



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

Hervé Acloque

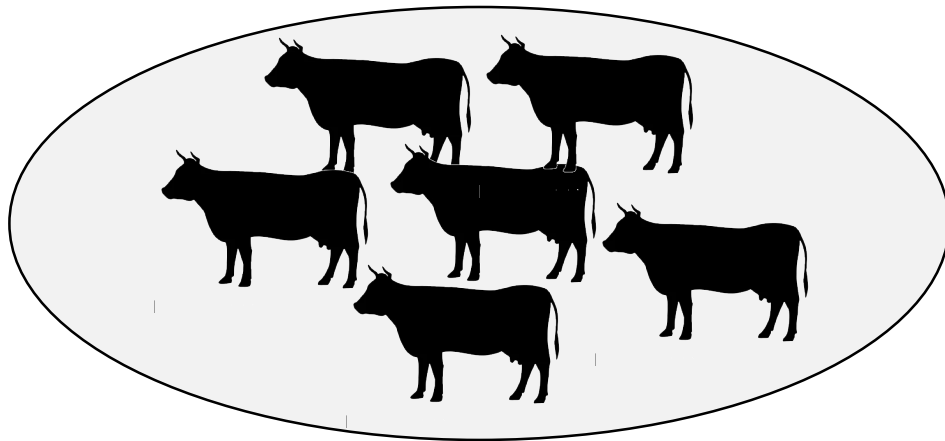
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➤ Pluripotent stem cells in livestock: a platform for high throughput phenotyping

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

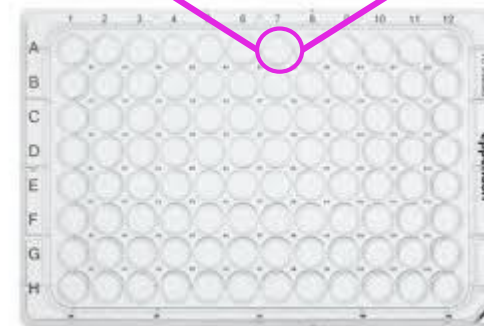
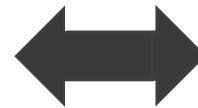
Reference population



Phenotypes

Genotypes

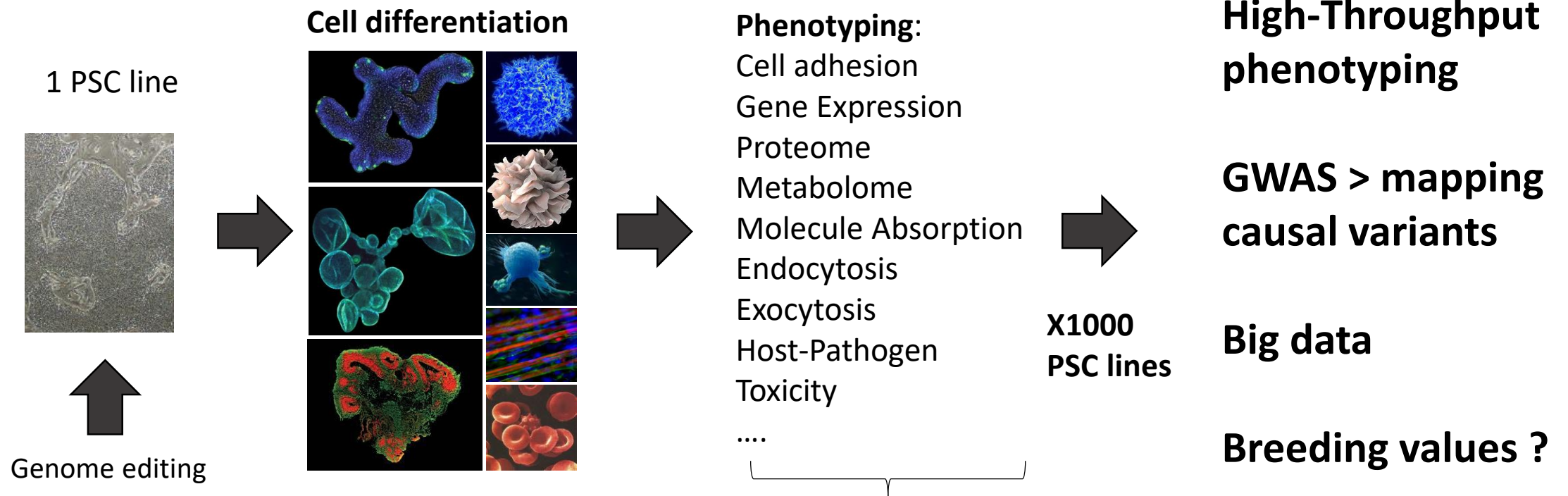
In vitro reference population (x1000) cell lines



Biobank of pluripotent stem cells

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

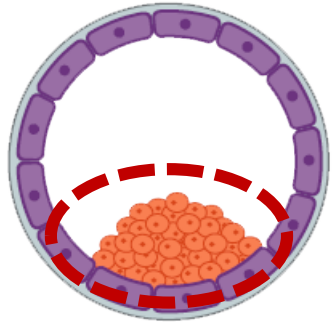
A challenge for animal production: phenotyping complex traits and predicting breeding values for those traits



Proxies or intermediate phenotypes not accessible on living animals



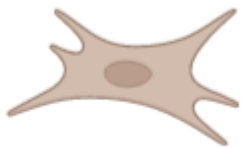
➤ Producing standardized true PSCs for livestock species is still challenging



