

Supplementary Material

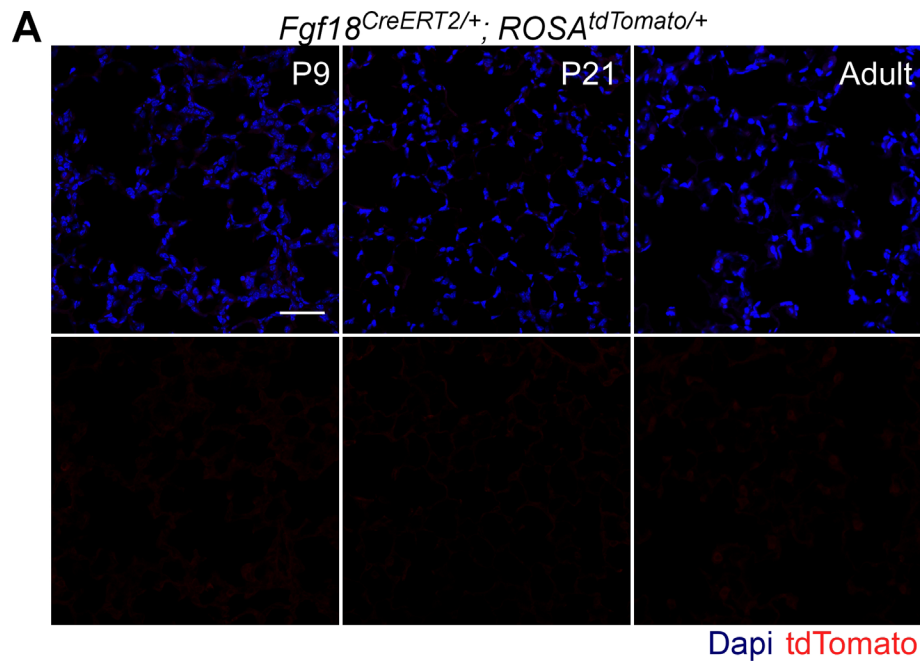


Figure S1

Fgf18^{CreERT2} does not induce recombination in the absence of tamoxifen. (A) Uninduced *Fgf18^{CreERT2/+}; ROSA^{tdTomato/+}* mice were collected at P9, P21, and in the adult showing epifluorescence for tdTomato⁺. Dapi (Blue). Scale bars: 50μm. n=2 for P9, P21, adult.

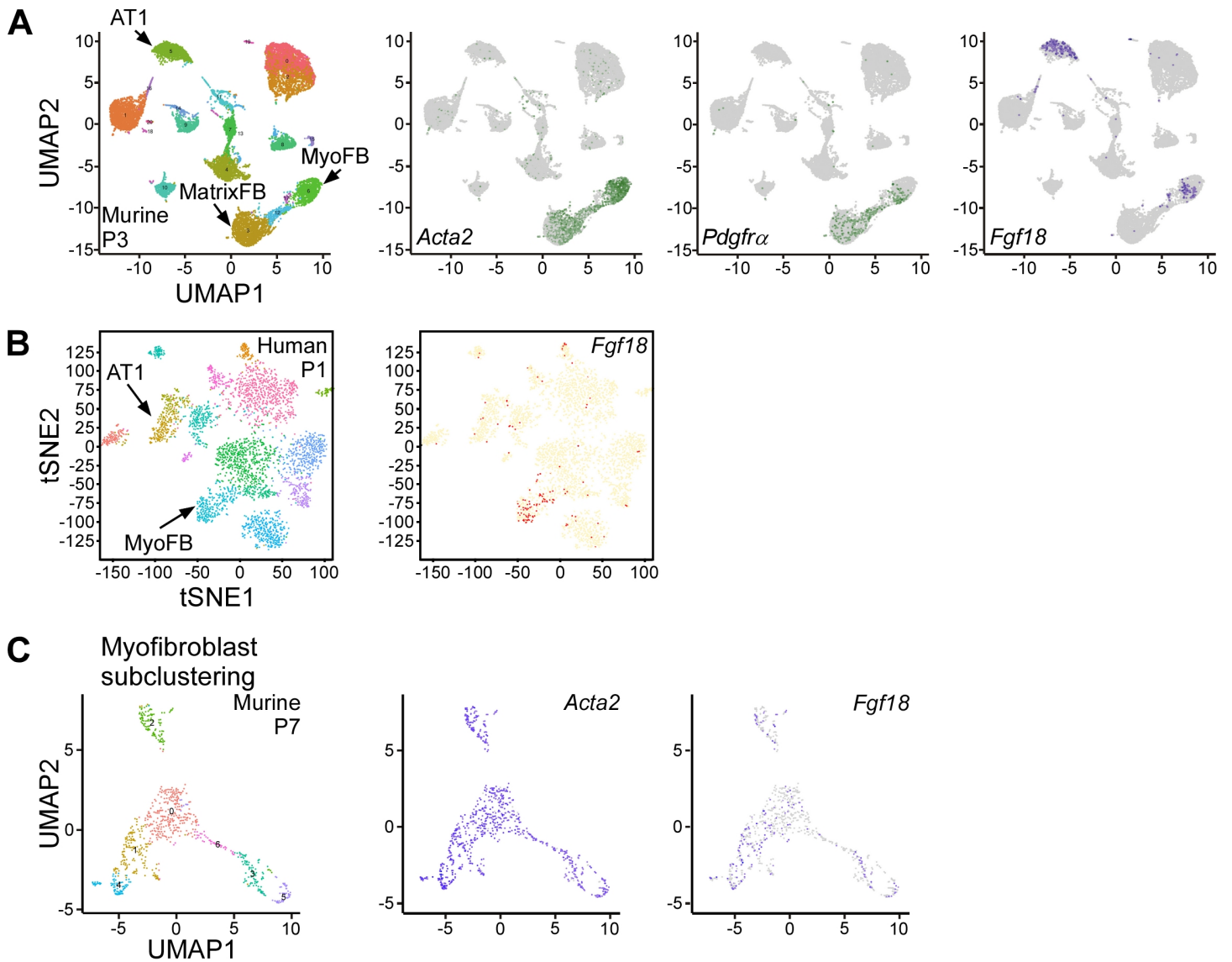


Figure S2

scRNA-seq of postnatal lung identifies *Fgf18* expression in alveolar myofibroblasts.

