

Alveolar stem cells transdifferentiate to drive bronchiolar regeneration

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The lung consists of two anatomically distinct compartments: the airways and the alveoli. Although airway epithelial stem cells are known to mobilize for alveolar regeneration, whether alveolar cells can reciprocally traverse these anatomical boundaries and contribute to bronchial repair remains unclear^{1–4}. Here we developed dual-recombinase-mediated lineage-tracing techniques in mice to demonstrate that alveolar type 2 (AT2) cells migrate into injured bronchioles and transdifferentiate into club cells and ciliated cells, thereby actively contributing to airway regeneration. Mechanistically, after bronchial injury, infiltrating immune cells secrete SPP1, establishing a chemotactic gradient directing cell migration. Peribronchiolar AT2 cells sense this SPP1 signal through the integrin ITGB1 and migrate towards the injured bronchioles. On arrival, these migrated AT2 cells receive Notch ligands from resident ciliated cells, leading to Notch pathway activation, which in turn drives their transdifferentiation into club cells. This transdifferentiation process is accompanied by an intermediate stage marked by *Cldn4* expression. Functional blockade of either SPP1 or ITGB1 impairs AT2 cell migration, whereas inhibition of Notch signalling prevents their transdifferentiation into club cells. Collectively, our findings reveal a cross-compartmental cellular mechanism for bronchiolar epithelial repair, expanding the current understanding of lung regenerative plasticity and potentially informing therapeutic strategies for airway injury.

Chronic and acute pulmonary diseases (such as chronic obstructive pulmonary disease, asthma, pneumonia and bronchiectasis) involve bronchial epithelial damage that impairs airway regeneration and worsens progression^{5–11}. Understanding the cellular and molecular mechanisms of the epithelial repair is therefore critical for targeted therapies. The lung epithelium comprises distinct compartments—trachea, bronchioles and alveoli—each with specialized progenitors that maintain homeostasis and repair^{5,12–14}. In the trachea, basal cells serve as multipotent stem cells^{15–21} that sustain the epithelial lining. The bronchioles contain club cells and neuroendocrine (NE) cells that drive local regeneration^{22–25}. The alveolar compartment relies on alveolar type II (AT2) cells that self-renew and differentiate into AT1 cells to maintain gas exchange^{26–31}. Bronchoalveolar stem cells (BASCs), which are located at the bronchioalveolar duct junction (BADJ) and co-express *Scgb1a1* and *Sftpc*, show multipotency across both bronchiolar and alveolar compartments^{32–35}. Recent studies also reveal club cell plasticity in alveolar repair^{1–4}.

While airway progenitors contribute to alveolar repair, the reciprocal capacity of alveolar cells to participate in bronchiolar regeneration remains unclear. In vitro studies suggest that human AT2 cells

can differentiate into airway basal cells³⁶ and transdifferentiate into bronchiolar secretory cells, a phenomenon that is also observed in non-human primate injury models³⁷. However, genetic manipulation limitations in these systems have prevented definitive in vivo evidence for this transcompartmental repair. In mouse models, conventional *Sftpc-creER* lineage tracing lacks the specificity to accurately track AT2 cell fate after bronchiolar injury, because both AT2 and BASCs express *Sftpc*³², resulting in simultaneous labelling. Given that BASCs actively contribute to bronchiolar repair^{33–35}, this co-labelling confounds interpretation of AT2 cell-specific contributions. Thus, new genetic tools for selective AT2 tracing are needed to conclusively determine whether alveolar cells contribute to bronchiolar regeneration in vivo.

In this study, we established an AT2-specific in vivo labelling system using a dual-gene-marker-mediated lineage tracing strategy. Using this approach in bronchiolar injury models, we found that AT2 cells can migrate across anatomical boundaries and transdifferentiate into functional club and ciliated cells within bronchioles, contributing to cross-compartmental epithelial repair. Mechanistically, this process depends on integrin- β 1-mediated migration and Notch-dependent fate

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specification. After bronchial injury, infiltrating immune cells secrete SPP1 to direct AT2 cell migration towards injured bronchioles, where residual ciliated cells activate Notch signalling to drive AT2-to-club transdifferentiation.

AT2 cells regenerate airway epithelium

We first identified the origin of bronchiolar regeneration after naphthalene injury. We observed SFTPC⁺ AT2 cells within the bronchiolar epithelium after injury, suggesting their potential involvement in repair (Extended Data Fig. 1a,b). To track AT2 cells, we crossed *Sftpc-creER* (ref. 38) with *R26-tdTomato* (*R26-tdT*) mice. At 4 weeks after injury, SFTPC-labelled cells migrated into bronchioles and transdifferentiate into club and AT1 cells (Extended Data Fig. 1c–e). However, SFTPC⁺ cells comprise two distinct cell types: SFTPC⁺ AT2 cells and SFTPC⁺SCGB1A1⁺ BASCs (Extended Data Fig. 1f,g). This distinction is crucial because BASCs, which are naphthalene resistant, can transdifferentiate into club cells after injury^{33–35} (Extended Data Fig. 1h–j). Thus, single-recombinase systems like *Sftpc-creER* cannot distinguish these populations, potentially confounding AT2-specific interpretation.

To determine AT2 cell-specific contributions, we first generated a *Scgb1a1-DTR-GFP* knock-in line by inserting diphtheria toxin receptor (DTR) and GFP sequence into the *Scgb1a1* locus (Extended Data Fig. 1k). In this system, all *Scgb1a1*-expressing cells (club cells and BASCs) should co-express GFP and DTR, enabling diphtheria toxin (DT)-mediated ablation. Immunostaining confirmed GFP/DTR expression in all SCGB1A1⁺ populations in vehicle-treated mice, while DT treatment effectively eliminated GFP⁺ cells, validating system functionality (Extended Data Fig. 1l–p). We next generated triple transgenic mice: *Sftpc-creER;R26-tdT;Scgb1a1-DTR-GFP* (Fig. 1a,b). As predicted, tamoxifen treatment labelled AT2 cells (tdT⁺ only), BASCs (tdT⁺GFP⁺) and club cells (GFP⁺ only). We refer to this system as AT2-tracer1. These mice were treated with tamoxifen and DT, and lung tissue was collected for analysis (Fig. 1c).

DT administration selectively eliminated GFP⁺ populations, leaving only tdT⁺ AT2 cells in alveoli (Fig. 1d,e). After 4 weeks of recovery, lineage tracing revealed that tdT-labelled AT2 cells migrated to bronchioles and generated tdT⁺GFP⁺ club cells (Fig. 1f). These AT2-derived cells transdifferentiated exclusively into E-cadherin (ECAD)-positive epithelial cells, including club cells, AT1 cells, ciliated cells and undifferentiated AT2 cells (Fig. 1g). Quantification showed that $77.42 \pm 4.71\%$ of new club cells originated from AT2 cells (Fig. 1h). AT2-derived cells in bronchioles comprised $38.18 \pm 4.55\%$ club cells, $14.61 \pm 2.62\%$ undifferentiated AT2 cells, $18.90 \pm 4.48\%$ AT1 cells and $0.27 \pm 0.07\%$ ciliated cells (Fig. 1i). AT2-derived tdT⁺GFP⁺ club cells often localized near residual ciliated cells, whereas ciliated-free areas were covered by non-club cells, including tdT⁺ flattened AT1-like cells (Extended Data Fig. 1q–t). We also detected rare AT2-derived GFP⁺SFTPC⁺ BASC-like cells at days 7, 14 and 28 after DT ($0.87 \pm 0.15\%$, $0.70 \pm 0.16\%$ and $0.33 \pm 0.08\%$, respectively; Extended Data Fig. 1u,v). These data provide conclusive evidence that AT2 cells actively migrate and participate in bronchiolar epithelial repair.

We further identified two migratory routes for tdT⁺ AT2-derived cells towards injured bronchioles: through the BADJ (route 1) and through bronchiolar wall channels (route 2) (Fig. 1j and Extended Data Fig. 2a–g). Immunostaining demonstrated that these channels were gaps in the bronchiolar wall, which were lined by tdT⁺ AT2-derived AT1 cells and connected the airway lumen to the alveolar spaces (Extended Data Fig. 2f–h). tdT⁺ coverage of bronchiolar epithelium increased over time (Extended Data Fig. 2e), and both routes remained observable throughout the time (Extended Data Fig. 2f,g). Quantitative analysis revealed that route 1 events increased rapidly from day 3 to 7, whereas route 2 events, though detectable at day 3, showed only a modest increase by day 7 (Fig. 1k). These data suggest that AT2 cells contribute mainly through route 1, with route 2 serving as a supplementary pathway (Fig. 1l).

To investigate whether AT2 cells contribute at the single-cell level, we generated *Sftpc-creER;R26-Confetti;Scgb1a1-DTR-GFP* mice (Extended Data Fig. 2i–k). Our results showed that individually labelled AT2 cells possess multipotent transdifferentiation capacity during bronchiolar repair, including club and AT1 cells (Extended Data Fig. 2k,l). Quantitative analysis showed AT2-derived bronchiolar populations comprised $33.69 \pm 7.90\%$ club, $17.17 \pm 3.42\%$ undifferentiated AT2 and $24.24 \pm 4.78\%$ AT1 cells (Extended Data Fig. 2m). Thus, single AT2 cells can transdifferentiate into multiple lineages during DT-induced bronchiolar repair (Extended Data Fig. 2n).

Regional airway regeneration by basal and NE cells

Basal cells, which serve as airway epithelial stem cells, are located primarily in the trachea, with minimal presence in the distal bronchioles, and have a critical role in epithelial regeneration^{15–18}. To trace basal cell fate, we used *p63-dreER;R26-RSR-tdT* (*R26-rox-stop-rox-tdTomato*) mice, in which proximal basal cells were efficiently labelled with tdT. Rare tdT⁺ basal cells were also detected in the distal bronchioles (Extended Data Fig. 3a–c). We then used *p63-dreER;R26-RSR-tdT;Scgb1a1-DTR-GFP* mice to simultaneously trace basal cells (tdT⁺) and ablate club cells (GFP⁺; Extended Data Fig. 3d). After tamoxifen induction and DT treatment, we analysed lungs after a 4-week repair period (Extended Data Fig. 3e). Immunostaining revealed that nearly all regenerated GFP⁺ club cells in the trachea were tdT⁺, confirming their basal cell origin. Moreover, rare basal cells in the distal bronchioles underwent clonal expansion and contributed a small number of club cells (Extended Data Fig. 3f,g). These findings indicate that basal cells primarily regenerate proximal airways. Furthermore, we detected SCGB3A2⁺SCGB1A1⁺ intralobular serous (IS) cells in the bronchioles, consistent with previous reports³⁹ (Extended Data Fig. 3h,i). These cells were rare in number but proliferated after DT injury, suggesting that they may also participate in club cell regeneration (Extended Data Fig. 3j–m).

As NE cells have been implicated in club cell regeneration^{24,25}, we tested their contribution after DT injury using *Ascl1-creER;R26-tdT;Scgb1a1-DTR-GFP* mice, which specifically label CGRP⁺ NE cells (tdT⁺) while ablating club cells (GFP⁺; Extended Data Fig. 3n–p). At 4 weeks after injury, immunostaining and quantification detected a small population of tdT⁺GFP⁺ club cells, accounting for $4.59 \pm 1.36\%$ of distal club cells (Extended Data Fig. 3q–s). Thus, NE cells contribute a small subset of club cells after injury (Extended Data Fig. 3t).

Together with previous findings, these results demonstrate that club cell regeneration is compartment specific, driven primarily by AT2 cells and basal cell, with minor contributions from NE cells or possible IS cells.

AT2 cells contribute to club cell regeneration

To study AT2-mediated regeneration physiologically, we used naphthalene instead of DT ablation. As naphthalene-resistant cells (such as BASCs) persist after injury^{22,33,40}, conventional single-recombinase AT2 tracing cannot be used due to potential confounding labelling. To overcome this, we used a dual-recombinase system with the nested reporter (*R26-NR*)⁴¹, in which *Dre-rox* and *Cre-loxP* recombinations activate ZsGreen (ZsG) and tdT, respectively⁴¹ (Fig. 2a). We generated *Scgb1a1-creER;Sftpc-dreER;R26-NR* mice to trace AT2 cells independently of BASCs. Here *Sftpc-dreER* labels all SCGB1A1⁺SFTPC⁺ AT2 cells with ZsG (termed AT2-tracer2), while *Scgb1a1-creER* marks SCGB1A1⁺SFTPC⁺ club cells and SCGB1A1⁺SFTPC⁺ BASCs with tdT (Fig. 2a,b). Tamoxifen induced efficient and specific ZsG labelling of AT2 cells, while BASCs converted to tdT (Extended Data Fig. 4a–c), validating system specificity.

We then administered tamoxifen and naphthalene to mice and analysed lungs at 4 weeks (short term) and 6 months (long term) after injury (Fig. 2c). Immunostaining revealed that $42.93 \pm 5.60\%$ of

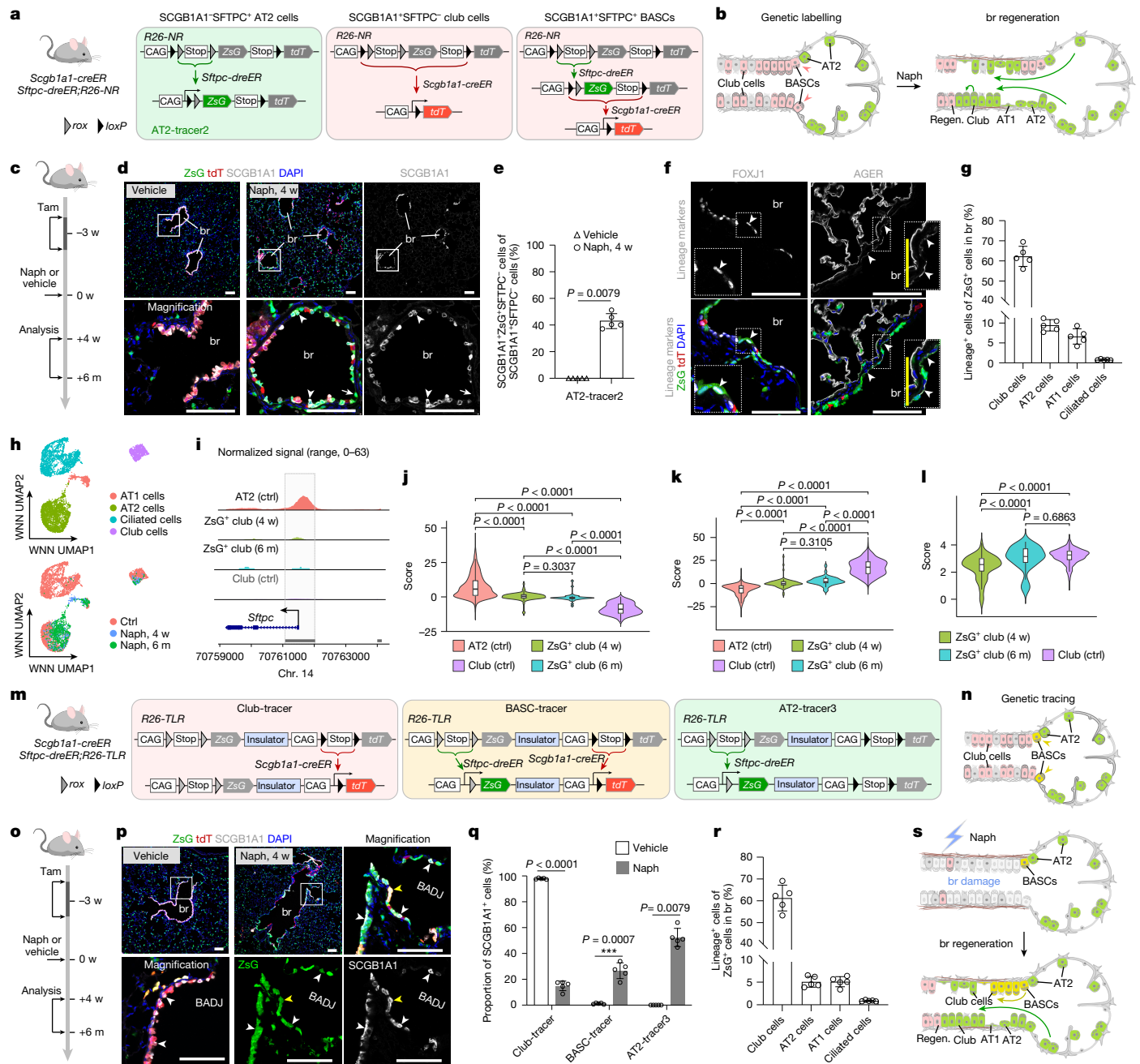


Fig. 2 | See next page for caption.

repair (Extended Data Fig. 4h–i). Single-cell multi-omics analysis further revealed that AT2-derived club cells converged with native airway cells at both transcriptional and epigenetic levels, and acquired corresponding functional features (Fig. 2h–i and Supplementary Fig. 1a). The AT2-specific epigenetic signature rapidly declined after transdifferentiation and remained low from 4 weeks to 6 months after injury, whereas the club-cell-specific epigenetic and secretory/immune signatures increased progressively during repair (Fig. 2j–l). Collectively, these data show that AT2 cells participate in bronchial epithelial repair after naphthalene injury (Fig. 2b).

To evaluate the relative contributions of club cell, AT2 cell and BASC progenitors to airway regeneration in a single mouse model, we used the *R26-TLR*³ dual-recombinase system, as regenerated club cells may originate from club cell expansion, BASCs or AT2 cell differentiation. Using *Scgb1a1-creER*; *Sftpc-dreER*; *R26-TLR* mice, we established three distinct tracing populations: club-tracer (tdT⁺ZsG⁺ club cells),

AT2-tracer3 (tdT⁺ZsG⁺ AT2 cells) and BASC-tracer (tdT⁺ZsG⁺ BASCs) (Fig. 2m,n). Immunostaining confirmed specific labelling (Extended Data Fig. 4m–o).

We analysed lungs at 4 weeks (short term) and 6 months (long term) after injury (Fig. 2o). At 4 weeks, quantitative analysis revealed that AT2-tracer3 contributed the majority (52.35 ± 7.09%) of regenerated club cells, followed by BASC-tracer (26.66 ± 6.15%) and club-tracer (14.89 ± 3.72%; Fig. 2p,q). Notably, AT2-tracer3-derived cells undergo cell proliferation and exhibit multiple differentiation potential, giving rise to club cells (61.30 ± 5.92%), AT2 cells (5.09 ± 1.19%), AT1 cells (5.10 ± 1.12%) and ciliated cells (0.87 ± 0.14%), but not mesenchymal cells (Fig. 2r and Extended Data Fig. 4p,q), consistent with our AT2-tracer2 data (Fig. 2g). At 6 months, these cell types remained within the bronchiolar epithelium (Extended Data Fig. 4r). These results establish AT2 cells as a major cellular source for distal bronchiolar epithelial repair (Fig. 2s).

Fig. 2 | AT2 cells regenerate club cells after naphthalene injury. **a,b**, Dual nested reporter system: ZsG labels AT2 cells (SFTPC⁺SCGB1A1⁺) and tdT labels club cells and BASCs (**a**), allowing tracing of ZsG⁺ AT2 cell fate after bronchiolar injury (**b**). The red arrowheads in **b** indicate BASCs. **c**, Schematic of the experimental design. **d**, Immunostaining for tdT, ZsG and SCGB1A1 on lung sections at 4 weeks after naphthalene (Naph) injury or vehicle. The white arrowheads indicate ZsG⁺SCGB1A1⁺ cells. **e**, Quantification of SCGB1A1⁺ club cells in bronchioles labelled by AT2-tracer2. Data are mean $\pm$ s.d. $n = 5$ mice. **f**, Immunostaining for tdT, ZsG, AGER and FOXJ1 at 4 weeks after naphthalene. The white arrowheads indicate ZsG⁺FOXJ1⁺ or ZsG⁺AGER⁺ cells. **g**, Quantification of AT2-tracer2 cells in bronchioles expressing SCGB1A1, SFTPC, AGER and FOXJ1. Data are mean $\pm$ s.d. $n = 5$ mice. **h**, Weighted nearest neighbour (WNN) uniform manifold approximation and projection (UMAP) of integrated scRNA-seq assay for transposase-accessible chromatin (ATAC) data, showing clusters of AT1, AT2, club and ciliated cells and their distribution across control, naphthalene injury at 4 weeks and naphthalene injury at 6 months. **i**, ATAC-seq tracks (pseudobulk) for *Sftpc* chromatin accessibility dynamics, showing progressive closure in AT2-derived club cells at 4 weeks and 6 months versus resident controls. **j–l**, Comparison of regenerated club cells at 4 weeks ($n = 86$ cells) and 6 months ($n = 41$ cells) against endogenous club cells ($n = 526$ cells), showing AT2-cell-specific (**j**) and club-cell-specific (**k**) chromatin accessibility

scores (based on differential peaks from homeostatic AT2 ($n = 1,095$ cells) versus club cells; and enrichment of club-related functional gene signatures (secretory and immune; **l**). The box plots show the median (centre line), 25th–75th percentiles (box limits) and $1.5 \times$ interquartile range (whiskers). $n = 1$ mouse. *P* values were determined using two-sided Kruskal–Wallis tests with Dunn’s and Benjamini–Hochberg correction. **m,n**, Intersectional genetic strategy (**m**), enabling simultaneous distinct labelling of club cells (tdT⁺, club-tracer), AT2 cells (ZsG⁺, AT2-tracer3) and BASCs (tdT⁺ZsG⁺, BASC-tracer) for lineage tracing (**n**). **o**, Experimental design. **p**, Immunostaining for tdT, ZsG and SCGB1A1 at 4 weeks after naphthalene injury or vehicle treatment. The white and yellow arrowheads indicate ZsG⁺SCGB1A1⁺ cells and ZsG⁺tdT⁺SCGB1A1⁺ cells, respectively. **q**, Quantification of SCGB1A1⁺ club cells labelled by club-tracer, BASC-tracer and AT2-tracer3, respectively. Data are mean $\pm$ s.d. $n = 5$ mice. **r**, Quantification of AT2-tracer3-labelled bronchiolar cells expressing SCGB1A1, SFTPC, AGER and FOXJ1 ($n = 5$ mice). Data are mean $\pm$ s.d. **s**, Diagram of the contribution of AT2-tracer3, club-tracer and BASC-tracer cells to bronchiolar regeneration. For **d**, **f** and **p**, scale bars, 100 μ m (white bars) and 50 μ m (yellow bars). Statistical analysis was performed using unpaired two-tailed Welch’s *t*-tests (club-tracer and BASC-tracer in **q**) and Mann–Whitney *U*-tests (**e** and AT2-tracer3 in **q**). Each immunostaining image is representative of 5 mice.

