SuperCellCyto: enabling efficient analysis of large scale cytometry datasets

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Abstract

The rapid advancements in cytometry technologies have enabled the quantification of up to 50 proteins across millions of cells at the single-cell resolution. The analysis of cytometry data necessitates the use of computational tools for tasks such as data integration, clustering, and dimensionality reduction. While numerous computational methods exist in the cytometry and single-cell RNA sequencing (scRNAseq) fields, many are hindered by extensive run times when processing large cytometry data containing millions of cells. Existing solutions, such as random subsampling, often prove inadequate as they risk excluding small, rare cell subsets. To address this, we propose a practical strategy that builds on the SuperCell framework from the scRNAseq field. The supercell concept involves grouping single cells with highly similar transcriptomic profiles, and has been shown to be an effective unit of analysis for scRNAseq data. We show that for cytometry datasets, there is no loss of information by grouping cells into supercells. Further, we demonstrate the effectiveness of our approach by conducting a series of downstream analyses on six publicly available cytometry datasets at the supercell level, and successfully replicating previous findings performed at the single cell level. We present a computationally efficient solution for transferring cell type labels from single-cell multiomics data which combines RNA with protein measurements, to a cytometry dataset, allowing for more precise cell type annotations. Our SuperCellCyto R package and the associated analysis workflows are available on our GitHub repositories (https://github.com/phipsonlab/SuperCellCyto and https://phipsonlab.github.io/SuperCellCyto-analysis/).

Introduction

Cytometry technologies, such as flow cytometry and mass cytometry, have revolutionised the field of cellular biology by enabling the high-throughput quantification of physical and biochemical characteristics of millions of cells at the single-cell level. These technologies, which can measure cellular dimensions, granularity, and the expression of over 40 proteins (markers), have found widespread application across diverse biological and medical research domains. For instance, they have been used to elucidate immune responses to infectious diseases [1], to investigate B cell behaviour in Multiple Sclerosis patients [2], and to study the human hematopoietic system [3].

Traditional cytometry data analysis typically employs manual gating, a process which delineates cell types by iteratively refining polygons on a series of two-dimensional scatter plots, with each plot illustrating the expression of two markers. However, this approach is intractable for identifying a vast variety of cell types due to the numerous combinations of two markers that must be examined [4]. To address this, a plethora of computational methods have been developed. These include methods for data pre-processing (such as CATALYST [5]), clustering (such as FlowSOM [6], Phenograph [7], X-shift [8]), dimensionality reduction (such as UMAP [9] and FITsne [10]), and differential expression or abundance analysis (such as Diffcyt [11] and Citrus [12]). Additionally, toolkit-like software libraries such as Spectre [4], CytofKit [13], and CyTOF workflow [14] provide function interfaces (wrappers) for a range of computational methods, thereby facilitating the creation of analysis workflows. Concurrently, the single-cell RNA sequencing (scRNAseq) field has seen an explosion in bioinformatics methods development, with more than 1,500 tools currently available [15], including Seurat [16]–[19], Bluster [20], Batchelor [21], and Speckle [22]. Remarkably, there are many computational analysis tasks that are shared between cytometry and scRNAseq data analysis, e.g., clustering and batch correction. However, methods to analyse cytometry and scRNAseq data have been mostly developed independent of one another. We believe there is an opportunity to take advantage of the many scRNAseq tools for analysing cytometry data.

Despite the robustness and diversity of existing computational methods, many face challenges in terms of scalability when processing large cytometry datasets [4]. This issue is particularly pronounced when using methods primarily developed for scRNAseq data [23]. Given that scRNAseq data typically encompassess fewer cells but a significantly higher number of features compared to cytometry data, these methods may require an excessive runtime when applied on cytometry data, making them impractical for efficient data analysis. Importantly, this issue is not confined to scRNAseq methods alone, and is also evident in some cytometry-specific methods [24]. Furthermore, many computational methods depend on parameters that significantly influence their performance, often necessitating time-consuming parameter tuning for optimal results. Given the continuous expansion of cytometry data, which now routinely includes millions of cells, the demand for more efficient methods is increasingly urgent.

Existing strategies to address these challenges include data reduction through random subsampling, which, although expediting analysis, may result in the loss of representation for rare cell populations. Alternative solutions such as method re-implementation or utilisation of

high-performance computing (HPC) platforms are often not feasible due to the need for specialised expertise and/or limited accessibility to HPC resources.

In this study, we present a strategy to mitigate these challenges by developing SuperCellCyto, an adaptation of the SuperCell R package [25]. Initially developed for scRNAseq data, the SuperCell method aggregates cells with similar transcriptomic profiles into "supercells" (also known as “metacells” in the scRNAseq literature [26]–[28]). This aggregation effectively reduces the dataset size by 10 to 50 times, while preserving biological diversity, thus alleviating the computational demands of downstream analysis. SuperCellCyto is a significant enhancement of the SuperCell package. Specifically, we have implemented within-sample supercell creation, preventing supercells from containing cells across multiple samples. Additionally, we have introduced parallel processing capabilities using a careful load balancing strategy, allowing the simultaneous creation of supercells across multiple samples, which significantly speeds up computational time.

We highlight supercells’ capacity to retain biological heterogeneity by aggregating single cells in previously annotated cytometry data into supercells and verify that each supercell predominantly comprises unique cell types. Furthermore, we showcase the viability of analysing supercells as a surrogate to single cells by conducting a series of downstream analyses on supercells derived from six publicly available cytometry datasets. These analyses encompass cell type identification, batch effect correction, differential expression and abundance analysis. Finally, we demonstrate the ability to annotate supercell-level cytometry data using a multiomics dataset that measures RNA and protein expression on the same cells (Cellular Indexing of Transcriptomes and Epitopes (CITEseq) [29]) as a reference. This allows us to exploit the rich cell type annotation derived from deep transcriptome sequencing. Altogether, our findings affirm that SuperCellCyto efficiently reduces dataset size, vastly reduces the computational burden of analysing large cytometry datasets, and maintains the integrity of downstream analyses. The SuperCellCyto R package is publicly available on GitHub (https://github.com/phipsonlab/SuperCellCyto), as well as the associated analysis workflow (https://phipsonlab.github.io/SuperCellCyto-analysis/), offering a valuable resource for the research community.

Results

Concurrent Generation of Supercells Across Multiple Samples Using SuperCellCyto

The SuperCellCyto R package is an extension of the SuperCell package adapted specifically for cytometry data. SuperCellCyto offers a practical approach to reduce the size of large cytometry data by grouping cells with similar marker expression into supercells. Figure 1A depicts the schematic overview of the generation of supercells using the SuperCellCyto R package. The process begins with performing a Principal Component Analysis (PCA) on the cytometry data to capture the main sources of variation. The number of principal components (PCs) is adjustable, with a default setting of 10, but can be increased up to the number of markers in the data. For datasets with fewer than 10 markers, the number of PCs is set to the number of markers in the data. Using the PCs, a k-nearest-neighbour (kNN) graph is constructed, with each node representing a single cell. A walktrap algorithm [30] is then applied to identify densely interconnected subgraphs or communities. This step involves performing a series of four-step random walks from each node, where a single step represents a transition from one node to another. The destination node for each step is selected randomly, and the probability of a random walk starting and ending at a given pair of nodes is used to determine their proximity. After computing these distances, nodes are iteratively merged, starting with each node in its own community, until a single community is formed, creating a dendrogram-like structure. This dendrogram is then cut at a specific level to yield the desired number of communities, referred to as supercells.

The number of supercells generated for a given dataset is controlled by the parameter gamma, which governs the granularity of the supercells and determines at which level the dendrogram is cut. Gamma is defined as the ratio of the number of cells to the number of supercells. Choosing the appropriate gamma value involves balancing the trade-off between the data compression level and the probability of supercells encompassing diverse cell types. A larger gamma value produces fewer supercells (higher compression level), but each supercell is more likely to contain more heterogeneous cell types. Conversely, a smaller gamma value yields more supercells (lower compression level), but each supercell is less likely to contain multiple different cell types. It is important to note that each supercell may contain a different number of cells, centred around the value of gamma. We found the default value of 20 to work well for most datasets. Critically, we have implemented within SuperCellCyto, the ability to adjust the number of supercells generated for a dataset without having to regenerate the kNN graph or rerunning the walktrap algorithm. This feature allows users to rapidly fine tune the data compression level as required.

The SuperCellCyto R package takes processed and appropriately transformed cytometry data as input, formatted as an R data.table or data.frame where each row represents a cell and each column denotes a marker. It generates, for each supercell, their marker expression, computed as the mean expression of all cells within the supercell, as well as a list detailing all the cells

each supercell encompasses (Figure 1B step 1-2). Importantly, SuperCellCyto is designed to process each sample independently, and we have introduced the ability to process multiple samples concurrently. This is facilitated through the use of the BiocParallel R package [31] and a custom load balancing strategy. Samples are first ordered based on their cell count, and parallel workers are then tasked to process these samples in descending order, starting with the sample that contains the most number of cells. This strategy allows workers processing smaller samples to be assigned additional samples, while those handling larger samples can concentrate on their tasks without being overburdened. This approach maximises throughput and minimises idle time, thereby enhancing overall efficiency.

Figure 1B illustrates our proposed workflow for an end-to-end cytometry data analysis, using supercells as the fundamental unit of analysis. The first step is to pre-process the cytometry data to exclude doublets and dead cells, transform the data using either ArcSinh transformation or Logicle [32] transformation, and create supercells from the cleaned transformed single cell level cytometry data. Once supercells have been created, almost all subsequent analysis tasks can be performed at the supercell level, including batch correction (if necessary), clustering and cell type annotation, and differential expression analysis. For differential abundance analysis, we strongly recommend expanding the annotated supercells to the single cell level and calculating cell type proportions from the single cell level data. Additionally, we also recommend discarding underrepresented clusters, that is, clusters that only captured a small number of cells from each sample. For differential expression and abundance analyses, existing R packages, including those from the scRNAseq field such as Limma [33], EdgeR [34]–[36], or Propeller [22], can be used.

