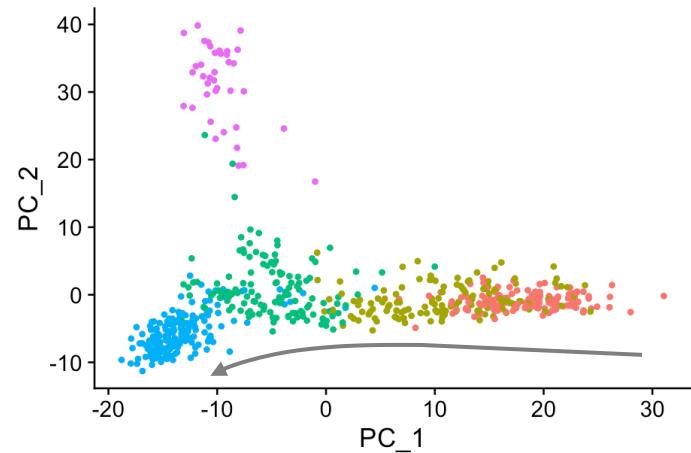
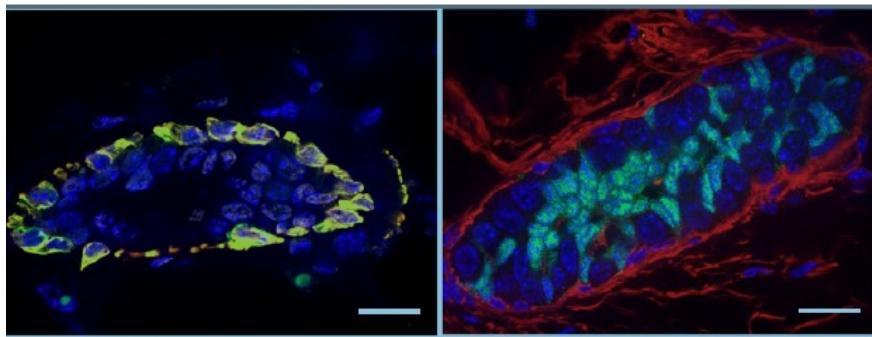


# Transcriptional mechanisms underlying a Notch-induced cell fate transition in the adult mouse mammary epithelium



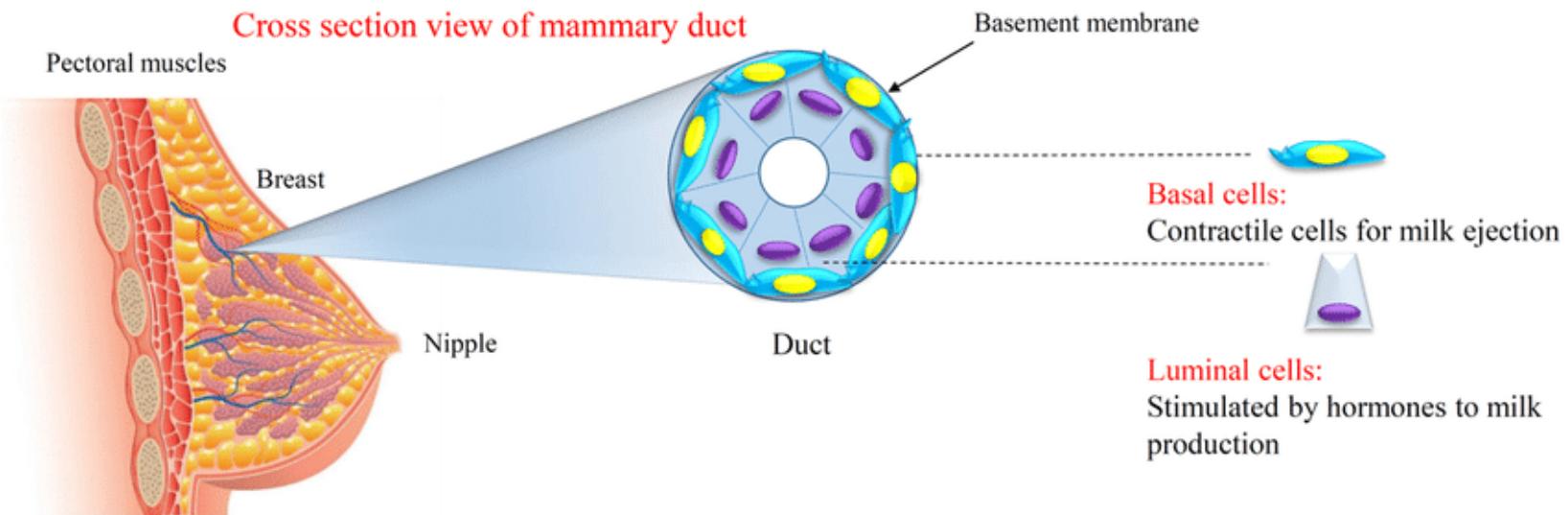
Calvin Rodrigues, PhD

Bioinformatician

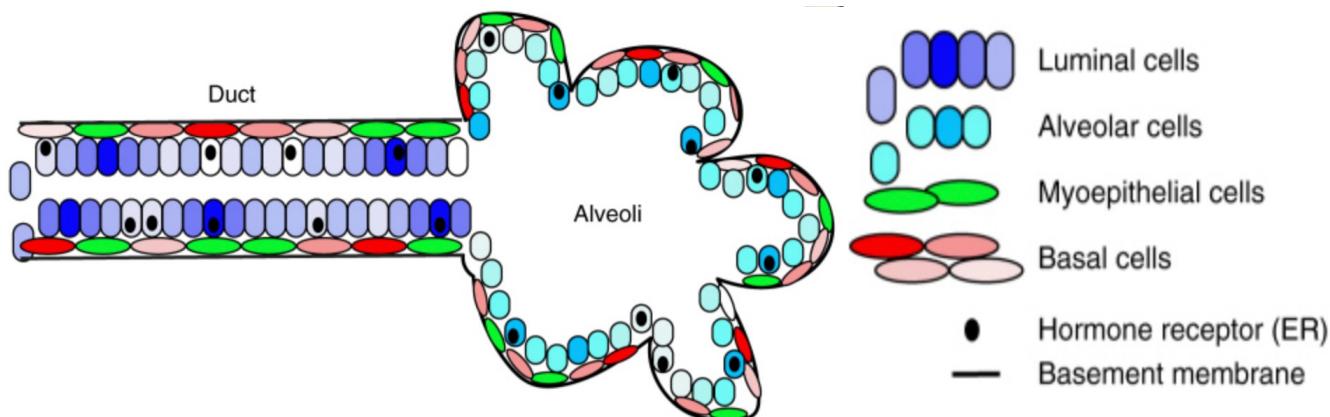
Silvia Fre + Raphaël Margueron labs, Curie Bioinfo Hub

Institut Curie, Paris

# Epithelial cell types in the Mammary gland



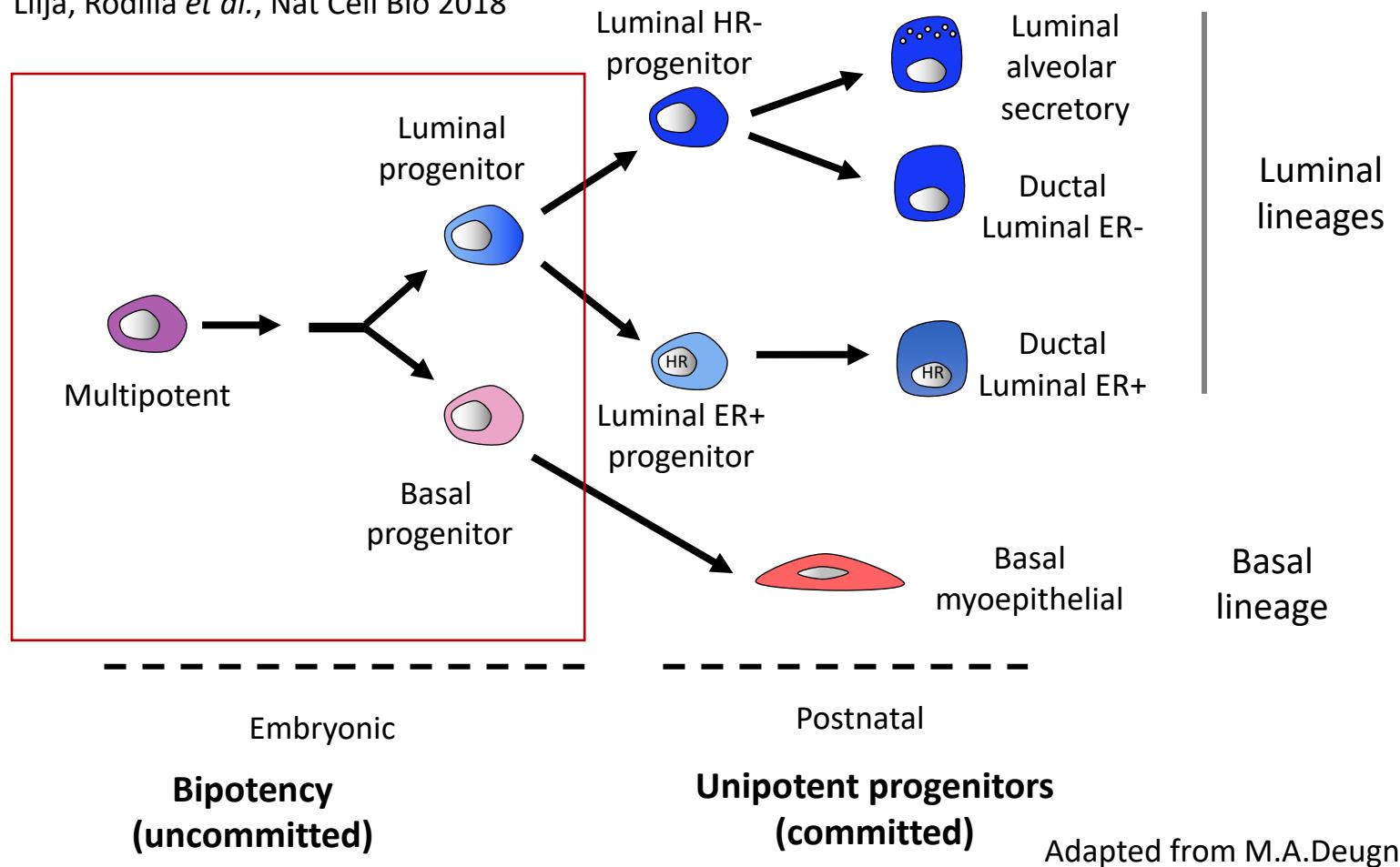
Serej et al., 2020



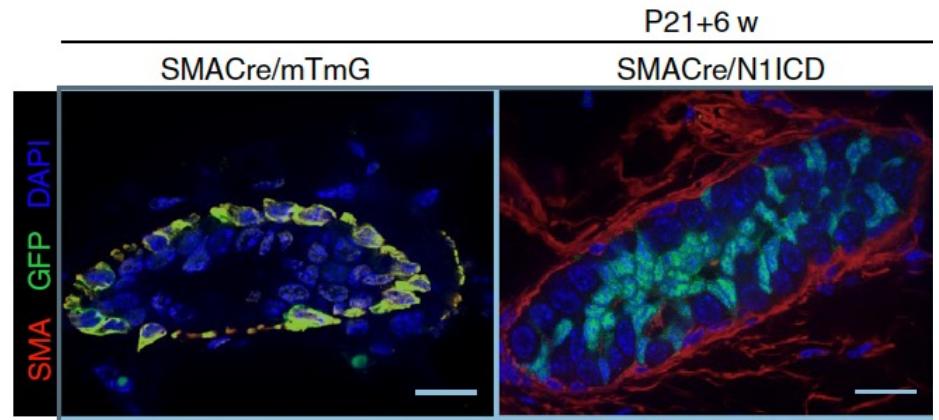
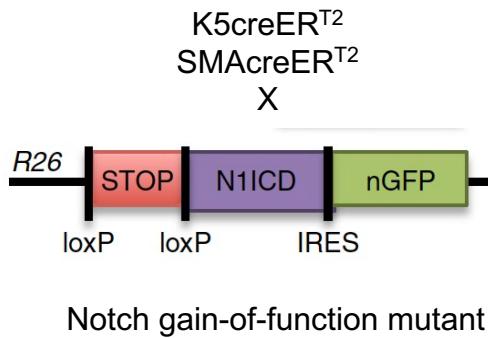
# Basal and luminal lineage commitment in epithelial cells occurs early during mouse mammary gland development

