Parent stem cells can serve as niches for their daughter cells

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Stem cells integrate inputs from multiple sources. Stem cell niches provide signals that promote stem cell maintenance3,5, while differentiated daughter cells are known to provide feedback signals to regulate stem cell replication and differentiation2,4. Recently, stem cells have been shown to regulate themselves using an autocrine mechanism. The existence of a ‘stem cell niche’ was first postulated by Schofield in 1978 to define local environments necessary for the maintenance of haematopoietic stem cells. Since then, an increasing body of work has focused on defining stem cell niches2,4. Yet little is known about how progenitor cell and differentiated cell numbers and proportions are maintained.

In the airway epithelium, basal cells function as stem/progenitor cells that can both self-renew and produce differentiated secretory cells and ciliated cells6,9. Secretory cells also act as transit-amplifying cells that eventually differentiate into post-mitotic ciliated cells2,3,6. Here we describe a mode of cell regulation in which adult mammalian stem/progenitor cells relay a forward signal to their own progeny. Surprisingly, this forward signal is shown to be necessary for daughter cell maintenance. Using a combination of cell ablation, lineage tracing and signalling pathway modulation, we show that airway basal stem/progenitor cells continuously supply a notch ligand to their daughter secretory cells. Without these forward signals, the secretory progenitor cell pool fails to be maintained and secretory cells execute a terminal differentiation program and convert into ciliated cells. Thus, a parent stem/progenitor cell can serve as a functional daughter cell niche.

To establish whether post-mitotic ciliated cells send a conventional feedback signal to regulate the replication of their parent stem and progenitor cells, we genetically ablated ciliated cells using FOXJ1-creER; LSL-DTA (Rosa26R-DTA) mice (hereafter referred to as FOXJ1-DTA) (Fig. 1a). Following ciliated cell ablation, the absolute numbers and morphology of secretory progenitor cells (SCGB1A1−) and basal stem/progenitor cells (CK5−) remained unchanged despite the ablation of 78.8% of ciliated cells (on day 5, a total of 24.29 ± 0.3% of all epithelial cells in control mice (identified with the nuclear marker 4’,6-diamidino-2-phenylindole, DAPI+) were FOXJ1+ ciliated cells versus 5.13 ± 0.4% in tamoxifen-treated mice (n = 3 mice)) (Fig. 1b, c and Extended Data Fig. 2a, b). Surprisingly, we did not observe the anticipated increase in stem or progenitor cell proliferation and/or their differentiation to replenish missing ciliated cells (Extended Data Fig. 2c–e). Even over extended periods of time, the rates of epithelial proliferation remained similar to those of uninjured controls (Extended Data Fig. 2d). The number of ciliated cells increased at a rate that corresponds to the normal rate of ciliated cell turnover (Fig. 1d). Following ciliated cell ablation, ciliated cell turnover occurs with a half-life of 149 days (Fig. 1e) which mirrors the reported steady-state half-life of approximately 6 months11. Additionally, the mesenchymal, haematopoietic, endothelial and smooth muscle cell populations appeared unchanged (Extended Data Fig. 2f, g).

Lacking evidence to support the presence of a feedback mechanism to restore ciliated cell numbers after ablation, we wondered whether basal stem/progenitor cells might regulate secretory daughter cell behaviour by regulating the differentiation of secretory cells into ciliated cells. Thus, we ablated basal cells and simultaneously traced the lineage of secretory progenitor cells using Scgb1a1-creER;LSL-YFP;CK5-rtTA;tetO/DTA mice (hereafter referred to as SCGB1A1-YFP;CK5-DTA), as previously described12 (Fig. 1f). In addition to the dedifferentiation of secretory cells we previously described following stem cell ablation12, we observed an increase in lineage-labelled yellow fluorescent protein (YFP+) cells expressing the ciliated cell marker FOXJ1 (8.1 ± 1.6% of YFP+ cells were FOXJ1+ in controls versus 42.4 ± 1.0% in experimental animals) and an accompanying decrease in YFP+ SCGB1A1+ secretory cells (88.5 ± 4% versus 45 ± 3%) (n = 3 mice) (Fig. 1g, h). We again observed that ~8% of lineage-labelled secretory cells dedifferentiated into basal cells as previously described12. Thus, we can now account for the fates of all lineage-labelled secretory cells after stem cell ablation, as the decrement in secretory cell lineage label (43.5%) is almost precisely equal to the combined increase in lineage-labelled ciliated and basal cells (34% and 8%, respectively). Importantly, lineage-labelled ciliated cells expressed c-MYB, a transcription factor required for ciliogenesis13,14 and acetylated tubulin (AcTub) confirming that secretory cells differentiated into mature ciliated cells (Extended Data Fig. 3a, b). These results were confirmed by flow cytometry (Extended Data Fig. 3c). In contrast to the changes in the tracheal epithelium in which the total number of ciliated cells increased twofold (625 ± 29 versus 1,208 ± 93 ciliated cells, representing 24.5 ± 1.5% and 61 ± 4.7% of total cells, respectively) (Extended Data Fig. 3d), the underlying mesenchyme remained unchanged in morphology and its complement of haematopoietic, endothelial and smooth muscle cells (Extended Data Fig. 3e, f).

As the Notch pathway has been shown to regulate ciliated versus secretory cell fate choices in the embryonic lung and regenerating adult airway epithelium15–20, we assessed the expression of Notch pathway components in each cell type of the adult homeostatic airway epithelium. Quantitative real time PCR analysis on purified airway epithelial cells revealed that the Notch1 receptor was highly expressed in basal stem/progenitor cells as previously reported18, Notch2 and Notch3 were significantly enriched in secretory progenitor cells, and Notch4 was not detected (n = 3 mice) (Fig. 2a and Extended Data Fig. 4a).

