1 **Release of Notch activity coordinated by IL-1β signalling confers differentiation**
2 **plasticity of airway progenitors via Fosl2 during alveolar regeneration**
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Abstract

While the acquisition of cellular plasticity in adult stem cells is essential for rapid regeneration after tissue injury, little is known about the underlying mechanisms governing this process. Our data reveal the coordination of airway progenitor differentiation plasticity by inflammatory signals during alveolar regeneration. Upon damage, IL-1β signalling-dependent modulation of Jag1/2 expression in ciliated cells results in the inhibition of Notch signalling in secretory cells, which drives reprogramming and acquisition of differentiation plasticity. We identify a transcription factor Fosl2/Fra2 for secretory cell fate conversion to alveolar type 2 (AT2) cells retaining the distinct genetic and epigenetic signatures of secretory lineages. We furthermore reveal that KDR/FLK-1^{+} human secretory cells display a conserved capacity to generate AT2 cells via Notch inhibition. Our results demonstrate the functional role of a IL-1β-Notch-Fosl2 axis for the fate decision of secretory cells during injury repair, proposing a potential therapeutic target for human lung alveolar regeneration.

48 **Main**

49 Tissue homeostasis is maintained by stem and progenitor cells that acquire a remarkable
50 potential for multi-lineage differentiation after tissue insults to allow for rapid regeneration of
51 damaged tissue[1,2]. These capacities depend on the nature and/or extent of injury, the repair
52 ability of resident stem/progenitor cells, and their local microenvironments. Dysregulation of
53 cellular plasticity, however, leads to lineage infidelity, and is implicated in various diseases[2].
54 Thus, to understand the process of tissue repair and regeneration in homeostasis and pathology,
55 it is important to elucidate how stem and progenitor cells sense damage signals and control
56 transient plasticity for fate conversion.

57

58 Lung tissue is maintained by several stem and progenitor cell types that reside in anatomically
59 distinct regions alongside the pulmonary axis[3,4]. In the distal lungs two main stem cell types,
60 secretory cells and alveolar type 2 (AT2) cells, separately maintain the airway and alveolar
61 epithelium compartments, respectively. In alveolar homeostasis and injury conditions, AT2
62 cells have been identified as the resident stem cells capable of self-renewal and differentiation
63 into alveolar type 1 (AT1) cells[5-7]. However, when alveolar integrity is severely disrupted
64 following lung injury, such as bleomycin or influenza infection, stem/progenitor cells localised
65 in the distal airway can contribute to alveolar regeneration by differentiating into AT2 cells[5,6,8-
66 12]. In particular, several lines of evidence reveal the capacity of *Scgb1a1*$^{+}$ airway secretory
67 cells, which exhibit restricted capacity to generate airway cells in homeostasis, to produce new
68 AT2 cells following severe lung injury[5,6]. However, little is known about the cellular events
69 and molecular cues which dictate how secretory cells lose their identity in response to alveolar
70 damage and subsequently acquire AT2 cell fate. Significantly, the chronic loss of alveolar
71 integrity is strongly associated with various human lung diseases. Thus, it is imperative to
72 understand whether there is a functionally conserved secretory cell population that readily
73 mediates human alveolar regeneration, and whether regulatory mechanisms are conserved
74 between human and mouse lungs.

75

76 Here, we establish chemically defined feeder-free *in vitro* organoid cultures wherein secretory
77 cells and AT2 cells are capable of expansion with a restricted differentiation capacity. This
78 platform enables us to perform robust interrogation of the molecular and cellular behaviour of
79 secretory and AT2 cells *in vitro*. By comparing the gene expression patterns in these organoids,
80 we identify the Notch signalling pathway as a pivotal regulator for the differentiation plasticity
81 of secretory cells into AT2 cells. We demonstrate the role of IL-1β-mediated inflammation in

82 ciliated cells in regulating this differentiation plasticity *in vivo*. The AP-1 transcription factor
83 Fosl2/Fra2 is defined as an essential transcription factor for AT2 cell conversion from secretory
84 cells in response to Notch signalling. Further, the distinct genetic and epigenetic signatures of
85 secretory-derived AT2 cells confer their functional difference compared to resident AT2 cells.
86 Lastly, we demonstrate that the role of Notch signalling in the transition of secretory cells to
87 AT2 cells is conserved in human airway organoids derived from KDR/FLK-1^{+} secretory cells.
88 Our study illustrates the precise molecular and cellular coordination required for secretory cell-
89 mediated alveolar regeneration, providing insights into both the potential regenerative roles of
90 these cells and regulatory networks in repairing alveolar destruction in lung disease.
91

92 **Results**

93 **Establishment of feeder-free organoids for distal stem cells**

94 We established a feeder-free organoid culture with defined factors which support the molecular
95 and functional identity of stem cells over long-term culture. Airway secretory cells expressing
96 *Scgb1a1* (*Secretoglobin 1a1*, also known as *CC10* or *CCSP*) were isolated from the distal lung
97 tissues of secretory cell reporter mice (*Scgb1a1-CreER$^{TM/+}$;R26R$^{tdTomato/+}$*) after tamoxifen
98 treatment[13] (Fig. 1a). Lineage-labelled secretory cells were embedded in Matrigel
99 supplemented with WNT3A, RSPO1 (R-spondin 1), EGF, FGF7, FGF10, and NOG (Noggin),
100 factors that are known to support the growth of human embryonic lung tip cells and progenitors
101 derived from foregut lineages[14-18] (Fig. 1b). Under this condition, secretory cells formed cyst-
102 like organoids that were capable of long-term expansion (>2 years) (Fig. 1b-d). We also
103 successfully generated organoids after limiting dilution with drops containing single cells (Fig.
104 1e), which serves as evidence of the clonogenicity and stem cell property of secretory cells
105 maintained in this condition. FGF10 was dispensable for organoid growth (Fig. 1b-d). Without
106 FGF7, however, secretory cells failed to form organoids (Fig. 1b,d). Furthermore, the absence
107 of factors supporting WNT activity resulted in deterioration of organoid expansion over
108 passages (Fig. 1b,d), indicating that both FGF7 and WNT signalling are essential for the self-
109 renewal of distal secretory cells. The initial organoids retained heterogeneous phenotypes,
110 including airway retaining secretory and ciliated cells, alveolar retaining AT2 and AT1 cells,
111 and mixed organoids retaining both airway and alveolar lineages, similar to previous findings
112 from the co-culture system[19] (Fig. 1f). Organoids expanded to form mainly mixed organoids
113 with budding-like morphology over multiple passages (Fig. 1g-i).
114

Like secretory cell-derived organoids (referred to here as SCOs), AT2 cells isolated from *Sftpc-CreER*$^{T2/+}$;*R26R*$^{tdTomato/+}$ reporter mice formed cyst-like organoids (Extended Data Fig. 1a,b). Consistent with previous studies[20-23], FGF7 and WNT activity were essential for forming and maintaining AT2 cell-derived organoids (referred to hereafter as ACOs), which contained both AT2 and AT1 cells (Extended Data Fig. 1b-f). ACOs were better maintained in the absence of FGF10 (Extended Data Fig. 1d). We also confirmed the clonogenic ability of AT2 cells by organoid culture with single cells (Extended Data Fig. 1e). These organoids expanded to form alveolar-like structures that were mainly composed of AT2 cells with little AT1 cell differentiation over multiple passages (Extended Data Fig. 1g,h). Taken together, we developed a feeder-free organoid culture system with chemically defined medium, wherein distal airway secretory cells and AT2 cells were maintained with stem cell activity in long-term cultures.

Differentiation of secretory cells by Notch signalling

To investigate the characteristics of SCOs and ACOs, we first performed bulk RNA-seq analysis. SCOs exhibited a distinct expression pattern enriched in secretory cell markers such as *Scgb1a1* and *Cyp2f2*, while AT2 cell markers including *Sftpc* and *Etv5* were highly expressed in ACOs (Fig. 2a,b). Notably, expression of genes related with Notch signalling, such as *Hes1* and *Nrarp*, was upregulated in SCOs compared to ACOs (Fig. 2a,b). Additional immunofluorescent (IF) staining also confirmed the expression of intracellular domain of Notch1 (N1ICD) in SCOs (Extended Data Fig. 2a). This observation prompted us to examine whether Notch signalling is involved in the identity and fate behaviour of secretory cells. Remarkably, AT2 cells were dramatically increased at the expense of secretory cells when we treated DAPT, a γ-secretase inhibitor, in SCOs compared to DMSO control (Fig. 2c). Further RNA-seq analysis also confirmed that DAPT-treated organoids displayed a strong upregulation of AT2 cell markers and a substantial reduction of secretory cell markers (Fig. 2d,e). Knockdown (KD) of *Rbpj* in SCOs promoted the differentiation of secretory cells into AT2 cells without DAPT treatment (Extended Data Fig. 2b,c), suggesting that inhibition of Notch signalling triggers the conversion of secretory cells into AT2 lineage.

To address this finding *in vivo*, we used mice carrying a conditional dominant-negative mutant of mastermind-like 1 (dnMAML$^{flox/flox}$)[24], which inhibits NICD-induced Notch signalling, crossed with *Scgb1a1-CreER*$^{TM/+}$;*R26R*$^{tdTomato/+}$. Control (*Scgb1a1-CreER*$^{TM/+}$;*R26R*$^{tdTomato/+}$) or dnMAML$^{flox/+}$ (*dnMAML*$^{flox/+}$;*Scgb1a1-CreER*$^{TM/+}$;*R26R*$^{tdTomato/+}$) mice were exposed to

PBS or bleomycin after tamoxifen treatment to induce alveolar injury (Fig. 2f). Inhibition of Notch activity by *dnMAML* expression was confirmed by assessing Hes1 expression (Extended Data Fig. 2d,e). Consistent with previous studies[25-27], the proportion of lineage-labelled ciliated cells was increased by Notch inhibition (Extended Data Fig. 2f,g). Frequency of lineage-labelled AT2 cells (6.97±13.12%) in dnMAML$^{flox/+}$ lungs was comparable to that seen in control lungs (6.23±1.09%) (Fig. 2g). However, we observed a substantial increase in lineage-labelled AT2 cells (56.39±12.91%) post injury in dnMAML$^{flox/+}$ lungs compared to control (25.24±8.08%) (Fig. 2h,i), suggesting that persistent inhibition of Notch signalling in secretory cells enhances their differentiation towards AT2 cells during injury repair. Further, organoids derived from *dnMAML*-expressing secretory cells enhanced the frequency of alveolar organoid formation and reduced occurrence of airway organoids (Extended Data Fig. 3). Pharmacological inhibition of Notch activity by DAPT treatment *in vivo* also showed a significant increase of AT2 cells derived from secretory cells (Extended Data Fig. 4).

