

Lecture 17: Single Cell Analysis (part IV)

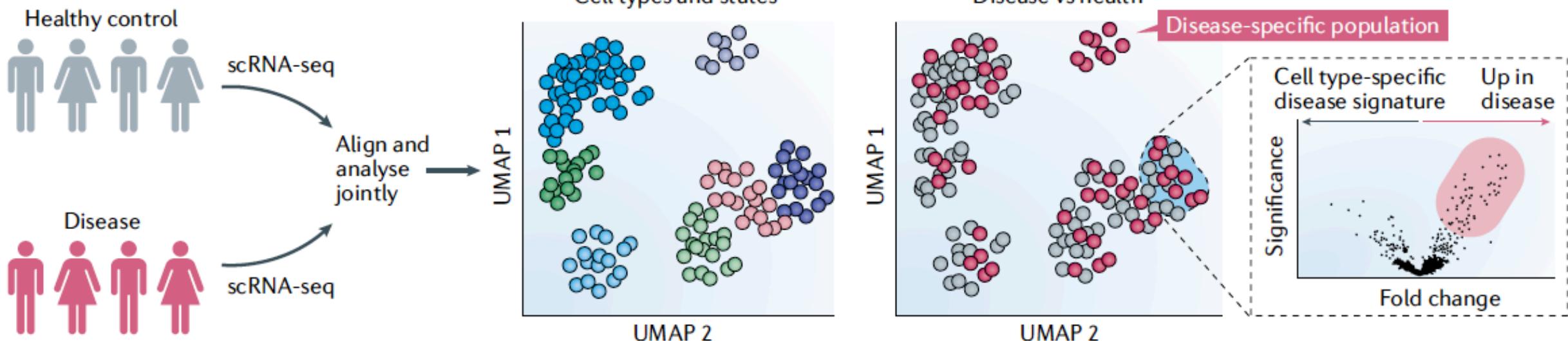
Prof. Daniel Kim

BME 110

Winter 2021

3.9.21

d Single-cell assessment of disease states



The Single Cell PORTAL is a BETA platform designed to reduce barriers and accelerate single-cell research. It features a collection of 321 studies involving 12,492,801 cells. The platform includes search functions for studies and genes, and a dedicated section for COVID-19 studies.

Single Cell PORTAL BETA

Help ▾ Sign In

Featuring 321 studies 12,492,801 cells

Reducing barriers and accelerating single-cell research

Search Studies

Search Genes

COVID-19 Studies

Enter keyword

Most Popular

Most Recent

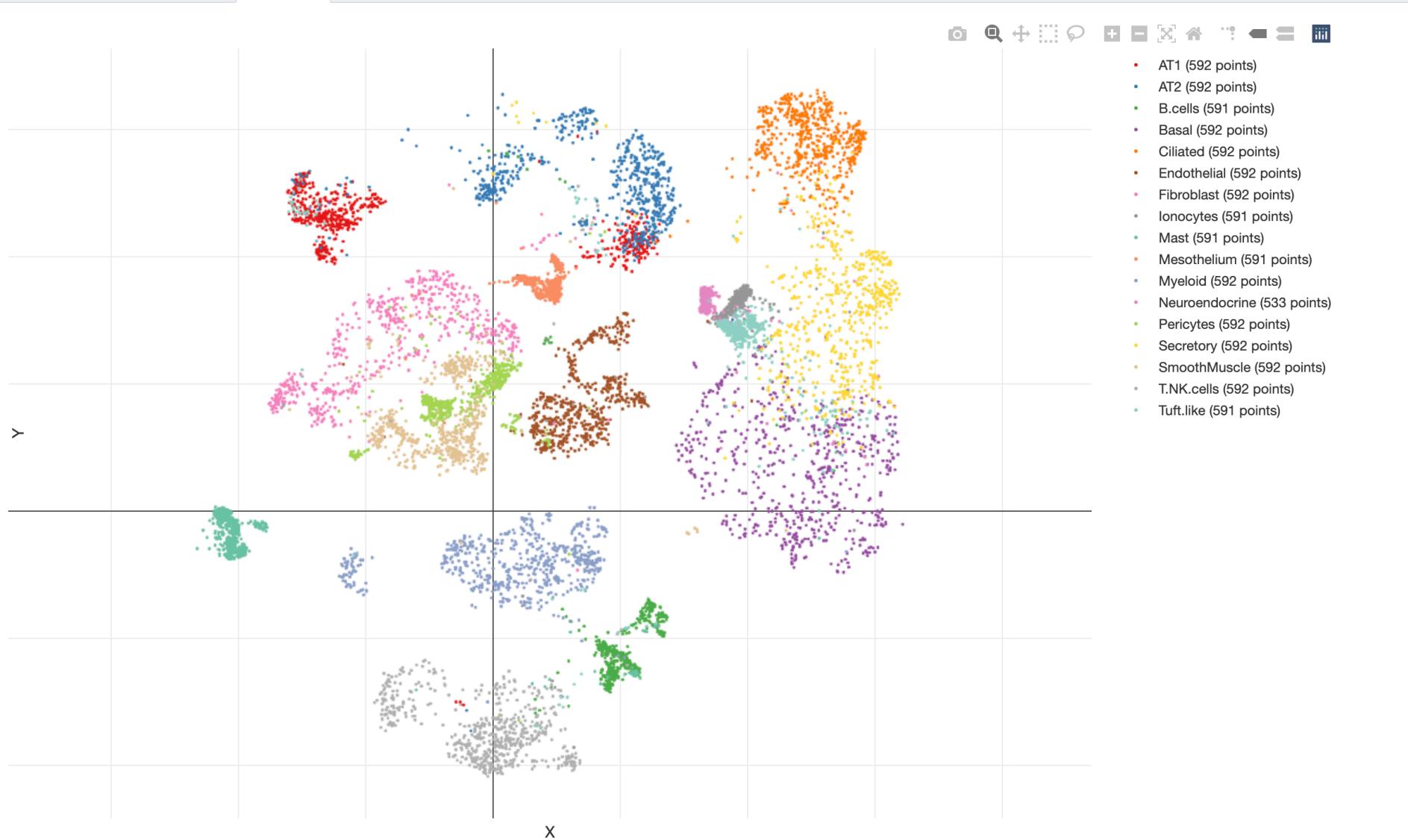
Advanced Search BETA

Study: HCA LungMAP COVID-19 Internal Nonsmokers Lung 277224 cells[Summary](#)[Explore](#)[Download](#)

Search genes



Clusters

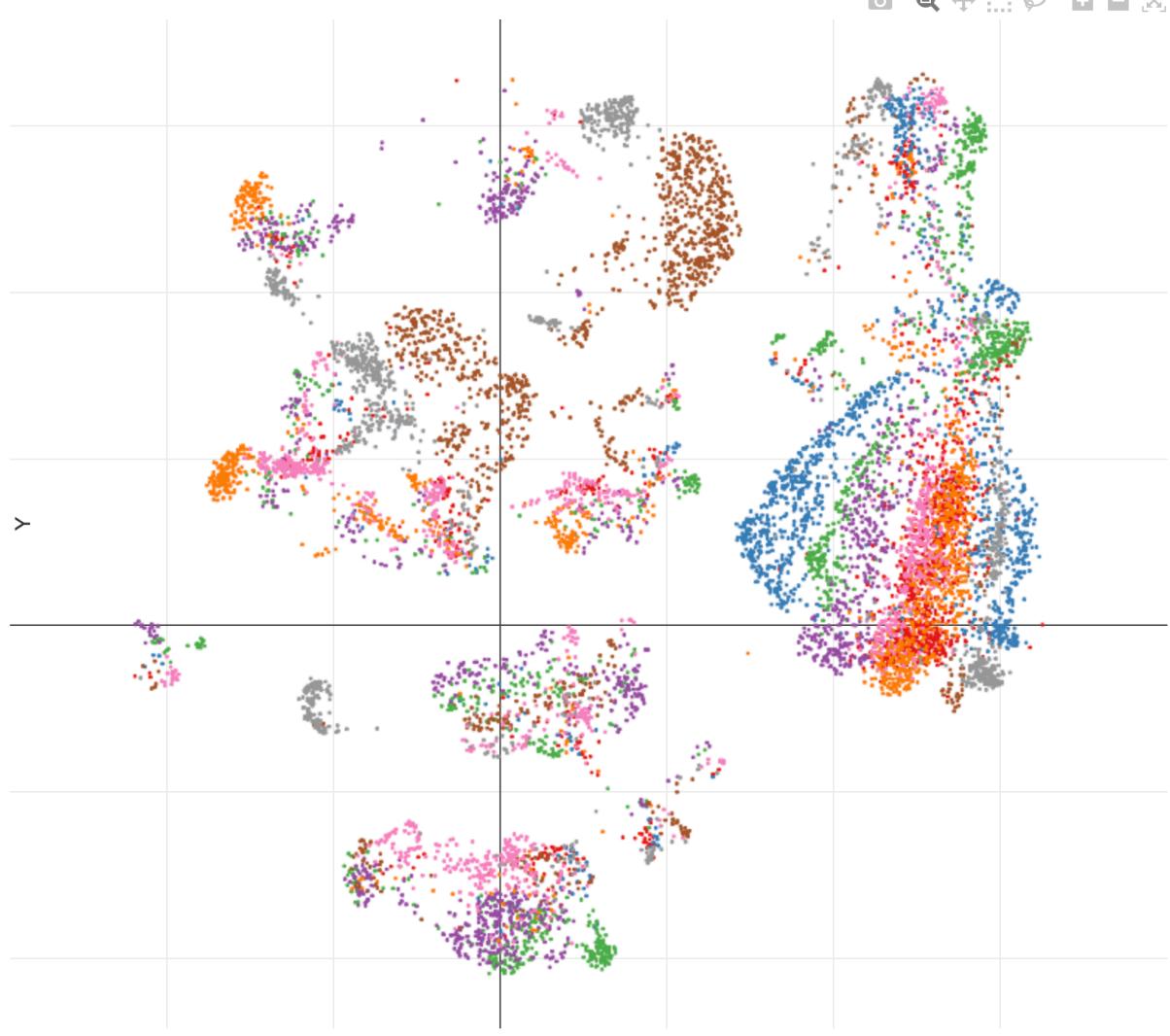
[View Options](#)

Study: HCA LungMAP COVID-19 Internal Nonsmokers Lung 277224 cells[Summary](#)[Explore](#)[Download](#)

Search genes



Clusters

[View Options](#)

- 0 (1250 points)
- 1 (1250 points)
- 2 (1250 points)
- 3 (1250 points)
- 4 (1250 points)
- 5 (1250 points)
- 6 (1250 points)
- 7 (1250 points)

[Load cluster](#)

UMAP

[Select annotation](#)

Patient_ID

[Subsampling threshold](#) ?

10000

[Distribution](#)[Scatter](#)[Heatmap](#)

Gene Expression for ACE2

[Summary](#)[Explore](#)[Download](#)

ACE2



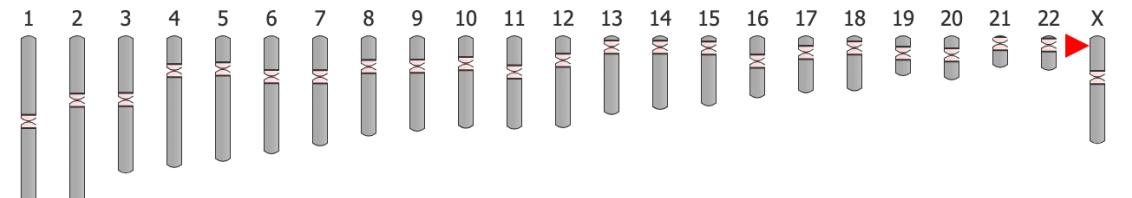
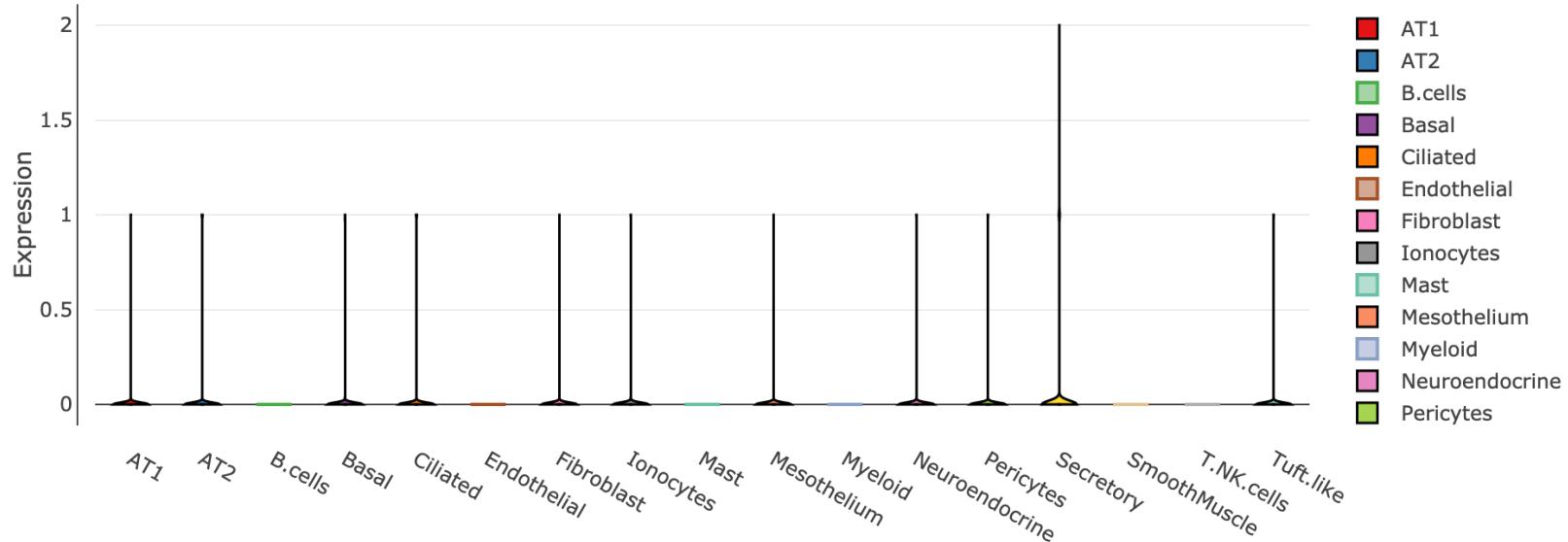
Distribution

Scatter

[View Options](#)**Related genes**

Click gene to search

- ▶ Interacting gene
- ▶ Paralogous gene
- ▶ Searched gene

**UMAP****Load cluster**

UMAP

Select annotation

original_cell_subset

Subsampling threshold ⓘ

10000

Distribution**Plot type** ⓘ

Violin plot

Data points ⓘ

All

 Toggle Annotations**Scatter****Heatmap**

Gene Expression for ACE2

Summary

Explore

Download

ACE2



Distribution

Scatter

View Options

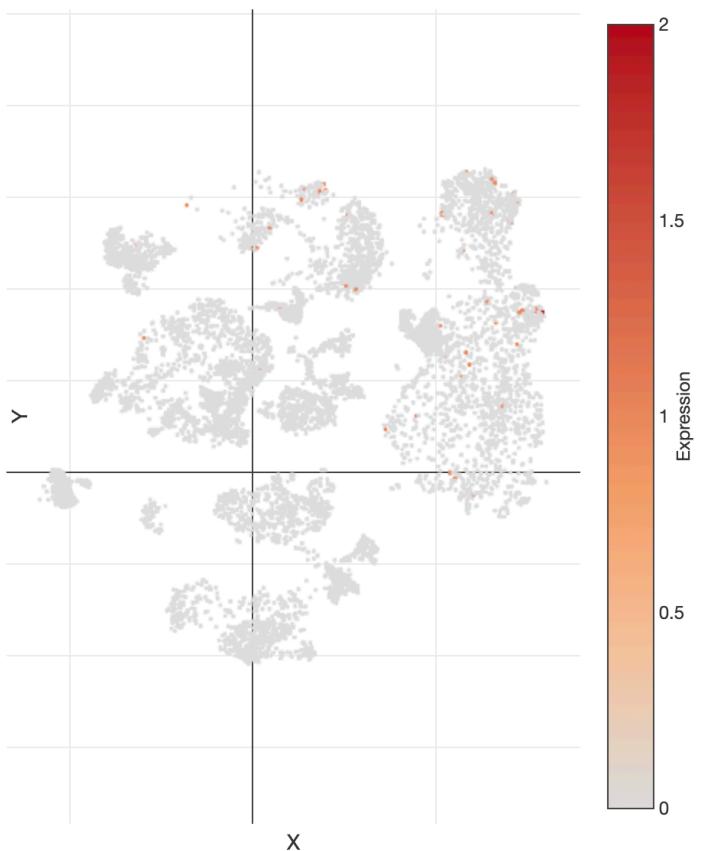
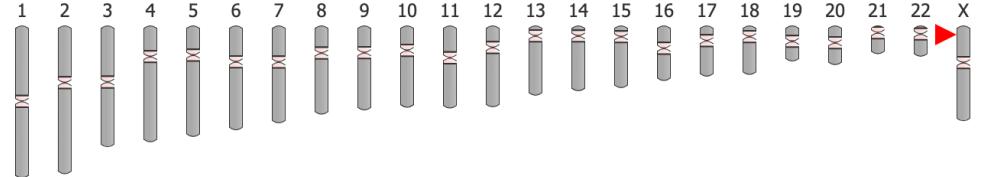
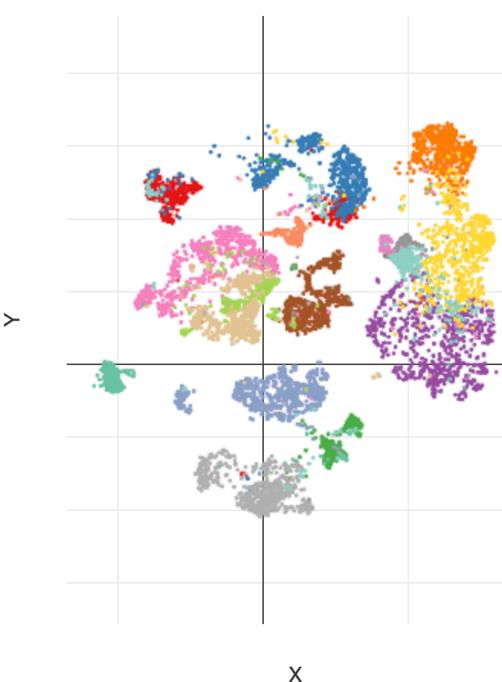
Related genes

Click gene to search

▶ Interacting gene

▶ Paralogous gene

▶ Searched gene

UMAP
original_cell_subset

Load cluster

UMAP

Select annotation

original_cell_subset

Subsampling threshold ?

