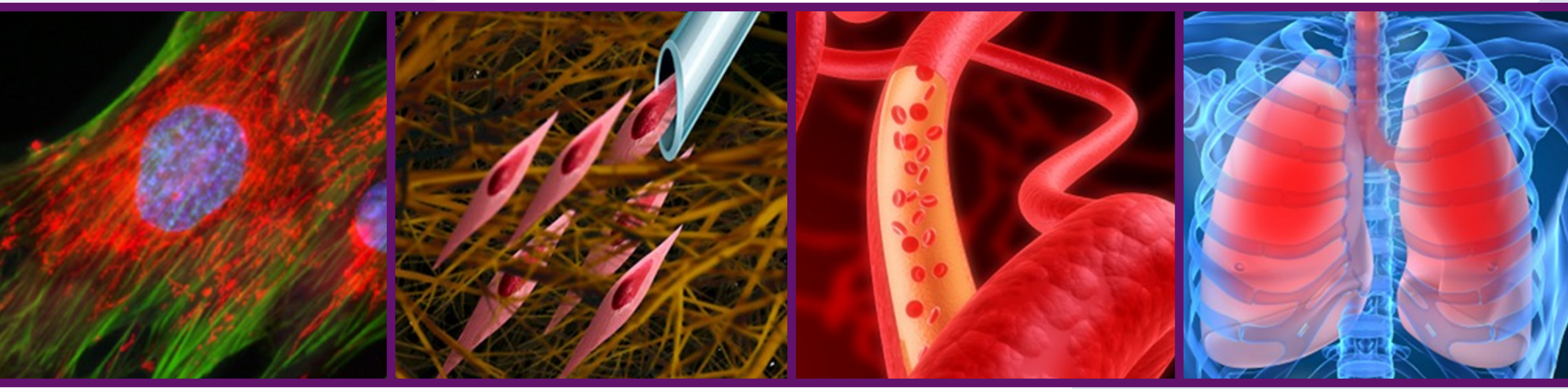


# Recent Progress in Whole Lung Engineering


Laura E Niklason MD, PhD

Founder and CEO
Humacyte Incorporated

Adjunct Professor
Professor of Anesthesia & Biomedical Engineering
Yale University

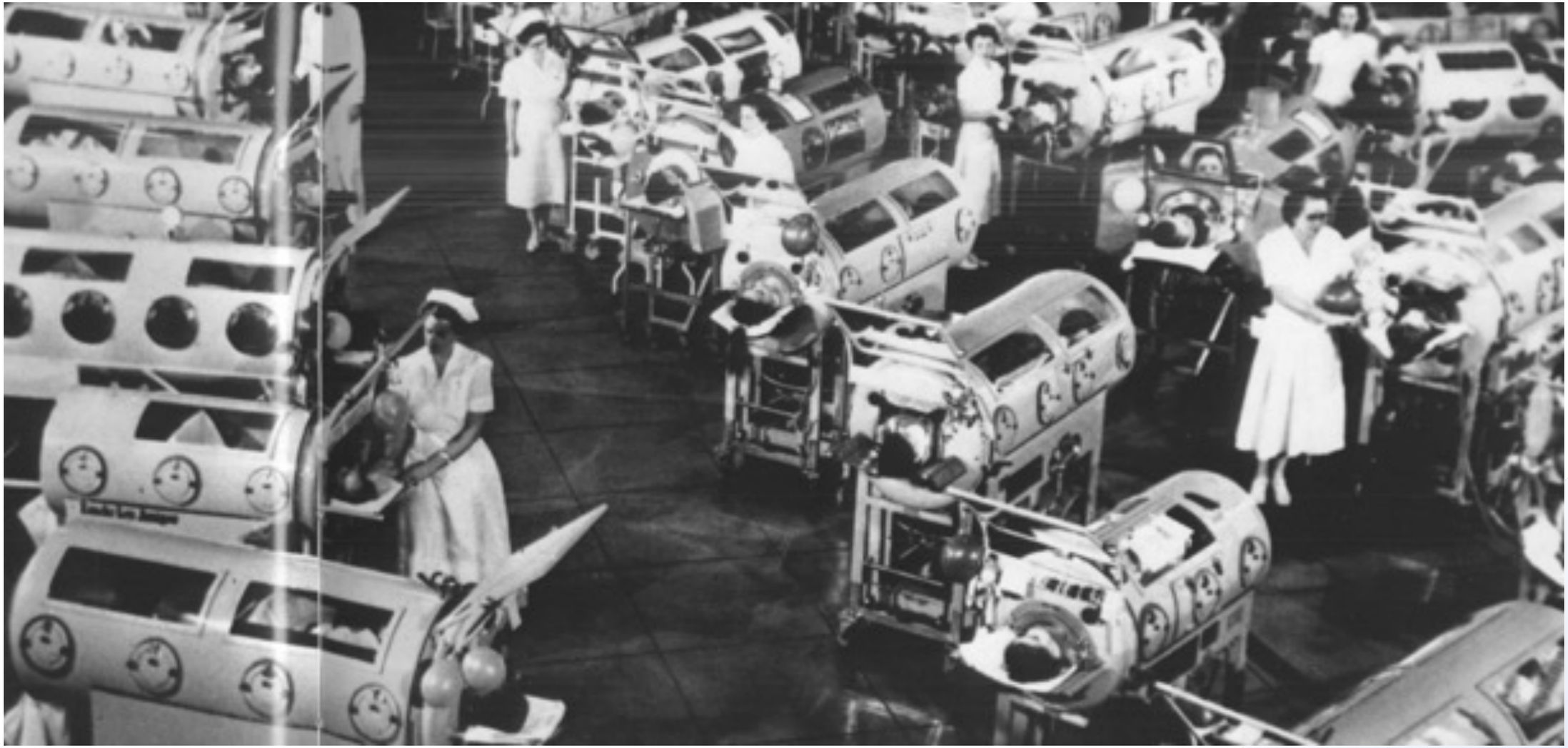
## CONFLICTS OF INTEREST & DISCLOSURES

Niklason is the CEO, Founder and a shareholder in Humacyte, Inc.

Nothing in this presentation should be construed as a claim regarding a medical product.

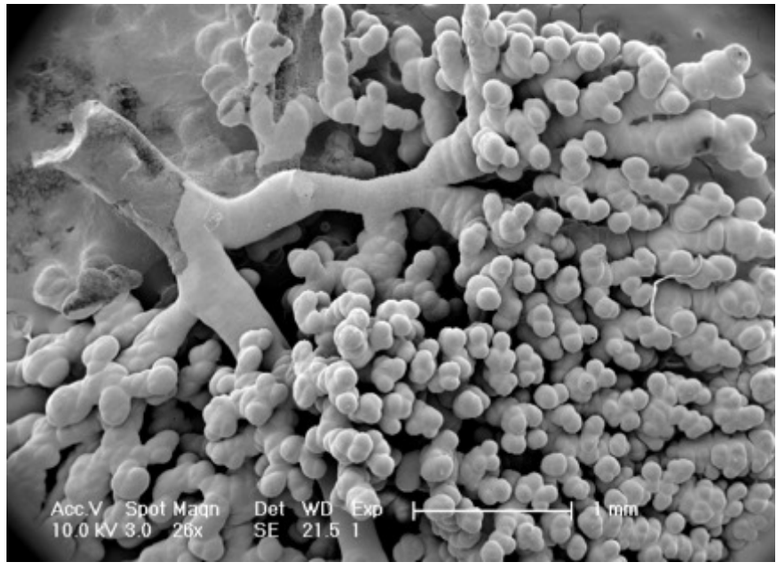
All of the information in this presentation relates solely to pre-clinical studies of engineered lung tissues.

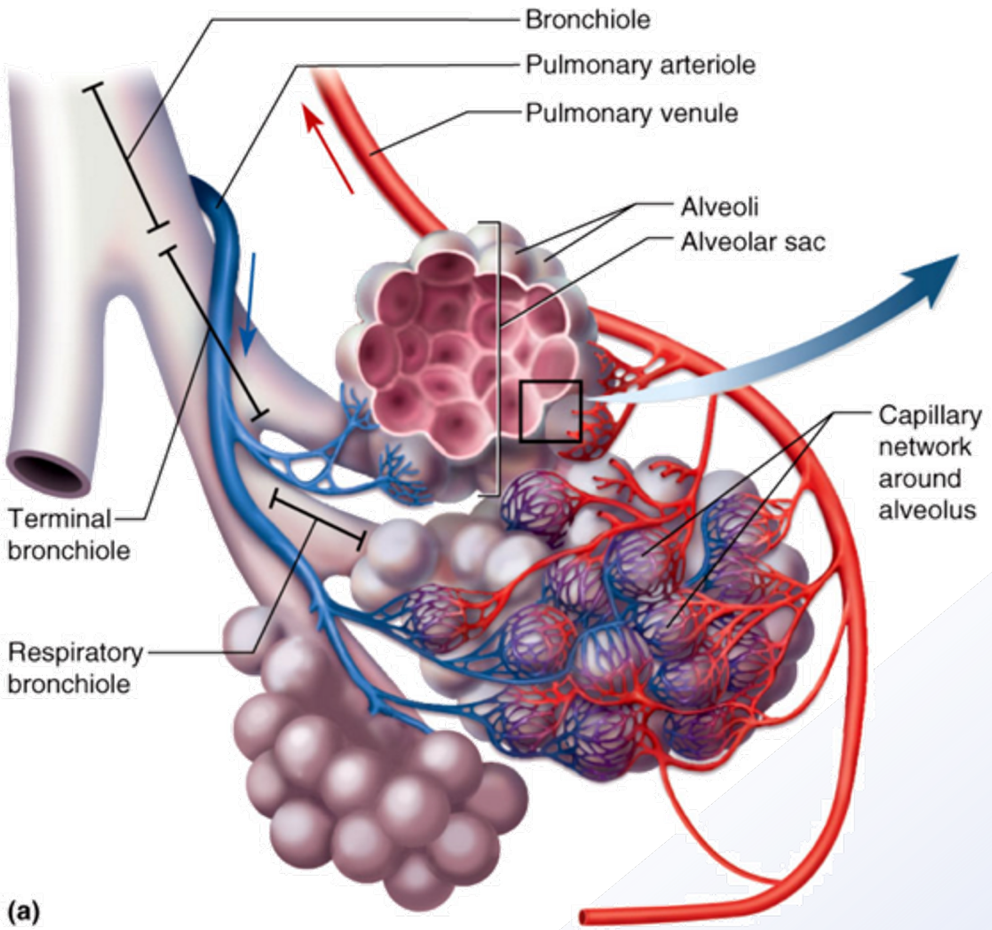
# FIRST ATTEMPT AT HUMAN LUNG “REPLACEMENT” – THE POLIO EPIDEMIC :



# DESIGN CRITERIA FOR ENGINEERED LUNG

- Adequate surface area for $O_2/CO_2$ exchange – acellular matrix.
- Maintain barrier function between blood and air – basement membrane, ATI and EC junctions
- Proper mechanics – not emphysematous or fibrotic
- Cellular components should be autologous, but hundreds of billions of cells – blood-type matched?



# HOW TO REPOPULATE THE LUNG MATRIX? MANY CELL TYPES…

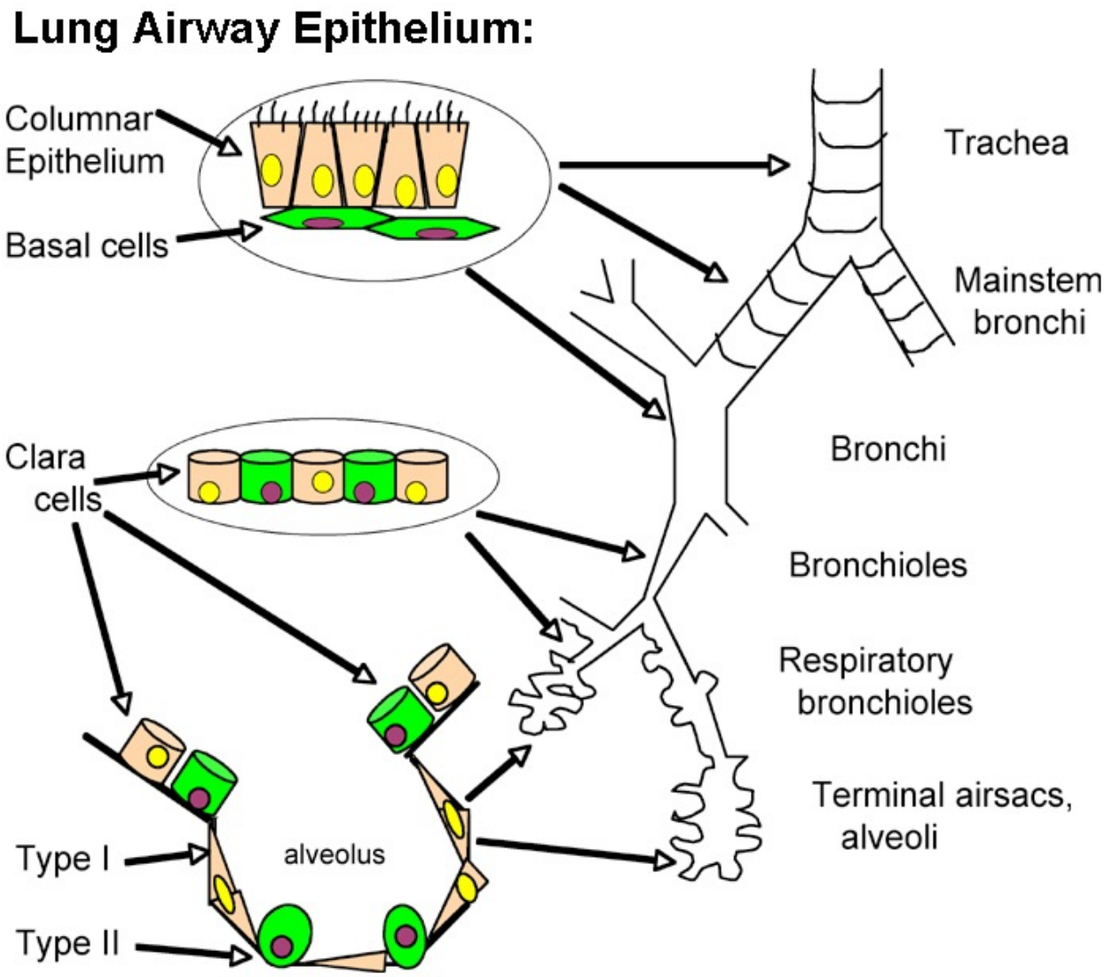
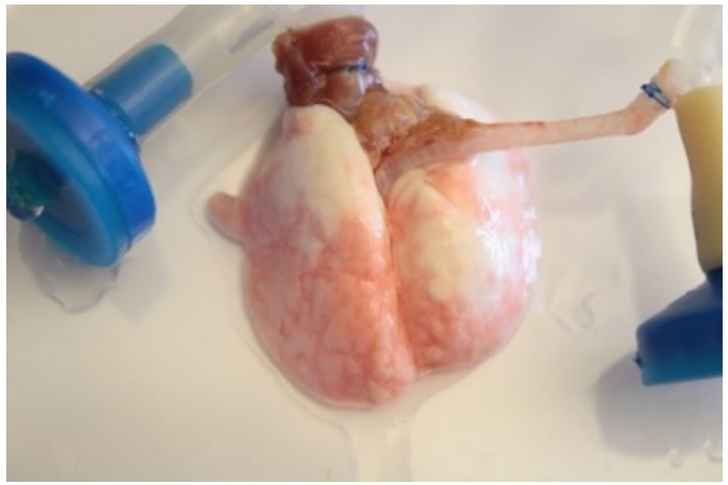


