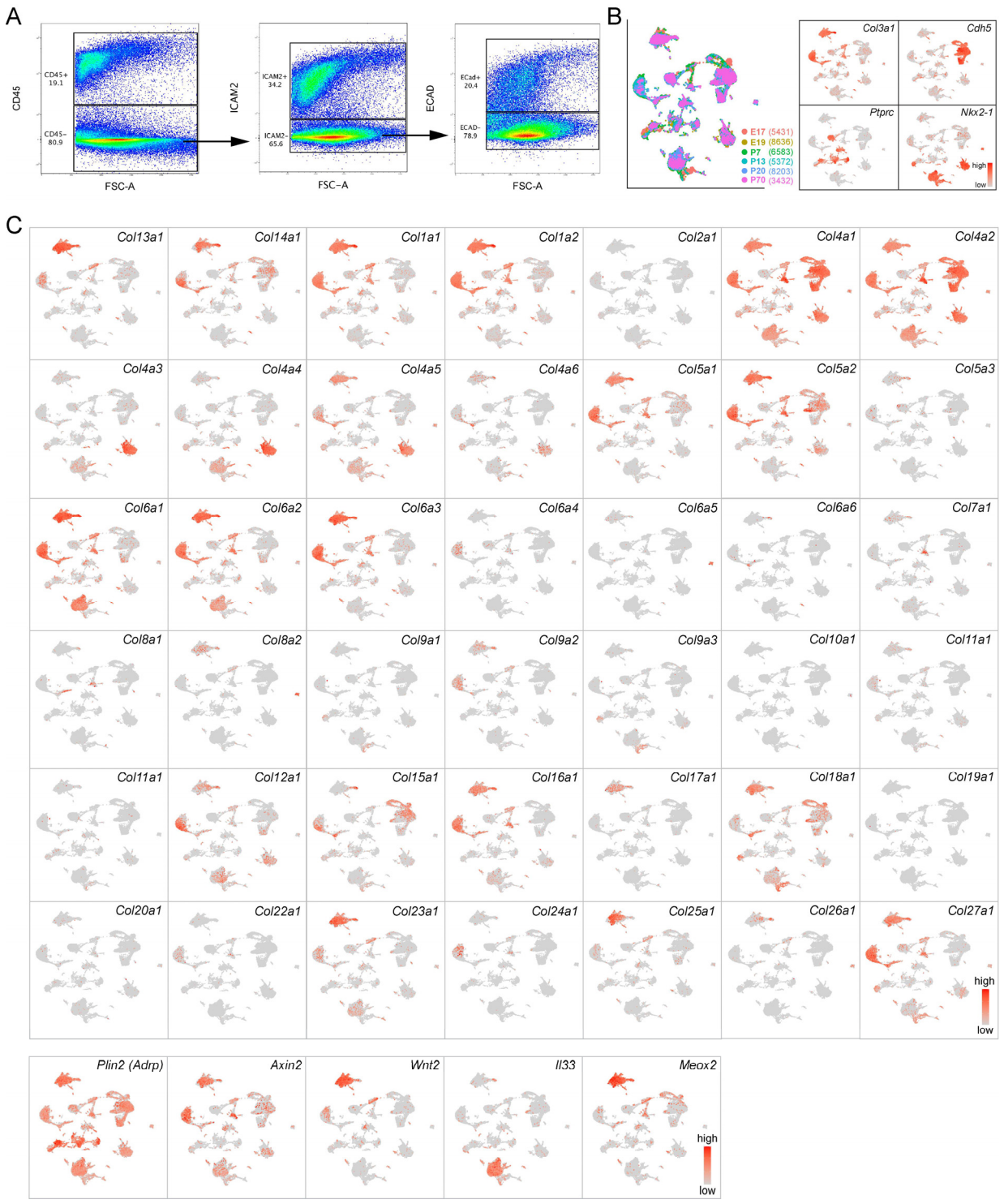

Fig. S1. Single-cell RNA-seq of mouse lungs.

(**A**) Representative FACS gating strategy to separate immune (CD45), endothelial (ICAM2), epithelial (ECAD), and mesenchymal (triple negative) cells.

(**B**) ScRNA-seq UMAP (cell number in parenthesis) and feature plots to identify mesenchymal (*Col3a1*), endothelial (*Cdh5*), immune (*Ptprc*), and epithelial (*Nkx2-1*) cells.

