Dedifferentiation of committed epithelial cells into stem cells in vivo

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Cellular plasticity contributes to the regenerative capacity of plants, invertebrates, teleost fishes and amphibians. In vertebrates, differentiated cells are known to revert into replicating progenitors, but these cells do not persist as stable stem cells. Here we present evidence that differentiated airway epithelial cells can revert into stable and functional stem cells in vivo. After the ablation of airway stem cells, we observed a surprising increase in the proliferation of committed secretory cells. Subsequent lineage tracing demonstrated that the luminal secretory cells had dedifferentiated into basal stem cells. Dedifferentiated cells were morphologically indistinguishable from stem cells and they functioned as well as their endogenous counterparts in repairing epithelial injury. Single secretory cells clonally dedifferentiated into multipotent stem cells when they were cultured ex vivo without basal stem cells. By contrast, direct contact with a single basal stem cell was sufficient to prevent secretory cell dedifferentiation. In analogy to classical descriptions of amphibian nuclear reprogramming, the propensity of committed cells to dedifferentiate is inversely correlated to their state of maturity. This capacity of committed cells to dedifferentiate into stem cells may have a more general role in the regeneration of many tissues and in multiple disease states, notably cancer.

The term dedifferentiation was first coined to describe the process in which cells of the urodele retinal pigment epithelium lose their differentiated properties to replace extirpated lens cells. Although not formally demonstrated, the term was used to suggest that differentiated epithelial cells reverted to a previous developmental stage before their subsequent differentiation into an alternative cell fate. Dedifferentiation has since been explored in plants, invertebrates, teleost fishes and amphibians. In vertebrates, differentiated cells are known to revert into replicating progenitors, but these cells do not persist as stable stem cells. Indeed, in murine hair follicle progenitor cells, the immediate differentiated progeny of epithelial stem cells are already resistant to dedifferentiation. Conversely, the undifferentiated secretory progenitors of the intestine that are the immediate progeny of intestinal stem cells are able to dedifferentiate into stem cells after injury, mimicking the capacity for dedifferentiation of the immediate progeny of Drosophila germline stem cells. Recently, using stringent lineage-tracing strategies, airway epithelial cells have been shown to be more plastic than recognized previously. In a separate study, differentiated secretory cells have been shown to give rise to very rare cells (0.34 ± 0.09%) that express basal cell markers after severe injury, but the properties of these rare basal-like cells were not studied and their functional capacity was not assessed. Here, we specifically sought to determine whether stably committed luminal cells could dedifferentiate into functional stem cells.

**Secretory cells replicate after stem cell ablation**

Airway basal stem cells have been shown to self-renew and differentiate into multiple airway epithelial cell types using genetic lineage tracing. Secretory cells are differentiated luminal cells that have both secretory and detoxifying functions. Secretory cells can also further differentiate into ciliated cells. To test whether secretory cells can dedifferentiate into stem cells, we ablated basal stem cells of the airway epithelium and simultaneously lineage traced the secretory cells of the same mouse. After the ablation of airway stem cells, we observed a surprising increase in the proliferation of committed secretory cells. Subsequent lineage tracing demonstrated that the luminal secretory cells had dedifferentiated into basal stem cells. Dedifferentiated cells were morphologically indistinguishable from stem cells and they functioned as well as their endogenous counterparts in repairing epithelial injury. Single secretory cells clonally dedifferentiated into multipotent stem cells when they were cultured ex vivo without basal stem cells. By contrast, direct contact with a single basal stem cell was sufficient to prevent secretory cell dedifferentiation. In analogy to classical descriptions of amphibian nuclear reprogramming, the propensity of committed cells to dedifferentiate is inversely correlated to their state of maturity. This capacity of committed cells to dedifferentiate into stem cells may have a more general role in the regeneration of many tissues and in multiple disease states, notably cancer.

