



Get relevant post-clustering analyses quickly

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We have no financial interest nor other relationships with any manufacturer(s) of commercial product(s) and/or providers of commercial services discussed in our presentation

Meeting day

- French Society of Cytometry
- CyTOF, Spectral, Data analysis
- [Web](#)



Association Française de Cytométrie

The poster features a background image of a grand, illuminated building at night, likely the Institut Pasteur in Lille. A large, semi-transparent portrait of Louis Pasteur is overlaid on the top left. The text is centered and reads: 'STAIRWAY TO HIGH-CONTENT - PART 1 - SUSPENSION CYTOMETRY'. Below this, it says 'Institut Pasteur LILLE' and 'THURSDAY 13 JUNE 2024'. In the top right corner, there is a logo for 'Institut Pasteur de Lille' with a portrait of Pasteur and the motto 'Vivre mieux, plus longtemps'. On the far right edge, there is a small vertical copyright notice: '© Ulla Delfino'.

CytoBatchNorm



O. Molendi-Coste

cytoBatchNorm

Create Bunch
Setup Batch
Tune Params
Process

Tune parameters | Review scaling | Review functions | Review bi-param plot | Tune

Define the amount of cells per FCS file
5000

Extract a sample of cells
Sample

Select the channel to process
CD8a

Batch adjust

Set the method to adjust batch effect
percentile_hi

Set the percentile to adjust batch effect
0.95

Exclude the zeroes from percentiles
 Apply to transformed intensities

Transform

Set the function
asinh

Set the cofactor
5

Graphical options

Normalized

Raw

Post-clustering analyses

- Clusters or populations => features
 - cell counts in every sample
 - MFI of selected markers in every sample
- Identify the clusters that have a feature that makes a difference according to the question
- Time consuming and error prone if not automated
- Few software to perform an automated analysis, e.g. R/diffcyt, or commercial software

Key points

- Data quality is important
 - How to control it?
- Results reporting is important
 - Underestimated

The right position?

