University of Pisa - Computational Health Lab 2023

Project presentation:
A Single Cell data analysis for Chron's Disease with COTAN

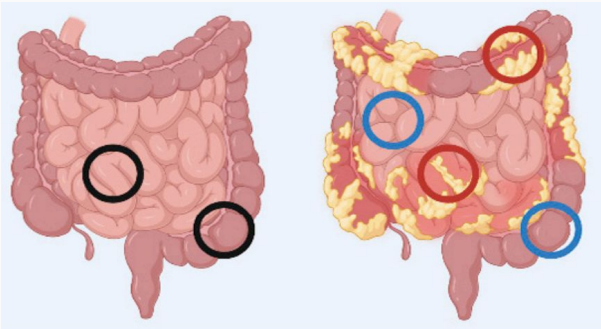
Ninniri Matteo (student ID: 543873)

Piras Andrea (student ID: 619640)

Outline

- Introduction to Chron’s Disease
- Datasets summary
- Data preprocessing with COTAN
- Clustering & making sense of it
- Gene set enrichment analysis
- Finding new markers
- Single patient analysis

Introduction to Chron’s disease

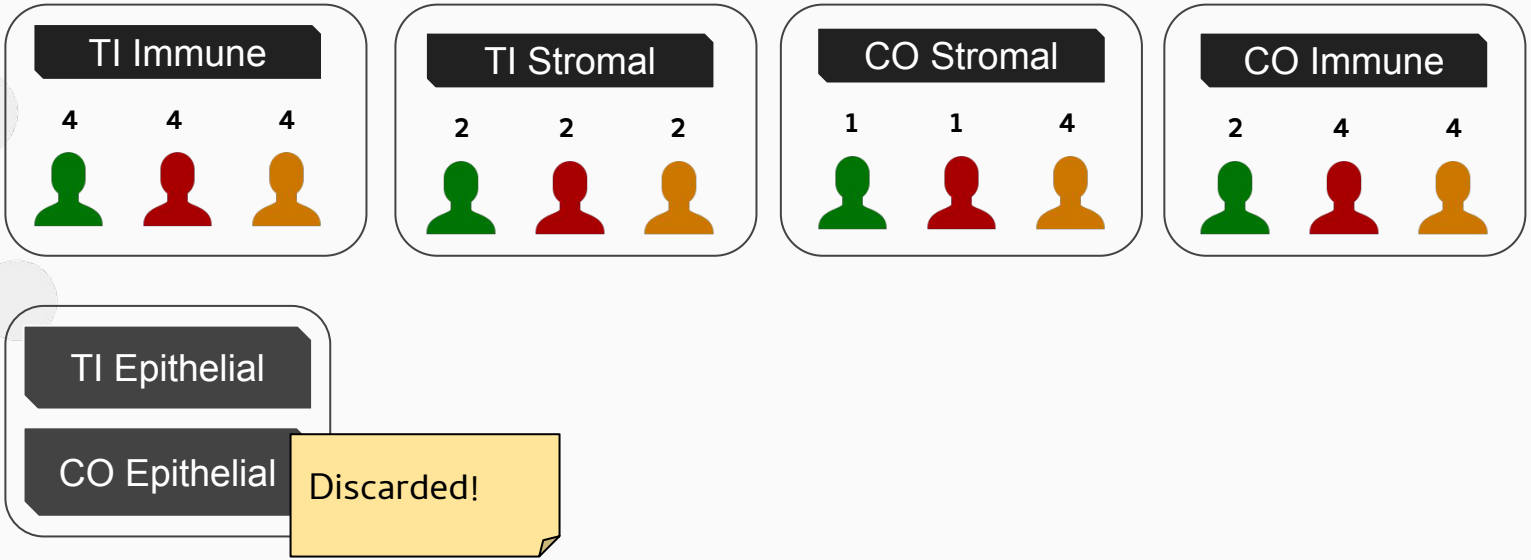


- Healthy
- Not inflamed
- Inflamed

- Chronic gastrointestinal autoimmune disease
- Understand the causes is very complex
- Single-cell RNA sequencing

Datasets summary

- 71 donors
- 25 Healthy
- 46 With the disease
- ~720.000 cells total
- Only samples with > 1000 cells