Signalling through the Notch2 receptor has previously been postulated to regulate secretory cell fate in the embryonic lung21, in inflammatory cytokine-induced goblet cell metaplasia22, and we have found it to be activated during secretory cell fate commitment during regeneration21. We found that steady-state nuclear Notch2 intracellular domain (N2ICD) expression was restricted to secretory progenitor
Secretory progenitor cells differentiate into ciliated cells following basal stem/progenitor cell ablation. a, Schematic representation of ciliated cell ablation. Ciliated, secretory and basal cells are shown in blue, pink and grey, respectively. b, Immunostaining for SCGB1A1 (green), FOXJ1 (red) and CK5 (cyan) on control (top) or tamoxifen (Tam)-treated FOXJ1-DTA mice (bottom) (n = 6 mice). c, Absolute number of each cell type in both groups (n = 3 mice). d, Percentage of FOXJ1+ cells per total DAPI+ cells over time (n = 3 mice). NS, not significant when compared to day 0 of the same group. e, Percentage of FOXJ1+ cells in tamoxifen treated mice (n = 3 mice). f, Schematic representation of secretory cell lineage labelling and basal cell ablation. g, Immunostaining for FOXJ1 (red), YFP (green) and CK5 (cyan) on inhaled (i)-PBS (top) or i-Dox (bottom) treated SCGB1A1-YFP; CK5-DTA mice (n = 3 mice). White arrowheads, lineage-labelled ciliated cells. h, Percentage of SCGB1A1+ and FOXJ1+ cells per total YFP+ cells. Nuclei, DAPI (4’,6-diamidino-2-phenylindole, blue), n = biological replicates/condition repeated twice (two independent experiments). **P < 0.01, ***P < 0.001. Error bars, means ± s.e.m. Scale bars, 20 μm.

Relative messenger RNA expression of Notch2 in sorted cells (n = 3 mice) (middle). Percentage of each cell type per total N2ICD+ cells (right).

b–e, Immunostaining for p63 (left) or FOXJ1 (right) (green), SSEA-1 (b) or SCGB1A1 (d) (cyan) and N2ICD (red). Percentage of N2ICD+ cells per total SSEA-1 (c) or SCGB1A1+ (e) cells (n = 3 mice). f, Immunostaining for eGFP (green) and N2ICD (red) in B1-eGFP mice. g, Percentage of N2ICD+ cells per total eGFP+ cells (n = 3 mice). Nuclei, DAPI (blue). White arrowheads, double-positive cells. Images are representative of n = 3 mice (biological replicates). *P < 0.05, **P < 0.001. Error bars, means ± s.e.m. Scale bars, 20 μm.

Figure 1

Figure 2

To determine whether secretory-cell-specific N2ICD transduces a putative basal cell signal that is required for the maintenance of the
secretory cell pool, we deleted Notch2 from secretory cells using Sgb1a1-creER;Lsl-YFP;Notch2fl/fl mice (hereafter referred to as Sgb1a1-Notch2fl/fl) (Fig. 3a). We first confirmed the efficient deletion of Notch2 and the downregulation of Hes1 and HeyL (Extended Data Fig. 7a–d). Upon Notch2 deletion, we observed that lineage-labelled cells ceased to express the secretory cell markers Sgb1a1 (95.6 ± 1.5% versus 23.8 ± 3%), Sgb3a2 (90.8 ± 1.3% versus 6.8 ± 1%) and SSEA-1 (88.2 ± 2.8% versus 22.7 ± 1%) and acquired the expression of the ciliated cell marker FoxJ1 (5.7 ± 2.1% versus 78 ± 0.7%), AcTub (3.7 ± 1.9% versus 57.6 ± 6%) and c-MYB (5.6 ± 0.4% versus 84.5 ± 2.3%) (n = 7 mice) (Fig. 3f, g and Extended Data Fig. 7e, f). The expression of secretory cell genes (Sgb1a1 and Sgb3a2) was consistently downregulated in lineage-labelled cells, while ciliated cell genes (FoxJ1 and c-myc) were upregulated (n = 3 mice) (Fig. 3h). Intriguingly, YFP staining was present in the actual cilia of lineage-labelled cells, consistent with the terminal differentiation of secretory cells into mature ciliated cells (Fig. 3f, i).

Flow cytometry analysis confirmed these cell fate transitions (Extended Data Fig. 7g, h) and also confirmed a lack of dedifferentiation of secretory cells into basal stem cells following Notch pathway modulation (Extended Data Fig. 7j, i). The observation that N2ICD and FoxJ1 expression remained mutually exclusive following Notch2 deletion also suggested a largely completed cell fate transition (Fig. 3i).

However, very rarely, YFP+ cells expressing both markers were observed, leading one to speculate that these rare cells are transient cells caught in the process of differentiating from a secretory cell into a ciliated cell (Extended Data Fig. 8a). Similarly, rare lineage-labelled cells also co-express SSEA-1 and FoxJ1 (Extended Data Fig. 8b). Furthermore, following Notch2 elimination, Ki67 and BrdU incorporation and rates of apoptosis remained unchanged (Extended Data Fig. 8c–g). Additionally, secretory cells directly differentiated into ciliated cells in the absence of proliferation since an insignificant (1.4 ± 1.7%) percentage of FoxJ1+ cells were BrdU+ following continuous BrdU administration (Extended Data Fig. 8d, e).

Together these data demonstrate that tonic Notch2 activity within secretory cells is required for the maintenance of secretory cells. Based upon the results of the basal cell ablation, we speculated that the Notch signalling centers are basal stem/progenitor cells. Consistent with prior studies, we found that Dll1 and Jag2 were expressed in basal stem/progenitor cells, while Jag1 was enriched in ciliated cells (Fig. 4a), and Dll3 and Dll4 were undetectable (data not shown). To remove the putative Notch signal arising from basal stem/progenitor cells, we deleted Mindbomb1 (Mib1) which is an E3 ubiquitin ligase required for the normal endocytic processing of all Notch ligands in basal cells using Cks5-rtTA; tet(O) Cre; Mindbomb1b/b mice (hereafter referred to as Cks5-b/b) (Fig. 4b). Upon efficient removal of Mib1 (93.3 ± 3.8% of basal cells) (Extended Data Fig. 9a, b), a decrease in Sgb1a1+ (42.8 ± 0.9% versus 26.2 ± 1.0%), Sgb3a2+ (44.6 ± 6.6% versus 62.6 ± 0.7%) and SSEA-1+ secretory cells (49.2 ± 2.6% versus 24.8 ± 1.1%) was accompanied by an increase in FoxJ1+ (30.1 ± 0.9% versus 36.1 ± 1.0%), AcTub+ (21.7 ± 0.7% versus 24.8 ± 0.7%), and c-MYB+ ciliated cells (30.8 ± 2.9% versus 56.2 ± 8.0%) (n = 4 mice) (Fig. 4c, d and Extended Data Fig. 9c, d). A corresponding significant decrease in the percentage of N2ICD+ secretory cells was observed (43.7 ± 1.7% versus 29.6 ± 0.8% of total epithelial cells) (Fig. 4e, f), confirming that Notch ligands emanating from stem cells are necessary for N2ICD activity in secretory cells. These results were confirmed by flow cytometry which additionally revealed that there were no changes in the abundance of basal cells (Extended Data Fig. 9e, f). Rates of proliferation and apoptosis were
also unchanged (Extended Data Fig. 9g–l) and a negligible amount (0.77 ± 1.5%) of FOXJ1+ cells were found to incorporate BrdU after continuous BrdU administration (Extended Data Fig. 9i, j). In addition, the cell fate changes described above continued to be present 5 weeks after Mib1 deletion (Extended Data Fig. 9m).