10000

▶ Distribution

▶ Scatter

▶ Heatmap

Based on HCA LungMap (Regev et al.), is ACE2 expression higher in the lung cells of smokers or non-smokers?

Which cell type in the colon has the highest levels of ACE2 expression?

Table 1 | SARS-CoV-2 infection in humans and in animal models

Trait	Organism
Virus replication	
Upper respiratory tract	Humans, mice, ferrets, non-human primates, mink, cats and bats
Lower respiratory tract	Humans, mice, hamsters, ferrets and non-human primates
Other organs	Humans (GI tract, CNS and kidney), hACE2 mice (CNS), hamsters, ferrets and non-human primates (GI tract)

Single cell resources at UCSC

Single cell experiments measure tens of thousands of gene loci in thousands of cells at the same time. That resulting data is challenging to visualize. The UCSC Genome Browser is adding published datasets as tracks and an entirely new data exploration tool:

■ UCSC Cell Browser

■ Tabula Muris

Interactively color dimensionality reduction plots by cell annotation or genes

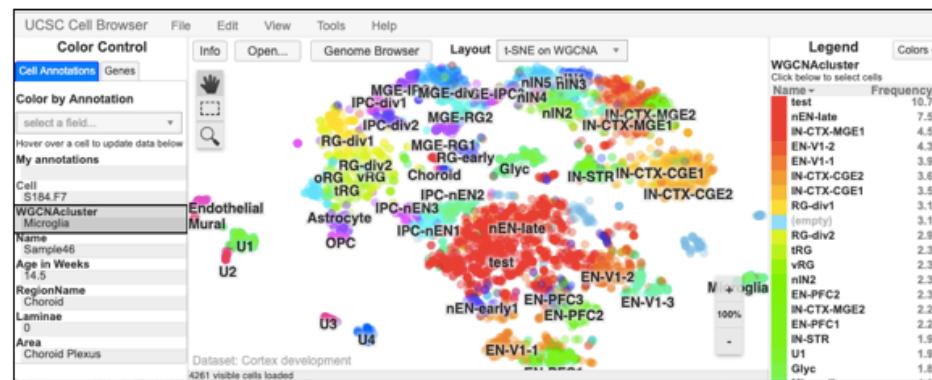
Genome Browser track showing single cell RNA-seq gene expression, genome coverage and splice sites

UCSC Cell Browser

The [UCSC Cell Browser](#) is a fast, lightweight viewer for single-cell data. You can:

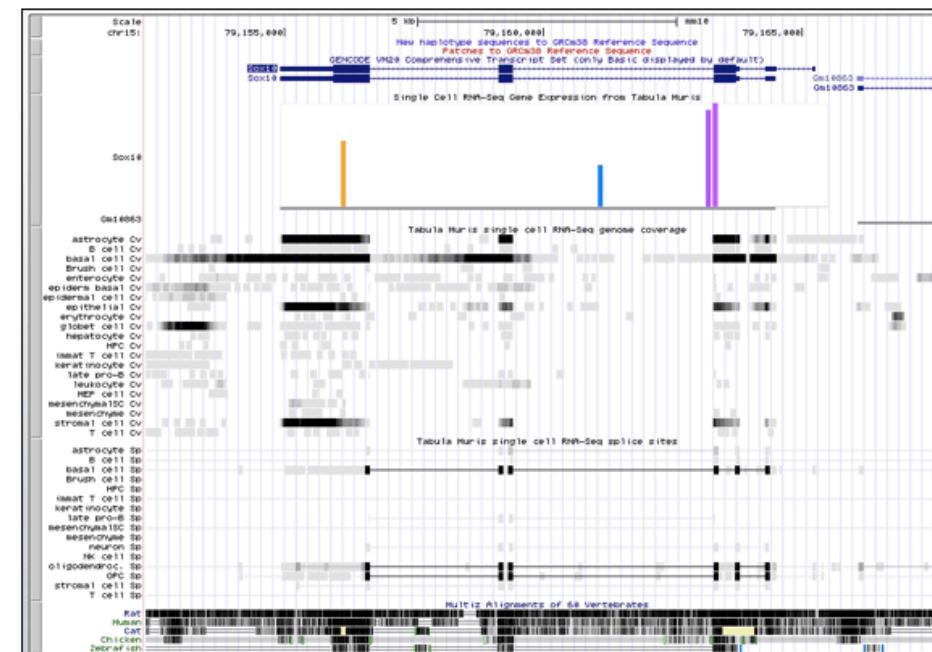
- View 2D plots of cells arranged by algorithms such as t-SNE or UMAP
- Color cells by metadata and gene expression
- View cluster marker genes and selected dataset-relevant genes
- View heatmap showing selected gene expression across clusters
- Rename clusters and add custom annotations to a selected set of cells

Contact us at cells@ucsc.edu to request new datasets or suggest improvements. To set up your own cell browser, follow our [installation and set up instructions](#).



Single cell datasets in the UCSC Genome Browser

Below is a screenshot of a [public session](#) that highlights the Tabula Muris gene expression track. The session shows the Sox10 locus. The gene expression track at the top shows that the gene is only well expressed in basal cells (orange), mammary gland (blue) and oligodendrocytes (pink). These cell type names are shown when you hover with the mouse over the bars on the genome browser. The coverage tracks below show that all exons are transcribed in these, but the last exon is also transcribed in stromal cells and some non-coding transcription is seen in goblet cells 3' of the gene. The splicing tracks below show that proper splicing is limited to the four cell types described above. This is also a non-coding region conserved in zebrafish and chicken.





Genomes

Genome Browser

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UCSC Genome Browser on Mouse Dec. 2011 (GRCm38/mm10) Assembly

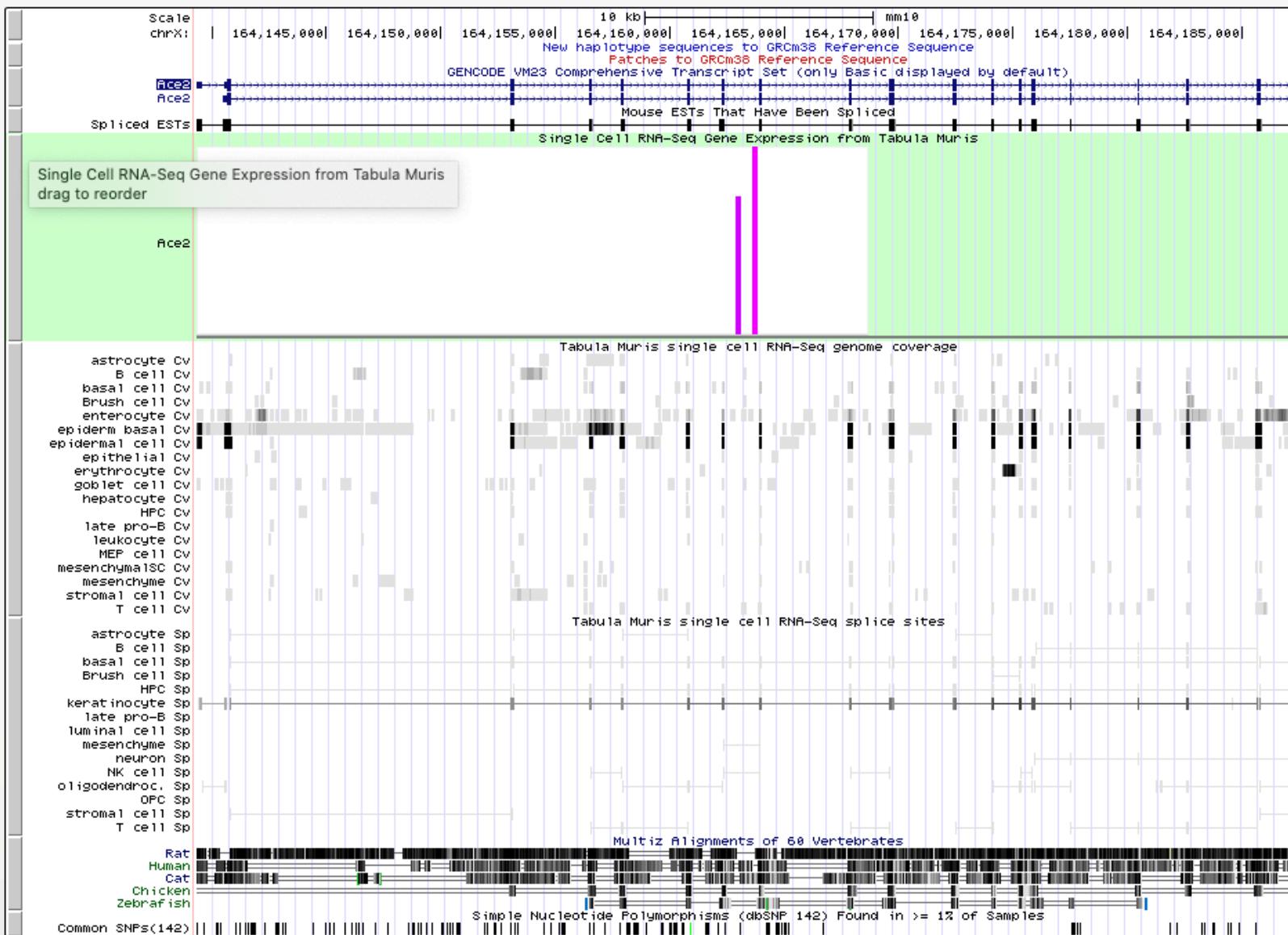
move <<< << < > >> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x 100x

chrX:164,139,332-164,188,420 49,089 bp.

enter position, gene symbol or search terms

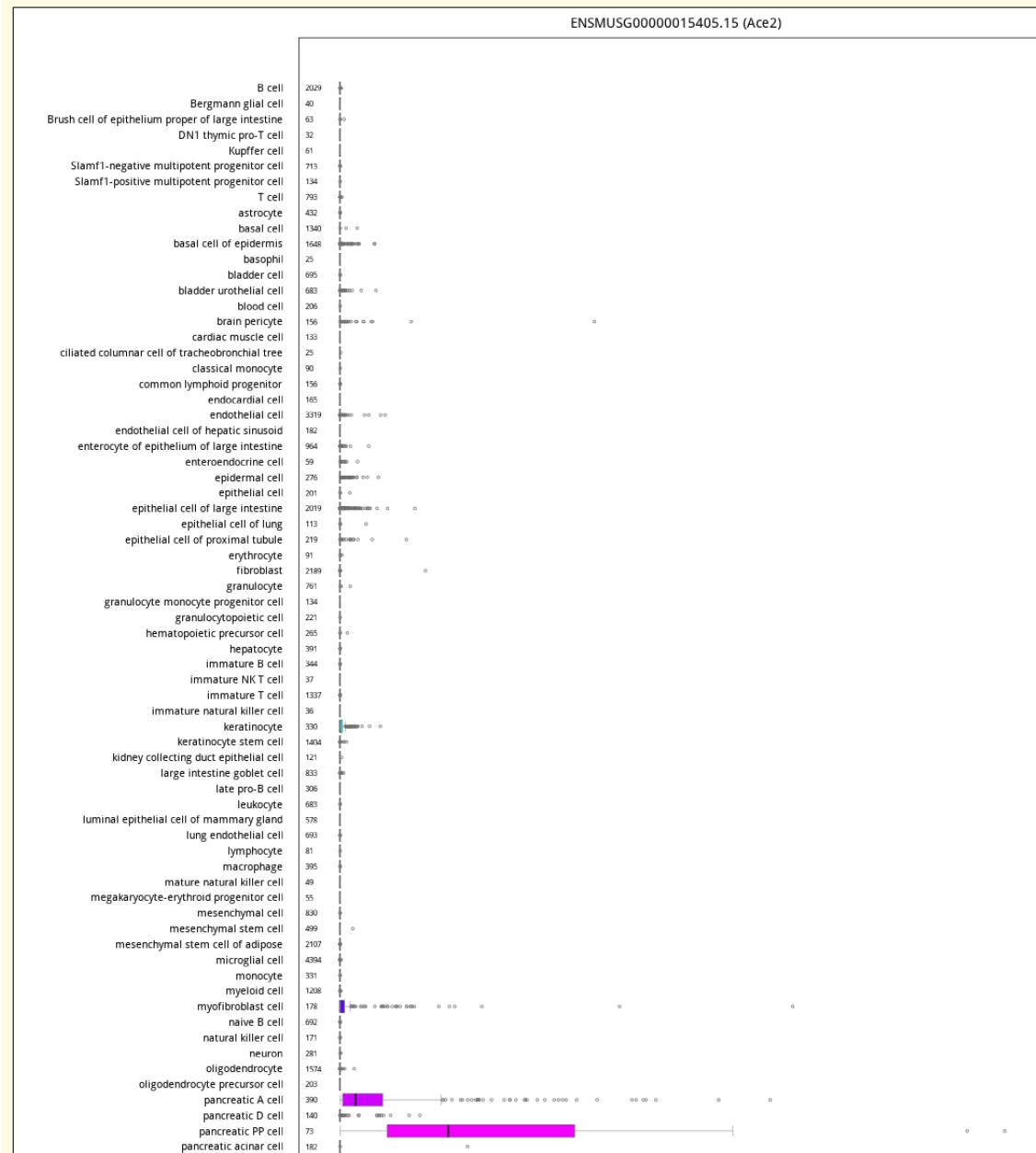
go

chrX (qF5) XqA1.1 XqA2 XqA4 XqA5 XqA6 XqA7.1 XqB XqC1 XqC3 XqD XqE1 XqE3 XqF1 XqF2 XqF3 XqF4 XqF5



Single Cell RNA-Seq Gene Expression from Tabula Muris (ENSMUSG00000015405.15)

Gene identifier: ENSMUSG00000015405.15
On UCSC Cell Browser: [Ace2](#)
Total all median values: 1601.00 TPM
Maximum median value: 1400.00 TPM in pancreatic_PP_cell
Score: 79
Genomic position: mm10 [chrX:164139332-164188420](#)
Strand: +



Single-cell RNA-Seq mRNA baseline

Number of cells: 1,144

Organism: *Homo sapiens*

Publication:

- Lawlor N, George J, Bolisetty M, Kursawe R, Sun L et al. (2017) [Single-cell transcriptomes identify human islet cell signatures and reveal cell-type-specific expression changes in type 2 diabetes](#).



