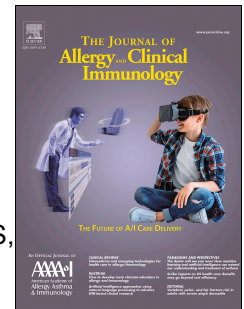


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Optimizing drug inhibition of IgE-mediated anaphylaxis in mice

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use of drugs to suppress anaphylaxis that was awarded to the University of Cincinnati and
could benefit from the licensing of this patent. FDF is a principal in a start-up company
that is trying to commercialize rapid desensitization with anti-FcεRIα monoclonal
antibodies. The other authors have no conflicts of interest to disclose.

24

25 **Abstract:**

26 *Background:* Administering allergens in increasing doses can temporarily suppress IgE-
27 mediated allergy and anaphylaxis by desensitizing mast cells and basophils; however,
28 allergen administration during desensitization therapy can itself induce allergic responses.
29 Several small molecule drugs and nutraceuticals have been used clinically and
30 experimentally to suppress these allergic responses.

31 *Objective:* To optimize drug inhibition of IgE-mediated anaphylaxis.

32 *Methods:* Several agents were tested individually and in combination for ability to
33 suppress IgE-mediated anaphylaxis in conventional mice, Fc ϵ RI α -humanized mice, and
34 reconstituted immunodeficient mice that have human mast cells and basophils.

35 Hypothermia was the read-out for anaphylaxis; therapeutic efficacy was measured by
36 degree of inhibition of hypothermia. Serum mouse mast cell protease 1 (MMCP1) level
37 was used to measure extent of mast cell degranulation.

38 *Results:* Histamine receptor 1 (HR1) antagonists, β -adrenergic agonists, and a spleen
39 tyrosine kinase (Syk) inhibitor were best at individually inhibiting IgE-mediated
40 anaphylaxis. A Bruton's tyrosine kinase (BTK) inhibitor, administered alone, only
41 inhibited hypothermia when Fc ϵ RI signaling was suboptimal. Combinations of these
42 agents could completely or nearly completely inhibit IgE-mediated hypothermia in these
43 models. Both Syk and BTK inhibition decreased mast cell degranulation, but only Syk
44 inhibition also blocked desensitization. Many other agents that are used clinically and
45 experimentally had little or no beneficial effect.

46 *Conclusion:* Combinations of an HR1 antagonist, a β -adrenergic agonist, and a Syk or a
47 BTK inhibitor protect best against IgE-mediated anaphylaxis, while an HR1 antagonist
48 plus a β -adrenergic agonist $\pm$ a BTK antagonist is optimal for inhibiting IgE-mediated
49 anaphylaxis without suppressing desensitization.

50

51 *Clinical implication:* The combined prophylactic use of a HR1-specific antihistamine and
52 a β -adrenergic receptor agonist can increase the safety of rapid desensitization.

53

54 *Capsule summary:* HR1 and BTK antagonists and β -adrenergic receptor agonists
55 additively protect against IgE-mediated anaphylaxis in a mouse model. Syk inhibition is
56 also protective, but inhibits mast cell desensitization.

57

58 *Key words:* Desensitization, Antihistamine, Syk, BTK, β -adrenergic agonist, mast cell,
59 mouse, humanized mouse, Fc ϵ RI

60

61 *Abbreviations used:* BTK, Bruton's tyrosine kinase; EM-95, rat IgG2a anti-mouse IgE
62 monoclonal antibody; FcεR1, the high affinity IgE receptor; HR1, histamine receptor 1;
63 HR2, histamine receptor 2; HR4, histamine receptor 4; mAb, monoclonal antibody;
64 MMCP1, mouse mast cell protease 1; PI3K, phosphoinositide 3-kinase; Syk, spleen
65 tyrosine kinase

Introduction:

The considerable and increasing prevalence of allergic disorders¹⁻⁵ has served as an impetus for the development of novel therapies. Several of these therapeutic approaches involve desensitization, the concept that exposure to allergen doses that are insufficient to cause symptoms temporarily increases the allergen dose required to elicit symptoms⁶⁻¹². For IgE-dependent allergy, desensitization is predominantly allergen-specific^{8, 13-16} and results from reversible allergen-induced changes in IgE/FcεRI expression^{17, 18}, actin conformation¹⁹, and signaling^{15, 18, 19} on mast cells and basophils. Unlike true tolerance, allergen sensitivity recurs unless allergen exposure is repeated at frequent intervals^{7, 9, 11, 19-22}. Despite this limitation, desensitization with escalating doses of allergen, administered intravenously, orally, sublingually, or transcutaneously, is currently being used effectively to treat drug and food allergy^{7, 23-29}.

Allergen desensitization, however, whether the relatively slow approach that is most frequently used to treat food allergy⁷ or the rapid approach used to treat drug allergy²⁵, is not without risk. Patients undergoing this therapy, like patients being treated with more prolonged courses of subcutaneous allergen that can induce more persistent tolerance by inducing blocking IgG antibodies and promoting regulatory T and B cell responses, can develop local or systemic adverse responses when the therapy induces excessive mast cell or basophil activation^{7, 12, 23, 27, 29-35}. To minimize these adverse responses during rapid desensitization for food allergy, it is common to treat patients prophylactically with corticosteroids, antihistamines that suppress H1 and H2 receptors, and leukotriene or leukotriene receptor antagonists³⁶⁻⁴². With some exceptions⁴²,

however, the choice of these drugs is based more on theoretical considerations than on experimental results that demonstrate efficacy.

To optimize such prophylactic drug therapy during desensitization, we have used conventional and humanized mouse models to test the ability of different agents to protect against IgE-mediated anaphylaxis. Our results demonstrate additive or synergistic protective effects of H1, but not H2 receptor antagonists, β -adrenergic agonists (but not epinephrine), a Bruton's tyrosine kinase (BTK) inhibitor, and a spleen tyrosine kinase (Syk) inhibitor (although the Syk inhibitor suppresses desensitization as well as anaphylaxis); in contrast, several other agents that have been described to protect against anaphylaxis had little or no protective effect against the development of hypothermia in our experiments.

99 **Methods:**

100 *Mice:* BALB/c and C57BL/6 mice were either purchased from Charles River or
 101 bred in house. Human FcεRIα transgenic, mouse FcεR1α-deficient mice on a BALB/c
 102 background⁴³ were a gift from Jean-Pierre Kinet, (Cambridge, MA). NOD/LtSz-SCID
 103 IL-2RG^{-/-}SGM3 (NSGS)⁴⁴ and NRG-SGM3 (NRGS) mice were obtained from James
 104 Mulloy and bred in-house. These mice were reconstituted with red blood cell-depleted,
 105 anti-human CD3 mAb (mAb OCT-3, BioXCell)-treated human cord blood cells as
 106 described⁴⁵. Animal work was approved by the Cincinnati Children's Hospital Research
 107 Foundation Institutional Animal Care and Use Committee and was conducted in accord
 108 with the National Institutes of Health guide for the care and use of Laboratory
 109 animals (NIH Publications No. 8023, revised 1978). Female mice, age 8 – 12 weeks,
 110 were used for experiments unless otherwise noted. Cages of mice were randomly
 111 assigned to different groups without a specific randomization protocol, with the
 112 exception that mice in different groups were age- and sex-matched.

113 *Reagents:* Rat IgG2a anti-mouse IgE mAb-secreting hybridoma cells (EM-95)⁴⁶,
 114 were a gift of Zelig Eshhar (Weizmann Institute, Rehovot, Israel). Mouse IgEαTNP
 115 mAb-secreting hybridoma cells (IgEL2a)⁴⁷ were purchased from the ATCC (Rockville,
 116 MD). Mouse IgG1 anti-human FcεR1α mAb-secreting hybridoma cells (15.1)⁴⁸ were a
 117 gift of Jean-Pierre Kinet. Mouse IgG1 anti-human IgD mAb-secreting hybridoma cells
 118 (CBDA-4E5-4C7) was a gift of John Kearny (Birmingham, AL). Mouse IgG1 anti-hen
 119 egg lysozyme mAb secreting hybridoma cells (HyHel-10) were a gift of S. Smith-Gill,
 120 Bethesda, MD)⁴⁹. We purchased mouse IgG2b anti-human FcεR1α mAb AER-37 (also
 121 called CRA-1) (Biolegend, San Diego, CA), doxepin hydrochloride (Sigma, St. Louis,

MO), triprolidine hydrochloride (Sigma, St. Louis, MO), formoterol fumarate dihydrate (Sigma, St. Louis, MO), terbutaline sulfate (Akorn Inc., Decatur, IL), albuterol sulfate (Sigma, St. Louis, MO), indacaterol maleate (Selleckchem, Houston, TX), adrenaline hydrochloride solution – 1 mg/ml (Par Pharmaceutical Cos. Ind., Spring Valley, NY), fostamatinib R788 (Selleckchem, Houston, TX), imatinib mesylate (Santa Cruz Biotechnology, Santa Cruz, CA), idelalisib (also known as CAL-101, GS-1101) (Selleckchem, Houston, TX), and ibrutinib (ChemieTek, Indianapolis, IN).

Trinitrophenyl (TNP)-labeled bovine serum albumin (BSA) was prepared by dissolving 1 gm of BSA in 5 ml of saline, then adding 500 μ l of 1M NaHCO₃ buffer, PH 9.6. A 200 mg/ml solution of TNP was made by dissolving 500 μ l TNP (picrylsulfonic acid) in 500 μ l DMSO. TNP/DMSO was added to the BSA solution while vortexing and the resulting solution was left overnight in the dark at room temperature. The resulting TNP-BSA was dialyzed 3 times against saline and stored at -80° C. TNP-ovalbumin (TNP-OVA) was made as previously described⁵⁰. Formoterol was dissolved in DMSO before dilution in normal saline. Idelalisib was dissolved at 30 mg/ml in 30% PEG 400/0.5% Tween80/5% propylene glycol. Table S1 summarizes the dose ranges tested, routes of administration, timing of administration, and vehicles used for the different agents tested; the same vehicle used to dissolve or suspend and agent was used as the control for that agent.

Determination of IgE expression by peritoneal mast cells: IgE staining of peritoneal mast cells (identified as c-kit⁺ IL-3R⁺ B220⁻ high side scatter cells)^r was determined by flow cytometry.

IL-4C: Mouse recombinant IL-4 (Peprotech, Rocky Hills, NJ) was mixed with BVD4-1D11.1 rat anti-mouse IL-4 mAb (purified from ascites) at a 1:5 weight ratio and

incubated for 2 minutes at room temperature, then diluted to the desired concentration with 1% autologous mouse serum in PBS.

Measurement of mouse mast cell protease 1 (MMCP1) levels: Serum levels of MMCP1 were measured in blood drawn 3 or 4 hours after challenge with an ELISA kit (eBioscience), according to the manufacturer's protocol.

Anaphylaxis: The severity of anaphylactic shock was assessed by decrease in rectal temperature, as measured by digital thermometry⁵¹.

Passive anaphylaxis model: Mice were sensitized by i.v. injection of 10 µg of IgE anti-TNP mAb, then challenged i.v. 24 hours later with 10 µg of TNP-BSA or TNP-OVA.

Generation of mouse anti-human FcεR1α mAbs: FcεR1α-deficient mice (BALB/c background) were immunized 3 times i.p. at 2 week intervals with 20 µg of the 176 N-terminal amino acid human FcεR1α ectodomain in 50% alum adjuvant. Once high titers of mouse IgG1 anti-human FcεR1α were detected by ELISA, the mice were injected i.v. with 2 µg of human FcεR1α ectodomain; two days later, their splenocytes were fused with a non-secreting mouse plasmacytoma cell and cloned. Five mouse IgG1 anti-human FcεR1α ectodomain mAb-secreting clones were selected by ELISA, followed by flow cytometric evaluation of their ability to stain peritoneal mast cells from mice that expressed human, but not mouse FcεR1α and their failure to stain peritoneal mast cells from normal BALB/c mice. Selected clones, including the clones IE7 and IB10 that are used in this paper, were expanded for mAb generation and grown as ascites in Pristane-primed BALB/c mice. IE7 and IB10 mAbs were purified from ascites by ammonium sulfate fractionation followed by DE-52 chromatography.

