Supplemental Information Inventory

1. Supplemental Figures and Legends

- Figure S1 (Related to Figure 1). Expression pattern of Hdac3 during lung development and assessment of cell proliferation and survival in the *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lungs.
- Figure S2 (Related to Figure 2). Examination of cell differentiation marker gene expression in *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lungs.
- Figure S3 (Related to Figure 3). qPCR analysis on isolated primary epithelial cells and the miRNA expression in the mesenchymal cells of *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lungs.
- Figure S4 (Related to Figure 4). qPCR analysis on the Tgf-β pathway in the mesenchyme of *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lungs and the effect of SB431542 and Tgf-β on p-Smad2 expression.
- Figure S5 (Related to Figure 5). Over-expression of miR-17-92 by the *R26*$^{MIR17-92}$ allele does not affect epithelial progenitor marker expression.
- Figure S6 (Related to Figure 6). Loss of epithelial miR-17-92 alleviates the defects of lung sacculation in the *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lungs.

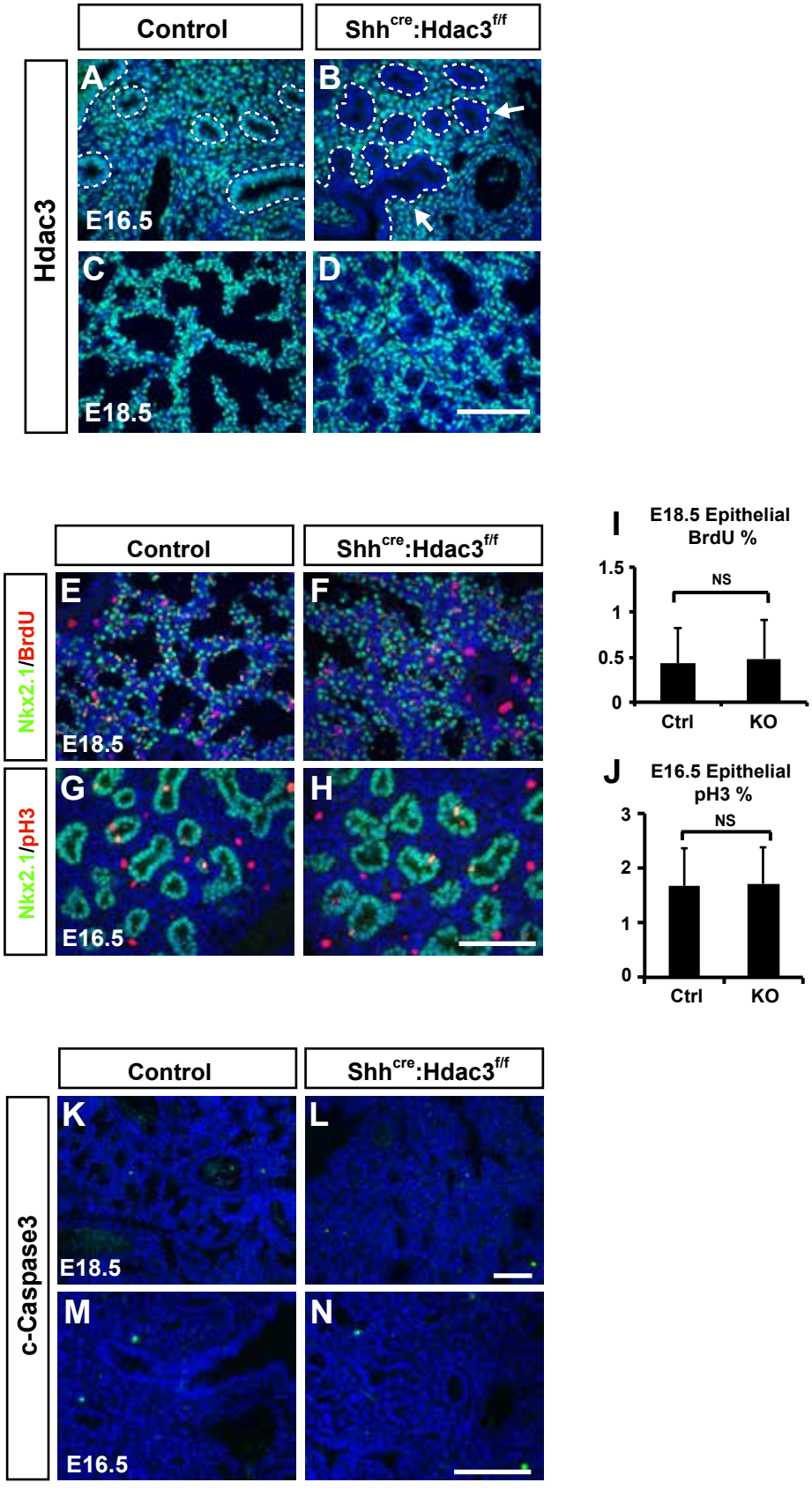
2. Supplemental Movies

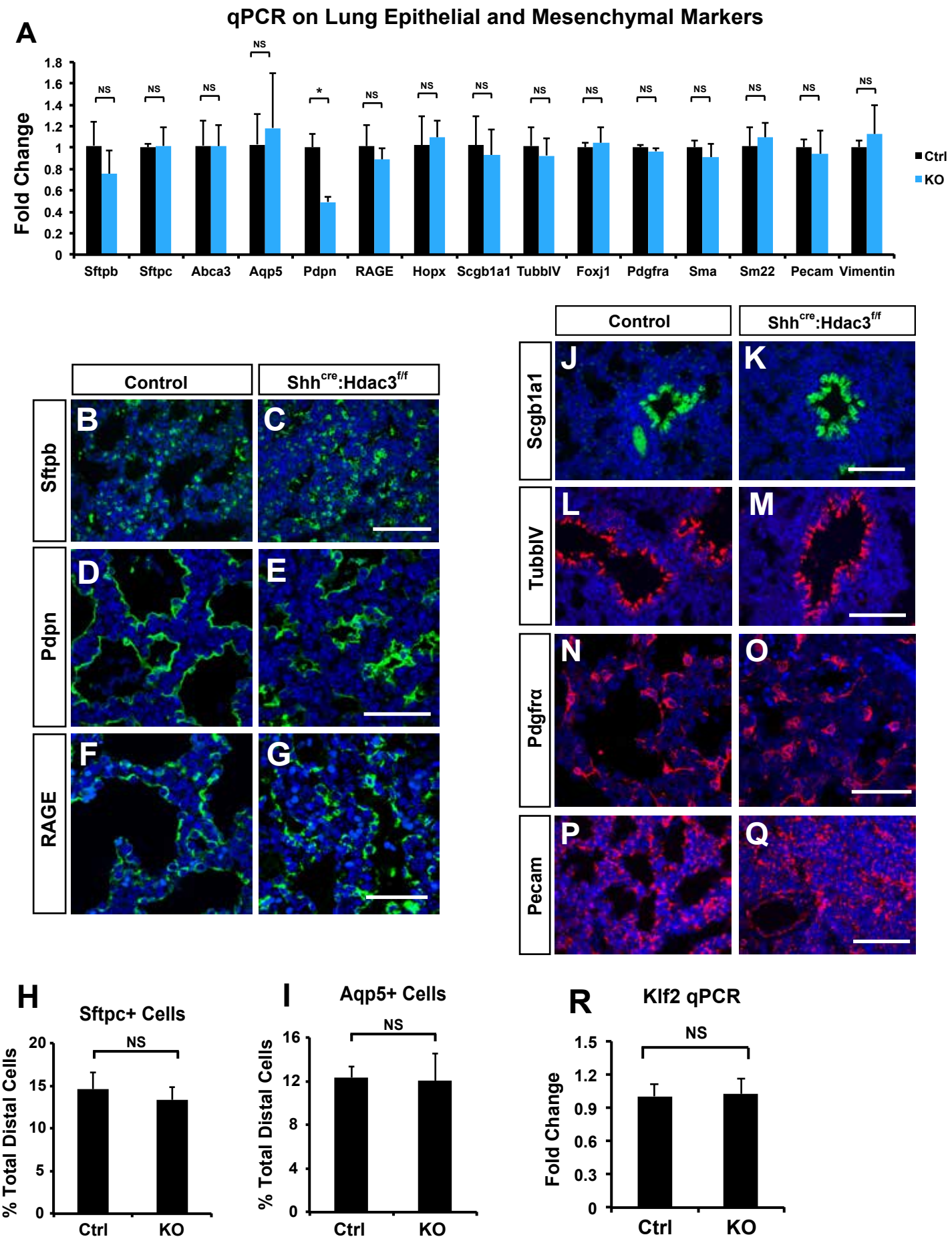
- Movie S1 (Related to Figure 1). 3D reconstruction of Aqp5 whole mount staining showing the distal saccular region of an E18.5 control lung.
- Movie S2 (Related to Figure 1). 3D reconstruction of Aqp5 whole mount staining showing the distal saccular region of an E18.5 *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lung.