CLDN4⁺ state mediates AT2-to-club-cell transition

We next performed single-cell RNA-seq analysis of AT2-tracer3, BASC-tracer and club-tracer cells from *Scgb1a1-creER; Sftpc-dreER;R26-TLR* mice at 4 weeks after naphthalene injury (Fig. 3a and Extended Data Fig. 5a). Analysis of AT2-tracer3 cells revealed five cell clusters: cluster 1 (mature AT2), cluster 2 (transient), cluster 3 (club-like), cluster 4 (pre-AT1) and cluster 5 (AT1) (Fig. 3b and Extended Data Fig. 5b,c). Pseudotime analysis revealed a bifurcating AT2-derived pathway through a CLDN4⁺ intermediate to club cells or pre-AT1 to AT1, confirming bipotency (Fig. 3b). Club cell specification correlated with *Sftpc* downregulation and *Scgb1a1* upregulation, with peak *Cldn4* in the transient state (Fig. 3c,d). Functional enrichment differed: transient cells upregulated wound response, apoptosis and migration (Fig. 3e), whereas club cells enriched xenobiotic metabolism, inflammation and Notch signalling (Fig. 3f).

scRNA-seq analysis of BASC-tracer cells revealed four distinct clusters: cluster 1 (canonical BASCs), cluster 2 (transient intermediates), cluster 3 (club cell) and cluster 4 (ciliated cells) (Extended Data Fig. 5d–f). Pseudotime analysis showed a unidirectional trajectory: BASCs to transient cells to club cells to ciliated cells (Extended Data Fig. 5e). Transcriptomically, the BASC-to-club-cell transition involved injury response, migration, naphthalene catabolism and Notch signalling, with glutathione metabolism additionally upregulated in mature club cells (Extended Data Fig. 5g,h). Transcriptomic comparison revealed key differences in AT2- versus BASC-to-club transdifferentiation (Extended Data Fig. 5i–l and Supplementary Table 1). At initiation, BASCs showed stronger club cell and weaker AT2 cell signatures compared with AT2 cells. In the transient state, BASCs maintained a higher club cell signature, but the Notch and PATS²⁹ signatures did not differ from AT2-tracer3 cells. Although both generated club cells, AT2-derived club cells retained AT2 and PATS expression signature, in contrast to BASC-derived counterparts. To assess origin-dependent long-term fate, we performed scRNA-seq analysis of lineage-traced cells at 6 months after injury (Fig. 3a). AT2-tracer3 yielded five clusters (residual AT2, regenerated club, ciliated, pre-AT1 and AT1), whereas BASC-tracer and club-tracer populations each gave three clusters (club, ciliated and goblet cells) (Extended Data Fig. 5m). Transient progenitors from acute repair were no longer detectable. Although AT2-derived club cells retained distinct signatures at 4 weeks, they converged with BASC- and club-cell-derived club cells by 6 months, losing residual PATS and alveolar traits (Extended Data Fig. 5n,o). Thus, prolonged repair resolves lineage-specific differences through maturation to

a common end point, consistent with previous epigenetic analyses (Fig. 2h–l).

Our findings suggest that the CLDN4⁺ intermediate state can transdifferentiate into club and AT1 cells after injury (Fig. 3d). To test this, we first generated *Cldn4-LSL-dre* (*Cldn4-loxP-stop-loxP-dre*) mice (Extended Data Fig. 6a). We also generated *Lamp3-creER* (AT2-tracer4) for efficient AT2 labelling (Extended Data Fig. 6b–e). We then generated *Lamp3-creER;Scgb1a1-DTR-GFP;Cldn4-LSL-dre;R26-RSR-LSL-tdT* mice to trace AT2-derived CLDN4⁺ transient cells (Fig. 3g,h). The reporter requires dual recombination: Cre excises LSL from both *Cldn4-LSL-dre* and the reporter and, subsequently, injury-induced *Cldn4* gene activation drives expression of Dre to excise RSR cassette, thereby labelling AT2-derived CLDN4⁺ cells with tdT (Fig. 3g). Club cells derived from these transient cells co-express tdT and GFP (yellow). Four weeks after DT injury, control (PBS treated) mice showed minimal tdT signal, whereas injured mice exhibited abundant tdT⁺GFP⁺ club cells (Fig. 3i). We also detected tdT⁺AGER⁺ AT1 cells and residual tdT⁺SFTPC⁺ AT2 cells in the bronchiolar epithelium (Fig. 3j). Quantification showed that $62.10 \pm 6.16\%$ of new club cells originated from CLDN4⁺ transient cells (Fig. 3k). Among tdT⁺ lineages, club cells, AT1 cells and AT2 cells accounted for $56.55 \pm 4.54\%$, $14.05 \pm 3.13\%$ and $7.48 \pm 2.06\%$, respectively (Fig. 3l). To further validate the functional role of CLDN4 during transition, we generated *Cldn4-flox* mice and crossed them with *Sftpc-creER;R26-tdT;Scgb1a1-DTR-GFP* mice (Fig. 3m and Extended Data Fig. 6f). At 4 weeks after DT, *Cldn4* knockout (KO) significantly reduced the proportion of AT2 cells contributing to club cells (control versus KO: $65.94 \pm 6.50\%$ versus $25.49 \pm 3.54\%$) and AT1 cells ($21.11 \pm 2.74\%$ versus $8.82 \pm 1.56\%$) (Fig. 3m–p and Extended Data Fig. 6g). Collectively, these data suggest that CLDN4⁺ intermediate cells derived from AT2 cells not only contribute to the bronchiolar epithelium but also functionally promote AT2-cell-mediated epithelial repair.

Notch signalling regulates AT2-to-club cell conversion

We found that Notch signalling is upregulated in AT2-tracer3-derived club cells and BASC-tracer-derived transient cells, suggesting its role in fate transition (Fig. 3f and Extended Data Fig. 5h). To functionally validate this observation, we developed a system combining lineage tracing with conditional *Rbpj* KO (*Rbpj^{rox/+}*) using Dre-rox recombination in SFTPC⁺ cells (Extended Data Fig. 6h). Using *Scgb1a1-creER;Sftpc-dreER;R26-TLR;Rbpj^{rox/rox}* (mutant) and *Scgb1a1-creER;Sftpc-dreER;R26-TLR;Rbpj^{rox/+}* (control) mice, we traced and disrupted

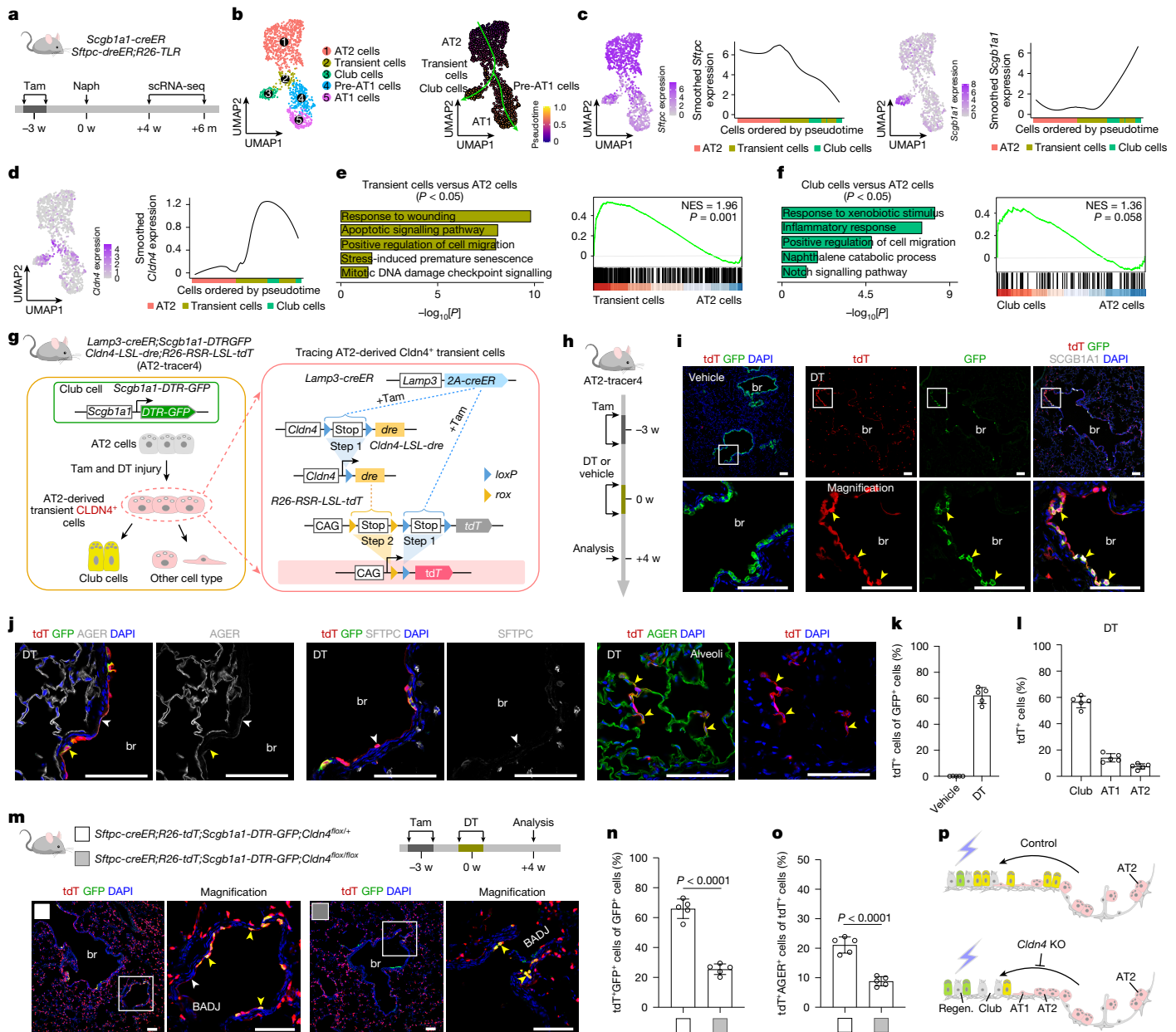


Fig. 3 | The CLDN4⁺ transient cell state mediates fate transition of AT2 to club cells. **a**, Schematic of the experimental strategy. **b**, UMAP embedding of AT2-tracer3 cells (ZsG⁺tdT) at 4 weeks after injury, coloured by five distinct clusters (left). Pseudotime trajectory of AT2-to-club-cell transdifferentiation (right). The arrows indicate inferred directionality; the colour scale reflects pseudotime progression. **c,d**, Expression dynamics of *Sftpc* and *Scgb1a1* (**c**) and *Cldn4* (**d**) during AT2-to-club-cell transdifferentiation. **e**, Enriched Gene Ontology (GO) terms ($P < 0.05$) for differentially expressed genes (DEGs) between transient and AT2 cells in AT2-tracer3 (left). Gene set enrichment analysis (GSEA) analysis indicates significant enrichment of the gene set 'positive regulation of cell migration' in the transient cells (right). NES, normalized enrichment score. **f**, Enriched GO terms ($P < 0.05$) for DEGs between club and AT2 cells in AT2-tracer3 (left). GSEA analysis indicates enrichment of the gene set 'Notch signalling pathway' in the club cells (right). **g**, Schematic of the strategy for specific tracing of CLDN4⁺ transient cells with *Lamp3-creER*; *Scgb1a1-DTR-GFP*; *Cldn4-LSL-dre*; *R26-RSR-LSL-tdT* mice. **h**, Schematic of the experimental design. **i,j**, Immunostaining of tdT and GFP on vehicle-treated lung sections (**i**, left) and of tdT, GFP and lineage markers (SCGB1A1 (**i**, right), and AGER and SFTPC (**j**)) on DT-treated lung sections. The yellow arrowheads

in **i** indicate tdT⁺GFP⁺SCGB1A1⁺ cells. The white arrowheads in **j** indicate tdT⁺AGER⁺ cells (left) or tdT⁺SFTPC⁺ cells (middle); the yellow arrowheads in **i** indicate tdT⁺GFP⁺AGER⁺ cells (left) or tdT⁺AGER⁺ cells (right). **k**, Quantification of the percentage of GFP⁺ club cells in bronchioles labelled by tdT in **i** ($n = 5$ mice). Data are mean $\pm$ s.d. **l**, Quantification of the percentage of tdT⁺ cells within bronchioles expressing GFP, SFTPC and AGER. $n = 5$ mice. Data are mean $\pm$ s.d. **m**, Schematic of the experimental design (top). Immunostaining of tdT and GFP on lung sections from control and mutant mice after DT injury (bottom). The white arrowheads indicate tdT⁺ cells; the yellow arrowheads indicate tdT⁺GFP⁺ cells. **n**, Quantification of the percentage of GFP⁺ cells in bronchioles labelled by tdT within bronchioles in **m**. Data are mean $\pm$ s.d. $n = 5$ mice. **o**, Quantification of the percentage of tdT⁺ cells expressing AGER within bronchioles. Data are mean $\pm$ s.d. $n = 5$ mice. **p**, Diagram showing that AT2-derived CLDN4⁺ intermediate cells contribute to bronchiolar epithelium and promote AT2-mediated epithelial repair. For **i**, **j** and **m**, scale bars, 100 μ m. Statistical analysis was performed using unpaired two-tailed Welch's *t*-tests (**n** and **o**). GO term enrichment was assessed using a one-sided hypergeometric test (**e** and **f**). GSEA significance was assessed using two-sided permutation tests (**e** and **f**). Each immunostaining image is representative of 5 mice.

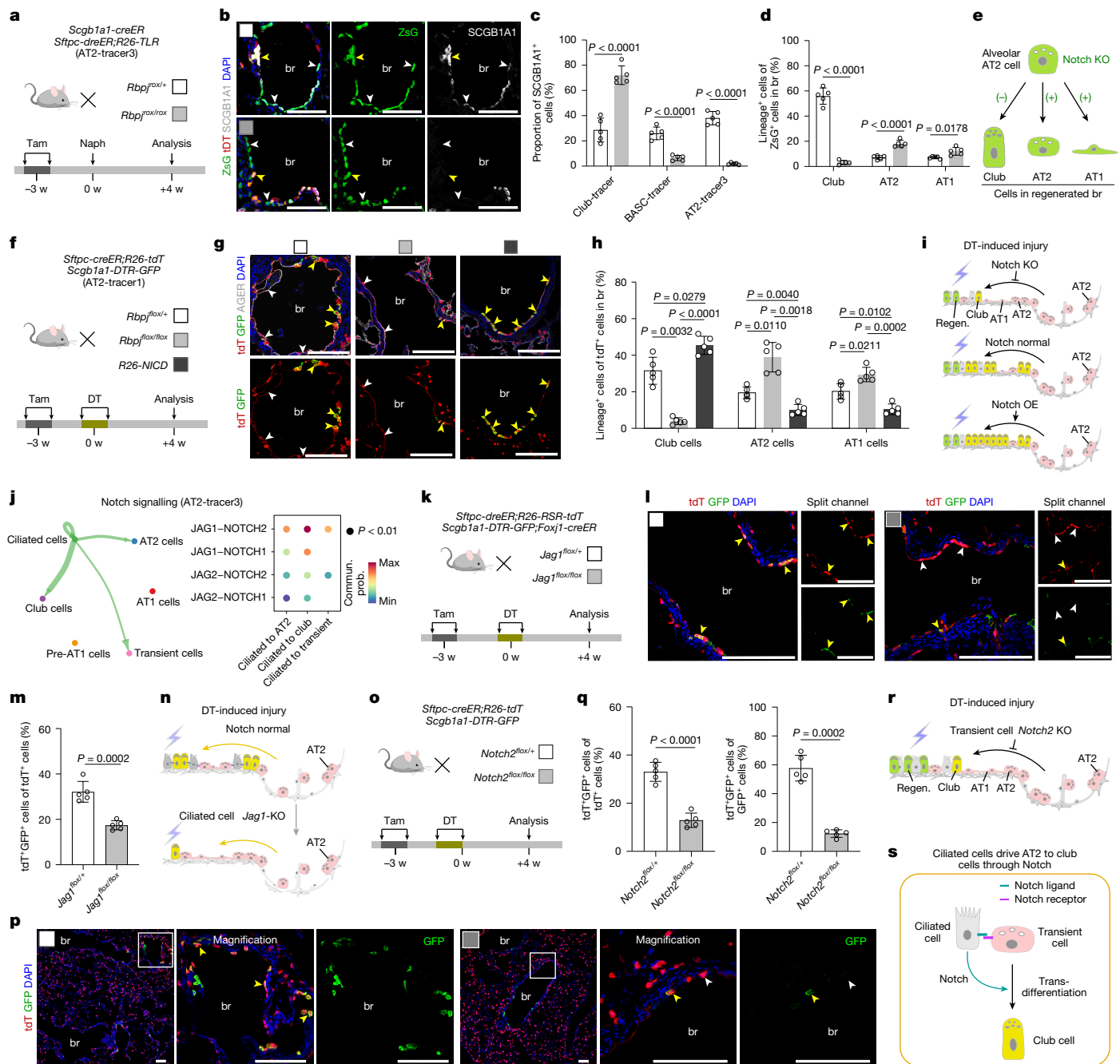


Fig. 4 | See next page for caption.

Notch signalling simultaneously in AT2-tracer3 and BASC-tracer cells (Fig. 4a and Extended Data Fig. 6i,j). After tamoxifen induction and naphthalene injury, a 4 week repair period study revealed that *Rbpj*-KO cells migrated to the bronchioles but did not express club cell markers, while retaining alveolar markers instead (Fig. 4b and Extended Data Fig. 6k). Notably, the proportion of club cells derived from AT2-tracer3 and BASC-tracer was significantly reduced in mutants than that in the controls (AT2-tracer3: $1.86 \pm 0.80\%$ versus $38.09 \pm 5.29\%$; BASC-tracer: $6.48 \pm 2.08\%$ versus $25.97 \pm 4.97\%$; Fig. 4c). Furthermore, among the bronchiolar cells from AT2-tracer3, mutants exhibited significantly fewer club cells compared with the controls ($3.16 \pm 1.60\%$ versus $55.89 \pm 6.47\%$), but more alveolar cells (AT2 cell: $18.14 \pm 2.70\%$ versus $7.12 \pm 1.62\%$; AT1 cell: $12.07 \pm 3.09\%$ versus $6.95 \pm 0.96\%$; Fig. 4d). Independent AT2-tracer2 tracing confirmed that *Rbpj*-deficient AT2 cells did not generate club cells during bronchiolar repair (Extended Data

Fig. 6l–n). The above results demonstrate that Notch signalling is essential for AT2-to-club cell conversion. *Rbpj* deletion not only blocks this transition but also leads to aberrant accumulation of alveolar cells in bronchioles (Fig. 4e).

To comprehensively assess Notch's role in modulating cell fate, we developed complementary loss- and gain-of-function models using DT-induced injury with AT2-tracer1. DT-induced club cell depletion caused bronchial wall thickening, which subsides as the club cells were repaired (Extended Data Fig. 6o,p). In loss-of-function experiments (*Sftpc-creER;R26-tdT;Scgb1a1-DTR-GFP;Rbpj^{flax/flax}* mice), *Rbpj* deletion in AT2 cells significantly reduced club cell formation (mutant ($3.95 \pm 1.83\%$) versus control ($31.54 \pm 7.39\%$)) while increasing alveolar differentiation (AT2: mutant ($38.92 \pm 8.08\%$) versus control ($19.50 \pm 3.23\%$); AT1: mutant ($29.46 \pm 3.95\%$) versus control ($20.26 \pm 4.25\%$); Fig. 4f–h and Extended Data Fig. 6q,r). Conversely, Notch overexpression

Fig. 4 | Notch signalling from ciliated cells regulates AT2-to-club-cell transition. **a**, Schematic of the experimental design. The colour key applies to **b**. **b**, Immunostaining for tdT, ZsG and SCGB1A1 on lung sections after naphthalene injury. The white arrowheads indicate ZsG⁺SCGB1A1⁺ cells (top) or ZsG⁺SCGB1A1⁺ cells (bottom); the yellow arrowheads indicate ZsG⁺tdT⁺SCGB1A1⁺ cells (top) or ZsG⁺tdT⁺SCGB1A1⁺ cells (bottom). **c**, Quantification of the percentage of SCGB1A1⁺ club cells from club-tracer, BASC-tracer and AT2-tracer3, respectively, after naphthalene injury. Data are mean $\pm$ s.d. $n = 5$ mice. **d**, Quantification of the percentage of AT2-tracer3 cells within bronchioles expressing SCGB1A1, SFTPC and AGER. Data are mean $\pm$ s.d. $n = 5$ mice. **e**, Diagram showing that the Notch signalling pathway regulates the transdifferentiation of AT2 cells after naphthalene injury. **f**, Schematic of the experimental design. The colour key applies to **g**. **g**, Immunostaining of tdT, GFP and AGER on lung sections after DT injury. The white arrowheads indicate tdT⁺AGER⁺ cells; the yellow arrowheads indicate tdT⁺GFP⁺ cells. **h**, Quantification of the percentage of AT2-tracer1-labelled cells within bronchioles expressing GFP, SFTPC and AGER after DT injury. Data are mean $\pm$ s.d. $n = 5$ mice. **i**, Cartoon image showing that the Notch pathway regulates the transdifferentiation of AT2 cells in DT injury. OE, overexpression. **j**, Inferred Notch signalling network from ciliated cells to other cell types in AT2-tracer3 (left). Right, significant ligand–receptor pairs

driving Notch signalling; *P* values were calculated using one-sided permutation tests. Commun. prob., communication probability. **k**, Schematic of the experimental design. The colour key applies to **l** and **m**. **l**, Immunostaining of tdT and GFP on lung sections after DT. The white arrowheads indicate tdT⁺GFP⁺ cells; the yellow arrowheads indicate tdT⁺GFP⁺ cells. **m**, Quantification of the percentage of bronchiolar tdT⁺ cells expressing GFP in **l**. Data are mean $\pm$ s.d. $n = 5$ mice. **n**, Diagram showing that *Jag1* knockdown in ciliated cells inhibits the AT2-to-club-cell transition. **o**, Schematic of the experimental design. The colour key applies to **p** and **q**. **p**, Immunostaining of tdT and GFP on lung sections after DT treatment. The white arrowheads indicate tdT⁺GFP⁺ cells; the yellow arrowheads indicate tdT⁺GFP⁺ cells. **q**, Quantification of the percentage of tdT⁺ cells expressing GFP (left) and of GFP⁺ club cells expressing tdT (right). Data are mean $\pm$ s.d. $n = 5$ mice. **r,s**, Diagram showing that AT2/transient cells recognize Notch ligands on ciliated cells through Notch receptors (**r**), which then transduce Notch signalling to regulate their trans-differentiation into club cells (**s**). For **b**, **g**, **l** and **p**, scale bars, 100 μ m. Statistical analysis was performed using unpaired two-tailed *t*-tests (club-tracer and BASC-tracer in **c**, AT2 cells and AT1 cells in **d**, **m** and **q** (left)), Welch's *t*-test (AT2-tracer3 in **c**, club cells in **d** and **q** (right)) and one-way analysis of variance (ANOVA) with Tukey's test (**h**). Each immunostaining image is representative of 5 mice.

(*Sftpc-creER;R26-tdT;Scgb1a1-DTR-GFP;R26-NICD*) enhanced AT2-to-club-cell conversion ($45.19 \pm 4.69\%$ in mutant) while suppressing alveolar fates (AT2: $10.07 \pm 3.03\%$ in mutant; AT1: $10.37 \pm 2.94\%$ in mutant; Fig. 4f–h and Extended Data Fig. 6q,r). These complementary approaches demonstrate that Notch signalling functions as a critical bidirectional regulator of AT2 cell fate decisions during bronchiolar repair (Fig. 4i). *Rbpj* KO increased bronchiolar wall thickness and reduced small airway ventilation (Extended Data Fig. 6s,t), indicating that Notch-mediated club cell regeneration is essential for structural and functional recovery. tdT⁺GFP⁺ AT2 cells sorted from DT-injured lungs did not transdifferentiate into club cells in club medium alone but did so substantially after Notch activation, indicating that achieving AT2-to-club conversion in vitro requires strong drivers such as Notch signalling (Extended Data Fig. 6u–z and Supplementary Fig. 1b).

Ciliated cells mediate AT2-to-club cell conversion

scRNA-seq analysis revealed that migrating AT2 cells require local Notch signals for club cell transdifferentiation (Fig. 3f). After injury, ciliated cells were the dominant Notch-ligand source (high JAG1/2) among diverse epithelia (Extended Data Fig. 7a–c). Interaction analysis confirmed JAG1/2–Notch1/2 signalling between ciliated and AT2-derived cells (Fig. 4j), with similar networks in BASC lineages (Extended Data Fig. 7d–f), suggesting a conserved mechanism. To functionally validate these findings, we developed a genetic system that combines AT2 tracing (*Sftpc-dreER;R26-RSR-tdT*, designated as AT2-tracer5) with conditional ablation of both club cells/BASCs (through *Scgb1a1-DTR-GFP*) and ciliated cells (through *Foxj1-creER;R26-iDTR*; Extended Data Fig. 7g–i). We then established two mouse groups: *Sftpc-dreER;R26-RSR-tdT;Scgb1a1-DTR-GFP* (control mice) and *Sftpc-dreER;R26-RSR-tdT;Scgb1a1-DTR-GFP;Foxj1-creER;R26-iDTR* (experimental mice). This design achieved ablation of club cells and BASCs in both groups, selective ciliated cell ablation in experimental mice and AT2 cell tracing in all animals. At 4 weeks after injury, ciliated ablation substantially reduced AT2-to-club-cell conversion (experimental: $3.84 \pm 2.25\%$; control: $25.71 \pm 4.87\%$; Extended Data Fig. 7i–l). Spatial analysis provided further mechanistic insights, showing that successfully converted AT2-derived club cells (tdT⁺GFP⁺) predominantly localized adjacent to ciliated cells, while unconverted AT2-derived cells (tdT⁺GFP⁺) typically lacked this close association (Extended Data Fig. 7m–o).

