

# Single Cell Sequencing

## SFRP2/DPP4 and FMO1/LSP1 Define Major Fibroblast Populations in Human Skin

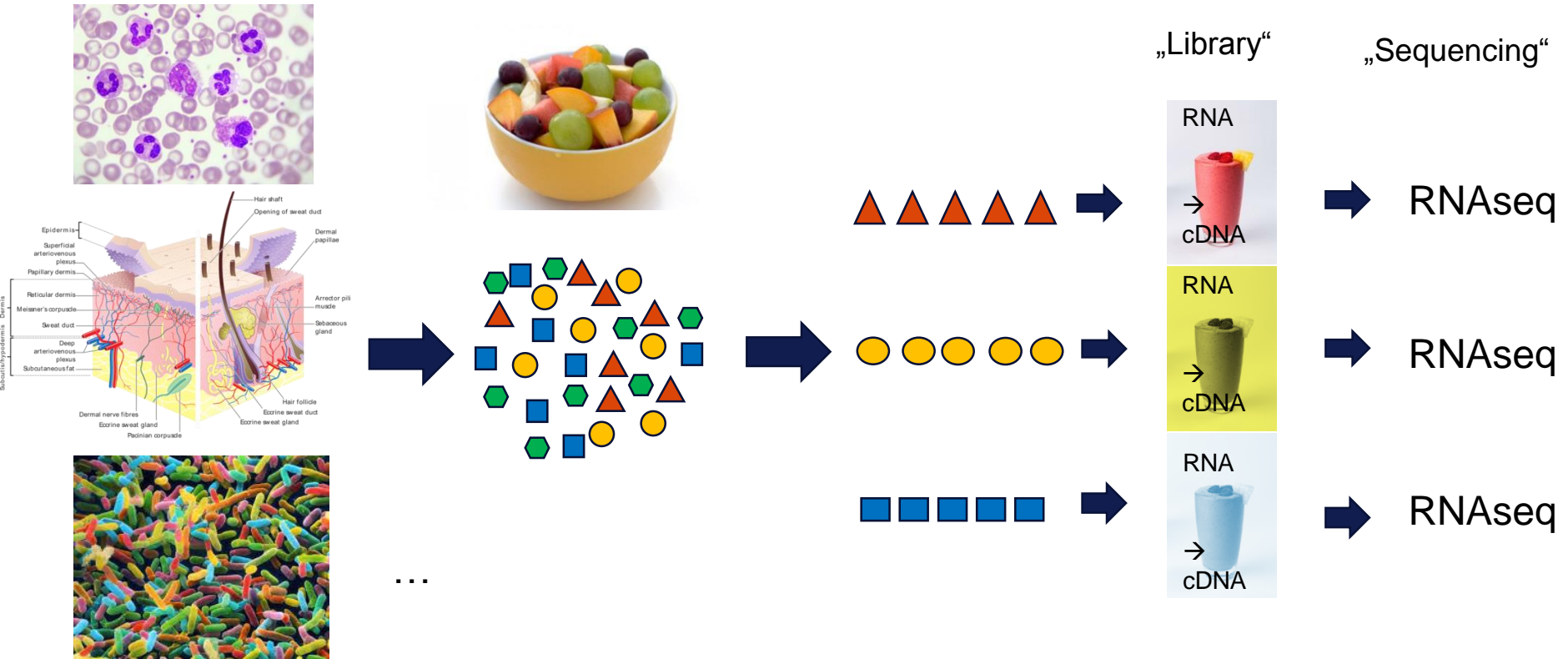
Tracy Tabib, Christina Morse, Ting Wang, Wei Chen and Robert Lafyatis

Journal of Investigative Dermatology (2018) 138, 802e810; [oi:10.1016/j.jid.2017.09.045](https://doi.org/10.1016/j.jid.2017.09.045)

Vera Vorstandlechner

22.10.2018

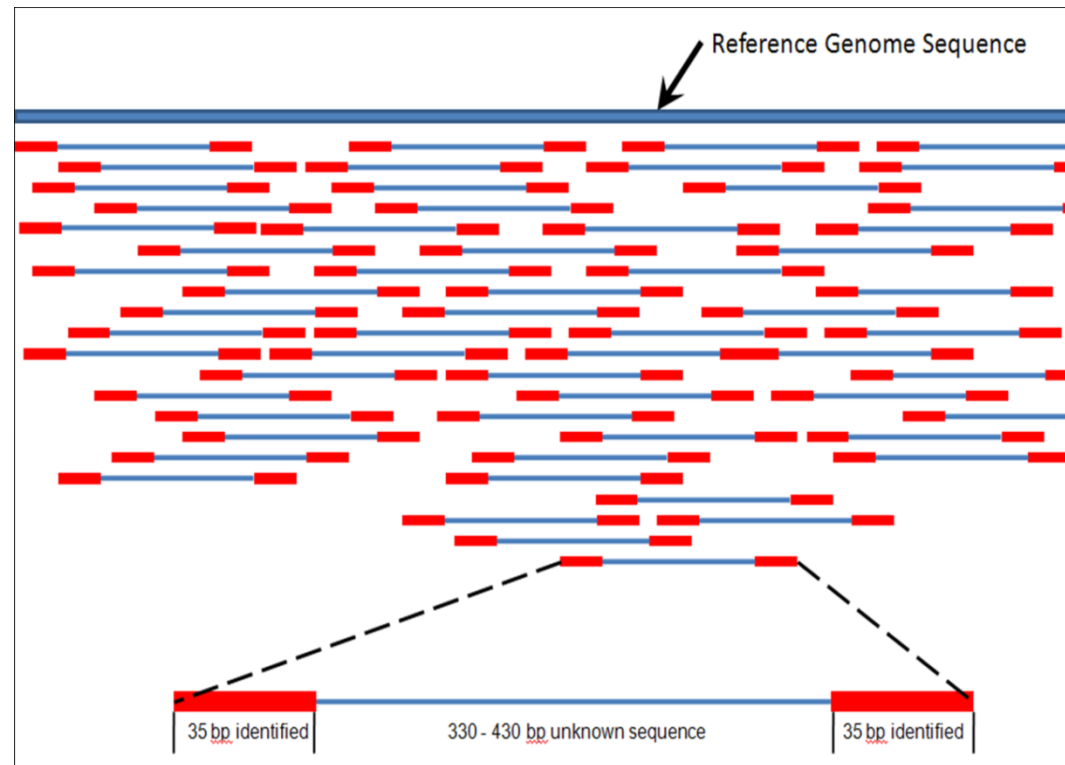
# RNAseq



- „**Transcriptome**“ = total amount of all mRNA present in a cell, = all genes transcribed at the moment
- cDNA = DNA processed from RNA using reverse transcriptase
- RNAseq = sequencing of the transcriptome from cDNA
- ~15.000 genes per sample

# High throughput sequencing

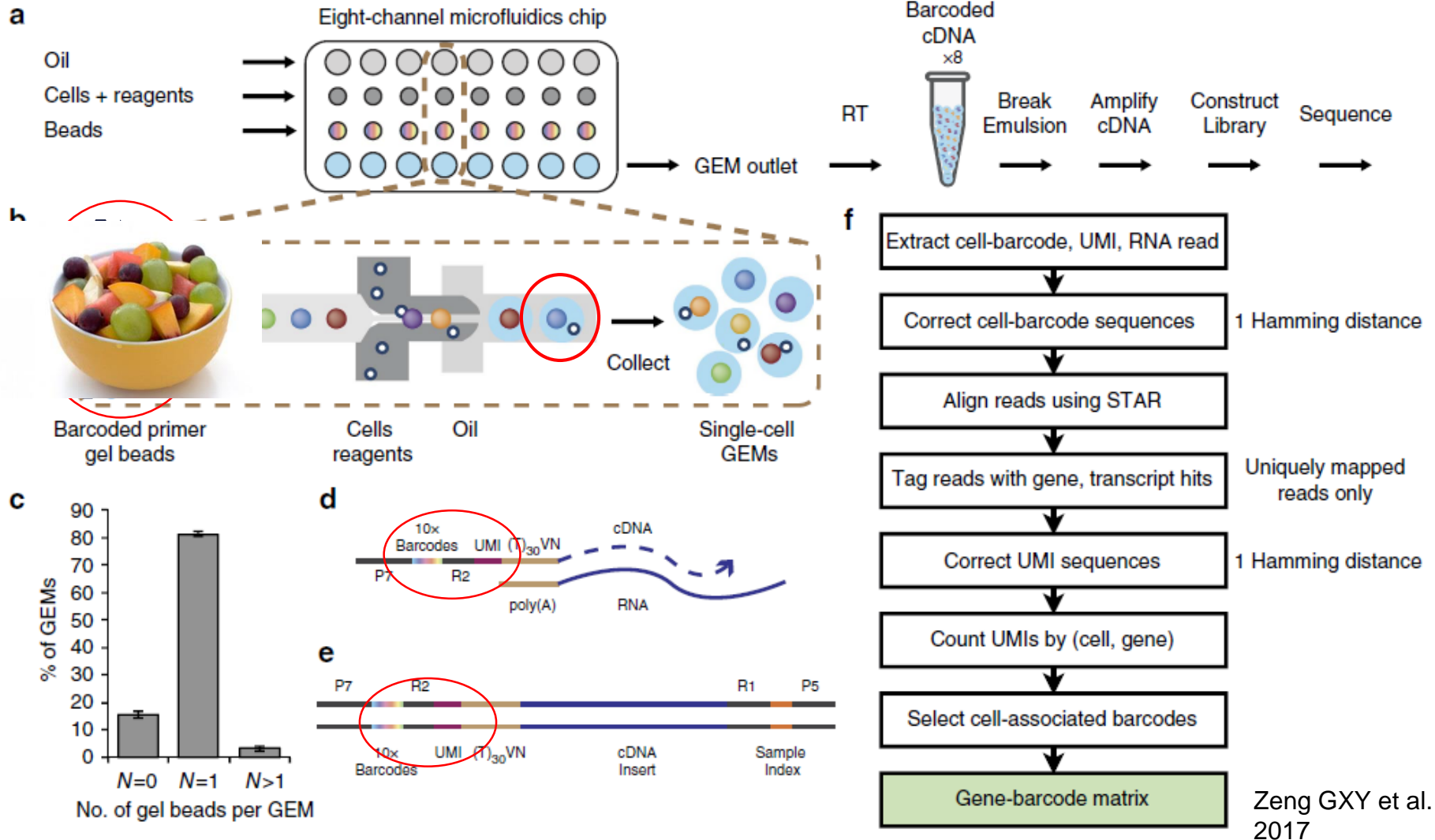
- „Next Generation Sequencing“
- Massively parallel sequencing
- ChIP-Seq
- Sequencing by synthesis (Illumina)
- ...



