Réseaux de gènes contrôlant la biologie de l'embryon de porc préimplantatoire et des cellules souches pluripotentes révélées par une approche multi-omiques à l'échelle cellule unique

Hervé Acloque

Gene networks controlling functional cell interactions in the pig embryo revealed by omics studies

Hervé Acloque
GABI Laboratory INRAE Jouy en Josas France
herve.acloque@inrae.fr

RÉPUBLIQUE FRANÇAISE
Liberté Égalité Fraternité

INRAE

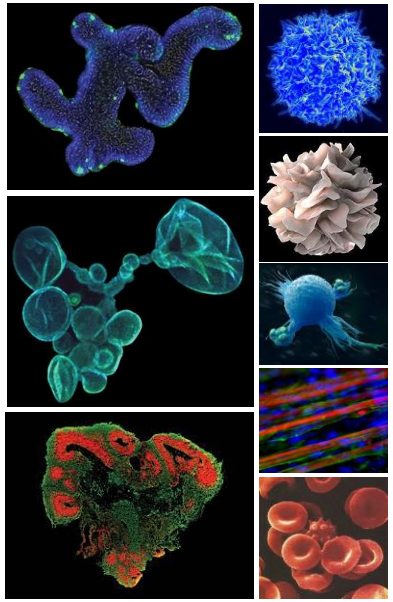
Pluripotent stem cells in livestock: a platform for high throughput phenotyping

A challenge for animal production: phenotyping complex traits and predicting breeding values for those traits

1 PSC line

Genome editing

Cell differentiation



Phenotyping:
Cell adhesion
Gene Expression
Proteome
Metabolome
Molecule Absorption
Endocytosis
Exocytosis
Host-Pathogen
Toxicity
....

Proxies or intermediate phenotypes not accessible on living animals

X1000 PSC lines

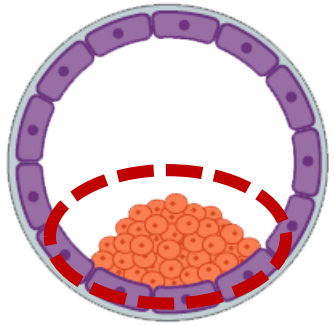
High-Throughput phenotyping

GWAS > mapping causal variants

Big data

Breeding values ?

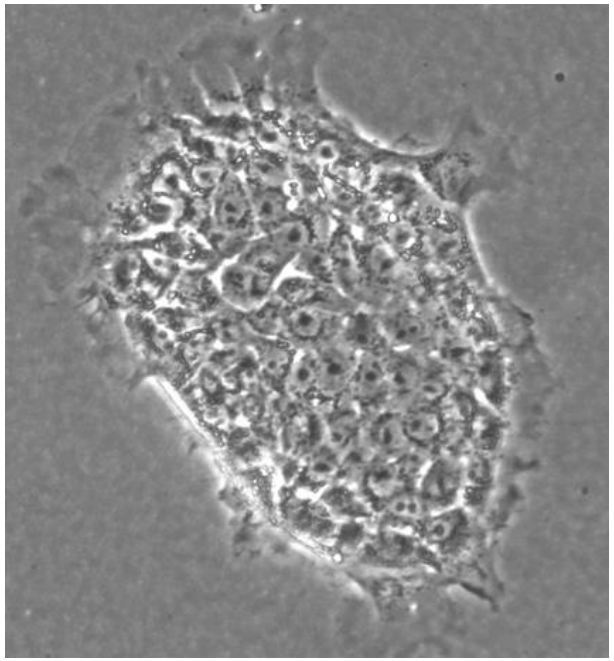
Producing standardized true PSCs for livestock species is still challenging



Amplification of Epiblast cells

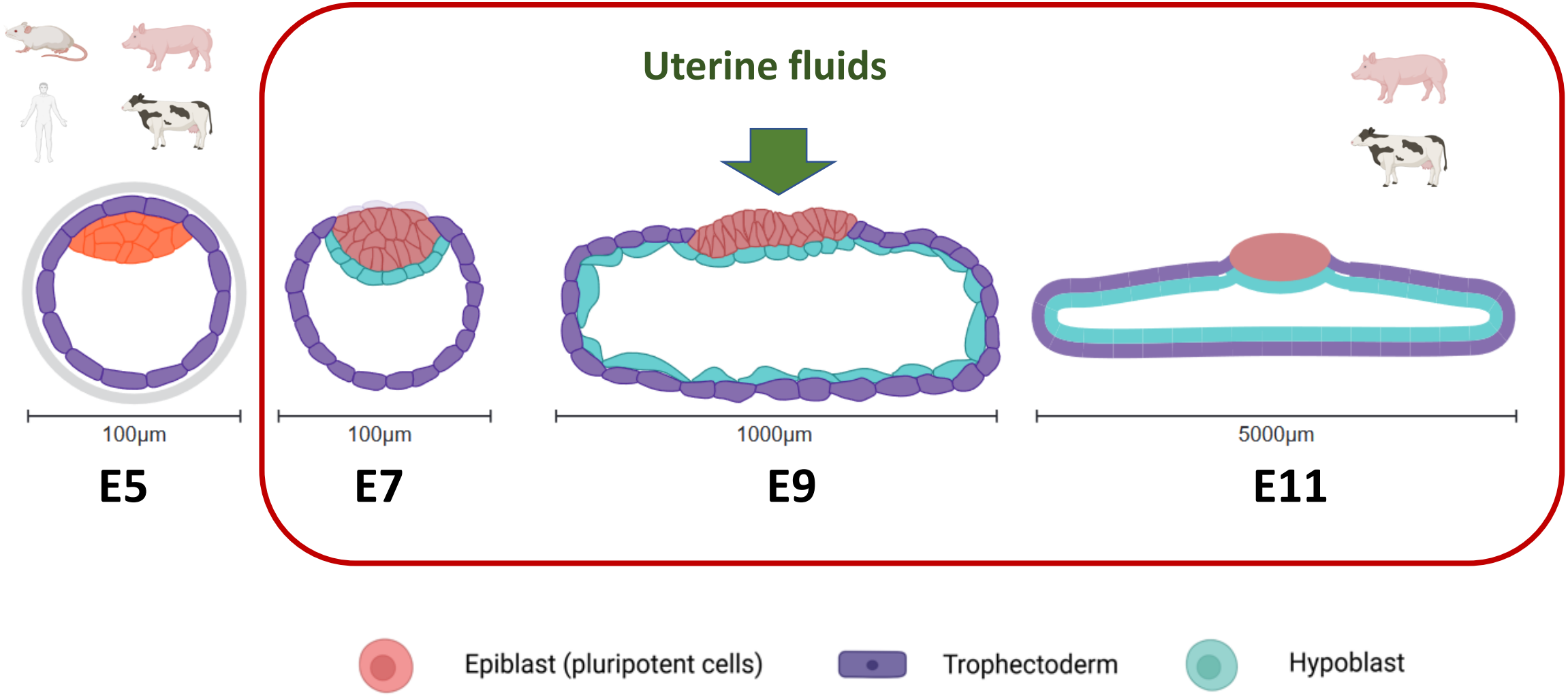
Chemically defined medium

Reprograming cocktail
(OCT4, SOX2, KLF4, MYC)

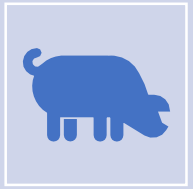


Heterogeneity ?
Stability overtime ?
Differentiation potential ?

What can we learn from the embryo ?



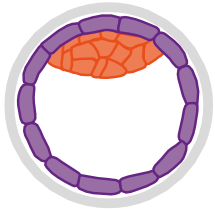
Working hypothesis



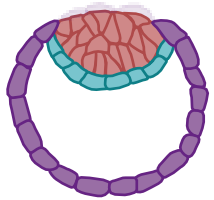
Paracrine regulations specific to pig embryonic development are not taken into consideration for the establishment of pig's pluripotent lines

