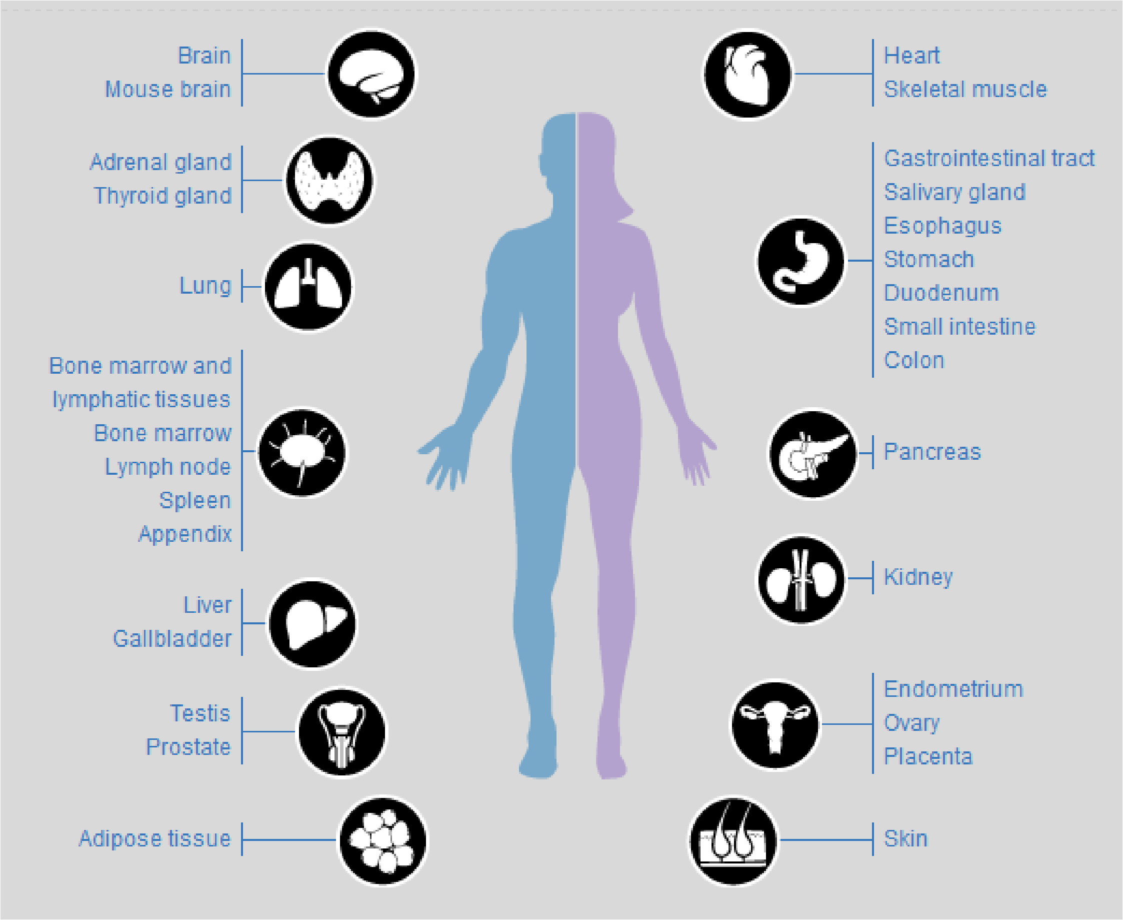
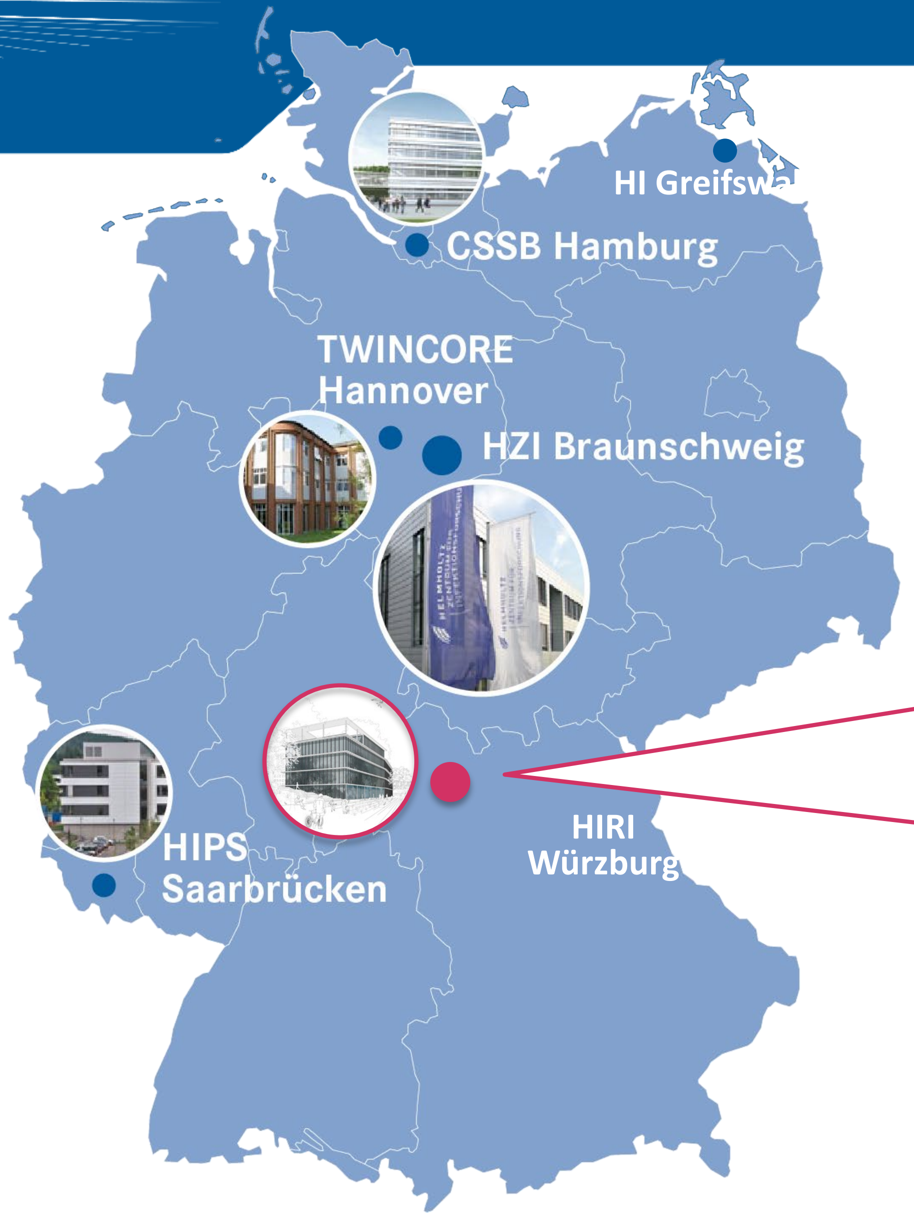


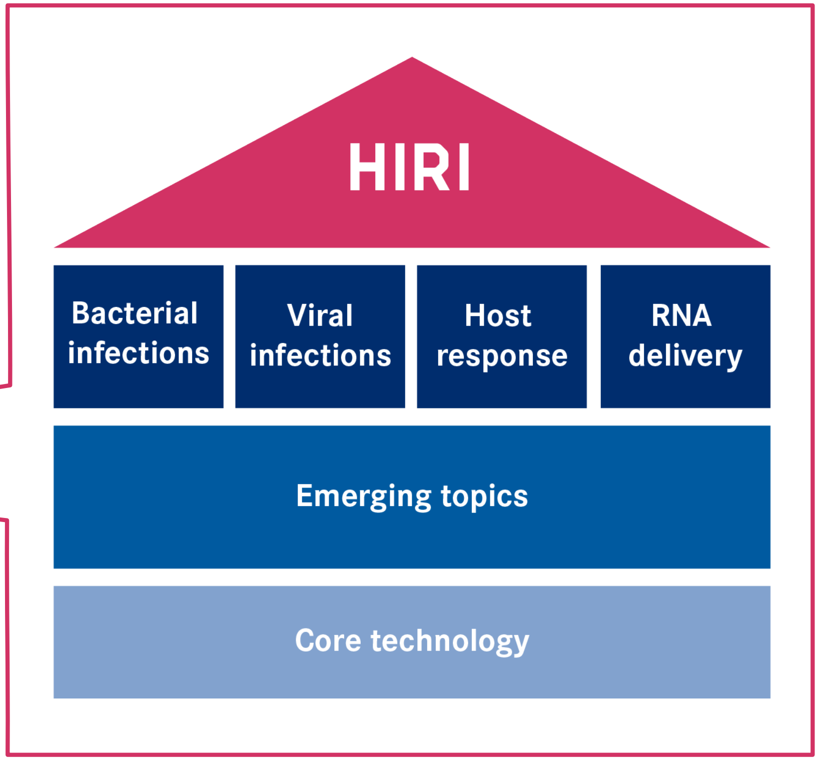
AE_Saliba

Single-Cell Biology course – Introduction – A.-Emmanuel Saliba

Winter Semester 2021 - 2022 | 2021-12-05

HIRI HELMHOLTZ
Institute for RNA-based Infection Research


HI Greifswa
CSSB Hamburg
TWINCORE
Hannover
HZI Braunschweig
HIPS
Saarbrücken
HIRI
Würzburg


HIRI
Bacterial infections
Viral infections
Host response
RNA delivery
Emerging topics
Core technology

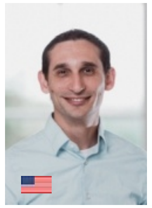
The Team

January 2017 – December 2021
HIRI grown to eight research groups

RNA Biology of Bacterial Infections

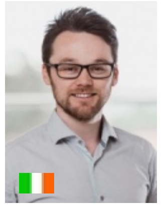
J. Vogel

RNA Synthetic Biology



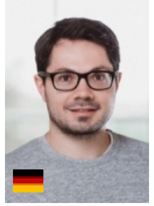
C. Beisel

Genome Architecture & Evolution of Viruses



R. Smyth

Host-Pathogen-Microbiota



A. Westermann

Single-cell Analysis

E. Saliba

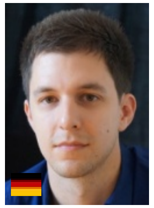
Recoding Mechanisms

N. Caliskan

Integrative Informatics

L. Barquist

Host lncRNAs



M. Munschauer

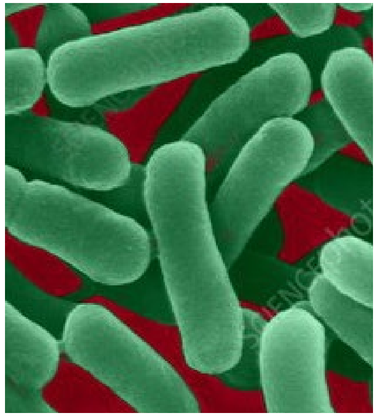
Saliba lab Laboratory pillars

1- Enabling technologies to decipher host-pathogen interactions at the single cell level

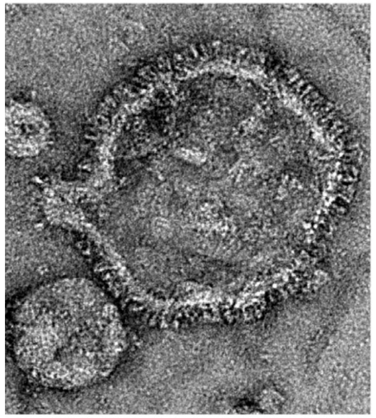
2- Deciphering how a *Salmonella enterica* antibiotic tolerant subpopulation leads to replase (and if)

3- Nailing down the host response and the cellular tropism of respiratory virus (RSV and SARS)

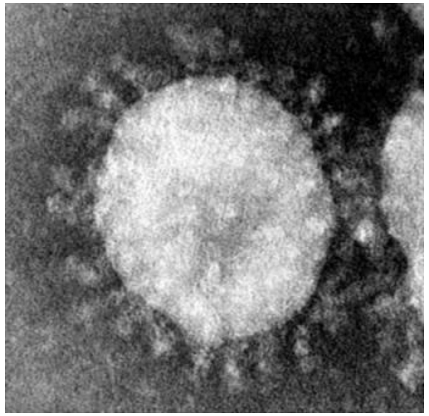
***Salmonella* Typhimurium**: Gram-, enteric pathogen, model for persistence



RSV (Respiratory syncytial virus) : single-stranded RNA virus major pathogen in children



SARS-CoV-2: betacoronavirus, positive-strand RNA viruses



LECTURE PLAN:

- Part 1 - Fundamentals in analyzing tissues and single cells
- Part 2 – Our latest research on COVID-19 lung damage

Who has already used a flow cytometer ?

Who knows what RNA-seq is?

Who has already coded?
(R, Python, C++, …)

What is short-read sequencing?

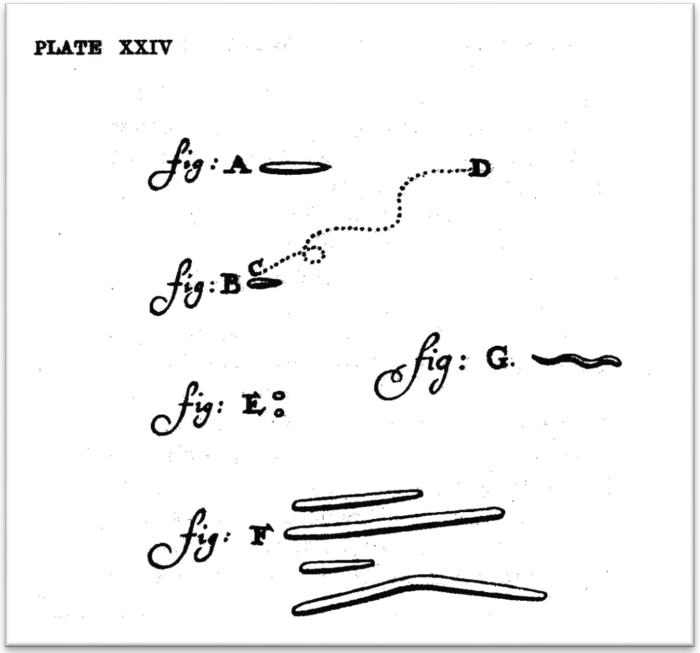
How many cells a human body has?

How many protein-coding genes in the human genome?

Who heard about ‘epigenomics’?

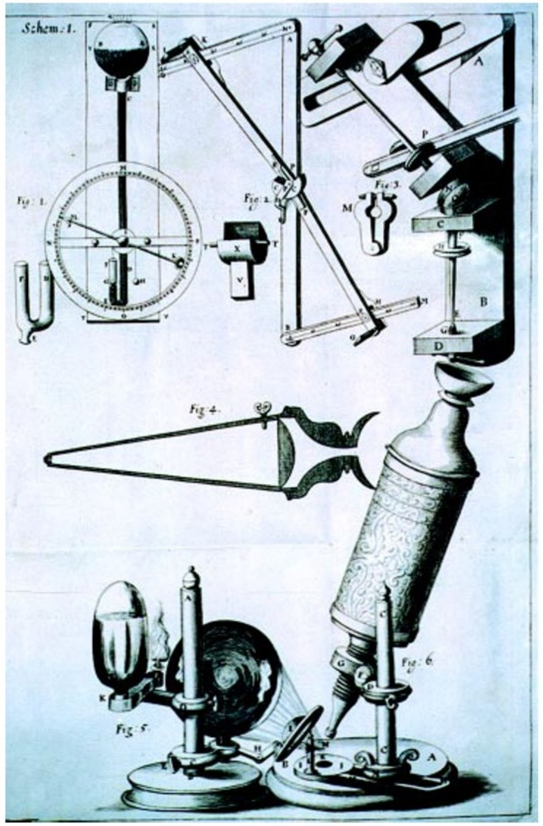
The first observation of *cell* structures

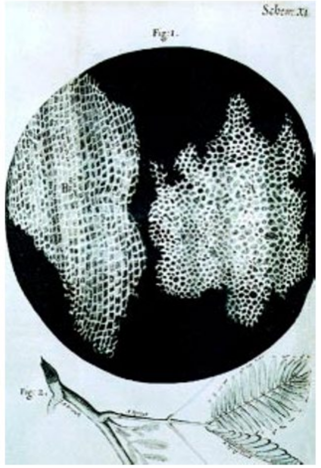
Antoni van Leenwenhoek (1632-1723)



→ Linked 'movement' to 'life'
→ Observation of living organisms

Robert Hooke (1635-1702)





→ Observed woods
→ Foster the word 'Cell'

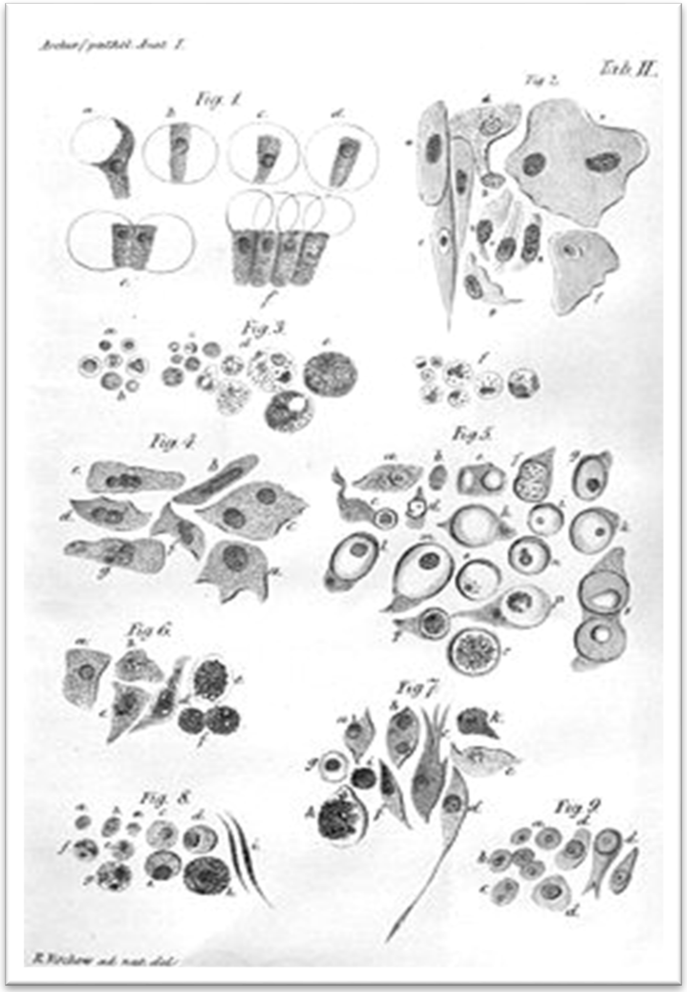
‘Cell Theory’
Omnis cellula e cellula ("all cells (come) from cells")

Rudolf Virchow
(1821-1902)

Matthias Schleiden
(1804-1881)

Theodor Schann
(1810-1882)

Array of new structures



Cell Theory

- *Cella* (Latin) = small room
- Organisms are composed of one or more cells
- Basic unit of structure and function in life
- Cells are derived from pre-existing cells