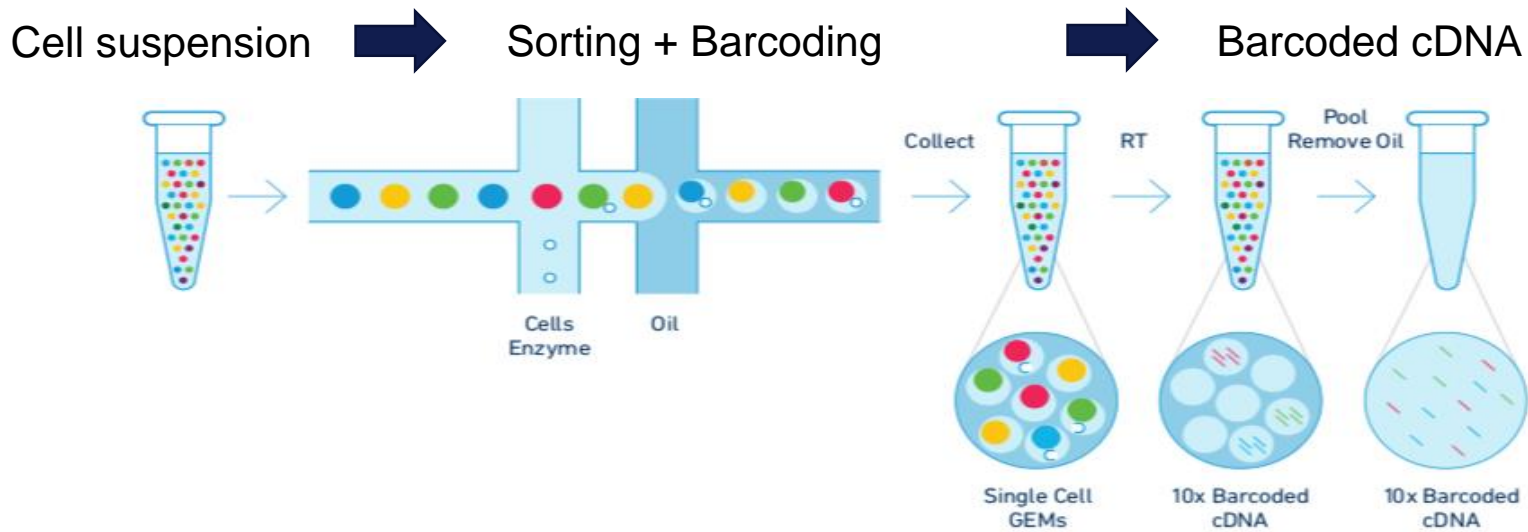
[Wikipedia.org/DNA-sequencing](http://Wikipedia.org/DNA-sequencing)

# Single cell sequencing

- (i) sequencing adapters and primers
- (ii) 14 bp barcode
- (iii) 10 bp randomer to index molecules (unique molecular identifier, UMI)
- (iv) an anchored 30 bp oligo-dT to prime polyadenylated RNA transcripts



# Barcoded Single Cell Gel Beads



- Input: Single cells in suspension + 10x Gel Beads and Reagents
- Output: Digital gene expression profiles from every partitioned cell

Transcriptional profiling of individual cells

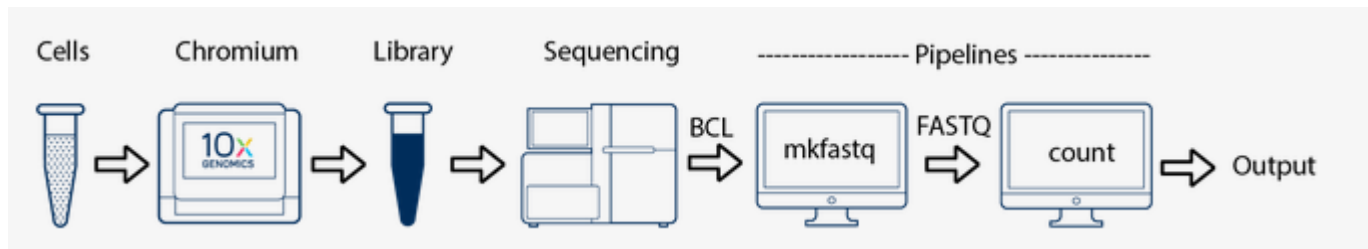


→ max. 10.000/sample → ~15.000 genes /sample

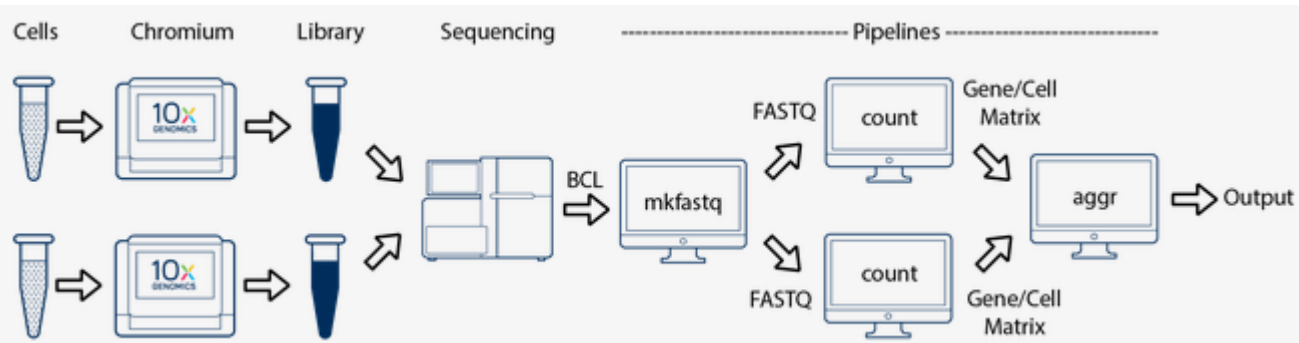
10xgenomics.com

# Data processing

- 10x Genomics: CellRanger pipelines
  - CellRanger mkfastq: demultiplexing of raw data
  - CellRanger count: alignment, filtering, barcode counting, and UMI counting, generate gene-barcode matrices, determine clusters, and perform gene expression analysis
  - CellRanger aggregate: aggregates outputs from several samples



# Data processing



## System Requirements

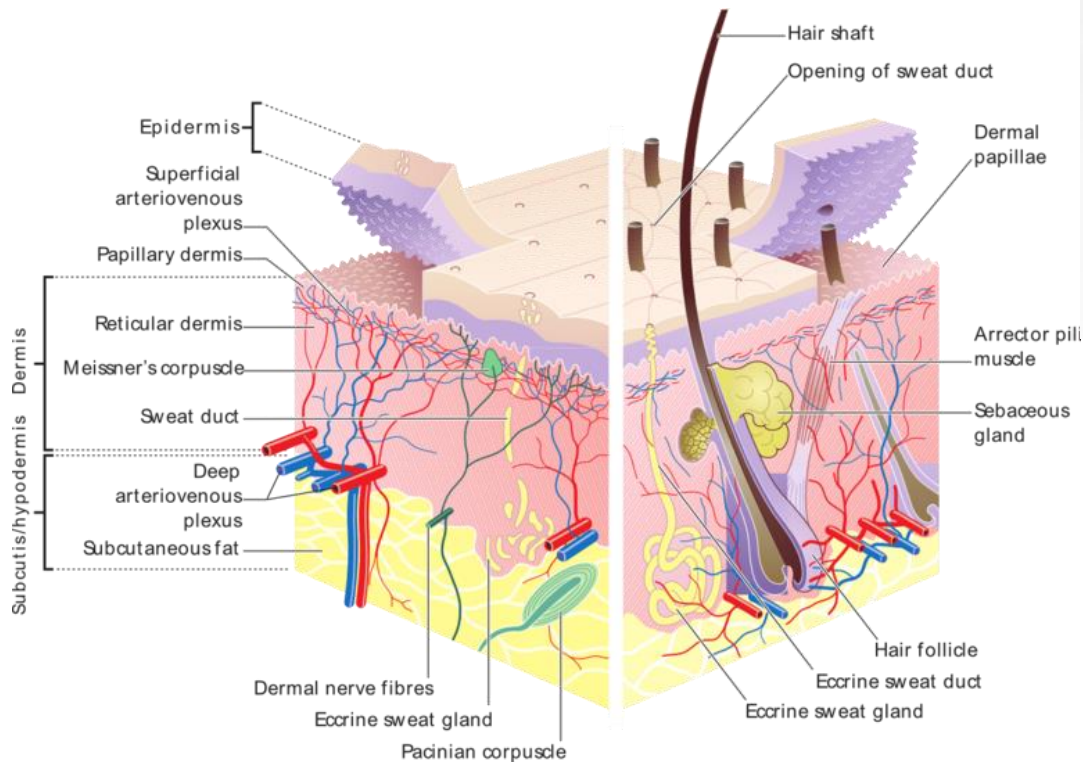
### Cell Ranger

Cell Ranger pipelines run on Linux systems that meet these minimum requirements:

- 8-core Intel or AMD processor (16 cores recommended)
- 64GB RAM (128GB recommended)
- 1TB free disk space
- 64-bit CentOS/RedHat 5.5 or Ubuntu 10.04

- Secondary analysis: R-package „Seurat“, Loupe Cell Browser

# Cells of the skin



[https://pl.wikipedia.org/wiki/Plik:Skin\\_layers.png](https://pl.wikipedia.org/wiki/Plik:Skin_layers.png)

## Epidermis

- Keratinocytes
- Langerhans-Cells
- Melanocytes
- Merkel-Cells

## Dermis

- Fibroblasts
- Endothelial cells
- Mast cells, granulocytes, monocytes, etc.



