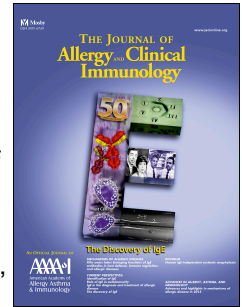


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Vascular endothelial specific IL-4R α –ABL1 kinase signaling axis regulates severity of IgE-mediated anaphylactic reactions

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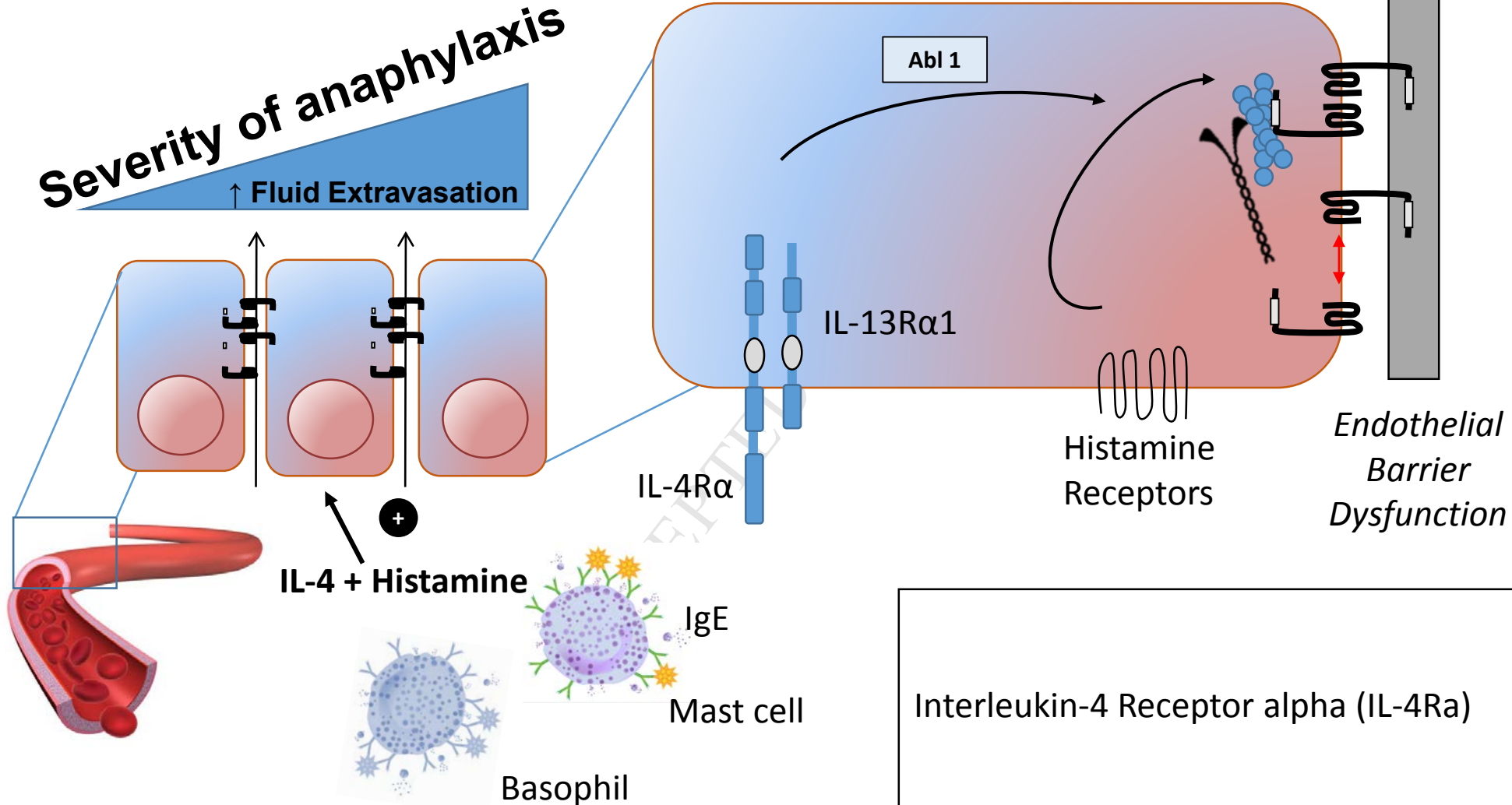
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Vascular endothelial specific IL-4R α signaling regulates severity of IgE-mediated anaphylactic reactions

Fluid
Extravasation

Severity of anaphylaxis
↑ Fluid Extravasation



**Vascular endothelial specific IL-4R α -ABL1 kinase signaling axis regulates severity
of IgE-mediated anaphylactic reactions**

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Abstract

Background: Severe IgE-mediated, food-induced anaphylactic reactions are characterized by pulmonary venous vasodilatation and fluid extravasation, which is thought to lead to the life-threatening anaphylactic phenotype. The underlying immunological and cellular processes involved in driving fluid extravasation and the severe anaphylactic phenotype are not fully elucidated.

Objective: To define the interaction and requirement of IL-4 and vascular endothelial (VE) IL-4R α chain signaling in histamine-ABL1-mediated VE dysfunction and fluid extravasation in the severity of IgE-mediated anaphylactic reactions in mice.

Methods: Mice deficient in VE IL-4R α and models of passive and active oral antigen- and IgE-induced anaphylaxis were employed to define the requirements of VE IL-4R α and ABL1 pathway in severe anaphylactic reactions. Human VE cell line (EA.hy926 cells) and pharmacologic (imatinib) and genetic approaches (shRNA knockdown of *IL4RA* and *ABL1*) were used to define the requirement of this pathway in VE barrier dysfunction.

Results: IL-4 exacerbation of histamine-induced hypovolemic shock in mice was dependent on VE expression of the IL-4R α . IL-4 and histamine induced ABL1 activation in human VE cells and VE barrier dysfunction was ABL1 dependent. Development of severe IgE-mediated hypovolemia and shock required VE-restricted ABL1 expression. Treatment of mice with a history of food-induced anaphylaxis with the ABL kinase inhibitor imatinib protected the mice from developing severe IgE-mediated anaphylaxis.

Conclusion: IL-4 amplifies IgE- and histamine-induced VE dysfunction, fluid extravasation, and severity of anaphylaxis via a VE IL-4R α -ABL1-dependent mechanism. These studies implicate an important contribution by the VE compartment in the severity of anaphylaxis and identify a new pathway for therapeutic intervention of IgE-mediated reactions.

Key Messages:

- IL-4 amplifies IgE- and histamine-induced hypovolemic shock.
- IL-4 amplification of the shock response is dependent on vascular endothelial expression of ABL1 and IL-4R α .
- Utilization of the ABL kinase inhibitor imatinib prevented the development of severe IgE-mediated anaphylaxis.

Capsule Summary:

IL-4-Histamine induced vascular endothelial cell ABL1 kinase signaling controls severity of IgE-mediated anaphylactic reactions.

Keywords:

IL-4R α chain; hypovolemic shock; IgE and Mast cells; food-induced anaphylaxis; histamine; vascular endothelial barrier dysfunction; ABL1 kinase.

Abbreviations:

HRP, Horseradish peroxidase; iIL-9Tg, intestinal interleukin 9 transgenic mice; IL-4C, Interleukin-4 complex; IL-4R α , Interleukin 4 receptor alpha chain; mMCPT-1, Mouse mast cells protease-1; OVA-TNP, Ovalbumin- 2,4,6-trinitrophenol; shRNA, Short hairpin RNA; TER, Transendothelial resistance; VE, Vascular endothelial;

Introduction

Anaphylaxis is a severe, life-threatening allergic reaction that affects both children and adults and males and females in the United States ¹. The most common inciting agents (33.2% of reactions) are foods, particularly peanuts and tree nuts, and food-induced anaphylaxis (FIA) hospitalization rates for children in US have more than doubled from 2000 to 2009 ².

A food-induced anaphylactic reaction encompasses a variety of symptoms that may affect one or more target organs including those of the gastrointestinal (GI), cutaneous, respiratory, and cardiovascular systems ³⁻⁵. In humans, compromise of either the cardiovascular or respiratory system defines a severe reaction ^{3,4}, and it is postulated that basophil- and mast cell (MC)-derived mediators, through inducing pulmonary venous vasodilatation and fluid extravasation, cause the respiratory and cardiovascular collapse that leads to the severe, life-threatening anaphylactic phenotype ⁶. Fluid extravasation in anaphylaxis is thought to be consequence of capillary fluid leak due to loss of the vascular endothelial (VE) barrier integrity, leading to the movement of fluids, electrolytes, and proteins from the vascular compartment into the interstitial spaces ⁷⁻⁹.

The VE barrier is maintained by adherens junction (AJ) and tight junction (TJ) proteins ¹⁰. The AJ proteins are the most ubiquitously expressed endothelial cell-cell junctional proteins and act as mechanical anchoring points that promote endothelial TJ protein-protein interactions and interjunctional integrity ¹¹. The TJ proteins are tethered to the actin cytoskeleton and seal the intercellular space, establishing the dense “fence” barrier preventing the bilateral apical-basolateral passage of ions, proteins, and lipids ¹¹. VE-cadherin is one of the first endothelial cell-specific molecules expressed and required for endothelial survival, blood vessel assembly, and stabilization ^{12,13}. VE-cadherin forms Ca²⁺-dependent homophilic interactions with adjacent endothelial cells via actin-linking catenin family proteins and the actin cytoskeleton, establishing the vascular barrier integrity ¹¹. The stability of the VE-cadherin–catenin–cytoskeleton complex is essential to maintaining endothelial barrier function ¹⁴ and disruption of these processes via receptor-signaling pathways including non-receptor kinases, including SRC, ABL1 and ARG and myosin light

chain kinase (MLCK) leads to VE-cadherin-mediated AJ disorganization or VE-cadherin internalization and loss of endothelial barrier integrity¹⁴⁻²⁰.

The cellular and molecular pathways that directly contribute to the severe anaphylaxis phenotype are unclear. Clinical studies have reported increased levels of IL-4 and histamine in the serum of human patients with severe anaphylaxis²¹, and histamine, but not IL-4, levels were associated with severe disease²² suggesting a possible role for these molecules in expression of the severe disease phenotype. We and others have demonstrated that symptoms of food-induced anaphylaxis in mice are dependent on IgE-MCs and histamine type I receptor signaling and that the severity of the reaction positively correlates with an increase in hemoconcentration (an indication for fluid extravasation and hypovolemic shock)²³⁻²⁶. In vitro experimental evidence suggests that IL-4 modulates VE barrier properties²⁷⁻²⁹ and we have demonstrated that IL-4 can interact with vasoactive mediators to increase hemoconcentration and the severity of anaphylaxis^{30, 31}. However, the cellular target of these IL-4-mediated effects and the underlying IL-4R α -dependent signaling processes involved in the amplification of histamine-induced VE barrier dysfunction and fluid extravasation in IgE-mediated reactions is not yet fully understood.

Herein, we examine the relationship between IL-4 and histamine in IgE-mediated vascular endothelial leak and hypovolemic shock. We show that IL-4 amplifies histamine-induced hypovolemic shock via VE IL-4R α chain-dependent process. Furthermore, we show that IL-4 and histamine stimulate ABL1 kinase activity in vascular endothelial cells and VE barrier dysfunction that was inhibited by pharmacologic and genetic ablation of ABL1 activity. Importantly, employing both passive and active models of food-induced anaphylaxis, treating mice with the ABL kinase inhibitor imatinib protected the mice from developing severe IgE-mediated anaphylactic phenotype after allergen exposure. These studies implicate an important contribution by the IL-4R α -ABL1-signaling pathway in the VE compartment in the severity of IgE- and histamine-induced anaphylaxis.