(**C**) ScRNA-seq feature plots showing matrix genes (top) in non-mesenchymal cells, wide-spread expression of a presumable lipofibroblast marker *Plin2* (also known as *Adrp*) and a Wnt-signaling target gene *Axin2*, and specific expression of *Wnt2*, *Il33*, and *Meox2* (bottom row).

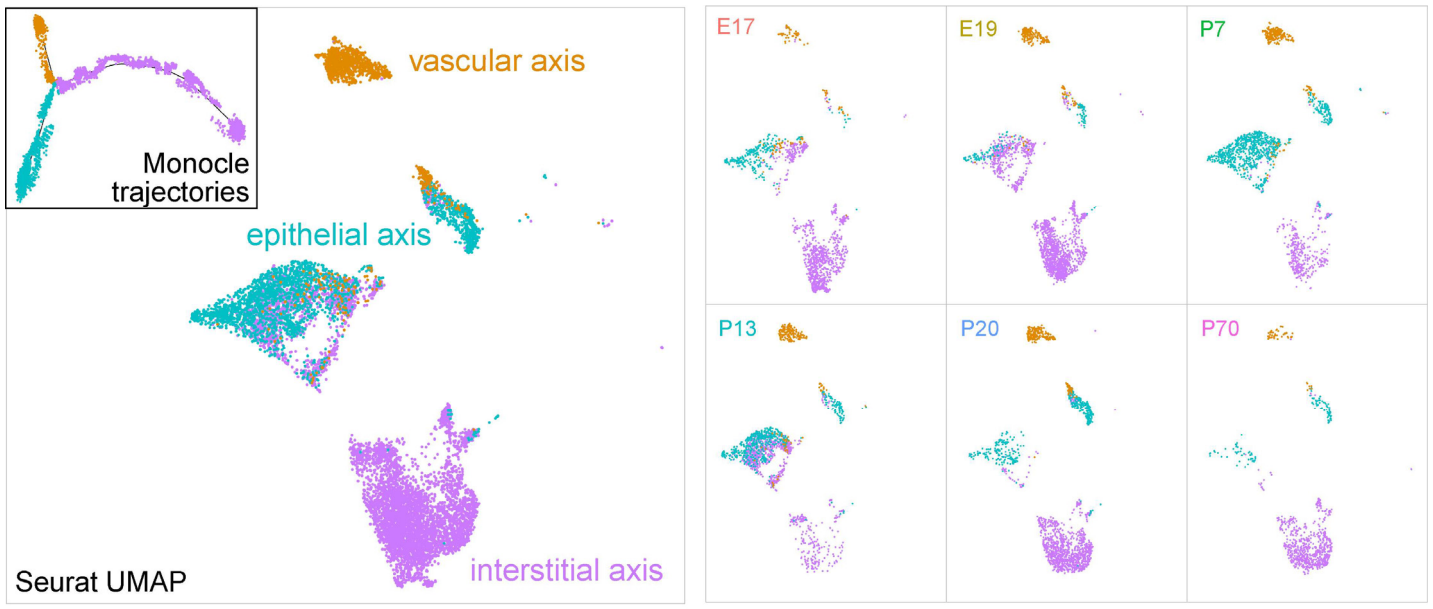

Fig. S2. Distribution of Monocle trajectories on Seurat UMAPs.

Cells in the 3 Monocle trajectories are colored on Seurat UMAPs corresponding to the vascular, epithelial, and interstitial axes.

