Supplemental Methods:

Naphthalene injury and BrdU labeling protocol: Naphthalene was freshly dissolved in corn oil, and 275 mg of naphthalene/kg body weight was injected i.p. into mice under anesthesia (to avoid injection into internal organs) with 2% isofluorane. Mice were also injected with 1 ml dextrose saline twice daily for the first two days following injury to avoid dehydration and increase survival. Slow cycling label-retaining cells (LRCs) were marked by injecting animals (i.p.) with 80 mg/kg of BrdU three times within the first 5 days following naphthalene injury (designated as day 1) as previously described—two BrdU labeling protocols were employed: 1) injection of BrdU on day 1, 2, and 3 was used to more selectively label the initial dividing pool of LRC progenitors following airway injury (this method was used only for comparisons of LRC localization to the SAE and SMGs between *CFTR*$^{+/-}$ and *CFTR*$^{-/-}$ mice) or 2) injection of BrdU on day 1, 3, and 5 was used to capture a larger number of LRCs in studies evaluating the phenotype of LRCs and capacity of LRCs to re-enter the cell cycle.

General immunofluorescence and immunohistochemical staining protocols: All immunofluorescent staining was performed on 10 μm frozen sections that were post-fixed in 4% paraformaldehyde for 30 minutes, followed by three rinses in PBS for 5 minutes each. Slides were placed into pre-heated 95^{o}C Citrate Epitope Retrieval Buffer and incubated for 20 minutes, and then allowed to slowly cool down for 2 hours at room temperature. For staining, the slides were rinsed in PBS three times for 5 minutes per rinse, incubated in PBS plus 10% donkey serum and 0.5% Triton-X-100 for 1 hr at room temperature, stained with primary antibody in PBS plus 1% donkey serum and 0.5% Triton-X-100 at 4°C overnight, and then stained with secondary antibody in PBS plus 1% donkey serum and 0.5% Triton-X-100 for 1 hr at room temperature. In the case of peptide competition studies for CGRP immunoreactivity, 100 ng of synthetic CGRP (Abcam ab47101; or an equivalent volume of diluent buffer alone) were preincubated with anti-CGRP antibody in 1 ml staining buffer for 2 hr prior to application to the tissue sections. Following staining, slides were rinsed in PBS three times for 5 minutes each, and then immersed in Vectashield Mounting Medium (Vector Labs H-1200) before coverslips were applied. Primary antibodies used for immunostaining included mouse

anti-BrdU at a 1:200 dilution (Roche 11170376001), rabbit anti-CGRP at a 1:4000 dilution (Sigma C8198), rabbit anti-Cyclin D1 (provided pre-diluted by the company; Abcam ab15196), rabbit anti-RAMP1 (1:500) (1), and rabbit anti-CLR (1:1000) (1). The secondary antibodies used for immunostaining were Texas Red-conjugated donkey anti-mouse antibody at a 1:250 dilution (Jackson ImmunoResearch 715-076-150), and FITC-conjugated donkey anti-rabbit secondary antibody at a 1:250 dilution (Jackson ImmunoResearch 711-095-152). Immunohistochemical staining for CGRP was performed on either frozen sections or paraffin sections due to higher autofluorescence in human, pig and ferret samples. In this case, an alkaline phosphatase-conjugated donkey anti-rabbit second antibody (Jackson ImmunoResearch, 711-056-152, 1:250) was used with a VECTOR Red Alkaline Phosphatase Substrate Kit (SK-5100, Vector Labs) and manufacturer's protocols.

Morphometric quantification of immunofluorescent staining: MetaMorph quantification software was used for all morphometric analyses, and the thresholds for all images within a dataset were adjusted using identical parameters. All fluorescent photomicrographs for a given dataset were acquired using identical camera and exposure settings on a Leica spinning-disk microscope. Collages encompassing the entire longitudinal axis of the trachea (C1-C13) were generated in Adobe Photoshop using the automatic overlap function from individual images obtained using a 20X objective; thresholds for all collages were adjusted simultaneously prior to quantification using the same parameters. For quantification of LRC localization, at least three animals from each genotype were quantified, and three sections were analyzed from each animal. Average values for the multiple quantified sections from each animal were used to calculate the mean +/-SEM between experimental groups. Longitudinal sections of the tracheas used for quantification were chosen on the basis of similar length and structure of the C1 cartilage ring, where the largest gland resides. Anatomic boundaries for quantification of the sectors for each cartilaginous ring included the midpoint between each cartilage ring. BrdU-labeled cells in the SAE or SMG that resided within each cartilage ring boundary were quantified using the Metamorph software, and the DAPI-stained channel as used to define the boundaries of the SAE and SMG regions. The LRC index is defined as the number of BrdU-positive cells in a given region of the cartilaginous ring, divided by the total number of BrdU-positive cells in the whole tracheal

section. Similarly, the LRC indexes for the SAE and SMGs are defined as the percentage of LRCs in either region relative to those in the entire trachea.