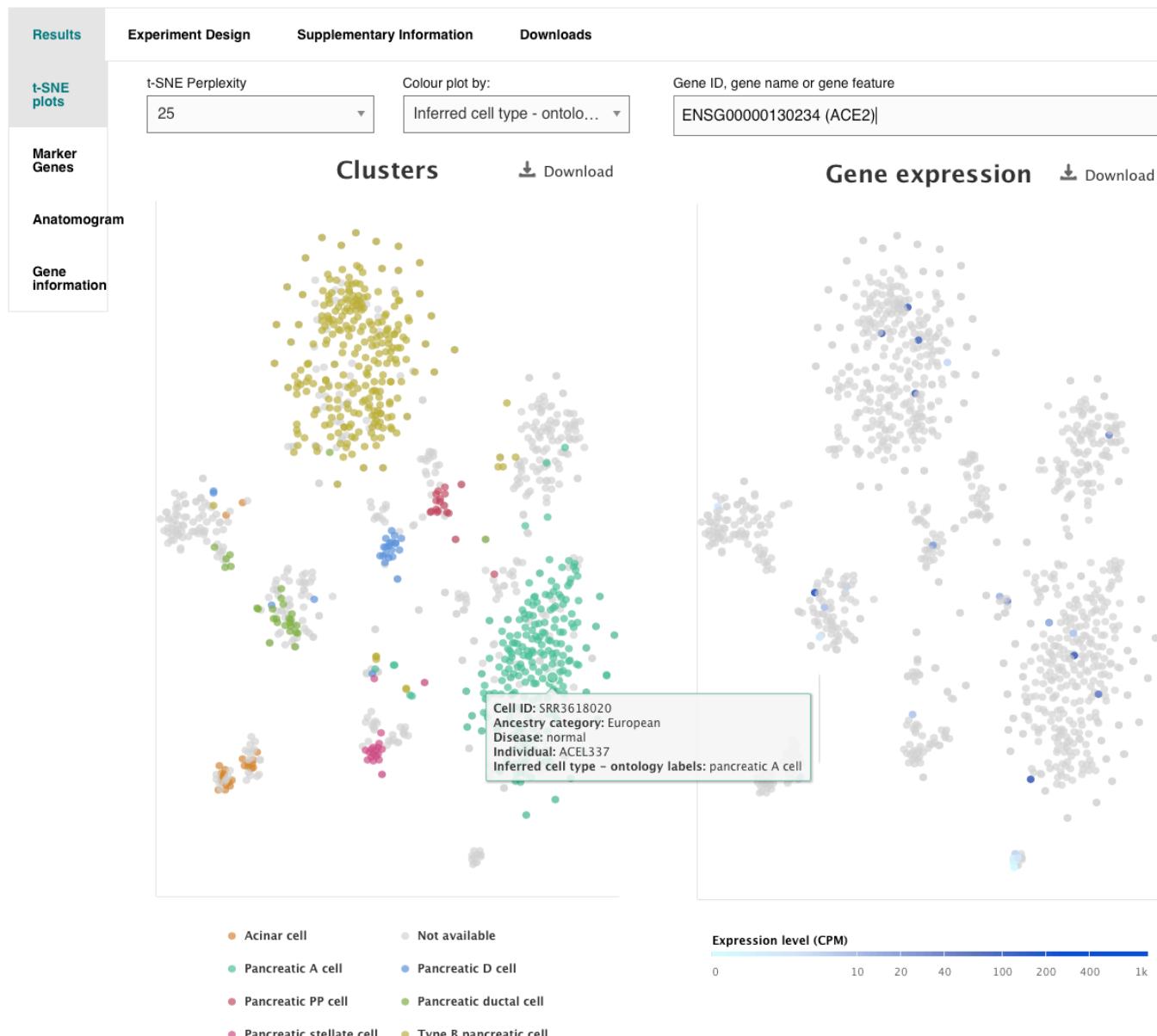
Single-cell RNA-Seq mRNA baseline

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Tabula Muris single cell RNA-Seq genome coverageTrack collection: ▲[Single Cell FACS RNA-Seq of 44,779 cells from Tabula Muris](#) **Description** **All tracks in this collection (3)****Display mode:** [Reset to defaults](#)**Type of graph:** **Track height:** pixels (range: 11 to 128)**Data view scaling:** **Always include zero:** **Vertical viewing range:** min: max: (range: 0 to 127)**Transform function:** Transform data points by: **Windowing function:** **Smoothing window:** pixels**Negate values:** **Draw y indicator lines:** at y = 0.0: at y = [Graph configuration help](#)**All subtracks:** **List subtracks:** [only selected/visible](#) [all](#) (19 of 81 selected)

<input type="checkbox"/> <input type="button" value="dense"/>	<input type="button" value="Configure"/>	acinar cell Cv	Schema
<input type="checkbox"/> <input type="button" value="dense"/>	<input type="button" value="Configure"/>	adipocyte Cv	Schema
<input checked="" type="checkbox"/> <input type="button" value="dense"/>	<input type="button" value="Configure"/>	astrocyte Cv	Schema
<input checked="" type="checkbox"/> <input type="button" value="dense"/>	<input type="button" value="Configure"/>	B cell Cv	Schema
<input checked="" type="checkbox"/> <input type="button" value="dense"/>	<input type="button" value="Configure"/>	basal cell Cv	Schema
<input type="checkbox"/> <input type="button" value="dense"/>	<input type="button" value="Configure"/>	basophil Cv	Schema
<input type="checkbox"/> <input type="button" value="dense"/>	<input type="button" value="Configure"/>	Bergmann glial Cv	Schema
<input type="checkbox"/> <input type="button" value="dense"/>	<input type="button" value="Configure"/>	bladder cell Cv	Schema
<input type="checkbox"/> <input type="button" value="dense"/>	<input type="button" value="Configure"/>	blood cell Cv	Schema
<input type="checkbox"/> <input type="button" value="dense"/>	<input type="button" value="Configure"/>	brain pericyte Cv	Schema
<input checked="" type="checkbox"/> <input type="button" value="dense"/>	<input type="button" value="Configure"/>	Brush cell Cv	Schema
<input type="checkbox"/> <input type="button" value="dense"/>	<input type="button" value="Configure"/>	cardiac muscle Cv	Schema
		pancreatic acinar cell Coverage (182 cells) ▾	Schema
		mesenchymal stem cell of adipose Coverage (2107 cells) ▾	Schema
		astrocyte Coverage (432 cells) ▾	Schema
		B cell Coverage (2029 cells) ▾	Schema
		basal cell Coverage (1340 cells) ▾	Schema
		basophil Coverage (25 cells) ▾	Schema
		Bergmann glial cell Coverage (40 cells) ▾	Schema
		bladder cell Coverage (695 cells) ▾	Schema
		blood cell Coverage (206 cells) ▾	Schema
		brain pericyte Coverage (156 cells) ▾	Schema
		Brush cell of epithelium proper of large intestine Coverage (63 cells) ▾	Schema
		cardiac muscle cell Coverage (133 cells) ▾	Schema

<input type="checkbox"/> dense	 Configure	L.I. epithelia Cv	epithelial cell of large intestine Coverage (2019 cells) ▾	Schema
<input type="checkbox"/> dense	 Configure	late pro-B Cv	late pro-B cell Coverage (306 cells) ▾	Schema
<input type="checkbox"/> dense	 Configure	leukocyte Cv	leukocyte Coverage (683 cells) ▾	Schema
<input type="checkbox"/> dense	 Configure	luminal cell Cv	luminal epithelial cell of mammary gland Coverage (578 cells) ▾	Schema
<input checked="" type="checkbox"/> full	 Configure	lung endothelia C	lung endothelial cell Coverage (693 cells) ▾	Schema
<input checked="" type="checkbox"/> full	 Configure	lung epithelia Cv	epithelial cell of lung Coverage (113 cells) ▾	Schema
<input type="checkbox"/> dense	 Configure	lymphocyte Cv	lymphocyte Coverage (81 cells) ▾	Schema
<input type="checkbox"/> dense	 Configure	macrophage Cv	macrophage Coverage (395 cells) ▾	Schema
<input type="checkbox"/> dense	 Configure	mature NK Cv	mature natural killer cell Coverage (49 cells) ▾	Schema
<input type="checkbox"/> dense	 Configure	MEP cell Cv	megakaryocyte-erythroid progenitor cell Coverage (55 cells) ▾	Schema
<input type="checkbox"/> dense	 Configure	mesenchymalSC Cv	mesenchymal stem cell Coverage (499 cells) ▾	Schema
<input type="checkbox"/> dense	 Configure	mesenchyme Cv	mesenchymal cell Coverage (830 cells) ▾	Schema
<input type="checkbox"/> dense	 Configure	microglia Cv	microglial cell Coverage (4394 cells) ▾	Schema

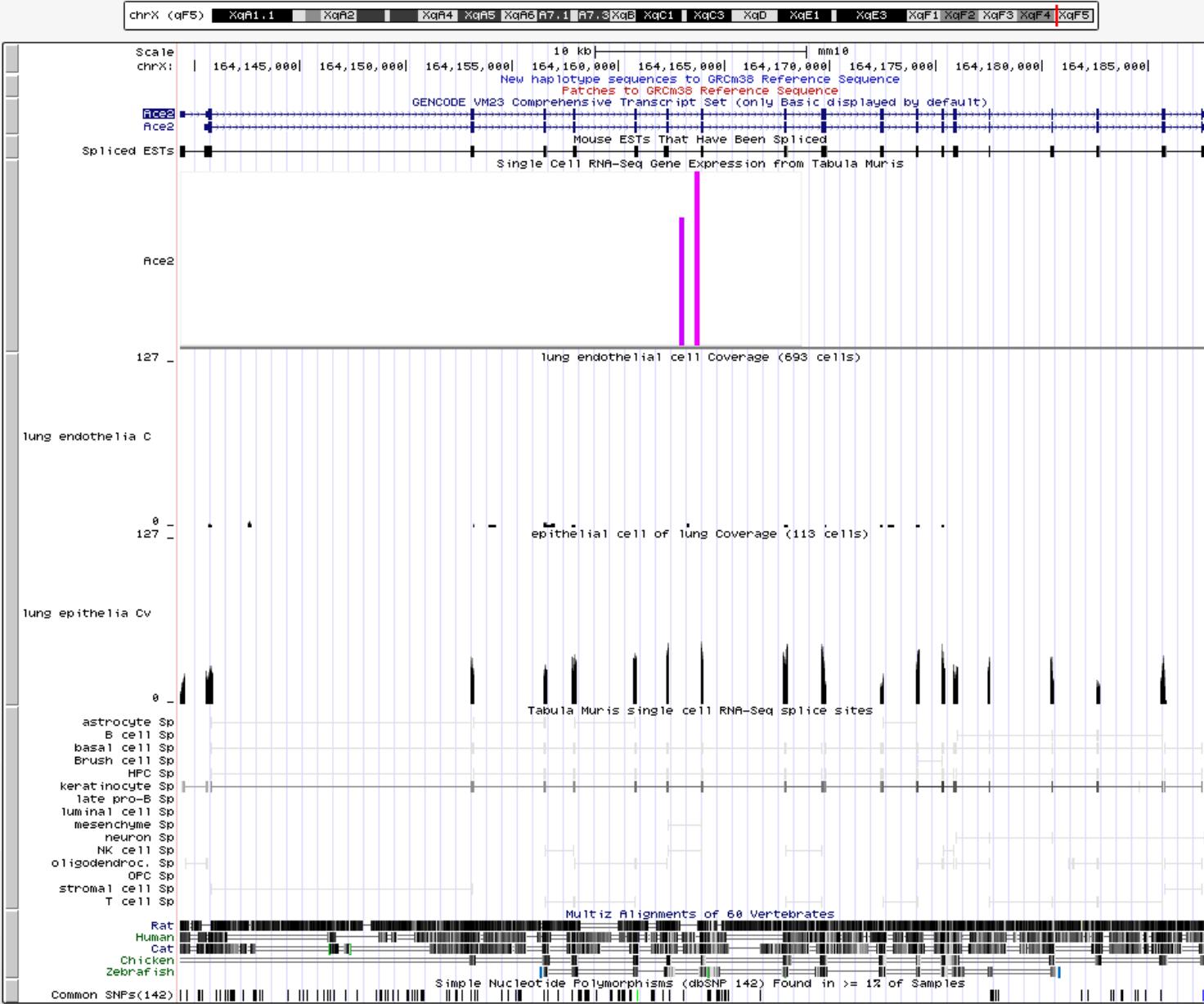
UCSC Genome Browser on Mouse Dec. 2011 (GRCm38/mm10) Assembly

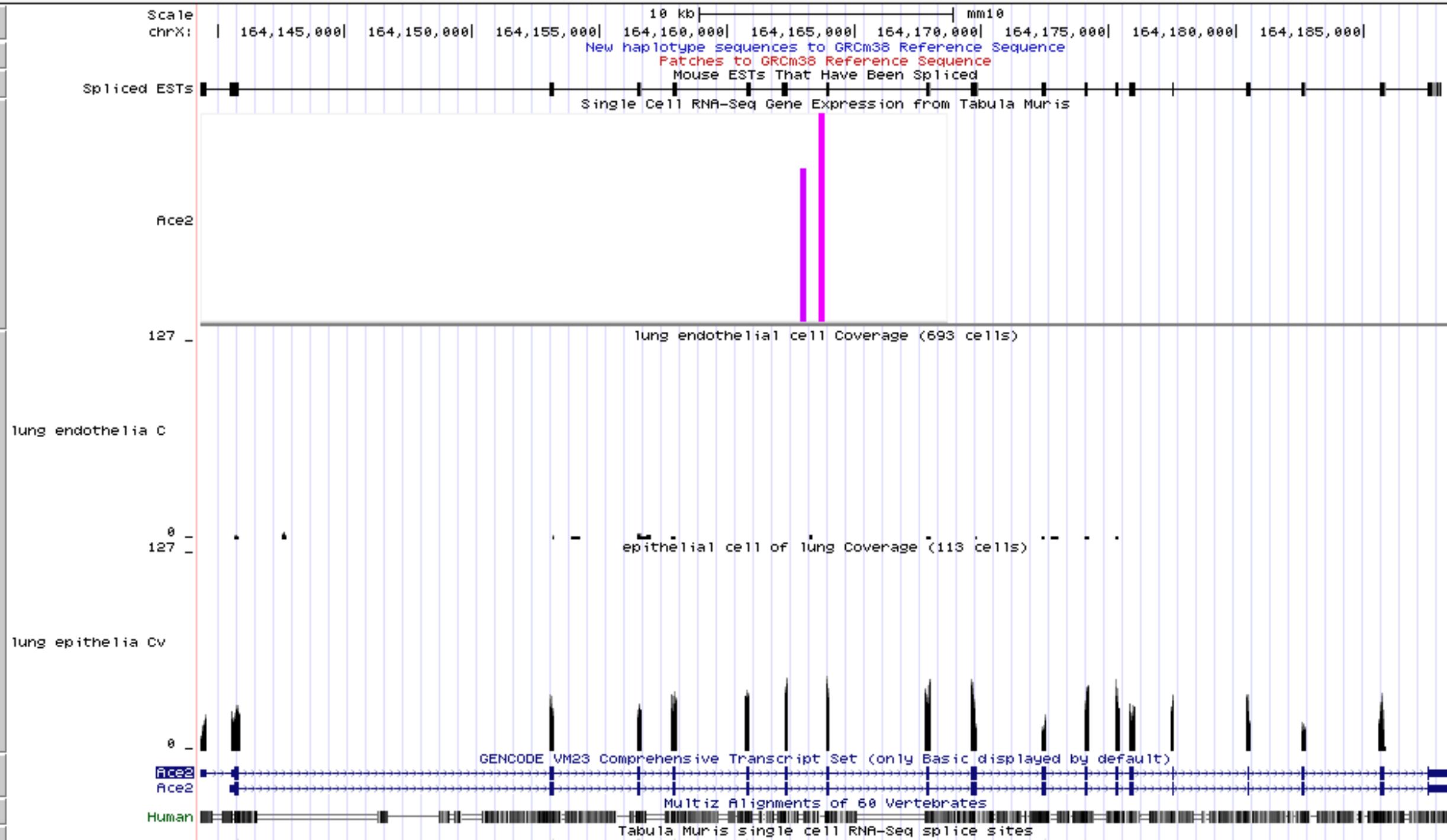
move <<< << < > >> >>> **zoom in** 1.5x 3x 10x **base** **zoom out** 1.5x 3x 10x 100x

chrX:164,139,332-164,188,420 49,089 bp.

