Supplementary Materials for

Multi-batch cytometry data integration for optimal immunophenotyping

Supplementary Figures

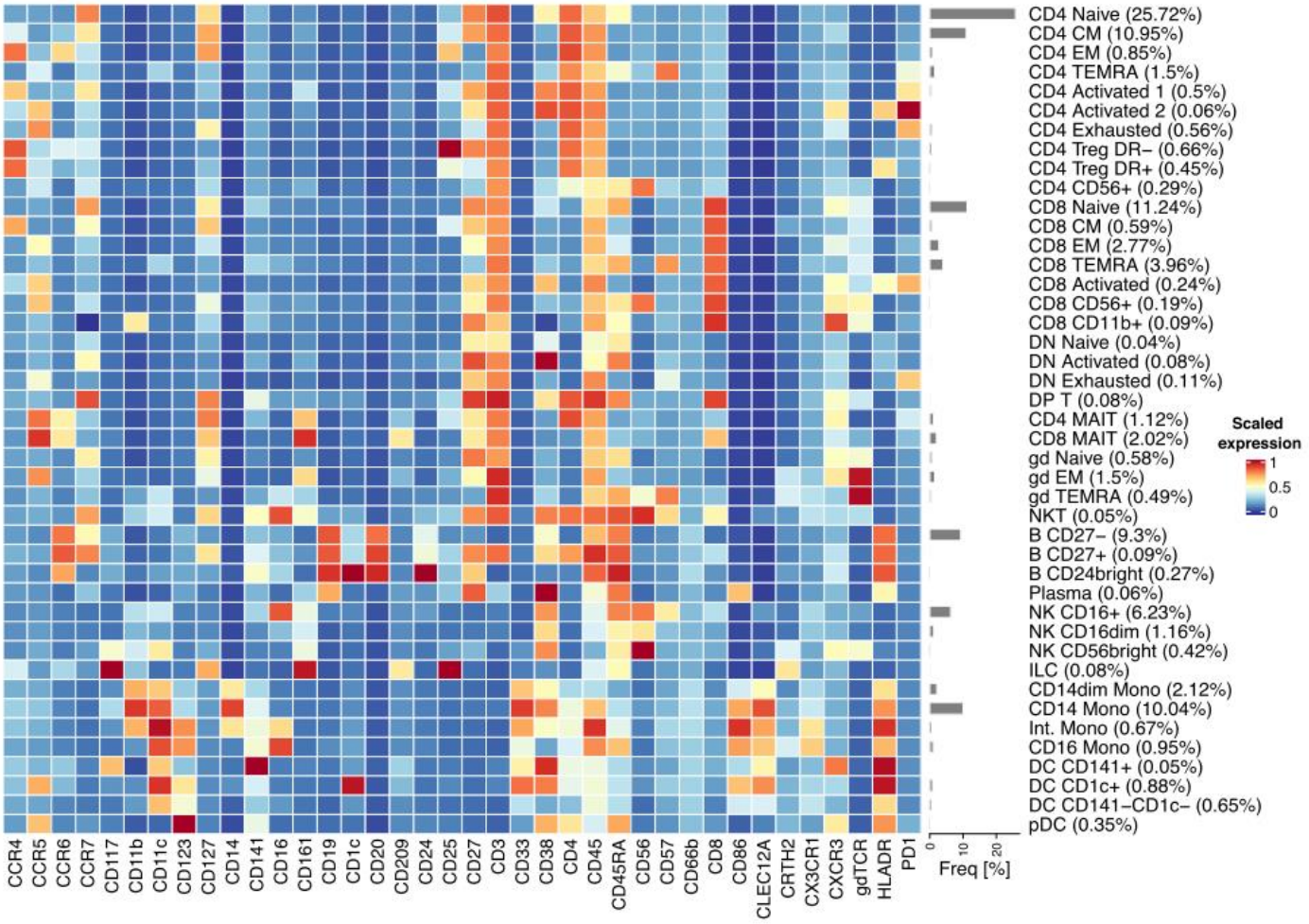

Figure S1. Cell-type identification for the in-house CyTOF dataset. After batch correction, the batch-corrected expression values were used for unsupervised clustering with FlowSOM. Initially, 60 clusters were generated. Clusters were then manually inspected to determine their identity. A summary heatmap of the scaled median expression levels of all markers for all cell subsets is shown. Clusters resembling granulocytes ($CD45^{-}CD66b^{+}$) were excluded from the heatmap analysis.