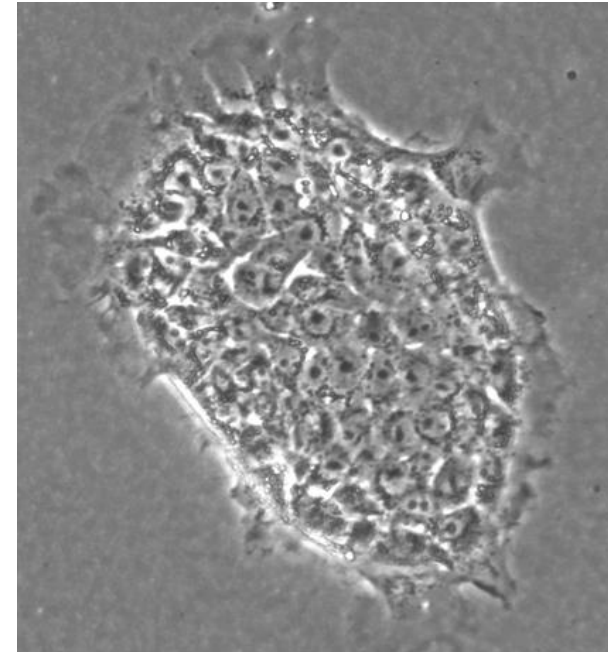
Amplification of **Epiblast cells**



Chemically defined medium

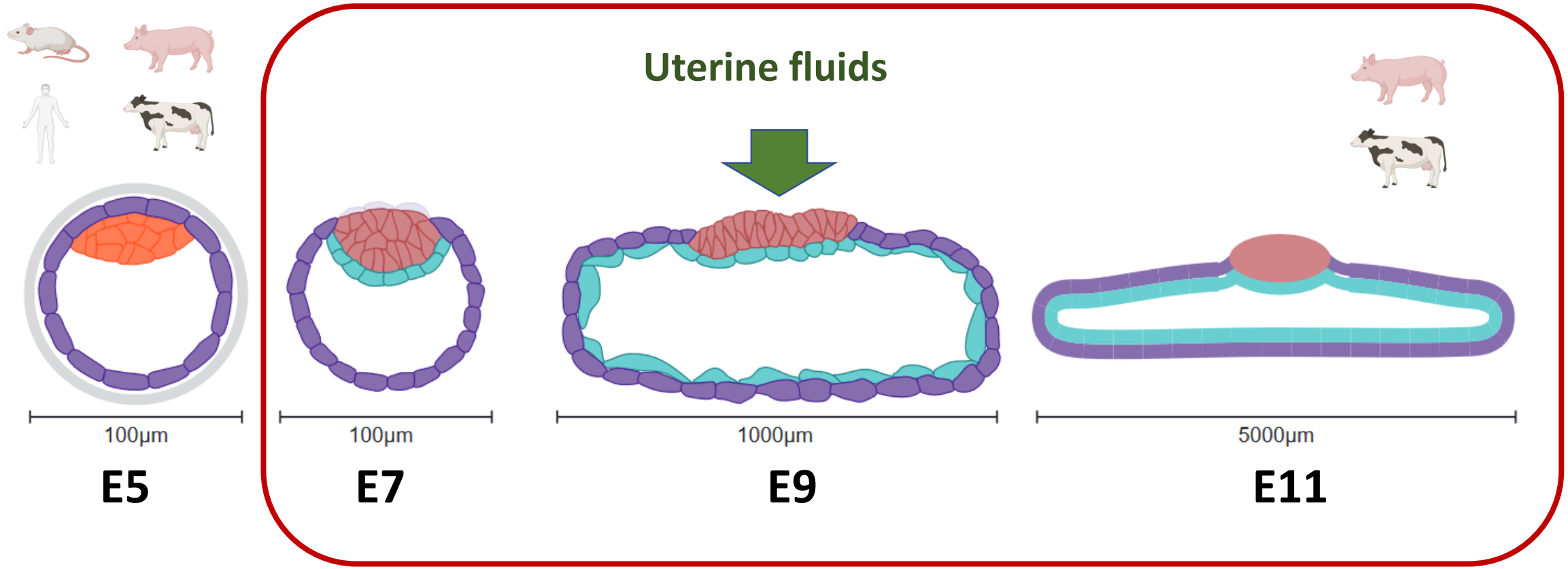


Reprogramming cocktail
(OCT4, SOX2, KLF4, MYC)



Heterogeneity ?
Stability overtime ?
Differentiation potential ?

➤ What can we learn from the embryo ?



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Gene networks controlling functional cell interactions in the pig embryo
29 August 2023 / EAAP meeting / Hervé Acloque

➤ Working hypothesis

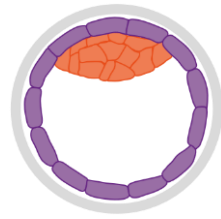


Paracrine regulations specific to pig embryonic development are not taken into consideration for the establishment of pig's pluripotent lines

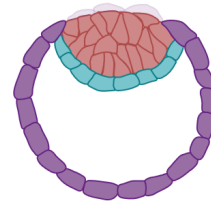


Embryonic regulatory networks are not necessarily conserved in mammals

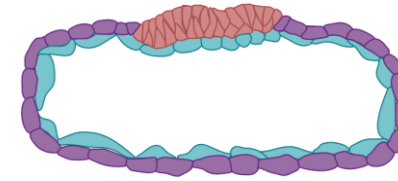
➤ Methods



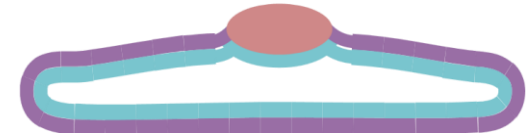
Early blastocyst **E5**



Hatched blastocyst **E7**



Spherical blastocyst **E9**

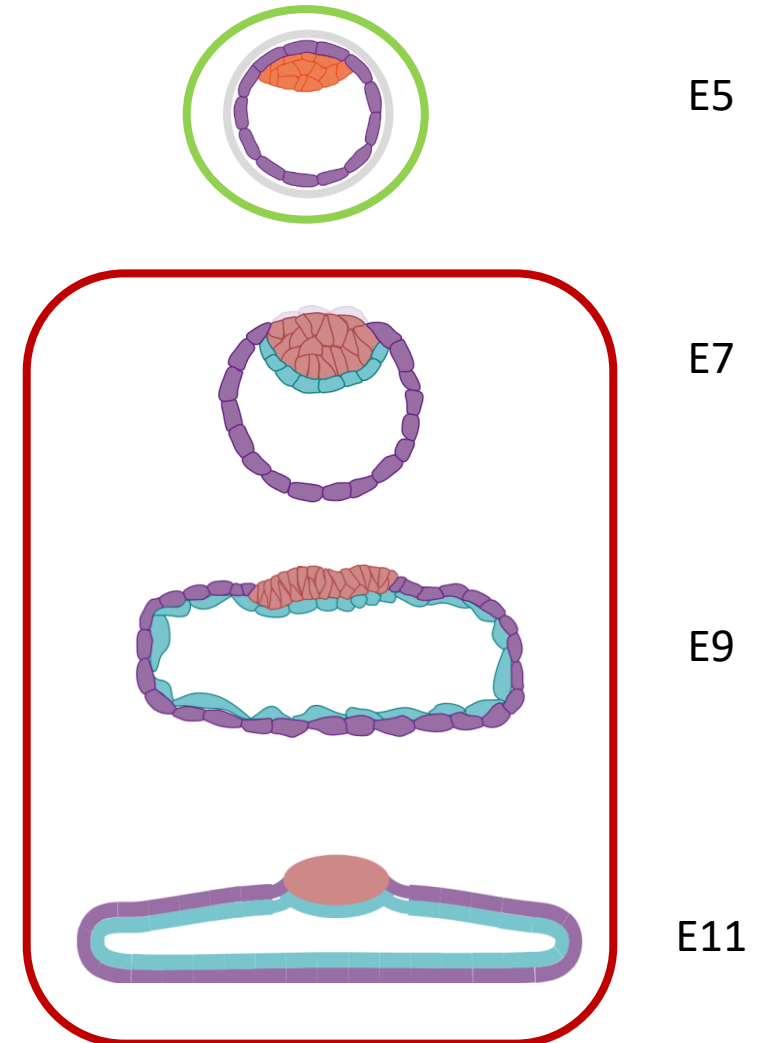
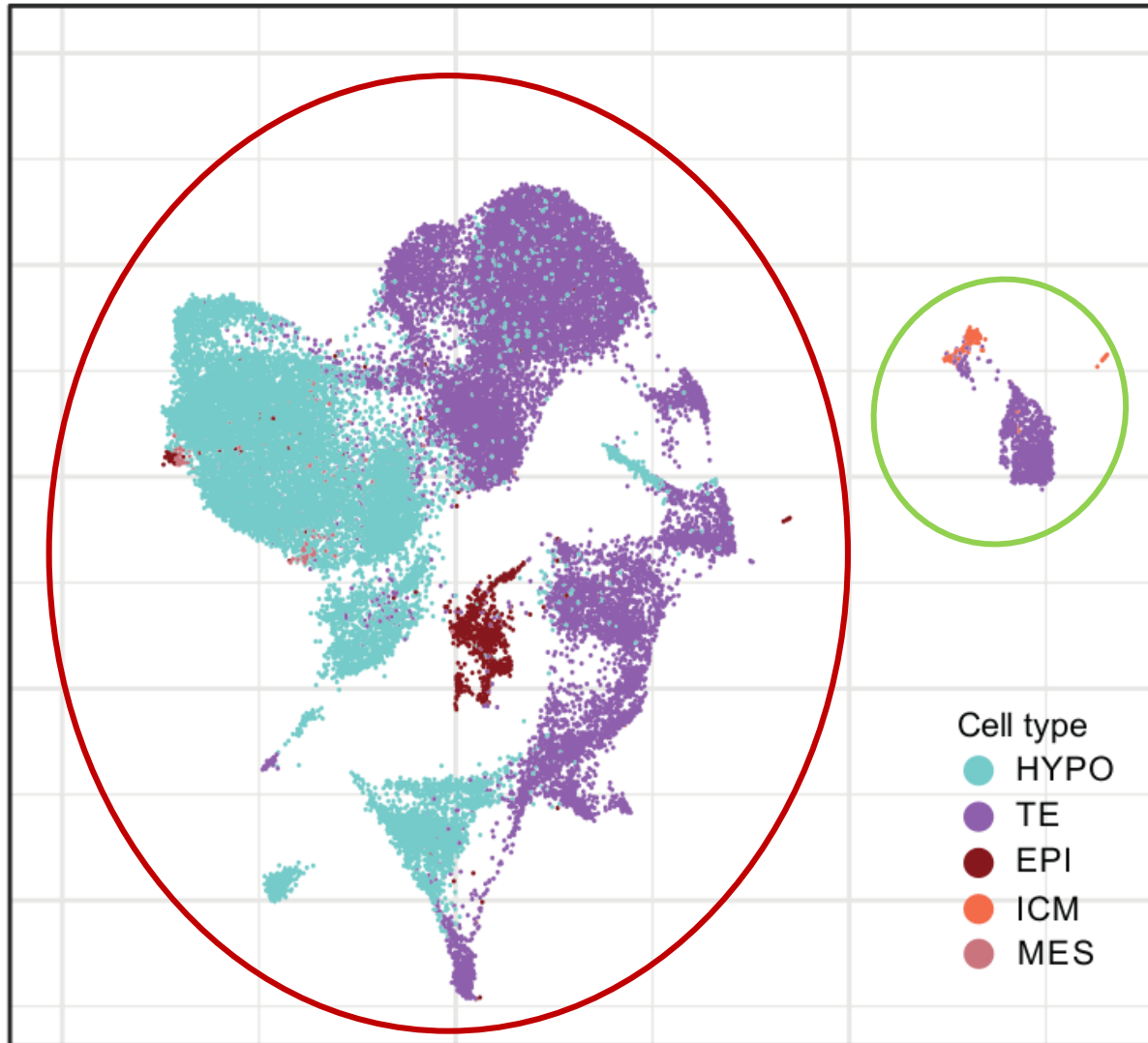


