Cell Reports, Volume *35*

Supplemental information

Alveolar epithelial cell fate is maintained in a spatially restricted manner to promote lung regeneration after acute injury

Derek C. Liberti, Madison M. Kremp, William A. Liberti III, Ian J. Penkala, Shanru Li, Su Zhou, and Edward E. Morrisey

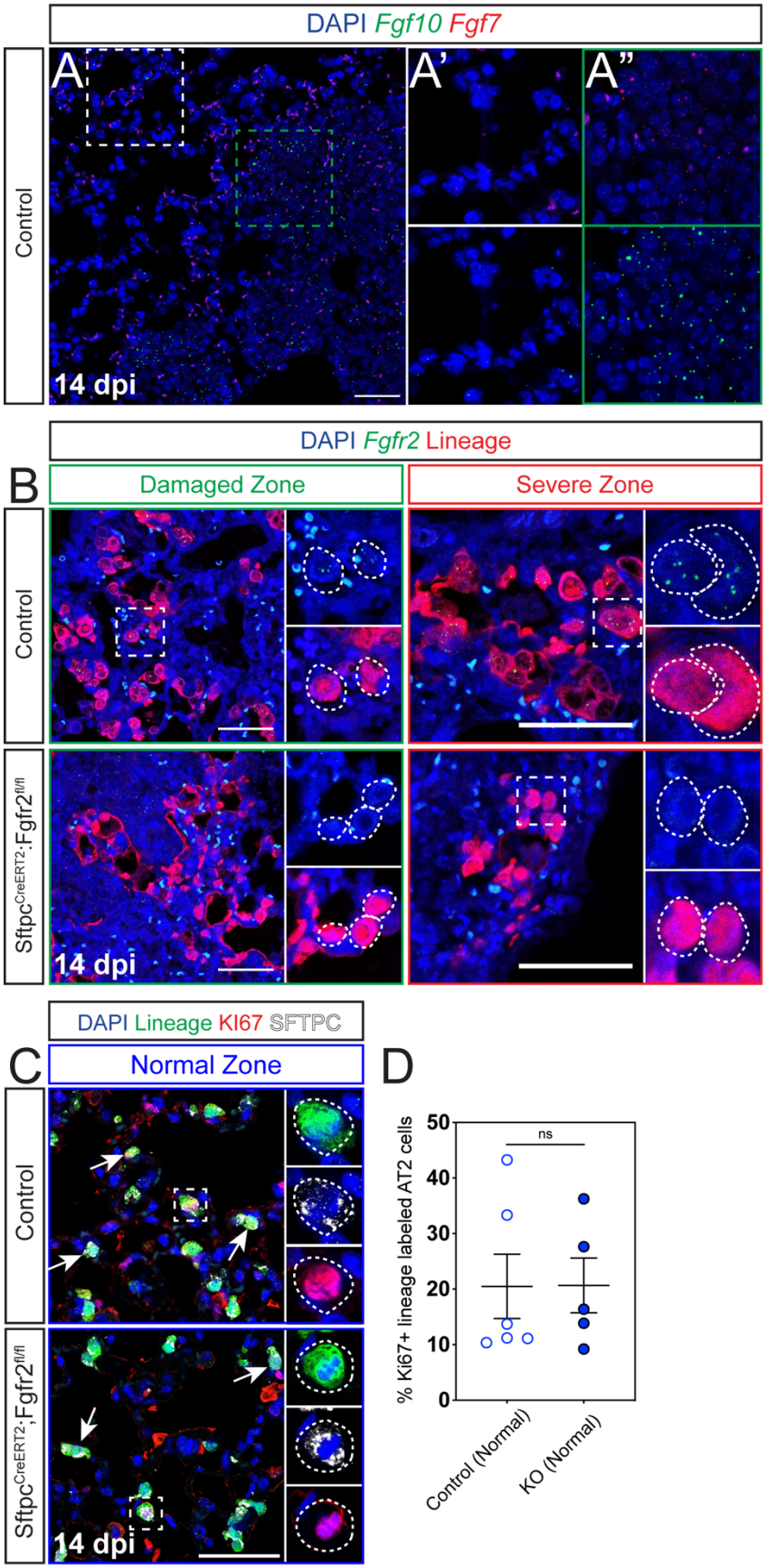
DAPI Fgf10 Fgf7
A
A'
A"
Control
14 dpi
DAPI Fgfr2 Lineage
B
Damaged Zone
Severe Zone
Control
SftpcCreERT2:Fgfr2fl/fl
14 dpi
DAPI Lineage KI67 SFTPC
C
Normal Zone
Control
SftpcCreERT2;Fgfr2fl/fl
14 dpi
D
% Ki67+ lineage labeled AT2 cells
50
40
30
20
10
0
ns
Control (Normal)
KO (Normal)