168 *Statistics:* Differences in temperature and concentrations of MMCP1 were
169 compared with a 2- or 3-way ANOVA or Kruskal-Wallis test, as appropriate, followed by
170 Tukey's honest significant difference test or Mann-Whitney U test, as appropriate
171 (GraphPad Prism 7.0; GraphPad Software, La Jolla, Calif). A 1-tailed test was used to
172 test hypotheses that a given treatment would decrease the temperature drop; otherwise a
173 2-tailed test was used. For line graphs showing development of hypothermia, statistical
174 analysis was performed on the maximum temperature drops for individual mice. A *p*
175 value of less than .05 was considered significant. For all figures, * = <0.05; ** = < 0.01;
176 *** = <0.001; **** = < 0.0001.

Results:

Effects of antihistamines: Initial experiments evaluated the ability of single small molecule inhibitors to suppress anaphylaxis in BALB/c mice injected i.v. with 20 µg of rat IgG2a anti-mouse IgE mAb (EM-95). In a total of 10 experiments, each of which had 4-6 mice/group, 200 µg of the histamine receptor 1 (HR1) antagonist triprolidine, injected i.p. 1 hr prior to i.v. EM-95 challenge, decreased the maximum temperature drop on average from $3.98 \pm 0.23^{\circ}\text{C}$ to $2.22 \pm 0.25^{\circ}\text{C}$ ($44.9 \pm 4.0\%$ suppression (mean $\pm$ SEM)), with a typical result shown in Fig. 1A. Doses of triprolidine <200 µg were less effective than the 200 µg dose, while doses > 200 µg had no additional suppressive effect; triprolidine injection 37 min or 75 min prior to EM-95 challenge were equally effective, but triprolidine injected 150 min prior to challenge was ineffective (Fig. S1). An H1 receptor antagonist that also suppresses the H2 receptor (doxepin⁵², 10 mg/kg), was no more effective than triprolidine at suppressing IgE-mediated hypothermia (Fig. 1B); an H2R antagonist (ranitidine) had no effect (Fig. 1C) and 25 mg/kg of ketotifen, which is thought to be both an H1R antagonist and a mast cell stabilizer⁵³, was considerably less effective than 200 µg of triprolidine at preventing IgE-mediated hypothermia (not shown).

Effects of β -adrenergic receptor agonists: Because β -adrenergic receptor agonists have been described to both suppress mast cell degranulation^{54, 55} and the effects of mast cell-generated mediators^{56, 57}, we tested the ability of drugs in this class to suppress IgE-mediated anaphylaxis; these drugs (formoterol, terbutaline, albuterol, and indacaterol) indeed dose-dependently protected against development of hypothermia (Fig. 2). In contrast, subcutaneous injection of 2 – 50 µg of epinephrine 5 minutes after antigen

challenge of mice primed with antigen-specific IgE failed to prevent hypothermia (Fig. S2). Intraperitoneal injection of 2 µg of epinephrine 5 minutes after Ag challenge also failed to reverse the development of hypothermia and i.p. injection of 10 or 50 µg of epinephrine was toxic, inducing hypothermia even in mice that received no other treatment (not shown). In contrast, s.c. injection of 25 µg of epinephrine 10 minutes prior to anti-IgE mAb challenge significantly decreased development of hypothermia, particularly when results were adjusted to eliminate the hypothermic effects directly induced by epinephrine (Fig. S3A).

Because we had evaluated a HR1 antagonist and β-adrenergic agonists solely as prophylactics (i.e.; injected prior to anti-IgE mAb challenge) and epinephrine has some effectiveness as a prophylactic, but not as a therapeutic (injected after anti-IgE mAb challenge) in our model, we evaluated whether a HR1 antagonist and/or a β-adrenergic agonist had any efficacy in our model as therapeutics. Results of this experiment (Fig. S3B) show significant efficacy, although less than in experiments that used these drugs prophylactically.

Effects of tyrosine kinase inhibitors: Because the tyrosine kinase Syk is involved in the initiation of IgE-mediated mast cell activation⁵⁸, we also tested the ability of a Syk inhibitor (fostamatinib), to block IgE-mediated hypothermia. This drug was consistently effective at a dose of 80 mg/kg, but not 40 mg/kg (typical results shown in Fig. 3A).

Unlike triprolidine and β-adrenergic agonists, fostamatinib suppressed mucosal mast cell degranulation, as shown by inhibition of serum levels of mouse mast cell protease 1 (MMCP1), a proteolytic enzyme that is released by degranulating mucosal mast cells⁵⁹ (Fig. 3B). The BTK inhibitor ibrutinib, at a single dose of 25 mg/kg, failed to suppress

IgE-mediated hypothermia in BALB/c mice, but almost completely suppressed IgE-mediated hypothermia in huFcεRIα mice, whose chimeric FcεRI is composed of human FcεRIα and mouse FcεRIβ and FcεRIγ⁴³ and which develop less severe hypothermia and less mast cell degranulation (lower serum MMCP1) than BALB/c mice in response to anti-IgE mAb (Fig. 3C and D). Ibrutinib significantly decreased the anti-IgE mAb-induced MMCP1 response in both BALB/c and huFcεRIα mice, but fully suppressed this response only in the huFcεRIα mice (Fig. 3D). In contrast to Syk and BTK inhibitors, other tyrosine kinase inhibitors by themselves had little or no suppressive effects on hypothermia or on mast cell degranulation at doses that inhibit their targets in vivo. These inhibitors included 5-80 mg/kg of the phosphoinositide 3-kinase (PI3K) P110δ inhibitor, idelalisib, and 1.25 mg/kg of the Abl/Kit inhibitor, imatinib (Fig. S4 and data not shown).

Lack of effects of additional agents: In addition to these tyrosine kinase antagonists, a large number of other drugs and agents that have been described to inhibit anaphylaxis⁶⁰⁻⁷⁰ in mice had little or no effect in our model as single agents when used at the concentration, route of administration, and timing specified in the previously described mouse studies. These included an H4R inhibitor (JNJ-777120, at 20 mg/kg), a natural phenol/phytoalexin (resveratrol, at 10 mg/kg), a flavonoid polyphenol (quercetin, at 50 mg/kg), an antioxidant flavanol (kaempferol (50 mg/kg), a natural phenol diarylheptanoid (curcumin, 50 mg/kg), corticosteroids, a mast cell stabilizer (cromolyn sodium (a single 300 µg dose), theophylline (5 mg/kg), a 5-lipoxygenase inhibitor, zileuton 50 mg/kg dissolved in DMSO and inoculated by oral gavage 1 hour and 24 hours prior to challenge), a leukotriene receptor (CysLT1) antagonist, montelukast (6 mg/kg inoculated subcutaneously 1 hour and 24 hours before challenge), a leukotriene D4

receptor antagonist (REV 5901, 250 μ g i.v. diluted in a 1:1000 solution of DMSO in saline 15 minutes prior to challenge), and a PAF antagonist (ABT-491, 2 μ g i.v.). In addition to these, serotonin receptor (5-HT_{1/2a/7}) antagonists (metergoline (200 μ g i.p. in 1% carboxymethylcellulose 30 minutes prior to challenge) and ketanserin (60 μ g i.p. 30 minutes prior to challenge) were also shown by us previously to fail to inhibit murine IgE-mediated anaphylaxis⁵⁰. The effects of all of the different agents tested are summarized in Table S2.

Additive or synergistic suppressive effects of an antihistamine, β -adrenergic agonists, and a Syk inhibitor. Upon finding that antihistamines, β -adrenergic agonists, and a Syk inhibitor were the most effective individual inhibitors of IgE-mediated murine anaphylaxis, studies were performed to determine: (1) whether combinations of these agents could additively or synergistically suppress anaphylaxis; and (2) whether the use of combinations would allow use of lower doses of a β -adrenergic agonist and a Syk inhibitor, which are more likely than an H1R blocker to have adverse effects. Results of these studies showed additive or synergistic effects of any two of these agents (Fig. 4). In some experiments, the use of all three agents almost totally blocked anaphylaxis (Fig. 4B). A similar result was observed in huFc ϵ RI α instead of mouse Fc ϵ RI α mice that were challenged with an activating anti-human Fc ϵ RI α mAb (Fig. S5A). In other experiments, three agents were no more effective than two (Fig. 4D and E). This was true even when mice were pre-treated with a long-acting formulation of IL-4⁷¹ to increase anaphylaxis severity⁷² (Fig. 4D). Although these studies were all performed with female BALB/c mice, the combination of triprolidine and albuterol was also more effective than either

drug alone at suppressing IgE-mediated hypothermia in BALB/c male and C57Bl/6 female mice (Fig. S6).

Additive and synergistic effects of different tyrosine kinase inhibitors. Because Syk, PI3K and BTK are all involved in FcεRI-mediated mast cell signaling⁷³ and Kit also promotes mast cell activation⁷⁴, we hypothesized that PI3K, BTK, and Kit inhibitors might act synergistically with a Syk inhibitor (fostamatinib) to suppress FcεRI-mediated mast cell activation, even if they had little or no effect by themselves. This appeared to be the case for each of these inhibitors (Fig. 5A-C), although the effects of the combination of fostamatinib and imatinib did not reach statistical significance ($p = 0.07$) and the combination of fostamatinib with another tyrosine kinase antagonist sometimes caused toxicity (reversible hypothermia) in the absence of FcεRI crosslinking (Fig. 5B and data not shown).

The ability of PI3K, BTK, and Kit inhibitors to enhance Syk inhibitor suppression of IgE-mediated anaphylaxis, even though they had little effect on their own in BALB/c mice, led us to evaluate whether the same kinase inhibitors would enhance suppression by an H1 antagonist or β -adrenergic receptor agonist in these mice. Results of these studies demonstrated increased inhibition when the BTK antagonist ibrutinib was combined with either triprolidine or albuterol, as compared to triprolidine or albuterol alone, while adding imatinib or idelalisib to triprolidine or albuterol had much less of an effect (Fig. 5D - F). The effects of all tested combinations of agents on IgE-mediated anaphylaxis are summarized in Table S3.

Tripolidine, indacaterol, and fostamatinib suppress IgE-mediated anaphylaxis in mice that have human mast cells and basophils. Because human mast cells and basophils

have somewhat different properties than the same cell types in mice^{21, 75-77}, we used immune-deficient, rehuIL-3-, rehuGM-CSF-, rehuSCF-producing NSGS mice that had been reconstituted for 2-3 months with T cell-depleted human cord blood cells to determine whether antihistamine/ β -adrenergic agonist/Syk inhibitor treatment could block IgE-mediated anaphylaxis that is mediated by human mast cells and basophils. These mice provide a particularly sensitive tool for studying IgE-mediated anaphylaxis, because they develop large numbers of human mast cells (along with smaller numbers of basophils); both cell types in these mice have abnormally high responsiveness to Fc ϵ RI crosslinking because of their high levels of three mast cell-stimulating cytokines^{78, 79}, which also increase responsiveness to mast cell-produced mediators (Khodoun and Finkelman, unpublished data). Indeed, intravenous injection of a relatively high dose of anti-IgE mAb (Fig. 6A), Ag following priming with Ag-specific IgE (Fig. 6B), or anti-huFc ϵ RI α mAb (Fig. 6C) typically kills these mice in <30 minutes, while injection of these mice with 100 – 500 ng of the same anti-Fc ϵ RI α mAb typically causes a 4-6°C temperature drop (Fig. S5B). Pre-treatment with the combination of triprolidine/indacaterol/fostamatinib usually prevented death (Fig. 6A, B, and C), and substantially inhibited the development of hypothermia in mice injected with Ag or the higher doses of anti-IgE mAb. Hypothermia was almost completely prevented by the combinations of triprolidine/indacaterol/fostamatinib or triprolidine/indacaterol when mice were challenged i.v. with the low dose of anti-Fc ϵ RI α mAb (Fig. S5B).

Fostamatinib, but not ibrutinib, suppresses mast cell/basophil desensitization.

