Hedgehog actively maintains adult lung quiescence and regulates repair and regeneration

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Postnatal tissue quiescence is thought to be a default state in the absence of a proliferative stimulus such as injury. Although previous studies have demonstrated that certain embryonic developmental programs are reactivated aberrantly in adult organs to drive repair and regeneration1–3, it is not well understood how quiescence is maintained in organs such as the lung, which displays a remarkably low level of cellular turnover4,5. Here we demonstrate that quiescence in the adult lung is an actively maintained state and is regulated by hedgehog signalling. Epithelial-specific deletion of sonic hedgehog (Shh) during postnatal homeostasis in the murine lung results in a proliferative expansion of the adjacent lung mesenchyme. Hedgehog signalling is initially downregulated during the acute phase of epithelial injury as the mesenchyme proliferates in response, but returns to baseline during injury resolution as quiescence is restored. Activation of hedgehog during acute epithelial injury attenuates the proliferative expansion of the lung mesenchyme, whereas inactivation of hedgehog signalling prevents the restoration of quiescence during injury resolution. Finally, we show that hedgehog also regulates epithelial quiescence and regeneration in response to injury via a mesenchymal feedback mechanism. These results demonstrate that epithelial–mesenchymal interactions coordinated by hedgehog actively maintain postnatal tissue homeostasis, and deregulation of hedgehog during injury leads to aberrant repair and regeneration in the lung.

The Hedgehog (Hh) pathway coordinates tissue–tissue interactions in multiple organs during embryonic development through paracrine activation of smoothened (Smo)-mediated downstream signalling events6–9. We have previously demonstrated that Shh expressed by nascent lung endoderm progenitors coordinates cardiopulmonary mesoderm progenitor differentiation into various cardiac and lung mesenchymal cell lineages8. To determine whether Hh signalling continues to be active in the postnatal adult lung, we used the ShhcreGFP reporter9 and our data show that Shh is expressed in the adult lung epithelium predominantly in the Sgb1a1+ club epithelial cells in the proximal airway (Fig. 1a), with scattered expression in ciliated epithelium (Extended Data Fig. 1a) and the Sftpc+ alveolar type II epithelial cells (Fig. 1b). The downstream transcriptional effector and target of hedgehog Gli1 (ref. 10), is expressed predominantly in mesenchymal cells adjacent to the proximal airway and pulmonary artery (Fig. 1c), with scattered expression in the alveolar interstitium as previously reported (Fig. 1d)11. Lineage tracing in the adult lung with Gli1creERT2:R26RmTmG animals12 showed that Gli1+ Hh-responsive cells express several mesenchymal markers including Pdgfra, Pdgfrb, vimentin, S100A4, and Col1a1 (Fig. 1e–h, Extended Data Fig. 1b, c). Gli1+ Hh-responsive mesenchymal cells do not contribute markedly to the smooth muscle lineage under homeostatic conditions, with the exception of rare venous smooth muscle within the proximal pulmonary venous myocardium (Extended Data Fig. 1d–i) and

Figure 1 | The lung epithelium signals to the adjacent mesenchyme via paracrine Hh signalling during normal homeostasis. a, b, The Shh ligand is expressed in airway epithelium marked by Sgb1a1 (a), with scattered expression in the Sftpc+ alveolar epithelium (b). c, d, The Gli1 LacZ reporter is expressed in the mesenchyme adjacent to the airway and pulmonary artery (c) with scattered activation in the alveolar interstitium (d). e–h, Lineage traced Gli1+ cells express Pdgfra+ (e), Pdgfrb+ (f), vimentin+ (g), and Coll1a1 (h). i–k, Lineage-traced Gli1+ cells do not expand in the adult lung after chase periods of 2 days (i), 4 weeks (j) and 12 weeks (k), with negligible expression of the cell cycle marker Ki67 (i–k, arrowheads). AW, airway; V, blood vessel. Scale bars, 100 μm. Images representative of 3 animals with 5 sections examined per animal.

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myofibroblasts during fibrotic injury (Extended Data Fig. 1j). Gli1

knockout; blue represents DAPI counterstaining. Scale bars, 100 μm;

Extended Data Fig. 3a–i). Adult Pdgfrb(S)K

*P < 0.05. Data represent n = 3 animals per group with 5 sections analysed per

animal. In vitro lung mesenchyme studies represent technical triplicates,

with BrdU assay representative of three separate experiments. One-sided
t-test used to determine statistical significance with centre value representing
the mean and error bar representing s.d.

