Cell Reports, Volume *42*

Supplemental information

Temporal and spatial staging of lung alveolar regeneration is determined by the grainyhead transcription factor *Tfcp2l1*

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SUPPLEMENTARY INFORMATION

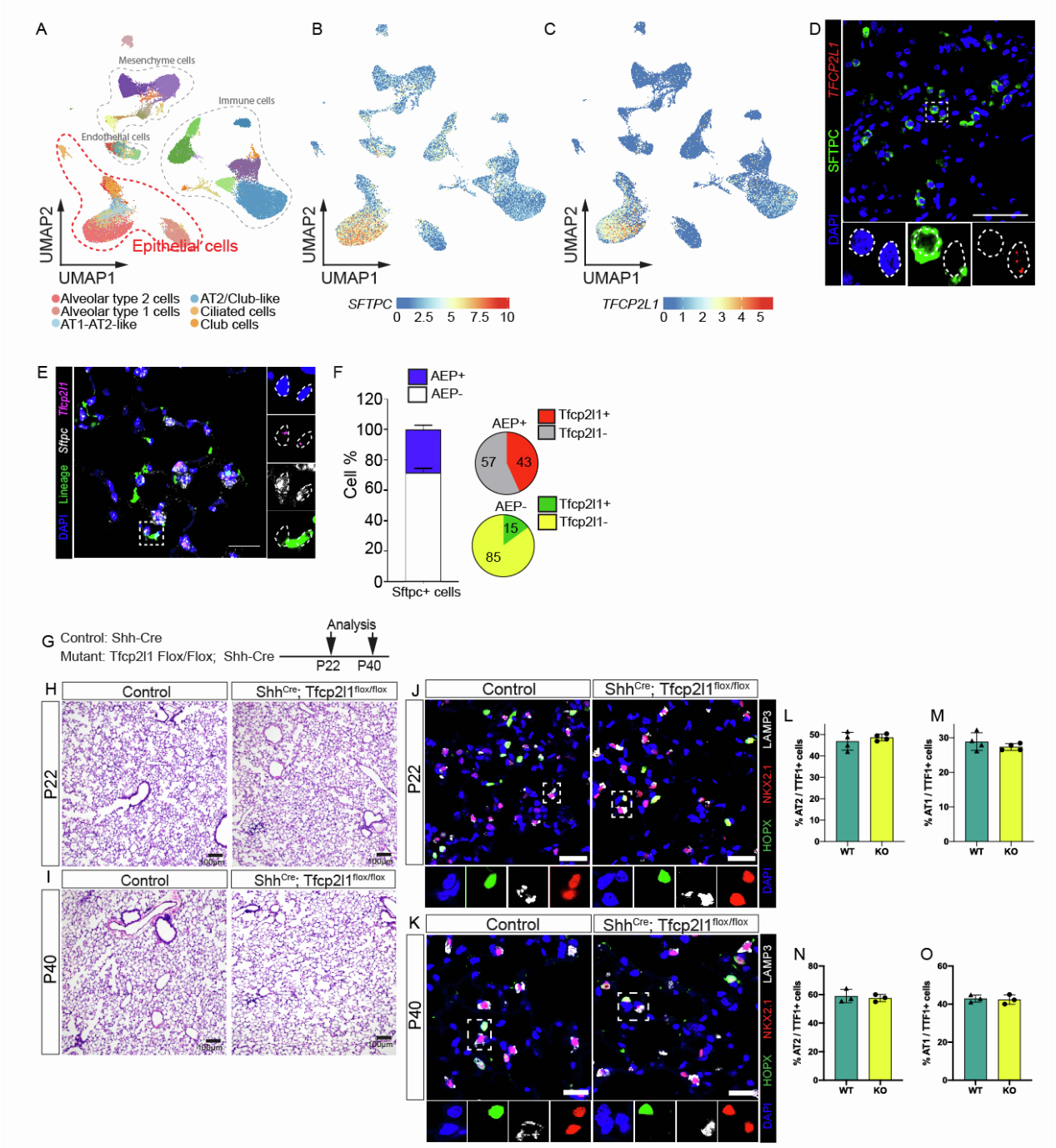


Figure S1: Human and mouse lung cell types expressing *TFCP2L1* or *Tfcp2l1* in human and mouse lung samples. (A-C) Human lung scRNA-seq data from the Human LungMap database (http://www.lungmap.net). (A) UMAP plot showing the main four cellular clusters from

human distal lung (B)) *SFTPC* UMAP plot (C) *TFCP2L*1 UMAP plot. (D) SFTPC immunostaining and *TFCP2l1* RNA scope performed on healthy human distal lung (n=2) (scale bar 20μm). (E) IHC for the Axin2 cell lineage marker EYFP and RNA scope for *Sftpc*, and *Tfcp2l1* in homeostasis mouse lung with highlighted areas to show co-staining of markers (scale bar 20μm). (F) Cell percentage quantification of AEP+ *Tfcp2l1*+ and AEP- *Tfcp2l1*+. n=3 mice (G) Experimental schematic to characterize the inactivation of *TFCP2L1* during mouse lung development. (H) H+E stain of mouse $Tfcp2l1^{fl/fl}$; Shh^{Cre} lung at P22 and P40 (I-J) LAMP3, HOPX and NKX2.1 immunostaining on mouse lung (scale bar 20μm) (I) P22 (n=4 per group) (J) P40 (n=3 per group). (K) AT2 cell quantification at P22. (L) AT1 cell quantification at P22. (M) AT2 cell quantification at P22. (N) AT1 cell quantification at P22.