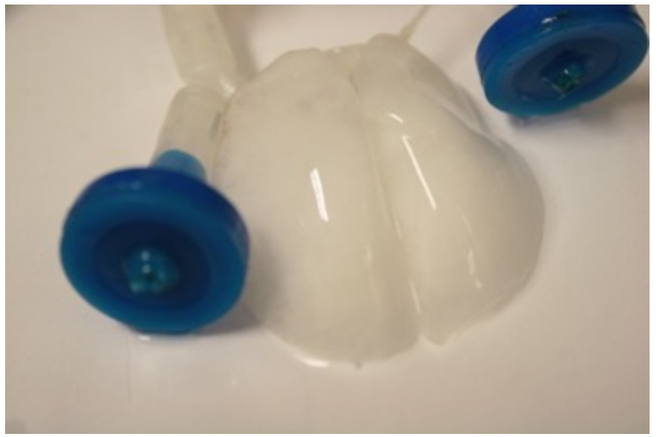

**Figure 1: Epithelial cells of the lung. Differentiated epithelial cells are orange, stem cells for each compartment are green.**

# PARADIGM FOR WHOLE LUNG REGENERATION

Donor Lung



Detergent Perfusion



Endothelium
Epithelium

Cell Seeding

Patient in Need

Mesenchyme
Immune?

Transplant

# CONSIDERATIONS OF SCALE FOR FUNCTIONAL LUNG ENGINEERING

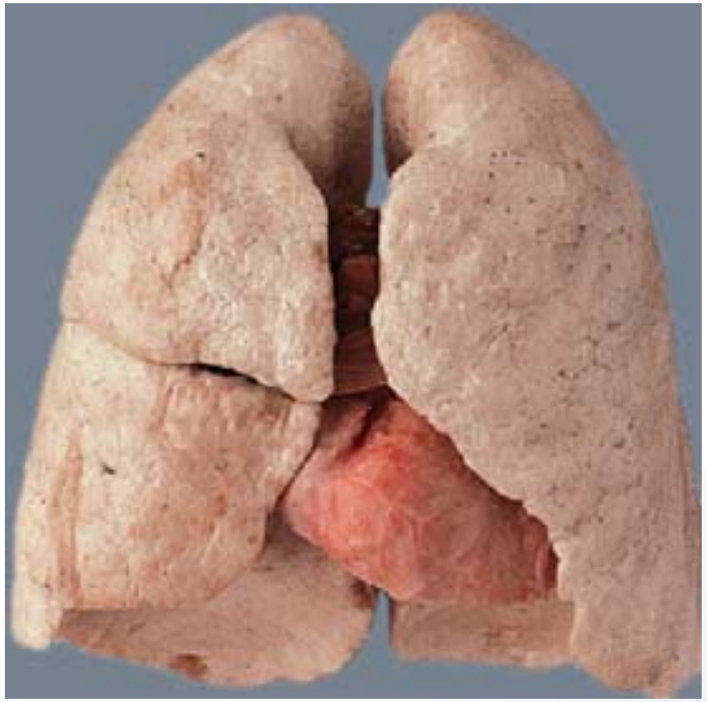
Average human lungs absorb 22 liters (~ 5 gallons) of pure oxygen ***per hour*** into the body.

This is accomplished via a collection of 200 million alveoli, having a surface area of a tennis court.

Adult human lungs have 23 generations of airway branching, all contained within a 5-liter volume.

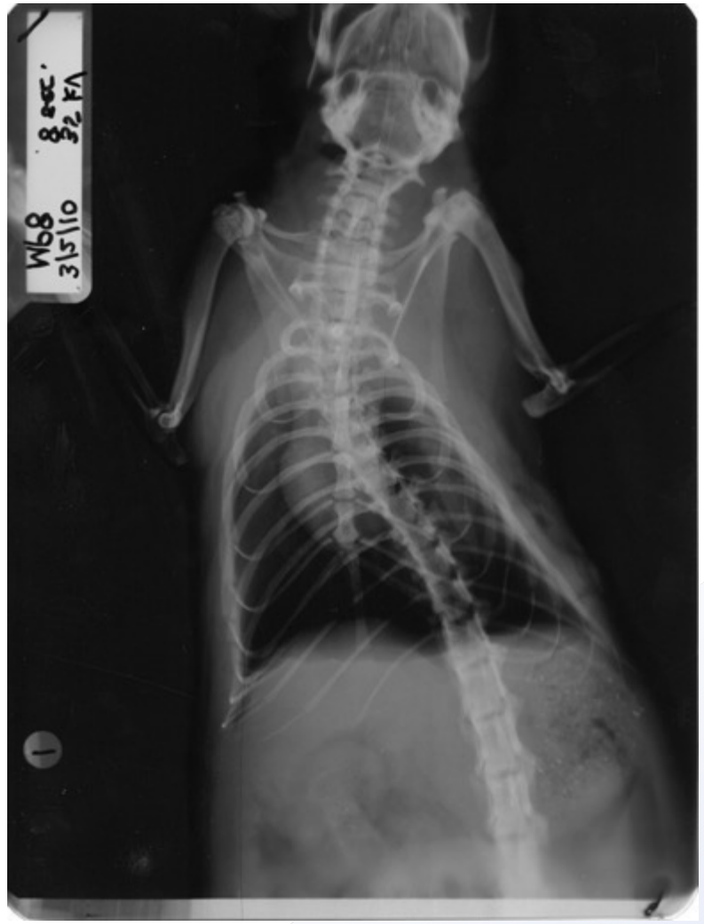
If an organoid is 200-microns, and if we can grow 100 organoids in a small dish, this means we would need ***2,000,000 dishes*** to provide the surface area of human lung.

Successful organ engineering requires both structural “engineering”, and also detailed understanding of cellular behaviors, especially at the alveolar level.



# ENGINEERED LUNG IMPLANTATION

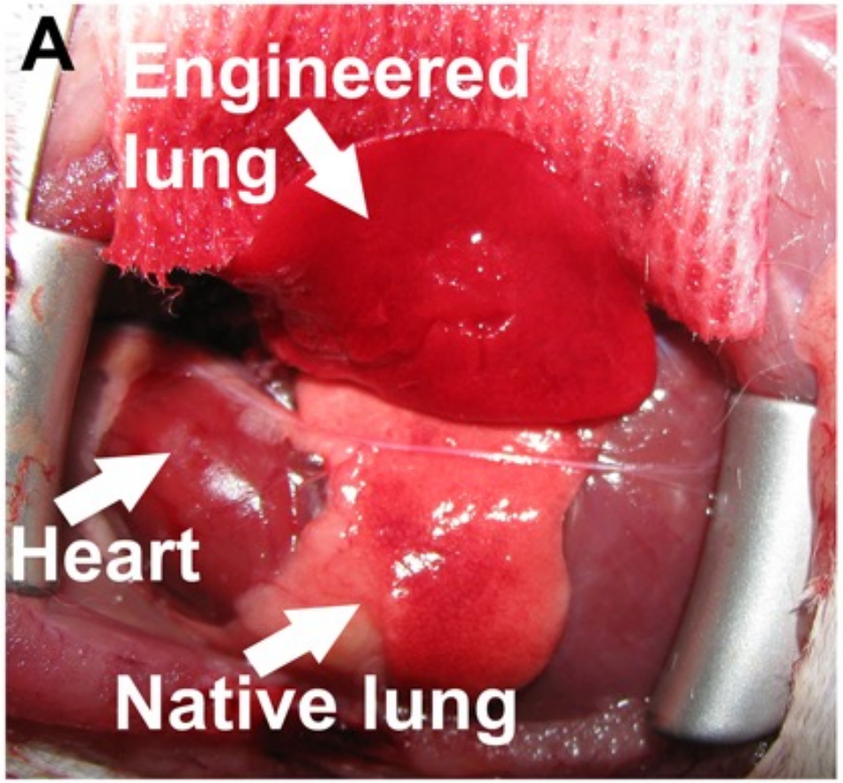
- Rat Neonatal epithelium
- Rat lung endothelium
- Cultured for 3-4 days
- Implanted as left lung orthotopic transplants

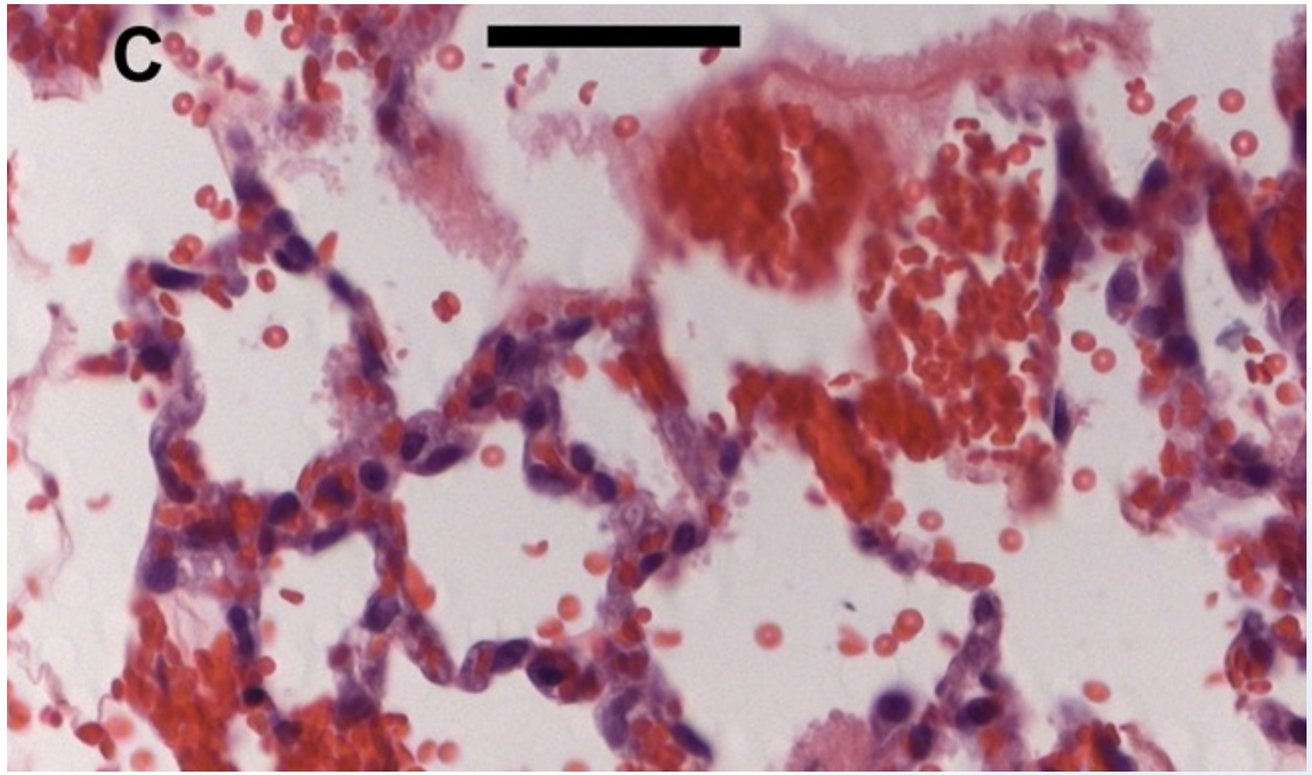


| Sample Location: | pH | $pO_2$ (mm Hg) | $O_2$ Sat (%) | $pCO_2$ (mm Hg) |
|---|---|---|---|---|
| Pulmonary Artery | 7.30 ± 0.06 | 27 ± 7 | 44 ± 20 | 41 ± 13 |
| Right Pulmonary Vein | 7.53 ± 0.08 | 634 ± 69 | 100 ± 0 | 20 ± 1 |
| Left (implant) Pulmonary Vein | 7.68 ± 0.28 | 283 ± 48 | 100 ± 0 | 11 ± 5 |
| Mixed Pulmonary Veins | 7.58 ± 0.08 | 495 ± 174 | 100 ± 0 | 18 ± 3 |

# INTRAVASCULAR CLOTTING

Intravascular clotting limited gas exchange.
Clotting after ~ 2 hours, despite heparin.
Alveolar barrier function incomplete.




