



Contents lists available at ScienceDirect

Allergy International

journal homepage: <http://www.elsevier.com/locate/alit>

Letter to the Editor

Resveratrol inhibits IgE binding and down-regulates intracellular phosphorylation of Syk following IgE aggregation on human basophils

Dear Editor,

Resveratrol is a natural bisphenol found predominantly in plants, including grapes, berries and peanuts. It has been studied extensively for its potential beneficial effects on human health, and its advantageous roles against cardiovascular diseases, aging, oxidative stress and inflammation have been biologically demonstrated.¹ Additionally, we showed that incubation of basophils with resveratrol suppressed degranulation and synthesis of leukotriene C4 induced by IgE aggregation *in vitro*, although the underlying mechanism(s) remain unclear.²

Degranulation of human basophils is thought to be one of the central mechanisms in acute allergic reactions such as anaphylaxis and acute exacerbation of asthma in human biology.³ Indeed, basophils play pivotal roles in allergic inflammation, mainly by rapidly releasing preformed chemical mediators such as histamine. In addition to their roles in acute allergic reactions, basophils were shown to be indispensable for allergic late-phase reactions.⁴ Thus, using a naturally occurring substance to suppress basophil functions would be an ideal method for preventing and curing allergic diseases. Also, elucidation of the underlying mechanism(s) may provide us clue(s) for new therapeutic strategies. In this study, we sought to determine the mechanism(s) by which resveratrol suppresses human basophil degranulation. The protocol for this study was reviewed and approved by the Institutional Ethics Review Board of National Hospital Organization Tokyo National Hospital. Informed consent was obtained from all blood donors.

Basophils were semi-purified from healthy individuals' blood by density centrifugation using Percoll solutions (GE Healthcare, Chicago, USA; 1.080 and 1.070 g/ml). Cells were incubated with resveratrol (Wako Pure Chemicals, Osaka, Japan) or control ethanol at the same concentration as used to prepare the highest concentration of resveratrol prior to IgE aggregation. After pretreatment with resveratrol or ethanol, cells were stained with specific antibodies for CD63 (IgG1 κ , clone H5C6) (BioLegend, San Diego, CA, USA), CD69 (IgG1 κ , clone FN50) (BioLegend), CD203c (IgG1, clone 97A6) (Immunotech, Marseille, France), Fc ϵ RI (IgG2b κ , clone AER-37) (eBioscience, San Diego, CA, USA) and phosphorylated Syk (IgG1 κ , clone I120-722) (BD Biosciences, San Jose, CA, USA) for analysis by FACSVerse (BD Biosciences). Cells highly positive for Fc ϵ RI were gated as basophils, and their mean fluorescence intensity (MFI) was determined. The data were analyzed using Flowjo (Tree Star Inc., Ashland, OR, USA), and statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test.

CD63 is a good marker for human basophil degranulation. It is expressed on the inner side of the vesicle membrane, and its expression on the outer side of the membrane is induced when the vesicle fuses to the plasma membrane due to IgE aggregation.⁵ In our experiment, we detected baseline expression of CD63 on resting basophils, i.e., basophils without IgE aggregation, by flow cytometry which was not altered with or without resveratrol (data not shown). In line with our previous report showing decreased histamine release from human basophils as a result of pretreatment with resveratrol, surface expression of CD63 by human basophils following stimulation with polyclonal goat anti-Human IgE (ϵ chain) (MBL, Nagoya, Japan) for 45 min at 37 °C was down-regulated upon treatment with resveratrol at 100 μ M (Fig. 1A and B; Supplementary Fig. 1). On the other hand, levels of surface-expressed CD69 and CD203c, two known basophil activation markers,⁶ did not change in response to resveratrol (data not shown). Based on those results, we conclude that resveratrol suppressed human basophils' expression of CD63, but not CD69 or CD203c.

