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$_2$O, and then fixed in 4% PFA overnight at 4°C. After fixation, the lungs were washed by cold PBS X 4 times in 2 hrs at 4°C, 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 65°C 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 °C 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 °C 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-PDGFRα-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-APC/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 cm³) 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 °C 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 °C 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) configuration. 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 $\pm$ 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’ TTGCAACGCTCTGTACATCAG 3’), *Mus_Ihh* (For: 5’ CTCTTGCCTACAAGCAGTCA 3’; Rev: 5’ CGTGTTTCCTCCTCGTCCTT 3’), *Mus_Shh* (For: 5’ AAAGCTGACCCCTTTAGCCTA 3’; Rev: 5’
TTCGGAGTTTCTTGTGATCTTCC 3’), *Mus_Gli1* (For: 5’

CCCCTTCCAATGAGAAGCCAT 3’; Rev: 5’ CGGACCATGCACTGTCTTCA 3’),

*Mus_Ptch1* (For: 5’ GGGGGTTCTCAATGGGACTGG 3’; Rev: 5’

CATTGGCTGAGACACCTCA 3’), *Mus_Ptch2* (For: 5’ CTCCGCACCTCATATCCTAGC 3’; Rev: 5’ TCCCAGGAAGAGCAACTTTGC 3’), *Mus_Hgf* (For: 5’

TCGGATAGGAGCCCAAGGA 3’; Rev: 5’ GCCGGGCTGAAAGAATCAAAG 3’),

*Mus_Ebf1* (For: 5’ GCATCCAACGGAGTGGAAG 3’; Rev: 5’

GATTTCGCCAGGTAGAAGGC 3’), *Mus_Foxg1* (For: 5’

TTCGGGACTGTTTGCTTG 3’; Rev: 5’ GTAGCAAAAGAGCTT CCTGCG 3’),

*Mus_Fst* (For: 5’ TGCTGCTACTCTGAGTCCAGTTC 3’; Rev: 5’

CTGCACAGATATGGCCGAGATG 3’; Rev: 5’ CCGGGTTATGTGAGCCCAA 3’),

*Mus_Wnt1* (For: 5’ TGATGTCTGGCCACCTTACCCACCCT ACC 3’; Rev: 5’

CCTCAGGATGGGAAAAAGGGT 3’), *Mus_Wnt2* (For: 5’ CTGATGACCACTGCAAGGGGG 3’; Rev: 5’ CCTGTAGCTCTCATATGACCACC 3’), *Mus_Wnt3a* (For: 5’

ATCTGGTGCTCTGGCCTGTG 3’; Rev: 5’ GGGCATGATCTCCACGTA GTG 3’),

*Mus_Wnt7b* (For: 5’ CTGAGCGCTGGTCTCTACC 3’; Rev: 5’

ATGACAATGCTCCAGGCTTCA 3’) and *Mus_Gapdh* (For: 5’

CCCAGCAAGAATCTGAGCAAGAG 3’; Rev: 5’

GGCCCCCTCTGGTATTAGGAGG GT 3’) was used as internal control. The human

primers used for this study includes: *Hs_GLI1* (For: 5’ CCCGGAGTGCA GTCAAGTT 3’; Rev: 5’ CCAGAGATGGGCTTAGGTG 3’), *Hs_PTCH1* (For: 5’

GCCGCGTTAATCCCAATTCC 3’; Rev: 5’ GCAGGGGCTTGTAAAACAGG 3’),
Hs_PTC2 (For: 5' CGCCGCCAGAGGTGATAC 3'; Rev: 5' CCACGGTCATGGAGGTAGTC 3'), Hs_DHH (For: 5' GTGCCGCTACTCTACAAGCA 3'; Rev: 5' TACAACGCTCGGTGTCATCAGG 3'), Hs_IHH (For: 5' CTCCGTCAGTCCGAGCAC 3'; Rev: 5' ATGAGCACATCGCTGAAGGT 3'), Hs_SHH (For: 5' GTGAAAGCAGGCAAGGAAAGGA 3'; Rev: 5' AAACTCTTGCTCCGTCACAC 3') and Hs_GAPDH (For: 5' AACGACCCCTTCATTGAC 3'; Rev: 5' TCCACGACATACTCAGC 3') was used as internal control.
Supplementary Figure 1. Wang et al.

A

Distal alveoli  Overlay  Proximal airway

Shh  Shh SCGB1A1

Distinct alveoli  Overlay

Shh  Shh SFTPC

B

Distal alveoli  Overlay  Proximal airway

Southern blot confirmation of BmtI fragment

C

Gli2creERT2-tdT/+;R26RYFP

Distal alveoli  Proximal airway

D

Hematopoietic  Endothelial  Epithelial

Gli2-tdTomato

Gli2-tdTomato

E

Pitch1LacZ+/+

Distal alveoli  Proximal airway
Supplemental Figure 2. Wang et al.

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: 5,615
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

C3-Mesothelium

C4-Airway Smooth Muscle

C1
C2
C3
C4

Exp. Level
Low
High

Gli2creERT2:tdT/+;R26RLacZ+

Gli2creERT2:tdT/+;R26RYFP+

Msln YFP

Mesothelium

X-gal ACTA2

Proximal airway

ASM

C1-Proximal fibroblasts

C2-Distal fibroblasts

Exp. Level
Low
High

C1
C2

Col1a1

Pdgfra

Tcf21

Fbln2

Thy1

Npnt
Supplemental Figure 3. Wang et al.

