scMerge: Integration of multiple single-cell transcriptomics datasets leveraging stable expression and pseudo-replication

Presented by

Dr Shila Ghazanfar

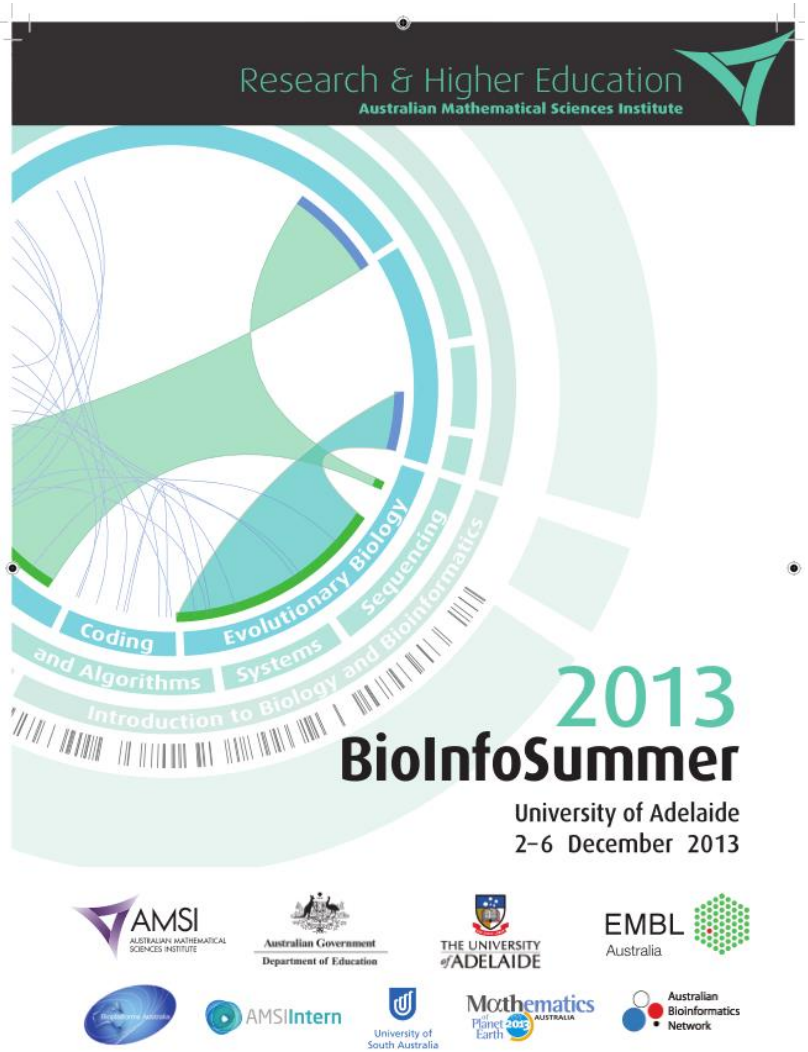
Judith and David Coffey LifeLab
Charles Perkins Centre

School of Mathematics and Statistics
The University of Sydney

@shazanfar

THE UNIVERSITY OF SYDNEY

A brief history



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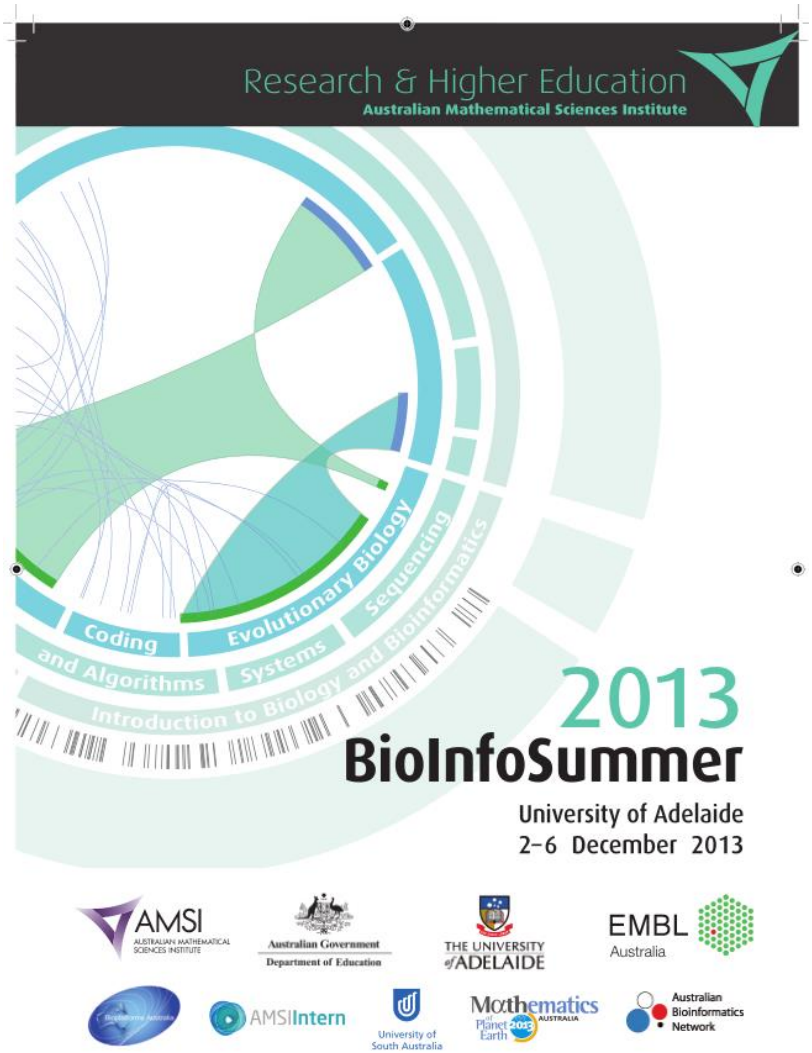

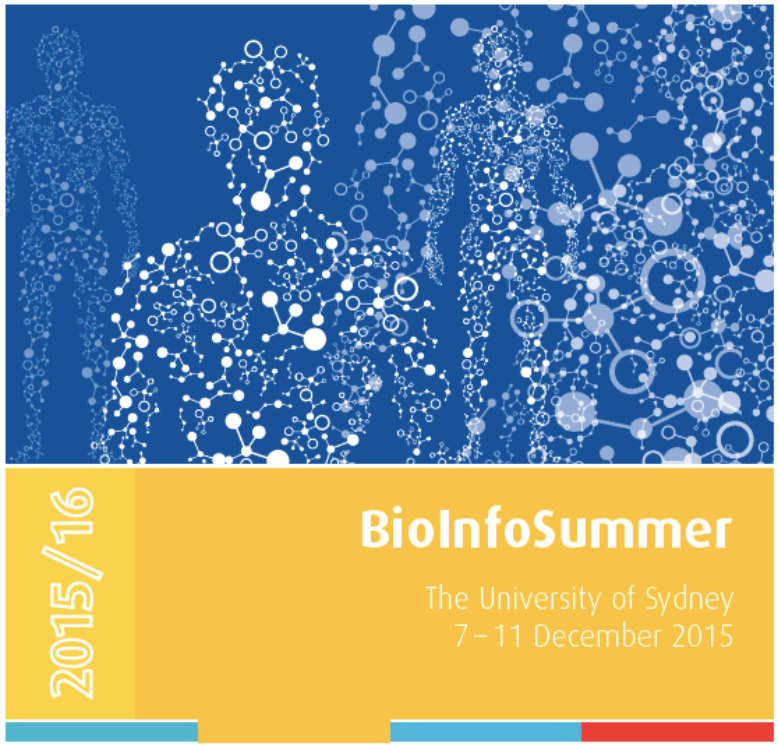

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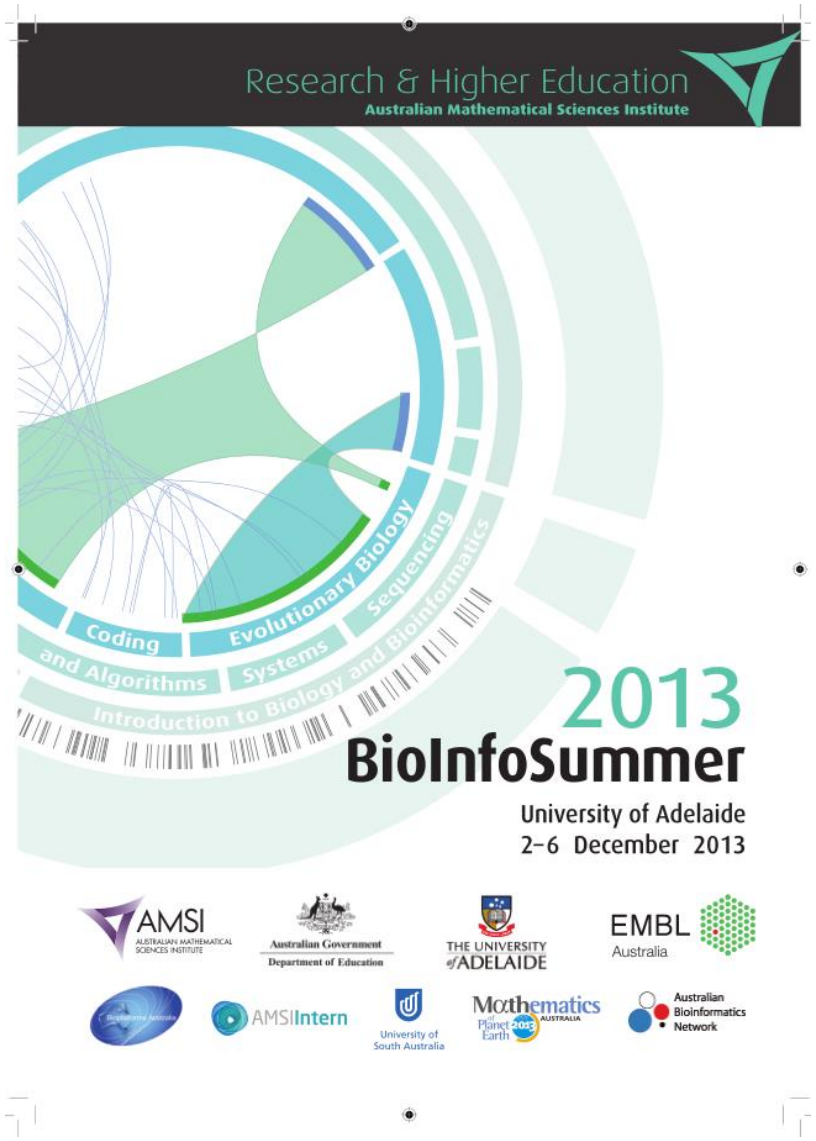
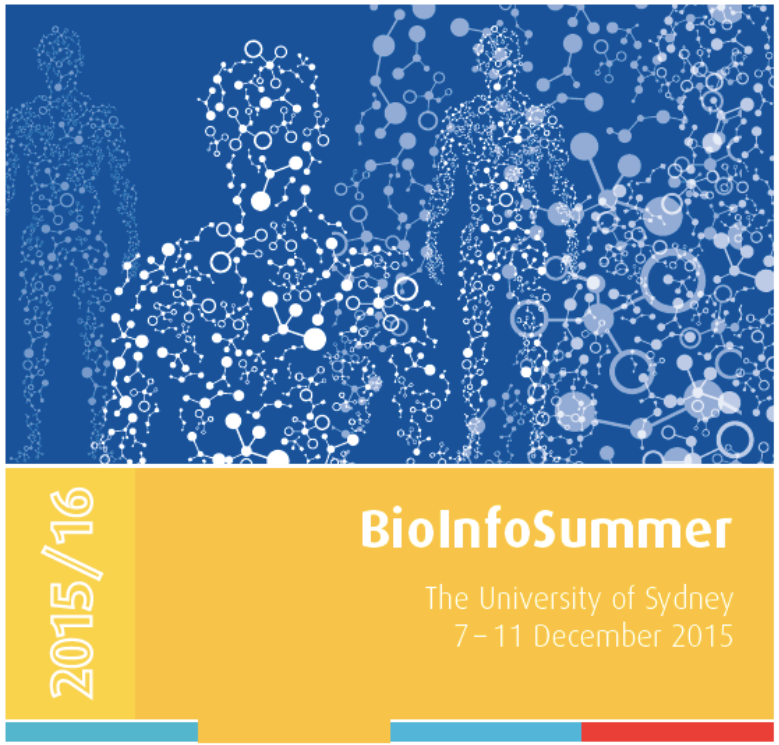
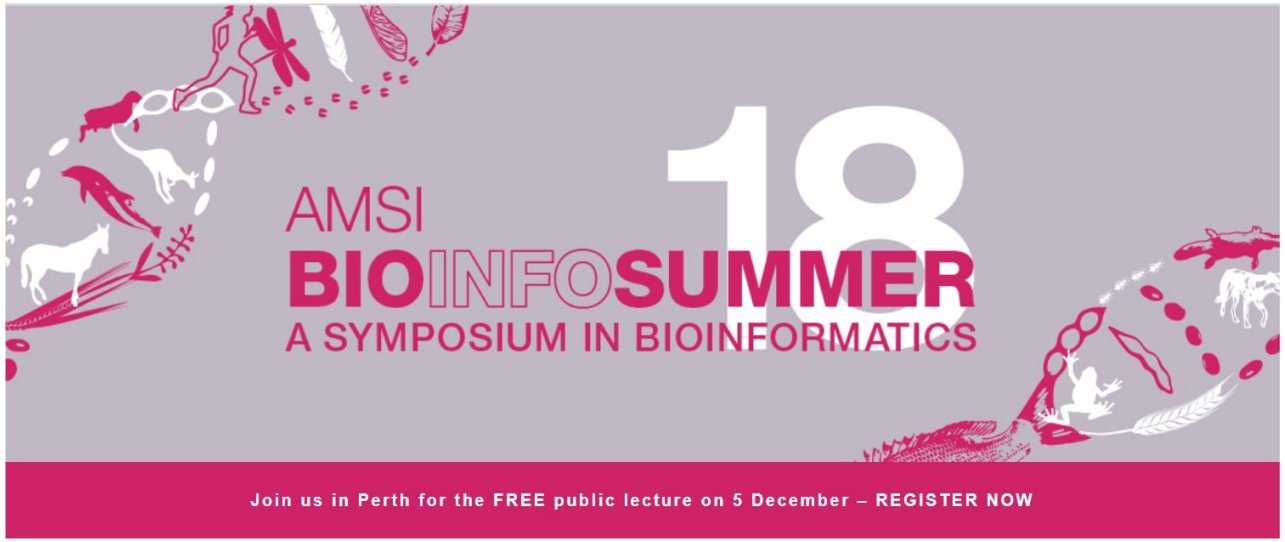




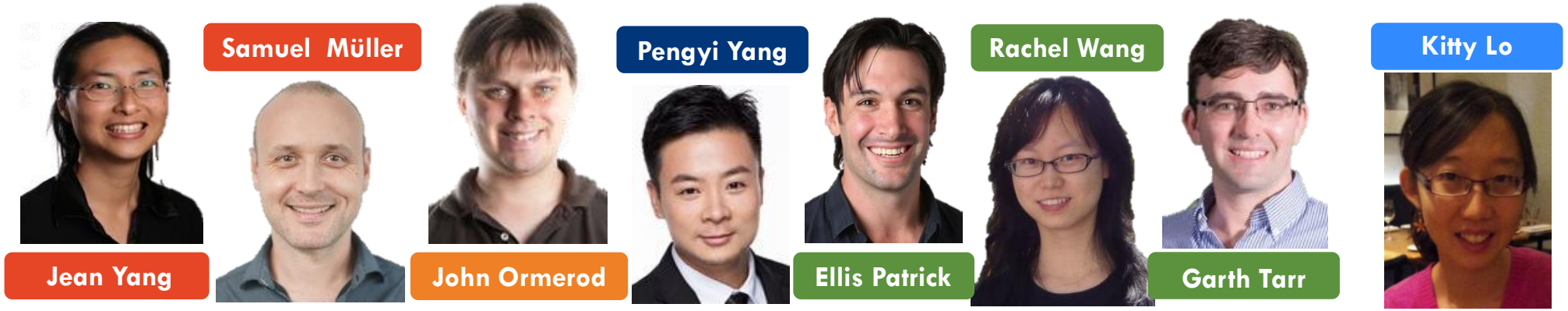


Sydney Precision Bioinformatics Research Group

THE UNIVERSITY OF SYDNEY

We share an interest in developing statistical and computational methodologies to tackle the foremost significant challenges posed by modern biology and medicine.

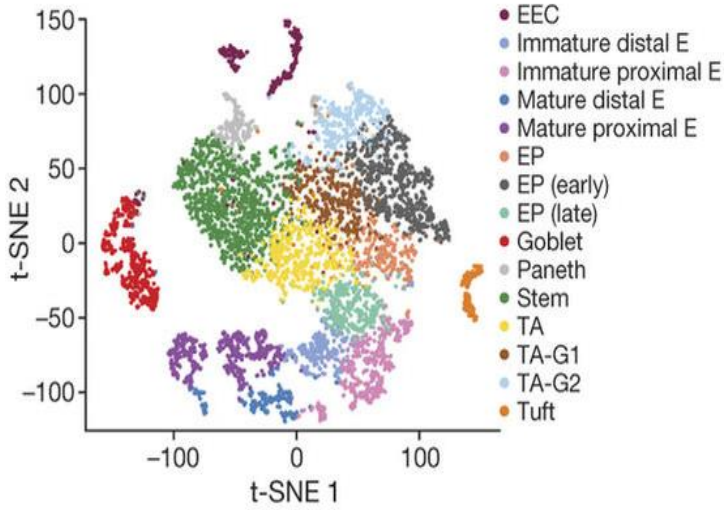
Meet our senior and junior research leaders …



… and senior research associates: 4; PhD candidates: 20; Honours and TSP students: 8

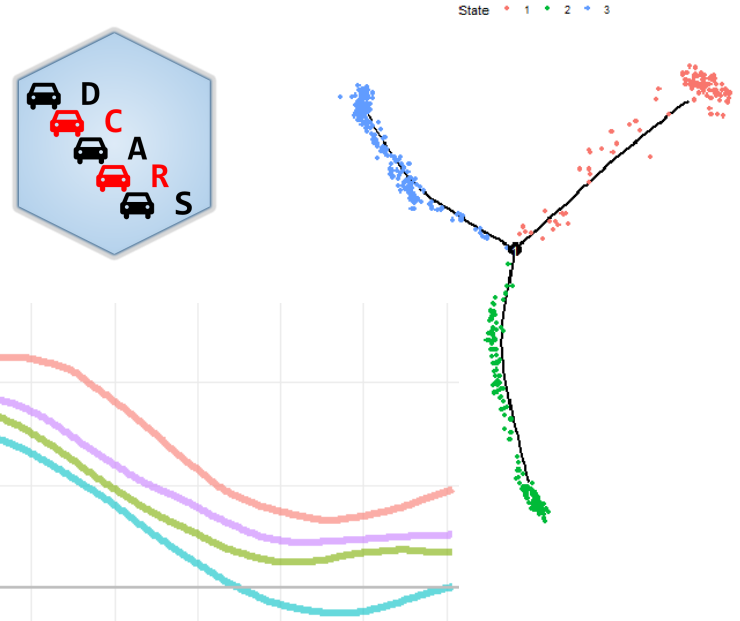
Find out more: http://www.maths.usyd.edu.au/bioinformatics/

Get interactive: http://shiny.maths.usyd.edu.au/



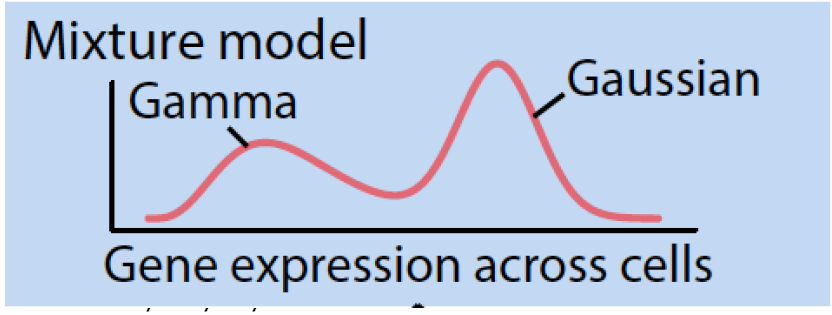
Clustering metrics

Sydney
Single cell

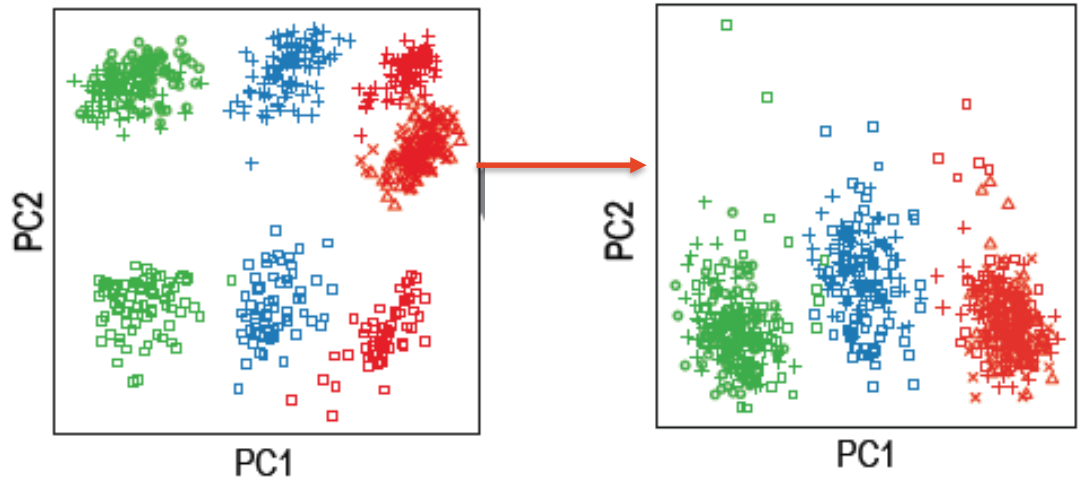


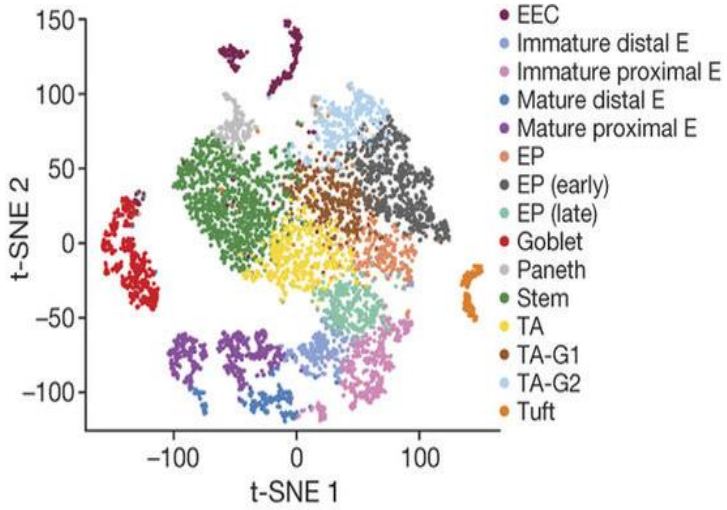
Differential correlation

Finding stably expressed genes



scMerge





Clustering metrics

Pengyi Yang

Taiyun Kim

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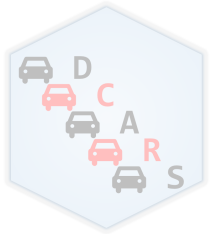

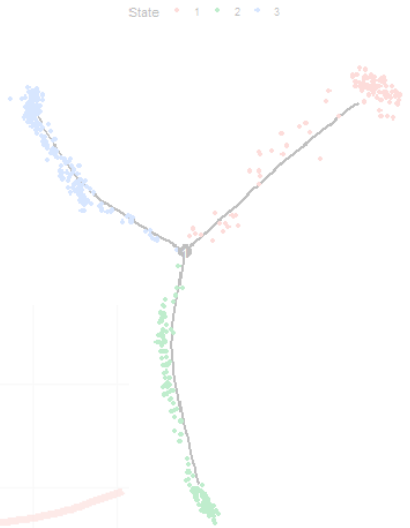

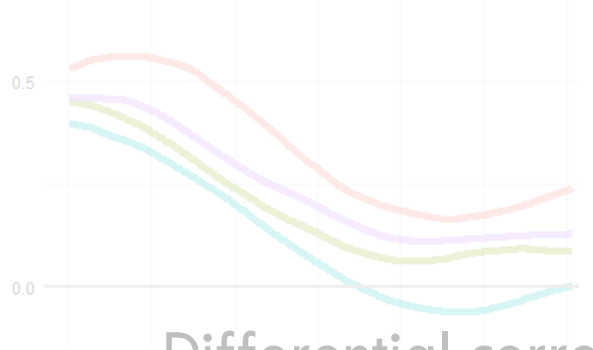

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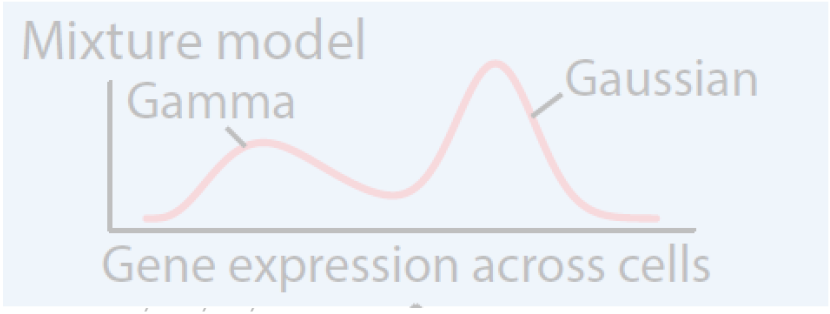

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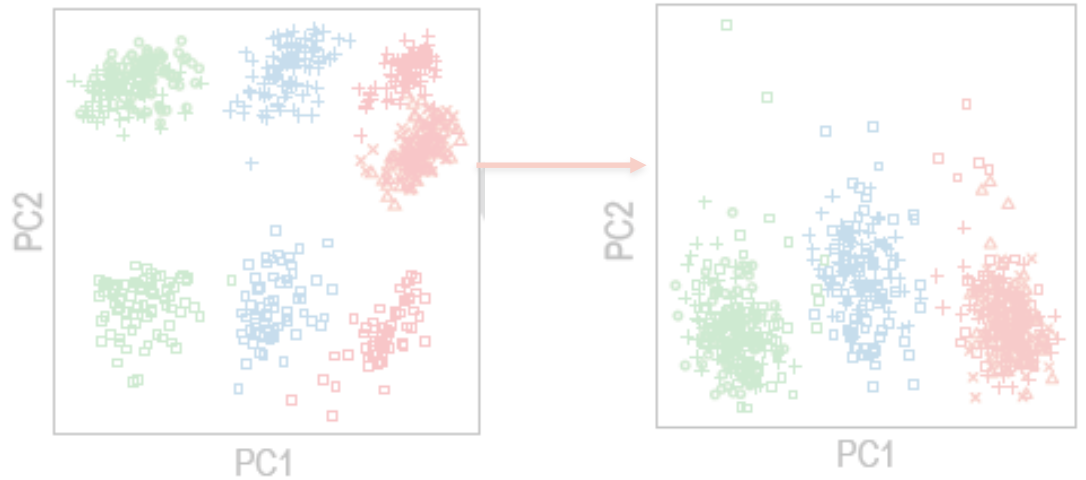

Key underlying aspect of clustering? Similarity metrics

- *k*-means
- Hierarchical
- RaceID
- SC3
- CIDR
- countClust
- RCA
- SIMLR

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$$s_{ij} = \sqrt{\sum_{g=1}^{G}(x_{ig} - x_{jg})^2};$$

Manhattan

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Maximum

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$$s_{ij} = \frac{\sum_{g=1}^{G}(x_{ig} - \bar{x}_i)(x_{jg} - \bar{x}_j)}{\sqrt{\sum_{g=1}^{G}(x_{ig} - \bar{x}_i)^2}\sqrt{\sum_{g=1}^{G}(x_{jg} - \bar{x}_j)^2}};$$

