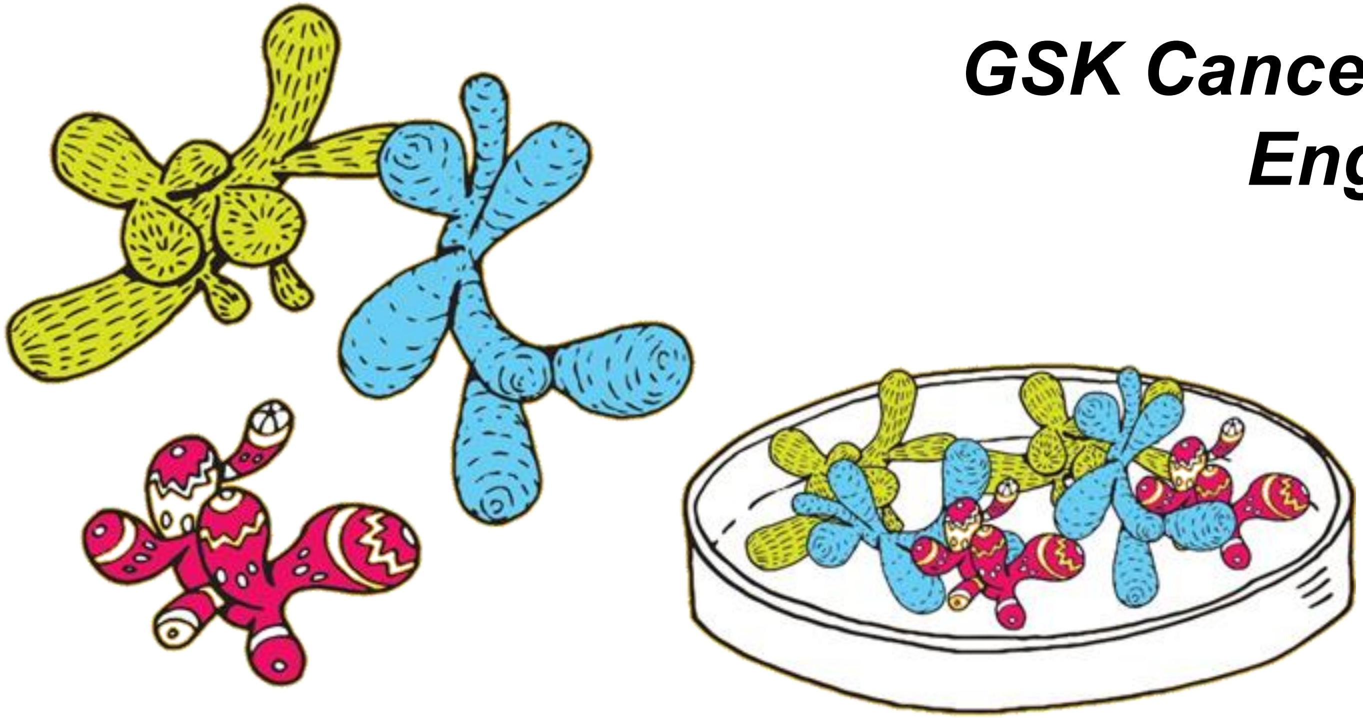
Multicellular platforms from organoids to tissue biology



GSK Cancer Engineering/ Genetic Engineering Course, 2025

Joo-Hyeon Lee, PhD.

Lecture content

Organoid Technology: From Stem Cells to Mini-Organs
•Concept and development of organoids
•Self-organization and key properties of organoids
•Applications in developmental biology and disease modeling

Engineering the Cellular Microenvironment
•Multicellular organoids (assembloids)
•Biomaterials and synthetic matrices
•Influence of physical and chemical cues on cell behavior
•Advanced hydrogels for 3D cell culture and organoid growth

Bridging Organoids and Complex Tissue Biology
•Single-cell analysis in organoid systems
•Organ-on-a-chip technologies
•Challenges and future directions in creating more complex tissue models

Lecture content

Discussion Paper:

Abilez et al. Gastruloids enable modeling of the earliest stages of *human cardiac and hepatic vascularization*. **Science**. 2025 Jun 5;388(6751):eadu9375. PMID: 40472086.

Miao et al. Co-development of mesoderm and endoderm enables *organotypic vascularization in lung and gut organoids*. **Cell**. 2025 Aug 7;188(16):4295-4313.e27. PMID: 40592324.

Review Paper:

Onesto MM, Kim JI, Pasca SP. Assembloid models of cell-cell interaction to study tissue and disease biology. Cell Stem Cell. 2024 Nov 7;31(11):1563-1573. PMID: 39454582.

What are “Organoids”?

An organoid is a miniaturized and simplified version of an organ produced *in vitro* in three dimensions that mimics the key functional, structural, and biological complexity of that organ.

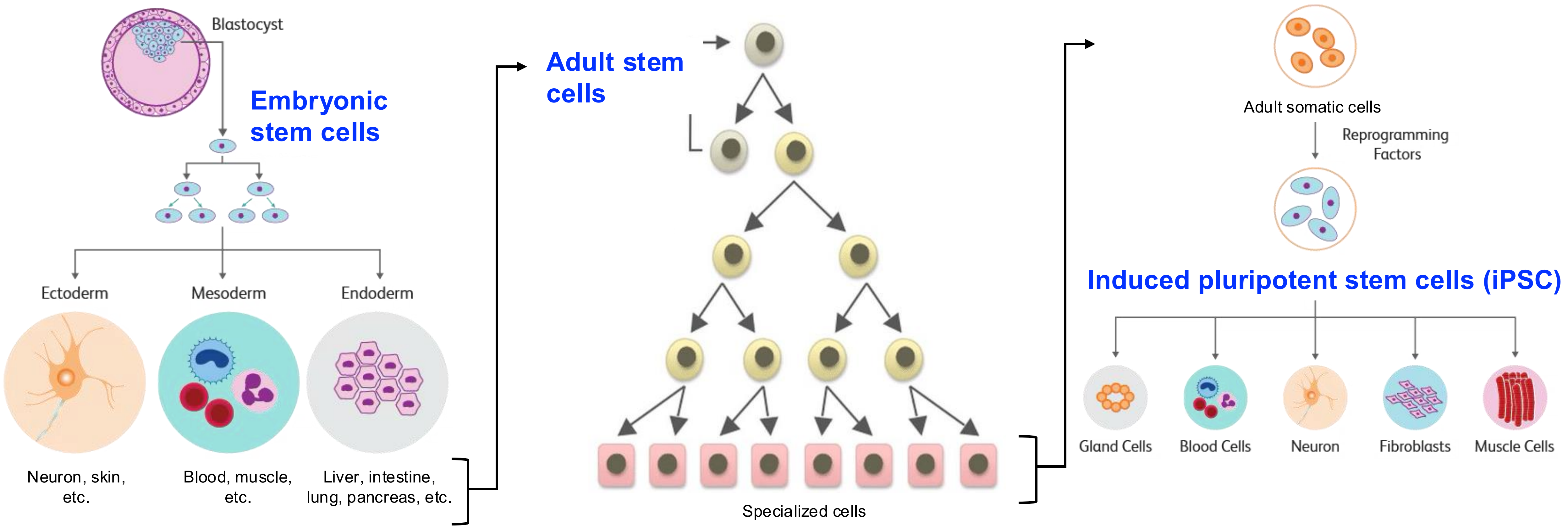
It is derived from one or a few cells from a tissue, embryonic stem cells, or induced pluripotent stem cells, which can self-organize in three-dimensional culture owing to their self-renewal and differentiation capacities.

“Stem cells”

Wikipedia

What are “Stem cells”?

Can we study how stem cells form, maintain and repair tissues using organoids?

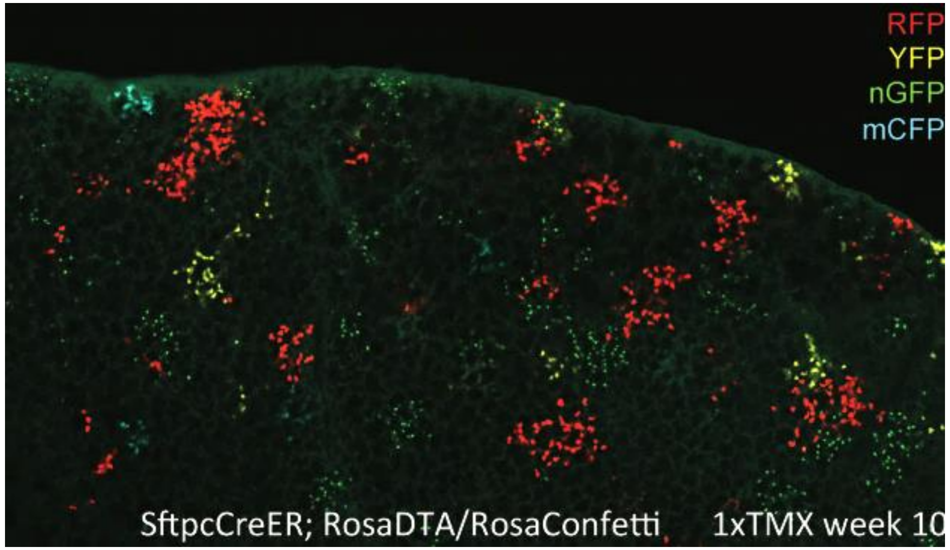


Why organoids?

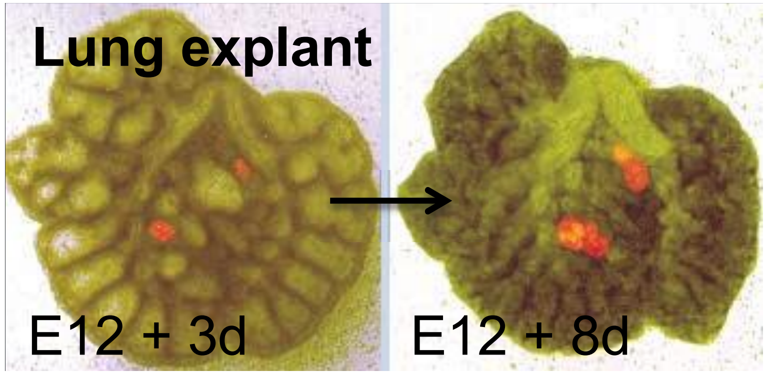
<u>Classical methods to study stem cells:</u>

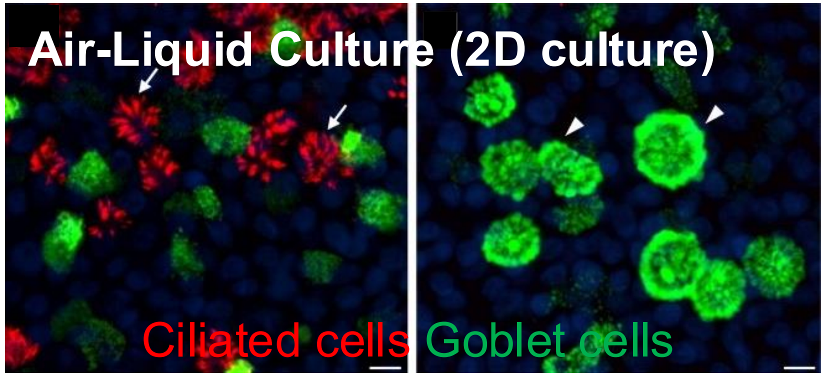
In vivo lineage tracing

Clonal analysis



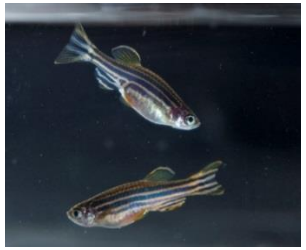
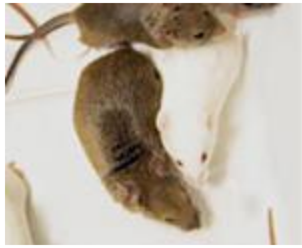
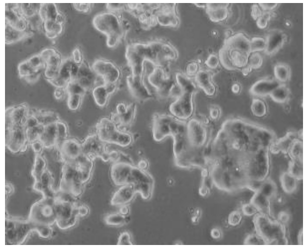
In vitro culture models





2D colony assay

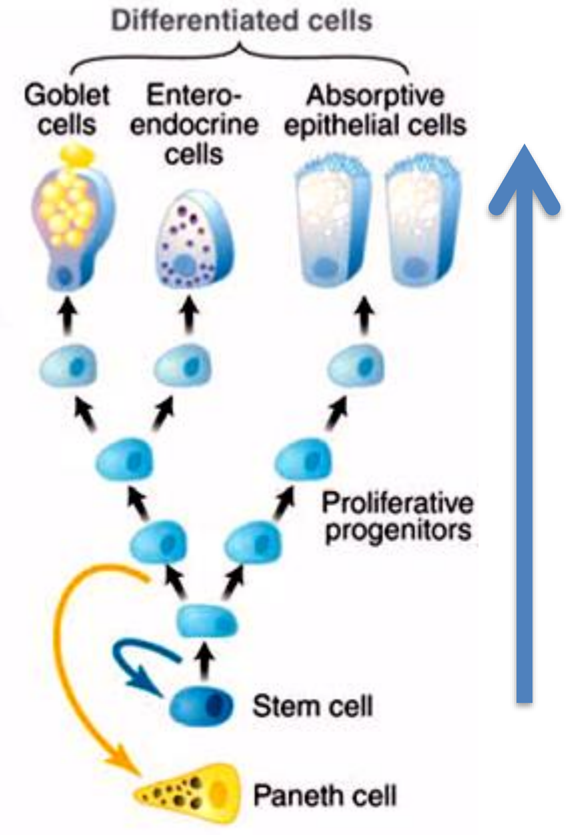
Why organoids as a model system?

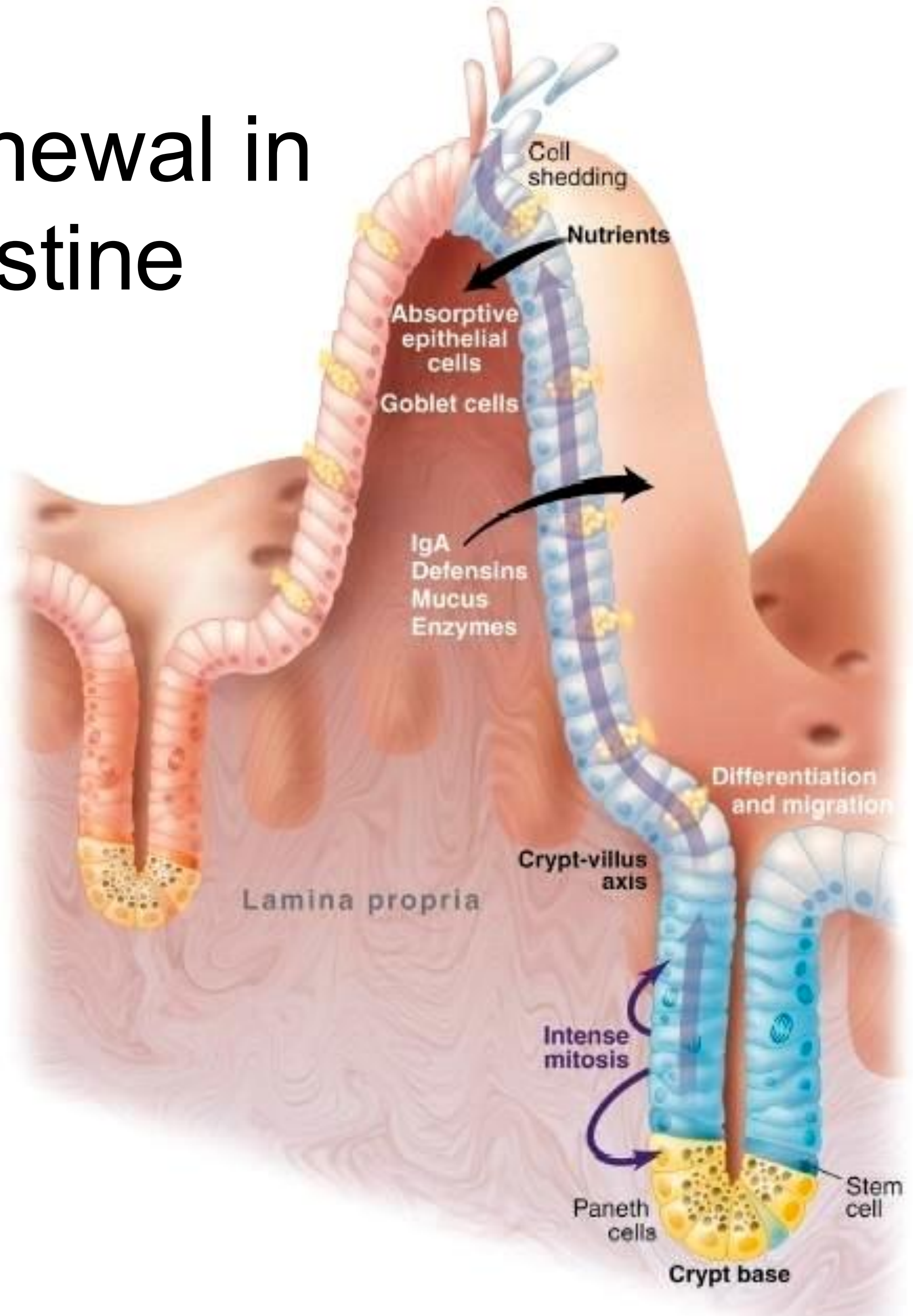
    

Human biology: requires a model system that mirrors human physiology

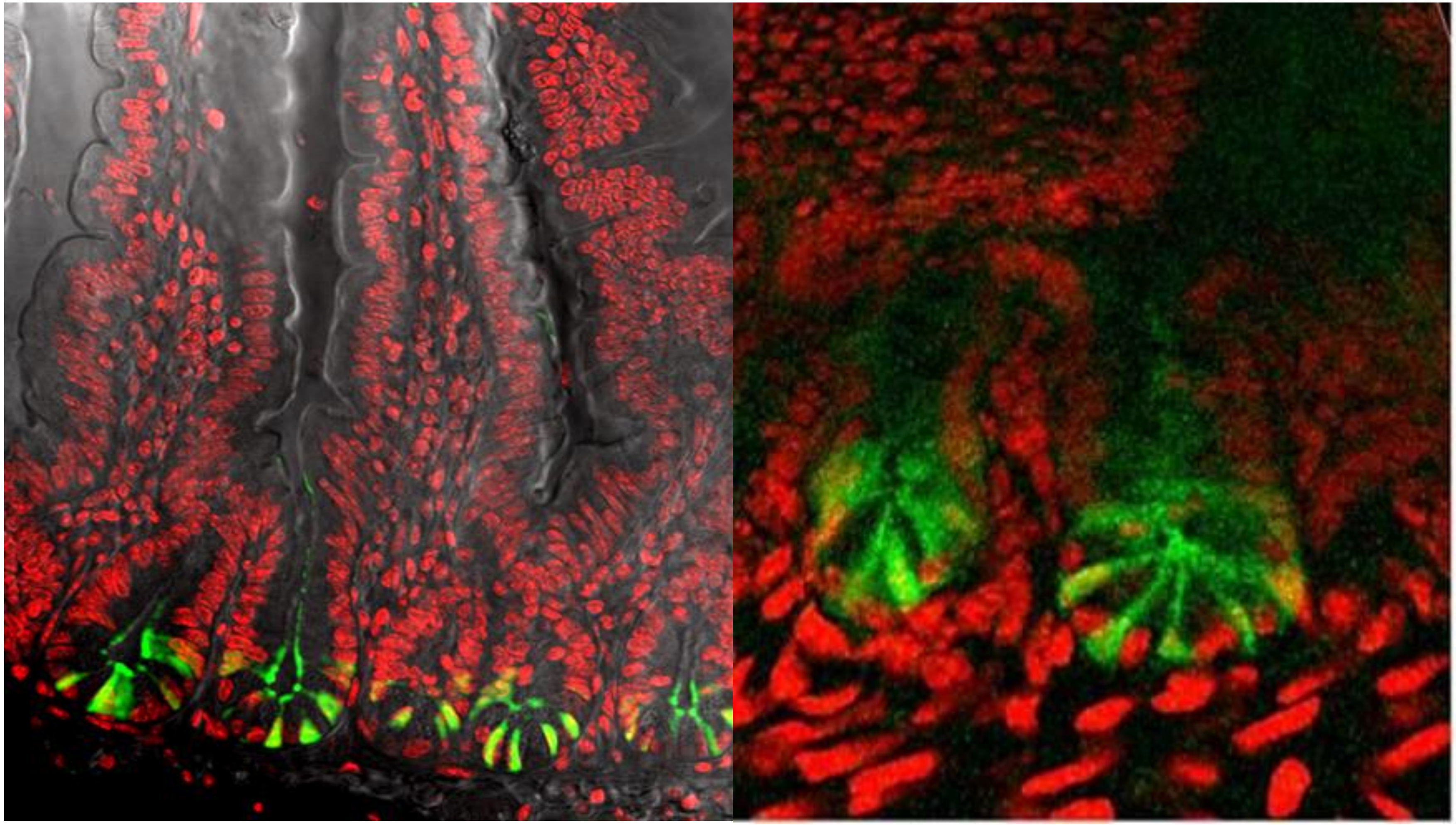
	Mouse	Cell line
		Not easy to predict *in*
Genetic Manipulation	It takes 3x-very long time.	Easy and Fast
Screening, Combinatory Treatment, Time Course Exp.	Difficult	Do-able

