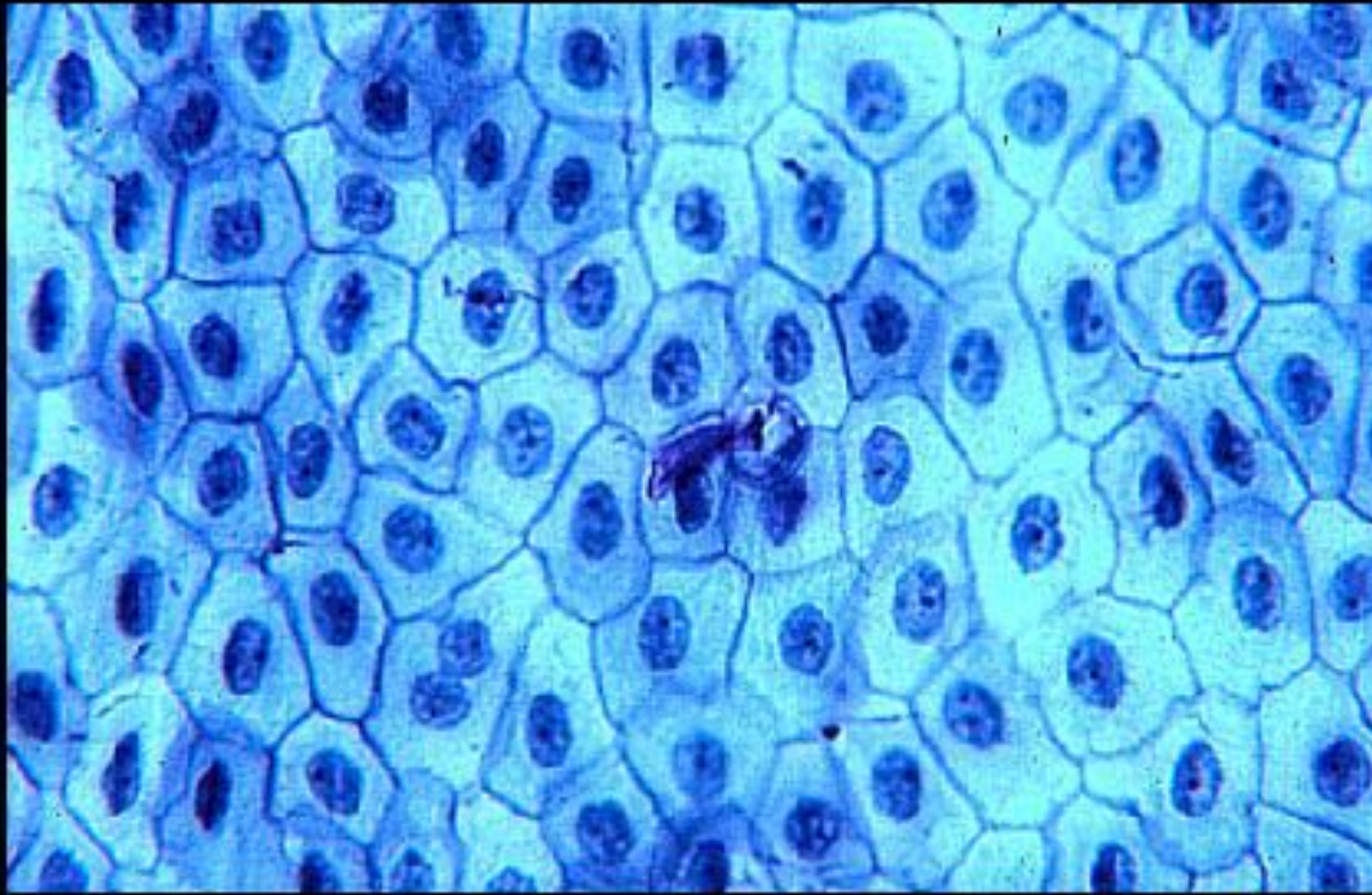


22 August 2018



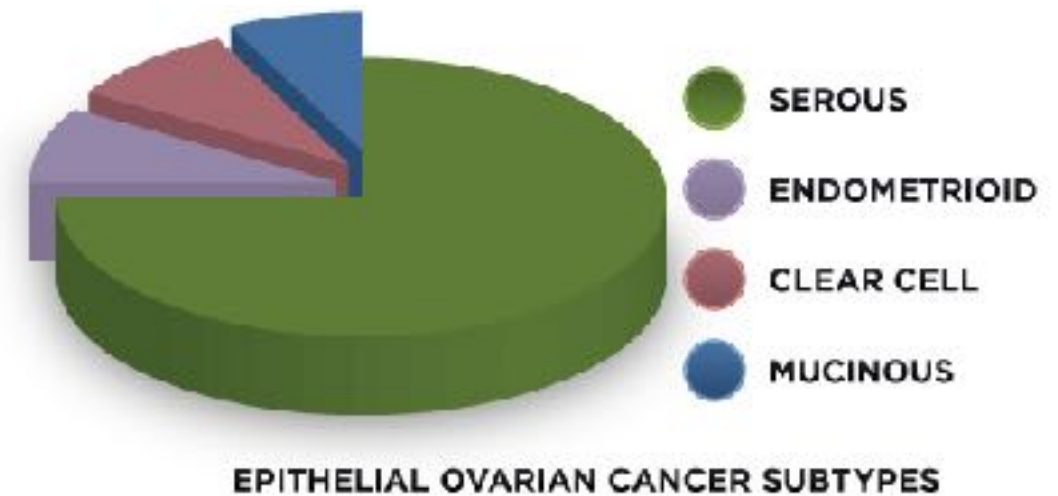
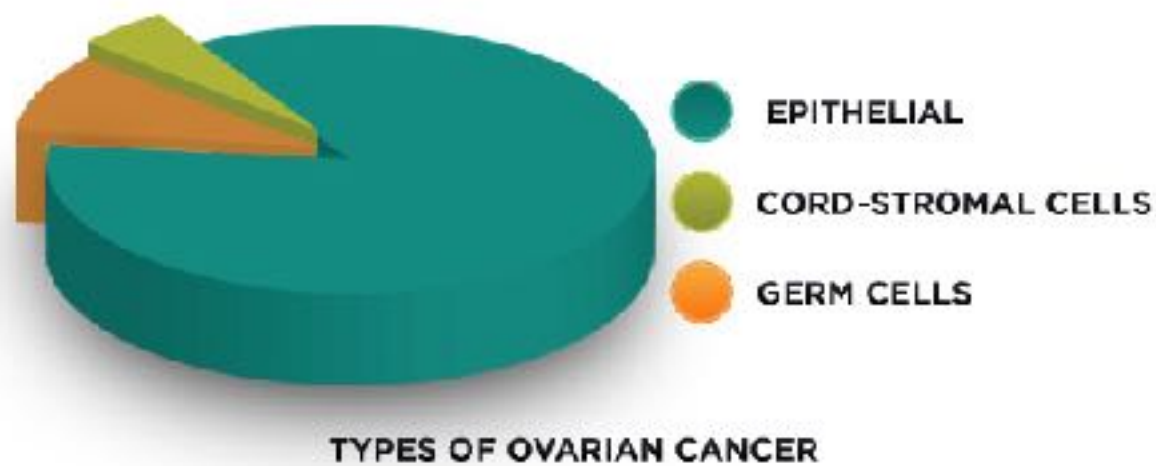
# Single Cell Study of the Chemoresistance in the Ovarian Cancer

*Alishayeva Saudat*

---

Mentor : Dr. Jieckun (Jackie) Yang  
Advisor: Assoc. Prof. Mazhar Adli

High Grade Serous Carcinoma is the most common type of ovarian cancers with the lowest survival rates.



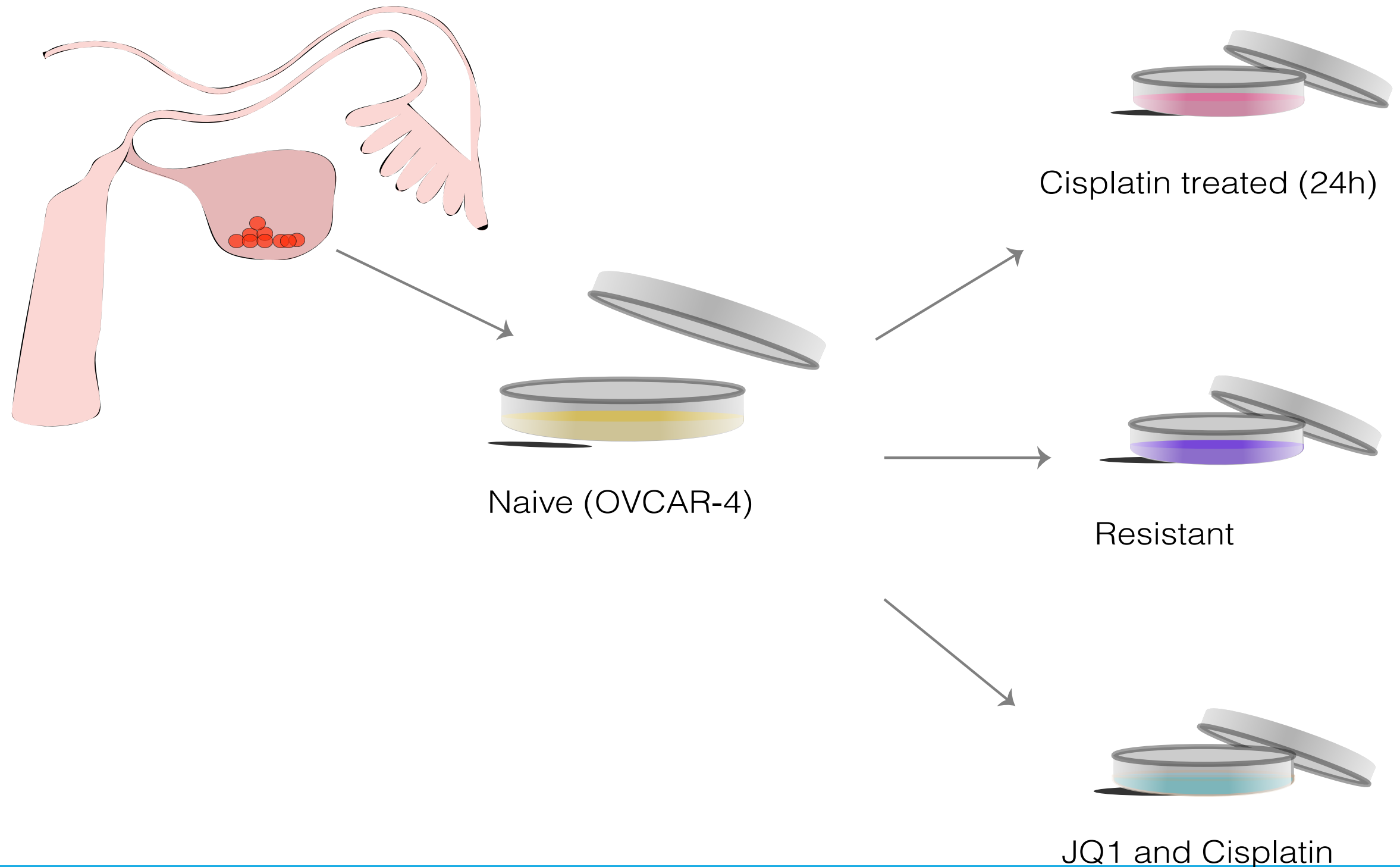
CREDITS:[https://www.researchgate.net/figure/Clinico-pathological-profiles-of-CSIOVDB-Pie-charts-or-histogram-showing-distribution-of\\_fig2\\_283640774](https://www.researchgate.net/figure/Clinico-pathological-profiles-of-CSIOVDB-Pie-charts-or-histogram-showing-distribution-of_fig2_283640774)

Hypothesis:

Chemoresistance is heterogenous population of cells transitioning towards a resistant state.

