UCDAVIS

Characterizing cell type-specific responses to stimuli using single cell RNA sequencing

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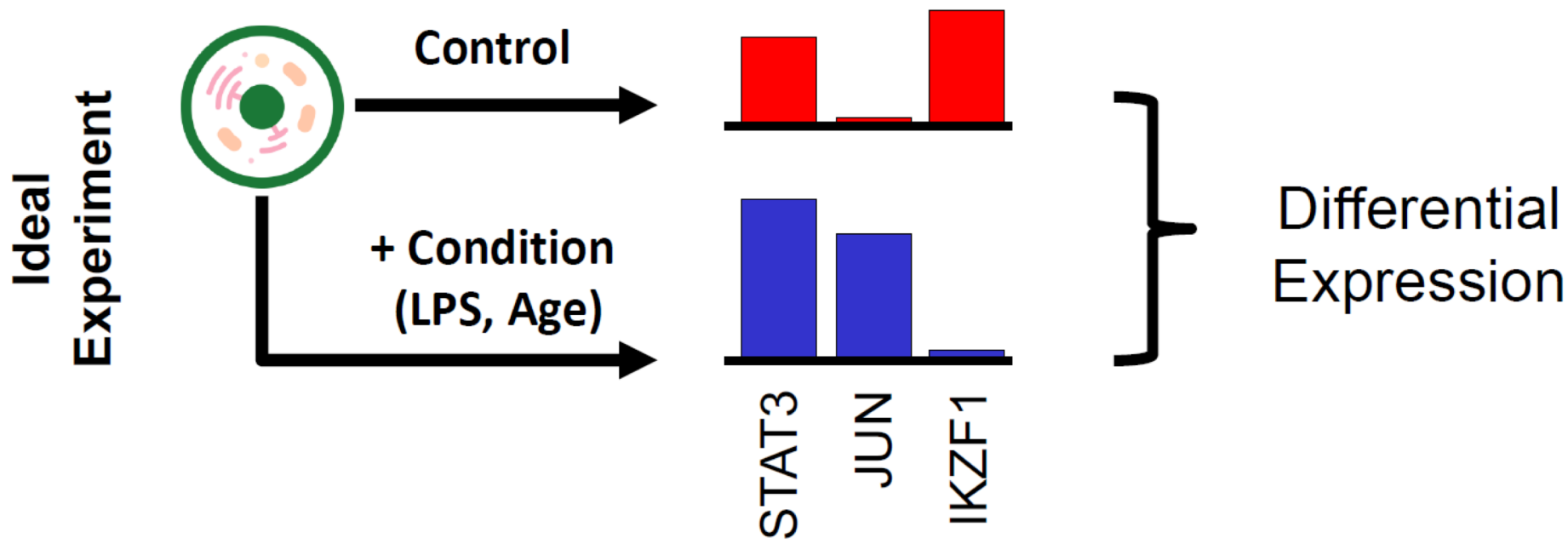
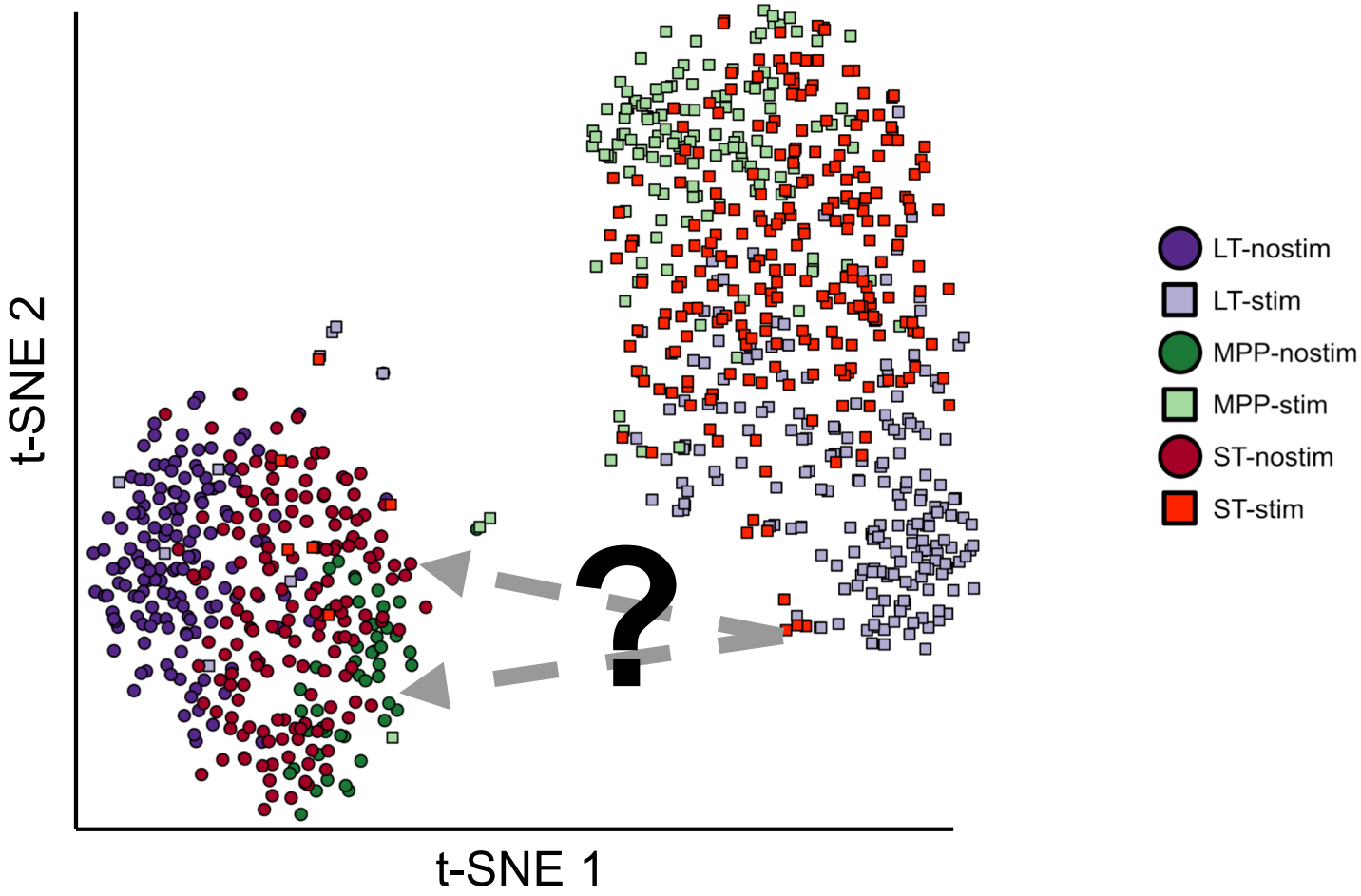
Ideal experiment for single cell perturbation

- In the ideal experiment, we would perform scRNA-seq on the same individual cell before and after stimulus.



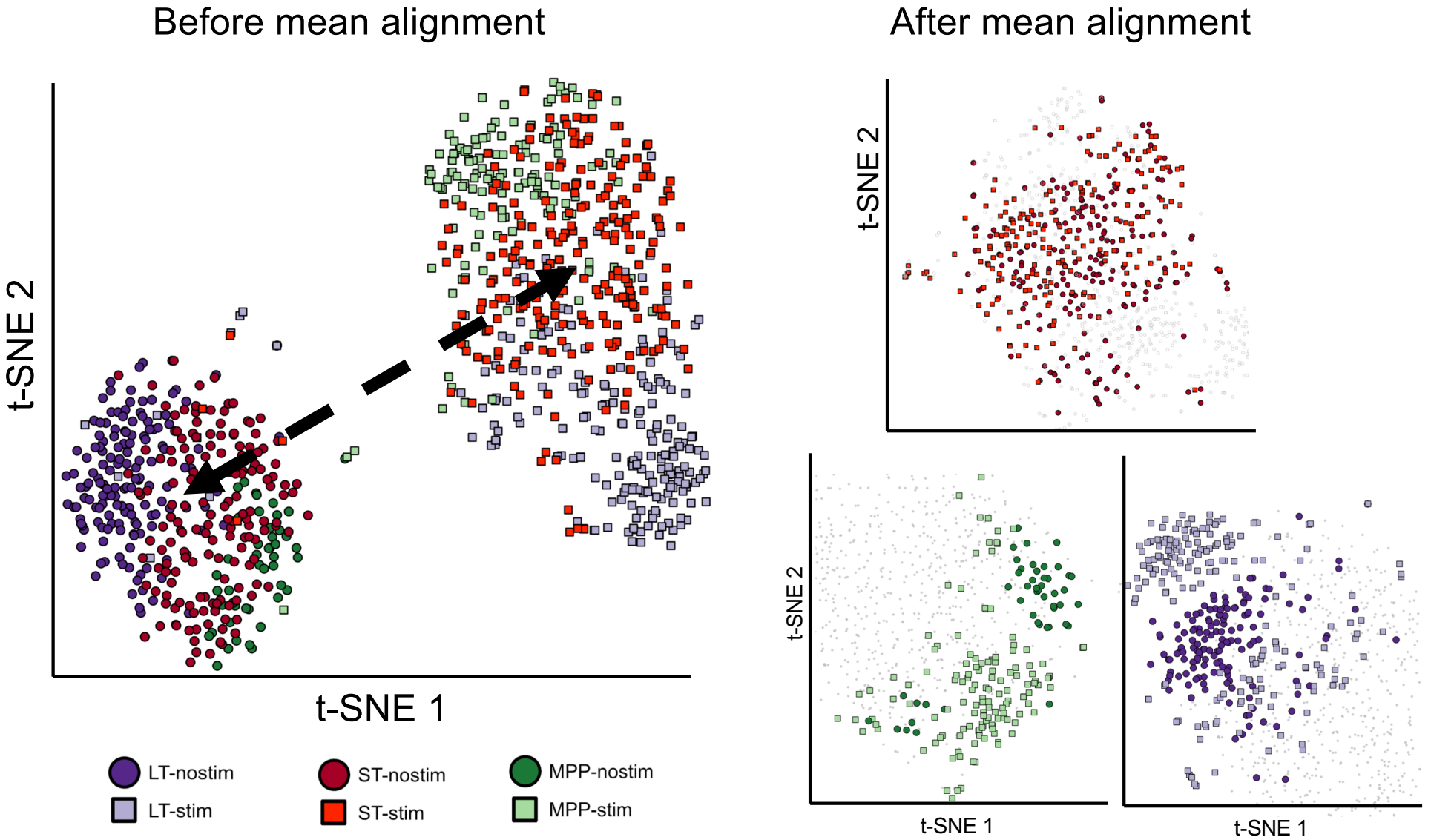
Alignment is the primary problem in perturbation studies

What was the state for each perturbed cell before exposure?

