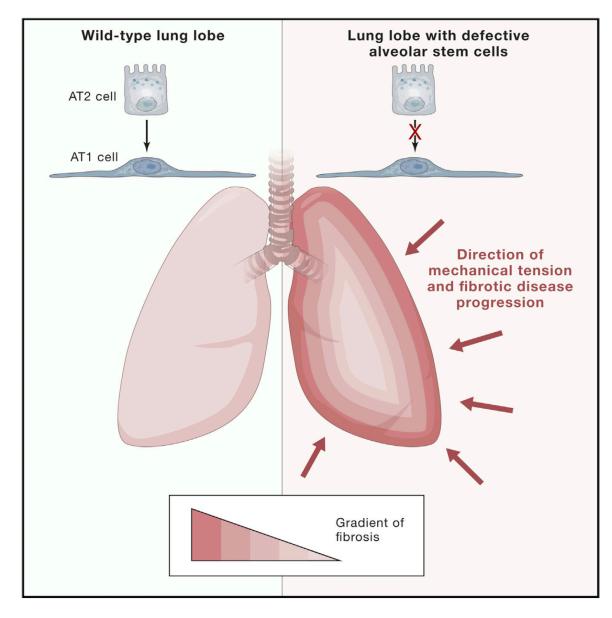
Mechanical tension on alveolar stem cells

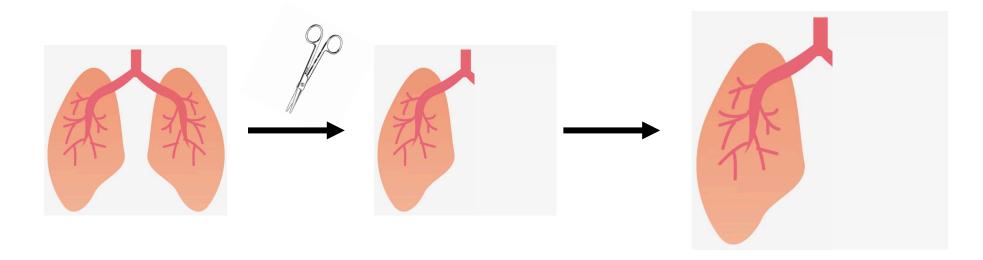
qBIO 01/29/2021

Mechanosensing in the lung

Defective alveolar stem cells and mechanical tension lead to spatially specific fibrosis

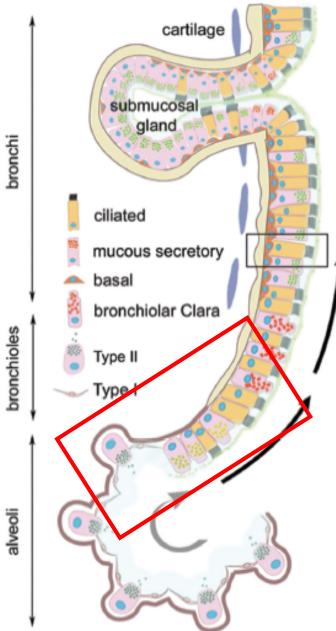


Lung regeneration*: Pneumonectomy (PNX) as a model of compensatory growth

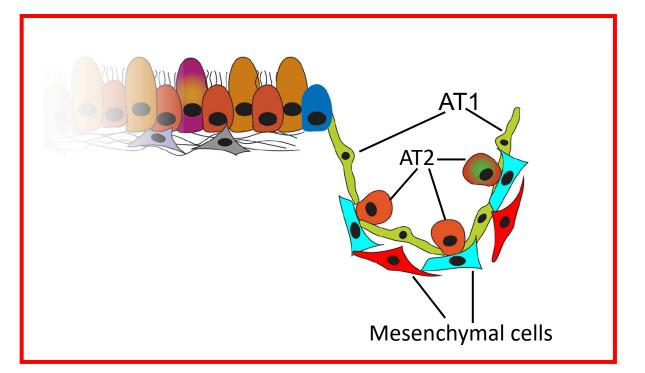


- 1) Unilateral removal of 1 lobe
- 2) Growth of remaining lobe

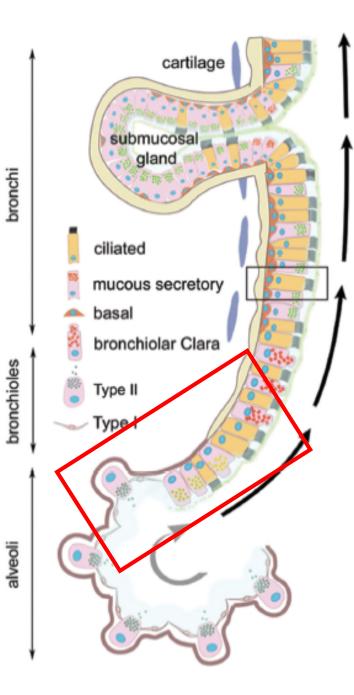
Which cells act as progenitor cells? What signals are important to activate progenitors?