Peterson et al, *Science 2010*

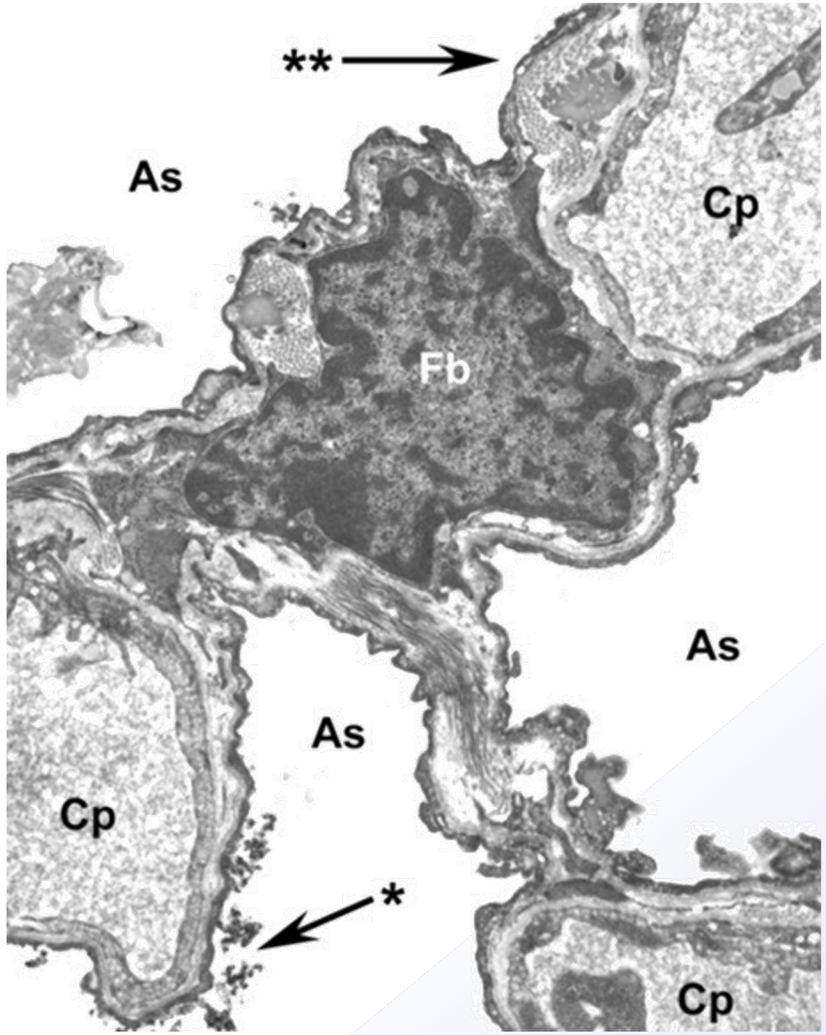
# RE-FOCUSED DESIGN CRITERIA – HOW TO ENGINEER THE ALVEOLUS

Barrier-forming and non-thrombotic endothelial lining of alveolar micro-vessels.

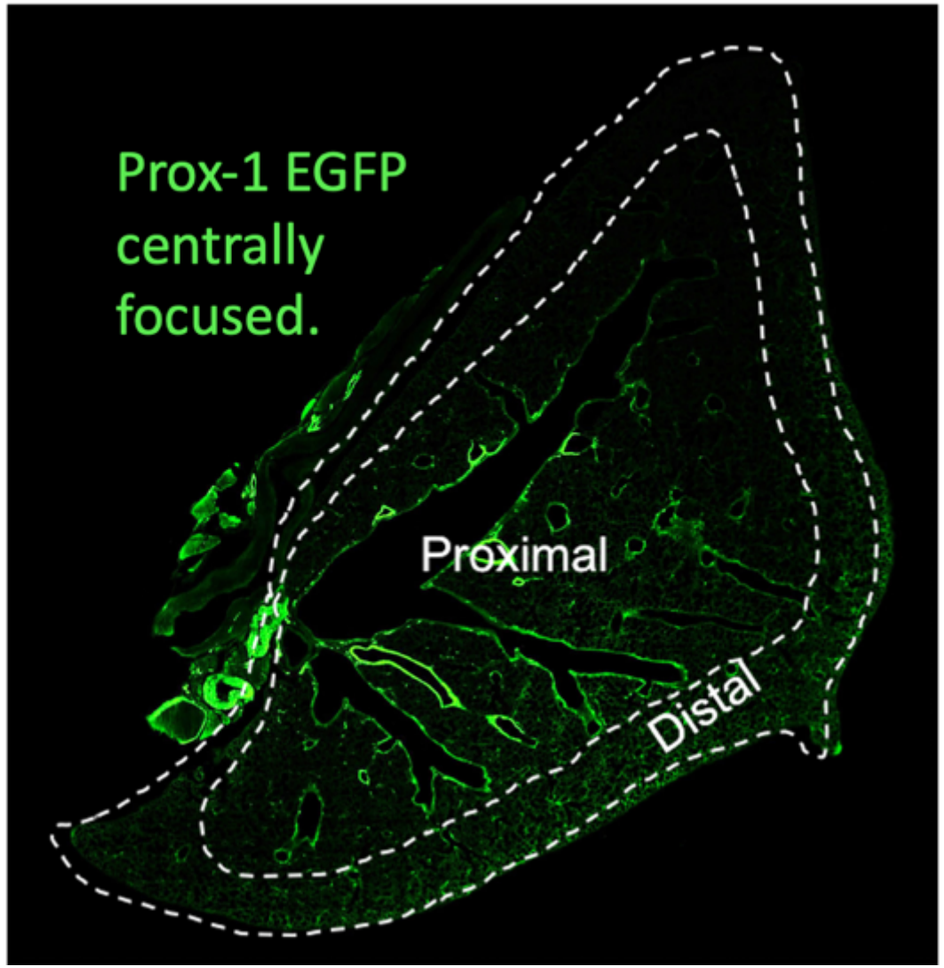
Alveolar epithelium providing surfactant (type II) and barrier function (type I) cells.

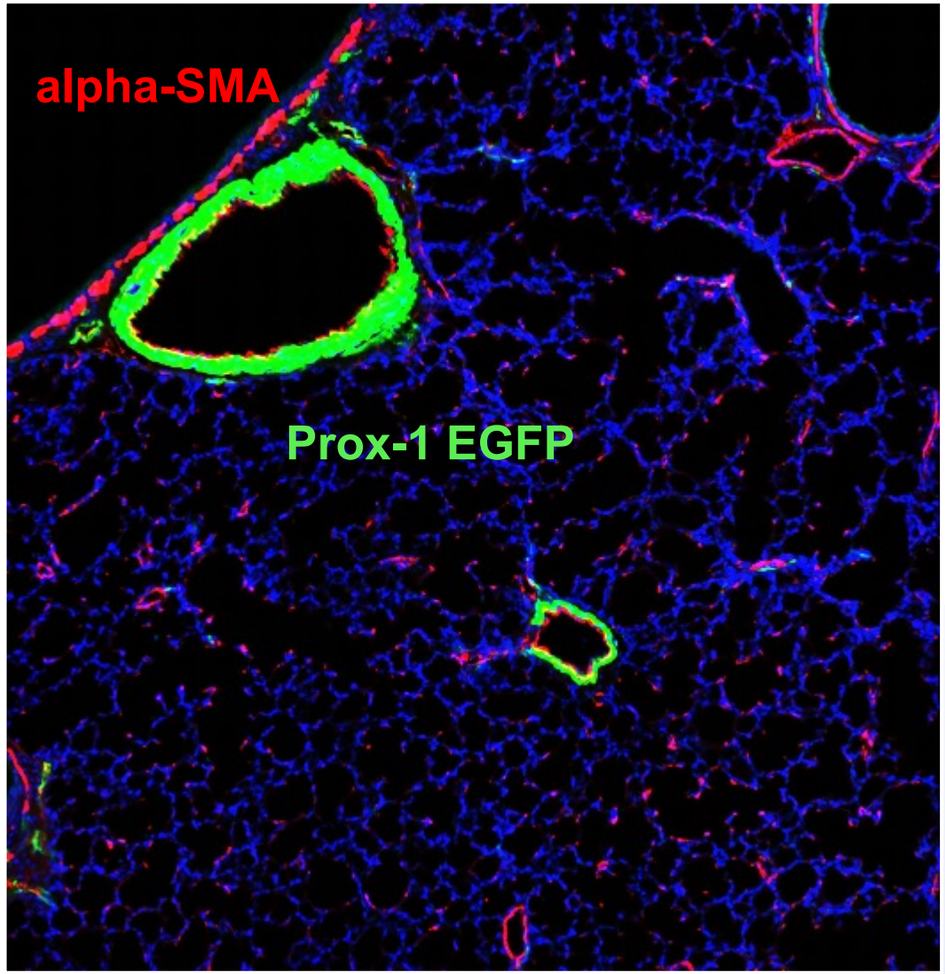
Mesenchyme to support cellular phenotype and matrix maintenance.

Cellular homeostasis of the alveolar niche, without active remodeling, fibrosis, or tissue destruction.

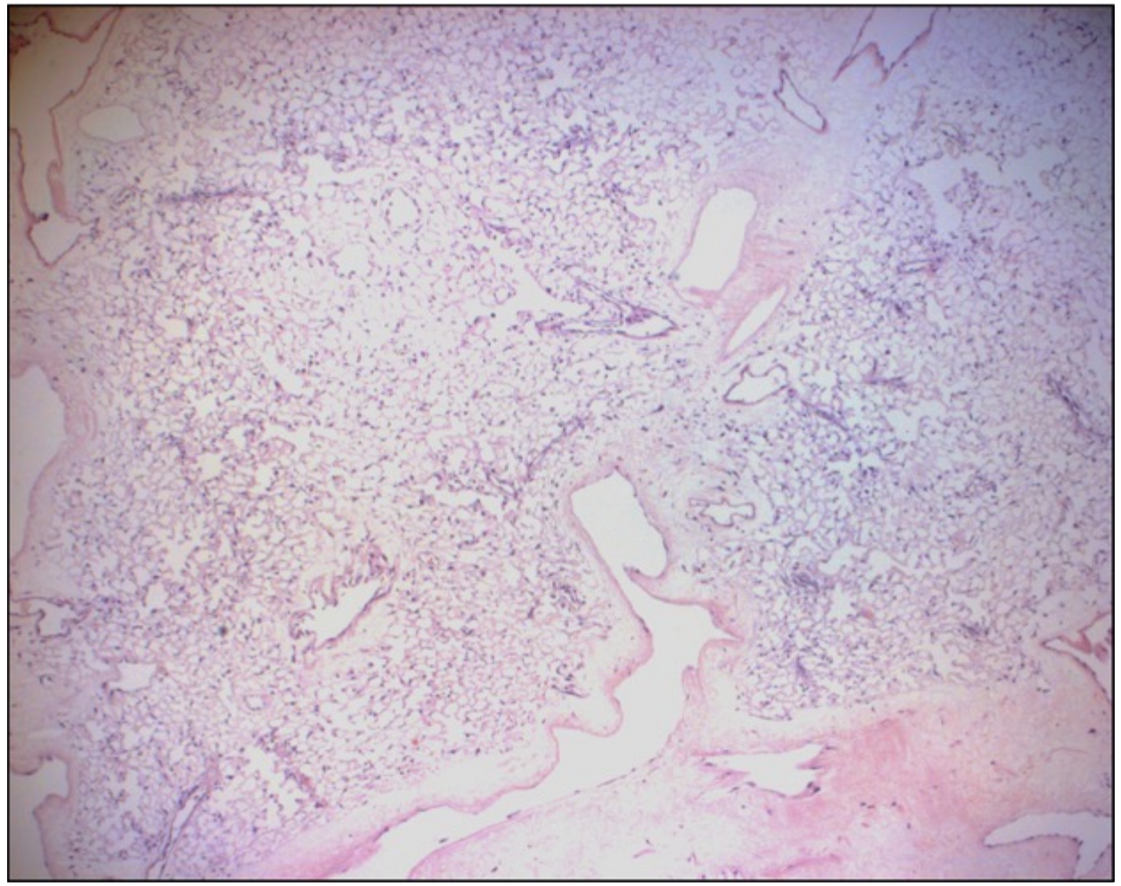


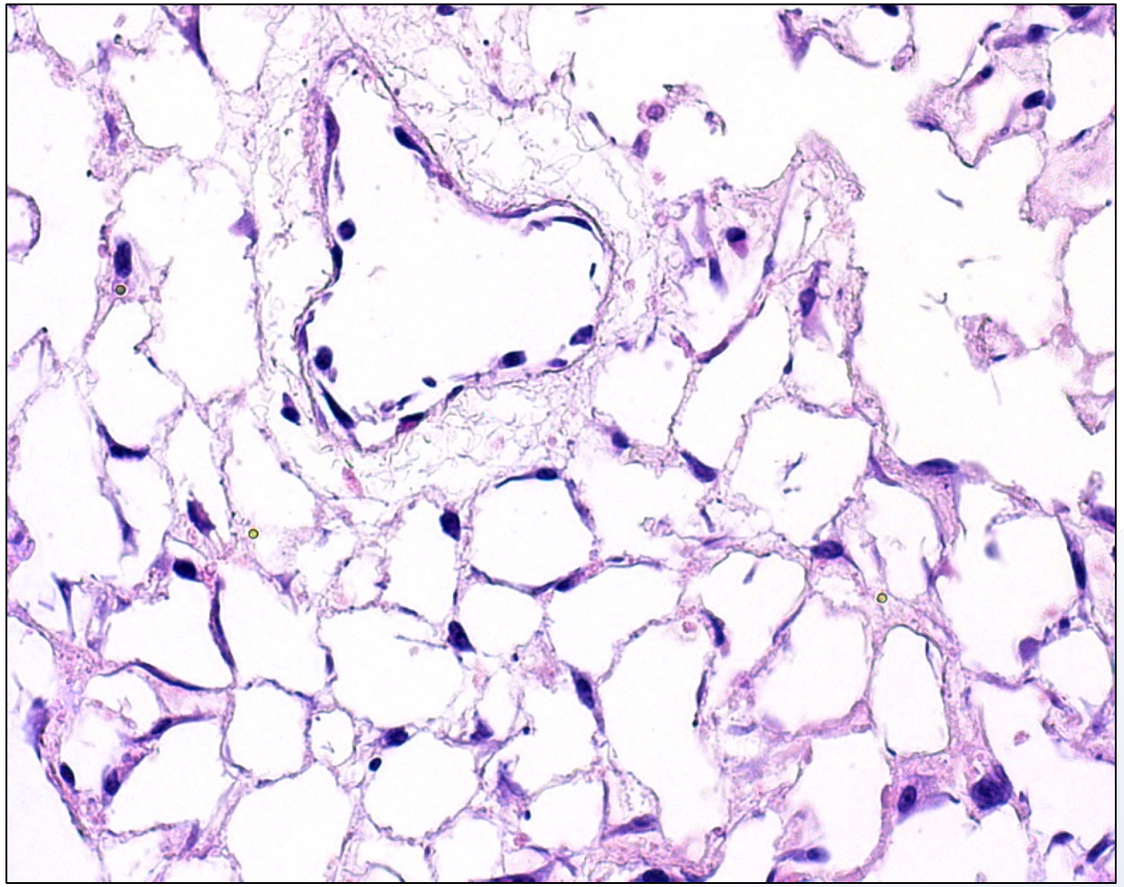
# THE VASCULAR SIDE: HOW TO GET NON-LYMPHATIC ENDOTHELIAL CELLS EGFP-LABELED LYMPHATIC ENDOTHELIUM IS LOCATED IN THE CENTRAL LUNG



