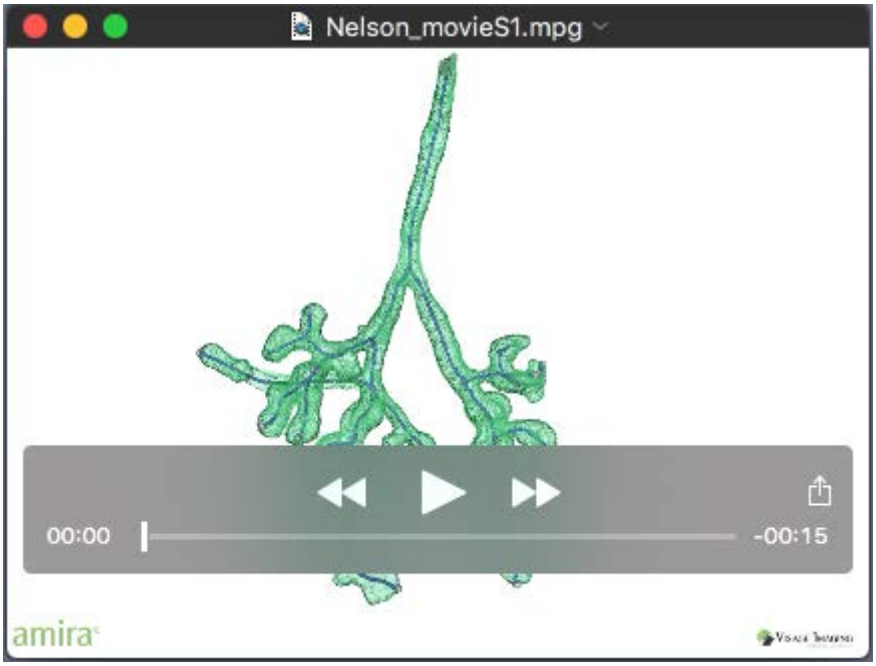
## Supplemental Material

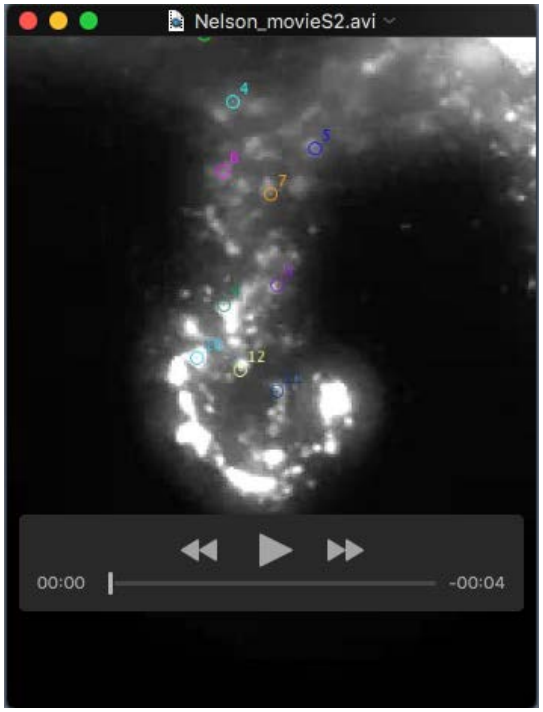
## Supplemental Table

| Gene | Primer Sequences |
|---|---|
| fgf7 | Forward: 5'-GCAATCAAAGGGGTGGAAAGTG-3'<br>Reverse: 5'-TCCATTTAGCTGATGCATAGGTG-3' |
| fgf10 | Forward: 5'-ACTTAGCCATGAACAAGAAGGG-3'<br>Reverse: 5'-TTTGCCTGCCATTGTGCTG-3' |
| sfta2 | Forward: 5'- CTGCCATCAGGGACCAATGT-3'<br>Reverse: 5'- AAGGCGTGTTCACTTCCCAA-3' |
| sftpc | Forward: 5'- GGAGCACCGGAAACTCAGAA -3'<br>Reverse: 5'- CTGGCTTATAGGCCGTCAGG -3' |
| muc1 | Forward: 5'- CCAAGCGTAGCCCCTATGAG -3'<br>Reverse: 5'- GTGGGGTGACTTGCTCCTAC -3' |
| 18S | Forward: 5'- TCAGATACCGTCGTAGTTC -3'<br>Reverse: 5'- CCTTTAAGTTTCAGCTTTGC -3' |