Epithelial self-renewal in the small intestine



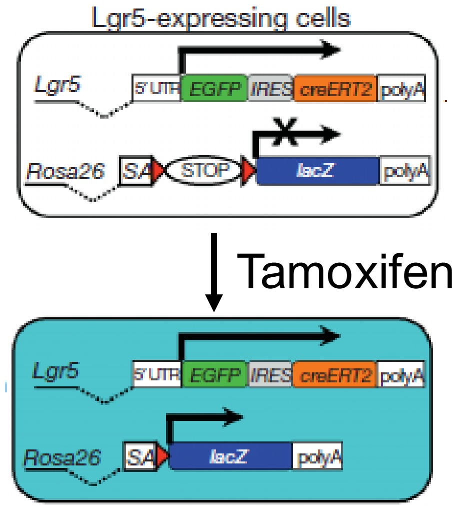


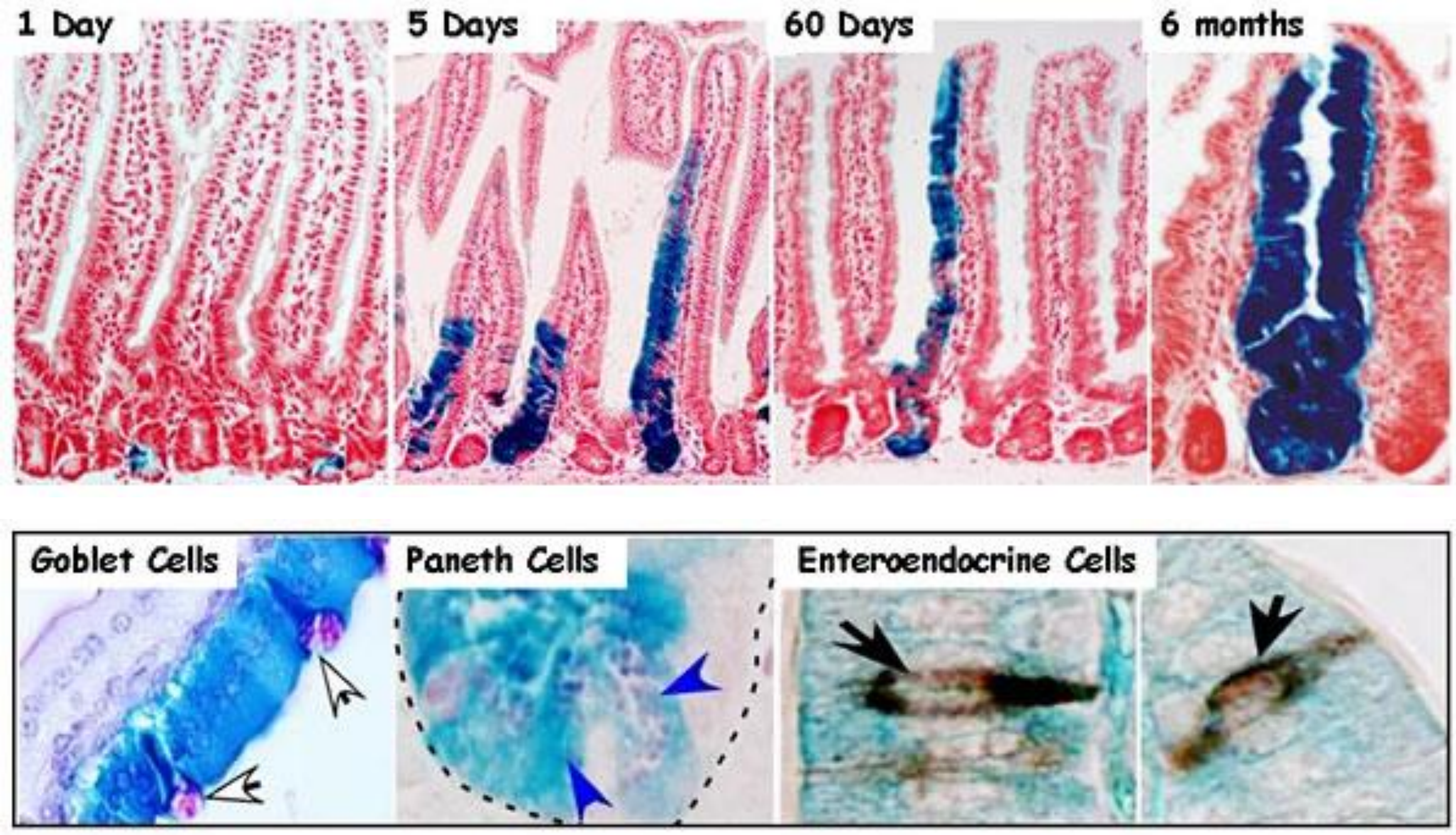
Lgr5+ stem cells in the intestine



Nick Barker

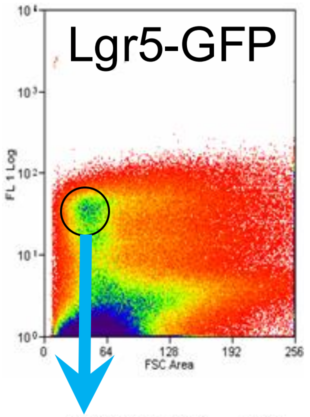
Lgr5+ stem cells in the intestine

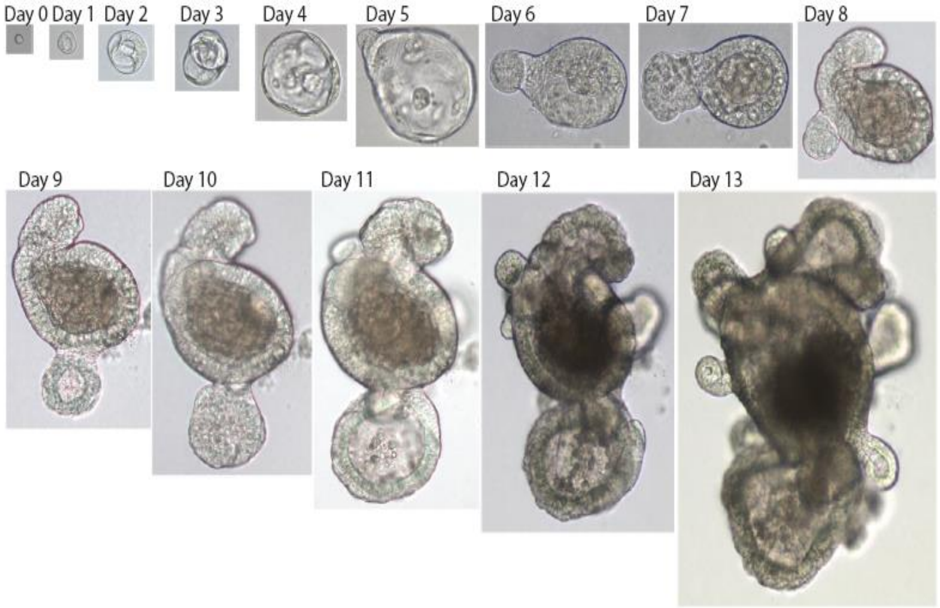


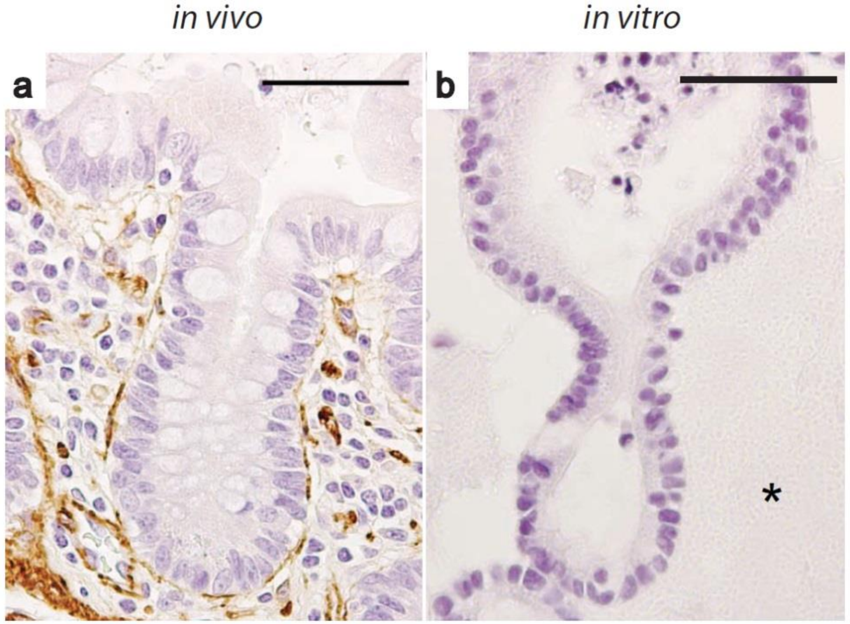


Nick Barker *et al*. Nature 2007

Intestinal organoids are stable primary culture system



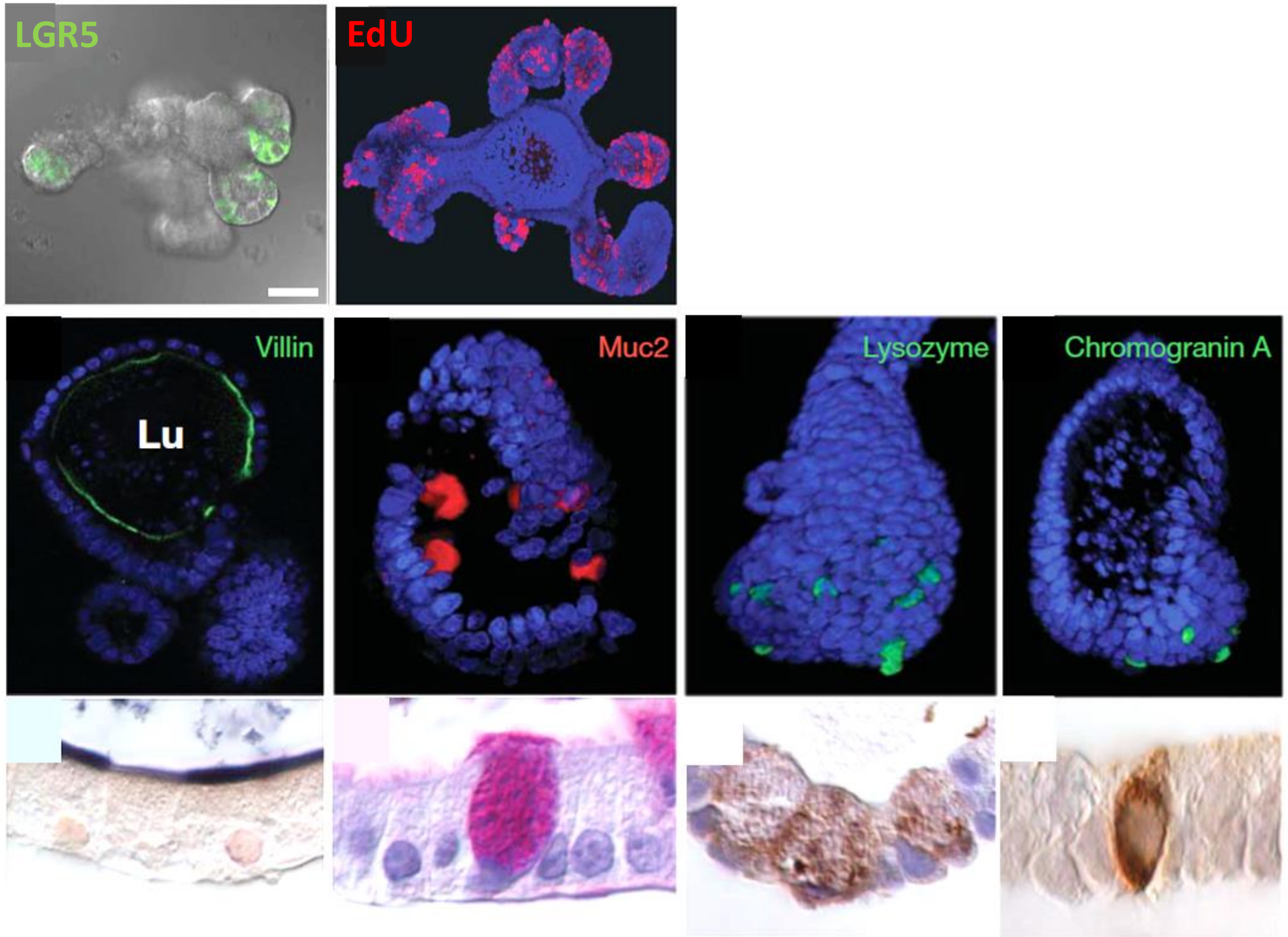




Single Lgr5-expressing intestinal stem cells self-organize to crypt-villus structures *in vitro* without necessity of a mesenchymal niche, making them the first **organoids**.

Toshiro Sato Nature 2009

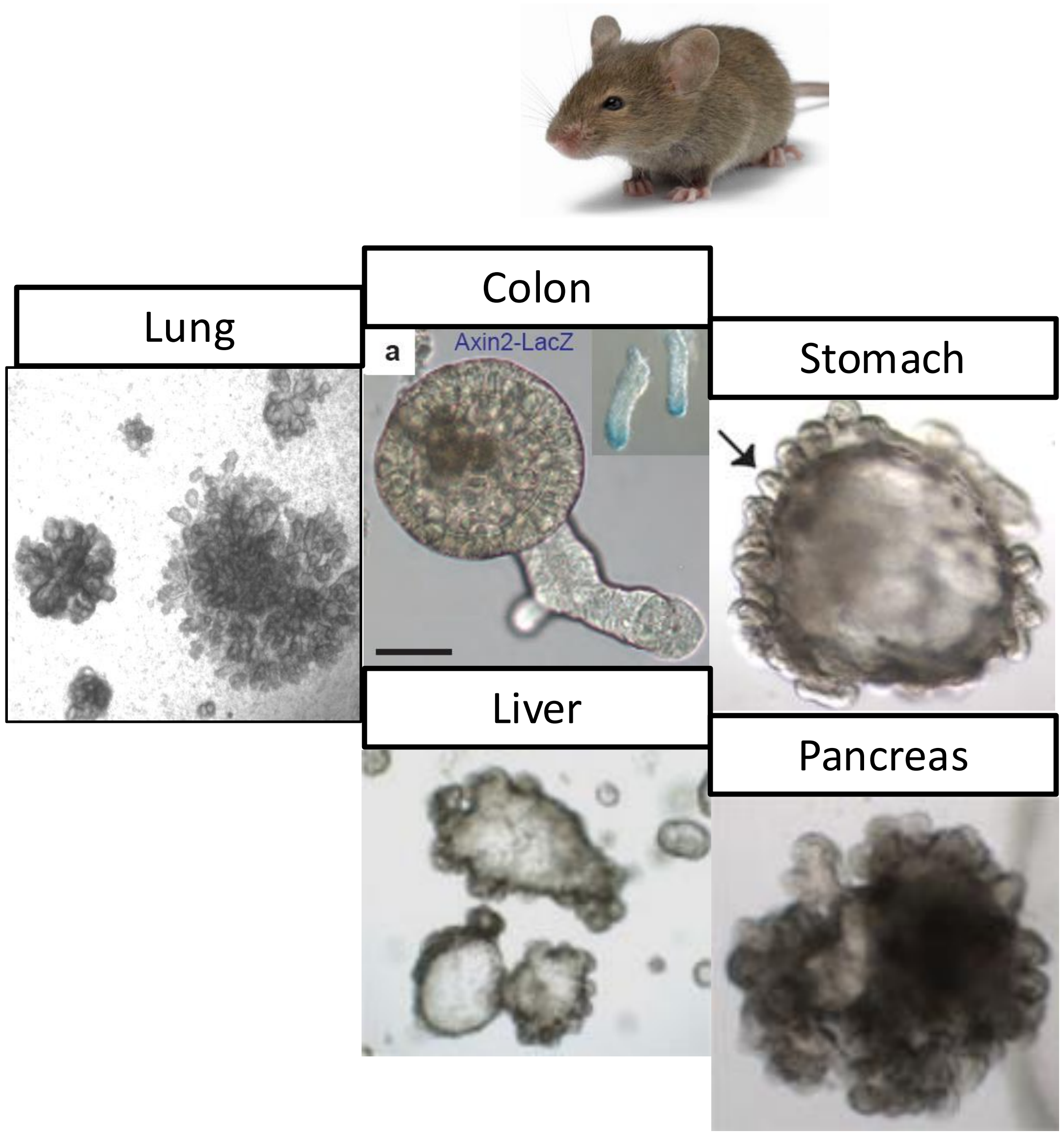
Intestinal organoids are self-organizing epithelial structures containing stem cells, progenitors and all differentiated cell types

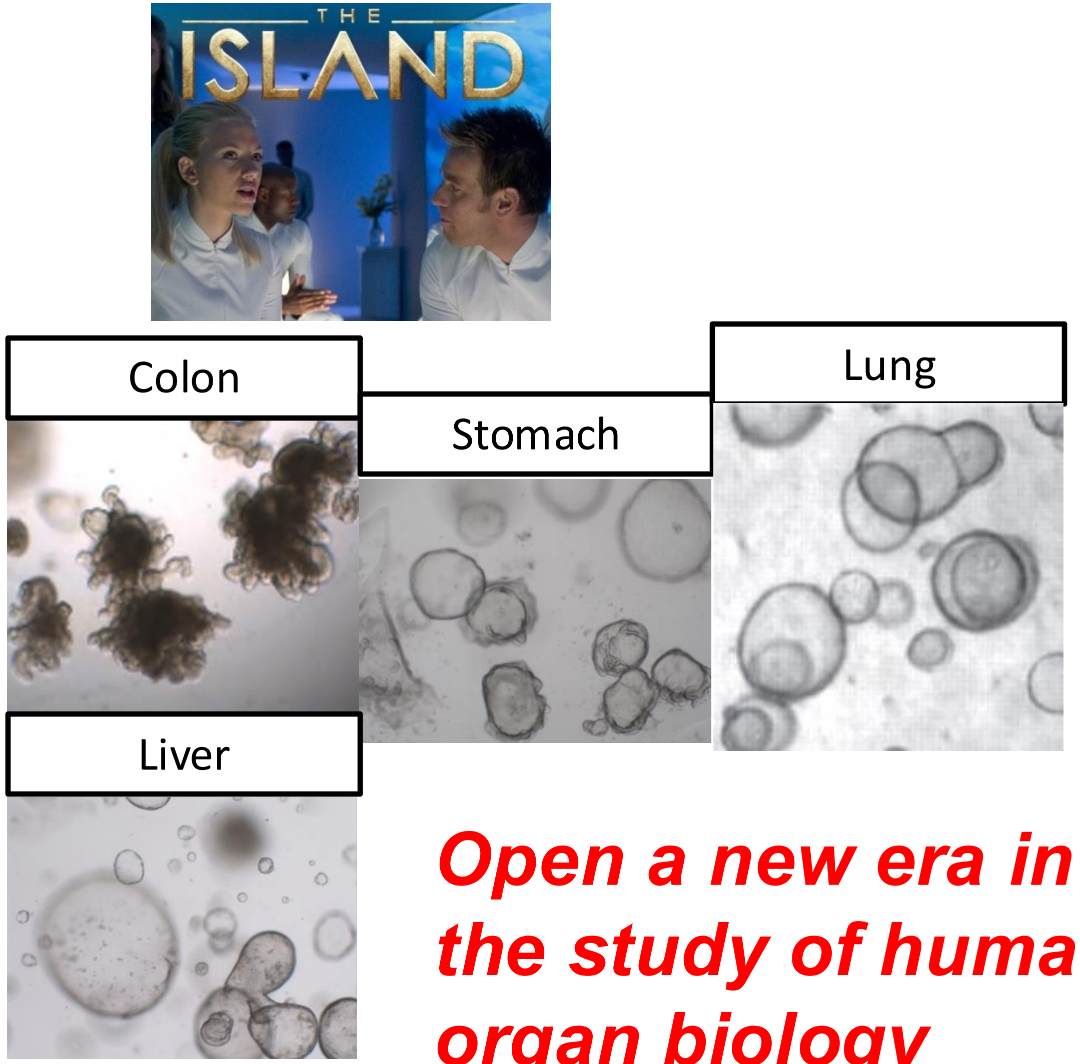


- Can be cultured longer than 1 year without major genetic changes (long-term longevity).
- Self-organizing structure with stem cells, progenitors and differentiated cell types