Given that *Scgb1a1*$^{+}$ lineage-labelled cells contained heterogeneous populations, including CC10^{+}SPC^{+} cells, we further evaluated the differentiation capacity of *Scgb1a1*$^{+}$ lineage-labelled cells after excluding lineage-labelled AT2 cells. Isolated lineage-labelled secretory cells (EpCAM^{+}Tomato^{+}MHCII^{-}) from control and dnMAML$^{flox/+}$ lungs were transplanted into the lungs after bleomycin injury (Extended Data Fig. 5a,b). Secretory cells isolated from dnMAML$^{flox/+}$ lungs showed enhanced ability to generate AT2 cells compared to control secretory cells (Extended Data Fig. 5c-h). Further, *dnMAML*-expressing secretory cells revealed the greater frequency of alveolar organoid formation compared to control secretory cells (Extended Data Fig. 5i). Together, our data strongly suggest that persistent inhibition of Notch signalling enhances the differentiation of secretory cells into AT2 cells post injury.

Impaired alveolar regeneration by sustained Notch activation

To further ask whether the constitutive Notch activation affects the cell fate of secretory cells upon injury, we used *Red2-Notch*^{N1ICD} mice, where constitutive *N1ICD* is specifically co-expressed in *tdimer2 red fluorescent protein* (RFP)$^{+}$ cells in the original confetti mouse line[28] (Fig. 3a). The other three lineage outcomes, namely YFP, GFP and CFP, all represent wild-type events. Isolated RFP^{+} cells formed organoids retaining only airway cells whereas YFP^{+} control cells generated three types of organoids in co-cultures (Fig. 3b-e). To further investigate the cellular behaviour of secretory cells with constitutive Notch activity *in vivo*, *Scgb1a1-CreER*$^{TM/+}$;*Red2-Notch*$^{N1ICD/+}$ mice were administered PBS or bleomycin injury (Fig. 3a). In

uninjured lungs, YFP^+ and RFP^+ cells were localised in airways with CC10 expression, and no lineage-labelled cells were observed in the alveolar region (Fig. 3f and Extended Data Fig. 5j). After bleomycin injury, we observed an increased frequency of YFP^+ lineage-labelled AT2 cells (Fig. 3g-i). However, an increase of RFP^+ lineage-labelled AT2 cells was significantly compromised after injury (Fig. 3g-i). Although RFP^+ cells also expanded post injury, they still maintained identity of secretory cells retaining CC10 expression (Fig. 3g-i).

We next sought to define the molecular identity and characteristics of YFP^+ or RFP^+ cells during alveolar regeneration. To this end, we carried out single-cell RNA-sequencing (scRNA-seq) analysis by isolating lineage-labelled (YFP^+ or RFP^+) and non-lineage-labelled ($YFP^–$ $RFP^–$) populations at day 28 post bleomycin injury (Extended Data Fig. 6a-c). To gain insight into the impact of Notch activity on lineage differentiation of secretory cells into AT2 cells, we further analysed the secretory and AT2 cell populations (Fig. 3j). This analysis uncovered four distinctive clusters, including three different types of secretory cells and an AT2 cell cluster (Fig. 3j,k). In addition to the known secretory cell populations, including $Scgb3a2^{high}$ canonical secretory cells (cluster 1) and $H2\text{-}K1^{high}$ cells (cluster 2)[8] sharing common secretory markers (e.g. *Cldn10*), we identified an uncharacterized population which we termed $Fstl1^+$ secretory cells (cluster 3; Fig. 3j,k). While cells in this cluster still retained the identity of secretory cells based on *Scgb1a1* expression, they showed lower expression levels of some secretory cell markers such as *Scgb3a2* and *Cldn10* (Fig. 3k). Moreover, this cluster was marked by specific gene expression such as *Id3* and *Porcn* (Fig. 3k). Interestingly, this population had elevated expression of *Cdkn1c*/p57, involved in cell cycle inhibition, which could indicate the quiescent characteristics of this cluster *in vivo* (Fig. 3k and Extended Data Fig. 6d,e). Consistent with lineage-tracing analysis, control YFP^+ cells contributed to alveolar regeneration by giving rise to AT2 cells post injury (Fig. 3l). However, RFP^+ cells were arrested at the stage of the $Fstl1^+$ population, causing impaired differentiation into AT2 cells (Fig. 3l). Pseudotime analysis revealed that the transition from secretory cells to AT2 cells is mediated via this cluster 3, suggesting that this population may function as a transitional intermediate in the lineage transition between secretory cells and AT2 cells, and inactivation of Notch activity is required for the transition of this intermediate state into AT2 cell fate (Extended Data Fig. 6f). Overall, our results indicate that sustained Notch activation causes defects in alveolar regeneration by blocking the fate conversion from secretory to AT2 cells.

Notch ligand expression in ciliated cells by IL-1β signals

217 Given that Notch signalling involves short-range cellular communication through direct
218 contact by receptor-ligand interaction, we examined which cells highly express the Notch
219 ligands in the airway. Our scRNA-seq analysis revealed that *Jag1* and *Jag2* are highly
220 expressed in ciliated cells, which reside adjacent to secretory cells, consistent with previous
221 studies[27,29,30] (Extended Data Fig. 7a,b). Immunohistochemistry (IHC) staining also confirmed
222 the high expression of Jag1 in ciliated cells (Extended Data Fig. 7c). Notably, Jag1 expression
223 was downregulated after bleomycin treatment, suggesting dynamic modulation of Notch
224 signalling by ciliated cells during injury repair (Extended Data Fig. 7c). Previously we showed
225 dynamic expression of IL-1β during alveolar regeneration[31]. Notably, *Il1r1*, a functional
226 receptor for IL-1β, is specifically expressed in ciliated cells, in addition to subsets of AT2 cells
227 (Extended Data Fig. 7d,e)[31]. The expression of *Jag1* and *Jag2* was significantly reduced in
228 *Il1r1*$^{+}$ ciliated cells (EpCAM^{+}*Il1r1*$^{+}$CD24^{high}) after bleomycin injury compared to PBS control
229 (Fig. 4a,b). Furthermore, isolated ciliated cells treated with IL-1β for 24 hours *in vitro* also
230 showed a significant reduction in the expression of *Jag1* and *Jag2* compared to PBS control,
231 suggesting that IL-1β signalling directly modulates the expression of Notch ligands in ciliated
232 cells after injury (Fig. 4c).
233

234 We next asked whether depletion of IL-1β signalling on ciliated cells impacts the fate
235 behaviour of secretory cells after injury *in vivo*. To delete *Il1r1* in ciliated cells and lineage-
236 trace secretory cells simultaneously, we established *Il1r1*$^{flox/flox}$;*Foxj1-CreER*^{T2} mice crossed
237 with *Scgb1a1-CreER*^{TM};*R26*^{RtdTomato} mice (Fig. 4d). Of note, in the distal airway, *Il1r1* only
238 marks ciliated cells, not secretory cells[31]. Moreover, ciliated cells are known to lack hallmark
239 features of progenitors including generation of other lineages such as secretory and AT2 cells,
240 which was also supported by our organoid assay (Extended Data Fig. 7f)[31-33]. Given these
241 pieces of evidence, we examined the expression of Notch ligands (*Jag1*, *Jag2*, *Dll1*, *Dll4*) in
242 ciliated cells of *Il1r1*$^{flox/flox}$;*Scgb1a1-CreER*$^{T2/+}$;*R26*$^{RtdTomato/+}$ lungs after PBS or bleomycin
243 treatment. qPCR analysis revealed significant downregulation of *Jag1/2* expression in ciliated
244 cells after injury, indicating a transient reduction in Notch activity as an initial step in secretory
245 cell-mediated regeneration after injury (Fig. 4e). However, ciliated cells of *Foxj1-*
246 *CreER*^{T2};*Il1r1*$^{flox/flox}$;*Scgb1a1-CreER*$^{T2/+}$;*R26*$^{RtdTomato/+}$ lungs failed to downregulate the
247 expression of these ligands after bleomycin treatment, suggesting that IL-1β signalling via
248 *Il1r1* mediates the reduction of *Jag1/2* expression in ciliated cells after injury (Fig. 4e).
249 Significantly, after injury, Notch activity was reduced in secretory cells, which was impaired

by *Il1r1* deletion in ciliated cells (Fig. 4f-h). We observed no discernible alteration in lineage-labelled cells by *Il1r1* deletion in ciliated cells in PBS control lungs (Extended Data Fig.7g-i). However, we found a significant reduction in the frequency of lineage-labelled AT2 cells in the lungs of *Foxj1-CreER*^{T2};*Il1r1*$^{flox/flox}$;*Scgb1a1-CreER*$^{T2/+}$;*R26*$^{RtdTomato/+}$ mice compared to the lungs of *Il1r1*$^{flox/flox}$;*Scgb1a1-CreER*$^{T2/+}$;*R26*$^{RtdTomato/+}$ mice after injury (Fig. 4i,j). Together, these data indicate that IL-1β signals build niche environments governing the expression of Notch ligands in ciliated cells, which is essential for the fate transition of secretory cells toward AT2 cells during alveolar regeneration.

