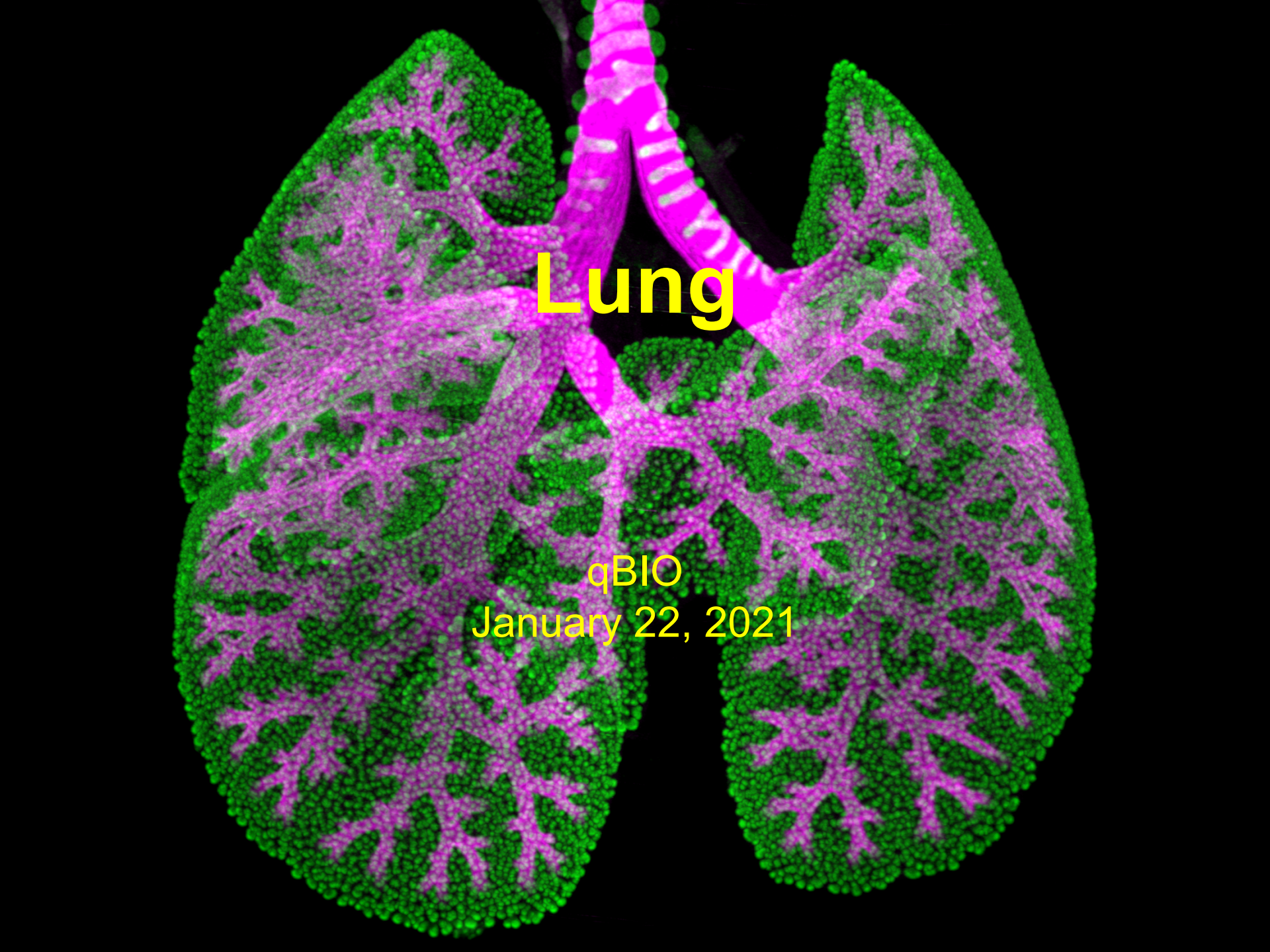
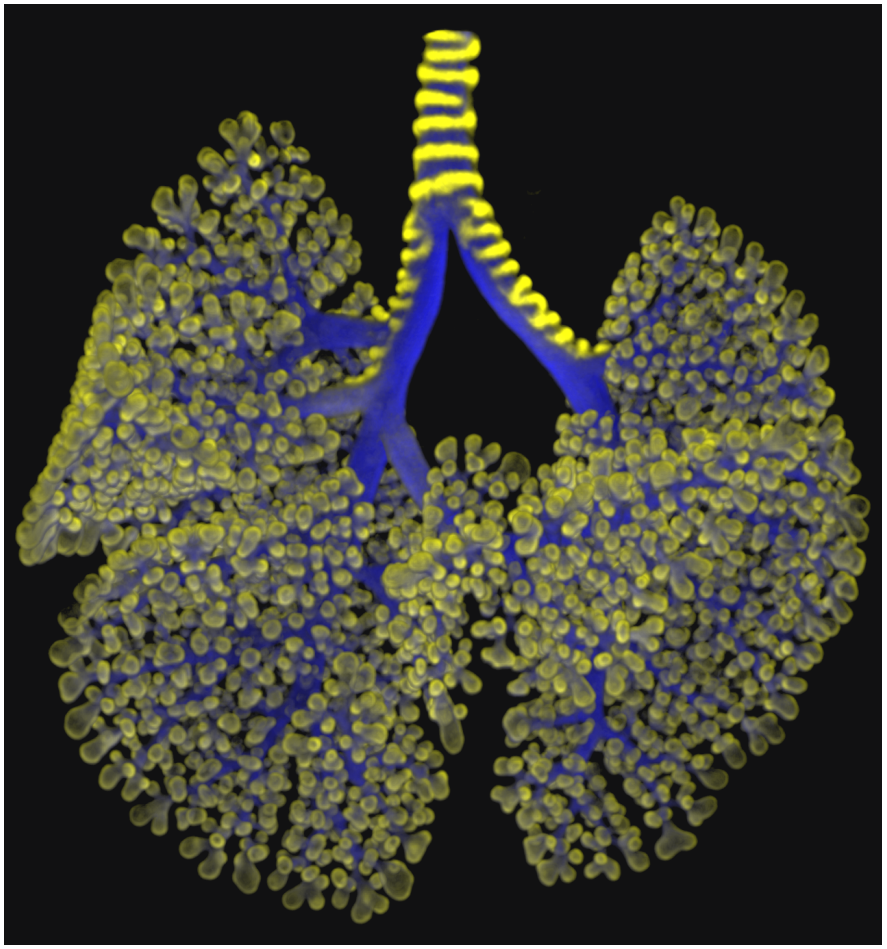

Lung
qBIO
January 22, 2021

Outline



-General and unique features of the lung

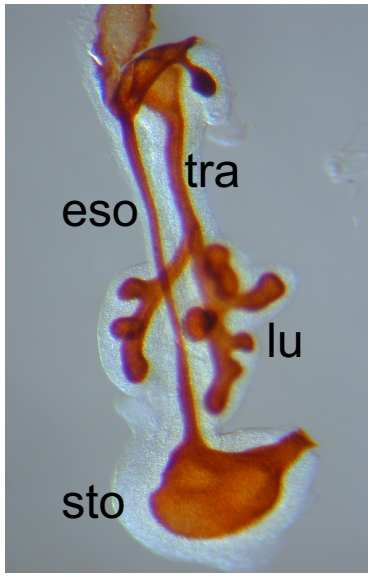
-Lung fate specification and iPSC-derived lung cells

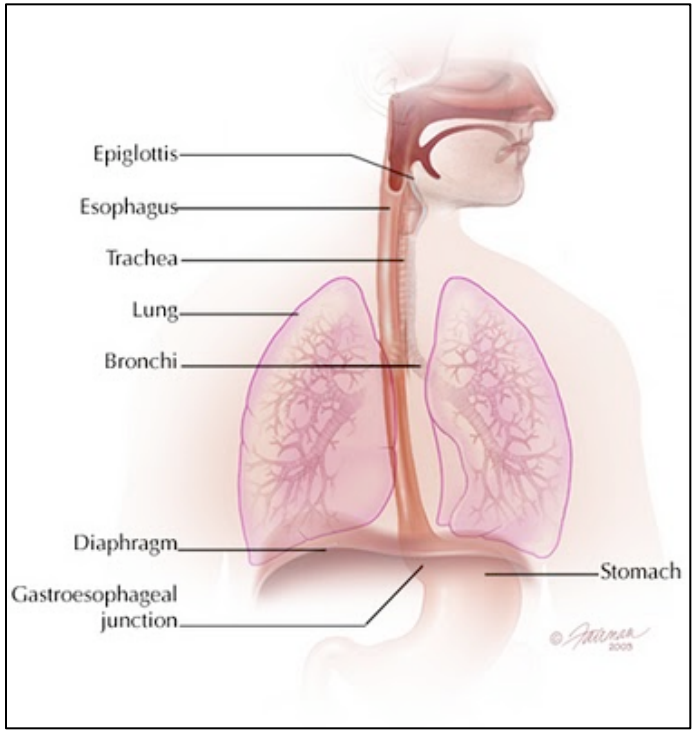
-Lung branching

-Airway and alveolar progenitor cells.

-Progenitors in regeneration

-Progenitors in injury repair





General Features

Respiratory organ=trachea+lung;

Largest branching organ;

Gas-exchange units: 600 million, surface area: 1,000ft^2;

Vital starting at birth;

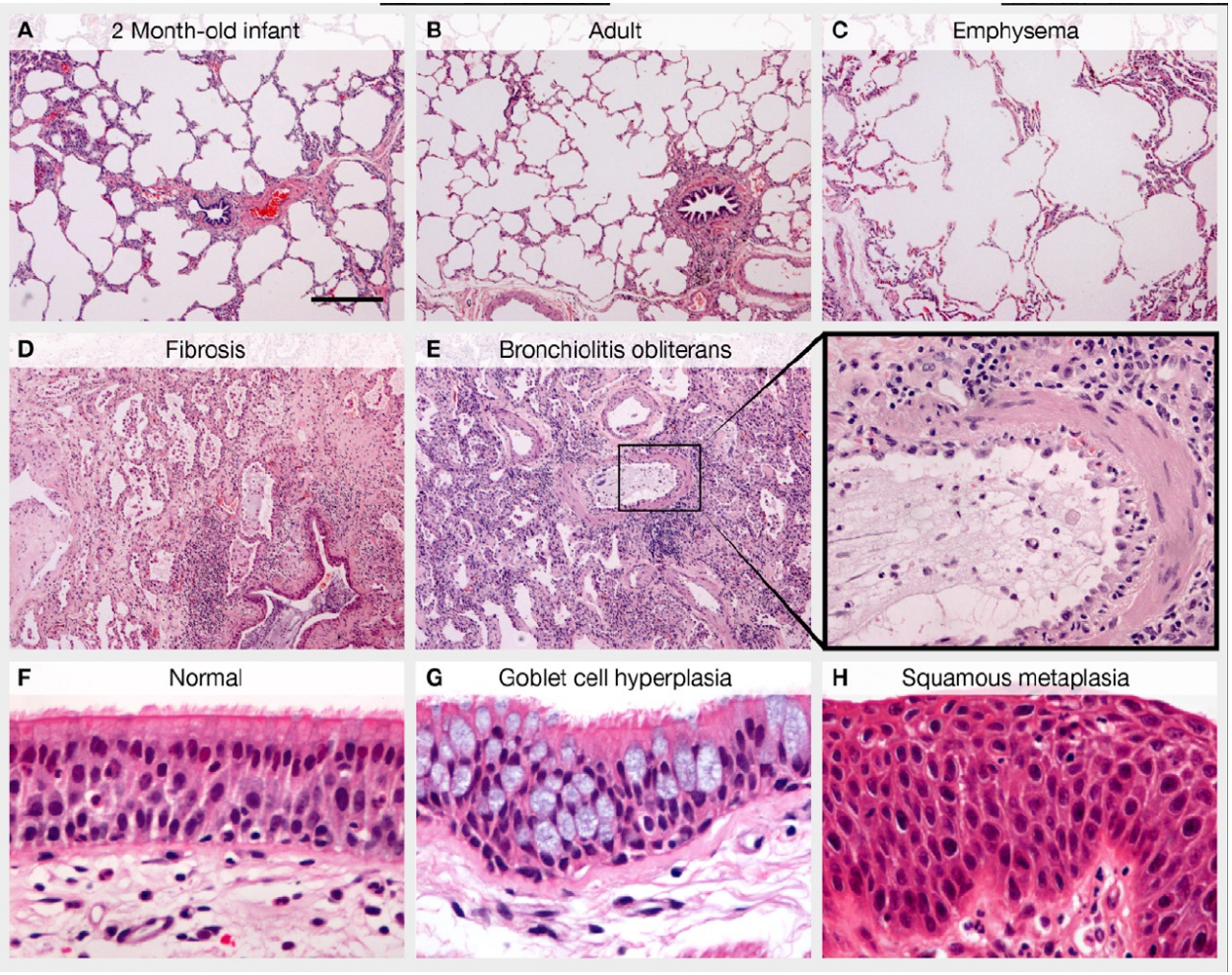
Per minute at resting, 5-8 litters of air pass in and out;

Slow turnover of cells;

Barrier to aerosol environment.

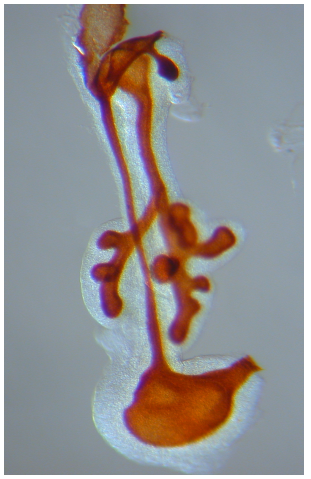
Lung Diseases

Asthma, emphysema, fibrosis, pulmonary hypertension, cancer, common site of cancer metastasis.

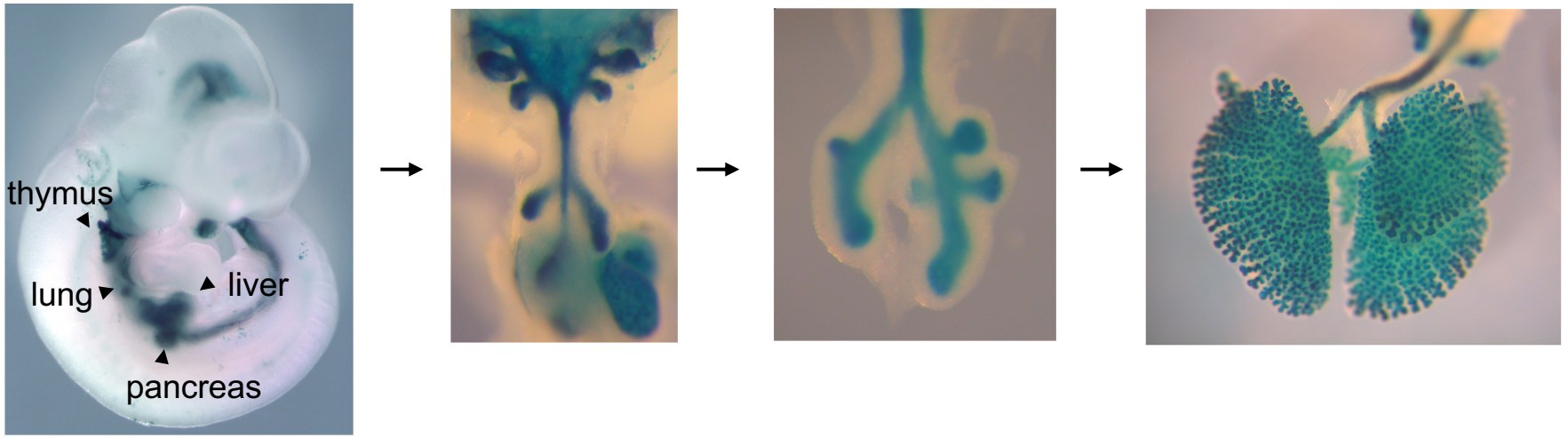


Hogan, Morrisey et al., 2014.

