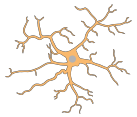
Which single-cell methods for which biological questions: strengths and weakness

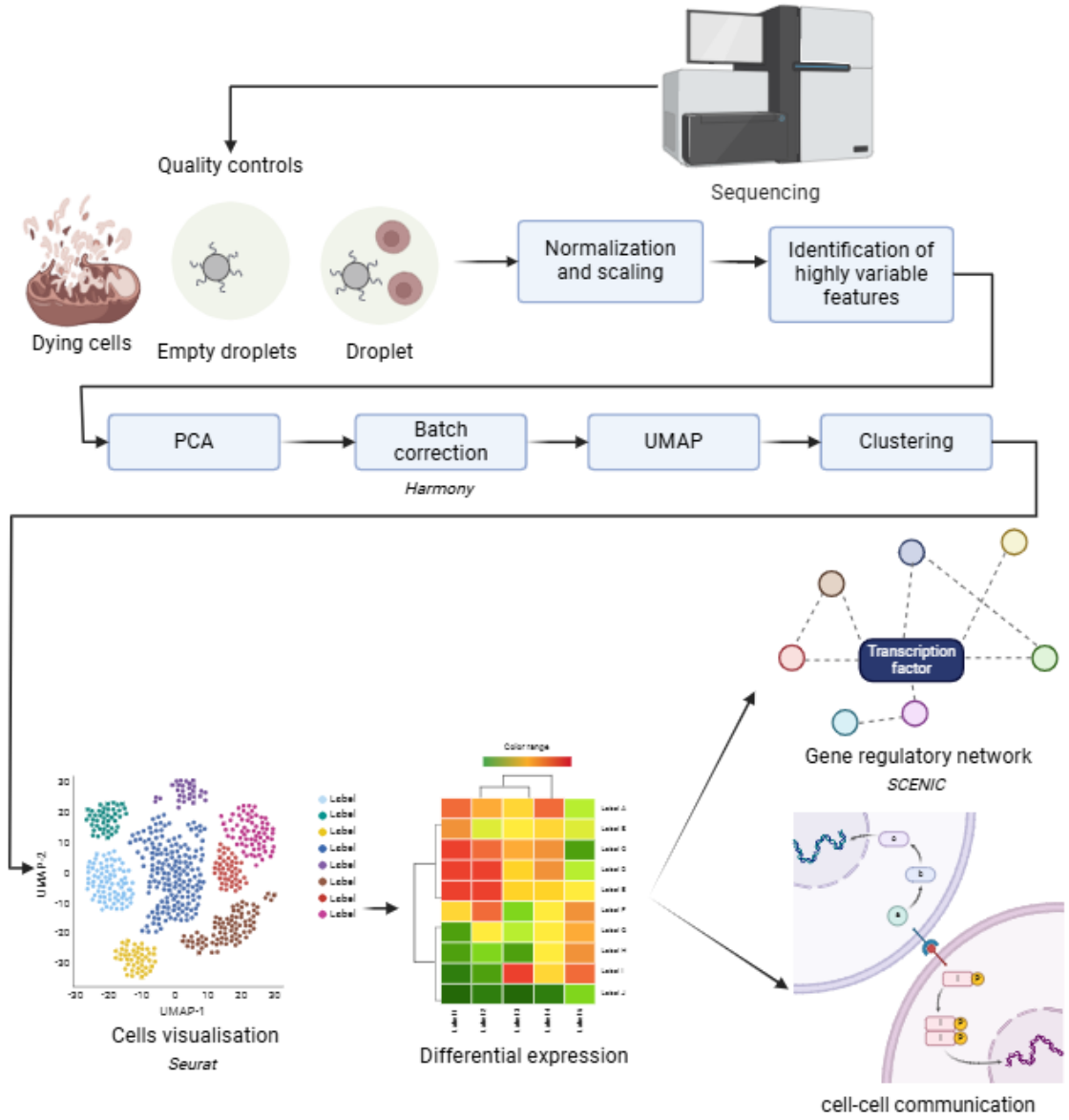
My journey



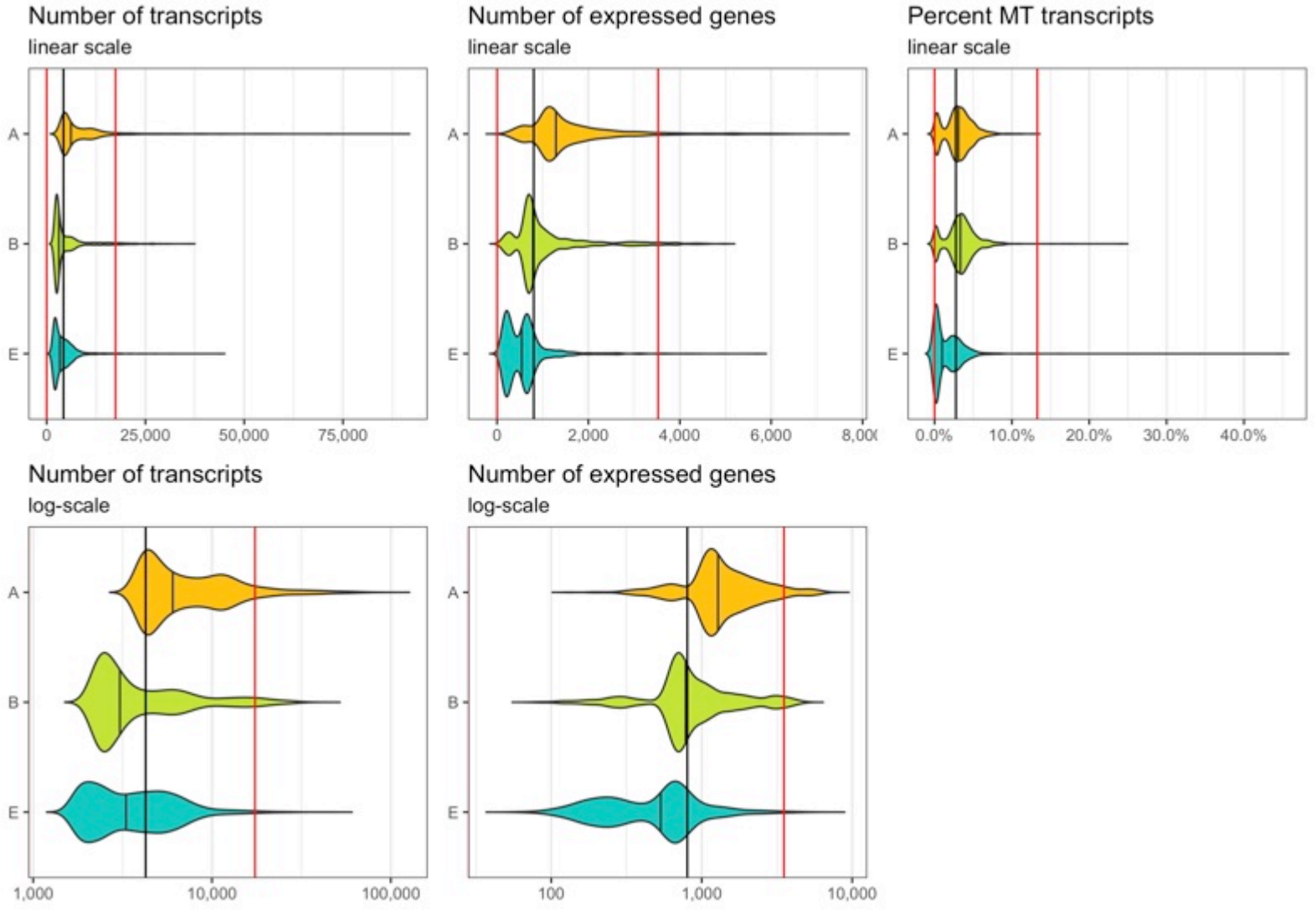


	scRNAseq	scMultiomics (RNAseq + ATACseq)	spatialRNAseq
Mice Microglia	X		
Pig embryo	X	X	
Pig stem cells		X	
Pig Melanoma	X		X
Mice Non neural brain cells	X		

