Introduction to Data Mining Publicly Available Single-Cell RNA-Sequencing Datasets

BMB Seminar

John Klement

June 4th, 2021

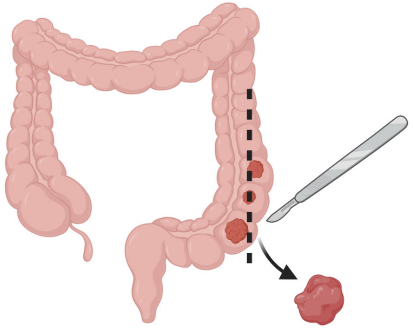
Outline

- High Level
  - How scRNAseq works
  - Recent ‘Add-ons’ to single-cell analysis
  - Sources of data
  - Seurat Platform
- Low level
  - Walkthrough using Colon HCA scRNAseq data
    - Acquiring Data
      - Preprocessing and QC
    - Analysis
    - Generating Figures

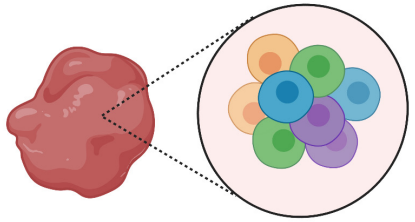
Misconceptions

- *I need a supercomputer to do any real analysis*
- *It will take days to analyze a single dataset*
- *I need to have extensive coding experience*
- *There's not a dataset relevant to the question that I am asking*

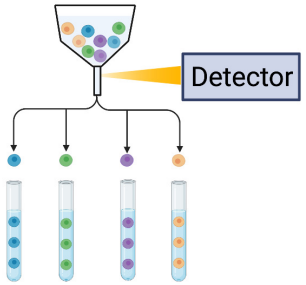
Brief Review of scRNAseq



1 Tissue dissection

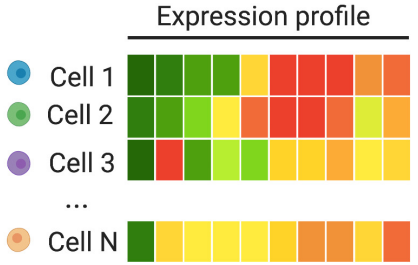


2 Cellular composition of the dissected tissue

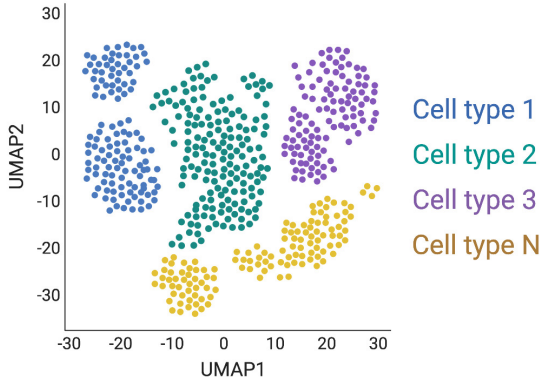


3 Single-cell sorting by FACS

4 Single-cell sequencing

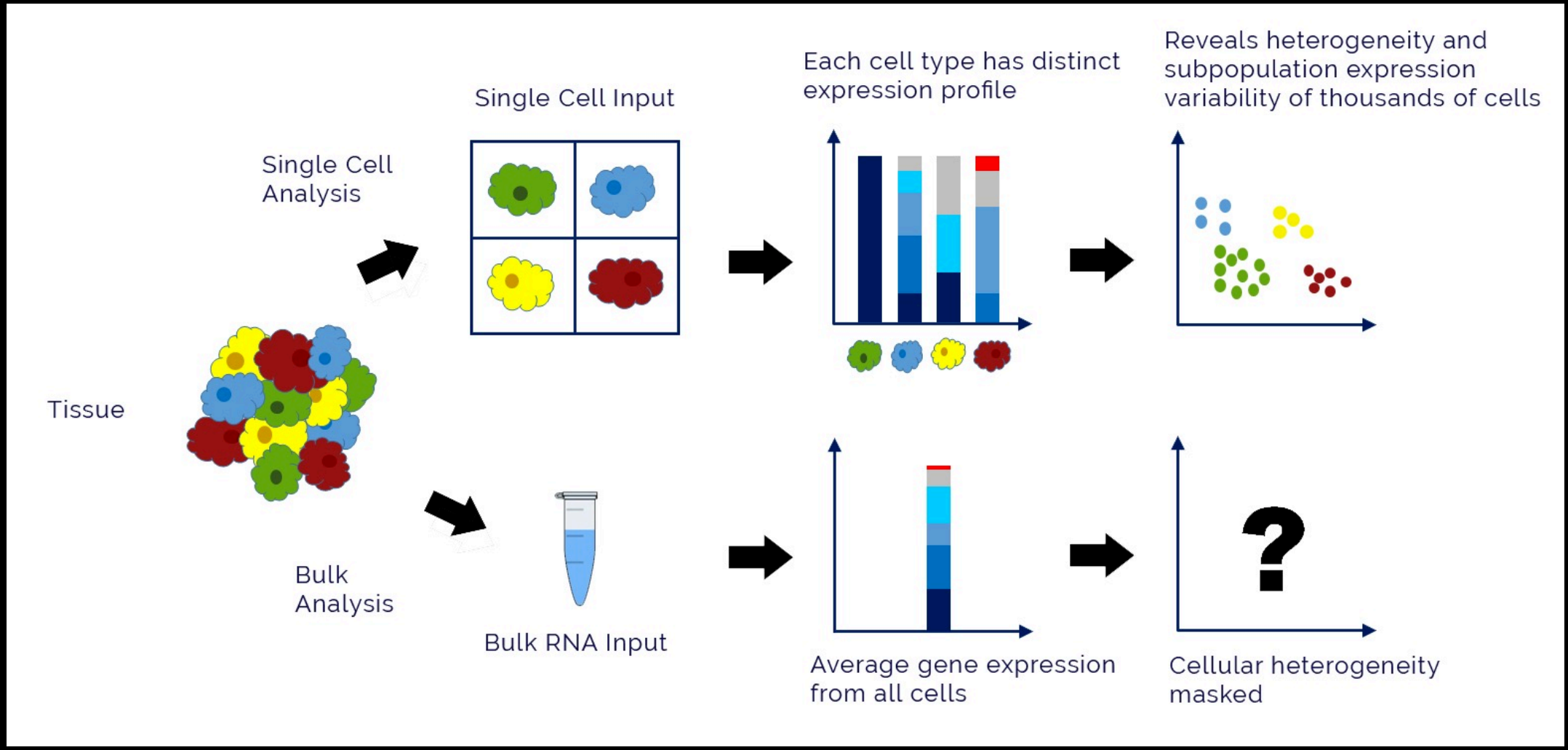


5 Single-cell expression profile

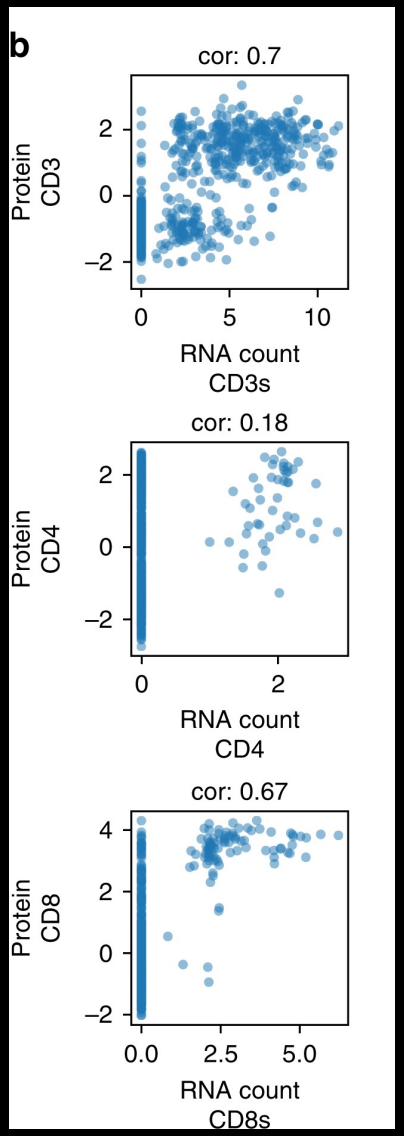


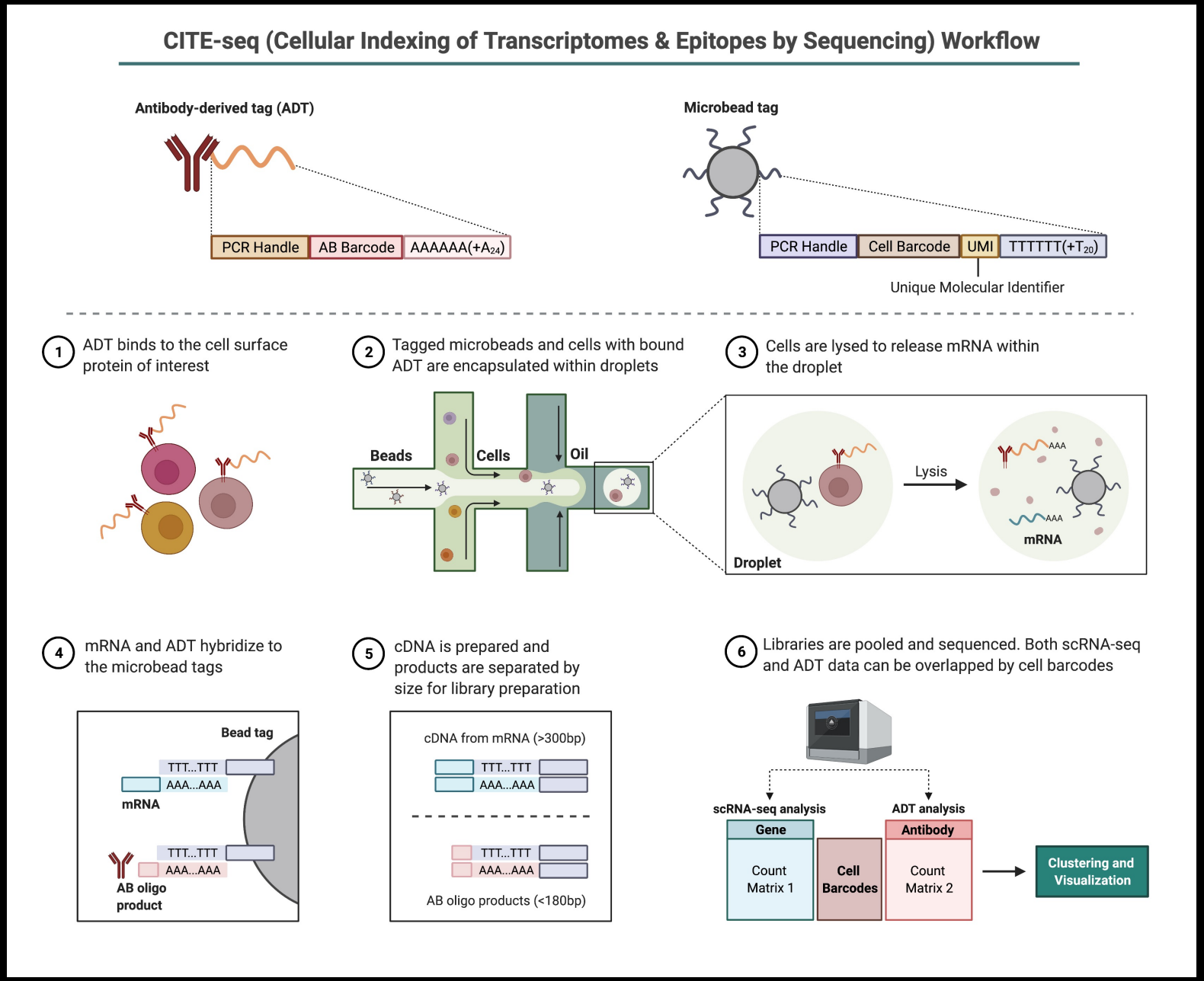
6 Clustering & cell type identification

Why is scRNAseq important?

