Supporting Information

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SI Materials and Methods

Detailed Protocol for Lung Cell Preparations and Flow Cytometry. Mouse lungs were dissected and rinsed in sterile PBS following removal of tracheas and extrapulmonary airways and were finely minced with sterile scissors and incubated in 3 mg/mL Collagenase Type I (Roche) in PBS (310 mOsm) in a volume of 2 mL per lung for 60 min at 37 °C in a shaking incubator. The resulting cell suspension was further disaggregated by trituration through an 18-gauge needle, diluted in PBS, filtered through a 40-μm nylon cell strainer (Falcon; Becton Dickinson) and washed twice in PBS supplemented with 2% vol/vol FCS or PBS-2% vol/vol Se by centrifugation (400 × *g*, 5 min, 10 °C).

Discontinuous density gradient centrifugation was employed to remove contaminating erythrocytes, neutrophils, and cell debris. Twenty-milliliter aliquots of lung cell suspension (two to three lungs per gradient) were layered on 10 mL of Nycoprep 1.077A (1.077 g/cm^3, 265 mOsm; Nycomed Pharma) (600 × *g*, 20 min, room temperature, brake off) in 50 mL of sterile polypropylene tubes. Low-density cells at the PBS-Nycoprep interface were collected and washed twice in excess PBS-2% Se. Cells were then resuspended and incubated in PBS-2% Se (5×10^7 cells/mL, 20 min on ice) in an optimally pretitered mixture of antibodies, including anti-CD45, anti-CD31, anti-Sca-1, anti-EpCAM, anti-CD49f, anti-CD24, anti-CD104, and relevant isotype controls (Biolegend). Labeled cells were washed in PBS-2% Se, resuspended at $5–10 \times 10^6$ cells/mL, and held on ice for flow cytometric analysis and sorting. Viability was determined by propidium iodide (1 μg/mL) staining, and doublets were excluded by forward scatter (height) vs. forward scatter (area) gating. For proSP-C analysis, cells were fixed using a Fix 'n' Perm kit (Invitrogen). Sorting was performed using a BD Influx cell sorter (Becton Dickinson) equipped with five lasers (488 nm 200 mW, 640 nm 40 mW, 561 nm 75 mW, 405 nm 50 mW, and 350 nm 100 mW) using a 90-μm nozzle at 30 psi. Analysis was done using a BD LSRII bench top analyzer (Becton Dickinson) equipped with four lasers (488 nm 100 mW, 640 nm 30 mW, 561 nm 40 mW, and 405 nm 50 mW). Data were analyzed using FlowJo (Tree Star).

In Situ Immunohistochemistry. Five-micrometer sections of adult mouse lungs fixed with paraformaldehyde (1%) fixed and embedded in optimal cutting temperature medium were incubated in blocking buffer (1 h, 5% wt/vol BSA, 1% skim milk, 0.05% Triton X-100 in PBS). Sections were then incubated overnight with biotinylated anti-CD104 in combination with goat anti-CCSP (Santa Cruz Biotechnology) or rabbit anti-proSP-C antibody (Millipore), washed in PBS (0.05% Tween 20), and then subjected to tyramide signal amplification with Alexafluor 488-labeled tyramide (Invitrogen). Sections were washed and then incubated with donkey anti-goat or anti-rabbit conjugated to Alexafluor 568 (Invitrogen) for 1 h and then washed. Nuclei were stained with DAPI, followed by rinsing and mounting in Vectashield mounting medium (Vecta Laboratories). Photomicrographic images were acquired using a Leica SP confocal microscope. Images were colored and overlaid using Adobe Photoshop (Adobe Systems).

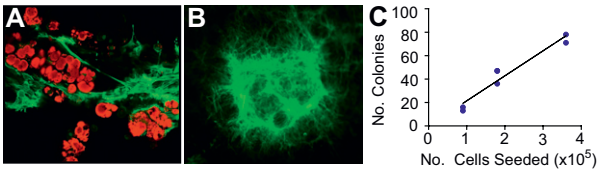

Fig. S1. Oil-Red O^{pos} and α-SMA^{pos} mesenchymal cells. (*A*) Oil-Red O (red) and α-SMA (green) staining of EpCAM^{neg} Sca-1^{pos} cells. (*B*) α-SMA (green) staining of mesenchymal cells wrapping around epithelial colonies when EpCAM^{pos} cells are cocultured with EpCAM^{neg} Sca-1^{pos} cells. (*C*) Cloning efficiency of CD45^{neg} CD31^{neg} Sca-1^{pos} cells with linear regression analysis showing that the number of cells plated is directly proportional to the number of colonies generated (r^2 = 0.9361, 1/slope = 4,688).