3. Supplemental Tables

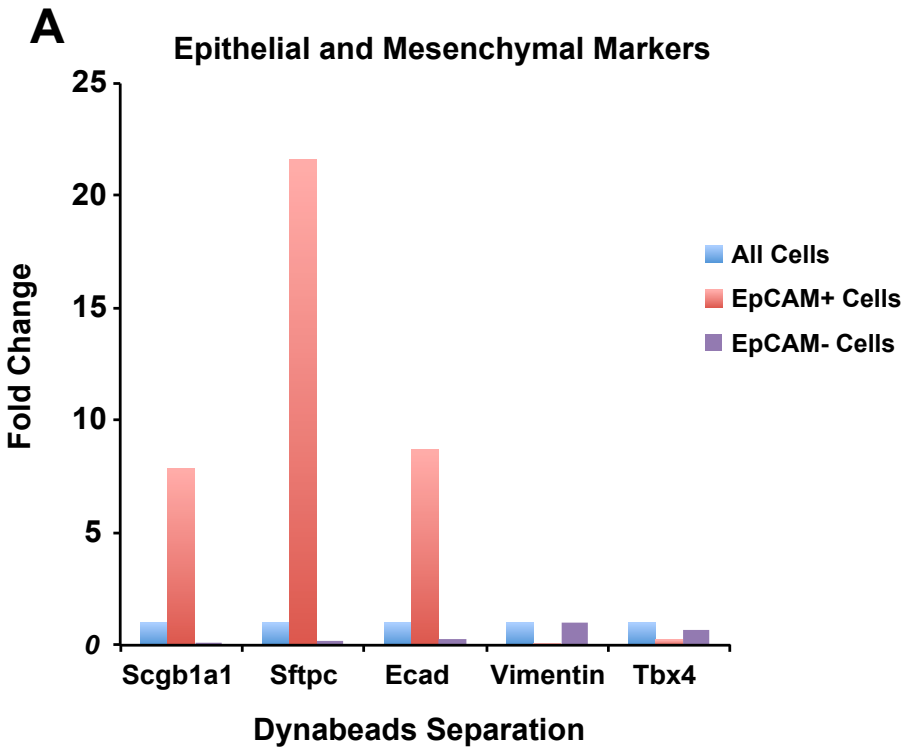
- Table S1 (Related to Figure 3). A list of all the differentially expressed genes in the microarray comparing control lungs and *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lungs at E18.5.
- Table S2 (Related to Figure 3). Result of SPIA pathway analysis on the common predicated targets of miR-17-92 and Dlk1-Dio3 miRNAs that were differentially expressed in the microarray data.
- Table S3 (Related to Figure 3). Result of SPIA pathway analysis on the predicated targets of all the differentially up-regulated miRNAs in the microarray data.
- Table S4 (Related to Figure 4) Result of SPIA pathway analysis on all the differentially down-regulated genes in the microarray data.

