

ORIGINAL ARTICLE

Basic and Translational Allergy Immunology

The soluble isoform of human FcεRI is an endogenous inhibitor of IgE-mediated mast cell responses

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Abstract

Background: The soluble isoform of FcεRI, the high-affinity IgE receptor (sFcεRI), is a protein of the IgE network with poorly defined functions.

Objective: To define cellular sources and signals that result in the production of human sFcεRI and study its *in vivo* functions.

Methods: FcεRI-transfected human cell lines (MeJuso), human monocyte-derived dendritic cells (moDCs), and murine bone marrow-derived mast cells (MC) were stimulated by FcεRI cross-linking and release of sFcεRI was analyzed (ELISA, Western Blot). Lysosomal-associated membrane protein 1 degranulation assays and human basophil activation tests (BATs) were used to study IgE-dependent activation. Recombinant sFcεRI (rsFcεRI) was used to assess its role in murine models of anaphylaxis with WT (wild-type) and IgE^{-/-} (IgE-deficient) mice.

Results: Antigen-specific cross-linking of IgE-loaded FcεRI on MeJuso cells that express the trimeric or tetrameric receptor isoform induced the production of sFcεRI. Using MCs and moDCs, we confirmed that IgE/FcεRI activation induces sFcεRI release. We demonstrated that generation of sFcεRI requires Src phosphorylation and endo/lysosomal acidification. In experimental mouse models, sFcεRI diminishes the severity of IgE-mediated anaphylaxis. BATs confirmed that, comparable to the anti-IgE monoclonal antibody omalizumab, sFcεRI is an inhibitor of the human innate IgE effector axis, implying that sFcεRI and omalizumab potentially inhibit each other *in vivo*.

Conclusion: sFcεRI is produced after antigen-specific IgE/FcεRI-mediated activation signals and functions as an endogenous inhibitor of IgE loading to FcεRI and IgE-mediated activation. Our results imply, therefore, that sFcεRI contributes to a negative regulatory feedback loop that aims at preventing overshooting responses after IgE-mediated immune activation.

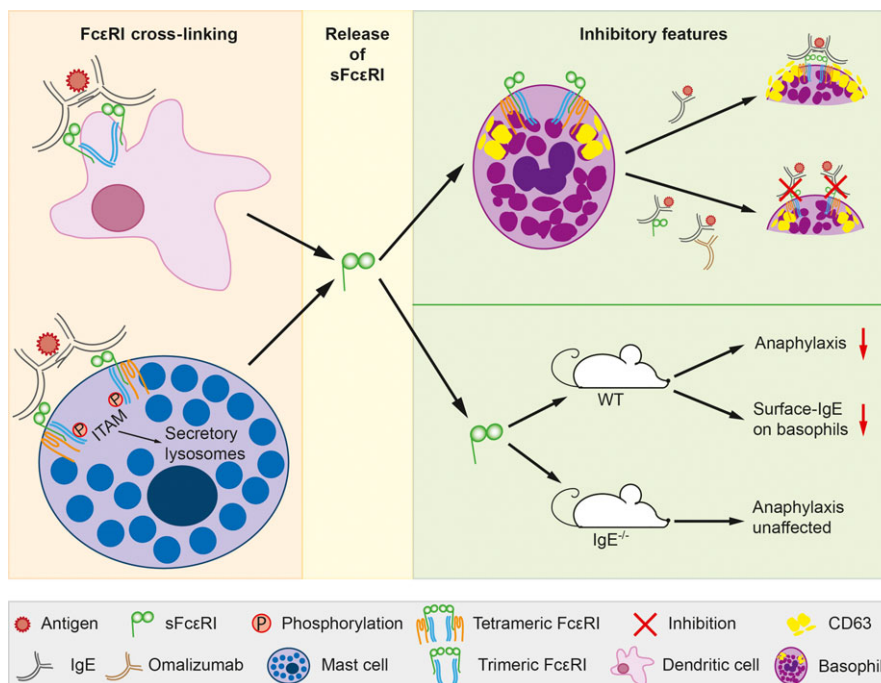
Abbreviations: Alum, aluminum hydroxide and magnesium hydroxide; BAT, basophil activation test; cIgE, chimeric humanized anti-NP immunoglobulin E; CSU, chronic spontaneous urticaria; DC, dendritic cell; DNP, dinitrophenyl; FcεRI, Fc epsilon receptor I, high-affinity IgE Fc receptor; fMLP, N-formyl-methionyl-leucyl-phenylalanine; GM-CSF, granulocyte-macrophage colony-stimulating factor; hFcεRI, human Fc epsilon receptor I, high-affinity IgE Fc receptor; i.p., intraperitoneal; IgE^{-/-}, Immunoglobulin E deficiency; IgE, Immunoglobulin E; IL-3, Interleukin-3; IL-4, interleukin-4; LAMP-1, lysosomal-associated membrane protein 1; MC, mast cell; mFcεRI, murine Fc epsilon receptor I, high-affinity IgE Fc receptor; MMP, matrix metalloproteinase; moDC, monocyte-derived dendritic cell; NP-BSA, 4-Hydroxy-3-nitrophenylacetyl bovine serum albumin; NP-OVA, 4-Hydroxy-3-nitrophenylacetyl ovalbumin; OVA, ovalbumin; PCA, passive cutaneous anaphylaxis; r.o., retro-orbital; rsFcεRI, recombinant human sFcεRI; SCF, stem cell factor; sFcεRI, soluble isoform of FcεRI; SPT, skin prick test; WT, wild type.

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Education; Mead Johnson Nutrition
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KEYWORDS

allergy, FcεRI, IgE receptor, mast cell, omalizumab



GRAPHICAL ABSTRACT

The soluble isoform of FcεRI, sFcεRI is released after antigen-specific cross-linking on monocyte-derived dendritic cells and murine bone marrow-derived mast cells, where Src phosphorylation and endo/lysosomal acidification are key events. Comparable to omalizumab, sFcεRI prevents IgE binding and activation on human basophils *in vitro*. In experimental mouse models, sFcεRI diminishes severity of IgE-mediated anaphylaxis and decreases surface IgE on basophils.

1 | INTRODUCTION

Allergen-specific cross-linking of IgE-loaded FcεRI on mast cells (MCs) and peripheral blood basophils is a central activation event in many types of allergies. Activation of this IgE/FcεRI effector axis is also pivotal to IgE hypersensitivity reactions which, when elicited by food, range from oral tingling and swelling to life-threatening anaphylaxis.^{1,2} For food allergy, no treatments other than allergen avoidance and emergency management with epinephrine, corticosteroids, and antihistamines are currently available to the general public. Therefore, there is a high priority to study the IgE/FcεRI pathway because such research offers opportunities to discover new therapeutic intervention targets for allergy and anaphylaxis.

FcεRI is a multimeric immune recognition receptor of the IgE network and has been studied in great detail due to its crucial role in allergy.^{3,4} The tetrameric isoform constitutes of the IgE-binding alpha chain, the beta chain, and the gamma chain dimer; the latter two subunits serving as signaling proteins.¹ Tetrameric FcεRI is constitutively expressed on the surface of immune cells of the innate IgE effector axis, such as MCs and basophils, in rodents and humans.

During the acute phase of an allergic response, the cell-bound fraction of antigen-specific IgE pool gets cross-linked and induces an activation cascade that results in the immediate release of preformed intracellular mediators such as histamine. Additionally, the innate cells start to produce inflammatory Th2-type cytokines that perpetuate allergic inflammation.^{4,5}

In humans, dendritic cells (DCs) constitutively express a trimeric isoform of FcεRI which has been described to execute regulatory functions by contributing to the clearance of serum IgE⁶ and by providing a feedback mechanism that restrains allergic tissue inflammation.^{7,8} The trimeric isoform of FcεRI can be induced by virus infections on murine DCs⁹ but is not constitutively expressed in wild-type (WT) mice. In addition to the two transmembrane FcεRI isoforms, a truncated version of the alpha chain, further referred to as soluble FcεRI (sFcεRI), has been described in human serum.^{10,11} Currently, the cellular source of sFcεRI in humans or the signaling requirements for its generation are poorly defined. Previous studies have shown that sFcεRI is released after IgE cross-linking and it prevents IgE binding to different FcεRI-expressing cell lines (MelJuso¹⁰ and RBL-2H3), and interferes with PCA (passive cutaneous

anaphylaxis) response in rats.^{10,12} Several open questions with regard to the biology of sFcεRI and its role within the IgE network³ remain to be addressed. Specifically, the signaling requirements for sFcεRI production have not been defined and, furthermore, it remains to be tested and how sFcεRI affects IgE-mediated anaphylaxis *in vivo*.

Omalizumab, a recombinant humanized anti-IgE monoclonal antibody, has been established as a treatment for uncontrolled allergic asthma and more recently for CSU, chronic spontaneous urticaria.¹³ Omalizumab binds to the Fc portion of IgE and has been shown to interact with free IgE that circulates in serum.¹⁴ This type of anti-IgE therapy leads to an increase in circulating total IgE levels and decreased free IgE accompanied by a decrease in IgE and FcεRI expression on DCs, MCs, and basophils.^{15–19} As omalizumab and sFcεRI can both interact with IgE in human serum, it is important to address if the anti-IgE antibody and the soluble IgE Fc receptor potentially overlap in their function.

Here, we show that cross-linking of tetrameric and trimeric surface FcεRI induces the production of the soluble receptor isoform in cell line models and primary human cells. In addition to IgE-mediated receptor activation, generation of sFcεRI depends on Src signals and requires endo/lysosomal acidification. Similar to omalizumab, sFcεRI inhibits FcεRI-mediated basophil activation, and experimental models of murine allergy showed that sFcεRI ameliorates antigen-induced anaphylaxis *in vivo*.

