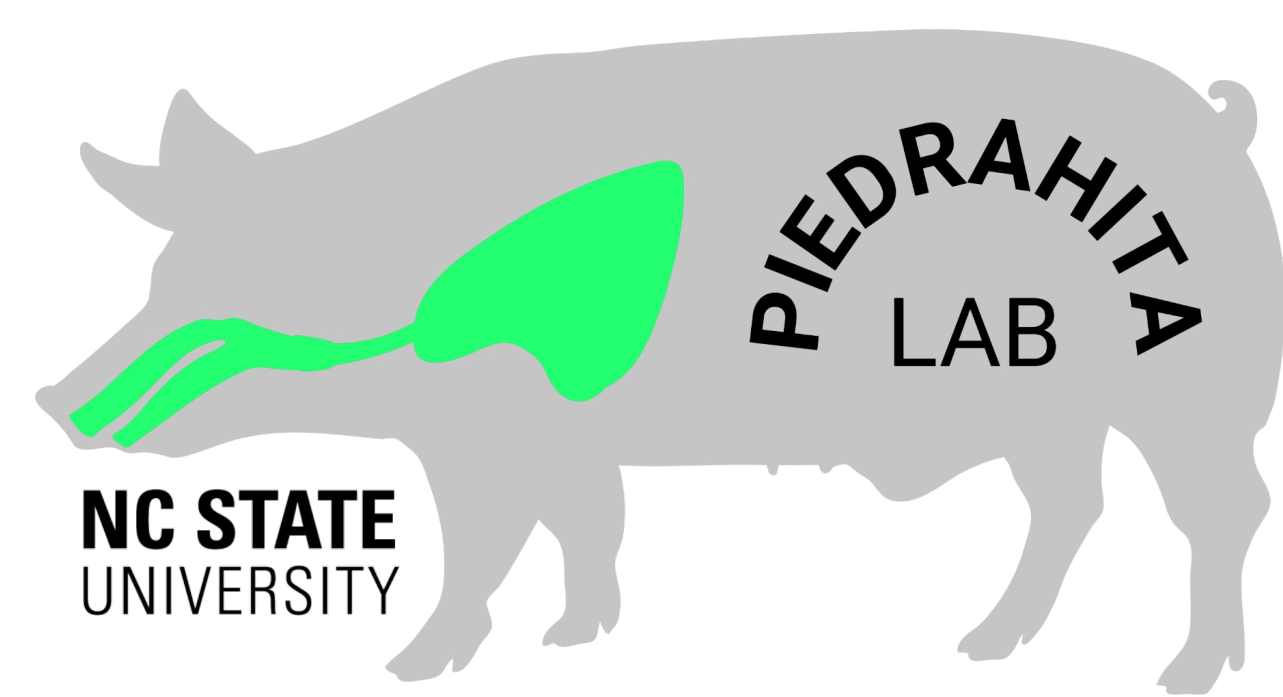




Age Associated Changes in LGR5+ Cell Distribution in Proximal and Distal Airways in Porcine Model

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Background

- LGR5+ Fibroblasts in the peribronchial region of both humans and pigs act as niche regulators, controlling basal epithelial stem cell fate through WNT and SHH signaling pathways¹.
- In preliminary work we noticed a decline of LGR5+ stromal population in aged pigs. As well as a lower overall percentage of LGR5 signaling in the proximal, large airways than the distal, small airways. This prompted us to ask the following question:

How does the density of this LGR5+ population remodel across the pulmonary axis during lung maturation?

Here we present our current progress on answering that question.