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Figure 1 | Secretory cells proliferate after basal cell ablation. a, Schematic representation of the ablation of CK5-expressing basal cells of the trachea. Secretory, ciliated and basal stem cells are shown in pink, blue and grey, respectively. b, Schematic of the timeline of i-Dox or i-PBS administration and tissue collection (C). c, Immunostaining for basal (p63 green) and CK5 (cyan) and secretory cells (SCGB1A1 green) in combination with Ki67 (red) on either i-PBS (top)- or i-Dox (bottom)-treated mice (n = 6). White arrows, Ki67+ cells. d, Quantification of the percentage of p63+ and SCGB1A1+ cells per total number of DAPI (4',6-diamidino-2-phenylindole)-stained epithelial cells in i-PBS or i-Dox groups. n = 3. e, Percentage of p63+ Ki67+ and SCGB1A1+ Ki67+ cells relative to total Ki67+ cells in i-PBS- and i-Dox (n = 3)-treated CK5-DTA mice. Nuclei, DAPI (blue). *P < 0.05, **P < 0.01 (two tailed and paired t-test). n = 3 (three mice per condition). Error bars, average ± s.e.m. Scale bars, 20 μm.

to the total population of replicating cells after ablation (Fig. 1c, e). Notably, there was a twofold increase in the numbers of replicating secretory cells (SCGB1A1+ Ki67+) in i-Dox-treated animals (51.29 ± 3.02%) as compared to i-PBS-treated animals (17.7 ± 2.68%) (Fig. 1c, e and Extended Data Fig. 3a). Consistent with the increased proliferation of differentiated secretory cells, Ki67 staining was specifically increased in the CK8 (also known as KRT8) suprabasal layer of the airway epithelium (Extended Data Fig. 3b). Thus, secretory cells are the predominant cells that replicate after stem cell ablation. Interestingly, occasional cells expressed both the basal cell marker CK5 and the secretory cell marker SCGB1A1 in i-Dox-treated mice (Extended Data Fig. 3c).

Secretory cells dedifferentiate in vivo

To lineage-label secretory cells before stem cell ablation, we generated quadruple transgenic mice: Sgb1A1-creERT2/LSL-YFP:CK5:rtTA-tet(O)DTA (hereafter referred to as SCGB1A1–YFP/CK5-DTA mice). Administration of tamoxifen to induce the CreER-mediated expression of the yellow fluorescent protein (YFP) label in secretory cells was followed by three doses of i-Dox to induce basal cell ablation (Fig. 2a). Lineage-labelled YFP+ secretory cells demonstrated increased rates of proliferation in i-Dox-treated animals as compared to i-PBS treated controls (Extended Data Fig. 3d, e). We identified YFP+ secretory-cell-derived cells that were morphologically indistinguishable from basal stem cells (Fig. 2b). In addition, we found that a subset of lineage-labelled cells expressed a suite of basal cell markers including CK5, NGFR, p63 and T1α (Fig. 2b and Extended Data Fig. 3f). Quantification revealed that 7.9 ± 2.08% of basal cells (585 CK5+ YFP+ cells out of 7,320 total CK5+ cells in i-Dox-treated animals, n = 6 mice) expressed a YFP lineage label, demonstrating that differentiated basal-like cells comprised a substantial fraction of the total stem cell pool. Dedifferentiated cells did not appear in PBS-treated controls (3 CK5+ YFP+ cells out of 7,558 total CK5+ cells counted (0.041 ± 0.028%; n = 6 mice)). Consistently, when the entire basal cell population is purified by flow cytometry, the YFP-lineage-labelled basal-like cells have lost the secretory cell surface marker SSEA-1 (Fig. 2c). Thus, dedifferentiating cells lose markers of secretory cell differentiation as they acquire markers of basal stem cells.

Secretory cells dedifferentiate ex vivo

Because our in vivo experiments demonstrated that secretory cells are stimulated to dedifferentiate by the ablation of basal stem cells, we wondered whether secretory cell dedifferentiation could be induced ex vivo when secretory cells were cultured in the absence of basal stem cells. We reasoned that such an assay would provide a platform for further determining whether the dedifferentiation process is actively suppressed by the presence of co-cultured basal stem cells. To assess this possibility, we isolated and sorted unlabelled basal stem cells and YFP+ secretory cells from SCGB1A1–YFP mice after tamoxifen
Mature secretory cells resist dedifferentiation