2 | MATERIALS AND METHODS

2.1 | Cells and culture conditions

Human melanoma-derived cell line MelJuso were transfected with FcεRIαγ, FcεRIαβγ, or empty vector (MelJuso-αγ, MelJuso-αβγ, and MelJuso-∅∅, respectively) cultured as previously described.^{10,20}

For the generation of monocyte-derived dendritic cells (moDCs), monocyte isolation from heparinized blood was performed with CD14 microbeads by MACS separation according to manufacturer's protocol (Miltenyi Biotec, Bergisch Gladbach, Germany). Monocytes were cultured in RPMI-medium supplemented with 10% FBS (Gibco Thermo Fisher Scientific, Waltham, MA, USA), 10 000 U/mL Pen-Strep (Gibco), 2 mM L-Glutamine, and 0.1 mM nonessential amino acids, with the addition of IL-4 and GM-CSF (1000 U/mL; PeproTech, Rocky Hill, CT, USA).

For the generation of murine bone marrow-derived mast cells (MCs), femurs of hFcεRIα+/mFcεRIα-/- mice were flushed and progenitor cells were cultured as described²¹ in RPMI-medium supplemented with 10% FBS, 10 000 U/mL Pen-Strep, 2 mM L-Glutamine, 50 μM β-mercaptoethanol, 10 ng/mL recombinant murine IL-3 and SCF (PeproTech).

2.2 | FcεRI cross-linking assays

MelJuso cells (5×10^5 cells), moDCs ($1.7\text{--}2.5 \times 10^6$ cells), and MCs (5×10^5 cells) were loaded overnight with clgE (from 0.1 to 10 μg/mL) and stimulated with NP-OVA or -BSA (from 0.1 to 100 μg/mL)

for 2–24 hours. Omalizumab (0.27–0.8 μM) and sFcεRI (25–500 nM) were incubated with clgE for 30 minutes prior to loading on MelJuso and basophils. Supernatants from cell cultures were collected between 2 and 24 hours after FcεRI cross-linking and analyzed by ELISA or Western Blot.

2.3 | Lysosomal-associated membrane protein 1 degranulation assays

Mature murine MCs (1×10^5 cells per condition), defined as at least 8-week-old cells with a purity of greater than 90% assessed by cell surface coexpression of mast cell markers c-Kit and FcεRIα by flow cytometry, were loaded overnight with clgE (500 ng/mL). The next day, cells were washed twice with medium, plated in a 96-well tissue culture plate (10^5 cells in 100 μL per well), and challenged for 10 minutes with a cocktail containing NP-BSA (100 ng/mL) as antigen for IgE-specific receptor activation, an anti-mouse lysosomal-associated membrane protein 1 (LAMP-1) antibody, and a viability dye.²² Before NP-BSA, 5–50 μM of PP2 (Sigma-Aldrich, Merck, St. Louis, MO, USA) or 20–50 μM of chloroquine (Sigma-Aldrich, Merck) were added for 30 minutes. IgG anti-human FcεRIα antibodies 19-1 and 15-1²³ were used (1 and 10 ng/mL) in the absence of clgE and NP-BSA for 2 hours. Following 2 washes with cold staining buffer, cells were acquired on a FACS Canto II flow cytometer and further analyzed using FlowJo v10 (Ashland, OR, USA).

2.4 | Murine anaphylaxis and testing of inhibitory features of sFcεRI *in vivo*

Age- and sex-matched BALB/c wild-type (WT) and IgE-deficient (IgE^{-/-}) mice²⁴ were allocated to experimental groups. Mice were passively ($n = 3$, 3–6 mice per group; total of 19 mice) sensitized with IgE anti-DNP (5 μg) in the presence or absence of human rsFcεRI (3 μg) by retro-orbital (r.o.) injections. Mice sensitized with heat-denatured IgE (dlgE) anti-DNP (3 hours at 65°C) were used as a control. Active sensitization was performed by intraperitoneal (i.p.) injections of OVA/Alum (100 μg) once a week for 3 weeks. Experimental groups received additional rsFcεRI (3 μg) by i.p. injection 24 hours prior challenge. Mice were challenged with DNP-OVA (100–200 μg) or OVA (500 μg) by i.p. injections for passive and active sensitization models, respectively. Mice challenged with PBS were used as a control. Drop of core body temperature was used as read-out for systemic anaphylaxis and was recorded up to 1 hour after challenge.^{25,26}

3 | RESULTS

3.1 | Antigen-mediated IgE cross-linking of tetrameric and trimeric FcεRI results in production of sFcεRI

To test whether sFcεRI was released by the tetrameric and/or trimeric isoform of FcεRI, we first used cell lines (MelJuso-αγ and MelJuso-αβγ¹⁰). MelJuso cell lines that stably express trimeric or tetrameric

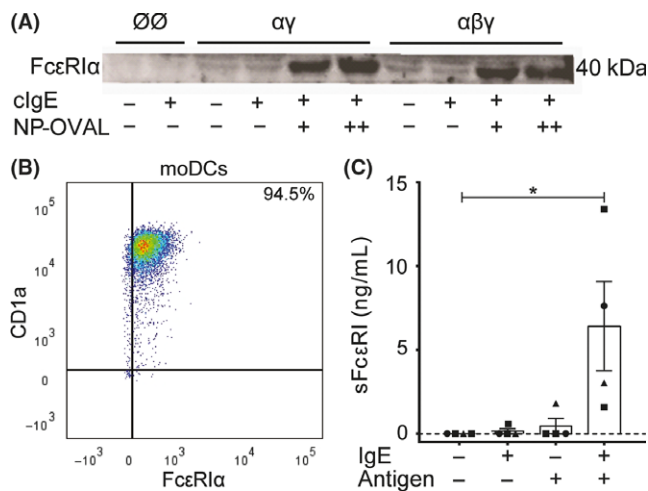


FIGURE 1 FcεRI cross-linking on moDCs induces sFcεRI production. (A) Detection of sFcεRI by Western blot analysis from MelJusoØØ/αγ/αβγ cell cultures. (B) A representative dot plot of mature monocyte-derived DCs. (C) Detection of sFcεRI by ELISA analysis from monocyte-derived DCs given by mean ± SEM. clgE (5–10 μg/mL) and NP-OVA (50–100 μg/mL). Individual points represent each donor where squares are males, circles females, and triangle unknown. Kruskal-Wallis test plus Dunn's multiple correction was performed where * $P < 0.05$

FcεRI have been published as suitable model to study IgE binding to FcεRI and receptor activation via antigen-specific IgE-mediated cross-linking.^{10,20} Cells were loaded with hapten NP-specific (4-Hydroxy-3-nitrophenylacetyl) chimeric IgE (clgE) overnight. After removal of excess IgE, cells were treated with haptenized bovine serum albumin (NP-BSA) or ovalbumin (NP-OVA) to activate IgE-loaded FcεRI. Supernatants from cell cultures were harvested and analyzed by Western blot (Figure 1A). Supernatants from MelJusoØØ cells served as negative controls as they neither express nor release sFcεRI. Low sFcεRI levels were detected in supernatants from unloaded and clgE-loaded MelJuso-αγ and MelJuso-αβγ cells and were significantly increased after cross-linking, which demonstrates that activation of both human receptor isoforms induces the production of sFcεRI.

To confirm our findings with human primary cells, we generated monocyte-derived DCs (moDCs) defined as CD1a⁺/FcεRIα⁺ cells by FACS analysis (Figure 1B). moDCs were loaded with clgE and activated as described for MelJuso cells earlier in the article. After receptor cross-linking, sFcεRI was found in supernatants from moDC cultures of four individual donors. Low amounts of sFcεRI were released from moDC from 1 of 4 donors in the absence of IgE-mediated activation. IgE-mediated cell activation induced or significantly increased the production of sFcεRI ($P < 0.05$) in all cultures (Figure 1C).

3.2 | Production of sFcεRI requires Src kinase activation and endo/lysosomal acidification

To understand the process of sFcεRI release, we used MelJuso-αγ cells in the presence of a potent inhibitor of matrix metalloproteinases (MMP); a class of proteases that has been described as

being responsible for the shedding of several surface proteins.^{27–29} Cells were loaded with clgE overnight and incubated with the inhibitor TAPI-2 for 30 minutes before receptor activation with NP-OVA (Figure S1A). Supernatants were harvested after 2 hours of activation and analyzed by ELISA. TAPI-2 treatment resulted in a non-significant reduction in sFcεRI production (Figure S1B). Our results indicate that MMPs might be partially involved but not solely responsible for the release of sFcεRI.

To further investigate signaling requirements for the production of sFcεRI, we generated bone marrow-derived MCs from mice that were humanized for the expression of the human alpha chain of FcεRI.^{21,25,30} These animals were generated on the murine FcεRIα^{-/-} background and, therefore, express FcεRI on MCs as a chimeric tetrameric receptor of the human alpha chain with the murine signal transducing receptor units. MCs, according to the literature³¹ and defined as c-Kit⁺/FcεRIα⁺, were loaded with clgE on the chimeric FcεRI and the receptor internalized after NP-BSA stimulation (Figure S2).