These results are consistent with the model that basal stem/progenitor cells send an essential signal to secretory progenitor cells, and this signal is necessary for the maintenance of the appropriate balance of cell types in the airway epithelium. As Jag2 is the most abundantly expressed ligand in basal stem cells (Fig. 4a), we knocked down Jag2 expression in vitro using short hairpin RNA (shRNA) lentiviral vectors (Extended Data Fig. 10a–c). This resulted in a decrease in Scgb1a1 and Scgb3a2 expression and an increase in Foxj1 and c-myc expression (Extended Data Fig. 10d), resembling the effects of in vivo Notch signalling disruption. To confirm that Jag2 is the signal emanating from basal stem/progenitor cells, we generated CK5-creER; LSL-YFP; Jagged2fl/fl mice (hereafter referred to as CK5-Jag2fl/fl) to genetically remove Jag2 from basal stem/progenitor cells in vivo (Fig. 4a), Jag2 deletion was confirmed (Extended Data Fig. 10e) and although the efficiency of recombination as judged by the number of YFP+ recombinated cells was approximately 10% (Extended Data Fig. 10f), the deletion caused a striking decrease in N2ICD+ suprabasal cells (43 ± 6.6% versus 17 ± 4.5% of total airway epithelial cells) (Fig. 4g, h) confirming that Jag2 is the basal cell signal responsible for activating N2ICD in secretory cells. We observed a consistent decrease in Scgb1a1+ (63 ± 2.1% versus 44.4 ± 3.3%), Scgb3a2+ (55 ± 7% versus 17.5 ± 0.5%) and Ssea-1 + secretory cells (42.8 ± 2% versus 21.8 ± 2%) and a concomitant increase in Foxj1+ (31.3 ± 3.6% versus 46.6 ± 2.2%), Actub+ (21.7 ± 2.1% versus 46.2 ± 3.9%) and c-MYB+ ciliated cells (28.2 ± 2.1% versus 49.6 ± 11.3%) (n = 5 mice) (Fig. 4i, j and Extended Data Fig. 10g, h). Results were also confirmed by flow cytometry (Extended Data Fig. 10i, j). Furthermore, we found no difference in the percentage of p63+ (also known as Trp63) basal cells (Extended Data Fig. 10k, l). N2ICD and FOXJ1 expression was mutually exclusive, consistent with a completed cell fate transition (Extended Data Fig. 10m, and there were no differences in overall proliferation and apoptosis (Extended Data Fig. 10n–r).

Together, our results show that basal stem/progenitor cells regulate the maintenance of their own progeny through a mechanism in which basal-stem-cell-produced Jag2 activates Notch2 in daughter secretory progenitor cells to prevent secretory cell differentiation into postmitotic ciliated cells (Extended Data Fig. 1).

Schofield first introduced the term niche to make sense of experimental evidence that suggested the presence of local environments necessary for the maintenance of haematopoietic stem cells. However, he was explicit in referring to stem cell niches. We now show that stem/progenitor cells themselves serve as ‘daughter cell niches’ (Extended Data Fig. 1c). We would like to suggest that reciprocal forms of niche-type regulation may be a general feature of many tissues in which stem, progenitor and differentiated cells might all regulate the maintenance of one another.

To serve as a progenitor cell niche, airway stem/progenitor cells use a ‘forward signal’ sent to their own progeny. We define a forward signal as a signal that is relayed from a parent cell to its daughter cell. Interestingly, in parallel to our mammalian example, in the fly mid gut, a forward Notch signal is sent from an intestinal stem cell to alter the fate choice of its own downstream progeny. However, from one setting to the next, Notch, with its myriad receptors and ligands, will inevitably be deployed in very divergent ways, even within the same tissue. For example, following injury, airway basal stem/progenitor cells use a mechanism akin to lateral inhibition to segregate their lineages, whereas pan-epithelial Jag2 deletion alters the distribution of airway progenitors in the embryonic airway epithelium, and in this context Notch3 is thought to be the relevant receptor. Notably, we identify Notch2 as the receiving receptor on secretory cells. N2ICD is, to the best of our knowledge, the first transcription factor that has
been found to be specific to steady-state adult airway secretory progenitor cells.

