

## Supplementary Materials for

### **The cysteinyl leukotriene 3 receptor regulates expansion of IL-25–producing airway brush cells leading to type 2 inflammation**

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#### **The PDF file includes:**

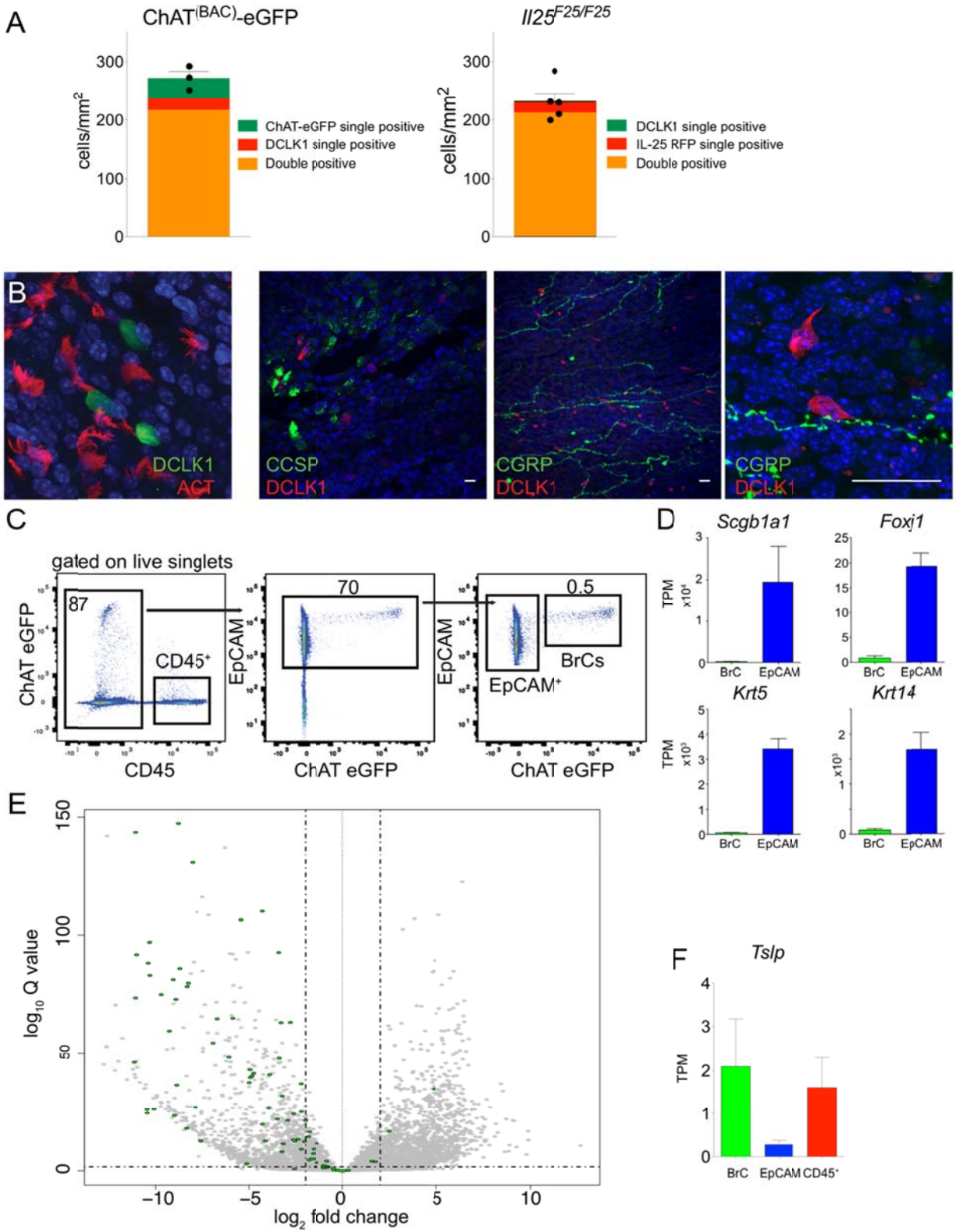
Fig. S1. Histological and flow cytometrical characterization of BrCs.  
Fig. S2. Colocalization of X-gal and DCLK1 in whole tracheal mounts.  
Fig. S3. Flow cytometry data and gating strategy for eosinophils, ILC2s, CD4 T cells, macrophages, and DCs.  
Fig. S4. LTE<sub>4</sub>-elicited airway inflammation is STAT6-dependent.  
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Table S1. Reagents.

