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Introduction

Persistent measurable residual disease (MRD) is highly predictive for acute myeloid leukemia (AML) relapse and has become one of the most important biomarkers for risk stratification, response assessment and treatment allocation. MRD detection can be performed with multiparametric flow cytometry (MRD-MFC) due to its applicability to most AML patients, short turnaround times, and widespread availability of markers and fluorochromes. This approach relies on the evaluation of abnormal expression of surface markers on leukemic blasts (leukaemia associated immunophenotypes, LAIPs). Such marker combinations are not present on normal hematopoietic cells or on normal maturation pathways and can be detected with high sensitivity in follow-up samples. Current challenges of MRD-MFC are represented by the high number of LAIPs described, complex multi-tube conventional flow cytometry panels, and their subjective identification by trained experts. Therefore, automated unsupervised analysis tools that overcome the subjectivity of expert analysis and faithfully capture rare AML cells in MRD samples are needed.

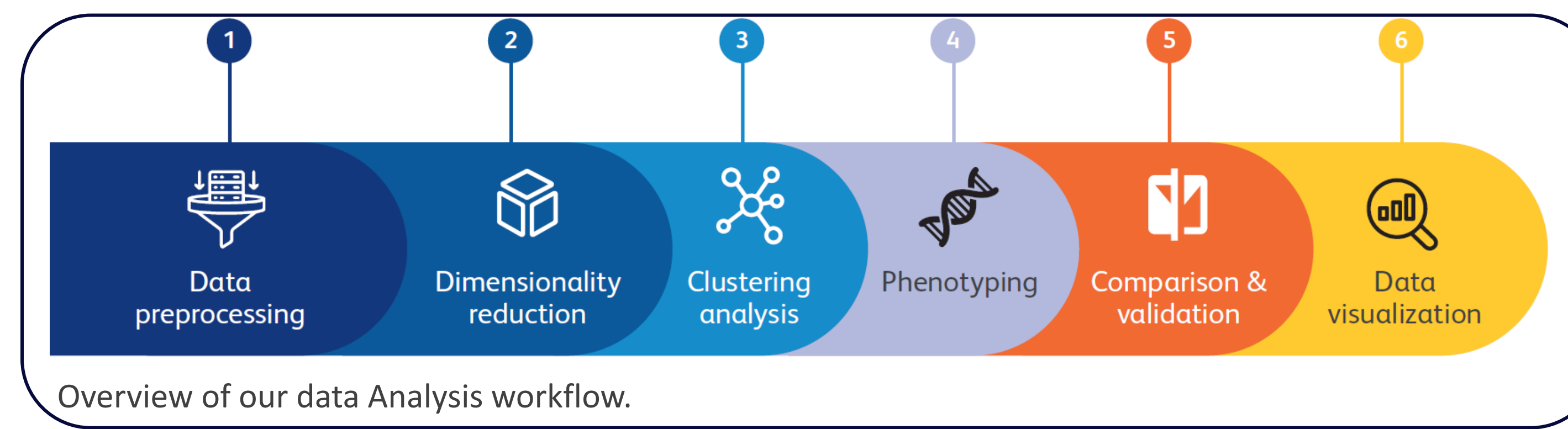
Methods

Disidentified leftover bone marrow (BM) samples from a cohort of subjects with confirmed LAIP for AML were utilized for this scientific research study with the informed consent of donors. Cases were selected and re-analysed using samples collected at different timepoints between disease onset, treatment and relapse by staining with the relevant combinations of antibodies (CD33, Anti-HLA-DR, CD19, CD7, CD45, CD117, CD13, CD14, CD34) in a polychromatic flow cytometry assay. This step served to define a leukaemia immunophenotypic fingerprint that, in turn, was used to track residual leukaemia cells (RLCs) across the different timepoints. An unsupervised analysis approach was applied on 17 different timepoints, including data preprocessing (compensation and scaling, QC using PeacoQC, metadata annotation and concatenation-1); tSNE dimensionality reduction (2), FlowSOM clustering with clustering QC (3) Phenotyping and final validation and comparison by using cluster explorer (4-5). The analysis was performed by using FlowJo™ v10.10.