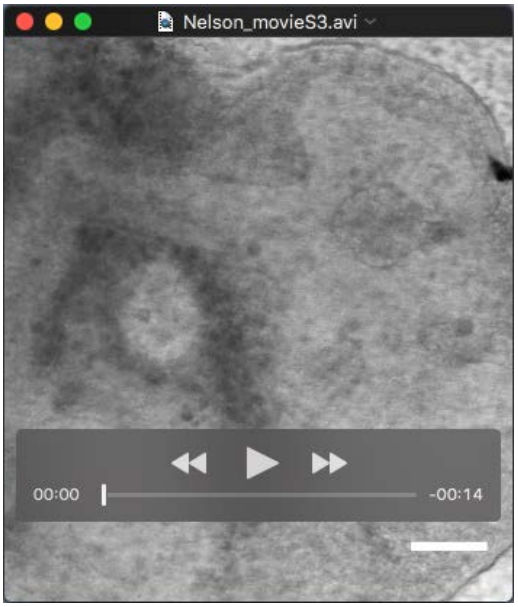
## Supplemental Movies




**Supplemental Movie 1.** Example 3D reconstruction of confocal image stacks using Amira software for lung explant presented in Figure 1E. Epithelium is shown in green; skeletonization of major branches is indicated by a blue line for clarity.



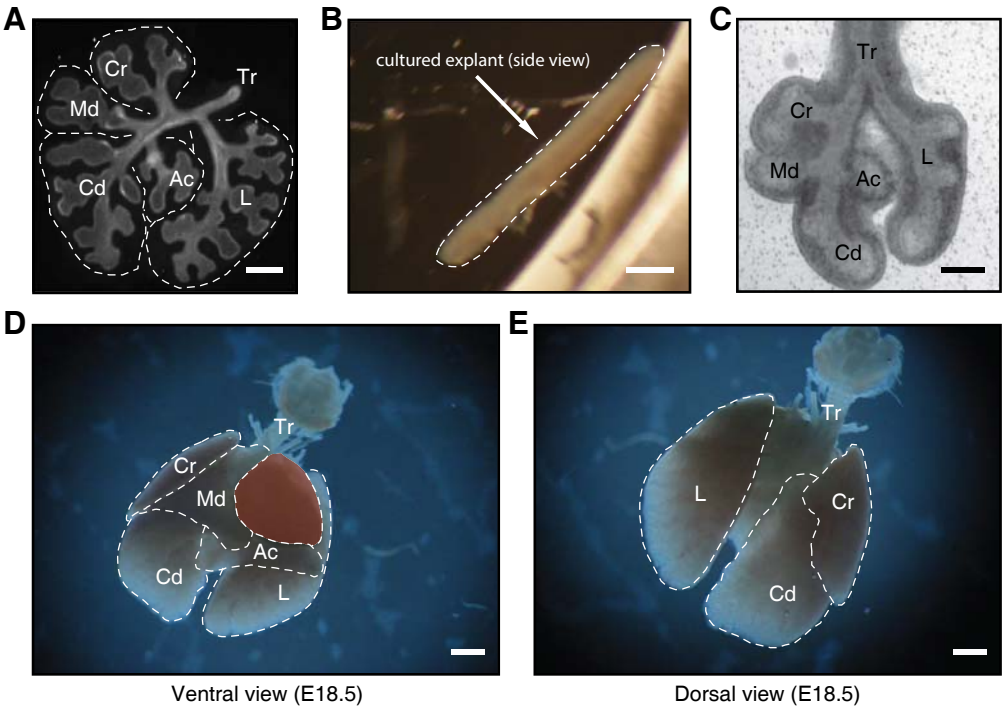

**Supplemental Movie 2.** Timelapse movie of the flow of fluorescent tracer particles within the lumen of the airways, as shown in Figure 3F.