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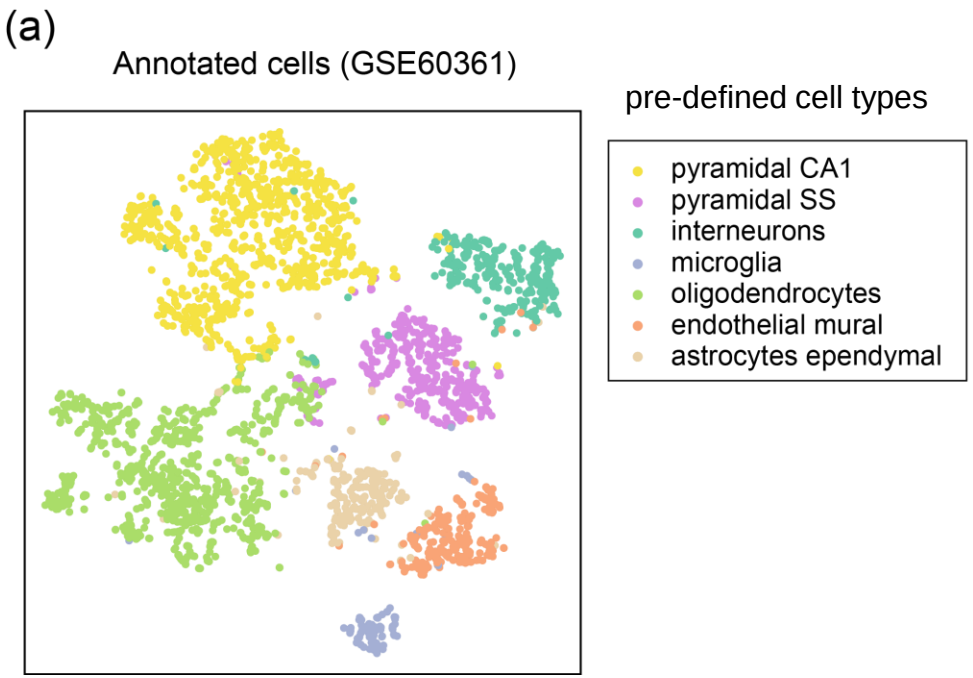
Correlation-based

k-means Clustering on GSE60361

k-means



Zeisel A, et al. *Science* 2015

k-means Clustering on GSE60361

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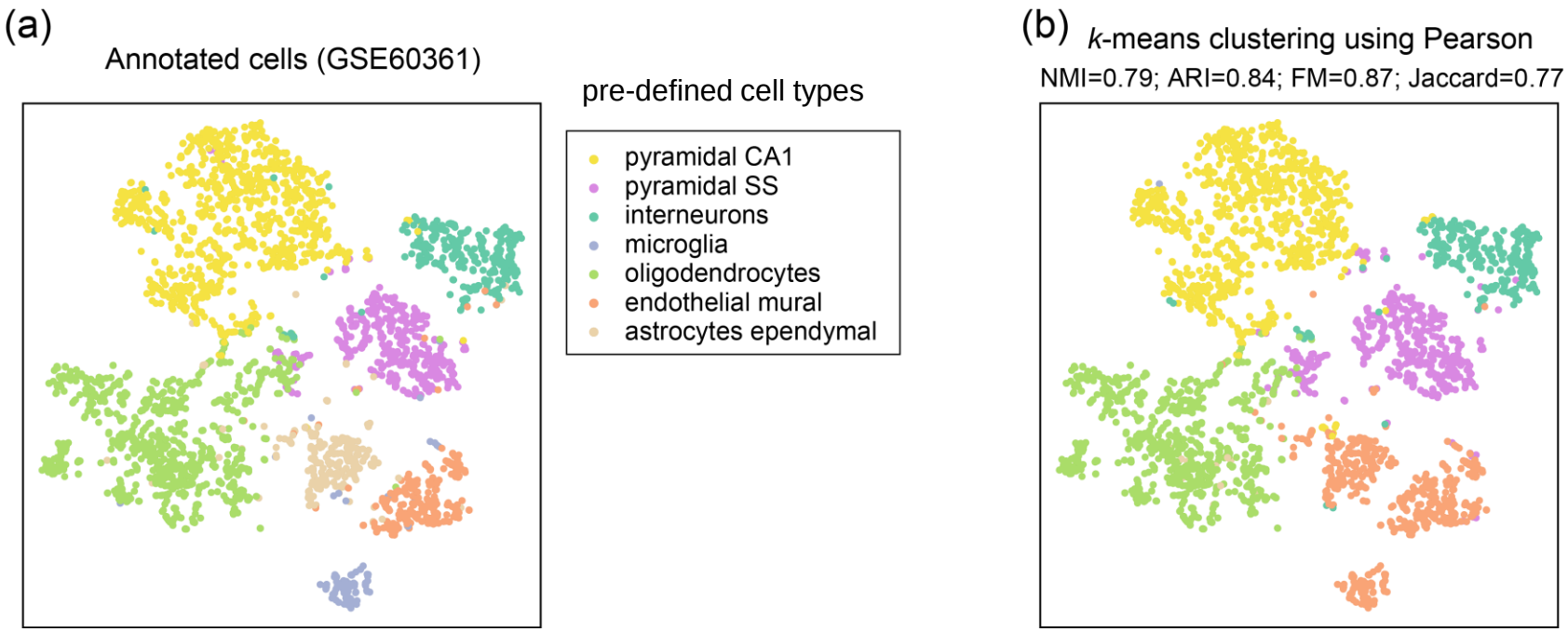

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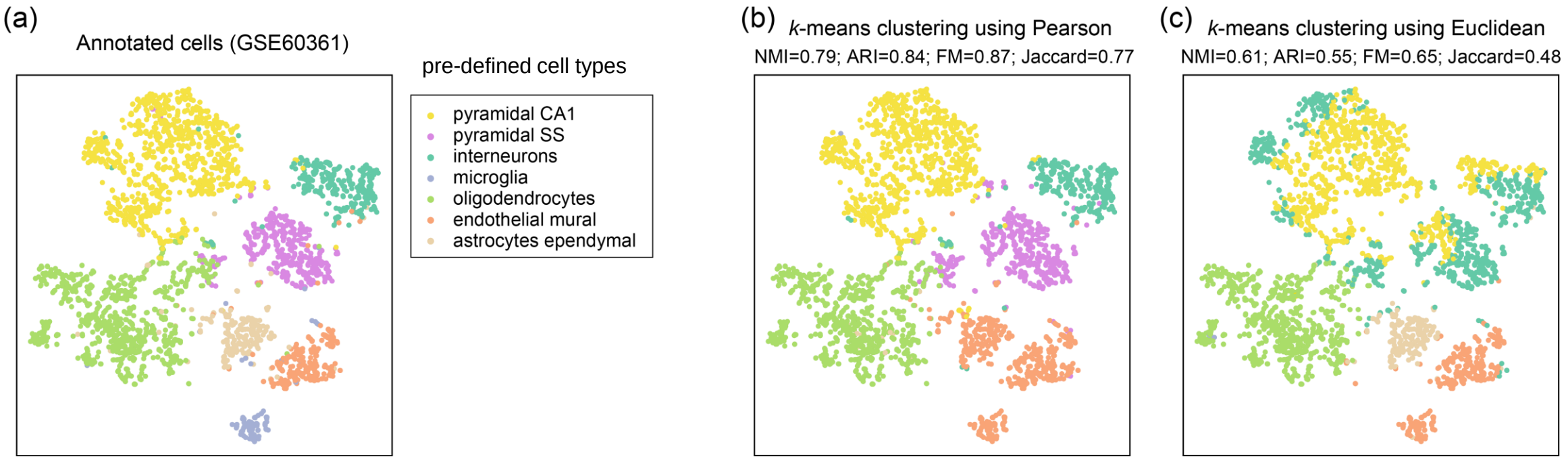

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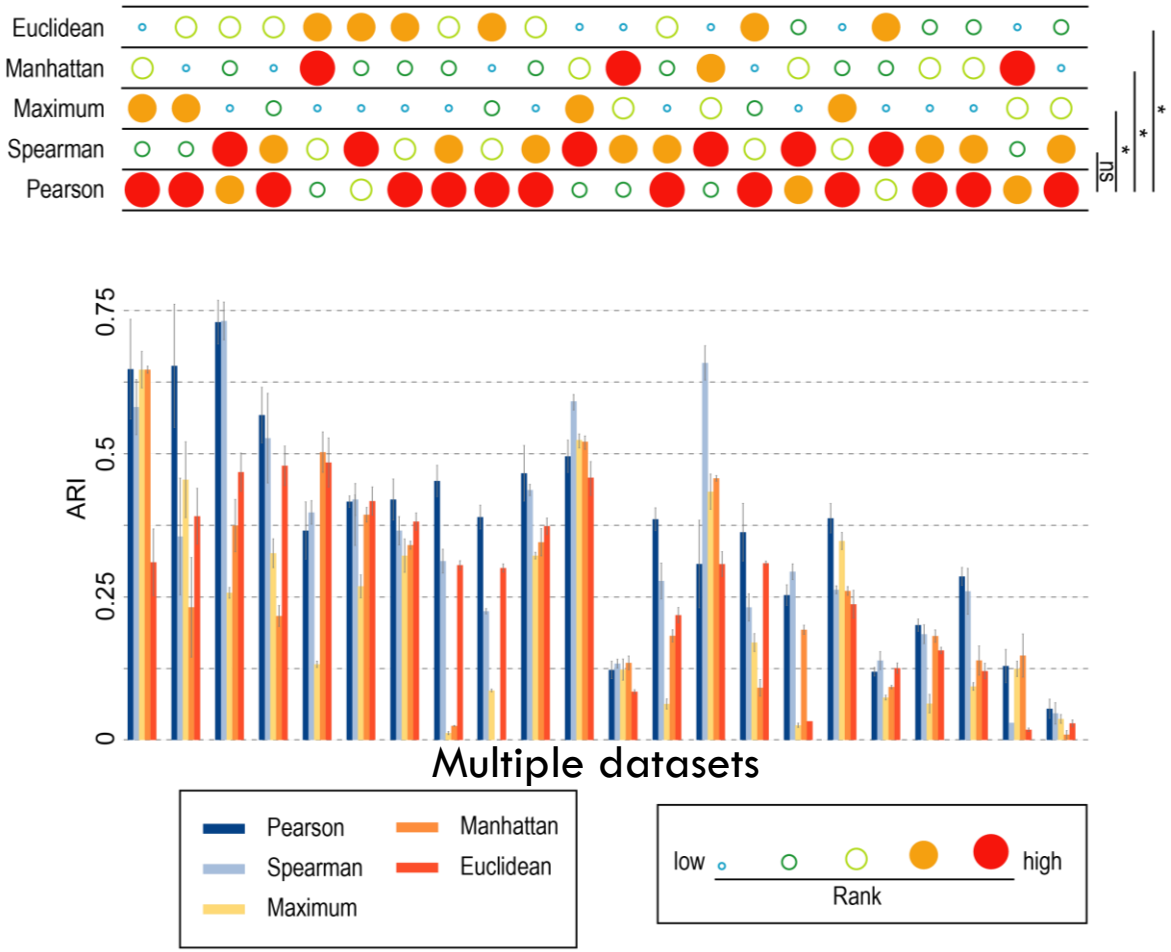
Evaluation results (against the pre-defined cell types)



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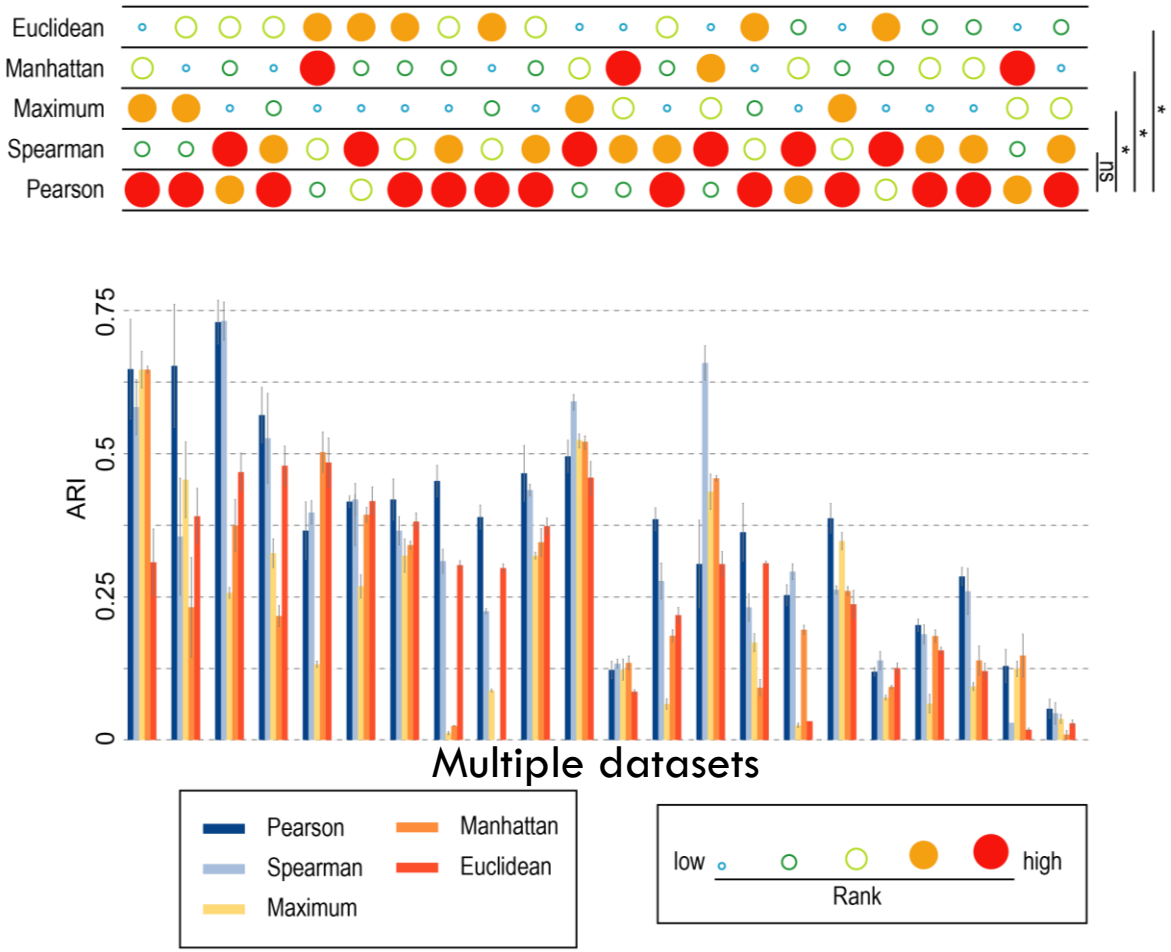


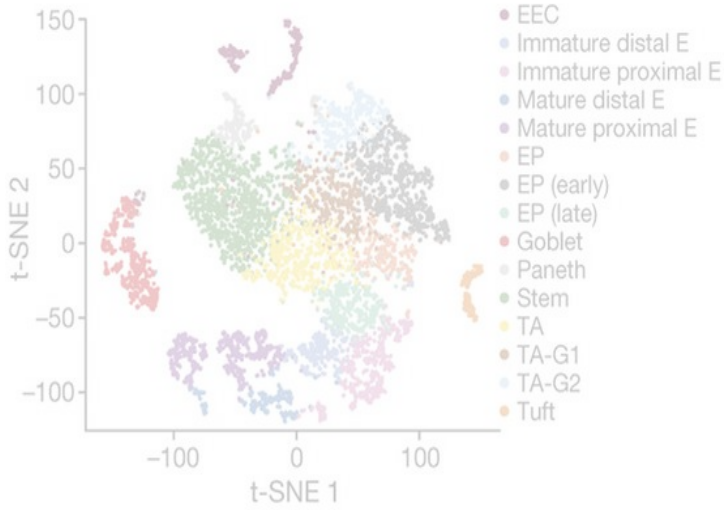
Impact of similarity metrics on single-cell RNA-seq data clustering

Taiyun Kim, Irene Rui Chen, Yingxin Lin, Andy Yi-Yang Wang, Jean Yee Hwa Yang, Pengyi Yang

Briefings in Bioinformatics, bby076,

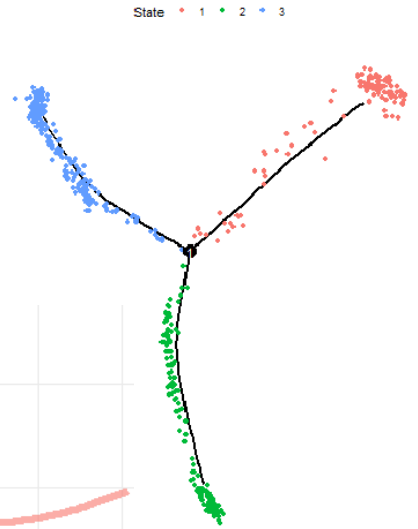
Pengyi Yang

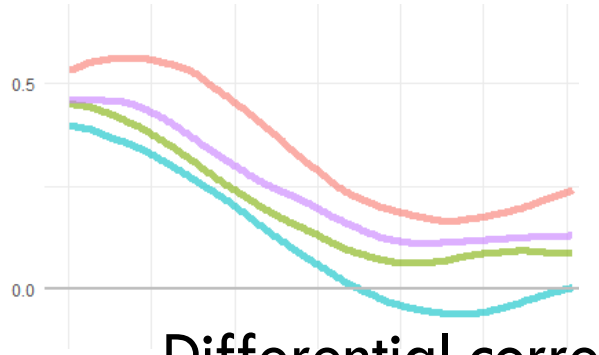
Taiyun Kim



Clustering metrics

Ellis Patrick





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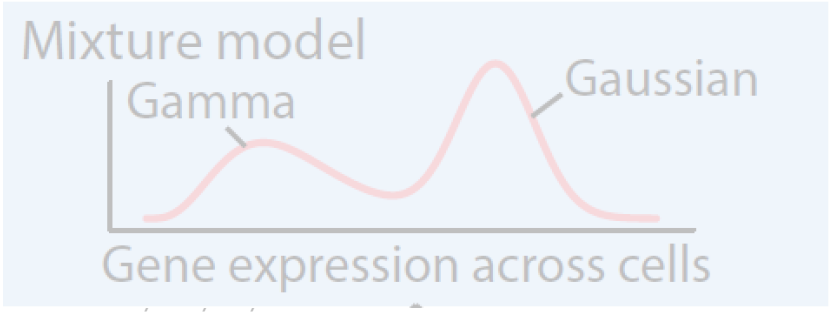

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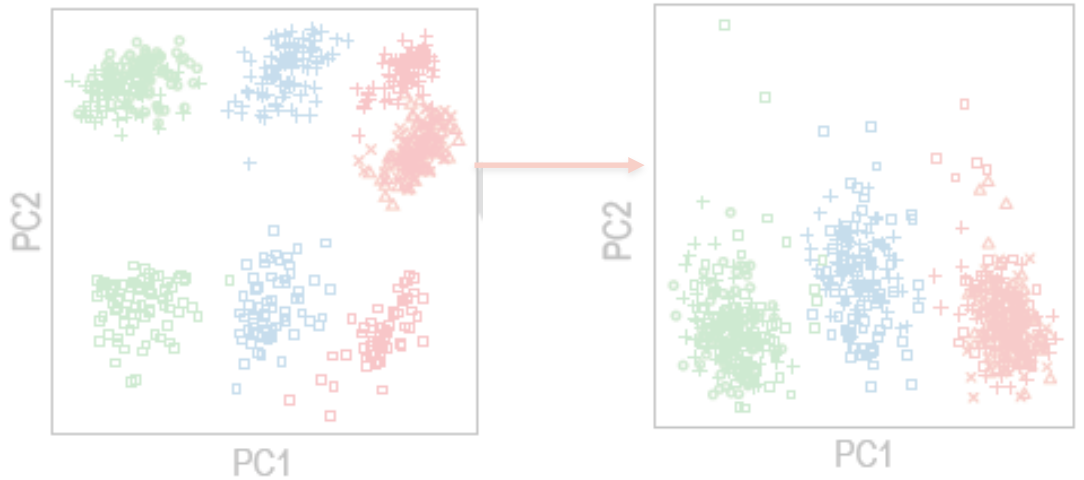
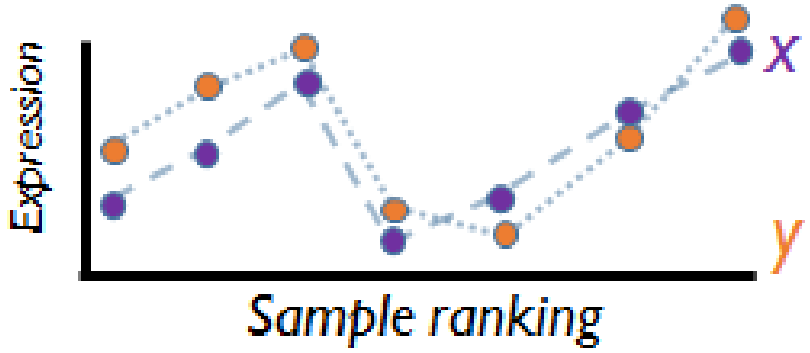


What is differential correlation?

Consider the expression of gene x and gene y



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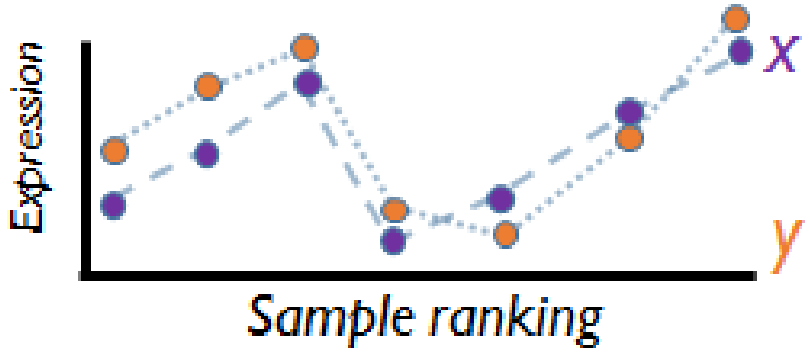

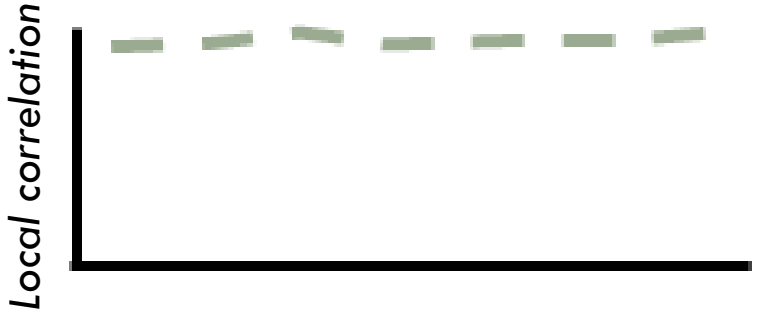

Constant correlation

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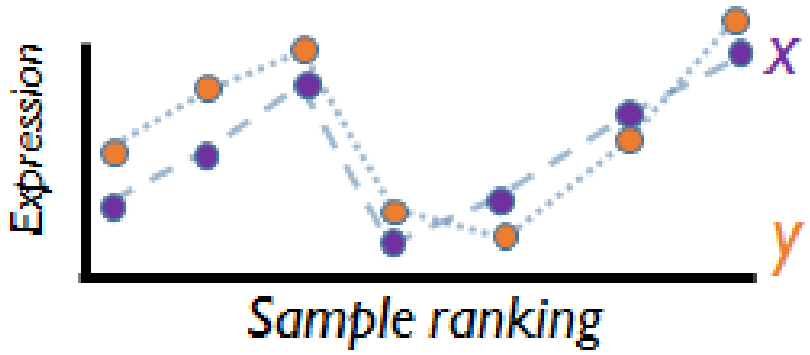
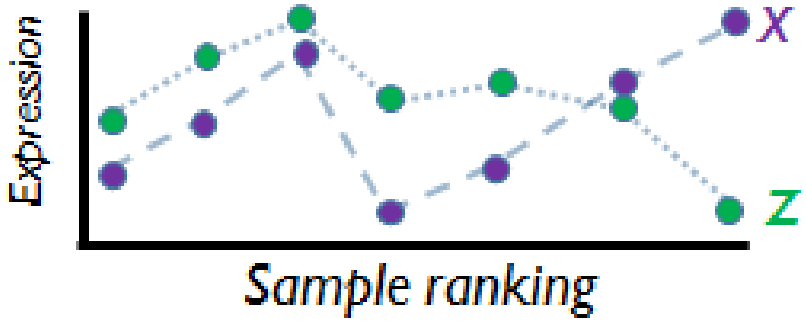
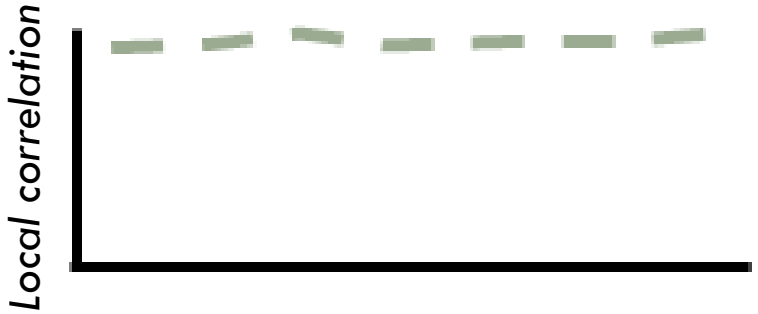


Now consider the expression of gene x and gene z





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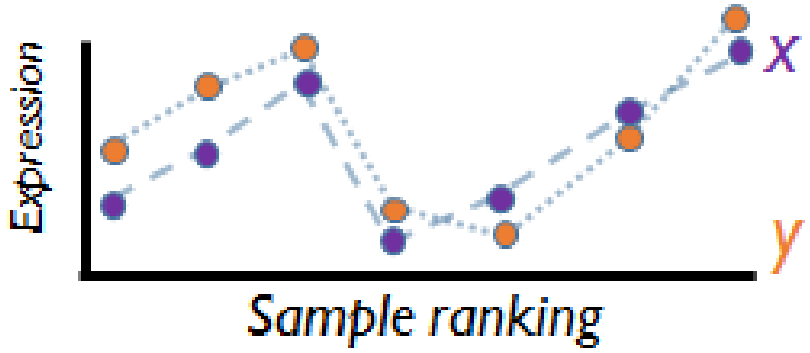

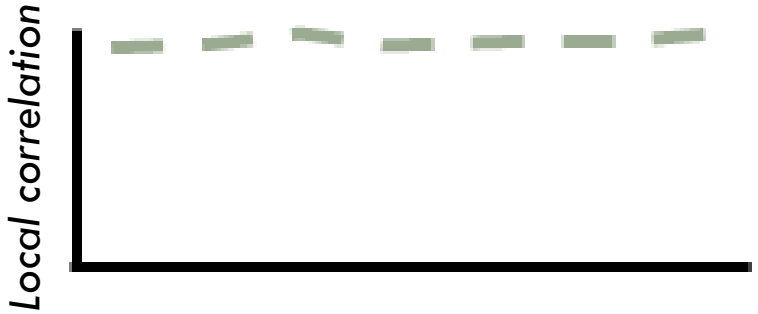

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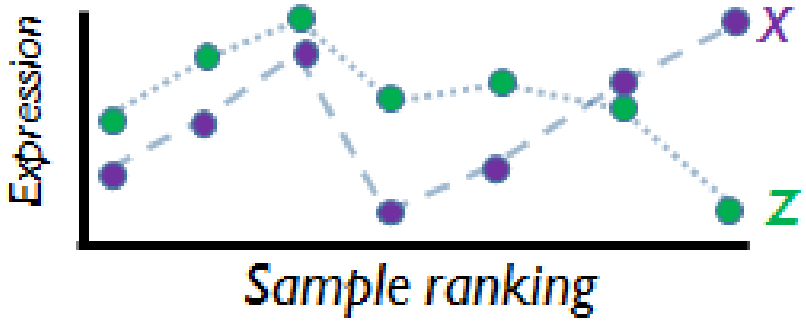
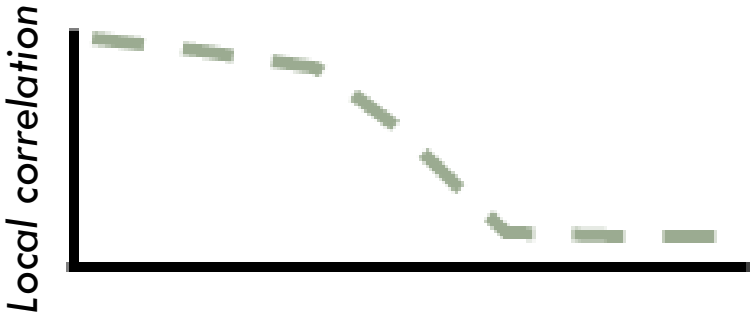




Differential correlation

Bioinformatics, 2018, 1–7
doi: 10.1093/bioinformatics/bty698
Advance Access Publication Date: 9 August 2018
Original Paper

OXFORD

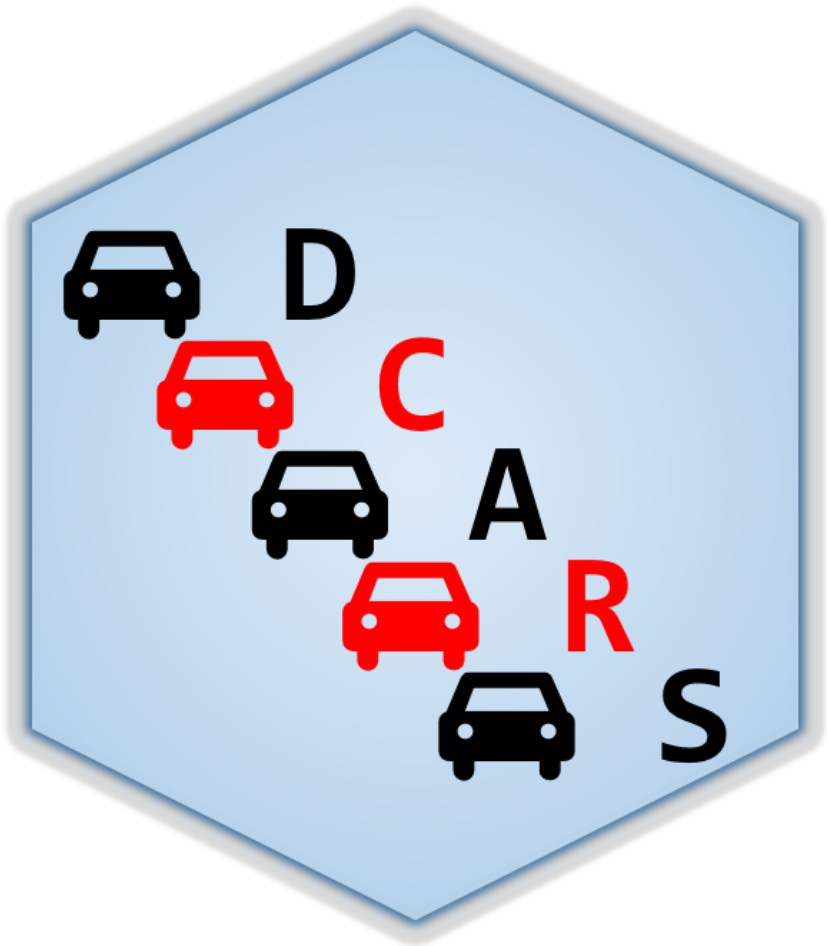
Systems biology

DCARS: differential correlation across ranked samples

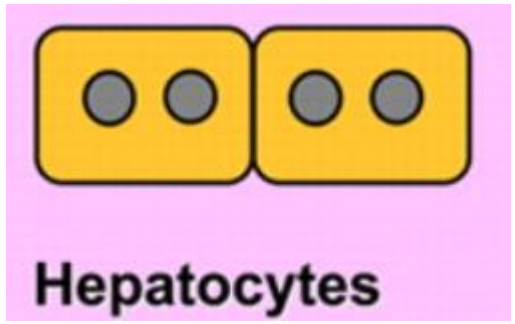
Shila Ghazanfar [1,2,*], Dario Strbenac[2], John T. Ormerod[2,3], Jean Y. H. Yang[2,1] and Ellis Patrick [2,4]