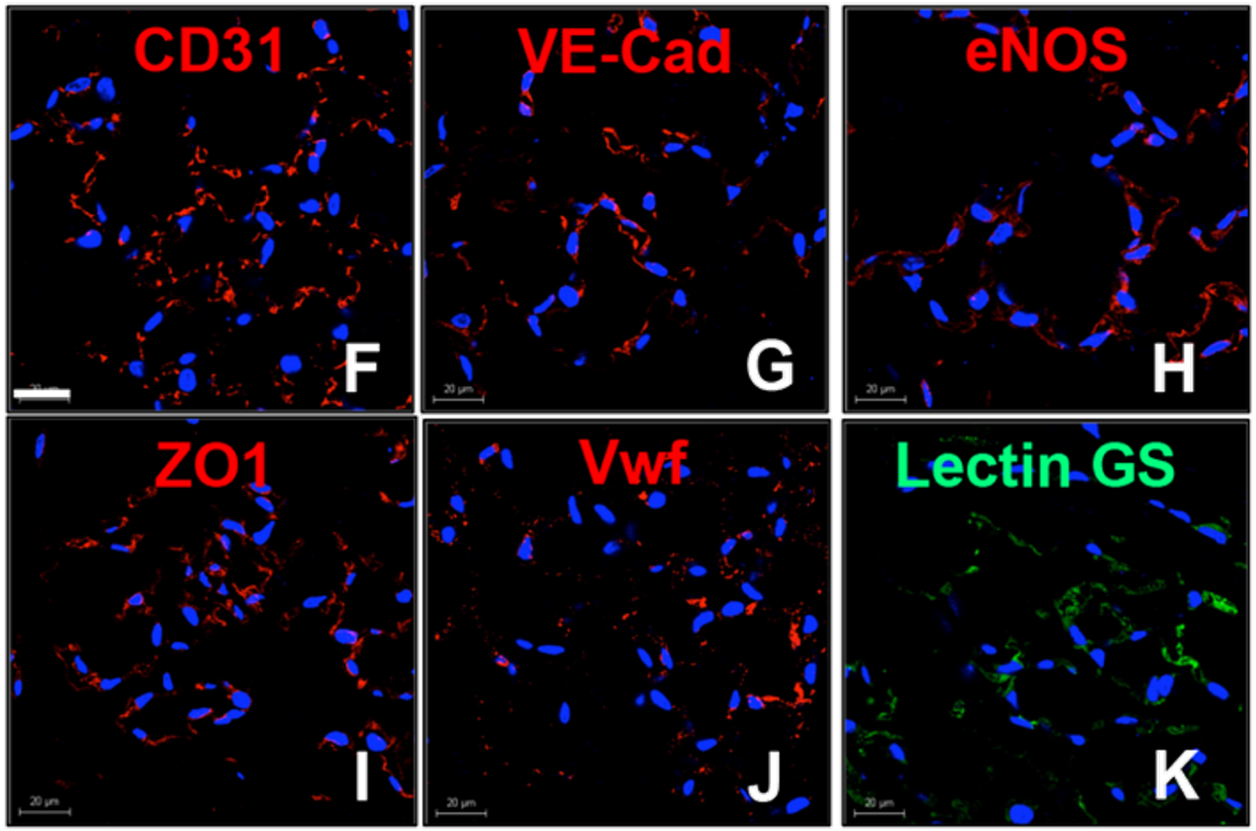



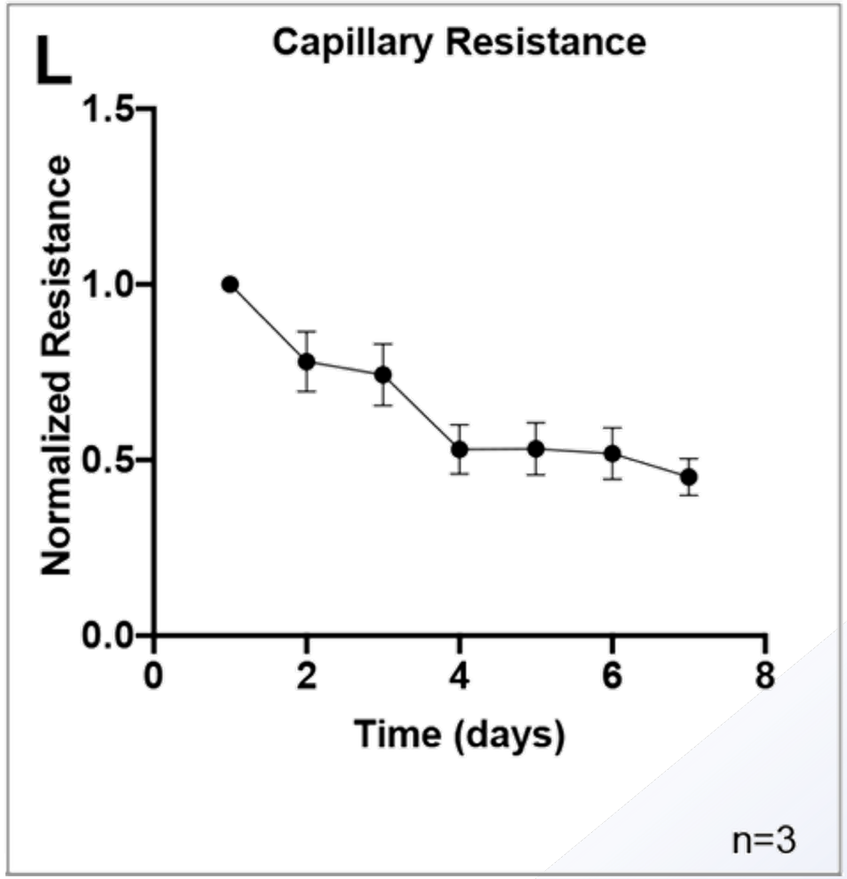
# LYMPH$^{low}$ PULMONARY MICROVASCULAR ENDOTHELIAL CELLS SEEDED ONTO LUNG SCAFFOLDS



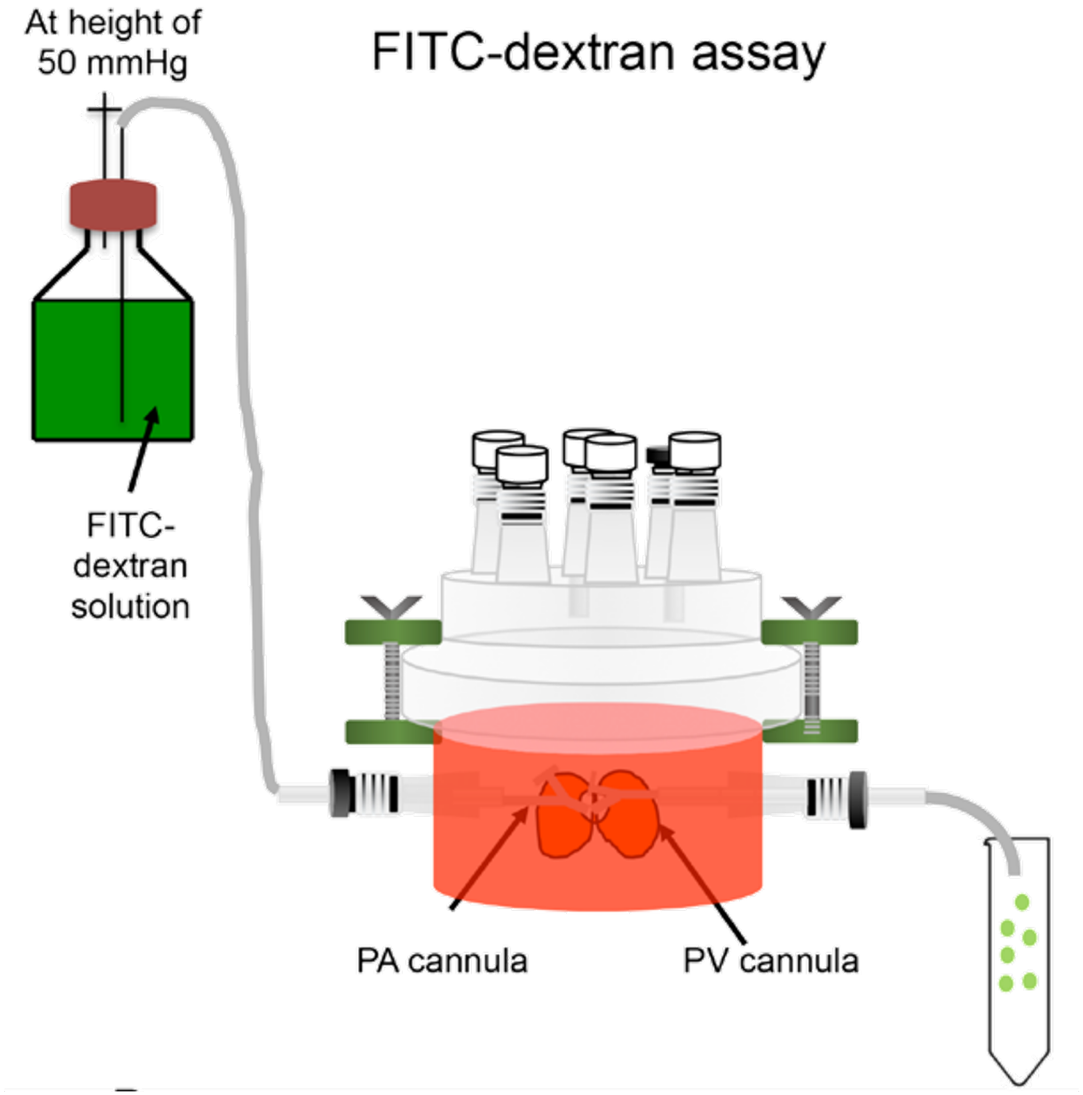


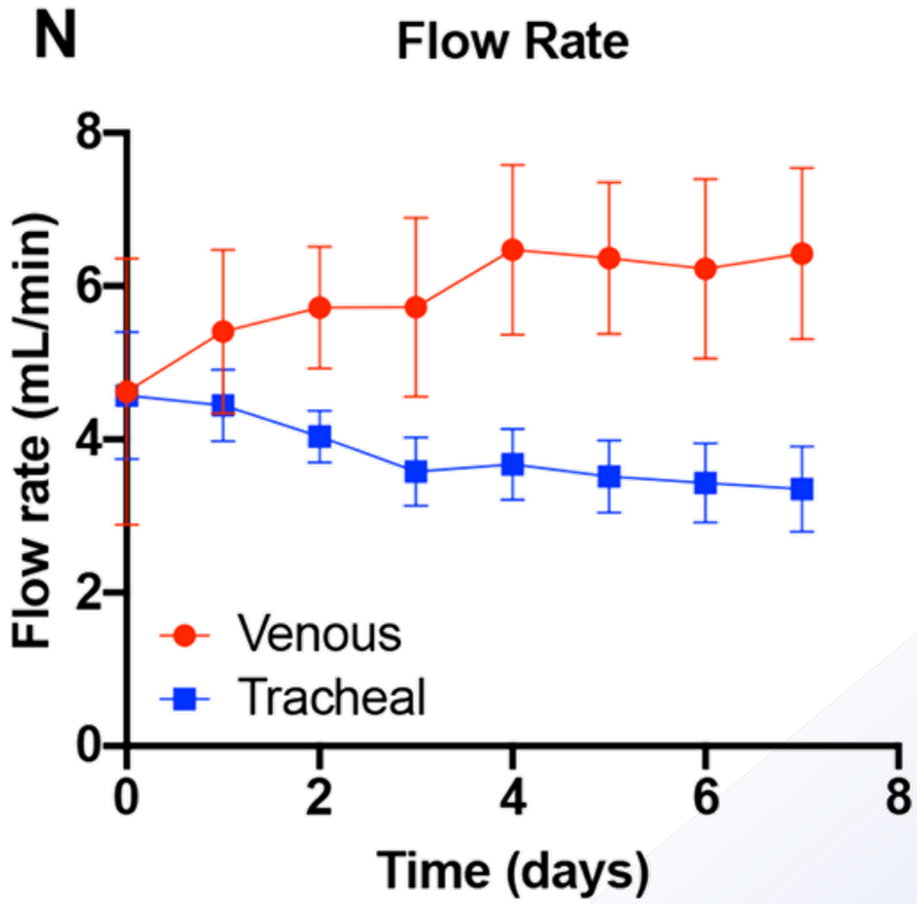
# ENDOTHELIAL CELLS EXPRESS TYPICAL MARKERS, AND REDUCE CAPILLARY RESISTANCE OVER TIME, IMPLYING COVERAGE OF THE CHANNELS



# ENDOTHELIAL CELLS ENHANCE BARRIER FUNCTION – INCREASE IN VENOUS OUTFLOW – IMPLYING SOME FUNCTIONALITY FOR ALVEOLAR PROTECTION