To determine whether all secretory cells have the potential to dedifferentiate or whether only a subset of secretory cells is endowed with this capacity, we attempted to subset this class of epithelial cells. To do so, we made use of a transgenic mouse strain that expresses enhanced green fluorescent protein (eGFP) specifically in secretory cells of the

Figure 3 | Secretory cells dedifferentiate in the absence of basal cells in an ex vivo sphere-forming assay. a, Schematic representation of lineage labelling, sorting and ex vivo sphere-forming assay. Schematic representation of different types of spheres anticipated from the basal and secretory cell-mixing assay. b, Quantification of the number of spheres formed relative to the number of cells seeded. c, Immunostaining for p63 (red; top) or CK5 (red; bottom) in combination with YFP (green). n = 3 (two replicates per condition). Error bars, average ± s.e.m. Scale bars, 20 μm.
airway epithelium driven by the promoter of the B1 subunit of the vacuolar H^+ -ATPase gene (Ap6v1b1; which we reasoned would be associated with mature secretory cells and hereafter refer to as B1-
GFP)\textsuperscript{25,26}. Co-immunostaining for SSEA-1 and GFP demonstrated the existence of three subpopulations of secretory cells: SSEA-1\textsuperscript{+} / GFP\textsuperscript{+}, SSEA-1\textsuperscript{+}/GFP\textsuperscript{−}, and SSEA-1\textsuperscript{−}/GFP\textsuperscript{+} (Extended Data Fig. 7a). Of note, all GFP\textsuperscript{+} cells are SCGB1A1\textsuperscript{+} secretory cells and none are CK5\textsuperscript{+} basal cells (Extended Data Fig. 7a). To define the cellular hierarchy of these three subsets of cells, we exposed the airway epithelium of B1-eGFP mice to sulphur dioxide (SO\textsubscript{2}) injury. In this injury model, SO\textsubscript{2} causes the complete sloughing of only the suprabasal differentiated cells. The remaining basal stem cells are left intact and start replicating within 24 h to give rise to a mature epithelium within 14 days. We found that single-positive SSEA-1\textsuperscript{+} cells appeared first on day 4 and then matured into double-positive SSEA-1\textsuperscript{+}/GFP\textsuperscript{+} cells on day 6 (Extended Data Fig. 7b) before the formation of any fully mature single-positive B1-eGFP\textsuperscript{+} cells evident in the fully mature homeostatic epithelium (Extended Data Fig. 7a, bottom panel, arrowheads). Using B1-eGFP mice, we performed sphere-forming assays with each of the three subsets of secretory cells (Fig. 4a). Intriguingly, all three subsets of cells formed similar large spheres and all these spheres contained basal-like cells (Fig. 4b–d). Interestingly, the sphere-forming ability of the three populations was inversely proportional to the relative maturity of the secretory cell subsets (Fig. 4d). Of note, most of the cell aggregates produced from the most mature SSEA-1\textsuperscript{−}/GFP\textsuperscript{+} secretory cell subset occurred as small cell clusters instead of spheres (Fig. 4c, e). Furthermore, these cell clusters did not contain CK5- or p63-expressing basal stem cells (Fig. 4c).

**Dedifferentiated cells stably persist**

To assess whether dedifferentiated stem-cell-like cells have the ability to self-renew and persist in vivo, we generated SCGB1A1–YFP/CK5-DTA mice that possessed lineage-labelled dedifferentiated basal-like cells as above and these mice were then maintained for 2 months before euthanization (Extended Data Fig. 8a). Dedifferentiated YFP\textsuperscript{+} CK5\textsuperscript{+} cells persisted and continued to represent a sizeable fraction of the stem cell pool (9.15% ± 0.41; n = 3). The relative pool size of dedifferentiated basal-like cells remained stable over the course of 2 months (dedifferentiated basal cells represented 8% of the stem cell pool immediately after dedifferentiation). In addition, triple immunostaining for CK5, YFP and K67 revealed that YFP\textsuperscript{+} CK5\textsuperscript{+} dedifferentiated basal-like cells have the same self-renewal rates as do their normal YFP\textsuperscript{−} CK5\textsuperscript{−} basal stem cell counterparts (Extended Data Fig. 8b, c).