(A) Visualization of scRNA-seq data by UMAP plots from postnatal murine lung cells isolated at P3. MyoFB and AT1 clusters are indicated by arrows (left). Panels for cells expressing individual genes are indicated by color (right): *Acta2* (green), *Pdgfra* (green), *Fgf18* (purple). (B) Visualization of scRNA-seq data by tSNE plots from human lung cells isolated at P1. MyoFB and AT1 clusters are indicated by arrows (left) and *Fgf18* expressing cells are indicated in red (right). (C) Subcluster analysis of P7 scRNA-seq data by UMAP performed on *Acta2* high clusters 1, 10, 11 (from Fig. 2I). Panels for cells expressing individual genes are indicated by color (right): *Acta2* (purple) and *Fgf18* (purple). scRNA-seq tSNE plots were generated from LungGENS (<https://research.cchmc.org/pbge/lunggens>).

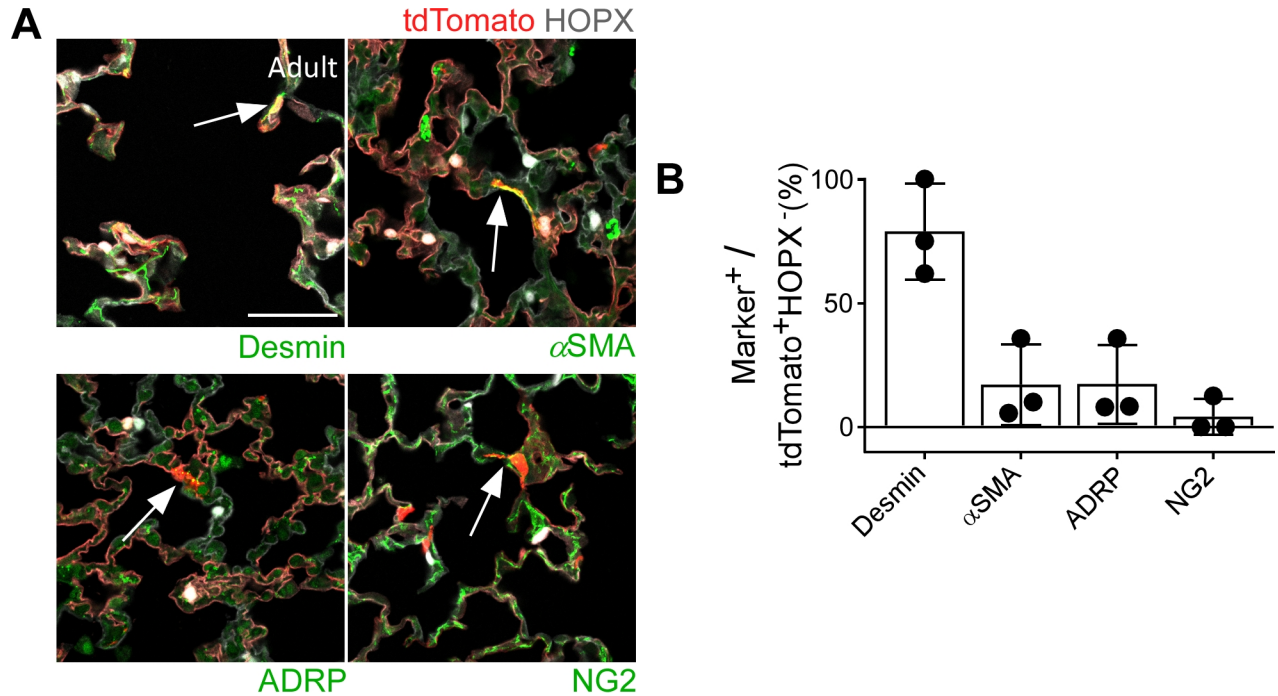


Figure S3

Few HOPX⁻ *Fgf18*^{Lineage} cells in alveolar region express differentiated mesenchymal markers in the adult. (A) *Fgf18*^{CreERT2/+}; *ROSA*^{tdTomato/+} animals were injected with Tam daily from P5-8. Lungs were analyzed in adults (11 weeks of age). Colocalization of tdTomato (red) with alveolar type 1 marker (HOPX, white), and mesenchymal markers (green: fibroblast, Desmin; myofibroblast, αSMA; lipofibroblast, ADRP; pericyte, NG2) in the alveolar region. (B) Quantification of the percentage of tdTomato⁺ cells that are positive for the indicated marker in lineage traced adult mice. Only HOPX⁻ cells were considered when counting mesenchyme marker⁺ cells. ADRP⁺ cells had multiple labeled puncta within the tomato signal. Scale bar: 50μm; Arrow indicates cells that are HOPX⁻ and tdTomato⁺, Marker⁺. n=3.