Toshiro Sato Nature 2009

Adult Stem Cell-derived Organoids for Human Biology

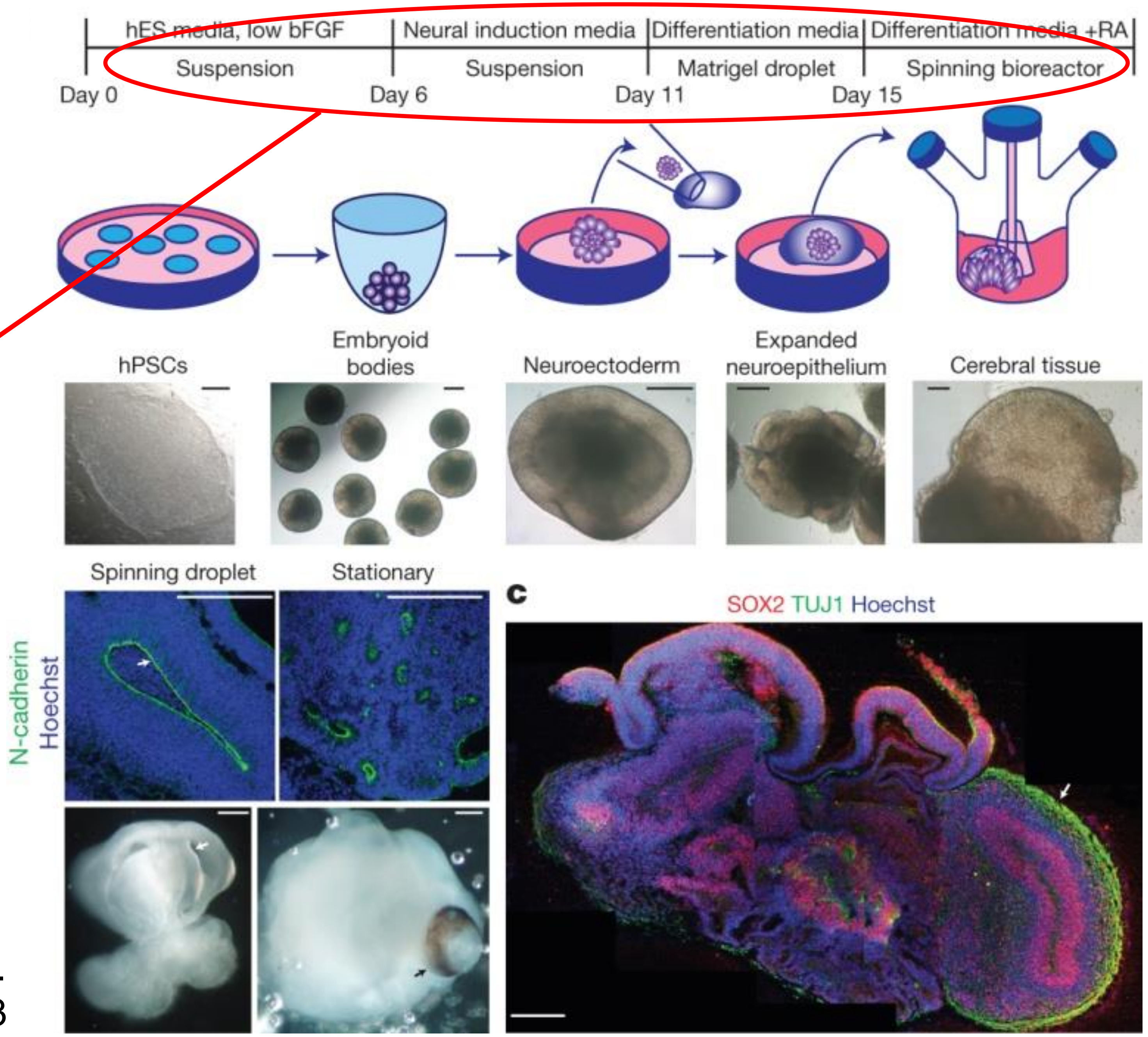




Open a new era in the study of human organ biology

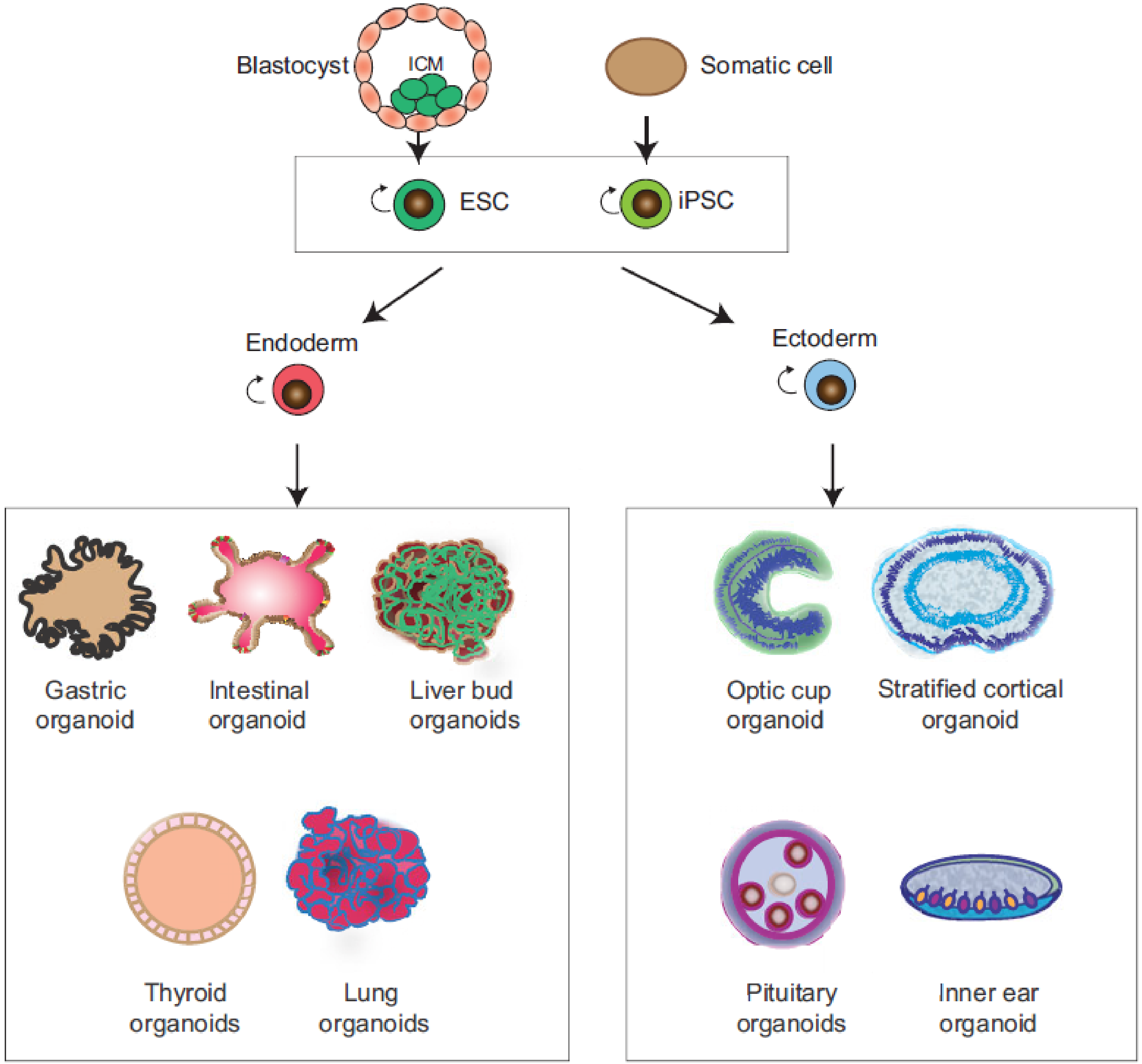
Human Embryonic Stem Cell-derived Cerebral Organoids

Establish culture conditions based on an understanding of the developmental process



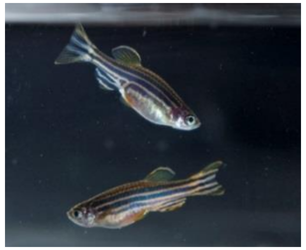
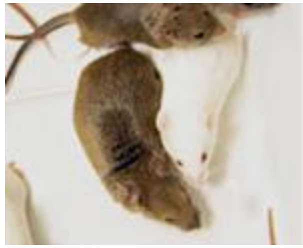
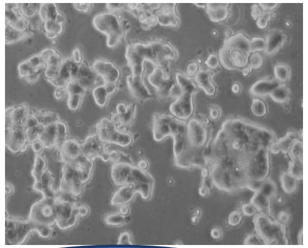
MA Lancaster et al.
Nature 2013

Pluripotent Stem Cell (ESC and iPSC)-derived Organoids



Why organoids as a model system?



Ex vivo system
: in vivo phenotype

Ever-expanding system
: as cell lines

?

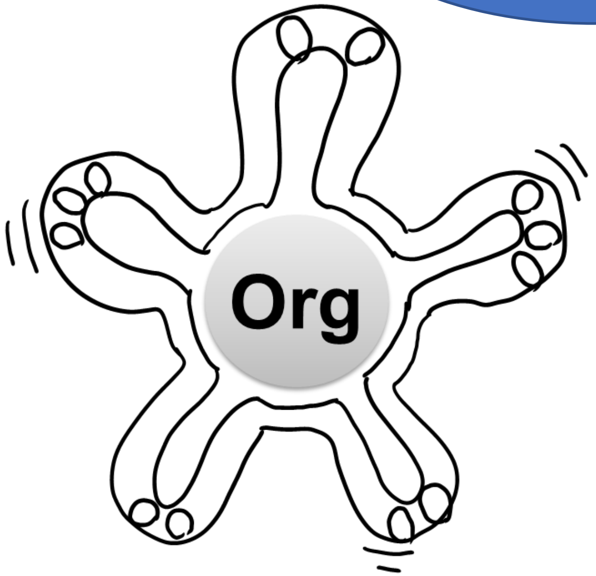
Easy to treat
: Ligand, Inhibitor etc.

Evolutional similarity
: Mouse (and Human)

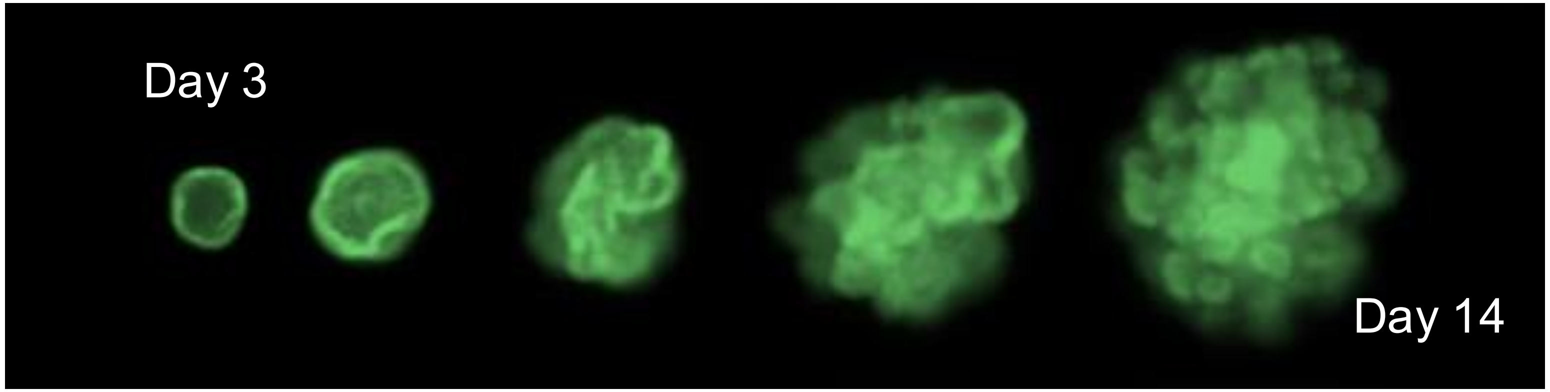
?

Experiment with genes

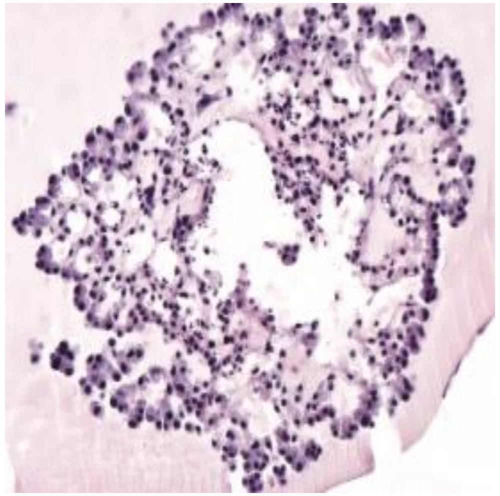
Cell therapy



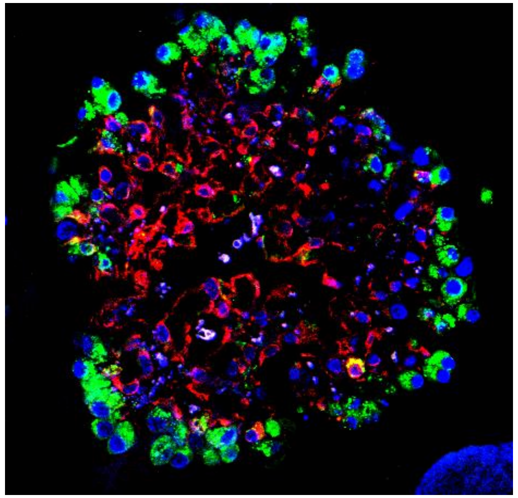
Lung Alveolar Organoids: tools for studying stem cell differentiation and cell-cell interactions



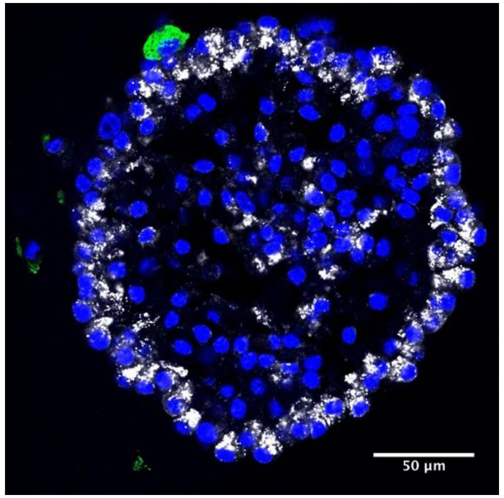
Self-organizing organoids: monitoring stem cell behaviors, which replicate regeneration processes



H&E



Sftpc (AT2: stem cells) Ager (AT1: differentiated cells) DAPI (nuclear)

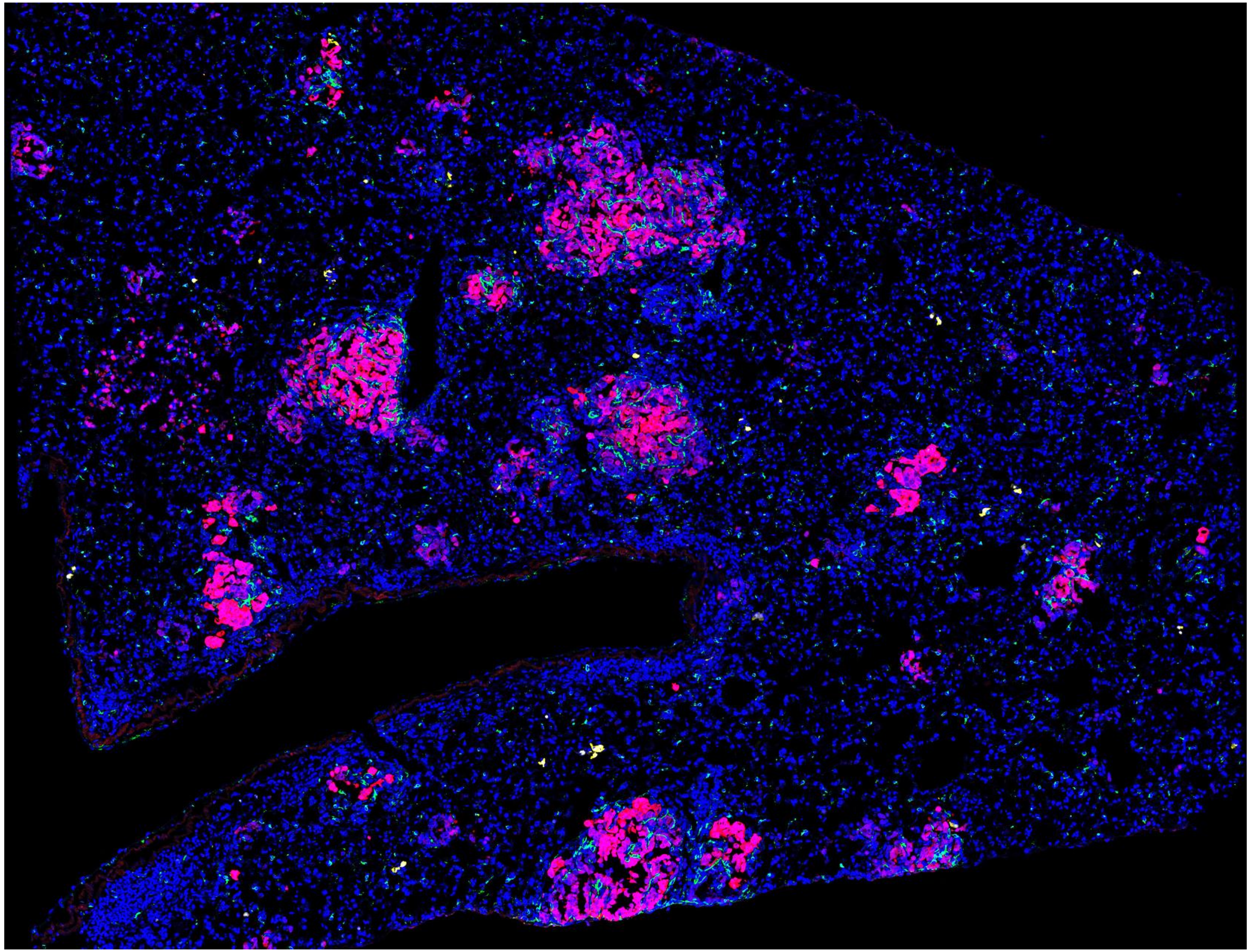


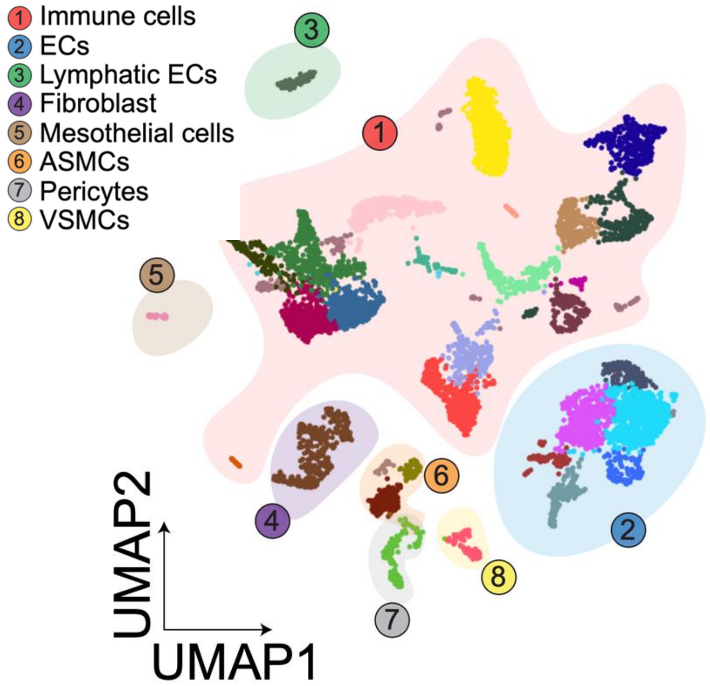
Sftpc (AT2: stem cells) Mac2 (macrophages) DAPI (nuclear)

Organoid co-cultures: deconstructing cell-cell communication during regeneration processes

Lee et al. Cell. 2014
Lee et al. Cell. 2017
Choi et al. Cell Stem Cell. 2020

Dynamic Evolution of Tumors and the Surrounding Microenvironment

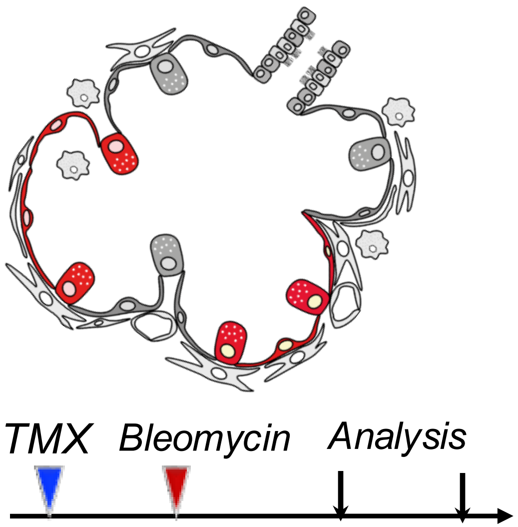


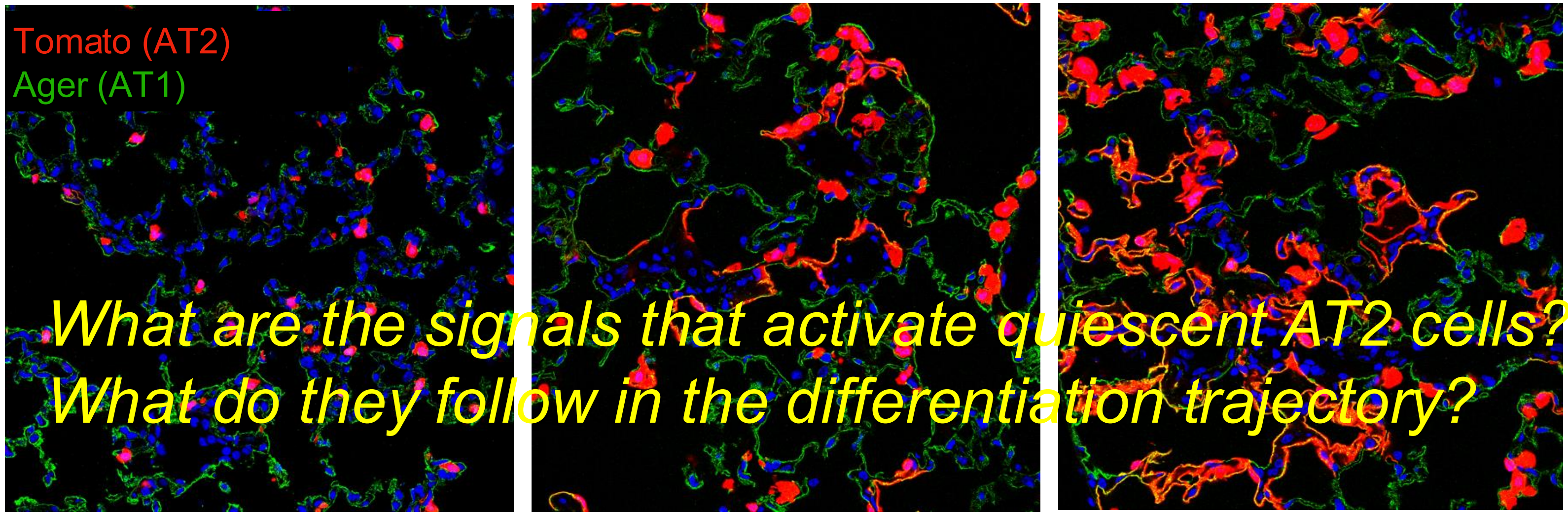


How to validate transcriptomic data from single-cell RNA-sequencing and spatial transcriptomics

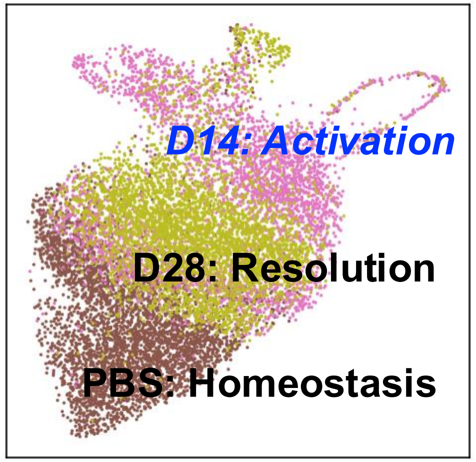
How do lung alveoli regenerate after injury?

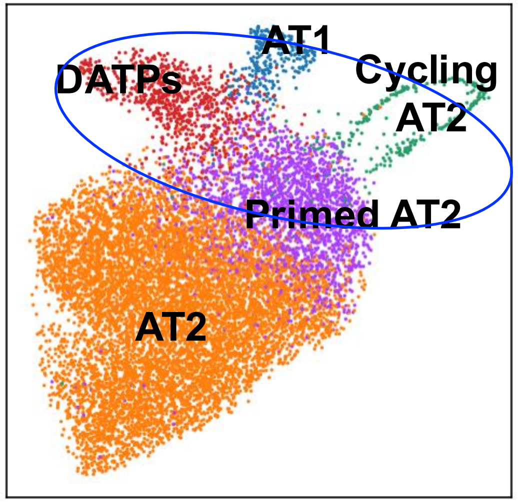
Sftpc-Cre^{ERT2}; Tom

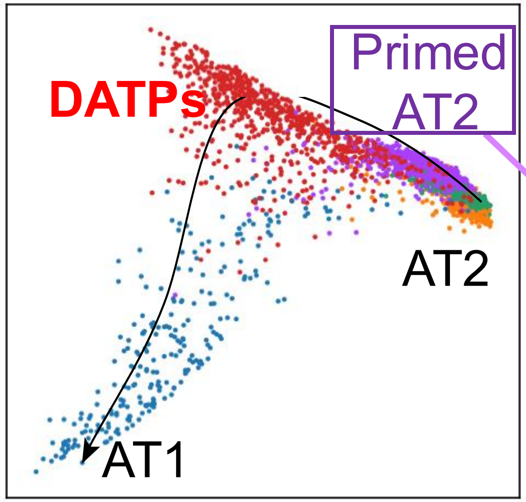




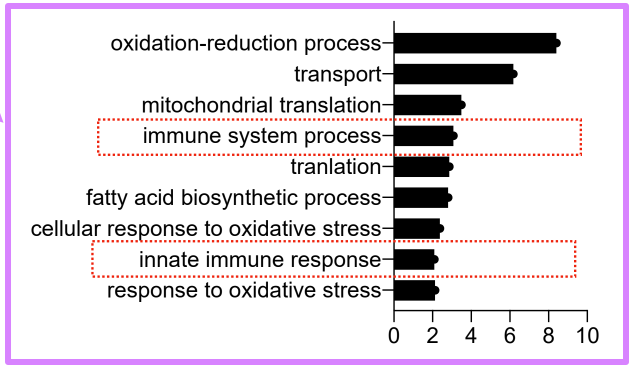
What are the signals that activate quiescent AT2 cells?
What do they follow in the differentiation trajectory?





