

A Framework for Confirming Axon-LGR5+ Fibroblast Signaling in Gene-edited 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.

Neural fibers have been observed aligning with LGR5+ Airway Fibroblasts¹. **What are the fiber types that are aligning with LGR5+ Airway Fibroblasts?**

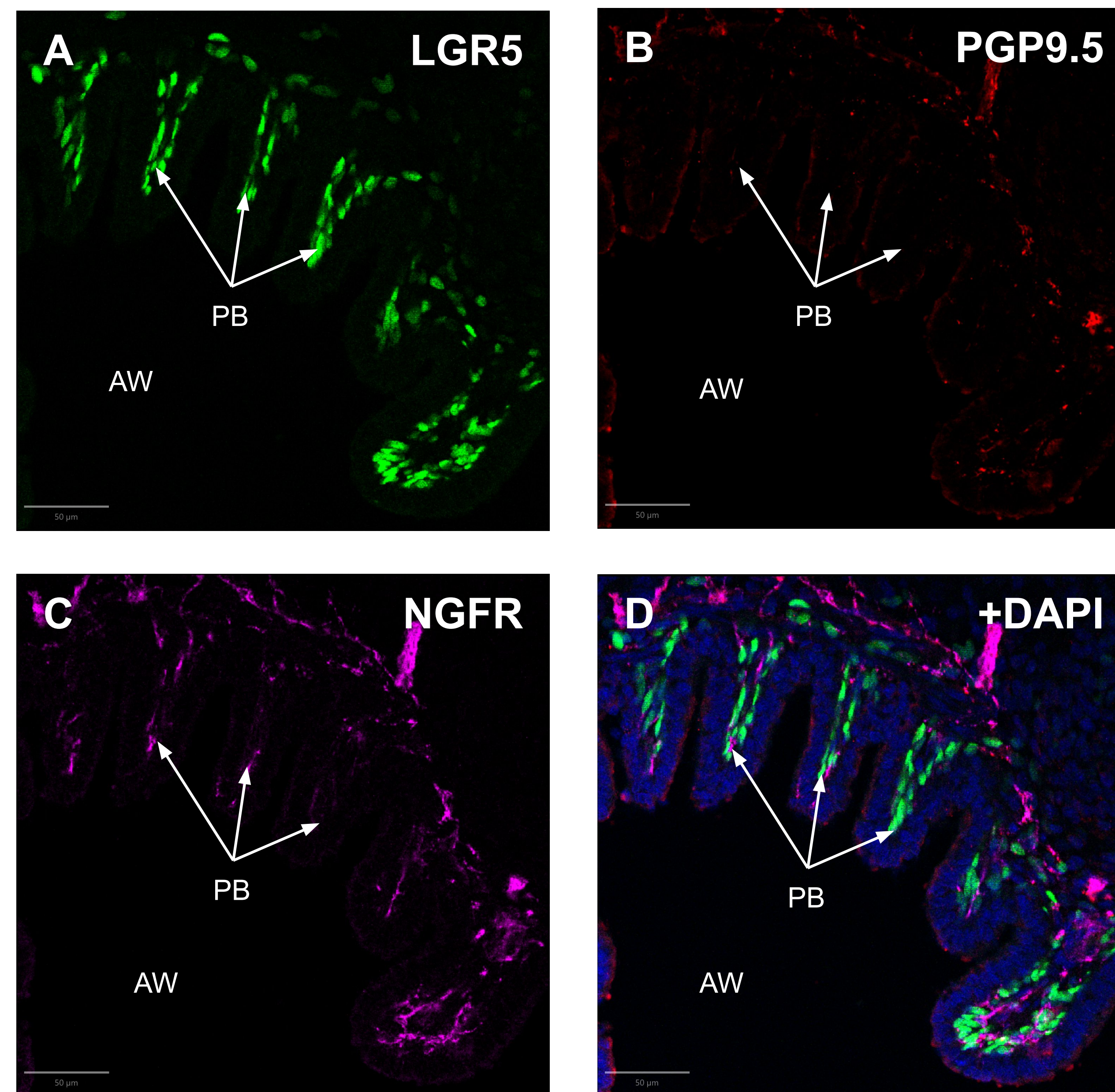


Fig. 1. Neural Fibers Align with LGR5+ Fibroblasts. (A) LGR5+ stromal cells. (B) PGP9.5 (Pan-Neuronal Marker). (C) NGFR (Nerve Growth Factor Receptor). (D) NGFR+ fibers are shown to align with LGR5+ fibroblasts. AW = Airway, PB = Peribronchial Region.

