Supporting Information

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SI Text

Endotoxin-Evoked Inflammatory Changes and Basal Airway Responsiveness. When analyzing the morphologic score values separately, although there were more infiltrating mononuclear cells in the knockouts ($sst_4^{-/-}$: 1.78 ± 0.11 vs. $sst_4^{+/+}$: 1.33 ± 0.21), the difference was not significant. The scores for the other 3 parameters, peribronchiolar/perivascular edema formation (2.11 ± 0.09 vs. 1.50 ± 0.24; $P = 0.032$), peribronchiolar granulocyte accumulation (2.25 ± 0.15 vs. 1.83 ± 0.16; $P = 0.041$), and goblet cell hyperplasia (1.5 ± 0.13 vs. 0.33 ± 0.12; $P = 0.016$) were significantly greater in mice lacking the sst_4 receptor.

Both baseline Penh and RL significantly increased after intranasal LPS instillation compared with PBS-treated, noninflamed control values ($sst_4^{+/+}$: 0.73 ± 0.1 arbitrary units vs. 4.83 ± 0.1 arbitrary units for Penh and 0.093 ± 0.005 vs. 0.143 ± 0.012 ΔcmH_2O * sec/mL for RL; $sst_4^{-/-}$: 0.74 ± 0.1 arbitrary units vs. 4.26 ± 0.5 arbitrary units for Penh and 0.092 ± 0.005 vs. 0.144 ± 0.012 ΔcmH_2O * sec/mL for RL), with no difference between the 2 mouse groups.

Direct Measurement of Airway Resistance of Anesthetized Mice. The stainless 18G tracheostomy cannula was passed through a hole in the Buxco whole-body plethysmograph. A connector was attached to this tube, with 2 ports connected to the inspiratory and expiratory sides of a ventilator (MiniVent Type 845; Hugo Sachs Electronik-Harvard Apparatus). Ventilation was achieved at 150 breaths per minute and a V_T of 0.2 mL with a positive end-expiratory pressure of 2–4 cm H_2O. The flow was measured by digital differentiation of the volume signal, and RL was continuously computed by fitting flow, volume, and pressure to an equation of motion using a recessive least squares algorithm (1, 2). Aerosolized carbachol was administered through bypass tubing, following a similar protocol described for the unrestrained measurement.

Measurement of MPO Activity and Cytokine Concentrations in the Lung. Lung pieces were homogenized with a polytrone homogenizer (Kika Lab Techniques) in 4 mL 20 mM potassium–phosphate buffer (pH 7.4) and centrifuged at 10,000 × *g* at 4 °C for 10 min. The pellet was resuspended in 4 mL 50 mM potassium–phosphate buffer containing 0.5% hexadecyltrimethyl-ammonium-bromide (pH 6.0) and centrifuged again. MPO enzyme activity of the samples was compared with a human standard MPO preparation (Sigma) using H_2O_2-3,3≪,5,5≪-tetramethyl-benzidine (Sigma). The optical density was measured with a microplate reader (Labsystems) at 620 nm, plotted, and MPO activity determined with the help of a calibration curve.

For cytokine measurements the lung samples were homogenized in 500 μL RPMI-1640 buffer (Biochrom) containing 50 μL PMSF (Sigma-Aldrich) at 11,000 × g for 2 min. The homogenates were centrifuged for 10 min at 8,944 × g and 4 °C.

Determination of Accumulated Inflammatory Cells and Cytokines from the BALF. The trachea of anesthetized $sst_4^{+/+}$ and $sst_4^{-/-}$ littermate mice was cannulated, and lavage was performed 4 times with 0.8 mL ice-cold PBS each time through the cannula. The retrieved BALF aliquots were pooled, their volume was measured, and centrifugation was performed (3). The pellet was resuspended in 0.5 mL staining buffer containing antimouse CD45 FITC leukocyte marker and propidium iodine to stain nonviable cells. After an incubation period of 30 min, the samples were washed twice and the number of inflammatory cells (granulocytes, lymphocytes, and macrophages) determined with a Partec CyFlow space flow cytometer. Differentiation was made on the basis of the size and granulation; cell numbers were calculated by FloMax software (Partec). The supernatants were used for ELISA measurement of 3 inflammatory cytokine (IL-1β, TNF-α, and IFN-γ) concentrations.