There is a small subset of naive cells that are transcriptionally more similar to resistant cells.

Methods: samples were exposed to the cisplatin or JQ1 drugs in short/long term manner and later sequenced with 10xGenomics.

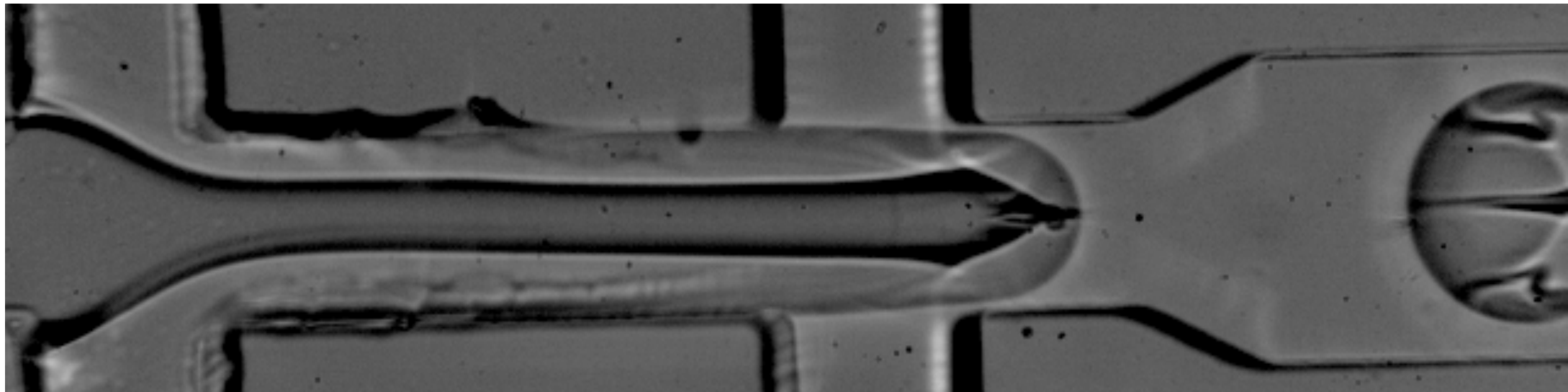
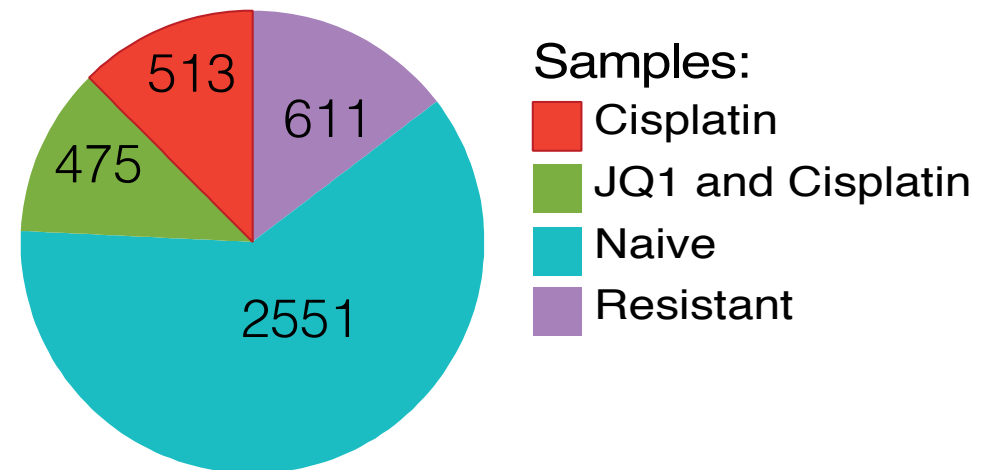




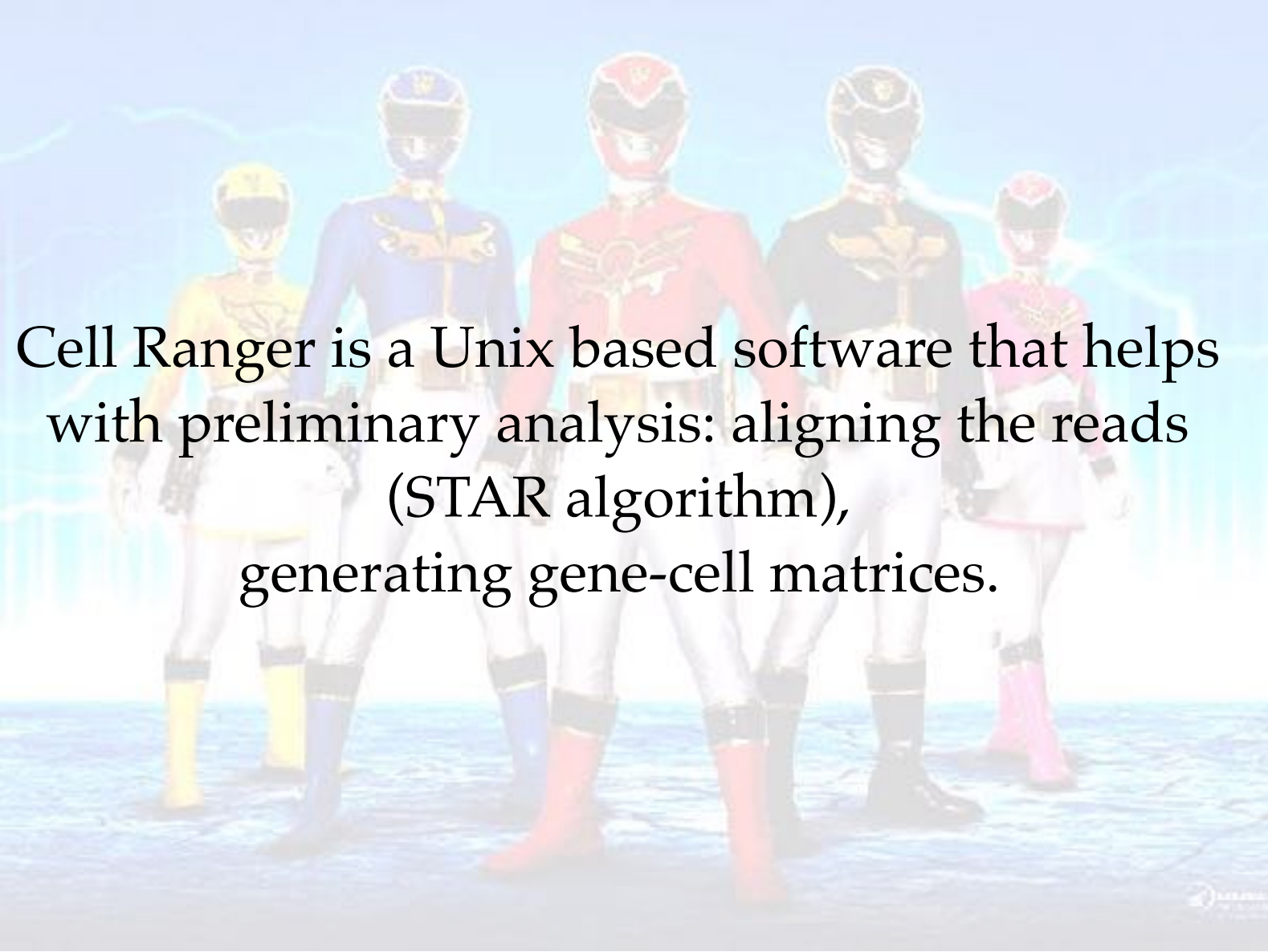
# 10xGenomics performs massive single cells profiling using microfluidic system.



Total: 4150 cells



# The sequenced reads were aligned using Cell Ranger

A background image showing five Ultraman characters standing in a row. From left to right: Ultraman Yellow, Ultraman Blue, Ultraman Red, Ultraman Black, and Ultraman Pink. They are all wearing their signature colored suits and helmets, standing on a blue, rocky surface against a light blue sky with faint clouds.

Cell Ranger is a Unix based software that helps with preliminary analysis: aligning the reads (STAR algorithm), generating gene-cell matrices.

The Cell Ranger output is the estimated number of cells and the transcription coverage data.