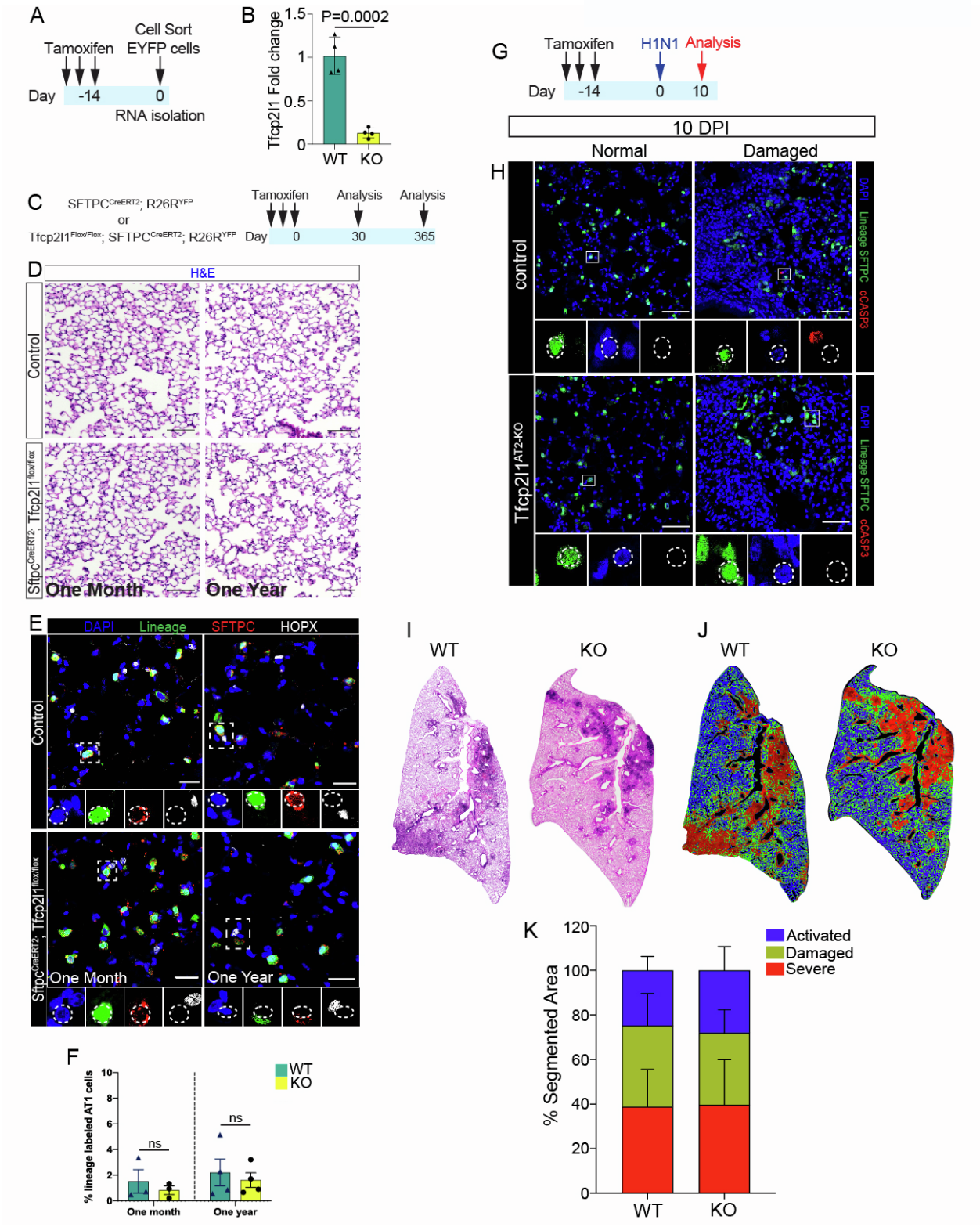

A
Tamoxifen
Cell Sort
EYFP cells
Day
-14
0
RNA isolation
B
P=0.0002
Tfcp2l1 Fold change
1.5
1
0.5
0
WT
KO
C
SFTPC^CreERT2; R26R^YFP
or
Tfcp2l1^Flox/Flox; SFTPC^CreERT2; R26R^YFP
Tamoxifen
Analysis
Analysis
Day
0
30
365
D
H&E
Control
Sftpc^CreERT2; Tfcp2l1^flox/flox
One Month
One Year
E
DAPI
Lineage
SFTPC
HOPX
Control
Sftpc^CreERT2; Tfcp2l1^flox/flox
One Month
One Year
F
WT
KO
% lineage labeled AT1 cells
10
8
6
4
2
0
ns
ns
One month
One year
G
Tamoxifen
H1N1
Analysis
Day
-14
0
10
10 DPI
Normal
Damaged
H
control
Tfcp2l1^AT2-KO
DAPI
Lineage
SFTPC
cCASP3
I
WT
KO
J
WT
KO
K
% Segmented Area
120
100
80
60
40
20
0
Activated
Damaged
Severe
WT
KO