Given the strong JAG1–NOTCH2 association, we deleted this pair respectively in ciliated and AT2-derived cells to test whether ciliated cells regulate AT2 fate through Notch (Extended Data Fig. 7p). First, we generated experimental (*Foxj1-creER;Jag1^{fllox/+}*) and control

(*Foxj1-creER;Jag1^{fllox/+}*) mice that also carried *Sftpc-dreER;R26-RSR-tdT;Scgb1a1-DTR-GFP* (Fig. 4k). After tamoxifen induction, *Jag1* was deleted in ciliated cells; AT2 cells were labelled with tdT, club cells with GFP and BASCs with both. DT was then administered to ablate club cells and BASCs (Fig. 4k). Immunostaining and quantification at 4 weeks after DT treatment showed that *Jag1* deletion significantly reduced AT2-derived club cells in the bronchioles (Fig. 4k–n and Extended Data Fig. 7q–s), demonstrating that ciliated cells regulate AT2-to-club-cell transdifferentiation through the Notch ligand *Jag1*. To investigate Notch2 function, we generated conditional *Notch2^{fllox}* mice (Extended Data Fig. 7t,u). For specific deletion in AT2-derived cells (including AT2 cells and transient cells), we produced experimental *Sftpc-creER;R26-tdT;Scgb1a1-DTR-GFP;Notch2^{fllox/fllox}* mice and controls with *Sftpc-creER;R26-tdT;Scgb1a1-DTR-GFP;Notch2^{fllox/+}* (Fig. 4o). Immunostaining at 4 weeks after DT treatment showed that *Notch2* deletion significantly inhibited AT2-to-club-cell transition (Fig. 4o–r and Extended Data Fig. 7v), consistent with the *Rbpj*-deletion results (Fig. 4h). Collectively, these findings demonstrate that Notch signalling from ciliated cells critically regulates AT2-to-club-cell transition (Fig. 4s).

SPP1 induces AT2 migration through integrin β 1

To investigate how AT2 cells migrate in response to bronchial injury, we performed scRNA-seq analysis of PBS- and DT-treated *Scgb1a1-DTR-GFP* lungs (Supplementary Fig. 1c). DT injury induced neutrophil infiltration and peribronchiolar fibroblast expansion, creating an inflammatory and stromal microenvironment (Extended Data Fig. 8a,b). CellChat analysis revealed global signalling increase after injury, with neutrophils, monocytes and peribronchiolar fibroblasts becoming major signal sources towards AT2 cells (Fig. 5a and Extended Data Fig. 8c). Among chemotactic pathways, SPP1 signalling from immune cells was robustly induced (Extended Data Fig. 8d). Peribronchiolar fibroblast-to-AT2 signals were dominated by structural, adhesion and morphogen pathways, rather than by generating migratory cues (Extended Data Fig. 8d–f). SPP1 is a secreted chemotactic factor that binds to integrins and CD44 to promote migration^{42–44}. DT injury significantly enhanced interactions between immune-derived SPP1 and integrin heterodimers on AT2 cells (Fig. 5b), correlating with upregulation of migration and S-phase gene signatures (Extended Data Fig. 8g,h and Supplementary Table 1). These data suggest that SPP1-mediated chemotaxis drives AT2 recruitment to injured bronchioles.

To gain spatial resolution, we performed spatial transcriptomics analysis of DT-injured lungs (at day 7) using wild-type mice as controls (Extended Data Fig. 8i). DT injury induced a localized approximately

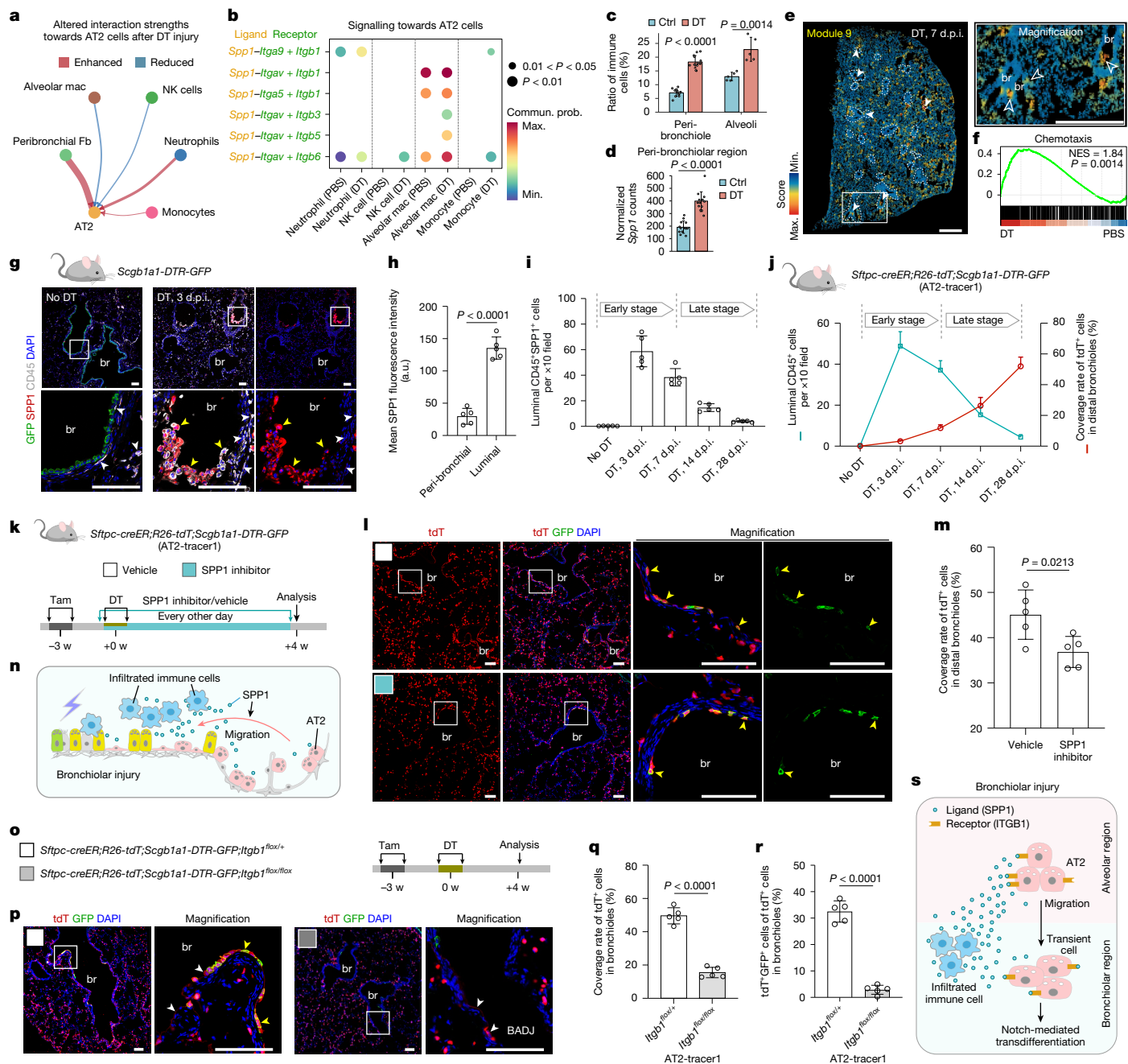


Fig. 5 | Infiltrating immune cells secrete SPP1 to direct AT2 cell migration towards the bronchioles after injury. **a**, Differential interaction network showing altered communication strengths towards AT2 cells after injury; edge thickness represents change magnitude; the red and blue lines indicate enhanced and reduced, respectively. Fb, fibroblasts; mac, macrophages; NK, natural killer. **b**, SPP1-integrin ligand–receptor pairs across immune-to-AT2-cell interactions. The dot size represents *P* values; colour represents the communication probability as determined using a one-sided permutation test. **c, d**, Immune cell analyses in DT versus control groups (*n* = 13 peri-broncholar and 5 alveolar areas from 1 mouse; as in Extended Data Fig. 8i): cell proportions (**c**) and *Spp1* expression (**d**) are shown. Statistical analysis was performed using two-sided Student's *t*-tests. Data are mean ± s.d. **e**, Spatial feature plots: module 9 (leukocyte activation/chemotaxis) is enriched in lumen and peribroncholar areas after DT. The white arrowheads indicate the module 9 high-expression region. d.p.i., days post-injury. **f**, GSEA of the chemotaxis gene signature in peri-broncholar immune cells (DT versus control). Statistical significance was assessed using two-sided permutation tests. **g**, Immunostaining for GFP, SPP1 and CD45 on lung sections (DT versus PBS). The white arrowheads indicate CD45⁺SPP1^{low} cells; the yellow arrowheads indicate CD45⁺SPP1^{high} cells. **h**, Quantification of the SPP1 mean fluorescence intensity of CD45⁺ cells at luminal or peri-broncholar

regions at 3 days after DT. Data are mean ± s.d. *n* = 5 mice. a.u., arbitrary units. **i**, Quantification of luminal CD45⁺SPP1⁺ cells per ×10 field over time after DT treatment. Data are mean ± s.d. *n* = 5 mice. **j**, Quantification of luminal CD45⁺ cells (left axis) and tdT⁺ coverage in distal bronchioles (right axis) in *Sftpc-creER;R26-tdT;Scgb1a1-DTR-GFP* mice at different timepoints after DT. Data are mean ± s.d. *n* = 5 mice. **k**, The experimental design. **l**, Immunostaining for tdT and GFP after DT with SPP1 inhibitor (bottom row) or vehicle (top row). The yellow arrowheads indicate tdT⁺GFP⁺ cells. **m**, Quantification of tdT⁺ coverage (AT2-tracer1) in distal bronchioles. Data are mean ± s.d. *n* = 5 mice. **n**, Diagram of immune cell secretion of SPP1 to promote AT2-derived cell migration to bronchioles. **o**, Experimental design. The colour key applies to **p–r**. **p**, Immunostaining for tdT and GFP after DT treatment. The white arrowheads indicate tdT⁺GFP⁺ cells; the yellow arrowheads indicate tdT⁺GFP⁺ cells. **q**, Quantification of AT2-tracer1 coverage in bronchioles. Data are mean ± s.d. *n* = 5 mice. **r**, Quantification of AT2-tracer1 cells expressing GFP within bronchioles. Data are mean ± s.d. *n* = 5 mice. **s**, Hypothesis: after injury, immune cells secrete SPP1, which binds to ITGB1 on AT2 cells, directing their migration to bronchioles, where they become transient cells for fate conversion. Scale bars, 100 μm (**g**, **l** and **p**) and 1 mm (**e**). Statistical analysis was performed using unpaired two-tailed *t*-tests (**h**, **m**, **q** and **r**). Each immunostaining image is representative of 5 mice.

threefold increase in peribronchiolar immune infiltration (Fig. 5c). Hotspot analysis revealed that, under homeostatic conditions, no modules associated with leukocyte activation or chemotaxis were enriched near bronchioles, whereas DT-injured lungs exhibited such modules (for example, module 9) concentrated in the bronchiolar lumen and peribronchiolar areas (Fig. 5d,e and Extended Data Fig. 8j,k). Module 9 contained pro-inflammatory and chemokine genes (*Spp1* and *Ccl6*). *Spp1* was upregulated in peribronchiolar immune cells after DT injury (Fig. 5d), and chemotaxis-related gene sets were enriched in these cells (Fig. 5f). Together, these spatial data indicate that bronchiolar injury triggers localized immune cell accumulation and secretion of chemotactic factors like SPP1, potentially establishing a gradient to direct AT2 migration.

To test this hypothesis, we validated SPP1 expression in immune cells by immunostaining. After DT injury, many CD45⁺ immune cells infiltrated the bronchiolar lumen and showed high SPP1 expression (Fig. 5g,h). Infiltration peaked within the first 7 days (early response stage) and then declined (late stage; Fig. 5i and Extended Data Fig. 9a,b). Using AT2-tracer1 mice, tdT⁺ AT2 lineage cell migration began during the early stage and coverage increased over time (Fig. 5j (red line) and Extended Data Fig. 9c,d). Immune infiltration peaked during the early phase (blue line), suggesting that SPP1 from these cells induces AT2 migration (Fig. 5j). Injury also induced *Mmp14* in AT2 cells, fibroblasts and SMCs, and *Mmp9* in immune cells, potentially degrading matrix to facilitate AT2 cell migration (Extended Data Fig. 9e–g). Indeed, at day 3 after injury, SPP1⁺MMP9⁺ immune cells were localized near migrating AT2-derived cells at both BADJ and channel sites (Extended Data Fig. 9h–j). Similar dynamics were observed after naphthalene injury (Extended Data Fig. 9k–p). We next performed scratch and Transwell assays to test whether infiltrating SPP1^{high} immune cells promote AT2 migration. Recombinant SPP1 significantly accelerated wound closure and enhanced Transwell migration of AT2 cell line (Extended Data Fig. 10a–d). Adding immune cells to the lower chamber also increased migration, an effect that was blocked by an SPP1 inhibitor (Extended Data Fig. 10e–g and Supplementary Fig. 1c). We then validated SPP1 function in vivo. In AT2-tracer1 mice, SPP1 inhibitor treatment significantly reduced bronchiolar coverage of tdT⁺ cells compared with the controls (Fig. 5k–n). Similarly, in naphthalene-injured AT2-tracer2 mice, SPP1 inhibition decreased ZsG⁺ cell coverage at day 28 (Extended Data Fig. 10h–j). Collectively, these experiments show that immune-derived SPP1 acts as a chemoattractant guiding AT2 cell migration to injured bronchioles (Fig. 5n).

scRNA-seq analysis revealed a robust interaction between *Spp1* and the integrin $\beta 1$ (*Itgb1*) expressed on AT2 cells (Fig. 5b). To test whether ITGB1 regulates AT2 migration, we performed in vitro scratch and Transwell assays. Addition of an anti-ITGB1 antibody significantly inhibited wound closure and reduced cell migration (Extended Data Fig. 10k–n). To extend these findings in vivo, we generated AT2-specific *Itgb1*-KO mice (*Sftpc-creER;R26-tdT;Scgb1a1-DTR-GFP;Itgb1^{fllox/flox}*). At 28 days after DT, *Itgb1* KO significantly reduced AT2 cell migration towards the bronchioles compared with in the control mice (*Sftpc-creER;R26-tdT;Scgb1a1-DTR-GFP;Itgb1^{fllox/+}*) (Fig. 5o–r). Moreover, *Itgb1*-KO mice showed increased bronchiolar wall thickness and impaired small airway ventilation (Extended Data Fig. 10o–s). These findings demonstrate that AT2 cells require ITGB1 to receive chemotactic cues from infiltrating immune cells, directing their migration to the bronchioles after injury (Fig. 5s).

Discussion

The widely used *Sftpc-creER* system labels both AT2 cells and BASCs, confounding specific analysis of AT2 cell contributions to bronchiolar repair. To address this, we developed five distinct complementary AT2-tracer systems and showed that AT2 cells participate in bronchiolar repair through trans-compartmental migration and transdifferentiation.

After injury, infiltrating immune cells secrete SPP1 to form a chemotactic gradient, probably acting through ITGB1 on AT2 cells to promote migration. Once at the injured site, they enter a transient intermediate state before transdifferentiating into club cells. This transdifferentiation depends on Notch signalling: AT2-derived cells sense Notch ligands from adjacent ciliated cells to activate club cell specification (Extended Data Fig. 10t). These findings underscore AT2 cell plasticity and expand their regenerative capacity beyond alveolar maintenance. Furthermore, KO of *Itgb1* or *Rbpj* in AT2 cells suppresses functional recovery, highlighting the importance of club cell regeneration for lung function.

In the DT model, AT2 cells migrate into bronchioles through BADJ and bronchiolar wall channels—although classification is somewhat subjective. By contrast, naphthalene injury induces only BADJ-mediated migration. We found that matrix metalloproteinases secreted by immune cells, AT2 cells and peribronchiolar fibroblasts may promote channel formation after DT injury, but direct evidence is lacking. Further studies are needed to elucidate the microenvironmental differences between these two injury models. Moreover, alveolar and bronchiolar epithelial regeneration are known to be regulated by niche cells such as fibroblasts, endothelial cells and immune cells^{27,30,45–49}. Whether other cell types or signalling pathways mediate AT2-to-club-cell transition remains to be investigated.

Our finding that AT2 cells respond to bronchiolar injury aligns with a recent report showing that airway injury triggers myeloid-mediated lung alveolar responses⁵⁰. Although AT2 cells and BASCs show distinct transdifferentiation kinetics during short-term repair, these differences resolve over time, with AT2-derived club cells acquiring full functional maturity—including progenitor capacity and ciliation—and undergoing stable epigenetic reprogramming to airway-specific profiles that resemble native cells. Owing to technical limitations, several questions remain: whether AT2 cells adopt a BASC-like intermediate state during transdifferentiation, and whether IS cells contribute to club cell regeneration. Both of these questions require lineage-tracing studies. Transcriptome sequencing combined with in vitro/in vivo validation revealed that immune-secreted SPP1 regulates AT2 migration. We propose that fibroblasts remodel ECM and provide structural support, potentially complementary to immune-driven chemotaxis.

Our findings have important implications for lung regeneration therapies^{5,13}. While current clinical approaches primarily target airway-resident stem cells (such as basal cells and secretory cells) for airway repair^{5,6,12}, our research suggests that AT2 cells could represent an alternative cellular source for airway regeneration, particularly given their ability to fully mature into functional club cells⁵. Moreover, the identification of Notch signalling as a key regulator of AT2-to-club-cell transdifferentiation reveals a potential therapeutic target for enhancing alveolar-to-bronchiolar repair. We found that ectopic activation of Notch signalling in AT2 cells promotes AT2-to-club-cell transdifferentiation in vitro, providing a cellular model for studying AT2 cell lineage conversion. However, mouse–human lung structural differences preclude direct extrapolation, leaving the existence of a conserved AT2-to-club program in humans unresolved^{37,51–53}.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-026-11127-w>.

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Methods

Mice

All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC, protocol SIBCB-S374-1702-001-C1) at the Center for Excellence in Molecular Cell Science, Chinese Academy of Sciences, and were performed in accordance with its guidelines. *Sftpc-creER*, *Sftpc-dreER*, *Scgb1a1-creER*, *Ager-creER*, *Hopx-2A-dreER*, *p63-dreER*, *Foxj1-creER*, *R26-NR*, *R26-TLR*, *R26-tdT*, *R26-RSR-tdT*, *R26-RSR-LSL-tdT*, *R26-Confetti*, *R26-iDTR*, *R26-NICD*, *Rbpj^{fllox/+}* and *Jag1^{fllox/+}* mouse lines were described previously^{3,22,33,38,41,54–61}. The *Scgb1a1-DTR-GFP*, *Ascl1-creER*, *Itgb1^{fllox/+}*, *Rbpj^{prox/+}*, *Lamp3-creER*, *Cldn4-LSL-dre*, *Cldn4^{fllox/+}* and *Notch2^{fllox/+}* mouse lines were generated by Shanghai Model Organisms Center (SMOC). For the *Scgb1a1-DTR-GFP* mouse line, the P2A-DTR-GFP sequence was inserted into the *Scgb1a1* locus by homologous recombination. For the *Ascl1-creER* mouse line, the *creER^{T2}* sequence was inserted into the *Ascl1* locus by homologous recombination. For the *Itgb1^{fllox/+}* mouse line, two *loxP* sites were added on either side of exon 3 of *Itgb1* by homologous recombination. For the *Rbpj^{prox/+}* mouse line, two *rox* sites were added on either side of exon 6 and exon 7 of *Rbpj* by homologous recombination. For the *Lamp3-creER* mouse line, the 2A-*creER* sequence was inserted into the *Lamp3* locus by homologous recombination. For the *Cldn4-LSL-dre* mouse line, the *loxP-stop-loxp-dre* sequence was inserted into the *Cldn4* locus by homologous recombination. For the *Cldn4^{fllox/+}* mouse line, two *loxP* sites were added on either side of exon 1 of *Cldn4* by homologous recombination. For the *Notch2^{fllox/+}* mouse line, two *loxP* sites were added on either side of exon 3 of *Notch2* by homologous recombination. All mice were housed and bred in the specific pathogen free (SPF) animal facility of the Center for Excellence in Molecular Cell Science, under controlled conditions at 22 ± 2 °C, 50 ± 10% relative humidity and a 12 h–12 h light–dark cycle, with ad libitum access to food and water. Depending on the experimental cohort, mice were used at either 7–20 weeks of age or 6 months of age, and all animals were maintained on the C57BL/6/ICR mixed background. Both male and female mice were randomly used for experiments. The mice were administrated 0.2 mg g⁻¹ tamoxifen (Sigma-Aldrich, T5648; ABCONE, T56488) dissolved in corn oil through oral gavage at the indicated timepoints. For clonal analysis, the dose of tamoxifen was reduced to 0.02 mg g⁻¹. The mice were injected intraperitoneally 10 ng g⁻¹ DT (Sigma-Aldrich, D0564-1MG) dissolved in PBS (Invitrogen, C10010500BT) for five times at indicated time to induce cell ablation.

Genomic PCR

Genomic DNA was prepared from the mouse tissues as previously described³. Mouse tissues were dissociated with lysis buffer (100 mM Tris HCl, pH 7.8, 5 mM EDTA, 0.2% SDS, 200 mM NaCl and 100 mg ml⁻¹ proteinase K) for 6–12 h at 55 °C, and then the supernatants with genomic DNA were collected to obtain DNA precipitation using isopropanol after centrifugation at maximum speed (21,130g) for 4 min. The DNA precipitation was then washed in 70% ethanol and air-dried, followed by dissolved with deionized water for PCR amplification.

Lung tissue collection and immunofluorescence staining

The lung tissues were collected as previously reported³. Mice were euthanized and then the blood within the lung was eliminated by perfusing 10–15 ml PBS through the right ventricle. Subsequently, the lungs were infused with 1.5 ml of 4% paraformaldehyde (PFA) through the trachea, and the collected tissue was fixed in 4% PFA for 1 h (h) at 4 °C, followed by washing three times in PBS. The lungs were then dehydrated overnight at 4 °C using 30% sucrose and then embedded in optimal cutting temperature (OCT) compound (Sakura). The lung tissues embedded with OCT could be stored at –80 °C for several months. For immunostaining, frozen sections (9–12 µm) were first prepared on negatively charged slides, which were air-dried at room temperature and subsequently washed several times with PBS. The slides were then incubated with 5%

PBS-stabilized serum and 0.2% Triton X-100 (PBSST buffer) for 30 min at room temperature, followed by overnight incubation with primary antibodies at 4 °C. Primary antibodies used were as follows: E-cadherin (R&D system, AF748, 1:500), SCGB1A1 (Santa Cruz, SC-9772, 1:200), SCGB1A1 (Abcam, ab213203, 1:300), SCGB3A2 (R&D system, AF3545-SP, 1:500), tdTomato (Rockland, 600-401-379, 1:1000), tdTomato (Rockland, 200-101-379, 1:500), GFP (Abcam, ab6662, 1:500), collagen IV (Millipore, Ab769, 1:500), GFP (Nacalai Tesque, 04404-84, 1:500), GFP (Invitrogen, A11122, 1:500), AGER (R&D system, MAB1179-100, 1:200), SFTPC (Millipore, AB3786, 1:200), ZsGreen (Clontech, 632474, 1:1,000), Ki-67 (Abcam, ab15580-100ug, 1:400), DTR (R&D system, AF-259-NA, 1:500), Ace-Tub (Sigma-Aldrich, T7451, 1:300), Krt5 (BioLegend, 905504, 1:500), CGRP (Sigma-Aldrich, C8198, 1:500), PDGFRα (R&D system, AF1062, 1:500), LAMP3 (NovusBio, 1006F7.05, 1:300), FOXJ1 (Abcam, ab235445, 1:300), T1A/PDPN (DSHB, 8.1.1, 1:100), HOPX (Abcam, ab307670, 1:100), JAGGED1 (Abcam, ab7771, 1:100), MMP9 (Thermo, MA5-15886, 1:200), SPPI/OPN (R&D system, AF808, 1:400), CD45 (eBioscience, 17-0451-82, 1:200).