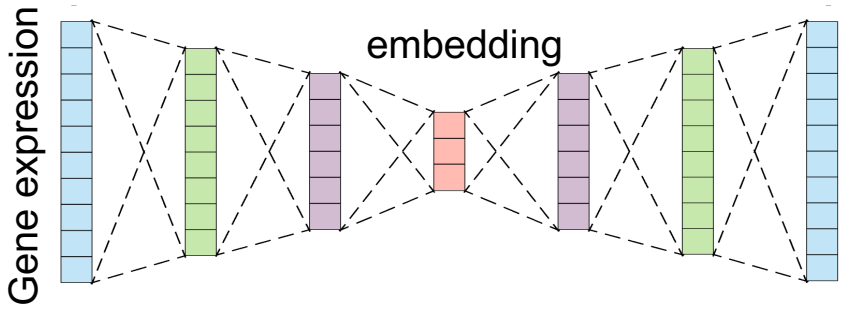


Cell type specific responses hinder alignment

- Problem similar to batch effect correction

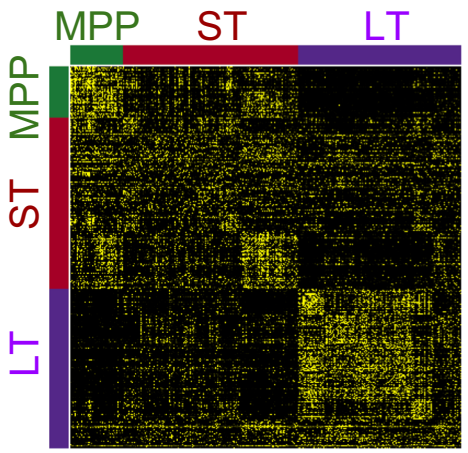


Unsupervised (optionally supervised) alignment outline

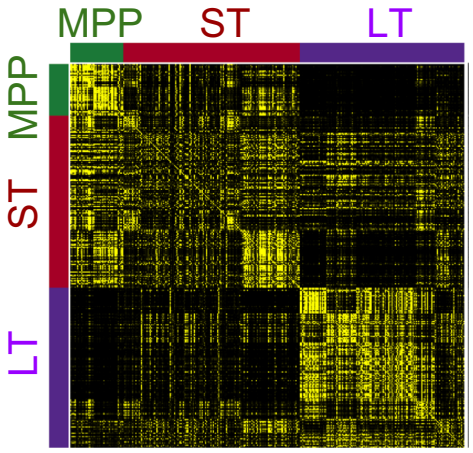


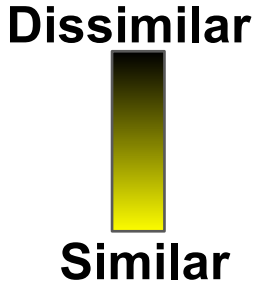
Cell-cell similarity

Gene expression



Embedding





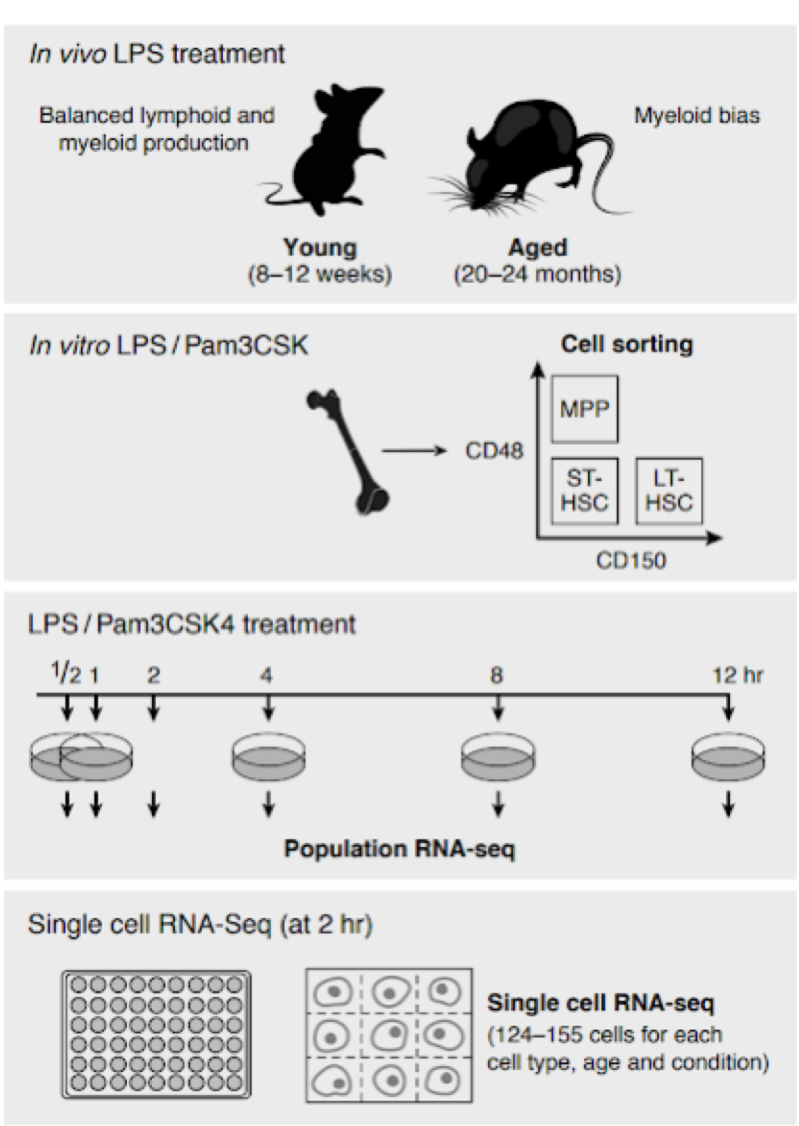
$$P^{\text{expr}}_{s_1}, P^{\text{expr}}_{s_2}$$

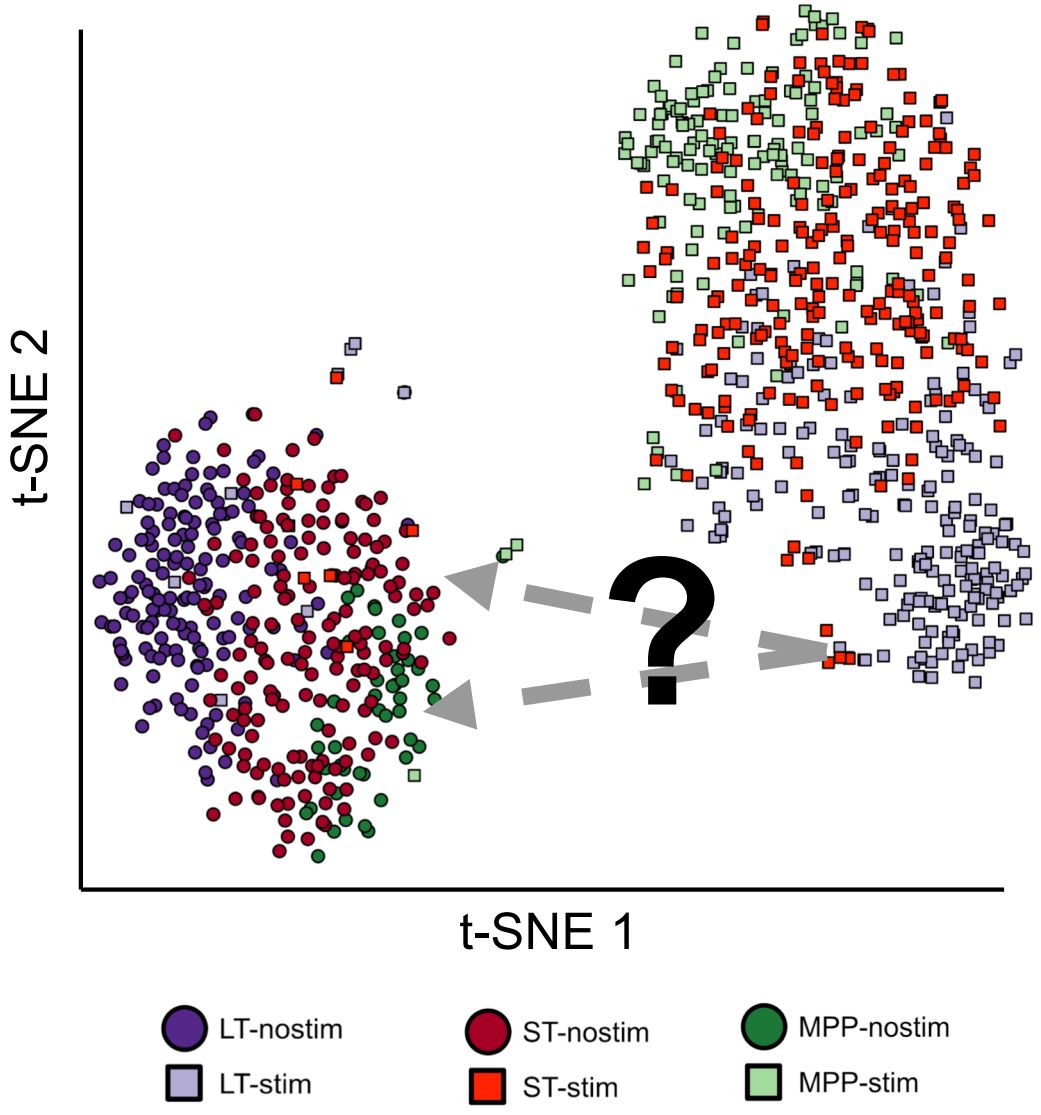
$$P^{\text{emb}}_{s_1 \to s_2}, P^{\text{emb}}_{s_2 \to s_1}$$

$$\text{x-entropy}(P^{\text{expr}}_{s_1}, P^{\text{emb}}_{s_1 \to s_2} P^{\text{emb}}_{s_2 \to s_1}) + \text{x-entropy}(P^{\text{expr}}_{s_2}, P^{\text{emb}}_{s_2 \to s_1} P^{\text{emb}}_{s_1 \to s_2}) + \lambda[L_2]$$

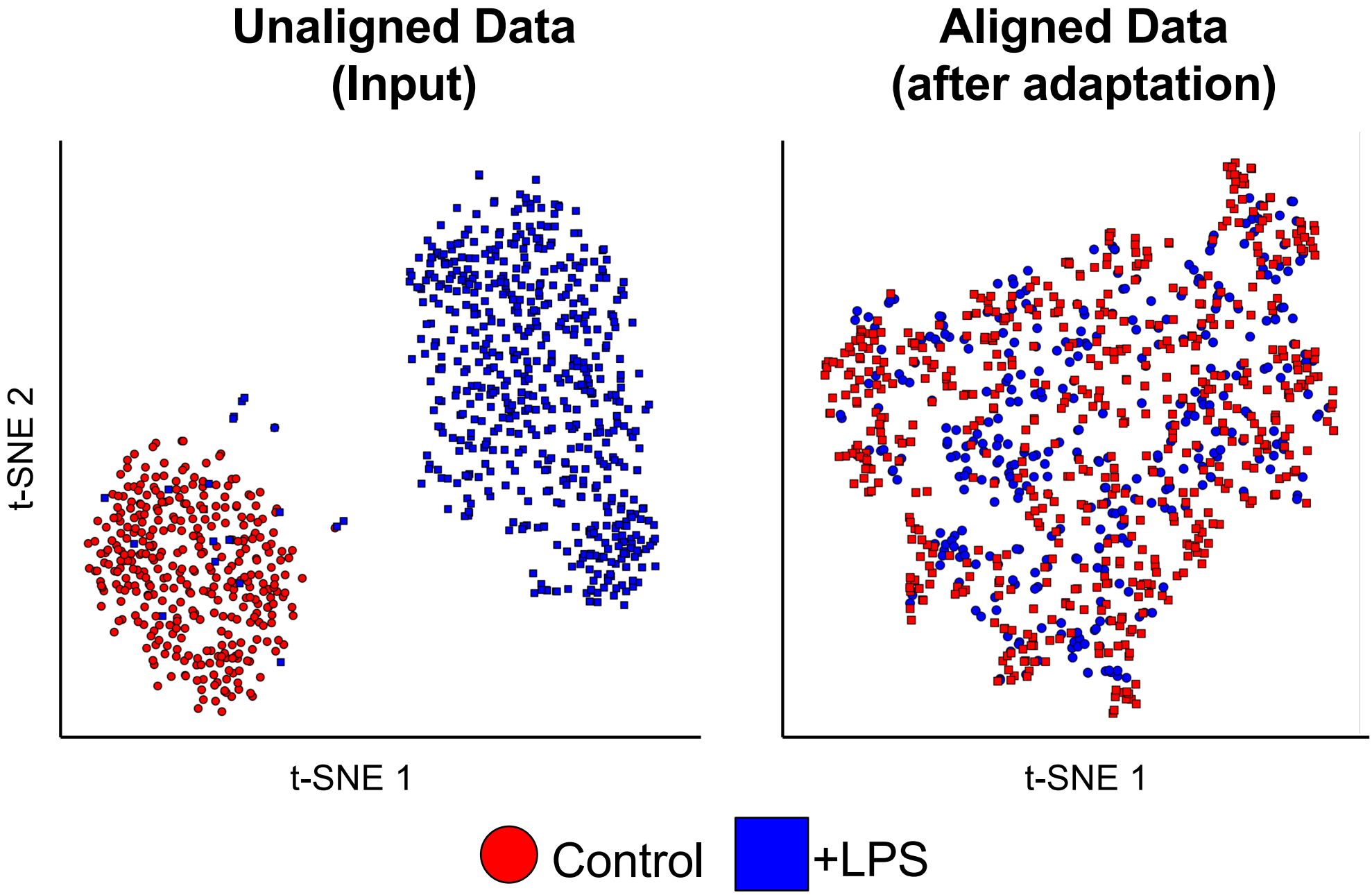
HSC Inflammation study: Mann et al. (2017)