More generally, we note that differentiated cells are commonly thought to send back signals to their respective stem and progenitor cells to regulate their proliferation and differentiation. However, this process is generally termed feedback regulation, and we were surprised not to see evidence of such a regulatory mechanism following ciliated cell ablation. More recently, self signals have been identified that mediate autocrine stem cell regulation. As we demonstrate the existence of a forward signal, we would like to suggest that ‘forward regulation’ by stem cells is likely to exist (Extended Data Fig. 1d). Although it is tempting to call this form of regulation ‘feed-forward regulation’ to contrast it with ‘feedback regulation’, this term has been used in control theory to denote a more complex form of regulation that involves three discrete entities that interact in a loop. Therefore, we opt to propose the simpler term ‘forward regulation’. To illustrate what we intend to suggest, we note that Notch signals in fly intestinal stem cells occur at varying levels of Notch activation that in turn determine daughter cell fate. Thus, the regulation of these forward Notch signals could be used to alter the distribution and ratio of daughter cell types. In our case, perhaps fluctuations in basal cell ligand levels determine the rate of ciliated cell turnover? And how would such forward signals be modulated following tissue injury? A recent study points to Notch2 as a receptor relevant to human asthma. Perhaps increasing basal cell ligand concentration is a mechanism used to engender the asthmatic epithelial phenotype in which secretory daughter cells differentiate into mucous-secreting goblet cells. Thus, we speculate that stem cells, using forward regulatory mechanisms, may orchestrate many tissue-wide changes, rather than merely acting as a source of new cells.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Animals. FOXJ1-creER1, CK5-mT1A, Sgb1al-creER4, tet(O)DTA1, Rosa26-RTA1 (JAX 000664), RBPjkfl/fl, LSL-YFP (JAX 006148), Mindbombp62, Notch2g9 (JAX 010525), Jag2f9 (JAX 006146), and C57BL/6J (JAX 000664) were previously described. Progeny of Sgb1al-creER and LSL-YFP crosses as well as CK5-tTA and tet(O)DTA crosses were subsequently mated to generate Sgb1al-creER;LSL-YFP;CK5-tTAtet(O)DTA mice. These mice were treated with tamoxifen and then with inhaled PBS (control) or inhaled doxycycline as previously described. Sgb1al-creER mice were crossed with RBPjkfl/fl mice to generate secretory-progenitor-specific Sgb1al-creER;RBPjkfl/fl conditional knockout mice. To allow for lineage tracing, these mice were crossed with LSL-YFP mice to generate Sgb1al-creER;LSL-YFP;RBPjkfl/fl mice. Tamoxifen was administered by intraperitoneal injection (2 mg per day) for five consecutive days to induce the Cre-mediated recombination. Similarly, Sgb1al-creER;LSL-YFP;Notch2g9 mice were generated and treated. CK5-tTA and tet(O)cre mice were crossed to generate CK5-tTAtet(O)cre mice. CK5-tTAtet(O)cre mice were crossed with Mindbombp62 mice to generate basal-stem-cell-specific CK5-tTAtet(O)Cre;Mindbombp62 conditional knockout mice. Doxycycline administration was performed through drinking water (1 mg per ml) for 2 weeks as described previously. Sgb1al-creER;LSL-YFP;Jag2g9 mice were generated and treated, in this case with 2 doses of tamoxifen, due to a higher sensitivity of this strain to the compound. Mice were euthanized 10 days after the last tamoxifen injection. Male 6–12-week-old mice were used for experiments, except in specific circumstances in which breeding limitations led to the use of females in the following strains: Sgb1al-creER;LSL-YFP;CK5-tTA tet(O)DTA and CK5-tTA tet(O)Cre;Mindbombp62 mice. Similarly aged mice were used for both control and treated animals. Controls include corn oil-treated FOXJ1-creER, tet(O)DTA mice, 1-PS treated Tam-induced Sgb1al-creER;LSL-YFP;CK5-tTA tet(O)DTA mice, Tam-treated Sgb1al-creER;LSL-YFP;RBPjkfl/fl mice, Tam-treated Sgb1al-creER;LSL-YFP;Notch2g9 mice, doxycycline-treated CK5-tTA tet(O)Cre;Mibi1+/ mice and Tam-treated CK5-tTA tet(O)Cre;Mindbombp62 mice and the cell ablation experiments that were repeated twice. All procedures and protocols were approved by the MGH Subcommittee on Research Animal Care in accordance with NIH guidelines.

Tissue preparation, immunohistochemistry and immunofluorescence. Mouse trachea were removed using sterile technique and then fixed in 4% paraformaldehyde for 2 h at 4 °C, washed with PBS, and transferred to a 30% sucrose solution overnight. Cryosections were embedded in OCT and cryosectioned as transverse 7-μm sections. Cryosections were stained with the exception of each experiment and all the experiments were repeated at least three times with the exception of CK5-tTA tet(O)Cre;Mindbombp62 and the cell ablation experiments that were repeated twice. All procedures and protocols were approved by the MGH Subcommittee on Research Animal Care in accordance with NIH guidelines.

Cell culture and viral transduction. Airway epithelial cells from tracheas were dissociated using papain solution as previously described. Briefly, following trachea removal, airway tissue was cut into small fragments and transferred to a 2 ml solution containing 1 ml 100 U of pre-activated papain (Worthington Biochemical Corporation, catalogue number 10003182) and 1 ml of activation buffer as per the manufacturer’s protocol. Tissue fragments were incubated on a shaking platform for 90 min at 37 °C. The cell suspension was passed through a 70-μm cell strainer to remove airway husks and pelleted for 5 min at 400g. The supernatant was aspirated and the pellet was resuspended in overnight solution (Worthington Biochemical Corporation, catalogue number 10003182) for 20 min at 4 °C to inactive residual papain activity. Dissociated cells were washed with the following antibodies: EpCam-PECy7 (1:50, 25-5791-80, ebiosciences); or EpCam-APC (1:50, 17-5791, ebiosciences); GSIJ4 (Griffonia Simplicifolia Isosolitin beta4)-Biotin (L210, Sigma); SSEA-1 eFluor 650NC (1:75, 95-8813-41, ebiosciences); CD24-PE (1:100, 553262, BD Pharmingen). Primary antibodies were incubated for 30 min in 2.5% FBS in PBS on ice. FACs and flow cytometry was performed on a BD FACS Aria II sorter at the CRM Flow Cytometry Core (Boston, MA). All aforementioned cell sortings were previously gated for EpCam to exclusively select epithelial cells. Of note, differences in the percentage of each airway epithelial cell type analysed by flow cytometry might differ from the quantitation performed by cell counting. This reflects the use of cell surface markers for flow cytometry (CD4 for ciliated cells) in contrast to cell counts based on the nuclear transcription factors (such as Foxj1 and c-MYB for ciliated cells). Additionally, flow cytometry involves enzymatic tracheal dissociation and cells may die in this process and some cell types might demonstrate differential viability following enzymatic dissociation. Sorted cells were lysed immediately in TRI Reagent (Sigma) and RNA was extracted as previously described. Data were analyzed on FlowJo Software (version 10).
cells were dissociated and seeded onto transwell membranes. After confluence, media was removed from the upper chamber. Mucociliary differentiation was performed with PneumaCult-ALI Medium (StemCell, 05001). Differentiation of airway basal cells on ALI was followed by directly visualizing beating cilia in real time after 8 days. One day after plating, mouse basal cells were infected with lentiviral vectors carrying shRNAs targeting mouse Jag2. Four different clones were obtained from Sigma (MISSION shRNA jagged2 NM_010588, clones TRCN0000028858, TRCN0000028871, TRCN0000028877, TRCN0000028906), and cloned into pLKO.1 vector (Addgene Plasmid 10878). Lentiviral production was performed in HEK293 cells following standard protocols. Concentrated viruses were used at a MOI of 6 to infect murine basal cells for 9 h at 37 °C in 5% CO₂, one day after plating. The cells were allowed to grow to confluence before being transferred onto transwell membranes. Then 23 days after ALI initiation, cells were washed, collected and sorted for GFP and cell specific markers. To assess the efficiency of shRNA Jag2 knockdown, non-purified infected cells were collected 72 h after infection and lysed in TRI Reagent.