Figure S2: Analysis of Tfcp2l1 deficient AT2 cells in adult homeostasis and post-acute viral injury. (A) Experimental schematic showing tamoxifen treatment to test Sftpc Cre-mediated Tfcp2l1 deletion in AT2 cells. (B) Relative gene expression analysis of Tfcp2l1 comparing control and Tfcp2l1AT2-KO mice. (C) Experimental schematic to characterize the long-term inactivation of *TFCP2L1* in AT2 adult mouse after one month and one year inactivation. (D) H+E stain of mouse $Tfcp2l1^{fl/fl}$; $Tfcp2l1^{Cre}$ lung after one month and one year after tamoxifen treatment. (E) IHC for the AT2 cell lineage marker EYFP, SFTPC, and HOPX after one month and one year tamoxifen administration (scale bar 20μm). (F) Quantification of AT1 cell differentiation after month tamoxifen administration (n=3 per group) and one year after tamoxifen administration (n=4 per group) (G) Experimental schematic to characterize AT2 lineage traced apoptosis at 10dpi. (H) IHC for the AT2 cell lineage marker EYFP and cCASP3 at 10dpi (scale bar 20μm). (I) H+E stain picture of mouse lung at 14 dpi. (I) H+E pictures representing the injury zones found at 14 dpi. (J) Cluster injury zone map generated from the H+E picture. (K) Quantification of the percentage segmented area per injury zone. (n=5 per group)