Material and Methods

Animals. Intestinal IL-9 transgenic (iIL9Tg) mice were generated as previously described³². WT BALB/c mice were originally provided by Charles River laboratories, (Wilmington, MA, USA) and bred in-house at Cincinnati Children's Hospital Medical Center (CCHMC) (Cincinnati, OH, USA). IL-4R α ^{Y709F} mice were obtained from Fred Finkelman, CCHMC³³. Cadherin-5^{Cre} mice (purchased from Jackson Laboratory) (Bar Harbor, ME, USA) and IL-4R α ^{fl/fl} mice (generously provided by Frank Brombacher, University of Cape Town, South Africa) and iIL9Tg were used to generate mice lacking IL-4R α in VE cells³⁴. Tie2^{Cre} mice (generously provided by Joseph E. Qualls, CCHMC) and Abl1^{fl/fl} mice³⁵ and iIL-9Tg mice were used to generate mice lacking ABL1 in VE cells³⁶. Age-, sex-, weight-matched littermates were used as controls in all experiments. The mice were maintained and bred in a clean barrier facility and were handled under an approved Institutional Animal Care and Use Committee protocols at CCHMC animal facility.

Oral Antigen-Induced Anaphylaxis. Six- to 8-week-old mice were sensitized and challenged as previously described²³. In some experiments, mice received imatinib mesylate (Santa Cruz Biotechnology, TX, USA) intraperitoneally (i.p.) (1.25 or 1.75 mg / 100 μ L distilled water per mouse) 2 h prior to OVA challenge.

Passive Anaphylaxis. Mice were injected intravenously (i.v.) with 20 μ g / 200 μ L of anti-IgE (IgG2a mAb to mouse IgE; EM-95) (Obtained from Fred Finkelman, CCHMC)³⁷. EM95 cross-linking to Fc ϵ R1 lead to MC and basophil activation and degranulation³⁸.

IL-4 and Histamine-Induced Anaphylaxis. Twenty-four hours before the experiments, mice were injected i.v. with IL-4C (recombinant, IL-4-neutralizing, anti-IL-4 monoclonal antibody [mAb] complex, 1:5 weight [1 μ g of IL-4 + 5 μ g anti-IL-4 mAb]) (anti-IL-4 mAb was obtained from Fred Finkelman, CCHMC) followed by histamine biphosphate monohydrate (Sigma-Aldrich, St. Louis, MO, USA) (0.4 or 2 mg / 200 μ L saline per mouse)^{31, 39}.

Passive Oral Antigen-Induced Anaphylaxis. Mice were primed and challenged as previously described²³. Mice received imatinib mesylate (Santa Cruz Biotechnology, TX,

USA) i.p.(1.25-1.75 mg / 100 μ L distilled water per mouse) 2 h prior to OVA–2, 4, 6-trinitrophenyl hapten (TNP) challenge.

Anaphylaxis Assessments. Hypothermia (significant loss of body temperature) and hypovolemia (increased in hemoconcentration) were used as an indication of anaphylactic reaction. Rectal temperature was taken with a rectal probe and a digital thermocouple thermometer (Model BAT-12; Physitemp Instruments, Clifton, NJ, USA). Temperature was taken prior to the challenge and then every 15 min for 30 or 60 min. For hemoconcentration assessment, blood was drawn by retro-orbital bleeding into heparinized capillary tubes and centrifuged for 5 min at 10,000 rpm. Hematocrit (percentage of packed red blood cell [RBC] volume) was calculated as previously described³¹.

Mast Cell Quantification. Jejunum and ileum were collected and processed for MC quantification by chloroacetate esterase (CAE) staining, as described previously²³.

ELISA Measurements. Mouse MC protease 1 (mMCPT-1) serum levels were measured by ELISA according to the manufacturer's instructions (eBioscience, San Diego, CA, USA).

Cell Lines and Culture. EA.hy926 cell line (human VE cell line) (ATCC, Manassas, VA, USA) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (Atlanta Biological), nonessential amino acids (0.1 mM), sodium pyruvate (1 mM), HEPES (10 mM), and 1X penicillin and streptomycin (50 μ g/mL) in a humidified incubator (5% CO₂, 37°C)^{40, 41}. All reagents were obtained from Gibco, Life Technologies (Waltham, MA, USA) unless stated otherwise.

ABL Kinase Activity Assay. 3x10⁶ cells/well were plated in a 6-well plate and grown for 24 h. Cells were pretreated with IL-4 (10 ng for 24 h; Peprotech, Rocky Hill, NJ, USA) before imatinib treatment (10-100 μ M for 3 h; Santa Cruz Biotechnology, TX, USA) followed by histamine (10 μ M for 30 min; Sigma–Aldrich, St. Louis, MO, USA) stimulation. To assess for ABL1 kinase activity, ABL1 was immunoprecipitated from treated EA.hy926 cells for a subsequent measurement of CRK-GST phosphorylation. The ABL1 activation assay was

performed as describe by Dudek *et al.*⁴².

In Vitro Permeability. EA.hy926 were plated on transwells (12-mm diameter, 0.4- μ m pore; Polyester Membrane Insert, Corning, NY, USA), cells were seeded and cultured until a monolayer formed. Transendothelial resistance (TER) was monitored with an EVOM/Endohm (WPI Inc, Sarasota, FL, USA), and endothelial monolayers with a TER >100 Ω /cm² were used for all experiments. Monolayers were mounted between the hemi-chambers of an Ussing apparatus (P2300 Ussing chamber, Physiologic instruments, CA, USA), and 0.33 cm² of cell monolayer was exposed to 6 mL of Krebs buffer at 37°C. The transendothelial potential difference was detected with two paired electrodes that contain 4% agar in 3M KCl. The electrodes were connected to a voltage clamp amplifier (VCC MC8, multichannel voltage/current clamp, Physiologic instruments, CA, USA). The electrode potential difference and fluid resistance were compensated before mounting the monolayer onto the chamber. To establish equilibrium, the potential difference was continuously monitored under open-circuit conditions for 15 min. Thereafter, the monolayers were voltage-clamped at 0 mV while continuously measuring the short circuit current (Isc). Voltage pulses (10-mV square waves sustained for 200 ms) were delivered every 20 s to yield a current response for calculating resistance across a mucosa (resistance) using Ohm's law. IL-4- (100 ng/mL; 24 h), histamine- (100 mM; 30 min), and vehicle-stimulated endothelial monolayers were placed in Ussing chambers, where TER was measured as described previously⁴³. To measure paracellular flux, horse radish peroxidase (HRP) solution was left for 30 min at room temperature (RT) before the assay. The assay was started by adding 4 μ g/mL of HRP to the apical side of the monolayers to measure flux from the apical to the basolateral direction. Afterward, aliquots of 250 μ L from the basolateral side were collected at 30, 60, 90, 120, 150, and 180 min. HRP was assayed colorimetrically (BD OptEIA™, CA, USA) according to the manufacturer's instructions.

Lentiviral Transduction. Bacterial glycerol stock (Sigma–Aldrich, St. Louis, MO, USA) containing shRNA plasmid DNA was used to generate lentiviral particles containing target shRNA. In brief, to expand the bacterial glycerol stock 1 μ L was incubated at 37°C for 30 min in terrific broth (TB) without antibiotic then cultured in Luria broth (LB) agar with

ampicillin (100 µg/ml). After an overnight incubation, a single colony was expanded by incubating with 3 mL of LB plus ampicillin for 8-10 h and then moved to 300 mL LB with ampicillin and incubated overnight. ShRNA plasmid DNA was extracted using QIAGEN Plasmid Maxi Kit (Hilden, Germany). Afterward, the lentivirus partials were produced by CCHMC's Viral Vector Core. Cells were transduced with 5 µL of concentrated lentiviral particles or control shRNA lentiviral particles containing an empty vector (PLKO.1). Transduction was carried out with media in the presence of 10 µg/mL of polybrene (Sigma-Aldrich, St. Louis, MO, USA). The cells were incubated for 24 h at 37°C, and then the media was replaced with fresh media. Cells were selected with 10 µg/mL puromycin (Gibco, Grand Island, NY, USA) two days after transduction.

Flow Cytometry. Mice were injected with IL-4C (1 µg of IL-4 + 5 µg anti-IL-4 mAb) or vehicle. Twenty-four hours later, mice were sacrificed, and the spleen was harvested. To prepare a single-cell suspension, the spleen was homogenized, and then the suspension was filtered with a 40-µm pore cell strainer. Afterward, the cell solution was centrifuged (5 min/1200 rpm). Red blood cells (RBCs) were lysed with RBC lysing buffer (Sigma-Aldrich, St. Louis, MO, USA) and incubated for 2 min at 37°C; the reaction was stopped using 30 mL 1X HBSS buffer (Gibco, Grand Island, NY, USA). Cells were centrifuge and re-suspended with 200 µL FACS buffer (0.1% sodium azide, 2 % fetal calf serum, and 1X PBS) at 2×10^6 cells/mL and stained with anti-mouse MHC II (I-A) FITC and anti-mouse/human CD45R/B220 PerCP/Cy5.5 antibodies (BioLegend, San Diego, CA, USA). Following fixation with 1% paraformaldehyde (30 min at 4°C), the cells were re-suspended in 200 µL FACS buffer. Flow cytometry data acquisition and analysis were performed on BD LSR Fortessa using FACS Diva software (BD Biosciences, Mountain View, CA, USA).

Immunofluorescent Assay. Antibodies and concentrations used were as follows: anti-IL-4Rα at 1:400 (Santa Cruz, # sc-28361), anti-MECA32 at 1:100 (Novous Biologicals, # NB100-77668). Immunofluorescence was done as previously described⁴⁴, except that the antigen retrieval step was done using a decloaking chamber for 30 s at 125°C.

Statistical Analysis. Data are expressed as mean $\pm$ standard deviation (SD), unless otherwise stated. In experiments comparing multiple experimental groups, statistical differences between groups were analyzed using the one/two-way ANOVA parametric test. In experiments comparing two experimental groups, statistical differences between groups were determined using a Student's t-test. A $P < 0.05$ was considered significant. Correlation analysis was performed using the Spearman's rank correlation coefficient. All analyses were performed using Prism 7.0 software (GraphPad Software Inc., San Diego, CA, USA).

Results

IL-4 enhances histamine-induced hypovolemic shock via vascular endothelium-dependent IL-4R α chain signaling

We first examined the effect of IL-4 on histamine-induced increase in hemoconcentration and hypothermia (as an indication for anaphylactic shock severity). WT mice were i.v. injected with IL-4C (a long-acting formula IL-4 complexed with anti-IL-4³⁹) or vehicle and 24 h later received an i.v. challenge of histamine. Treatment of mice with IL-4C alone did not impact hemoconcentration nor impact body temperature (Figure 1). In contrast, histamine alone induced an increase in hemoconcentration that was associated with hypothermia (Figure 1). Notably, this response was potentiated by pretreatment with IL-4C, suggesting that IL-4 amplifies histamine-induced hypovolemic shock (Figure 1). To determine whether IL-4 amplification of the histamine response was via direct modulation of VE barrier function, we examined the effect of histamine and IL-4 on the human VE cell line EA.hy926^{40, 41}. We show that TER decreased and flux of HRP (40 kDa) increased in histamine-stimulated compared to unstimulated EA.Hy926 cells (Figure 2A-B). Importantly, the histamine-induced increase in endothelial cell permeability was enhanced by pretreatment with IL-4 (Figure 2A-B). Collectively, these data suggest that IL-4 amplifies histamine-induced VE permeability through a direct activation of VE cells.