The sections were then incubated with Alexa-conjugated secondary antibodies for 30 min at room temperature and nuclei were counterstained with 4'6-diamidino-2-phenylindole (DAPI, Vector lab). Second antibodies were listed as follows: Alexa donkey anti-rabbit 555 (Invitrogen, A31572, 1:1,000), Alexa donkey anti-rabbit 488 (Invitrogen, A21206, 1:1,000), Alexa donkey anti-rabbit 647 (Invitrogen, A31573, 1:1,000), Alexa donkey anti-goat 488 (Invitrogen, A11055, 1:1,000), Alexa donkey anti-goat 555 (Invitrogen, A21432, 1:1,000), Alexa donkey anti-goat 647 (Invitrogen, A21447, 1:1,000), Alexa donkey anti-mouse 647 (Invitrogen, A31571, 1:1,000), Alexa donkey anti-mouse 488 (Invitrogen, A21202, 1:1,000), Alexa donkey anti-rat 647 (Invitrogen, A21247, 1:1,000), Alexa donkey anti-rat 488 (Invitrogen, A21208, 1:1,000). For weak signals, the horseradish-peroxidase-conjugated secondary antibodies were used for signal amplification using a Tyramide signal amplification kit (PerkinElmer). Finally, the slides washed in PBS followed by mounting with medium. Images were obtained using the Olympus FV1200, FV4000 confocal system and the Zeiss 880 confocal system. Images were analysed using ImageJ (NIH, v.2.3.0/1.53q).

Lung tissue dissociation and flow cytometry

Lung epithelium was isolated according to previously established protocols³. The mice were euthanized, and the lungs were perfused with cold PBS through the right ventricle to eliminate blood cells, followed by the injection of protease solution consisting of collagenase type I (500 U ml⁻¹; Gibco 17100–017), elastase (4 U ml⁻¹; Worthington Biochemical Corporation LS002279), dispase (5 U ml⁻¹; BD Biosciences 354235) and DNase I (0.33 U ml⁻¹; Worthington Biochemical, LS002139) in DMEM (Gibco, 11965092) through the trachea. Subsequently, the lungs were minced and incubated in 4 ml of protease solution for 30 min at 37 °C with continuous agitation. The tissue fragments were then dissociated by pipetting during the digestion process, followed by adding equal volume of the DMEM/F12 with 10% FBS to terminate the digestion. After being centrifuged at 1,000g for 5 min at 4 °C and washing with DMEM, the precipitate was resuspended in 3 ml enzyme mixture of 0.1% Trypsin-EDTA (Gibco, 25200072) containing DNase I and incubated for 20 min at 37 °C with agitation.

The digestion was then terminated by addition of DMEM/F12 with 10% FBS, after which the cells were sequentially filtered through 100 µm strainers and centrifuged at 1000g for 5 min at 4 °C. The red blood cells were lysed by incubation of red blood cell lysis buffer (2–5 ml, eBioscience, 00-4333-57) at room temperature for 5 min. The process was ended by adding 8–10 ml PBS and then centrifuging at 1,000g for 5 min at 4 °C. The precipitate was resuspended in block solution (eBioscience, 14-0161, 1:100) for 5 min to block nonspecific antigens before further staining. The cells were then incubated with primary antibodies at 4 °C for 30 min. The following antibodies were used: CD45 APC-eFluor 780 (eBioscience, 47-0451-82, 1:200), Ter-119 APC-eFluor 780 (eBioscience, 47-5921-82, 1:200). Next, the cells were washed with PBS, followed by

centrifugation. Subsequently, the cells were resuspended in PBS supplemented with DAPI and DNase I.

Bulk AT2 cells were excluded using Lyso-Tracker by staining the lysosomes in AT2 cells. For Lyso-Tracker staining, the cells were resuspended in DMEM containing LysoTracker (Invitrogen, Lyso-Tracker Deep Red, L12492) and incubated at 37 °C for 10 to 15 min. The cells were next centrifuged at 1,000g for 5 min at 4 °C, and then washed with PBS. Subsequently, the cell pellets were resuspended in PBS supplemented with DAPI and DNase I. For scRNA-seq, the AT2-tracer3, BASC-tracer and club-tracer cells were sorted from *Scgb1a1-creER;Sftpc-dreER;R26-TLR* lungs at 4 weeks and 6 months after naphthalene injury. For scATAC-seq, the AT2-tracer2 cells were sorted from *Scgb1a1-creER;Sftpc-dreER;R26-NR* lungs at 4 weeks and 6 months after naphthalene injury. All cells in the single-cell suspension were negatively selected for CD45 and Ter119, and then gated the tdT^+ZsG^- cells as club-tracer, tdT^+ZsG^+ cells as BASC-tracer and $\text{tdT}^+\text{ZsG}^+\text{Lyso-Tracker}^{\text{low}}$ cells as AT2-tracer3. The Sony MA900 and BD FACS Aria systems were used for flow cell sorting. Fluorescence-activated cell sorting (FACS) data were analysed using FlowJo v.10.4 (FlowJo). Representative gating strategies used in this study are shown in Supplementary Fig. 1.

In vitro organoid culture

Mouse AT2 or club organoid experiments were performed as previously described^{2,62}. tdT^+GFP^- AT2 cells or tdT^+GFP^+ club cells were sorted from *Sftpc-creER;R26-tdT;Scgb1a1-DTR-GFP* mice. Freshly sorted cells were resuspended in AddMEM/F12 (Gibco, 12634010) after centrifugation and counted to calculate the cell number. For each droplet, 8,000–12,000 cells in 30 μl AddMEM/F12 were mixed gently with Matrigel (Corning, 354230) at a ratio of 1:1 by pipetting. Then, 60 μl of the mixture was placed at the bottom of the each well of a 24-well culture plate, or 30 μl was placed into each well of LabTek 8 chamber slides (Thermo Fisher Scientific, 177445), respectively. Next, the plates or 8-chamber slides were placed in a 37 °C incubator for 20–30 min to solidify the Matrigel, followed by addition of 600 μl culture medium per well. AT2 culture medium was prepared as previously reported⁶²: Advanced DMEM/F12 supplemented with N2 (Gibco, 17502048), B27 (Gibco, 17504044), antibiotic–antimycotic (Gibco, 15240062), 15 mM HEPES (Gibco, 15630080), GlutaMAX (Gibco, 35050061), 1.25 mM *N*-acetyl-L-cysteine and 10 μM SB431542 (ABCam, Ab120163), 3 μM CHIR99021 (StemCell, 72052), 1 μM BIRB796 (StemCell, 72682), insulin-transferrin-selenium (Thermo Fisher Scientific, 41400045), 50 ng ml^{-1} human EGF (Thermo Fisher Scientific, PHG0313), 10 ng ml^{-1} mouse FGF10 (R&D, 6224-FG-025), 10 ng ml^{-1} mouse noggin (Thermo Fisher Scientific, 250-38) and 5 $\mu\text{g ml}^{-1}$ heparin. For the first 4 days, 10 μM Y-27632 (Selleck, S1049) and 10 ng ml^{-1} mouse IL-1 β (BioLegend, 575104) was additionally added. Club cell culture medium was prepared according to manufacturer's instructions: bioGenous Mouse Airway Organoid Basal Medium A supplemented with Supplement B and Supplement C (bioGenous, K2203-MA). The medium was changed every other day. Organoids were captured using the Olympus CKX53 microscope and collected at day 7–10. Analysis of colony-forming efficiency and size of organoids was performed at 7 days after plating.

Whole-organoid immunostaining

Organoids were cultured in LabTek 8 chamber slides (Thermo Fisher Scientific, 177445) and fixed with 500 μl of 4% PFA for 30 min at room temperature. Wells were gently washed three times with PBS for 5 min each at room temperature. For immunostaining, organoids were blocked with 0.5% Triton X-100 in PBS containing 5% NDS for 30 min at room temperature. Primary antibodies diluted in 0.5% Triton X-100 in PBS were added to each well and incubated at 4 °C overnight. After three washes with PBS, secondary antibody incubation was performed for 2 h at room temperature. For organoid fixation and sectioning, organoids were fixed with 4% PFA at 4 °C for 2 h or at room temperature for 1 h, respectively, followed by washing with PBS. For cryosectioning,

organoids from submersion cultures were either embedded directly in OCT or first immersed in 1% agarose and then embedded in OCT before sectioning (8–10 μm).

Wound healing assay

Migration assay was performed and developed as previously reported⁶³. In brief, MLE-12 (Sunnecell, SNL-414) were seeded into 24-well culture plates and cultured until they reached 80% confluence. A sterile 200 μl pipette tip was used to gently scratch the cell monolayer in a straight line across the centre of each well, with the long axis of the tip held perpendicular to the well surface to ensure a uniform gap width. After scratching, wells were washed gently with PBS and replenished with fresh medium with or without indicated treatments: the SPP1 groups were supplemented with SPP1 (1 $\mu\text{g ml}^{-1}$, MedChemExpress, HY-P78358, resolved in 1% BSA to obtain 500 ng ml^{-1} solution) and the anti-ITGB1 groups were supplemented with ITGB1 antibody (1:100, Millipore, MAB1997), and control groups were supplemented with an equivalent volume of 1% BSA. Images of the same scratch area were captured at 0 h and 24 h (end point) using the Olympus CKX53 microscope. The wound area (px^2) was measured using ImageJ software (NIH) at 0 h (A_0) and at the end point (A_1). The percentage of wound closure was calculated using the formula: wound closure rate (%) = $(1 - A_1/A_0) \times 100\%$. Each condition was tested in triplicate, and five independent experiments were performed. Results are presented as mean $\pm$ s.d.

Transwell assay

The migratory capacity of MLE-12 was assessed using 24-well Transwell inserts with 8.0- μm -pore-size polycarbonate membranes (Corning, 353097).

For migration assays, $2-3 \times 10^5$ epithelial cells were resuspended in 150 μl of DMEM/F12 containing with 1% FBS and seeded into the upper chamber. The lower chamber was filled with 600 μl of complete medium (DMEM/F12 with 10% FBS) with or without the indicated treatments (recombinant SPP1 or anti-ITGB1 antibody).

For co-culture experiments, primary CD45⁺ lung immune cells were sorted using FACS and 2×10^5 cells were seeded into the bottom of 24-well plate in complete medium: RPMI-1640 (Thermo Fisher Scientific, 22400089) with 10% FBS (with or without SPP1 inhibitor (100 μM , R&D, AF808)). Cells were incubated for 2–6 h before the addition of the Transwell insert to allow macrophage adherence. Subsequently, $2-3 \times 10^5$ epithelial cells were resuspended in 150 μl of RPMI-1640 containing 1% FBS and seeded into the 24-well Transwell insert.

After incubation at 37 °C in 5% CO₂ for 16 h, non-migrated cells on the upper surface of the membrane were gently removed with a cotton swab. Migrated cells that attached to the lower surface were fixed with 4% PFA for 15–30 min at room temperature and stained with 0.5% crystal violet. For quantification, five randomly selected fields per membrane were imaged using the Olympus IX73 microscope. The area of migrated cells was measured using ImageJ software (NIH), and the results were expressed as the relative coverage rate, defined as the migrated area in the experimental group normalized to that in the control group (control = 1). Each condition was tested in triplicate, and at least five independent experiments were performed. The results are presented as the mean $\pm$ s.d. of migrated cells per field.

3D reconstruction

Three-dimensional image data processing was performed using Imaris software (v.9.5.1) (Bitplane, <http://www.bitplane.com/Imaris/Imaris>). The Surface module was used for 3D reconstruction. Reconstructed surfaces were visualized and analysed for morphological features as indicated.

RNA preparation and RT-qPCR

The total RNA of isolated cells sorted by FACS was extracted using Trizol reagent (Invitrogen, 15596018) according to the manufacturer's

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protocol. The Prime Script RT kit (Takara, RR047A) was used to generate cDNA using the reverse transcription (RT) procedure. Quantitative PCR (qPCR) was performed with SYBR Green qPCR master mix (Thermo Fisher Scientific, 4367659) and QuantStudio 6 Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific). The *Gapdh* expression level was used as the internal control in this paper.

The primer sequences for qPCR were as follows: forward primer of *Rbpj*: ACTGTTCAATCGCTTCGGT; reverse primer of *Rbpj*: TGACAGTCTGCCCCGAATGG; forward primer of *Itgb1*: CAAGCAGGCCCCAATTGTGG; reverse primer of *Itgb1*: GAAGCAGCTGTGTGGTTGG; forward primer of *Notch2*: GGACTGCCAATACTCCACCT; reverse primer of *Notch2*: GCTTCATTTTCGCAGGGAT; forward primer of *Cldn4*: CTTCATCGGCACCAACATCG; reverse primer of *Cldn4*: GATGACCATAAGGGCTCGGG.

Naphthalene-induced lung injury

Bronchiolar injury was induced by naphthalene as previously described³³. Naphthalene (Sigma-Aldrich, 84679) was freshly dissolved in sterile corn oil (25 mg ml⁻¹). For severe bronchiolar injury, the adult mice were treated with 500 mg per kg naphthalene or vehicle (corn oil) by intraperitoneal injection. The lung tissues were collected at the indicated time.

In vivo SPP1 inhibition

For in vivo inhibition of SPP1, the SPP1/OPN expression inhibitor (HY-146064; MedChemExpress) was administered to mice through intraperitoneal injection at a dose of 5 mg per kg body weight once every other day for 4 consecutive weeks. The compound was first dissolved in DMSO (Sigma-Aldrich, D2650) and then diluted with a vehicle comprising PEG300 (HY-Y0873; MedChemExpress), Tween-80 (HY-Y1891; MedChemExpress) and PBS to a final concentration of 2.5 mg ml⁻¹. Control mice received an equal volume of vehicle alone.

Pulmonary function and airway responsiveness measurements

All animal procedures were performed in accordance with institutional guidelines and approved protocols. Mice were deeply anesthetized with an intraperitoneal injection of tribromoethanol (Thermo Fisher Scientific, T48402-25G, 250 mg per kg) to ensure adequate anaesthesia throughout the procedure. After confirmation of loss of pedal reflex, a tracheotomy was performed, and the mice were placed into a whole-body plethysmograph (EMMS) connected to a flow measurement system for baseline lung function assessment. Functional residual capacity, forced vital capacity, as well as chord compliance were measured. For airway responsiveness measurements, under the same deep anaesthesia, mice were connected to a resistance and compliance system (EMMS) through the plethysmograph. Mice were challenged with increasing doses of aerosolized acetylcholine (Sigma-Aldrich, A6625): 0, 8, 16, 32, 64, 128 and 256 mg ml⁻¹, each for 3 min. Lung resistance was recorded and expressed as the percentage change from the baseline lung resistance measured during nebulization of PBS. Data acquisition was performed using eDacq (v.1.9.9) software. At the end of the experiments, animals were euthanized under deep anaesthesia by an approved method.

Single-cell library preparation

For single-cell 5' transcriptomic profiling, FACS-purified cell suspensions were processed using the Chromium Next GEM Single Cell 5' Reagent Kit (v2 or v3 Chemistry). Specifically, cell suspensions were loaded into the 10x Chromium controller for gel bead-in-emulsion (GEM) generation. Reverse transcription, cDNA amplification and library construction were conducted in accordance with the manufacturer's instructions.

For single-cell multiome analysis, nuclei were isolated from FACS-sorted cells according to the manufacturer's instructions (10x Genomics). Joint profiling of chromatin accessibility (ATAC) and gene expression (RNA) was performed on the Chromium platform. Specifically,

nuclei were subjected to an in-bulk transposition reaction using the Chromium Next GEM Single Cell Multiome ATAC+Gene Expression Kit. After transposition, transposed nuclei were loaded for GEM generation. This system allows for the simultaneous capture of ATAC fragments and poly-adenylated mRNA from the same individual nucleus through unique barcoding. ATAC and GEX (cDNA) libraries were independently amplified and indexed as per the standard 10x Genomics protocol.

Libraries were pooled and sequenced on the Illumina NovaSeq 6000 platform in paired-end 150 bp mode. Each library was sequenced to a total depth of approximately 200 GB of raw data, ensuring an average sequencing depth exceeding 50,000 read pairs per cell/nucleus.

Stereo-seq library construction

Fresh lung tissue was collected following intratracheal instillation of pre-chilled 20% Tissue-Tek OCT (Sakura, 4583) in sterile 1× PBS, then washed with 4 °C PBS. Lung lobes were dissected and immediately frozen on dry ice. The tissue was subsequently transferred to and stored in a -80 °C freezer. Before sectioning, the lung tissue was rewarmed to -20 °C. The commercialized Stereo-seq protocol was performed as previously described (201ST13114, Stereo-seq Kit for FF, v.1.3, STOmics)⁶⁴. In brief, 10 µm cryosections were adhered to Stereo-seq ChipT and incubated at 37 °C for 5 min. The sections were then fixed in methanol (Sigma-Aldrich, 34860-1L-R) for 30 min, followed by permeabilization at 37 °C for 12 min. After washing with 0.1× SSC (Thermo Fisher Scientific, Invitrogen, AM9770) supplemented with 2 U µl⁻¹ RNase inhibitor (Thermo Fisher Scientific, E00382), the released RNA was captured by oligo probes and subjected to reverse transcription for 2 h. cDNA was then released from the chip by incubation at 55 °C for 10 min, denatured, amplified and purified using 0.8× SPRIselect (Beckman, B23318), and quantified with the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Invitrogen, Q32854). The Stereo-seq Library Preparation Kit v1.1 was used for library construction according to the manufacturer's instructions. A total of 100 ng cDNA was used as template. After cDNA multiple displacement amplification and single-stranded DNA (ssDNA) purification, the ssDNA concentration was quantified using the Qubit ssDNA Assay Kit (Thermo Fisher Scientific, Invitrogen, Q32856). After purification, the product underwent a second round of amplification. Subsequently, the PCR product was subjected to double-sided magnetic bead selection using room-temperature equilibrated beads at ratios of 1:0.55 and 1:0.15. Sequencing was performed on the MGI Tech (DNBSEQ-T7) platform.

Single-cell transcriptomic data processing and analysis

Raw sequencing reads were trimmed using Trim Galore (v0.6.7) with the parameters '--quality 20 --illumina --stringency 13 --length 150 --paired' to remove adapter sequences and filter low-quality reads. The cleaned reads were then aligned to a customized mm10 mouse reference genome using Cell Ranger (v.6.1.1) 'cellranger count' with the default parameters to generate gene expression matrices.

Downstream analysis was conducted using Seurat (v.4.1.2). To ensure high-quality transcriptomic profiles, we excluded cells with fewer than 1,000 detected genes and a mitochondrial gene content exceeding 10%. For lineage-specific analysis, fluorescence-labelled populations, comprising AT2-tracer3, BASC-tracer and club-tracer cells, were identified and extracted based on reporter transcript levels.

The filtered data were normalized to adjust for sequencing depth variations, after which highly variable genes were systematically identified. To mitigate technical biases, scaled expression matrices were generated by regressing out potential technical confounders, including cell cycle effects, mitochondrial content and sequencing depth. Dimensionality reduction was performed using principal component analysis (PCA) based on highly variable genes. The cells were then projected into UMAP space with the top significant principal components (PCs) as input. Subsequently, cell clusters were identified through unsupervised clustering analysis. Potential outlier cells were identified

and subsequently exclude from downstream analyses using DBSCAN (v1.1-10) with empirically determined parameters. Cluster-specific marker genes were determined through differential expression analysis, followed by cell type annotation using known lung epithelial gene signatures. To integrate multiple datasets, we used a canonical correlation analysis-based integration to harmonize cross-batch variations while preserving biological heterogeneity.

Cell differentiation trajectories were reconstructed by applying diffusion map analysis (destiny v.3.20.0) to infer pseudotemporal ordering. For functional enrichment analyses, we performed Gene Ontology (GO) and KEGG terms enrichment analyses using R package limma (v.3.62.1) and clusterProfiler (v.4.14.4). Cell type signatures were derived from published datasets: club/AT2 cells³³, pre-AT1 transitional cells (PATS)^{29,65} and Notch signalling genes (GO:0007219). Enrichment scores were calculated as the mean of z-score normalized expression of signature genes.

Intercellular signalling networks were systematically inferred using CellChat (v.1.6.1) following the standard pipeline with default parameter. In brief, we used the standard mouse signalling database to compute communication probabilities and identify significant signalling hubs. Separate CellChat objects were constructed for different experimental conditions and subsequently merged to enable comparative analysis.

Single-cell multiome sequencing and analysis

After sequencing, raw fastq files were processed using Cell Ranger ARC (v.2.1.0) against the customized mm39 reference genome. Initial quality control was performed using Signac (v.1.16.0) and Seurat (v.5.3.0). Nuclei were filtered based on standard quality control metrics for downstream analysis. Specifically, we retained only those nuclei with a total fragment count between 1,000 and 150,000 and total read count ranging from 800 to 75,000. Furthermore, the analysis was restricted to nuclei exhibiting a mitochondrial transcript proportion of less than 20%, and a transcription start site (TSS) enrichment score exceeding 4.

To resolve cell identities across homeostatic and injured conditions, we performed cross-modality integration. For the RNA modality, data were normalized using the SCTransform function, and integration anchors were identified in the PCA space. For the ATAC modality, peak calling was performed on each sample, and a unified peak set was generated by merging and reducing overlapping genomic ranges. Term frequency-inverse document frequency normalization and latent semantic indexing were applied for ATAC dimensionality reduction. We used WNN analysis to construct a multimodal graph based on both integrated SCT and integrated latent semantic indexing embeddings, followed by UMAP visualization and clustering.

Lineage-specific epigenetic signatures were defined by identifying differentially accessible regions between homeostatic AT2 cells and native club cells using the FindMarkers function. Single-cell epigenetic signature scores were computed using the AddChromatinModule function, which aggregates normalized signals from lineage-specific peaks while controlling for technical biases.

Transcriptional maturation of AT2-derived cells was assessed using the AddModuleScore function. Representative gene sets were curated for native club cell functions: secretory and immune (such as *Scgb1a1*, *Scgb3a2* and *Muc5b*).

Spatial transcriptomic analysis

Spatial transcriptomic libraries were generated using the Stereo-seq platform as indicated above. Raw data were processed to generate coordinate-annotated gene expression matrices at cellular resolution using SAW (v.8.2.2) and downstream analysis was primarily conducted using Stereopy (v.1.6.2). To ensure high-quality data, cells were filtered based on the following criteria: a minimum of two hundred total counts, at least ten detected genes and a mitochondrial content of no more than 6%. Cells characterized by high haemoglobin levels, specifically those with a haemoglobin content reaching or exceeding 5%, were excluded

from the analysis to minimize potential contamination or artefacts. Gene-level filtration was performed using pyBioMart to retain only protein-coding genes, while removing genes located on X and Y chromosomes to minimize sex-linked bias.

Filtered count matrices were normalized using scTransform function. Highly variable genes were identified, and PCA was performed. A neighbourhood graph was constructed in the PCA space (dim = 50), followed by UMAP visualization and Leiden clustering. Cell identities were manually annotated by comparing cluster-specific marker genes with canonical lineage markers.

To investigate spatially defined microenvironments, we utilized a Lasso strategy to capture specific anatomical regions. Genomic coordinates were mapped back to the cellular bins to categorize nuclei into bronchiolar regions and alveolar regions. For robust comparative analysis, we generated pseudobulk expression profiles by aggregating raw counts from immune cells localized within the bronchiolar regions. Differential expression analysis was performed using the DESeq2 (v.1.46.0). GSEA and GO enrichment were conducted to identify functional pathways associated with spatially restricted injury responses.

To identify gene expression patterns that are spatially informative independent of predefined clusters, we used the Hotspot algorithm⁶⁶. This approach defines informative genes as those of which the transcriptional variation aligns with the spatial proximity of cells. We assessed spatial autocorrelation to identify genes with non-random spatial distributions across the tissue. Subsequently, significant spatial modules were defined by grouping genes that exhibited high local correlations, representing coordinated expression variation among spatially neighbouring cells. These module scores were then projected onto spatial coordinates to visualize regional functional heterogeneity.