Significance

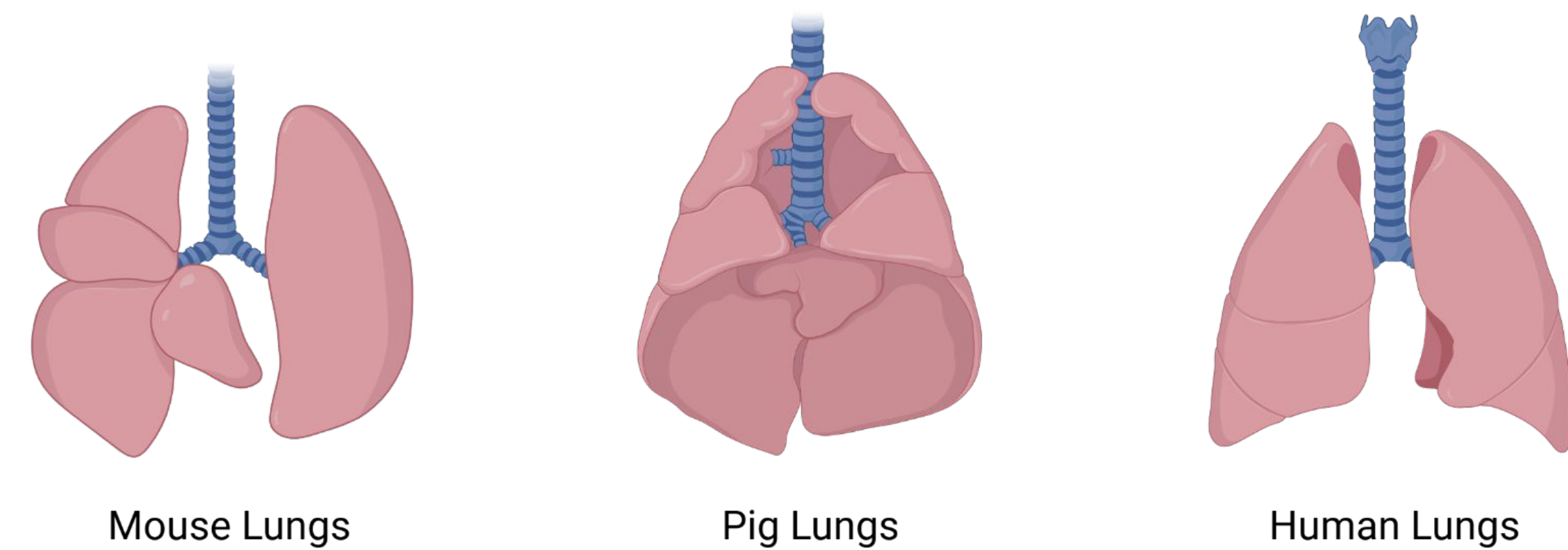


Fig. 1. Translational Research. Pig lung anatomy is similar to that of humans, making pigs a valuable large animal model for understanding lung injury and repair.

Defining Structures

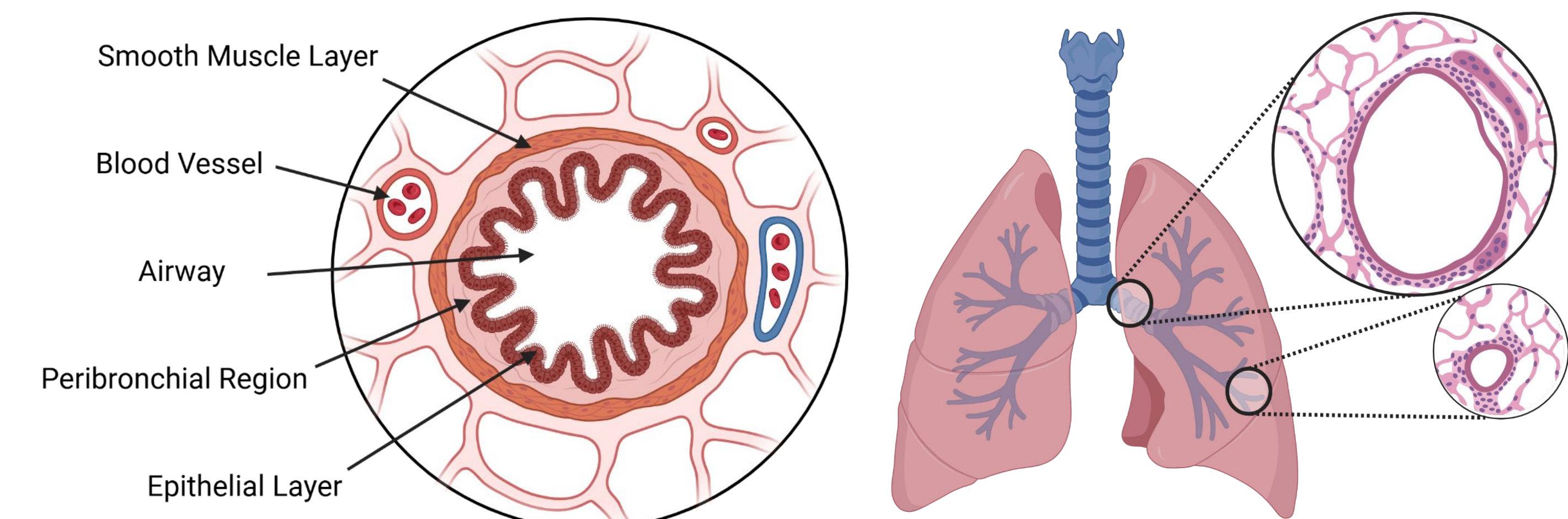


Fig. 2. Large Airway Diagram. Peribronchial region lies between smooth muscle layer and epithelial layer. Airway diameter is measured from basement membrane. Cartilage serves as a biological marker to distinguish proximal bronchi from distal bronchioles.