To identify the requirement of IL-4 signaling on VE cells in the exacerbation of histamine-induced hypovolemic shock in vivo, we took a transgenic approach using the VE-specific promoter of the junction protein cadherin-5 (also called VE-cadherin)³⁴. Cadherin-5^{Cre} IL-4R α ^{fl/fl} mice (referred to as VE ^{Δ IL-4R α}) lack the expression of the IL-4R α chain on the VE compartment, whereas Cadherin-5^{WT} IL-4R α ^{fl/fl} mice (referred to as VE^{IL-4R α WT}) were used as a WT control (Supplemental Figure S1). Mice received an i.v. injection of either vehicle or IL-4C and 24 h later received an i.v. injection of histamine; evidence of hypovolemic shock and hypothermia were evaluated (Figure 3A). I.v. administration of histamine induced hypothermia and increased hemoconcentration in both VE^{IL-4R α WT} and VE ^{Δ IL-4R α} (Figure 3B-C). Pretreating VE^{IL-4R α WT} with IL-4C enhanced histamine-induced hypothermia and hemoconcentration (Figure 3B-C). However, the IL-4C amplification of the histamine-induced hypovolemic shock was ablated in VE ^{Δ IL-4R α} mice (Figure 3B-C). Consistent with

previous studies, we found that IL-4C alone had no effect on body temperature nor on hemoconcentration³¹ (Figure 3B-C). To confirm IL-4C activity in VE^{ΔIL-4Rα} mice, we evaluated the expression of MHC II on B cells (B220⁺) after IL-4C treatment. We showed that the expression of MHC II on B cells increased in IL-4C-treated compared to vehicle-treated mice (Supplemental Figure S2). Notably, IL-4C treatment induced a comparable increase in MHC II expression in the VE IL-4Rα^{WT} and VE^{ΔIL-4Rα} mice (Supplemental Figure S2). These studies indicate that IL-4 amplification of histamine-induced hypovolemic shock was dependent on IL-4 signaling on the VE compartment.

Interestingly, clinical and experimental studies indicate that gain-of-function mutations in the IL-4Rα chain have been associated with increased susceptibility to atopic disease and enhanced allergic inflammatory responses⁴⁵⁻⁵⁰. Given the observation that IL-4/IL-4Rα signaling plays an important role in histamine-induced hypovolemic shock, we hypothesized that mice with a gain-of-function in IL-4 receptor signaling would have increased severity of histamine-induced hypovolemic shock. To test this, we employed IL-4Rα^{Y709F} mice³³, which have an activating variant IL-4Rα chain (Figure 3D-F). Histamine challenge of IL-4Rα^{Y709F} mice induced a hypovolemic shock response equivalent to that observed in WT mice (Figure 3E and F). IL-4C treatment amplified histamine-induced hypothermia and hypovolemic shock in both IL-4Rα^{Y709F} and WT BALB/c mice (Figure 3E and F). Notably, we observed a significantly greater IL-4C-induced amplification of the histamine-induced hypothermic response in the IL-4Rα^{Y709F} mice than BALB/c mice, and this increased hypothermic response was associated with increased hemoconcentration (Figure 3E and F). These data indicate that enhanced IL-4Rα signaling can exacerbate histamine- and IL-4-induced hypovolemic shock.

Vascular endothelial IL-4Rα chain deletion attenuated hypovolemic shock induced by passive oral antigen-induced anaphylaxis

To confirm the requirement of IL-4Rα chain expression on VE in IgE/MC-mediated anaphylaxis, we used a passive oral antigen-triggered IgE-mediated anaphylaxis model⁵¹. Cadherin-5^{Cre} IL-4Rα^{fl/fl} mice were backcrossed on the iIL-9Tg background (referred to as iIL-9Tg VE^{ΔIL-4Rα}). iIL-9Tg VE^{ΔIL-4Rα} mice and iIL-9Tg VE^{IL-4Rα WT} mice were injected with

anti-TNP IgE with IL-4C and then administered TNP-OVA 24 h later (Figure 4A). Oral gavage of TNP-OVA increased hemoconcentration and induced shock in both iIL-9Tg $VE^{IL-4R\alpha WT}$ and iIL-9Tg $VE^{\Delta IL-4R\alpha}$ mice treated with anti-TNP IgE (Figure 4B-C). Notably, pretreatment of the iIL-9Tg $VE^{IL-4R\alpha WT}$ mice with IL-4C exacerbated hypothermia and hemoconcentration; however, the IL-4 enhancement was absent in iIL-9Tg $VE^{\Delta IL-4R\alpha}$ mice (Figure 4B-C). The reduced disease in iIL-9Tg $VE^{\Delta IL-4R\alpha}$ mice could not be explained by decreased MC activation or levels or immunoglobulin levels, as serum mMCPT-1 levels, intestinal MC levels, and immunoglobulin (Ig) (IgE and IgG1) were comparable between groups (Figure 4D; Supplemental Figure S3A-E). Collectively, these data indicate that IL-4 enhancement of passive oral antigen-triggered anaphylaxis is dependent on the VE IL-4R α chain.

Histamine and IL-4 modulation of paracellular leakage in the human vascular endothelial cell line (EA.hy926) is ABL1 kinase-dependent

We were next interested in defining the signaling pathways involved in IL-4C amplification of the histamine-induced VE dysfunction. Recent studies have identified roles for members of the ABL family of non-receptor tyrosine kinases (ABL1 and ARG) and RhoA and ROCK in histamine-induced VE leakage; therefore, we examined histamine and IL-4C induction of ABL1 kinase activity^{15, 52}. Employing the immortalized human endothelial cell line (EA.hy926), we firstly examined ABL1 kinase activity after histamine and IL-4C stimulation. We found that histamine or IL-4C alone stimulated ABL1 kinase activity as indicated by GST-CRK (a known ABL1 substrate) phosphorylation (Figure 5A). Notably, EA.hy926 cell costimulation with both histamine and IL-4C amplified ABL1 kinase activity (Figure 5A). To determine the requirement of ABL1 kinase in the histamine/IL-4C-induced barrier function, we used a pharmacologic (imatinib) and shRNA ABL1 kinase knockdown approach. Histamine stimulation of EA.hy926 cells reduced the TER in a dose dependent manner (Supplemental Figure S5A). Pretreatment of EA.hy926 cells with Imatinib antagonized histamine-induced reduction in TER and paracellular HRP-flux (Supplemental Figure S4B and C). Pretreatment of EA.hy926 cells with imatinib blocked IL-4 amplification of histamine-induced VE barrier dysfunction in a dose-dependent manner (Figure 5C-D). Notably, the decreased VE response in the presence of imatinib was associated with

decrease of ABL1 kinase activity (Figure 5B). Consistent with these observations, pretreating shRNA *ABL1*–transduced EA.hy926 cells with histamine or histamine and IL-4 did not induce VE barrier dysfunction (Figure 5F-G). Taken together, these findings suggest that histamine-induced, IL-4–enhanced paracellular leakage is dependent on the VE ABL kinase, ABL1 kinase.

Vascular endothelial ABL1 kinase involvement in passive anaphylaxis in iIL-9Tg VE^{ΔABL1} mice

We next examined the requirement of the VE ABL1 kinase in passive anaphylaxis by employing mice lacking ABL1 expression in the VE compartment. Tie2^{Cre} transgenic mice have been used as a genetic tool for the analyses of endothelial cell–specific gene targeting³⁶. Therefore, we employed Tie2^{Cre} ABL1^{f/f} mice on an iIL9Tg background (referred to as iIL-9Tg VE^{ΔABL1}) and WT control iIL9Tg Tie2^{WT} ABL1^{f/f} (referred to as iIL-9Tg VE^{ABL1 WT}). iIL-9Tg VE^{ΔABL1} mice and iIL-9Tg VE^{ABL1 WT} mice received an i.p. injection with vehicle or imatinib 30 min before i.v. injection with anti-IgE mAb (EM95) (Supplemental Figure S5A). In iIL-9Tg VE^{ABL1 WT} mice, i.v. anti-IgE mAb induced hypothermia and increased hemoconcentration, with these changes being sensitive to imatinib inhibition (Supplemental Figure S5B-C). Importantly, iIL-9Tg VE^{ΔABL1} mice showed an attenuated hypothermia that was comparable to the hypothermia observed in iIL-9Tg VE^{ABL1 WT} treated with imatinib (Supplemental Figure 5B). Importantly, levels of MC activation and degranulation were similar between groups (Supplemental Figure S5D). Collectively, these data indicate a requirement for VE ABL1 in hypovolemic shock induced by IgE.

ABL kinase inhibitor attenuated passive oral antigen–induced anaphylaxis

To determine whether the ABL kinase inhibitors could suppress the development of symptoms of oral antigen–induced IgE/MC-mediated reactions in mice, we examined the effect of imatinib in a passive oral antigen–triggered, IgE-mediated anaphylaxis model²³. Evidence of anaphylaxis was evaluated in iIL-9Tg mice who received an i.v. injection of anti-TNP IgE and 24 h later received either vehicle or imatinib (16-70 μg/kg per mice; 2 h) and subsequently received oral gavage with TNP-OVA (Figure 6A). In vehicle-treated mice, oral challenge of TNP-OVA induced shock, which was associated with an increase in

hemoconcentration (Figure 6B-C). Pretreating mice with imatinib prior to TNP-OVA administration protected the mice from hypothermia and hemoconcentration (Figure 6B-C). Importantly, imatinib-mediated protection was not associated with reduced MC degranulation as levels of the mMCPT-1 were comparable between all groups (Figure 6D).

Imatinib is known to target c-KIT, which is required for optimum MC degranulation^{53, 54}. Though mMCPT-1 levels were not significantly different between groups, the levels of mMCPT-1 in the Imatinib-treated mice were lower than vehicle-treated mice (Figure 6D). To confirm that the imatinib-mediated protective effects were not due to decreased MC degranulation, we examined the relationship between mMCPT-1 levels and the onset of hypothermic response (Figure 6E). We show that 10 out of 11 mice in the vehicle-treated, TNP-OVA-challenged group with mMCPT-1 >10,000 ng/mL were hypothermic ($\geq 1.5^\circ\text{C}$ temperature loss) and that no mice (0/6) with <10,000 ng/mL mMCPT-1 developed hypothermia (Figure 6E-F). Therefore, if we set a cut-off threshold of $\geq 10,000$ ng/mL mMCPT-1 as a level of MC degranulation sufficient to induce symptoms of anaphylaxis, this provides us with $\geq 90\%$ confidence that mice with $\geq 10,000$ ng/mL mMCPT-1 will develop evidence of anaphylaxis. Examining mMCPT-1 levels from the imatinib-treated, TNP-OVA-challenged group revealed that 10/13 mice had $\geq 10,000$ ng/mL mMCPT-1 and were protected from hypothermic response ($\geq 1.5^\circ\text{C}$ temperature loss) (Figure 6E-F). Therefore, the protection from hypothermia in those mice is unlikely due to an effect on MC degranulation. mMCPT-1 is a surrogate for MC degranulation and the hypothermic response is histamine-dependent; we therefore examined histamine levels after TNP-OVA administration in anti-TNP IgE-primed mice treated with vehicle or imatinib. TNP-OVA administration induced a significant increase in histamine levels in the vehicle-treated group, which was comparable with histamine levels in the imatinib-treated group (Figure 6G). These studies suggest that imatinib protection is mainly via inhibiting ABL kinase activity independent of MC degranulation. Collectively, these data indicate that imatinib can reduce the severity of IgE/MC-driven anaphylaxis and that this effect is independent of MC degranulation.