Embryonic regulatory networks are not necessarily conserved in mammals

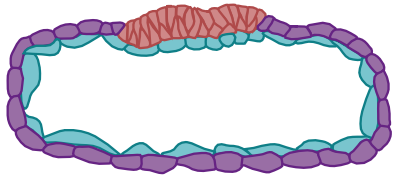
Methods



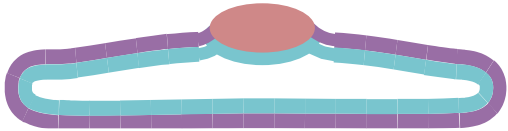
Early blastocyst **E5**



Hatched blastocyst **E7**



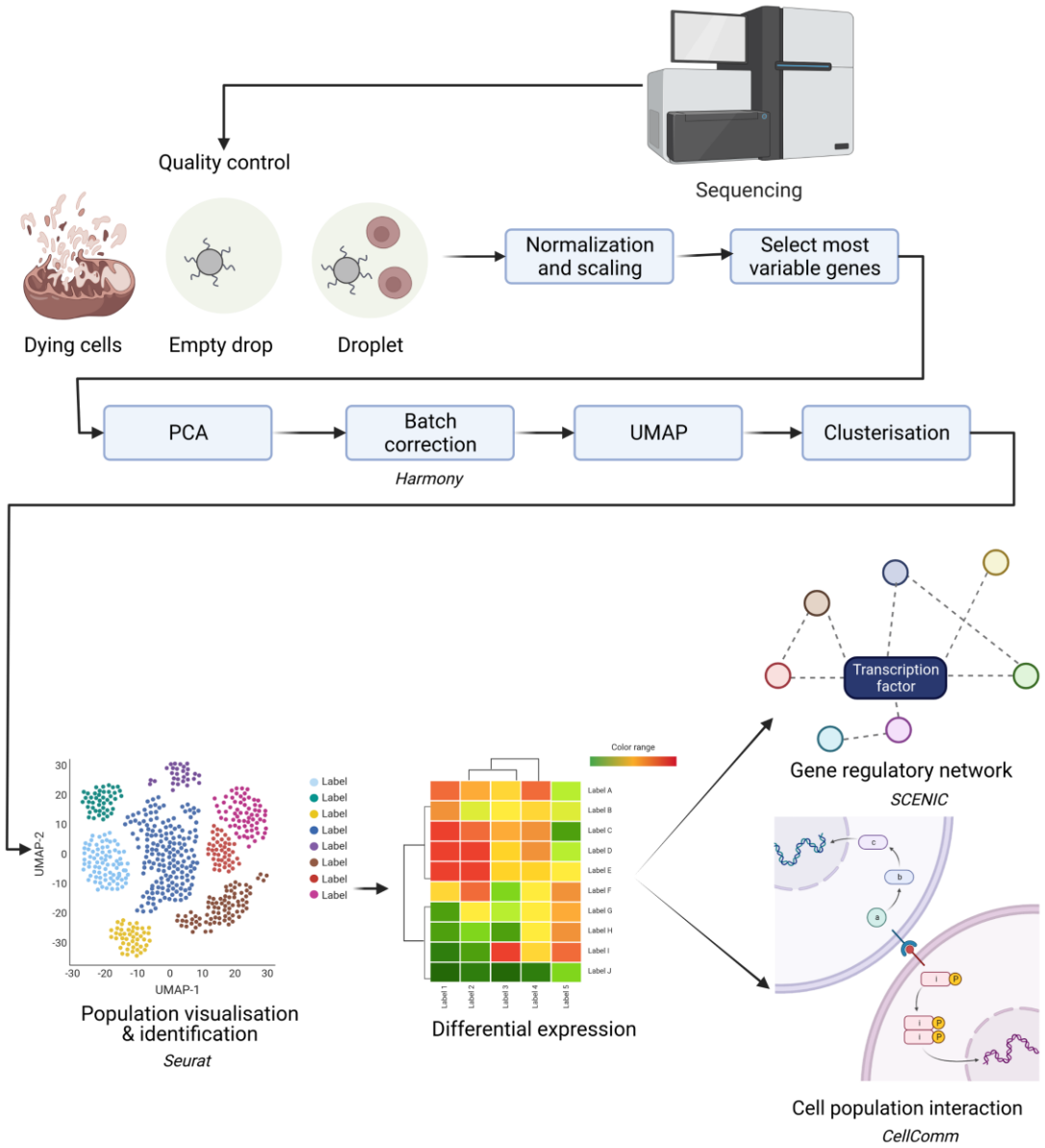
Spherical blastocyst **E9**



Ovoid blastocyst **E11**

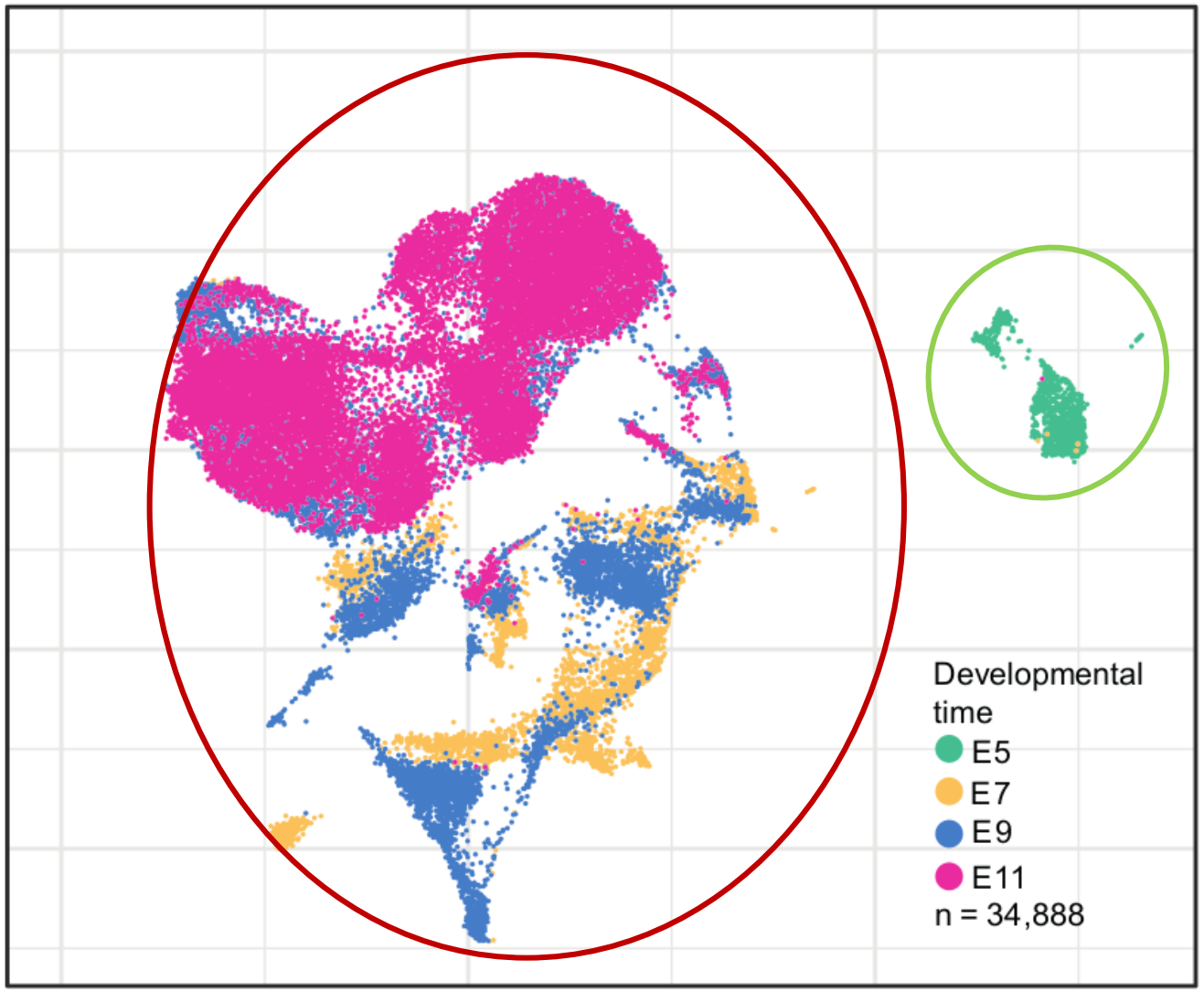
Single-cell RNAseq	2 libraries (~2000 cells each)	4 libraries (~1000 cells each)	4 libraries (~3000 cells each)	2 libraries (~6000 cells each)
Uterine fluids	8 sows	4 sows	3 sows	3 sows
Single-cell multiomics (scATAC-seq + scRNA-seq)	0	1 library (~2000 cell's nucleus)	2 libraries (~4000 cell's nucleus)	4 libraries (~8000 cell's nucleus)

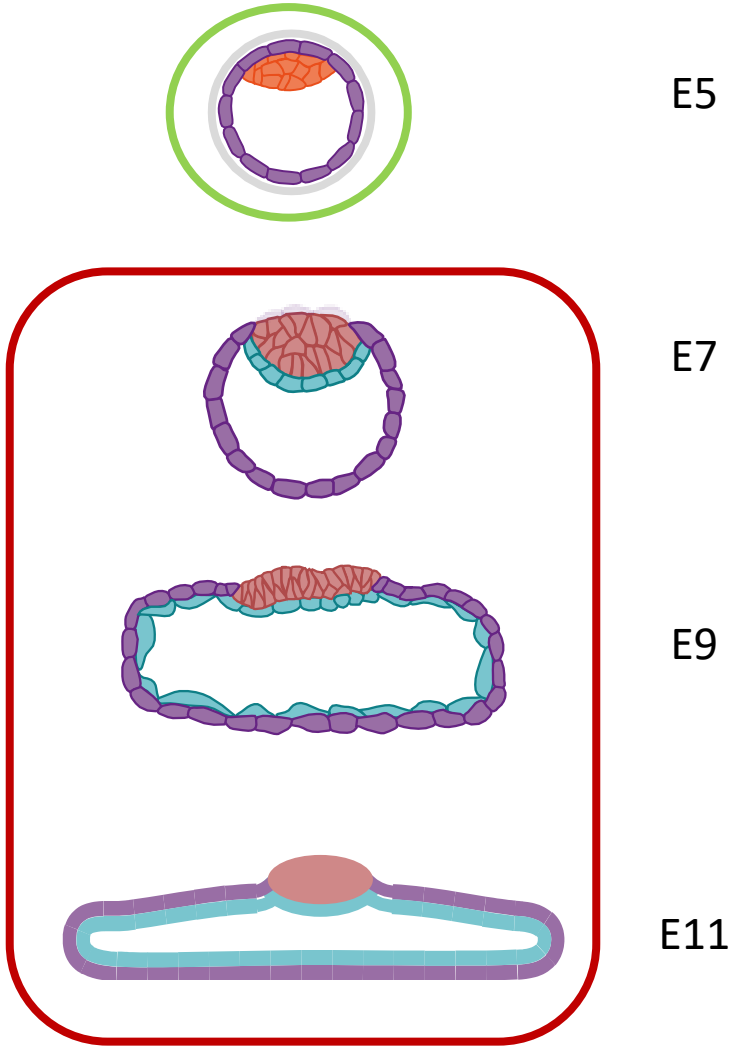
Methods


Quality control
Sequencing
Dying cells
Empty drop
Droplet
Normalization and scaling
Select most variable genes
PCA
Batch correction
Harmony
UMAP
Clusterisation
Transcription factor
Gene regulatory network
SCENIC
UMAP-2
UMAP-1
Population visualisation & identification
Seurat
Differential expression
Cell population interaction
CellComm

scRNAseq allows the identification of cell populations constituting the embryo

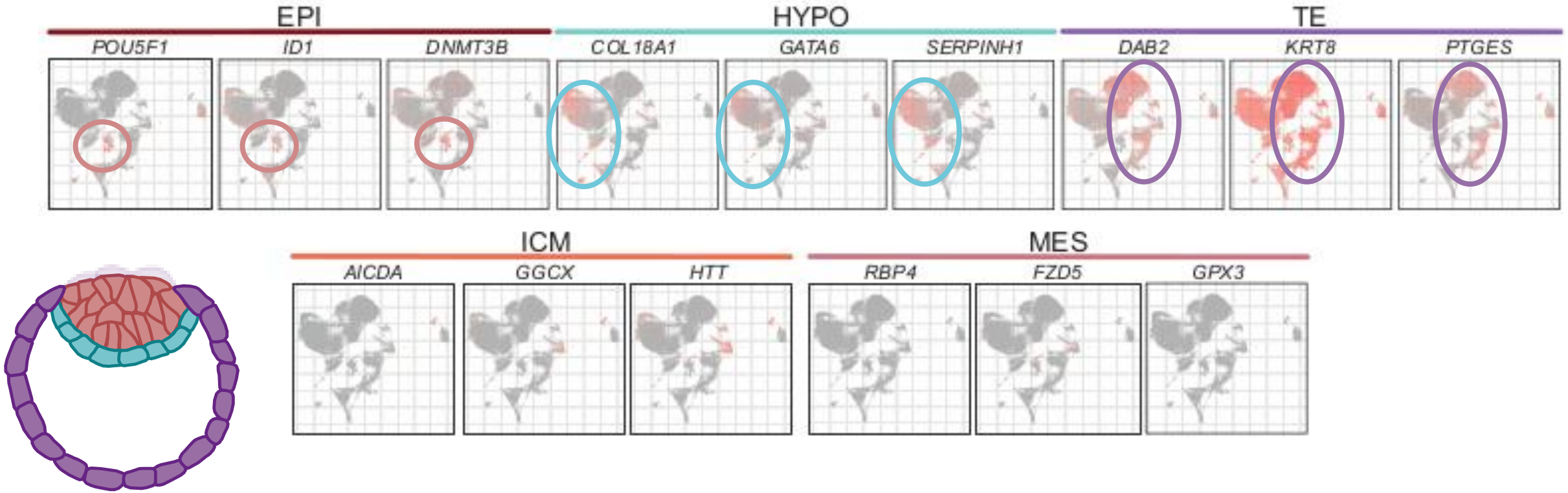
UMAP
of **34,888 cells**





Dufour et al. submitted; https://doi.org/10.1101/2023.05.30.542847

scRNAseq allows the identification of cell populations constituting the embryo

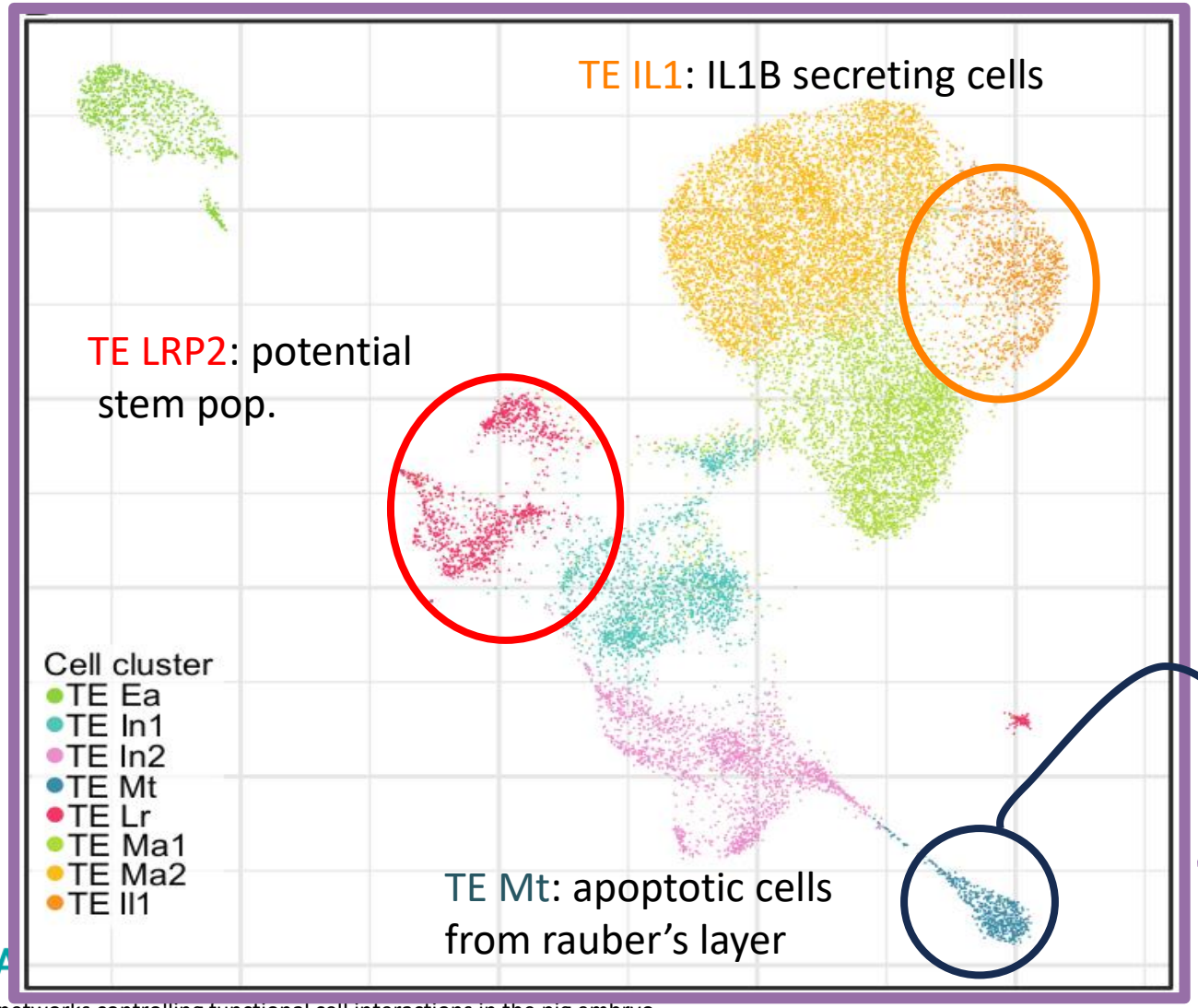


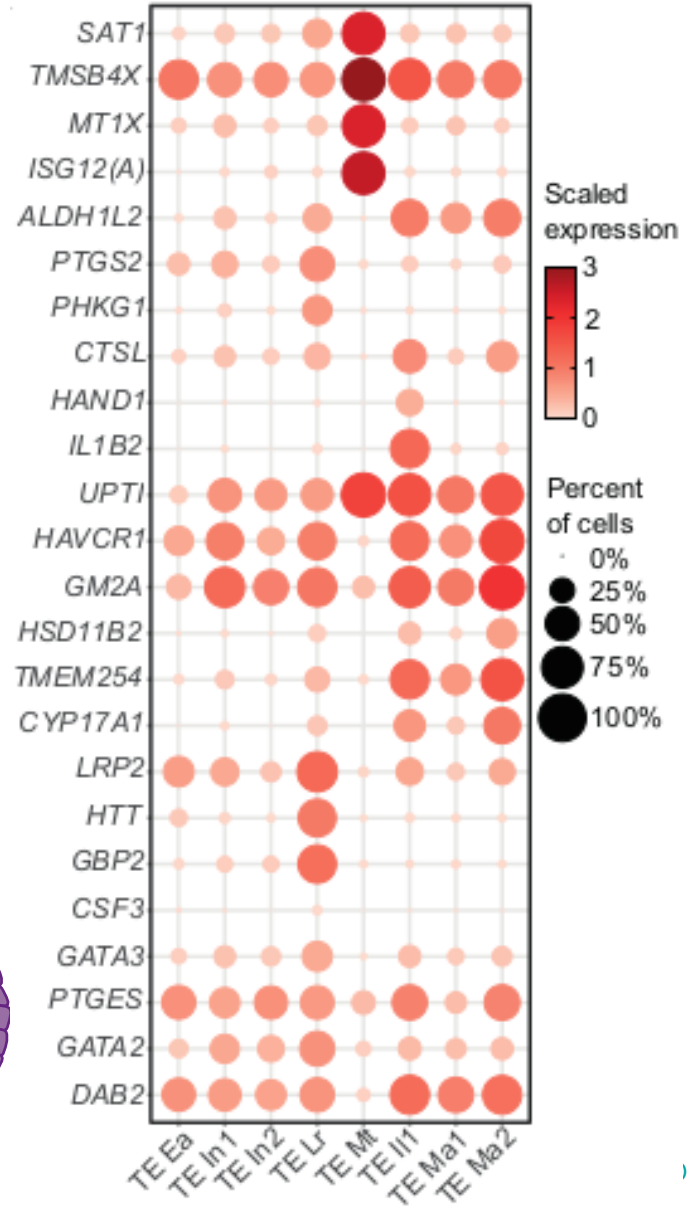
Dufour et al. submitted; https://doi.org/10.1101/2023.05.30.542847