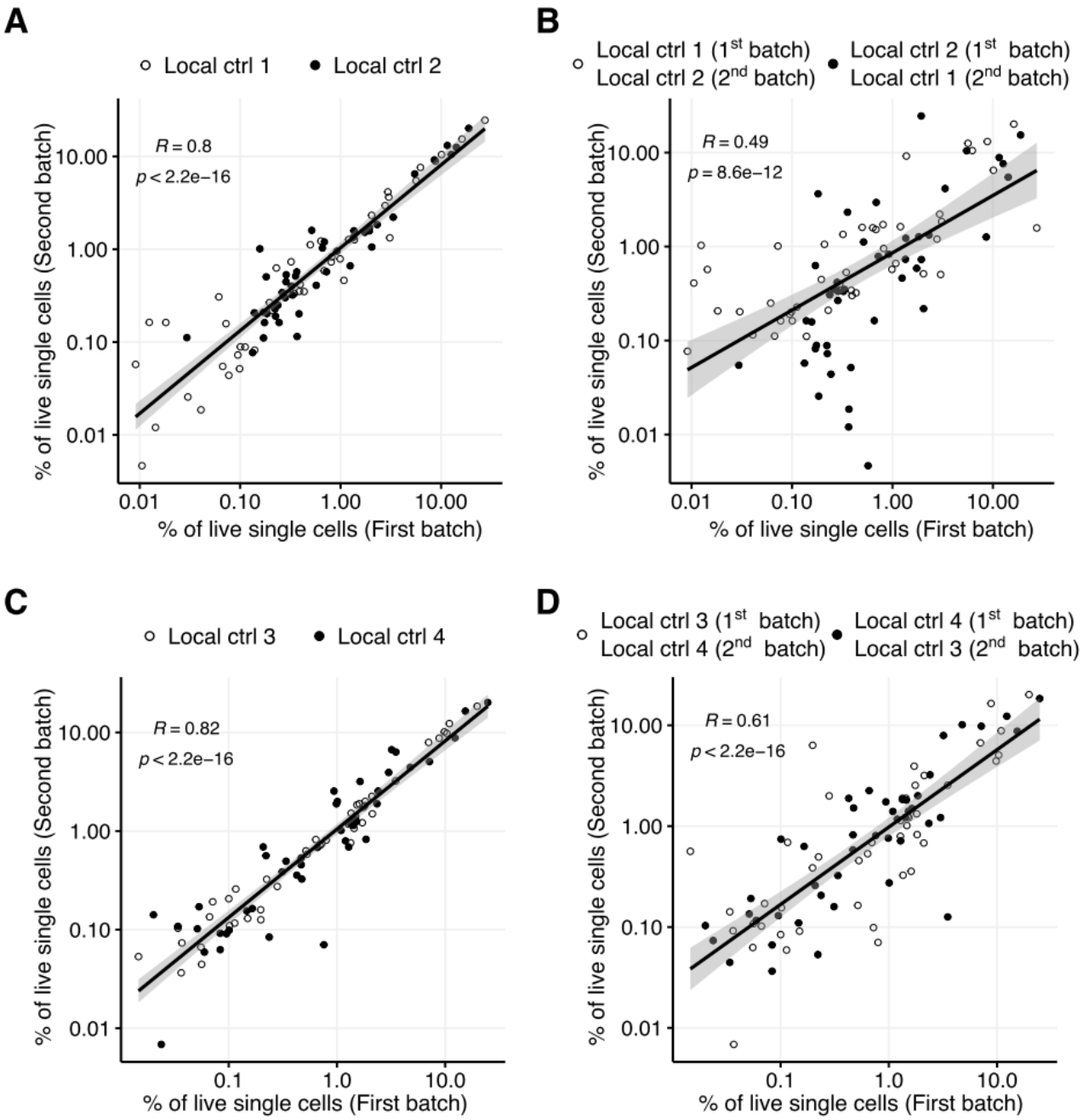

Figure S2. Analysis of the correlation between technical and biological replicates. From the in-house CyTOF dataset, we selected two healthy local controls analyzed as technical replicates (*i.e.*, experiments performed on different dates with aliquots of the same PBMC samples), and another two healthy local controls analyzed as biological replicates (*i.e.*, experiments performed on different dates using PBMC samples obtained on different occasions from the same donors). We examined the consistency of the predicted immunophenotypes between these replicates, by assessing the correlation between relative frequencies among live single cells (excluding $CD45^{-}CD66b^{+}$ granulocytes) of each of the cell types defined by unsupervised clustering followed by manual refinement. We also assessed the "background-level" correlation by intentionally inverting the donors in the second batch. (A) Technical replicates. (B) Dummy analysis of technical replicates in which two donors were intentionally inverted in the second batch. (C) Biological replicates. (D) Dummy analysis of biological replicates in which two donors were intentionally inverted in the second batch. *R*, Kendall's correlation coefficient.

