Figure S5

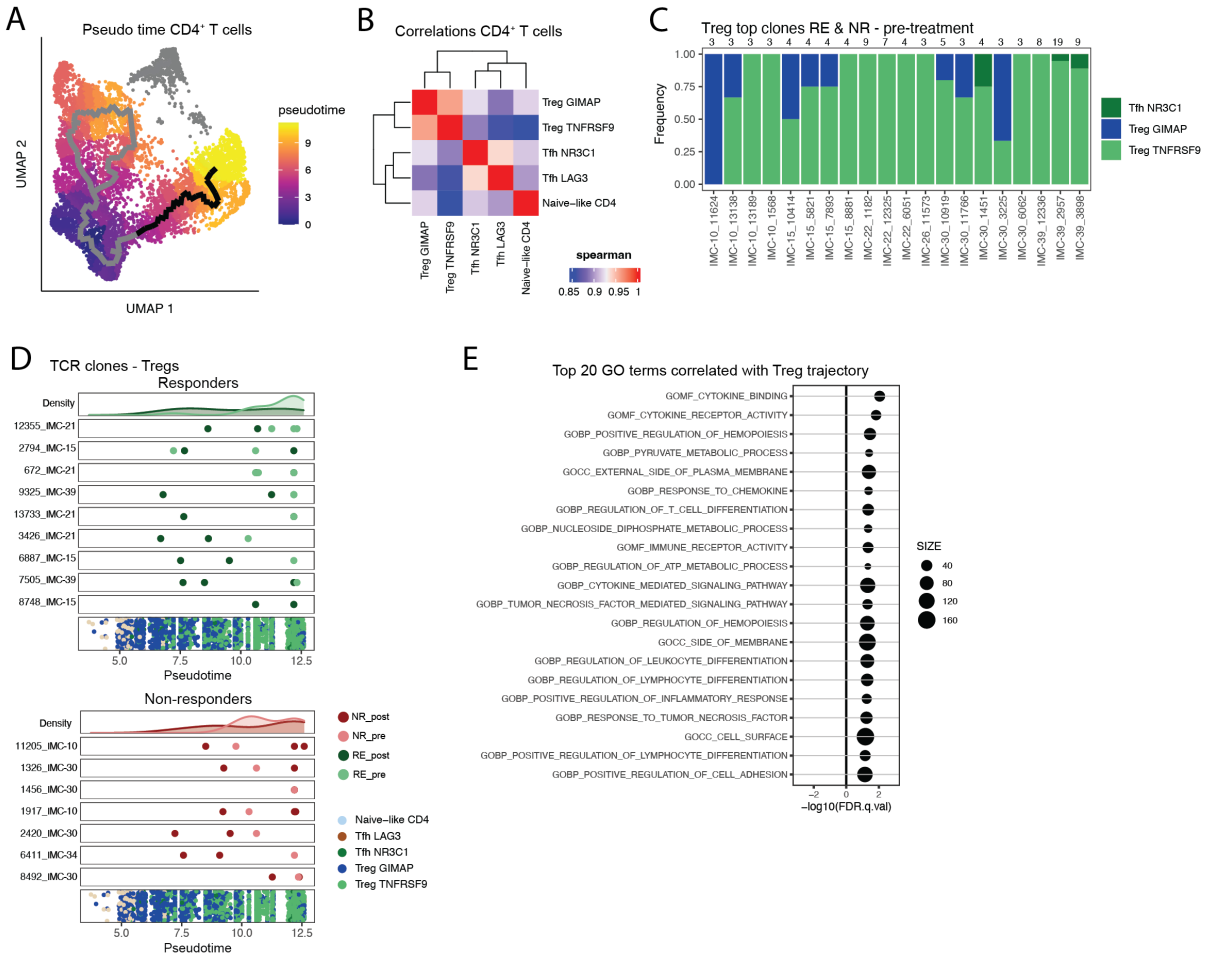


**Figure S5. Dual ICB-induced changes in activation state of Tregs**

A) Pseudo time colored scaling plotted on top of the CD4$^+$ T cell trajectory, in which the start of the trajectory is located at the naïve-like CD4 T cell compartment. The trajectory across the two Treg subsets is depicted in black, while the remainder of the trajectory is depicted in grey. Note that the trajectory represents transcriptional relatedness between cell pools, and hence does not necessarily reflect a direct differentiation path between cell pools. B) Correlation analysis of gene expression of feature genes between cell states in the CD4$^+$ T cell compartment. Correlations were calculated using a spearman's rank correlation. C) Composition of cell states of the top TCR clones abundant in the Treg compartment in pre-treatment biopsies of responding and non-responding patients. Colors represent cell states. D) Cells from individual TCR clones that occurred > 2 times per biopsy, or of which the TCR clone expanded upon treatment, projected onto the Treg trajectory in pseudo time in pre- and on-

treatment biopsies of responding and non-responding patients. Top plot shows the density of TCR clones is biopsies from responding or non-responding patients over pseudotime. Bottom scatter plot represents cell states in pseudo time, color coding of cell states as described in Figure 4A. E) Top 20 GO terms enriched in the Treg trajectory, using gene set enrichment on the genes in the trajectory scored by correlation with the Treg trajectory. Size of the data points reflects gene set size.