Although 80 mg/kg of fostamatinib suppresses anaphylaxis and 40 mg/kg of this Syk inhibitor enhances the abilities of triprolidine and β -adrenergic agonists to suppress

anaphylaxis, fostamatinib treatment was associated in some anti-Fc ϵ RI α or anti-IgE mAb-challenged mice with a late decrease in temperature that occurred once the effects of this drug had worn off (Fig. 6C). Because this late exacerbation of hypothermia was not seen in mice that did not receive a Syk inhibitor (data not shown), this result raised the possibility that Syk inhibition might interfere with Fc ϵ RI-mediated mast cell desensitization. To evaluate this possibility, huFc ϵ RI α transgenic mice were sensitized with IgE anti-TNP mAb, then treated the next day with anti-huFc ϵ RI α mAb or isotype-control mAb in the presence or absence of 40 mg/kg of fostamatinib. These mice were challenged 4 hours after that with 10 μ g of TNP-BSA and evaluated for the development of hypothermia (Fig. 7A). Hypothermia failed to develop in the anti-Fc ϵ RI α mAb-treated mice that had not received fostamatinib, but was only partially suppressed in those that had received this drug (Fig. 7B). Fostamatinib had no significant effect on the ~7-fold decrease in mast cell IgE expression that was caused by anti-Fc ϵ RI α mAb treatment (Fig. 7C). Thus, Syk inhibition appears to inhibit mast cell desensitization in vivo. In contrast, inhibition of BTK, which is also involved in induction of mast cell degranulation, had little or no suppressive effect on IgE-mediated anaphylaxis (hypothermia) in our model (Fig. 7D and E; the difference in timing between the fostamatinib and ibrutinib studies was necessary to allow the serum ibrutinib concentration to decrease to a level that would no longer directly suppress anaphylaxis in huFc ϵ RI α mice).

Discussion:

The considerable and increasing prevalence of IgE-mediated allergic disorders, including drug allergy, food allergy, venom allergy and chronic urticaria, has encouraged the development of safe and effective therapies for these disorders. Rapid desensitization is one therapeutic approach that has been increasingly used, predominantly to treat drug allergy, but also to some extent as part of strategy to treat food allergy. In this approach, allergic patients are administered rapidly increasing quantities of the relevant allergen or allergen-containing substance, starting with a dose that is insufficient to elicit clinically apparent signs, symptoms and basophil/mast cell degranulation, and ending with a dose that is hopefully sufficient to temporarily desensitize these cells to exposure to a fully therapeutic dose of a drug or to typically ingested quantities of a food. We have extended this approach by demonstrating that rapid desensitization can be applied in a “polyclonal,” Ag-non-specific way in mice by treating them with serially increasing doses of a mAb to the IgE-binding chain of the basophil and mast cell high affinity IgE receptor, FcεRIα⁸⁰⁻⁸². In addition to its ability to desensitize to all IgE-mediated anaphylactic reactions, this approach has safety and efficacy advantages over desensitization with Ag, an advantage that most likely reflects the lack of pre-existing IgG Abs to anti-FcεRIα mAb and the longer in vivo half-life of IgG Abs than most allergens. At least two mechanisms are involved in mast cell desensitization in our model: suppression of FcεRI signaling and depletion of mast cell/basophil IgE and FcεRI⁸⁰.

The use of rapid desensitization with either Ag or anti-FcεRIα mAb is not without risk, because excessive crosslinking of FcεRI by either agent can cause the same IgE-

mediated reactions that the approach is designed to prevent. Indeed, such reactions have occurred frequently during rapidly desensitization for drug allergy and can be sufficiently severe to require epinephrine injection^{83, 84}. For this reason, allergy researchers, particularly the group headed by Marianna Castells, have used combinations of drugs to suppress allergic reactions that can occur during rapid drug desensitization^{15, 36-39, 42}. These drugs, which include H1R- and H2R-specific antihistamines, aspirin, the leukotriene antagonist monteleukast, adrenocorticosteroids and opioids, provide some protection against frequently encountered adverse reactions, including pruritis, urticaria, bronchospasm, angioedema, flushing, and fever. However, there has not been a systematic evaluation of potentially protective agents in an animal model of anaphylaxis that could be thoroughly studied and rigorously controlled and there have not been any reports of drug prophylaxis against allergic reactions to anti-FcεRIα mAb. Consequently, we undertook the mouse studies that are described in this paper.

Using: (a) conventional mice and humanized mice that express human FcεRIα on mouse mast cells and basophils or that have human mast cells and basophils, (b) high dose anti-IgE mAb, anti-FcεRIα mAb, or IgE anti-TNP mAb followed by TNP-OVA or TNP-BSA to trigger anaphylaxis, and (c) shock (measured as hypothermia) as a readout for anaphylaxis, we identified agents that were effective by themselves and in combination, agents that were effective only in combination, and agents for which we found no evidence of efficacy. The first, most efficacious group, included H1R-specific antihistamines, β-adrenergic agonists, and an inhibitor of the tyrosine kinase, Syk. In addition to their abilities to partially suppress anaphylaxis by themselves, our results demonstrate additive and synergistic effects that completely or nearly completely prevent

hypothermia. This most likely reflects the different mechanisms of action of these three therapeutics: blocking the effect of mast cell/basophil-released histamine on HR1; β -adrenergic suppression of increases in vascular permeability through direct effects on vascular endothelial cells⁸⁵; and inhibition of mast cell degranulation by blocking a tyrosine kinase, Syk, that has a critical role in this process (notably, the Syk inhibitor is the only one of these agents that suppresses mast cell degranulation, as evaluated by serum MMCP1 levels). Importantly, a considerable increase in efficacy was seen when any two agents were combined and treatment with drug combinations allowed the use of lower doses of individual drugs, which should decrease drug toxicity. Although an HR1 antagonist and β -adrenergic agonist had some ability to decrease the loss in core body temperature when injected 5 min after anti-IgE mAb challenge, this effect was considerably less than when these drugs were used prophylactically.

Agents in the second group had little or no efficacy by themselves at the doses used (higher doses were toxic) in BALB/c mice, but amplified the protective effect of Syk inhibition. These agents include three tyrosine kinase antagonists: imatinib, which suppresses Kit (required for mast cell development and survival⁸⁶), idelalisib, which suppresses P110 δ (the δ isoform of PI3K, which is involved in Fc ϵ RI signaling^{87, 88}), and ibrutinib, which suppresses BTK (also important in Fc ϵ RI signaling⁸⁹). Thus, the combined use of tyrosine kinase inhibitors that suppress Fc ϵ RI signaling at different stages appears to synergistically inhibit Fc ϵ RI-mediated mast cell degranulation, just as suppressing different steps in anaphylaxis pathogenesis (mast cell degranulation, histamine binding to the HR1, mediator-induced increases in vascular permeability) additively or synergistically suppresses anaphylaxis. Ibrutinib was unique among these

tyrosine kinase antagonists in acting synergistically with an HR1 inhibitor and a β -adrenergic receptor agonist to suppress IgE-mediated anaphylaxis. Unlike Syk activation, the downstream activation of BTK is not an absolute requirement for mast cell degranulation because of the presence of alternative pathways. The importance of the BTK pathway becomes apparent in our model, however, when other inhibitors prevent compensation for a delay or moderate decrease in mast cell degranulation and in mice that express a chimeric Fc ϵ RI that appears to signal less potently than wild-type Fc ϵ RI.

A second generation BTK inhibitor, acalabrutinib, has recently been shown by Dispenza et al. to suppress Ag-induced, IgE-mediated anaphylaxis in passively sensitized human cord blood-reconstituted immunodeficient mice, that produce human mast cells, although this suppression can be overcome by increasing the dose of the challenge Ag⁹⁰. Potential differences in the effectiveness of acalabrutinib vs. ibrutinib at suppressing BTK, potential differences in the susceptibility of human vs. mouse BTK to suppression by these drugs, potential differences in the potency of Ag vs. anti-IgE mAb at inducing the BTK-independent pathway of mast cell degranulation, and the longer period of treatment of mice prior to Ag challenge by Dispenza et al. than in our study might explain the more effective BTK inhibitor suppression of mast cell degranulation in the Dispenza study than in this paper. Regardless of mechanism, the ability of BTK inhibitors to at least partially suppress mast cell degranulation by themselves and to synergize with inhibitors that work through different mechanisms, without inhibiting mast cell desensitization, suggests a potential for prophylactic use during desensitization. However, ibrutinib is considerably more expensive than HR1 antagonists and β -

adrenergic agonists and it is not yet known whether it can enhance the suppressive effect of an HR1 antagonist/ β -adrenergic receptor agonist combination.

The third group of agents tested had little or no efficacy at suppressing anti-Fc ϵ RI α mAb- or anti-IgE mAb-induced hypothermia by themselves or when combined with an HR1-specific antihistamine. This group includes HR2 and HR4 inhibitors, cromolyn, theophylline, zileuton, montelukast, a PAF antagonist, a bradykinin receptor 2 antagonist, an inhibitor of plasma kallikrein, and serotonin receptor antagonists, in addition to a number of nutraceuticals that have been described by others to suppress anaphylaxis in mouse models. Surprisingly, epinephrine was without efficacy as a therapeutic in our model, although it had some efficacy as a prophylactic. At lower doses, epinephrine had no obvious toxic effects but failed to alter hypothermia development when injected after anti-IgE mAb challenge, while higher doses induced hypothermia by themselves, most likely by activating α -adrenergic receptors sufficiently to decrease cardiac output by increasing arterial resistance. This possible explanation is consistent with the greater effect of α -adrenergic stimulation in mice than in humans. Although epinephrine's α -adrenergic receptor-mediated vasoconstriction is thought to increase recovery from human anaphylaxis by enhancing its β -adrenergic receptor-mediated increases in heart rate and contractility, the α -adrenergic receptor-related adverse effects of this drug make it unsuitable for prophylactic use^{91, 92}.

The most important practical consequence of our work is evidence that adding a non-toxic dose of a β -adrenergic agonist to an HR1-specific antihistamine provides considerably better protection against Fc ϵ R1-mediated anaphylaxis than the HR1-specific antihistamine alone. Several β -adrenergic agonists are FDA-approved drugs and these

drugs are easily available, relatively inexpensive, and have been used for many years to treat asthma. Consequently, their use with antihistamines for prophylaxis during rapid desensitization seems reasonable and practical. In contrast, the clinical use of a Syk inhibitor, such as fostamatinib, as prophylaxis during rapid desensitization may be problematic. Fostamatinib, while approved for use in immune thrombocytopenic purpura, is expensive and appears to block mast cell desensitization. The last issue might not be a problem for very short term suppression of anaphylaxis, but would likely be problematic for use during rapid desensitization, which depends, at least in part, on temporary inhibition of mast cell signaling⁸⁰. In contrast to our in vivo observation, previous in vitro studies found that FcεRI-mediated mast cell and basophil desensitization was not blocked by a Syk inhibitor⁹³. This apparent discrepancy probably represents an in vivo/in vitro difference, although the possibility that it reflects the use of different Syk inhibitors in the in vitro and in vivo studies cannot be excluded (the inhibitor that was used for the in vitro studies is no longer available).

One strength of our paper is its evidence that prophylaxis with two or three drugs has similar suppressive effects on FcεRI-mediated anaphylaxis in human cord blood-reconstituted NSGS and NRGS mice, which have human mast cells and basophils, as it has in normal mice. Substantial inhibition (albeit incomplete) was observed in these reconstituted mice even though transgenic production of human stem cell factor, IL-3 and GM-CSF in this model causes the production of a large number of human mast cells, partially activates these cells, and increases sensitivity to histamine.

