Supplemental Materials and Methods

Histology and immunofluorescence staining

Mouse right ventricles were perfused with 1 ml PBS and the lungs were inflated with 4% paraformaldehyde (PFA) at a constant pressure of 25 cm H_2O, and then fixed in 4% PFA overnight at 4ºC. After fixation, the lungs were washed by cold PBS X 4 times in 2 hrs at 4ºC, and dehydrated in a series of increasing ethanol concentration washes (30%, 50%, 70%, 95% and 100%). The dehydrated lungs were incubated with Xylene for 1 hr at RT and with paraffin at 65ºC for 90 min X 2 times, and then embed in paraffin and sectioned. Antibodies used were rabbit anti-ACTA2 (1:200, Abcam, ab5694), rat anti-Brdu (1:200, Abcam, ab6326, Clone# BU1/75 (ICR1)), chicken anti-GFP (1:200, Aves Labs, GFP-1020), mouse anti-MSLN-Alexa Fluor 647 (1:50, Santa Cruz, sc-33672, Clone# K1), rabbit anti-SFTPC (1:250, Millipore, ab3786), goat anti-SCGB1A1 (1:500, Santa Cruz, sc-9772, Clone# T-18), rabbit anti-cleaved caspase-3 (1:200, CST, 9664T, Clone# 5A1E) and rabbit anti-MET (1:50, Santa Cruz, sc-10, Clone# C-12). LacZ staining of lungs was performed as previously described (58). For human lung slides, mouse anti-THY1-PE (1:200, R&D Systems, FAB2067P, Clone# Thy-1A1) and mouse anti-ITGA8 antibodies (1:200, Clone# YZ-3 recombinant antibody generated in the Yokosaki lab) (59) were utilized. X-gal staining was visualized with both light microscopy and confocal microscopy using excitation (633 nm)/emission range (650-770 nm) (60).

Western blotting

Mice lungs were lysed with tissue protein extraction buffer with protease and phosphatase inhibitor cocktail (Thermo). Western blotting analysis was performed as described previously (61). Antibodies used were mouse GAPDH (1:2000, Santa Cruz, sc-32233, Clone# 6C5), rabbit anti-MET (1:1000, Santa Cruz, sc-10, Clone# C-12) and rabbit anti-phospho-MET (1:1000, Invitrogen, 44-888G). Densitometry was quantified using Fiji software. See complete unedited blots in the supplemental material.

Bronchoalveolar lavage fluid (BALF) collection

BALF was obtained by inserting a 20-gauge catheter into the trachea through which 1 ml of cold PBS was flushed back and forth 3 times. Total white blood cells were quantified using a Coulter counter (Beckman Coulter), and the cell types were determined by cytospin preparation (Cytospin 3; Thermo Electron Corp) and Diff-Quick staining.

Fluorescence activated cell sorting (FACS) and analysis

Whole mouse lung was dissected from adult animals and tracheally perfused with a digestion cocktail of Collagenase Type I (225 U/ml, Thermo Fisher), Dispase (15 U/ml, Thermo Fisher) and Dnase (50 U/ml, Sigma) and removed from the chest. For FACS analysis of immune cells, a digestion cocktail of Liberase TM (40 µg/mL, Sigma) and Dnase (50 U/ml, Sigma) in HBSS was used to dissociate the lung. The lung was further diced with razor blades and the mixture was incubated for 45 mins at 37 ºC and vortexed intermittently. The mixture was then washed with FACS buffer (2% FBS in DMEM-F12). The mixture was passed through a 70 µm cell strainer and resuspended in RBC lysis buffer, before passing through a 40 µm cell strainer. Cells suspensions were incubated

with the appropriate antibodies in FACS buffer for 30 min at 4 ºC and washed with FACS buffer. The following antibodies were used at 1:200 for staining: rat anti-CD45-PE-Cy7 (Thermo Fisher, 25-0451-82, Clone# 30-F11), rat anti-EPCAM-BV421 (Thermo Fisher, BDB563214, Clone# G8.8), rat anti-CD31-PerCP-eFluor 710 (Thermo Fisher, 46-0311-80, Clone# 390), mouse anti-ITGA8-APC (59), rat anti-LY6A-APC-Cy7 (Biolegend, 108126, Clone# D7), rat anti-LY6C-APC (Biolegend, 128016, Clone# HK1.4), rat anti-PDGFRa-APC (Thermo Fisher, 17-1401-81, Clone# APA5), Armenian hamster anti-CD3e-APC (Biolegend, 100312, Clone# 145-2C11), rat anti-CD4-BV711 (Biolegend, 100549, Clone# RM4-5), rat anti-CD19-PE/Dazzle 594 (Biolegend, 115554, Clone# 6D5), Armenian Hamster anti-CD11C-PE/Cy7 (Biolegend, 117318, Clone# N418), rat anti-CD11b-Pacific Blue (Biolegend, 101224, Clone# M1/70), rat anti-LY6G/LY6C-APC/Cy7 (Biolegend, 108424, Clone# RB6-8C5), rat anti-Siglec-F-BV786 (BD, 740956, Clone# E50-2440), mouse anti-NK-1.1-BV650 (Biolegend, 108736, Clone# PK136), and rat anti-CD45-BUV395 (BD, 564279, Clone# 30-F11). For human lung, a piece of it (~10 cm^3) was dissected from the whole lung and washed with HBSS X 4 times in 15 min. The piece of lung was further diced with razor blades and was added into the digestion cocktail of Collagenase Type I (225 U/ml, Thermo Fisher), Dispase (15 U/ml, Thermo Fisher) and Dnase (100 U/ml, Sigma). The mixture was incubated for 2 h at 37 ºC and vortexed intermittently. The mixture was then liquefied with a blender and passed through 4X4 gauze, a 100 µm and a 70 µm cell strainer. The mixture was resuspended in RBC lysis buffer, before passing through a 40 µm cell strainer. Cells suspensions were incubated with the appropriate antibodies in FACS buffer for 30 min at 4 ºC and washed with FACS buffer. The following antibodies were used at 1:200 for staining: mouse anti-