scRNAseq data provide cues to better understand the biology of the pig embryo

Identify new subpopulations

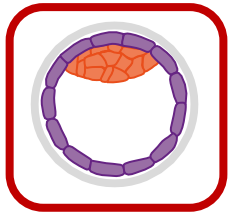
UMAP of
18,239 TE cells



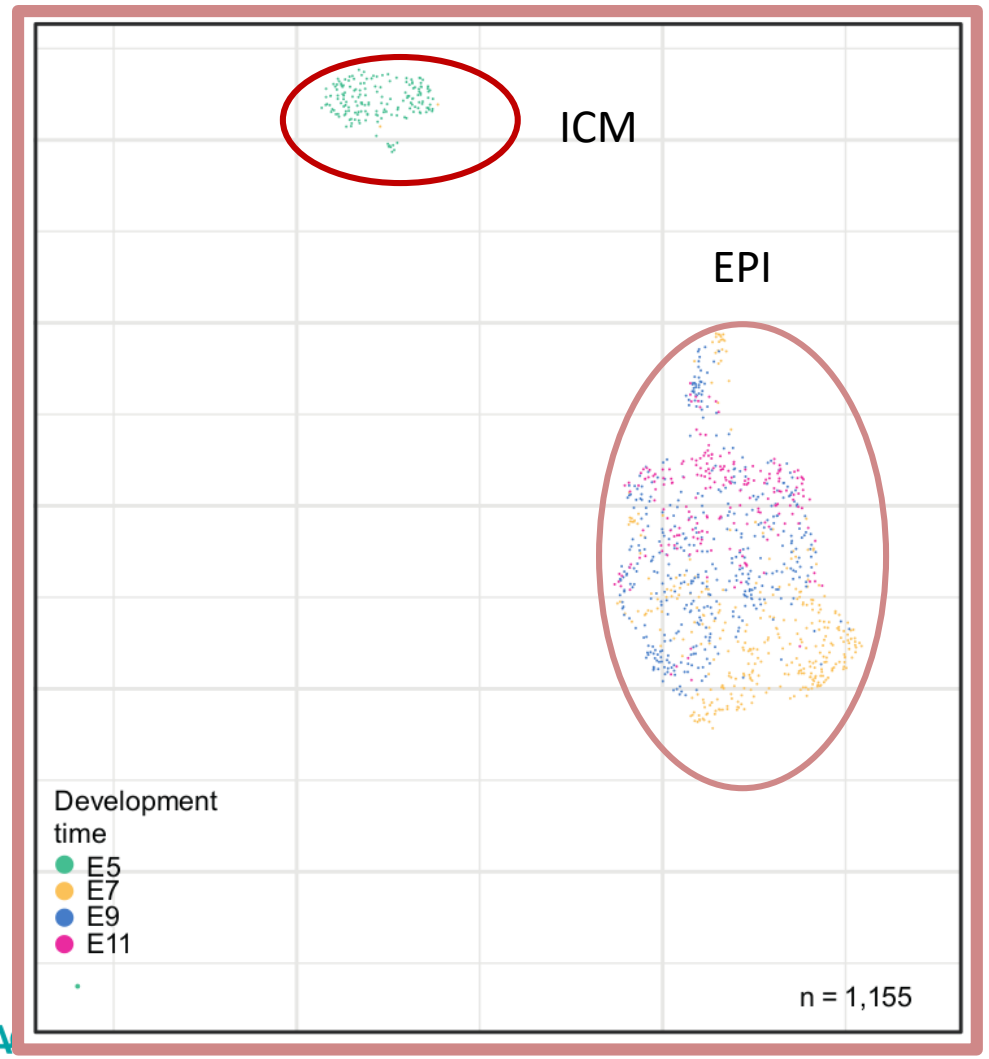


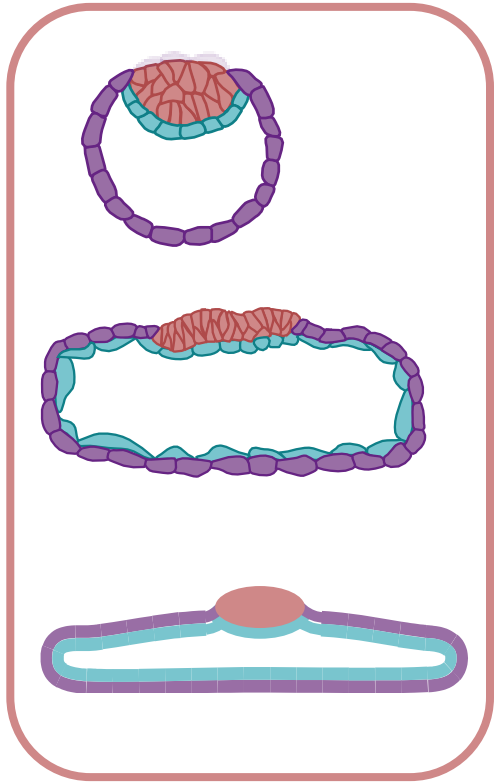
scRNAseq data provide cues to better understand the biology of the pig embryo