Enter position, gene symbol or search terms

g





Choose Cell Browser Dataset

Overview

Cortex development	smartseq2	4.3k	Open	
HCA Datasets via Xena	9 datasets		Open	
Adult Pancreas	smartseq2	4.0k	Open	
Autism	10x	105k	Open	
Alexandria Project from the Shalek Lab	2 collections	3 datasets	Open	
Lifespan Nasal Atlas	3 datasets		Open	
Mouse Hematopoietic Stem Cells	2 datasets		Open	
Glioblastoma	33k		Open	
Head and Neck Cancer	smartseq2	3.6k	Open	
Melanoma	Drop-Seq	10x	3 datasets	Open
Choroid Plexus Organoids	32k		Open	
Macrophage Development	1.2k		Open	
Mouse Nervous System	20 datasets		Open	
Ciona Larva	2 datasets		Open	
Lung in Pulmonary Fibrosis vs Control	114k		Open	
Heart of Cells	7 datasets		Open	
COVID-19 and Influenza Immunophenotyping	60k		Open	
Multimodal PBMC	2 datasets		Open	
Mouse Developing Brain	292k		Open	
Airway Macrophage Diversity	49k		Open	
Human Cerebellar Development	69k		Open	
Drosophila Ovary	7 datasets		Open	

Overview

UCSC Cell Browser Intro

The UCSC Cell Browser is an interactive viewer for single-cell expression. You can find a few datasets converted at UCSC in the list on the left.

You can also set one up yourself, by [installing the package](#). Exporters to create a Cell Browser from your own data are integrated into [Seurat](#) or [Scanpy](#) and we provide one for [CellRanger](#) and for [text files](#).

We are very happy about bug reports or feedback: cells@ucsc.edu.

Or open an issue in our [GitHub Repo](#)

News

November 17, 2020

- [Radial Glia in Early Brain](#) - exploring the diversity of radial glia, neuroepithelial cells, and various progenitors in the developing human brain
- [Single-cell atlas of human leptomeningeal metastasis](#) - looking at the tumor micro-environment for leptomeningeal metastasis in 5 different patients
- [Alexandria Project from the Shalek Lab](#) - collection of datasets looking at expression of ACE2/TMPRSS2 in a few different, non-COVID-19 contexts

November 12, 2020

New pip release! We've put out a new version of the cellbrowser package, v0.7.15. Install it via [pip install --upgrade cellbrowser](#). This new version includes some general bug fixes and a few new features (e.g. you can add `&openDialog=1` to URL to open dataset description by default).

November 4, 2020

We just posted a pre-print about the UCSC Cell Browser on [bioRxiv](#)!

New datasets added:

- [COVID-19 Immunological Response](#) - immune response of patients with different severities of COVID-19
- [Engraftable Hematopoietic Stem Cells](#) - looks at fetal liver cells to study the engraftment potential of hematopoietic stem cells, also includes surface protein measurements
- [Aging Human Skin](#) - a look at how expression patterns and cell types change in human skin as we age
- [Oligodendrocyte lineage in Development](#) - study of how oligodendrocytes develop in the mouse brain over time
- [Oligodendrocyte Lineage in Multiple Sclerosis](#) - discovery of a lineage of oligodendrocyte cells unique to experimental autoimmune encephalomyelitis (EAE), a mouse model for MS
- [Mouse Oligodendrocyte Heterogeneity](#) - exploration of the various oligodendrocytes present in the mouse central nervous system
- [Human Cellular Landscape](#) - Microwell-Seq across many organs in human
- [Mouse Cell Atlas](#) - Microwell-Seq of multiple organs in mouse
- [Drosophila Ovary](#) - development of ovary in Drosophila, includes developmental trajectories of specific ovary parts (e.g. polar stalk)

cells.ucsc.edu

Select one dataset from the collection "HCA LungMAP COVID-19 Integrated Analysis"

The collection 'HCA LungMAP COVID-19 Integrated Analysis' contains 24 datasets. Double-click or click 'Open' below. To move between datasets later in the cell browser, use the 'Collection' dropdown.

Go back to: [All Datasets](#)

Overview

Lung (cross-tissue)	44k	Open
Lung Fibrosis	31k	Open
Lung (Reyfman et al. 2018.)	37k	Open
Lung (Misharin. Unpublished 2020.)	18k	Open
Internal Nonsmokers Lung	277k	Open
Internal Smokers Lung	97k	Open
Lung (Morse et al. 2019.)	22k	Open
Adipose	11k	Open
Bone Marrow	284k	Open
Cord Blood	264k	Open
Enteric Nervous System (ENS)	134k	Open
Esophagus Mucosa (cross-tissue)	33k	Open
Esophagus Muscularis (cross-tissue)	43k	Open
Heart (cross-tissue)	40k	Open
Diabetic Kidney	75k	Open
Liver	13k	Open
Pancreas	2.6k	Open
Pancreatic ductal adenocarcinoma (PDAC) naive	102k	Open
Pancreatic ductal adenocarcinoma (PDAC) treated	60k	Open
Placenta (Suryawanshi et al. 2018.)	14k	Open
Prostate (Cross Tissue)	38k	Open
Skeletal Muscle (Cross Tissue)	36k	Open
Skin (Cross Tissue)	6.8k	Open
Breast (Cross Tissue)	18k	Open

Abstract**Methods****Data Download****HCA-LungMAP COVID-19 Integrated Analysis**

Datasets from [HCA-LungMAP COVID-19 Integrated Analysis](#).

From [Muus et al:](#)

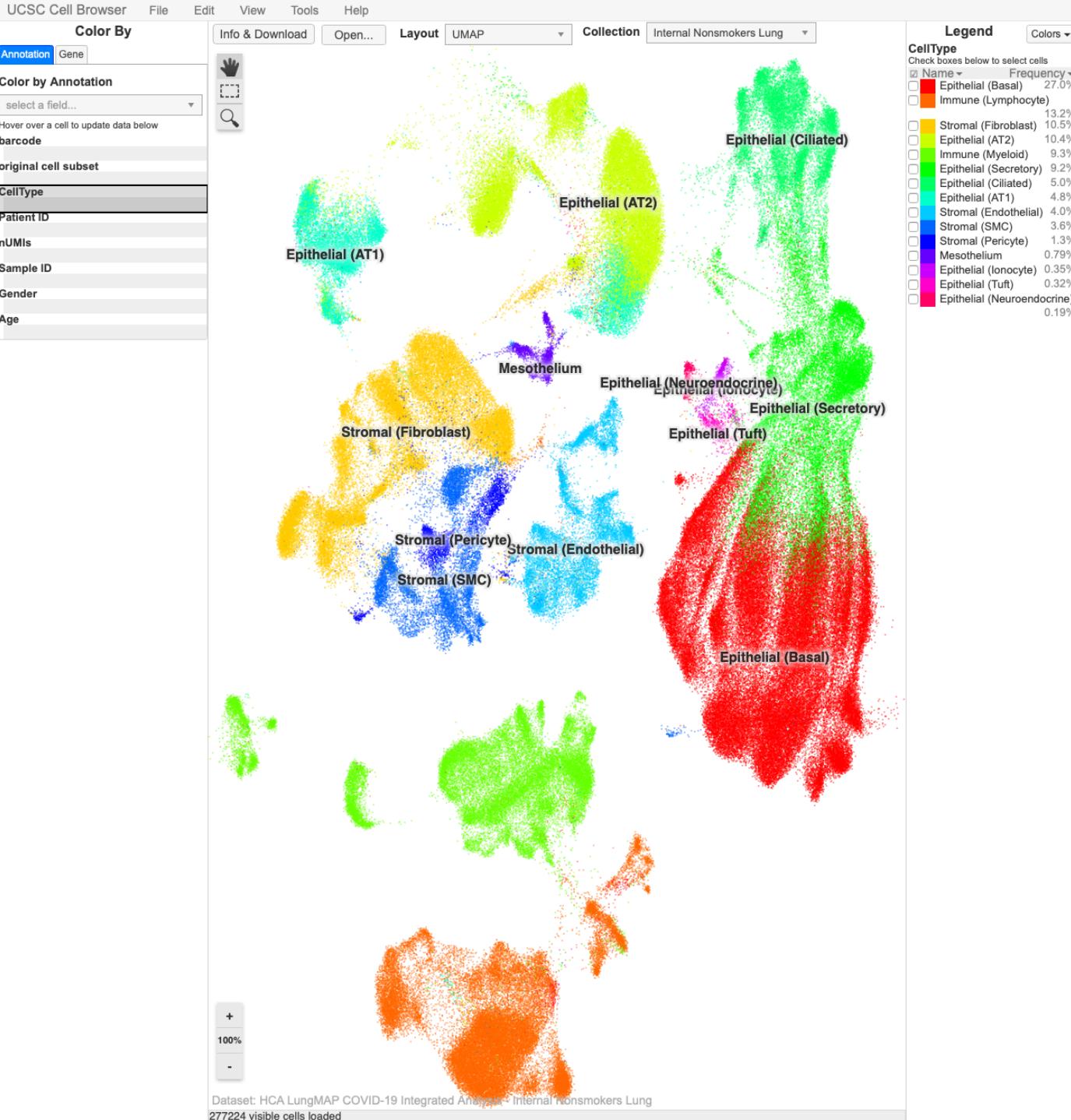
The COVID-19 pandemic, caused by the novel coronavirus SARS-CoV-2, creates an urgent need for identifying molecular mechanisms that mediate viral entry, propagation, and tissue pathology. Cell membrane bound angiotensin-converting enzyme 2 (ACE2) and associated proteases, transmembrane protease serine 2 (TMPRSS2) and Cathepsin L (CTSL), were previously identified as mediators of SARS-CoV2 cellular entry. Here, we assess the cell type-specific RNA expression of ACE2, TMPRSS2, and CTS defense against COVID-19. We performed an integrated analysis of 107 single-cell and single-nucleus RNA-Seq studies, including 22 lung and airways datasets (16 unpublished), and 85 datasets from other diverse organs. Joint expression of ACE2 and the accessory proteases identifies specific subsets of respiratory epithelial cells as putative targets of viral infection in the nasal passages, airways, and alveoli. Cells that co-express ACE2 and proteases are also identified in cells from other organs, some of which have been associated with COVID-19 transmission or pathology, including gut enterocytes, corneal epithelial cells, cardiomyocytes, heart pericytes, olfactory sustentacular cells, and renal epithelial cells. Performing the first meta-analyses of scRNA-seq studies, we analyzed 1,176,683 cells from 282 nasal, airway, and lung parenchyma samples from 164 donors spanning fetal, childhood, adult, and elderly age groups, associate increased levels of ACE2, TMPRSS2, and CTS defense against COVID-19. We found that ACE2+TMPRSS2+ cells in nasal, lung, and gut tissues, including genes that may mediate viral entry, subtend key immune functions, and mediate epithelial-macrophage cross-talk. Amongst these are IL6, its receptor and co-receptor, IL1R, TNF response pathways, and complement genes. Cell type specificity in the lung and airways and smoking effects were conserved in mice. Our analyses suggest that differences in the cell type-specific expression of mediators of SARS-CoV-2 viral entry may be responsible for aspects of COVID-19 epidemiology and clinical course, and point to putative molecular pathways involved in disease susceptibility and pathogenesis.

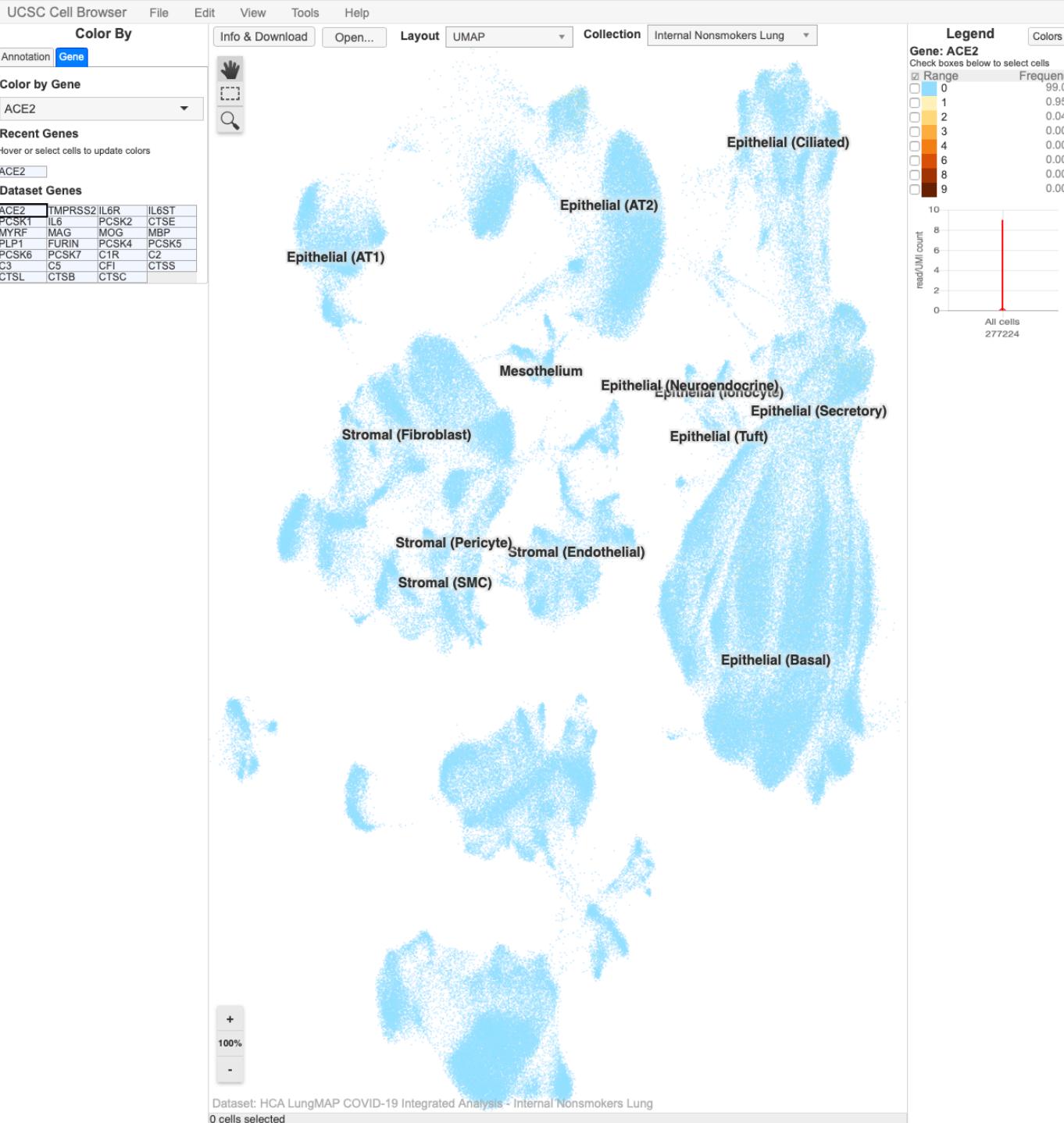
Publication: [Muus et al. 2020. bioRxiv](#).

