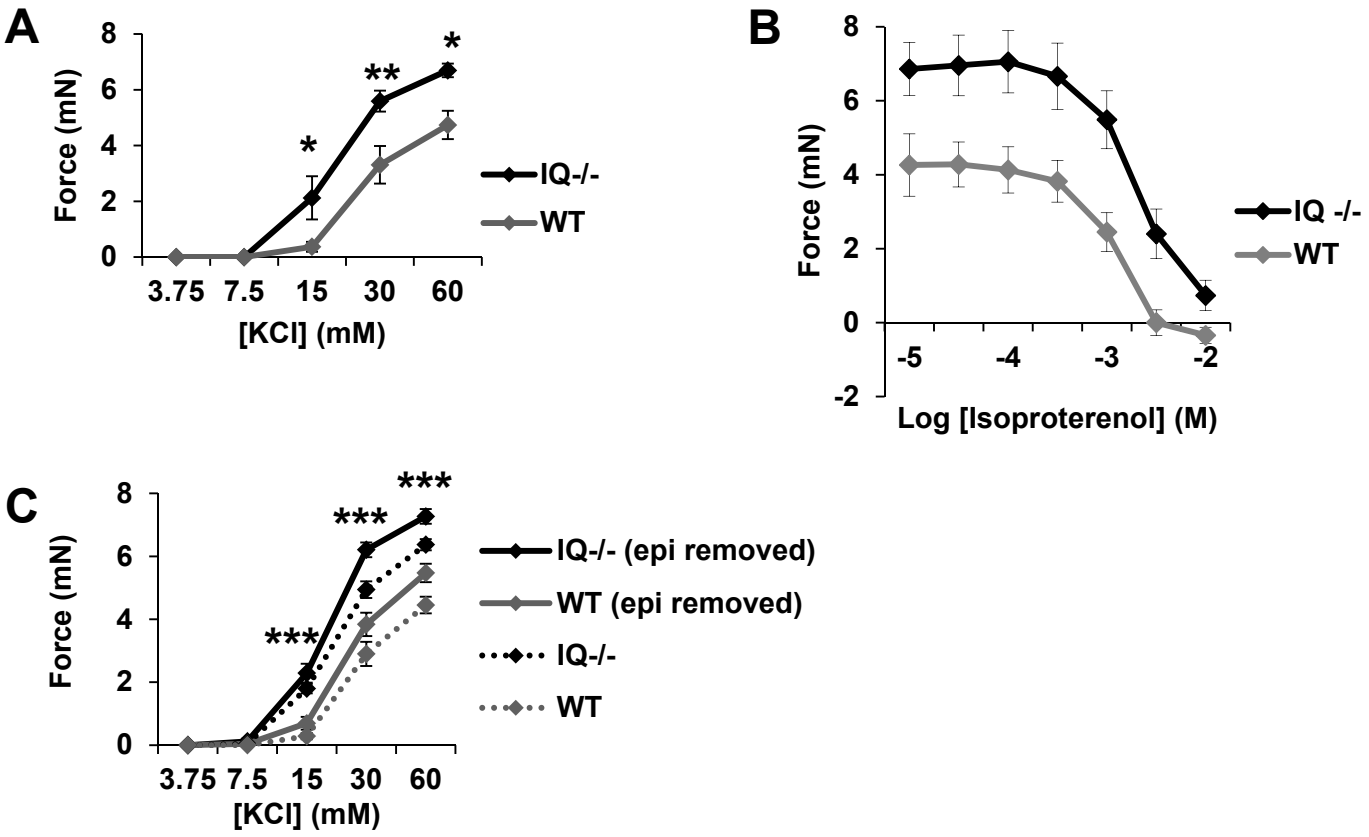
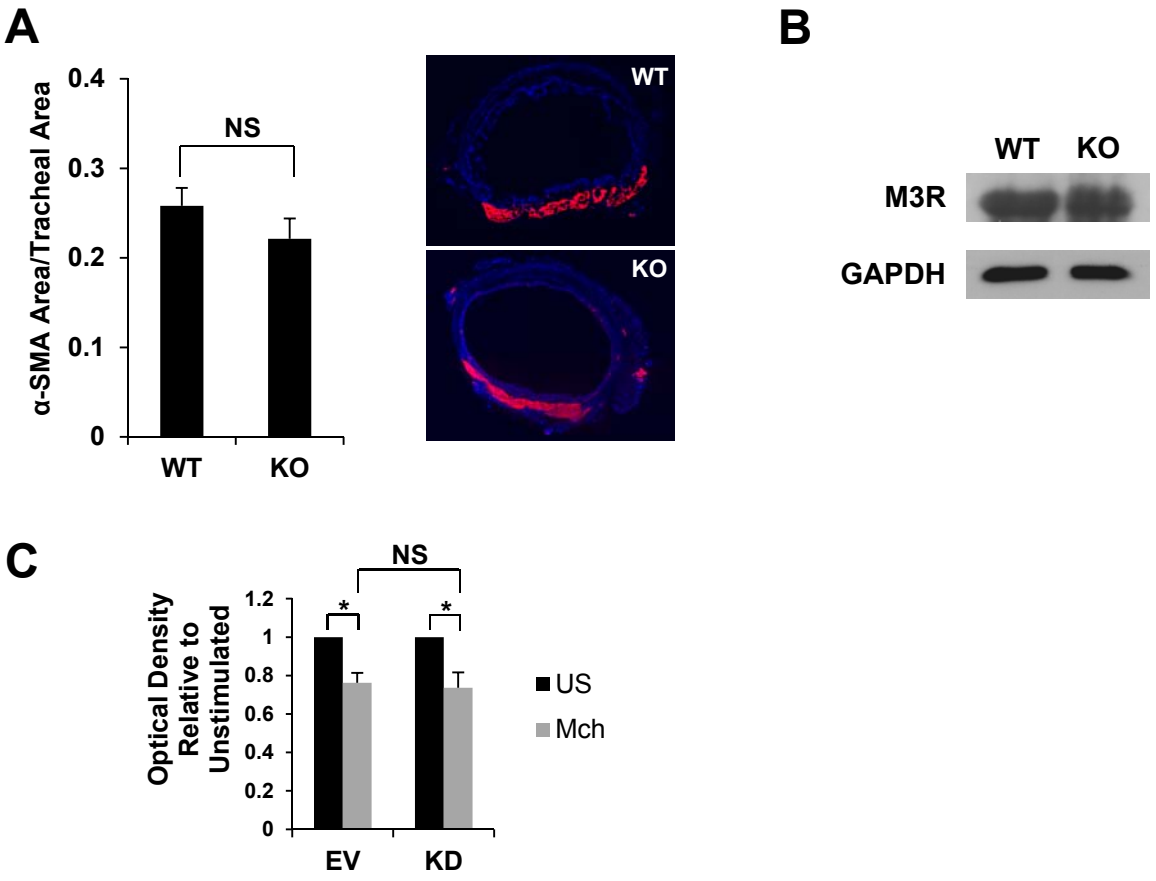
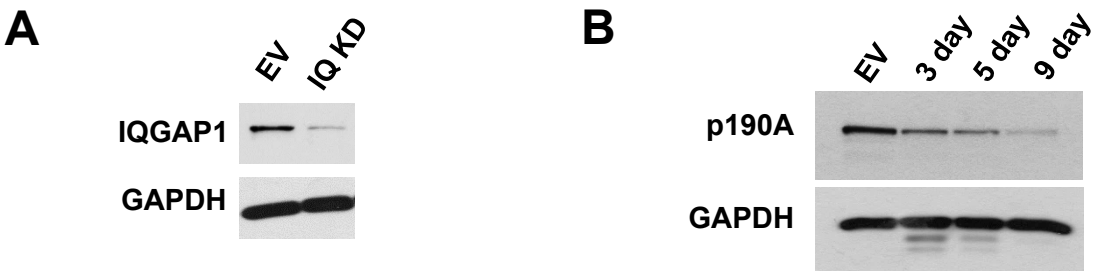


