Supplemental Materials Legend

Table S1. List of antibodies used in this study.

Table S2. Demographics and baseline clinical characteristics of patients with or without severe irAE.

Table S3. List of all toxicities included in this study.

Figure S1. Manual gating strategy for PBMCs in this study. TEM: Effector Memory T cell; TCM: Central Memory T cell; Treg: regulatory T cell; TEMRA: CD45RA+ effector memory T cell; DNT: double-negative ($CD4^-CD8^-$) T cells; DPT: double-positive ($CD4^+CD8^+$) T cells; Mono: monocytes; NK: natural killer cell; NKT: natural killer T cell; pDC: plasmacytoid dendritic cell; cDC: classical dendritic cell. Representative gating strategy from n=1 randomly selected sample.

Figure S2. $CD4^+$ T cell clusters found with unsupervised analysis to be associated with severe irAE. **A.** Uniform Manifold Approximation and Projection (UMAP) visualizing the FlowSOM-guided clustering of $CD4^+$ T cells. Circled clusters were found to be significantly associated with severe irAE. **B.** Heatmap of relevant functional marker expression in each $CD4^+$ T cell cluster, scaled to relative expression of each marker within the cluster group. **C.** UMAPs of individual functional marker expression within $CD4^+$ T cell clusters. **D.** Frequencies of $CD4^+$ TEM cluster 30 as percentage of non-granulocytes in patients with or without severe irAE at irAE-max. **E.** Frequencies of $CD4^+$ TCM cluster 31 as percentage of non-granulocytes in patients with or without severe irAE at irAE-max. ** = false discovery rate (fdr)<0.01 as determined by Significance Analysis of Microarray (SAM). Clusters were created based on all PBMC samples across timepoints and lines of therapy. N of lines of therapy per comparison: n=10 no severe irAE, n=16 severe irAE at baseline; n=9 no severe irAE, n=20 severe irAE at irAE-max.

Figure S3. NK cell clusters found with unsupervised analysis to be associated with severe irAE. **A.** UMAP visualizing the FlowSOM-guided clustering of NK cells. Circled clusters were found to be significantly associated with severe irAE. **B.** Heatmap of relevant functional marker expression in each NK cell cluster, scaled to relative expression of each marker within the

cluster group. **C.** UMAPs of individual functional marker expression within NK cell clusters. **D.** Frequencies of $CD56^{high}$ NK cell cluster 59 as percentage of non-granulocytes in patients with or without severe irAE at irAE-max. ** = fdr<0.01 as determined by SAM. Clusters were created based on all PBMC samples across timepoints and lines of therapy. N of lines of therapy per comparison: n=9 no severe irAE, n=20 severe irAE at irAE-max.

Figure S4. B cell clusters found with unsupervised analysis to be associated with severe irAE. **A.** UMAP visualizing the FlowSOM-guided clustering of B cells. The circled cluster was found to be significantly associated with severe irAE. **B.** Heatmap of relevant functional marker expression in each B cell cluster, scaled to relative expression of each marker within the cluster group. **C.** UMAPs of individual functional marker expression within B cell clusters. **D.** Frequencies of memory B cell cluster 38 as percentage of non-granulocytes in patients with or without severe irAE at pre-irAE. ** = fdr<0.01 as determined by SAM. Clusters were created based on all PBMC samples across timepoints and lines of therapy. N of lines of therapy per comparison: n=9 no severe irAE, n=6 severe irAE at pre-irAE.

Figure S5. Non-$CD4^+$ T cell clusters found with unsupervised analysis to be associated with severe irAE. **A.** UMAP visualizing the FlowSOM-guided clustering of non-$CD4^+$ T cells. Circled clusters were found to be significantly associated with severe irAE. **B.** Heatmap of relevant functional marker expression in each non-$CD4^+$ T cell cluster, scaled to relative expression of each marker within the cluster group. **C.** UMAPs of individual functional marker expression within non-$CD4^+$ T cell clusters. **D.** Frequencies of $CD8^+$ TCM clusters 43 and 44, and $CD8^+$ TEM cluster 56 as percentage of non-granulocytes in patients with or without severe irAE at irAE-max. ** = fdr<0.01 as determined by SAM. Clusters were created based on all PBMC samples across timepoints and lines of therapy. N of lines of therapy per comparison: n=9 no severe irAE, n=20 severe irAE at irAE-max.