CD45-APC-Cy7 (BioLegend, 3304014, Clone# HI30), mouse anti-CD31-APC-Cy7 (BioLegend, 303120-BL, Clone# WM59), mouse anti-CD11b-APC-Cy7 (BD Biosciences, 557754, Clone# ICRF44), mouse anti- EPCAM-APC-Cy7 (BioLegend, 324233, Clone# 9C4), mouse anti- EPCAM-PE (BioLegend, 324206, Clone# 9C4) mouse anti-THY1-PE (R&D Systems, FAB2067P, Clone # Thy-1A1) and mouse anti-ITGA8-APC (Clone# YZ-3) (59). DAPI (0.2 µg/ml) and DRAQ7 (CST, 1:1000) was used to exclude dead cells. Doublets and dead cells were excluded based on forward scatter, side scatter and DAPI/DRAQ7 fluorescence. Cells were sorted into FACS buffer. FACS analysis was performed by FACSDiva (BD Biosciences) and FlowJo (TreeStar) softwares.

Bulk RNA library preparation and HiSeq sequencing

The RNA was extracted by PicoPure RNA Isolation Kit (Applied Biosystems) from the sorted GLI2+ cells, and the amount and quality of extracted RNA was measured by RNA 6000 Pico Kit (Agilent). The downstream library preparation and sequencing was performed at GENEWIZ, LLC (South Plainfield, NJ, USA). RNA sequencing library preparation used the NEBNext Ultra RNA Library Prep Kit for Illumina by following manufacturer's recommendations (NEB, Ipswich, MA, USA). Briefly, mRNA was first enriched with Oligod(T) beads. Enriched mRNAs were fragmented for 15 minutes at 94°C. First strand and second strand cDNA were subsequently synthesized. cDNA fragments were end repaired and adenylated at 3'ends, and universal adapter was ligated to cDNA fragments, followed by index addition and library enrichment with limited cycle PCR. Sequencing libraries were validated on the Agilent TapeStation (Agilent Technologies, Palo Alto, CA, USA), and quantified by using Qubit 2.0 Fluorometer

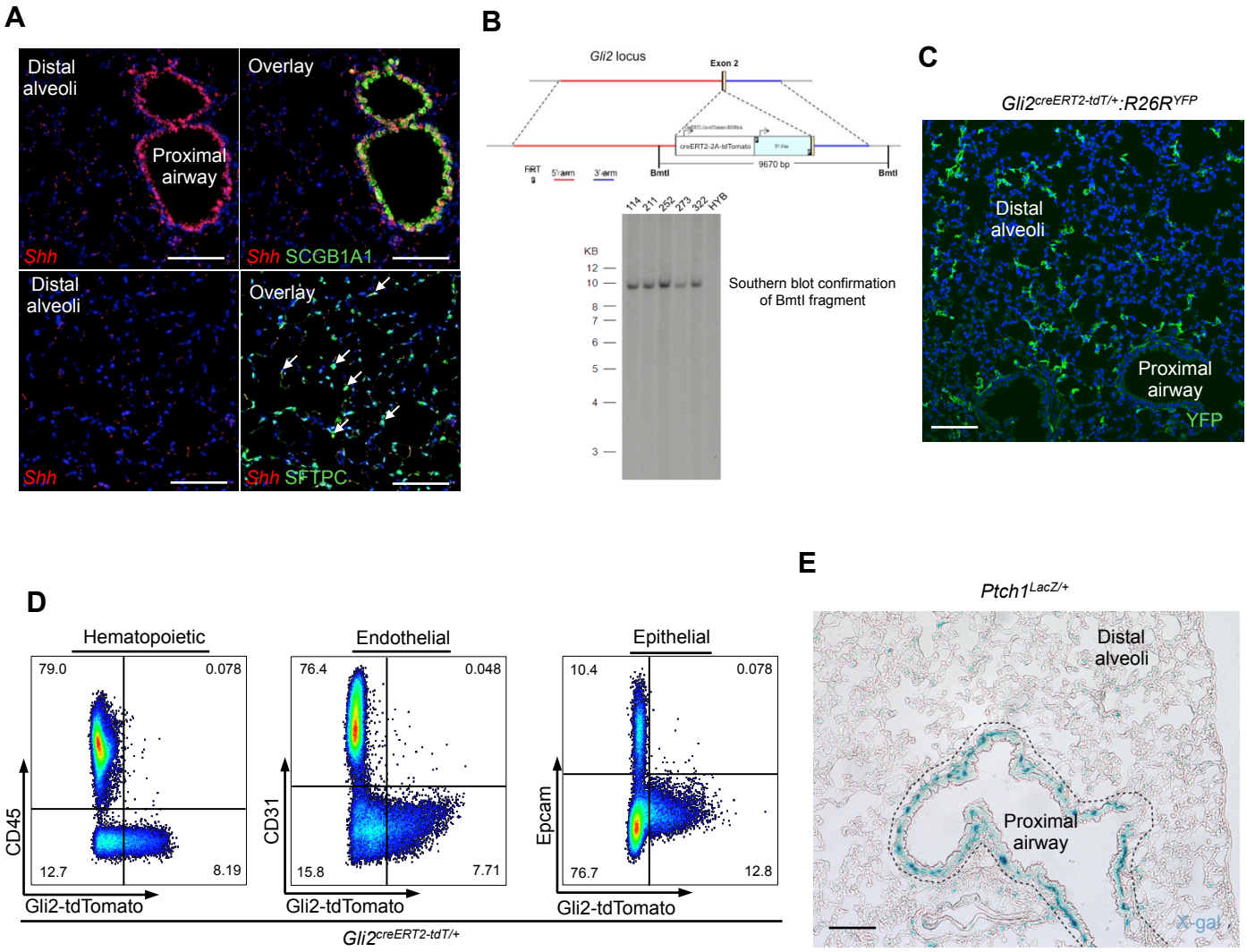
(Invitrogen, Carlsbad, CA) as well as by quantitative PCR (Applied Biosystems, Carlsbad, CA, USA). The sequencing libraries were multiplexed and clustered on one lane of a flowcell. After clustering, the flowcell was loaded on the Illumina HiSeq instrument according to manufacturer's instructions. The samples were sequenced using a 2x150 Paired End (PE) conFigureuration. Image analysis and base calling were conducted by the HiSeq Control Software (HCS). Raw sequence data (.bcl files) generated from Illumina HiSeq was converted into fastq files and de-multiplexed using Illumina's bcl2fastq 2.17 software. One mis-match was allowed for index sequence identification.