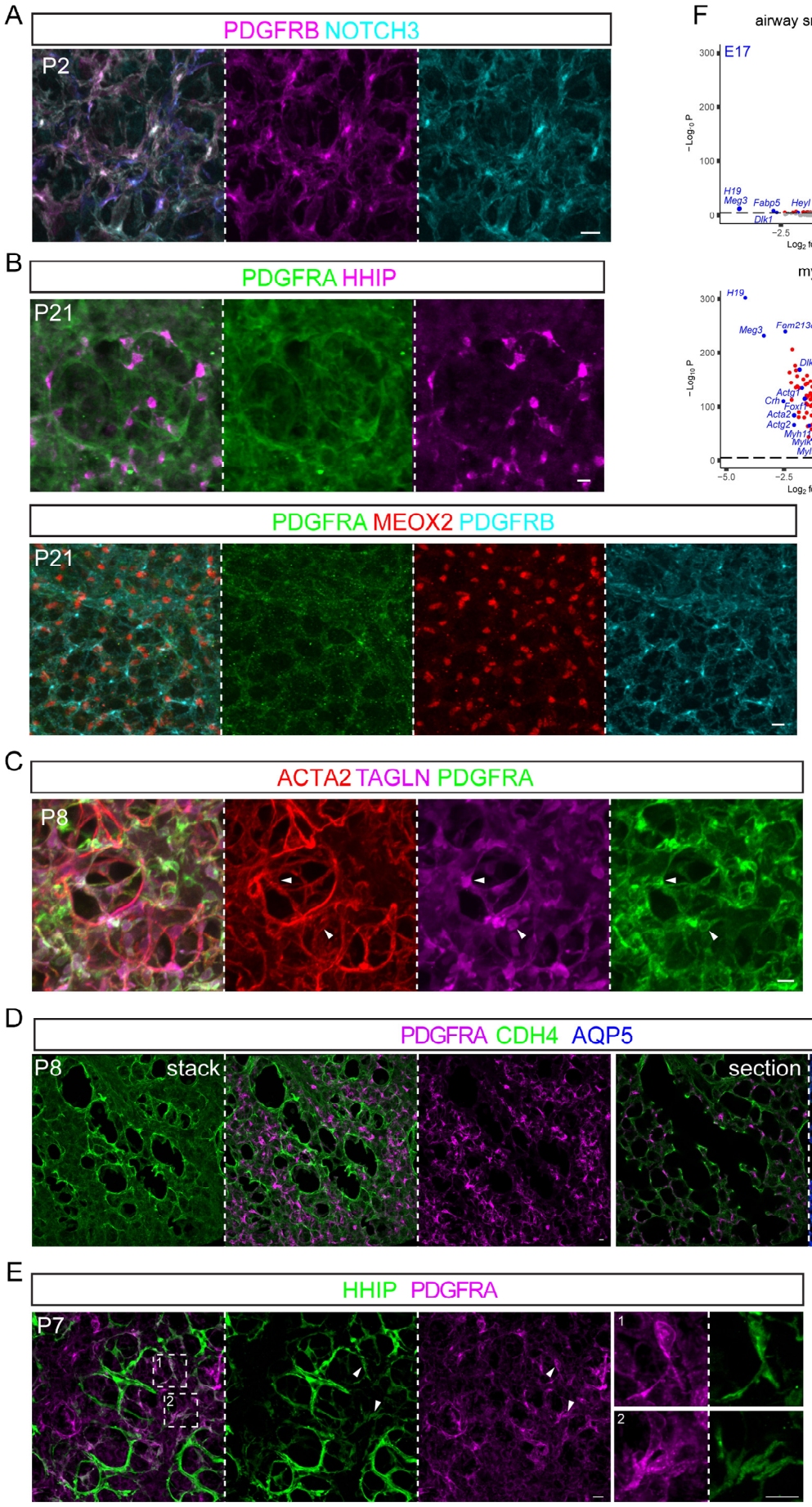

Fig. S3. Further analysis of mesenchymal cell markers.

(**A**) PDGFRB and NOTCH3 are co-expressed in pericytes and have perinuclear accumulation. Scale: 10 um.

(**B**) PDGFRA staining in MEOX2+ cells in the mature lung is diffuse and has no perinuclear accumulation, as compared to perinuclear HHIP staining in ductal myofibroblasts (top) and perinuclear PDGFRB staining in pericytes (bottom). Scale: 10 um.

(**C**) ACTA2 staining does not reliably mark the cell nucleus, in contrast to TAGLN and PDGFRA (arrowhead). Scale: 10 um.

(**D**) Stack (left) and section (right) views of alveolar ducts (AQP5, an AT1 cell marker) extending toward the lung lateral edge and surrounded by CDH4+ ductal myofibroblasts, distinct from PDGFRA + alveolar myofibroblasts. Scale: 10 um.

(**E**) Occasional HHIP and PDGFRA double positive cells (arrowhead) are possibly intermediates between ductal and alveolar myofibroblasts. Scale: 10 um.

(**F**) Volcano plots comparing over time (P13 versus E17) airway smooth muscle cells and myofibroblasts. See Table S3 for full data.