ABL kinase inhibitor attenuated pre-existing oral antigen-induced anaphylaxis

Next, we examined the effect of the ABL kinases pharmacologic antagonist, imatinib, on mice with established food allergy²³. BALB/c WT mice were primed i.p. with OVA-alum and subsequently challenged by repeated oral gavage with OVA (Figure 7A). By the 6th allergen challenge, mice developed symptoms of food induced-anaphylaxis, including hypothermia and diarrhea^{51, 55, 56}. Prior to the 7th challenge, mice were stratified into two groups, with some mice receiving vehicle while others were pretreated with imatinib (50-75 µg/kg i.p.; 2 h) and then subsequently challenged with OVA (Figure 7A). Oral antigen challenge induced hypothermia in vehicle-treated mice (Figure 7B-C). In contrast, pretreating mice with imatinib protected the mice from anaphylactic shock; the level of hypothermia and diarrhea were significantly reduced in the imatinib-treated mice compared to the vehicle-treated mice (Figure 7B-D). Notably, the protection from hypothermia was associated with reduced hemoconcentration (Figure 7E) in the presence of comparable MC degranulation (mMCPT-1 levels) (Figure 7F), suggesting that imatinib attenuates histamine-induced fluid extravasation. Collectively, these studies indicate that pretreating mice with imatinib can protect from the development of a severe food-induced reaction in mice with a previous history of severe reactions.

Discussion

Clinical studies suggest a relationship between increased levels of IL-4 and histamine and severity of anaphylaxis^{22, 57, 58}. This was supported animal-based studies indicating that IL-4 can exacerbate IgE-mediated reactions³¹. IL-4 alone has been shown to induce vascular endothelial barrier dysfunction *in vitro*^{27, 28}. However, the cellular target of IL-4 action and the underlying IL-4R α -dependent signaling processes involved in the amplification of the IgE-MC-histamine driven VE barrier dysfunction and fluid extravasation was not previously fully understood.

Herein, we show that: 1) IL-4 increased histamine-induced fluid extravasation, and this increase positively correlated with anaphylaxis severity (hypovolemic shock); 2) IL-4 enhancement of histamine- and IgE/MC-induced systemic anaphylaxis symptoms was dependent on signaling through the VE IL-4R α chain; 3) IL-4 enhanced histamine-induced ABL1 activity and VE barrier dysfunction in human VE cells, which could be antagonized by ABL kinase inhibition; and 4) pharmacologic inhibition of ABL kinases by imatinib protected mice from passive and established oral antigen-induced anaphylaxis via suppressing VE barrier dysfunction and fluid extravasation.

^{59, 60}

Clinical evidence supports possible involvement of hypovolemia and distributive and cardiogenic shock in human anaphylaxis⁷, however mounting evidence identifies hypovolemic shock as an important contributor to the cardiovascular collapse that leads to the severe, life-threatening anaphylactic phenotype.⁵⁹⁻⁶¹ Consistent with these observations, aggressive fluid resuscitation has been shown to be an effective treatment for anaphylaxis occurring under anesthesia^{8, 60}. Similarly, In the mouse, anaphylaxis causes fluid extravasation, leading to severe hypovolemia and fatal tissue hypoxia^{62, 63}. IgE-mediated MC activation promotes the secretion of vasoactive amines (histamine and serotonin) and lipid mediators (leukotrienes, prostaglandins) that are thought to induce VE dysfunction and the hypovolemic shock^{64, 65}. Consistent with this observation, histamine treatment of mice increases hemoconcentration and hypovolemia-induced shock³¹ and conversely, treating mice with H1R antagonists with or without concurrent H2R blockade attenuates IgE/MC-mediated hypovolemic shock^{25, 66}. Herein, we demonstrate that IL-4

can amplify the IgE-mediated, histamine-induced fluid extravasation through priming of the vascular endothelial compartment. Previous studies have revealed an important role for IL-4 and IL-4R α signaling in IgE-mediated anaphylaxis; however, this was predominantly thought to be through its effect on the hematopoietic compartment driving IgE-isotype switch and the associated CD4⁺ Th2 and mast cell response in anaphylaxis^{31, 67-69}. We show that IL-4 also acts on the non-hematopoietic compartment, namely the VE to increase the sensitivity of the VE to histamine. This is supported by our observation, that levels of mMCPT-1 were comparable between iIL-9Tg VE ^{Δ IL4R α} and iIL-9Tg VE^{IL-4R α WT} even though the iIL-9Tg VE^{IL-4R α WT} mice experienced greater hypovolemic shock. Notably, the mice lacking the expression of the IL-4R α chain on their VE developed histamine-induced anaphylactic shock; however, they were protected from the IL-4-induced amplification of anaphylaxis symptoms, suggesting that VE IL-4R α chain signaling is not required for histamine-induced hypovolemia but exacerbates the histamine-dependent component.

IL-4 can signal through either the type I (IL-4R α chain and γ c chain) and type II (IL-4R α chain and IL-13R α 1 chain) IL-4 receptor pathways. The previous demonstration of IL-4-enhanced anaphylaxis in RAG2/ γ c-deficient mice (which lack B cells, T cells, MCs, eosinophils, and NK cells) indicates the involvement of the IL-4 type II receptor and not the IL-4 type I receptor³¹. Interestingly, mutations in the *IL4RA* gene have been associated with susceptibility to atopic disease⁴⁵⁻⁵⁰. Importantly, gain-of-function mutations in the IL-4R α chain (E375A, S478P, or Q551R) have been linked with enhanced allergic inflammatory responses including increased MCs and elevated IgE levels⁴⁵⁻⁵⁰; Consistent with these observations, murine-based studies have revealed that mutations in the IL-4R α that promote gain-of-function (IL-4R α ^{Y709F}, IL-4R α ^{Q576R}, and IL-4R α ^{Y500F}) led to enhanced allergic inflammatory phenotypes including asthma, IgE-mediated responses, and food allergy^{33, 70-73}. We show that pretreating IL-4R α ^{Y709F} mice with IL-4 enhanced histamine-induced hypovolemic shock, suggesting that gain-of-function mutations can increase severity of food-induced anaphylaxis. Relevant to this study, Burton *et al.* and Mathias *et al.* demonstrated that IL-4R α ^{Y709F} mice have an enhanced sensitivity to food allergens and that the enhanced IgE/MC-mediated systemic anaphylaxis in IL-4R α ^{Y709F} mice is due to a direct

effect on MCs^{71, 74}. Of note, Burton *et al.* showed that IL-4R α ^{Y709F} mice had a similar responsiveness to histamine to that of WT mice, suggesting no role for gain-of-function mutations in the IL-4R α chain in severity of anaphylaxis⁷⁴. However, these studies were performed in the absence of IL-4 treatment. We show enhanced histamine-induced anaphylaxis with exogenous IL-4 treatment in IL-4R α ^{Y709F} mice, suggesting that gain-of-function mutations in IL-4R α signaling can affect not only hematopoietic cell function but also non-hematopoietic cell function and severity of food-induced reactions.

We have previously demonstrated that the onset of a severe food-induced anaphylaxis in mice requires increased type 2 cytokines, food-specific IgE, and an intestinal mastocytosis²³. Antigen sensitization and repeated food antigen challenge of sensitized mice promotes an intestinal antigen-specific CD4⁺ Th2 cell / ILC2 response, which is thought to drive IL-9 producing mucosal MCs (MMC9 cells) and lead to the intestinal mastocytosis^{75, 76}. We speculate that the repeated antigen challenge promotes heightened IL-4 levels, leading to the increased sensitivity of the VE compartment to MC-derived mediators. The cellular source of IL-4 during these allergic conditions is likely to be CD4⁺ T cells and basophils^{77, 78}. Repeated oral antigen exposure drives the antigen-specific CD4⁺ type 2 response and generation of IL-4-dependent, antigen-specific IgE and mastocytosis^{75, 76, 79}. Concurrently, oral antigen exposure will also lead to IgE-Fc ϵ RI ligation on basophils and MCs. The demonstration that IgE-mediated IL-4 responses are dependent on Fc ϵ RI and can occur independently of MCs but not basophils suggest that basophils are another likely source of IL-4^{77, 78, 80}. Studies of mediators in human anaphylaxis revealed that in patients with anaphylaxis, histamine levels peaked during emergency department arrival and that histamine levels correlated with reaction severity²². From our observations, one would predict that IL-4 levels should also correlate with reaction severity; however, IL-4 has previously been reported to not correlate with reaction severity. Interestingly, the most sensitive known *in vivo* effect of IL-4 is upregulation of B cell MHC II expression^{39, 81}, and IL-4 doses barely capable of inducing an increase in B cell class II MHC expression have been shown to be sufficient to enhance anaphylaxis³¹. These data suggest that incremental changes in IL-4 may be sufficient to enhance histamine-induced VE dysfunction and anaphylaxis severity and may explain the lack of association between IL-4

levels and severity of anaphylaxis in humans.

The mechanism by which IL-4 modulates histamine responses is not fully elucidated. We show that IL-4 amplifies histamine-induced ABL1 kinase activation and that blockade of ABL1 kinase genetically or pharmacologically abrogated histamine-induced VE dysfunction and leak, suggesting that IL-4 modulation of histamine-induced responses is likely through increased ABL1 activity. The ABL family of non-receptor tyrosine kinases, including both ABL1 and ARG, have been implicated in the regulation of endothelial barrier function⁶. Notably, Chislock *et al.* demonstrated a requirement for activation of the ABL kinases in endothelial permeability induced by thrombin and Vascular endothelial growth factor (VEGF) in human microvascular endothelial cells (HMVECs)¹⁵. VE-cadherin is a critical requirement for assembly and stability of the VE-cadherin–catenin–cytoskeleton complex and maintenance of endothelial barrier function^{12, 13, 82}. Histamine is thought to destabilize the VE-cadherin–catenin complex through activating protein kinase C (PKC) and Rho-associated protein kinase (ROCK) and subsequent downstream activation of myosin light chain (MLC), resulting in reduced endothelial cell adhesiveness and increased paracellular permeability⁸³. Pharmacologic and genetic blockade of ABL1 has been shown to reduce myosin light chain kinase (MLCK) phosphorylation and VE barrier dysfunction after VEGF and thrombin stimulation¹⁵.

Collectively, our data suggest that blockade of ABL1 kinase-dependent processes does not ablate anaphylaxis but rather dramatically reduces the severity of the IgE-mediated reaction. Based upon these observations, we suggest that inhibiting ABL1 kinase-dependent processes may be a therapeutic target for diminishing or preventing severe IgE-mediated anaphylactic reactions and could be used in conjunction with desensitization protocols such as sublingual immunotherapy and oral immunotherapy to minimize the risk of possible severe adverse reactions. Recently, there has been significant development of second- and third-generation tyrosine kinase inhibitors (TKIs) for chronic myeloid leukemia (CML), including second-generation TKIs (dasatinib, nilotinib, and bosutinib) and third-generation TKIs (Ponatinib and GNF-2, -5, and -7) that act as ATP-binding site competitive inhibitors and allosteric ABL inhibitors that have distinct efficacy, specificity, and long-term

safety^{84, 85}. Given that TKIs such as Imatinib bind and inhibit c-KIT-dependent TK signaling which is essential for MC development and maturation, an added advantage of these class of inhibitors is that they can target both MC levels and the VE compartment to attenuate IgE/MC-mediated reactions⁸⁶⁻⁸⁸. Consistent with this argument, long-term treatment with imatinib caused a profound MC deficiency in mice and patients with PH⁺ CML⁸⁹. Furthermore, a recent study examined the clinical utilization of Imatinib for treatment of severe refractory asthmatic patients and demonstrated that Imatinib had excellent efficacy, reducing mast cell counts and activation and asthma symptoms⁹⁰.