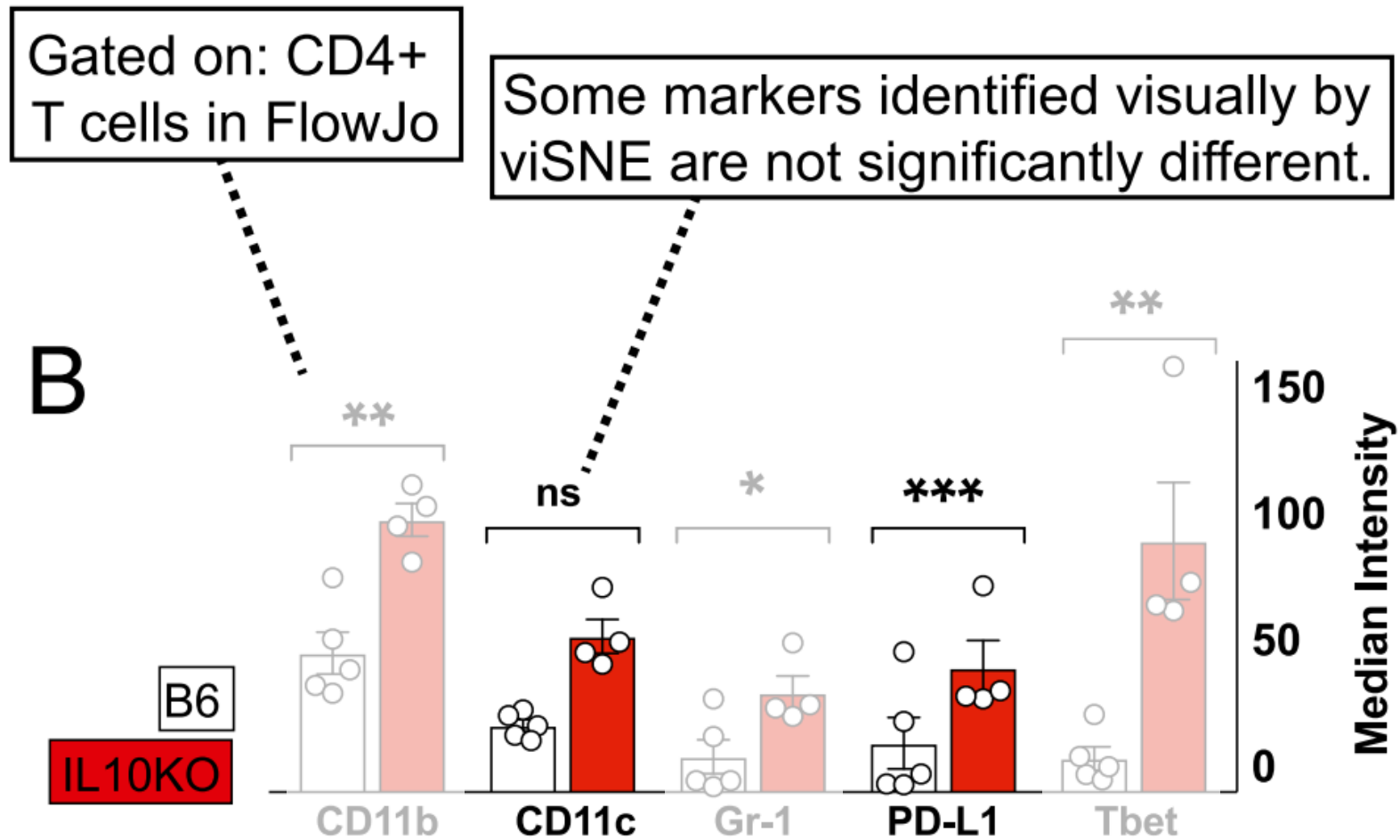


Fig. 4B, Kimball et al., 2018

Errors in figures

- automate p-values drawing
- automate points labelling

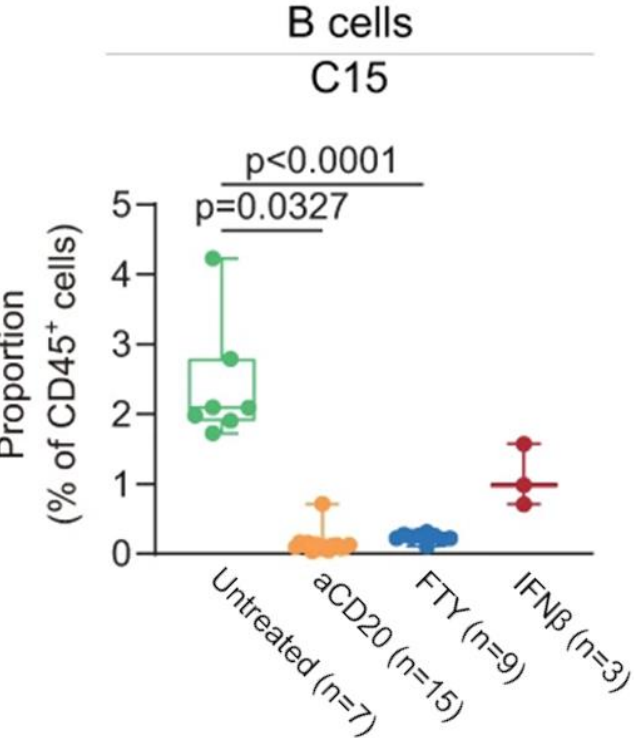


Fig. 1D, Wang, 2023

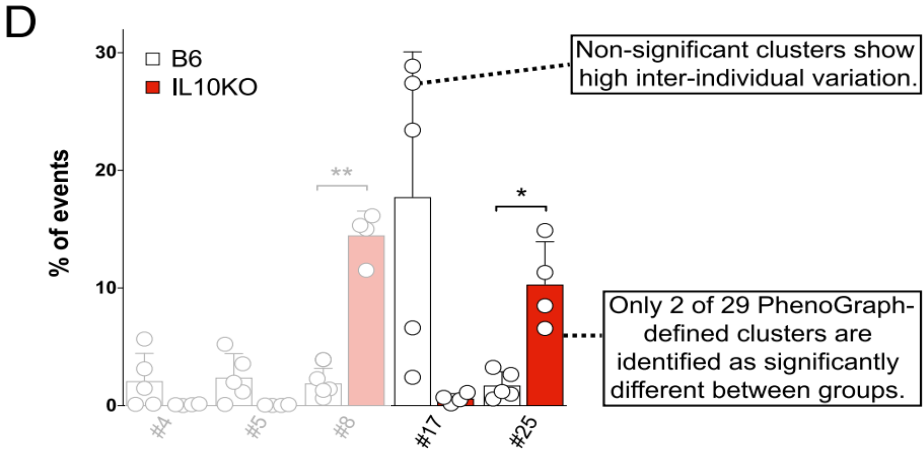


Fig.3D, Kimball, 2018

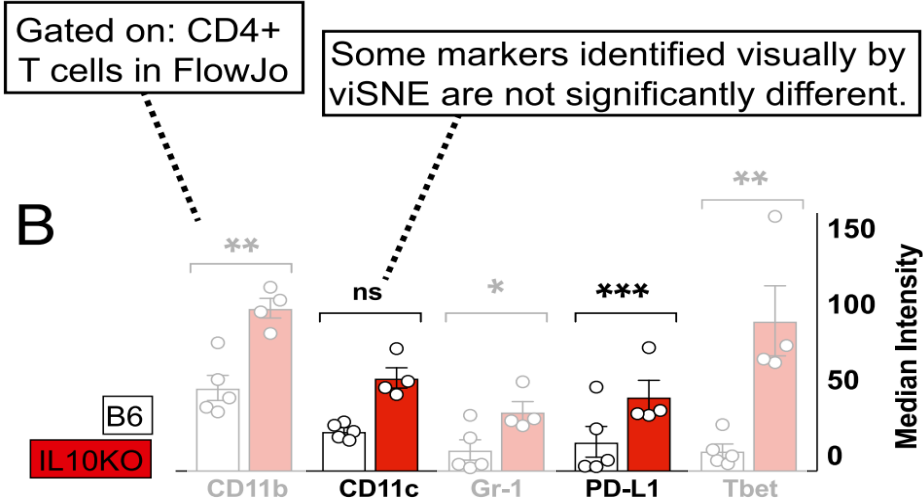


Fig. 4B, Kimball, 2018

Objectives

- Control quality of features
- Reliable graphics
- Differential Abundance (percentage)
- Differential State (MFI)

- Import
 - cluster features and FCS annotations
 - from various software
- Graphical User Interface

analycyte

- Control quality of features
- Differential Abundance (percentage)
- Differential State (MFI)
- Reliable graphics

- Import
 - cluster features and FCS annotations
 - from **OMIQ, FlowJo, text files**
- Graphical User Interface (**R/Shiny**)

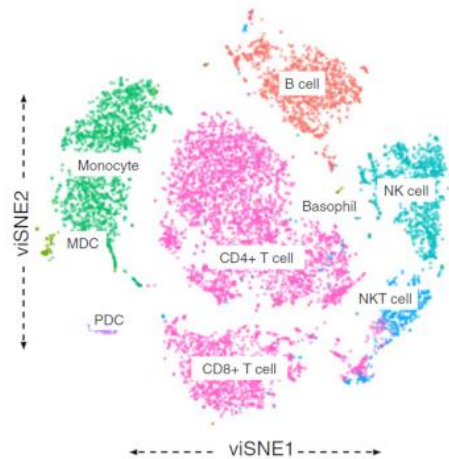
**ready-to-use
reports**







E. Lohmann



A-S. Chrétien



Comprehensive innate immune profiling of chikungunya virus infection in pediatric cases

Daniela Michlmayr^{1,†}, Theodore R Pak^{2,†} , Adeeb H Rahman^{2,3}, El-Ad David Amir^{2,3}, Eun-Young Kim⁴ , Seunghee Kim-Schulze^{2,3}, Maria Suprun⁵, Michael G Stewart⁴, Guajira P Thomas⁴, Angel Balmaseda⁶, Li Wang², Jun Zhu² , Mayte Suárez-Fariñas^{2,5}, Steven M Wolinsky⁴, Andrew Kasarskis² & Eva Harris^{1,*} 

We performed whole-blood RNA-seq, 37-plex **mass cytometry** of peripheral blood mononuclear cells (**PBMCs**), and serum cytokine measurements of **acute- and convalescent-phase** samples obtained from **42 children** naturally infected with CHIKV. Semi-supervised classification and clustering of single-cell events into **57 sub-communities** of canonical

leukocyte phenotypes revealed a monocyte-driven response to acute infection, with the greatest expansions in “intermediate” **CD14++CD16+ monocytes** and an **activated subpopulation of CD14+ monocytes**. Increases in acute-phase CHIKV envelope protein E2 expression were highest for monocytes and dendritic cells.

GUI

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> history

Choose a Project:
demo_chkv

[+ Add Project](#)

Cell Set:
No cell set

Feature Set:
0823E3E4

- Dashboard
- Dataset
- Export Project
- Expert View
- Session information

[Close Session](#)

[Delete Session](#)

Dataset

NUMBER OF FCS
86

NUMBER OF CLUSTERS
29

NUMBER OF MARKERS
37

NUMBER OF CELLS
1069654

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FCS information table

fcs_id	filename	acqdate	patient_id	conc
1 1758 Acute	160406_EHA001_1758_1_Patients_Acute.fcs.astrolabe.fcs	2016-04-06	1758	Acu
2 1758 Conv	160406_EHA001_1758_1_Patients_Conv.fcs.astrolabe.fcs	2016-04-06	1758	Coi
3 1760 Acute	160406_EHA001_1760_1_Patients_Acute.fcs.astrolabe.fcs	2016-04-06	1760	Acu
4 1760 Conv	160406_EHA001_1760_1_Patients_Conv.fcs.astrolabe.fcs	2016-04-06	1760	Coi
5 1773 Acute	160406_EHA001_1773_1_Patients_Acute.fcs.astrolabe.fcs	2016-04-06	1773	Acu
6 1773 Conv	160406_EHA001_1773_1_Patients_Conv.fcs.astrolabe.fcs	2016-04-06	1773	Coi
7 1785 Acute	160406_EHA001_1785_1_Patients_Acute.fcs.astrolabe.fcs	2016-04-06	1785	Acu
8 1785 Conv	160406_EHA001_1785_1_Patients_Conv.fcs.astrolabe.fcs	2016-04-06	1785	Coi
9 1790 Acute	160406_EHA001_1790_1_Patients_Acute.fcs.astrolabe.fcs	2016-04-06	1790	Acu
10 1790 Conv	160406_EHA001_1790_1_Patients_Conv.fcs.astrolabe.fcs	2016-04-06	1790	Coi