### Estimated Number of Cells

4,556

Post-Normalization Mean  
Reads per Cell

159,775

Median Genes per Cell

5,601

### Sequencing

Pre-Normalization Number of Reads

727,937,919

Post-Normalization Number of Reads

727,937,919

### Aggregation

Naive Fraction of Reads Kept

100.0%

JQ1andCis Fraction of Reads Kept

100.0%

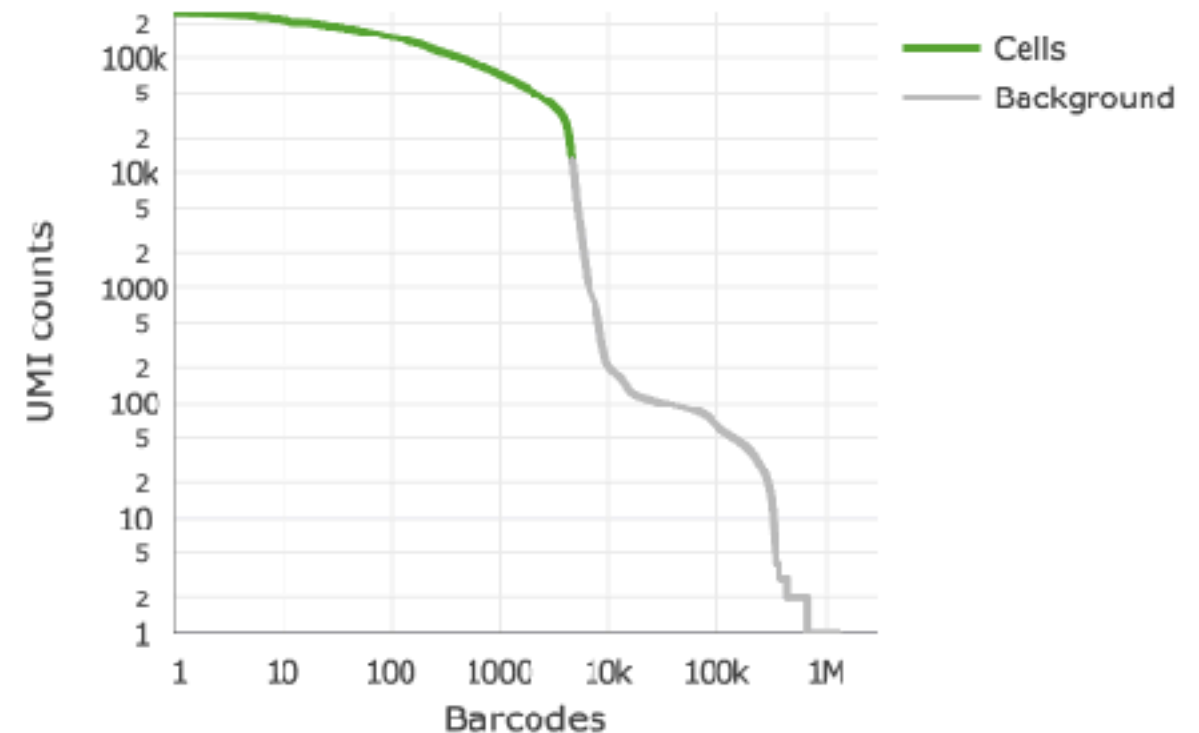
Cisplatin Fraction of Reads Kept

100.0%

Resistant Fraction of Reads Kept

100.0%

### Cells



Estimated Number of Cells	4,556
Fraction Reads in Cells	90.2%
Pre-Normalization Mean Reads per Cell	159,775
Post-Normalization Mean Reads per Cell	159,775
Median Genes per Cell	5,601
Median UMI Counts per Cell	47,164

# Quality Control (QC) was performed using Seurat package

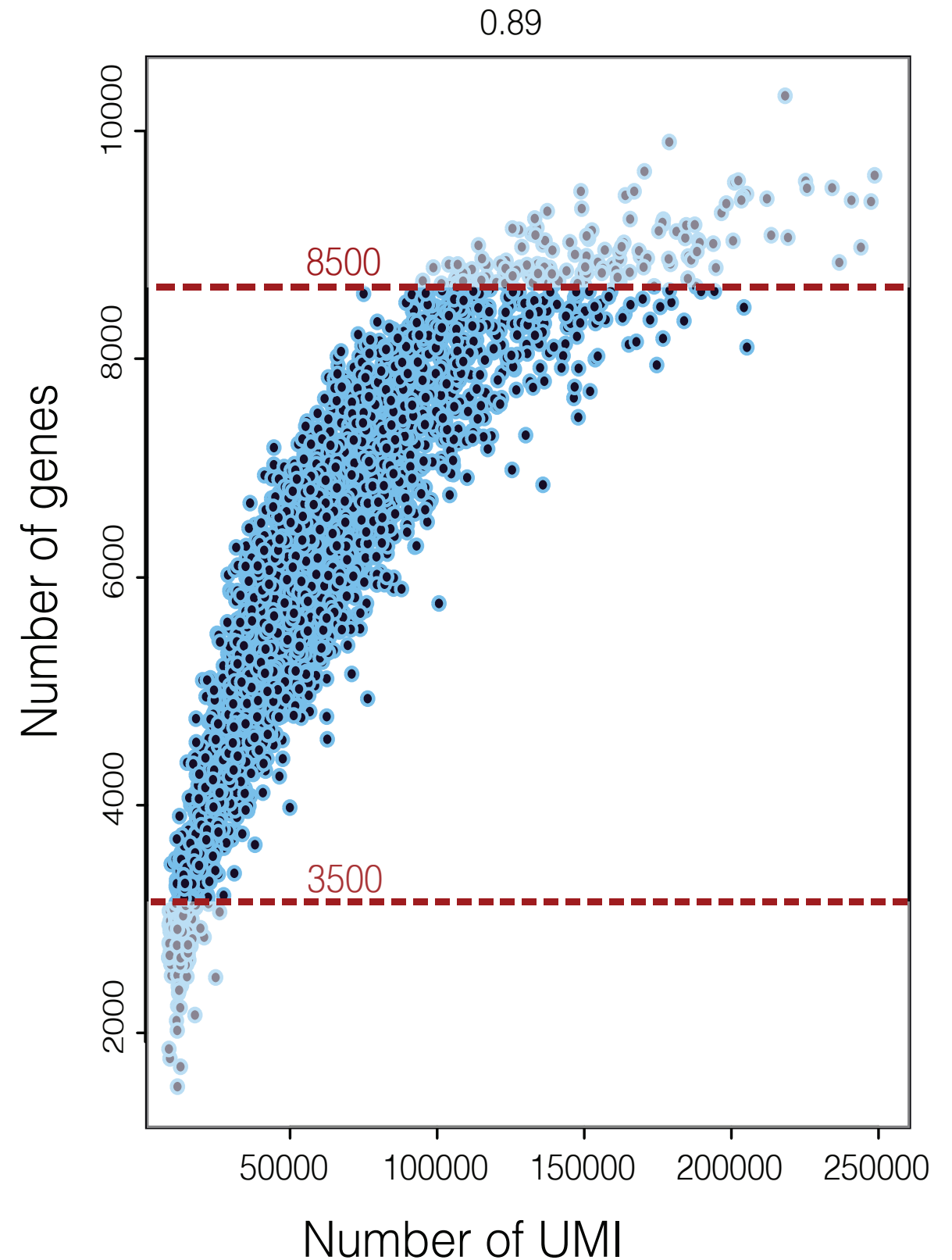
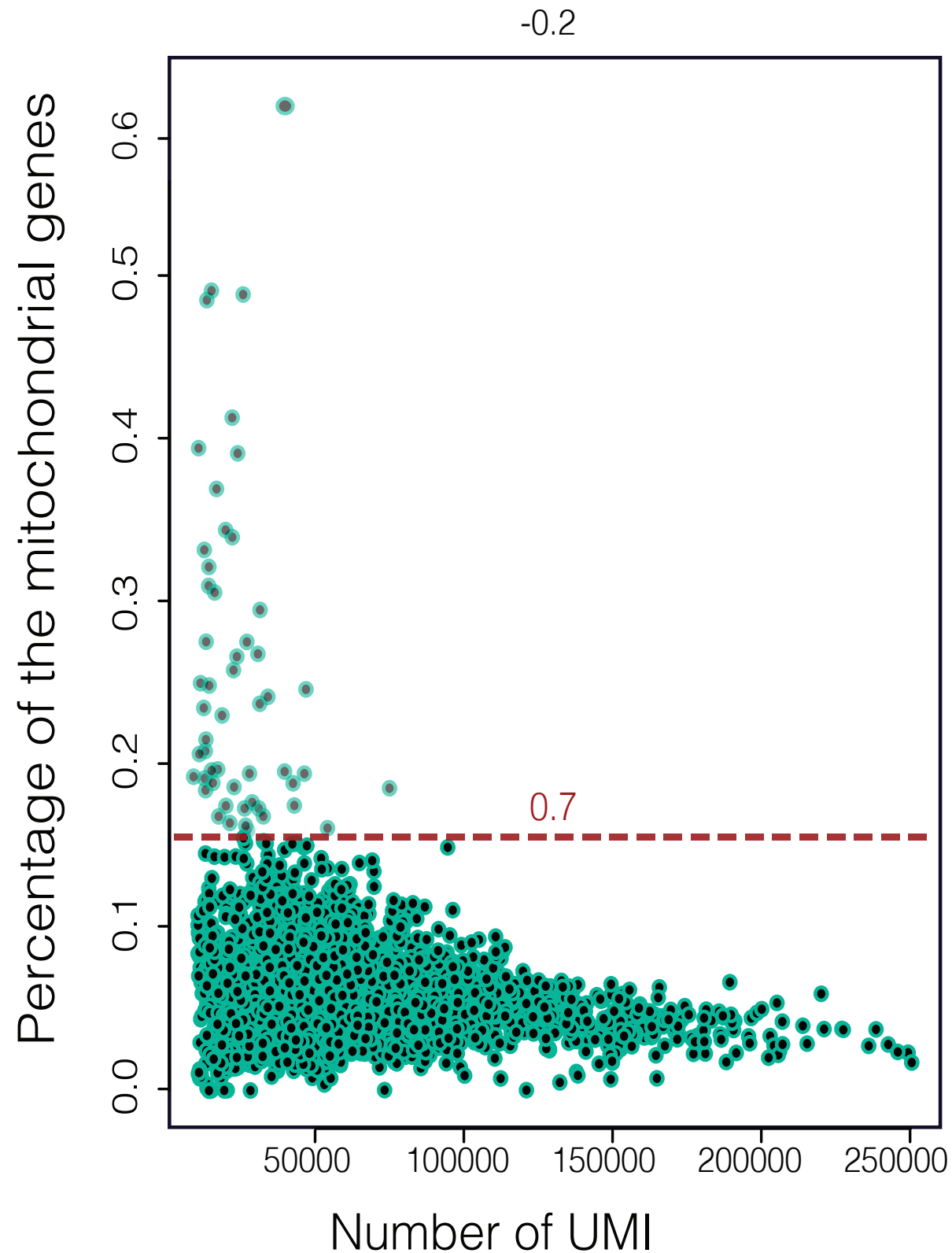


Seurat [Satija Lab]