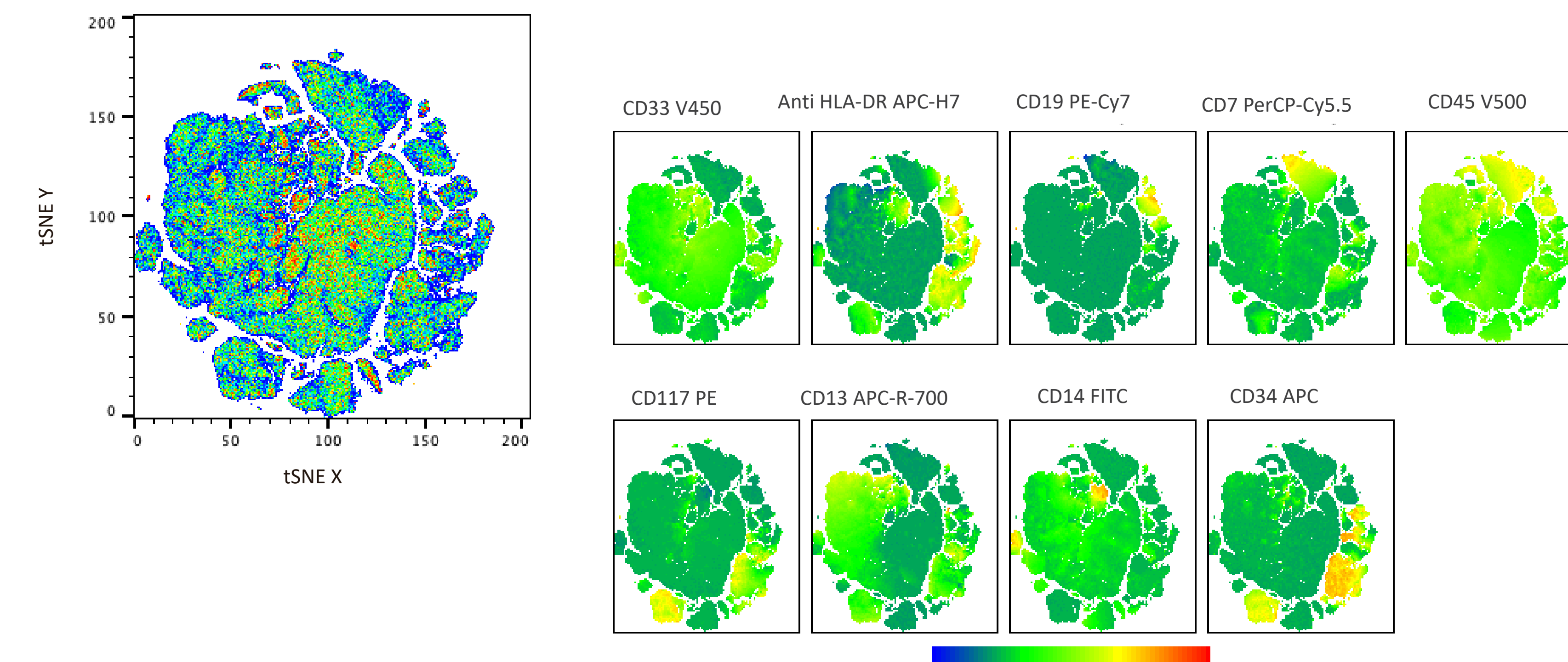
Results

Rare cell populations with aberrant immunophenotypic patterns may represent a challenge for conventional analysis approaches using manual gating strategies. In this scientific research study, we applied a structured workflow using unsupervised computational analysis to evaluate disidentified bone marrow samples of subjects with leukemia associated immunophenotypes in different timepoints from disease onset to relapse. This approach allowed the characterization of an aberrant cluster identified as Pop12 (4) present at the timepoints of diagnosis, post-consolidation, post-transplant increase, immunosuppressive therapy suspension and relapse (5 and 6). Our results suggest that structured unsupervised analysis may be useful for detecting rare cell populations in complex samples such as BM of AML subjects and useful for studying aberrant immunophenotypic patterns over time.