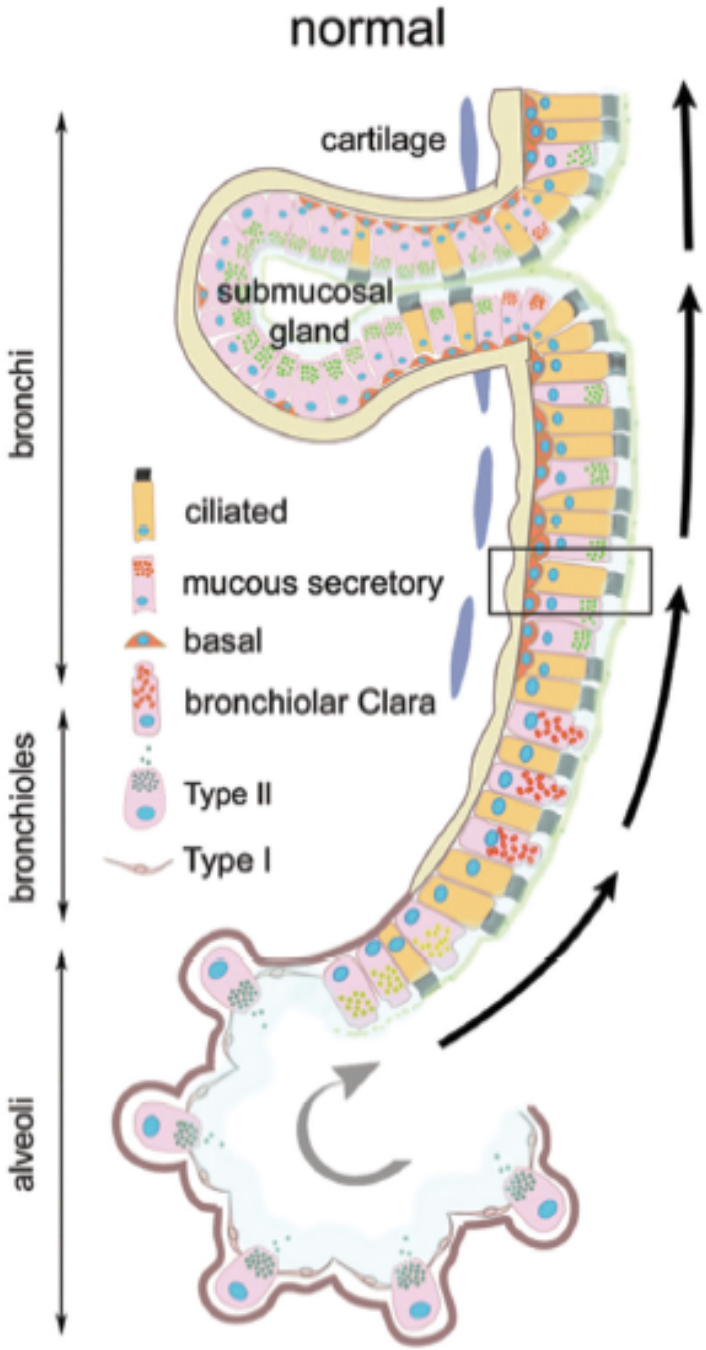
Progression of Lung Development

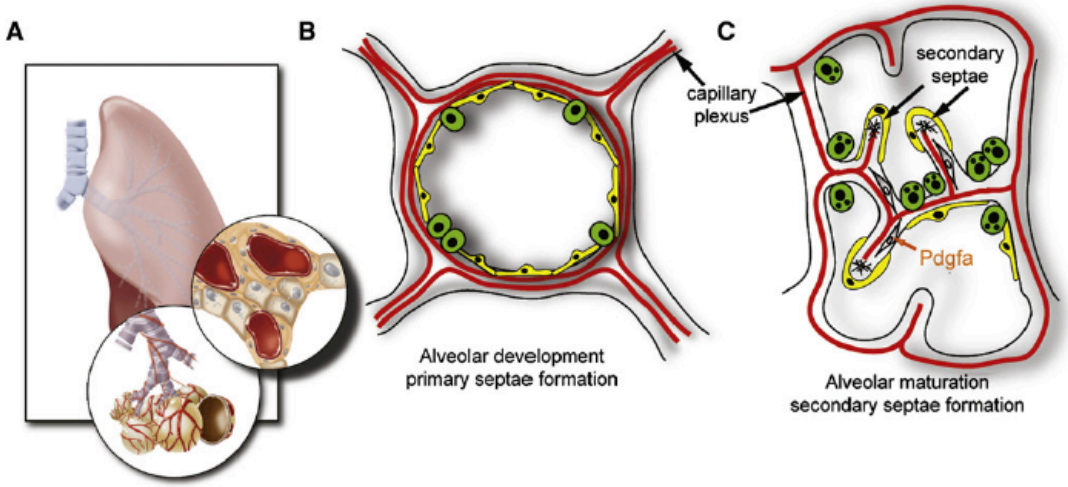


Specification Initiation Branching Morphogenesis

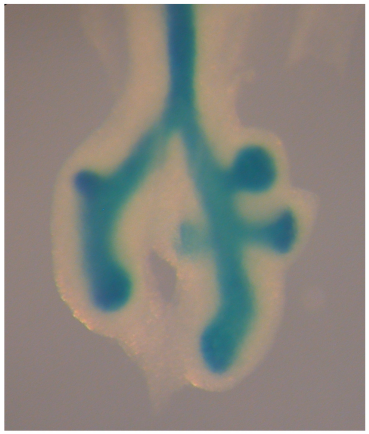


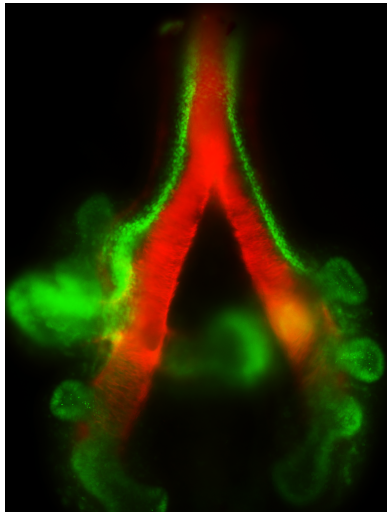
Lung epithelium patterning

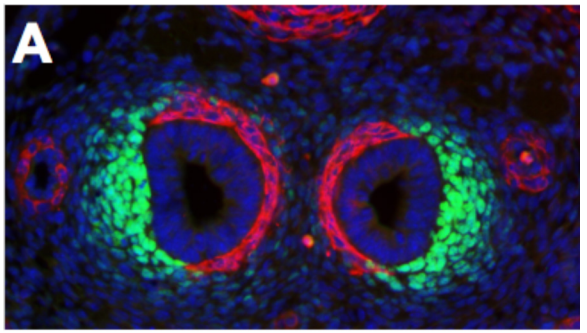


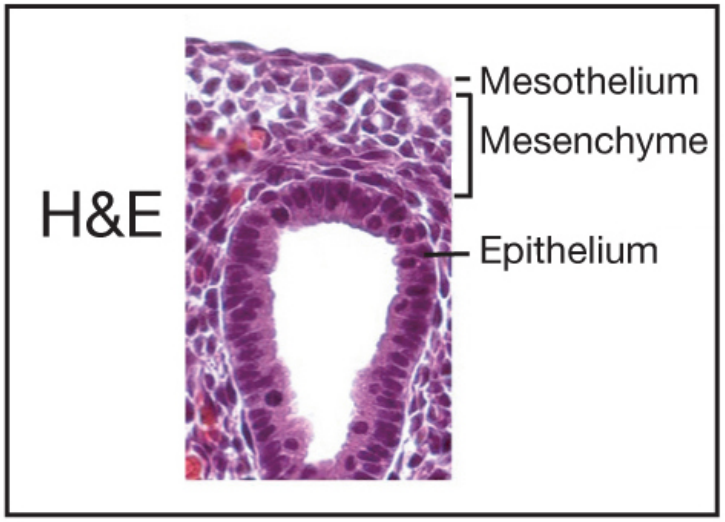


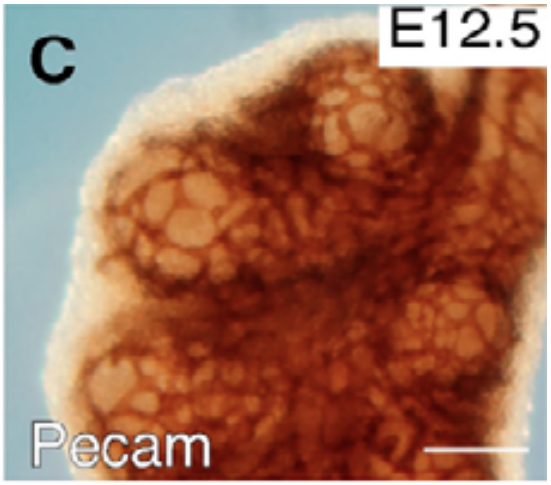
Lung mesenchyme and endothelium







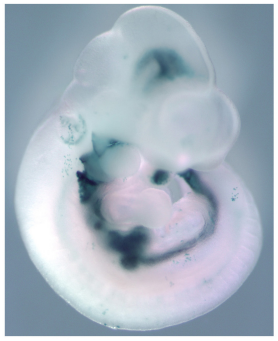




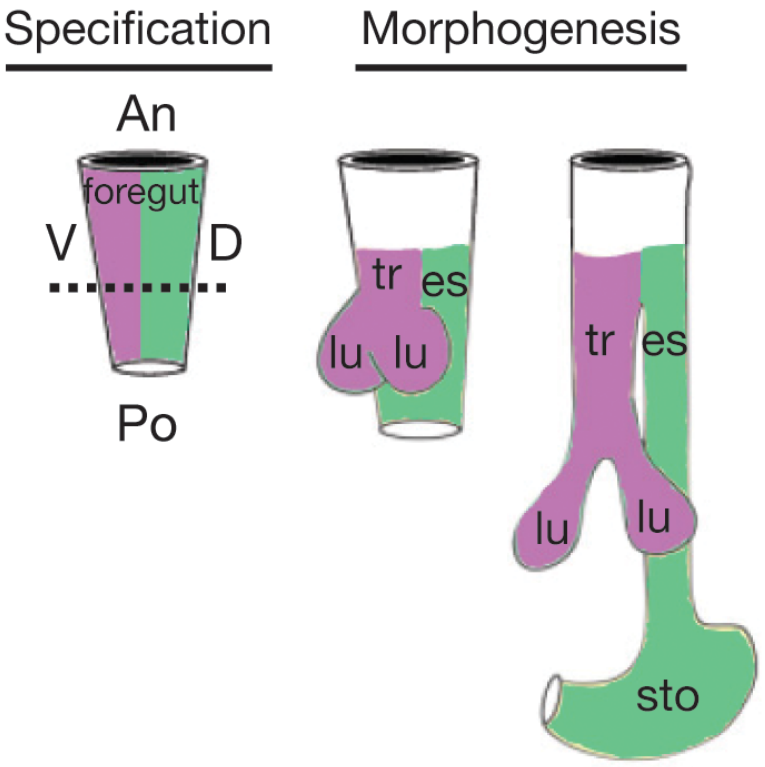
Neural innervation of the lung

Jamie Barr, Sun lab

Lung Fate Specification



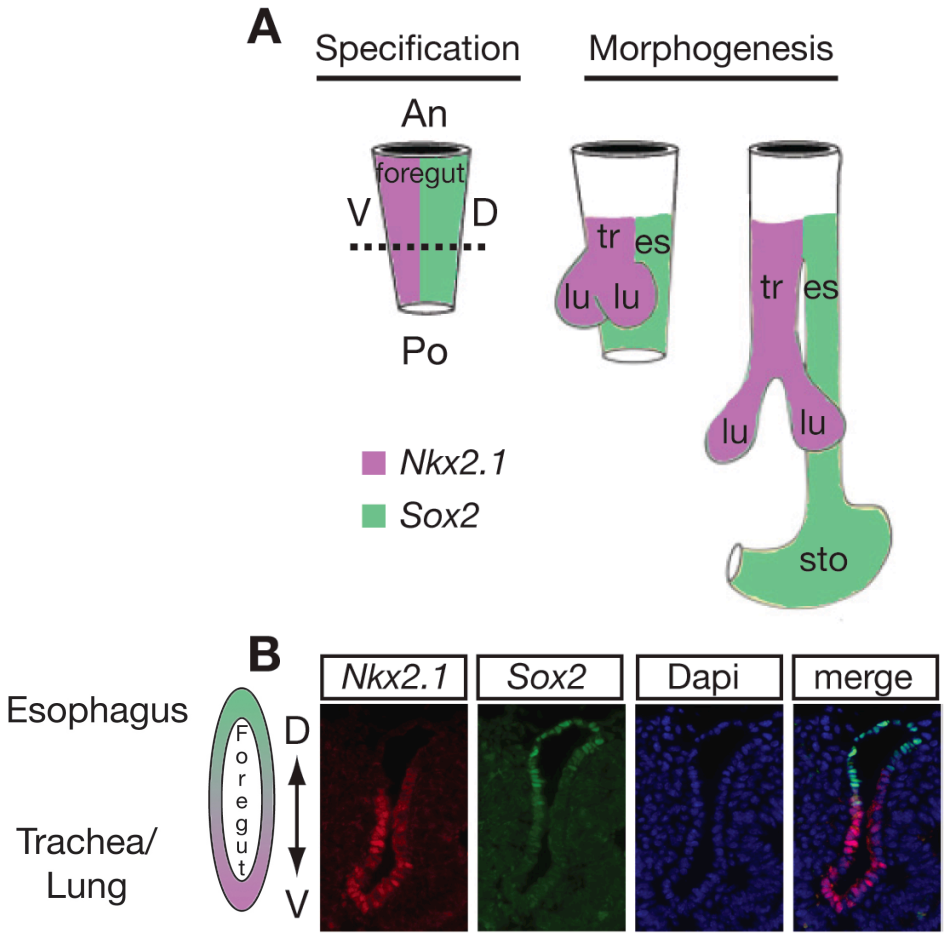
Specification of the respiratory fate



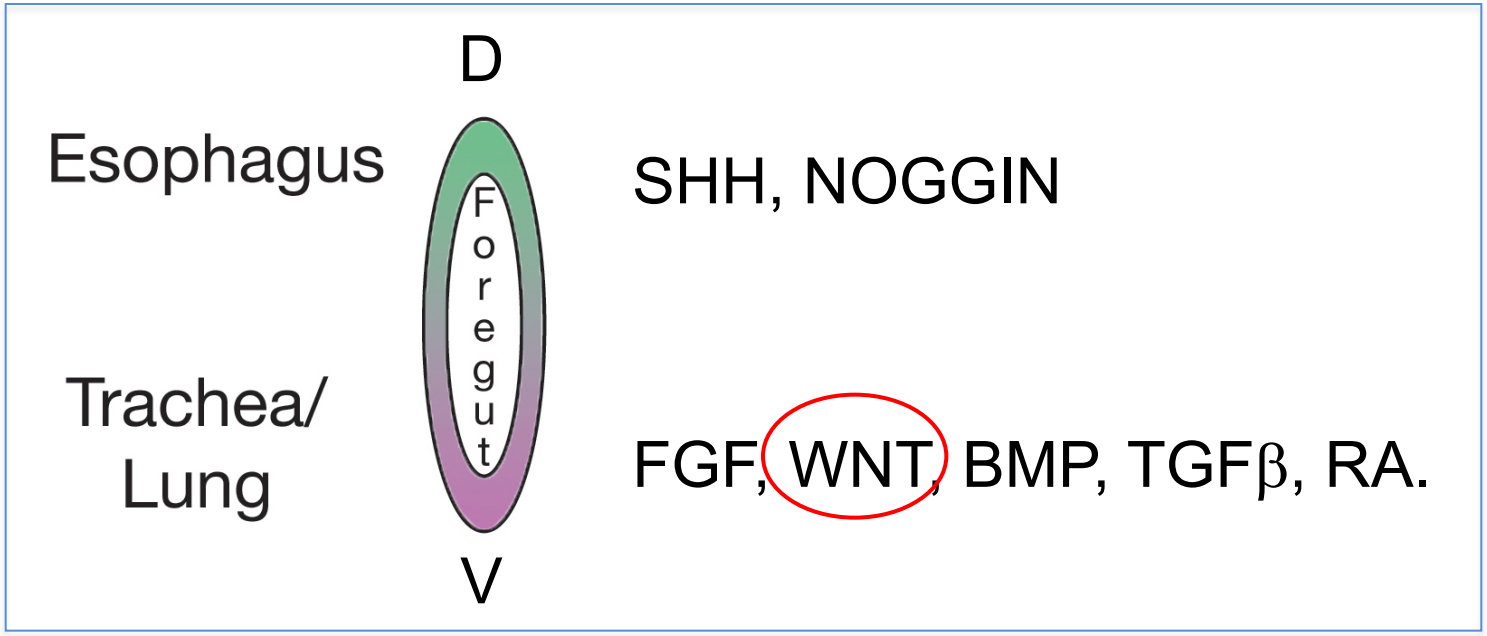
Cell fate “flavors”:
Respiratory vs Digestive.

Question:
What drives the specification of distinct respiratory vs digestive lineages?

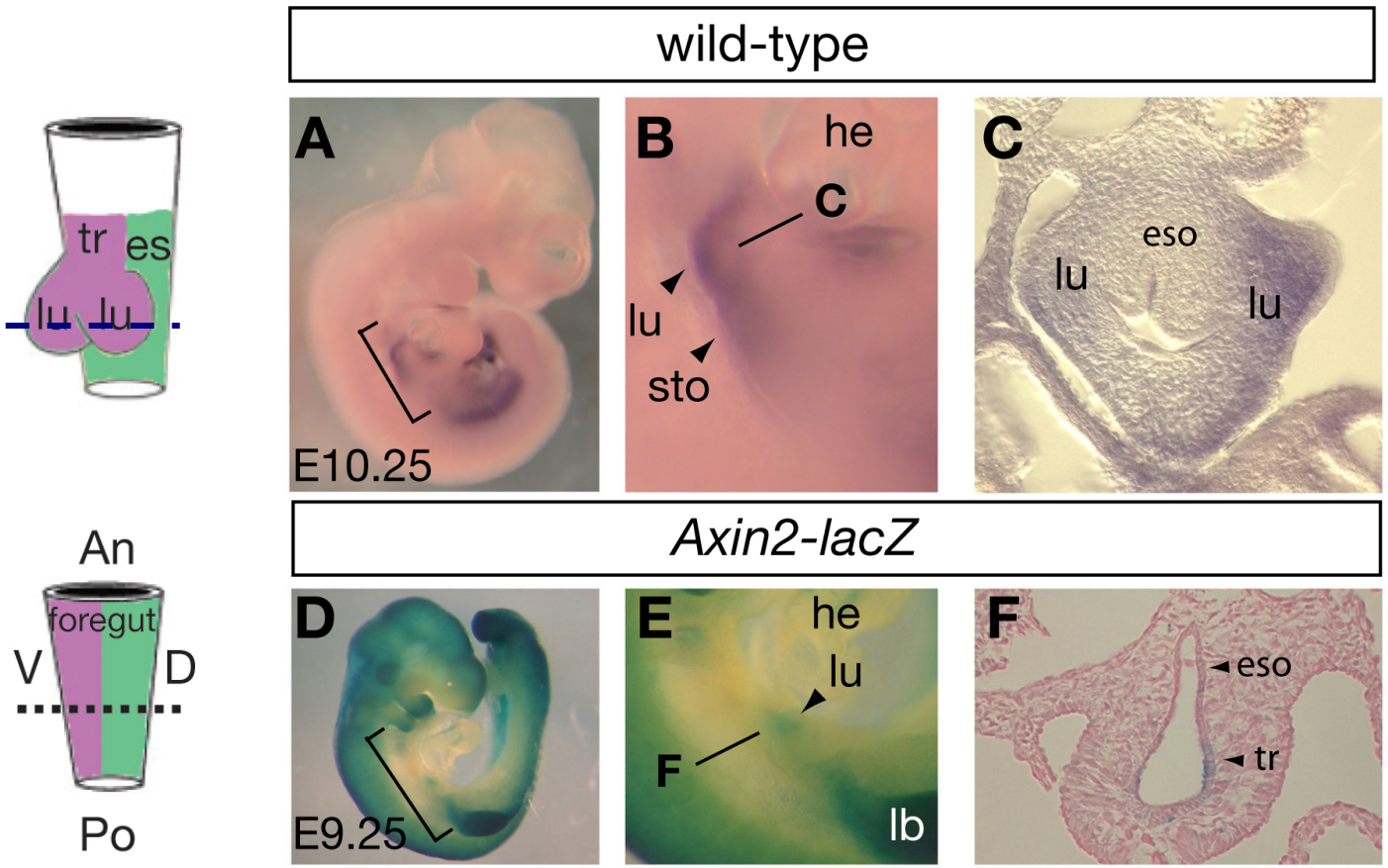
Distinct markers for respiratory vs digestive fates



Foregut is a hub for signaling pathways

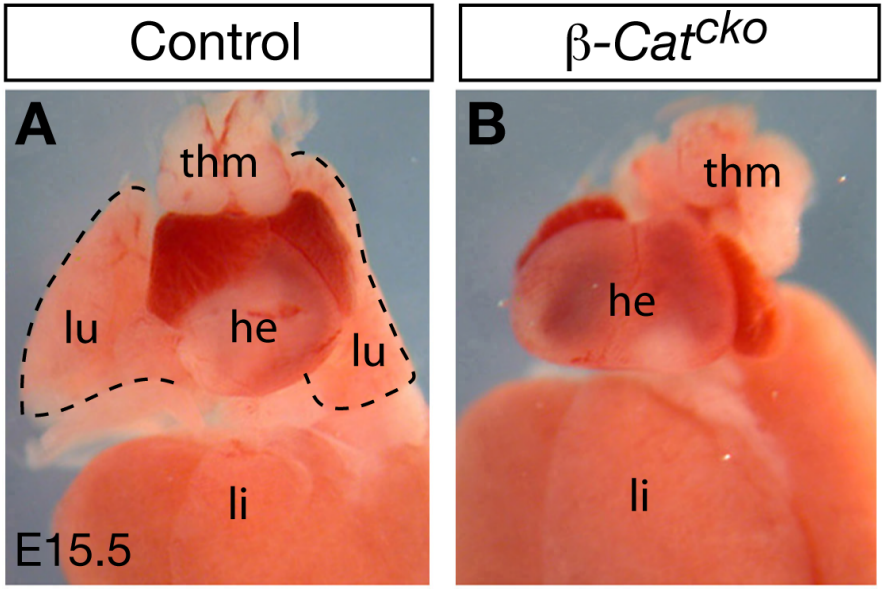


WNT is active in ventral foregut



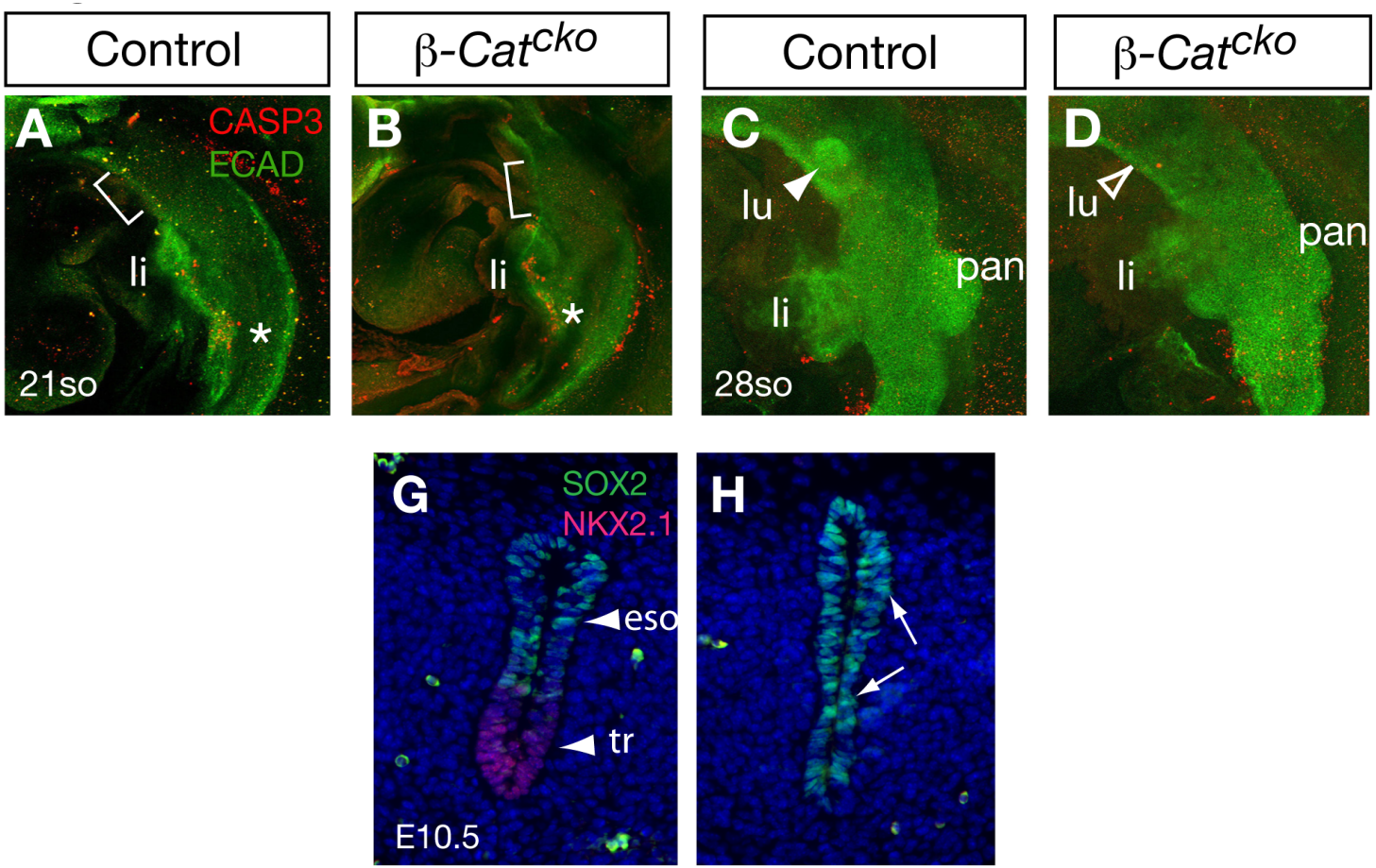
Harris and Sun et al., 2009.

Inactivation of β-Catenin leads to trachea and lung agenesis



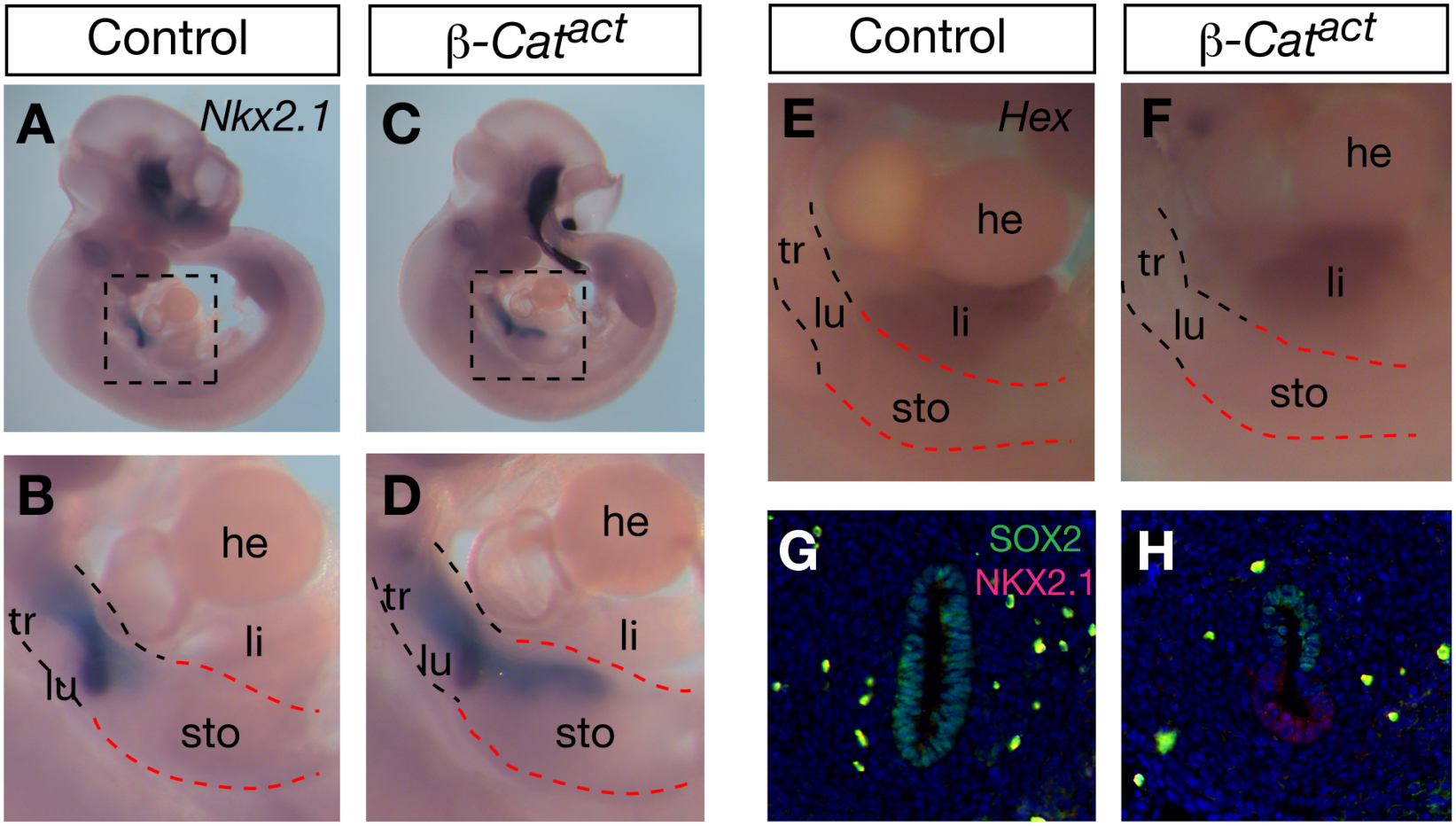
Harris and Sun et al., 2009.

WNT/β-Catenin signaling is necessary for promoting respiratory fate



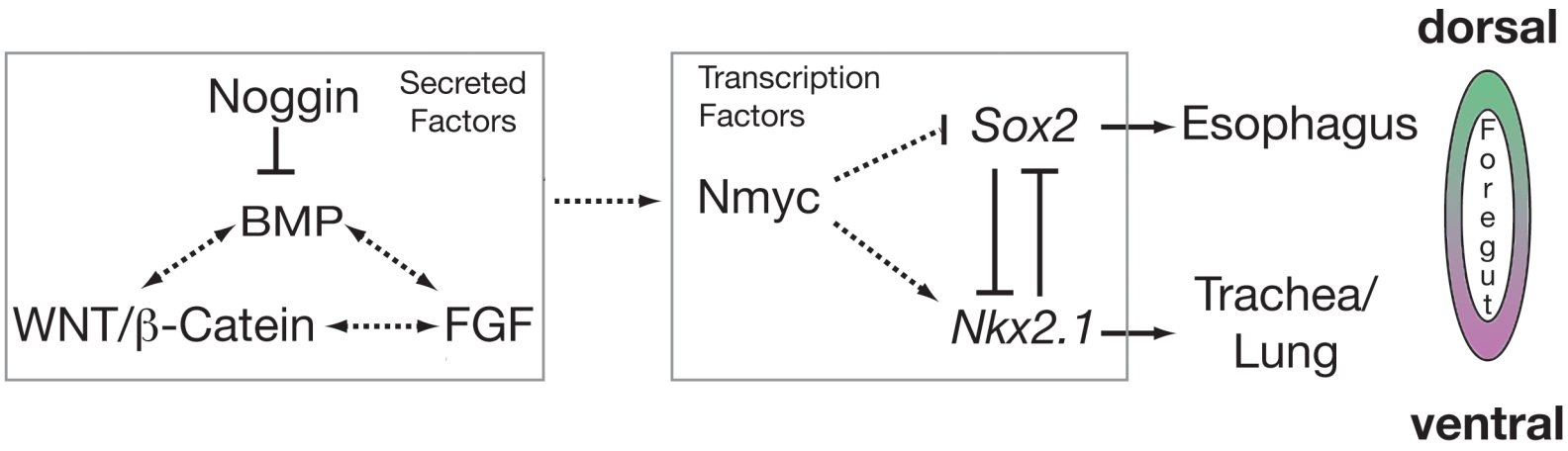
Harris and Sun et al., 2009.