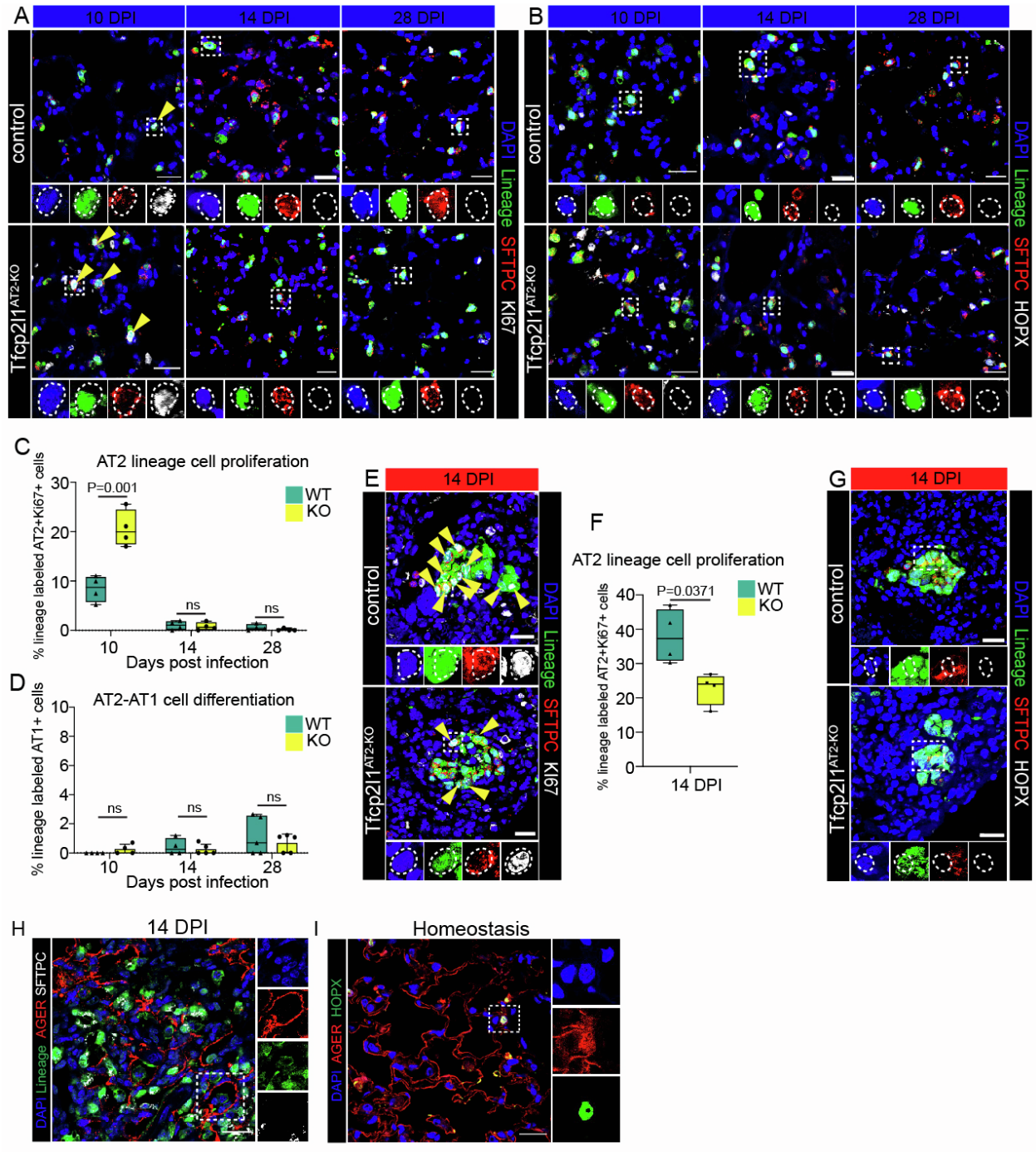
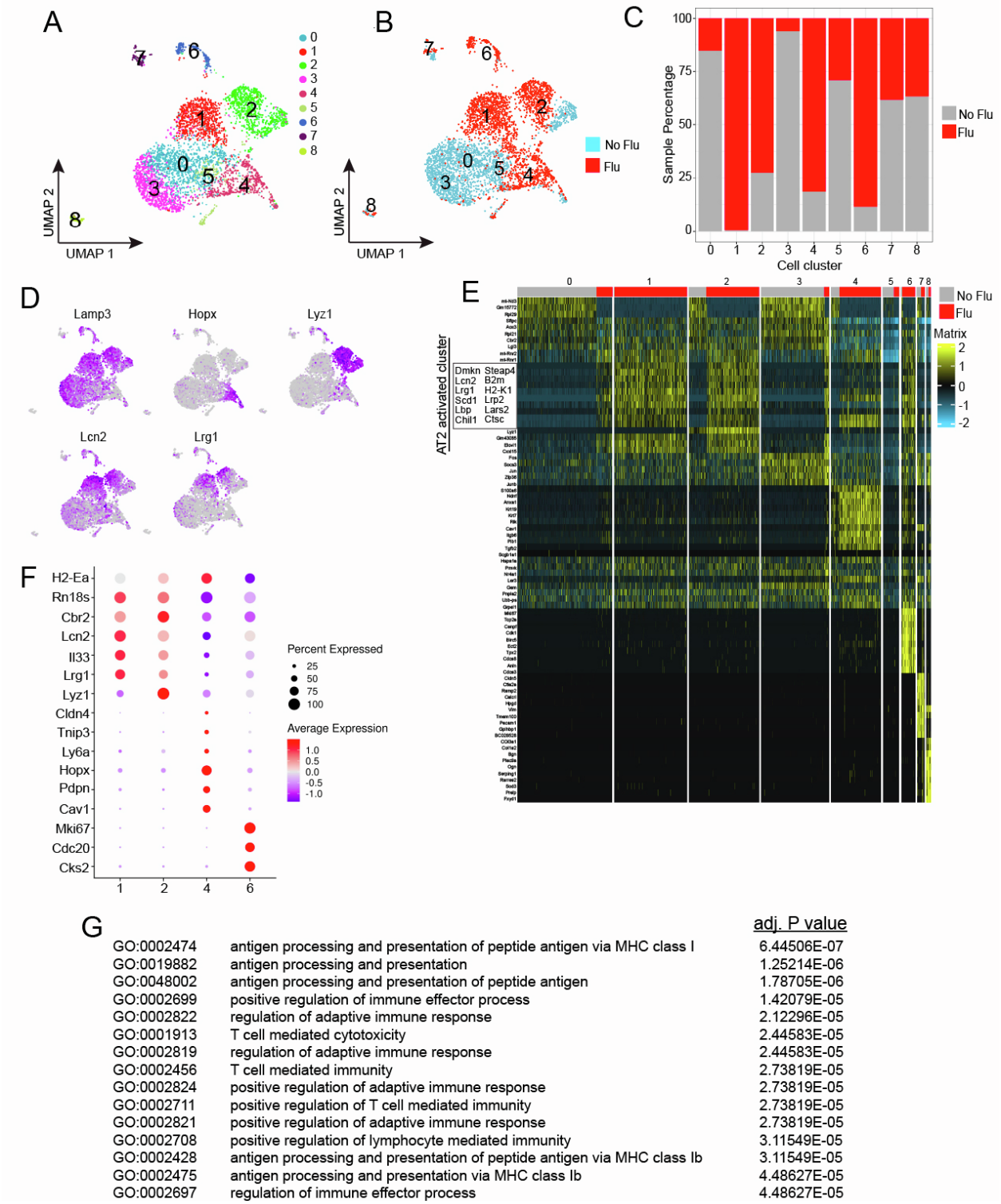


Figure S3: AT2 cell differentiation and proliferation analysis post-acute viral injury. (A) IHC for the AT2 cell lineage marker EYFP, SFTPC, and Ki67 in normal zones at 10, 14 and 28 dpi with highlighted areas to show co-staining of markers (scale bar 20μm). (B) IHC for the AT2 cell lineage marker EYFP, SFTPC, and HOPX in normal zones at 10, 14 and 28 dpi with highlighted areas to show co-staining of markers (scale bar 20μm) (C) Quantification of lineage traced

proliferative AT2 cells in normal zones at 10, 14 and 28 dpi. (n ≥ 4 per group) (D) Quantification of derived AT1 cells from lineage traced AT2 cells in normal zones at 10, 14 and 28 dpi. (n ≥ 4 per group) (E) IHC for the AT2 cell lineage marker EYFP, SFTPC, and Ki67 in severe zones at 14 dpi with highlighted areas to show co-staining of markers (scale bar 20μm). (F) Quantification of lineage traced proliferative AT2 cells in severe zones at 14 dpi. (n = 4 per group). (G) IHC for the AT2 cell lineage marker EYFP, SFTPC, and HOPX in severe zones at 14 dpi with highlighted areas to show co-staining of markers (scale bar 20μm). (H) IHC for the AT2 cell lineage marker EYFP, SFTPC, and AGER in severe zones at 14 dpi with highlighted areas to show co-staining of markers (scale bar 20μm). (I) IHC for the AT1 cell marker AGER and HOPX in adult homeostasis lung with highlighted areas to show co-staining of markers (scale bar 20μm). All quantification data are represented as mean ± SEM. Two-tailed t tests p values shown.