#### **Other Supplementary Material for this manuscript includes the following:**

(available at [immunology.sciencemag.org/cgi/content/full/3/28/eaat9453/DC1](http://immunology.sciencemag.org/cgi/content/full/3/28/eaat9453/DC1))

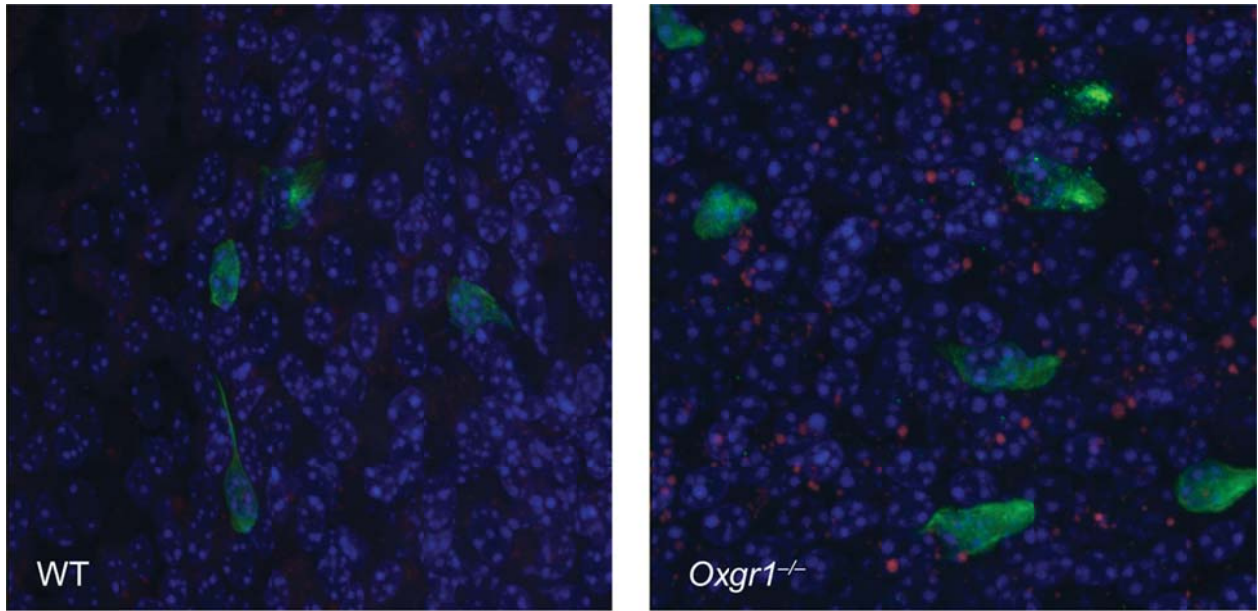
Table S2. Raw data (Excel file).