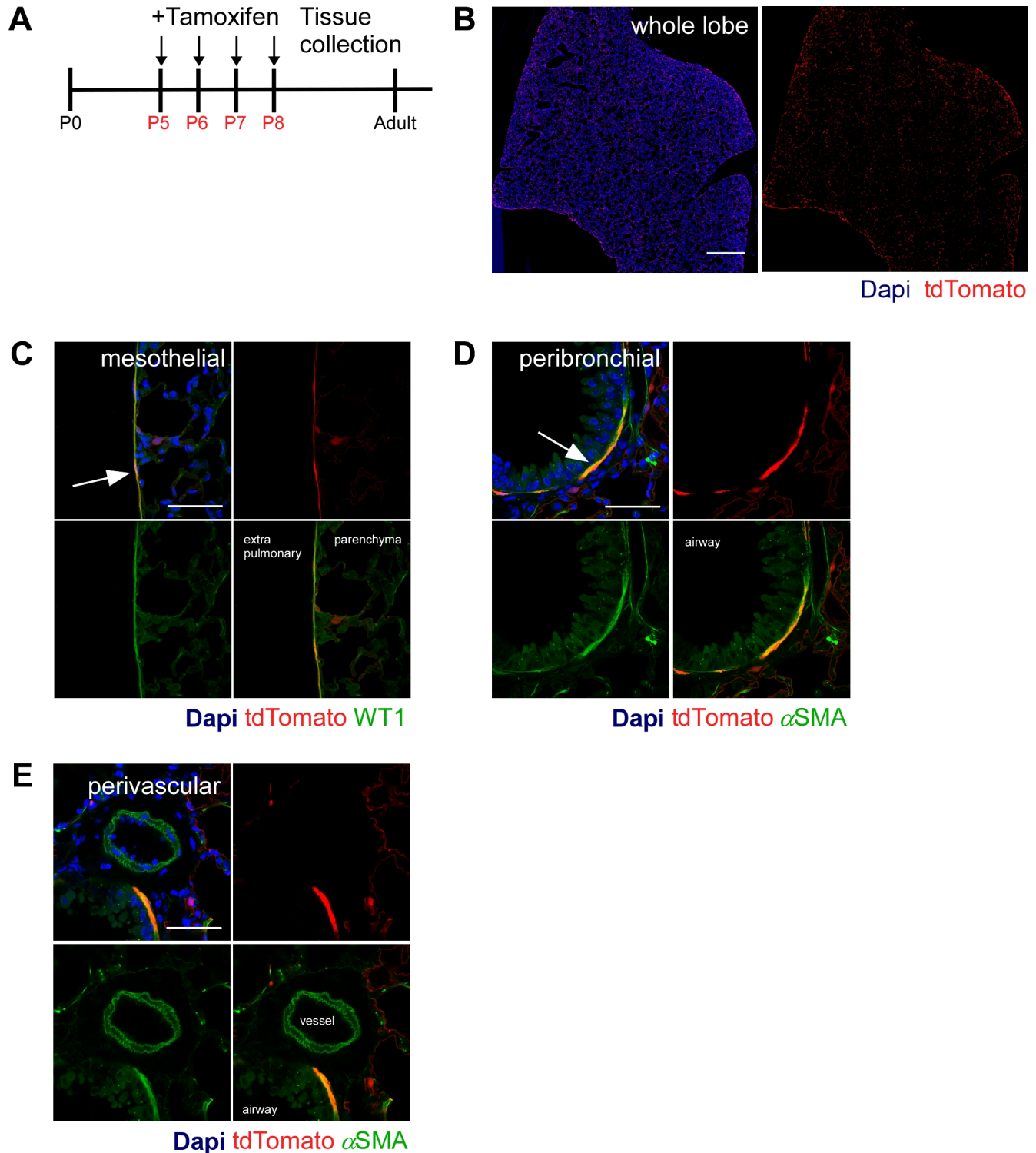


Figure S4

***Fgf18*^{Lineage} in mesothelial and peribronchial regions are maintained into the adult.** (A) *Fgf18*^{CreERT2/+}; *ROSA*^{tdTomato/+} animals were injected with Tam daily from P5-8. Lungs were analyzed in adults (11 weeks of age). (B) Whole lobe section showing tdTomato epifluorescence. (C-E) Colocalization of tdTomato (red) with (C) mesothelial marker WT1 (green) and the smooth muscle marker α SMA (green) in the (D) peribronchial and (E) perivascular region. Dapi (Blue). Scale bars: C, D, E, 50 μ m; B, 1mm. n=2 (C). Arrow indicates colocalization of signal.