G

		adj. P value
GO:0002474	antigen processing and presentation of peptide antigen via MHC class I	6.44506E-07
GO:0019882	antigen processing and presentation	1.25214E-06
GO:0048002	antigen processing and presentation of peptide antigen	1.78705E-06
GO:0002699	positive regulation of immune effector process	1.42079E-05
GO:0002822	regulation of adaptive immune response	2.12296E-05
GO:0001913	T cell mediated cytotoxicity	2.44583E-05
GO:0002819	regulation of adaptive immune response	2.44583E-05
GO:0002456	T cell mediated immunity	2.73819E-05
GO:0002824	positive regulation of adaptive immune response	2.73819E-05
GO:0002711	positive regulation of T cell mediated immunity	2.73819E-05
GO:0002821	positive regulation of adaptive immune response	2.73819E-05
GO:0002708	positive regulation of lymphocyte mediated immunity	3.11549E-05
GO:0002428	antigen processing and presentation of peptide antigen via MHC class Ib	3.11549E-05
GO:0002475	antigen processing and presentation via MHC class Ib	4.48627E-05
GO:0002697	regulation of immune effector process	4.48627E-05

Figure S4: Single-cell transcriptional analysis of mouse AT2 lineage traced cells pre and post-viral injury. (A-B) Merged scRNA-seq data, showing the UMAP plot of control AT2 cells isolated from mice not infected with influenza (No flu) compared to AT2 cells isolated 14dpi (Flu)

(n=1 per library). **(A)** Cell cluster **(B)** Mouse AT2-no flu and AT2-flu sample distribution in different cell clusters. **(C)** Cell percentage distribution per cell cluster. **(D)** Feature plots showing distribution and expression of Lamp3, Hopx, Lyz1, Lcn2, and Lrg1 in mouse AT2-no flu and AT2-flu merged at 14dpi. **(E)** Heat map showing differential expressed genes per cell cluster comparing AT2-no flu and AT2-flu. **(F)** Dot plot visualization showing unique gene expression patterns in clusters 1, 2, 4, and 6. **(G)** GO analysis of clusters one and two.