Our observations, however, have four important limitations. First, agents that have little or no ability to inhibit mast cell degranulation in mice may have considerable

ability to inhibit mast cell degranulation in other species, including humans. In this regard, cromolyn has been shown to have greater ability to suppress mast cell degranulation in rats than in mice⁹⁴. Second, even mice that have human mast cells have mouse, rather than human tissues that respond to mast cell-released mediators; these may respond differently than human tissues. Our failure to observe a therapeutic effect of epinephrine may be an example of this. Third, our interpretations about the mechanisms responsible for the effects of some of our inhibitors are complicated by the incomplete specificity of some of these inhibitors. For example, fostamatinib inhibits some tyrosine kinases, including the src-family kinases, JAK1, JAK3, c-Kit, and Flt 3 in addition to Syk⁹⁵. Consequently, we cannot totally eliminate the possibility that these off-target effects of fostamatinib contribute to its inhibition of mast cell degranulation and/or mast cell desensitization. We think this unlikely, however, because fostamatinib inhibits Syk 5-times more potently than it inhibits the other tyrosine kinases when studied in vitro on mouse mast cells⁵⁸ while a fostamatinib dose (40 mg/kg) that is barely able to inhibit Syk-dependent mast cell degranulation in vivo significantly suppresses mast cell desensitization (Fig. 7A-C). Fourth, our murine models, even those with human mast cells and basophils, do not develop detectable IgE-mediated disease other than the development of shock (e.g.; urticaria, bronchospasm, flushing, angioedema, and fever). Consequently, drugs that suppress these disease features, but do not suppress shock (which is predominantly mediated in mice by vascular leak), will not be found to be efficacious in our models. This may explain why some of the drugs found useful by Castells and her colleagues for IgE-mediated features other than shock, including flushing and bronchospasm⁴², had no efficacy in our model. Thus, our observation that β -

493 adrenergic agonists are useful for preventing shock during rapid desensitization is more
494 likely to be human-relevant than the failure of several other agents to ameliorate
495 anaphylaxis in our models. We look forward to clinical trials that evaluate the usefulness
496 of adding relatively small doses of β -adrenergic agonists to antihistamines during rapid
497 drug desensitization and, we hope in the future, rapid desensitization with anti-Fc ϵ RI α
498 mAbs.

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Figure legends:

Figure 1. H₁ antagonists antihistamines partially protect against anti-IgE mAb-induced hypothermia. A and B: BALB/c mice (6/group) were injected i.p. with 200 µg of triprolidine or vehicle, followed 1 hour later by i.v. injection of saline (no treatment) or 20 µg of anti-mouse IgE mAb and followed for 60 minutes for development of hypothermia. Kruskal-Wallis followed by Whitney-Mann U test. B: Doxepin (10 mg/kg injected i.p. 5 minutes before challenge) and triprolidine protect BALB/c mice (6/group) against anti-IgE mAb-induced hypothermia to the same extent. Kruskal-Wallis followed by Whitney-Mann U test. C. BALB/c mice (5-6/group) were injected i.p. with saline, 200 µg of triprolidine, and/or 1 mg/kg of ranitidine, followed 1 hr later by i.v. injection of saline (no treatment) or 20 µg of anti-mouse IgE mAb and followed for 60 minutes for development of hypothermia. Ranitidine did not significantly suppress IgE-mediated hypothermia; triprolidine + ranitidine did not suppress hypothermia significantly > triprolidine alone. Kruskal-Wallis followed by Mann-Whitney U test. For all figures, * = <0.05; ** = < 0.01; *** = <0.001; **** = < 0.0001.

Figure 2. β -adrenergic agonists partially protect against anti-IgE mAb induced hypothermia. BALB/c mice (4-6/group) were pretreated with or without formoterol (A), terbutaline (B), albuterol (C) or indacaterol (D), challenged i.v. with 20 µg of anti-mouse IgE mAb and followed for 60 min for development of hypothermia. Formoterol was injected i.v. 37 minutes prior to challenge; terbutaline was injected s.c. 30 minutes prior to challenge; albuterol was injected i.p. 30 minutes prior to challenge; indacaterol was injected i.v. 30 min prior to challenge. Statistical tests: A-D. Kruskal-Wallis followed by Whitney-Mann U test.

Figure 3. Syk and BTK dependence of anaphylaxis and mast cell degranulation. A. In 2 separate experiments, BALB/c mice (6/group) were pre-treated by i.p. injection with vehicle or 40 or 80 mg/kg of fostamatinib, then challenged i.v. with 20 µg of anti-mouse IgE mAb and followed 60 minutes for hypothermia. B. In 2 separate experiments, BALB/c mice (4-6/group) were treated 1 hour prior to challenge with vehicle, triprolidine, 80 mg/kg of fostamatinib, or 2.5 mg/kg s.c. of indacaterol and challenged i.v. with 20 µg of anti-mouse IgE mAb. Mice were bled 4 hours after challenge and serum MMCP1 determined by ELISA. C. BALB/c and huFcεRIα mice (5/group) were pre-treated by i.p. injection with vehicle or 25 mg/kg ibrutinib and challenged 30 min later i.v. with saline or 20 µg of anti-mouse IgE mAb, then followed 60 minutes for hypothermia. Both temperature curves and maximum decreases in temperature are shown. Similar results were observed in a second experiment (not shown). D. In the same experiment shown in C, mice were bled 4 hr after anti-IgE mAb or saline challenge and serum MMCP1 levels determined by ELISA. Statistical tests: A: Whitney-Mann U test. B - D: Kruskal-Wallis followed by Whitney-Mann U test.

Figure 4. Inhibition of IgE-mediated anaphylaxis with combinations of an antihistamine, a β -adrenergic agonist, and a Syk inhibitor. BALB/c mice (5-6/group) were pretreated with the agents shown and challenged i.v. with 20 µg of anti-mouse IgE mAb, then followed for 60 minutes for development of hypothermia. All mice were treated with IL-4C that contained 1 µg of IL-4 24 hours prior to challenge for experiments shown in panel D. For all panels: Kruskal-Wallis followed by Whitney-Mann U test.

Figure 5. Synergistic inhibition of IgE-mediated anaphylaxis by tyrosine kinase inhibitors. BALB/c mice (4-6/group) were pre-treated i.p. 30 minutes prior to challenge

with vehicle, fostamatinib, imatinib, idelalisib, or ibrutinib, or combinations of fostamatinib with each of the other tyrosine kinase inhibitors, then challenged i.v. with 20 μ g of anti-IgE mAb and followed for 60 minutes for development of hypothermia. Statistical tests: A: Kruskal-Wallis + Whitney-Mann U (2-tailed); B – C: 2-way ANOVA + THSD. D - F: Kruskal-Wallis + Whitney-Mann U (1-tail) with adjustment for multiple groups.

Figure 6. Inhibition of IgE/Fc ϵ RI-mediated anaphylaxis in humanized mice by a combination of an antihistamine, a β -adrenergic agonist, and a Syk inhibitor. Non-reconstituted (C) or human cord blood-reconstituted (A-D) NSGS mice (3-4/group) were: A) pre-treated with vehicle (no drugs) or a combination of 80 mg/kg of fostamatinib, 2.5 mg/kg of indacaterol and 200 μ g of triprolidine and challenged i.v. with 10 μ g of an anti-huFc ϵ RI α mAb; B) primed i.v. with 10 μ g mouse IgE anti-TNP mAb, pre-treated with vehicle or a combination of 80 mg/kg of fostamatinib, 2.5 mg/kg of indacaterol and 200 μ g of triprolidine and challenged i.v. with 10 μ g of TNP-OVA; or C) pre-treated with vehicle or 40 mg/kg of fostamatinib, 2.5 mg/kg of indacaterol and 200 μ g of triprolidine and challenged i.v. with 50 μ g of an anti-huFc ϵ RI α mAb. Statistical tests: Fisher's exact test for comparison of mortality.

Figure 7. Fostamatinib inhibits mast cells and/or basophil desensitization. A and B) In 2 pooled experiments, a total of 12 huFc ϵ RI α transgenic mice/group were sensitized i.v. with 10 μ g of mouse IgE anti-TNP mAb (which binds avidly to huFc ϵ RI α), and pre-treated the next day with 40 mg/kg of fostamatinib or vehicle, followed 30 min later by i.v. injection of 100 μ g of 15.1 anti-huFc ϵ RI α mAb or isotype control mAb. Four hours later, mice were challenged i.v. with 10 μ g of TNP-BSA and followed for 60 minutes for

development of hypothermia. C. In a separate experiment (5 mice/group), peritoneal mast cells were analyzed for membrane expression of mouse IgE by flow cytometry 24 hours after anti-Fc ϵ RI α mAb injection. Fostamatinib was used at 80 mg/kg. D and E. The ability of ibrutinib to suppress mast cell desensitization in vivo by 15.1 anti-Fc ϵ RI α mAb was tested, using the diagrammed protocol. 6 mice/group. Statistical tests: A: Kruskal-Wallis followed by Whitney-Mann U test. B: Kruskal-Wallis and 1-tailed Whitney-Mann U test with correction for multiple comparisons.

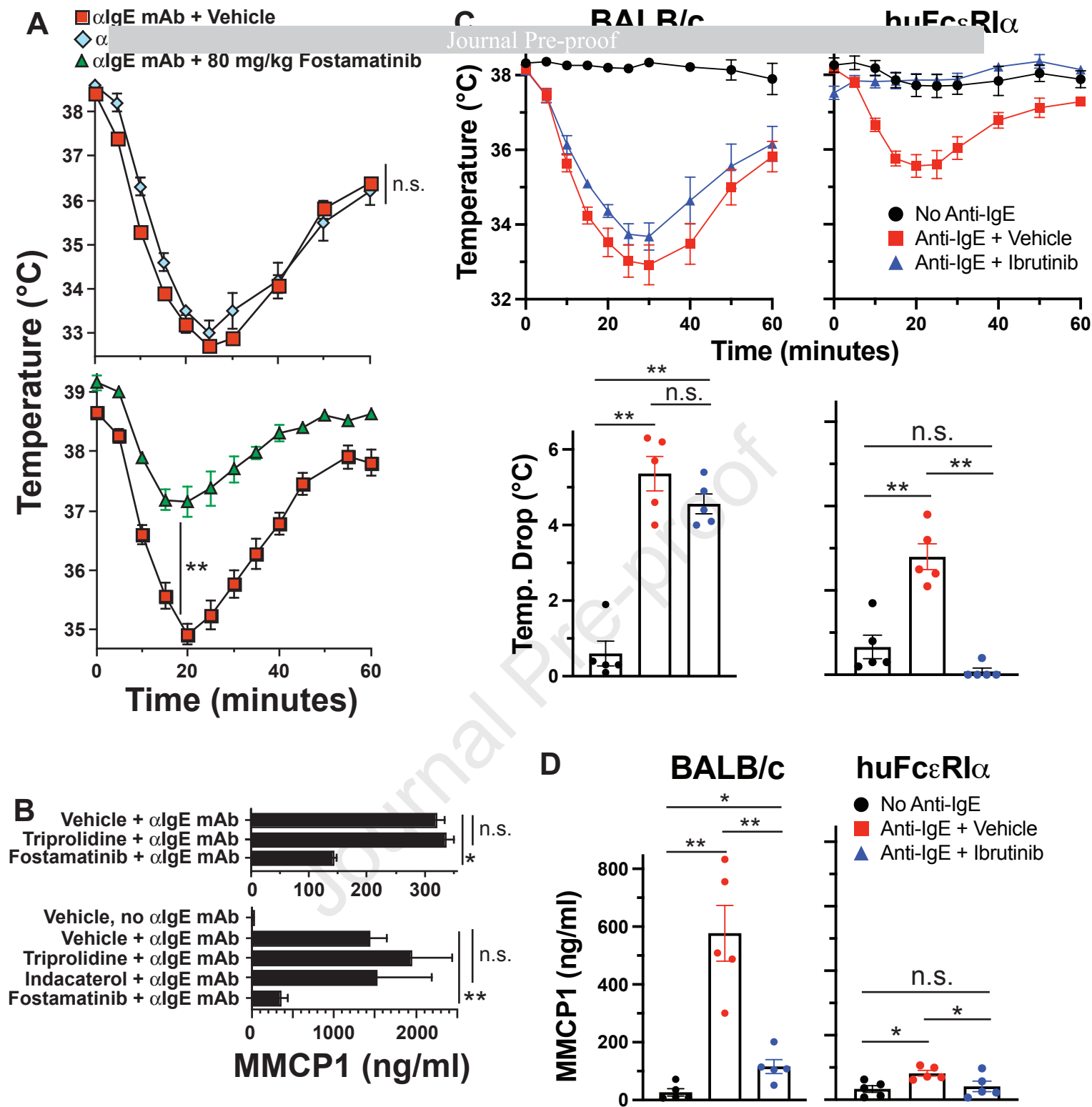
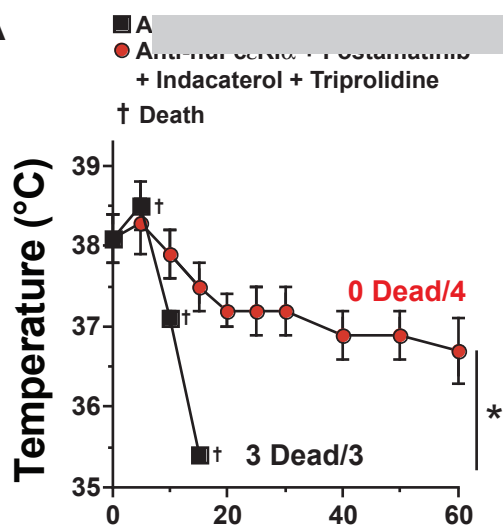
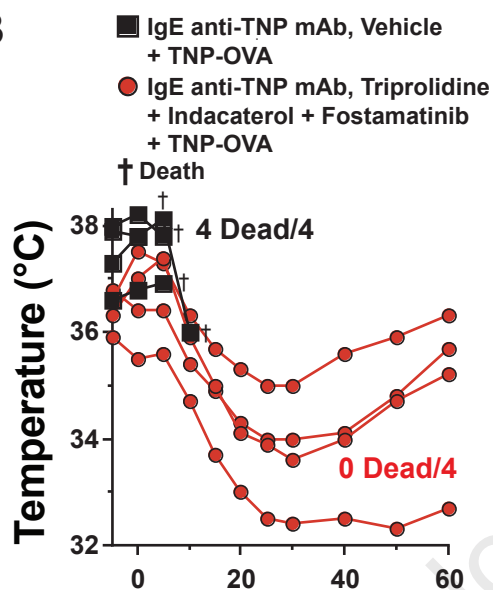