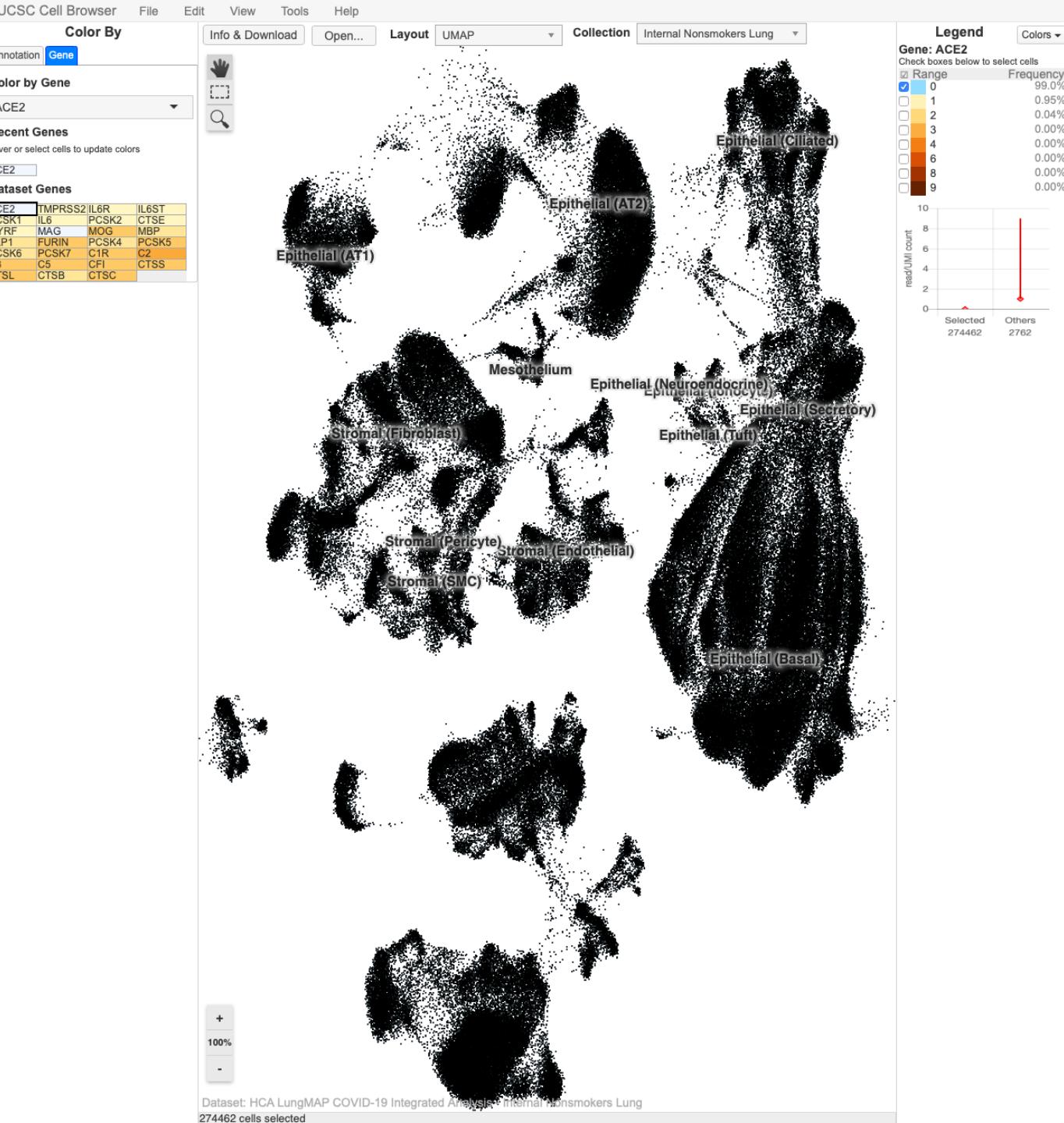
Website: [HCA COVID-19 Integrated Analysis](#)

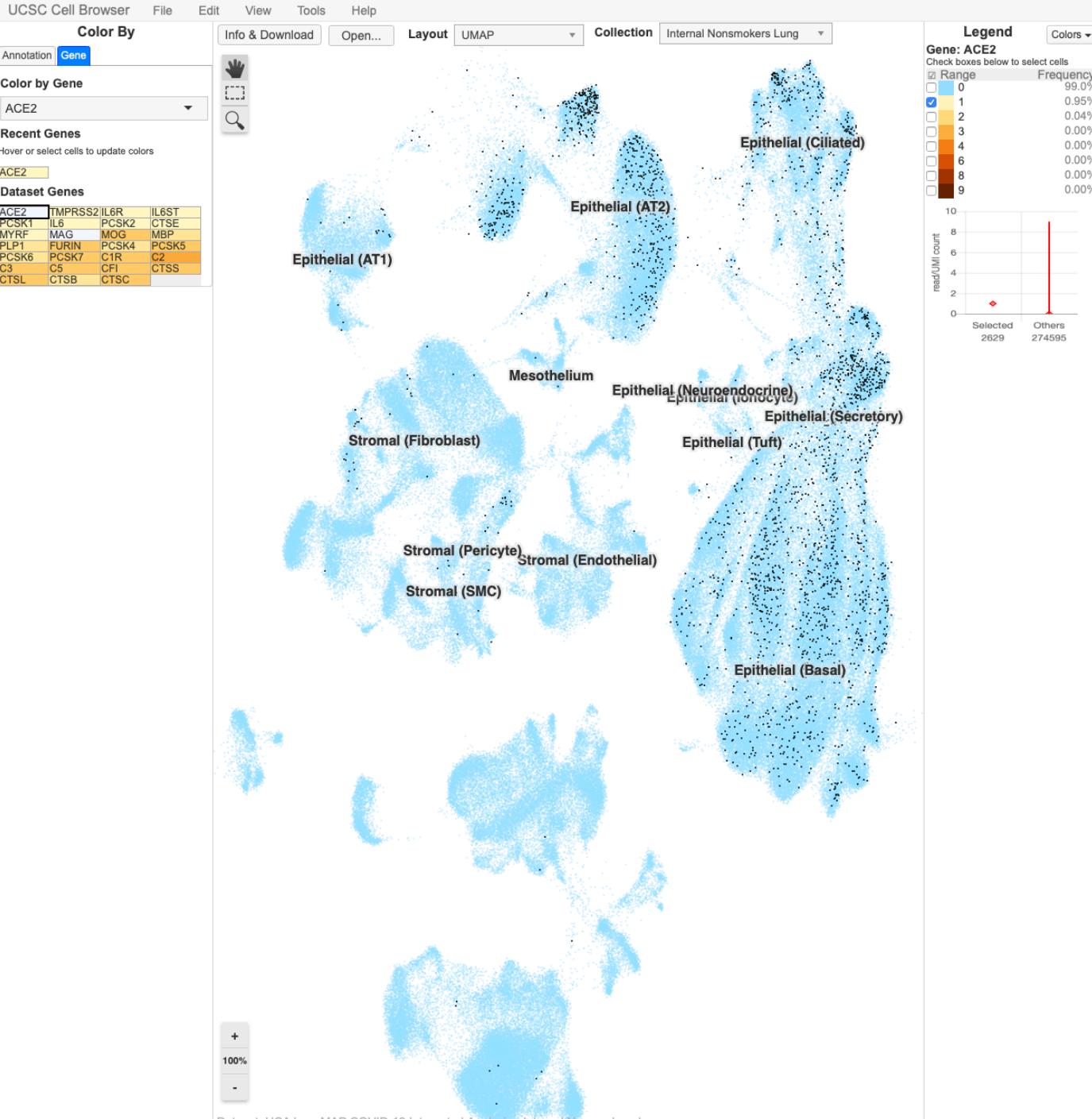
Direct link to this plot for manuscripts: <https://hca-lungmap-integrated.cells.ucsc.edu>

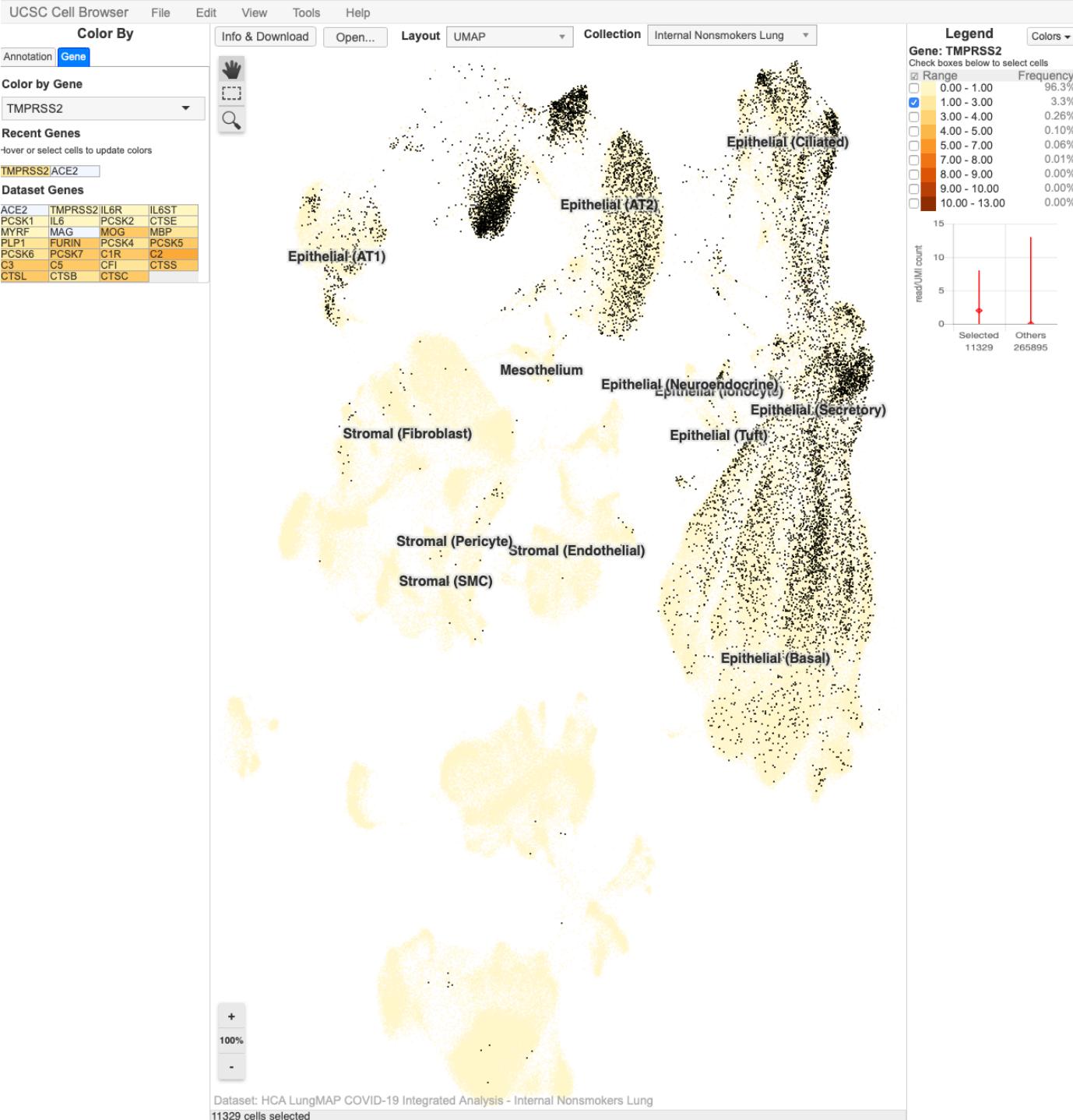
Cell Browser dataset ID: hca-lungmap-integrated