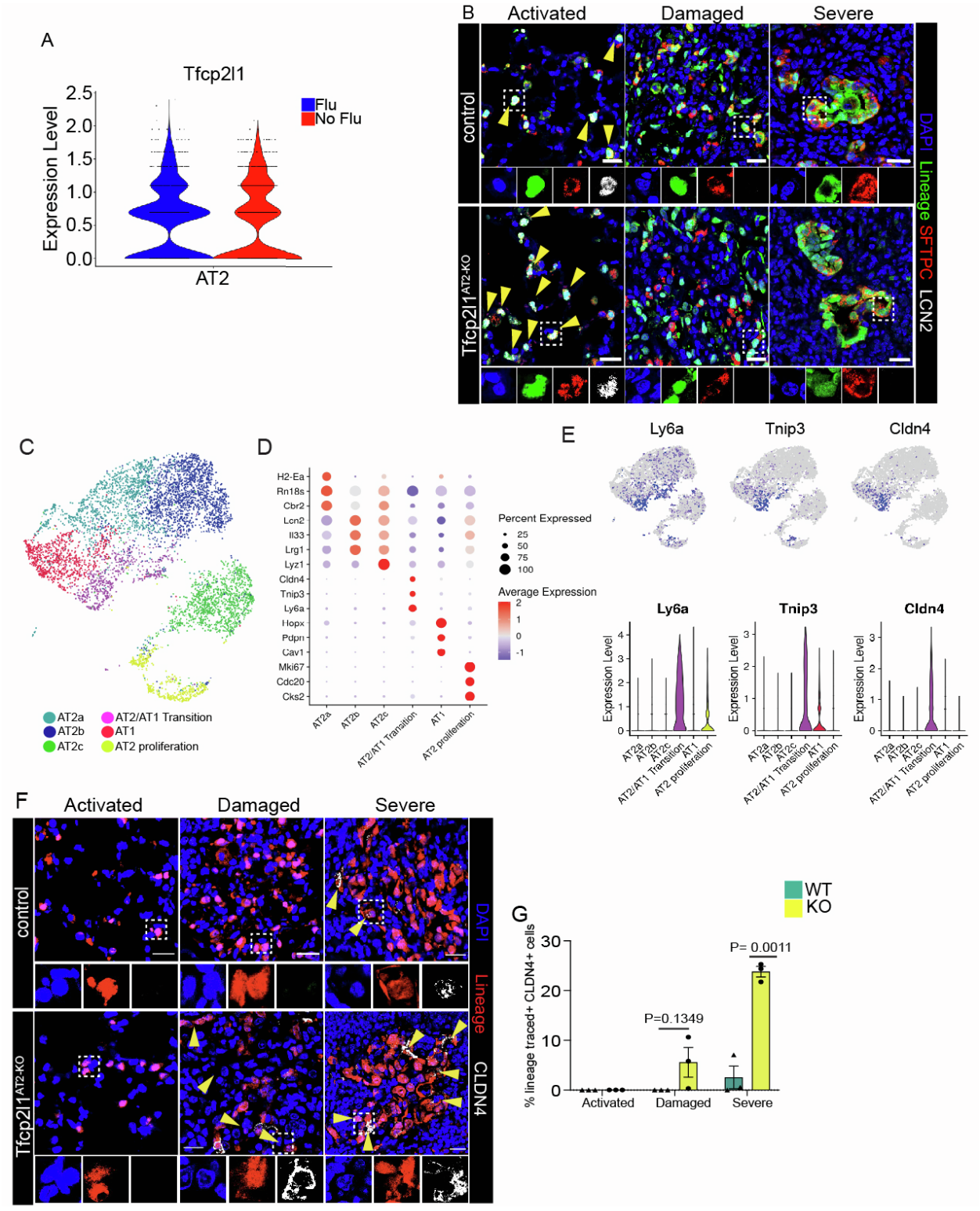


Figure S5: AT2-AT1 cell transition analysis post-viral injury. (A) Tfcp2l1 gene expression represented as a violin at 14 dpi vs no flu sample. (B) IHC for the AT2 cell lineage marker EYFP, SFTPC, and LCN2 in activated, damaged and severe zones 14dpi, white boxes point at zoomed

areas, yellow arrow indicated LCN2 positive cells in AT2 cells (scale bar 20μm). (C) Merged scRNA-seq data, showing UMAP plot of control and Tfcp2l1AT2-KO mice 14dpi. **(D)** Dot plot visualization showing unique gene expression pattern in merged AT2 control and Tfcp2l1AT2-KO mice 14dpi. **(E)** Top. Feature plots showing distribution and expression of Ly6a, Tnip3, and Cldn4. Bottom. Gene expression of Ly6a, Tnip3, and Cldn4 per cell cluster showed using violin plots in merged AT2 control and Tfcp2l1AT2-KO mice 14dpi. **(F)** IHC for the AT2 cell lineage marker EYFP and CLDN4 in activated, damaged severe zones at 14dpi, white boxes point at zoomed areas, yellow arrow indicated CLDN4 positive cells in lineage traced cells (scale bar 20μm). **(G)** Quantification CLDN4+ lineage traced cells is activated, damaged severe zones at 14dpi. All quantification data are represented as mean ± SEM. Two-tailed t-tests not significant; $p \leq 0.05$ n= 3 mice per group.