Ovoid blastocyst **E11**

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)

➤ scRNAseq allows the identification of cell populations constituting the embryo

UMAP of 34,888 cells

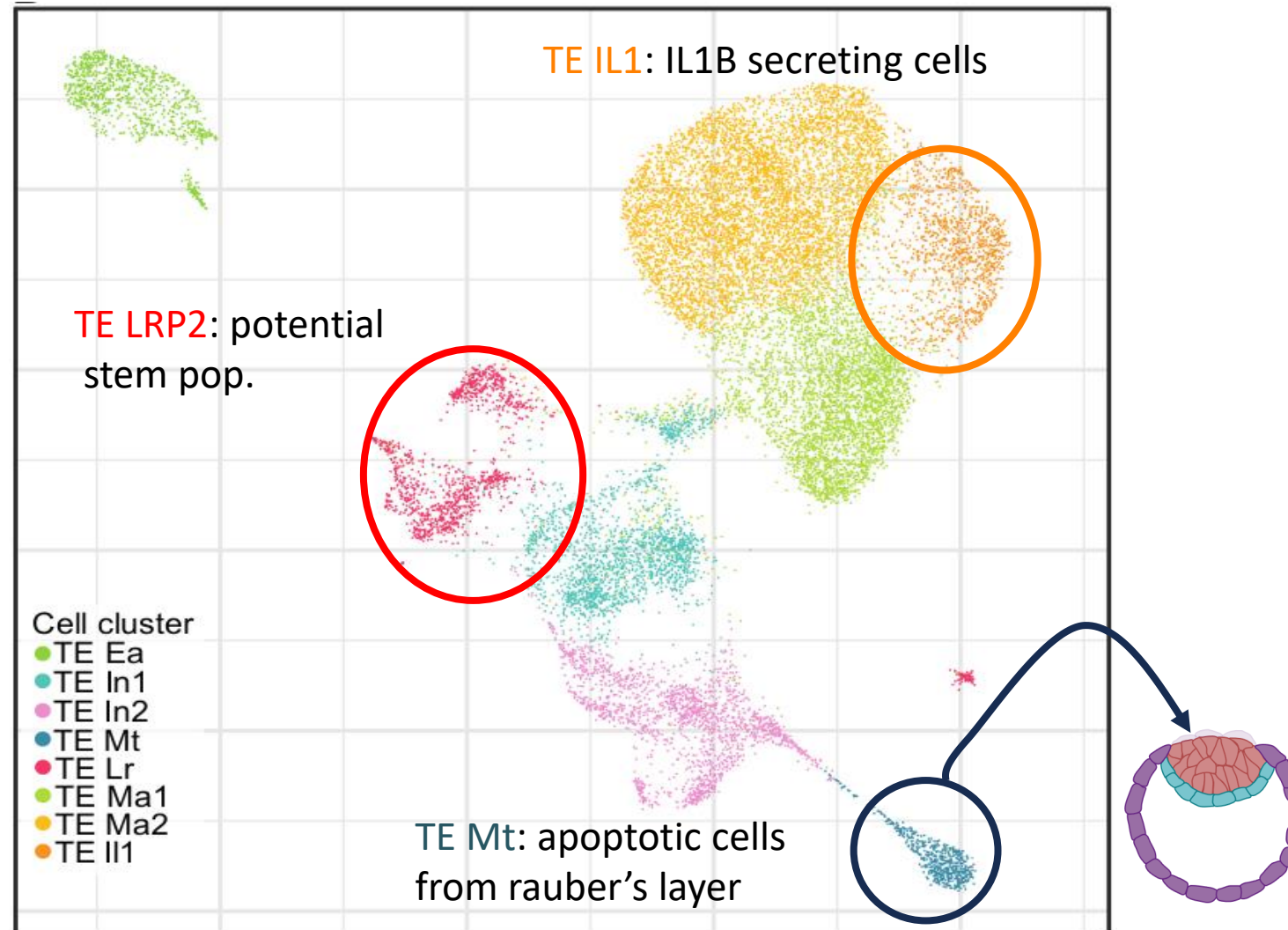


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➤ scRNAseq data provide cues to better understand the biology of the pig embryo