**Dedifferentiated cells are functional stem cells**

To assess the functional stem cell capacity of dedifferentiated basal-like cells, we generated SCGB1A1–YFP/CK5-DTA mice that possessed lineage-labelled dedifferentiated basal-like cells and then exposed these animals to two forms of physiologic airway injury (Fig. 5a, b). First, a toxin-induced airway injury with inhaled SO\textsubscript{2} was used to efficiently denude suprabasal cells from the airway epithelium, leaving behind a single layer of basal cells, some of which were derived from labelled secretory cells that had dedifferentiated (marked by YFP) (Extended Data Fig. 9a). The epithelium fully regenerated in 14 days as expected and immunofluorescence analysis for YFP in combination with CK5 (basal cell), SCGB1A1 (secretory cell) and FOXJ1 (ciliated cell) revealed that YFP\textsuperscript{+} cells contributed to all three epithelial cell lineages in the form of scattered YFP\textsuperscript{+} patches (Fig. 5c, top panels). To further scrutinize the functional potential of our dedifferentiated basal-like cells, we used influenza viral infection as a second physiologic injury model (Extended Data Fig. 9b)\textsuperscript{27}. We again observed that dedifferentiated basal-like cells participate in regeneration by giving rise to all three epithelial cell types of the airway (Fig. 5c, bottom panels). Similarly, sorted dedifferentiated cells that were produced either in vivo or ex vivo could be serially passaged in culture and differentiated into mature airway epithelium (Fig. 5d–f and Extended Data Fig. 10a–c).

Furthermore, we asked whether individual dedifferentiated basal-like cells are multipotent (that is, able to give rise to ciliated, secretory and basal cells) or unipotent (that is, able to give rise to only one of the cell types). To address this issue, we cultured individual dedifferentiated cells and then performed an air–liquid interface culture using these clonally derived stem cells. Immunofluorescence analyses for basal, secretory and ciliated cell markers revealed that most of the clonally derived basal-like cells (11 out of 13 clones) are multipotent (Extended Data Fig. 10d). Intriguingly, rare clones give rise to only

![Figure 4](image-url)
maturity of the differentiated cells. This is analogous to the results of stem and committed cells may have been ‘designed’ to ensure differentiation occurs through direct contact, even with a single stem cell. In our example, the prevention of secretory cell dedifferentiation has many implications for tissue biology in general, as toxic or infectious insult. Cellular reservoirs of regenerative capacity may allow a more effective repair of injury. The ability of basal stem cells to prevent the dedifferentiation of secretory cells to revert into stem cells was not assessed. Here we show the capacity of fully differentiated villin-positive intestinal secretory cells to revert back to a stem cell state after injury. Notably, the capacity of fully differentiated villin-positive intestinal secretory cells to revert into stem cells was not assessed. Here we show that fully committed secretory cells respond to stem cell ablation by proliferating and converting into functional epithelial stem cells. Our study points to an alternative cellular mechanism through which tissues can regenerate after stem cell loss. The existence of multiple cellular reservoirs of regenerative capacity may allow a more effective reparative response when one or the other cell type is damaged by a toxic or infectious insult.

The ability of basal stem cells to prevent the dedifferentiation of secretory cells has many implications for tissue biology in general, as stem cells and their progeny can now be seen to reciprocally modulate one another to regulate their relative ratios and thereby overall tissue architecture. In our example, the prevention of secretory cell dedifferentiation occurs through direct contact, even with a single stem cell. This mechanism ensures a precise and local control of epithelial architecture. More generally, it suggests that the reciprocal interactions of stem and committed cells may have been ‘designed’ to ensure a robust self-organizing property in diverse tissue types. Furthermore, the ability of differentiated cells to acquire stem cell properties seems to be inversely proportional to the degree of the maturity of the differentiated cells. This is analogous to the results seen in amphibian nuclear reprogramming in which nuclei from more mature cells were less easily reprogrammed than those of their immature counterparts. The capacity to segregate secretory cells according to their maturity and associated ability to resist dedifferentiation provides an ideal in vivo experimental model for dissecting the molecular mechanisms through which cells might lock their identity as they mature. Finally, our findings have broad implications for cancer biology, as our results point to an underlying physiologic form of cell plasticity that could be co-opted in the process of tumorigenesis. Indeed, some lung cancers seem to be able to resist chemotherapy by using a lineage conversion into a different tumour subtype.