Morphometric analysis was also used to quantify the total intensity of CGRP immunofluorescent staining in the SMGs found in the C1-C2 region. As in the case of BrdU staining, collages were generated from images taken using a 20X objective, after which simultaneous setting of the threshold was performed for all samples. Metamorph quantification of DAPI-staining was used to demarcate the SMG boundaries, and the number of DAPI-positive nuclei and the intensity of CGRP staining were quantified using identical software parameters. Four groups of animals were quantified including: uninjured *CFTR*$^{-/-}$ mice; uninjured *CFTR*$^{+/-}$ mice; naphthalene-injured (5 days prior to staining for CGRP) *CFTR*$^{-/-}$ mice; and naphthalene-injured (5 days prior to staining for CGRP) *CFTR*$^{+/-}$ mice. The index of CGRP expression was calculated as the total intensity divided by total nuclei in the region quantified.

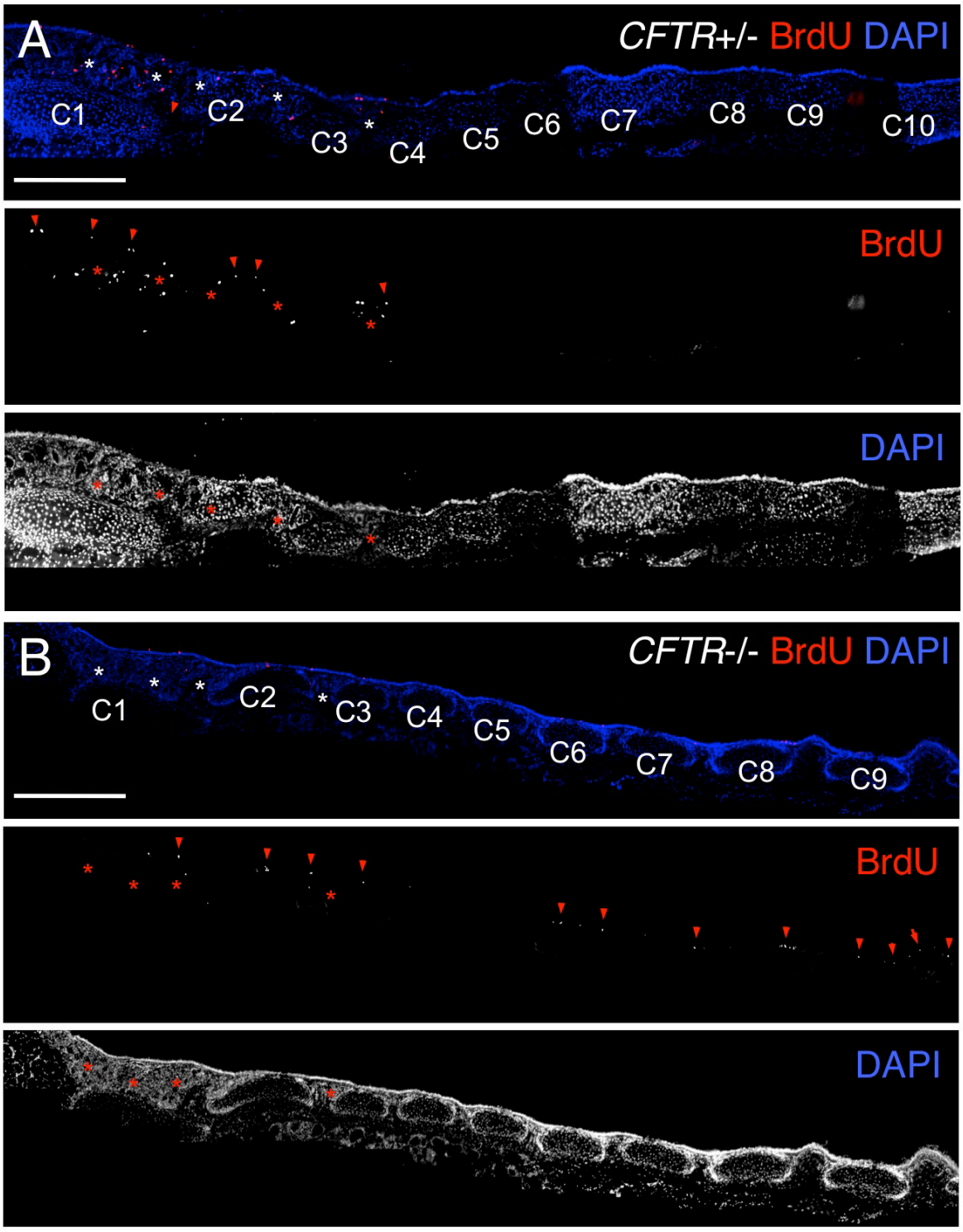
Enzyme-linked immunosorbent assay (ELISA) for CGRP in tracheal lysates: Tracheal-tissue homogenates were generated from the C1-C3 region of the trachea, and used for ELISA-based quantification of CGRP expression. Four groups of animals were quantified including: uninjured *CFTR*$^{-/-}$ mice (N=10 animals); uninjured CFTR$^{+/-}$ mice (N=10 animals); naphthalene-injured (5 days prior to tracheal harvest) *CFTR*$^{-/-}$ mice (N=5 animals); and naphthalene-injured (5 days prior to tracheal harvest) *CFTR*$^{+/-}$ mice (N=5 animals). Because of the low abundance of CGRP, tracheal samples were pooled into groups representing 5 animals each, and analyzed in triplicate by ELISA. Tracheal segments were rapidly removed, snap frozen, and pulverized to a powder under liquid nitrogen. A cytoplasmic extract was generated using an Extraction Kit (#40010) from Active Motif, and used in ELISAs. 100 μg of total protein was diluted in 100 μl of 50 mM carbonate buffer, and was subsequently applied onto the EIA/RIA Stripwell plates (COSTAR #24107021) overnight at 4^{o}C. On the second day, wells were washed 4 times in PBS followed by incubation in blocking buffer containing 3% BSA for 1 hour at room temperature. After three washes in PBS plus 0.5% BSA and 0.05% Tween20, the wells were incubated with primary antibody for 2 hours at room temperature, after which the appropriate secondary antibody was added and incubations proceeded for an additional hour at room temperature. Tetramethylbenzemidine (TMB) (Sigma T0440) and H_2O_2