WNT/ β-Catenin signaling is sufficient to induce respiratory fate

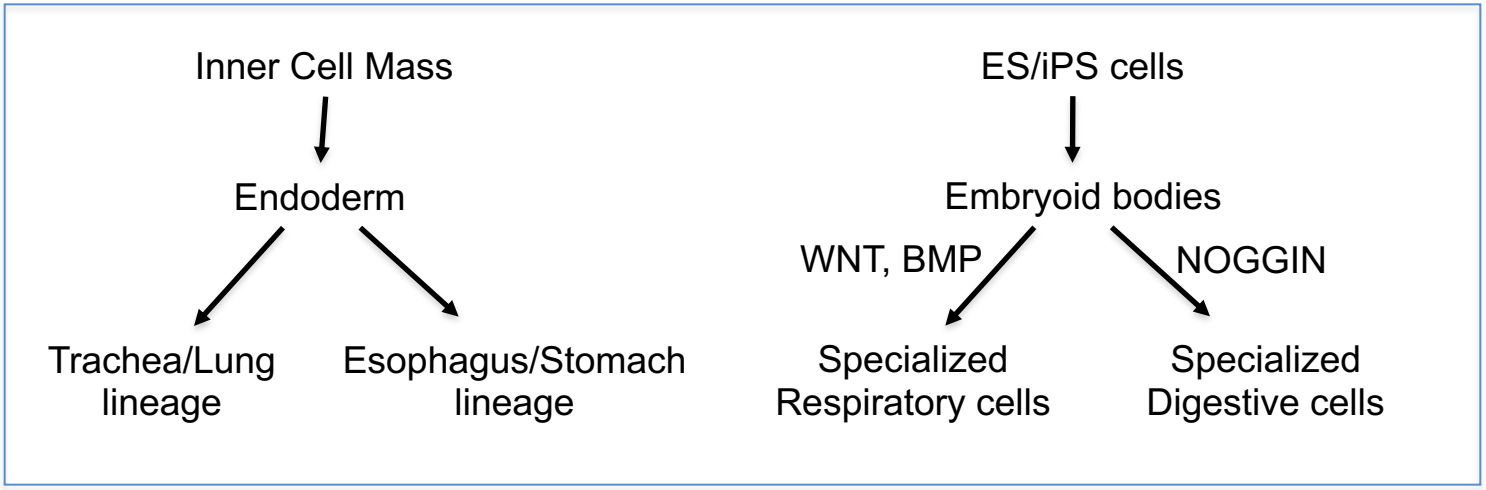


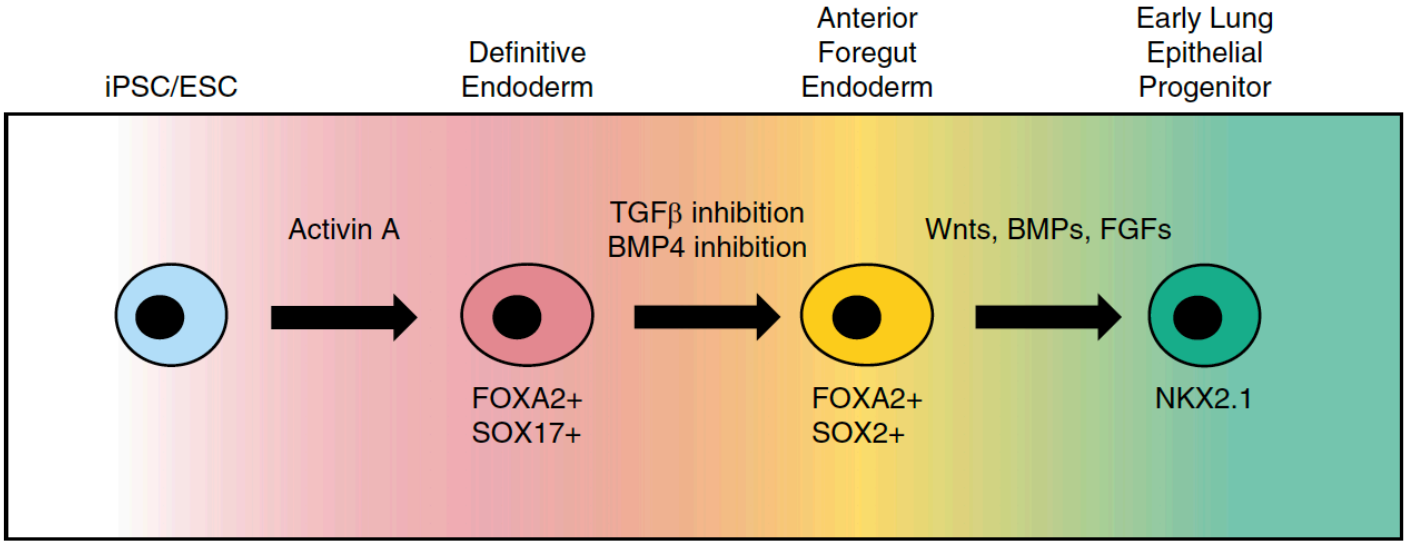
Harris and Sun et al., 2009.

Dissect pathway crosstalk and genetic circuitry



Recapitulating development in a dish

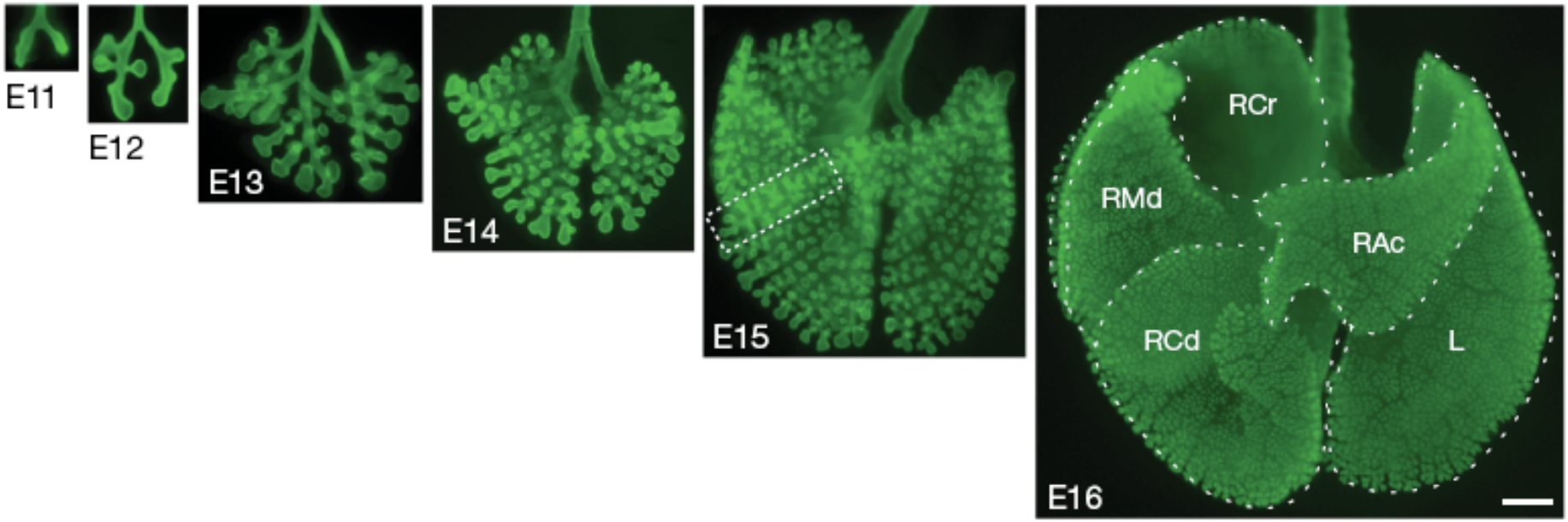




Hawkins and Kotton et al., 2017.

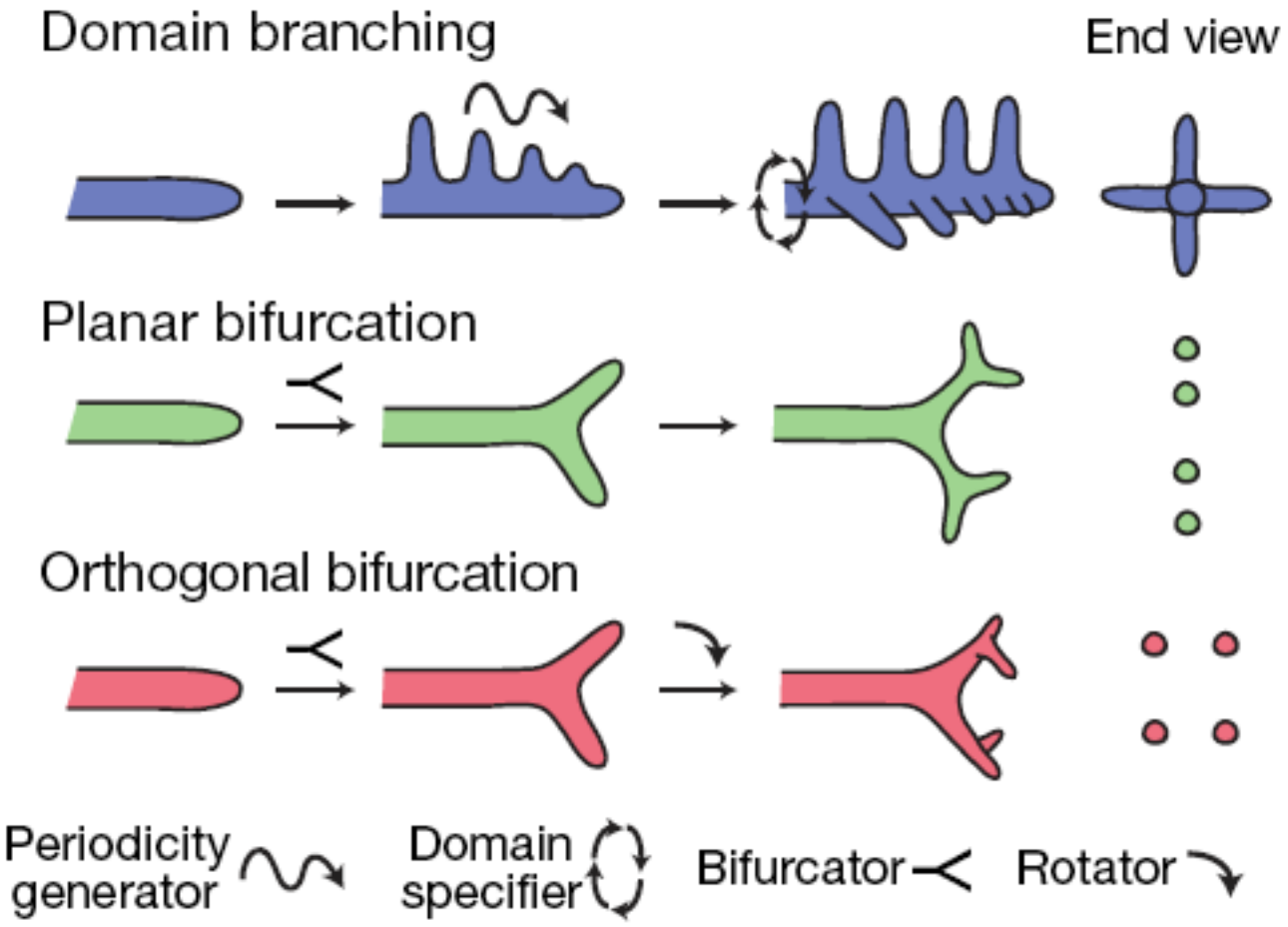
Lung Branching

Entire Lung Branching program



Metzger, 2008.

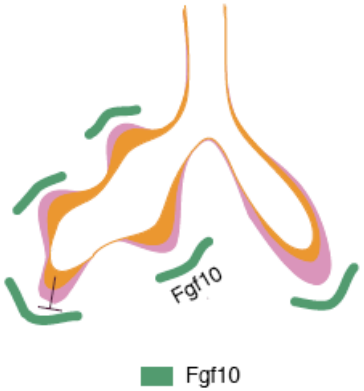
Branching Subroutines

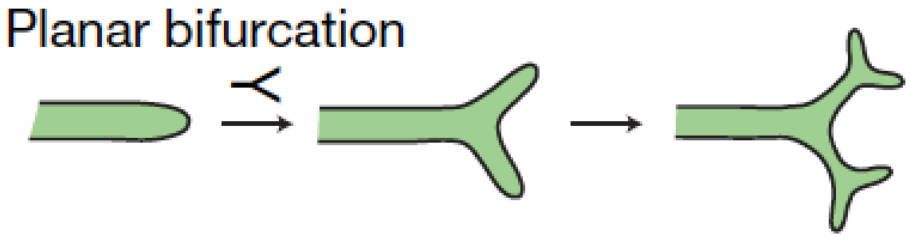


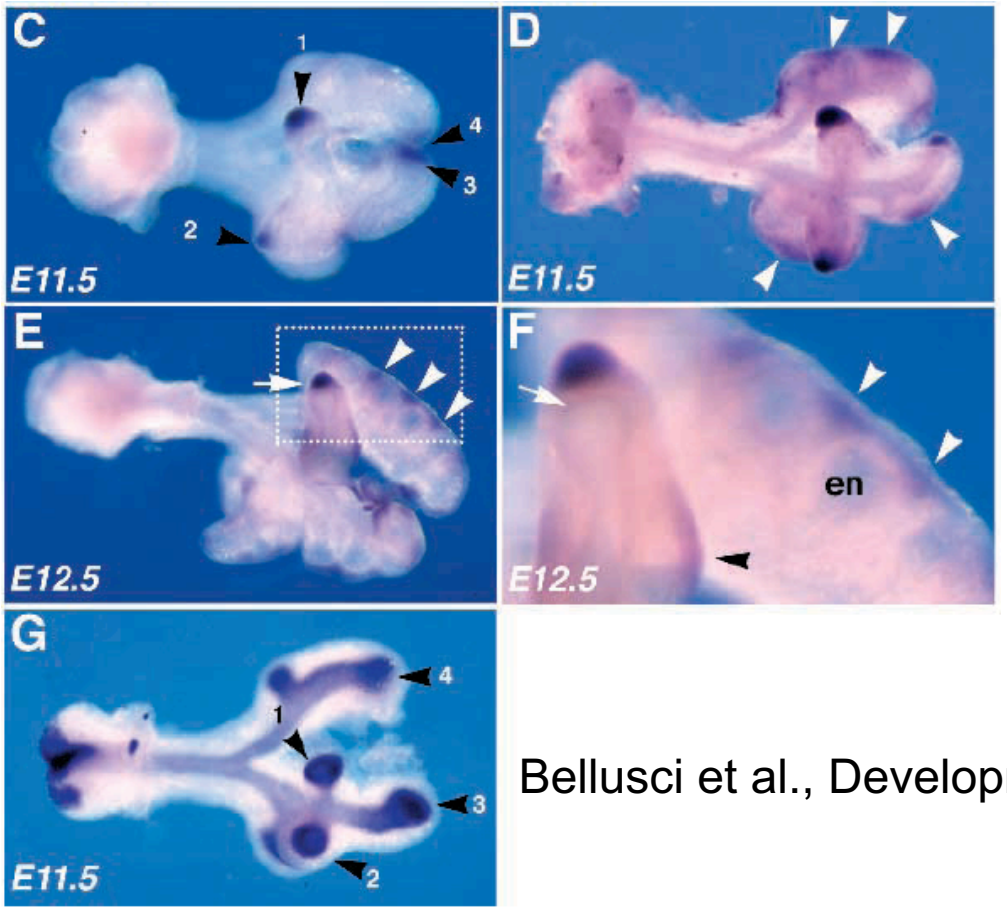
Metzger, 2008.

Signaling Feedback Loop in Lung Branching

Fgf10 is expressed at branch destination

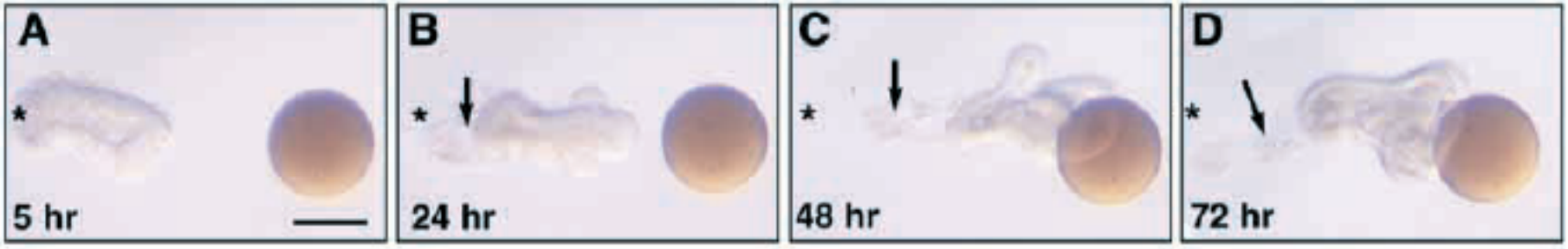






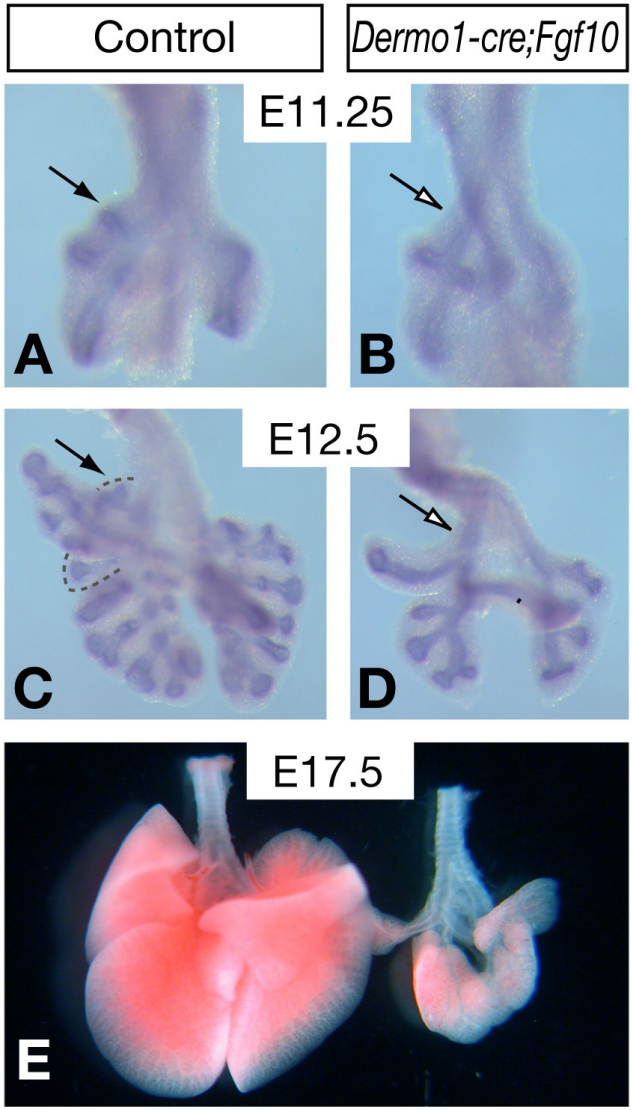
Bellusci et al., Development, 1997.

FGF10 functions as a chemoattractant for epithelial branches



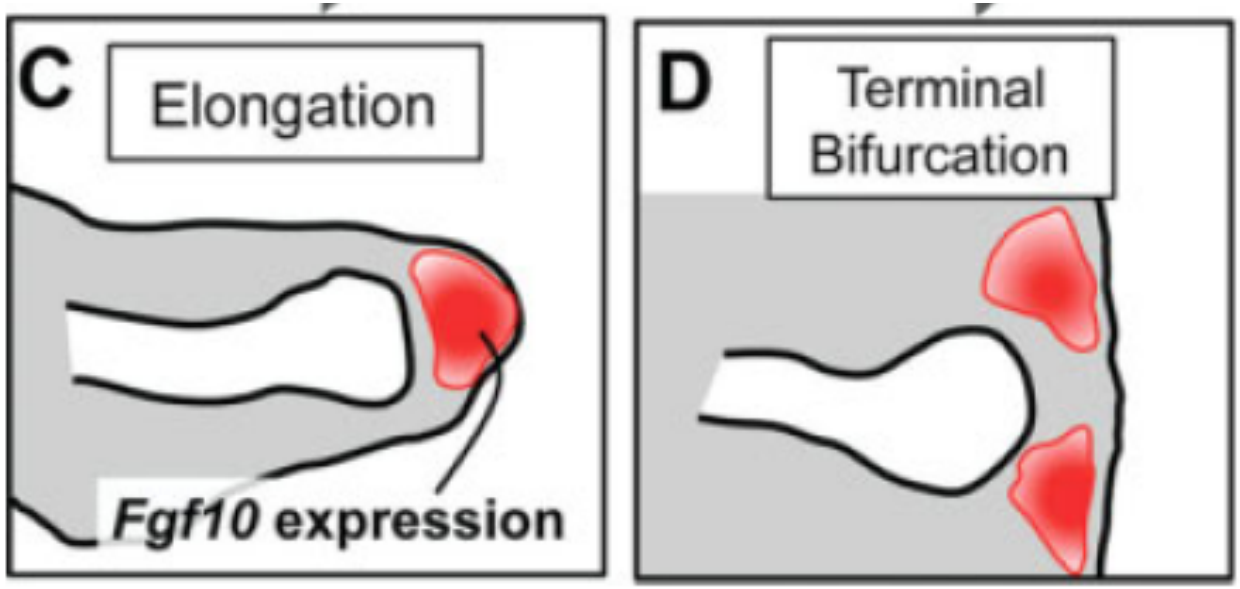
Weaver et al., Development 2000.

Fgf10 is essential for branching

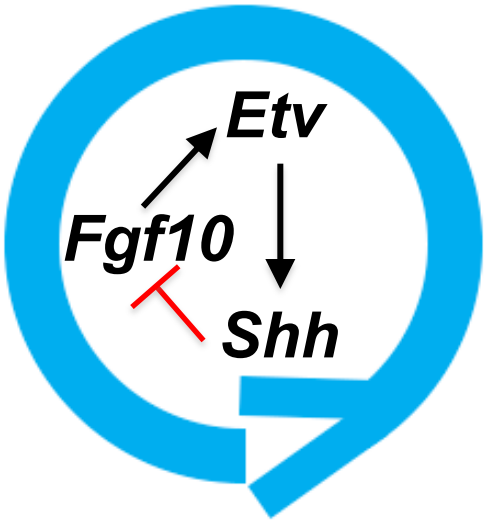


Abler, Sun et al., Dev Dyn, 2009.

FGF10-SHH feedback loop for bifurcation



Hirashima et al., 2009.



Herriges and Sun et al., DevCell, 2015.

