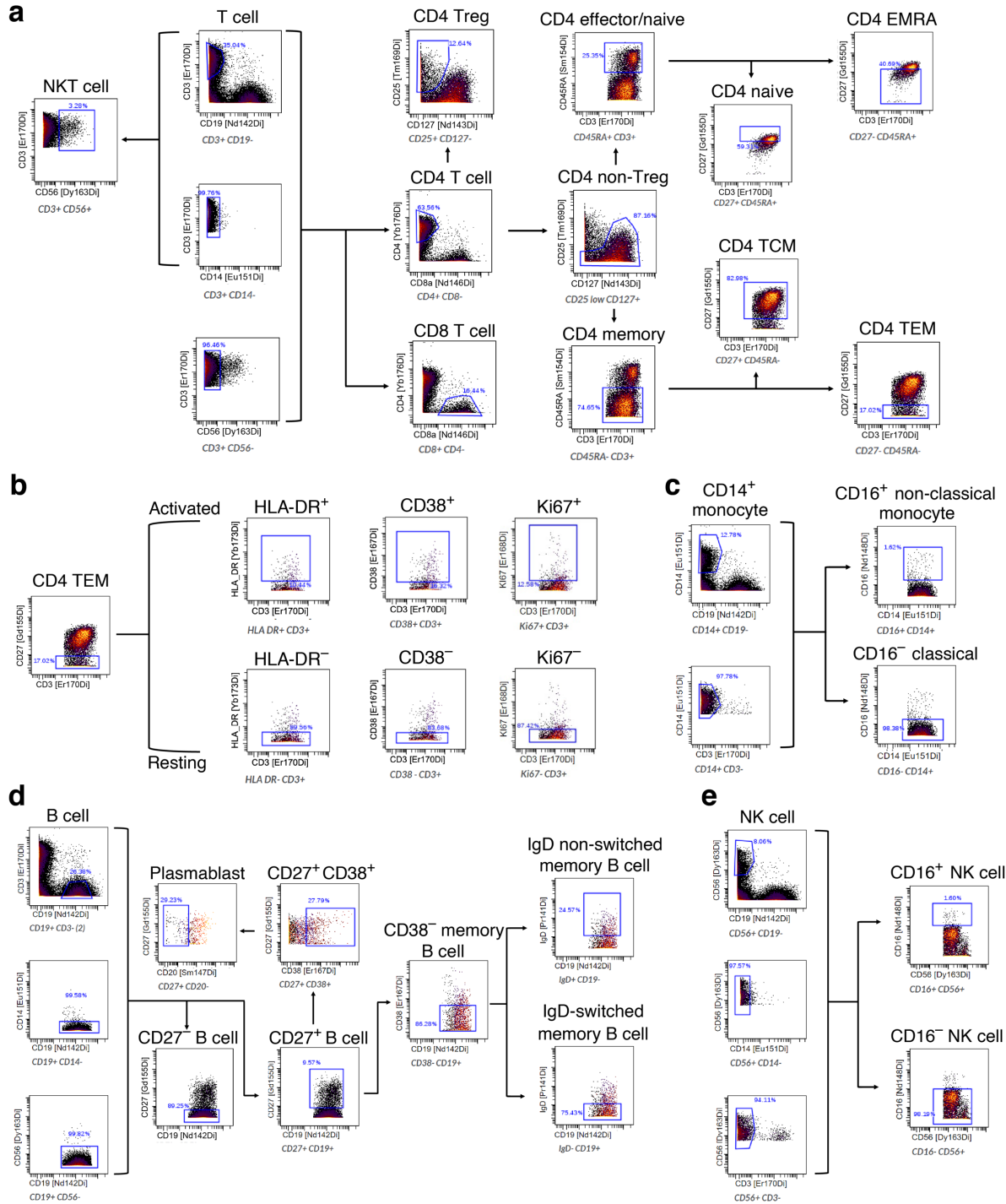## Supplementary information

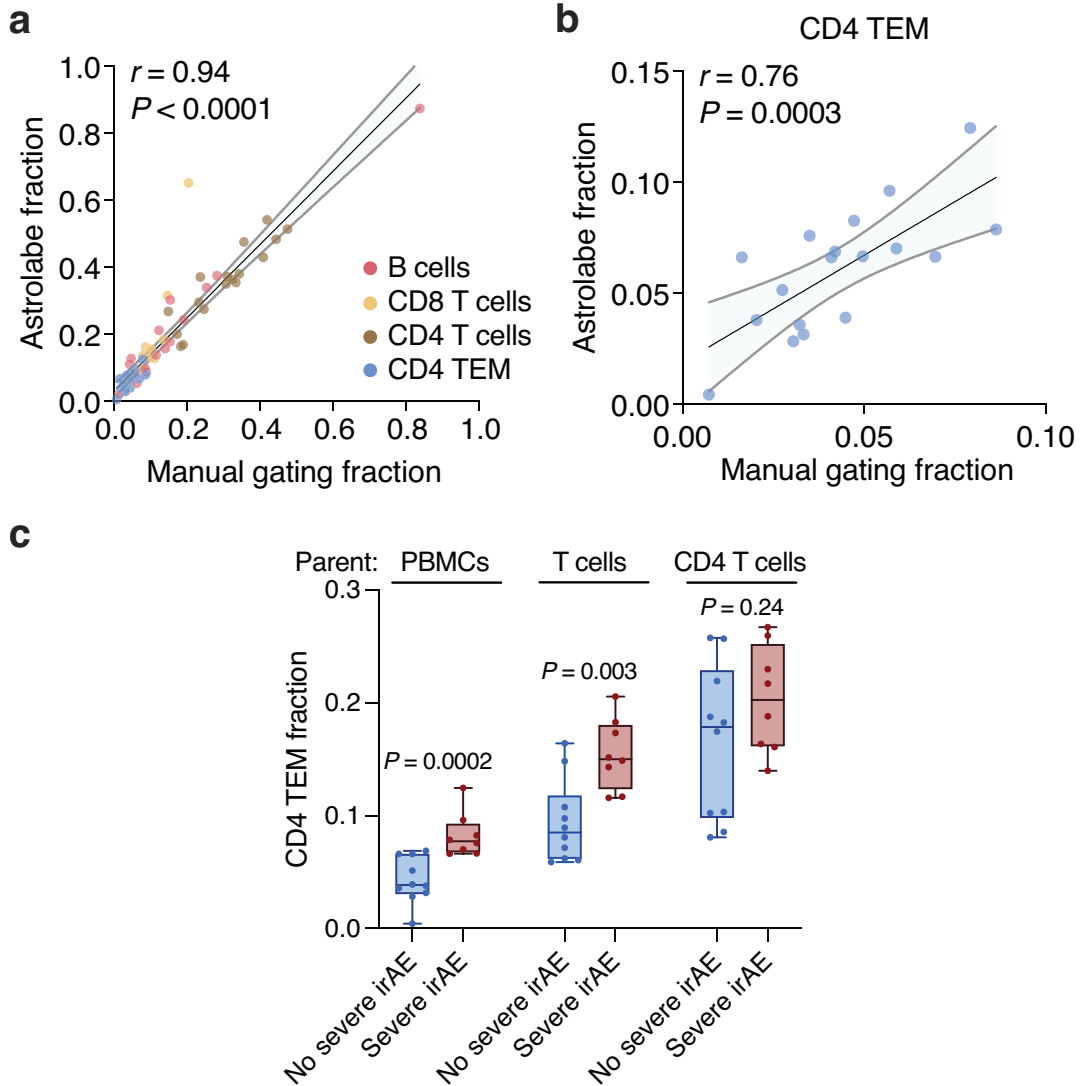# T cell characteristics associated with toxicity to immune checkpoint blockade in patients with melanoma
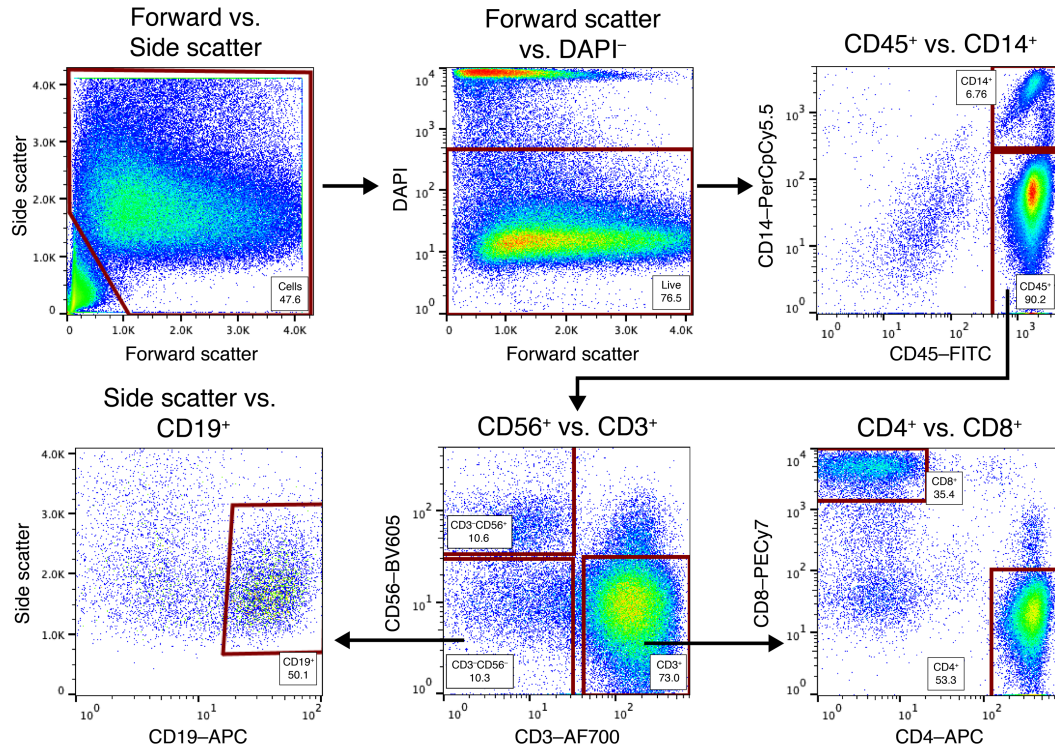
In the format provided by the authors and unedited

Supplementary Figure 1: Representative CyTOF gating hierarchies and labeling strategy. a–e, Gating hierarchies and staining results for major lineages and phenotypic states profiled by CyTOF from pretreatment PBMCs (**Supplementary Tables 1–5**; **Methods**). **a**, CD4 T cell subsets and NKT cells. All CD4 T cell subsets except T regulatory cells (Tregs) were gated analogously for CD8 T cells. TCM, central memory T cell; TEM, effector memory T cell; EMRA, CD45RA^{+} terminally differentiated