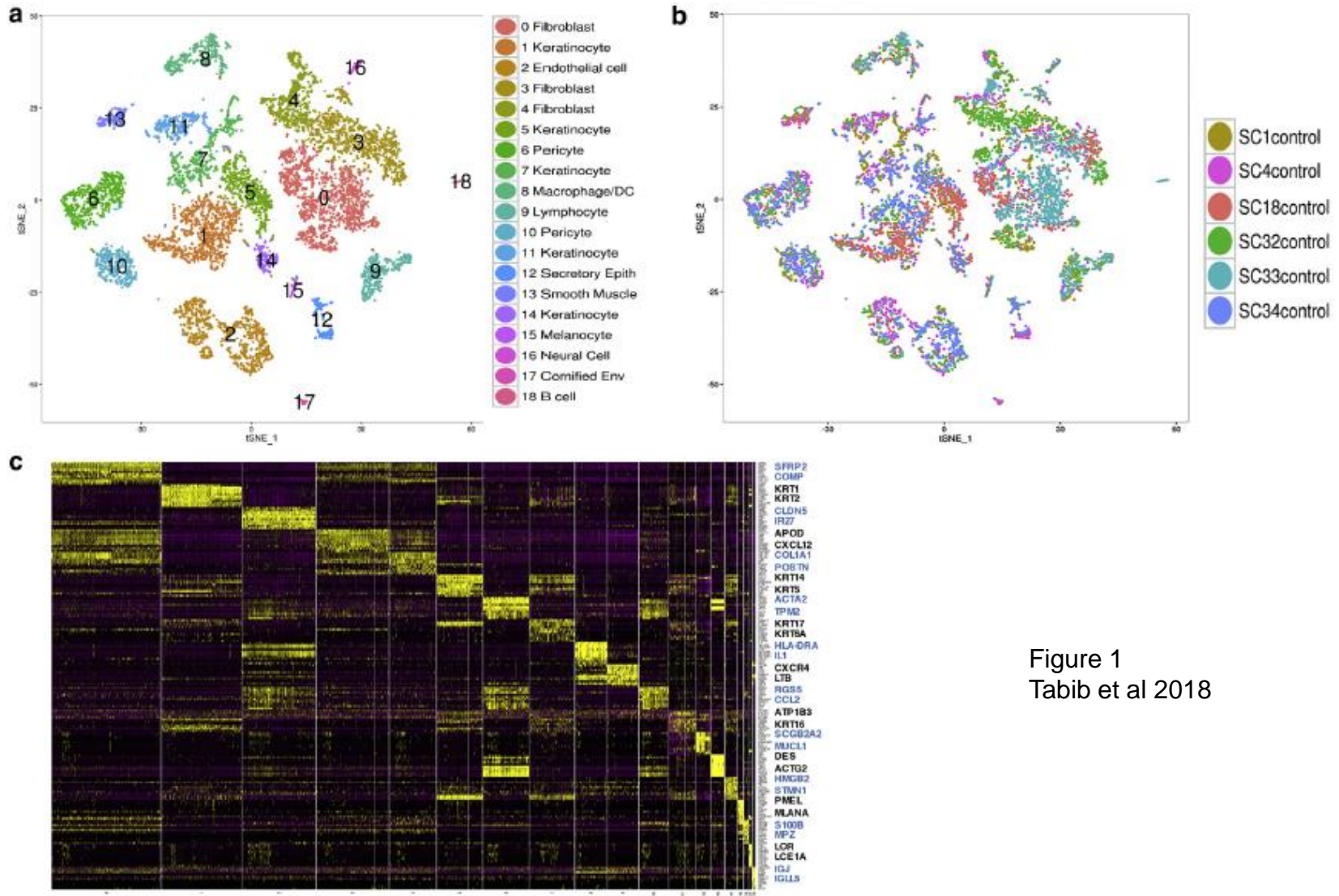


Figure 1  
Tabib et al 2018

# Feature plots of cluster markers

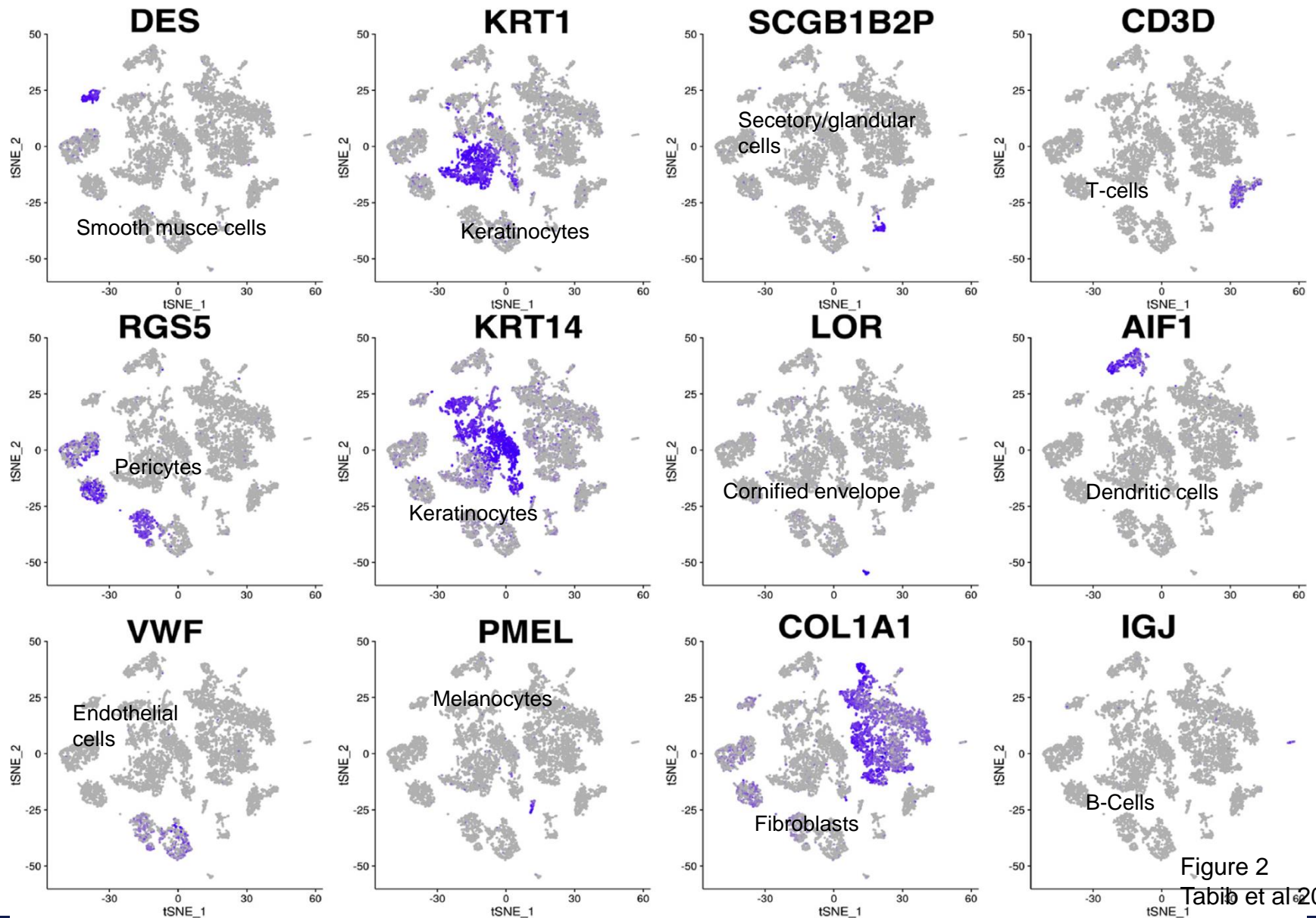


Figure 2  
Tabib et al 2018

# Hierarchical Clustering of fibroblasts

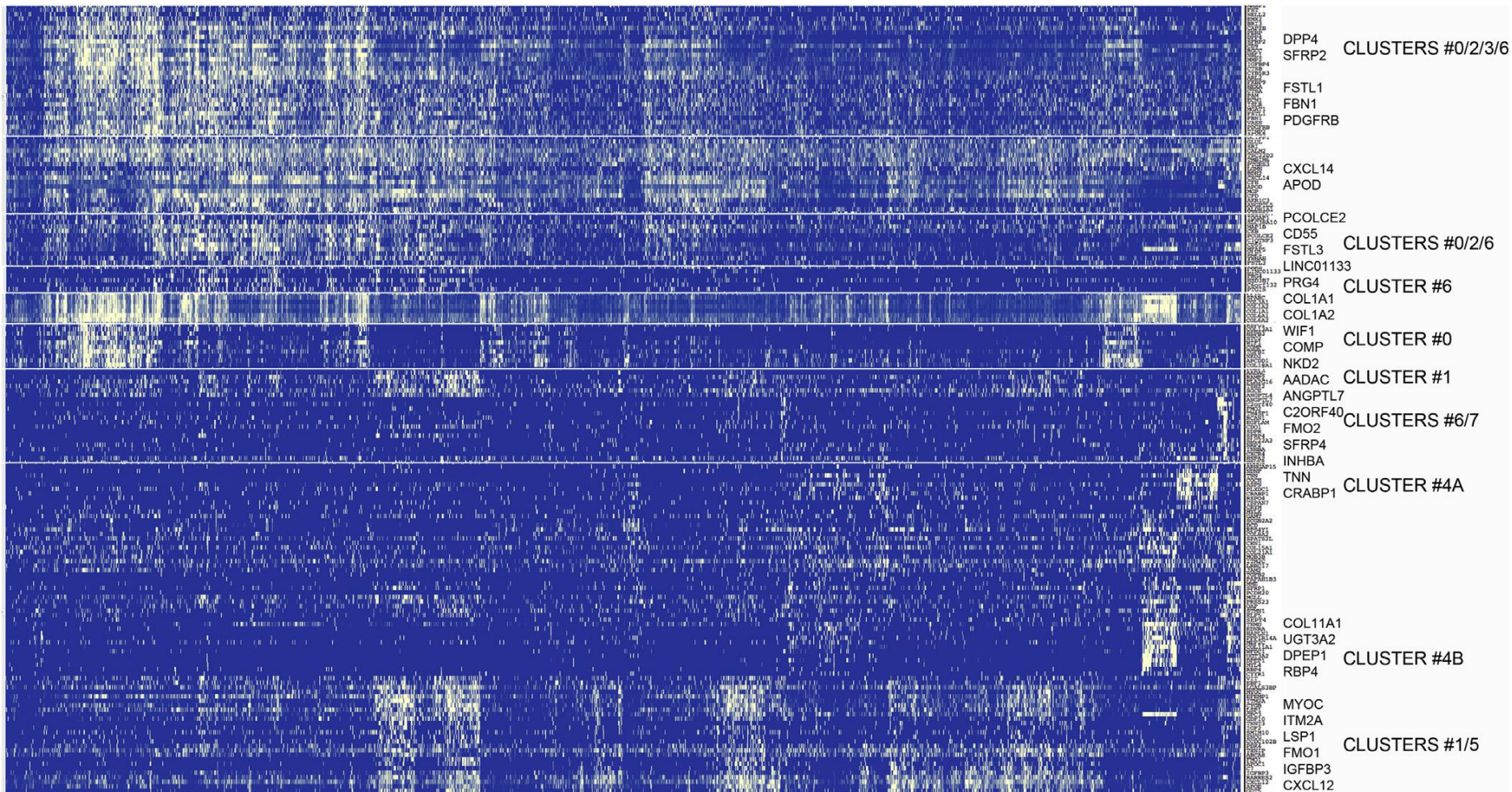
