

Accepted Manuscript

Basophil-derived interleukin-4 promotes epicutaneous antigen sensitization concomitant with the development of food allergy

Maryam Hussain, MSc, Loïc Borcard, MSc, Kevin P. Walsh, PhD, Maria Pena Rodriguez, BSc, Christoph Mueller, PhD, Brian S. Kim, MD, Masato Kubo, PhD, David Artis, PhD, Mario Noti, PhD

PII: S0091-6749(17)30566-3

DOI: [10.1016/j.jaci.2017.02.035](https://doi.org/10.1016/j.jaci.2017.02.035)

Reference: YMAI 12728

To appear in: *Journal of Allergy and Clinical Immunology*

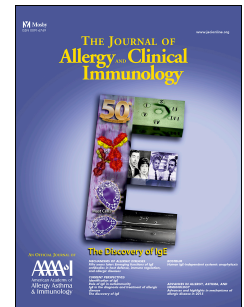
Received Date: 14 October 2016

Revised Date: 13 January 2017

Accepted Date: 13 February 2017

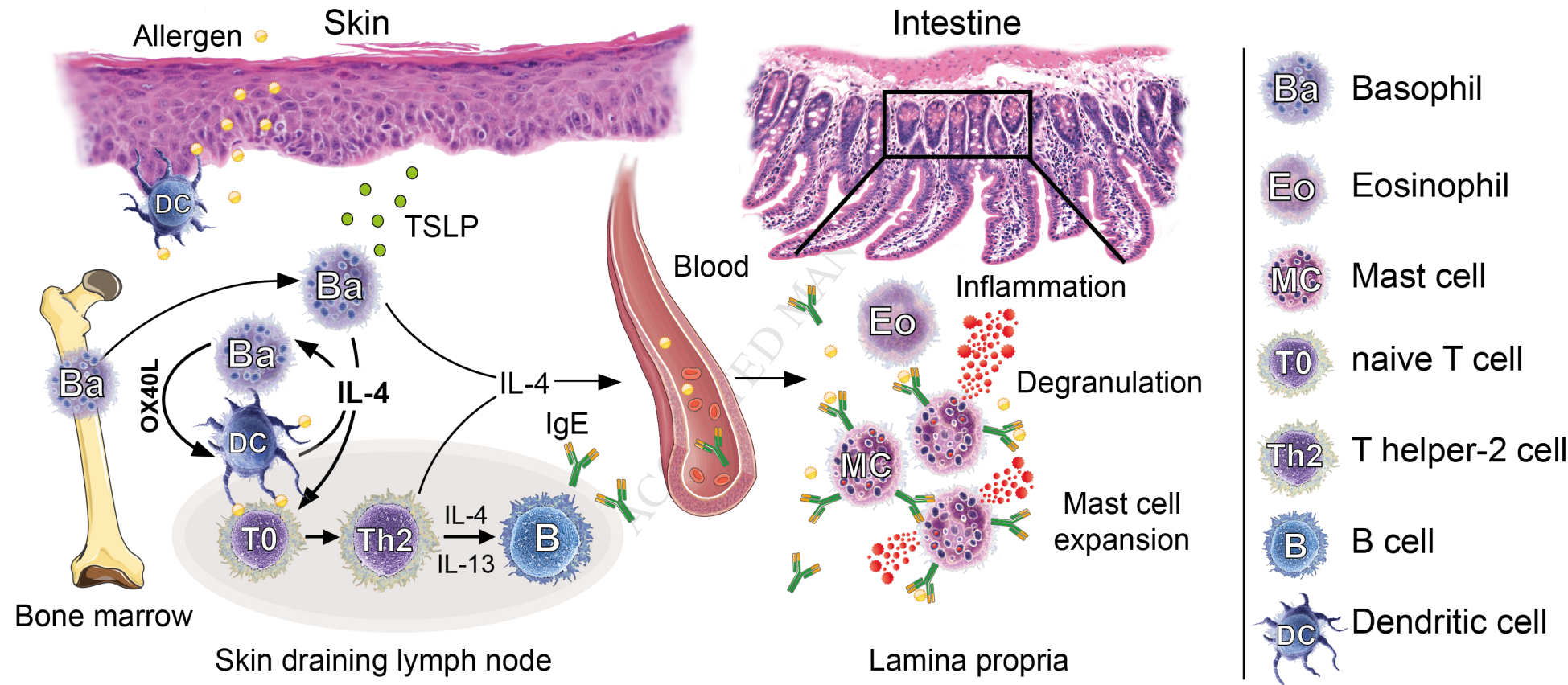
Please cite this article as: Hussain M, Borcard L, Walsh KP, Pena Rodriguez M, Mueller C, Kim BS, Kubo M, Artis D, Noti M, Basophil-derived interleukin-4 promotes epicutaneous antigen sensitization concomitant with the development of food allergy, *Journal of Allergy and Clinical Immunology* (2017), doi: 10.1016/j.jaci.2017.02.035.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



Epicutaneous sensitization

Oral allergen exposure



Basophil-derived interleukin-4 promotes epicutaneous antigen sensitization concomitant with the development of food allergy

Maryam Hussain, MSc^{1*}, Loïc Borcard, MSc^{1*}, Kevin P. Walsh, PhD², Maria Pena Rodriguez, BSc¹, Christoph Mueller, PhD¹, Brian S. Kim, MD³, Masato Kubo, PhD⁴, David Artis, PhD⁵, Mario Noti, PhD¹.

¹Institute of Pathology, Division of Experimental Pathology, University of Bern, Bern, Switzerland

²Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, USA

³Division of Dermatology, Department of Medicine, Washington University School of Medicine, St. Louis, USA

⁴Division of Molecular Pathology, Research Institute for Biomedical Science, Laboratory for Cytokine Regulation, RIKEN Center for Integrative Medical Sciences (IMS), RIKEN Yokohama Institute, Tokyo University of Science, Yamazaki, Noda-shi, Chiba, Japan

⁵Jill Roberts Institute for Research in Inflammatory Bowel Disease, Joan and Sanford I. Weill Department of Medicine, Department of Microbiology and Immunology, Weill Cornell Medical College, Cornell University, New York, NY 10021, USA

* these authors contributed equally

Research support:

Research in the Noti lab is supported by the Swiss National Science Foundation (PZ00P3_154777 to M.N.) and the Olga Mayenfisch Stiftung (to M.N.).

The authors declare no conflict of interest

Correspondence to:

Mario Noti, PhD

Institute of Pathology

Division of Experimental Pathology

University of Bern

CH-3008 Bern, Switzerland

E-mail: mario.noti@pathology.unibe.ch

Key words

IgE-mediated food allergy, atopic dermatitis; epicutaneous sensitization; T_H2 polarization; thymic stromal lymphopoietin; basophils; mast cells; IgE; IL-4.

Abbreviations used

AD, Atopic dermatitis

DC, Dendritic cell

OVA, Ovalbumin

TSLP, thymic stromal lymphopoietin

IL-4, Interleukin-4

Ig, immunoglobulin

MMCP, mouse mast cell protease

eGFP, Enhanced green fluorescent protein

ILC2, Group 2 innate lymphoid cell

CSFE, Carboxyfluorescein succinimidyl ester

WT, wild-type.

Clinical implications

Targeting the TSLP-basophil-IL-4 axis in patients with AD might offer a novel therapeutic approach for early intervention strategies to prevent the development of food allergies.

ABSTRACT

Background: Exaggerated TSLP production and infiltration of basophils are associated with the pathogenesis of atopic dermatitis (AD), a recognized risk factor for the development of food allergies. While TSLP and basophils have been implicated to promote food-induced allergic disorders in response to epicutaneous sensitization, the mechanisms by which TSLP-elicited basophils guide the progression of allergic inflammation in the skin to distant mucosal sites such as the gastrointestinal tract are poorly understood.

Objective: We sought to test the role of basophil-intrinsic IL-4 production in T_H2 sensitization to food antigens in the skin and effector food allergic responses in the gut.

Methods: Mice were epicutaneously sensitized with ovalbumin on an AD-like skin lesion, followed by intra-gastric antigen challenge to induce IgE-mediated food allergy. The requirement for basophil-derived IL-4 production for T_H2 polarization and the pathogenesis of IgE-mediated food allergy was assessed *in vitro* by co-culture experiments with naïve T cells and *in vivo* using *IL-4 3'UTR* mice that selectively lack IL-4 production in basophils.

Results: Epicutaneous food antigen sensitization is associated with the infiltration of IL-4 competent innate immune cells to the skin with basophils and eosinophils representing the predominant populations. In contrast to basophils, absence of eosinophils did not alter disease outcome. Co-culture of IL-4 competent basophils together with dendritic cells and naïve T cells was sufficient to promote T_H2 polarization in an IL-4 dependent manner *in vitro*, while absence of basophil-intrinsic IL-4 production *in vivo* was associated with reduced food allergic responses.

Conclusion: TSLP-elicited basophils promote epicutaneous sensitization to food antigens and subsequent IgE-mediated food allergy via IL-4. Strategies to target the TSLP-basophil-IL4 axis in patients with AD may lead to innovative therapies that can prevent the progression of allergies to distant mucosal sites.

INTRODUCTION

Food allergies have risen to epidemic proportions and result in significant morbidity and mortality^{1,2}. Despite their increased prevalence, current therapeutic strategies are limited by our incomplete understanding of the immunological events that initiate and propagate type 2 inflammation^{3,4}. Classically, the development of food allergies is thought to occur via sensitization to antigens that results in the priming of CD4⁺ T-helper type 2 (T_H2) cells, characterized by their expression of interleukin (IL)-4, IL-5, IL-9 and IL-13. Through the production of IL-4, T_H2 cells promote the development of antigen-specific IgE via class-switching of B cells, which ultimately mediates effector responses via mast cells in the setting of food allergy⁵⁻⁷. However, it remains unclear how antigen specific T_H2 responses are initiated given that T_H2 polarization often relies on IL-4 signaling and that naïve T cells are a poor source of IL-4⁸. One prominent hypothesis is that innate immune cell populations including basophils, eosinophils, mast cells or Group 2 innate lymphoid cells (ILC2) represent an early source of non-T cell derived IL-4 that favor T_H2 sensitization to food antigens^{9,10}.

Atopic dermatitis (AD) is a known risk factor for the development of food allergies^{11,12}. Skin barrier defects can contribute to the development of allergic inflammation at multiple epithelial barrier surfaces through epicutaneous antigen sensitization¹³⁻¹⁵, a process that is often referred to as the 'atopic march.' However, how this cascade of inflammatory events at the skin leads to the orchestration of an IgE-dependent food allergy process in the gut remains incompletely understood. To examine this, we have recently established an animal model in which food antigen sensitization on an AD-like skin lesion predisposes mice to IgE-mediated intestinal food allergy^{16,17}. Employing this model system, we demonstrated that basophils infiltrate the skin in a TSLP-dependent manner, promote T_H2 sensitization to food antigens and the development of IgE-mediated food allergy. Although TSLP-elicited basophils were both necessary and sufficient for the pathogenesis of food allergy, the precise molecular mechanisms by which basophils promote disease are not well understood. Importantly, our previous studies demonstrated that TSLP-elicited basophils rather than T_H2 cells are the dominant source of IL-4 in inflamed allergic dermatitis skin¹⁸ suggesting that TSLP-elicited basophils through their production of IL-4 may mediate T_H2 sensitization to food antigens and the development of IgE-mediated food allergy.

Here, employing an established model of epicutaneous food antigen sensitization to promote IgE-mediated food allergy, we demonstrate that IL-4-competent innate immune cells consisting of basophils and

eosinophils readily accumulate in the skin in response to epicutaneous food antigen sensitization. While our previous studies identified TSLP-elicited basophils to be important for the development of food allergy, IL-4 competent eosinophils were dispensable for both T_H2 sensitization to food antigens and the development of food allergy. Our data further show that co-culture of IL-4-competent TSLP-elicited basophils together with dendritic cells (DCs) and naïve T cells are sufficient to promote T_H2 polarization in an IL-4 dependent manner *in vitro*, while absence of basophil-intrinsic IL-4 production *in vivo* limits T_H2 responses and the development of IgE-mediated intestinal food allergy. These results identify an important role for basophil-intrinsic IL-4 production in epicutaneous T_H2 sensitization to food antigens and associated pathogenesis of IgE-mediated food allergy. Together, this study provides important new insights into the therapeutic potential of targeting the TSLP-basophil-IL-4 axis in the context of AD and associated food allergy development.