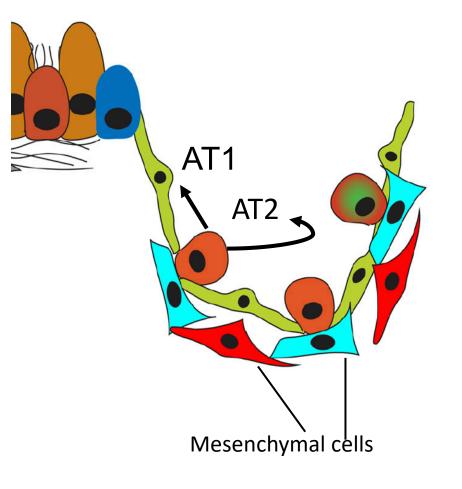
Alveolar Epithelial Cells: AT1 and AT2



alveoli



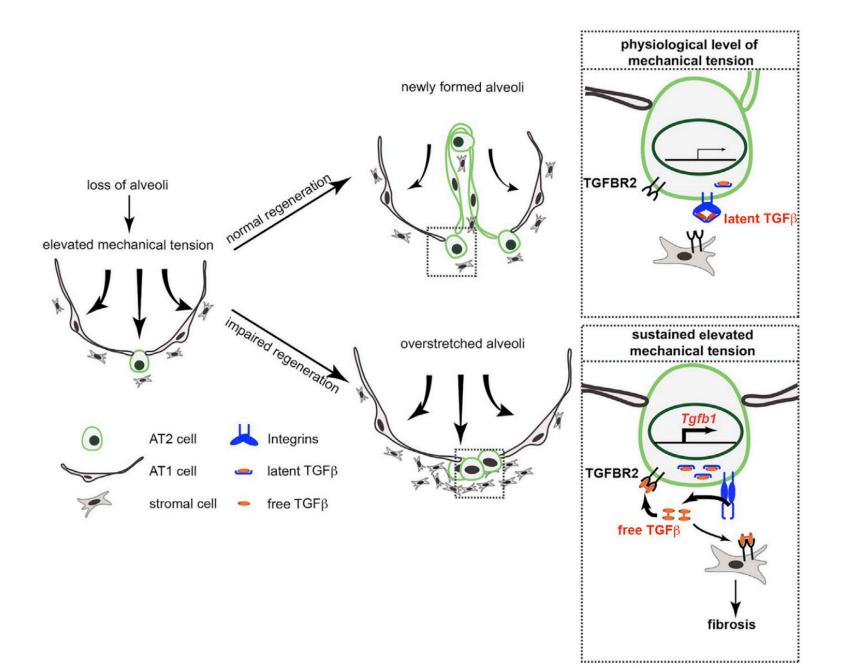
Alveolar Epithelial Cells: AT2 Progenitor Function



Prosthesis Mechanical tension on Mechanical tension off Cdc42 Cdc4 actin polymerization JNK p38 JNK p38 YAP YAP YAP alveolar alveolar ≁ regeneration regeneration \sim Pulmonary alveolar type 2 cell Pulmonary alveolar type 2 cell

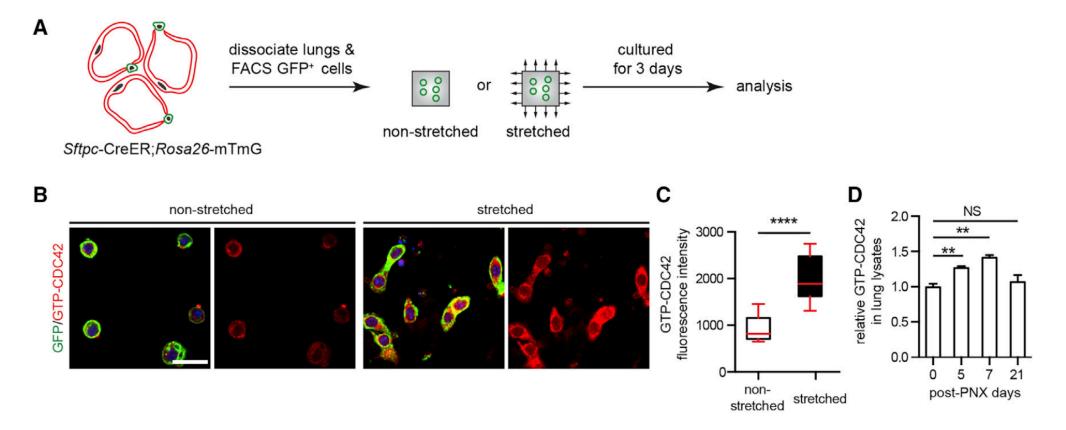
Cdc42 signaling cascade is required for AT2 regenerative response to mechanical tension

MAPK-Mediated YAP Activation Controls Mechanical-Tension-Induced Pulmonary Alveolar Regeneration Cell Reports, 2016 Mechanical tension induced AT2 signaling can lead to regeneration or progressive pulmonary fibrosis



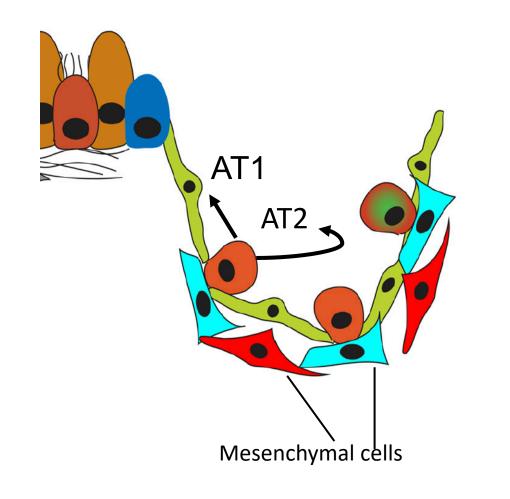
Is Cdc42 required for AT2 cell mechanosensing?