- Alignment of single cell perturbation study

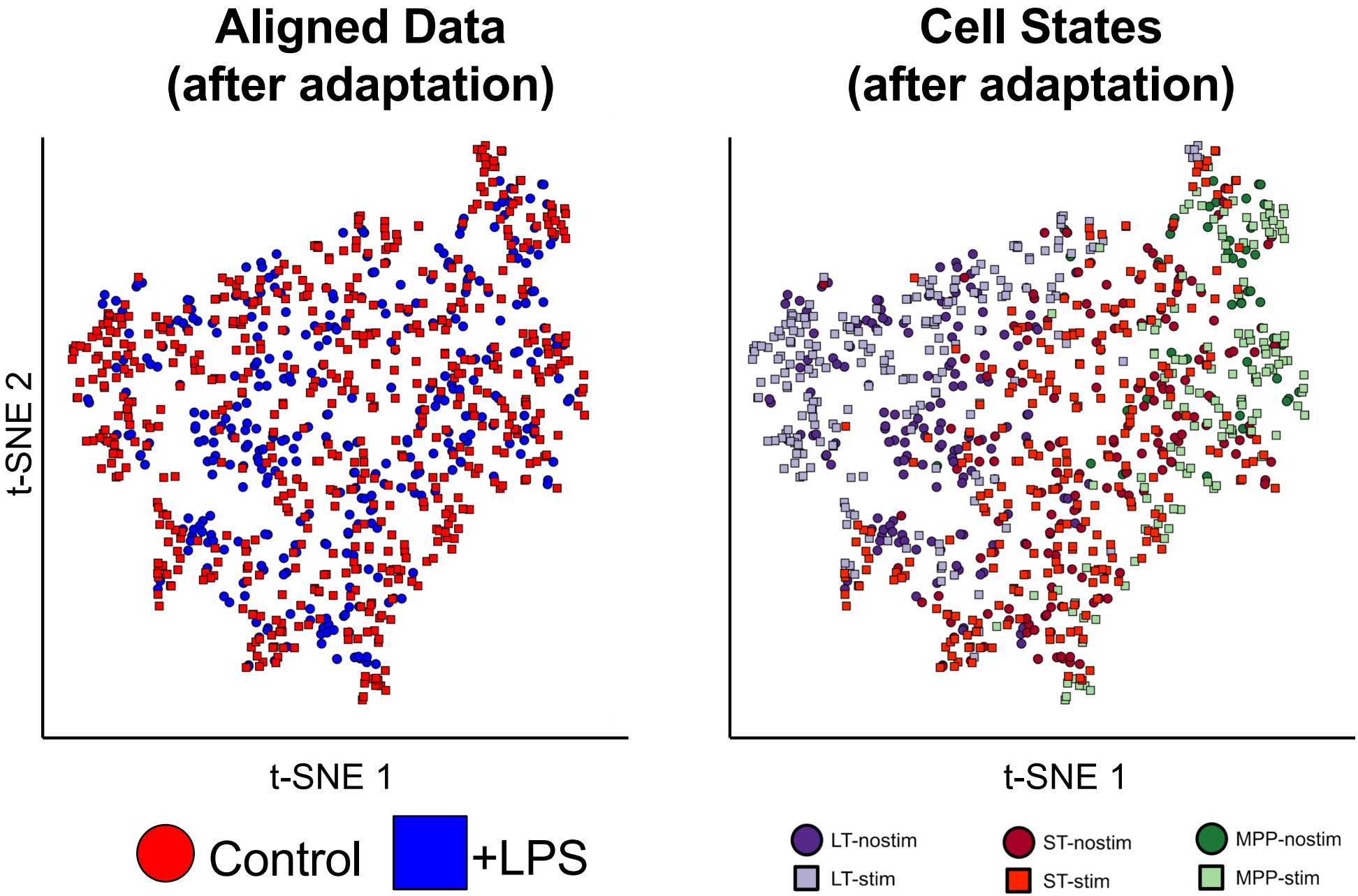




Alignment of HSCs leads to mixing of control, perturbation

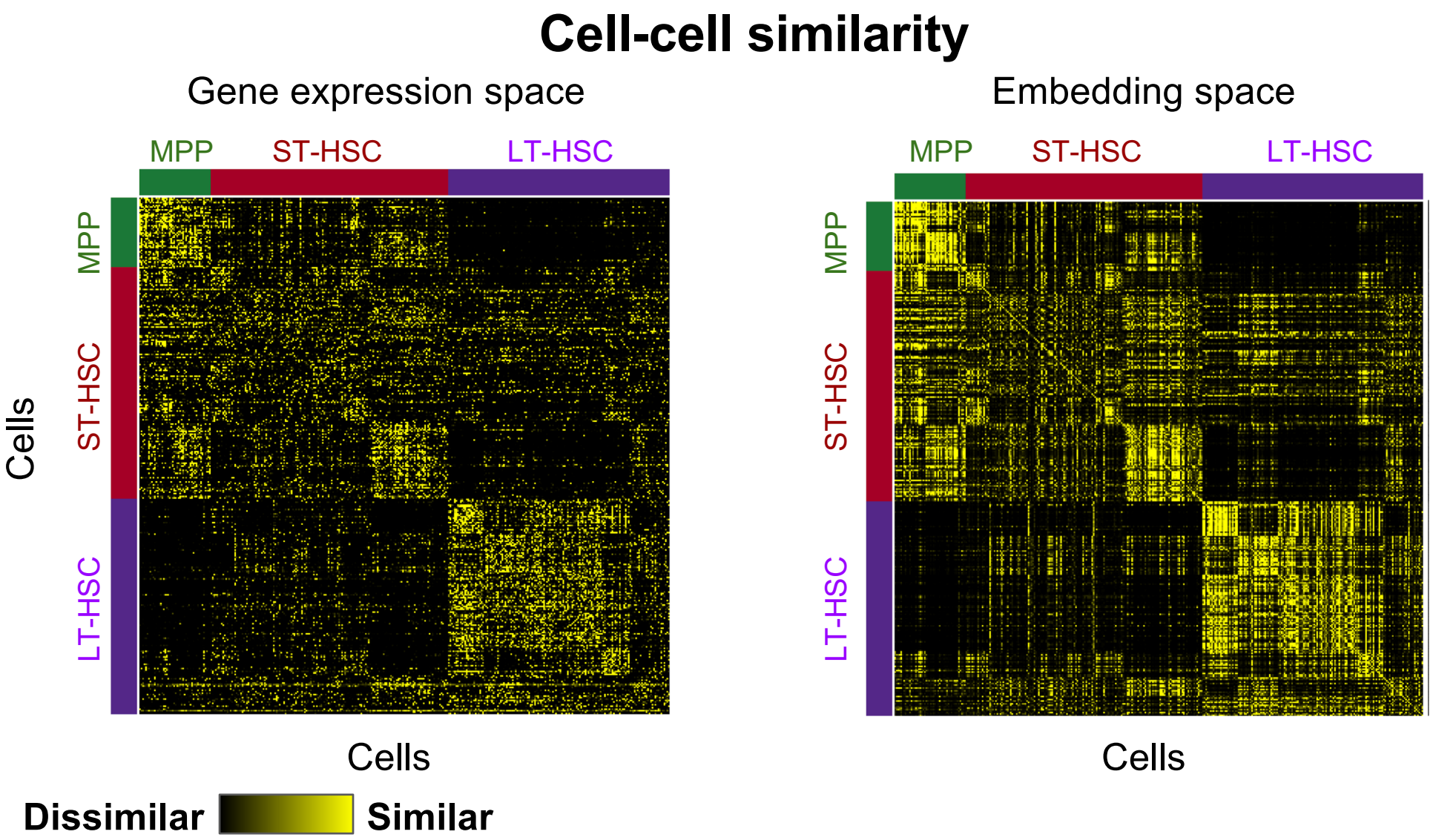


Unsupervised alignment matches cell types

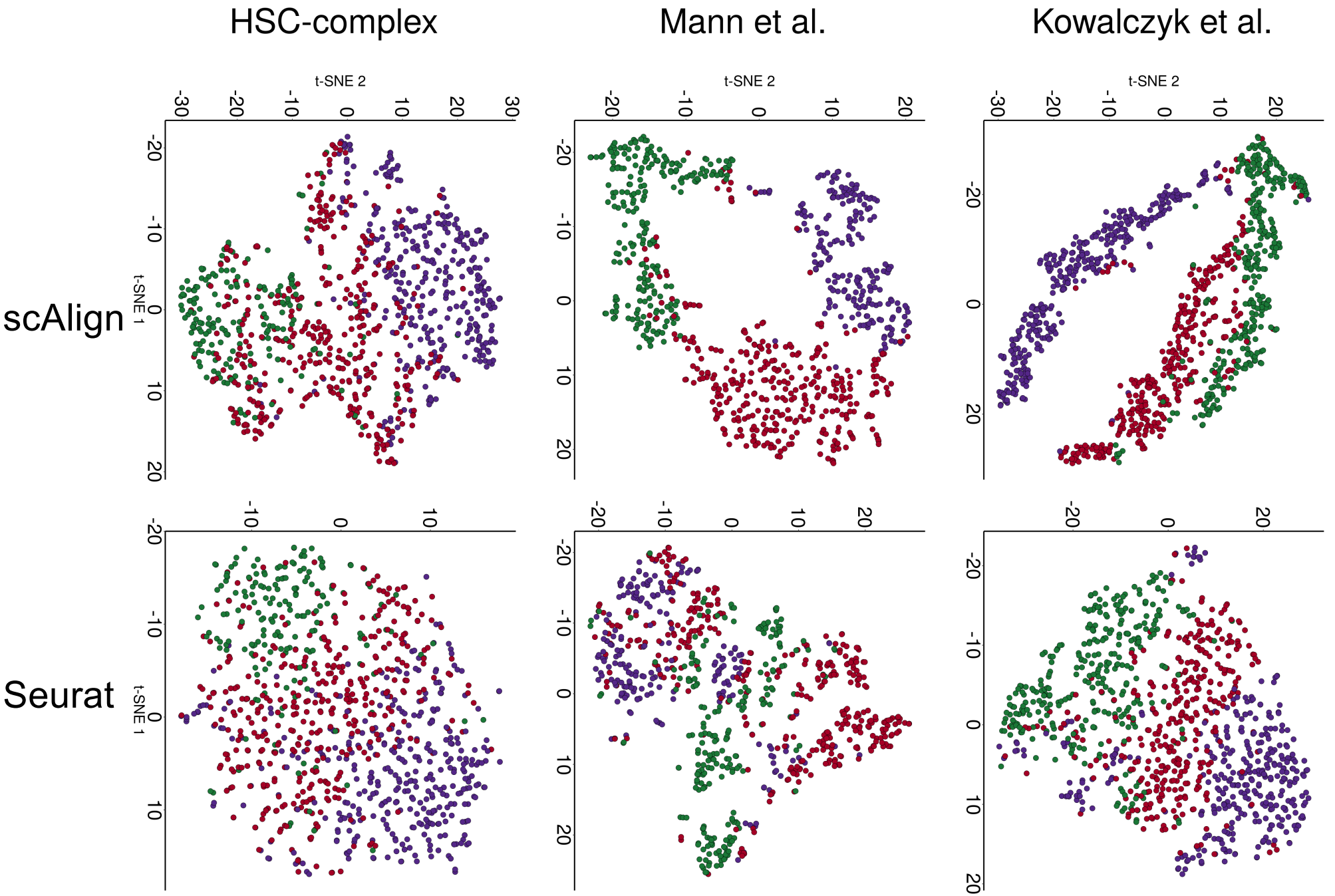


Alignment preserves similarity between cells

- ”compression” accentuates the similarity matrix

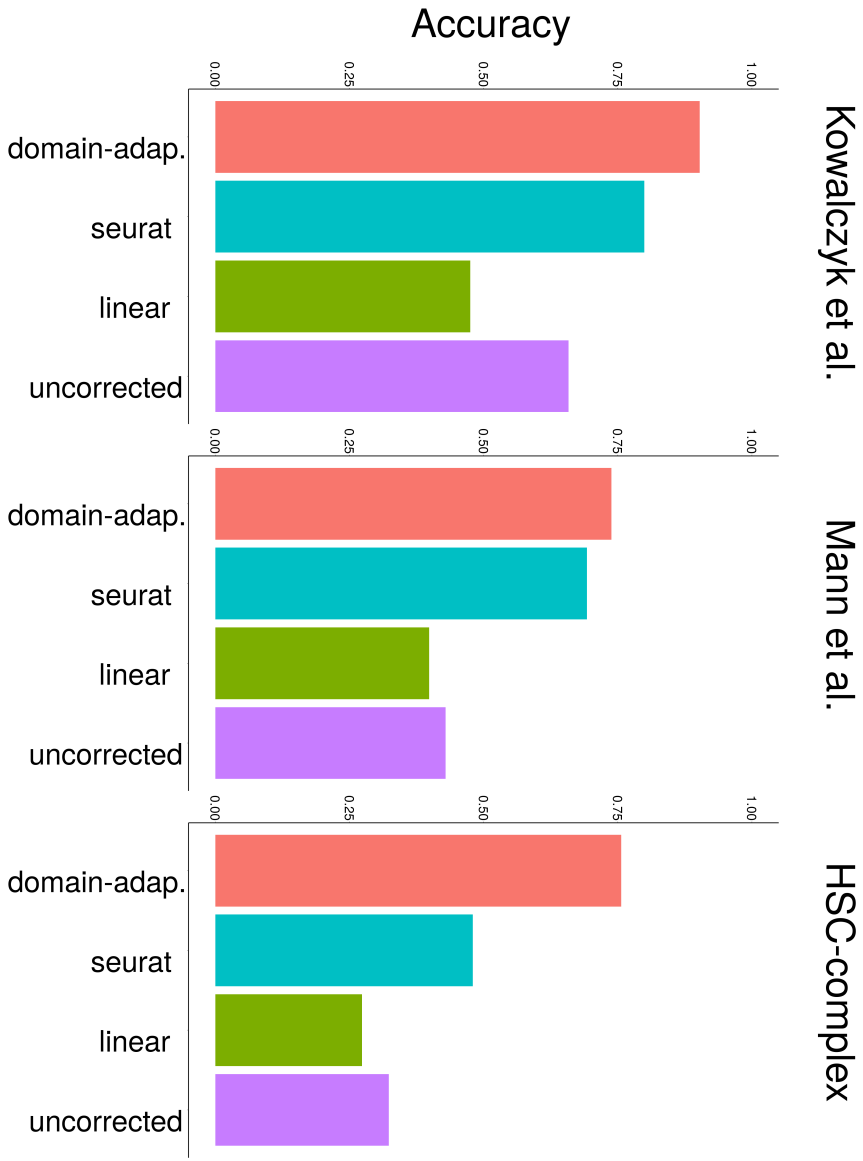


Domain adaptation aligns cell types with heterogeneous response



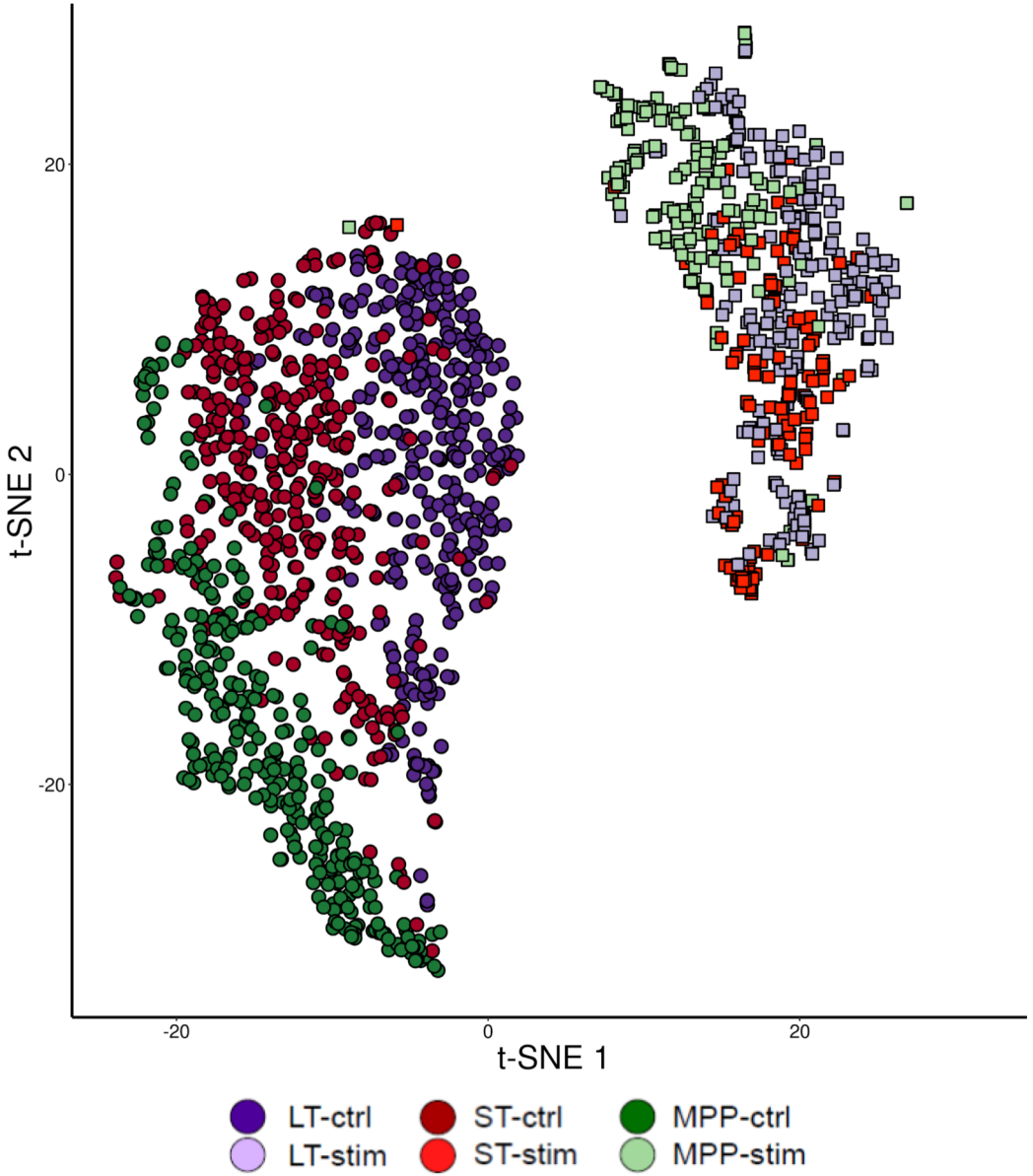