Identify new subpopulations

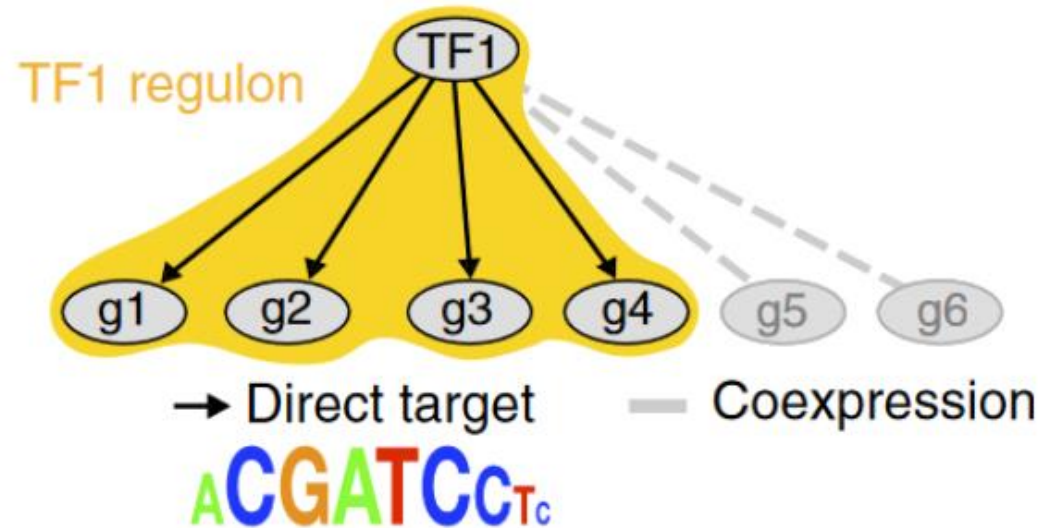
UMAP of 18,239 TE cells



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

Identify modules of gene regulation

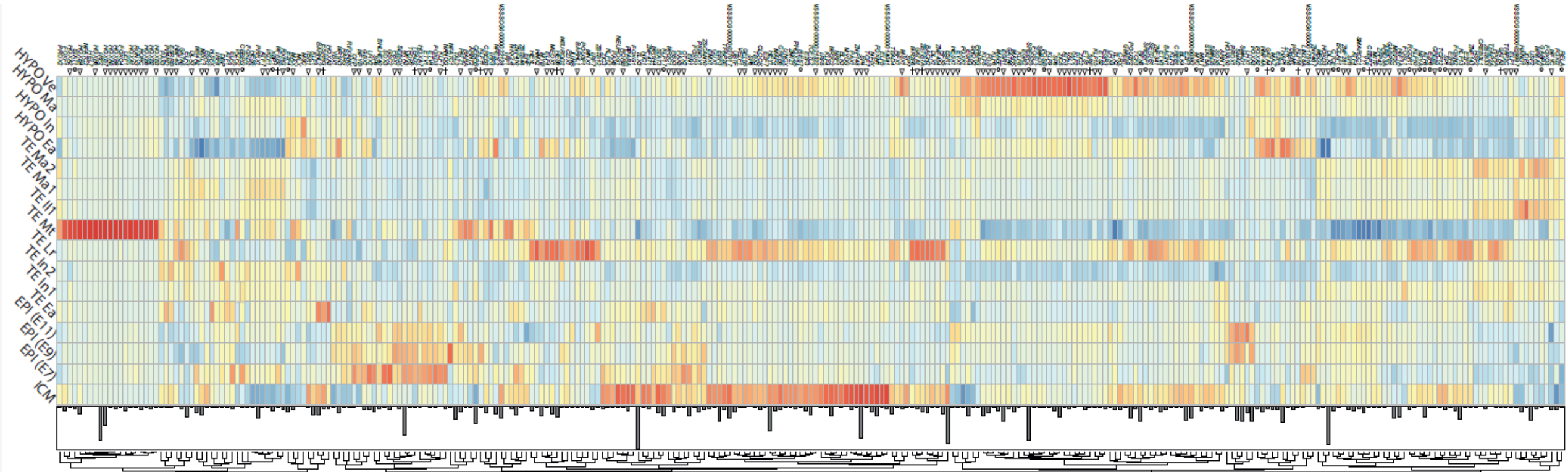
Regulons
(gene regulatory network)



Aibar et al. 2017

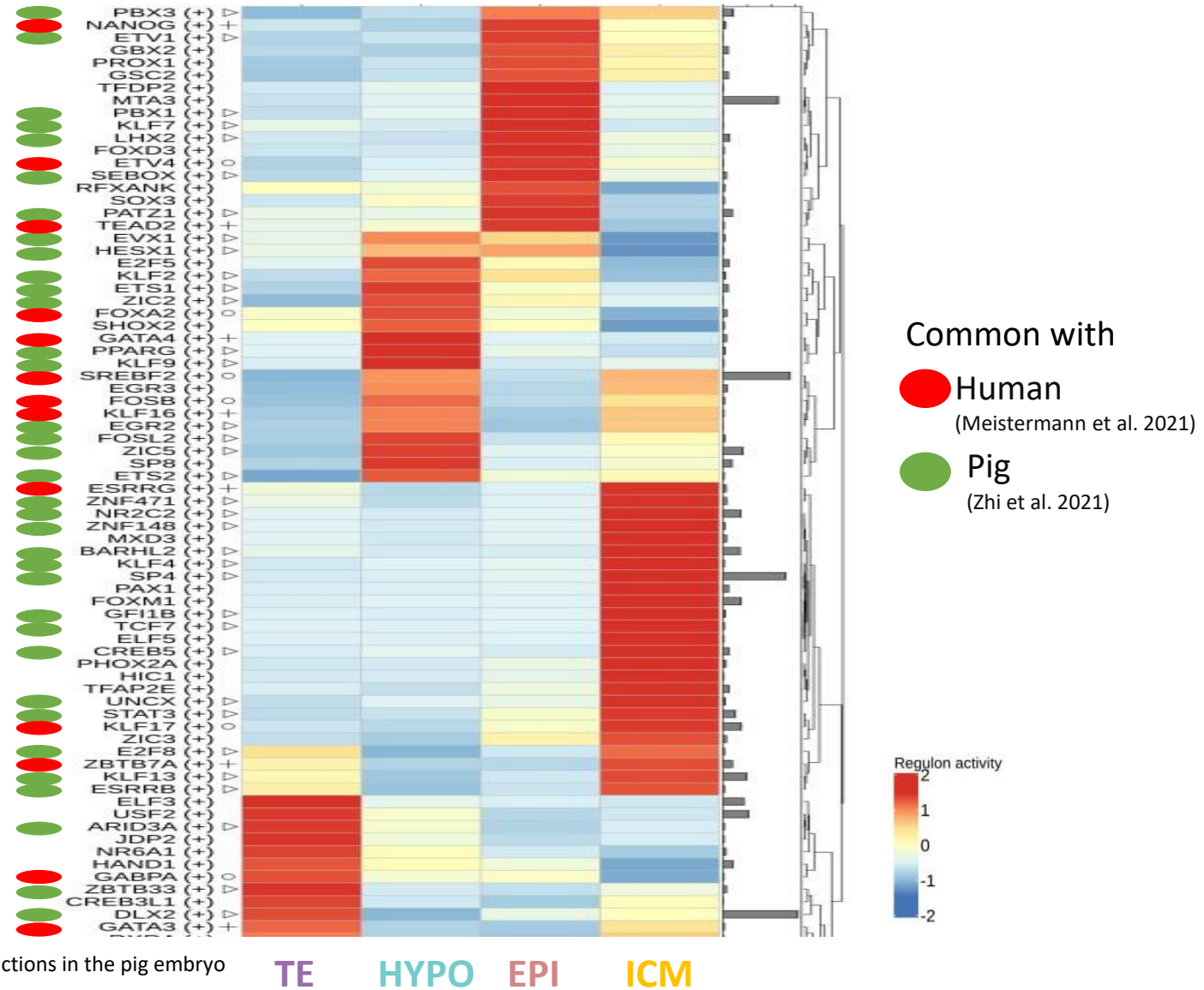
➤ scRNAseq data provide cues to better understand the biology of the pig embryo