Supercells Preserve Biological Heterogeneity and Facilitate Efficient Cell Type Identification

We assessed whether supercells could preserve the biological diversity inherent in a cytometry dataset, and whether the clustering of supercells could expedite the process of cell type identification without compromising accuracy. We generated supercells for two publicly available cytometry datasets, Levine_32dim [7] and Samusik_all [8] (Supplementary Material Table S1) using a range of gamma values (Supplementary Material Table S2 and Supplementary Material Figure S1). For gamma set to 20 (the default), the Samusik_all dataset was reduced from 841,644 single cells to 42,082 supercells, an approximately 20 fold reduction.

Thereafter, we clustered the supercells using FlowSOM [6], a popular clustering algorithm for cytometry data, and Louvain [37], a popular clustering algorithm for analysing scRNAseq data. For both algorithms, we broadly explored their parameter space, namely the grid size and the number of metaclusters for FlowSOM, and the parameter *k* for Louvain (see Supplementary Material Table S3 for the list of values explored). Using the cell type annotation acquired through a manual gating process performed by the authors of the datasets as the ground truth, we evaluated the quality of the supercells by using two metrics; purity and Adjusted Rand Index (ARI). Purity quantifies the homogeneity of cell types within each supercell by measuring the proportion of the most dominant cell type within a given supercell. ARI measures the similarity

between the clustering of supercells and the manually gated cell type annotation. Additionally, for ARI, we also measured the concordance between the clustering of supercells and the clustering of single cells (see Materials and Methods section for more details).

Figure 2A-B illustrates the distribution of supercells' purity scores across all gamma values. For both datasets, we observed very high mean purity scores across all gamma values (mean purity > 0.9, Figure 2A), with the vast proportion of supercells attaining a purity score of 1 (Figure 2B). A small percentage of supercells (0.04% for Levine_32dim and 0.59% for Samusik_all across all gamma values) obtained purity < 0.5. Altogether, this indicates that most supercells are composed of either only or mostly a single cell type. As the gamma value increases, we observe a slight decline in both the mean purity score and the proportion of supercells obtaining a purity score of 1 (Figure 2A and 2B), consistent with the fact that larger gamma values result in a higher likelihood of each supercell capturing multiple cell types. Lastly, we compared the distribution of marker expression between the supercells and single cells, and found most of them to be almost identical (Supplementary Material Figure S2).

Upon examining the ARI computed between the supercell clustering and the cell type annotation (Figure 2C), as well as between the clustering of supercells and single cells (Figure 2D), we observed high scores across all datasets, clustering algorithms, and gamma values. Similar to the trend observed for purity scores, the ARI scores also exhibited a slight decline as the gamma value increased, likely due to each supercell encompassing a more diverse set of cell types. We noted a larger variation in ARI scores obtained for FlowSOM clustering results compared to those obtained for Louvain clustering results, potentially due to the broader range of parameter settings explored for FlowSOM (Supplementary Material Table S3).

These results collectively demonstrate that supercells can vastly improve clustering efficiency while effectively preserving the biological heterogeneity within a dataset, thereby enabling accurate clustering and identification of cell types.

Identifying Rare B Cells Subsets by Clustering Supercells

Downstream analysis of cytometry data routinely involves clustering cells and subsequently manually annotating them based on their marker expression to determine the cell types they represent. While we have demonstrated that SuperCellCyto can maintain fidelity of cell types compared to cell type annotation obtained through manual gating, we next sought to verify whether we can faithfully replicate the traditional clustering and annotation process, and identify rare cell populations at the supercell level. We applied SuperCellCyto and Louvain clustering to a large flow cytometry dataset profiling more than 8 million B cells in healthy human bone marrow samples (Oetjen_bcells data [38], Supplementary Material Table 1).

Our analysis workflow consists of steps 1, 2, and 4 in Figure 1B. First, we ran SuperCellCyto with gamma set to 20 to reduce the dataset to 415,711 supercells. We next clustered the supercells using the Louvain clustering algorithm. Based on the clustering results and known marker expression for B cells (Figure 2E and Supplementary Material Note S1), we successfully identified all the B cell subsets present in the dataset, including the extremely rare plasma cells

of which there are only 162 cells (0.002%) present at the single cell level (Figure 2F and Supplementary Material Figure S3A). To further validate the cell type annotation conducted at the supercell level, for each cell type, we expanded the supercells into individual cells and examined their marker expression profiles. We found them to be consistent with the manual gating scheme previously used by Oetjen et al. to identify the B cell subsets (Supplementary Material Figure S3B and Note S1).

Mitigating Batch Effects in the Integration of Multi-Batch Cytometry Data at the Supercell Level

Cytometry experiments often generate datasets comprising millions of cells across several batches. This can introduce technical variation, known as batch effects, between samples from different batches. Batch effects stem from differences in experimental conditions and/or instruments [4]. Before proceeding to downstream analyses like clustering or differential expression or abundance analyses, it is imperative to rectify these batch effects using batch effect correction methods such as CytofRUV [39] or cyCombine [40] (for more details, see Materials and Methods).

To demonstrate the feasibility of correcting batch effects at the supercell level, we applied CytofRUV and cyCombine to a large mass cytometry dataset profiling the peripheral blood of healthy controls (HC) and Chronic Lymphocytic Leukaemia patients (CLL) (Trussart_cytofruv data [39], Supplementary Material Table 1). This dataset consists of 8,589,739 cells and 12 paired samples profiled across two batches (each batch profiles one of the paired samples), yielding a total of 24 samples (Figure 3A). Our analysis workflow consists of steps 1-3 in Figure 1B. We applied SuperCellCyto with gamma set to 20 to reduce the data to 429,488 supercells, followed by CytofRUV and cyCombine batch correction. The effectiveness of the batch correction methods was assessed through Multidimensional Scaling (MDS) analysis and comparison of the Earth Mover Distance (EMD) metric for each marker across the two batches.

Figure 3A presents the MDS plots at the sample level for uncorrected single cells, uncorrected supercells, and CytofRUV- and cyCombine-corrected supercells. In both uncorrected single cells and supercells, dimension 1 distinguishes CLL samples from HC samples, while dimension 2 separates the batches, indicating the presence of strong batch effects even at the supercell level. However, in the batch-corrected supercells, while dimension 1 continues to differentiate the CLL samples from the HC samples, dimension 2 no longer separates the samples based on their batch. This observation is further supported by the UMAP plots of uncorrected and corrected supercells, which demonstrate reduced batch-based separation following correction (Supplementary Material Figure S4).

We compared the marker expression distribution between the two batches for uncorrected, CytofRUV-corrected, and cyCombine-corrected supercells, and found them to be more similar to one another following batch effect correction (Supplementary Material Figure S5). This was particularly evident for CD14, CD45RA and PUMA markers (Figure 3B).

The EMD calculated for the uncorrected and corrected supercells are shown in Supplementary Material Figure S6. Generally, we observed a reduction in EMD score for both CytofRUV- and cyCombine-corrected supercells, indicating effective batch effect correction.

In summary, our analysis demonstrates that while batch effects are present at both the single cell and supercell level, they can be effectively corrected using batch effect correction methods such as CytofRUV and cyCombine following summarisation of single cells to supercells with SuperCellCyto.

Recovery of Differentially Expressed Cell State Markers Across Stimulated and Unstimulated Human Peripheral Blood Cells

For cytometry data analyses, subsequent downstream analysis steps following clustering and cell type annotation typically involve identifying cell state markers that are differentially expressed across different experimental groups or treatments. In this analysis, we assess whether a differential expression analysis performed at the supercell level can recapitulate previously published findings obtained by performing differential expression analysis using the Diffcyt algorithm [11] at the single cell level. Specifically, we analysed a publicly available mass cytometry dataset quantifying the immune cells in stimulated and unstimulated human peripheral blood cells (BCR_XL dataset [41], Supplementary Material Table 1). Notably, this is a paired experimental design, with each of the 8 independent samples, obtained from 8 different individuals, contributing to both stimulated and unstimulated samples (16 samples in total). Importantly, this dataset was previously analysed using the Diffcyt algorithm to identify the cell state markers that were differentially expressed between the stimulated samples (BCR-XL group) and the unstimulated samples (Reference group). We refer readers to the Materials and Methods section for more information on the dataset. Our aim was to replicate these findings using a combination of SuperCellCyto and the Limma R package [33].

Our analysis workflow consists of steps 1, 2, 4, and 5 in Figure 1B. First, we used SuperCellCyto (gamma = 20) to generate 8,641 supercells from 172,791 cells (step 1-2). We then annotated the supercells with the corresponding cell type labels (step 4) and calculated the mean expression of each cell state marker for every sample and cell type combination, akin to the pseudobulk approach commonly used in scRNAseq differential expression analysis. Next we used the Limma R package to test for expression differences between the stimulated and unstimulated samples, for each cell type separately, accounting for the paired experimental design (step 5, see Materials and Methods section for more details). Given the availability of cell type annotation, we slightly modified step 4 by annotating each supercell with the label of the most abundant cell type it encompasses, as opposed to clustering the supercells (refer to the Materials and Methods section for more details).

Our workflow successfully identified several cell state markers which show strong differential expression between the stimulated and unstimulated groups (False Discovery Rate (FDR) <= 0.05, Figure 4A-B and Supplementary Material Figure S7). Our findings were consistent with those identified by Diffcyt, including elevated expression of pS6, pPlcg2, pErk, and pAkt in B

cells in the stimulated group, along with reduced expression of pNFkB in the stimulated group (Figure 4A). We also recapitulated the Diffcyt results in CD4 T cells and Natural Killer (NK) cells, with significant differences in the expression of pBtk and pNFkB in CD4 T cells between the stimulated and unstimulated groups (Figure 4B), and distinct differences in the expression of pBtk, pSlp76, and pNFkB in NK cells between the stimulated and unstimulated groups (Supplementary Material Figure S7).

Identification of Differentially Abundant Rare Monocyte Subsets in Melanoma Patients

In this analysis, we investigated the capacity to conduct differential abundance analysis using supercells. We applied SuperCellCyto and Propeller [22] to a mass cytometry dataset quantifying the baseline samples (pre-treatment) of melanoma patients who subsequently either responded (R) or did not respond (NR) to an anti-PD1 immunotherapy (Anti_PD1 dataset [42], Supplementary Material Table 1). There are 20 samples in total (10 responders and 10 non-responders). The objective of this analysis was to identify a rare subset of monocytes, characterised as CD14+, CD33+, HLA-DRhi, ICAM-1+, CD64+, CD141+, CD86+, CD11c+, CD38+, PD-L1+, CD11b+, whose abundance correlates strongly with the patient's response status to anti-PD1 immunotherapy [11], [42].