Domain adaptation aligns cell types with heterogeneous response

- Accuracy: how well cells of the same type cluster together.



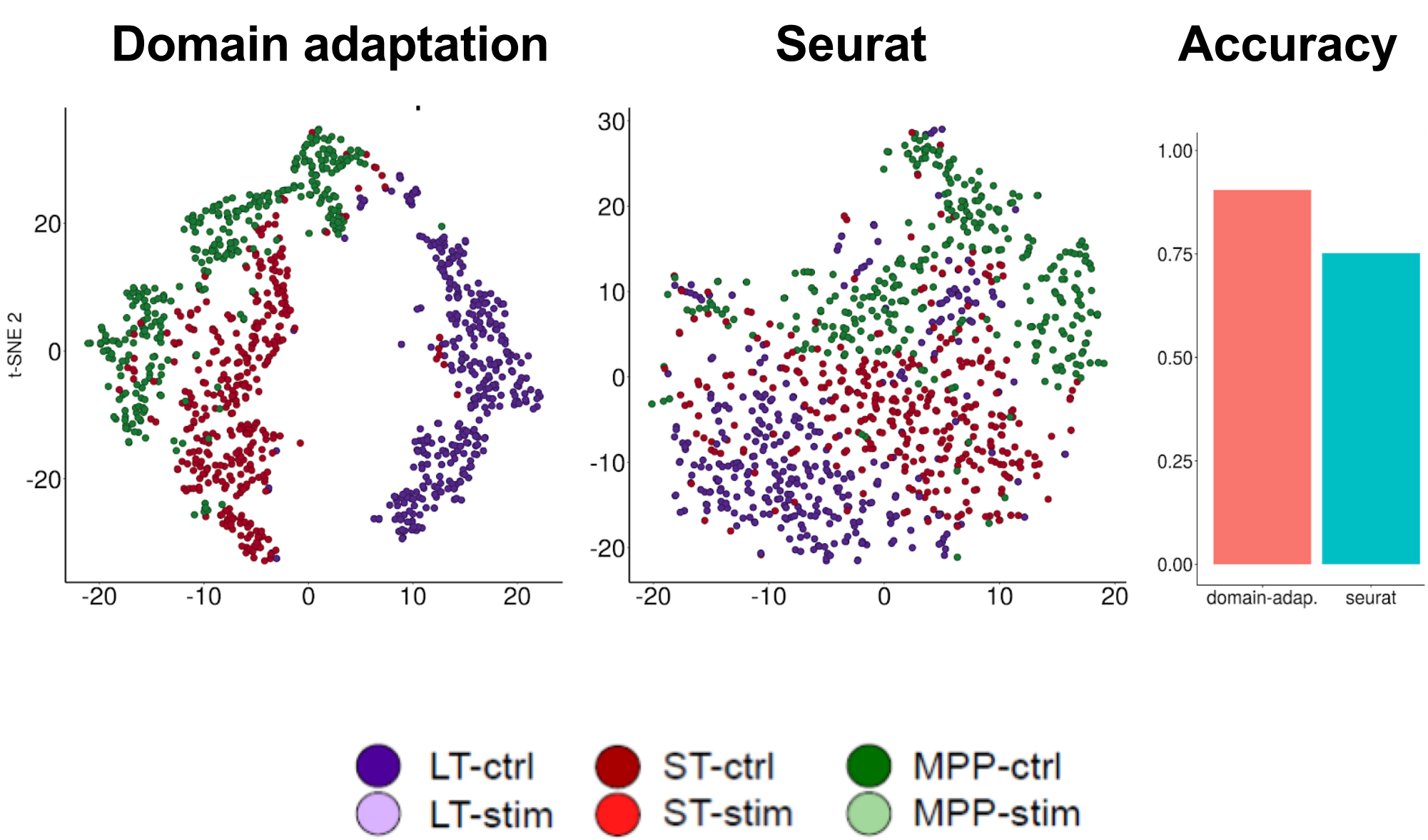
Aligning hematopoietic cells from young and old mice

Single-cell RNA-seq reveals changes in cell cycle and differentiation programs upon aging of hematopoietic stem cells. - **Kowalczyk et al. 2015**



Aviv Regev
(Broad)

Aligning hematopoietic cells from young and old mice

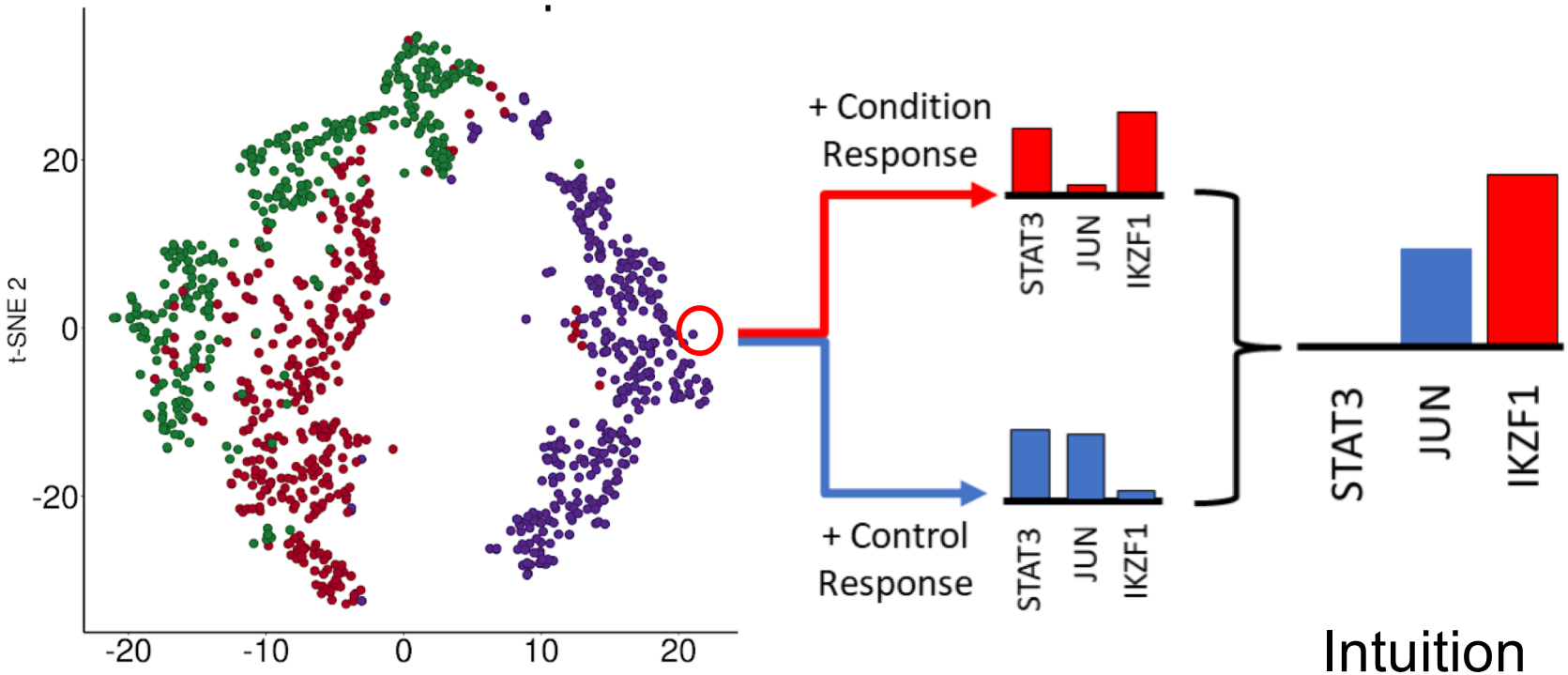


Paired-representation of each cell for differential expr.

