, are shown. Bottom: Heatmap depicting the median log fold changes of each cell state in responding and non-responding patients. Patient IMC-04, who received anti-PD-1 monotherapy, and patients for which matched data was lacking were excluded from analysis (IMC-38 and IMC-12). A two-tailed Mann–Whitney U test was performed between the pre- and on-treatment cell fractions. (°p < 0.2, ∗p < 0.05). C) Quantification of the abundance of Treg, $CD8^+$ dysfunctional, naïve-like $CD8^+$ T cell, and transitional T cell subsets in pre- and on-treatment biopsies of non-responding patients, depicted as fraction of total T cells. Patient IMC-04, who received anti-PD-1 monotherapy, and patients for which matched data was lacking were excluded from analysis (IMC-38 and IMC-12). Boxes show the median and 25^{th} and 75^{th} percentiles. Whiskers depict 1.5 × IQR and data points represent individual patients, sized by the number of cells that underlie that data point. A Paired Wilcoxon signed ranks test was performed. D) Ratio between $Treg_{TNFRSF9}$ and $Treg_{GIMAP}$ (top panel), and CD8-$dysf_{GZMB}$ and CD8-$dysf_{ZNF683}$ (bottom panel) cells in pre- and on-treatment samples of responding and non-responding patients. The ratio of $Treg_{TNFRSF9}$/$Treg_{GIMAP}$ and CD8-$dysf_{GZMB}$ /CD8-$dysf_{ZNF683}$ show a significant switch in pre- versus on-treatment samples in responders (*P*<0.01 and *P*<0.01, respectively), while not in non-responders (*P*=0.69 and *P*=0.56, respectively). Colors represent patient response. A Paired Wilcoxon signed ranks test was performed. E) Abundance of Naïve-like CD4 T cells and Tfh cells in pre- and on-treatment biopsies of responding and non-responding patients. Patients for which matched data was lacking were excluded from analysis (IMC-38 and IMC-12), data points from IMC-27 are marked in yellow. Boxes, whiskers, size of data points as described in B. A Paired Wilcoxon signed ranks test was performed. F) Differentially expressed genes between the NK_{FGFBP2} and NK_{KLRC1} cell subsets across all biopsies. Genes present in the cytotoxic (mature) NK cell signature and the less cytotoxic ($CD56_{bright}$) signature[33] are highlighted in red and yellow, respectively. Mann Whitney U test with false detection

rate (FDR) correction was used to determine p-values. G) Abundance of NK cells in pre- and on-treatment biopsies of non-responding patients. Boxes, whiskers, size data points as described in B and D. A Paired Wilcoxon signed ranks test was performed. H) Differentially expressed genes in NK cells in pre- and on-treatment biopsies of non-responding and responding patients. Genes from the cytotoxic (mature) NK cell signature and from the less cytotoxic ($CD56_{bright}$) signature are highlighted in red and yellow, respectively. Mann Whitney U test with false detection rate (FDR) correction was used to determine p-values. I) Composition of cell states of the top TCR clones in the transitional $CD8^+$ cell compartment in pre- and on-treatment biopsies of responding and non-responding patients. Colors represent cell state. J) Number of cells in top transitional $CD8^+$ T cell clones in pre- and on-treatment biopsies of responding and non-responding patients. A Paired Wilcoxon signed ranks test was performed.