Quantitative RT-PCR

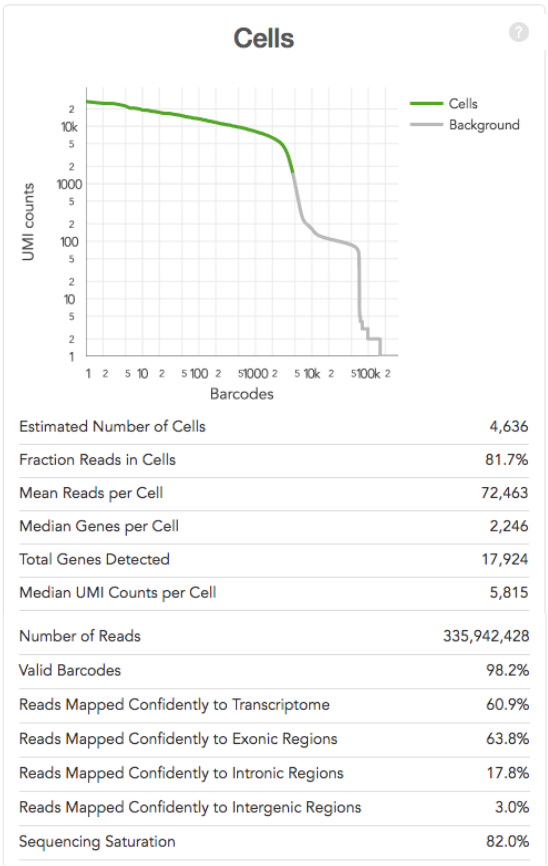
Total RNA was isolated from fresh sorted or cultured primary lung fibroblasts using the PicoPure RNA Isolation Kit (Applied Biosystems) or the RNeasy kit (QIAGEN), following the manufacturers' protocols. cDNA was synthesized from total RNA using the SuperScript Strand Synthesis System (Invitrogen). Quantitative real-time PCR (qPCR) was performed using the SYBR Green system (Thermo Fisher). Relative gene expression levels after qPCR were defined using the $\Delta\Delta$Ct method and normalizing to GAPDH. Data are shown as the average of a minimum of three biological replicates for each genotype/condition ± SEM. Statistical analysis was done using the Student's t-test. The mouse primers used in this study includes: *Mus_Dhh* (For: 5' AGCAACTTGTGCCTCTGCTA 3'; Rev: 5' TTGCAACGCTCTGTCATCAG 3'), *Mus_Ihh* (For: 5' CTCTTGCCTACAAGCAGTTCA 3'; Rev: 5' CCGTGTTCTCCTCGTCCTT 3'), *Mus_Shh* (For: 5' AAAGCTGACCCCTTTAGCCTA 3'; Rev: 5'

TTCGGAGTTTCTTGTGATCTTCC 3'), *Mus_Gli1* (For: 5' CCCACTCCAATGAGAAGCCAT 3'; Rev: 5' CGGACCATGCACTGTCTTCA 3'), *Mus_Ptch1* (For: 5' GGGGGTTCTCAATGGACTGG 3'; Rev: 5' CATTGGCTGGAGACACCTCA 3'), *Mus_Ptch2* (For: 5' CTCCGCACCTCATATCCTAGC 3'; Rev: 5' TCCCAGGAAGAGCACTTTGC 3'), *Mus_Hgf* (For: 5' TCGGATAGGAGCCACAAGGA 3'; Rev: 5' GCCGGGCTGAAAGAATCAAAG 3'), *Mus_Ebf1* (For: 5' GCATCCAACGGAGTGGAAG 3'; Rev: 5' GATTTCCGCAGGTTAGAAGGC 3'), *Mus_Foxg1* (For: 5' TTCGGGACTGTTTGGGTCTG 3'; Rev: 5' GTAGCAAAAGAGCTT CCTGCG 3'), *Mus_Fst* (For: 5' TGCTGCTACTCTGCCAGTTC 3'; Rev: 5' GTGCTGCAACACTCTTCCTTG 3'), *Mus_Nkx6-1* (For: 5' CTGCACAGTATGGCCGAGATG 3'; Rev: 5' CCGGGTTATGTGAGCCCAA 3'), *Mus_Wnt1* (For: 5' TGATGTTTGCCCACCCTACC 3'; Rev: 5' CCTCAGGATGGCAAAAGGGT 3'), *Mus_Wnt2* (For: 5' CTGATGTAGACGCAAGGGGG 3'; Rev: 5' CCTGTAGCTCTCATGTACCACC 3'), *Mus_Wnt3a* (For: 5' ATCTGGTGGTCCTTGGCTGT 3'; Rev: 5' GGGCATGATCTCCACGTAGT 3'), *Mus_Wnt7b* (For: 5' CTGAGCGTGGTCCTACCG 3'; Rev: 5' ATGACAATGCTCCGAGCTTCA 3') and *Mus_Gapdh* (For: 5' CCCCAGCAAGGACACTGAGCAAGAG 3'; Rev: 5' GGCCCCTCCTGTTATTATGGGGGT 3') was used as internal control. The human primers used for this study includes: *Hs_GLI1* (For: 5' CCCGGAGTGCAGTCAAGTT 3'; Rev: 5' CCAGAGATGGGCTCATGGTG 3'), *Hs_PTCH1* (For: 5' GCCGCGTTAATCCCAATTCC 3'; Rev: 5' GCAGGGGCTTGTAAAACAGC 3'),

Hs_PTCH2 (For: 5’ CGCCGCCAGAGGTGATAC 3’; Rev: 5’ CCACGGTCATGGAGGTAGTC 3’), *Hs_DHH* (For: 5’ GTGCCGCTACTCTACAAGCA 3’; Rev: 5’ TACAACGCTCGGTCATCAGG 3’), *Hs_IHH* (For: 5’ CTCCGTCAAGTCCGAGCAC 3’; Rev: 5’ ATGAGCACATCGCTGAAGGT 3’), *Hs_SHH* (For: 5’ GTGAAAGCAGGCAAGGAAAGGA 3’; Rev: 5’ AAACTCTTGGCTCCGTCAAC 3’) and *Hs_GAPDH* (For: 5’ AACGACCCCTTCATTGAC 3’; Rev: 5’ TCCACGACATACTCAGCAC 3’) was used as internal control.