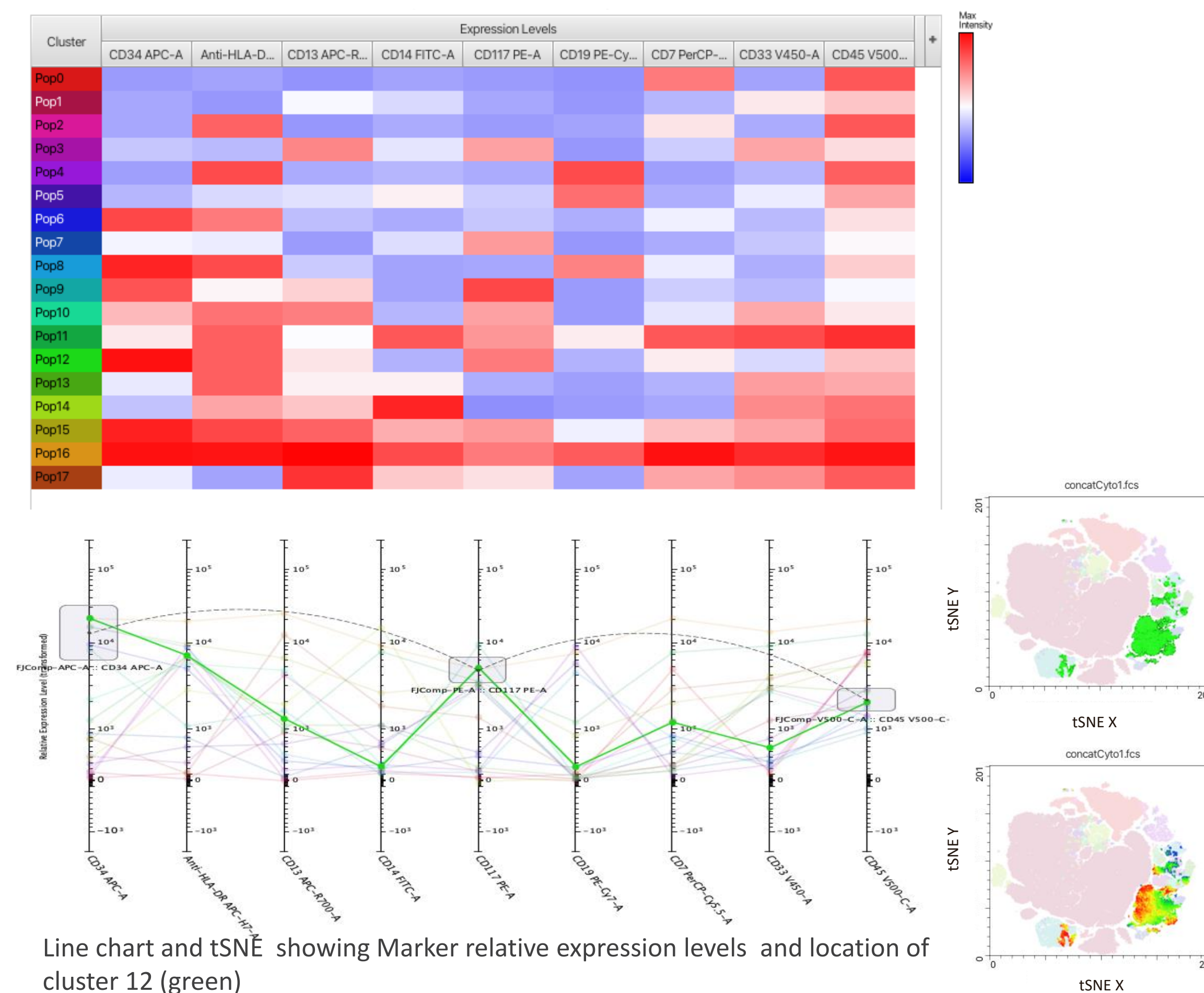
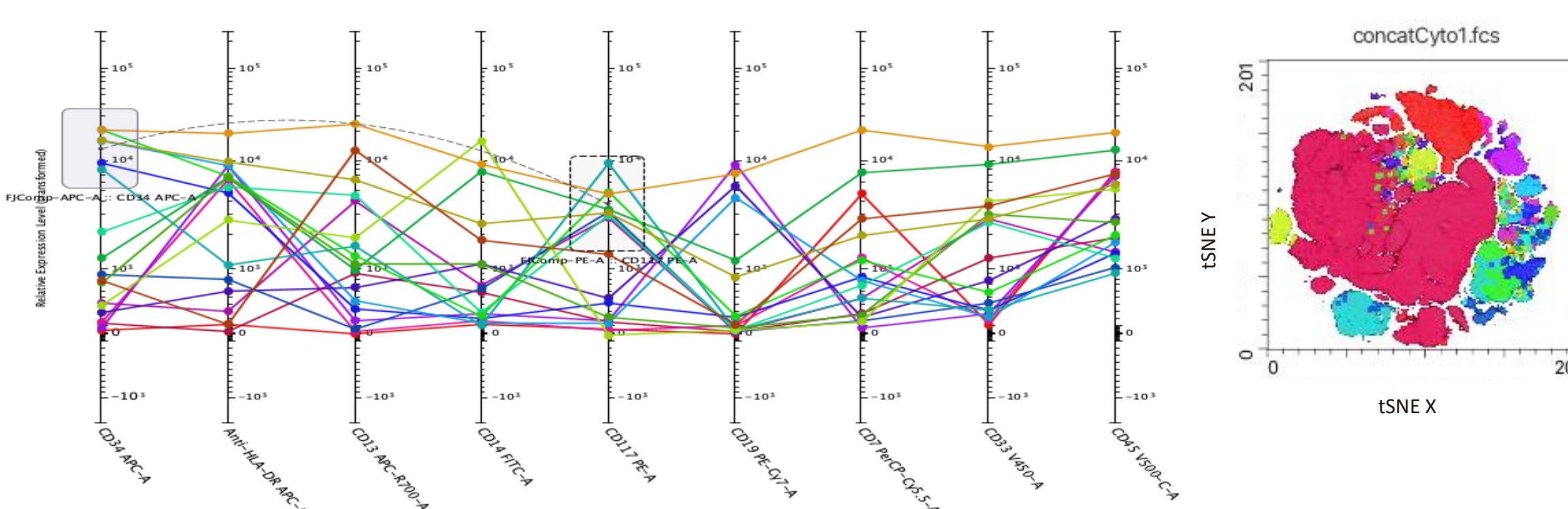