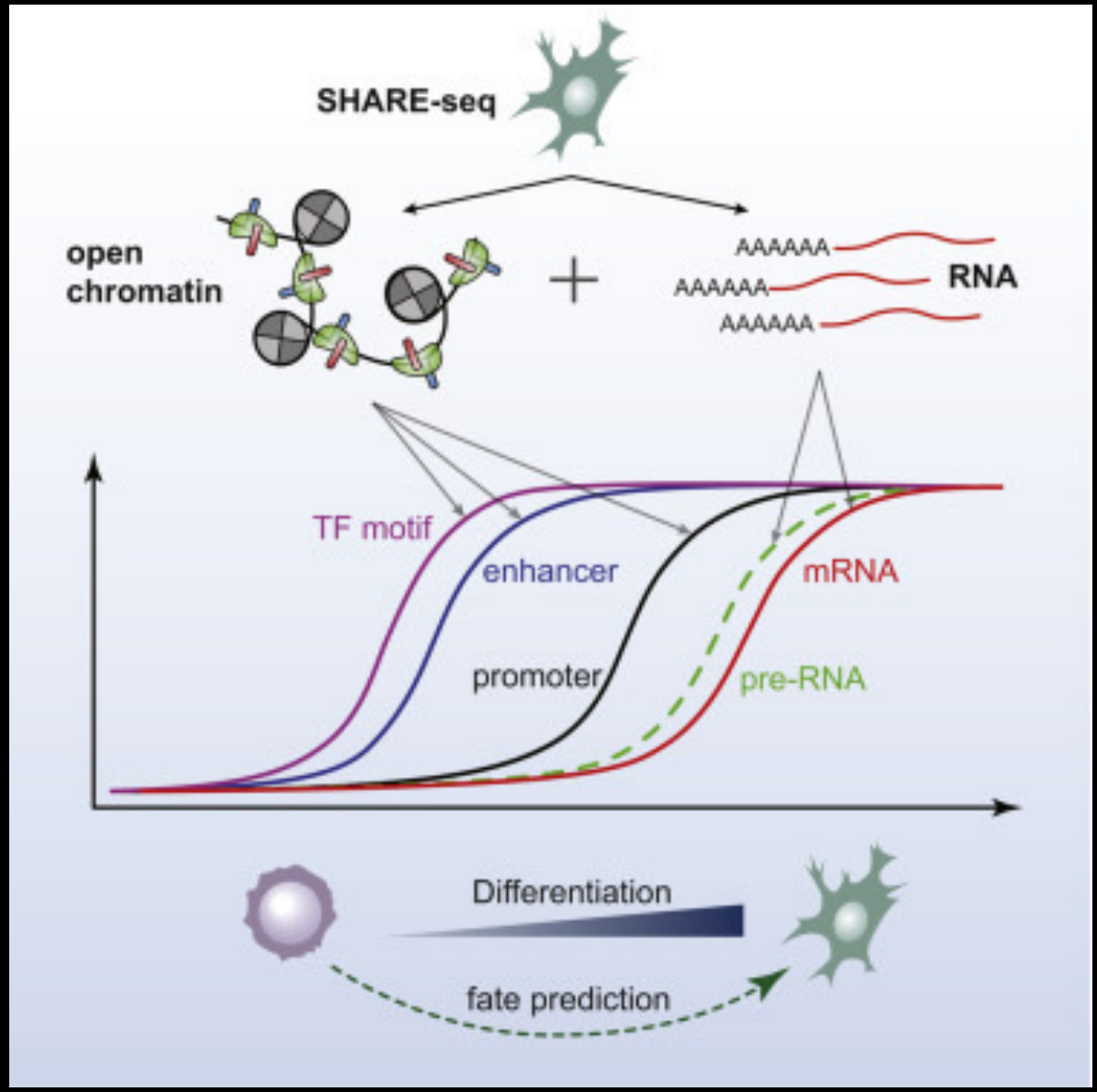


CITE/REAP-seq





Epigenetic scRNAseq/SHARE-Seq

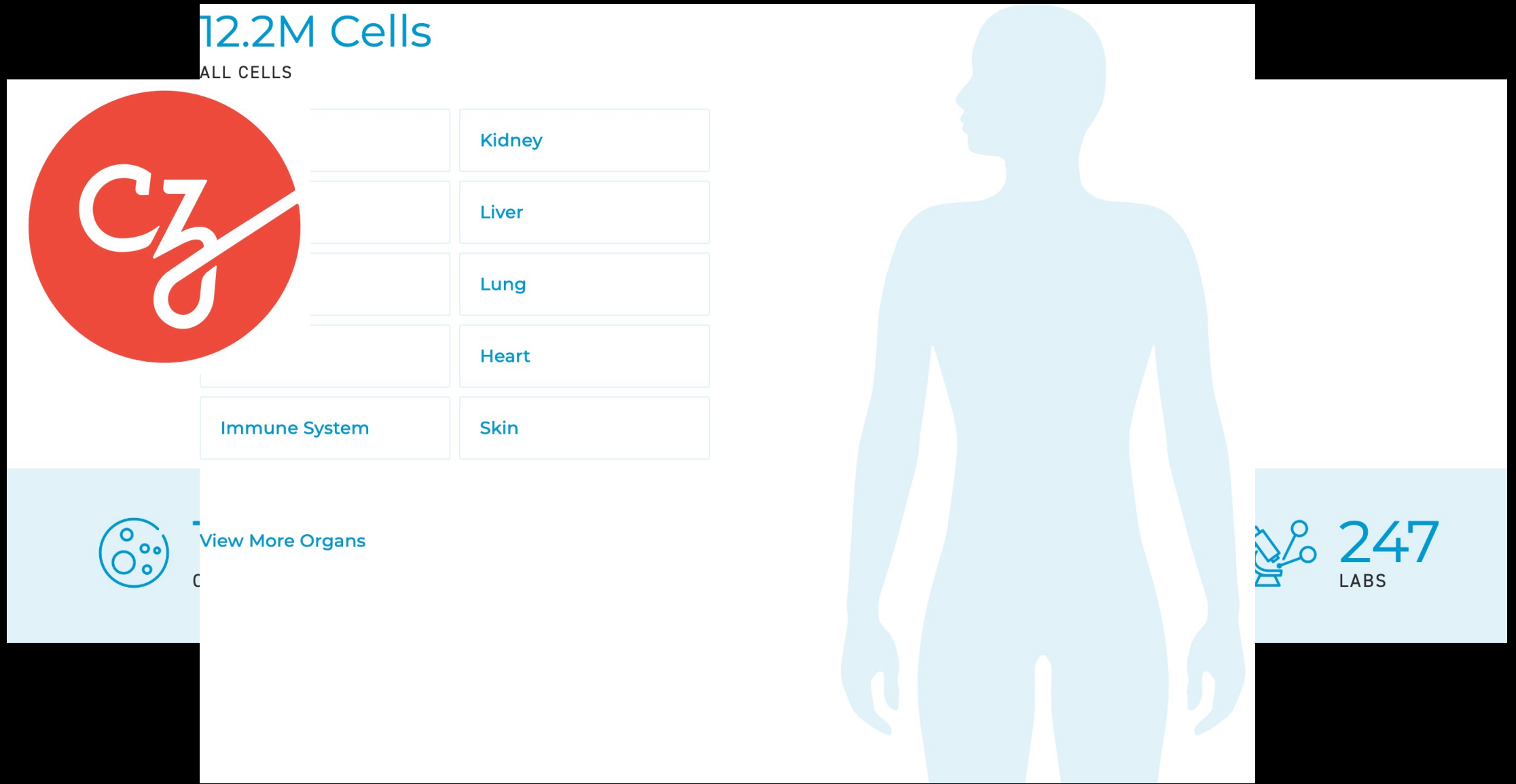


ScRNAseq Drawbacks

- Co$t
- Tissue Procurement/Isolation
- Appropriate QC is often complex

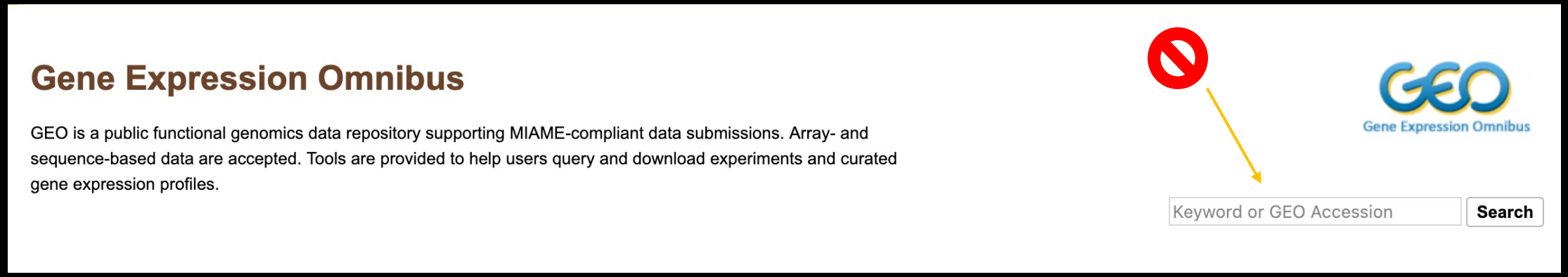
Public scRNAseq datasets are widely available and easily accessible

Human Cell Atlas



Using NIH GEO

- Access: https://www.ncbi.nlm.nih.gov/geo



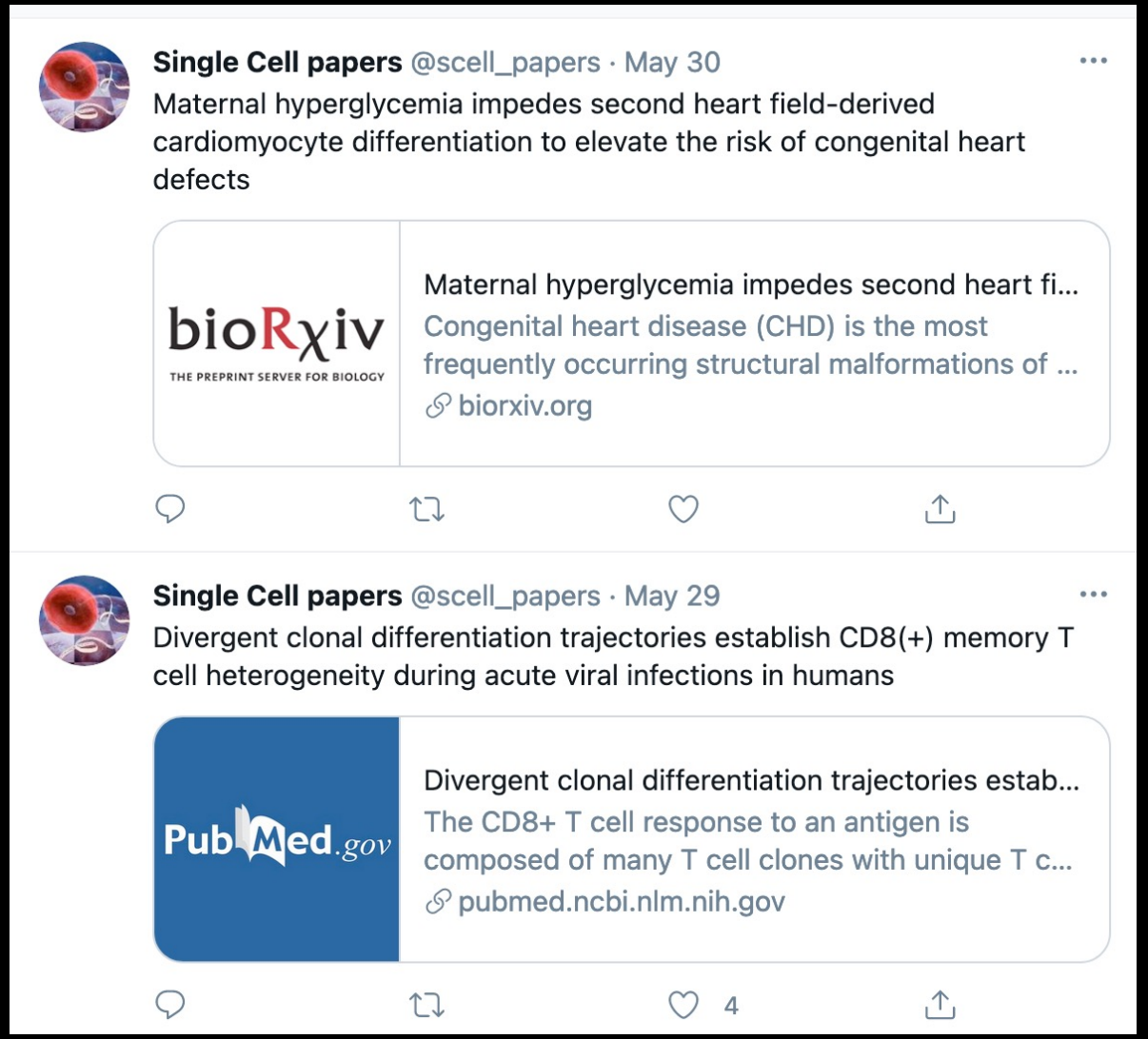
From Manuscripts

Data availability

The scRNA-seq data generated for this study have been deposited in ArrayExpress under E-MTAB-8656. The reference genome sequence was downloaded from Ensembl (http://www.ensembl.org/Mus_musculus) and used for alignment of the scRNA-seq data. To evaluate stem cell priming, scRNA-seq data were obtained from the Single Cell Portal (https://portals.broadinstitute.org/single_cell/study/small-intestinal-epithelium) and used to define gene sets for differentiated sub-lineages of epithelial cells. The lists of marker genes used to annotate types of epithelial, mesenchymal and immune cells in Fig. 3b, f and Extended Data Figs. 6d, 8a–c are given in Supplementary Tables 1, 3. Gene sets used in Fig. 3d, e and Extended Data Fig. 7a, b, k–m are provided in Supplementary Table 2. Source data are provided with this paper.