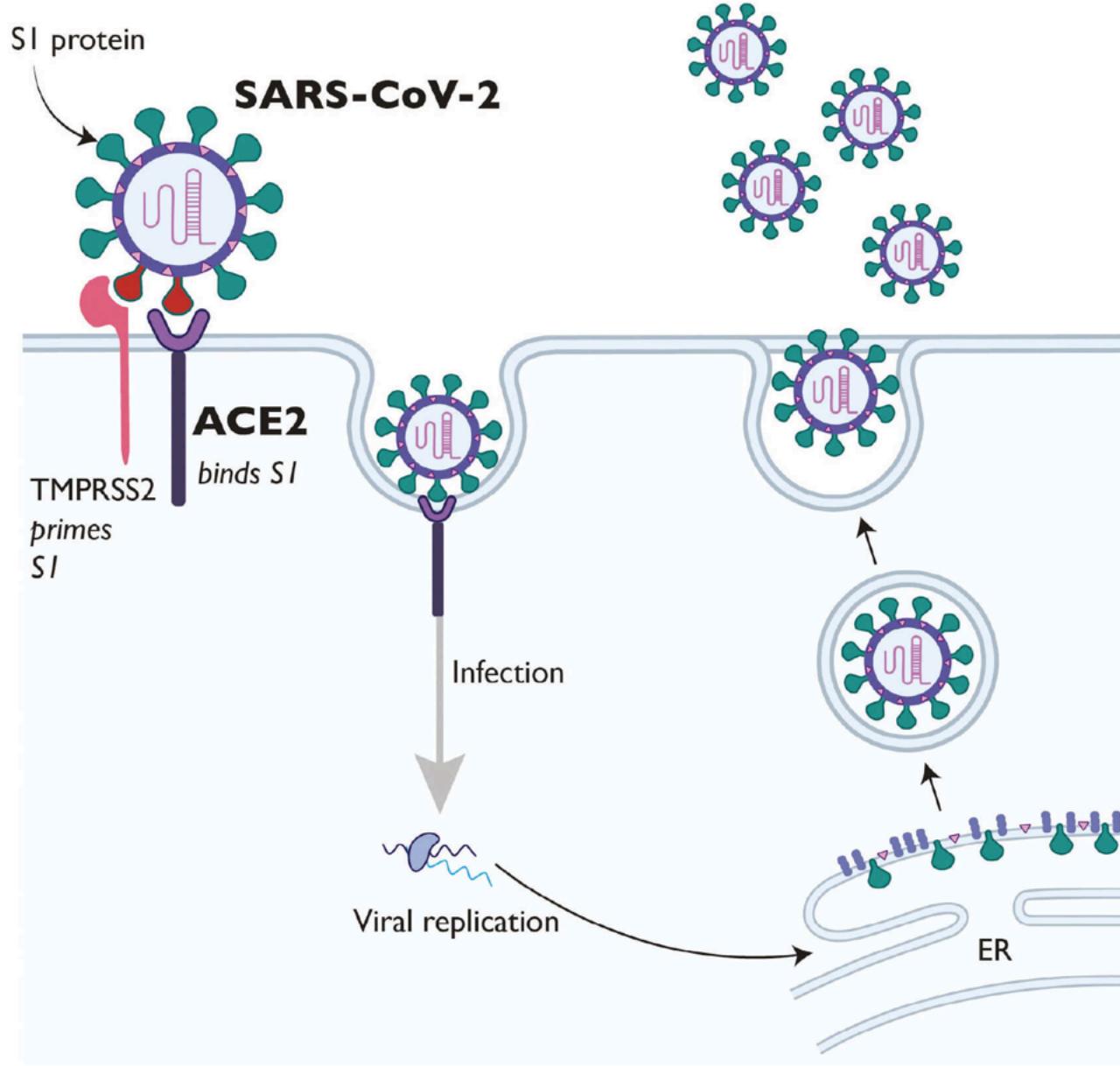








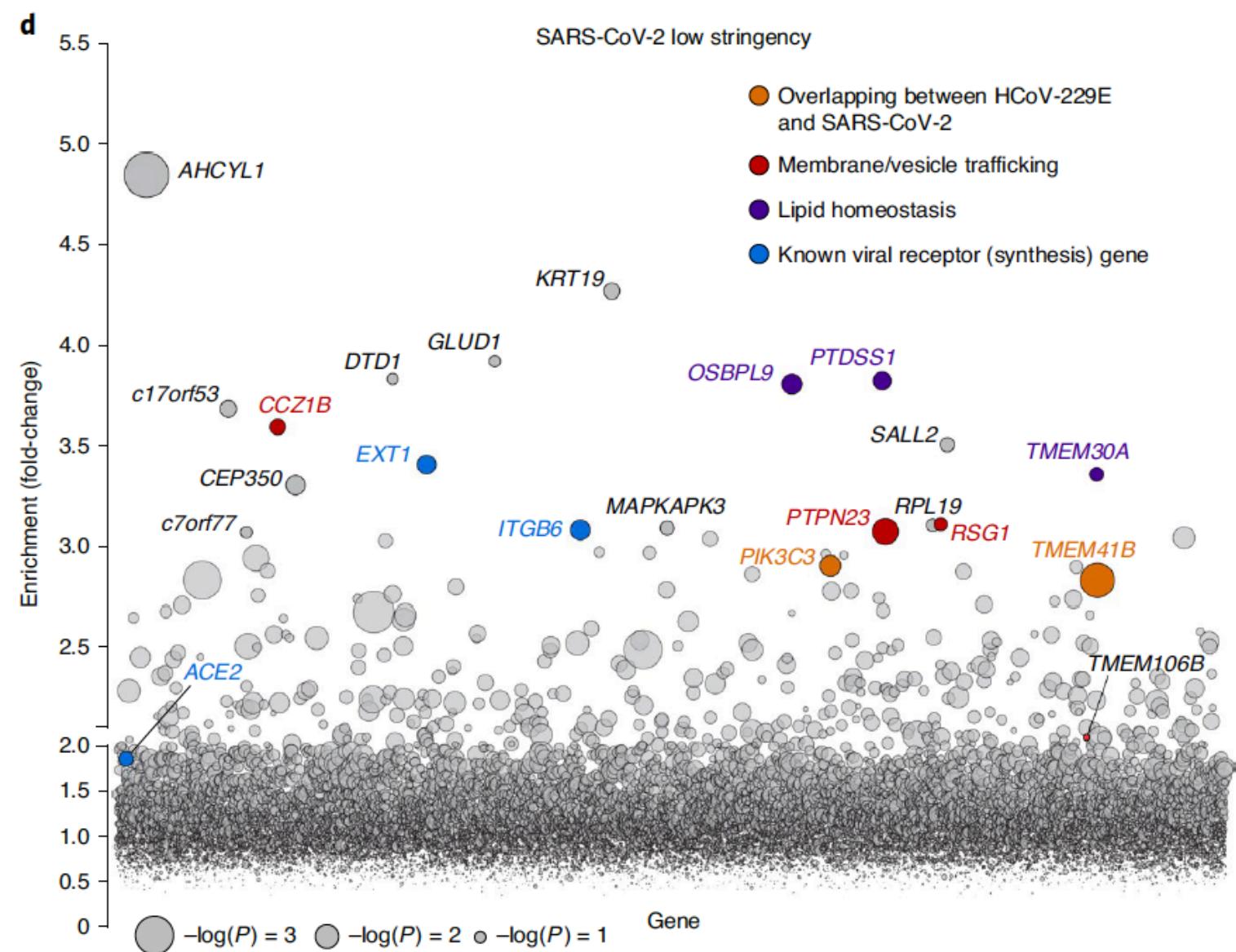
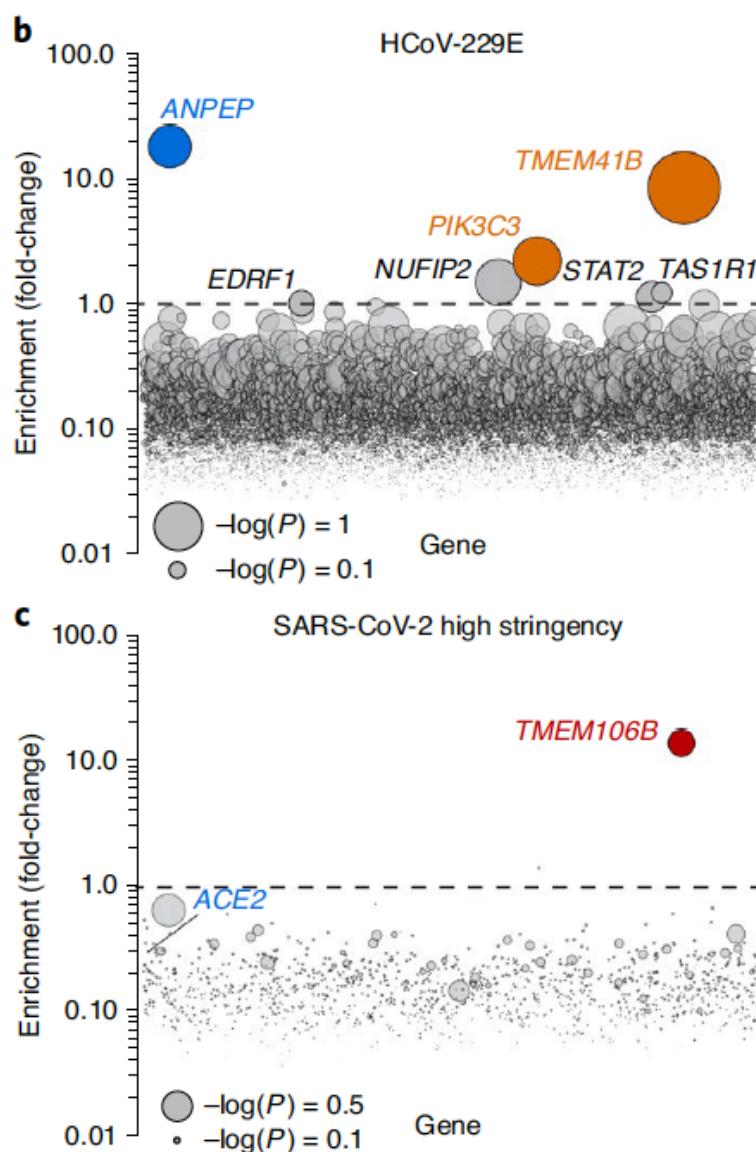


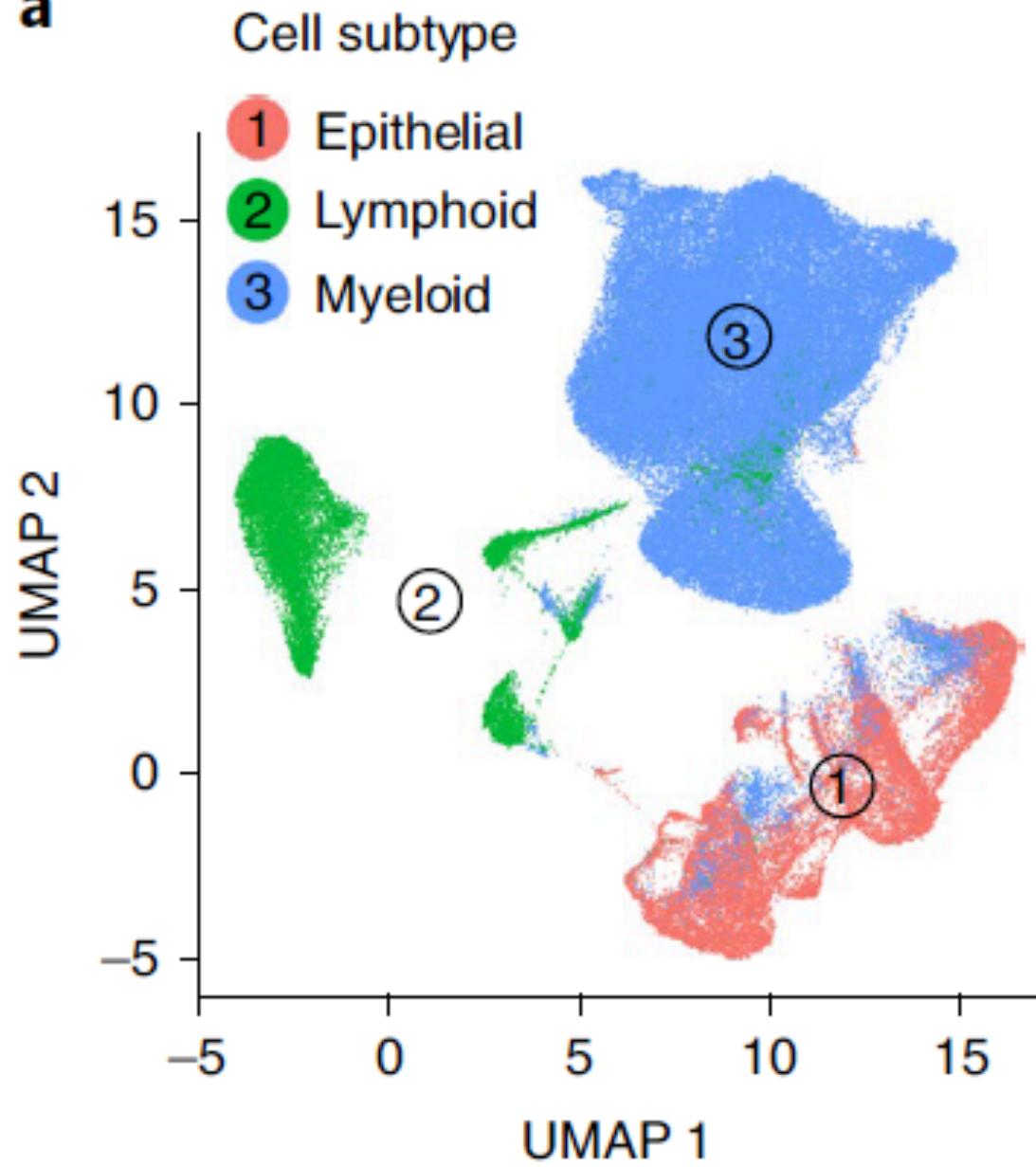


How does AT2 cell frequency change in smokers vs non-smokers?

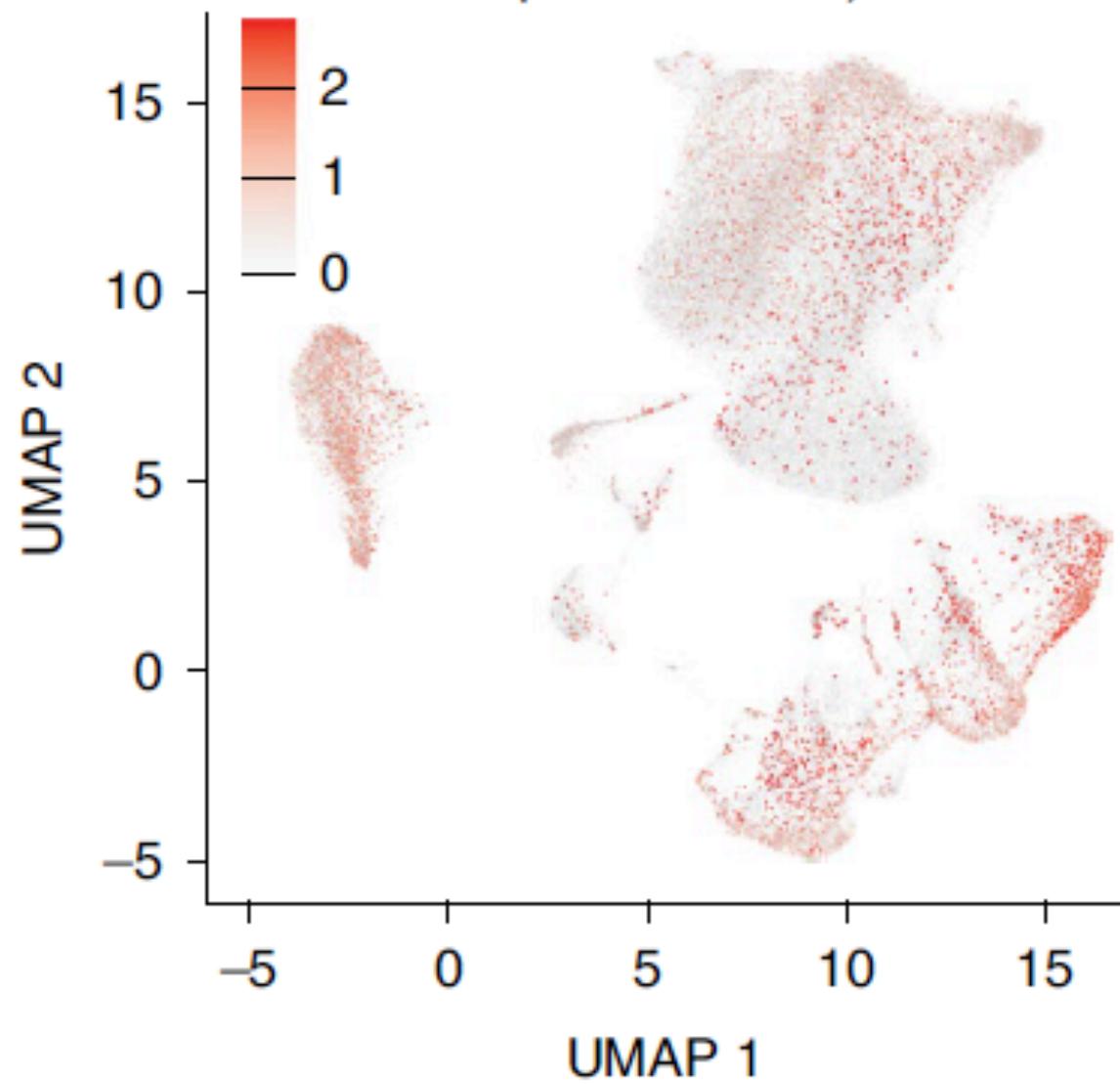
How does ACE2 expression change?

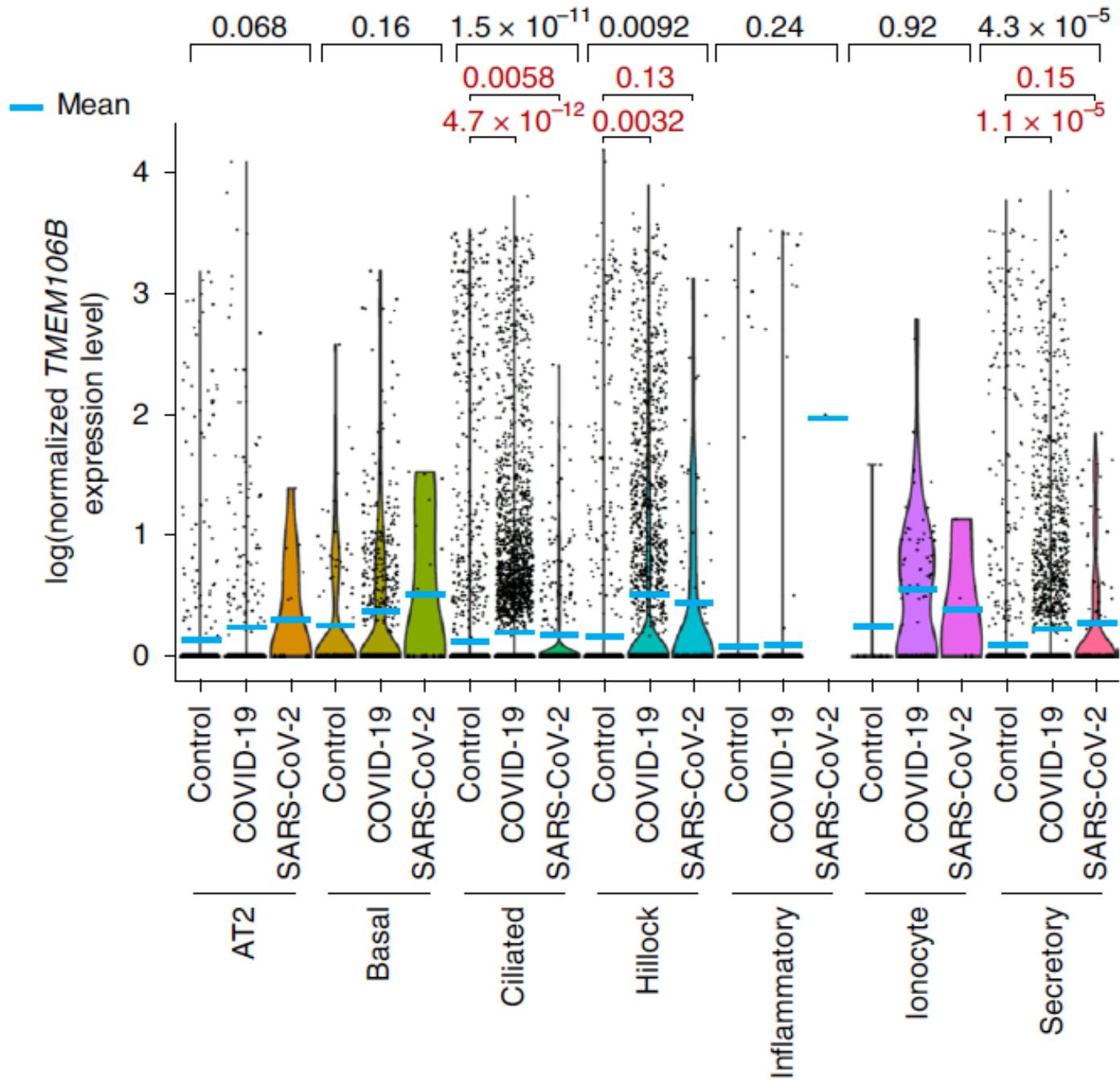
How does TMPRSS2 expression change?



a

log(normalized *TMEM106B* expression level)







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Gene expression profiling of SARS-CoV-1/2 infected human cell lines at single-cell level

Single-cell RNA-Seq mRNA baseline

Number of cells: 197,537

Organism: *Homo sapiens*



Immunophenotyping of COVID-19 and Influenza Underscores the Association of Type I IFN Response in Severe COVID-19

Single-cell RNA-Seq mRNA baseline

Number of cells: 77,689

Organism: *Homo sapiens*

Publication:

- Lee JS, Park S, Jeong HW, Ahn JY, Choi SJ et al. (2020) *Immunophenotyping of COVID-19 and influenza highlights the role of type I interferons in development of severe COVID-19.*