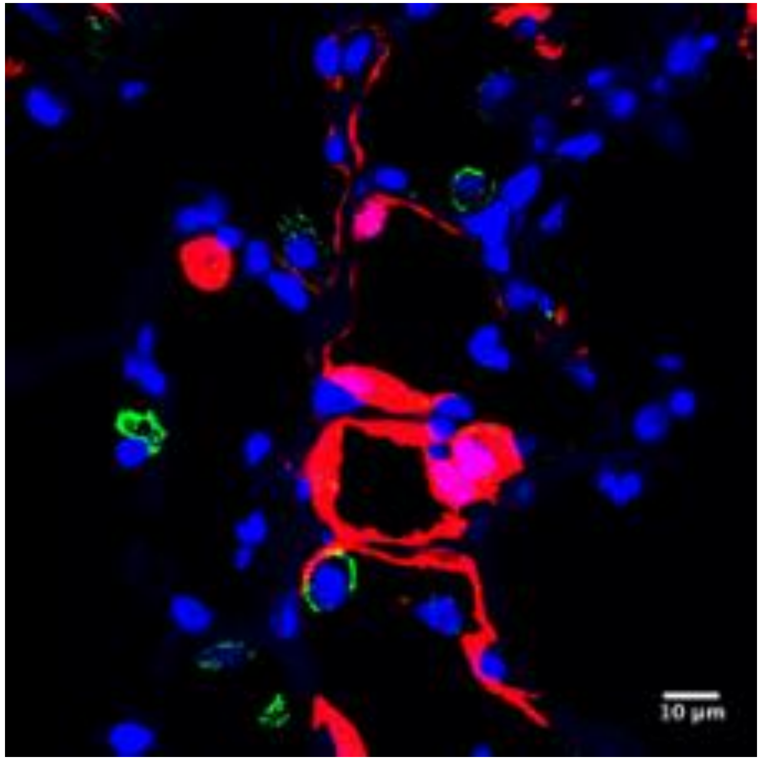


DATPs: Damage-Associated Transient Progenitors



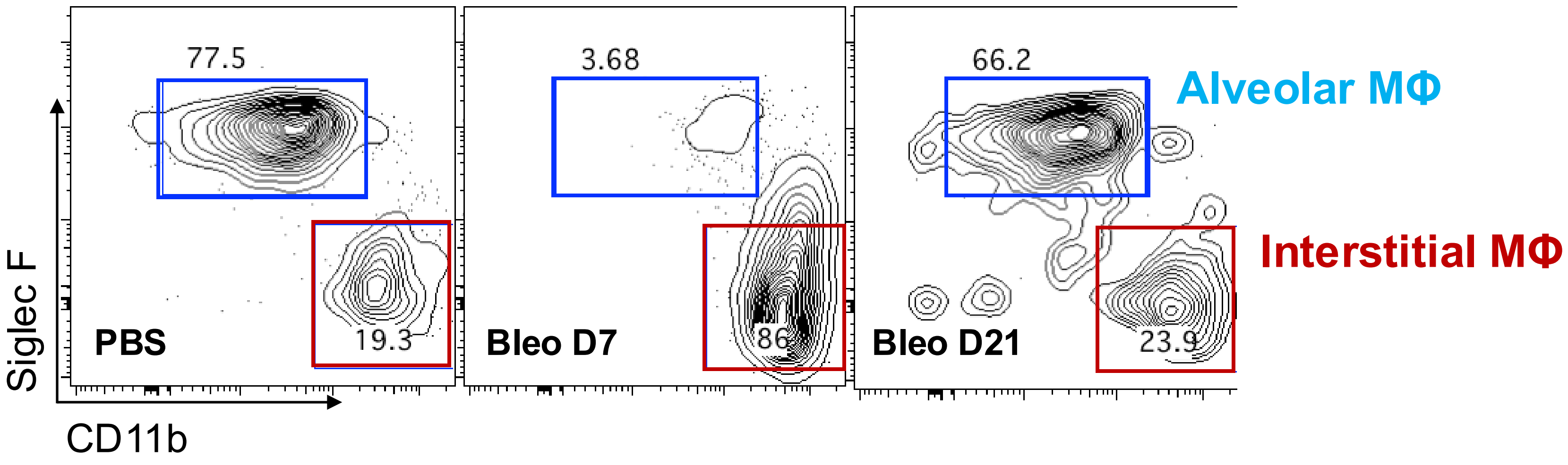
Dynamic changes in macrophages during injury repair

Sftpc-Cre^{ERT2}; Tom



Tomato (AT2) F4/80 (Macrophage)

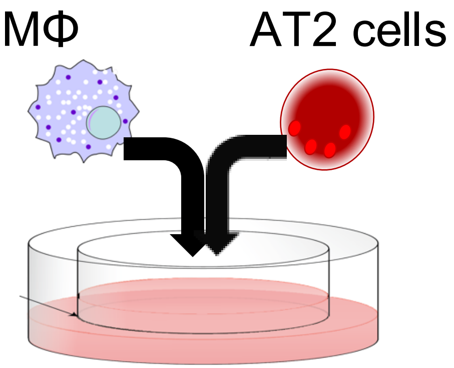
Gated on CD45+Gr1−CD64+

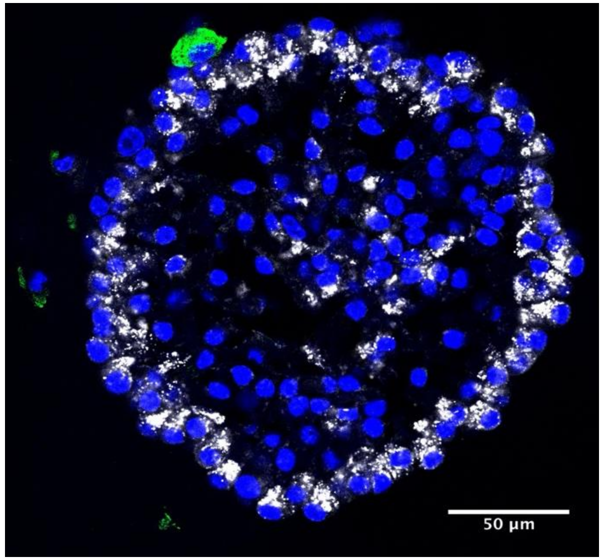


Choi et al. Cell Stem Cell 2020

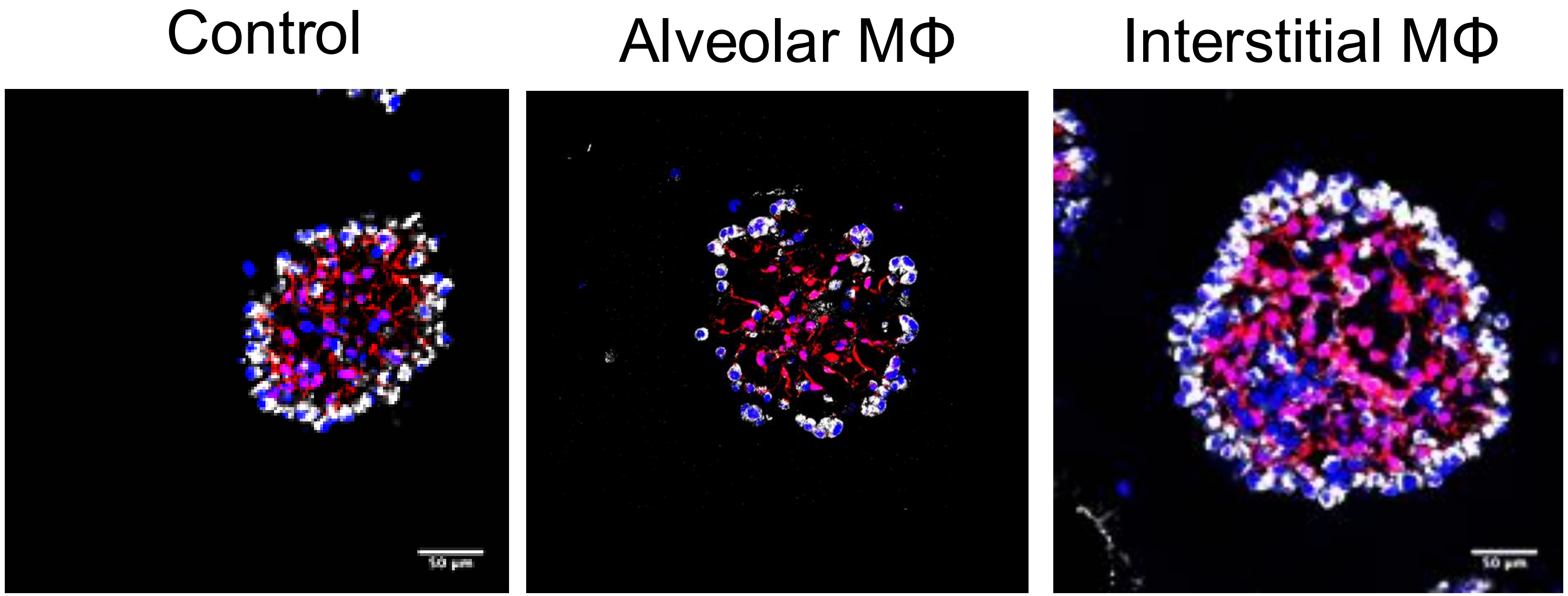
Does macrophage affect AT2 cell behavior?

<u>Ex vivo organoid co-culture</u>





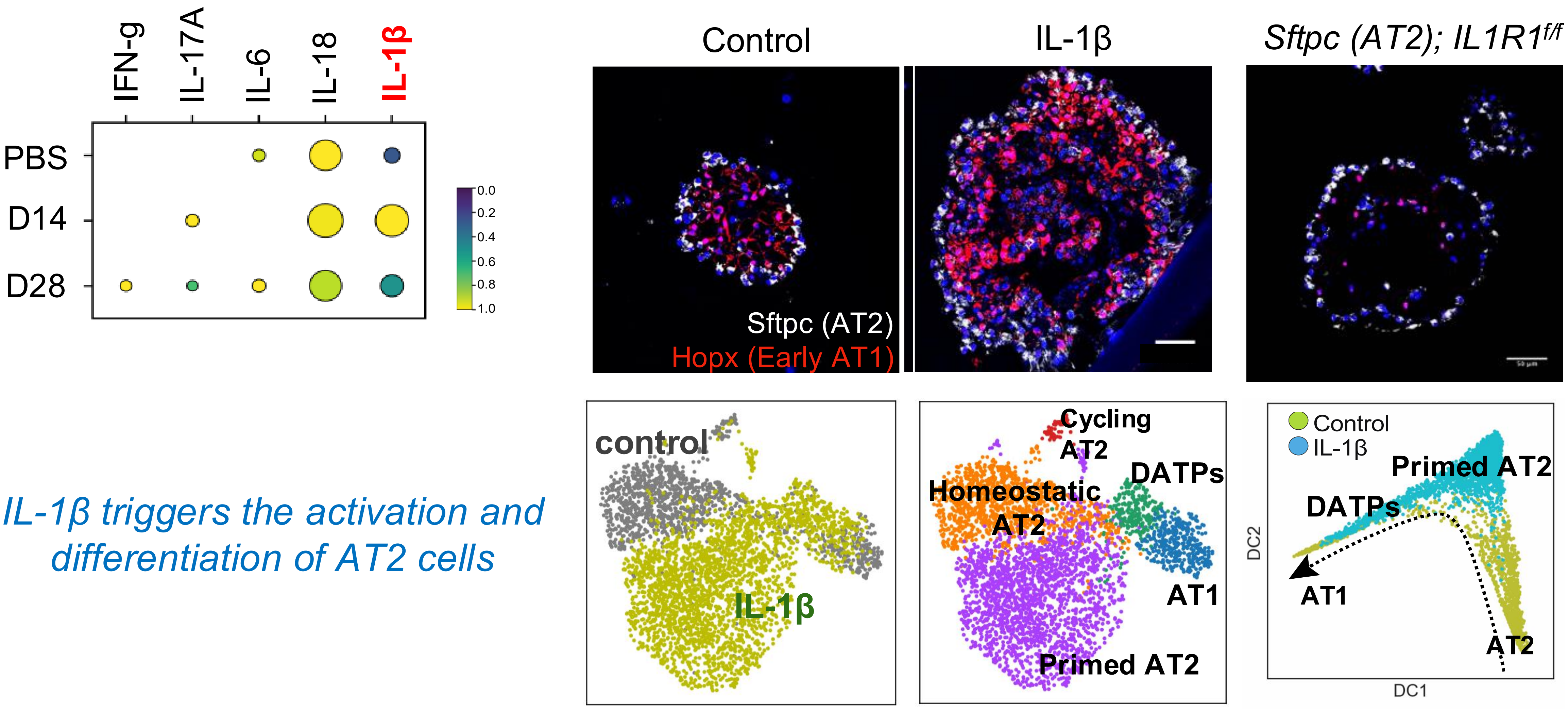
Sftpc (AT2) Mac2 (MΦ) DAPI



Sftpc (AT2) Hopx (Early AT1) DAPI

Choi et al. Cell Stem Cell 2020

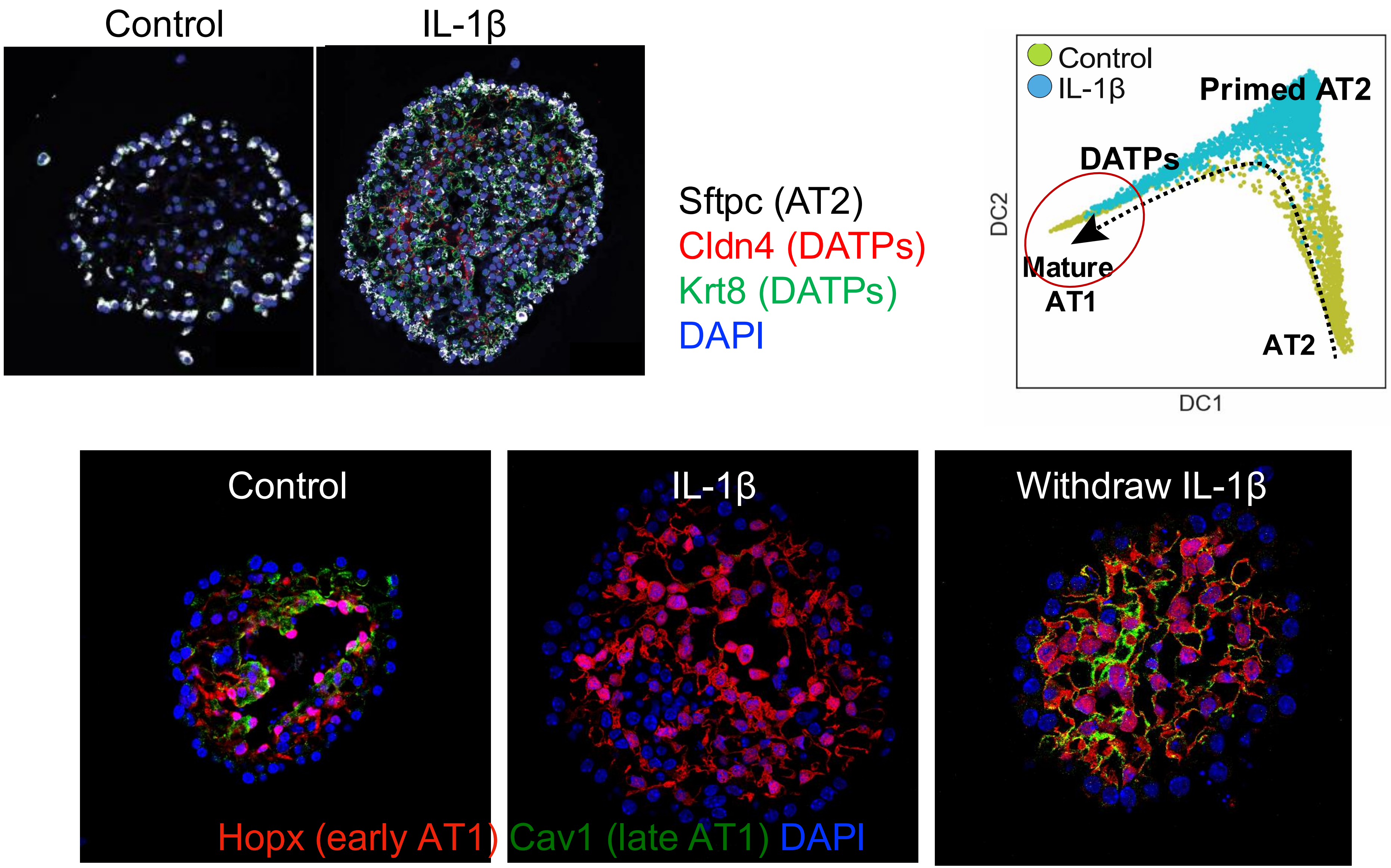
How do inflammatory signals affect stem cell fate or state?



IL-1β triggers the activation and differentiation of AT2 cells

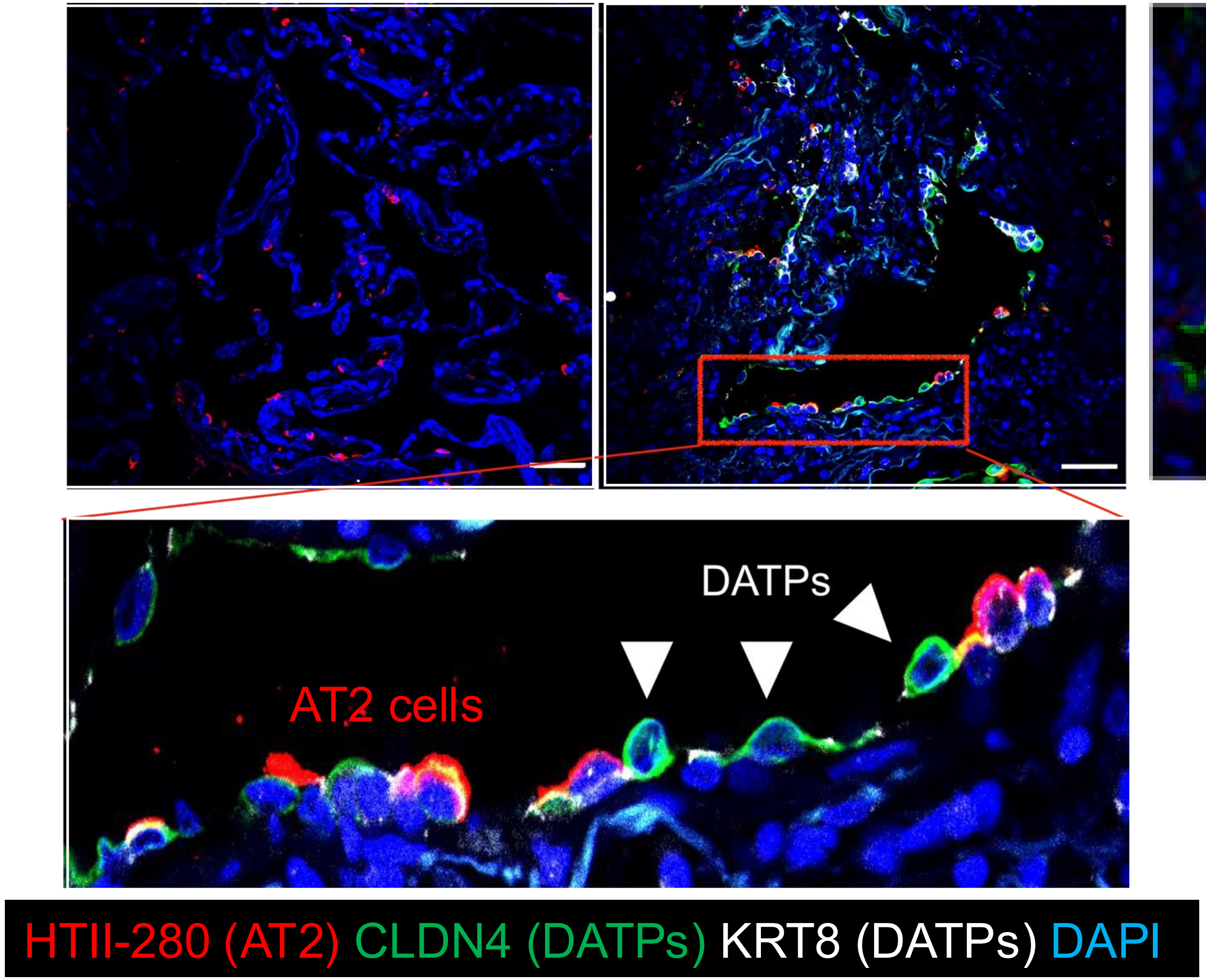
Choi et al. Cell Stem Cell 2020

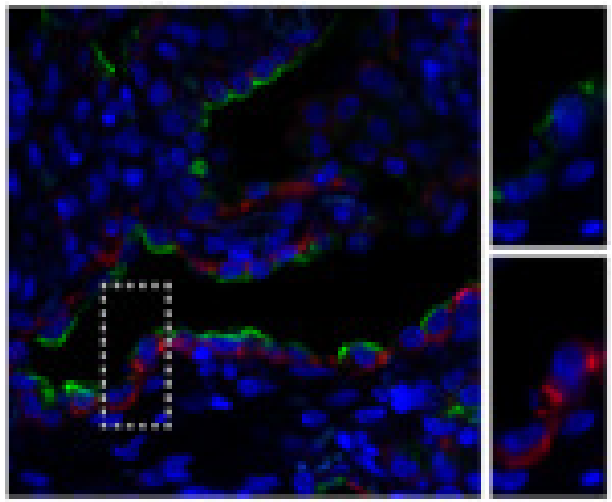
Sustained IL-1β blocks the transition from DATPs to mature AT1

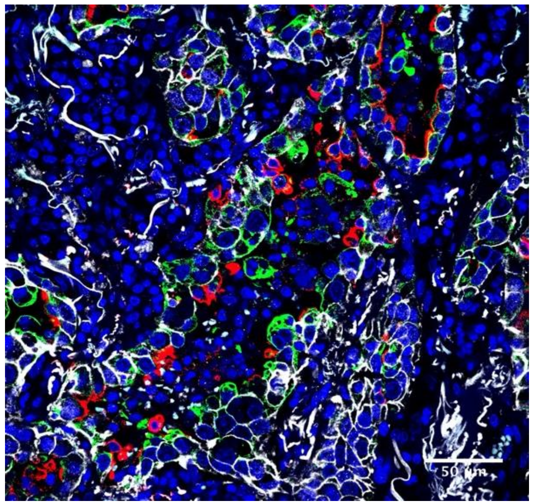


Choi et al. Cell Stem Cell 2020

Emergence of DATP-like cells in injured human lungs: *Impaired regeneration causes lung disease?*