Epithelial basal and luminal lineage commitment occurs by E15

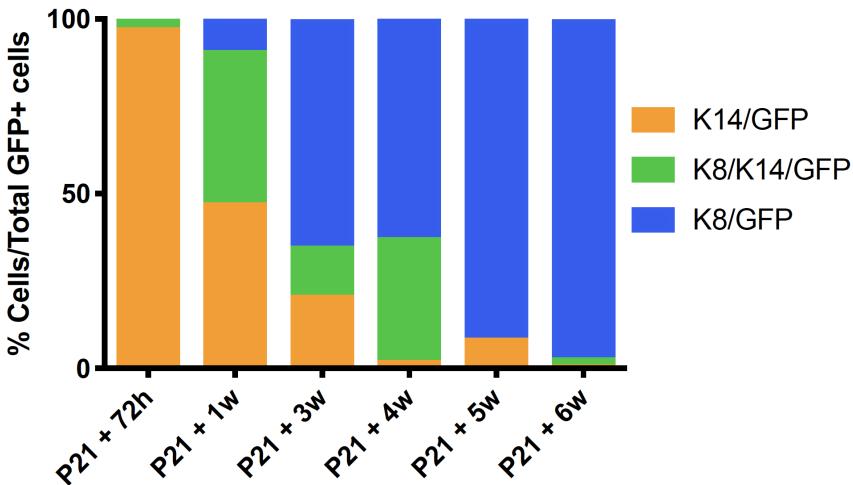
Lilja, Rodilla *et al.*, Nat Cell Bio 2018



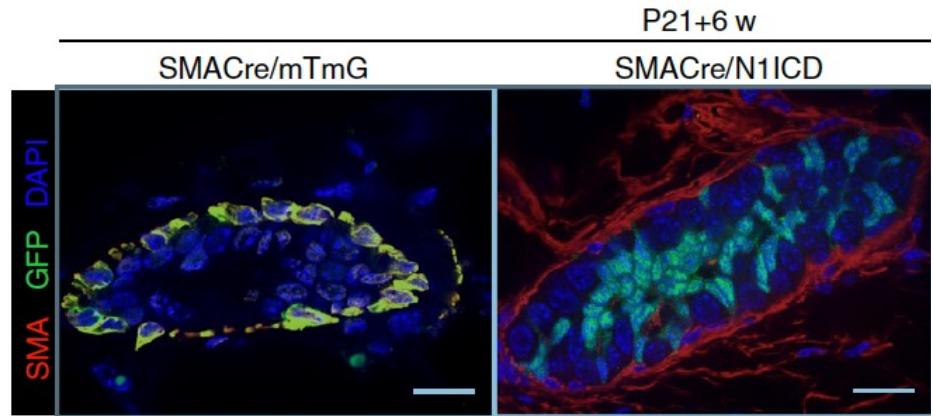
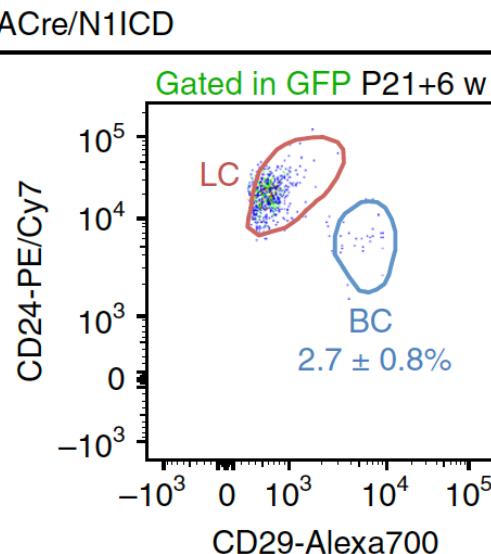
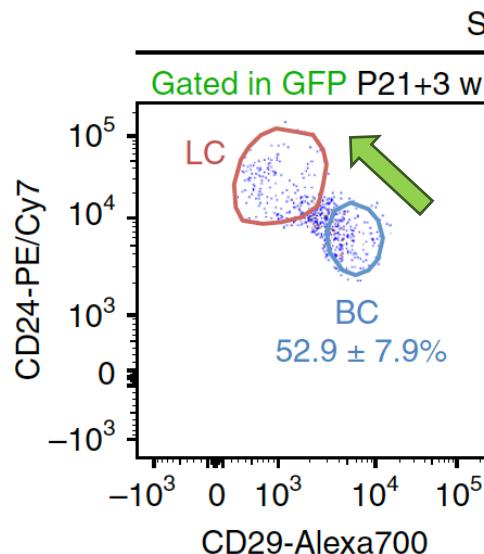
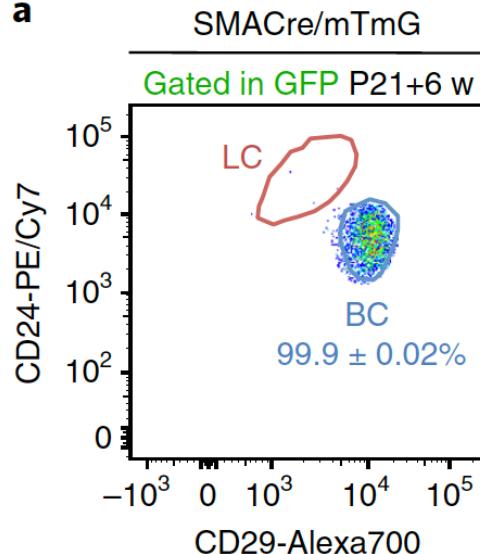
## Activated Notch signaling in adult basal cells Leads to a switch from **basal** to **luminal** cell fate



# Activated Notch signaling in adult basal cells Leads to a switch from **basal** to **luminal** cell fate

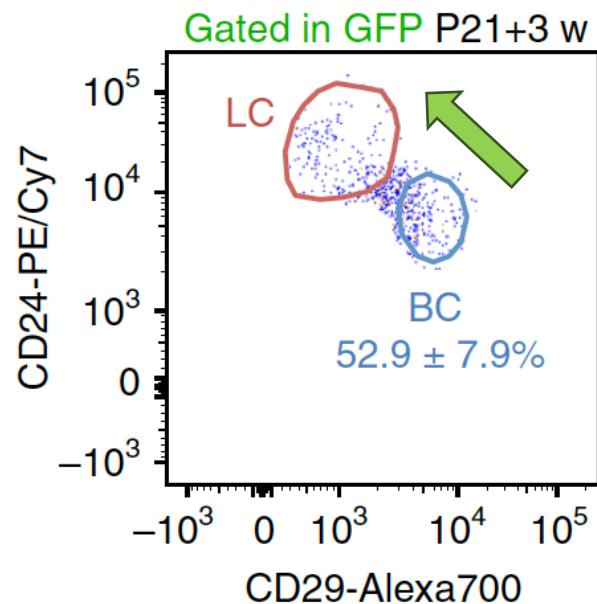


**a**

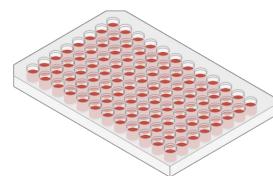
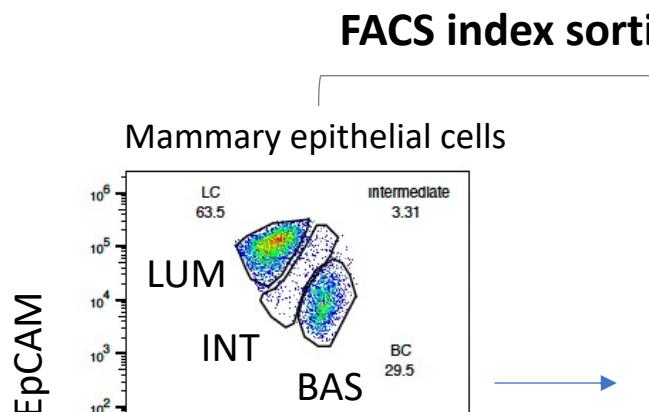
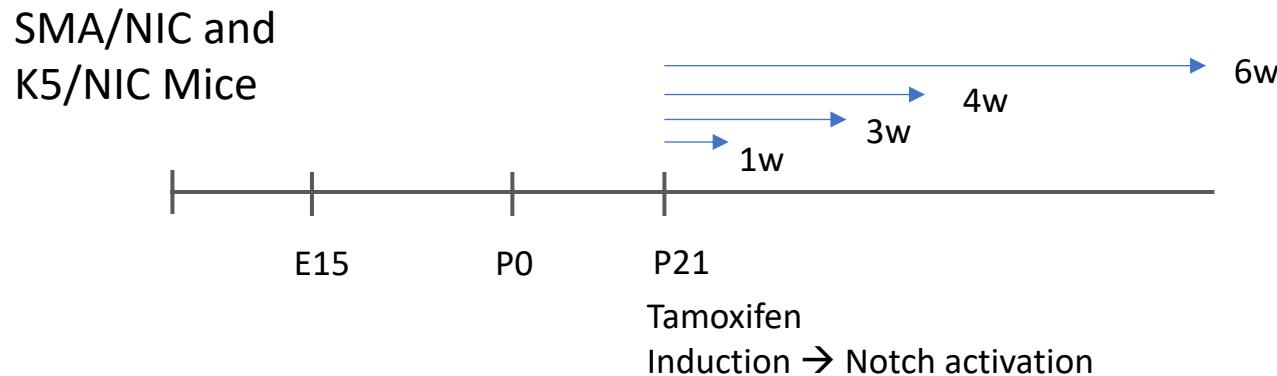


## Do intermediate cells pass through a transcriptional state which resembles early development?

- Do intermediate cells show **transcriptional similarities** with earlier **developmental timepoints**?
- What **transcriptional mechanisms** are involved in the cell fate switch?