To further elucidate the mechanism underlying this phenomenon, we sought to examine the effect of resveratrol on IgE binding to human basophils. We first used lactate buffer (pH 3.9) for 1 min to strip surface-bound IgE from Percoll-separated basophils⁷ and then incubated the cells with resveratrol or control ethanol for 30 min at 37 °C. Basophils were then incubated with Alexa Fluor 647-conjugated polyclonal human IgE (Dendritics, Lyon, France), followed by incubation with FITC-conjugated anti-human Fc ϵ RI (IgG2b κ , clone AER-37) (eBioscience) for 30 min. Newly-bound IgE was semi-quantitated by flow cytometry. Binding of IgE to human basophils seemed to be slightly down-regulated by resveratrol at 100 and 200 μ M (Fig. 2A), and reached statistical significance with resveratrol at 100 μ M when analyzed on the basis of MFI (%) (Fig. 2B). To rule out the possibility of cytotoxicity due to the treatment with lactate buffer, we confirmed down-regulation of IgE binding by using an IgE-naive basophilic cell line, KU812 (Japanese Collection of Research Bioresources Cell Bank, National Institutes of Biomedical Innovation, Health and Nutrition, Osaka, Japan). Similarly, resveratrol treatment at 200 μ M significantly suppressed the bound IgE level (Supplementary Fig. 2). On the other hand, the expression level of basophil-surface Fc ϵ RI did not change (Fig. 2C). Therefore, resveratrol seemed to inhibit IgE binding to basophils without changing the Fc ϵ RI expression level. The mechanism underlying this inhibition of IgE binding to basophils is unclear, but it may involve a conformational change in IgE or Fc ϵ RI, or suppression of turnover of Fc ϵ RI on human basophils.

Peer review under responsibility of Japanese Society of Allergy.

<http://dx.doi.org/10.1016/j.alit.2017.06.015>1323–8930/Copyright © 2017, Japanese Society of Allergy. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

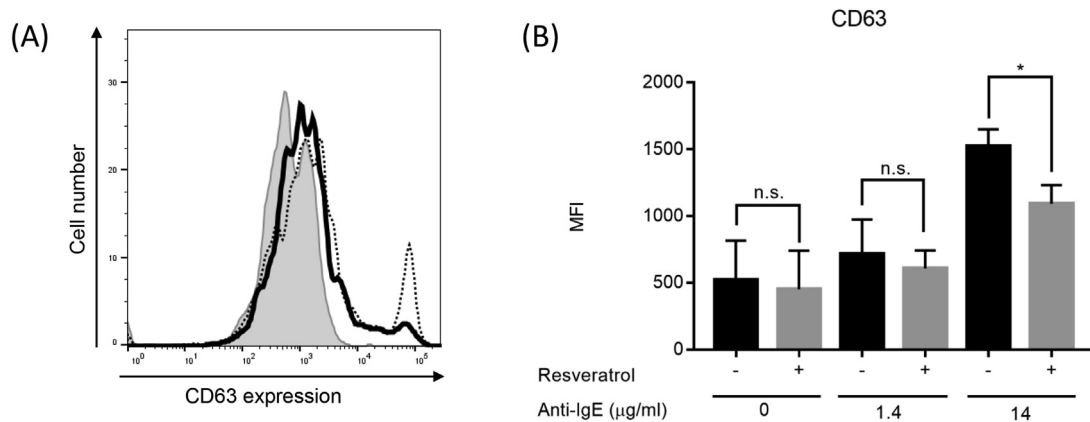


Fig. 1. CD63 expression by peripheral blood basophils following IgE aggregation. (A) Percoll-separated basophils were incubated with resveratrol (bold line) at 100 μ M or control ethanol (dotted line) for 30 min at 37 °C followed by stimulation with goat anti-human IgE antibody, and then analyzed by flow cytometry. The shaded area indicates cells stained with the control antibody. Data are representative of four separate experiments showing similar results. (B) The MFIs of CD63 expression in (A) are shown (basophils treated with control ethanol: black bars; and resveratrol: gray bars). Bars represent the SD ($n = 4-5$). n.s., not significant; * $p < 0.05$ vs. cells pretreated with ethanol.