Mathematical modeling of branching

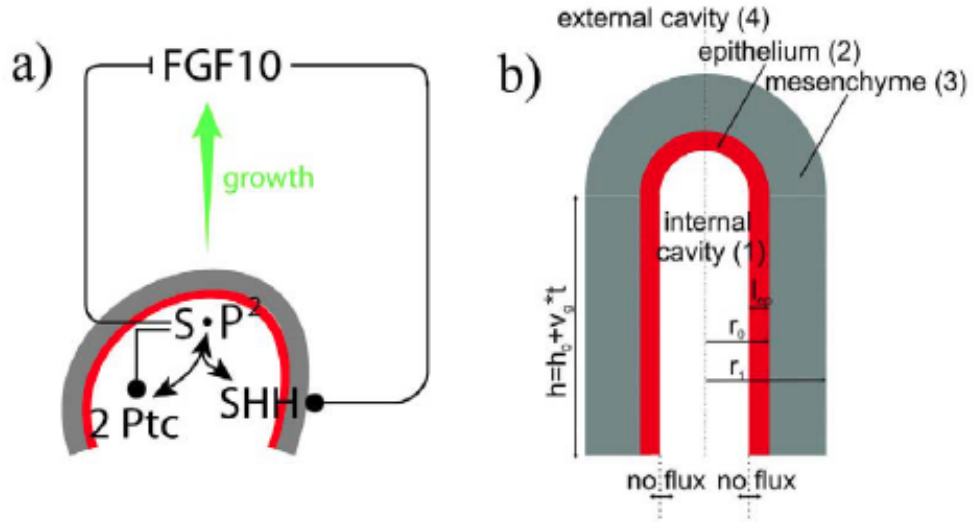
OPEN ACCESS Freely available online

PLoS COMPUTATIONAL BIOLOGY

Branch Mode Selection during Early Lung Development

Denis Menshykau[1], Conradin Kraemer[1], Dagmar Iber[1,2]*

1 Department for Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland, 2 SIB, Basel, Switzerland



$$\dot{S} = D_S \Delta S + \rho_S \frac{F^n}{F^n + 1} - \delta_S S - \delta_C P^2 S$$

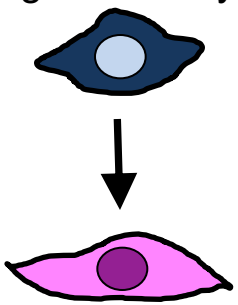
$$\dot{P} = D_P \Delta P + \rho_P - \delta_P P + (v - 2\delta_C) P^2 S$$

$$\dot{F} = \Delta F + \rho_F \frac{1}{(P^2 S)^n + 1} - \delta_F F$$

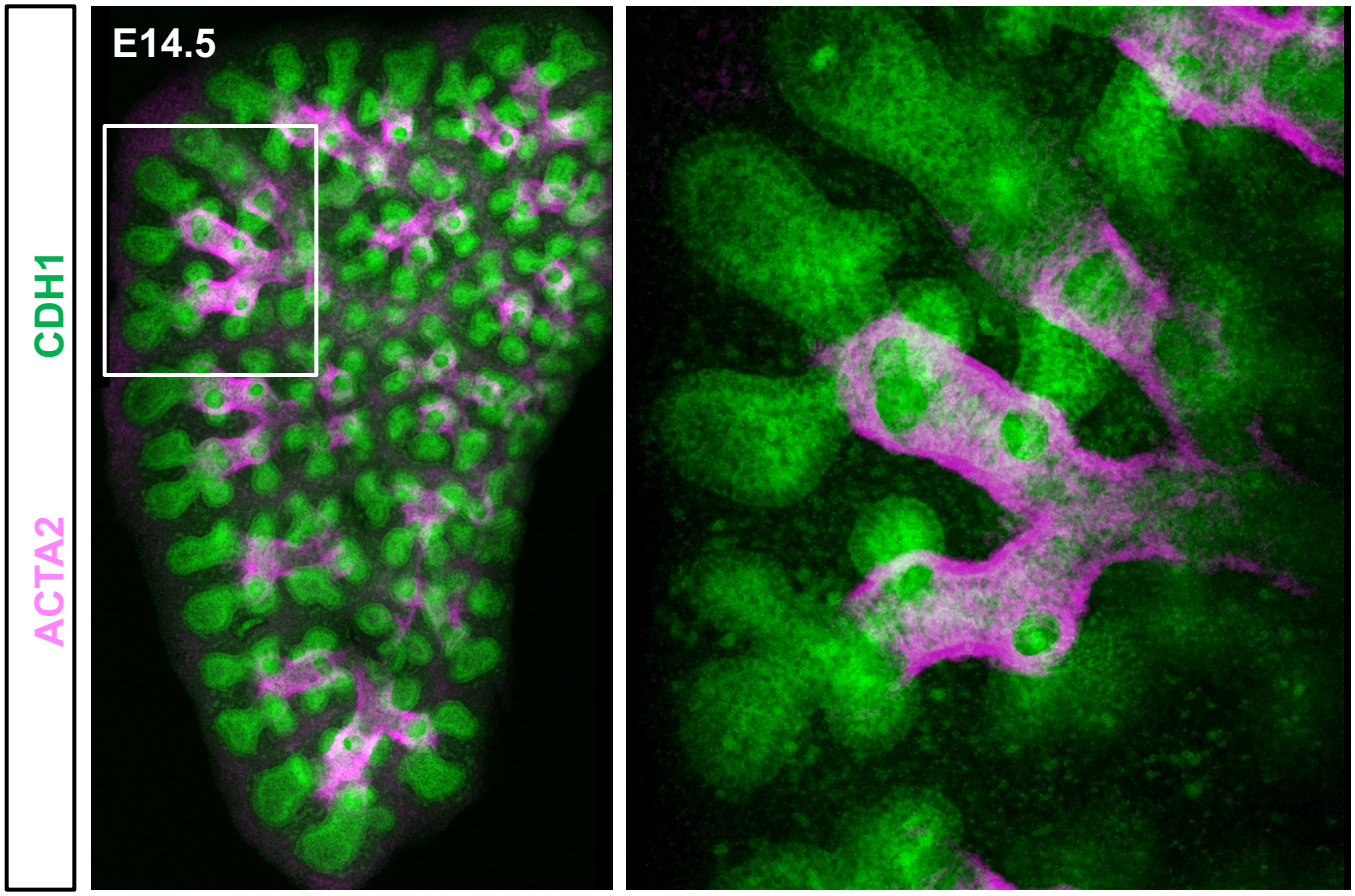
Airway Smooth Muscle Cells in Lung Branching

Airway smooth muscle surrounds the airway epithelium

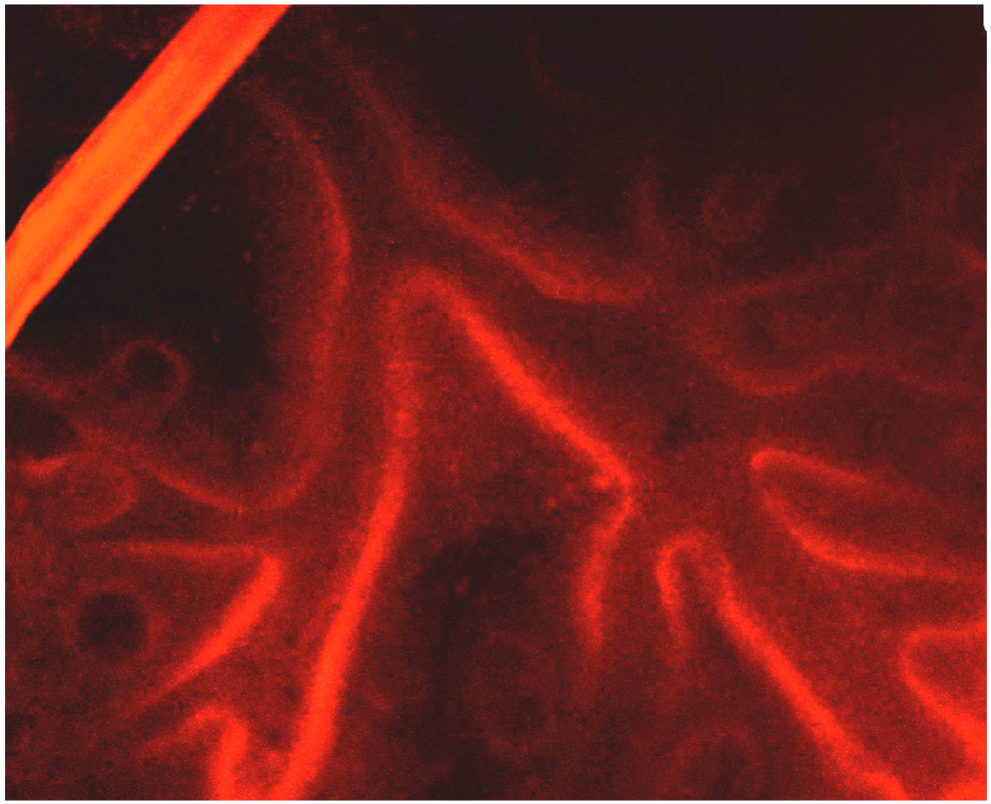
Undifferentiated Lung Mesenchyme



Airway Smooth Muscle



Prevailing hypothesis: Airway smooth muscle promotes lung branching



Airway Smooth Muscle

↓

Airway Smooth Muscle Contraction

↓

Airway Peristalsis

↓

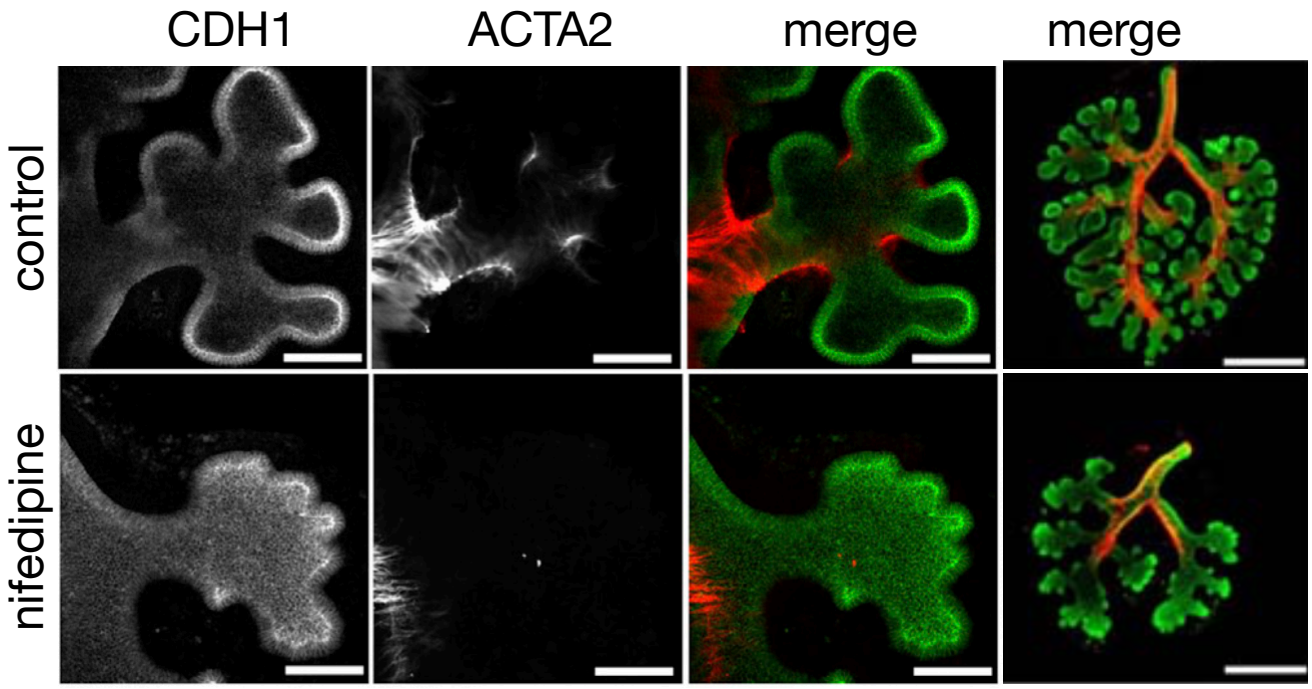
Airway Pressure

↓

Lung Branching

Brennan et al. *PLOS One* 2013

Chemical inhibition of smooth muscle contraction disrupts epithelial branching *ex vivo*



Kim and Nelson et. al. *Developmental Cell,* 2015
Goodwin and Nelson et al. *Development* 2019

Airway Smooth Muscle

↓

Airway Smooth Muscle Contraction

↓

Airway Peristalsis

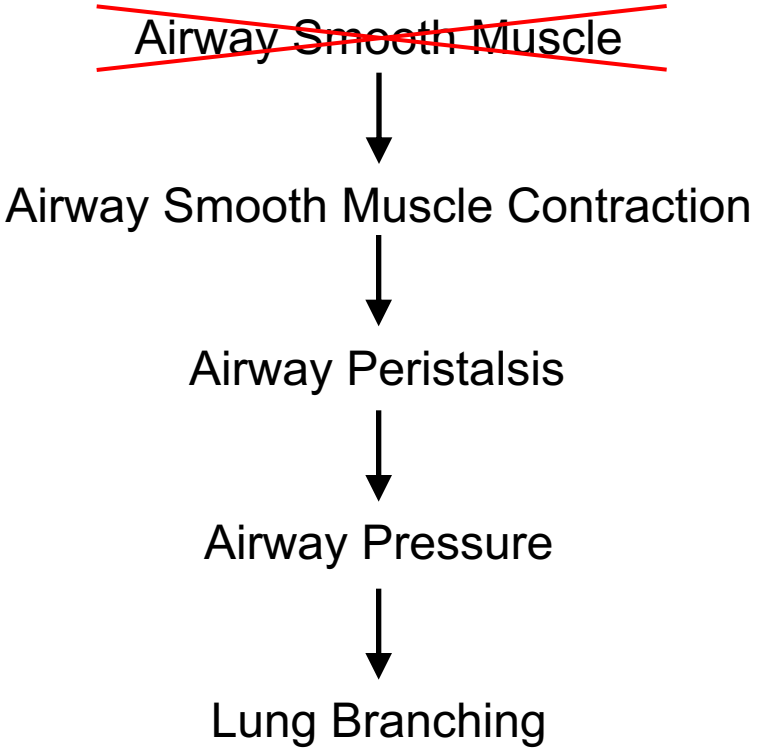
↓

Airway Pressure

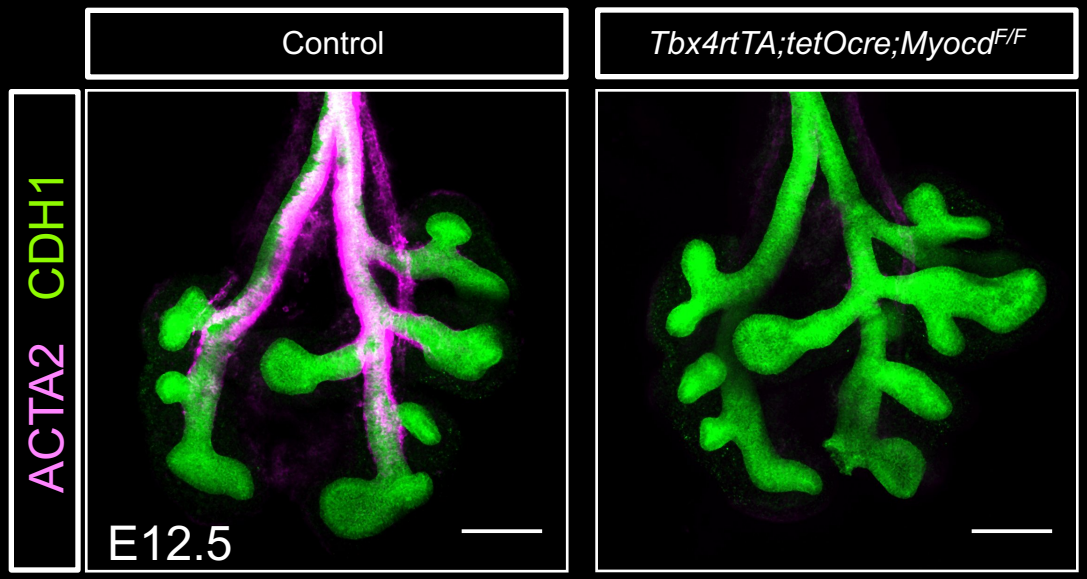
↓

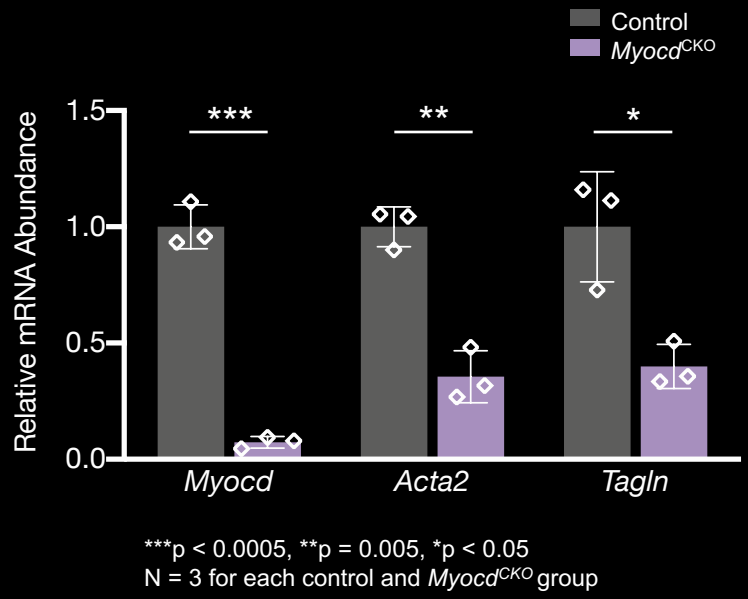
Lung Branching

In vivo testing of the prevailing hypothesis



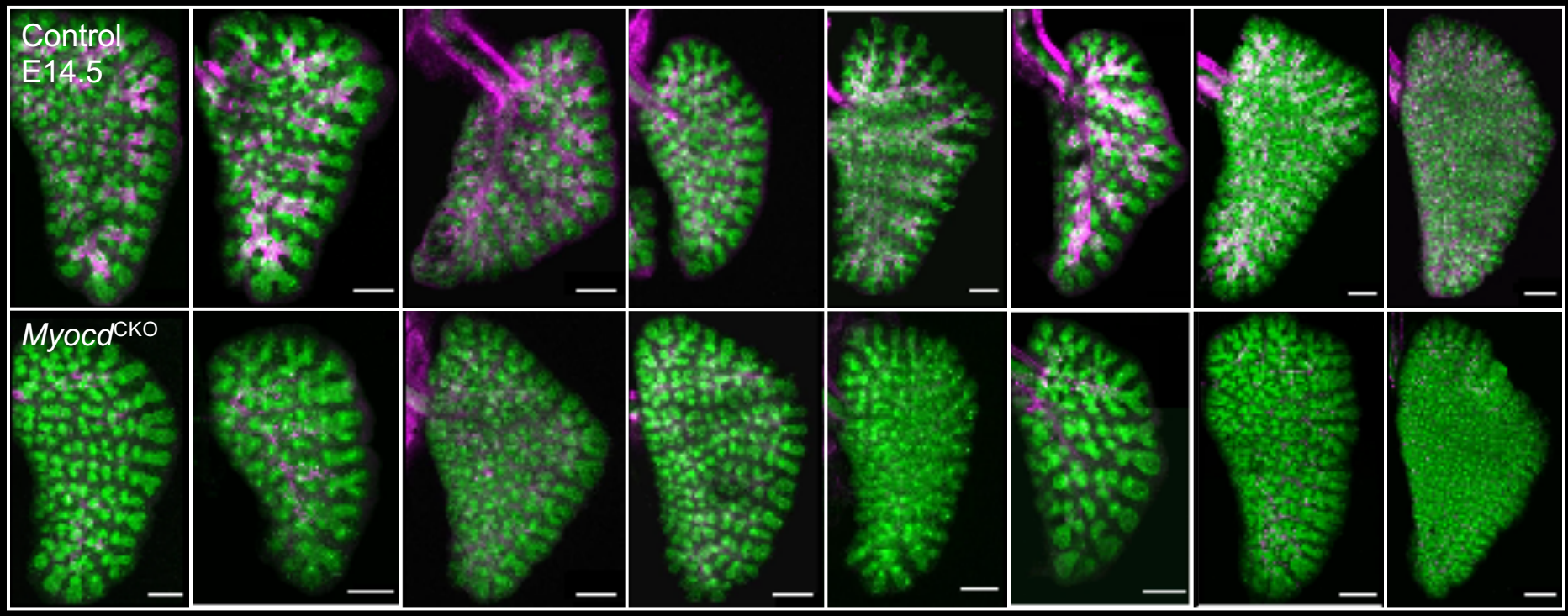
Myocd^{CKO} inactivation led to loss of airway smooth muscle



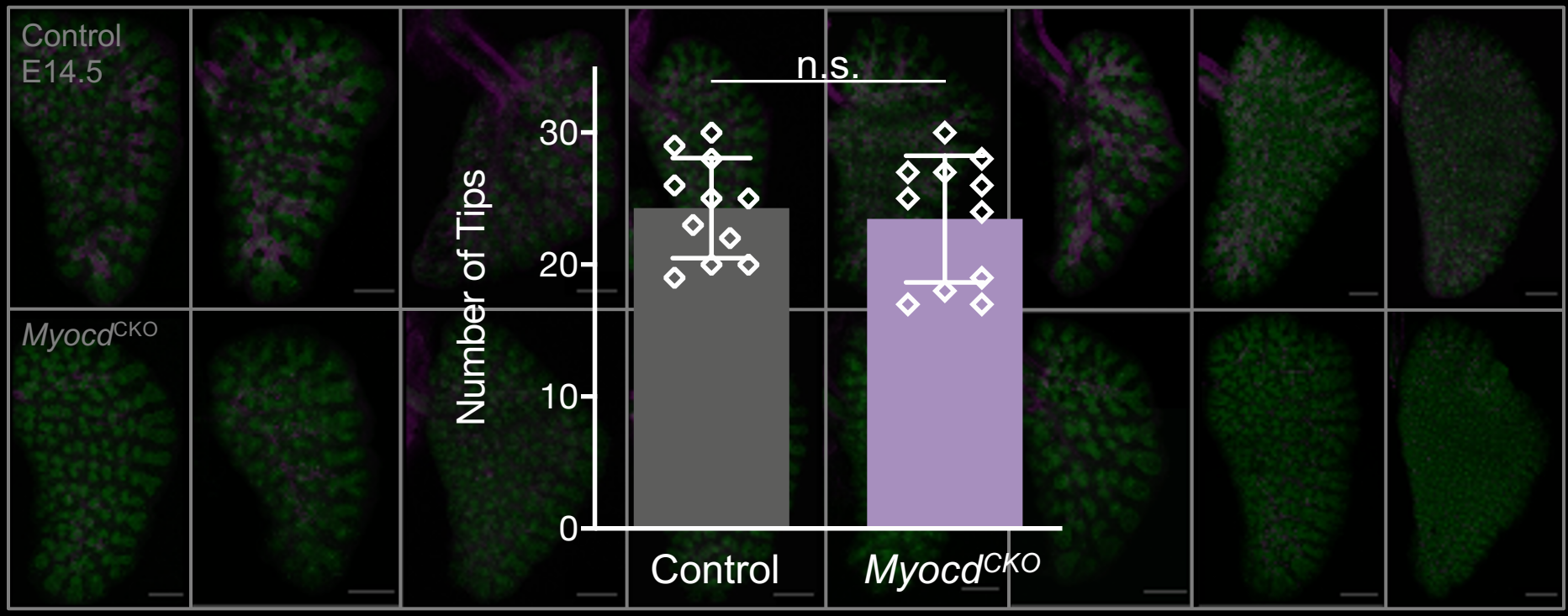


***p < 0.0005, **p = 0.005, *p < 0.05
N = 3 for each control and *Myocd*^{CKO} group

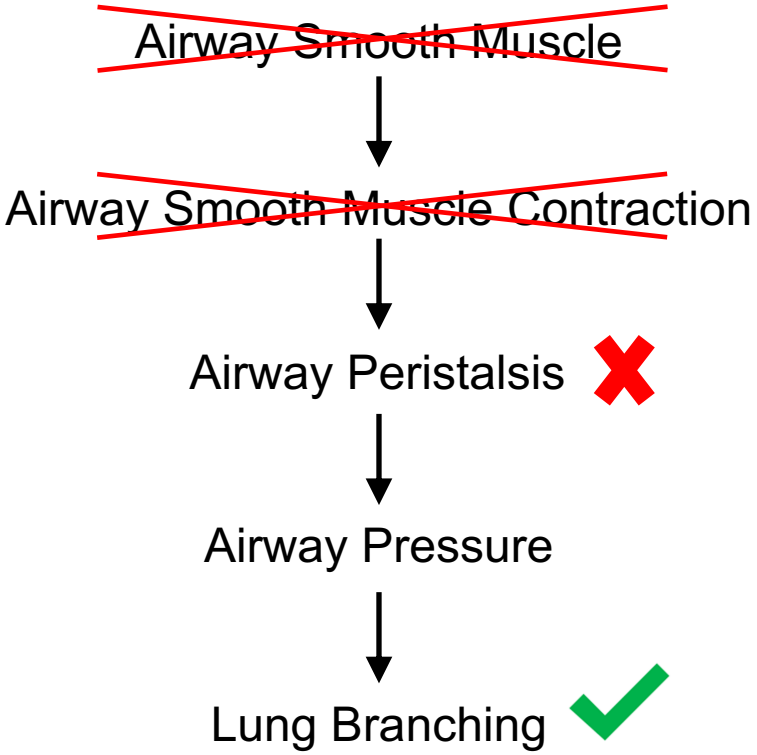
Inhibiting airway smooth muscle differentiation does not disrupt lung epithelial branching

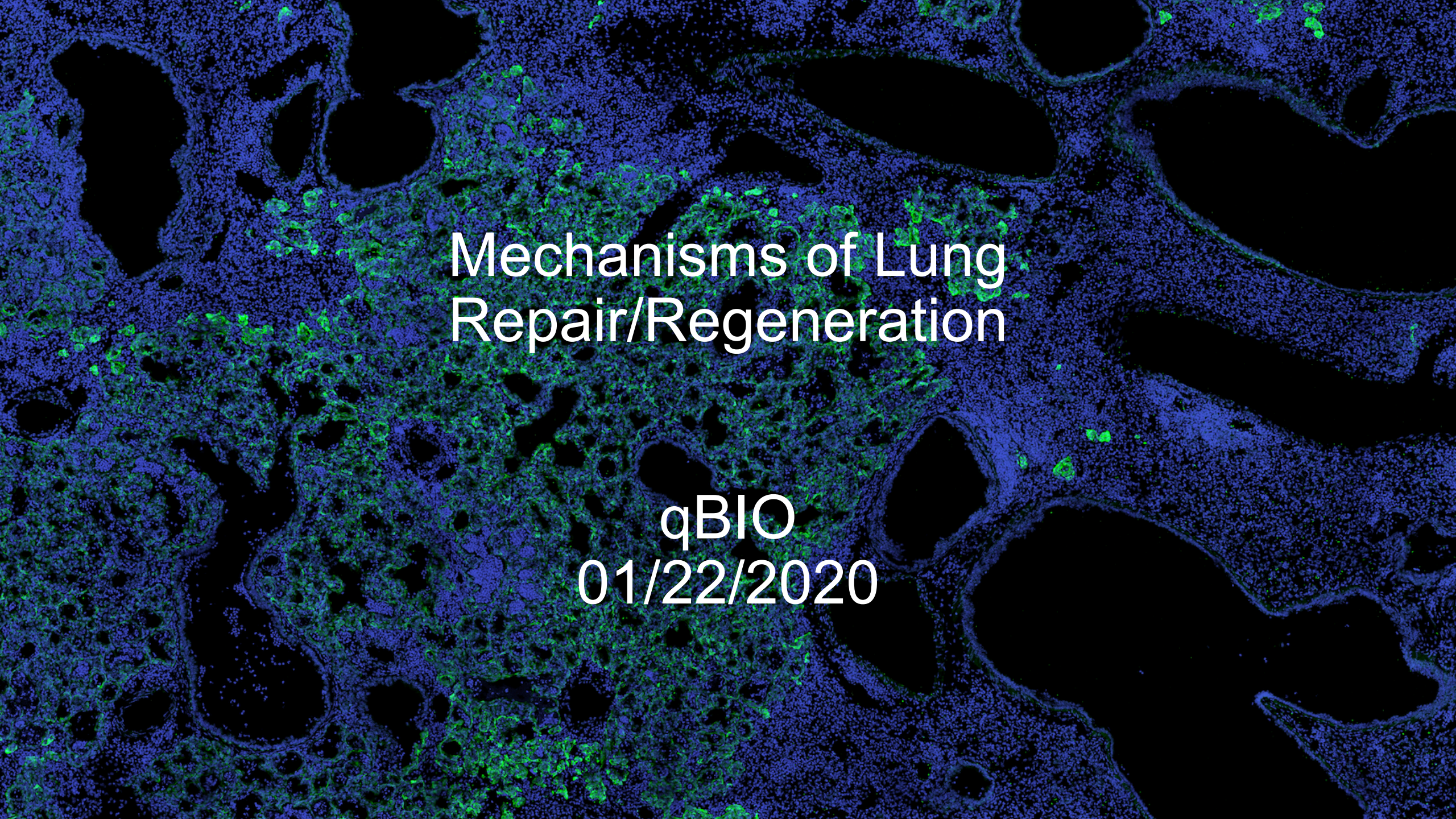


Inhibiting airway smooth muscle differentiation does not disrupt lung epithelial branching