In conclusion, we show that IL-4 enhances histamine responses through the VE IL-4R α chain by modulating VE function and amplifying vascular leakage and anaphylaxis phenotypes. We demonstrated that IgE-mediated fluid extravasation and hypovolemic shock in mice are mediated by a histamine-induced, VE ABL kinase-dependent mechanism, possibly via ABL1 kinase. These studies identify that targeting of the ABL1-sensitive VE compartment may be a new pathway for therapeutic intervention and prevention of food-induced severe and life-threatening anaphylaxis and that repurposing TKI inhibitors such as imatinib may prevent food-induced reactions.

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Figure Legends**Figure 1: IL-4 enhancement of histamine-induced shock negatively correlates with fluid extravasation.**

VE^{IL-4Rα^{WT}} (iIL-9^{WT} Cadherin-5^{WT} IL-4Rα^{fl/fl}) mice were injected i.v. with (0.2 mL) of vehicle or IL-4C (1 μg of IL-4 + 5 μg anti-IL-4 mAb) and then challenged i.v. 24 h later with vehicle or histamine (0.4 mg / 200 μL). Shown is Spearman's rank correlation coefficient between hematocrit percentage and the maximum temperature change from 0 to 30 minutes. Each circle represents an individual mouse, n = 3 - 11 per group.

Figure 2: IL-4 amplifies histamine-induced vascular endothelial barrier dysfunction.

Percent reduction in transendothelial resistance (TER) (A) and horseradish peroxidase (HRP) flux (B) of vehicle- or IL-4-treated EA.hy926 cells following histamine stimulation. EA.hy926 monolayers were pretreated with vehicle or IL-4 (100 ng/mL) for 24 hrs and stimulated with histamine (100 μM for 30 min) or vehicle and TER and HRP flux were measured as described in methods. Data are represented as the mean ± SD; n = 3 wells per group from n = 2 independent experiments (A, B). **** P < 0.0001, *** P < 0.0001, ** P < 0.01, ns > 0.05.

Figure 3: Loss and amplification of vascular endothelial IL-4Rα signaling alters IL-4 enhancement of histamine-induced hypovolemic shock.

Experimental regimen (A and D), Maximum Temperature Change (B and E) and Hemacrit (%) (C and F) in iIL-9Tg VE^{ΔIL-4Rα} and iIL-9Tg VE^{IL-4Rα^{WT}} mice (A – C) and IL-4Rα^{Y709F} and IL-4Rα^{WT} mice (D – F) following i.v. injection with vehicle or IL-4C and i.v. challenged with histamine (0.4 mg). Data are represented as the mean ± SD; n = 3 - 10 (B) and n = 3 - 6 mice (C) and n = 9 - 13 mice (E and F) per group from n = 3 - 4 independent experiments. **** P < 0.0001, ** P < 0.001, * P < 0.05, ns > 0.05.

Figure 4: Vascular endothelial IL-4Rα chain deletion attenuated hypovolemic shock in a passive oral antigen anaphylaxis model.

Experimental regimen (A), Maximum Temperature Change (B) Hemacrit (%) (C) and serum mMCPT-1 levels (D) in iIL-9Tg VE^{ΔIL-4Rα} and iIL-9Tg VE^{IL-4Rα^{WT}} mice injected i.v. with IL-4C mixed with anti-TNP IgE and then oral gavaged the next day with TNP-OVA. Data are represented as the mean ± SD; n = 3 - 13 mice per group from n = 4 independent experiments. **** P < 0.0001, *** P < 0.001, ** P < 0.01, * P < 0.05, ns > 0.05.

Figure 5: Histamine and IL-4 induced-vascular endothelial barrier dysfunction is ABL1 kinase dependent.

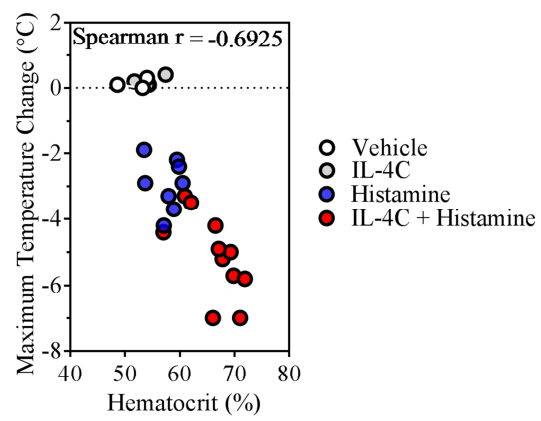
ABL1 kinase activity in vehicle or IL-4 stimulated EA.hy926 cells following (A) histamine stimulation and (B) in the presence of Imatinib. TER (C and F) and HRP-flux (D and G) of EA.hy926 cells and EA.hy926 cells transduced with ABL1 shRNA or empty vector (PLKO-1) pretreated with IL-4 (10 ng/mL) and stimulated with histamine in the presence and absence of imatinib (100 μM). (E) ABL1 levels in EA.hy926 transduced with ABL1 shRNA lentiviral particles or an empty vector (PLKO-1) detected by western blot. (C and D; F and G) Data are represented as the mean ± SD; n = 3 wells per group from two representative experiments. **** P < 0.0001, *** P < 0.001, * P < 0.05, ns > 0.05.

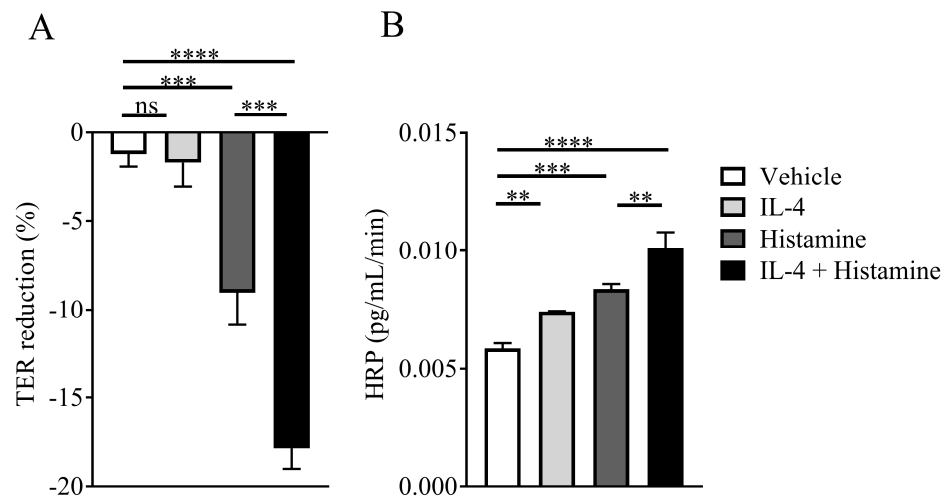
Figure 6: Pharmacologic inhibition of ABL kinases attenuated IgE-mediated hypovolemic shock in a passive oral antigen anaphylaxis model.

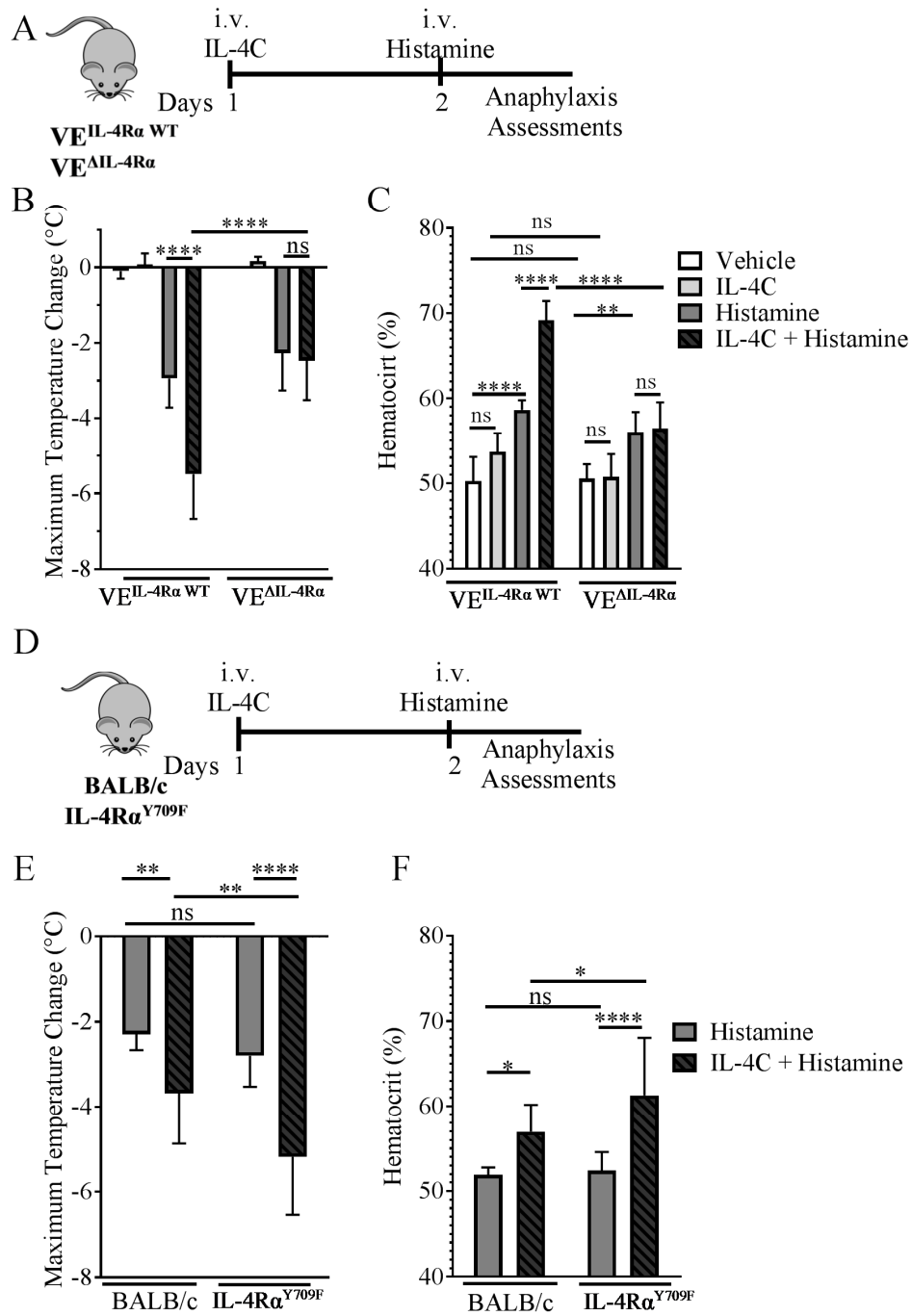
Experimental regimen (A), Maximum Temperature Change (B), Hemacrit (%) (C), and serum mMCPT-1 levels (D) in iIL-9Tg mice injected i.v. with anti-TNP IgE (20 µg) and o.g. with TNP-OVA. (E) mMCPT-1 values verse temperature change following oral challenge and (F) Fisher's exact test analysis. (G) Serum histamine levels in iIL-9Tg mice following anti-TNP IgE and o.g. with TNP-OVA in the presence and absence of Imatinib. Data are represented as the mean ± SD; n = 3 - 9 mice per group (B-D) from n = 3 experiments and n = 5 - 6 mice per group (G). **** P < 0.0001, *** P < 0.001, ** P < 0.01, * P < 0.05, ns > 0.05.

Figure 7: Established food-induced anaphylaxis is attenuated with ABL kinase inhibitor.