**Supplemental Movie 3.** Treatment with nifedipine blocks airway smooth muscle contraction.

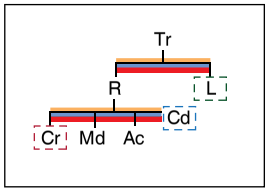
## Supplemental Figures

# Supplemental Figure 1




**Supplemental Figure 1:** (A) E-cadherin staining of airways from an explant cultured for 48 hours on a floating porous membrane. (B) Side profile view of a fixed lung explant cultured on a floating porous membrane, demonstrating that the organ grows and develops into a planar structure. Lobes of the murine lung labeled for explants at (C) early stages of development (~*E*12.5) and (D, E) late gestation (*E*18.5). Tr, trachea; L, left lobe; Cr, cranial lobe; Md, middle lobe; Cd, caudal lobe; Ac, accessory lobe; dotted lines in panels A, D, and E outline the boundaries of the lobes. Scale bars: 200 μm in A, B, and C; 500 μm in D and E.

# Supplemental Figure 2

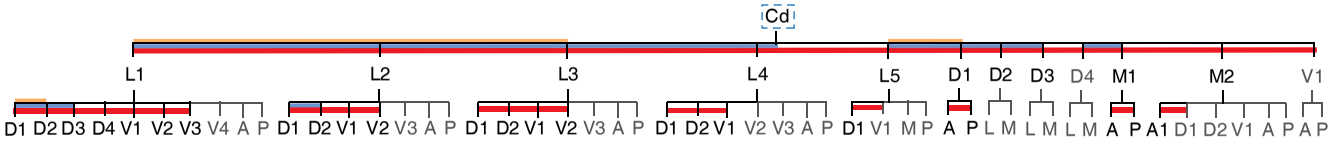


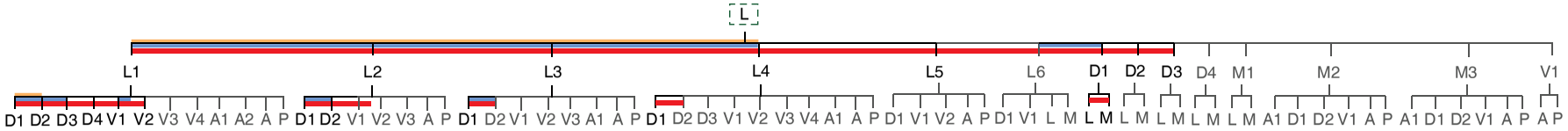

ΔP = 20 Pa, ΔP = 100 Pa, ΔP = 200 Pa, *in vivo*

Cr See Figure 2C

# terminal branches per lobe

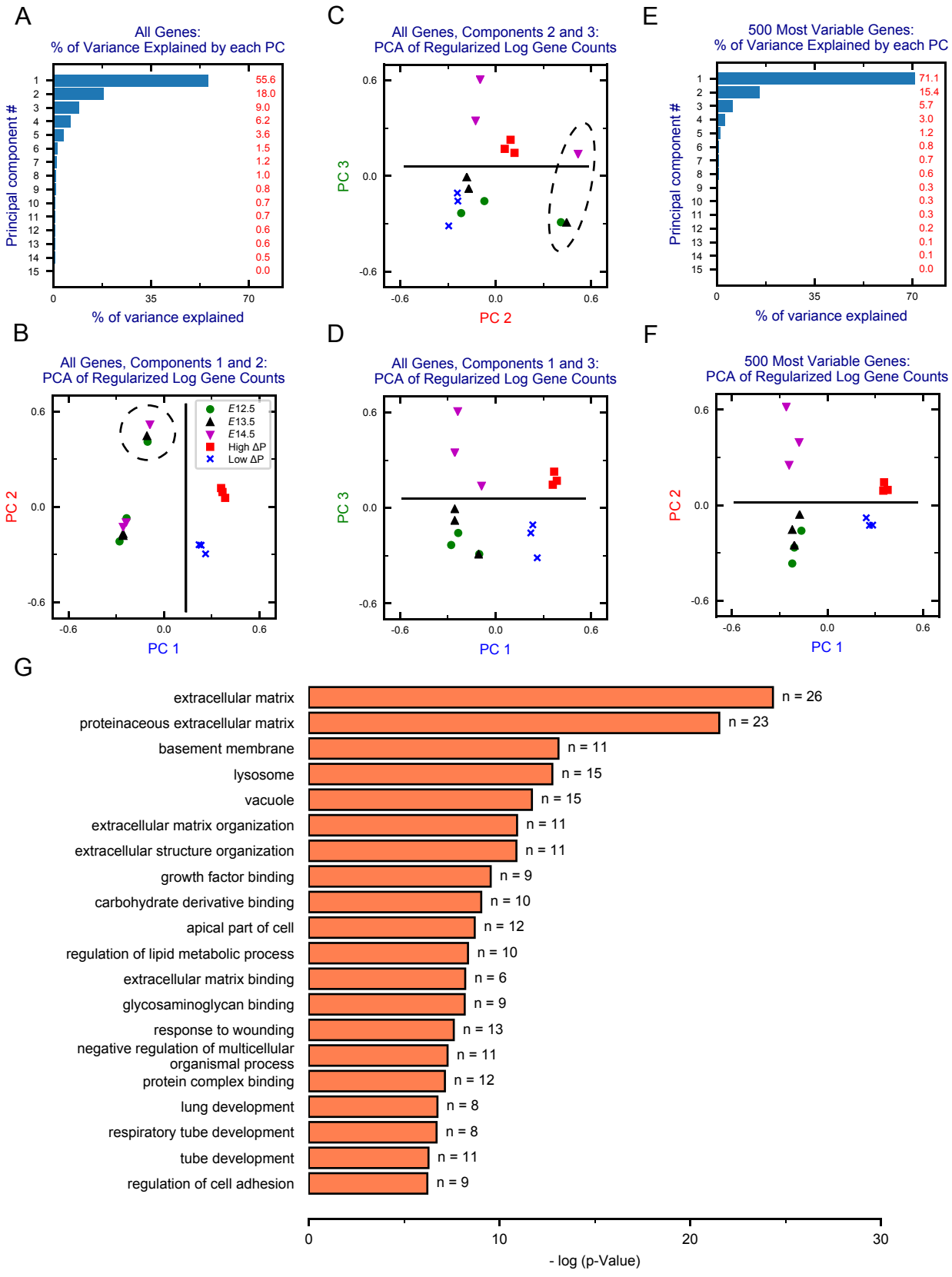
| lobe \ ΔP | 20 Pa | 100 Pa | 200 Pa |
|---|---|---|---|
| Cr | 6 | 12 | 16 |
| Cd | 6 | 13 | 24 |
| L | 5 | 10 | 15 |