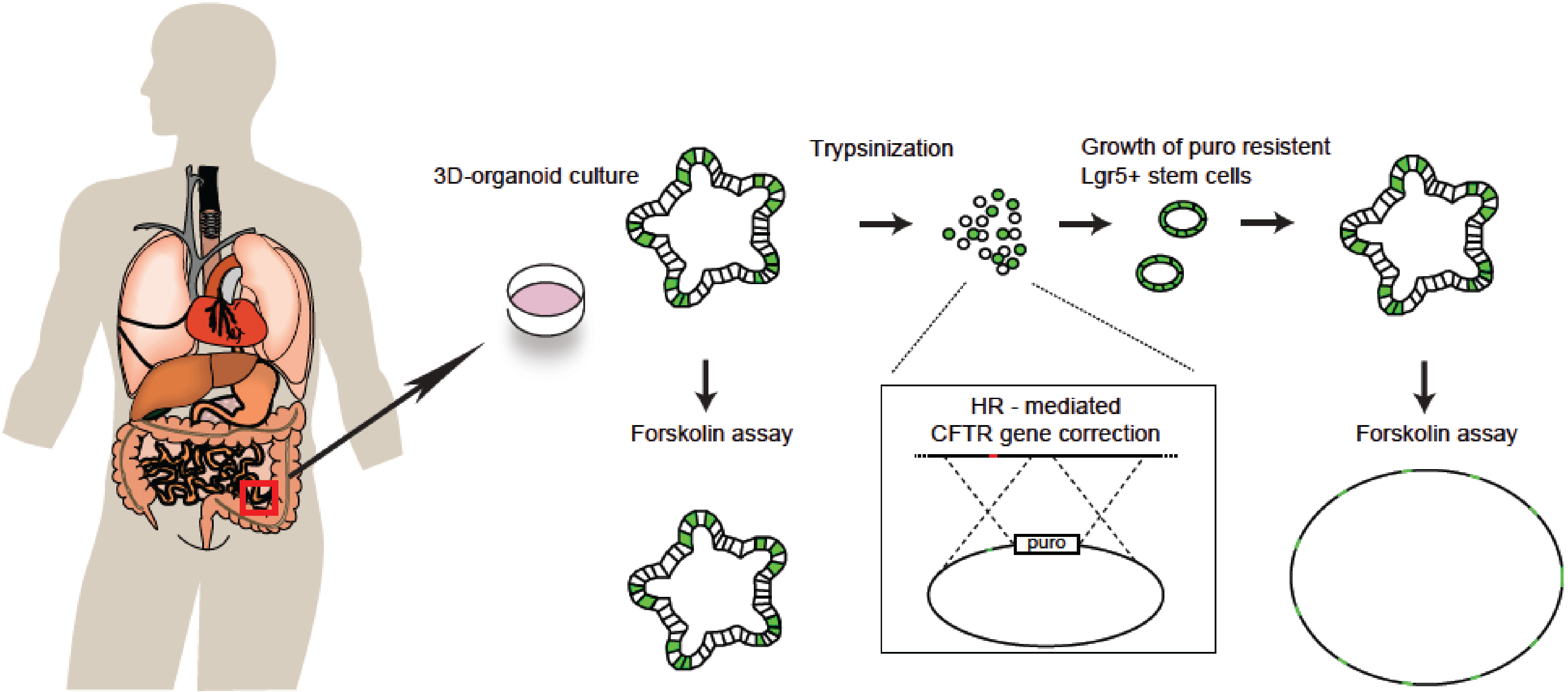






Choi et al. Cell Stem Cell. 2020
Chen et al. Cell Res. 2020
Strunz et al. Nat. Comm. 2020
Kobayashi et al. Nat Cell Biol. 2020
Marjanovic et al. Cancer Cell. 2020
Melms et al. Nature. 2021
Delorey et al. Nature. 2021
Many more…

Functional repair of the *CFTR* locus in primary colon stem cells of a cystic fibrosis patient

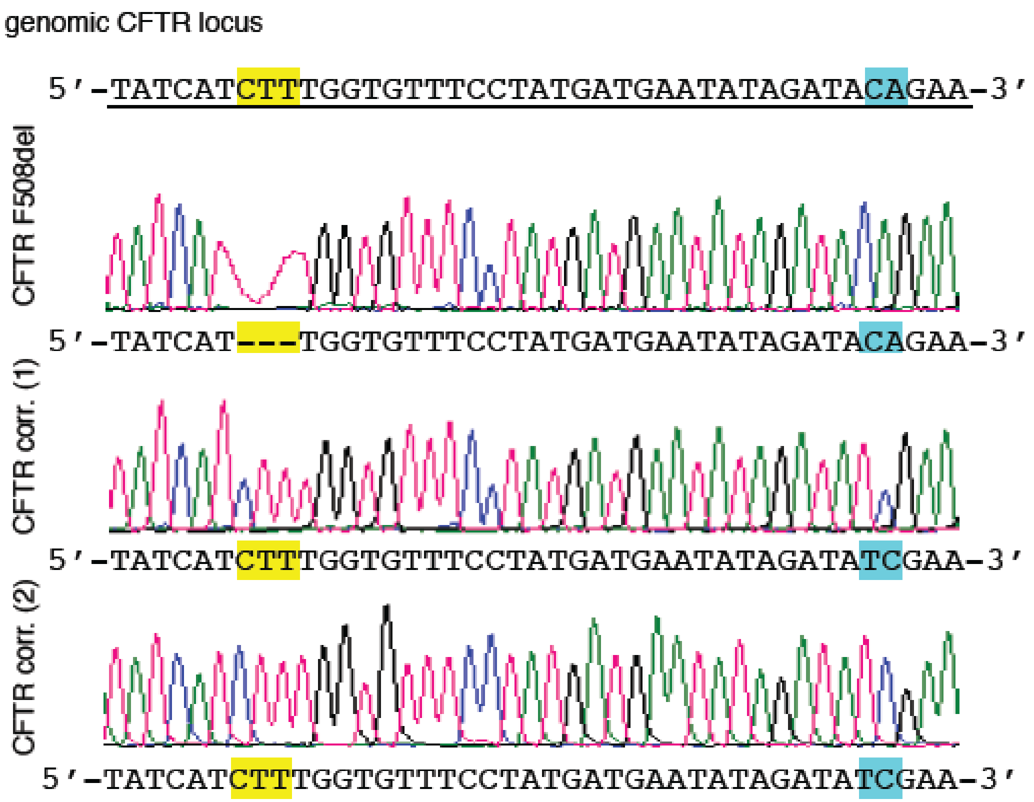


CFTR encodes an anion channel essential for fluid and electrolyte homeostasis of epithelia. Mutations in this receptor cause cystic fibrosis (CF), a disease that leads to the accumulation of viscous mucus in the pulmonary and gastrointestinal tract.

G Schwank, *et al*. Cell Stem Cell 2013

Functional repair of the *CFTR* locus in primary colon stem cells of a cystic fibrosis patient

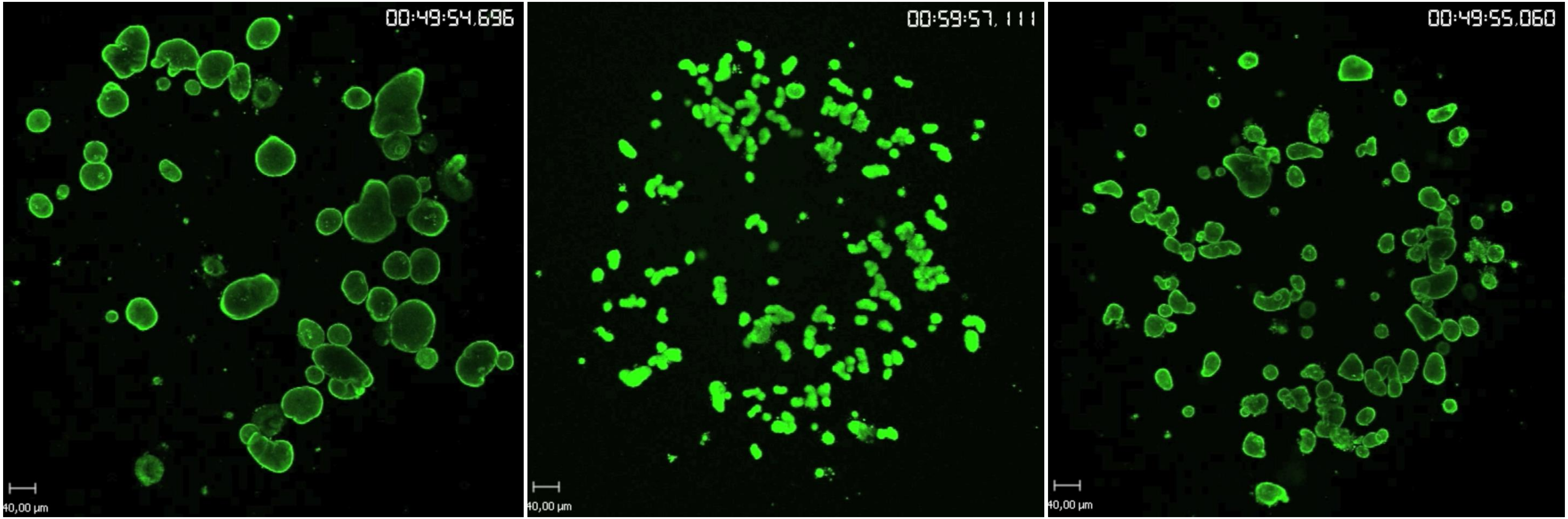
CRISPR/Cas9 gene editing in patient-derived organoids



Two patients were homozygous for the most common *CFTR* mutation, a deletion of phenylalanine at position 508 (CFTR F508 del) in exon 11, which causes misfolding, endoplasmic reticulum retention, and early degradation of the CFTR protein.

Gene correction for cystic fibrosis

Wt human CFTR opened with forskolin	Mutant CFTR does not open	Gene corrected CFTR opened with forskolin



Forskolin assay: forskolin induces CFTR-dependent fluid secretion into the organoid lumen that leads to a rapid increase of the whole organoid area that can be quantified by time-lapse live cell microscopy

Significance of this achievement

The key test used is the forskolin-induced swelling (FIS) assay in patient-derived organoids, which measures CFTR function. This test shows:

- Whether CFTR is functional
- How well different drugs might work
- Patient-specific responses

These tests are particularly valuable because:

- They can test drugs before giving them to patients
- They help predict which treatments will work best
- They can justify expensive treatments to insurers
- They reduce trial-and-error in treatment selection

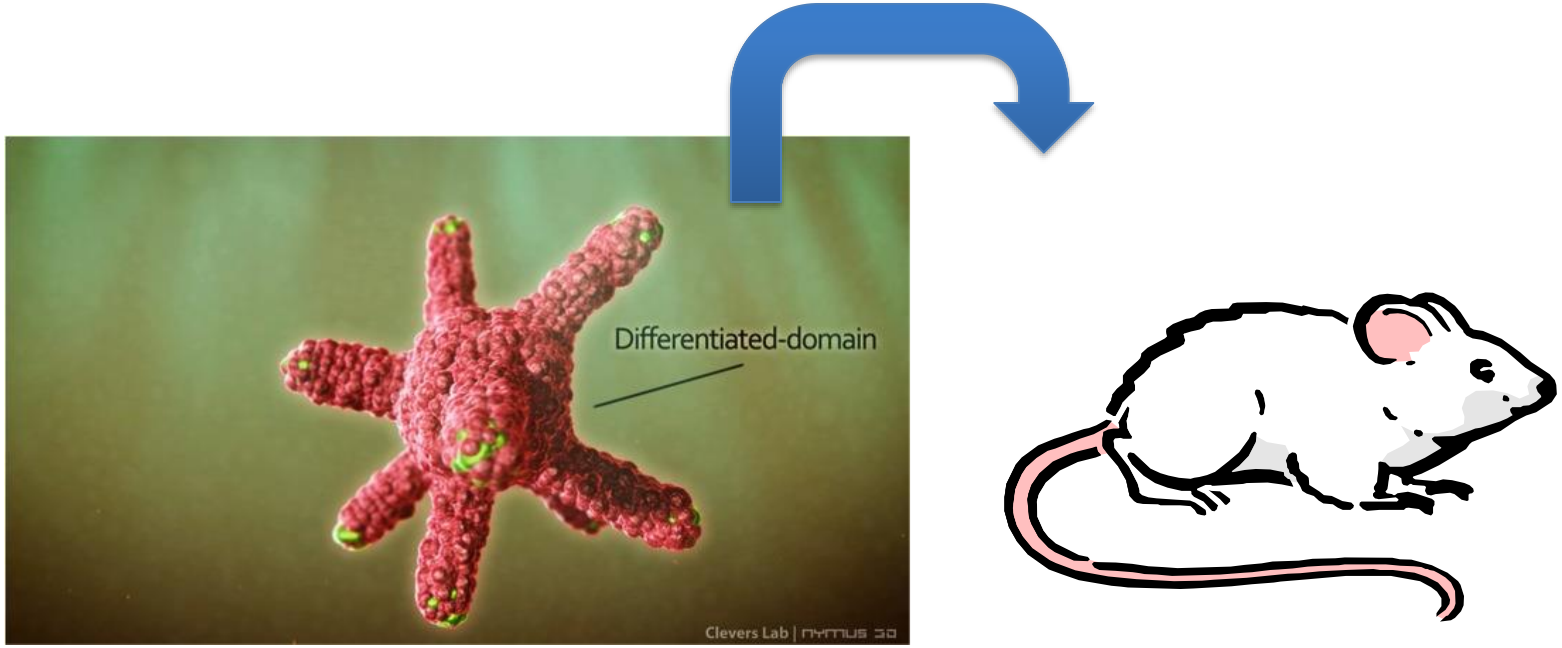
Broad applications of this assay

In Industry/Pharma:
- •Drug screening and development
- •Testing CFTR modulators
- •Predicting drug responses
- •Developing personalized treatments

In Hospitals/Clinics:
- •Patient-specific drug testing
- •Treatment response prediction
- •Diagnostic tool for CF variants
- •Guiding treatment decisions

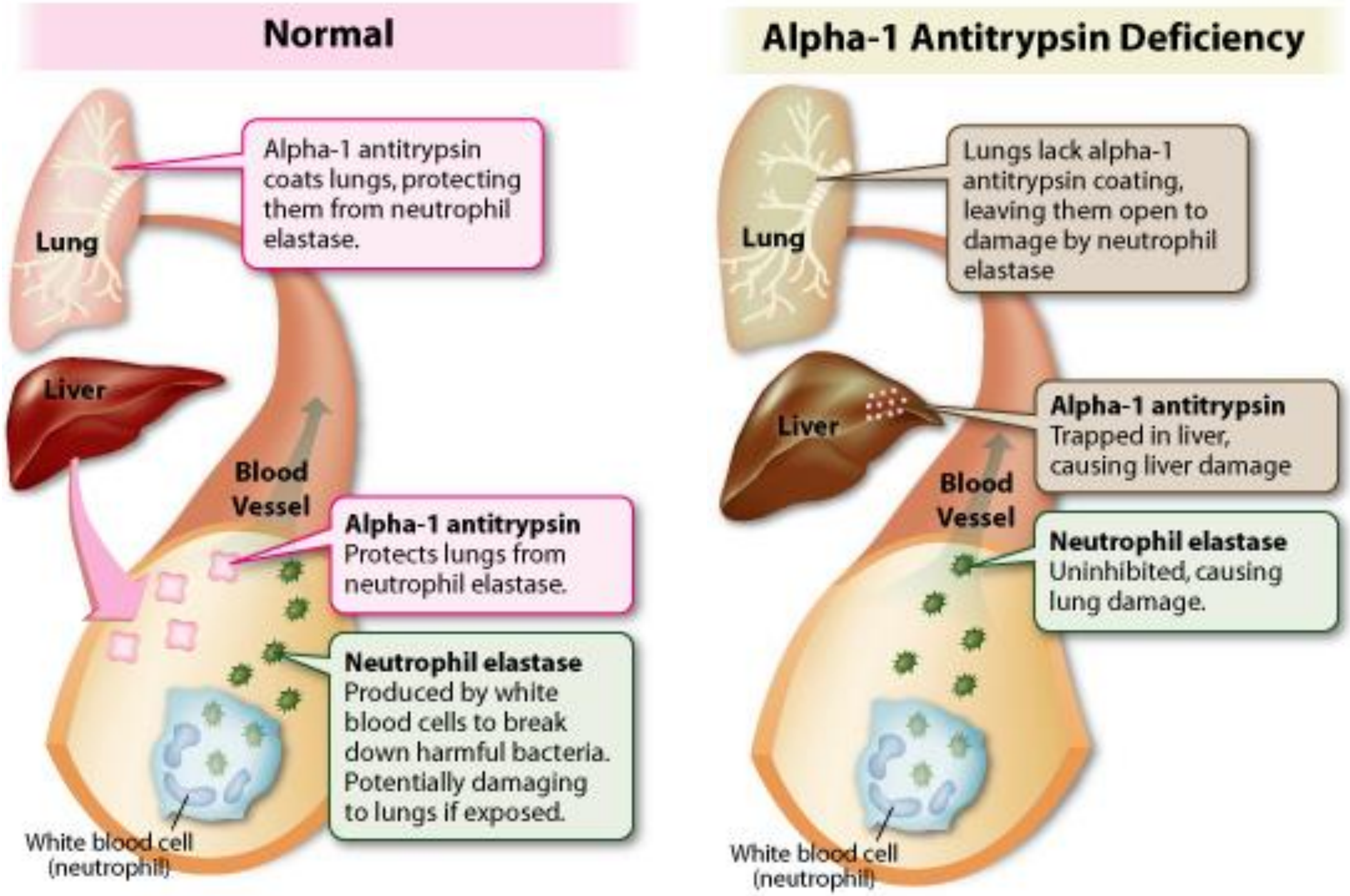
In Health Insurance:
- •Supporting coverage decisions for expensive CF drugs
- •Providing evidence for treatment efficacy
- •Determining drug reimbursement
- •Cost-effectiveness assessment

Can we transplant organoids grown from a single stem cell (resource for cell therapy)?



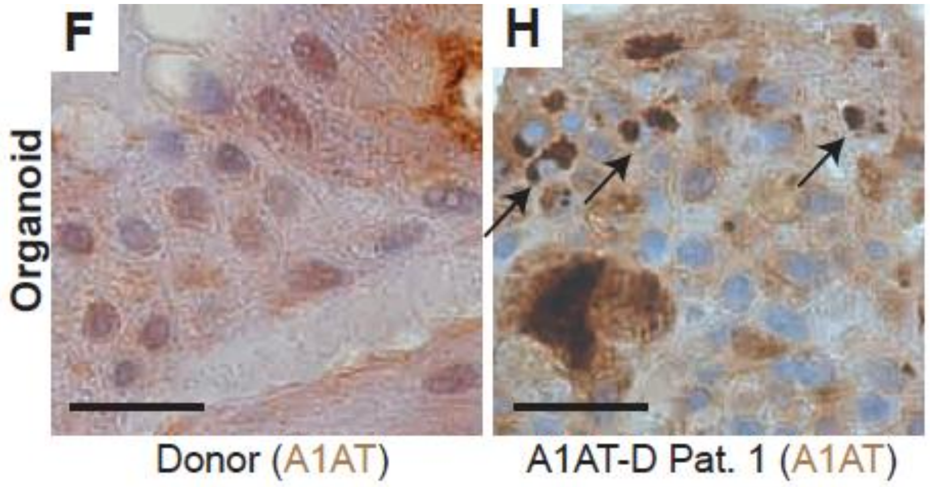
Transplantation of organoids grown from stem cells

Functional repair of the *A1AT* deficiency in human iPSCs derived from patients



The resulting inclusions cause cirrhosis/ emphysema for which the only current therapy is transplantation.

A1AT-D culture (120 days)

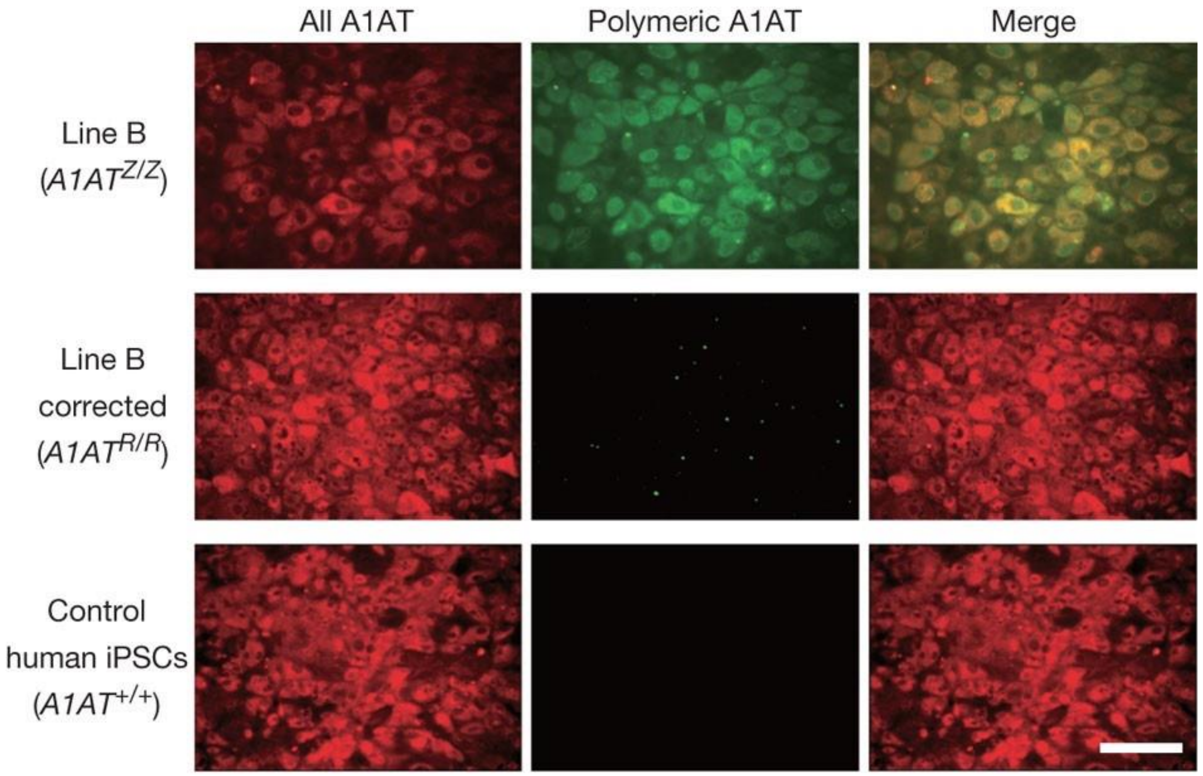


A1AT-deficiency derived organoids phenocopy liver disease in culture

Functional analysis of restored A1AT in corrected iPSC-derived hepatocyte-like cells (gene and cell therapy)