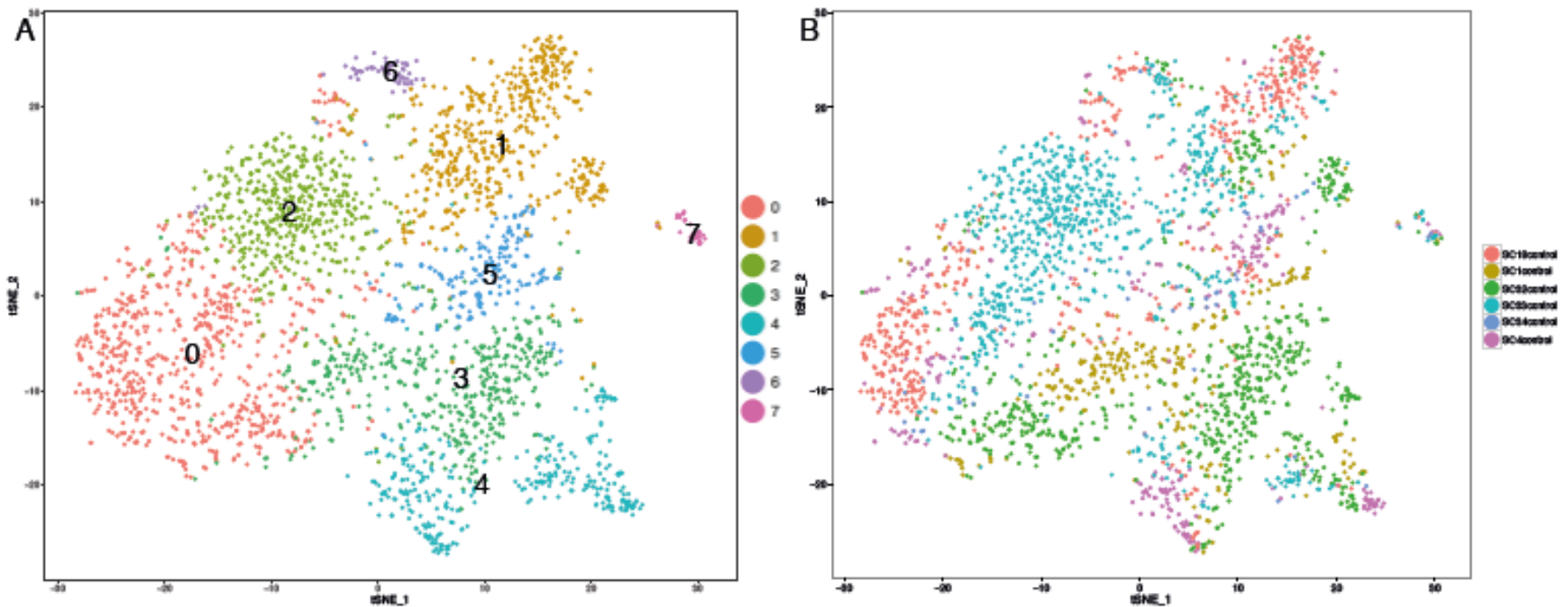


Figure 3  
Tabib et al 2018

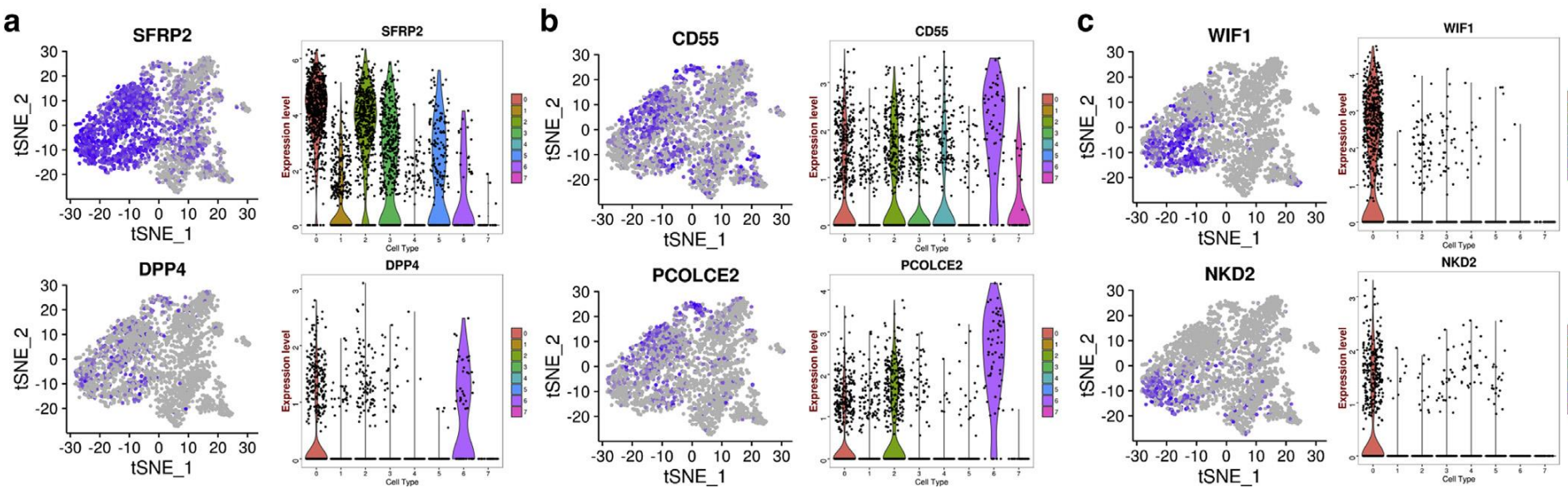
# Re-Running Clustering for fibroblasts only



Fibroblasts = cells expressing Col1A1, Col1A2 & PDGFRA

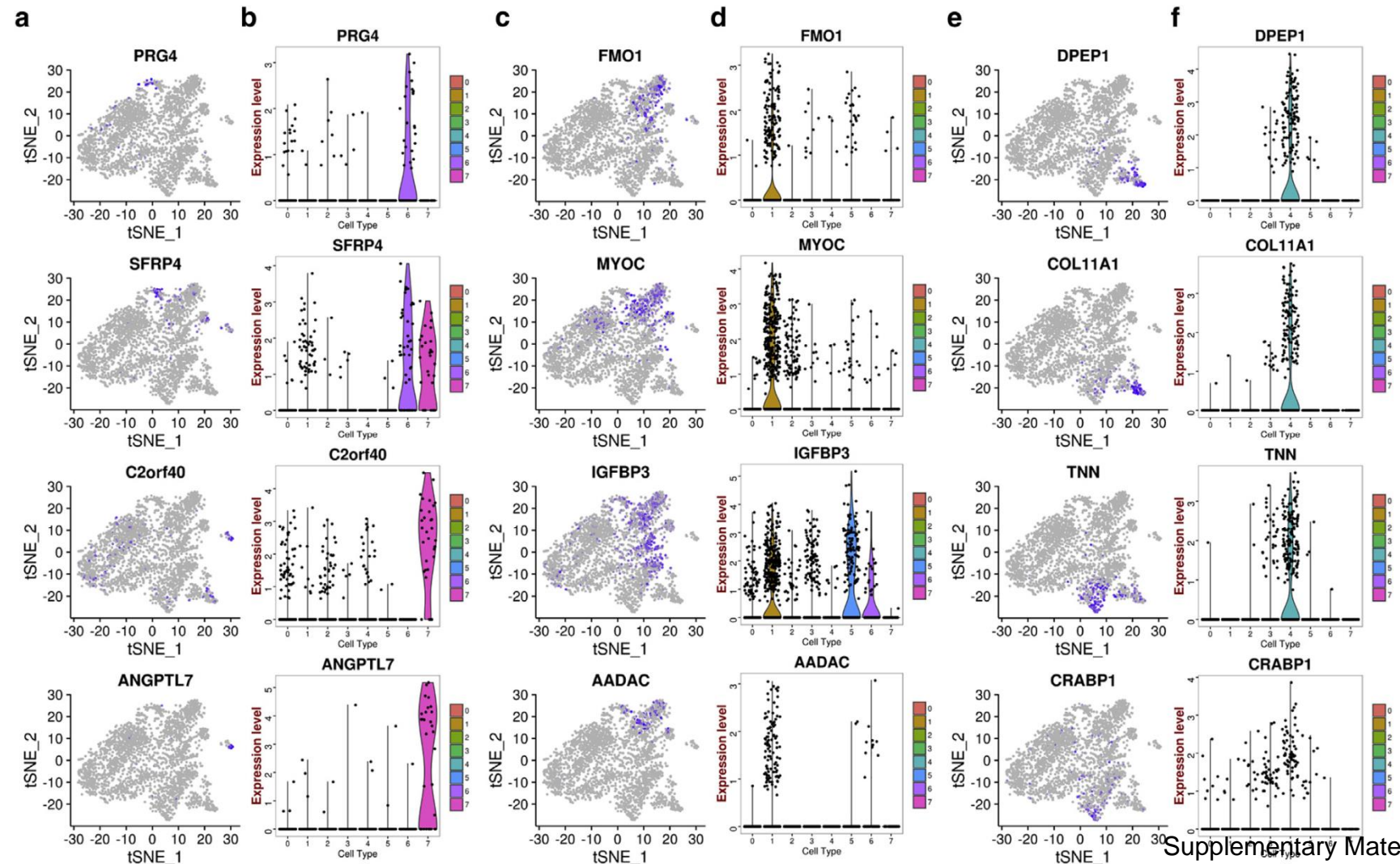
Supplementary Material  
Tabib et al 2018