# Single-cell transcription profiling of basal-luminal transition: Using Smart-Seq2 with FACS indexing



### Smart-seq2 scRNA-Seq

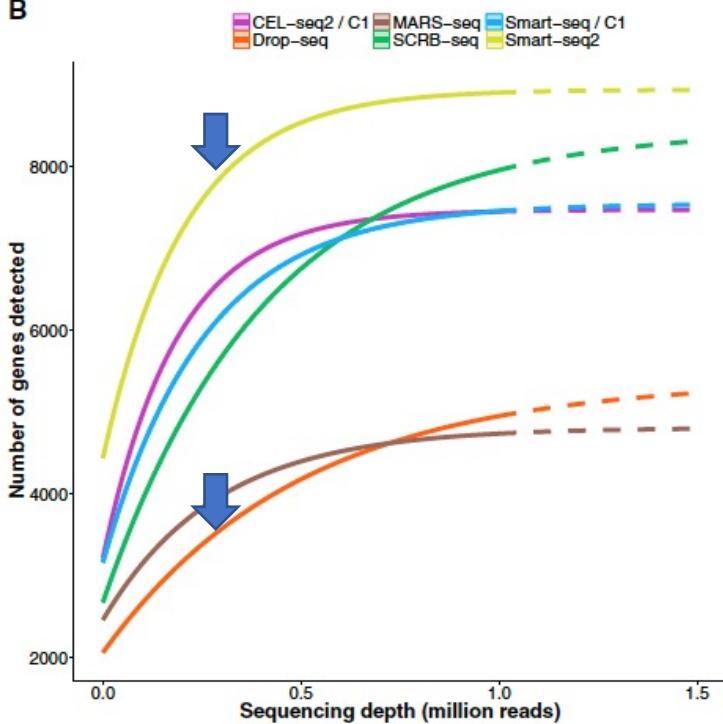
- Index: Retains FACS info.
- Can integrate with analysis – e.g. cluster identification
- Allows low cell #

Experiments: Candice M. , Meghan P.  
Smart-Seq2 by Berthold Göttgens group

# Smart-seq2 scRNA-Seq

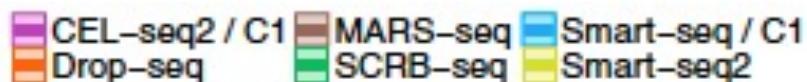
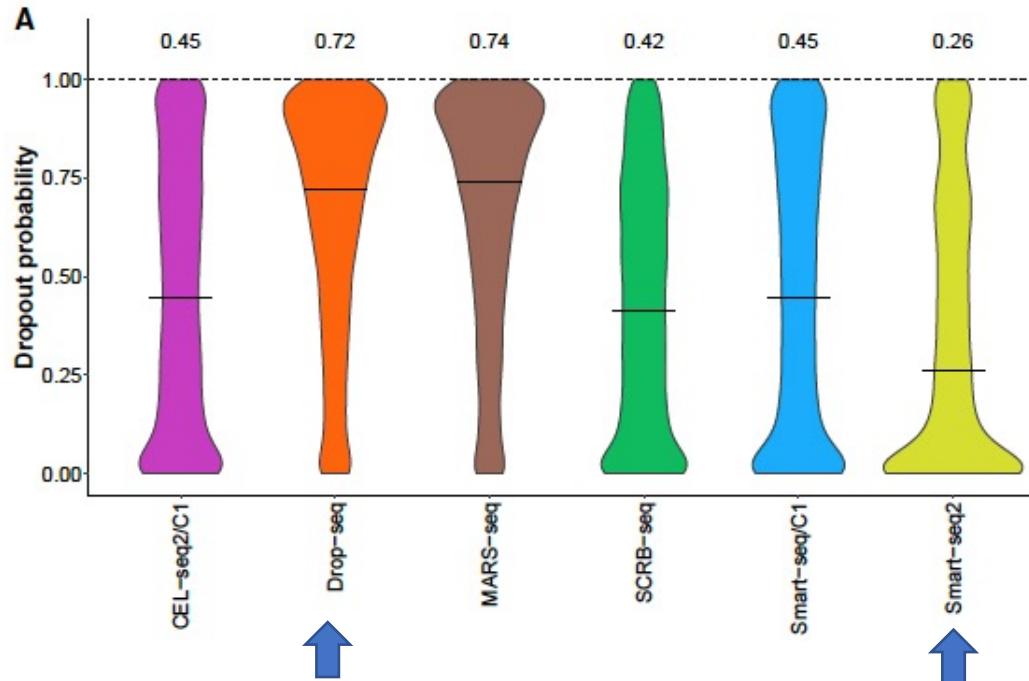
Median number of genes  
detected per cell

B



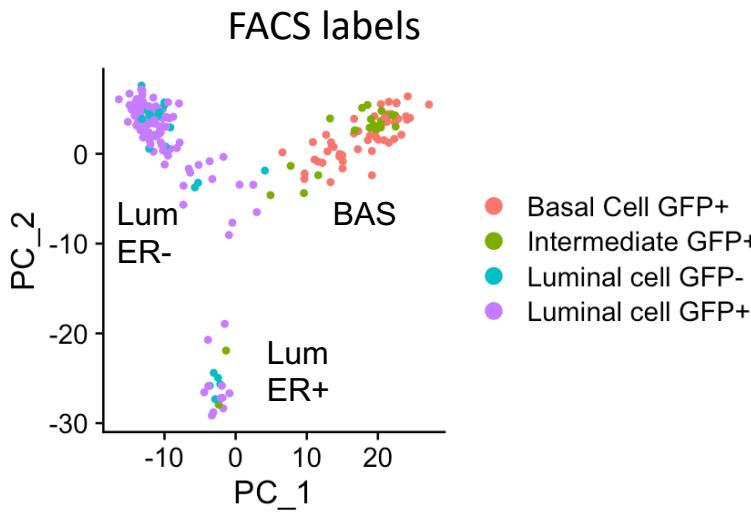
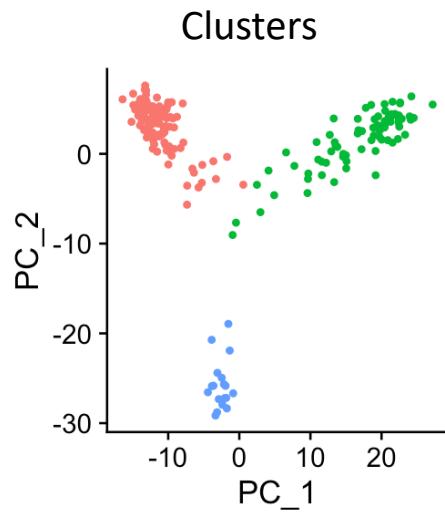
Sequencing precision – dropout rates

A

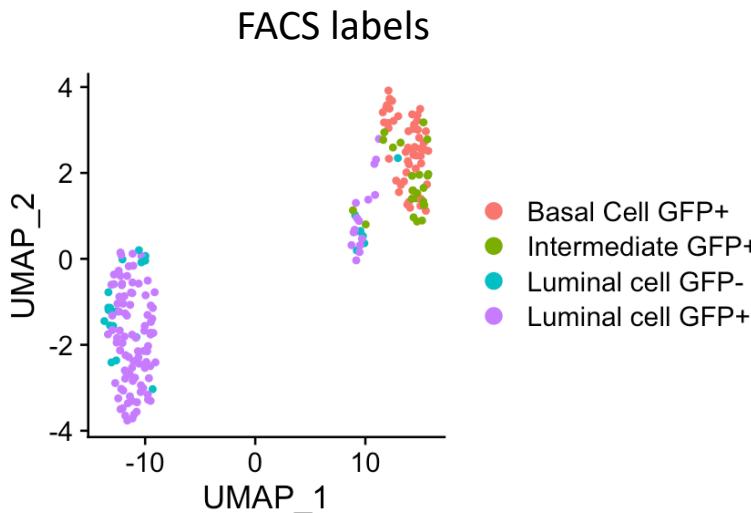
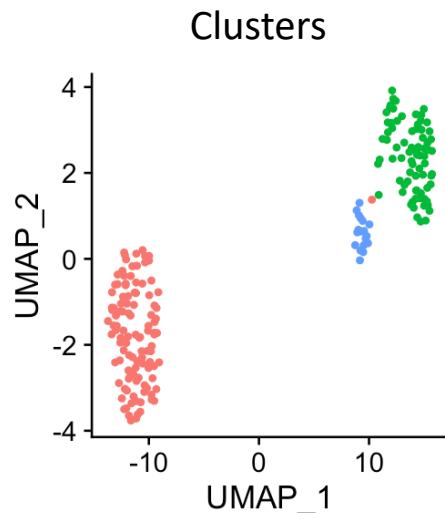


Smart-seq2 – No UMIs

## Visualising the cell fate transition

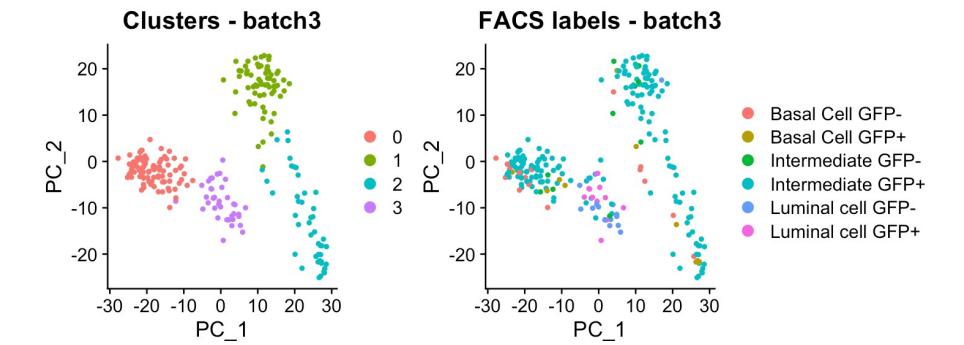
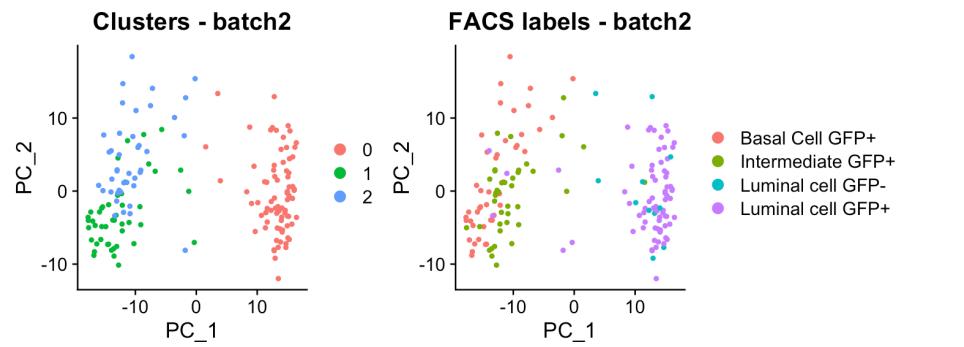
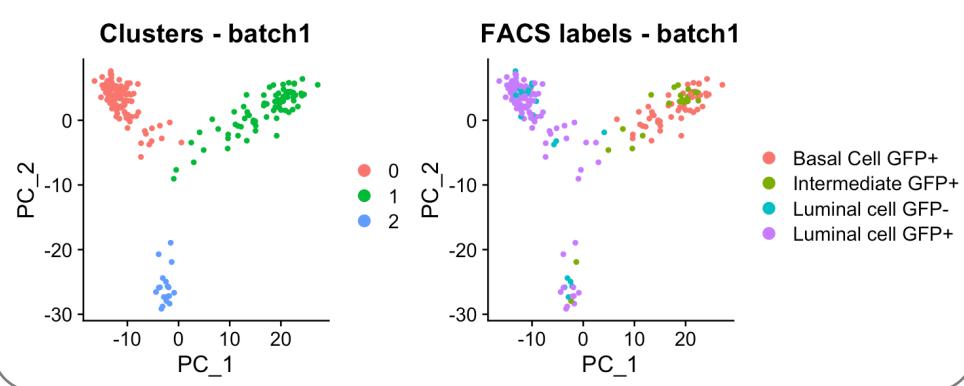