Figure 2 | Postnatal activation of Hh signalling is required to maintain
lung mesenchymal quiescence. a–d, m. Deletion of Shh from airway

epithelium increases proliferation in the mesenchyme surrounding the

airway. e–h, n. Deletion of Smo within Gli1

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Gli1\(^+\) Hh-responsive mesenchyme (Fig. 2q–s). Activation of Hh signalling in isolated lung mesenchymal cells in vitro (derived from UBC\(^{Gli1\creERT2:R26RmTmG}\) animals) attenuates the proliferation induced by exogenous Pdgf-BB (platelet-derived growth factor-BB) (Extended Data Fig. 4). Next, we assessed the expression of Hh signalling components during airway epithelial injury with naphthalene\(^{18}\). Acute naphthalene injury caused a reduction in Hh activation as assessed by decreased Gli1\(^{LacZ}\) reporter activity in the mesenchyme surrounding the airway, reduced expression of Shh and Gli1 transcripts, and decreased expression of GFP in the Shh\(^{CreGFP}\) reporter (Fig. 3a–g, Extended Data Figs 5 and 6). Chronic repetitive bleomycin caused a similar reduction in Hh activation following injury (Extended Data Figs 5 and 6). Thus, Hh signalling is downregulated in response to epithelial injury in the lung, and is not upregulated as has been previously reported\(^{19,20}\). Of note, these results correlate with the loss of Shh-expressing epithelium after injury.

To assess the behaviour of Gli1\(^+\) lung cells after epithelial injury, we exposed Gli1\(^{CreERT2:R26RmTmG}\) adult animals to tamoxifen followed by a one-week washout period before inducing lung epithelial injury with naphthalene. Hh-activated Gli1\(^+\) lung cells rapidly undergo proliferative expansion after naphthalene injury (Fig. 3h–i). Utilizing the Gli1\(^{CreERT2:R26RmTmG}\) mice for stochastic multicolour clonal analysis, we demonstrate that individual Gli1\(^+\) cells clonally expand after naphthalene injury (Fig. 3m–q). Reconstitution of Hh activation with SmoM2 during acute epithelial injury attenuates the normal expansion of mesenchyme following injury (Fig. 3r–v). In the bleomycin injury model, Hh signalling is also downregulated within Gli1\(^+\) mesenchymal cells after injury, which is similarly attenuated by the expression of activated SmoM2 (Extended Data Fig. 6).

Despite an initial reduction in Hh activation during naphthalene injury, Shh and Gli1 expression return to homeostatic levels three months following injury (Fig. 4a–e) as the Shh-expressing bronchial epithelium is reconstituted (Extended Data Fig. 7a–d). Mesenchymal

**Figure 3** Hh signalling modulates the acute mesenchymal response to epithelial injury. a–f, Naphthalene injury downregulates Gli1\(^{lox/lox}\) expression (n = 2 animals per group) and g, Shh and Gli1 expression noted by quantitative PCR (n = 3 animals per group). NS, not significant. h–l, Gli1\(^+\) lung cells undergo proliferative expansion shortly after naphthalene injury as measured by Ki67\(^+\) expression. m–q, Clonal analysis of Gli1\(^+\) lung cells at single cell resolution demonstrates clonal expansion three days after injury.
Hh signalling modulates restoration of quiescence during injury resolution in the lung. a–c, Hh activation returns to homeostatic levels three months after naphthalene injury in Gli1lacZ lungs (n = 3 animals per group, 5 sections analysed per animal). d, e, Shh and Gli1 expression returns to normal as noted by qPCR (n = 3 animals per group). f–m, Gli1^+ lung cells undergo proliferative expansion shortly after naphthalene injury but return to quiescence by 2–3 months after injury. n–q, s. Conditional deletion of Smo in lineage-traced Gli1^+ lung cells prevents the restoration of mesenchymal quiescence. t. Relationship between Hh expression and activation and lung mesenchymal proliferation as plotted over time during injury and resolution. AW, airway; V, blood vessel. Scale bars, 100 µm; *P < 0.05. Data represent n = 3 animals per group with 5 sections analysed per animal unless otherwise noted. Error bars, mean ± s.d.