Fig. 3. Pulmonary Axis. Large proximal airways fork and become smaller distal airways as they approach the alveoli. Large airways have cartilage, well defined muscle and more peribronchial space.

Age Categories

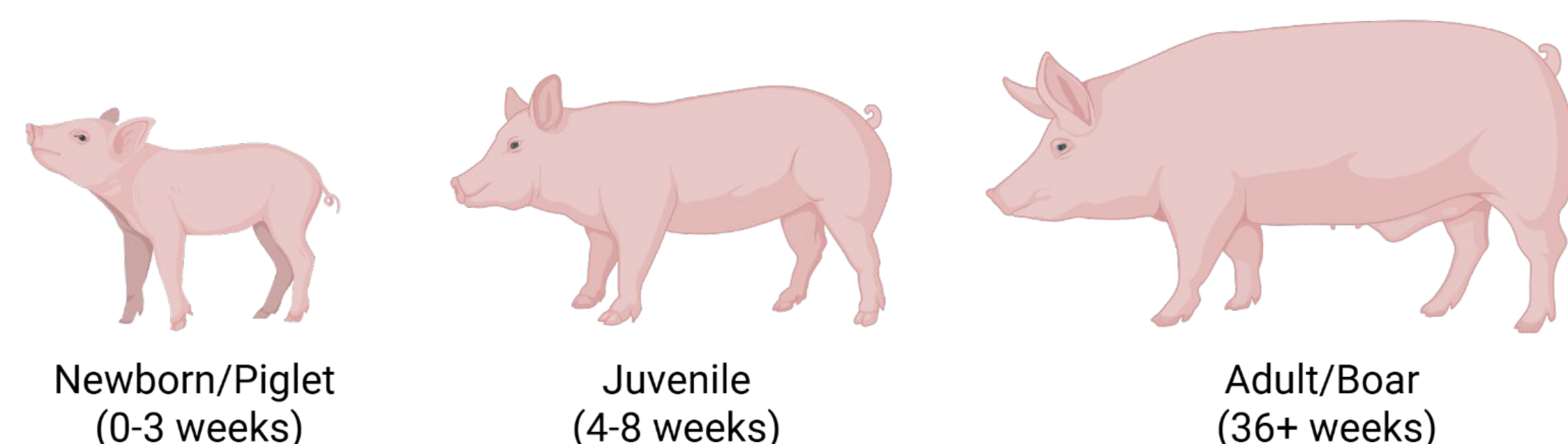


Fig. 4. Pig Age Groups. Newborns, juveniles, boars used in this study.

Methods

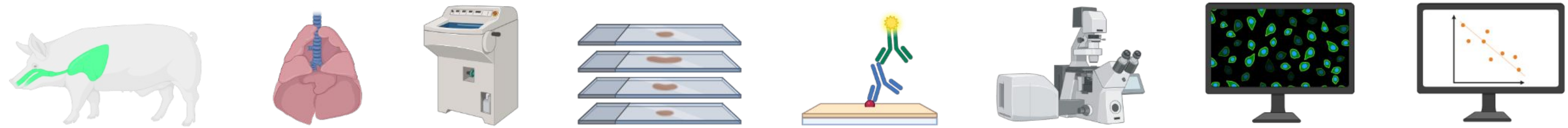


Fig. 5. Process. Gene-edited LGR5-H2B-GFP porcine lungs were sectioned and fixed, encased in OCT or wax, then sliced. Slides were immunohistochemically stained with DAPI, ASMA and EPCAM. Z-stack images were taken with confocal microscopy. Imaged airways were analyzed, measured and cell counted in QuPath. Data was analyzed using two-way ANOVA. All graphics were produced in Biorender.