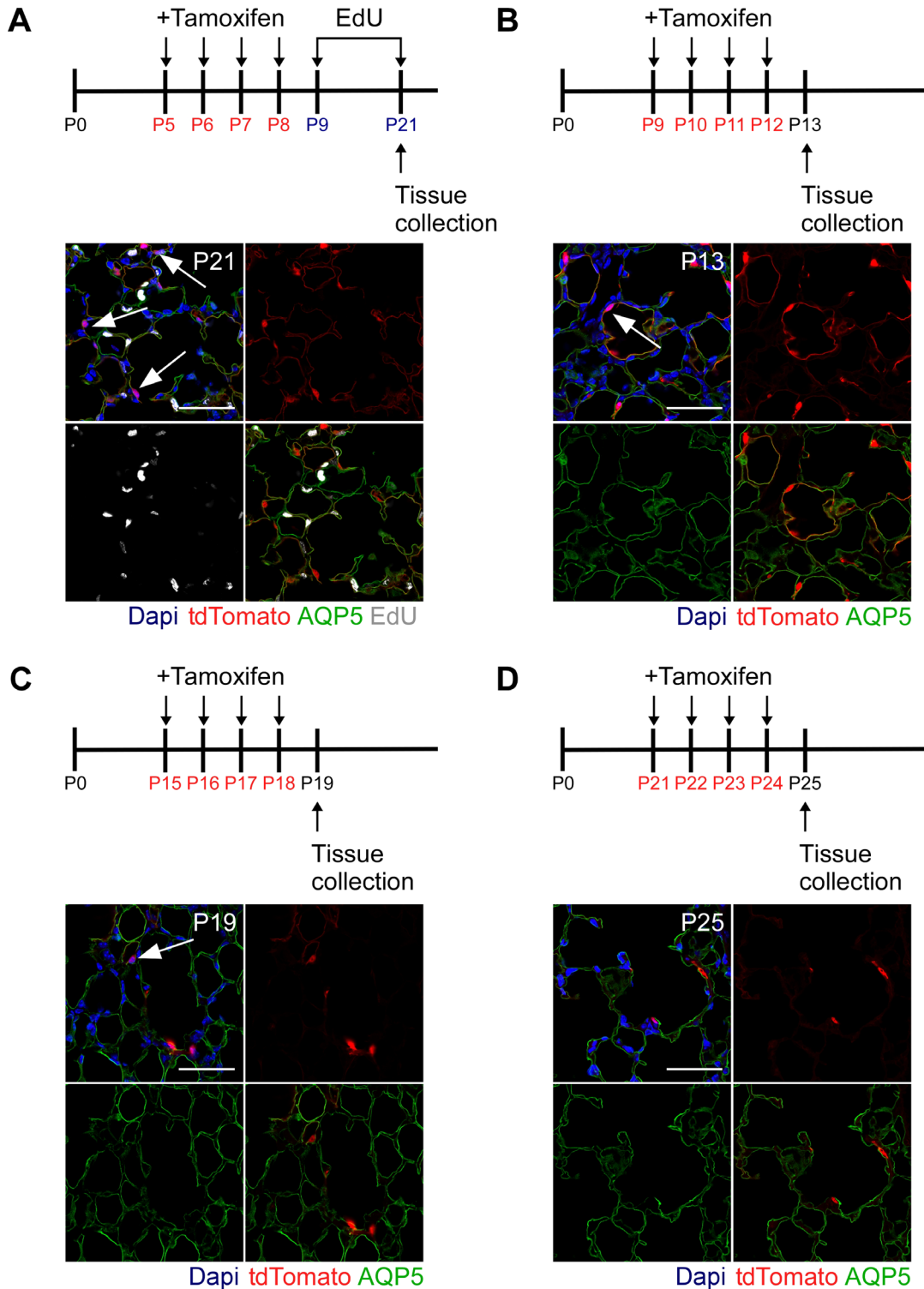


Figure S5

***Fgf18*^{Lineage} labeled alveolar type 1 cells do not proliferate but *Fgf18*^{CreERT2} is capable of inducing recombination in alveolar type 1 cells past postnatal day 9.** (A) *Fgf18*^{CreERT2/+}; *ROSA*^{tdTomato/+} mice were injected with Tam daily from P5-8. Animals were injected with EdU daily from P9-P21. Mice were analyzed at P21. Colocalization of tdTomato (red) with the alveolar type 1 membrane marker AQP5 (green), and EdU incorporation (white). (B-D) *Fgf18*^{CreERT2/+}; *ROSA*^{tdTomato/+} mice were injected with Tam daily from (B) P9-12 and collected on P13, (C) P15-18 and collected on P19, or (D) P21-24 and collected on P25. Colocalization of tdTomato (red) with the alveolar type 1 membrane marker AQP5 (green). Dapi (Blue). Scale bars: 50µm. Arrows indicate tdTomato⁺ cell that display alveolar type 1 morphology.

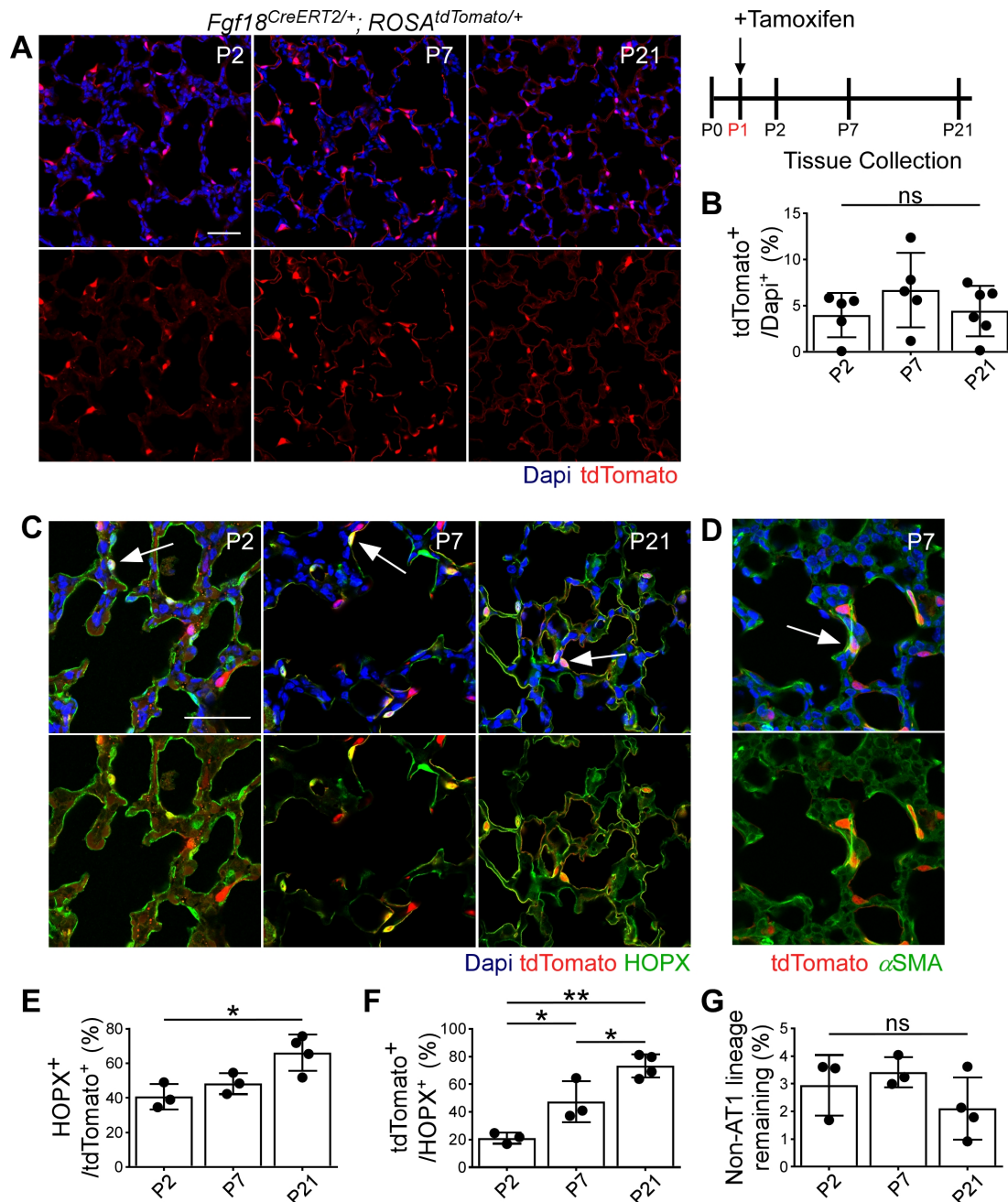


Figure S6

***Fgf18*^{Lineage} induction on P1 labels alveolar type 1 cells and few alveolar myofibroblasts.** (A) *Fgf18^{CreERT2/+}; ROSA^{tdTomato/+}* mice were injected with Tam on P1 and collected at P2, P7, and P21. (B) Quantification of the percentage of Dapi⁺ cells in the alveolar region that are tdTomato⁺. One-way ANOVA, $p = 0.36$, $n = 5$ (P2), $n = 6$ (P7), $n = 6$ (P21). (C-D) Colocalization of tdTomato (red) in lineage traced mice with: (C) alveolar type 1 marker HOPX (green) and (D) alveolar myofibroblast marker α SMA (green). (E) Quantification of the percentage of tdTomato⁺ cells that are HOPX⁺ in lineage traced mice. One-way ANOVA, $p = 0.015$. * $\alpha < 0.05$, Tukey's HSD, $n=3$ (P2, P7), $n=4$ (P21)(F). Quantification of the percentage of HOPX⁺ cells that are tdTomato⁺ in lineage traced mice. One-way ANOVA, $p = 7.0 \times 10^{-4}$. * $\alpha < 0.05$, Tukey's HSD. ** $\alpha < 0.01$, Tukey's HSD, $n=3$ (P2, P7), $n=4$ (P21). (G) Quantification of the percentage of remaining mesenchymal tdTomato⁺ cells in lineage traced mice. Remaining mesenchymal lineage were calculated from the percentage of tdTomato⁺/Dapi⁺ (Fig. S6B) with alveolar type 1 labeled cells removed (Fig. 6SE). One-way ANOVA, $p = 0.27$, $n=3$ (P2, P7), $n=4$ (P21). Dapi (Blue). Scale bars: 50 μ m. Error bars, mean \pm SD. ns, indicates not statistically significant.