Statistical analysis. The standard error of the mean was calculated from the average of the indicated number of samples in each case (n = biological replicates/condition/experiment). All the experiments were repeated at least three times with the exception of CK5-rtTA;tet(O)Cre;Mindbomb1fl/fl and the cell ablation experiments that were repeated twice. Data was compared among groups using the Student’s t-test (unpaired, two-tailed test). A P value of less than 0.05 was considered significant. The analysis was performed with Prism software (Graphpad Prism version 5.0a).

Data reporting. No statistical methods were used to predetermine sample size. Experiments were performed completely blinded, being repeated by two different investigators and some of the experiments were repeated without knowing which samples were analysed.

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Extended Data Figure 1 | Parent stem/progenitor cells can serve as niches for their own daughter cells. a, Schematic representation of the airway epithelial cell lineage. Basal stem/progenitor cells give rise to secretory progenitor cells that, in turn, give rise to terminally differentiated ciliated cells. b, Basal cells expressing Notch ligands provide a tonic forward Notch signal to neighbouring secretory daughter cells. Blocking this forward signal prevents Notch activation in secretory cells and results in their differentiation into ciliated cells. c, A schematic of the traditional arrangement of a stem cell that is maintained in a stem cell niche (left) and a schematic that further illustrates that stem cells can themselves serve as daughter cell niches, in which the parent stem cell itself is required for the maintenance of its own progeny (right). d, Schematic of the types of signals that occur between cells within a lineage and the theoretical modes of cell regulation that they imply. Blue arrows indicate a lineage relationship. Red arrows represent signals.
Extended Data Figure 2 | Ablation of ciliated cells has no effect on airway cell proliferation, mesenchymal cell types, mesenchymal morphology and airway stem and progenitor cell replication over time. 

**a** Immunostaining for basal (CK5 (green)), ciliated (FOXJ1 (red)), and secretory cells (SCGB1A1 (white)) on either control (left panels) or tamoxifen (Tam)-treated FOXJ1-DTA mice (right panels) 5, 15, 30, 45, 60 and 150 days after ciliated cell ablation (\(n = 3\) mice). 

**b** Quantification of absolute cell numbers of basal CK5\(^+\) cells (top graph) and secretory SCGB1A1\(^+\) cells (bottom graph) per trachea on control (black bars) or Tam-treated (white bars) mice over time (\(n = 3\) mice). 

**c** Immunostaining for ciliated cells (FOXJ1 (green)) and proliferating cells (Ki67 (red)) on either control (upper panel) or Tam-treated (lower panel) mice at day 3 (\(n = 6\) mice). On the right, quantification of the percentage of Ki67\(^+\) cells per total DAPI\(^+\) cells in tracheal sections from control (C) or Tam treated mice (\(n = 3\) mice). 

**d** Quantification of the percentage of proliferating Ki67\(^+\) cells relative to total DAPI\(^+\) cells in control (black bars) or Tam-treated (white bars) mice over time (\(n = 3\) mice). 

**e** Immunostaining for ciliated cells (AcTub (green)) and cells that have undergone proliferation (BrdU (red)) on either control (upper panel) or Tam-treated mice (lower panel) at day 3 (\(n = 6\) mice). On the right, quantification of the percentage of BrdU\(^+\) cells per total DAPI\(^+\) cells in tracheal sections from control (C) or Tam treated mice (\(n = 3\) mice). 

**f** Haematoxylin & eosin (H&E) staining of tracheal sections 3 days after ciliated cell ablation. 

**g** Immunostaining for CD45\(^+\) haematopoietic cells (left panels), CD31\(^+\) endothelial cells (middle panels) and SMA\(^+\) smooth muscle cells (right panels) (green) three days after cell ablation (\(n = 6\) mice). Nuclei stained with DAPI (blue). The ns indicates that the cell number comparisons are not statistically significant. \(n = \) biological replicates/condition (two independent experiments). Data shown in the graphs are means \(\pm\) s.e.m. Scale bar, 20 \(\mu\)m.
Extended Data Figure 3 | Basal stem/progenitor cell ablation promotes the differentiation of secretory cells into ciliated cells without affecting the mesenchyme. a, Immunostaining for YFP lineage label (green) and the ciliated cell marker c-MYB (red) in SCGB1A1-YFP; CK5-DTA mice (n = 3 mice). White arrowheads point to double positive cells. b, Immunostaining for YFP lineage label (green) and the ciliated cell marker AcTub (red) using SCGB1A1-YFP; CK5-DTA mice (n = 3 mice). c, Flow cytometry analysis for lineage-labelled YFP$^+$ cells (x axis) and CD24$^+$ ciliated cells (y axis) cells from control iPBS-treated or doxycycline-treated SCG1A1-YFP; CK5-DTA mice. d, Quantification of the percentage of FOXJ1$^+$ cells per total DAPI$^+$ cells in tracheal sections from control iPBS-treated or iDOX-treated SCG1A1-YFP; CK5-DTA mice (n = 3 mice). On the right, absolute numbers of FOXJ1$^+$ cells per tracheal section (n = 3 mice). e, H&E staining of tracheal sections following basal cell ablation. f, Immunostaining for CD45$^+$ haematopoietic cells (left panels), CD31$^+$ endothelial cells (middle panels) and SMA$^+$ smooth muscle cells (right panels) (green) in control or basal cell-ablated trachea (n = 3 mice). All analyses were performed 3 days after cell ablation. Nuclei stained with DAPI (blue). n = biological replicates/condition (two independent experiments). **P < 0.01. Data shown in the graphs are means ± s.e.m. Scale bar, 20 μm.
Extended Data Figure 4 | Characterization of Notch pathway components in the steady-state murine tracheal epithelium. 