Our analysis workflow consists of steps 1, 2, 3, 4, 6 in Figure 1B. Firstly, we used SuperCellCyto (gamma= 20) to generate 4,286 supercells from 85,715 cells. We then used cyCombine to integrate the two batches together (Figure 4C-D), and clustered the batch-corrected supercells using FlowSOM (see Materials and Methods for more details). We then identified the clusters representing the rare monocyte subset based on the median expression of the aforementioned rare monocyte subset’s signatory markers (see Supplementary Material Figure S8A). For differential abundance analysis, we strongly recommend expanding the supercells back to single cells for more accurate cell type proportion calculation as each supercell contains different numbers of cells. Once we expanded the supercells back to single cells, we retained only clusters that contained more than three cells from each sample, and performed a differential abundance test using Propeller, accounting for the batch. For comparison, we also applied Propeller directly on the supercells without expanding them back to single cells. For consistency, we only compared clusters that were retained by the filtering process.

Using the heatmap depicting the median expression of markers for each cluster (Supplementary Material Figure S8A), we identified the rare monocyte subset as cluster 10. We found a statistically significant shift in abundance for cluster 10 between the responder (R) and non-responder (NR) groups, with responders exhibiting a higher proportion of these rare monocyte subsets (FDR <= 0.05, Figure 4E).

Comparing the statistical test outcomes performed at the single cell and supercell level, we found that the difference in the abundance of supercells for the rare monocyte subset (cluster 10) was also statistically significant at the FDR threshold of 0.05 (Supplementary Figure S8B,

Figure 4F). While this finding is consistent with the test outcome obtained from the single cell level abundance test, broadly, we observed variations in the FDR values obtained for each cluster (Figure 4F). Neither the single cell nor the supercell level tests consistently yielded higher or lower FDR. Hence, our recommendation is to perform differential abundance analysis at the single cell level for the most accurate results.

Efficient Cell Type Label Transfer Between CITEseq and Cytometry Data

In this analysis, we investigated the potential for automating the annotation of cell types in cytometry data using previously annotated CITEseq data. Our workflow involves generating supercells from the cytometry data, subsetting the supercell and CITEseq data to only the common markers, and subsequently performing the label transfer process from the CITEseq data to the supercells, using either Seurat rPCA [16] or the Harmony alignment algorithm [43] combined with a kNN classifier (Harmony plus kNN). For comparison, we also performed the cell type label transfer at the single cell level. We demonstrate the effectiveness of the workflow by transferring the cell type annotation from the CITEseq data generated by Triana et.al [44] to the Levine_32dim mass cytometry dataset (see Supplementary Material Table 1).

Figure 5 and Supplementary Material Figure S9 depict, for each cell type label in the cytometry data (actual label), the proportion of cells assigned the cell type label in the CITEseq data (predicted label) by either Seurat rPCA or Harmony plus kNN respectively. Both Seurat rPCA and the Harmony plus kNN approach demonstrated good correspondence between the annotations where over 70% of cells were accurately matched with the corresponding predicted label.

Excellent correspondence was observed for CD16+ NK cells, Plasma cells, CD4 T cells, CD8 T cells, and Mature Naive B cells. Specifically, a large proportion of CD16+ NK cells were classified as CD56dimCD16+ NK cells and Plasma B cells as Plasma cells. CD4 T cells, CD8 T cells, and the Mature B cells were each broken down into distinct subpopulations, namely for CD4 T cells; CD4+ memory T cells, Naive CD4+ T cells, CD4+ Cytotoxic T cells. For CD8 T cells; CD8+CD103+ tissue resident memory T cells, CD8+ central memory T cells, CD8+ effector memory T cells, CD8+ Naive T cells. For Mature B cells; Mature Naive B cells and Non-switched or Class-switched Memory B cells.

For Plasmacytoid Dendritic Cells (pDC)s, Monocytes, and subsets of Hematopoietic Stem Cells (HSCs) and Progenitors (HSPCs), we obtained a moderate correspondence. 27% - 39% of PDCs were assigned as PDCs while 30 - 49% of Monocytes were assigned as Classical Monocytes. Similarly 60 - 78% of CD34+CD38lo HSCs were assigned as HSCs & MPPs. Interestingly, for CD34+CD38+CD123+ HSCPs, rPCA and Harmony plus kNN assigned a significant proportion of them as pDC progenitors (57% and 67% respectively). This is feasible as CD123 is commonly used for identifying pDCs [45] while CD34+ and CD38+ cells are commonly identified as Common Lymphoid Progenitor [46], the precursor of dendritic cells. Additionally, 20% - 40% of CD34+CD38+CD123- HSCPs were assigned as early erythroid

progenitors which is plausible as previous study showed early erythroid progenitors did not express CD123 protein [47].

While the majority of cell types exhibited good correspondence, there were instances of incorrect predictions. For example, the majority of CD16- NK cells were assigned as CD56dimCD16+ NK cells instead of CD56brightCD16- NK cells. Additionally, most Pre B cells were assigned as either Pro B cells or Immature B cells, with only a small fraction (11.7%) correctly assigned as small pre-B cells by the Harmony plus kNN approach (Supplementary Material Figure S9). Basophils were classified as either PDC progenitors, late erythroid progenitors, or PDCs, which is not surprising due to the absence of Basophils in the CITEseq data.

We also observed differences between the predictions provided by rPCA and Harmony plus kNN approach. For instance, a significantly larger proportion of Pro B cells were correctly annotated by rPCA (92%) compared to Harmony plus kNN approach (13%). Additionally, a larger proportion of CD34+CD38+CD123- HSPCs were assigned as Lymphoid Primed Multipotent Progenitors by Harmony plus kNN (31%) compared to rPCA (1%).

We compared the results from directly transferring the CITEseq labels to the single cells in cytometry data against those obtained from performing the transfer to the supercells (Figure 5 and Supplementary Material Figure S10 for Seurat rPCA, Supplementary Material Figure S9 and S11 for Harmony plus kNN). For both approaches, we found a strong correspondence between the results.

In conclusion, these results demonstrate the feasibility of employing computational methods such as Seurat rPCA and Harmony plus kNN for transfering cell type labels from an annotated CITEseq data to a cytometry data, thereby facilitating automated cell type annotation.

Computational Efficiency Gains with SuperCellCyto

In this analysis, we examine the time taken to create supercells, and subsequently compare the time taken to perform several analysis steps, such as clustering, batch correction, and cell type label transfer, on both supercells and single cells. For clustering and batch correction, we broadly explored the algorithms' parameter space. By contrasting these timings, we underscore the potential advantages of performing downstream analyses on supercells over single cells in terms of computational efficiency.

Figure 6A illustrates the time taken to create supercells for 6 different cytometry datasets. These datasets contain a wide variety of number cells, ranging from tens of thousands to millions (Supplementary Material Table 1). Supercell creation for datasets containing less than one million cells took less than 3 minutes. For datasets comprising over 8 million cells, the time taken was 161 to 216 minutes.

For clustering Levine_32dim, Samusik_all, and Oetjen_bcells datasets (see Supplementary Material Table 1 for more details on the datasets) using Louvain, clustering supercells instead of

single cells resulted in significant time savings (Figure 6B). For Levine_32dim, clustering supercells took < 1 minute while clustering single cells took 4 to 19 minutes. For the Samusik_all datasets, clustering supercells took < 5 minutes while clustering single cells took 56 to 140 minutes. For the Oetjen_bcells dataset, which comprises over 8 million cells, we observed stark differences. Clustering single cells took 41 minutes to 35 hours, whereas clustering supercells took 0.5 to 45 minutes.

Similarly, for FlowSOM clustering, we also observed improvement in run time for both the Levine_32dim and Samusik_all datasets when clustering supercells (Supplementary Material Figure S12).

Comparing the time taken to run batch correction (Figure 6C) on the Trussart_cytofruv dataset (Supplementary Material Table 1), performing batch correction at the supercell level took less than 2 minutes, whereas correcting batch effects at the single cell level could take up to 19 minutes. Importantly, we observed a 14 fold reduction in the amount of memory taken to run CytofRUV at the supercell level compared to the single cell level (~3GB vs ~42GB, Figure 6D).

Lastly, transferring cell type labels from CITEseq data to supercells generated for a cytometry dataset resulted in 4 to 5 fold improvement in run times. Specifically, transferring these labels to supercells generated for the Levine_32dim cytometry data took 9.6 minutes with Seurat rPCA and 1 minute with Harmony plus kNN. On the other hand, transferring these labels to the single cells in the same dataset (Levine_32dim) took 37 minutes using Seurat rPCA and 5.4 minutes with Harmony plus kNN.

Discussion

The increasing size and complexity of cytometry data has rendered traditional manual gating inadequate, necessitating the adoption of computational methods for data analysis. A myriad of computational methods designed for cytometry and scRNAseq data analysis offer robust, data-driven, and diverse analysis workflows. However, many of these tools, particularly those developed for scRNAseq, often require extensive processing time due to the vast number of cells typically found in cytometry data. When algorithm parameters need to be adjusted and analysis workflows repeated, the computational time required to analyse large cytometry datasets with millions of cells can increase to days and even weeks.

To address this challenge, we propose a strategy for reducing large cytometry datasets that can drastically reduce computational time from hours to seconds. We developed an R package, SuperCellCyto, which leverages and extends the SuperCell R package to aggregate phenotypically similar cells based on their marker expressions. We further enhanced this approach by introducing the ability to process multiple samples concurrently using a custom load balancing strategy. Utilising this framework, we generated supercells for six publicly available cytometry datasets and successfully performed various downstream analyses. These included clustering, cell type annotation, differential expression and abundance analysis, and batch correction. Moreover, we demonstrated the ability to efficiently transfer cell type labels

from CITEseq data to cytometry data. This was achieved by harnessing the capabilities of SuperCellCyto in conjunction with Seurat rPCA and Harmony combined with a kNN classifier. Our findings underscore the feasibility and effectiveness of our proposed strategy in managing the increasing complexity of cytometry data analysis.

In cytometry, markers are typically classified into two categories: cell type markers and cell state markers. Cell type markers serve to identify distinct cell types, whereas cell state markers are employed to investigate cell states. For most analyses, we recommend generating supercells using all available markers, as opposed to exclusively using cell type markers. This approach minimises the risk of creating supercells that encompass cells of identical type but differing states, thereby enhancing the reliability and accuracy of downstream analyses. Importantly, SuperCellCyto offers the flexibility to generate supercells based on either cell type markers or cell state markers exclusively, catering to the specific requirements of different analyses.