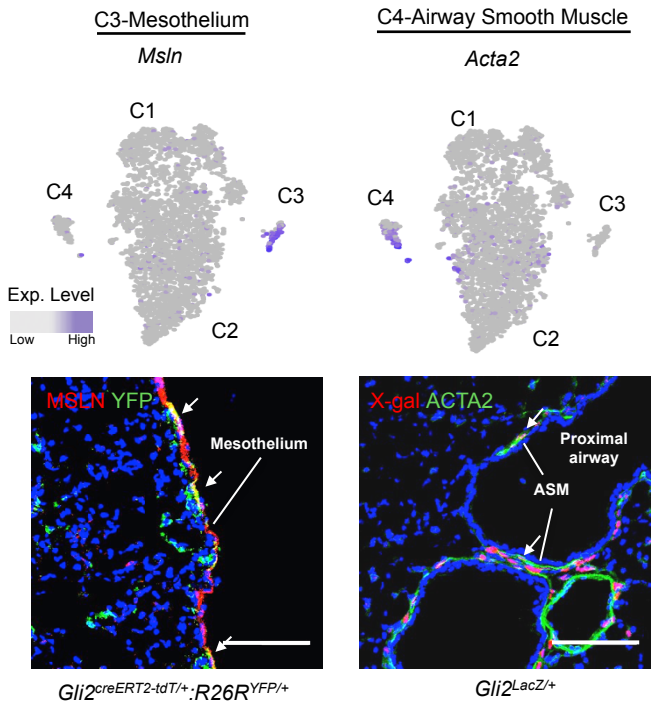

A
Distal alveoli
Proximal airway
Overlay
Shh
Shh SCGB1A1
Distal alveoli
Overlay
Shh
Shh SFTPC
B
Gli2 locus
Exon 2
creERT2-2A-tdTomato
FRT
5' arm
3' arm
BmtI
9670 bp
BmtI
114
211
252
273
322
HYB
KB
12
10
8
7
6
5
4
3
Southern blot confirmation of BmtI fragment
C
Gli2creERT2-tdT/+:R26RYFP
Distal alveoli
Proximal airway
YFP
D
Hematopoietic
Endothelial
Epithelial
79.0
0.078
12.7
8.19
76.4
0.048
15.8
7.71
10.4
0.078
76.7
12.8
CD45
CD31
Epcam
Gli2-tdTomato
Gli2-tdTomato
Gli2-tdTomato
Gli2creERT2-tdT/+
E
Ptch1LacZ/+
Distal alveoli
Proximal airway
X-gal

A

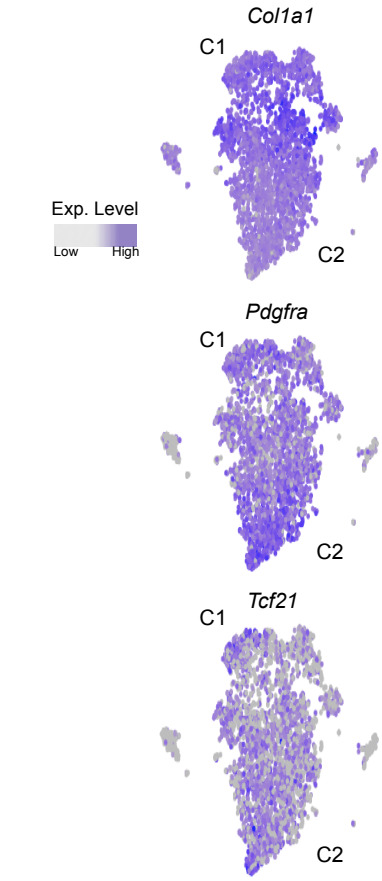


Estimated Number of Cells	4,636
Fraction Reads in Cells	81.7%
Mean Reads per Cell	72,463
Median Genes per Cell	2,246
Total Genes Detected	17,924
Median UMI Counts per Cell	5,815
Number of Reads	335,942,428
Valid Barcodes	98.2%
Reads Mapped Confidently to Transcriptome	60.9%
Reads Mapped Confidently to Exonic Regions	63.8%
Reads Mapped Confidently to Intronic Regions	17.8%
Reads Mapped Confidently to Intergenic Regions	3.0%
Sequencing Saturation	82.0%