Fig. 3

A



B



C

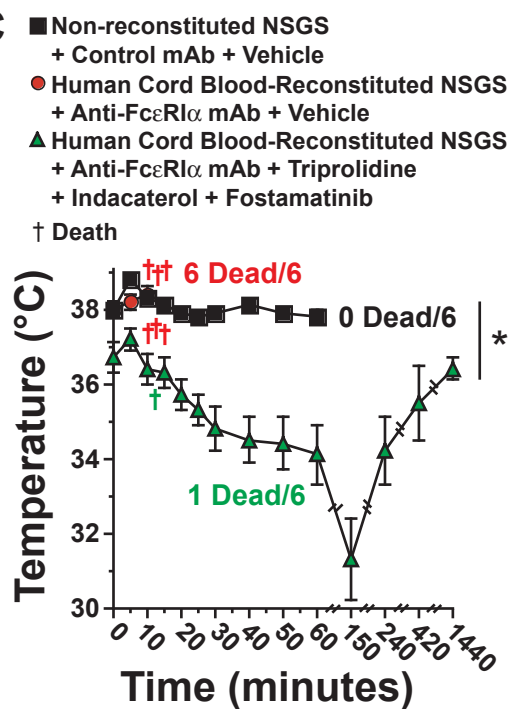


Fig. 6

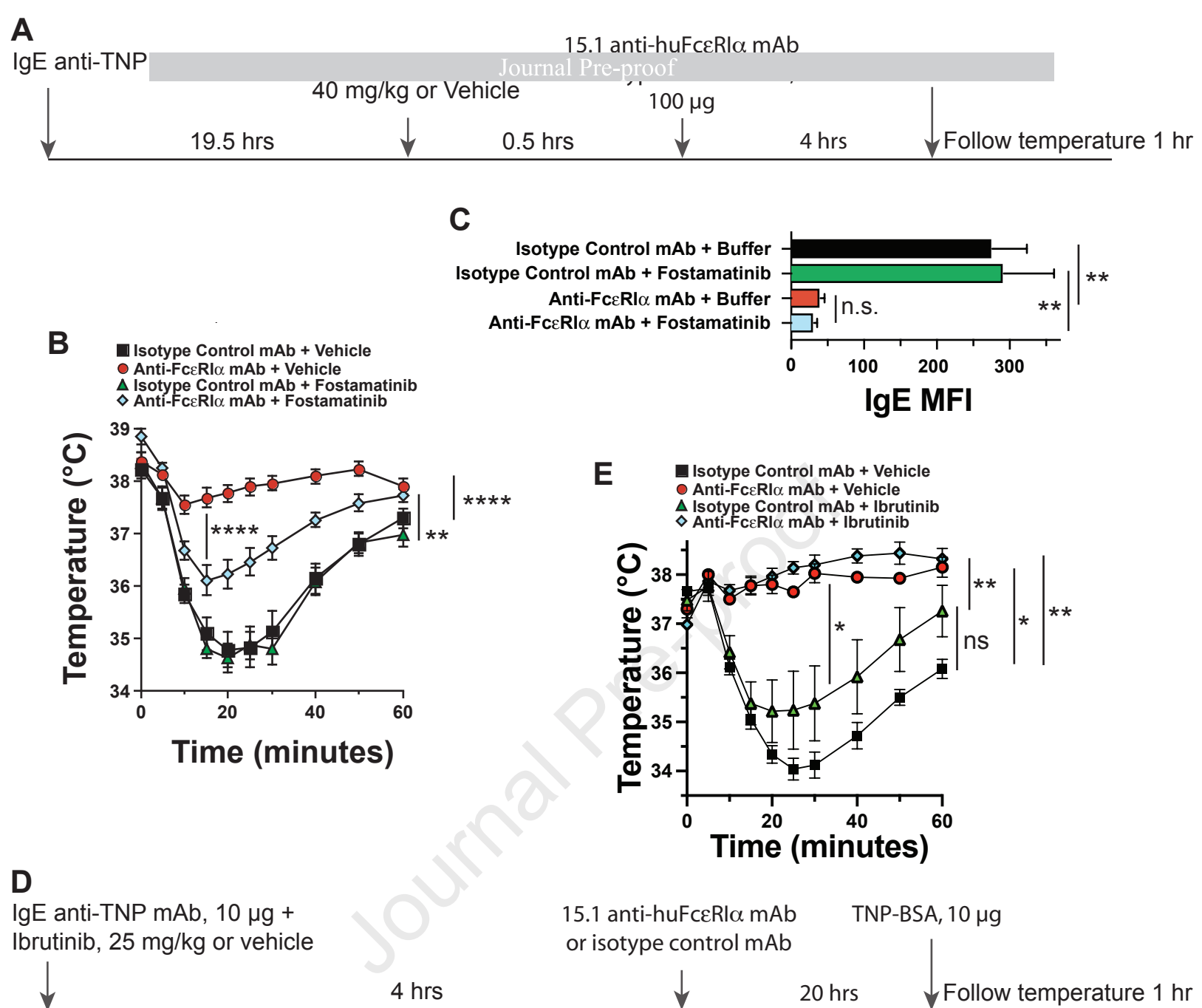


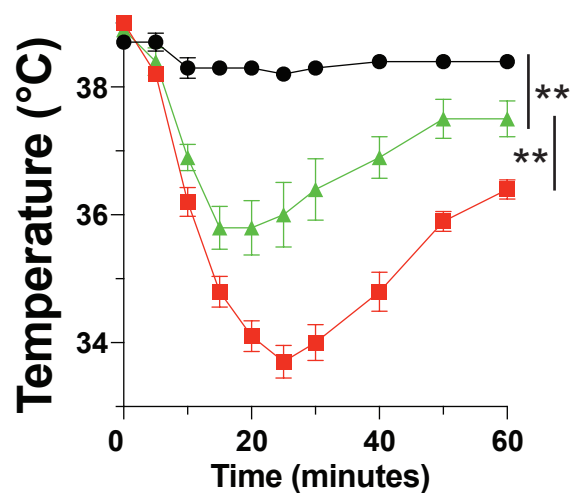
Fig. 7

A

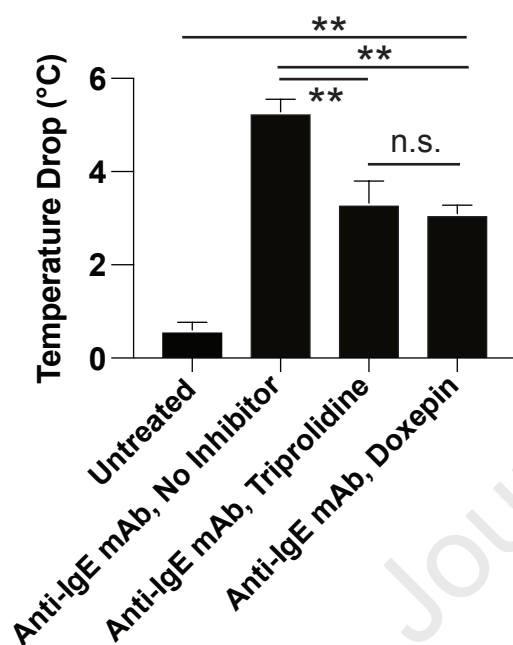
● Untreated

■ Ar

Journal Pre-proof

▲ Anti-IgE mAb, 200 μ g Triprolidine

B



C

● No Anti-IgE mAb

■ Anti-IgE mAb

▲ Anti-IgE mAb + Triprolidine

▼ Anti-IgE mAb + Ranitidine

◆ Anti-IgE mAb + Triprolidine + Ranitidine

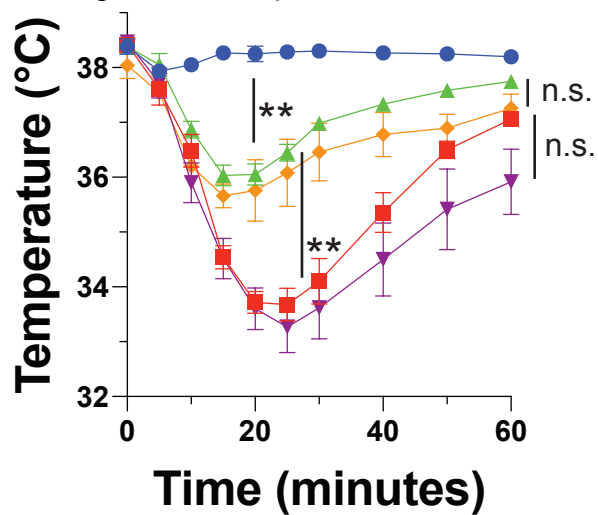


Fig. 1

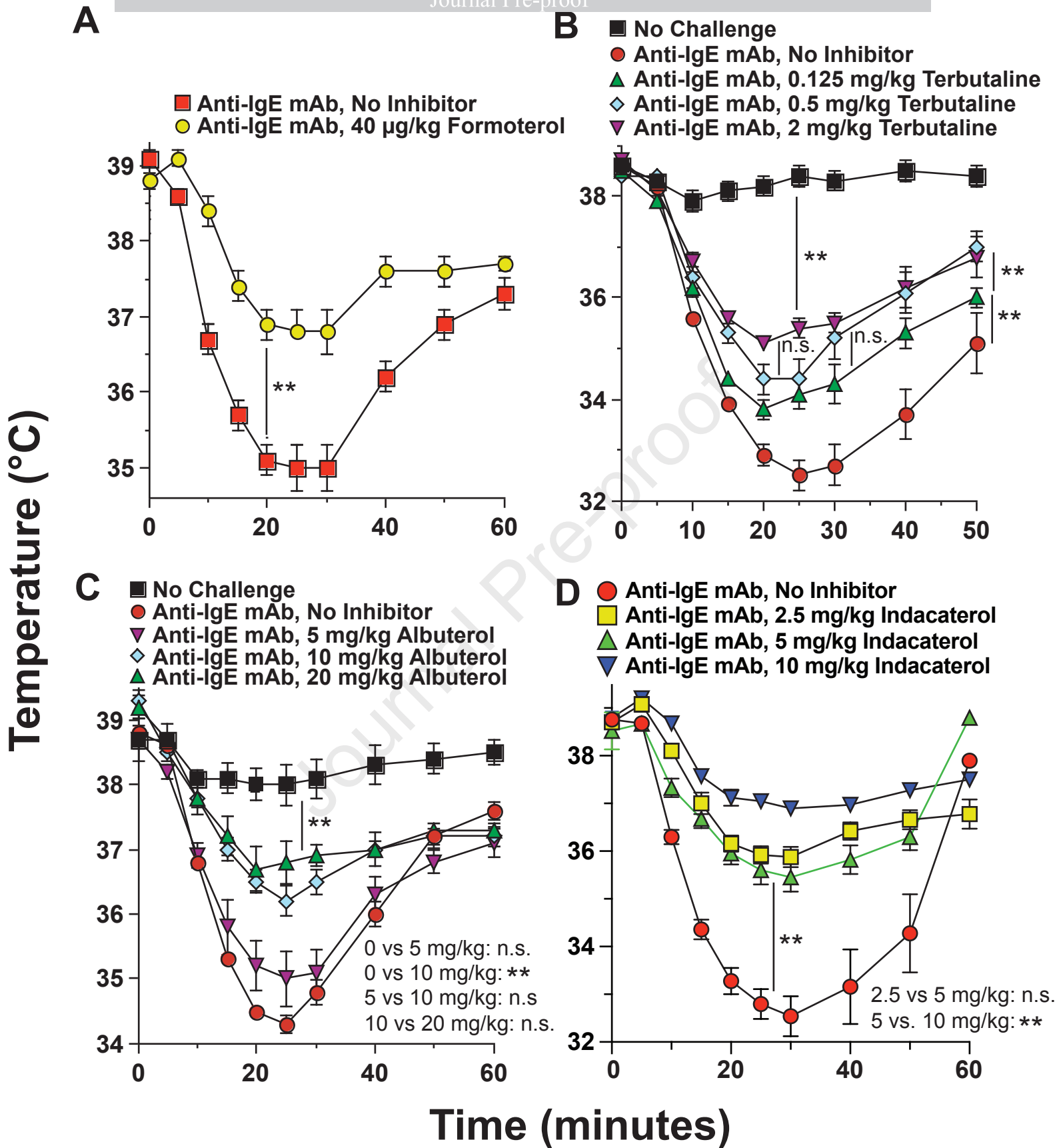
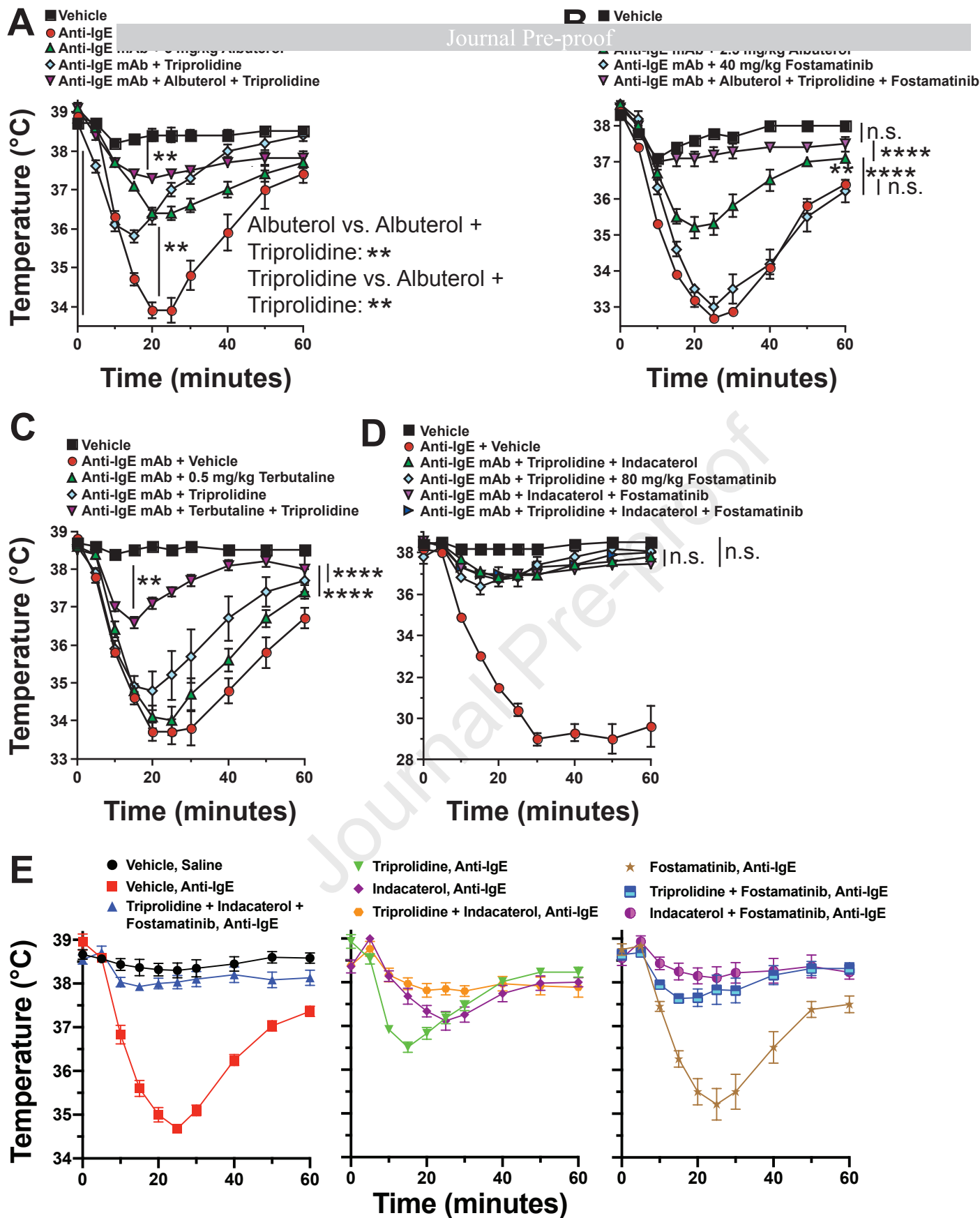


Fig. 2



Vehicle, Saline vs. Anti-IgE + Triprolidine + Indacaterol: n.s.
 Vehicle, Saline vs. Anti-IgE + Triprolidine + Fostamatinib: ns
 Vehicle, Saline vs. Anti-IgE + Indacaterol + Fostamatinib: n.s.
 Vehicle, Saline vs. Anti-IgE + Triprolidine + Fostamatinib + Indacaterol: n.s.
 Anti-IgE + any 2 drugs vs. Anti-IgE + 3 drugs: n.s.
 Anti-IgE + Indacaterol vs. Anti-IgE + Indacaterol + Fostamatinib: .0023
 Anti-IgE + Indacaterol vs. Anti-IgE + Indacaterol + Triprolidine: .036
 Anti-IgE + Triprolidine vs. Anti-IgE + Triprolidine + Indacaterol: .0005
 Anti-IgE + Triprolidine vs. Anti-IgE + Triprolidine + Fostamatinib: .0032
 Anti-IgE + Fostamatinib vs. Anti-IgE + Indacaterol + Fostamatinib: <.0001