IgE aggregation resulting in crosslinking of Fc ϵ RI induces a cascade of phosphorylation-dependent intracellular events in basophils that are predominantly regulated by spleen tyrosine kinase (Syk).⁸ Finally, to elucidate the intracellular signals that may be modified by resveratrol, we examined activation of spleen tyrosine

kinase (Syk) by resveratrol following stimulation with anti-IgE antibody. For flow cytometry, Percoll-separated basophils were fixed with Fix Buffer I (BD Biosciences) and then stained with FITC-conjugated anti-human Fc ϵ RI α . The cells were permeabilized with Perm Buffer II (BD Biosciences), followed by staining with Alexa

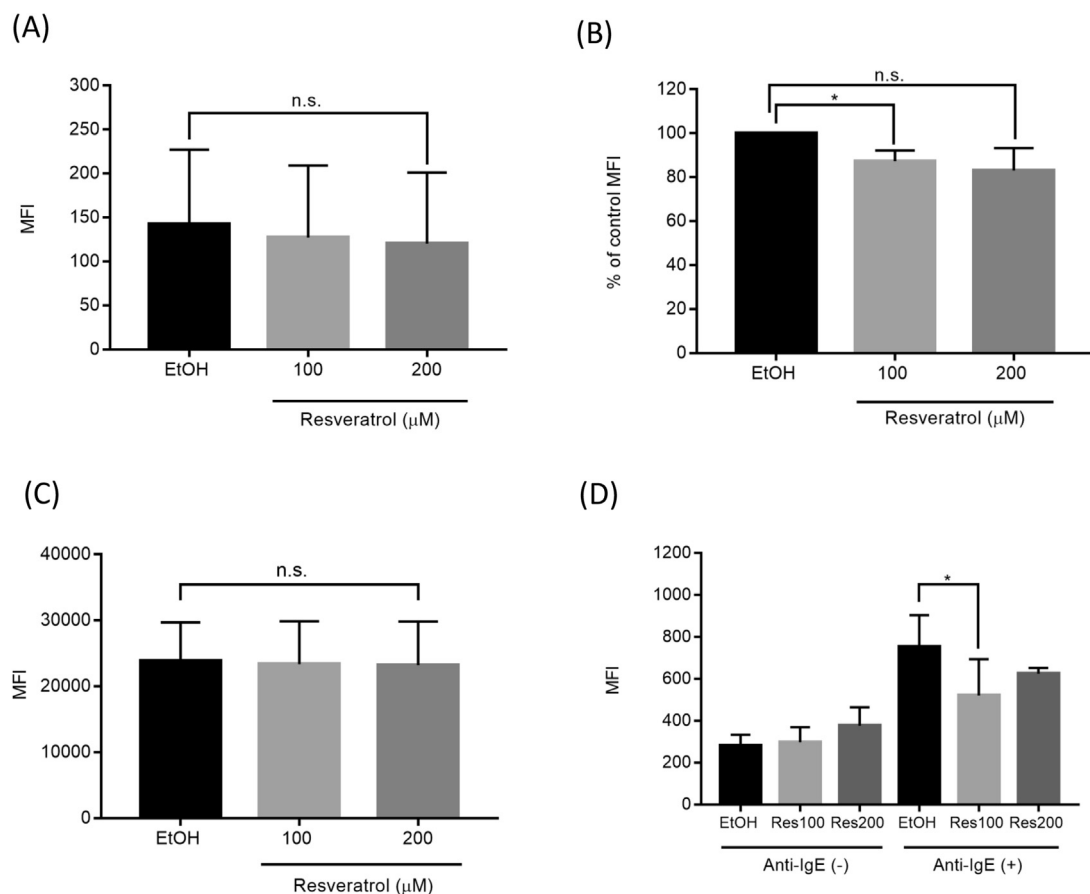


Fig. 2. IgE binding, expression levels of Fc ϵ RI and phosphorylated Syk by peripheral blood basophils. Existing IgE on basophils was stripped with lactate buffer, and then the cells were incubated with resveratrol (Res) at the indicated concentrations or control ethanol (EtOH) for 30 min at 37 °C (EtOH: black bars; Res 100 μ M: light gray bars; Res 200 μ M: dark gray bars). By using Alexa Fluor 647-conjugated polyclonal human IgE, basophil-bound IgE was semi-quantitated by flow cytometry, and the MFI (A) and % of MFI (B) to that of the cells pretreated with EtOH (control MFI) are shown. Bars represent the SD ($n = 4-5$). n.s., not significant; * $p < 0.05$ vs. cells pretreated with ethanol. (C) Percoll-separated basophils were stained with FITC-conjugated anti-Fc ϵ RI antibody, and the MFIs are shown. Bars represent the SD ($n = 4-5$). n.s., not significant. (D) Percoll-separated basophils were pretreated with resveratrol at the indicated concentrations (μ M) or with control ethanol (EtOH) for 30 min at 37 °C, followed by stimulation with goat anti-human IgE antibody. The cells were then stained for intracellular phosphorylated Syk. They were analyzed by flow cytometry, and the MFIs are shown. Bars represent the SD ($n = 4-5$). n.s., not significant; * $p < 0.05$ vs. cells pretreated with ethanol.