The granularity of supercells is governed by the gamma parameter. A larger gamma value results in the generation of fewer supercells, each potentially encompassing a greater number of cells, whereas a smaller gamma value yields more supercells, each containing fewer cells. The selection of an appropriate gamma value is contingent upon the specific analysis objectives and the desired level of data compression. Generally, we found a gamma value of 20 strikes a good balance between preserving cell type diversity and reducing data size.

One key advantage of using SuperCellCyto for an analysis, is that the creation of supercells will typically only need to be run once or twice at the outset. This allows subsequent downstream algorithms, which often need to be run multiple times with varying parameters, to benefit from the performance gains offered by supercells. If, after creating supercells once, there is a need to change the number of supercells produced, there is no need to rebuild the kNN graph or rerun the Walktrap algorithm. We provided a function within SuperCellCyto which will recut the dendrogram produced by Walktrap algorithm based on a new gamma parameter.

Future research could explore strategies to further improve the processing time required to generate supercells. This could involve accelerating certain internal calculations by leveraging the multicore processing capabilities commonly available in contemporary computers or harnessing the power of Graphics Processing Units (GPUs).

We have successfully demonstrated the ability to mitigate batch effects at the supercell level using CytofRUV and cyCombine. However, it is important to note that batch correction executed at the supercell level does not translate to correction at the single cell level. Consequently, for downstream analyses that compare marker expression and necessitate the expansion of specific supercells back to the single cell level, batch correction must be reapplied to these single cells.

When performing differential abundance analysis, we noted slight differences in results when running Propeller at the supercell and single cell levels. These differences could potentially be attributed to the fact that each supercell does not encapsulate an identical number of cells. The gamma parameter primarily dictates the number of supercells generated rather than the number

of cells encompassed within each supercell. Consequently, we advocate for the expansion of supercells back to single cells prior to undertaking differential abundance analysis for the most accurate and reliable analysis.

We observed that in certain cases, the time savings from processing supercells as opposed to single cells can be marginal, for instance when clustering supercells using FlowSOM. However, it is crucial to recognise that not all algorithms (or parameter settings) exhibit the same level of efficiency. Many can substantially benefit from processing supercells instead of single cells. For instance, when running Louvain clustering, the time taken to cluster supercells was significantly less than the time taken to cluster single cells. Similarly, we also observed a significant reduction in memory consumption when correcting batch effects at the supercell level compared to single cells using cytofRUV.

In SuperCellCyto, supercells are generated using single cell kNN graphs combined with the Walktrap algorithm. However, various other types of graphs and community detection algorithms are available. Future work could investigate a plug and play functionality which allows users to select their preferred type of graph to use (e.g., shared k nearest neighbour graphs or self organising map) and subsequently the algorithms to identify densely connected subgraphs (e.g., Louvain, Leiden, or hierarchical clustering). Notably, the SuperCell R package is not the only approach currently available for creating supercells, or metacells, from scRNAseq data. Future research could explore and compare other methods, such as SEAcells [28], MetaCell [26], [27], and GeoSketch [48], with a specific focus on their efficacy in compressing cytometry data.

Lastly, in our assessment, we demonstrated the ability to perform cell type label transfer from an annotated CITEseq dataset to a mass cytometry dataset using Seurat rPCA and Harmony in conjunction with a kNN classifier. This area presents substantial opportunities for further exploration and development. Recent promising new methods, such as StabMap [49], Seurat CCA [17], and MultiMAP [50], could potentially further improve automated cell type annotation between cytometry and CITEseq data.

Materials and Methods

Datasets

The Levine_32dim dataset is a mass cytometry data that profiles healthy human bone marrow. It comprises two samples and 32 markers, with a total of 265,627 cells. Of these cells, 39% (104,184) were assigned 14 cell types using manual gating.

The Samusik_all dataset is a mass cytometry data that profiles healthy mice bone marrow. It includes 10 samples and 39 markers, with a total of 841,644 cells. Of these, 61% (514,386) were assigned 24 cell types using manual gating.

The Oetjen_bcell data profiles healthy human bone marrow using flow cytometry. The dataset contains 13 markers, 22 samples, and 18,648,656 events. While no cell type annotation is provided for each cell, the manual gating scheme previously used by the authors to identify the B cell subsets was made publicly available and subsequently used to label the clusters. The manual gating scheme is available in the Supplementary Material Note S1.

The BCR_XL dataset consists of 8 paired samples of healthy human peripheral blood cells. These samples are either stimulated with B cell receptor / FC receptor cross-linker (BCR-XL) or left unstimulated [41].

The Anti_PD1 dataset quantifies immune cell subsets in the peripheral blood of melanoma patients subsequently treated with anti-PD1 immunotherapy. It contains 20 samples taken from melanoma patients prior to undergoing anti-PD1 immunotherapy treatment. The samples were divided into two groups: those who subsequently responded to the treatment (responder or R) and those who did not (non-responder or NR), and quantified across two batches (batch 23 and batch 29). There are, in total, 11 samples from the responder group, and 9 samples from the non-responder group.

The Trussart_cytofruv dataset quantifies the peripheral blood mononuclear cells of 9 healthy individuals and 3 Chronic Lymphocytic Leukaemia patients (CLL). The dataset contains 12 paired samples (1 paired sample per patient) processed over 2 batches, with each batch containing 1 sample from every patient. The dataset includes 31 markers, specifically 19 cell type markers and 12 cell state markers.

The CITEseq dataset profiles the mononuclear bone marrow cells from hip aspirates of healthy young and old adults using 97 antibodies and targeted sequencing. The dataset includes 10 samples and 49,057 cells.

Analysis workflows

Data Transformation

All mass cytometry datasets were transformed using an inverse hyperbolic sine (arc-sinh) transformation with a co-factor of 5 before the generation of supercells or any subsequent downstream analyses. Similarly, the flow cytometry dataset underwent arc-sinh transformation with a co-factor of 150. For the CITEseq data, a Centred Log Ratio transformation was used to transform the protein expression.

Cell Type Identification for Annotated Mass Cytometry Data

For the Levine_32dim and Samusik_all datasets, SuperCellCyto was used to generate supercells, using gamma values that ranged from 5 to 50, in increments of 5. Following this, the supercells were clustered using the FlowSOM and Louvain clustering algorithms. An extensive exploration of the parameter space was conducted for both clustering algorithms. Specifically, for FlowSOM, the grid size and the number of metaclusters were varied, while for Louvain, the

parameter k, which governs the number of nearest neighbours considered when constructing the single cell network graph, was varied. The specific range of values explored for each parameter is documented in Supplementary Material Table S3.

Cell Type Identification for Flow Cytometry Data

For the Oetjen_bcell dataset, an initial step of manual gating was performed using CytoExplorer [51] to exclude debris and doublets. This resulted in over 8.3 million single live cells (refer to Supplementary Material Figure S13 for the manual gating scheme). Following this, SuperCellCyto was used to generate supercells, with the gamma parameter set to 20, yielding 415,711 supercells. These supercells were subsequently clustered using the Louvain clustering algorithm with the parameter k set to 3, based on the expression of markers used in the manual gating strategy by Oetjen et.al to identify the B cell subsets (see Supplementary Note S1) [38], namely CD19, CD45, CD10, CD20, CD27, CD21, CD38, and CD138. Clusters were then annotated with B cell subsets based on the manual gating scheme provided in the original publication of the datasets [38] (see Supplementary Material Note S1). Approximately 32% of the supercells expressed CD45 and CD19. The remaining supercells were CD45- and CD19- (68%) and were excluded from Figure 2E-F and Supplementary Material Figure S3B.

Differential Expression and Abundance Analysis

For the BCR_XL dataset, SuperCellCyto (with the gamma parameter set to 20) was used to generate 8,641 supercells. Using the cell type annotations provided with the dataset, the supercells were annotated based on the cell type that was most abundantly represented within each supercell. Subsequently, the mean expression of all cell state markers was computed for each combination of sample and cell type. Following this, Limma was applied to each cell type. Specifically, a linear model was first fitted using Limma's *lmFit* function, followed by the application of Limma's *eBayes* and *treat* (*fc* parameter set to 1.1) functions.

For the Anti_PD1 dataset, SuperCellCyto was used (with the gamma parameter set to 20) to generate 4,286 supercells. These supercells were then batch corrected using cyCombine (grid size set to 4x4) clustered using FlowSOM using 20x20 grid size and 50 metaclusters. We then performed a differential abundance test (taking the batch into account) using Propeller. Propeller was run either on the single cells expanded from the supercells or directly on the supercells. Importantly, we tested only clusters which captured more than 3 cells from each sample for both the single cell and supercell level tests.

Batch Correction

For the Trussart_cytofruv dataset, an initial preprocessing step was carried out using the CATALYST R package and the R scripts provided with the dataset [39]. Following this, SuperCellCyto was employed (with the gamma parameter set to 20) to generate 429,488 supercells from over 8.5 million cells.

Subsequently, CytofRUV (with parameter k set to 5 and samples from CLL2 and HC1 patients used as the pseudo-replicates) [39] and cyCombine (with grid size set to 8x8) [40] were run, to integrate the two batches. CytofRUV is a Remove Unwanted Variation (RUV)-based method [52], specifically the RUV-III method [53], for identifying and removing unwanted technical variations such as batch effect. CyCombine [40] is a cytometry data integration method based on the Empirical Bayes method ComBat [54].

Cell Type Label Transfer

SuperCellCyto, with a gamma parameter set to 20, was used to generate 13,282 supercells for the Levine_32dim dataset. Both the CITEseq data and the supercells were subsequently subsetted to retain only the markers common to both, resulting in a total of 21 shared markers.

The datasets were then processed by Seurat rPCA which first identified the transfer anchor between the two datasets, and thereafter transferred the cell type annotation from the CITEseq data to the supercells.

In the case of the Harmony and kNN approach, Harmony was employed to align the CITEseq data and the supercells. Following this, a k-Nearest-Neighbour classifier (with the k parameter set to 1) was trained on the CITEseq data and subsequently used to classify the supercells.