Mazzarello 1999 *Nat Cell Biol*

From Genotypes to Phenotypes

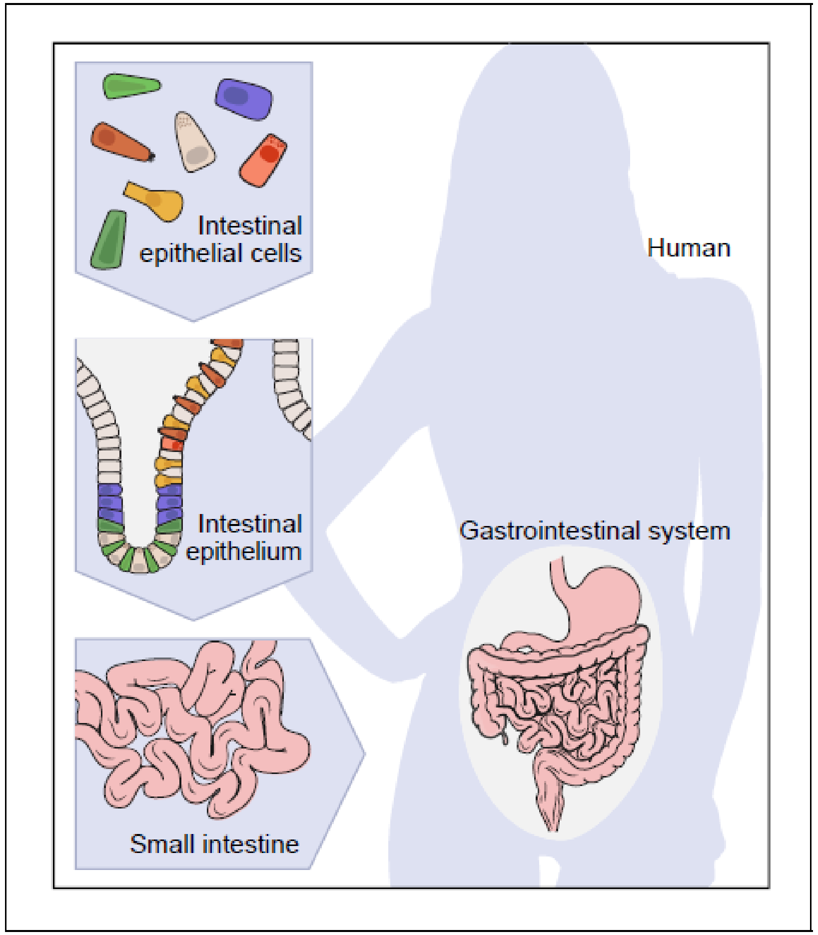


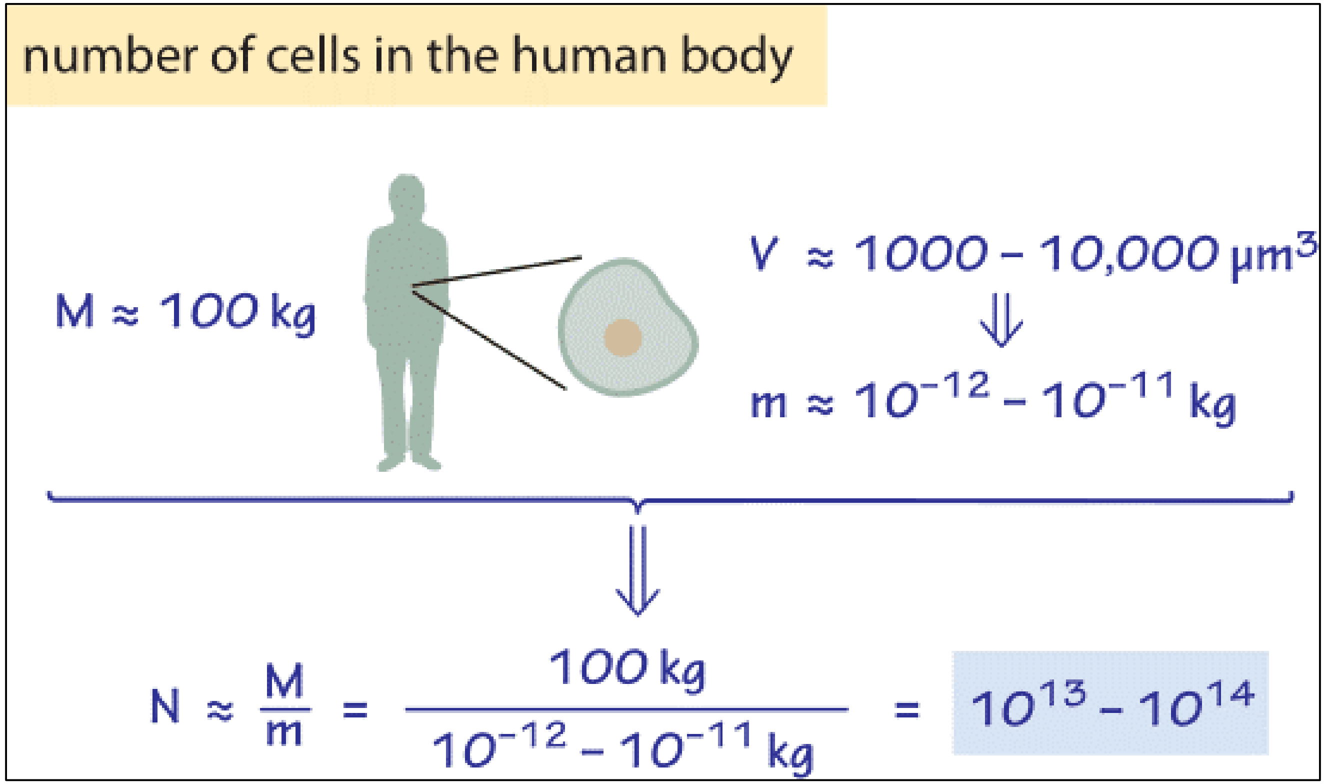
Figure 1. A hierarchical view of human anatomy. A graphical depiction of the anatomical hierarchy from organs (such as the gut), to tissues (such as the epithelium in the crypt in the small intestine), to their constituent cells (such as epithelial, immune, stromal and neural cells).

- Physiology emerges from normal cell functions and intercellular interactions.
- Genotypes gives phenotypes through the intermediate of cells (the functional units).
- Each cell regulates its own program of gene expression.

Regev et al 2017 *eLife*

Number of cells in a human body?

Rough estimate
‘Order of magnitude‘



Interested to know more

Ann Hum Biol. 2013 Nov-Dec;40(6):463-71. doi: 10.3109/03014460.2013.807878. Epub 2013 Jul 5.

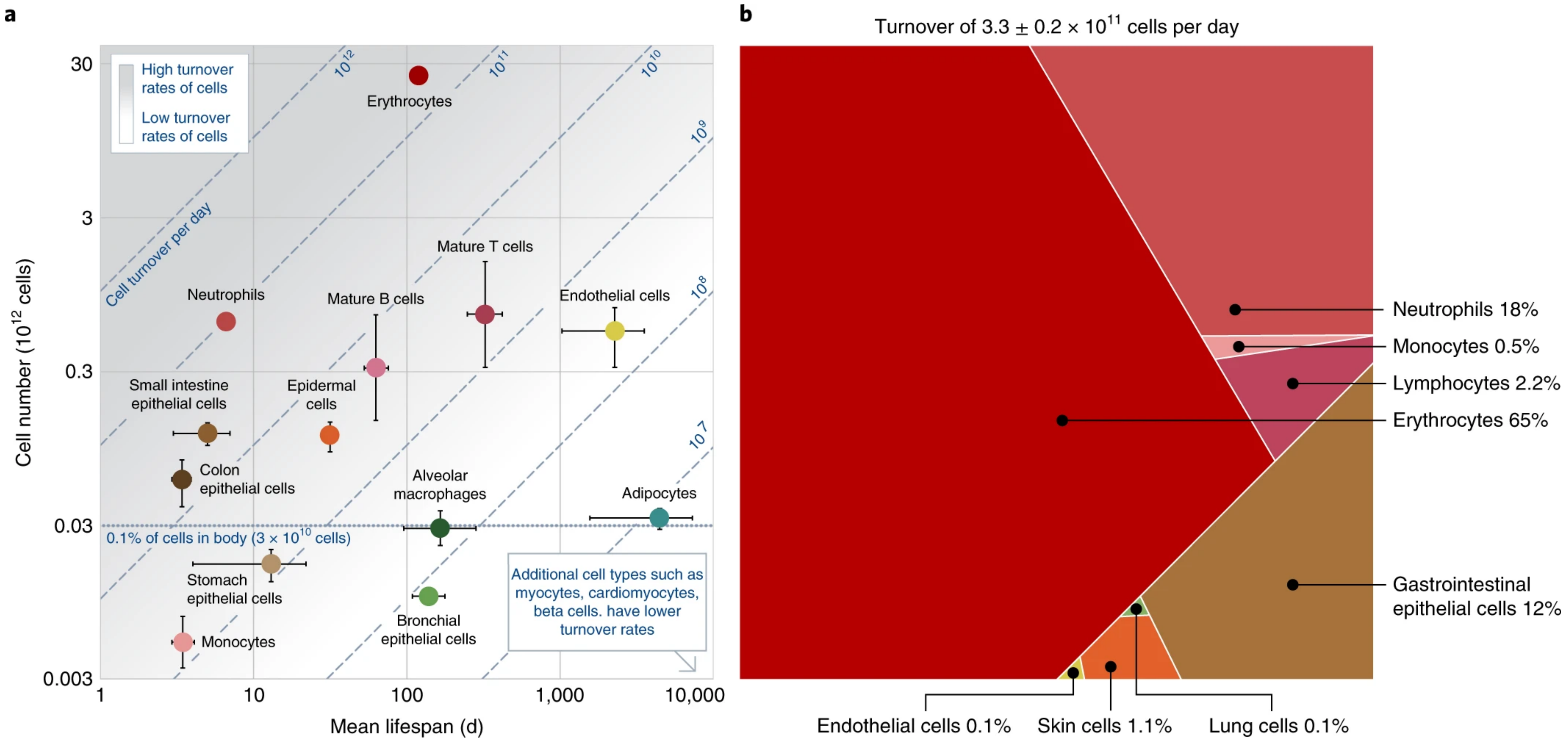
An estimation of the number of cells in the human body.

Bianconi E[1], Piovesan A, Facchin F, Beraudi A, Casadei R, Frabetti F, Vitale L, Pelleri MC, Tassani S, Piva F, Perez-Amodio S, Strippoli P, Canaider S.

Author information

1 Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna , Bologna , Italy .

Cell lifespan and turnover in a human body?



Sender and Milo, 2021 *Nature Medicine*

How to define cellular identities?

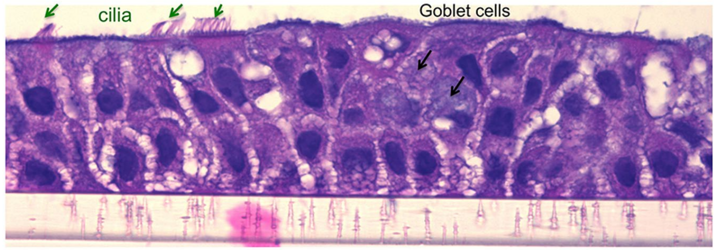
- Shape
- Localization
- Localization relation to other cells
- Biological functions
- Molecular components

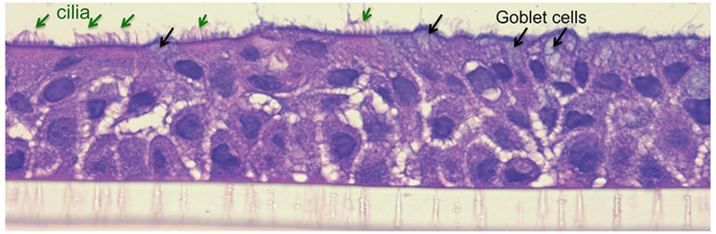
Histology - Chemical dyes
Tissue sections/ Cell shapes

Hematoxylin : nucleic staining
Eosin : extracellular matrix and cytoplasm

Spleen section

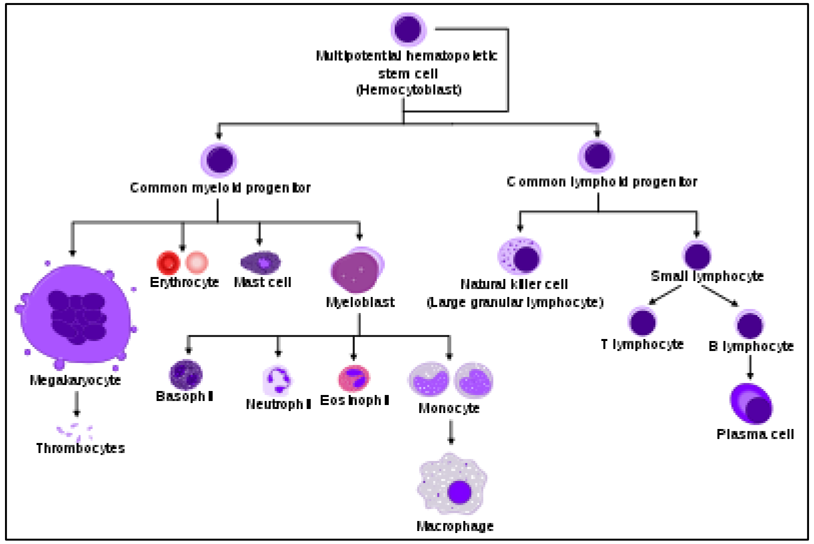
Airway interface

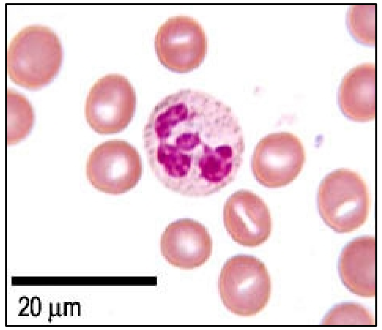




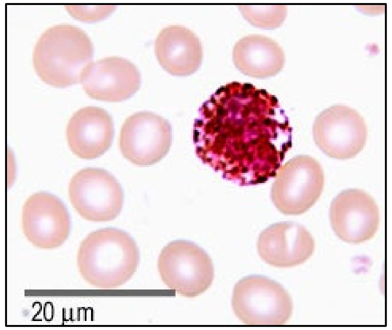
https://doi.org/10.1371/journal.pone.0196256.g001

Prototypical example: Haematopoiesis





Neutrophils

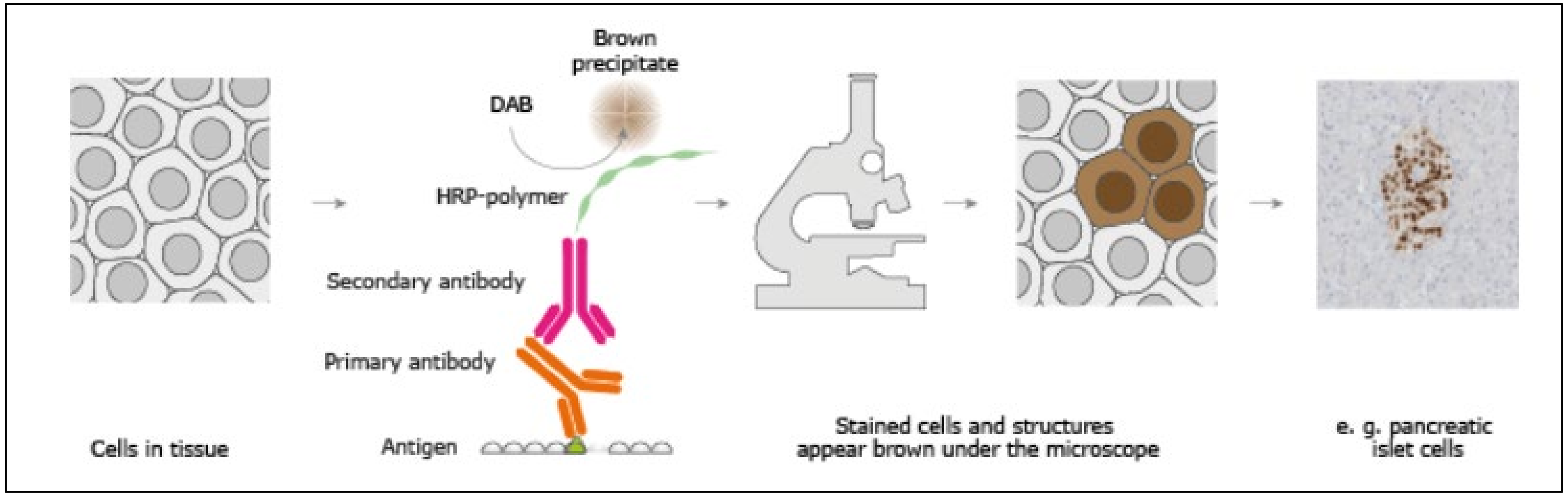


Basophils

Lymphocytes

https://www.histology.leeds.ac.uk/

Immunohistochemistry

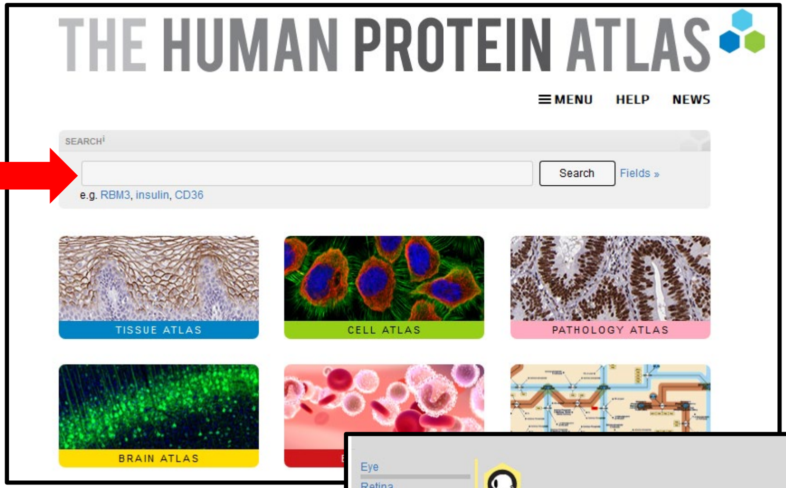


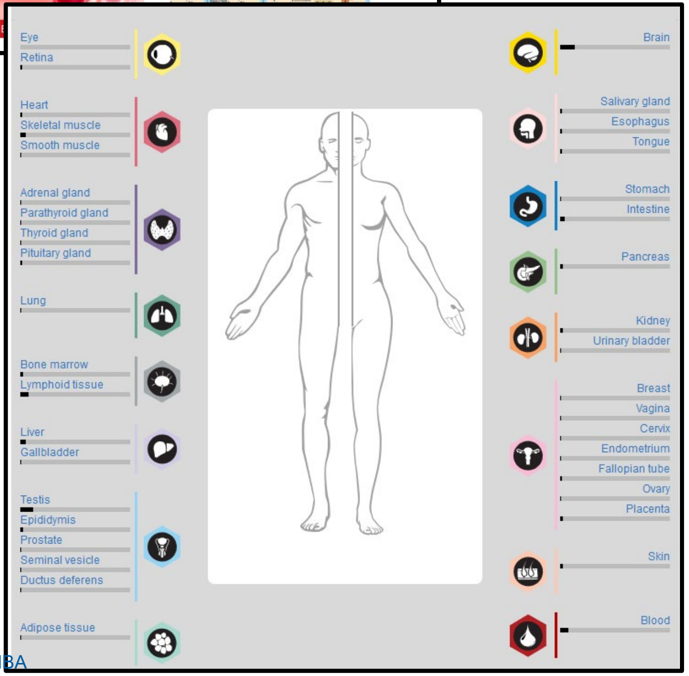
www.proteinatlas.org

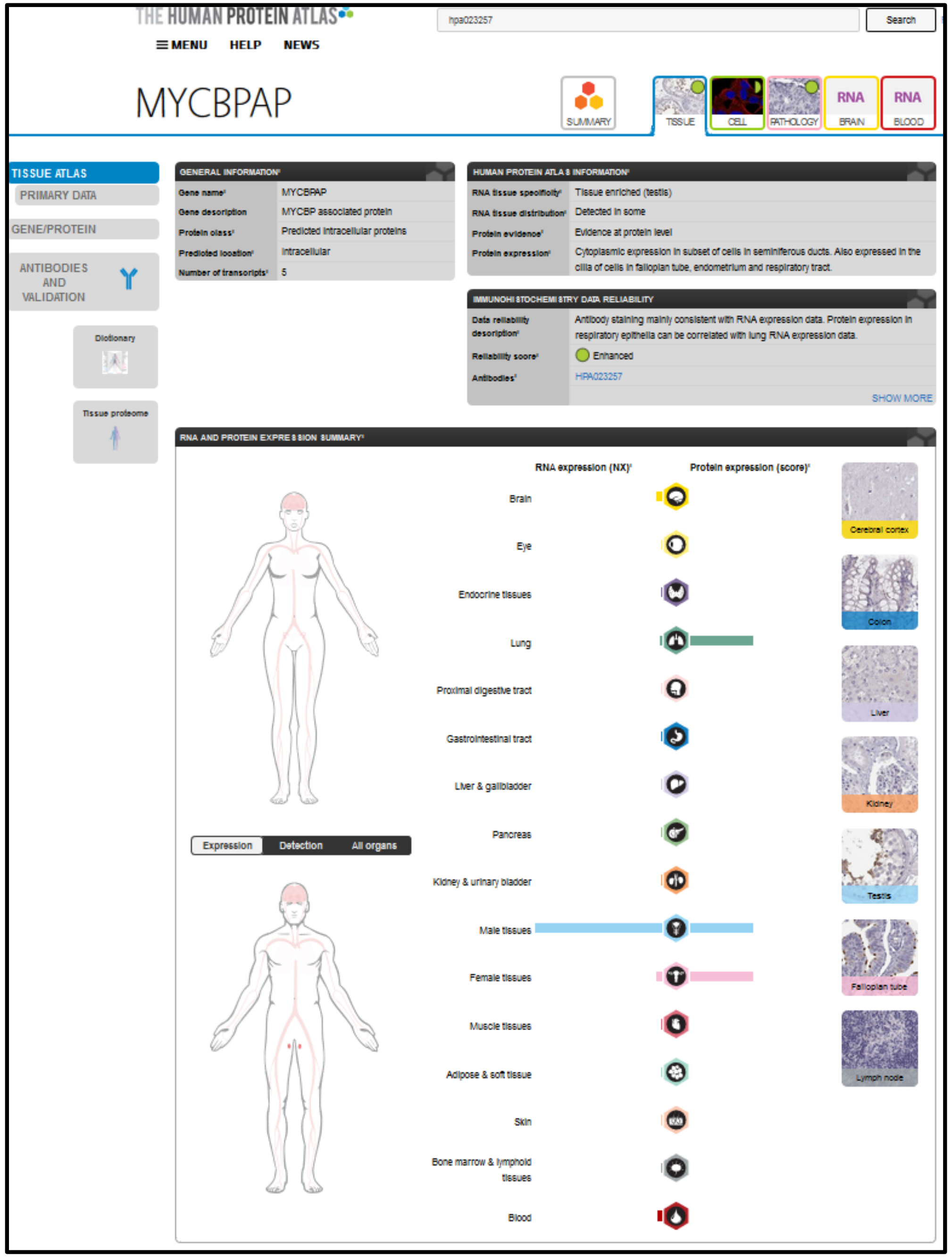
- Detection of epitopes expressed by a single protein-target within a tissue sample using a “primary antibody”
  - A “secondary antibody” capable of binding the primary antibody with high specificity is added
  - The secondary antibodies then carry an enzyme for example horseradish peroxidase (HRP)
    - HRP catalyzes the conversion of chromogenic substrates into colored products