LAMP-1 degranulation assays were used as read-out for IgE/FcεRI-mediated MC activation (Figure 2A). In line with previous studies, FcεRI internalized, dependent on the used cross-linking conditions, with an average degranulation rate of 40% (Figure 2B). Comparable to human moDCs, human sFcεRI was detected in supernatants of murine-humanized MCs 2 hours after antigen-specific cross-linking (Figure 2C). Release of sFcεRI in the supernatant was also correlated with β-hexosaminidase release (data not shown), another common marker of MCs activation.³² To further assess the production of sFcεRI after receptor activation, we cross-linked with the human alpha chain-specific antibodies, 19-1 and 15-1, which target two different epitopes of the alpha chain of the FcεRI which are both located close to the IgE-binding site and induce cross-linking in an IgE-independent manner.³³ Antibody-mediated cross-linking of the receptor did not provide a signal that increased the production of sFcεRI, at least not at the concentration tested in our assay (Figure 2C).

The signaling cascade of tetrameric FcεRI is far better understood than that of the trimeric isoform. Therefore, we further investigated the signaling requirements for sFcεRI production with MCs from FcεRIα-humanized mice. IgE-mediated activation of MCs triggers a series of FcεRI signaling events starting with phosphorylation of ITAM domains by Src kinases, specifically Lyn and Syk, followed by receptor internalization and degradation by the endo/lysosomal compartment.^{34,35} Consequently, we tested the effects of the Src inhibitor PP2 and the endo/lysosomal acidification inhibitor chloroquine on the production of sFcεRI by MCs. By blocking Src kinases,^{36,37} the ITAM-dependent signaling cascade after receptor cross-linking will be stopped. When receptor activation cross-linking occurred in the presence of PP2, degranulation and sFcεRI production (24 hours after activation) were significantly inhibited (Figure 2). A significant inhibition of sFcεRI production was also observed in the presence of chloroquine, an acidification inhibitor, which impairs receptor internalization and degradation (Figure 2D and E). When both inhibitors were combined, MC degranulation and sFcεRI production were completely abrogated.

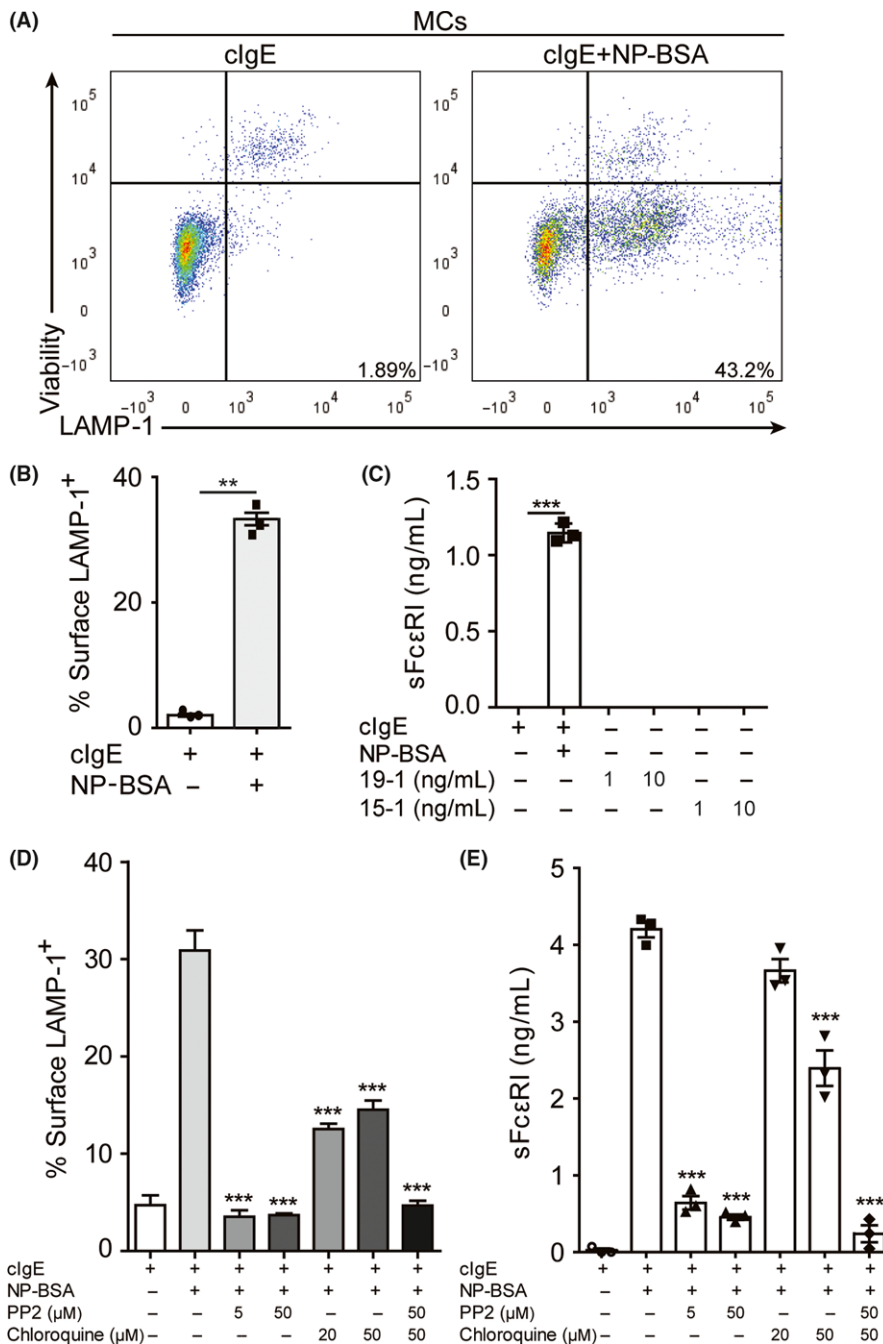


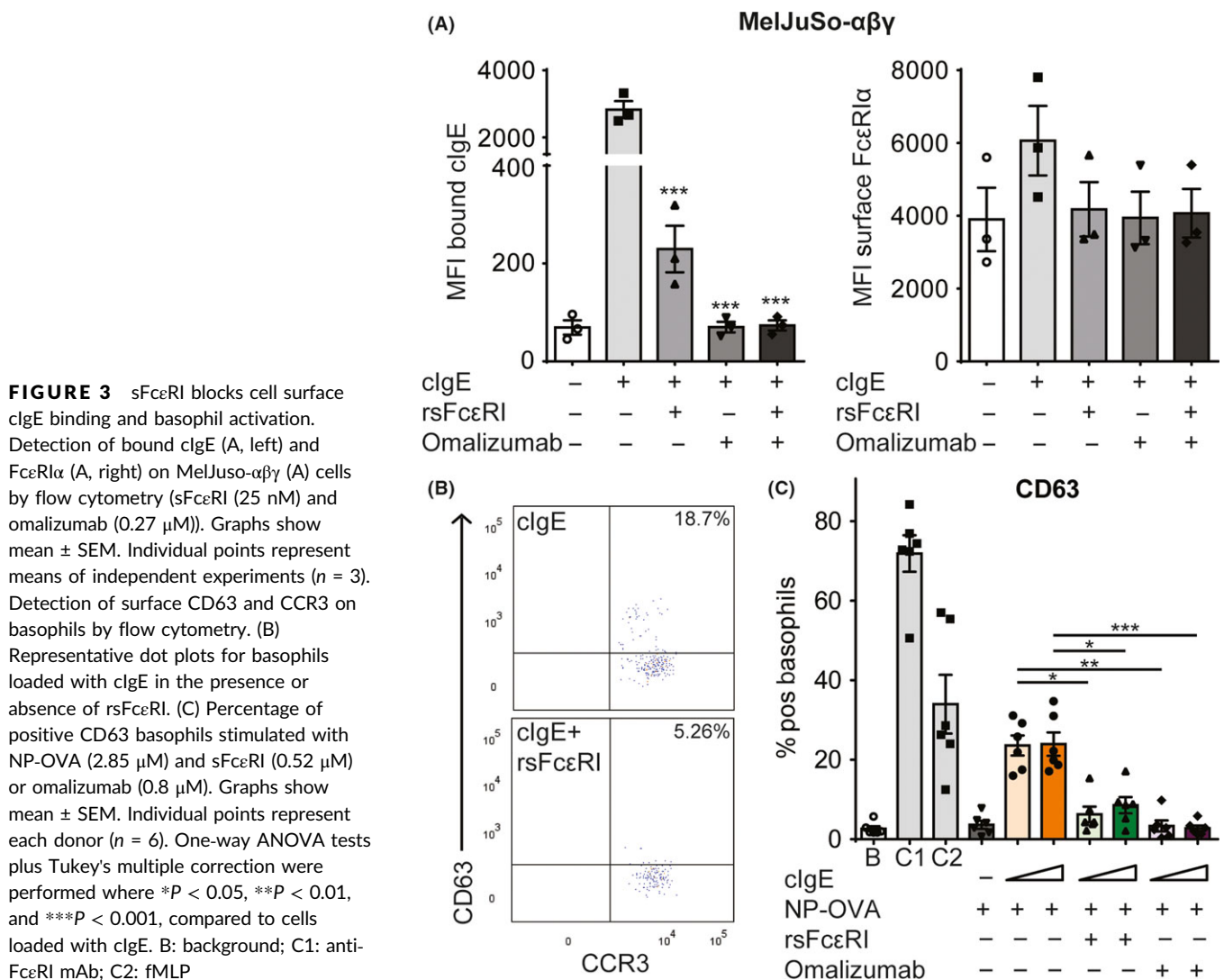
FIGURE 2 Src kinase activation and endo/lysosomal acidification are required for sFcεRI production. Detection of surface LAMP-1 expression on bone marrow MCs by flow cytometry (A, B, D) and sFcεRI in supernatants by ELISA (C, E). (A) Representative dot plots of MCs from LAMP-1 degranulation assays. (B and D) Quantification of percentage of surface LAMP-1⁺ cells. (C and E) It shows total sFcεRI levels from supernatant of MCs cultures. MCs were loaded overnight with clgE (500 ng/mL) and added NP-BSA (100 ng/mL) for 10 minutes for LAMP-1 degranulation assays. PP2 and chloroquine were added for 30 minutes prior to NP-BSA. In parallel, MCs were loaded overnight with clgE and added NP-BSA, 19-1, or 15-1 for 2 (19-1 and 15-1) or 24 hours before supernatants were harvested. Graphs show mean ± SEM where paired *t* test (B) or one-way ANOVA tests plus Tukey's multiple correction (C-E) was performed, where ***P* < 0.01 and ****P* < 0.001 compared to cells incubated with clgE and NP-BSA