Finally, each supercell was expanded, and the cell type label of the corresponding supercell was assigned to the resulting single cells. Importantly, for evaluation purposes, only the cells for which manually gated cell type annotations were available were included in the analysis.

Runtime Benchmarking

Supercells were generated on a 2022 MacBook Pro laptop equipped with an M2 chip and 24GB of RAM. FlowSOM clustering and Louvain clustering for Levine_32dim and Samusik_all datasets, and cyCombine were run on the aforementioned MacBook Pro.

Louvain clustering for the Oetjen_bcells data and CytofRUV was run on a HPC platform using Nextflow pipelines. The amount of RAM and CPUs allocated are specified within the Nextflow scripts.

Runtimes were measured using the *tictoc* R package [55]. Each operation was repeated twice to ensure consistency, and the mean duration was reported.

Evaluation Metrics

Purity and Adjusted Rand Index

To evaluate the quality of the generated supercells and the results of the clustering process, two metrics were used: purity and the Adjusted Rand Index (ARI). The purity metric quantifies the degree to which each supercell is composed of a singular cell type, with values spanning from 0 to 1. A purity value of 1 indicates that a supercell is entirely composed of cells from a single cell type.

ARI measures the agreement between the clustering results and a known ground truth, taking into account the influence of chance. ARI value can range from -1 to 1, with a score of 1 signifying maximum agreement between the clustering results and the ground truth. Conversely, ARI values of 0 and -1 suggest that the agreement is either the same or worse than what would be achieved by random chance.

Notably, purity and ARI scores were computed only for cells for which cell type annotations were available, which constituted 39% of cells for the Levine_32dim dataset and 61% of cells for the Samusik_all dataset.

To compute the ARI score, each supercell was expanded to the single cell level, ensuring that each cell within a given supercell was assigned the cluster label of the supercell. Finally, for both datasets, two types of ground truths were used: the cell type annotation provided with the dataset, and the clustering results obtained by clustering the single cells using the same combination of parameter values.

Earth Mover Distance

The Earth Mover's Distance (EMD) metric was employed to evaluate the efficacy of batch effect correction algorithms by quantifying the dissimilarity in the distribution of markers across different batches. Following a successful batch correction, the EMD score for any given marker in the dataset is anticipated to be lower than the score obtained prior to the application of the batch effect correction algorithm.

In our evaluation, the EMD score was calculated for each paired sample at the supercell level. Initially, the distribution of each marker was obtained by binning the data into bins of size 0.1. Subsequently, for each marker and paired samples, the EMD score was computed to compare the differences in the distribution of the marker values.

Availability of Data and Materials

All analysis workflow is available as a workflowr [56] website (https://phipsonlab.github.io/SuperCellCyto-analysis/), with the original source code available on GitHub (https://github.com/phipsonlab/SuperCellCyto-analysis). The SuperCellCyto

implementation is available as an R package on GitHub (https://github.com/phipsonlab/SuperCellCyto).

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Figure Legends

Figure 1. The SuperCellCyto framework. (A) Schematic overview of the SuperCellCyto R package for generating supercells from cytometry data. (B) Proposed workflow for an end-to-end cytometry data analysis using supercells as the fundamental unit of analysis. The workflow begins with the generation of supercells using SuperCellCyto from cleaned and transformed cytometry data. If necessary, batch correction is then performed at the supercell level. Supercells are then clustered and annotated based on the cell type they represent. Differential expression analysis can be performed using existing tools such as Limma following aggregation of supercells at the sample and cell type level. Differential abundance analysis is performed after expanding supercells back to single cells using existing tools such as Propeller.

Figure 2. SuperCellCyto preserves biological heterogeneity. (A) Mean purity of supercells generated using varying gamma values for Levine_32dim and Samusik_all datasets. (B) Distribution of supercell purity for Levine_32dim and Samusik_all datasets. (C) Adjusted Rand Index (ARI) illustrating the agreement between supercell clustering and manually gated cell type annotation for Levine_32dim and Samusik_all datasets. (D) ARI comparison between supercell and single-cell clustering. (E) Identification of rare B cell subsets through clustering of supercells. Median expression of cell type markers across supercells for each cluster and B cell subsets for the Oetjen_bcell dataset. (F) The abundance of B cell subsets identified by supercell clustering. Cluster 46 represents the extremely rare Plasma cells which make up only 0.002% of the cells. Only clusters annotated with B cell subsets are shown.

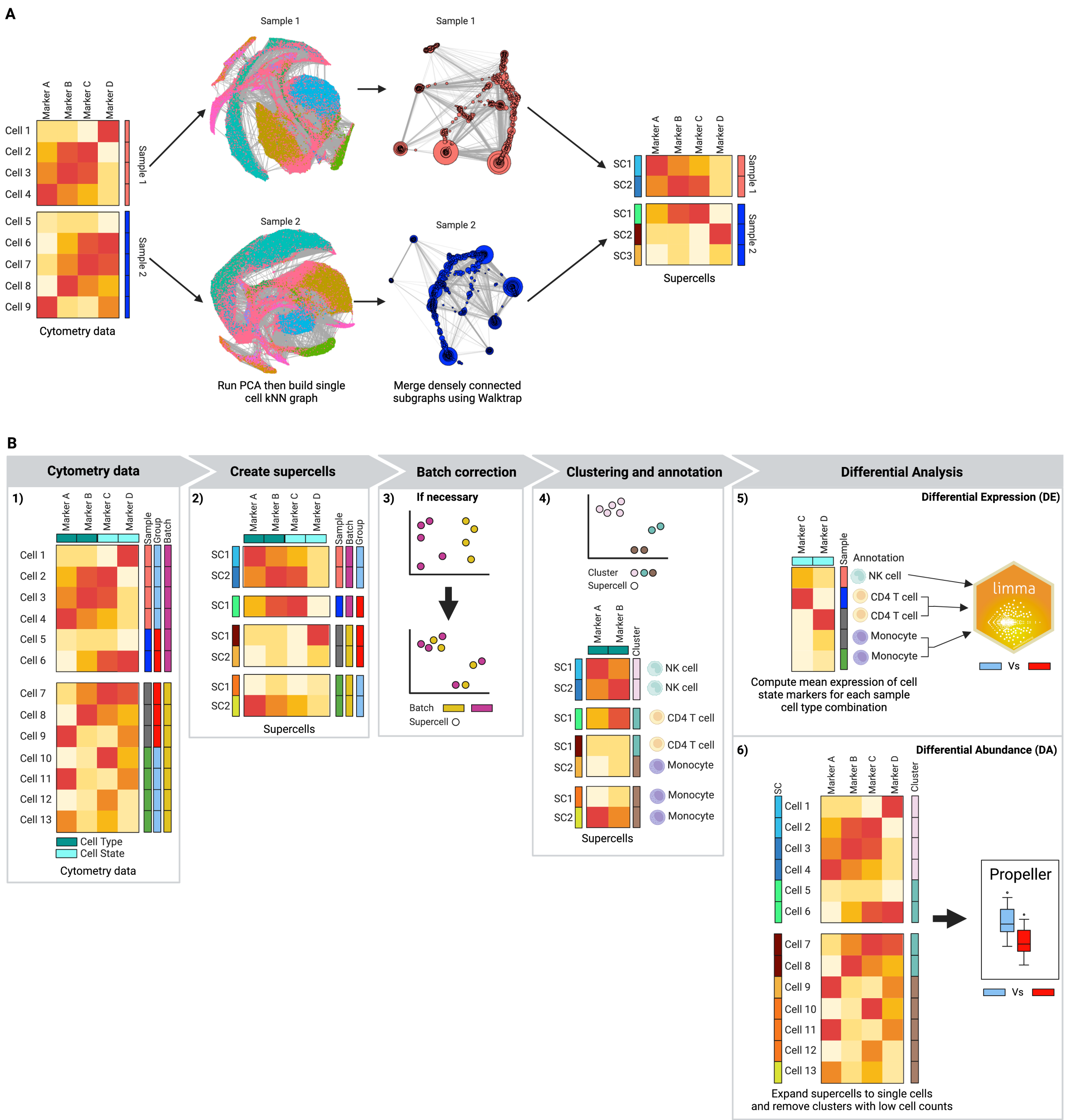
Figure 3. Application of batch correction techniques at the supercell level. (A) A Multidimensional Scaling (MDS) plot showcasing the variation in uncorrected single cells, supercells, and supercells that have been batch corrected using the CytofRUV and cyCombine algorithms for the Trussart_cytofruv dataset. Each point represents a sample. (B) Distribution of CD14, CD45RA, and PUMA expression for uncorrected, CytofRUV-, and cyCombine-corrected supercells for Trussart_cytofRUV dataset. The distribution of the marker expressions are highly concordant between batch 1 and 2 following batch correction.