PCA gives an intuitive visualisation of cell fate transition



UMAP is helpful to separate cell clusters, especially those existing in different PCs

Batch1

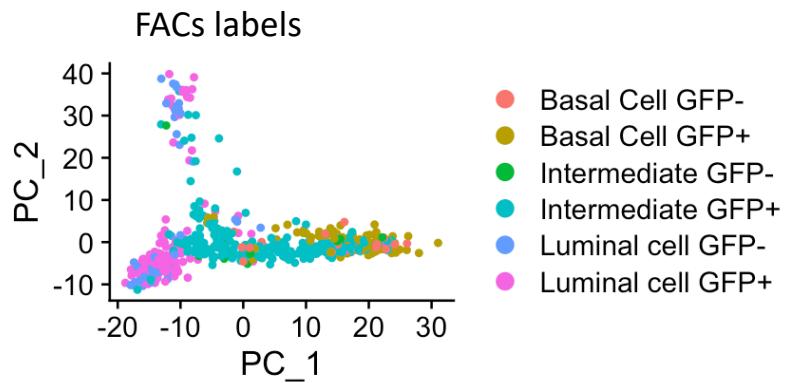
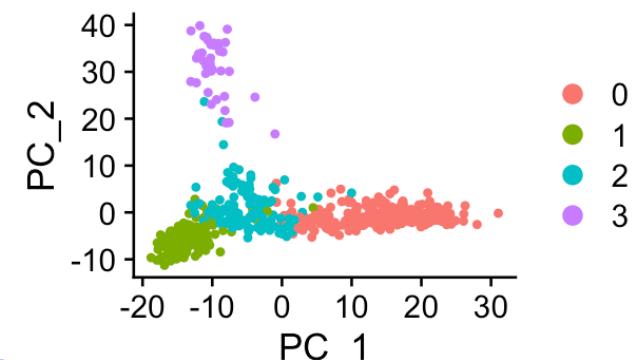


## Seurat Integration

Select **batch1** as a **reference**

during integration →

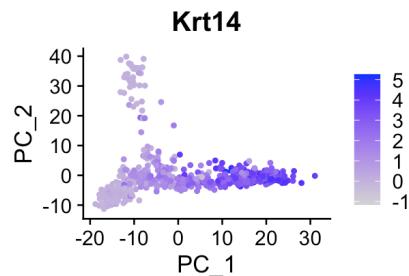
Balanced representation of basal, intermediate and luminal cells



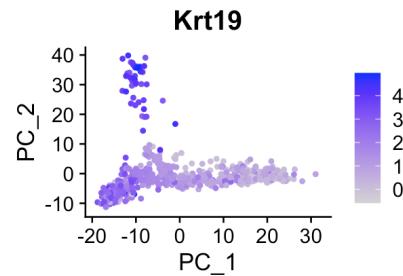
Intermediate cells in central positions during transition

# Smart-seq2 data: Identifying cell clusters

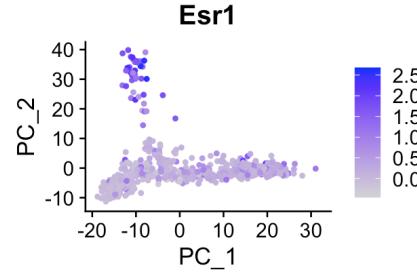
Basal markers



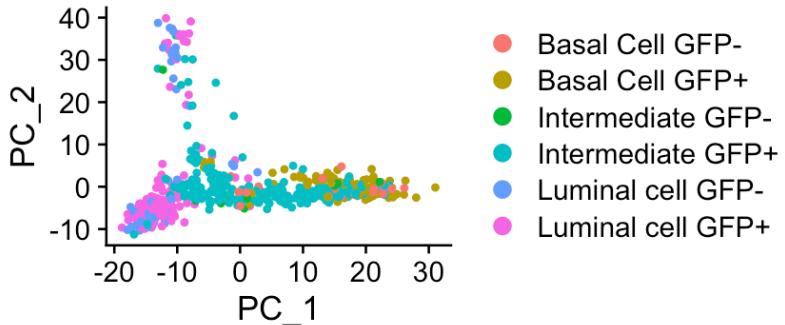
Luminal Markers



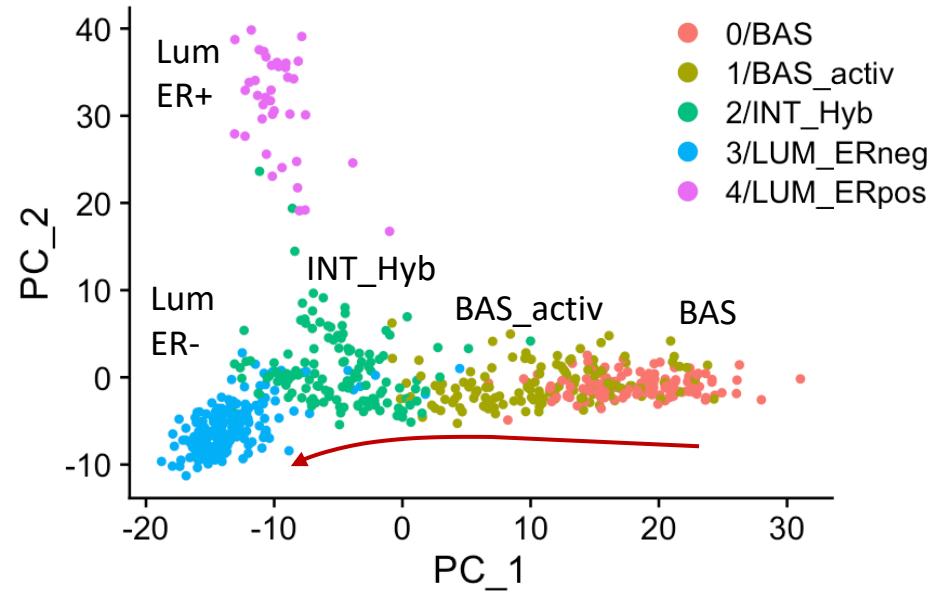
Luminal ER+ markers



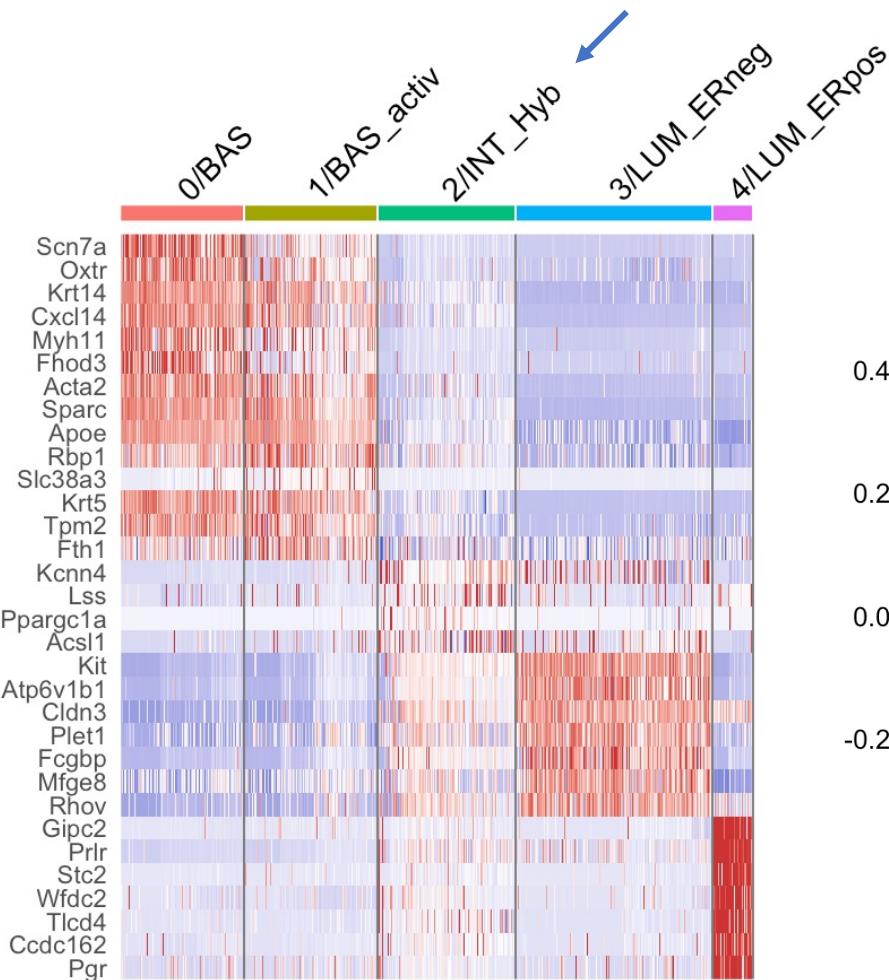
FACS labels (from Indexing)



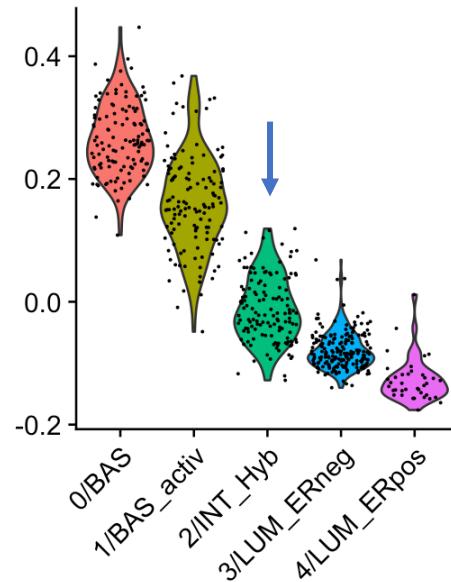
Smart-NIC clusters



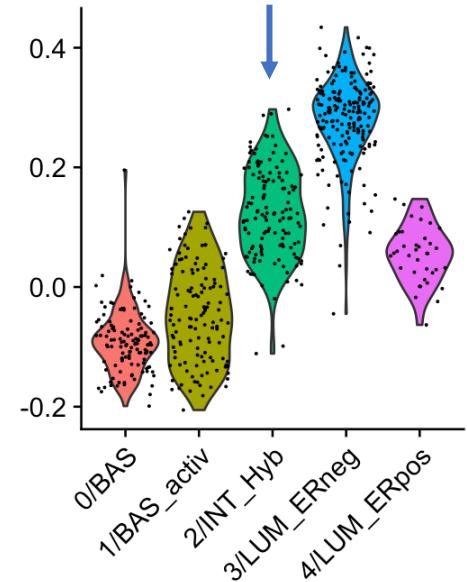
## Intermediate cells show a hybrid basal-luminal gene expression profile