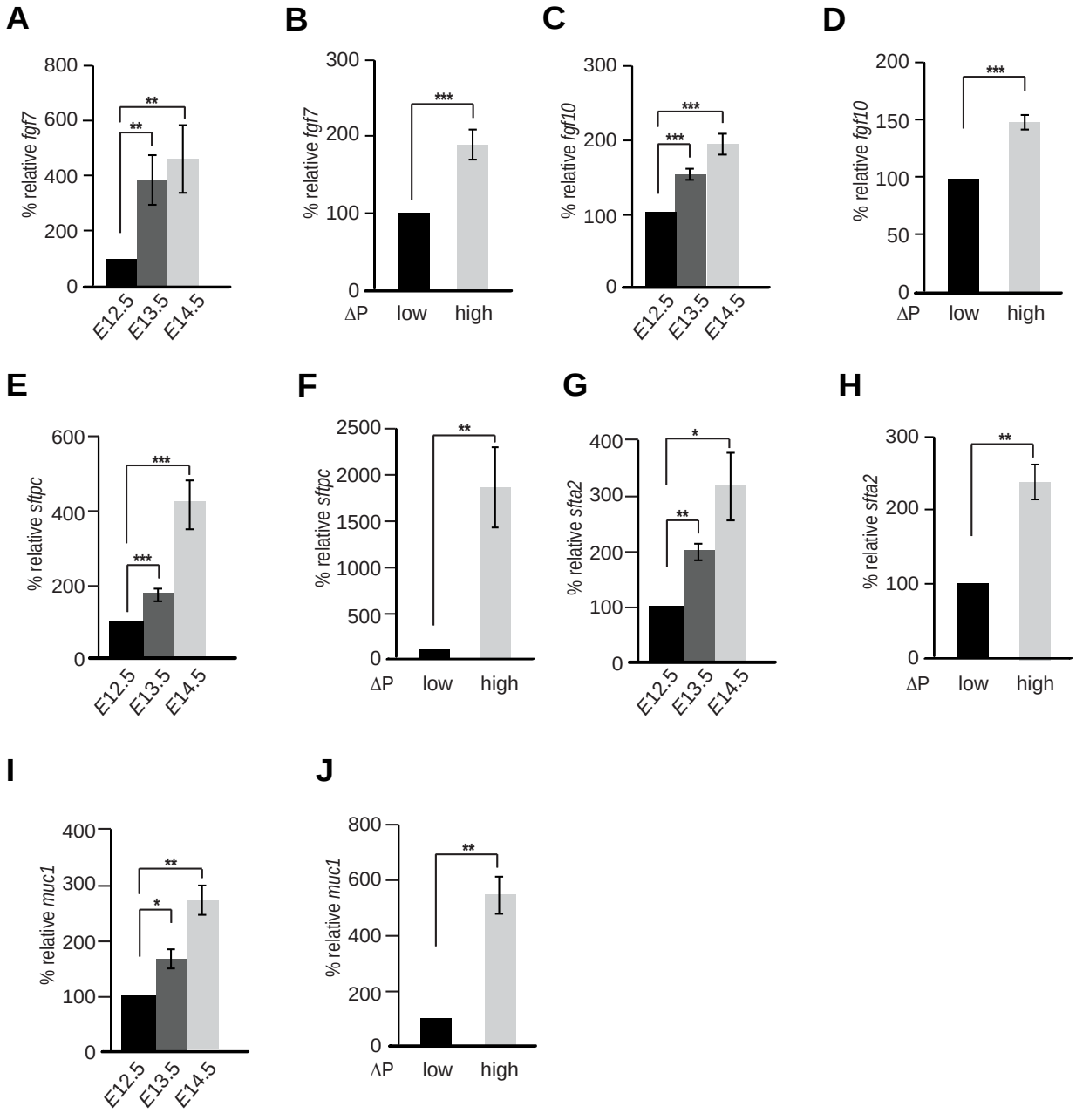
**Supplemental Figure 2:** Lineage diagrams for explants cultured unde ΔP of 20 Pa (orange), 100 Pa (blue), and 200 Pa (black) compared to late gestation explants *in vivo* (gray). Table shows number of branches per lobe for explants held under each ΔP.