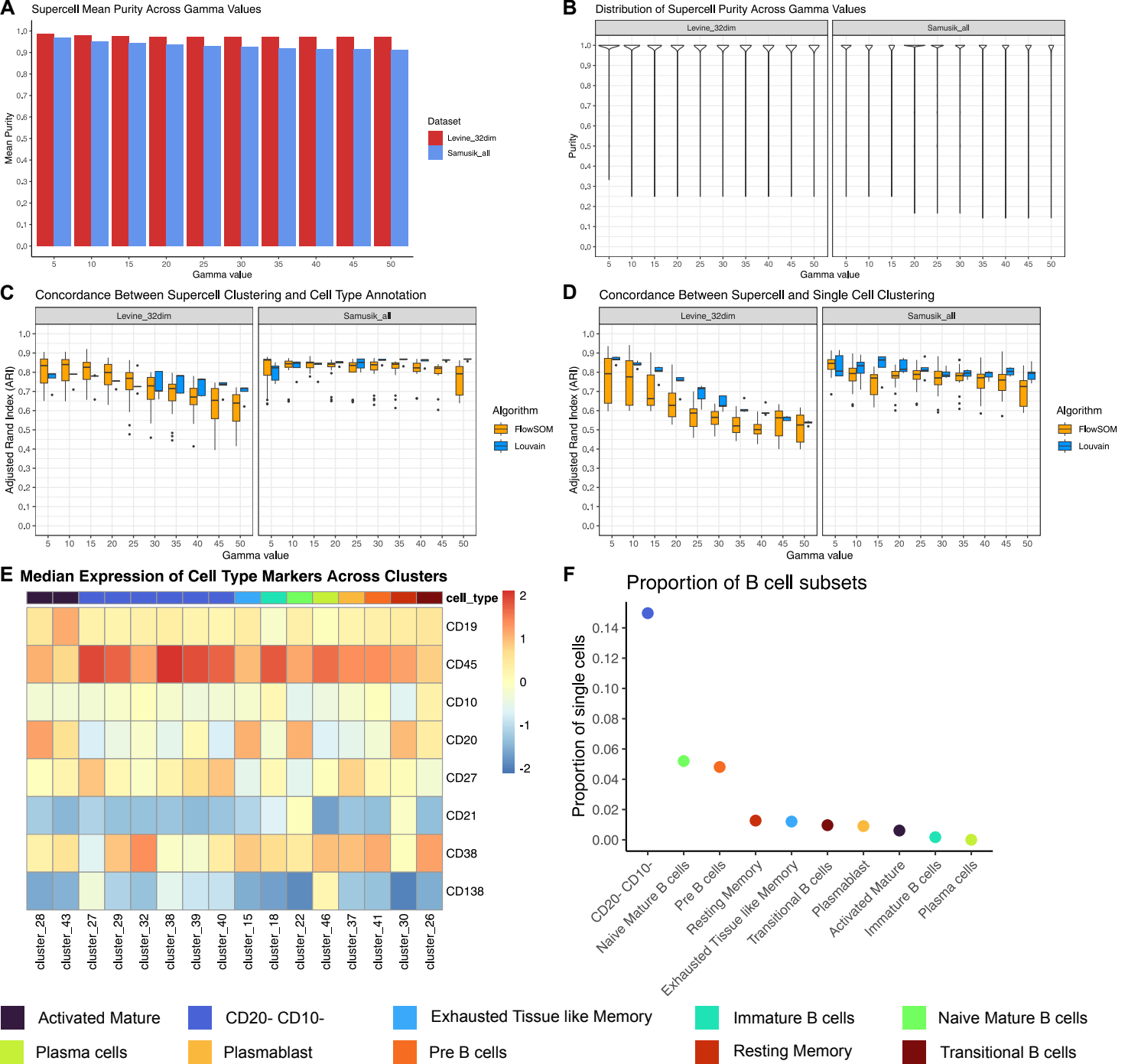
Figure 4. Differential expression and abundance analysis using supercells (A) - (B) Heatmaps illustrate the scaled and centred median expression of cell state markers, calculated for each sample across the supercells, for B cells and CD4 T cells in the BCR_XL dataset. Each sample (row) is annotated according to its stimulation status, either with B cell receptor / FC receptor cross-linker (BCR-XL) or unstimulated. Each marker (column) is annotated based on its statistical significance as determined by Limma at a 5% False Discovery Rate (FDR_sig). (C) - (F) Differential abundance analysis following supercell creation for the Anti_PD1 dataset. (C) A Multidimensional Scaling (MDS) plot showcasing the variance in samples following cyCombine correction. (D) Distribution of marker expression for cyCombine-corrected supercells. (E) The proportion of single cells for the rare monocyte subset cluster (cluster_10). (F) Comparison of FDR obtained by running Propeller at the single cell or supercell level. The y-axis represents the

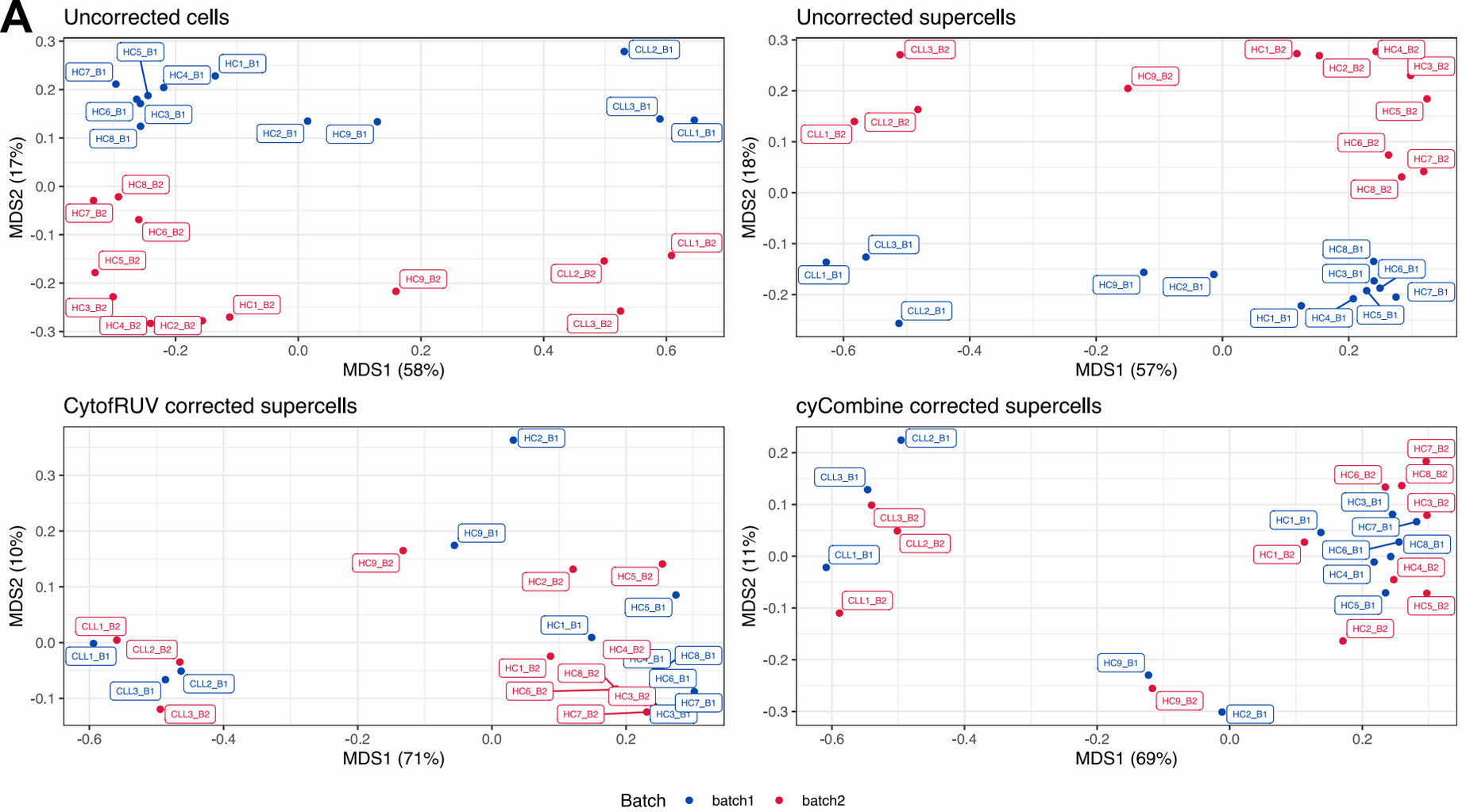
-log10 transformed FDR, with lower FDR (more significant) corresponding to higher -log10 values. The red dotted line shows an equivalent FDR of 0.1.

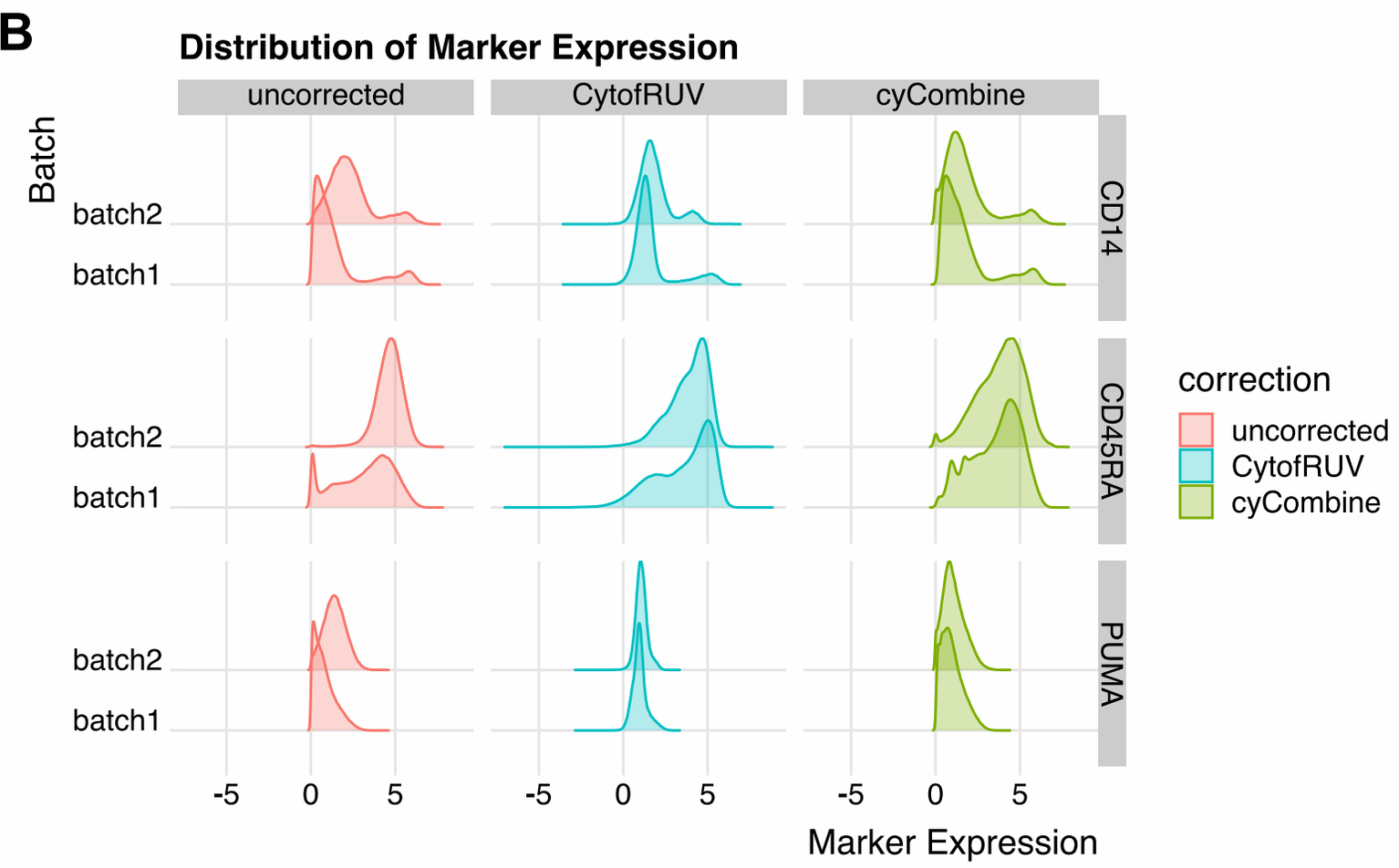
Figure 5. Performance evaluation of cell type label transfer from a CITEseq dataset to supercells. Supercells were generated for the Levine_32dim cytometry dataset and label transfer was performed using Seurat rPCA. The size and colour of each circle represent the proportion of a specific cell type (indicated by the actual label assigned through manual gating) assigned a given predicted label in the CITEseq dataset. An empty cell in the plot signifies that no cells of a given cell type were assigned the corresponding predicted label.