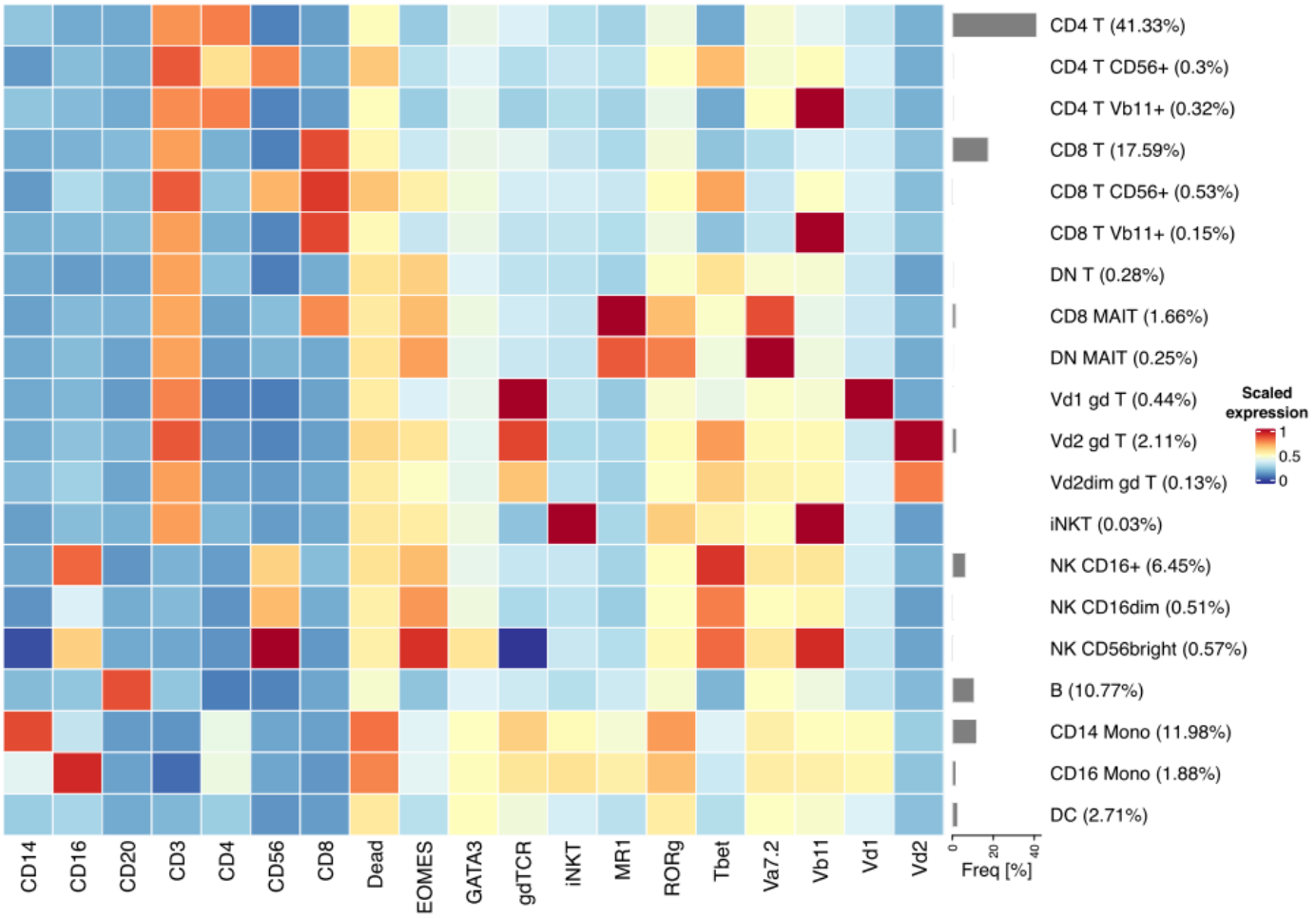

Figure S3. Cell-type identification for the in-house spectral flow cytometry dataset. The datasets consist of data for PBMCs from 11 healthy controls and three patients (two patients with FAS deficiency and one patient with a *STAT3* gain-of-function mutation) studied in two batches of experiments. The two batches were integrated with Harmony, using default parameters. The FlowSOM-guided clustering initially identified 60 clusters. Clusters were then manually inspected and annotated. A heatmap representing scaled median expression levels is shown.