# Gene expression in fibroblast subpopulations



Supplementary Material  
Tabib et al 2018

# Gene expression in fibroblast subpopulations



Supplementary Material  
Tabib et al 2018

# IF staining in normal skin showing fibroblast subpopulations

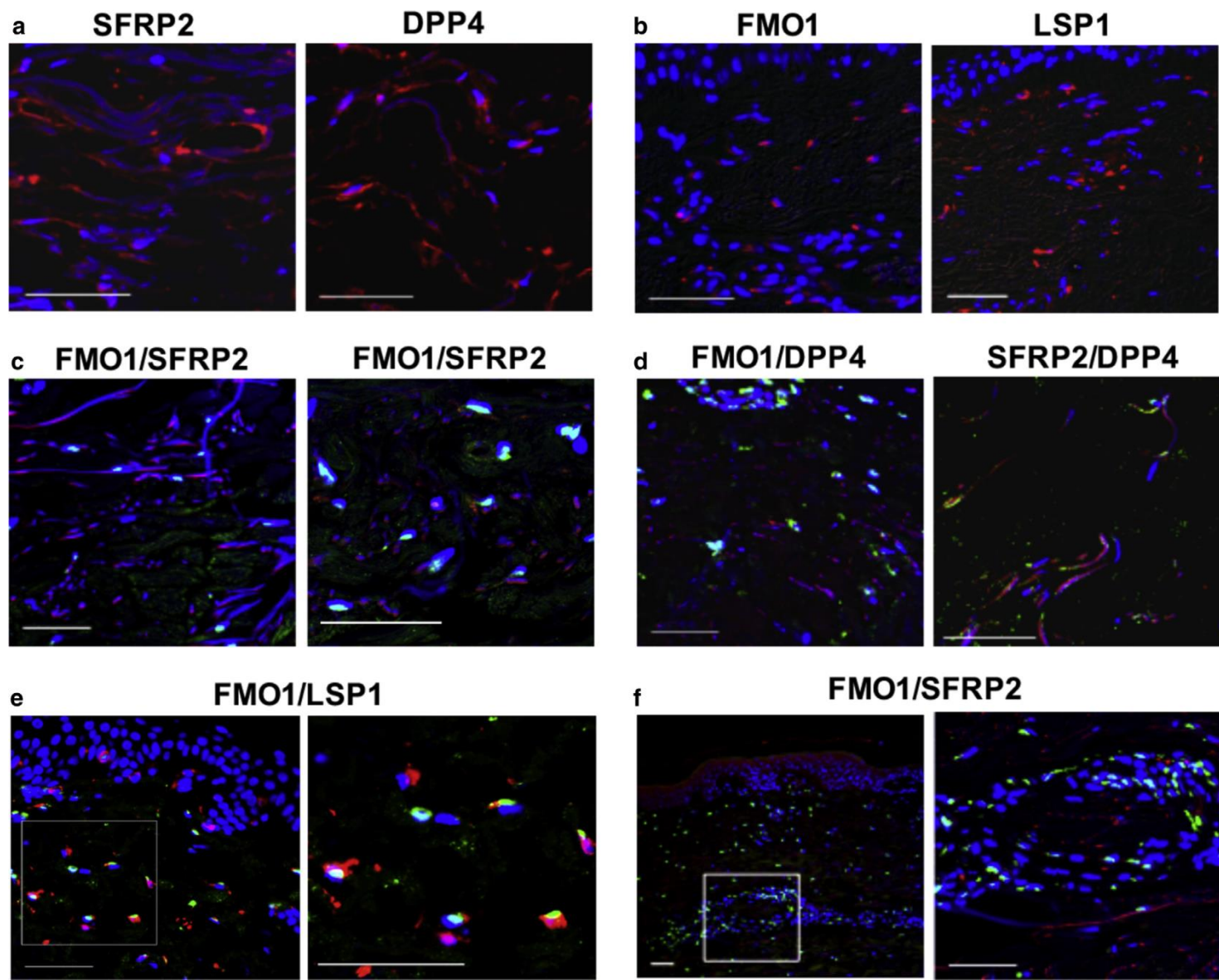


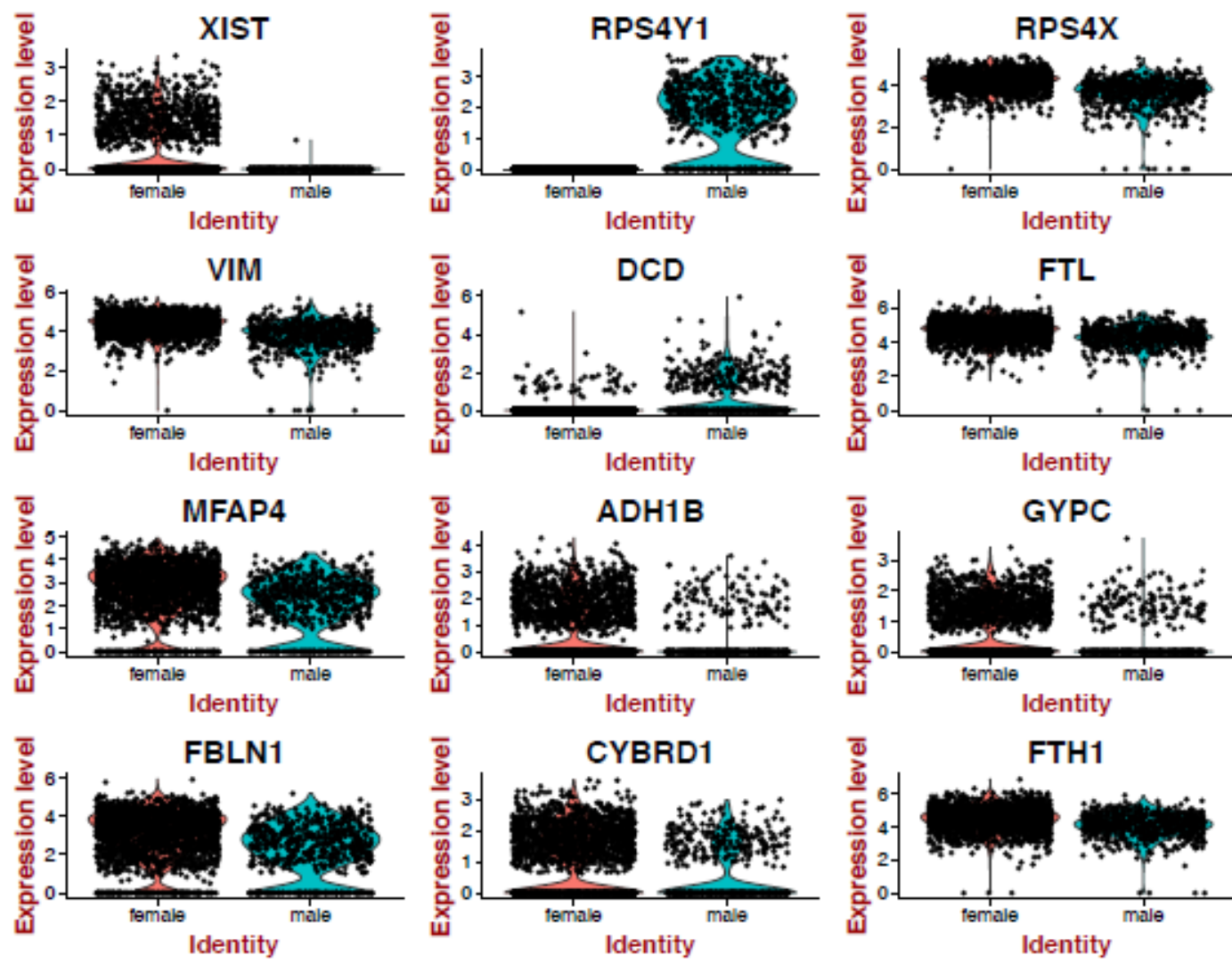
Figure 4  
Tabib et al  
2018

# Differentially expressed genes per cluster

Supple					
Statist	0	0.706	0.084	1	KRT2
differe	0	0.981	0.119	1	KRT1
cluster	0	0.996	0.239	1	DMKN
remain	0	0.987	0.508	1	KRT10
p_val	0	0.901	0.112	1	KRTDAP
0	0	0.981	0.13	1	LY6D
0	0	0.878	0.153	1	LYPD3
0	0	0.952	0.229	1	SFN
0	0	0.944	0.15	1	LGALS7B
0	0	0.797	0.073	1	SBSN
0	0	0.997	0.443	1	PERP
0	0	0.984	0.225	1	S100A14
0	0	0.888	0.162	1	DSP
0	0	0.794	0.07	1	RHOV
0	0	0.93	0.195	1	AQP3
0	0	0.9	0.266	1	TACSTD2
0	0	0.725	0.067	1	KLK11
0	0	0.668	0.074	1	CLDN4
0	0	0.676	0.078	1	LGALS7
0	0	0.673	0.063	1	DSG1
0	0	0.669	0.071	1	PKP1
0	0	0.725	0.127	1	SERPINB5
0	0	0.846	0.288	1	DEGS1
0	0	0.709	0.07	1	NSG1
0	0	0.885	0.201	1	FXYD3

