Multiomics analysis of pig pre-implantation embryo

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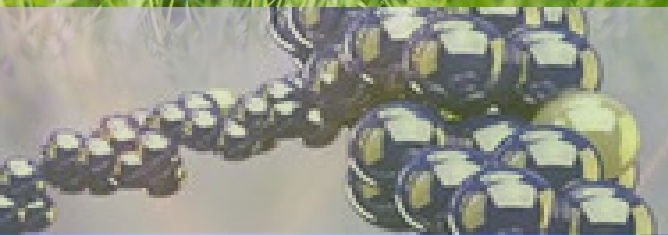


Micr genomics 2023

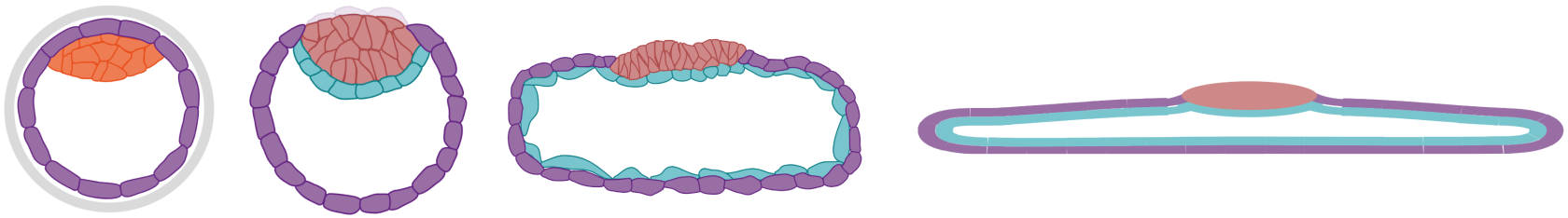
INRAE

UNIVERSITÀ DEGLI STUDI FIRENZE

Problematics

- The use of pluripotent stem cells in livestock is still limited by technological hurdles
- The optimised modes of obtaining and maintaining pluripotent stem cells for rodents and humans are not adequate for the porcine species

Working hypothesis



Early blastocyst | Hatched blastocyst | Spherical blastocyst | Ovoid blastocyst

100µm | 100µm | 1000µm | 5000µm

Time post fertilisation (days)

Pig	6	7-8	9-10	11-12 — 16 = implantation
Cow	6-7	8	9-10	11-13 — 20 = implantation
Mouse	3.5	4	5.5 = implantation	
Human	5.5	6.5	8 = implantation	

Trophectoderm

Epiblast (pluripotent cells)

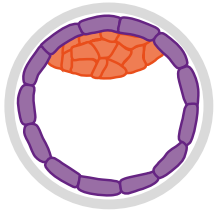
Hypoblast

Working hypothesis

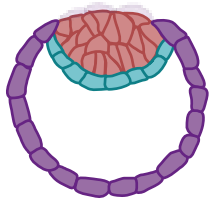
Paracrine regulations specific to pig embryonic development are not taken into consideration for the establishment of a pig's pluripotent lines

Embryonic pluripotency regulatory networks are not necessarily conserved in mammals