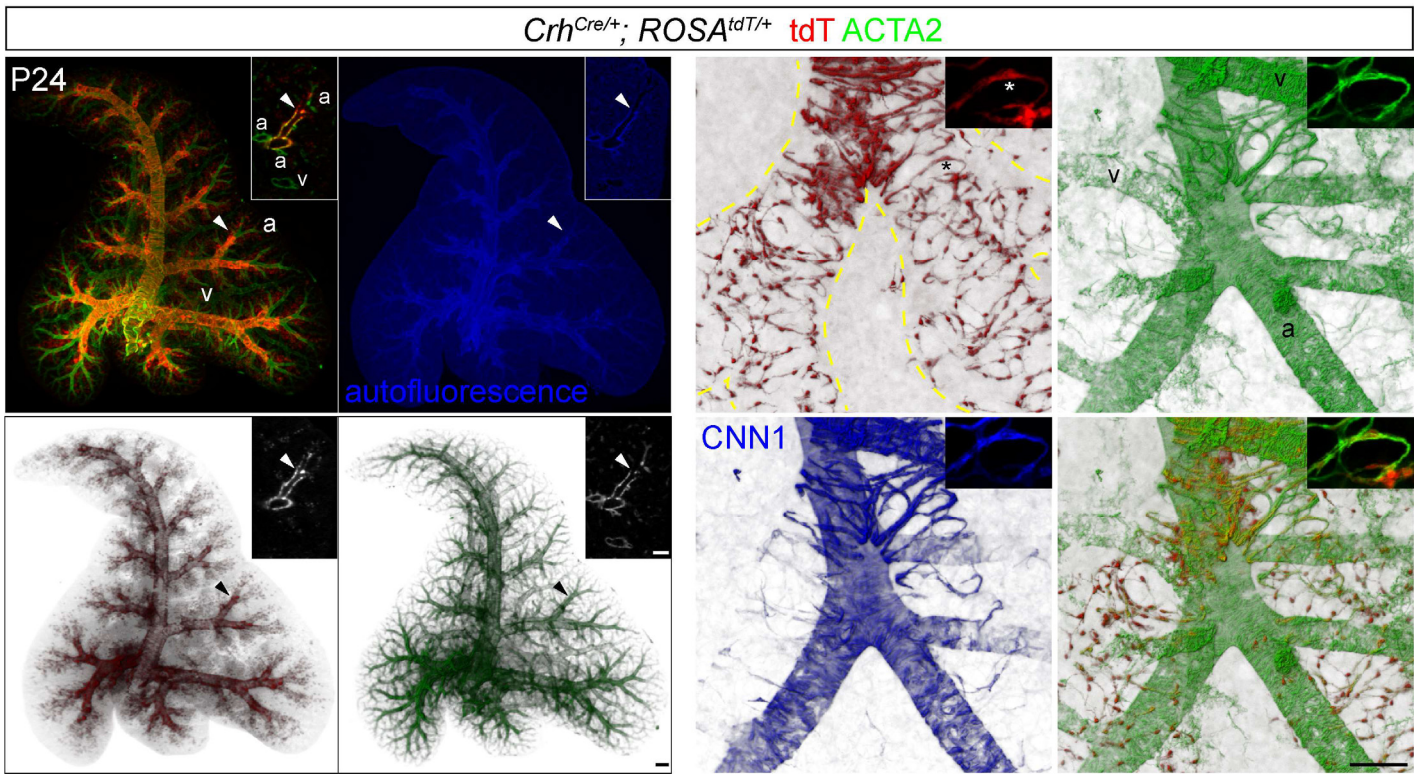

Fig. S4. Characterization of a *Crh*^{Cre} driver.

Left: Optical projection tomography images showing that *Crh*^{Cre} labeled cells surround airways and alveolar ducts (arrowhead, bronchoalveolar duct junction recognizable by the abrupt thinning of the epithelium autofluorescence), but not arterioles (a) and venules (v). Scale: 250 um.

Right: *Crh*^{Cre} labeled ductal myofibroblasts extend beyond the airways and express a low level of contractile proteins ACTA2 and CNN1. Asterisk: nucleus of the cell-of-interest. Imaris normal shading view is used when appropriate. Scale: 100 um.