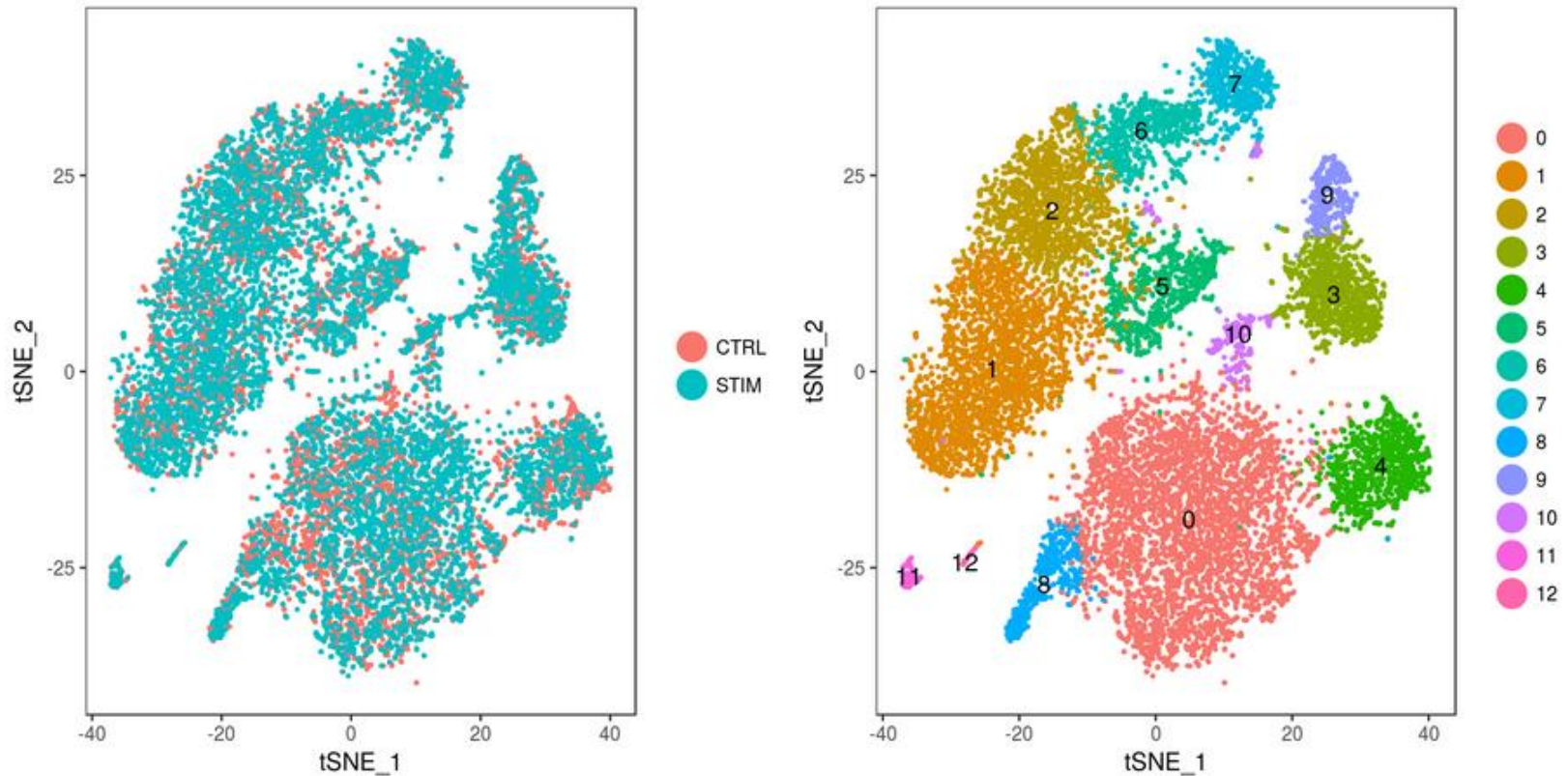


# Significant genes female vs. male



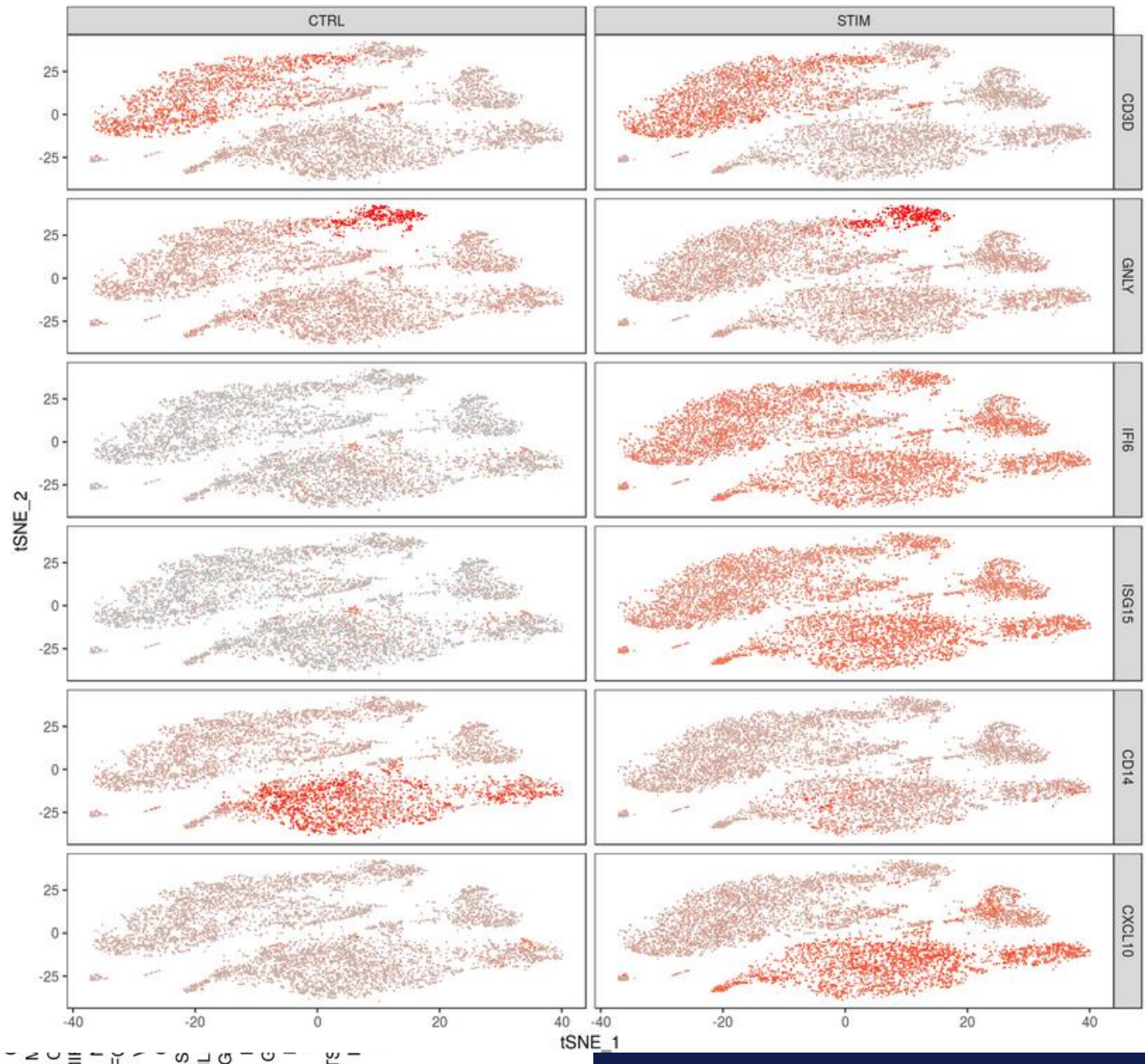
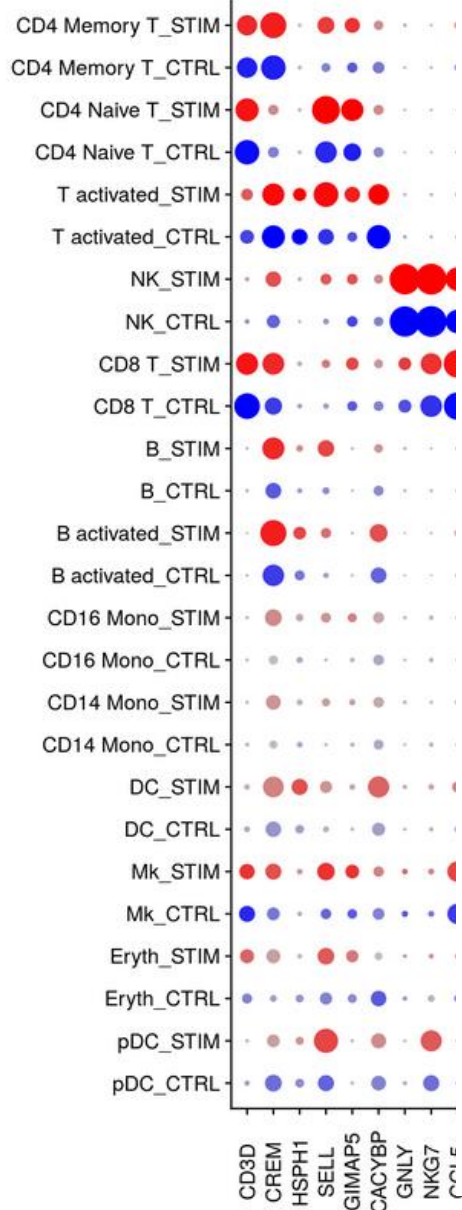
Supplementary Material  
Tabib et al 2018