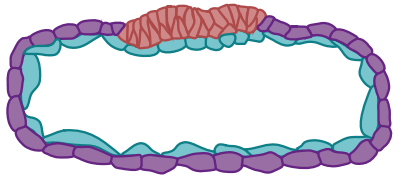
Methods



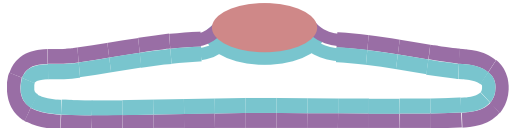
Early blastocyst



Hatched blastocyst



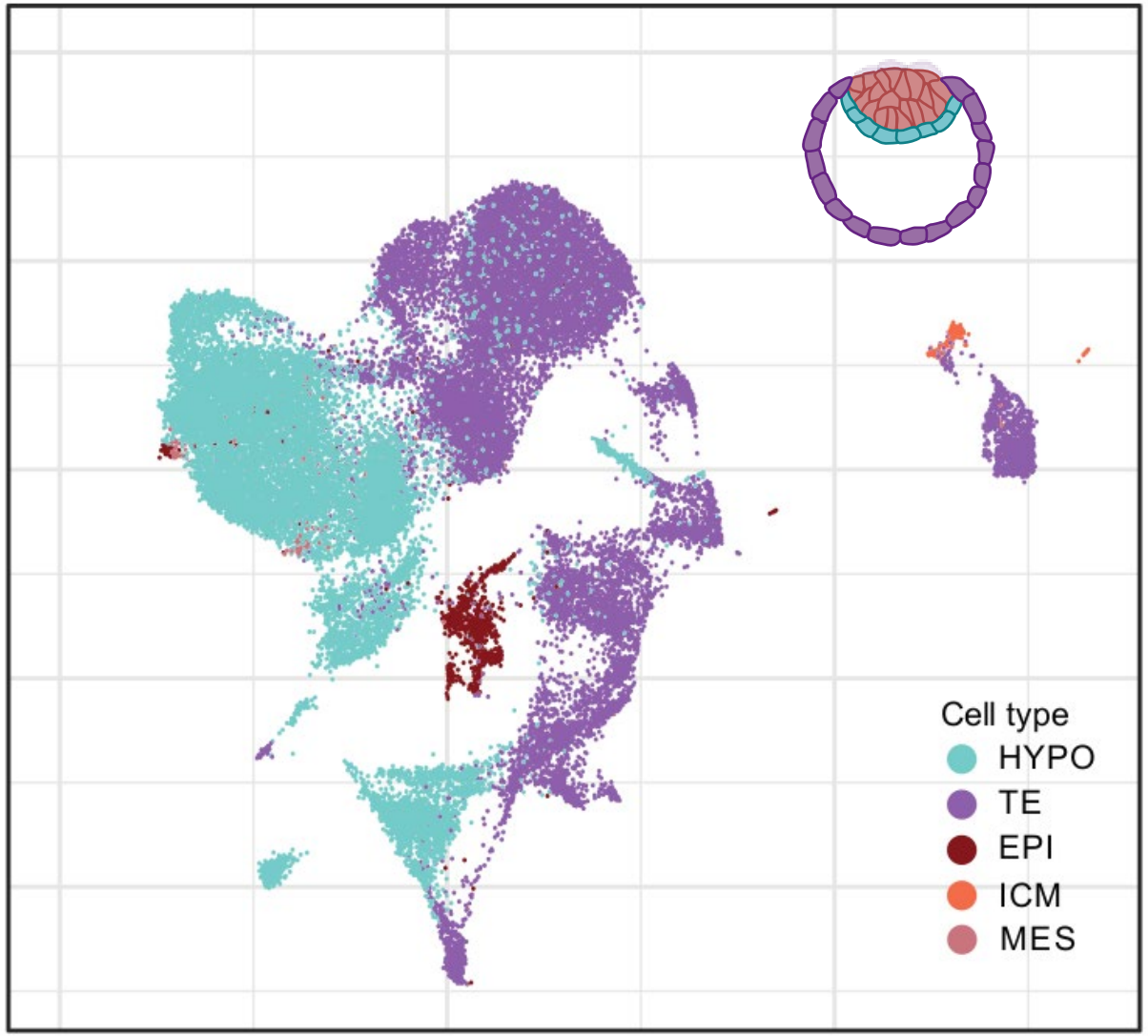
Spherical blastocyst



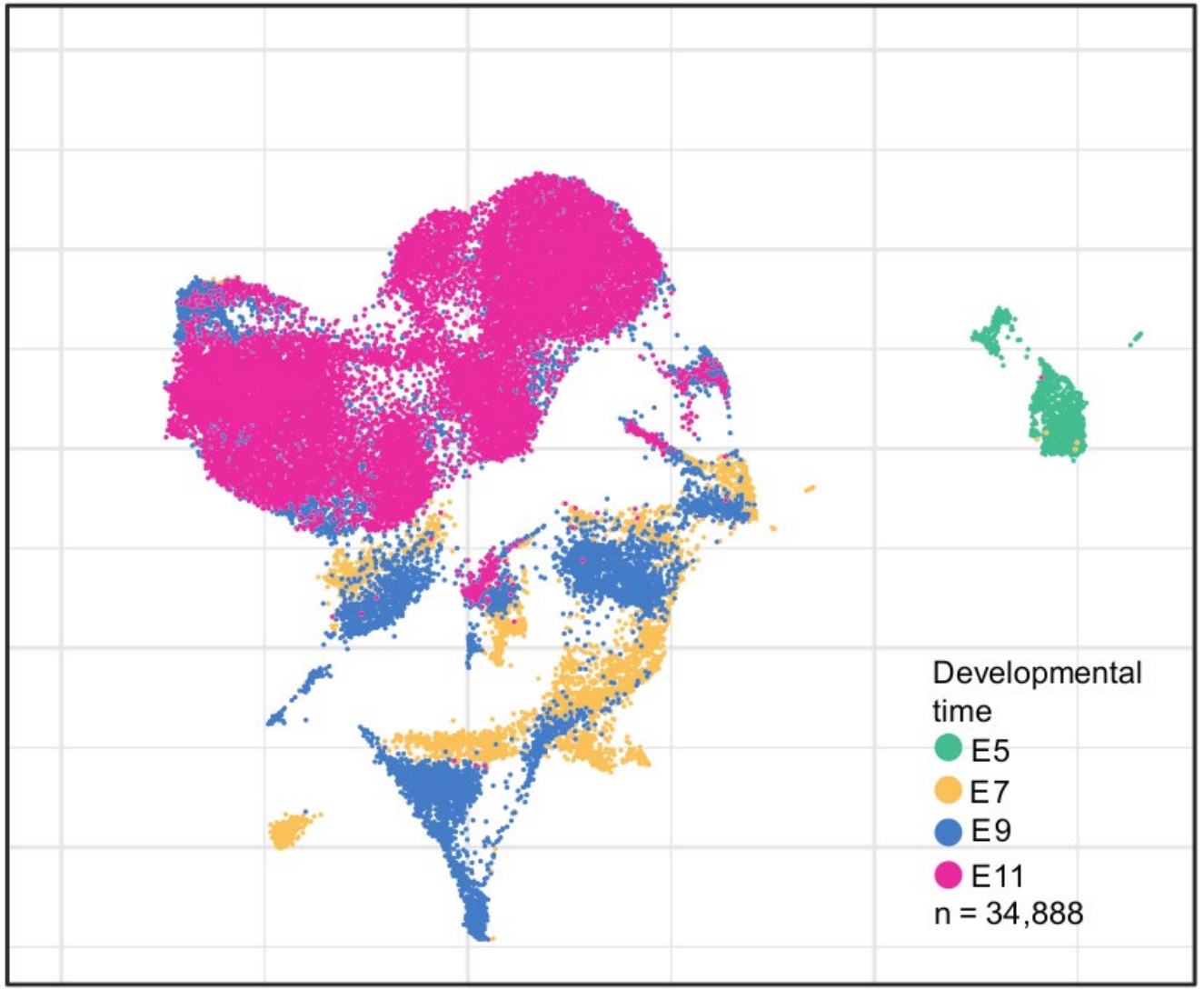
Ovoid blastocyst

Single-cell RNAseq (10x Genomics)	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 (10x Genomics) (scATAC-seq + scRNA-seq)	0	1 library (~2000 cell's nuclei)	2 libraries (~4000 cell's nuclei)	4 libraries (~8000 cell's nuclei)