Airway smooth muscle is dispensable for lung branching morphogenesis in vivo



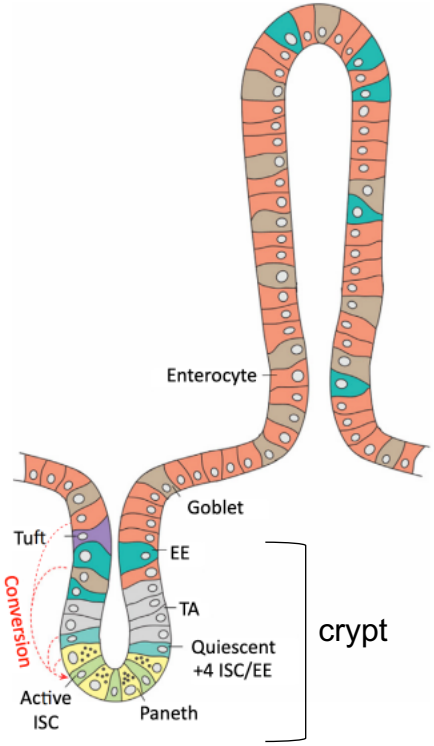


Mechanisms of Lung Repair/Regeneration

qBIO
01/22/2020

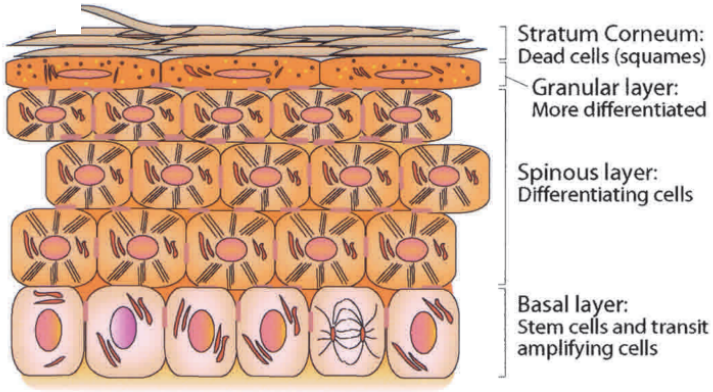
Respiratory epithelial cell turnover is relatively slow at homeostasis

Intestinal crypt



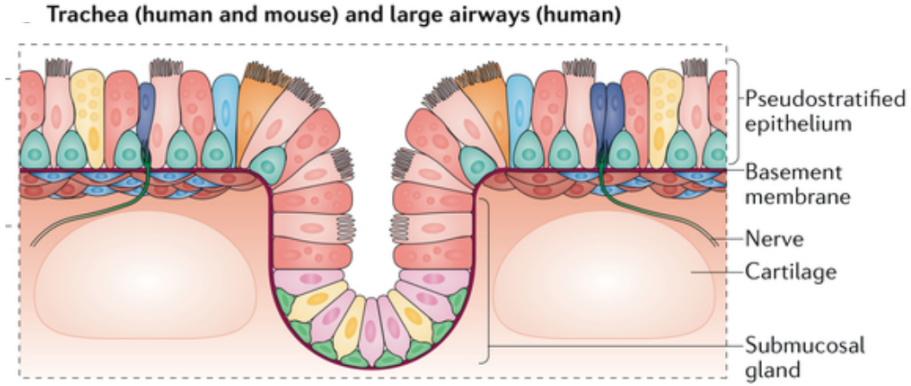
- Turnover of mouse gut epithelial cells ~ 4 days

Skin epidermis

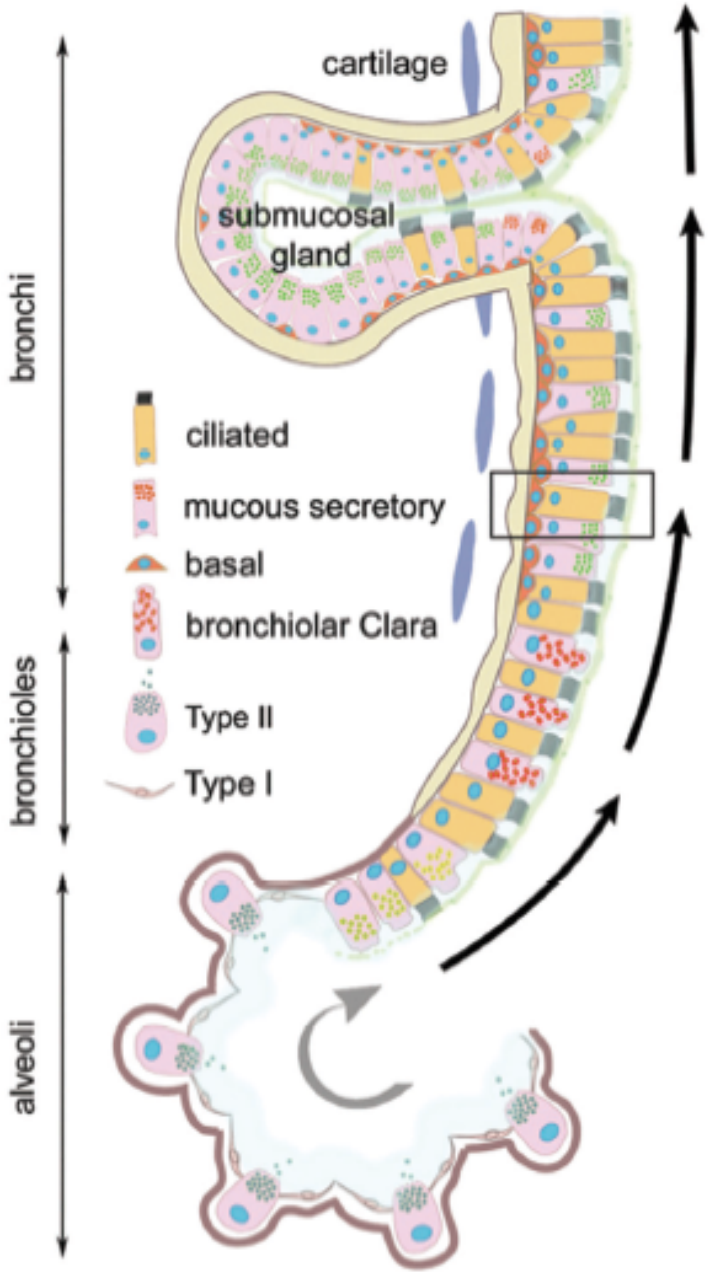


- Turnover of mouse skin ~2 weeks

Trachea-bronchial epithelial cells



- Turnover of mouse Trachea-bronchial epithelial cells ~4 months

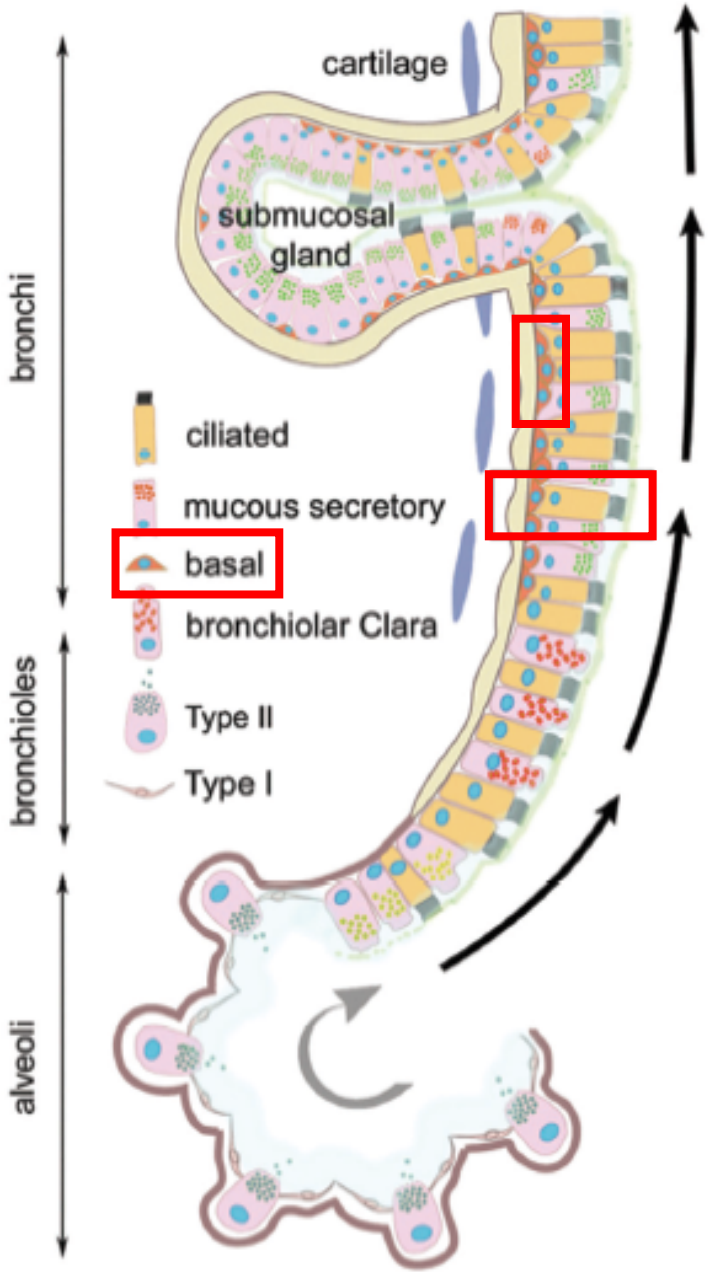


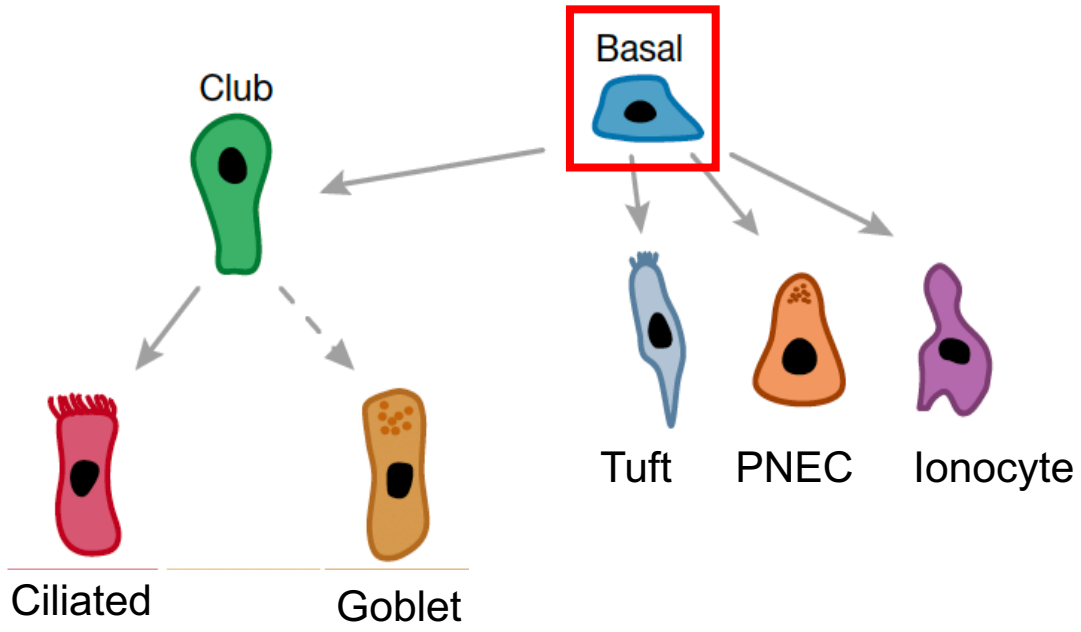
Epithelial progenitor cells within the lung

1. Basal cells (airway)

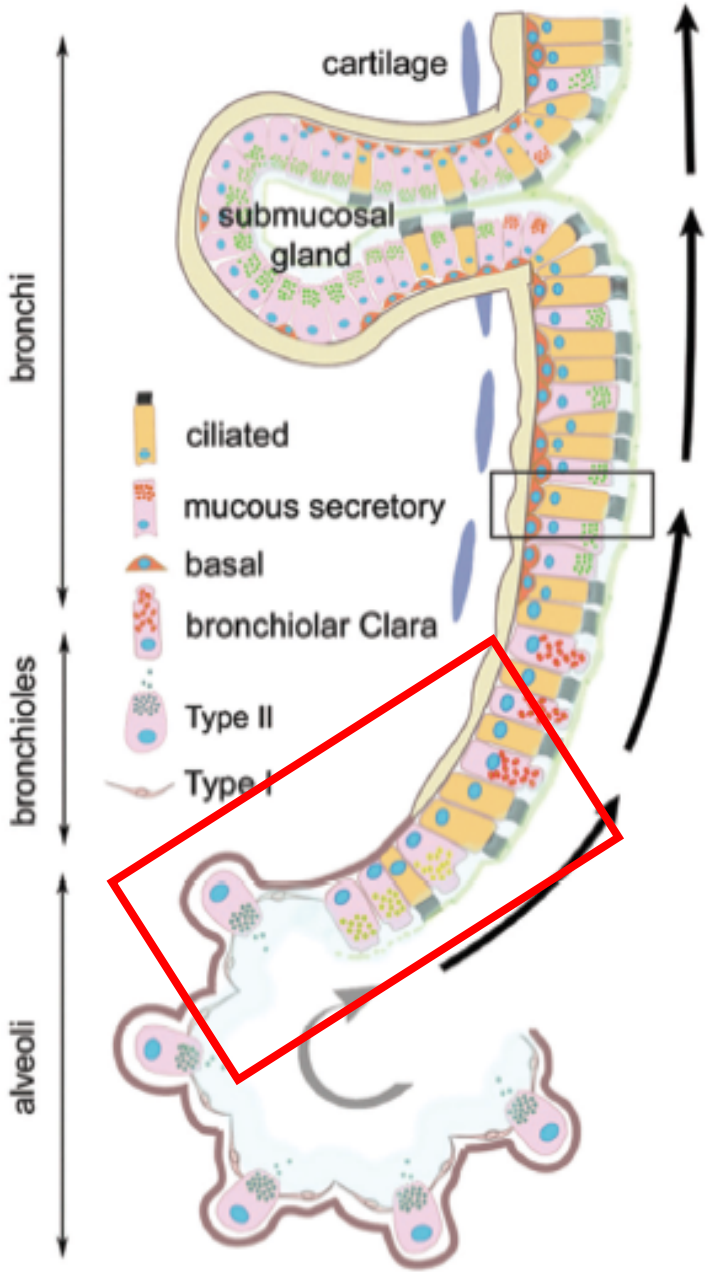
2. Alveolar type 2 cells (alveolar compartment)

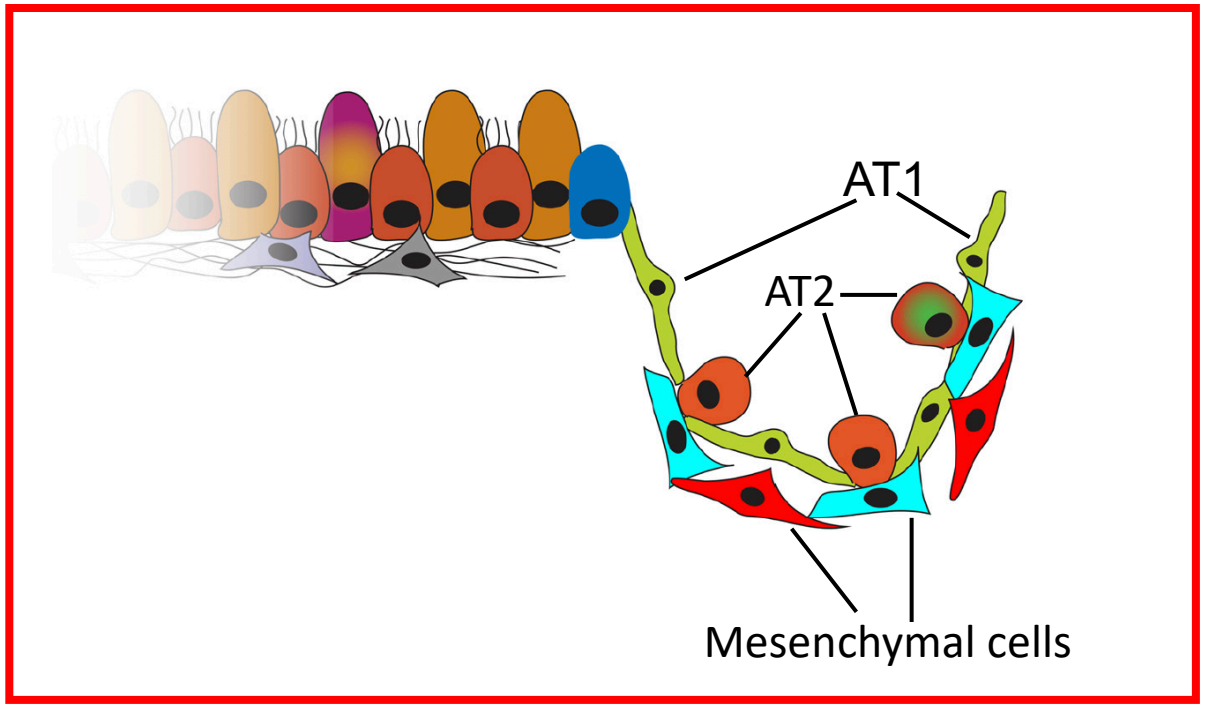
1. Airway Basal Cells



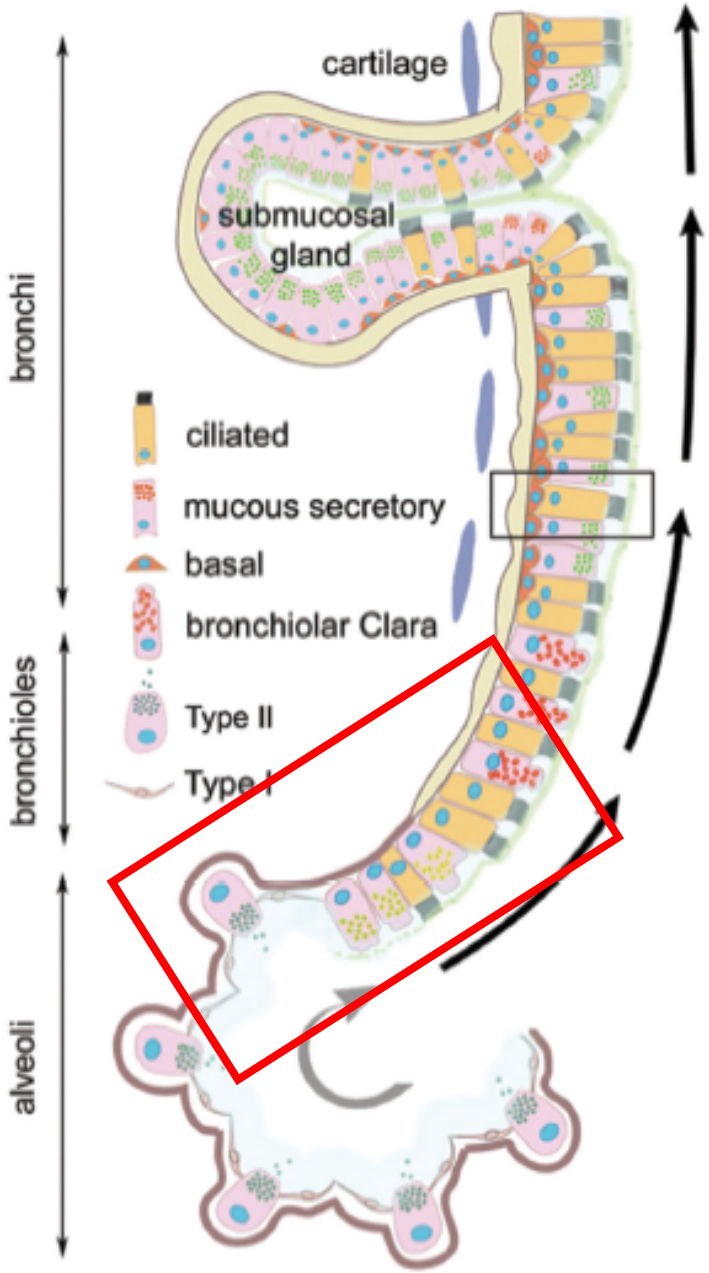


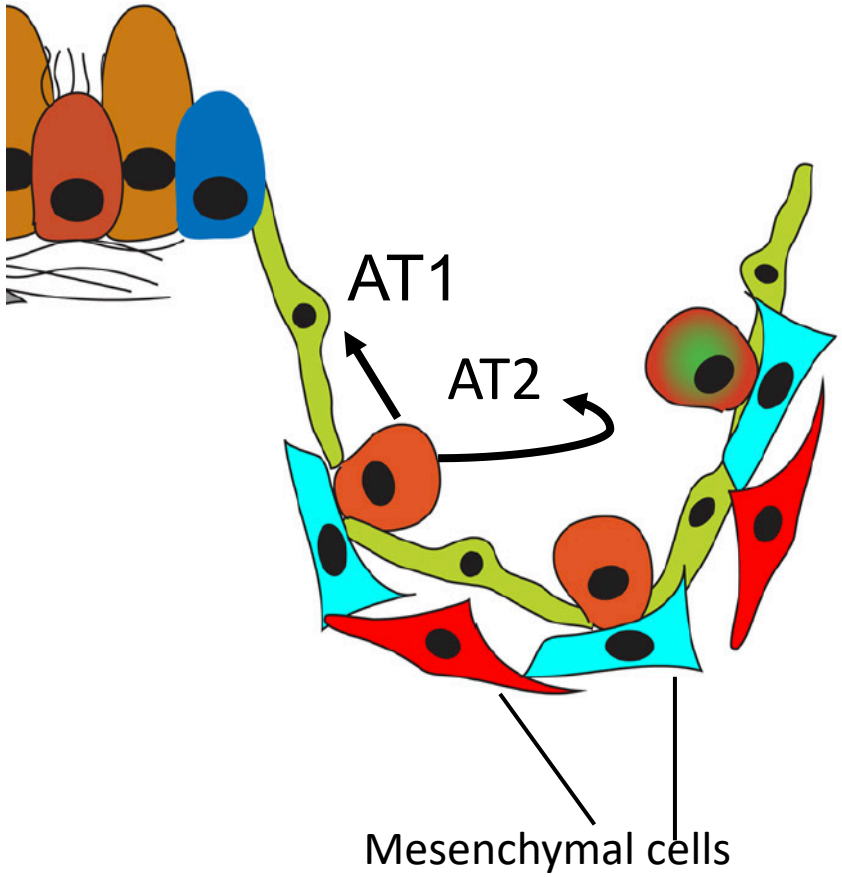
2. Alveolar Epithelial Cells: AT1 and AT2