Transplanted gene corrected organoids into the liver

Alb-uPA$^{+/+}$;*Rag2*$^{-/-}$; *Il2rg*$^{-/-}$ mice

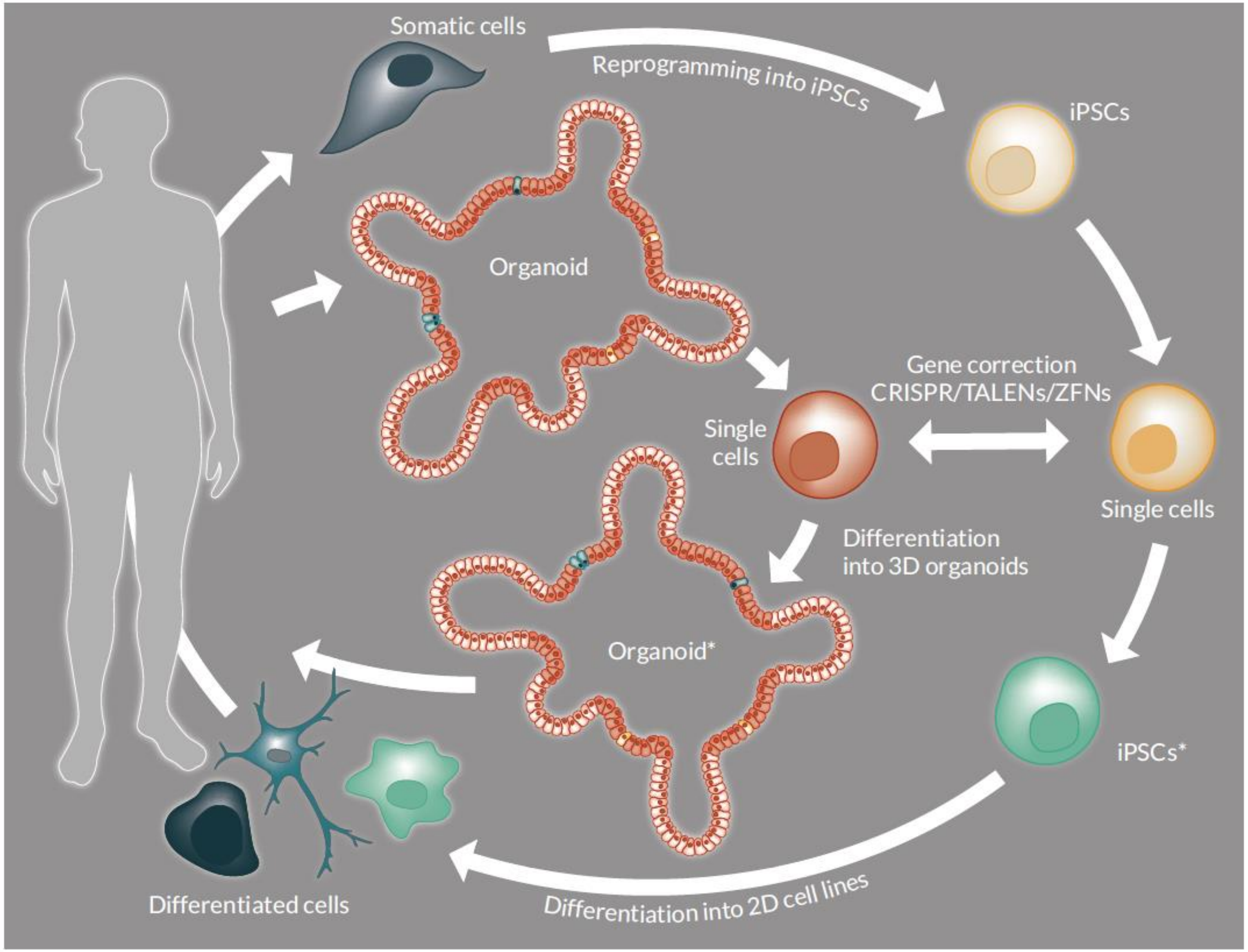


Genetic correction of the *Z* mutation resulted in functional restoration of A1AT in patient-derived cells

Yusa et al. Nature 2011

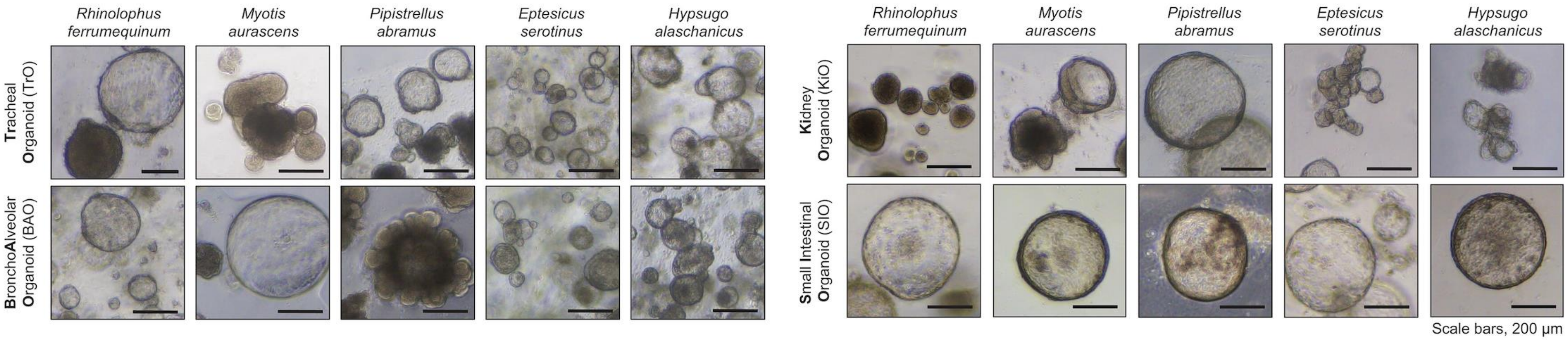
Gene editing and cell therapy

Schematic illustrating the routes of gene-edited cell therapy.



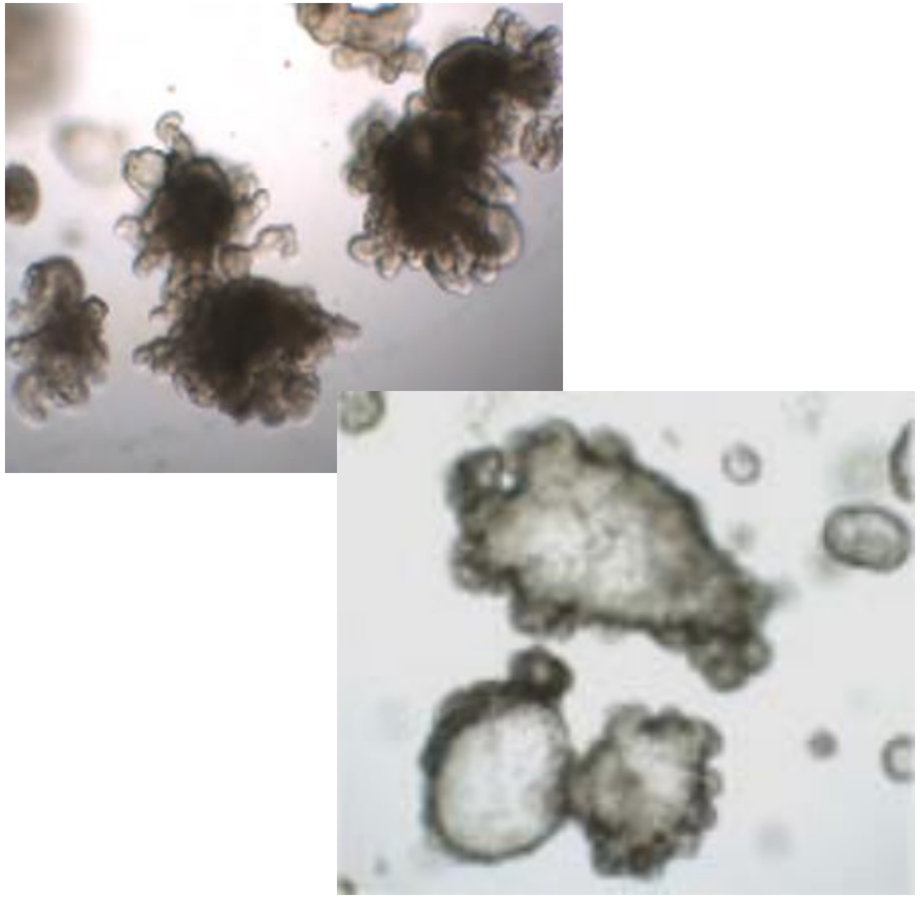
Expanded utility belt for tackling bat viruses

Multispecies, multiorgan bat organoids

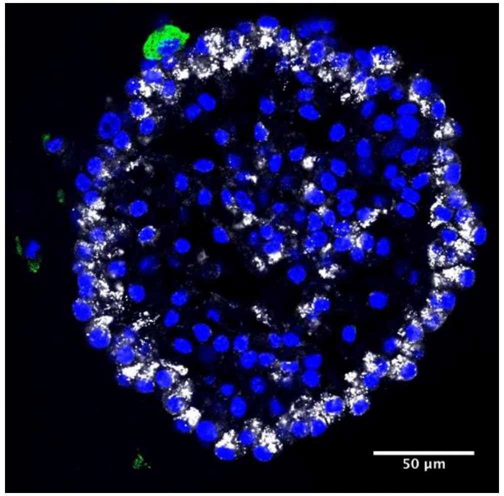


Kim et al. Science. 2025

Bridging Organoids and Complex Tissue Biology



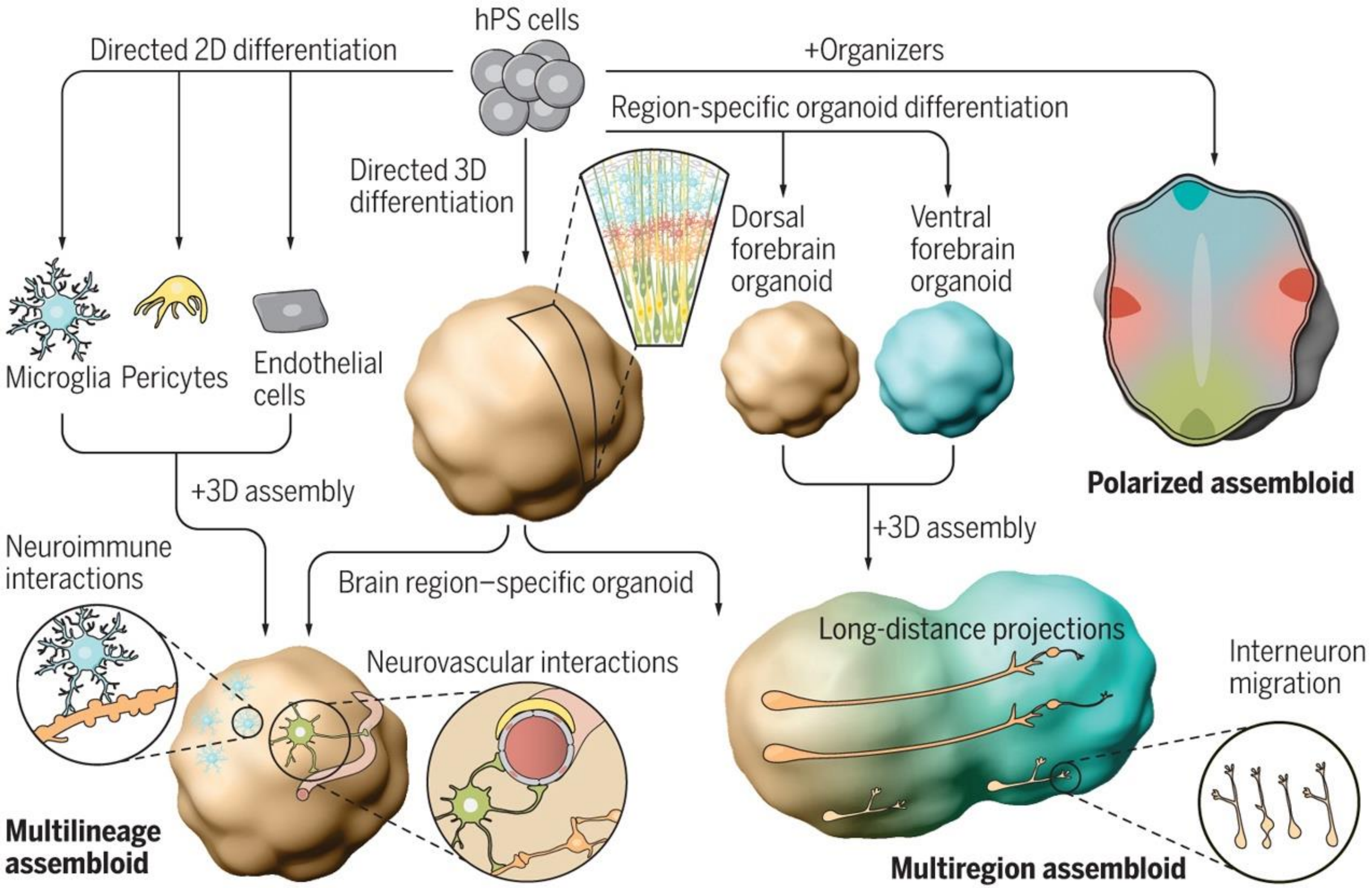
Limitation: lack of cellular and mechanical complexity of tissues



Co-culture of epithelial stem cells and their stromal cells

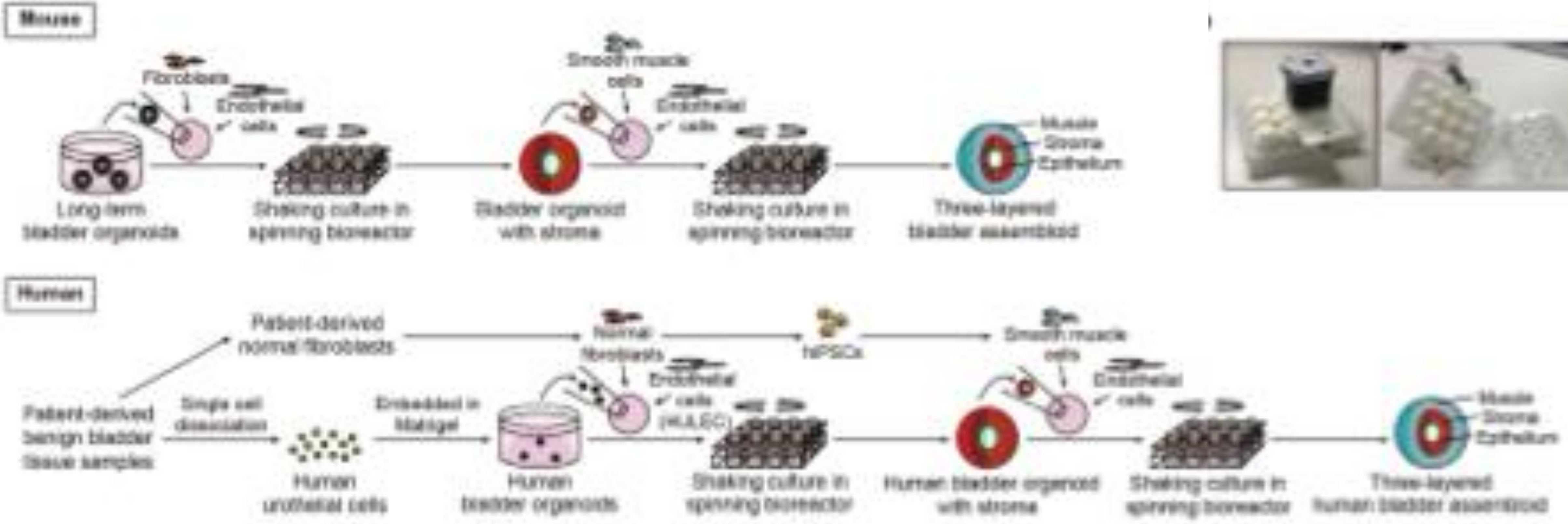
Sftpc (AT2: stem cells)
Mac2 (macrophages)
DAPI (nuclear)