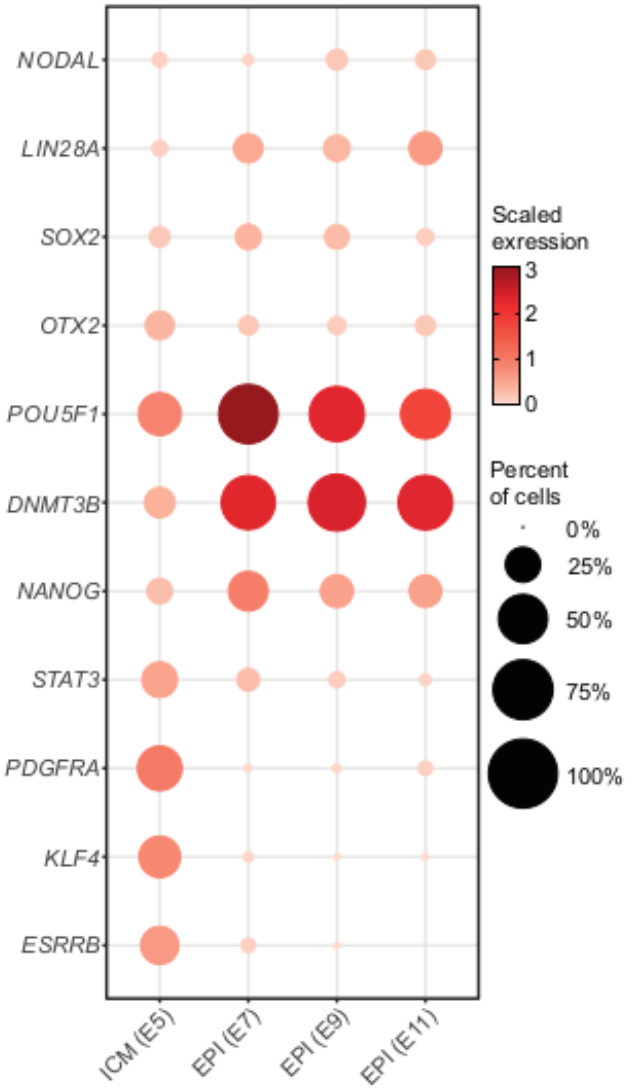
Identify subpopulations



UMAP of **1’155 EPI cells**



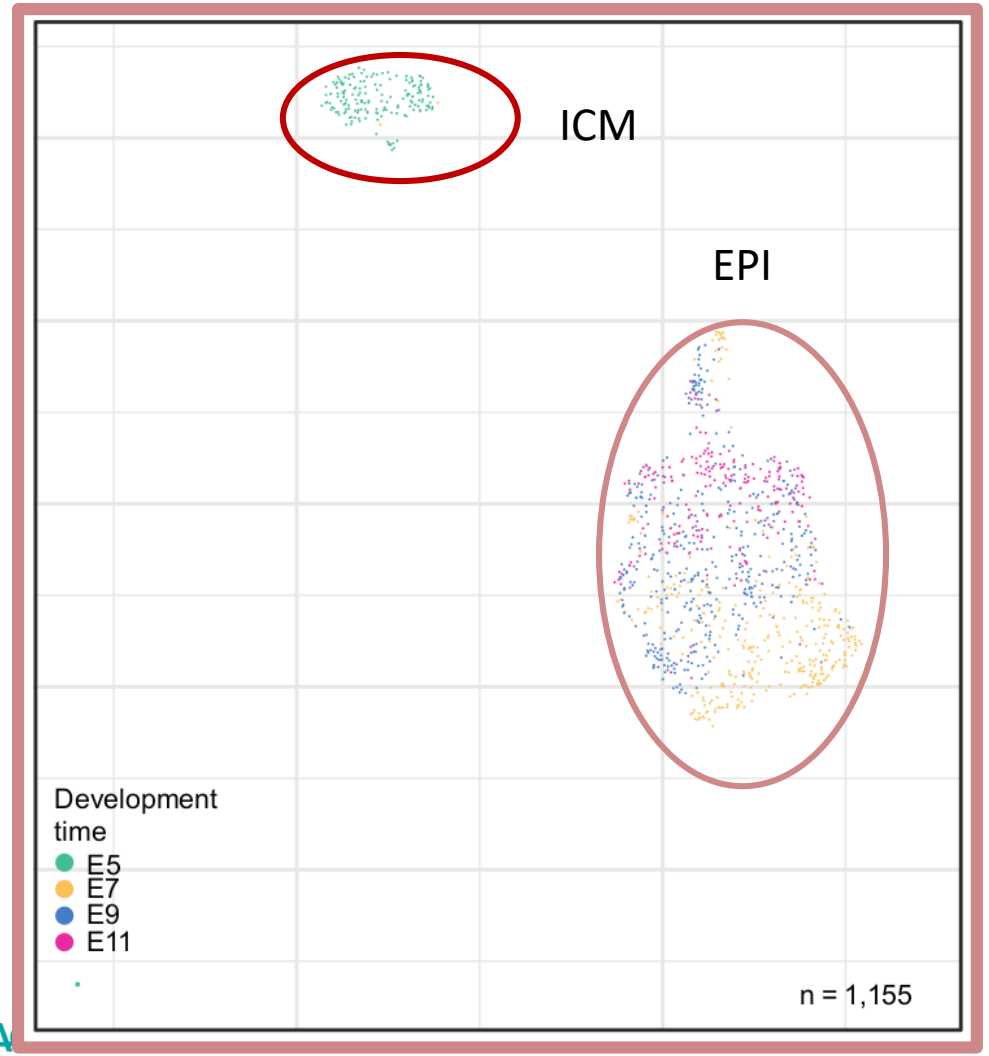


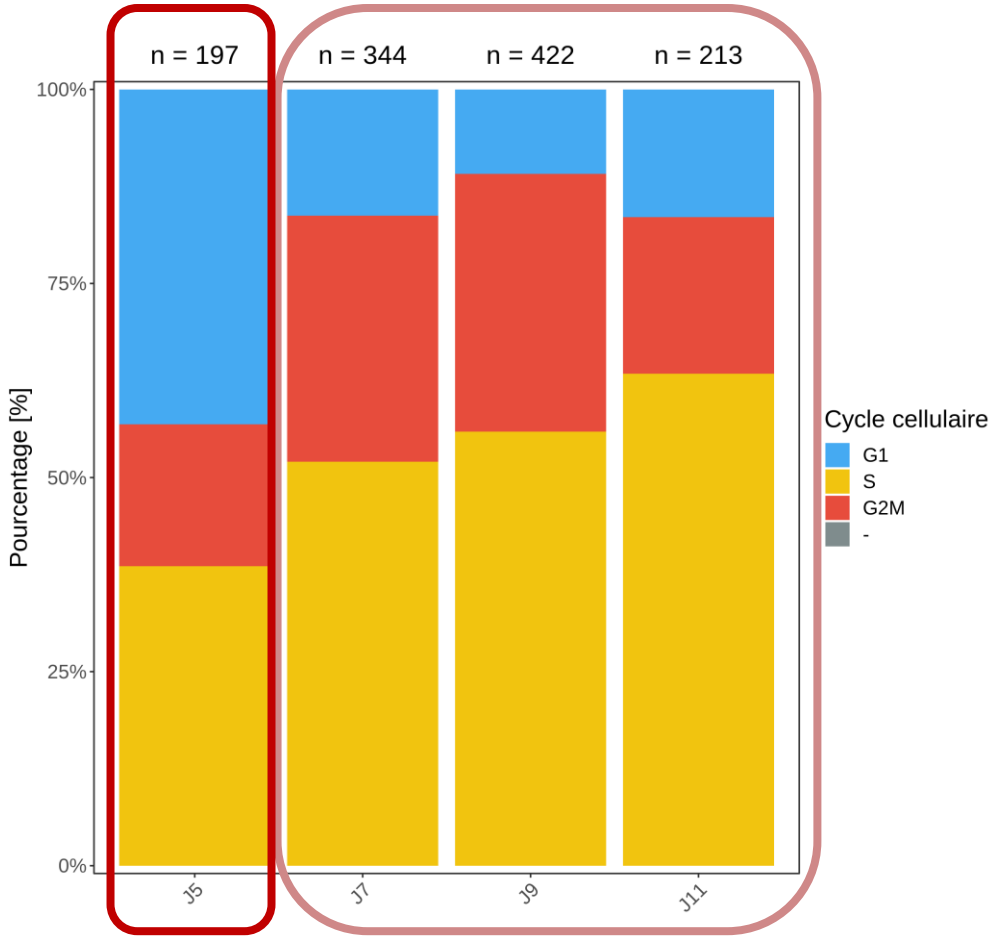


scRNAseq data provide cues to better understand the biology of the pig embryo

Identify subpopulations

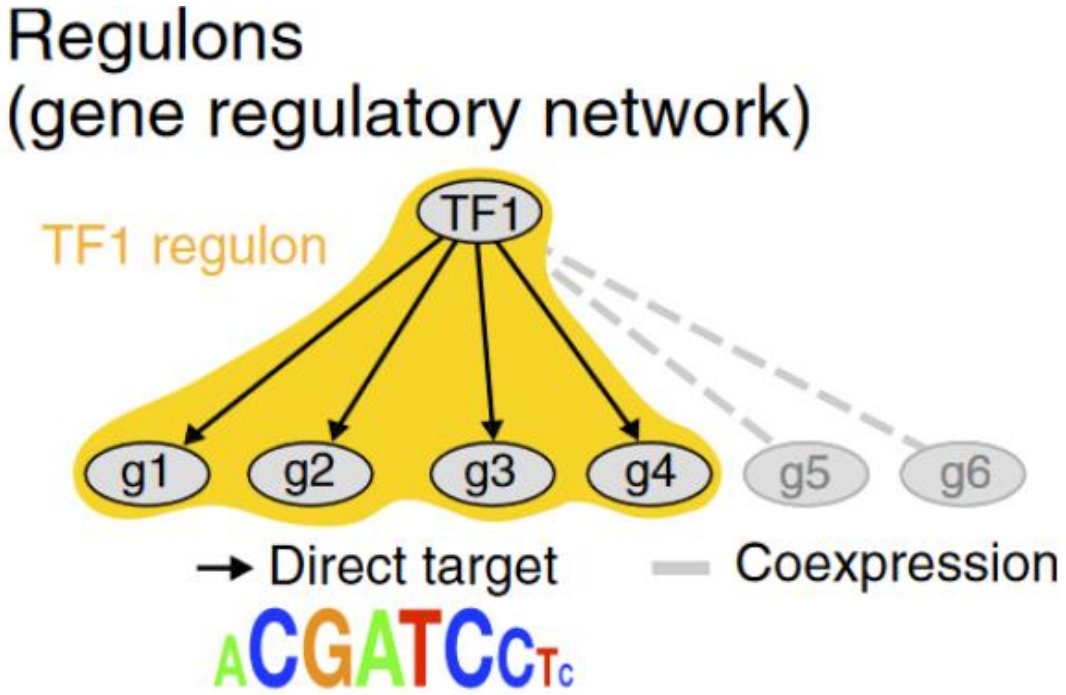
UMAP of **1’155 EPI cells**





scRNAseq data provide cues to better understand the biology of the pig embryo

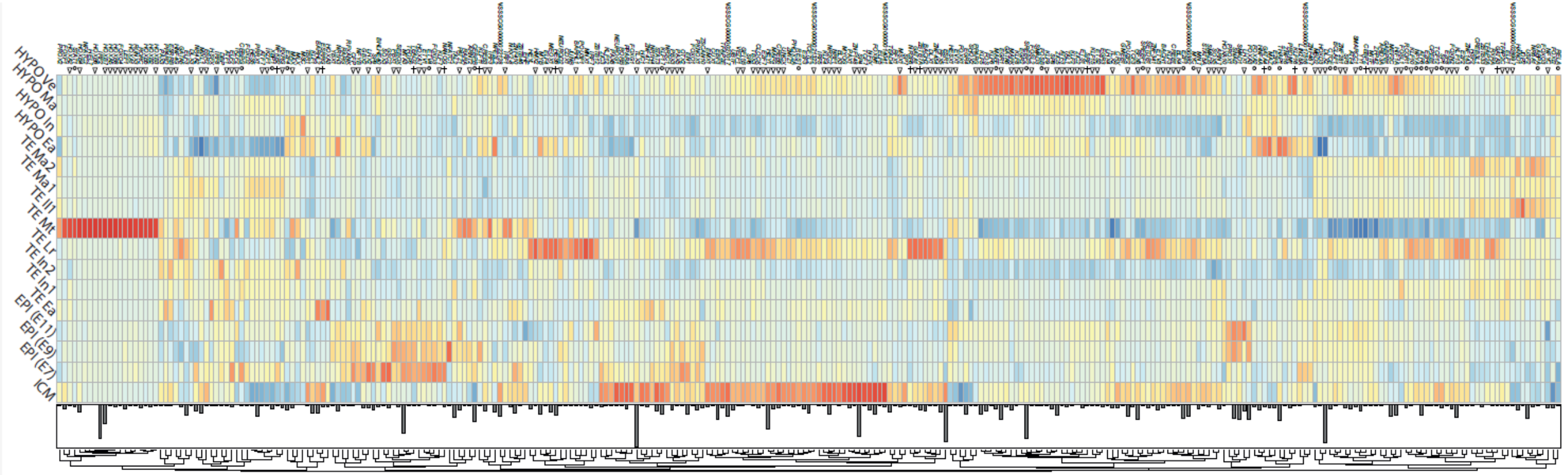
Identify modules of gene regulation



Aibar et al. 2017

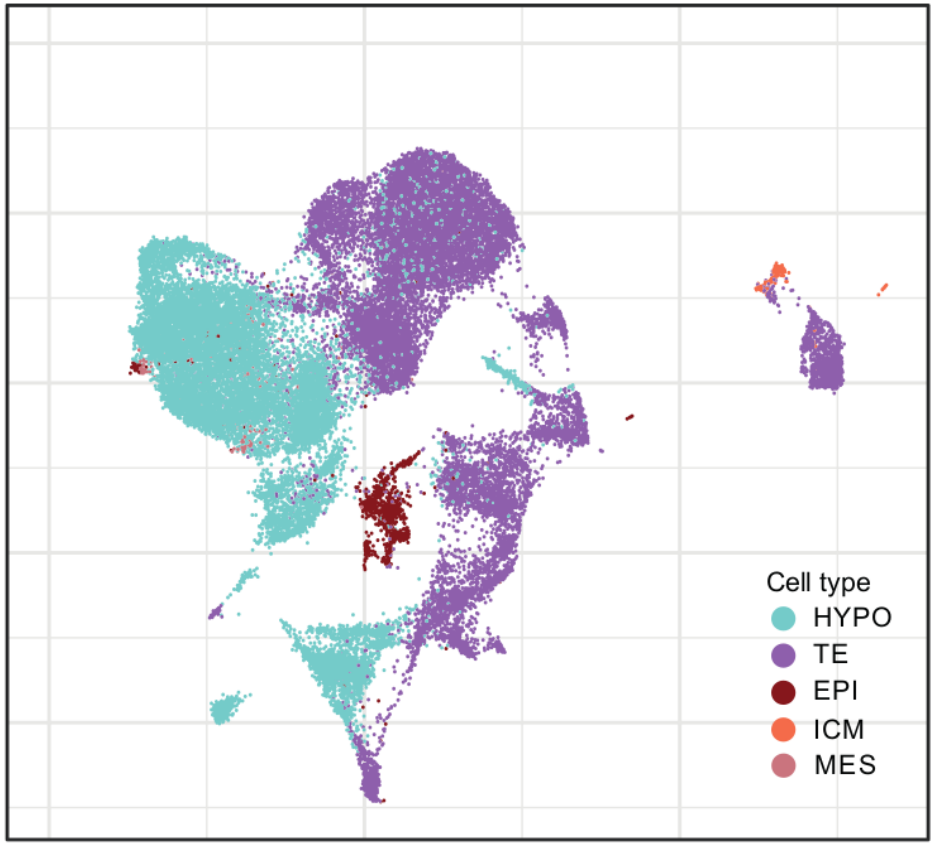
scRNAseq data provide cues to better understand the biology of the pig embryo