The protein atlas

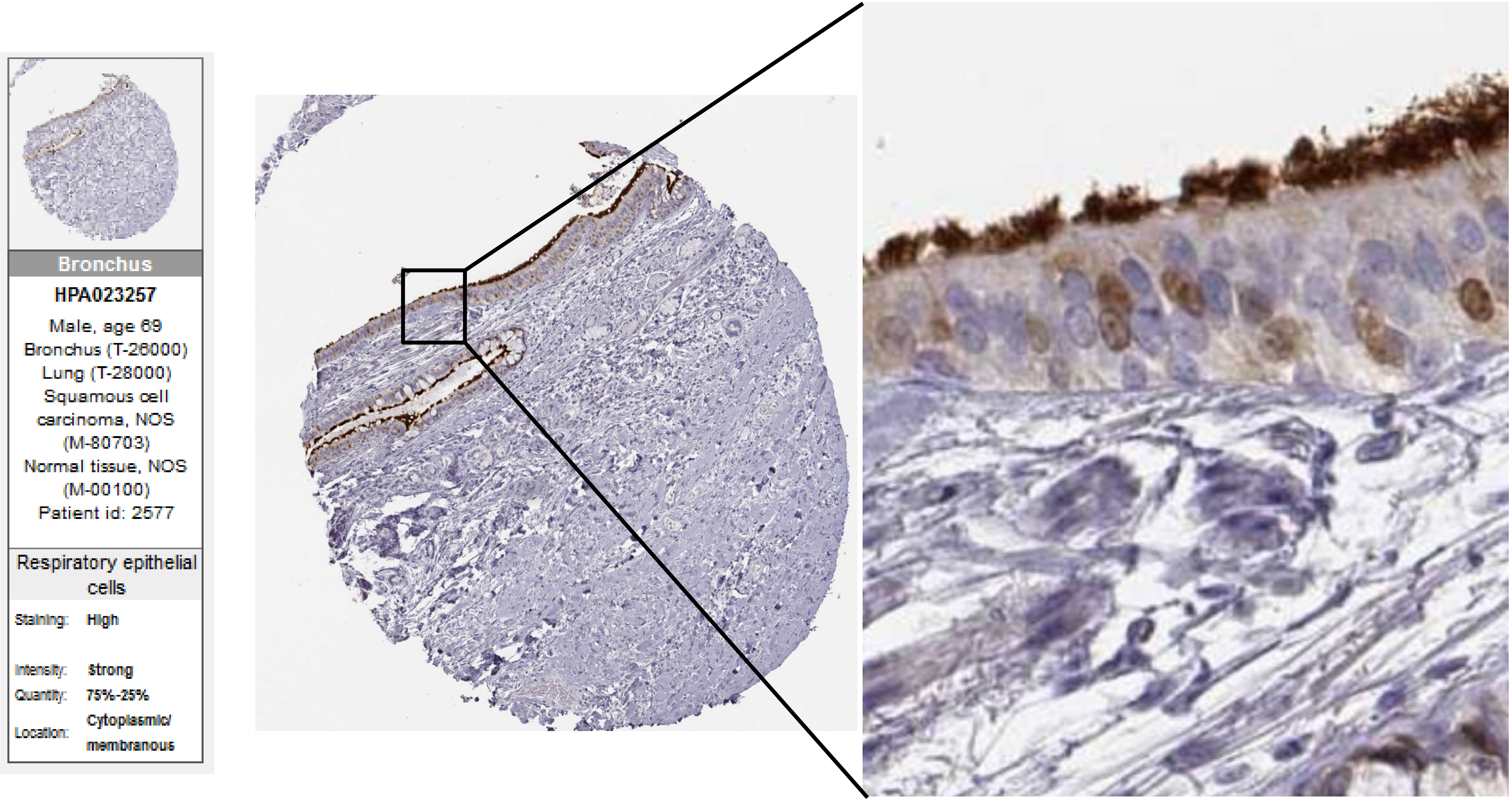
www.proteinatlas.org/







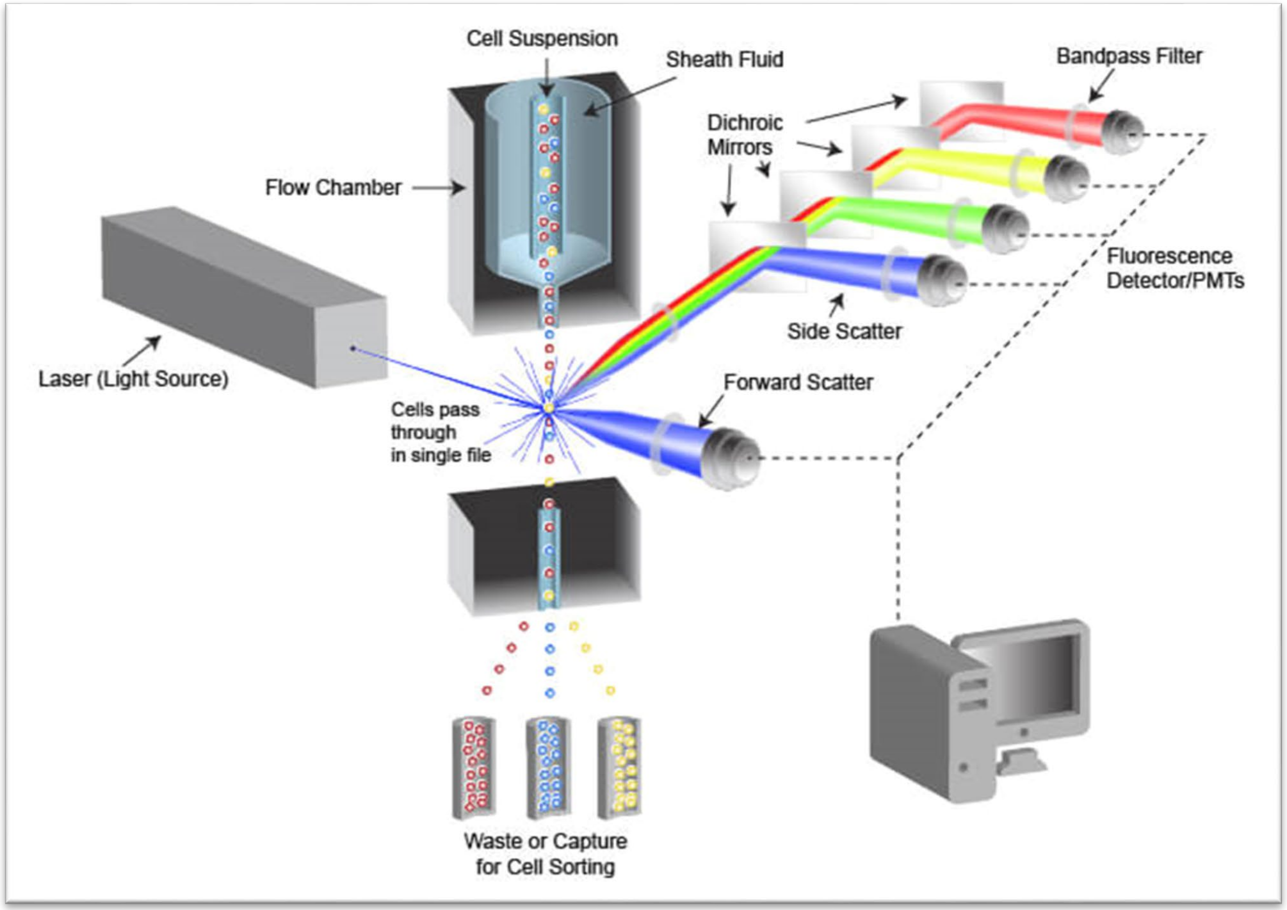
Thul PJ et al 2017 *Science*



HPA023257 = antibody targeting MYCBPAP

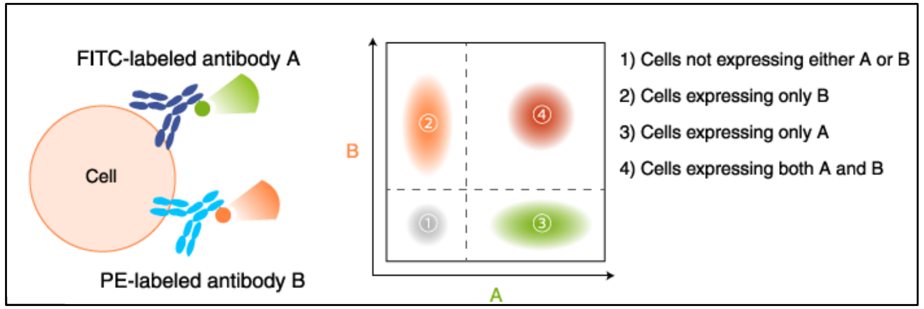
Flow cytometry

Single cell analysis

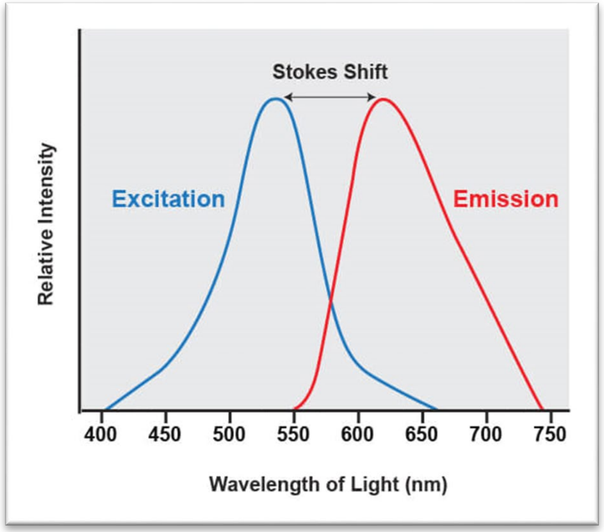


https://www.cellsignal.com

Multimodal labeling



Fluorescence spectra

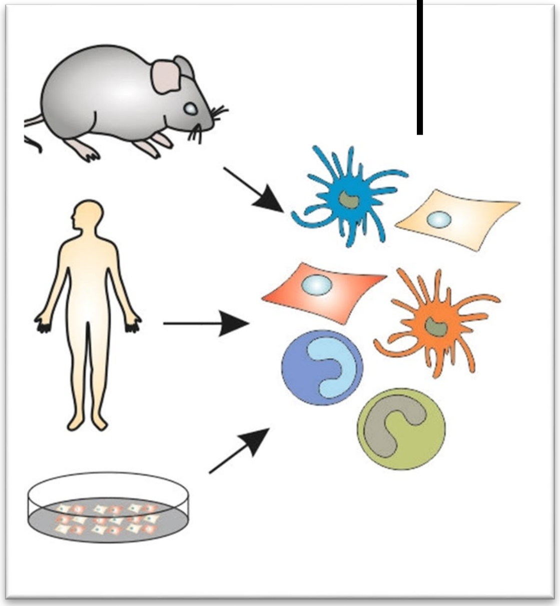


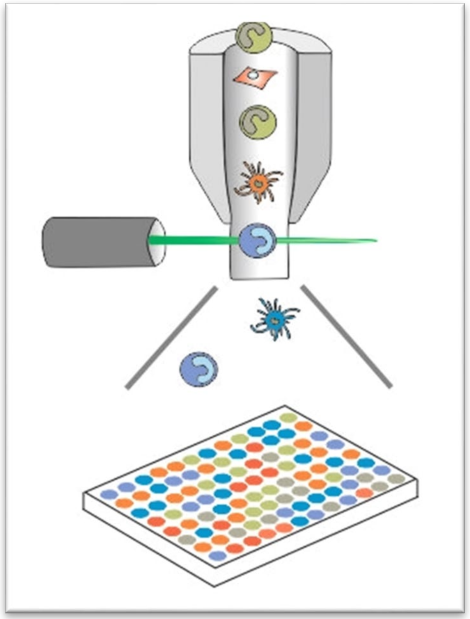
- Calculate spectral recovery
  - Apply compensation

Flow cytometry and FACS (Fluorescence-activated cell sorting)

Dissociate in single-cell suspension

Analyse and Sort





One can sort down to a single-cell (efficiency >99%)

<u>Challenges:</u>

- Overlapping fluorescent signals
- Strong autofluorescence of the cells
- False positive rate (for rare populations)
- Sorting cells < 10^{-4} start to be difficult

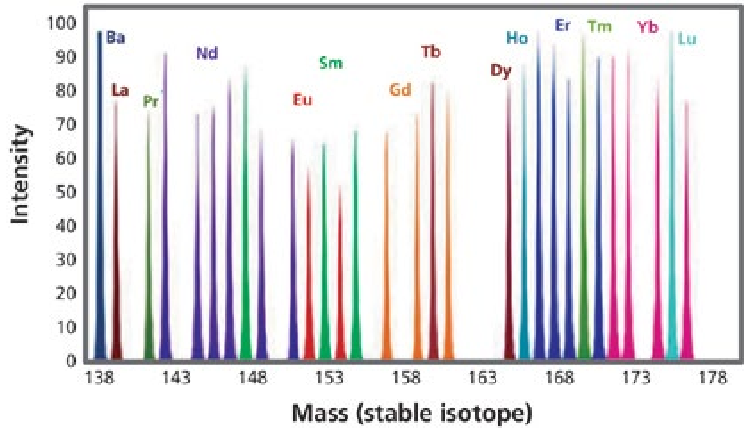
<u>Index Sorting:</u>

<u>(only when you sort single-cells)</u>

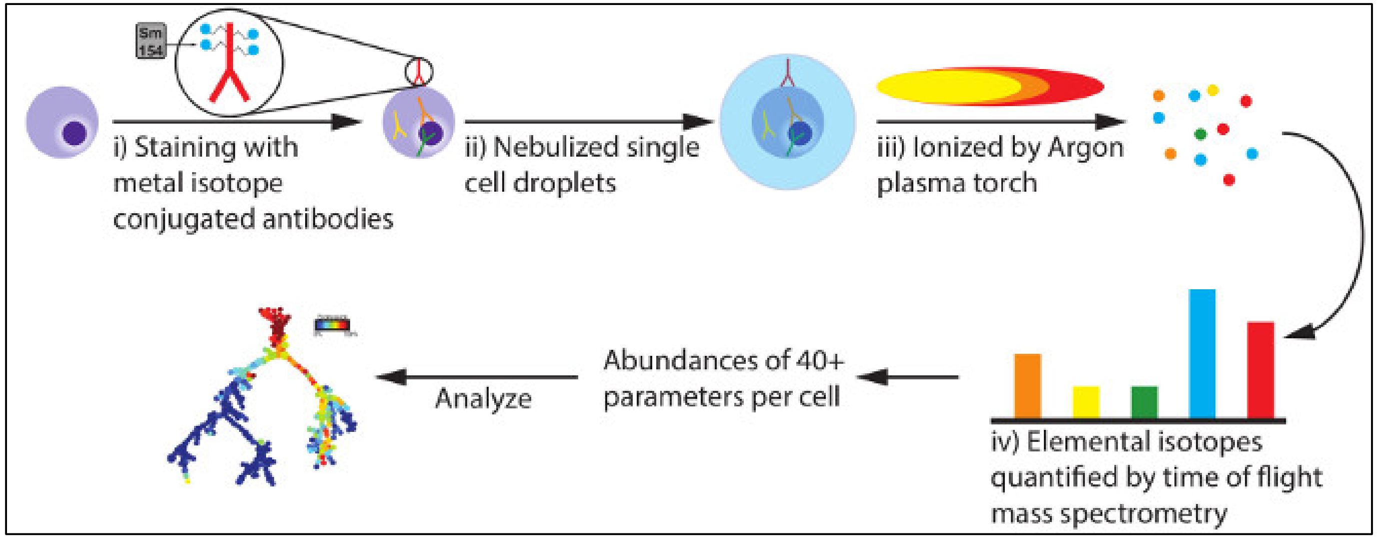
- Record the fluorescence signals associated to the sorting position

The ultimate flow cytometry - Mass cytometry

Isotope conjugated antibodies



Principle of mass cytometry



McCarthy et al 2017 *JOVE*

- ➢ Up to 100 signals simultaneously
- ➢ Suffer from a poor sensitivity
- ➢ Still rely on the production of antibodies

Limitations in defining cell identities based on a restricted set of markers

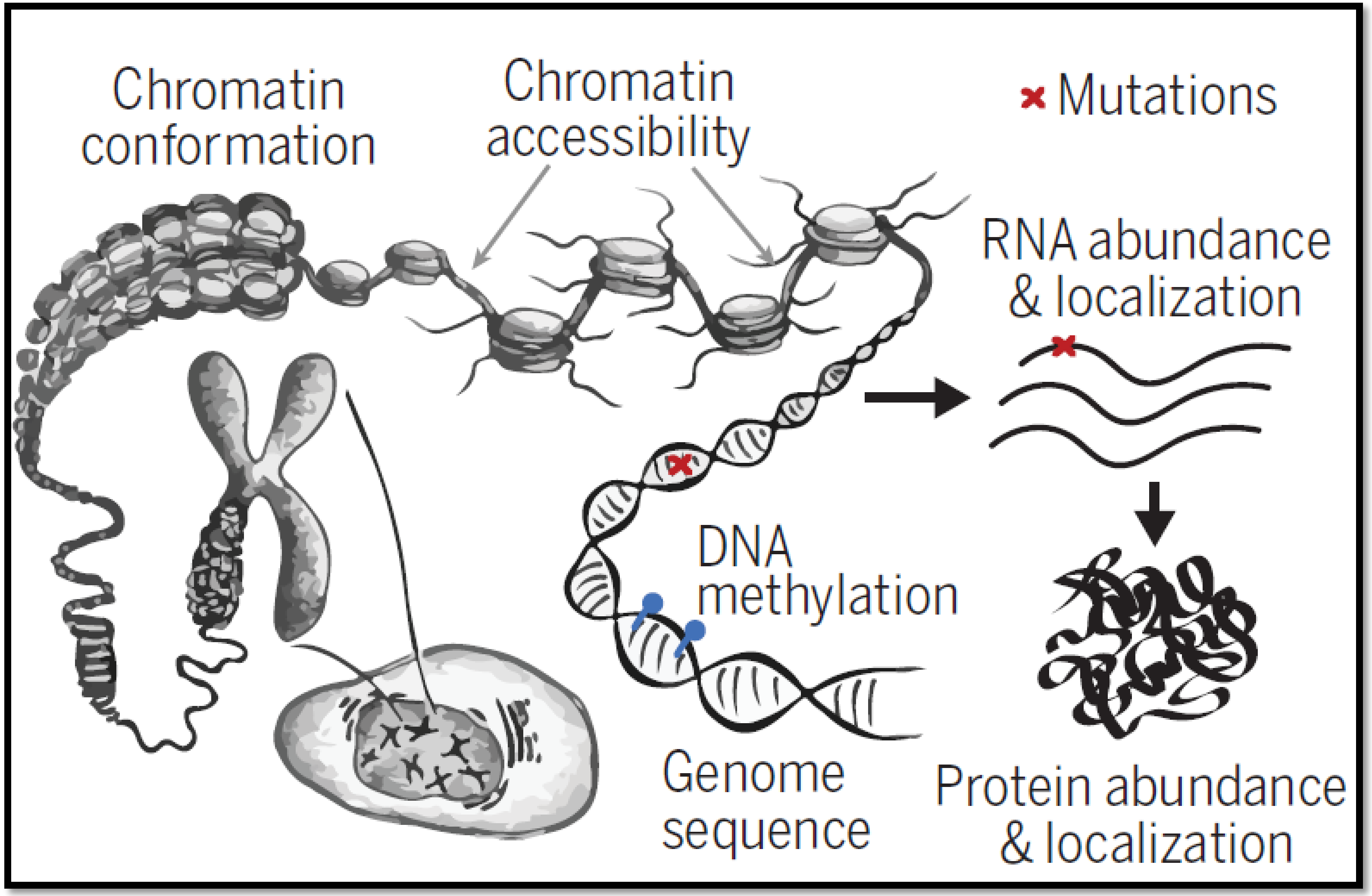
- ➢ Distinct molecular phenotypes ~ distinct functions (example: immune cells)
- ➢ So far cell definition is empirical and …
- ➢ …biased (based on an *a priori* knowledge)…
- ➢ Use features that can apply to many cell types

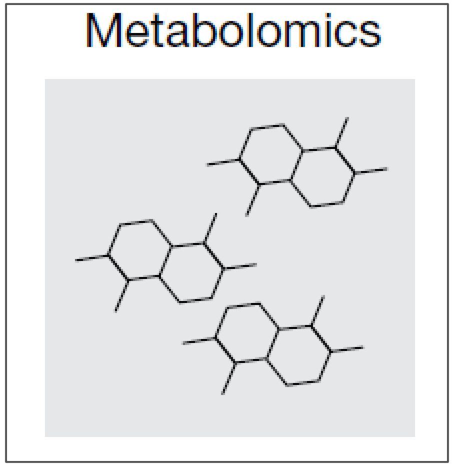
Can we get a data-driven/systematic definition of a cell?