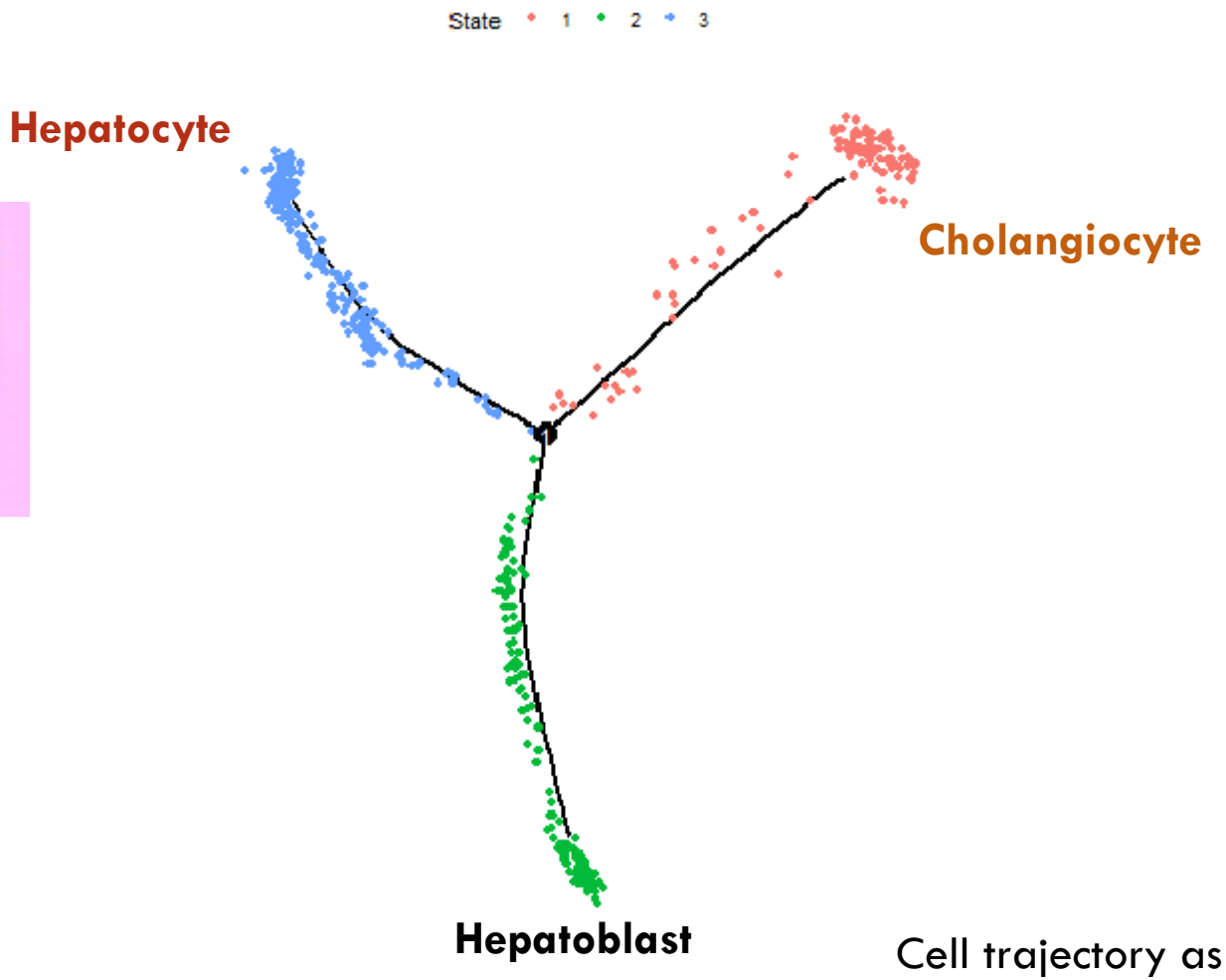
Installation

```
# Install the development version from GitHub:
# install.packages("devtools")
devtools::install_github("shazanfar/DCARS")
library(DCARS)
```



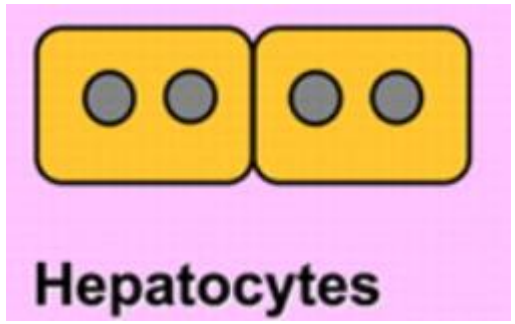
Differential correlation across pseudotime

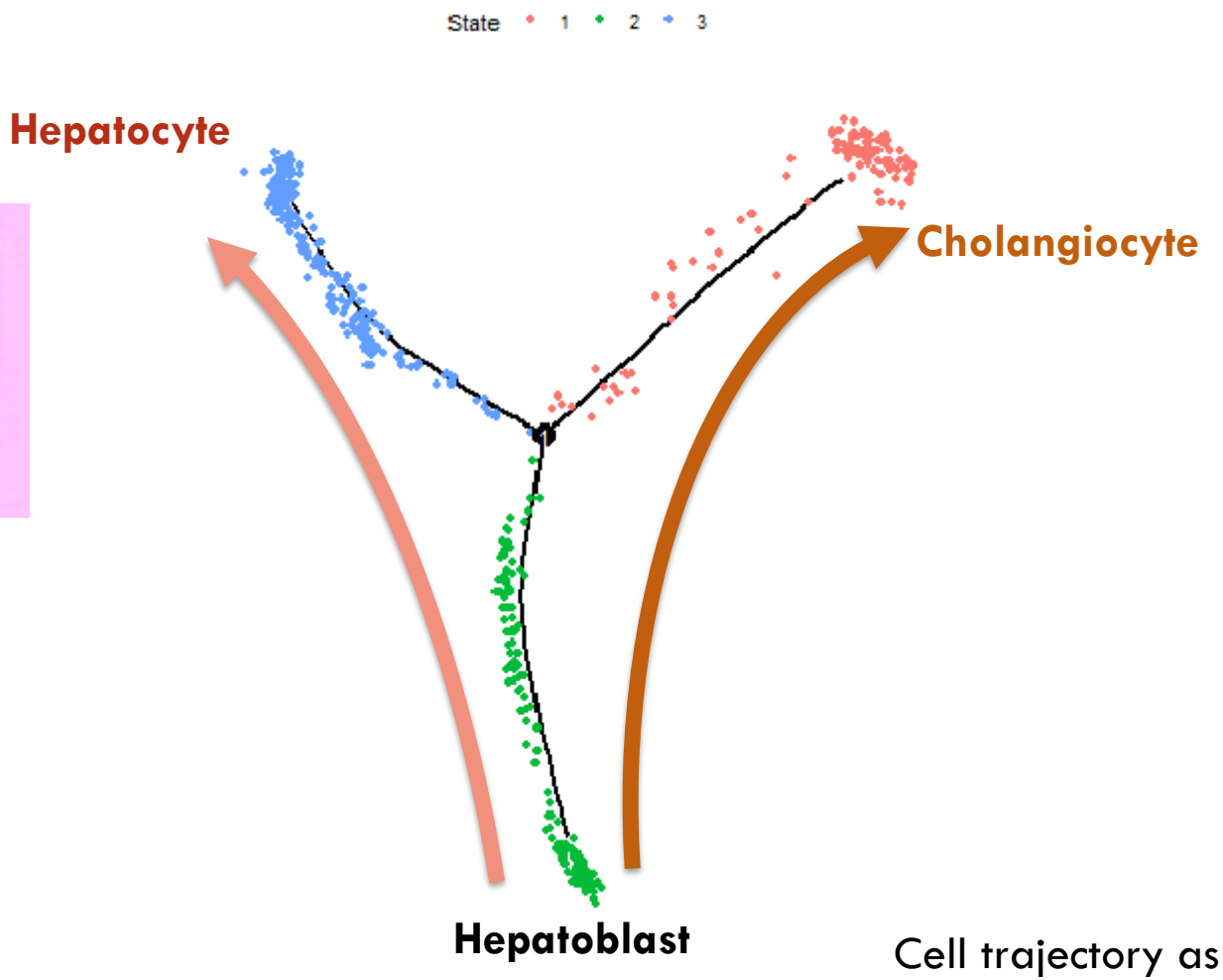




Cell trajectory as estimated using Monocle 2

Differential correlation across pseudotime





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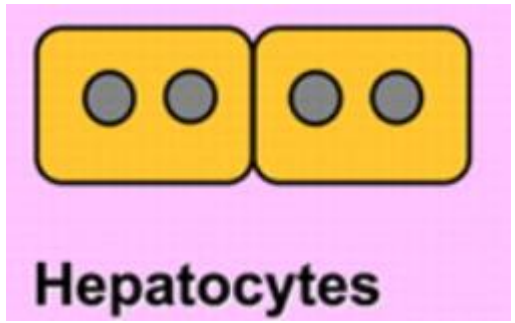
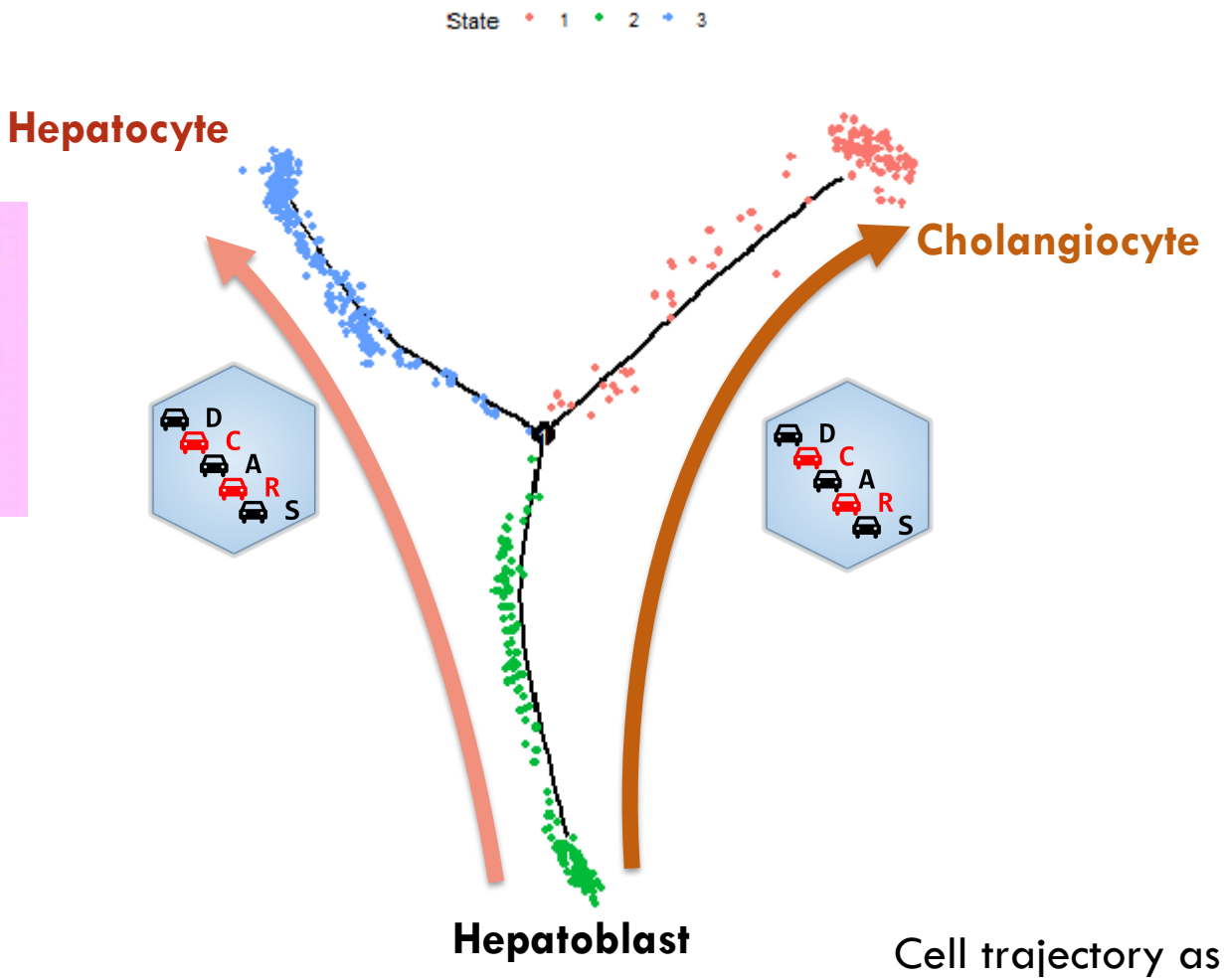
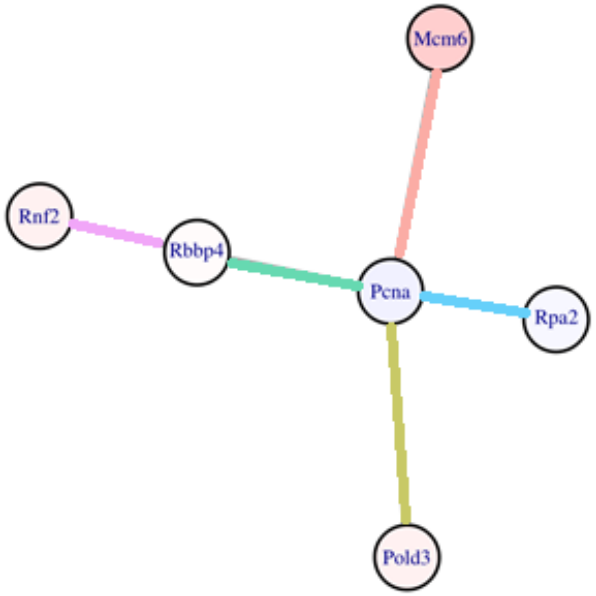
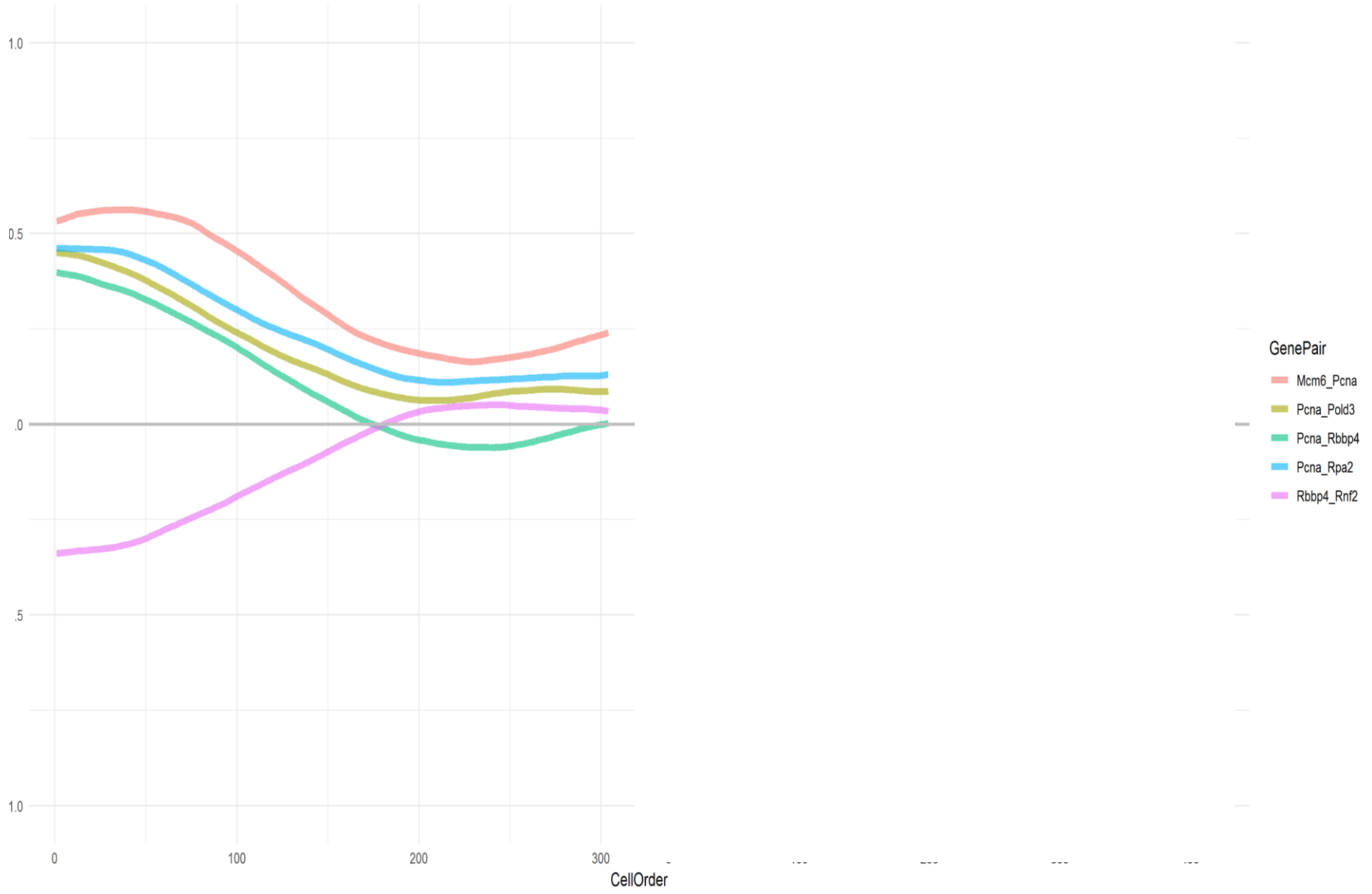




Cell trajectory as estimated using Monocle 2

Pcna: DNA replication, cell cycle. Correlation lost during differentiation





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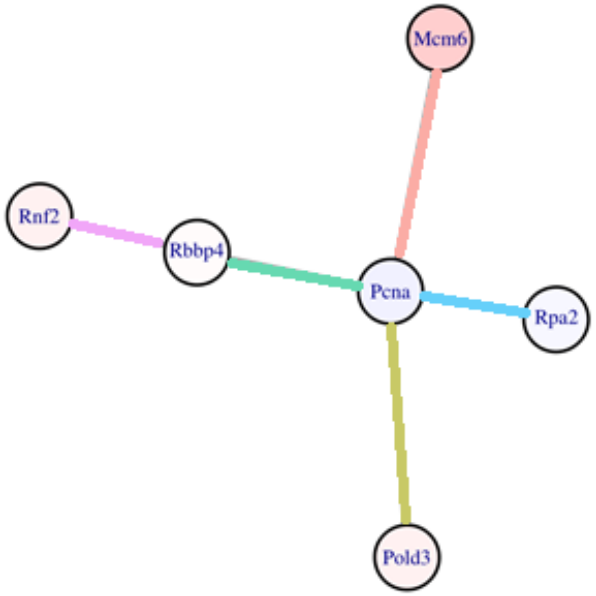

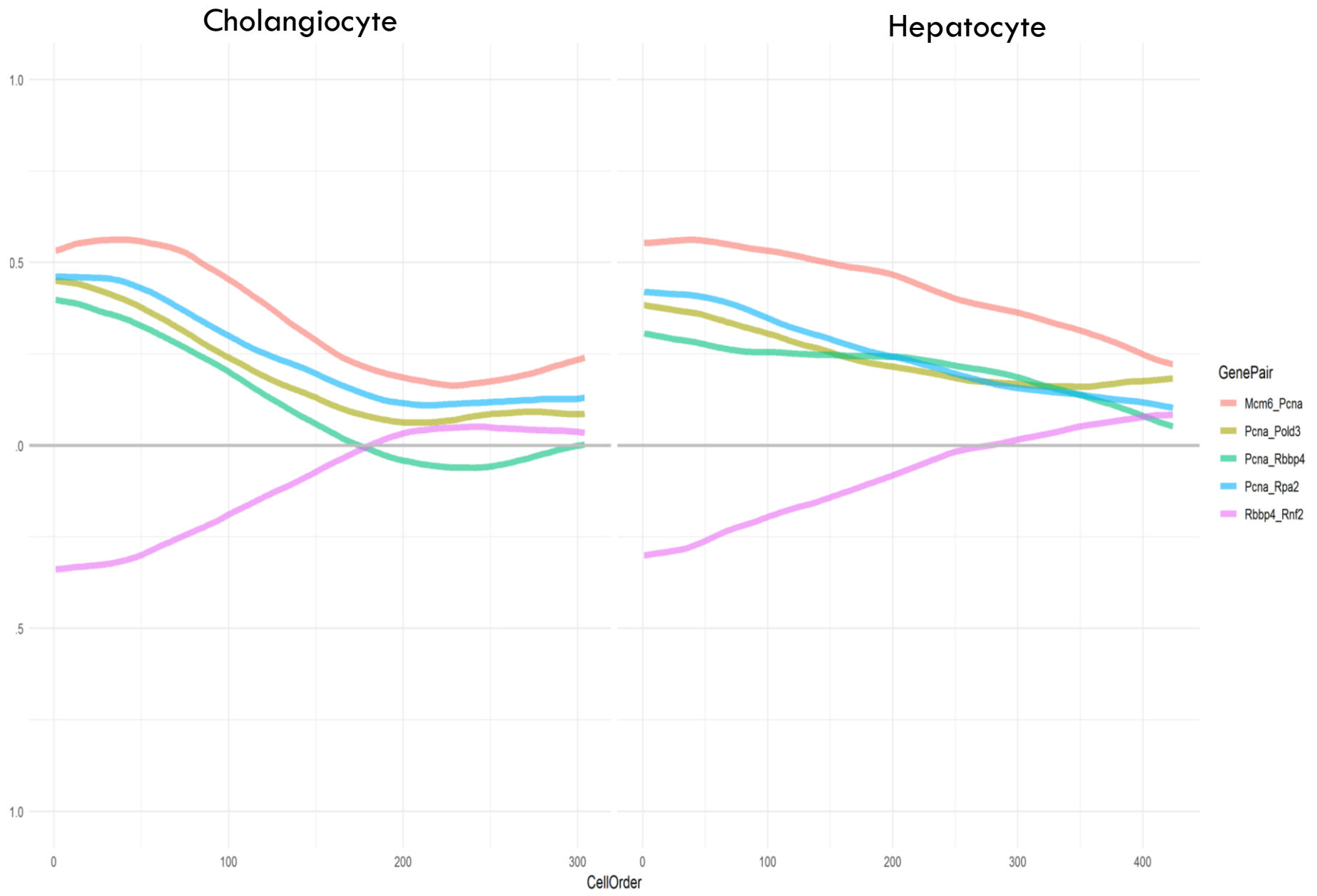

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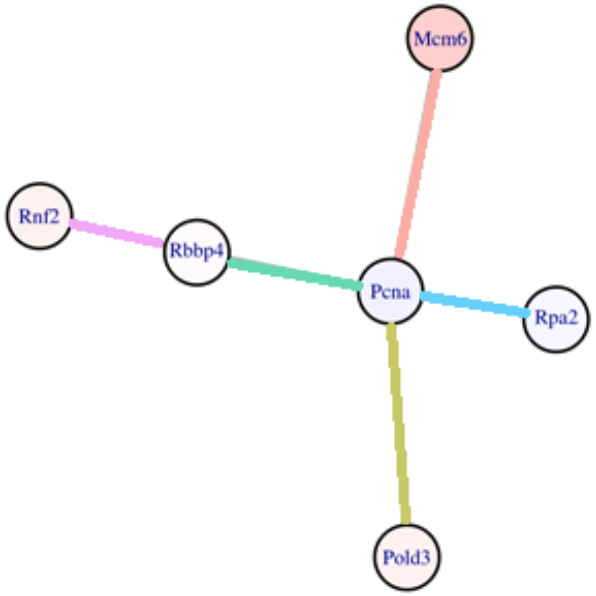
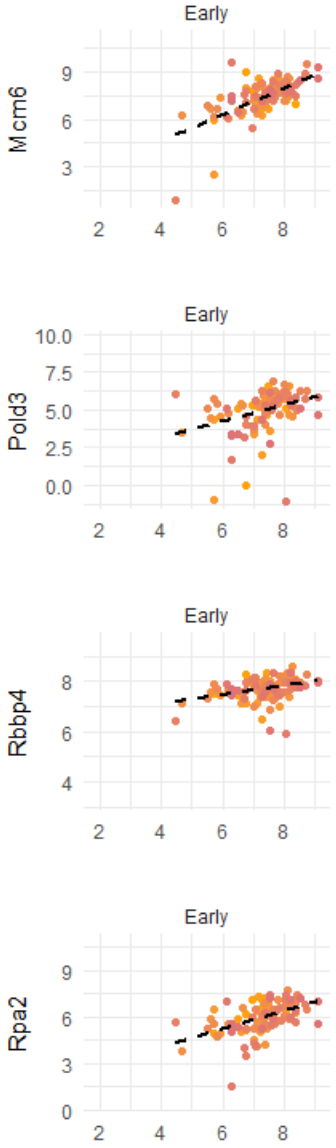


Cholangiocyte



Hepatocyte

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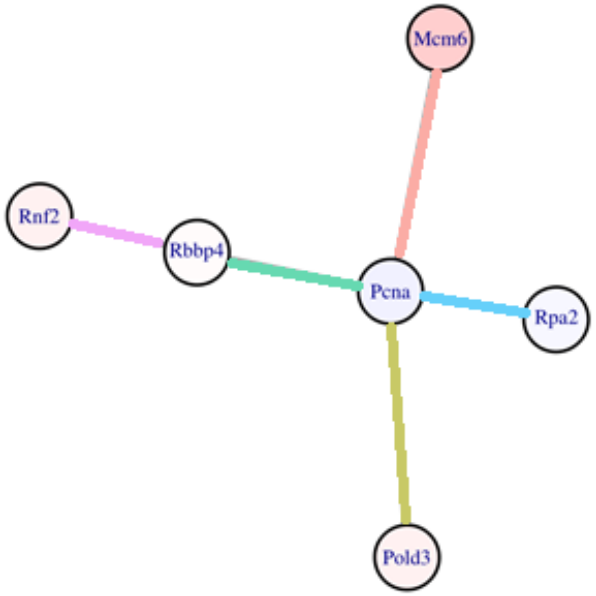

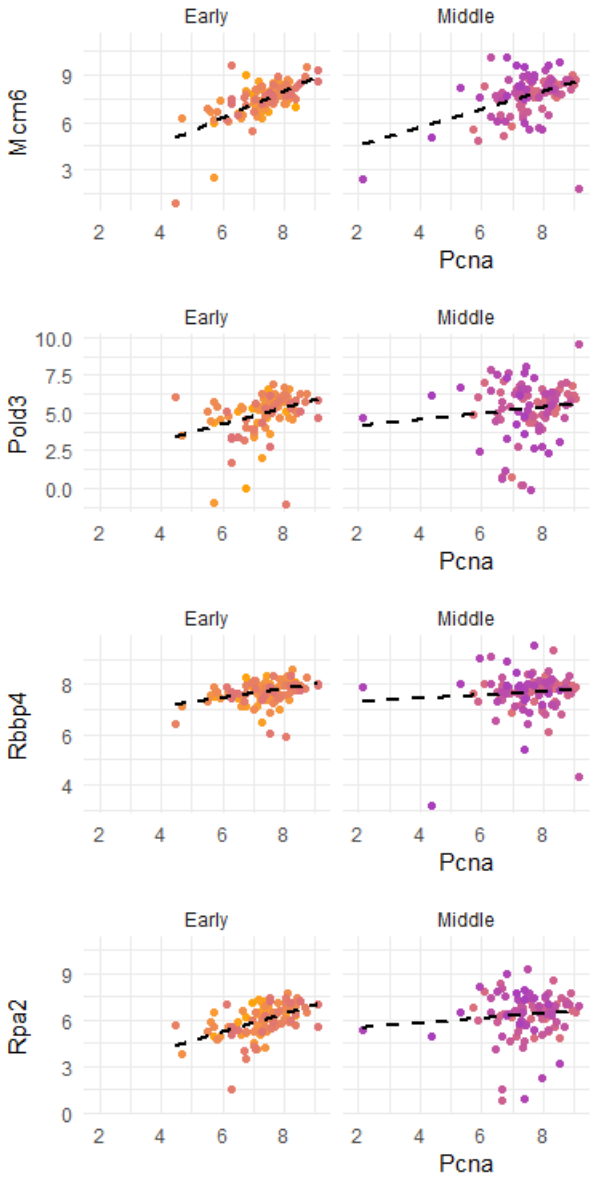

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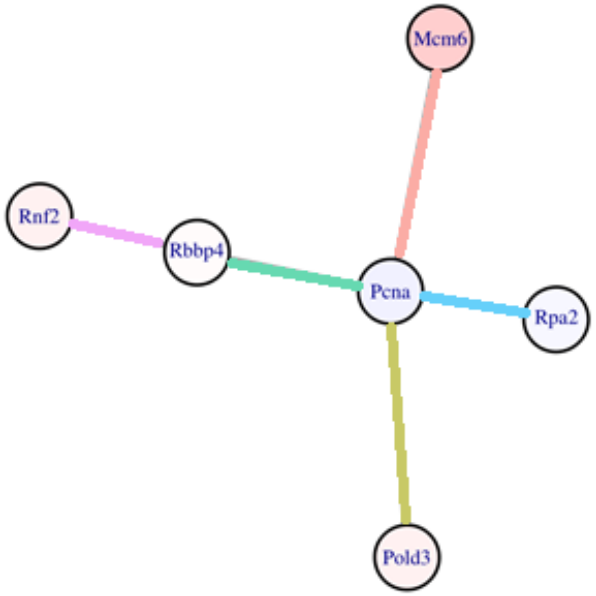