METHODS

Mice

Age, sex, and strain-matched control mice were used in all experiments. Wild-type (WT) C57BL/6, WT BALB/c, C57BL/6 *IL-4*^{-/-}, C57BL/6 *4get*, C57BL/6 OTII, BALB/c *ΔdblGATA* (provided by Wolf-Dietrich Hardt, ETH Zürich), and C57BL/6 *IL-4* 3'UTR mice (provided by Masato Kubo, Japan) were bred and housed in specific pathogen-free (SPF) conditions at the central animal facility of the Medical School of the University of Bern. All animal experiments were performed in compliance with the Swiss law and were approved by the animal experimentation committee of the Canton of Bern.

Food allergy model

Mice were treated daily for 14 consecutive days with 2 nmol of MC903 (calcipotriol; Tocris Bioscience, Bristol, United Kingdom) in 20 μ L of 100% EtOH on ears. 100 μ g of ovalbumin (OVA) in PBS was applied on dry ears post calcipotriol treatment. As a vehicle control, the same volume of EtOH and OVA was applied. All mice were challenged intra-gastrically with 50 mg of OVA on days 14 and 17.5 and killed 12 hours after the final oral antigen challenge. Starting 20 minutes after oral antigen challenge, mice were individually observed for a period of 30 minutes and a clinical allergy score was assessed as described previously¹⁶. Investigators were blind to the treatment group conditions when scoring for allergy symptoms. Ear thickness was measured at indicated time points following treatment by measuring the entire width of

the ear at the widest point in microns from the top of one stratum corneum to the top of the stratum corneum on the opposite side of the ear.

Flow cytometry

At necropsy, single cell suspensions of small intestinal lamina propria, the skin, skin draining lymph nodes and the spleen were isolated. Cell preparations were incubated with Aqua Live/Dead Fixable Dye (Invitrogen, Carlsbad, Calif) for dead cell exclusion and stained with anti-mouse fluorochrome-conjugated mAbs against CD3 ϵ (145-2C11), CD4 (GK1.5), CD5, CD8, CD19 (1D3), CD11c (N418), CD40 (1C10), CD86 (GL-1), OX40L (RM134L), NK1.1 (PK136), CD19 (6D5), IgE, Fc ϵ RI (MAR-1), CD45 (30-F11), CD49b (DX5), c-Kit (2B8). Mast cells were identified as live, CD45⁺, Lin⁻ (CD3⁻, CD5⁻, CD19⁻, CD11c⁻, and NK1.1⁻), c-Kit⁺, and IgE⁺ or Fc ϵ R1⁺ cells, eosinophils were identified as live, Lin⁻ (CD3⁻, CD5⁻, CD19⁻, NK1.1⁻), CD45⁺, SSC^{high}, SiglecF⁺ cells, and basophils were identified as live CD45⁺, Lin⁻ (CD3⁻, CD5⁻, CD19⁻, CD11c⁻, and NK1.1⁻), c-Kit⁻ cells co-expressing CD49b and IgE. For staining against intracellular antigens, cells were stimulated for 5 h with 50 ng/mL PMA and 500 ng/mL ionomycin in the presence of 10 μ g/mL brefeldin A (Sigma Aldrich). Cells were first stained with antibodies against cell surface antigens and then following fixation with paraformaldehyde and saponin permeabilization with fluorochrome-labelled anti-IFN- γ and anti-IL-4 antibodies. All samples were acquired on a LSRII or LSRII SORP (BD Biosciences, San Diego, USA) and analyzed using FlowJo software (Tree Star, Ashland, USA).

Basophil-DC-T cell co-cultures

To generate TSLP-dependent basophils *in vitro*, bone marrow cells from WT C57BL/6 mice (5x10⁶ cells/ml) were cultured in Mast Cell Medium (RPMI 1640, 15% FBS, 100 U/ml penicillin, 100 μ g/ml streptomycin, 2.9 mg/ml glutamine, 50 mM 2-ME, 1 mM sodium pyruvate, 1X nonessential amino acids, 10 mM HEPES) containing recombinant TSLP (1 μ g/ml; Amgen) for 5 days with medium replenished daily. To generate bone marrow derived DCs, bone marrow cells (0.5x10⁶ cells/ml) were cultured in standard medium with recombinant GM-CSF (20 ng/ml; Peprotech) for 10 days. Sort-purified basophils (1x10³-1x10⁵ cells/ml) were co-cultured with sort purified splenic OTII CD4⁺ T cells (1x10⁶ cells/ml), OVA 323-339 peptide (1 μ g/ml; New England Peptide) and bone marrow derived DCs (1x10⁵ cells/ml) for 4 days. Where indicated, DCs were stimulated with LPS (100 ng/ml; Enzo Life Sciences) or Papain (100 μ g/ml; Calbiochem) and the 96 well trans-well system used was supplied by Corning.

ELISA, histology, real-time RT-PCR

Measurement of TSLP in the serum was performed with a commercially available DuoSet ELISA kit (eBioscience). Total and antigen-specific serum IgE responses were measured, as described previously¹⁶. For histologic analysis, tissues were fixed in 4% paraformaldehyde and embedded in paraffin, and sections were stained with hematoxylin and eosin. Intestinal mast cells were stained with Naphtol AS-D Chloroacetate specific esterase (Sigma Aldrich) according to the manufacturer's instructions. RNA was isolated from indicated tissues using TRIzol according to the manufacturer's instructions (Molecular Research Centre, Cincinnati, USA). Genomic DNA was digested with DNase I, (Ambion) and cDNA was generated using the Superscript III kit (Life Technologies). Samples were run on an Applied Biosystems 7500 Real-time PCR system using Quantitect Primer Assays (Qiagen). Results were normalized to the housekeeping genes β -actin or *Gapdh*, and the $\Delta\Delta C_t$ method was employed for all analyses.

CD4 T cell depletion

For CD4 T cell depletion, mice were injected i.p. on indicated days with 200 μ g of anti-CD4 antibody (GK1.5).

Statistics

Data were analyzed using Graphpad Prism 5. Results are shown as mean $\pm$ standard error of the mean (SEM). Statistical significance was determined using, Students *t*-test, one-way ANOVA with Tukey's multiple or Dunn's multiple comparison test. Results were considered significant at $P \leq 0.05$. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$; **** $P < 0.001$. The number of experiments performed is indicated at the end of each figure legend.

RESULTS

Epicutaneous food antigen sensitization is associated with the infiltration of IL-4 competent innate immune cells. IL-4 is an important player in the development of protective immunity to helminth parasites but also contributes to allergic inflammatory disorders⁸. The requirement for IL-4 in mediating food-induced allergic diseases has been demonstrated in animal models as targeting IL-4 signaling often prevents the development of disease (¹⁹, **Supplementary Figure 1**) while enhanced IL-4 receptor signaling has a disease promoting effect²⁰. Given the importance of IL-4 signaling in the polarization of antigenic T_H2 cells and the pathogenesis of IgE-mediated food allergy, we comprehensively assessed the cellular sources of IL-4 in the context of epicutaneous antigen sensitization. To do so, IL-4 reporter (*4get*) mice²¹ were treated topically with the vitamin D analogue calcipotriol (MC903) in presence of OVA to induce an AD-like skin lesion¹⁶. Frequencies and total numbers of IL-4 eGFP expressing cells in the skin were assessed by flow cytometry on indicated days (**Figure 1A**). In response to epicutaneous food antigen sensitization, a population of non-T cell IL-4 eGFP⁺ immune cells expanded over time (**Figure 1B**). Flow cytometric analysis of the CD3⁻ IL-4 eGFP⁺ cell population revealed an expansion of eosinophils (SiglecF⁺) and a marked accumulation of a c-Kit⁻ SiglecF⁻ population in the skin (**Figure 1C**) while total numbers of c-Kit⁺ cells (marker for mast cells) did not change over time. Further characterization of the c-Kit⁻ SiglecF⁻ population revealed a cell surface phenotype consistent with that of basophils, characterized by the combined expression of CD49b and IgE (**Figure 1D**). Together, our data indicate that eosinophils and

basophils are the primary sources of IL-4 in the context of a developing AD-like skin lesion and may promote optimal T_H2 polarization by providing an early source of IL-4.

Eosinophils are dispensable for both epicutaneous food antigen sensitization and the development of IgE-mediated food allergy.

Basophils infiltrate the skin in response to mechanical or immunological stress and are key orchestrators in the recruitment of eosinophils to sites of allergic inflammation, a hallmark of atopic disorders^{22,23}. In the context of food allergies, recent studies have demonstrated that ablation of basophil responses resulted in a significant decline in the recruitment of eosinophils to sites of antigen sensitization and protected mice from the development of IgE-dependent and -independent food-induced allergic disorders^{16,24,25}. Given that both, basophils and eosinophils are potent innate sources of IL-4²⁶ and that eosinophils often outnumber basophils at inflammatory sites it is likely that basophil-mediated infiltration of eosinophils to the skin may promote food antigen sensitization and the development of IgE-mediated food allergy. To test the functional role of eosinophils in epicutaneous food antigen sensitization and associated development of IgE-mediated food hypersensitivity, $\Delta db/GATA$ mice deficient in eosinophils were epicutaneously sensitized with the model food antigen ovalbumin (OVA) on a developing AD-like skin lesion prior to oral food antigen challenge (**Figure 2A**). Similar to eosinophil-sufficient mice, $\Delta db/GATA$ mice exhibited pronounced ear swelling (**Figure 2B**), histopathological changes associated with AD-like inflammation (**Figure 2C**) and dermal infiltration of basophils in response to topical calcipotriol treatment (**Figure 2D**). Oral food antigen challenge of epicutaneously sensitized wild-type (WT) and $\Delta db/GATA$ mice resulted in comparable measures of food allergy signs as assessed by a clinical allergy score (**Figure 2E**). Further, in response to antigen feeding, OVA-specific IgE (**Figure 2F**) and mouse mast cell protease (MMCP) -1 levels in the serum (**Figure 2G**) did not significantly differ in food allergic eosinophil-sufficient- or -deficient mice. As intestinal mast cell numbers often correlate with disease severity in models of experimental food allergy²⁰, we assessed mast cell accumulation in the small intestinal lamina propria of food allergic mice. Flow cytometry was used to quantify mast cells (CD45⁺, Lin⁻, SiglecF⁻ c-Kit⁺, IgE⁺) in the small intestinal lamina propria of control or food allergic WT and $\Delta db/GATA$ mice revealing a comparable increment in mast cell frequencies in both WT and $\Delta db/GATA$ food allergic mice (**Figure 2H,I**). Visualization and enumeration of mast cells in chloroacetate esterase stained jejunal sections corroborated flow cytometric observations with comparable mast cell accumulation predominantly in the small intestinal lamina propria of WT and $\Delta db/GATA$ food allergic mice (**Figure 2 J,K**). Together,

despite a significant infiltration of IL-4 competent eosinophils in response to AD-associated inflammation, eosinophils were dispensable for the pathogenesis of IgE-mediated food allergy.

Basophil-derived IL-4 is required for optimal T_H2 polarization *in vitro*. Depletion of TSLP-elicited basophils has been reported to reduce T_H2 cell induction in mouse models of both helminth infection and allergic inflammation^{16,24,27,28}. Although depletion of eosinophils did not significantly alter the development of type-2 immune mediated food allergic responses, targeted depletion of basophils has been shown to impair T_H2 sensitization to food antigens and the onset of intestinal food allergy²⁴. These findings provoke the hypothesis that basophils may directly modulate T_H2 response independently of eosinophils. To test how basophils promote T_H2 polarization, we sort purified TSLP-elicited basophils from bone marrow cultures and co-cultured basophils or dendritic cells (DC) with CFSE labeled $CD4^+$ OT-II T cells. While DCs cultured with OVA induced robust proliferation of OT-II T cells, TSLP-elicited basophils alone were not capable of promoting antigen-specific T cell proliferation as assessed by CFSE dilution (**Supplementary Figure 2**). Given the importance of IL-4 in T_H2 polarization, we next assessed whether basophil-intrinsic IL-4 production is required for optimal T_H2 cell induction *in vitro*. To do so, we co-cultured TSLP-elicited basophils from the bone marrow of either wild-type- or IL-4-deficient mice together with DCs and $CD4^+$ OTII T cells. As a control, DCs were co-cultured with $CD4^+$ OTII T cells alone (**Figure 3A**). IL-4 as a marker of T_H2 polarization and IFN- γ production as a measure of T_H1 differentiation were assessed in T cells in response to antigen stimulation. In line with previous reports^{29,30}, IL-4 sufficient basophils drove robust T_H2 cell induction in the presence of antigen and DCs with increasing basophil numbers resulting in higher IL-4⁺ T cell percentages as well as corresponding decreases in T cell specific IFN- γ production (**Figure 3B**). In contrast, IL-4-deficient basophils were unable to promote IL-4 expression by $CD4^+$ T cells, suggesting that basophil-intrinsic IL-4 secretion is critical for optimal T_H2 polarization *in vitro* (**Figure 3C**). Strikingly, basophils were capable of limiting IFN- γ expression by $CD4^+$ T cells independent of IL-4 signaling suggesting that other basophil-derived factors may regulate T_H1 polarization (**Figure 3C**).