Fate conversion of secretory cells into AT2 cell by Fosl2

To investigate the epigenetic regulation that mediates the differentiation of secretory cells into AT2 cells, we performed ATAC-seq (Assay for Transposase-Accessible Chromatin with high-throughput sequencing) with secretory and secretory-derived AT2 cells (referred to as sAT2 from here). We generated *Sftpc-dsRed*$^{IRES\text{-}DTR}$ AT2 reporter mice to monitor SPC-expressing AT2 cells and crossed them with the *Scgb1a1-CreER*^{TM};*R26R*^{fGFP} mice to trace cells derived from secretory cells after injury (Fig. 5a). For ATAC-seq analysis, we isolated lineage-labelled secretory (GFP^{+}dsRed^{-}) and sAT2 (GFP^{+}dsRed^{+}) cells at day 28 post injury (Fig. 5a,b and Extended Data Fig. 7j). While approximately 45,548 peaks were common, 9297 and 7316 peaks were mapped as cell-type specific to secretory and sAT2 cells, respectively (Fig. 5c and Extended Data Fig. 8a-c). Analysis of Gene Ontology (GO) terms with the genes associated with cell-type specific ATAC-seq peaks revealed the distinct characteristics of secretory or AT2 cells (Extended Data Fig. 8d). Indeed, known-secretory cell markers, such as *Gabrp*, were associated with secretory cell specific-differential peaks, whereas AT2 cell markers, such as *Etv5*, were associated with sAT2 cell-specific peaks (Extended Data Fig. 8e). Motif analysis of DNA binding-site showed that sAT2-enriched chromatin contains motifs for key transcriptional factors (TFs) associated with lung development, including Nkx2-1 and Fosl2[34,35] (Fig. 5d). We compared the expression of TFs obtained from motif analysis with RNA-seq data of control and DAPT-treated SCOs (Fig. 2d). TFs in sAT2-enriched open chromatin were also highly expressed in DAPT-treated organoids, whereas the expression levels of TFs identified in secretory cell-restricted motif were higher in control organoids (Fig. 5e and Extended Data Fig. 8f). To interrogate the key TFs that are required for AT2 cell differentiation, we established feeder-free SCOs with KD of five TFs (*Srebf2*, *Fosl2*, *Rbpjl*, *Cebpa*, *Etv5*) selected from motif analysis or a list of differentially expressed genes in RNA-seq analysis. Notably, KD of *Fosl2/Fra2* significantly blocked the increase of AT2 cell markers,

284 including *Sftpc* and *Etv5*, by DAPT treatment whereas the downregulation of secretory cell
285 markers such as *Scgb1a1*was not altered (Fig. 5f and Extended Data Fig. 8g,h). Further IF
286 analysis also confirmed impaired induction of AT2 cells in *Fosl2*-deficient secretory organoids
287 treated with DAPT (Fig. 5g). Interestingly, we found that *Fosl2* KD markedly increased Act-
288 Tub^{+} ciliated cells in DAPT-treated organoids (Fig. 5g). These data suggest that Fosl2 is a
289 critical mediator regulating the fate conversion of secretory cells to AT2 lineage upon Notch
290 inhibition.
291

292 **Distinct character of airway-derived AT2 cells**

293 While AT2 cells serve as stem cells to maintain the alveolar epithelium during both
294 homeostasis and regeneration, secretory cells are the major source of replenishing alveolar
295 lineages after severe alveolar damage[5,6]. However, it remains unexplored whether sAT2 cells
296 generated after injury-repair are genetically or functionally identical to resident AT2 cells
297 (hereafter referred to as rAT2 cells) in alveoli. To address this question, we further analysed
298 the AT2 cell population in our scRNA-seq (Fig. 3j) of *Scgb1a1-CreER*$^{TM/+}$;*Red2-Notch*$^{N1ICD/+}$
299 mice challenged by bleomycin injury and found that secretory lineage-labelled AT2 (sAT2,
300 YFP^{+}) and non-lineage-labelled AT2 (rAT2, RFP^{-}YFP^{-}) cells were indeed segregated (Fig. 6a).
301 Notably, sAT2 cells still retained higher expression of many secretory cell markers such as
302 *Scgb1a1* and *Sox2* while the expression of AT2 markers such as *Sftpc* and *Etv5* was comparable
303 to that seen in rAT2 cells (Fig. 6b,c). We then examined the epigenetic signatures of sAT2 and
304 rAT2 cells via ATAC-seq on isolated GFP^{+}dsRed^{+} (sAT2) and GFP^{-}dsRed^{+}(rAT2) cells from
305 *Scgb1a1-CreER*$^{TM/+}$;*R26R*$^{fGFP/+}$;*Sftpc-dsRed*$^{IRES-DTR/+}$ mice treated with bleomycin injury at
306 day 28 (Extended Data Fig. 9a,b). We identified 52,497 shared open regions from sAT2 and
307 rAT2 cells and 429 differential open regions of sAT2 cells (Extended Data Fig. 9c). Most
308 sAT2-specific open regions were located far away from the transcription start sites (TSS),
309 suggesting that the differential peaks co-localised with contained distal enhancer regions
310 (Extended Data Fig. 9d). Consistent with genetic signatures, the regulatory regions surrounding
311 secretory cell marker loci, including *Scgb1a1* and *Sox2*, showed accessible chromatin
312 signatures in sAT2 cells, much like in secretory cells (Fig. 6d). Importantly, we found the
313 distinctive feature of an apoptosis-related signature in sAT2 cells (Extended Data Fig. 9e).
314 Genes involved in the negative regulation of apoptotic processes (GO:0043066) were also more
315 highly expressed in sAT2 than rAT2 cells in our scRNA-seq analysis (Extended Data Fig. 9f,g).
316 We confirmed that the *Nr4a2* locus, one of the most critical anti-apoptotic regulators in the
317 lung, is open in sAT2 cells, but not in rAT2 cells[36,37] (Extended Data Fig. 9h). Furthermore,

sAT2 cells showed higher expression of *Slc7a11*, which is a master regulator for redox homeostasis and essential for survival in response to cellular stress such as cystine deficiency, which can lead to cell death, referred to as ferroptosis[38,39] (Fig. 6b).

To further interrogate the functional differences between sAT2 and rAT2 cells, we established organoid co-cultures of $GFP^{+}dsRed^{+}$ (sAT2) and $GFP^{-}dsRed^{+}$ (rAT2) cells isolated from *Scgb1a1-CreER*$^{M2/+}$;*R26R*$^{fGFP/+}$;*Sftpc-dsRed*$^{IRES\text{-}DTR/+}$ mice at 2 months post bleomycin injury. Consistent with previous research[40], rAT2 cells lost colony forming efficiency (CFE) after serial passaging (Fig. 6e,f). However, sAT2 cells formed stable organoids without loss of CFE over multiple passages (Fig. 6e,f). Moreover, sAT2-derived organoids revealed a greater ratio of AT1 versus AT2 cells compared to that of rAT2-derived organoids, suggesting enhanced differentiation capacity into AT1 lineages (Extended Data Fig. 9i-k). We were also able to detect sAT2 cells in the lungs of *Scgb1a1-CreER*$^{TM/+}$;*R26R*$^{fGFP/+}$;*Sftpc-dsRed* mice for least at 3 months post bleomycin injury (Extended Data Fig. 9l,m). Together, these data indicate that AT2 cells derived from secretory cells during alveolar regeneration remain functionally and epigenetically distinct compared to alveolar resident AT2 cells.

Conserved differentiation potential of human secretory cells

We asked whether secretory cells in the human distal lungs have the potential to convert into AT2 cells. By combining analysis of our scRNA-seq and ATAC-seq analysis, we identified a surface marker *Kdr/Flk-1* that is specifically expressed in mouse secretory cells (Extended Data Fig.10a,b), which is also supported by previous studies[41,42]. Flow cytometry analysis confirmed that Kdr expression specifically marks secretory cells in the mouse lung (Extended Data Fig.10c-e). We further confirmed the expression of KDR in secretory cells of human distal lung tissue, which is also supported by recent scRNA-seq data[43,44] (Fig. 7a and Extended Data Fig. 10f,g). In combination with a human AT2 cell-specific surface marker HTII-280, we were able to sort $KDR^{+}HTII\text{-}280^{-}$ cells by flow cytometry analysis (Fig. 7b). Further analysis of cytospin and qPCR analysis on freshly isolated $KDR^{-}HTII\text{-}280^{+}$ and $KDR^{+}HTII\text{-}280^{-}$ populations revealed that $CC10^{+}$ secretory cells were specifically enriched in the KDR^{+} population (Fig. 7c-e). $KDR^{+}HTII\text{-}280^{-}$ population could form organoids consisting of secretory cells and ciliated cells, with a few basal cells in our culture conditions (Fig. 7f, **See Method**).

Given that human secretory cells also showed higher Notch activity (e.g. *HES1* expression) similar to mouse tissue (Extended Data Fig. 10f), we inhibited Notch activity by treating organoids derived from $KDR^{+}HTII\text{-}280^{-}$ cells with DAPT to investigate the differentiation potential of human secretory cells into AT2 cells. Notably, Notch inhibition promoted the generation of AT2 cells at the expense of secretory cells in DAPT-treated organoids (Fig. 7g-j). We barely observed any significant alterations in other lineages such as ciliated cells and basal cells (Extended Data Fig. 10h-j). Overall, our data suggest that KDR^{+} secretory cells share a conserved key molecular component of Notch signalling in governing alveolar regeneration of human and mouse lungs.

Discussion

Spatiotemporal dynamics of stem and progenitor cells ensure the rapid and efficient process of tissue repair for the reconstruction of epithelial integrity and function. In response to severe lung injury, secretory cells localised in the distal airway mobilise and differentiate into AT2 cells to compensate for the loss of alveolar epithelium and restore alveolar function. However, the cellular events and regulatory networks directing the differentiation plasticity of secretory cells, and the identity of the functional niches during this process, have been largely elusive. Particularly, how secretory cells are activated and acquire differentiation plasticity is unknown. Here, our data reveal two sequential stages of secretory cell fate conversion into AT2 cells during alveolar regeneration. Loss of Notch activity mediated by IL-1β signalling in ciliated cells reprograms the secretory cells to lose their identity upon injury. Fosl2/Fra2-mediated AP-1 transcription factor then drives the fate conversion of secretory cells into AT2 cells. Furthermore, using scRNA-seq and ATAC-seq analysis, we identified that secretory-derived AT2 cells retain the airway lineage identity in both a genetic and epigenetic manner, and have greater functional capacity of long-term maintenance *in vitro* compared to resident AT2 cells. By identifying a surface marker KDR/FLK-1 of human secretory cells, we propose that functionally equivalent human secretory cells are capable of contributing to alveolar lineages post injury via Notch signalling.