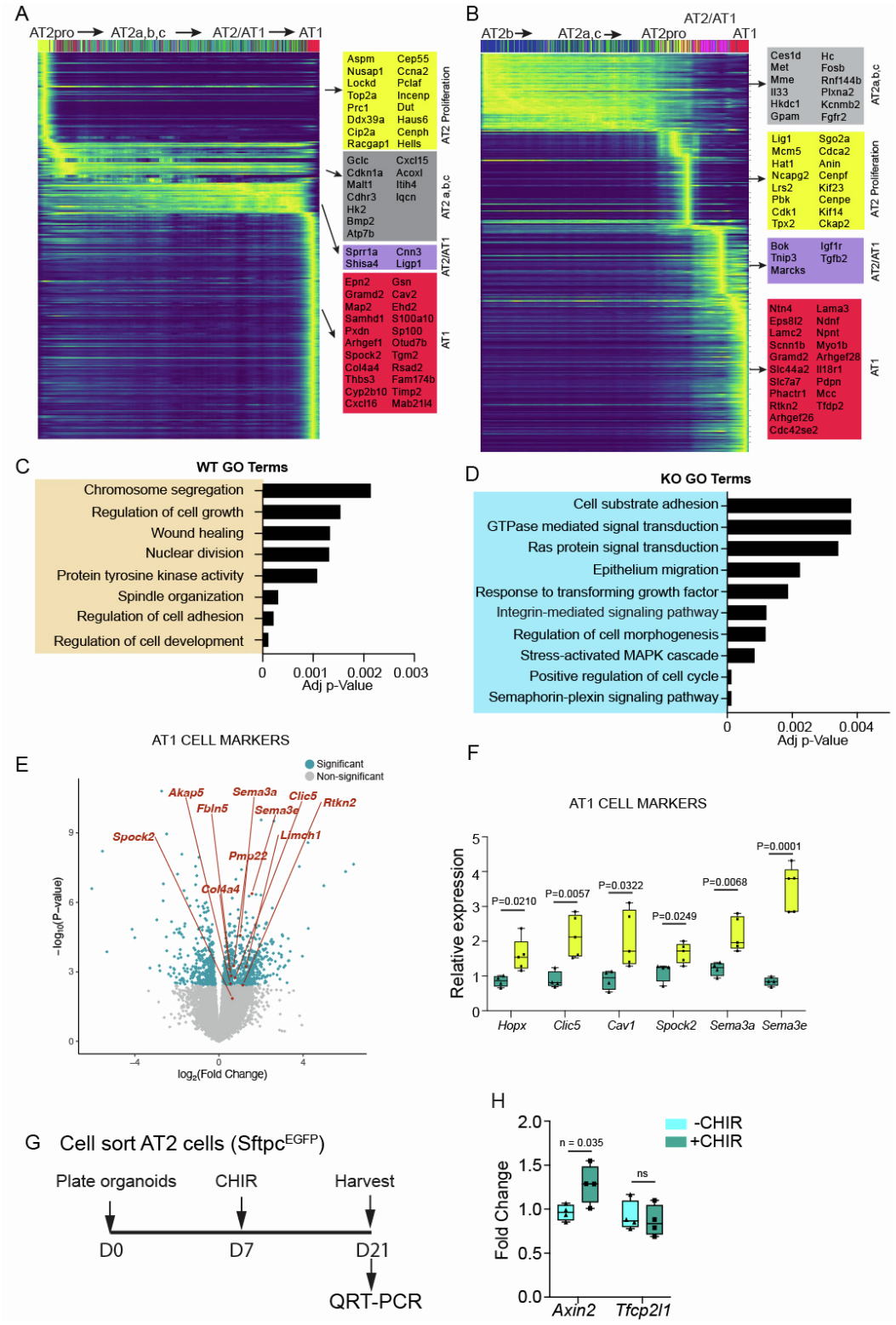


Figure S6: Transcriptional changes of AT2 control and Tfcp2l1 deficient cell population 14 days post-viral injury. (A-B) Density histograms displaying the distribution of AT2 cell states based on latent time of lineage traced control and Tfcp2l1AT2-KO AT2 cells at 14 dpi, displaying

gene signature specific for each category. **(C-D)** GO enrichment analysis from putative genes in latent time analysis **(C)** Control **(D)** Tfcp2l1AT2-KO. **(E)** AT1 cell markers represented in volcano plot from RNA-seq data showing control and Tfcp2l1AT2-KO mice at 14 dpi (adjusted p-value <0.05) dark grey dots show statistically significant different genes between control and Tfcp2l1AT2-KO **(F)** Relative gene expression validation for AT1 cell target genes. (G) Experimental schematic showing the days for CHIR treatment in AT2 organoids and plan for analysis. (H) Relative gene expression for *Axin2* and *Tfcp2l1 on* AT2 organoids with or without CHIR treatment. Two-tailed t-tests not significant; p≤ 0.05 (n=4 per group)