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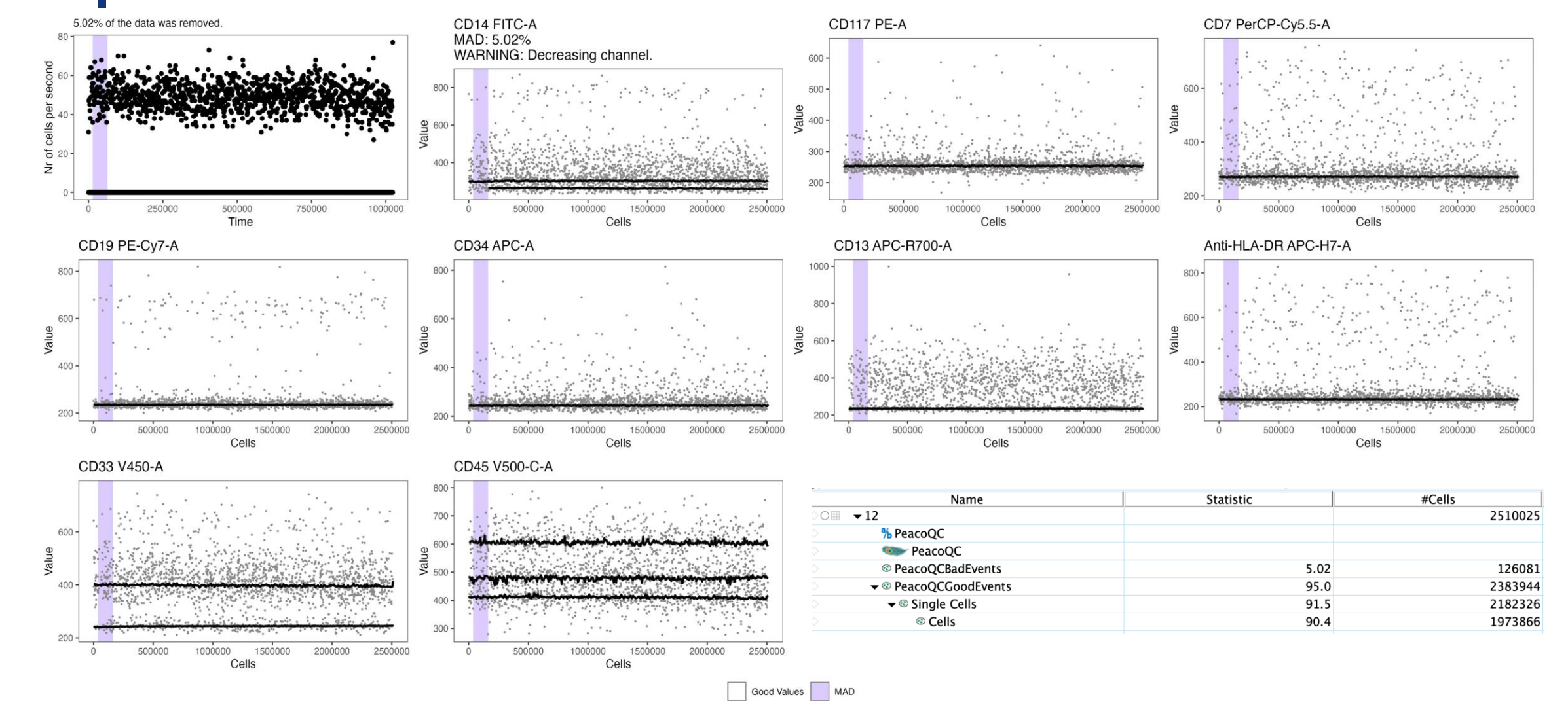
2 Dimensionality reduction by using tSNE and Heat map antigen expression orientation.