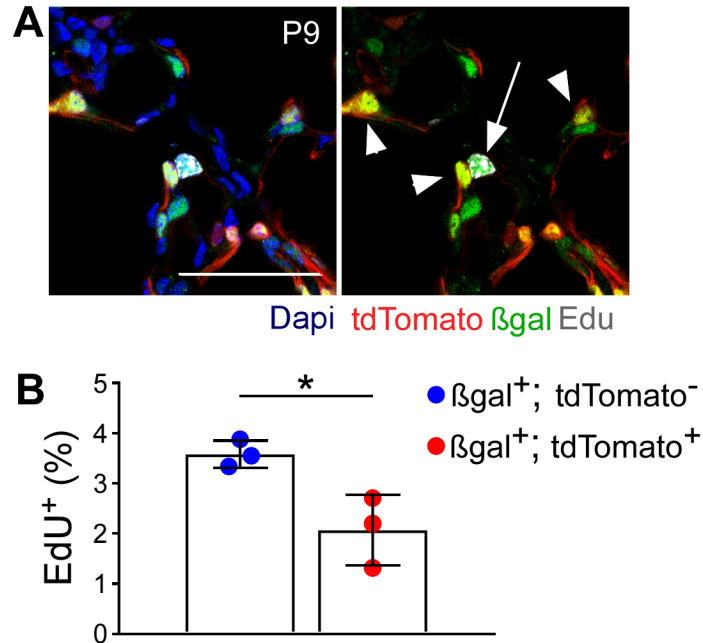


Figure S7

Alveolar lipofibroblasts are more proliferative than alveolar myofibroblasts at P9. (A) EdU incorporation in P9 *Fgf18*^{CreERT2/+}; *ROSA*^{tdTomato/+}; *Gli1*^{LacZ/+} mice. Arrow indicates a proliferating tdTomato⁻, βgal⁺ cell (lipofibroblast). Arrowheads indicate non-proliferating tdTomato⁺, βgal⁺ cells (myofibroblast). (B) Quantification of proliferation in tdTomato⁻, βgal⁺ cells vs tdTomato⁺, βgal⁺ cells. Student's t-test, **p* < 0.05. *n*=3. Dapi (Blue). Scale bars: 50μm. Error bars, mean ± SD.