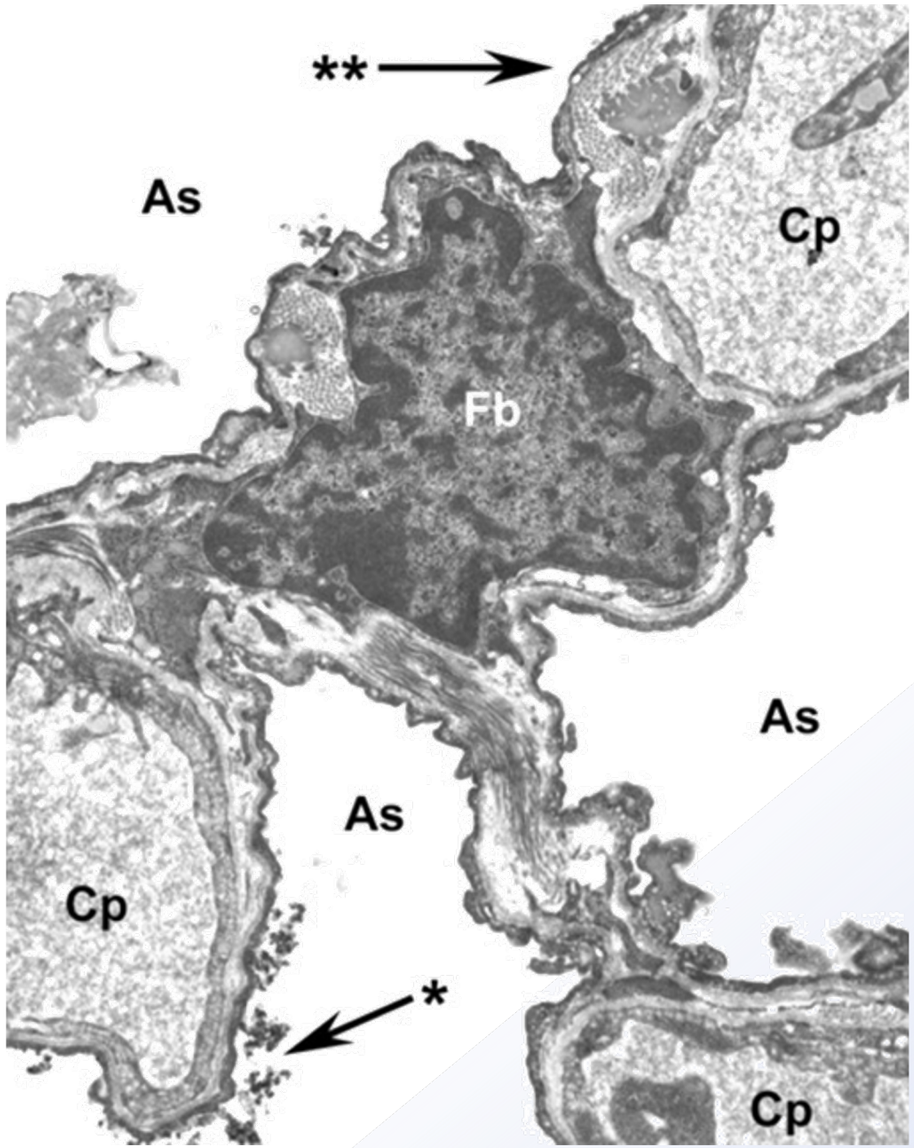




n=3

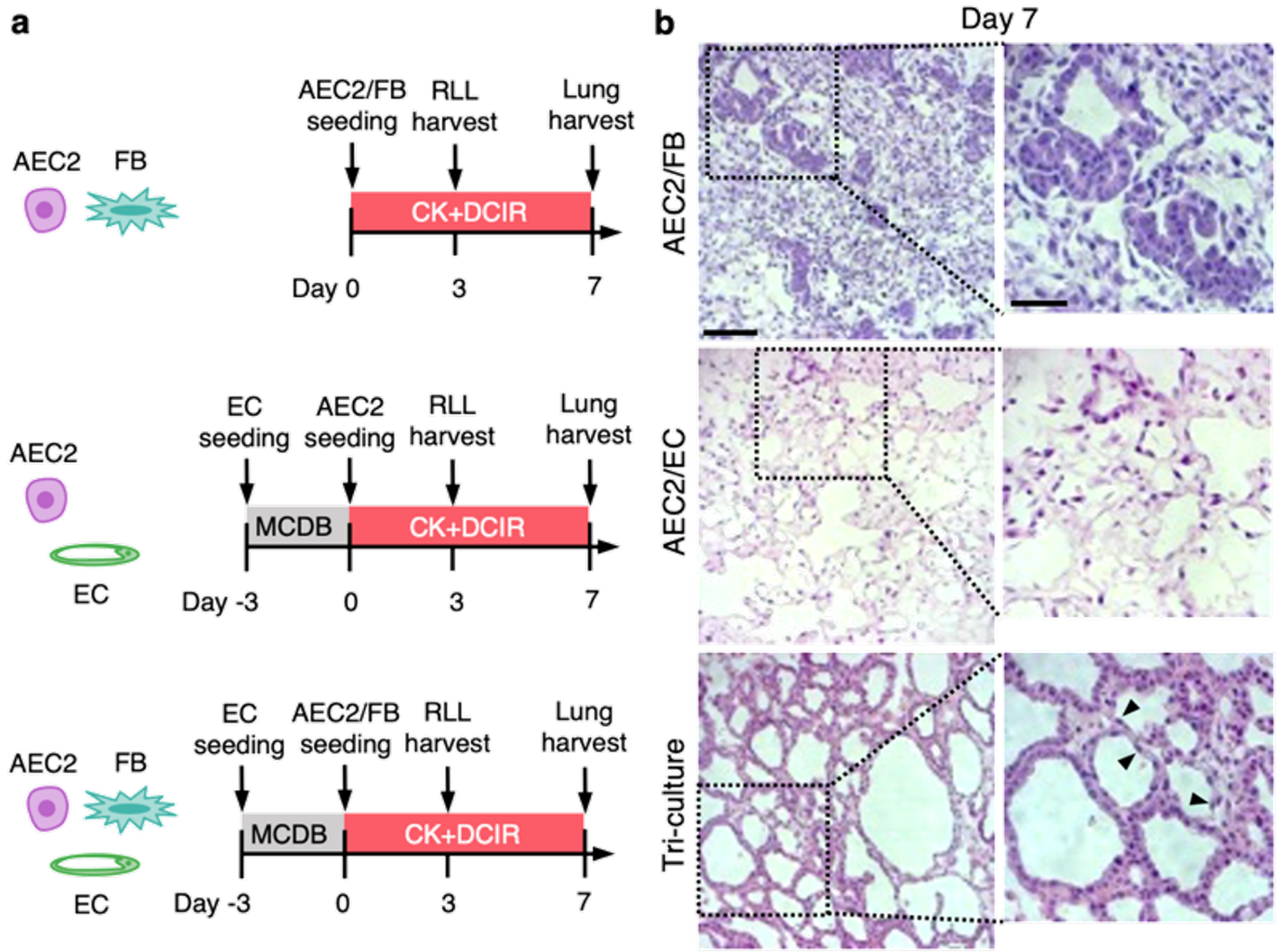
## HOWEVER, ENDOTHELIUM ALONE CANNOT RECONSTITUTE LUNG BARRIER FUNCTION

Alveolar type I epithelium provides greater barrier to solutes and does the vascular endothelium under normal conditions.

Furthermore, alveolar epithelium has extensive cross-talk with vascular endothelium, meaning an absence of epithelium in the lung scaffold leads to abnormal EC phenotype and function.



# RECONSTRUCTING THE EPITHELIUM OF THE ALVEOLUS – TYPE II CELLS: HOW TO SUPPORT REPOPULATION AND DIFFERENTIATION

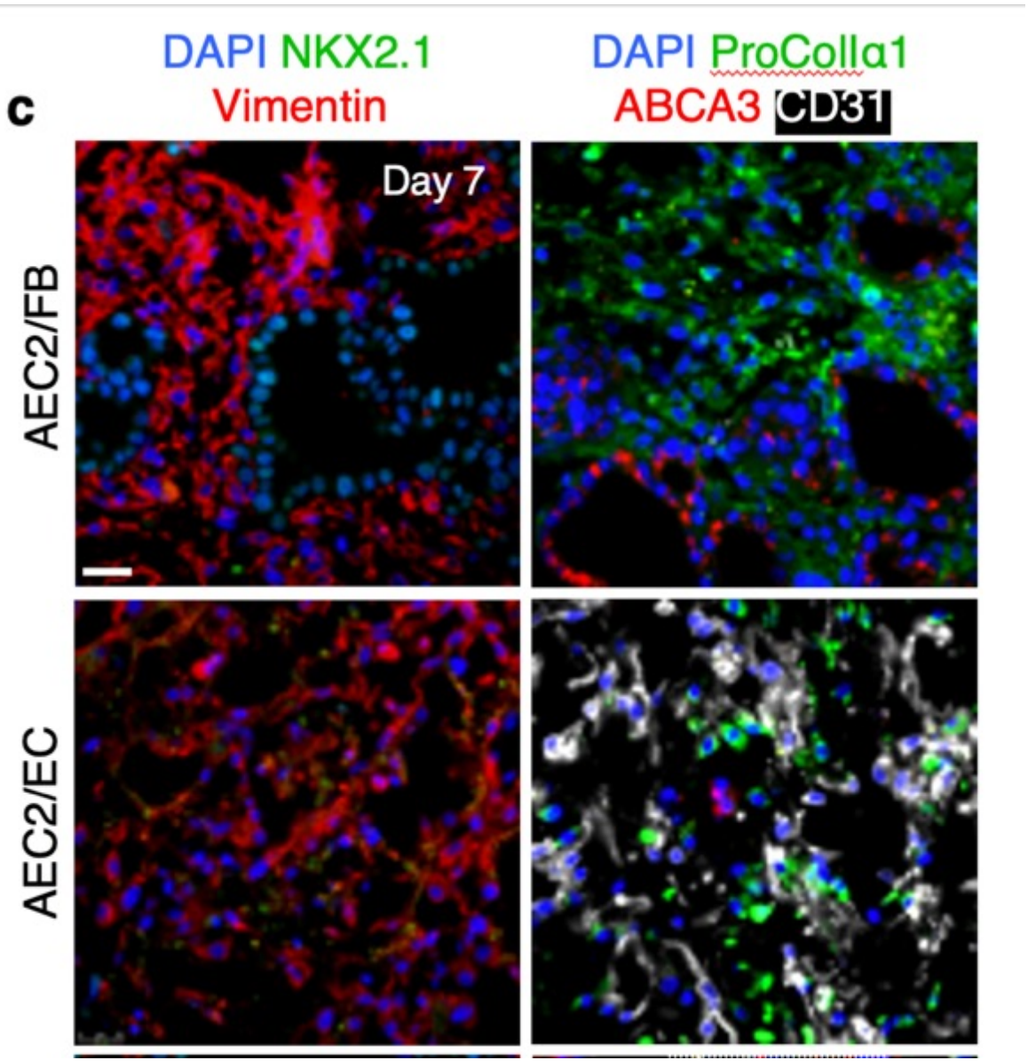


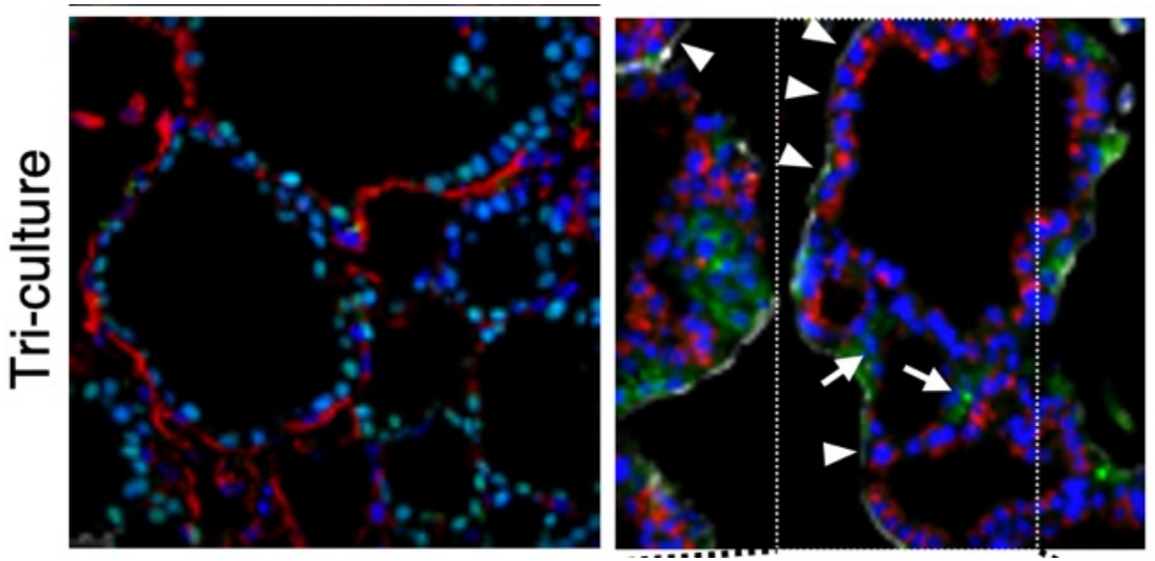

AEC2 (type II epithelium) with fibroblasts is disorganized.

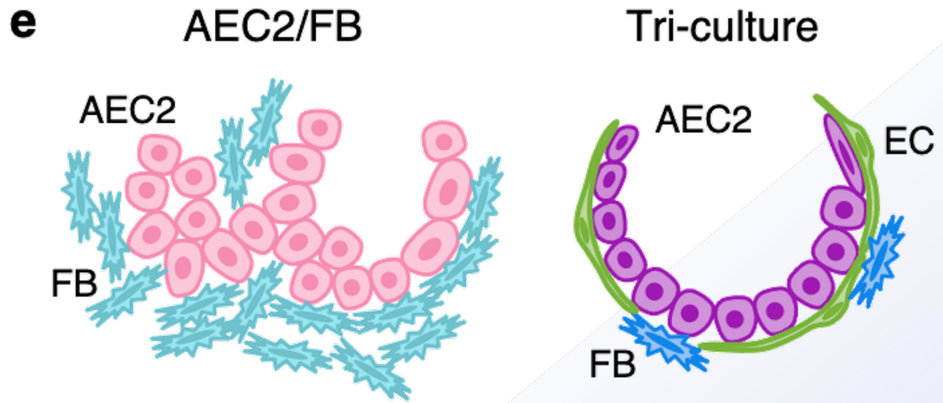
AEC2 (type II epithelium) with endothelium is sparse, few living cells.