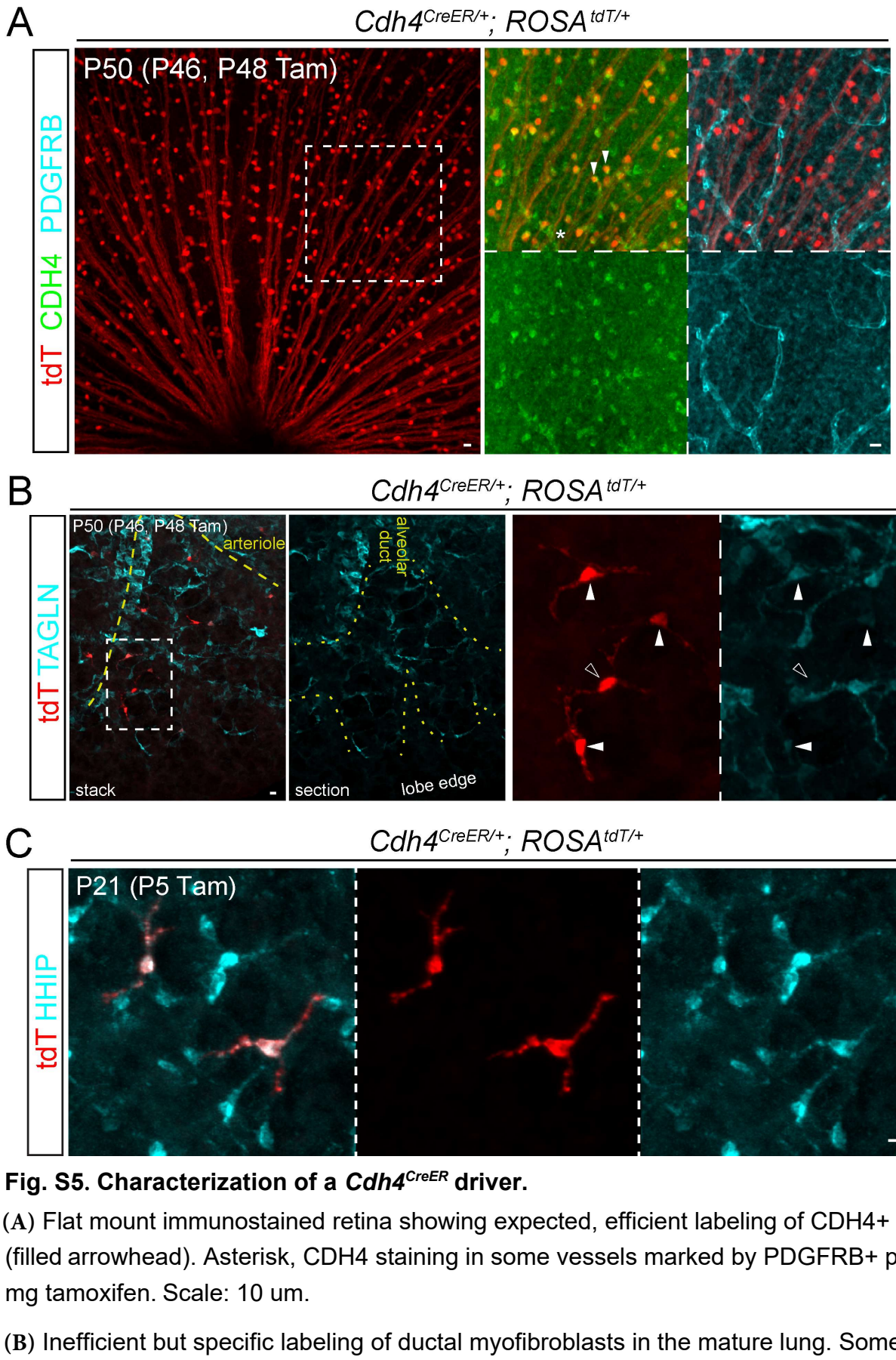

Fig. S5. Characterization of a *Cdh4*^{CreER} driver.

(**A**) Flat mount immunostained retina showing expected, efficient labeling of CDH4+ ganglion cells (filled arrowhead). Asterisk, CDH4 staining in some vessels marked by PDGFRB+ pericytes. Tam, 3 mg tamoxifen. Scale: 10 um.

(**B**) Inefficient but specific labeling of ductal myofibroblasts in the mature lung. Some ductal myofibroblasts have reduced TAGLN (filled versus open arrowhead). Tam, 3 mg tamoxifen. Scale: 10 um.

(**C**) Lineage-traced ductal myofibroblasts expressing HHIP. Tam, 500 ug tamoxifen. Scale: 10 um.