## Supplementary Materials

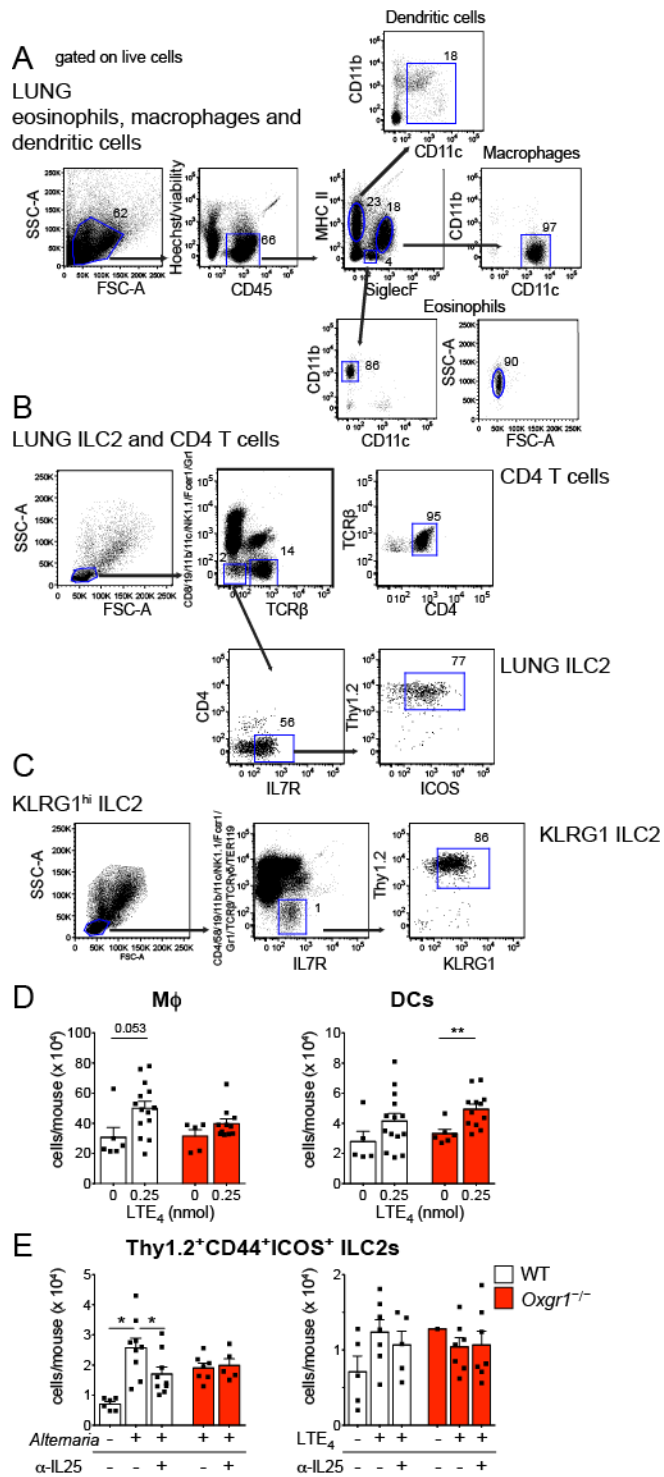


**Fig. S1. Histological and flow cytometrical characterization of BrCs.**

(A) Quantitation of subsets of BrCs expressing DCLK1, ChAT<sup>BAC</sup>-eGFP mice and IL-25 RFP in ChAT<sup>(BAC)</sup>-eGFP mice and *Il25*<sup>F25/F25</sup> mice, respectively. The number of single and double positive was counted in unchallenged mice. (B) Immunofluorescence demonstrating lack of co-expression between DCLK1 and either acetylated tubulin (ACT, marking ciliated cells), Clara cell secretory protein (CCSP, marking Clara/club cells) or calcitonin gene-related peptide (CGRP, marking both neuroendocrine cells and peptidergic neurons). CGRP-positive nerve endings are closely intertwined with DCLK1<sup>+</sup> cells (right panel). Nuclear staining with Hoechst 33342 (blue). Scale bar = 25 μm (C) Gating strategy for isolation of BrCs, EpCAM<sup>+</sup> EpCs and CD45<sup>+</sup> cells from single cell tracheal suspensions. (D) Selected EpC-specific genes differentially expressed in EpCAM<sup>+</sup> cells compared to BrCs. Y-axis indicates average of normalized transcripts per million (TPM). (E) Enrichment of intestinal tuft cells signature genes (marked in green) in tracheal BrCs (19) presented on a volcano plot generated as shown in Fig. 1E. Tracheal BrCs show increased expression for 74/94 intestinal tuft cell signature genes at a FDR < 0.05, 59 of which had a fold change > 4 (P=1.2e<sup>-54</sup>, hypergeometric cumulative distribution, upper tail). (F) TSLP expression in tracheal BrCs, EpCAM<sup>+</sup> EpCs and CD45<sup>+</sup> cells. Y-axis indicates average of normalized transcripts per million (TPM).