Fluor 488-conjugated anti-Syk antibody (IgG1κ, clone I120-722) (BD Biosciences) and analysis using FACSVerse. As a result, pretreatment with resveratrol at 100 μM significantly suppressed the expression level of phosphorylated Syk (Fig. 2D; Supplementary Fig. 3). Thus, we assume that down-regulated degranulation of basophils by resveratrol treatment is at least partly due to suppressed phosphorylation of an intracellular signaling molecule, Syk. However, degranulation of basophils is complete within as little as 10 min after IgE aggregation, while phosphorylation of Syk is known to peak at 30 min.⁹ Therefore, we are not able to conclude that suppressed phosphorylation of Syk is the only mechanism underlying the effect of resveratrol in down-regulating basophil degranulation.

There is accumulating evidence showing beneficial roles of resveratrol in protecting against cardiovascular, aging-associated, neurodegenerative and metabolic diseases, as well as cancers and other disorders through activation of SIRT 1, a critical deacetylase that affects the acetylation status of p53, forkhead proteins and DNA repair enzymes.¹ Moreover, resveratrol showed anti-inflammatory effects in a mouse model of allergic asthma and suppressed airway hyperresponsiveness.¹⁰ Natural food-derived organic substances, including resveratrol, that exhibit health benefits appear attractive and promising. However, previous studies and our present report indicate that the concentration of resveratrol necessary for actual benefits is quite high, and unlikely to be obtained from daily consumption of food. It may be reasonable to use nutritional supplements with precisely defined contents of resveratrol in combination with existing therapies.

In conclusion, suppression of binding of IgE on basophils' surface as well as suppression of phosphorylation of Syk are possible mechanisms underlying decreased basophil activation due to resveratrol treatment. Further research is needed to elucidate the mechanism underlying resveratrol's suppression of human basophil degranulation.

Conflict of interest

The authors have no conflict of interest to declare.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.alit.2017.06.015>.

Sayaka Arakawa^{a,b}, Maho Suzukawa^{a,*}, Sayaka Igarashi^a, Hiroto Matsui^a, Masao Yamaguchi^c, Takahide Nagase^b, Ken Ohta^a

^a National Hospital Organization, Tokyo National Hospital, Tokyo, Japan

^b Department of Respiratory Medicine, University of Tokyo, Tokyo, Japan

^c Division of Respiratory Medicine and Allergy, Department of Medicine, Teikyo University School of Medicine, Tokyo, Japan

* Corresponding author. Center for Pulmonary Diseases, Clinical Research Center, National Hospital Organization, Tokyo National Hospital, 3-1-1 Takeoka, Kiyose-Shi, Tokyo 204-8585, Japan.

E-mail address: fueta-tyk@umin.ac.jp (M. Suzukawa).