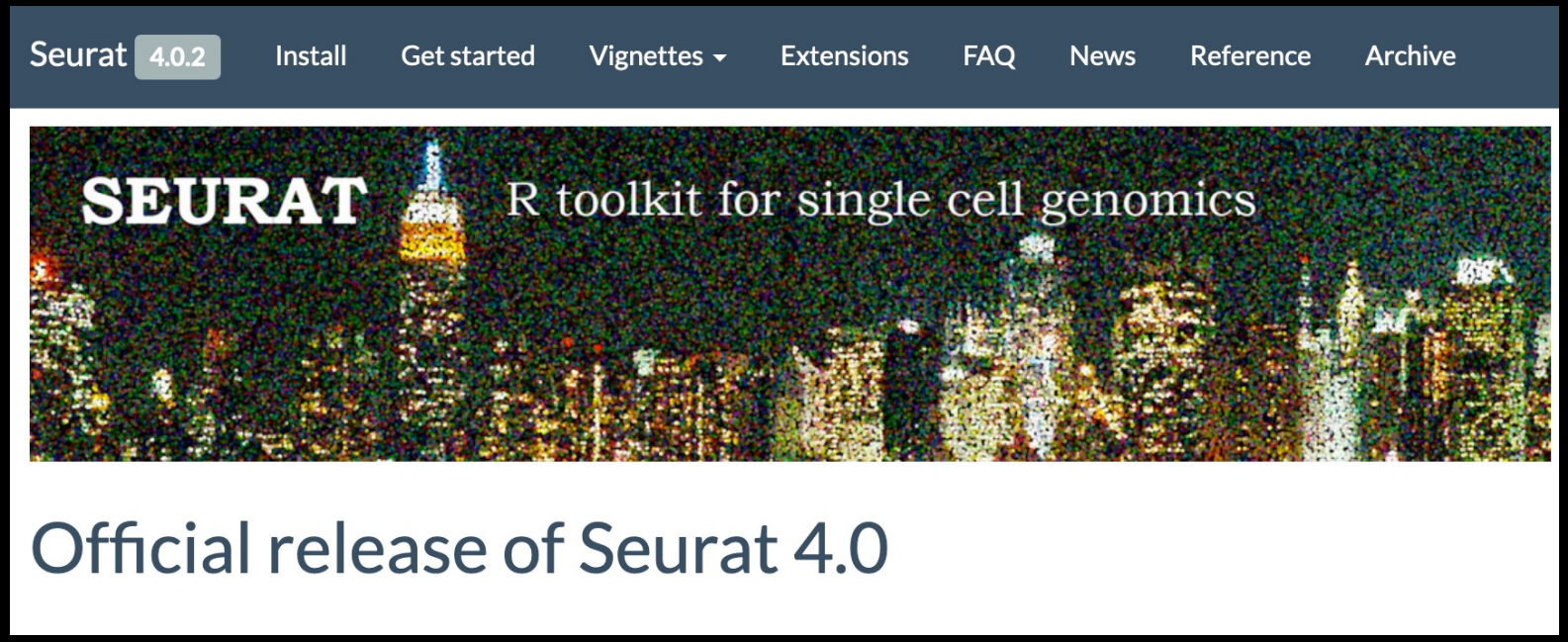
Other Useful Sources

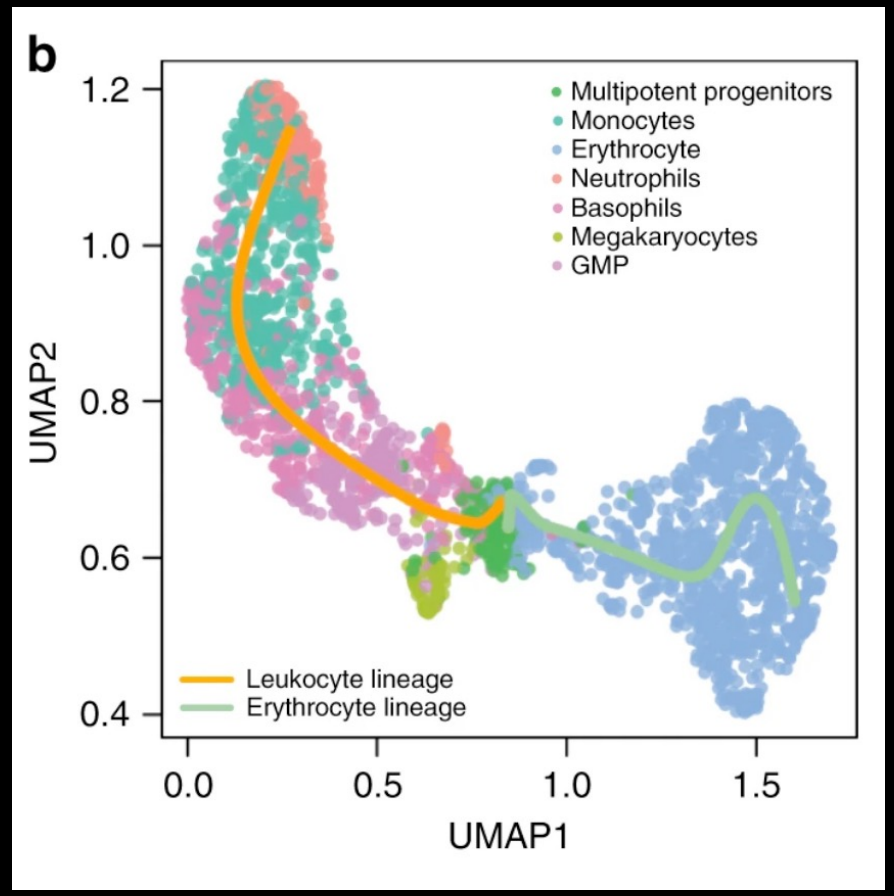
- https://twitter.com/scell_papers



How to analyze

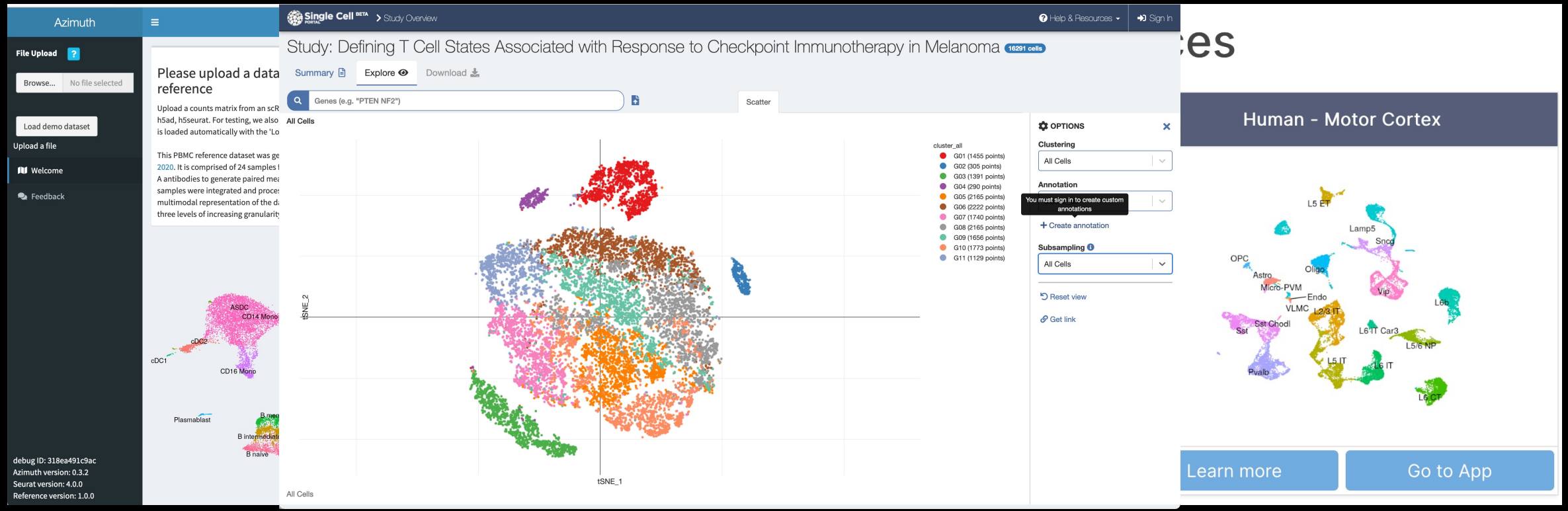
- Seurat
- Main alternative: Monocle





How to analyze

- Web-based platforms
  - Azimuth: https://azimuth.hubmapconsortium.org/
  - Single Cell Portal : https://singlecell.broadinstitute.org/single_cell



Step-by-step walkthrough

- Installing Software
- Downloading datafiles
  - Human Cell Atlas
  - NIH GEO
- Setting up workspace in RStudio
- Inputting downloaded datafiles
- Analyzing scRNAseq data with Seurat
- Generating Figures

Installing Software

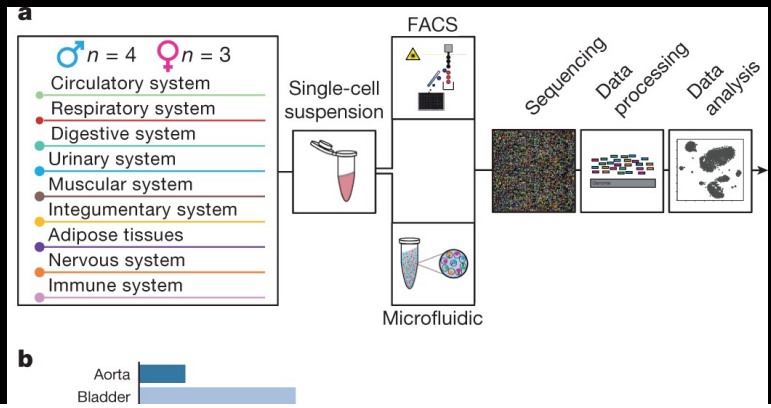
- Minimum Hardware Requirements
  - 16GB RAM
  - i5 Processor

1. Install R
2. Install Rstudio
3. Install Rtools
   - Windows only

- R Learning Resources
  - Coursera
  - EdX
  - Melbourne
  - Data Science for Immunologists (book)

Downloading Files - HCA

- Access the HCA Data Portal
- Tabula Muris
  - Large Intestine



Article | Published: 03 October 2018

FACS_matrices.zip	Mus musculus	adip aort blac bon brai diap hea kidr larg liver lung mar mus pan skin sple thyr tong trac

Project Information

Project Metadata

Project Matrices

External Resources

Tabula Muris: Transcriptomic characterization of 20 organs and tissues from Mus musculus at single cell resolution

Select Project

Project Information

Description

We have created a compendium of single cell transcriptome data from the model organism Mus musculus comprising more than 100,000 cells from 20 organs and tissues. These data represent a new resource for cell biology, revealing gene expression in poorly characterized cell populations and allowing for direct and controlled comparison of gene expression in cell types shared between tissues, such as T-lymphocytes and endothelial cells from distinct anatomical locations. Two distinct technical approaches were used for most tissues: one approach, microfluidic droplet-based 3'-end counting, enabled the survey of thousands of cells at relatively low coverage, while the other, FACS-based full length transcript analysis, enabled characterization of cell types with high sensitivity and coverage. The cumulative data provide the foundation for an atlas of transcriptomic cell biology.

Downloading Files - GEO

NCBI

Gene Expression Omnibus

COVID-19 is an emerging, rapidly evolving situation.
Get the latest public health information from CDC: https://www.coronavirus.gov .
Get the latest research from NIH: https://www.nih.gov/coronavirus.
Find NCBI SARS-CoV-2 literature, sequence, and clinical content: https://www.ncbi.nlm.nih.gov/sars-cov-2/.