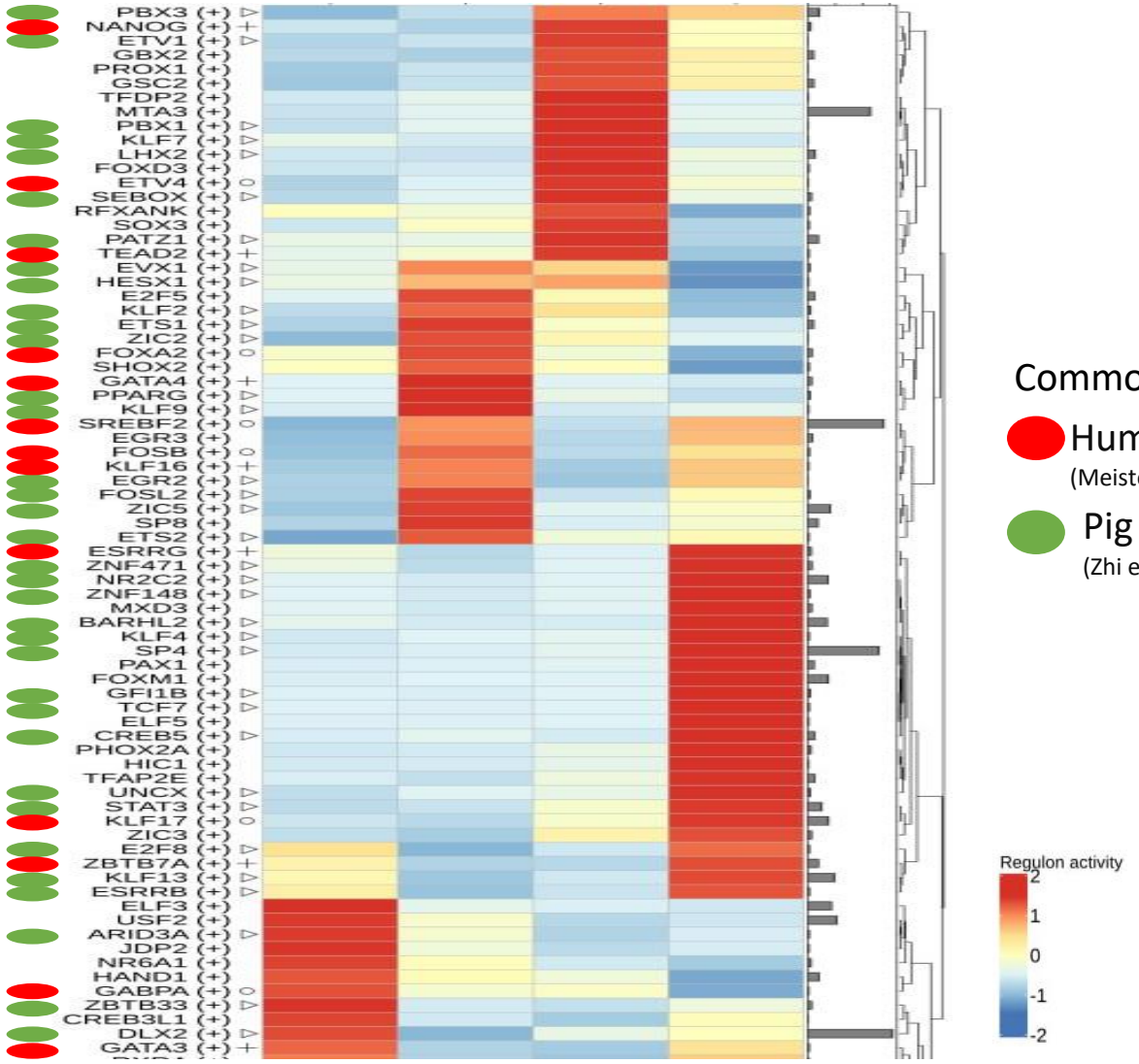
Identify modules of gene regulation



scRNAseq data provide cues to better understand the biology of the pig embryo

Identify modules of gene regulation

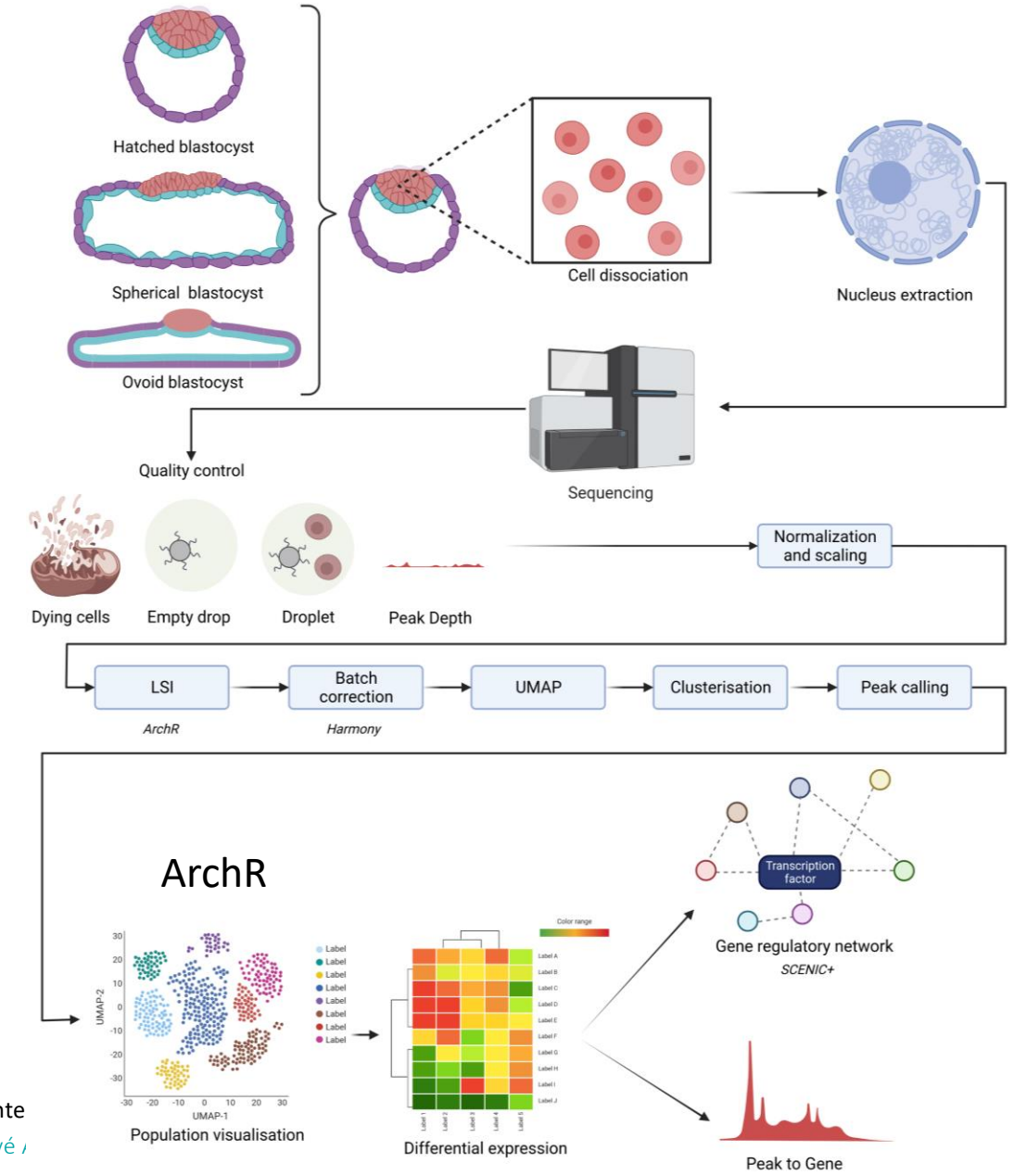




Common with

- Human (Meistermann et al. 2021)
- Pig (Zhi et al. 2021)

scOMICS: adding a layer of information to refine module of gene regulation



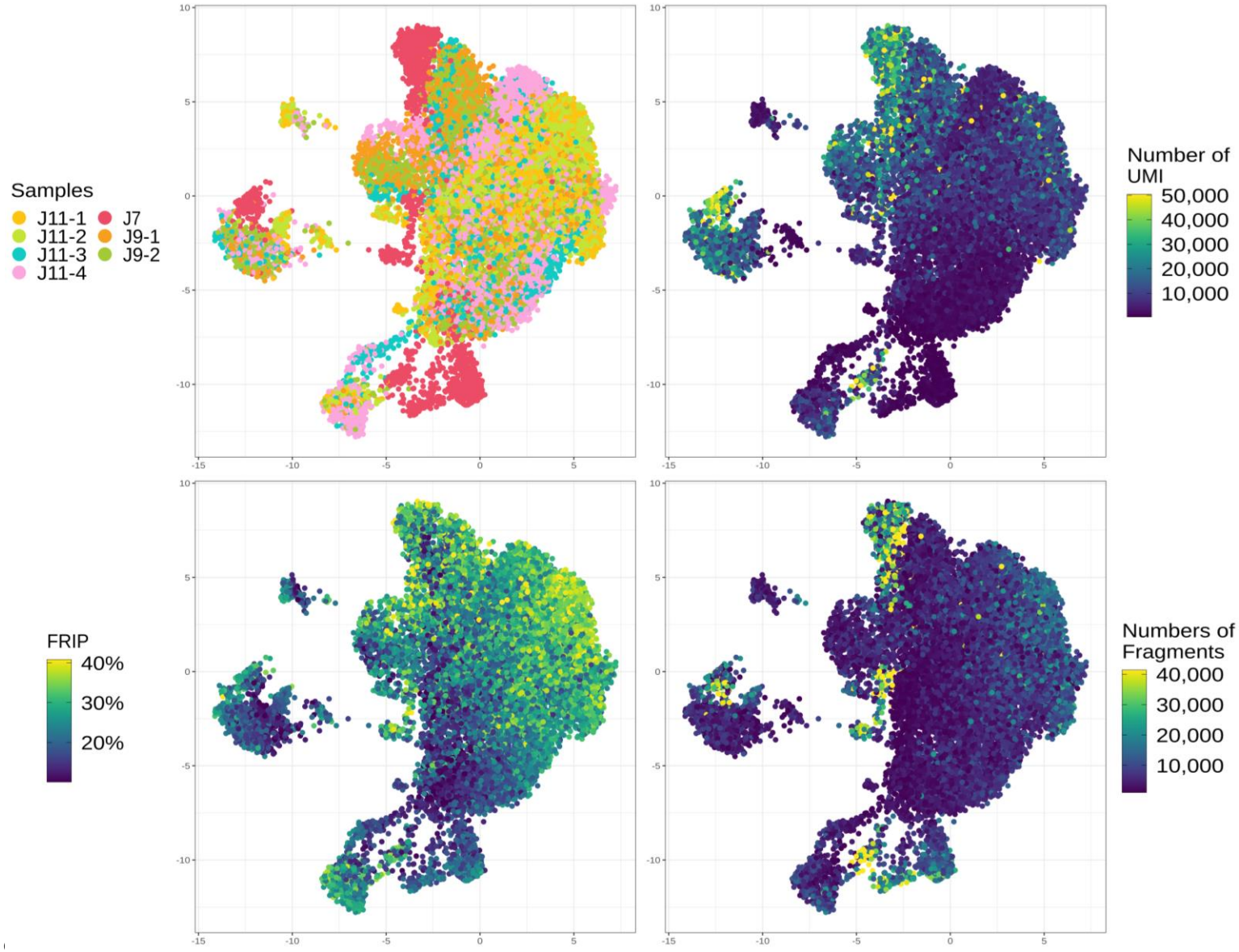
scOMICS: adding a layer of information to refine module of gene regulation

Quality Control

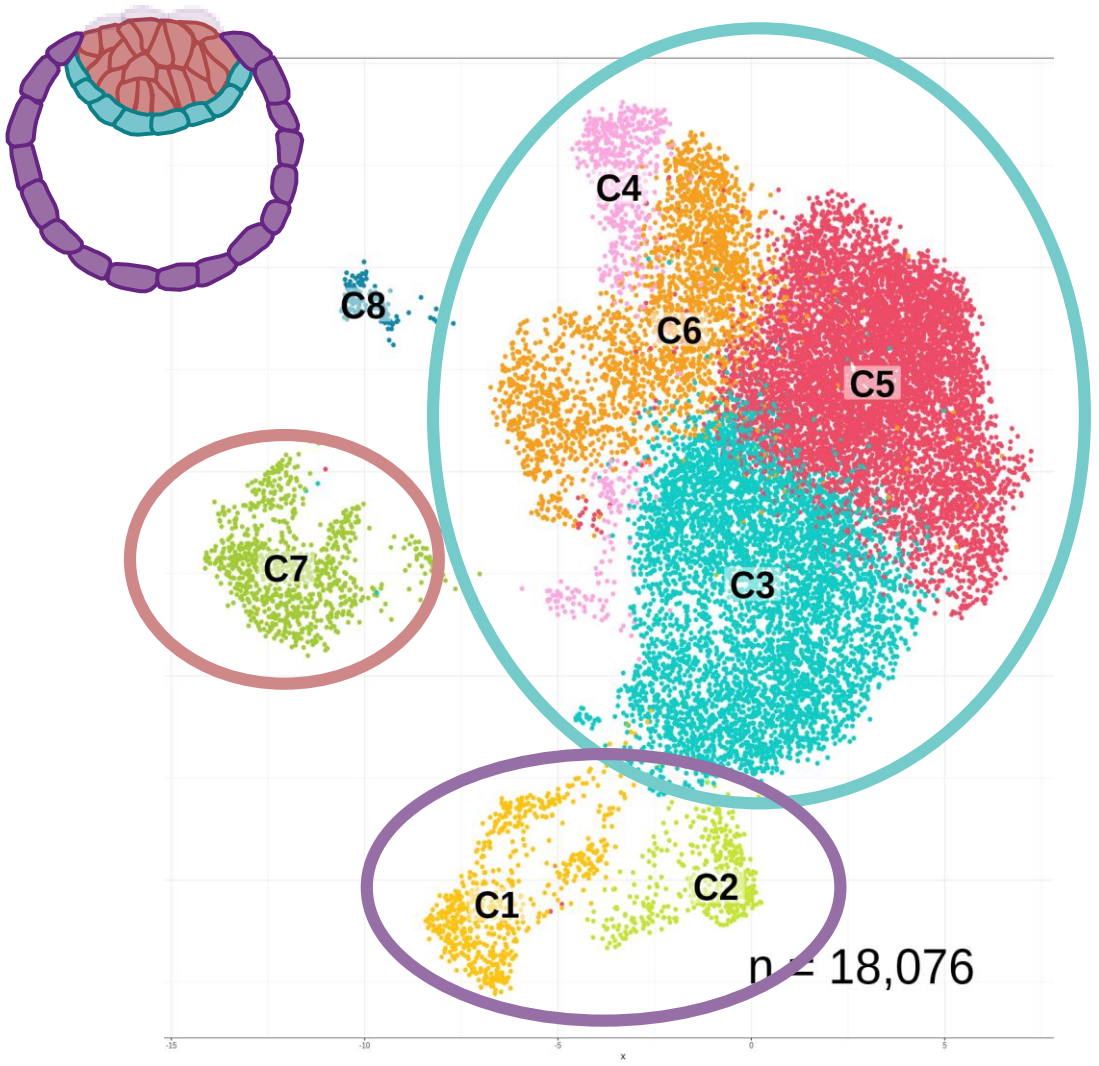
- Remove cells without both information (RNA+ATAC)
- Remove doublets (~5%)

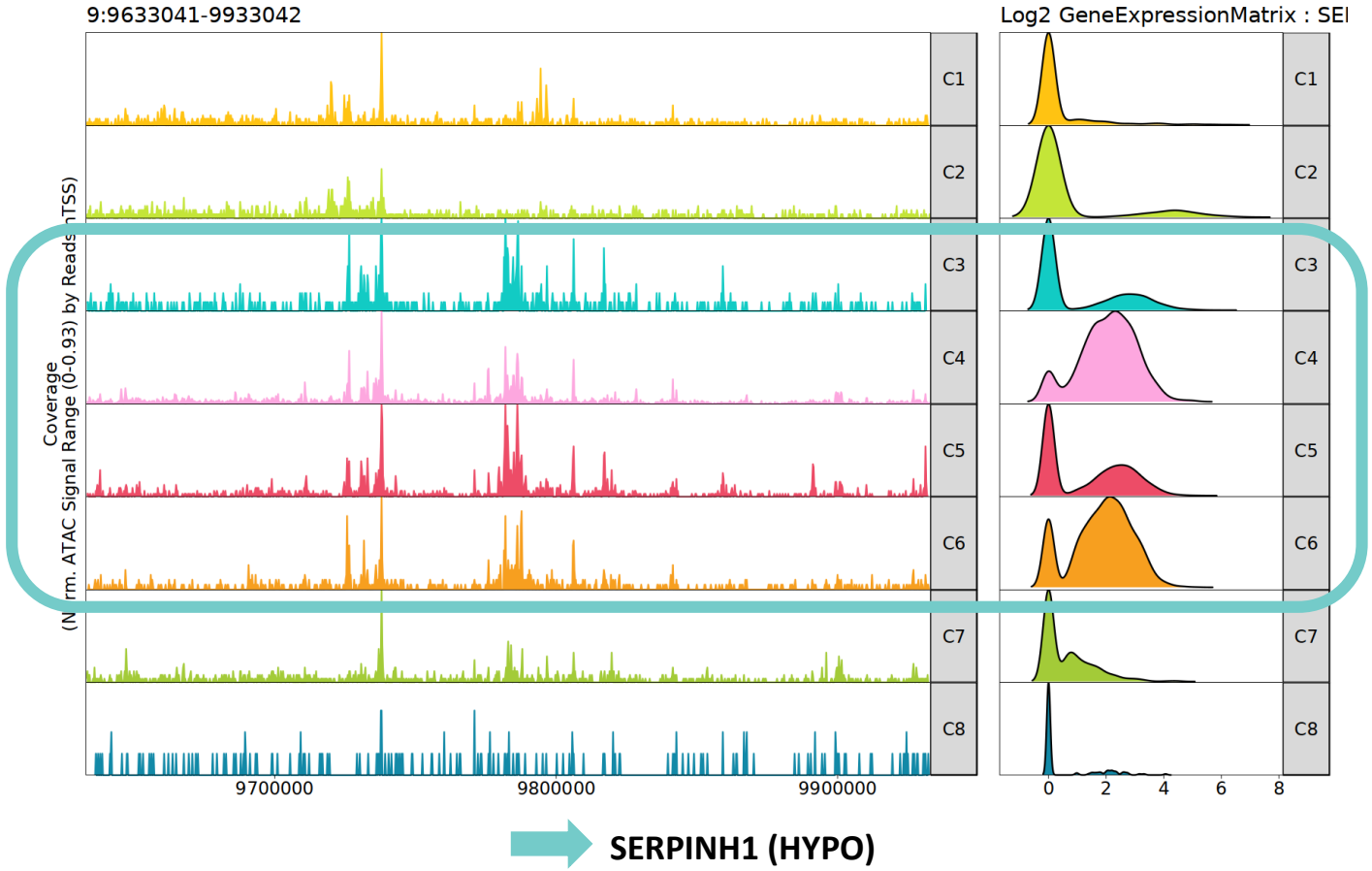
Select cells based on:

- UMIs number
- Fragments number
- FRIP score



scOMICS: adding a layer of information to refine module of gene regulation

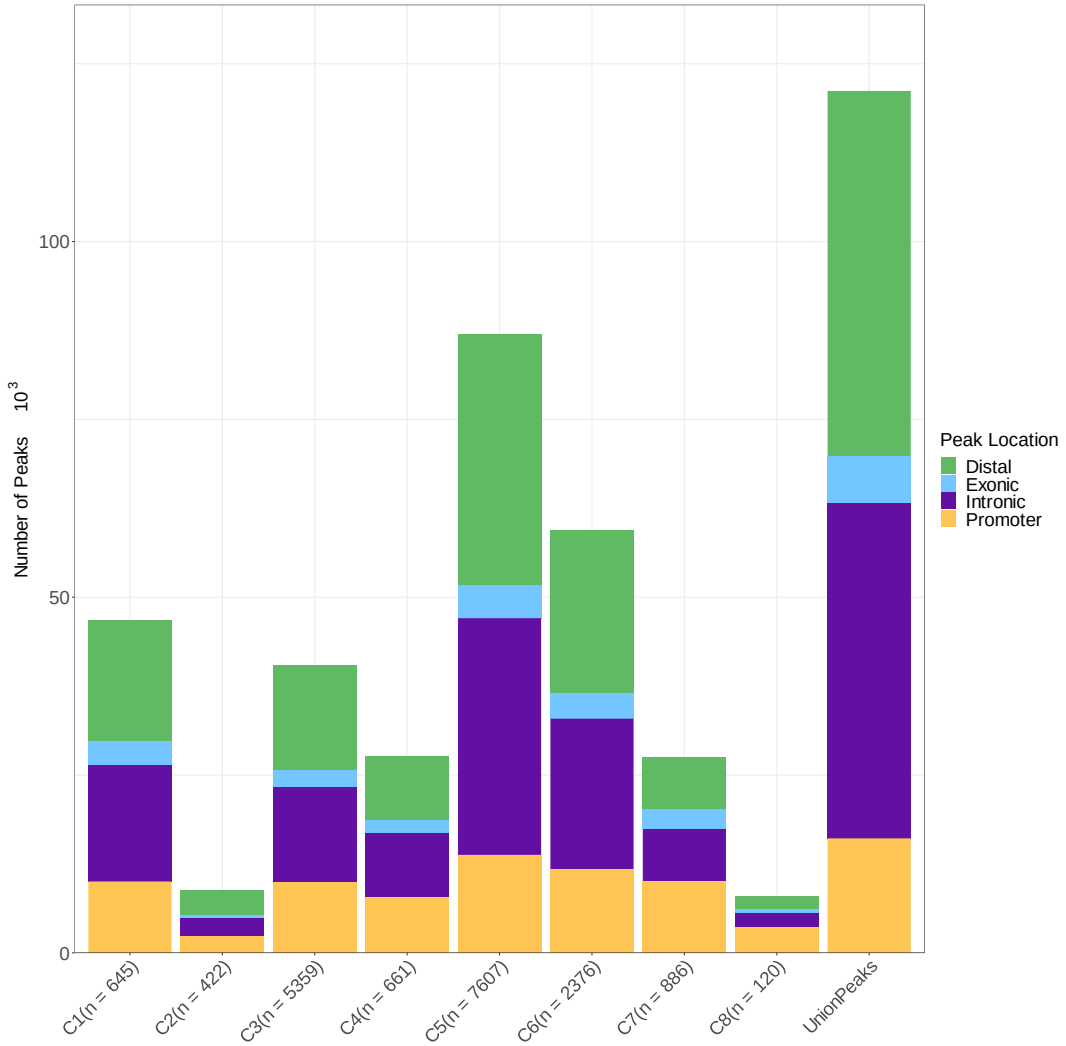




Dufour et al. unpublished

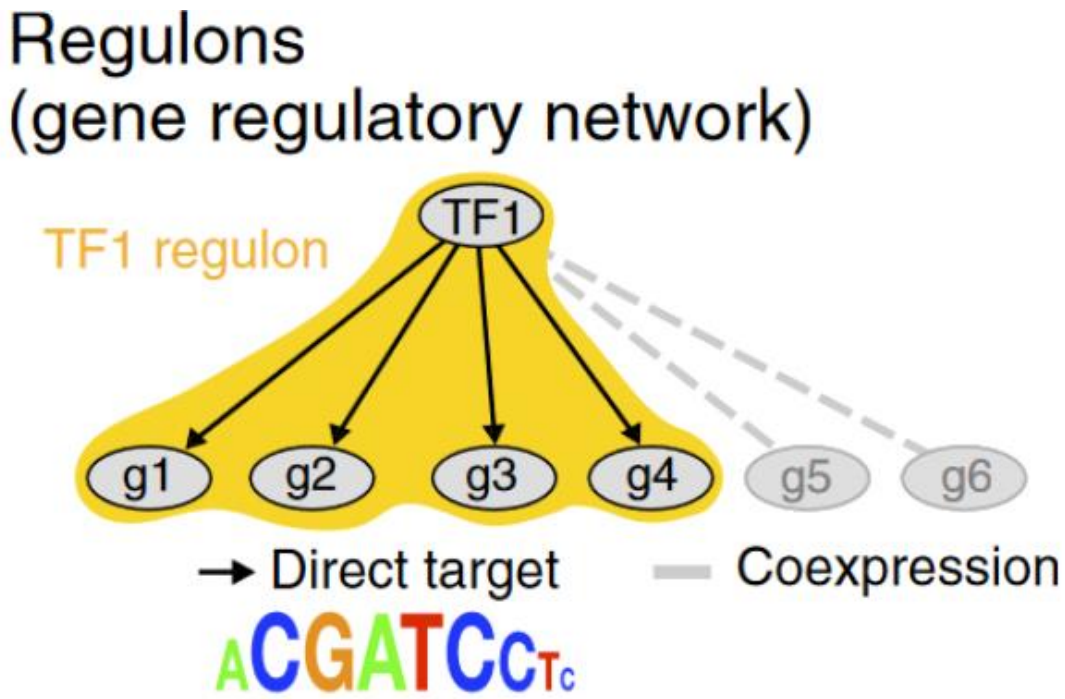
scOMICS: adding a layer of information to refine module of gene regulation

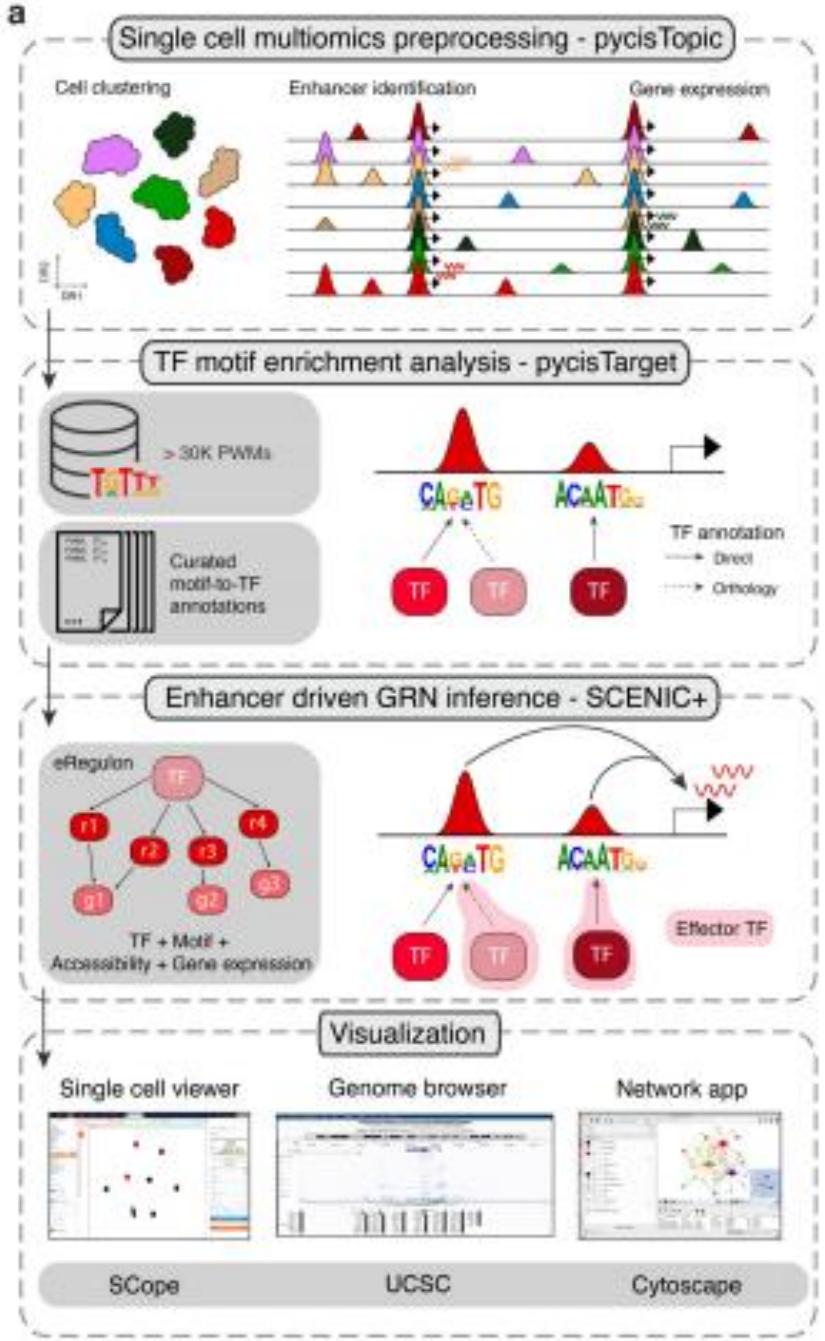
Peaks' localization



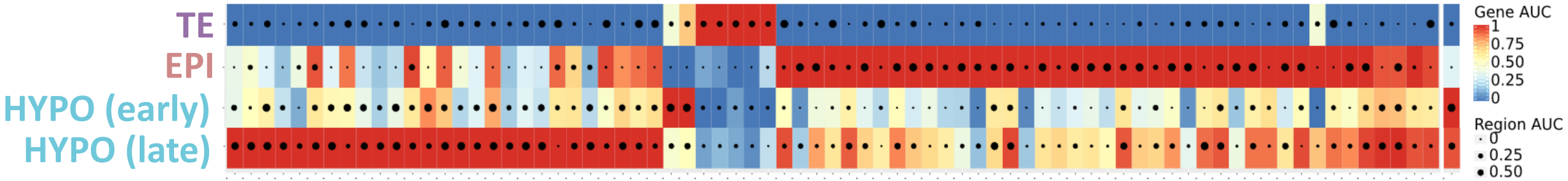
scOMICS: adding a layer of information to refine module of gene regulation