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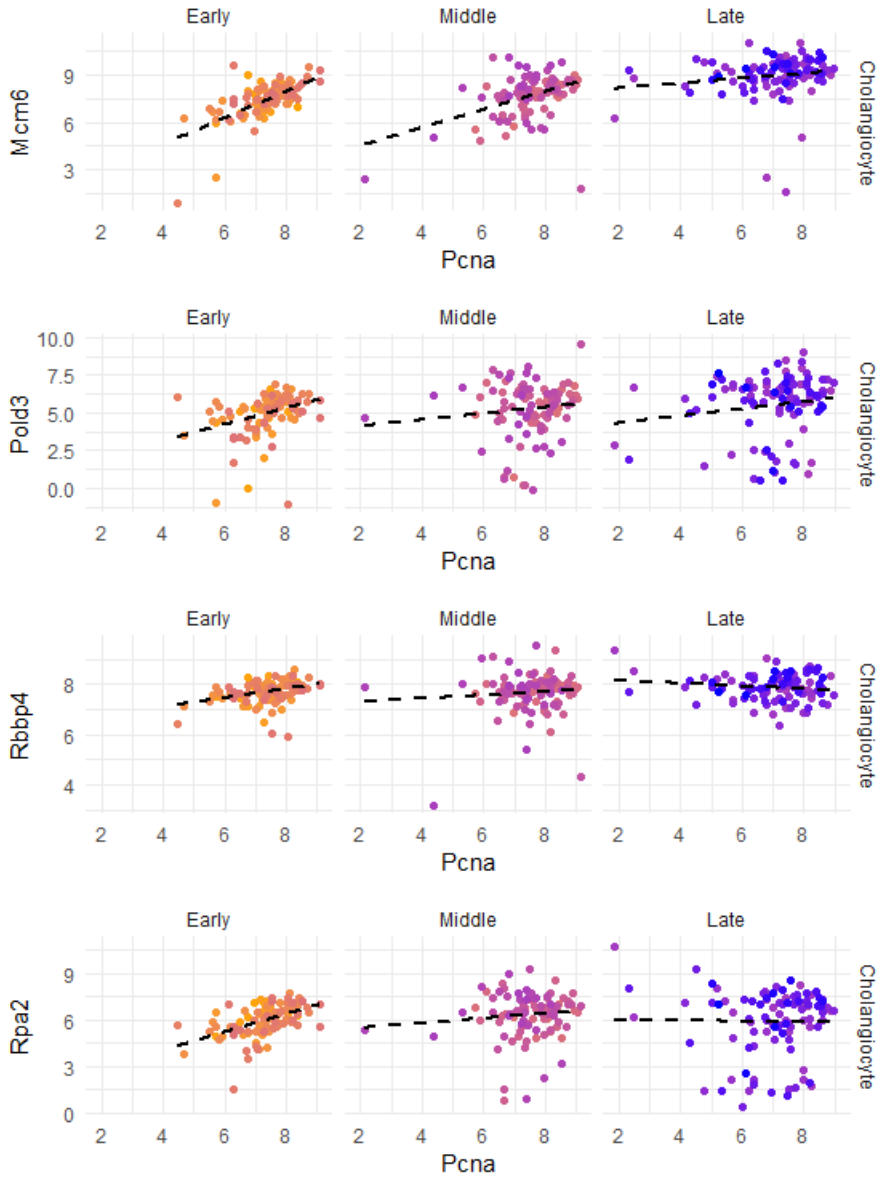

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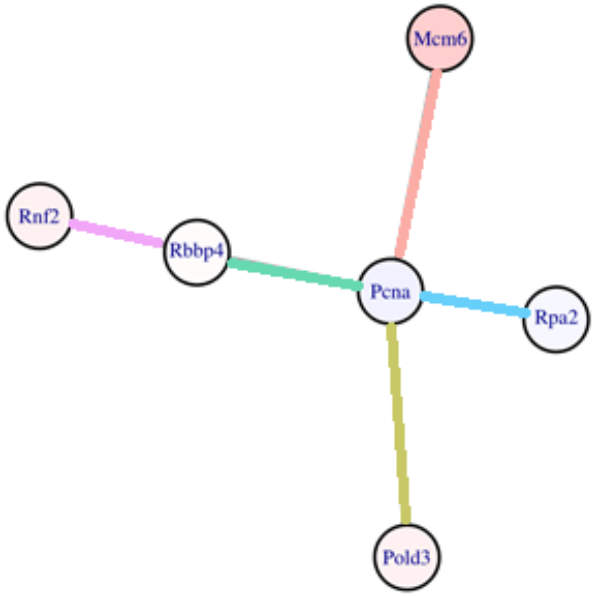

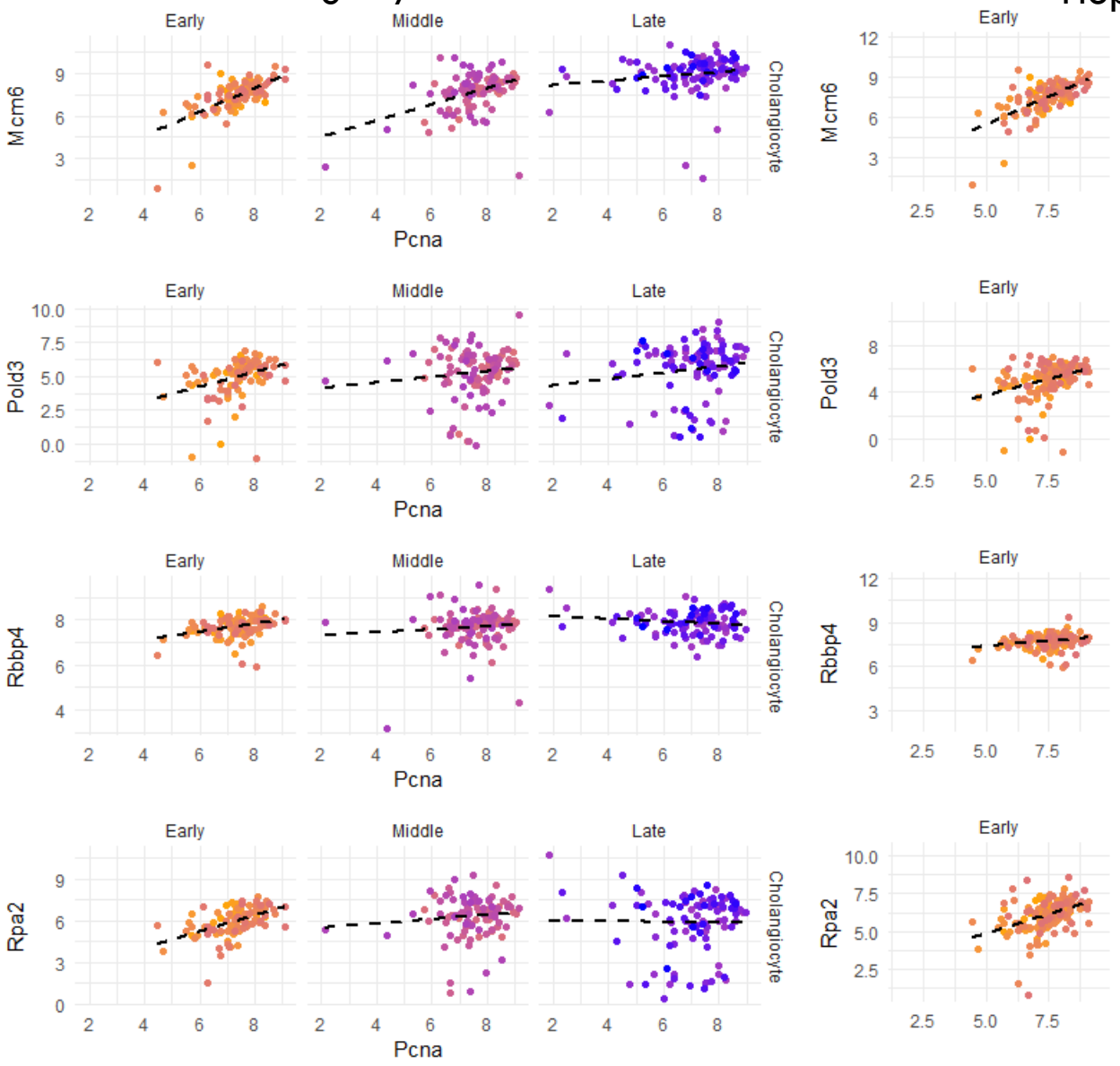

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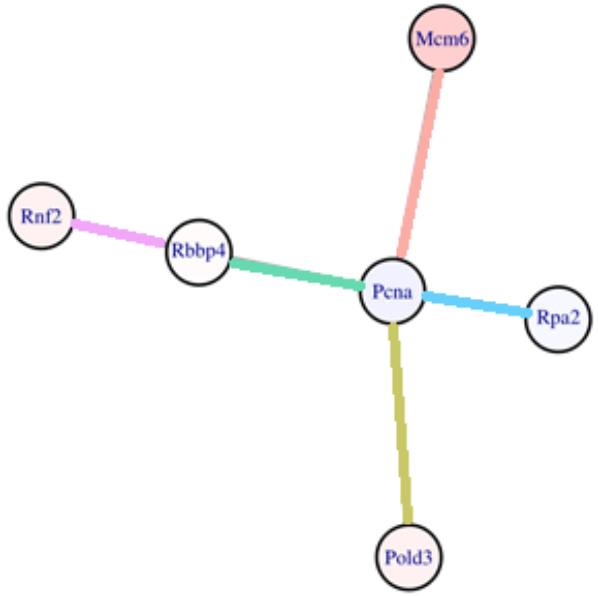

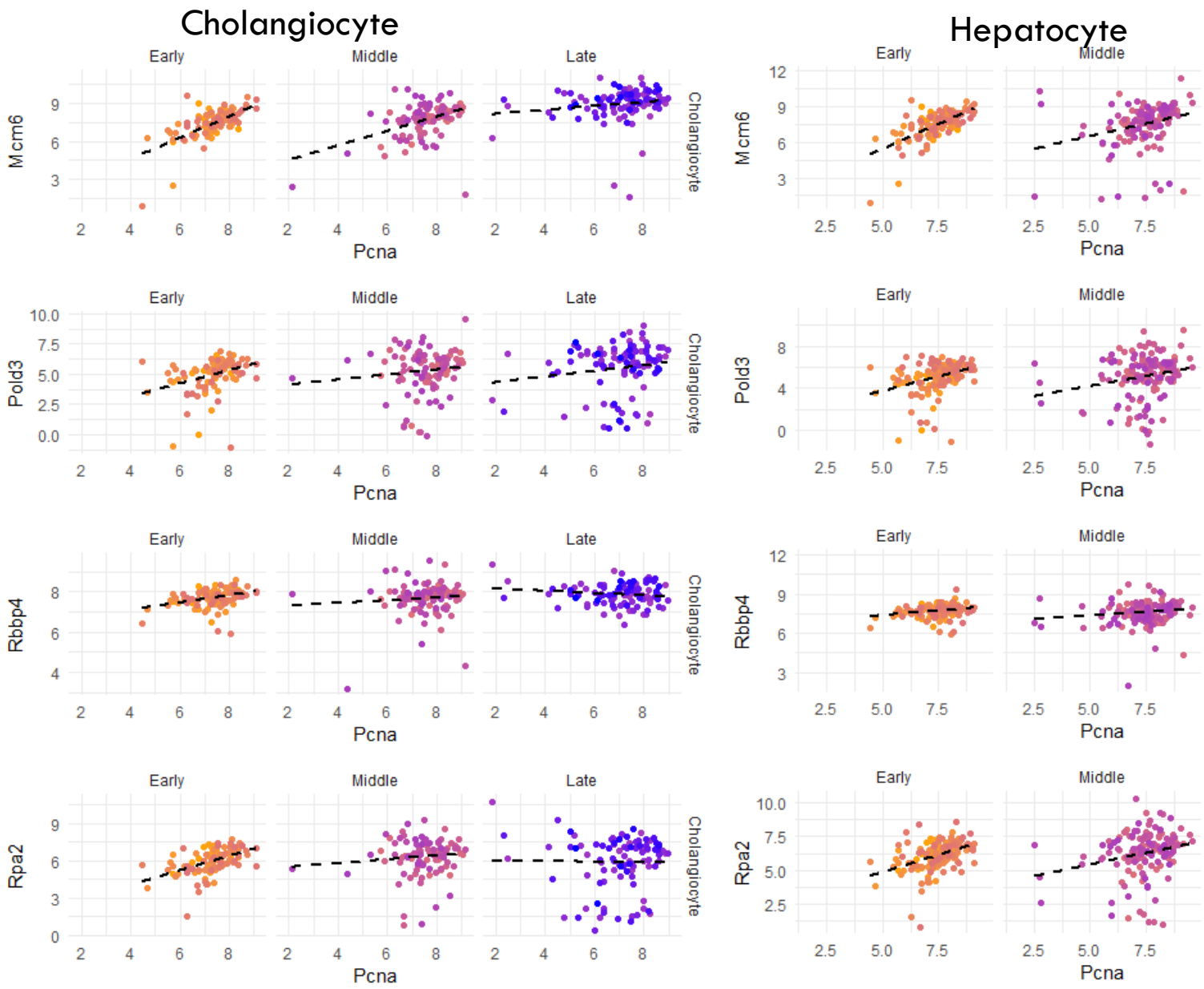

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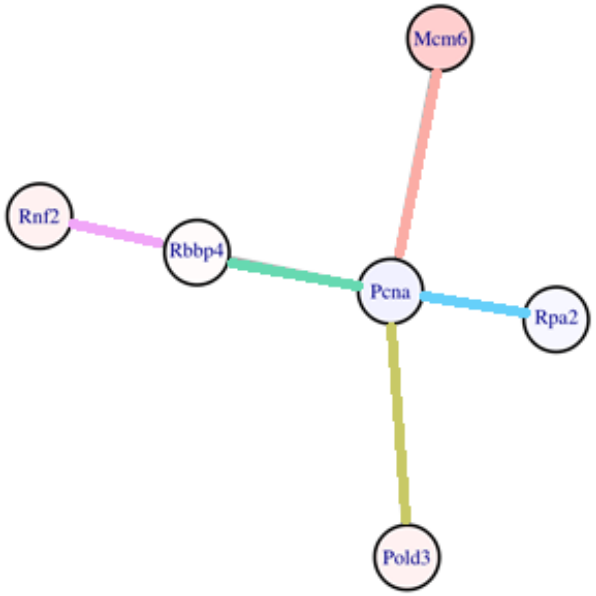
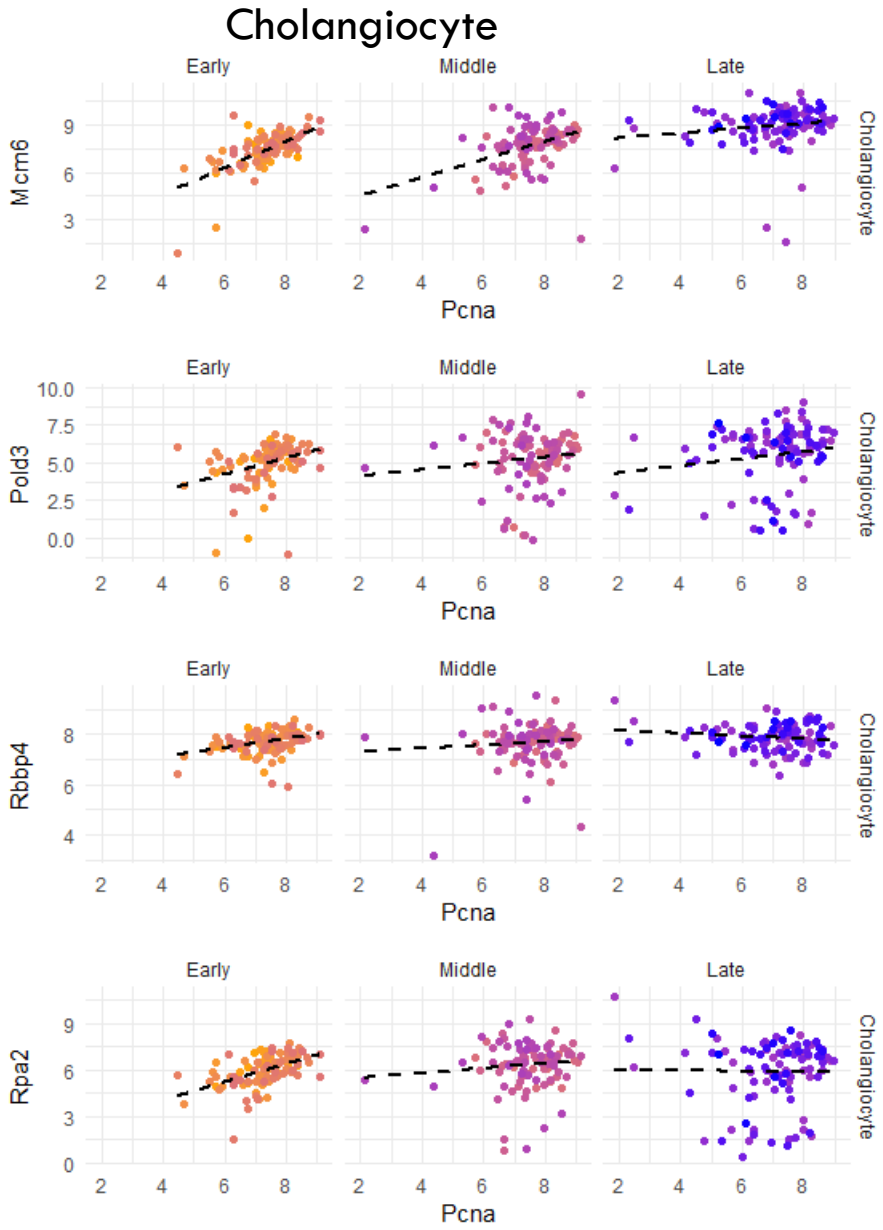
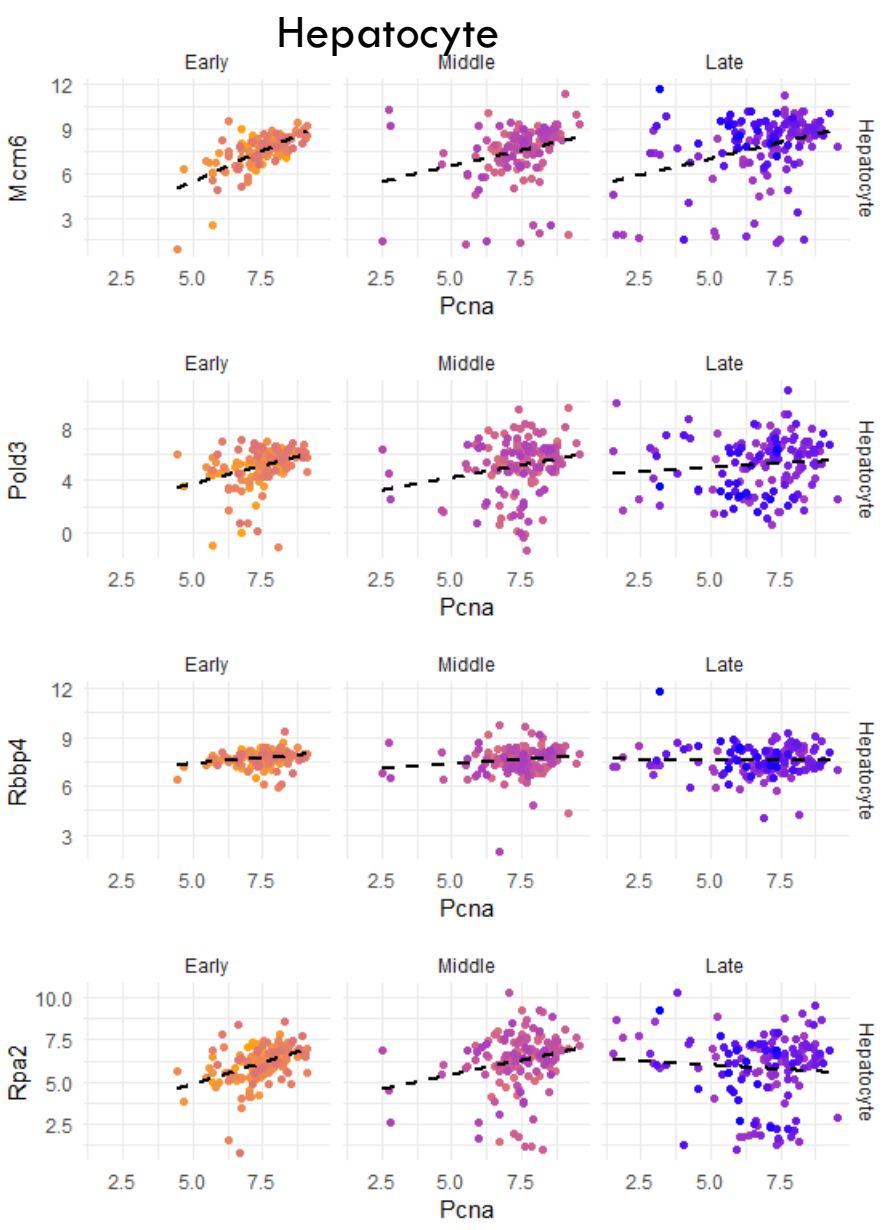
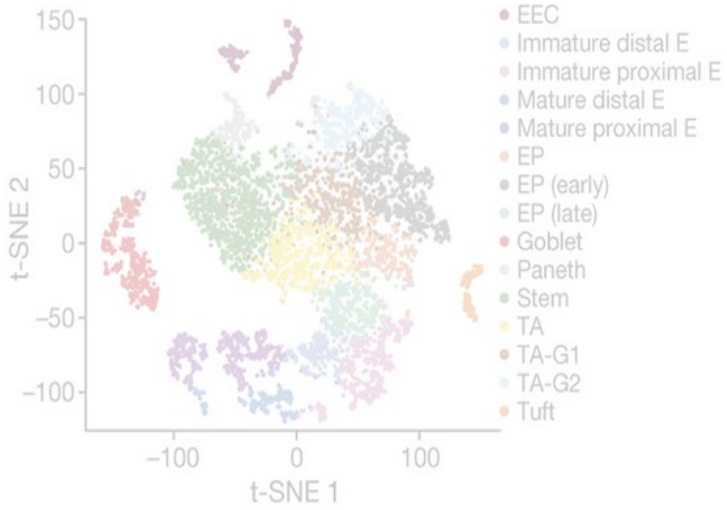






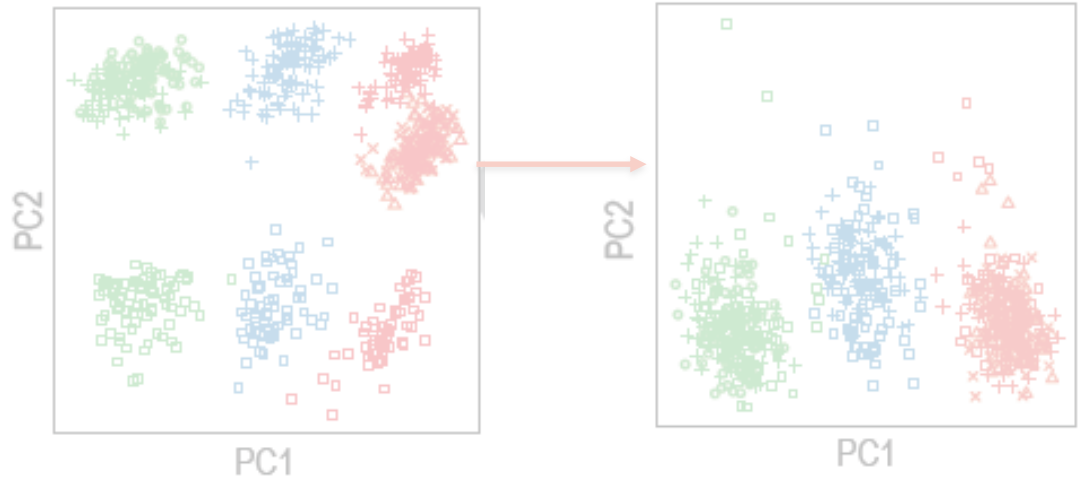


Clustering metrics

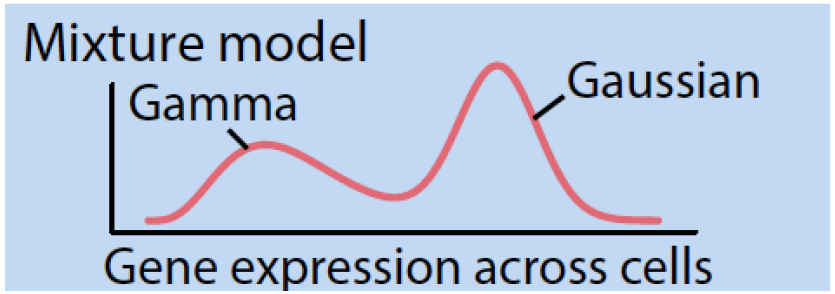
Differential correlation

Sydney
Single cell

scMerge

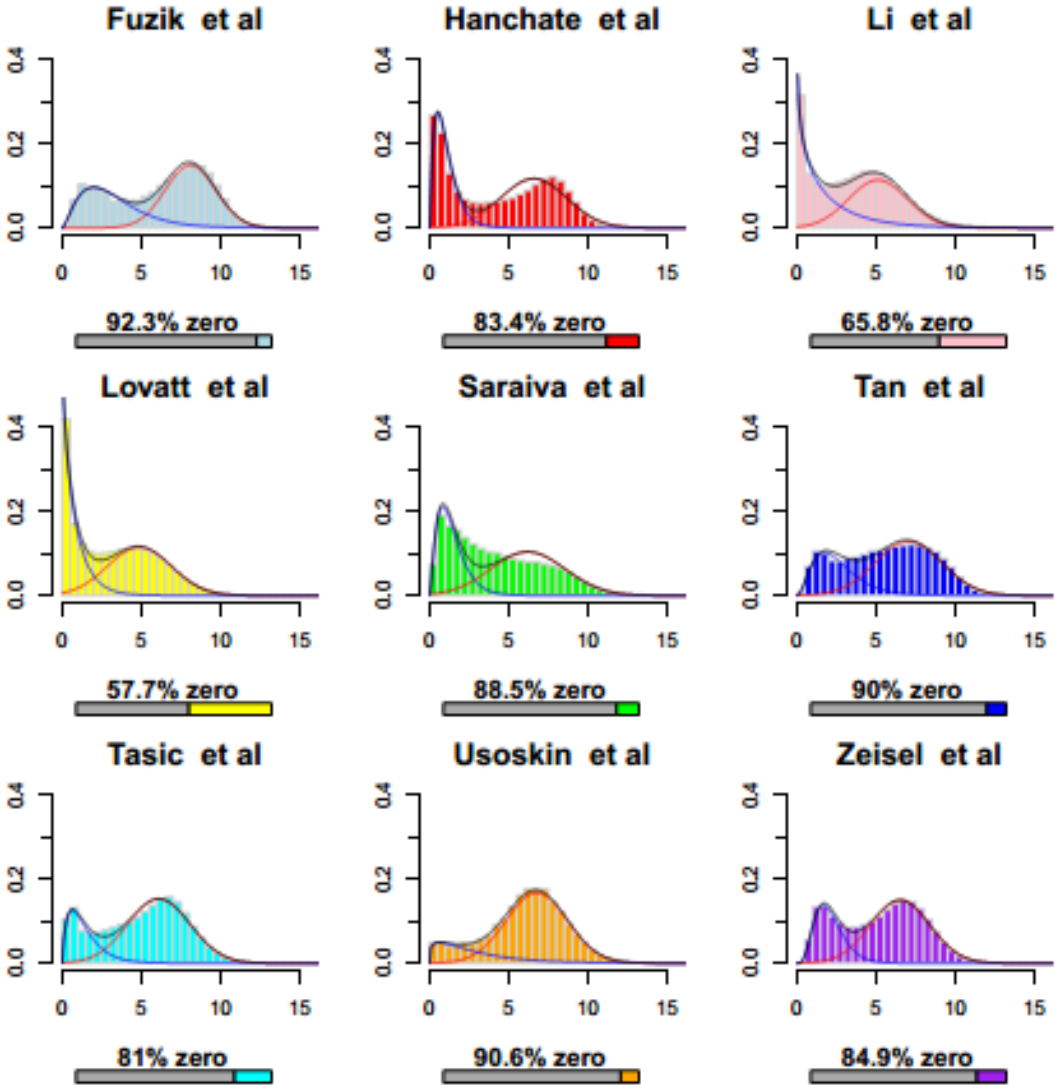


Finding stably expressed genes



Yingxin Lin

Previous work on mixture modelling



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BMC Syst Biol. 2016; 10(Suppl 5): 127.
Published online 2016 Dec 5. doi: 10.1186/s12918-016-0370-4
PMCID: PMC5249008
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Integrated single cell data analysis reveals cell specific networks and novel coactivation markers