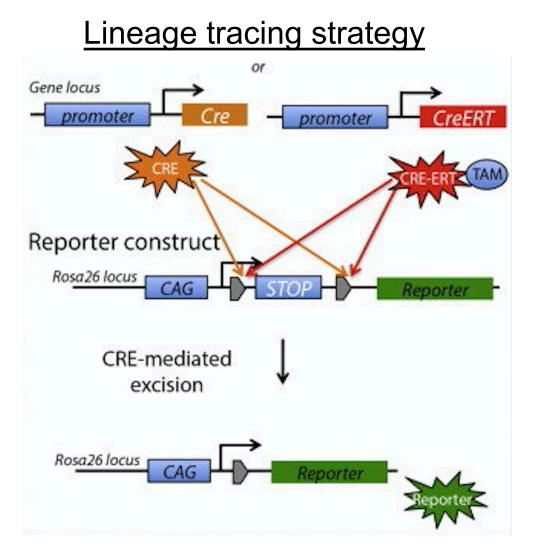
Figure 1



Activation of Cdc42 in stretched AT2 cells and after PNX

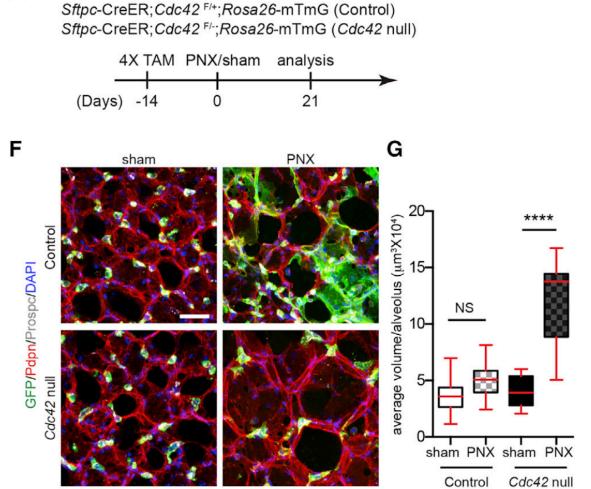
Is Cdc42 required for AT2 cell differentiation to AT1 cell?





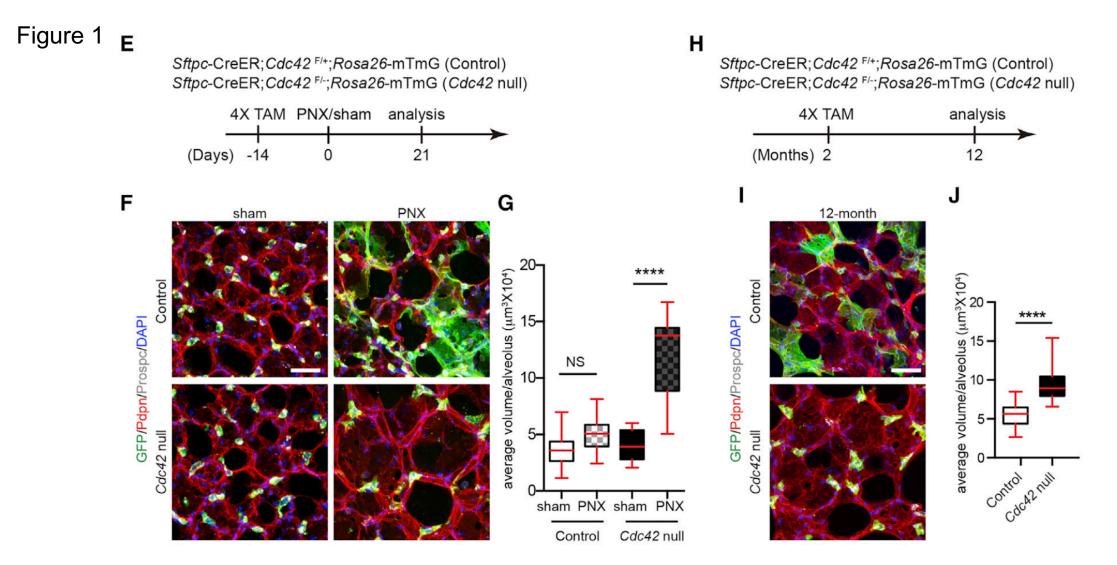
Is Cdc42 required for AT2 cell differentiation to AT1 cell?





Cdc42 required for AT2 regeneration of alveolar compartment after PNX

Is Cdc42 required for AT2 cell differentiation to AT1 cell?



Cdc42 required for AT2 regeneration of alveolar compartment after PNX AT2 progenitor function prevents alveolar simplification after PNX or with aging

What are the long-term consequences of failed AT2 regeneration following PNX?

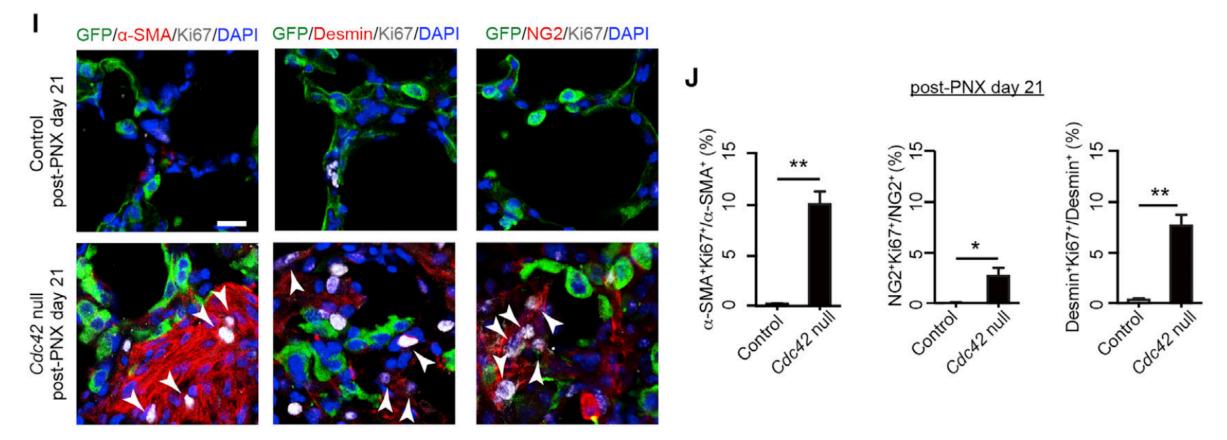
Α 4X TAM PNX analysis Sftpc-CreER;Cdc42 F/+;Rosa26-mTmG (Control) Sftpc-CreER;Cdc42 F/-;Rosa26-mTmG (Cdc42 null) (Days) -14 180 0 В Е С D F P<0.001 relative hydroxyproline - 0.1 - 0.2 - 0.1 - 0.2 - 0.1 - 0.2 -100 *** survival proportion (%) forced vital capacity (ml) Control 1.27 0.03-100 100 **** 80-(n=23) lung compliance (ml/cmH₂O) 0.9aO 60- SaO_2 (%) 75 0.02-0.6-40-(mmHg) 0.01-Cdc42 null 0.3-20-25 (n=33) 0.00 0.0 Control null 0 Control null Cdc42 null Cdc42 null Control Control Control Cdc42 mill 100 150 200 0 50 post-PNX days G Н PNX day 21 sham sham PNX day 14 PNX day 21 PNX day 45 end-point Cdc42 null Control