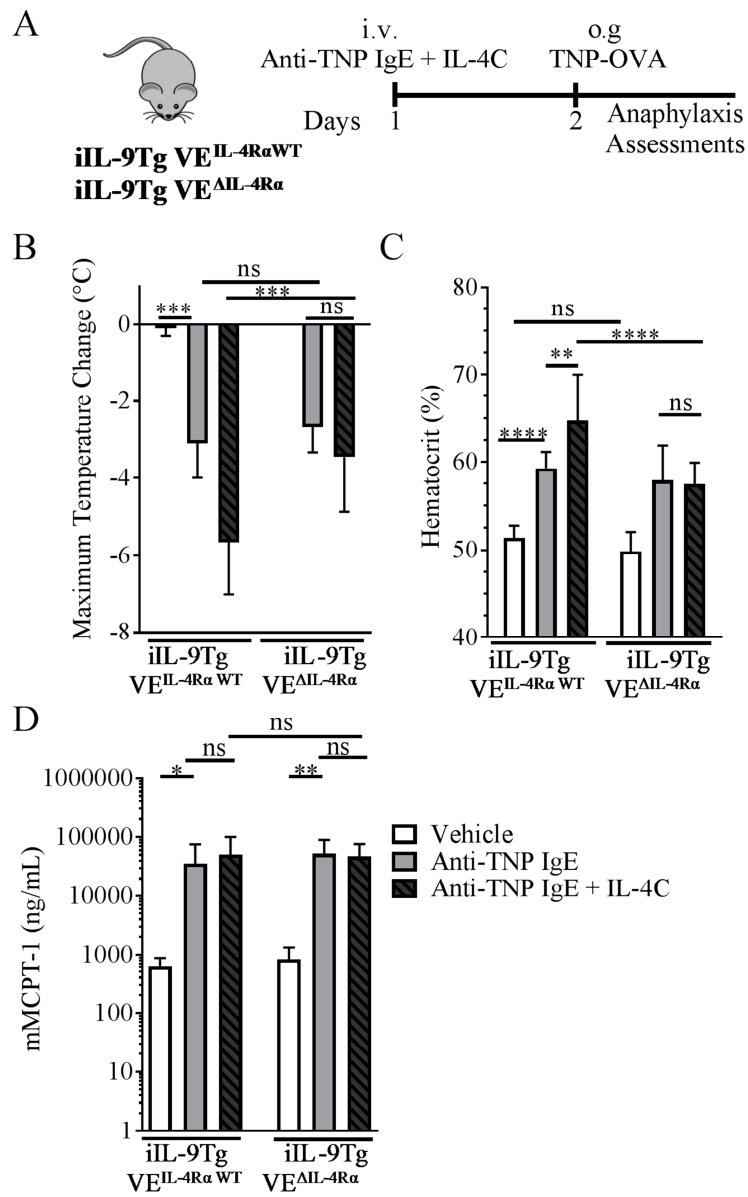
Experimental regimen (A), Maximum temperature change at the 6th and 7th challenge (± Imatinib) (B) the 7th challenge ± imatinib (C), percentage of mice with diarrhea (D), Hemacrit (%) (E) and serum mMCPT-1 levels (F) of OVA-sensitized WT BALB/c mice treated ± imatinib (1.27-1.75 mg/mice). Data are represented as the mean ± SD; n = 8 mice per group (D) and n = 7 - 8 per group except baseline n = 3 mice per group (E, F) from n = 2 experiments. (D) Ratio indicate number of mice with diarrhea : total number of mice. **** P < 0.0001, ** P < 0.01, * P < 0.05, ns > 0.05.