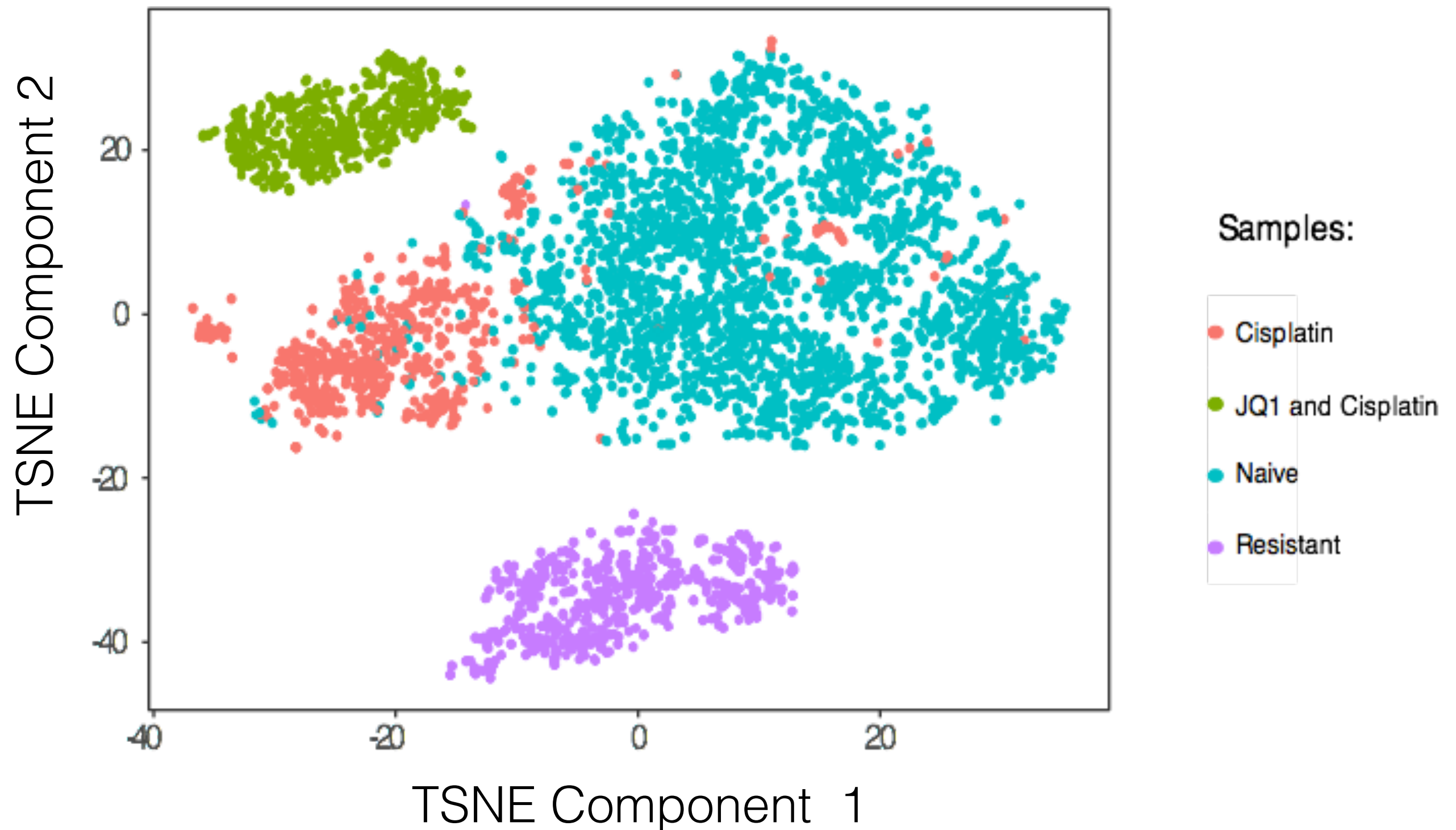
- Designed for sc RNA-seq exploratory analysis
- Helps to perform heterogeneity analysis in single cell transcriptome data



We filtered out the low quality cells according to the mitochondrial noise and their genetic profiles.



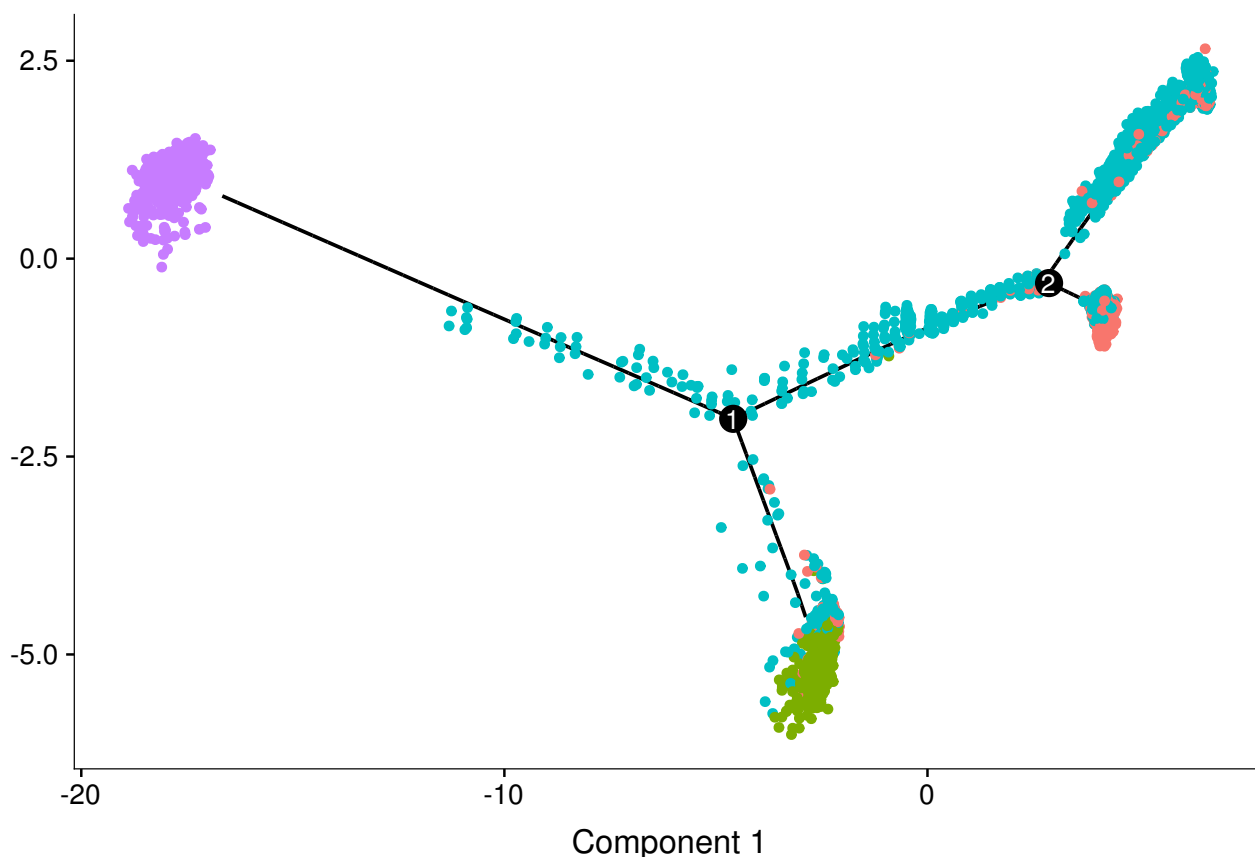
The PCA based clustering showed that some Naive and Cisplatin samples exhibit inter—(60 PCs were used)



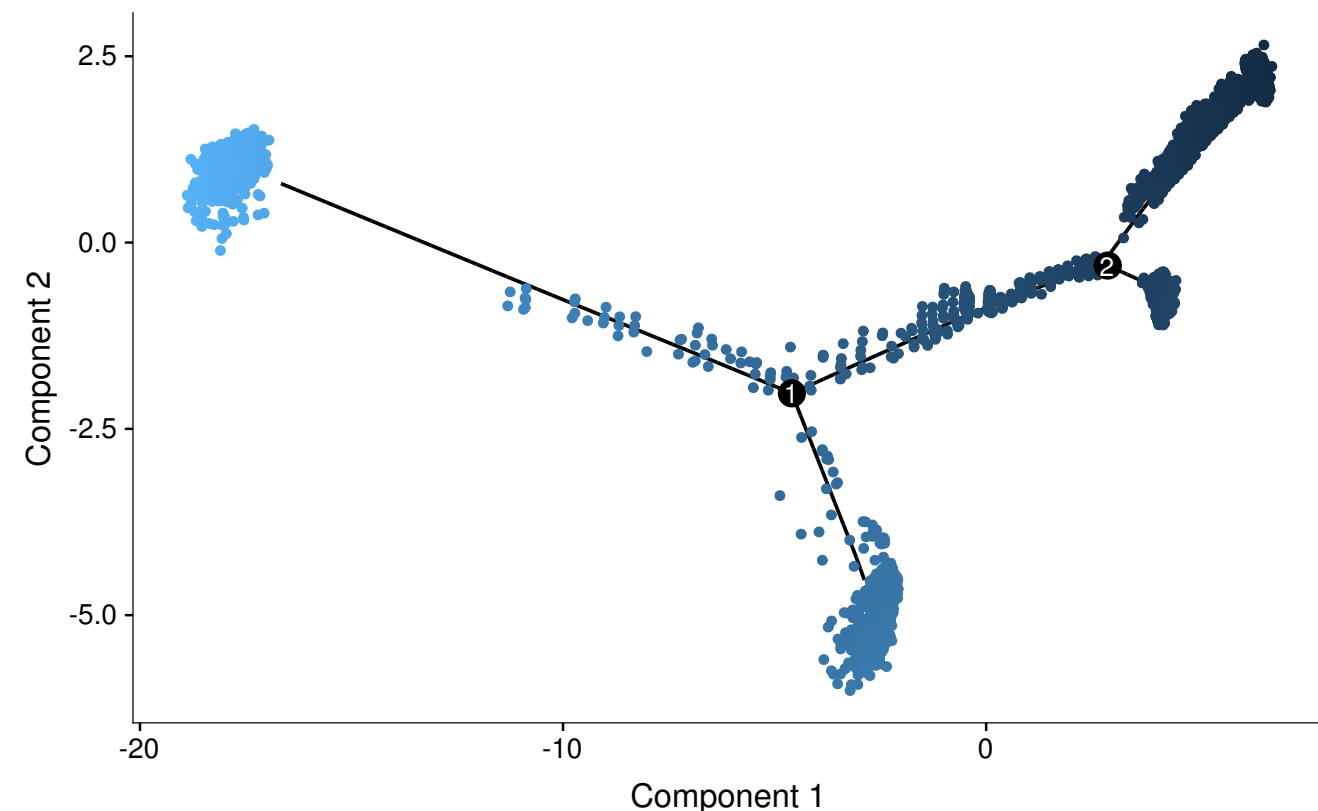
# Main Goals for the Trajectory analysis [Monocle - Trapnell Lab]:

- Build single-cell trajectories according to the “pseudotime” of the differentiating cells.
- Find cell fate decisions and the genes regulated as they are made.