Decreased lung function and progression of outside-in fibrosis

Figure 2

What cell types in the lung mediate fibrosis following PNX when AT2 cell regeneration is compromised?

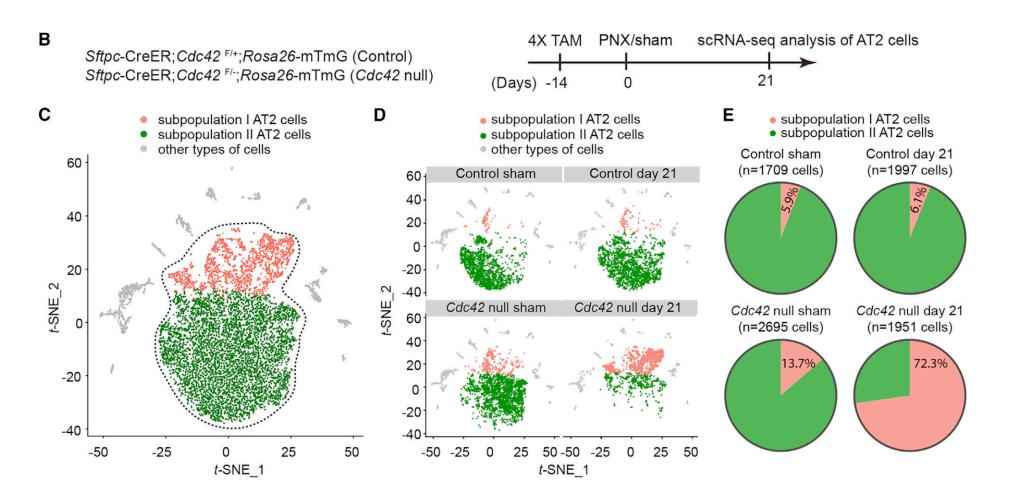
Figure 2



Mesenchymal myofibrolast proliferation contributes to fibrotic phenotype

What molecular changes are observed in AT2 cells that fail to differentiate into AT1 cells after PNX?

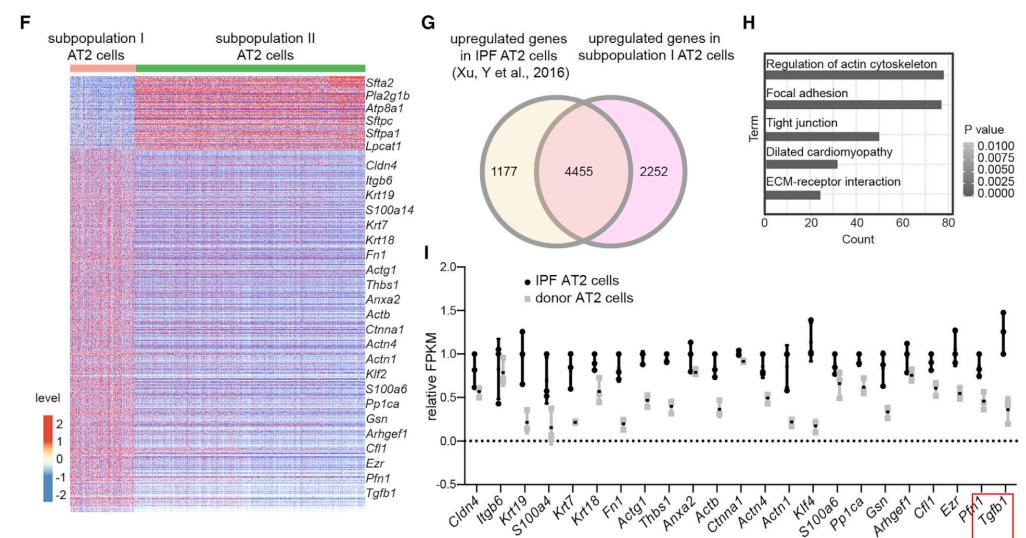
Figure 3



There are 2 populations of AT2 cells; subpopulation I is increased in the mutant following PNX

What are the molecular characteristics of subpopulation I AT2 cells?

