Supporting Information

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SI Methods

Collection of Bronchoalvelolar Lavage (BAL) Fluid and Leukocyte Analysis. After completion of the airway physiology measurements BAL was performed by cannulation of the trachea and gentle instillation/aspiration (3 times) of 1.0 mL PBS with 0.1% BSA and protease inhibitor (Roche). The lavage fluid was centrifuged and the supernatant was stored at -80 °C for later assessment of cytokines, chemokines, and neuropeptides. The cell pellet was treated with red blood cell lysis buffer (BD Biosciences), washed and resuspended in 200 μL PBS. Total cell counts were determined with a hematology analyzer (Scil Vet ABC). Cells were centrifuged onto cytoslides (Cytospin 3, Shandon Inc.) and stained with Diff-Quick (Dade-Behring Inc.). Differential cell counts were obtained by microscopic counting of a minimum of 200 cells/slide using standard morphological and staining criteria.

Lung Histology and Inflammation Scoring. Following lavage, lungs were prepared for histology by perfusing the animal via the right ventricle with 20 mL PBS. Lungs were then inflated with 1.0 ml of 10% buffered formalin through a tracheostomy tube and immersed in 10% formalin for at least 24 h. Fixed lungs were embedded in paraffin, sectioned at 5-μm thickness, and stained with hematoxylin and eosin (H&E). For scoring of inflammation, numbers of eosin-stained inflammatory cells in areas around 4 randomly picked bronchovascular bundles were counted. Cell counts were divided by area size and averaged for 4 mice per group each. Images were digitally photographed at 40× magnification (Olympus BX41, Canon Powershot A80, National Institutes of Health ImageJ software).

EIA Assay for OVA-specific IgE. OVA-specific IgE levels were determined by ELISA using microplates coated with OVA and blocked with BSA. Diluted serum samples obtained from blood collected by cardiac puncture (after airway responsiveness measurements and BALF collection) were added to each well. Bound IgE was detected with biotinylated anti-mouse IgE (R35–118) and developed using streptavidin-conjugated horseradish peroxidase and TMB as a substrate (all reagents from BD Biosciences PharMingen). The absorbance was measured at 450 nm.

Quantitative Real-time PCR. Lungs and DRGs were surgical removed and white cells were collected from BAL by centrifugation. CD4 T_h2 cells were purified as previously described (1). Tissue and cells were frozen in liquid nitrogen. Total RNA was isolated from tissue homogenates using RNeasy Mini Kit 50 (Qiagen). cDNA synthesis was performed with the High Capacity RNA-to-cDNA Kit (Applied Biosystems). Quantitative PCR was performed using TaqMan Gene Expression Assays (Applied Biosystems). Typically, 20-μL reactions contained 10 μL TaqMan Fast Universal PCR Master Mix (2×), 1 μL of the specific TaqMan assay, 1 μL cDNA, and water to 20 μL. Cycling parameters were 52 °C for 2 min for activation and 10 min initial setup at 95 °C, followed by 40 cycles of 95 °C for 15 s and 1 min at 60 °C (ABI 7500 Fast, Applied Biosystems). Each sample was run in triplicate and normalized to GADPH gene expression. C_T values were determined using ABI PRISM software and averaged. Relative quantification was determined by the $\Delta\Delta C_T$ method (2). The TaqMan Gene Expression Assays included specific primers and FAM/MGB probes for mGAPDH [endogenous Control (4352932E)], mTRPA1 (Mm00625268_m1), mMucin 5 subtypes A and C (Mm01276735_m1), and mIL-5 (Mm99999063_m1).

Data Analysis and Statistics. Data were analyzed using Origin 8 (OriginLab Corp.) and SPSS (SPSS Inc.) software. Cell differentials, qPCR, IgE and cytokine data were analyzed by Student's *t*-test for independent samples. Airway forced oscillation data were analyzed by repeated-measures ANOVA, followed by Bonferroni post-hoc analysis. CN-induced neuropeptide release was analyzed using ANOVA followed by Bonferroni (for CGRP) or Fisher LSD (SP and NKA) post-hoc tests. NKA-levels in BAL of naïve and OVA-treated mice were analyzed using ANOVA followed by Bonferroni post-hoc test. Error bars, SEM.

1. Niu N, et al. (2007) A novel pathway that regulates inflammatory disease in the respiratory tract. *J Immunol* 178:3846–3855.

2. Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc* 3:1101–1108.