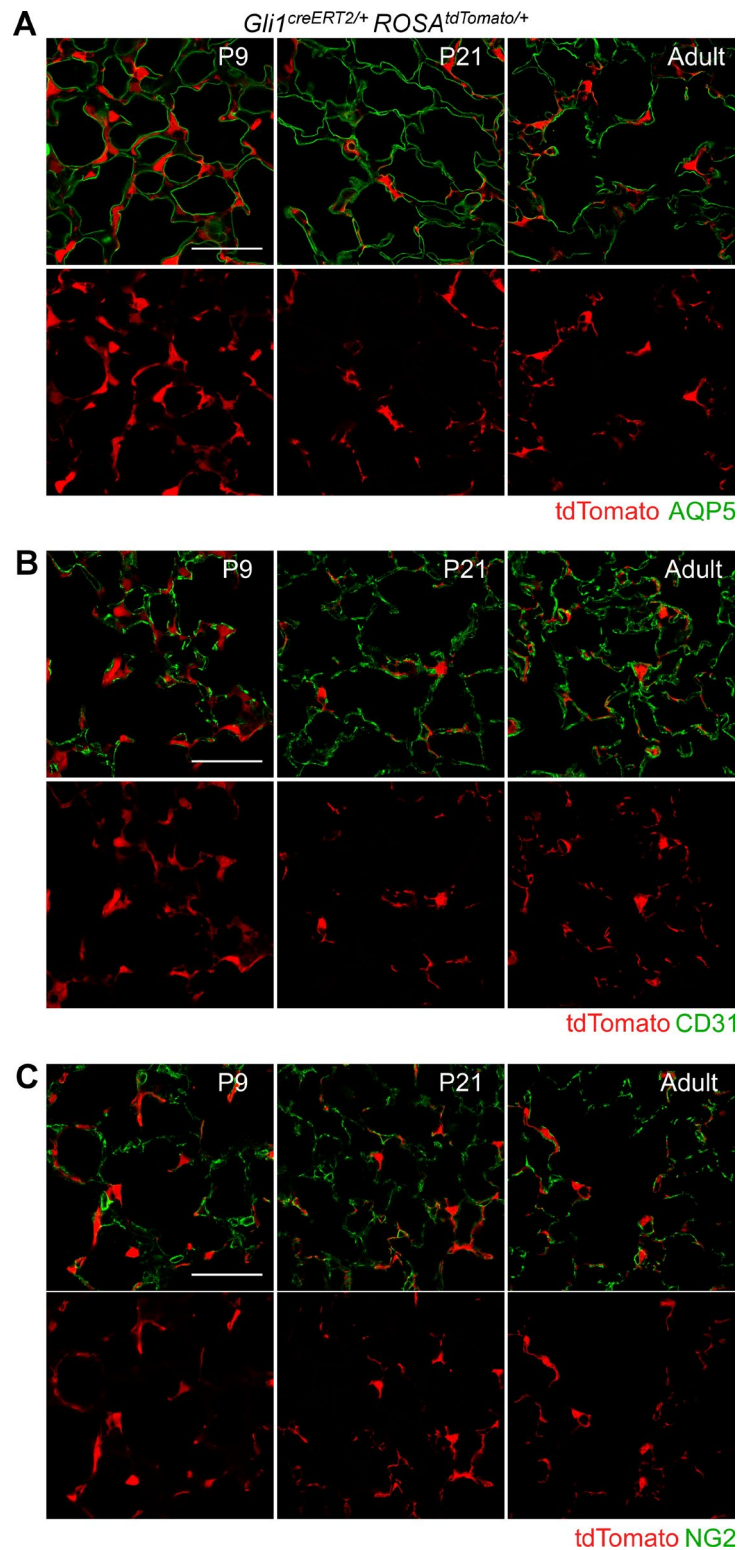


Figure S8

***Gli1^{Lineage}* does not contribute to endothelial cells, epithelial cells, or pericytes.** (A-C) *Gli1^{CreERT2/+}; ROSA^{tdTomato/+}* mice were injected with Tam daily from P5-P8 and collected throughout postnatal lung development and in the adult (8 weeks of age). Colocalization of tdTomato (red) with the (A) alveolar type 1 marker AQP5 (green), (B) the endothelial marker CD31 (green), and (C) the pericyte marker NG2 (green) at P9, P21, and adult. Scale bars: 50 μ m.

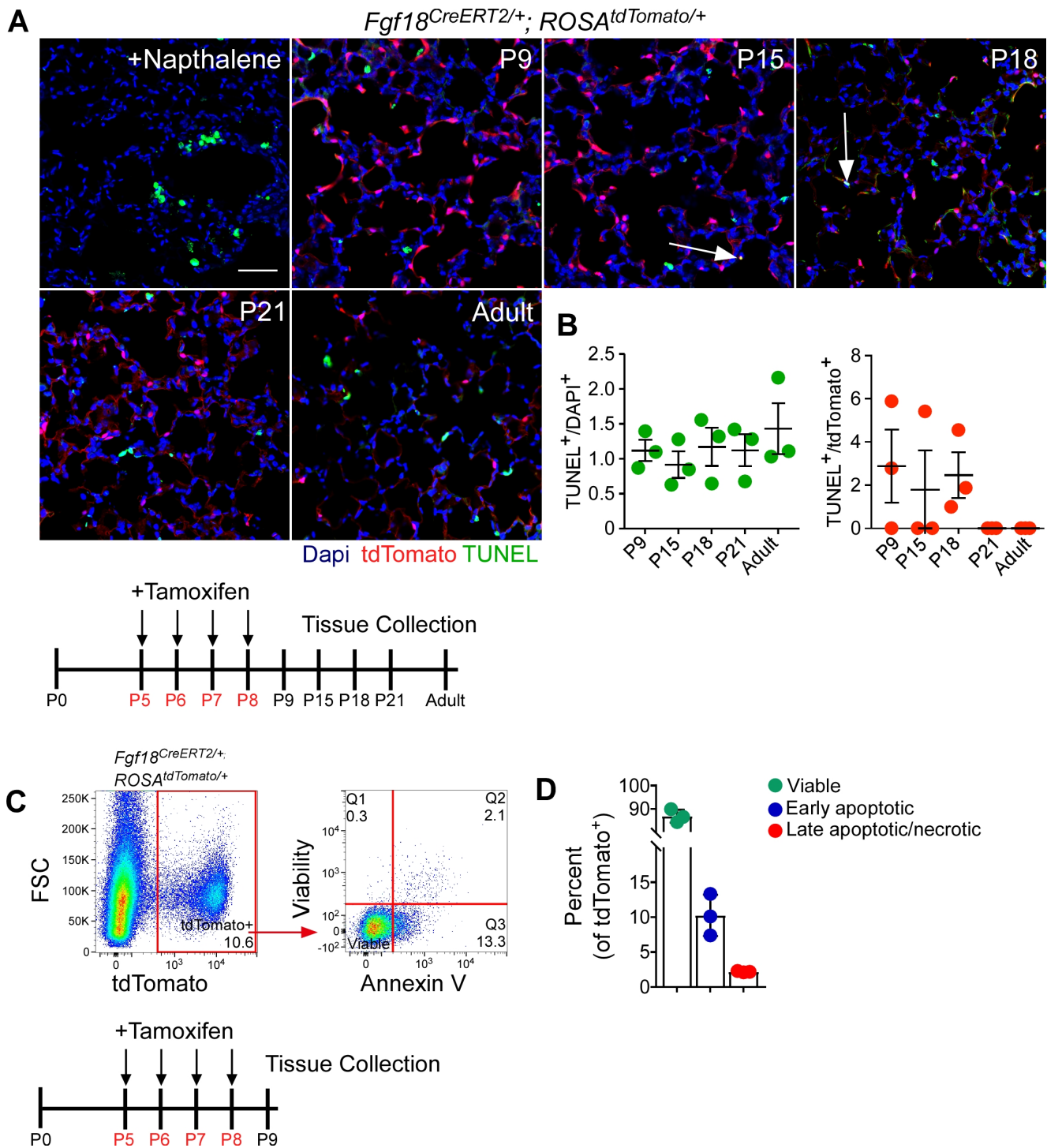


Figure S9

Limited apoptosis is detected in the *Fgf18*^{Lineage} throughout lung development. (A) *Fgf18^{CreERT2/+}; ROSA^{tdTomato/+}* mice were injected with Tam daily from P5-8 and collected throughout postnatal lung development. As a positive control, a wildtype mouse was injected with naphthalene to specifically kill Club cells. tdTomato lineage labeled cells (red); TUNEL positive cells (green). (B) Quantification of the percentage of TUNEL⁺/Dapi⁺ and TUNEL⁺/tdTomato⁺ cells in the alveolar region. Scale bar: 50µm. n=1 for naphthalene control. Arrows indicate colocalization. (C) *Fgf18^{CreERT2/+}; ROSA^{tdTomato/+}* mice were injected with Tam daily from P5-8 and collected on P9 for fluorescence-activated cell sorting. tdTomato⁺ cells were gated into quadrants based on staining of the viability dye (Zombie Violet™) and apoptosis marker, Annexin V. Quadrants were used to determine cell state. Bottom left, viable; bottom right, early apoptotic; top right; late apoptotic/necrotic. (D) Quantification of percentage of tdTomato⁺ cells in each quadrant. Dapi (Blue). Error bars, mean ± SD.