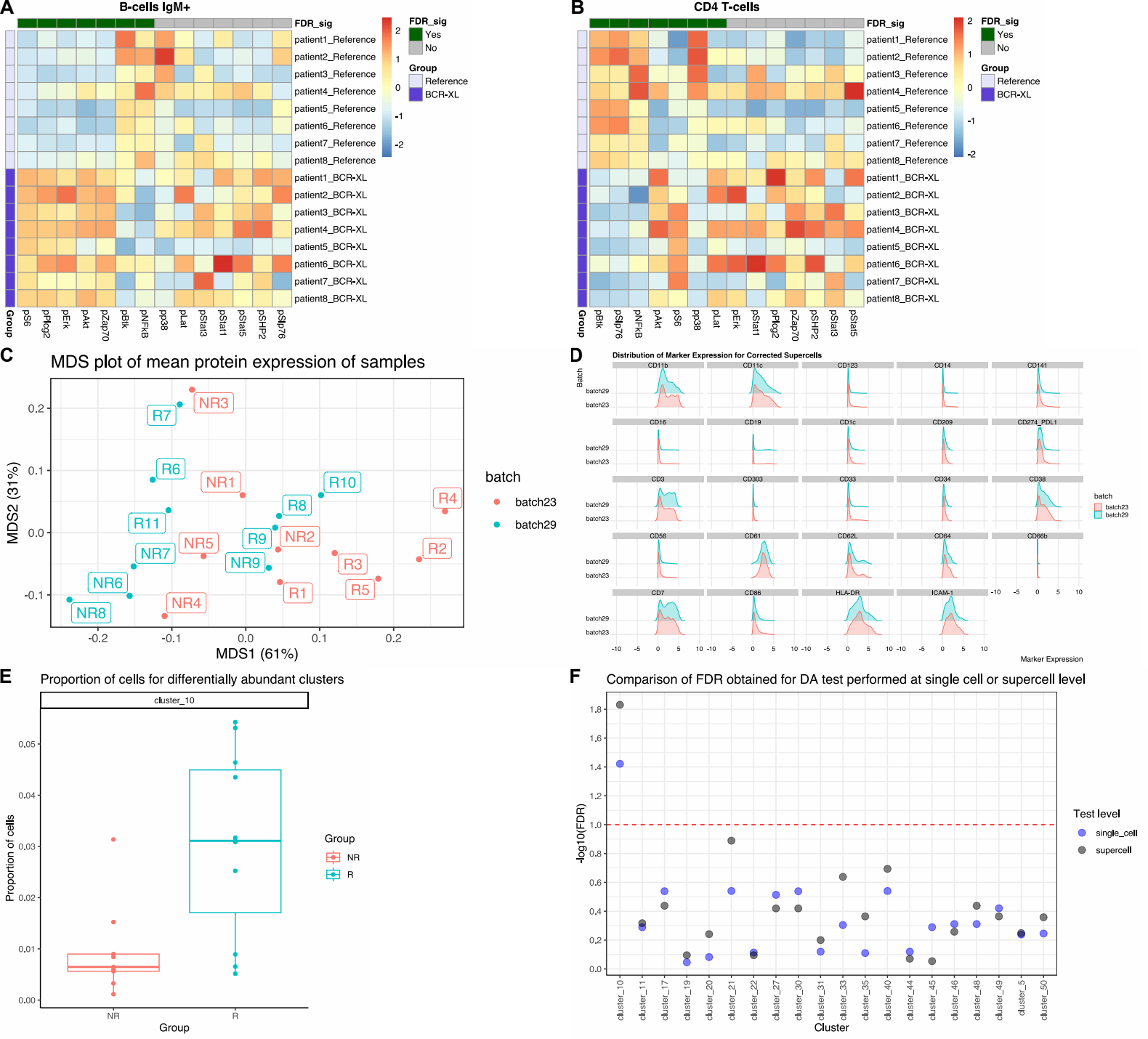
Figure 6. Evaluating the run time of SuperCellCyto and subsequent downstream analyses. (A) The runtime of SuperCellCyto for six cytometry datasets, measured in minutes. (B) The runtime of Louvain clustering for Levine_32dim, Oetjen_bcells, and Samusik_all datasets, measured in minutes. (C) The runtime of cyCombine and CytofRUV for correcting batch effects in Trussart_cytofRUV dataset. For both (B) and (C), x-axis denotes whether the clustering was done on single cells or supercells created using various gamma values. (D) The memory consumption of CytofRUV for correcting batch effects in Trussart_cytofRUV dataset either at the single cell (orange) or supercell level (blue).

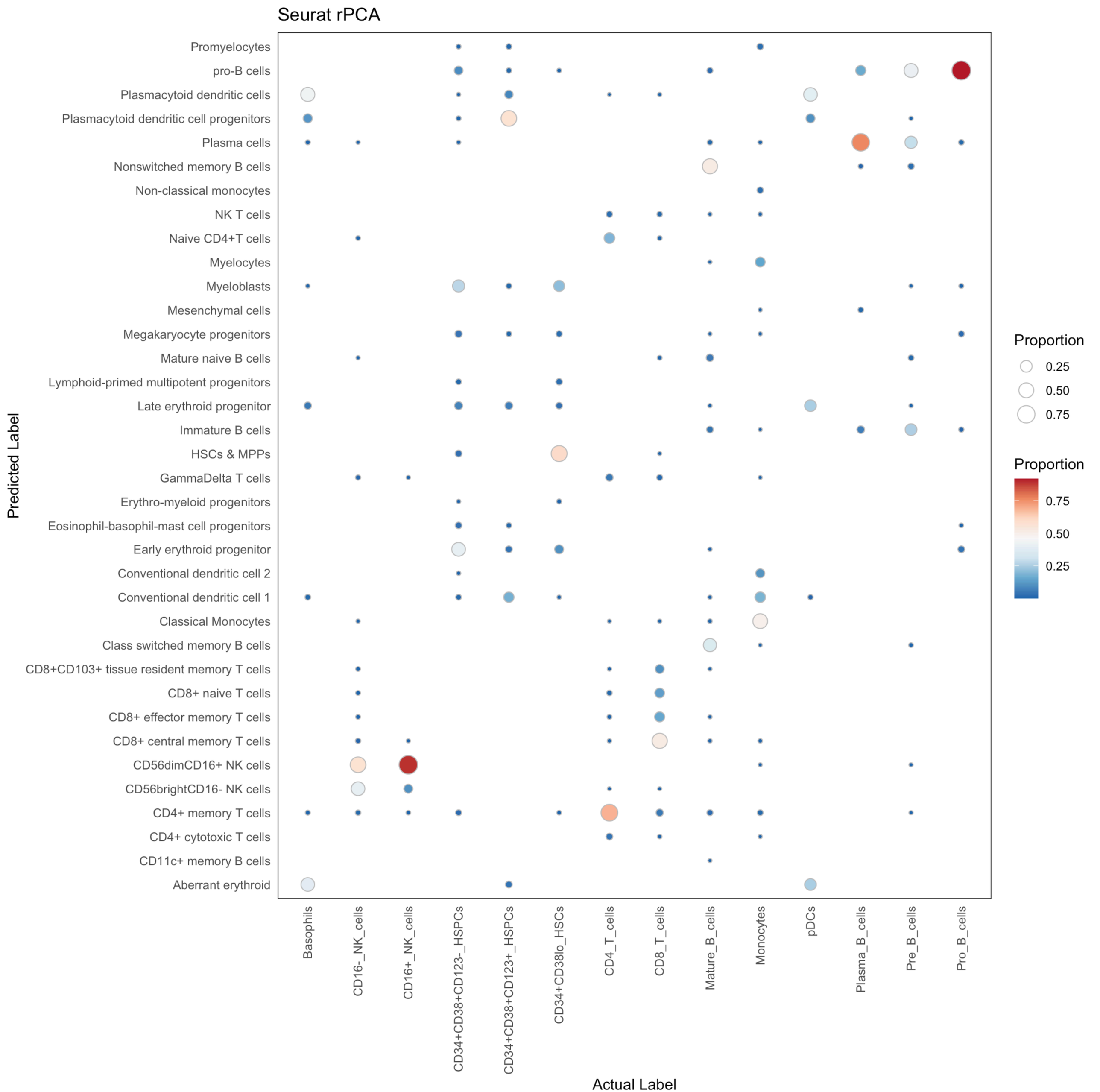

A
Marker A
Marker B
Marker C
Marker D
Cell 1
Cell 2
Cell 3
Cell 4
Cell 5
Cell 6
Cell 7
Cell 8
Cell 9
Sample 1
Sample 2
Cytometry data
Sample 1
Sample 2
Run PCA then build single cell kNN graph
Merge densely connected subgraphs using Walktrap
SC1
SC2
SC3
Supercells
B
Cytometry data
Create supercells
Batch correction
Clustering and annotation
Differential Analysis
1)
2)
3)
4)
5)
6)
Sample
Group
Batch
Cell 10
Cell 11
Cell 12
Cell 13
Cell Type
Cell State
If necessary
Batch
Supercell
Cluster
NK cell
CD4 T cell
Monocyte
Differential Expression (DE)
Annotation
limma
Vs
Compute mean expression of cell state markers for each sample cell type combination
Differential Abundance (DA)
SC
Propeller
Expand supercells to single cells and remove clusters with low cell counts

