

# Age-Associated Changes in Proximal-to-Distal LGR5+ Stromal Cell Distribution in the Porcine Lung

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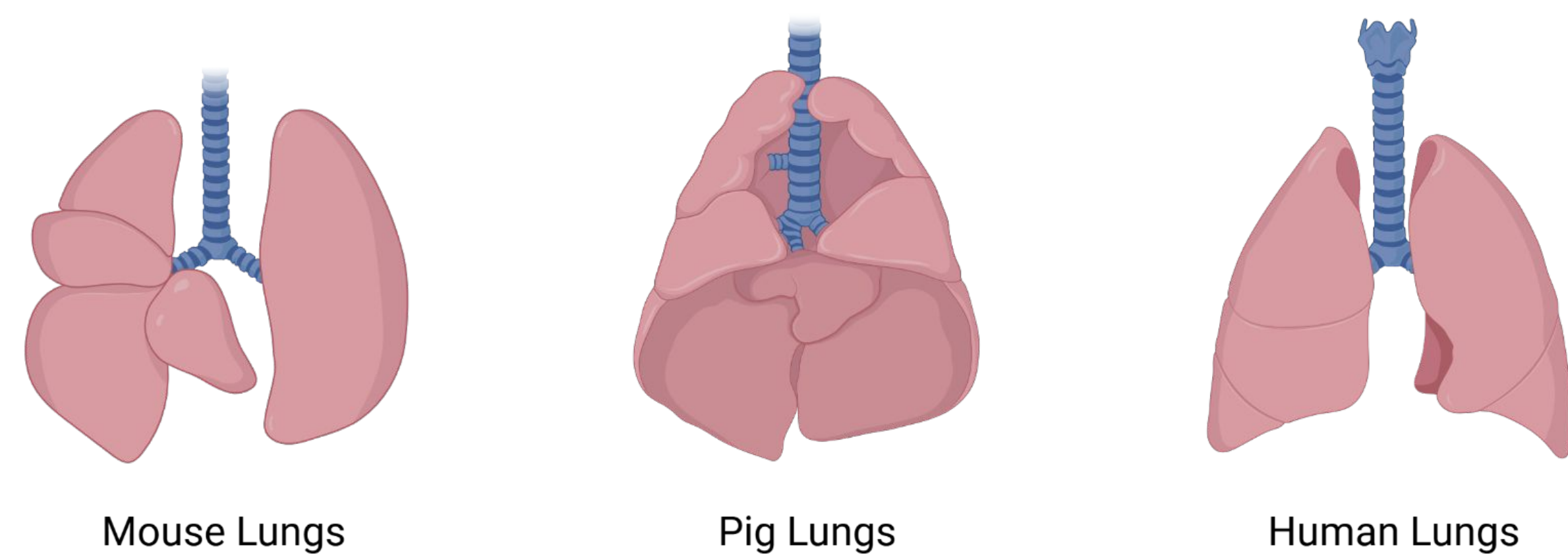
## Background

- 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 pathways<sup>1</sup>.
- 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?**