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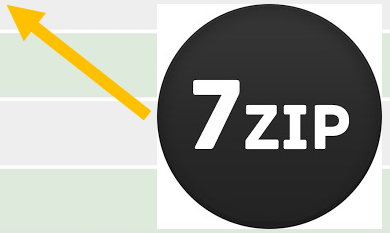
Scope: Self Format: HTML Amount: Quick GEO accession: GSE109774 GO

Series GSE109774 Query DataSets for GSE109774

Status	Public on Mar 19, 2018
Title	Tabula Muris: Transcriptomic characterization of 20 organs and tissues from Mus musculus at single cell resolution
Organism	Mus musculus
Experiment type	Expression profiling by high throughput sequencing
Summary	We have created a resource of single cell transcriptome data from the model organism Mus musculus. Contributor: The Tabula Muris Consortium The full list of contributors to this dataset can be found in the corresponding publication.
Overall design	Single cell RNA sequencing of single cells across 20 tissues of 3 month aged mice
Citation(s)	Tabula Muris Consortium., Overall coordination., Logistical coordination., Organ collection and processing. et al. Single-cell transcriptomics of 20 mouse organs creates a Tabula Muris. *Nature* 2018 Oct;562(7727):367-372. PMID: 30283141

Supplementary file	Size	Download	File type/resource
GSE109774_Bladder.tar.gz	114.9 Mb	(ftp)(http)	TAR
GSE109774_Brain_Microglia.tar.gz	315.3 Mb	(ftp)(http)	TAR
GSE109774_Brain_Neurons.tar.gz	382.0 Mb	(ftp)(http)	TAR
GSE109774_Colon.tar.gz	288.8 Mb	(ftp)(http)	TAR
GSE109774_Fat.tar.gz	395.2 Mb	(ftp)(http)	TAR
GSE109774_Heart.tar.gz	468.4 Mb	(ftp)(http)	TAR
GSE109774_Kidney.tar.gz	55.2 Mb	(ftp)(http)	TAR
GSE109774_Liver.tar.gz	65.7 Mb	(ftp)(http)	TAR
GSE109774_Lung.tar.gz	128.3 Mb	(ftp)(http)	TAR
GSE109774_Mammary.tar.gz	183.4 Mb	(ftp)(http)	TAR
GSE109774_Marrow.tar.gz	364.7 Mb	(ftp)(http)	TAR
GSE109774_Muscle.tar.gz	139.6 Mb	(ftp)(http)	TAR
GSE109774_Pancreas.tar.gz	135.1 Mb	(ftp)(http)	TAR
GSE109774_RAW.tar	374.5 Mb	(http) (custom)	TAR (of TAR)
GSE109774_Skin.tar.gz	169.2 Mb	(ftp)(http)	TAR
GSE109774_Spleen.tar.gz	112.8 Mb	(ftp)(http)	TAR
GSE109774_Thymus.tar.gz	103.5 Mb	(ftp)(http)	TAR
GSE109774_Tongue.tar.gz	102.4 Mb	(ftp)(http)	TAR
GSE109774_Trachea.tar.gz	95.9 Mb	(ftp)(http)	TAR
GSE109774_list_of_SRR_accessions_and_raw_filenames.txt.gz	774.0 Kb	(ftp)(http)	TXT



Installing Software Packages in R

- General script for installing given package
  - install.packages('NAME OF PACKAGE')
- Some packages require downloading and compilation from Github
  - Github: online code repository

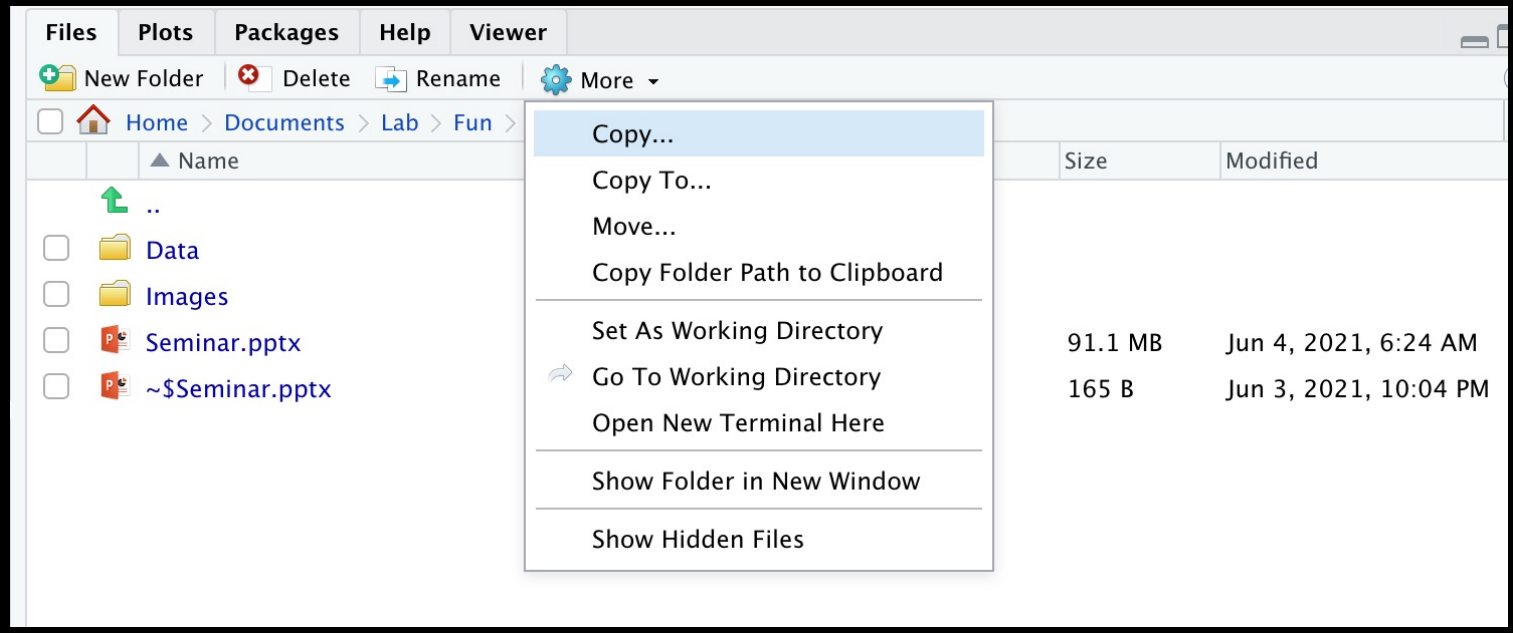
```
#install packages available from CRAN
install.packages('Seurat', 'tidyverse', 'msigdbr', 'ggpubr')

#install CelliD from bioconductor
if (!requireNamespace("BiocManager", quietly = TRUE))
  install.packages("BiocManager")
##The following initializes usage of Bioc devel
BiocManager::install(version='devel')
BiocManager::install("CelliD")
```

Set up environment

- Load Packages
  - library(PACKAGE NAME)
- Set Workspace
  - Simplest way is to navigate using RStudio

```
#load packages in workspace
library(Seurat)
library(tidyverse)
library(CelliD)
library(ggpubr)
library(msigdbr)
```

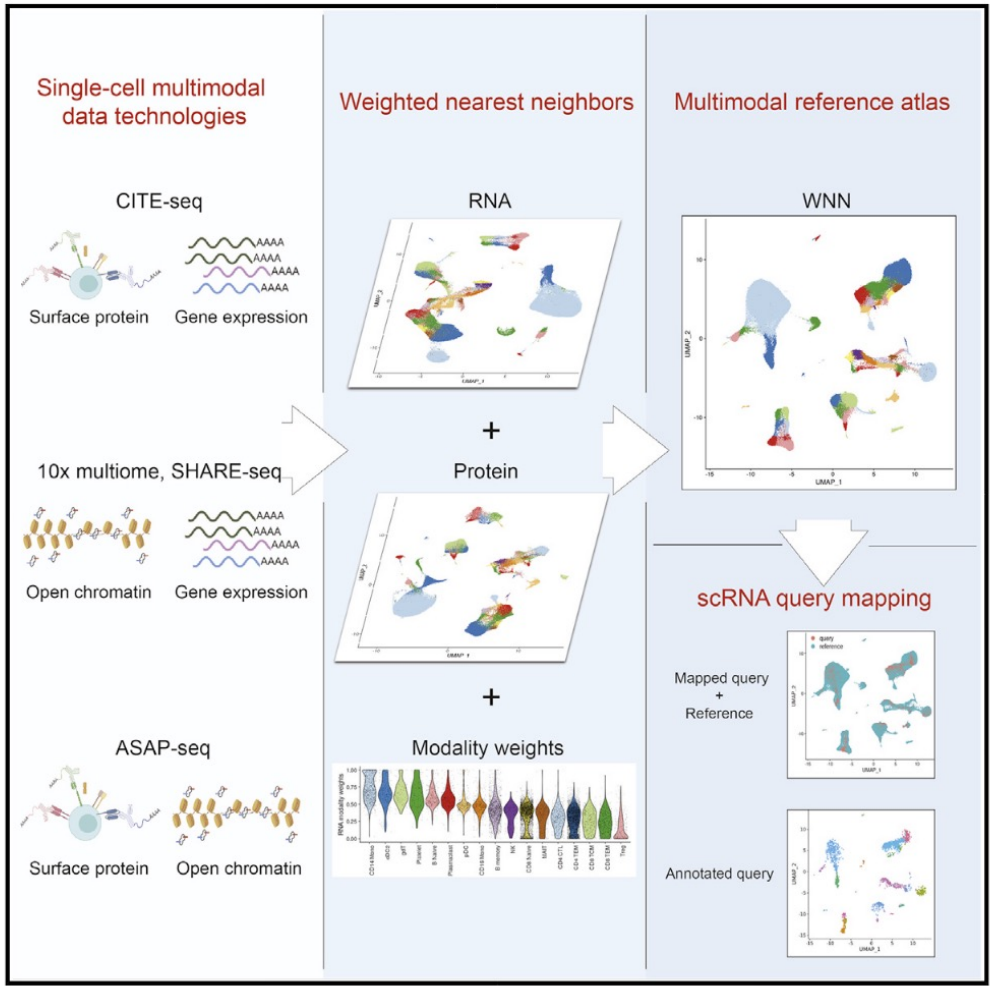


Seurat

- Website
- Installation
- Tutorial

Integrated analysis of multimodal single-cell data

Graphical abstract



Authors

Yuhan Hao, Stephanie Hao, Erica Andersen-Nissen, ..., Raphael Gottardo, Peter Smibert, Rahul Satija

Correspondence

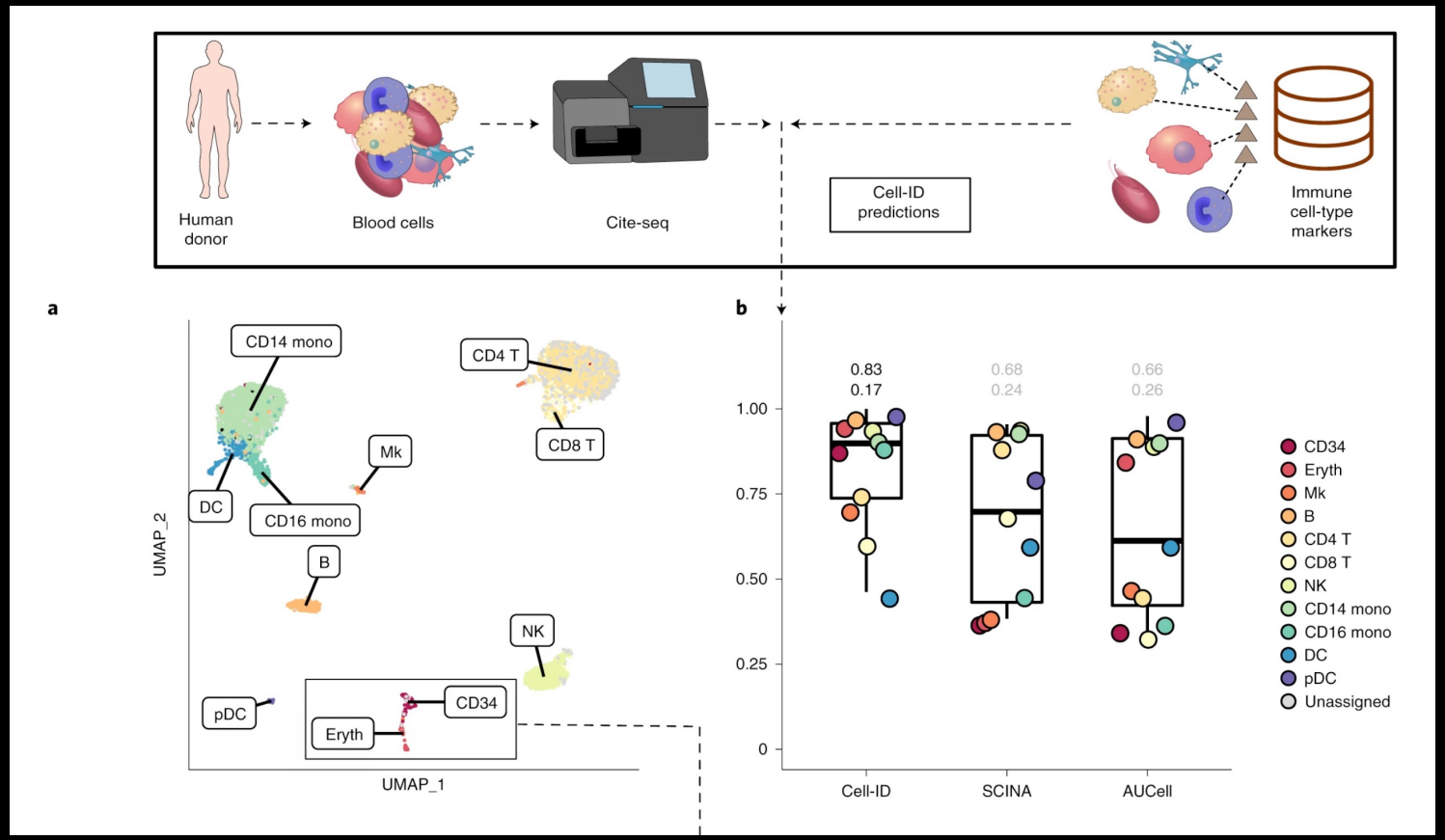
rsatija@nygenome.org (R.S.), smibertp@gmail.com (P.S.)