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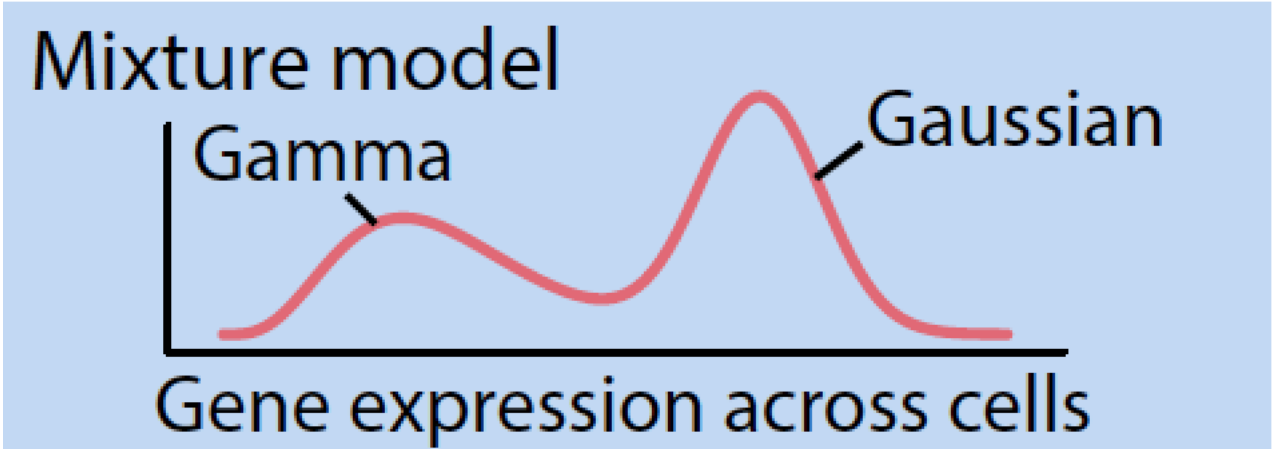

Ghazanfar et al BMC Systems Biology 2016

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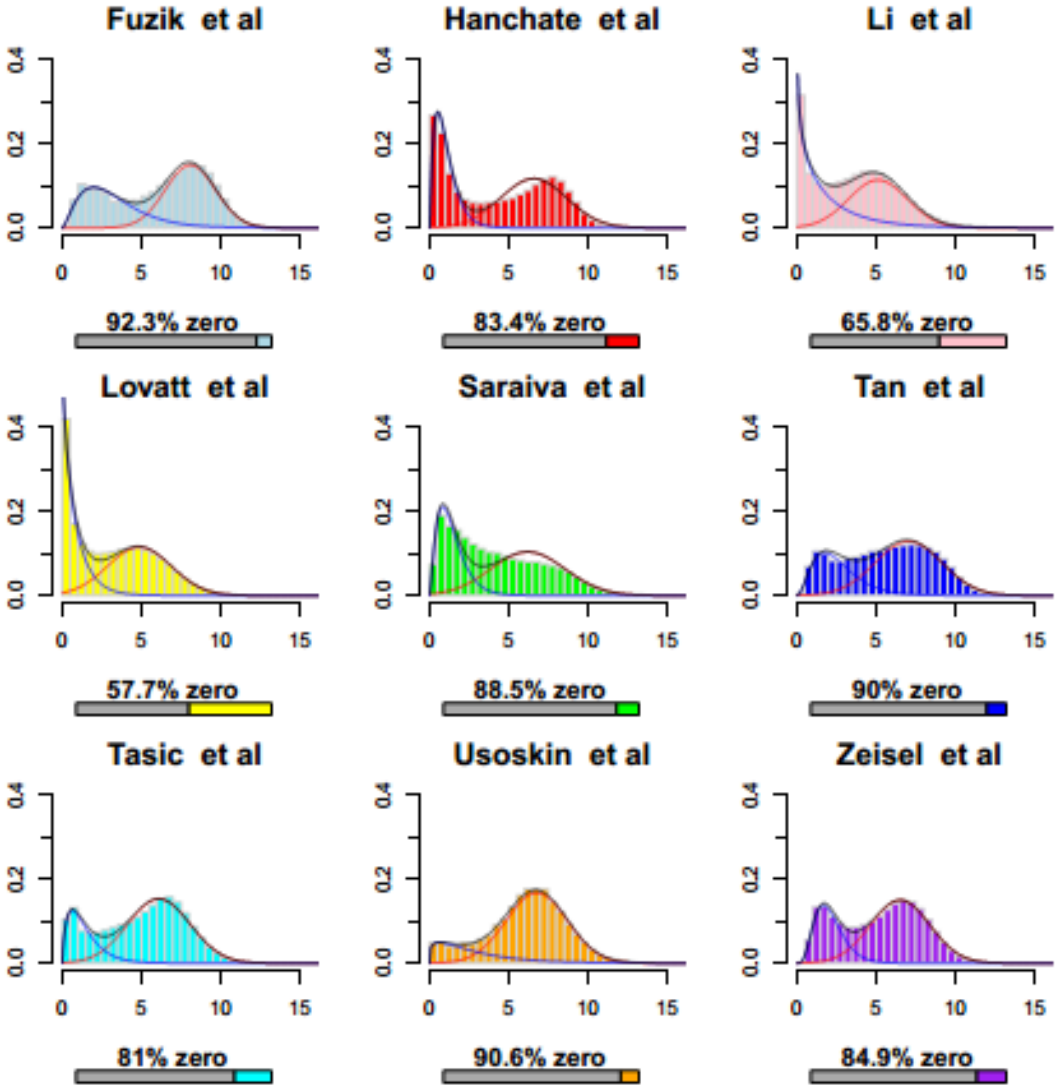

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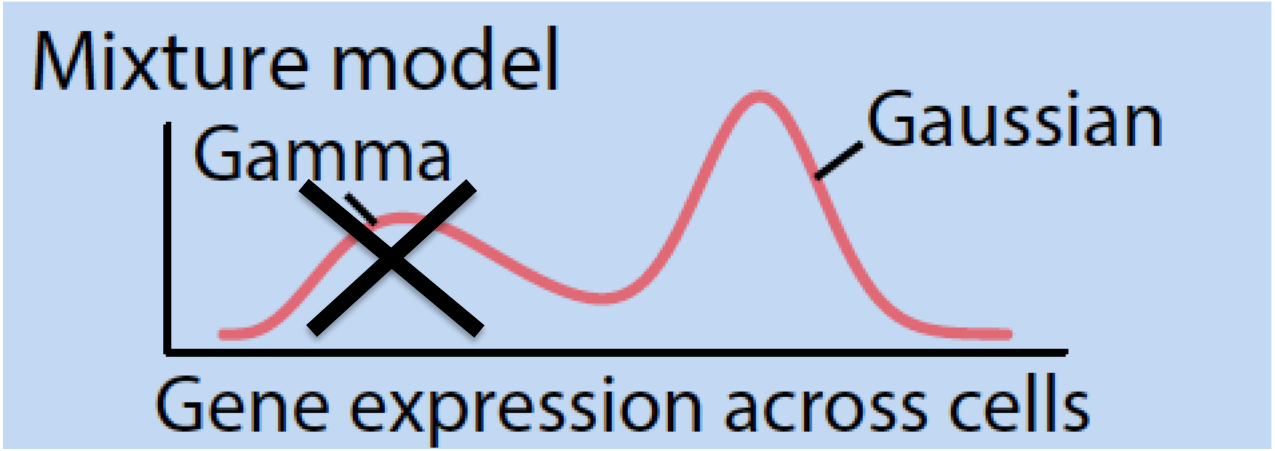


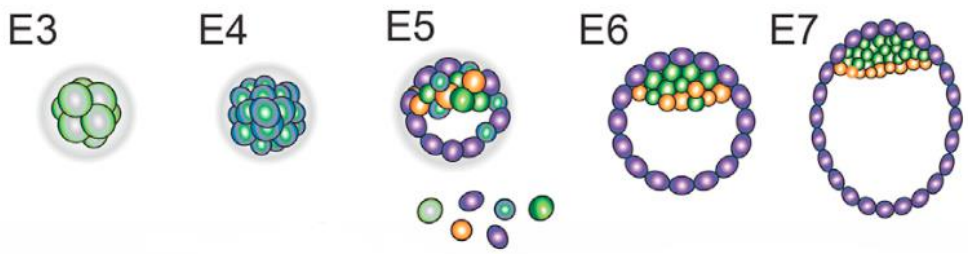
Ghazanfar et al BMC Systems Biology 2016

Establishing a good set of stably expressed genes

Datasets with wide ranges of cell types

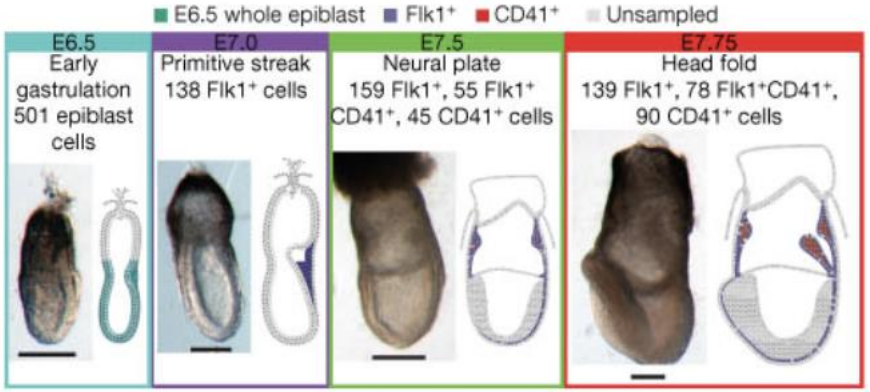
Human early developmental dataset (Petropoulos et al.):

1529 cells from five timepoints: E3, E4, E5, E6, E7



Mouse early developmental dataset (Scialdone et al.):

1205 cells from four timepoints: E6.5, E7.0, E7.5, E7.75



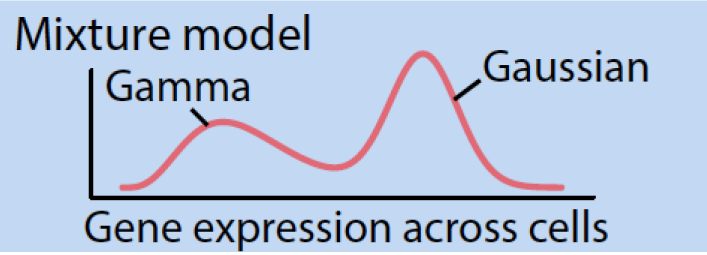
Building features for stably expressed genes

- Mixture Model:
  - **Mixing Proportion** of the second component (λ_i)
  - **Standard deviation** of the second component (σ_i)

- **Zero Proportion** of each gene across all the cells (ω_i)

- **F-statistics** from one-way ANOVA (F_i):

$$\log_2 \text{FPKM} \sim \text{Any conditions or known cell types}$$



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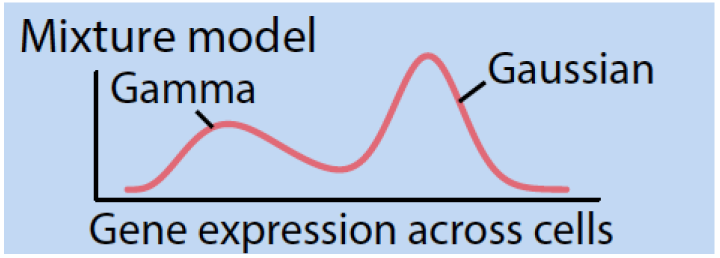
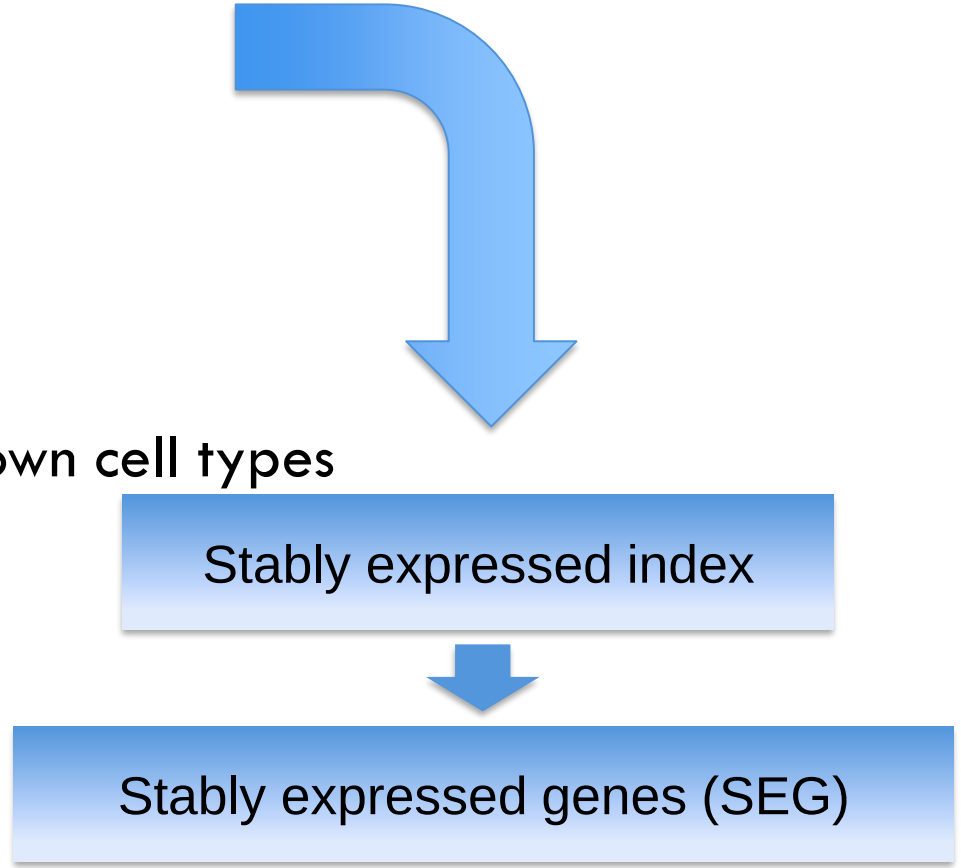
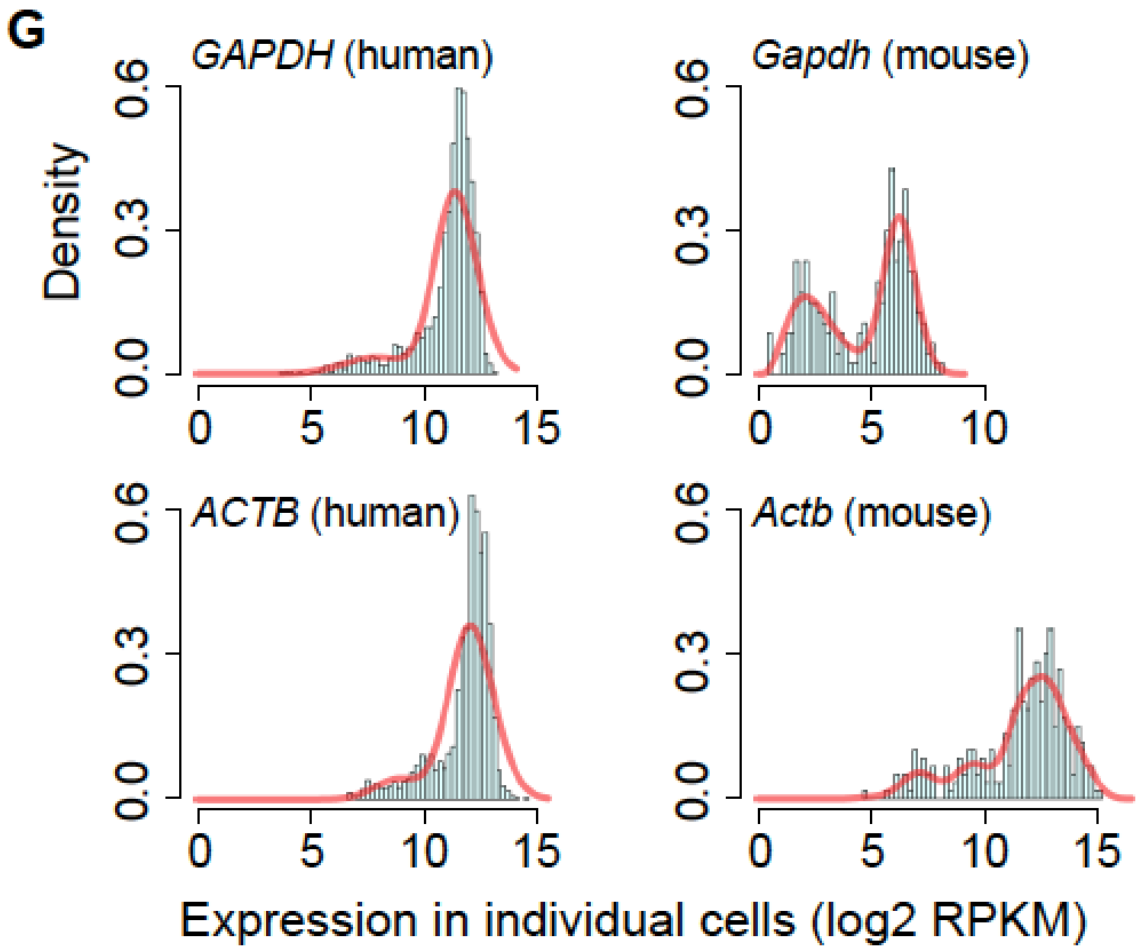


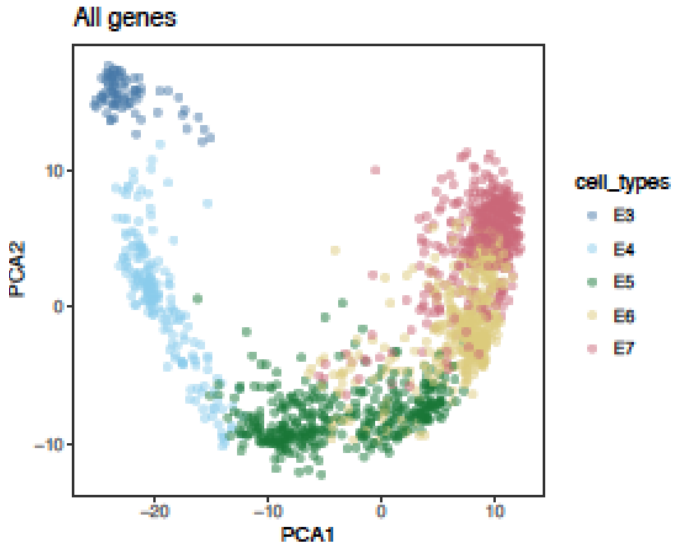


What about GAPDH?



Evaluation of scSEG (PCA plot) - Human early developmental dataset (Petropoulos et al)

ALL genes



House-keeping genes based on microarray data

House keeping genes based on bulk RNA-seq

Proposed scSEG

Eisenberg and Levanon 2013. Human housekeeping genes, revisited. Trends Genet 29 :

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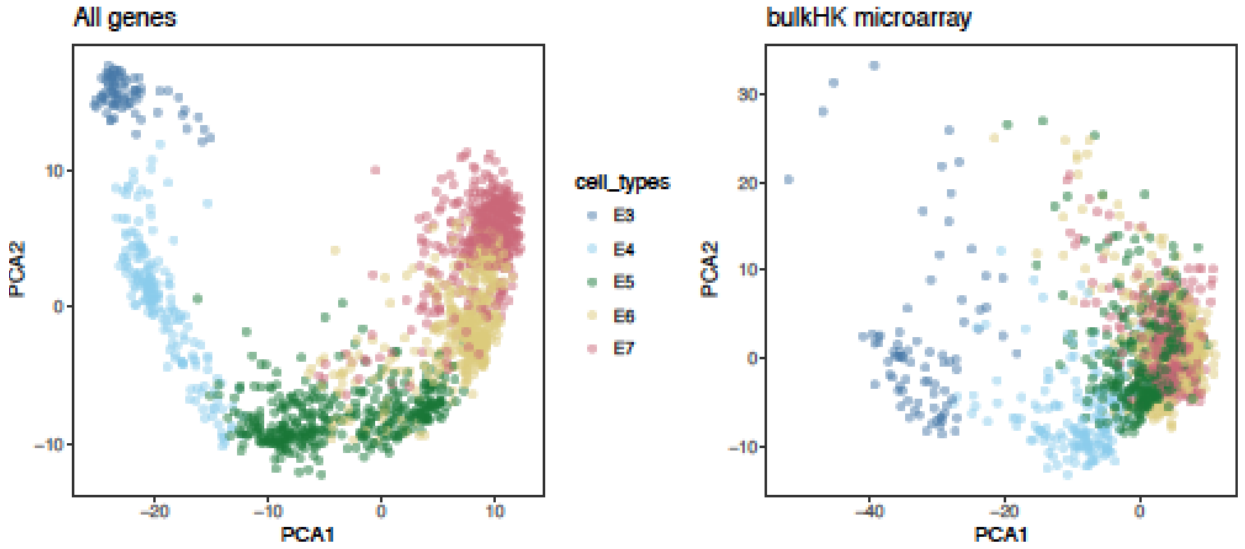

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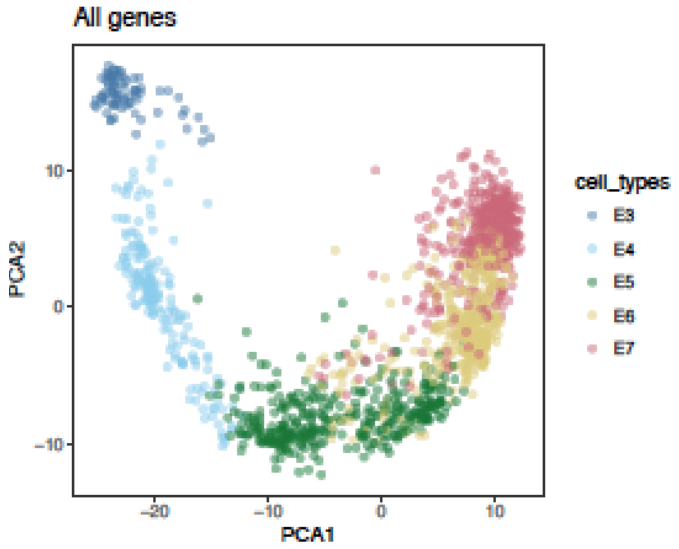

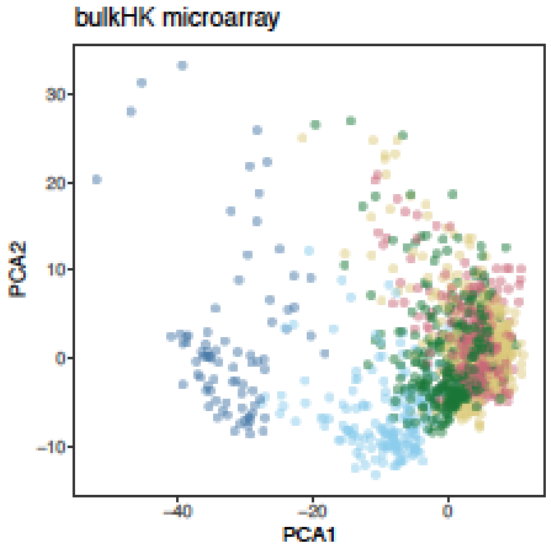

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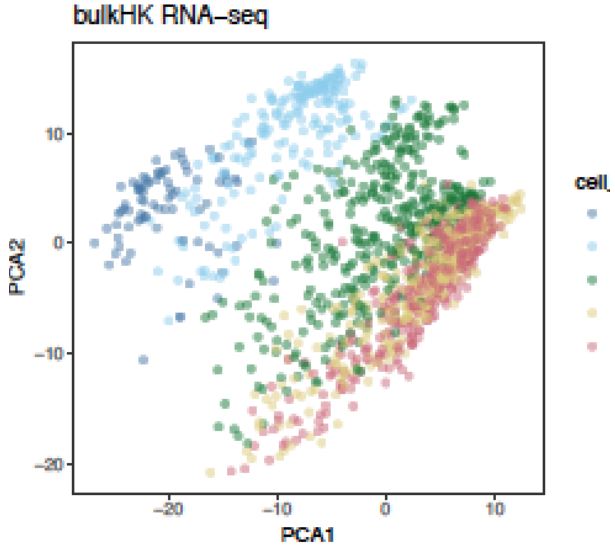

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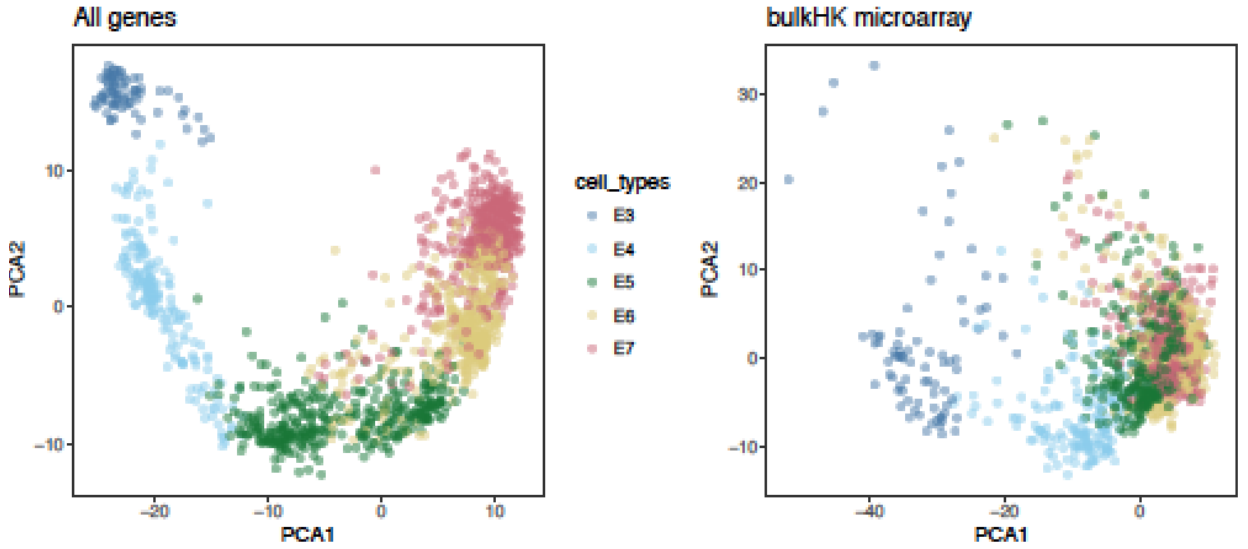

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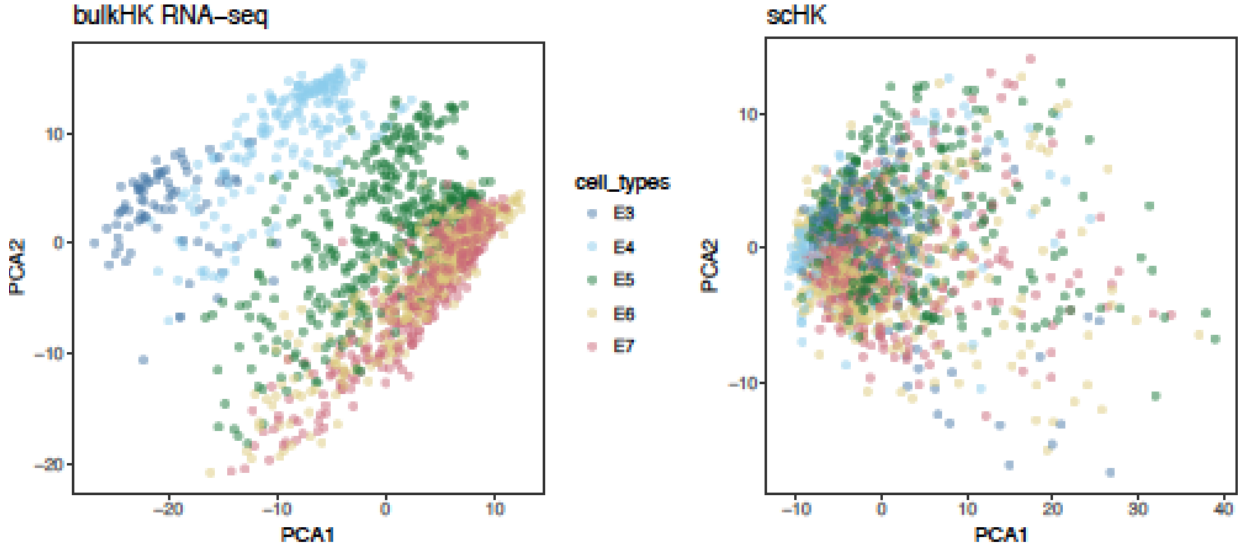

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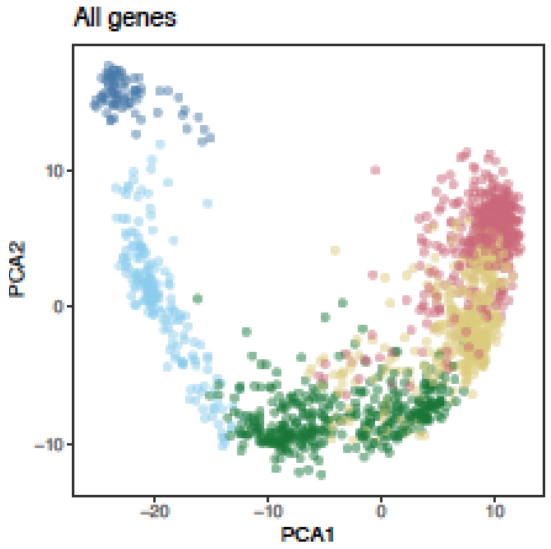
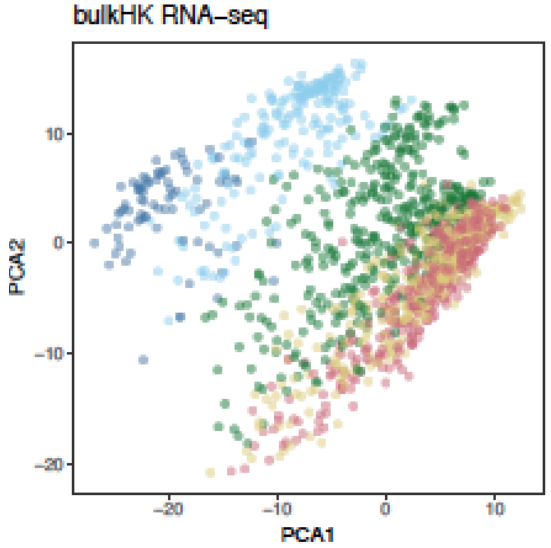
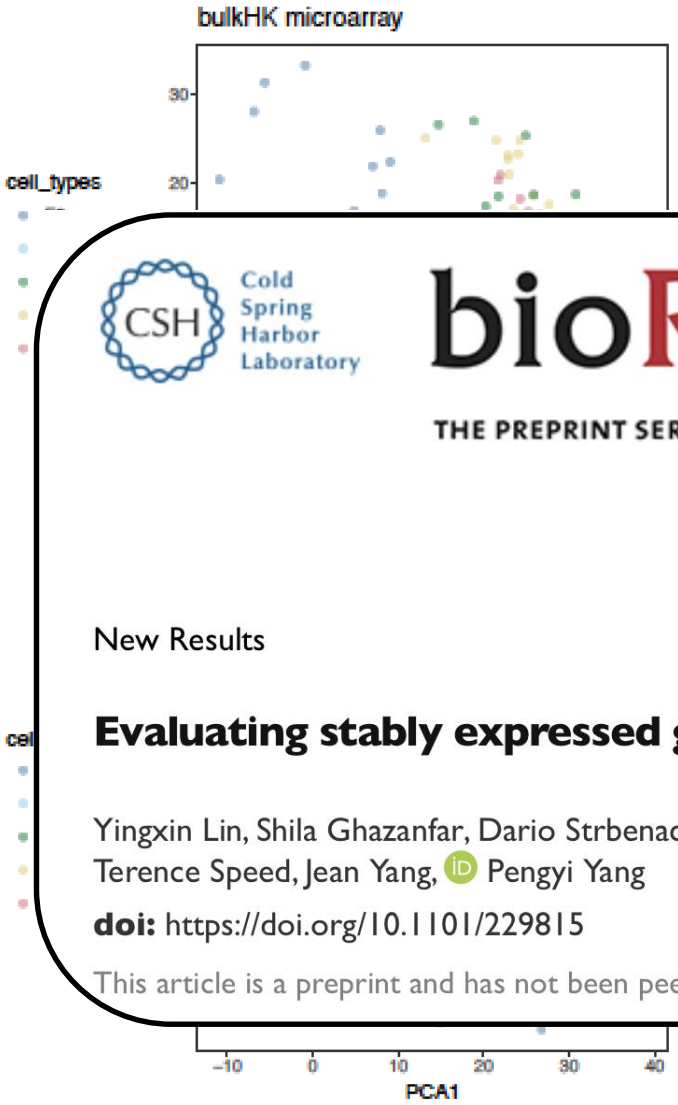


House keeping genes based on bulk RNA-seq





House-keeping genes based on microarray data

Cold Spring Harbor Laboratory

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New Results

Evaluating stably expressed genes in single cells

Yingxin Lin, Shila Ghazanfar, Dario Strbenac, Andy Wang, Ellis Patrick, Dave Lin, Terence Speed, Jean Yang, Pengyi Yang

doi: https://doi.org/10.1101/229815

This article is a preprint and has not been peer-reviewed [what does this mean?].