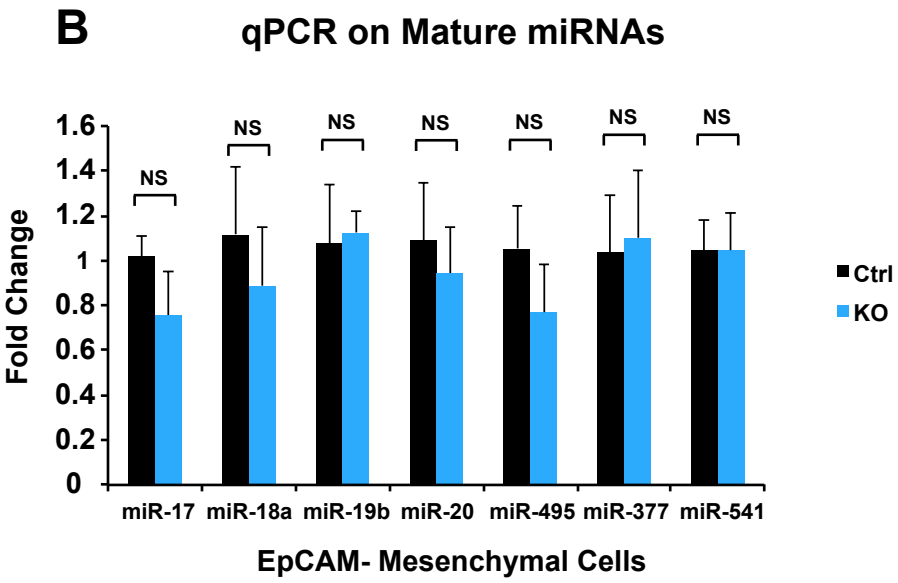
4. Supplemental Experimental Procedure

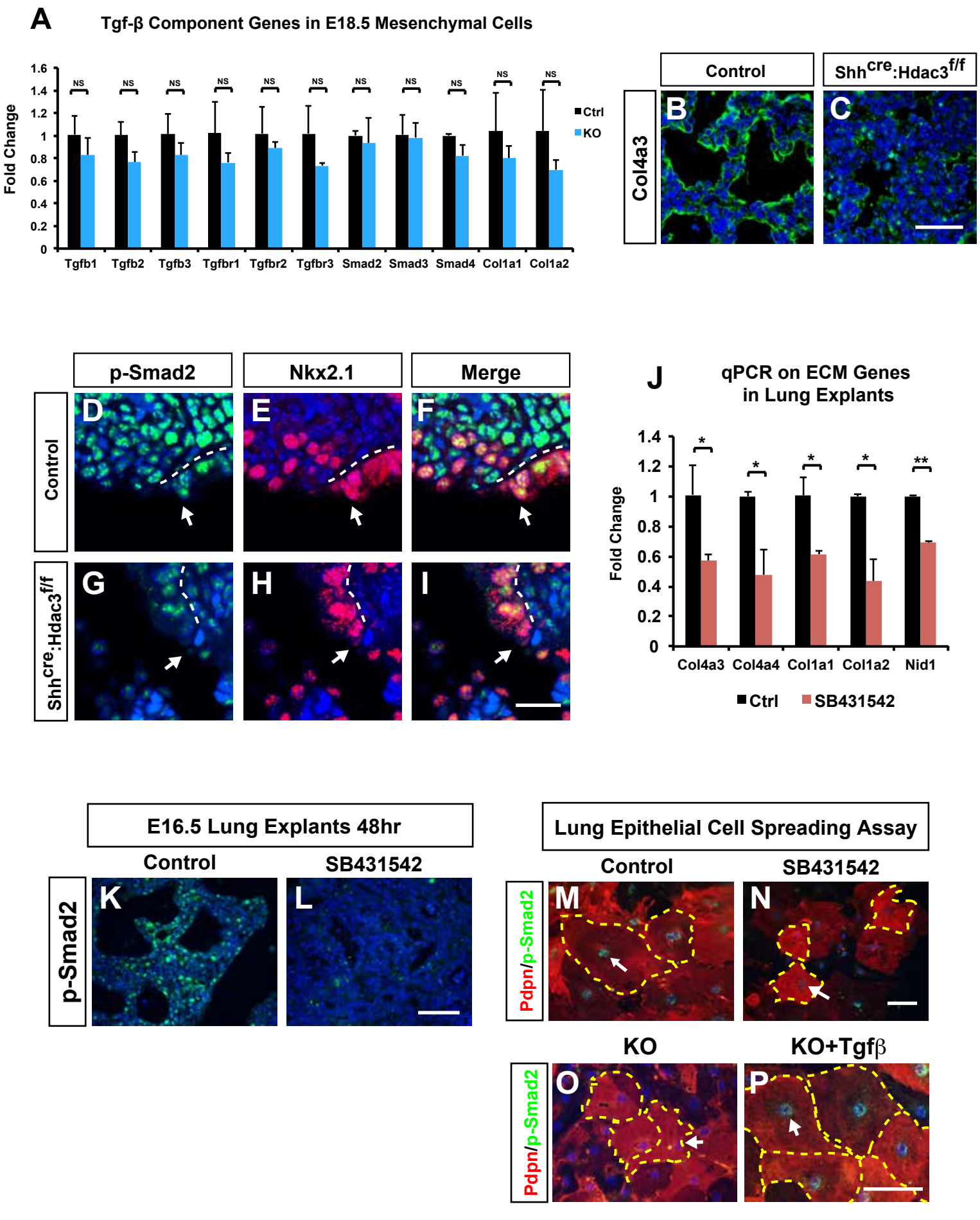

Control
Shh^cre:Hdac3^f/f
Hdac3
A
B
E16.5
C
D
E18.5
Control
Shh^cre:Hdac3^f/f
Nkx2.1/BrdU
E
F
E18.5
Nkx2.1/pH3
G
H
E16.5
I
E18.5 Epithelial BrdU %
1.5
1
0.5
0
NS
Ctrl
KO
J
E16.5 Epithelial pH3 %
3
2
1
0
NS
Ctrl
KO
Control
Shh^cre:Hdac3^f/f
c-Caspase3
K
L
E18.5
M
N
E16.5

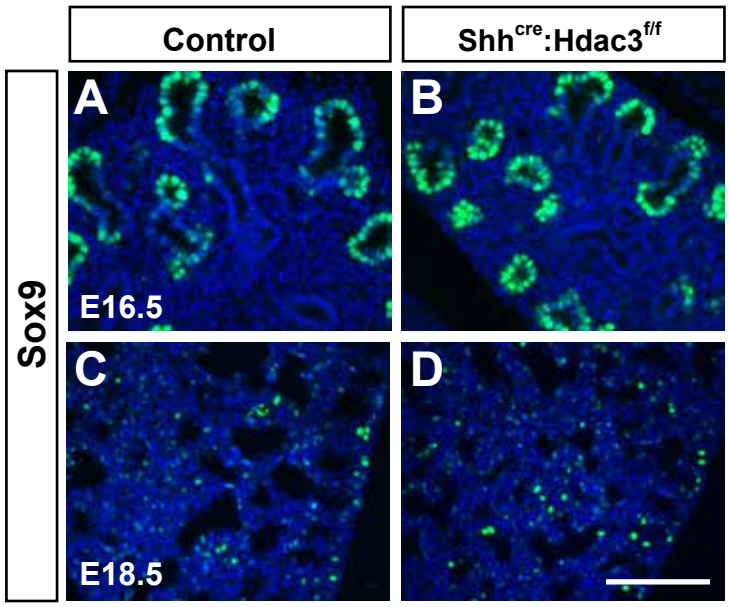

A
qPCR on Lung Epithelial and Mesenchymal Markers
Fold Change
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
NS
NS
NS
NS
*
NS
NS
NS
NS
NS
NS
NS
NS
NS
NS
Sftpb
Sftpc
Abca3
Aqp5
Pdpn
RAGE
Hopx
Scgb1a1
TubbIV
Foxj1
Pdgfra
Sma
Sm22
Pecam
Vimentin
Ctrl
KO
Control
Shh^cre:Hdac3^f/f
Sftpb
B
C
Pdpn
D
E
RAGE
F
G
Control
Shh^cre:Hdac3^f/f
Scgb1a1
J
K
TubbIV
L
M
Pdgfrα
N
O
Pecam
P
Q
H
Sftpc+ Cells
% Total Distal Cells
20
15
10
5
0
NS
Ctrl
KO
I
Aqp5+ Cells
% Total Distal Cells
16
12
8
4
0
NS
Ctrl
KO
R
Klf2 qPCR
Fold Change
1.5
1.2
0.9
0.6
0.3
0
NS
Ctrl
KO