Basophils and DCs undergo reciprocal interactions to promote T_H2 differentiation *in vitro*. We next investigated the mechanisms by which DCs and TSLP-elicited basophils co-operate to induce T_H2 cell differentiation. To address whether cell-to-cell contact is required, we utilized a trans-well system. As observed before, co-culture of TSLP-elicited basophils with DCs and T cells in the same well resulted in the induction of IL-4 and inhibition of IFN- γ by $CD4^+$ T cells (**Figure 4A**). However, physical separation of basophils from DCs and T cells resulted in reduced T_H2 cell polarization as indicated by a decrease in IL-4

expression and an increase in IFN- γ expression by CD4⁺ T cells when compared with cultures where basophils were not physically separated from the other cell types (**Figure 4B**). Intriguingly, when DCs were added to TSLP-elicited basophils in the trans-well and cultured as before with CD4⁺ OT-II T cells, T_H2 polarizing conditions were more favorable than when basophils were alone in the trans-well (**Figure 4B**). Together these data demonstrate that DCs and TSLP-elicited basophils co-operate directly through contact-dependent cell-cell interactions to drive optimal T_H2 cell polarization *in vitro*.

Given that DCs and TSLP-elicited basophils cooperate in promoting T_H2 cell differentiation via contact-dependent mechanisms, we next investigated potential reciprocal interactions between DCs and basophils. To do so, we co-cultured TSLP-elicited basophils and DCs together for 24 h and assessed surface expression levels of co-stimulatory molecules on DCs involved in T cell activation. Incubation of DCs with the TLR4 agonist LPS or the cysteine protease papain resulted in the up-regulation of CD86, CD40 and OX40L expression, a co-stimulatory marker that is required for optimal T_H2 cell induction³¹ (**Figure 4C, and Supplementary Figure 3A,B**). Conversely, co-culture of DCs with TSLP-elicited basophils had no effect on CD86 or CD40 expression on DCs (**Supplementary Figure 3A,B**) but did increase OX40L expression (**Figure 4C**). Importantly, physical separation of TSLP-elicited basophils from DCs using a trans-well system completely ablated the up-regulation of OX40L expression on DCs thereby demonstrating that this observation occurs independently of soluble factors and requires cell to cell contact (**Figure 4C**). In addition, we investigated the effects of DCs on TSLP-elicited basophils and found that basophils that were co-cultured with DCs produced significantly higher levels of IL-4 than basophils cultured alone (**Figure 4D**). Using intracellular flow cytometry, we confirmed that basophils were the sole source of IL-4 in these co-cultures (data not shown). Taken together, these data highlight important interactions between DCs and TSLP-elicited basophils whereby the two cell types co-operate synergistically to induce optimal T_H2 cell differentiation *in vitro*.

Basophil-intrinsic IL-4 production promotes epicutaneous antigen sensitization and the development of IgE-mediated food allergy. While our *in vitro* studies strongly suggest an important role for basophil-derived IL-4 production in the polarization of T_H2 cells, we wanted to address the role of basophil-intrinsic IL-4 in T_H2 sensitization to food antigens and associated development of IgE-mediated food allergic responses *in vivo*. To do so, WT or *IL-4-3'UTR* mice that exclusively lack IL-4 production in basophils³² were epicutaneously sensitized to OVA as described before¹⁶. In the absence of basophil-derived IL-4 production, *IL-4-3'UTR* mice exhibited reduced skin inflammation as assessed histologically

(**Figure 5A**) and measured by ear swelling compared to WT mice (**Figure 5B**). Consistent with reduced skin inflammation, we observed a significant reduction in the frequencies of basophils (CD45⁺, Lin⁻, c-Kit⁻, CD49b⁺, IgE⁺) isolated from the skin of *IL-4-3'UTR* mice compared to controls (**Figure 5C, D**) that were characterized by a lower mean fluorescence intensity (MFI) of basophil-bound IgE (**Figure 5E**). Further, type-2 cytokine production of *ex vivo*-stimulated skin-draining lymph node cells was significantly higher in WT controls compared to *IL-4-3'UTR* mice (**Figure 5F**) suggesting that basophil-intrinsic IL-4 production may contribute to epicutaneous food antigen sensitization. To assess whether reduced T_H2 sensitization in *IL-4-3'UTR* mice alters disease outcome of IgE-mediated food allergy, WT and *IL-4-3'UTR* skin sensitized mice were challenged intra-gastrically with OVA. Strikingly, while WT mice exhibited an increased allergy score in response to oral food antigen challenge, *IL-4-3'UTR* mice demonstrated with reduced clinical signs of food allergy in response to oral food antigen challenge (**Figure 6A**). In the absence of basophil-intrinsic IL-4 production, serum antigen-specific IgE- (**Figure 6B**) and MMCP-1 levels (**Figure 6C**) were significantly reduced in *IL-4-3'UTR* mice compared to their WT counterparts, while serum levels of TSLP did not differ between groups (**Figure 6D**). Given that small intestinal mast cell numbers correlate with symptom severity in mouse models of IgE-mediated food allergy^{16,20} we next assessed the accumulation of mast cells in the small intestine. Consistent with a reduction in symptom severity (**Figure 6A**), small intestinal mast cell accumulation was significantly reduced in *IL-4-3'UTR* mice compared to WT controls as assessed by flow cytometry (**Figure 6E, F**). *Mmcp1* expression levels in the small intestine (**Figure 6G**) as well as visualization and enumeration of chloroacetate esterase positive mast cells in small intestinal tissue sections (**Figure 6H, I**) corroborated our flow cytometric findings with *IL-4-3'UTR* mice exhibiting a significantly reduced accumulation of mast cells in the small intestine. Of note, targeting basophil responses post epicutaneous food allergen sensitization did not result in the depletion of intestinal mast cells. These data indicate that reduced intestinal mast cell burden in experimental settings where basophils are depleted during the sensitization phase is not an off-target effect of basophil depletion models on intestinal mast cells (**Supplementary Figure 4**). Together, our findings provide first time evidence that basophil-intrinsic IL-4 production favors epicutaneous food antigen sensitization and promotes the pathogenesis of IgE-mediated experimental food allergy.

DISCUSSION

The present study provides four conceptual advances that broaden our understanding on how TSLP-elicited basophils promote epicutaneous T_H2 sensitization to food antigens and concomitant development of food allergy. First, we demonstrate that eosinophils and basophils are the predominant IL-4 competent cells accumulating in the skin in response to epicutaneous food antigen sensitization. Second, we find that in contrast to basophils, eosinophils are dispensable for the development of food allergy in response to epicutaneous sensitization to food antigens. Third, we identify that TSLP-elicited basophils and DCs undergo reciprocal interactions to promote optimal T_H2 polarization in an IL-4 dependent manner. Fourth, we highlight that basophil-derived IL-4 is critical for both skin sensitization to food antigens and the development of IgE-mediated food allergy. Collectively, these findings indicate that basophil-intrinsic IL-4 is important to promote the progression of allergic inflammation from the skin to distant mucosal sites such as the gastrointestinal tract.

Basophils, representing less than 1% of peripheral blood leukocytes, have been shown to play crucial and non-redundant roles in the immune system^{33,34}. The finding that basophils readily generate large quantities of T_H2 cytokines including IL-4 has provided new insights into the possible role of basophils in providing immunity to pathogens and the development of allergic disease³⁵⁻³⁷. Only until recently, basophils have been demonstrated to contribute to the pathogenesis of food-mediated allergic disorders by regulating

other immune cells including T cells, B cells or mast cells likely by their production of IL-4^{16,24,25,38}. Basophils have been historically thought to be late phase effector cells that migrate to inflammatory sites in response to established T_H2 immune responses. However, recent studies have highlighted heterogeneity in basophil populations with TSLP-elicited basophils playing a central role in the induction of T_H2 cytokine mediated immunity and inflammation and IL-3-elicited basophils exhibiting more classical basophil functions such as degranulation and histamine production in response to FcεRI cross-linking²⁸. While IL-3 is predominantly produced by activated T cells, TSLP is readily accessible from stressed epithelial cells resulting in rapid TSLP-mediated basophil infiltration to sites of inflammation likely to promote a T_H2 permissive environment³⁹. As previous studies highlighted that IL-3-elicited basophils from bone marrow progenitors were able to induce T_H2 polarization in the presence of DCs and cognate antigen in an IL-4 dependent manner⁴⁰, we here tested T_H2 polarizing capacities of TSLP-elicited basophils. Similar to IL-3 elicited basophils, we demonstrate that TSLP-dependent basophils are not sufficient to induce antigen-specific T_H2 polarization on their own but do co-operate with DCs to effectively drive T_H2 cell differentiation in an IL-4 dependent manner. Surprisingly, while T cells co-cultured with DCs and IL-4-deficient basophils were not able to efficiently generate IL-4, T cell-derived IFNγ production as a readout for T_H1 polarization production was reduced in response to co-culture with both IL-4 sufficient- and -deficient basophils. Exogenous administration of recombinant IL-4 to IL-4-deficient basophil-DC-T cell co-cultures suppressed T cell-derived IFNγ production only partially, suggesting that other factors in combination with soluble IL-4 are responsible for a global suppression of IFNγ from activated T cells in this system (data not shown). Consistent with previous reports we show that direct cell-to-cell contact between DCs and TSLP-dependent basophils results in the up-regulation of OX40L expression on DCs³¹. Strikingly, co-culture of basophils with DCs alone had a significant positive effect on basophil intrinsic IL-4 production highlighting a previously unrecognized crosstalk between basophils and DCs. Similar to studies demonstrating that co-operation exists between granulocytes and DCs in driving T_H1 immune responses⁴¹, our findings show that reciprocal and cooperative interactions between TSLP-dependent basophils and DCs are necessary for optimal T_H2 cell induction *in vitro*.

Atopic disorders are often associated with the presence of IL-4 competent innate immune cells in the inflammatory infiltrate and elevated numbers in the circulation often appear to correlate with disease activity⁴². Studies in humans and mice demonstrated that IL-4 competent cells including eosinophils, basophils or ILC2 accumulate in lesional AD skin - likely to promote a T_H2 permissive environment^{18,43,44}. Given the

importance of IL-4 for T_H2 polarization and AD being a risk factor for the development of food allergy, we here assessed the recruitment of IL-4 competent immune cells in the skin in response to AD-like disease development. We found that in early stages of AD-associated skin inflammation the majority of IL-4 competent immune cells are of non T cell origin with eosinophils and basophils representing the major populations. Although we and others have reported a critical role for TSLP-elicited basophils in the pathogenesis of food-induced allergic disorders^{16,24}, the mechanisms by which TSLP-elicited basophils promote disease are poorly understood. Given that basophil ablation in these experimental settings was associated with a decline in eosinophil recruitment to sites of inflammation, it was critical to assess whether eosinophils are downstream effector cells of basophils. Importantly, eosinophils can rapidly secrete pre-formed T_H2-initiating cytokines that may contribute to T_H2 polarization and associated antigen sensitization⁴⁵. Surprisingly, despite a significant accumulation of IL-4-competent eosinophils to sites of inflammation, absence of eosinophils did not alter AD-like skin lesion quality nor the magnitude of food allergic responses. Although we have not ruled out the contribution of eosinophils in later stages of disease, these data suggest that immediate food hypersensitivity responses are not dependent on eosinophils but rely on basophils in this experimental regime. While a previous study by Chu et al. elegantly demonstrated the requirement for indigenous eosinophils for the induction of T_H2 immunity to oral antigen, the nature of antigen sensitization, adjuvant used or composition of the microbiota may explain differences observed in these model systems⁴⁶.

Recent studies demonstrated the requirement for ILC2-derived IL-4 to promote immunity to parasites and the development of allergic inflammation^{47,48}. In contrast to these studies, we did not observe an accumulation of IL-4 competent ILC2s at site of antigen sensitization or challenge in our model system (data not shown). Our previous studies demonstrated that basophil responses precede ILC2 responses and that basophils are the dominant IL-4-enhanced GFP-expressing cell type in AD associated skin inflammation¹⁸. Importantly, basophil-mediated ILC2 expansion in the context of AD associated skin inflammation was driven by IL-4. Similar findings were reported in a model of asthma in which mice reconstituted with IL-4-deficient basophils had fewer ILC2s in the lung, demonstrating that IL-4 from basophils is necessary for optimal ILC2 expansion *in vivo*³². Despite ILC2s accumulate in the skin in response to topical calcipotriol treatment^{18,43}, these innate cells are not a primary source of IL-4 in our experimental regimen and may thus promote local tissue inflammation rather than T_H2 polarization. Therefore, ILC2s may be critical in amplifying the effects of basophils on additional effector cells by their

potent production of cytokines and chemokines. However, additional studies are required to investigate the importance of the basophil-ILC2 axis in epicutaneous T_H2 sensitization to food antigens and development of IgE-mediated food allergy.