**Discussion**

Here, using lineage tracing, we have presented evidence that fully differentiated cells can revert into stable and functional basal stem cells. By contrast, in the hair follicle, the committed progeny of skin stem cells do not revert into stem cells when the skin stem cells are ablated. However, in the murine intestinal epithelium, undifferentiated secretory progenitors that are the immediate villin-negative progeny of stem cells can revert back to a stem cell state after injury. Notably, the capacity of fully differentiated villin-positive intestinal secretory cells to revert into stem cells was not assessed. Here we show that fully committed secretory cells respond to stem cell ablation by proliferating and converting into functional epithelial stem cells. Our study points to an alternative cellular mechanism through which tissues can regenerate after stem cell loss. The existence of multiple cellular reservoirs of regenerative capacity may allow a more effective reparative response when one or the other cell type is damaged by a toxic or infectious insult.

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**METHODS SUMMARY**

*CK5-hTA*<sup>23</sup>, *Scgb1a1-creER*<sup>16</sup>, *tet(O)DTA*<sup>11</sup> and *B1-eGFP*<sup>25,26</sup> mice were described previously. Corn oil or tamoxifen (2 mg per 20 g body weight) were intraperitoneally injected for five consecutive days. Aerosolized PBS or doxycycline was administered by inhalation. SO<sub>2</sub> injury models have been reported previously. Mice were anesthetized and infected with a sublethal dose of influenza by intranasal injection for five consecutive days. Aerosolized PBS or doxycycline was administered by inhalation. SO<sub>2</sub> injury models have been reported previously. Mice were anesthetized and infected with a sublethal dose of influenza by intranasal injection as described previously. Sphere culture and staining was performed as described previously. Immunofluorescence and cell sorting were performed using standard protocols.

**Online Content** Any additional Methods, 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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Author Contributions P.R.T. designed and performed experiments and wrote the manuscript; H.M., A.P.-S., R.Z., M.P., B.M.L. and V.V. performed influenza infection experiments; A.S. provided doxycycline; B.D.M. reviewed the manuscript; J.R. suggested and co-designed the study and co-wrote the manuscript with P.R.T.

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METHODS

Mouse models. CK5-rtTA2, Scgb1a1-creER2, tet(O)DTA3 and B1-eGFP23,24 mice were described previously. Rosa26R-eYFP (Gt(Rosa)26Sor<tm1(YFP)J]), mice (stock no. 006148) and tet(tet(O)H1T2B2/GFP)/Teflu; (stock no. 005104) were purchased from The Jackson Laboratory. CK5-rtTA females were crossed to tet(O)DTA males to generate a double-transgenic mouse (CK5-DTA) that expresses DTA protein upon doxycycline administration. Aerosolized doxycycline or PBS was administered as described previously24. For secretory cell lineage tracing after basal cell ablation, we crossed male Scgb1a1-creER/Rosa26R-YFP to CK5-DTA female mice to generate quadruple (SCGB1A1–YFP/CK5-DTA)-transgenic mice. To label secretory cells, we injected tamoxifen intraperitoneally (2 mg per 20 g body weight) for five consecutive days to induce the Cre-mediated excision of a stop codon and subsequent expression of YFP. Both male and female mice were used for experiments. 6–12-week-old mice were used for experiments. Similar aged mice were used for both control and treated animals. We analysed at least three mice per condition in each experiment. The MGH Subcommittee on Research Animal Care approved animal protocols in accordance with NIH guidelines.