Statistical analyses

Mice were randomly assigned to experimental groups, with no blinding of sample processing or data collection. Mice that died in the experiments were not used for analysis. For quantification, all five lung lobes from each mouse were analysed. From each mouse, we examined at least ten non-consecutive lung tissue sections. Within each section, a minimum of five 10× fields containing airways was analysed, resulting in the assessment of at least 50 fields per mouse. Representative images are shown from at least three independent biological replicates, which yielded similar results. For statistical analysis, the data from all fields for each mouse were first averaged to generate a single composite value per animal (for example, the percentage of lineage-positive cells). Thus, in the lineage tracing study, the statistical sample size (n) refers to the number of mice. For single-cell and spatial transcriptomics analyses, n refers to the number of cells or fields, respectively, as indicated in the corresponding figure legends. Normality (Shapiro–Wilk test) and homogeneity of variance (F -test) were assessed before analyses. Data from ≥ 3 independent experiments are presented as mean $\pm$ s.d. Initially, data were subjected to comparison across groups by conducting a Shapiro–Wilk normality test and an equal variance test. Two-group comparisons were performed using unpaired two-tailed Student's t -tests (equal variance) or Welch's t -tests (unequal variance); non-normal data were analysed using Mann–Whitney U -tests. Multigroup comparisons were performed using one-way ANOVA with Tukey's test (normal data) or Kruskal–Wallis test with Dunn's test (non-normal data). All data were acquired in this study from at least three independent experiments and presented as mean values $\pm$ s.d. as indicated in figure legends. Specific quantification methods and P values are indicated in the figures and legends; $P < 0.05$ was considered to be significant. All analyses used GraphPad Prism (v.9.3.1), R (v.4.4.2) and Python (v.3.8.18). All illustrations in this study were created by Photoline software (v.23.02).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The raw sequencing data generated in this study have been deposited in the Genome Sequence Archive (GSA) under accession PRJCA039649. Source data are provided with this paper.

Code availability

The codes executing the analysis workflow are available at Zenodo⁶⁷ with persistent access at <https://doi.org/10.5281/zenodo.21768825>.

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Author contributions K.L. and B.Z. conceived and designed the study and wrote the manuscript; K.L., Z. Liu., X.M., M.T., H.J., W.P. and H.Z. performed the experiments and contributed to data interpretation. K.L. and X.M. performed the in vitro cellular experiments. Z. Liu, M.T., Z.W. and X.Y. performed single-cell and spatial transcriptomics analyses. K.L., F.D. and C.L. conducted pulmonary function measurements. S.Y., Y.Z., Z. Lv, X.L., H.J., W.P. and H.Z. managed mouse breeding, genotyping and colony maintenance. F.L. and P.S. provided expert oversight and contributed to the writing of the manuscript. B.Z. supervised the project, coordinated collaboration among authors and assumed overall responsibility for the study. All of the authors approved the final manuscript.

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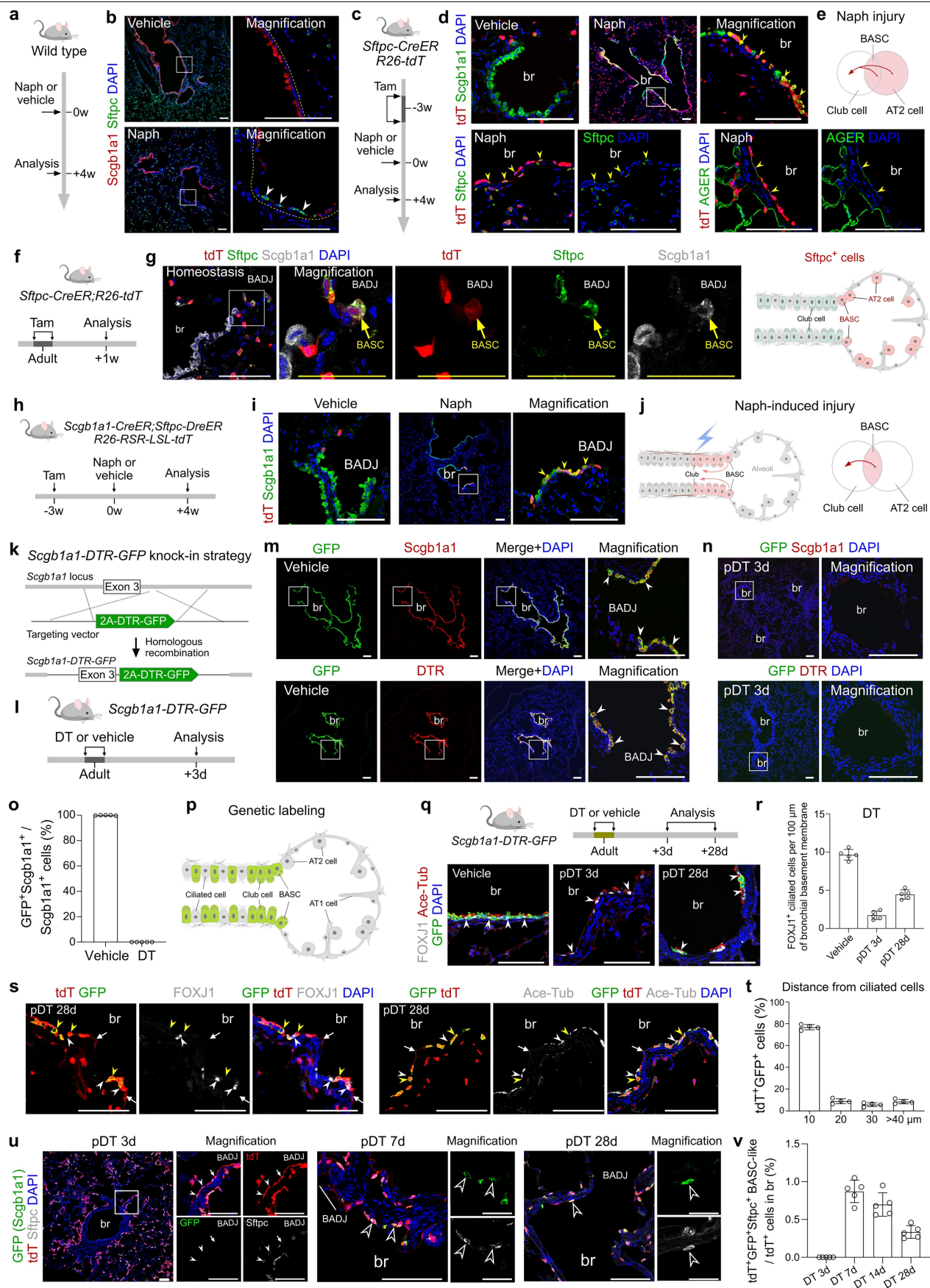
Additional information

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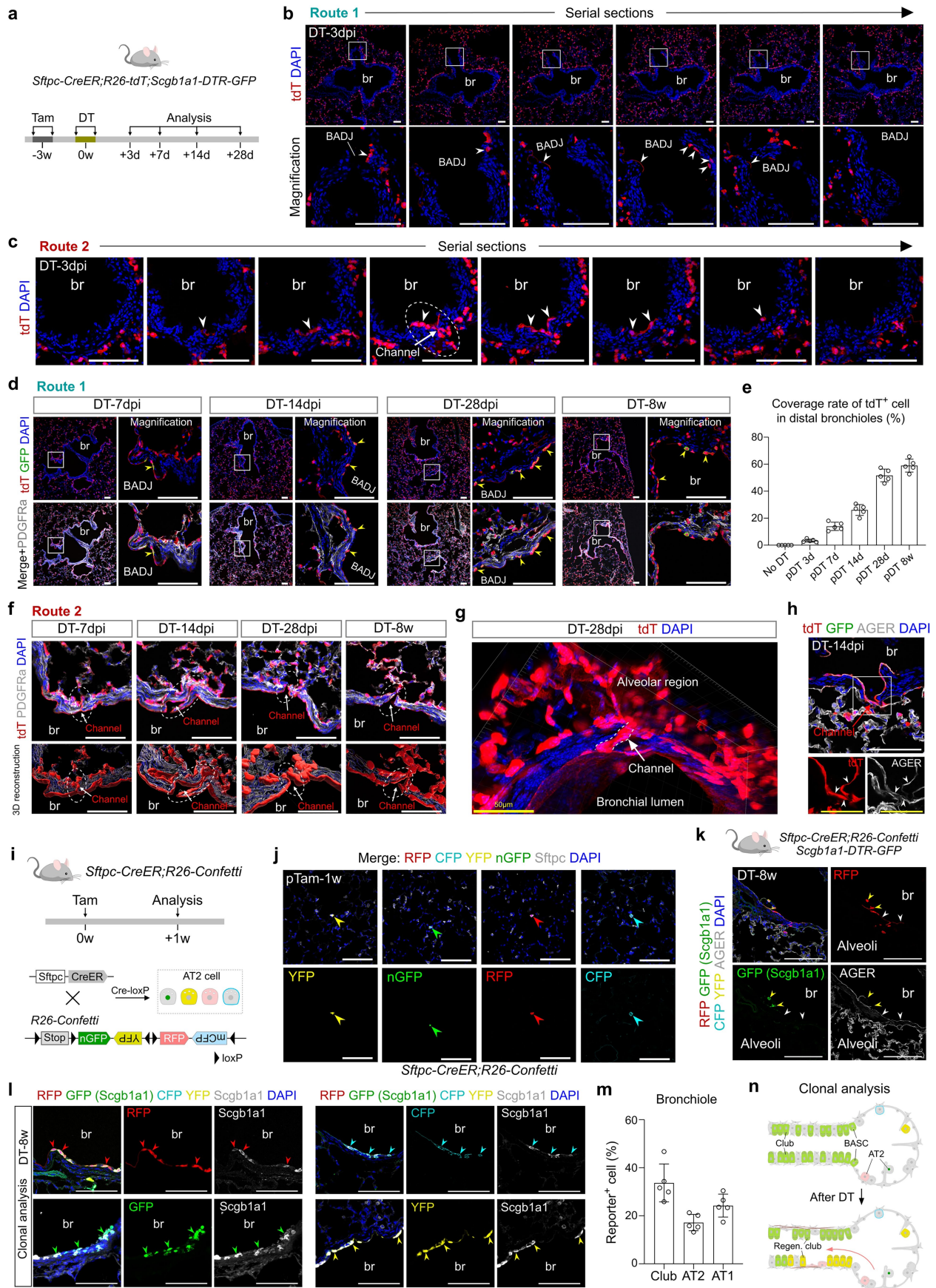


Extended Data Fig. 1 | See next page for caption.

Article

Extended Data Fig. 1 | Sftpc⁺ AT2 cells contribute to club cells after DT-induced bronchiolar injury. **a**, A schematic diagram illustrating the experimental design. **b**, Immunostaining for Scgb1a1 and Sftpc on lung sections of wild type mice following Naph-induced lung injury. White arrowheads indicate Sftpc⁺ cells. **c**, A schematic diagram illustrating the experimental design. **d**, Immunostaining for tdT, Scgb1a1, Sftpc, and AGER on lung sections following naphthalene (Naph) injury. Yellow arrowheads indicate tdT⁺Sftpc⁺ AT2 cells or tdT⁺AGER⁺ AT1 cells. **e**, A cartoon image showing that Sftpc⁺ cells (AT2 cell and BASC) contribute club cells after Naph injury. **f**, A schematic diagram illustrating the experimental design. **g**, Immunostaining for tdT, Sftpc, and Scgb1a1 on lung sections (left). Yellow arrowheads, tdT⁺Sftpc⁺Scgb1a1⁺ BASCs. A cartoon image showing *Sftpc-CreER*-mediated labelling of both BASCs and AT2 cells (right). **h**, A schematic diagram illustrating the experimental design. **i**, Immunostaining for tdT and Scgb1a1 on lung sections after Naph or vehicle treatment. Yellow arrowheads, tdT⁺Scgb1a1⁺ club cells. **j**, A cartoon image showing that BASCs contribute to club cells after Naph injury. **k**, A schematic diagram showing the knock-in strategy for *Scgb1a1-DTR-GFP* mice. **l**, Schematic of experimental design. **m,n**, Immunostaining for GFP, Scgb1a1, and DTR on lung sections after vehicle (PBS, **m**) or DT (**n**) treatment. White arrowheads indicate GFP⁺Scgb1a1⁺ and GFP⁺DTR⁺ club cells. **o**, Quantification of the percentage of

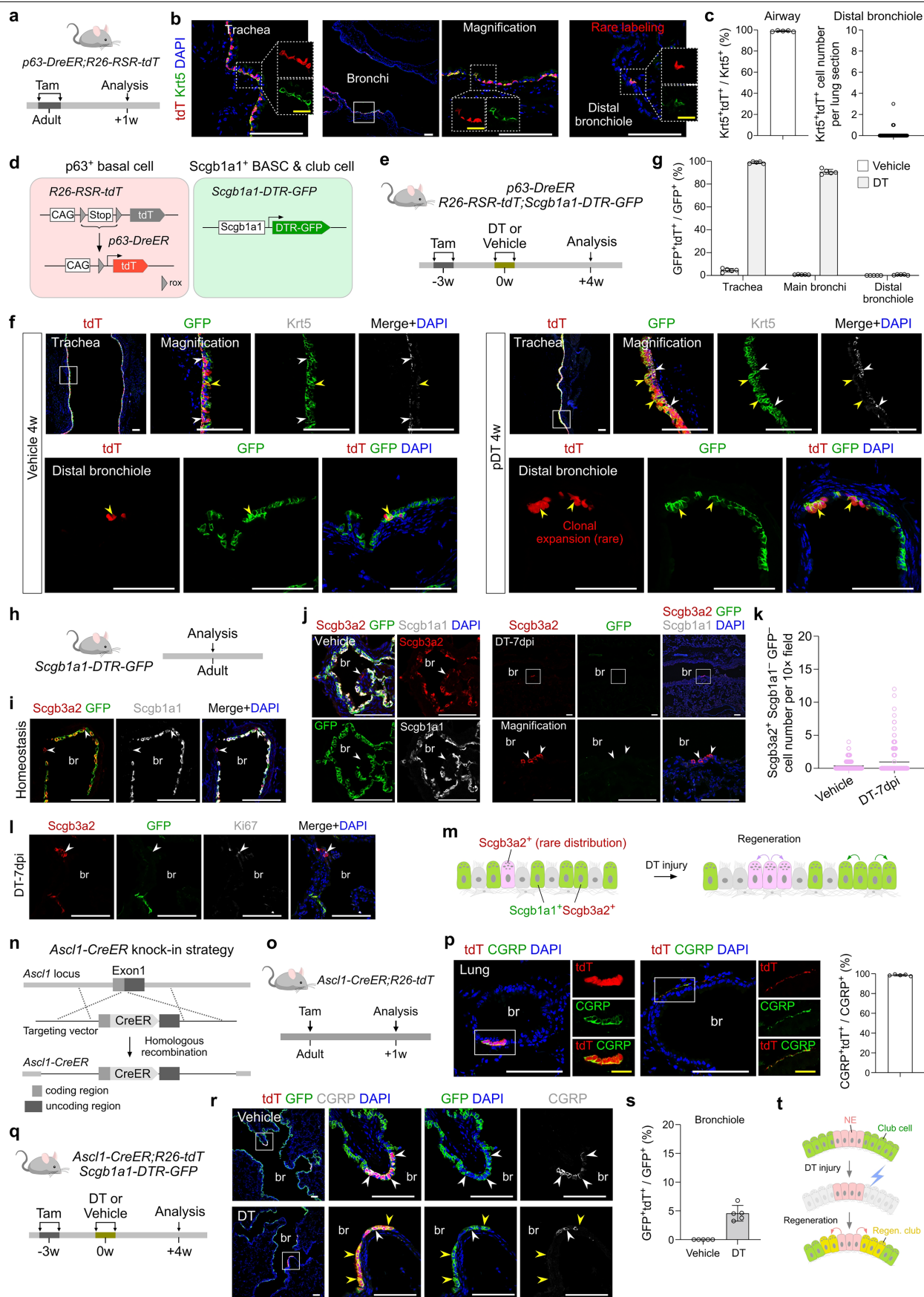
GFP⁺Scgb1a1⁺ club cells in (**m,n**). Data are mean $\pm$ SD. n = 5 mice. **p**, A cartoon image showing Scgb1a1⁺ club cells and BASCs are GFP⁺ in *Scgb1a1-DTR-GFP* mice. **q**, Schematic of experimental design (upper panel). Immunostaining for GFP, FOXJ1, and Ace-Tub on lung sections (lower panel). White arrowheads indicate FOXJ1⁺Ace-Tub⁺ ciliated cells. **r**, Quantification of FOXJ1⁺ cell number per 100 μ m of bronchiolar basement membrane. Data are mean $\pm$ SD; n = 5 mice. **s**, Immunostaining for tdT, GFP, FOXJ1, and Ace-Tub on lung sections of AT2-tracer1 mice after DT injury. Yellow arrowheads indicate tdT⁺GFP⁺ club cells; white arrowheads indicate tdT⁺FOXJ1⁺ or tdT⁺Ace-Tub⁺ ciliated cells; white arrows indicate AT1 cells. **t**, Quantification of the percentage of tdT⁺GFP⁺ cells with different distances from the ciliated cells. Data are mean $\pm$ SD; n = 4 mice. **u**, Immunostaining for tdT, GFP, and Sftpc on lung sections from AT2-tracer1 mice at different timepoints post-DT. White arrowheads indicate tdT⁺Sftpc⁺GFP⁺ AT2 cells; white arrows indicate tdT⁺Sftpc⁺GFP⁺ cells; white open arrowheads indicate tdT⁺GFP⁺Sftpc⁺ BASC-like cells. **v**, Quantification of the percentage of tdT⁺ bronchiolar cells that are BASC-like. Data are mean $\pm$ SD; n = 5 mice. Naph, naphthalene. DT, diphtheria toxin. br, bronchiole. BADJ, bronchoalveolar duct junction. Scale: white bars, 100 μ m; yellow bars, 50 μ m. Images in panels b, d, g, i, m, n, q, and u are representative of 5 independent biological samples, and panels of 4 samples.



Extended Data Fig. 2 | See next page for caption.

Extended Data Fig. 2 | AT2 cells enter bronchioles via BADJ and bronchiolar wall channels after DT injury. **a**, A schematic diagram illustrating the experimental design. **b,c**, Immunostaining for tdT on serial lung sections from AT2-tracer1 mice at 3 days post-DT, showing route 1 (b) and route 2 (c). White arrowheads indicate tdT⁺ AT2-lineage cells in both panels. **d**, Immunostaining of tdT, GFP, and PDGFR α on lung sections at different timepoints post-DT. Yellow arrowheads indicate tdT⁺GFP⁺ club cells. **e**, Quantification of the coverage rate of tdT⁺ in distal bronchioles at different timepoints post-DT. Data are mean $\pm$ SD; n = 5 mice. **f**, Immunostaining for tdT and PDGFR α , and 3D reconstruction on lung sections at different timepoints post-DT. White arrowheads and dashed circles indicate channels. **g**, Immunostaining and 3D imaging of 150- μ m-thick lung sections reveal tdT⁺ AT2-derived cells forming a channel that bridges the alveolar and bronchiolar compartments. **h**, Immunostaining for tdT, GFP, and AGER on lung sections at day 14 post-DT. White arrowheads indicate tdT⁺AGER⁺ cells within the channel. **i**, A schematic diagram illustrating the experimental design of clonal analysis. Single Sftpc⁺

cell could be labelled by RFP, nGFP, CFP, or YFP after Tam. **j**, Immunostaining of RFP, GFP, CFP, YFP, and Sftpc on lung sections. **k**, Immunostaining for RFP, GFP, CFP, YFP, and AGER on lung sections of *Sftpc-CreER;R26-Confetti;Scgb1a1-DTR-GFP* mice at 8 weeks post-DT. Yellow arrowheads indicate tdT⁺GFP⁺ club cells; white arrowheads indicate tdT⁺AGER⁺ AT1 cells. **l**, Immunostaining for RFP, GFP, CFP, YFP, and Scgb1a1 on lung sections. Red arrowheads, RFP⁺GFP⁺Scgb1a1⁺ club cells. Green arrowheads, nGFP⁺GFP⁺Scgb1a1⁺ club cells. Blue arrowheads, CFP⁺GFP⁺Scgb1a1⁺ club cells. Yellow arrowheads, YFP⁺GFP⁺Scgb1a1⁺ club cells. **m**, Quantification of the percentage of single reporter⁺ cells within bronchioles expressing Scgb1a1, Sftpc, and AGER. Data are mean $\pm$ SD; n = 5 mice. **n**, A cartoon image showing that the single AT2 cell could contribute to club cells, AT2 cells, and AT1 cells of bronchiolar region after DT injury. br, bronchiole. BADJ, bronchoalveolar duct junction. Scale: white bars, 100 μ m; yellow bars, 50 μ m. Each image is representative of 5 individual biological samples.



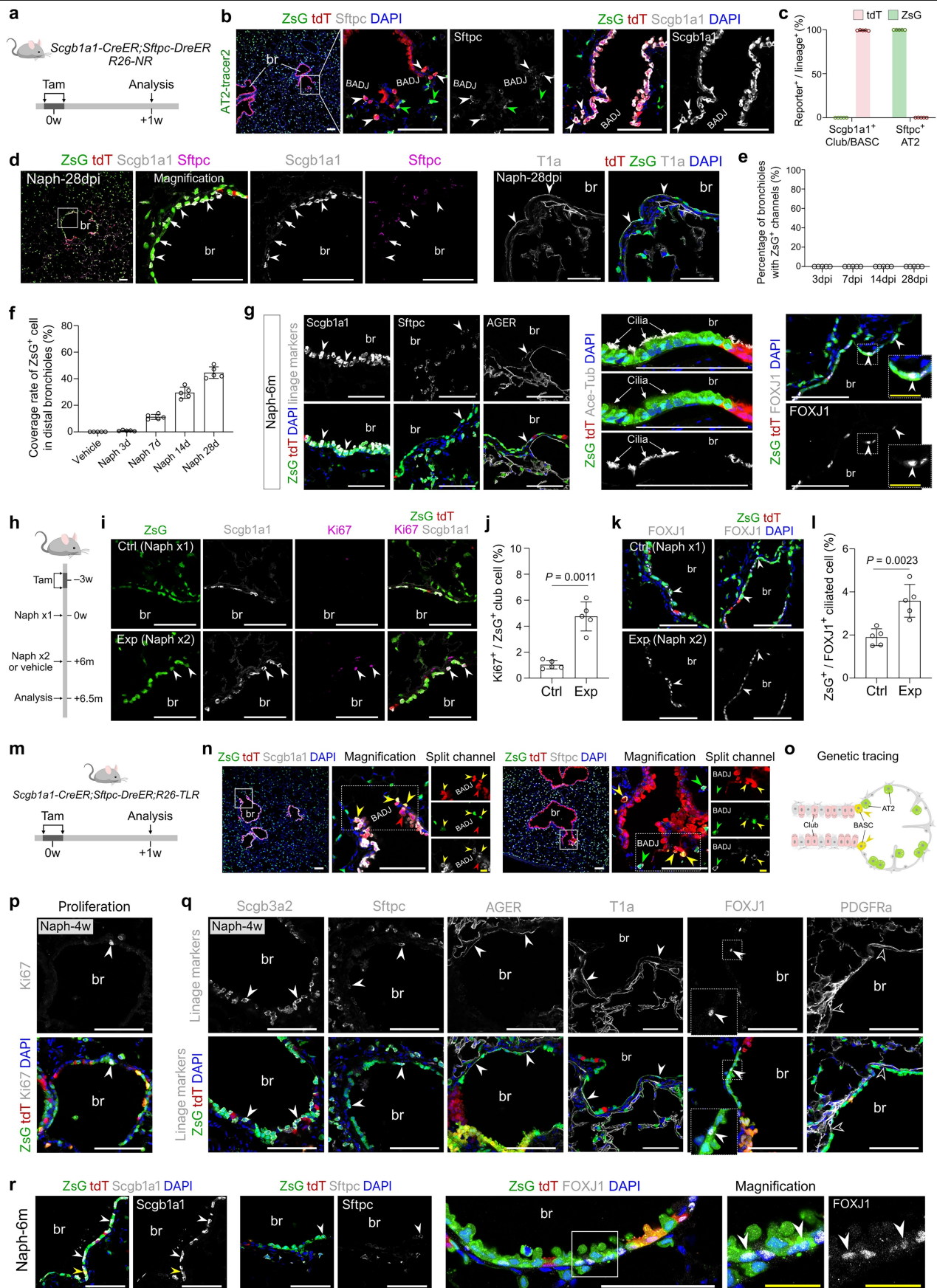
Extended Data Fig. 3 | See next page for caption.