Measurement of Inflammatory Cytokines in the Ear. Changes of cytokine profile during delayed-type hypersensitivity reaction were determined by mouse cytokine cytometric bead array (Becton Dickinson Biosciences). Ears were homogenized in 1 mL RPMI-1640 medium (Sigma-Aldrich) and 1% PMSF (Sigma-Aldrich) mixture at 4 °C and centrifuged at 10,000 × *g* for 10 min at 4 °C. The cytometric bead array kit contains 5 bead populations with distinct fluorescent intensities that have been coated with capture antibodies specific for IL-2, IL-4, IL-5, IFN-γ, and TNF-α proteins. Fifty microliters of mixed capture beads, 50 μL ear sample, and 50 μL phycoerythrin-labeled detection antibody solution were added into an assay tube and counted by flow cytometry. Data were evaluated by FCS Express software, version 3 (De Novo Software). IL-1β was measured with ELISA as described above for the lung samples.

1. Helyes Z, et al. (2007) Role of transient receptor potential vanilloid 1 receptors in endotoxin-induced airway inflammation in the mouse. *Am J Physiol Lung Cell Mol Physiol* 292:L1173–L1181.
2. Elekes K, et al. (2007) Role of capsaicin-sensitive afferents and sensory neuropeptides in endotoxin-induced airway inflammation and consequent bronchial hyperreactivity in the mouse. *Regul Pept* 141:44–54.
3. Elekes K, et al. (2008) Inhibitory effects of synthetic somatostatin receptor subtype 4 agonists on acute and chronic airway inflammation and hyperreactivity in the mouse. *Eur J Pharmacol* 578:313–322.

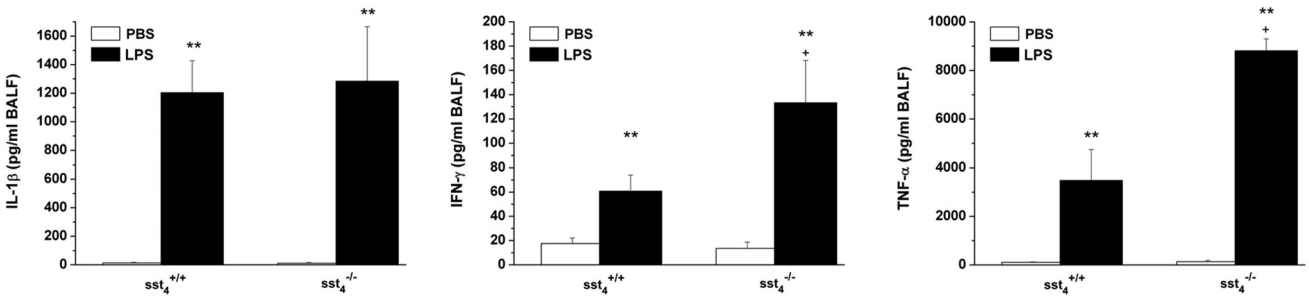

Fig. S1. Inflammatory cytokine concentrations in the BALF 24 h after intranasal endotoxin administration in $sst_4^{+/+}$ and $sst_4^{-/-}$ littermate mice. Columns show means ± SEM of $n = 6$ mice per group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the respective PBS-treated mice; $^{+}P < 0.05$, $^{++}P < 0.01$ vs. the $sst_4^{+/+}$ group (one-way ANOVA plus Bonferroni's *t* test).