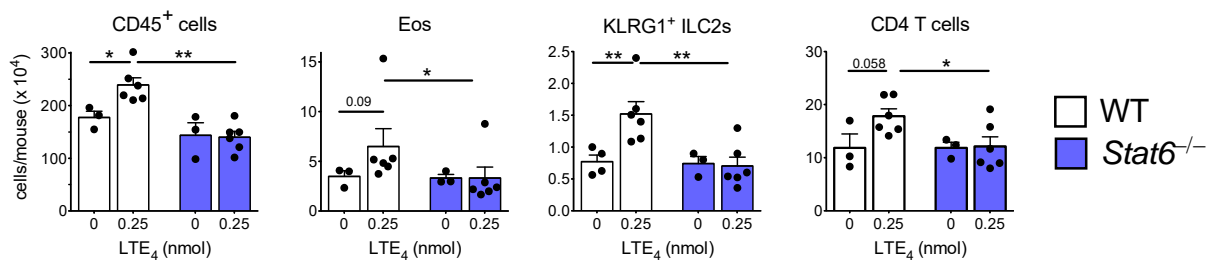
**Fig. S2. Colocalization of X-gal and DCLK1 in whole tracheal mounts.** Co-localization of X-gal reactivity (red) and DCLK1 (green) in tracheal whole mounts of naive *Oxgr1*<sup>-/-</sup> mice which carry the targeted insertion of *lacZ*, encoding *E. coli*  $\beta$ -galactosidase.



**Fig. S3. Flow cytometry data and gating strategy for eosinophils, ILC2s, CD4 T cells, macrophages, and DCs.** Recruitment and expansion of dendritic cells, macrophages, CD44<sup>+</sup>Thy1.2<sup>+</sup> ILC2 in response to LTE<sub>4</sub> and *Alternaria*.