# Kang HM et al. 2018



Tutorial: Stimulated and unstimulated PBMCs:  
[https://satijalab.org/seurat/immune\\_alignment.html](https://satijalab.org/seurat/immune_alignment.html)

# Kang HM et al 2019



# Li L. et al., 2018

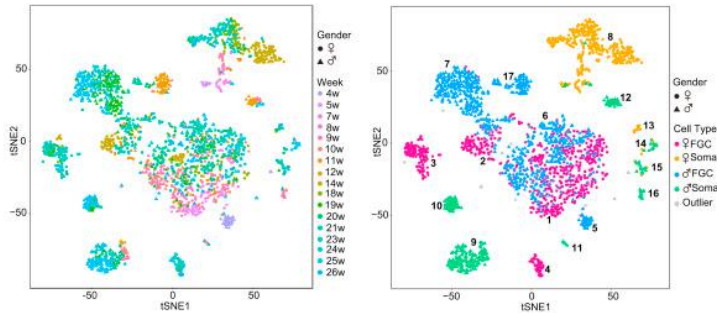
A

Week	5w		7w		8w		10w		11w		12w		14w		18w		20w		23w		24w		26w		Total
Embryo	twin-e1	twin-e2	e1	e1	e1	e1	e1	e1	e1	e1	e1	e1	e1	e1	twin-e1	twin-e2	twin-e1	twin-e2	twin-e1	twin-e2	twin-e1	twin-e2	e1	e1	732
No. of ♀ FGC	34	15	34	41	50	30	48	75	31	34	47	59	64	52	46	52	20	20	20	20	20	20	20	20	310
No. of ♀ Soma	13	17	12	3	16	11	2	94	24	44	29	18	19	27	10	10	16	367							2,167

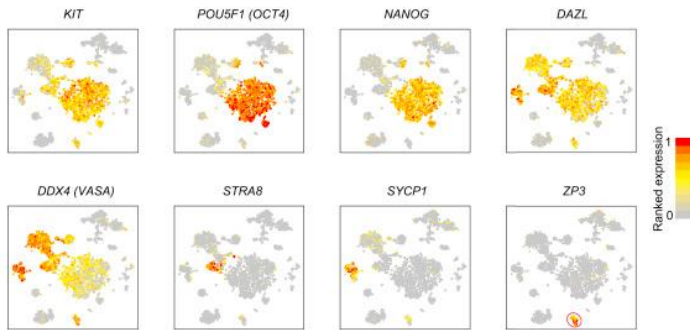
  

Week	4w		9w		10w		12w		19w		20w		21w		25w		Total
Embryo	e1	e1	e1	e2	e1	twin-e1	twin-e2	e1	twin-e1	twin-e2	e3	e1	e1	e1	e1	758	
No. of ♂ FGC	37	49	43	3	55	72	72	78	65	76	9	199	310			2,167	
No. of ♂ Soma	4	25	2	16	30	20	18	10	20	1	17	147				2,167	

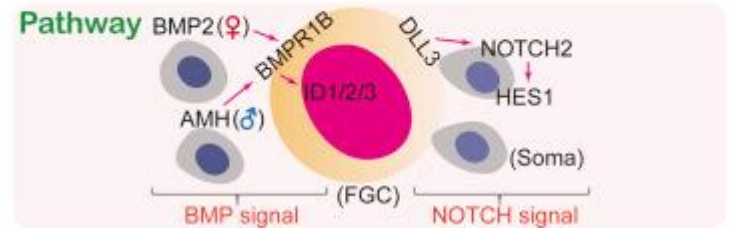
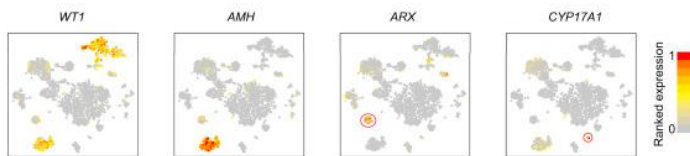
B



C

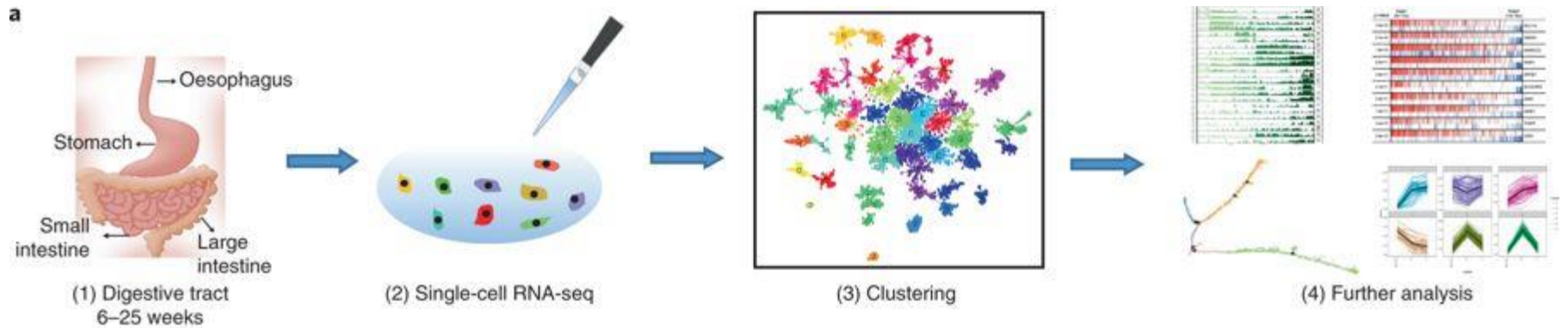


D



“Single-Cell RNA-Seq Analysis Maps Development of Human Germline Cells and Gonadal Niche Interactions”