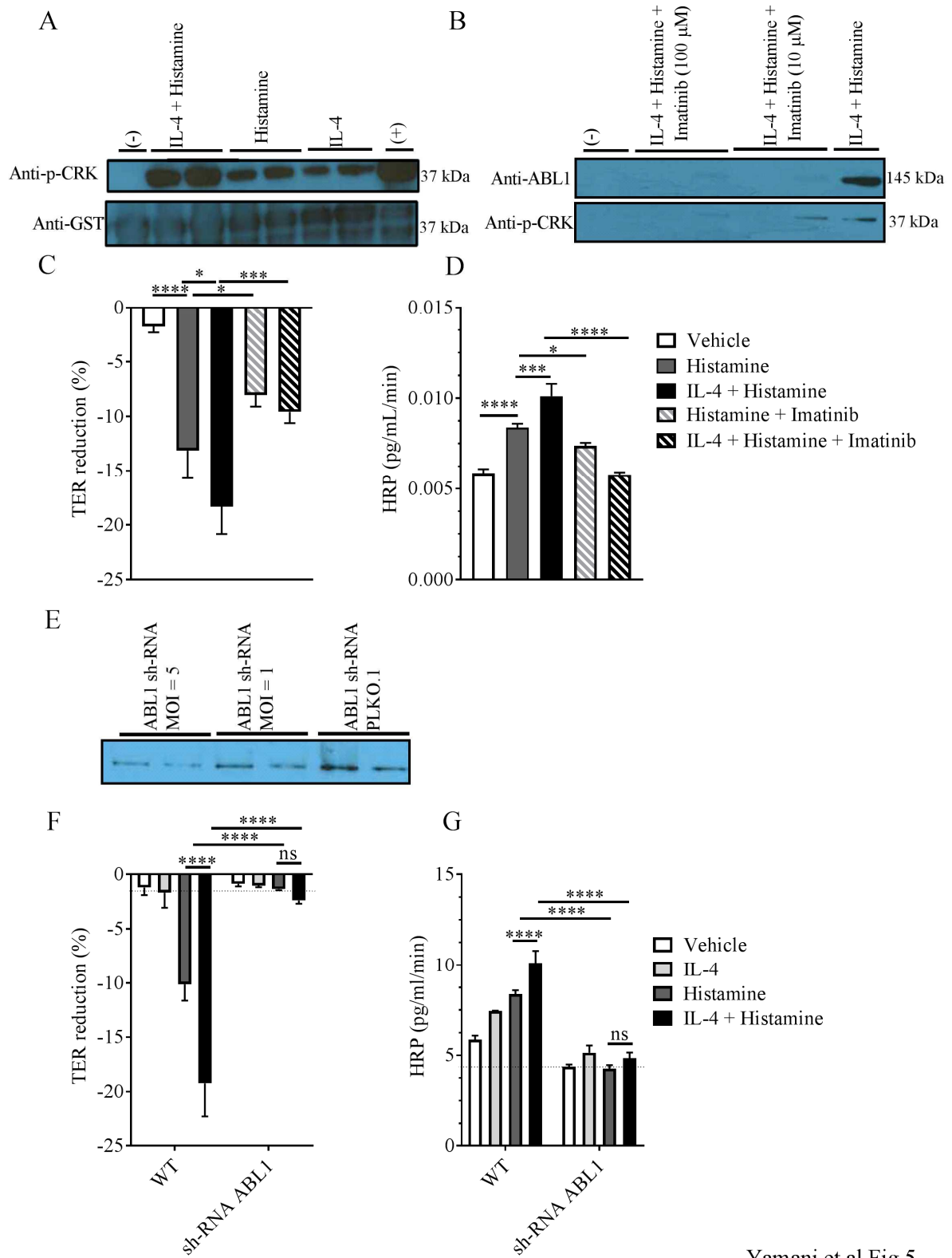




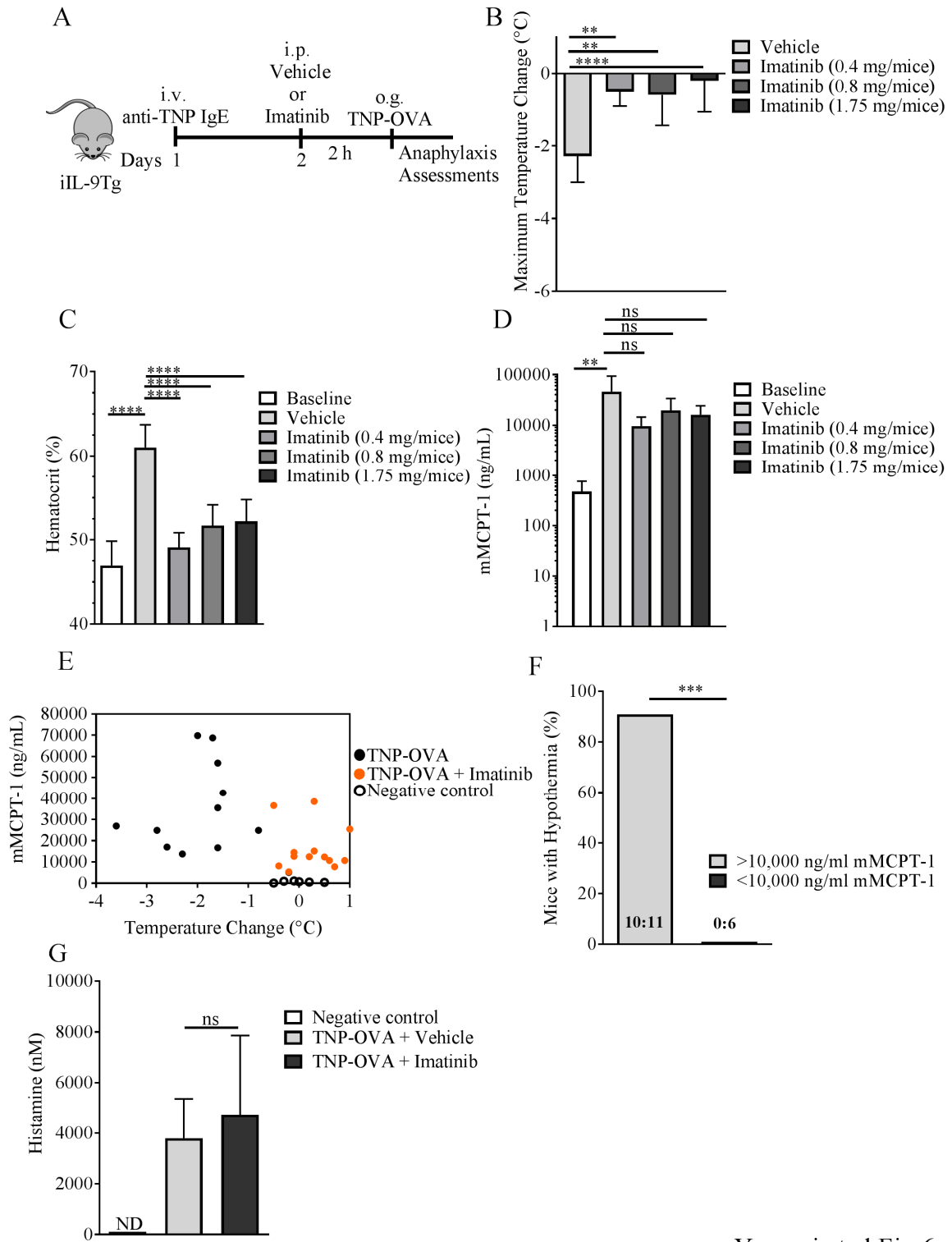


Yamani et al fig 3

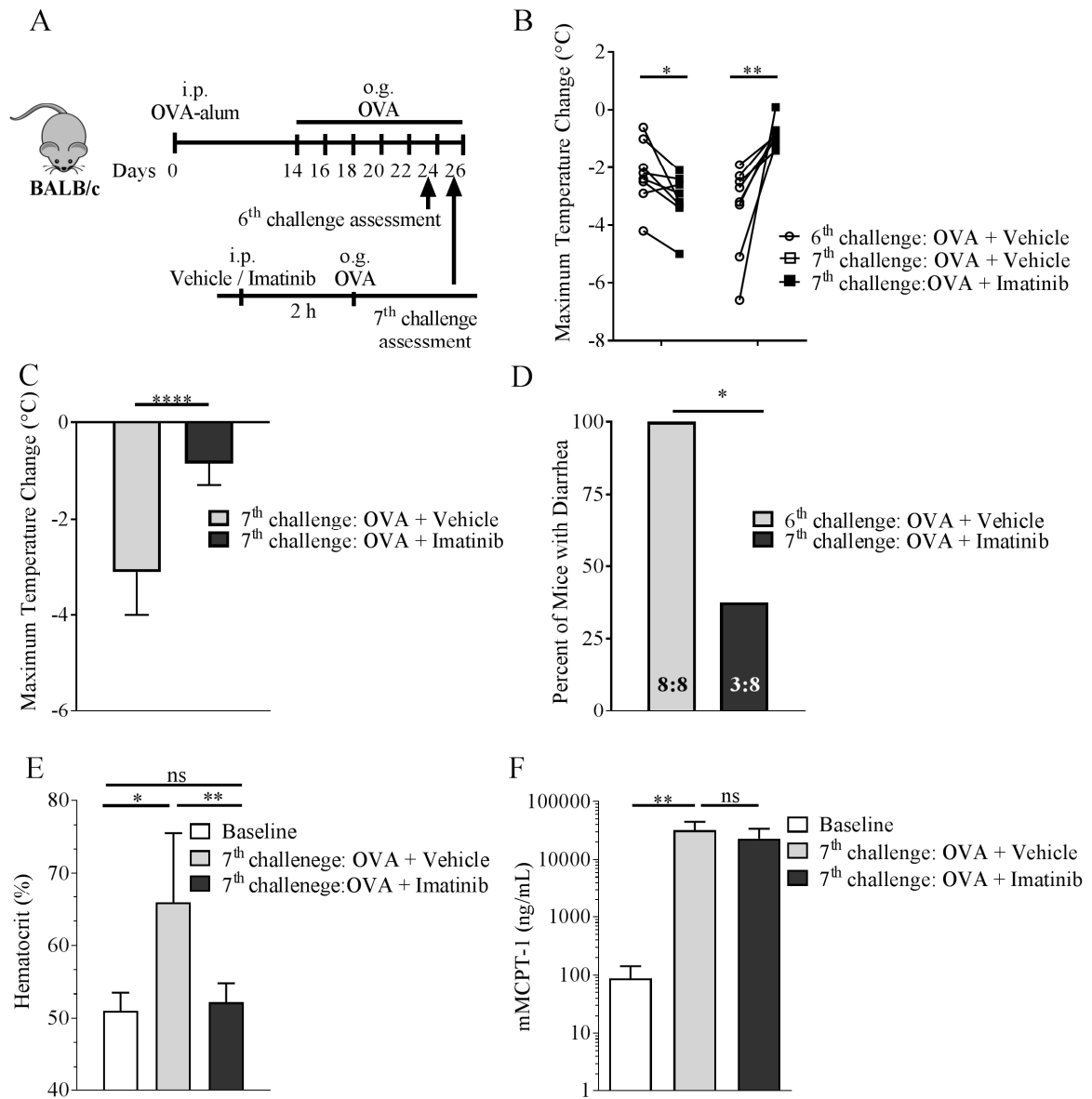




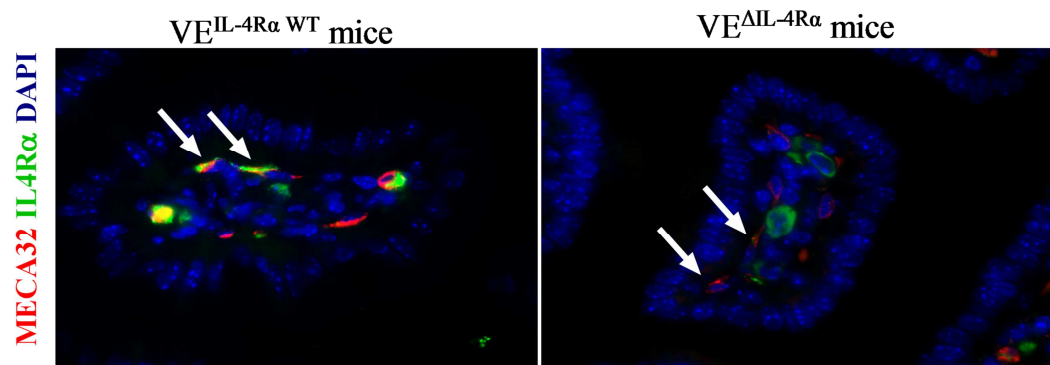
Yamani et al Fig 5

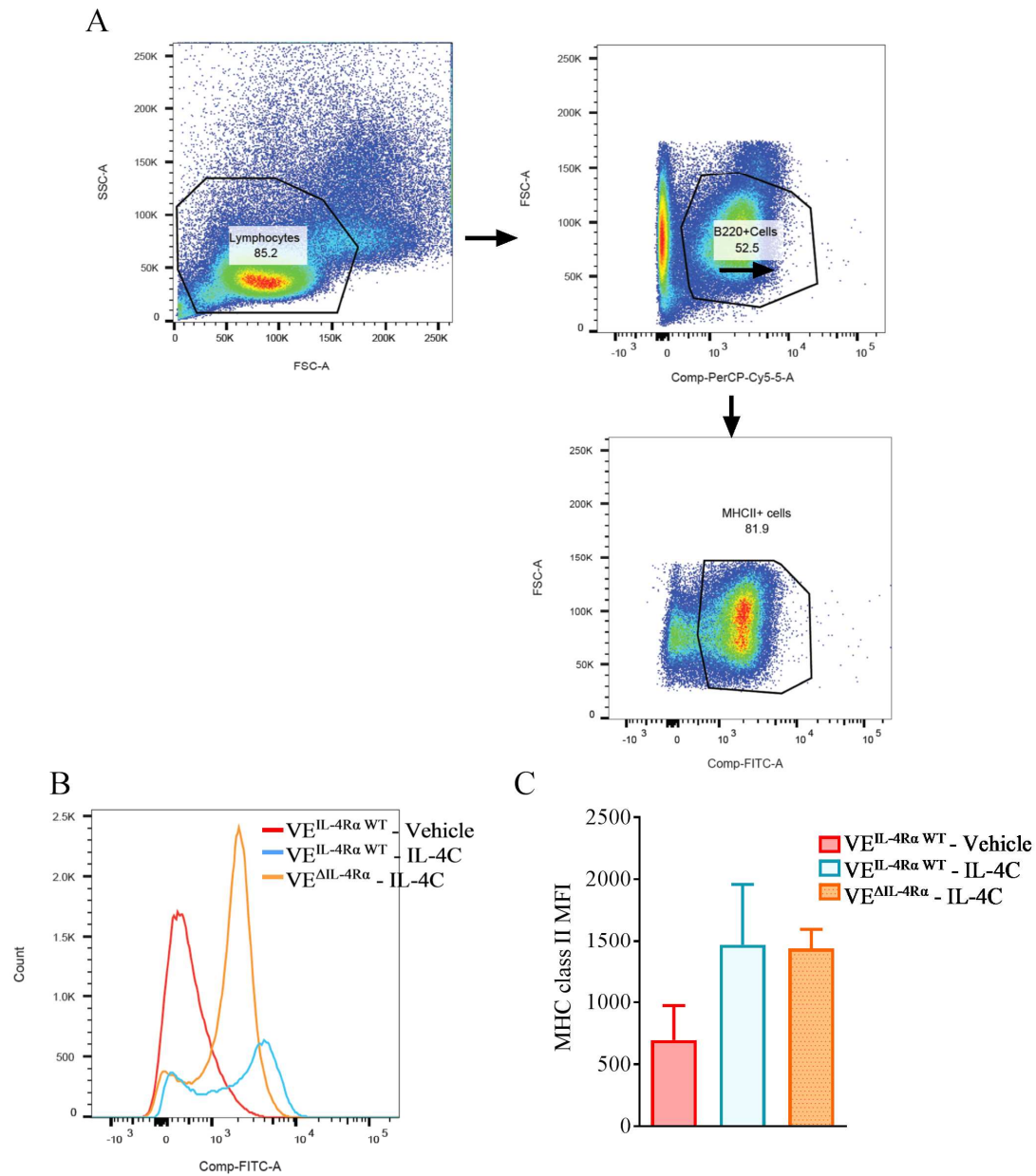


Yamani et al Fig 6

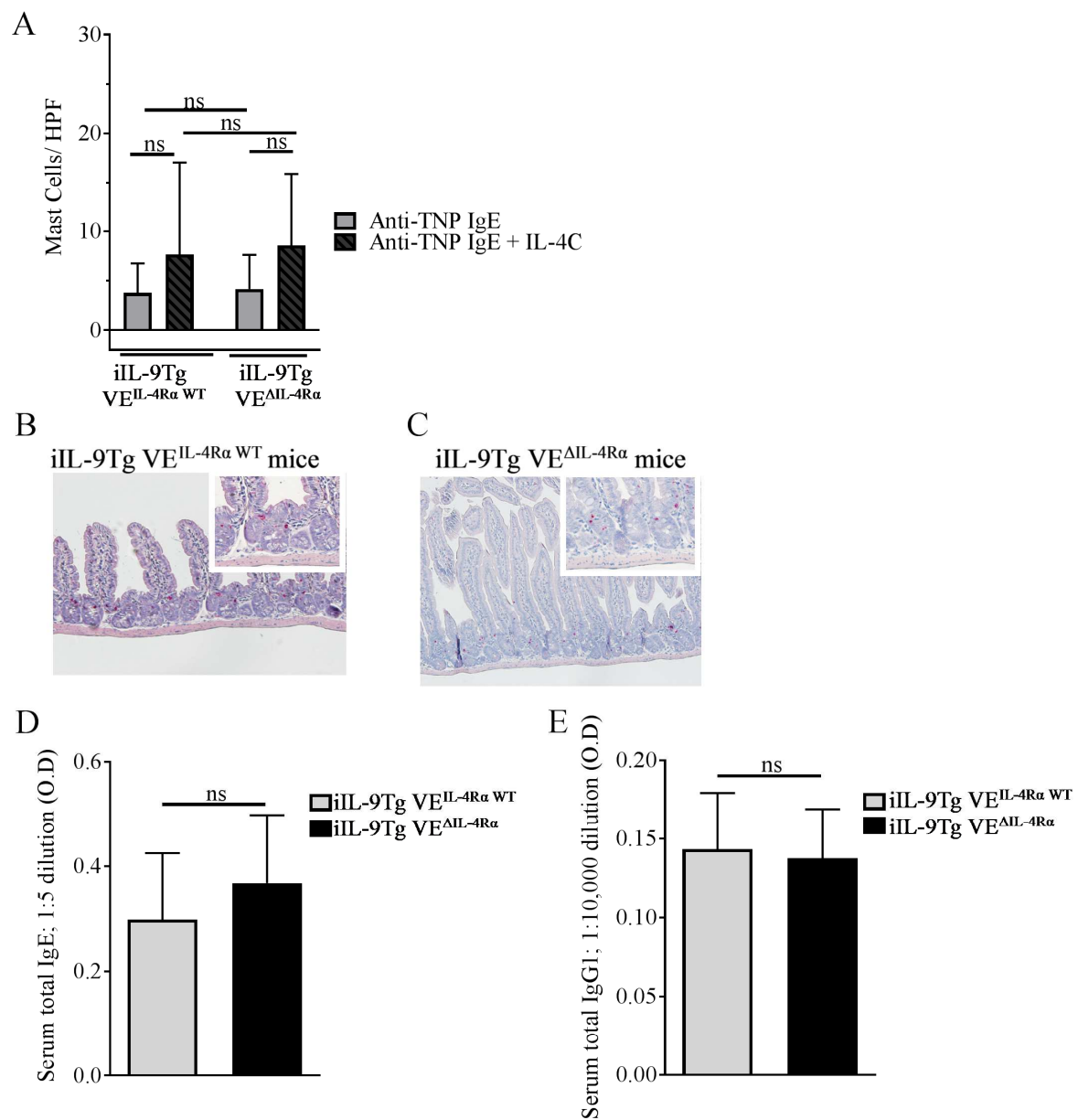


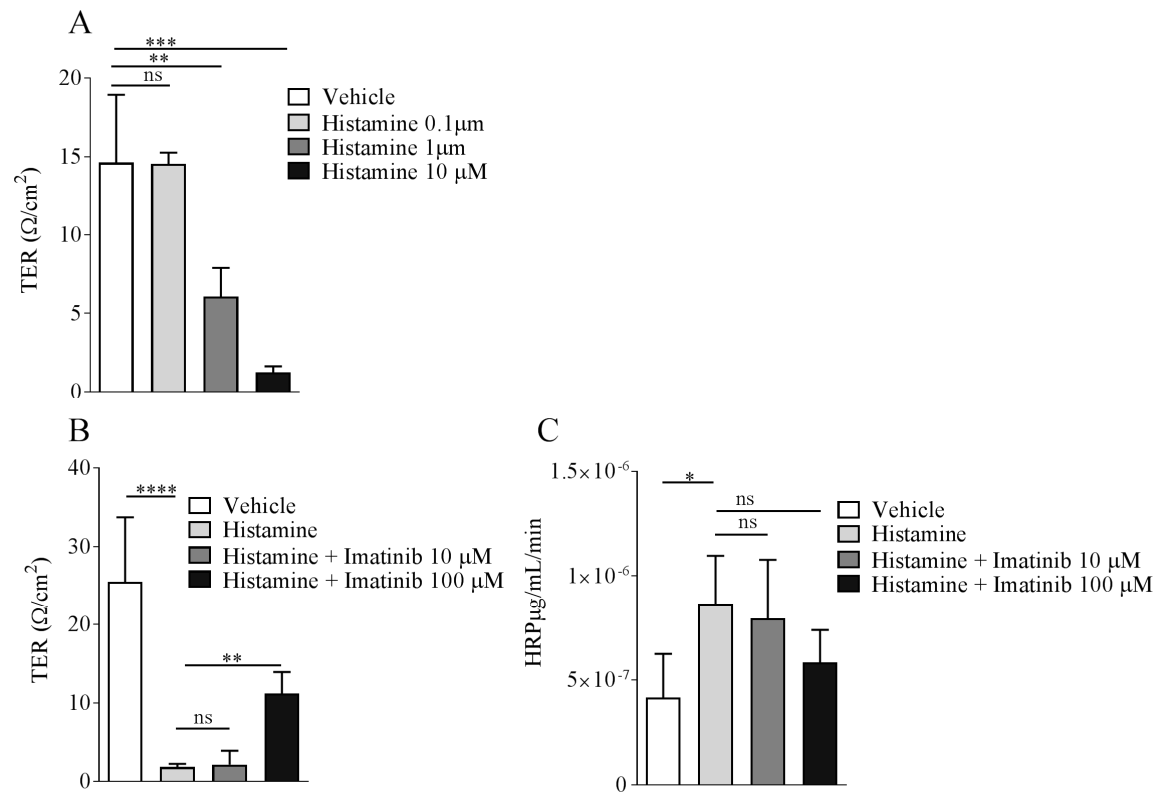
Yamani et al Fig 7

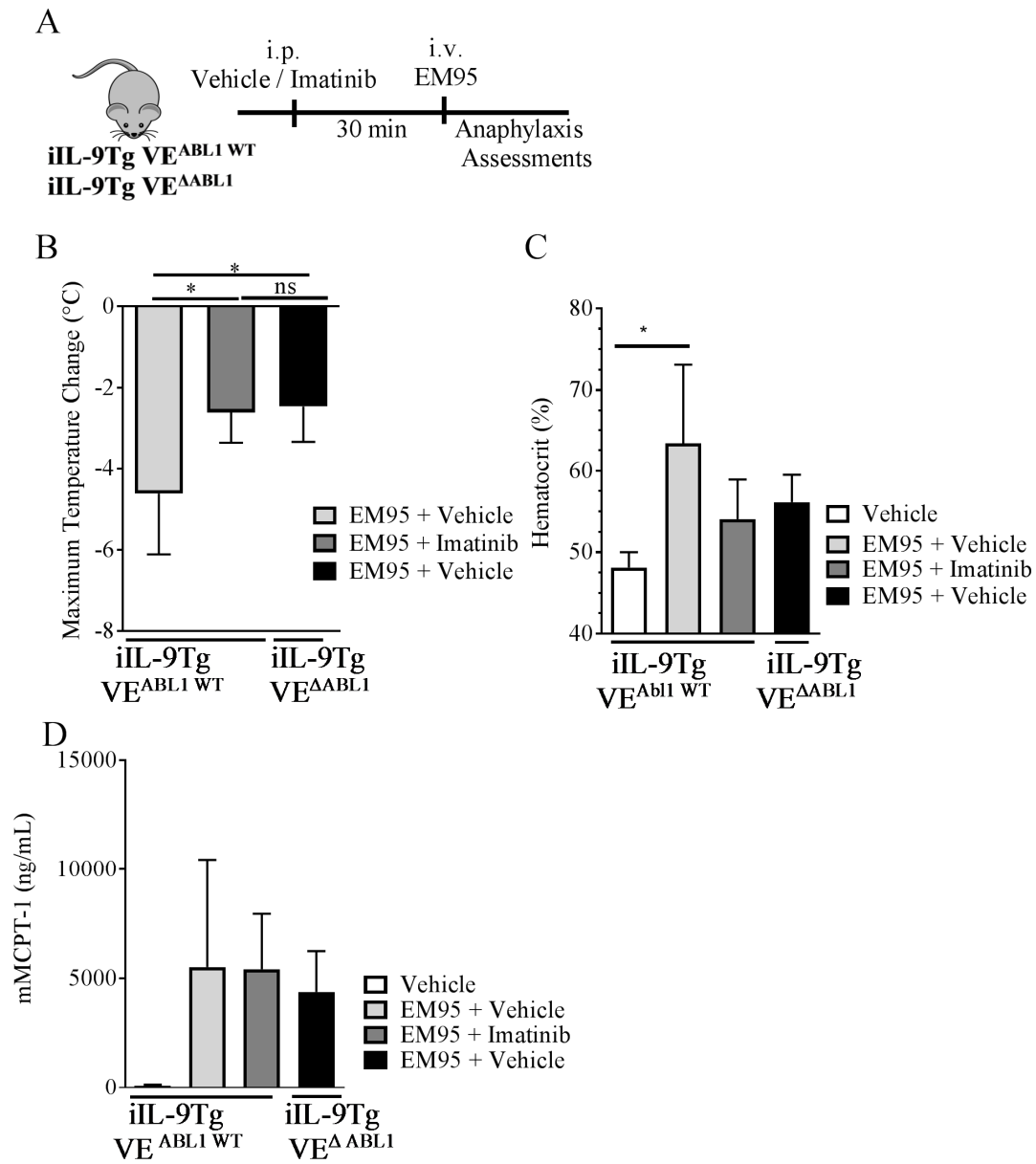




Yamani et al supp fig 2







Supplemental Figure Legends**Figure S1: $VE^{\Delta IL-4R\alpha}$ mice lack the expression of IL-4R α chain on VE cells.**

Immunofluorescence images of jejunum from $VE^{IL-4R\alpha^{WT}}$ and $VE^{\Delta IL-4R\alpha}$ mice. Anti- Panendothelial Cell Antigen staining with anti-rat monoclonal PLVAP antibody (MECA32) (red) identifies endothelial cells; anti-IL-4R α staining (shown in green) was performed with anti-IL-4R α (Santa Cruz, # sc-28361) and DAPI staining is shown in blue. White arrows show the presence of IL-4R α chain staining on endothelial cells of $VE^{IL-4R\alpha^{WT}}$ mice and its absence on endothelial cells of $VE^{\Delta IL-4R\alpha}$ mice.

Figure S2: IL-4C is biologically active in the $VE^{\Delta IL-4R\alpha}$ mice.

Representative flow plots show the gating of the B220/MHC II population from lymphocytes (A). Histogram (B). Median fluorescence intensity (MFI) of FITC on PerCp/Cy5.5+ cells determined by flow cytometry (C). $VE^{IL-4R\alpha^{WT}}$ and $VE^{\Delta IL-4R\alpha}$ mice were injected i.v. with vehicle or IL-4C (1 μ g of IL-4 + 5 μ g anti-IL-4 mAb). Mice were sacrificed the next day, and spleen cells were stained with anti-mouse MHC II (I-A), FITC, and anti-mouse/human CD45R/B220 PerCP/Cy5.5+ antibody. Data are represented as the mean $\pm$ SD; = 2 mice per group from n = 1 experiment.

Figure S3: The effect of vascular endothelial IL-4R α chain on hypovolemic shock is mast cell and immunoglobulin independent.