Showing 1 to 10 of 86 entries

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1 2 3 4 5 ... 9 Next

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Cluster information table

cluster_id	n_cells	CellSubset
1	52418	B Cell (CD27-)
10	104785	CD4+ T Cell (Naive)
11	22851	CD4+ T Cell (Treg)
12	66642	CD4- CD8- T Cell
13	15246	CD8+ T Cell (Central Memory)
14	2564	CD8+ T Cell (Effector Memory)
15	13593	CD8+ T Cell (EMRA)
16	97722	CD8+ T Cell (Naive)
17	10169	CM- HLADR+_unassigned
18	23598	CM-_unassigned

Showing 1 to 10 of 29 entries

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Marker information table

fcs_colname	desc	range	minRange	maxRange	marker_class
In113Di	CD57	1734	0	1733	state
In115Di	CD45	573	0	572	state
Nd142Di	CD19	676	0	675	type
Nd143Di	CD45RA	1137	0	1136	type

QC report

analycyte ☰ > history 🔌

Choose a Project:
demo_chkv ▼
[+ Add Project](#)

Cell Set:
No cell set ▼

Feature Set:
0823ECE7 ▼

[Dashboard](#)
[Dataset](#)
[Export Project](#)
[Expert View](#) <
[Session information](#)
[Close Session](#)
[Delete Session](#)
↻

Select an action:
Nothing selected ▼

Import R Object × Quality Control ×

Control Quality

Project of Reference: demo_chkv Folder of Reference: 0823ECE7

[Details](#) +

General Parameters

Condition: ⓘ
cond ▼

Batch: ⓘ
acqdate ▼

Supplementary annotation (multiple choices): ⓘ
Gender ▼

Annotation Column: ⓘ
CellSubset ▼

Thresholds

Minimum cells treshold:

ON/OFF

Add code info Show the summary tables
 Log on graphs Show the Presence/Absence heatmap
 Sort on graphs

See in right panel Open in new tab

[⚙️ RUN THE REPORT](#)

Quality Control Report

demo_chkv

AUTHOR Eugénie Lohmann AFFILIATION CRCM (CIBI Group)

PUBLISHED April 29, 2024

[Report Parameters](#) >

This document has been formatted using `knitr`¹ and `quarto`².

1 Features summary

[Samples informations](#) [Metacluster informations](#)
[Markers informations](#)

Sample table with information on conditions (**cond**), batch (**acqdate**), ncells (**Number of Cells**)

[Copy](#) [CSV](#) [Excel](#) [Print](#)

Show entries

Search:

percent.cluster ↕	Number.of.Cells ↕	fcs_id ↕	
All	All	All	All
82.76	7944	1	160406_EH/
89.66	13727	2	160406_EH.
89.66	12080	3	160406_EH.

QC report

1 Features summary

Samples informations

Metacluster informations

Markers informations

Sample table with information on conditions (**cond**), batch (**acqdate**), ncells (**Number of Cells**)

Copy CSV Excel Print

Show entries

Search:

percent.cluster ⚡	Number.of.Cells ⚡	fcs_id ⚡	filename
All	All	All	All
82.76	7944	1	160406_EHA001_1758_1_Patients_Acute.fcs.astrola
89.66	13727	2	160406_EHA001_1758_1_Patients_Conv.fcs.astrola
89.66	12080	3	160406_EHA001_1760_1_Patients_Acute.fcs.astrola
93.1	11500	4	160406_EHA001_1760_1_Patients_Conv.fcs.astrola
86.21	6981	5	160406_EHA001_1773_1_Patients_Acute.fcs.astrola

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- **percent.cluster** : Percentage of clusters with cells (> 10 cells) in this fcs. [see 1](#)

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- [7 Abundance Heatmap](#)
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QC report

2 Presence / Absence data

With a minimum of 10 per cluster.