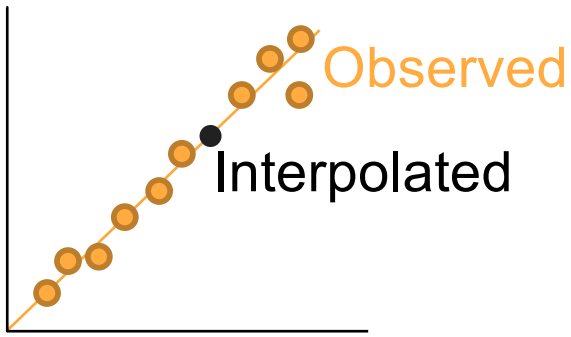
Research steps:

(1) Alignment of single cell data across conditions.

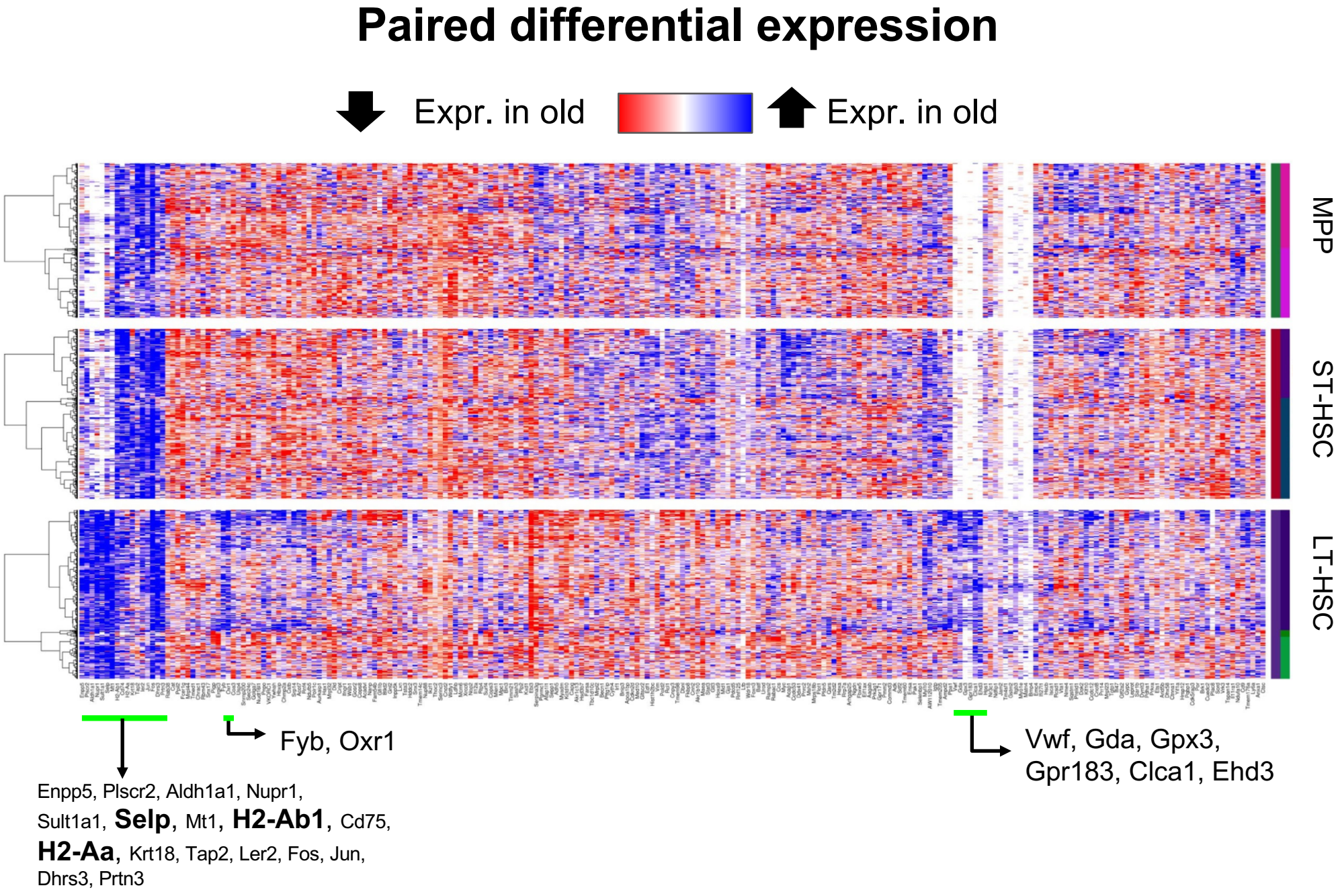
(2) Decode cells from joint state space back to different conditions.



Intuition

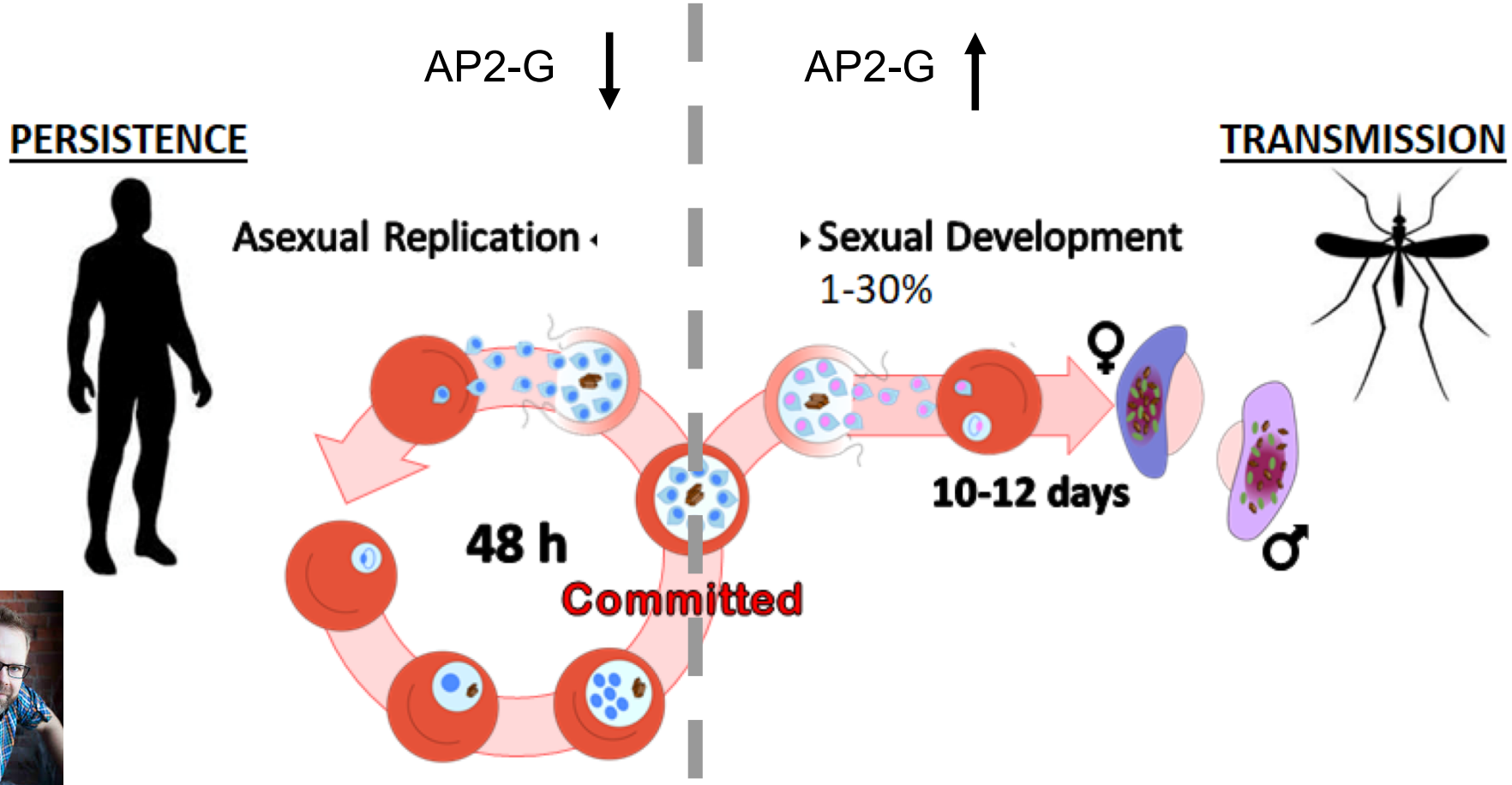


Paired differential expression reveals HSC subpopulations



Sexual commitment in malaria

- Differential “trajectories”
- Pathogens balance transmission with persistence.
- AP2-G is a master switch of commitment.

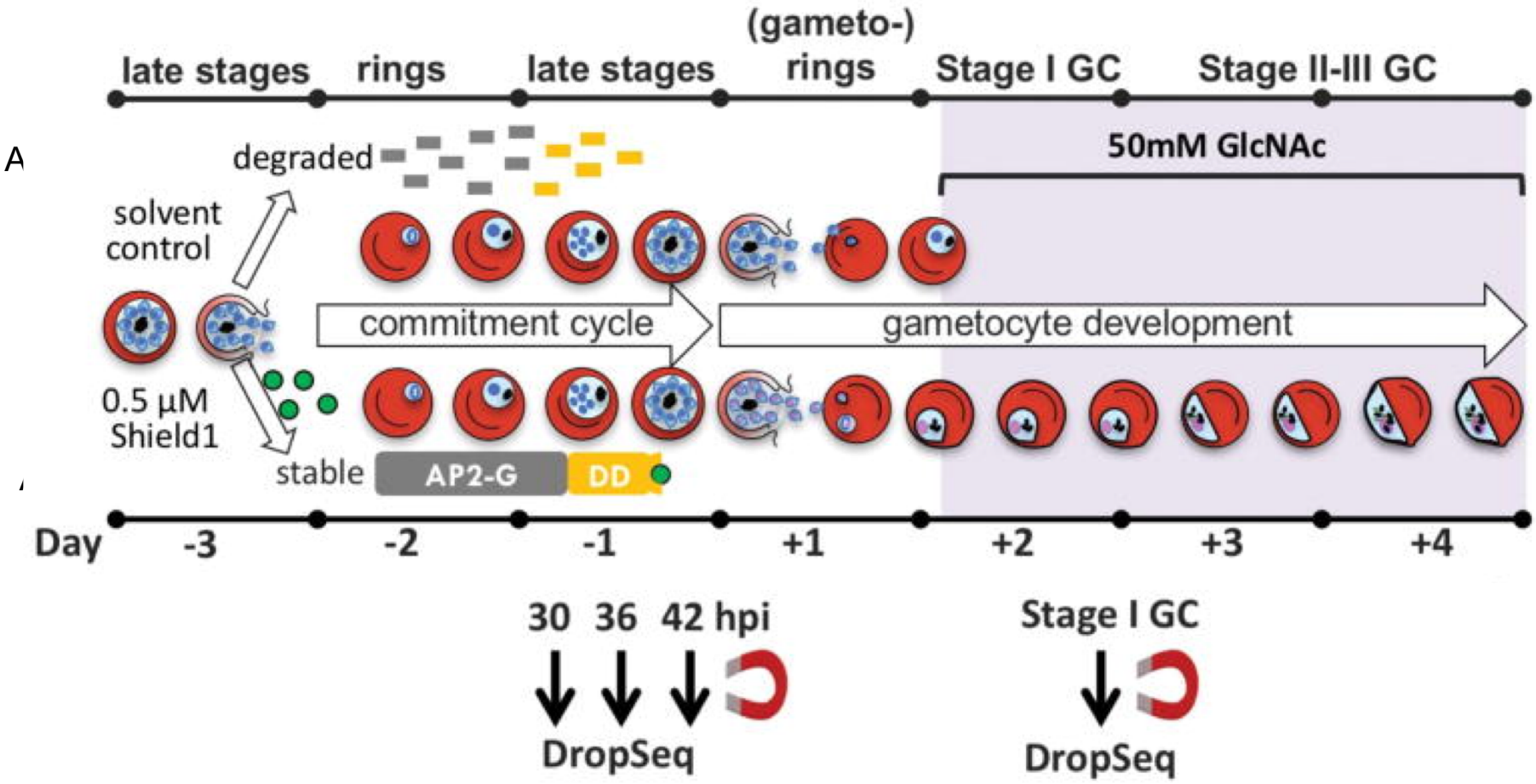


Bruce et al. *Parasitology* 1990
Silvestrini et al. *Parasitology* 2000

Bjorn Kafsack
(Cornell)