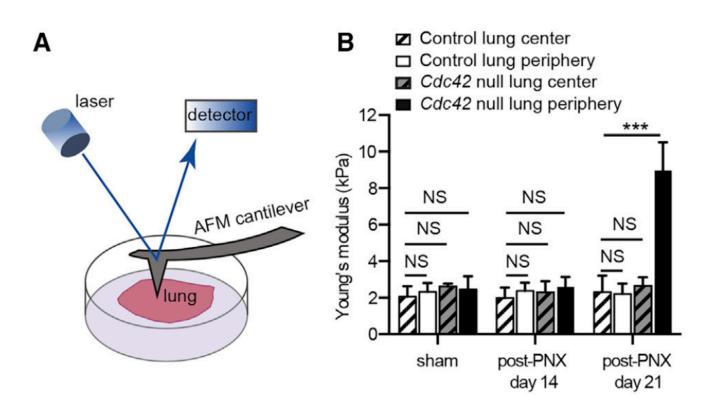
Figure 3



> 2/3 of upregulated genes in subpopulation I are also upregulated in IPF

Is increased mechanical tension responsible for the progression of lung fibrosis?

Figure 4



Increased tissue stiffness at the periphery during outside-in lung fibrosis

Is increased mechanical tension responsible for the progression of lung fibrosis?

180

NS

Gsn

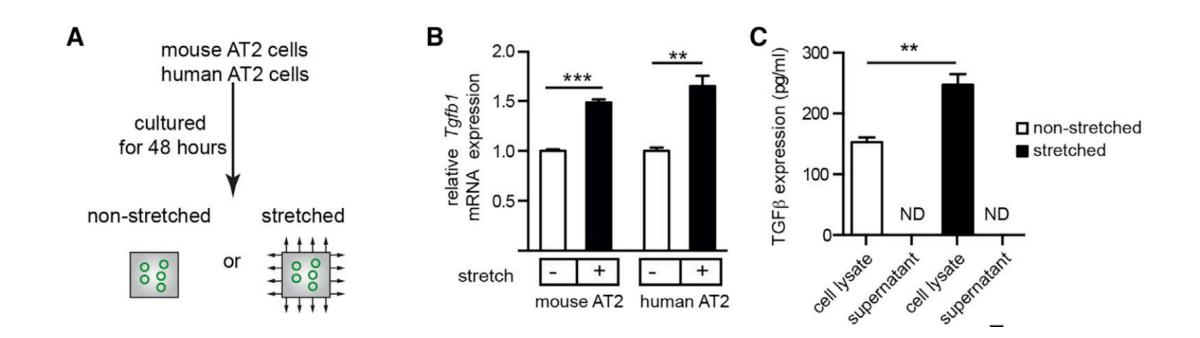
D 4X TAM PNX prosthesis analysis Sftpc-CreER;Cdc42 F/+;Rosa26-mTmG (Control) Sftpc-CreER;Cdc42 F/-;Rosa26-mTmG (Cdc42 null) (Days)-14 0 14 21 Е F G Control NS Cdc42 null -prosthesis *** 20-Control+prosthesis Cdc42 null+prosthesis average volume/alveolus (μm³X10⁴) Cdc42 null +prosthesis relative mRNA expression GFP/Pdpn/Prospc/DAPI 15-NS post-PNX day 21 NS 10-NS NS *** 5-Control Cdc42 null +prosthesis +prosthesis Actb Actn1 Pfn1 Ezr н J ⊕ Cdc42 null -prosthesis Cdc42 null mice at post-PNX day 180 Cdc42 null +prosthesis 2.5relative hydroxyproline -prosthesis +prosthesis P<0.01 2.0-100 survival proportion (%) +prosthesis 1.5-80-(n=10) 1.0-60-0.5-40--prosthesis 0 20-Cdc42 null sis (n=19) Calca2 null sis 0-50 100 150 200 0 post-PNX days

Reducing mechanical tension leads to rescue of fibrotic phenotype

Figure 4

What signaling pathways mediate mechanical tension induced fibrosis?

Figure 5



Stretched AT2 cells express Tgf-B locally

What signaling pathways mediate mechanical tension induced fibrosis?

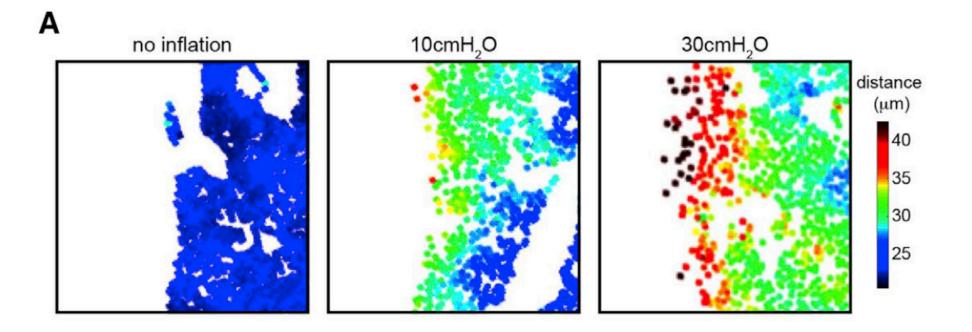
**** Ε p-SMAD2⁺ AT2 cells (%) 100 **** **** 80. D non-stretched stretched stretched+anti-TGFβ stretched+GSK3008348 60-GFP/p-SMAD2/DAPI 40-20mouse stretch + + anti-TGF_β + -GSK3008348 G **** p-SMAD2⁺ AT2 cells (%) **** F 80stretched+GSK3008348 non-stretched stretched stretched+anti-TGFβ HTII-280/p-SMAD2/DAPI 60-40-human 20-stretch + + anti-TGF_β + -GSK3008348