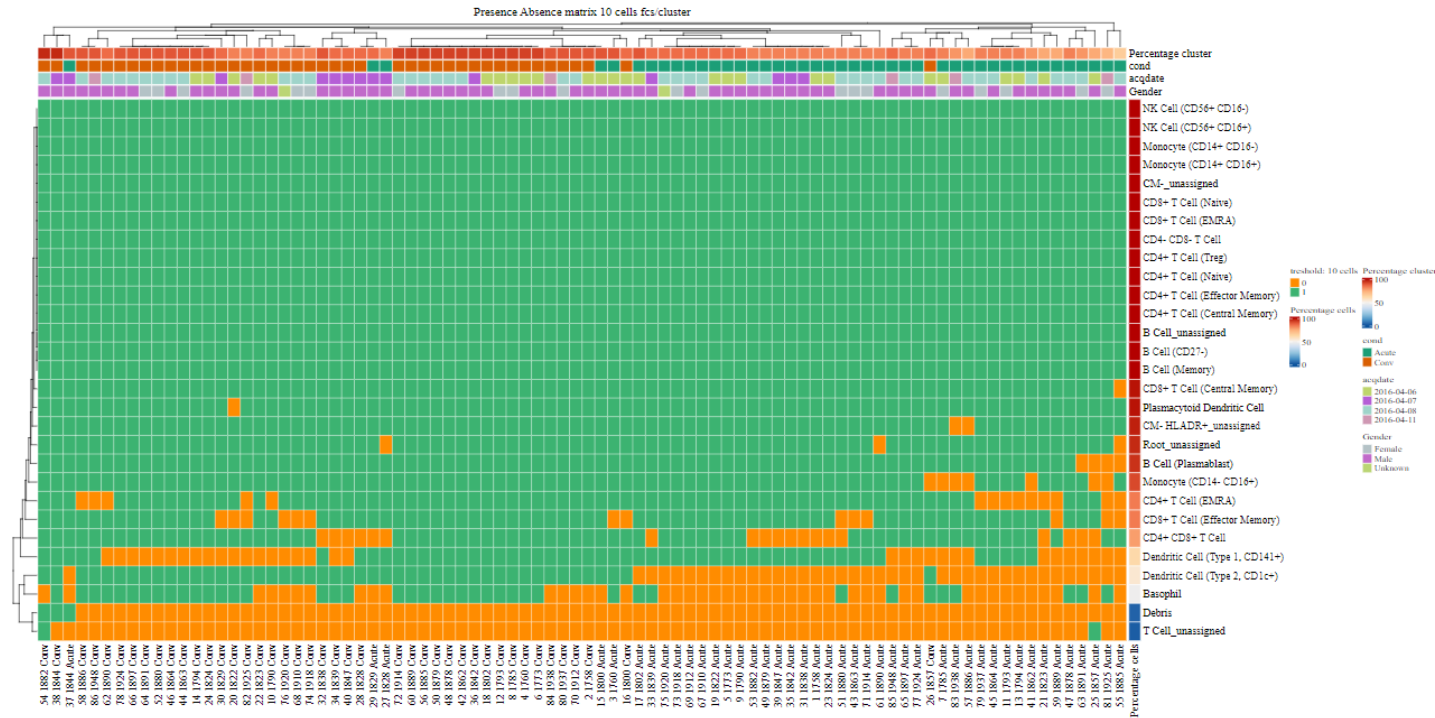


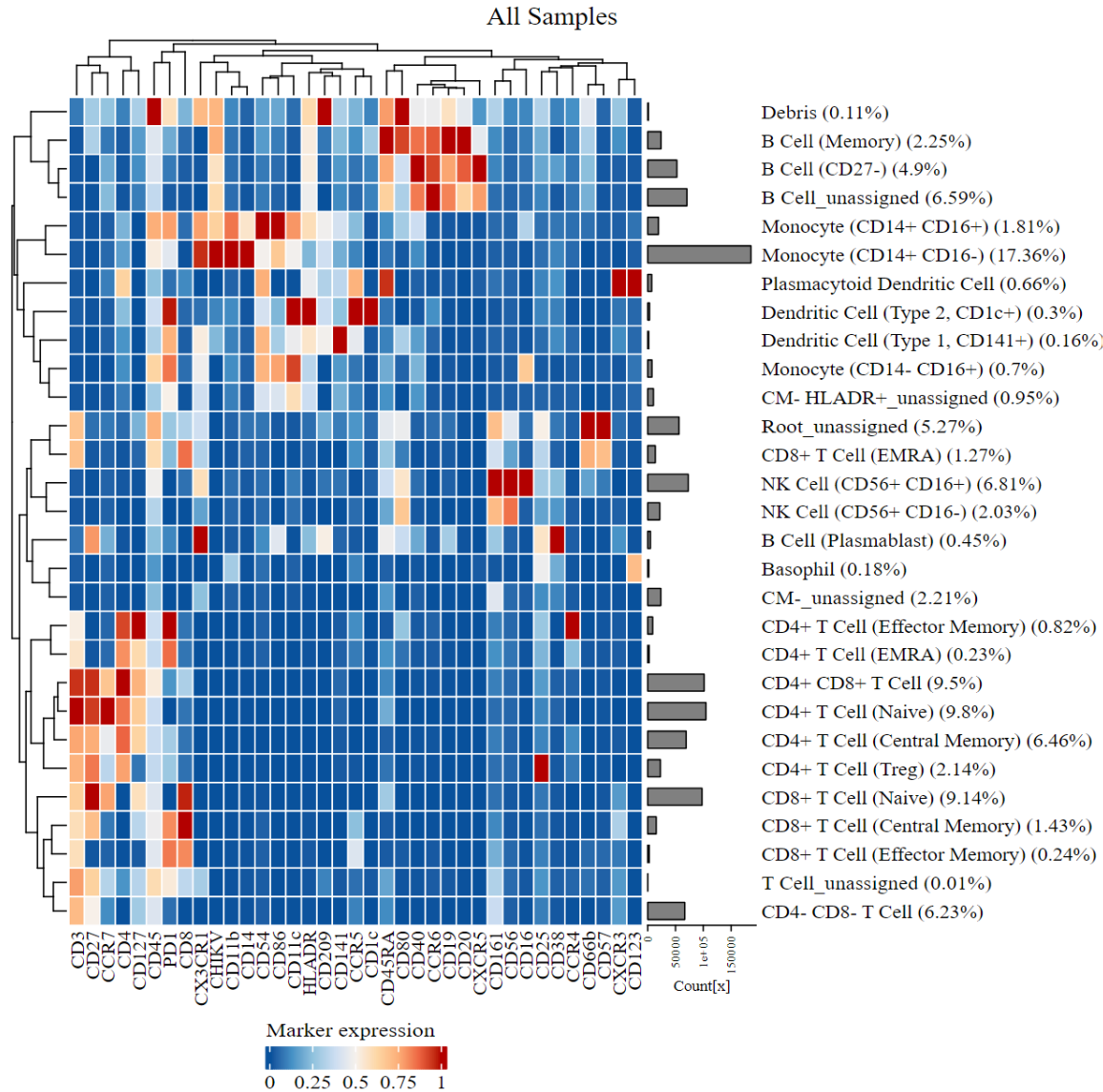
Figure 1: Heatmap Presence Absence

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