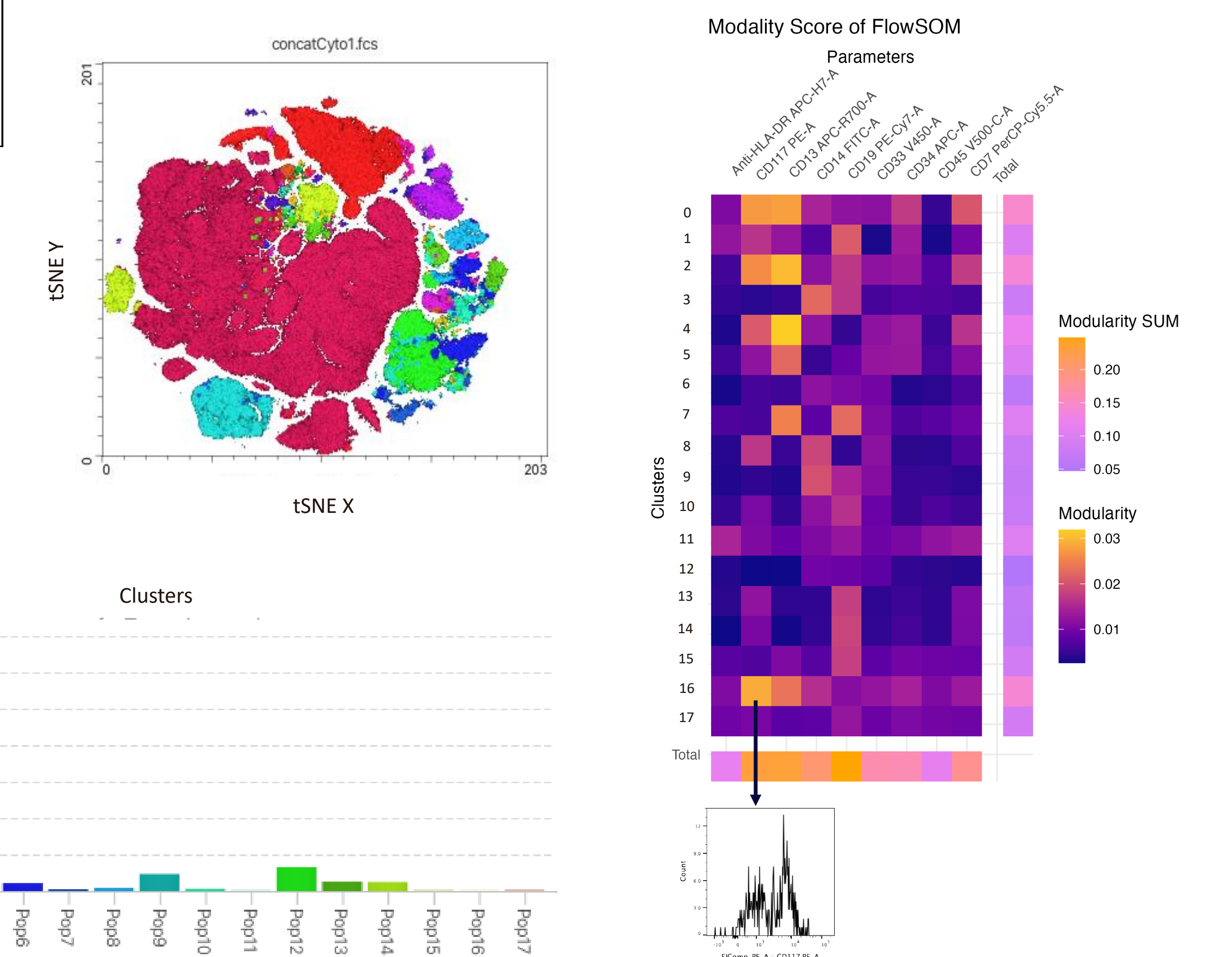
4 Phenotyping. CD 34^{hi} and CD 117+ clusters were selected. Line chart showing markers relative expression level and MFI Heatmap for each cluster.



1 Preprocessing step: compensation and scaling (not shown), data QC by using PeacoQC, export of good events as a new fcs file, metadata annotation and concatenation.



3 Clustering analysis by using FlowSOM returned 18 different clusters. Bar chart showing clusters size in % of events. Clusters QC was evaluated by calculating modality score. Example of "bad cluster" with histogram showing Cluster 16 having multimodal distribution for CD117.



5 Phenotyping and Comparison: FlowSOM Cluster Heatmap table. Timepoints comparison showing cluster frequencies. Cluster 12 is highly represented at TP0 (Diagnosis) and TP13 (Relapse)



6 Validation: Cluster 12 is present at relevant timepoints.