sample • Cisplatin • JQ1andCis • Naive • Resistant

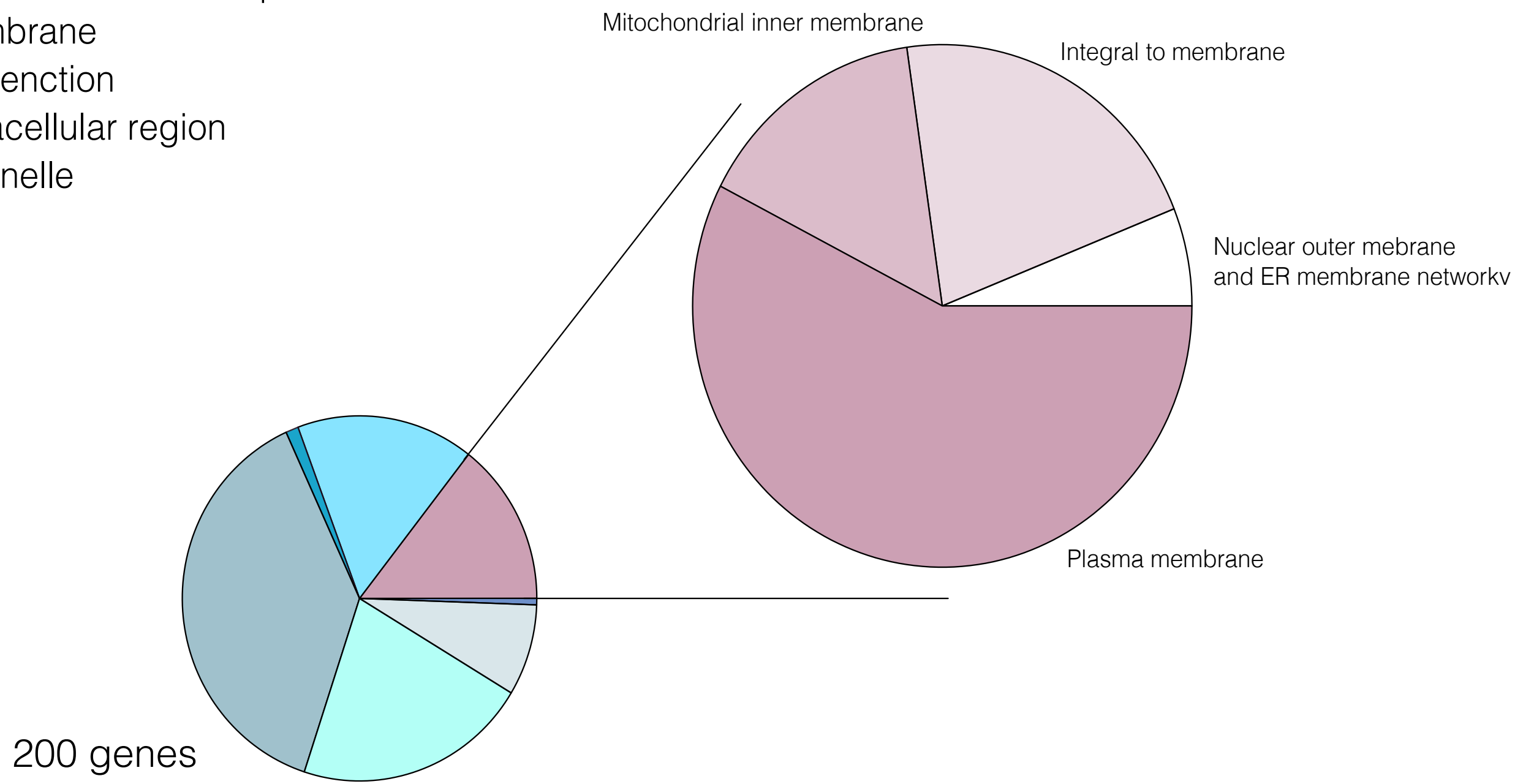


Pseudotime 0 5 10 15 20 25

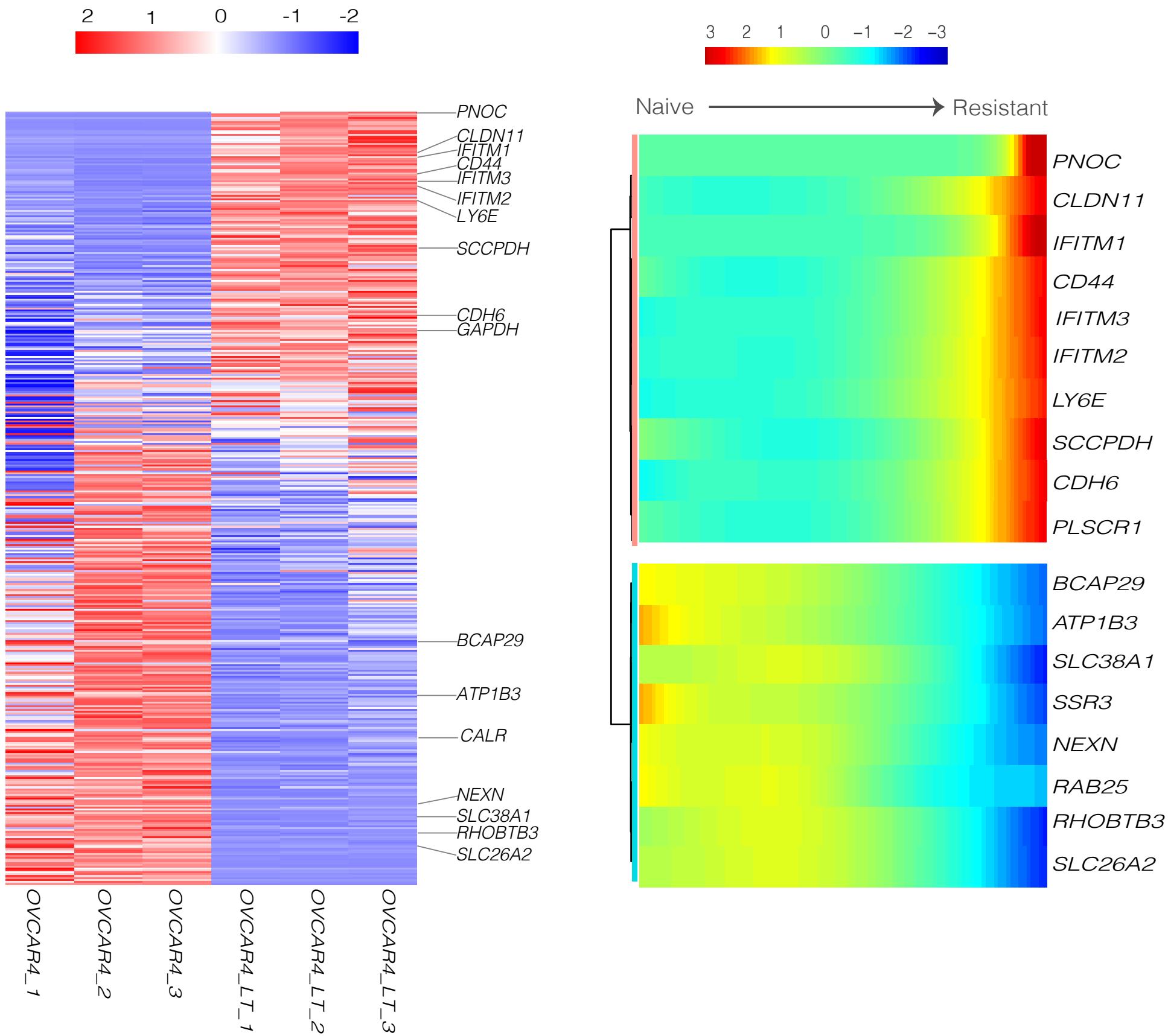


# GEO CLASSIFICATION IN THE PANTHER SHOWS THE CELLULAR LOCATION OF THE GENES OF THE INTEREST.

- cell part
- extracellular matrix
- macromolecular complex
- membrane
- cell jention
- extracellular region
- organelle

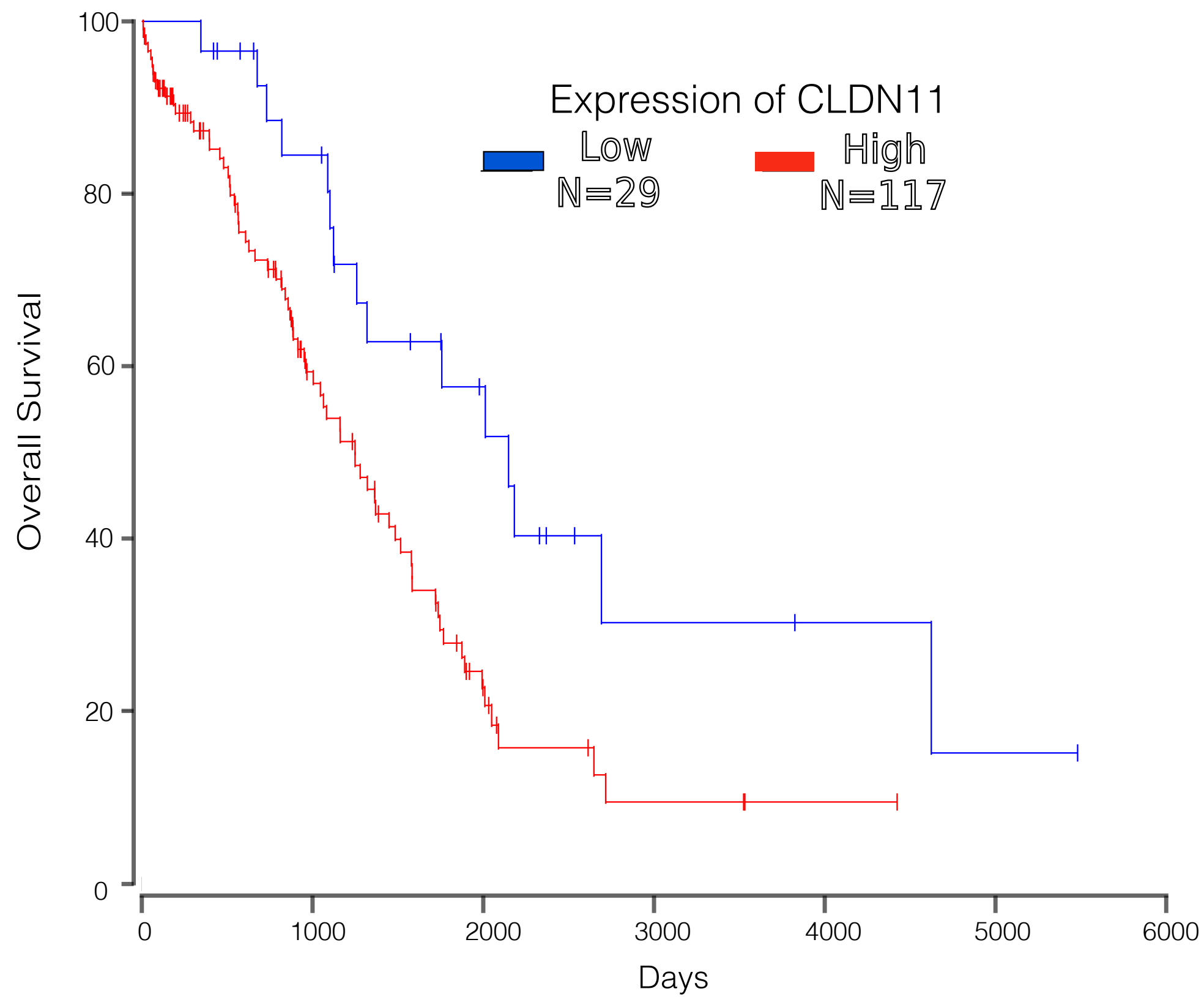


The cell surface proteins are constitutively upregulated in the bulk RNA seq data