In brief

A framework that allows for the integration of multiple data types using single cells is applied to understand distinct immune cell states, previously unidentified immune populations, and to interpret immune responses to vaccinations.

Cell-ID

- Repository
  - Contains installation instructions, documentation, etc.
- Allows for:
  - Identification of cell-type at single-cell, rather than cluster, level
  - Easy method to score cells for gene signature expression



Preparing data files for input

- Creation of Seurat object requires two datasets
  - Gene expression matrix
    - Rows: Cells
    - Columns: Genes
  - Cell Metadata
    - Rows: Cells
    - Columns: Information re: identity of cell
      - E.g. tissue, identity, treatment, etc.

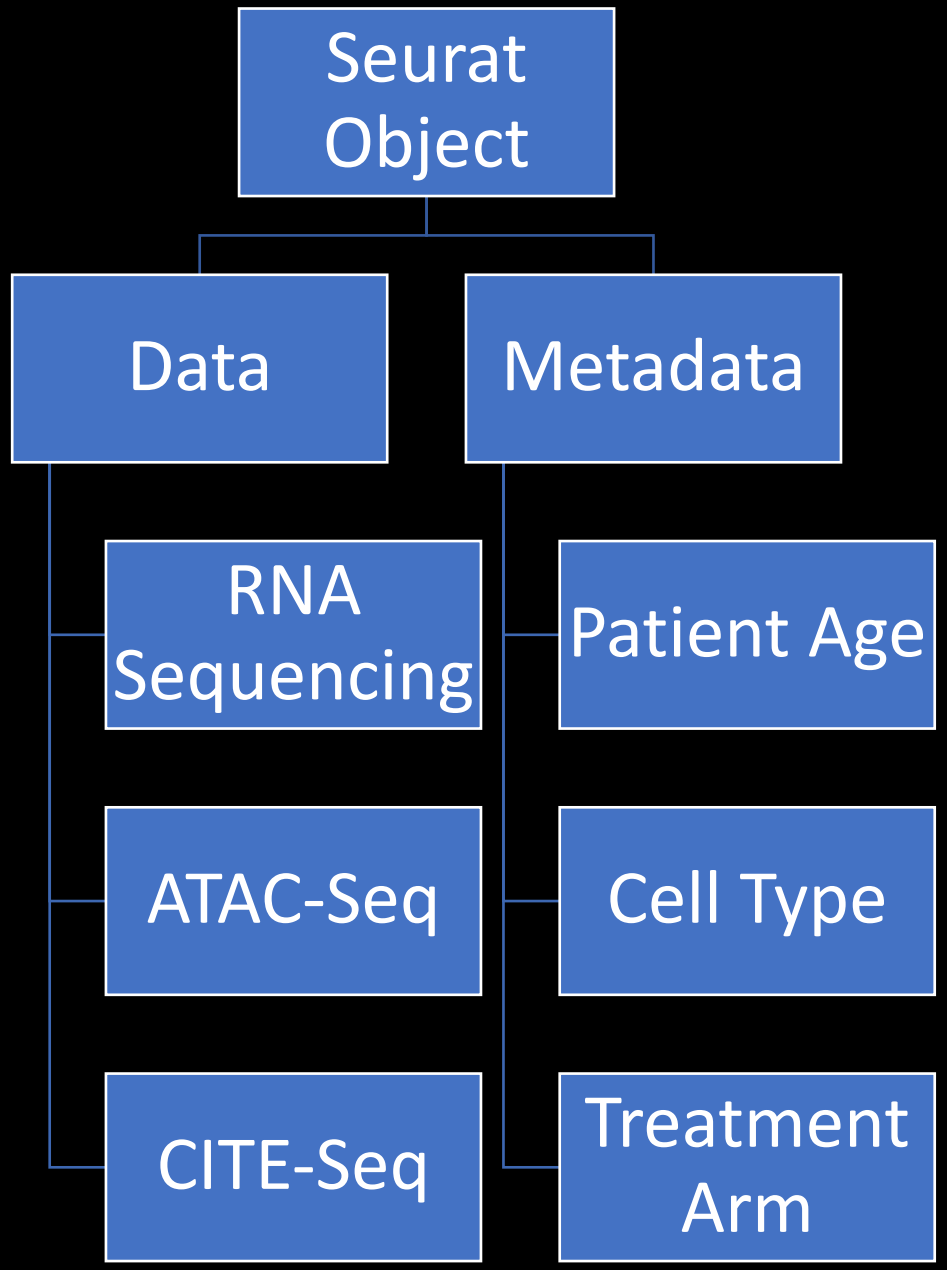
	Irf8	*Myc*	*Hyal1*
Cell.1	5	2	1
Cell.2	0	0	2
Cell.3	3	4	6

	Age	*Gender*	*Tissue*
Cell.1	54	M	C
Cell.2	44	F	B
Cell.3	36	F	C

Seurat Objects

- Contain both assay and metadata
  - Assay: RNA, protein, DNA, etc.
  - Metadata: age, cell source, etc.
    - Accessible with $ operator: Colon$age

```
#Create Seurat object
##read in Colon expression dataset; note read_csv used as file csv
colon <- read_csv("FACS/Large_Intestine-counts.csv")
##set cell to rowname
colon <- tibble::column_to_rownames(colon, var = "X1")
##filter out rownames that do not encode for protein in mice
colon <- colon[rownames(colon) %in% MgProteinCodingGenes,]
##read in metadata
colon.anno <- read_csv("annotations_facs.csv")
##set cell to rowname
colon.anno <- tibble::column_to_rownames(colon.anno, var = "cell")
##Create seurat object, filtering out genes that are not expressed in >=5 cells,
##or cells that do not express at least 200 genes
colon <- CreateSeuratObject(counts = colon, meta.data = colon.anno,
                            min.cells = 5, min.features = 200)
```



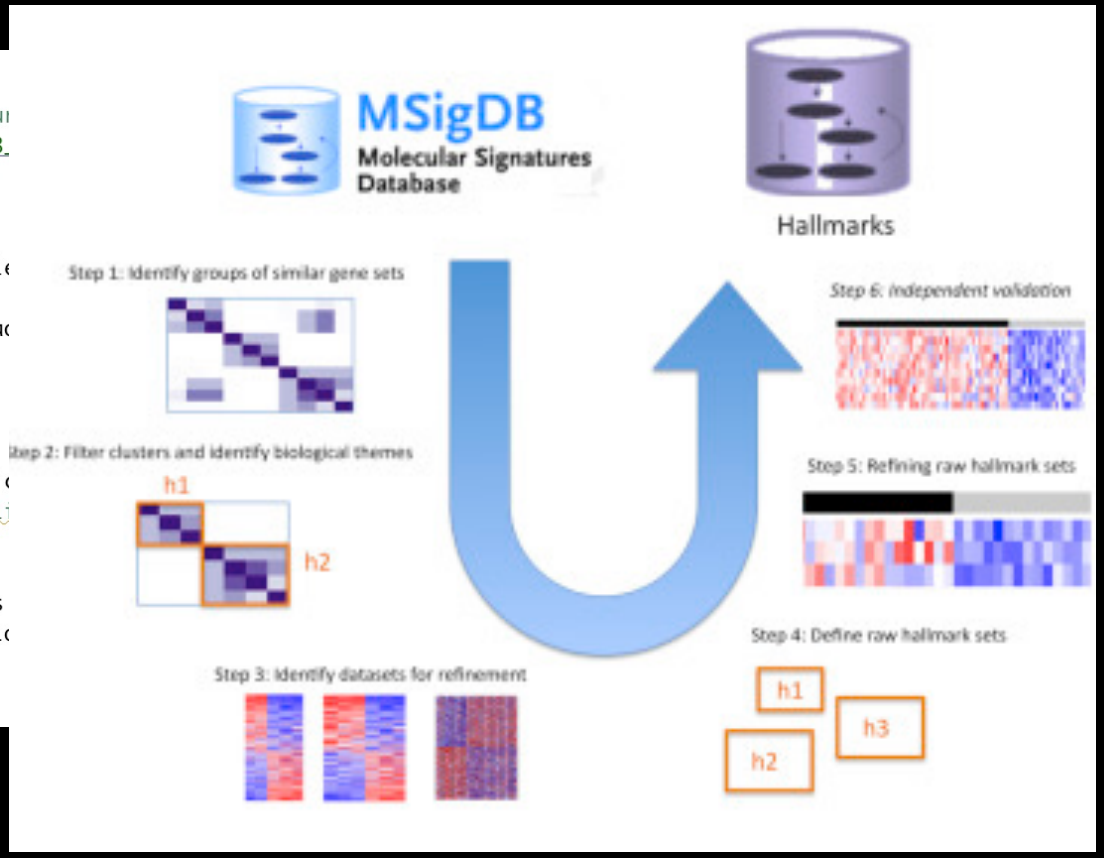
Analyze Seurat Object

```
#Process files according to standard Seurat pipeline, scaling out mitochondrial reads
#so as not to be used in defining variable features for downstream analysis
colon <- colon %>%
  Seurat::NormalizeData() %>%
  Seurat::FindVariableFeatures() %>%
  Seurat::ScaleData(vars.to.regress = c("percent.mt"))
colon <- Seurat::RunPCA(colon, npcs = 100, features = VariableFeatures(colon))
##Use Elbowplot to find 'inflection' point in PCs to use as cutoff
ElbowPlot(colon, ndims = 100)
##Number of dims = number of PCs used; if you wanted to use first 50 PCs, would imput 1:50 instead of 1:30
colon <- FindNeighbors(colon, dims = 1:30)
##Increasing resolution will increase the number of clusters identified by Seurat
colon <- FindClusters(colon, resolution = 0.5)
colon <- RunUMAP(colon, dims = 1:30)
```