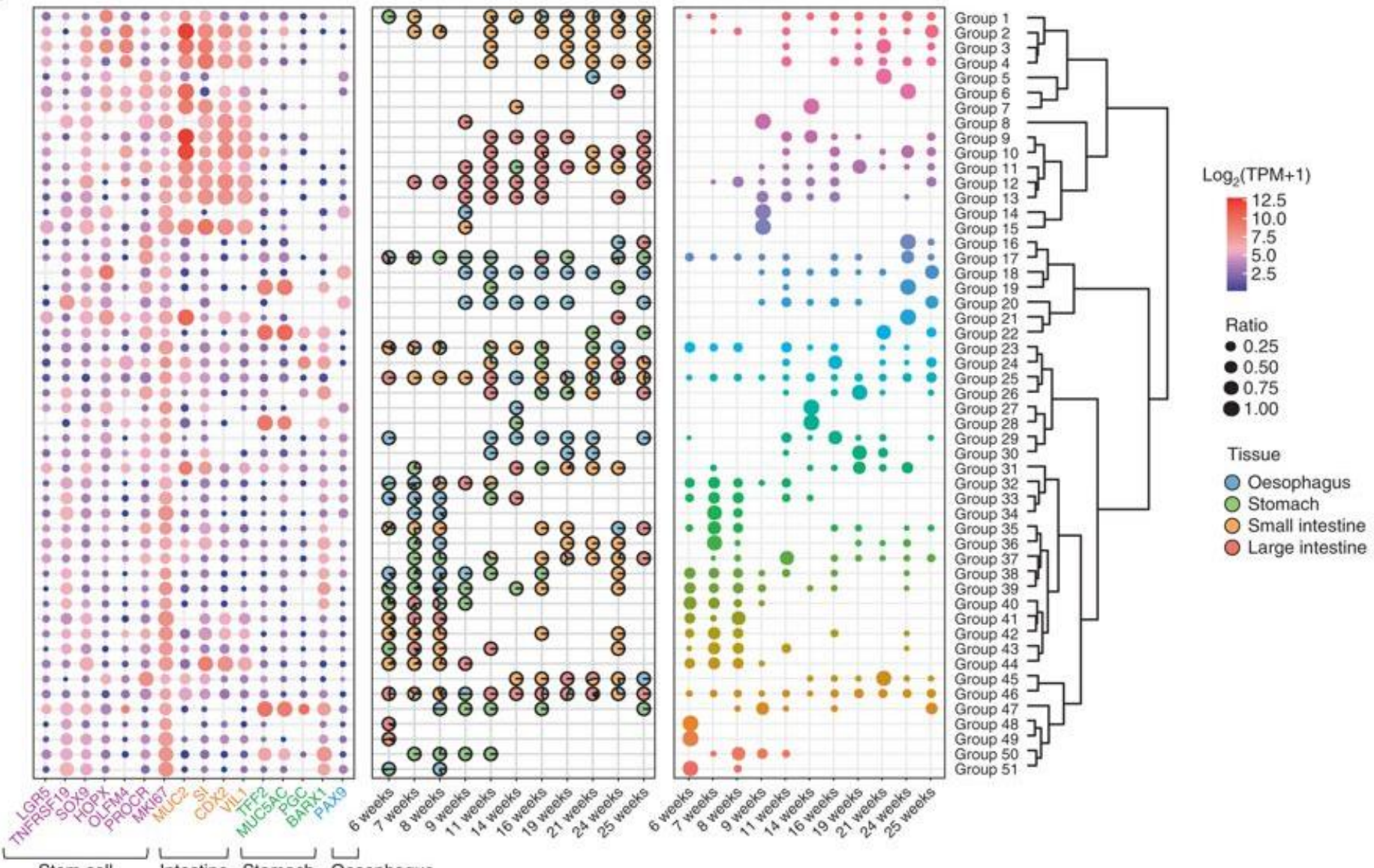
# Gao S. et al 2018

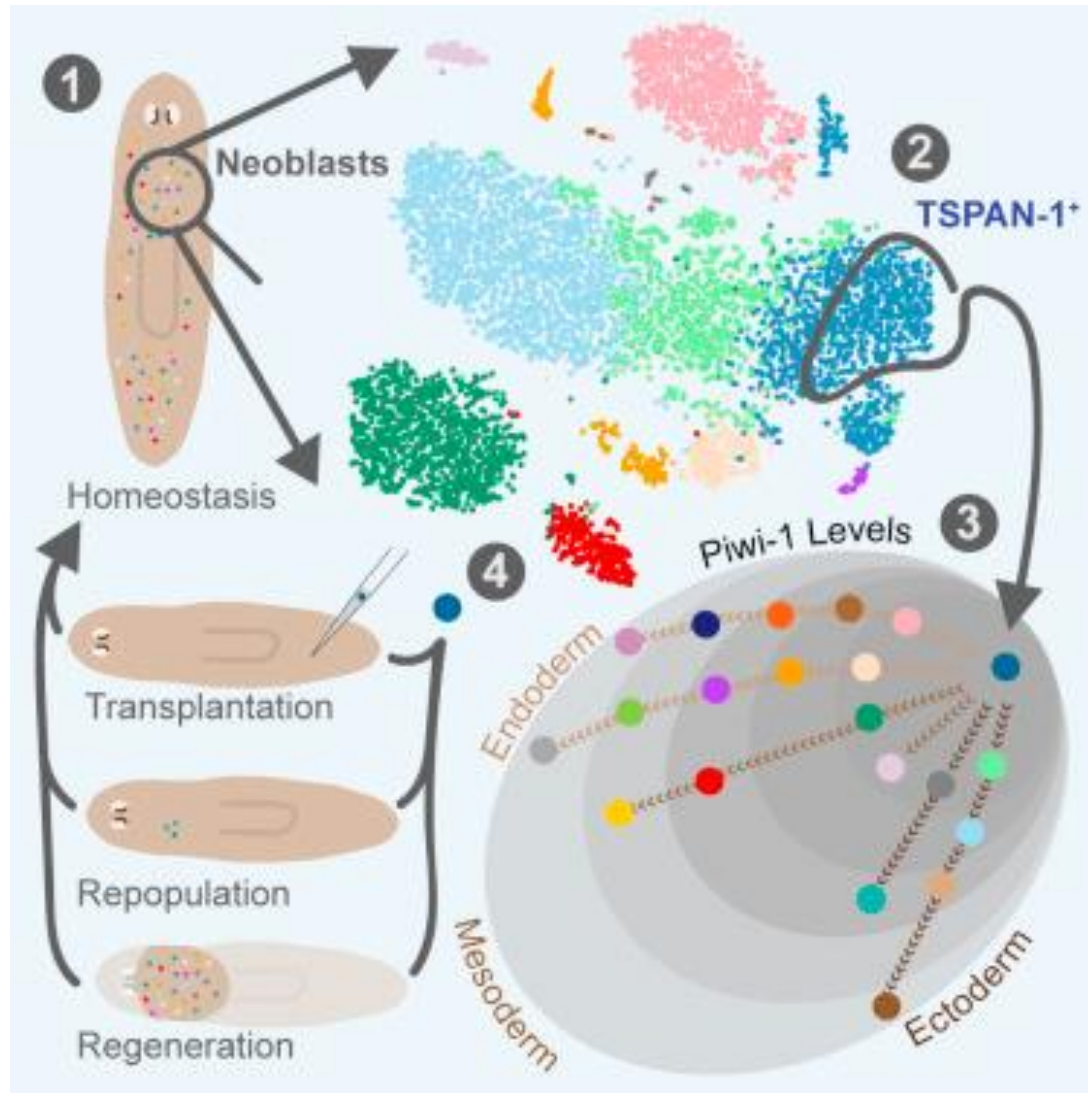


“Tracing the temporal-spatial transcriptome landscapes of the human fetal digestive tract using single-cell RNA-sequencing”

# Gao S. et al 2018

c

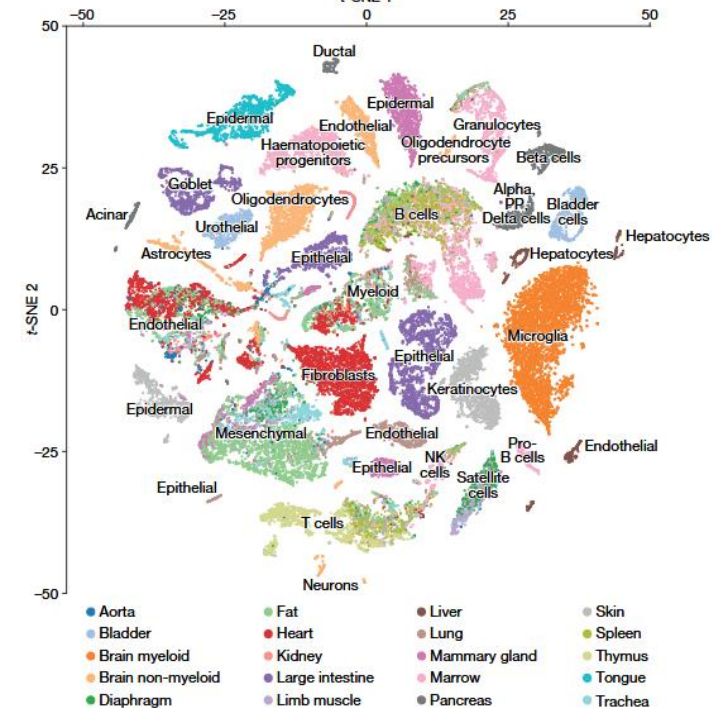
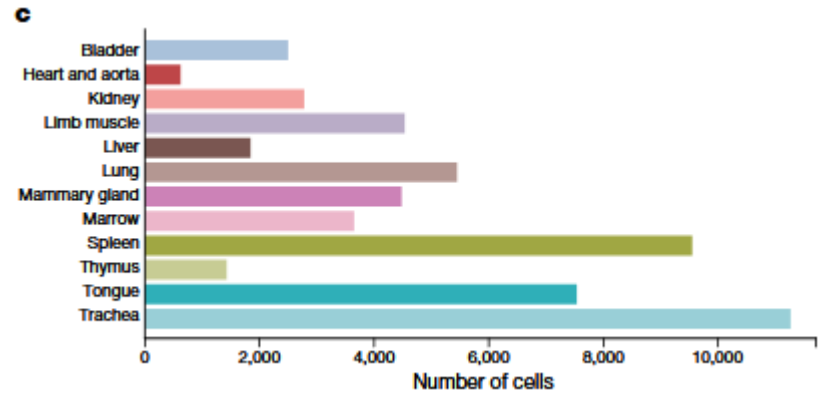
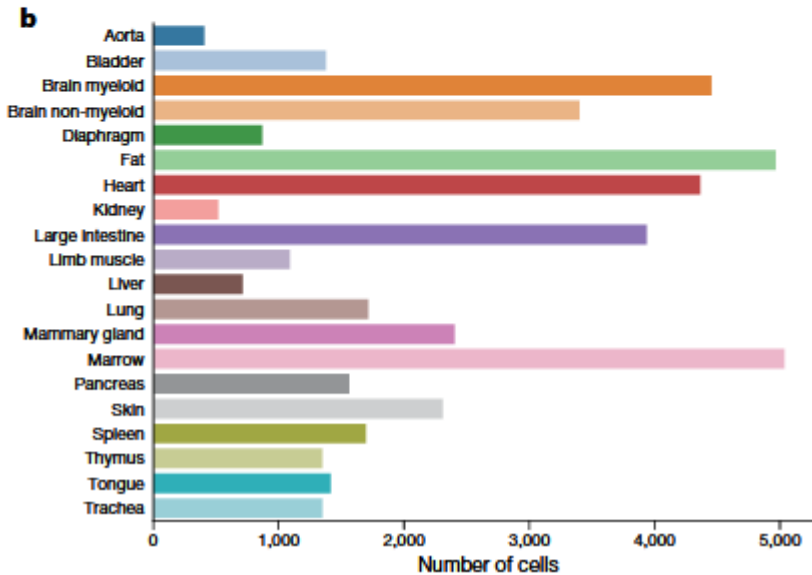
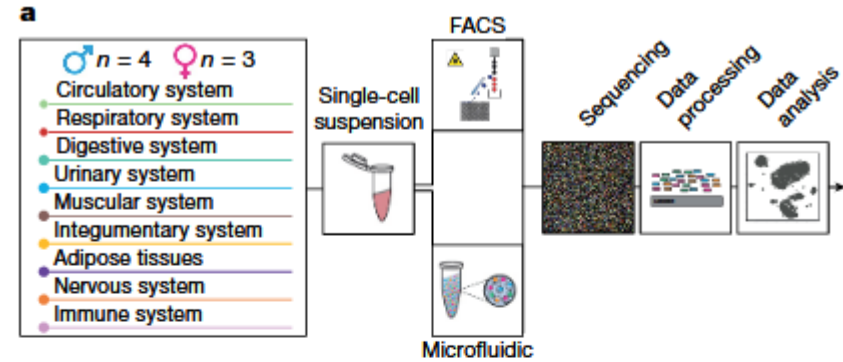




“Prospectively Isolated Tetraspanin+ Neoblasts Are Adult Pluripotent Stem Cells Underlying Planaria Regeneration”

# Tabula muris: Single cell transcriptomics from 20 mouse organs

*Tabula muris consortium, 2018*





# Conclusion

- New perspectives for high-resolution genetic analyses
- Applications:
  - Single cell genomics (e.g. microbiome)
  - Single cell transcriptomics: **gene expression**, immune profiling,
  - Single cell epigenetics
  - Linked-reads genomics: whole genome-sequencing, exome sequencing, de novo assembly
- Complex bioinformatic process and data visualization
- For developmental studies, substance-effect studies, microbiome screening

# Discussion

- Pros/Cons?
- Applicability
- Applications
- Future directions
- ...

Questions? Thank you!

# References

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