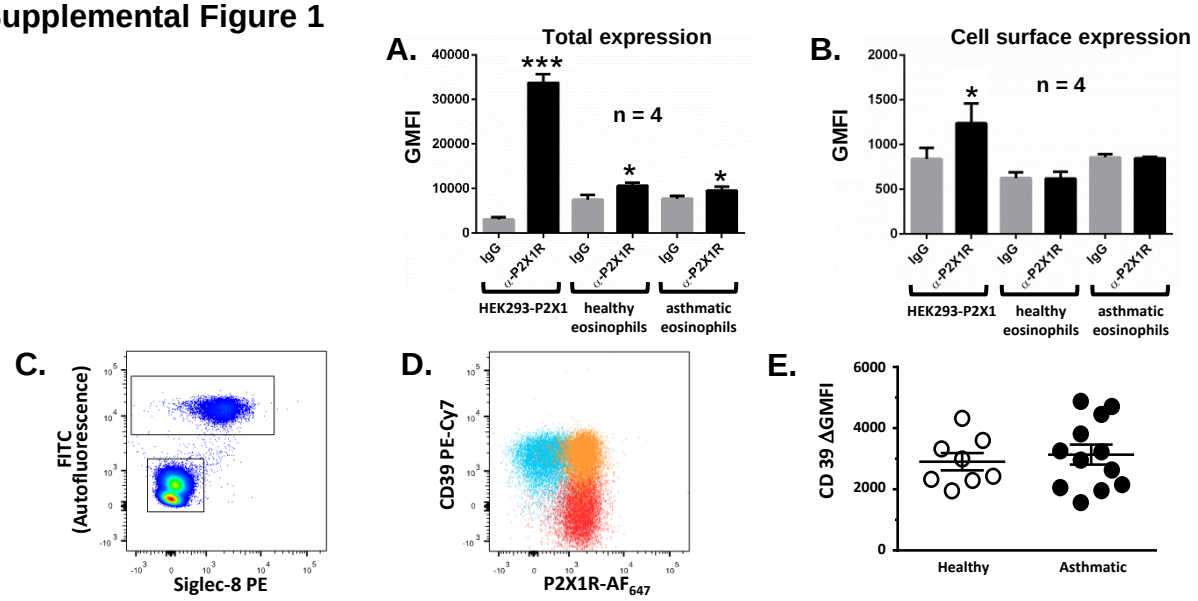
Supplemental Table 1. List of primers used for classical RT-PCR and qPCR

Genes (human)	Forward primer	Reverse primer
Classical RT-PCR[1]		
β-actin	5'-TGGTGGGCATGGGTCAGAAG-3'	5'-GTCCCGGCCAGCCAGGTCCAG-3'
P2X1	5'-CGTCATCGGGTGGGTGTTTCTCTA-3'	5'-AGGGCGCGGGATGTCGTCA-3'
P2X2	5'-GGGCCCCGAGAGCTCCATCATC-3'	5'-GCAGGCAGGTCCAGGTCACAGTCC-3'
P2X3	5'-ACTGGCCGCTGCGTGAACTACA-3'	5'-CACGTCGAAGCGGATGCCAAAAG-3'
P2X4	5'-CGGCACCCACAGCAACGGAGTCT-3'	5'-TGTATCGAGGCGGCGGAAGGAGTA-3'
P2X5	5'-GGCCCCAAGAACCACTACTGC-3'	5'-CCTCGGCCTCCTGGGAACTGTCT-3'
P2X6	5'-AGCCCCTACTGTCCCGTGTTCC-3'	5'-GCCTTGGCCTCCTCATACTTTGTC-3'
P2X7	5'-CCGGCCACAACTACACCACGAG-3'	5'-GGCCAGACCGAAGTAGGAGAGG-3'
q-PCR[2]		
β-actin	5'-TCCTATGTGGGCGACGAG-3'	5'-ATGGCTGGGGTGTTGAAG-3'
PPIB (cyclophilin B)	5'-CGTCTTCTTCCTGCTGCTG-3'	5'-AGCCAAATCCTTTCTCTCCTG-3'
RPL13A1	5'-CTGCCCCACAAAACCAAG-3'	5'-TCTCTTTCCTCTTCTCCTCCAG-3'
P2X1	5'-CTGGTGGAGGAGGTGAATG-3'	5'-AAGTTGAAGCCTGGGGAGAG-3'
P2X2	5'-CATCGGGGTCATTATCAAC-3'	5'-CAGTCGCACAGGAAGGAG-3'
P2X3	5'-GACCCTTTCTGCCCCATC-3'	5'-CACTGCCATTTTCCATTTTG-3'
P2X4	5'-GGAGAACGCAGGACACAG-3'	5'-CCTTCCCAAACACAATGATG-3'
P2X5	5'-TGGTCGTATGGGTGTTCCTG-3'	5'-TGCCTTCATTCTCAGCACAG-3'
P2X6	5'-CCCAAGTTCAGGGCAGATG-3'	5'-GAAGGTGACTGTGTTTTTGATG-3'
P2X7	5'-TACATCGGCTCAACCCTCTC-3'	5'-GCAGGTCTTGGGACTTCTTG-3'