Results

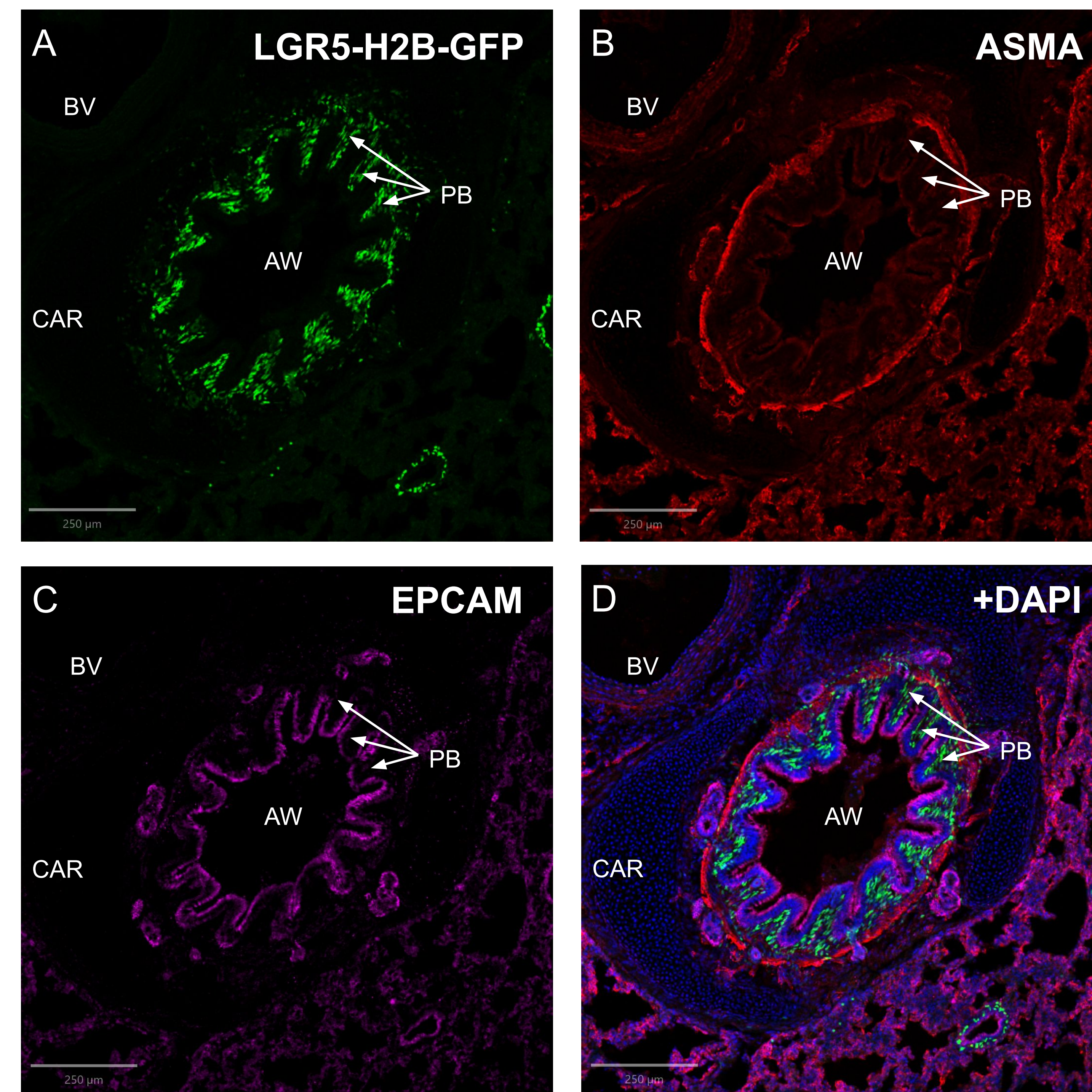


Figure 6. Immunofluorescent Characterization of the Newborn Porcine Peribronchial Niche. (A) LGR5-H2B-GFP defines the LGR5+ stromal fibroblast population. (B) ASMA defines the smooth muscle layer. (C) EPCAM defines the epithelial layer. (D) Merged composite with DAPI. Key anatomical landmarks include the airway lumen (AW), adjacent blood vessels (BV), and structural cartilage (CAR). The area of study, the peribronchial region (PB), is found between the EPCAM+ and ASMA+ layers.

Conclusions

- LGR5+ fibroblasts are the dominant stromal population of the peribronchial niche in early development.
- Distal bronchioles (lacking cartilage) have a more diverse spread of LGR5+ cells in days 161 and 287, suggesting retained regenerative capacity in some small airways and early differentiation in others.