SO2 and influenza infection induced injury. SO2 injury models have been previously reported19. In brief, mice were exposed to 500 p.p.m. of SO2 for 3 h containing TGF-β/BMP4/WNT antagonist cocktails and 5 μM Rock inhibitor Y-27632 (Selleckch, S1049). To initiate air–liquid interface cultures, airway basal stem 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 stem cells on an air–liquid interface was followed by directly visualizing beating cilia in real time after 10–14 days. For clonal culture assays, dedifferentiated basal-like cells (GSI-β YFP ) were sorted and plated on collagen-coated plates at low cell density to obtain individual colonies. Individual colonies were isolated using microscopic visualization of single colonies followed by trypsin treatment and pipette-assisted aspiration. Individual colony-derived cells were maintained and expanded separately and used for air–liquid interface culture. Sphere culture was performedas described previously20. In brief, 50 μl of 1:4 cold Matrigel/MTEC-plus medium was layered on an 8-well chamber slide (Thermo Scientific, cat. no. 177402) and incubated at 37 °C for 10 min to solidify the Matrigel. Sorted cells were mixed in 2% Matrigel in MTEC-plus and plated on pre-coated 8-well chamber slide at a density of 4,000 cells per well. For mixing assays, sorted cells were seeded at a density of 6,000 cells per well. In each experiment, three independent wells were used for each condition tested. Medium was changed every other day for 9 days. For transwells cultures, cells were suspended in MTEC-plus medium and plated on transwell inserts at a cell density of 6,000 cells per well. Medium was changed every day for 7 days. For basal-cell reporter assays, cells were treated with 1 μg ml−1 doxycycline either at 24 h after plating or just 24 h before collection. For serial passaging of spheres, medium from culture wells was aspirated, washed with PBS, and then treated with trypsin-EFDA (0.25%) for 2 min. Trypsin was inactivated and dissociated cells were collected and centrifuged at 350g for 3 min at 4 °C. Cells were re-seeded at a 1:20 dilution in Matrigel for the next round of sphere culture.

Statistical analysis. The standard error of the mean was calculated from the average of at least three independent tracheal samples unless otherwise mentioned. Data were compared among groups using the Student’s t-test (unpaired, two-tailed). A P value of less than 0.05 was considered significant.
**Extended Data Figure 1** | Schematic representation of the dedifferentiation of luminal secretory cells into functional basal stem cells. a. Differentiated luminal secretory cells are labelled with a YFP lineage tag in a homeostatic airway epithelium. Basal stem cells are then ablated using diphtheria toxin. In response, lineage-labelled secretory cells dedifferentiate into cells that morphologically resemble basal cells and express basal stem cell markers. These dedifferentiated basal-like cells respond to physiologically relevant toxic and infectious injury and serve as multipotent stem cells during epithelial regeneration. The inset depicts the different cell types of the airway epithelium. b, Graphical representation of the dedifferentiation potential of differentiated basal-like cells. x axis represents the maturity of a secretory cell; y axis represents the propensity for dedifferentiation to a basal-like cell. The propensity to dedifferentiate is inversely correlated to the maturity of the secretory cell.
Extended Data Figure 2 | Inhaled doxycycline efficiently ablates basal stem cells of the airway epithelium.  

**a**, Schematic representation of basal-cell-specific ablation using i-Dox. 

**b**, Co-labelling of p63 (green) and CK5 (red) on tracheal sections CK5-DTA mice that received either i-PBS (top) or i-Dox (middle and bottom panels show 2 and 3 doses of i-Dox, respectively). 