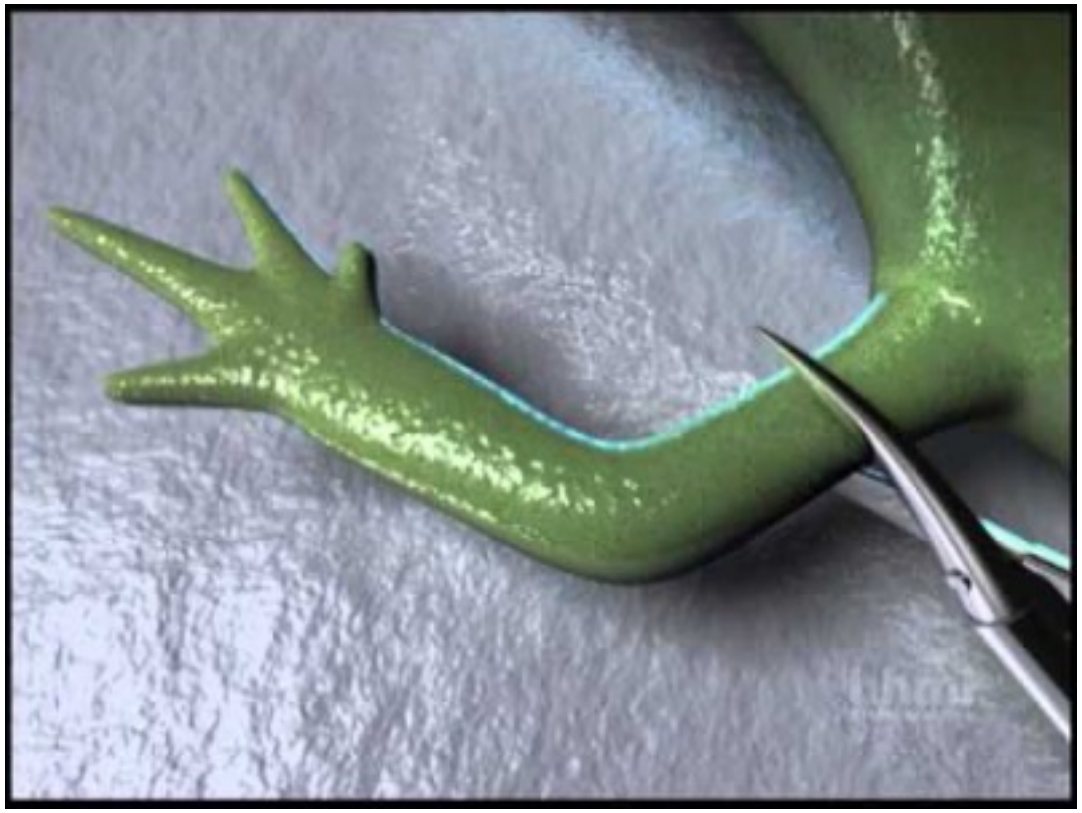
2. Alveolar Epithelial Cells: AT2 Progenitor Function





2 Activation Settings for Progenitor Cell Function: Regeneration vs Repair

1. Regeneration: recapitulation of structurally and cellular normal tissue, the ideal form of healing.
Renewing, restoring



2 Activation Settings for Progenitor Cell Function: Regeneration vs Repair

1. **Regeneration:** recapitulation of structurally and cellular normal tissue, the ideal form of healing.
   Renewing, restoring

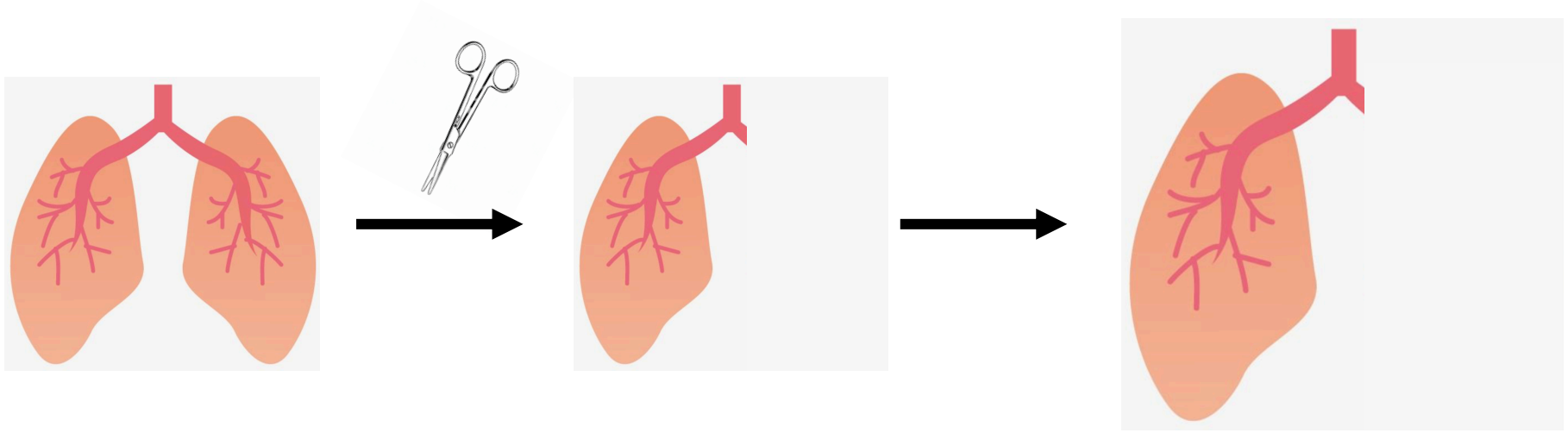
2. **Repair:** when complete regeneration cannot be achieved, and instead regeneration is combined with aberrant tissue and scar formation caused in part by a dysplastic response.
   Fix, mend

2 Activation Settings for Progenitor Cell Function: Regeneration vs Repair

2 Stories on Lung Injury Repair:

1) **Regeneration: Compensatory growth following pneumonectomy**
2) Repair: Influenza injury of the lung

Lung regeneration*: Pneumonectomy (PNX) as a model of compensatory growth

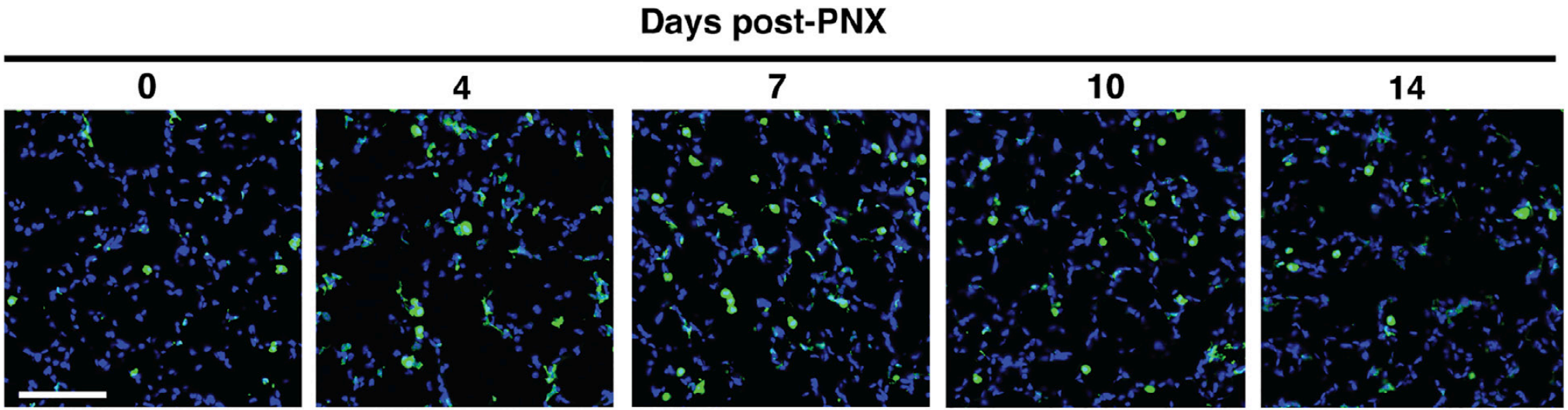


1) Unilateral removal of 1 lobe
2) Growth of remaining lobe

Which cells act as progenitor cells?
What signals are important to activate progenitors?

PNX as a model of compensatory growth

What changes are observed in the lung during the growth stage?



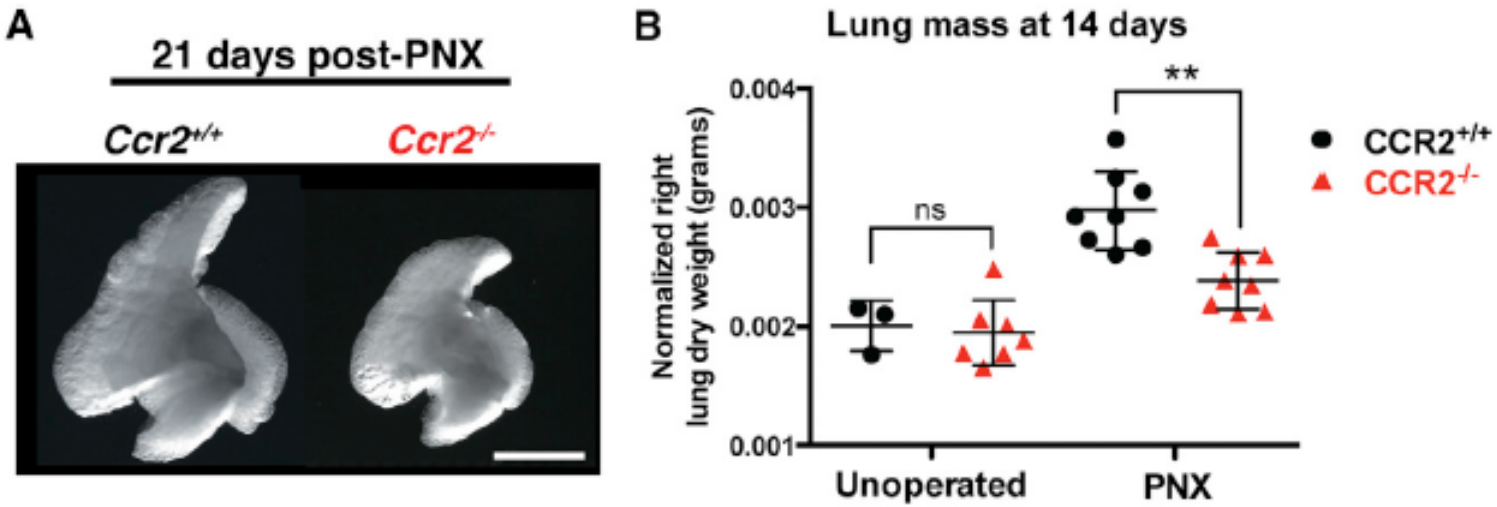
Macrophages and monocytes

Monocytes are recruited into the lung from circulation
Is this immune population important for regeneration?

Recruited monocytes and Th2 cytokine signaling promote lung regeneration following pneumonectomy
Cell Stem Cell, July 2018

PNX as a model of compensatory growth

CCL2-CCR2 signaling is important for monocyte recruitment
Is this functionally important for growth following PNX?

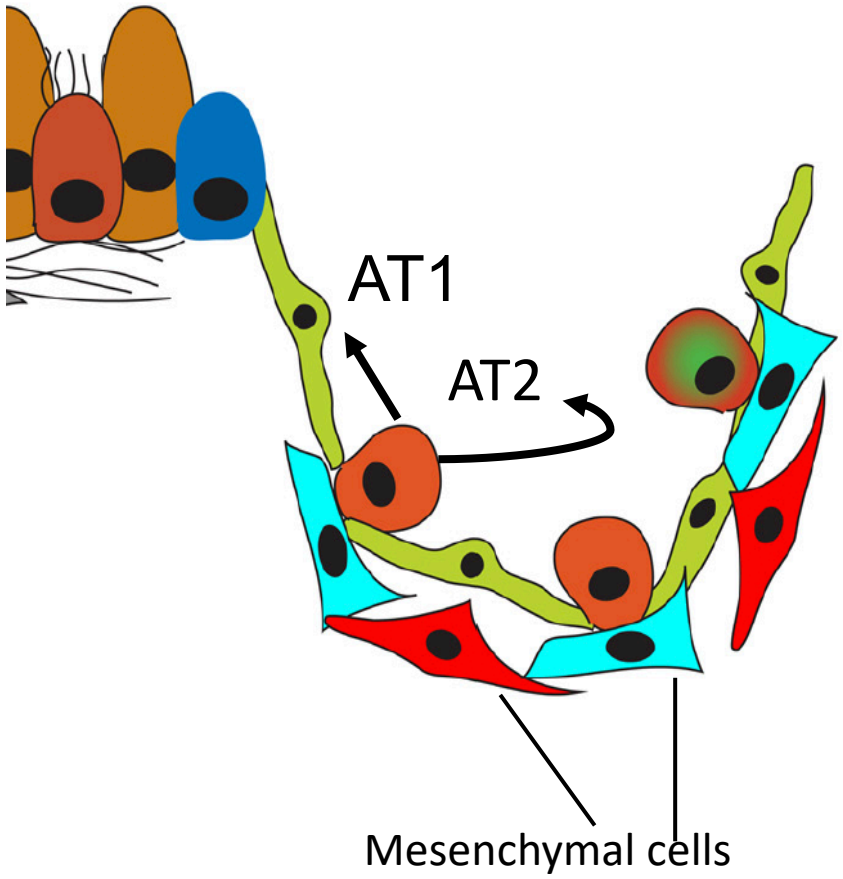


How do recruited monocytes positively regulate growth following PNX?

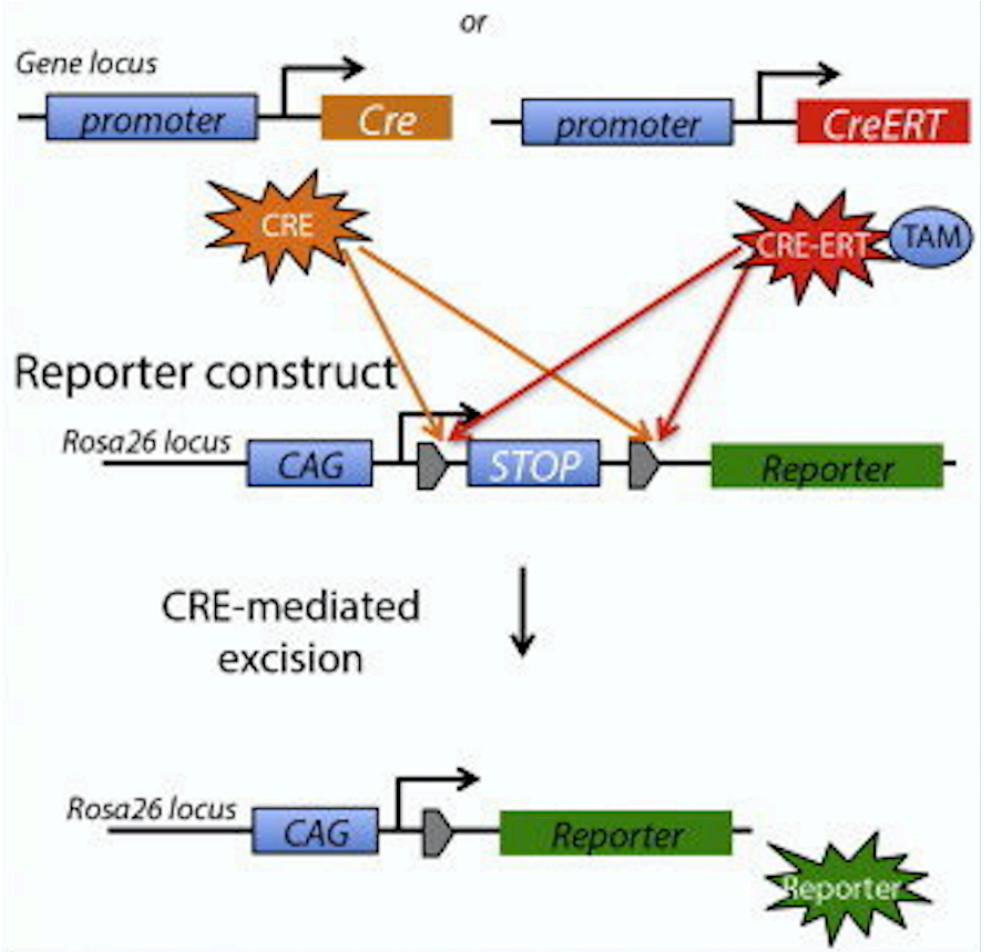
Recruited monocytes and Th2 cytokine signaling promote lung regeneration following pneumonectomy
Cell Stem Cell, July 2018

PNX as a model of compensatory growth

How do recruited monocytes positively regulate growth following PNX?



Lineage tracing strategy



PNX as a model of compensatory growth

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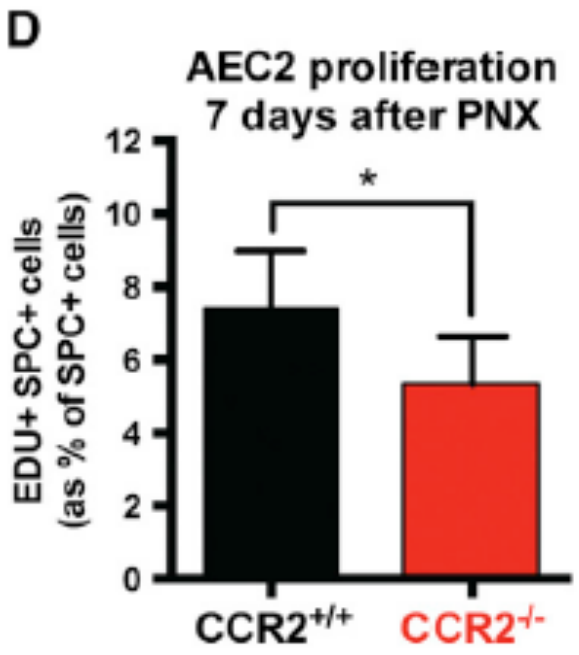
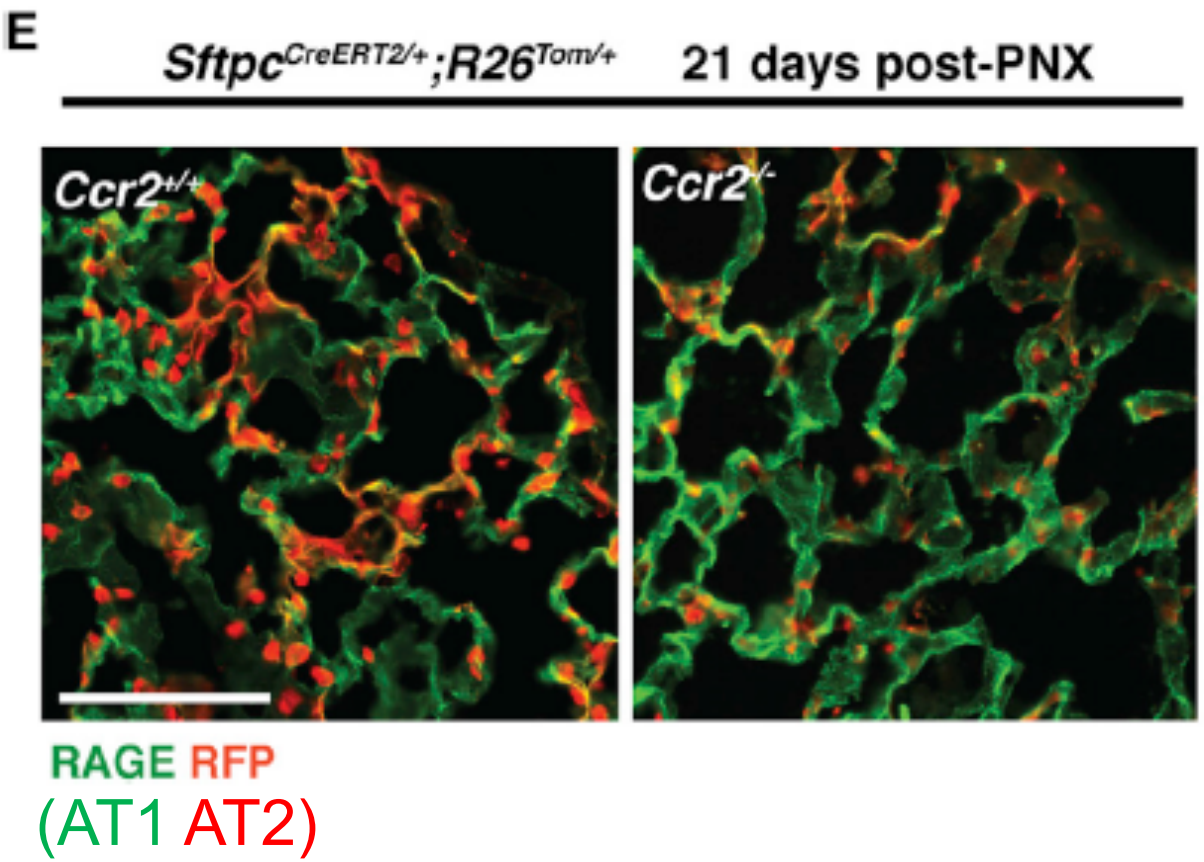
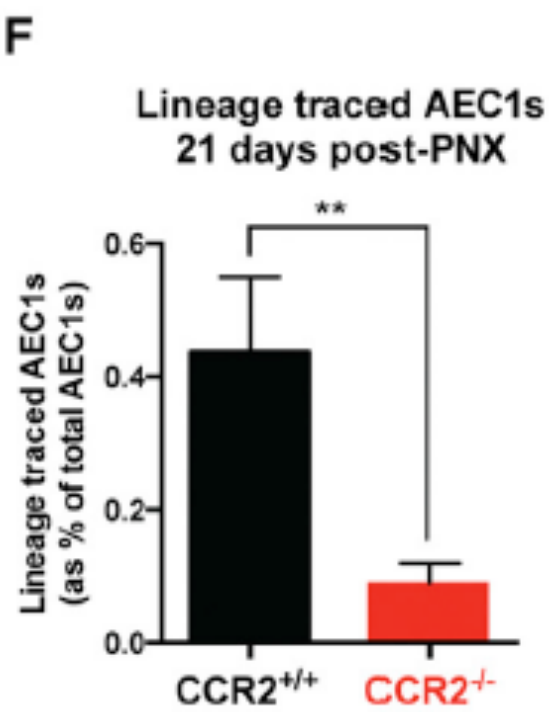






Recruited monocytes and Th2 cytokine signaling promote lung regeneration following pneumonectomy
Cell Stem Cell, July 2018