were then used as substrates to develop color, and the reactions were terminated using stopping buffer containing hydrochloric acid. In this assay, the primary antibody was goat anti-CGRP antibody at 1:1000 dilution (Abcam ab36001). The secondary antibody was horseradish peroxidase (HRP)-conjugated Donkey anti-goat antibody at 1:5000 dilution (Santa Cruz sc-2033).

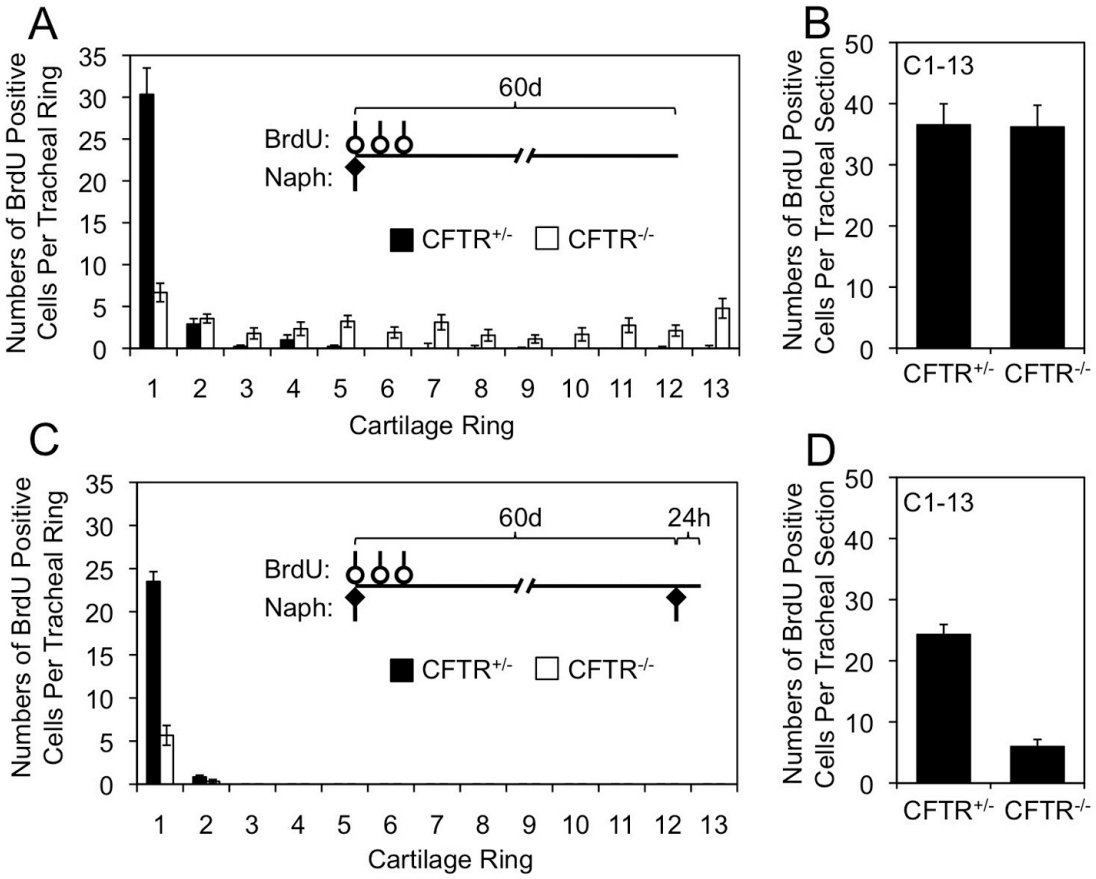
References

1. Lennerz, J.K., Ruhle, V., Ceppa, E.P., Neuhuber, W.L., Bunnett, N.W., Grady, E.F., and Messlinger, K. 2008. Calcitonin receptor-like receptor (CLR), receptor activity-modifying protein 1 (RAMP1), and calcitonin gene-related peptide (CGRP) immunoreactivity in the rat trigeminovascular system: differences between peripheral and central CGRP receptor distribution. *J Comp Neurol* 507:1277-1299.