Single-cell analysis workflow

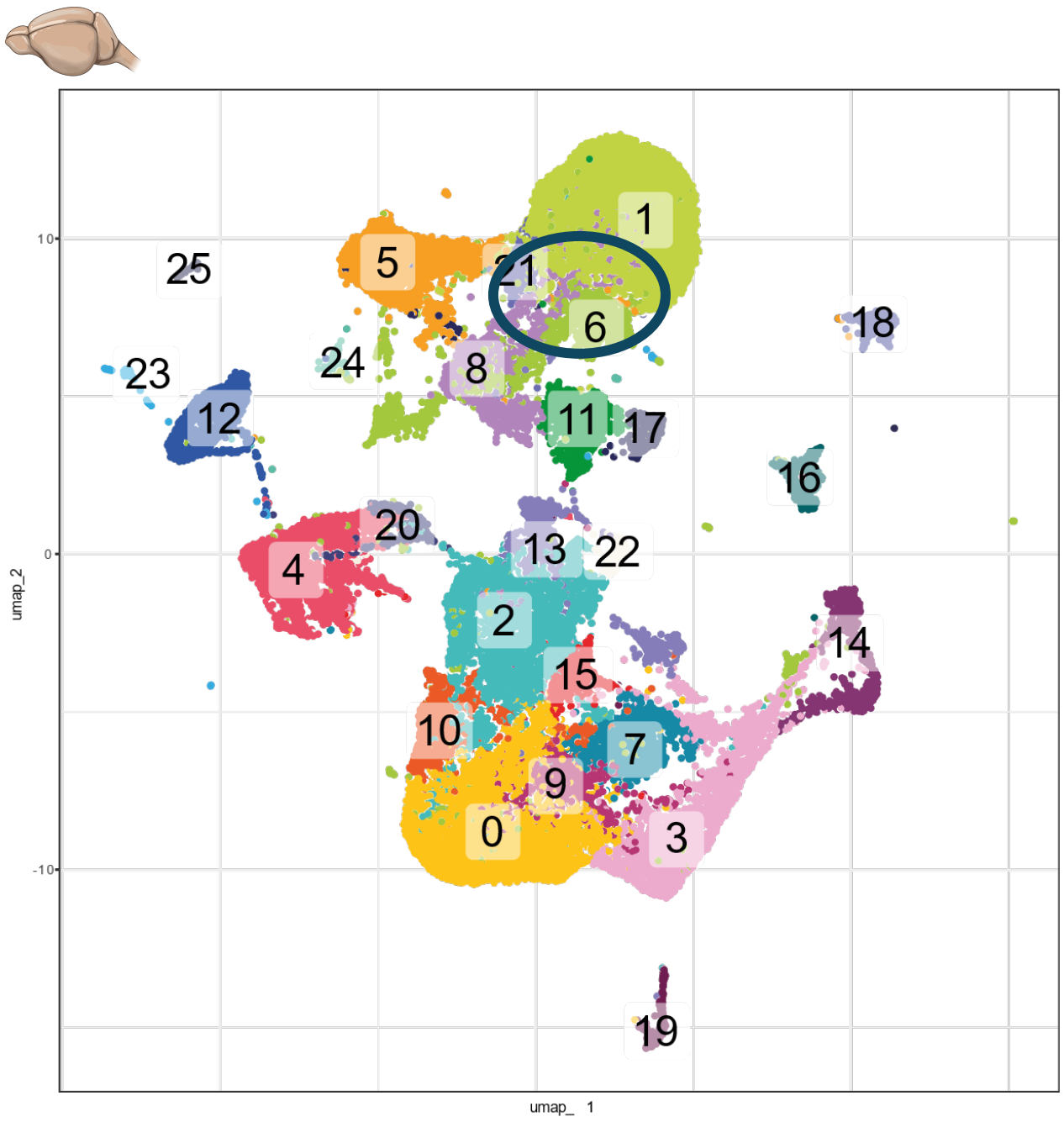


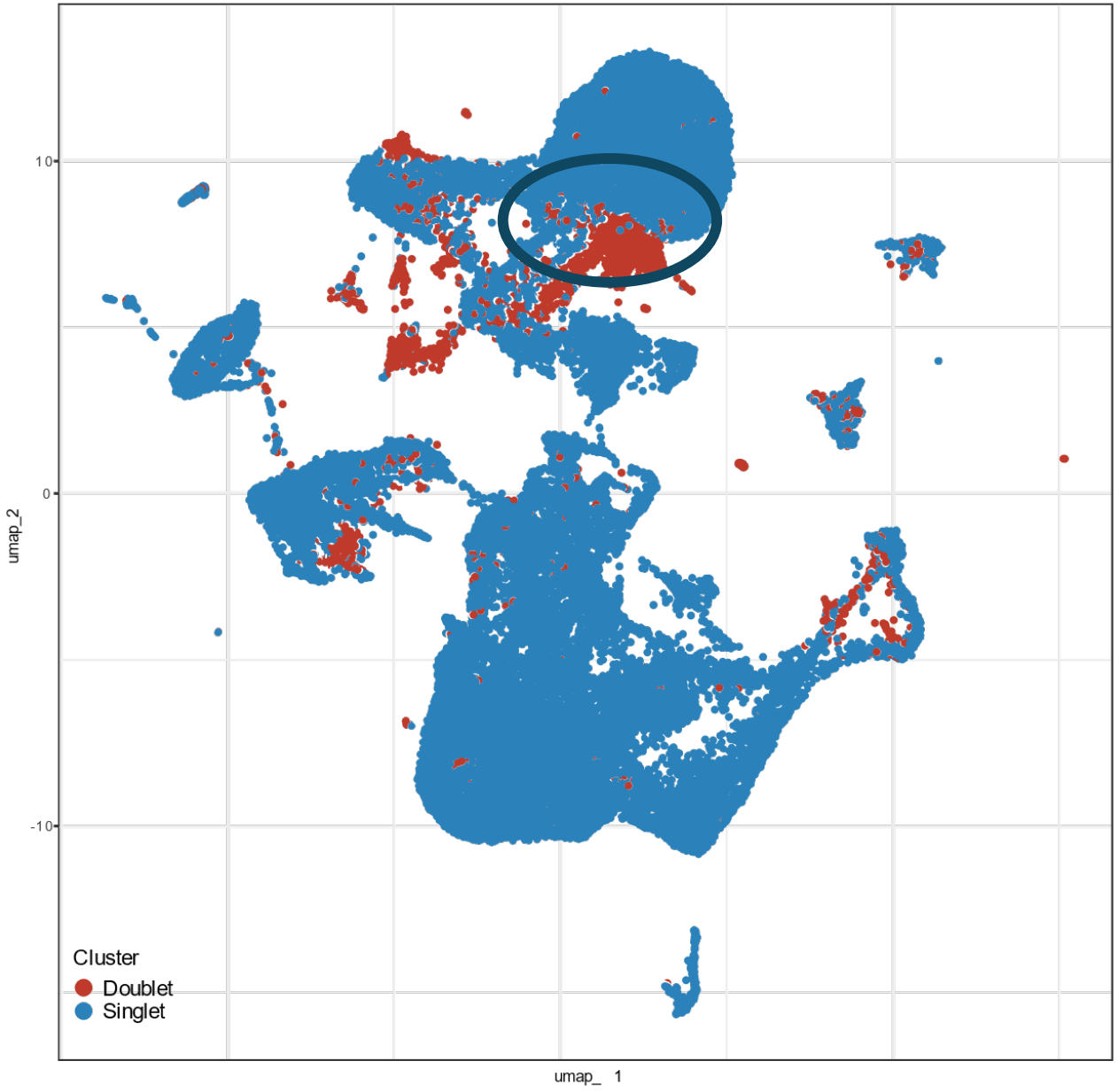
Filter for QC metrics



Doublet removal approach

- Cell properties and dissociation methods can increase doublet numbers
- The proportion of doublets differs from technology ex : InDrops, 10x, smartseq ...


1
5
21
6
25
18
24
8
23
12
11
17
16
20
13
22
4
2
14
15
10
7
9
0
3
19
10
0
-10
umap_2
umap_ 1