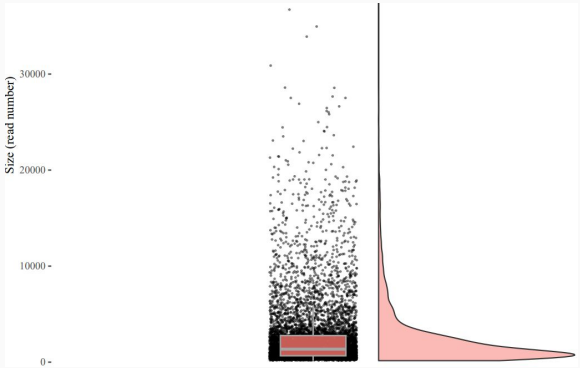


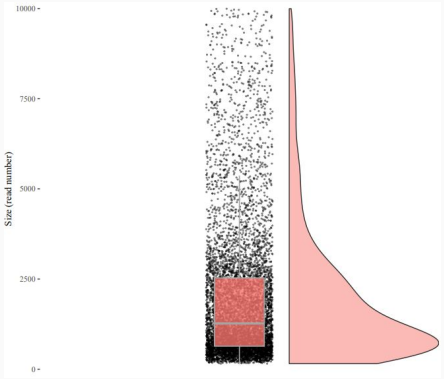
Data preprocessing with COTAN- Cells and genes