Identification of the main cell populations



Visualisation of cells within their lineage

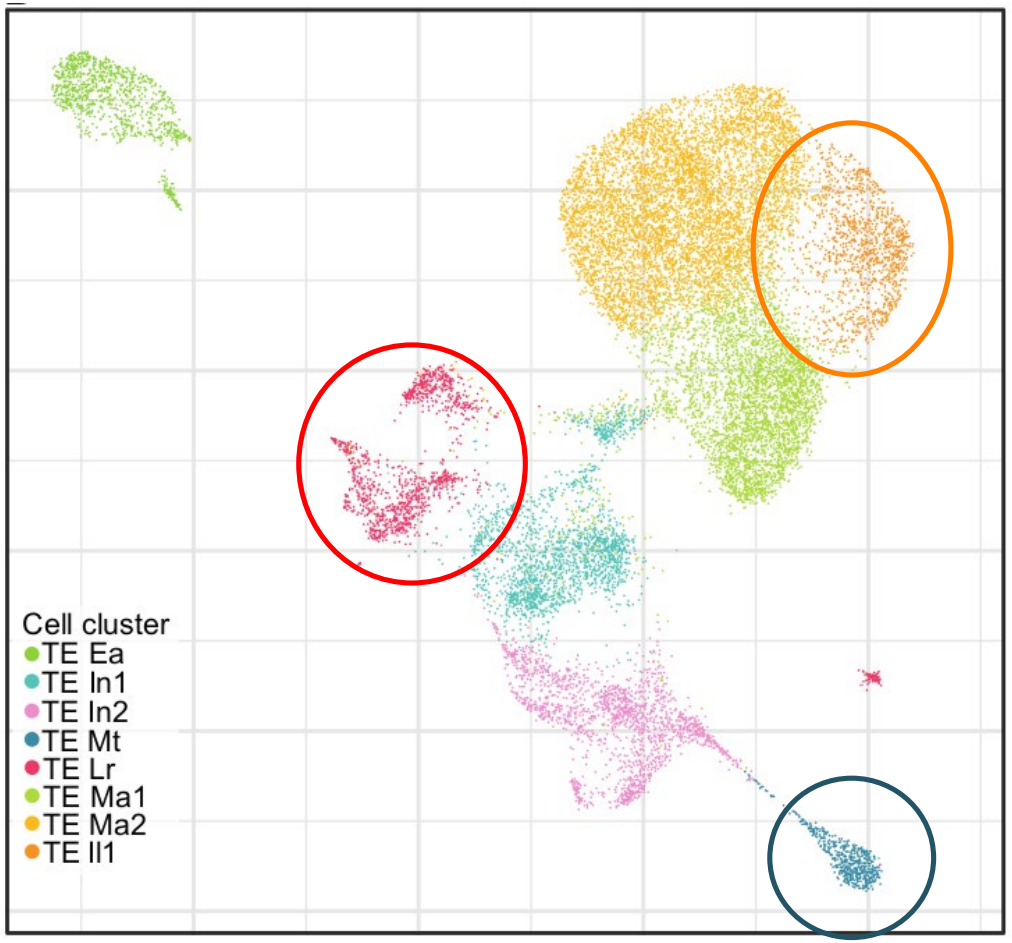


Visualisation of cells within their stages

Dufour et al. bioRxiv doi.org/10.1101/2023.05.30.542847

Caracterisation of sub-populations

- Our analysis allowed us to characterise sub-populations
- For the TE, we identified three new subpopulations:
  - TE IL1: IL1 secreting cells
  - TE LR: potential stem cell pop.
  - TE Mt: apoptotic cells that may correspond to the polar TE also named rauber's layer

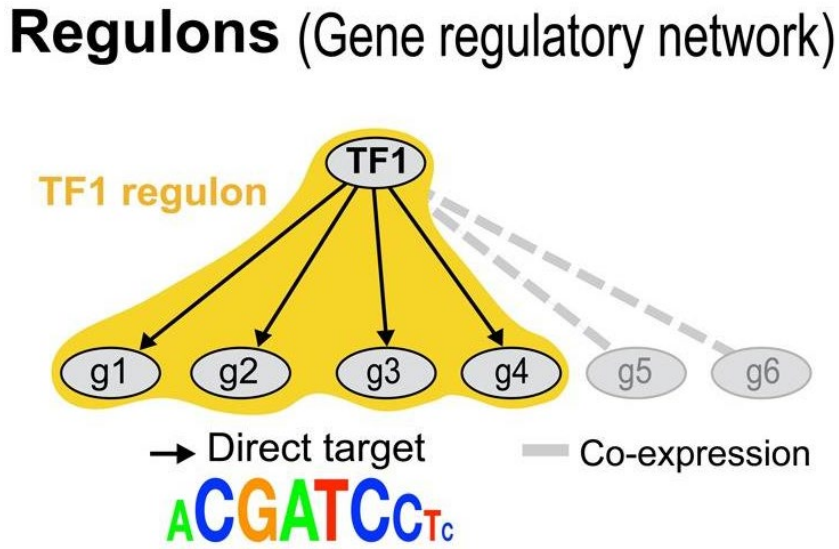


Visualisation of trophectoderm cells within their clusters

Dufour et al. bioRxiv doi.org/10.1101/2023.05.30.542847

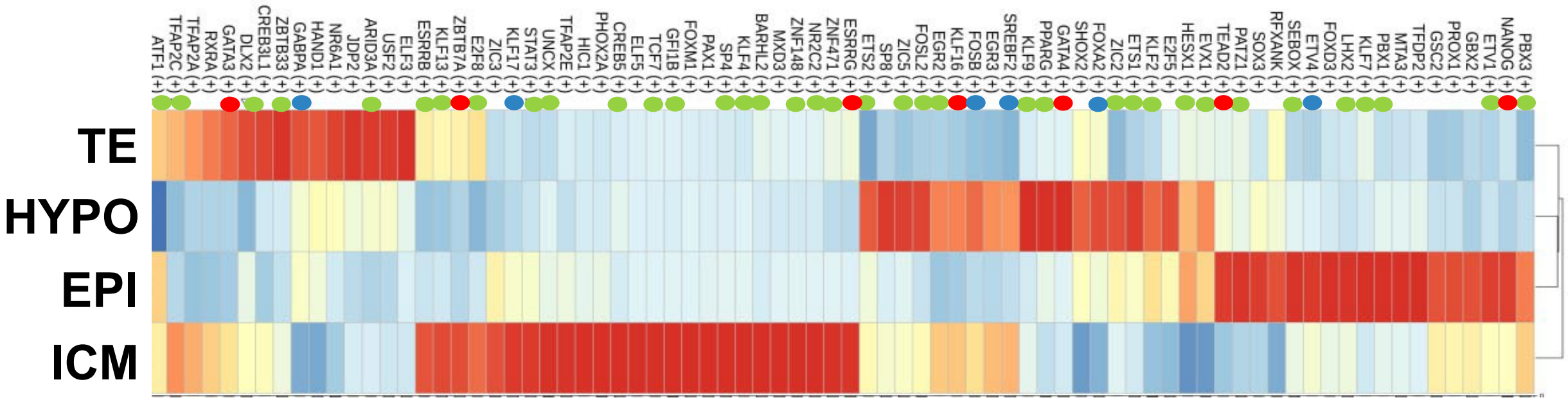
Identification of modules of regulation (regulon)