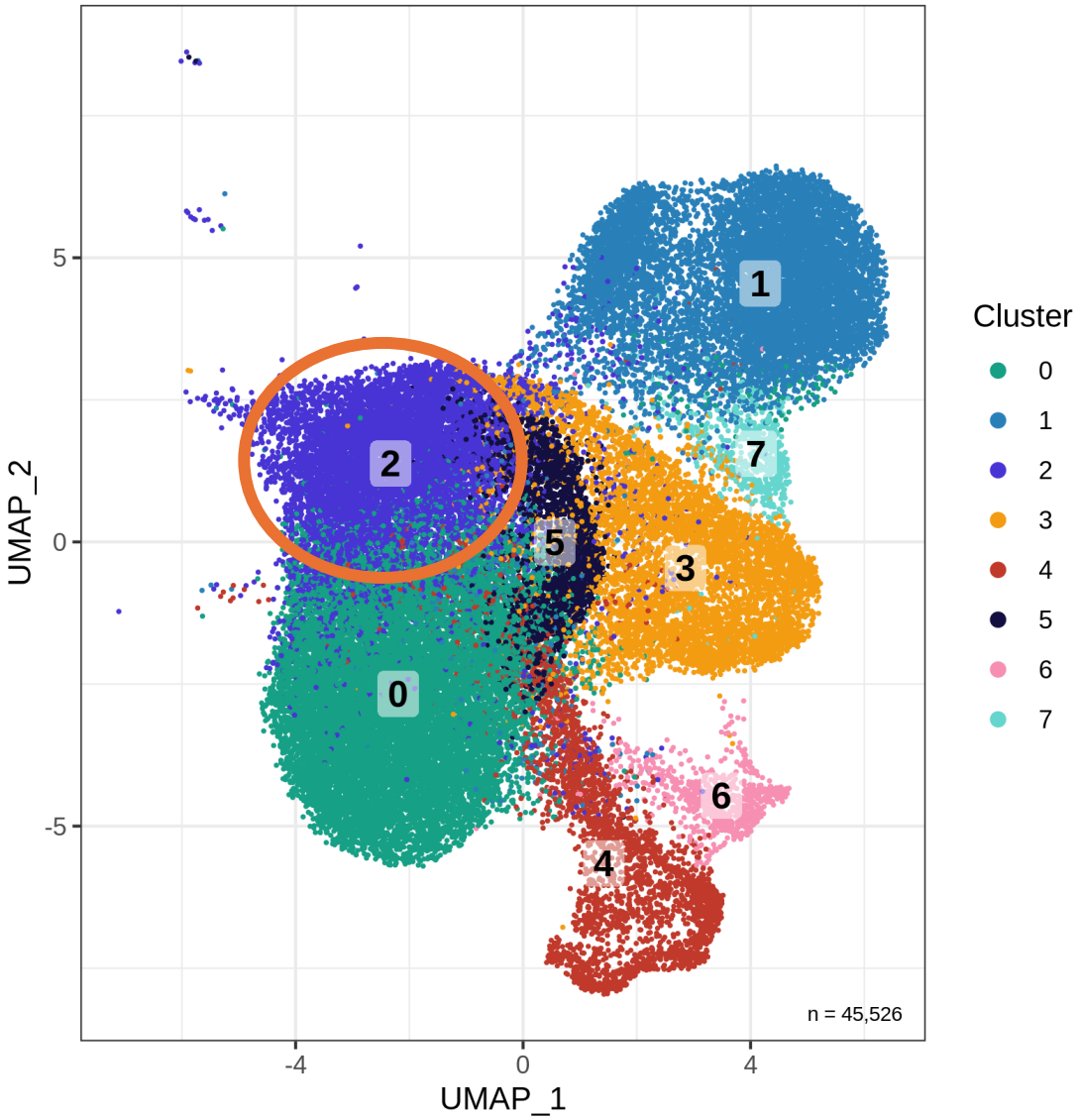

10
0
-10
umap_2
umap_ 1
Cluster
Doublet
Singlet

Normalization and gene selection

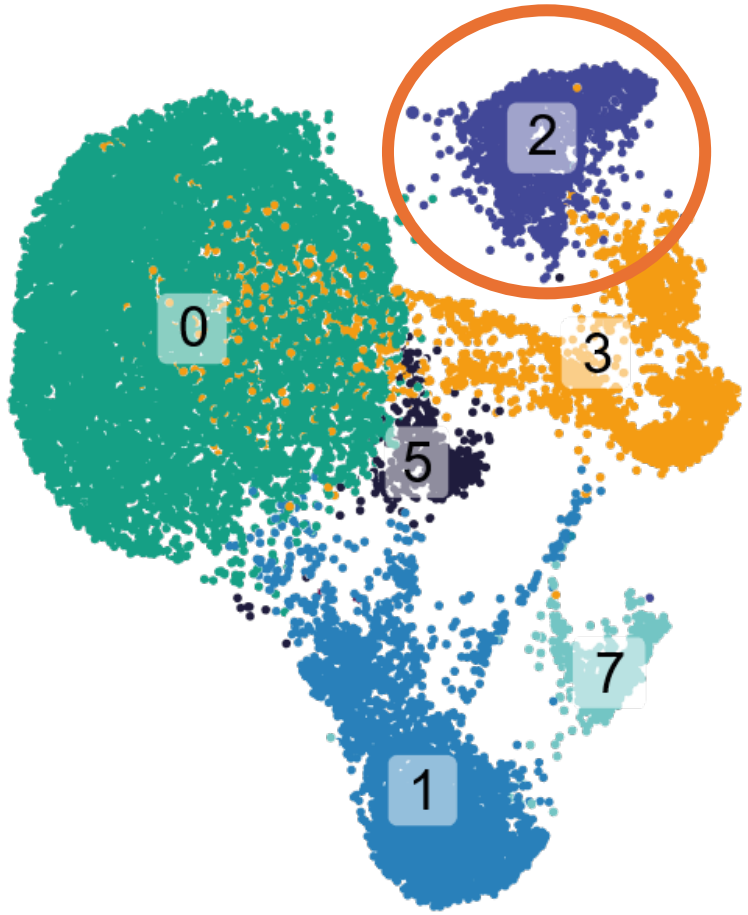
- Two main normalization methods : log normalization and SCTransform
- SCTransform is required for most methods in SpatialRNAseq

- Gene selection will differ depending on the diversity of cells (atlas vs FACS sorted)

Noise effect on UMAP and clusterisation



InDrops (more noise)



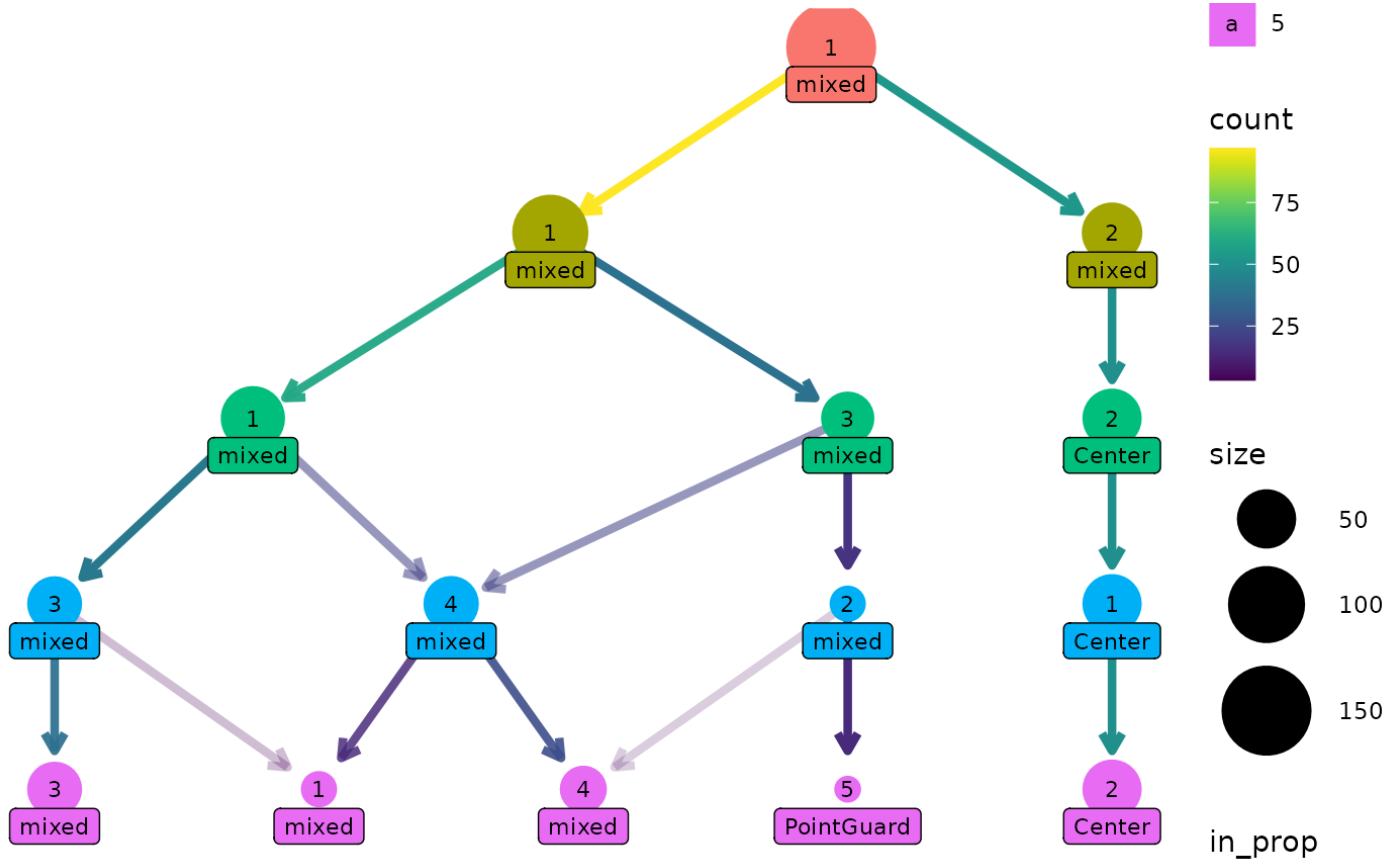
10x Flex (less noise)

Single-cell give bad proportion

- All cells don’t have the same survival rates throughout the experiment (ex : trophectoderm cells don’t like nuclei isolation)
- You can induce biases in tissue section (ex: melanoma)