Identify modules of gene regulation

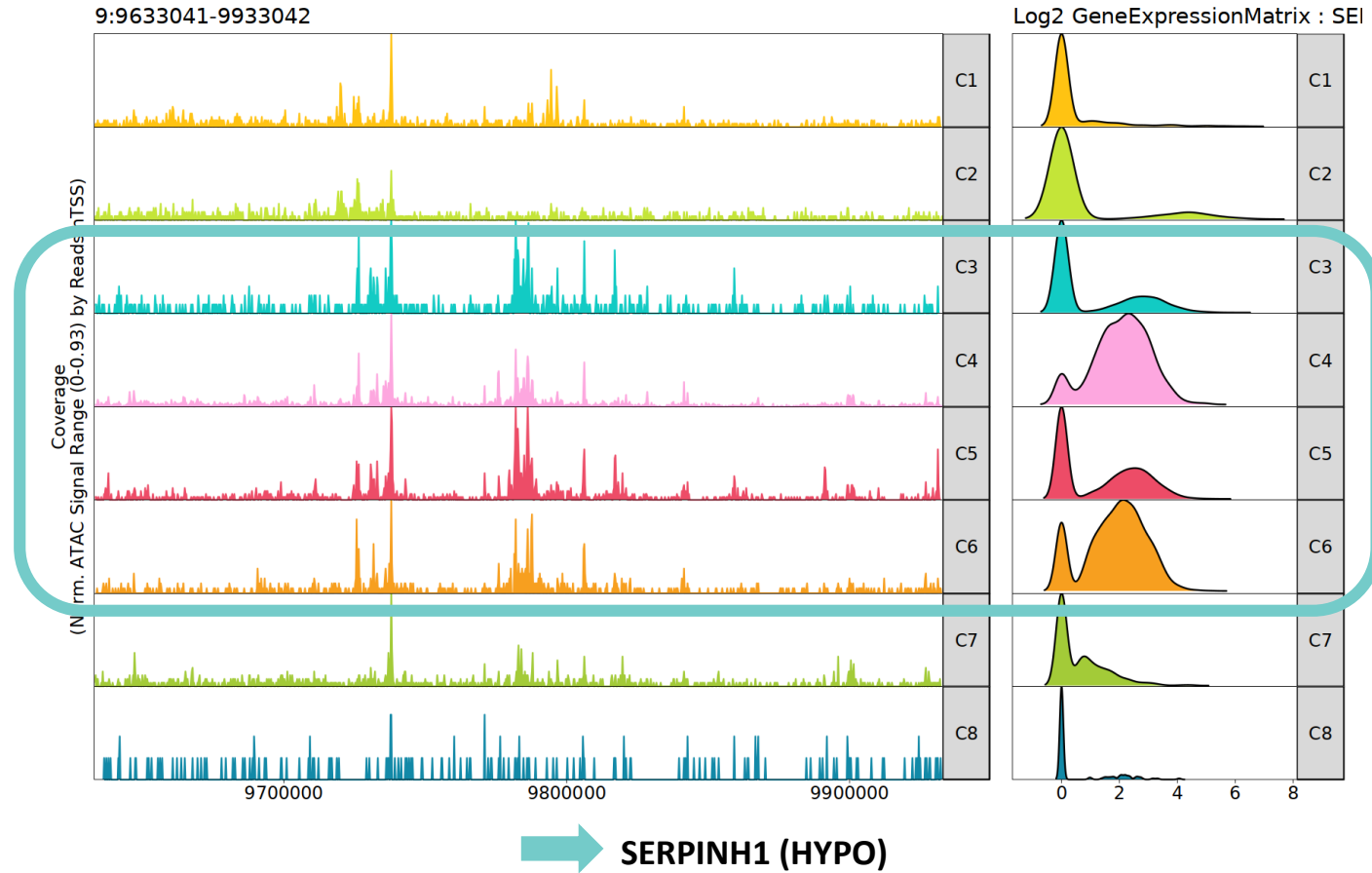
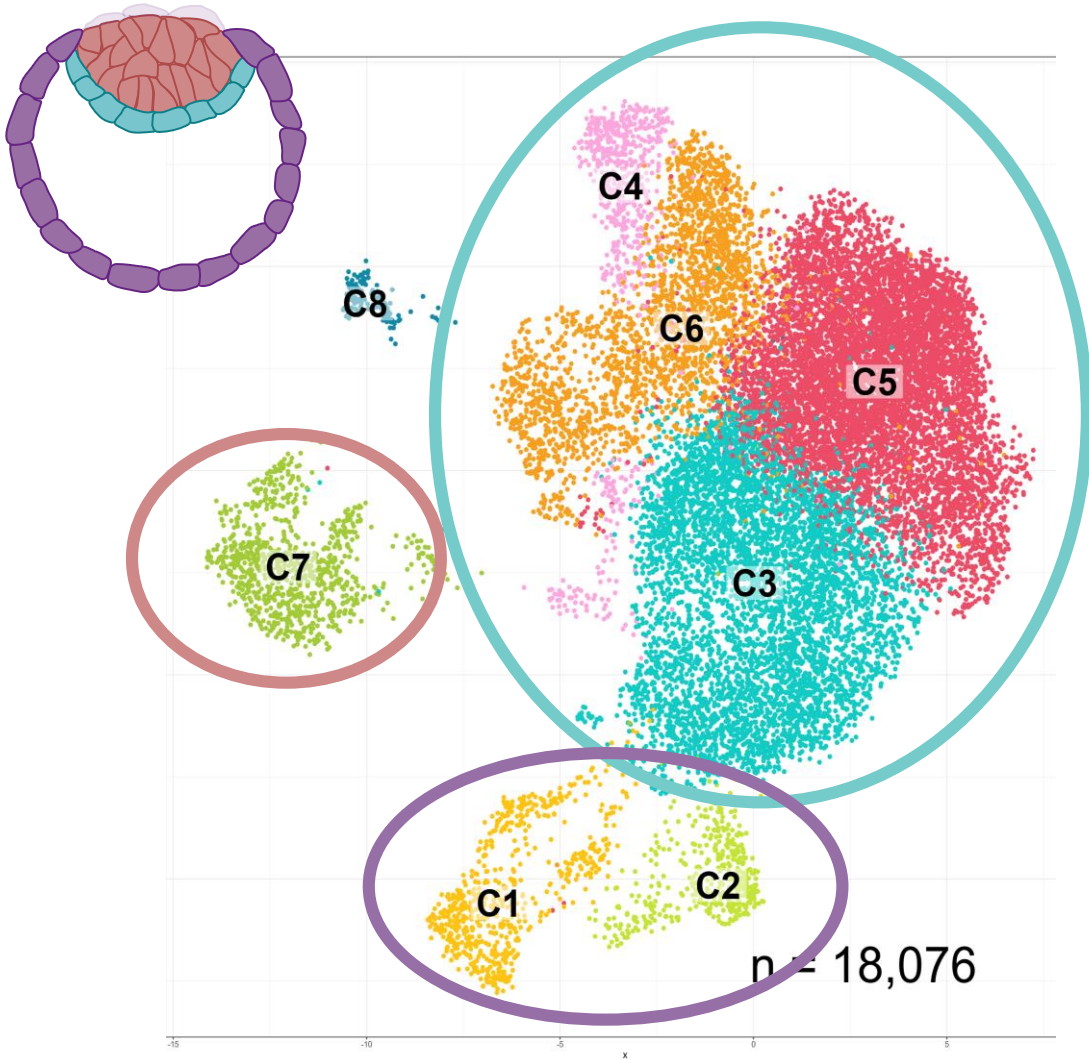


➤ scRNAseq data provide cues to better understand the biology of the pig embryo

Identify modules of gene regulation

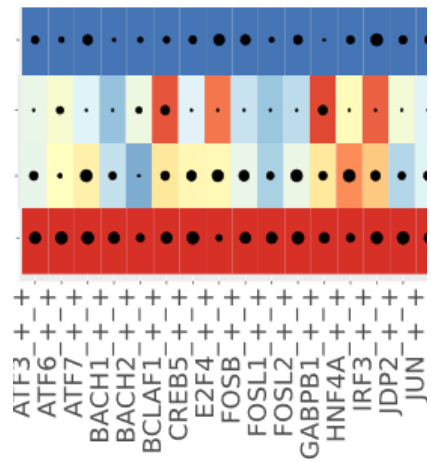
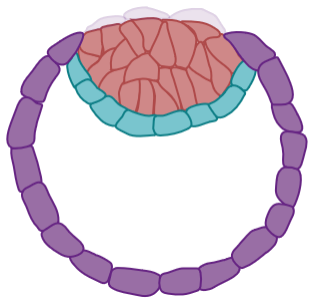


➤ scOMICS: adding a layer of information to refine module of gene regulation

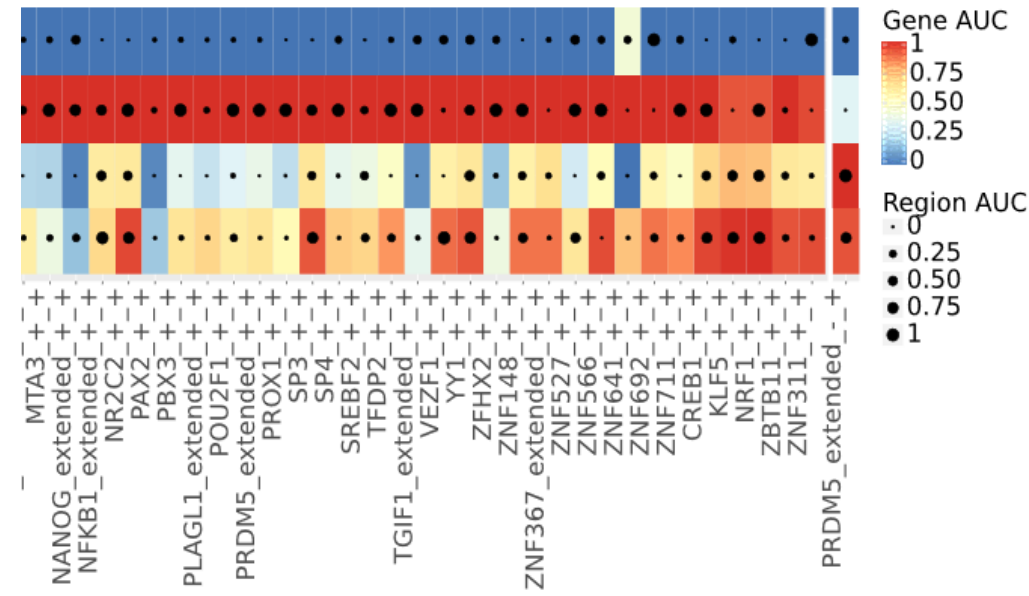
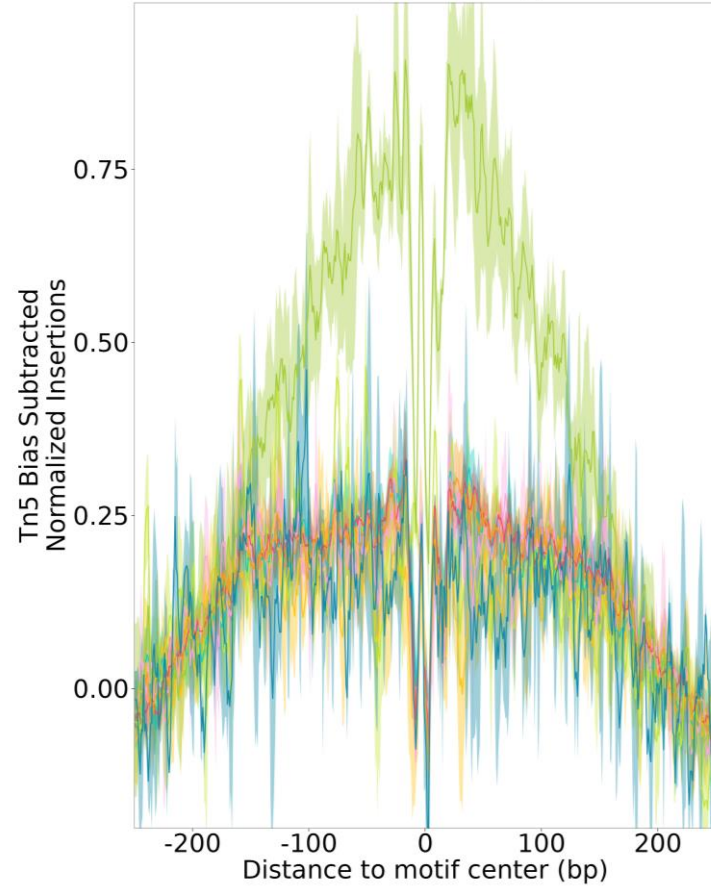