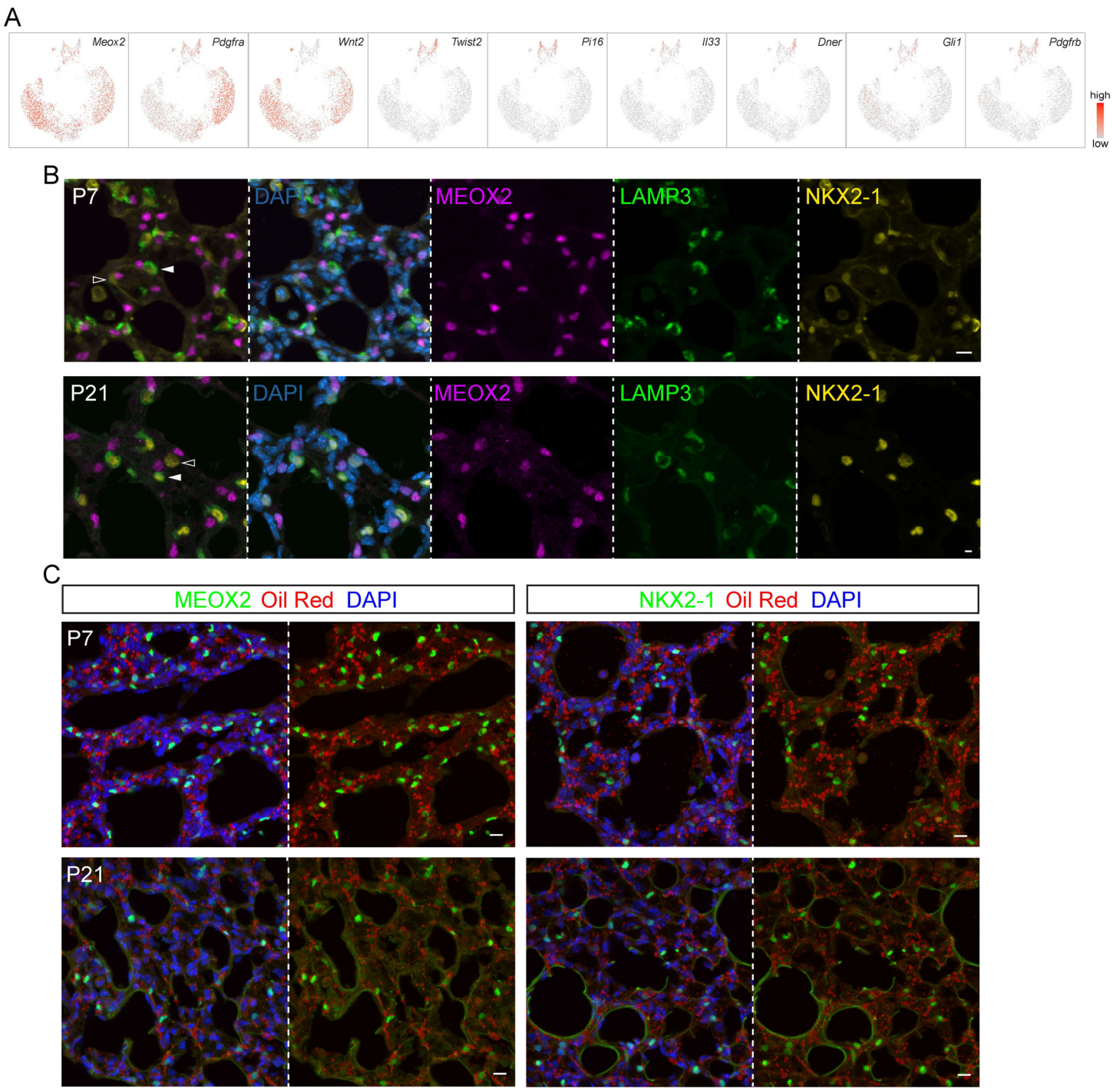

Fig. S6. Localization of MEOX2+ interstitial cells and oil red stained cells.

(**A**) ScRNA-seq feature plots of interstitial cells. See UMAP in Fig. 5A.

(**B**) Immunostaining images showing that MEOX2+ cells are not closer to alveolar type 2 cell nuclei (filled arrowhead; LAMP3+ NKX2-1+) than to alveolar type 1 cell nuclei (open arrowhead; LAMP3- NKX2-1+) or any other nuclei (DAPI). Scale: 10 um.

(**C**) Oil red stained lipid droplets are wide-spread and not specific to MEOX2+ interstitial cells or NKX2-1+ epithelial cells. Scale: 10 um.