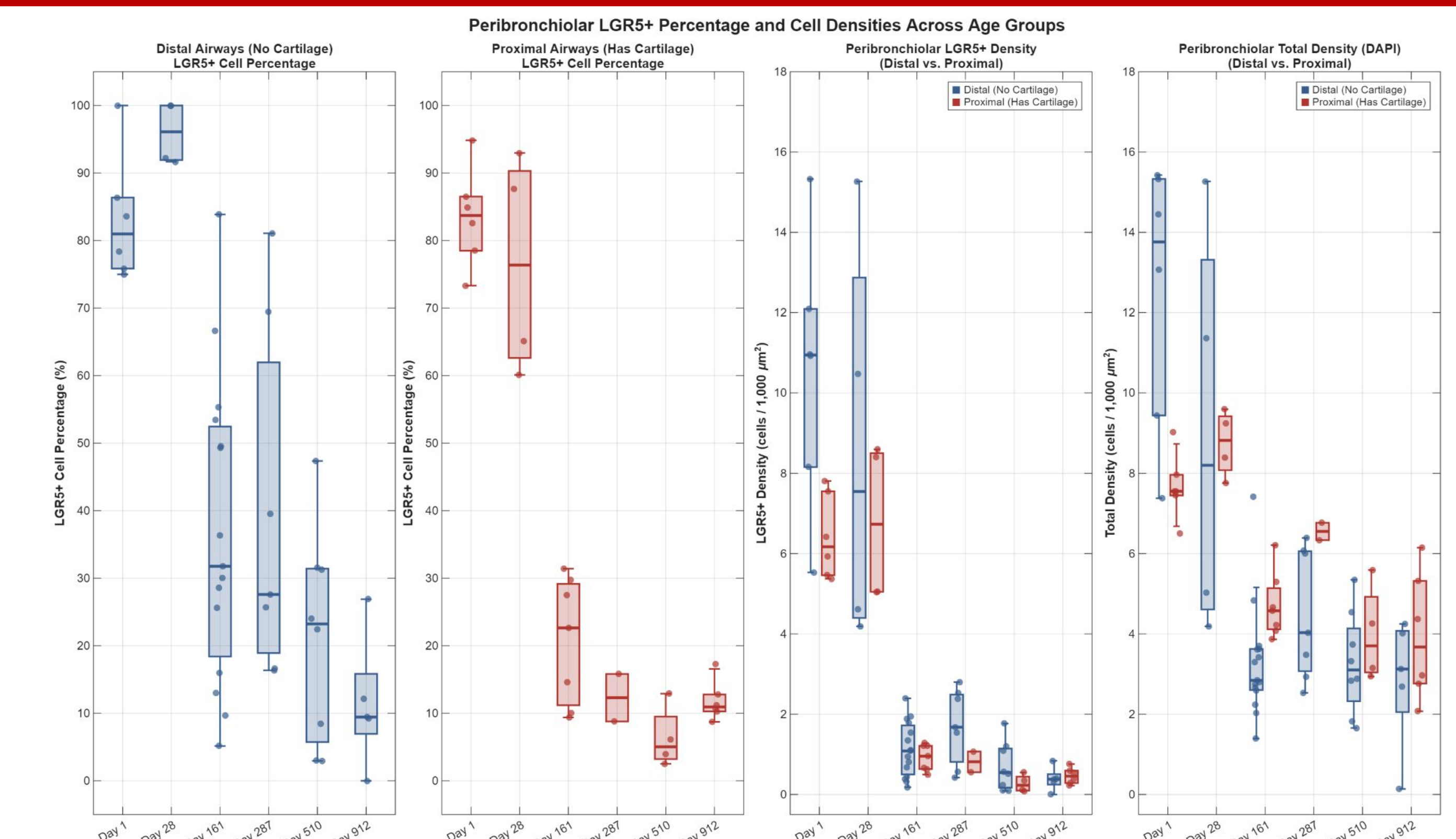


Fig. 7. Spatial Distribution Analysis. LGR5+ cell percentage shows an 86.0% depletion from day 1 to day 912 ($p = 4.04 \times 10^{-13}$). LGR5+ cell density exhibits a 20x drop ($p = 1.17 \times 10^{-17}$) compared to only a 2.8x drop in total DAPI cell density ($p = 1.28 \times 10^{-11}$) during maturation.