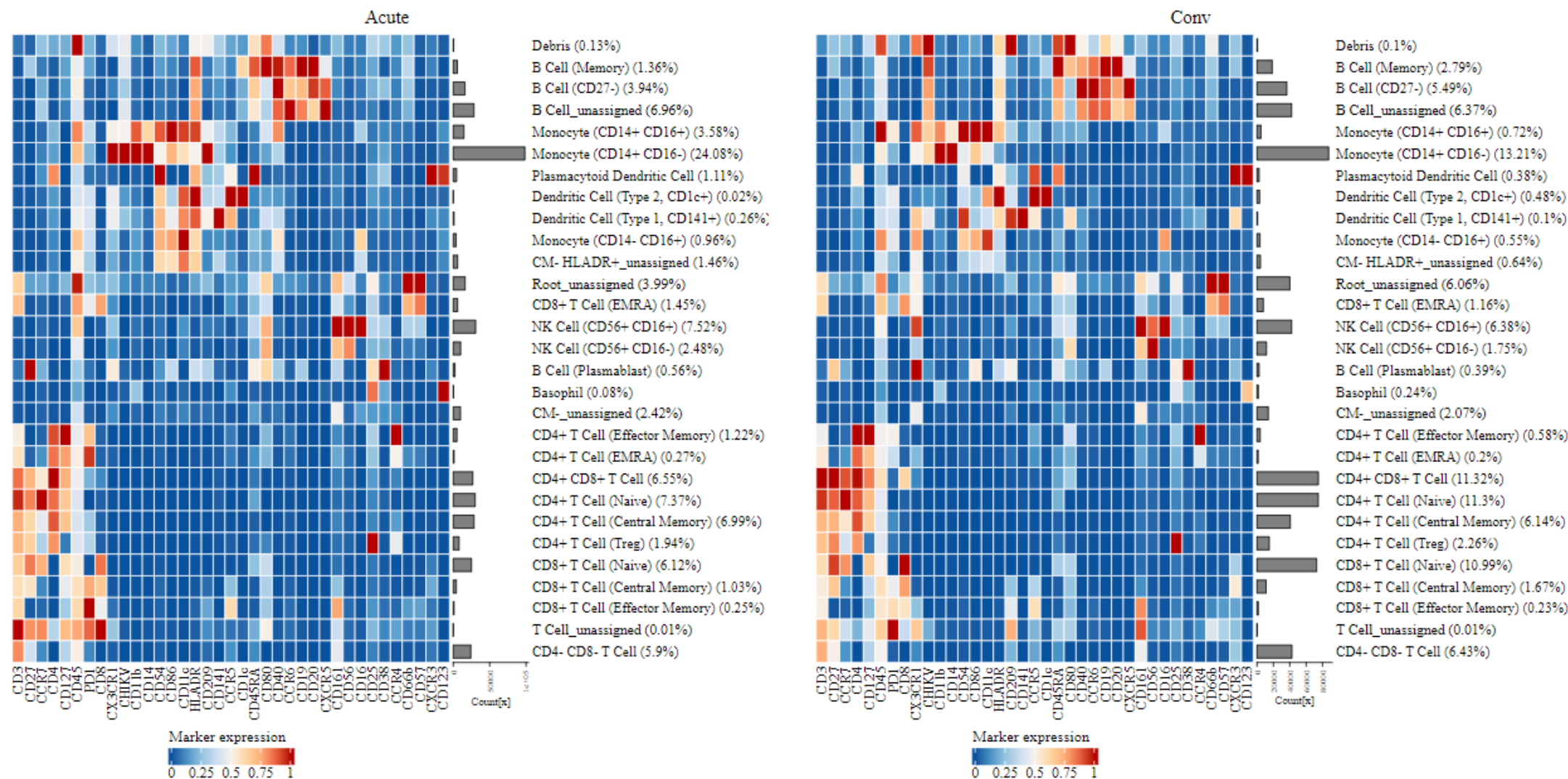
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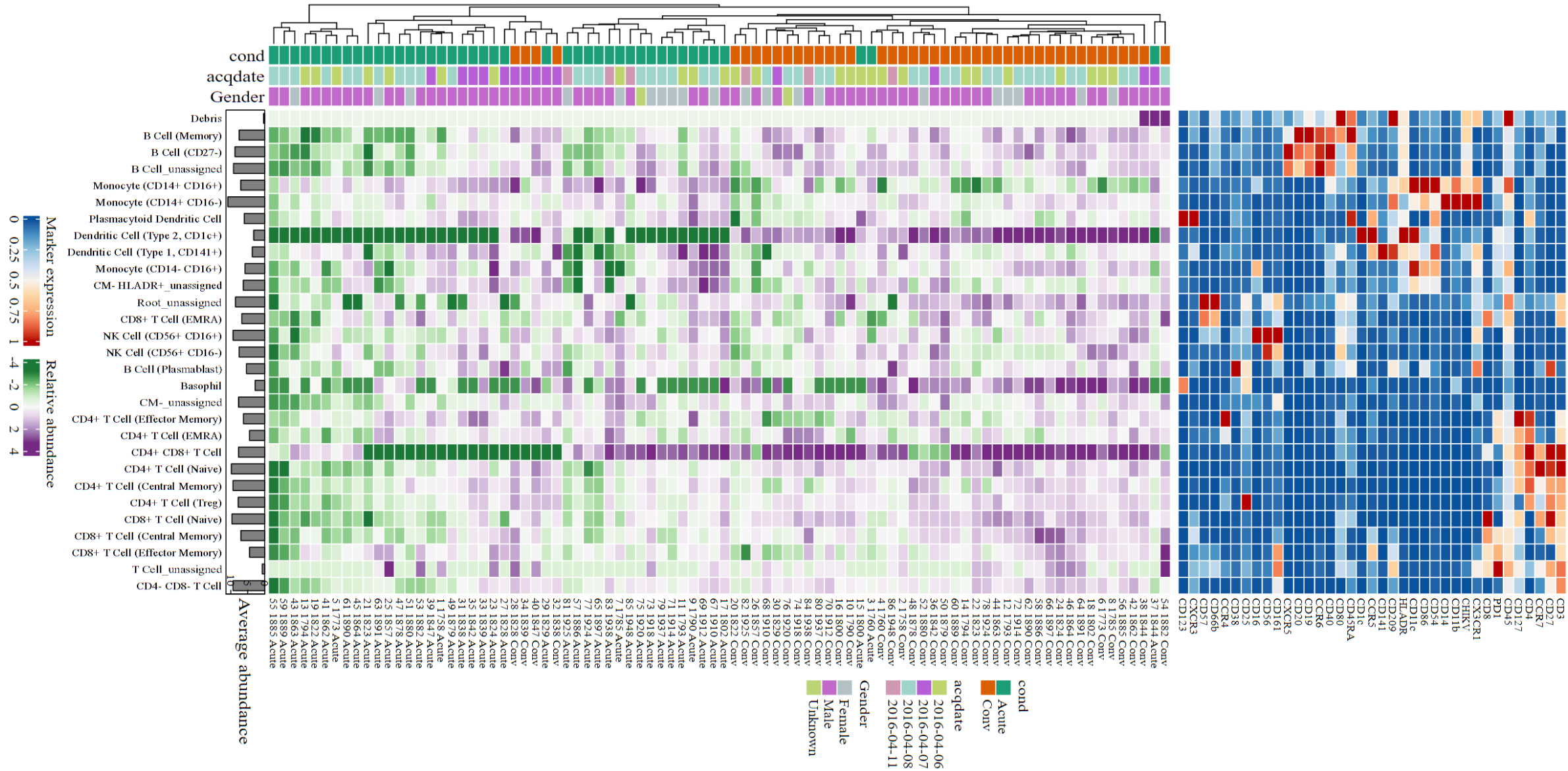
5 MFI's Heatmap