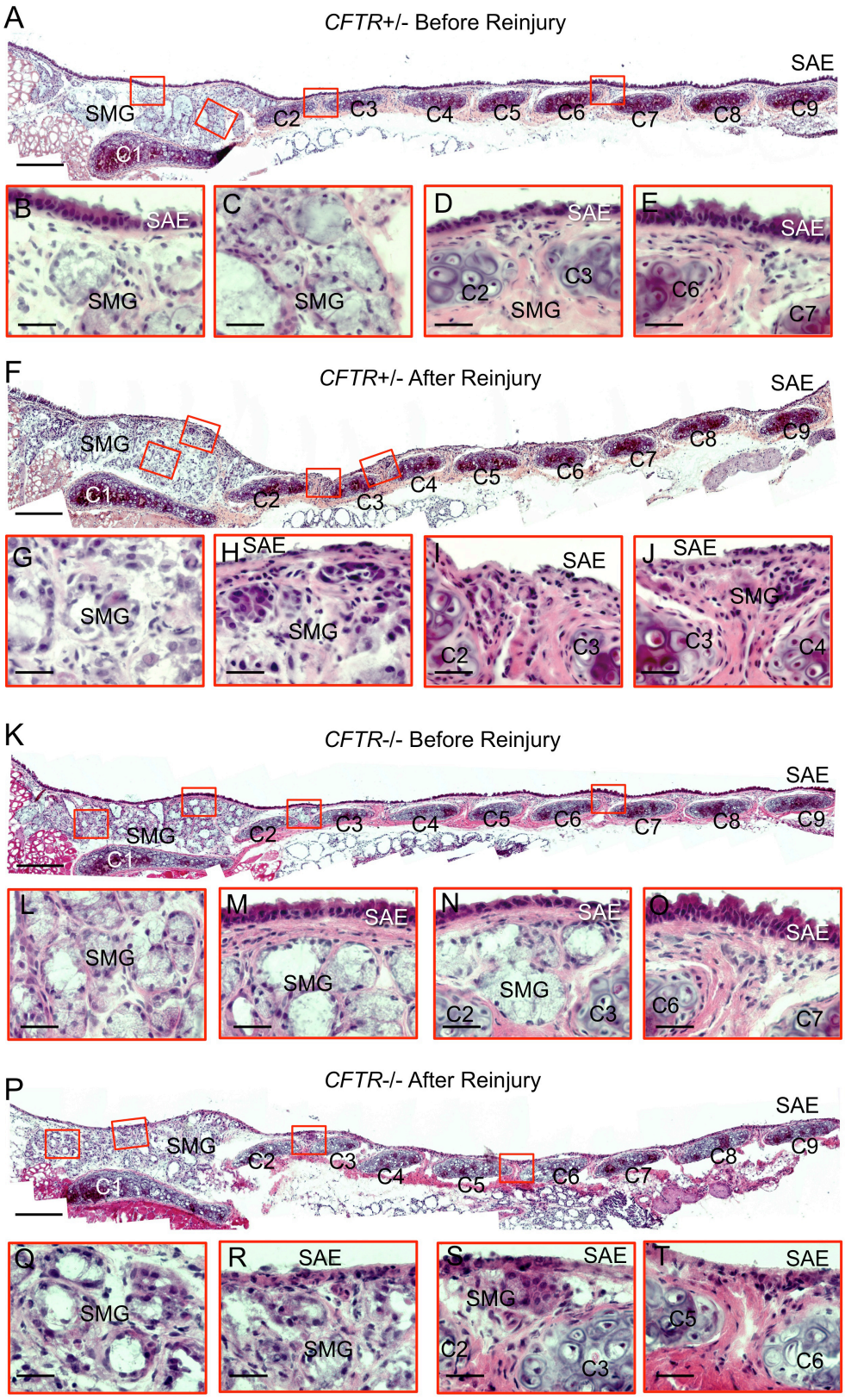
Supplemental Data



Supplemental Figure 1. Naphthalene injury promotes LRC relocation from SMGs to the tracheal SAE in *CFTR* knockout mice. Naphthalene-injured mice were injected with BrdU three times during the first week following injury. At 60 days post-injury, tracheal sections were stained for BrdU (red). Collages of BrdU-stained tracheas from (A) $CFTR^{+/-}$ and (B) $CFTR^{-/-}$ naphthalene-injured mice. The collages were generated from 20x photomicrographs and all BrdU images were captured using identical exposure times. DAPI was used to mark nuclei (blue). The cartilaginous rings of each tracheal section (C1-C10) are labeled in the color images. Red and white asterisks mark SMGs; red arrowheads mark BrdU-positive cells in the SAE. Micron bars = 200 μm.



Supplemental Figure 2. Quantification of tracheal LRCs following a second naphthalene injury. Mice were injured with naphthalene and labeled with BrdU on days 1, 3, and 5 post-injury. At 60 days post-injury, tracheas were either (A, B) harvested directly for analysis of LRCs or (C, D) mice were re-injured with naphthalene, euthanized 24 hrs later, and tracheas harvest for LRC analysis. Longitudinal tracheal sections were then stained for BrdU. (A, C) Quantification of LRCs along the distal axis of tracheal sections from *CFTR*$^{+/-}$ and *CFTR*$^{-/-}$ mice (A) prior to and (C) following second injury. For quantification purposes the midpoint between each cartilaginous ring (C1-C13) was designated as the boundary for quantification. Results depict the mean +/-SEM number of LRCs per tracheal ring in a single section (N=6-9). Inset in each panel shows the protocol for labeling and injury. (B, D) Average number of total LRCs (+/-SEM) in a single longitudinal tracheal section (C1-13) from *CFTR*$^{+/-}$ and *CFTR*$^{-/-}$ mice (B) prior to and (D) following second injury. Results in panels B and D are the sum of LRCs from data represented in panels A and C, respectively.



Supplemental Figure 3. Histopathology of *CFTR*$^{+/-}$ and *CFTR*$^{-/-}$ mouse tracheas before and after a second naphthalene injury. Longitudinal H&E stained tracheal sections from (A-J) *CFTR*$^{+/-}$ and (K-T) *CFTR*$^{-/-}$ mice before and after a second injury with naphthalene. Mice were treated identically to that described in the Supplemental Figure 2 legend. Panels B-E, G-J, L-O, and Q-T are higher-magnification views of the boxed regions in the collages shown in panels A, F, K, and P, respectively. Cartilaginous rings are marked C1-C9. SAE = surface airway epithelium; SMG = submucosal glands. Micron bars: A, F, K, and P = 200 μm; B-E, G-J, L-O, Q-T = 25 μm.