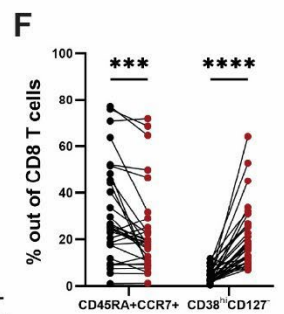
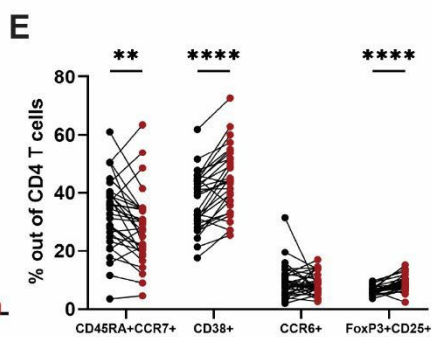
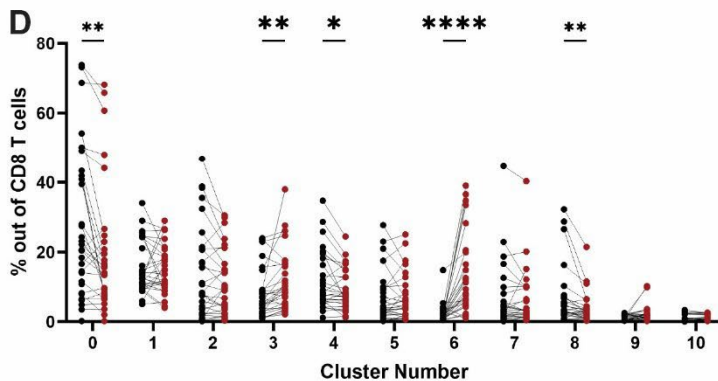
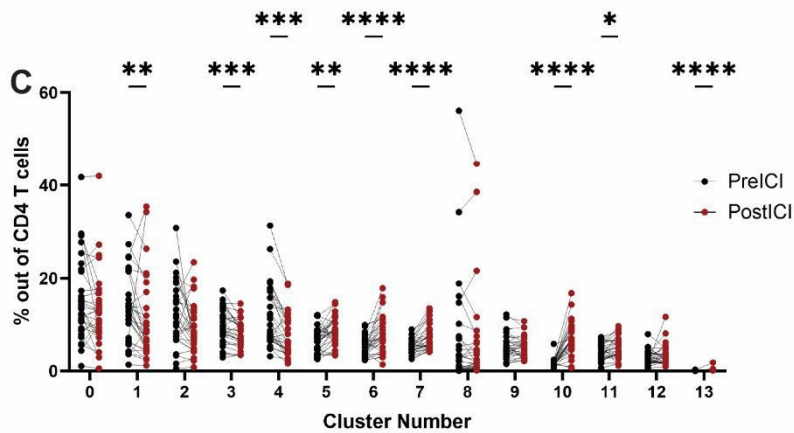
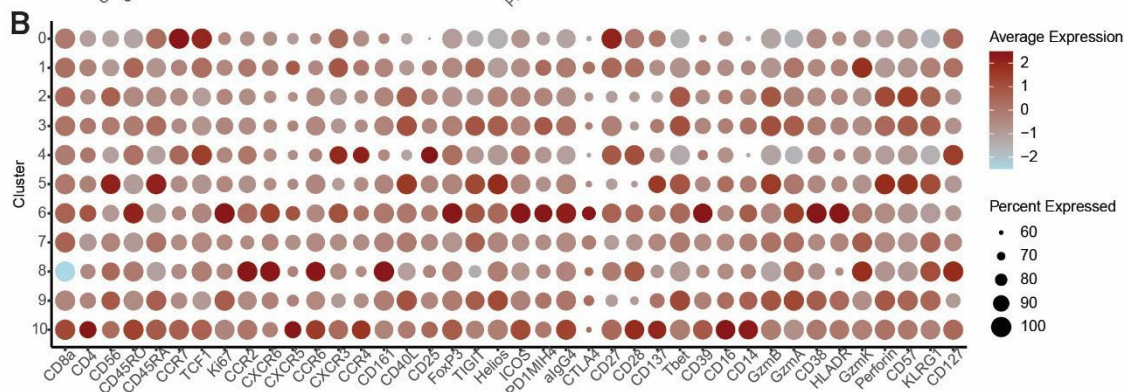
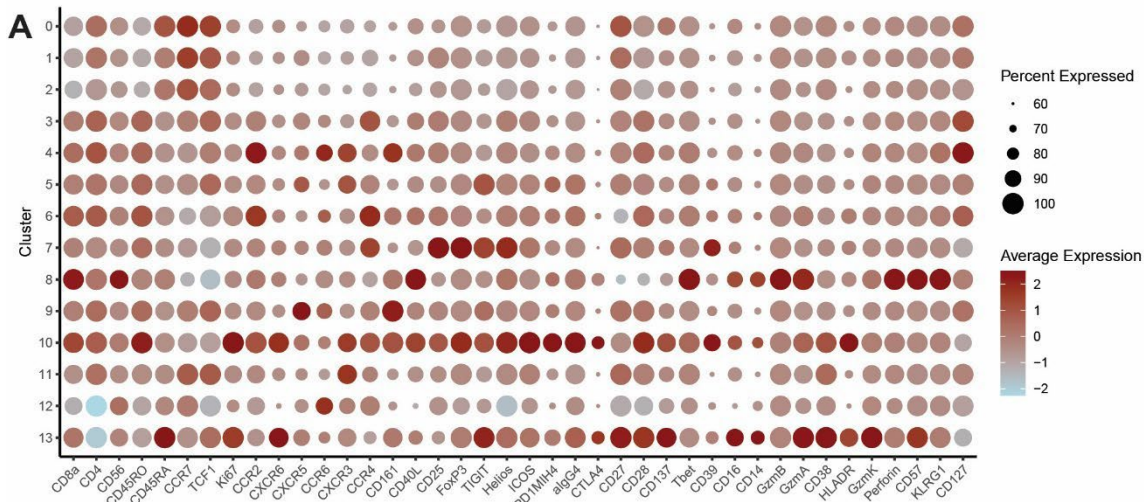
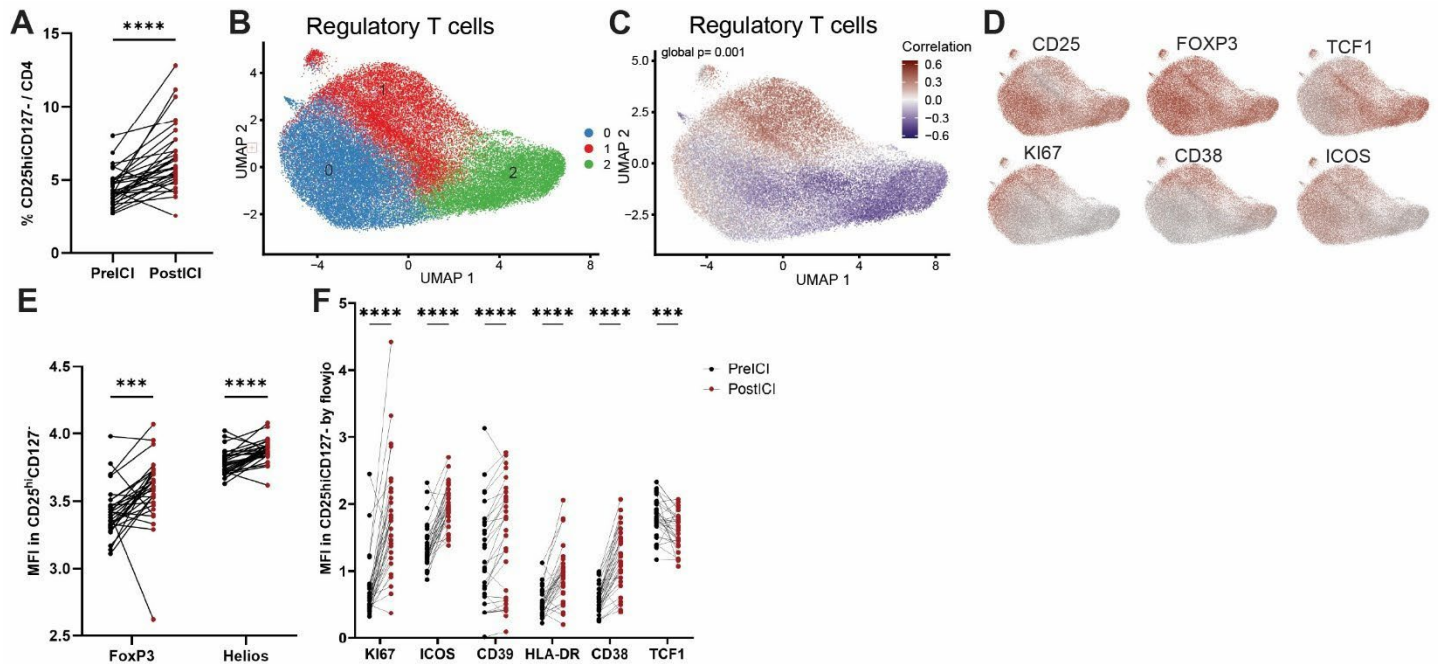


indicated populations. F) Paired analysis of frequency of plasmacytoid dendritic cells (gated as CD3CD19-CD14-CD11C-BDCA2+CD123+) before vs after treatment per patient. A G, H) Analysis of frequency of indicated B cell populations analyzed by flow cytometry before treatment per patient in indicated populations. Frequencies of B cells in patients with low and