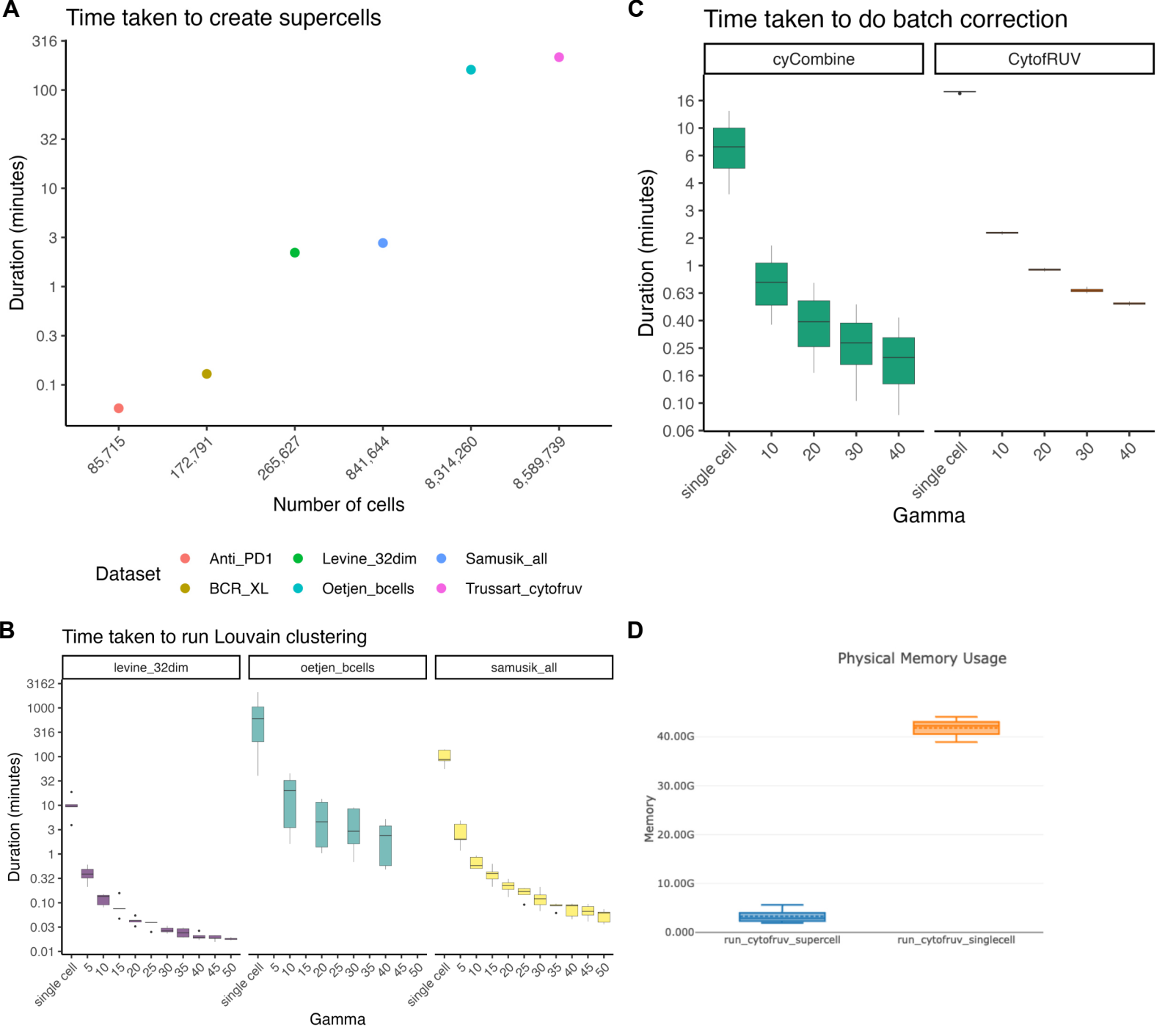

A
Supercell Mean Purity Across Gamma Values
Mean Purity
Gamma value
Dataset
Levine_32dim
Samusik_all
B
Distribution of Supercell Purity Across Gamma Values
Levine_32dim
Samusik_all
Purity
Gamma value
C
Concordance Between Supercell Clustering and Cell Type Annotation
Levine_32dim
Samusik_all
Adjusted Rand Index (ARI)
Gamma value
Algorithm
FlowSOM
Louvain
D
Concordance Between Supercell and Single Cell Clustering
Levine_32dim
Samusik_all
Adjusted Rand Index (ARI)
Gamma value
Algorithm
FlowSOM
Louvain
E
Median Expression of Cell Type Markers Across Clusters
cell_type
CD19
CD45
CD10
CD20
CD27
CD21
CD38
CD138
cluster_28
cluster_43
cluster_27
cluster_29
cluster_32
cluster_38
cluster_39
cluster_40
cluster_15
cluster_18
cluster_22
cluster_46
cluster_37
cluster_41
cluster_30
cluster_26
F
Proportion of B cell subsets
Proportion of single cells
CD20- CD10-
Naive Mature B cells
Pre B cells
Resting Memory
Exhausted Tissue like Memory
Transitional B cells
Plasmablast
Activated Mature
Immature B cells
Plasma cells
Activated Mature
CD20- CD10-
Exhausted Tissue like Memory
Immature B cells
Naive Mature B cells
Plasma cells
Plasmablast
Pre B cells
Resting Memory
Transitional B cells


A
Uncorrected cells
MDS1 (58%)
MDS2 (17%)
Uncorrected supercells
MDS1 (57%)
MDS2 (18%)
CytofRUV corrected supercells
MDS1 (71%)
MDS2 (10%)
cyCombine corrected supercells
MDS1 (69%)
MDS2 (11%)
Batch
batch1
batch2


B
Distribution of Marker Expression
uncorrected
CytofRUV
cyCombine
Batch
batch2
batch1
CD14
CD45RA
PUMA
Marker Expression
correction
uncorrected
CytofRUV
cyCombine


A
B-cells IgM+
FDR_sig
Yes
No
Group
Reference
BCR-XL
patient1_Reference
patient2_Reference
patient3_Reference
patient4_Reference
patient5_Reference
patient6_Reference
patient7_Reference
patient8_Reference
patient1_BCR-XL
patient2_BCR-XL
patient3_BCR-XL
patient4_BCR-XL
patient5_BCR-XL
patient6_BCR-XL
patient7_BCR-XL
patient8_BCR-XL
Group
pS6
pPlcg2
pErk
pAkt
pZap70
pBlk
pNFkB
pp38
pLat
pStat3
pStat1
pStat5
pSHP2
pStb76
B
CD4 T-cells
pBlk
pStb76
pNFkB
pAkt
pS6
pp38
pLat
pErk
pStat1
pPlcg2
pZap70
pSHP2
pStat3
pStat5
C
MDS plot of mean protein expression of samples
MDS2 (31%)
MDS1 (61%)
batch
batch23
batch29
R1
R2
R3
R4
R5
R6
R7
R8
R9
R10
R11
NR1
NR2
NR3
NR4
NR5
NR6
NR7
NR8
NR9
D
Distribution of Marker Expression for Corrected Supercells
Batch
CD11b
CD11c
CD123
CD14
CD141
CD16
CD19
CD1c
CD209
CD274_PDL1
CD3
CD303
CD33
CD34
CD38
CD56
CD61
CD62L
CD64
CD66b
CD7
CD86
HLA-DR
ICAM-1
Marker Expression
E
Proportion of cells for differentially abundant clusters
cluster_10
Proportion of cells
Group
NR
R
F
Comparison of FDR obtained for DA test performed at single cell or supercell level
-log10(FDR)
Cluster
Test level
single_cell
supercell
cluster_10
cluster_11
cluster_17
cluster_19
cluster_20
cluster_21
cluster_22
cluster_27
cluster_30
cluster_31
cluster_33
cluster_35
cluster_40
cluster_44
cluster_45
cluster_46
cluster_48
cluster_49
cluster_5
cluster_50


Seurat rPCA
Predicted Label
Promyelocytes
pro-B cells
Plasmacytoid dendritic cells
Plasmacytoid dendritic cell progenitors
Plasma cells
Nonswitched memory B cells
Non-classical monocytes
NK T cells
Naive CD4+T cells
Myelocytes
Myeloblasts
Mesenchymal cells
Megakaryocyte progenitors
Mature naive B cells
Lymphoid-primed multipotent progenitors
Late erythroid progenitor
Immature B cells
HSCs & MPPs
GammaDelta T cells
Erythro-myeloid progenitors
Eosinophil-basophil-mast cell progenitors
Early erythroid progenitor
Conventional dendritic cell 2
Conventional dendritic cell 1
Classical Monocytes
Class switched memory B cells
CD8+CD103+ tissue resident memory T cells
CD8+ naive T cells
CD8+ effector memory T cells
CD8+ central memory T cells
CD56dimCD16+ NK cells
CD56brightCD16- NK cells
CD4+ memory T cells
CD4+ cytotoxic T cells
CD11c+ memory B cells
Aberrant erythroid
Basophils
CD16-_NK_cells
CD16+_NK_cells
CD34+CD38+CD123-_HSPCs
CD34+CD38+CD123+_HSPCs
CD34+CD38lo_HSCs
CD4_T_cells
CD8_T_cells
Mature_B_cells
Monocytes
pDCs
Plasma_B_cells
Pre_B_cells
Pro_B_cells
Actual Label
Proportion
0.25
0.50
0.75
Proportion
0.75
0.50
0.25


A
Time taken to create supercells
Duration (minutes)
316
100
32
10
3
1
0.3
0.1
85,715
172,791
265,627
841,644
8,314,260
8,589,739
Number of cells
Dataset
Anti_PD1
Levine_32dim
Samusik_all
BCR_XL
Oetjen_bcells
Trussart_cytofruv
C
Time taken to do batch correction
cyCombine
CytofRUV
Duration (minutes)
16
10
6
4
3
2
1
0.63
0.40
0.25
0.16
0.10
0.06
single cell
10
20
30
40
Gamma
B
Time taken to run Louvain clustering
levine_32dim
oetjen_bcells
samusik_all
Duration (minutes)
3162
1000
316
100
32
10
3
1
0.32
0.10
0.03
0.01
single cell
5
10
15
20
25
30
35
40
45
50
Gamma
D
Physical Memory Usage
Memory
40.00G
30.00G
20.00G
10.00G
0.000
run_cytofruv_supercell
run_cytofruv_singlecell