To test a potential role for basophil-intrinsic IL-4 production in the context of food allergy, we sensitized *IL-4 3'UTR* mice that exclusively lack IL-4 production from basophils on an AD-like skin lesion prior to oral antigen feeding. Our findings illustrate that several cardinal features of IgE-mediated experimental food allergy, including T_H2 sensitization to food antigens, IgE synthesis and mucosal mast cell expansion are significantly reduced in *IL-4 3'UTR* mice compared to WT controls. These data suggest that basophil-derived IL-4 production contributes to the pathogenesis of IgE-mediated food allergy by inducing antigen specific T cell responses. Interestingly, selective ablation of IL-4 in basophils was associated with reduced skin inflammation and infiltration of basophils in *IL-4 3'UTR* mice compared to WT controls suggesting that the local inflammatory environment further promotes basophil expansion. Importantly and consistent with previous reports ²⁵, we find that depletion of $CD4^+$ T cells in the context of epicutaneous antigen sensitization limits IgE production and protects mice from disease onset (**Supplementary Figure 5**). Together, we propose a model in which basophil-derived IL-4 production is important for the polarization of antigen specific T_H2 cells that figure as pathogenic effector cells in the setting of IgE-mediated experimental food allergy.

Despite the fact that basophils have been found in skin biopsies of AD patients and experimental studies have demonstrated the requirement for TSLP-elicited basophils in the pathogenesis of AD and food-induced allergic disorders, no basophil-specific therapeutics have been tested in humans to date. Of note, administration of benralizumab, a humanized anti-IL-5R α antibody significantly reduced asthma symptoms and lung function in patients with severe asthma by targeting eosinophils and basophils ^{49,50}. However, whether the beneficial effects of benralizumab can be attributed to the depletion of basophils needs further investigation. With AD being a risk factor for asthma and food allergy development, targeting the key factors that promote basophil activation and expansion in patients with AD may represent one approach to paralyze the allergic response to food antigens at multiple levels. In fact, clinical trials using dupilumab, a monoclonal antibody to a subunit of the IL-4 receptor (IL-4R α), show promising disease limiting results in patients with AD and asthma ⁵¹⁻⁵³. Whether IL-4 receptor targeting in humans will result in the prevention of the atopic march from AD to food allergy, asthma and allergic rhinitis remains to be determined through

prospective clinical trials. In conclusion, using an experimental model of food allergy that recapitulates many characteristics of human disease our previous results and those reported herein provide an important rationale for targeting the TSLP-basophil-IL-4 axis in the context of AD to prevent its possible complications in the gastrointestinal tract.

Acknowledgments

We thank members of the Noti and Mueller lab for critical reading of the manuscript. Further, we thank the DKF Flow Cytometry and Cell Sorting Core for technical advice and support, the Animal Care taker team of the Medical Faculty of the University of Bern and the Clean Mouse Facility (CMF) Bern.

FIGURE LEGENDS

Figure 1. Epicutaneous food antigen sensitization is associated with the infiltration of IL-4 competent innate immune cells. **A**, Kinetics of IL-4eGFP⁺ skin infiltrating cells in response to topical MC903+OVA treatment. **B**, Frequencies of Siglec-F⁺, c-Kit⁺ and double negative CD3⁻ IL-4 eGFP⁺ cells in the skin. **C**, Total numbers of CD3⁻ IL-4eGFP⁺ Siglec-F⁺ c-Kit⁻, c-Kit⁺ Siglec-F⁻ and double negative cells in the skin. **D**, Frequencies of basophils (CD49b⁺, IgE⁺) in the Siglec-F⁻ c-Kit⁻ cell fraction in panel C. Basophils were gated as live, singlet, CD45⁺, Lin⁻, c-Kit⁻, Siglec-F⁻ cells co-expressing CD49b and IgE. Data are representative of 3 independent experiments with 3 to 5 mice per group. Error bars indicate the mean $\pm$ SD. ** $P < 0.01$; *** $P < 0.005$.

Figure 2. Absence of eosinophils does not alter disease pathogenesis of IgE-mediated food allergy. **A**, Experimental protocol. **B**, Ear swelling. **C**, Hematoxylin and eosin staining from the skin. Scale bar = 100 μ m. **D**, Numbers of skin infiltrating basophils. **E**, Allergy score. **F**, OVA-specific serum IgE levels. **G**, serum MMCP-1 levels. **H**, Representative FACS plots of mast cells in the small intestinal lamina propria. Mast cells were gated as live, CD45⁺, lin⁻ (CD3⁻, CD5⁻, CD19⁻, CD11c⁻, and NK1.1⁻), Siglec-F⁻ cells co-expressing c-Kit and IgE. **I**, Quantification of mast cell frequencies in the small intestinal lamina propria. **J**, Chloroacetate esterase staining of mast cells from the jejunum. Scale bar = 100 μ m. **K**, Quantification of chloroacetate esterase positive mast cells. Data are representative of 3 independent experiments with 4 to 5 mice per group. Solid bars represent WT animals, open bars Δ dblGATA mice. Error bars indicate the mean $\pm$ SD. *i.g.*, intra-gastric; *ND*, no disease. *ns*, not significant.

Figure 3. Basophil-derived IL-4 production promotes optimal T_H2 polarization *in vitro*.

Bone marrow from WT or IL-4^{-/-} mice was cultured for 4 d with rTSLP and sort-purified basophils were co-cultured with DCs alone or with DCs and OTII T cells in the presence of OVA peptide for 4 d. **A**, Frequencies of IL-4⁺ and IFN γ ⁺ CD4⁺ OT II T cells co-cultured with DCs alone. Frequencies of IL-4⁺ and IFN γ ⁺ CD4⁺ OT II T cells co-cultured with DCs and increasing numbers of IL-4 sufficient- (**B**) or IL-4 deficient (**C**) basophils are shown. Data are representative of one experiment out of three.

Figure 4. Basophils and DCs undergo reciprocal interactions to promote T_H2 differentiation in vitro. **A**, Frequencies of OVA-peptide stimulated IL-4⁺ and IFN γ ⁺ CD4⁺ OTII T cells co-cultured with DCs alone. **B**, Frequencies of OVA-peptide stimulated IL-4⁺ and IFN γ ⁺ CD4⁺ OTII T cells co-cultured with DCs together or separated by trans-well from basophils alone or basophils and DCs. **C**, Surface expression of OX40L (MFI) on DCs stimulated with LPS, papain or DCs co-incubated together or separated from basophils by transwell. Grey histograms: DCs incubated with medium alone. Bold black lines: treated DCs. **D**, IL-4 protein levels in supernatants of DC and basophil co-cultures. Data shown are representative of one experiment out of three. TW, trans-well. ***P* < 0.01.

Figure 5. Basophil-intrinsic IL-4 production regulates epicutaneous antigen sensitization. **A**, Hematoxylin and eosin staining from the skin. **B**, Ear swelling. **C**, Representative FACS plots of basophils in the skin. Basophils were gated as live, CD45⁺, lin⁻ (CD3⁻, CD5⁻, CD19⁻, CD11c⁻, and NK1.1⁻), c-Kit⁻ cells co-expressing CD49b and IgE. **D**, Quantification of basophil frequencies in the skin. **E**, Mean fluorescent intensity (MFI) of basophil-bound IgE. **F**, IL-5 and IL-13 production from re-stimulated skin-draining lymph nodes. Data are representative of 3 independent experiments with 4 to 6 mice per group. Solid bars represent WT animals, open bars *IL-4* 3'UTR mice. Error bars indicate the mean $\pm$ SD. **P* < 0.05; ***P* < 0.01.

Figure 6. Basophil-intrinsic IL-4 production promotes the development of IgE-mediated food allergy. **A**, Allergy score. **B**, OVA-specific serum IgE levels. **C**, serum MMCP-1 levels. **D**, serum TSLP levels. **E**, Representative FACS plots of mast cells in the small intestinal lamina propria. Mast cells were gated as live, CD45⁺, lin⁻ (CD3⁻, CD5⁻, CD19⁻, CD11c⁻, and NK1.1⁻) cells co-expressing c-Kit and IgE. **F**, Quantification of mast cell frequencies in the small intestinal lamina propria. **G**, mRNA expression levels of *Mmcp1* from the jejunum. **H**, Chloroacetate esterase staining of mast cells from the jejunum. **K**, Quantification of chloroacetate esterase positive mast cells. Data are representative of 3 independent experiments with 4 to 6 mice per group. Solid bars represent WT animals, open bars *IL-4* 3'UTR mice. Error bars indicate the mean $\pm$ SD. ** *P* < 0.01; ****P* < 0.005.

REFERENCES

1. Sicherer SH, Sampson HA. Food allergy: Epidemiology, pathogenesis, diagnosis, and treatment. *J Allergy Clin Immunol* 2014;133:291–307–quiz308.
2. Hadley C. Food allergies on the rise? Determining the prevalence of food allergies, and how quickly it is increasing, is the first step in tackling the problem. *EMBO Rep* 2006;7:1080–3.
3. Burks AW, Tang M, Sicherer S, Muraro A, Eigenmann PA, Ebisawa M, et al. ICON: food allergy. *J Allergy Clin Immunol* 2012;129:906–20.
4. Pulendran B, Artis D. New paradigms in type 2 immunity. *Science* 2012;337:431–5.
5. Berin MC, Mayer L. Immunophysiology of experimental food allergy. *Mucosal Immunol* 2009;2:24–32.
6. Berin MC. Pathogenesis of IgE-mediated food allergy. *Clin Exp Allergy* 2015;45:1483–96.
7. Chinthrajah RS, Hernandez JD, Boyd SD, Galli SJ, Nadeau KC. Molecular and cellular mechanisms of food allergy and food tolerance. *J Allergy Clin Immunol* 2016;137:984–97.
8. Paul WE, Zhu J. How are T(H)2-type immune responses initiated and amplified? *Nat Rev Immunol* 2010;10:225–35.
9. Caubet J-C, Masilamani M, Rivers NA, Mayer L, Sampson HA. Potential non-T cells source of interleukin-4 in food allergy. *Pediatr Allergy Immunol* 2014;25:243–9.
10. Hussain M, Epstein MM, Noti M. Experimental food allergy models to study the role of innate immune cells as initiators of allergen specific Th2 immune responses. *Drug Discovery Today Disease Models*. In press.
11. Brough HA, Liu AH, Sicherer S, Makinson K, Douiri A, Brown SJ, et al. Atopic dermatitis increases the effect of exposure to peanut antigen in dust on peanut sensitization and likely peanut allergy. *J Allergy Clin Immunol* 2015;135:164–70.
12. Tsakok T, Marrs T, Mohsin M, Baron S, Toit du G, Till S, et al. Does atopic dermatitis cause food allergy? A systematic review. *J Allergy Clin Immunol* 2016;137:1071–8.
13. Han H, Xu W, Headley MB, Jessup HK, Lee KS, Omori M, et al. Thymic stromal lymphopoietin (TSLP)-mediated dermal inflammation aggravates experimental asthma. *Mucosal Immunol* 2012;5:342–51.
14. Bartnikas LM, Gurish MF, Burton OT, Leisten S, Janssen E, Oettgen HC, et al. Epicutaneous sensitization results in IgE-dependent intestinal mast cell expansion and food-induced anaphylaxis. *J Allergy Clin Immunol* 2013;131:451–60.e1–6.
15. Leyva-Castillo JM, Hener P, Jiang H, Li M. TSLP produced by keratinocytes promotes allergen sensitization through skin and thereby triggers atopic march in mice. *J Invest Dermatol* 2013;133:154–63.
16. Noti M, Kim BS, Siracusa MC, Rak GD, Kubo M, Moghaddam AE, et al. Exposure to food allergens through inflamed skin promotes intestinal food allergy through the thymic stromal lymphopoietin-basophil axis. *J Allergy Clin Immunol* 2014;133:1390–9–1399.e1–6.
17. Moghaddam AE, Hillson WR, Noti M, Gartlan KH, Johnson S, Thomas B, et al. Dry roasting