Original

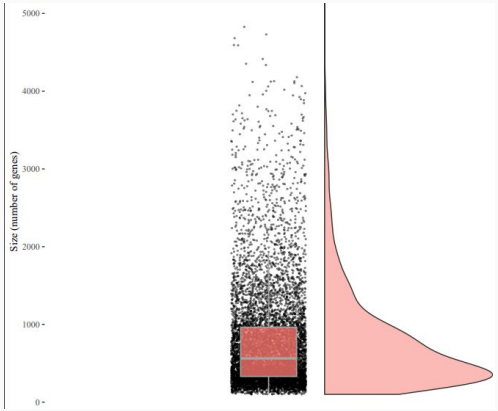
After cut

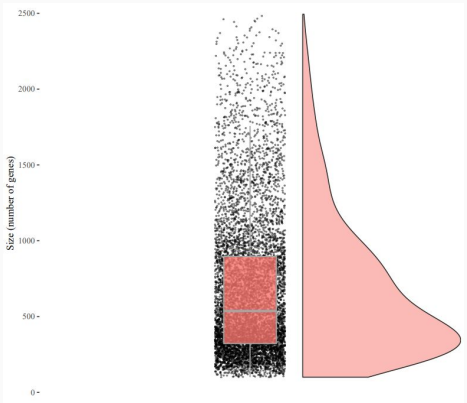
Cell count





Gene count



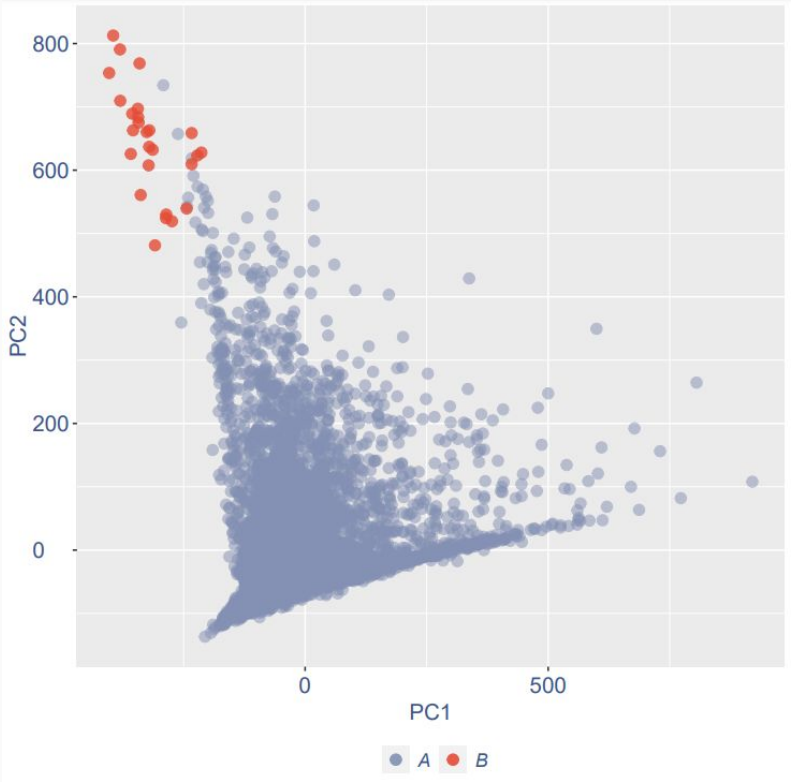


Patient: TI Immune I104689

Data preprocessing - Cluster B

Original

Cluster B removal

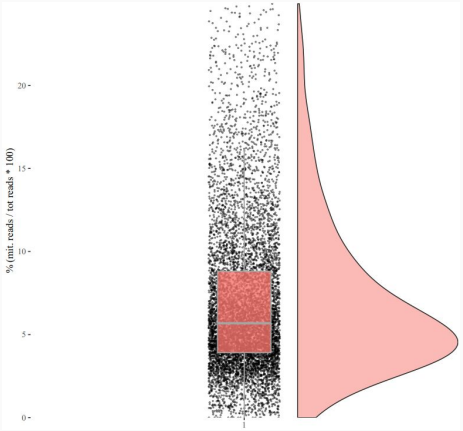


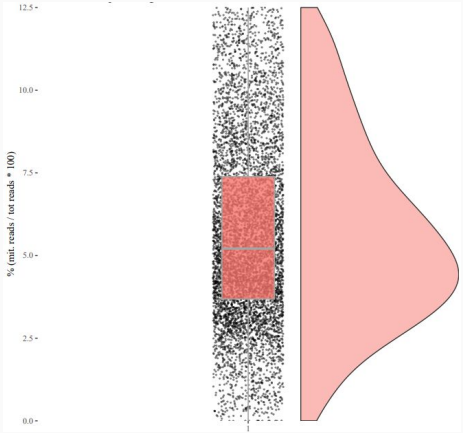
Data preprocessing - Mitochondrial and UDE

Original

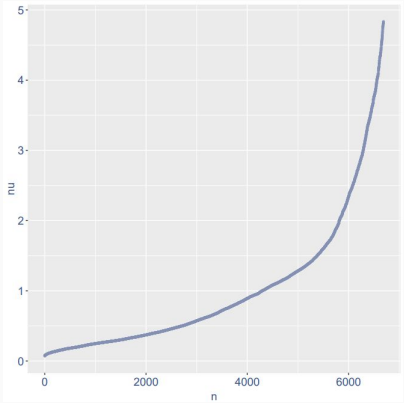
After cut

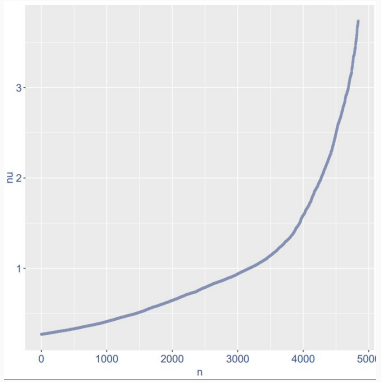
Mitochondrial percentage





UDE (UMI efficiency detection)