In this study, we uncovered the role of Notch signalling as a key regulator in reprogramming secretory cells to acquire differentiation plasticity post alveolar injury. Constitutive activation of Notch signalling substantially blocked AT2 cell differentiation from secretory cells post injury, by arresting cells at the intermediate state expressing high levels of *Cdkn1c/p57* expression. Notably, the intermediate population is specifically marked by the expression of

385 *Fstl1* that has been known to antagonize BMP signalling[45]. Given the functional role of BMP
386 signalling in facilitating proximal epithelium with a concurrent reduction in the distal
387 epithelium during lung development[45,46], impaired alveolar regeneration in constitutive Notch
388 activity could be connected to compromised proximal-distal patterning. It would be interesting
389 to further define the presence of *Fstl1*-like populations in lung development, and whether
390 alveolar regeneration reflects the developmental stages.
391
392 We identified Fra2/Fosl2, which together comprise the heterodimeric AP-1 transcription factor,
393 as an essential driver for the fate conversion of secretory cells into AT2 lineages. Further,
394 previous study also showed that Fosl2 is a functional target of Rbpj, a key mediator of Notch
395 signalling, and several regulatory regions of AT2 markers, including *Sftpc*, are directly
396 occupied by *Fosl2*[47]. Importantly, *Fosl2*-deficient secretory cells failed to acquire AT2 cell
397 differentiation in the presence of DAPT, however decreased secretory cells were not recovered.
398 Further, *Fosl2* KD didn't influence the cell fate of secretory cells without Notch inhibition.
399 These data suggest that Notch inhibition coordinates the cell fate decisions of secretory cells
400 into AT2 lineages in two stages: 1) loss of secretory cell identity and 2) acquisition of
401 transcriptional programmes for AT2 cell differentiation (Extended Data Fig. 10k). Our data
402 revealed that Fosl2 is required for AT2 cell conversion, but dispensable for the loss of secretory
403 cell identity. Other candidates, such as *Foxc2* and *Six1*, that occupy the regulatory region of
404 secretory cell-specific genes and are substantially downregulated by Notch inhibition (Fig.5d),
405 could be tested for potential roles in regulating the maintenance of secretory cell identity.
406
407 Notch inhibition has been reported to promote the differentiation of secretory cells into ciliated
408 cells in lung development and homeostatic adult lungs, although there are some differences in
409 the incidence of ciliated cell differentiation, potentially due to different contexts or model
410 systems[25-27]. In this study, we demonstrated that, during alveolar regeneration after injury,
411 Notch inhibition facilitated the fate conversion of secretory cells into AT2 cells via *IL-1β-*
412 *Notch-Fosl2* axis. How does *Fosl2* regulate this process? Interestingly, withdrawals of WNT
413 signalling-inducing factors, including Wnt3a and R-spondin-1, in our organoid cultures,
414 showed a dramatic increase in ciliated cells at the expense of secretory cells in DAPT-treated
415 organoids (Extended Data Fig.10l). AT2 cells were barely observed in these organoids
416 (Extended Data Fig.10l). This result suggests that Notch and WNT signalling likely function
417 cooperatively in secretory cells differentiating to AT2 cells. Indeed, previous studies showed
418 increased expression of Wnt ligands in mesenchymal cells during alveolar regeneration after

419 injury[19,48]. Further analysis of scRNA-seq from our previous study[31] revealed that Wnt ligands,
420 such as *Wnt5a* and *Wnt7b*, were highly upregulated at day 14 and returned back to homeostatic
421 levels at day 28 post bleomycin injury (Extended Data Fig.10m). Notably, however, *Fosl2*-KD
422 secretory cells failed to generate AT2 cells even in the presence of Wnt activity (Fig. 5g). Given
423 these results, it is likely that increased Wnt ligands from mesenchymal cells, in parallel with
424 Notch-mediated Fosl2 activity, may cooperatively confer differentiation potential of secretory
425 cells into AT2 lineages, via independent regulatory axes, during alveolar injury repair. While
426 Fosl2 was identified as a key factor in the axis of Notch signalling, further identification of
427 interacting partners regulated by Wnt signalling will uncover the molecular networks
428 governing the fate decision of secretory cells into ciliated or AT2 cells in homeostasis and
429 regeneration.
430
431 We found that secretory cell-derived AT2 (sAT2) cells display distinct features compared to
432 resident AT2 (rAT2) cells. sAT2 cells still retained some airway lineage identity based on the
433 higher expression of secretory cell markers such as *Sox2*, whereas most genetic and epigenetic
434 signatures of genes, including canonical AT2 cell markers such as *Sftpc*, are shared with rAT2
435 cells. Notably, sAT2 cells are capable of long-term self-renewal compared to rAT2 cells in *in*
436 *vitro* organoids, which may be attributed to the enriched signatures of anti-apoptotic functions
437 such as *Slc7a11* and *Nr4a2* in sAT2 cells compared to rAT2 cells. sAT2 cells may have a
438 higher tolerance for severe alveolar insults than rAT2 cells, enabling them to replenish
439 damaged alveolar epithelium more efficiently. Notably, in chronic lung diseases such as
440 pulmonary fibrosis and lung cancer, the destruction of alveolar structure with bronchiolization
441 is a common feature[49,50]. Moreover, AT2 cells expressing airway-restricted genes such as Sox2
442 were also seen in fibrosis patients[51]. Given our findings, it would be interesting to explore
443 further whether the genetic and epigenetic memory of sAT2 cells contributes to this pathologic
444 phenotype after injury resolution.
445
446 The loss of alveolar integrity is a life-threatening and key pathologic feature of various chronic
447 lung diseases. Recent studies suggested the regenerative potential of human AT2 cells using
448 an *in vitro* organoid model, yet their capacity to generate new alveolar epithelium seems
449 limited[22,23]. In particular, the functional capacity of human airway secretory cells to contribute
450 to alveolar lineages has never been explored, largely due to the lack of information for isolation
451 and culture of secretory cells *in vitro*. Here we provide the potential progenitor capacity of
452 secretory cells in self-renewal and generation of AT2 cells, by establishing a feeder-free

453 organoid culture of human secretory cells. We furthermore demonstrated that KDR$^+$ secretory
454 cells in human lungs have the differentiation plasticity to give rise to AT2 cells, which is also
455 regulated by Notch signalling, as we demonstrated in mouse lungs. Our findings provide a solid
456 foundation for further studies of the potential role of these cells in chronic lung diseases.
457
458 In summary, our results identify the molecular and cellular mechanisms of the cross-
459 compartment contribution of airway secretory cells to the regeneration of the alveolar
460 epithelium after lung injury. The presence of conserved markers and ease of cultivation of both
461 mouse and human secretory cells provide a unique opportunity for mechanistic studies to shed
462 light on human lung progenitor cell biology and assist in developing treatments for acute and
463 chronic lung diseases. Our study also provides clues for the potential therapeutic targets of
464 Notch signalling in lung diseases caused by defective and dysregulated alveolar regeneration.

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655

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658 analysis; Y.J.J. performed and analysed bulk RNA-seq and ATAC-seq data; C.D. designed and
659 performed lineage-tracing analysis of *Red2-Notch*^{N1ICD} mice, provided valuable comments on
660 the manuscript; E. I. generated the *Sftpc-IRES-DTR-P2A-dsRed* targeting vector; K.V.E.
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665
666 **Competing Interests Statement:** The authors declare that they have no competing interests.
667
668 **Figure Legends**
669 **Figure 1. Establishment of feeder-free organoids derived from distal secretory cells.**
670 **a,** Experimental design for isolation of *Scgb1a1* lineage-labelled cells. *Scgb1a1* lineage-
671 labelled cells were isolated at day 4 after final tamoxifen treatment. **b,** Representative bright-
672 field or fluorescent images of organoids derived from lineage-labelled *Tomato^{+}Scgb1a1^{+}* cells
673 in indicated conditions; complete medium (See **Methods**) with WNT3A, RSPO1 (R-spondin
674 1), EGF, FGF7, FGF10, and NOG (Noggin), withdrawal of indicated factors (−FGF10, −FGF7,
675 or −WNT3A/RSPO1). Scale bar, 2,000μm. **c, d,** Statistical quantification of colony forming
676 efficiency (n=6) (**c**) and passaging numbers (n=5) (**d**) of organoids. Each individual dot
677 represents one biological replicate and data are presented as mean and s.e.m. n.s; not significant.
678 **e,** Representative serial bright-field images of a lung organoid growing originated from single
679 *Tomato^{+}Scgb1a1^{+}* cells at the indicated time points. Magnifications: X20 (day 0 and 4), X10
680 (day4-21), and X4 (day 24 and 30). Scale bars, 200μm. **f,** Representative immunofluorescence
681 (IF) images of three distinctive types of organoids derived from *Tomato^{+}Scgb1a1^{+}* cells at the
682 establishment under feeder-free condition with complete culture medium; Airway organoids
683 retaining CC10^{+} secretory and Act-Tub^{+} ciliated cells (left), Alveolar organoids retaining SPC^{+}
684 AT2 and HOPX^{+} AT1 cells (middle), and Mixed organoids retaining both CC10^{+} secretory and
685 SPC^{+} AT2 cells (right). CC10 (for secretory cells, red or green), Acetylated-Tubulin (Act-Tub,
686 for ciliated cells, green), SPC (for AT2 cells, white), HOPX (for AT1 cells, white), and DAPI
687 (blue). Scale bars, 50μm. **g,** Representative bright-field images of organoids under feeder-free
688 condition with complete culture medium at passage 5. Insets show high-power view. Scale bars,
689 2,000μm. **h,** Representative IF images of mixed organoids cultured in complete medium at
690 passage 5. CC10 (green), Act-Tub (red), SPC (white, left), p63 (for basal cells, white, right),
691 and DAPI (blue). Insets (1, 2) show high-power view. Scale bars, 50μm. **i,** Quantification of
692 each organoid types in complete medium at passage 0 and over passage 5; Airway organoids
693 (CC10^{+}SPC^{-}; red), Alveolar organoids (CC10^{-}SPC^{+}; blue), and Mixed organoids (CC10^{+}SPC^{+};
694 grey). Data are presented as mean and s.e.m. (n=3 independent experiment). Statistical analysis
695 was performed using two-tailed unpaired Student's t test; ***p=0.002.
696

697 **Figure 2. Enhanced differentiation of secretory cells into AT2 cells by inhibition of Notch**
698 **activity upon lung injury.**