- enhances peanut-induced allergic sensitization across mucosal and cutaneous routes in mice. *J Allergy Clin Immunol* 2014;134:1453–6.
18. Kim BS, Wang K, Siracusa MC, Saenz SA, Brestoff JR, Monticelli LA, et al. Basophils promote innate lymphoid cell responses in inflamed skin. *J Immunol* 2014;193:3717–25.
19. Brandt EB, Munitz A, Orekov T, Mingler MK, McBride M, Finkelman FD, et al. Targeting IL-4/IL-13 signaling to alleviate oral allergen-induced diarrhea. *J Allergy Clin Immunol* 2009;123:53–8.
20. Burton OT, Darling AR, Zhou JS, Noval-Rivas M, Jones TG, Gurish MF, et al. Direct effects of IL-4 on mast cells drive their intestinal expansion and increase susceptibility to anaphylaxis in a murine model of food allergy. *Mucosal Immunol* 2013;6:740–50.
21. Mohrs M, Shinkai K, Mohrs K, Locksley RM. Analysis of type 2 immunity in vivo with a bicistronic IL-4 reporter. *Immunity* 2001;15:303–11.
22. Cheng LE, Sullivan BM, Retana LE, Allen CDC, Liang H-E, Locksley RM. IgE-activated basophils regulate eosinophil tissue entry by modulating endothelial function. *J Exp Med* 2015;212:513–24.
23. Nakashima C, Otsuka A, Kitoh A, Honda T, Egawa G, Nakajima S, et al. Basophils regulate the recruitment of eosinophils in a murine model of irritant contact dermatitis. *J Allergy Clin Immunol* 2014;134:100–7.
24. Noti M, Wojno EDT, Kim BS, Siracusa MC, Giacomini PR, Nair MG, et al. Thymic stromal lymphopoietin-elicited basophil responses promote eosinophilic esophagitis. *Nat Med* 2013;19:1005–13.
25. Han H, Thelen TD, Comeau MR, Ziegler SF. Thymic stromal lymphopoietin-mediated epicutaneous inflammation promotes acute diarrhea and anaphylaxis. *J Clin Invest* 2014;124:5442–52.
26. Gessner A, Mohrs K, Mohrs M. Mast cells, basophils, and eosinophils acquire constitutive IL-4 and IL-13 transcripts during lineage differentiation that are sufficient for rapid cytokine production. *J Immunol* 2005;174:1063–72.
27. Giacomini PR, Siracusa MC, Walsh KP, Grenier RK, Kubo M, Comeau MR, et al. Thymic stromal lymphopoietin-dependent basophils promote Th2 cytokine responses following intestinal helminth infection. *J Immunol* 2012;189:4371–8.
28. Siracusa MC, Saenz SA, Hill DA, Kim BS, Headley MB, Doering TA, et al. TSLP promotes interleukin-3-independent basophil haematopoiesis and type 2 inflammation. *Nature* 2011;477:229–33.
29. Otsuka A, Nakajima S, Kubo M, Egawa G, Honda T, Kitoh A, et al. Basophils are required for the induction of Th2 immunity to haptens and peptide antigens. *Nat Commun* 2013;4:1739.
30. Wakahara K, Van VQ, Baba N, Bégin P, Rubio M, Delespesse G, et al. Basophils are recruited to inflamed lungs and exacerbate memory Th2 responses in mice and humans. *Allergy* 2013;68:180–9.
31. Leyva-Castillo JM, Hener P, Michea P, Karasuyama H, Chan S, Soumelis V, et al. Skin thymic stromal lymphopoietin initiates Th2 responses through an orchestrated immune cascade. *Nat Commun* 2013;4:2847.
32. Motomura Y, Morita H, Moro K, Nakae S, Artis D, Endo TA, et al. Basophil-derived interleukin-4 controls the function of natural helper cells, a member of ILC2s, in lung inflammation. *Immunity* 2014;40:758–71.
33. Karasuyama H, Mukai K, Obata K, Tsujimura Y, Wada T. Nonredundant roles of basophils in immunity. *Annu Rev Immunol* 2011;29:45–69.
34. Sullivan BM, Locksley RM. Basophils: a nonredundant contributor to host immunity. *Immunity*

- 672 2009;30:12–20.
- 673 35. Voehringer D, Shinkai K, Locksley RM. Type 2 immunity reflects orchestrated recruitment of cells
674 committed to IL-4 production. *Immunity* 2004;20:267–77.
- 675 36. Min B, Prout M, Hu-Li J, Zhu J, Jankovic D, Morgan ES, et al. Basophils produce IL-4 and
676 accumulate in tissues after infection with a Th2-inducing parasite. *J Exp Med* 2004;200:507–17.
- 677 37. Schroeder JT, Lichtenstein LM, Roche EM, Xiao H, Liu MC. IL-4 production by human basophils
678 found in the lung following segmental allergen challenge. *J Allergy Clin Immunol* 2001;107:265–71.
- 679 38. Muto T, Fukuoka A, Kabashima K, Ziegler SF, Nakanishi K, Matsushita K, et al. The role of
680 basophils and proallergic cytokines, TSLP and IL-33, in cutaneously sensitized food allergy. *Int*
681 *Immunol* 2014;26:539–49.
- 682 39. Oetjen LK, Noti M, Kim BS. New insights into basophil heterogeneity. *Semin Immunopathol*
683 2016;;1–13.
- 684 40. Oh K, Shen T, Le Gros G, Min B. Induction of Th2 type immunity in a mouse system reveals a novel
685 immunoregulatory role of basophils. *Blood* 2007;109:2921–7.
- 686 41. van Gisbergen KPJM, Sanchez-Hernandez M, Geijtenbeek TBH, van Kooyk Y. Neutrophils mediate
687 immune modulation of dendritic cells through glycosylation-dependent interactions between Mac-1
688 and DC-SIGN. *J Exp Med* 2005;201:1281–92.
- 689 42. Stone KD, Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. *J Allergy Clin*
690 *Immunol* 2010;125:S73–80.
- 691 43. Kim BS, Siracusa MC, Saenz SA, Noti M, Monticelli LA, Sonnenberg GF, et al. TSLP elicits IL-33-
692 independent innate lymphoid cell responses to promote skin inflammation. *Sci Transl Med*
693 2013;5:170ra16–6.
- 694 44. Roediger B, Kyle R, Yip KH, Sumaria N, Guy TV, Kim BS, et al. Cutaneous immunosurveillance and
695 regulation of inflammation by group 2 innate lymphoid cells. *Nat Immunol* 2013;14:564–73.
- 696 45. Lacy P, Stow JL. Cytokine release from innate immune cells: association with diverse membrane
697 trafficking pathways. *Blood* 2011;118:9–18.
- 698 46. Chu DK, Jimenez-Saiz R, Verschoor CP, Walker TD, Goncharova S, Llop-Guevara A, et al.
699 Indigenous enteric eosinophils control DCs to initiate a primary Th2 immune response in vivo. *J Exp*
700 *Med* 2014;211:1657–72.
- 701 47. Noval Rivas M, Burton OT, Oettgen HC, Chatila T. IL-4 production by group 2 innate lymphoid cells
702 promotes food allergy by blocking regulatory T-cell function. *Journal of Allergy and Clinical*
703 *Immunology* 2016;
- 704 48. Pelly VS, Kannan Y, Coomes SM, Entwistle LJ, Rückerl D, Seddon B, et al. IL-4-producing ILC2s
705 are required for the differentiation of TH2 cells following *Heligmosomoides polygyrus* infection.
706 *Mucosal Immunol* 2016;
- 707 49. Laviolette M, Gossage DL, Gauvreau G, Leigh R, Olivenstein R, Katial R, et al. Effects of
708 benralizumab on airway eosinophils in asthmatic patients with sputum eosinophilia. *J Allergy Clin*
709 *Immunol* 2013;132:1086–1096.e5.
- 710 50. Ghazi A, Trikha A, Calhoun WJ. Benralizumab--a humanized mAb to IL-5R α with enhanced
711 antibody-dependent cell-mediated cytotoxicity--a novel approach for the treatment of asthma.
712 *Expert Opin Biol Ther* 2012;12:113–8.
- 713 51. Beck LA, Thaçi D, Hamilton JD, Graham NM, Bieber T, Rocklin R, et al. Dupilumab treatment in
714 adults with moderate-to-severe atopic dermatitis. *N Engl J Med* 2014;371:130–9.

52. Thaçi D, Simpson EL, Beck LA, Bieber T, Blauvelt A, Papp K, et al. Efficacy and safety of dupilumab in adults with moderate-to-severe atopic dermatitis inadequately controlled by topical treatments: a randomised, placebo-controlled, dose-ranging phase 2b trial. *Lancet* 2016;387:40–52.
53. Wenzel S, Castro M, Corren J, Maspero J, Wang L, Zhang B, et al. Dupilumab efficacy and safety in adults with uncontrolled persistent asthma despite use of medium-to-high-dose inhaled corticosteroids plus a long-acting β 2 agonist: a randomised double-blind placebo-controlled pivotal phase 2b dose-ranging trial. *Lancet* 2016;388:31–44.