References

1. Weiskirchen S, Weiskirchen R. Resveratrol: how much wine do you have to drink to stay healthy? *Adv Nutr* 2016;**7**:706–18.
2. Tanaka Y, Yamaguchi M, Suzukawa M, Arai H, Nagase H, Ohta K. Modulation of human basophil activation by resveratrol. *Allergol Int* 2015;**64**(Suppl):S80–2.
3. Hirai K, Morita Y, Miyamoto T. Hemopoietic growth factors regulate basophil function and viability. *Immunol Ser* 1992;**57**:587–600.
4. Karasuyama H, Obata K, Wada T, Tsujimura Y, Mukai K. Newly appreciated roles for basophils in allergy and protective immunity. *Allergy* 2011;**66**:1133–41.
5. Knol EF, Koenderman L, Mul FP, Verhoeven AJ, Roos D. Differential activation of human basophils by anti-IgE and formyl-methionyl-leucyl-phenylalanine. Indications for protein kinase C-dependent and -independent activation pathways. *Eur J Immunol* 1991;**21**:881–5.
6. Gober LM, Eckman JA, Sterba PM, Vasagar K, Schroeder JT, Golden DB, et al. Expression of activation markers on basophils in a controlled model of anaphylaxis. *J Allergy Clin Immunol* 2007;**119**:1181–8.
7. Suzukawa M, Komiya A, Koketsu R, Kawakami A, Kimura M, Nito T, et al. Three cases of ortho-phthalaldehyde-induced anaphylaxis after laryngoscopy: detection of specific IgE in serum. *Allergol Int* 2007;**56**:313–6.
8. Sanderson MP, Wex E, Kono T, Uto K, Schnapp A. Syk and Lyn mediate distinct Syk phosphorylation events in FcεRI-signal transduction: implications for regulation of IgE-mediated degranulation. *Mol Immunol* 2010;**48**:171–8.
9. Miura K, Lavens-Phillips S, MacGlashan Jr DW. Localizing a control region in the pathway to leukotriene C4 secretion following stimulation of human basophils with anti-IgE antibody. *J Immunol* 2001;**167**:7027–37.
10. Lee M, Kim S, Kwon OK, Oh SR, Lee HK, Ahn K. Anti-inflammatory and anti-asthmatic effects of resveratrol, a polyphenolic stilbene, in a mouse model of allergic asthma. *Int Immunopharmacol* 2009;**9**:418–24.