Defining Structures

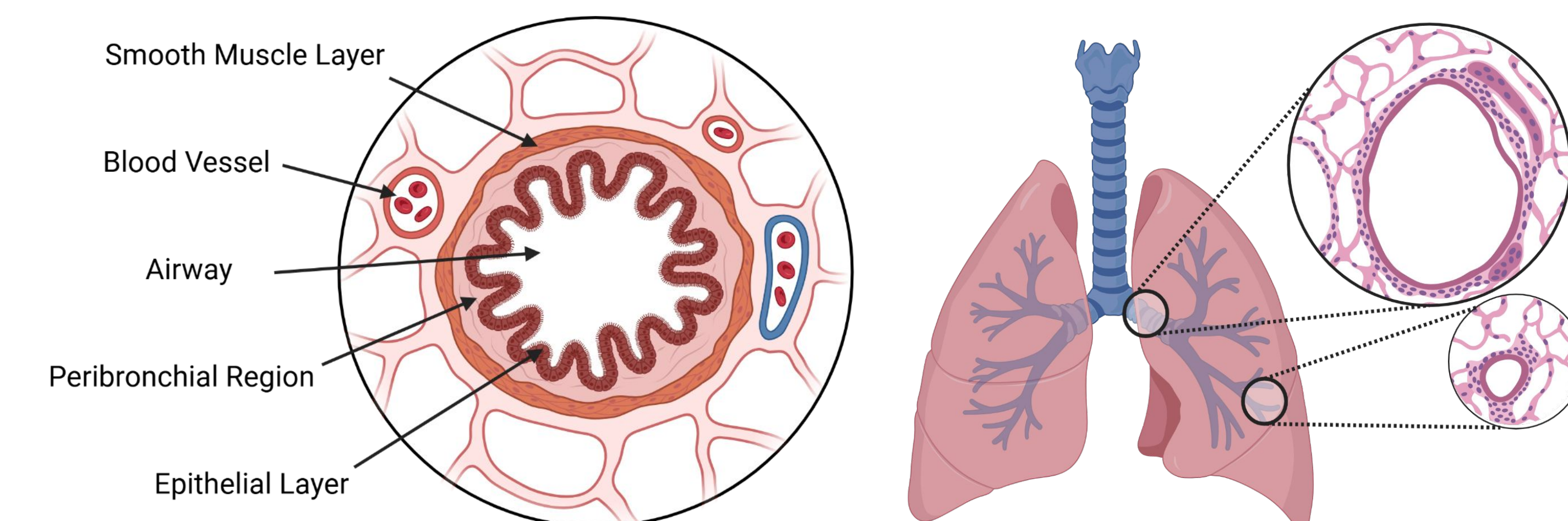


Fig. 2. Large Airway Diagram. Peribronchial region lies between smooth muscle layer and epithelial layer. Airway diameter is measured from basement membrane. Cartilage serves as a biological marker to distinguish proximal bronchi from distal bronchioles.

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.

Methods and Approach

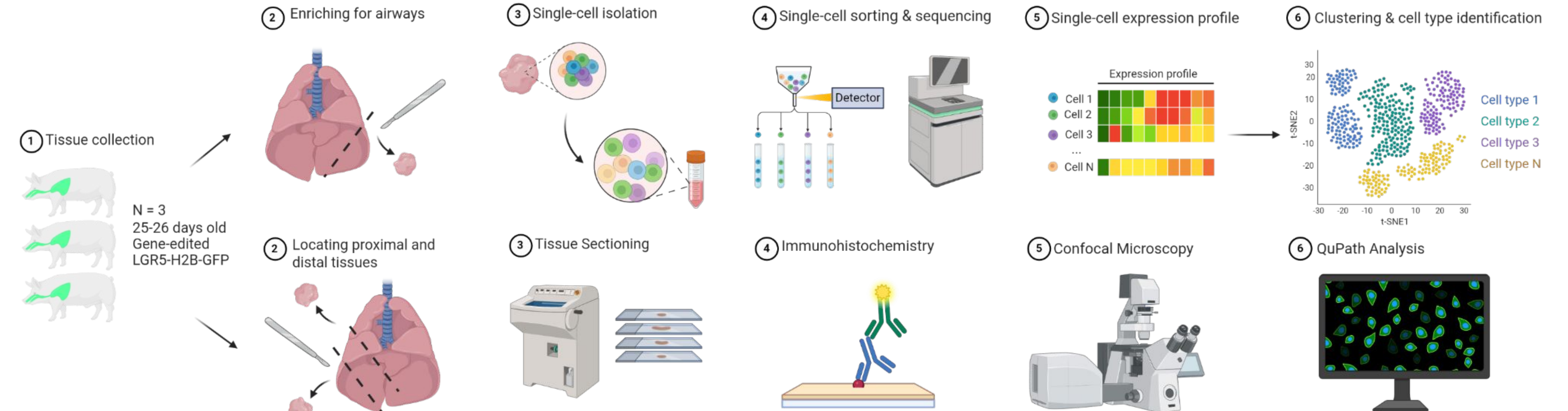


Fig. 4. Process. Three gene-edited pigs were collected and processed similarly. One side of the lungs were used for single-cell sequencing while the other side was used for immunohistochemical imaging. All graphics were produced in Biorender.