- Identification of regulons: aggregation of transcription factors and genes with a common TF-binding motif with SCENIC
- Meta-analyse using human and pig datasets



Aibar et al, 2017

Identification of modules of regulation (regulon)



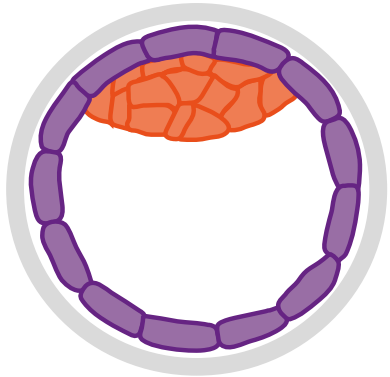
- New regulons poorly known for the biology of embryonic and extra-embryonic cells: *TFPD2 (EPI)*, *GABPBA (TE)*, *FOSL2 (HYPO)*

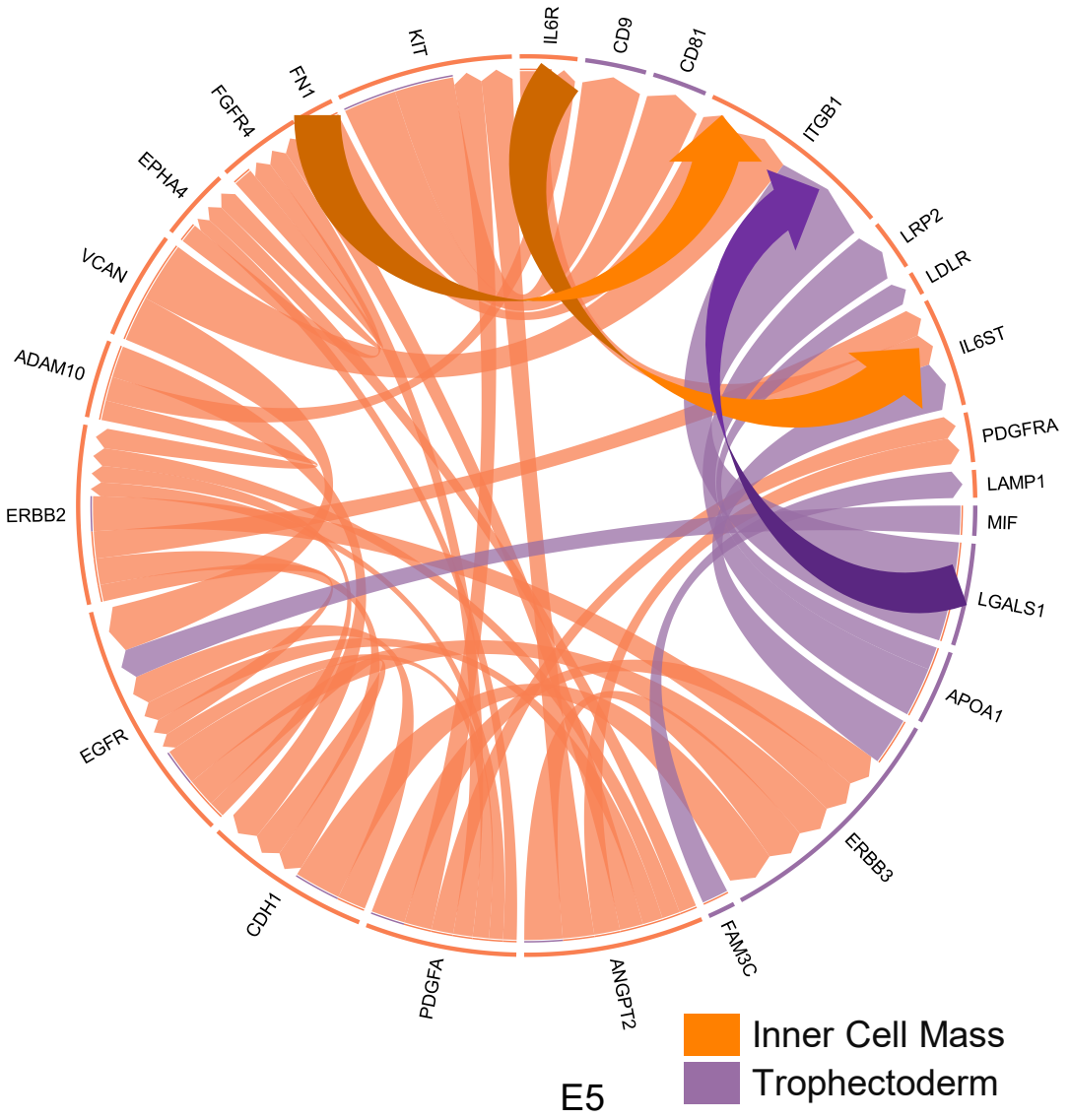
Commons with :

- Humans
- Pigs
- Both

Identification of Cell-Cell communication

- Allow us to retrieve known pathways in pigs (Jak-Stat): autocrine
- Less known pathways like *ITGB1* (autocrine and paracrine)