[1].β-actin gene was used as reference gene for the classical RT-PCR. The reactions consisted of 40 cycles at 94°C for 45 s, 55°C (P2X2, 5 & 7) or 58°C (P2X1, 3, 4 & 6) for 45 s, and at 72°C for 60 s. For analysis, all RT-PCR products were run on 1.5% agarose gel containing ethidium bromide.

[2].β-actin, PPIB (cyclophilin B) and RPL13A1 genes were used as reference genes for the qPCR. The reactions consisted of 38 cycles at 95°C for 15 s, 60°C for 15 s, and at 72°C for 30 s.

Supplemental Figure 1



Assessment of human eosinophil P2X1 receptor and CD39 protein expression levels by flow cytometry:

A. and B. Extracellular and intracellular (total) P2X1 receptor expression levels were measured in eosinophils and HEK293 cells stably transfected with P2X1 tagged with GFP (HEK293-P2X1) using polyclonal rabbit antibodies (4 µg/ml) raised against extracellular (P2X1-eAb) (#APR-022, Alomone labs, Jerusalem, Israel) and intracellular (P2X1-inAb) (#APR-001, Alomone labs) P2X1 receptor epitopes. All flow cytometry acquisitions were performed using a CSnT checked BD FACS Canto (Becton Dickinson, Oxford, UK) using FACSDiva software (Becton Dickinson) and reported as geometric mean fluorescence intensity (GMFI). Statistics were performed using the Student paired t-test.

Total P2X1 receptor protein level in human eosinophils and HEK293-P2X1 (positive control) cells were assessed following cell fixation [250 µl of intracellular fixation buffer (eBioscience, Ltd., Hatfield, UK) was added to cells ($5x10^4$) and incubated for 20 min at 4°C] and permeabilisation (eBioscience, Ltd.). After washing, the cells were incubated with P2X1-inAb or rabbit IgG for 30 min at room temperature, washed again and stained with 1 µg/ml AF_{647}–goat anti-rabbit IgG (H+L) (Fab_2 fragment) (#A21246, Life Technologies Ltd.) on ice, all in the presence of the permeabilisation buffer. Following washing, the cells were resuspended in 400µl of Fluorfix (Biolegend UK Ltd., London, UK) buffer for flow cytometry acquisition.

Both healthy (GMFI of 10693±613 against 7513±1184 for P2X1 antibody and IgG control respectively, p=0.0238 n=4) and asthmatic (GMFI of 9558±936 versus 7747±602 for P2X-inAb and IgG control respectively, p=0.0207, n=4) eosinophils exhibited total P2X1 receptor protein expression **(A)**. However, there was no difference in the level of total P2X1 receptor protein expression between eosinophils from healthy and asthmatic donors. In comparison, HEK293-P2X1, used here as a positive control for the expression of P2X1 receptor protein, expressed a high level of P2X1 receptor protein (GMFI of 33801±1835 versus 3132±448 for P2X1 antibody and IgG control respectively, p=0.0002, n=4) **(A)**.