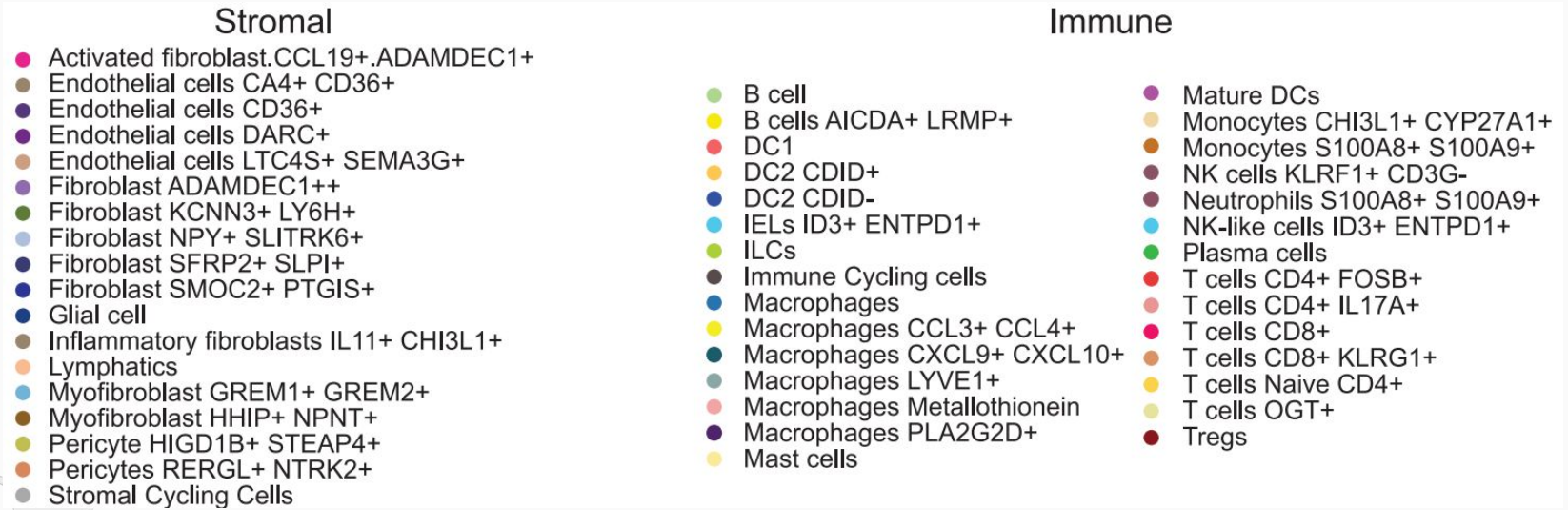


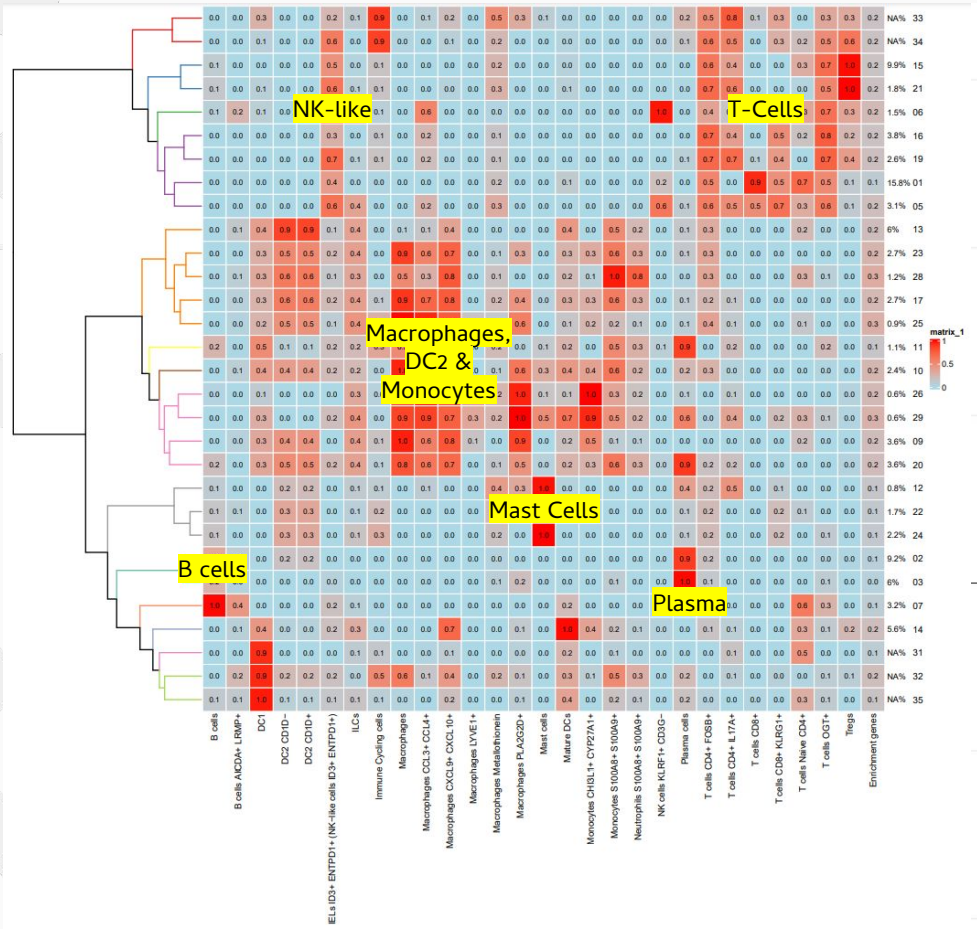
Patient: TI Immune I104689