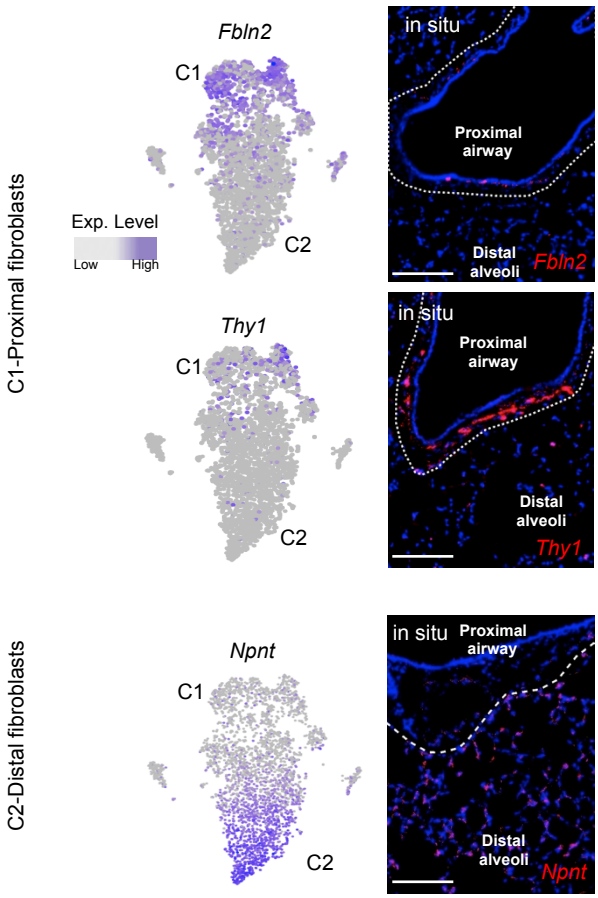
B



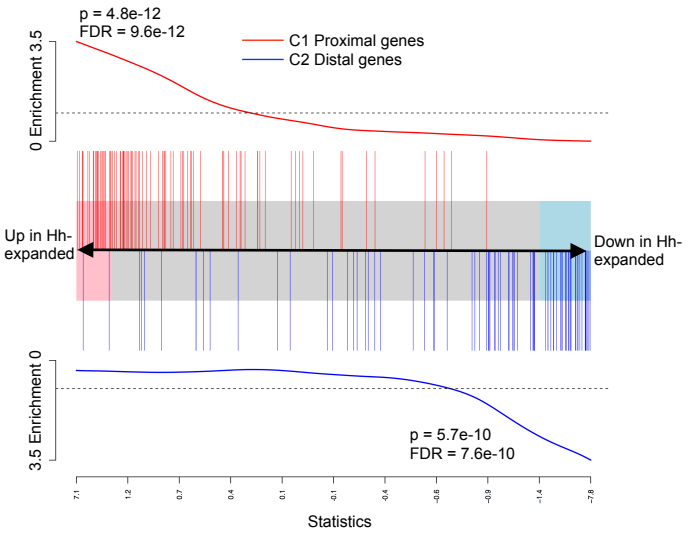
C



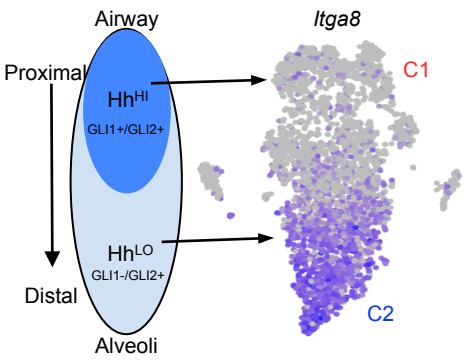
D

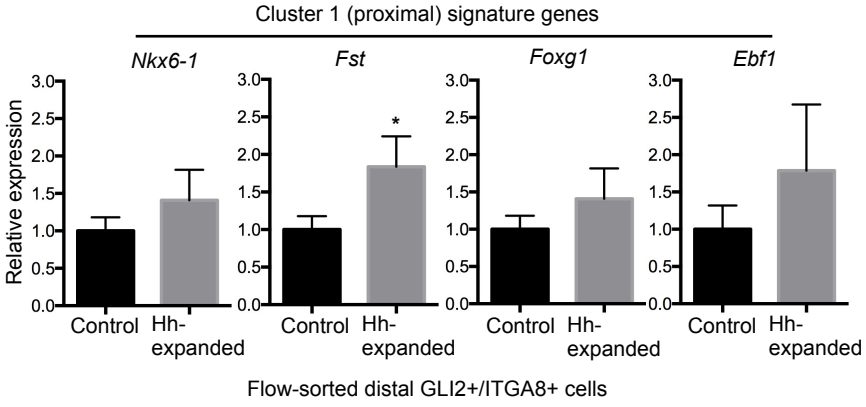


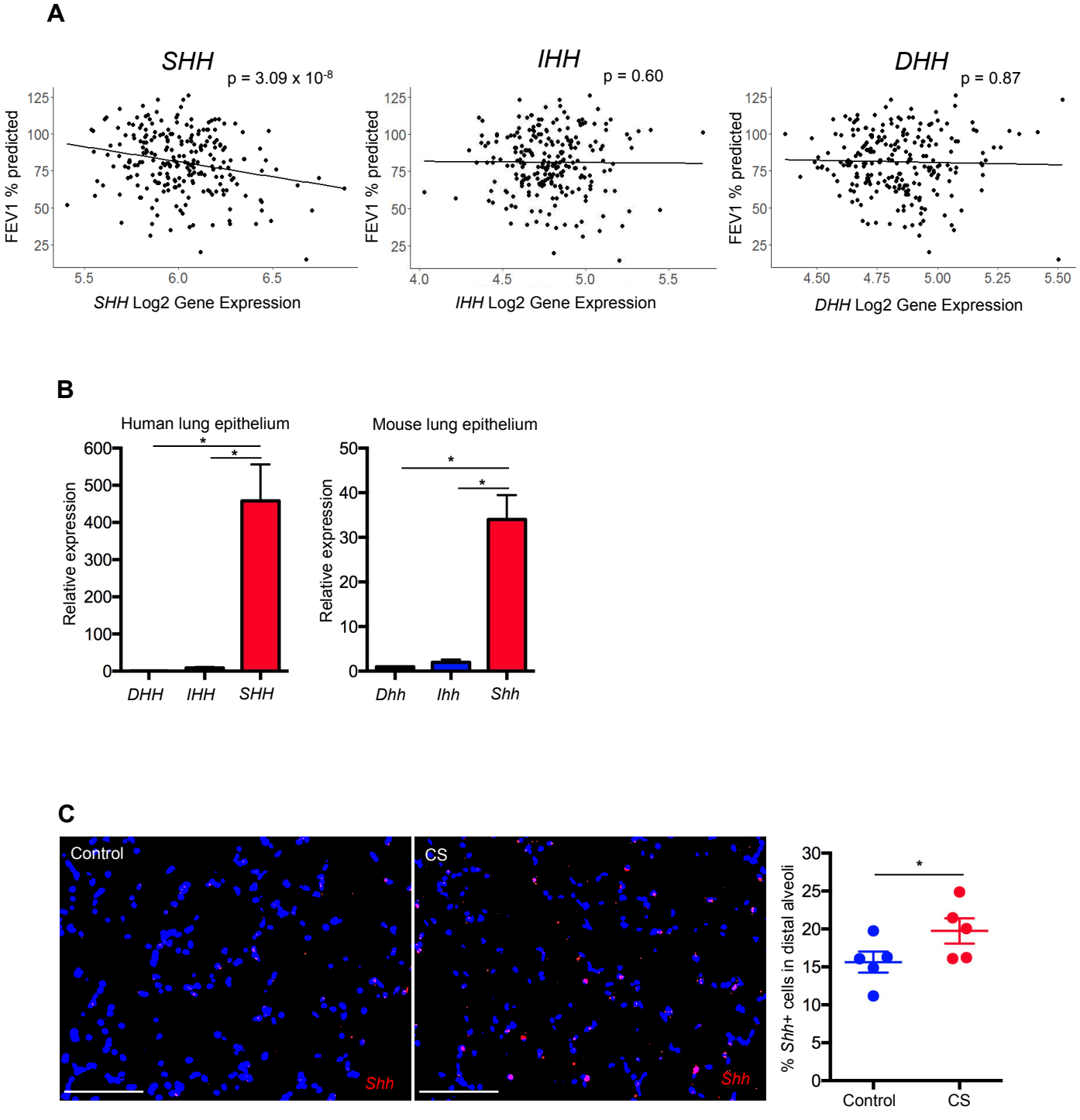