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Extended Data Fig. 3 | Basal cells and NE cells contribute to club cell regeneration post-DT injury.

a, A schematic diagram illustrating the experimental design. **b**, Immunostaining for tdT and Krt5 on lung sections of *p63-DreER;R26-RSR-tdT* mice after Tam. **c**, Quantification of the percentage of Krt5⁺ cells expressing tdT (left panel, *n* = 5 mice, each individual dot represents one mouse). Quantification of cell number of Krt5⁺ cells expressing tdT per lung section (right panel, *n* = 4 mice, each individual dot represents one lung section). Data are mean ± SD. **d**, Schematic diagrams illustrating the strategy for tracing of p63⁺ basal cells. **e**, A schematic diagram illustrating the experimental design. **f**, Immunostaining for tdT, GFP, and Krt5 on lung sections of *p63-DreER;R26-RSR-tdT;Scgb1a1-DTR-GFP* mice at 4 weeks post-DT or vehicle (PBS) treatment. White arrowheads indicate tdT⁺Krt5⁺ basal cells; yellow arrowheads indicate tdT⁺GFP⁺ club cells. **g**, Quantification of the percentage of GFP⁺ club cells in trachea, main bronchi, and terminal bronchiole expressing tdT. Data are mean ± SD; *n* = 5 mice. **h**, A schematic diagram illustrating the experimental design. **i**, Immunostaining for Scgb3a2, GFP and Scgb1a1 on lung sections of *Scgb1a1-DTR-GFP* mice. White arrowheads indicate Scgb3a2⁺GFP⁺ Scgb1a1⁺ cells. **j**, Immunostaining of Scgb3a2, GFP and Scgb1a1 on lung sections of *Scgb1a1-DTR-GFP* mice after DT or vehicle (PBS) treatment. White arrowheads indicate Scgb3a2⁺GFP⁺ Scgb1a1⁺ cells. **k**, Quantification of the cell number of

Scgb3a2⁺GFP⁺ Scgb1a1⁺ cells per 10× field. Data are mean ± SD. Each individual dot represents one 10× field. *n* = 150 10× fields from 3 mice per group. **l**, Immunostaining for Scgb3a2, GFP and Ki67 on lung sections. **m**, A cartoon depicts rare Scgb3a2⁺Scgb1a1⁺ cells (also GFP⁺) distributed in the bronchiolar epithelium, which proliferate in response to DT-induced injury. **n**, A schematic diagram showing the knock-in strategy of *Ascl1-CreER* mice. **o**, A schematic diagram illustrating the experimental design. **p**, Immunostaining for tdT and CGRP on lung sections of *Ascl1-CreER;R26-tdT* mice after Tam (left). Quantification of the percentage of CGRP⁺ NE cells expressing tdT (right). Data are mean ± SD; *n* = 5 mice. **q**, A schematic diagram illustrating the experimental design. **r**, Immunostaining for tdT, GFP, and CGRP on lung sections of *Ascl1-CreER;R26-tdT;Scgb1a1-DTR-GFP* mice at 4 weeks post-DT or vehicle (PBS) treatment. White arrowheads indicate tdT⁺CGRP⁺ NE cells; yellow arrowheads indicate tdT⁺GFP⁺ club cells. **s**, Quantification of the percentage of GFP⁺ club cells expressing tdT in (**r**). Data are mean ± SD; *n* = 5 mice. **t**, A cartoon image illustrating regional regeneration of club cells by NE cells at bronchiolar branchpoint post-DT injury. Tam, tamoxifen. DT, diphtheria toxin. Scale: white bars, 100 μm; yellow bars, 25 μm. Images in panels b, f, i, l, p, and r are representative of 5 independent biological samples, and panel j of 3 samples.

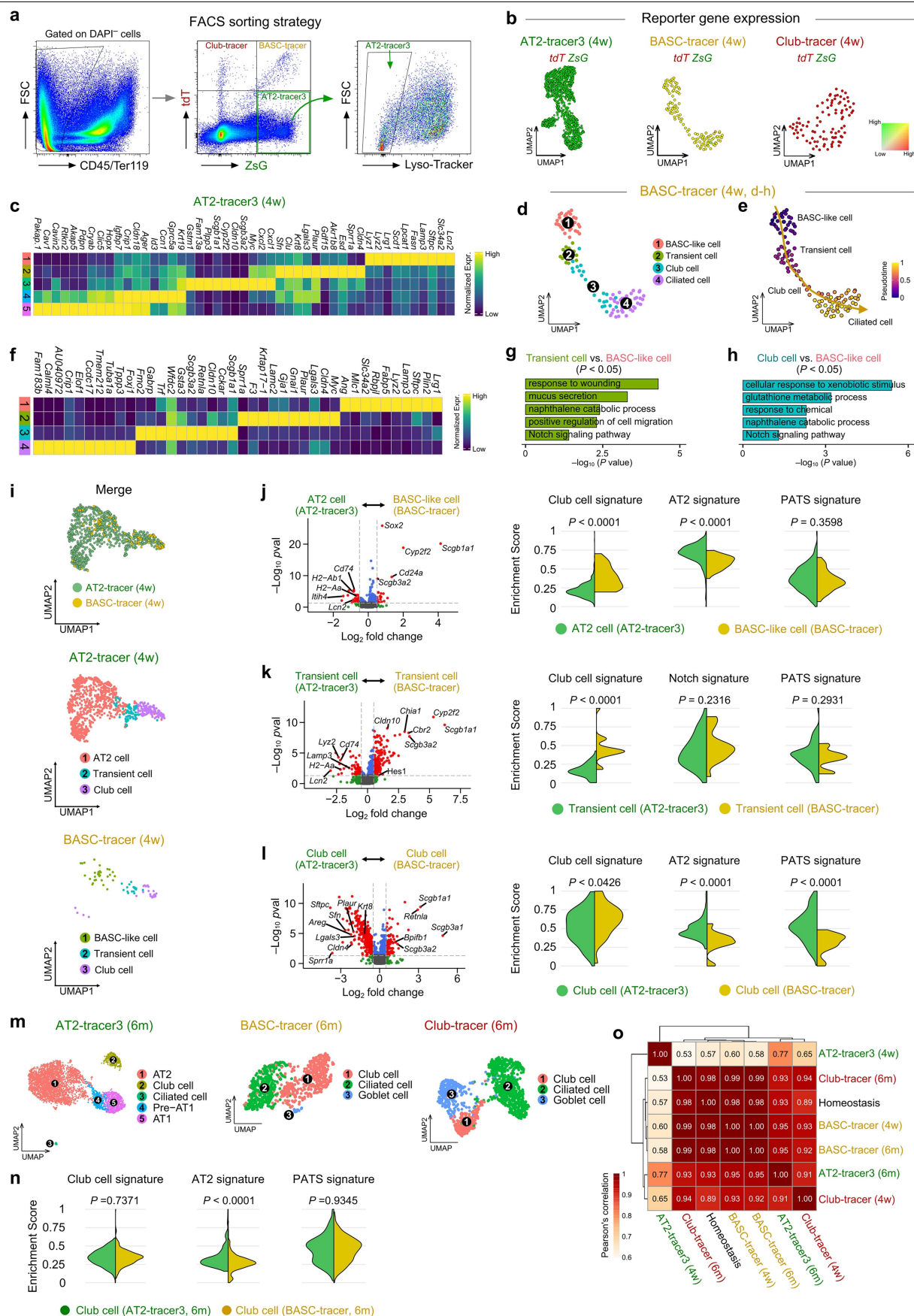


Extended Data Fig. 4 | See next page for caption.

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Extended Data Fig. 4 | AT2 cells regenerate bronchiolar epithelial cells following naphthalene injury. **a**, A schematic diagram illustrating the experimental design. **b**, Immunostaining for tdT, ZsG, Scgb1a1, and Sftpc on lung sections after Tam. White arrowheads indicate tdT⁺ZsG⁺Sftpc⁺ BASCs and tdT⁺ZsG⁺Scgb1a1⁺ BASCs. Green arrowheads indicate tdT⁺ZsG⁺Sftpc⁺ AT2 cells. **c**, Quantification of the percentage of Scgb1a1⁺ cells (club cells and BASCs) and AT2 cells labelled by tdT and ZsG, respectively. Data are mean ± SD; n = 5 mice. **d**, Immunostaining for tdT, ZsG, Scgb1a1 and Sftpc on lung sections at 4 weeks post-Naph (left). White arrowheads indicate tdT⁺ZsG⁺Scgb1a1⁺Sftpc⁺ club cells; White arrows indicate tdT⁺ZsG⁺Scgb1a1⁺Sftpc⁺ AT2 cells. Immunostaining for tdT, ZsG, and T1a on lung sections at 4 weeks post-Naph (right). White arrowheads indicate ZsG⁺T1a⁺ AT1 cells. **e**, Quantification of the percentage of bronchioles with ZsG⁺ channels at different timepoints post-Naph. Data are mean ± SD; n = 5 mice. **f**, Quantification of the coverage rate of ZsG⁺ cell in distal bronchioles at different timepoints after naphthalene injury or not. Data are mean ± SD; n = 5 mice. **g**, Immunostaining for tdT, ZsG, Scgb1a1, Sftpc, AGER, Ace-Tub, and FOXJ1 on lung sections at 6 months post-Naph. White arrowheads indicate tdT⁺ZsG⁺Scgb1a1⁺Sftpc⁺ club cells, tdT⁺ZsG⁺Sftpc⁺ AT2 cells, or tdT⁺ZsG⁺AGER⁺ AT1 cells; White arrows indicate tdT⁺ZsG⁺Ace-Tub⁺ or tdT⁺ZsG⁺FOXJ1⁺ ciliated cells **h**, A schematic diagram illustrating the experimental design. **i**, Immunostaining for tdT, ZsG, Scgb1a1 and Ki67 on lung sections of *Scgb1a1-CreER;Sftpc-DreER;R26-NR* mice subjected to one or two rounds of

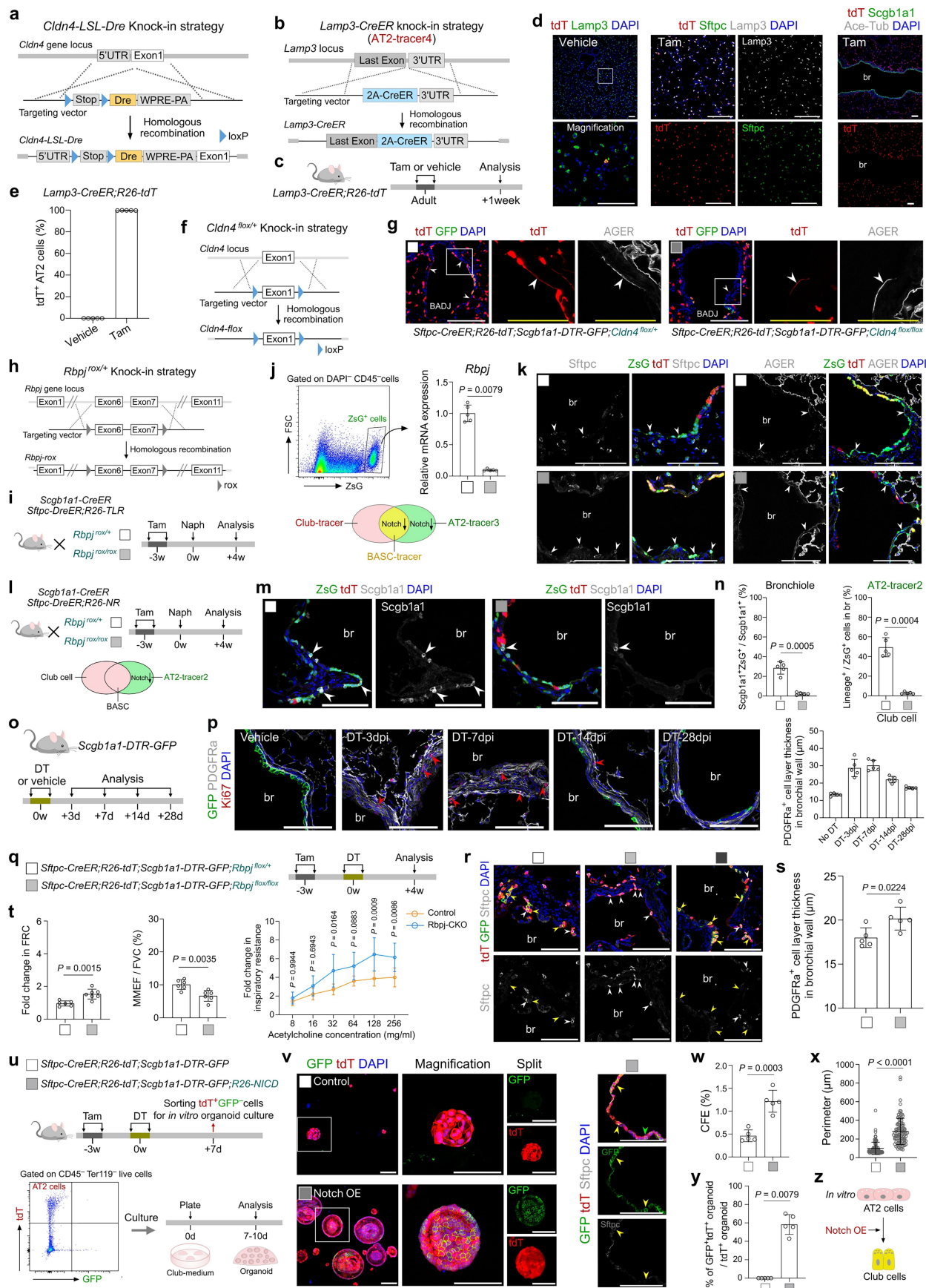
naphthalene injury. White arrowheads indicate ZsG⁺Scgb1a1⁺Ki67⁺ club cells. **j**, Quantification of the percentage of ZsG⁺ club cells co-expressing Ki67 in (**i**). Data are mean ± SD; n = 5 mice. Statistical analysis was performed using unpaired two-tailed Welch's *t*-test. **k**, Immunostaining of tdT, ZsG and FOXJ1 on lung sections after naphthalene injury. White arrowheads indicate ZsG⁺FOXJ1⁺ ciliated cells. **l**, Quantification of the percentage of FOXJ1⁺ ciliated cells expressing ZsG after naphthalene injury in (**k**). Data are mean ± SD; n = 5 mice. Statistical analysis was performed using unpaired two-tailed *t*-test. **m**, A schematic diagram illustrating the experimental design. **n**, Immunostaining for tdT, ZsG, Scgb1a1, and Sftpc on lung sections after Tam. Yellow arrowheads indicate tdT⁺ZsG⁺Sftpc⁺ and tdT⁺ZsG⁺Scgb1a1⁺ BASCs. Red arrowheads indicate tdT⁺ZsG⁺Scgb1a1⁺ club cells. Green arrowheads indicate tdT⁺ZsG⁺Sftpc⁺ AT2 cells. **o**, A cartoon image showing that the club cells, BASCs, and AT2 cells were simultaneously and distinctly labelled as red, yellow, and green signals after Tam. **p,q**, Immunostaining for tdT, ZsG, and Ki67 (**p**), as well as Scgb3a2, Sftpc, AGER, T1a, FOXJ1, and PDGFRα (**q**) on lung sections at 4 weeks post-Naph. **r**, Immunostaining of tdT, ZsG, Scgb1a1, Sftpc, and FOXJ1 on lung sections at 6 months post-Naph. White arrowheads indicate tdT⁺ZsG⁺Scgb1a1⁺ club cells, tdT⁺ZsG⁺Sftpc⁺ AT2 cells, and tdT⁺ZsG⁺FOXJ1⁺ ciliated cells. Tam, tamoxifen. Naph, naphthalene. br, bronchiole. Scale: white bars, 100 μm; yellow bars, 25 μm. Each image is representative of 5 individual biological samples.



Article

Extended Data Fig. 5 | Single-cell transcriptomic profiling of murine lung epithelial cells after naphthalene injury. **a**, FACS gating strategy for AT2-tracer3, BASC-tracer, and Club-tracer cells. All cells in the single-cell suspension were first negatively selected for CD45 and Ter119, and then gated the tdT⁺ZsG⁻ cells as Club-tracer, tdT⁺ZsG⁺ cells as BASC-tracer. Since the most cells in AT2-tracer3 were AT2 cells, then the tdT⁺ZsG⁺ cells were negatively selected for Lyso-tracker (targeting AT2 cells) to enrich the non-AT2 cells (such as intermediate cells, club cells) in AT2-tracer3. **b**, Co-expression of *tdT* (red gradient) and *ZsG* (green gradient) in AT2-tracer3, BASC-tracer, and Club-tracer cells. **c**, Heatmap of differential expression genes (DEGs) across AT2-tracer3 clusters. **d**, UMAP embedding of BASC-tracer cells (ZsG⁺tdT⁺) at 4 weeks post-injury, coloured by four distinct clusters. **e**, Pseudotime trajectory of BASC-to-club cell differentiation. Arrow indicates inferred directionality; colour scale reflects pseudotime progression. **f**, Heatmap of DEGs across BASC-tracer cluster. **g**, Enriched GO terms ($P < 0.05$) for DEGs between transient and BASC-like cells in BASC-tracer. Statistical significance was assessed using a one-sided hypergeometric test. **h**, Enriched GO terms ($P < 0.05$) of DEGs between club and BASC-like cells in BASC-tracer. Statistical significance was assessed using a one-sided hypergeometric test. **i**, Integrated UMAP embedding of AT2-tracer3 and BASC-tracer cells (4 weeks), annotated by origin (top) and cell type (middle/bottom). **j**, Volcano plot of DEGs between AT2-tracer3-derived AT2 cells and BASC-tracer-derived BASC-like cells (left). Violin plot of enrichment scores of club signature, AT2 signature, and PATS signature in AT2 cells from AT2-tracer3

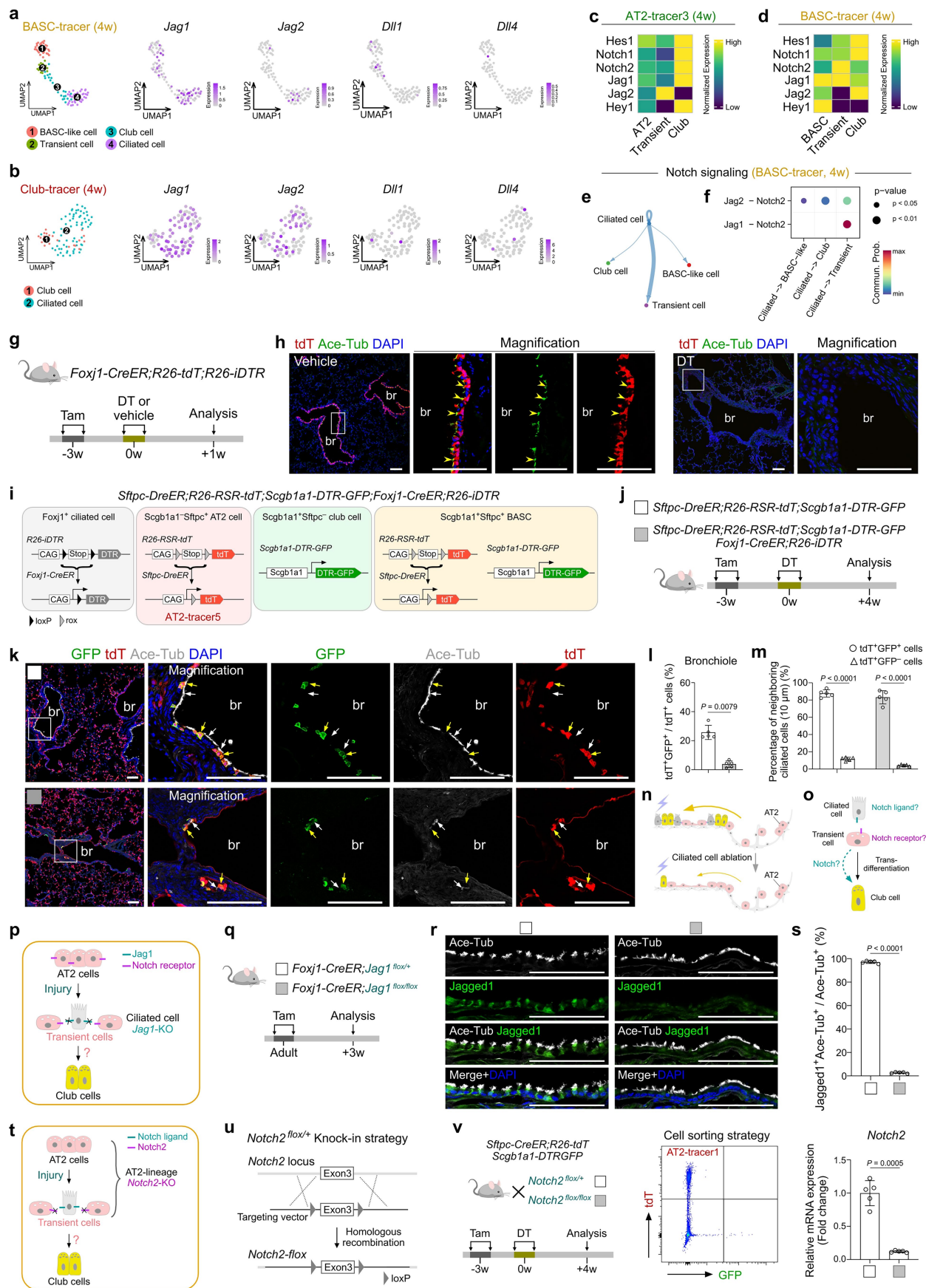
and in BASC-like cells from BASC-tracer (right). Statistical significance was assessed using the two-sided Mann-Whitney tests in both plots. **k**, Volcano plot of DEGs between the transient cells from AT2-tracer3 and BASC-tracer (left). Violin plot of enrichment scores of club signature, Notch signature, and PATS signature in transient cells from AT2-tracer3 and BASC-tracer (right). Statistical significance was assessed using the two-sided Student's *t*-test (Notch and PATS signature) or Mann-Whitney tests (club signature). **l**, Volcano plot of DEGs between the club cells from AT2-tracer3 and BASC-tracer (left). Violin plot of enrichment scores of club signature, AT2 signature, and PATS signature in club cells from AT2-tracer3 and BASC-tracer (right). Statistical significance was assessed using the two-sided Student's *t*-test (club signature) or Mann-Whitney tests (AT2 and PATS signature). **m**, At 6 months post-injury, UMAP embedding of the three tracer-labelled populations reveals distinct clustering patterns: AT2-tracer3 cells (ZsG⁺tdT⁺) segregate into five clusters, while BASC-tracer (ZsG⁺tdT⁺) and Club-tracer (ZsG⁻tdT⁺) each resolve into three clusters. **n**, Violin plot of enrichment scores of club signature, AT2 signature, and PATS signature in club cells from AT2-tracer3 and BASC-tracer at 6 months post-injury. Statistical analysis was performed by two-sided Mann-Whitney test. DEGs, differential expression genes. **o**, Pearson correlation of gene expression among club cells from AT2-tracer3, BASC-tracer, and Club-tracer during homeostasis and at 4 weeks and 6 months post-Naph. Single-cell transcriptomic data were each derived from one biological sample.



Extended Data Fig. 6 | See next page for caption.