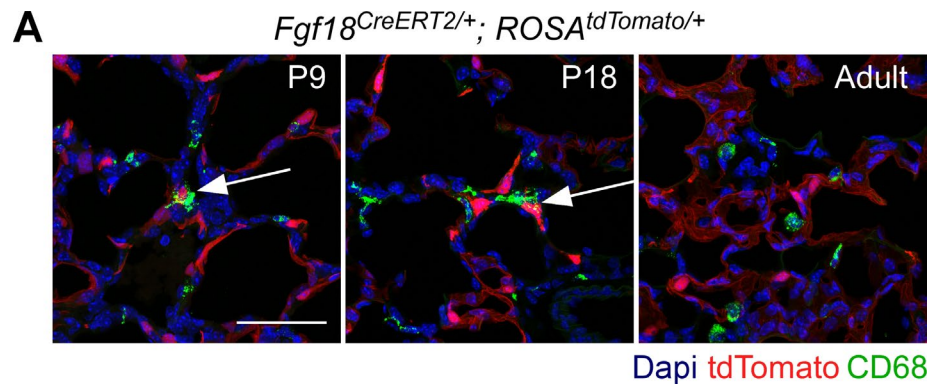


Figure S10

***Fgf18*^{Lineage} shows proximity to macrophages.** (A) $Fgf18^{CreERT2/+}; ROSA^{tdTomato/+}$ mice were injected with Tam daily from P5-8 and analyzed at P9, P18 and in the adult (11 weeks of age). Localization of tdTomato (red) with pan macrophage marker CD68 (green). Dapi (Blue). Scale bar: 50 μ m. Images compiled as maximum projection Z-stacks.

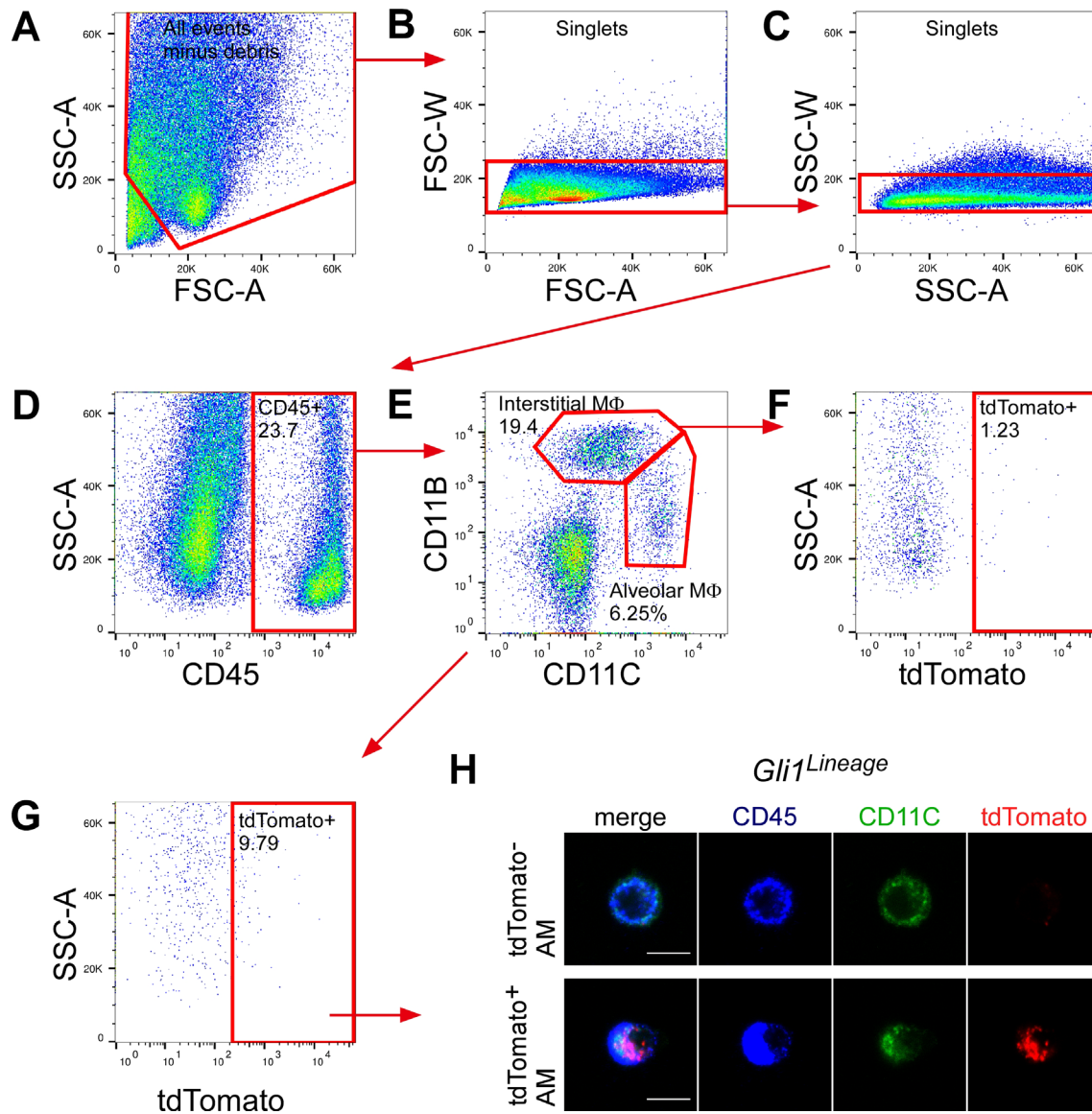


Figure S11

Alveolar macrophages phagocytose particles from *Gli1* labeled cells. (A-G) *Gli1*^{CreERT2/+}; *ROSA*^{tdTomato/+} mice were injected with Tam daily from P5-8 and analyzed on P13 by fluorescence-activated cell sorting. Sorted cells were gated against (A) debris and (B-C) doublets. (D-G) Sequential gating strategy to identify populations of tdTomato⁺ interstitial macrophages (CD45⁺ CD11B⁺ CD11C⁻) and alveolar macrophages (CD45⁺ CD11B⁻ CD11C⁺). (H) Fluorescence microscopy image of flow sorted cells from pooled P13 tdTomato⁻ and tdTomato⁺ alveolar macrophages. Scale bars: 10 μm.