A

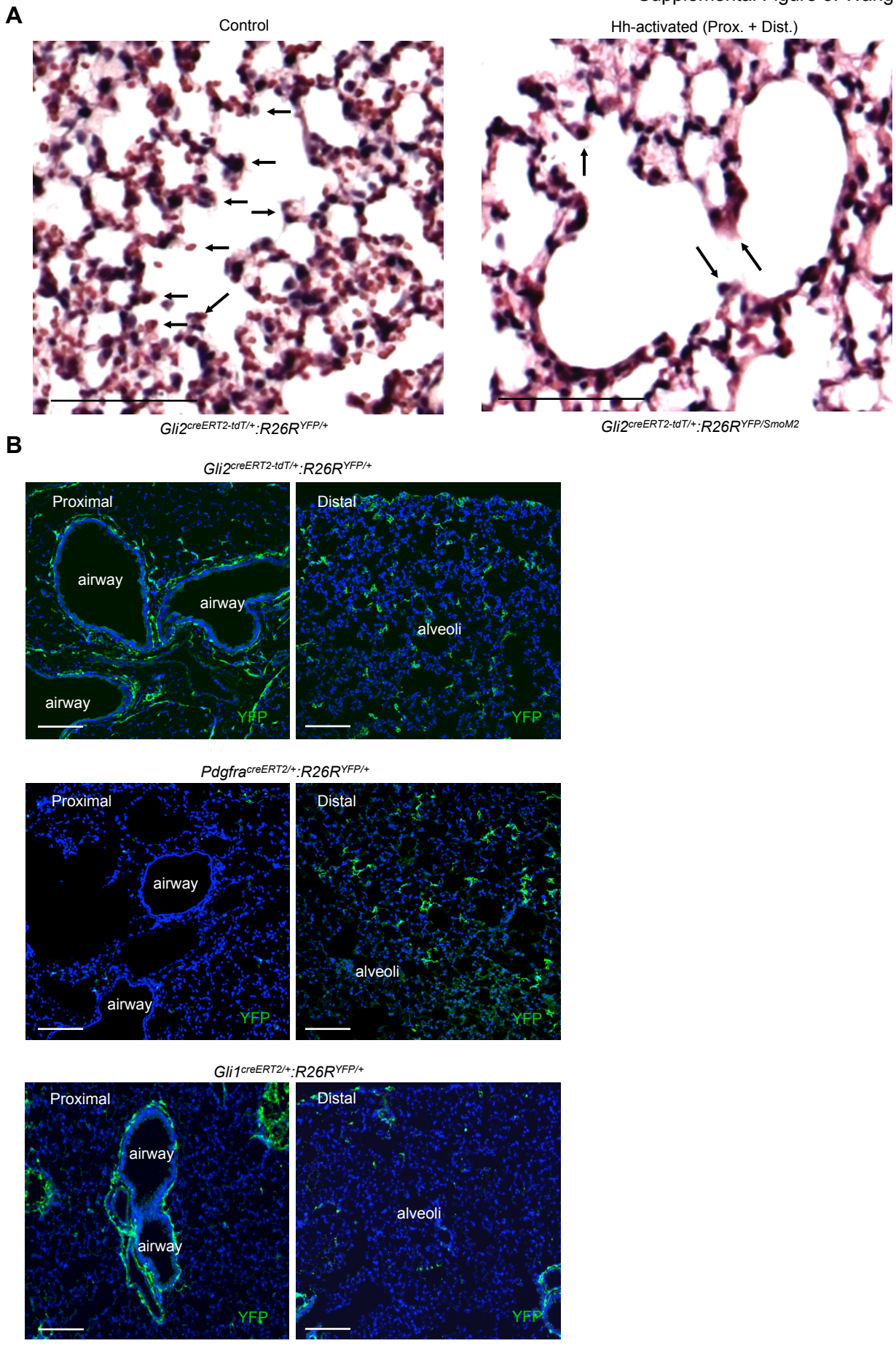

p = 4.8e-12
FDR = 9.6e-12
C1 Proximal genes
C2 Distal genes
0 Enrichment 3.5
Up in Hh-expanded
Down in Hh-expanded
3.5 Enrichment 0
p = 5.7e-10
FDR = 7.6e-10
Statistics