Anti-IgE + Fostamatinib vs. Anti-IgE + Triprolidine + Fostamatinib: <.0001

Fig. 4

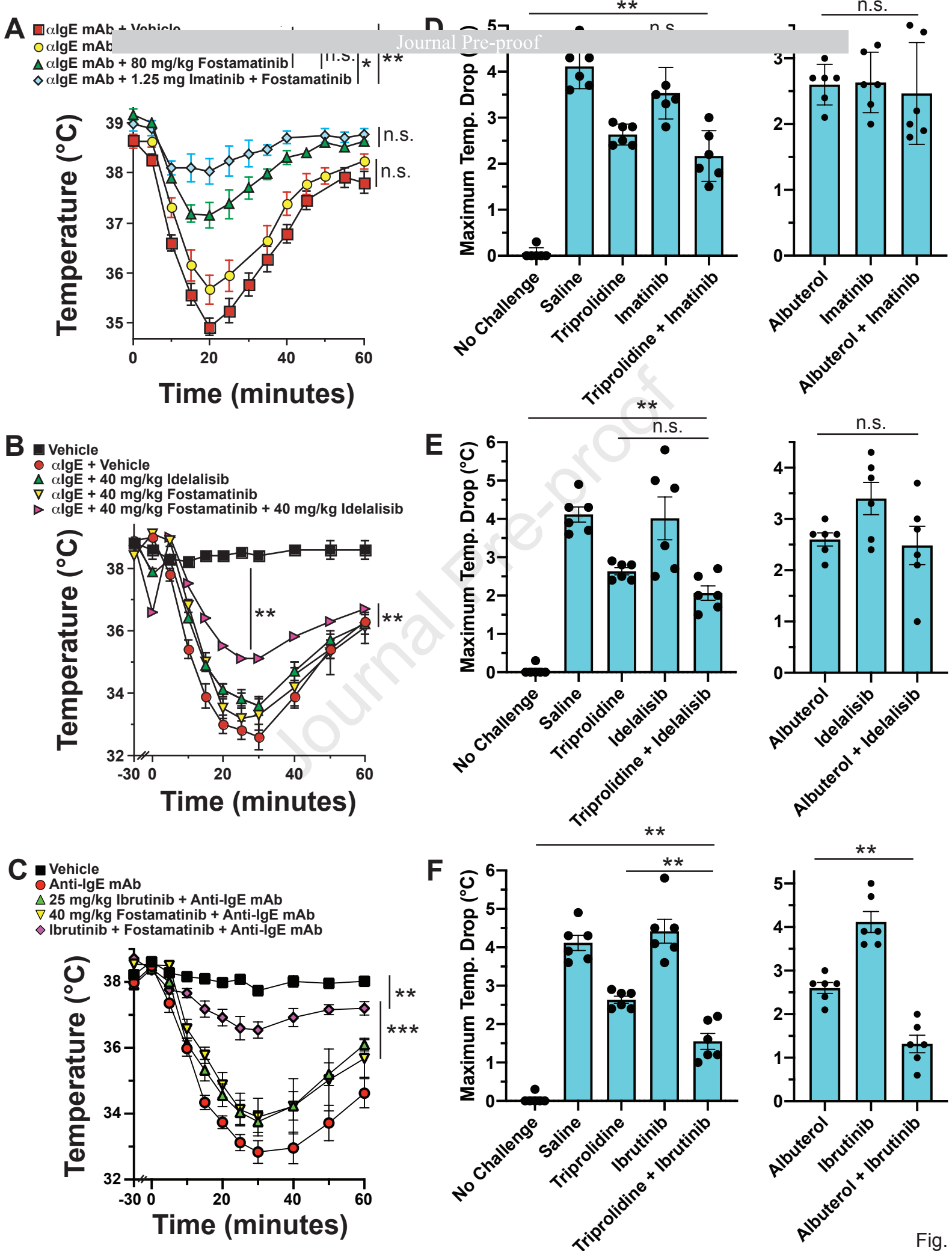


Fig. 5

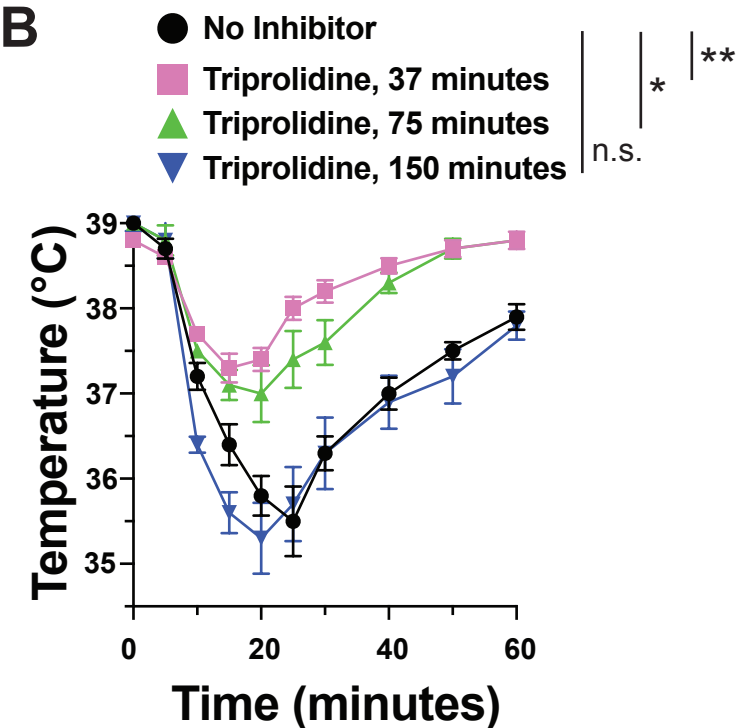
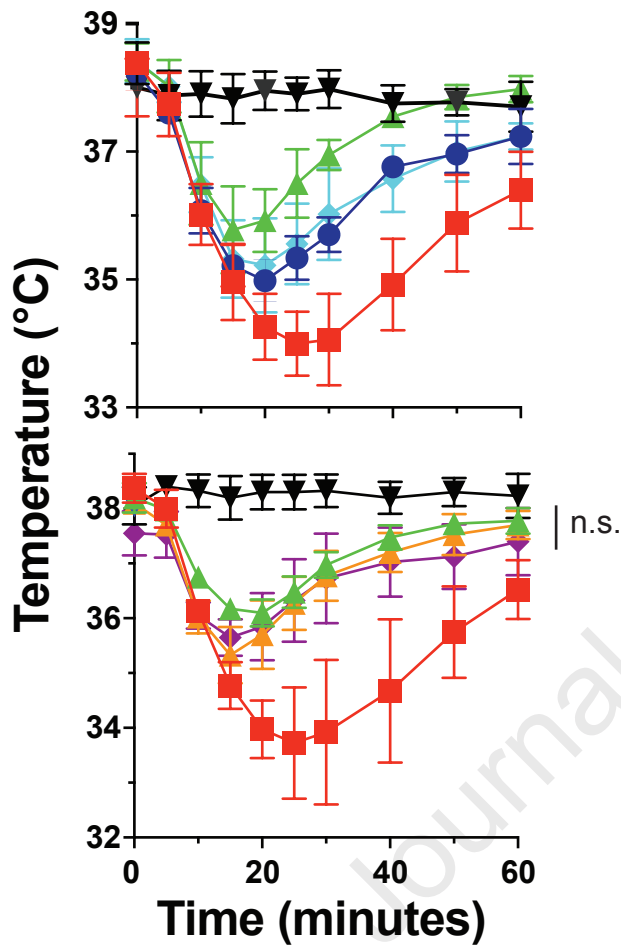
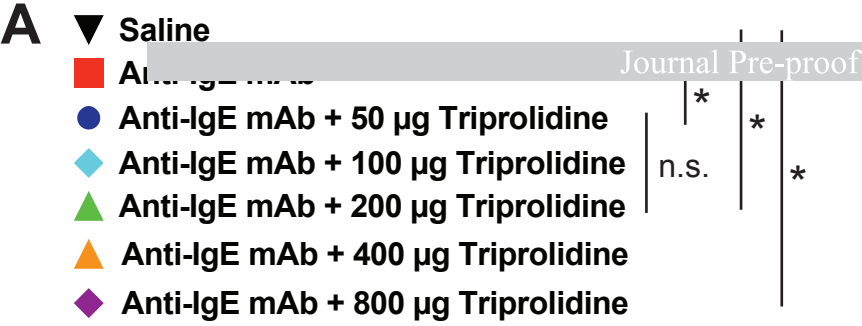
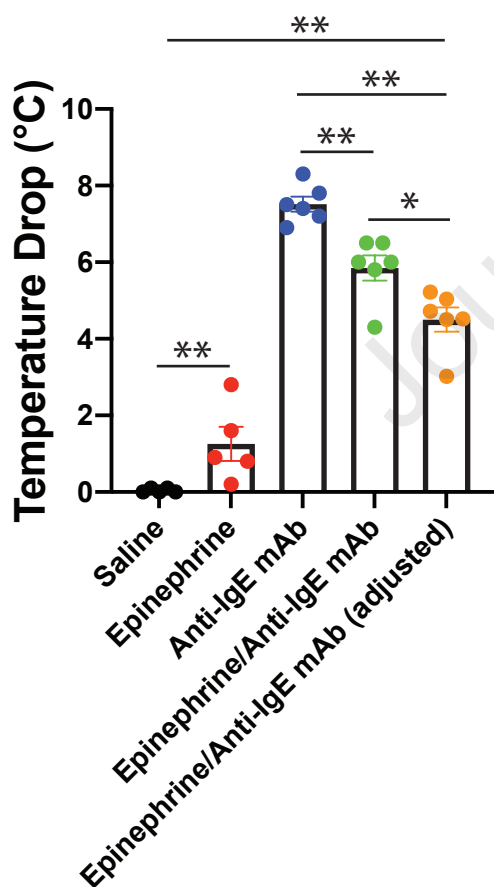
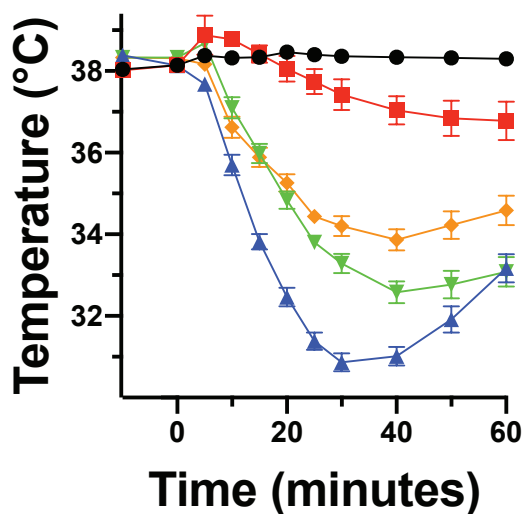
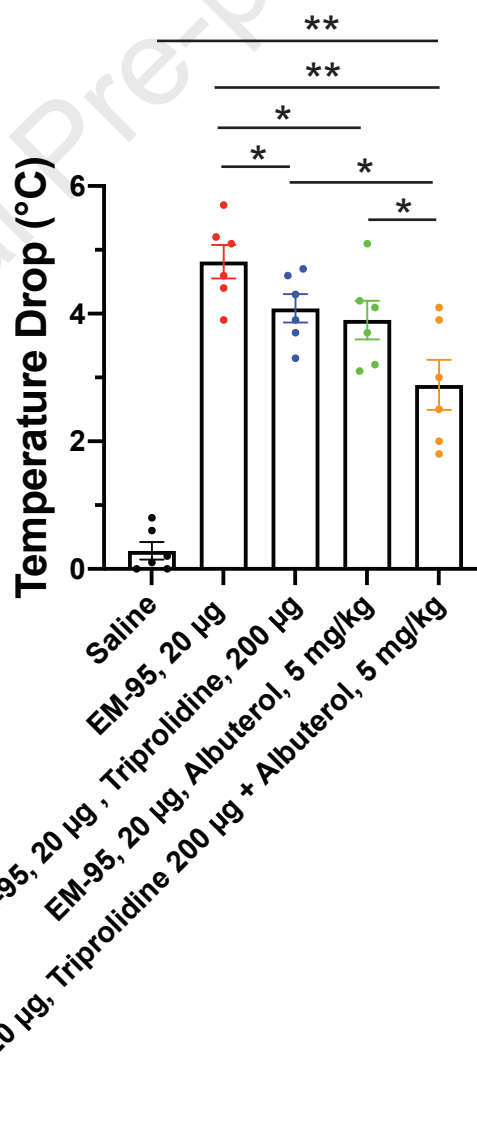
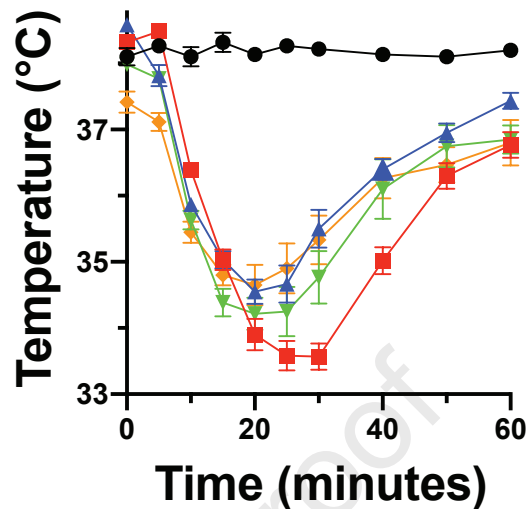


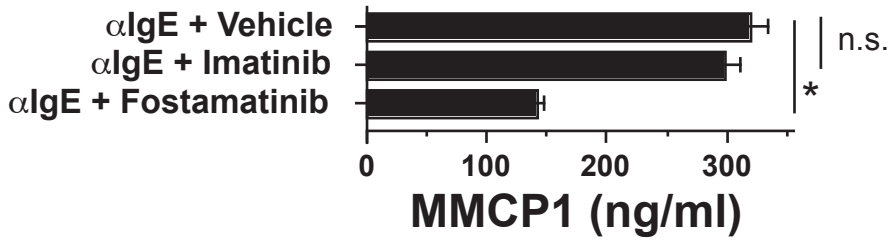