AEC2 with ***both*** fibroblasts ***and*** endothelium leads to rapid alveolar filling with type II epithelium (!!)

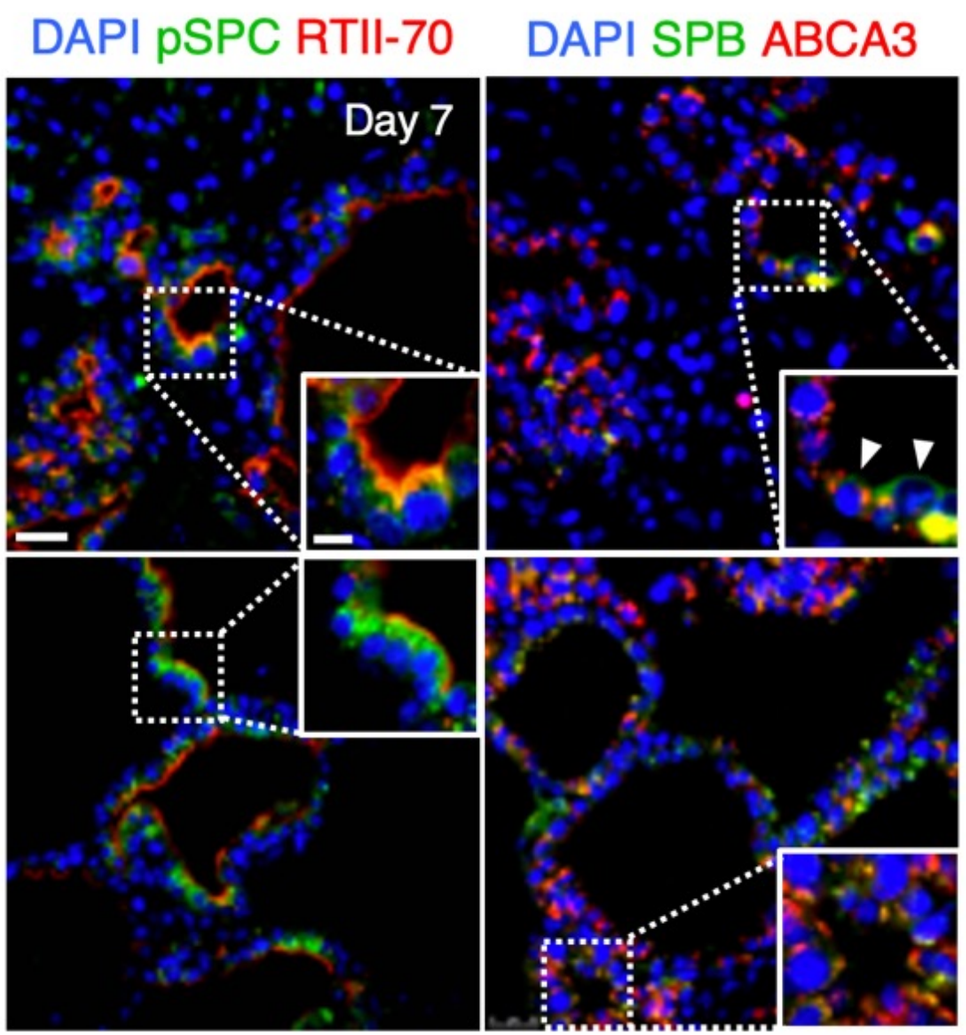
# FIBROBLASTS AND ECS CONTRIBUTE TO EPITHELIAL ORGANIZATION INTO ALVEOLAR COMMUNITIES

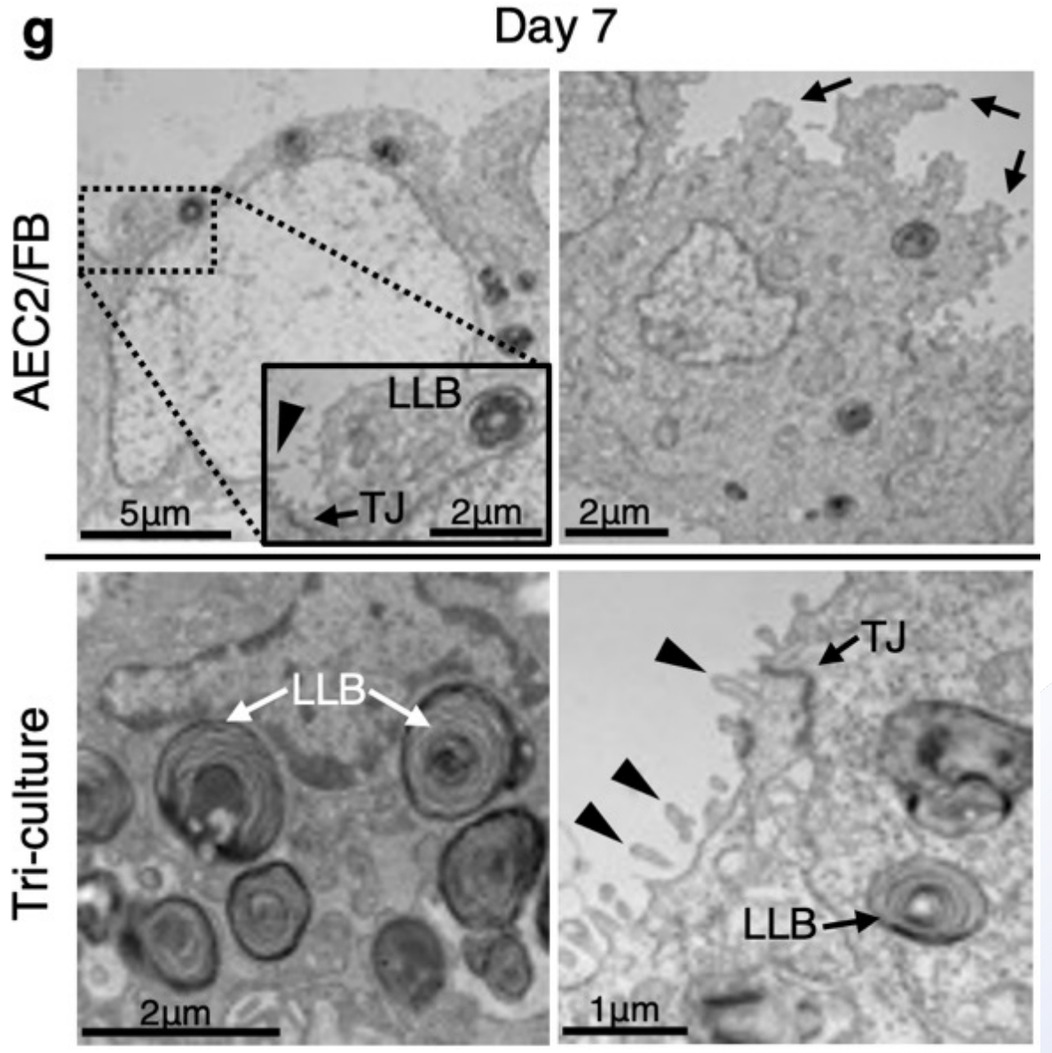


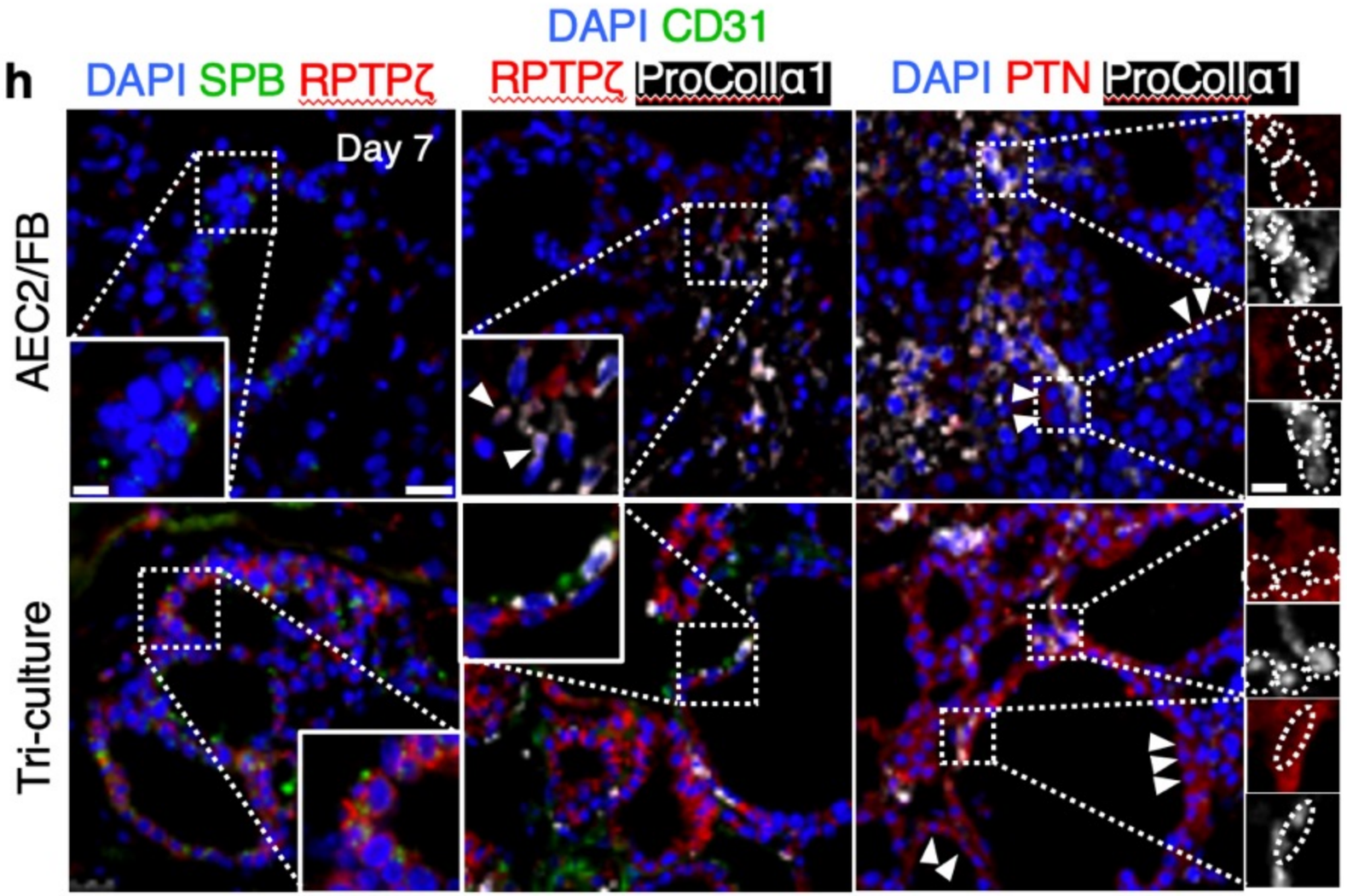



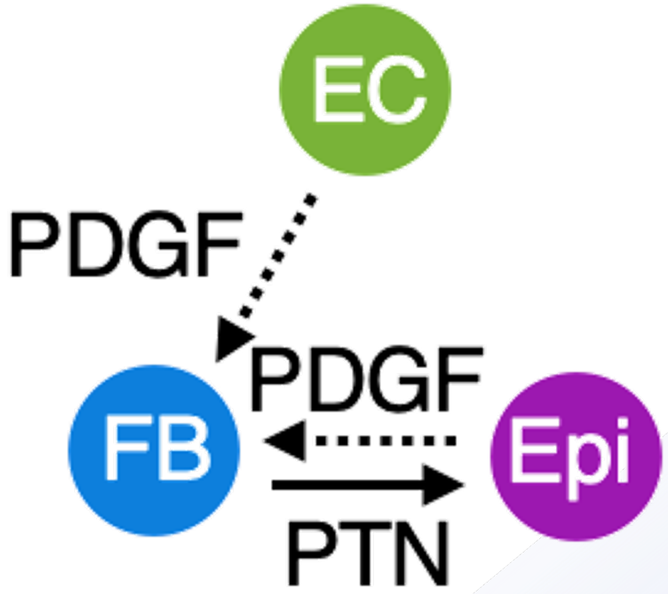
# EPITHELIUM MAKES MORE SURFACTANT IN PRESENCE OF FIBROBLASTS AND ENDOTHELIAL CELLS – LAMELLAR BODIES FILLED WITH SURFACTANT PROTEIN C