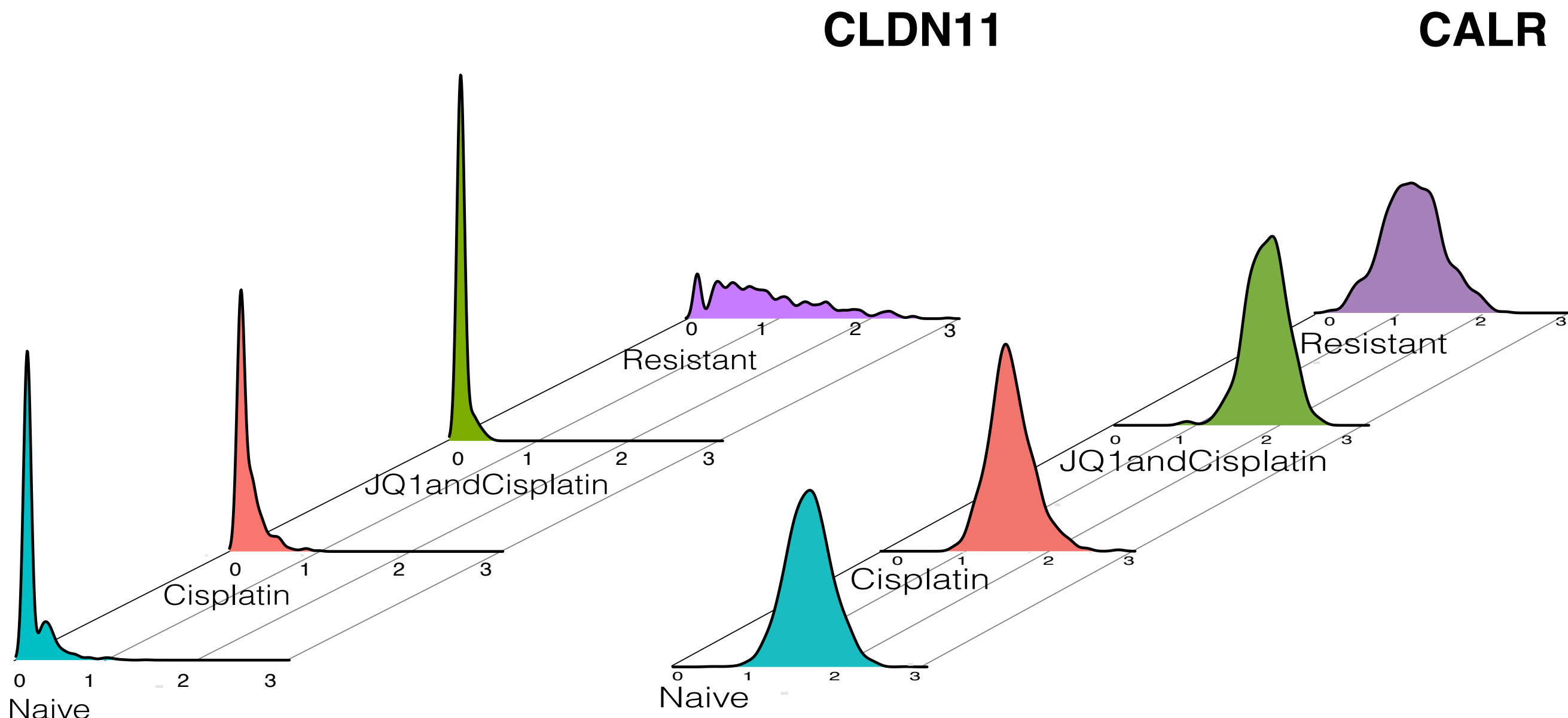




Provisional data from TCGA shows when patients that express CLDN11 at more than -0.3—  
have significantly less chances to survive.

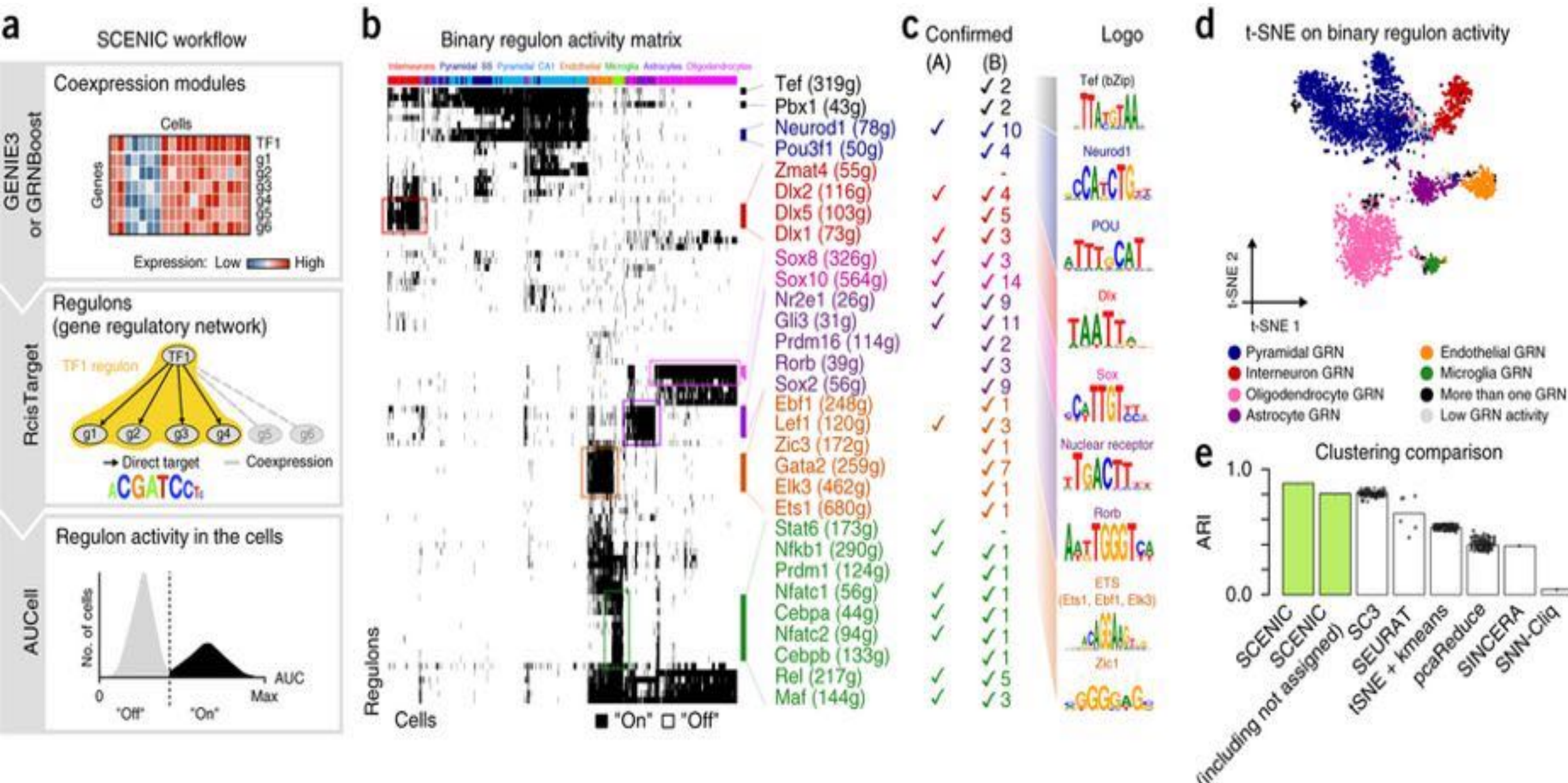


# The relative expression of CLDN11 and CALR across the samples



# SCENIC

Powerful tool that allows to infer gene regulatory networks (GRN) and identify cell states.



## Objective and key results (OKR):

- Do the SCENIC analysis
- Order antibodies and make the flow cytometry experiment to sort the cells out.
- If we can distinguish the Naive cells with resistant gene expression profiles we can test them for the resistance







You all are great scientists. It was a great chance to learn from you...Thank you...