Annotate Cell Identity

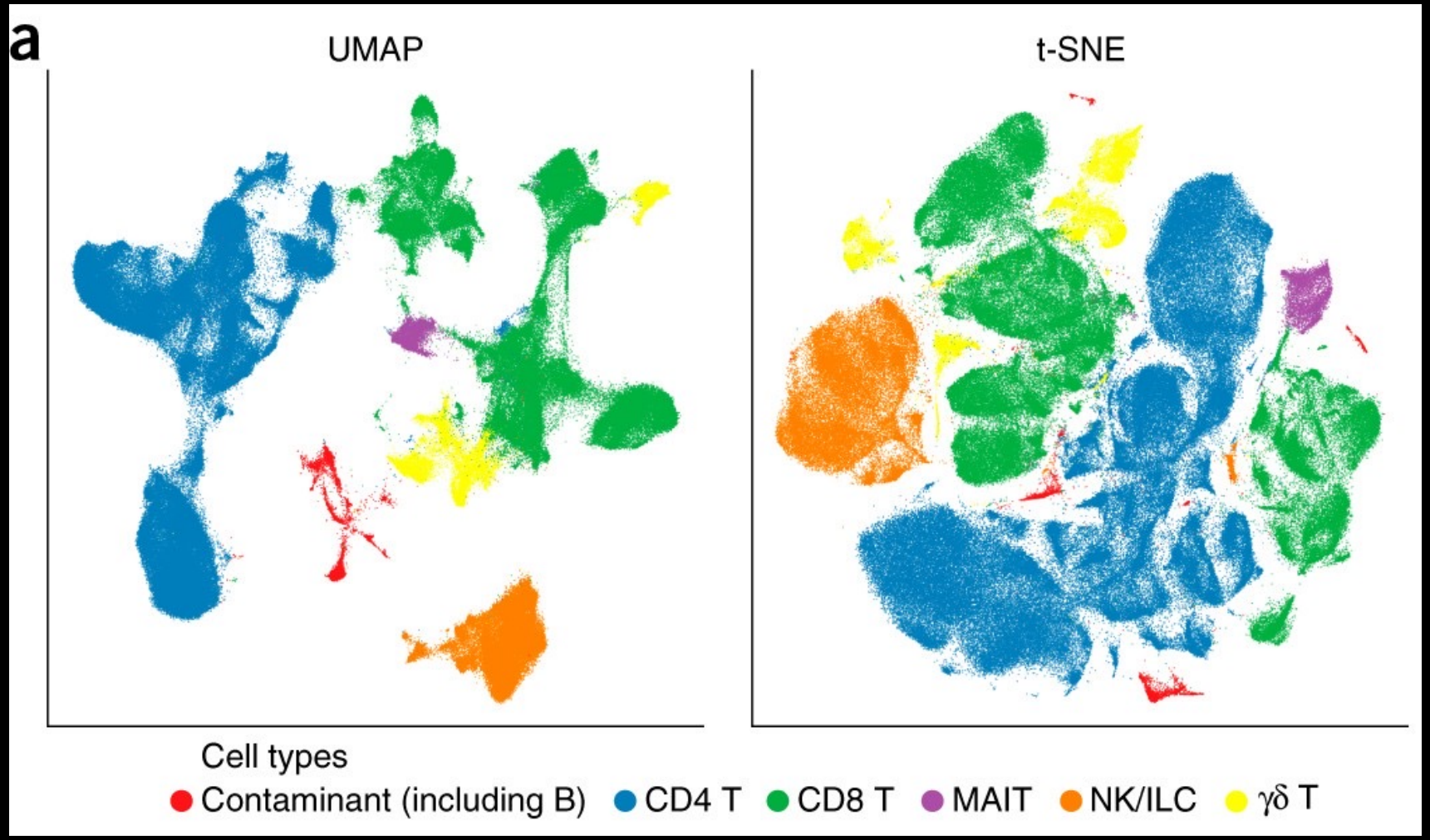
```r
#Score cells for HALLMARK signat
##downloads hallmark signatures
msigdf <- msigdbr::msigdbr(speci
##creates hallmark signatures ir
HallmarkDF <- split(x = msigdf$g
HallmarkDF <- as.list(HallmarkDF
##scores cells against hallmark
HGT_Hallmark <- RunCellHGT(color
##loads scores back into Seurat
colon@assays[["Hallmark"]] <- Cr
DefaultAssay(colon) <- "Hallmark
```

```r
#label cells with CellID
##reads in Panglao dataset containing cell specific signatur
panglao <- read_tsv("https://panglaodb.se/markers/PanglaoDB_
##filtering dataset for signatuers associated for GI tract
panglao_colon <- panglao %>% filter(organ == "GI tract")
##restricting to mouse specific genes
panglao_colon <- panglao_colon %>%  filter(str_detect(specie
##ensuring signatures are calcualted same way as our enes
panglao_colon$`official gene symbol` <- str_to_title(panglao
##converts dataframe to list
panglao_colon <- panglao_colon %>%
  group_by(`cell type`) %>%
  summarise(geneset = list(`official gene symbol`))
colon_gs <- setNames(panglao_colon$geneset, panglao_colon$`c
##creates MCA (multiple correspondence analysis dimensionali
colon <- RunMCA(colon)
##Runs prediction algorithm
HGT_colon_gs <- RunCellHGT(colon, pathways = colon_gs, dims
colon_gs_prediction <- rownames(HGT_colon_gs)[apply(HGT_colo
##adds annotations to Seurat object
colon$colon_gs_prediction <- colon_gs_prediction
```

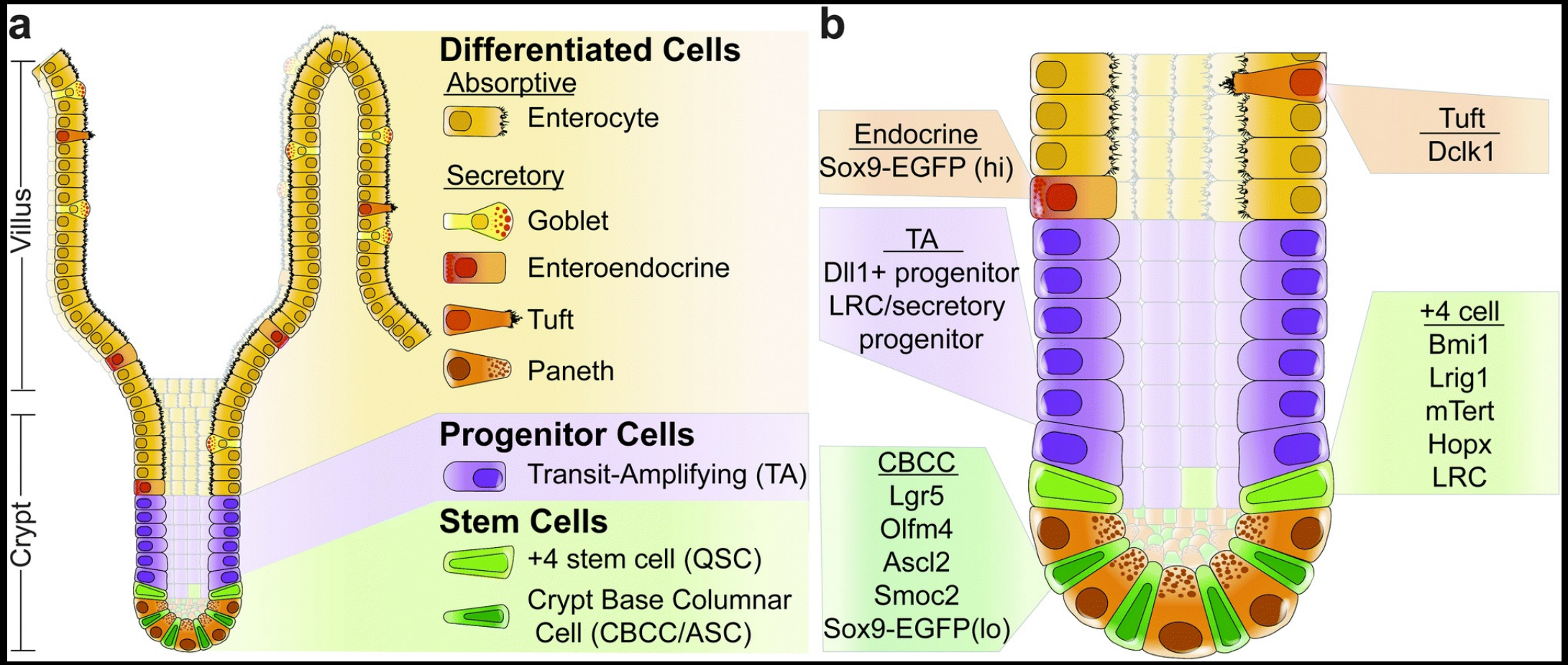


What is UMAP?

- **UMAP**: Uniform Manifold Approximation and Projection
  - X/Y axis: Dimensional Representations
  - Dot: Cell
  - Clusters: Similar cells