3.3 | Human sFcεRI blocks loading of IgE on cell surface FcεRI and inhibits basophil activation

The ability of sFcεRI to interfere with IgE loading on the receptor was first tested on MelJuso cells. When MelJuso-αγ and MelJuso-αβγ cells were loaded with clgE in the presence of either endogenously produced sFcεRI or recombinant sFcεRI (rsFcεRI), the binding of IgE to the cell surface was impaired in a dose-dependent manner (Figure S3). The effect of endogenously produced and recombinant sFcεRI on loading of surface receptor was comparable. MelJusoØØ cells were used as negative control and showed no binding of IgE.

As sFcεRI seemed to have a strong blocking effect on IgE binding to the surface receptor, we compared its effect to widely studied humanized monoclonal antibody omalizumab, which binds to IgE Fc and blocks the FcεRI-binding site.^{15,17} Using the same setting, the inhibitory effect of rsFcεRI was comparable to omalizumab (Figure 3A and Figure S4).

To assess functional consequences of the inhibition of IgE binding, we performed modified basophil activation tests. Surface IgE was stripped from human basophils from healthy donors. Thereafter, basophils were reloaded with clgE in the presence or absence of rsFcεRI or omalizumab and stimulated with NP-OVA. CD63 expression was used as read-out for basophil activation (Figure 3B and C).



Compared to unstimulated basophils (Figure S5), the expression levels of CD63 increased after activation of clgE-loaded basophils (Figure 3C). Basophil activation was diminished by rsFcεRI and omalizumab (Figure 3C). A significant inhibition was achieved by sFcεRI (0.5 μM), and the effect was comparable to omalizumab (0.8 μM) despite the lower concentration. Two positive controls, FcεRI-dependent and -independent (C1 and C2, respectively), were used to ensure viability of basophils.

3.4 | sFcεRI is an in vivo modulator of IgE-mediated immune responses

Our next aim was to investigate how the presence of sFcεRI in serum would affect IgE-mediated anaphylaxis. It is important to note here that murine IgE can bind to human FcεRIα and murine FcεRIα with comparable binding kinetics.^{30,38} WT BALB/c mice were passively sensitized by r.o. injections using murine IgE anti-DNP (dinitrophenyl) in the presence or absence of human rsFcεRI 24 hours before a challenge with DNP-OVA (Figure 4A and Figure S6A for the characterization of rsFcεRI). Challenge-induced drop in core body

temperature was observed in IgE-sensitized mice. Importantly, animals that were sensitized in the presence of rsFcεRI responded with a significantly diminished drop in temperature (Figure 4B). Mice that were injected with IgE and rsFcεRI, but not challenged and animals that received only rsFcεRI were used as specificity controls and did not show alterations of body temperature (Figure 4C). An experimental condition in which we used heat-dIgE for passive sensitization confirmed that our experimental settings specifically monitor IgE-mediated anaphylaxis (Figure 4C).

Symptoms of murine anaphylaxis observed in passive sensitization experiments are commonly rather moderate.³⁹ As a complementary approach, we therefore addressed whether rsFcεRI would be able to improve symptoms of anaphylaxis after systemic immunization. WT BALB/c mice were sensitized with OVA/Alum (ovalbumin/aluminum hydroxide and magnesium hydroxide) injections once a week for 3 weeks. The experimental group received a single rsFcεRI injection 24 hours before antigen-challenge while the control group was injected with PBS alone (Figure 4D). Animals responded to antigen-challenge with a pronounced temperature drop from which they started to recover after 40 minutes (Figure 4E and Figure S6).

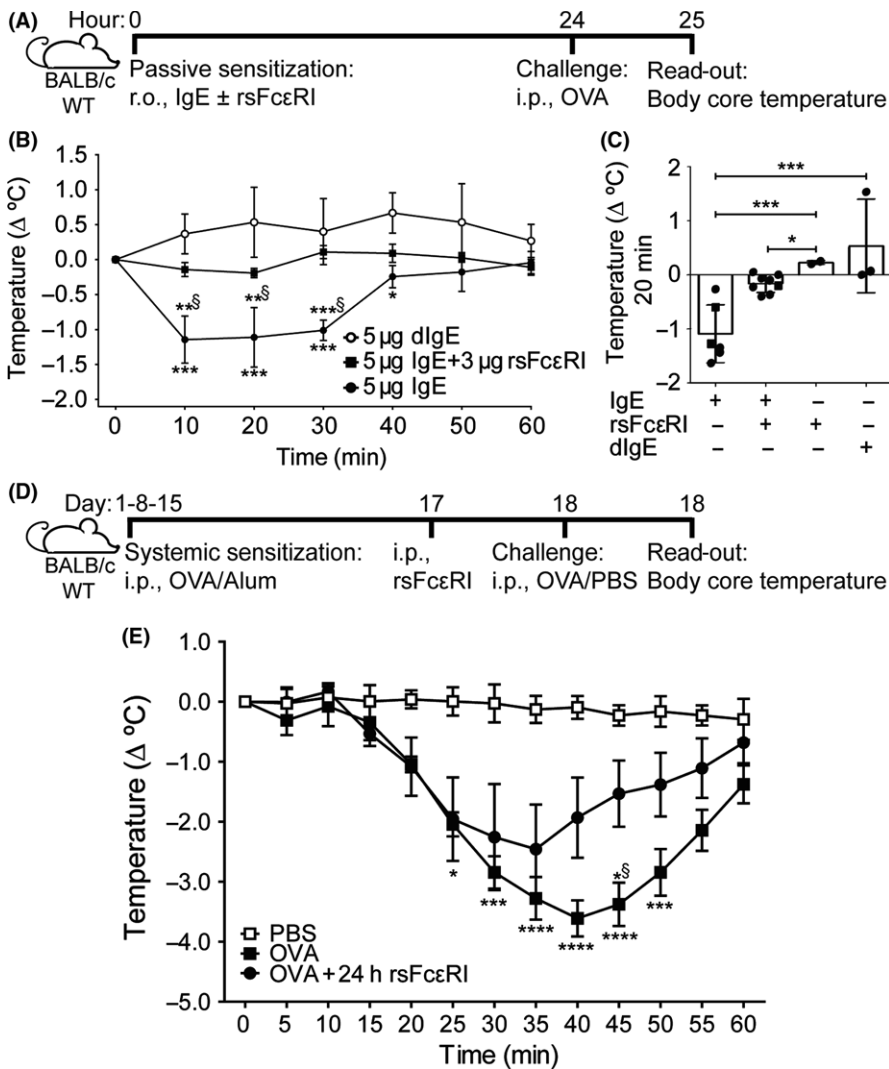


FIGURE 4 sFcεRI prevents systemic sensitization and improves recovery. Analysis of core body temperature from passively (A-C) and systemically (D, E) sensitized mice. (A) Passive sensitization and challenge strategy. (B) Time course of core body temperature drop in sensitized mice that were challenged in the absence or presence of rsFcεRI. Representative experiment, average temperature of 3-6 mice per time point. (C) Composite graph of temperature drops at the 20-minute time point following DNP-OVA challenge, from three independent experiments ($n = 3$, total of 19 mice). (D) Th2-type sensitization and challenge strategy. (E) Time course of body temperature drop in sensitized mice that were challenged with OVA in the absence or presence of rsFcεRI 24 hours before challenge. rsFcεRI (3 μ g), IgE anti-DNP (5 μ g), OVA/Alum (100 μ g), and OVA (100-500 μ g). Graphs show mean $\pm$ SEM with $n = 3$ -6/group where one-way ANOVA test and Tukey's multiple correction (C), or two-way ANOVA tests and Tukey's (B) or Bonferroni's (E) multiple correction were performed; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, and **** $P < 0.0001$. * represent statistics between IgE and dIgE (B), and PBS and OVA (E) groups. § represent statistics between IgE anti-DNP and IgE anti-DNP + rsFcεRI (B), and OVA and OVA+24h rsFcεRI (E) groups