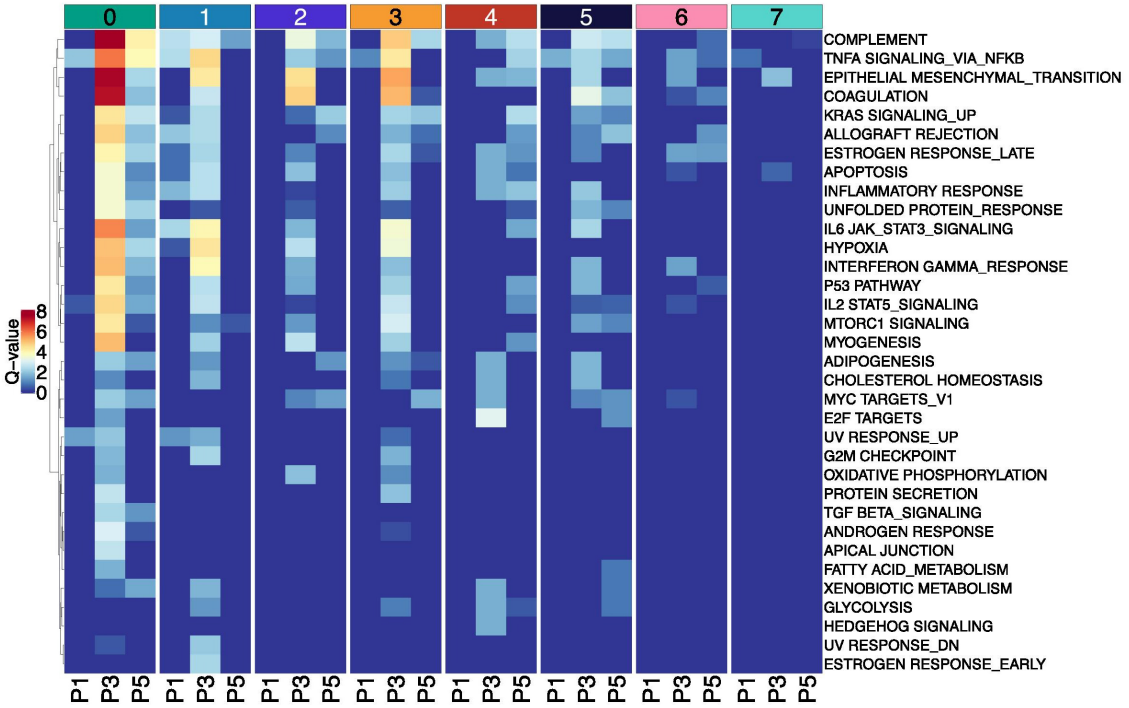
Clusterisation

- Depends on the levels of sub-populations you want to describe
- It depends on the cell annotation you will use
- You can use clustree (R package) to help you



Downstream possibility in scRNAseq

DEG & Functional enrichment



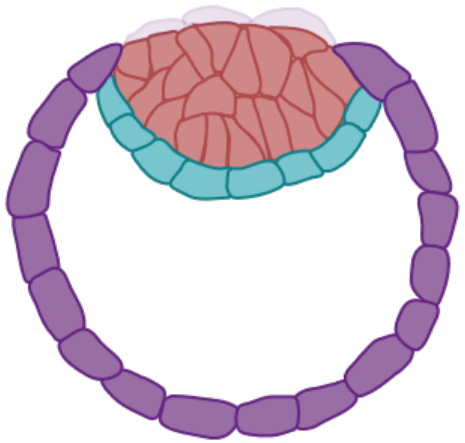
C0 *Sparc* Microglia
C1 BAMs
C2 *Crybb1* Primitive Microglia
C3 Proliferative Microglia
C4 Neutrophil granulocytes
C5 *Spp1* Microglia
C6 Monocytes
C7 Proliferative BAMs

- Different enrichment methods : AUCell, GSVA, GSEA
- SCPA for pathway analysis between condition or pseudotimes

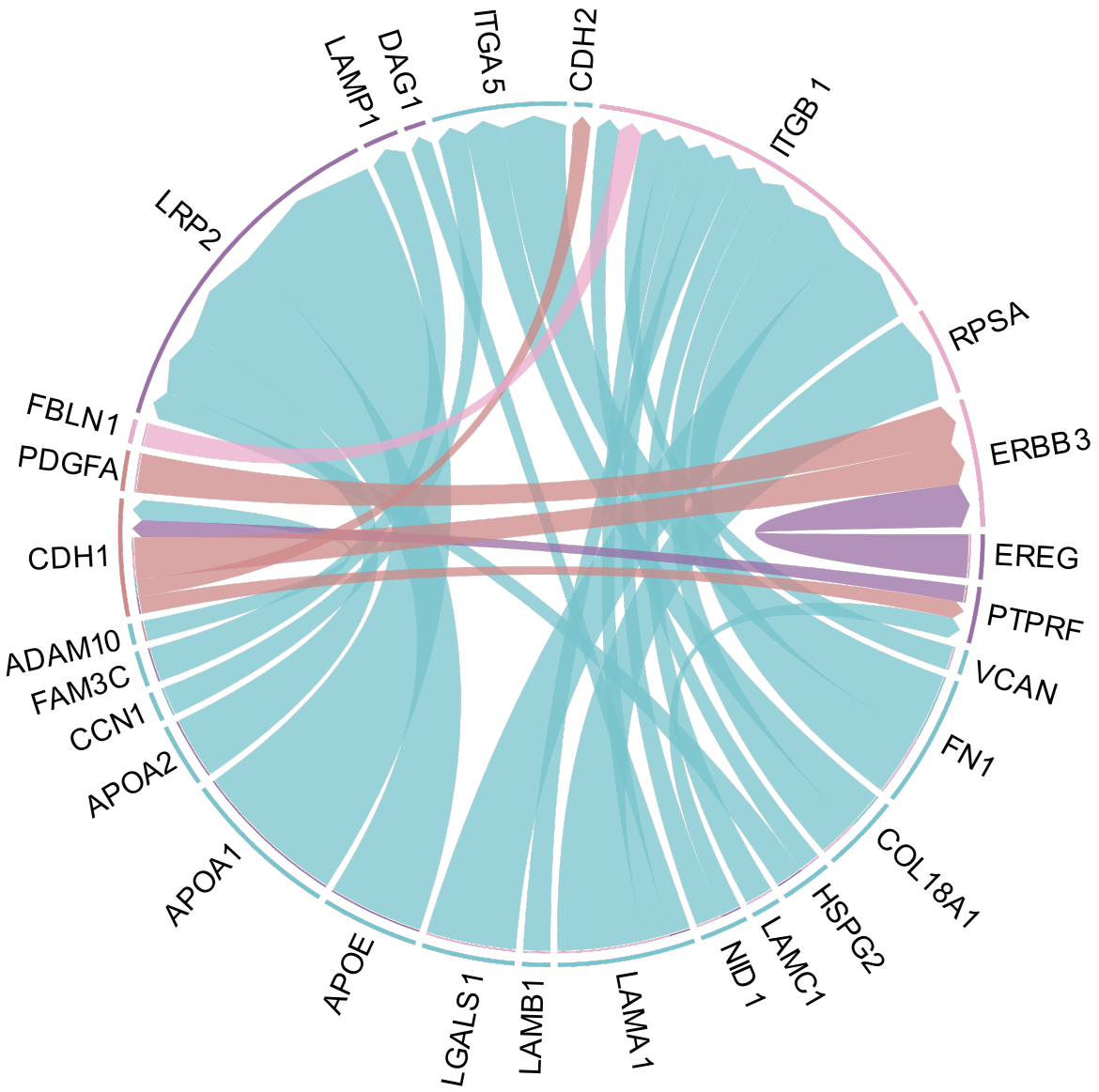
Dufour et al 2025.