➤ Added value of omics data: selection of active regulons

TE
EPI
HYPO (early)
HYPO (late)



None hocomoco__NANOG_HUMAN.H11M

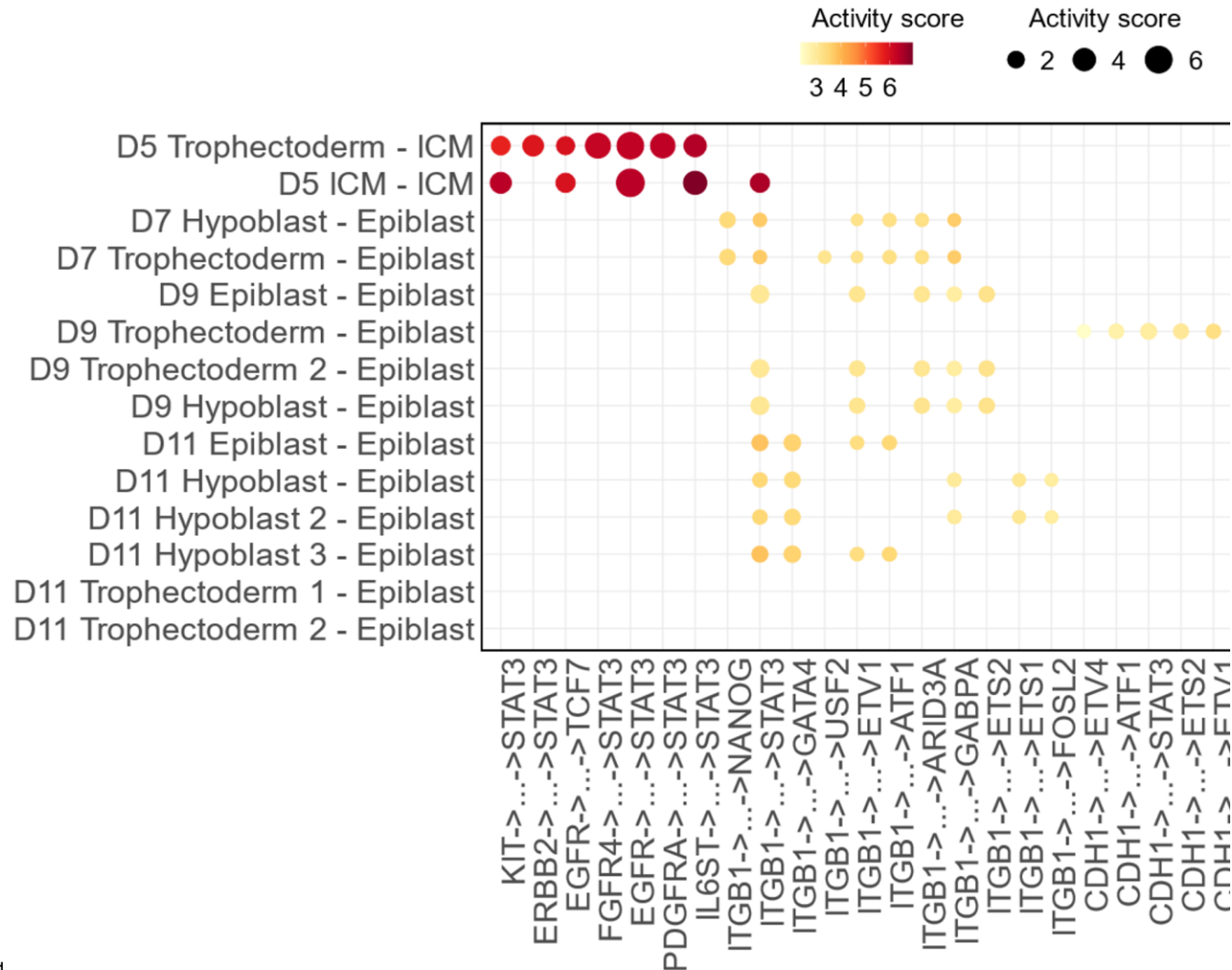


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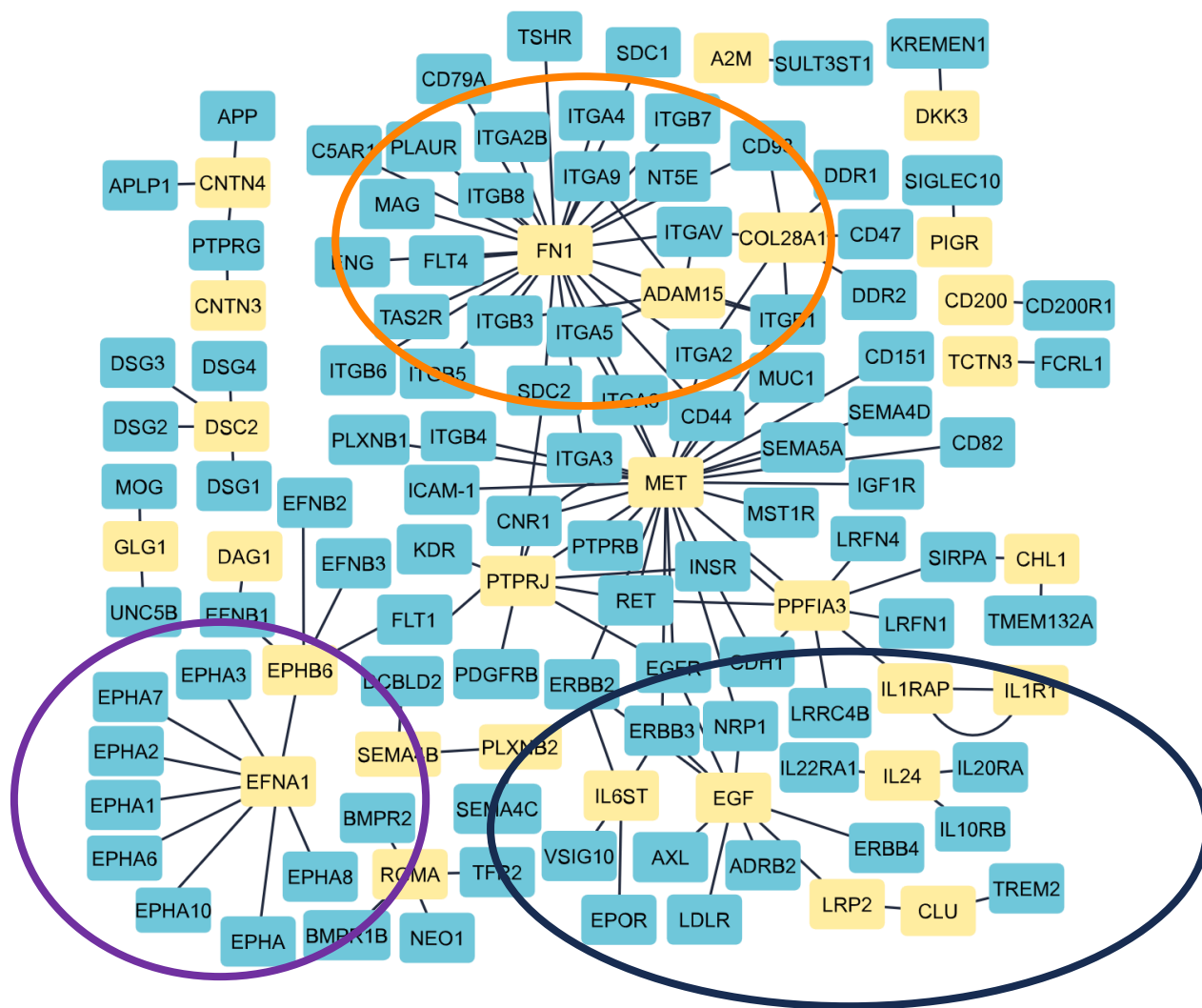
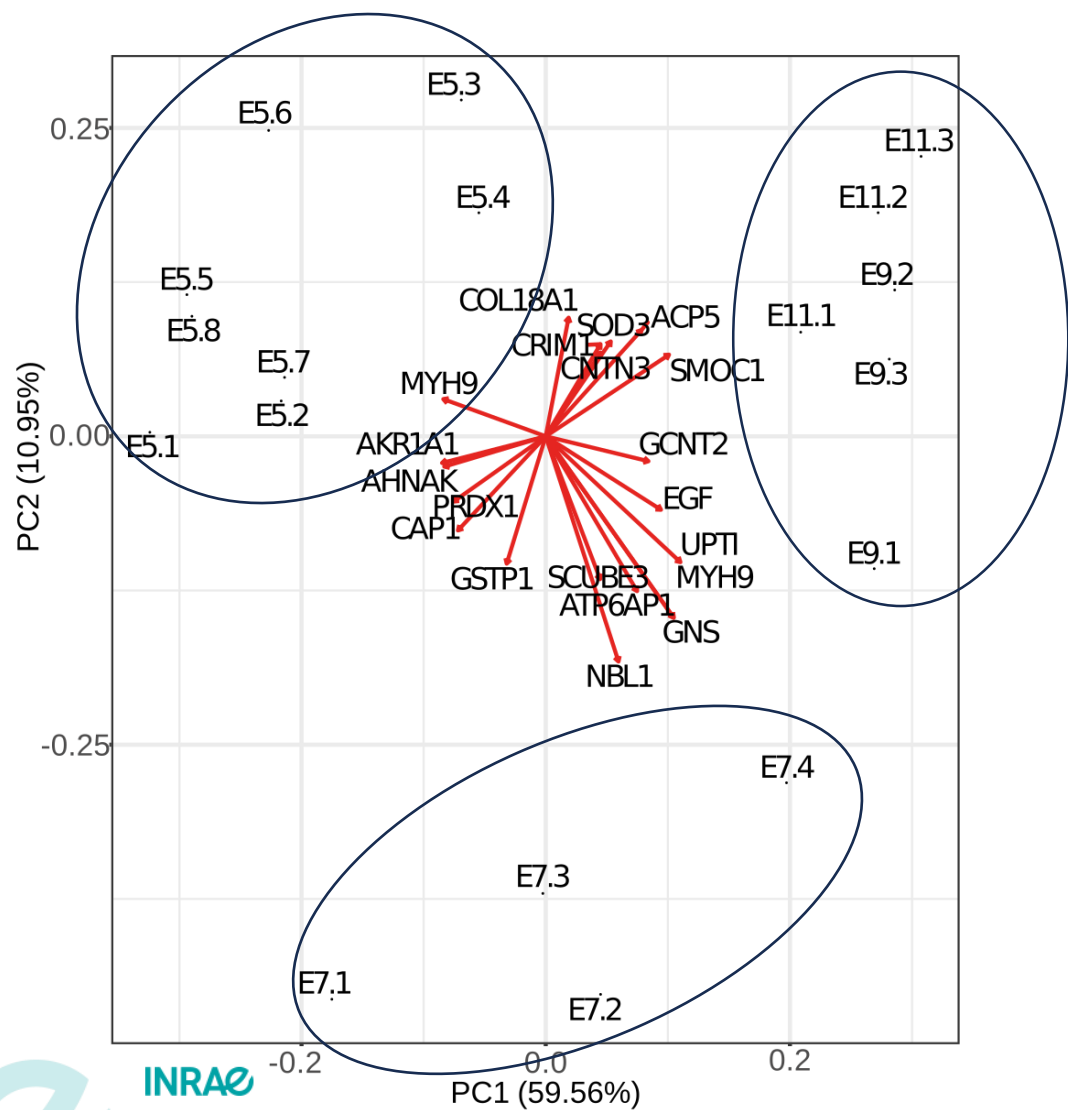
Dufour et al. unpublished

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

From ligand/receptor interactions to modules of gene regulation