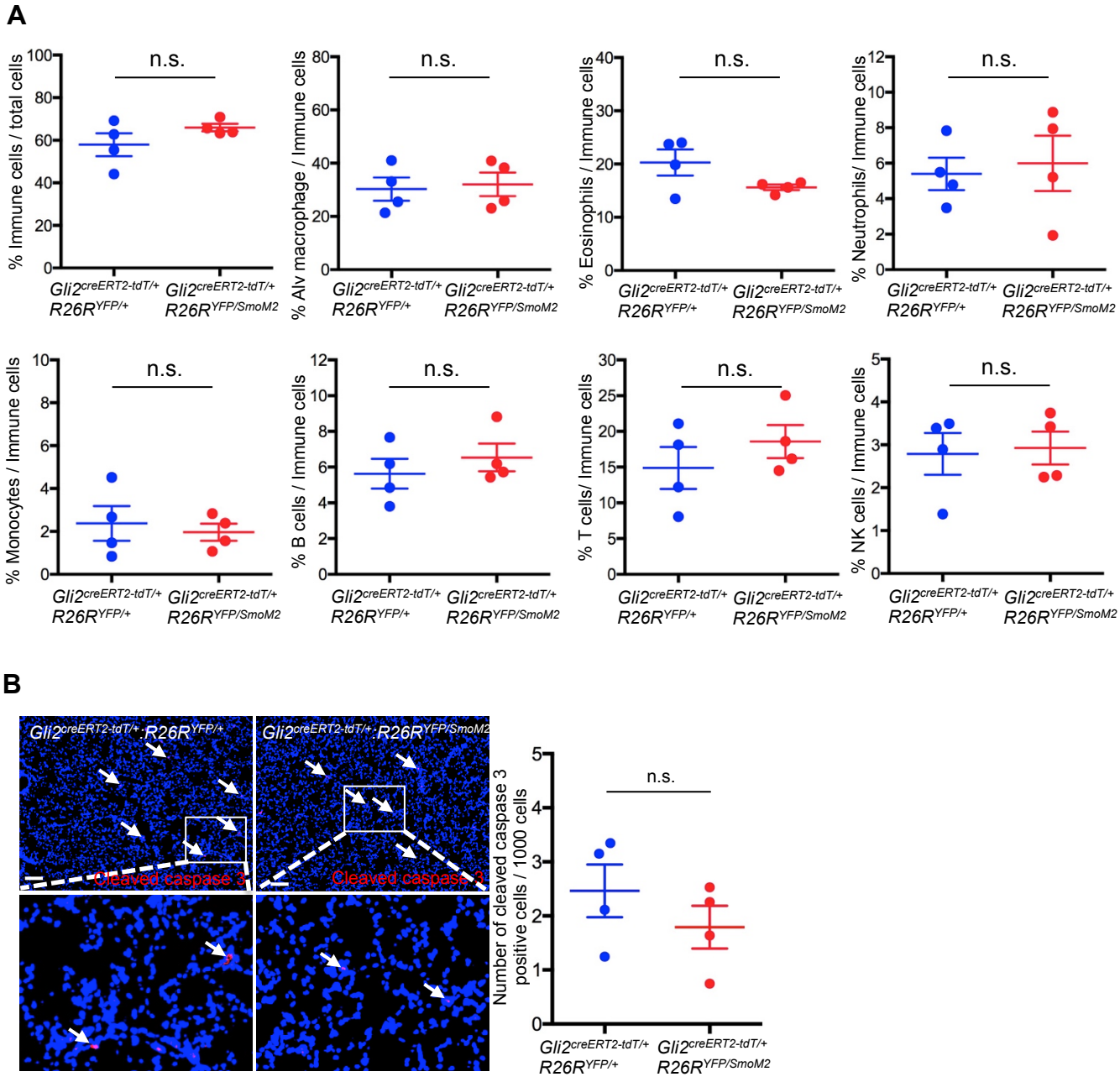
B

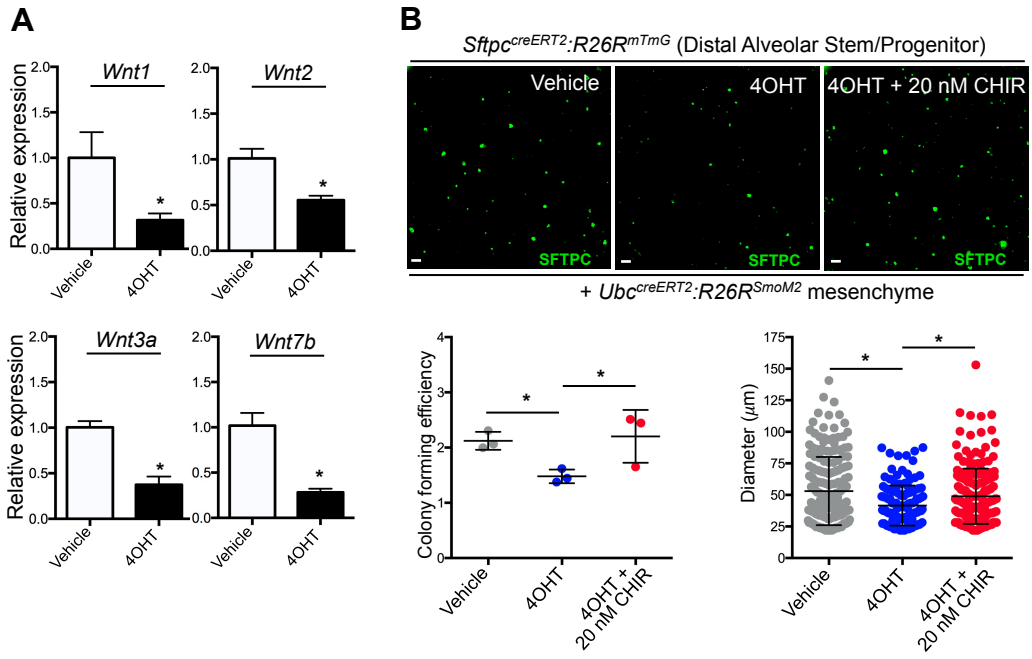

Airway
Proximal
Distal
Alveoli
HhHI
GLI1+/GLI2+
HhLO
GLI1-/GLI2+
Itga8
C1
C2


Cluster 1 (proximal) signature genes
Nkx6-1
Fst
Foxg1
Ebf1
Relative expression
Control
Hh-expanded
*
Flow-sorted distal GLI2+/ITGA8+ cells