Can we differentiate cell types (persistent) and cell states (transitory)

Can we have an atlas of all cells in a body ?

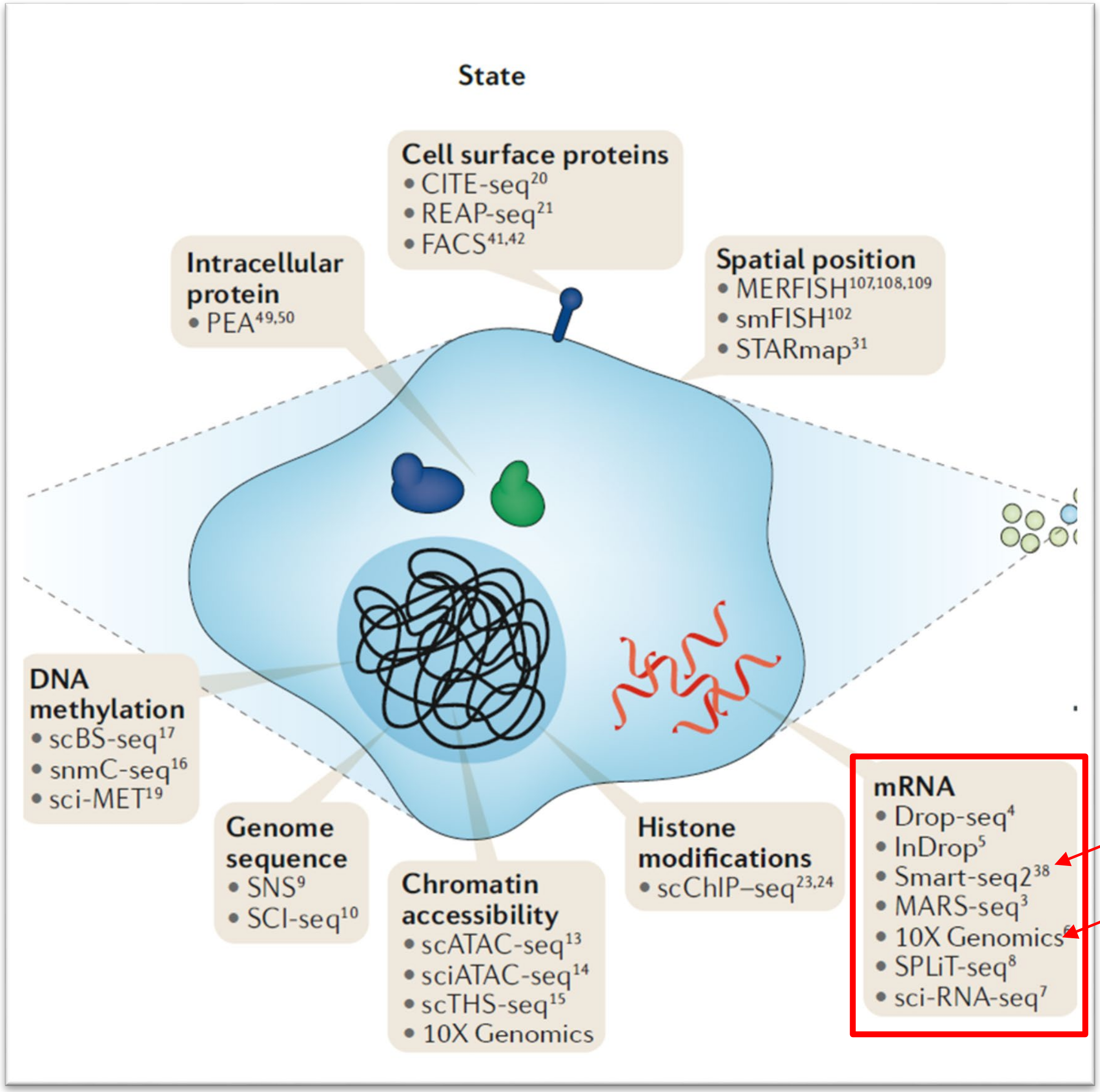
Describing a single cell with '–omics' methods





Camp et al. 2019 *Science*

Describing a single cell with '–omics' methods



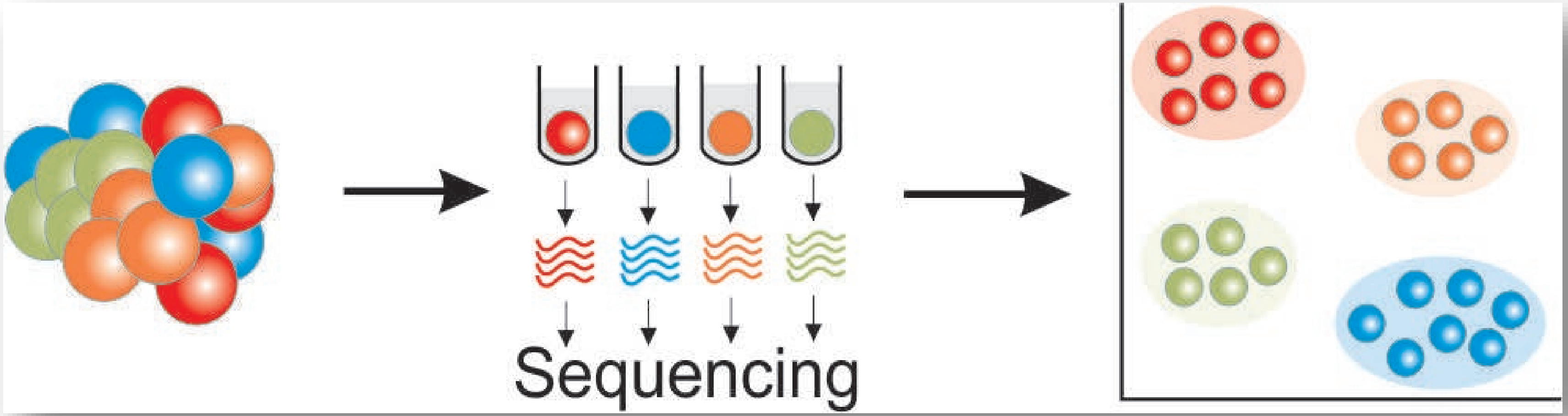
Stuart and Satija 2019 *Nat Rev Gen*

Principle of single-cell analysis

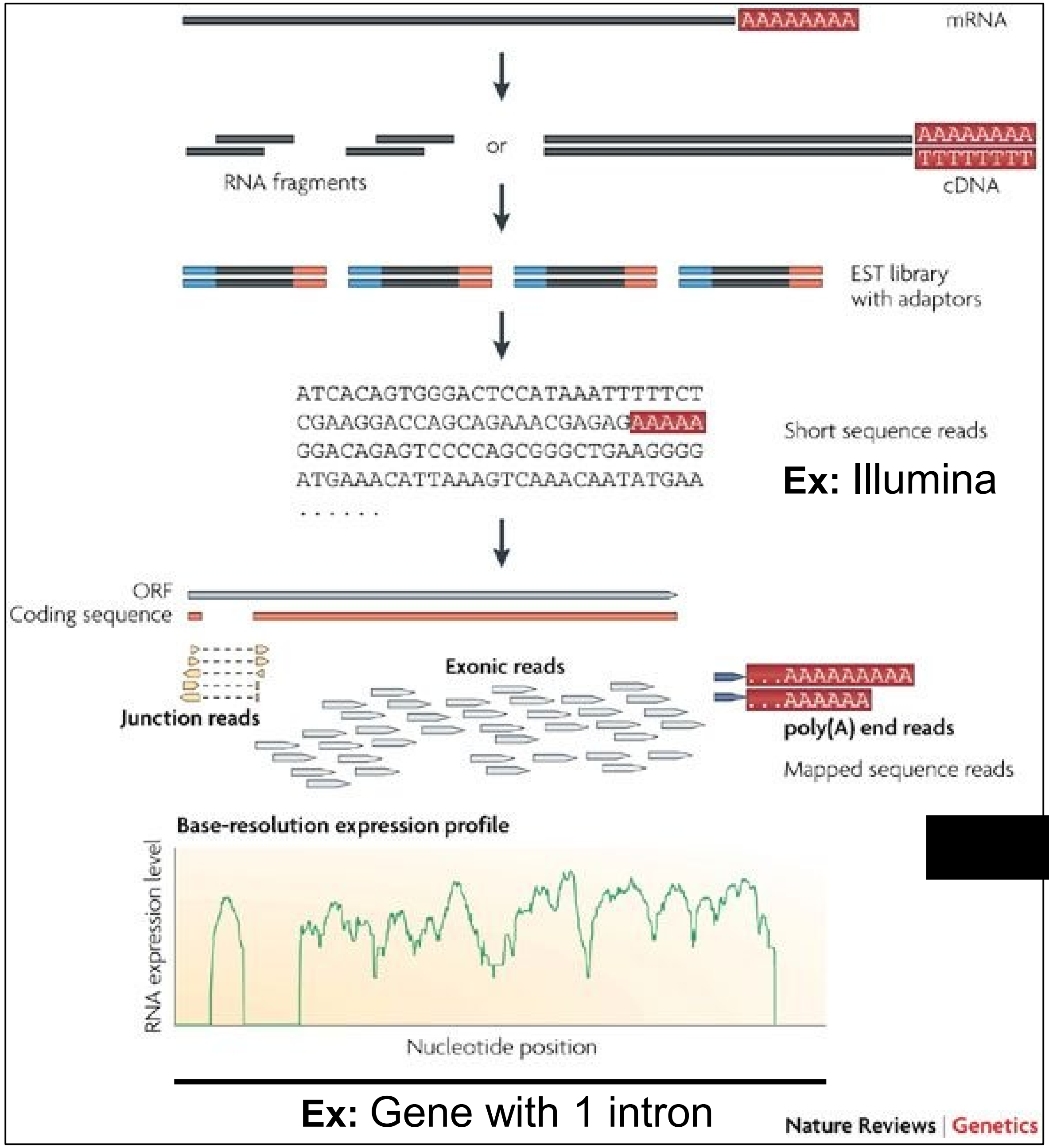
Population

Isolation for omics-analyses

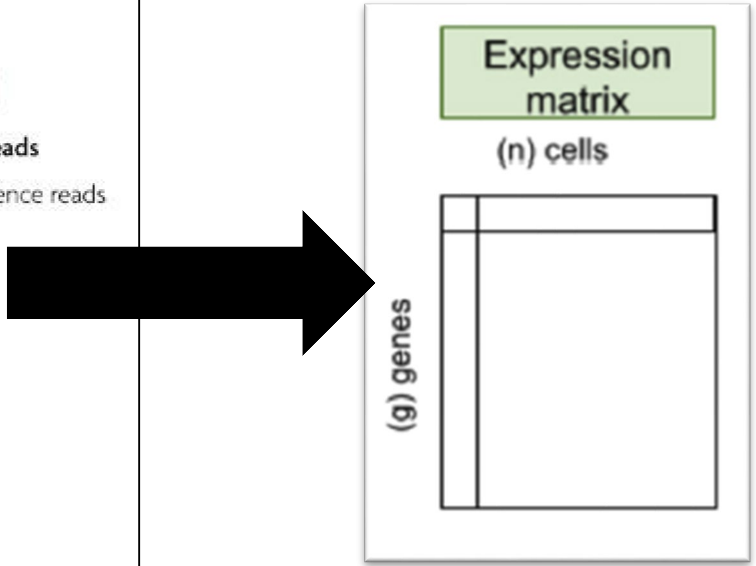
Data-driven mapping and identity determination

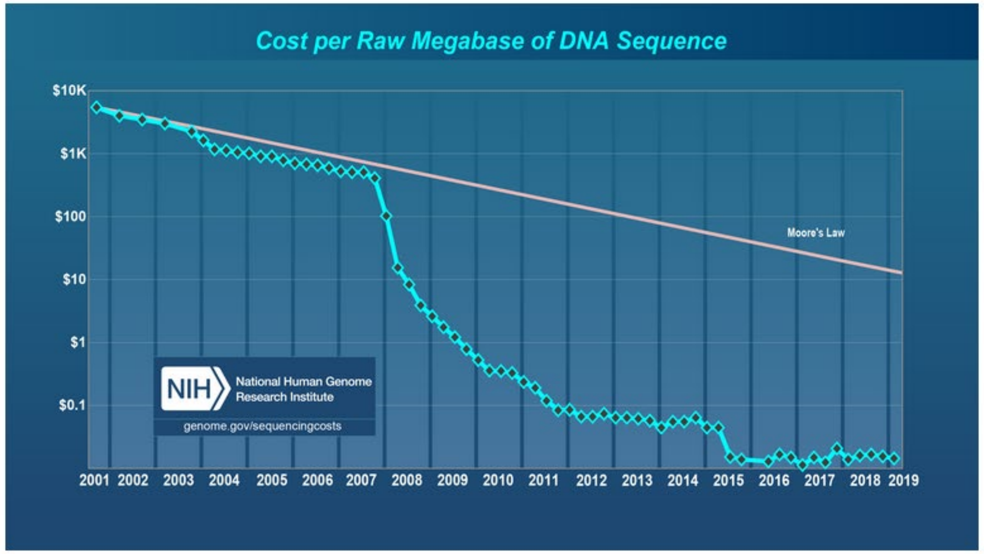


RNA-seq



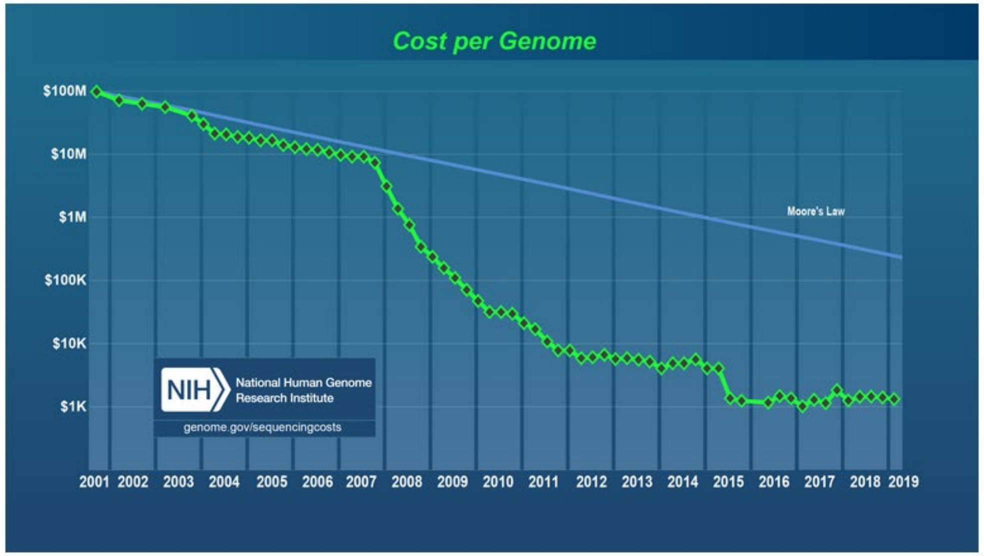
Snyder and co 2009 *Nat Rev Gen*





Sequencing cost per Mb - 2019

Mb: a million bases

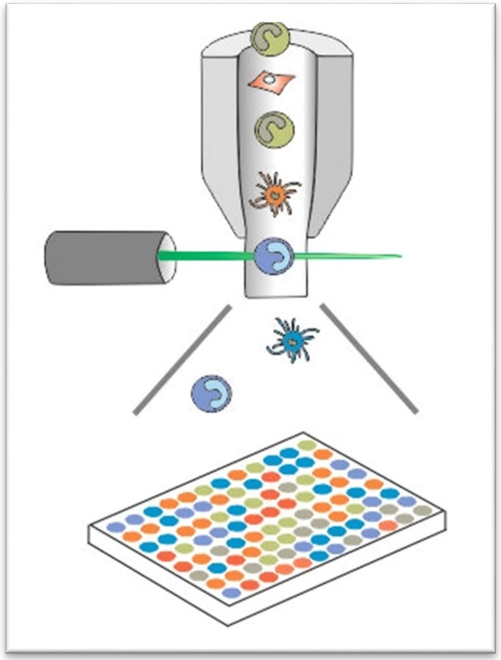


Sequencing cost per genome - 2019

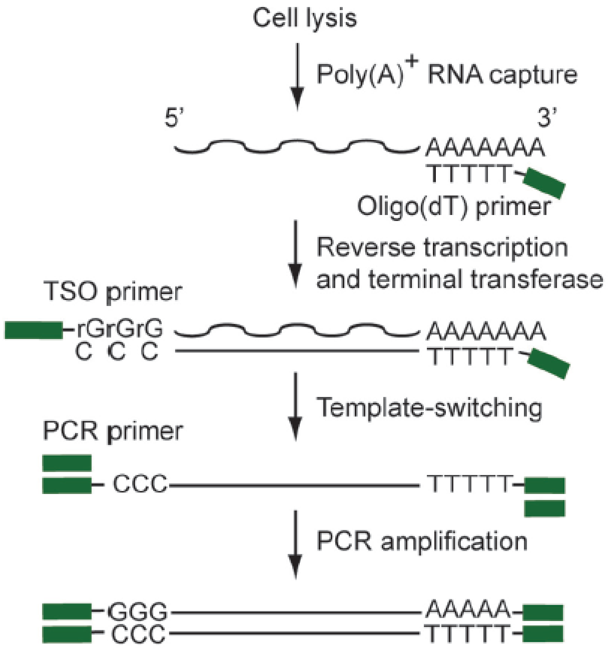
Illumina Nova-seq
up to 48 genomes

Basic scRNA-seq protocol : Smart-seq (also called deep scRNA-seq)