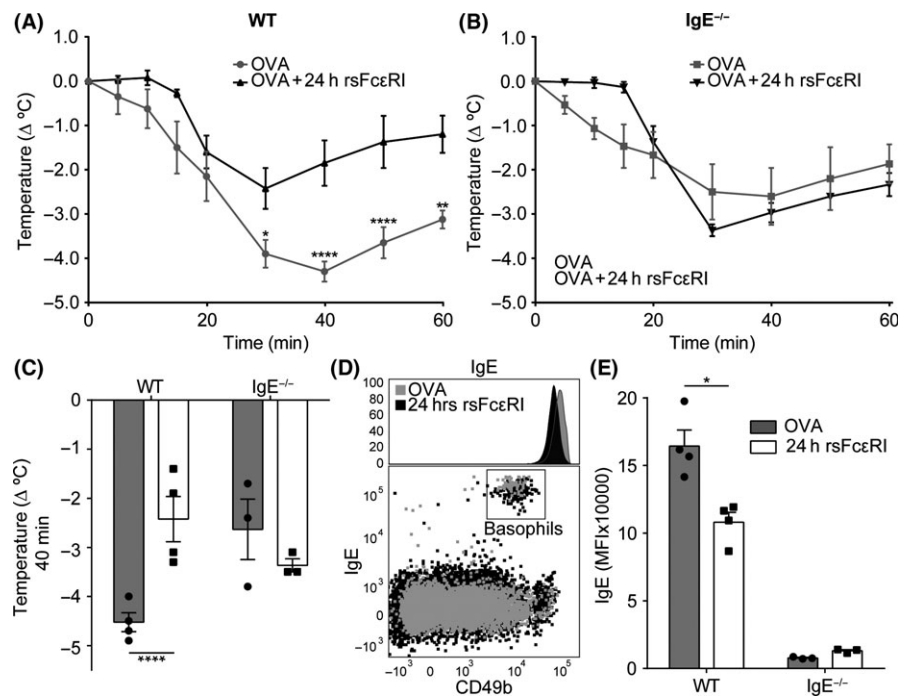
Temperature of mice that were injected with rsFcεRI 24 hours before challenge dropped significantly less and animals started to recover as early as 20 minutes after challenge.

To assess whether sFcεRI specifically impairs IgE-mediated anaphylaxis, we performed side-by-side experiments in WT and IgE-deficient (IgE^{-/-}) animals. Both strains were sensitized and challenged using the experimental outline detailed in Figure 4D. We confirmed that the temperature of WT mice that received a single dose of rsFcεRI 24 hours before challenge suffered from less pronounced anaphylaxis than untreated WT animals (Figure 5A and C). In IgE^{-/-} animals, in which other pathways than IgE-mediated FcεRI cross-linking on MCs are responsible for the induction of anaphylaxis,^{40,41} the protective effect of rsFcεRI was not observed (Figure 5B and C). When measuring IgE on peripheral blood basophils, we found that basophils from WT mice that were treated with rsFcεRI expressed lower levels of surface IgE than controls (Figure 5D and E). As expected, basophils from IgE^{-/-} mice had no surface IgE (Figure 5E). In summary, this set of experiments confirms that sFcεRI specifically affects anaphylaxis induced by the IgE/FcεRI-mediated activation of the innate effector axis of allergy.^{7,34}

4 | DISCUSSION

Here, we demonstrated that sFcεRI release occurs for the tetrameric isoform when expressed on MelJuso cells and from murine-humanized MCs. Most importantly, this study describes sFcεRI release from human moDCs for the first time. We further delineate the requirements for sFcεRI release, such as antigen-specific receptor cross-linking, phosphorylation, internalization, and intracellular endo/lysosomal acidification. We cannot completely exclude the effect of proteinase-induced shedding, which might also contribute in the release of sFcεRI found in the presence of Src kinase or endo/lysosomal acidification inhibitors in MCs. Furthermore, our data indicate that sFcεRI may prevent binding of IgE to FcεRI and thereby, impairs IgE-mediated effector cell activation. This effect was further assessed showing amelioration of anaphylaxis. Our findings, together with the similarities in effect between sFcεRI and omalizumab, provide evidence that sFcεRI is an endogenous modulator of IgE-mediated responses and the induction of its production might be a potential strategy to modulate allergic responses.

FIGURE 5 Recombinants FcεRI prevents anaphylaxis and removes surface IgE from basophils. Analysis of rectal temperature from systemically sensitized mice (A-C). (A and B) Comparison of the effect of rsFcεRI treatment in a time course of core temperature drop in WT (A) or IgE^{-/-} (B) mice. (C) Maximum temperature drop of WT and IgE^{-/-} mice 40 minutes after OVA challenge. (D) A representative histogram and a dot plot of peripheral blood basophils from WT mice with and without rsFcεRI treatment. (E) MFI of surface-bound IgE on basophils with and without rsFcεRI treatment. rsFcεRI (3 μg), OVA/Alum (100 μg), and OVA (500 μg). Graphs show mean ± SEM with *n* = 3-4/group where two-way ANOVA tests and Tukey's multiple correction (A-C) and unpaired *t* test (E) were performed; **P* < 0.05; *****P* < 0.0001



It has been previously described that sFcεRI can be found in serum, almost uniquely bound to IgE.⁴² Our results suggest that both DCs and MCs are potential sources of sFcεRI in serum. However, we cannot exclude that other cell types that express either trimeric or tetrameric FcεRI, such as basophils, eosinophils, macrophages, or Langerhans cells, additionally contribute to the serum levels of sFcεRI. Moreover, FcεRI expression can be induced on neutrophils⁴³ in allergic asthma and murine DCs after viral infection,⁹ which might also have an effect on sFcεRI in these particular conditions.

It has been previously shown that sFcεRI can prevent IgE binding on MelJuso-αγ¹⁰ and RBL-2H3¹² cells. Here, we have shown that, *in vitro*, sFcεRI binds to IgE leading to the formation of sFcεRI/IgE complexes, thus lowering the capacity of free IgE to bind to and subsequently activate cell surface FcεRI on MelJuso-αβγ cells and human basophils. We propose the process of sFcεRI release to be a protective mechanism against subsequent allergen-mediated FcεRI/IgE cross-linking and sustained cellular activation. This hypothesis is supported by *in vivo* experiments in mouse models of anaphylaxis in the current publication. When rsFcεRI was injected at the time of sensitization, it was sufficient to protect from anaphylaxis. If rsFcεRI was injected in a systemic sensitization model, it resulted in a diminished severity and improved recovery of IgE-mediated anaphylaxis, as well as a decrease in bound IgE to basophils. An additional argument for the specificity of the inhibitor activity of sFcεRI for the IgE/FcεRI-mediated pathway of anaphylaxis is derived from the observation that no amelioration of IgE-independent anaphylaxis^{40,41} was observed in IgE^{-/-} animals. The strong immune response triggered in the active sensitization model, compared to the passive sensitization model, might explain why we still observe a temperature drop in the presence of rsFcεRI, which suggests that the IgE:rsFcεRI ratio may be important. These results imply that sFcεRI is an *in vivo* modulator of IgE-mediated responses and support

our proposed mechanism of a negative regulatory feedback loop. It is tempting to speculate that sFcεRI could be used as an adjuvant medication during oral immune therapy to prevent immediate responses during therapeutic antigen exposure.

Omalizumab, the humanized anti-IgE antibody, has proven to be a successful treatment for diverse IgE-mediated diseases such as severe allergic asthma, rhinitis, or chronic spontaneous urticaria.^{16,19,44-51} In addition, omalizumab has occasionally been used for the treatment of food allergy in combination with food-specific immunotherapy and successfully reduced IgE-mediated side effects.^{19,52,53} Furthermore, studies based on IgE-trapping molecules have shown potential to modulate IgE-mediated responses, such as anti-IgE designed ankyrin repeat proteins, which show inhibition of basophil activation and release of proinflammatory mediators,⁵⁴ and PepE that prevents passive systemic anaphylaxis.²⁶

Based on the similarities between omalizumab and sFcεRI, and the emerging research on IgE-trapping strategies, we compared both molecules, and both could effectively inhibit IgE binding to the receptor as well as IgE-mediated basophil activation. This effect was observed even at low concentrations of sFcεRI as compared to omalizumab, indicating a high-affinity and inhibitory capacity of sFcεRI. Our data suggest that both molecules have a similar functional effect as they compete for the same binding domain on IgE, indicating that monitoring of sFcεRI prior to omalizumab therapy might be necessary to identify patients in which the serum IgE-binding site is already occupied by an endogenous inhibitor. However, further research on circulating sFcεRI/IgE complexes and human data are needed to fully understand this mechanism.

In conclusion, although the possible implication of sFcεRI as an interference molecule in the IgE network has been considered and

partially investigated, we provide strong evidence that human sFcεRI is released from moDCs and MCs, and it might be part of a negative regulatory feedback loop to prevent detrimental IgE-mediated immune activation. The development of strategies to boost endogenous sFcεRI levels might be a potential therapeutic strategy for prevention and treatment of IgE-mediated allergies.

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CONFLICTS OF INTEREST

The authors confirm that there is no known conflict of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

AUTHOR CONTRIBUTIONS

SMR, LE, KS, SCD, BFS, BB, EF, and ZS designed experiments, performed research, and analyzed data. HCO provided mice and designed in vivo experiments. LP and TJ generated recombinant protein for in vivo experiments. SMR, EF, and ZS wrote the first draft of the manuscript. All coauthors contributed to writing the final manuscript and approved its last version.