When examining whole blood depleted of RBC from COVID-19 patients,

- 1. What cell type does not seem to express TMEM106B?**

- 2. Is TMEM106B a marker of cells from COVID-19 patients?**

- 3. Are blood cells likely to be infected by SARS-CoV-2?**

RNA-seq of RBC depleted whole blood from COVID-19 patients and controls

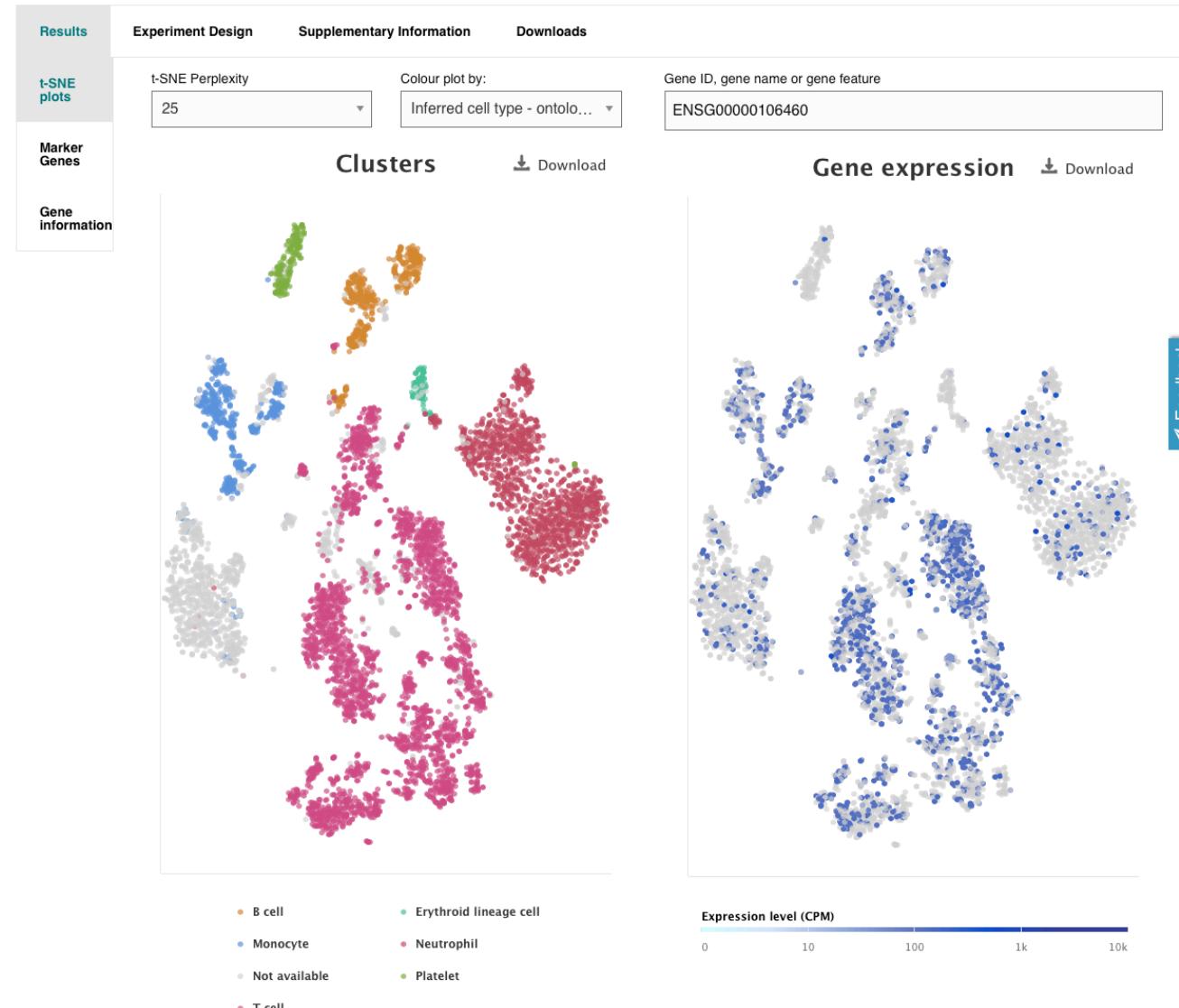
Single-cell RNA-Seq mRNA baseline

Number of cells: 6,177

Organism: *Homo sapiens*

Publication:

- Silvin A, Chapuis N, Dunsmore G, Goubelet AG, Dubuisson A et al. (2020) [Elevated Calprotectin and Abnormal Myeloid Cell Subsets Discriminate Severe from Mild COVID-19.](#)



RNA-seq of RBC depleted whole blood from COVID-19 patients and controls

Single-cell RNA-Seq mRNA baseline

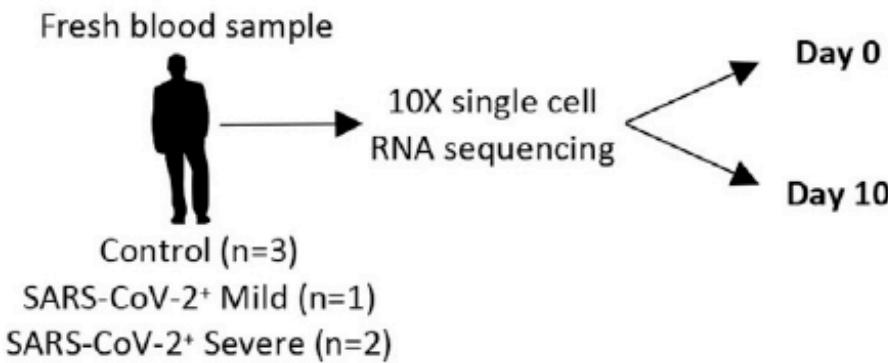
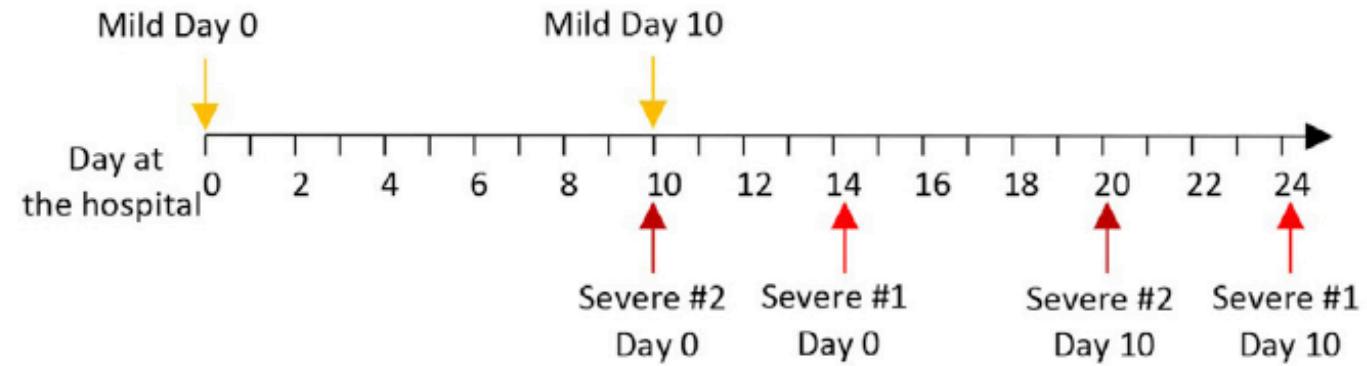
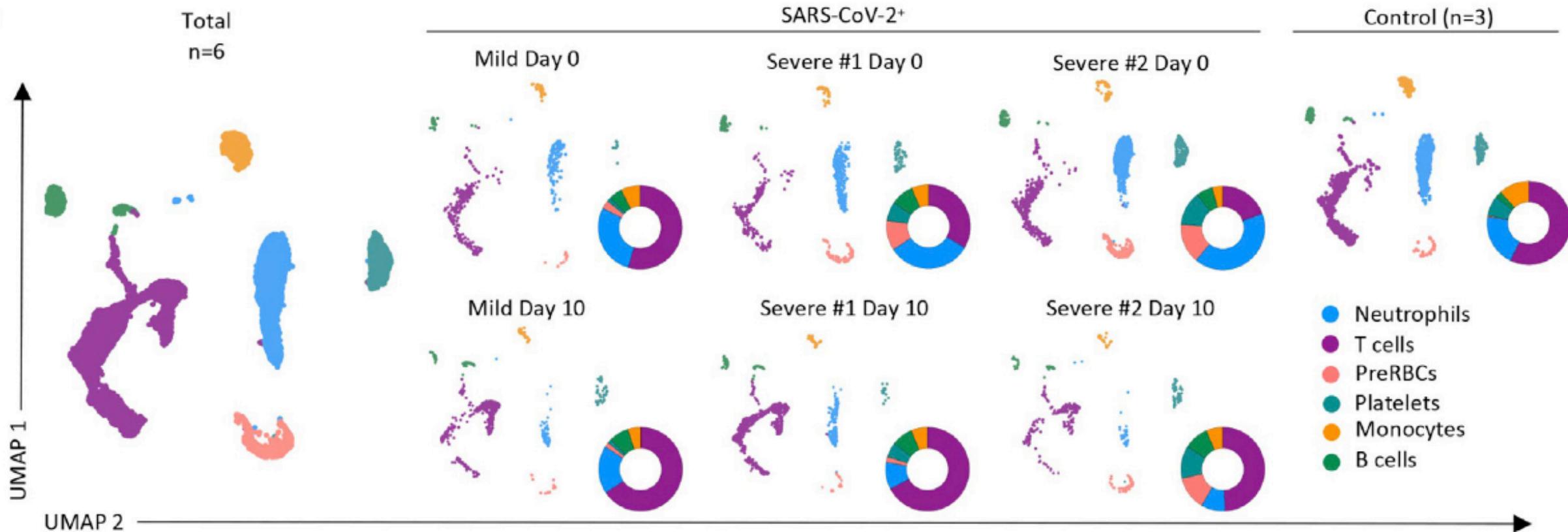
Number of cells: 6,177

Organism: *Homo sapiens*

Publication:

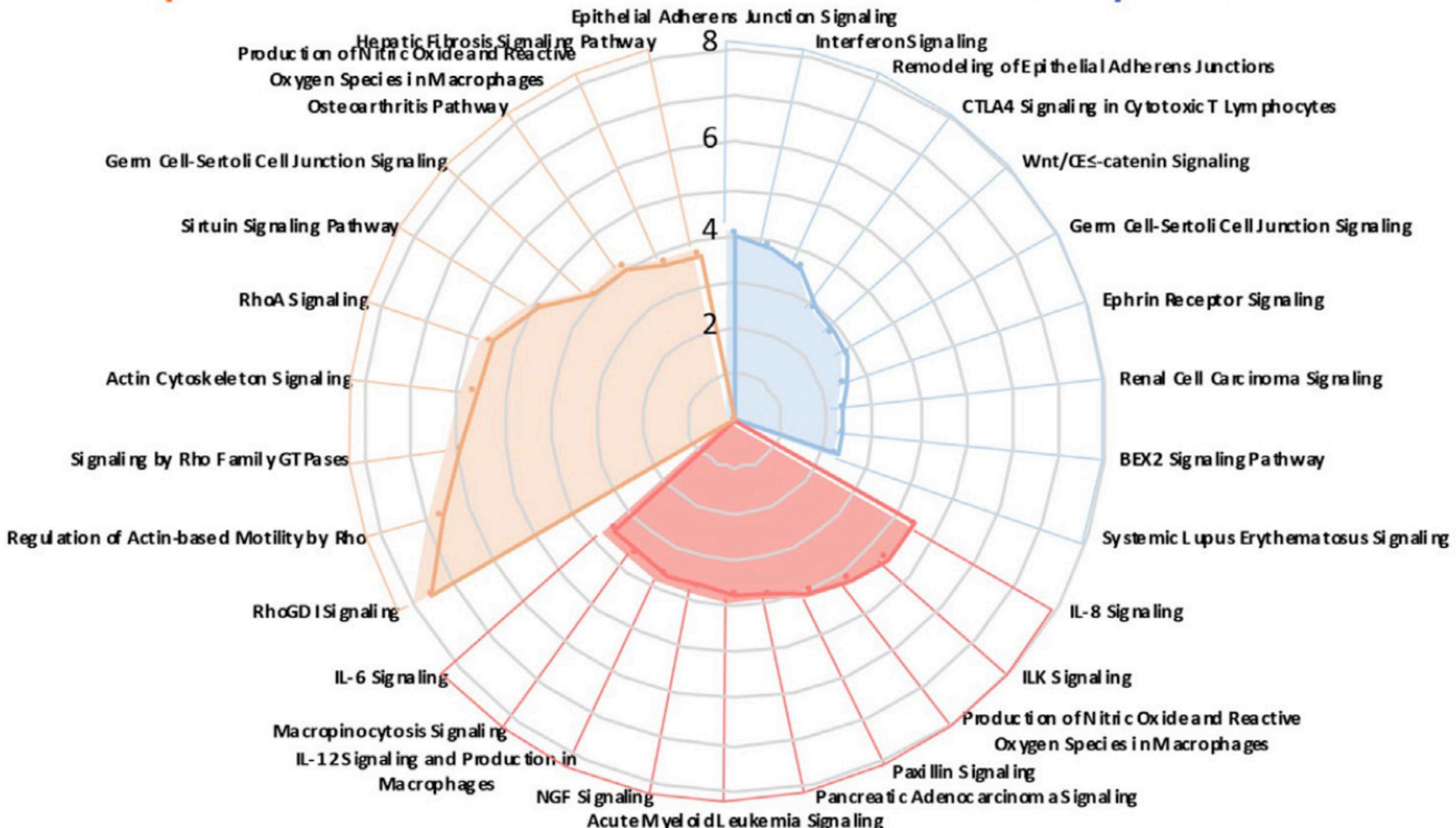
- Silvin A, Chapuis N, Dunsmore G, Goubet AG, Dubuisson A et al. (2020) [Elevated Calprotectin and Abnormal Myeloid Cell Subsets Discriminate Severe from Mild COVID-19.](#)

[Read publication](#)