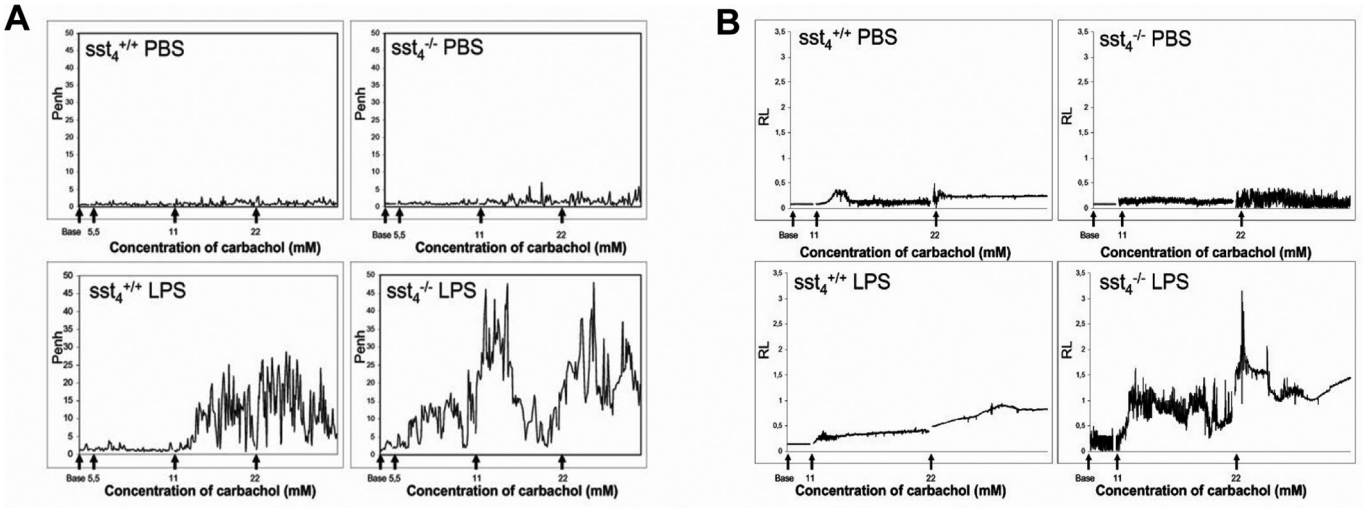

Fig. S2. Representative original (*A*) Penh and (*B*) RL recordings of intact and intranasal endotoxin-treated $sst_4^{+/+}$ and $sst_4^{-/-}$ mice, obtained with whole-body plethysmography in freely moving, unrestrained mice and anesthetized, tracheotomized mice, respectively. Aerosolized saline (baseline) and increasing concentrations of carbachol were nebulized into the chamber for 50 sec to induce bronchoconstriction 24 h after LPS (or PBS) administration.

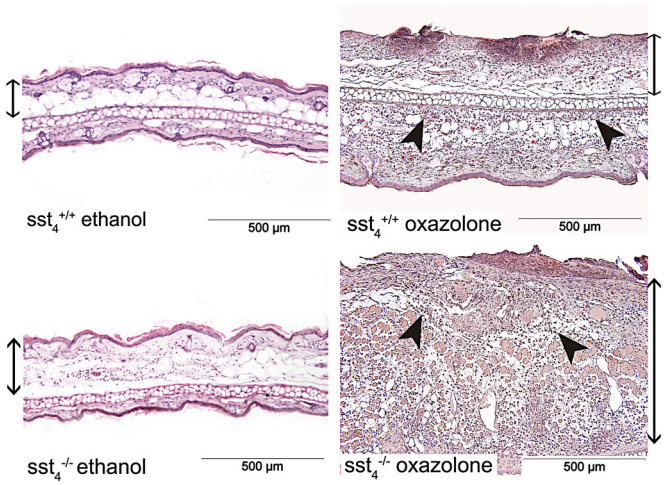

Fig. S3. Representative light micrographs (hematoxylin and eosin staining, ×200) showing morphologic features of allergic contact dermatitis 48 h after oxazolone smearing on the ears of sensitized $sst_4^{+/+}$ and $sst_4^{-/-}$ mice (vertical arrows show the extent of the ear swelling, arrowheads point to the accumulated inflammatory cells).