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SUPPORTING INFORMATION

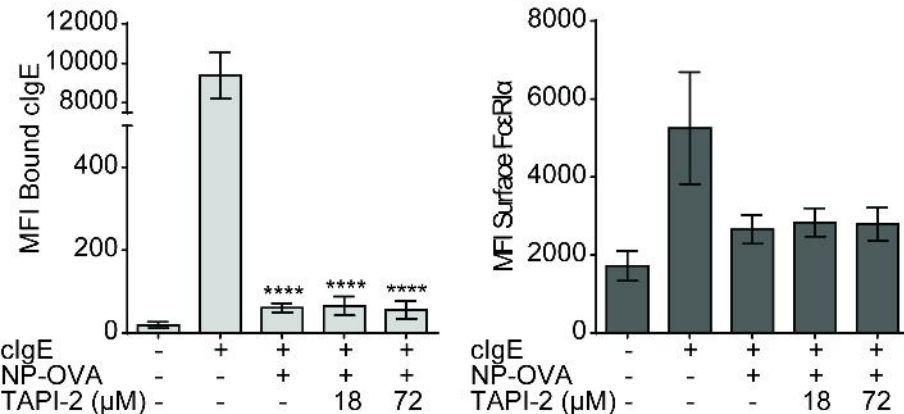
Additional supporting information may be found online in the Supporting Information section at the end of the article.

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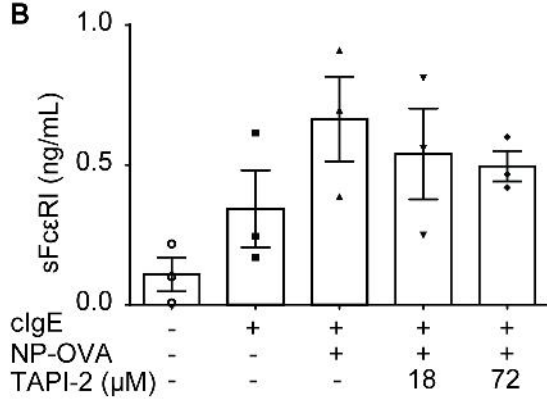
Supplementary Figure 1

A

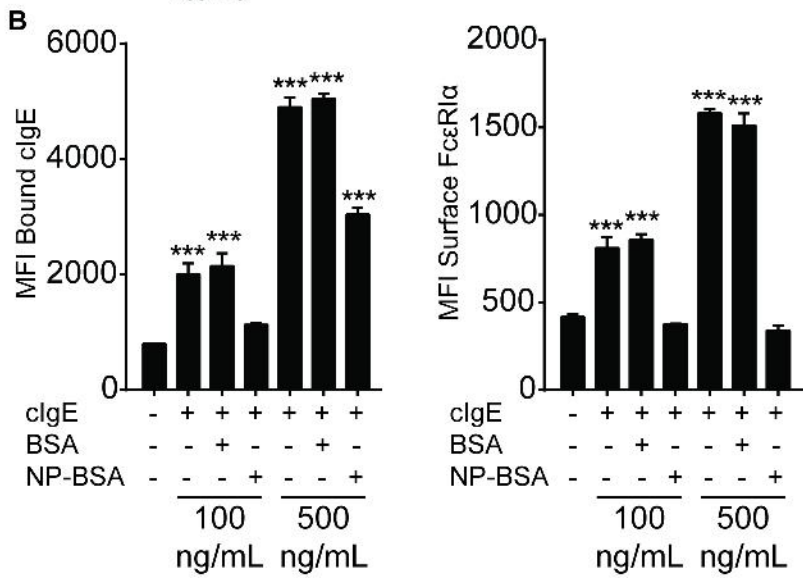
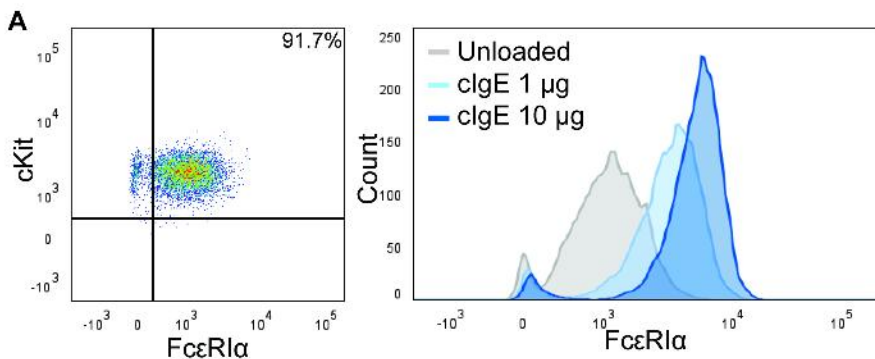
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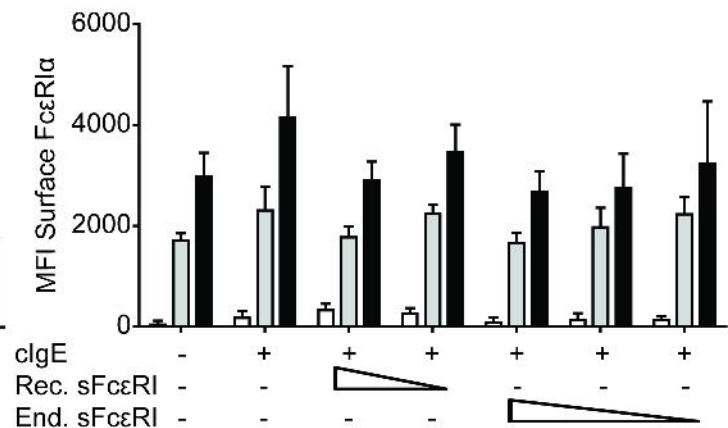
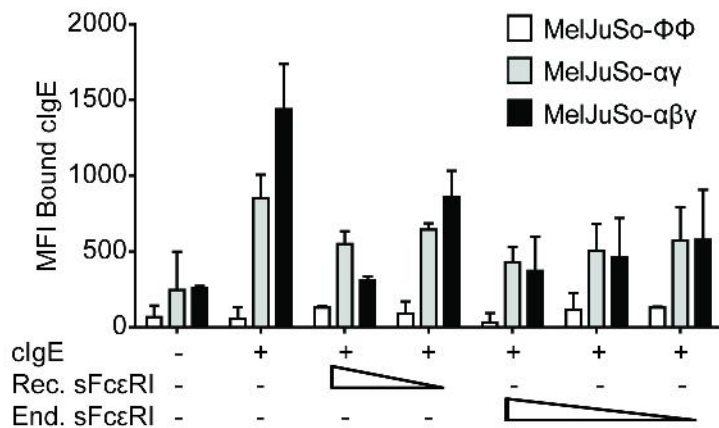
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Supplementary Figure 2

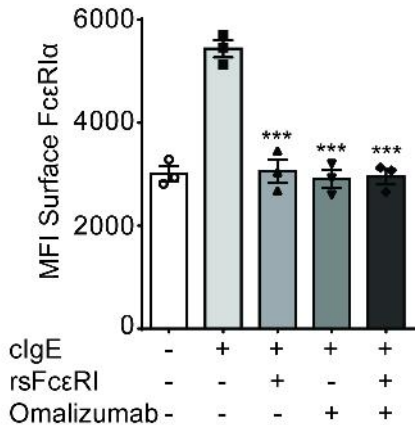
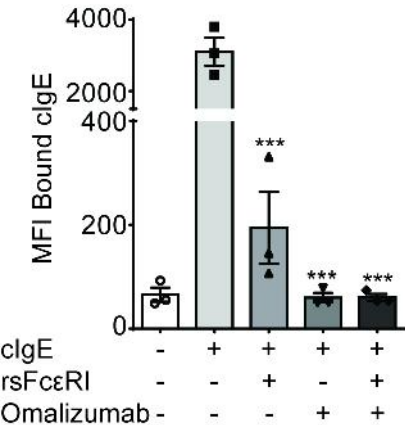


Supplementary Figure 3

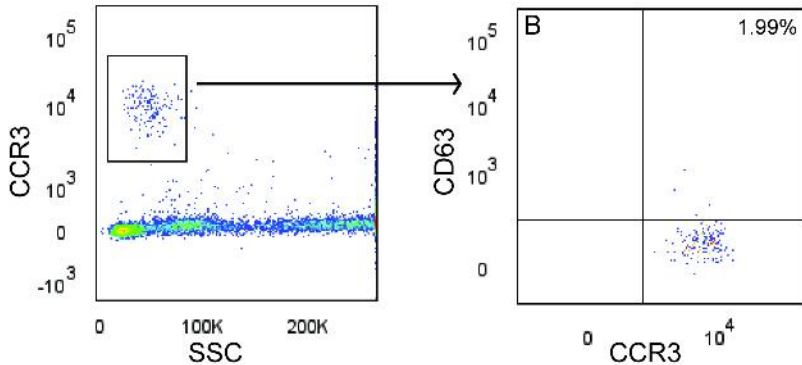


Supplementary Figure 4

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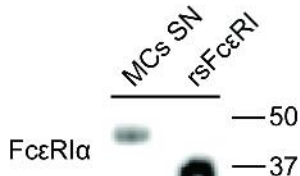


Supplementary Figure 5

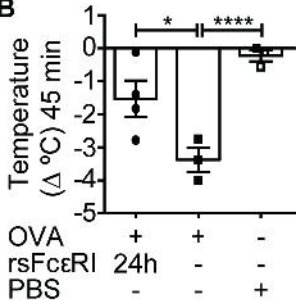


Supplementary Figure 6

A



B



1 ONLINE REPOSITORY

2 METHODS

3 sFcεRI ELISA

4 Levels of soluble FcεRI (sFcεRI) from supernatants were measured by a commercial ELISA
5 according to manufacturer's protocol (BMS2101, Thermo Fisher Scientific, Waltham, MA,
6 USA).