Interactions between populations

- CellChat, NicheNet, CellPhone …



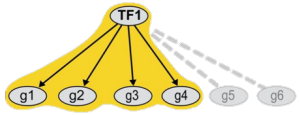
Epiblast
Trophectoderm
Hypoblast
No preference

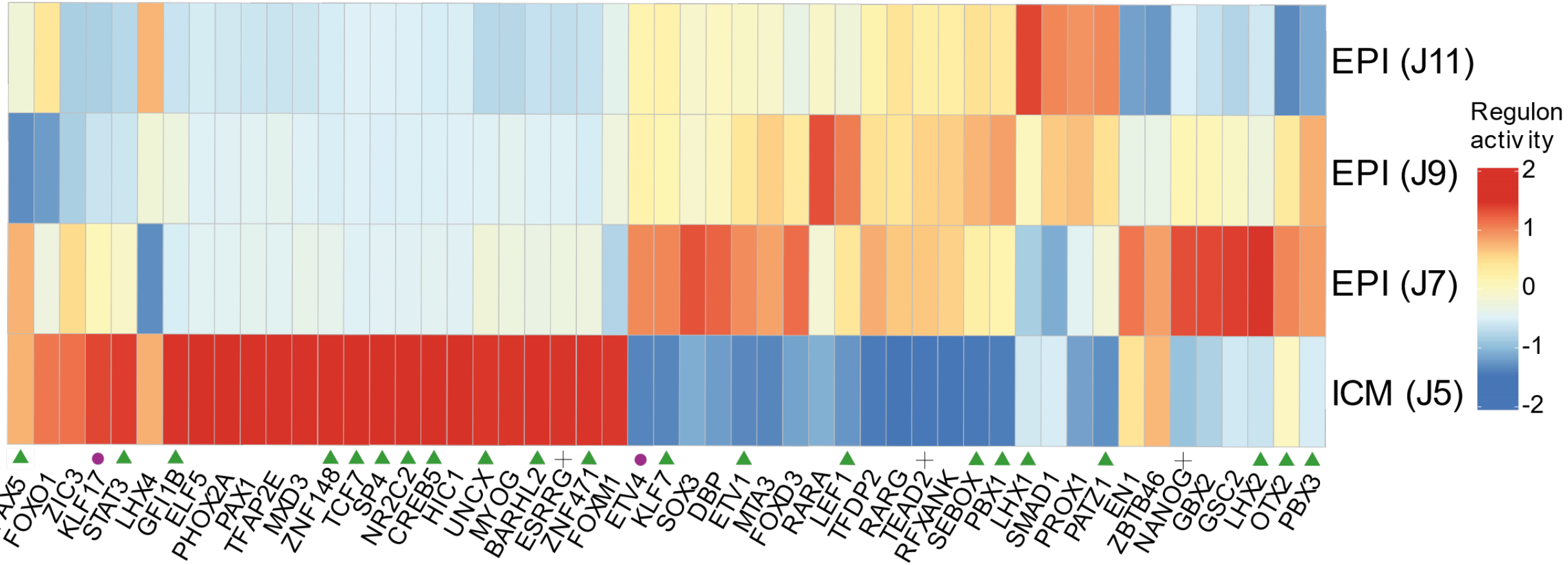


Dufour et al 2024.

Chordiagram of interactions between populations at D9

Gene regulatory networks

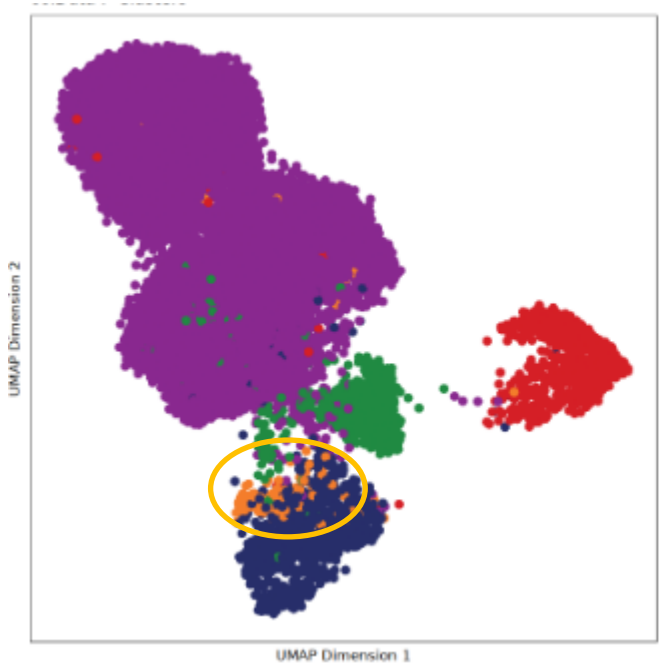




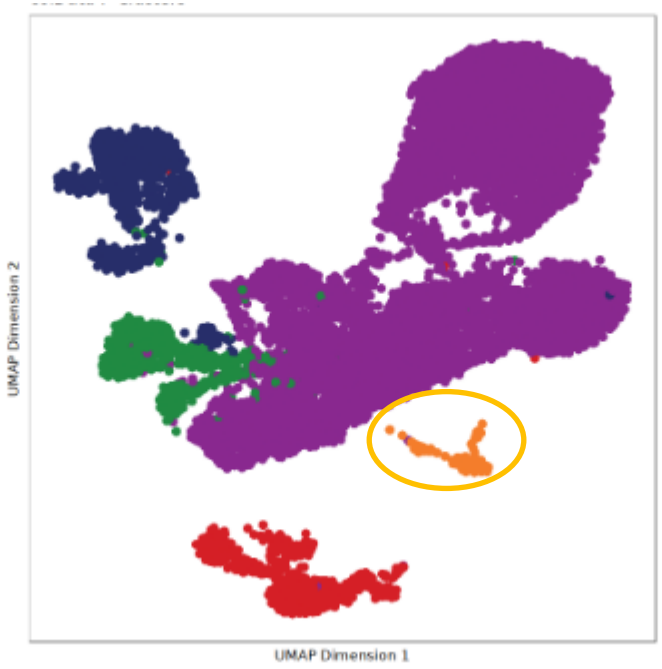
Combined heatmap of Regulons identified in our data

Dufour et al 2024.

Downstream possibility
scMultiomics (RNAseq+ATACseq)