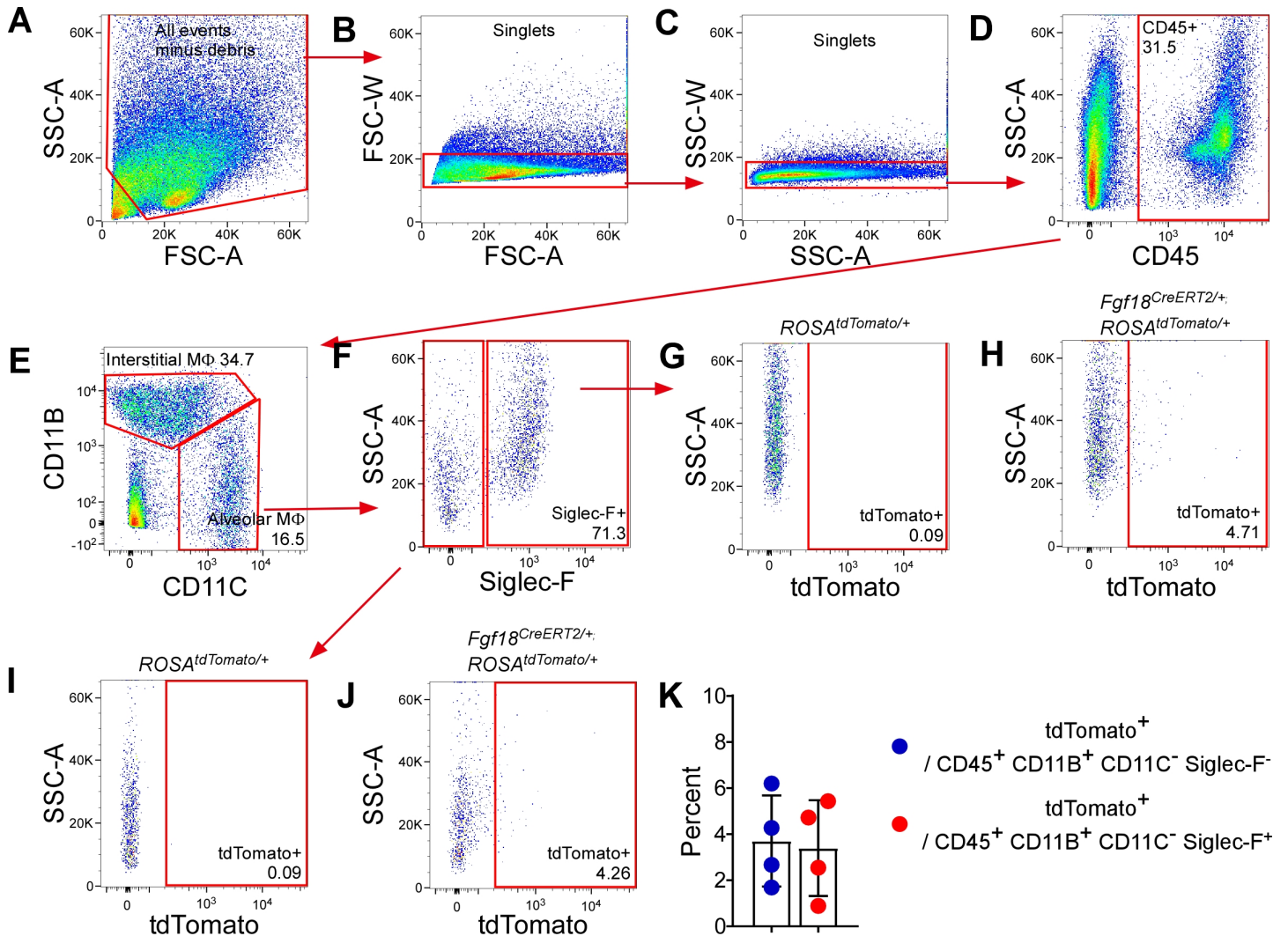
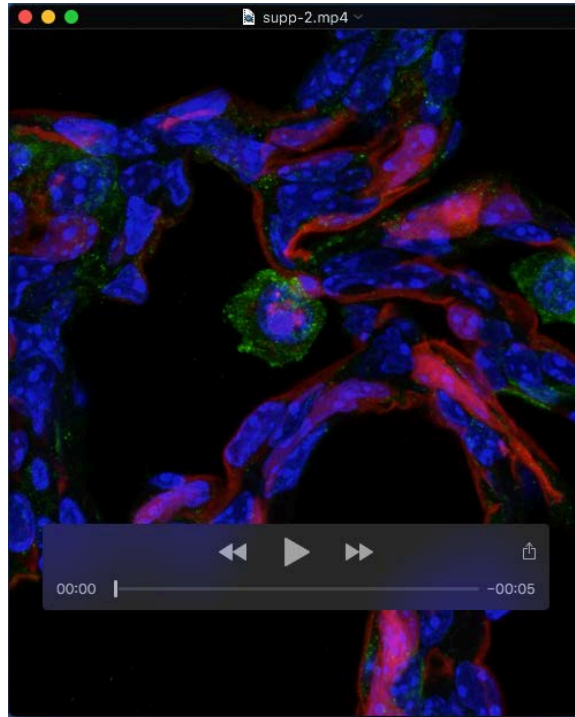


Figure S12

Alveolar macrophages and dendritic cells phagocytose particles from *Fgf18* labeled cells. (A-G) *Fgf18^{CreERT2/+}; ROSA^{tdTomato/+}* animals were injected with Tam daily from P5-8 and analyzed on P9 by fluorescence-activated cell sorting. Sorted cells were gated against (A) debris and (B-C) doublets. (D-J) Sequential gating strategy to identify populations of tdTomato⁺ alveolar macrophages (CD45⁺ CD11B⁻ CD11C⁺ Siglec-F⁺) and dendritic cells (CD45⁺ CD11B⁻ CD11C⁺ Siglec-F⁻). Fluorescence minus one (FMO) control stained with all antibodies but without the tdTomato fluorophore were used as a negative control (G, I). (H) Quantification of the percentage of (G) alveolar macrophages (CD45⁺ CD11B⁻ CD11C⁺ Siglec-F⁺) and dendritic cells (CD45⁺ CD11B⁻ CD11C⁺ Siglec-F⁻) that were gated as tdTomato⁺.



Movie 1

***Fgf18*^{CreERT2/+}; *ROSA*^{tdTomato/+} mice were injected with Tam daily from P5-8 and collected at P9. 3D reconstruction of *Fgf18*^{Lineage} cells (red) with pan macrophage marker F4/80 (green) showing Dapi⁺, tdTomato⁺ particles within a macrophage. Dapi (Blue).**