**Basal score**



**Luminal ER- score**



Signatures from Kendrick *et al.*, 2008

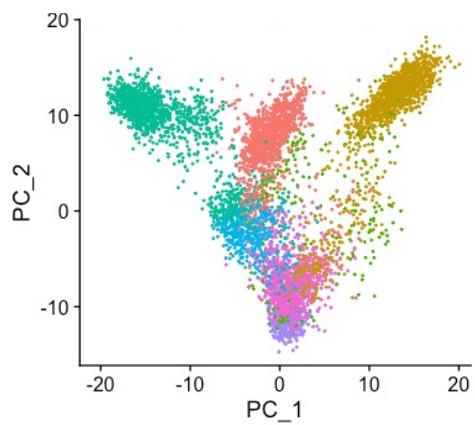
Also moderately higher mito., ribosomal gene exp.  
→ suggesting higher metabolic activity

# Do intermediate clusters show transcriptional similarities with earlier stages of development?

Integration with published datasets:

**Giraddi *et al.*, 2018**

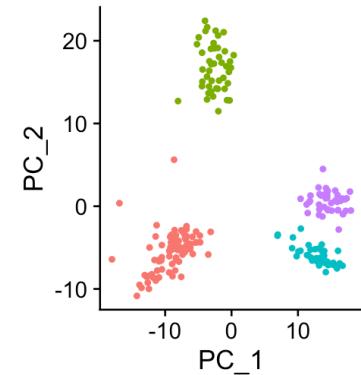
Wahl group



E16, E18, P4  
Adult

**Wuidart *et al.*, 2018**

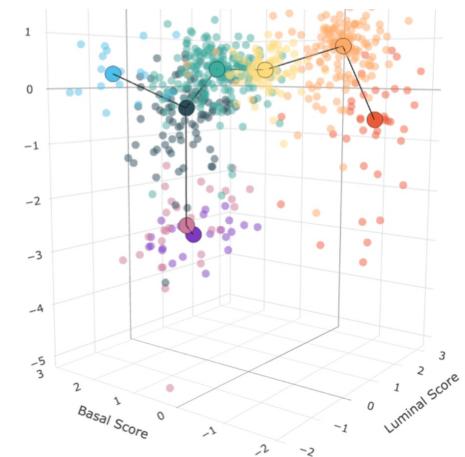
Blanpain group



E14  
Adult

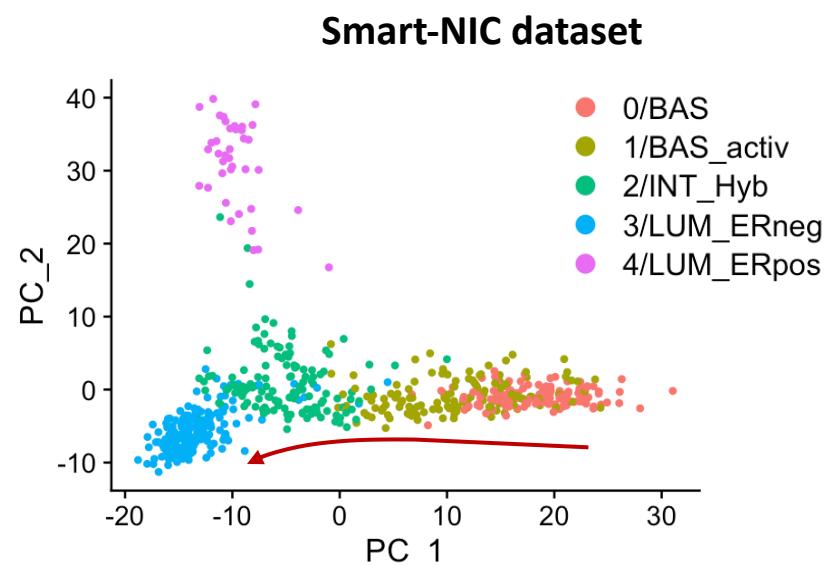
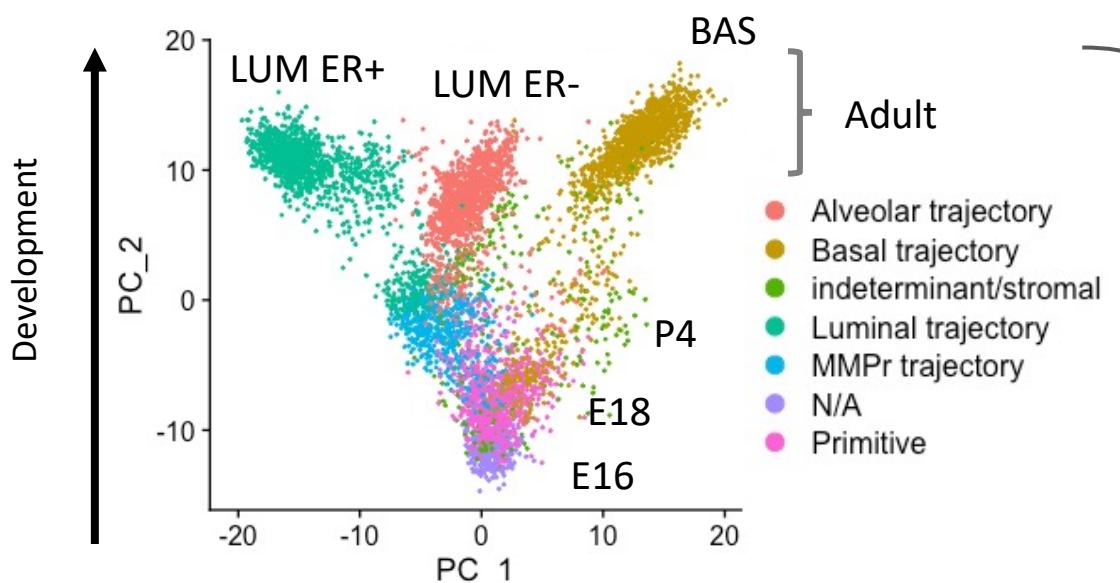
**Carabaña *et al.*, BioRxiv 2022**

Fre group

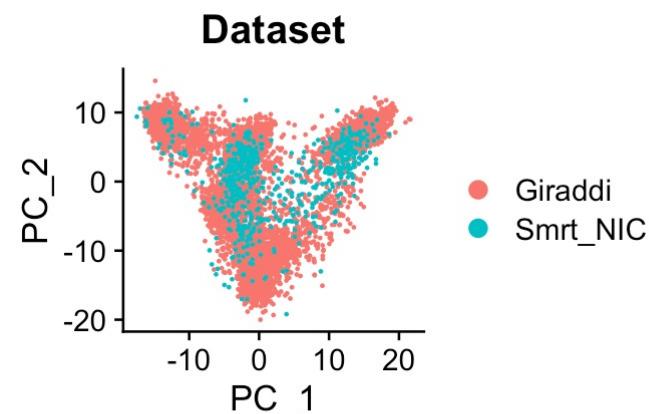


E13, E14, **E15**, P0

## Giraddi dataset: trajectory-labelled clusters



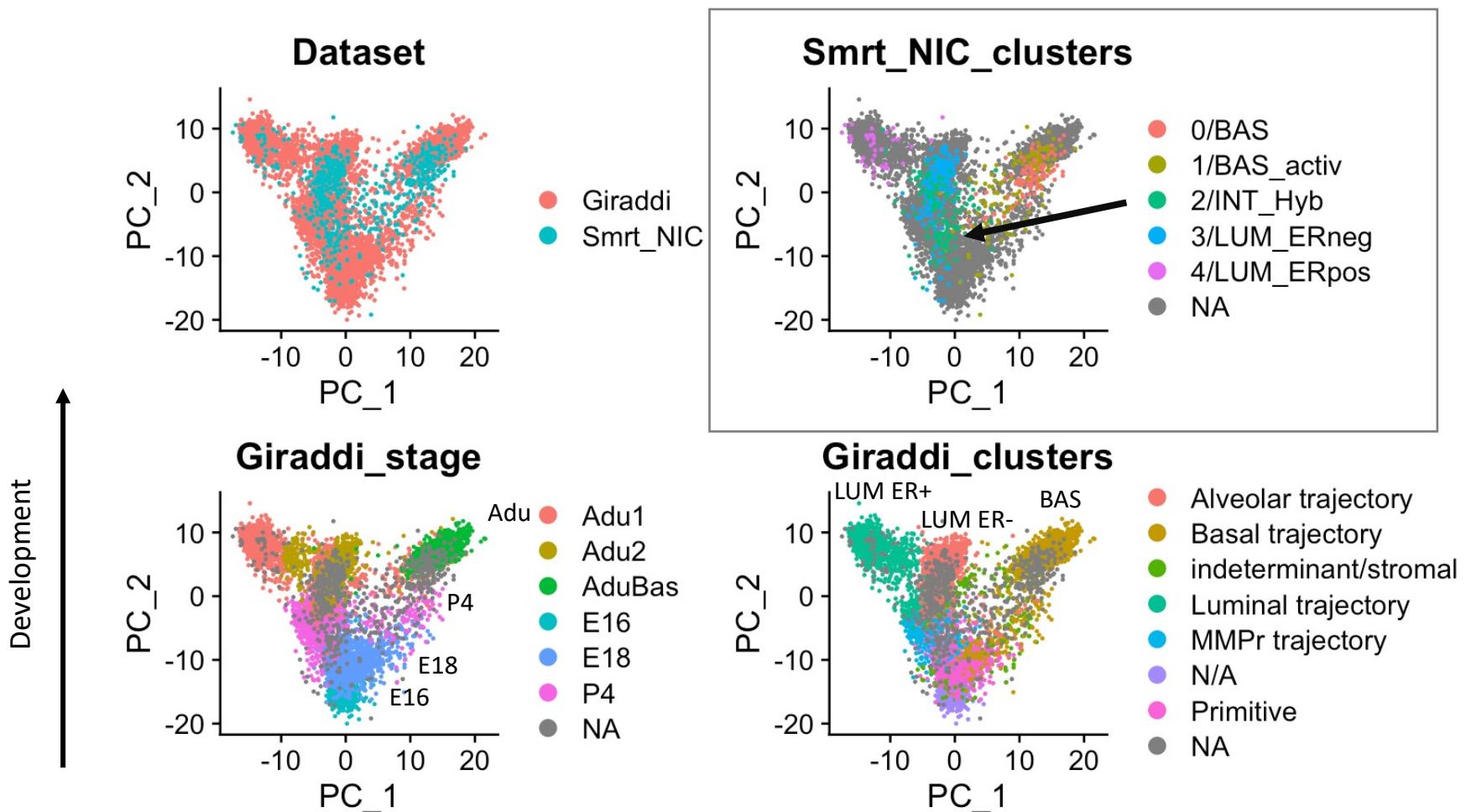
## Integration of Smrt- NIC and Giraddi datasets