699 **a,** A heatmap showing normalised expression data for secretory and AT2 cell markers that were
700 differentially expressed in secretory cell-derived organoids (SCOs) or AT2 cell-derived
701 organoids (ACOs) cultured with defined media at passage 10. Values are z-scores. **b,** Gene set
702 enrichment analysis (GSEA) showing the gene activity of the three gene sets for secretory cells
703 (top), AT2 cells (middle), and Notch signalling pathway-related in SCOs or ACOs **c,**
704 Representative IF (top) and H&E (bottom) images of organoids in treatment with DMSO
705 (control) and DAPT at day 14. DAPT (20µM) were treated every other day during organoid
706 culture. CC10 (green), Acetylated-Tubulin (Act-Tub, red), and SPC (white), and DAPI (blue).
707 Scale bar, 50µm. **d,** A heatmap showing normalised expression data for Notch signalling
708 related, secretory and AT2 cell marker genes that were differentially expressed in SCOs with
709 or without DAPT treatment for 14 days. Values are z-scores. **e,** GSEA with two gene sets
710 representing markers for secretory (left) and AT2 cells (right) in SCOs with or without DAPT
711 treatment. **f,** Experimental design of lineage-tracing analysis for contribution of secretory cells
712 into alveolar lineages by inhibition of Notch signalling using *Scgb1a1-*
713 *CreER*$^{TM/+}$;*R26R*$^{tdTomato/+}$ and *dnMAML*$^{flox/+}$;*Scgb1a1-CreER*$^{TM/+}$;*R26R*$^{tdTomato/+}$ mice after
714 bleomycin (Bleo) injury. Specific time points for tamoxifen injection and tissue analysis are
715 indicated. **g, h,** Representative IF images showing the derivation of *Scgb1a1* lineage-labelled
716 AT2 cells in PBS-treated control (**g**) or bleomycin-treated (**h**) mice at day 28. Tomato (for
717 *Scgb1a1* lineage, red), SPC (white), and DAPI (blue). Scale bar, 100µm. **i,** Statistical
718 quantification of *Scgb1a1*$^{+}$ lineage-labelled AT2 cells (n=14 sections (PBS), n=14 sections
719 (Bleo), pooled from 2 mice for *Scgb1a1-CreER*$^{TM/+}$;*R26R*$^{tdTomato/+}$; n=26 sections (PBS) and
720 n=31 sections (Bleo), pooled from 4 mice for *dnMAML*$^{flox/+}$;*Scgb1a1-*
721 *CreER*$^{TM/+}$;*R26R*$^{tdTomato/+}$). Data are presented as mean ± s.e.m. Statistical analysis was
722 performed using two-way ANOVA; ***p<0.0001.
723

724 **Figure 3. Impaired contribution of secretory cells into AT2 cell regeneration by sustained**
725 **Notch activity.**
726 **a,** Experimental design of *ex vivo* organoid co-culture with stromal cells, lineage-tracing
727 analysis, and scRNA-seq analysis using *Scgb1a1-CreER*$^{TM/+}$*; Red2-Notch*$^{N1ICD/+}$ mice after
728 bleomycin injury. Specific time points for tamoxifen injection and analysis for tissue and
729 scRNA-seq are indicated. **b, c**, Representative bright-field images (**b**) and colony forming

730 efficiency (CFE) of organoids derived from control YFP^{+} (left) and *N1ICD*-expresing RFP^{+}
731 (right) cells. Scale bar, 2,000μm. Data are presented as mean and s.e.m. Statistical analysis
732 (n=7 biological replicates for YFP, n=8 biological replicates for RFP) was performed using
733 two-tailed unpaired Student's t test; *p=0.0141. **d,** Representative IF images of three distinctive
734 types of organoids in (**b**); Airway organoids (CC10^{+}SPC^{-}, 1), Alveolar organoids (CC10^{-}SPC^{+},
735 2), and Mixed organoids (CC10^{+}SPC^{+}, 3). CC10 (secretory cells, red), Act-Tub (ciliated cells,
736 green), SPC (AT2 cells, white), and DAPI (blue). Insets (1 and 3) show high-power view. Scale
737 bars, 50μm. **e,** Quantification of each organoid types in (**d**), Data are presented as mean and
738 s.e.m (n=3 biological replicates). ***p<0.0001 (Student's t test). **f,** Representative IF images
739 showing the derivation of YFP^{+} or RFP^{+} cells in control mice at day 28 post PBS treatment.
740 YFP (yellow), RFP (red), CC10 (white), and DAPI (blue). Scale bar, 50μm. **g,** Representative
741 IF images showing the derivation of YFP^{+} or RFP^{+} cells at day 28 post bleomycin treatment.
742 YFP (yellow), RFP (red), SPC (top, white), CC10 (bottom, white), and DAPI (blue).
743 Arrowhead points to RFP^{+} cells. Scale bar, 50μm. **h, i,** Statistical quantification of CC10^{+}
744 secretory (**h**) and SPC^{+} AT2 (**i**) cells derived from YFP^{+} or RFP^{+} cells at day 28 post PBS or
745 bleomycin treatment. Data (n=3 biological replicates) are presented as mean ± s.e.m.
746 **p=0.025 (**h**), p=0.0052 (**i**) from Student's t test. **j,** Secretory and AT2 cell population were
747 further analysed from scRNA-seq results (Extended Data Fig. 7b). Number of cells in the
748 individual cluster is depicted in the figure. **k,** Gene expression of key markers in each
749 distinctive cluster. **l,** Quantification (left) and UMAP (right) of distribution of each cluster
750 across indicated lineage-labelled cells after injury.
751
752 **Figure 4. Regulation of Notch ligand expression in ciliated cells by IL-1β signalling.**
753 **a,** Experimental design for isolation of *Il1r1*-expressing ciliated cells. **b,** qPCR analysis
754 showing the expression of Notch ligands in ciliated cells (EpCAM^{+}*Il1r1*$^{+}$CD24^{high}). Data are
755 presented as mean ± s.e.m. (n= 4 biological replicates). ***p=0.0004 (Jag1), ***p<0.0001
756 (Jag2) using Student's t test. **c,** qPCR analysis showing the expression of *Jag1* (n=3 biological
757 replicates) and *Jag2* (n=4 biological replicates) in cultured ciliated cells treated with PBS or
758 IL-1β. Data are presented as mean ± s.e.m. **p=0.0016, ***p<0.0003 using Student's t test. **d,**
759 Experimental design for lineage-tracing analysis of secretory cells after PBS or bleomycin
760 treatment using *Scgb1a1-CreER*$^{TM/+}$;*Il1r1*$^{flox/flox}$;*R26R*$^{tdTomato/+}$ or *Foxj1-CreER*^{T2};*Scgb1a1-*
761 *CreER*$^{TM/+}$;*Il1r1*$^{flox/flox}$; *R26R*$^{tdTomato/+}$ mice. **e,** qPCR analysis showing the expression of *Jag1*
762 and *Jag2* in isolated ciliated cells (Tom^{+}EpCAM^{+}CD24^{high}) after treatment of PBS or

763 bleomycin. P-values using Student's t test are indicated in the figure, n=4 biological replicates,
764 n.s; not significant. **f,** Representative IF images showing Hes1 expression: Tomato (for
765 *Scgb1a1* lineage, red), Hes1 (white), and DAPI (blue). Insets show high-power view of Hes1
766 expression. Scale bars, 50μm. **g,** Flow cytometry sorting strategy for lineage-labelled secretory
767 cells after PBS or bleomycin injury. EpCAM^{+}CD24^{-} cells gated from EpCAM^{+}Tomato^{+} were
768 used for isolation of lineage-labelled secretory cells and qPCR analysis. **h,** qPCR analysis of
769 *Hes1* and *Nrarp* from isolated lineage-labelled secretory cells in (**g**). n=2, 4, and 3 individual
770 experimental mice. Data are presented as mean ± s.e.m. P-values using Student's t test are
771 indicated in the figure. **i,** Representative IF images showing the derivation of *Scgb1a1*$^{+}$ lineage-
772 labelled AT2 cells in PBS-treated control or bleomycin-treated mice at day 28. Tomato (for
773 *Scgb1a1* lineage, red), SPC (white), and DAPI (blue). White boxed insets show high-power
774 view (right panel). Scale bar, 100μm. **j,** Statistical quantification of Tomato^{+}SPC^{+} AT2 cells at
775 day 28 post bleomycin treatment in (**f**). n=30 sections, pooled from 4 mice (*Scgb1a1-*
776 *CreER*$^{TM/+}$;*Il1r1*$^{flox/flox}$;*R26R*$^{tdTomato/+}$) and n=27 sections, pooled from 3 mice (*Foxj1-*
777 *CreER*^{T2};*Scgb1a1-CreER*$^{TM/+}$;*Il1r1*$^{flox/flox}$;*R26R*$^{tdTomato/+}$). Data are presented as mean ± s.e.m.
778 Statistical analysis was performed using two-way ANOVA; ***p<0.0001.
779

780 **Figure 5**. **Fosl2/Fra2-mediated AP-1 activity is required for the fate conversion of**
781 **secretory cells into AT2 cells**.