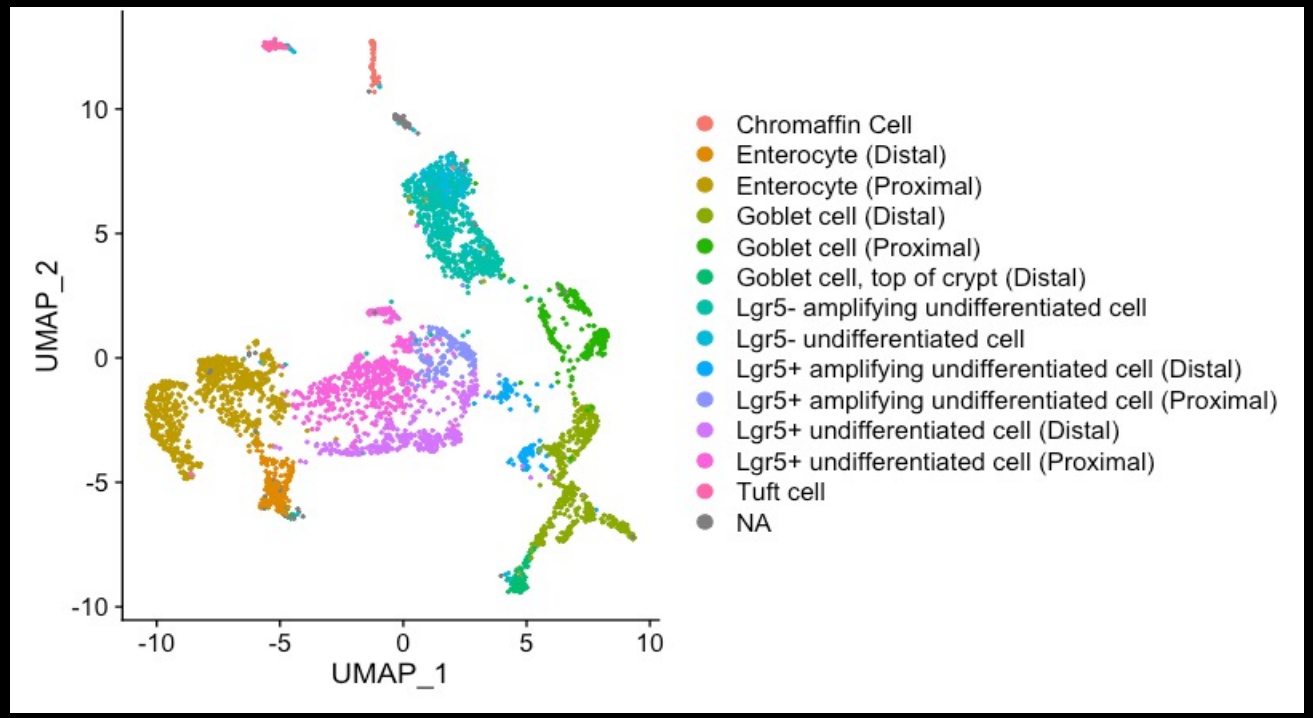


Colon Crypt Organization

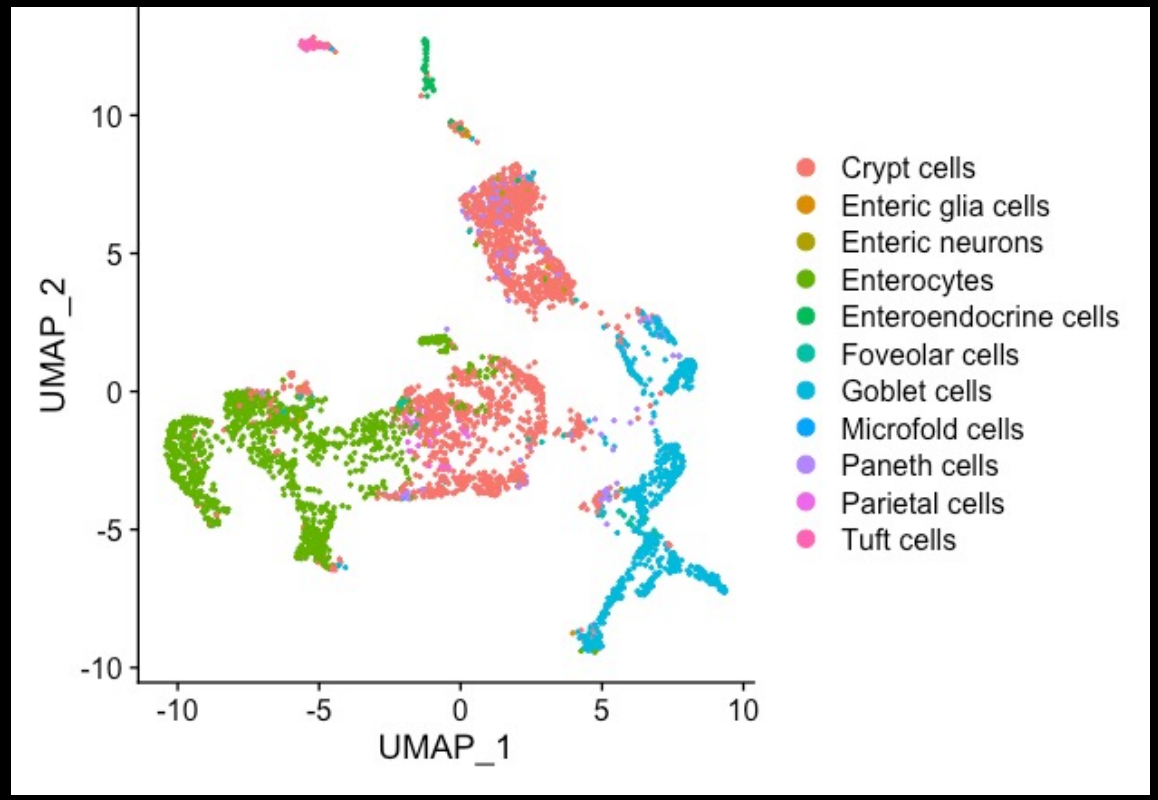


Analyze Cell Identity

Tabula Muris Annotations



Cell-ID Annotations



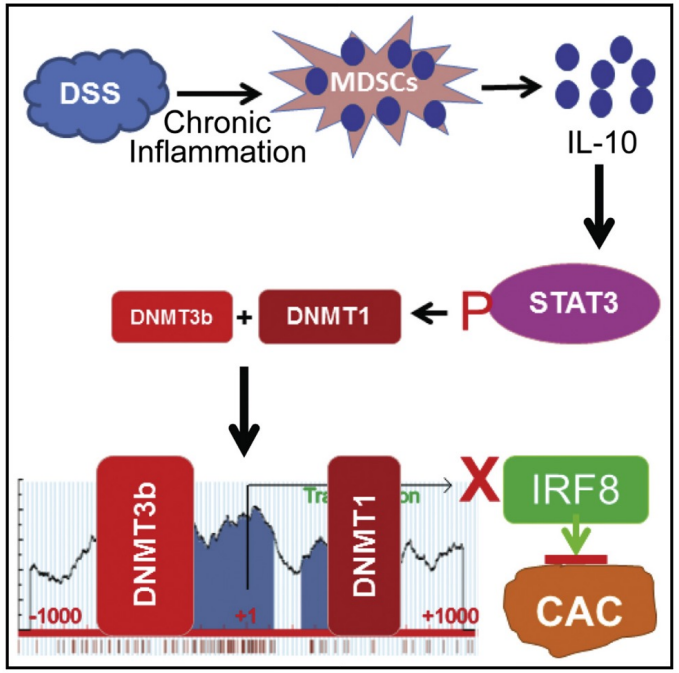
Analyze Gene Expression

Article

Cell Reports

Myeloid-Derived Suppressor Cells Produce IL-10 to Elicit DNMT3b-Dependent IRF8 Silencing to Promote Colitis-Associated Colon Tumorigenesis

Graphical Abstract



Authors

Mohammed L. Ibrahim, John D. Klement, Chunwan Lu, ..., Phillip J. Buckhaults, Herbert C. Morse III, Kebin Liu

Correspondence

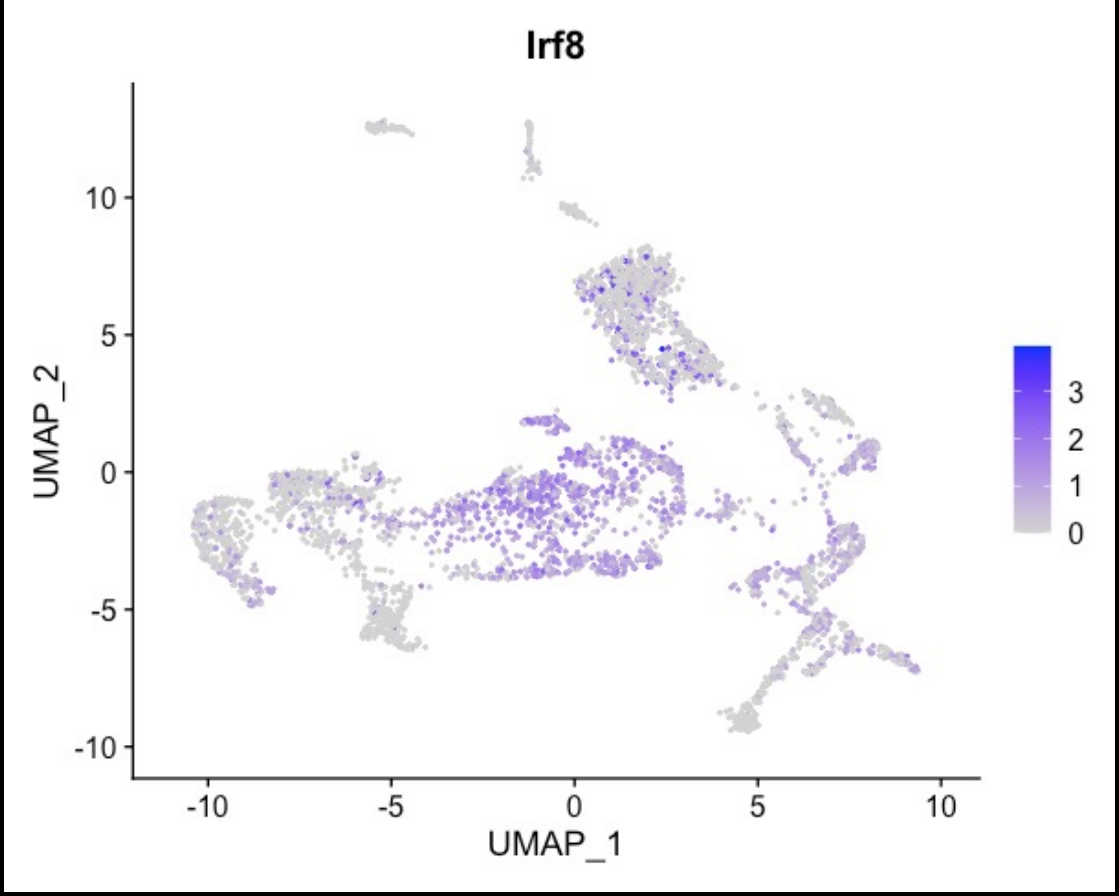
kliu@augusta.edu

In Brief

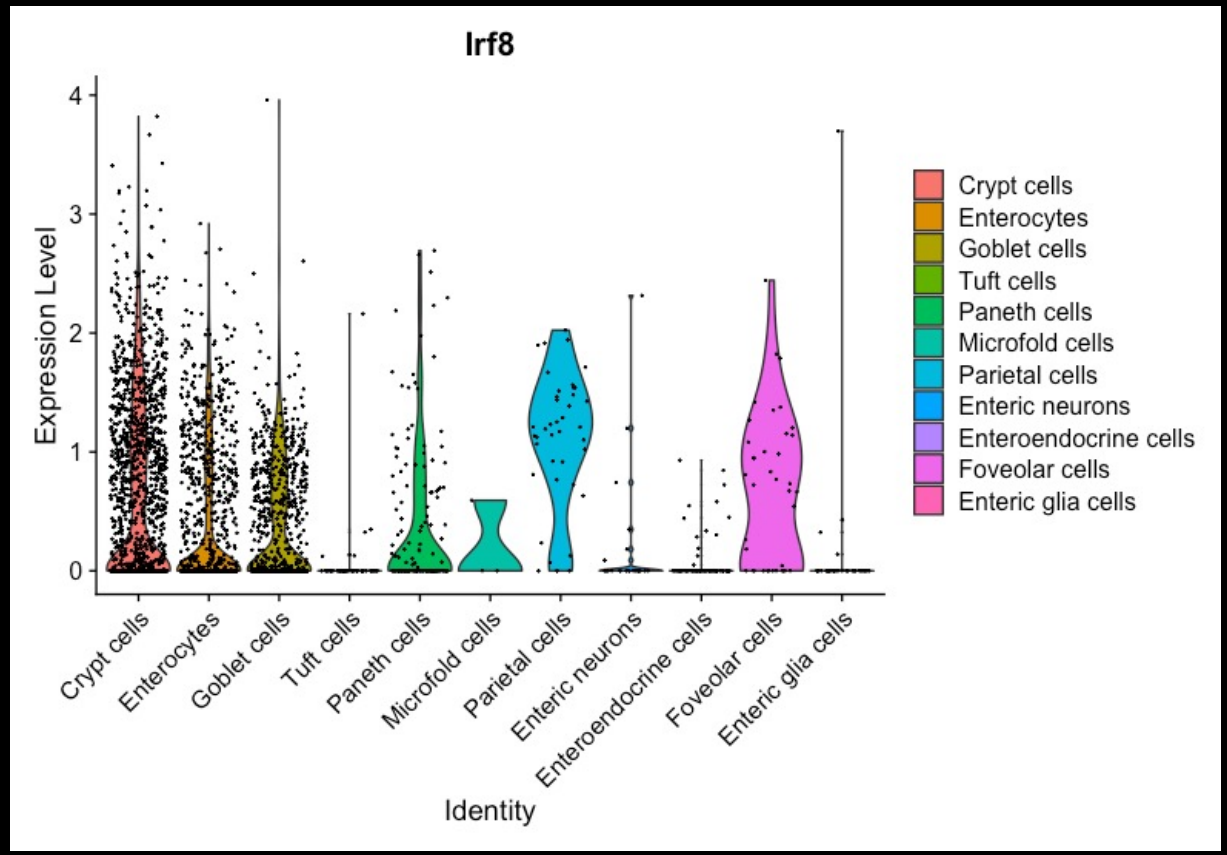
Ibrahim et al. report that chronic inflammation induces colonic accumulation of myeloid-derived suppressor cells (MDSCs) that upregulates IL-10. IL-10 directly regulates STAT3 activation to upregulate DNMT3b to silence tumor suppressor IRF8 in colonic epithelial cells. The MDSC-IL-10-STAT3-DNMT3b-IRF8 pathway links chronic inflammation to colon cancer initiation.

How does IRF8 mediate its function as a tumor suppressor?

Where is IRF8 expressed?