QC report



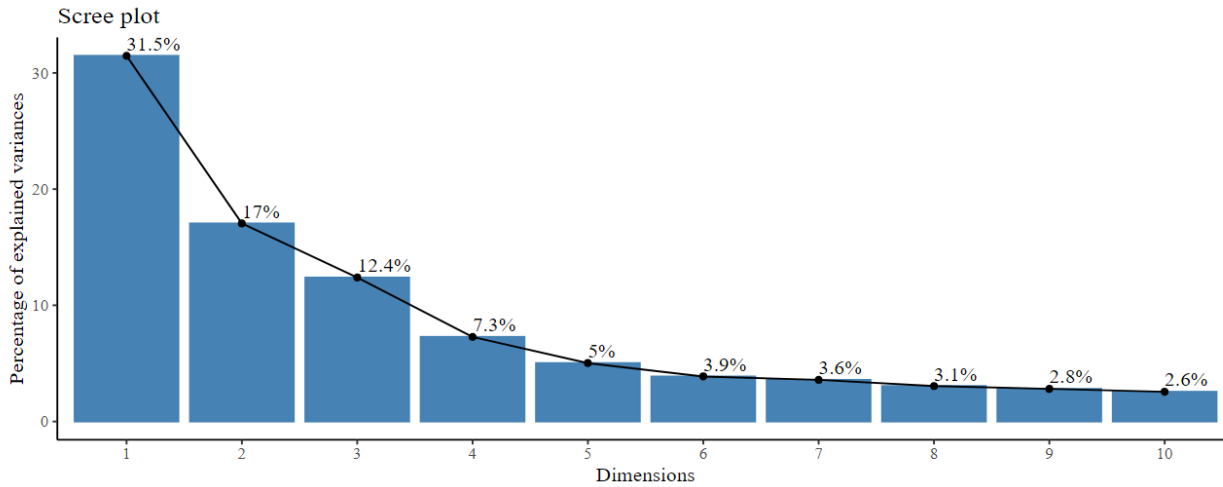
QC report



QC report

8 PCA of samples using percentages per cluster

8.1 Barplot of variance of each PC



8.2 Dim.1 vs Dim.2

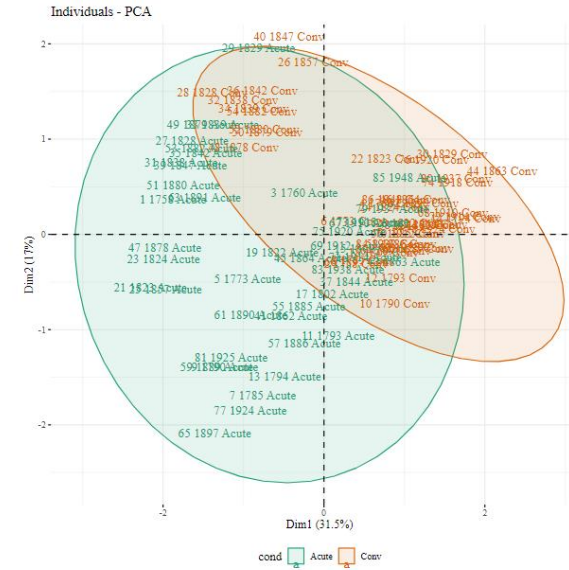


Figure 12: Dim.1 vs Dim.2 by cond

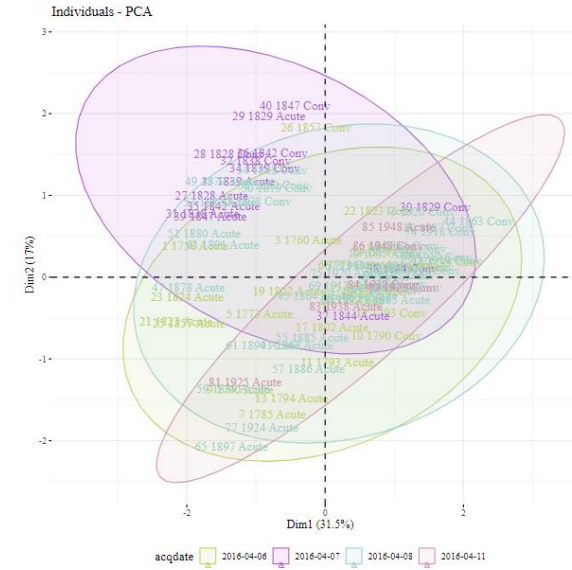


Figure 13: Dim.1 vs Dim.2 by acqdate

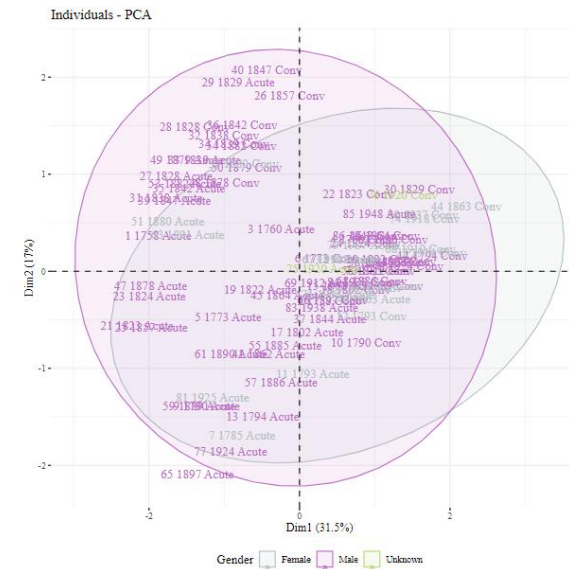


Figure 14: Dim.1 vs Dim.2 by Gender

QC report

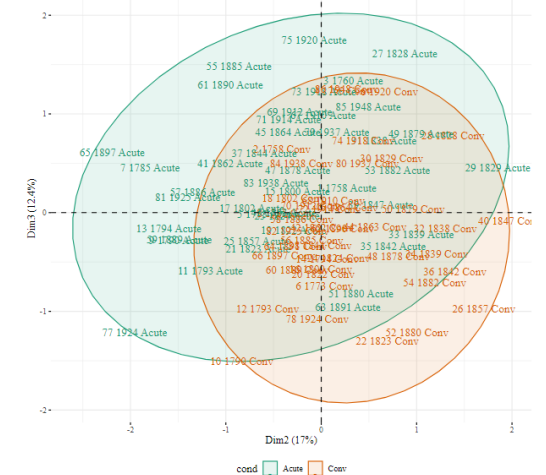
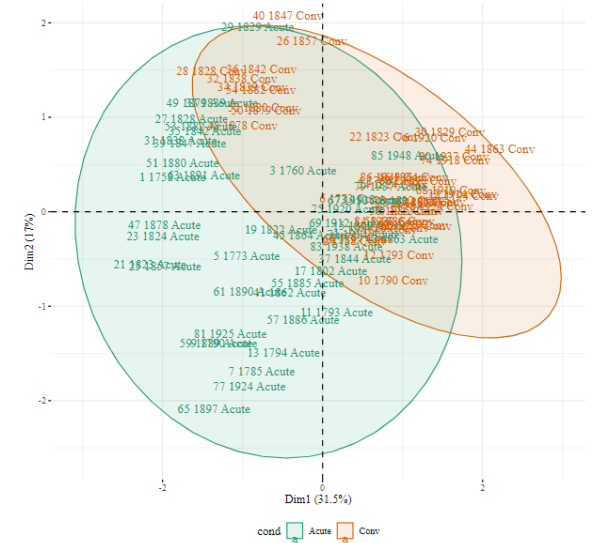
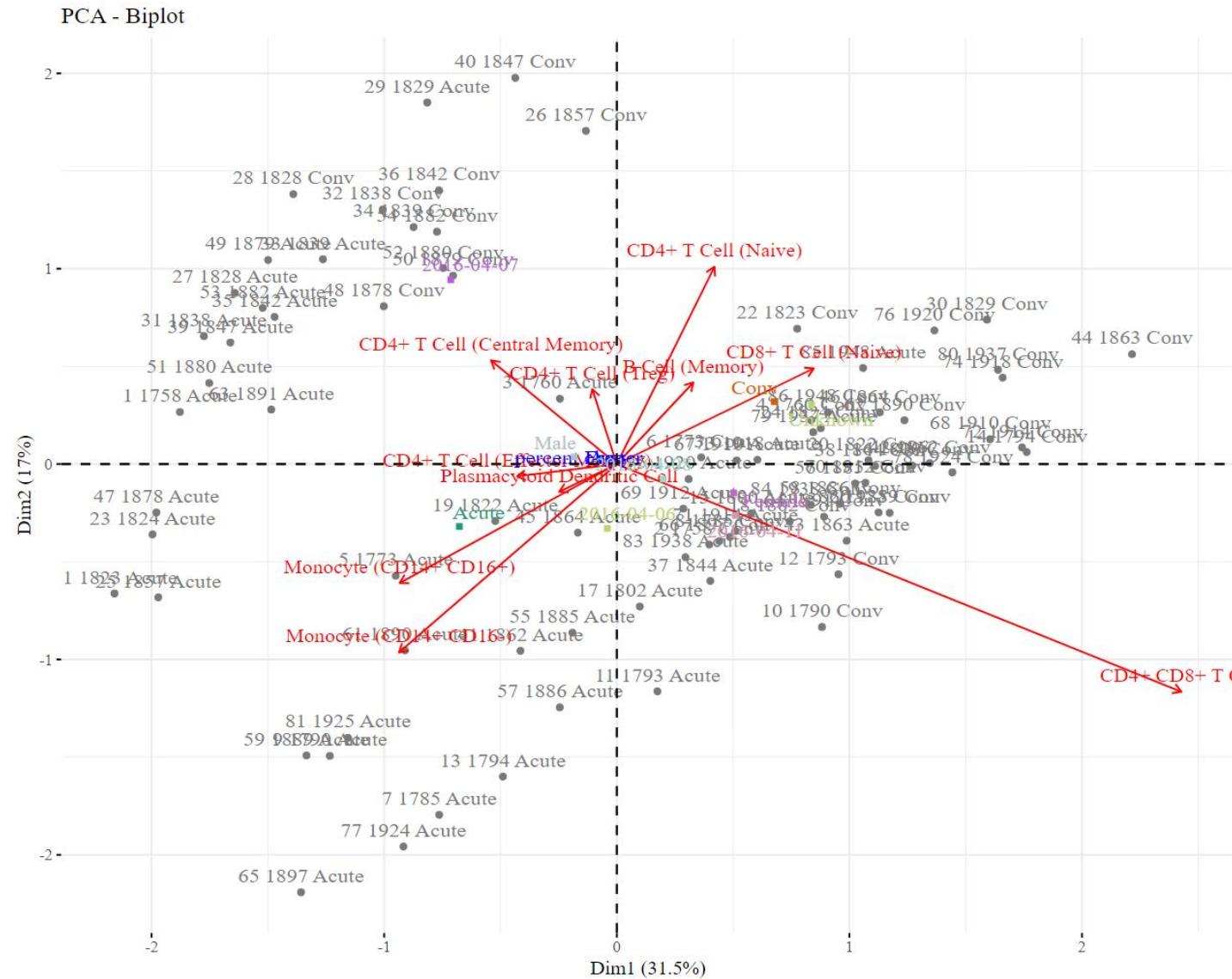