Figure 4. Hh signalling modulates restoration of quiescence during injury resolution in the lung. a–c, Hh activation returns to homeostatic levels three months after naphthalene injury in Gli1lacZ lungs (n = 3 animals per group, 5 sections analysed per animal) and d, e. Shh and Gli1 expression returns to normal as noted by qPCR (n = 3 animals per group). f–m, Gli1^+ lung cells undergo proliferative expansion shortly after naphthalene injury but return to quiescence by 2–3 months after injury. n–q, s. Conditional deletion of Smo in lineage-traced Gli1^+ lung cells prevents the restoration of mesenchymal quiescence. t. Relationship between Hh expression and activation and lung mesenchymal proliferation as plotted over time during injury and resolution. AW, airway; V, blood vessel. Scale bars, 100 µm; *P < 0.05. Data represent n = 3 animals per group with 5 sections analysed per animal unless otherwise noted. Error bars, mean ± s.d.
Figure 5 | Hh signalling regulates epithelial quiescence via mesenchymal feedback. a–f. Deletion of Shh from the proximal secretory epithelium and deletion of Smo within cells derived from Pdgfrα+ mesenchyme increase proliferation of Scgb1a1+ club cells during homeostasis, g–i. Inducible deletion of Smo within Gli1+ lung mesenchymal cells results in a non-significant trend towards increased Scgb1a1+ club cell proliferation. j–m. Activation of Smo2 in Gli1+ mesenchyme results in impaired regeneration of Scgb1a1+ cells after naphthalene injury, whereas deletion of Smo in the mesenchyme induces excessive expansion of Scgb1a1+ cells resulting in bronchial hyperplasia. AW, airspace. Scale bars, 100 μm; *P < 0.05. Data represent n = 3 animals per group with 5 sections analysed per animal. Error bars, mean ± s.d.

size of the epithelial colonies (Extended Data Fig. 7i–n). These data show that Hh promotes epithelial quiescence via a mesenchymal feedback mechanism, possibly by downregulating stromal factors necessary for epithelial proliferation.

In this study, we have demonstrated that the lung epithelium actively maintains mesenchymal quiescence through paracrine Hh signalling, which also regulates a feedback loop to maintain epithelial quiescence. This finding stands in contrast to the known role of Shh in promoting cell proliferation during tissue development as well as its role in promoting tumorigenesis in adults. While previous reports have suggested that Hh signalling is pro-mitogenic in the adult lung11,19,20, our study is the first report to utilize multiple genetic models to assess Hh function in the adult lung in vivo and in vitro. Our data indicate that certain signalling pathways such as Hh maintain a balance between proliferation and quiescence during lung homeostasis and regeneration. Our studies reveal that disruption of this balance upon injury can lead to changes in expansion of the mesenchyme, which may disrupt epithelial regeneration after injury or in disease (Extended Data Fig. 8).

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Author Contributions T.P. and E.E.M. designed the overall experimental strategy. T.P. and R.S.K. performed lineage tracing and animal injury experiments. T.P. and D.B.F. performed in vitro BrdU and organoid experiments. T.W. performed right heart catherization on the animals. S.Z.C. and M.M.L. performed histology and immunohistochemistry. T.P., D.B.F., K.S.R., M.P.M. and E.E.M. analysed intravital data. T.P. and E.E.M. wrote and edited the manuscript with input from all authors.

Author Information The Gene Expression Omnibus accession number for the microarray data produced in these studies is GSE68201. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to E.E.M. (enorrisse@mail.med.upenn.edu) or T.P. (ben.peng@ucsf.edu).
METHODS

No statistical methods were used to predetermine sample size. Animals used were maintained in accordance with the institutional animal care committee guidelines at the University of Pennsylvania. Animals were housed and treated in accordance with the IACUC protocol approved by the University of Pennsylvania. Animals between the ages of 8–12 weeks old were used for the experiments with balance of gender between groups. Tamoxifen (Sigma) was dissolved in corn oil and administered intraperitoneally at 200 mg kg\(^{-1}\) per day \(\times 3\) days for lineage tracing studies, with the exception of clonal analysis studies with the R26R\(^{OP} \text{Cre}\) reporter, where only one dose of tamoxifen was given at 200 mg kg\(^{-1}\).