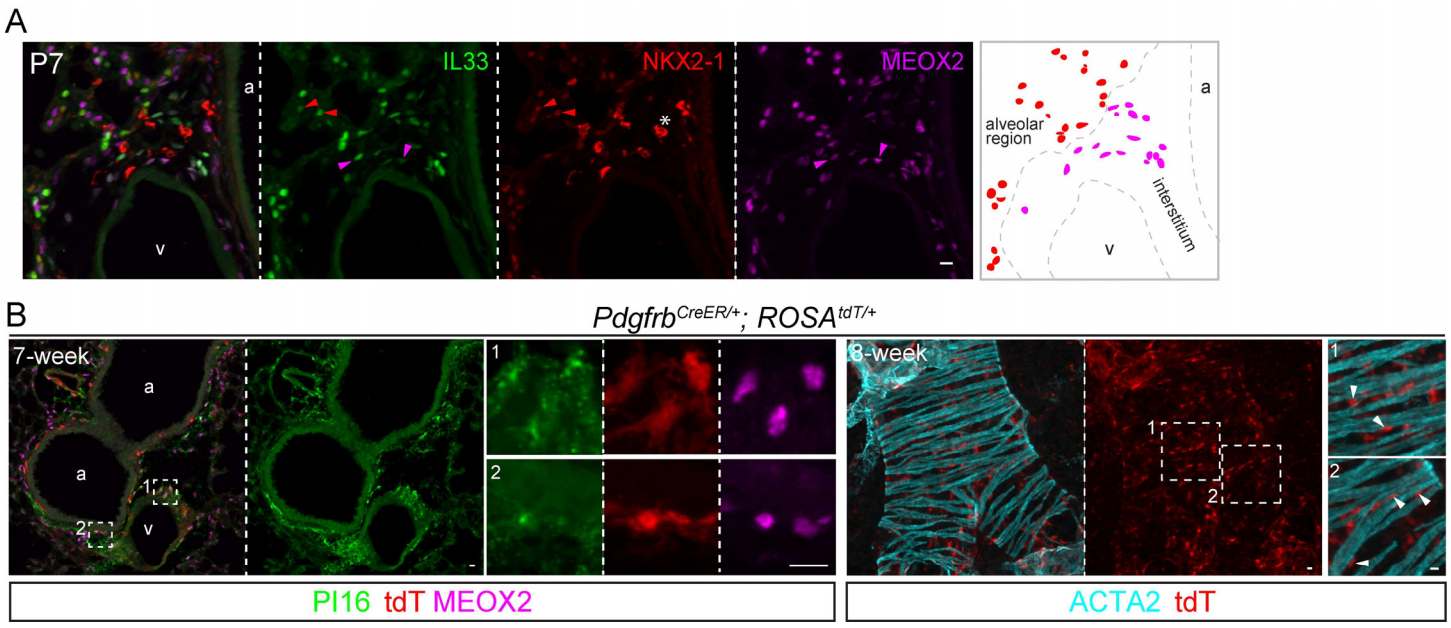

Fig. S7. Further characterization of proximal MEOX2+ interstitial cells.

(A) Immunostaining images and diagram showing that IL33 marks MEOX2+ cells in the bronchovascular bundle (a, airway; v, vessel), but NKX2-1 epithelial cells in the alveolar region. Asterisk, stained immune cells from the mouse NKX2-1 antibody. Scale: 10 um.

(B) Section (left) and wholemount (right) immunostaining images to show that *Pdgfrb*^{CreER} labeled proximal interstitial cells express PI16 and MEOX2 and are between ACTA2+ airway smooth muscle cells (a, airway; v, vessel). 3 mg tamoxifen was administrated 48 hr before lung harvest. Scale: 10 um.