Figure 15: Dim.1 vs Dim.2

QC report

9 PCA of samples using MFI

9.1 Dim.1 vs Dim.2

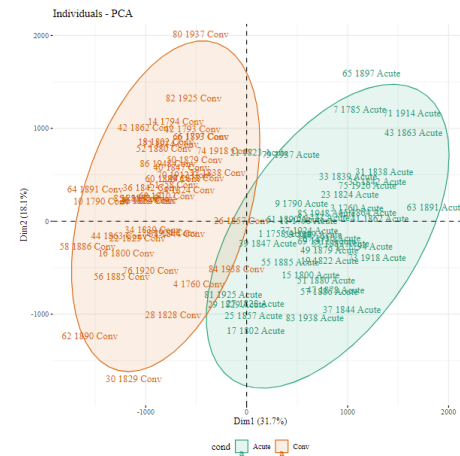


Figure 25: Dim.1 vs Dim.2 by cond

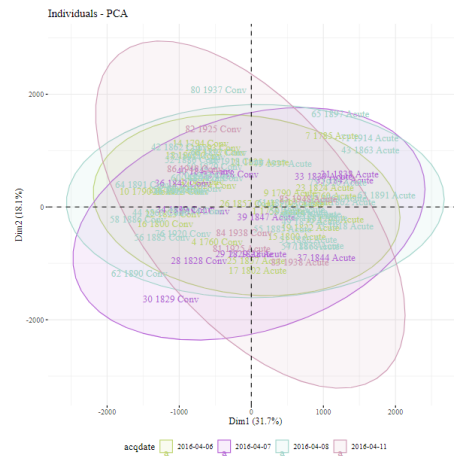


Figure 26: Dim.1 vs Dim.2 by acqdate

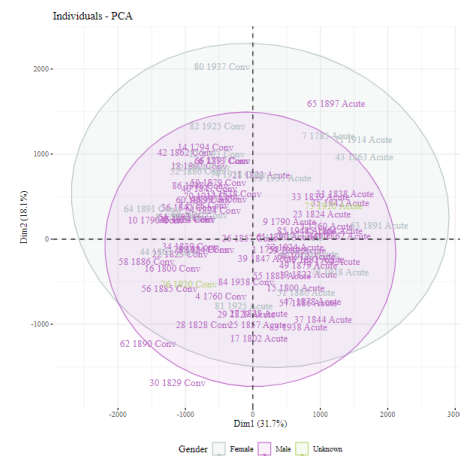


Figure 27: Dim.1 vs Dim.2 by Gender

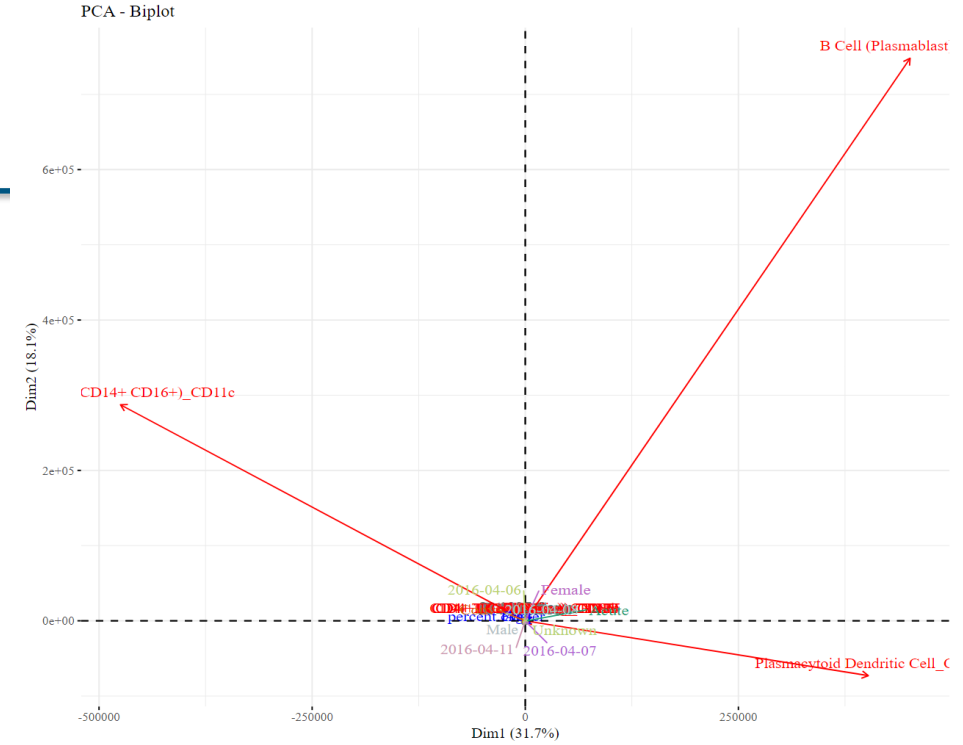


Figure 28: Dim.1 vs Dim.2

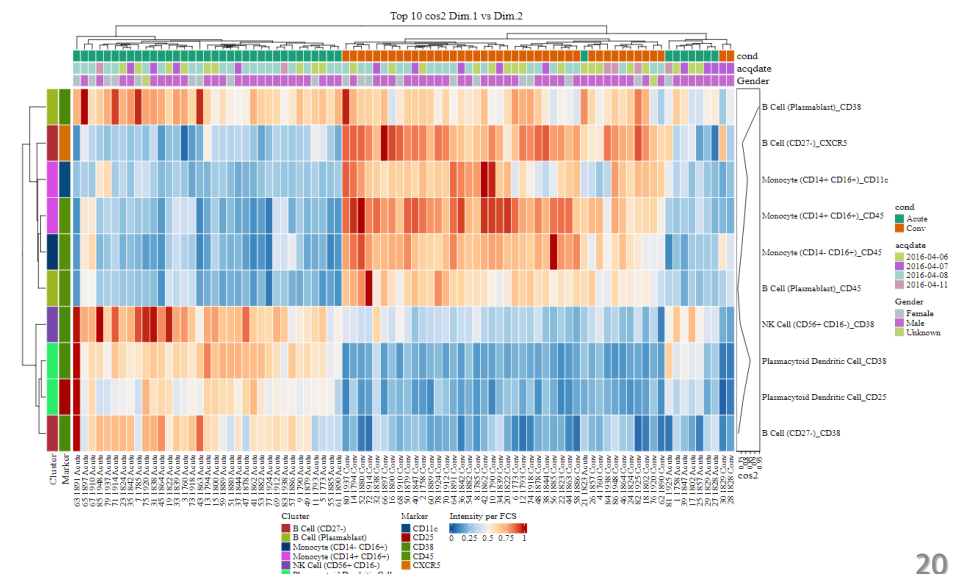


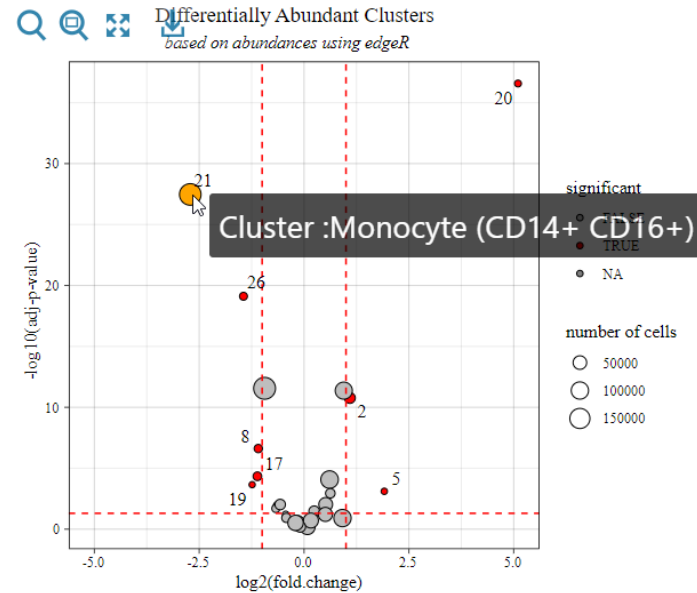
Figure 29: Heatmap Top 10 cos2 for :Dim.1 vs Dim.2

DA report

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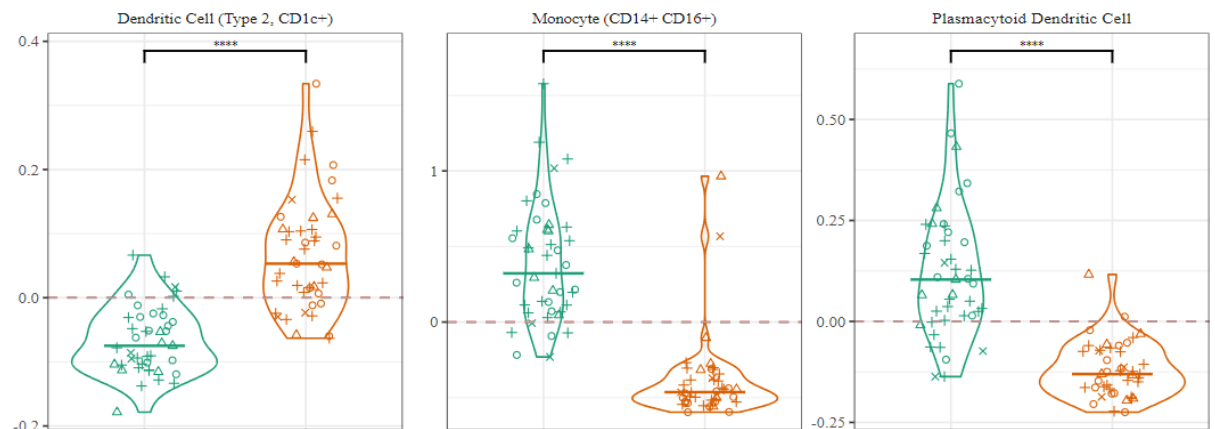
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- 6 VOOM
- 7 GLMM
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5.1.1 volcano and abundance plot



5.1.2 violin plot

relative perCellCountsNorm of each clusters by acqdate



DS report

5 limma

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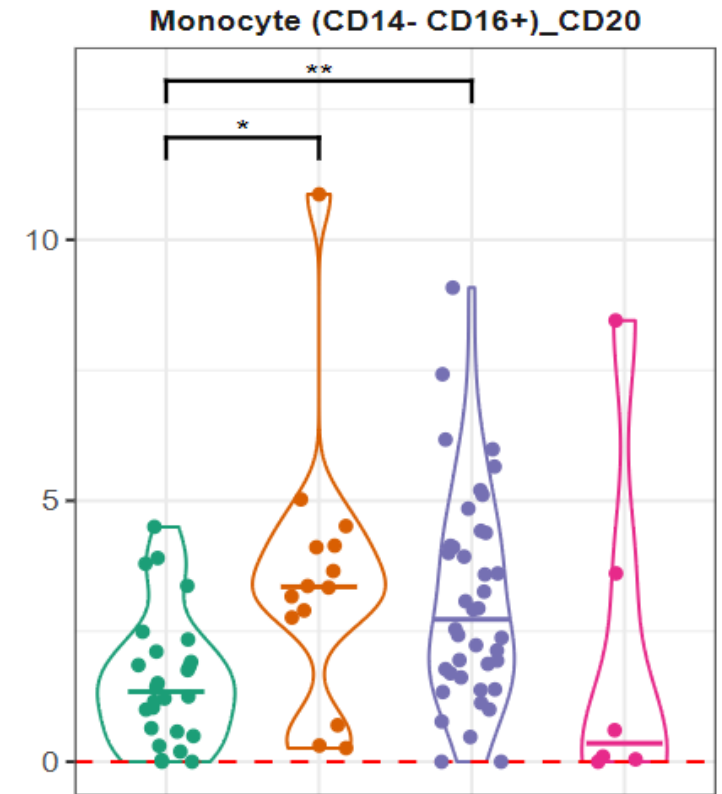
cluster_id	CellSubset	marker_id	reference	contrast	logFC	explicit FC