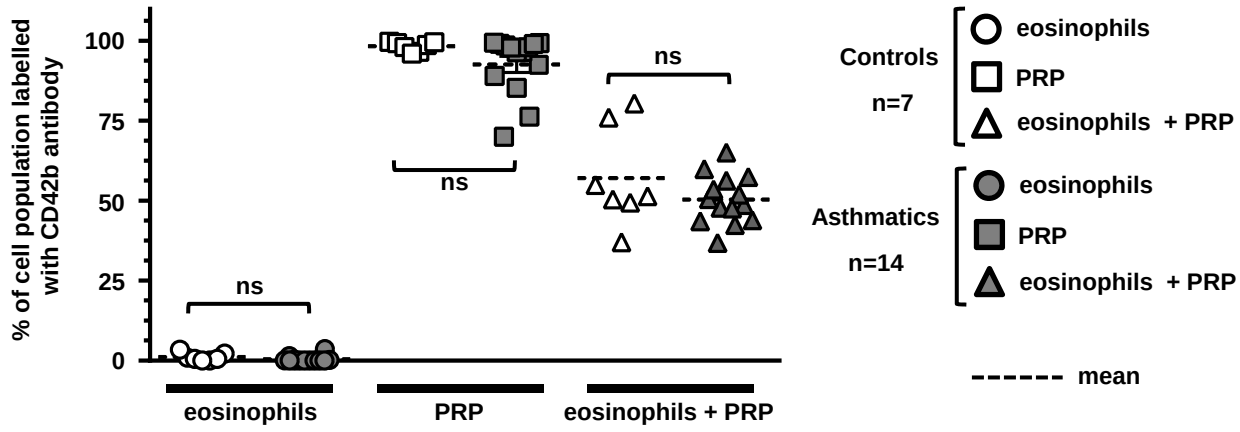
For P2X1 receptor cell surface expression measurement, 50 µl of cell suspension ($5x10^4$ cells) were incubated for 15 min at 4◦C in presence of human IgG (9.4 µg/ml)(Sigma-Aldrich) to block non-specific binding of primary antibodies. Cells were then incubated either with P2X1-eAb or a control rabbit IgG (#X0936, Agilent Technologies, Dako Glostrup, Denmark) at room temperature for 30 min. Cells were subsequently washed and stained with 1 µg/ml of an AF_{647}–goat anti-rabbit IgG (H+L) as described above. Following washing, cells were resuspended in 400µl FluorFix buffer (Biolegend UK Ltd., London, UK) prior to flow cytometry.

P2X1 protein surface expression in eosinophils from healthy (GMFI of 622±76 versus 625±63 for P2X1-eAb and IgG control respectively, n=4) and asthmatic (GMFI of 847±17 versus 858±34 for P2X1 antibody and IgG control respectively, n=4) donors was not detected **(B)**. In comparison cell surface P2X1 receptor protein was detected in HEK239-P2X1 (GMFI of 1239±222 versus 839±126 for P2X1-eAb and IgG control respectively, p=0.0360, n=4) though greatly reduced compared to cell total expression **(B)**.

While we expect P2X1 receptor protein expression at the eosinophil cell surface to be low due to the small amplitudes of the currents measured, flow cytometry may not be an approach sensitive enough to measure minute receptor expression levels on per cell basis. In addition, we cannot rule out that the antibody directed against the extracellular epitope itself may not be optimal for the measurement of the weakly expressed P2X1 protein.

C, D and E. Eosinophil P2X1 receptor and CD39 expression levels were measured from whole blood. See Protocol in main manuscript "*Flow cytometric detection of eosinophil P2X1 total protein expression*" (**C**).
The geometric mean fluorescence intensity (ΔGMFI) of Eosinophil Siglec-8, P2X1R and CD39 expression (or relevant controls) was calculated. Compared to FMO (Red in **D**) and polyclonal controls (Blue in **D**), Eosinophils expressed both CD39 and P2X1R (Orange in **D**), respectively. Total CD39 ΔGMFI (2905±284 vs 3138±325) fluorescence values were similar between healthy (n=8) and asthmatic (n=12), respectively **(E)**.

Supplemental Figure 2



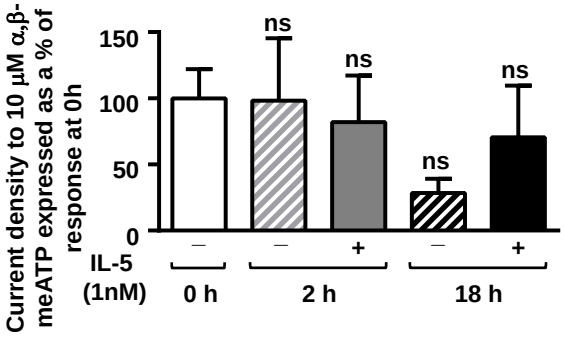
Platelets do not bind to eosinophils in our eosinophil preparations:

To detect whether platelets were bound to eosinophils, purified eosinophils ($1x10^5$/100 µl) were incubated with PE-conjugated mouse anti-human CD42b (clone HIP-1, BioLegend UK Ltd.) or PE-conjugated IgG_{1k} isotype control (clone MOPC-21, BioLegend UK Ltd) at the same final concentration (0.125µg/ml) in PBS (+2% AB serum, Invitrogen) for 30 minutes at room temperature. Subsequently, 400µl of PBS were added and at least 10,000 eosinophil events were acquired immediately on a BD FACSCanto flow cytometer using FACSDiva software. As a positive control, we collected autologous platelet rich plasma (PRP)[1] and stained PRP ($3x10^6$ platelets, volume adjusted to 100µl with PBS) using the same protocol as described above (at least 10,000 platelet events were acquired). We also verified that platelets can bind to eosinophils using our experimental approach by co-incubating the purified eosinophils and PRP ($1x10^5$ and $3x10^6$ cells in 100µl for eosinophils and platelets respectively) and 10,000 eosinophil events were acquired on a BD FACSCanto flow cytometer. For both cell populations, we measured cell associated fluorescence in the PE channel. The data shown are expressed as % of cells positive for CD42b following subtraction of the isotype control treated cells . Statistics were performed using the Mann-Whitney test.

Jawień *et al.* have previously reported the binding of platelets to eosinophils, therefore raising the possibility of cross-contamination of our preparation of eosinophil RNA with platelet RNA[2,3]. To determine if our eosinophil preparations were contaminated with platelets, the cell suspensions were incubated with the platelet-selective antibody anti-CD42b and analysed by flow cytometry. Platelets were not detected in the eosinophil preparations from healthy or asthmatic donors [1.1±0.5% and 0.4±0.3% of cells were CD42b-positively labelled for healthy (n=7) and asthmatic (n=14), respectively]. In contrast, high level of CD42b expression was observed in platelet rich plasma [98±1% and 93±3% of cells were CD42b-positively labelled for healthy (n=7) and asthmatic (n=14), respectively]. In accordance with Jawień *et al*., the co-incubation of eosinophils and PRP resulted in the binding of platelets to eosinophils ([57±6% and 50±2% of eosinophils were CD42b-positively labelled for healthy (n=7) and asthmatic (n=14), respectively]. These data suggest that our eosinophil preparations are not contaminated with platelets.

1. Rolf MG, Brearley CA, Mahaut-Smith MP. Platelet shape change evoked by selective activation of P2X1 purinoceptors with alpha,beta-methylene ATP. Thromb Haemost. 2001;85(2):303-8.
2. Jawien J, Lomnicka M, Korbut R, Chlopicki S. The involvement of adhesion molecules and lipid mediators in the adhesion of human platelets to eosinophils. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society. 2005;56(4):637-48. Epub 2006/01/05.
3. Jawien J, Chlopicki S, Gryglewski RJ. Interactions between human platelets and eosinophils are mediated by selectin-P. Polish journal of pharmacology. 2002;54(2):157-60. Epub 2002/07/26.

Supplemental Figure 3



IL-5 treatment has no effect on eosinophil P2X1 receptor currents:

As would be expected[1], the asthmatic donors used in this study had a mild eosinophilia ($0.49\pm0.12x10^6$ cells/ml, n=22, compared to $0.14\pm0.03x10^6$ cells/ml in healthy donors, n=32, p=0.0002). This is thought to be due to excess production of IL-5 and other eosinophil growth factors. We therefore tested whether the reduced current in eosinophils from asthmatic donors could be reproduced *in vitro* in eosinophils from healthy subjects by treating them with IL-5. Eosinophils from healthy donors were plated into 24 well plates (10^6 cells/ml) and treated or not with IL-5 (1 nM) for 0, 2 or 18h at 37ºC in a humidified atmosphere of 5% CO_2 before recordings. IL-5 had no effect on 10 μM α,β-meATP-induced P2X1 receptor currents after 2h (98±47% for control and 82±35% for IL-5 of response at time "0") or 18 h treatment (22±11% for control and 71±39% for IL-5) (at time "0", 100±22%)(n=3). The survival of IL-5-treated (97±2%) or non-treated eosinophils (97±2%, n=3) remained unaffected. Friedman test ANOVA followed by Dunn's multiple comparisons test were used and significance was accepted at $p<0.05$.

1. Rothenberg ME, Hogan SP. The Eosinophil. Annu Rev Immunol. 2005.