Figure 1

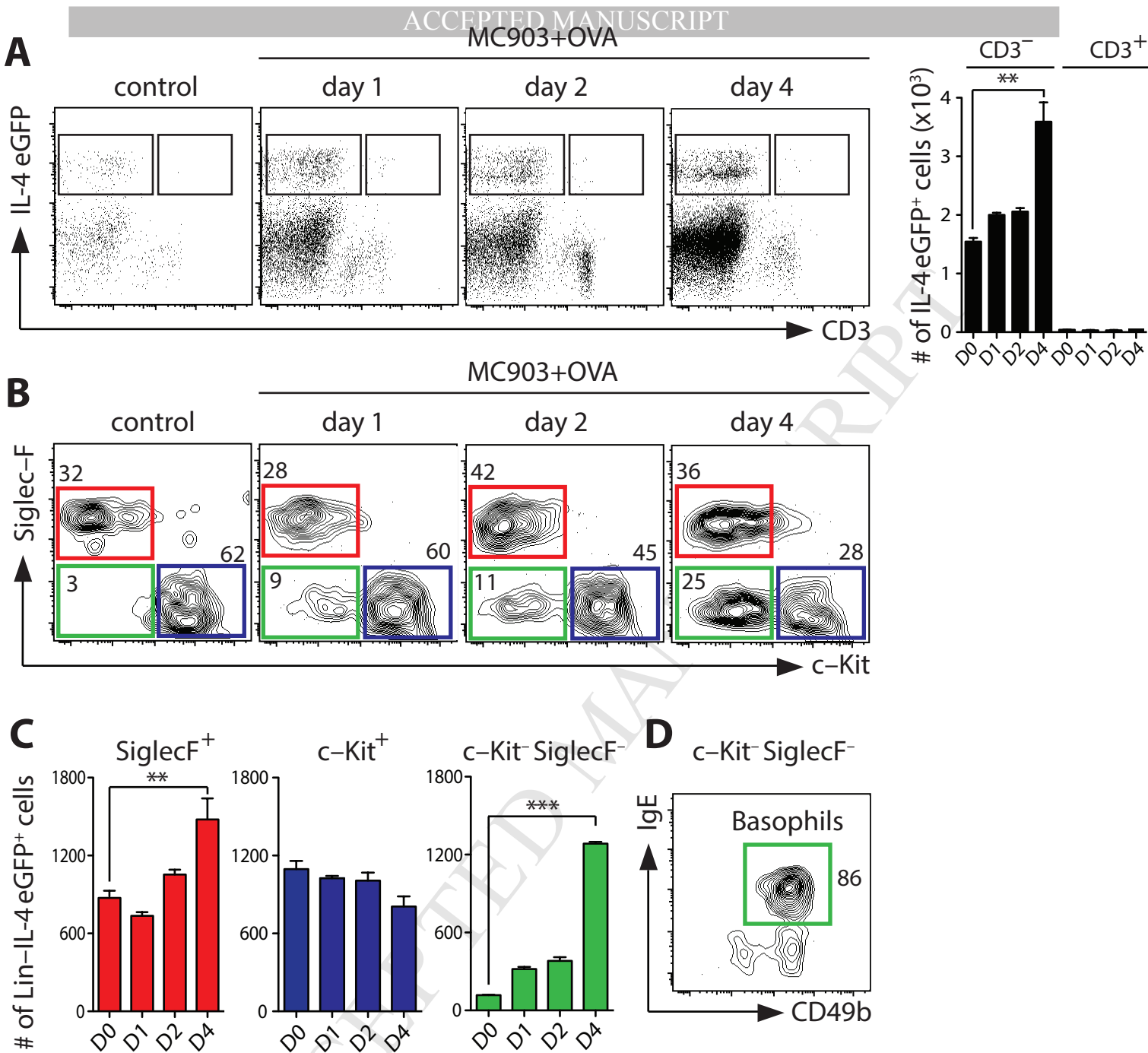


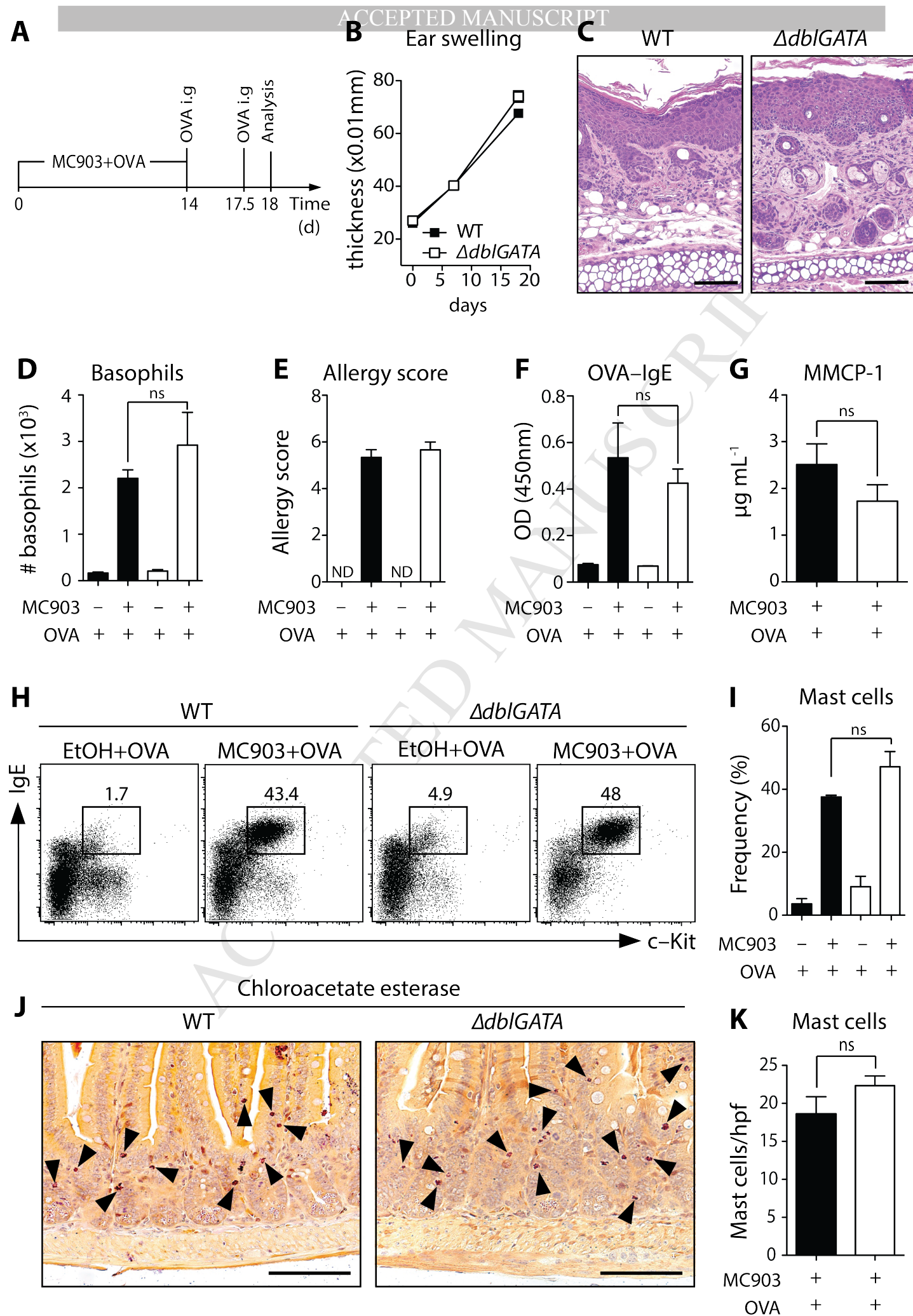
Figure 2

Figure 3

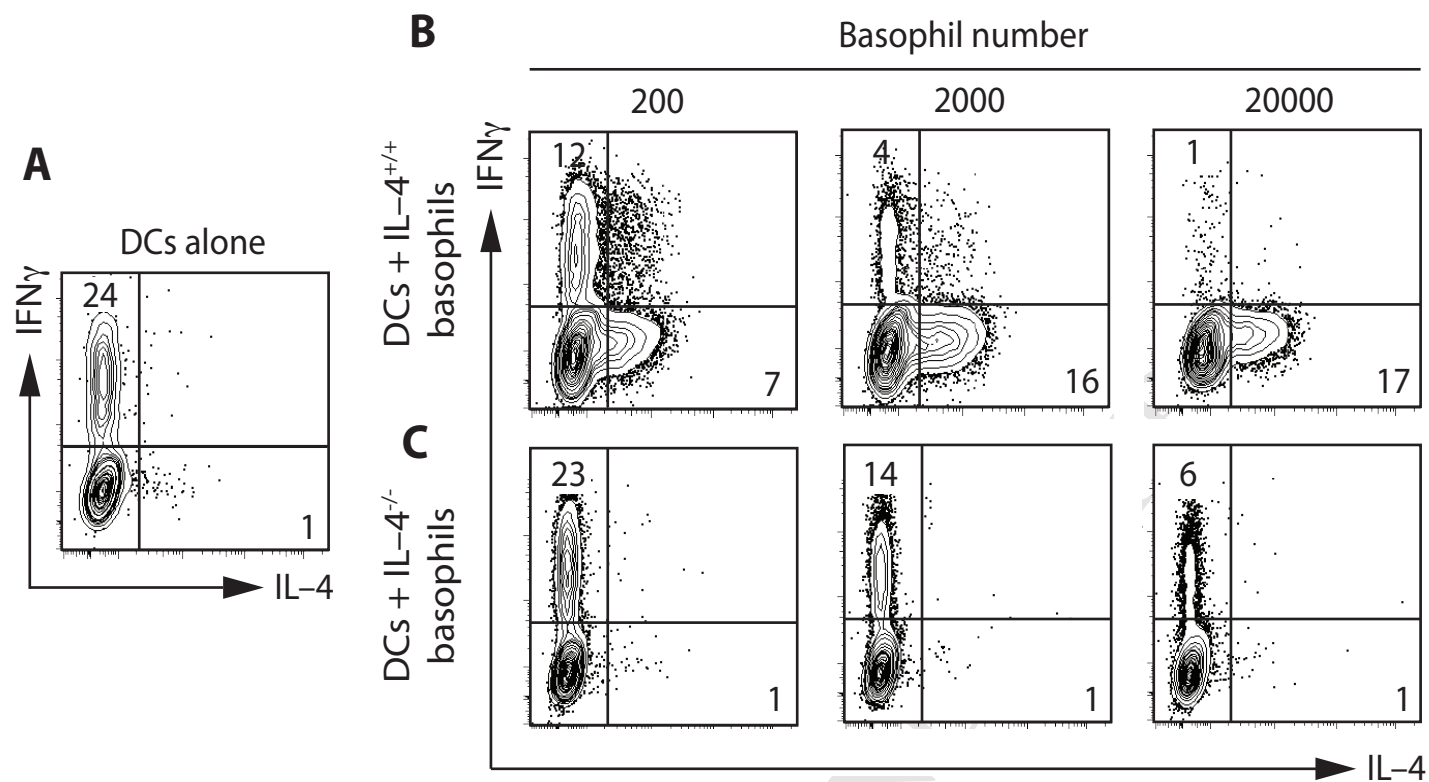


Figure 4

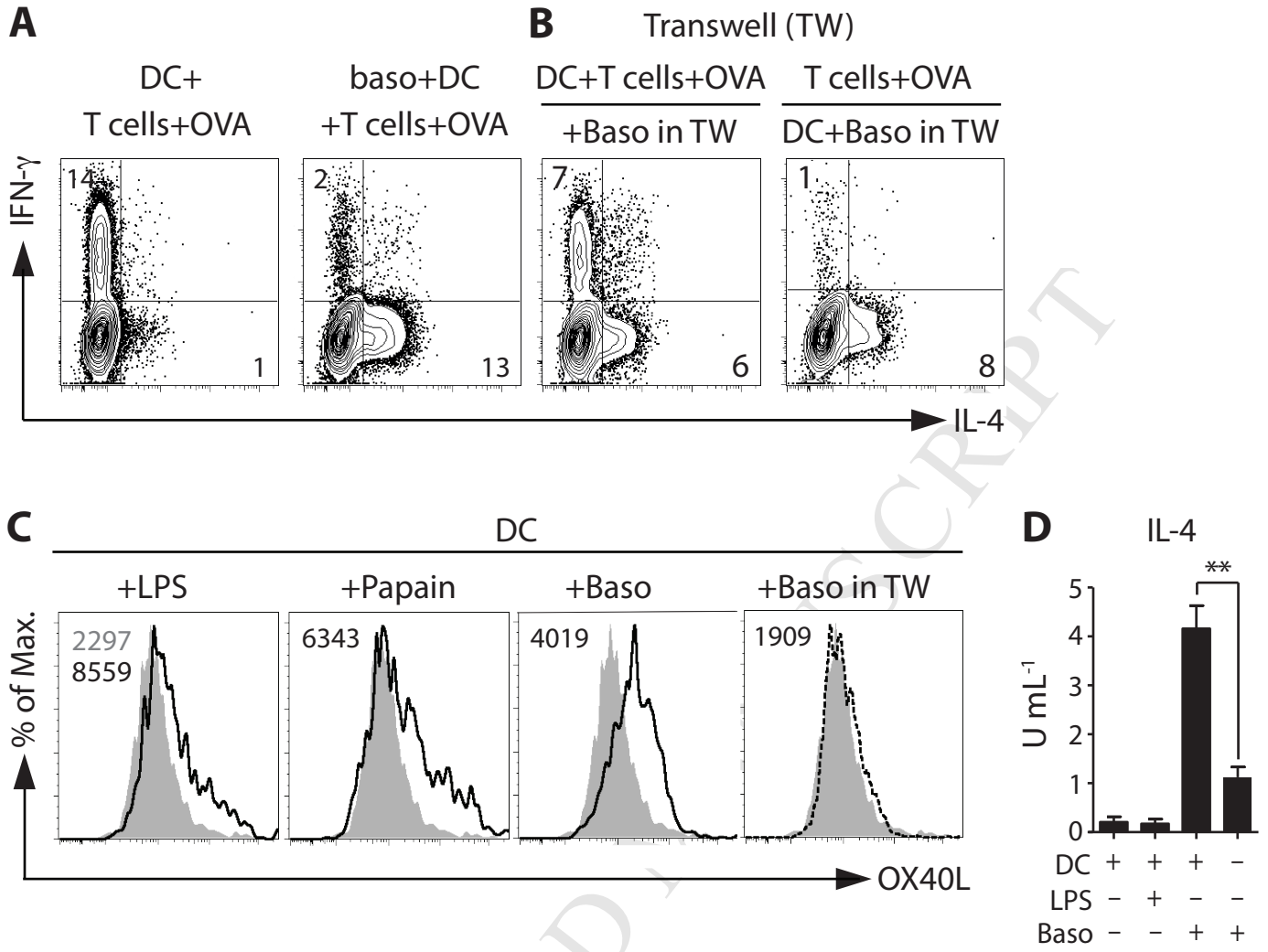


Figure 5

ACCEPTED MANUSCRIPT

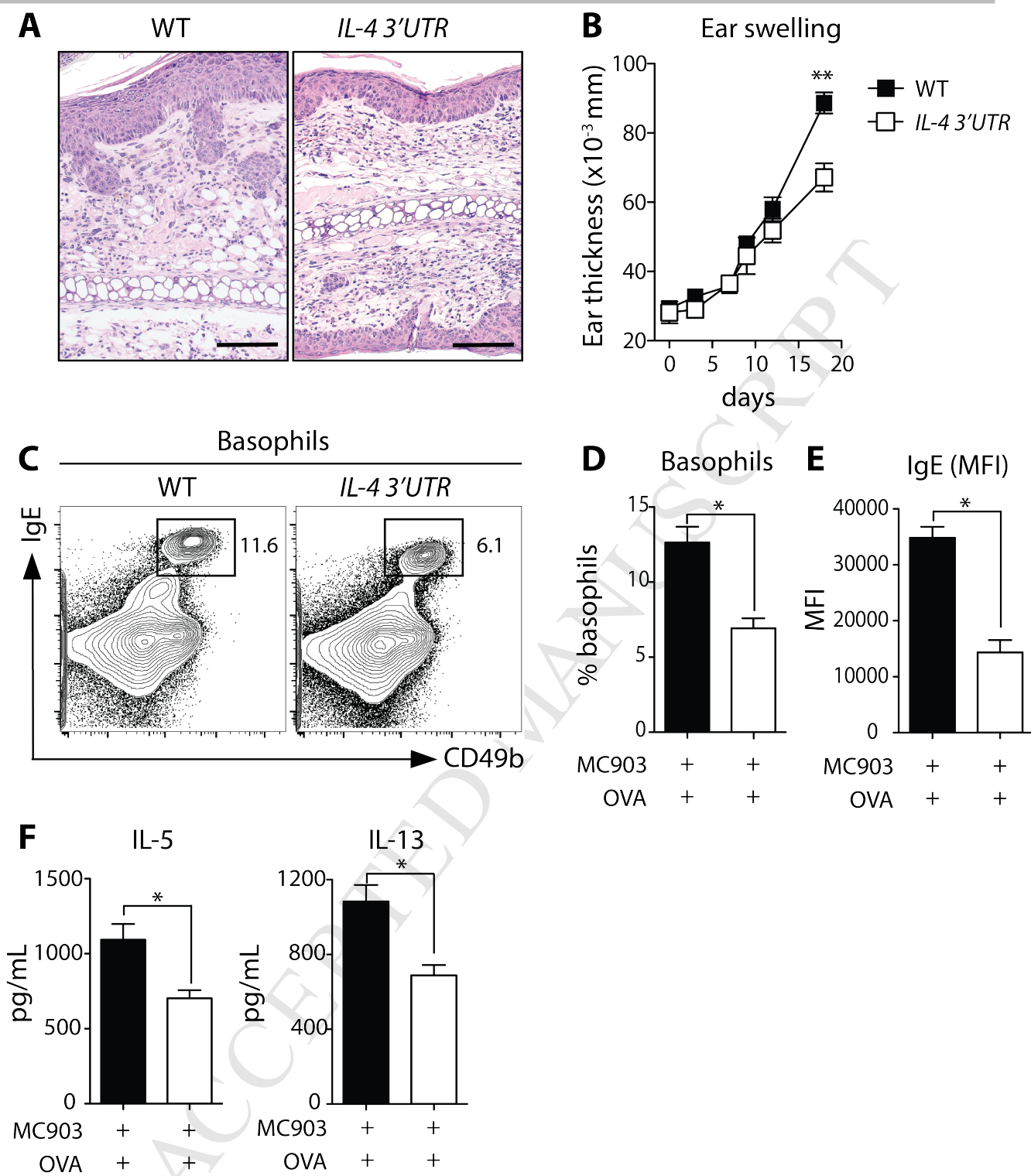
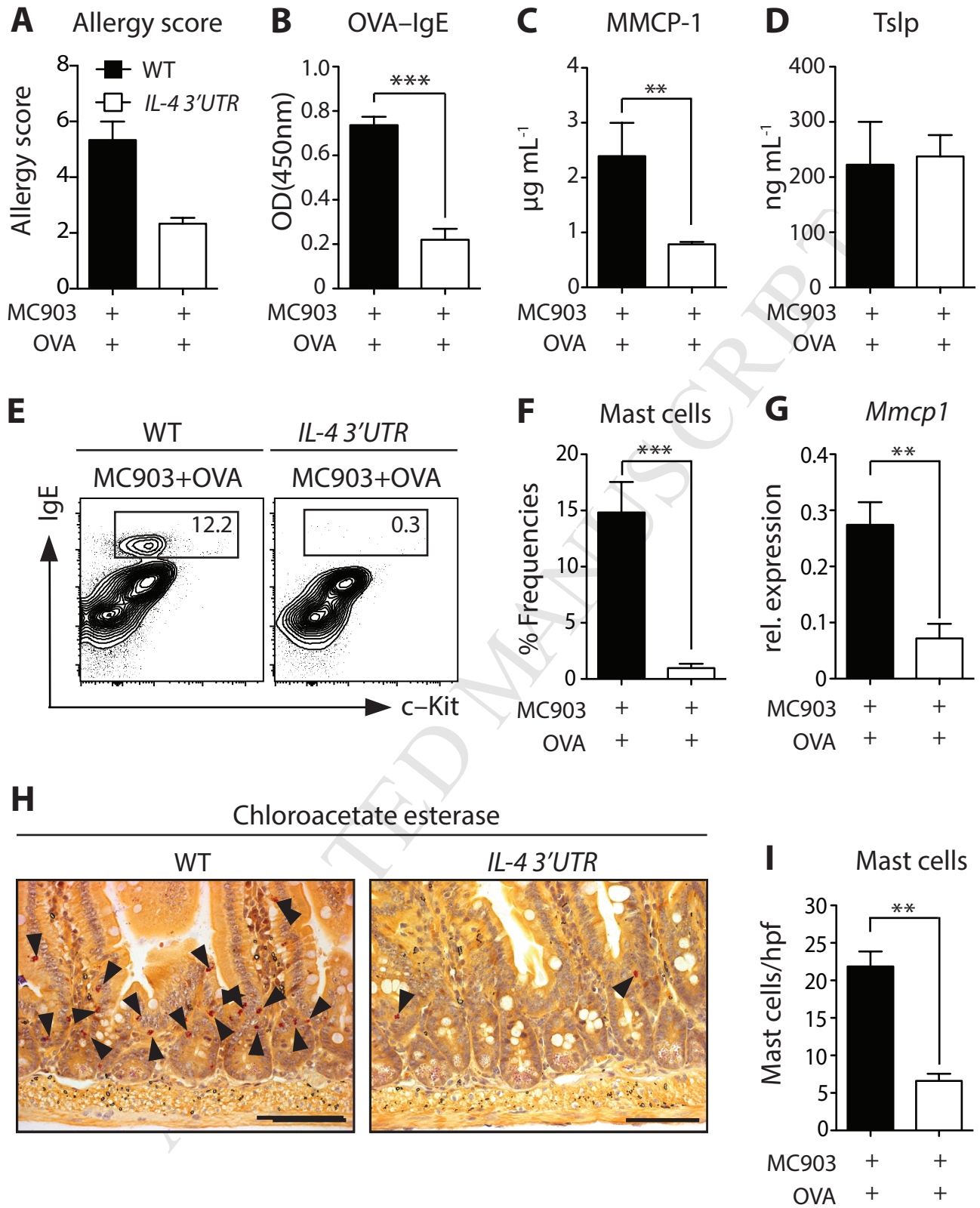


Figure 6

ACCEPTED MANUSCRIPT



Supplementary Figure 1. IL-4 is critical for the pathogenesis of IgE-mediated food allergy. **A**, Allergy score.

B, OVA-specific serum IgE levels. **C**, Mast cell frequencies in the small intestine as assessed by flow cytometry. **D**, *Mcpt1* expression levels in the small intestinal jejunum. Data are representative of 3 independent experiments with 3-4 mice per group. Error bars indicate the mean $\pm$ SD. ND, no disease. *** $P < 0.005$.

Supplementary Figure 2. T cell proliferation assay with DCs or TSLP-elicited basophils.

Proliferation of OT-II T cells stimulated with OVA in the presence of DCs or TSLP-elicited basophils as assessed by CFSE dilution. Data are representative of one experiment out of three.

Supplementary Figure 3. DC surface expression of CD80 and CD40 in response to LPS, Papain or co-incubation with TSLP-elicited basophils. BMDCs (2×10^4 cells) were stimulated with LPS (100 ng/ml), Papain (25 μ g/ml) or co-incubated with *in vitro* TSLP-elicited basophils (2×10^4 cells) for 24 hrs. DC surface marker expression was then determined by flow cytometry for CD80 (**A**) and CD40 (**B**). Grey histograms represent DCs that were incubated with medium alone. Bold black lines represent treated DCs. Numbers indicate mean fluorescence intensity. Data shown are representative of one experiment of three.