Advanced organoid models: *Assembloids*



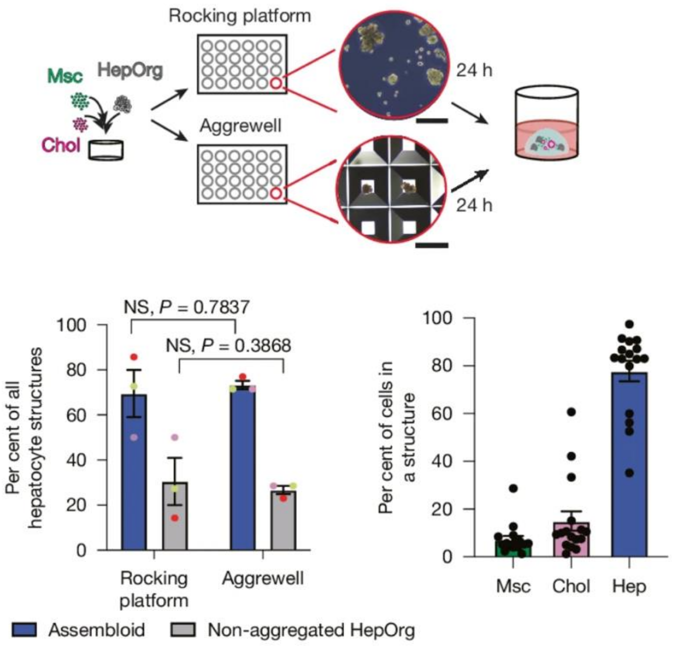
Sergiu P. Pasca. Science 2019

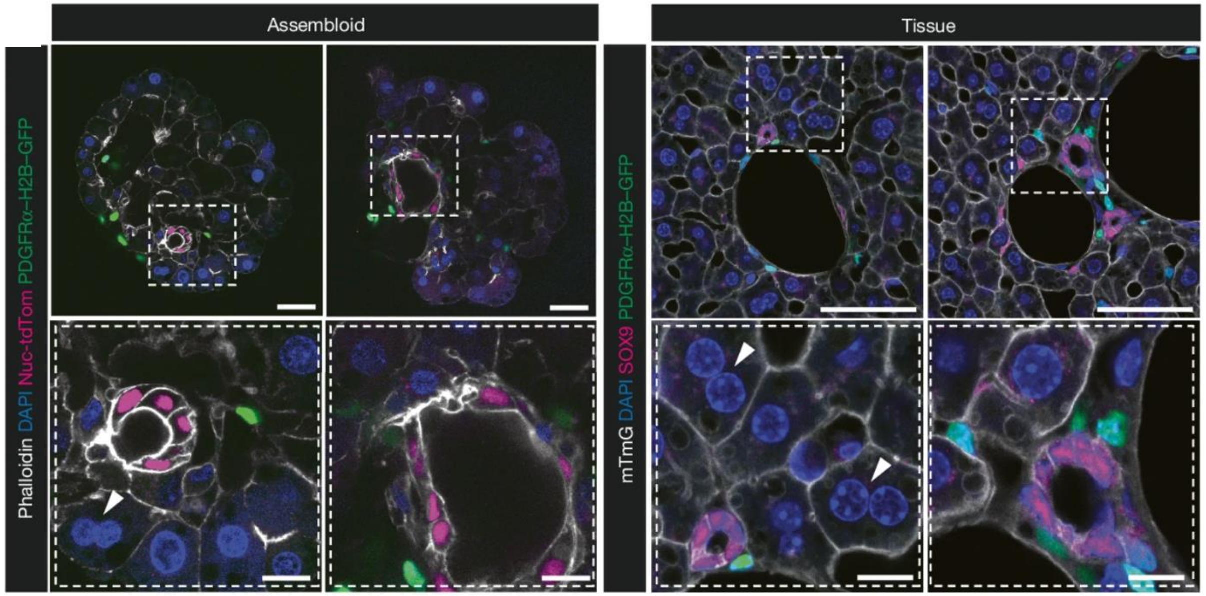
Advanced organoid models: *Assembloids*

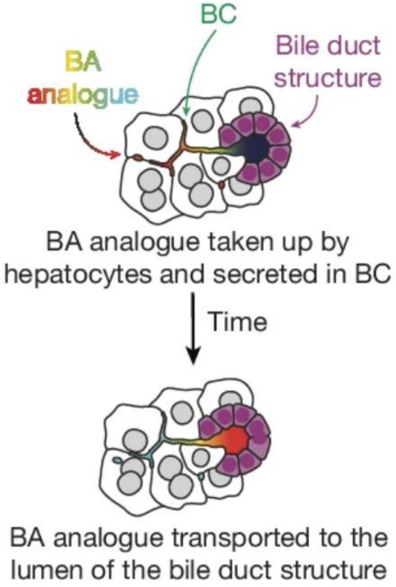


Kim et al. Nature 2020

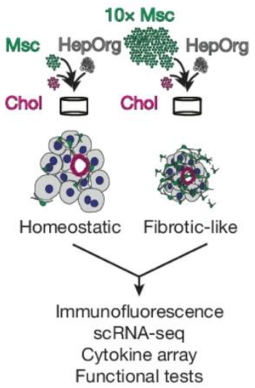
Advanced organoid models: *Assembloids*

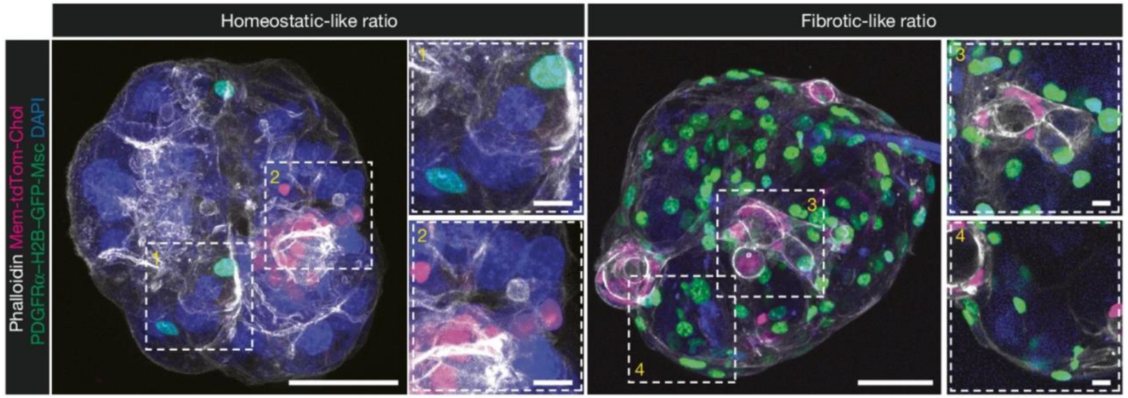






<u>*Modeling fibrosis using assembloids*</u>

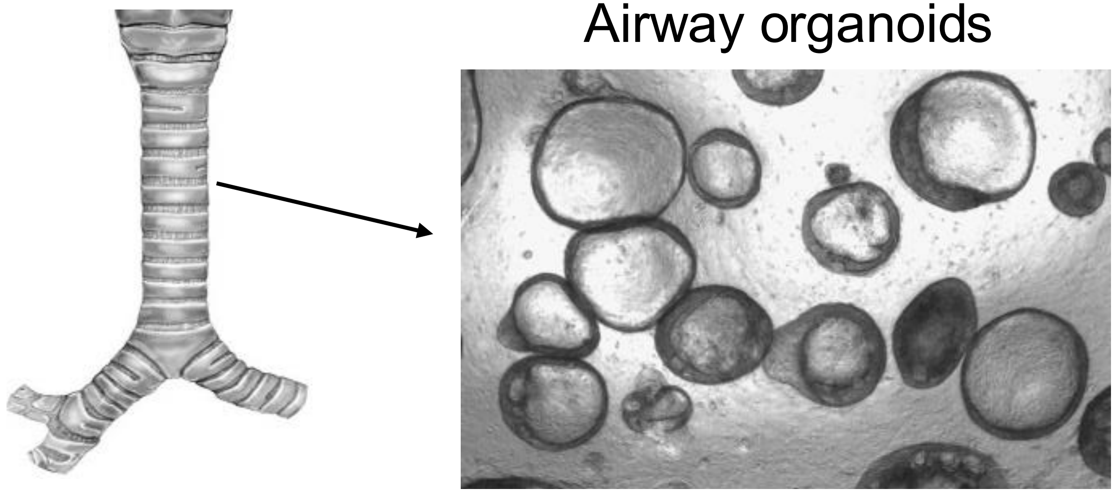




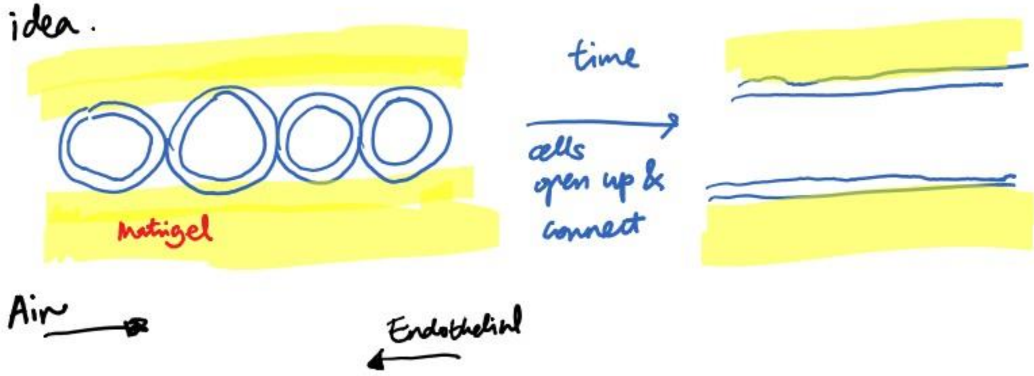
Dowbaj et al. Nature 2025

Advanced organoid models: *Macroscale tubular structure*

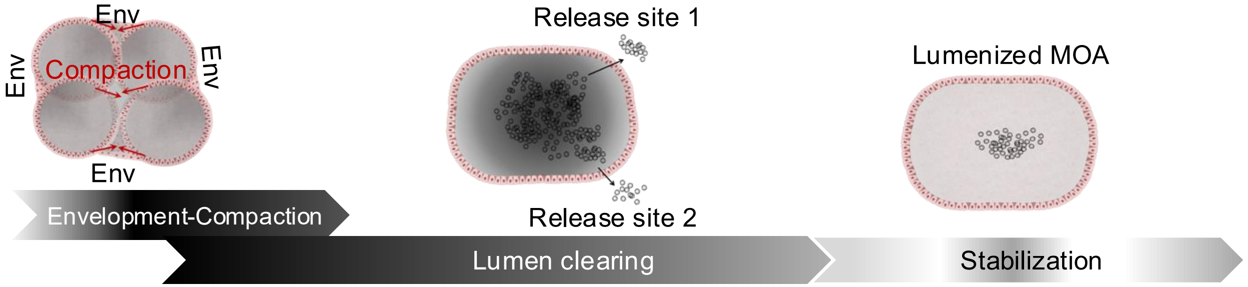
Airway organoids



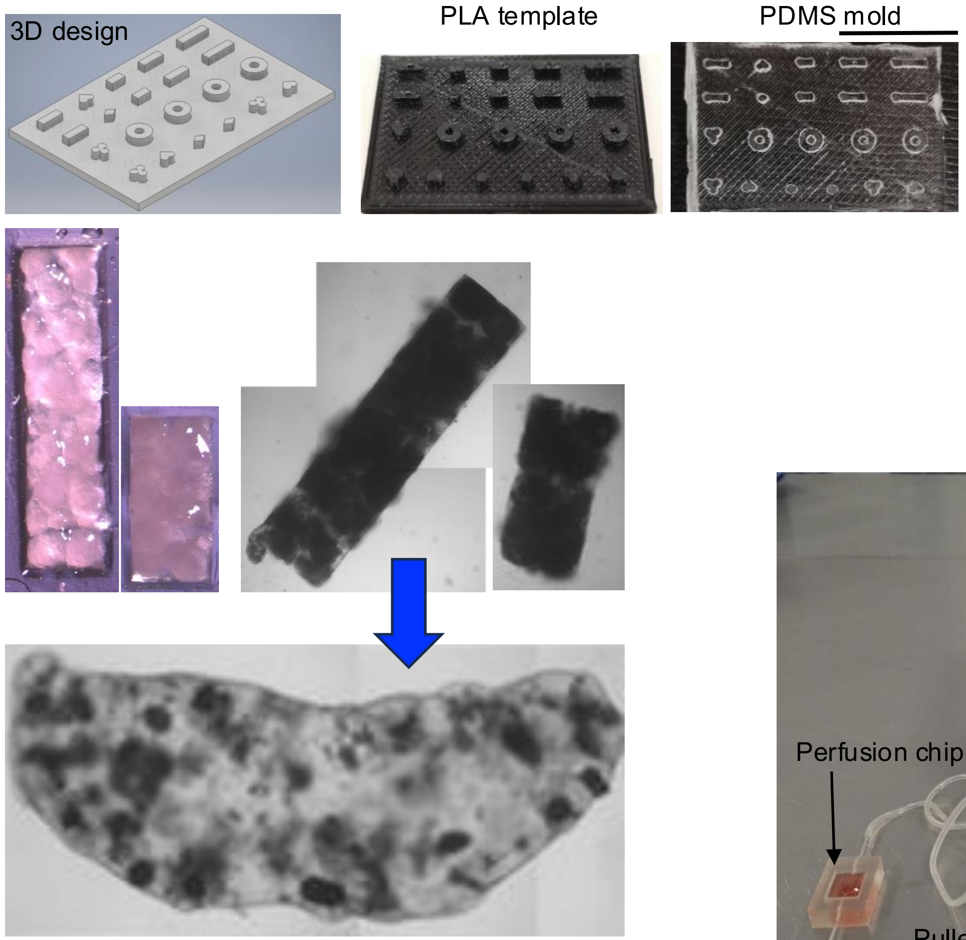
Can we build trachea?



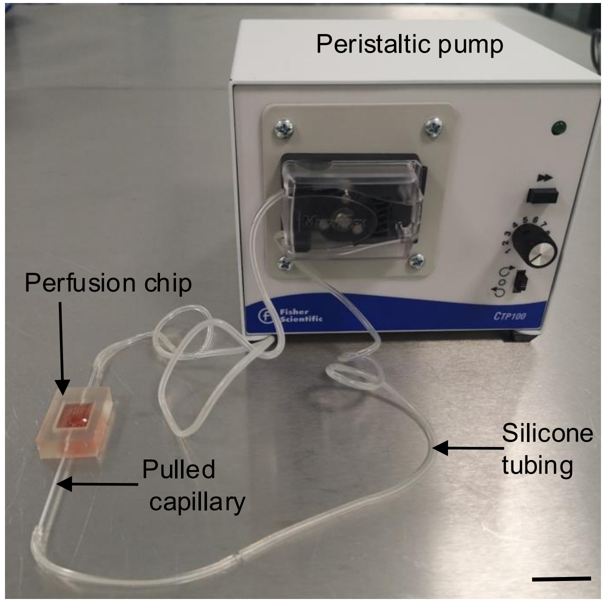
Self-organizing tubes



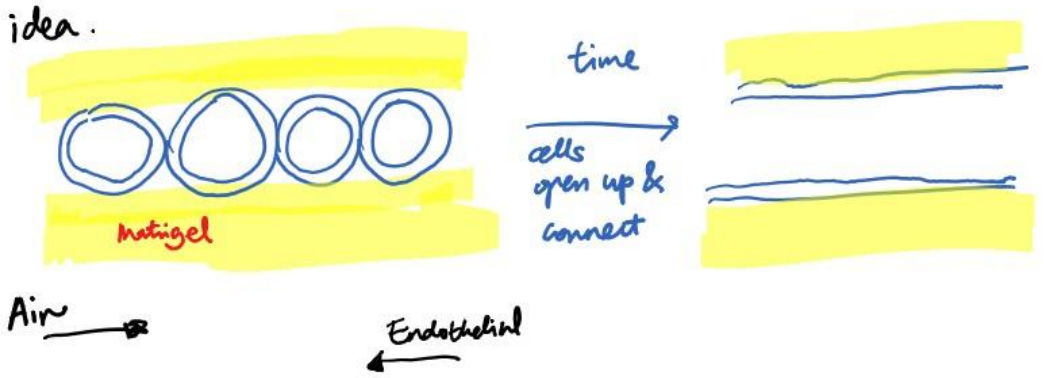
Advanced organoid models: *Macroscale tubular structure*

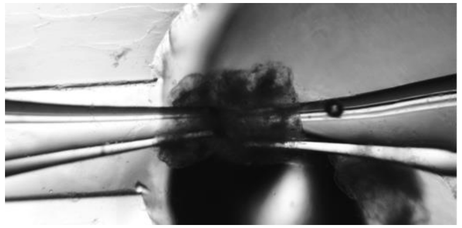


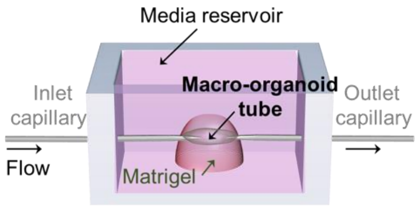
Patterned self-organizing tubes



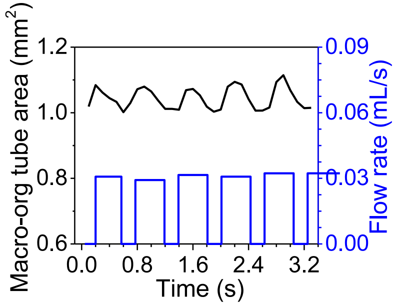
Can we build trachea?







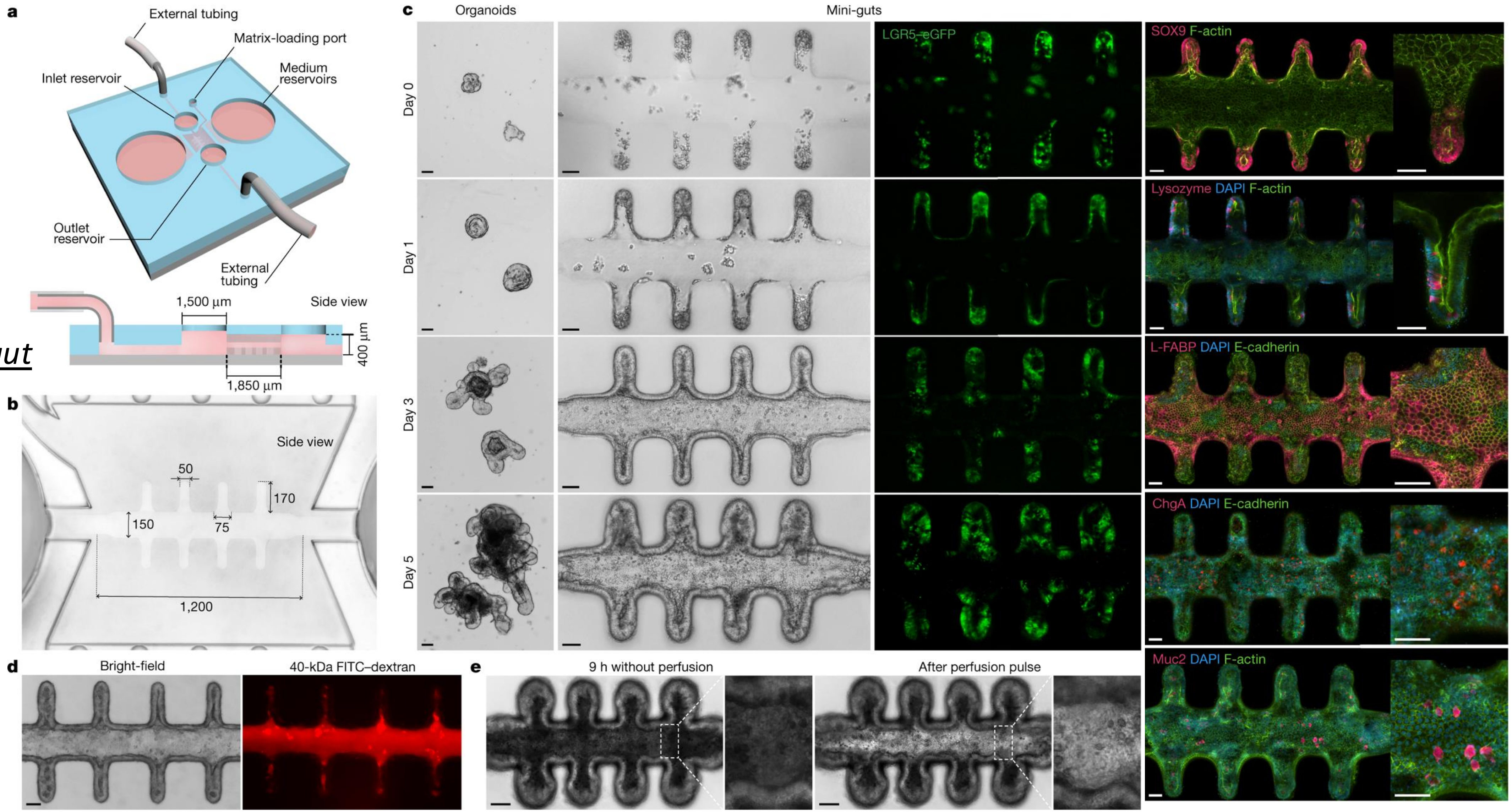
Flow-able macro-organoid tube



Air-flow connected airway tubes

Liu et al. Ad Sci. 2021

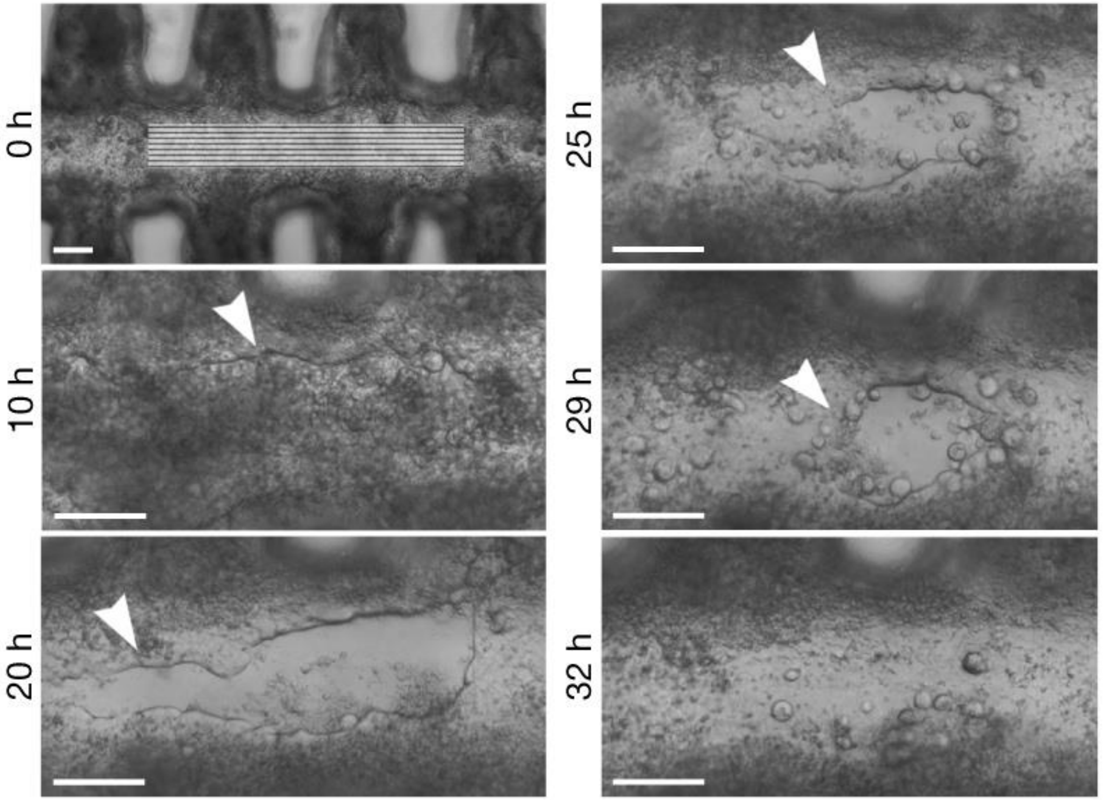
Advanced organoid models: *Macroscale tubular structure*



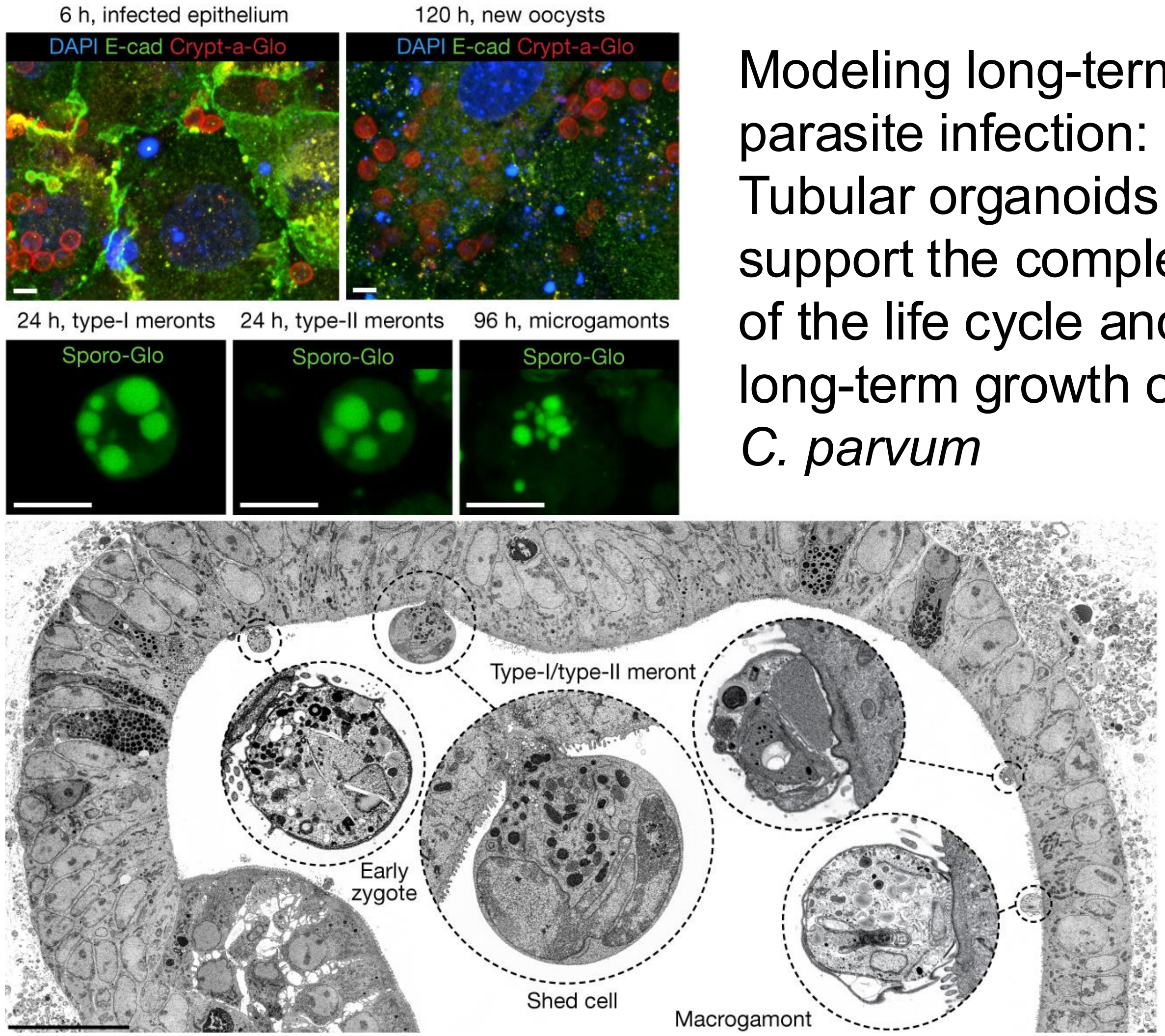
Mini-gut

Nikolaev et al. Nature 2020

Advanced organoid models: *Macroscale tubular structure*



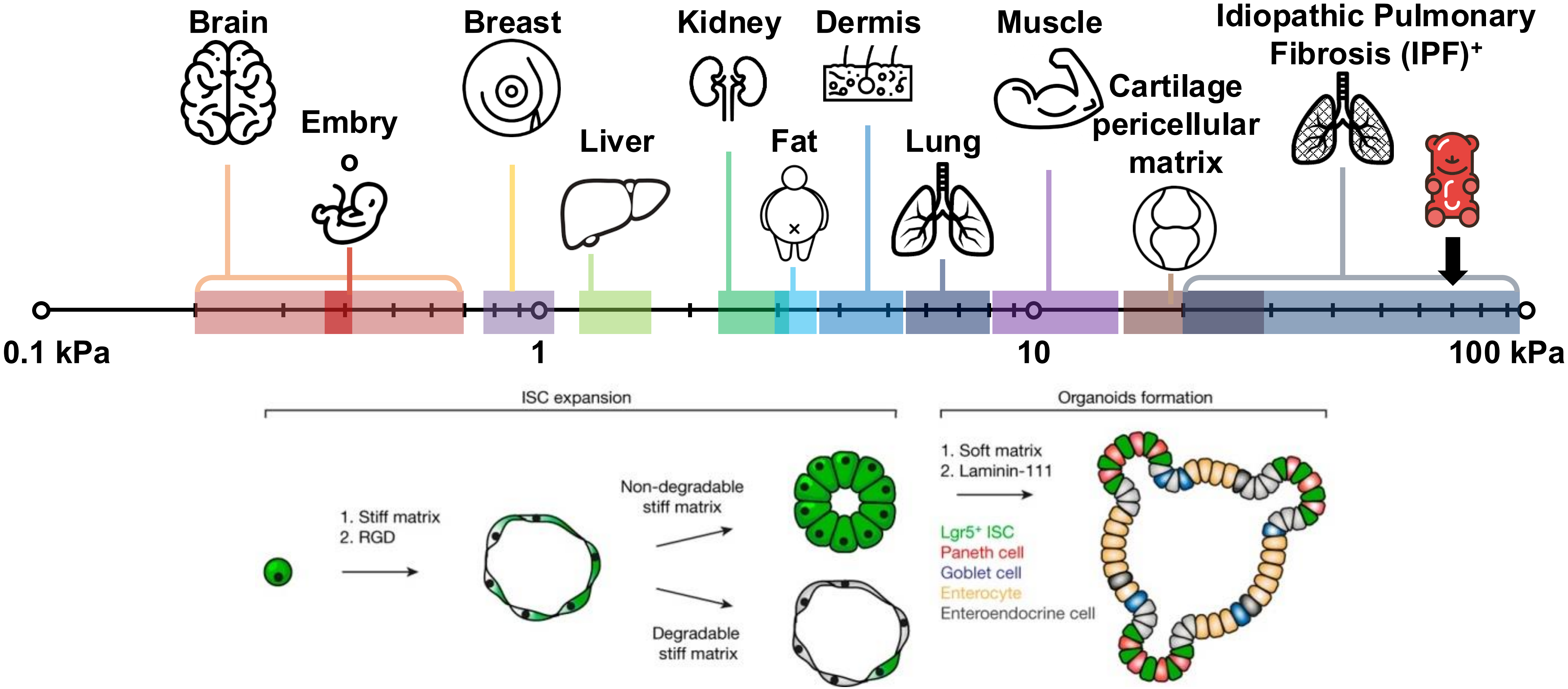
Real-time monitoring of the injury repair process at the tissue level