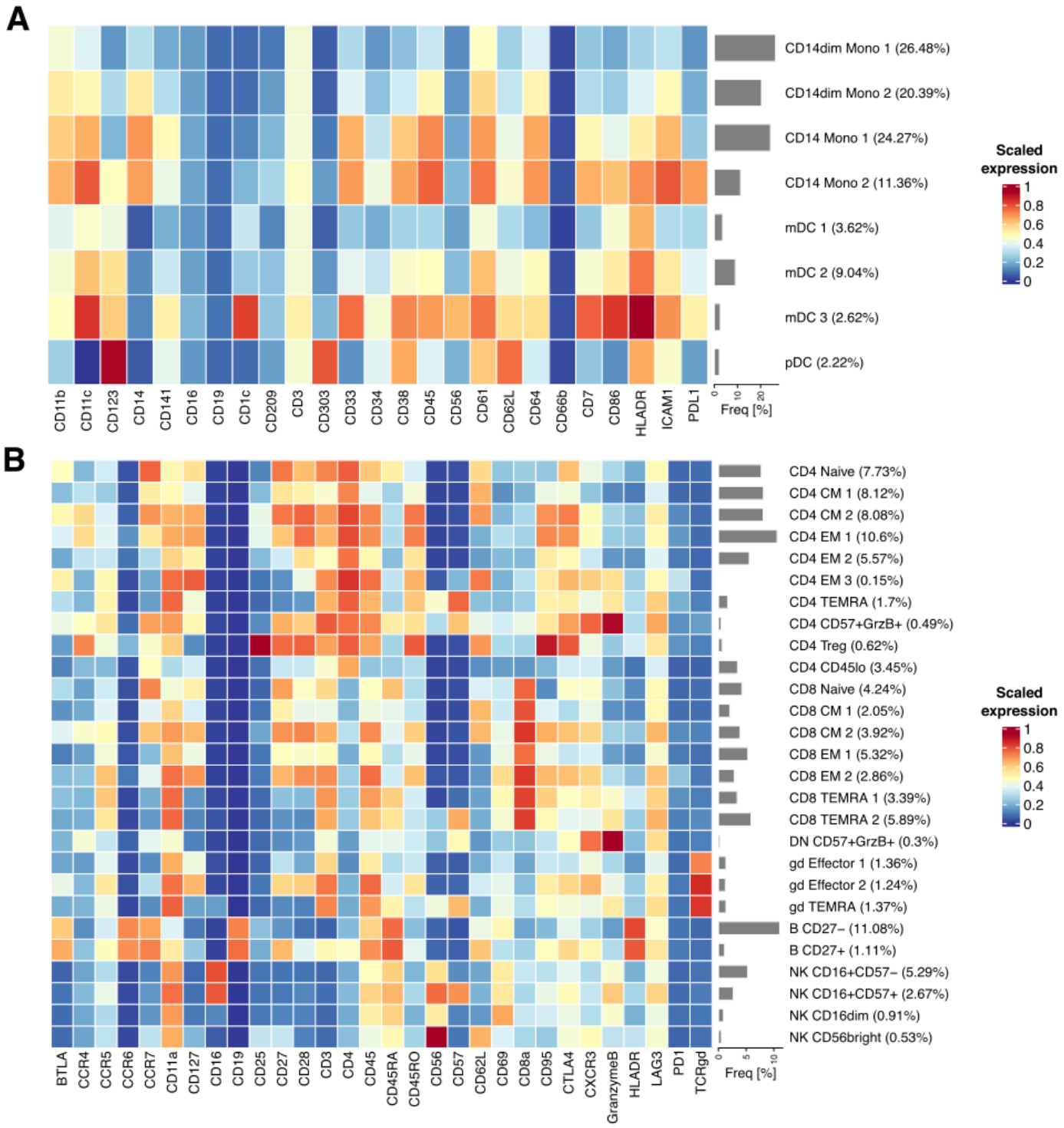

Figure S4. Cell-type identification for the CyTOF datasets for patients treated with PD-1 blockade. The datasets were downloaded from the FlowRepository (FR-FCM-ZY34). The datasets consist of PBMC samples from 10 healthy controls and 20 patients with stage IV melanoma before and after PD-1 blockade immunotherapy (*N*=11 and 9 for responders and non-responders, respectively) stained with three different antibody panels. The two batches of experiments (*i.e.*, discovery and validation cohorts) were integrated with Harmony. The FlowSOM-guided clustering initially identified 40 clusters. Clusters were then manually inspected and annotated. A heatmap representing scaled median expression levels is shown. (A) Panel 3 for myeloid cells. (B) Panel 1 for lymphocyte subsets.