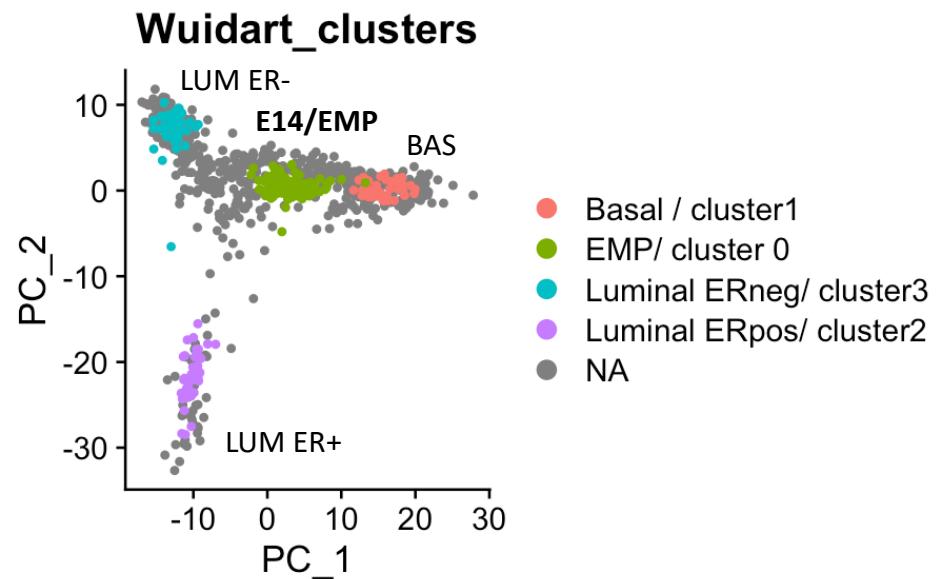
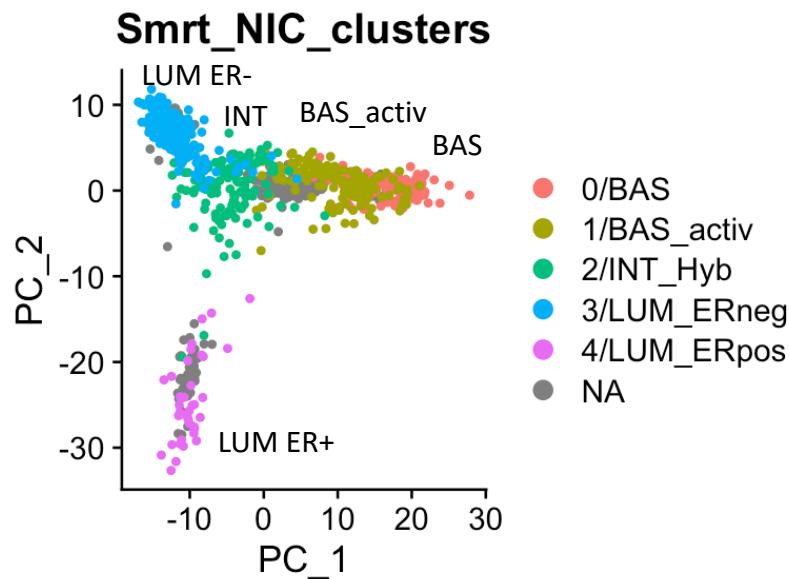
Dataset analysed from:  
Giraddi *et al.*, 2018

# Intermediate clusters show transcriptional similarities with earlier developmental stages – E18, P4

## 1. Integration of Giraddi and NIC Smart-seq2 datasets

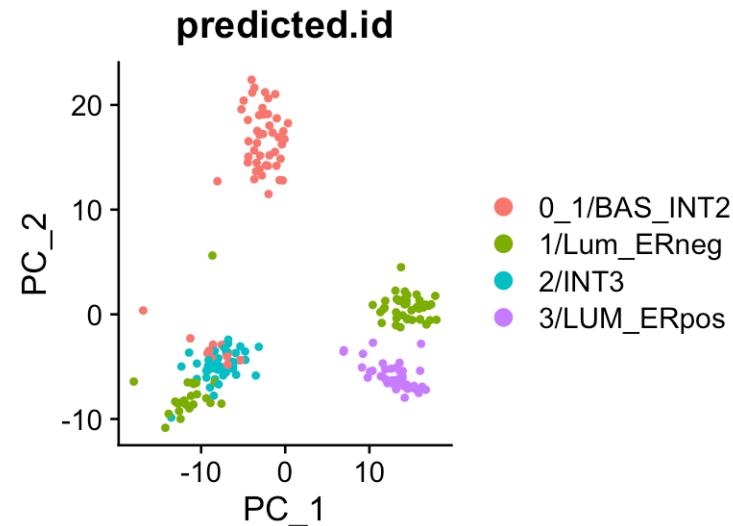
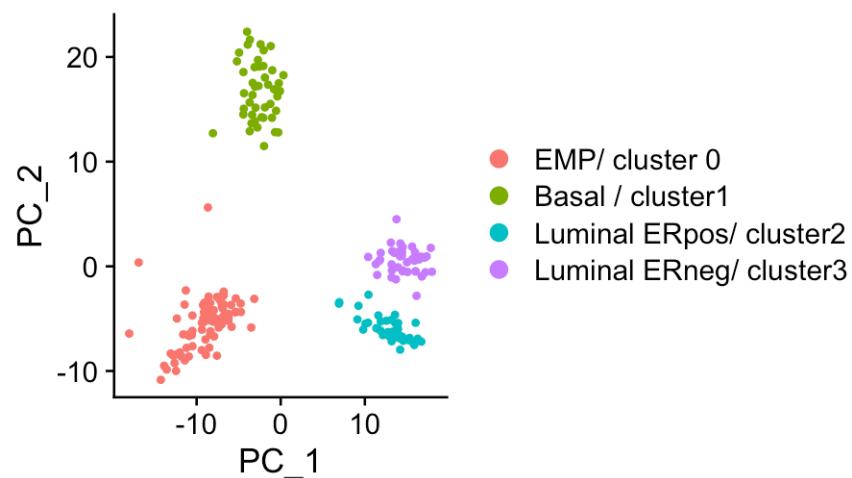


## Integration of Smart-seq2 NIC and Wuidart Smart-seq2 datasets

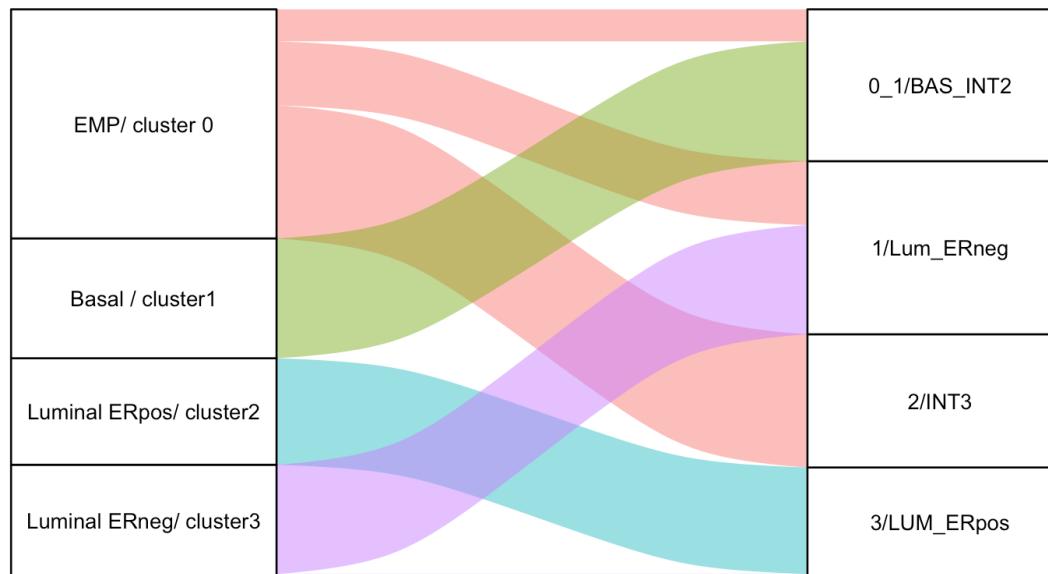


Dataset analysed from:  
Wuidart *et al.*, 2018

## Label transfer: Wuidart dataset- NIC smartseq2 as a reference (labels from NIC)



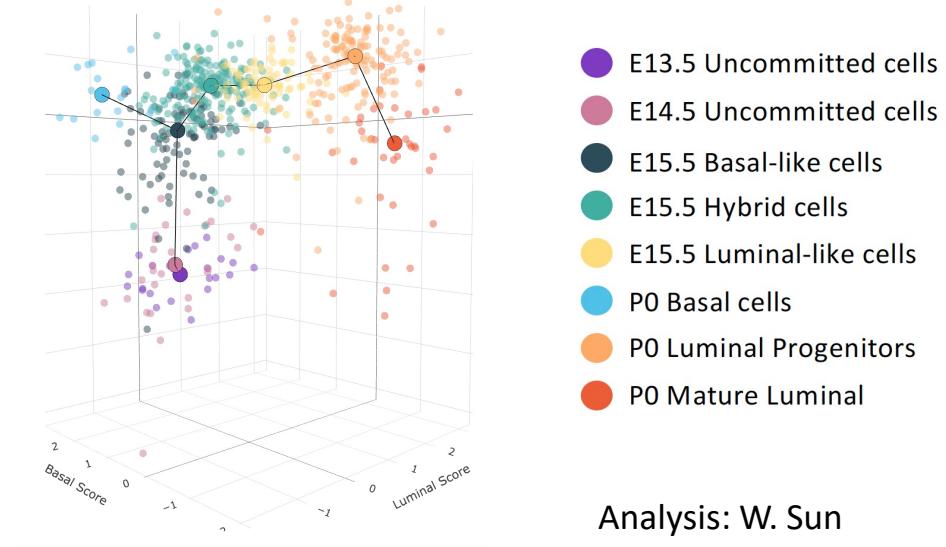
Wuidart cluster



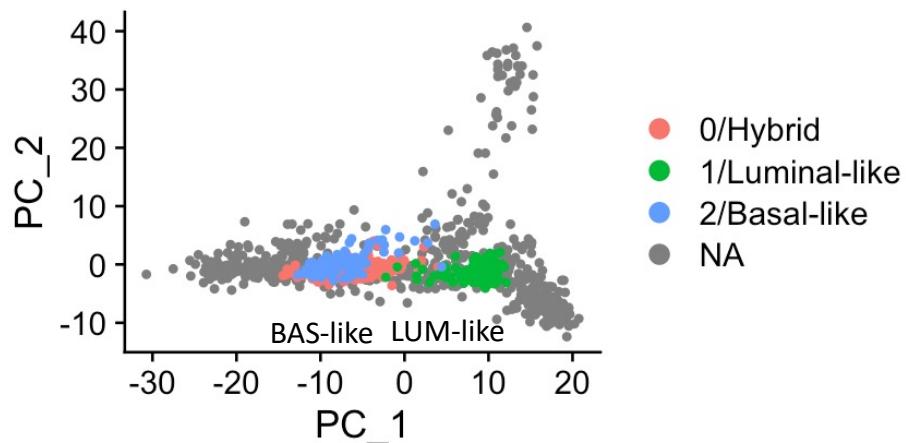
**Intermediate clusters show transcriptional similarities with earlier developmental stages –E15**