PNX as a model of compensatory growth

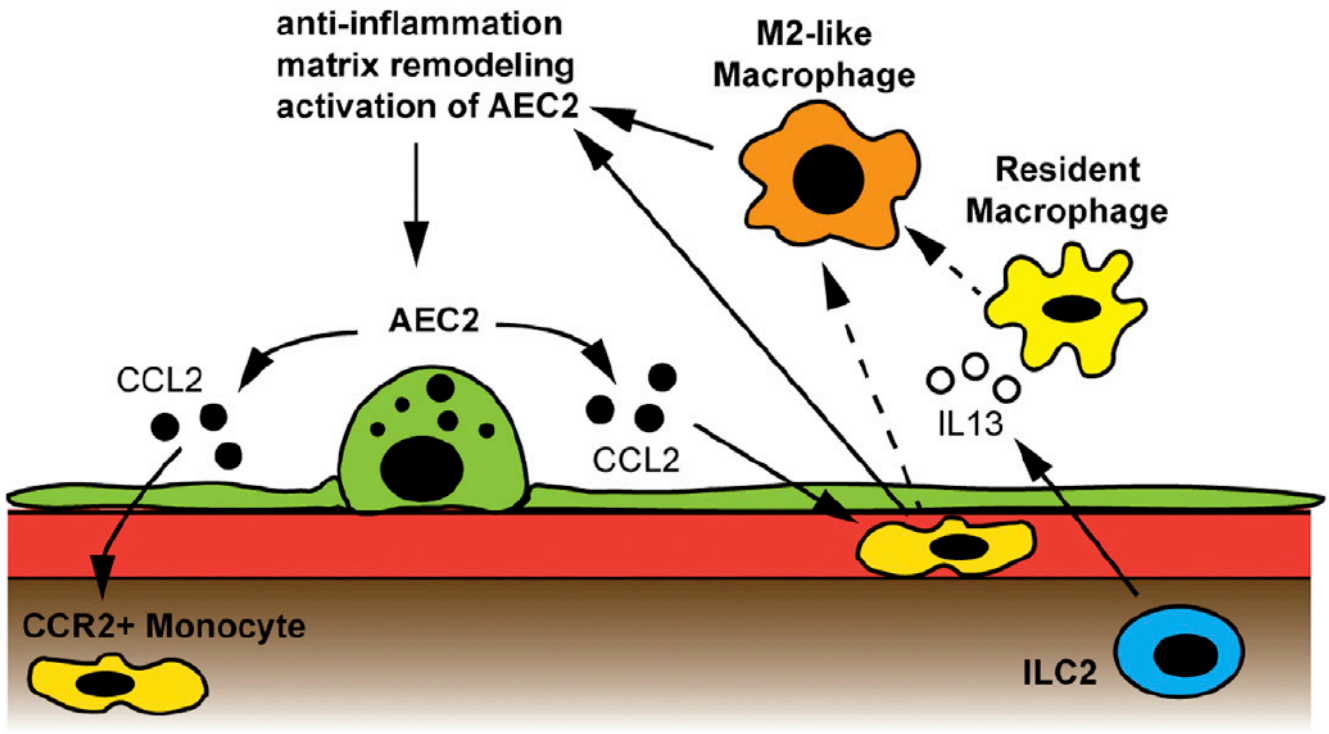
CCL2-CCR2 dependent recruitment

↓

Polarization of tissue resident macrophages

↓

Activation of AT2 progenitor function



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Cell Stem Cell, July 2018

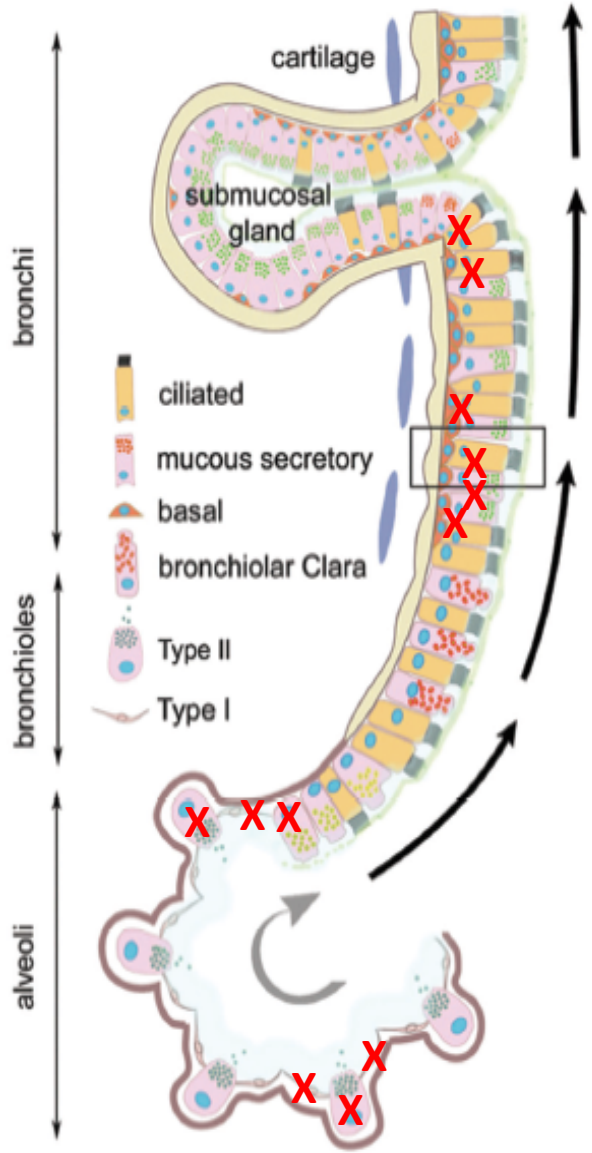
2 Activation Settings for Progenitor Cell Function: Regeneration vs Repair

2 Stories on Lung Injury Repair:

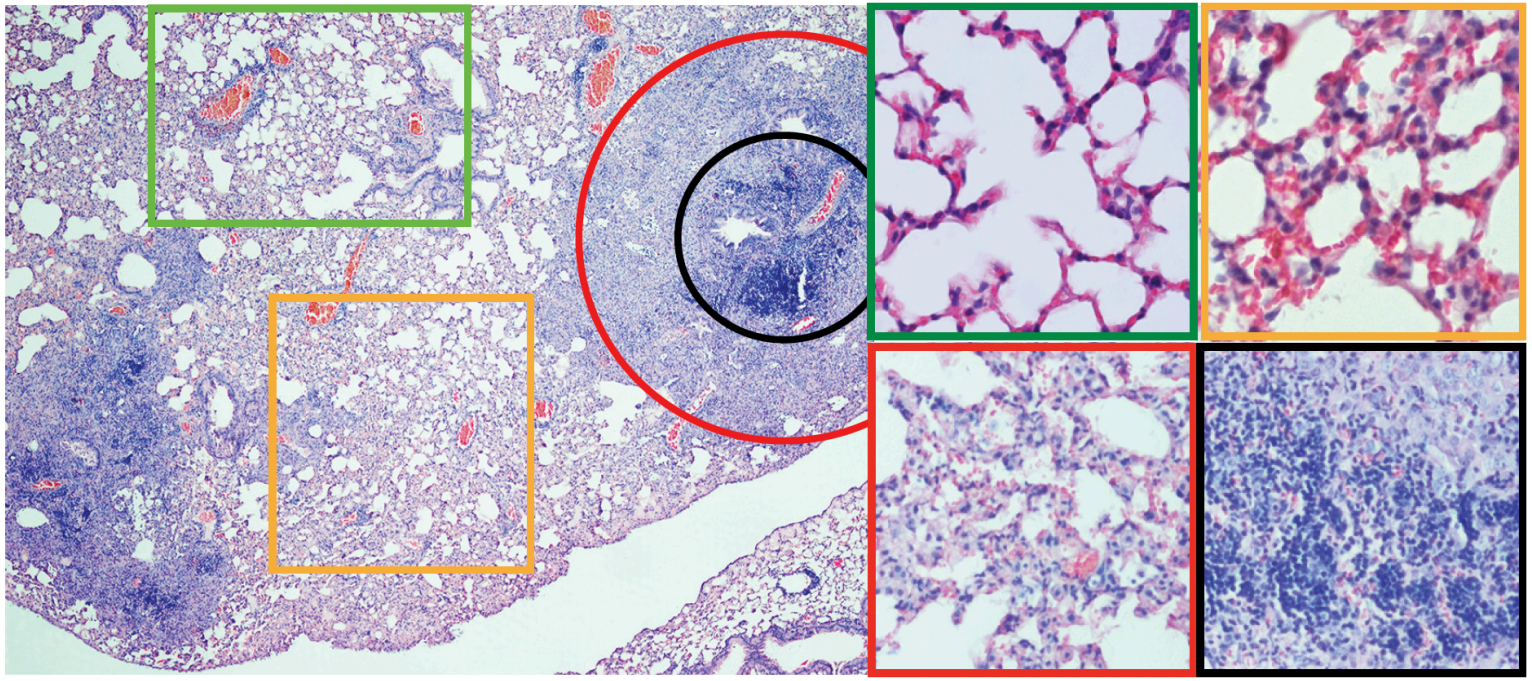
1) Regeneration: Compensatory growth following pneumonectomy

2) **Repair: Influenza injury of the lung**

Lung repair after flu: progenitor activation and dysplastic response



Zones of injury

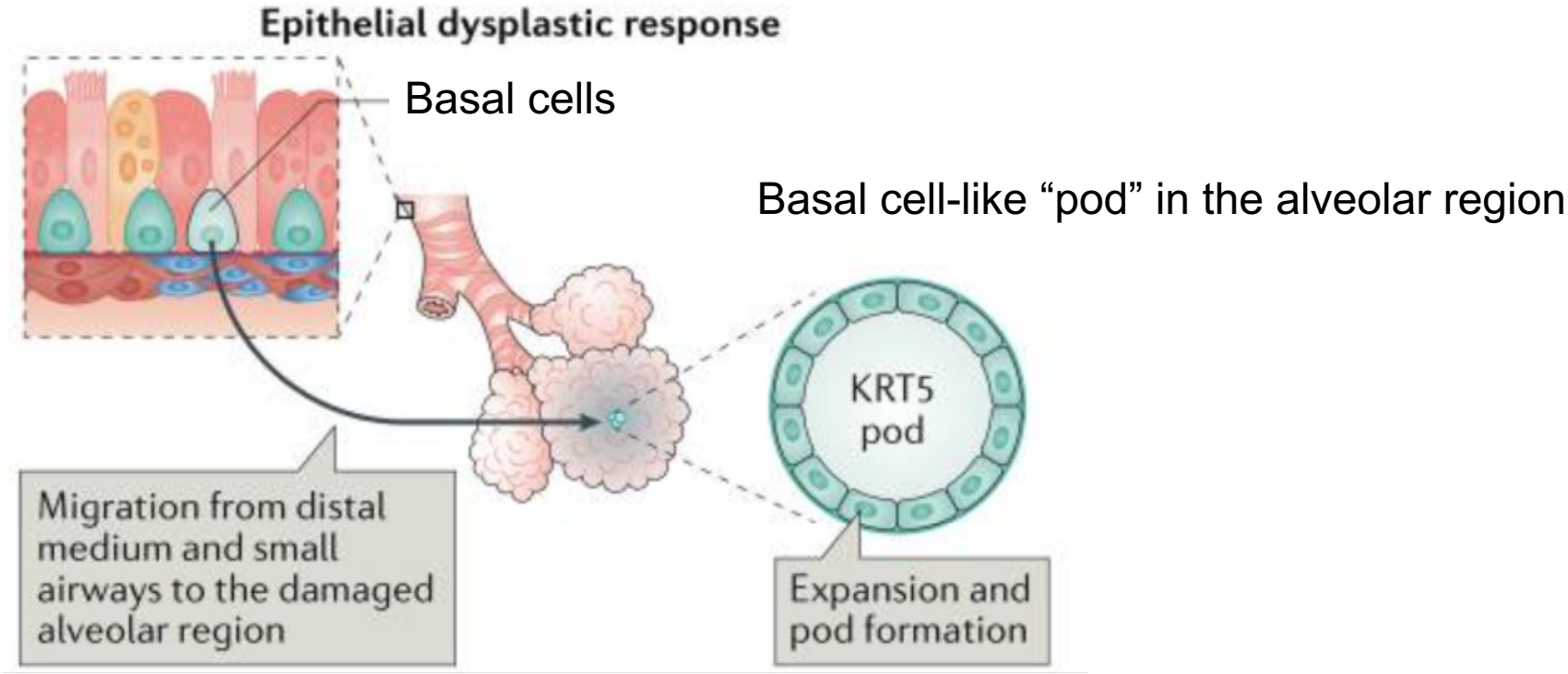


Zone 1	Zone 2	Zone 3	Zone 4
Normal	Minor Injury	Injured	Severe injury

Regeneration of the lung alveolus by an evolutionary conserved epithelial progenitor
Nature, March 2018

What is the nature of repair within different zones of injury?

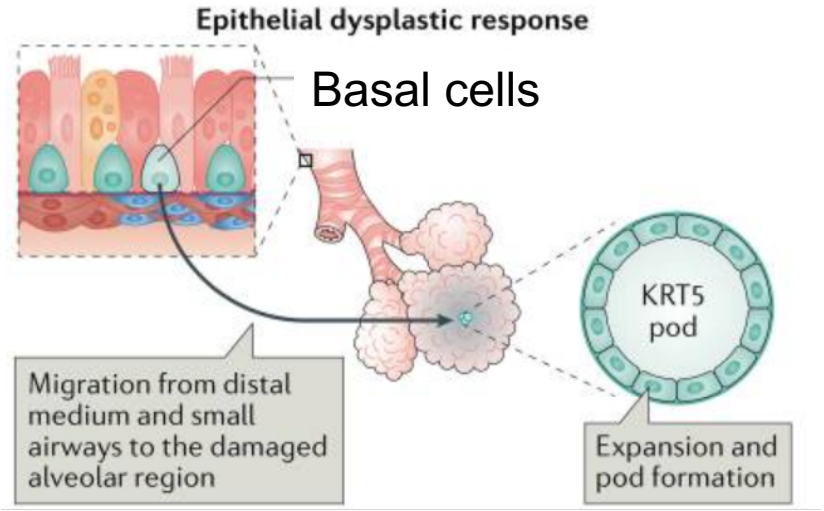
Zone 4, Severe Injury: Dysplastic response



Activation of airway basal cells to seal the wound;
Scarring leads to permanent loss of gas exchange surface area

What is the nature of repair within different zones of injury?

Zone 4, Severe Injury: Dysplastic response



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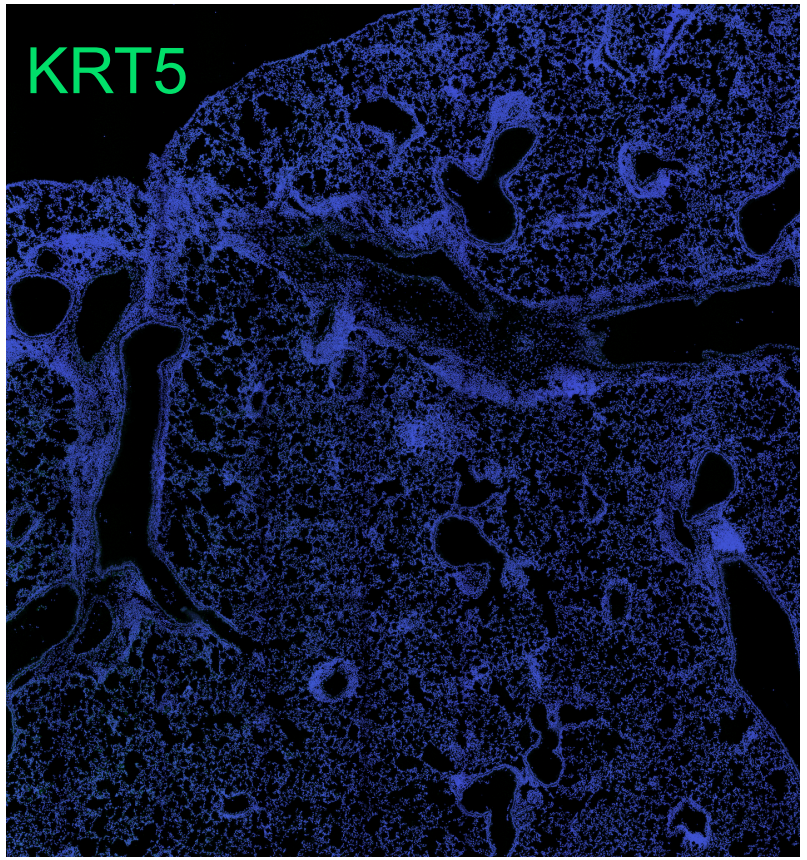
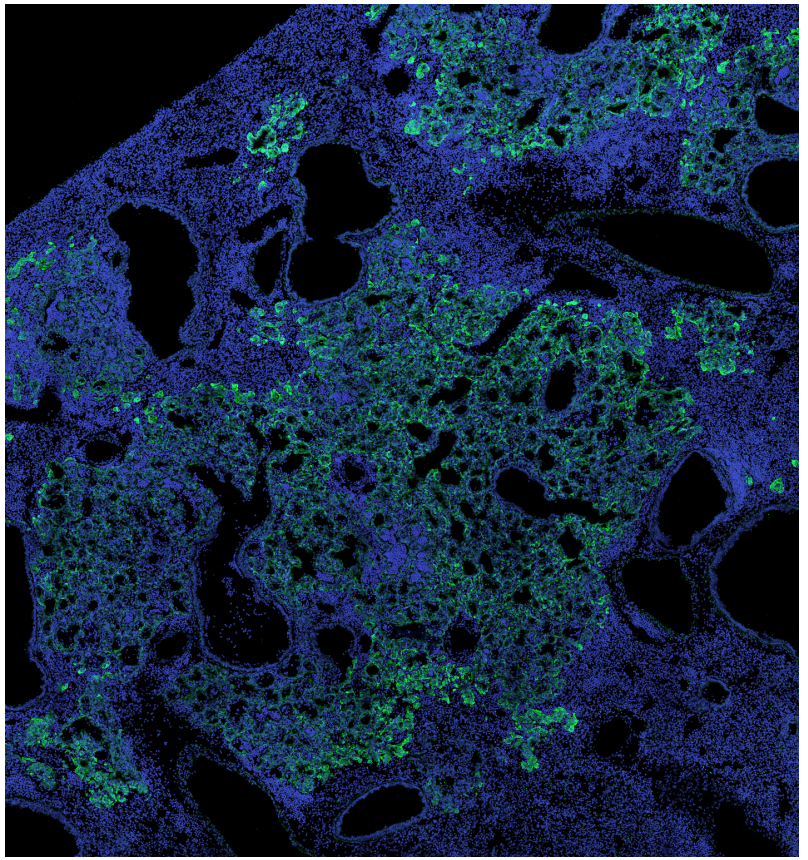


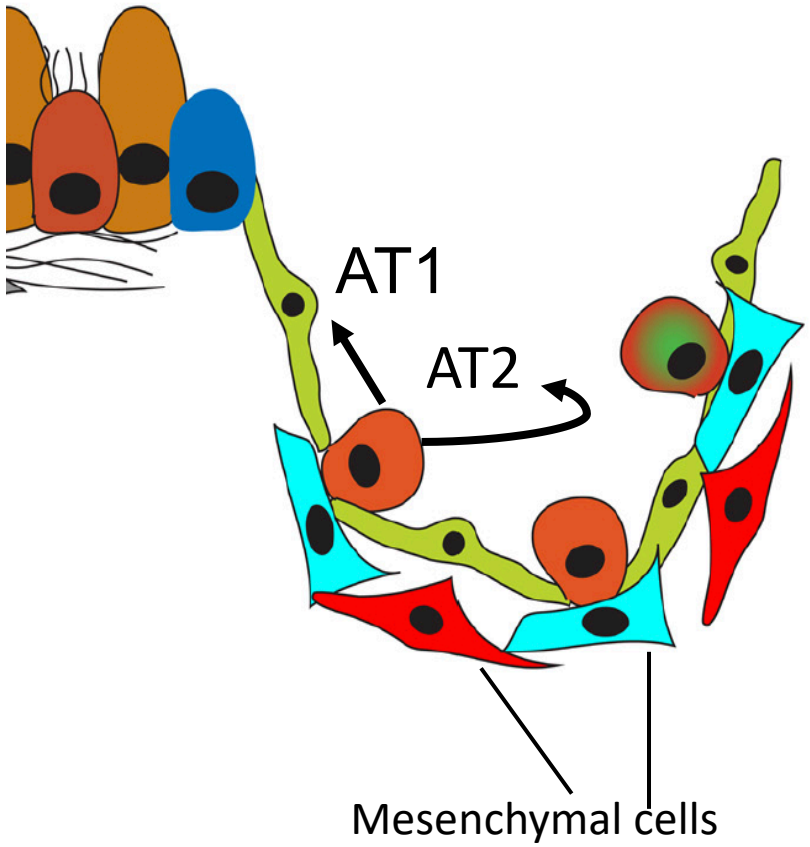
Infected



Activation of airway basal cells to seal the wound;
Scarring leads to permanent loss of gas exchange surface area

What is the nature of repair within different zones of injury?

Zone 3, Injured: Progenitor Activation



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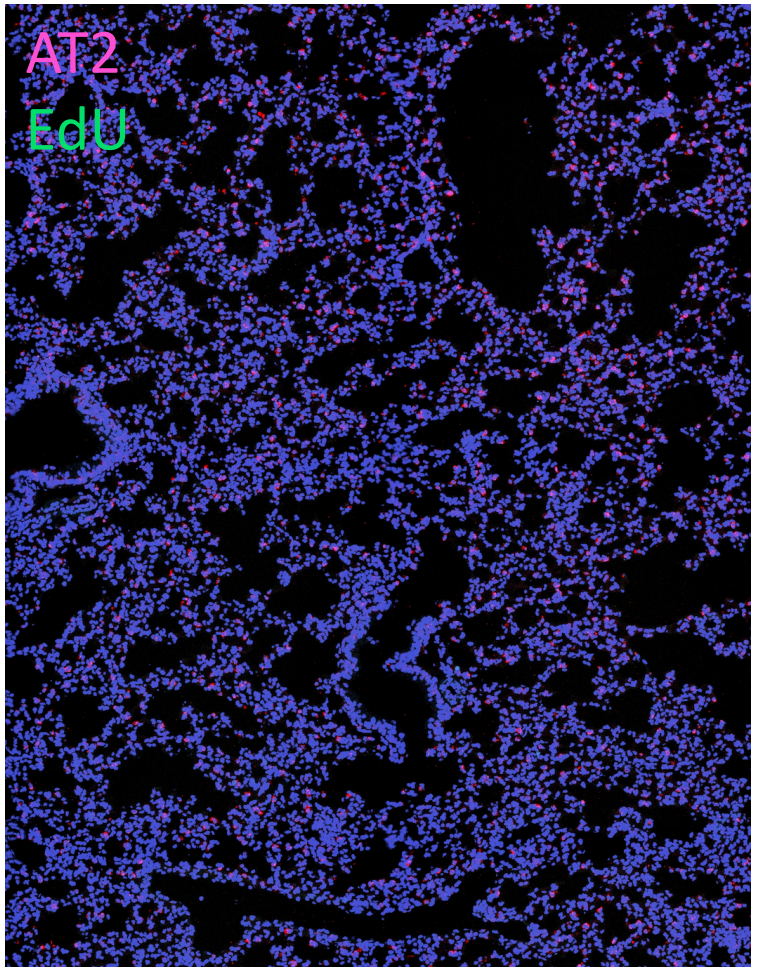
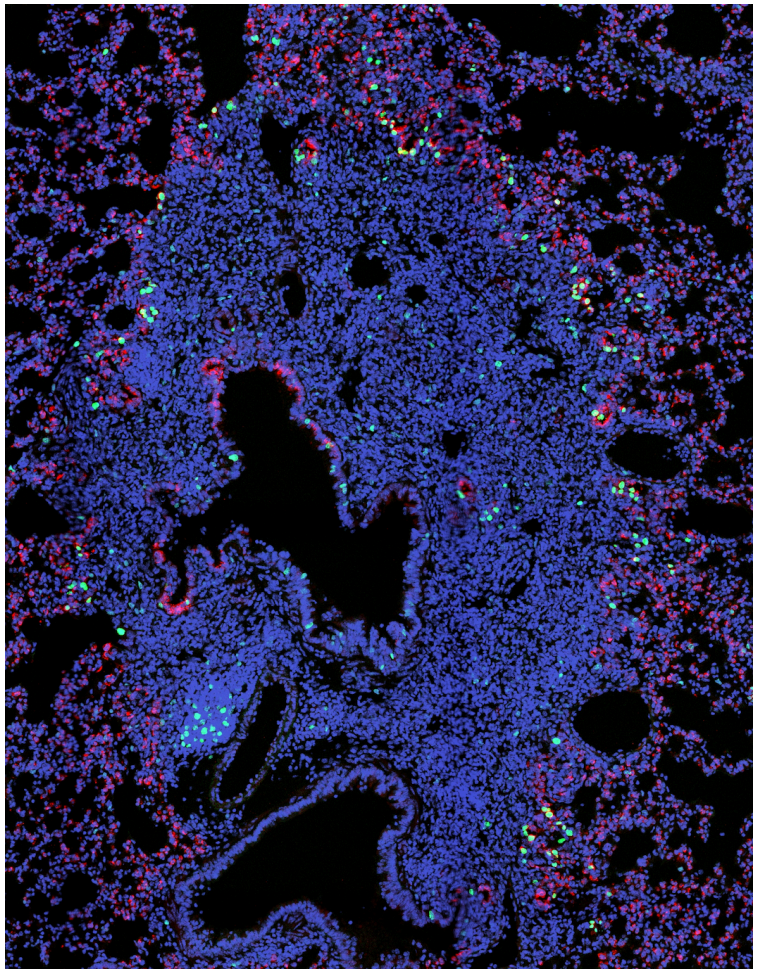


Infected

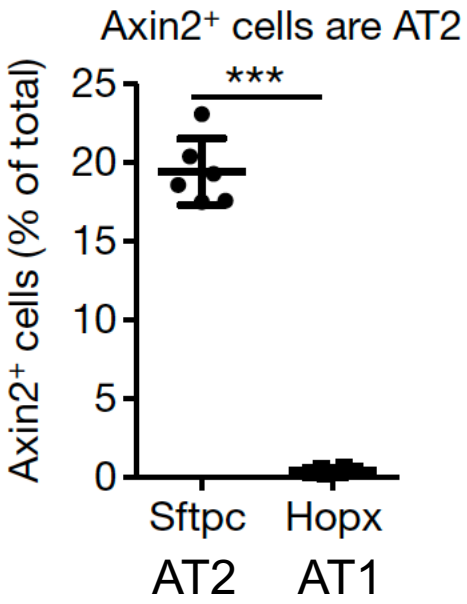


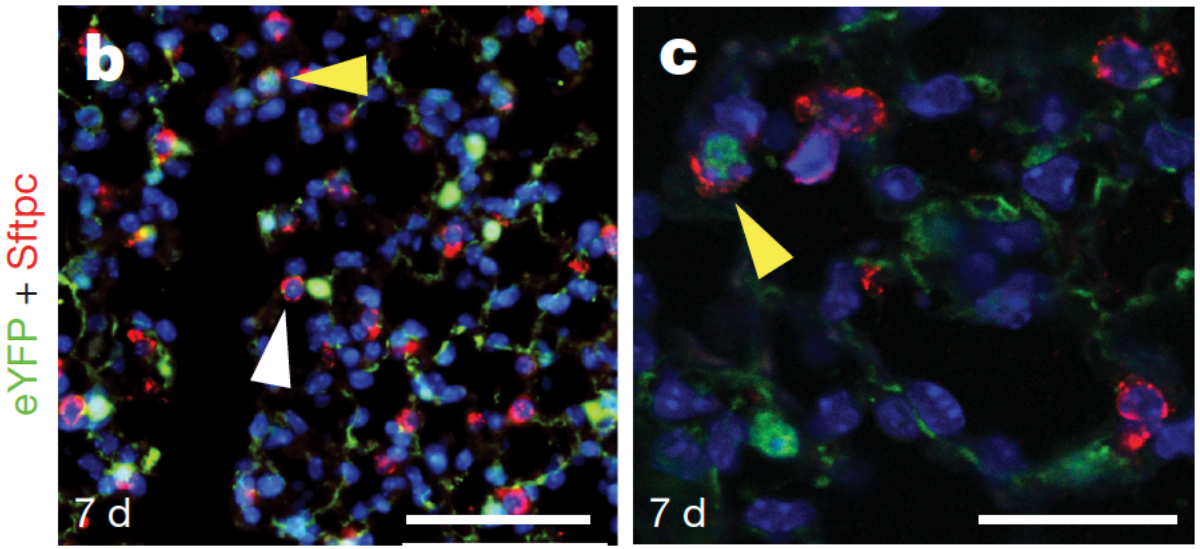
Lung repair after flu

What signals are important to activate facultative progenitor cells after flu?