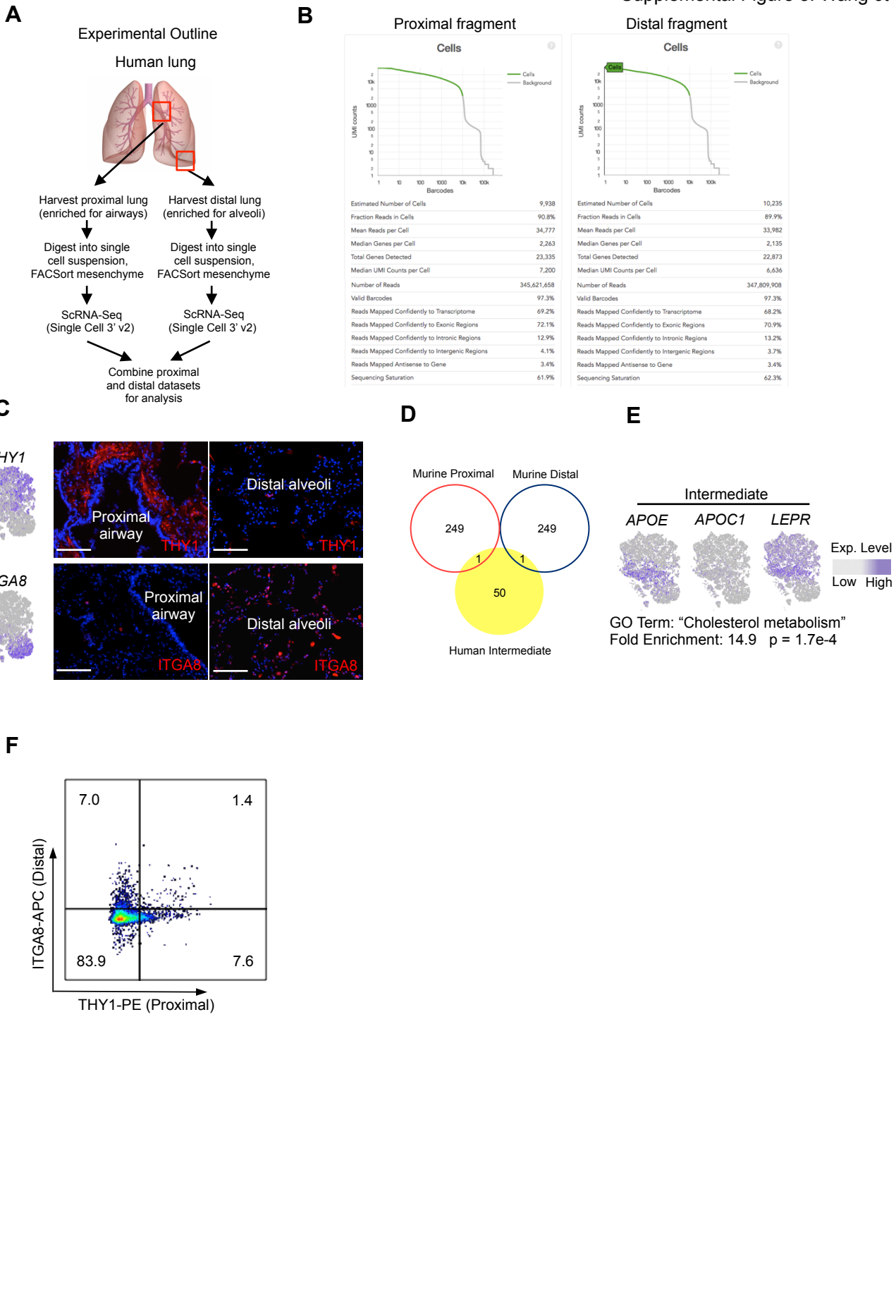

A
SHH
p = 3.09 x 10-8
IHH
p = 0.60
DHH
p = 0.87
FEV1 % predicted
SHH Log2 Gene Expression
IHH Log2 Gene Expression
DHH Log2 Gene Expression
B
Human lung epithelium
Mouse lung epithelium
Relative expression
DHH
IHH
SHH
Dhh
Ihh
Shh
C
Control
CS
Shh
% Shh+ cells in distal alveoli


A
Control
Hh-activated (Prox. + Dist.)
Gli2creERT2-tdT/+:R26RYFP/+
Gli2creERT2-tdT/+:R26RYFP/SmoM2
B
Gli2creERT2-tdT/+:R26RYFP/+
Proximal
airway
airway
airway
YFP
Distal
alveoli
YFP
Pdgfracre ERT2/+:R26RYFP/+
Proximal
airway
airway
YFP
Distal
alveoli
YFP
Gli1creERT2/+:R26RYFP/+
Proximal
airway
airway
YFP
Distal
alveoli
YFP


A
% Immune cells / total cells
% Alv macrophage / Immune cells
% Eosinophils / Immune cells
% Neutrophils/ Immune cells
% Monocytes / Immune cells
% B cells / Immune cells
% T cells/ Immune cells
% NK cells / Immune cells
n.s.
Gli2creERT2-tdT/+ R26RYFP/+
Gli2creERT2-tdT/+ R26RYFP/SmoM2
B
Gli2creERT2-tdT/+ ·R26RYFP/+
Gli2creERT2-tdT/+ ·R26RYFP/SmoM2
Cleaved caspase 3
Number of cleaved caspase 3 positive cells / 1000 cells


A
Wnt1
Wnt2
Wnt3a
Wnt7b
Relative expression
Vehicle
4OHT
B
Sftpc^creERT2:R26R^mTmG (Distal Alveolar Stem/Progenitor)
Vehicle
4OHT
4OHT + 20 nM CHIR
SFTPC
+ Ubc^creERT2:R26R^SmoM2 mesenchyme
Colony forming efficiency
Diameter (μm)
4OHT + 20 nM CHIR

Supplemental Figure 8. Wang *et al.*



Unedited Wetern blotting figure for Figure 5C. Wang *et al*.