## 2. Integration of Smart-seq2 NIC and Carabaña E15 datasets

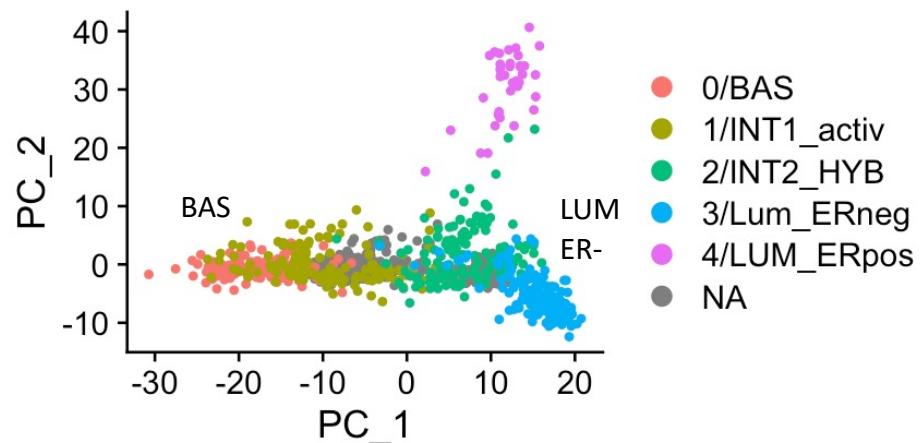
Dataset: Carabaña *et al.*, BioRxiv 2022



**E15\_clusters**

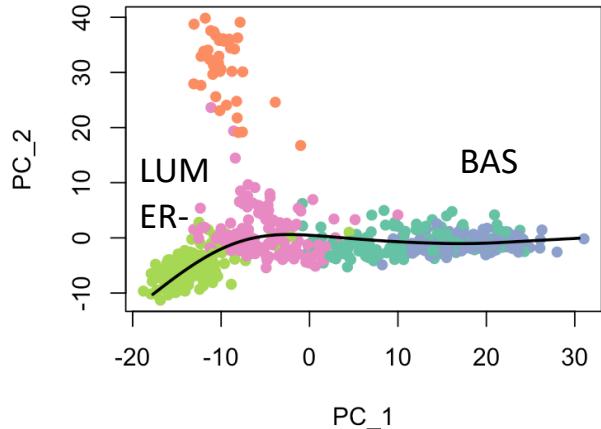


**Smrt\_NIC\_clusters**

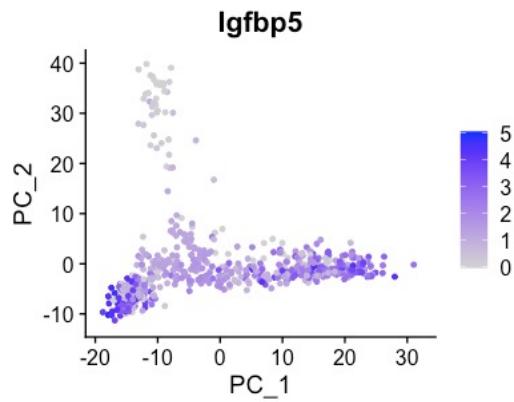


## Which genes are altered along the cell fate transition?

Basal to Luminal ER-ve trajectory

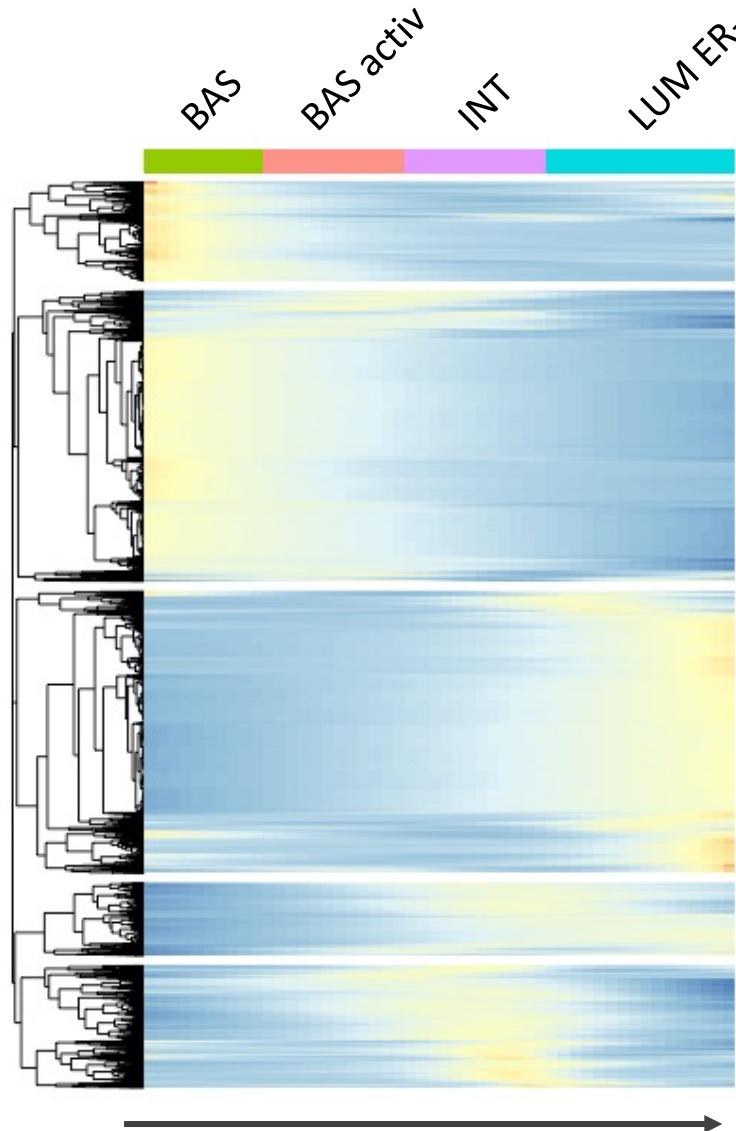


Slingshot trajectory analysis



A few genes show modulation in intermediate states → Could impact cell fate transition?

Tradeseq- genes which correlate with trajectory



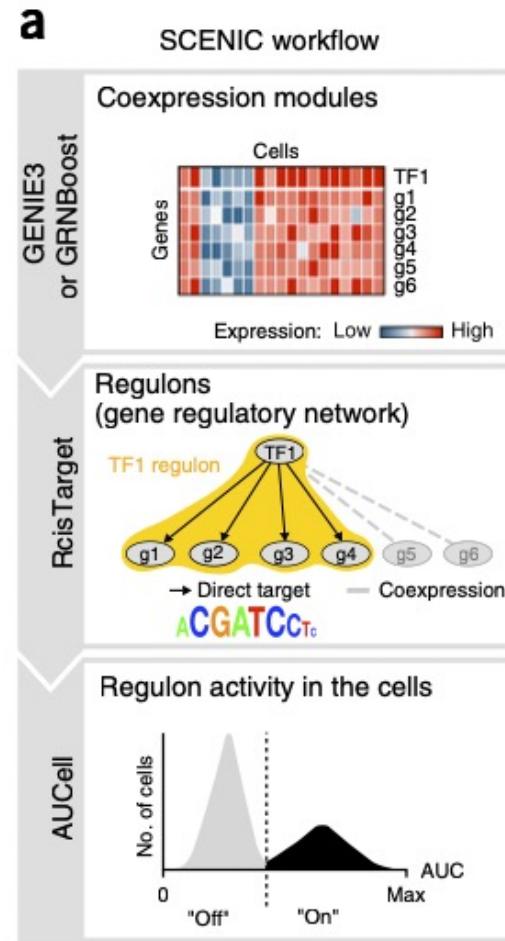
Gene expression in cells ordered by trajectory

# Which TFs could drive this cell fate transition?

SCENIC: Assess Gene regulatory networks GRNs/ TF activity in each cell, in scRNA-Seq data → to infer cell states

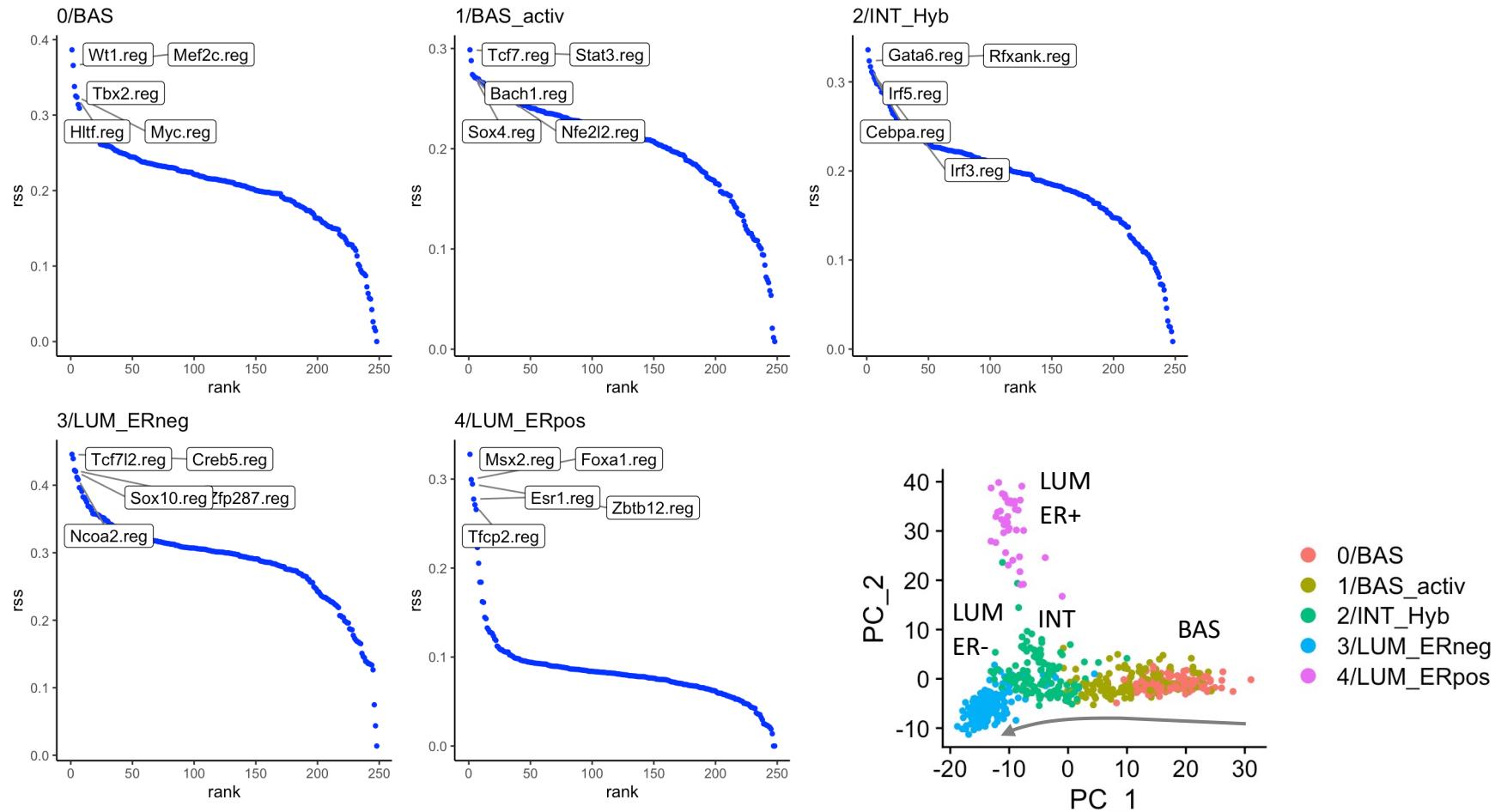
## SCENIC: single-cell regulatory network inference and clustering