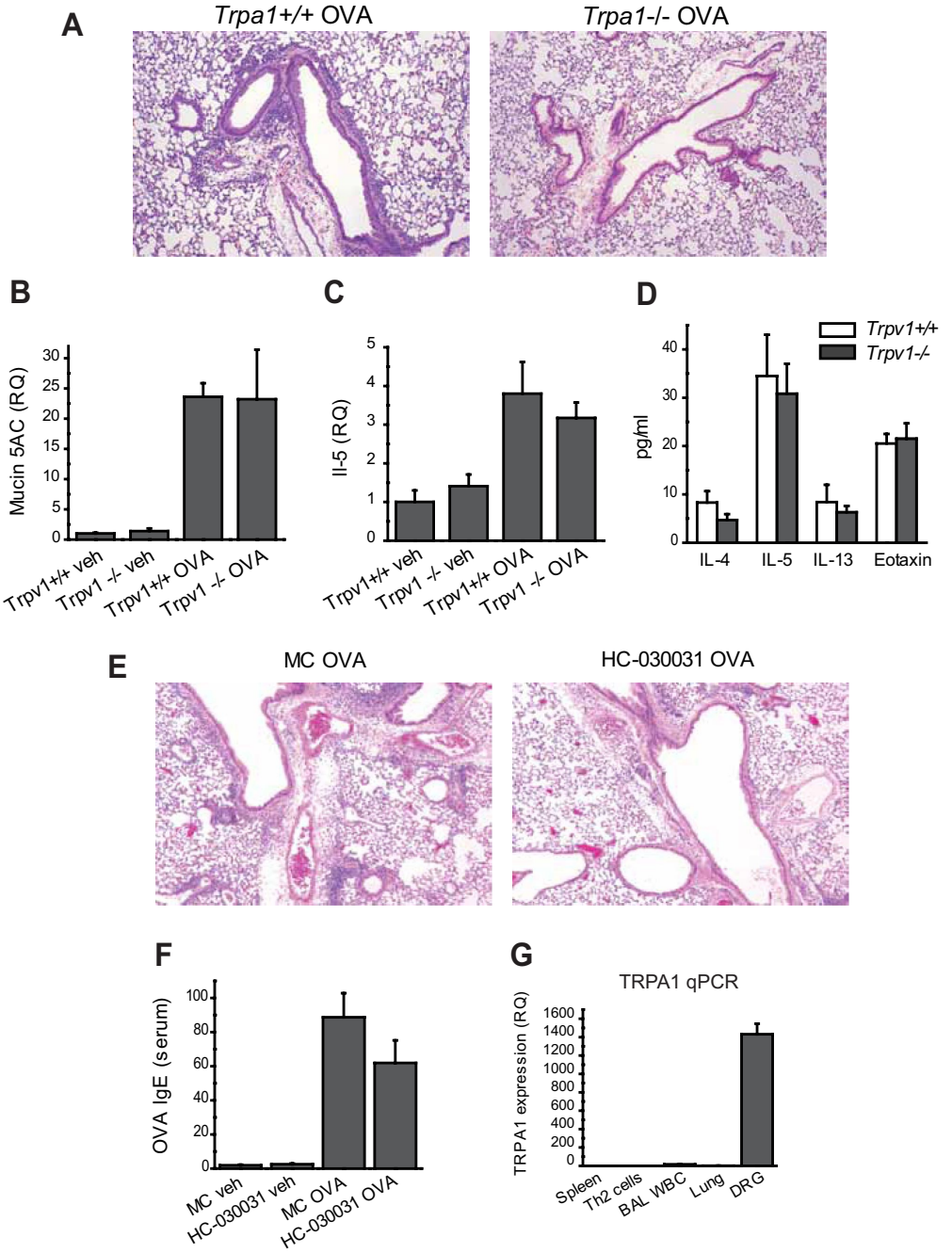

Fig. S1. (*A*) Marked infiltration of inflammatory leukocytes (purple) and thickened goblet cell layers in H&E-stained lung sections of OVA-challenged wild-type mice (left). In contrast, sections of OVA-challenged Trpa1-/- mice showed little leukocyte infiltration and less goblet cell hyperplasia (right) (magnification 20×). (*B*) Comparison of relative quantities (RQ) of mucin5ac gene transcript in wild-type and TRPV1-deficient mice, as determined by Taqman real-time quantitative PCR of whole mouse lung cDNA. OVA-challenged wild-type mice (Trpv1+/+ OVA) show strong induction of mucin5ac transcription. Mucin5ac induction is similarly elevated in in Trpv1-/- OVA mice. GAPDH transcript levels were used for normalization as endogenous control ($n = 4$ mice/group). (*C*) Relative quantities (RQ) of interleukin 5 (IL-5) gene transcript, as determined by Taqman real-time quantitative PCR of whole mouse lung cDNA. OVA-challenged Trpv1-/- mice show similarly strong increases in IL-5 gene transcription than wild-type mice. GAPDH transcript levels were used for normalization as endogenous control ($n = 4$ mice/group). (*D*) Similar peptide concentrations of T_h2 cytokines, IL-4, IL-5, IL-13, and eotaxin in bronchoalveolar lavage fluid (BALF) of OVA-challenged wild-type and Trpv1-/- mice ($n = 4$ mice/group). (*E*) Histological analysis of lung sections stained with H&E showing decreased eosinophilic inflammation in mice treated with the TRPA1 antagonist HC-030031 (right) compared with mice treated with the vehicle methyl cellulose (MC) (left) (magnification 20×). (*F*) IgE levels in blood serum collected from PBS- and OVA-challenged mice treated with HC-030031 or methyl cellulose (MC). Mouse groups as in Fig. 3*A*. (*G*) Averaged relative quantities (RQ) of Trpa1 gene transcript, examined by Taqman real time quantitative PCR of whole mouse spleen cDNA, purified Th2 lymphocyte cDNA, lung cDNA, and BAL white blood cell cDNA compared with dorsal root ganglia cDNA (DRG) ($n = 3–6$ mice per group).