Sequencing of conditional knockdown of AP2-G

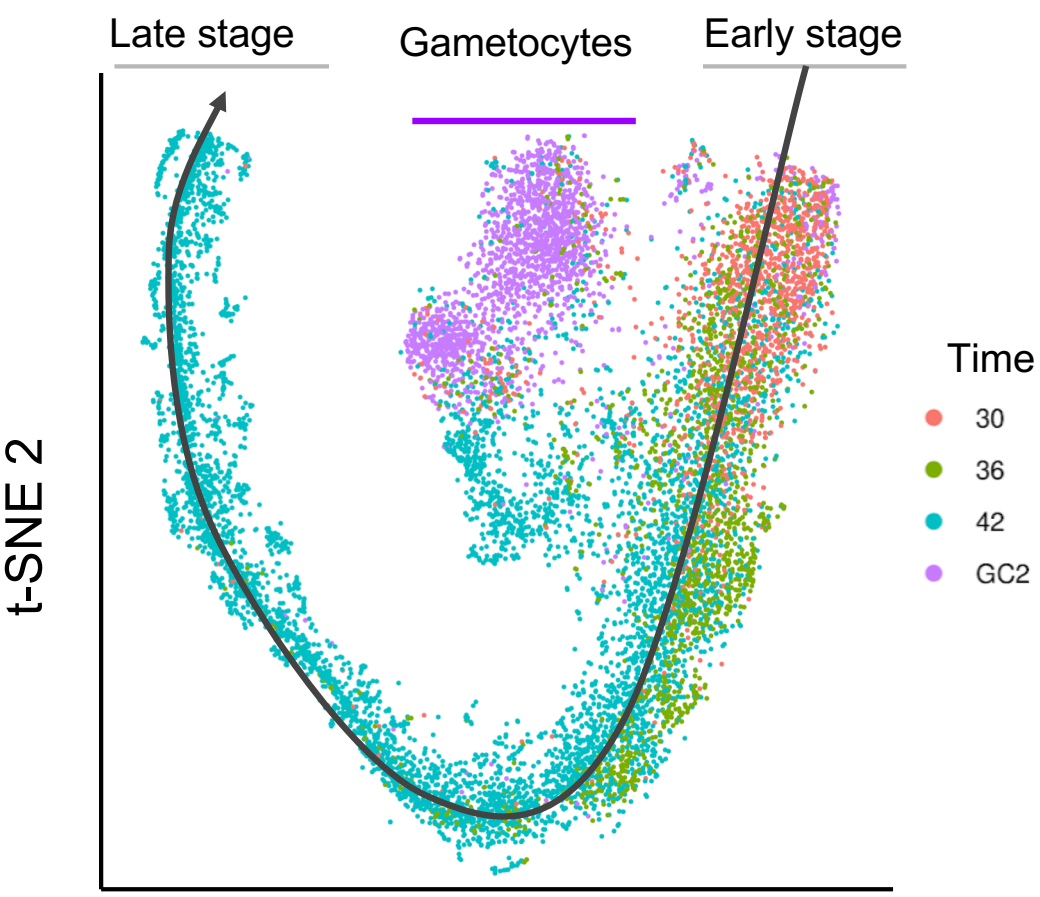
- Data collected at 3 time points and gametocytes
- AP2-G (ON) and AP2-G (OFF) cell conditions



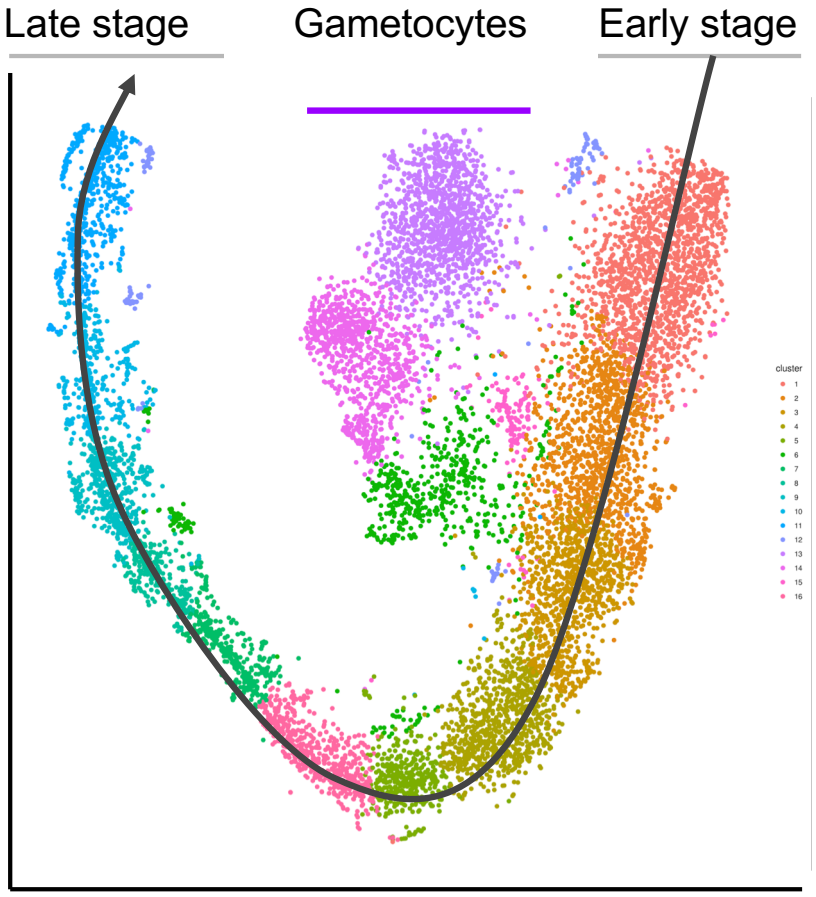
Cell position driven by cell cycle state