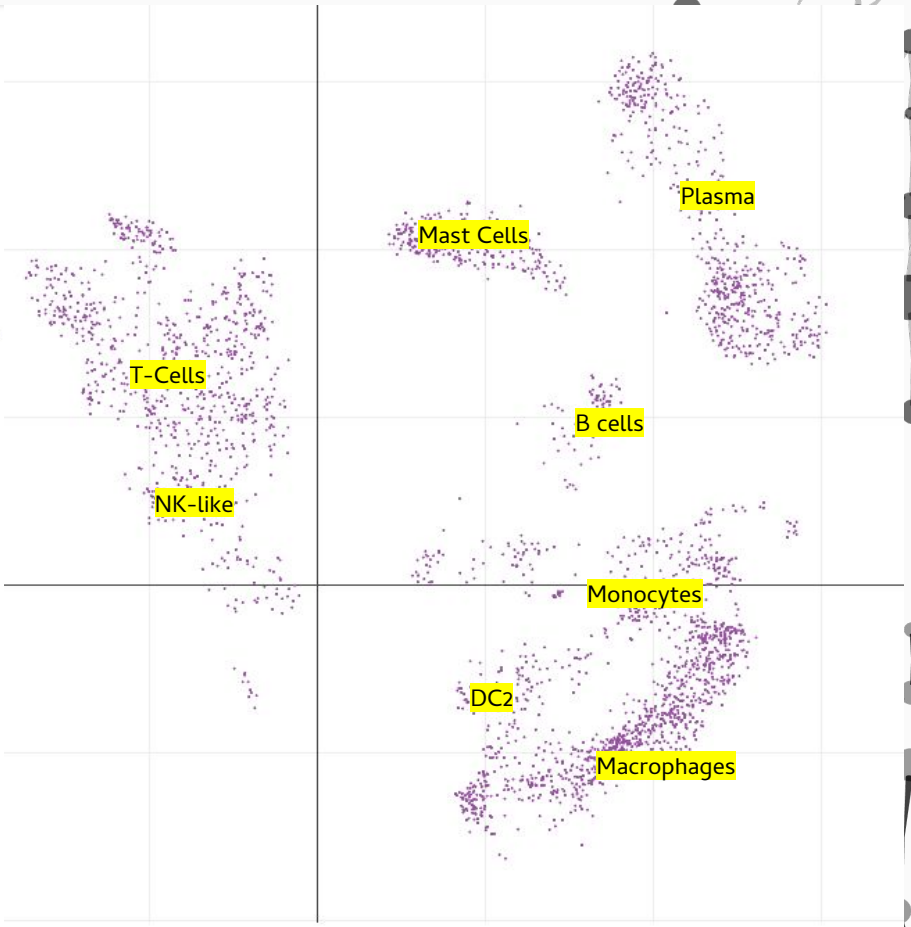
Making sense of the cell clusters

Two approaches:

- Literature search
- Use well known cell types

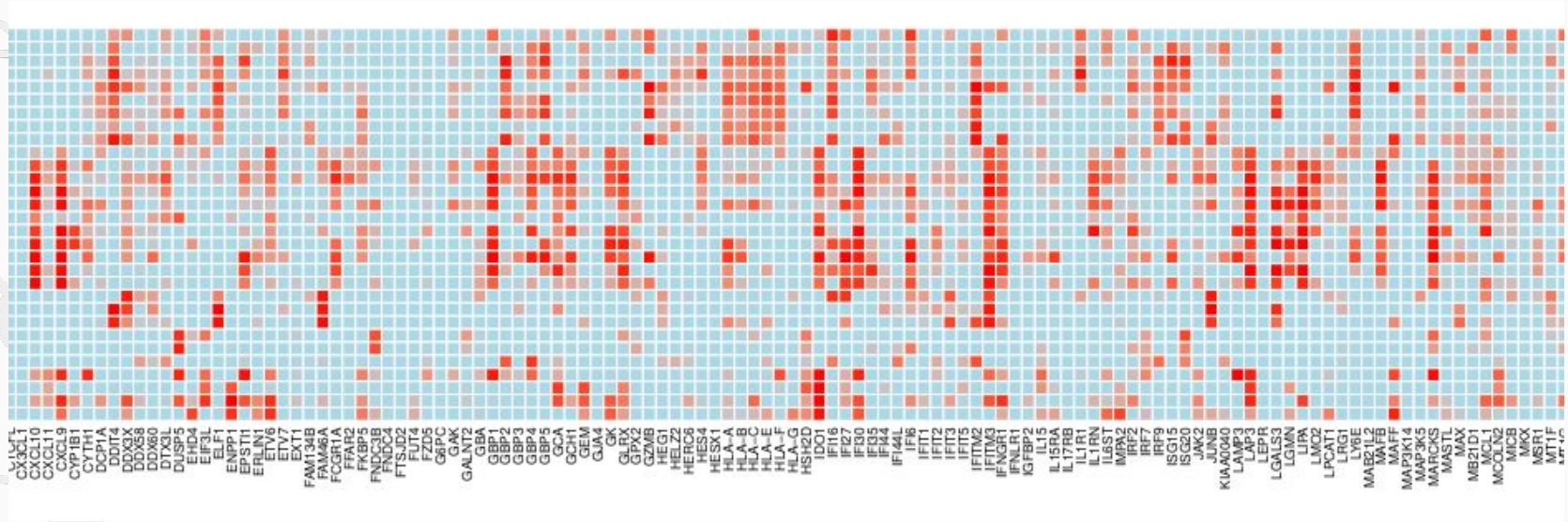






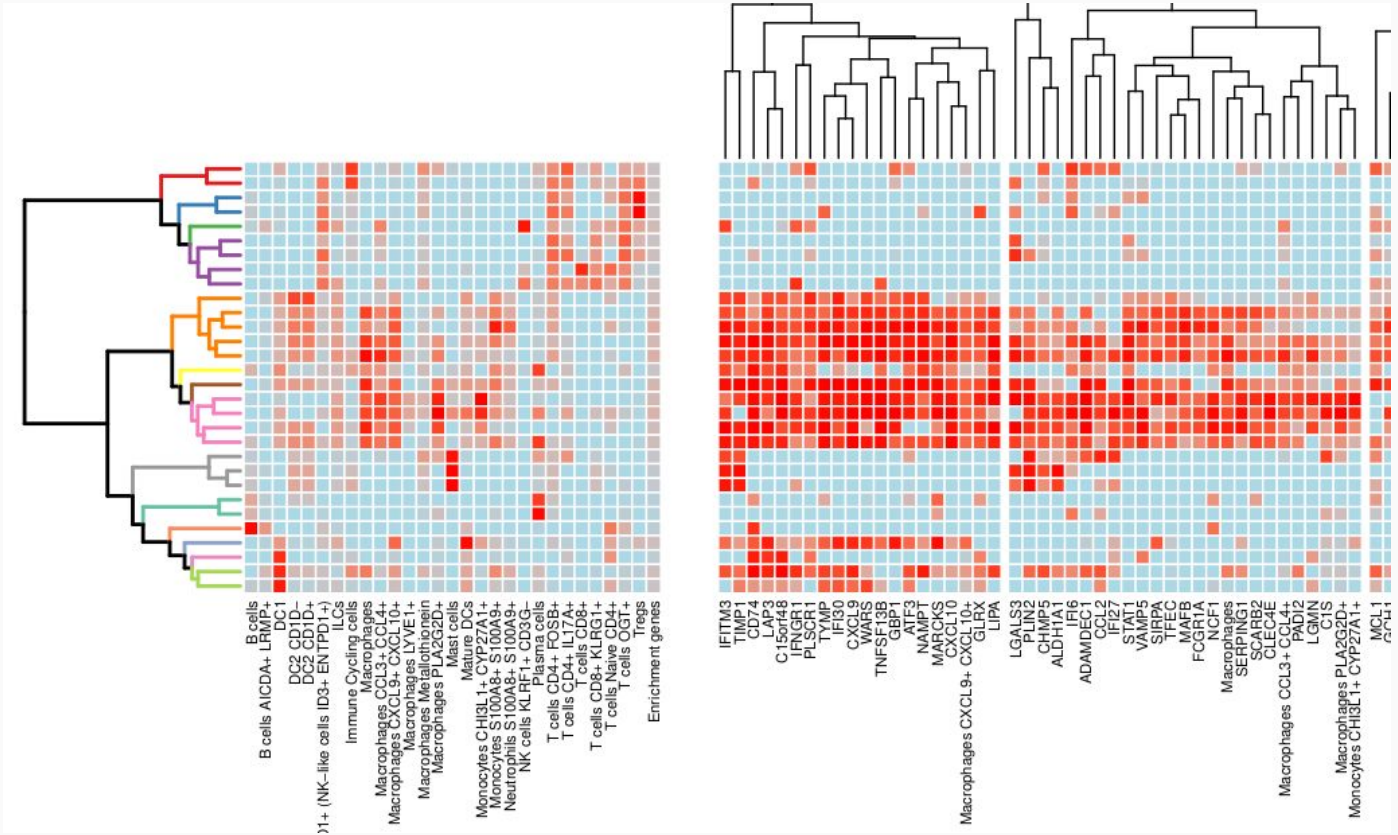
Patient: TI Immune l104689

Gene Enrichment



Patient: TI Immune I104689

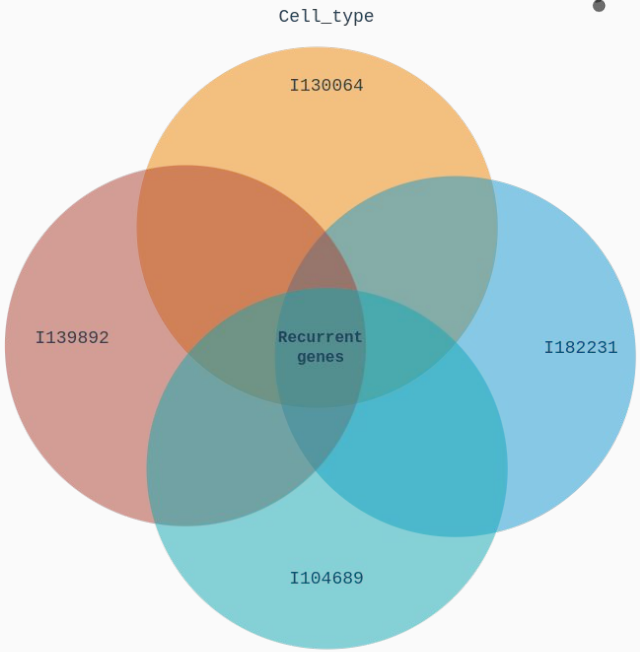
Gene Enrichment



Patient: TI Immune I104689

How to spot new disease markers?

1. Get multiple patients
2. Clusterize their enrichment
3. For each cell type:
    a. Intersect the cluster where they appear



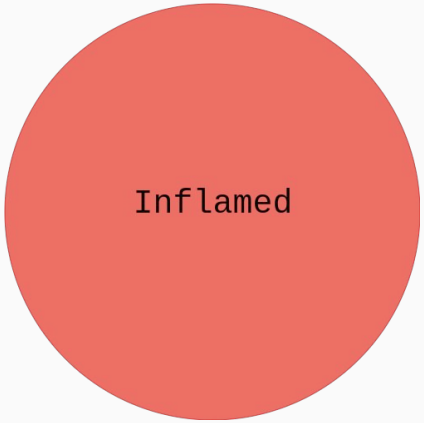