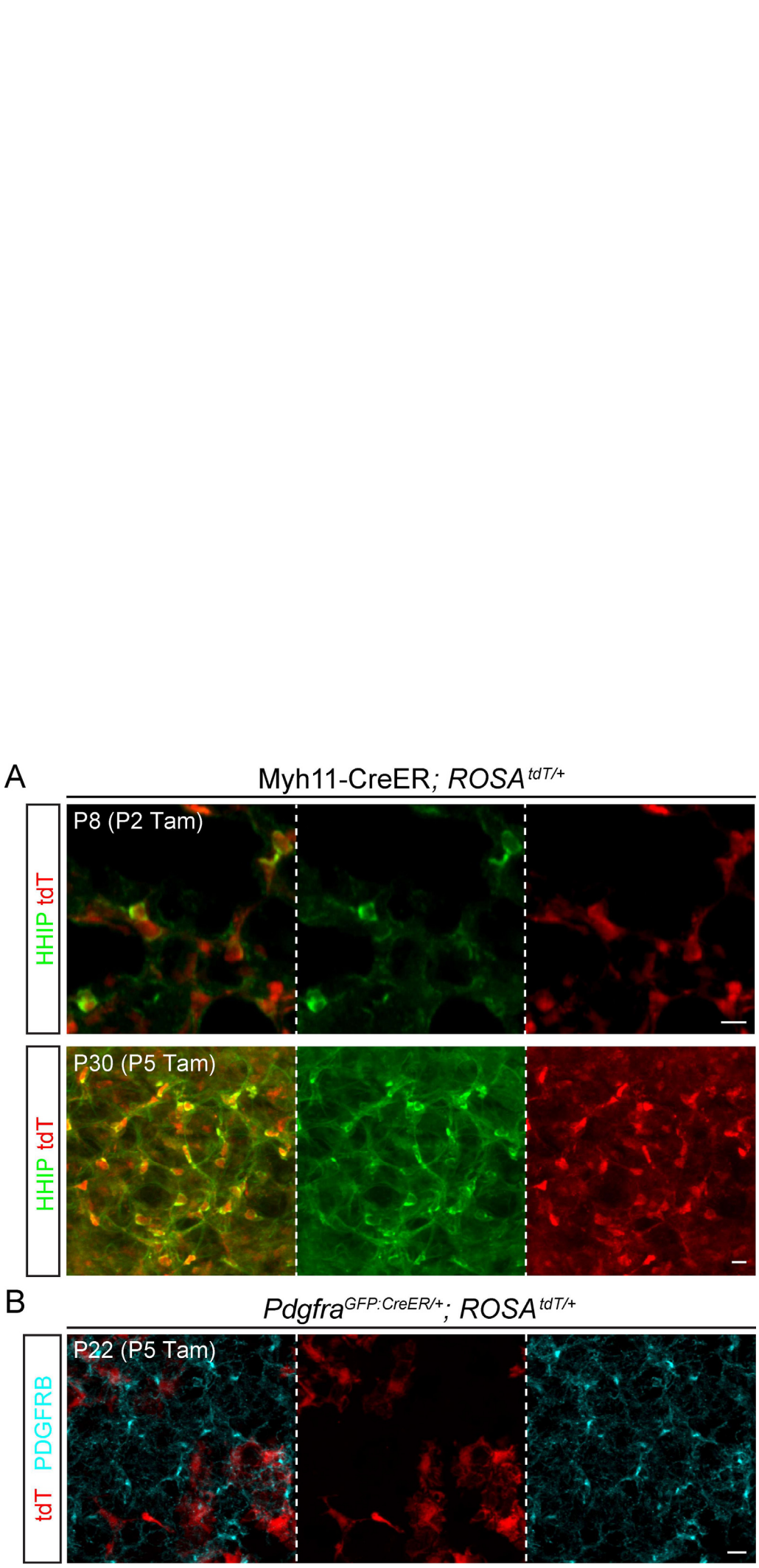

Fig. S8. Myh11-CreER labels ductal myofibroblasts.

(A) Immunostaining images showing Myh11-CreER labels HHIP+ ductal myofibroblasts in the neonatal lung (top), which persist in the mature lung (bottom). Tam, 300 ug tamoxifen. Scale: 10 um.

(B) Immunostaining images showing *Pdgfra*$^{GFP:CreER}$ labeled cells do not trace into pericytes (PDGFRB). Tam, 300 ug tamoxifen. Scale: 10 um.

Table S1. Markers for the 24 clusters in Figure 1C.

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TableS2. Markers for cell populations in the vascular axis (worksheet 1) and dot plot (worksheet 2) in Figure 2A.

Click here to download Table S2

Table S3. Markers for cell populations in the epithelial axis in Figure 3A (worksheet 1) and dot plot in Figure 4A (worksheet 2). Comparisons of airway smooth muscle cells and myofibroblasts over time (P13 versus E17; Fig. S3F) are included in worksheets 3 and 4.

Click here to download Table S3

Table S4. Markers for cell populations in the interstitial axis (worksheet 1) and dot plot (worksheet 2) in Figure 5A.

Click here to download Table S4

Table S5. Cell quantification for Myh11-CreER, *Pdgfra*$^{GFP:CreER}$, and *Pdgfrb*^{CreER} drivers.

Click here to download Table S5

Supplementary file 1. R script for scRNA-seq analysis.

Click here to download Supplementary file 1