Viral cells self-organized based on time post infection

Time Point

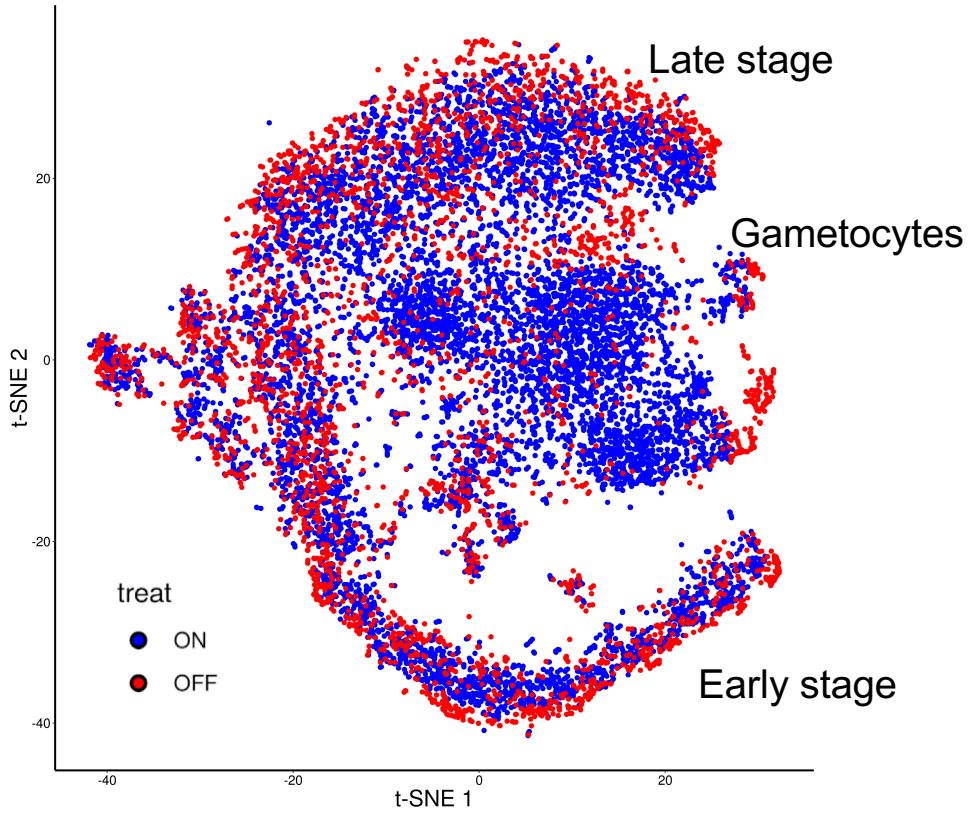


Clustering

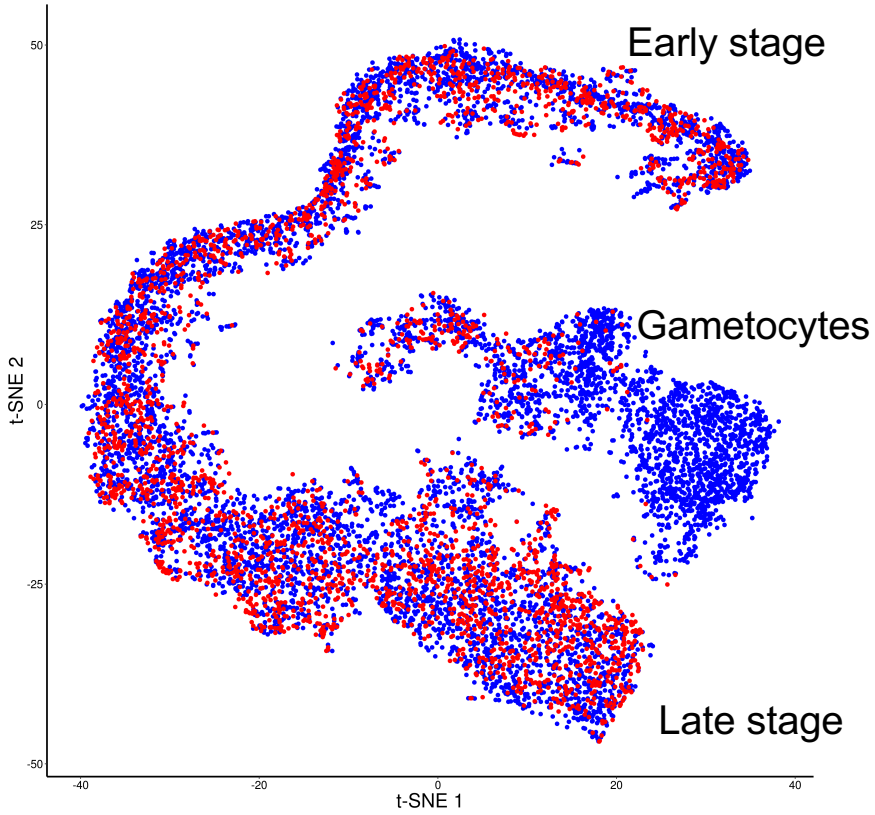


Alignment of malaria AP2-G+/- cells preserves AP2-G ON-specific gametocytes

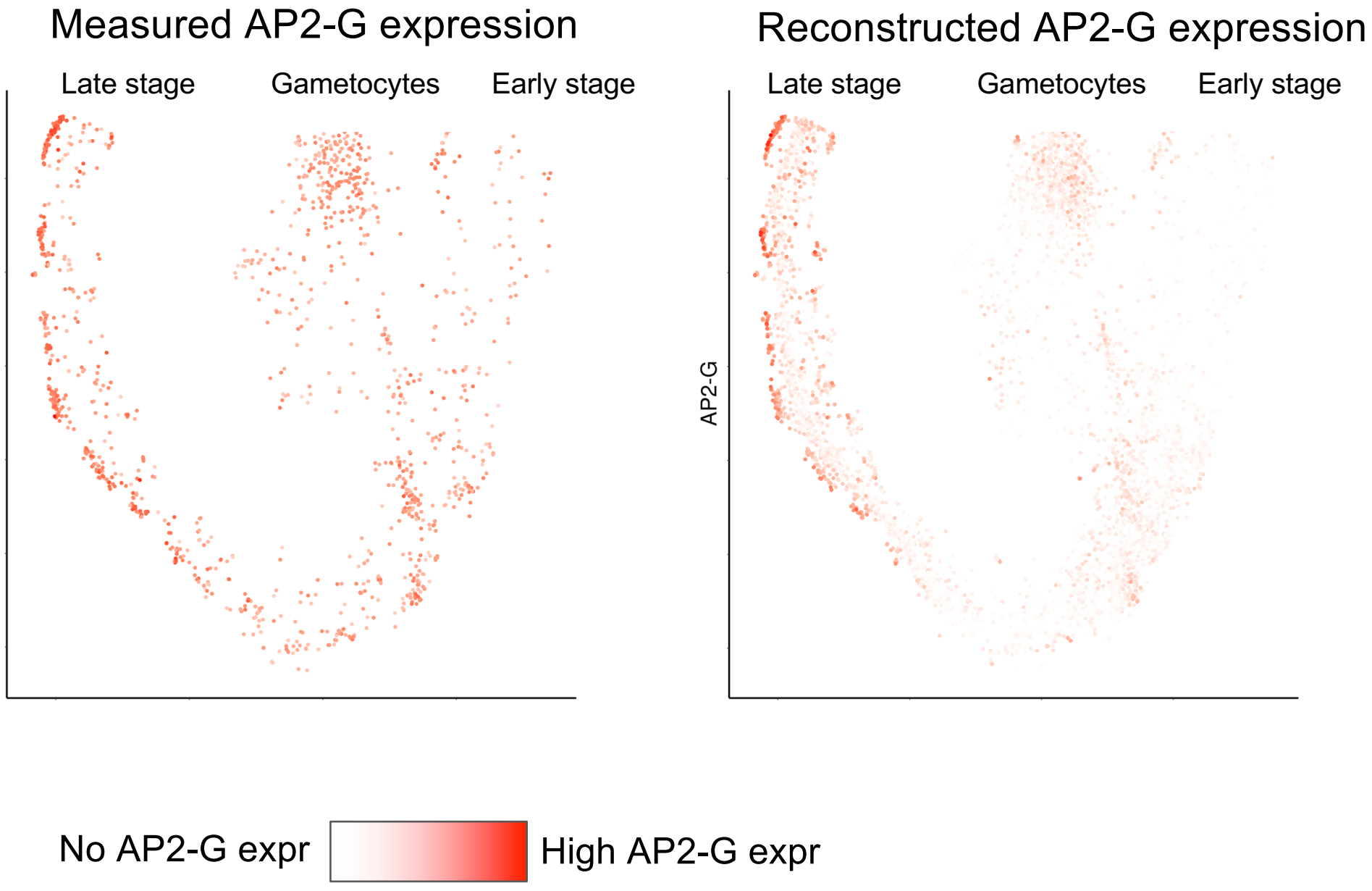
Seurat



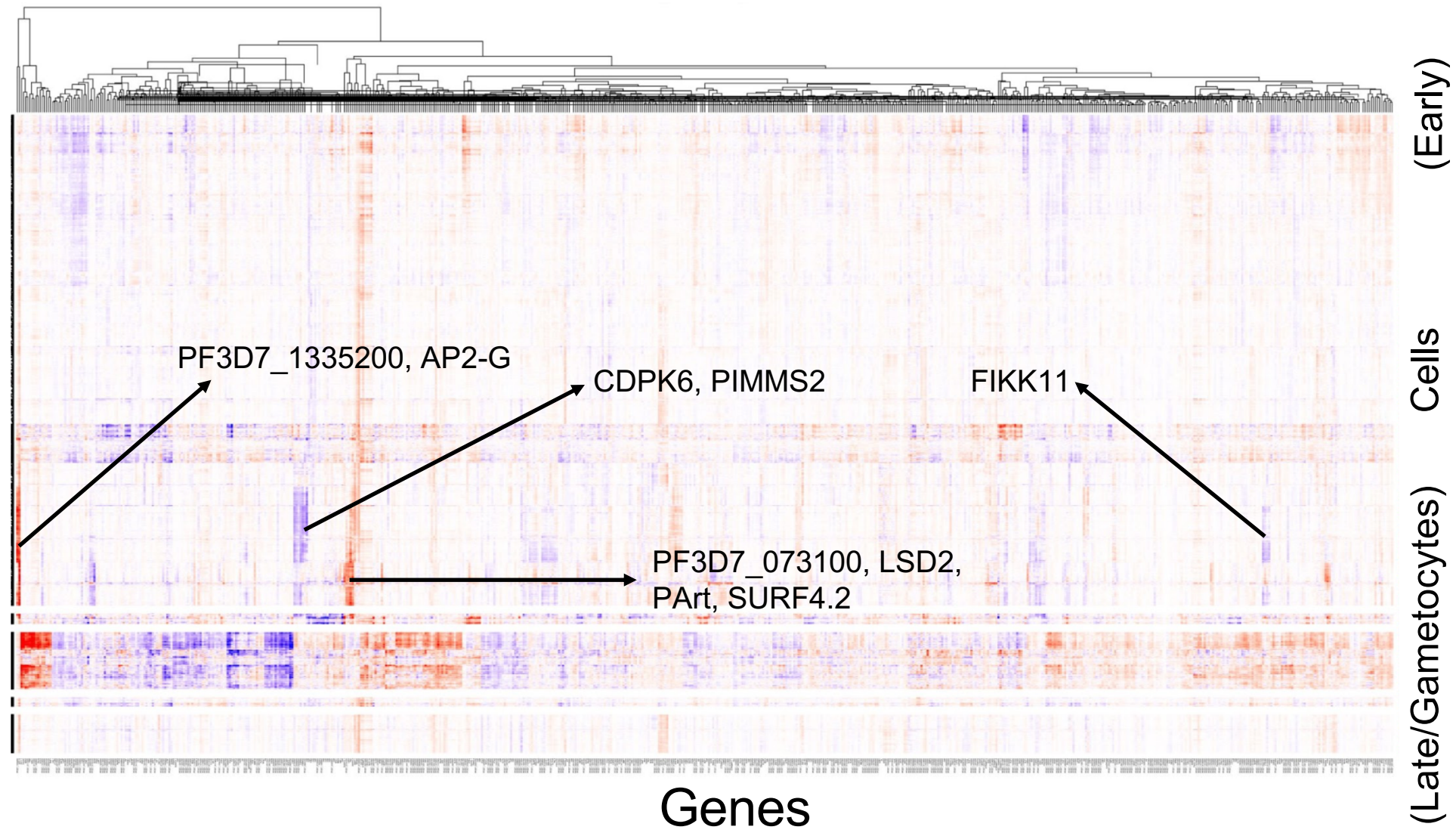
scAlign



Compression algorithm smooths gene expression observations



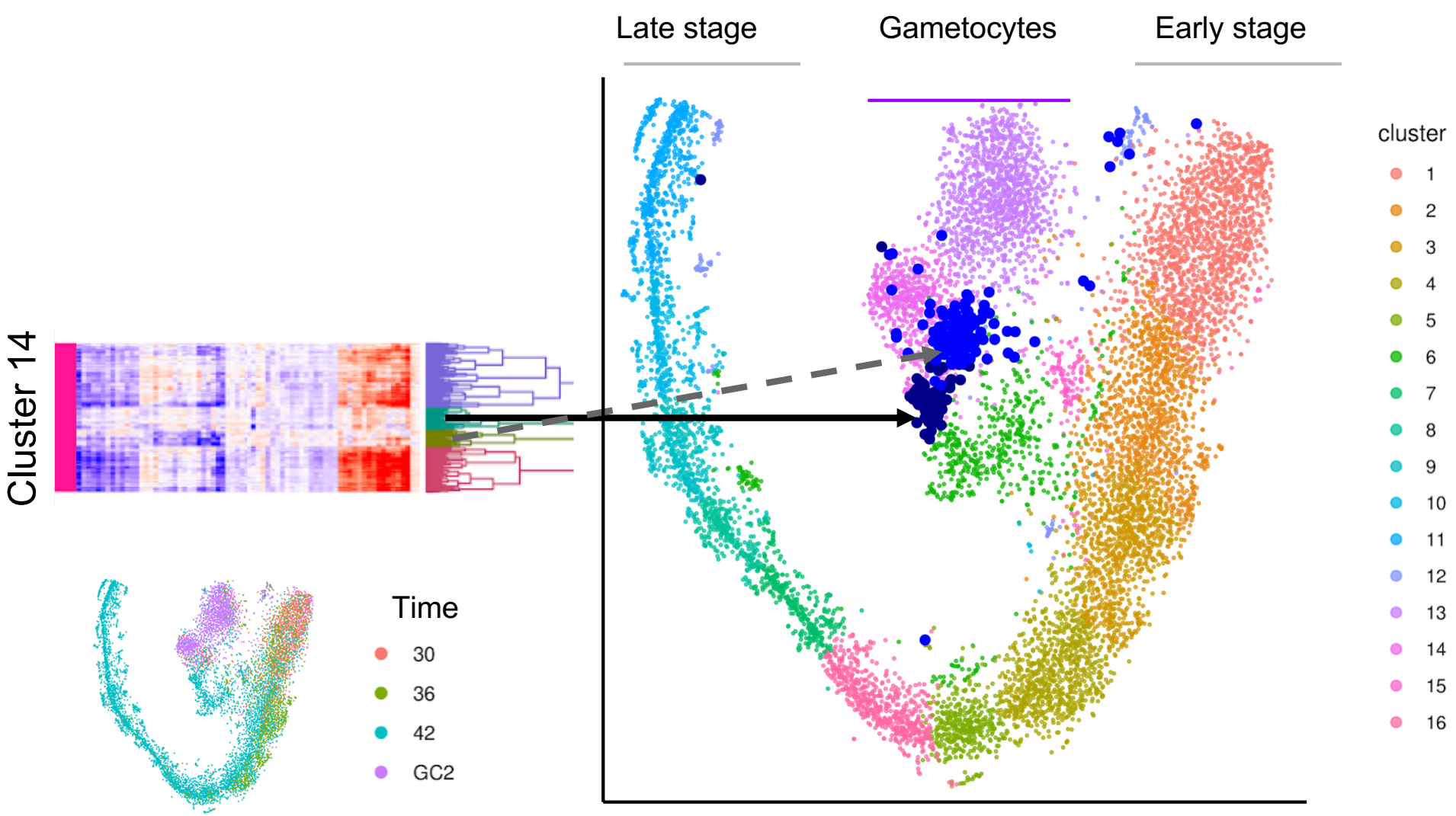
Paired-DE identifies AP2-G related changes near gametocyte formation