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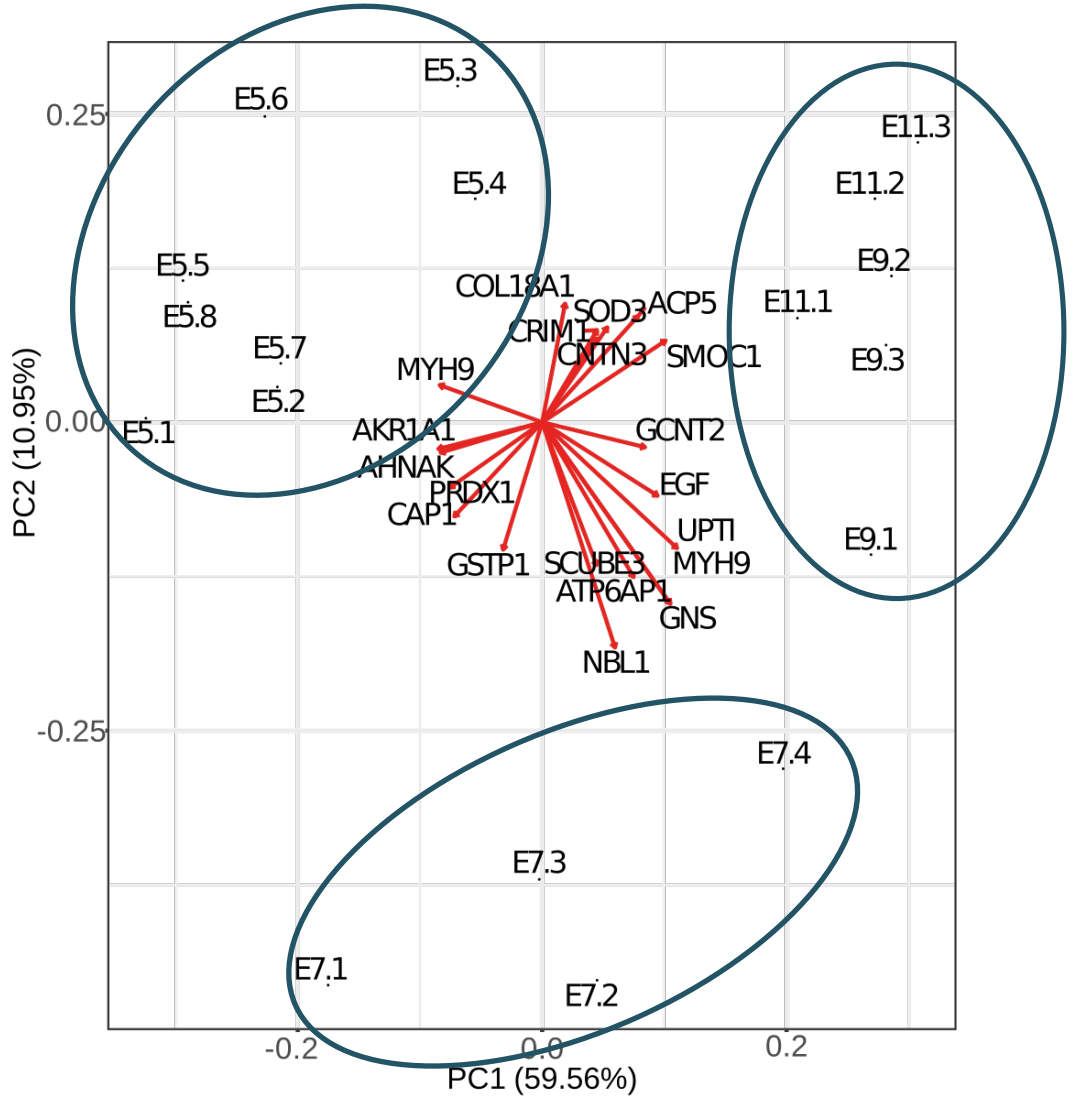
Proteomics fluids analysis

- 18 Samples from the same sows as scRNAseq embryos
- Separation of fluids according to the stage

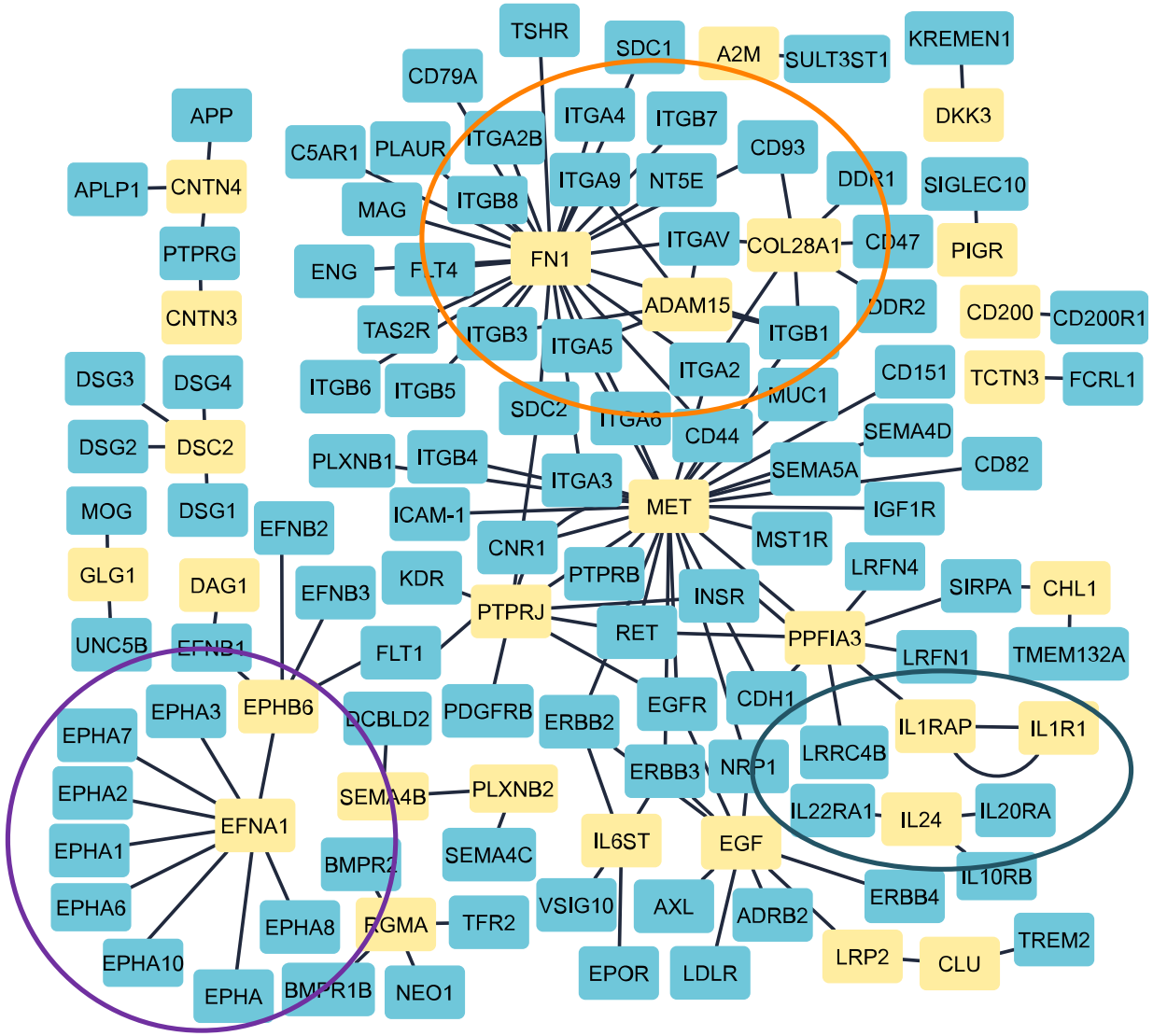


Visualization of uterine fluid samples

Interaction between uterine fluids and single-cell data

- Connect fluid proteomes and cell surface receptors from transcriptomics
- Known mechanism of interplay between endometrium and embryo via interleukin: IL1
- Importance of ECM components: FN1 and COL
- EPHA/EFNA signalling pathways