a, Relative mRNA expression of Notch1, Notch3 and Notch4 assessed by qRT–PCR in pure sorted populations of airway epithelial cells (n = 3 mice). Relative expression is normalized to baseline transcript levels in secretory progenitor cells. b, Immunostaining for N1ICD (red) in combination with the basal cell marker p63 (top panel), the secretory cell marker SSEA-1 (middle panel) and the ciliated cell marker FOXJ1 (bottom panel) (green). c, Immunostaining for N3ICD (red) in combination with the basal cell marker podoplanin (PDPN) (top panel), the secretory cell marker SSEA-1 (middle panel) and the ciliated cell marker FOXJ1 (bottom panel) (green). d, Relative mRNA expression of Hes1, Hey1 and HeyL assessed by qRT–PCR in pure sorted populations of airway epithelial cells (n = 3 mice). Relative expression is normalized to baseline transcript levels in secretory progenitor cells. 

**P < 0.01; ***P < 0.001. nd indicates lack of detection. Data shown in the graphs are means ± s.e.m. Nuclei stained with DAPI (blue). White arrowheads point to double positive cells. Scale bar, 20 μm.
Extended Data Figure 5 | Downregulation of Notch signalling transduction following RBPjk deletion in secretory progenitor cells induces their conversion into ciliated cells. a, Immunostaining for lineage-labelled YFP$^+$ cells (green) in combination with RBPjk (red) in Tam-treated SCGB1A1-RBPjk$^{fl/1}$ control mice (upper panels) and Tam-treated SCGB1A1-RBPjk$^{fl/4}$ mice (lower panels). White arrowheads point to lineage-labelled RBPjk$^+$ cells. The yellow arrows point to lineage-labelled cells that have not undergone recombination. b, Quantification of the percentage of RBPjk$^+$ cells per total YFP$^+$ cells at experimental day 15 following tamoxifen administration to SCGB1A1-RBPjk$^{fl/1}$ control (black bar) and SCGB1A1-RBPjk$^{fl/4}$ mice (white bar) ($n = 6$ mice). c, Relative mRNA expression of Notch signalling component genes (RBPjk, Hes1, HeyL) analysed by qRT–PCR in sorted YFP$^+$ cells from Tam-treated SCGB1A1-RBPjk$^{fl/1}$ control mice (black bars) ($n = 3$ mice) and Tam-treated SCGB1A1-RBPjk$^{fl/4}$ mice (white bars) ($n = 4$ mice). Relative expression is normalized to baseline transcript levels in YFP$^+$ control cells. d, Immunostaining for YFP lineage label (green) and the secretory progenitor cell markers SCGB3A2 (left panels) and SSEA-1 (right panels) (red) in Tam-treated SCGB1A1-RBPjk$^{fl/1}$ control (top panels) and SCGB1A1-RBPjk$^{fl/4}$ mice (bottom panels). White arrowheads point to lineage-labelled secretory RBPjk$^+$ cells. e, Immunostaining for YFP lineage label (green) and the ciliated cell markers AcTub (left panels) and c-MYB (right panels) (red) in Tam-treated SCGB1A1-RBPjk$^{fl/1}$ control (top panels) and SCGB1A1-RBPjk$^{fl/4}$ mice (bottom panels). White arrowheads point to lineage-labelled secretory cells that differentiated into ciliated cells following RBPjk deletion. f, Immunostaining for lineage-labelled YFP$^+$ cells (green) and the basal cell marker CK5 (red) on either Tam-treated SCGB1A1-RBPjk$^{fl/1}$ control mice (upper panel) or Tam-treated SCGB1A1-RBPjk$^{fl/4}$ mice (lower panel). g, Quantification of the percentage of CK5$^+$ cells per total YFP$^+$ cells in Tam-treated SCGB1A1-RBPjk$^{fl/1}$ control (black bar) and SCGB1A1-RBPjk$^{fl/4}$ mice (white bar) ($n = 6$ mice). h, Flow cytometry analysis of EpCAM$^+$ YFP$^+$ CD24$^+$ lineage-labelled ciliated cells and EpCAM$^+$ YFP$^+$ CD24$^-$ SSEA-1$^+$ lineage-labelled secretory cells or EpCAM$^+$ YFP$^+$ CD24$^-$ GSI$^+$ lineage-labelled basal cells in airways from either control or Tam-treated SCGB1A1-RBPjk$^{fl/4}$ mice. i, Quantification of the percentage of epithelial (EpCAM$^+$) lineage-labelled (YFP$^+$) basal, secretory and ciliated cells in either Tam-treated SCGB1A1-RBPjk$^{fl/1}$ control or SCGB1A1-RBPjk$^{fl/4}$ mice by flow cytometry ($n = 3$ mice). The analysis was performed 10 days after the last tamoxifen injection. Images are representative of $n = 6$ mice per condition (biological replicates) repeated three times. Nuclei stained with DAPI (blue). **$P < 0.01$; ***$P < 0.001$. Data shown in the graphs are means ± s.e.m. Scale bar, 20 $\mu$m.
Extended Data Figure 6 | Lineage-labelled ciliated cells demonstrate long term persistence after RBPjk deletion without a change in epithelial cell proliferation and apoptosis. a–d. Immunostaining for the lineage label YFP (green) in combination with the secretory cell markers SCGB1A1 (a), SCGB3A2 (b) or the ciliated cell markers FOXJ1 (c) and AcTub (d) (red) on either Tam-treated SCGB1A1-RBPjkfl/+ control mice (upper panels) or Tam-treated SCGB1A1-RBPjkfl/fl mice (lower panels) 30 days after the last tamoxifen injection (n = 3 mice). White arrowheads point to lineage-labelled ciliated cells. 