Supplemental Figure 1. (A) Force of contraction was measured for tracheal rings in response to potassium chloride (KCl). Data are mean ± SEM. *n* = 6 per group. *p≤0.05, **p≤0.01, ***p≤0.001 for comparisons of KO versus wild type. (B) Force of contraction was measured in tracheal rings pre-contracted with 10^{-4} M methacholine followed by increasing doses of isoproterenol. Data are mean ± SEM. *n* = 5 per group. (C) Force of contraction in response to KCl was measured for tracheal rings with and without removal of the epithelium (*n* = 6 per group). Data are mean ± SEM. *n* = 6 per group. *p≤0.05, **p≤0.01, ***p≤0.001 for comparisons of KO versus wild type.



Supplemental Figure 2. (A) Tracheal sections were stained with alpha smooth muscle actin antibody (α-SMA; red) and DAPI (blue). α-SMA was divided by total trachea area marked by DAPI stain for each section and plotted as a quotient (*n* = 4 per group). Two representative photomicrographs are shown (2.5x objective). NS=nonsignificant. (B) Western blot for M3 muscarinic receptor (M3R) in mouse posterior tracheal lysates. A representative blot of three replicates is shown. (C) Human airway smooth muscle cells with (KD) and without (empty vector=EV) IQGAP1 knockdown were analyzed by IP1 accumulation assay. In the competitive ELISA, lower optical density (OD) represents higher levels of endogenous IP1 (US=unstimulated; Mch=methacholine). A quotient of OD for each condition divided by OD for a corresponding unstimulated condition was computed. Data are mean quotients ± SEM. *p≤0.05



Supplemental Figure 3. (A) shRNA knockdown of IQGAP1 was verified by Western blot with lysates of human airway smooth muscle cells (EV=empty vector). (B) Time course of shRNA knockdown of p190A-RhoGAP was done by Western blot using lysates of human airway smooth muscle cells. Results are representative of at least three individual experiments.

Methods Supplement (page 1/3)

Antibodies. Mouse and rabbit anti-IQGAP1 antibodies were purchased from Millipore (clone AF4) and Santa Cruz Biotechnology. Mouse anti-RhoA antibody was purchased from Cytoskeleton (catalogue number ARH03). Rabbit IgG was purchased from Millipore (catalogue number 12-370). Rabbit phospho-myosin light chain, myosin light chain, phospho-myosin light chain phosphatase 1, p190A-RhoGAP, and GAPDH antibodies were purchased from Cell Signaling Technology. Goat anti-p190A-RhoGAP and rabbit anti-M3 muscarinic receptor antibodies were purchased from Santa Cruz Biosciences (cat no. 131536). Mouse anti-α-smooth muscle actin antibody was purchased from Sigma (clone 1A4).

Cells. Human airway smooth muscle cells and media were purchased from Lonza, cultured according to the vendor's instructions, and used between passage 5 and 10.

Mice. Mice functionally deficient in IQGAP1 have been previously characterized (1). Mice used for all the experiments were 6-8 weeks old and housed under specific pathogen-free conditions in the Animal Barrier Facility at UCSF.

Sensitization and challenge. Mice were sensitized on days 0, 7, and 14 by intraperitoneal injection of 50 μg OVA/1 mg alum in a total of 200 μl saline. Control mice received an equal volume of alum only. Mice were lightly anesthetized with isoflurane inhalation and challenged with intranasal OVA (100 μg in 30 μl saline) or saline on days 21–23.

Measurement of airway response to acetylcholine. 24 hours after the last challenge, pulmonary resistance was measured in response to a range of concentrations of intravenous acetylcholine using the forced oscillation technique with the FlexiVent system (SCIREQ) as previously described (2).

Determination of OVA specific IgE. Serum samples were analyzed for OVA-specific IgE using an ELISA. Briefly, wells were coated with OVA (Sigma-Aldrich), and a biotinylated IgE antibody (BD Biosciences, clone R35-118) was used to analyze blood samples for IgE.