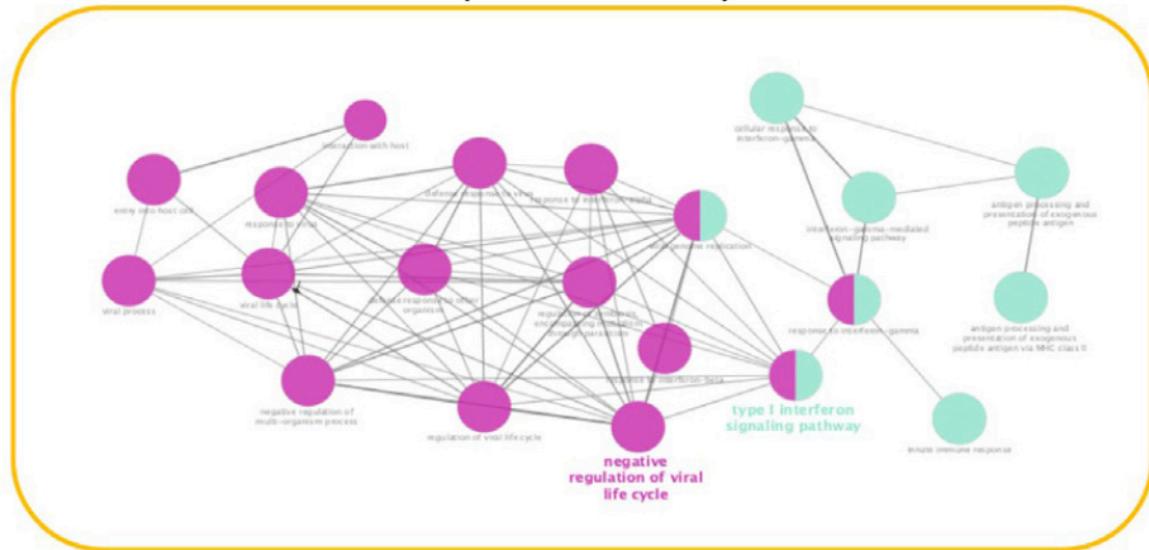
A**B****C**

Severe patient #2

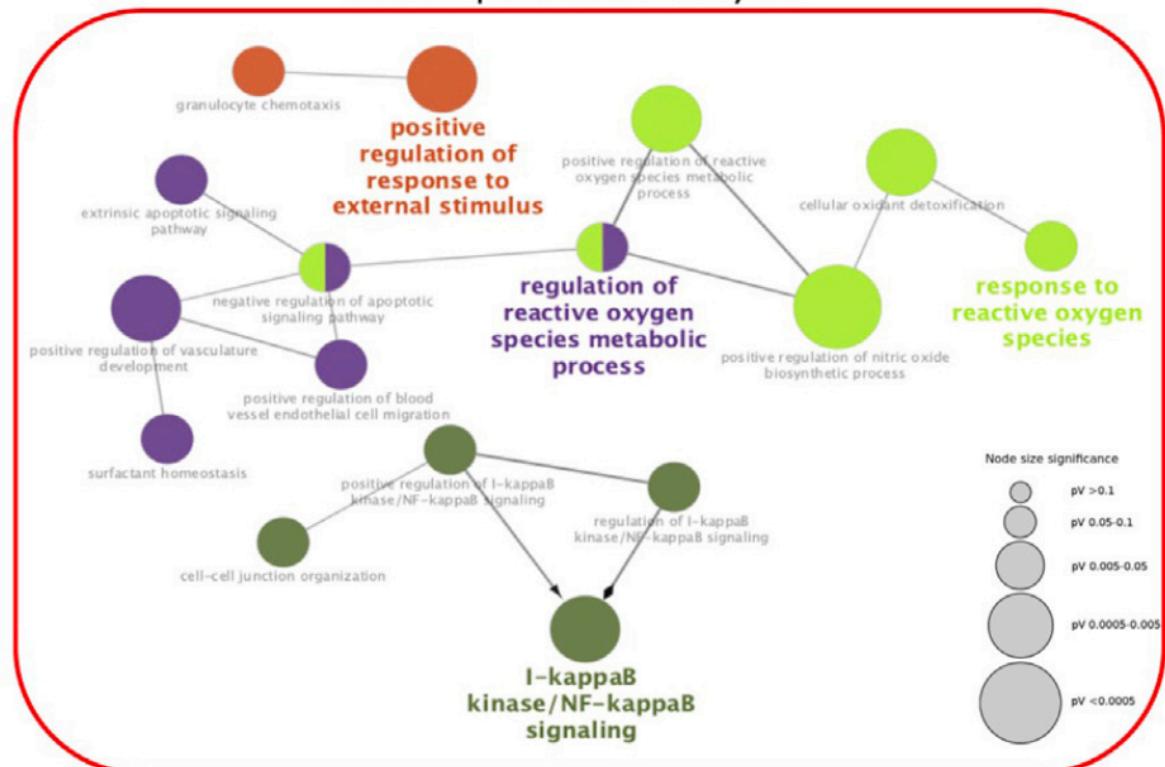
Mild patient



Mild patient monocytes

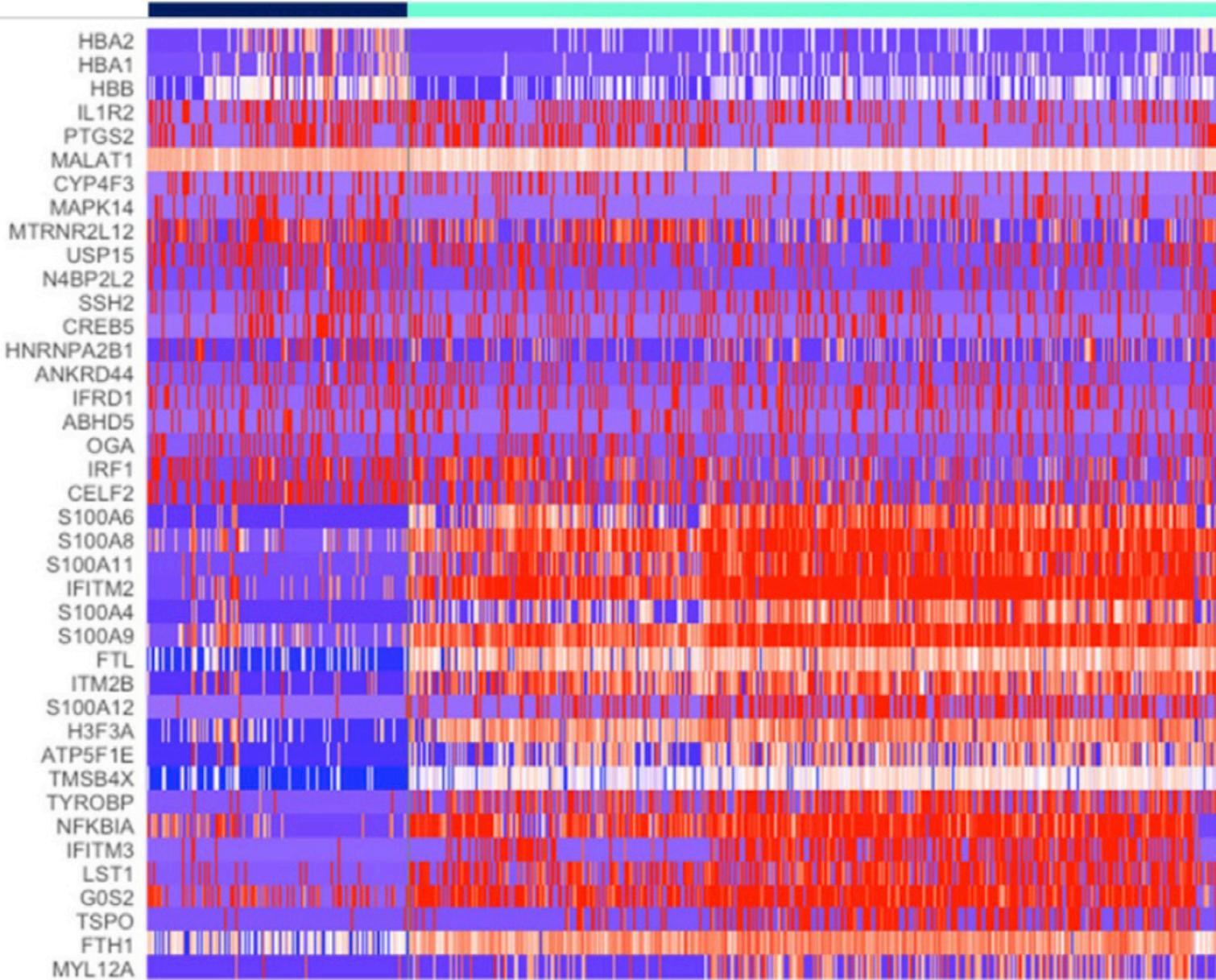


Severe patient monocytes



Cluster 1

Cluster 2

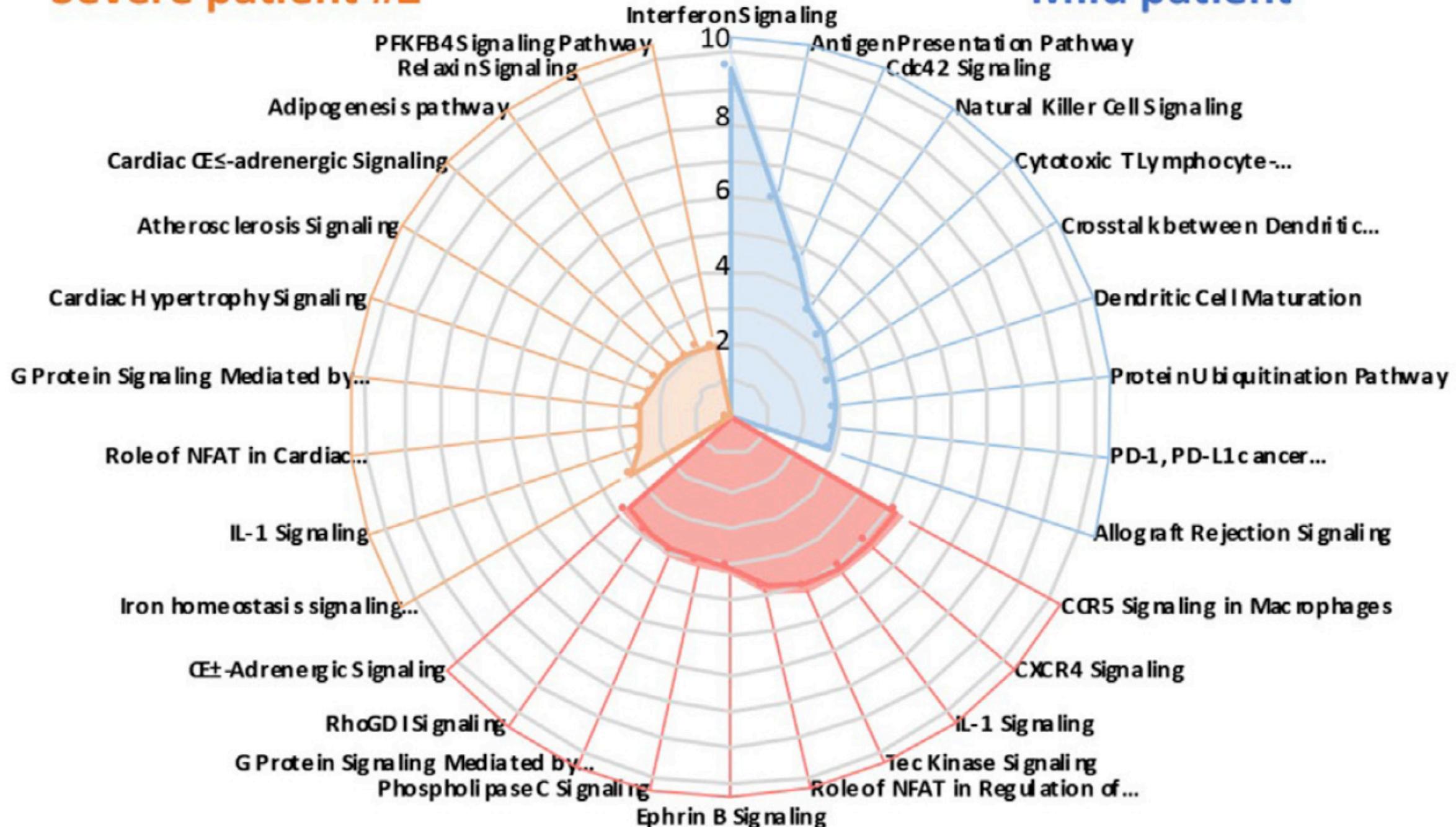


Expression



Severe patient #2

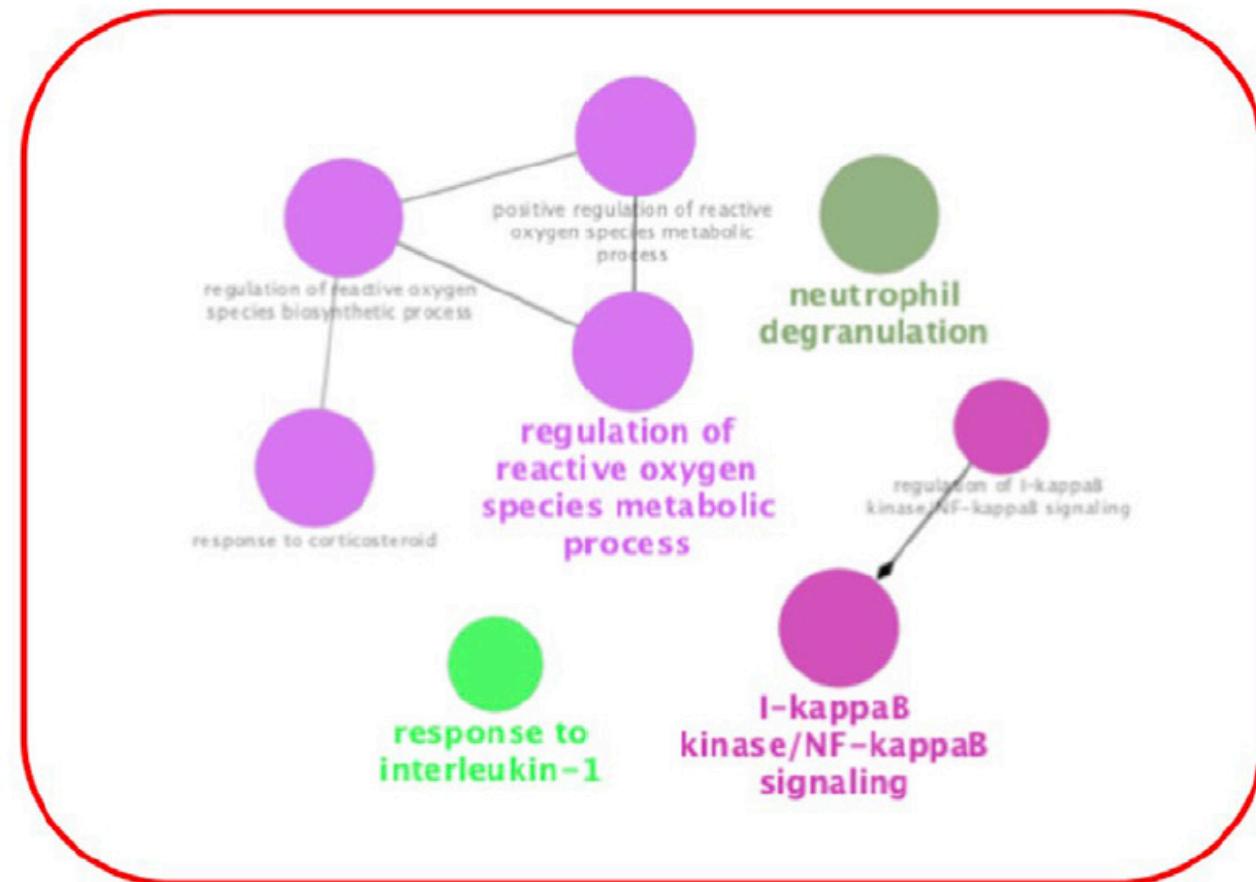
Mild patient



Mild patient neutrophils

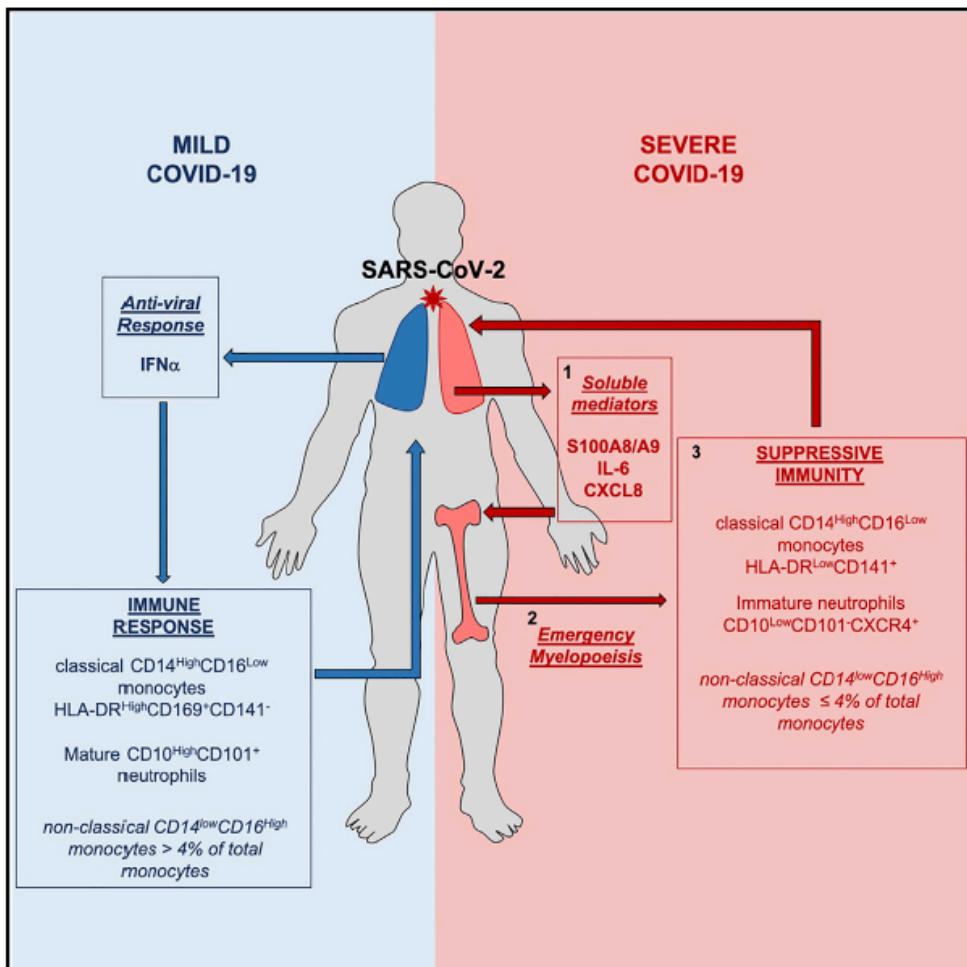


Severe patient neutrophils



Elevated Calprotectin and Abnormal Myeloid Cell Subsets Discriminate Severe from Mild COVID-19

Graphical Abstract



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In Brief

An analysis of patients with severe COVID-19 reveals immature neutrophil and non-classical monocyte pools, with levels of the protein calprotectin linked to disease severity.