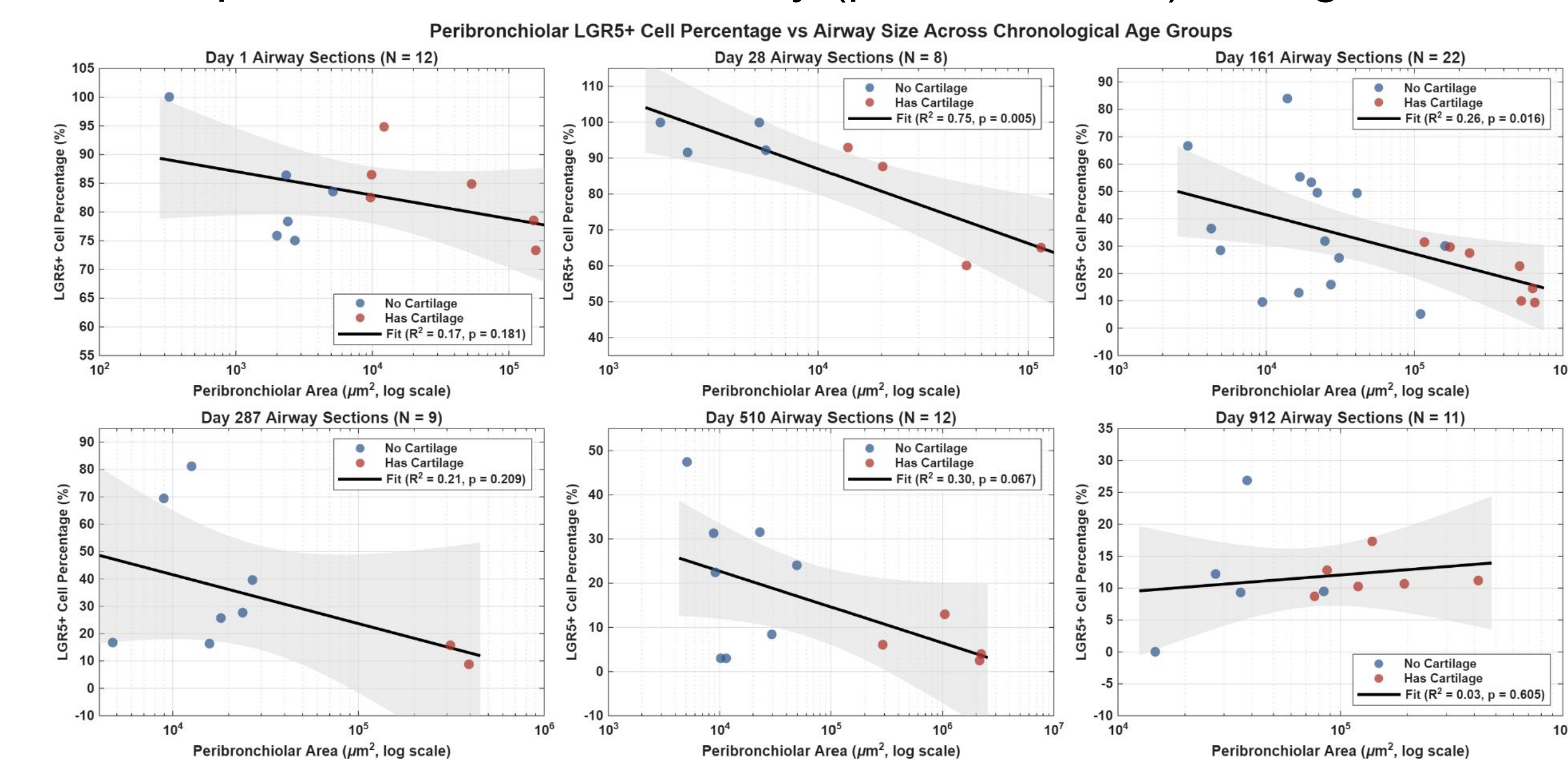


Fig. 8. Variation in Proximal/Distal Airways With Measured Area. When controlling for differences in LGR5+ percentage due to age. The high variation in distal airway percentages can be visualized.

Future Directions

Incorporation of more ages and airways. Building out our understanding of this loss of LGR5 in the peribronchial region, potentially explaining reduced regenerative capacity in older animals.

References

- Polkoff, K. M., Lampe, R., Gupta, N. K., Murphy, Y., Chung, J., Carter, A., Simon, J. M., Gleason, K., Moatti, A., Murthy, P. K., Edwards, L., Greenbaum, A., Tata, A., Tata, P. R., & Piedrahita, J. A. (2025). Novel porcine model reveals two distinct LGR5 cell types during lung development and homeostasis. *American Journal of Respiratory Cell and Molecular Biology*, 72(5), 496–509.