Figure S1: Fgf signaling varies across injured areas, related to Figure 3.
(A) RNA FISH demonstrating the enrichment of *Fgf7* and *Fgf10* in injured areas of the lung. Dashed white box indicates a non-morphologically perturbed region, dashed green box indicates a morphologically perturbed region (scale bar: 50 μm). (A') Zoomed in view of the dashed white box region. (A'') Zoomed in view of the dashed green box region.
(B) RNA FISH for *Fgfr2* and immunohistochemistry for the lineage marker EYFP, showing the loss of *Fgfr2* in *Sftpc*^{CreERT2}*;Fgfr2*$^{fl/fl}$*;R26R*^{EYFP} animals in both damaged and severe zones. Dashed white lines demarcate lineage labelled AT2 cells (scale bars: 50 μm).
(C) Immunohistochemical staining for the lineage trace marker EYFP, KI67, and SFTPC in control and *Fgfr2*-deficient lungs in normal zones. Dashed white lines mark KI67+ lineage traced AT2 cells (scale bar: 50 μm).
(D) Quantification of the percentage of KI67+ AT2 cells reveals no difference between control and mutant lungs.
All quantification data are represented as mean ± SEM. Two-tailed t-test: ns: not significant, n=5-6 mice per group.

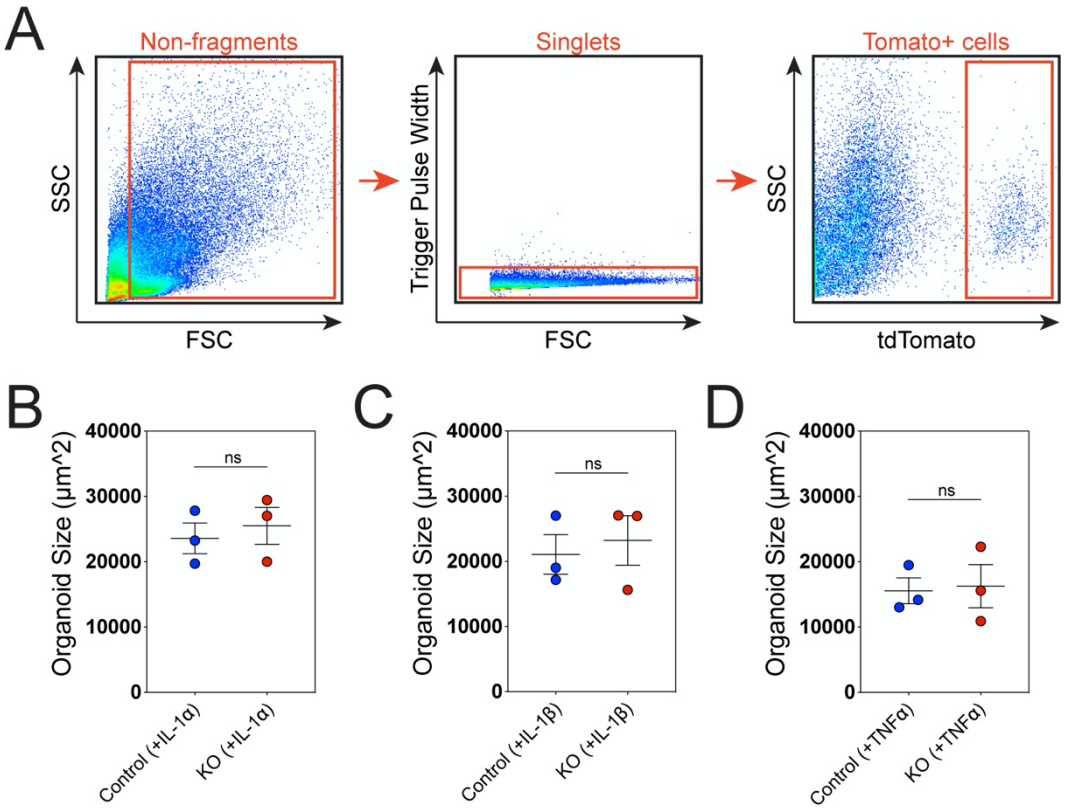