# Supplemental Figure 3



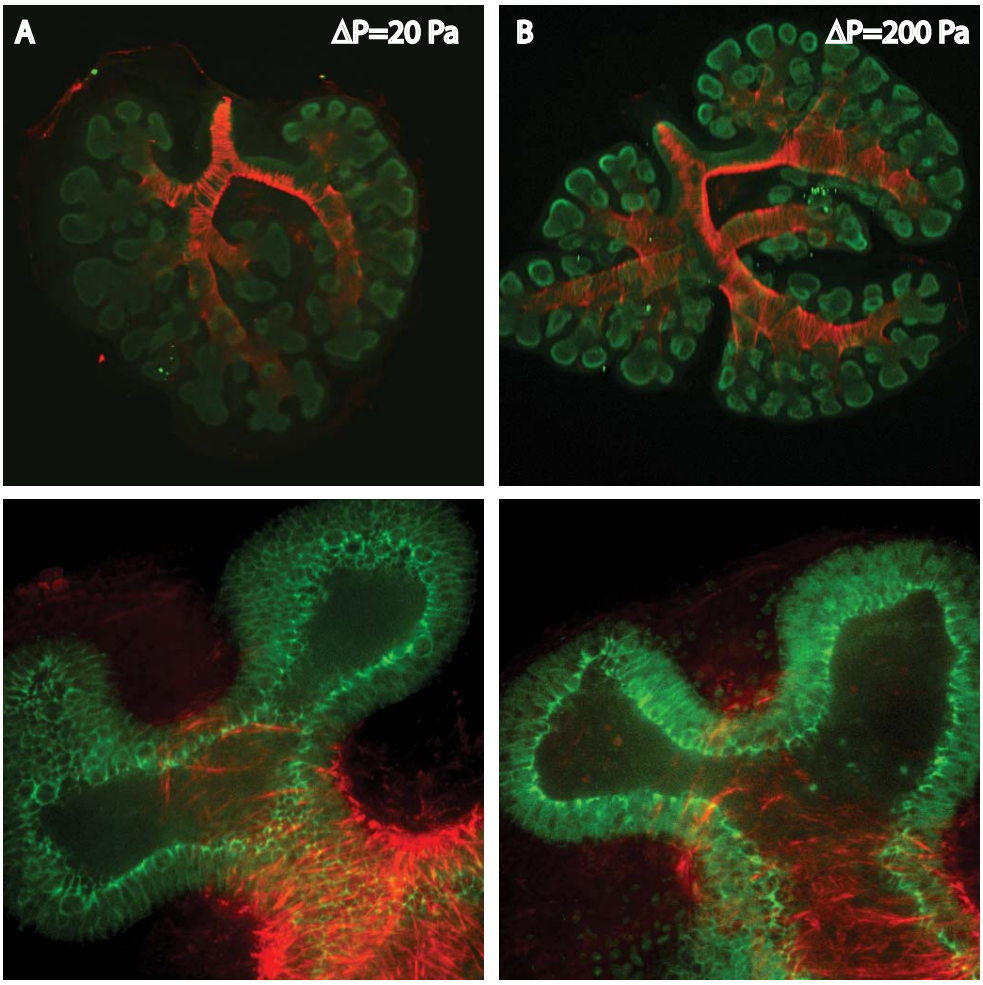
**Supplemental Figure 3:** (A-D) Principal component analysis on all genes from three biological replicates of each condition. (A) The percent of variance explained by each of the 15 principal components. (B) First and second principal components of each sample. The vertical line illustrates the separation along principal component 1 between lungs *in utero* and those cultured in microfluidic chest cavities. The dashed circle marks a batch effect in the second principal component for three samples that were sequenced at a different time than the others. (C) Second and third principal components of each sample. The horizontal line illustrates how *E*14.5 and high ΔP lungs separate from *E*12.5, *E*13.5, and low ΔP lungs along the third principal component. The dashed oval is the same as the circle in (B). (E-F) PCA analysis on the 500 genes with the highest variance across each condition. (E) The percent of variance explained by each principal component. (F) The first two principal components. The horizontal line illustrates how *E*14.5 and high ΔP lungs separate from *E*12.5, *E*13.5, and low ΔP lungs along the second principal component. (G) Top 20 gene ontology groups enriched among genes significantly increased in lungs both at *E*14.5 (vs *E*12.5) and under high (vs low) ΔP. n = number of genes from each group in this overlap (out of 216 total).

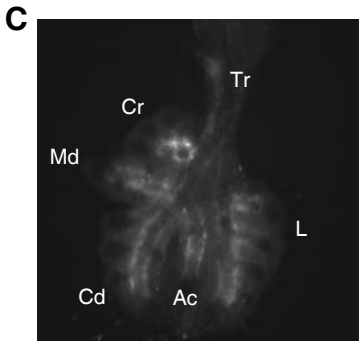
## Supplemental Figure 4

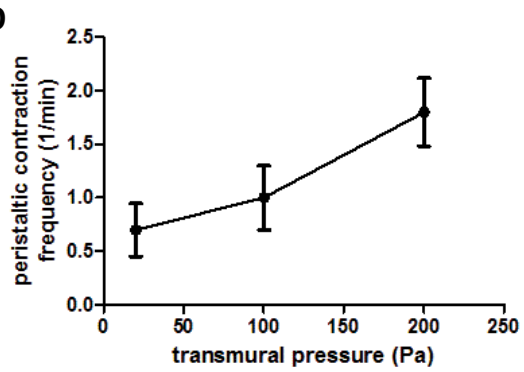



**Supplemental Figure 4:** High transmural pressure induces expression of genes that drive airway morphogenesis and maturation. Relative transcript levels of (A, B) *fgf7*, (C, D) *fgf10*, (E, F) *sftpc*, (G, H) *sfta2*, and (I, J) *muc1* for lungs isolated at *E*12.5, *E*13.5, and *E*14.5 or held under low or high ΔP. Error bars represent s.e.m. for experiments conducted in triplicate. (*) P<0.05; (**) P<0.01; (***) P<0.001.