Figure S6. Manually gated immune population frequencies in PBMCs of patients with or without severe irAE. **A.** Median relative frequencies of various immune populations in PBMCs of patients with or without severe irAE at different timepoints. ** = fdr<0.01 comparing patients with severe irAE to patients without severe irAE, as determined by SAM. **B.** Frequencies of $CD161^+$ $CD4^+$ T cells as percentage of $CD4^+$ T cells in patients with or without severe irAE at different timepoints. ** = fdr<0.01 as determined by SAM. n.s. = not significantly significant. **C.** Example of a paired comparison in cell population frequencies between irAE-max and baseline in patients with severe irAE and patients without severe irAE, as summarized in **Figure 2 E-F**.

Shown is the comparison in $CD38^+$ $CD8^+$ TCM cells as percentage of $CD8^+$ TCM. ** = fdr<0.01 as determined by SAM. n.s. = not significant. N of lines of therapy per comparison: n=10 no severe irAE, n=16 severe irAE at baseline; n=9 no severe irAE, n=6 severe irAE at pre-irAE; n=9 no severe irAE, n=20 severe irAE at irAE-max.

Figure S7. Contribution of individual irAE categories to the difference in $CD161^+$ $CD4^+$ T cells observed in association with severe irAE. Shown are frequencies of $CD161^+$ $CD4^+$ T cells as percentage of $CD4^+$ T cells in patients with or without severe irAE at baseline and irAE-max. No individual irAE category was significantly different compared to patients with no severe irAE. N of lines of therapy per comparison: n=10 no severe irAE, n=2 gastrointestinal irAE, n=8 hepatobiliary irAE, n=4 musculoskeletal irAE, n=2 pituitary irAE, n=1 respiratory irAE, n=3 cutaneous irAE, n=1 T1DM at baseline; n=9 no severe irAE, n=3 gastrointestinal irAE, n=11 hepatobiliary irAE, n=5 musculoskeletal irAE, n=3 pituitary irAE, n=1 respiratory irAE, n=6 cutaneous irAE, n=2 T1DM at irAE-max.

Figure S8. Contribution of BRAF mutation status to the difference in $CD161^+$ $CD4^+$ T cells observed in association with severe irAE. Shown are frequencies of $CD161^+$ $CD4^+$ T cells as percentage of $CD4^+$ T cells in patients with or without severe irAE and with a BRAF mutation (BRAF-Mut) or without a BRAF mutation (BRAF-WT) at baseline and irAE-max. * = p-value<0.05 as determined by Kruskal-Wallis test with post-hoc Wilcoxon Rank Sum pairwise analysis. n.s. = not significant. N of lines of therapy per comparison: n=5 no severe irAE BRAF-WT, n=6 severe irAE BRAF-WT, n=4 no severe irAE BRAF-Mut, n=7 severe irAE BRAF-Mut at baseline; n=5 no severe irAE BRAF-WT, n=9 severe irAE BRAF-WT, n=3 no severe irAE BRAF-Mut, n=8 severe irAE BRAF-Mut at irAE-max.

Figure S9. PMBC clusters found with unsupervised analysis and additional manually gated populations associated with clinical benefit. **A.** Frequencies of $CD16^+$ NK cell clusters 50, 71 and 72 as percentage of non-granulocytes in patients with or without clinical benefit at irAE-max. **B.** Frequencies of CD74+ pDCs as percentage of pDCs in patients with or without clinical benefit at irAE-max. ** = fdr<0.01 as determined by SAM. N of lines of therapy per comparison: n=6 no clinical benefit, n=18 clinical benefit at irAE-max.