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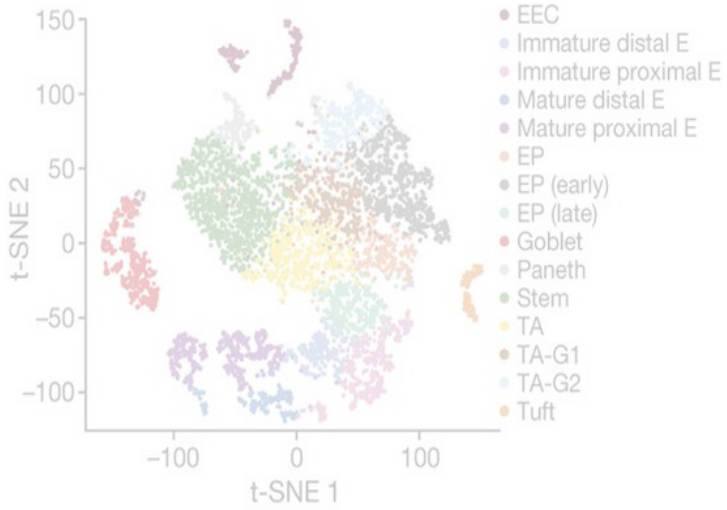
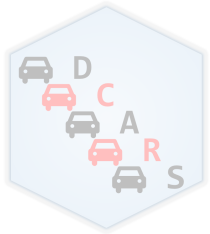
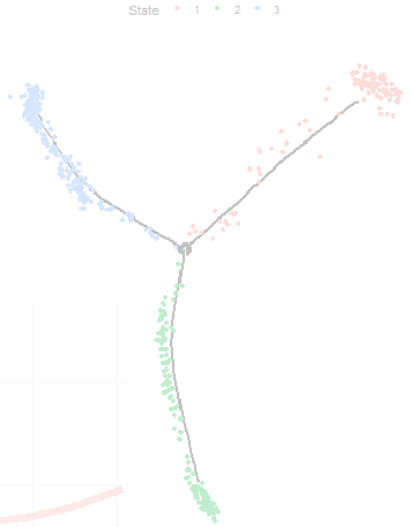
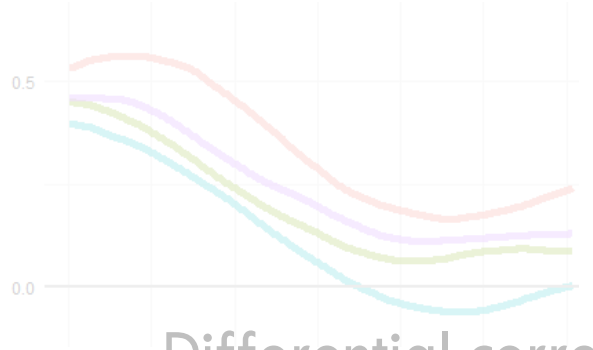


Clustering metrics



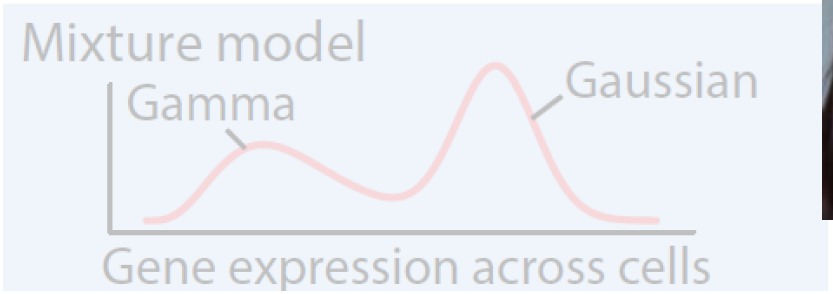




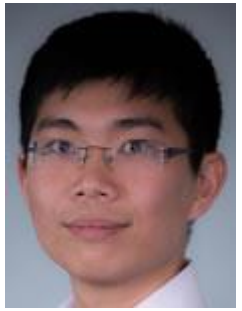
Differential correlation

Sydney Single cell

Finding stably expressed ge

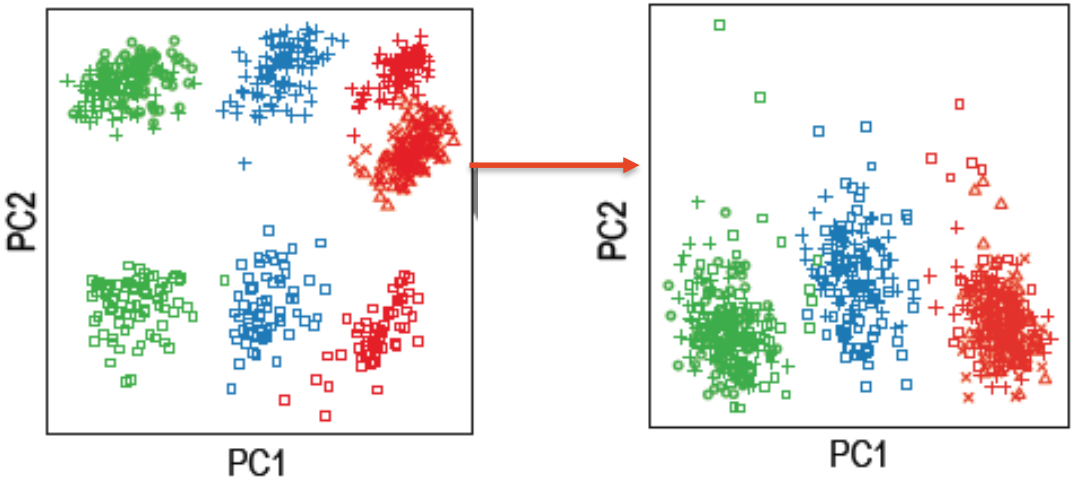


Yingxin Lin



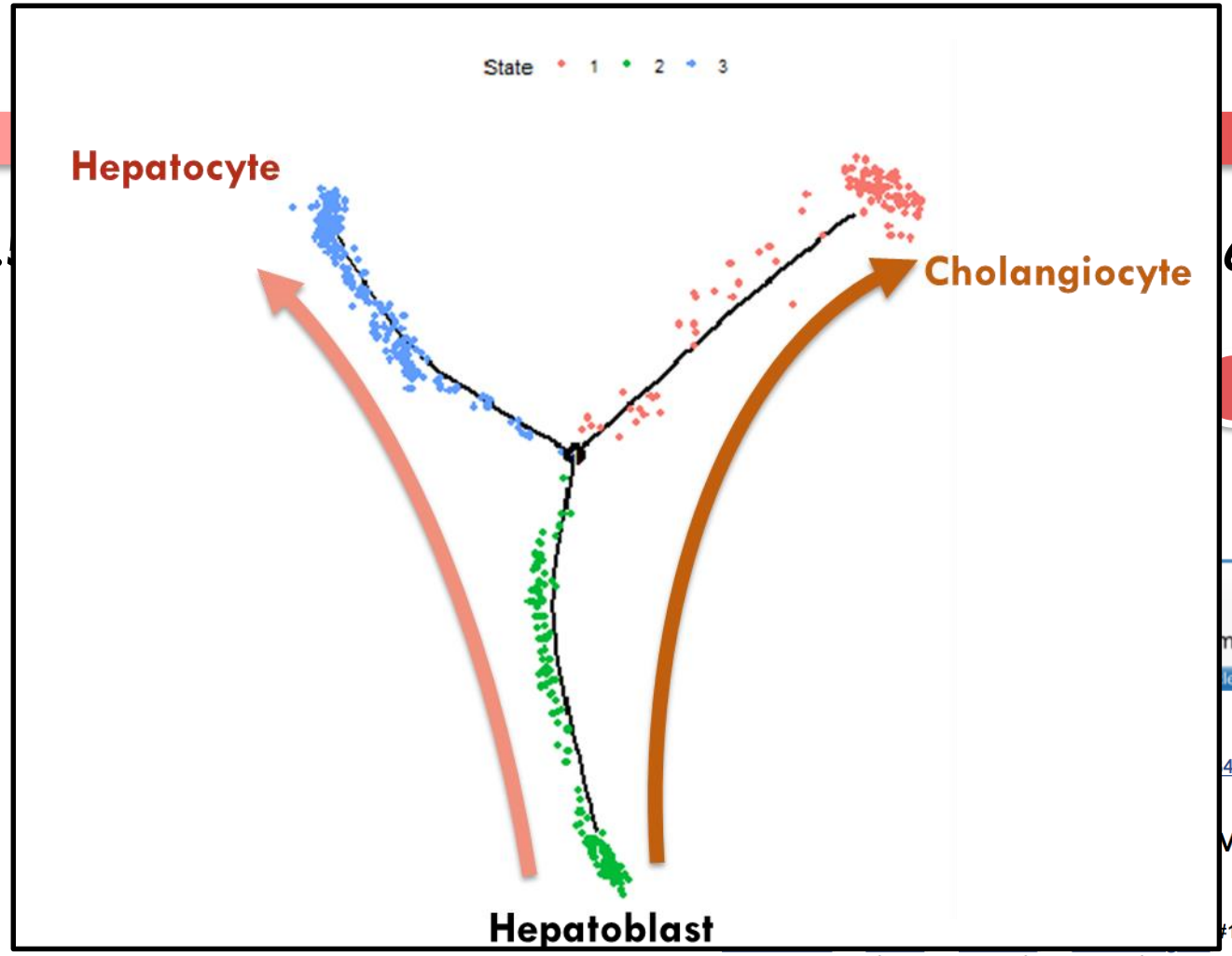
Kevin Wang

scMerge



Liver fetal development time course datasets – integrating multiple datasets

GSE87795
Su et al.



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PMCID: PMC5715535
PMID: 29202695

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veals dynamic trajectories during

#1 Gangcai Xie,[2] Jean Y. H. Yang,[3] Chong-Chao Wu,[1] Xiao-Fang Cui,[1] Kun-Yan He,[1] Qing Luo,[1] Yu-Lan Qu,[1] Na Wang,[1] Lan Wang,[1] and Ze-Guang Han[1,4]

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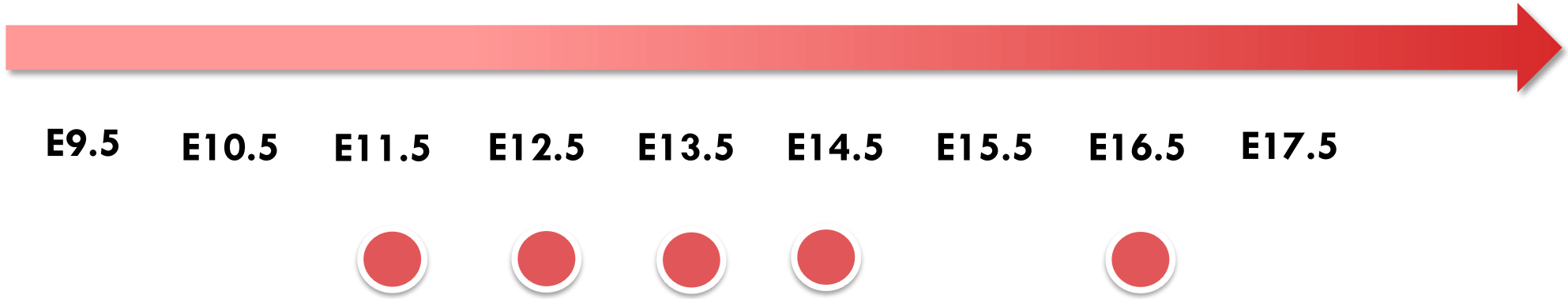
Liver fetal development time course datasets – integrating multiple datasets



GSE87795
Su et al.

BMC Genomics. 2017; 18: 946.
Published online 2017 Dec 4. doi: 10.1186/s12864-017-4342-x
PMCID: PMC5715535
PMID: 29202695

Single-cell RNA-Seq analysis reveals dynamic trajectories during mouse liver development

Xianbin Su,[#1] Yi Shi,[#1] Xin Zou,[#1] Zhao-Ning Lu,[#1] Gangcai Xie,[2] Jean Y. H. Yang,[3] Chong-Chao Wu,[1] Xiao-Fang Cui,[1] Kun-Yan He,[1] Qing Luo,[1] Yu-Lan Qu,[1] Na Wang,[1] Lan Wang,[1] and Ze-Guang Han[1,4]

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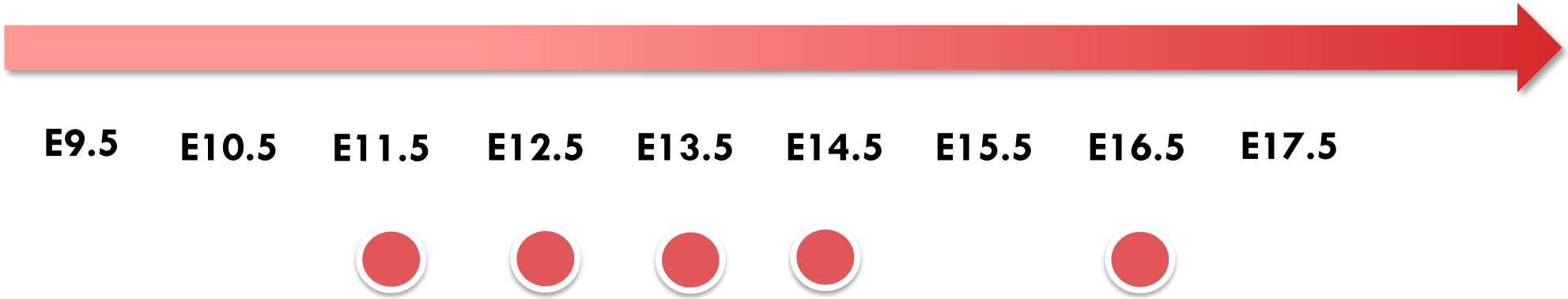

GSE87795
Su et al.

LETTER

doi:10.1038/nature22796

Multilineage communication regulates human liver bud development from pluripotency

J. Gray Camp[1]*, Keisuke Sekine[2]*, Tobias Gerber[1], Henry Loeffler-Wirth[3], Hans Binder[3], Malgorzata Gac[1], Sabina Kanton[1], Jorge Kageyama[1], Georg Damm[4,5], Daniel Seehofer[4,5], Lenka Belicova[6], Marc Bickle[6], Rico Barsacchi[6], Ryo Okuda[2], Emi Yoshizawa[2], Masaki Kimura[2], Hiroaki Ayabe[2], Hideki Taniguchi[2], Takanori Takebe[2,7] & Barbara Treutlein[1,6]

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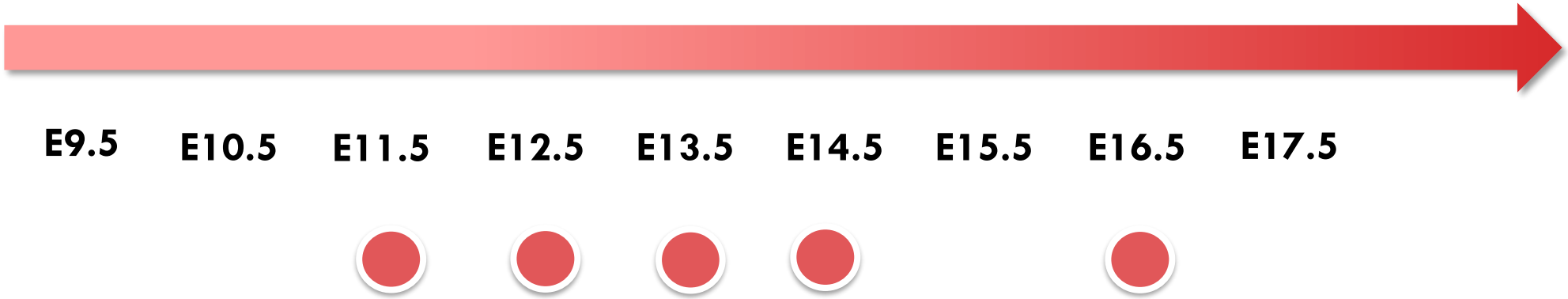
Liver fetal development time course datasets – integrating multiple datasets



GSE87795
Su et al.

HEPATOLOGY
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LET

A Single-Cell Transcriptomic Analysis Reveals Precise Pathways and Regulatory Mechanisms Underlying Hepatoblast Differentiation

Li Yang,[1,2*] Wei-Hua Wang,[1,2*] Wei-Lin Qiu,[1,3*] Zhen Guo,[1] Erfei Bi,[4] and Cheng-Ran Xu[1]

Multilin
bud dev

J. Gray Camp[1*], Kei
Jorge Kageyama[1], C
Emi Yoshizawa[2], M

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946.
4. doi: 10.1186/s12864-017-4342-x
PMCID: PMC5715535
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A-Seq analysis reveals dynamic trajectories during
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an He,[1] Qing Luo,[1] Yu-Lan Qu,[1] Na Wang,[1] Lan Wang,[1] and Ze-Guang Han[1,4]

Liver fetal development time course datasets – integrating multiple datasets

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GSE87795
Su et al.

HEPATOLOGY

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J. Gray Camp[1]*, Kei
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A Single-Cell Transcriptom
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Dong et al. Genome Biology (2018) 19:31
https://doi.org/10.1186/s13059-018-1416-2

Genome Biology

RESEARCH ARTICLE Open Access

Single-cell RNA-seq analysis unveils a prevalent epithelial/mesenchymal hybrid state during mouse organogenesis

Ji Dong[1,2†], Yuqiong Hu[1,2,3†], Xiaoying Fan[1,2†], Xinglong Wu[1,2,3†], Yunuo Mao[1,2], Boqiang Hu[1,2], Hongshan Guo[1,2], Lu Wen[1,2] and Fuchou Tang[1,2,3*]

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PMCID: PMC5715535
PMID: 29202695

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Xin Zou,[#1] Zhao-Ning Lu,[#1] Gangcai Xie,[2] Jean Y. H. Yang,[3] Chong-Chao Wu,[1]
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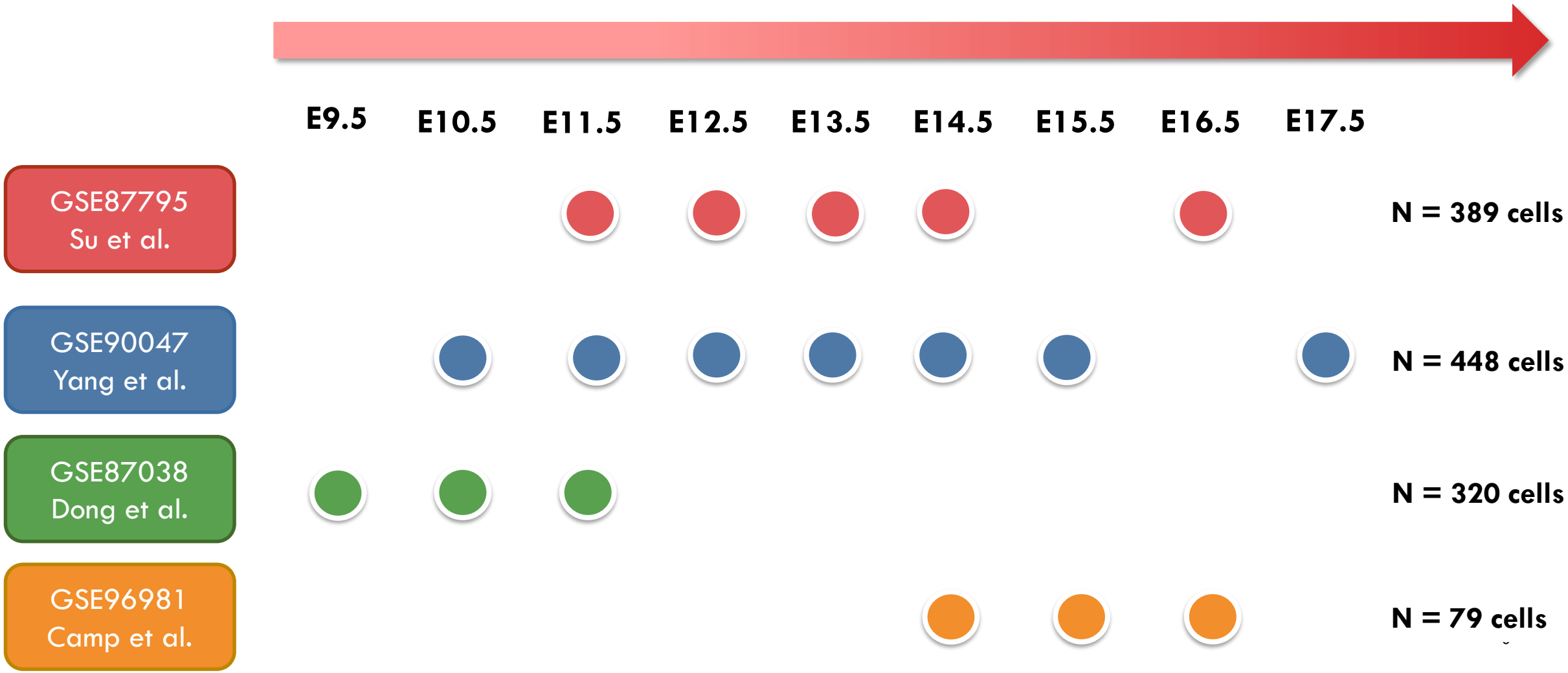
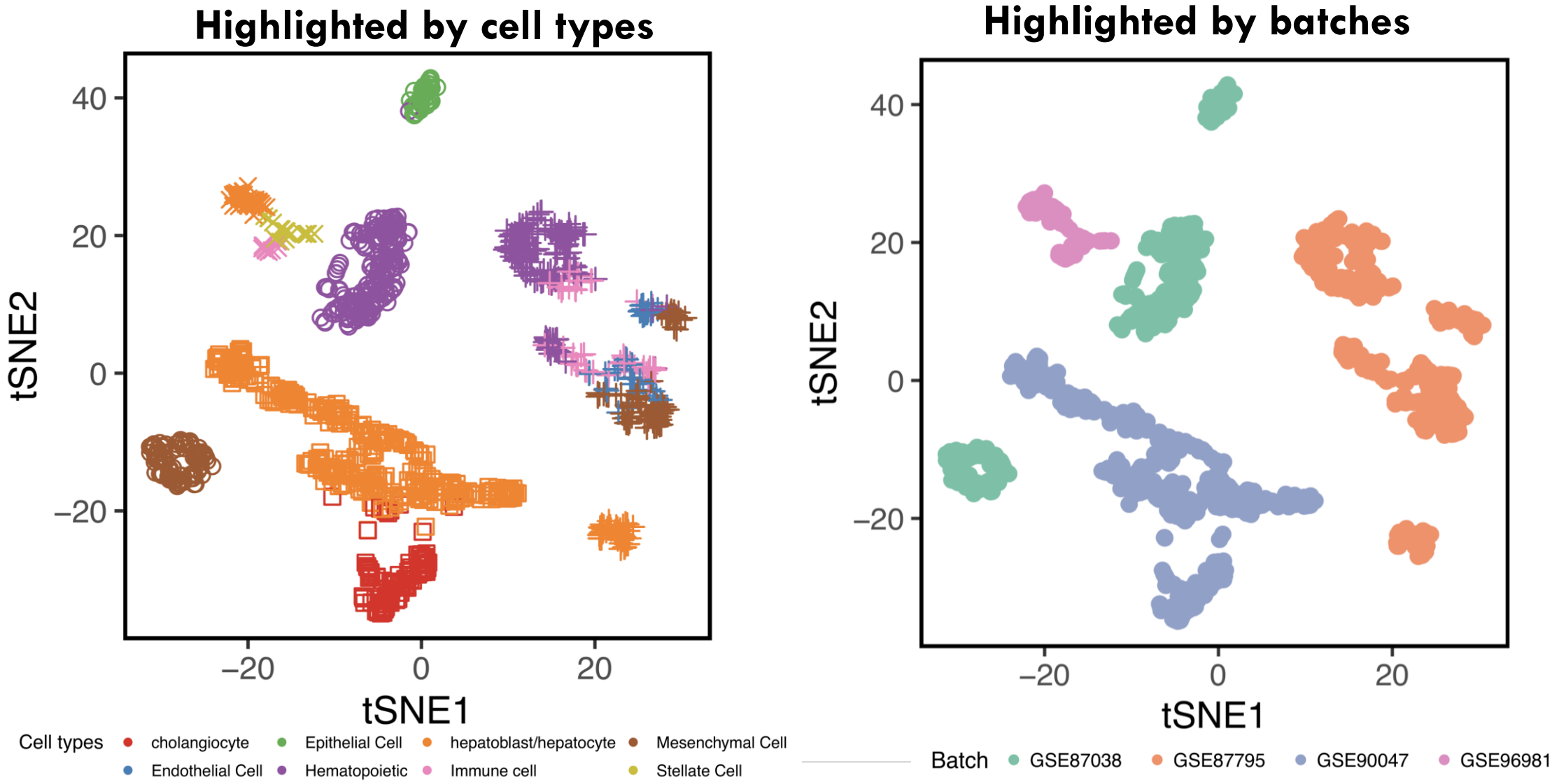
Liver fetal development time course datasets