All	All	All	All	All	All	All
22	Monocyte (CD14+ CD16-)	CD25	Acute	Conv	-2.452	-5.47
22	Monocyte (CD14+ CD16-)	CCR7	Acute	Conv	-6.657	-100
8	CD4+ T Cell (Effector Memory)	CD45	Acute	Conv	91.224	2.8913
22	Monocyte (CD14+ CD16-)	CD209	Acute	Conv	-3.08	-8.4
22	Monocyte (CD14+ CD16-)	CHIKV	Acute	Conv	-6.89	-118
22	Monocyte (CD14+ CD16-)	CD11c	Acute	Conv	235.168	6.2023
7	CD4+ T Cell (Central Memory)	CD45	Acute	Conv	73.722	1.5579
3	B Cell (Plasmablast)	CD45	Acute	Conv	45.216	4.0876
11	CD4+ T Cell (Treg)	CD45	Acute	Conv	67.598	2.2346
22	Monocyte (CD14+ CD16-)	CD123	Acute	Conv	-16.793	-1.1352

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analycyte

- Quality control
- Comparison 1-vs-1, all-vs-ref
- Automatically placed p-values
- Reproducible HTML reports
 - based on Quarto templates
- Interactive plots and SVG graphics



analycyte

- <https://i-cyto.github.io>
- docker image & R packages
- demo reports
- current presentation

- documentation
- multi-variate analyses (ML)