- Wnt signaling

Axin2–creERT2; Rosa-floxed-eYFP

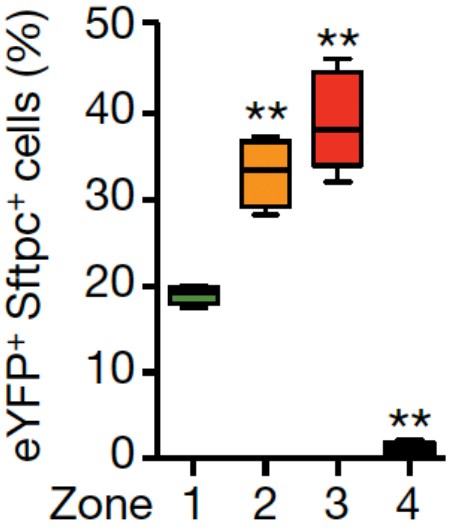




Wnt-responsive cells

AT2 cells

After flu



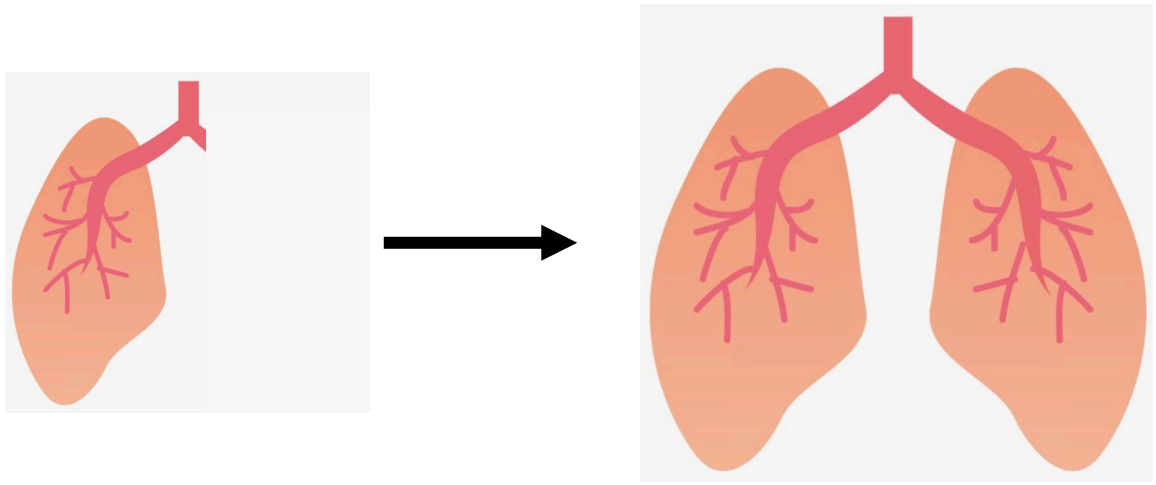
Regeneration of the lung alveolus by an evolutionary conserved epithelial progenitor
Nature, March 2018

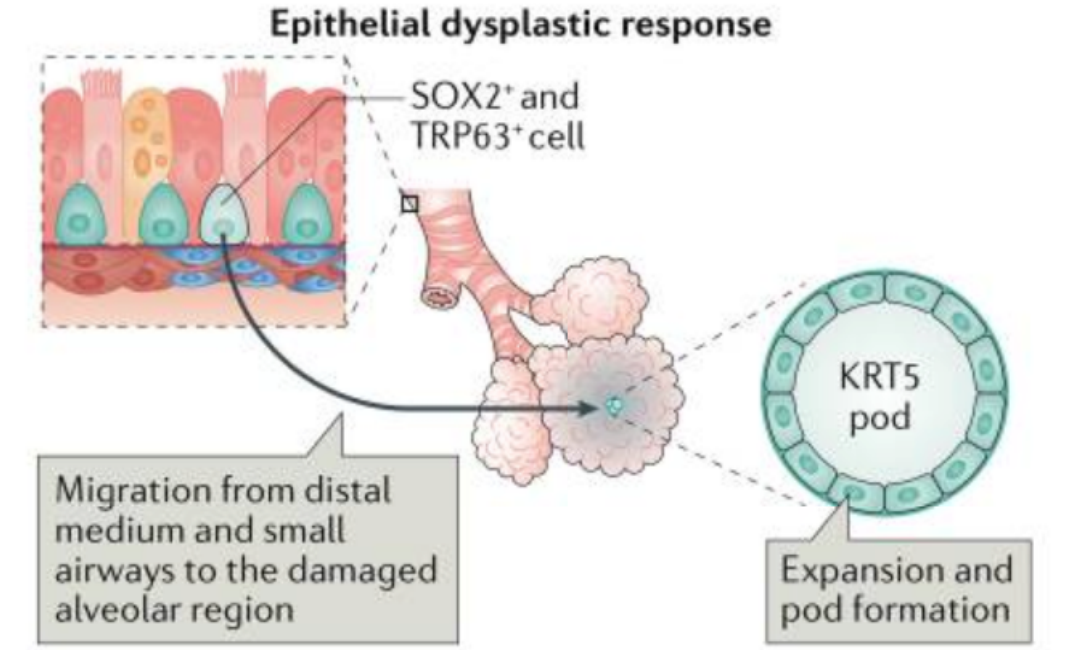
Lung repair after flu

Wnt responsive alveolar epithelial progenitor (AEP) cells

Are these progenitor cells conserved in human?

Regeneration vs Repair

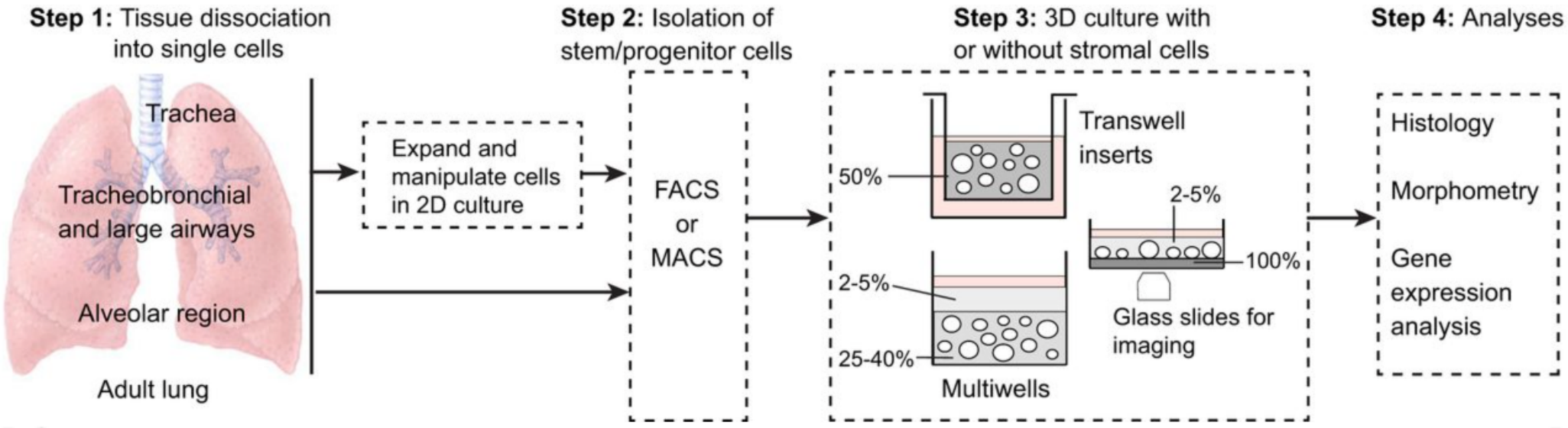




Lung repair after flu

Are AEPs conserved in human?

Lung Organoids



Lung repair after flu

Are AEPs conserved in human?

Lung Organoids

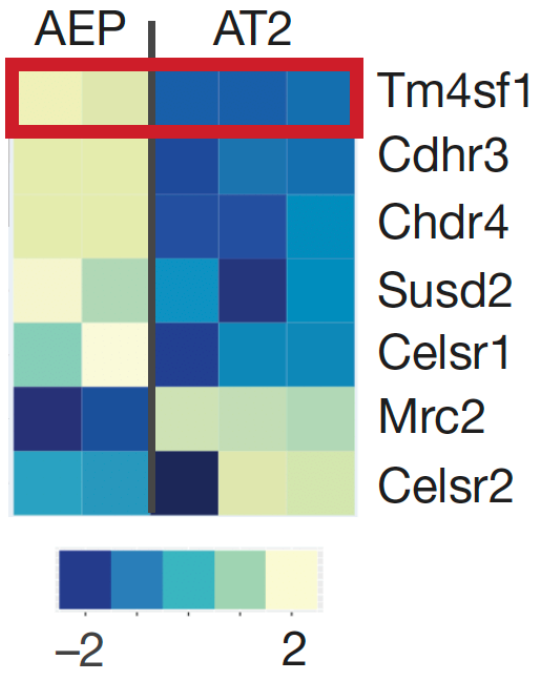
AEP AT2

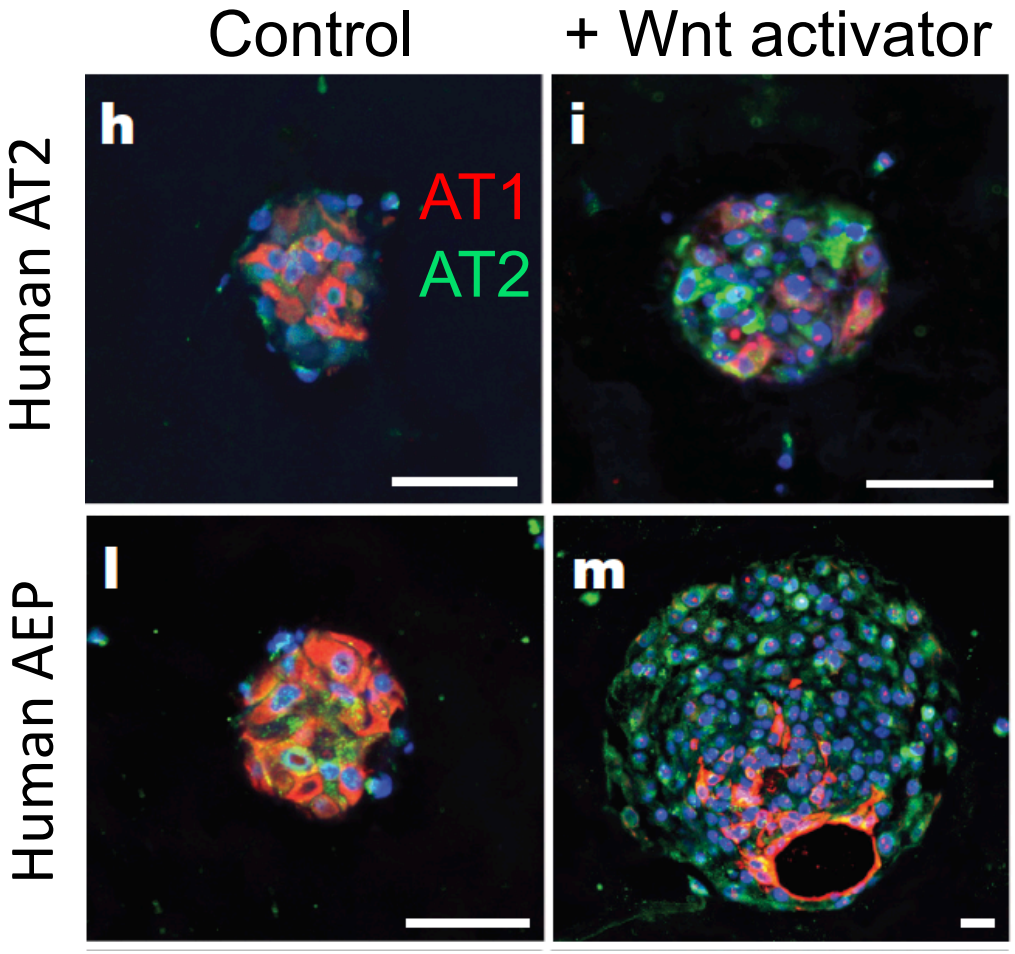
RNA-seq

Differential cell surface protein expression

Commercial available antibodies

FACS + Immunohistochemistry





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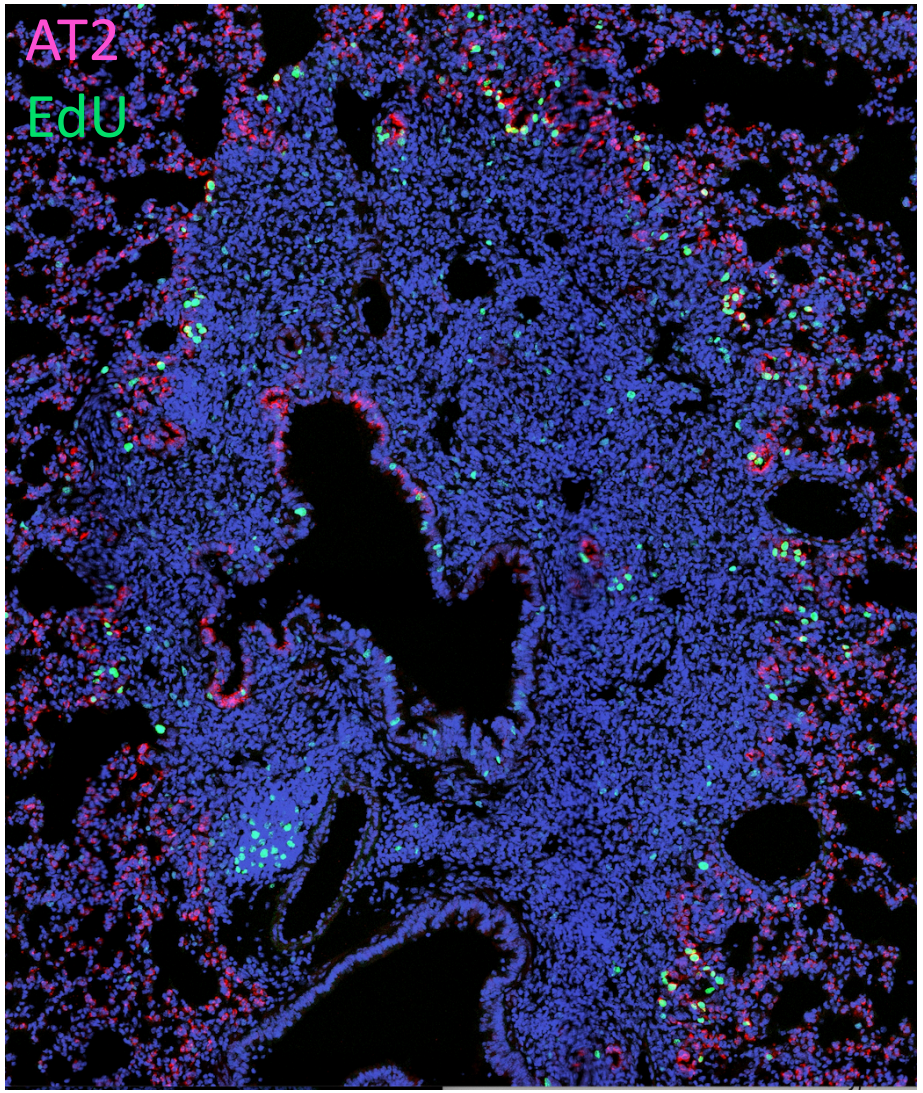
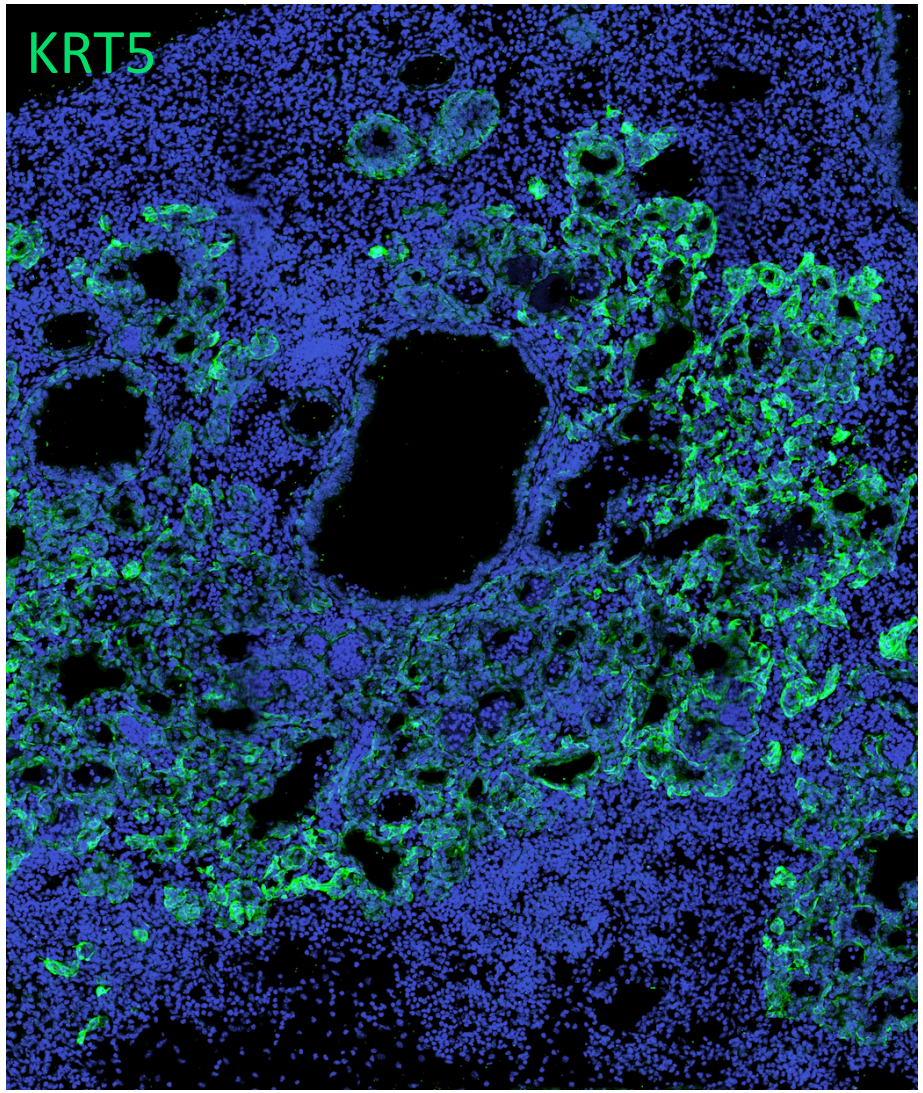
Nature, March 2018

Lung repair after flu

Injured Zone
Activation of Regenerative Potential



Severely Injured Zone
Dysplastic Repair



Questions about lung regeneration/repair?

j1barr@health.ucsd.edu
Sun lab postdoc

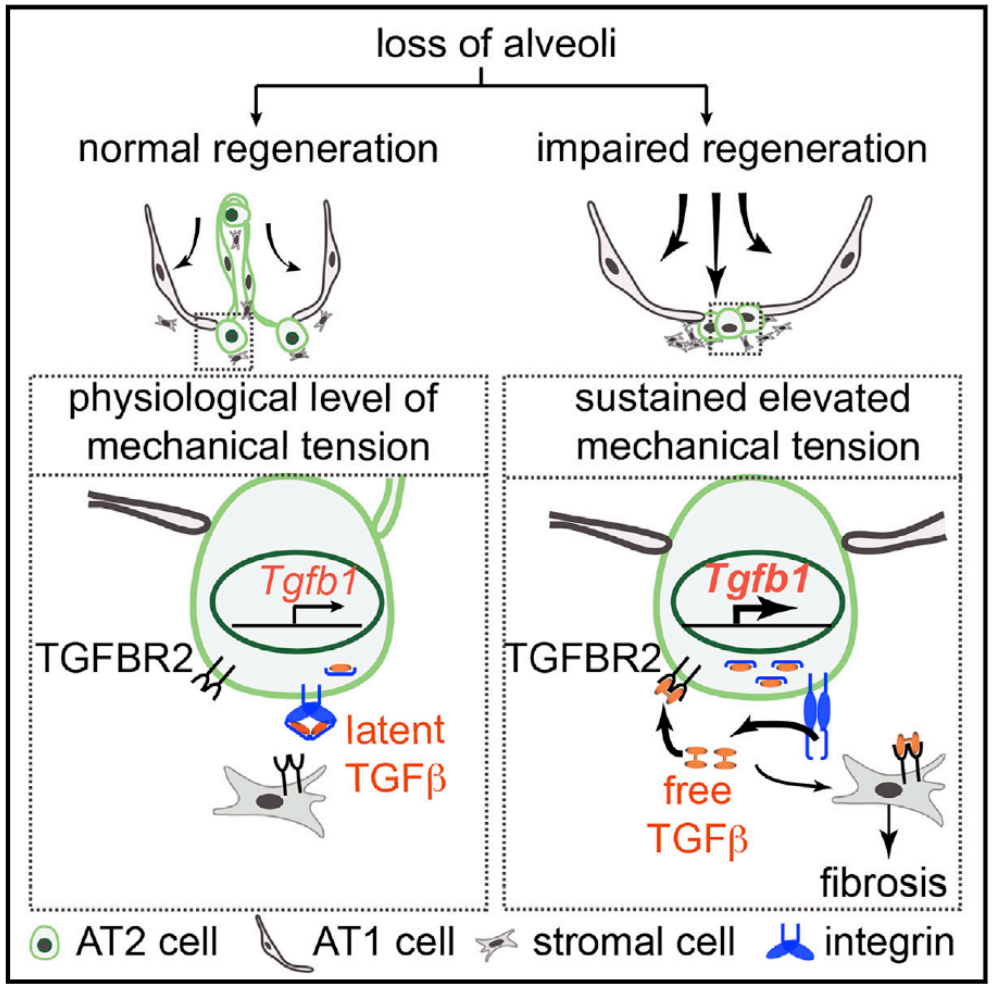
Recruited monocytes and Th2 cytokine signaling promote lung regeneration following pneumonectomy
AJ Lechner, IH Driver, J Lee, CM Conroy, A Nagle, RM Locksley, and JR Rock
Cell Stem Cell, July 2018

Regeneration of the lung alveolus by an evolutionary conserved epithelial progenitor
WJ Zacharias, DB Frank, JA Zepp, MP Morley, FA Alkhaleel, J Kong, S Zhou, E Cantu, EE Morrisey
Nature, March 2018

Cell

Progressive Pulmonary Fibrosis Is Caused by Elevated Mechanical Tension on Alveolar Stem Cells

Graphical Abstract



Authors

Huijuan Wu, Yuanyuan Yu, Huanwei Huang, ..., Huaping Dai, Jingyu Chen, Nan Tang

Correspondence

chenjy@wuxiph.com (J.C.), tangnan@nibs.ac.cn (N.T.)

In Brief

By investigating links between impaired alveolar regeneration and progressive pulmonary fibrosis, Wu et al. found that the periphery-to-center progression of the most common type of lung fibrosis is driven by sustained elevated mechanical tension that activates a TGF-β signaling loop in alveolar stem cells.