Assessment of pulmonary inflammation and mucus production. Total and differential cell percentages were determined by hemocytometer and by light microscopic evaluation of more than 300 cells per slide as previously described (2). After lavage, lungs were inflated with 10% buffered formalin to 25 cm H_2O and transferred to 10% buffered formalin. 5-μm sections were stained with H&E for semiquantitative assessment of inflammation and periodic acid–Schiff reagent (PAS) for evaluation of mucus production. To quantify inflammation, H&E-stained lung sections were de-identified for blinding and scored for peribronchial and perivascular inflammatory cell infiltration: grade 0, no infiltration; grade 1, <25% of examined area; grade 2, 25-50%; grade 3, 51-75%; and grade 4, >75%. To quantify goblet cell hyperplasia, PAS stained lung sections were de-identified for blinding and scored for the percentage of PAS positive cells among airway epithelial cells: grade 0: none; grade 1 <25% of airway epithelial cells; grade 2, 25-50%; grade 3, 51-75%; and grade 4, >75%.

Measurement of tracheal smooth muscle contractility. Tracheal ring contraction studies were performed as described previously (3). In some cases, the epithelium was first debrided mechanically with a p10 catheter (BD Biosciences) abraded with 60 grit sandpaper, as previously described (3). Epithelial removal was confirmed for each individual ring by microscopic inspection of H&E-stained sections. For relaxation studies, rings precontracted with 10^{-6} M methacholine were treated with increasing doses of isoproterenol (Sigma).

Methods Supplement (page 2/3)

Assessment of RhoA Activation. The RhoA activation assay was performed according to the manufacturer's instructions (Cytoskeleton). Briefly, smooth muscle dissected from posterior mouse trachea was homogenized in lysis buffer (50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 0.5 M NaCl, 1% Triton X-100, and protease and phosphatase inhibitor cocktail (Thermo)). The supernatants of these or lysates of cultured smooth muscle cells were collected after centrifugation and incubated with GST-rhotekin bound to glutathione-agarose beads at 4°C for 1 h. The beads were washed with a wash buffer containing 25 mM Tris, pH 7.5, 30 mM $MgCl_2$, and 40 mM NaCl. GTP-bound RhoA was detected by immunoblot.

Cell Transfection. Human airway smooth muscle cells were transfected with wild type RhoA-flag (Addgene) or GFP (PSicoR) by electroporation using Basic Nucleofector Kit for Primary Smooth Muscle Cells (Lonza) according to the manufacturer's instructions using Amaxa Nucleofector II device (program A-033) (Amaxa Biosystems, Cologne, Germany).

Immunoblots. Smooth muscle dissected from mouse tracheas or cultured cells were homogenized in lysis buffer (50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 150 mM NaCl, 1% Triton X-100, 10 mM NaF, 1 mM Na_3VO_4) with protease and phosphatase inhibitor cocktail (Thermo). Lysates were resolved by SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Millipore). Membranes were blocked for 1 h with 5% skim milk or 5% BSA in tris buffered saline with tween-20, incubated at room temperature for 2 h with primary antibodies, washed in tris buffered saline with tween-20, incubated for 1 h with peroxidase-conjugated secondary antibody, washed in tris buffered saline with tween-20, and developed with ECL plus (Perkin Elmer) prior to exposure to film (Denville Scientific).

Immunoprecipitation. Human airway smooth muscle cells were lysed in 1% Triton X-100, 25 mM Tris·HCl, 125 mM NaCl, 10 mM EDTA, and protease and phosphatase inhibitor cocktail (Thermo) and incubated with IQGAP1 antibody (Millipore) or p190ARhoGAP antibody (Cell Signaling) for 1 hour at 4°C with rotation (70 rpm). Protein G sepharose beads were then added with incubation for a further 1 hour. Samples were washed four times with 1 mL of lysis buffer and eluted with reducing sample buffer, resolved by SDS-PAGE, and analyzed by immunoblot.

Tracheal Immunofluorescence. Mouse trachea were dissected and embedded in OCT. 10 uM sections were prepared using Leica Cryostat CM1850. The sections were fixed with 1% paraformaldehyde, permeabilized with 0.3% triton X-100, blocked with 10% serum, and incubated with primary antibody overnight. This was followed by PBS washes, incubation with the appropriate secondary antibody for 1 hour, and PBS washes. Sections were then mounted in mounting medium with DAPI and visualized with a Leica DM5000B epifluorescence microscope.