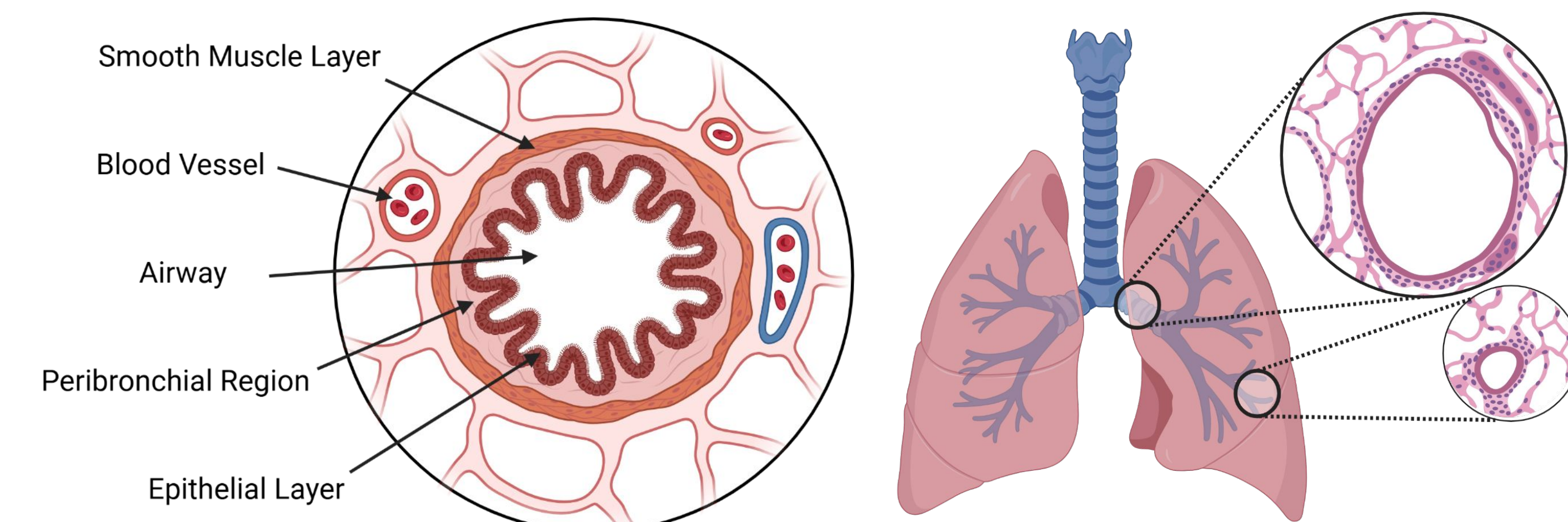
Here we present our current progress on answering that question.

## 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.

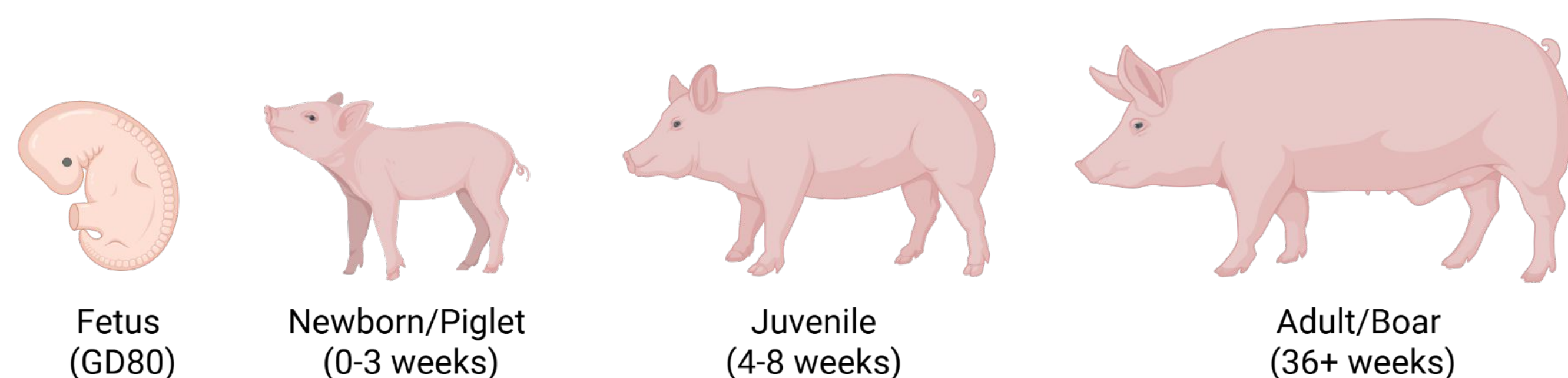
## Defining Structures



**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.

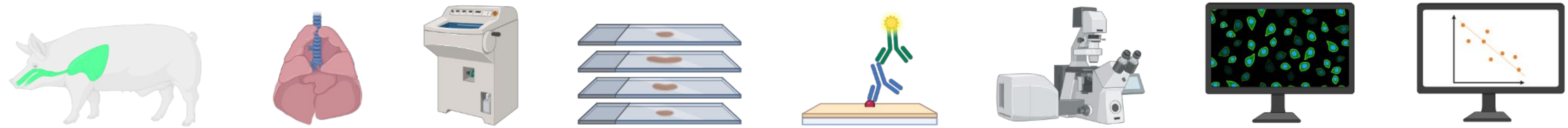
**Fig. 3. Pulmonary Axis.** Large proximal airways fork and become smaller distal airways as they approach the alveoli. Large airways have cartilage, well defined muscle and more peribronchial space.