To obtain the genes exclusive to a condition, just subtract the union of the other sets from it

- Healthy markers = Healthy / (Not infl. ∪ Infl.)
- Not infl. markers = Not infl. / (Healthy. ∪ Infl.)
- Infl. markers = Infl. / (Healthy ∪ Infl.)

Results

Terminal Ileum Immune dataset: Inflamed samples

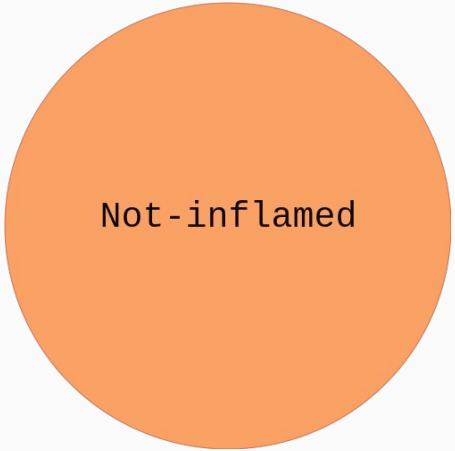
- IELs ID3+ ENTPD1+
  - ANXA2R
- Monocytes S100A8+ S100A9+
  - THBD
- Neutrophils S100A8+ S100A9+
  - TCF7L2
- Plasma cells
  - CD38, COMMD3
- T cells CD4+ FOSB+ & T cells OGT+
  - SOCS1;SLFN5;RARRES3