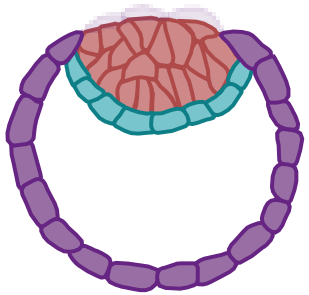
Identification of regulons By SCENIC+





Added value of omics data: selection of active regulons

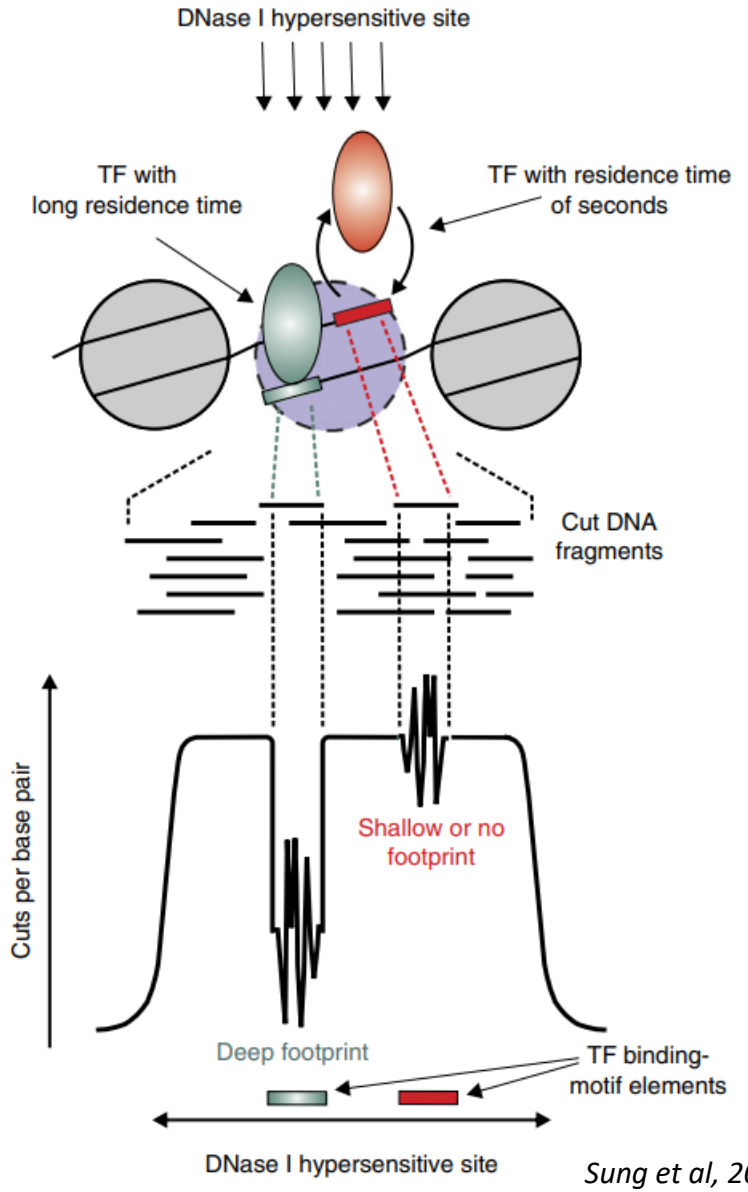




Dufour et al. unpublished

Added value of omics data: selection of active regulons

Detection of TFs' footprint

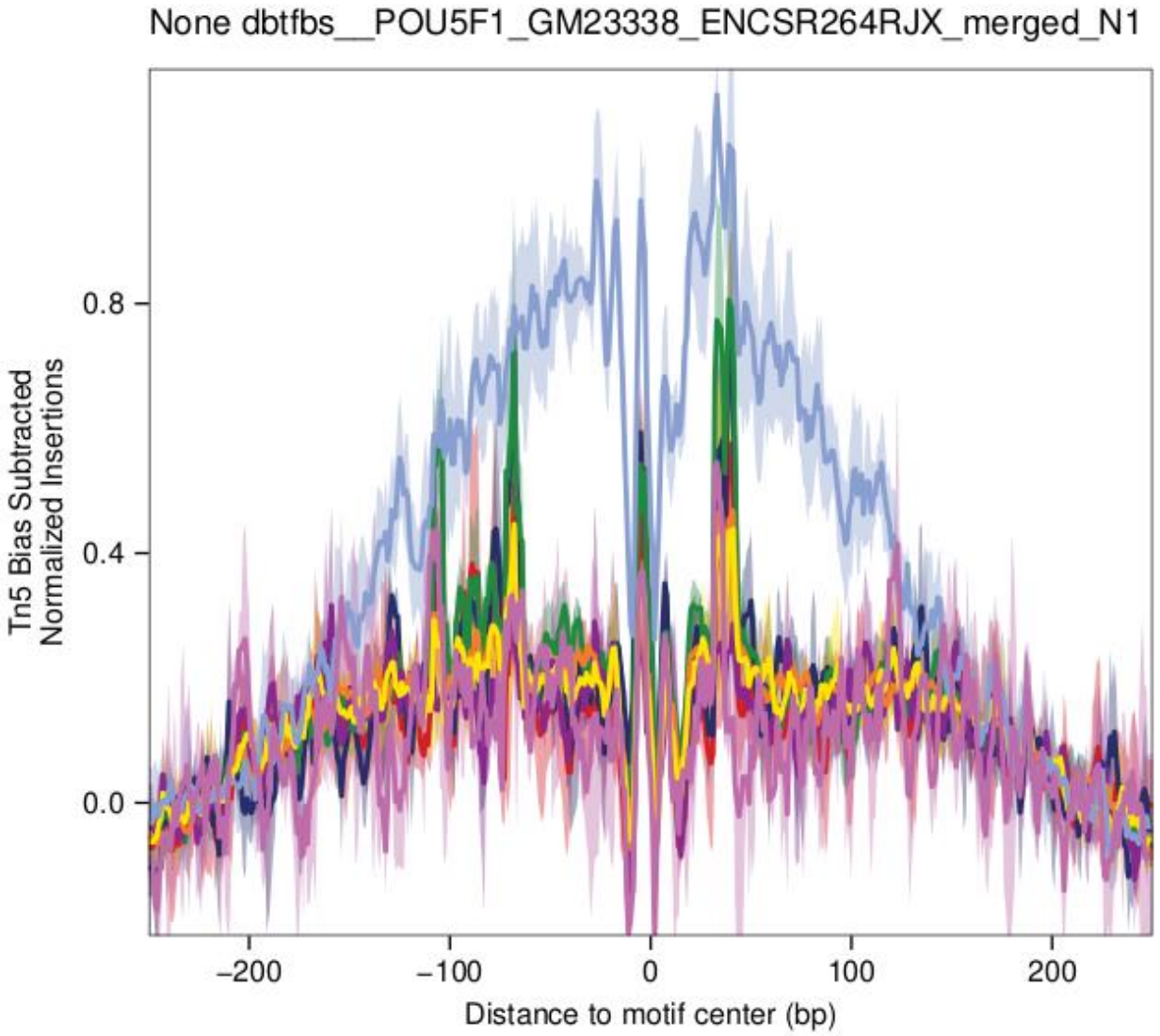


Sung et al, 2016

Dufour et al. unpublished

Added value of omics data: selection of active regulons

Detection of TFs' footprint

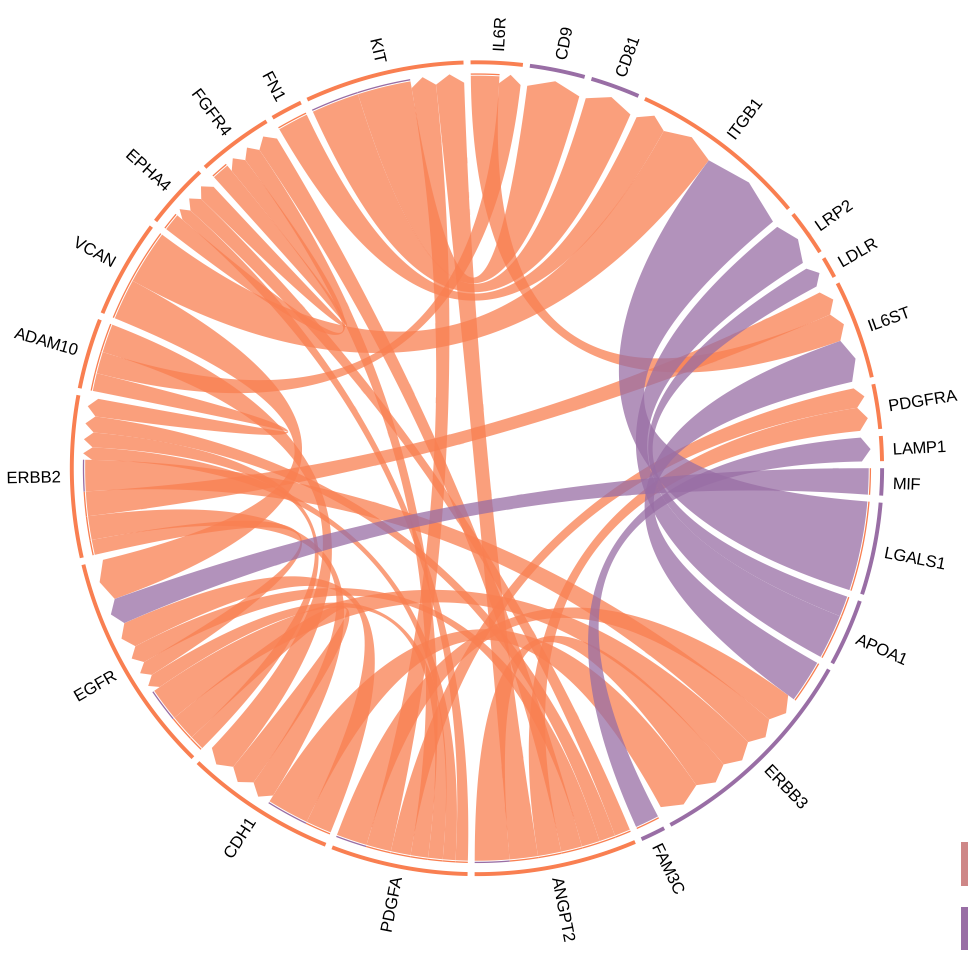


Dufour et al. unpublished

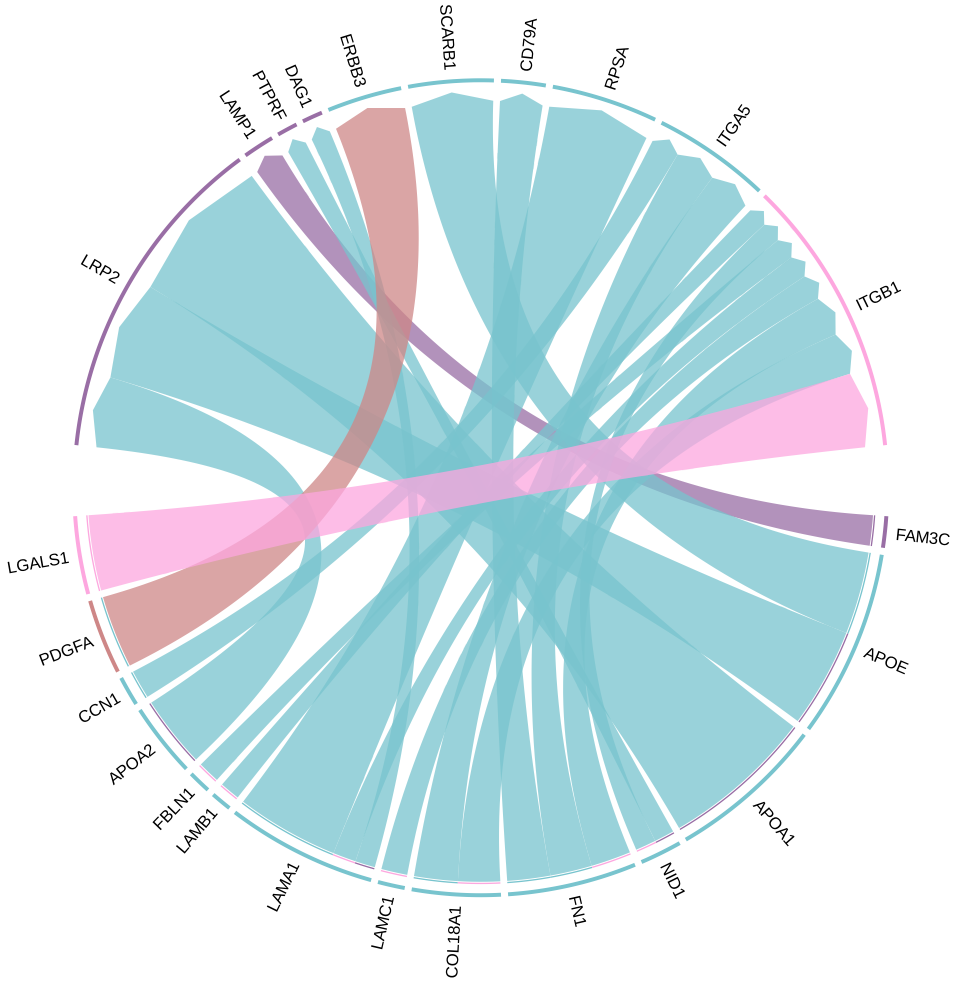
Sung et al, 2016

scRNAseq data provide cues to better understand the biology of the pig embryo

From ligand/receptor interactions to modules of gene regulation



E5

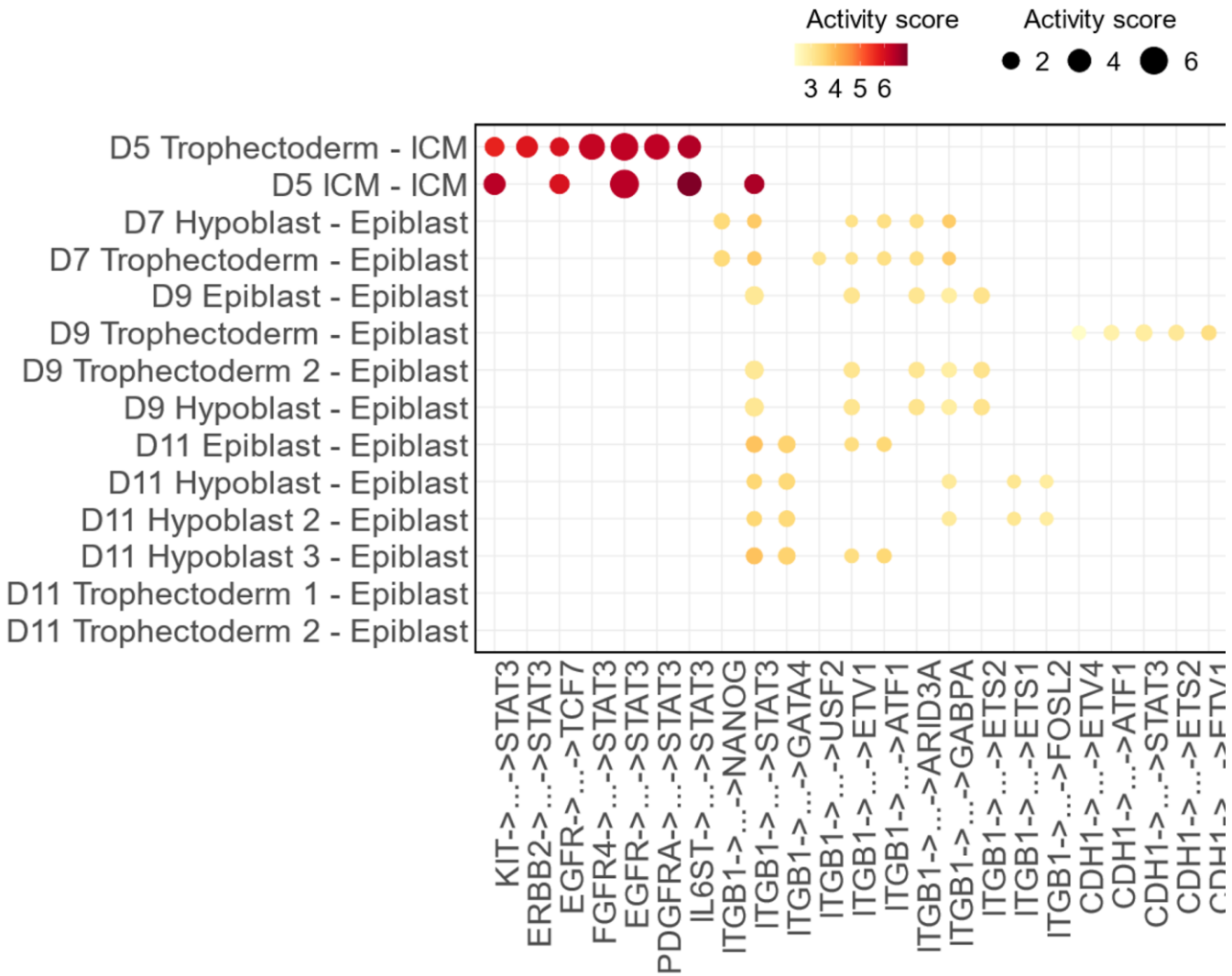


E7

- Epiblast
- Trophectoderm
- Hypoblast
- Pas de préférence

scRNAseq data provide cues to better understand the biology of the pig embryo

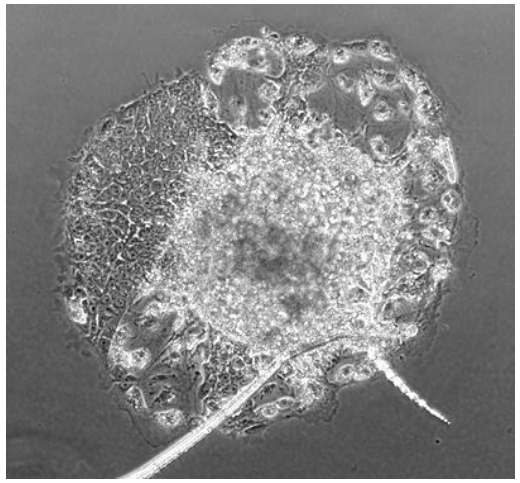
From ligand/receptor interactions to modules of gene regulation

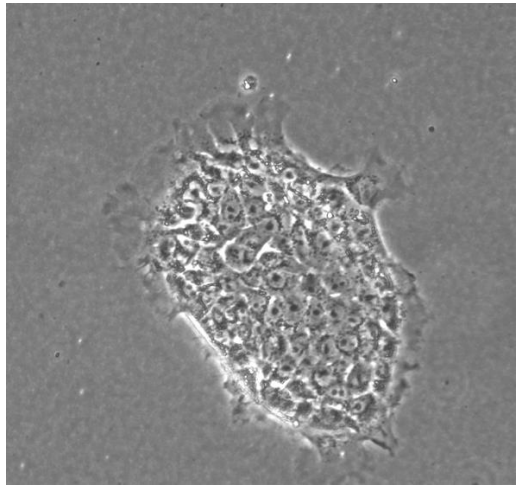


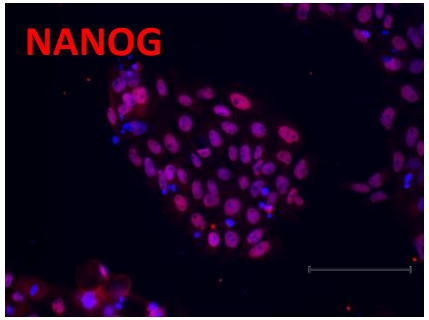
Dufour et al. submitted; https://doi.org/10.1101/2023.05.30.542847

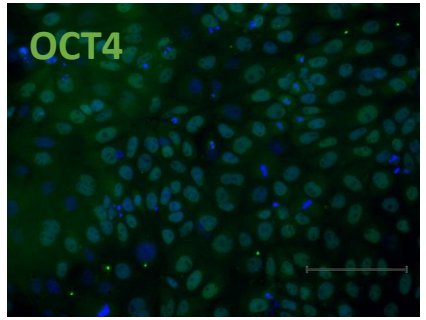
Omics plus-value: detection of poised states in pig ESCs

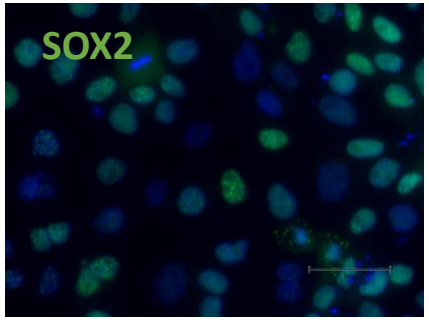
Pig ESCs