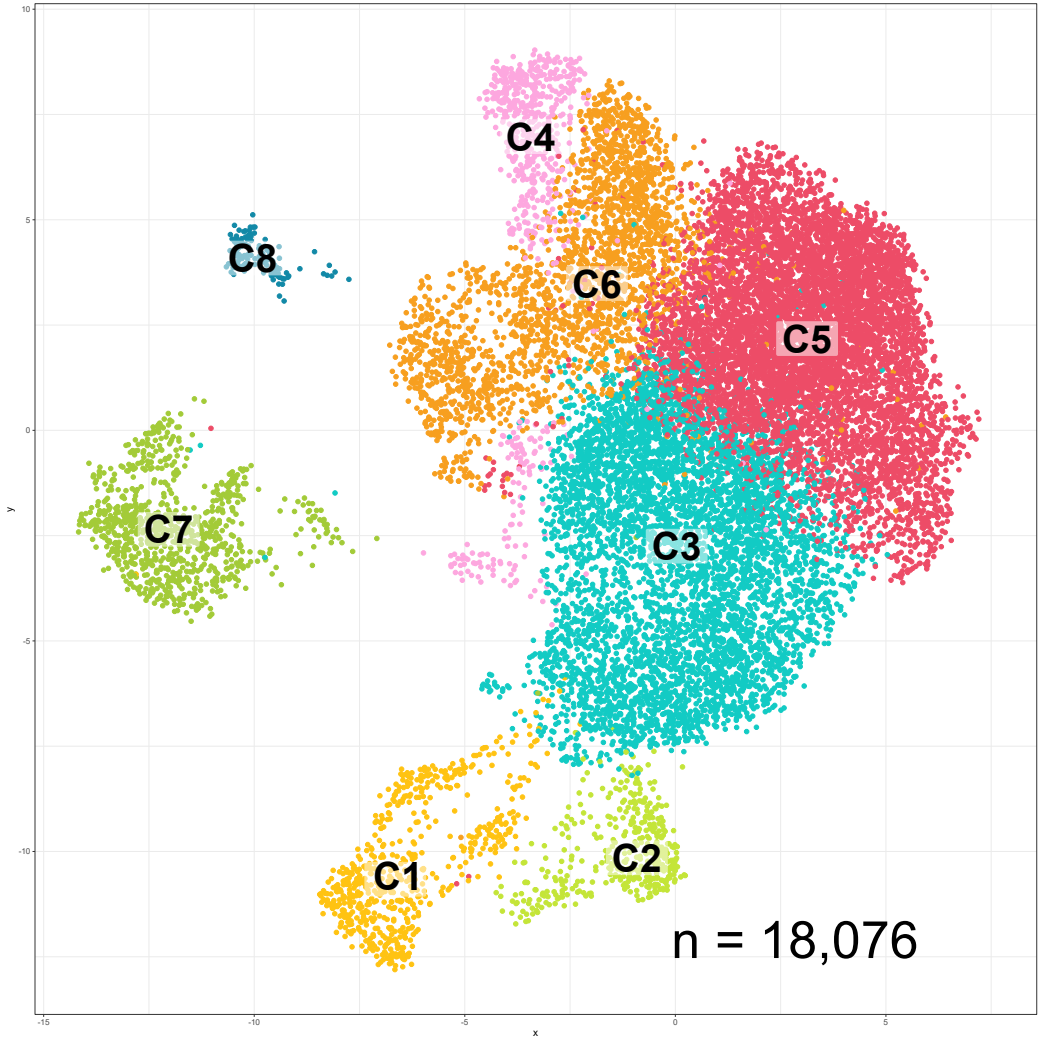
Ligand: uterine fluid
Receptor: trophectoderm or epiblast