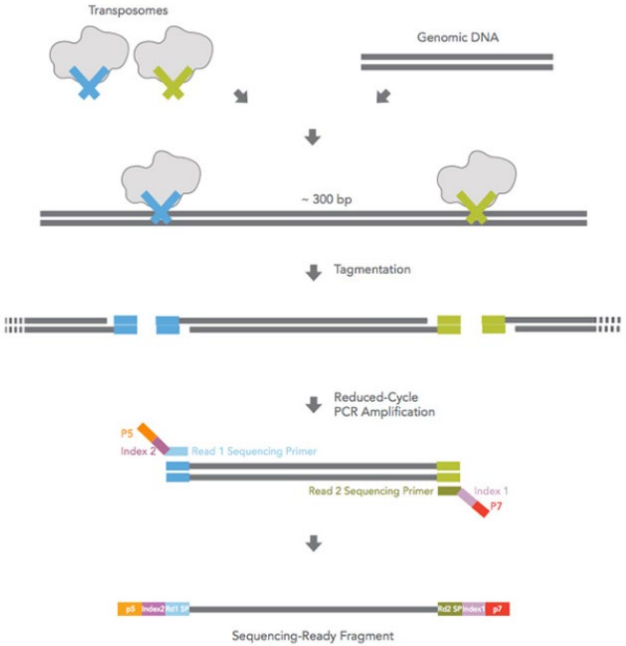
Single cell sorting on a plate



Step1: Template switching



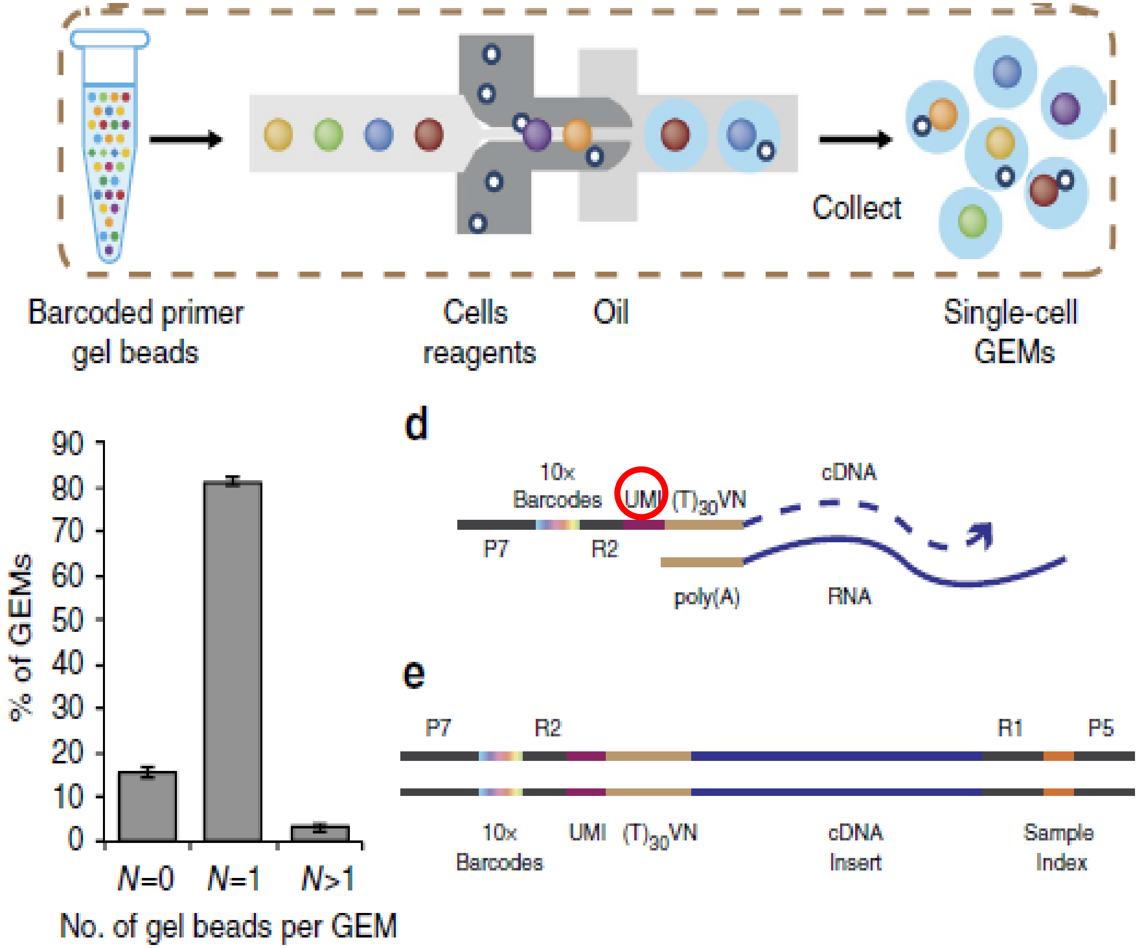
Step2: Tagmentation + indexing



Challenges:

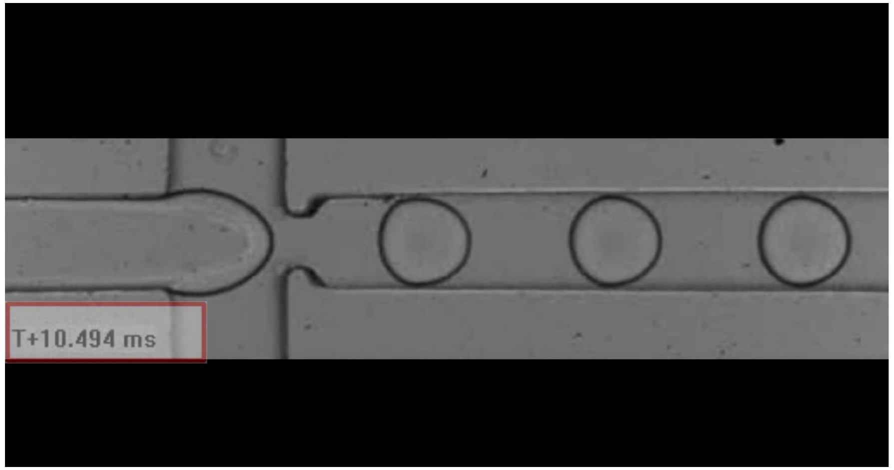
- ➢ Pretty demanding protocol
- ➢ Limited multiplexing because of indexing (few hundred cells)
- ➢ Limited compatibility with Nova-seq (index hopping)

Basic scRNA-seq protocol : droplet-based (10x Chromium)



Bielas et al 2017 *Nat Comm*

Single cell encapsulating into droplets



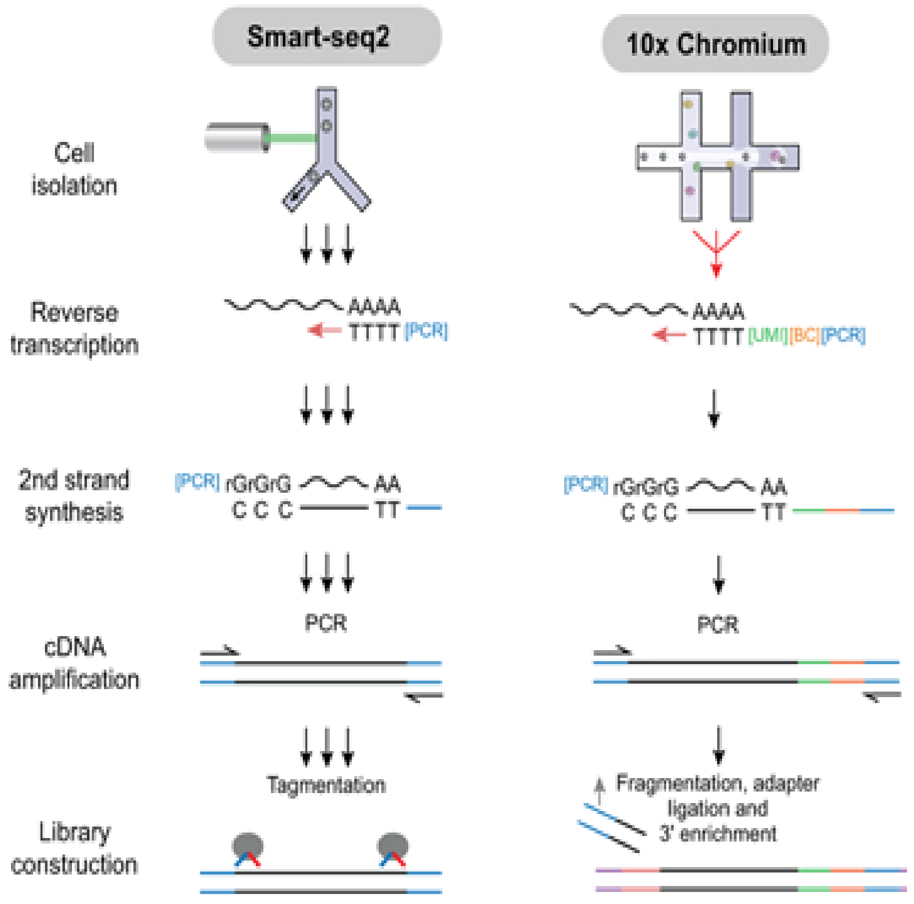
Hyperlinked image

<u>Features:</u>

- ➢ Thousands of cells in parallel
- ➢ UMI-based (minimizes PCR biases)
- ➢ UMI-based (prevents having the full-length transcript)

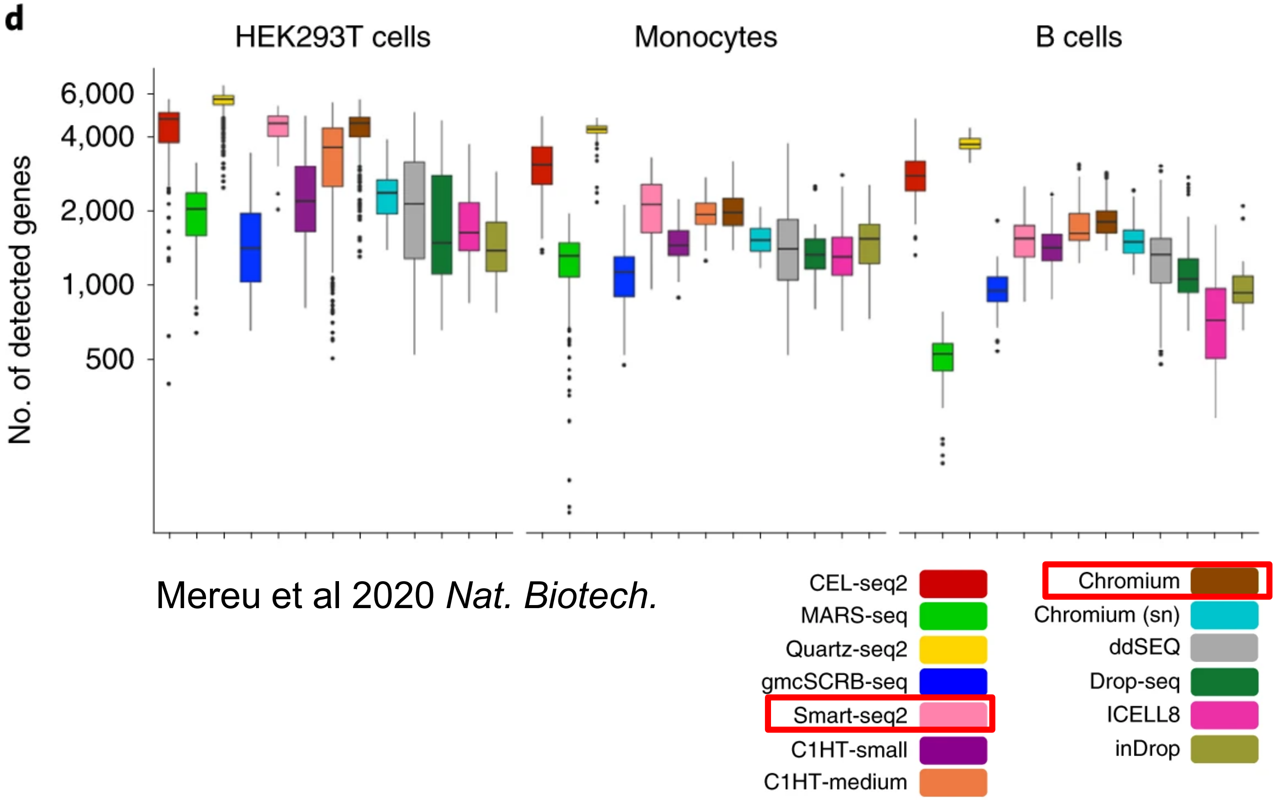
Comparison Smart-seq / 10x Chromium

Library prep.



Ding et al 2020 *Nat Biotech*

Sensitivity



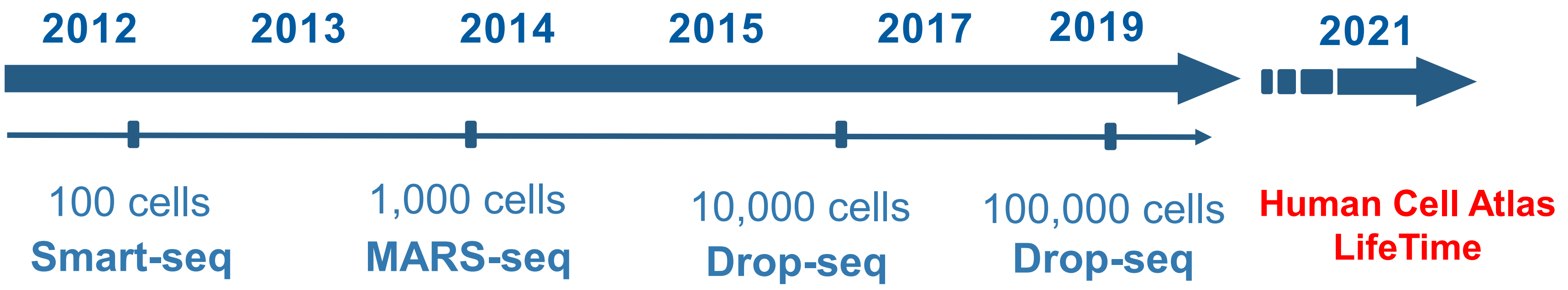
Mereu et al 2020 *Nat. Biotech.*

Main Features:

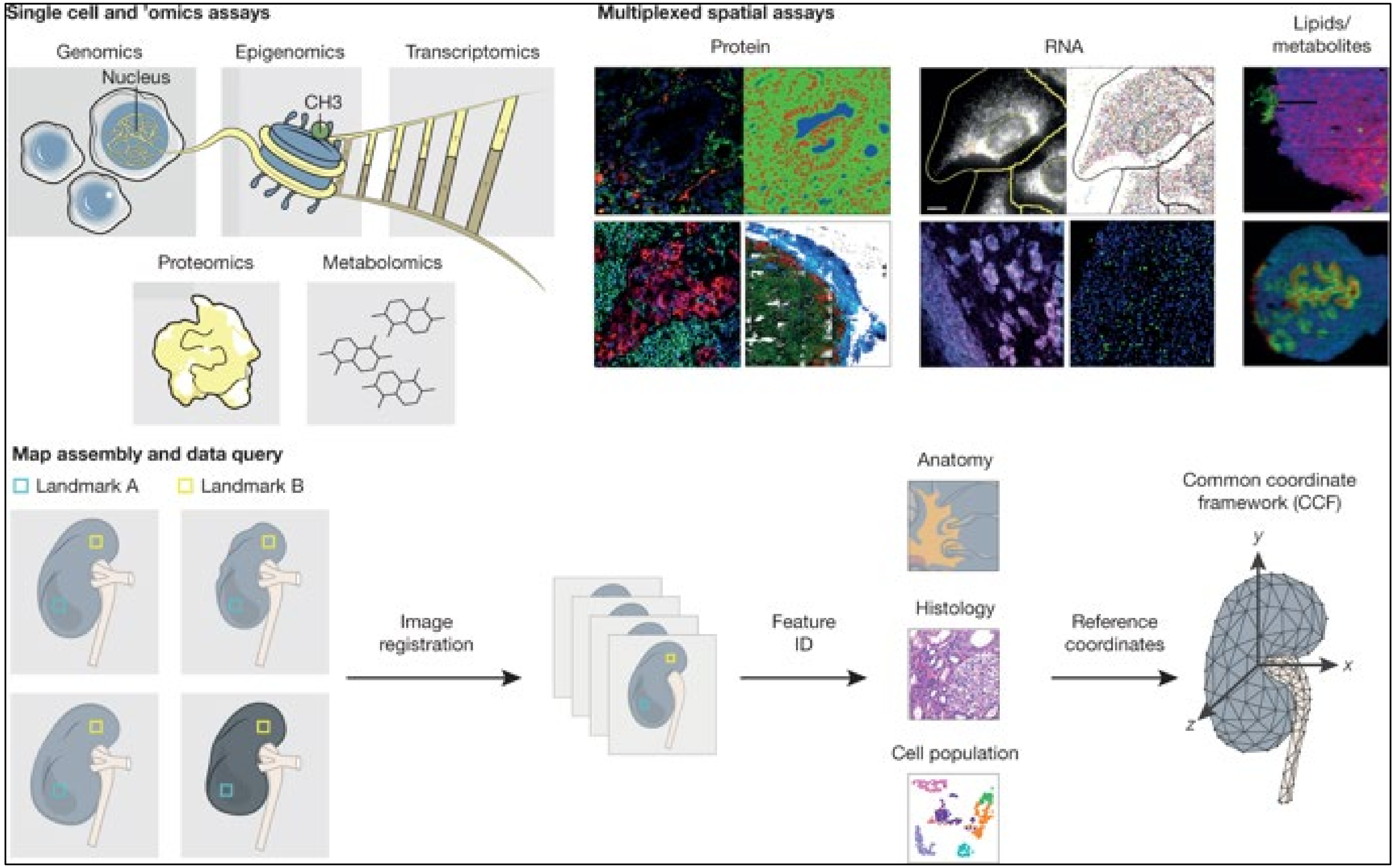
- ➢ Smart-seq: full-length and higher sensitivity
- ➢ 10x : UMI and higher throughput

Towards a whole organism in single cells

Timeline



3D maps of tissues at the single cell resolution



HuBMAP Consortium 2019 *Nature*

International initiatives to resolve 3D maps of tissues

Human Cell Atlas (US)
https://www.humancellatlas.org/

LifeTime
https://lifetime-fetflagship.eu/

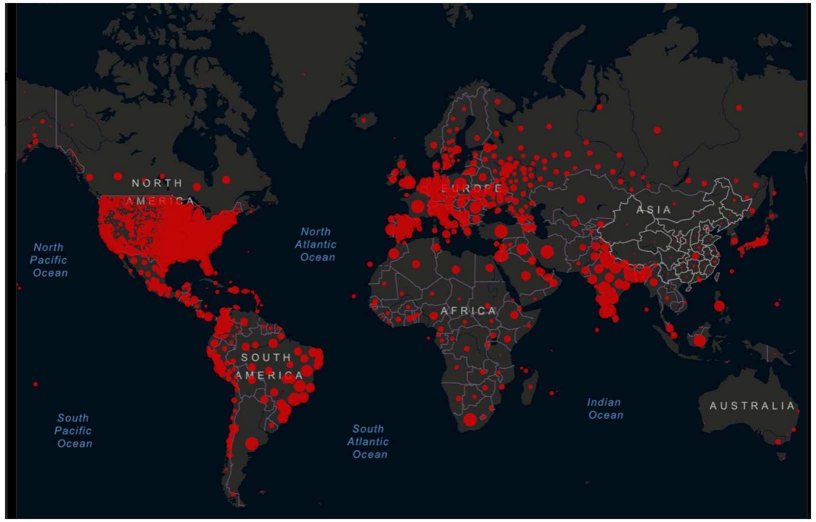
HuBMAP
https://commonfund.nih.gov/hubmap

LECTURE PLAN:

- Part 1 - Fundamentals in analyzing tissues and single cells
- Part 2 – Our latest research on COVID-19 lung damage