Histological analysis. Mouse lungs were inflated and fixed in 2% paraformaldehyde, dehydrated in a series of increasing ethanol concentration washes, embedded in paraffin and sectioned. Antibodies used were anti-sm22\(^{\alpha}\) (goat anti-SM22\(^{\alpha}\) 1:200 Abcam), S100A4 (rabbit anti-S100A4 1:200 Abcam). LacZ staining of lungs (mouse anti-PO4-Histone H3 1:200 Cell Signaling), TubbIV (mouse anti-TubbIV 1:200 Bioconductor), collagen type 1 (rabbit anti-Col1 1:50 Abcam), Ki67 (rabbit anti-Ki67 1:200 Abcam), vimentin (rabbit anti-vimentin 1:100 Cell Signaling), Pdgf-BB (mouse, R&D) was added and cultured for another 24 h. BrdU was then added to the media for 24 h of Pdgf-BB incubation and BrdU incorporation was assayed after four hours according to manufacturer instructions (Cell Signalling Technology, BrdU cell proliferation assay kit).

Bronchial organoid formation assay. GFP\(^{\text{bronchial}}\) epithelial cells were FACs sorted from Scgb1a1\(^{\text{R26R\text{GFP}}}\) lungs and co-cultured with lung mesenchyme isolated from UBC\(^{\text{CreERT2}}\); R26R\(^{GFP}\) mice and plated at 1 \(\times 10^{3}\) cells per well in 96 well plates and grown for 4 days with vehicle or 1 \(\mu\)g mL\(^{-1}\) of 4-OH-tamoxifen until cells became confluent. Cells were then incubated in serum-free DMEM F12 for 24 h before Pdgf-BB (mouse, R&D) was added and cultured for another 24 h. BrdU was then added to the media until 24 h of BrdU incorporation was assayed after four hours according to manufacturer instructions (Cell Signalling Technology, BrdU cell proliferation assay kit).

Microarray. Primary lung mesenchymal cells were isolated from UBC\(^{\text{CreERT2}}\); R26R\(^{GFP}\) adult mice and grown in DMEM-F12 plus 10% fetal calf serum. The cells were treated with vehicle or 1 \(\mu\)g mL\(^{-1}\) of 4-OH-tamoxifen in DMEM F12 without serum and total RNA was isolated after 48 h. Biotinylated cRNA probe libraries were generated from these RNA samples and assayed with the Affymetrix Mouse Gene 2.0ST genechip. Microarray data were analysed using the Oligo package available at the Bioconductor Website (http://www.bioconductor.org). The raw data were background-corrected by the robust multichip average (RMA) method and then normalized by an invariant set method. Genes with 85% correlation with an expression signal above the negative control probes were considered detectable or present. Differential gene expression analysis between control and mutant mice was analysed by the Limma package available at the Bioconductor Website. P values were adjusted for multiple comparison using a false discovery rate. GO enrichment analysis was performed using the Bioconductor package topGO. The Gene Expression Omnibus accession number for the microarray data produced in these studies is GSE68201.

Cell proliferation assay. Lung mesenchymal cells were isolated from UBC\(^{\text{CreERT2}}\); R26R\(^{GFP}\) mice and plated at 1 \(\times 10^{3}\) cells per well in 96 well plates and grown for 4 days with vehicle or 1 \(\mu\)g mL\(^{-1}\) of 4-OH-tamoxifen until cells became confluent. Cells were then incubated in serum-free DMEM F12 for 24 h before Pdgf-BB (mouse, R&D) and BrdU was added and cultured for another 24 h. BrdU was then added to the media until 24 h of BrdU incorporation was assayed after four hours according to manufacturer instructions (Cell Signalling Technology, BrdU cell proliferation assay kit).

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**Gli1creERT2::R26R{mTmG}**

**b**  
AW

**c**  
AW

**d**  
AW

**e**  
AW

**f**  
AW

**g**  
V

**i**  
PV

**j**  
Gli1creERT2::R26R{mTmG}

**k**

**Gli1creERT2::R26R{tdTomato}**

**GFP/TubbIV**

**bleomycin**

**4 weeks**

**Tm x3**

**Analyze**

**GFP/SMA**

**Q1**  
1.23%  
10^6

**Q2**  
0.335%  
10^6

**Q4**  
95.0%  
10^6

**Q3**  
0.00%  
10^6

**rat IgG2b Isotype PE-Cy7**

**CD45 PE-Cy7**

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Extended Data Figure 1 | Characterization of Hh signalling in the lung.  