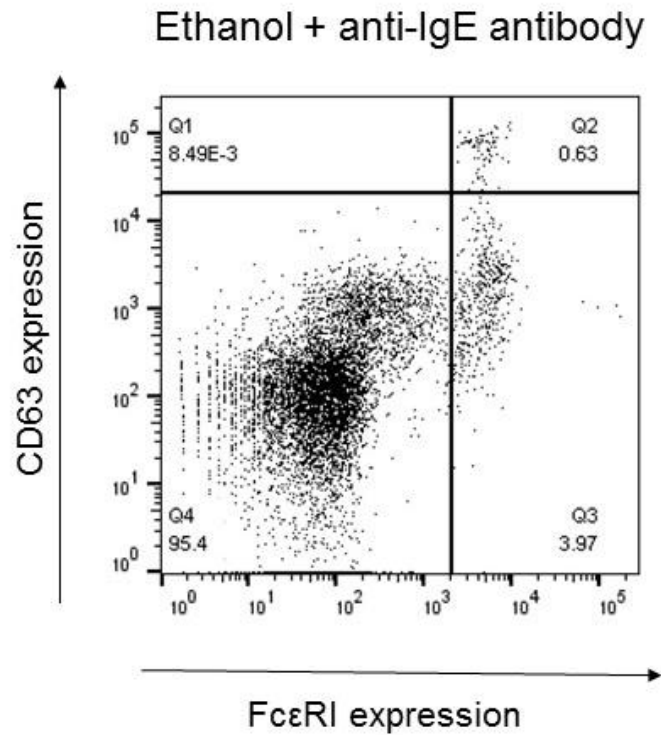
Received 30 January 2017

Received in revised form 7 June 2017

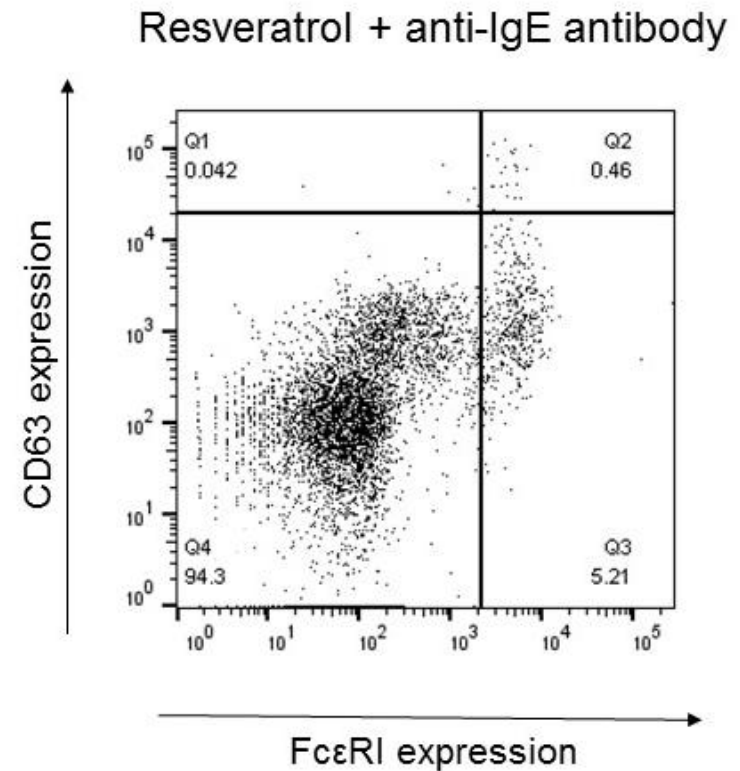
Accepted 12 June 2017

Available online xxx

A



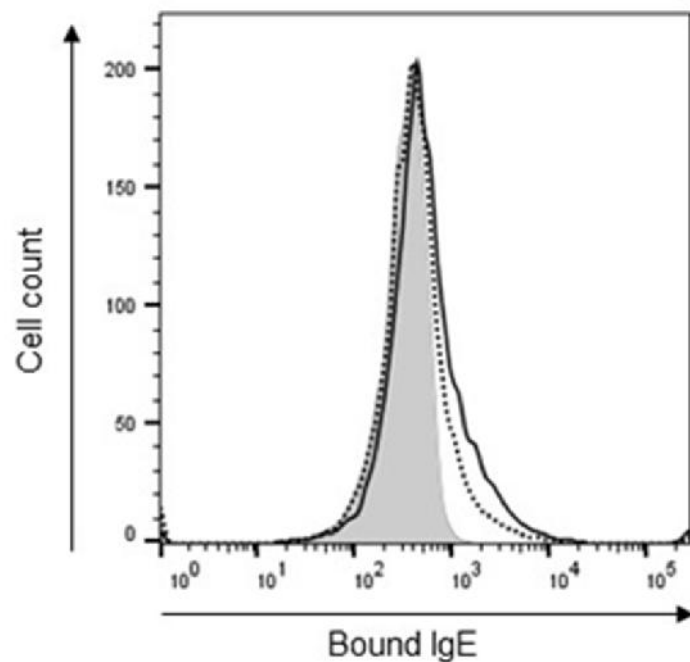
B



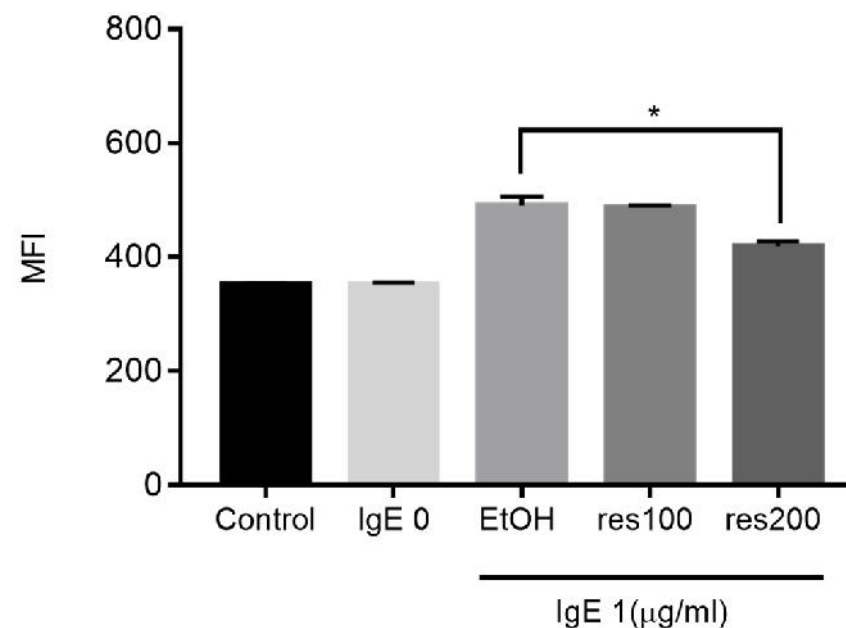
Supplementary Fig. 1

Expression levels of CD63 by peripheral blood basophils. Dot plots of CD63 and FcεRI expression with (B) and without (A) resveratrol are shown. Basophils were identified as FcεRI-high cells (Q2 and 3). Percentages of total cells in the dot plot are shown in each quadrant. Data are representative of four separate experiments yielding similar results.