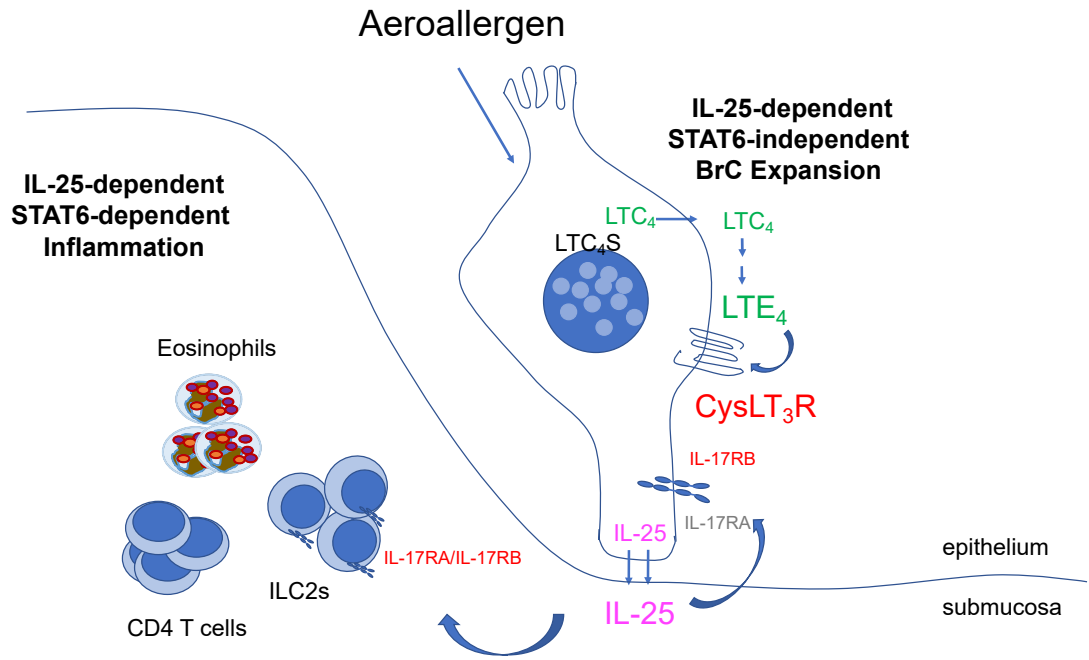
Representative gating for lung eosinophils, macrophages and dendritic cells (A), (B), KLRG1<sup>+</sup> ILC2 (C); (D) DCs (CD45<sup>+</sup>SiglecF<sup>-</sup>MHCII<sup>+</sup>CD11c<sup>+</sup>) and macrophages (CD45<sup>+</sup>SiglecF<sup>+</sup>MHCII<sup>+</sup>CD11b<sup>-</sup>CD11c<sup>+</sup>) in LTE<sub>4</sub>-challenged WT and *Oxgr1*<sup>-/-</sup> mice. (E) WT and *Oxgr1*<sup>-/-</sup> mice

were given 4 daily doses of 0 or 0.25 nmol intranasal LTE<sub>4</sub> or a single intranasal dose of *Alternaria* in conjunction with 100 µg of IL25 antibody or rat IgG1k on days 0 and 2 i.p. Flow cytometry analysis of ILC2s (CD45<sup>+</sup>lin<sup>-</sup>Thy1.2<sup>+</sup>CD44<sup>+</sup>ICOS<sup>+</sup>) 24 h after last LTE<sub>4</sub> dose or 3 days after *Alternaria*.



**Fig. S4. LTE<sub>4</sub>-elicited airway inflammation is STAT6-dependent.**

LTE<sub>4</sub>-elicited airway inflammation is STAT6-dependent. WT and *Stat6*<sup>-/-</sup> mice were given 4 daily doses of 0 or 0.25 nmol intranasal LTE<sub>4</sub> and analyzed 24 h after the last inhalation. Flow cytometry analysis of dispersed lung cells showing CD45<sup>+</sup> cells, eosinophils (CD45<sup>+</sup>SiglecF<sup>+</sup>CD11b<sup>+</sup>CD11c<sup>-</sup>SSC<sup>hi</sup>), ILC2s (CD45<sup>+</sup>lin<sup>-</sup>IL7R<sup>+</sup>KLRG1<sup>+</sup>), and CD4<sup>+</sup> T cells (CD45<sup>+</sup>TCRb<sup>+</sup>CD4<sup>+</sup>). Data are combined from two experiments.



**Fig. S5. IL-25–dependent BrC expansion and lung inflammation schema.** *Alternaria* and *Df* aeroallergens elicit  $LTC_4$  generation through the biosynthetic activity of  $LTC_4$  Synthase. After generation in activated hematopoietic cells or possibly airway BrCs,  $LTC_4$  is exported extracellularly and converted to  $LTE_4$ . Both endogenously generated and exogenously delivered  $LTE_4$  activate  $CysLT_3R$  to induce  $IL-25$ -dependent BrC expansion in the epithelium. BrC expansion is independent of the  $IL-13$ -elicited transcription factor  $STAT6$ , which may reflect an autocrine effect of  $LTE_4$  and  $IL-25$ . In the submucosa,  $IL-25$  activates  $ILC2s$  to generate  $IL-13$  and, signaling through  $STAT6$ , leads to both  $ILC2$  expansion and recruitment of eosinophils and  $CD4$  T cells.