Intestinal MC counts (A), photomicrograph of CAE-stained intestinal section of iIL-9Tg $VE^{IL-4R\alpha^{WT}}$ (B) and iIL-9Tg $VE^{\Delta IL-4R\alpha}$ (C), serum Total serum IgE (D). Total serum IgG1 (E) of iIL-9Tg $VE^{IL-4R\alpha^{WT}}$ and iIL-9Tg $VE^{\Delta IL-4R\alpha}$ mice following treatment with anti-TNP IgE and with IL-4C or vehicle and with TNP-OVA. Serum was collected from untreated iIL-9Tg $VE^{IL-4R\alpha^{WT}}$ and iIL-9Tg $VE^{\Delta IL-4R\alpha}$ mice. Data are represented as the mean $\pm$ SD; n = 4-5 mice per group (A, D-E). Photomicrograph 10X magnification; insert, 40X magnifications (B-C). ns > 0.05.

Figure S4: Histamine-induced VE-permeability in EA.hy926 cells is inhibited by Imatinib.

(A) Confluent monolayers of cell line EA.hy 296 (EVOM >100 ohms) were mounted in Ussing chambers and stimulated with different doses of histamine (0.1 – 10 μ M) for 30 min and TER was determined using Ohms law. EA.hy 296 cells were pretreated with different dose of imatinib (0 – 100 μ M) for 3 h, mounted in Ussing Chambers and treated with histamine (100 μ M for 30 min) and TER (B) and HRP flux (C) was determined. Data are represented as the mean $\pm$ SD; n = 3 wells per group. **** P < 0.0001, *** P < 0.001, ** P < 0.01, * P < 0.05, ns > 0.05.

Figure S5: Hypovolemic shock induced by EM95 is dependent on VE ABL1.

Experimental regimen (A), Maximum temperature change (B), Hematocrit percentage (C), and mMCPT-1 (D) in iIL-9Tg $VE^{\Delta ABL1}$ (iIL-9 ^{WT} Tie2 ^{cre} ABL1 $^{fl/fl}$) and iIL-9Tg $VE^{ABL1^{WT}}$ (iIL-9 ^{WT} Tie2 ^{WT} ABL1 $^{fl/fl}$; used as a WT control.) i.p. treated

with vehicle or imatinib (1.25 mg/mice) 30 min before EM95 (i.v. 2 mg / 200 mL). iIL-9Tg VE^{ΔAb1} received EM95 only. Data are represented as the mean ± SD; n = 4-6 mice per group from n = 3 experiments. * P < 0.05, ns > 0.05.