high severity irAEs are compared. IK) Change in frequency of B cell populations after - before treatment. L) Frequency of plasmacytoid dendritic cells (gated as CD3-CD19-CD14-CD11C-BDCA2+CD123+). M) Change in frequency of plasmacytoid dendritic cells after - before treatment. Red bars indicate patients with high severity irAEs, and blue bars indicate patients with low severity irAEs. -F: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by Wilcoxon paired test. G-M: All plots are not significant by Mann Whitney test.

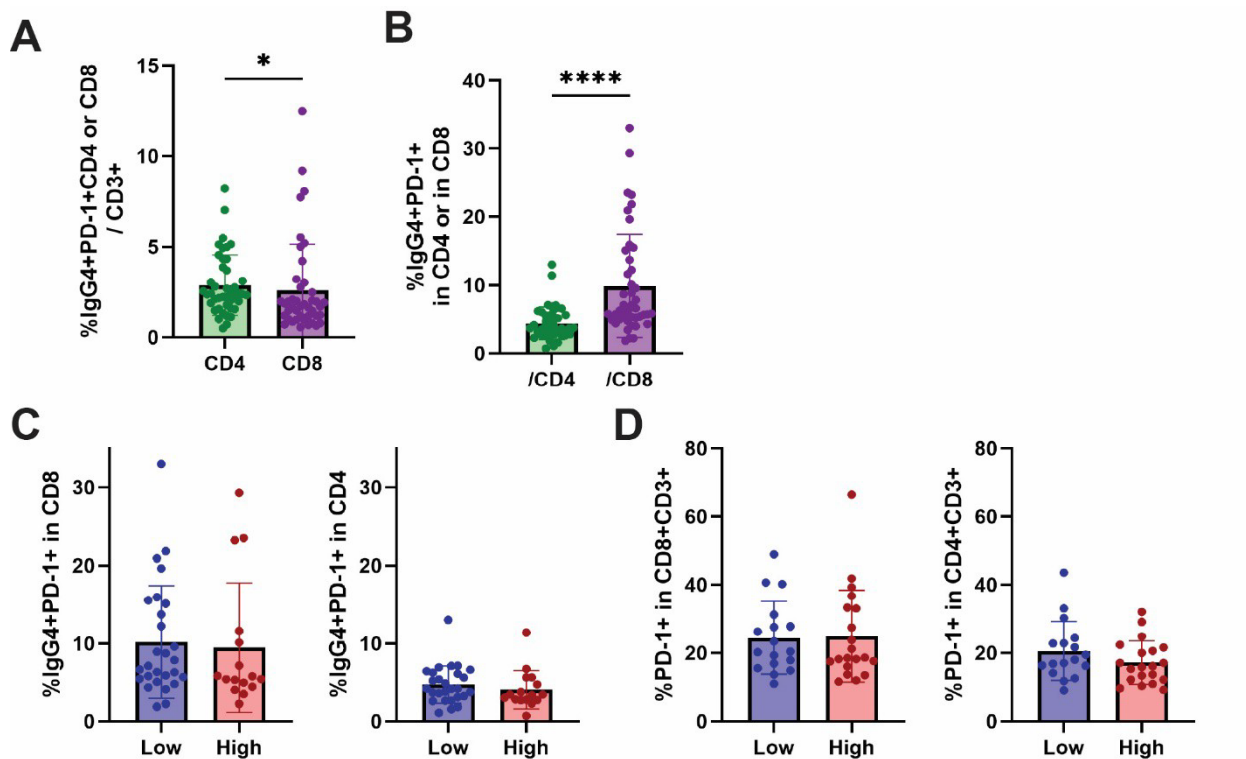


Supplemental Figure 3