effector memory T cell. **b**, Activated vs. resting CD4 TEM cells. **c**, Monocyte subsets. **d**, B cell subsets. **e**, NK cell subsets.

Supplementary Figure 2: Comparison of automated and manual cell state quantitation from CyTOF data. Scatter plot showing the concordance in frequencies between automated gating (Astrolabe) and manual gating for the indicated peripheral blood cell types. Concordance was assessed by Pearson correlation and linear regression (95% confidence band is shown). A two-sided t test was used to assess statistical significance. **b**, Same as panel a but for CD4 TEM cells. A representative gating scheme for CD4 TEM is provided in **Supplementary Fig. 1a**. Concordance was assessed by Pearson correlation and linear regression (95% confidence band is shown). A two-sided t test was used to assess statistical significance. **c,** Association of pretreatment CD4 TEM abundance with severe irAE development when expressed as a fraction of total PBMCs, total T cells, or CD4 T cells. Box center lines, bounds of the box, and whiskers denote medians, 1st and 3rd quartiles, and minimum and maximum values, respectively. Data in a–c are from patients analyzed by CyTOF in **Fig. 1** (*n* = 18; **Supplementary Tables 1–3**).

Supplementary Figure 3: Representative flow cytometry gating hierarchies and labeling strategy after excluding putative doublets. Gating hierarchies and staining results for B cells, NK cells, CD4 T cells, and CD8 T cells enumerated from healthy donor peripheral blood mononuclear cells as a function of total lymphocytes (see also **Extended Data Fig. 5e**).