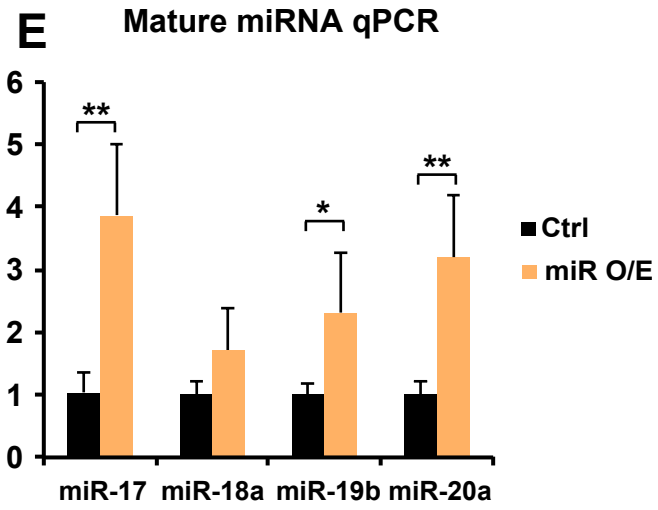
Supplemental Figure 3. Wang et. al.

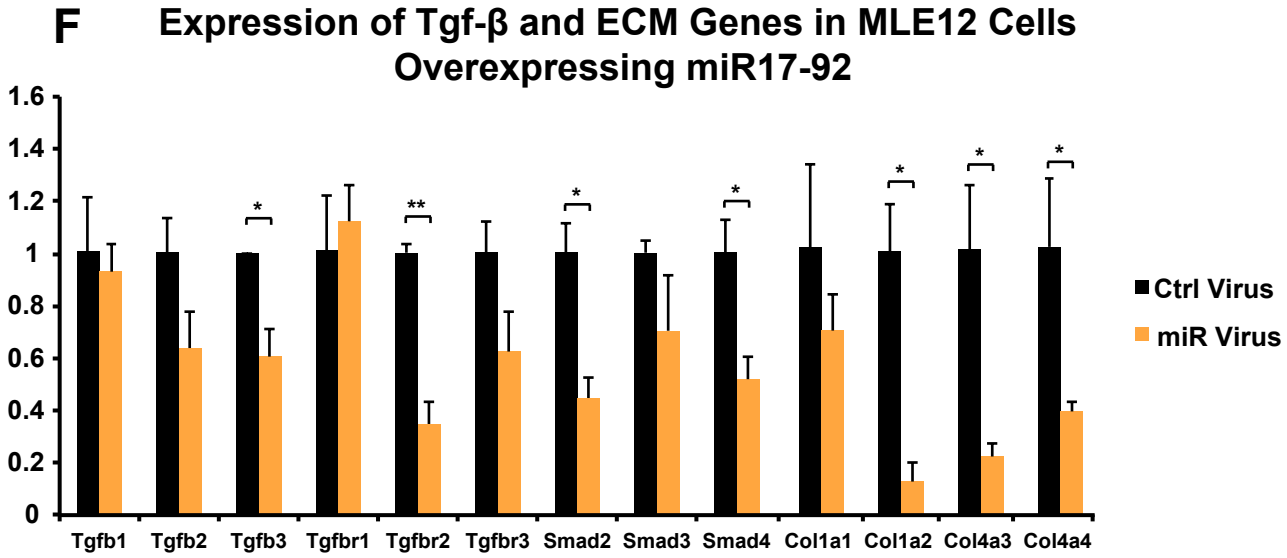


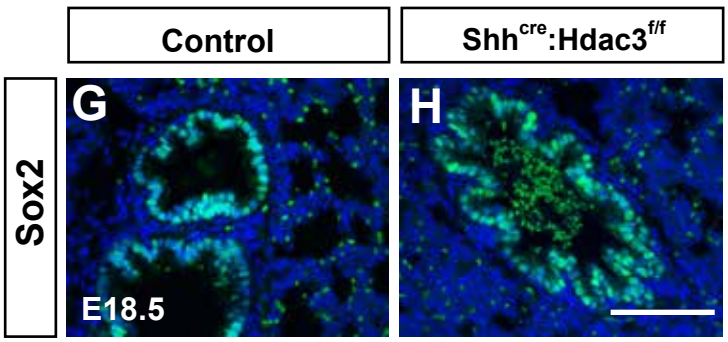


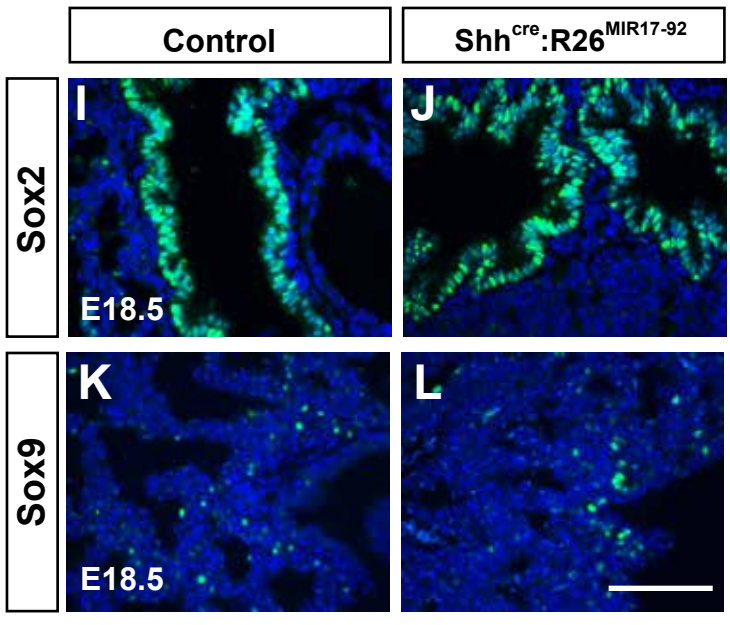

A
Tgf-β Component Genes in E18.5 Mesenchymal Cells
Fold Change
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
NS
Ctrl
KO
Tgfb1
Tgfb2
Tgfb3
Tgfbr1
Tgfbr2
Tgfbr3
Smad2
Smad3
Smad4
Col1a1
Col1a2
Control
Shh^cre:Hdac3^f/f
Col4a3
B
C
p-Smad2
Nkx2.1
Merge
Control
Shh^cre:Hdac3^f/f
D
E
F
G
H
I
J
qPCR on ECM Genes
in Lung Explants
Fold Change
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
**
Col4a3
Col4a4
Col1a1
Col1a2
Nid1
Ctrl
SB431542
E16.5 Lung Explants 48hr
Control
SB431542
p-Smad2
K
L
Lung Epithelial Cell Spreading Assay
Control
SB431542
Pdpn/p-Smad2
M
N
KO
KO+Tgfβ
Pdpn/p-Smad2
O
P


Control
Shh^{cre}:Hdac3$^{f/f}$
Sox9
A
B
C
D
E16.5
E18.5

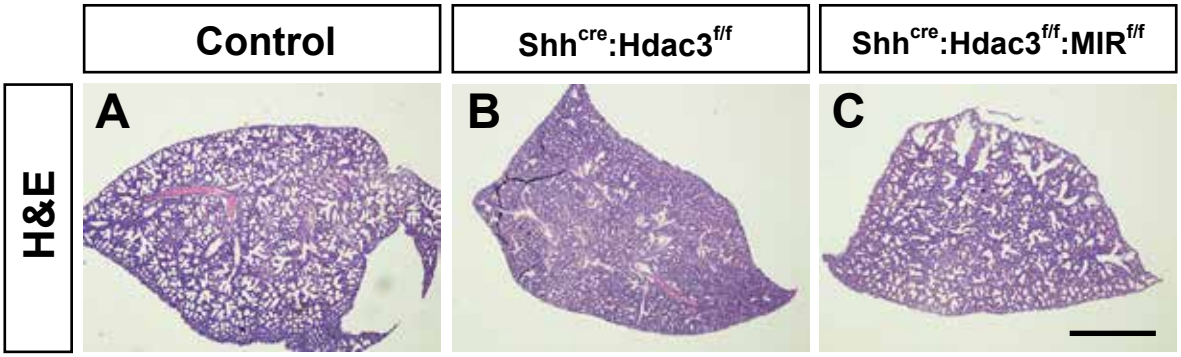

E
Mature miRNA qPCR
6
5
4
3
2
1
0
**
*
**
Ctrl
miR O/E
miR-17
miR-18a
miR-19b
miR-20a


F
Expression of Tgf-β and ECM Genes in MLE12 Cells
Overexpressing miR17-92
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Ctrl Virus
miR Virus
Tgfb1
Tgfb2
Tgfb3
Tgfbr1
Tgfbr2
Tgfbr3
Smad2
Smad3
Smad4
Col1a1
Col1a2
Col4a3
Col4a4


Control
Shh^{cre}:Hdac3$^{f/f}$
Sox2
G
H
E18.5


Control
Shh^{cre}:R26$^{MIR17-92}$
Sox2
I
J
E18.5
Sox9
K
L
E18.5

Supplemental Figure 6. Wang et. al.



SUPPLEMENTAL FIGURE LEGENDS

Figure S1 (Related to Figure 1). Expression pattern of Hdac3 during lung development and assessment of cell proliferation and survival in the *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lungs.

(A-D) Hdac3 is highly expressed in lung epithelium and mesenchyme at both E16.5 and E18.5 (A and C). Hdac3 is efficiently deleted in the epithelial cells of *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lungs (arrows). White dotted lines outline the airway epithelium.

(E-J) Assessment of cell proliferation by BrdU incorporation at E18.5 and phospho-histone H3 staining at E16.5 reveals no significant changes in epithelial cell proliferation in the *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lungs.

(K-N) Assessment of cell survival by cleaved-Caspase 3 staining shows no significant difference in cell apoptosis in the *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lungs.

Ctrl=Control; KO= *Shh*^{cre}:*Hdac3*$^{f/f}$. Two tail student's t test: NS=not significant. n=3. Quantification data are represented as mean ± SD. Scale Bars: 100μm.

Figure S2 (Related to Figure 2). Examination of cell differentiation marker gene expression in *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lungs.

(A) qPCR analyses on multiple proximal and distal airway epithelial differentiation markers and mesenchymal markers show no significant difference in the *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lungs, except Pdpn which is also expressed in the lymphatic endothelial cells and shows a moderate decrease.