7 Reagents

8 For *in vitro* experiments, chimeric humanized anti-NIP IgE (cIgE) (MCA333S clone JW8/1
9 Serotec BioRad, Hercules, CA, USA) and its hapten-specific antigen NP-OVA/BSA (N-
10 50051 and N-5050L, Biosearch Technologies, Petaluma, CA, USA) were used at different
11 concentrations (0.1-40 µg/mL and 1-10000 ng/mL respectively). Omalizumab solutions
12 (Novartis, Basel, Switzerland) were used in different dilutions (from 40 µg/mL to 1 ng/mL) in
13 Dulbecco's phosphate-buffered saline (DPBS). TAPI-2 solutions (Santa Cruz
14 Biotechnologies, Dallas, TX, USA) were used at different concentrations (18 and 72 µM) for
15 30 minutes before NP-OVA stimulation. Endogenously produced sFcεRI was purified from
16 MelJuso cell supernatants, analyzed by ELISA and Western Blot, and used at different
17 dilutions (4-10 ng/mL). Recombinant human sFcεRI (rsFcεRI) was either purchased
18 (MyBioSource, San Diego, CA, USA) or provided by the laboratory of Theodore Jardetzky,
19 PhD (Department of Structural Biology in the School of Medicine at Stanford University) for
20 *in vivo* experiments previously described (1).

21 Flow cytometry analysis

22 Cells were harvested, washed and centrifuged at 330 g at 4°C with staining-buffer (DPBS 1X
23 supplemented with 2% FBS and 0.1% NaN₃). Cells were stained for 15 minutes at 4°C with
24 NP-PE (Biosearch Technologies) for cIgE detection, CRA-1-FITC or -APC (mouse IgG2b,K
25 anti-human FcεRIα, clone AER-37) and MAR-1-APC (hamster IgG anti-murine FcεRIα)
26 (BioLegend, San Diego, CA, USA) for human and murine FcεRIα detection respectively,
27 IgE-PE (BioLegend) for murine IgE detection, CD49b-Pacific Blue™ (BioLegend) for
28 murine basophil characterization, CD1a-APC (mouse IgG1, K anti-human CD1a, clone
29 HI149) (BioLegend) for dendritic cell characterization, c-Kit-FITC (BioLegend) for mast cell
30 characterization, CD107a-PE/Cy7 (BioLegend), and Fixable Viability Dye eFlour™ 660
31 (eBioscience, Thermo Fisher Scientific) for LAMP-1 assays. Basophils (50 µL whole blood)

were IgE-stripped with lactic acid (pH=3.9) for 5 minutes and loaded with cIgE in presence or absence of recombinant sFcεRI (rsFcεRI) or omalizumab for 1 hour at 37°C. Basophil stimulation (NP-OVA (4-Hydroxy-3-nitrophenylacetyl ovalbumin), 2.85 μM) and staining with CD63-FITC and CCR3-PE were performed according to manufacturer's protocol (Flow CAST, Bühlmann, Schönenbuch, Switzerland). Data was acquired on a FACS-Canto II (BD Bioscience) flow cytometer and analysis was performed by FlowJo v10.

Patient information

Allergic patients or adult healthy volunteers were recruited and blood was collected for generation of monocyte-derived dendritic cells (moDCs) or basophil activation tests. Allergic patients were defined by >0.35 kU_A/L specific IgE levels or >3 mm wheal size in skin prick test (SPT), and any of the following clinical symptoms: allergic asthma, rhinitis, conjunctivitis, atopic dermatitis, and food allergy. Healthy volunteers had no allergic symptoms. A total of 10 donors were used in the analysis.

All patients included in the study gave written informed consent and were recruited after approved application from the Ethics Commission, Medical University of Vienna (EK Nr: 1015/2017 and EK Nr: 079/2009).

Immunoblot analysis

Supernatants from cell cultures and rsFcεRI were separated on 12% SDS minigels under reducing or non-reducing conditions and transferred on nitrocellulose or PVDF membranes by wet-transfer. Membranes were probed for FcεRIα with mouse IgG2b,K anti-human FcεRIα, clone AER-37 CRA-1 (1:1000) (BioLegend) or 19-1 (2) (1:500), followed by goat anti-mouse-HRP (Santa Cruz Biotechnologies). Membranes were developed with an enhanced chemiluminescence (ECL) Western Blotting substrate (Thermo Fisher Scientific) and detected by a Fusion FX imaging platform.

Mice

6-8-wk-old BALB/c male and female mice were either purchased from The Jackson Laboratory or bred in the animal facility at Boston Children's Hospital (Boston, MA). IgE deficient (IgE^{-/-}) mice on the *Il4ra*^{F709} BALB/c background were described previously (3). Mice were all housed in specific pathogen-free conditions according to the National Institutes of Health (NIH) and all experiments were performed with cohoused and littermate controlled cohorts that contained both genders. All animal studies were approved by the Boston

Children's Hospital Institutional Animal Care and Use Committee. For passive sensitization model the following reagents were used: murine IgE-anti-DNP (D8406 clone SPE-7, Sigma-Aldrich, Merck, St. Louis, MO, USA), DNP-OVA (D-5051, Biosearch Technologies) and human rsFcεRI (laboratory of Theodore Jardetzky). For active sensitization model the following reagents were used: OVA (A7641, Sigma-Aldrich), Imject® Alum (77161, Thermo Fisher Scientific) and human rsFcεRI (laboratory of Theodore Jardetzky).

Statistical analyses

All statistical analyses were performed using Prism 5 and 6 (GraphPad Software), and results are shown as mean ± SEM of the indicated number of individual data points or independent experiments (at least three). Statistical analysis was performed using Kruskal-Wallis test plus Dunn's multiple correction for comparison of two groups, 1way ANOVA test plus Tukey's multiple correction for comparison of more than three or more unmatched groups, and 2way ANOVA test plus Tukey's or Bonferroni's multiple correction for multiple comparisons between three or more groups; *p* values ≤ 0.05 were considered significant.

FIGURE LEGENDS

Supplementary Figure 1. TAPI-2 partially blunts sFcεRI release from MelJuso-αγ. Panel A shows MFI of bound cIgE (left) and surface FcεRIα (right) on MelJuso-αγ cells. Panel B shows sFcεRI levels detected by ELISA. Cells were loaded overnight with cIgE (1 μg/mL), and incubated with TAPI-2 (18-72 μM) for 30 minutes before addition of NP-OVA (50 μg/mL) for 2 hours. Graphs show mean ± SEM of independent experiments (n=3). 1way ANOVA tests plus Tukey's multiple correction were performed where ****p<0.0001 compared to the second condition.

Supplementary Figure 2. Chimeric FcεRI binds cIgE and internalizes after crosslinking. Analysis of receptor loading and internalization on mature bone marrow MCs by flow cytometry. Panel A shows a representative dot plot of MCs, and histogram of cIgE-loading (1 and 10 μg). Panel B shows the MFI of surface bound cIgE (left) and surface FcεRIα (right) on MCs. Cells were loaded overnight with cIgE (100 or 500 ng/mL) and BSA or NP-BSA (100 ng/mL) was added for 2 hours. Graphs show mean ± SEM of independent experiments (n=2). 1way ANOVA tests plus Tukey's multiple correction was performed where ***p<0.001 compared to the first condition.

Supplementary Figure 3. Endogenously produced and recombinant sFcεRI inhibit cIgE binding and crosslinking. Detection of bound cIgE (left) and surface FcεRIα (right) on MelJuso-00/αγ/αβγ cells. Endogenous sFcεRI was harvested and purified from MelJuso cell cultures. Cells were loaded overnight with cIgE (1 μg/mL) in presence or absence of endogenous or recombinant (2.5-25 nM) sFcεRI. NP-OVA (50 μg/mL) was added for 3-5 hours. Graphs show mean ± SEM of independent experiments (n=3-6). 1way ANOVA tests plus Tukey's multiple correction were performed where *p<0.05, **p<0.01, and ***p<0.001 compared to the second condition.

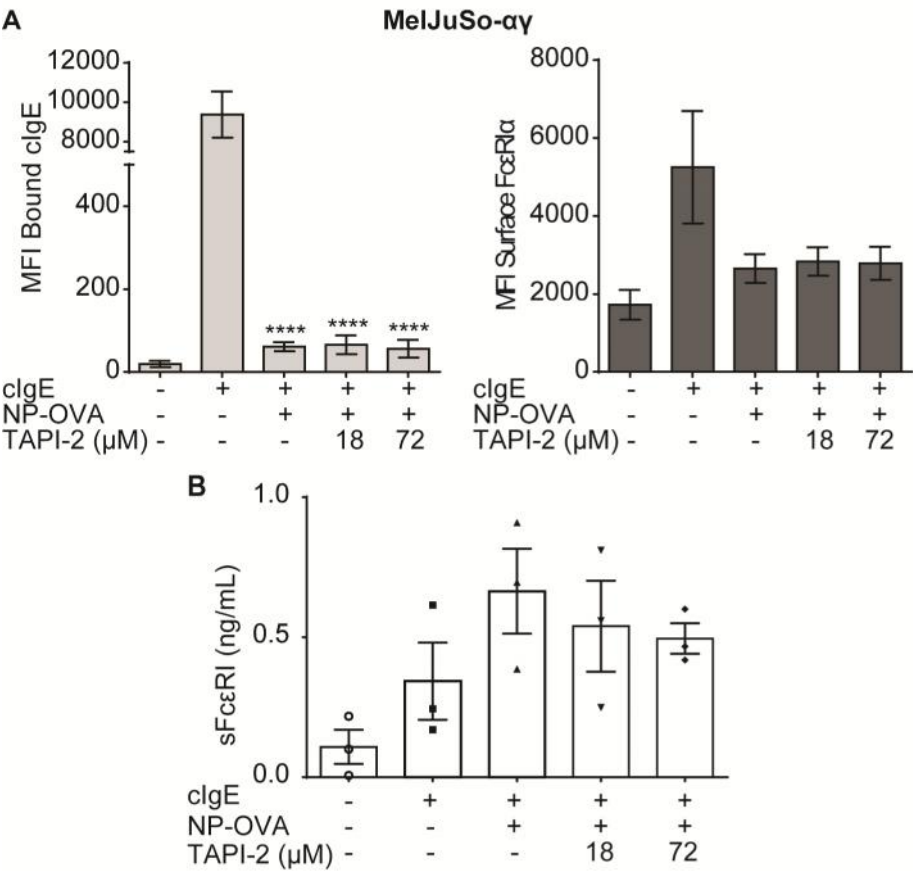
Supplementary Figure 4. sFcεRI blocks cell surface cIgE binding. Detection of bound cIgE (left) and FcεRIα (right) on MelJuso-αβγ cells by flow cytometry (sFcεRI (25 nM) and omalizumab (0.27 μM)). Graphs show mean ± SEM. Individual points represent means of independent experiments (n=3). 1way ANOVA tests plus Tukey's multiple correction were performed where *p<0.05, **p<0.01, and ***p<0.001, compared to cells loaded with cIgE.