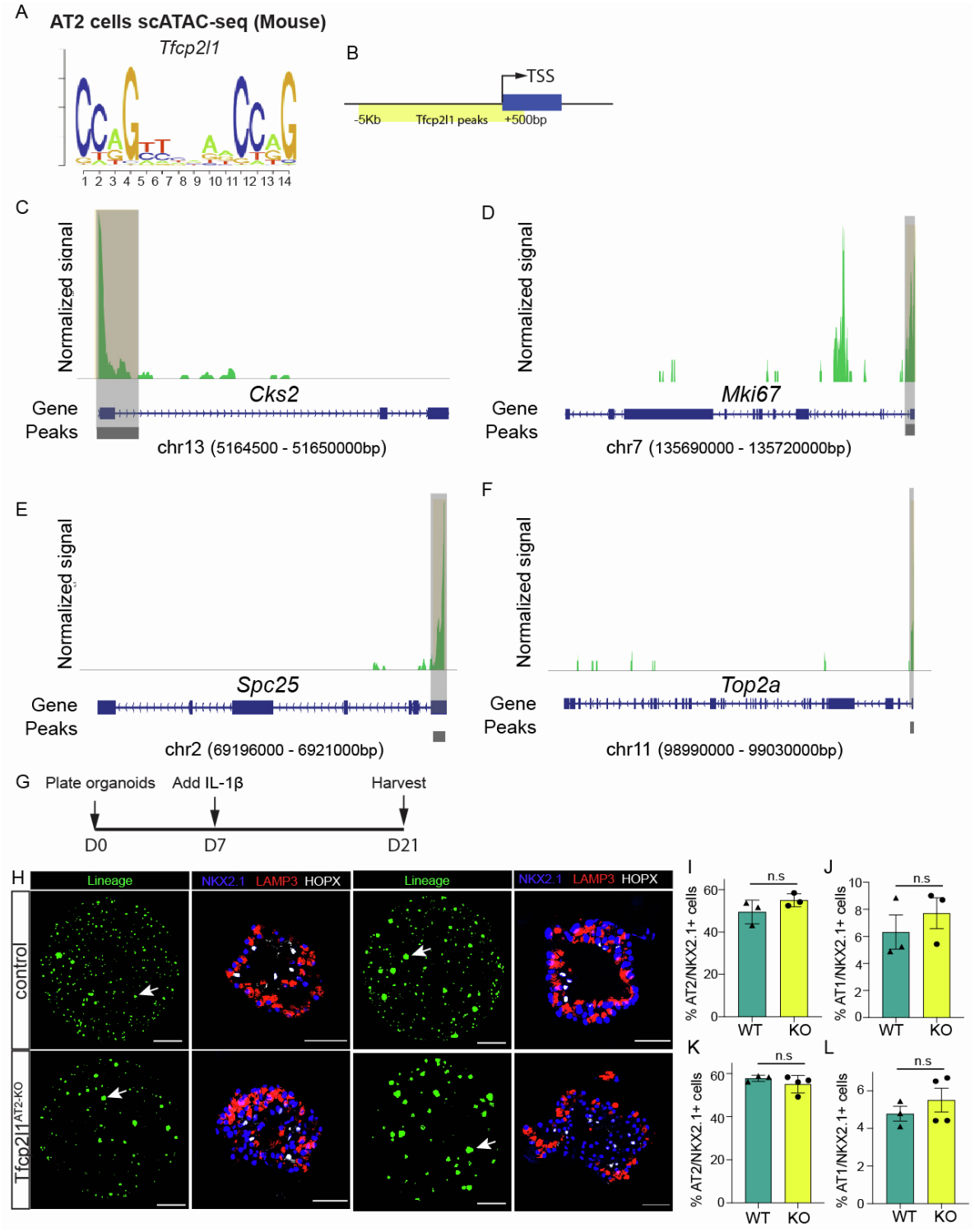


Figure S7: Mouse alveolar single-cell ATAC-seq and AT2 alveolar organoids. (A) *TFCP2l1* motif binding site. (B) Genomic regions scan for *TFCP2L1* peaks looking between -5Kb and +500bp from Transcription Start Site (TSS) in genes related with cell proliferation and inflammatory signaling. (C-F) *TFCP2L1* peaks genes related with cell proliferation in AT2 cells.

(C) *Cks2.* (D) Mki67. (E) *Spc25*. (F) *Top2a.* (G) Experimental schematic showing the days for IL-1β treatment in AT2 organoids. (H) Endogenous AT2 lineage traced EYFP reporter and IHC for LAMP3, HOPX and NKX2.1 markers to examine AT2 control and Tfcp2l1$^{AT2\text{-}KO}$ after 21 days in culture (scale bar for endogenous reporter 1000μm and for IHC picture 50μm). (I-J) Quantification of AT2 cells over total NKX2.1. (I) Without IL-1β. (J) with IL-1β. (K-L) Quantification of AT1 cells over total NKX2.1 (K) Without IL-1β (L) with IL-1β

Table S1. Q-PCR list of primers used in this study

Gene	Forward	Reverse
Tbp	5'-CCTTGTACCCTTCACCAATGAC-3'	5'-ACAGCCAAGATTCACGGTAGA-3'
Cdc20	5'-GGAGGTGACCGCTTTATCCC-3'	5'-CCAGGCTTTCTGATGCTCCT-3'
Cdkn3	5'-CCCTGATACATTGTTACGGAGGA-3'	5'-CTCGAAGGCTGTCTATGGCTT-3'
Top2a	5'-AACAAAGGGACCCAAAAATGTCT-3'	5'-TGTGTTCAACAACAGGGATTCC-3'
Kif2	5'-CTTCACGCACGCCTGTTTC-3'	5'-CTCCTTCGATCCATTCCACCG-3'
AurKb	5'-CAGAAGGAGAACGCCTACCC-3'	5'-GAGAGCAAGCGCAGATGTC-3'
Il7r	5'-AAAGTCCGATCCATTCCCCAT-3'	5'-CCATCCTCCTTGATTCTTGGGT-3'
Il1r1	5'-GTGCTACTGGGGCTCATTTGT-3'	5'-GGAGTAAGAGGACACTTGCGAAT-3'
Ccr7	5'-TGTACGAGTCGGTGTGCTTC-3'	5'-GGTAGGTATCCGTCATGGTCTTG-3'
Il1a	5'-TCTATGATGCAAGCTATGGCTCA-3'	5'-CGGCTCTCCTTGAAGGTGA-3'
Cd69	5'-CCCTTGGGCTGTGTTAATAGTG-3'	5'-AACTTCTCGTACAAGCCTGGG-3'
Sema3e	5'-AGGCTACGCCTGTCACATAAA-3'	5'-CCGTTCTTGATACTCATCCAGC-3'
Sema3a	5'-CACTGGGATTGCCTGTCTTTT-3'	5'-TGGCACATTGTTCTTTCCGTTT-3'
Clic5	5'-TCAATGGGGATGTGAAGACAGA-3'	5'-GGTGTTAGATTCCCGGTGTTTT-3'
Cav1	5'-GCGACCCCAAGCATCTCAA-3'	5'-ATGCCGTCGAAACTGTGTGT-3'
Spock2	5'-ACCCCCGGCAATTTCATGG-3'	5'-TGTCTTCCCAGCTCTTGATGTAA-3'
Tfcp2l1	5'-AGAGCATCTGCATTCATTCAGG-3'	5'-AAAAGGCACTCCCTTCTCACC-3'
Axin2	5'-AACCTATGCCCGTTTCCTCTA-3'	5'-GAGTGTAAAGACTTGGTCCACC-3'