Downstream target of Tgf-B signaling upregulated in AT2 cells

Figure 5

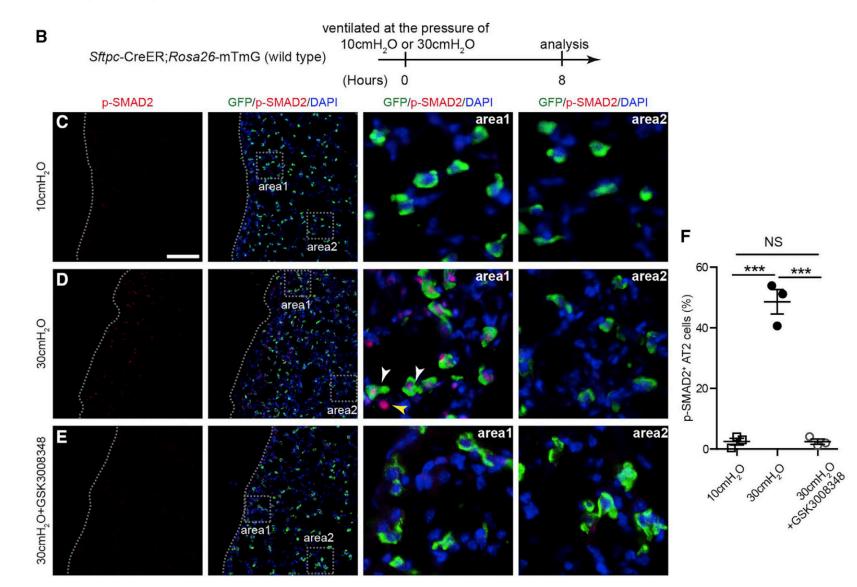
Is mechanical tension spatially regulated within the lung?

Figure 6



Peripheral alveoli are more stretched at high inflation pressure

What signaling pathways mediate mechanical tension induced fibrosis?

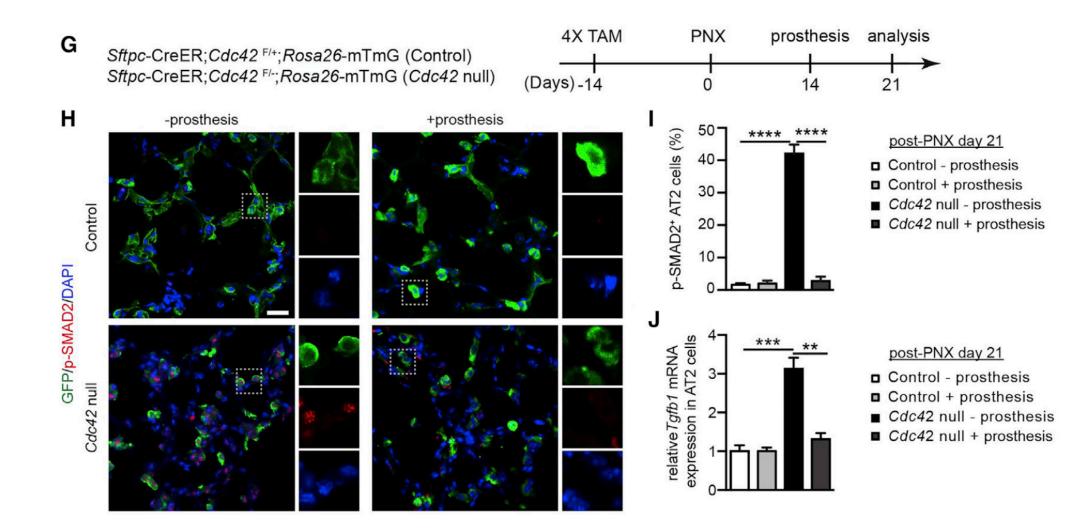


Tgf-B signaling is activated at the periphery at high inflation pressure

Figure 6

What signaling pathways mediate mechanical tension induced fibrosis?