A) DotPlot representation of all mass cytometry markers in CD4⁺ T cells subset from PBMCs (see Figure 2A-C). B) Dot Plot representation of all mass cytometry markers in CD8⁺ T cells subset from PBMCs (see Figure 2D-F). C) Paired analysis of frequency of all CD4 T cell and D) CD8 T cell clusters before vs after treatment per patient. test. E) Paired analysis of CD4 and F) CD8 T cells analyzed by biaxial gating. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ by Wilcoxon paired analysis.



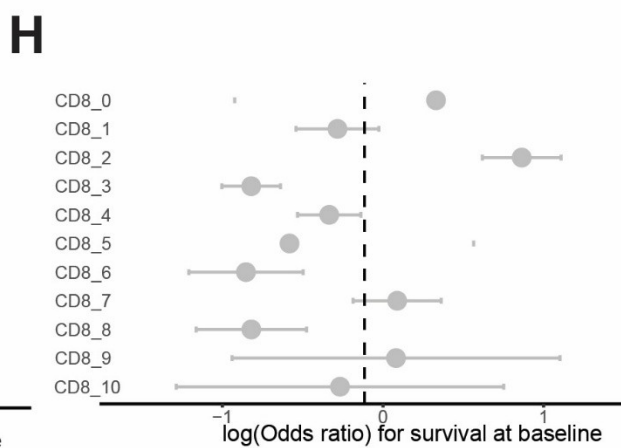
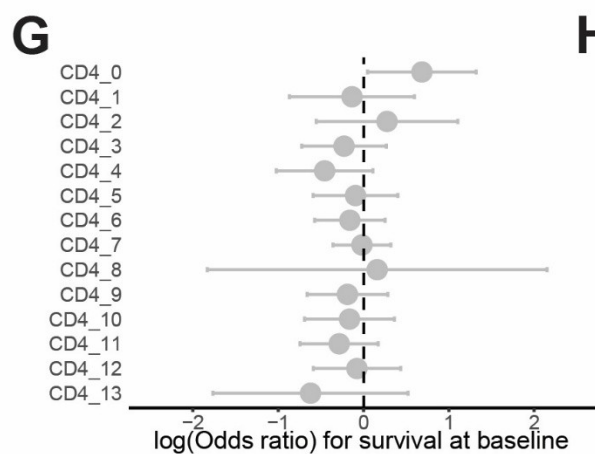
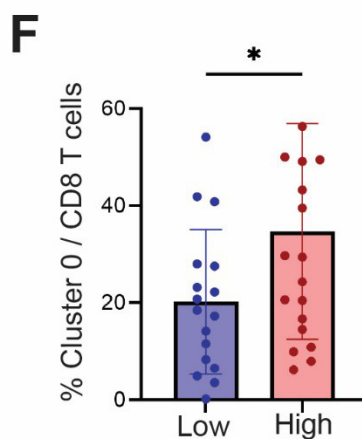
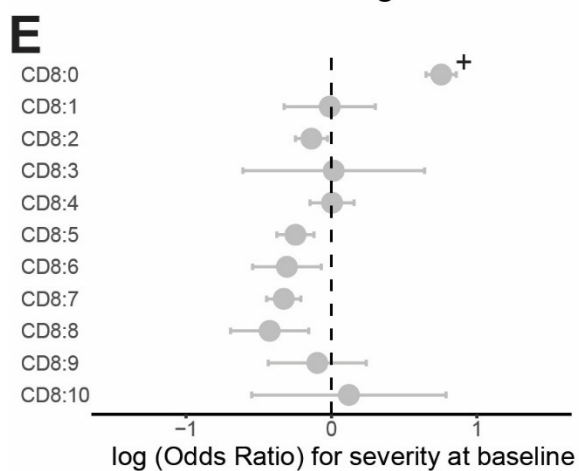
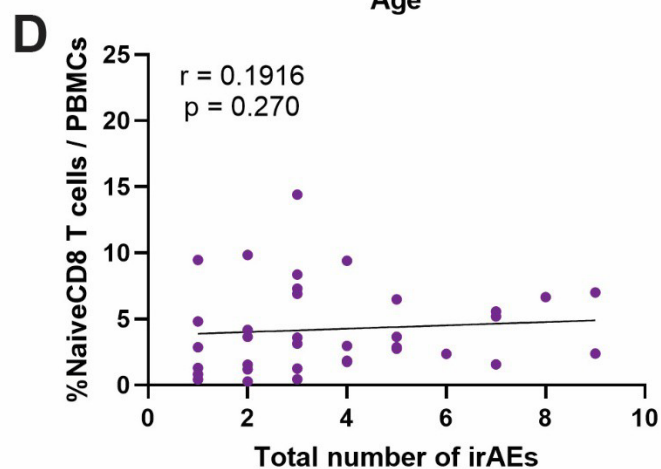