Interactions between uterine fluid and trophectoderm receptors

Adding a layer of information using multiomics to strengthen our conclusions

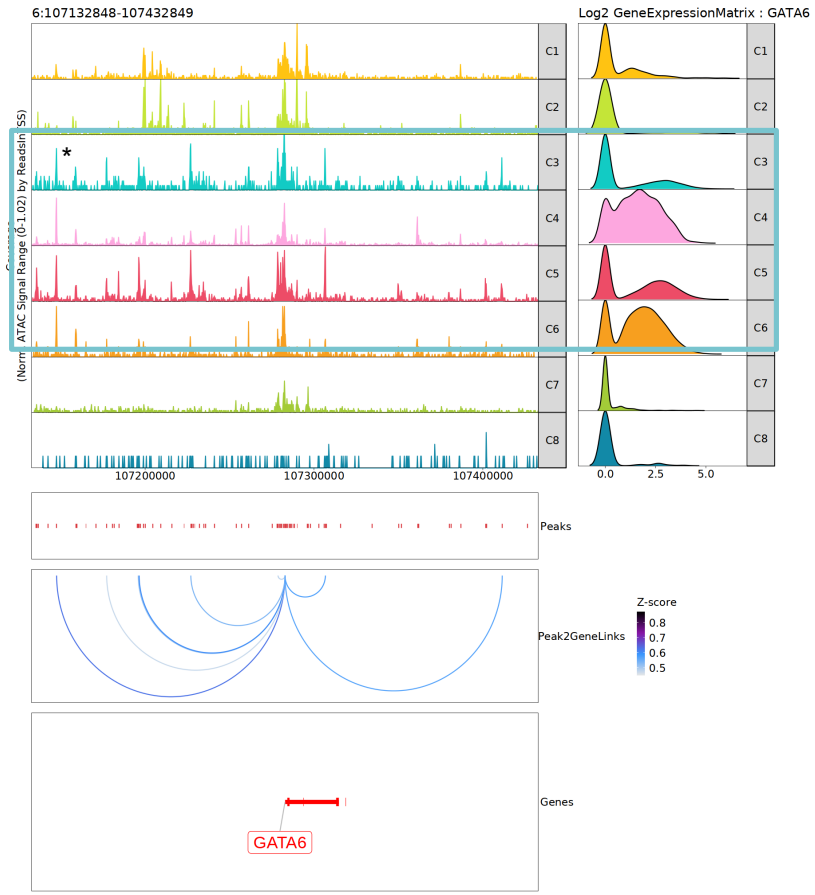
ScMultiOmics analysis



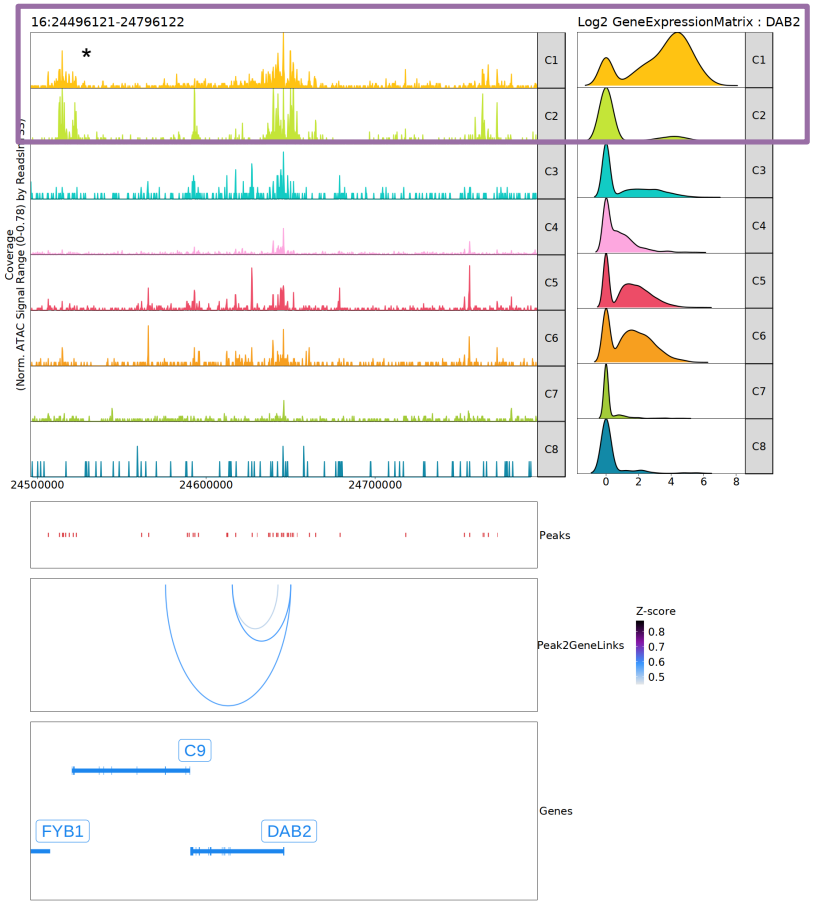
Visualisation of cells within their clusters

Clusters' Identification

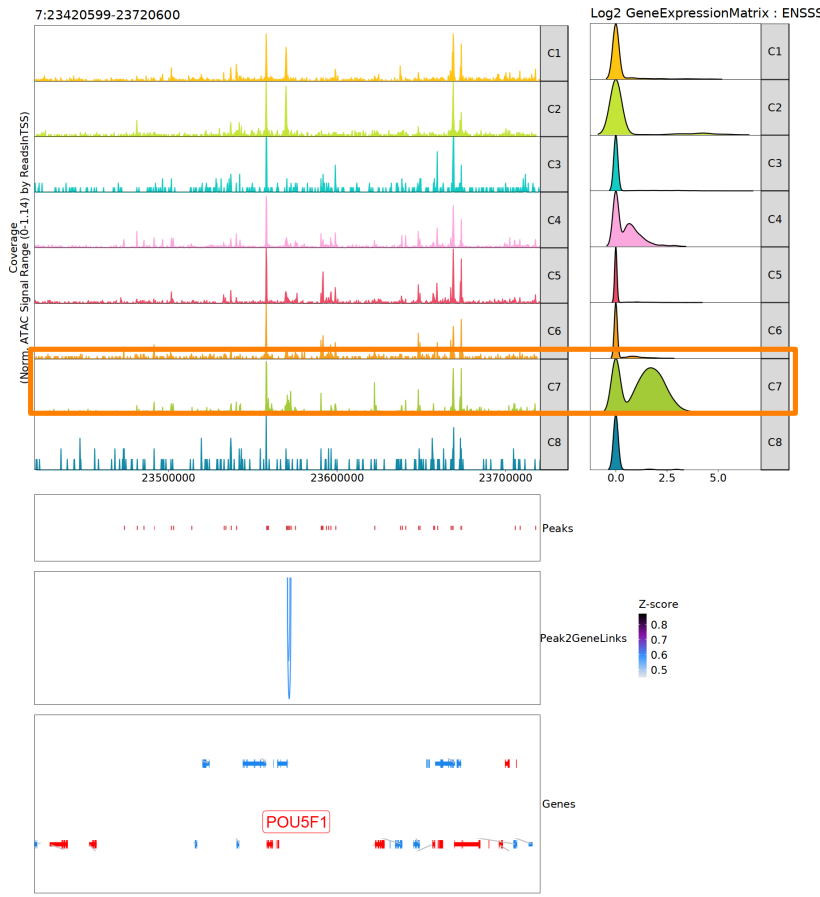
GATA6 (HYPO)



