# **Supplementary Material**

## **Supplementary Methods**

## **Dimensionality reduction**

For sample-wise dimensionality reduction, data were arcsinh-transformed as described above and MFI values were calculated as the median fluorescence intensity per channel of the cell type of interest belonging to one sample. For the analyses performed, only MFI values of fluorescent channels (therefore excluding scatter-, autofluorescence- and time-channel) without prior scaling of the data were used. Principal component analysis (PCA) was performed using the scikit-learn implementation (v. 1.3.0) using standard settings and computing three components. The data were visualized using seaborn (v. 0.12.2). For single cell dimensionality reduction, PCA was performed using only transformed data of fluorescent channels without prior scaling. The first 20 PCA components were used to compute a neighbourhood graph using scanpy (v. 1.9.3, standard settings)[17], that was then used to compute the UMAP representation of the data using umap-learn (v. 0.5.0) [16]. The data were visualized using seaborn.

### **Fold-change calculation**

To compute differential expression, the arcsinh-fold change was computed. As cytometry data can contain negative values, classical log2-fold-change calculations are not applicable. For the analysis performed, median fluorescence intensity (MFI) values of non-transformed data were calculated. Using the manually assigned cofactors, the MFI values were arcsinh-transformed and the fold change was calculated as the mean ratio of the arcsinh-transformed values of group1 divided by the arcsinh-transformed values of group2. The *P*-value was assigned using a Kruskal test on the transformed values where each sample represented one data point.

### **Sample correlation analysis**

For the sample correlation analysis, the median fluorescence values of arcsinh-transformed data were calculated as above and correlated using the Pearson correlation as implemented in the pandas (v. 2.1.1) library. For clustering, Euclidean pairwise distances of each sample represented by their Pearson correlation coefficient was calculated and hierarchical clustering, resulting in a linkage matrix, was performed using Scipy (v. 1.11.3). To calculate metaclusters, the cut tree with a manually defined number of metaclusters of the calculated linkage matrix was obtained using Scipy.