(B-I) Immunostaining of AT2 cell marker Sftpb (B and C), AT1 cell marker Pdpn and RAGE (D-G), club cell marker Scgb1a1 (J and K) and ciliated cell marker TubbIV (L and

M) shows no differences in major epithelial lineage commitment in the *Shh*^{cre}:*Hdac3*$^{f/f}$ lungs.

(H and I) Quantification of the AT2 (Sftpc+) and AT1 (Aqp5+) cell numbers in the distal lung shows no significant alterations of these two epithelial lineages in the *Shh*^{cre}:*Hdac3*$^{f/f}$ lungs.

(N-Q) Immunostaining of fibroblast marker Pdgfrα and vascular endothelial marker Pecam shows no differences in the mesenchymal cell lineage development in the *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lungs.

(R) qPCR analysis shows that Klf2 expression is not significantly changed in the *Shh*^{cre}:*Hdac3*$^{f/f}$ lungs.

Ctrl=Control; KO= *Shh*^{cre}:*Hdac3*$^{f/f}$. Two tail student's t test: *p<0.05; NS=not significant. n=3. qPCR data are represented as mean ± SD. Scale Bars: C, K, M and Q=100μm; E, G and O=50μm.

Figure S3 (Related to Figure 3). qPCR analysis on isolated primary epithelial cells and the miRNA expression in the mesenchymal cells of *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lungs.

(A) qPCR analysis on several epithelial and mesenchymal markers shows an enrichment of epithelial cell lineage and a depletion of mesenchymal cell lineage in the Dynabeads isolated E18.5 primary lung epithelial cells.

(B) qPCR analysis on mature miRNAs including miR-17-92 and miRNAs in the *Dlk1-Dio3* locus reveals no significant expression difference in the mesenchymal cells of

Shh^{cre}:*Hdac3*$^{f/f}$ mutant lungs. Ctrl=Control; KO= *Shh*^{cre}:*Hdac3*$^{f/f}$. Two tail student's t test: NS=not significant. n=3. qPCR data are represented as mean ± SD.

Figure S4 (Related to Figure 4). qPCR analysis on the Tgf-β pathway in the mesenchyme of *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lungs and the effect of SB431542 and Tgf-β on p-Smad2 expression.

(A) qPCR analysis on multiple components of Tgf-β pathway shows no significant differences in their expression levels in the mesenchyme of *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lungs.

(B and C) Immunostaining shows that Col4a3 is significantly down-regulated in the distal region of the *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lungs.

(D-I) Co-staining of p-Smad2 and Nkx2.1 shows that p-Smad2 level is decreased at the bronchoalveolar duct junctions *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lungs.

(J) Lung explants treated with SB431542 shows reduced expression of several cell-ECM genes.

(K-N) p-Smad2 level is decreased after SB431542 treatment in the lung explant culture and epithelial cell culture.

(O-P) p-Smad2 level is increased after treatment of Tgf-β ligands in the *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lung epithelial culture.

Ctrl=Control; KO= *Shh*^{cre}:*Hdac3*$^{f/f}$. Two tail student's t test: NS=not significant. n=3. qPCR data are represented as mean ± SD. Scale bars: I=20μm; C and L=50μm; N and P=100μm.

Figure S5 (Related to Figure 5). Over-expression of miR-17-92 by the *R26*$^{MIR17-92}$ allele does not affect epithelial progenitor marker expression.

(A-D) Immunostaining for Sox9 expression shows that distal lung progenitor cells are not altered in *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lungs at E16.5 or E18.5.

(E) qPCR analysis shows that miR-17-92 miRNA expression levels are elevated in the *Shh*^{cre}:*R26*$^{MIR17-92}$ lung epithelial cells by a similar amount to those of *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lungs.

(F) qPCR analysis shows that MLE12 cells infected by lentivirus over-expressing miR-17-92 have a significant reduction in Tgf-β pathway components and genes involved in cell-ECM interaction.

(G and H) Proximal epithelial progenitor marker Sox2 expression is not changed in the *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lungs.

(I-L) Sox2 and Sox9 expressions are not changed in the *Shh*^{cre}:*R26*$^{MIR17-92}$ lungs.

Ctrl=Control; miR O/E= *Shh*^{cre}:*R26*$^{MIR17-92}$. Two tail student's t test: *p<0.05; **p<0.01; n=3. qPCR data are represented as mean ± SD. Scale Bars: 100μm.

Figure S6 (Related to Figure 6). Loss of epithelial miR-17-92 alleviates the defects of lung sacculation in the *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lungs.

(A-C) Low magnification of H&E staining shows an overall improvement of lung sacculation defects in *Shh*^{cre}:*Hdac3*$^{f/f}$:MIR17-92$^{f/f}$ lungs compared to *Shh*^{cre}:*Hdac3*$^{f/f}$ lungs.

Scale bar=2mm.

Table S1 (Related to Figure 3). A list of all the differentially expressed genes in the microarray comparing control lungs and *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lungs at E18.5.

Table S2 (Related to Figure 3). Result of SPIA pathway analysis on the common predicated targets of miR-17-92 and Dlk1-Dio3 miRNAs that were differentially expressed in the microarray data.