A



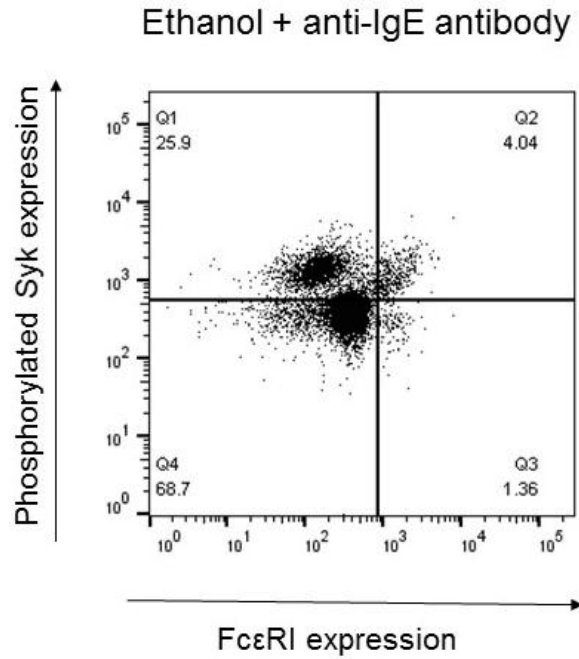
B



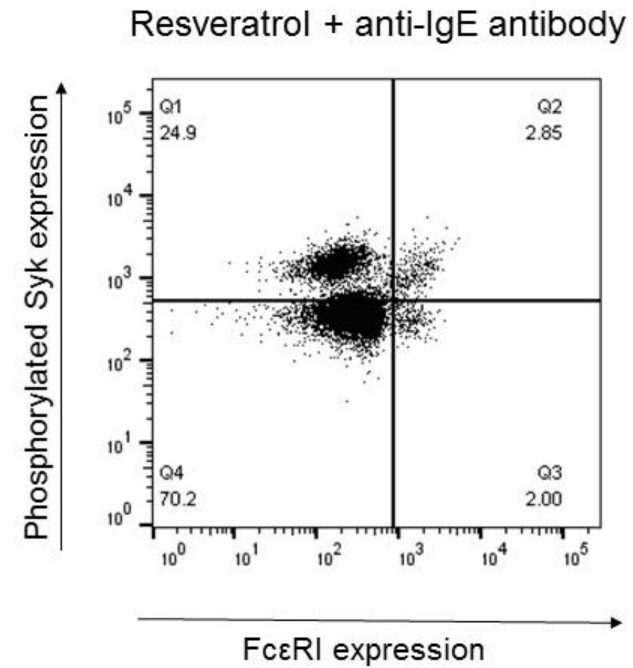
Supplementary Fig. 2

IgE binding by using a basophilic cell line, KU812. (A) Bound IgE levels on KU812 cells are shown. Cells preincubated with resveratrol at 200 μM (dotted line) or control ethanol (solid line), and cells stained with isotype control antibody (shaded area) followed by incubation with IgE at 1 $\mu\text{g}/\text{ml}$ for 17 h are shown. Data are representative of four separate experiments yielding similar results. (B) MFI of bound IgE levels on KU812 cells with resveratrol (res) at indicated concentrations (μM), control ethanol (EtOH), cells stained with isotype control antibody (control) and cells without incubation with IgE are shown ($n=4$). * $p < 0.05$.

A



B



Supplementary Fig. 3

Expression levels of phosphorylated Syk by peripheral blood basophils. Dot plots of phosphorylated Syk and FcεRI expression with (B) and without (A) resveratrol are shown. Basophils were identified as FcεRI-high cells (Q2 and 3). Percentages of total cells in the dot plot are shown in each quadrant. Data are representative of four separate experiments yielding similar results.