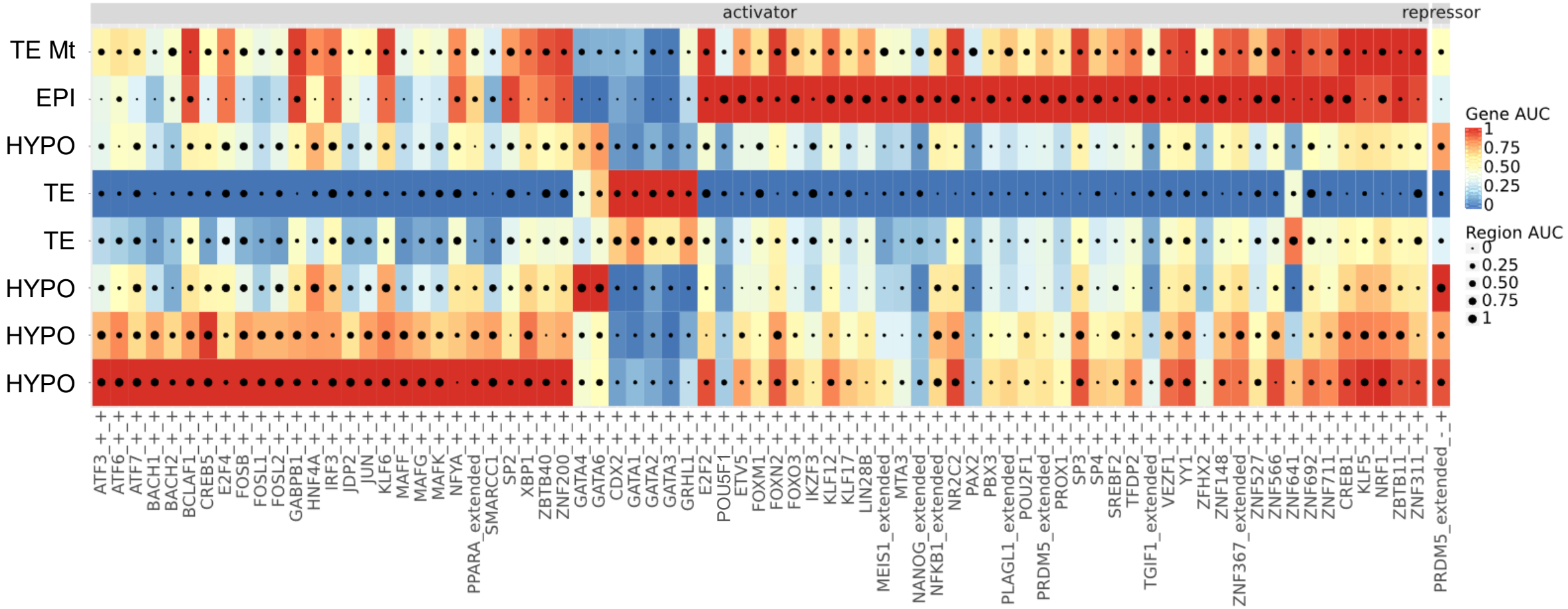
DAB2 (TE)



POU5F1 (EPI)



Identification of regulons using SCENIC+

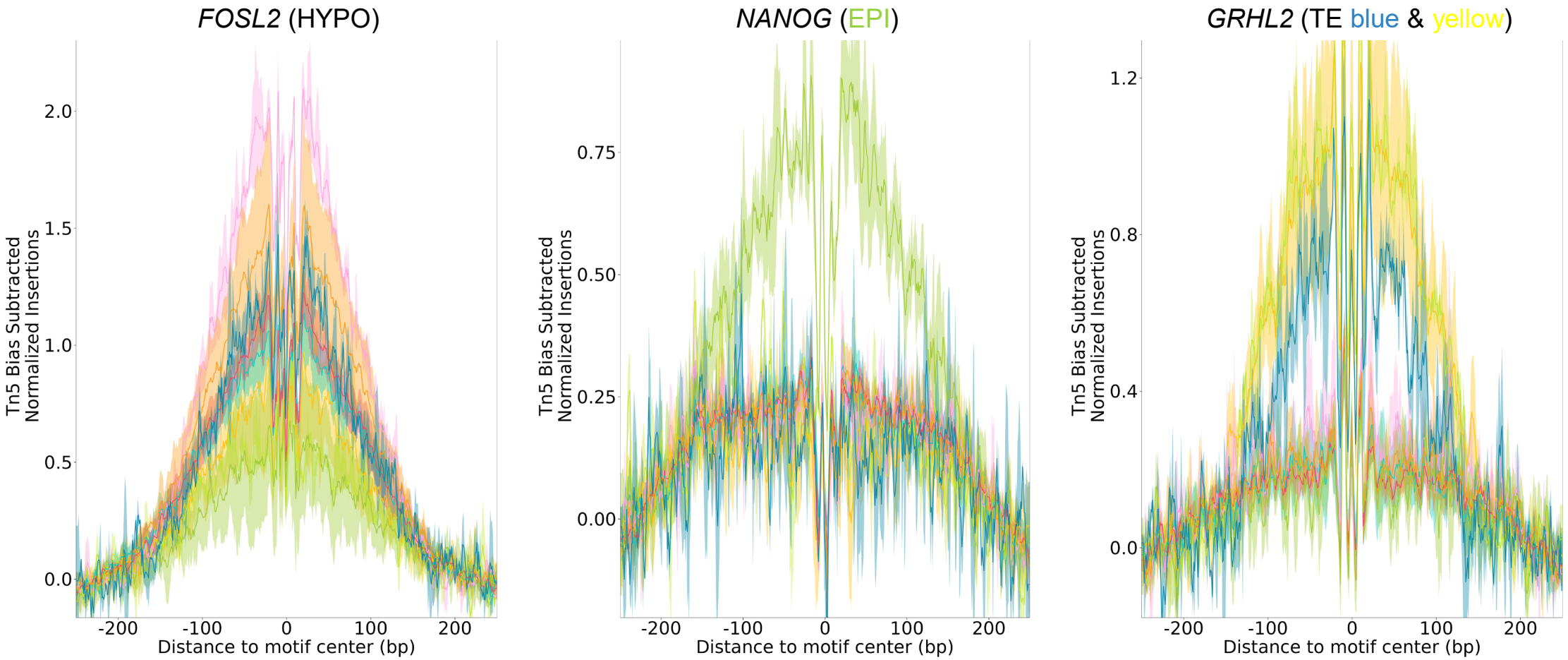


Activity score of regulons identified in terms of decay and gene expression

- We refined our previously identified (65% similarity) and literature knowledge (*CDX2*, *POU5F1*…)
- We connect those regulons to regions accessibility

Footprints confirm the presence of key TFs for each cell population

- Footprint match eRegulons and literature knowledge
- Give another level of detail with TF persistence



Conclusions

- We identified new TE populations in the pig blastocyst
- Identification of key paracrine and autocrine interactions between EPI, TE and uterine fluids
- Multiomics allowed us to refined our regulons identification
- Better characterisation of regulons with regions accesibility and motif Footprint

Thanks to

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- Cyril Kurylo, GenPHySE UMR1318 INRAE
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