Table S3 (Related to Figure 3). Result of SPIA pathway analysis on the predicated targets of all the differentially up-regulated miRNAs in the microarray data.

Table S4 (Related to Figure 4) Result of SPIA pathway analysis on all the differentially down-regulated genes in the microarray data.

SUPPLEMENTAL MOVIES

Movie S1 (Related to Figure 1). 3D reconstruction of Aqp5 whole mount staining showing the distal saccular region of an E18.5 control lung.

Movie S2 (Related to Figure 1). 3D reconstruction of Aqp5 whole mount staining showing the distal saccular region of an E18.5 *Shh*^{cre}:*Hdac3*$^{f/f}$ mutant lung.

SUPPLEMENTAL EXPERIMENTAL PROCEDURE

We used the following primers to perform qPCR analysis in our studies (sequences listed from 5' to 3'):

Gene	Forward	Reverse
Gapdh	AAATGGTGAAGGTCGGTGTGAACG	ATCTCCACTTTGCCACTGC
Scgb1a1	ATACCCTCCCACAAGAGACCAGGATA	ACACAGGGCAGTGACAAGGCTTTA
Foxj1	AGTGGATCACGGACAACTTCTGCT	TTCTCCCGAGGCACTTTGATGAAG
TubbIV	AACCCGGCACCATGGACTCTGT	TGCCTGCTCCGGATTGACCAAATA
Aqp5	ATGAACCCAGCCCGATCTTT	ACGATCGGTCCTACCCAGAAG
Pdpn	AGGTACAGGAGACGGCATGGT	CCAGAGGTGCCTTGCCAGTA
Sftpc	ACCCTGTGTGGAGAGCTACCA	TTTGCGGAGGGTCTTTCCT
Sftpb	TAGCCCTCTGCAGTGCTTCCAAA	AGCTGGGACATACAGACTGACACA
Abca3	TTCATCACCTGATGGCGGTGAAC	ACGCATGATGGCTTTGTCTACAGC
Tbx4	AAGGTGGGGCTGCATGAGAAGGA	GAACATCCTCCTGCCGGCCTT
Vimentin	TGACCTTGAACGGAAAGTGG	GGACATGCTGTTCCTGAATCT
Ecad	CCGTCCTGCCAATCCTGATGA	ACTGCCCTCGTAATCGAACACCAA
Tgfb1	CTGAACCAAGGAGACGGAATAC	GGGCTGATCCCGTTGATTT
Tgfb2	GTACCTTCGTGCCGTCTAATAA	GTGCCATCAATACCTGCAAATC
Tgfb3	CGCTACATAGGTGGCAAGAA	CAAGTTGGACTCTCTCCTCAAC
Tgfbr1	GTTCCGAGAGGCAGAGATTTAT	CGTCCATGTCCCATTGTCTT
Tgfbr2	GCTTCTCCCAAGTGTGTCAT	GACTGCTGGTGGTGTATTCTT
Tgfbr3	CTGGTGTGGCATGTGAAGA	TCCTCTGTTTCTGCTGTCAAG
Smad2	GATGACTACACCCACTCCATTC	ATATCCAGGTGGTGGTGTTTC
Smad3	CCTGAGTGAAGATGGAGAAACC	CTGGCTGTAGGTCCAAGTTATT
Smad4	GCAGAGTAATGCTCCAAGTATGT	CGAATGTCCTTCAGTGGGTAAG
Col1a1	TGACCGATGGATTCCCGTTC	GCAGTGATAGGTGATGTTCTGG
Col1a2	GATGTTGAACTTGTTGCTGAGG	CAATGATTGTCTTGCCCCATTC
Col4a3	CTCTCGAACCCTATATTAGCAGATG	ACGGAGGGATAGCAGTAGTT
Col4a4	CCCTGGATCAGATGTGATATACT	CTCCTTTGGCTCCTCTTCTT
Itga7	GGATTCCGAGGTGCGATTT	ACATCCAGATTGAAGGCGATAG
Itgb1	CTCCGGCCAGAAGACATTAC	GGGTAATCTTCAGCCCTCTTG
Nidogen 1	GCACTCCTATGTGGTGATGAA	ATCCGATGATGCCTCCAATG
Nidogen 2	CACCGAGGACAGTTTCCATTAC	TCAATTCTGCTGTGGCCTTC
Pdgfra	TGAGGGAGAGAAACAAACGGAGGA	AGCTCCTGAGACCTTCTCCTTCTA
SMA	ATTGTGCTGGACTCTGGAGATGGT	TGATGTCACGGACAATCTCACGCT
SM22	TCTAATGGCTTTGGGCAGTTTGGC	TTTGAAGGCCAATGACGTGCTTCC
CD31	ATTCCTCAGGCTCGGGTCTT	CCATGCACCTTCACCTCGTA

We used the following primers for ChIP assays in our studies:

Gene	Forward	Reverse
miR17-92	GGAGGTCGGAAGTACTTTGTTT	AAGGACCATGTGGGTGAATG
Mirg	GGTGCACAGGTTGTATCTTCT	CCGATTCTGACGGACTATCTTG
Intergenic Region	TGGCGGGTTTATGCTAAGTGAGGA	ACAGGTGTTGGAGGAAACTGTCCA