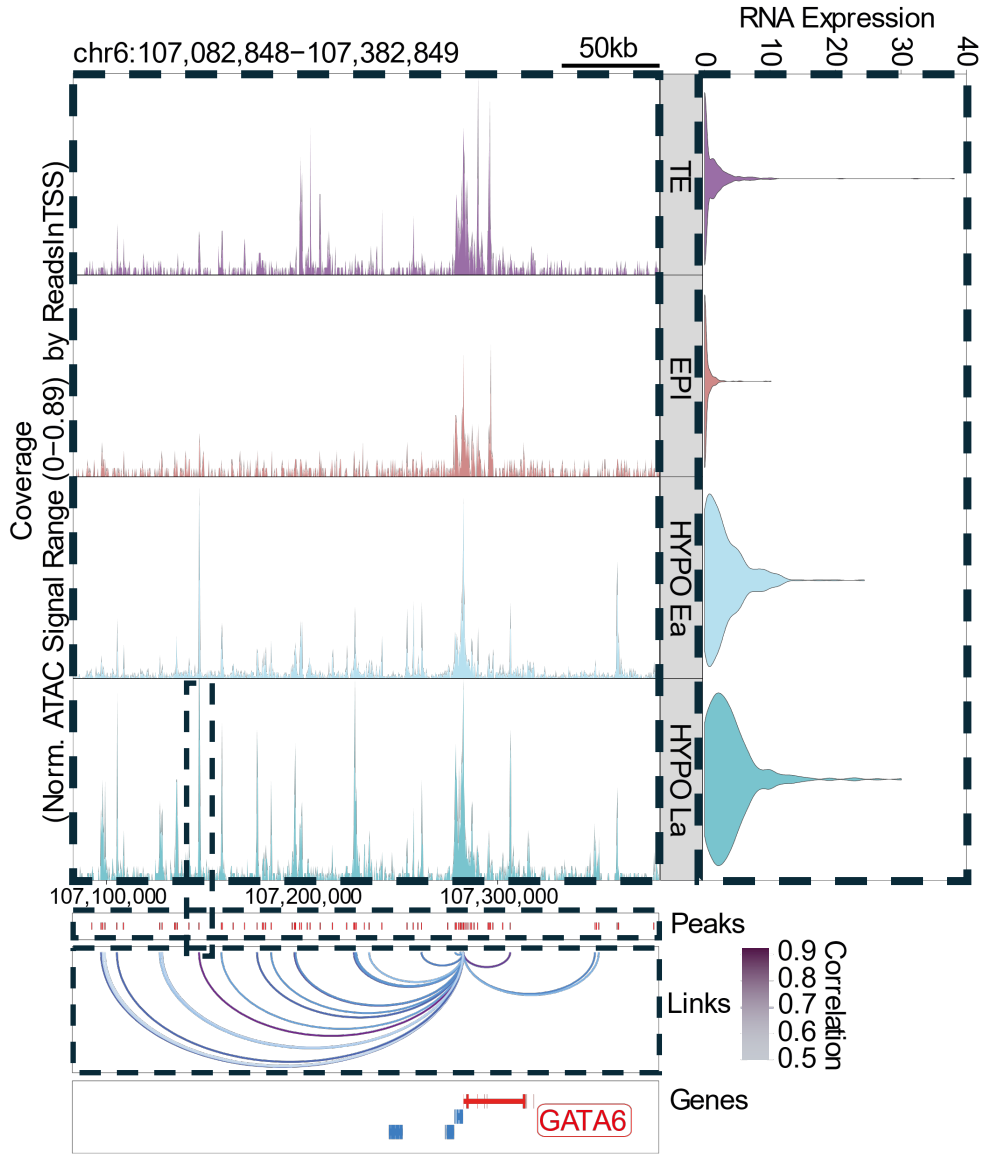
scATACseq



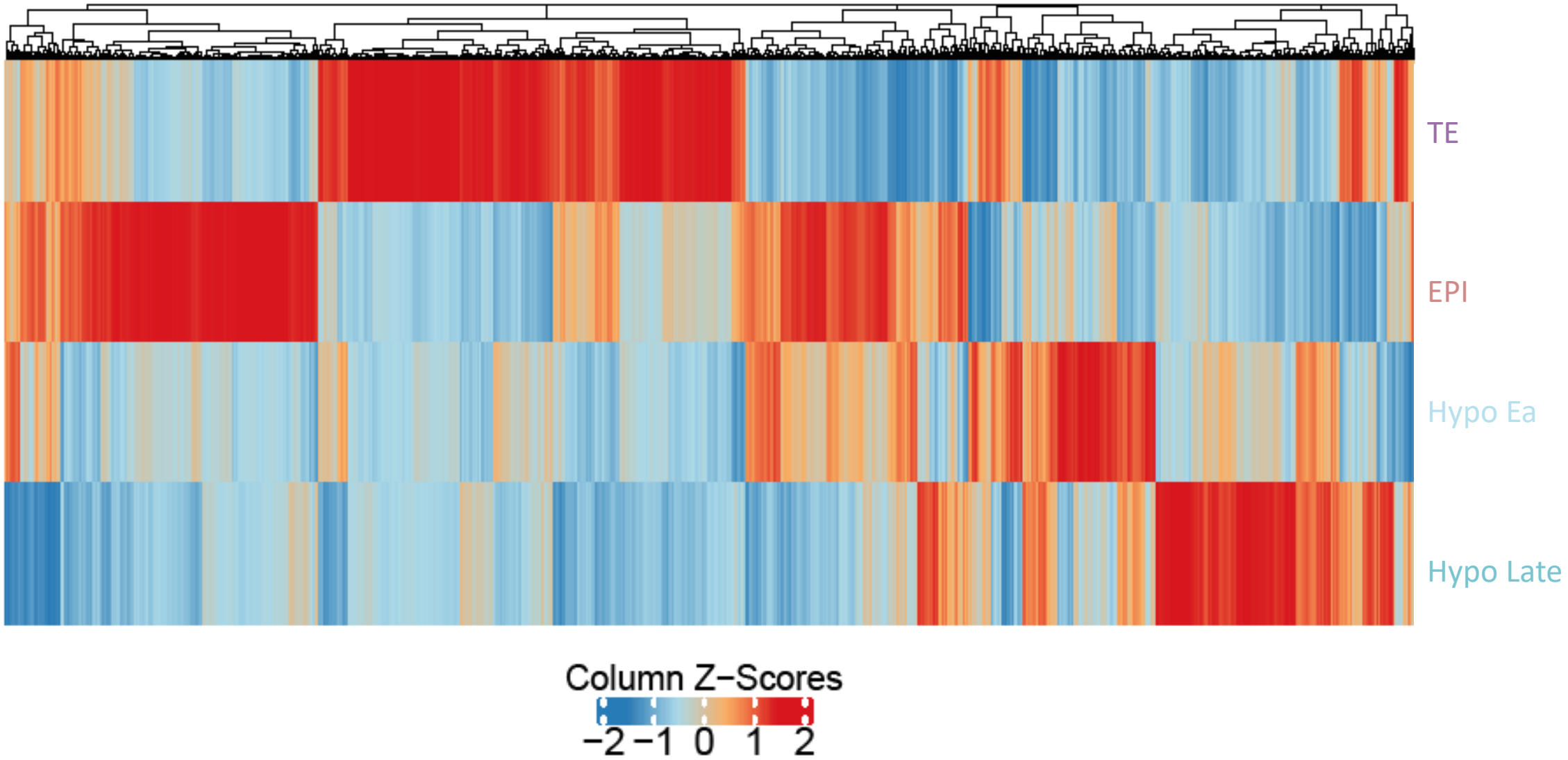
scRNAseq

scRNAseq +
scATACseq

Example gene : GATA6

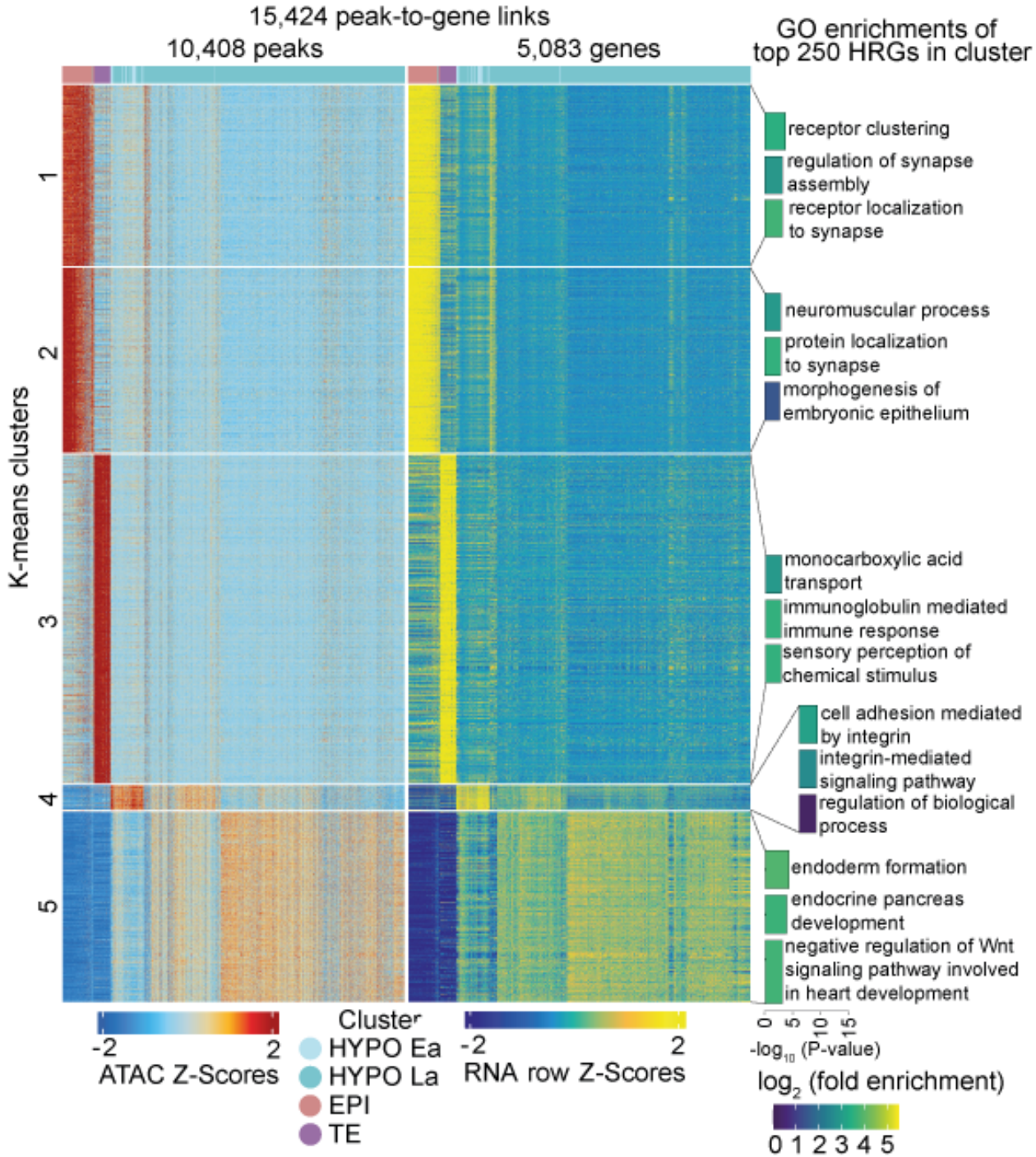


Difference in accessibility of promoter regions

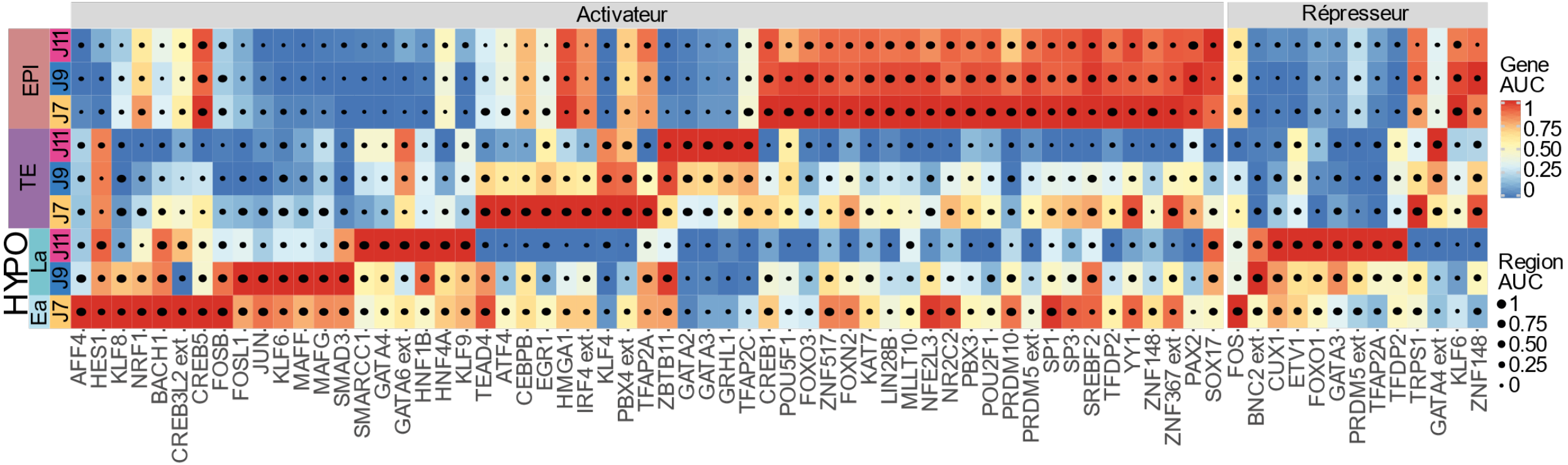


Heatmap of differential promoter regions