**Table S1. Reagents.**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
Anti mouse/human DCLK1 (Polyclonal Rabbit Ig)	Abcam	cat# ab37994
Anti mouse/human DCLK1 (Polyclonal Rabbit Ig)	Abcam	cat# ab31704
Anti mouse Ga-gustducin (Polyclonal Rabbit Ig)	Santa Cruz Biotechnology	cat# sc-395
Anti mouse/human acetylated tubulin (Monoclonal Mouse Ig)	Santa Cruz Biotechnology	cat#sc-23950
CC-10 (clara cell secretory protein; T-18)	Santa Cruz Biotechnology	cat#sc-9772
Anti mouse/rat CGRP (Polyclonal goat Ig)	Abcam	cat# ab36001
Living Colors® DsRed Polyclonal Antibody	Takara Bio/Clontech	cat# 632496
Anti-GFP (Polyclonal goat Ig)	Abcam	cat# ab5450
Donkey serum	Jackson Immuno Research	Cat# 017-000-121
Rabbit serum	Thermo/Invitrogen	cat# ab31883
LEAF-purified anti-mouse IL-25	Biolegend	cat#514404
LEAF-purified rat IgG1k	Biolegend	cat#400427
Anti-mouse CD45 (30F-11) Pacific Blue	Biolegend	cat#103126
Anti-mouse KLRG1 (MAFA/2F1) APC	Biolegend	cat#138411
Anti-mouse CD4 (RM4-5) FITC	Biolegend	cat#130308
Anti-mouse CD8 (53-6.7) FITC	Biolegend	cat#100706
Anti-mouse CD19 (6D5) FITC	Biolegend	cat#115506
Anti-mouse CD11b (M1/70) FITC	Biolegend	cat#101206
Anti-mouse FcεRIα (MAR-1) FITC	Biolegend	cat#134306
Anti-mouse CD11c (N418) FITC	Biolegend	cat#117306
Anti-mouse NK1.1 (PK136) FITC	Biolegend	cat#108706
Anti-mouse Gr1 (RB6-8C5) FITC	Biolegend	cat#108406
Anti-mouse IL7R (A7R34) PE Cy7	Biolegend	cat#135014
Anti-mouse TCRb (H57-597) FITC	Biolegend	cat#109206
Anti-mouse TCRγδ (GL3) FITC	Biolegend	cat#118106
Anti-mouse CD44 (IM7) APC Cy7	Biolegend	cat#103028
Anti-mouse Thy1.2 (53-2.1) PerCP Cy5	Biolegend	cat#140322
Anti-mouse ICOS (15F9) FITC	Biolegend	cat#107706
Anti-mouse CD5 FITC	Biolegend	cat#100606