Proximity Ligation* Assay *(PLA). Duolink™ proximity ligation assay (Sigma) was performed according to the manufacturer's instructions. Human airway smooth muscle cells cultured on gelatin-coated glass coverslips were fixed with 4% paraformaldehyde, permeabilized with 0.5% triton X-100, and blocked with 10% goat serum (Jackson) and 1% BSA (Sigma) in PBS. The coverslips or fixed tracheal sections were incubated with two primary antibodies (rabbit and mouse antibodies each specific to one of the two targets) at room temperature for 1 hour, followed by PBS wash. PLA probes were then incubated for 1 hour at room temperature followed by wash with Buffer A (0.01 M Tris, 0.15 M NaCl, 0.05% Tween 20, pH=7.40). A ligase reaction was performed at 37º for 30 minutes, followed by wash with Buffer A. Polymerase reaction was performed with fluorophore containing buffer at 37º for 100 minutes followed by wash in Buffer B (0.2 M Tris and 0.1 M NaCl, pH=7.5) and then 1:100 dilution of Buffer B in water. Coverslips were mounted in Duolink mounting medium with DAPI and visualized with a Leica DM5000B epifluorescence microscope.

Methods Supplement (page 3/3)

Lentiviral shRNA Knockdown. PsicoOligomaker 1.5 (web.mit.edu/jacks-lab) was used to select short hairpin RNA (shRNA) targets from the 3′ untranslated region of human p190A-RhoGAP mRNA and to generate DNA oligomers for annealing and ligation into the lentiviral expression vector PSicoR (Addgene) followed by lentivirus production by the University of California, San Francisco (UCSF) Lentiviral RNAi Core. Human airway smooth muscle cells were inoculated and cultured for 10 days for adequate knockdown prior to use in experiments. The p190A-RhoGAP 3' untranslated region target GGAAGAAACCACAGAAAGA was selected for functional experiments based on efficient knockdown by Western blot. The IQGAP1 3′ untranslated region target GCAAAGACCTAGCCAACAA was employed as before (4).

IP1 Accumulation Assay. IP1 accumulation assay (Cisbio) was performed according to the manufacturer's instructions. Human airway smooth muscle cells lentivirally infected for shRNA knockdown of IQGAP1 or with empty vector were cultured for 24 hours followed by treatment in some cases with 10^{-4} M methacholine. Cells were lysed and analyzed by ELISA.

Airway Biopsies from Human Subjects. Bronchoscopy with endobronchial biopsy was performed in asthmatic and healthy control subjects as part of a study of the effects of short-term ozone exposure on airway inflammation at UCSF Human Exposure Laboratory, as previously described (5). All subjects were recruited by public advertisement and included if they were nonsmokers and were able to perform moderately strenuous exercise. Asthmatic subjects were defined by self-report of physician-diagnosed asthma and airway hyperresponsiveness to inhaled methacholine (provocative concentration of methacholine resulting in a 20% decrease in FEV_1 compared with baseline [PC_{20}] ≤8.0 mg/ml). Healthy control subjects were defined by no history of asthma and a PC_{20} >8.0 mg/ml to inhaled methacholine. Exclusion criteria included history of cardiac or pulmonary diseases other than asthma or any history of autoimmune disorders. Subjects were excluded if they received any oral steroid medications in the previous 3 months or inhaled steroids in the 2 weeks before testing. Endobronchial biopsy specimens were embedded in OCT compound, frozen in a dry ice/ethanol bath, and stored at −80°C.

Ozone Exposure Methods. Ozone exposure took place in a chamber ventilated with filtered air at 20°C and 50% relative humidity to which ozone was added, as previously described (5). Briefly, the experimental protocol involved exposure to 200 ppb ozone for 4 h. The subjects exercised for first 30 min of each hour to achieve a target minute ventilation of 25 L/min/m^2 of body surface area, and then rested for the remaining 30 min of the hour. Subjects remained inside the chamber for the entire 4-h exposure period. The subjects were then discharged home and returned 18 hours after the end of exposure on the following day to undergo the bronchoscopy procedures.

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