tSNE of liver fetal development time course datasets



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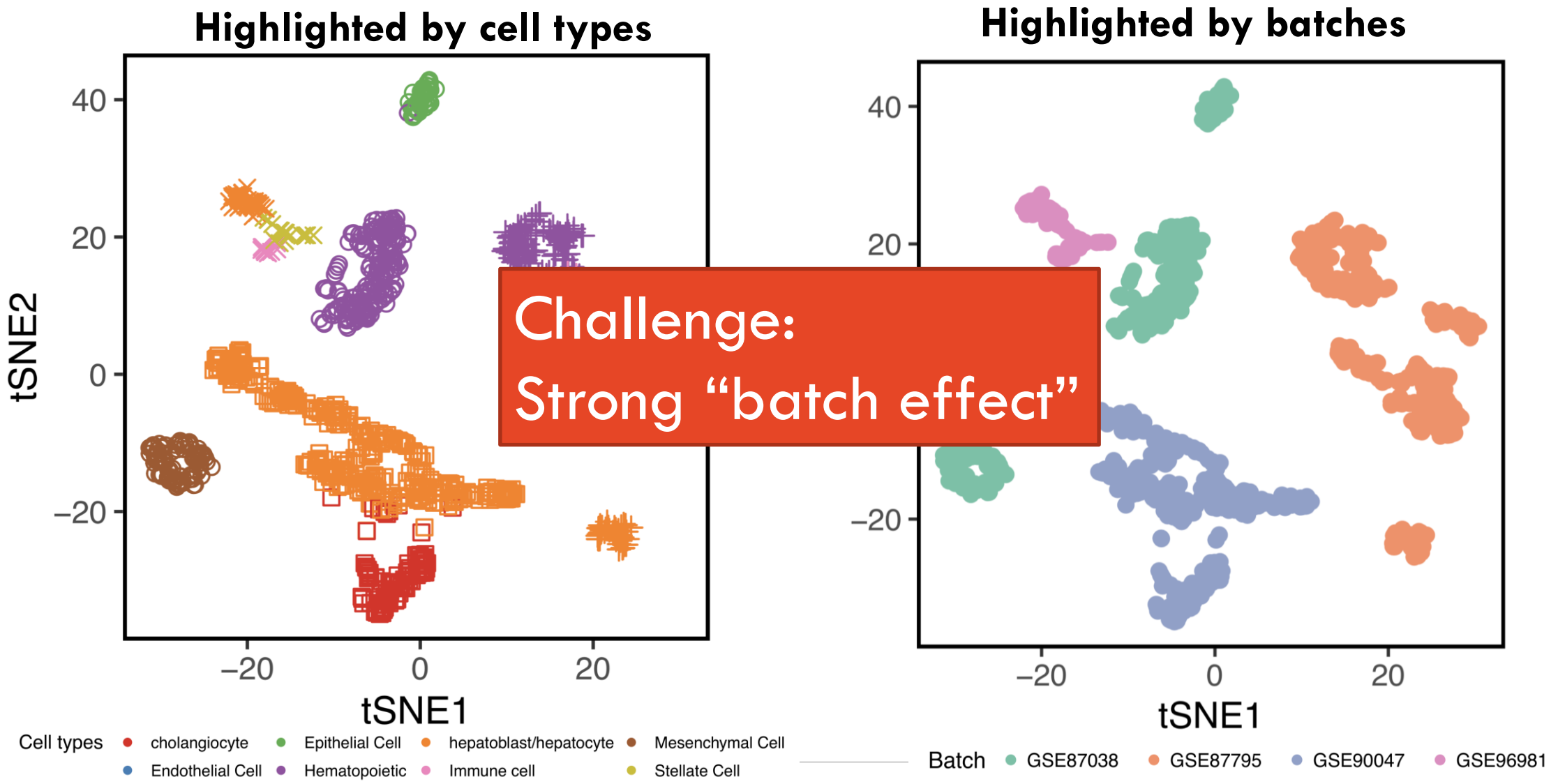
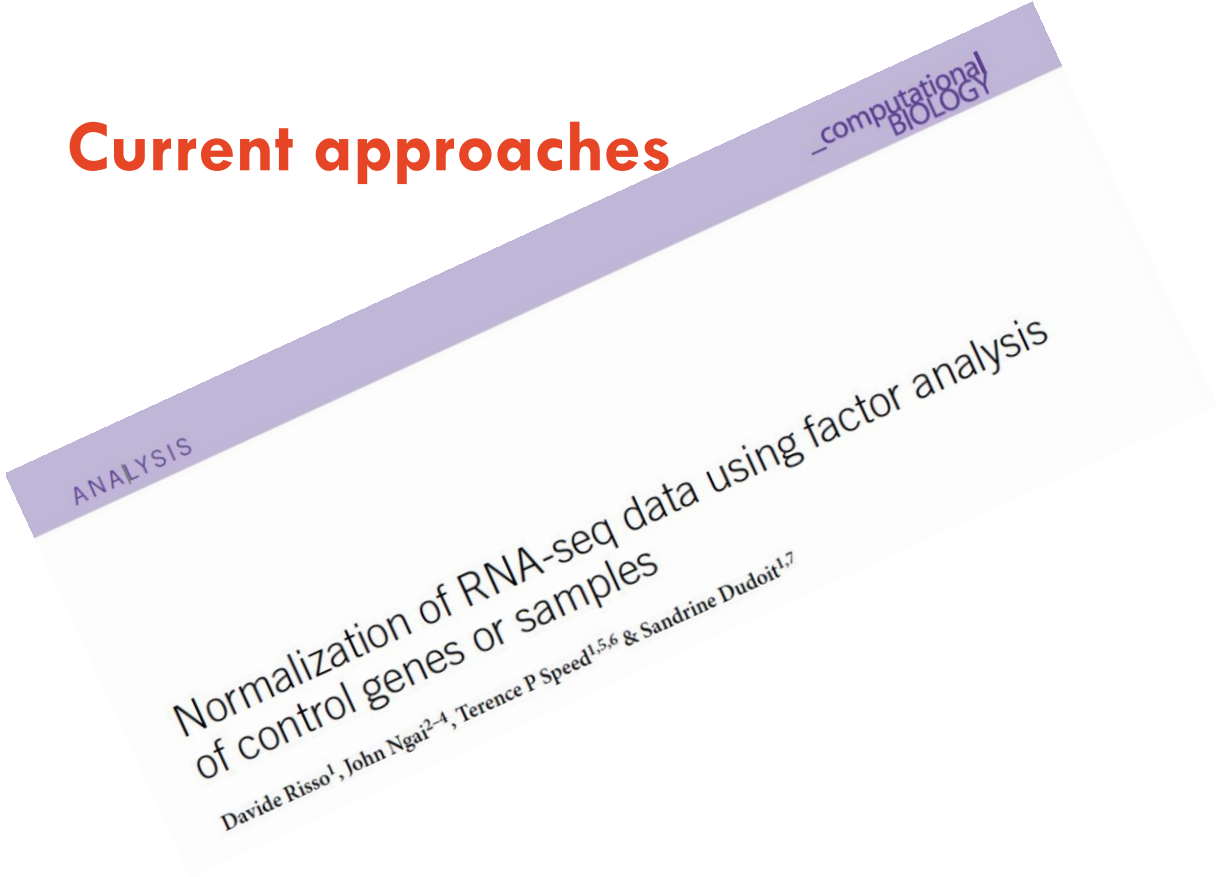


Current approaches

Current approaches



Current approaches

ANALYSIS

_computational BIOLOGY

Normalization of RNA-seq data using factor analysis of control genes or samples

Davide Risso[1], John Ngai[2–4], Terence P Speed[1,5,6] & Sandrine Dudoit[1,7]

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Adjusting batch effects in microarray expression data using empirical Bayes methods FREE

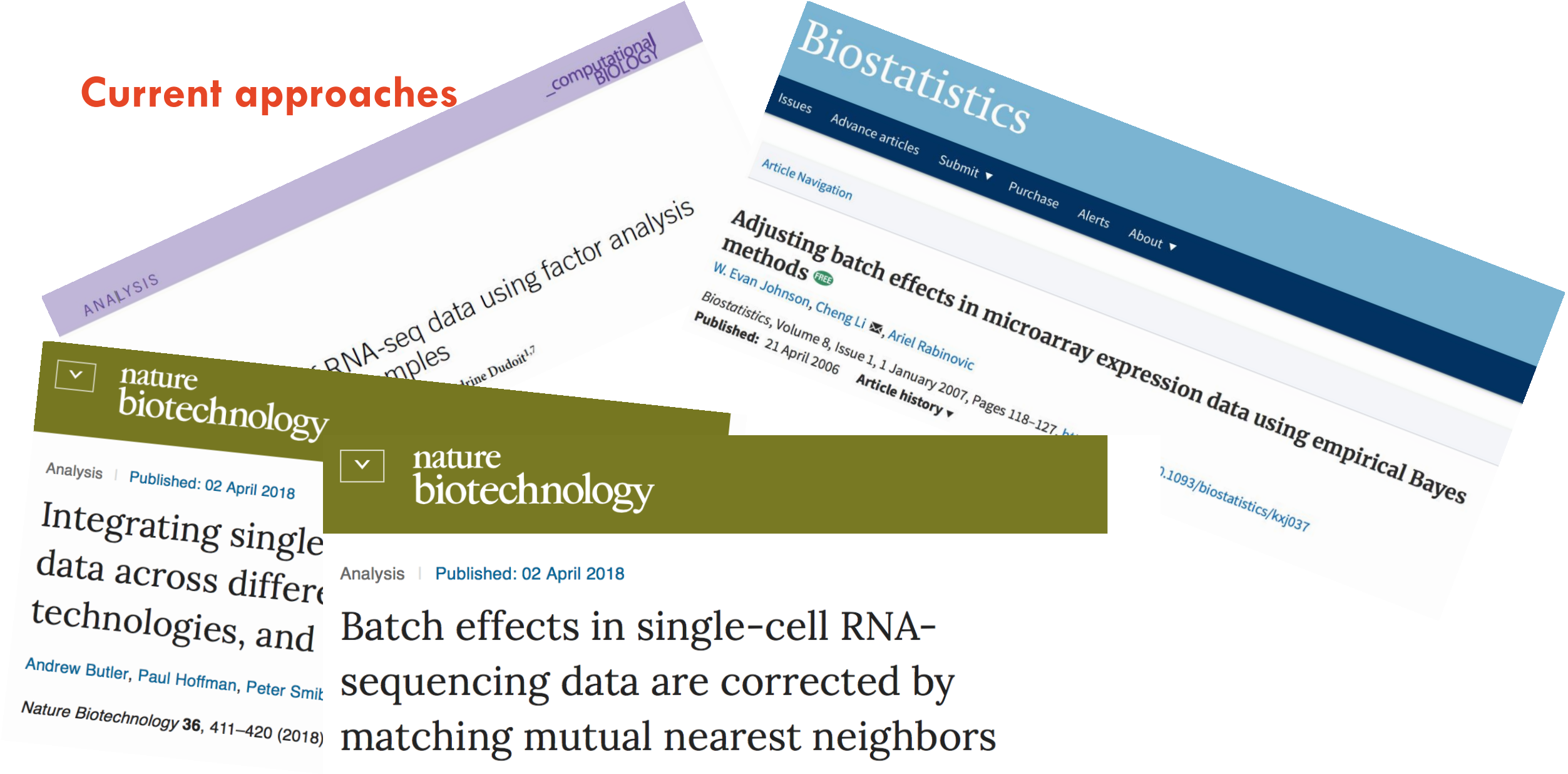
W. Evan Johnson, Cheng Li, Ariel Rabinovic

Biostatistics, Volume 8, Issue 1, 1 January 2007, Pages 118–127, https://doi.org/10.1093/biostatistics/kxj037

Published: 21 April 2006 **Article history**

Current approaches

Current approaches



Current approaches

scMerge

THE UNIVERSITY OF SYDNEY

Breaking observed data into components

For *n* cells with data collected for *m* genes

$$Y_{m\times n} = X_{m\times p}\beta_{p\times n} + W_{m\times k}\alpha_{k\times n} + \epsilon_{m\times n}$$

The data we observe

Biologically relevant variation
cell types
p *wanted* variables

Unwanted variation
batch and technical effects
k *unwanted* variables

Random noise

scMerge: algorithm

New Results

A new normalization for the Nanostring nCounter gene expression assay

Ramyar Molania, Johann A Gagnon-Bartsch, Alexander Dobrovic, Terence P Speed

doi: https://doi.org/10.1101/374173

RUVIII

$$Y_{m\times n} = X_{m\times p}\beta_{p\times n} + W_{m\times k}\alpha_{k\times n} + \epsilon_{m\times n}$$

Estimate unwanted variation

scMerge: algorithm

Negative controls

Genes with stable expression level are not affected by factors of interest i.e.,
Stably expressed across different cell types or developmental stages.

RUVIII

$$Y_{m\times n} = X_{m\times p}\beta_{p\times n} + W_{m\times k}\alpha_{k\times n} + \epsilon_{m\times n}$$

Estimated by negative controls

scMerge: algorithm

Negative controls

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$$Y_{m\times n} = X_{m} \beta_{p\times n} + W_{m\times k}\alpha_{k\times n} + \epsilon_{m\times n}$$

Estimated by negative controls

scMerge: algorithm

Single-cell stably expressed genes (SEG)

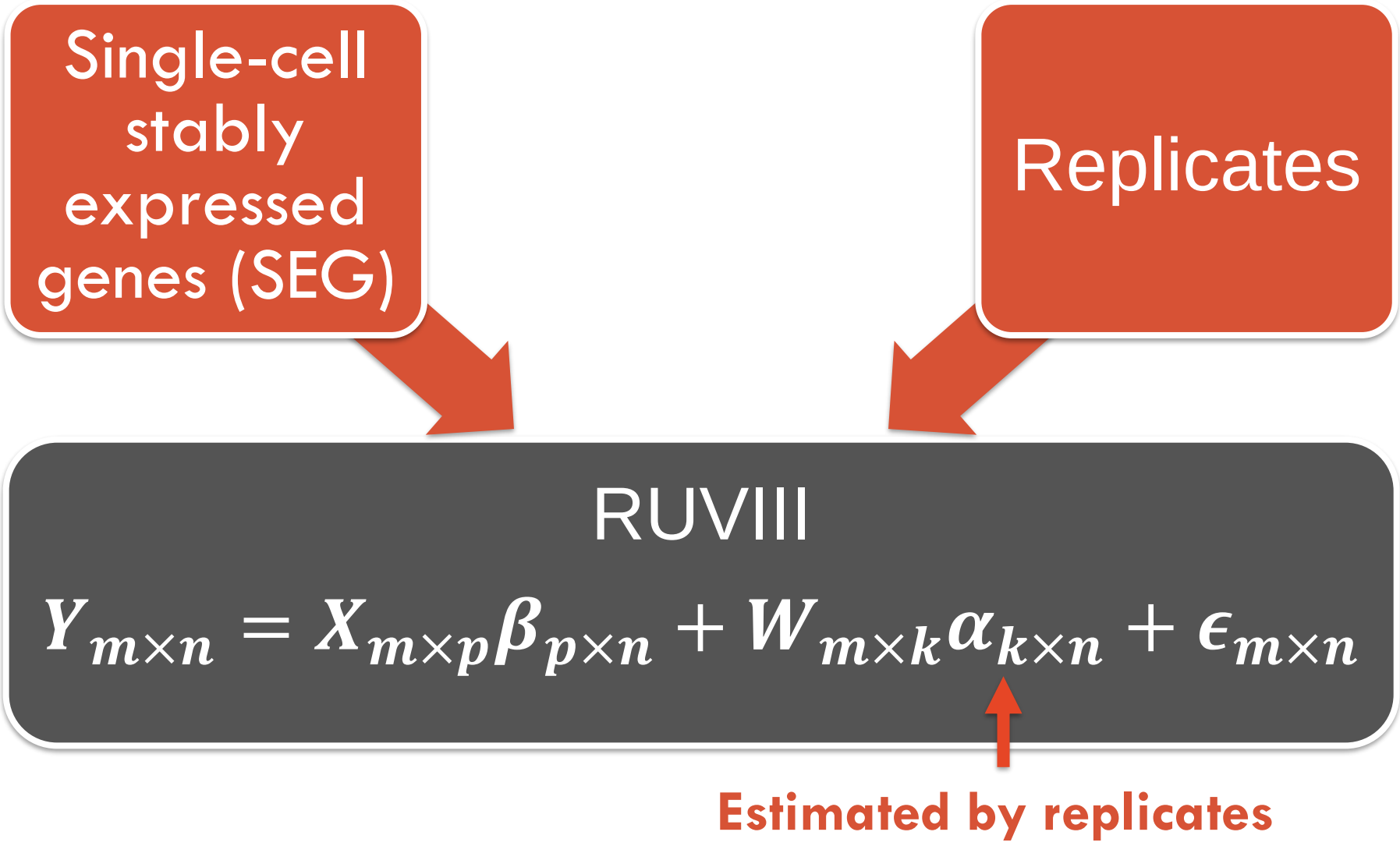
Genes with stable expression level are not affected by factors of interest i.e.,
Stably expressed across different cell types or developmental stages.

RUVIII

$$Y_{m\times n} = X_{m} \beta_{p\times n} + W_{m\times k}\alpha_{k\times n} + \epsilon_{m\times n}$$

Estimated by negative controls

scMerge: algorithm



scMerge: algorithm

Sin
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But cells don't have replicates!

RUVIII

$$Y_{m\times n} = X_{m\times p}\beta_{p\times n} + W_{m\times k}\alpha_{k\times n} + \epsilon_{m\times n}$$

Estimated by replicates

scMerge: algorithm

Sin
s
exp
gen

But cells don't have replicates!

$Y_{m\times$

Estimate pseudo-replicates

Estimated by replicates

scMerge: algorithm

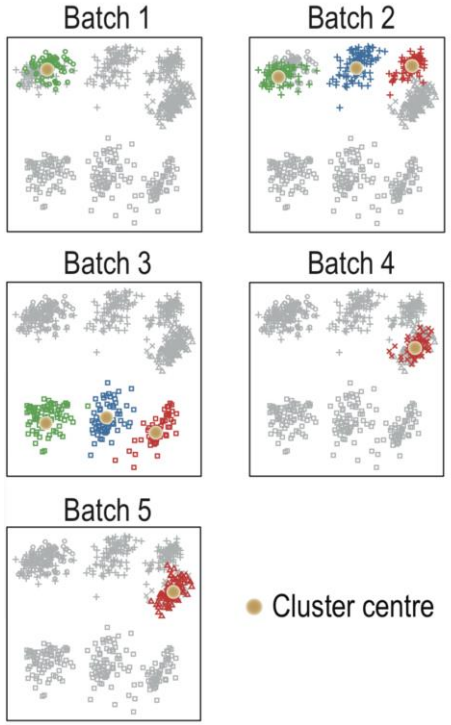
Idea:

- Consider cells from the same cell type as a set of pseudo-replicates
- Match the cell types across different batches

Pseudo-replicates

scMerge: algorithm

Clustering for each batch
(k-means by default)

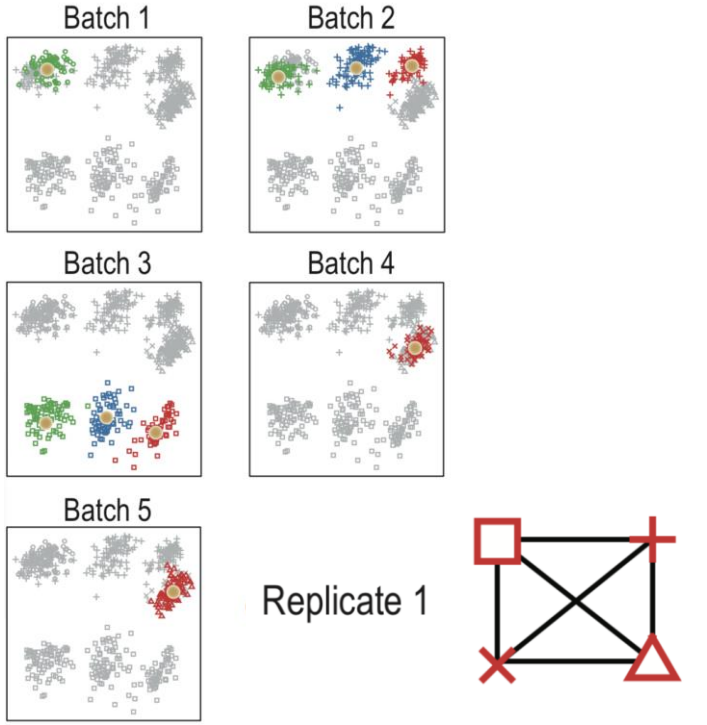


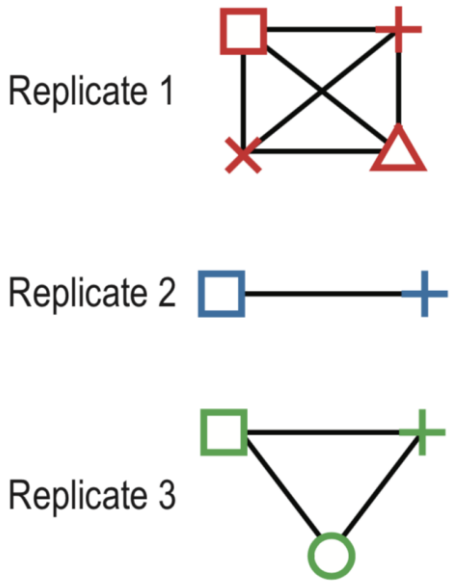
Pseudo-replicates

scMerge: algorithm

Clustering for each batch
(k-means by default)

Find **Mutual Nearest Clusters** as pseudo-replicates





Pseudo-replicates

scMerge: algorithm

Clustering for each batch
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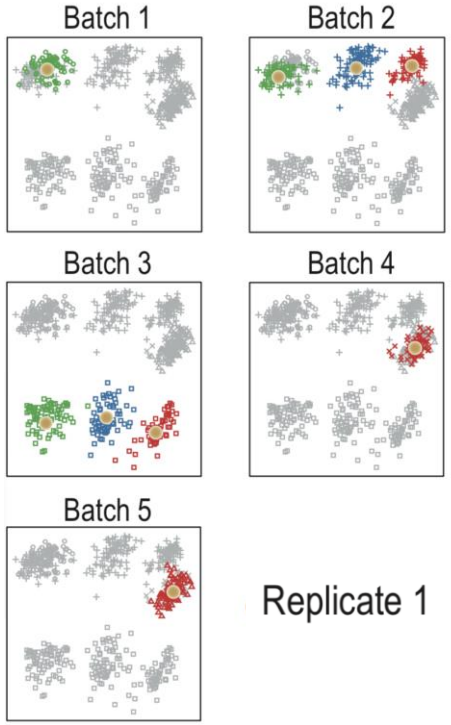

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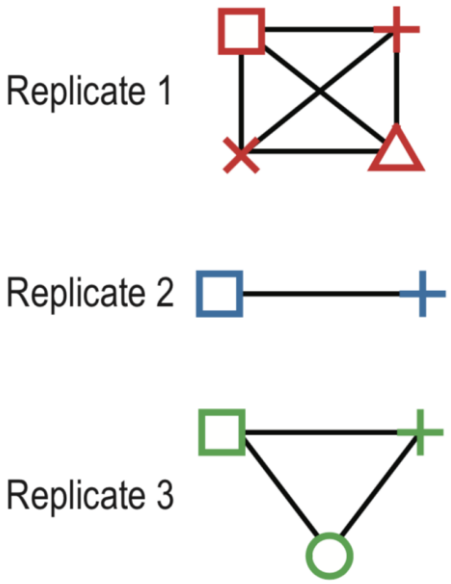
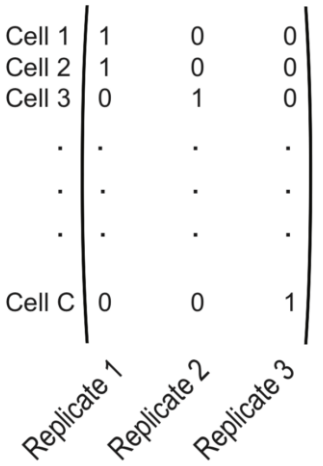
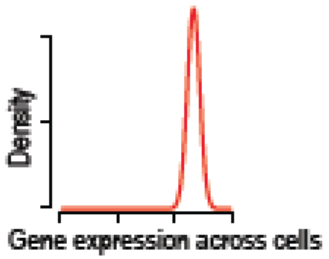


Pseudo-replicates

Frame as pseudo-replicate information

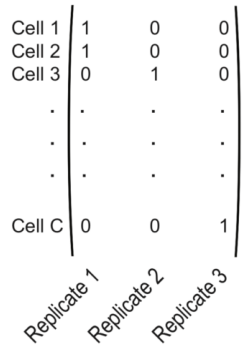


scMerge: algorithm



Single-cell stably expressed genes (SEG)

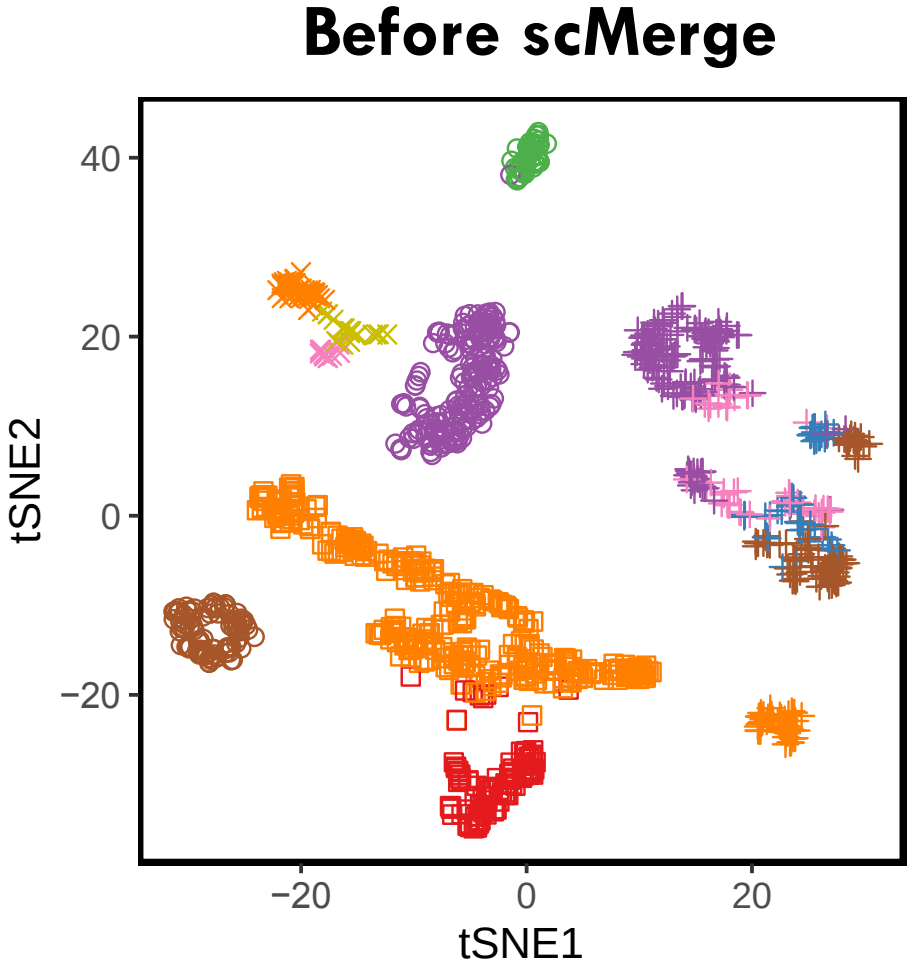
Pseudo-replicates

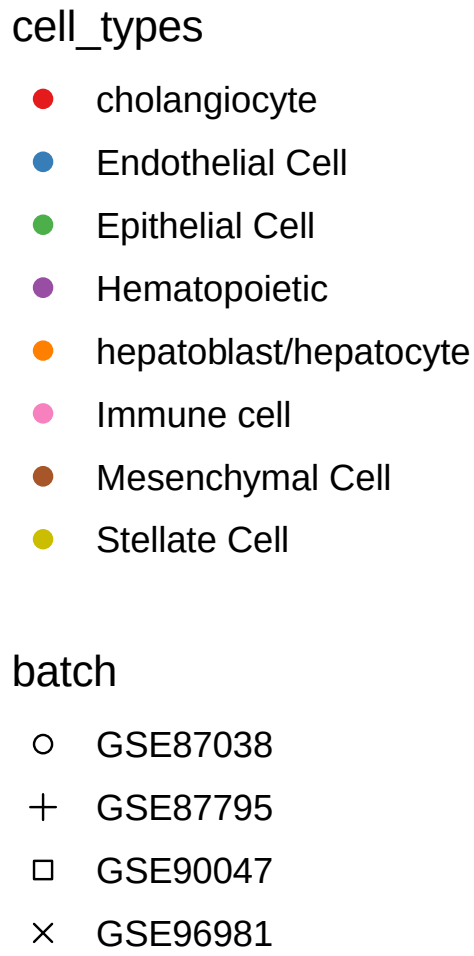


RUVIII

$$Y_{m\times n} = X_{m\times p}\beta_{p\times n} + W_{m\times k}\alpha_{k\times n} + \epsilon_{m\times n}$$

Coming back to our motivational data –
Liver fetal development time course datasets