## Age Categories



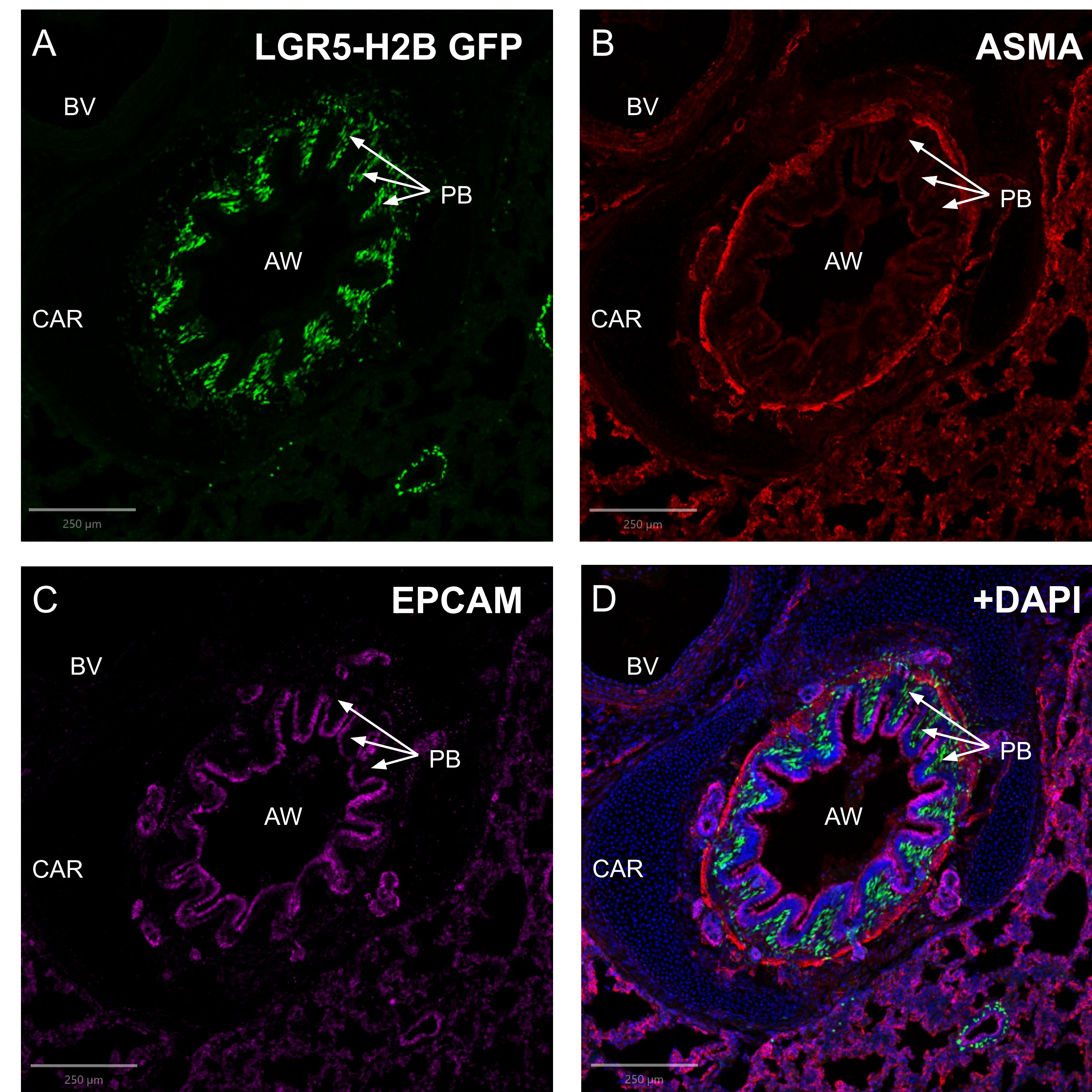
**Fig. 4. Pig Age Groups.** Newborn and juvenile pigs used in this study.

## Methods



**Fig. 5. Process.** Gene-edited LGR5-H2BGFP 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 with linear regression and t-tests. All graphics were produced in Biorender.