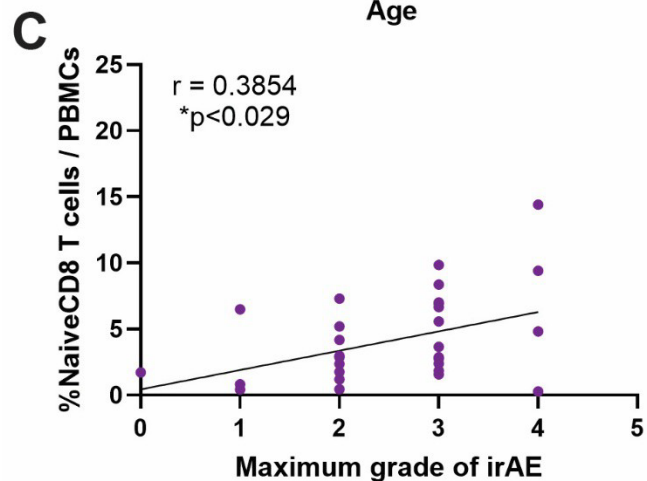
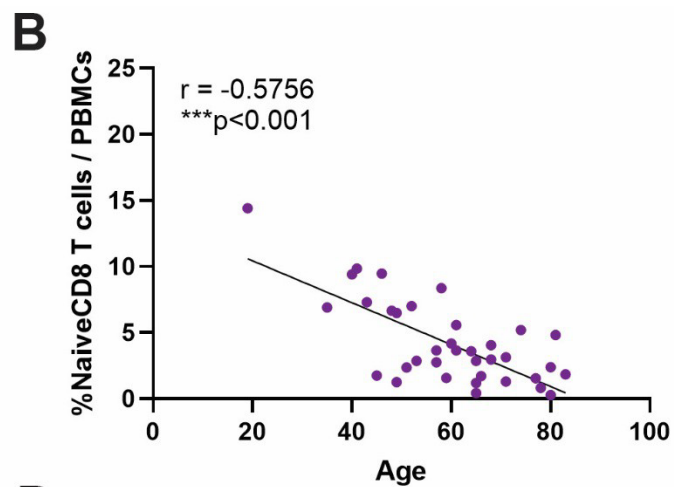
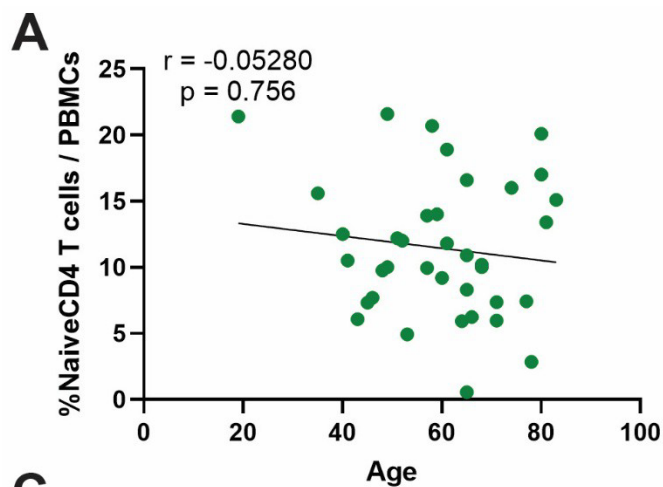
Supplemental Figure 4 A) Paired analysis of frequency of CD25^{hi}CD127⁻ CD4 T cells before and after ICI therapy by biaxial gating. B) UMAP representation following unsupervised clustering of CD4 regulatory T cells. C) CNA analysis of before treatment samples vs after treatment samples. Global p value = 0.001. D) Feature plots of the indicated markers on the Treg UMAP. E) Paired analysis of Median Fluorescent Intensity of FoxP3 and Helios or F) Ki67, ICOS, CD39, HLA-DR, and CD38 in CD25^{hi}CD127⁻ CD4 T cells before and after ICI therapy. *** $p < 0.001$, **** $p < 0.0001$ by Wilcoxon paired analysis.