a, A small number of TubbIV$^+$ ciliated cells express GFP in the ShhcreGFP reporter.  
b, c, Gli1$^+$ Hh activated cells co-label with S100A4, a fibroblast marker.  
d–g, Lineage tracing of Gli1$^+$ Hh activated cells shows little to no co-localization of GFP$^+$ cells with the airway smooth muscle (d, e) or the vascular smooth muscle of the adjacent pulmonary artery (f, g).  
h, i, The rare exception occurs in the pulmonary vein where Gli1$^+$ Hh activated cells contribute to the venous smooth muscle that is surrounded by the venous myocardium of the proximal pulmonary vein.  
j, Gli1$^+$ cells also generate myofibroblasts after fibrotic injury such as that induced by bleomycin.  
k, Lung Gli1$^+$ cells do not contribute to cells of haematopoietic lineage as marked by CD45.  
AW, airway; V, blood vessel; PV, pulmonary vein. Scale bars, 100 µm. Images representative of 3 animals with 5 sections examined per animal.
Extended Data Figure 2 | Conditional deletion of Shh from the adult airway epithelium increases proliferation in the adjacent mesenchyme. **a,** The Scgb1a1cre driver predominantly marks the airway epithelium in the adult lung when crossed with the R26RmTmG reporter. **b,** Whole-lung messenger RNA transcript analysis reveals efficient deletion of Shh transcripts in the Scgb1a1cre:Shhflox/flox animals compared to controls (Shhflox/flox) (**n** = 4 animals). **c–h,** Deletion of Shh from the airway epithelium resulted in an increased expression of proliferative markers, PCNA and phospho-histone H3 (PH3) in the mesenchyme surrounding the airways in Scgb1a1cre:Shhflox/flox mutants (d, f, h, **n** = 4 animals) versus controls (Scgb1a1cre:Shhflox/+). (c, e, g, **n** = 3 animals). AW, airway; V, blood vessel. Scale bars, 100 μm. *P < 0.05. Error bars, mean ± s.d.
Extended Data Figure 3 | Conditional deletion of Smo from Pdgfrb-derived mesenchyme increases mesenchymal proliferation at the epithelial-mesenchymal interface and vascular remodelling. a–d, Deletion of Smo from Pdgfrb-derived mesenchyme resulted in increased expression of proliferative markers, PCNA and PH3 in the mesenchyme at the epithelial-mesenchymal interface of Pdgfrbcre:Smoflox/flox mutants (b, d) versus controls (Pdgfrb Cre:Smoflox/1) (a, c). e–i, Pdgfrbcre:Smoflox/flox;R26RmTmG/1 mutants exhibit increased Ki67+ cells within lineage traced GFP+ cells in the alveoli compared to controls (n = 4 animals per group). j, k, Aged Pdgfrbcre:Smoflox/flox;R26RmTmG/1 mutants (>6 months old) spontaneously develop pulmonary hypertension with increased right ventricular systolic pressure (j, n = 3 animals per group) and right ventricle wall thickness (k, l). Scale bars, 100 μm. *P < 0.05. Error bars, mean ± s.d.
**Gli1**

Relative expression

Vehicle 4-OHT

**Pdgfr(α)GFP**

%Ki67+GFP+/Ki67+

**LETTER RESEARCH**

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Extended Data Figure 4 | Characterization of isolated lung mesenchyme.

a–h, Isolated mesenchymal cells in vitro predominantly express Pdgfra and Pdgfrb (c, d, compared to isotype control a), but not epithelial marker, Epcam (b, compared to isotype control a), endothelial marker, CD31 (f, compared to isotype control e), nor haematopoietic marker, CD45 (h, compared to isotype control g). i, Expression of the constitutively active form of Smo (SmoM2) by 4-OH-tamoxifen induction significantly upregulates Gli1 expression in the isolated lung mesenchyme after 48 h. j, GFP staining of the PdgfraGFP reporter demonstrates that Pdgfra+ cells are expressed broadly in the lung. k, Activation of Smo in isolated lung mesenchymal cells leads to reduced expression of cell cycle progression genes. l, Hh activation of lung mesenchyme with SmoM2 attenuated the proliferation induced by Pdgf-BB ligand in vitro as assayed by BrdU incorporation. m–p, s, Expression of activated Pdgfrb(PdgfrbGOF) within Gli1+ cells resulted in their proliferative expansion. q–s, Concurrent activation of SmoM2 attenuated the proliferative expansion induced by PdgfrbGOF (n = 3 animals per group). Scale bars, 100 μm. Error bars, mean ± s.d.
Extended Data Figure 5 | Shh expression is decreased with bleomycin and naphthalene injury to the airway. a–g, Repetitive bleomycin injury after one month or single-dose naphthalene injury after three days reduced GFP expression in the airways of the Shh\textsuperscript{creGFP} reporter compared to controls. Data represent n = 2 animals per group with 5 sections analysed per animal, AW, airway. Scale bars, 100 μm. *P < 0.05.
Gli1lacZ

(a) vehicle
(b) Bleomycin

(c) vehicle
(d) Bleomycin

(e) Bar graph showing xgal quantification of Shh and Gli1.