Urgent need to find new therapeutic avenues to treat infection diseases

COVID-19 cases: 1 year



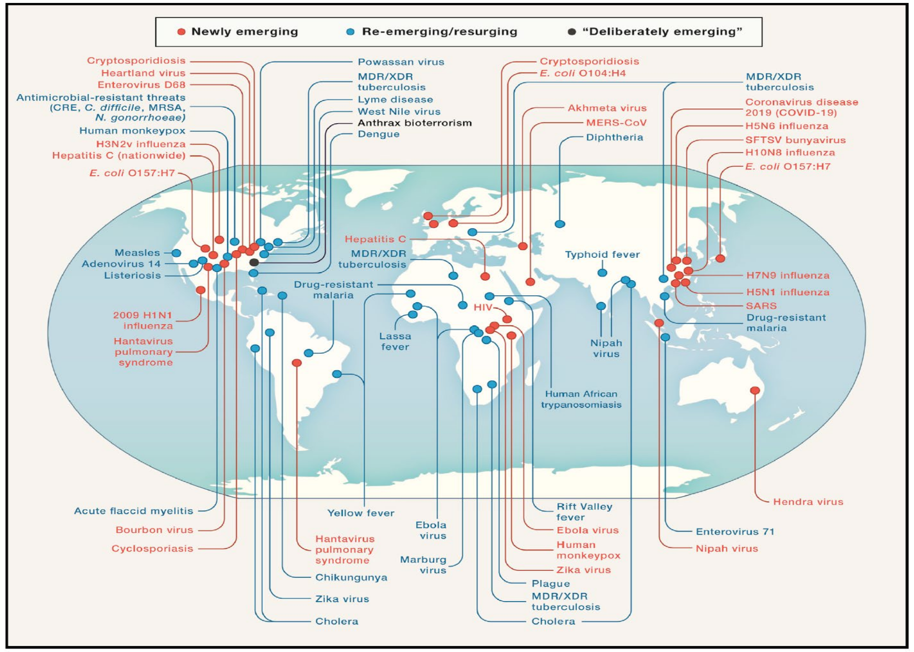
John Hopkins COVID dashboard Dong et al. 2020 *Lancet Infect Dis*

Total Deaths

5,218,935

Antibiotic resistance

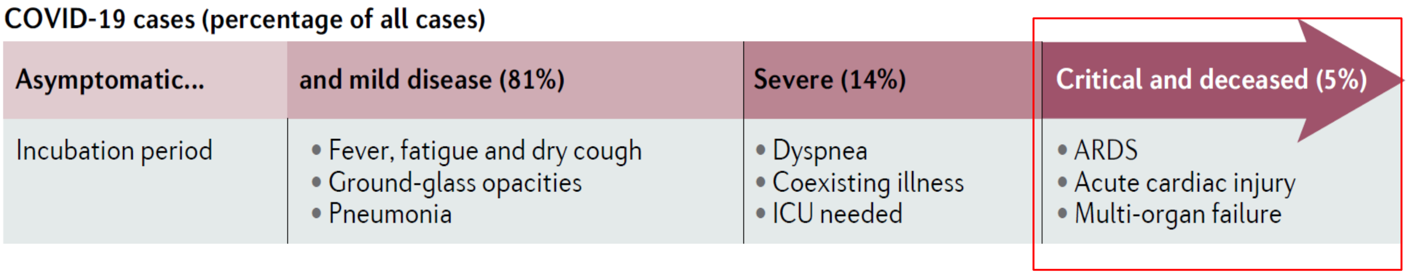
Recent Emerging Infectious Diseases (1981 to 2020)



Morens and Fauci 2020 *Cell*

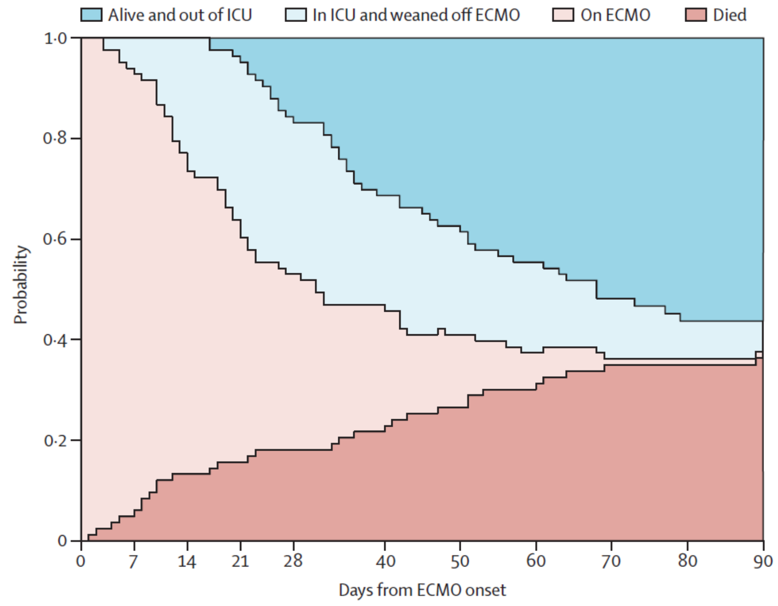
Clinical course and pathophysiology of COVID-19

Acute respiratory manifestations
Severe ARDS COVID-19 (WHO scale 6 to 8)
Mechanical ventillation
ECMO, Death



Hu et al. 2021 *Nat Rev Micro*
WHO R&D Blueprint - COVID-19 Therapeutic Trial Synopsis

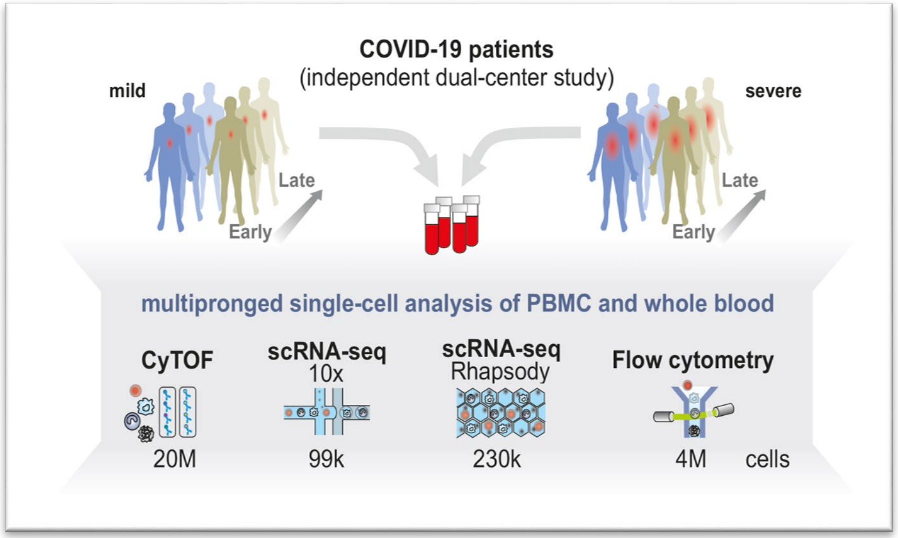
Fatality rate

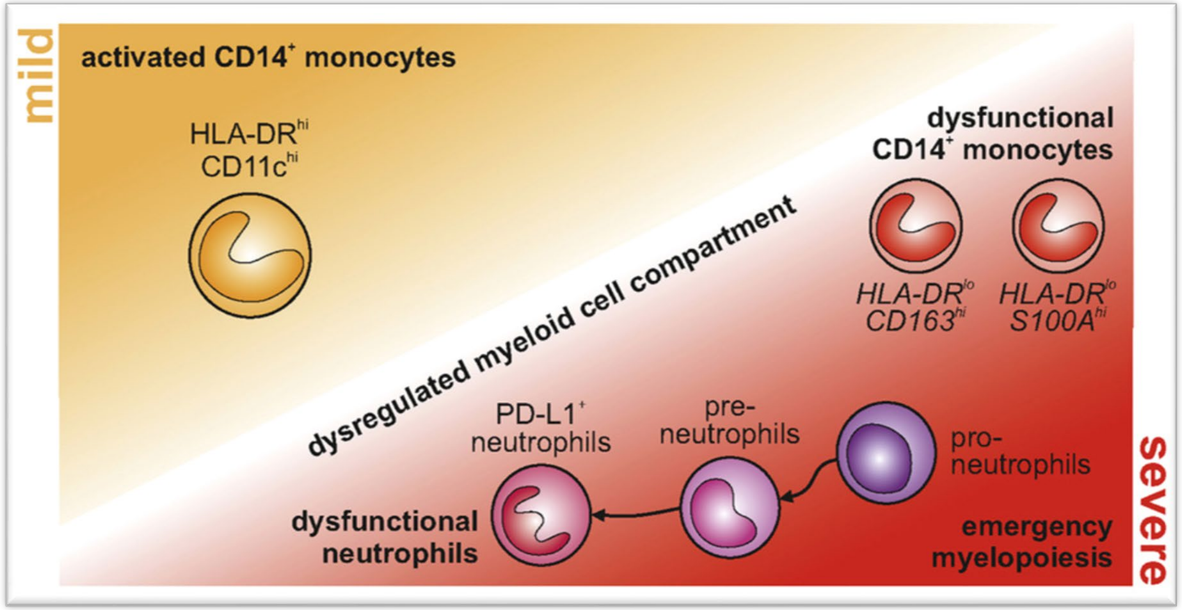


Schmidt et al. 2020 *Lancet Respir Med*
Osuchowski et al. 2021 *Lancet Respir Med*

What are the dysregulatory host responses to SARS-CoV-2 ?

Systemic response: Suppressive myeloid cells are a hallmark of severe COVID-19

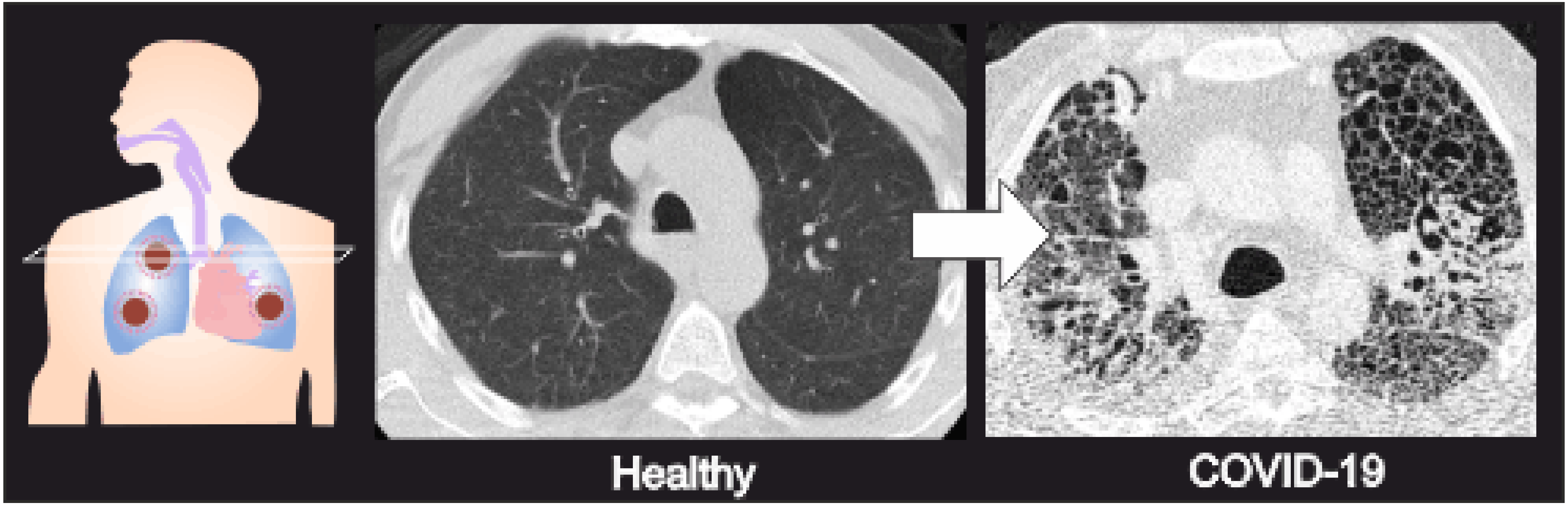




- Dual center study (Berlin and Bonn)
- 53 COVID-19 patients, 161 samples
  ca. 25 million single cells analysed
- Single-cell RNA-seq
  and single-cell proteomics

- Mild COVID-19 is marked by inflammatory CD14+ monocytes
- Dysfunctional CD14+ monocytes in severe COVID-19
- Emergency myelopoiesis with immature and dysfunctional neutrophils in severe COVID-19

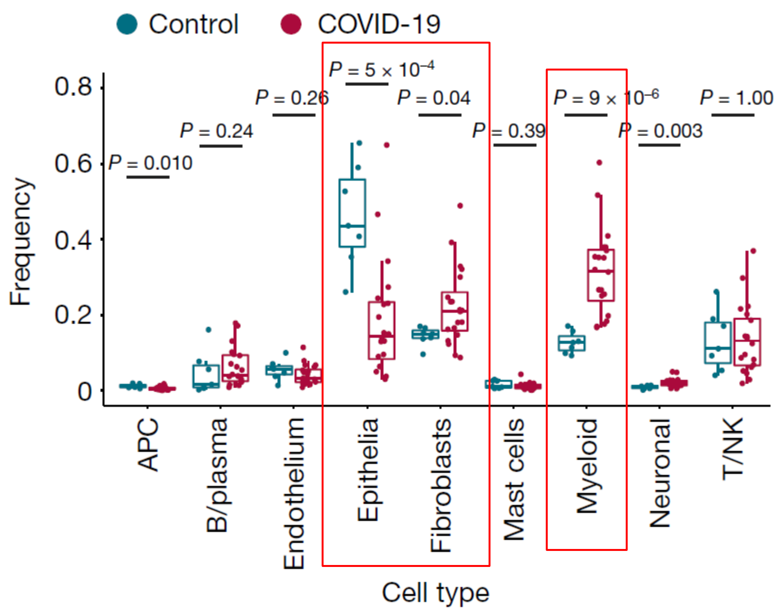
Schulte-Schrepping et al. 2020 *Cell*

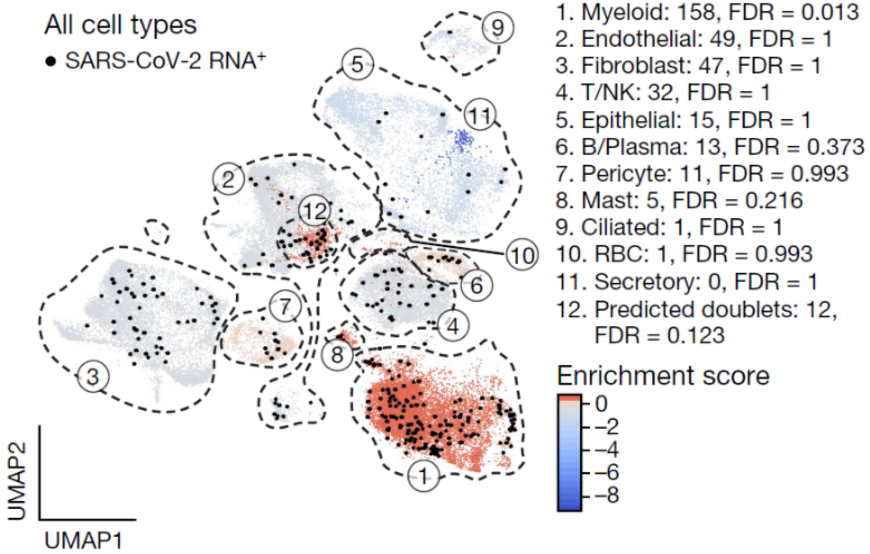

Healthy
COVID-19

Local response in the lung in severe COVID-19 patients

Article
A molecular single-cell lung atlas of lethal COVID-19
Melms et al. 2021 *Nature*

Article
COVID-19 tissue atlases reveal SARS-CoV-2 pathology and cellular targets
Delorey et al. 2021 *Nature*



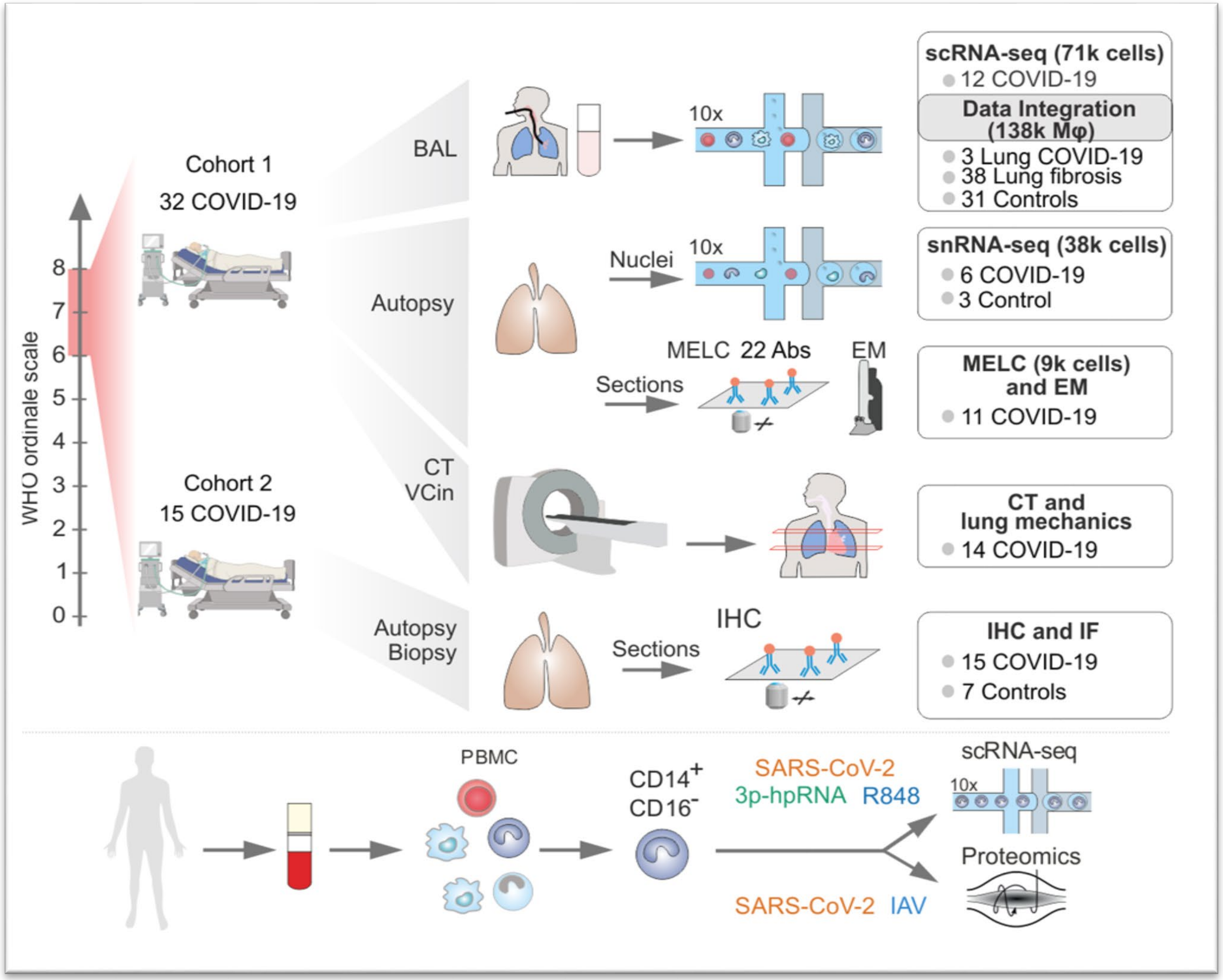


- ➢ Lung infiltration with dysregulated monocytes and macrophages
- ➢ Remodeling of lung epithelium