Association of accessibility of regulatory regions with gene expression

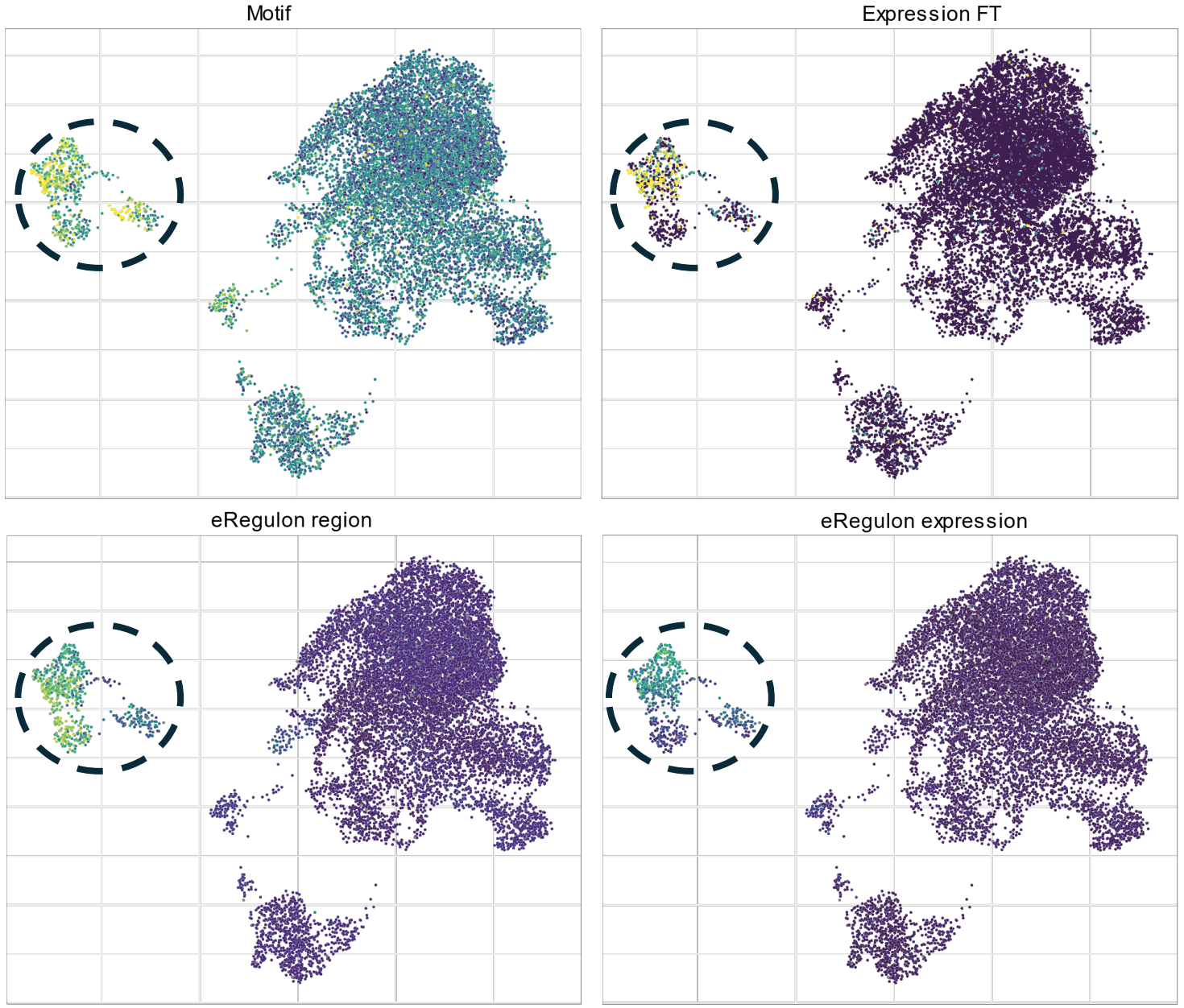


Gene regulatory networks

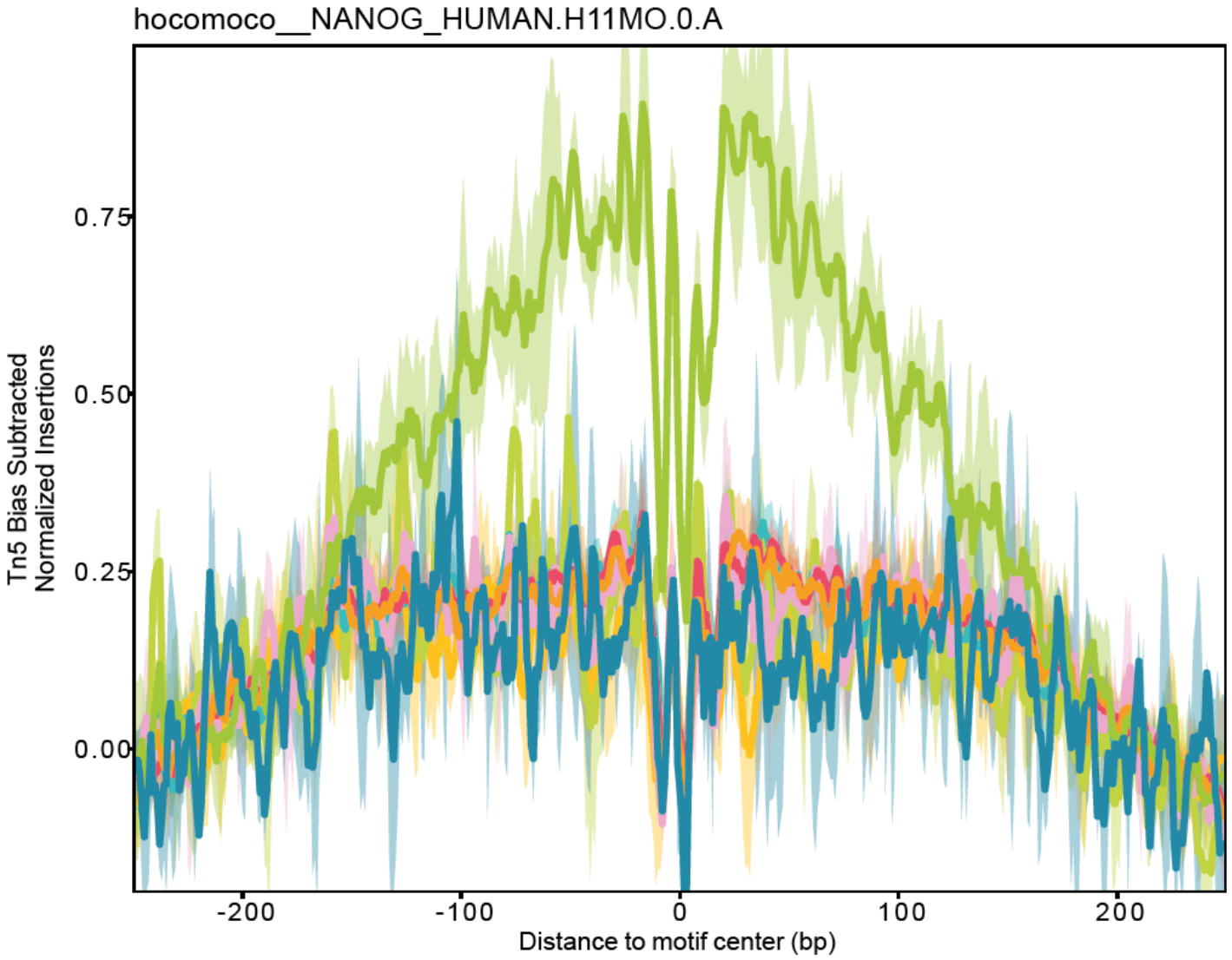


Combined heatmap of eRegulons identified in our data

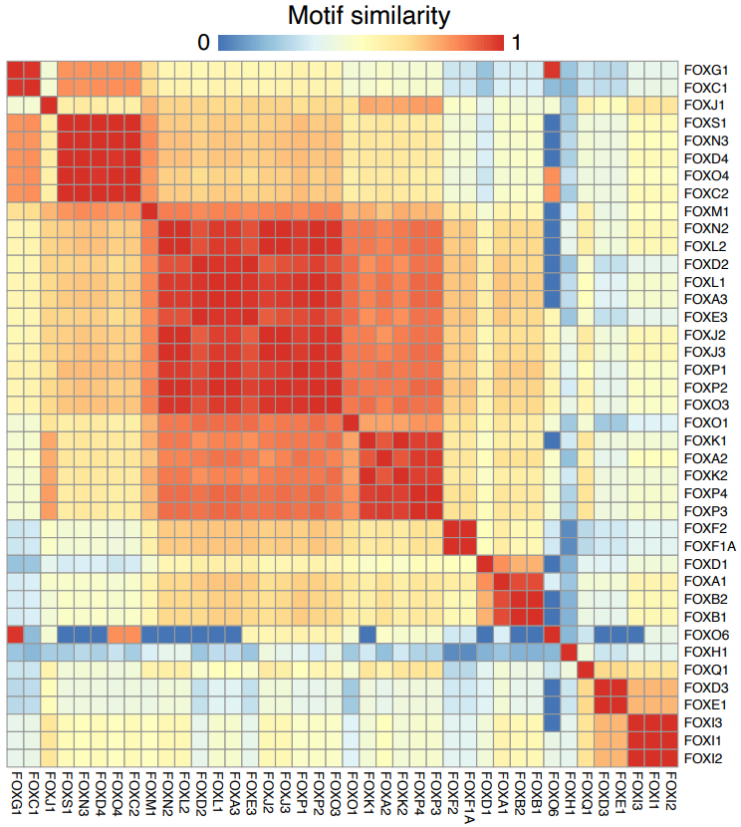
Example transcription factor : GRHL1

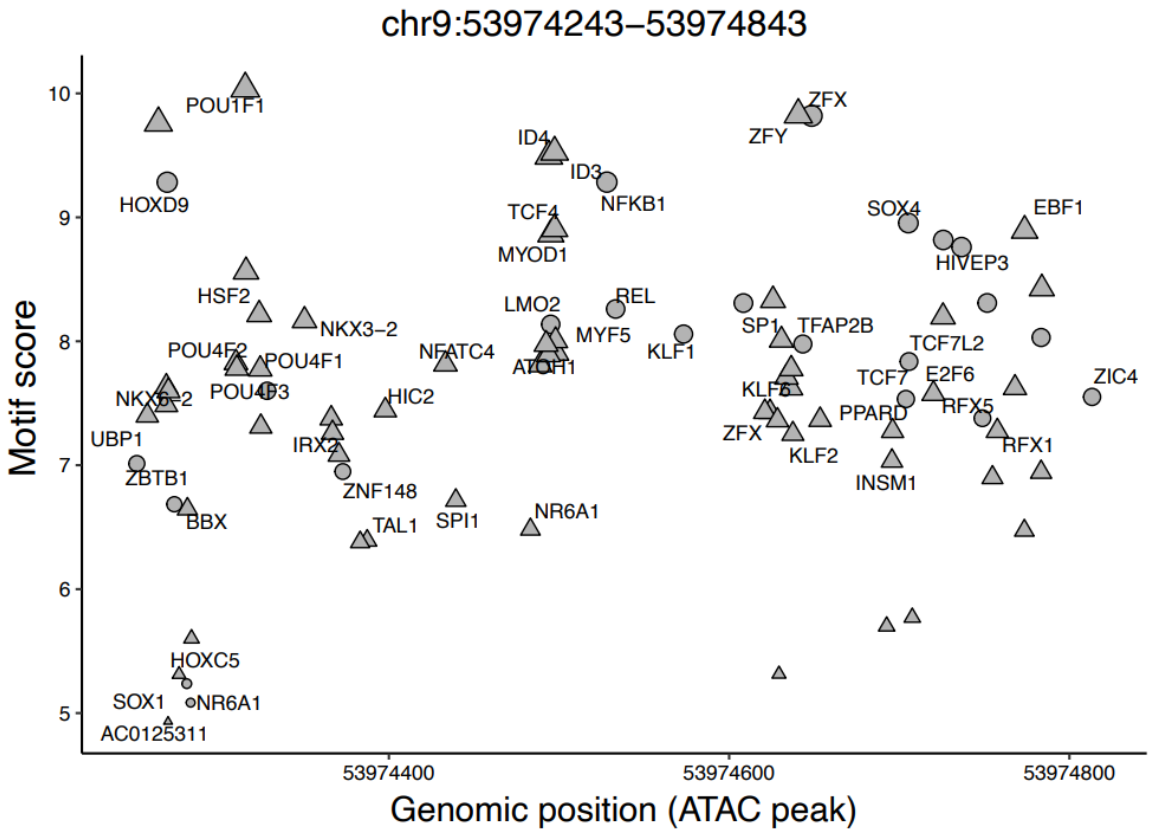


Motif Footprint