(f) Vehicle
(g) Bleomycin

(h) Vehicle
(i) Bleomycin

(j) Vehicle
(k) Bleomycin

(l) Vehicle
(m) Bleomycin

(n) Graph showing % Ki67+GFP+/Ki67+ in vehicle and bleomycin conditions.

(o) Graph showing % Ki67+GFP+/Ki67+ in bleomycin conditions.

(p) Vehicle
(q) 2 months post bleo

(r) Graph showing relative expression of Shh and Gli1 in vehicle and bleomycin conditions.

(s) DAPI/Col1a1
(t) 4 weeks
(u) 2 months

(v) Vehicle

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Extended Data Figure 6 | Hedgehog modulates mesenchymal response to bleomycin injury.  
a–e, Chronic repetitive injury to the lung epithelium with bleomycin over 4 weeks downregulates Gli1 expression in the mesenchyme adjacent to the airways in Gli1lacZ lungs as noted by histochemical staining for β-galactosidase activity (a–d) and as noted using X-gal quantification and qPCR analysis of Shh and Gli1 expression after four weeks of repetitive injury (e, n = 2 animals per group).  
f–i, Lineage-traced, Gli1Hh activated lung mesenchymal cells undergo proliferative expansion after repetitive bleomycin injury with an increase in Ki67+ mesenchymal cells (n = 4 animals per group).  
j–m, Expression of SmoM2 within lineage traced Gli1+ lung mesenchymal cells attenuates the proliferative expansion that normally follows repetitive bleomycin injury (n = 3 animals per group).  
p–v, Gli1 expression remains reduced 2 months after the end of bleomycin treatment (p–r, n = 2 animals per group), which might be due to the persistent scarring that is observed after repetitive bleomycin injury (s–v). AW, airway; V, blood vessel. Scale bars, 100 μm; *P < 0.05. Error bars, mean ± s.d.
Extended Data Figure 7 | Airway epithelium is able to regenerate in vitro and in vivo. 

a, b, d, Scgb1a1<sup>−</sup> secretory epithelium is initially depleted 3 days following naphthalene injury while TubbIV<sup>+</sup> ciliated epithelium remains relatively intact (n = 3 animals per group). c, d, However, 2 months after the initial naphthalene injury, Scgb1a1<sup>−</sup> secretory epithelium repopulates the airway and the ratio of secretory/ciliated cells is restored to levels before injury. e,f, GFP<sup>+</sup> bronchial epithelial cells isolated from Scgb1a1<sup>cre:R26RmTmG</sup> animals were cultured in the presence or absence of isolated lung mesenchymal cells, and only those co-cultured with lung mesenchymal cells were able to form organoids. g,h, Examples of the 3-dimensional structures formed by the bronchial organoids (g,h). Sgcb1a1<sup>−</sup> derived organoids predominantly express markers of secretory airway differentiation, including Scgb1a1 and Nkx2.1 (i,j), while a minority expresses markers of alveolar epithelial lineage including Sftpc (k). l–n, Co-culture of lung mesenchyme and bronchial epithelium induces organoid formation (l), which is inhibited in number (m) and colony size (n) with activation of Hh in the mesenchyme. AW, airway. Scale bars, 100 μm. *P < 0.05, n = 3 animals per group for injury time points. Error bars, mean ± s.d. In vitro organoid studies represent technical quadruplicates, with > 140 randomly selected clones analysed for size per group.
Extended Data Figure 8 | Hh signalling mediates both mesenchymal and epithelial quiescence during homeostasis and injury repair in the lung. The lung epithelium actively maintains mesenchymal quiescence through paracrine Hh signalling, which also regulates a feedback loop to maintain epithelial quiescence. Epithelial injury leads to downregulation of Hh signalling and loss of mesenchymal quiescence, which in turn stimulates epithelial regeneration to replete the airway epithelium until homeostasis is re-established.