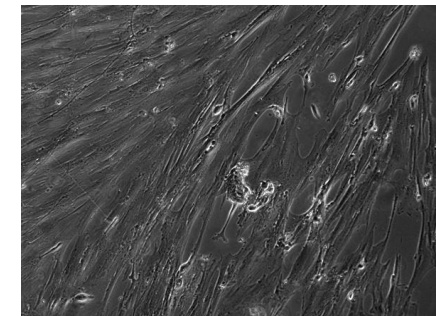
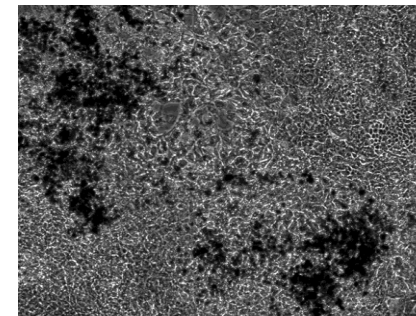
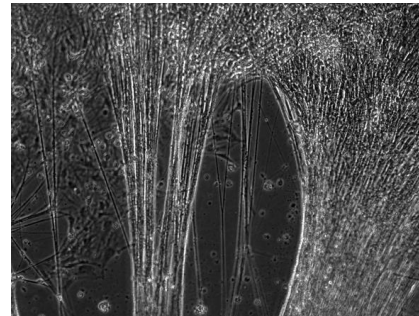
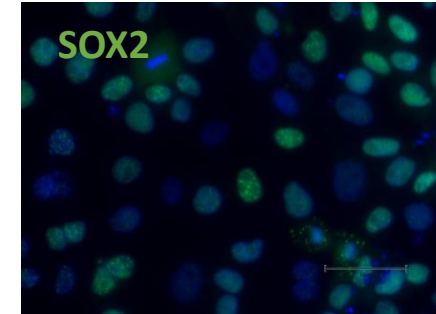
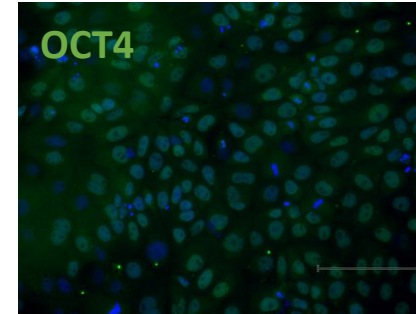
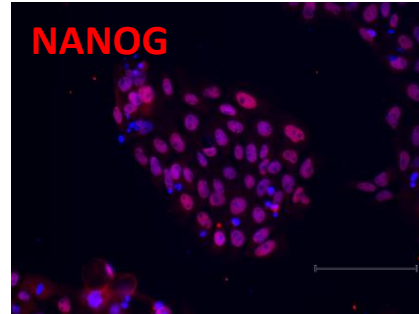
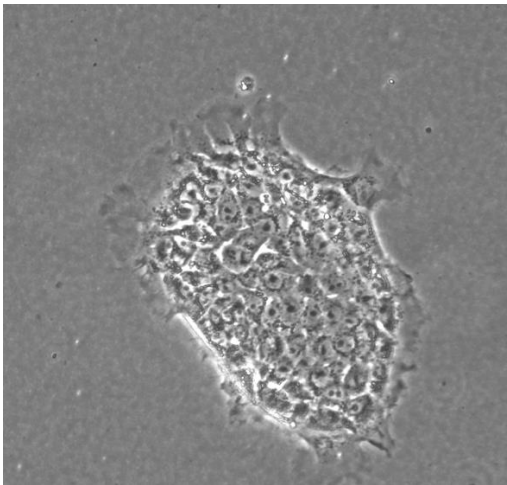
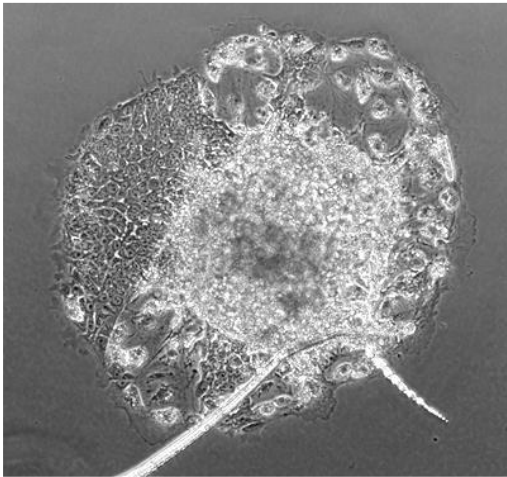
➤ Adding the fourth dimension: input from uterine fluids proteomics



