Extended Data figure legends

Extended Data Fig. 1. Schematic representation of the dedifferentiation of luminal secretory cells into functional basal stem cells.

a, Differentiated luminal secretory cells are labeled with a YFP lineage tag in a homeostatic airway epithelium. Basal stem cells are then ablated using diphtheria toxin. In response, lineage-labeled 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 Fig. 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-labeling of p63 (green) and CK5 (red) on tracheal sections CK5-DTA mice that received either i-

PBS (uppermost panel) or i-dox (middle and lower panels show 2 and 3 doses of i-dox respectively). c, Co-labeling 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, 1229±65.45; CK5, 1376±25.23), 2-doses of i-Dox (p63, 690±35.13; CK5, 716±12.44), or 3-doses of i-Dox (p63, 262±29.5; CK5, 255±46.82). y-axis represents the absolute numbers of basal cells (from 3 independent tracheal sections). Dox(2) and Dox(3) refer to 2 and 3 doses of doxycycline inhalation respectively. i-Dox, inhaled doxycycline. Nuclei - DAPI (blue). n=3 (2 mice per condition). Error bars, average±s.e.m. Scale bar 20μm.

Extended Data Fig. 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 co-localization of Ki67 in SCGB1A1. b, Quantification of the percentage of Ki67+CK8+ double positive cells per total Ki67+ cells from i-PBS (23.74%±6.76) and i-Dox (63.22%±4.14) treated CK5-DTA mice. c, Co-labeling 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%±7.15) or i-PBS (9.65%±2.12). f, Co-labeling of YFP (green) with p63 or T1α (red) on tracheal sections of Scgb1a1-YFP/CK5-DTA mice that were either treated with i-PBS (upper panels) or i-

Dox (lower panels). White arrows, double positive cells. i-Dox, inhaled doxycycline; i-PBS, inhaled PBS. Nuclei - DAPI (blue). n=3 (3 mice per condition). Error bars, average±s.e.m. Scale bar 20μm.

Extended Data Fig. 4. Dissociation and fluorescence activated cell sorting of airway epithelial cells a, Schematic representation of tracheal epithelial cell dissociation from secretory cell lineage-labeled mice (Scgb1a1-CreER/LSL-YFP) after 5 doses of tamoxifen. Of the total epithelial cells, EpCAM+ CD24- cells were gated to remove ciliated cells. Then, YFP+ secretory cells and GSIb4+ basal cells were sorted. b, Of the total epithelial cells, EpCAM+ CD24- cells were gated to remove ciliated cells (left plot). Then, YFP+ secretory cells were separated from GSIb4+ basal cells (middle plot). Sorted YFP+ cells were also marked by the secretory cell marker SSEA1 as expected for a pure population of Scg1a1+ cells (right plot).

Extended Data Fig. 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-SSEA1+ secretory cells. Sorted SSEA1+ 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 SSEA1+ 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 SSEA1+ secretory

cells did not express GFP. c, Immunofluorescence staining for p63 (red; upper panels) or CK5 (red; lower panels) in combination with H2BGFP (green; all panels) on sorted secretory cells that were either cultured as matrigel spheres (left panels) or on transwells (right panels). Immunofluorescence analysis confirmed that H2BGFP+ cells expressed p63 and CK5, again confirming that secretory cells dedifferentiate in culture. Nuclei - DAPI (blue). n=3 (2 replicates per condition). Scale bar 20μm.

Extended Data Fig. 6. Sorted lineage labeled 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 labeling, sorting and subsequent culturing in matrigel or on transwell membranes. b, Cell colonies obtained from early passage cultures of YFP+ secretory cell-derived cells on transwell membranes. c, Immunostaining for CK5 (red), p63 (magenta), and YFP (green) on passage-5 basal cell colonies from *ex vivo* dedifferentiated cells. d, Schematic showing that YFP+ secretory cell-derived spheres from Scgb1a1-CreER/LSL-YFP mice were serially passaged for 5 generations. e, Quantification of the sphere-forming efficiency: P1 (2.86%±0.65), 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^{+} basal-like cells. g, Immunostaining for CK5 (red), p63 (magenta), and YFP (green) on passage 5 cell colonies from *in vivo* dedifferentiated cells Nuclei - DAPI (blue). n=3 (2 replicates per condition). Error bars, average±s.e.m. Scale bar 20μm.

Extended Data Fig. 7. B1-EGFP transgenic mice express GFP in mature subsets of secretory cells.

a, Co-labeling of GFP (green) with CK5 or SCGB1A1 or SSEA1 (all in red) on large airways sections derived from adult B1-EGFP transgenic mice at homeostasis. White arrows indicate SSEA1+ B1-EGFP-, while white arrowheads point to cells that are B1-EGFP+ SSEA1- (lower panel). b, B1-EGFP trachea were stained for GFP (green) and SSEA1 (red) on day 4 and day 6 post sulfur dioxide (SO2)-induced injury. Nuclei - DAPI (blue). n=3 (2 mice per condition/time point). Scale bar 20μm.

Extended Data Fig. 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+YFP+ (10.16%±2.57) (green bar) and wild type CK5+YFP- (9.25%±0.79) (black bar) stem cells out of the total CK5+ 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. i-Dox, inhaled doxycycline. Nuclei - DAPI (blue). n=3 (2 mice per condition). Error bars, average±s.e.m. Scale bar 20μm.