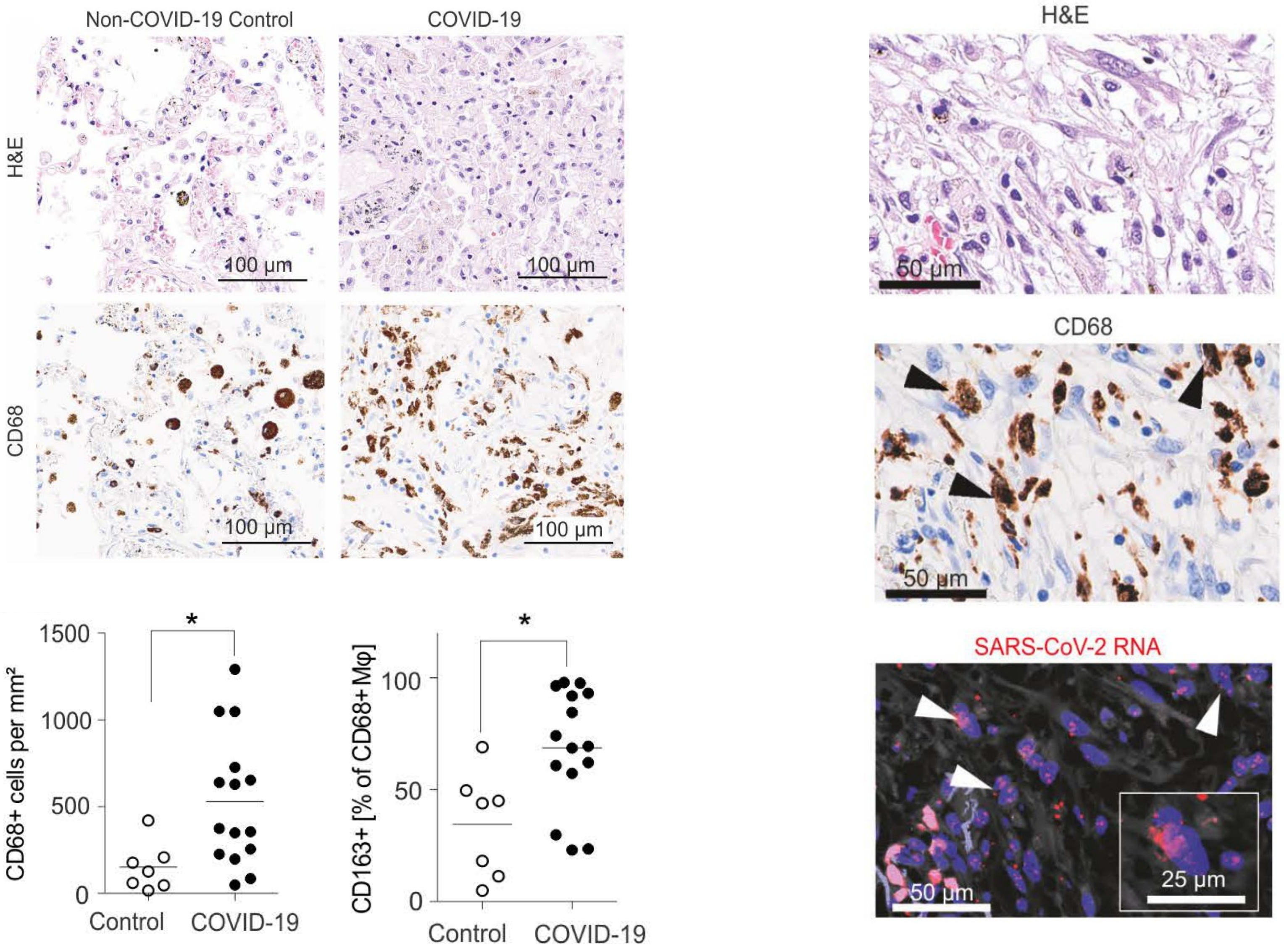
Studying Lung injury undelying COVID-19-induced ‘acute respiratory distress syndrome’ (ARDS)

Part 1
In the clinics

Part 2
Ex vivo infection

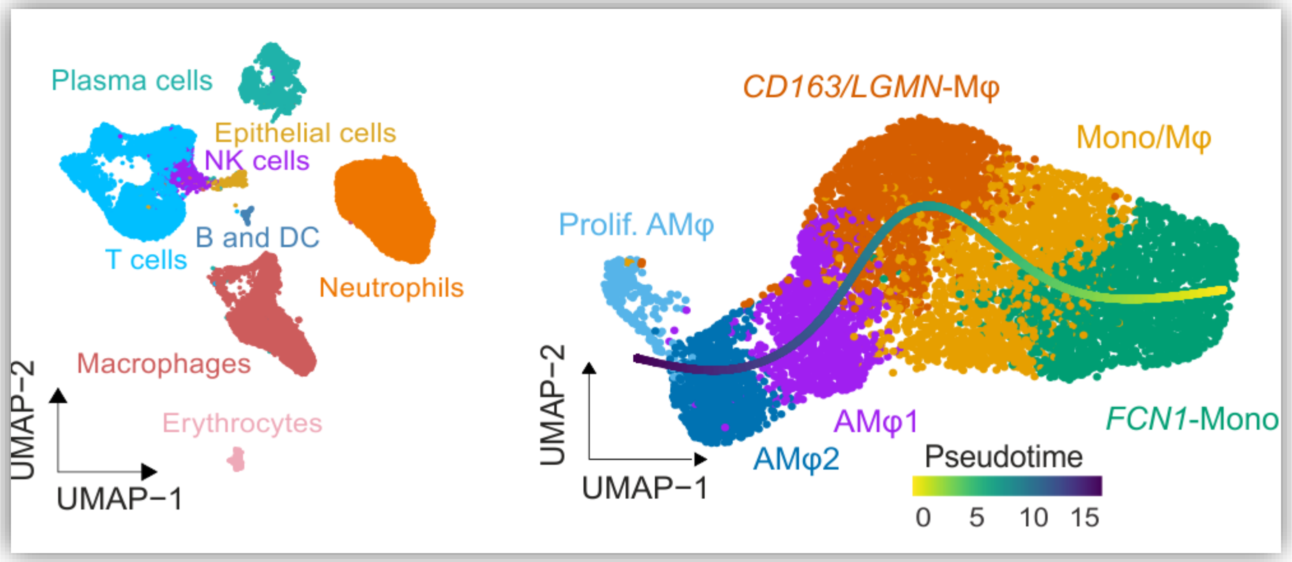


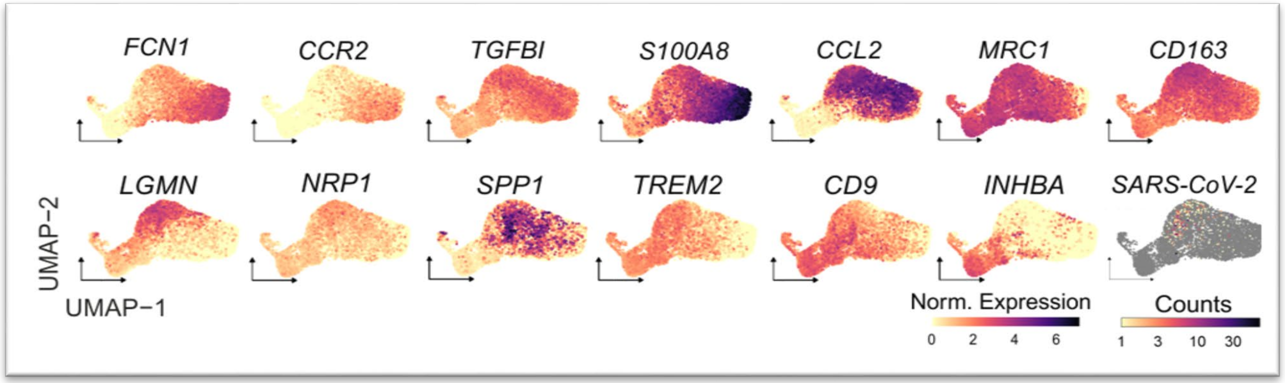
CD163+ macrophages accumulate in the lung