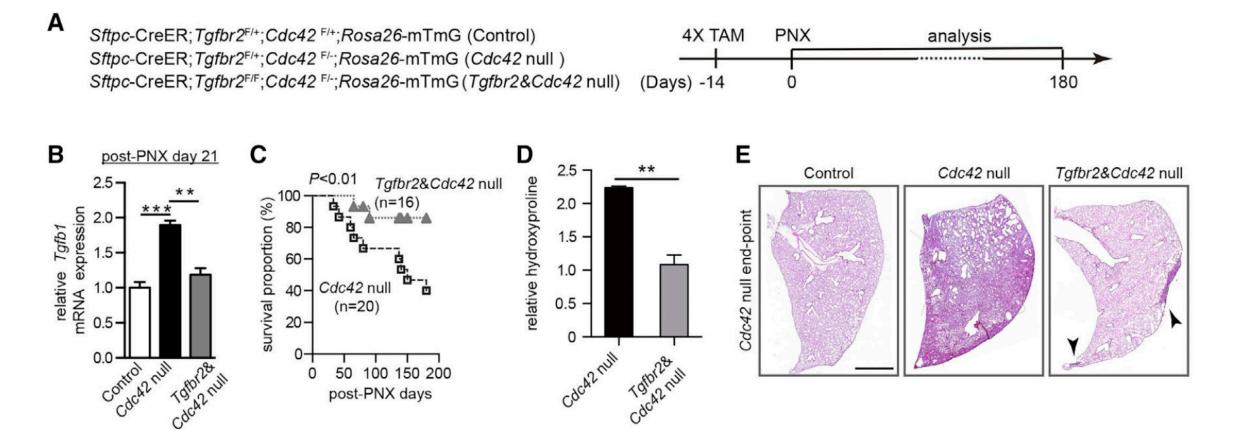
Figure 6



Tgf-B signaling in AT2 cells is activated in fibrosis model

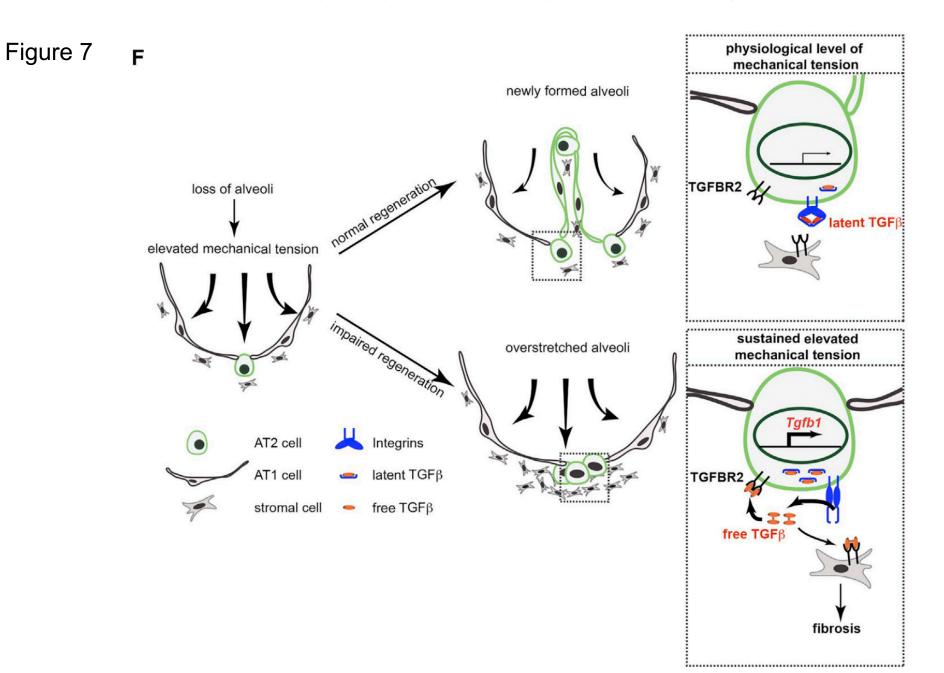
Is Tgf-B signaling required for progression of lung fibrosis?

Figure 7



Loss of Tgf-B signaling rescues fibrotic phenotype due to failed alveolar regeneration following PNX

Mechanical tension induces AT2 signaling can lead to regeneration or progressive pulmonary fibrosis



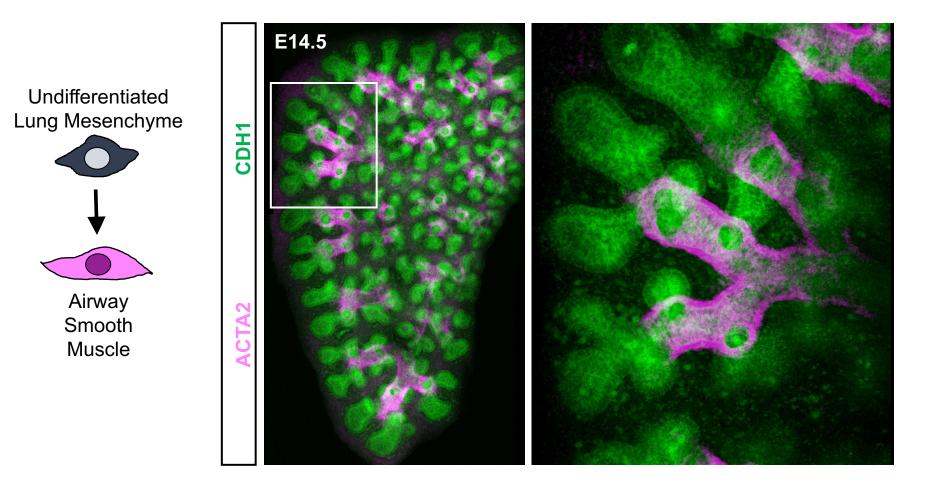
Discussion Questions

- What niche signals are important for AT2 stem cell response to mechanical tension?
- What's the signal from AT2 cells to fibrotic cells?
- What regenerative pathways are important to protect the lung from fibrosis during aging?
- Why does AT2 regenerative ability decrease with age?
- Are there treatments that can reduce mechanical stress and prevent fibrosis?

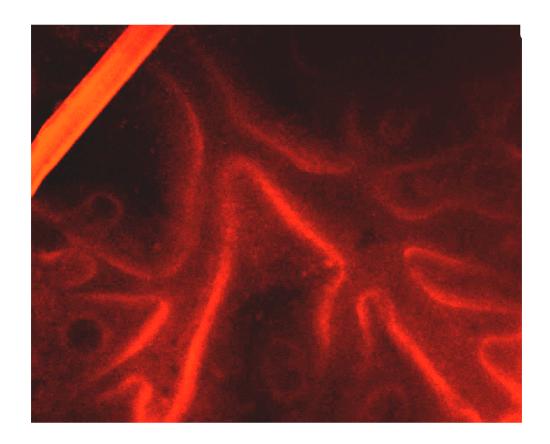
Questions about lung regeneration/repair?

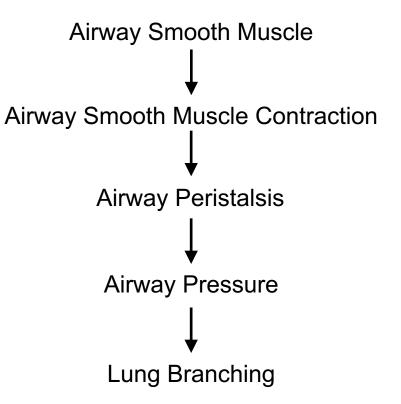
j1barr@health.ucsd.edu Sun lab postdoc Airway Smooth Muscle Cells in Lung Branching