Extended Data Fig. 6 | Notch signalling regulates AT2-to-club cell conversion during bronchiolar repair. **a**, Schematic showing the knock-in strategy of *Cldn4-LSL-Dre* mice by homologous recombination. **b**, Schematic showing the knock-in strategy of *Lamp3-CreER* mice by homologous recombination. **c**, A schematic diagram illustrating the experimental design. **d**, Immunostaining for tdT, Sftpc, Lamp3, Scgb1a1, and Ace-Tub on lung sections of *Lamp3-CreER; R26-tdT* mice after Tam or vehicle (corn oil) treatment. **e**, Quantification of the percentage of Lamp3⁺ cells expressing tdT. Data are mean $\pm$ SD; n = 5 mice. **f**, Schematic showing the knock-in strategy of *Cldn4-flox* mice by homologous recombination. **g**, Immunostaining of tdT, GFP, and AGER on lung sections post-DT injury. White arrowheads indicate tdT⁺AGER⁺ AT1 cells. **h**, Schematic showing the knock-in strategy of *Rbpj-rox* mice by homologous recombination. **i**, A schematic diagram illustrating the experimental design by using AT2-tracer3. **j**, FACS gating strategy for RT-qPCR (left). RT-qPCR analysis of *Rbpj* using sorted ZsG⁺ cells (right). Data are mean $\pm$ SD; n = 5 mice. Statistical analysis was performed using Mann-Whitney test. **k**, Immunostaining for tdT, ZsG and Sftpc or AGER on lung sections after naphthalene treatment. White arrowheads indicate ZsG⁺Sftpc⁺ AT2 cells (left panel) or ZsG⁺AGER⁺ AT1 cells (right panel). **l**, A schematic diagram illustrating the experimental design by using AT2-tracer2. **m**, Immunostaining of tdT, ZsG, and Scgb1a1 on lung sections after naphthalene injury. White arrowheads indicate tdT⁺ZsG⁺Scgb1a1⁺ club cells. **n**, Quantification of the percentage of Scgb1a1⁺ club cells labelled by AT2-tracer2 (left). Quantification of the percentage of AT2-tracer2 cells within bronchioles expressing Scgb1a1 (right). Data are mean $\pm$ SD; n = 5 mice; Statistical analysis was performed using unpaired two-tailed Welch's *t*-test. **o**, A schematic diagram illustrating the experimental design. **p**, Immunostaining for GFP, PDGFR α , and Ki67 on lung sections of *Scgb1a1-DTR-GFP* mice at different timepoints post-DT (left). Quantification of the thickness of PDGFR α ⁺ cell

layer in peri-bronchiolar region (right). Data are mean $\pm$ SD; n = 5 mice. Red arrowheads indicate PDGFR α ⁺Ki67⁺ cells. **q**, A schematic diagram illustrating the experimental design. **r**, Immunostaining of tdT, GFP, and Sftpc on lung sections after DT injury. These three groups correspond to main Fig. 4f–i. White arrowheads indicate tdT⁺Sftpc⁺ AT2 cells. Yellow arrowheads indicate tdT⁺GFP⁺ club cells. **s**, Quantification of the thickness of PDGFR α ⁺ cell layer in bronchioles. Data are mean $\pm$ SD; n = 5 mice. Statistical analysis was performed using unpaired two-tailed *t*-test. **t**, Quantification of the fold change in FRC (left; data are mean $\pm$ SD; control: n = 6 mice; experimental: n = 7 mice). Quantification the percentage of MMEF/FVC (middle; data are mean $\pm$ SD; 6 mice per group). Quantification the fold change in inspiratory resistance (right; data are mean $\pm$ SD; control, n = 6 mice; experimental, n = 7 mice). Statistical analysis was performed using unpaired two-tailed *t*-test (left and middle panel) or two way ANOVA with Šidák's multiple comparisons test (right panel). **u**, A schematic diagram illustrating the experimental design. **v**, Whole organoid immunostaining of tdT, GFP, and Sftpc on control and Notch-OE groups. **w**, Quantification of the percentage of CFE of organoids in (v). Data are mean $\pm$ SD; n = 5 mice. Statistical analysis was performed using unpaired two-tailed *t*-test. **x**, Quantification of the perimeter of organoids in (v). Each individual dot represents one organoid. Data are mean $\pm$ SD; n = 5 mice; Statistical analysis was performed using unpaired two-tailed Welch's *t*-test. **y**, Quantification of the percentage of tdT⁺ organoids expressing GFP. Data are mean $\pm$ SD; n = 5 mice; Statistical analysis was performed by two-tailed Mann-Whitney test. **z**, A cartoon image showing that the genetic overexpression of NICD promotes the transition of AT2 to club cells in vitro. FRC, Functional residual capacity. MMEF, Maximal mid-expiratory flow. CFU, colony-forming unit. CFE, colony-forming efficiency. Scale: white bars, 100 μ m; yellow bars, 50 μ m. Each image is representative of 5 individual biological samples.



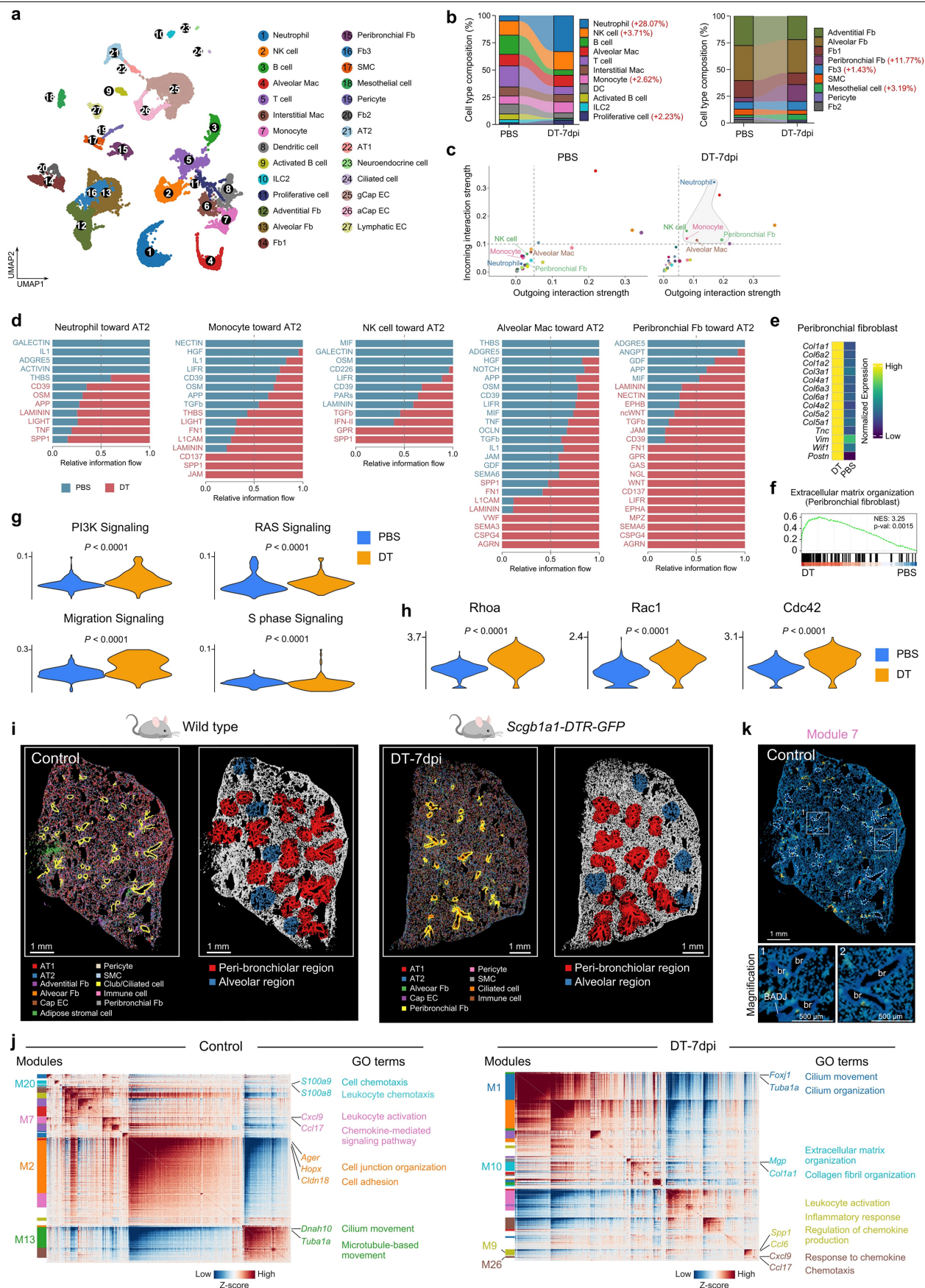
Extended Data Fig. 7 | See next page for caption.

Article

Extended Data Fig. 7 | Ciliated cells participate in regulating the

transdifferentiation of AT2 to club cells. a, b, The expression of Notch ligands (*Jag1*, *Jag2*, *Dll1*, *Dll4*) in BASC-tracer (**a**) and Club-tracer (**b**) cells. **c, d**, Heatmap displaying the expression of Notch signalling-related genes across different clusters of AT2-tracer3 cells (**c**) and BASC-tracer cells (**d**) at 4 weeks post-naphthalene injury. **e**, Interaction network plot showing inferred Notch signalling networks from ciliated cell to other cell types in BASC-tracer. Circle sizes scale with cell group abundance; edge widths denote the communication probabilities. **f**, Dot plot showing significant ligand-receptor pairs mediating Notch signalling sending from ciliated cell to other cell types in BASC-tracer. Dot colour and size represent communication probability and significance. *P*-values are computed from one-sided permutation test. **g**, A schematic diagram illustrating the experimental design. **h**, Immunostaining for tdT and Ace-Tub on lung sections of *Foxj1-CreER;R26-tdT;R26-iDTR* mice after tam and DT/vehicle (PBS) treatment. Yellow arrowheads indicate tdT⁺ Ace-Tub⁺ ciliated cells. **i**, Schematic diagrams illustrating the strategy for tracing *Sftpc*⁺*Scgb1a1*⁺ AT2 cells (AT2-tracer5) and simultaneous clearing of ciliated cells, club cells and BASCs that express DTR. **j**, A schematic diagram illustrating the experimental design by using AT2-tracer5. **k**, Immunostaining for tdT, GFP, and Ace-Tub on lung sections after DT treatment. Yellow arrows indicate tdT⁺GFP⁺ club cells. White arrowheads indicate Ace-Tub⁺ ciliated cells. **l**, Quantification of the percentage of tdT⁺ cells within bronchioles expressing GFP after DT injury. Data are mean ± SD; n = 5 mice. Statistical significance was assessed using the two-sided Mann-Whitney tests. **m**, Quantification of the percentage of bronchiolar

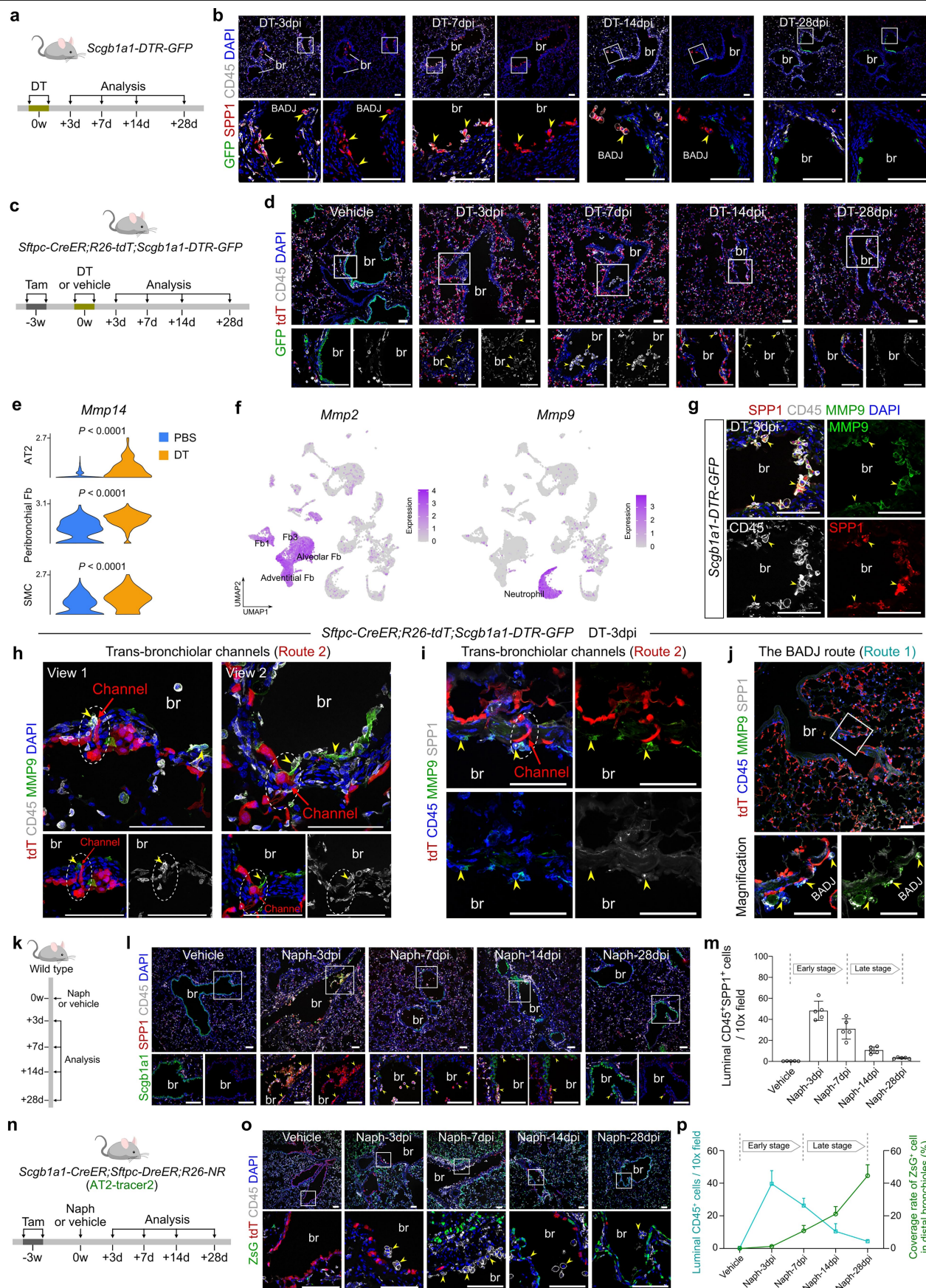
tdT⁺GFP⁺ club cells and tdT⁺GFP⁺ cells neighbouring ciliated cells (within 10 µm). Data are mean ± SD; n = 5 mice; Statistical analysis was performed using unpaired two-tailed *t*-test (left control group) or Welch's *t*-test (right experimental group). **n**, A cartoon image illustrating that ablation of ciliated cells inhibits the transition of AT2 cells into club cells. **o**, Cartoon illustrating a hypothesis that signalling between ciliated cells and transient cells via Notch ligands and receptors regulates the transition of AT2 to club cells. **p**, A cartoon image showing whether *Jag1* knockdown in ciliated cells affects the AT2-to-club transition. **q**, A schematic diagram illustrating the experimental design. **r**, Immunostaining for Jagged1 and Ace-Tub on lung sections of *Foxj1-CreER;Jag1^{fllox/+}* mice (control group) or *Foxj1-CreER;Jag1^{fllox/fllox}* (experimental group) mice after Tam treatment. **s**, Quantification of the percentage of Ace-Tub⁺ ciliated cells expressing Jagged1 in (**r**). Data are mean ± SD; n = 5 mice. Statistical analysis was performed using unpaired two-tailed *t*-test. **t**, A cartoon image showing whether *Notch2* knockdown in AT2-derived cells affects the AT2-to-club transition. **u**, Schematic showing the knock-in strategy of *Notch2^{fllox}* mice by homologous recombination. **v**, A schematic diagram illustrating the experimental design. FACS gating strategy and RT-qPCR of *Notch2* using sorted tdT⁺ cells from *Sftpc-CreER;R26-tdT;Scgb1a1-DTR-GFP;Notch2^{fllox/+}* mice (control group) and *Sftpc-CreER;R26-tdT;Scgb1a1-DTR-GFP;Notch2^{fllox/fllox}* mice (experimental group) after Tam. Data are mean ± SD; n = 5 mice; Statistical analysis was performed using unpaired two-tailed Welch's *t*-test. DT, diphtheria toxin. Ace-Tub, Acetylated tubulin. Scale bars, 100 µm. Each image is representative of 5 individual biological samples.



Extended Data Fig. 8 | See next page for caption.

Extended Data Fig. 8 | Single-cell and spatial transcriptomic profiling of murine lung cells after DT injury. **a**, UMAP visualization of integrated lung cells, identifying 27 distinct clusters including epithelial, endothelial, stromal, and immune lineages. **b**, Stacked bar plots showing the relative proportions of immune (left) and stromal (right) subpopulations in PBS versus DT-treated lungs. **c**, Scatter plots illustrating the incoming and outgoing interaction strengths for all identified cell populations in PBS-treated (left) and DT-treated (right) lungs. Selective cell types, including neutrophils, monocyte, NK cells, alveolar macrophages and peribronchiolar fibroblasts, exhibit distinct elevations in both signalling input and output following DT-mediated injury (indicated by labels and grey enclosure). **d**, Bar plots showing the relative information flow of significant signalling pathways from diverse donor populations toward AT2 cells. **e**, Heatmap of differentially expressed genes (DEGs) in peribronchiolar fibroblasts from PBS-treated versus DT-treated lungs. **f**, GSEA analysis indicates significant enrichment of the gene set “extracellular matrix organization” in peribronchiolar fibroblasts from DT-treated lungs compared to PBS controls. Statistical significance was assessed via two-sided permutation test. **g**, Violin plots comparing signalling scores for PI3K, RAS, migration, and S-phase gene signatures in AT2 cells

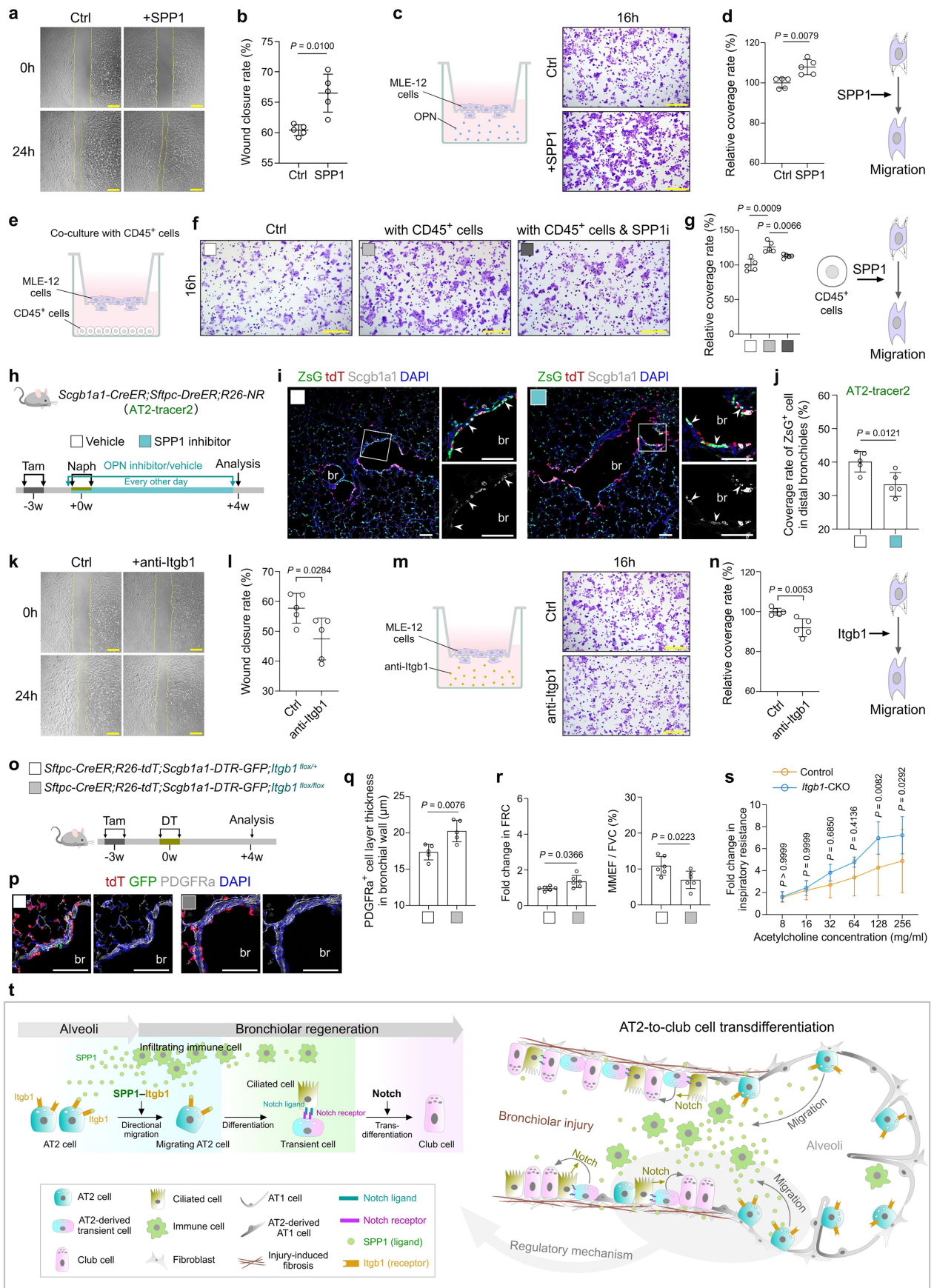
between PBS and DT conditions. Statistical significance was assessed using the two-sided Mann-Whitney test. **h**, Violin plots showing the significant upregulated expression of Rhoa, Rac1, and Cdc42 in AT2 cells following DT treatment. Statistical significance was assessed using the two-sided Mann-Whitney tests. **i**, Lung sections from *Scgb1a1-DTR-GFP* mice at day 7 post-DT injury were used for spatial transcriptomic sequencing. Lungs from wild-type mice served as controls. Spatial cell-type distribution maps of lung sections at 7 days post-DT and wild-type (control) mice generated by Stereo-seq. Regional segmentation defined the “peribronchiolar region” (red, within ~200 μ m of the bronchioles) and the “alveolar region” (blue) based on cell-type enrichment and morphological features. **j**, Spatially co-expressed gene modules identified by spatial hotspot analysis in control and DT-treated lungs. Heatmaps displaying the Z-score of local correlations among genes, with colour bars indicating distinct co-expressed modules (M). Key genes and enriched GO terms are annotated for representative modules (left). **k**, Spatial feature plots visualize module scores and reveal localized activation of inflammatory and chemotactic programs within the bronchiolar regions of the DT group compared with the control group. Single-cell and spatial transcriptomic data were each derived from one biological sample.



Extended Data Fig. 9 | See next page for caption.

Extended Data Fig. 9 | Immune cell infiltration into the bronchiolar region post-injury. **a**, A schematic diagram illustrating the experimental design. **b**, Immunostaining for GFP, SPP1, and CD45 on lung sections of *Scgb1a1-DTR-GFP* mice at different timepoints after DT treatment. Yellow arrowheads indicate SPP1⁺CD45⁺ immune cells. **c**, A schematic diagram illustrating the experimental design. **d**, Immunostaining for tdT, GFP, and CD45 on lung sections of *Sftpc-CreER;R26-tdT;Scgb1a1-DTR-GFP* mice at different timepoints after DT or vehicle (PBS) treatment. Yellow arrowheads indicate CD45⁺ immune cells in bronchioles. **e**, Violin plots showing the significant induction of *Mmp14* expression in AT2 cells, peribronchiolar fibroblast (Fb), and smooth muscle cell (SMC) following DT-mediated injury. Statistical significance was assessed using the two-sided Mann-Whitney test. **f**, The expression distribution of *Mmp2* and *Mmp9* across diverse cell populations. **g**, Immunostaining for SPP1, MMP9 and CD45 on lung sections of *Scgb1a1-DTR-GFP* mice post-DT. Yellow arrowheads indicate SPP1⁺CD45⁺MMP9⁺ immune cells. **h-j**, Immunostaining for tdT, MMP9, SPP1, and CD45 on lung sections of *Sftpc-CreER;R26-tdT;Scgb1a1-DTR-GFP* mice

post-DT. White dashed circles and red arrows indicate the channels across the bronchiolar wall. Yellow arrowheads indicate CD45⁺MMP9⁺ or SPP1⁺CD45⁺MMP9⁺ immune cells. **k**, A schematic diagram illustrating the experimental design. **l**, Immunostaining for *Scgb1a1*, SPP1, and CD45 on lung sections of wild type mice at different timepoints after naphthalene or vehicle treatment. Yellow arrowheads indicate SPP1⁺CD45⁺ immune cells. **m**, Quantification of the number of luminal CD45⁺SPP1⁺ cells per 10× field at different timepoints after naphthalene injury. Data are mean ± SD; n = 5 mice. **n**, A schematic diagram illustrating the experimental design. **o**, Immunostaining for tdT, ZsG, and CD45 on lung sections of *Scgb1a1-CreER;Sftpc-DreER;R26-NR* mice at different timepoints after naphthalene or vehicle treatment. Yellow arrowheads indicate CD45⁺ immune cells. **p**, Quantification of the number of luminal CD45⁺ cells (left axis) and the coverage rate of ZsG⁺ cells in distal bronchioles (right axis) per 10× field in (**o**). Data are mean ± SD; n = 5 mice. DT, diphtheria toxin. Tam, tamoxifen. br, bronchiole. Scale bars, 100 μm. Each image is representative of 5 individual biological samples.