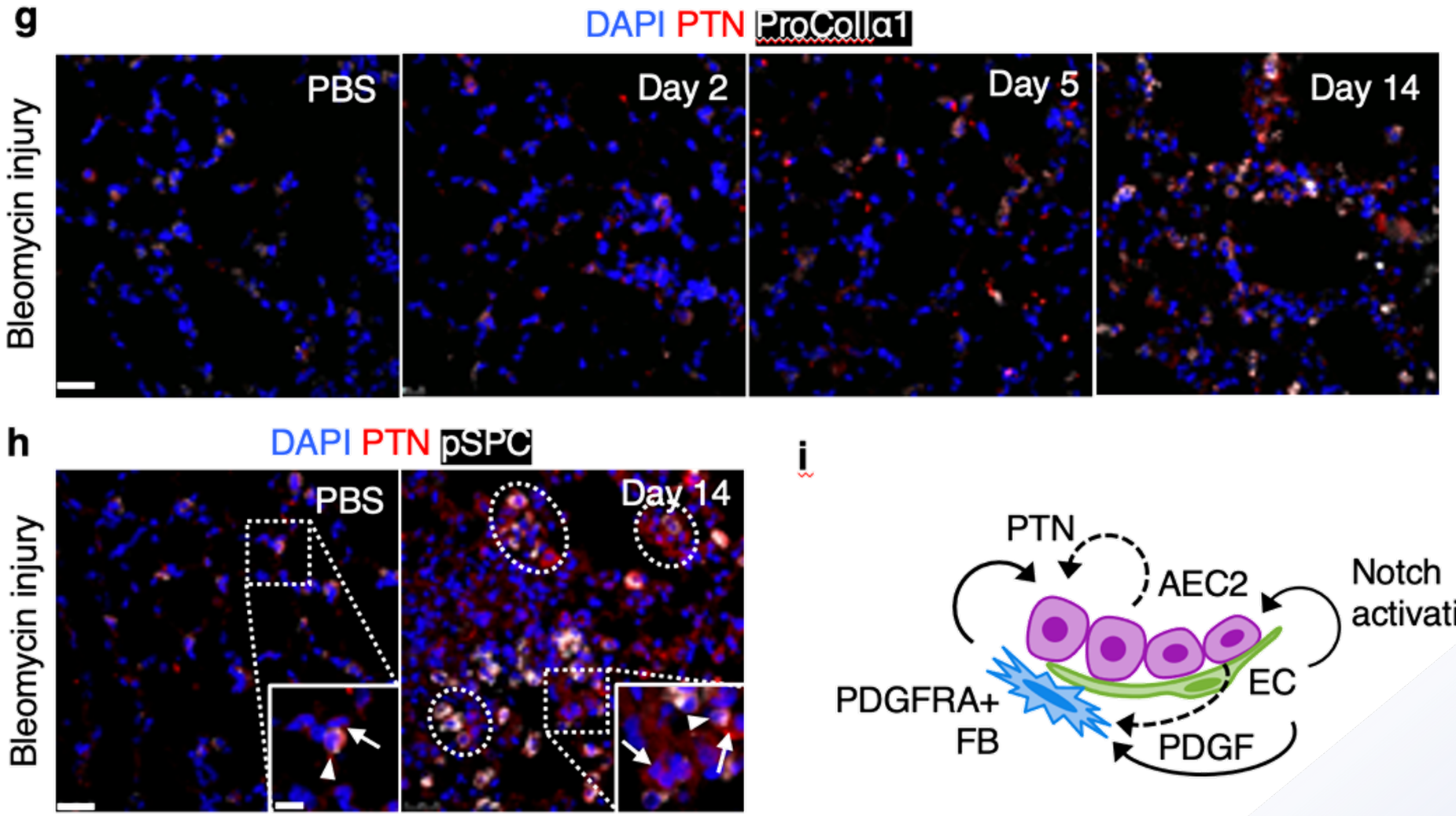



# IN AEC2-FIBROBLAST-ENDOTHELIAL TRI-CULTURES, PLEIOTROPHIN AND PDGF MAY CREATE COMMUNICATION BETWEEN CELL TYPES



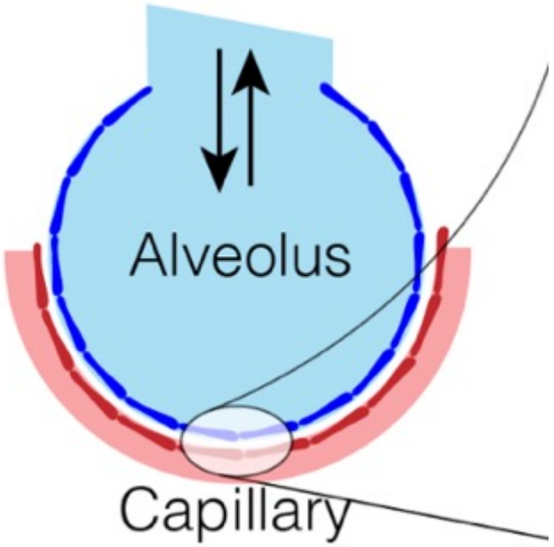
# PLEIOTROPHIN ALSO ACTS *IN VIVO* AFTER SEVERE LUNG INJURY, POSSIBLY DRIVING AEC2 REPLICATION AND REPAIR

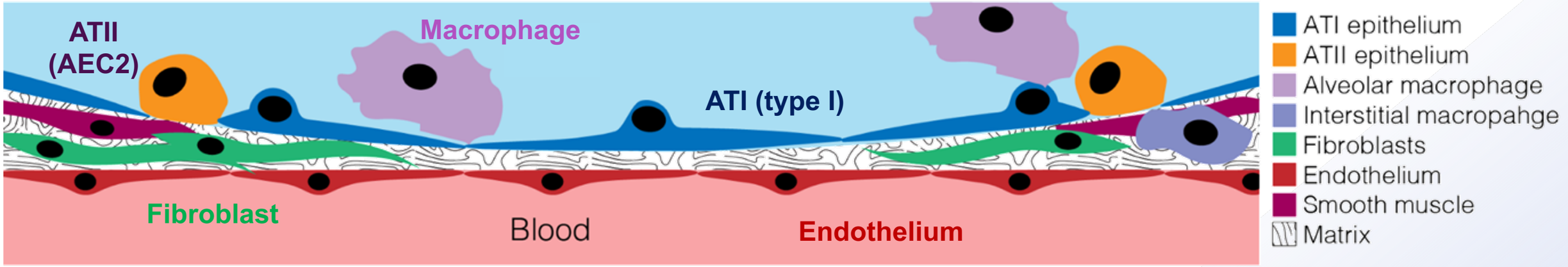


# THE ALVEOLAR ENVIRONMENT IS COMPLEX, HAVING MULTIPLE CELL-CELL COMMUNICATION AXES

The alveolus is a stereotyped, repeating structure containing roughly 6-7 cell types:  Type I, Type II, lipofibroblast, matrix fibroblast, endothelium, and macrophage (2 types - alveolar and interstitial).

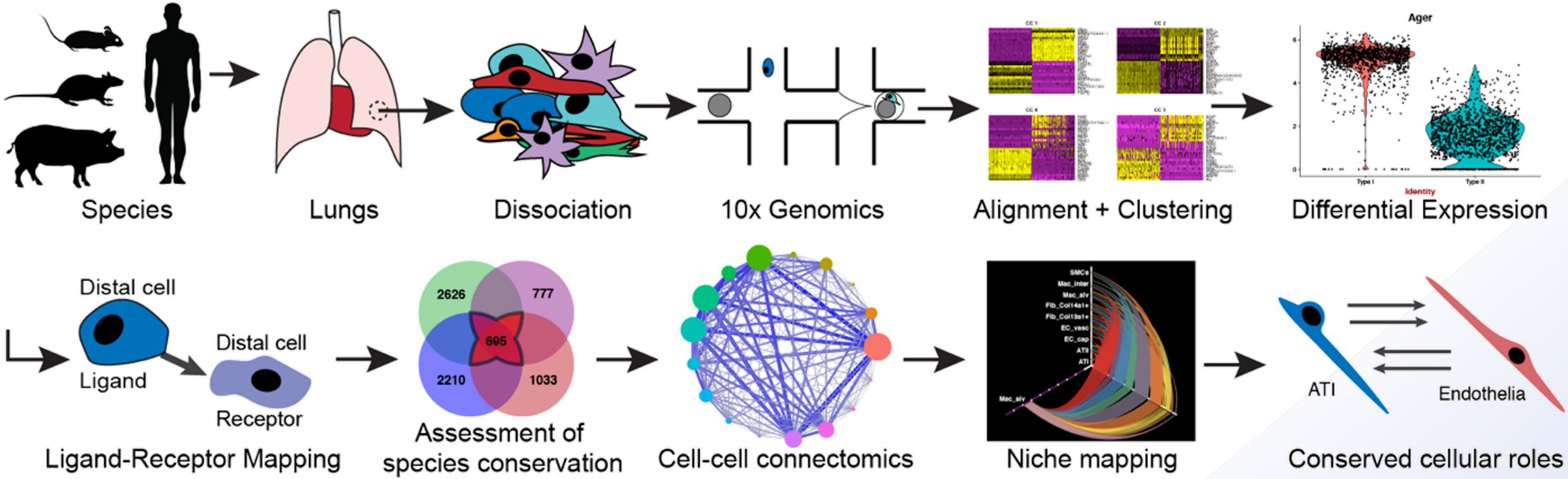
At quiescence, there is likely a species-conserved set of signals and cross-talk between these cell types that maintains barrier, alveolar patency, and gas exchange.



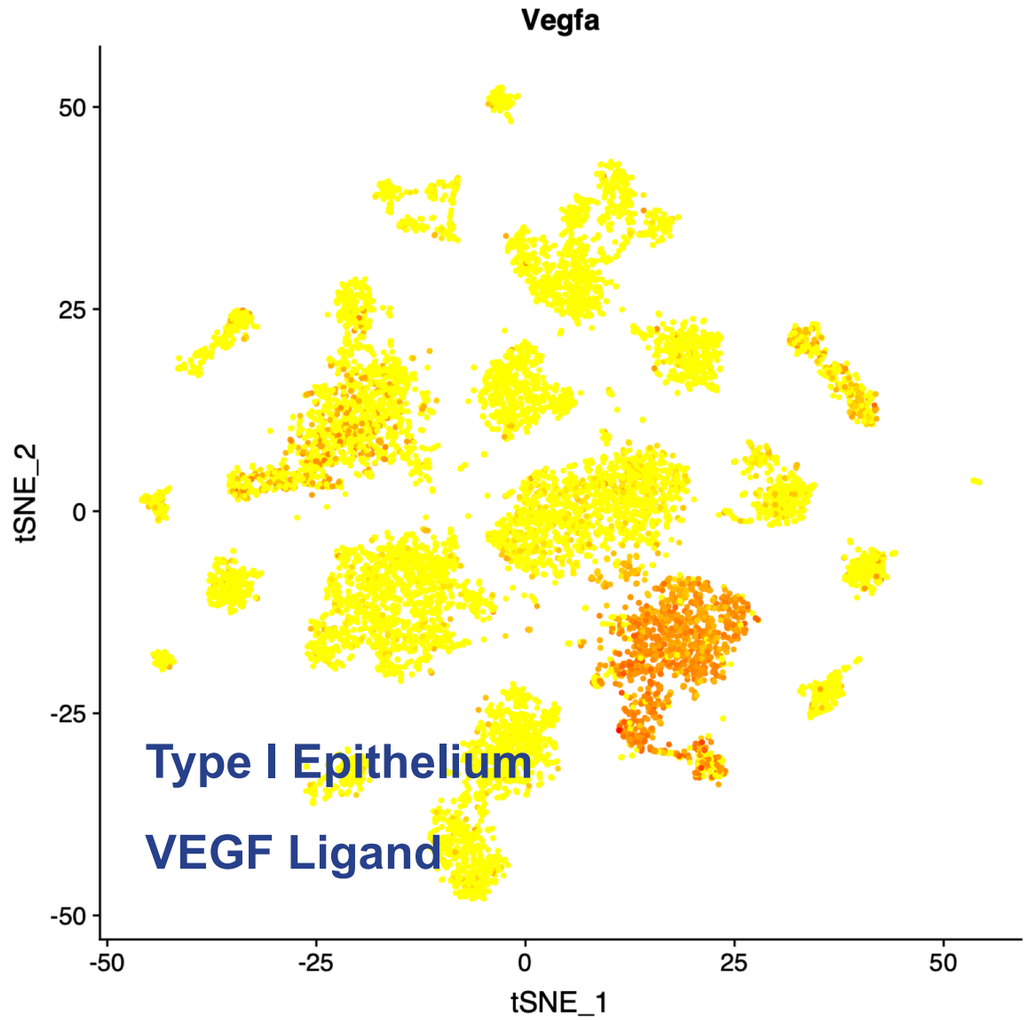



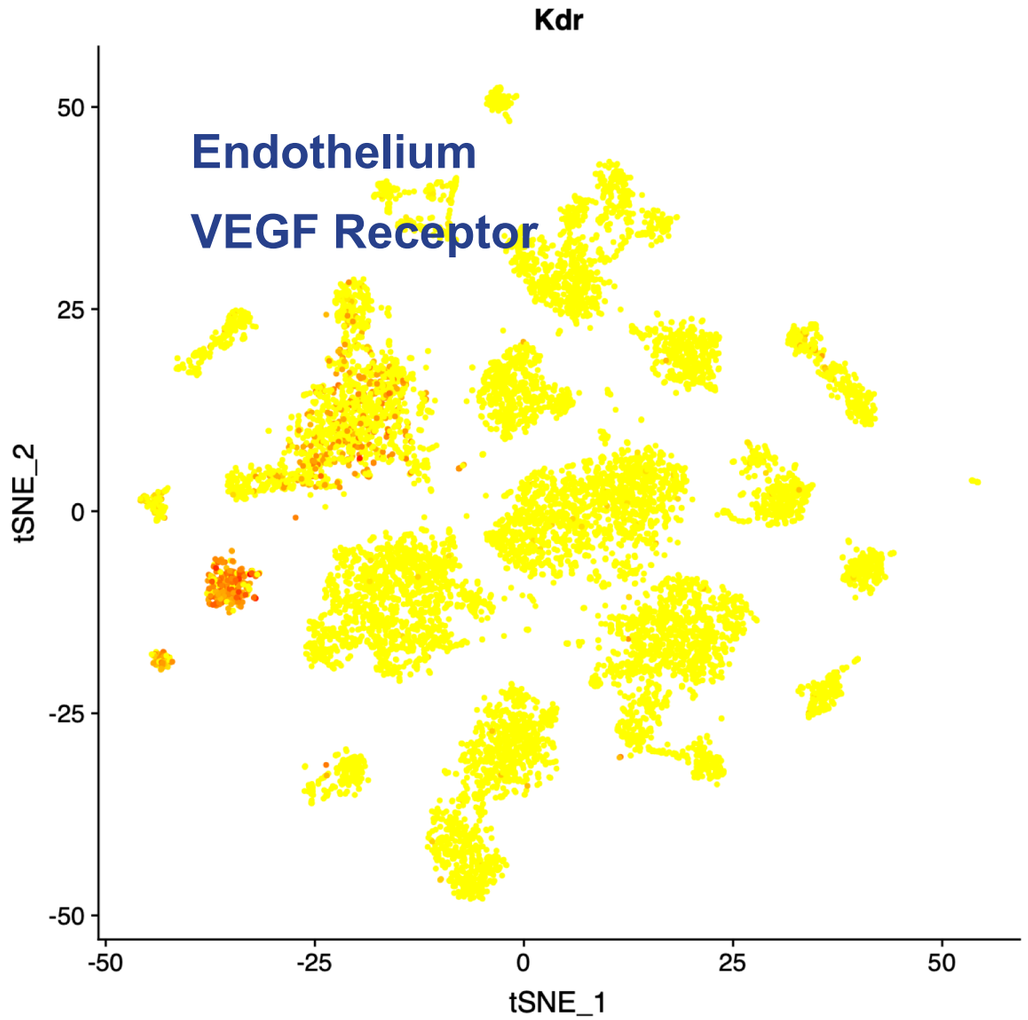
# CROSS-SPECIES ANALYSIS METHOD BY scRNA-SEQUENCING

Since alveolar geometry is tightly conserved from mouse to rat to pig to human,
we speculated that some patterns of communication within the alveolar niche must also be conserved.