Preliminary Results

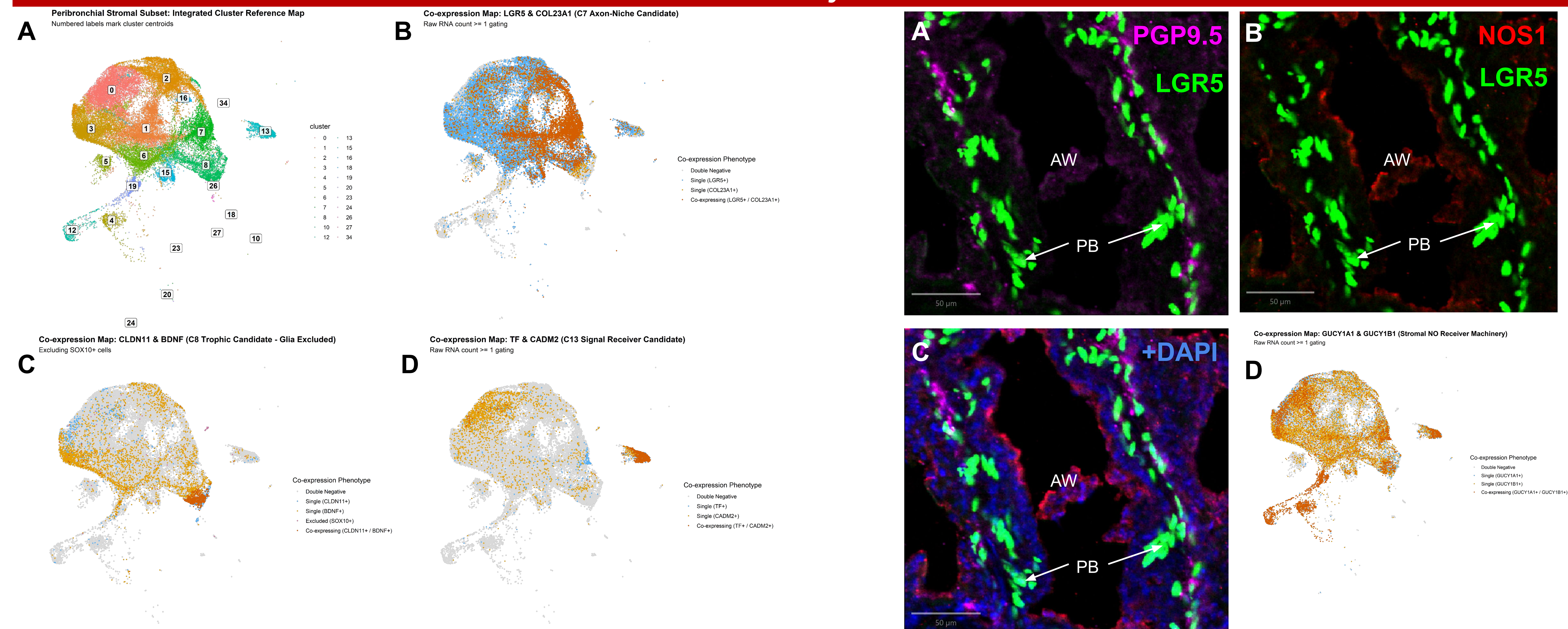


Figure 5. UMAPS. (A) Cluster reference map. (B) Cluster 7: LGR5+/COL23A1+. (C) Cluster 8: CLDN11+/BDNF+, excluding SOX10+ (Schwann cell marker). (D) Cluster 13:TF+/CADM2+. As neurons don't make it through single-cell isolation, pinning down the receptors that pick up neuronal signals on LGR5+ fibroblasts is ideal.

Conclusions

- Integrated scRNA-seq co-expression gating successfully prioritizes candidate neuro-stromal ligand-receptor pairs for spatial validation.
- Nitrgenic fibers are non-specific to LGR5+ stromal fibroblasts, as LGR5- stromal fibroblasts have the required receptors to receive nitric oxide signaling and downstream machinery to use it.

Figure 6. Investigating Possibility of Nitrgenic Fiber Identity. (A) LGR5+ cells and PGP9.5 Pan-Neuronal Marker. (B) LGR5+ cells and NOS1 a marker of nitrgenic fibers. (C) Merged composite with DAPI. Key anatomical landmarks include the airway lumen (AW) and peribronchial region (PB). (D) GUCY1A1+/GUCY1B1+ co-expression.

Future Directions

Screen peribronchiolar scRNA-Seq data set for peptidergic and adrenergic receptor markers to identify the specific neurochemical identity contacting LGR5+ fibroblasts.

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.