Macrophages adopt a damage response and tissue remodelling

Bronchoalveolar lavage / scRNA-seq

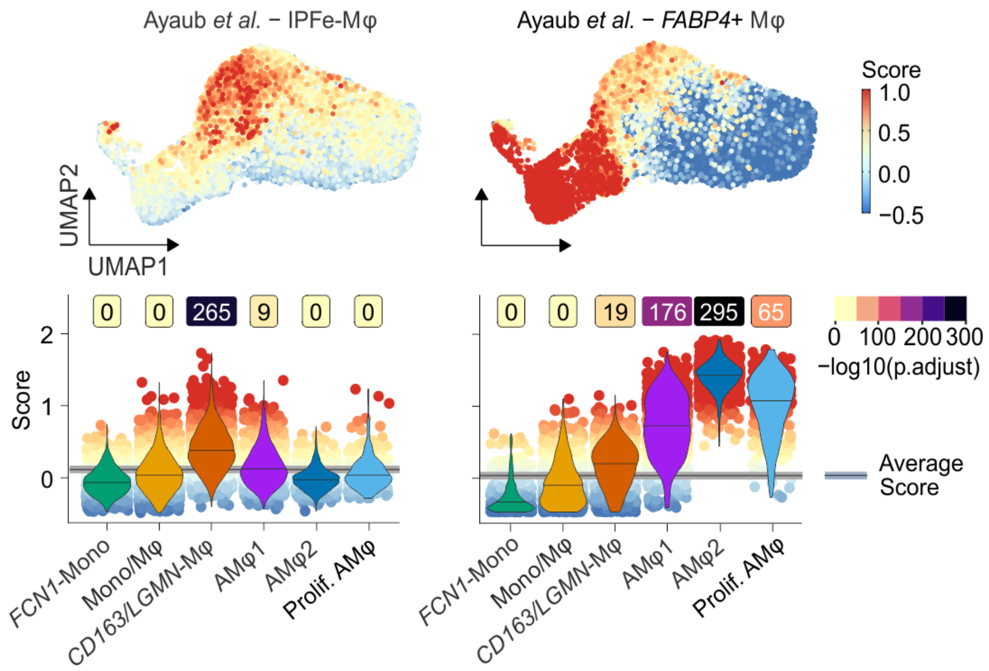


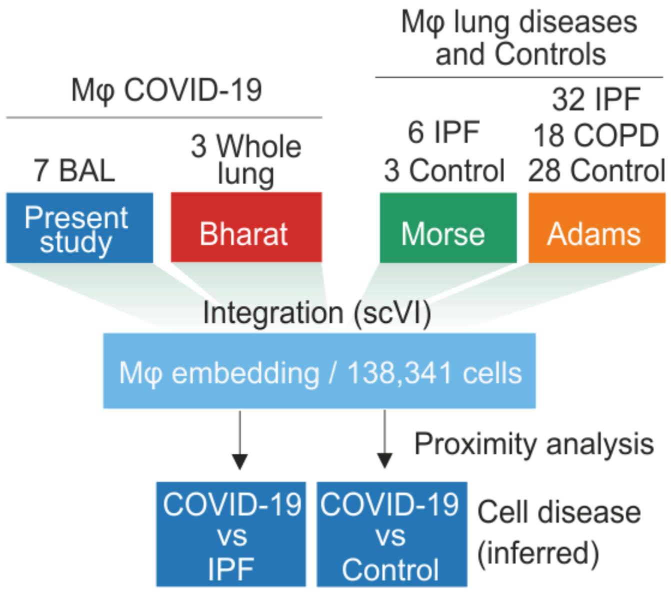


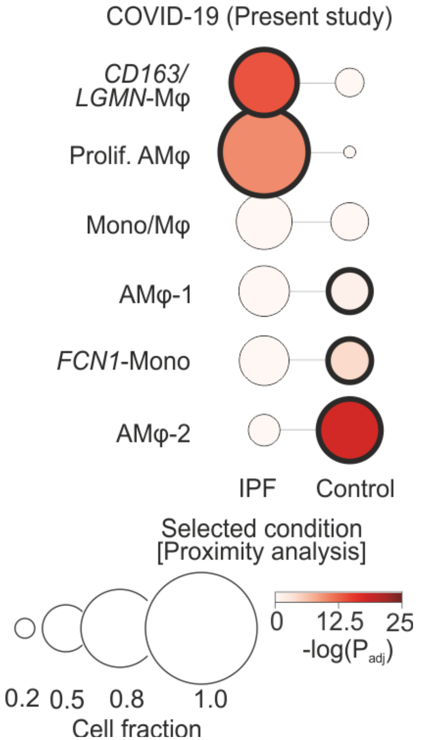
Oliver Dietrich

CD163/LGMN-Mφ resemble a profibrotic macrophage phenotype

Comparison and integration with the IPF Cell Atlas



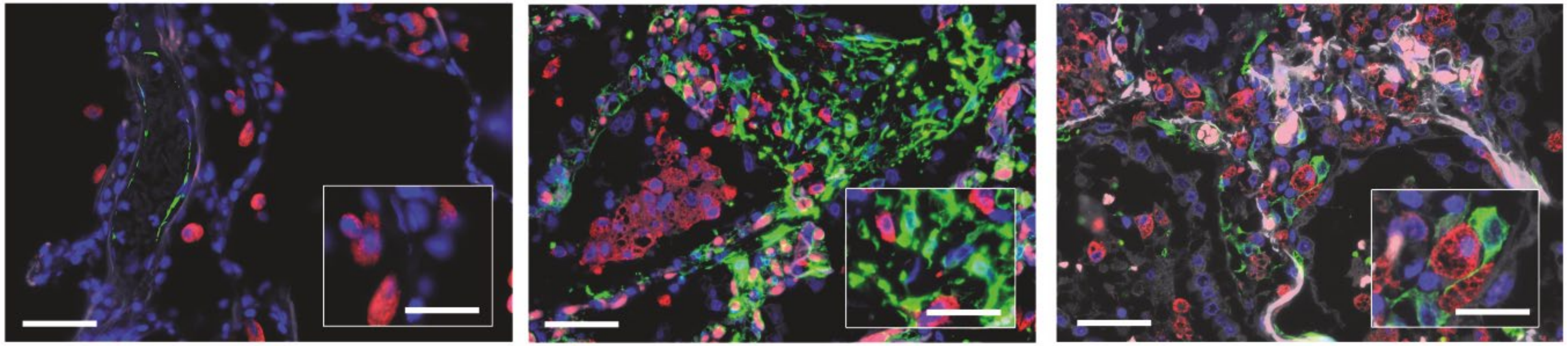




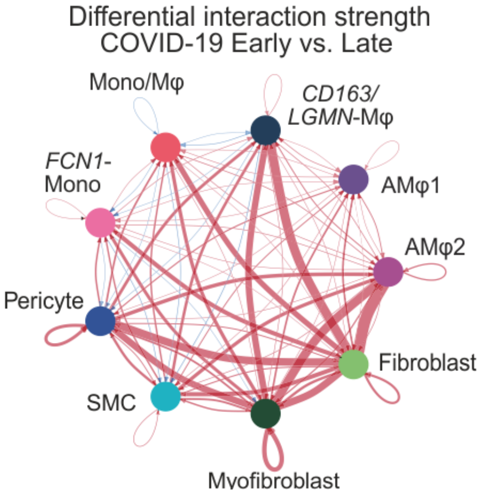
Collaboration with Fabian Theis lab

Macrophages-fibroblast interactions in COVID-19 lungs

Healthy | COVID-19

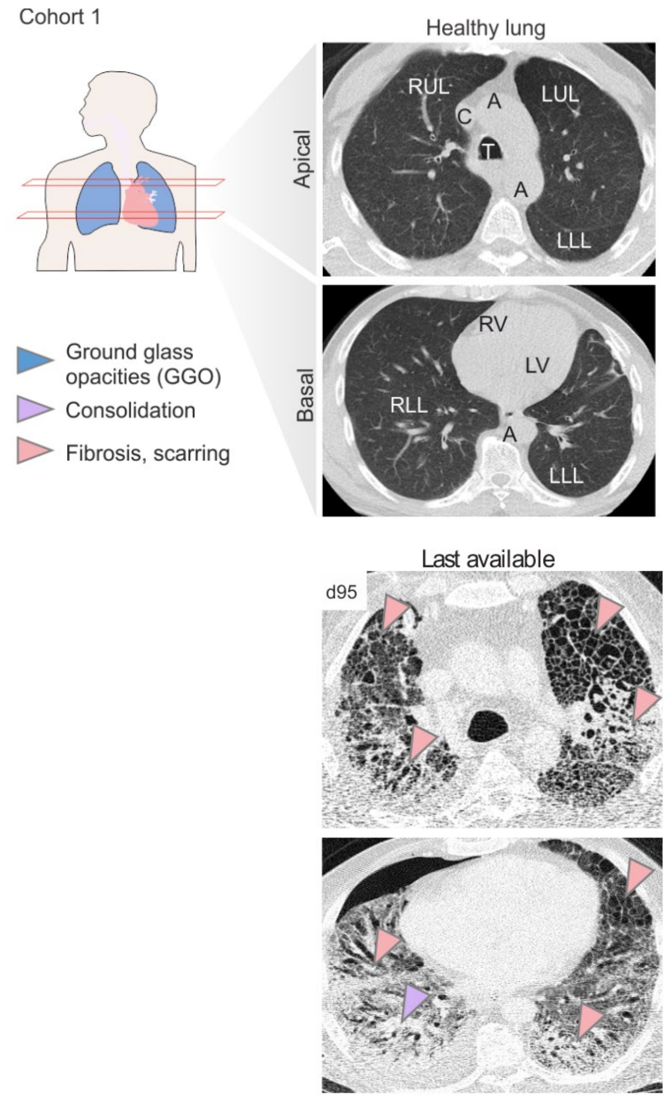


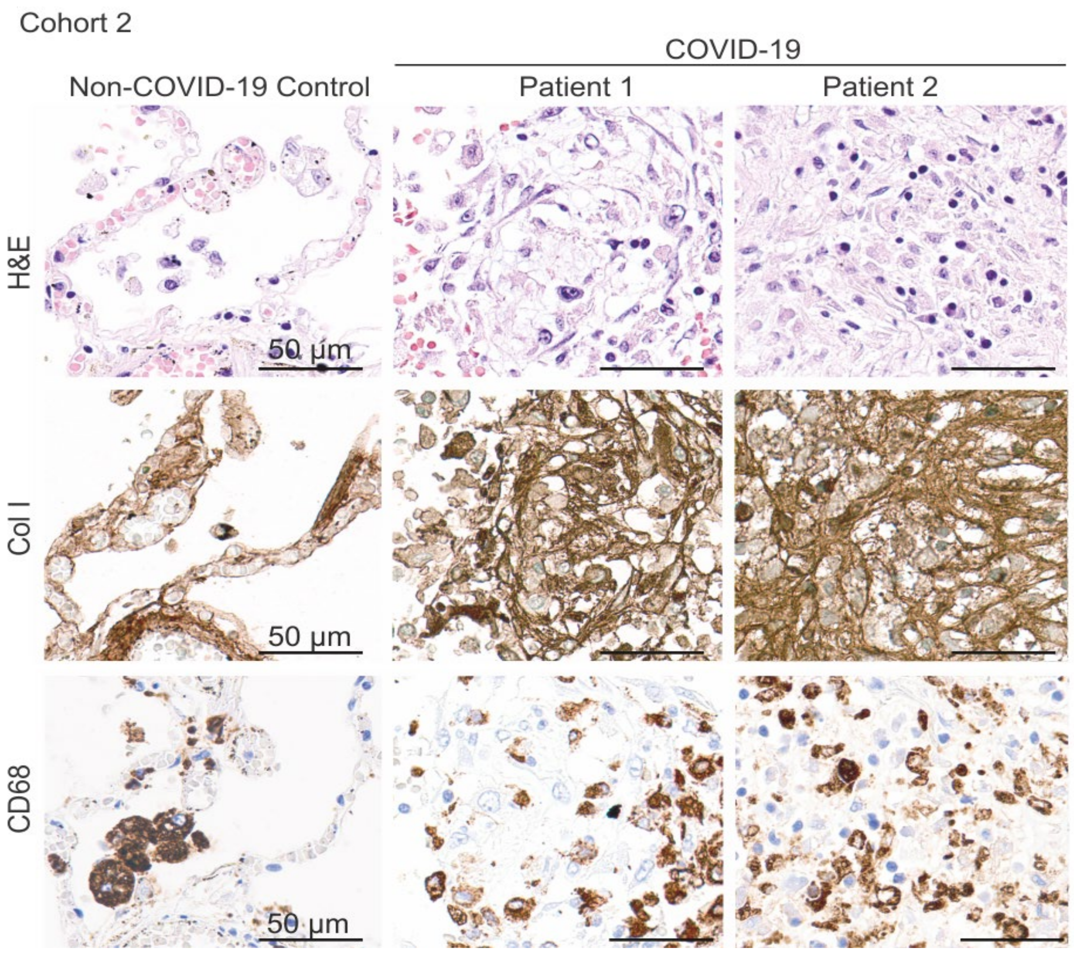
CD68 / SM22 / DAPI / Autofluorescence

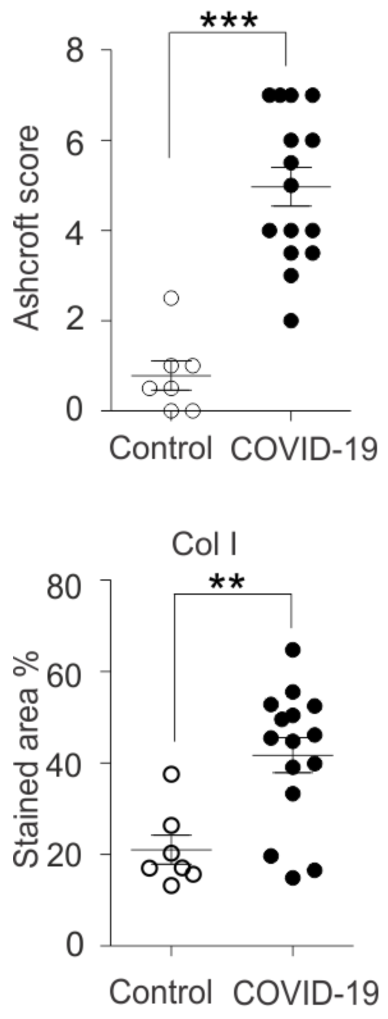


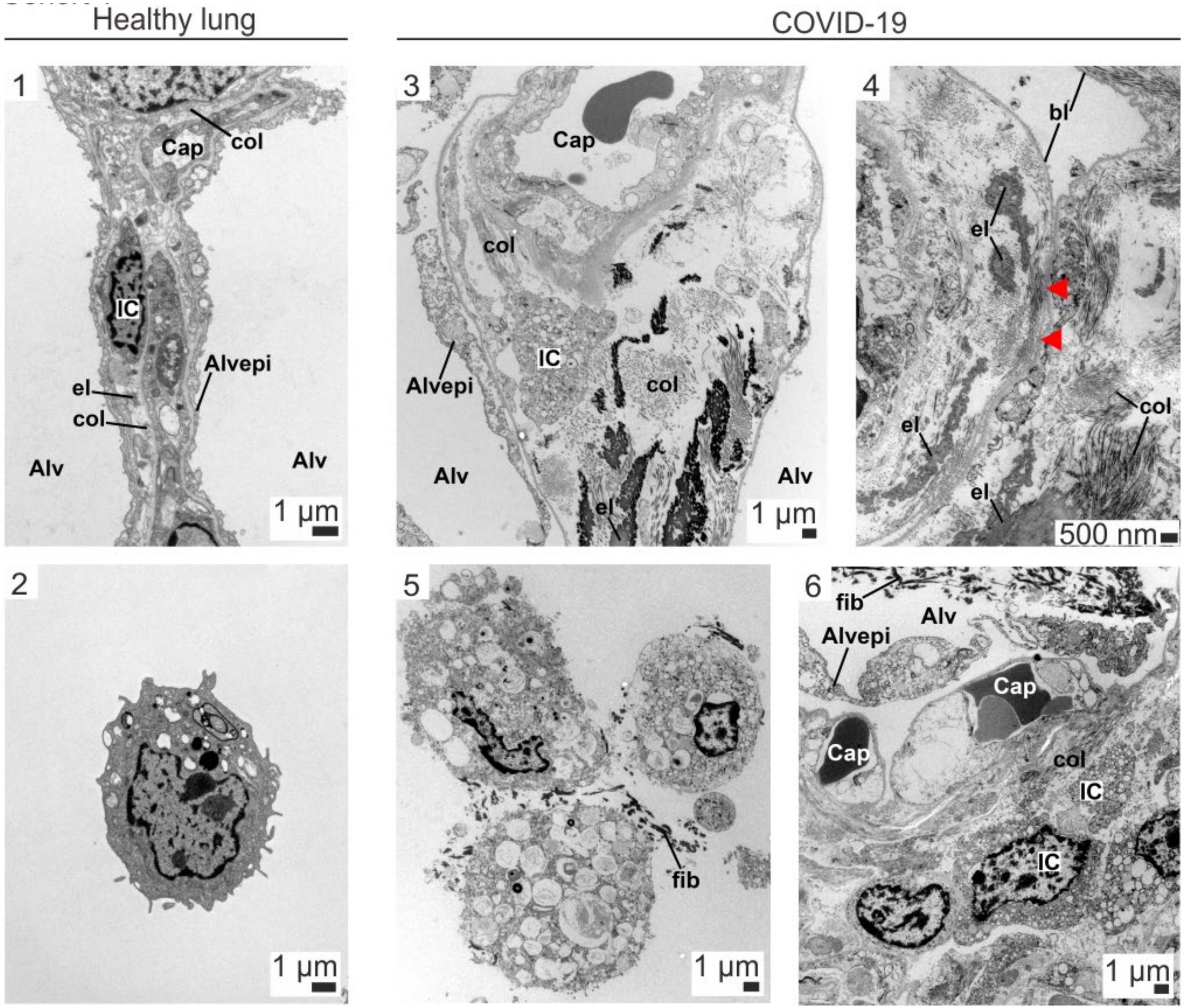
Collaboration with Peter Boor (Aachen) and Roland Eils (Berlin) labs

Severe COVID-19 induces pronounced fibroproliferative ARDS









Collaboration with Matthias Ochs, Berlin

Fibrosis as a hallmark of COVID-19-induced ARDS

Science Translational Medicine

Lung transplantation for patients with severe COVID-19

Ankit Bharat[1*], Melissa Querrey[1], Nikolay S. Markov[2], Samuel Kim[1], Chitaru Kurihara[1], Rafael Garza-Castillon[1], Adwaiy Manerikar[1], Ali Shilatifard[3], Rade Tomic[2], Yuliya Politanska[2], Hiam Abdala-Valencia[2], Anjana V. Yeldandi[4], Jon W. Lomasney[4], Alexander V. Misharin[2], G.R. Scott Budinger[2]

[1]Division of Thoracic Surgery, Northwestern Memorial Hospital, Feinberg School of Medicine, Northwestern University, Chicago, Illinois 60611, USA. [2]Division of Pulmonary and Critical Care Medicine, Northwestern Memorial Hospital, Feinberg School of Medicine, Northwestern University, Chicago, Illinois 60611, USA. [3]Department of Biochemistry and Molecular Genetics, Northwestern Memorial Hospital, Feinberg School of Medicine, Northwestern University, Chicago, Illinois 60611, USA. [4]Department of Pathology, Northwestern Memorial Hospital, Feinberg School of Medicine, Northwestern University, Chicago, Illinois 60611, USA.

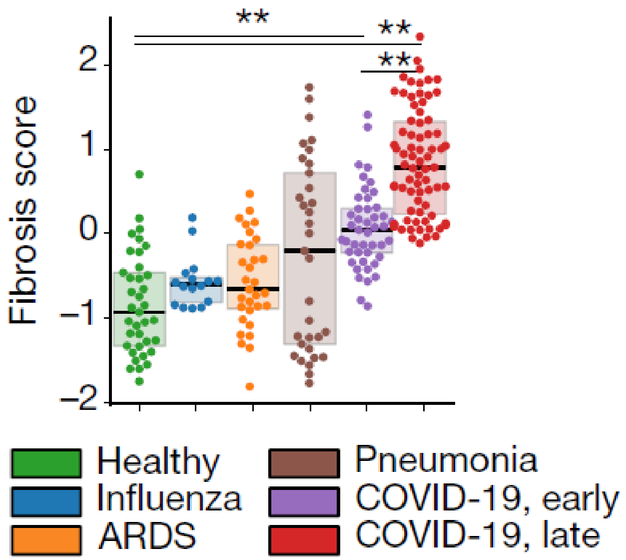
*Corresponding author. Email: abharat@nm.org

- Extensive evidence of injury and fibrosis that resembled end-stage pulmonary fibrosis

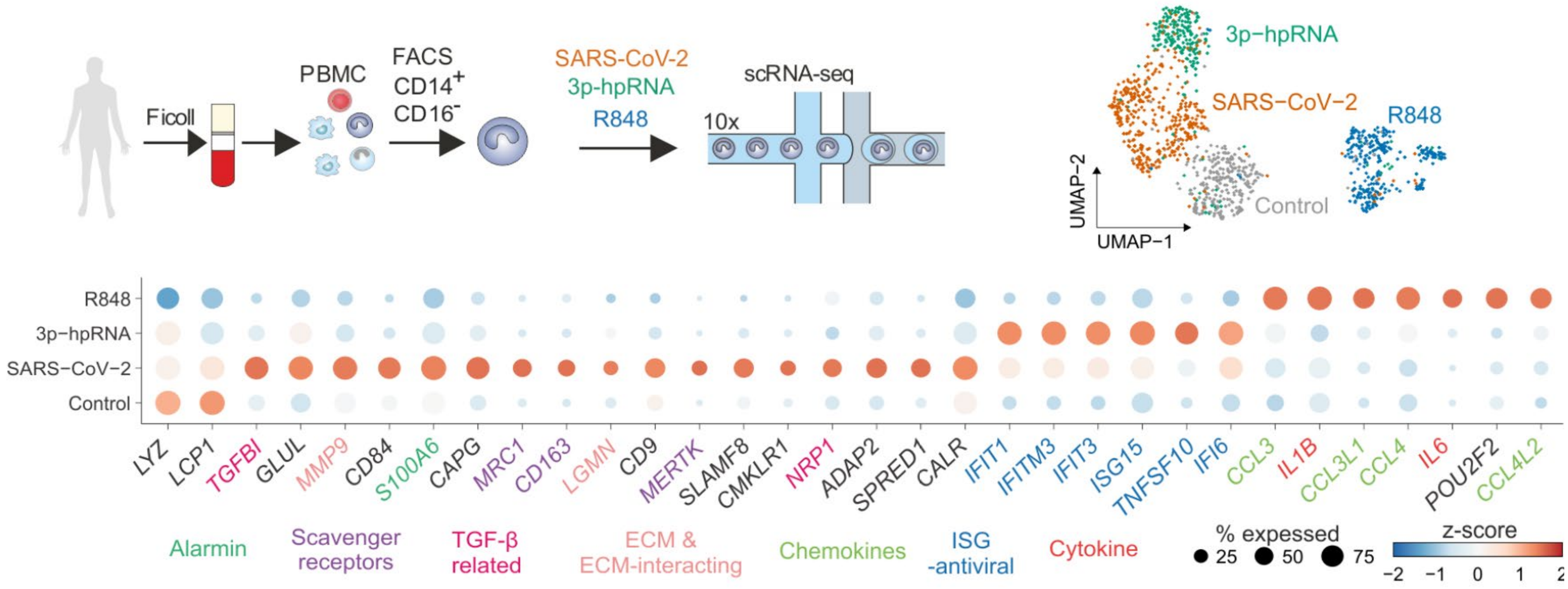
Article

The spatial landscape of lung pathology during COVID-19 progression

Rendeiro et al. 2021 *Nature*

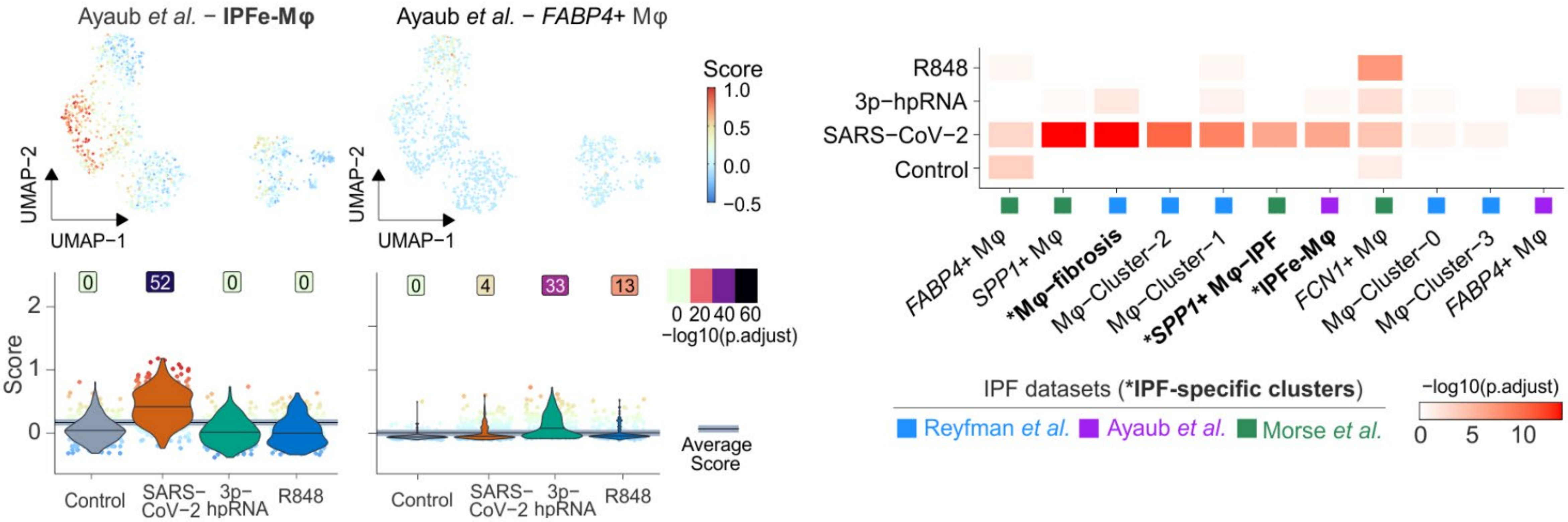


Ex vivo classical monocytes stimulation



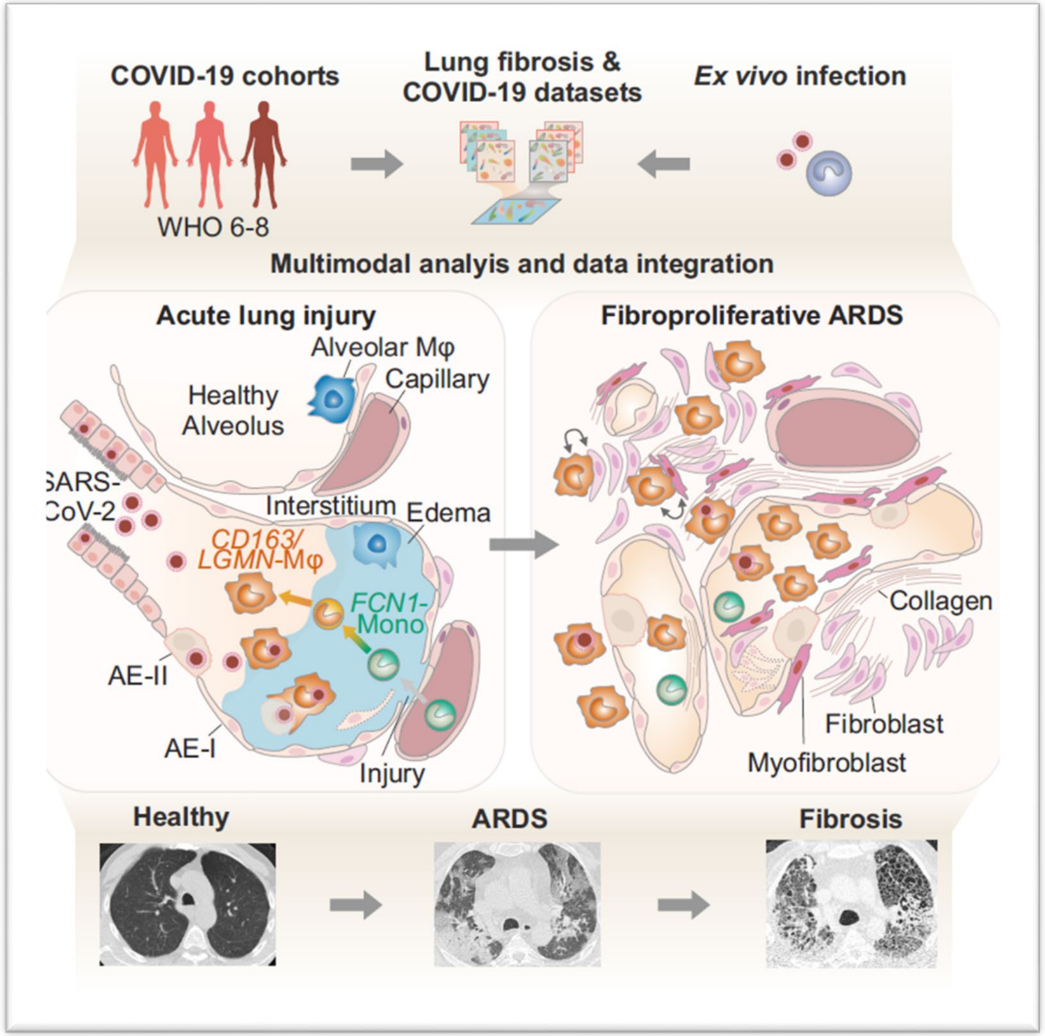
SARS-CoV-2 triggers a profibrotic gene expression profile in monocytes

Ex vivo classical monocytes stimulation



SARS-CoV-2 triggers a profibrotic gene expression profile in monocytes
(further validated by Mass Spec)

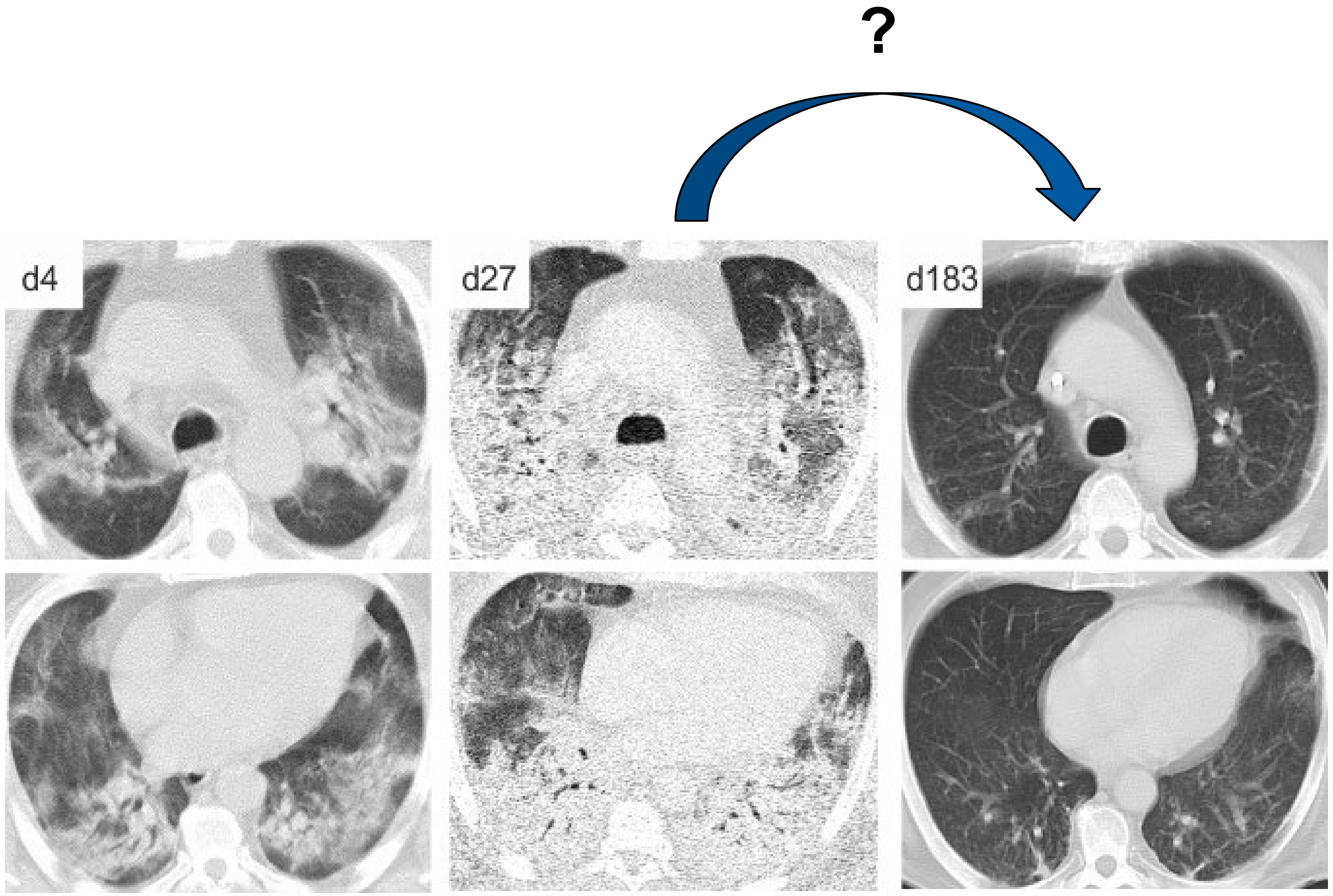
Take home messages



- ➢ Monocyte-derived macrophages accumulate in the lung in COVID-19 ARDS
- ➢ Macrophages in COVID-19 express genes associated with profibrotic functions
- ➢ Patients with severe COVID-19 ARDS display hallmarks of pulmonary fibrosis
- ➢ SARS-CoV-2 induces a profibrotic transcriptome and proteome profile in macrophages

Wendisch, Dietrich, Mari, von Stillfried et al. *Cell*

Take home messages and open questions



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Yang Li, CiiM HZI, Hannover
Bowen Zhang, CiiM HZI, Hannover

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FOR COVID
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3D INFECT
Tissue Infection Symposium

DeCOI
Deutsche COVID-19 OMICS Initiative