Figure S2: Comparison of isolated *Fgfr2*-deficient and control AT2 cells treated with inflammatory cytokines, related to Figure 4.
(A) Representative sort gates used to isolate AT2 cells using the *Sftpc*^{CreERT2} driven *R26R*^{tdTomato} reporter (SSC: side scatter; FSC: forward scatter).
(B-D) Organoid size at 21 days of culture after 14 days of cytokine treatment.
All quantification data are represented as mean ± SEM. Two-tailed t-tests: ns: not significant, n=3 mice per group.

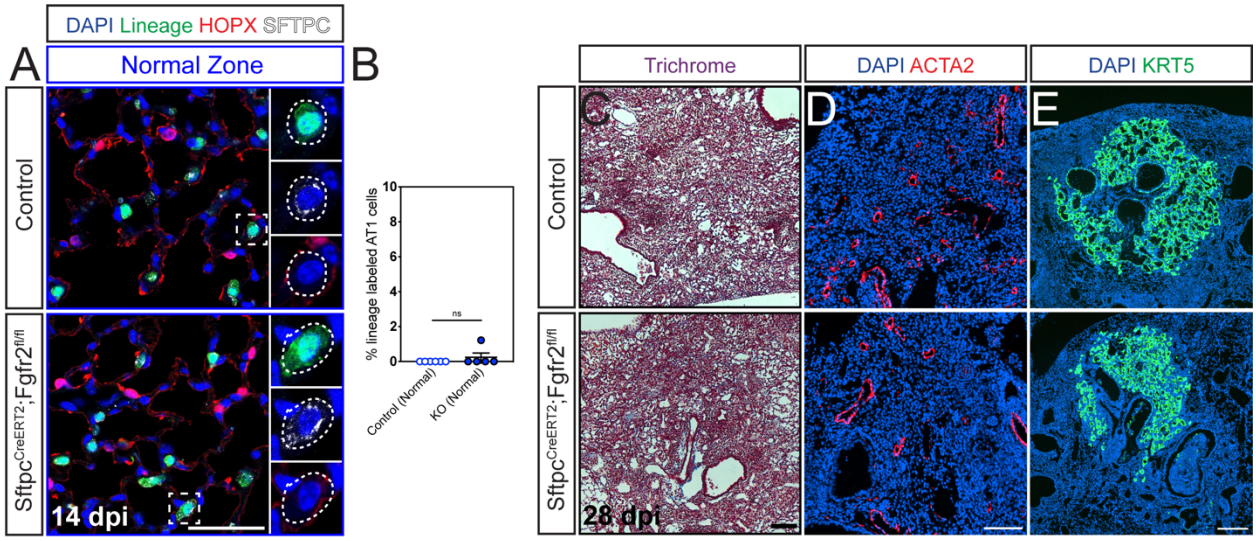

Figure S3: AT2-AT1 cell differentiation is rare in normal zones 14 dpi, related to Figure 5.
(A) Immunohistochemical staining for the lineage trace marker EYFP, HOPX, and SFTPC in control and *Fgfr2*-deficient lungs in normal zones at 14 dpi. White dashed lines mark SFTPC+ lineage traced AT2 cells (scale bar: 50 μm).
(B) Quantification reveals almost no lineage traced AT1 cells in normal zones and no difference between control and mutant lungs.
(C) Masson's trichrome staining of control and mutant lungs 28 dpi (scale bar: 100 μm).
(D and E) Immunohistochemical staining of control and mutant lungs for ACTA2 and KRT5 28 dpi (scale bars: 100 μm).
All quantification data are represented as mean ± SEM. Two-tailed t-test: ns: not significant, n=5-6 mice per group.