782 **a,** Experimental design for isolation of secretory (GFP^{+}dsRed^{-}) and secretory-derived AT2
783 (sAT2, GFP^{+}dsRed^{+}) cells using *Scgb1a1-CreER*$^{TM/+}$;*R26R*$^{fGFP/+}$;*Sftpc-dsRed*$^{IRES-DTR/+}$ mouse
784 post bleomycin injury. Specific time points for tamoxifen injection and isolation of cells are
785 indicated. **b,** Representative flow cytometry analysis for isolation of secretory (GFP^{+}dsRed^{-})
786 and sAT2 (GFP^{+}dsRed^{+}) cells. **c,** A heatmap showing ATAC-seq peaks representing open
787 chromatin regions in secretory cells (left) and sAT2 cells (right). Secretory-specific, shared,
788 and sAT2-specific peaks are shown. Regions of a pie chart presenting the proportion of
789 secretory-specific (blue), sAT2-specific (red), and shared regions (grey). **d,** Top five motif
790 matrices and transcription factors predicted by the HOMER *de novo* motif analysis using
791 secretory-specific and sAT2-specific open regions. **e,** A heatmap showing the expression levels
792 of transcription factors predicted by the motif analysis (Fig. 5d) in secretory cell-derived
793 organoids (SCOs) with or without DAPT treatment (20μM). **f,** qPCR analysis of the markers
794 for secretory (*Scgb1a1* and *Gabrp*) and AT2 cells (*Sftpc*, *Etv5*, *Ly6c1*, and *Lpcat1*) in SCOs
795 with or without DAPT treatment after knockdown of control (white), Rbpjl (black), Fosl2 (red),

796 and Srebf2 (blue). n=5 biological replicates (for SPC expression), n=4 (for other markers)
797 except Srebf2 KD samples. Data are presented as mean ± s.e.m. Statistical analysis was
798 performed using two-tailed unpaired Student's t test; P-values are indicated in the figure. **g,**
799 Representative IF images of secretory cell-derived control KD or *Fosl2* KD organoids treated
800 with DMSO or DAPT (20μM). Single cells dissociated from SCOs at passage 10-12 were
801 cultured in feeder-free condition for 7 days and further maintained with DMSO or DAPT for
802 another 7 days. CC10 (white, left and right panels; green, middle panel), SPC (white, middle
803 panels), Krt5 (green, left and right panels), Act-Tub (red), and DAPI (blue). Scale bars, 50μm.

804 **Figure 6. Distinctive features of secretory-derived AT2 cells.**
805 **a,** UMAP visualisation of two distinctive AT2 cell clusters sAT2 (secretory cell-derived AT2,
806 $YFP^{+}SPC^{+}$ cluster); rAT2 (resident AT2, $YFP^{-}RFP^{-}$ non-lineage labelled SPC^{+} cluster) from
807 scRNA-seq analysis of *Scgb1a1-CreER*$^{TM/+}$;*Red2-Notch*$^{N1ICD/+}$ mice in **Fig. 3j**. **b,** Gene
808 expression of key markers in sAT2 and rAT2 cell clusters. **c,** UMAP visualisation of the log-
809 transformed ($\log_{10}$(TPM+1)), normalised expression of selected marker genes (*Sftpc* and *Etv5*
810 for AT2 cells. *Scgb1a1* and *Cyp2f2* for secretory cells) in distinctive clusters shown in (**a**). **d,**
811 Signal track images showing open regions of markers for secretory (Scgb1a1 and Sox2) and
812 AT2 cells (Lyz2 and Sftpc) mapped in secretory (blue), sAT2 (red), and rAT2 cells (green)
813 from the result of **Fig. 5c**. **e,** Representative bright-field images of organoids derived from
814 rAT2 (top) or sAT2 (bottom) cells: Experiment scheme for labelling with tamoxifen is same
815 as **Fig. 5a**. *Scgb1a1-CreER*$^{TM/+}$;*R26R*$^{fGFP/+}$;*Sftpc-dsRed*$^{IRES\text{-}DTR/+}$ mice was given four doses of
816 tamoxifen followed by bleomycin injury. Isolated $GFP^{+}dsRed^{+}$ (sAT2) and $GFP^{-}dsRed^{+}$ (rAT2)
817 cells at 2 months post bleomycin injury were co-cultured with stromal cells with 1:5 ratio.
818 Images show the organoids at passage 0 and 4. Scale bar, 2,000μm. **f,** Statistical quantification
819 of colony forming efficiency (CFE) of indicated organoids. Each individual dot represents
820 individual biological replicate (n=6 and 8 for Passage 0, n=10 for passage 2, and n=2 for
821 passage 4) and data are presented as mean ± s.e.m. Statistical analysis was performed using
822 two-tailed unpaired Student's t test; P-values are indicated in the figure.
823

824 **Figure 7**. **Differentiation plasticity of secretory cells into AT2 cells by inhibition of Notch**
825 **signalling in human lungs**.
826 **a,** IF images showing the expression of KDR in secretory cells in the lung from normal donors.
827 CC10 (red), KDR (white), and DAPI (blue). White boxed insets show high-power view. Scale
828 bar, 50μm and 10μm (inset). **b,** Representative flow cytometry analysis for isolation of

KDR$^+$HTII-280$^-$ or KDR$^-$HTII-280$^+$ cells. **c,** Representative IF images of cytospin staining from freshly sorted KDR$^-$HTII-280$^+$ or KDR$^+$HTII-280$^-$ population. SPC (for AT2 cells, red), p63 (for basal cells, white), CC10 (for secretory cells, green) and DAPI (blue). Scale bars, 100μm. **d,** Quantification of SPC$^+$, CC10$^+$, and Act-Tub$^+$ cells revealed in cytospin staining from KDR$^-$HTII-280$^+$ or KDR$^+$HTII-280$^-$ cells in **Fig. 7c**. **e,** qPCR analysis of the markers for secretory (*SCGB1A1* and *SCGB3A2*) and AT2 (*SFTPC*, *ETV5*, and *LAMP3*) cells in freshly isolated KDR$^+$HTII-280$^-$ (red bars) or KDR$^-$HTII-280$^+$ (white bars) cells. Individual dots represent individual experiments (n=2 biological replicates). **f,** Representative IF images of KDR$^+$ cell-derived organoids at first passage. CC10 (green), Act-Tub (red), KRT5 (white), and DAPI (blue). Scale bar, 50μm. **g,** Representative bright-field images of organoids derived from KDR$^+$HTII-280$^-$ cells with or without DAPT treatment (20μM) at passage 1. Scale bar, 2,000μm. **h**, qPCR analysis of the markers for secretory cells (*SCGB1A1* and *SCGB3A2*) and AT2 cells (*SFTPC*, *ETV5*, and *LAMP3*) in organoids derived KDR$^+$HTII-280$^-$ cells without (white bars) or with DAPT treatment (red bars). Each individual dot represents individual biological replicate (n=3) and data are presented as mean ± s.e.m. Statistical analysis was performed using two-tailed unpaired Student's t test; P-values are indicated in the figure. **i**, Representative IF images of KDR$^+$ cell-derived organoids treated with DMSO or DAPT (20μM). CC10 (green), SPC (red), and DAPI (blue). Scale bar, 50μm. **j**, Quantification of the frequency of SPC$^+$ cells in the control or DAPT treated organoids. Each individual dot represents one organoid and data are presented as mean ± s.e.m (n=20 organoids, pooled from 3 mice). Statistical analysis was performed using two-tailed unpaired Student's t test; $p<0.0001$.

850

Methods

This study complies with all relevant ethical regulations for which approval of the institutional review board and the ethics committee of the University of Cambridge and Cambridge Stem Cell Institute.

Animals. *Scgb1a1-CreER*^{TM}[13], *Sftpc-CreER*^{T2}[5], *Foxj1-CreER*^{T2}[32], *Red2-Notch*^{N1ICD}[28], *Rosa26R-CAG-fGFP*[13], *Rosa26R-lox-stop-lox-tdTomato*[52], and *Il1r1*$^{flox/flox}$[53] mice have been described and are available from Jackson Laboratory. Il1r1-P2A-eGFP-IRES-CreER^{T2} (*Il1r1-CreER*^{T2})[31]. To monitor SPC-expressing AT2 cells, we generated Sftpc-IRES-DTR-P2A-dsRed (*Sftpc-dsRed*$^{IRES\text{-}DTR}$) reporter mouse where IRES-DTR-P2A-dsRed construct is inserted into 3'-UTR region of endogenous Sftpc gene. Mice for the lineage tracing and injury experiments were on a C57BL/6 background and 6-10 week old mice were used for most of the experiments described in this study. Mice were bred and maintained under specific-pathogen-free conditions at Gurdon Institute of University of Cambridge under the guidance of UK Home Office project license PC7F8AE82.

Cell lines. HEK129T cells were purchased from ATCC (CRL-3216) and maintained in Dulbecco's Modified Eagle medium (DMEM) with 10% FBS (Gibco) and antibiotics (penicillin and streptomycin).

Tamoxifen. Tamoxifen (Sigma) was dissolved in Mazola corn oil (Sigma) in a 20 mg/ml stock solution. 0.2 mg/g body weight tamoxifen was given via intraperitoneal (IP) injection. The numbers and date of treatment are indicated in the individual figures of each experimental scheme.

Bleomycin administration. 6-10 week-old mice were anesthetised via inhalation of isoflurane for approximately 3 mins. The mice were positioned on the intratracheal intubation stand, and bleomycin (1.25 U/kg) or PBS were delivered intratracheally by a catheter (22G). During the procedure anaesthesia was maintained by isoflurane and oxygen delivery.

Mouse lung tissue dissociation and flow cytometry. Lung tissues were dissociated with a collagenase/dispase solution as previously described[31]. Briefly, after lungs were cleared by perfusion with cold PBS, 2 ml of dispase (BD Biosciences, 50Uml) was instilled into the lungs