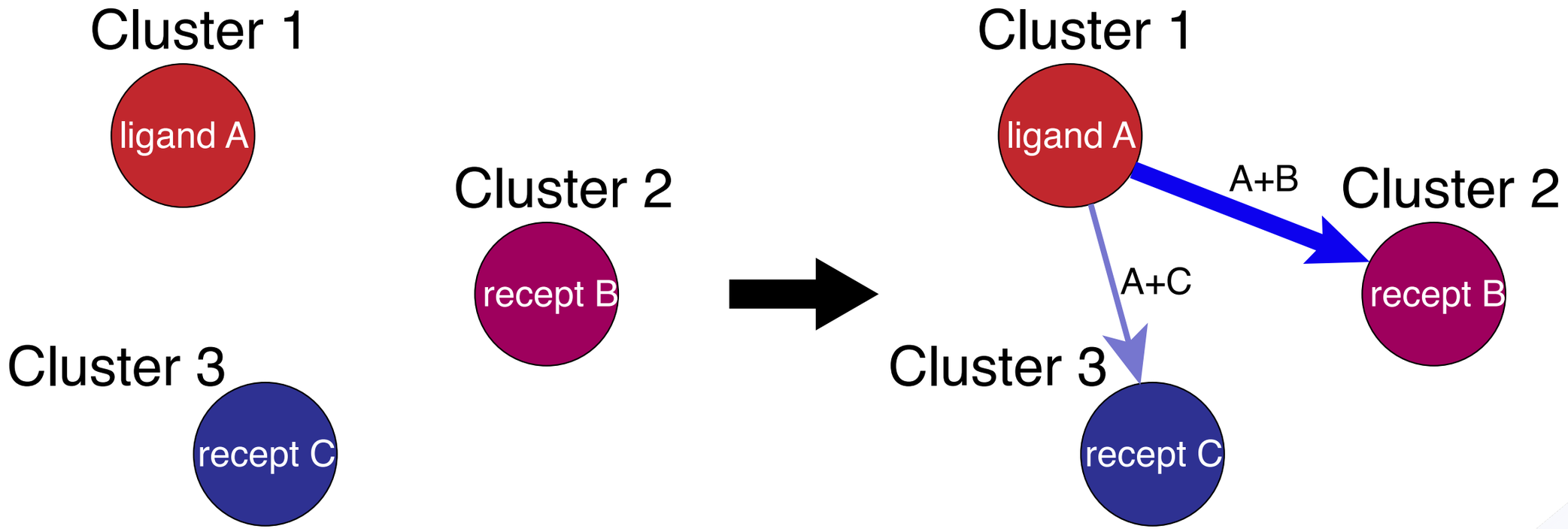


# IDENTIFYING ALVEOLAR LIGAND-RECEPTOR PAIRS – HIGHLY CONSERVED MECHANISM ACROSS SPECIES.



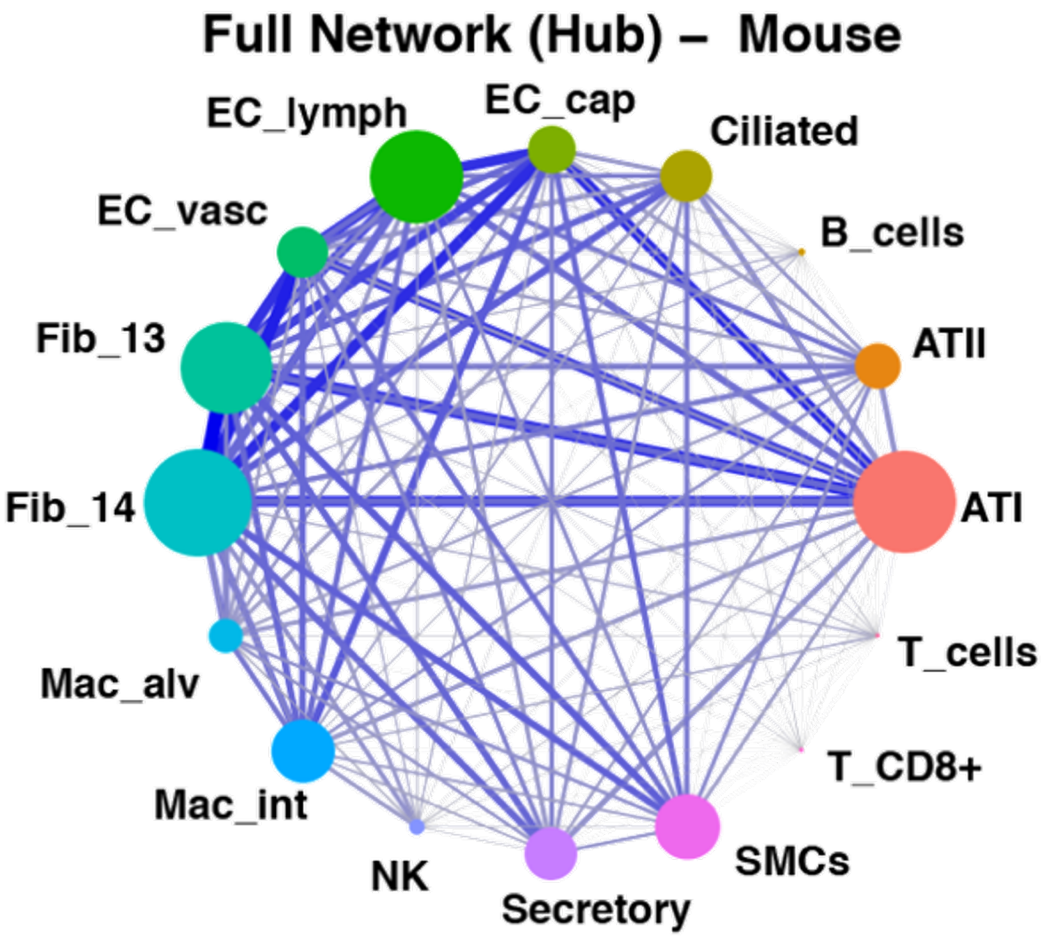



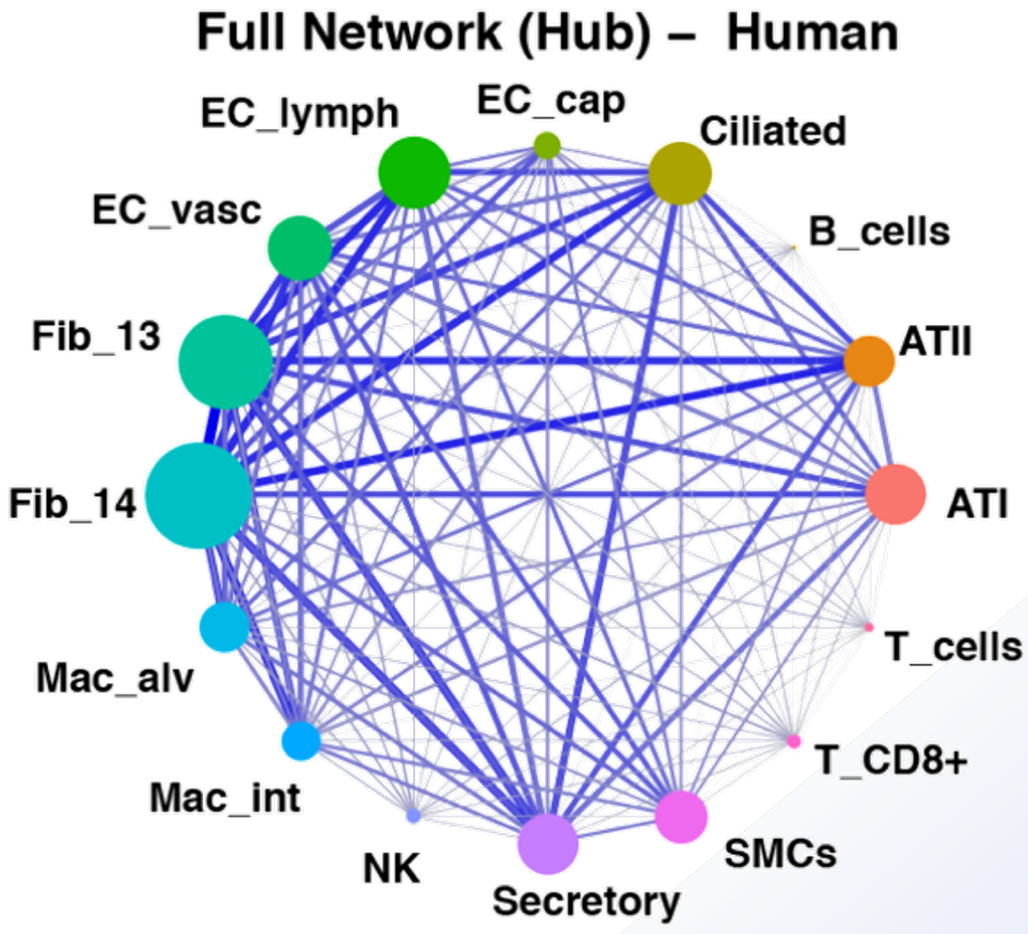
## EXTENDING LIGAND-RECEPTOR INTERACTIONS AND WEIGHTING




**FANTOM5 Mapping:** Functional annotation of mammalian genome, along with regulatory pathways.
**Threshold:** >5% expression for cells within a cluster
**Significance Value:** $p<0.05$ (Wilcoxon Rank test)

# OVERALL ALVEOLAR SIGNALING TOPOLOGY CONSERVED BETWEEN MOUSE AND HUMAN – “LUNG-NESS”



## CONCLUSIONS FROM CROSS-SPECIES ALVEOLAR NICHE WORK

- Inter-individual variability in single-cell data is tolerable – even for human samples.
- The alveolus in the mammal is a fairly conserved structure, with similar cell types, locations, and functions.
- With suitable thresholding and significance criteria, it is possible to identify ***conserved alveolar signaling patterns across wide phylogenetic ranges***.

- Our goal is to identify those interactions that are most important for critical alveolar cell functions, to include
  - Barrier function (cell-cell junctions)
  - Anti-thrombosis (endothelial phenotype)
  - Surfactant production (lung compliance)
  - Macrophage interactions/function

***We anticipate that these studies will unveil a "roadmap" for building a functional lung alveolus.***

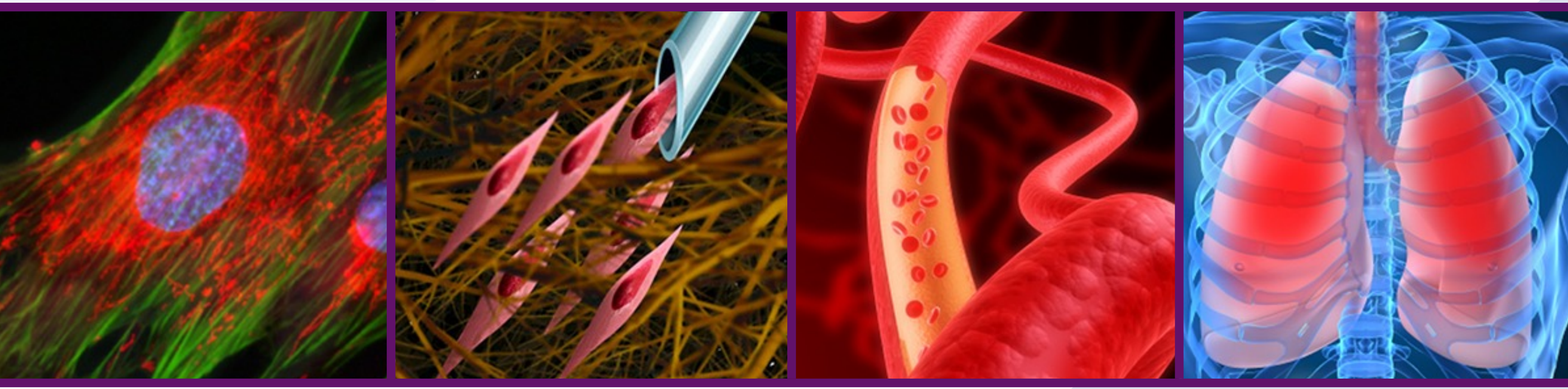
# LUNG, TRACHEA, VASCULAR REGENERATION

- Tom Petersen MD, PhD
- Allison Greaney MS
- Hong Qian PhD
- Andrew Le PhD
- Elizabeth Calle MD, PhD
- Alexander Engler MS
- Juan Wang PhD
- Mehmet Kural PhD
- Edward Han MD
- Pavlina Baevova

**Yifan Yuan PhD**
**Katie Leiby MS**
**Sam Raredon PhD**

National Heart Lung and Blood Institute
People Science Health

Connecticut Innovations



# Recent Progress in Whole Lung Engineering


Laura E Niklason MD, PhD

Founder and CEO
Humacyte Incorporated

Adjunct Professor
Professor of Anesthesia & Biomedical Engineering
Yale University