Sara Aibar<sup>1,2</sup> , Carmen Bravo González-Blas<sup>1,2</sup> , Thomas Moerman<sup>3,4</sup> , VÂN ANH HUYNH-THU<sup>5</sup>, Hana Imrichová<sup>1,2</sup> , Gert Hulselmans<sup>1,2</sup> , Florian Rambow<sup>6,7</sup>, Jean-Christophe Marine<sup>6,7</sup>, Pierre Geurts<sup>5</sup>, Jan Aerts<sup>3,4</sup> , Joost van den Oord<sup>8</sup>, Zeynep Kalender Atak<sup>1,2</sup> , Jasper Wouters<sup>1,2,8</sup>  & Stein Aerts<sup>1,2</sup> 



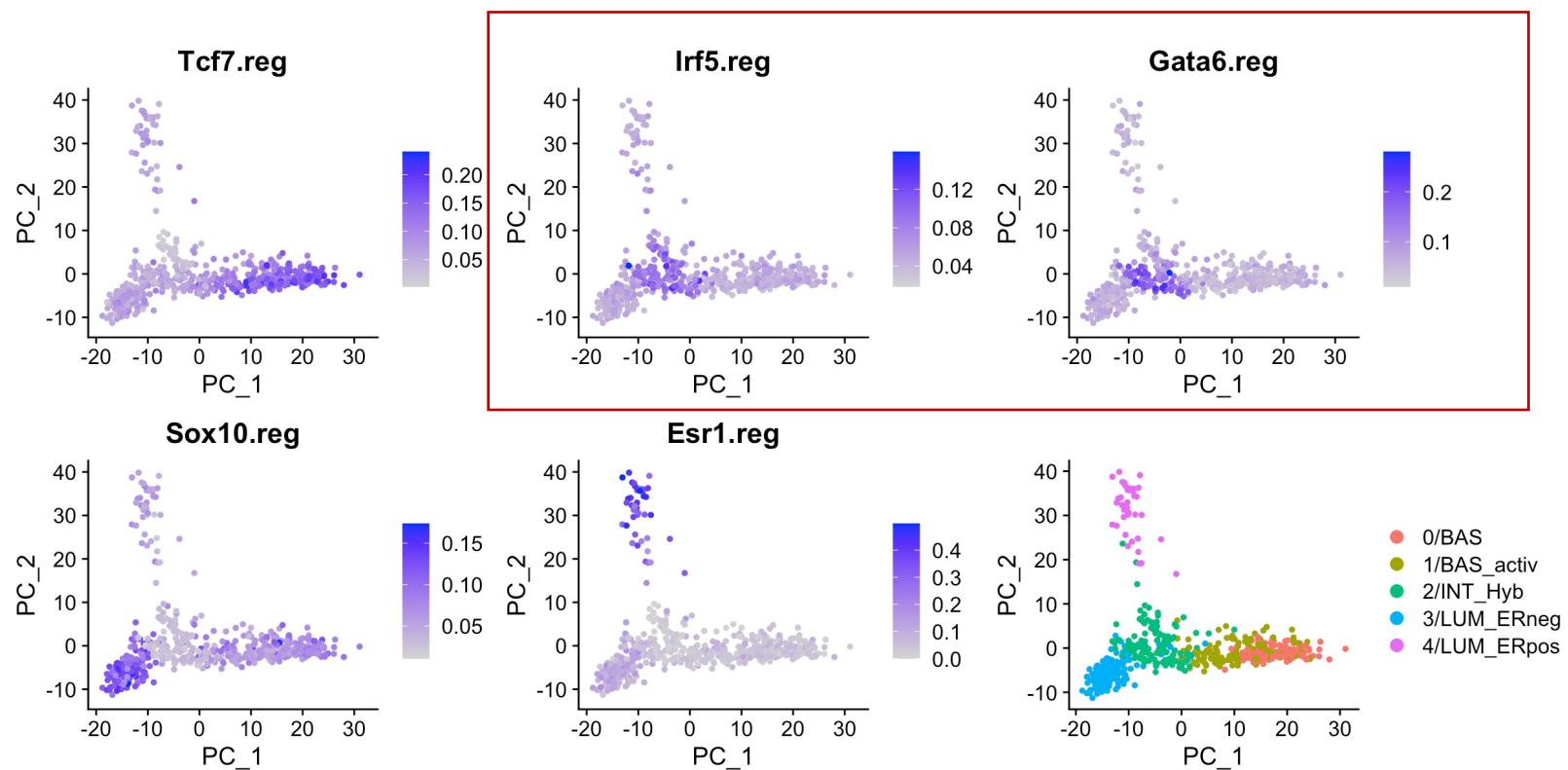
Aibar *et al.*, 2017

# SCENIC analysis – RSS- Regulon Specificity Score



RSS- Testing which regulon's activity are specific to different clusters

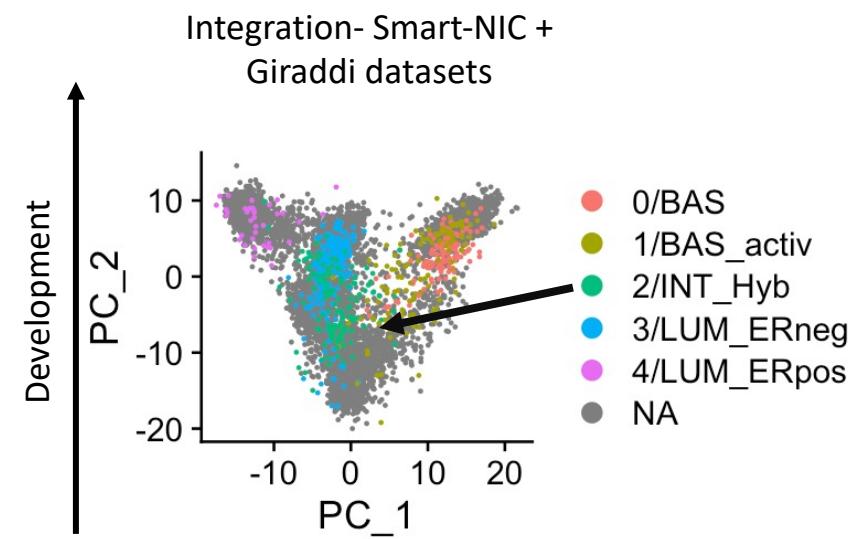
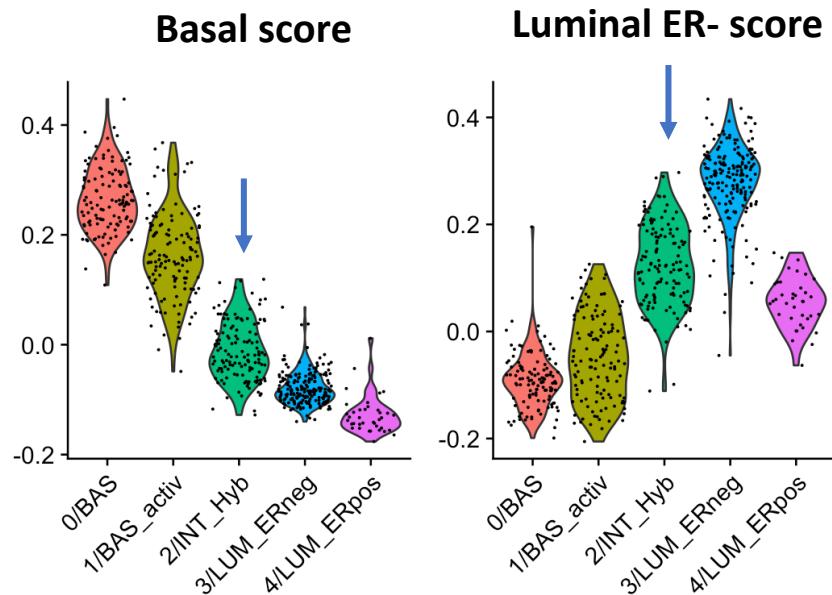
## SCENIC analysis – visualising TF regulon activity



## Summary:

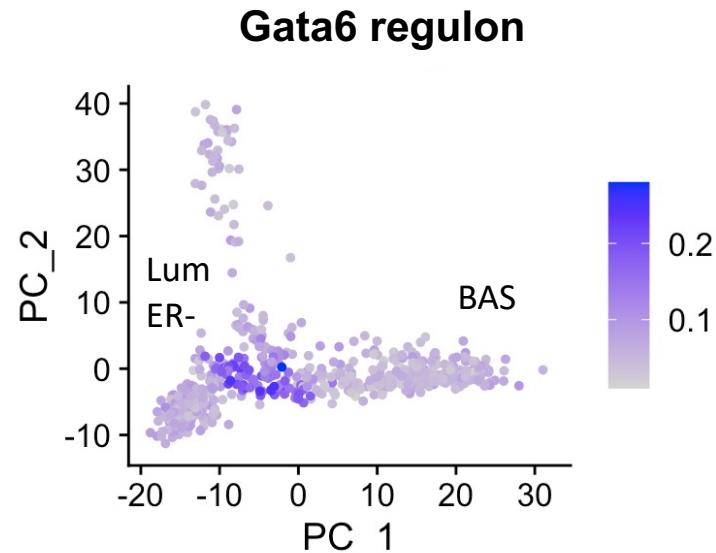
During Notch-induced basal-luminal cell fate transition; Intermediate cells show:

- A **hybrid basal-luminal** gene expression profile,
  - Some **transcriptional similarities with early developmental stages** (Giraddi E18/P4, Carabaña E15, Wuidart E14 datasets)
- (but they do not express distinct embryonic genes)



## Ongoing directions:

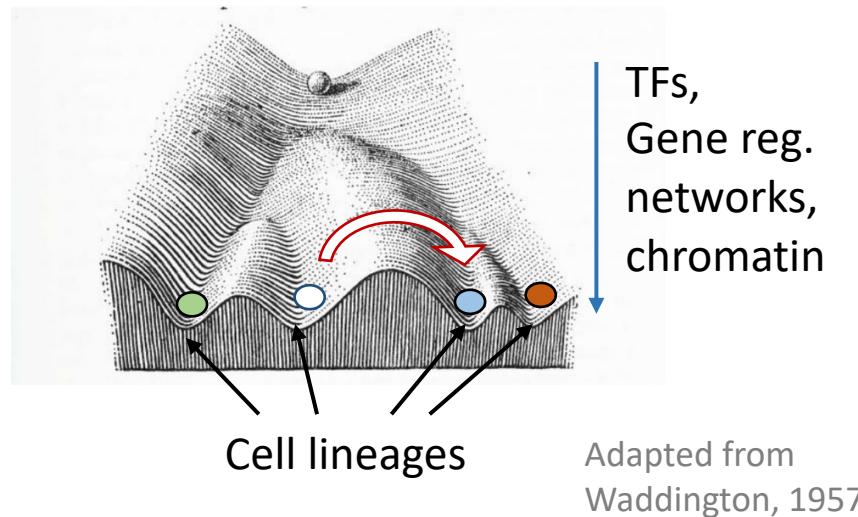
- TF activity changes during transition- SCENIC.
- Functional testing of candidate genes → impacts on cell fate/transition? Candice, Atifeh



## Perspectives:

Genes which affect transition – implications in:

- Directing lineage commitment
- Dere regulation of cell identity in cancers



# Acknowledgements

## Team Fre

Silvia Fre

Candice Merle

Meghan Perkins

Fre lab

## Curie Bioinformatics platform

Nicolas Servant

Curie Bioinformatics platform,

Curie Bioinformatics Hub,

Berthold Göttgens team

## Team Margueron

Raphaël Margueron

Margueron lab

**Thank you!**