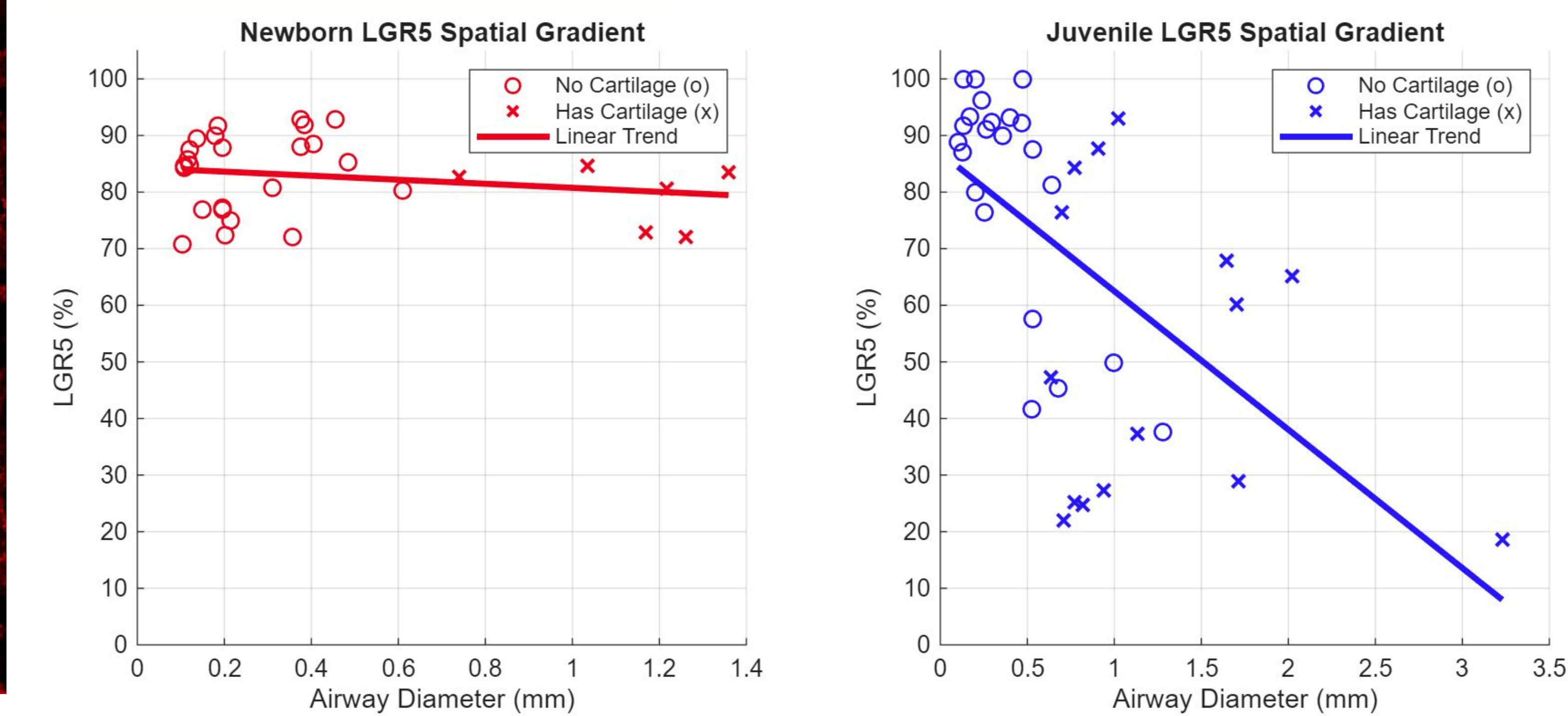
## Results



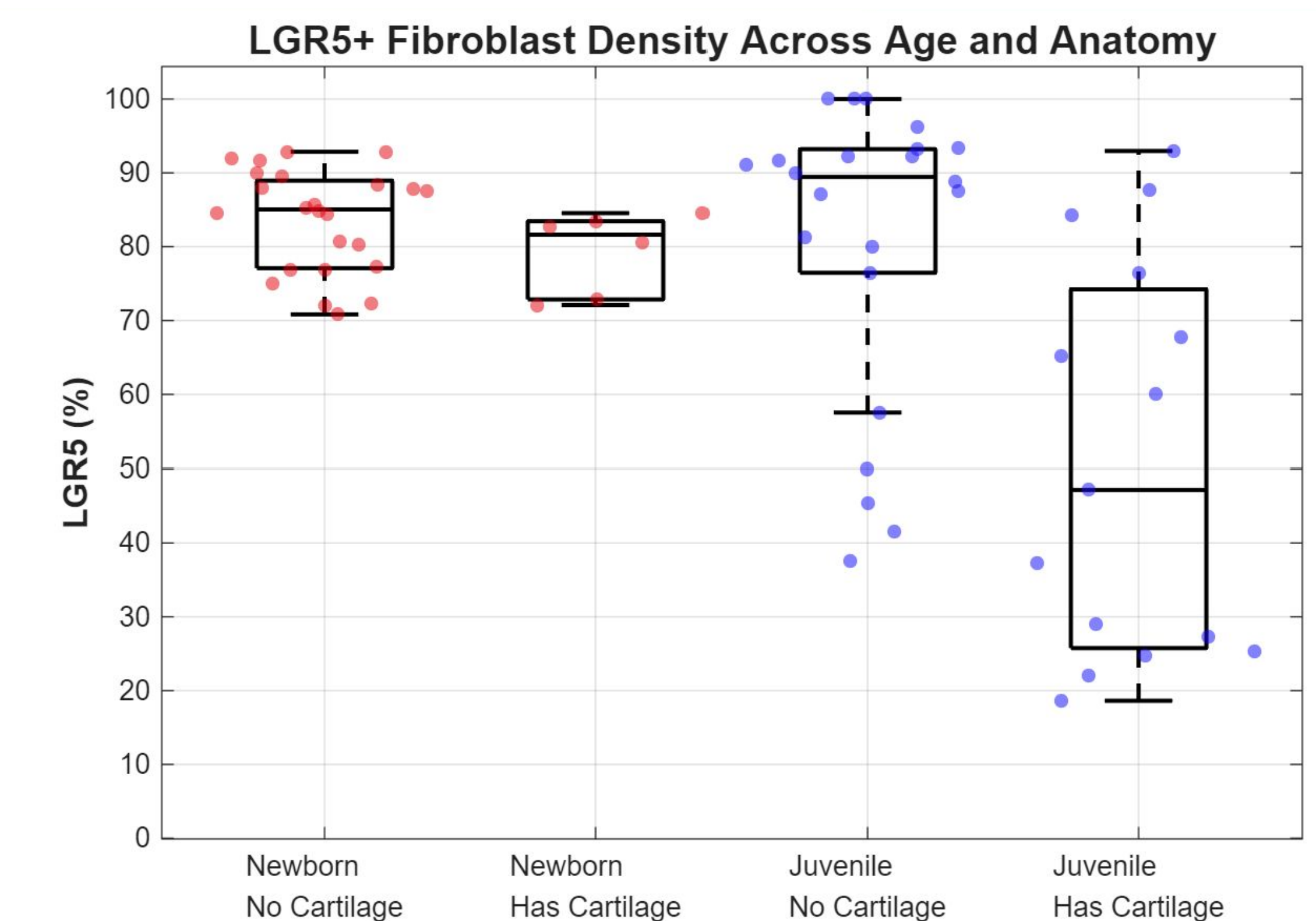
**Figure 6. Immunofluorescent Characterization of the Newborn Porcine Peribronchial Niche.** (A) LGR5-H2BGFP defines the LGR5+ stromal fibroblast population. (B) ASMA defines the smooth muscle layer. (C) EPCAM defines the epithelial layer. (D) Merged composite with DAPI. Key anatomical landmarks include 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, especially during early development.
- Distal bronchioles (lacking cartilage) serve as signaling-dense reservoirs (~80% LGR5+), suggesting these regions possess the highest innate repair capacity, regardless of age.



**Fig. 7. Spatial Distribution Analysis.** Identified a developmental transition from spatial uniformity in newborns ( $p=0.28$ ) to significant differences in juveniles ( $p=0.0001$ ).



**Fig. 8. Comparative Niche Density.** While newborns maintain a high-density LGR5+ niche across all regions, juvenile lungs exhibit a targeted ~30% depletion in cartilaginous airways ( $p=0.0005$ ).

## Future Directions

Incorporation of fetal and adult lung tissue to determine if the LGR5+ population diminishes in the distal bronchioles of aged lungs, potentially explaining reduced regenerative capacity in older organisms.

## 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.