**c**, Co-labelling of NGFR (green) and T1α (red) i-PBS- or i-Dox-treated mice. 

**d**, Quantification of the number of p63\(^+\) (black bar) and CK5\(^+\) (grey bar) basal cells from CK5-DTA animals treated with PBS (p63, 1,229 ± 65.45; CK5, 1,376 ± 25.23), two doses of i-Dox (p63, 690 ± 35.13; CK5, 716 ± 12.44) or three doses of i-Dox (p63, 262 ± 29.5; CK5, 255 ± 46.82); y axis represents the absolute numbers of basal cells (from three independent tracheal sections). Dox(2) and Dox (3) refer to two and three doses of doxycycline inhalation, respectively. \(n=3\) (two mice per condition). Error bars, average ± s.e.m. Scale bars, 20 μm.
Extended Data Figure 3 | Secretory cells begin to express proliferation and stem cell markers and undergo dedifferentiation following basal cell ablation. a, Orthogonal confocal optical sections of SCGB1A1 (green), CK5 (cyan) and Ki67 (red) XY and XZ planes are shown to demonstrate the colocalization of Ki67 and SCGB1A1. b, Quantification of the percentage of Ki67\(^+\) CK8\(^-\) double-positive cells per total Ki67\(^+\) cells from i-PBS (23.74\(\pm\)6.76)- and i-Dox (63.22\(\pm\)4.14)-treated CK5-DTA mice. c, Co-labelling of CK5 and SCGB1A1 in i-Dox-treated CK5-DTA mice. White arrows, double-positive cells. d, Immunostaining for YFP (green) and Ki67 (red) on sections from SCGB1A1–YFP/CK5-DTA mice. White arrows indicate YFP\(^+\) Ki67\(^+\) cells. e, Quantification of the percentage of YFP\(^+\) Ki67\(^+\) cells per total Ki67\(^+\) cells in SCGB1A1–YFP/CK5-DTA mice that were treated with either i-Dox (31.74\(\pm\)7.15) or i-PBS (9.65\(\pm\)2.12). f, Co-labelling of YFP (green) with p63 or T1\(_a\) (red) on tracheal sections of SCGB1A1–YFP/CK5-DTA mice that were either treated with i-PBS (top) or i-Dox (bottom). White arrows, double-positive cells. n = 3 (three mice per condition). Error bars, average \(\pm\) s.e.m. Scale bars, 20\(\mu\)m.
Extended Data Figure 4 | Dissociation and fluorescence activated cell sorting of airway epithelial cells. 

**a**, Schematic representation of tracheal epithelial cell dissociation from secretory cell lineage-labelled mice (SCGB1A1-CreER/LSL-YFP) after five doses of tamoxifen. Of the total epithelial cells, EPCAM$^+$ CD24$^-$ cells were gated to remove ciliated cells. Then YFP$^+$ secretory cells and GSI$^+$ basal cells were sorted. 

**b**, Of the total epithelial cells, EPCAM$^+$ CD24$^-$ cells were gated to remove ciliated cells (left). Then, YFP$^+$ secretory cells were separated from GSI$^+$ basal cells (middle). Sorted YFP$^+$ cells were also marked by the secretory cell marker SSEA-1 as expected for a pure population of SCG1A1$^+$ cells (right).
Extended Data Figure 5 | Sorted secretory cells dedifferentiate into basal-like self-renewing stem cells upon ex vivo culture. a, Schematic representation of tracheal epithelial cell dissociation from basal cell reporter mice (CK5-rtTA/tet(O)H2BGFP) followed by sorting of GFP–SSEA-1 secretory cells. Sorted SSEA-1 cells were grown as spheres in Matrigel or plated on transwell membranes. Doxycycline was administered and cells were monitored for the initiation of GFP expression.

b, Fluorescence-activated cell sorting of SSEA-1 cells from basal cell reporter mice (CK5-rtTA/tet(O)H2BGFP). Arrows indicate gating windows. EPCAM is a pan-epithelial marker used to exclude non-epithelial lineages. Sorted SSEA-1 secretory cells did not express GFP. c, Immunofluorescence staining for p63 (red; top) or CK5 (red; bottom) in combination with H2B–GFP (green; all panels) on sorted secretory cells that were either cultured as Matrigel spheres (left) or on transwells (right). Immunofluorescence analysis confirmed that H2B–GFP+ cells expressed p63 and CK5, again confirming that secretory cells dedifferentiate in culture. n = 3 (two replicates per condition). Scale bars, 20 µm.
Extended Data Figure 6 | Sorted lineage-labelled secretory cells undergo dedifferentiation ex vivo, express basal stem cell markers, and can be serially passaged, as can secretory cells that underwent dedifferentiation in vivo.