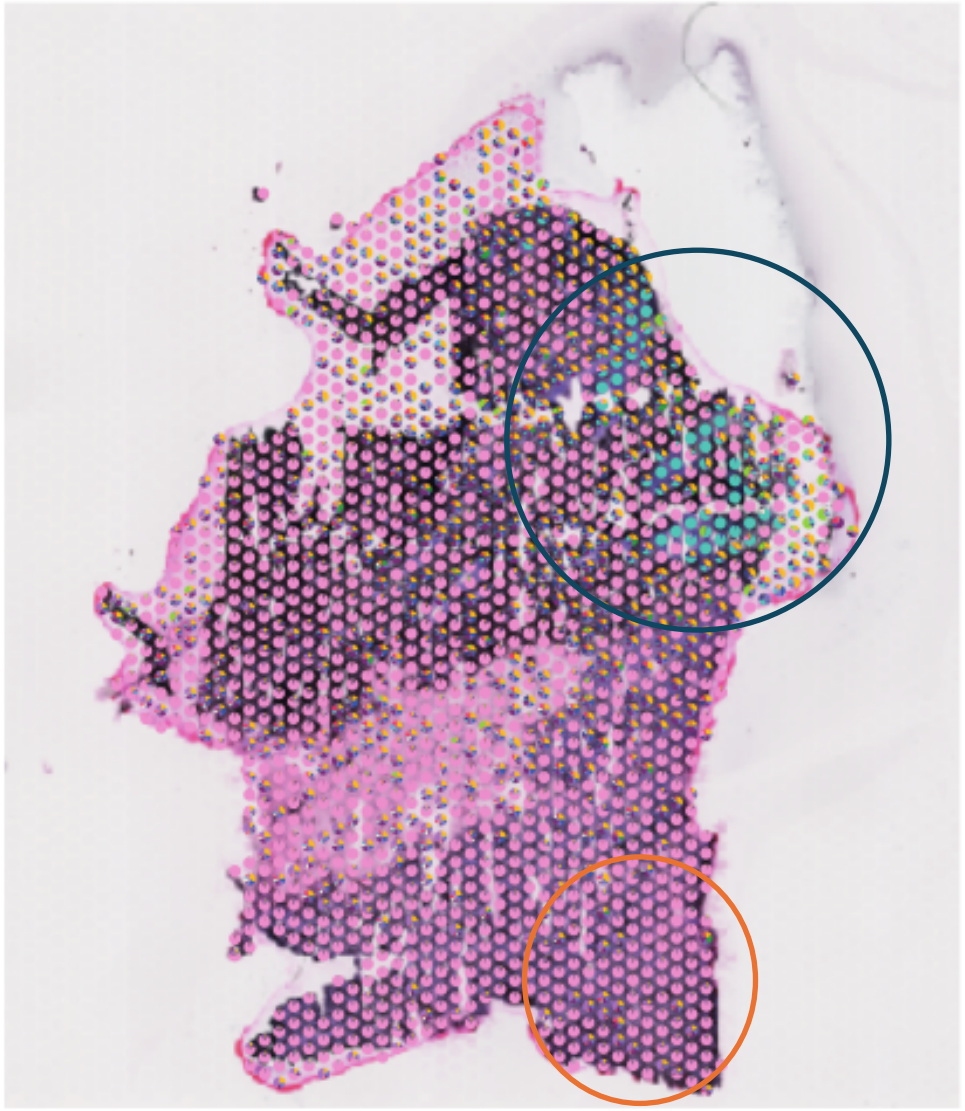
Motif can be difficult to infers





SpatialRNAseq

- Much more challenging experiments
- Less resolution and covered genes
- Difficulty in annotating complex spot
- Low-quality area



Conclusions

	DEG & Functionnal enrichment	Gene Regulatory Network	Cell Interactions
scRNAseq	✓	✓	✓
scMultiomics	✓	✓	✓
SpatialRNAseq	✓	–	✓