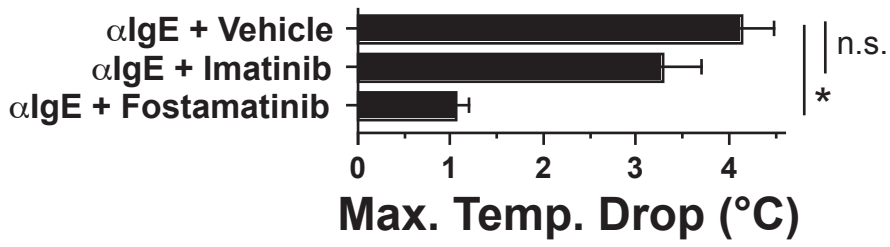
Fig. S1

- Saline
- Epinephrine
- ▲ Anti-IgE mAb
- ▼ Epinephrine/Anti-IgE mAb
- ◆ Epinephrine/Anti-IgE mAb (adjusted)



- Saline
- EM-95, 20 μ g
- ▲ EM-95, 20 μ g, Triprolidine, 200 μ g
- ▼ EM-95, 20 μ g, Albuterol, 5 mg/kg
- ◆ EM-95, 20 μ g, Triprolidine 200 μ g, Albuterol, 5 mg/kg





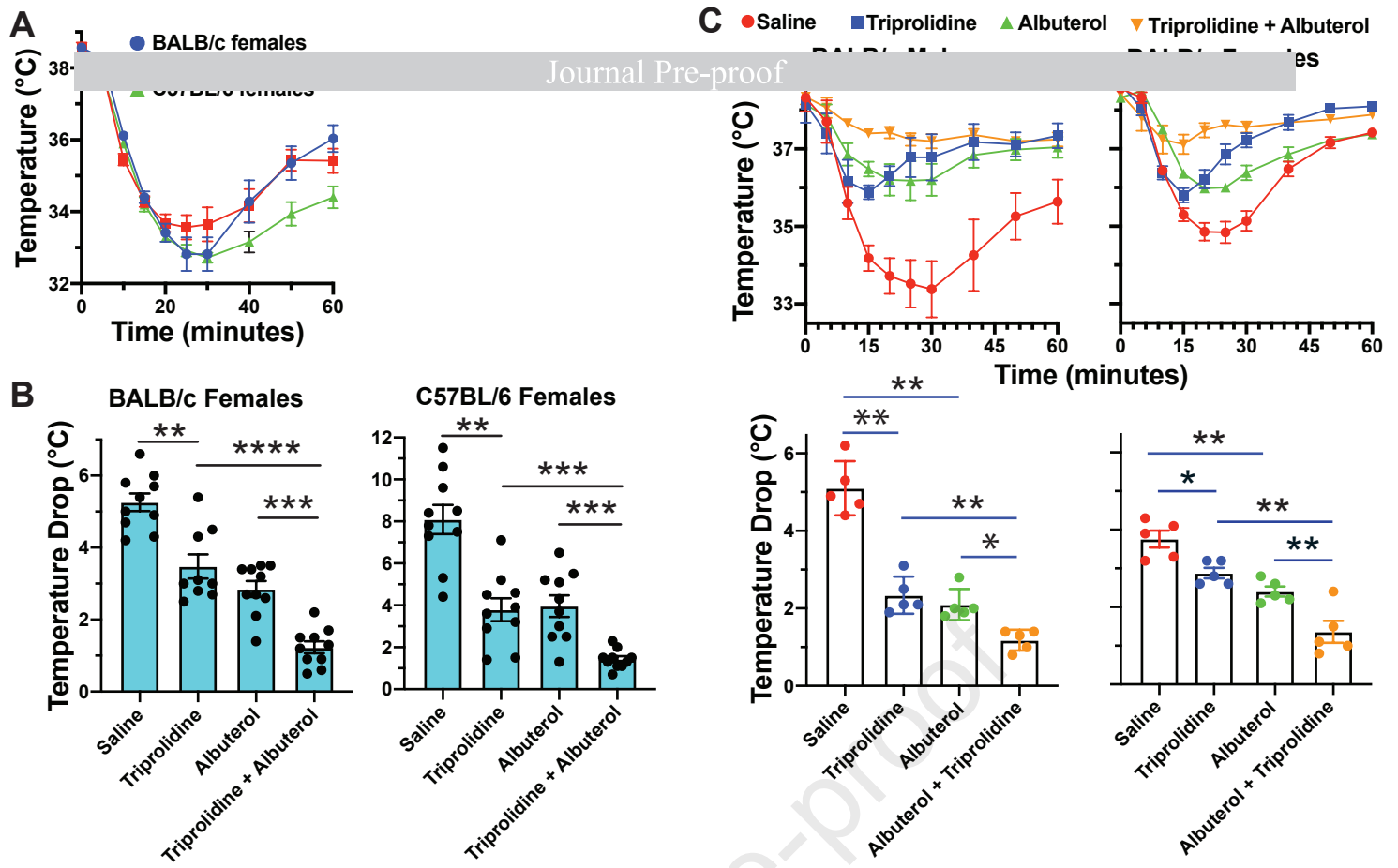


Fig. S6

Supplemental Table 1. Agents tested for inhibition of IgE-mediated anaphylaxis.