884 through the trachea until the lungs inflated. Each lobe was dissected and minced into small
885 pieces in a conical tube containing 3 ml of PBS, 60 µl of collagenase/dispase (Roche), and 7.5
886 µl of 1% DNase I (Sigma) followed by rotating incubation for 45 min at 37°C. The cells were
887 then filtered sequentially through 100- and 40-µm strainers and centrifuged at 1500 rpm for 5
888 min at 4°C. The cell pellet was resuspended in 1ml of RBC lysis buffer (ACK buffer, 0.15M
889 NH4Cl, 10mM KHCO3, 0.1mM EDTA) and lysed for 2 min at room temperature. 8 ml basic
890 F12 media (GIBCO) was added and 500µl of FBS (Hyclone) was slowly added in the bottom
891 of tube. Cells were centrifuged at 1500 rpm for 5 min at 4°C. The cell pellet was resuspended
892 in PF10 buffer (PBS with 10% FBS) for further staining. The antibodies used were as follows:
893 CD45 (30-F11)-APC or -APC-Cy7 (BD Biosciences), CD31 (MEC13.3)-APC (BD
894 Biosciences), EpCAM (G8.8)-PE-Cy7 or FITC (BioLegend), and CD24 (M1/69)-APC
895 (eBioscience), MHC-II (I-A/I-E, M5)-FITC or -APC-Cy7 (eBioscience), and CD309/Kdr/Flk-
896 1 (7D4-6)-APC (BioLegend). 4', 6-diamidino-2-phenylindole (DAPI) (Sigma) was used to
897 eliminate dead cells. Data were acquired on LSRII analyser (BD Bioscience) and then analysed
898 with FlowJo software (Tree Star).
899
900 **Human lung tissue dissociation and flow cytometry**. Human lung tissues were cleared by
901 perfusion with cold PBS and cut into small pieces. Tissue was transferred to 10 ml of digestion
902 buffers (2 ml of dispase II (Sigma), 100 µl of collagenase/dispase (Roche), 100 µl of 1% DNase
903 I (Sigma), and 7.8 ml of PBS), followed by rotating incubation for 1hr at 37°C. The cells were
904 then filtered through 40 µm strainers and centrifuged at 1500 rpm for 5min at 4°C. The cell
905 pellet was resuspended in 1ml of RBC lysis buffer and lysed for 5 min at room temperature.
906 10ml basic F12 media (GIBCO) was added and 500µl of FBS (Hyclone) was slowly added in
907 the bottom of tube. Cells were centrifuged at 1500rpm for 5 min at 4°C. The cell pellet was
908 resuspended in PF10 buffer for further staining. The antibodies used were as follows: CD45
909 (2D1)-APC (BioLegend), CD31 (VM59)-APC (BioLegend), EpCAM (9C4)- FITC
910 (BioLegend), HTII-280-mouse IgM (Terrace Biotech), Purified CD309/KDR (A16085H),
911 anti-mouse IgG1 (RMG1-1)-APC-Cy7 (BioLegend), and anti-mouse IgM (Il/41)-PE
912 (eBioscience). 4', 6-diamidino-2-phenylindole (DAPI) (Sigma) was used to eliminate dead
913 cells. Flexible BD Influx^{TM} cell sorter were used for the sorting at Cambridge NIHR BRC Cell
914 Phenotyping Hub and data were analysed with FlowJo software (Tree Star).
915
916 **Human Tissues**. For the establishment of human lung organoids and histological analysis,
917 human lung tissues from deidentified lungs not required for transplantation were obtained from

918 adult donors with no pathologies from Papworth Hospital Research Tissue Bank (T02233).
919 Human lung tissues were also obtained from patients undergoing lobectomy surgery at
920 University College London with written informed consent from approval of the ethical
921 committee (UCL:06/Q0505/12). Appropriate Human Tissue Act (HTA) guidance was
922 followed.
923
924 ***In vitro* feeder-free organoid culture and passages**. Freshly sorted lineage-labelled cells from
925 *Scgb1a1-CreER*$^{TM/+}$;*R26R*$^{tdTomato/+}$ or *Sftpc-CreER*^{T2};*R26R*$^{tdTomato/+}$ mice were resuspended in
926 basic medium (AdDMEM/F12 (Invitrogen) supplemented with B27 (Invitrogen), 1mM *N*-
927 Acetylcysteine (Sigma), 10 mM Nicotinamide (Sigma)). Cells were mixed with growth factor-
928 reduced (GFR)-Matrigel (BD Biosciences) at a ratio of 1:1. A 100 µl mixture was placed in a
929 24-well Transwell insert with a 0.4 µm pore (Corning). Approximately 10×10^3 epithelial cells
930 were seeded in each insert. After GFR-Matrigel formed a gel, 500 µl of culture medium (basic
931 medium supplemented with the growth factors: 50 ng/ml murine EGF (Life Technology), 100
932 ng/ml human FGF7/KGF (Peprotech), 100 ng/ml human FGF10 (Peprotech), 50% WNT3A-
933 conditioned media (provided by Tissue Core Facility of Cambridge Stem Cell Institute), 10%
934 RSPO1-conditioned media (provided by Tissue Core Facility of Cambridge Stem Cell
935 Institute), 100 ng/ml Noggin (Peprotech)) was placed in the lower chamber. Medium was
936 changed every other day and ROCK inhibitor Y27632 (10 µM, Sigma) was added in the
937 medium for the first 2 days of culture. Passage was performed once per 2 weeks. For passages,
938 organoids were removed from the Matrigel by incubation with dispase (1 mg/ml) for 40 mins
939 at 37 °C, followed by dissociation into single cells using trypLE (Gibco) treatment for 5min at
940 37°C. $5\sim10\times10^3$ cells were transferred to fresh GFR-Matrigel in 24-well Transwell insert. For
941 organoid culture from a single cell with limiting dilution, FACS-sorted cells were plated into
942 48-well plates (Corning) with one cell per well. Every dilution was replicated in 48-well plates
943 (Corning) for two independent experiments. Single cells imbedded in Matrigel were monitored
944 at microscope with RFP channels to check the expression of Tomato expression.
945
946 For human KDR$^+$ cell culture in organoids, approximately 10×10^3 epithelial cells were
947 resuspended in a 20 µl of 100% GFR-Matrigel and seeded in 48 well plates, followed by 30
948 min incubation at 37 °C for solidification. Then, 250µl of airway cell culture medium (basic
949 medium supplemented with the growth factors: murine EGF (50 ng/ml, Life Technology),
950 human FGF7/KGF (100 ng/ml, Peprotech), human FGF10 (100 ng/ml, Peprotech), Noggin
951 (100 ng/ml, Peprotech), A83-01 (1µM, Tocris), SB202190 (1µM, Tocris) was added to each

952 well. To avoid the growth of fungal and bacterial infection, 250 ng/mL Amphotericin B and
953 50 μg/mL gentamicin were added to culture medium. For culture with DAPT treatment in
954 Fig.7g, organoids cultured for 8 days with airway cell culture medium were followed by
955 inclusion of CHIR99021 (2μM, Tocris) for additional 10 days with or without DAPT (20μM).
956 Medium was changed every 3-4 days and ROCK inhibitor Y27632 (10μM, Sigma) was added
957 in the medium for the first 4 days of culture. Passage was performed once per 3 weeks.
958
959 **Knockdown construct and retroviral infection to organoids**. For sequence-specific
960 knockdown of candidate genes, target sequences were cloned into MSCV-LTRmiR30-PIG
961 (LMP, Open Biosystems) retroviral vectors or pLKO.1-puro lentiviral vector. To generate
962 retroviruses for infection into cells, the HEK293T cells were transfected using a standard
963 calcium phosphate protocol with vectors expressing GFP alone (Control-RV), GFP plus short-
964 hairpin against target genes. Viral supernatants were harvested 2 days later. Secretory-derived
965 organoids of passages between 15 and 20 were prepared after recovery from GFR-Matrigel
966 (BD Biosciences) by treatment of dispase (1 mg/ml) for 40 min at 37°C, followed by
967 dissociation into single cells using trypLE (Gibco) treatment for 5 min at 37°C. Single cells
968 dissociated from established organoids were infected with the viral supernatants in the presence
969 of polybrene (Sigma, 8μg/ml) by spin infection for 90 min at 2400 rpm at 32°C. This procedure
970 was repeated twice every day. Short-hairpin sequences for target genes are listed in
971 Supplementary Table 1.
972
973 ***In vitro* lung organoid co-culture with established stromal cells**. Freshly sorted lineage-
974 labelled cells were resuspended in culture medium (3D basic medium (DMEM/F12, Gibco)
975 supplemented with 10% FBS. (Gibco) and ITS (Insulin-Transferrin-Selenium, Corning)), and
976 mixed with cultured lung stromal cells negatively isolated by microbeads of CD326/EpCAM,
977 CD45, and CD31 via MACS (Miltenyi Biotech), followed by resuspension in GFR-Matrigel
978 (BD Biosciences) at a ratio of 1:5. A 100 μl mixture was placed in a 24-well Transwell insert
979 with a 0.4 μm pore (Corning). Approximately 5×10^3 epithelial cells were seeded in each insert.
980 500 μl of culture medium was placed in the lower chamber, and medium was changed every
981 other day. ROCK inhibitor Y27632 (10μM, Sigma) was added in the medium for the first
982 2 days of culture. Analysis of colony forming efficiency and size of organoids was performed
983 at 14 days after plating if there is no specific description.
984

985 ***In vitro* culture of ciliated cells for IL-1β treatment.** $CD24^{high}Tomato^{+}$ ciliated cells were
986 isolated from *Il1r1-CreER^{T2}* mice at day 4 after four doses of tamoxifen treatment. Purified
987 20,000 cells were embedded in GFR-Matrigel with PBS or IL-1β (10ng/ml) for 24 hrs. RNA
988 was isolated to analyse the expression of *Jag1* or *Jag2*.
989
990 **Transplantation of *Scgb1a1*$^{+}$ lineage-labelled cells**. Freshly sorted 20,000 cells of
991 $CD45^{-}EpCAM^{+}Tomato^{+}MHCII^{-}$ cells from *Scgb1a1-CreER$^{TM/+}$;R26R$^{tdTomato/+}$* or
992 *dnMAML$^{flox/+}$;Scgb1a1-CreER$^{TM/+}$;R26R$^{tdTomato/+}$* mice were mixed with lung stromal cells
993 isolated from WT mice (20,000 cells) to support epithelial cell survival during engraftment,
994 and were transplanted into WT C57BL/6 mice at day 7 after bleomycin injury (1.25 U/kg).
995 Lungs were analysed at day 14 post injury to determine the differentiation of engrafted cells.
996
997 **Quantitative RT-PCR.** Total RNA was isolated using a Qiagen RNeasy Mini-plus Kit
998 according the manufacturer's instructions. Equivalent quantities of total RNA were reverse-
999 transcribed with SuperScript IV cDNA synthesis kit (Life Technology). Diluted cDNA was
1000 analysed by real-time PCR (StepOnePlus; Applied Biosystem). Pre-designed probe sets
1001 (Thermo Fisher Scientific) were used as follows: human SCGB1A1 (Hs00171092_m1), human
1002 SFTPC (Hs00951326_g1), and Human FOXJ1 (Hs00230964_m1). ACTB expression
1003 (Hs01060665_g1) was used to normalise samples using the ΔCt method. Sybr green assays
1004 were also used for human or mouse gene expression with SYBR Green Master Mix (2x,
1005 Thermo Fisher Scientific). Primer sequences are listed in Supplementary Table 2:
1006
1007 **Cytospin, Immunofluorescence, and immunohistochemistry**. Mouse lung tissues were
1008 perfused, inflated, and fixed with 4% PFA for 2 hrs at 4°C. Cryosections (8-12 μm) and paraffin
1009 sections (7 μm) were used for histology and Immunofluorescence (IF) analysis. Cultured
1010 colonies from organoids were fixed with 4% PFA for 2 hrs at room temperature followed by
1011 immobilisation with Histogel (Thermo Scientific) for paraffin embedding. For cytospin
1012 staining, isolated 2,000 cells were resuspended in 250 μl of PBS supplemented with 10% FBS,
1013 followed by spinning in pre-wet cytospin funnels at 600 rpm for 5min. Sectioned lung tissues
1014 or colonies were stained with hematoxylin and eosin (H&E) or immunostained: after antigen
1015 retrieval with citric acid (0.01M, pH 6.0), blocking was performed with 5% normal donkey
1016 serum in 0.2% Triton-X/PBS at room temperature for 1hr. Primary antibodies were incubated
1017 overnight at 4°C at the indicated dilutions: Goat anti-CCSP/CC10 (T-18) (1:200, Santa Cruz
1018 Biotechnology Inc., sc-9772), Mouse anti-CCSP/CC10 (E11) (1:200, Santa Cruz