Results

Terminal Ileum Immune dataset: Non-inflamed samples

- B cells
  - MX1, HSH2D, AIM2, STAP1
- B cells AICDA+ LRMP+
  - MX1, HSH2D, AIM2
- DC1
  - LAMP3
- DC2 CD1D- & DC2 CD1D+
  - PLSCR1

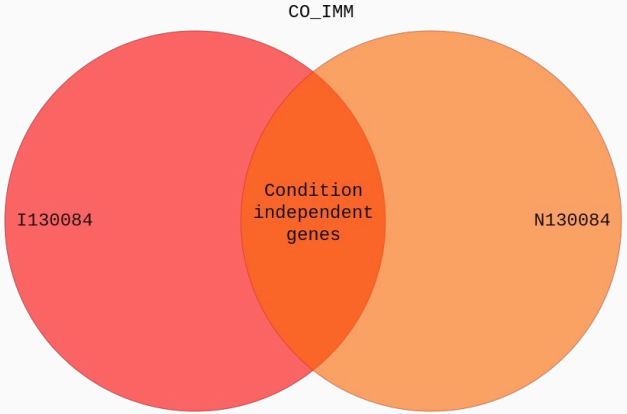


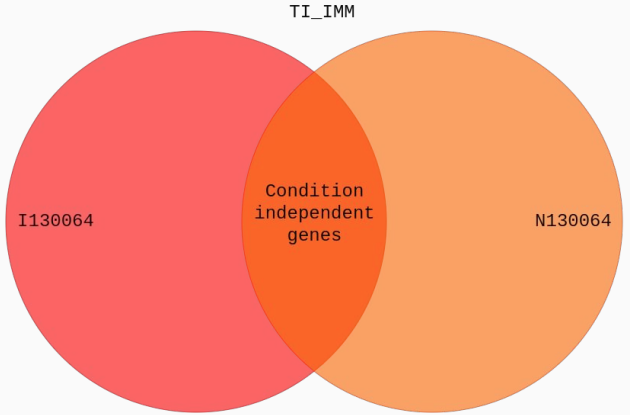
Results

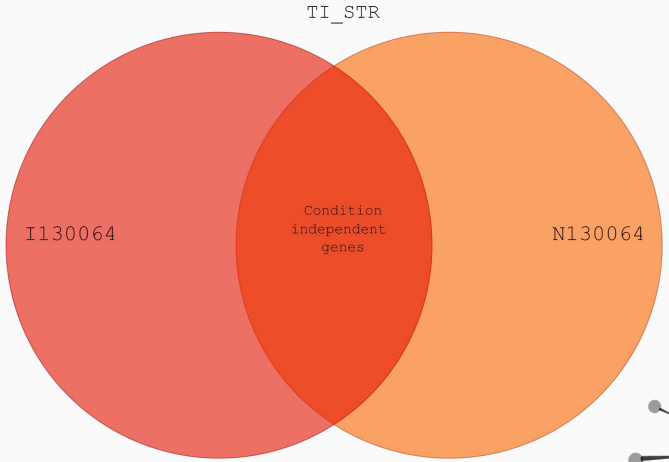
Terminal Ileum Immune dataset: healthy controls

- B cells & B cells AICDA+ LRMP+
  - NCF1, PXK
- Macrophages CCL3+ CCL4+
  - LIPA
- Macrophages CXCL9+ CXCL10+
  - CASP7
- Macrophages LYVE1+
  - FCGR1A
- Monocytes CHI3L1+ CYP27A1+
  - NDC80, HELZ2, IL15RA STARD5, SLC16A1, PI4K2B
- T cells CD8+ & T cells CD8+ KLRG1+:
  - CCL5, GZMBCCL5, GZMB
- T cells Naive CD4+
  - SUN2, SOCS1, SLFN5, FAM134B, ANXA2R
- Tregs
  - SUN2, SOCS1, SLFN5, FAM134B
- T cells CD4+ IL17A+
  - DDX58

Patient-specific markers







Future developments

- Better metrics for clustering
- More K-means iterations for the enrichment (column_km_repeats)

Thank you for the attention!

How to determine the optimal number of clusters?

1. Enumerate all the cluster combinations
2. For each combination:
    2.1. For each cell type:
        2.1.1. Get the clusters where the cell appears (one for each patient)
        2.1.2. Jaccard
    2.2. Average the scores
3. Optimal combination: the one with the highest average Jaccard