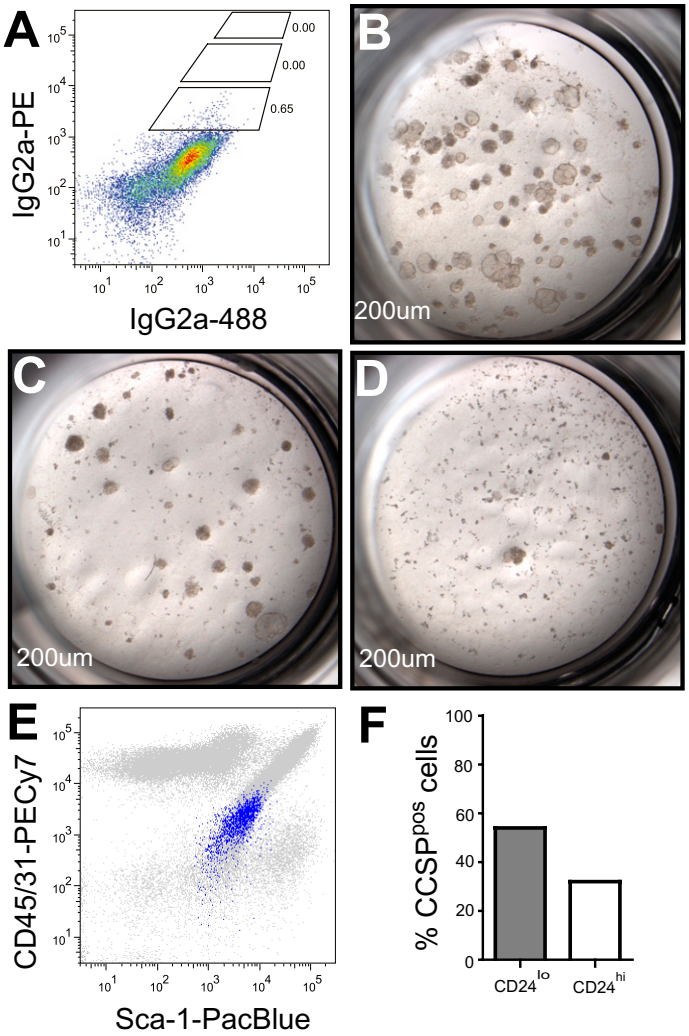

Fig. S2. Characterization of EpCAMhi CD24^{low} and EpCAM^{hi} CD24^{hi} cells. Isotype control (*A*), EpCAM^{hi} CD49f^{pos} (*B*), EpCAM^{med} CD49f^{low} (*C*), and EpCAM^{low} CD49f$^{neg/low}$ (*D*) cell cultures (cocultured with EpCAM^{neg} Sca-1^{pos} mesenchymal cells). (*E*) Backgating of EpCAM^{hi} cells (blue) onto whole lung digest (gray). (*F*) Percentage of CCSP^{pos} cells in EpCAM^{hi} CD24^{low} and CD24^{hi} cell fractions.

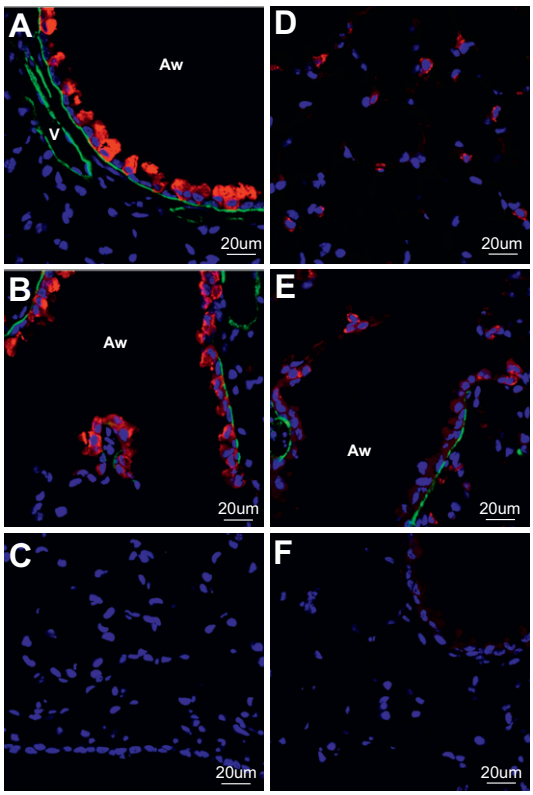

Fig. S3. Immunolocalization of CD104^{pos} cells to the distal airway in situ. Fluorescent photomicrographs of CD104 (green) and CCSP (red) staining in sections of distal airway (*A*) and a BADJ (*B*). (*C*) Isotype control. CD104 (green) and proSP-C (red) staining in sections of alveoli (*D*) and a BADJ (*E*). (*F*) Isotype control. DAPI staining of nuclei is shown in blue. Aw, airway; V, blood vessel.

Table S1. Applied Biosystems TaqMan gene expression assays

Protein	Gene	Assay
SP-C	*Sftpc*	Mm00488144_m1
SP-A	*Sftpa1*	Mm00499170_m1
SP-B	*Sftpb*	Mm00455681_m1
CCSP	*Scgb1a1*	Mm00442046_m1
ABCA3	*Abca3*	Mm00550501_m1
CEBP/α	*Cebpa*	Mm00514283_s1
CGRP	*Calca*	Mm00801463_g1
P63	*Trp63*	Mm00495788_m1
FoxJ1	*Foxj1*	Mm00807215_m1
Sox2	*Sox2*	Mm00488369_s1
ENaC	*Scnn1g*	Mm00441228_m1
MUC5AC	*Muc5ac*	Mm01276725_g1
MUC1	*Muc1*	Mm00449604_m1
Cftr	*Cftr*	Mm00445197_m1