Supplementary Figure 4. Basophil depletion post epicutaneous food allergen sensitization does not result in complete ablation of mast cells in the small intestinal lamina propria. **A**, Experimental protocol. Basophils were depleted in Baso-DTR mice post epicutaneous food allergen sensitization by intraperitoneal injection of diphtheria toxin (DT) on days 15, 16 and 17. As a control Baso-DTR mice were injected with PBS. **B**, Frequencies of basophils in the spleen of control treated (no DT treatment) or DT-treated Baso-DTR mice. Basophils were gated as live, CD45⁺, lin⁻ (CD3⁻, CD5⁻, CD19⁻, CD11c⁻, SiglecF⁻ and NK1.1⁻), c-Kit⁺ cells co-expressing IgE and CD49b. **C**, Mast cell frequencies

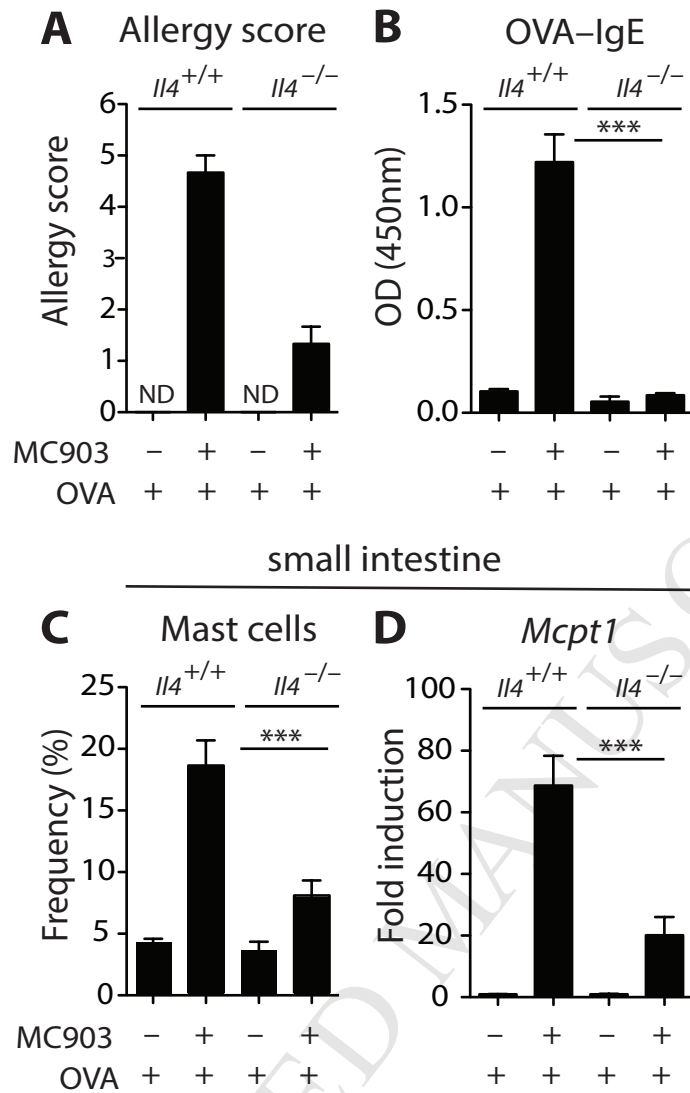
in the small intestinal lamina propria of Baso-DTR mice treated with PBS (no DT treatment) or DT on days 15, 16 and 17. Mast cells were gated as live, CD45⁺, lin⁻ (CD3⁻, CD5⁻, CD19⁻, CD11c⁻, SiglecF⁻ and NK1.1⁻) cells co-expressing c-Kit and IgE. Data are representative for $n=3$ (Baso-DTR no DT treatment) and $n=4$ mice (Baso-DTR + DT treatment).

Supplementary Figure 5. Depletion of CD4 T cells during the course of epicutaneous food allergen sensitization protects mice from IgE-mediated food allergy. **A**, Experimental protocol **B**, CD4 T cell frequencies in skin draining lymph nodes. **C**, IgE serum levels. **D**, Allergy score. Data are representative

of 2 independent experiments with 3-4 mice per group. Error bars indicate the mean $\pm$ SD. ** $P < 0.01$, *** $P < 0.005$.