a, Schematic representation of secretory cell labelling, sorting and subsequent culturing in Matrigel or on transwell membranes. b, Cell colonies obtained from early passage cultures of YFP<sup>+</sup> secretory-cell-derived cells on transwell membranes. c, Immunostaining for CK5 (red), p63 (magenta) and YFP (green) on passage-five basal cell colonies from ex vivo-dedifferentiated cells. d, Schematic showing that YFP<sup>+</sup> secretory-cell-derived spheres from SCGB1A1-CreER/LSL-YFP mice were serially passaged for five generations. e, Quantification of the sphere-forming efficiency: P1 (2.86% ± 0.60), P2 (3.36% ± 0.6), P3 (2.31% ± 0.32), P4 (2.75% ± 0.69) and P5 (2.7% ± 0.94). x axis, number of passages. y axis, percentage of spheres formed. f, Schematic representation of in vivo dedifferentiation followed by the sorting and culturing of YFP<sup>+</sup> basal-like cells. g, Immunostaining for CK5 (red), p63 (magenta) and YFP (green) on passage-five cell colonies from in vivo-dedifferentiated cells. n = 3 (two replicates per condition). Error bars, average ± s.e.m. Scale bars, 20 μm.
Extended Data Figure 7 | B1–eGFP transgenic mice express GFP in mature subsets of secretory cells. a, Co-labelling of GFP (green) with CK5 or SCGB1A1 or SSEA-1 (all in red) on large airways sections derived from adult B1–eGFP transgenic mice at homeostasis. White arrows indicate SSEA-1⁺ B1–eGFP⁺, whereas white arrowheads point to cells that are B1–eGFP⁺ SSEA-1⁻ (bottom). b, B1–eGFP trachea were stained for GFP (green) and SSEA-1 (red) on day 4 and day 6 after SO₂-induced injury. n = 3 (two mice per condition per time point). Scale bars, 20 µm.
Extended Data Figure 8 | Dedifferentiated basal-like stem cells are stable and self-renew to the same degree as endogenous basal stem cells.

**a**, Schematic representation of the dedifferentiation protocol to assess the ability of basal-like stem cells to persist and self-renew for 2 months.

**b**, Immunofluorescence staining for YFP (green) in combination with CK5 (cyan) or Ki67 (red) on sections from SCGB1A1–YFP/CK5-DTA mice 2 months after basal cell ablation.

**c**, Quantification of the percentage of proliferating dedifferentiated CK5<sup>+</sup> YFP<sup>+</sup> (10.16% ± 2.57) (green bar) and wild-type CK5<sup>+</sup> YFP<sup>−</sup> (9.25% ± 0.79) (black bar) stem cells out of the total CK5<sup>+</sup> stem cell population in the large airways of SCGB1A1–YFP/CK5-DTA mice 2 months after basal cell ablation. The white arrow points to a proliferating dedifferentiated basal-like stem cell. *n* = 3 (two mice per condition). Error bars, average ± s.e.m. Scale bar, 20 µm.
Extended Data Figure 9 | SO₂- and influenza-induced injury efficiently removes the suprabasal cells of the airway epithelium.

**a**, Immunofluorescence staining for CK5 (red) and YFP (green) in SCGB1A1–YFP/CK5-DTA airway epithelium 24 h after SO₂ inhalation. Only a single layer of basal cells persists after injury. White arrows, CK5⁻YFP⁺ double-positive cells (yellow).

**b**, Immunofluorescence analysis of basal cells (CK5, red) and secretory cells (SCGB1A1, green) from uninjured (top), 72 hours post-infection (h.p.i.; middle) and 14 days post-infection (d.p.i.; bottom). White arrow, secretory cell debris (in green). n = 3 (two replicates per condition). Scale bars, 20 μm.
Extended Data Figure 10 | *Ex vivo*-dedifferentiated cells can be clonally expanded and give rise to a complete airway epithelium in air–liquid interface cultures. a, Schematic representation of the *ex vivo* dedifferentiation of secretory cells, serial passage and differentiation. b, Whole-mount immunostaining for YFP (green) in combination with CK5 or p63 or acetylated (AC)-tubulin (red). c, Co-labelling of YFP (green) with CK5 or p63 or SCGB1A1 or FOXJ1 (red) on cross sections of clonally derived epithelium. Scale bars, 20 μm.