Anti-mouse TER119 FITC	Biolegend	cat#116206
Anti-mouse Siglec F (1RVM44N) eFluor 710	eBioscience	cat#46-1702-82
Anti-mouse I-A/I-E(M5/114.15.2) FITC	Biolegend	cat#107606
Anti-mouse CD11c (N418) APC	Biolegend	cat#117310
Anti-mouse/human CD11b (M1/70) APC Cy7	Biolegend	cat#101226
Anti-mouse TCRb (H57-597) APC Cy7	Biolegend	cat#109220
Anti-mouse CD4 (RM4-5) APC	Biolegend	cat#100516
Anti-mouse Epcam (G8.8)	Biolegend	cat#118214
donkey anti-rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 594	Life Technologies/Molecular Probes	cat#A-21207
donkey anti-rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Life Technologies/Molecular Probes	cat#A-21206
donkey anti-goat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Life Technologies/Molecular Probes	cat#A-11055
donkey anti-mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 594	Life Technologies/Molecular Probes	cat#A-21202
TruStain fcX anti-mouse CD16/32	Biolegend	cat# 101320
Chemicals, Peptides, and Recombinant Proteins		
LTE <sub>4</sub>	Cayman chemicals	cat#20410
Alternaria alternata culture filtrate (lot# 151774)	Greer Laboratories	cat#XPM1C3A25
Dermatophagoides farinae (lot# 245566)	Greer Laboratories	cat#XPB64D3A25
Mouse (Type 1) Oxgr1 ISH probe (NM 001001490)	Affymetrix	cat#VB1-11300-VT
Hoechst 33342	Sigma-Aldrich	cat#14533
X-gal	Sigma-Aldrich	cat#3117073001
Vectastain ABC-AP kit	Vector	cat#AK-5000
Collagenase IV	Worthington	cat#LS004188
DNase I	Sigma-Aldrich	cat# 10104159001
Dispase	Gibco	cat# 17105041
HEPES-Tyrode's Buffer Without Calcium	Boston BioProducts	cat# PY-912
Tyrode's Solution (HEPES-Buffered)	Boston BioProducts	cat# BSS-355
L-Cysteine	Sigma-Aldrich	cat# C7352
Papain from papaya latex	Sigma-Aldrich	cat# P3125
Leupeptin trifluoroacetate salt	Sigma-Aldrich	cat# L2023
Propidium iodide	Sigma-Aldrich	cat# P4170

Brilliant II SYBR Green QPCR Master Mix with Low ROX	Agilent Technologies	cat# 600830
TCL buffer	Qiagen	cat# 1031576
RNAprotect Cell Reagent	Qiagen	cat# 76526
Primers		
RT <sup>2</sup> qPCR Primer Assay for Mouse IL25	Qiagen	cat# PPM05427F
RT <sup>2</sup> qPCR Primer Assay for Mouse Oxgr1	Qiagen	cat# PPM31430A
RT <sup>2</sup> qPCR Primer Assay for Mouse Cysltr1	Qiagen	cat# PPM04827A
RT <sup>2</sup> qPCR Primer Assay for Mouse Gnat3	Qiagen	cat# PPM41042A
RT <sup>2</sup> qPCR Primer Assay for Mouse Pou2f3	Qiagen	cat# PPM 2520B
RT <sup>2</sup> qPCR Primer Assay for Mouse 18S	Qiagen	cat# PPM57735E
Primers for Mouse Cysltr2 cysLT2-gtS (sense); 5'- CACCGTCTGCTACCTGCTGATCAT-3' cysLT2-gtAS (anti-sense); 5'- GGCCTTATGTAGCACATCCCC-3'	Sigma-Aldrich	
Critical Commercial Assays		
viewRNA ISH Tissue 1-Plex assay kit	Affymetrix	cat#QVT0050
Experimental Models: Organisms/Strains		
Oxgr1 <sup>tm1(KOMP)Mlg</sup>	KOMP	VG10698
Ltc4s <sup>-/-</sup>	Yoshihide Kanaoka	N/A
B6.129S2(C)-Stat6< tm1Gru>/J	The Jackson Laboratory	002828
Chat <sup>BAC</sup> -eGFP (B6.Cg-Tg(RP23-268L19-EGFP)2Mik/J)	The Jackson Laboratory	007902
IL25F25/F25	Jakob von Moltke/Richard Locksley	(14)
Software and Algorithms		
FlowJo v.8	FlowJo, LLC	<a href="https://www.flowjo.com">https://www.flowjo.com</a>
Prism 5	GraphPad Software	<a href="https://www.graphpad.com/scientific-software/prism/">https://www.graphpad.com/scientific-software/prism/</a>
FIJI (ImageJ)	National Institutes of Health	<a href="https://imagej.nih.gov/ij/">https://imagej.nih.gov/ij/</a>
R and R studio		<a href="http://www.r-project.com/">http://www.r-project.com/</a>