e. Quantification of the percentage of each cell type per YFP+ cells on either control mice (black bars) or Tam-treated SCGB1A1-RBPjkfl/+ mice (white bars) at day 30. 
f. Quantification of the percentage of ciliated FOXJ1+ cells that incorporate BrdU after continuous BrdU administration to Tam-treated SCGB1A1-RBPjkfl/fl mice (n = 3 mice). 
g. Immunostaining for Ki67 (red) to assess overall proliferation in either Tam-treated SCGB1A1-RBPjkfl/+ control mice (upper panel) or Tam-treated SCGB1A1-RBPjkfl/fl mice (lower panel) (n = 3 mice). h. Immunostaining for FOXJ1 (green) and BrdU (red) in combination with YFP (cyan) (h) or alone (i) on Tam-treated SCGB1A1-RBPjkfl/fl mice that received continuous BrdU (n = 3 mice). 

i. Immunostaining to detect apoptotic cells by TUNEL assay (red) in combination with YFP lineage-labelled cells (green) in either Tam-treated SCGB1A1-RBPjkfl/+ control mice (upper panel) or Tam-treated SCGB1A1-RBPjkfl/fl mice (lower panel) (n = 3 mice). k. Immunostaining for activated caspase3 (green) in control and Tam-treated SCGB1A1-RBPjkfl/fl mice (n = 3 mice). f–k, Analysis conducted 10 days after induction. Nuclei stained with DAPI (blue). n = biological replicates per condition. ***P < 0.001. Data shown in the graph are means ± s.e.m. Scale bar, 20 μm.
Extended Data Figure 7 | Efficient deletion of Notch2 in secretory progenitor cells and its effect on cell type distribution. a, Relative mRNA expression of Notch2 in YFP<sup>+</sup> cells from Tam-treated SCGB1A1-Notch2<sup>+/+</sup> control mice and Tam-treated SCGB1A1-Notch2<sup>Δ/Δ</sup> experimental mice assessed by qRT–PCR (n = 3 mice). b, Relative mRNA expression of the Notch target genes (Hes1, HeyL) in YFP<sup>+</sup> cells from control mice and Tam-treated SCGB1A1-Notch2<sup>Δ/Δ</sup> experimental mice (n = 3 mice). Relative expression is normalized to baseline transcript levels in lineage-labelled YFP<sup>+</sup> control cells. c, Immunostaining for lineage label YFP (green) in combination with N2ICD (red) on control mice (Tam-treated SCGB1A1-Notch2<sup>+/+</sup>) and experimental airways (Tam-treated SCGB1A1-Notch2<sup>Δ/Δ</sup>). White arrowheads point to lineage-labelled cells that had lost Notch2 and therefore do not show N2ICD expression. d, Quantification of the percentage of N2ICD<sup>+</sup> cells per total YFP<sup>+</sup> cells (n = 3 mice). e, Immunostaining for YFP lineage label (green) and the secretory progenitor cell markers SCGB3A2 (left panels) and SSEA-1 (right panels) (red) in control (top panels) and experimental (bottom panels) mice. f, Immunostaining for YFP lineage label (green) and the ciliated cell markers AcTub (left panels) and c-MYB (right panels) (red) in control (top panels) and experimental (bottom panels) mice. g, Flow cytometry analysis of EpCAM<sup>+</sup> YFP<sup>+</sup> CD24<sup>+</sup> lineage-labelled secretory cells and EpCAM<sup>+</sup> YFP<sup>+</sup> CD24<sup>+</sup> SSEA-1<sup>+</sup> lineage-labelled secretory cells or EpCAM<sup>+</sup> YFP<sup>+</sup> CD24<sup>+</sup> GSI<sup>+1</sup> lineage-labelled basal cells in airways from either Tam-treated SCGB1A1-Notch2<sup>+/+</sup> control mice or Tam-treated SCGB1A1-Notch2<sup>Δ/Δ</sup> mice. h, Quantification of the percentage of epithelial (EpCAM<sup>+</sup>) lineage-labelled (YFP<sup>+</sup>) basal, secretory and ciliated cells in either Tam-treated SCGB1A1-Notch2<sup>+/+</sup> control (n = 4 mice) or SCGB1A1-Notch2<sup>Δ/Δ</sup> mice (n = 6 mice) by flow cytometry. i, Immunostaining for the basal cell transcription factor p63 (red) on control or SCGB1A1-Notch2<sup>Δ/Δ</sup> airways. j, Quantification of the percentage of p63<sup>+</sup> cells per total DAPI<sup>+</sup> cells on tracheal sections from control or experimental mice (n = 7 mice) by flow cytometry. Analysis performed 10 days after induction. Images are representative of n = 7 mice per condition (biological replicates) repeated three times (three independent experiments). Nuclei stained with DAPI (blue). *P < 0.05; ***P < 0.001. Data shown in the graphs are means ± s.e.m. Scale bar, 20 μm.
Extended Data Figure 8 | Proliferation and apoptosis following deletion of Notch2 in secretory progenitor cells. **a**, Immunostaining for lineage label YFP (green), FOXJ1 (cyan) and N2ICD (red) in Tam-treated SCGB1A1-Notch2fl/fl mice. White arrowhead points to a lineage-labelled cell co-expressing markers for secretory and ciliated cell fates. The inset shows the single stain for FOXJ1 of the indicated region. **b**, Immunostaining for lineage label YFP (green), FOXJ1 (cyan) and SSEA-1 (red) in Tam-treated SCGB1A1-Notch2fl/fl mice. White arrowhead points to a lineage-labelled transitional cell. **c**, Immunostaining for BrdU (green), p63 (red) and Ki67 (cyan) to assess overall proliferation on either Tam-treated SCGB1A1-Notch2fl/fl control mice (upper panels) or Tam-treated SCGB1A1-Notch2fl/fl mice (lower panels). **d**, Quantification of the percentage of ciliated FOXJ1+ cells that incorporate BrdU after continuous BrdU administration to Tam-treated SCGB1A1-Notch2fl/fl mice (n = 4 mice). **e**, Immunostaining for FOXJ1 (green) and BrdU (red) on Tam-treated SCGB1A1-Notch2fl/fl mice that received continuous BrdU (n = 4 mice). **f**, Immunostaining to detect apoptotic cells by TUNEL assay (green) on either Tam-treated SCGB1A1-Notch2fl/fl control mice (upper panel) or Tam-treated SCGB1A1-Notch2fl/fl mice (lower panel). **g**, Immunostaining for YFP (green) in combination with activated caspase3 (red) on control mice (upper panel) or Tam-treated SCGB1A1-Notch2fl/fl mice (lower panel). Analysis performed 10 days after induction. Images are representative of n = 7 mice per condition (biological replicates) repeated three times (three independent experiments). Nuclei stained with DAPI (blue). Scale bar, 20 μm.