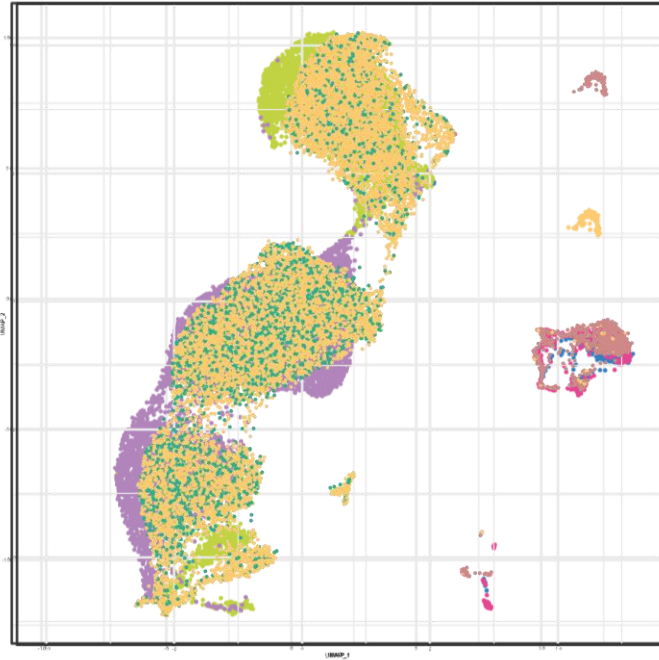
Interactions between **uterine fluid ligands** and **trophoblast receptors (E9/E11)**

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

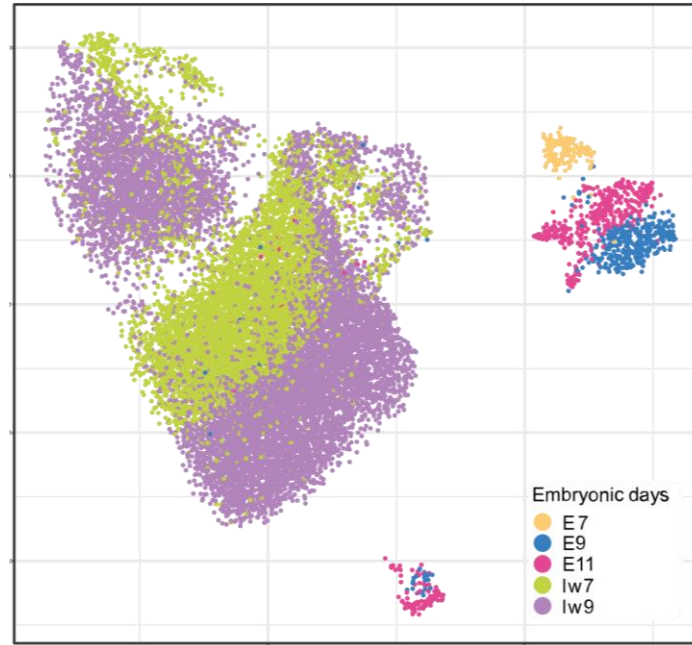
Pig ESCs



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

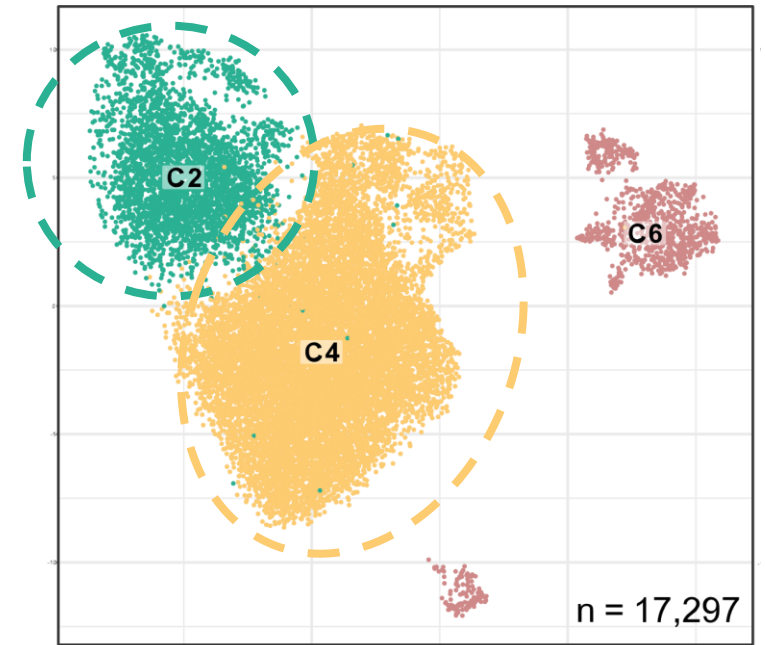


scRNAseq



scOMICS

Cells poised toward a neural fate
ZNF521



Cells poised toward non neural
ectoderm & mesendoderm fate
GRHL2



➤ 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



➤ Acknowledgements

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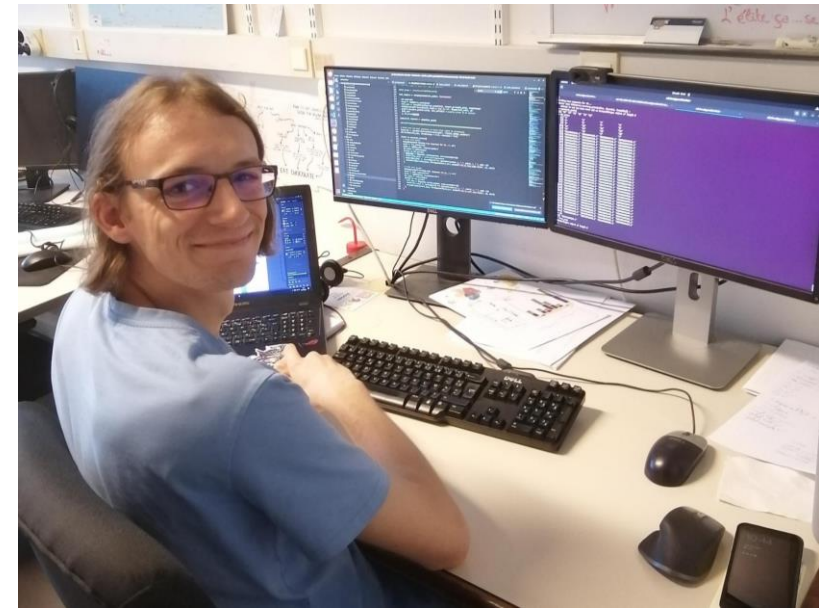
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➤ Confirming modules of gene regulation using footprints