# References:

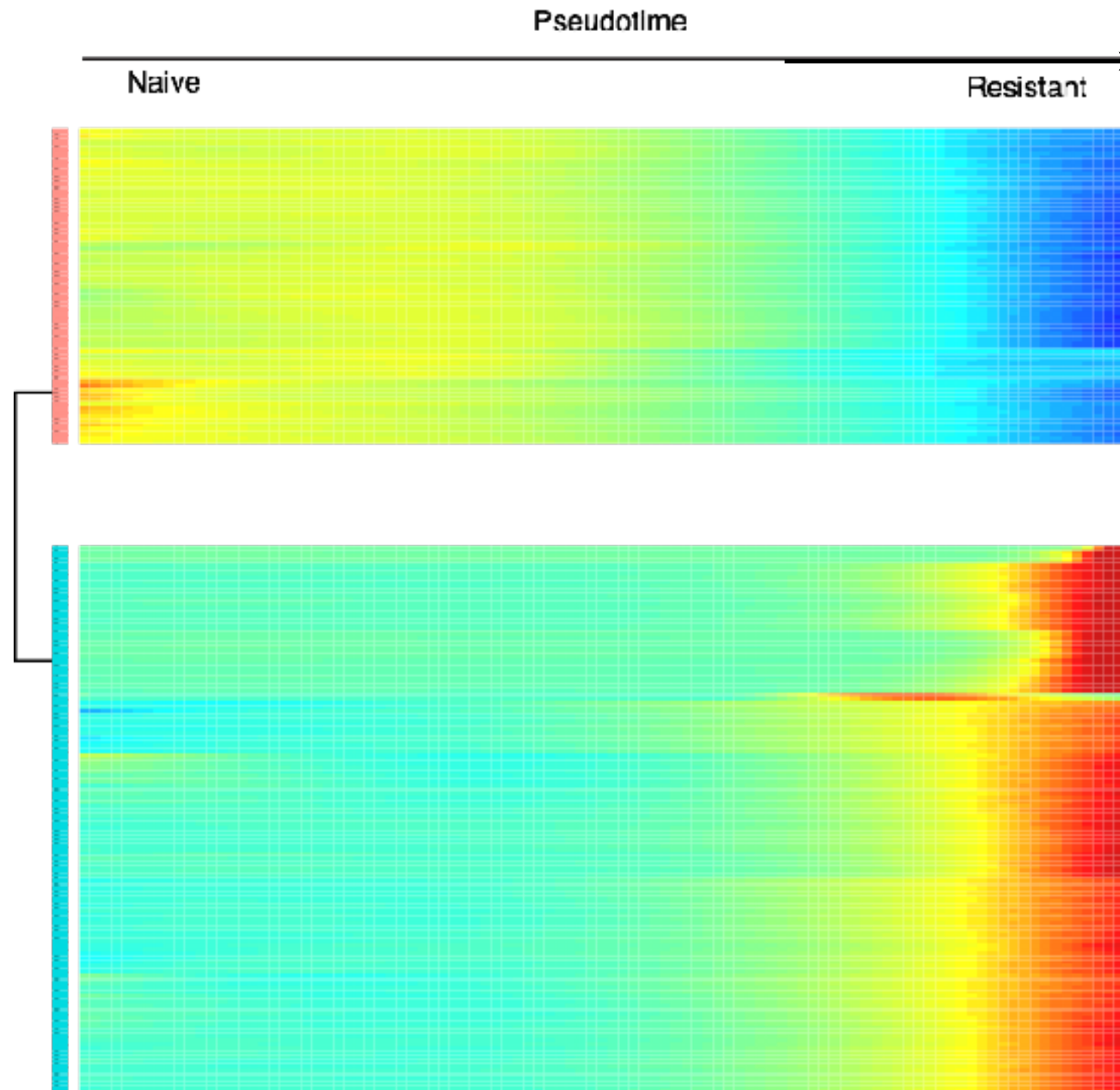
---

SCENIC: single-cell regulatory network inference and clustering

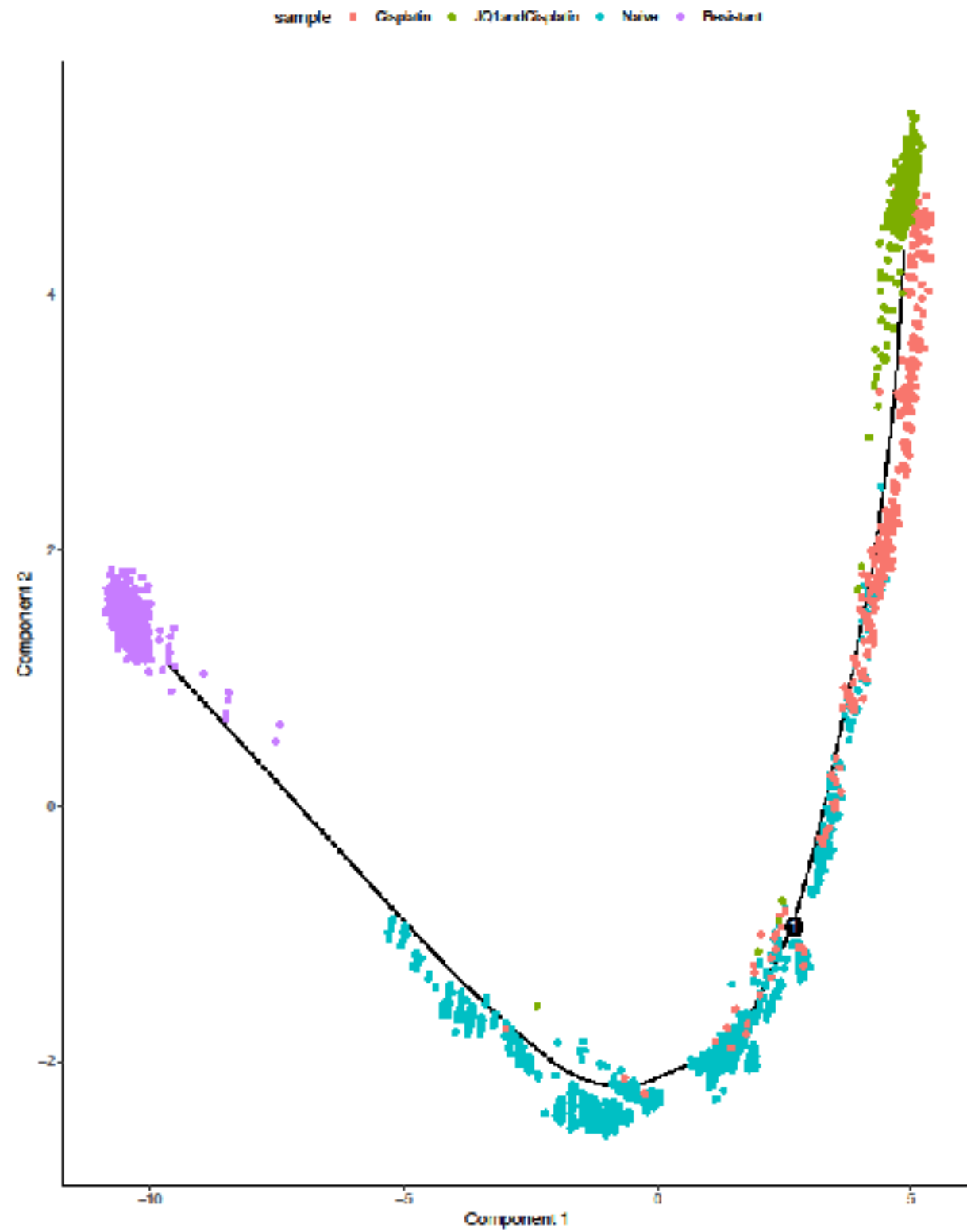
Sara Aibar, Carmen Bravo González-Blas, Thomas Moerman, Vân Anh Huynh-Thu, Hana Imrichova, Gert Hulselmans, Florian Rambow, Jean-Christophe Marine, Pierre Geurts, Jan Aerts, Joost van den Oord, Zeynep Kalender Atak, Jasper Wouters & Stein Aerts

Nature Methods volume 14, pages 1083–1086 (2017)

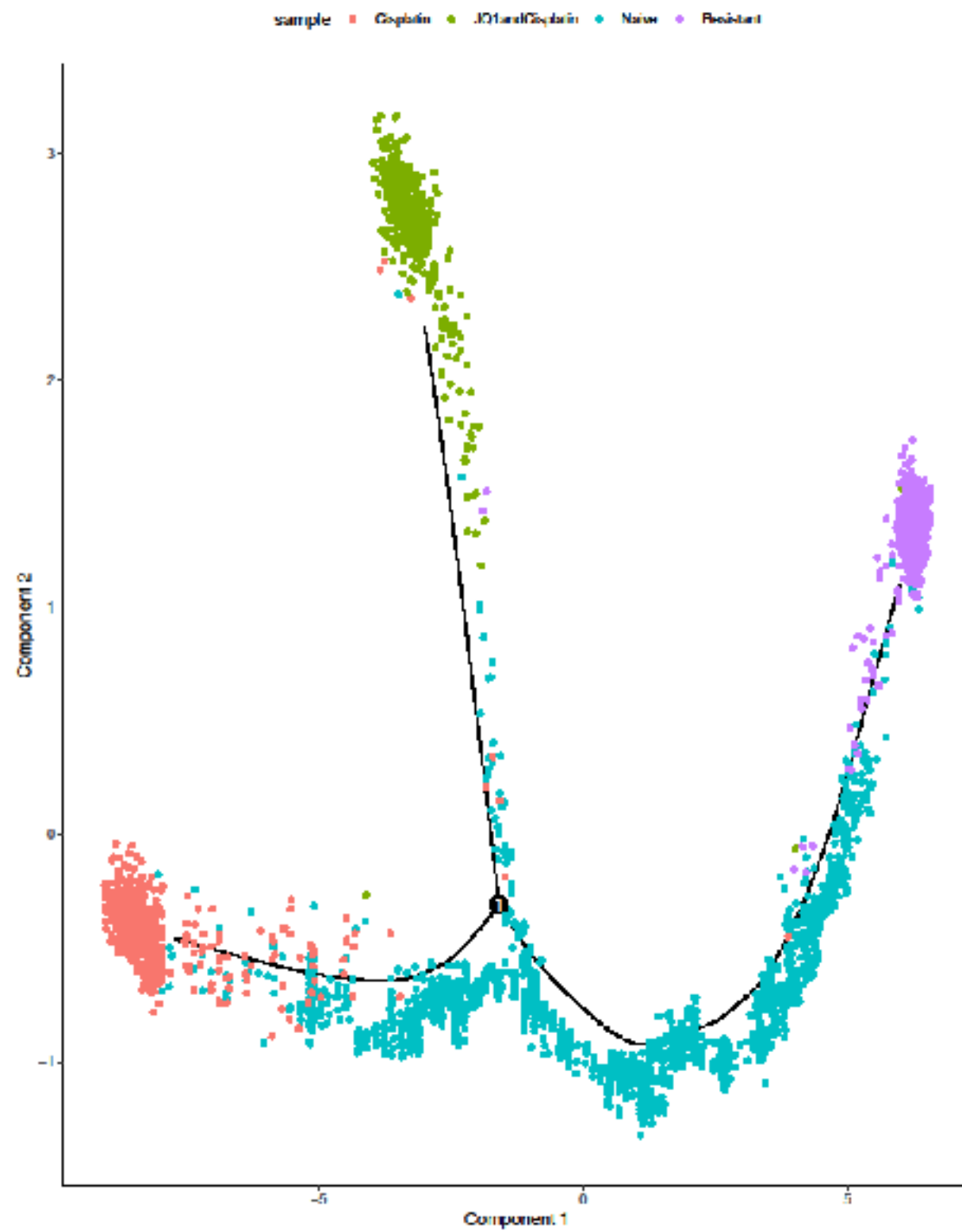
Proper number of the genes were selected by limiting the FDR to 0.