Supplemental Figure 5

A) Frequency of IgG4⁺PD-1⁺ CD4⁺ or CD8⁺ T cells out of CD3⁺ T cells by gating of mass cytometry analysis in the post-treatment samples. B) Frequency of IgG4⁺PD-1⁺ out of CD4⁺ or CD8⁺ T cells by gating of mass cytometry analysis in the post-treatment samples. C) Frequency of IgG4⁺PD-1⁺ out of CD4⁺ (right) or CD8⁺ (left) T cells in patients with low or high severity of irAE by gating of mass cytometry analysis. D)

Frequency of total PD-1⁺ out of CD4⁺ (right) or CD8⁺ (left) T cells in pre-treatment samples from patients with low or high severity of irAE by gating of mass cytometry analysis.



Supplemental Figure 6

A) Spearman correlation of proportion of gated naïve CD4⁺ T cells (CD19⁻CD14⁻CD3⁺CD4⁺CD45RA⁺CCR7⁺) with age. B) Spearman correlation of proportion of gated naïve CD8⁺ T cells (CD19⁻CD14⁻CD3⁺CD8⁺CD45RA⁺CCR7⁺) with age, C) maximum grade of irAE, and D) total number of irAEs diagnosed. E) MASC analysis of CD8 T cell clusters (see Figure 2). + indicates $p < .05$, adj $p = 0.5$. F) Proportion of cluster 0 out of CD8 T cells. $p < 0.05$ by Mann Whitney test. G) MASC analysis for overall survival in CD4⁺ and H) CD8⁺ T cells before treatment (survived $n = 5$, not survived $n = 29$).