Coming back to our motivational data –
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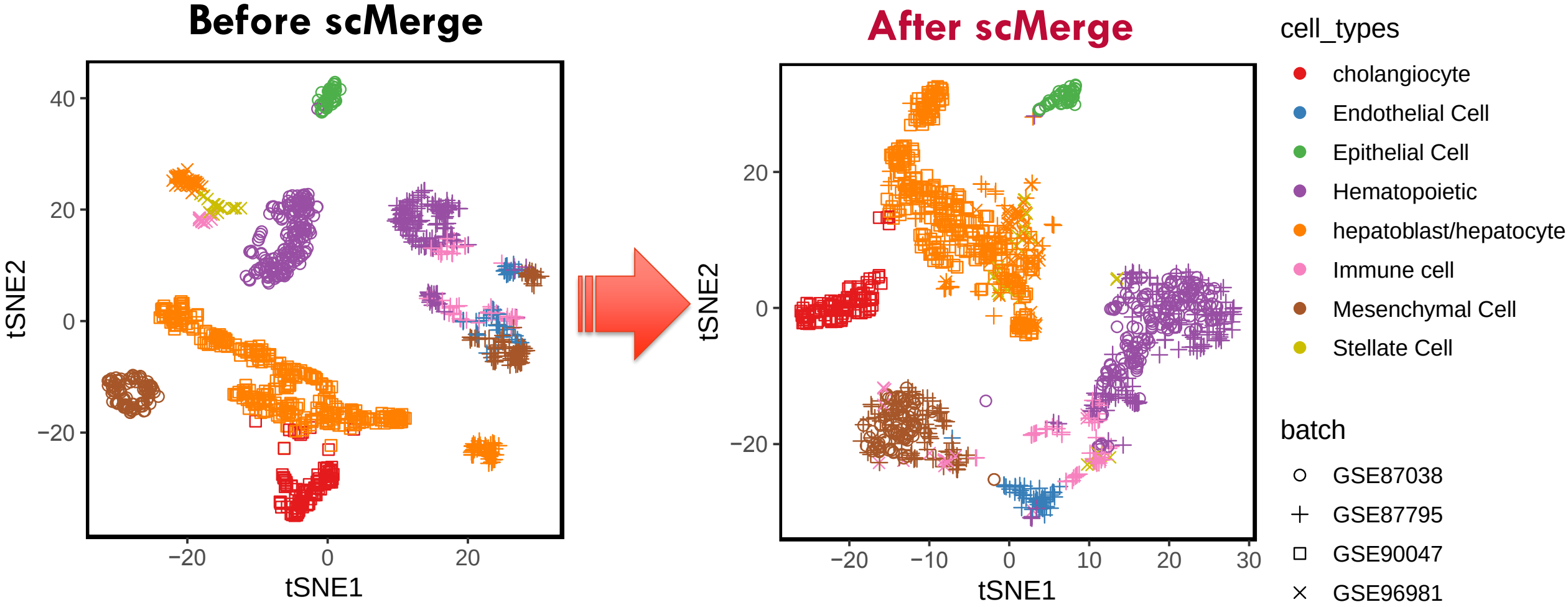
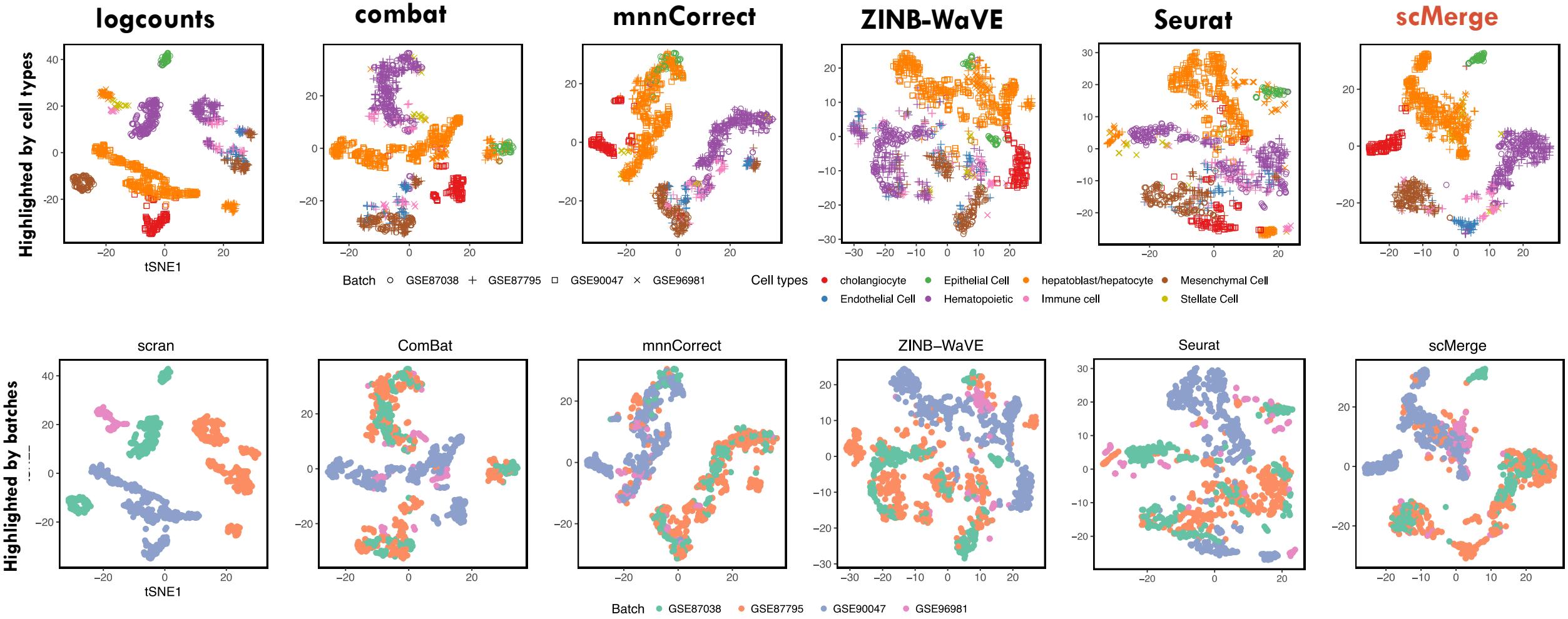


Results: liver datasets tSNE



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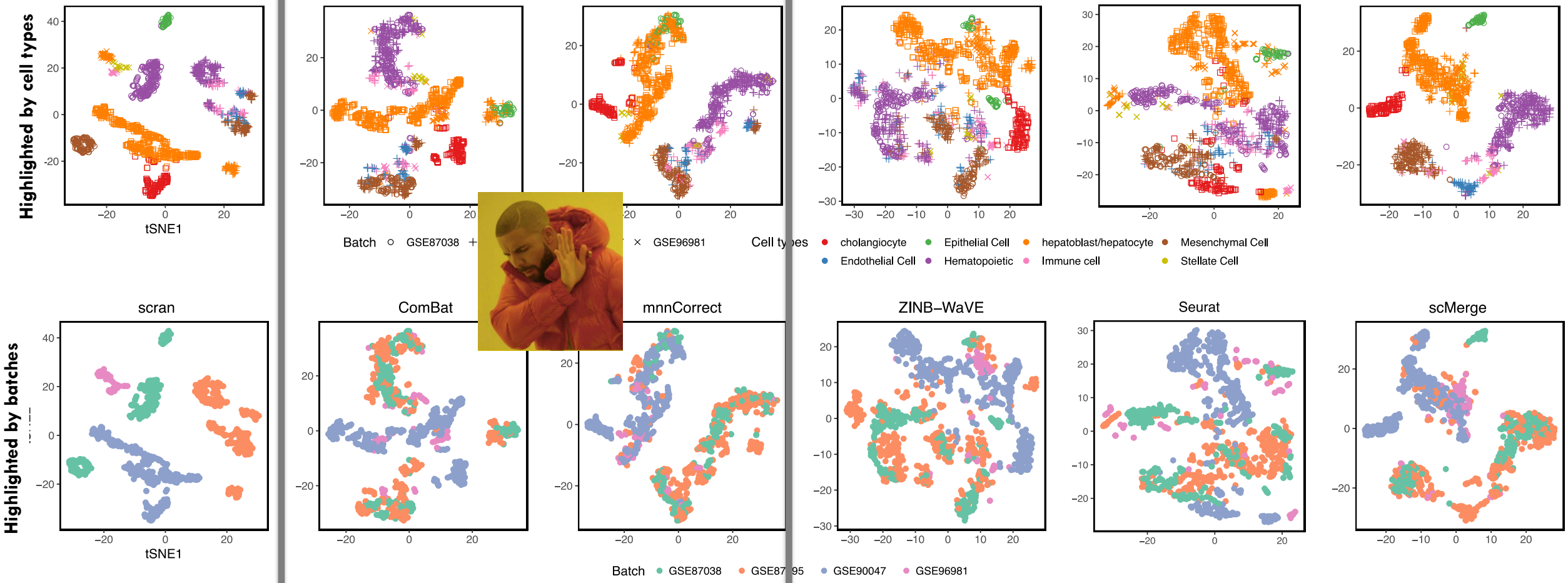

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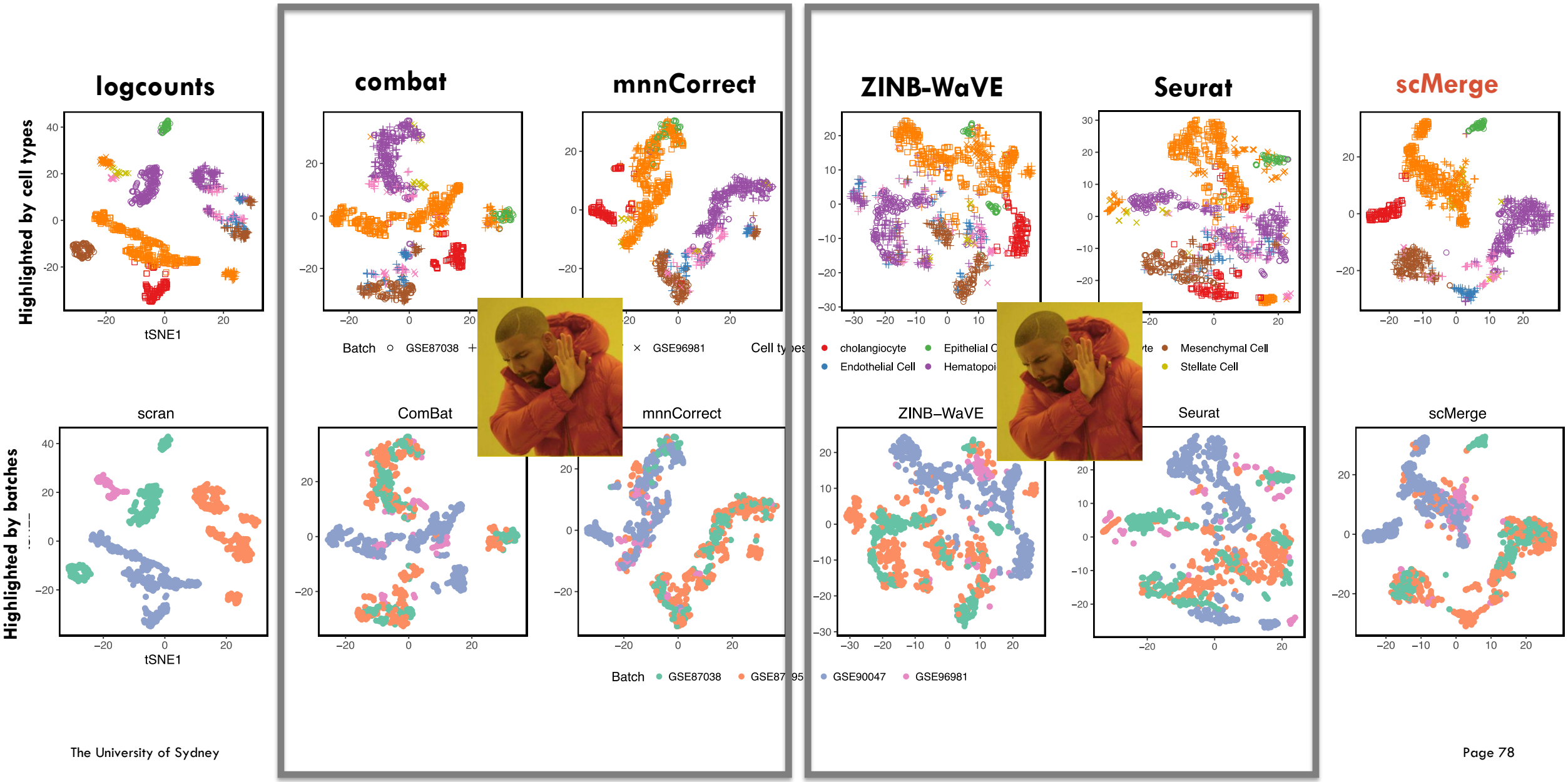

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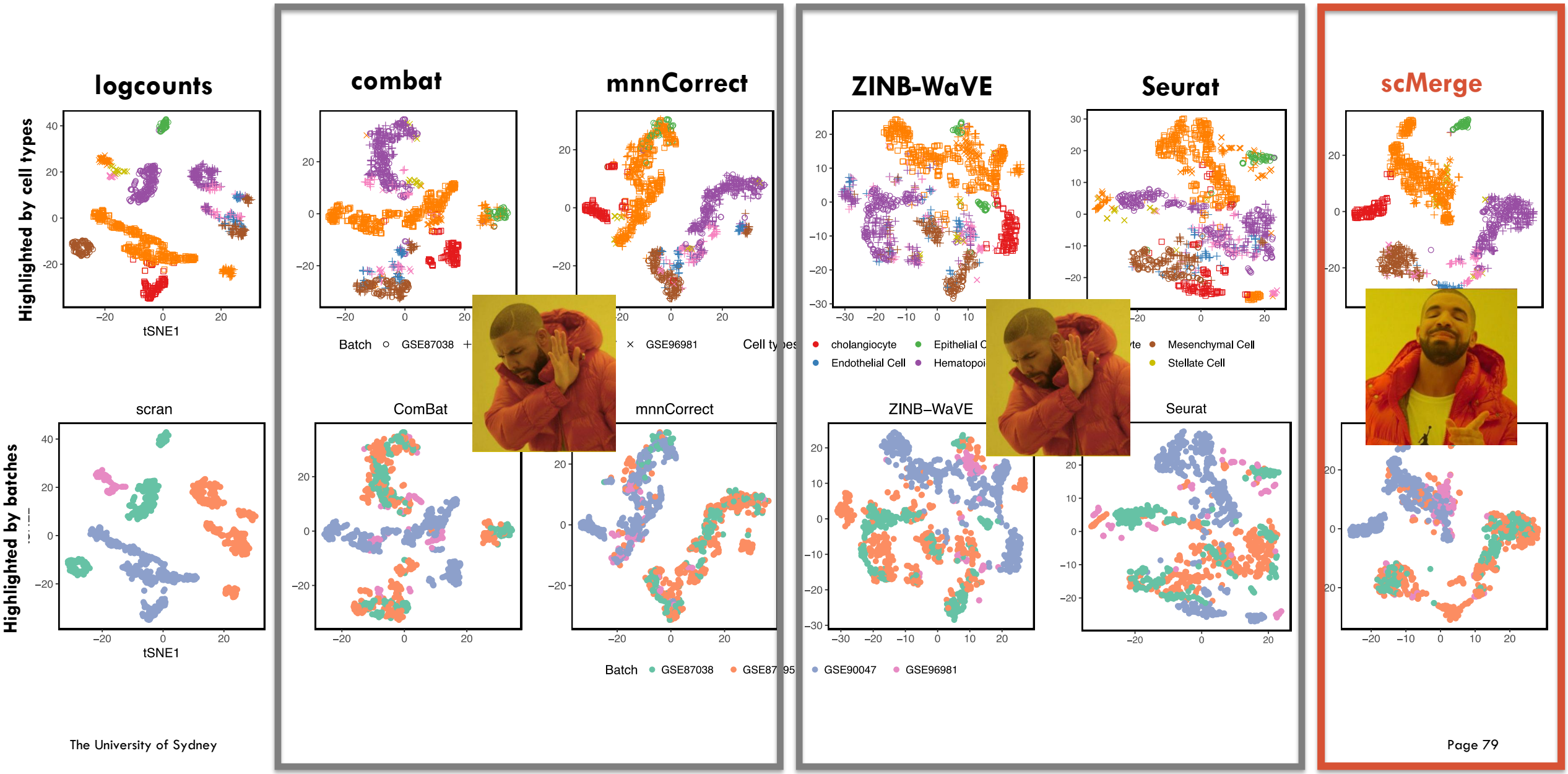
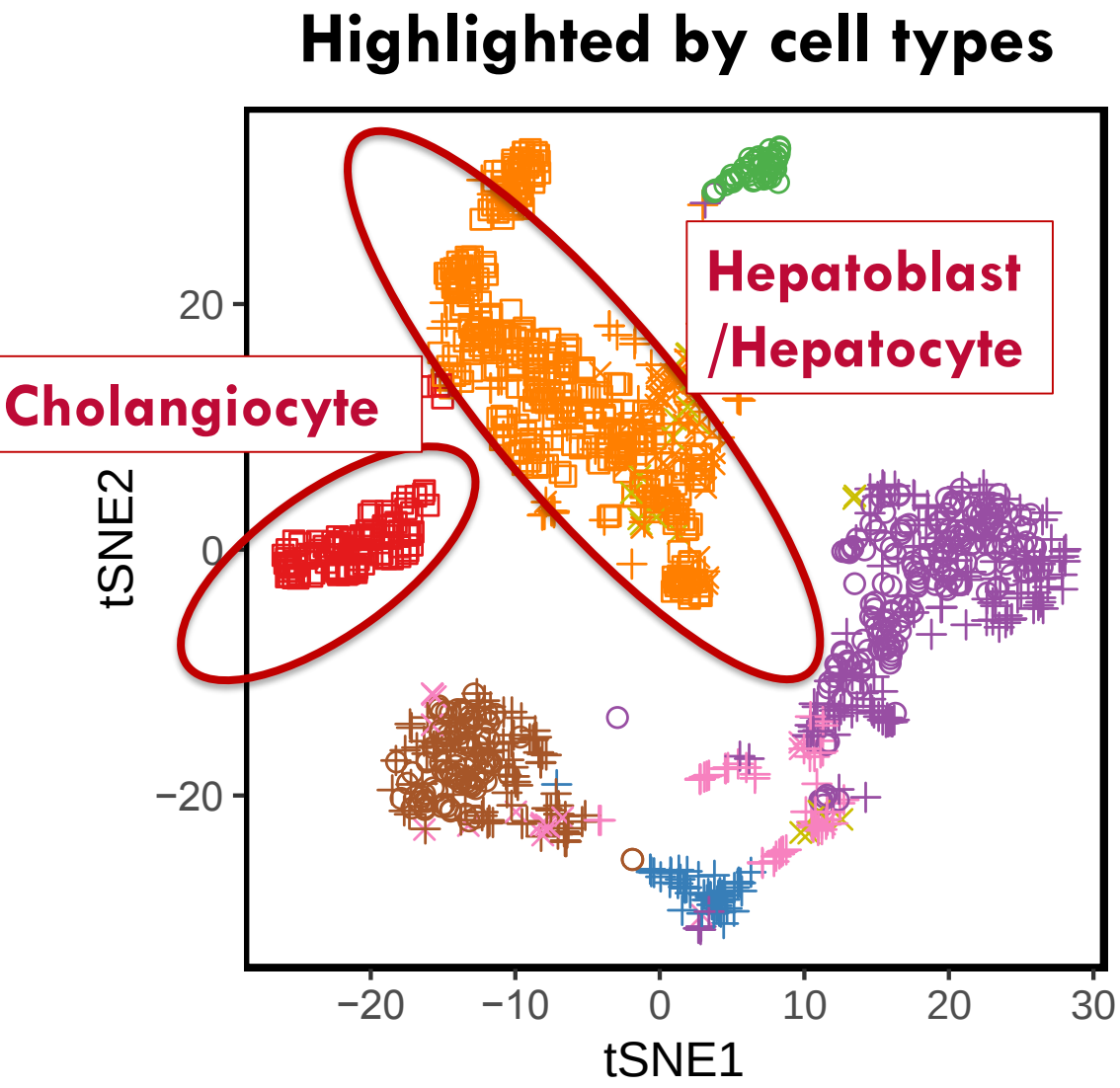
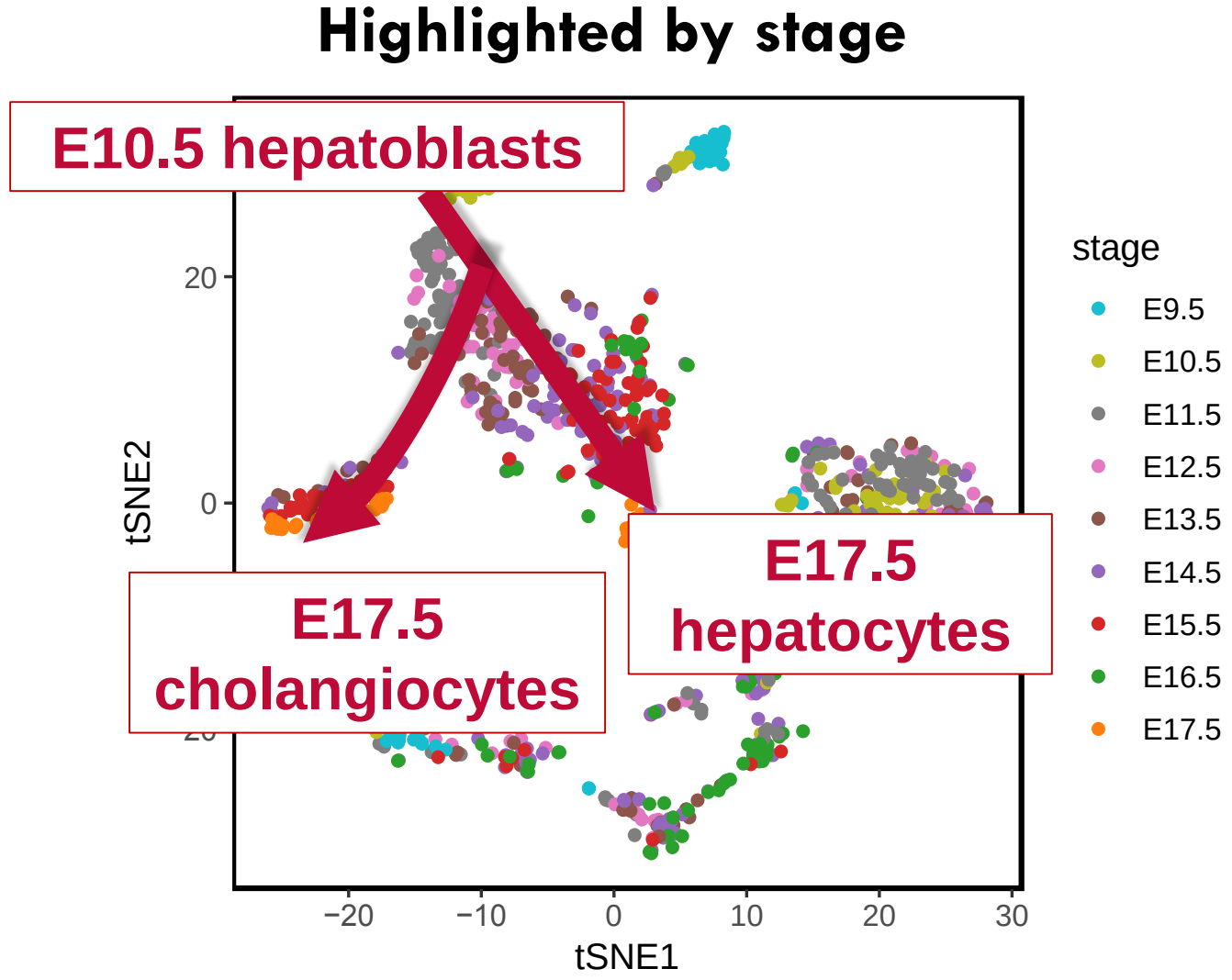


Results: liver datasets – tSNE retains rough trajectory





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New Results

scMerge: Integration of multiple single-cell transcriptomics datasets leveraging stable expression and pseudo-replication

Yingxin Lin, Shila Ghazanfar, Kevin Wang, Johann A. Gagnon-Bartsch, Kitty K. Lo, Xianbin Su, Ze-Guang Han, John T. Ormerod, Terence P. Speed, Pengyi Yang, Jean Y. H. Yang

doi: https://doi.org/10.1101/393280

scMerge R package and website:

https://sydneybiox.github.io/scMerge/

scMerge 0.1.14 | Vignette | Reference | Case Study

scMerge

`scMerge` is a R package for merging and normalising single-cell RNA-Seq datasets.

Installation

The installation process could take up to 5 minutes, depending if you have some of the packages pre-installed.

```
# Some CRAN packages required by scMerge
install.packages(c("ruv", "rsvd", "igraph", "pdist", "proxy", "foreach", "doSNOW", "distr", "Rcpp", "RcppEi
devtools::install_github("theislab/kBET")

# Some BioConductor packages required by scMerge
# try http:// if https:// URLs are not supported
source("https://bioconductor.org/biocLite.R")
biocLite(c("SingleCellExperiment", "M3Drop"))


# Installing scMerge and the data files using
devtools::install_github("SydneyBioX/scMerge.data")
devtools::install_github("SydneyBioX/scMerge")
```

Vignette

You can find the vignette at our website: https://sydneybiox.github.io/scMerge/index.html.

More information

Thursday workshop!

bioRxiv:
https://www.biorxiv.org/content/early/2018/09/12/393280

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New Results

scMerge: Integration of multiple single-cell transcriptomics datasets leveraging stable expression and pseudo-replication

Yingxin Lin, Shila Ghazanfar, Kevin Wang, Johann A. Gagnon-Bartsch, Kitty K. Lo, Xianbin Su, Ze-Guang Han, John T. Ormerod, Terence P. Speed, Pengyi Yang, Jean Y. H. Yang

doi: https://doi.org/10.1101/393280

scMerge R package and website:
https://sydneybiox.github.io/scMerge/

scMerge 0.1.14 | Vignette | Reference | Case Study

scMerge

`scMerge` is a R package for merging and normalising single-cell RNA-Seq datasets.

Installation

The installation process could take up to 5 minutes, depending if you have some of the packages pre-installed.

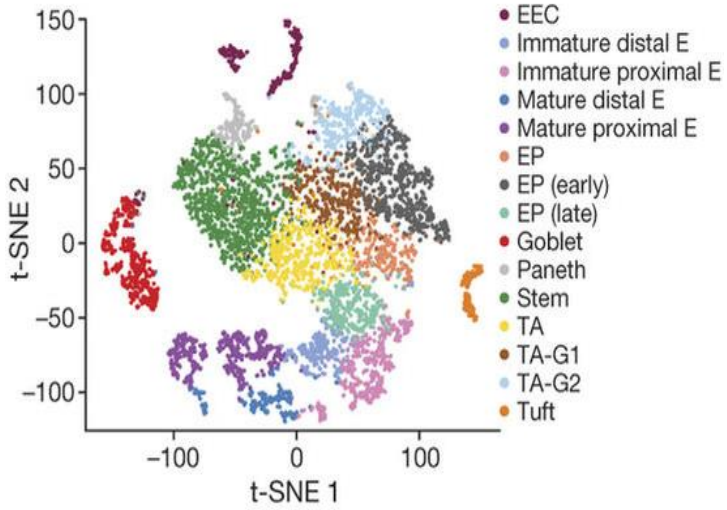
```
# Some CRAN packages required by scMerge
install.packages(c("ruv", "rsvd", "igraph", "pdist", "proxy", "foreach", "doSNOW", "distr", "Rcpp", "RcppEi
devtools::install_github("theislab/kBET")

# Some BioConductor packages required by scMerge
# try http:// if https:// URLs are not supported
source("https://bioconductor.org/biocLite.R")
biocLite(c("SingleCellExperiment", "M3Drop"))


# Installing scMerge and the data files using
devtools::install_github("SydneyBioX/scMerge.data")
devtools::install_github("SydneyBioX/scMerge")
```

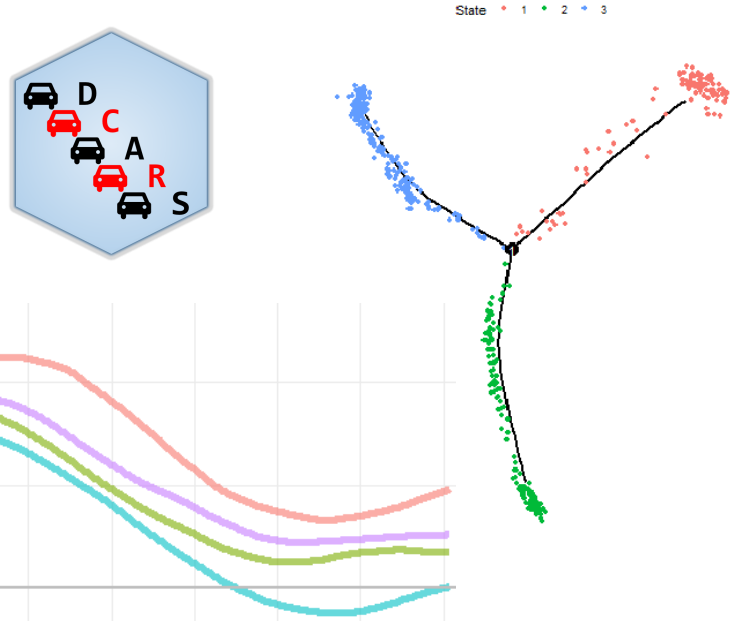
Vignette

You can find the vignette at our website: https://sydneybiox.github.io/scMerge/index.html.



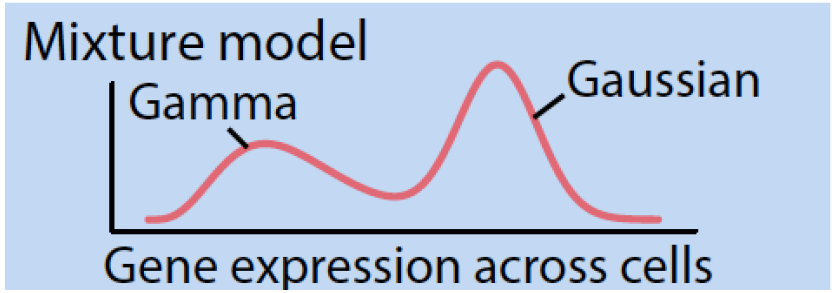
Clustering metrics

Sydney
Single cell

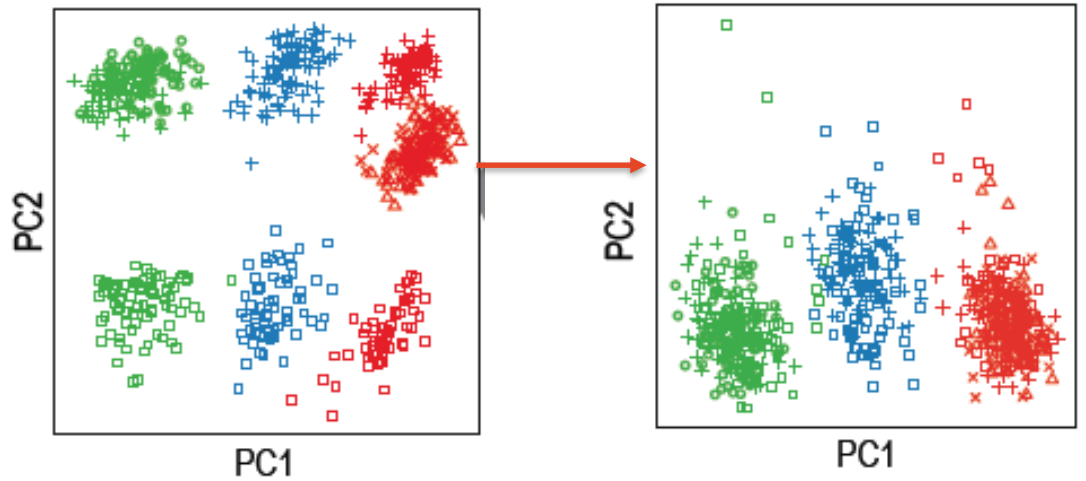


Differential correlation

Finding stably expressed genes



scMerge



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