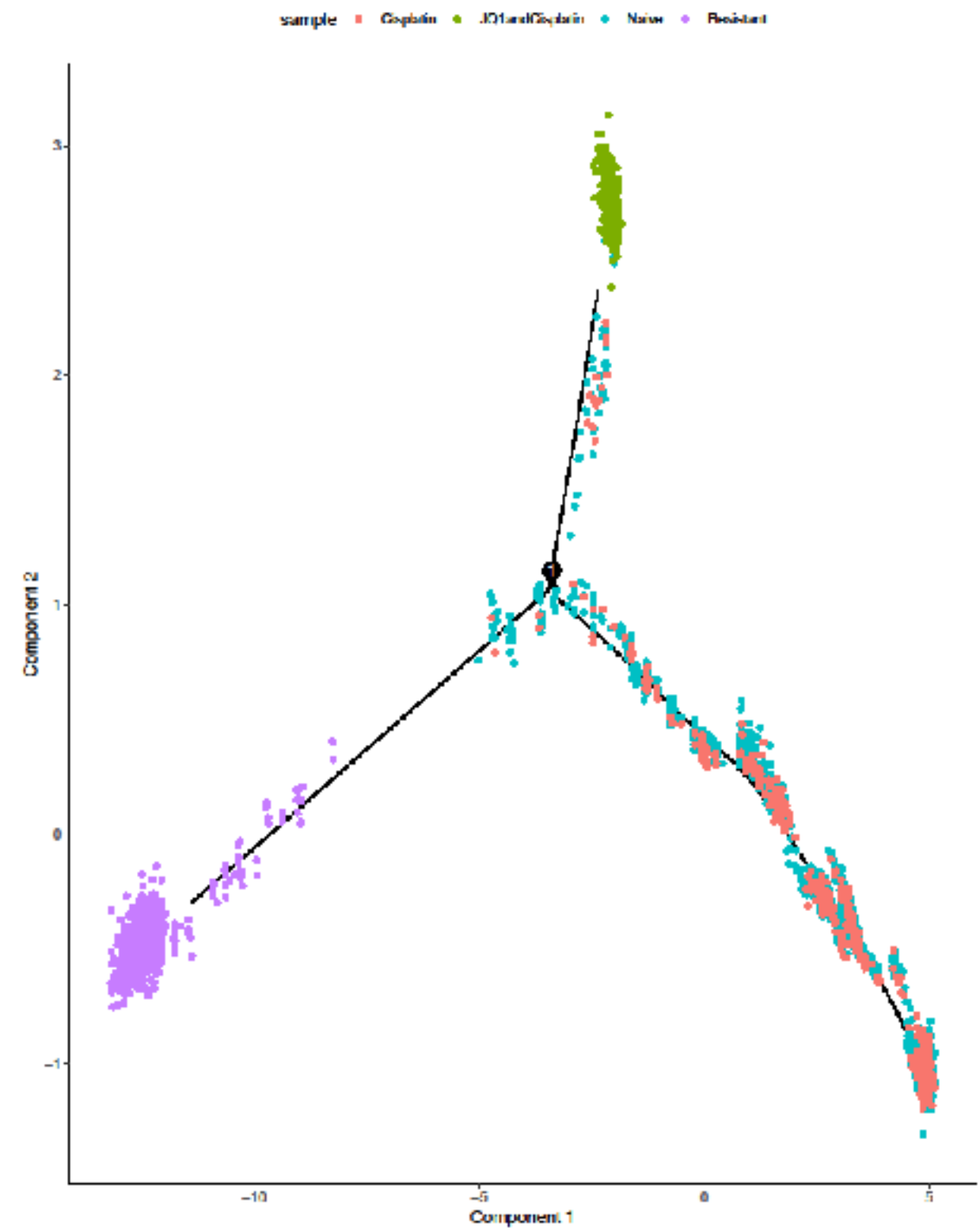
## All genes



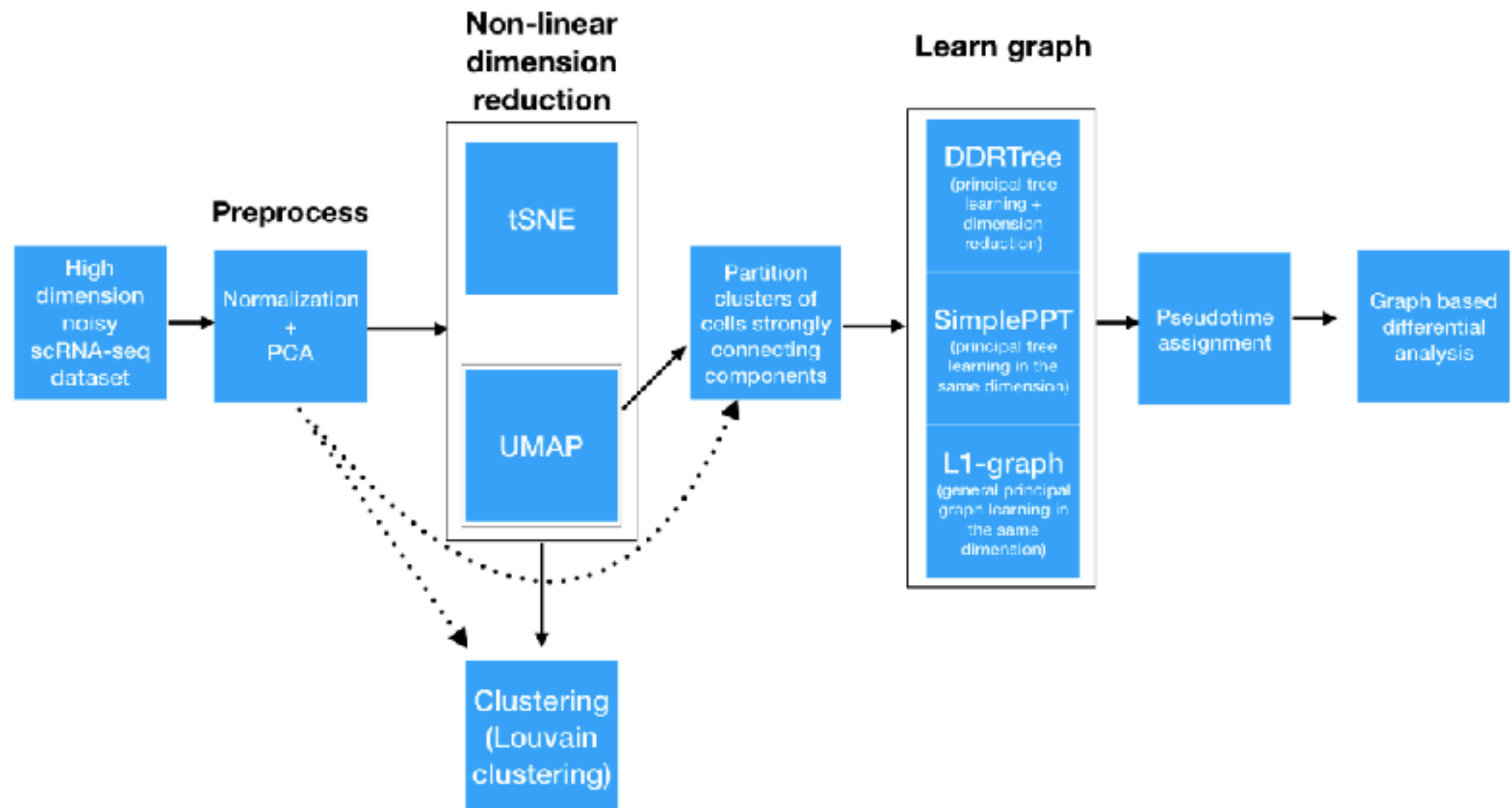
CN genes



NR genes



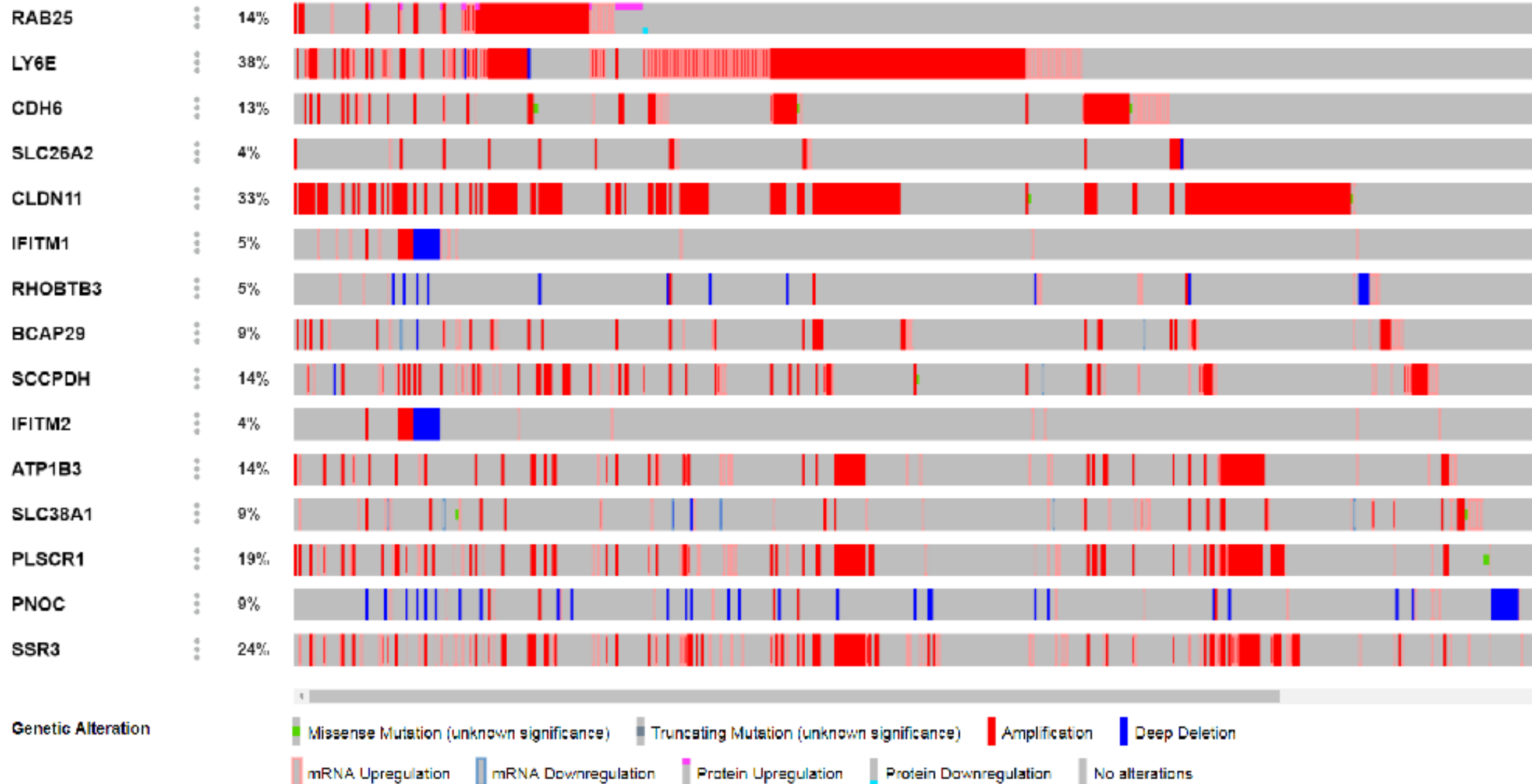
# The Monocle 3 workflow





# cBIOportal analysis – finding the variation in the patient survival data

Altered in 467 (79%) of 594 sequenced cases/patients (594 total)



## Dimensionality reduction algorithms in Monocle 2

- DDRTree
- SimplePTT
- tSNE

## Dimensionality reduction algorithm in Monocle 3

- UMAP -> does not allow alignment of different supergroups on the same trajectory