Supplementary Figure 5. Gating strategy for human basophils. Detection of surface CCR3 and CD63 on basophils by flow cytometry. Representative dot plot of PBMCs isolated from healthy or allergic volunteers (n=3) gated as CCR3+/SSC_{low}. CD63+ gate was adjusted according to donor's background. B: background.

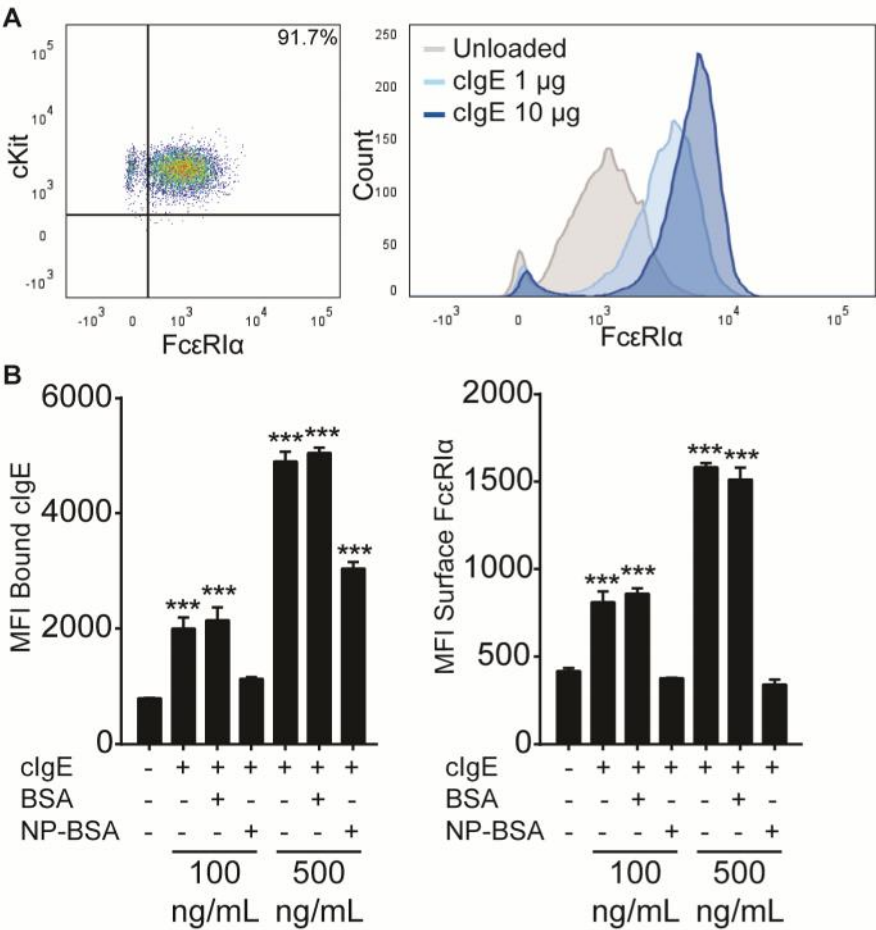
Supplementary Figure 6. sFcεRI prevents systemic anaphylaxis and improves recovery in actively-sensitized mice. Detection of recombinant sFcεRI and in MCs cultures by Western Blot (A) and analysis of core body temperature from systemically sensitized mice (B, C). Panel A shows a representative Western Blot analysis of sFcεRI from BMMCs cultures or recombinantly produced. Panel B shows temperature drops at the 45 min time point. Graphs shown mean ± SEM where individual points represent each mouse. Graphs shown mean ± SEM of each group (n=3-4 mice per group). 1way ANOVA tests plus Tukey's multiple correction were performed where *p<0.05 and ****p<0.0001.

FIGURES

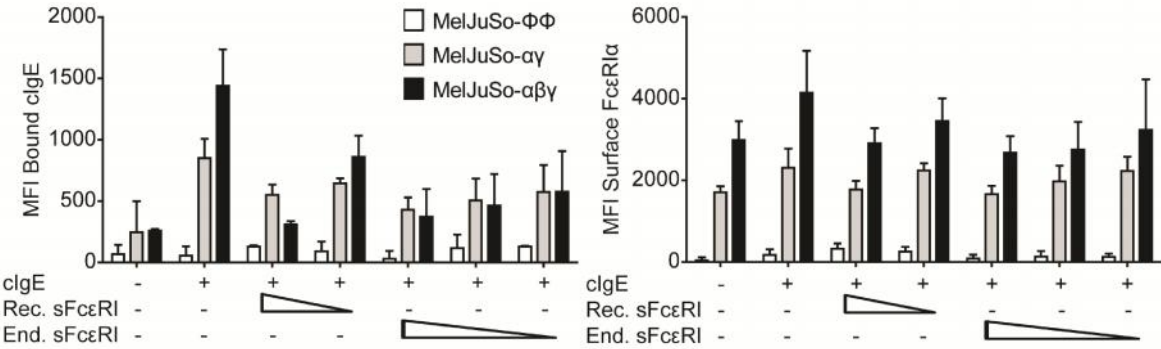
Supplementary Figure 1



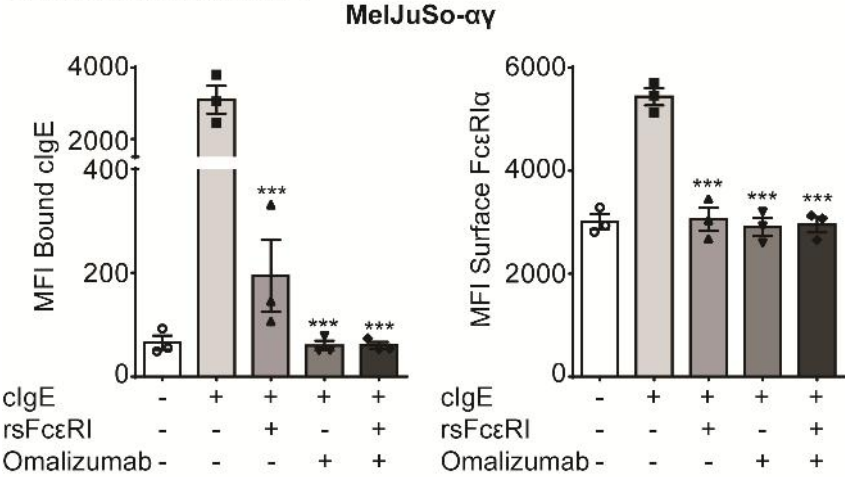
Supplementary Figure 2



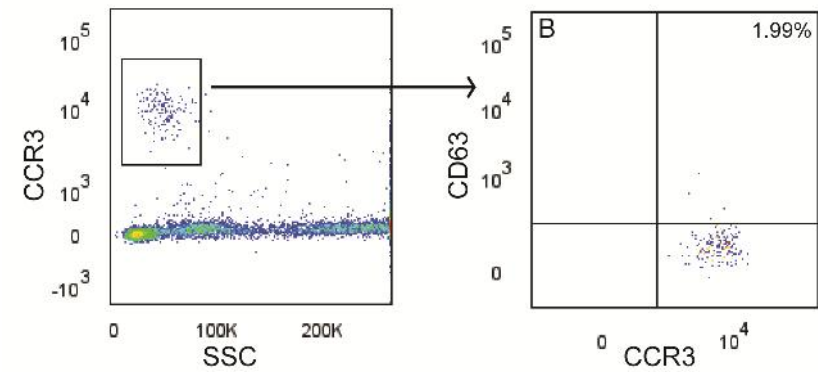
Supplementary Figure 3



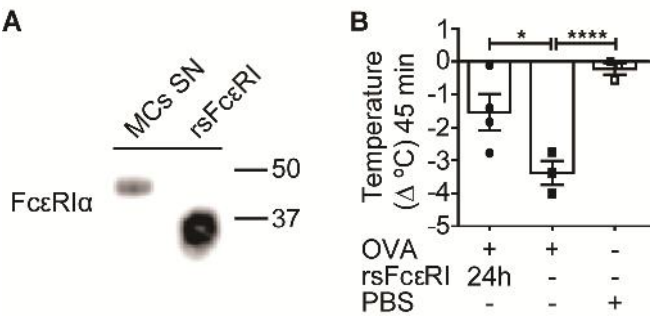
Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6



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