## Supplemental Figure 5

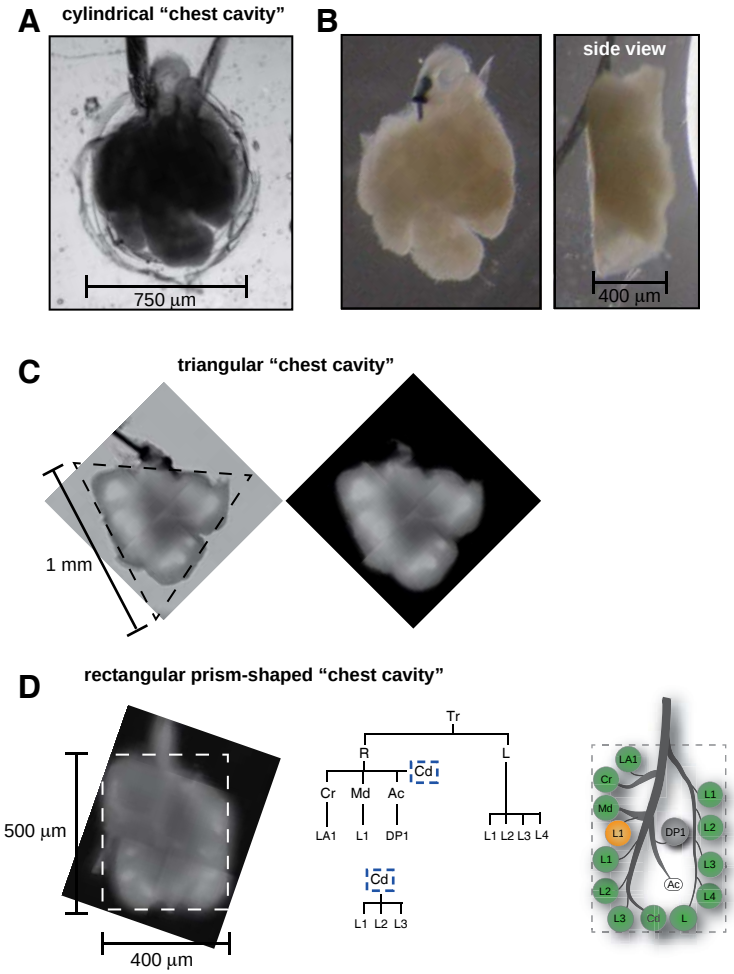


**Supplemental Figure 5:** Transmural pressure enhances smooth muscle development. (A, B) Immunofluorescence staining of E-cadherin (green) and αSMA (red) in explants cultured under ΔP of 20 or 200 Pa. (C) Image of an explanted lung from αSMA-RFP transgenic mouse embryo. This fluorescence signal delineates the developing airway smooth muscle at the outer boundaries of the airways and enables the observation of airway contractions. (D) Peristaltic contraction frequency of the proximal airways as a function of transmural pressure.

## Supplemental Figure 6




**Supplemental Figure 6:** The shape of the chest cavity determines the gross morphology of the lung. (A) Lung explant cultured for 48 hours within a cylindrical chest cavity and (B) brightfield images of front and side view of a representative explant. (C) Brightfield image and fluorescent image (E-cadherin) of a lung explant cultured within an equilateral triangle-shaped chest cavity (1-mm per side, 400 µm deep). (D) Fluorescent image of a lung explant cultured within a rectangular prism-shaped chest cavity (500 µm x 400 µm x 400 µm deep) and the corresponding lineage diagram and schematic showing the final location of the branches within the cavity.