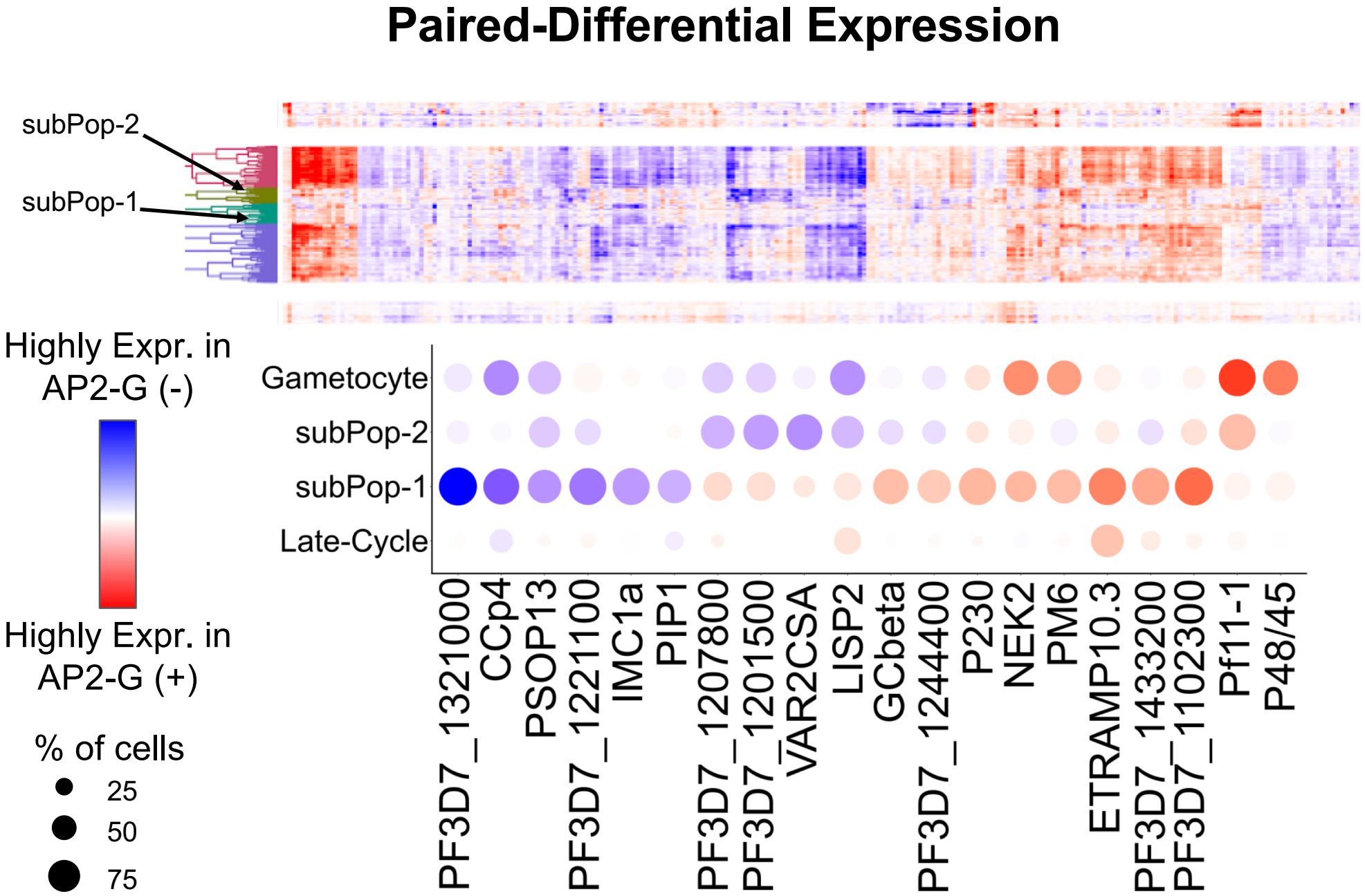
Highly expr. in AP2-G (+)

Highly expr. in AP2-G (-)

Subpopulations lie near reproduction commitment



Paired-DE defines substructure near commitment

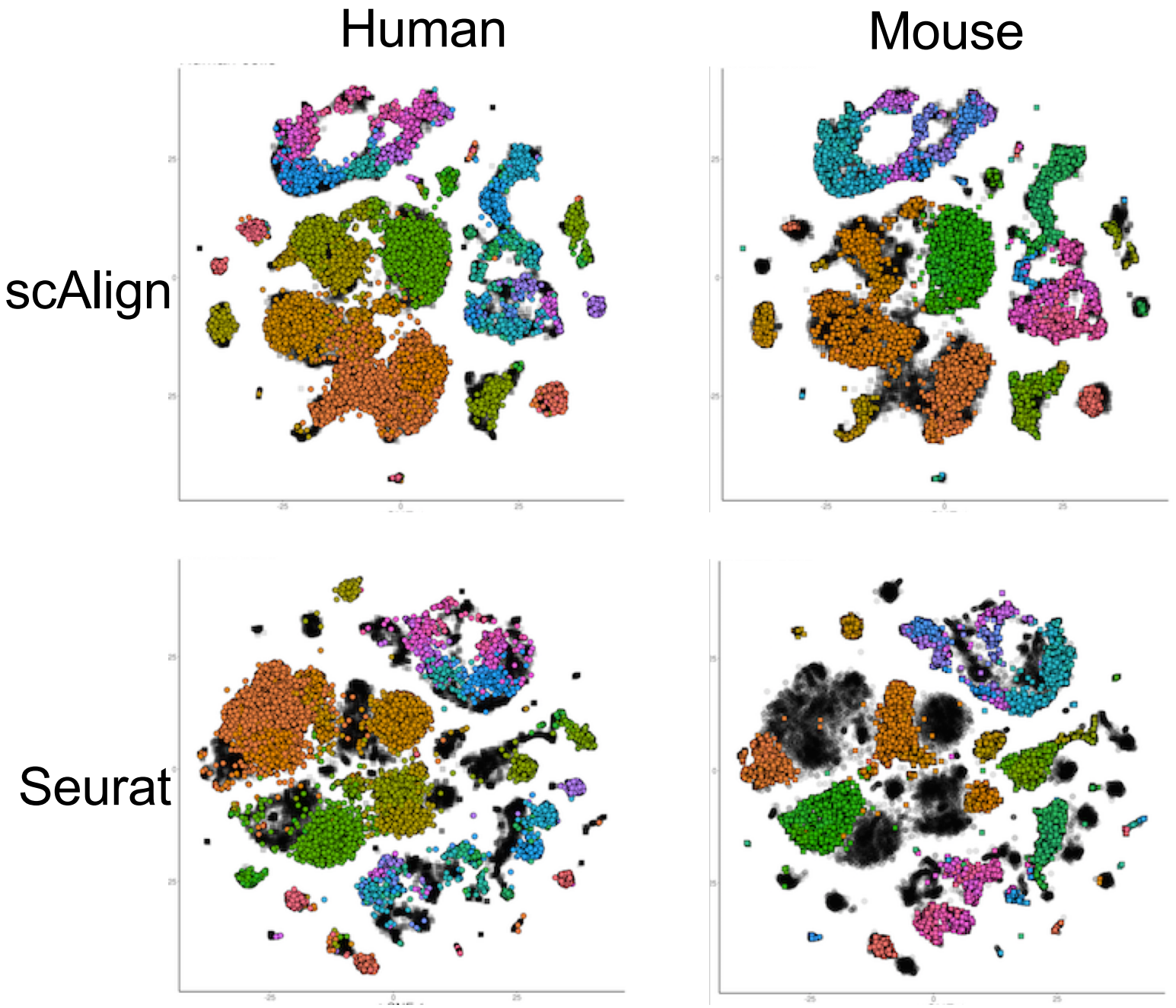


Species can be treated as a perturbation

- Human middle temporal gyrus (~70 cell types)
- Mouse primary visual cortex (~90 cell types)

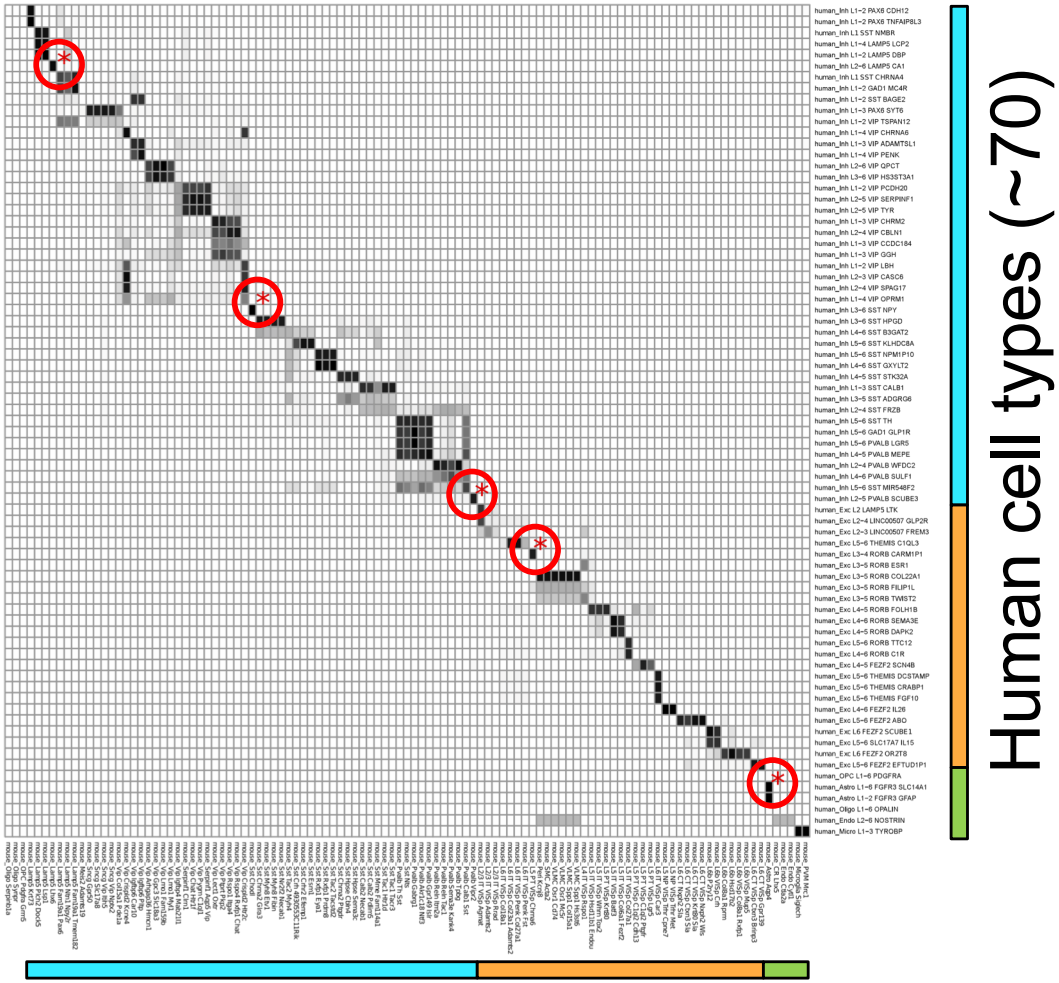
Trygve Bakken

Ed Lein



Most cell types have predicted functional homologs

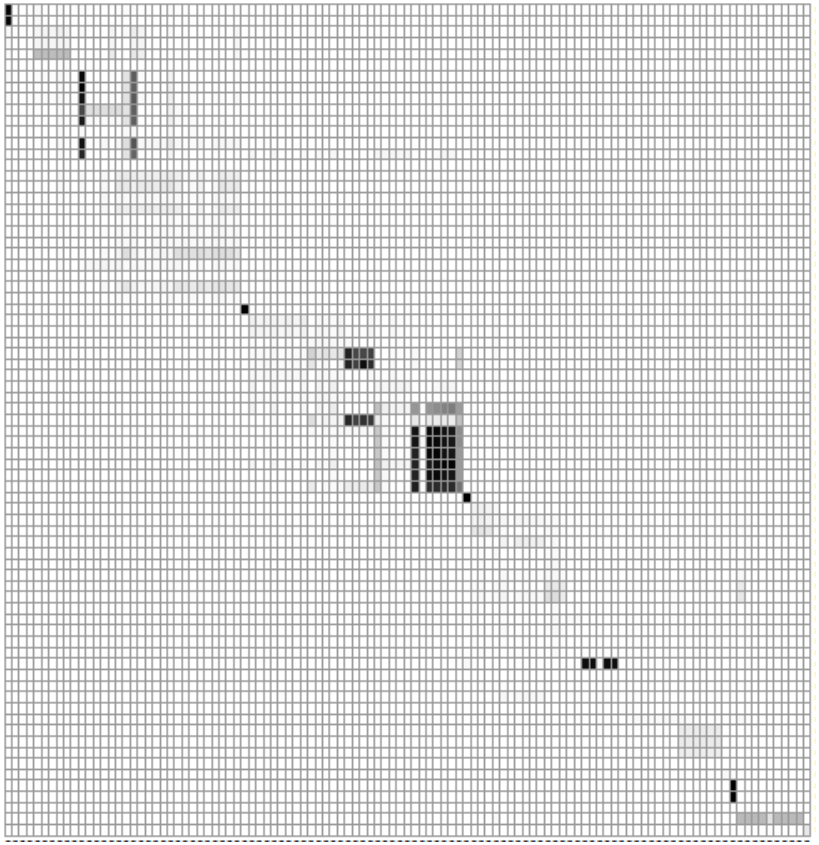
scAlign



Human cell types (~70)

Mouse cell types (~90)

Seurat



Mouse cell types (~90)

Open challenges

- How can you test whether alignment makes sense in the first place?
- How ”close” do cells across condition have to be, to be recognizable?
- How do we measure the statistical significance of paired differential expression?
- Can we use this to detect batch effects, systematic differences in features in other types of data?

Acknowledgements

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CHAN ZUCKERBERG INITIATIVE

HUMAN CELL ATLAS

Training Program in Molecular and Cellular Biology