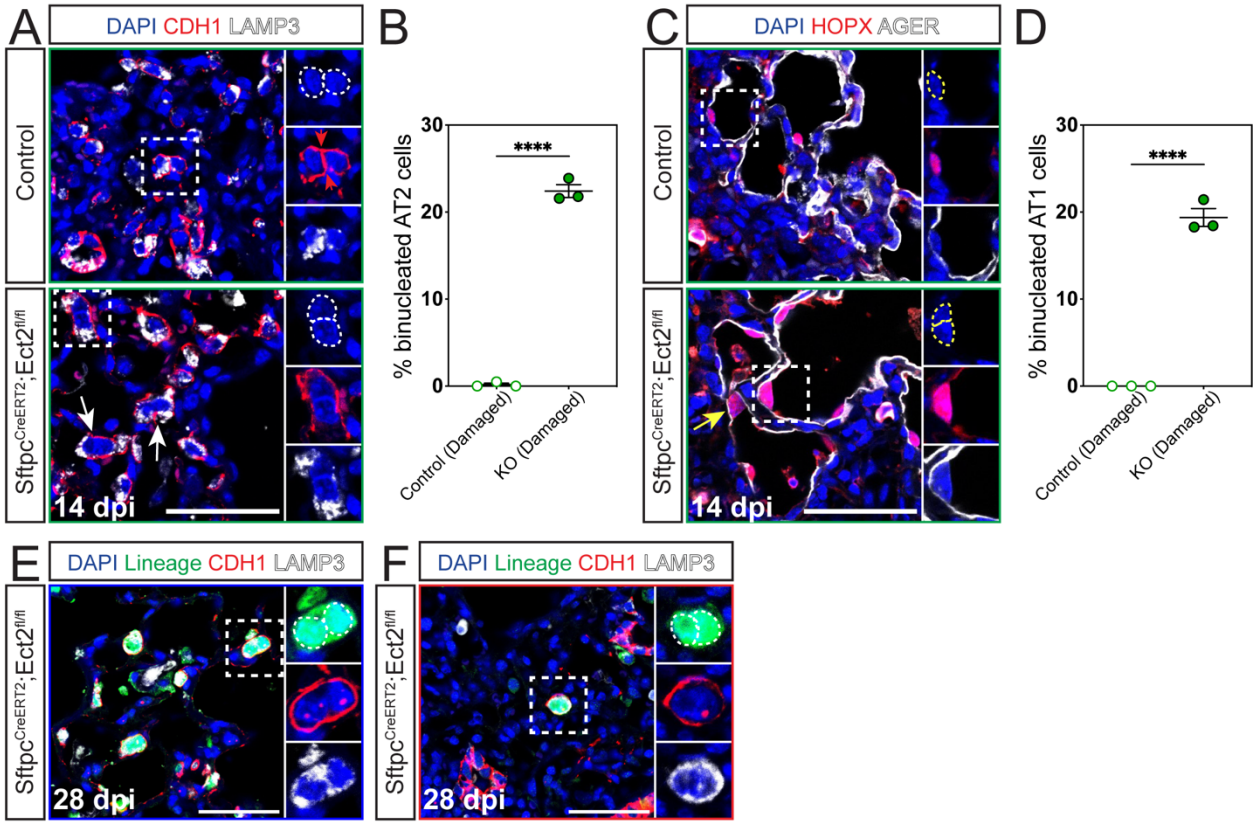

Figure S4: Analysis of binucleation of AT2 and AT2-derived AT1 cells 14 dpi, related to Figure 6.

(A and B) Immunohistochemistry for CDH1 and LAMP3 at 14 dpi. Quantification shows the percentage of binucleated AT2 cells in damaged zones in *Ect2*-deficient and control lungs. Zoomed in regions marked by dashed white boxes and individual AT2 cell nuclei marked by dashed white circles. Quantification shows the percentage of binucleated AT2 cells in control versus mutant lungs (scale bar: 50 μm).

(C and D) Immunohistochemistry for HOPX and AGER at 14 dpi. Quantification shows the percentage of binucleated AT1 cells in damaged zones in *Ect2*-deficient and control lungs. Zoomed in regions marked by dashed white boxes, yellow arrow indicates binucleated AT1 cell, and individual AT1 cell nuclei marked by dashed yellow circles. Quantification shows the percentage of binucleated AT1 cells in control versus mutant lungs (scale bar: 50 μm).

(E and F) Immunohistochemistry for the lineage marker EYFP, CDH1, and LAMP3 in *Ect2*-deficient lungs at 28 dpi in (E) normal and (F) severe zones. Zoomed in regions marked by dashed white boxes and individual AT2 cell nuclei marked by dashed white circles (scale bars: 50 μm).

All quantification data are represented as mean ± SEM. Two-tailed t-tests: **** $P \leq 0.0001$, n=3 mice per group.