1019 Biotechnology Inc., sc-365992), Goat anti-SP-C (1:200, Santa Cruz Biotechnology Inc., sc-
1020 7706), Rabbit anti-pro-SP-C (1:300, Millipore, AB3786), Mouse anti-Acetylated Tubulin (6-
1021 11B-1) (1:300, Sigma-Aldrich, T7451), Hamster anti-PDPN (1:300, DSHB, 8.1.1), Rabbit
1022 anti-KRT5 (1:300, BioLegend, 905501), Mouse anti-P63 (1:200, Abcam, ab735), Rabbit anti-
1023 PORCN (1:100, Abcam, ab201793), Rabbit anti-P57/KIP2 (1:100, Abcam, ab75974), Rabbit
1024 anti-RFP (1:250, Rockland, 600–401379), Rabbit anti-HOPX (1:100, Santa Cruz
1025 Biotechnology Inc., sc-30216), Rabbit anti-HES1 (1:200, D6P2U, #11988, Cell Signaling),
1026 Rabbit anti-NOTCH1 (1:50, Abcam, ab52627), Mouse IgM anti-HT2-280 (1:300, Terrace
1027 Biotech, TB-27AHT2-280), Rat anti-human SCGB1A1/CC10 (1:200, R&D system,
1028 MAB4218), Rabbit anti-KDR/VEGFR2 (1:100, Cell Signaling, 2479). Alexa Fluor-coupled
1029 secondary antibodies (1:500, Invitrogen) were incubated at room temperature for 60 min. After
1030 antibody staining, nuclei were stained with DAPI (1:1000, Sigma) and sections were embedded
1031 in RapiClear® (SUNJin Lab). Fluorescence images were acquired using a confocal microscope
1032 (Leica TCS SP5). All the images were further processed with Fiji software. For
1033 immunohistochemical staining of Jag1, Anti-human JAG1 antibody was used (1:100, Santa
1034 Cruz Biotechnology Inc., sc-390177). Slides were developed by using mouse IgG
1035 VECTASTAIN Elite ABC kit (Vector Laboratories). Slides were counterstained with
1036 haematoxylin.
1037
1038 **Cell counting and image analysis.** Sections included in cell scoring analysis for lung tissue
1039 were acquired using Leica TCS SP5 confocal microscope. At least six different sections under
1040 10X magnification including at least 8 different alveolar regions from individual mice indicated
1041 in the figures per group were used. Cell counts were performed on ImageJ using the 'Cell
1042 Counter' plug-in and the performer was blinded to the specimen genotype and condition. At
1043 least two step sections (30μm apart) per individual well were used for quantification of
1044 organoids.
1045
1046 **RNA-sequencing analysis**. RNA-seq were performed for global gene expression profiles in
1047 ACOs, and SCOs with or without DAPT treatment. Total RNAs were extracted using the
1048 RNeasy Plus Mini Kit following the manufacturer's instructions. 100 ng of total RNA was used
1049 to generate RNA-seq library using NEBNext Ultra RNA Library prep kit (NEB, E7530L)
1050 according to the vendor's protocol. Briefly, mRNAs were purified from total RNAs with
1051 Magnetic mRNA Isolation Kit (oligo(dT) beads) and fragmented. First and second strand
1052 cDNAs were synthesized subsequently. The double strand cDNAs were purified, and then the

ends of cDNAs were repaired and ligated with sample-specific barcodes. RNA-seq libraries were sequenced using an Illumina NextSeq 500 machine. Single-ends reads from RNA-seq were aligned to the reference mouse genome (mm10) using STAR (v2.5.2b). The python package HTSeq (https://htseq.readthedocs.io/en/master/) was used to generate read counts for each gene. The read counts were analysed by the R package DESeq2 (v1.28.0)[54] and regularized log transformed using the rlog function. Adjusted p-values (p.adj) for DEG were determined by Benjamini and Hochberg correction. The p.adj < 0.01 required to consider differentially expressed genes. Heat maps were generated using Java Treeview (v1.1.6r4).

ATAC-sequencing analysis. The ATAC-seq assay was performed in two biological replicates for each sample of 50,000 FACS-purified cells. The quality of data from ATAC-seq was tested using FASTQC. The adapter sequences were contained in raw data. Therefore, NGmerge[55] was used for adapter trimming. 150 bp paired-ends adapter trimmed reads were aligned against the mouse genome assembly (mm10) using bowtie2 (v2.3.4.1). We performed peak calling using MACS2 (v2.1.2) with default parameters for paired ends. Statistically significant differential open chromatin regions were determined using MAnorm (v1.1.4)[56], which normalises read density levels and calculates p-values by MA plot methods. The heat maps and the Spearman correlation map of ATAC-seq signals were generated using deepTool2[57]. Genome browser images were generated using the Integrative Genomics Viewer (IGV) 2.7.2[58] with bedGraph files processed from MACS2. The ATAC-seq peaks were mapped to the region surrounding 20 Kb up- and 2 Kb down-stream of the TSS of all genes from refFlat file (mm10, UCSC). All assigned genomic features from one open region were used. To describe the distribution of genes, a promoter was defined as a region within 2 Kb from the TSS, a proximal promoter region was defined as a region between 2 Kb and 20 Kb upstream from the TSS. Mapping sites other than promoter, proximal promoter, exon, or intron were considered as intergenic target loci. To identify the cell-type specific enriched motifs in the differential open chromatin regions from ATAC-seq data, we performed motif enrichment analysis using the findMotifsGenome.pl program in the HOMER software (v4.11)[59]. The regions were adjusted to the same size with 200 bp centred on each differential peak.

Gene ontology (GO) analysis. The gene ontology (GO) term enrichment analysis was performed using GREAT (v4.0.4)[60] with mouse genome assembly (mm10), and whole genomes for background regions in default setting from ATAC-seq data. Gene Ontology tool[61,62] was used for a set of gene target of the differential open regions.

Gene set enrichment analysis (GSEA). GSEA was carried out by using the Gene Ontology term gene sets provided by the Mouse Genome Informatics website (http://www.informatics.jax.org)[63]. Entire detectable genes derived from RNA-seq were used for GSEA. We followed the standard GSEA user guide (http://www.broadinstitute.org/gsea/doc/GSEAUserGuideFrame.html).

scRNA-seq library preparation and sequencing. For lineage-labelled cells from *Red2-Notch*^{N1ICD} mice, YFP^{+}CD45^{-}CD31^{-}EpCAM^{+} or RFP^{+}CD45^{-}CD31^{-}EpCAM^{+} cells were sorted at day 28 post bleomycin injury (4 mice were pooled for each experiment). For non-lineage-labelled cells isolated from *Red2-Notch*^{N1ICD} mice in parallel with experiment of lineage-labelled cells, we combined the cells of EpCAM^{+}RFP^{-}YFP^{-} and EpCAM^{-} population with a ratio of 1:1, respectively. The resulting cell suspension were submitted as separate samples to be barcoded for the droplet-encapsulation single-cell RNA-seq experiments using the Chromium Controller (10X Genomics). Single cell cDNA synthesis, amplification, and sequencing libraries preparation were performed using the Single Cell 3' Reagent Kit as per the 10x Genomics protocol.

Alignment, quantification and quality control of scRNA-seq data. Droplet-based sequencing data were aligned using the Cell Ranger Single-Cell Software Suite (version 2.0.2, 10x Genomics Inc) with the *Mus musculus* genome (GRCm38) (official Cell Ranger reference, version 1.2.0), as previously described[31]. Cells were filtered by custom cutoff (more than 500 and less than 7000 detected genes, more than 2000 UMI count) to remove potential empty droplets and doublets. Downstream analysis included data normalisation, highly variable gene detection, log transformation, principal component analysis, neighbourhood graph generation and Louvain graph-based clustering, which was done by python package scanpy (version 1.5.1)[64] using default parameters.

Statistics and reproducibility. Statistical analyses were performed with Prism software package version 7.0 (GraphPad). Statistical significance was calculated using two-tailed unpaired Student's t test or Two-way ANOVA. Specific test methods and P-values are indicated in figure and legends. Sample size for animal experiments was determined based upon pilot experiments. Mice cohort size was designed to be sufficient to enable accurate determination of statistical significance. No animals were excluded from the statistical analysis.

1121 Mice were randomly assigned to treatment or control groups, while ensuring inclusion criteria
1122 based on gender and age. Animal studies were not performed in a blinded fashion. The
1123 number of animals shown in each figure is indicated in the legends as $n = x$ mice per group.
1124 Data shown are representative from at least duplicates or more than three independent
1125 experiments, or combined from three or more independent experiments as noted and analysed
1126 as mean ± s.e.m.
1127
1128 **Data Availability**
1129 ATAC-seq, RNA-seq and scRNA-seq data that support the findings of this study have been
1130 deposited in the Gene Expression Omnibus (GEO) under accession codes GSE153677 (RNA-
1131 seq and ATACC-seq for organoids) and GSE154218 (scRNA-seq). Previous published
1132 sequencing data that were re-analysed here are available under accession code
1133 GAS000010043444[44], GSE135893[43], and GSE144553[31]. All other data supporting the findings
1134 of this study are available from the corresponding author on reasonable request.
1135