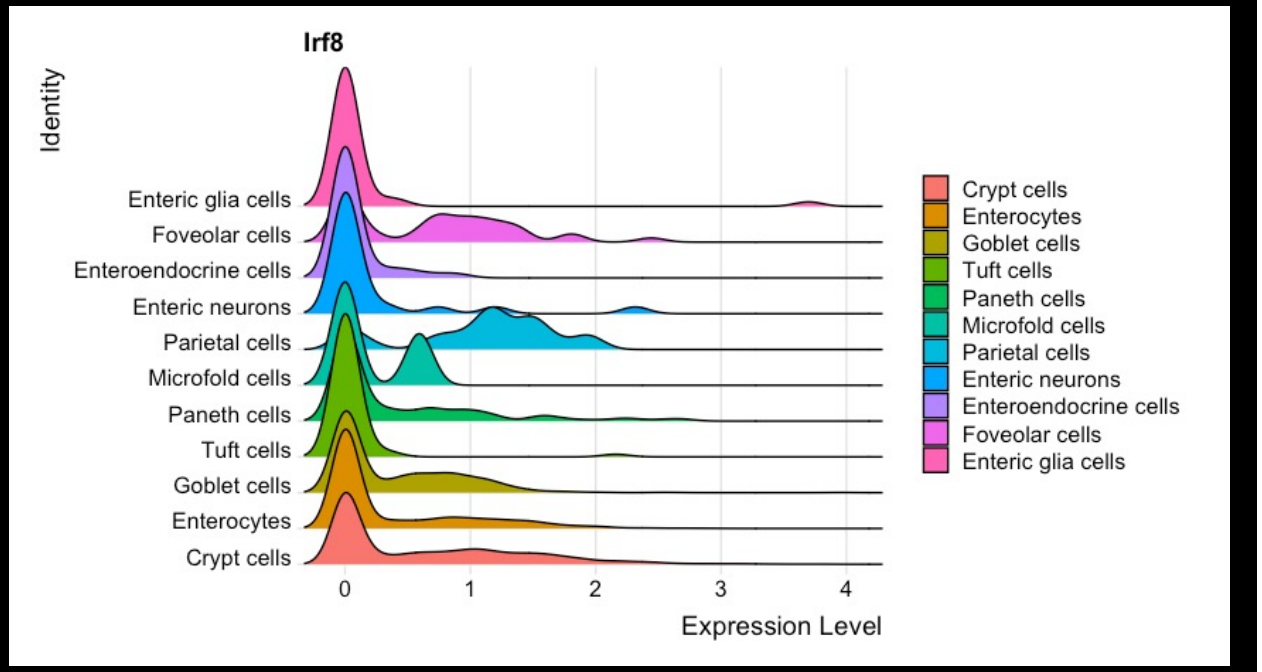



```
FeaturePlot(colon, "Irf8")
```

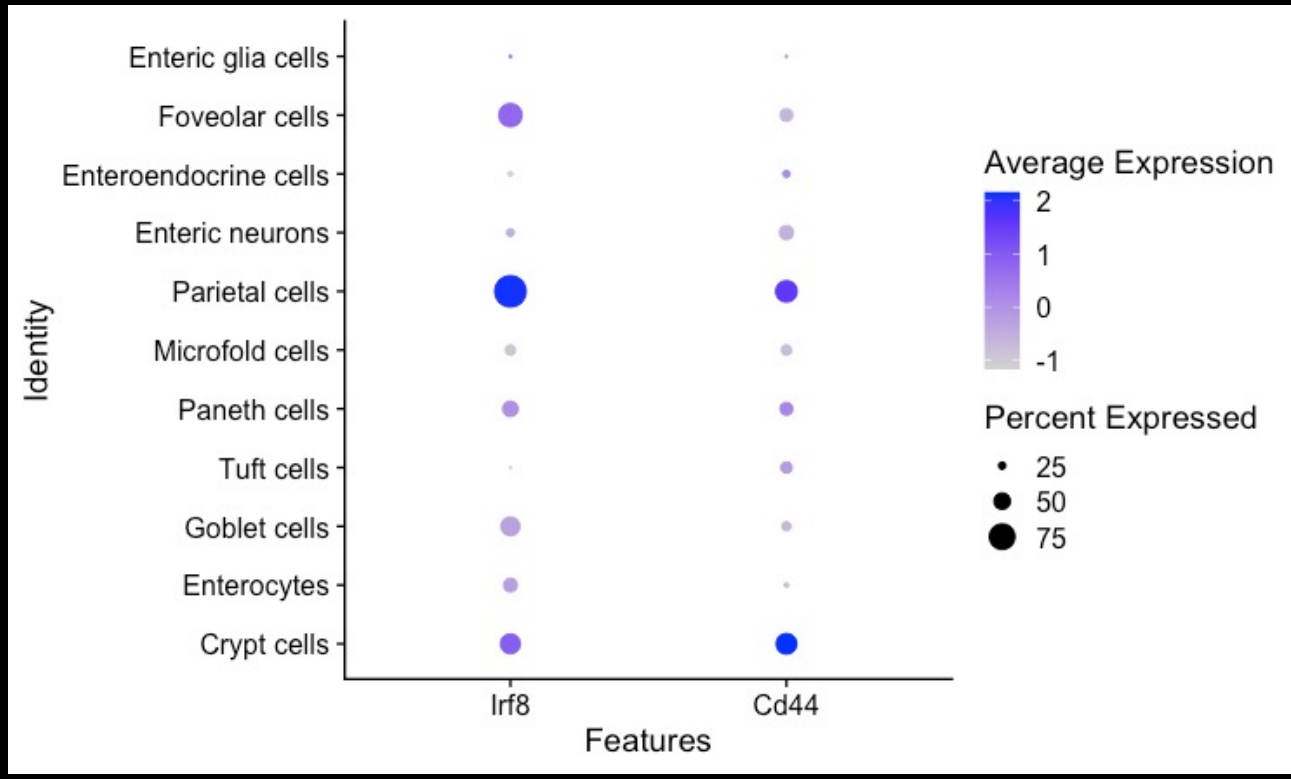



```
VlnPlot(colon, "Irf8")
```

Where is IRF8 expressed?

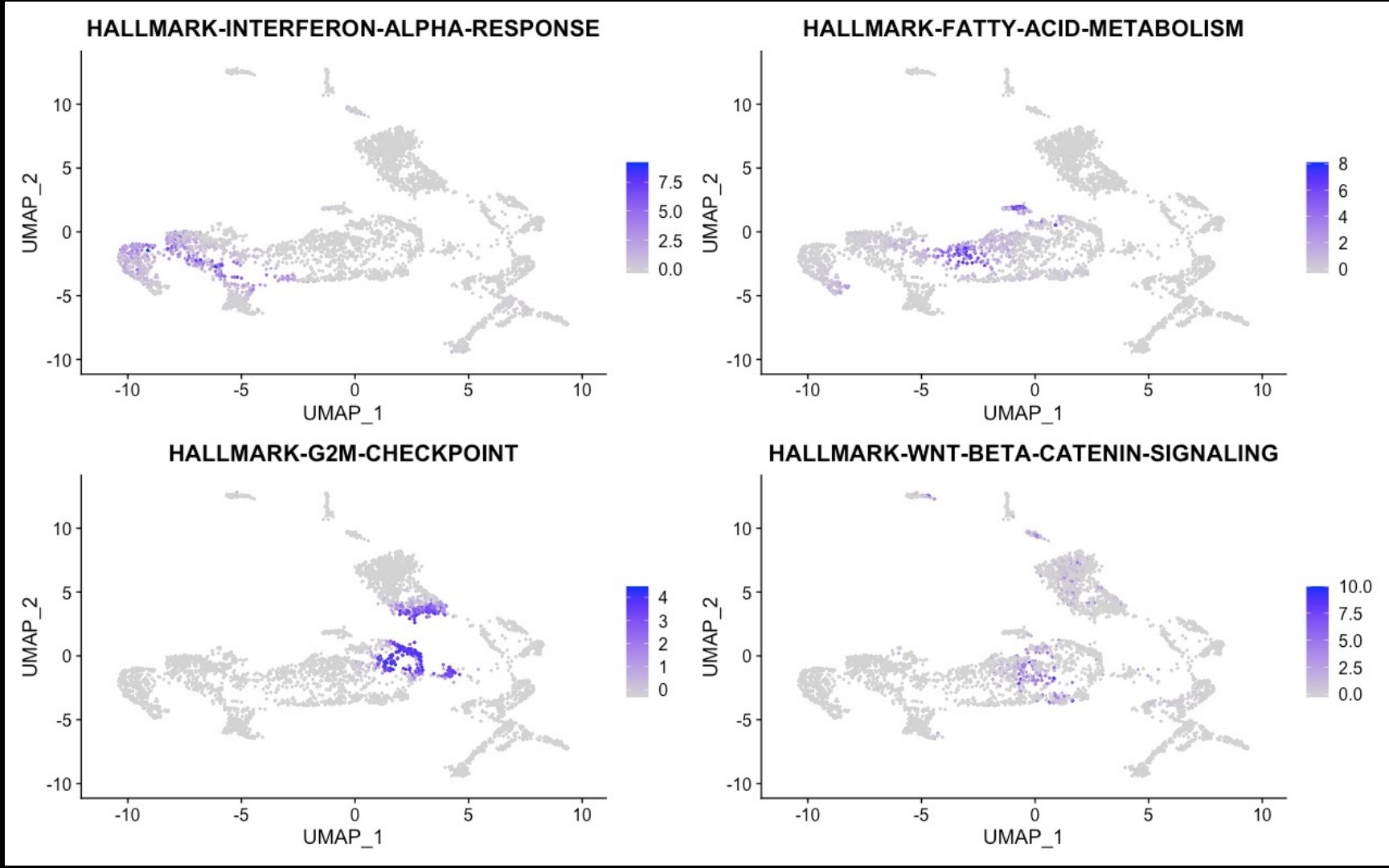



```
RidgePlot(colon, "Irf8")
```




```
DotPlot(colon, features = c("Irf8", "Cd44"))
```

Analyze Gene Signature Expression

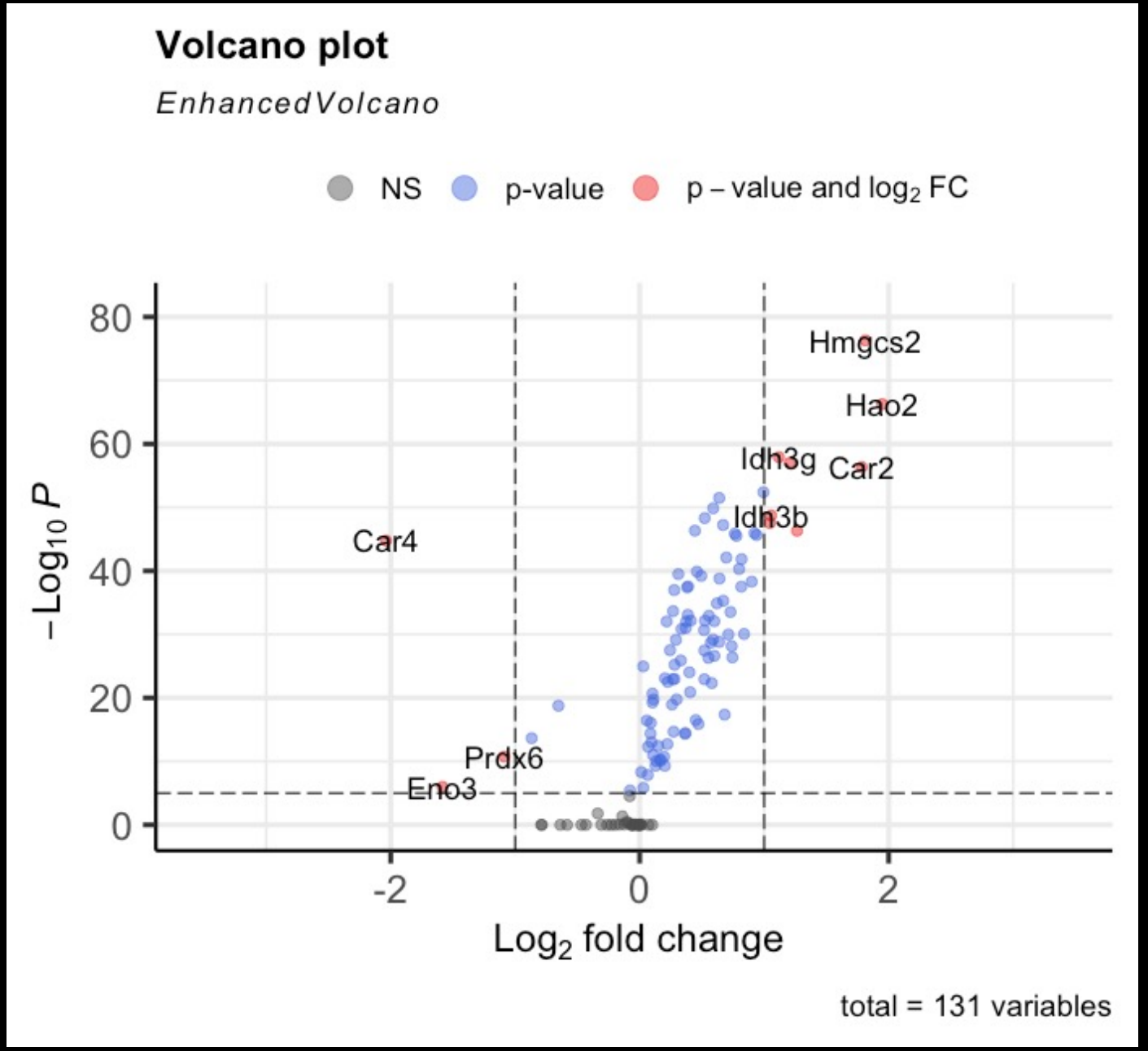


Is there is a difference between IRF8+ and IRF8- crypt cells?

10 lines of code…

```r
#Vln Plot differences
Idents(colon) <- "colon_gs_prediction"
DefaultAssay(colon) <- "RNA"
FeaturePlot(colon, "Irf8")
entero <- subset(colon, idents = "Crypt Cells")
irf8.mean <- mean(entero[["RNA"]]@data["Irf8",])
entero$irf8 <- entero[["RNA"]]@data["Irf8",] > irf8.mean
DefaultAssay(entero) <- "Hallmark"
Idents(entero) <- "irf8"
irf8.markers <- Seurat::FindMarkers(entero, ident.1 = T, ident.2 = F)
DoHeatmap(entero, features = entero@assays[["Hallmark"]]@counts@Dimnames[[1]],
          slot = "scale.data", label = F) + viridis::scale_fill_viridis()
```

What genes drive the difference in FA Oxidation?



Misconceptions

- *I need a supercomputer to do any real analysis*
  - Most analyses can be run on desktop computers that we use in lab
- *It will take days to analyze a single dataset*
  - Generally can be accomplished in minutes to hours
- *I need to have extensive coding experience*
  - Use of pre-made packages in R make data analysis and visualization easy
- *There's not a dataset relevant to the question that I am asking*
  - With hundreds of human and mouse datasets, it's likely that there is an easily accessible dataset suitable for exploratory analysis

PowerPoint and all script used to generate figures

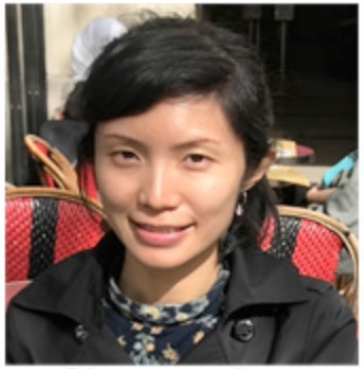
https://github.com/jdklement/Seminar_210604

Email: jklement@augusta.edu

Thank you!

Dr. Kebin Liu, PI

Dafeng Yang
Lab Manager



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