Extended Data Fig. 10 | See next page for caption.

Extended Data Fig. 10 | SPP1 and Itgb1 are involved in regulating AT2 cell migration.

a, Brightfield images of MLE-12 cells cultured with or without SPP1 at 0 h and 24 h after scratch. **b**, Quantification of wound closure rate in (**a**). Data are mean $\pm$ SD; $n = 5$ independent experiments. Statistical analysis was performed using unpaired two-tailed Welch's t -test. **c**, A cartoon image showing the Transwell assay design (left panel). Brightfield images of lower surface of Transwell inserts using MLE-12 cells cultured with or without SPP1 (right panel). **d**, Quantification of relative coverage rate in the Transwell assay (left panel). Data are mean $\pm$ SD; $n = 5$ independent experiments. Statistical significance was assessed using the two-sided Mann-Whitney test. A cartoon image showing that SPP1 promotes the migration of MLE-12 cells (right panel). **e**, A cartoon image showing the Transwell assay design. **f**, Brightfield images of lower surface of Transwell inserts using MLE-12 cells cultured with or without CD45⁺ cells (sorted from *Scgb1a1-DTR-GFP* at 7 days post-DT injury or cultured with CD45⁺ cells and SPP1 inhibitor (SPP1i). **g**, Quantification of relative coverage rate in the Transwell assay. Data are mean $\pm$ SD; $n = 5$ independent experiments. Statistical analysis was performed using unpaired two-tailed t -test. A cartoon image showing that CD45⁺ cells secrete SPP1 to promote the migration of MLE-12 cells (right panel). **h**, A schematic diagram illustrating the experimental design. **i**, Immunostaining for tdT, ZsG, and *Scgb1a1* on lung sections. White arrowheads indicate ZsG⁺*Scgb1a1*⁺ club cells. **j**, Quantification of the coverage rate of ZsG⁺ cells in distal bronchioles. Data are mean $\pm$ SD; $n = 5$ mice. Statistical analysis was performed using unpaired two-tailed t -test. **k**, Brightfield images of MLE-12 cell line cultured with or without anti-Itgb1 antibody at 0 h and 24 h after scratch. **l**, Quantification of wound closure rate in (**k**). Data are presented as mean $\pm$ SD; $n = 5$ independent experiments. Statistical analysis was performed

using unpaired two-tailed t -test. **m**, A cartoon image showing the Transwell assay design (left panel). Brightfield images of lower surface of Transwell insert using MLE-12 cell line cultured with or without anti-Itgb1 antibody (right panel). **n**, Quantification of relative coverage rate in the Transwell assay (left panel). Data are mean $\pm$ SD; $n = 5$ independent experiments. Statistical analysis was performed using unpaired two-tailed t -test. A cartoon image showing that Itgb1 promote the migration of MLE-12 (right panel). **o**, A schematic diagram illustrating the experimental design. **p**, Immunostaining for tdT, GFP, and PDGFR α on lung sections post-DT. **q**, Quantification of the thickness of peribronchiolar PDGFR α ⁺ cell layer in (**p**). Data are mean $\pm$ SD; $n = 5$ mice. Statistical analysis was performed using unpaired two-tailed t -test. **r**, Quantification of the fold change in FRC ($n = 6$ mice) and the percentage of MMEF/FVC ($n = 6$ mice. $*P = 0.0223$). Data are mean $\pm$ SD. Statistical analysis was performed using unpaired two-tailed Welch's t -test (for FRC) or t -test (for MMEF/FVC). **s**, Quantification the fold change in inspiratory resistance (Control group, $n = 6$ mice; Experimental group, $n = 7$ mice). Data are mean $\pm$ SD. Statistical analysis was performed using two-way ANOVA with Šidák's multiple comparisons test. **t**, Cartoon hypothesis: In homeostasis, club cells line the bronchiolar epithelium, while AT2 cells reside in the alveoli. Upon club cell injury, immune cells infiltrate and secrete SPP1, generating a concentration gradient that radiates outward from the bronchioles. Peribronchiolar AT2 cells detect SPP1 via Itgb1 and migrate toward the bronchioles. Once there, they receive Notch ligands from ciliated cells, which activate Notch signalling and promote their transdifferentiation into club cells. FRC, functional residual capacity. MMEF, Maximal mid-expiratory flow. Ace-Tub, Acetylated tubulin. Scale bars: white, 100 μ m; yellow, 500 μ m. Each image is representative of 5 independent experiments.

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Give P values as exact values whenever suitable.
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Software and code

Policy information about [availability of computer code](#)

Data collection	Immunofluorescent images were acquired using Olympus FV1200, FV4000 confocal system, and Zeiss 880 confocal system. Quantitative RT-PCR data was acquired using QuantStudio 6 Real-Time PCR System (Applied Biosystems, ThermoFisher). FACS analysis was acquired using Sony MA900 and BD FACS Aria. Pulmonary function and airway responsiveness measurement data were acquired using a whole-body plethysmograph (EMMS, Hants, UK) with the Resistance and Compliance System and the Flow Measurement system. Immunoelectron microscopy images were acquired using a Tecnai G2 Spirit transmission electron microscope (FEI).
Data analysis	Immunofluorescent images analysis using ImageJ (NIH, version: 2.3.0/1.53q) and Imaris (version: 9.5.1) software. FACS data analysis using FlowJo version 10.4. Statistical analysis used GraphPad Prism (v9.3.1) and R. Adaptor sequences and low-quality bases were trimmed from the raw data using Trim Galore version 0.6.7. The custom reference generation and alignment for single cell RNA-seq were performed using Cell Ranger version 6.1.1. The downstream analysis were conducted in R version 4.4.2. The following R packages were used for data analysis. Seurat_4.1.2 DBSCAN_1.1-10 destiny_3.20.0 limma_3.62.1 clusterProfiler_4.14.4 CellChat_1.6.1 Python (v3.8.18) eDacq (v1.9.9) software Signac (v.1.16.0) and Seurat (v.5.3.0) Cell Ranger ARC (v.2.1.0) SAW (v.8.2.2) Stereopy (v.1.6.2) Zenodo web-link: https://zenodo.org/records/15333044 FACS data were analyzed using FlowJo 10.4 (FlowJo LLC)

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All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

The raw sequencing data generated in this study are deposited in Genome Sequence Archive (GSA; <https://ngdc.cncb.ac.cn/gsa/>) under accession PRJCA039649. Source data are provided with this paper. The codes executing the analysis workflow are available on Zenodo with persistent access through DOI: <https://doi.org/10.5281/zenodo.21768825>.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	NA
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical method was used to predetermine the sample size. Sample sizes were chosen based on prior experience and pilot experiments for detecting statistically significant differences between conditions. For all in vivo and in vitro experiments, a minimum of four biological replicates ($n \geq 4$) was used. In compliance with IACUC guidelines, for in vivo experiments, a minimal number of animals with balanced sex was used to achieve statistically meaningful results. All specific sample sizes are indicated in the corresponding figure legends.
Data exclusions	No data was excluded from the analyses.
Replication	All in vivo mouse experiments and in vitro cell culture experiments included a minimum of three biological replicates, whereas single-cell transcriptomics and spatial transcriptomics analyses were carried out with one biological replicate per condition. Sample size is indicated in each figure and figure legend.
Randomization	Both adult male and female mice were randomly assigned to in vivo experimental groups. For in vitro experiments, random allocation was not applicable. Samples were equally distributed from a single homogenized pool, and all culture conditions (passage number, medium batch, seeding density, temperature, treatment duration) were standardized across groups. Thus, no additional covariate control was required, and randomization was not performed as it was not relevant to these assays.
Blinding	No blinding was applied as the same investigators performed the experiments and analyzed the data. The injury groups compared to uninjured groups are readily apparent.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

The species, application, manufacturer, catalog number, dilution ratio, and official validation website links for the primary antibodies used in immunofluorescence staining are as follows:

Goat, anti-E-cadherin (R&D system, AF748, 1:500), https://www.rndsystems.com/cn/products/human-mouse-e-cadherin-antibody_af748
 Goat, anti-Scgb1a1 (Santa Cruz, SC-9772, 1:200), <https://www.scbt.com/p/cc10-antibody-t-18>
 Rabbit, anti-Scgb1a1 (Abcam, ab213203, 1:300), <https://www.abcam.cn/uteroglobin-antibody-epr19846-ab213203.html>
 Goat, anti-Scgb3a2 (R&D system, AF3545-SP, 1:500), https://www.rndsystems.com/cn/products/human-ugrp1-scgb3a2-antibody_af3545
 Rabbit, anti-tdTomato (Rockland, 600-401-379, 1:1000), <http://www.rockland-inc.com/Product.aspx?id=34801>
 Goat, anti-tdTomato (Rockland, 200-101-379, 1:500), <https://www.rockland.com/categories/primary-antibodies/rfp-antibody-pre-adsorbed-600-401-379/>
 Goat, anti-GFP (Abcam, ab6662, 1:500), <http://www.abcam.cn/gfp-antibody-fitc-ab6662.html>
 Goat, anti-Collagen IV (Millipore, Ab769, 1:500), https://www.merckmillipore.com/CN/zh/product/Anti-Collagen-Type-IV-Antibody,MM_NF-AB769?ReferrerURL=https%3A%2F%2Fwww.bing.com%2F&bd=1
 Rat, anti-GFP (nacalai tesque, 04404-84, 1:500), http://www.nacalai.co.jp/global/reagent/Antibodies/GFP_Rat.html
 Rabbit, anti-GFP (Invitrogen, A11122, 1:500), <https://www.thermofisher.com/antibody/product/GFP-Tag-Antibody-Polyclonal/A-11122>
 Rat, anti-AGER (R&D system, MAB1179-100, 1:200), https://www.rndsystems.com/cn/products/mouse-rat-rage-antibody-175410_mab1179#product-citations
 Rabbit, anti-Sftpc (Millipore, AB3786, 1:200), [http://www.millipore.com/publications.nsf/a73664f9f981af8c852569b9005b4e4ee/e158402e5325b78c852573060076d773/\\$FILE/AB3786.pdf](http://www.millipore.com/publications.nsf/a73664f9f981af8c852569b9005b4e4ee/e158402e5325b78c852573060076d773/$FILE/AB3786.pdf)
 Rabbit, anti-ZsGreen (Clontech, 632474, 1:1000), http://www.clontech.com/CN/Products/Fluorescent_Proteins_and_Reporters/Fluorescent_Protein_Antibodies/ibcGetAttachment.jsp?citemId=27624&fileId=6464523&sitex=10022:22372:US
 Rabbit, anti-Ki67 (Abcam, ab15580-100ug, 1:400), <https://www.abcam.com/en-us/products/primary-antibodies/ki67-antibody-ab15580>
 Goat, anti-DTR (R&D system, AF-259-NA, 1:500), https://www.rndsystems.com/cn/products/human-hb-egf-antibody_af-259-na
 Mouse, anti-Ace-Tub (Sigma, T7451, 1:300), <https://www.sigmaaldrich.cn/CN/zh/product/sigma/t7451>
 Rabbit, anti-Krt5 (Biolegend, 905504, 1:500), <https://www.biolegend.com/en-gb/products/purified-anti-keratin-5-antibody-13378?GroupId=GROUP26>
 Rabbit, anti-CGRP (Sigma, C8198, 1:500), <https://www.sigmaaldrich.com/TW/en/product/sigma/c8198>
 Goat, anti-PDGFRa (R&D system, AF1062, 1:500), https://www.rndsystems.com/cn/products/mouse-pdgfr-alpha-antibody_af1062
 Rat, anti-Lamp3 (NovusBio, 1006F7.05, 1:300), <https://www.labome.com/product/Novus-Biologicals/DDX0192P-100.html>
 Rabbit, anti-FOXJ1 (Abcam, ab235445, 1:300), <https://www.abcam.cn/products/primary-antibodies/foxj1-antibody-epr21874-ab235445>
 Hamster, anti-T1a/Pdpn (DSHB, 8.1.1, 1:100), http://dshb.biology.uiowa.edu/8-1-1_2
 Rabbit, anti-Hopx (Abcam, ab307670, 1:100), <https://www.scbt.com/p/hop-antibody-e-1>
 Rabbit, anti-Jagged1 (Abcam, ab7771, 1:100), <https://www.abcam.cn/products/primary-antibodies/jagged1-antibody-ab7771>
 Mouse, anti-MMP9 (Thermo, MA5-15886, 1:200), <https://www.thermofisher.cn/cn/zh/antibody/product/MMP9-Antibody-clone-5G3-Monoclonal/MA5-15886>
 Goat, anti-SPP1/OPN (R&D system, AF808, 1:400), https://www.rndsystems.com/cn/products/mouse-osteopontin-opn-antibody_af808
 Rat, anti-CD45 (eBioscience, 17-0451-82, 1:200), <https://www.thermofisher.com/antibody/product/CD45-Antibody-clone-30-F11-Monoclonal/17-0451-82>
 Rat, anti-Integrin beta 1 (CD29) (Sigma/Millipore, MAB1997, 1:100), <https://www.sigmaaldrich.cn/CN/zh/product/mm/mab1997>

Secondary antibodies:

Alexa donkey anti-rabbit 555 (Invitrogen, A31572, 1:1000), <https://www.thermofisher.cn/cn/zh/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31572>
 Alexa donkey anti-rabbit 488 (Invitrogen, A21206, 1:1000), <https://www.thermofisher.cn/cn/zh/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21206>
 Alexa donkey anti-rabbit 647 (Invitrogen, A31573, 1:1000), <https://www.thermofisher.cn/cn/zh/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31573>
 Alexa donkey anti-goat 488 (Invitrogen, A11055, 1:1000), <https://www.thermofisher.cn/cn/zh/antibody/product/Donkey-anti-Goat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11055>
 Alexa donkey anti-goat 555 (Invitrogen, A21432, 1:1000), <https://www.thermofisher.cn/cn/zh/antibody/product/Donkey-anti-Goat->

IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21432
 Alexa donkey anti-goat 647 (Invitrogen, A21447, 1:1000), <https://www.thermofisher.cn/cn/zh/antibody/product/Donkey-anti-Goat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21447>
 Alexa donkey anti-mouse 647 (Invitrogen, A31571, 1:1000), <https://www.thermofisher.cn/cn/zh/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31571>
 Alexa donkey a-mouse 488 (Invitrogen, A21202, 1:1000), <https://www.thermofisher.cn/cn/zh/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21202>
 Alexa donkey anti-rat 647 (Invitrogen, A21247, 1:1000), <https://www.thermofisher.cn/cn/zh/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21247>
 Alexa donkey anti-rat 488 (Invitrogen, A21208, 1:1000), <https://www.thermofisher.cn/cn/zh/antibody/product/Donkey-anti-Rat-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21208>

For FACS, antibodies:

CD45 APC-eFluor 780 (eBioscience, 47-0451-82, 1:200), <https://www.thermofisher.com/cn/zh/antibody/product/CD45-Antibody-clone-30-F11-Monoclonal/47-0451-82>
 Ter-119 APC-eFluor 780 (eBioscience, 47-5921-82, 1:200), <https://www.thermofisher.com/cn/zh/antibody/product/CD45-Antibody-clone-30-F11-Monoclonal/47-0451-82>

Validation

All antibodies used in this study were validated by the manufacturer to confirm their specificity and suitability for the intended applications. The detailed validation methods and data can be accessed through the provided links to the manufacturer's websites.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	MLE-12 cell line (sunncell, catalog number: SNL-414), Web-link: https://www.sunncell.com.cn/product/SNL-414
Authentication	The MLE-12 cell line (SNL-414, ATCC CVCL-3751) was authenticated by the supplier via mouse species-specific STR profiling. No further authentication was performed in our laboratory.
Mycoplasma contamination	The MLE-12 cell line has been tested negative for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	The MLE-12 cell line used in this study is not listed in the ICLAC Register of Misidentified Cell Lines (version 14)

Animals and other research organisms

Policy information about [studies involving animals; ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC, protocol #SIBCB-S374-1702-001-C1) at the Center for Excellence in Molecular Cell Science, Chinese Academy of Sciences, and were performed in accordance with its guidelines. All mice used in this paper were performed on 7–20 weeks old and were kept in a C57BL6/ICR mixed background. Both male and female mice were randomly used for experiments. Sftpc-CreER, Sftpc-DreER, Scgb1a1-CreER, Ager-CreER, Hopx-2A-DreER, p63-DreER, Foxj1-CreER, R26-NR, R26-TLR, R26-tdT, R26-RSR-tdT, R26-RSR-LSL-tdT, R26-Confetti, R26-iDTR, R26-NICD, Rbpj flox/+, Jag1 flox/+ mice lines were previously described, references found in Materials and Methods. The Scgb1a1-DTR-GFP, Ascl1-CreER, Itgb1 flox/+, Rbpj rox/+, Lamp3-CreER, Cldn4-LSL-Dre, Cldn4 flox/+, and Notch2 flox/+ mice lines were generated by Shanghai Model Organisms Center, Inc. (SMOC). For the Scgb1a1-DTR-GFP mouse line, the P2A-Diphtheria Toxin Receptor (DTR)-GFP sequence was inserted into the Scgb1a1 gene locus via homologous recombination. For the Ascl1-CreER mouse line, the CreERT2 sequence was inserted into the Ascl1 gene locus via homologous recombination. For the Itgb1flox/+ mouse line, two loxP sites were added on either side of Exon3 of Itgb1 gene via homologous recombination. For the Rbpjrox/+ mouse line, two rox sites were added on either side of Exon6 and Exon7 of Rbpj gene via homologous recombination. For the Lamp3-CreER mouse line, the 2A-CreER sequence was inserted into the Lamp3 gene locus via homologous recombination. For the Cldn4-LSL-Dre mouse line, the loxP-stop-loxP-Dre sequence was inserted into the Cldn4 gene locus via homologous recombination. For the Cldn4flox/+ mouse line, two loxP sites were added on either side of Exon1 of Cldn4 gene via homologous recombination. For the Notch2flox/+ mouse line, two loxP sites were added on either side of Exon3 of Notch2 gene via homologous recombination. All mice were housed and bred in the Specific Pathogen Free (SPF) animal facility of the Center for Excellence in Molecular Cell Science, under controlled conditions at 22 ± 2 °C, 50 ± 10 % relative humidity, and a 12-h light/dark cycle, with ad libitum access to food and water. Depending on the experimental cohort, mice were used at either 7–20 weeks of age or 6 months of age, and all animals were maintained on a C57BL6/ICR mixed background. Both male and female mice were randomly used for experiments.
Wild animals	No wild animals were used in this study.
Reporting on sex	No findings applied only to one sex. Both male and female mice were used in this study.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	All animal experiments were conducted in accordance with the procedures approved by the Institutional Animal Care and Use Committee (IACUC) (protocol #SIBCB-S374-1702-001-C1) at the Center for Excellence in Molecular Cell Science, Chinese Academy of Sciences.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

The mice were euthanized, and the lungs were perfused with cold PBS through the right ventricle to eliminate blood cells, followed by the injection of protease solution consisting of collagenase type I (500 U/mL; Gibco 17100-017), elastase (4 U/mL; Worthington Biochemical Corporation LS002279), dispase (5 U/mL; BD Biosciences 354235), and DNase I (0.33 U/mL; Worthington Biochemical Corporation LS002139) in DMEM (Gibco, 11965092) through the trachea. Subsequently, the lungs were minced and incubated in 4 mL of protease solution for 30 minutes at 37°C with continuous agitation. The tissue fragments were then dissociated by pipetting during the digestion process, followed by adding equal volume of the DMEM/F12 with 10% FBS to terminate the digestion. After being centrifuged at 1000 × g for 5 minutes at 4°C and washing with DMEM, the precipitate was resuspended in 3 mL enzyme mixture of 0.1% Trypsin-EDTA (Gibco, 25200072) containing DNase I and incubated for 20 minutes at 37°C with agitation. Next, the digestion was terminated by addition of DMEM/F12 with 10% FBS, after which the cells were sequentially filtered through 100 µm strainers and centrifuged at 1000 × g for 5 minutes at 4°C. The red blood cells were lysed by incubation of red blood cell (RBC) lysis buffer (2–5 mL, eBioscience, 00-4333-57) at room temperature for 5 minutes. The process was ended by adding 8–10 mL PBS and then centrifuge at 1000 × g for 5 minutes at 4°C. The precipitate was resuspended in block solution (eBioscience, 14-0161, 1:100) for 5 minutes to block nonspecific antigens prior to further staining. Then the cells were incubated with primary antibodies at 4°C for 30 minutes. The following antibodies were utilized: CD45 APC-eFluor 780 (eBioscience, 47-0451-82, 1:200), Ter-119 APC-eFluor 780 (eBioscience, 47-5921-82, 1:200). Afterwards, the cells were washed with PBS, followed by centrifugation. Subsequently, the cells were resuspended in PBS supplemented with DAPI and DNase I. Bulk AT2 cells were excluded using Lyso-Tracker by staining the lysosomes in AT2 cells. For Lyso-Tracker staining, the cells were resuspended in DMEM containing LysoTracker (Invitrogen, Lyso-Tracker Deep Red, L12492) and incubated at 37°C for 10 to 15 minutes. Then the cells were centrifuged at 1000 × g for 5 minutes at 4°C, followed by washing with PBS. Subsequently, the cell pellets were resuspended in PBS supplemented with DAPI and DNase I. For scRNA-seq, the AT2-tracer3, BASC-tracer, and Club-tracer cells were sorted from Scgb1a1-CreER;Sftpc-DreER;R26-TLR lungs at 4 weeks and 6 months post-naphthalene injury. For scATAC-seq, the AT2-tracer2 cells were sorted from Scgb1a1-CreER;Sftpc-DreER;R26-NR lungs at 4 weeks and 6 months post-naphthalene injury. All cells in the single-cell suspension were negatively selected for CD45 and Ter119, and then gated the tdT+ZsG– cells as Club-tracer, tdT+ZsG+ cells as BASC-tracer, and tdT–ZsG+Lyso-Trackerlow cells as AT2-tracer3. Sony MA900 and BD FACS Aria were utilized for flow cell sorting. FACS data were analyzed using FlowJo 10.4 (FlowJo LLC). Representative gating strategies used in this study are shown in Supplementary Fig. 1.

Instrument	Sony MA900; BD FACS Aria
Software	FlowJo 10.4
Cell population abundance	The proportion of Club-tracer cells sorted in live CD45– Ter119– cells was about 0.4%. The proportion of BASC-tracer cells sorted in live CD45– Ter119– cells was about 0.3%. The proportion of AT2-tracer3 cells sorted in live CD45– Ter119– Lyso-Tracker-low cells was about 5.1%.

Gating strategy

Preliminary FSC/SSC gating were performed for all samples. The AT2-tracer3 cells were defined by DAPI– CD45– Ter119– ZsG + Lyso-Tracker low cells. The BASC-tracer cells were defined by DAPI– CD45– Ter119– ZsG+ tdT+ cells. The Club-tracer cells were defined by DAPI– CD45– Ter119– ZsG– tdT+ cells. Positive and negative staining cells populations were defined by the fluorescence-minus-one method. Representative gating strategies used in this study are shown in Supplementary Fig. 1.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.