Supplementary Figure 1

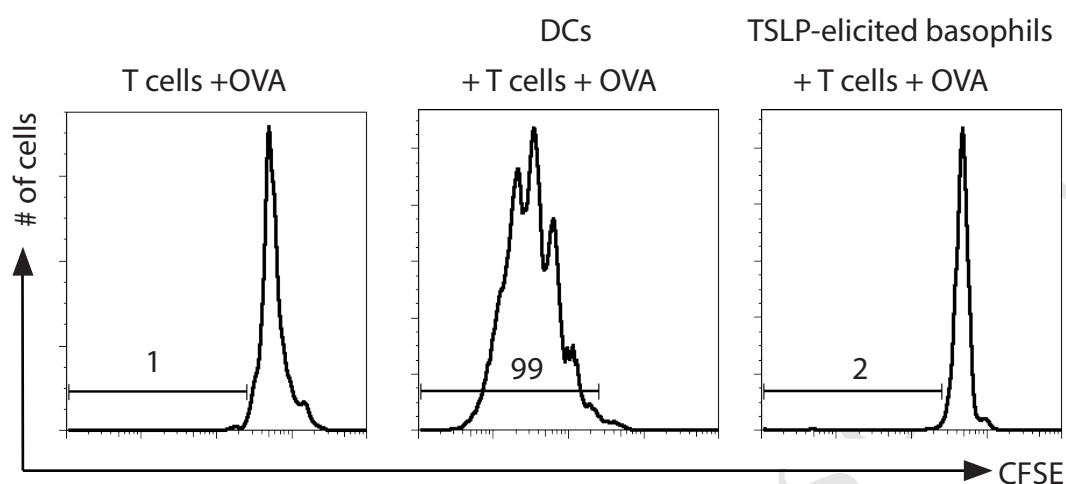
ACCEPTED MANUSCRIPT



Supplementary Figure 1. IL-4 is critical for the pathogenesis of IgE-mediated food allergy. **A**, Allergy score. **B**, OVA-specific serum IgE levels. **C**, Mast cell frequencies in the small intestine as assessed by flow cytometry. **D**, *Mcpt1* expression levels in the small intestinal jejunum. Data are representative of 3 independent experiments with 3-4 mice per group. Error bars indicate the mean $\pm$ SD. ND, no disease. *** $P < 0.005$.

Supplementary Figure 2

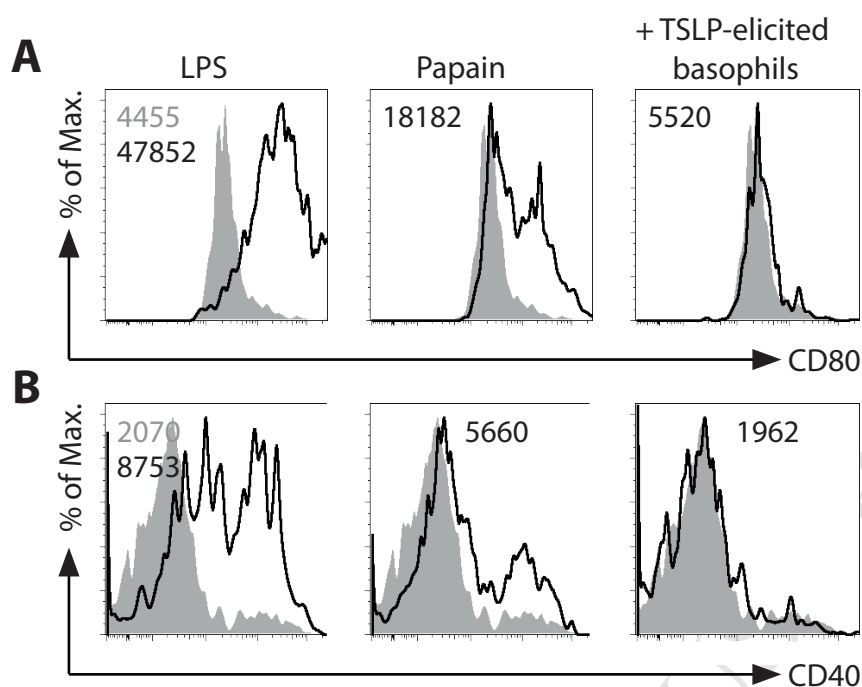
ACCEPTED MANUSCRIPT



Supplementary Figure 2. T cell proliferation assay with DCs or TSLP-elicited basophils. Proliferation of OT-II T cells stimulated with OVA in the presence of DCs or TSLP-elicited basophils as assessed by CFSE dilution. Data are representative of one experiment out of three.

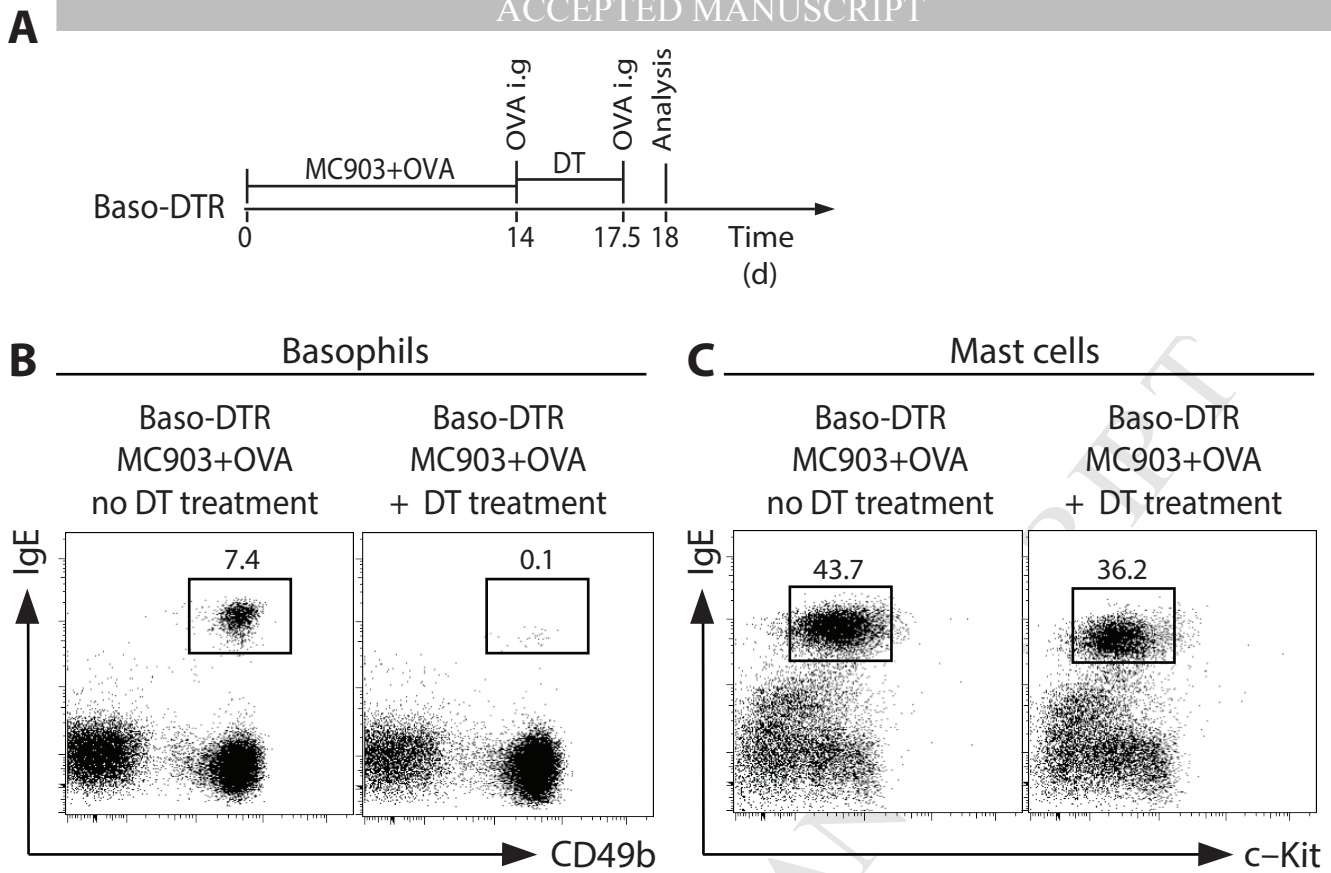
Supplementary Figure 3

ACCEPTED MANUSCRIPT



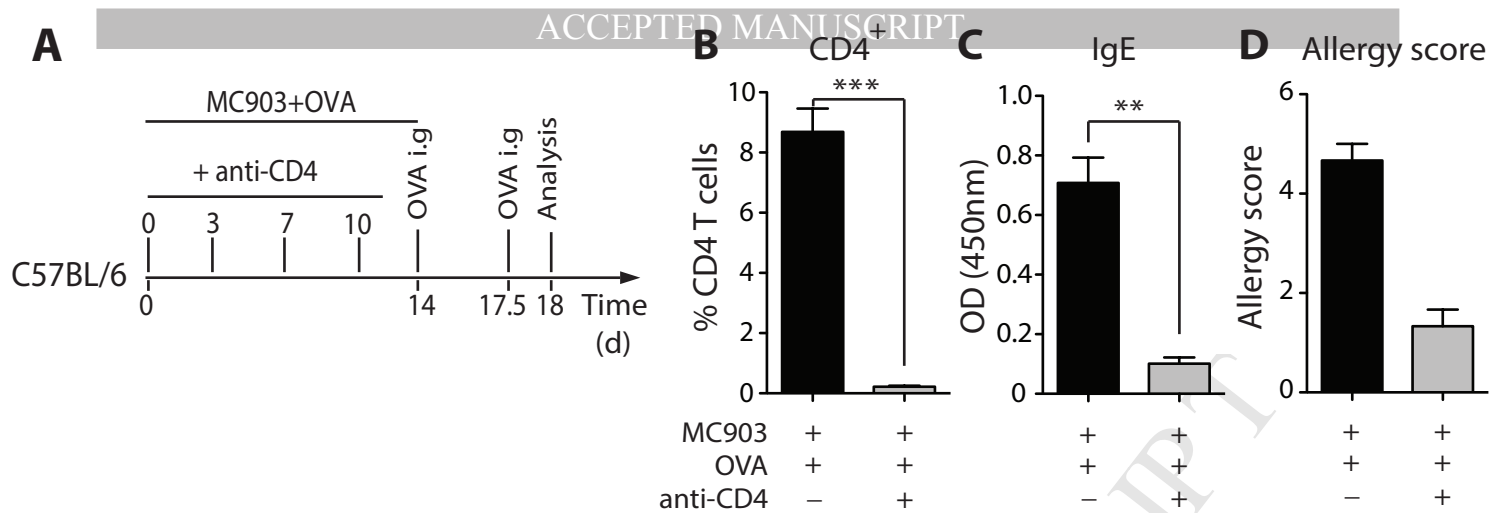
Supplementary Figure 3. DC surface expression of CD80 and CD40 in response to LPS, Papain or co-incubation with TSLP-elicited basophils. BMDCs (2×10^4 cells) were stimulated with LPS (100 ng/ml), Papain (25 μ g/ml) or co-incubated with *in vitro* TSLP-elicited basophils (2×10^4 cells) for 24 hrs. DC surface marker expression was then determined by flow cytometry for CD80 (**A**) and CD40 (**B**). Grey histograms represent DCs that were incubated with medium alone. Bold black lines represent treated DCs. Numbers indicate mean fluorescence intensity. Data shown are representative of one experiment of three.

Supplementary Figure 4



Supplementary Figure 4. Basophil depletion post epicutaneous food allergen sensitization does not result in complete ablation of mast cells in the small intestinal lamina propria. **A**, Experimental protocol. Basophils were depleted in Baso-DTR mice post epicutaneous food allergen sensitization by intraperitoneal injection of diptheria toxin (DT) on days 15, 16 and 17. As a control Baso-DTR mice were injected with PBS. **B**, Frequencies of basophils in the spleen of control treated (no DT treatment) or DT-treated Baso-DTR mice. Basophils were gated as live, CD45⁺, lin⁻ (CD3⁻, CD5⁻, CD19⁻, CD11c⁻, SiglecF⁻ and NK1.1⁻), c-Kit⁻ cells co-expressing IgE and CD49b. **C**, Mast cell frequencies in the small intestinal lamina propria of Baso-DTR mice treated with PBS (no DT treatment) or DT on days 15, 16 and 17. Mast cells were gated as live, CD45⁺, lin⁻ (CD3⁻, CD5⁻, CD19⁻, CD11c⁻, SiglecF⁻ and NK1.1⁻) cells co-expressing c-Kit and IgE. Data are representative for $n=3$ (Baso-DTR no DT treatment) and $n=4$ mice (Baso-DTR + DT treatment).

Supplementary Figure 5



Supplementary Figure 5. Depletion of CD4 T cells during the course of epicutaneous food allergen sensitization protects mice from IgE-mediated food allergy. **A**, Experimental protocol **B**, CD4 T cell frequencies in skin draining lymph nodes. **C**, IgE serum levels. **D**, Allergy score. Data are representative of 2 independent experiments with 3-4 mice per group. Error bars indicate the mean $\pm$ SD. $**P < 0.01$, $***P < 0.005$.