Modeling long-term parasite infection: Tubular organoids support the completion of the life cycle and long-term growth of *C. parvum*

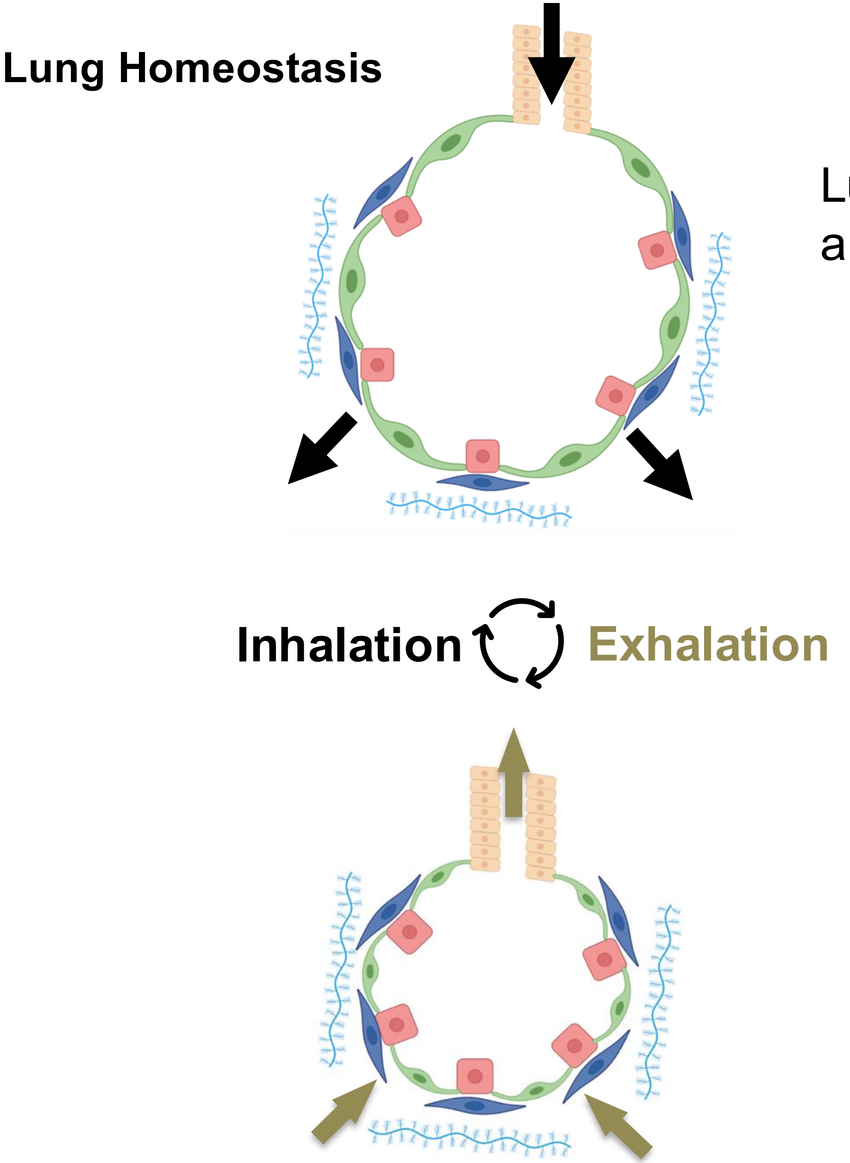
Nikolaev et al. Nature 2020

Advanced organoid models: *Tunable matrices*



N Gjorevski et al. Nature 2016

Advanced organoid models: *Tunable matrices*



Lung posses viscoelasticity: lung's respond to stress (Force/Area) in a time dependent manner

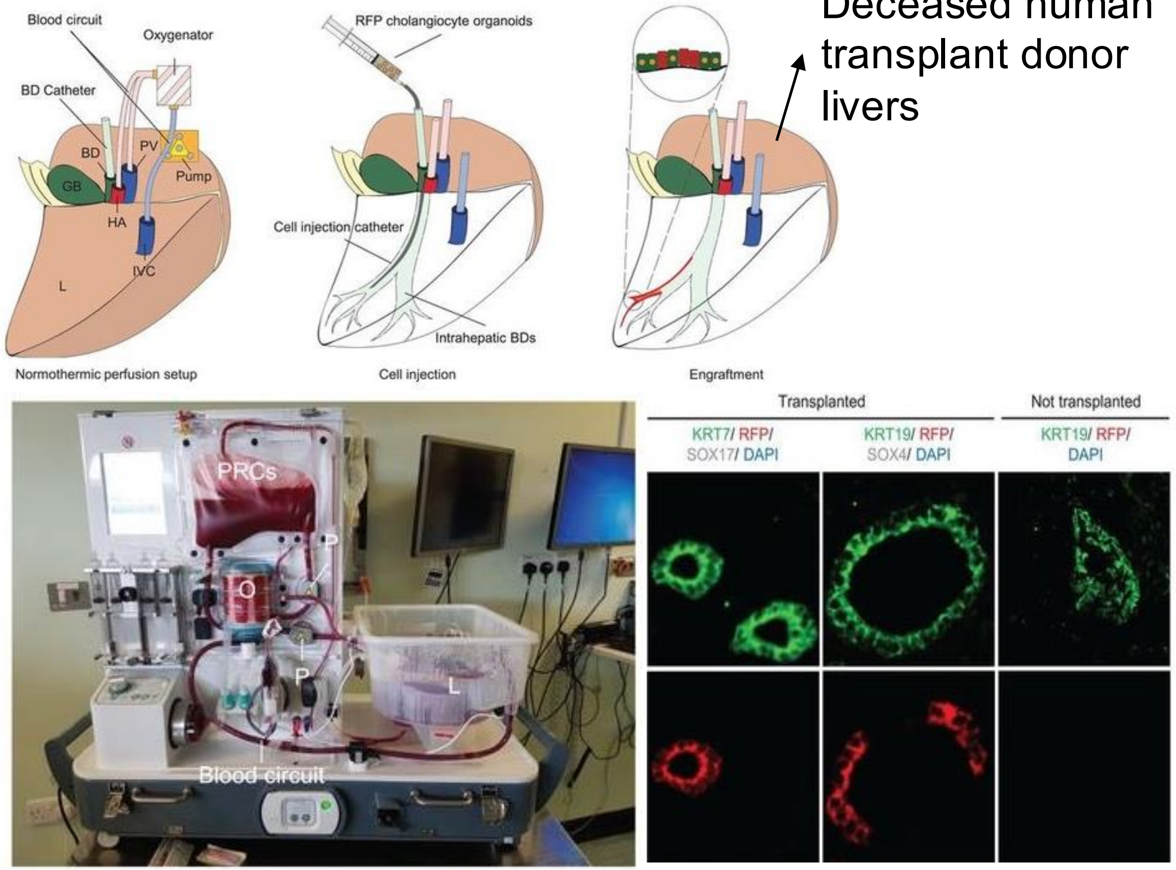
Instantenous elastic deformation

Time-dependent viscous deformation (Stress relaxation)

Building a tissue *in vitro* : Normothermic perfusion

Ex vivo human liver culture

Deceased human transplant donor livers



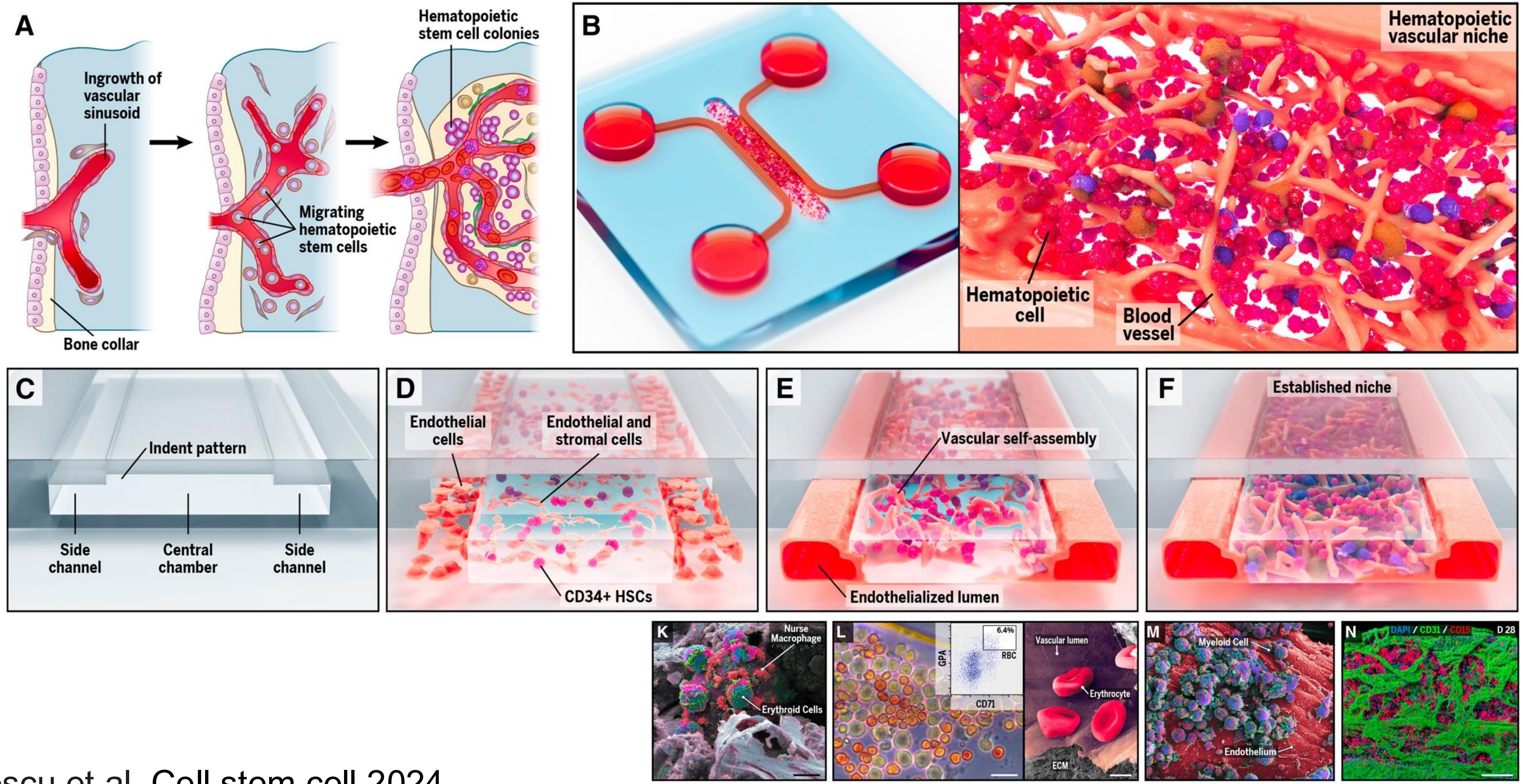
Sampaziotis et al. Science. 2021

Normothermic perfusion: cardiopulmonary bypass technology to keep organs in a physiological state by simulating body temperature and providing oxygen and nutrients.

Technical Challenges:

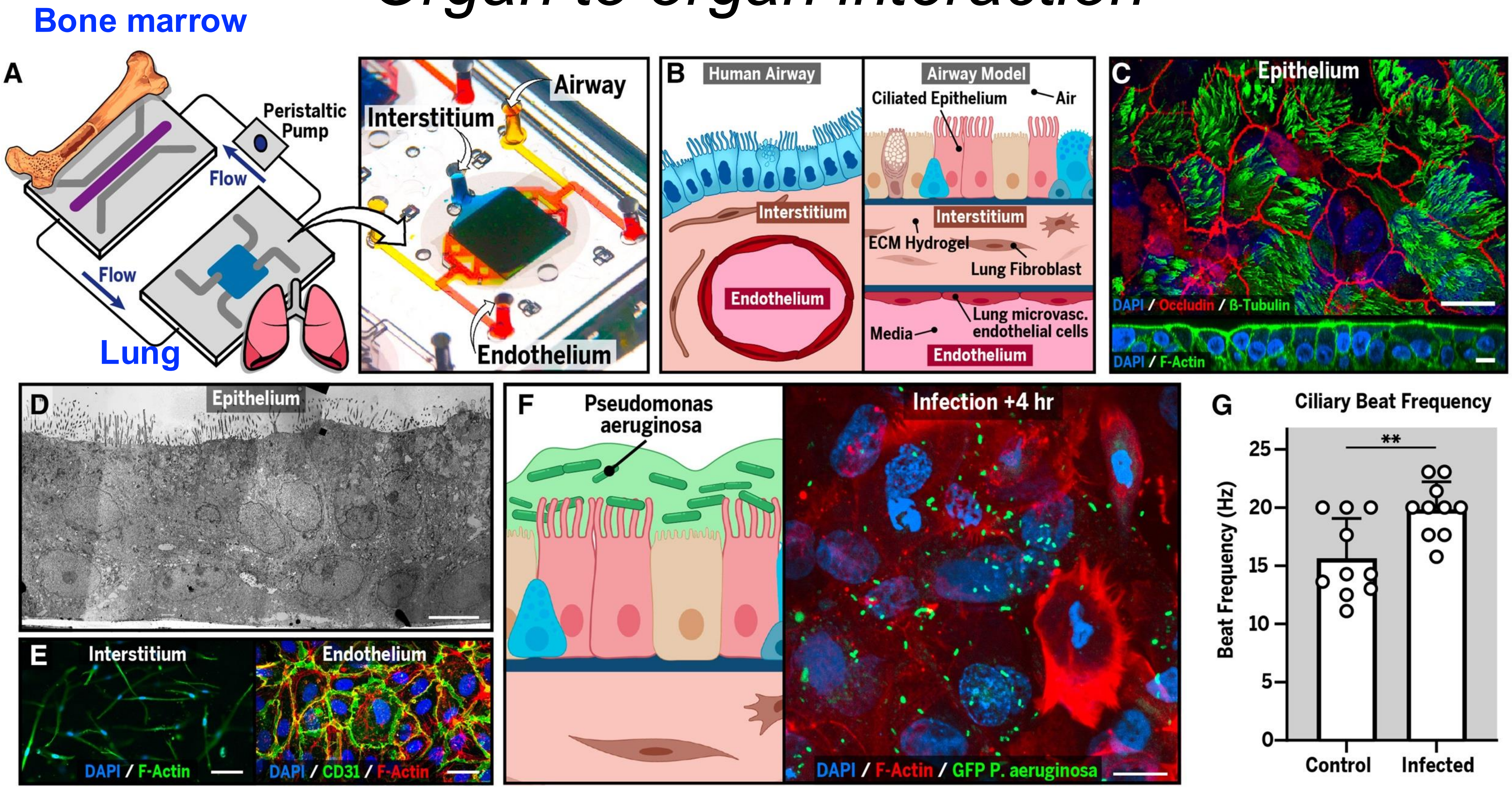
- Availability & Accessibility
- Reproducibility
- Long-term maintenance

Building a tissue *in vitro* : *Organ-on-a-chip*
Hematopoietic vascular niche



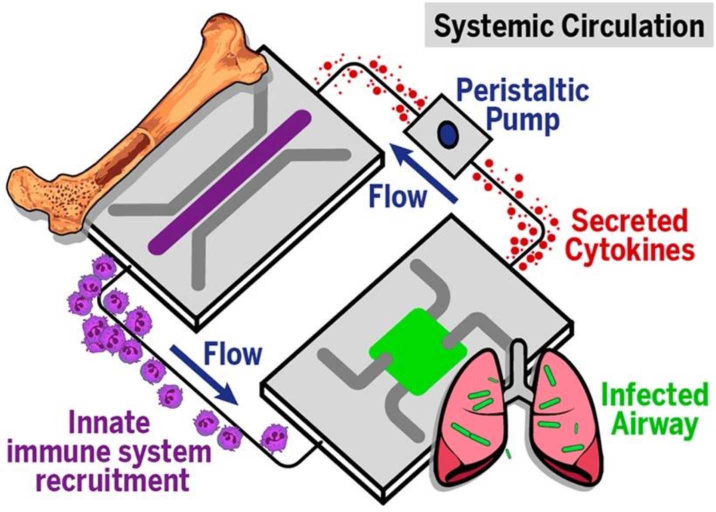
Georgescu et al. Cell stem cell 2024

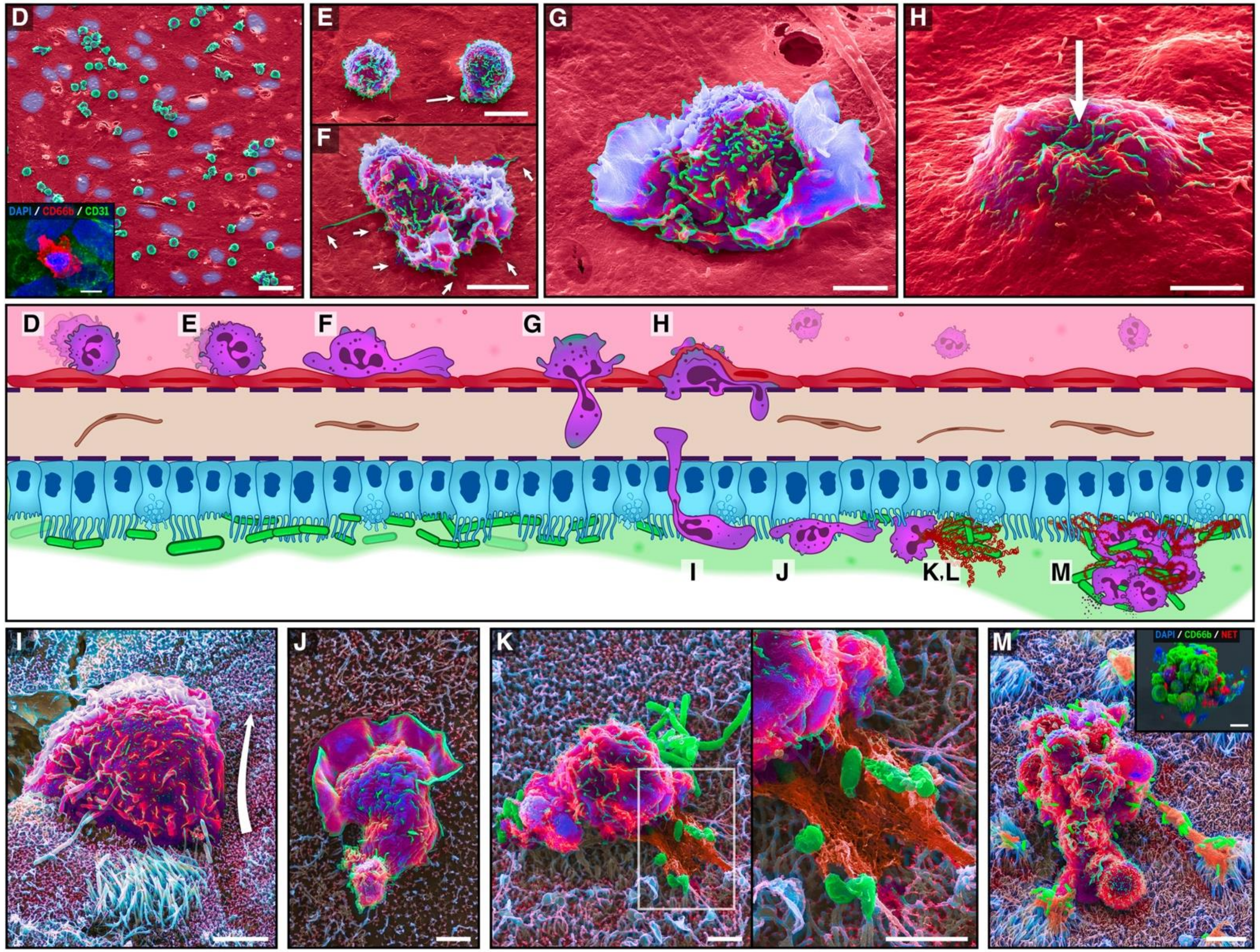
Building a tissue *in vitro* : *Organ-on-a-chip*
Organ to organ interaction



Georgescu et al. Cell stem cell 2024

Building a tissue *in vitro* : *Organ-on-a-chip*
Organ to organ interaction





Neutrophil-mediated innate immune responses to bacterial infection in the lung-bone marrow multiorgan model

Georgescu et al. Cell stem cell 2024

Building a tissue *in vitro* : Bottom to Top

- Structural, molecular, and functional features of in vivo tissues and organs
- Complexity for proper function of tissues and organs (organoids – lack cellular organization and organ-supportive tissues)
- Geometry for in vivo tissue structure and function (organoids – heterogeneous in size, shape, cellular composition)
- Functional assay for quantifiability and reproducibility (organoids – highly variable)
- Physiologically relevant scaffold/ matrices
- Systemic organ to organ interactions

Questions to be discussed….

- What are the current limitations of 3D organoid systems and how can organs-on-a chip devices help to overcome them?

- How do we balance scaling up organoids with ensuring accurate tissue representation?

- Development of cell type/organ/disease specific functional assays (quantifiable and reproducible).

- What are the challenges of applying omics readouts to scaled-up experiments with many samples, biologic/inter-individual variation, function/phenotype varies over time points, etc. and how do we overcome those?

Discussion Papers:
Organ-specific vascularized organoid engineering

Abilez et al. Gastruloids enable modeling of the earliest stages of *human cardiac and hepatic vascularization*. **Science**. 2025 Jun 5;388(6751):eadu9375. PMID: 40472086.

Miao et al. Co-development of mesoderm and endoderm enables *organotypic vascularization in lung and gut organoids*. **Cell**. 2025 Aug 7;188(16):4295-4313.e27. PMID: 40592324.

Limitation of this study?

Questions: leej49@mkscc.org