Extended Data Figure 9 | Loss of Notch ligands in basal stem cells promotes secretory cell differentiation into ciliated cells without affecting proliferation or apoptosis. a, Quantification of the percentage of basal PDPN$^+$ cells that express Mib1 (left graph) on either Dox-treated CK5-Mib1$^{+/+}$ control mice or Dox-treated CK5-Mib1$^{fl/fl}$ mice ($n = 4$ mice). Right graph, percentage of basal cells in which Mib1 was deleted in Dox-treated CK5-Mib1$^{fl/fl}$ mice ($n = 4$ mice). b, Immunostaining for Mib1 (red) and the basal cell marker CK5 (green). White arrowheads point to Mib1$^+$ basal cells. c, Immunostaining for the secretory cell markers SCGB3A2 (left panels) and SSEA-1 (right panels) (red) in control (top panels) and experimental (bottom panels) mice. d, Immunostaining for the ciliated cell markers AcTub (left panels) and c-MYB (right panels) (green) in control (top panels) and experimental (bottom panels) mice. e, Flow cytometry analysis of EpCAM$^+$ CD24$^+$ ciliated cells and EpCAM$^+$ SSEA-1$^+$ secretory cells from control and experimental mice. f, Percentage of epithelial (EpCAM$^+$) basal, secretory and ciliated cells on both groups by flow cytometry ($n = 3$ mice). g, Immunostaining for Ki67 (green) and the secretory cell marker SCGB1A1 (red) on control (top panel) or Dox-treated CK5-Mib1$^{fl/fl}$ mice (bottom panel). h, Immunostaining for BrdU (green) in combination with the basal cell transcription factor p63 (red) on both groups. i, Immunostaining for FOXJ1 (green) and BrdU (red) on Dox-treated CK5-Mib1$^{fl/fl}$ mice that received continuous BrdU. j, Percentage of ciliated FOXJ1$^+$ cells that incorporate BrdU after continuous BrdU administration to Dox-treated CK5-Mib1$^{fl/fl}$ mice ($n = 4$ mice). k, Immunostaining to detect apoptotic cells by TUNEL assay (green) on either control (upper panel) or experimental mice (lower panel). l, Immunostaining for activated caspase3 (green) on both groups. m, Immunostaining for N2ICD (red), SCGB1A1 and SCGB3A2 (red), or FOXJ1 and AcTub (green) in control (top panels) or experimental mice (bottom panels) after five weeks of continuous doxycycline treatment ($n = 4$ mice). a–l, Analysis performed 2 weeks after the beginning of Dox induction. Images are representative of $n = 4$ mice per condition (biological replicates) repeated twice. *$P < 0.05$; ***$P < 0.001$. Data shown in the graphs are means ± s.e.m. Nuclei, DAPI (blue). Scale bar, 20 μm.
Extended Data Figure 10 | Disruption of Jag2 in basal stem/progenitor cells causes the differentiation of secretory progenitor cells into ciliated cells without affecting proliferation or apoptosis. a, Schematic representation of Jag2 inhibition using lentiviruses (LV) carrying shRNAs. Infected GFP+ cells were cultured in ALI culture system for 23 days, when they were collected, sorted and analysed. b, Relative mRNA expression of Jag2 in tracheal epithelial cells infected with mock vector (control) or with vectors carrying 4 different shRNAs targeting Jag2 (shJag2 877) after 23 days in ALI. c, Relative mRNA expression of the secretory genes (Scgb1a1 and Scgb3a2) and the ciliated cell genes (FoxJ1 and c-myb) in mock (black bars) and shJag2 877 (grey bars) infected cells 23 days after ALI initiation. Relative expression is normalized to baseline transcript levels in mock-infected cells. d, Percentage of p63+ cells per total DAPI+ cells on both groups. e, Immunostaining for FOXJ1 (green), N2ICD (red) and SCGB1A1 (cyan). f, Immunostaining for BrdU (green), p63 (red) and Ki67 (cyan) in either control (upper panels) or experimental mice (lower panels). g, Flow cytometry analysis of EpCAM+CD24+ ciliated cells and EpCAM+SSEA-1+ secretory cells in control and experimental mice. h, Immunostaining for AcTub (left panels) and c-MYB (right panels) (red) in combination with YFP (green) in control (top panels) and experimental (bottom panels) mice. i, Flow cytometry analysis of EpCAM+CD24+ ciliated cells and EpCAM+SSEA-1+ secretory cells in control and experimental mice. j, Percentage of epithelial (EpCAM+) basal, secretory and ciliated cells from both groups assessed by flow cytometry (n = 3 mice). k, Immunostaining for p63 (red) on control (top panel) and experimental mice (bottom panel). l, Immunostaining for FOXJ1 (green) and BrdU (red) on Tam-treated CK5-Jag2fl/fl mice that received continuous BrdU (n = 3 mice). m, Immunostaining to detect apoptotic cells by TUNEL assay (green) on both groups. n, Immunostaining for YFP (green) in combination with activated caspase3 (red) on control (upper panel) or experimental mice (lower panel). o, Analysis performed 10 days after induction. Images are representative of n = 5 mice per condition (biological replicates) repeated three times. *P < 0.05; **P < 0.01; ***P < 0.001. Data shown in the graphs are means ± s.e.m. Nuclei, DAPI (blue). Scale bar, 20 μm.