Agent	Dose Range	Route	Min. Pre-Challenge	Vehicle
Fostamatinib	2.5, 5, 10, 20, 40, or 80 mg/kg	i.p.	30, 37, 60, 75, 120, 150	0.1% carboxymethylcellulose
Imatinib	1.25 mg	i.p.	30	saline
Triprolidine	50, 100, 200, 400 or 800 µg	i.p.	30, 60, or 120	saline
JNJ-777120	20 mg/kg	s.c.	30 mins	4.4% DMSO in saline
Resveratrol	10 mg/kg	o.g.	60	2% DMSO in saline
Formoterol	10, 20, or 40 µg/kg	i.v.	10, 37, 75, or 150	.6% DMSO in saline
Theophylline	5 mg/kg	i.p.	30	saline
Cromolyn	300 µg	i.p.	30	saline
Curcumin	50 mg/kg	o.g.	60	7.3% DMSO in saline
Kaempferol	50 mg/kg	o.g.	60	7.3% DMSO in saline
Quercetin	50 mg/kg	o.g.	60	7.3% DMSO in saline
Indacaterol	2.5, 5, or 10 mg/kg or 2.5 mg/kg	i.v. s.c.	30 37, 60, 75, 150, 300, or 600	2.3% DMSO in saline 0.55% DMSO in saline
Doxepin	10 mg/kg	i.p.	5	saline
Terbutaline	0.5 or 2 mg/kg	s.c.	30	saline
Albuterol	0.078125, 0.15625, 0.312, 0.625, 1.25, 2.5, 5, 10, or 20 mg/kg	i.p.	30	saline

Idelalisib	5, 10, 20, 40 or 80 mg/kg	i.p.	30	30% polyethylene glycol 400 / 0.5% Tween 80 / 5% propylene glycol
Ibrutinib	10 or 25 mg/kg	i.p.	30	1.8% DMSO in saline
Ketotifen fumate	25mg/kg	i.p.	30	25% DMSO in saline
Zileuton	50 mg/kg	o.g.	24 or 60	23.8% DMSO in saline
Montelukast	6 mg/kg	s.c.	24 or 60	16.7% DMSO in saline
Ranitidine	100 mg/kg	i.p.	30	PBS
Epinephrine	2, 10 or 50 µg	s.c.	5 (post-challenge)	saline

Supplemental Table2. Effects of individual agents on IgE-mediated anaphylaxis

Target	Inhibitor/Agonist	Effect on FcεRI-Mediated Induction of Hypothermia
Histamine R1	Triprolidine Doxepin ¹ Ketofen ²	Considerable dose-related inhibition Considerable inhibition, similar to triprolidine Less effective inhibition than triprolidine or doxepin
Histamine R2	Ranitidine	No effect
Histamine R4	JNJ-777120	tendency towards mild inhibition, but not significant
β-adrenergic R	<i>Formoterol</i> <i>Terbutaline</i> <i>Albuterol</i> <i>Indacaterol</i>	Considerable dose-related inhibition Considerable dose-related inhibition Considerable dose-related inhibition Considerable dose-related inhibition
α- and β-adrenergic R	<i>Epinephrine</i>	Direct toxic effect, induces hypothermia
Syk	Fostamatinib ³	Considerable dose-related inhibition
Bruton's tyrosine kinase (BTK)	Ibrutinib	Little or no effect
PI3K P110δ	Idelalisib	Little or no effect
Abl/Kit	Imatinib	Little or no effect
Mast cells	Cromolyn sodium	Little or no effect
5-lipoxygenase	Zileuton	Little or no effect
CysLT1	Montelukast	Little or no effect

Leukotriene D4R	REV 5901	Little or no effect
PAFR	ABT-491	Little or no effect
5-HTR _{1/2/2a/7}	Metergoline Ketanserin	Little or no effect
Corticosteroid R	Dexamethasone	Little or no effect
Multiple targets and anti-oxidant effects	Resveratrol (phenol/phytoalexin)	Little or no effect
	Quercetin (flavonoid polyphenol)	Little or no effect
	Kaempferol (antioxidant flavanol)	Little or no effect
	Curcumin (phenol diarylheptanoid)	Little or no effect
Multiple targets	Theophylline	Moderate inhibitory effect

¹Inhibits both H1R and H2R

²Inhibits H1R and 5-HTRs

³Also suppresses FcεRI desensitization

Supplemental Table 3. Effects of combinations of agents on IgE-mediated anaphylaxis.

Drug Combination	Targets¹	Suppressive Effect on FcεRI-Mediated Hypothermia
Triprolidine + ranitidine	H1R, H2R	= triprolidine alone
Triprolidine + JNJ-777120	H1R, H4R	tendency to be > triprolidine alone, but not significant
Triprolidine + albuterol	H1R, β -AR	> either drug alone
Triprolidine + indacaterol	H1R, β -AR	> either drug alone
Triprolidine + terbutaline	H1R, β -AR	> either drug alone
Triprolidine + fostamatinib	H1R, Syk	> either drug alone
Triprolidine + idelalisib	H1R, PI3K P110 δ	= triprolidine alone
Triprolidine + ibrutinib	H1R, BTK	> either drug alone
Triprolidine + imatinib	H1R, Abl/Kit	= triprolidine alone
Albuterol + idelalisib	β -AR, PI3K P110 δ	= albuterol alone
Albuterol + ibrutinib	β -AR, BTK	> either drug alone
Albuterol + imatinib	β -AR, Abl/Kit	= albuterol alone
Indacaterol + fostamatinib	β -AR, Syk	> either drug alone
Triprolidine + indacaterol + fostamatinib	H1R, β -AR, Syk	Similar to any of the 2 drug combinations
Triprolidine + albuterol + fostamatinib	H1R, β -AR, Syk	Similar to triprolidine + albuterol

Imatinib + fostamatinib	Abl/Kit; Syk	> either drug alone
Idelalisib + fostamatinib	PI3K P110 δ Syk	> either drug alone
Ibrutinib + fostamatinib	BTK, Syk	> either drug alone

¹Plain type signifies antagonist; italic type signifies agonist

Supplemental figure legends:

Figure S1. Triprolidine dose-response (A, 4-5 mice/group) and kinetic data (B, 4-5 mice/group) establish that the maximum ability of this antihistamine to inhibit IgE-mediated anaphylaxis is achieved by administration of at least 200 µg/mouse 30 – 60 minutes prior to challenge.

Statistics: Kruskal-Wallis and 1-tailed Whitney-Mann U test with correction for multiple comparisons. For all supplemental figures, * = <0.05; ** = < 0.01; *** = <0.001; **** = < 0.0001.

Figure S2. Epinephrine fails to inhibit the development of IgE-mediated anaphylaxis in BALB/c mice. BALB/c mice (4/group) were primed by injection of 10 µg of IgE anti-TNP mAb and challenged i.v. the next day with 10 µg of TNP-BSA. Five minutes after challenge mice were injected s.c. with saline or 2, 10, or 50 µg of epinephrine and followed for 1 hour for development of hypothermia. No significant differences (Kruskal-Wallis).

Figure S3. Prophylactic effect of epinephrine and therapeutic effects of triprolidine and albuterol on IgE-mediated anaphylaxis. A. BALB/c mice (5-6/group) were injected s.c. with saline or 25 µg of epinephrine. 10 min later they were injected i.v. with saline or 20 µg of EM-95 mouse anti-IgE mAb and rectal temperatures were followed for 60 min. To adjust for the direct effects of epinephrine on rectal temperature, deviations from baseline in mice injected with epinephrine alone (red line) were subtracted for each time point from temperatures in mice injected with epinephrine + anti-IgE mAb (green line). Adjusted temperatures are shown in orange line. B. BALB/c mice (6/group) were injected i.v. with saline or 20 µg of EM-95 anti-mouse IgE mAb. 5 min later some mice were injected i.p. with the doses of albuterol and/or triprolidine shown. Rectal temperatures were followed for 60 min after anti-mouse IgE mAb injection. Statistics: Kruskal-Wallis and Mann-Whitney U test.

Figure S4. Fostamatinib, but not imatinib, idelalisib, or ibrutinib provides acute protection against anaphylaxis in BALB/c mice. BALB/c mice (4-6/group) were pre-treated with vehicle, imatinib (1.25 mg/kg), fostamatinib (80 mg/kg), idelalisib, or ibrutinib (10 mg/kg) and challenged i.v. with 20 µg of anti-mouse IgE mAb. Mice were followed for 60 minutes for development of hypothermia; sera were obtained from some mice 4 hours after challenge and MMCP1 levels were determined by ELISA. Statistical tests: 1-way ANOVA with 1-tailed Whitney-Mann U-test with correction for multiple comparisons.

Figure S5. Combined antihistamine (triprolidine), β -adrenergic agonist (albuterol), and Syk inhibitor (fostamatinib) treatment protects highly susceptible mice from $Fc\epsilon RI$ -mediated anaphylaxis. A. huFc $\epsilon RI\alpha$ transgenic mice (5/group) were pre-treated with vehicle or a combination of triprolidine, albuterol and fostamatinib and challenged i.v. with 50 µg of AER-37 anti-huFc $\epsilon RI\alpha$ or isotype-control mAb, then followed for 60 minutes for development of hypothermia. Statistical tests: Kruskal-Wallis and 1-tailed Whitney-Mann U test with correction for multiple comparisons. B. reNSGS mice were pre-treated with vehicle, 2.5 mg/kg of indacaterol + 200 µg of triprolidine; or indacaterol, triprolidine + 40 mg/kg of fostamatinib and challenged i.v. with 500 ng of anti-huFc $\epsilon RI\alpha$ mAb. Mice were followed for 60 min for rectal temperature. Statistical tests: 2- or 3-way ANOVA + THSD. Statistical tests: 3-way ANOVA and THSD.

Figure S6. The combination of albuterol and triprolidine enhances protection against IgE-mediated anaphylaxis in male and female BALB/c mice and female C57Bl/6 mice. A. Anti-mouse IgE mAb induces anaphylaxis in male and female BALB/c mice and female C57Bl/6 mice. B. The combination of albuterol, 5 mg/kg i.p. and triprolidine, 200 µg i.p. decreases hypothermia induced by 20 µg EM-95 anti-IgE mAb in both BALB/c and C57Bl/6 female mice. Combination

of 2 experiments, total 10 mice/group. C. The combination of albuterol, 5 mg/kg i.p. and triprolidine, 200 μ g i.p. decreases hypothermia induced by 20 μ g of EM-95 anti-IgE mAb in both BALB/c male and female mice. 5 mice/group. Statistical test: Kruskal-Wallis and 1-tailed Whitney-Mann U test with correction for multiple comparisons.