A

p = 4.8e-12
FDR = 9.6e-12

C1 Proximal genes
C2 Distal genes

p = 5.7e-10
FDR = 7.6e-10

B

Cluster 1 (proximal) signature genes

Nkx6-1
Fst
Fogx1
Ebf1

Flow-sorted distal GLI2+/ITGA8+ cells
Supplemental Figure 4. Wang et al.

A

FEV1 % predicted vs. Log2 Gene Expression for SHH, IHH, and DHH.

SHH: $p = 3.09 \times 10^{-8}$

IHH: $p = 0.60$

DHH: $p = 0.87$

B

Relative expression of DHH, IHH, and SHH in human and mouse lung epithelium.

C

Image showing % Shh+ cells in distal alveoli in control and CS conditions.

Control vs. CS comparison with % Shh+ cells in distal alveoli.
Supplemental Figure 5. Wang et al.

A

Control

Hh-activated (Prox. + Dist.)

Gli2\textsuperscript{creERT2-tdT/+:R26R\textsuperscript{YFP/+}}

Gli2\textsuperscript{creERT2-tdT/+:R26R\textsuperscript{YFP/SmoM2}}

B

Gli2\textsuperscript{creERT2-tdT/+:R26R\textsuperscript{YFP/+}}

Pdgfra\textsuperscript{creERT2/+:R26R\textsuperscript{YFP/+}}

Gli1\textsuperscript{creERT2/+:R26R\textsuperscript{YFP/+}}

Proximal

Distal

airway

alveoli

YFP

Proximal

Distal

airway

alveoli

YFP

Proximal

Distal

airway

alveoli

YFP

Proximal

Distal

airway

alveoli

YFP
A

Supplemental Figure 6. Wang et al.

Gli2creERT2-tdT/+
R26RYFP/+  
Gli2creERT2-tdT/+
R26RYFP/SmoM2

% immune cells / total cells

Gli2creERT2-tdT/+
R26RYFP/+  
Gli2creERT2-tdT/+
R26RYFP/SmoM2

% macrophage / immune cells

Gli2creERT2-tdT/+
R26RYFP/+  
Gli2creERT2-tdT/+
R26RYFP/SmoM2

% eosinophils / immune cells

Gli2creERT2-tdT/+
R26RYFP/+  
Gli2creERT2-tdT/+
R26RYFP/SmoM2

% neutrophils / immune cells

Gli2creERT2-tdT/+
R26RYFP/+  
Gli2creERT2-tdT/+
R26RYFP/SmoM2

% monocyttes / immune cells

Gli2creERT2-tdT/+
R26RYFP/+  
Gli2creERT2-tdT/+
R26RYFP/SmoM2

% B cells / immune cells

Gli2creERT2-tdT/+
R26RYFP/+  
Gli2creERT2-tdT/+
R26RYFP/SmoM2

% T cells / immune cells

Gli2creERT2-tdT/+
R26RYFP/+  
Gli2creERT2-tdT/+
R26RYFP/SmoM2

% NK cells / immune cells

B

Gli2creERT2-tdT/+
R26RYFP/+  
Gli2creERT2-tdT/+
R26RYFP/SmoM2

Number of cleaved caspase 3

positive cells / 1000 cells
Supplemental Figure 7. Wang et al.

**A**

- **Wnt1**
- **Wnt2**
- **Wnt3a**
- **Wnt7b**

Relative expression:

- Vehicle: 1.0
- 4OHT: 0.5
- Vehicle: 0.5
- 4OHT: 0

**B**

*Stpc*creERT2:*R26R<sub>TmG</sub> (Distal Alveolar Stem/Progenitor)

- Vehicle
- 4OHT
- 4OHT + 20 nM CHIR

*Ubc*creERT2:*R26R<sub>SmoM2</sub> mesenchyme

Colony forming efficiency

- Vehicle: 4
- 4OHT: 2
- 4OHT + 20 nM CHIR: 1

Diameter (μm)

- Vehicle: 20
- 4OHT: 15
- 4OHT + 20 nM CHIR: 10
**Experimental Outline**

Human lung

Harvest proximal lung (enriched for airways)

Digest into single cell suspension, FACSort mesenchyme

ScRNA-Seq (Single Cell 3' v2)

Combine proximal and distal datasets for analysis

Harvest distal lung (enriched for alveoli)

Digest into single cell suspension, FACSort mesenchyme

ScRNA-Seq (Single Cell 3' v2)

**Supplemental Figure 8. Wang et al.**

**Proximal fragment**

- Cells
- Background

**Distal fragment**

- Cells
- Background

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<tr>
<th>Parameter</th>
<th>Proximal</th>
<th>Distal</th>
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<tbody>
<tr>
<td>Estimated Number of Cells</td>
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<td>Fraction Reads in Cells</td>
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<tr>
<td>Mean Reads per Cell</td>
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<td>Median Genes per Cell</td>
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<td>Total Genes Detected</td>
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<td>Median UMIs per Cell</td>
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<td>Reads Mapped to Antisense to Sense</td>
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<td>Sequencing Sensitivity</td>
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**THY1-PE (Proximal)**

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<td>ITGA8</td>
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<tr>
<td>ITGA8</td>
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**GO Term: “Cholesterol metabolism”**

Fold Enrichment: 14.9  p = 1.7e-4