Airway smooth muscle surrounds the airway epithelium

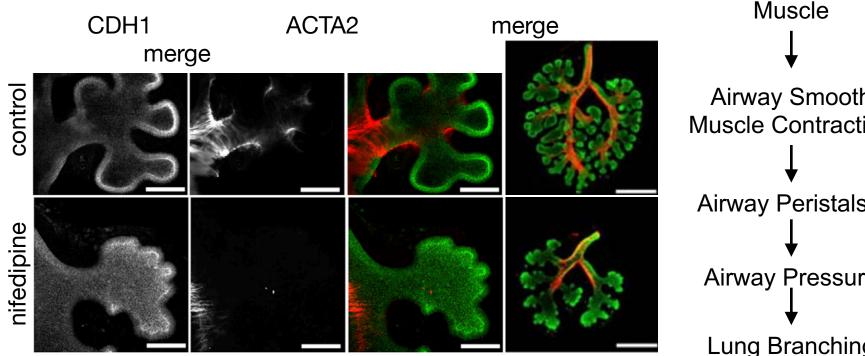


Prevailing hypothesis: Airway smooth muscle promotes lung branching





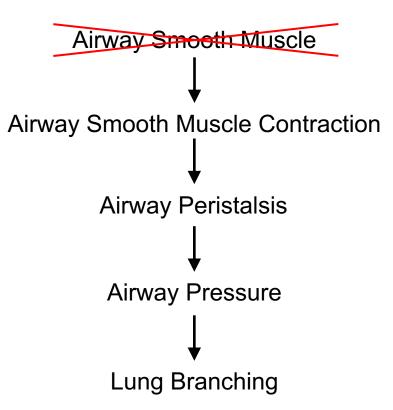
Chemical inhibition of smooth muscle contraction disrupts epithelial branching ex vivo



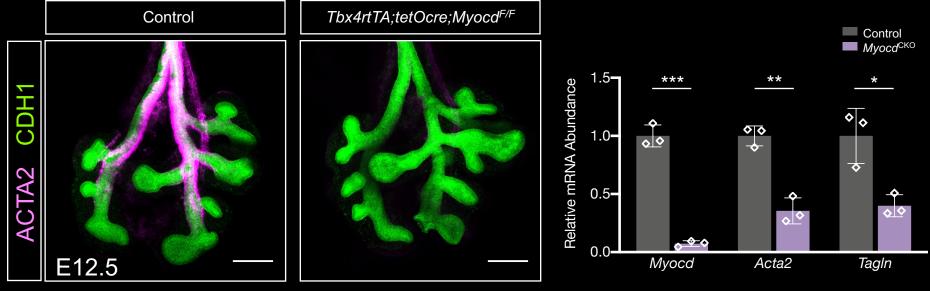
Kim and Nelson et. al. Developmental Cell, 2015 Goodwin and Nelson et al. Development 2019

Airway Smooth Muscle Airway Smooth **Muscle Contraction Airway Peristalsis** Airway Pressure Lung Branching

In vivo testing of the prevailing hypothesis

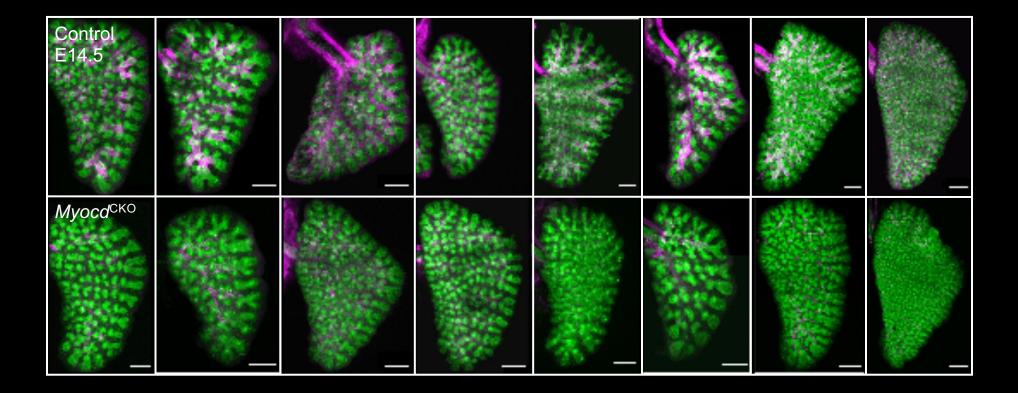


Myocd^{CKO} inactivation led to loss of airway smooth muscle

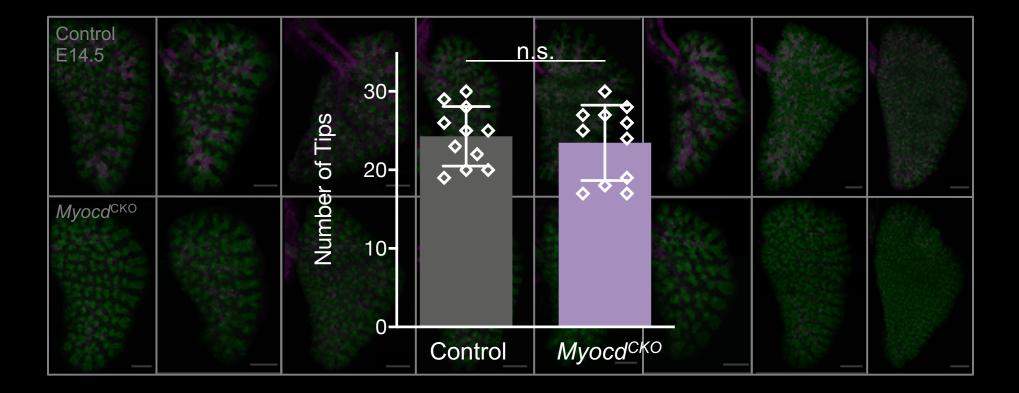


***p < 0.0005, **p = 0.005, *p < 0.05 N = 3 for each control and *Myocd^{CKO}* group

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