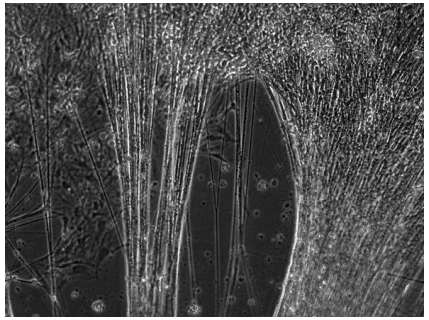


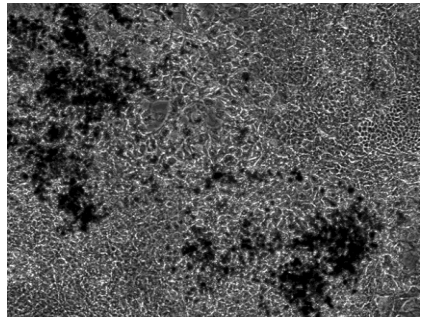


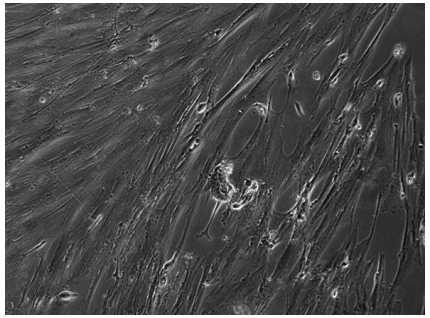










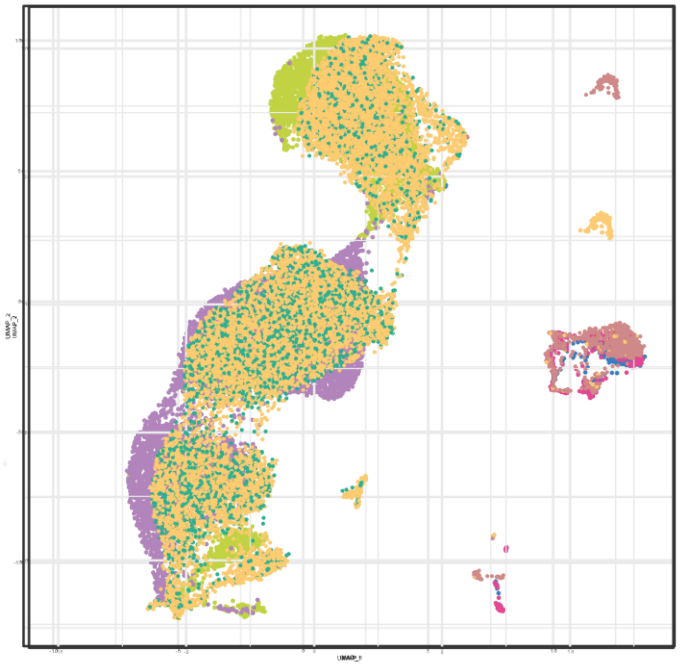


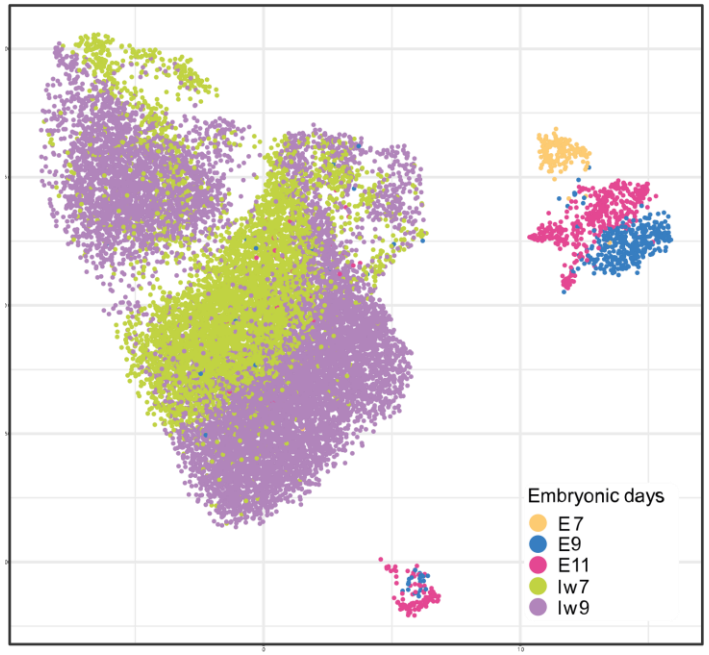
INRAE

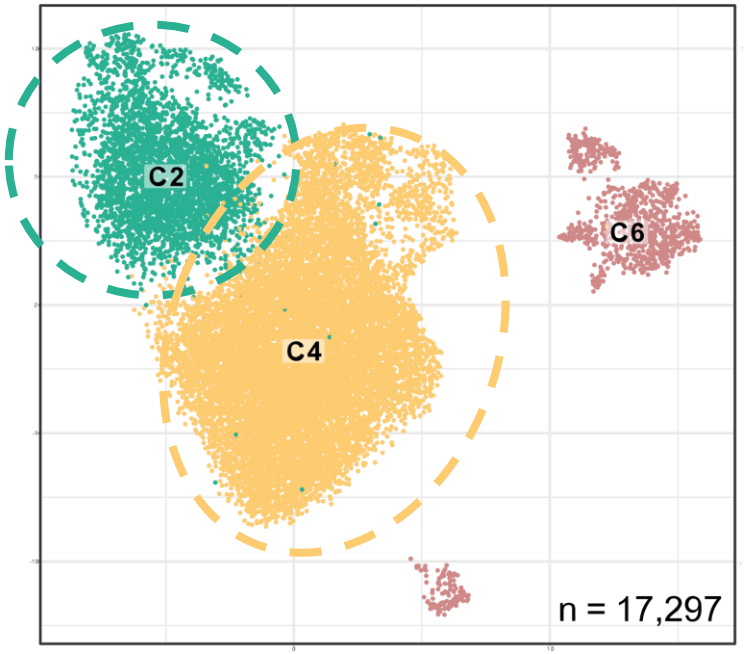
Dufour et al. unpublished

Omics plus-value: detection of poised states in pig ESCs

Cells poised toward a neural fate







scRNAseq

scOMICS

Cells poised toward non neural ectoderm & mesendoderm fate

Dufour et al. unpublished

Conclusions

Added values of omics vs gene expression:

- Identification of potential molecular interactions between embryonic cells and uterine fluids
- Better characterisation of gene regulatory networks at work in embryonic cells
- Validation of candidate regulons with motif footprints
- Detection of cell states not detectable by looking at gene expression only

Aknowledgements

Special thanks to Adrien Dufour (PhD student) and to people from the GALAC team in Jouy en Josas,

And to our collaborators:

- Sylvain Foissac and Cyril Kurylo, GenPHySE UMR1318 INRAE
- Jérome Artus, UA09 Inserm
- Yoann Bailly, Patrick Manceau, Stéphane Ferchaud, UE GenESI INRAE
- Thomas Fröhlich, GeneZentrum LMU, Munich, Allemagne
- Ramiro Alberio, University of Nottingham, UK
- Frederic Martins and Claire Koechly, GeT-PlaGe, INRAE

Our funders: