SUPPLEMENT VIDEOS

V1: The movement of a human eosinophil (white cell) on the surface of V-ctrl transduced HUVEC monolayer. HUVECs were transduced with V-ctrl and exposed to freshly isolated human eosinophils. The movement of eosinophils on the surface of the transduced HUVECs (μm/min) after binding were recorded by video microscopy.

V2: The movement of a human eosinophil (white cell) on the surface of V-miR-1 transduced HUVEC monolayer. HUVECs were transduced with V-miR-1 and exposed to freshly isolated human eosinophils. The movement of eosinophils on the surface of the transduced HUVECs (μm/min) after binding were recorded by video microscopy.

SUPPLEMENTAL METHODS:

Sample processing for the asthma cohort

Sputum and blood eosinophils were counted by blinded researchers and using a hemocytometer after staining, as described previously.[1] For miR-1 measurement, RNA was extracted from the banked frozen serum samples using Trizol extraction method (Thermo Fisher Scientific, US) followed by phenol/chloroform extraction method, as per the manufacture protocol. Two additional chloroform purification steps were performed before precipitating the RNA. The concentration and quality of these RNA samples were determined using Nanodrop spectrophotometer (Thermo Scientific) and RNA ScreenTape System (Agilent), respectively.

CRS biopsies

The nasal pathology tissues used for this study were removed by sinus endoscopy from the sites of inflammation in the CRS patients and included tissue from two or more of the maxillary, frontal, sphenoid or ethmoid sinuses. After removal, the tissues were immediately transferred to a culture medium (RPMI without phenol red) on ice and within 3 hours of removal, transferred to our lab for further processing. For RNA extraction, tissues were immediately suspended in Trizol and stored at -80 C until the RNA extraction started. Total RNA was extracted using miRNeasy purification kit (Qiagen). Following the RNA extraction, concentration of RNA samples was determined using Nanodrop spectrophotometer (Thermo Scientific) and the integrity of the RNA was checked by determining RIN (RNA integrity number) using RNA ScreenTape System (Agilent). Only RNA samples with RIN numbers higher than 7 were accepted for further experimentation and analyses. Tissue eosinophilia was determined by a clinical histopathologist by counting eosinophils in three high power fields (HPF) after staining the formalin-fixed paraffin-embedded (FFPE) tissue sections with hematoxylin and eosin (H&E). The eosinophil counts on

CRS tissues were further confirmed by immunohistochemistry with anti-EPX (NBP2-13967, Novus biologicals) antibody using ImmPRESS reagents (Vector laboratories) The eosinophil counts obtained after immunostaining was comparable with counts after H&E staining. We used eosinophil counts with anti-EPX immunostaining for our associations testing.

Ex vivo Lung Culture

Histologically normal lung tissue samples were obtained from patients who underwent surgical resection for lung masses at the Yale Cancer Center. All tissue samples were used after approval from the Yale Human Investigation Committee that approved the design of the study and patient consent forms (HIC protocol # 1103008160). Patients were consented for gene expression measurements before undergoing the surgical procedure. Fresh samples were kept in DMEM (Dulbecco Minimal Essential Medium) supplemented with 10% fetal Bovine Serum (FBS) at 4 degrees for up to 12 hours before being delivered to the lab for ex vivo culture. The lung tissue was cut into approximately 3-4mm size pieces and cultured in M199 (Life Technologies), 20% FBS (Gibco, Life Technologies) at 37 C. For IL-13 stimulation, tissues were treated with 1 or 10ng/ml of human recombinant IL-13 (R&D Biosystems) and harvested after 24 hours. Tissues were stored in Trizol at -80 C until RNA extraction. Total RNA samples were isolated using Trizol followed by phenol/chloroform extraction, per the manufacturer's protocol. The concentration and quality of these RNA samples were determined using Nanodrop spectrophotometer (Thermo Scientific).

Magnetic-Activated Cell Sorting (MACS) for endothelial isolation

Endothelial (CD31+, CD45-) epithelial (CD326+, CD45-) and hematopoietic (CD45+) cells were isolated from normal human lung tissues stimulated with IL-13 (or control PBS) and from OVA challenged transgenic (or wildtype) mice. For single cell suspension, lung tissues were minced and

passed through 70µm filter using an ice-cold media. After centrifugation, the cell pellet was washed and resuspended in cold separation buffer, containing PBS, 0.5% bovine serum albumin (BSA) and 2 mM EDTA, incubated for 15 min at 4°C with anti CD45 microbeads (Miltenyi Biotec). After labelling, cells were washed to remove unbound beads by adding 1 ml of separation buffer, followed by centrifugation at 300 × g for 10 min. Cells were then resuspended in 1 ml of separation buffer and applied onto a MACS Column (LD type, Miltenyi Biotec) placed in the magnetic field of a MACS Separator (Miltenyi Biotec). The flow-through was collected as the unlabelled negative fraction (CD45-). The column was then washed three times with 3 ml of separation buffer and the retained magnetically labelled cells were flushed out with 1 ml of separation buffer as the positive fraction (CD45+). Endothelial (CD31+, CD45-) fractions were obtained from labelling CD45- cells with anti-CD31 microbeads and passing through the MACS column (MS type). Similarly, epithelial positive (CD326+, CD45-) fractions were obtained from labelling CD45- cells with anti-CD 326 (EpCAM) microbeads

Allergen sensitization and challenge in mice

Six to 8 week-old C57 female BL6 mice were sensitized and challenged with OVA and HDM as described previously.[2] In the HDM model, mice were sensitized by delivery of ~1 µg DerP1 (Greer Labs) per mouse through intraperitoneal (or intranasal) routes. Lentiviral vectors were delivered through intranasal route 2 weeks before the challenge steps in OVA and HDM models. In transgenic mice, miR-1 transgene was induced at least 4 days before the antigenic challenge by adding Doxycycline to the drinking water.[3]

BAL and lung tissue analyses

BAL fluids were collected, processed, stained and analyzed or cytology performed as described previously.[2] BAL cytokines were measured using Bioplex (biorad) or U-plex (MSD) according to

the manufacturer's instructions. A Nikon DS Ri2 microscope was used for all the histometric measurements. Lung inflammation and mucus metaplasia were quantified as described previously[2] using formalin-fixed paraffin-embedded sections stained with hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS), respectively. Tissue eosinophilia was quantified by counting of eosinophils/bronchovascular areas in sections stained with Congo Red and expressed as the number of the eosinophils /mm2, as described previously.[2]

Measurement of AHR

24 hours after the last challenge, pulmonary resistance was measured by the forced oscillation technique in response to increasing concentrations of methacholine using the FlexiVent system (SCIREQ, Canada) as previously described.[2] Briefly, mice were anesthetized with 20 mg/kg pentobarbital and 1.8 g/kg urethane. In the parallel experiment, the adequacy of the dose of the administered sedative for sedation up to four hours were determined. Once the mice were completely anaesthetized, tracheostomy was performed, and a tubing adaptor (20 gauge) was used to cannulate the trachea. The mice were injected with 0.4 mg/kg pancuronium bromide and then attached to a rodent ventilator and pulmonary mechanics analyzer (FlexiVent; SCIREQ, Canada) and ventilated at a tidal volume of 10 ml/kg, a frequency of 150 breaths/minute, and 2 cm H_2O positive end-expiratory pressure. Each mouse was challenged with saline (0 mg/ml) followed by increasing concentrations of methacholine aerosol (10–100 mg/ml) generated with an in-line nebulizer and administered directly through the ventilator for 10 seconds. Mean airway resistance (cmH2O.s/ml) was measured and plotted by the machine at each methacholine concentration.

Eosinophil isolation

In order to choose the best isolation method for our assays we performed a comparison between the rapid method with the use of chemotactic peptide[4] and magnetic antibody sorting-negative

selection method (MACSxpress, Miltenyi). In the rapid isolation method, whole blood was collected from the healthy donors (15-20ml each) with large bore butterfly needles and drawn into a syringe containing citrate phosphate dextrose (CPD), a 6% HMW Dextran and f-Met-Leu-Phe (10^{-6} to 10^{-7} mol/L). After 45-60 minutes, plasma and red blood cells were separated, and plasma was removed carefully with the help of butterfly needle tubing into a fresh tube to avoid RBC contamination. After 6 min of centrifugation the supernatant was discarded and the cell pellet was resuspended in PBS and then added to Histopaque 1077 and 1190 (Sigma-Aldrich). The cell suspension was then centrifuged twice, the supernatants were discarded, and the eosinophil buffy layer was resuspended in 0.01% glucose solution (in PBS with Mg+ and Ca+) at a concentration of $1x10^5$ cells/mL as determined by hemocytometer counts. Contaminating red blood cells were lysed using RBC lysis solution.(Sigma Aldrich) The purity of the isolated eosinophils was determined by HEMA 3 staining (usually >95%). Trypan blue staining (Life technologies) was used to monitor the viability of the isolated eosinophils (usually >98%). In the negative selection method an antibody cocktail was used for isolation of eosinophils from whole blood, as per the manufacture's protocol (MACSxpress, Miltenyi). The viability and purity of eosinophils were ~92% and >95%, respectively. The main contaminants were red blood cells, and they were lysed using blood lysis solution. The yield with magnetic method was ~10-15% lower than the rapid method. Since in our hands the rapid method had higher yield and slightly higher viability and purity, we chose to use this method for our experiments.

Adhesion Assays

Eosinophil adhesion and movement were measured as described previously[5, 6] with some modifications. Briefly, HUVECs were transduced with v-miR-1 or control (v-ctrl), seeded on 25mm coverslips (Fisher Scientific) and grown to confluence to form a monolayer (cobblestone

morphology was visually determined). These cells were then starved in M199 (Life Technologies), 2% FBS (Gibco, Life Technologies) for 4 hours before being treated with human recombinant IL-13 (R&D) at 1ng/ml or vehicle. Adhesion assay was performed 24 hours after IL-13 stimulation. Eosinophils were isolated on the day of the adhesion experiment as described above, diluted to a concentration of $1x10^5$ cells/mL in PBS (with Mg+ and Ca+) and glucose and 1ml of this suspension was added to the apical surface of an enclosed Sykes-Moore adhesion chamber. The chambers were then incubated for 500 seconds at 37°C, after which the number of eosinophils on the endothelial surface were counted. Next, the chambers were inverted for 500 seconds to detach the non-adherent eosinophils. Firmly adherent eosinophils were then counted, and % adherent cells were calculated.

For measuring the eosinophil movement on the HUVEC, freshly isolated eosinophils (about $1x10^5$ cells/ml) were injected into Sykes-Moore adhesion chamber containing the endothelial monolayer. After allowing the solution to settle, a time-lapse sequence was captured using a Nikon Eclipse TE2000-U microscope and NIS-Elements imaging software. Images were taken at 15-second intervals over 15 minutes and were analyzed using ImageJ software (NIH). Eosinophils were tracked using the Manual Tracking plug-in and trajectories were plotted using X, Y-coordinates and average velocity was calculated for each eosinophil.

miRISC Recruitment Analysis

HUVECs were purchased from the Yale Vascular Biology and Therapeutics tissue culture core facility, grown in 10-cm plates and transduced with lenti miR-1. 36-48 h after transduction, cells were checked for fluorescence and then collected by scraping in cold PBS, spun down (300 g for 10 min at 4°C), and resuspended in 250 µl of cold lysis buffer (10 mM Hepes, pH 7.4, 150 mM KCl, 3 mM MgCl2, 0.2 mM DTT, 10% glycerol, 0.5% NP-40, and 1 mM PMSF). Part of this

lysate was kept as “input fraction,” and the rest was immunoprecipitated with anti-Ago2 antibody (Sigma-Aldrich) and dynabeads protein A (Life Technologies). The beads were directly added to TRIzol after washing three times (wash buffer: 0.1M NaP pH 8.0) and the RNA extracted with Phenol-chloroform extraction protocol.

REFERENCES FOR SUPPLEMENTARY METHODS

1. Yan X, Chu JH, Gomez J, Koenigs M, Holm C, He X, et al. Noninvasive analysis of the sputum transcriptome discriminates clinical phenotypes of asthma. Am J Respir Crit Care Med 2015; 191:1116-25.
2. Takyar S, Vasavada H, Zhang JG, Ahangari F, Niu N, Liu Q, et al. VEGF controls lung Th2 inflammation via the miR-1-Mpl (myeloproliferative leukemia virus oncogene)-P-selectin axis. J Exp Med 2013; 210:1993-2010.
3. Korde A, Jin L, Zhang JG, Ramaswamy A, Hu B, Kolahian S, et al. Lung Endothelial MicroRNA-1 Regulates Tumor Growth and Angiogenesis. Am J Respir Crit Care Med 2017; 196:1443-55.
4. Roberts RL, Gallin JI. Rapid method for isolation of normal human peripheral blood eosinophils on discontinuous Percoll gradients and comparison with neutrophils. Blood 1985; 65:433-40.
5. Ayres-Sander CE, Lauridsen H, Maier CL, Sava P, Pober JS, Gonzalez AL. Transendothelial migration enables subsequent transmigration of neutrophils through underlying pericytes. PloS one 2013; 8:e60025.
6. Lauridsen HM, Pober JS, Gonzalez AL. A composite model of the human postcapillary venule for investigation of microvascular leukocyte recruitment. The FASEB Journal 2014; 28:1166-80.

E1a.

Characteristics of Asthma cohort		
	Control Subjects (n = 11)	**Asthma Patients (n = 59)**
Age (year)		
Mean (Range)	36.8 (26-68)	44.5 (14-75)
Gender		
Male	6 (54.54%)	17 (27.87%)
Female	5 (45.45%)	42 (71.18%)
BMI [kg/m^2] (Mean, SD)	23.53 ± 2.9	29.34 ± 7.03
History of atopy, n (%)	6 (54.54%)	54 (91.52%)
Hospitalization for asthma (Lifetime), n (%)	NA	23 (38.98%)
Hospitalization for asthma (past year), n (%)	NA	10 (16.94%)
ACT score (Mean, SD)	NA	15.87 ± 6.403
FEV_1-% of predicted value (Mean, SD)	95.46 ± 11.83	77.74 ± 24.31
FVC-% of predicted value (Mean, SD)	101.45 ± 14.9	88.24 ± 21.39
OCS use	0 (0 %)	6 (10.17%)
ICS use	0 (0%)	40 (67.79%)

E1b.

Characteristics of CRS cohorts		
	Low Eosinophils (n = 20)	**High Eosinophils (n = 20)**
Age (year)		
Mean (range)	48.15 (19-83)	46 (21-67)
Gender		
Male	13 (65%)	9 (45%)
Female	7 (35%)	11 (55%)
Presence of Polyp	5 (25%)	5 (25%)
*OCS use	4 (21.05%)	6 (33.33%)
*ICS use	12 (63.15%)	16 (88.88%)
*Co-existence of Asthma	2 (10.52%)	6 (33.33%)

*Data available for n=19 for low eosinophils and n=18 for high eosinophils

Table I: Clinical correlations of miR-1 levels in the Asthma patients	
Variable	n=70
Age	P = 0.4158, r= -0.0988
Gender	P = 0.351
Atopy	P = 0.4711
Hospitalization for asthma (lifetime)	P = 0.0047, r = -0.3339
Hospitalization for asthma (past year)	P = 0.0163, r = -0.2862
ACT score	P = 0.0158, r = 0.2875
*FEV1 % predicted	P = 0.0007, r = 0.3996
*FVC % predicted	P = 0.0008, r = 0.3961
OCS use	P = 0.2082, r = -0.1523
ICS use	P = 0.0039, r = -0.3409
**Sputum cytology	
Eosinophils	P = 0.0132, r = -0.3037
Neutrophils	P = 0.0778, r = -0.2187
Macrophages	P = 0.8875, r = -0.01774
Lymphocytes	P = 0.9132, r = 0.01368

*Data available for n=68, **Data available for n=66.
ACT score- asthma control test score, FEV1- forced expiratory volume in the first second, FVC- forced vital capacity, OCS- oral corticosteroid, ICS- Inhaled corticosteroid
r : Spearman correlation coefficient. P : p-value for the comparison

SUPPLEMENTARY FIGURE LEGENDS

(Supplemental figure E1): a) Association of miR-1 and eosinophils in CRS tissues. Eosinophils were counted after H&E staining. (n=40, r = -5784, P<0.00001 Spearman). Representative images of CRS tissues after H&E (b) and immunohistochemistry with anti-EPX antibody (c) (400X magnification).

(Supplemental figure E2): Lung tissue samples from one subject was fractionated by magnetic immune sorting into immune (CD45+), epithelial (CD326+, CD45-); and endothelial (CD31+, CD45-) fractions after being cultured and treated with recombinant IL-13 (10ng/ml) or PBS (control). MiR-1/18s levels were measured, normalized to its respective control, and presented as $2^{-\Delta\Delta Ct}$ (n=1).

(Supplemental figure E3) Wild type C57BL/6 mice were sensitized with OVA and received intranasal v-miR-1 or v-ctrl vector 2 weeks before OVA aerosol challenge. (a) Cytokines were measured in the BAL of these mice using Biorad Luminex method. (n=8, *P=0.0328). **(b)** mRNA measured in RNA isolated from lungs of V-miR-1 or V-ctrl received mice after OVA challenge (n=4, P= 0.33, 0.34, 0.32, 0.42). Data were assessed by Student's unpaired t-test

(Supplemental figure E4) MiR-1 transgenic (miR-1 tg) and wildtype mice were sensitized with OVA and then received booster after a week. These mice were put on doxycycline to induce vascular-specific miR-1 transgene in transgenic, 14 days after sensitization and then after a week, challenged for 3 days. **(a)** Cytokines were measured in the BAL of these mice using Biorad Luminex. (n=5 or 7, P=ns). **(b)** mRNA measured in RNA isolated from lungs of WT or miR-1 TG mice after OVA challenge. (n= 5 WT and 10 miR-1 TG, P= 0.35, 0.44, 0.41, 0.072). Data were assessed by Student's unpaired t-test.

(Supplemental figure E5) MiR-1 tg and wildtype mice were sensitized intranasally with 1 μg HDM (Der P1) or PBS on days 1. Mice were challenged intranasally with 2 μg HDM (Der P1) on days 10, 11, 12 and 14. These mice were put on doxycycline to induce vascular-specific miR-1 transgene on day 9 until the sacrifice day 15. (a) Cytokines were measured in the BAL of these mice using MSD multiplex. (n=4 or 12, P=ns). **(b)** mRNA measured in RNA isolated from lungs of WT or miR-1 TG mice after HDM challenge. (n= 7 WT and 16 miR-1 TG, P= 0.88, 0.7, 0.68, 0.7) Data were assessed by Student's unpaired t-test

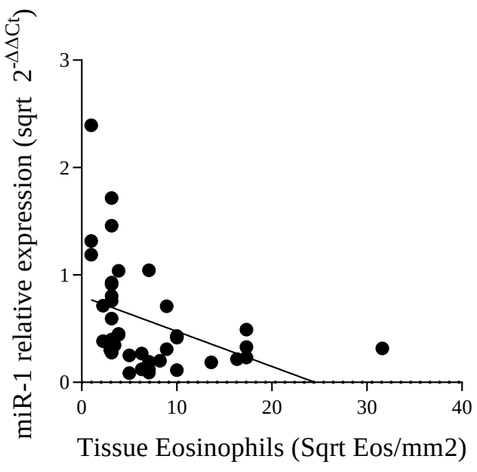
Supplemental figure E6: The effect of miR-1 on eosinophil-endothelial interaction. HUVECs were transduced with V-miR-1 or V-ctrl and then exposed to eosinophils freshly isolated by negative selection method from healthy human subjects. Eosinophil adhesion assay in Sykes-Moore adhesion chamber. The

percentages of eosinophils bound to the HUVEC surface were plotted for each group (n=11, data from 2 experiments. *p=0.0305, Mann-Whitney test) Error bars represent SEM.

(Supplemental figure E7): HUVECs were starved for 3 hrs and then treated with increasing doses of human recombinant IL13 for 3 hr. P-selectin expression on cell surface was measured by flow cytometry. Left panel is showing histogram for SELP levels at the surface of HUVECs after stimulation with various concentrations of human recombinant IL-13. Percentage of SELP positive cell count was plotted in the right panel (n=3 *P<0.01 and **P<0.001).

(Supplemental figure E8): mRNA expression levels for T2 genes in the high- and low-eosinophil CRS samples described in figure 1a. **(a)** POSTN (Periostin): n= 14, and 15; P=0.0769 (**b)** CSF2: n=13, and 15; P=0.2539 **(c)** IL-6: n=18, and 17; P=0.3865 **(d)** IL-4: n=20, and 20; P=0.3547 **(e)** IL-5: n=20, and 20; P=0.3273 and **(f)** IL-13: n= 20, and 18; P=0.2637. In each graph, mean and SEM are represented as red line and error bars respectively. N depicts the number of samples in each group (low- and high-eosinophil) for which data was available. P depicts p-values assessed by Mann-Whitney test. POSTN, Periostin; CSF2, Colony Stimulating Factor 2; IL-6, Interleukin 6; IL-4, Interleukin 4; IL-5, Interleukin 5; IL-13, Interleukin 13

a



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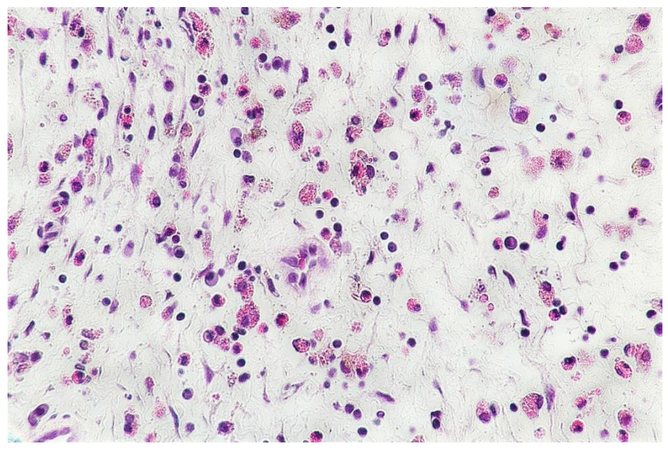
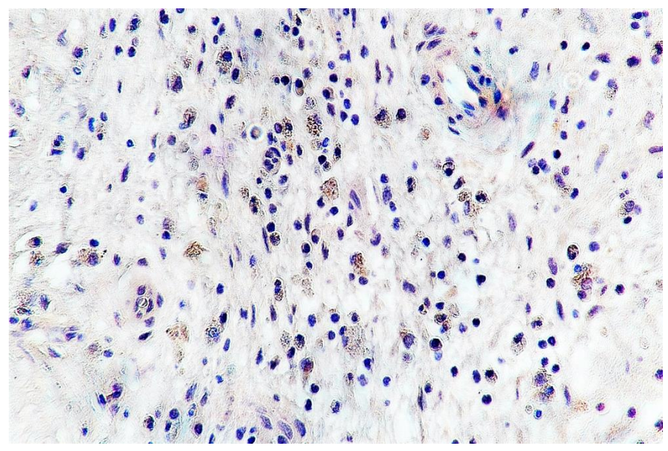


H&E

c



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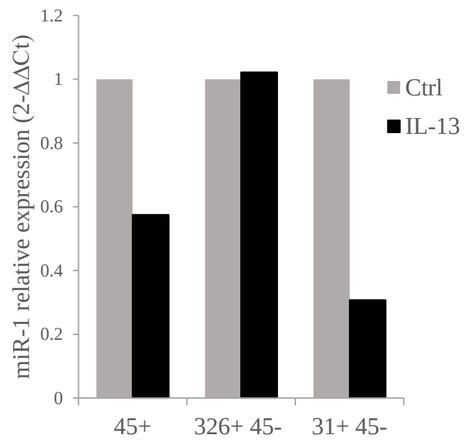
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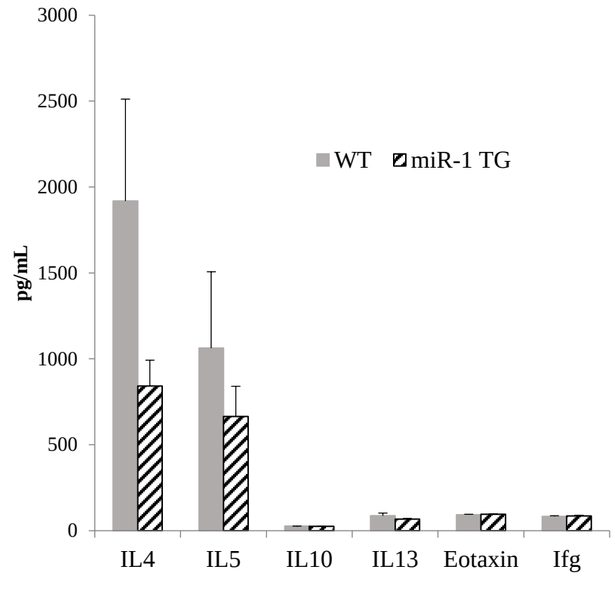
0.8
0.6
0.4
0.2
0
miR-1 relative expression (2-ΔΔCt)
Ctrl
IL-13
45+
326+ 45-
31+ 45-

a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2500
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1500
1000
500
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pg/mL
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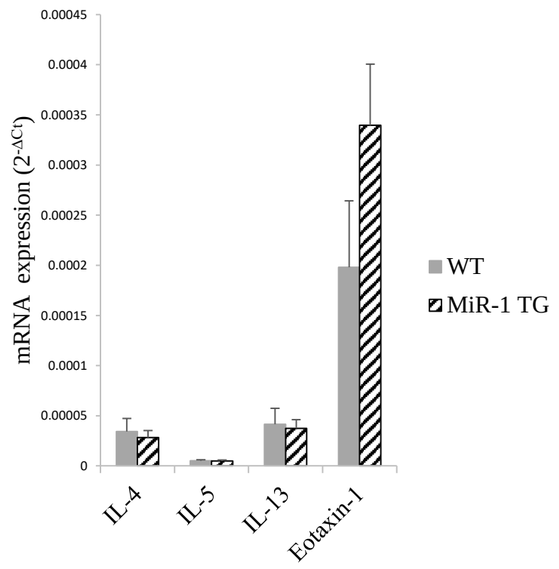
IL4
IL5
IL10
IL13
Eotaxin
Ifg

b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0.00035
0.0003
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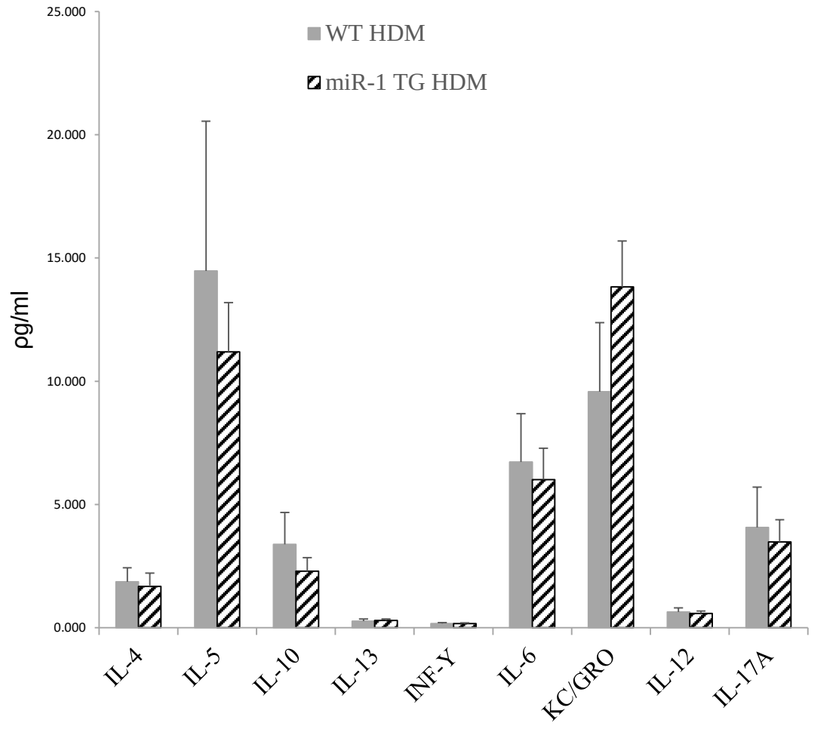
WT
MiR-1 TG
IL-4
IL-5
IL-13
Eotaxin-1

a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20.000
15.000
10.000
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pg/ml
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miR-1 TG HDM
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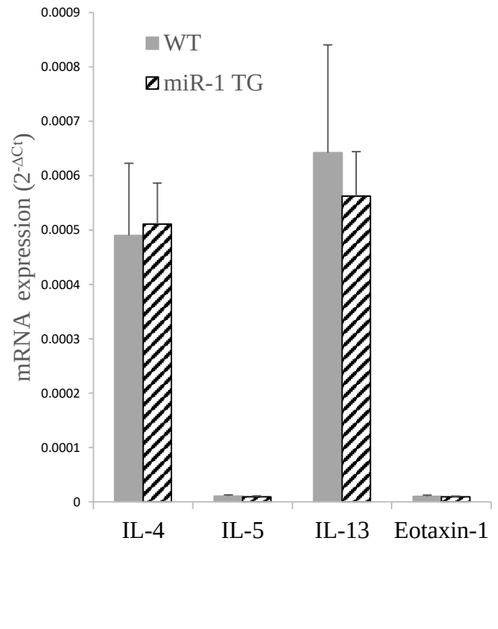
KC/GRO
IL-12
IL-17A

b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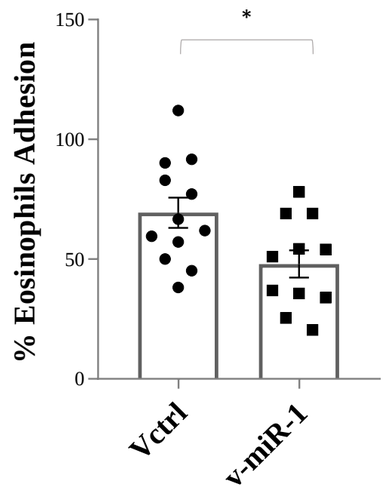
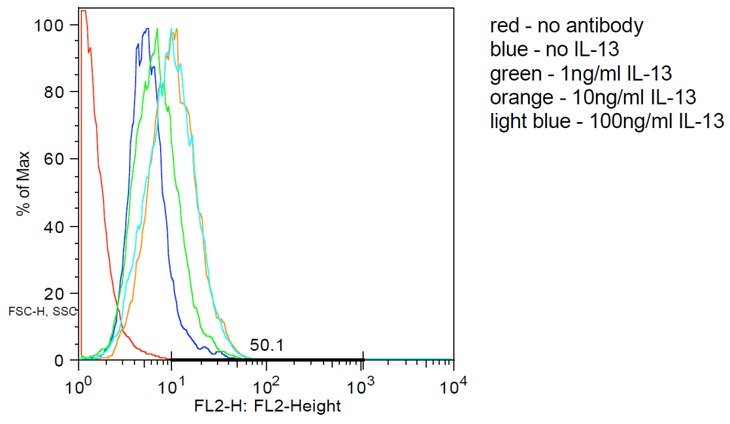
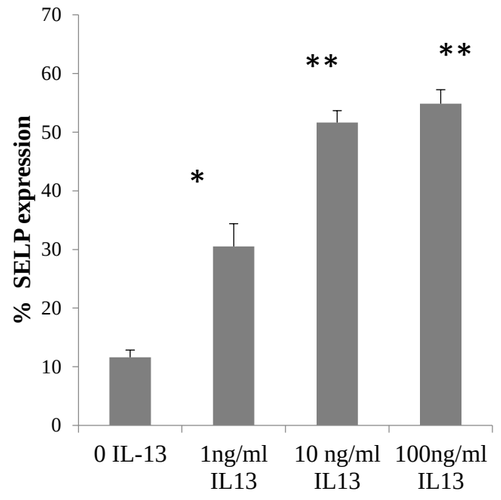
miR-1 TG
IL-4
IL-5
IL-13
Eotaxin-1

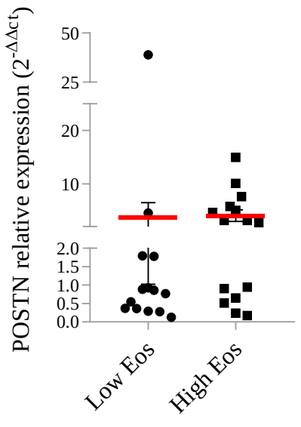

% Eosinophils Adhesion
150
100
50
0
*
Vctrl
v-miR-1



red - no antibody
blue - no IL-13
green - 1ng/ml IL-13
orange - 10ng/ml IL-13
light blue - 100ng/ml IL-13



a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25
20
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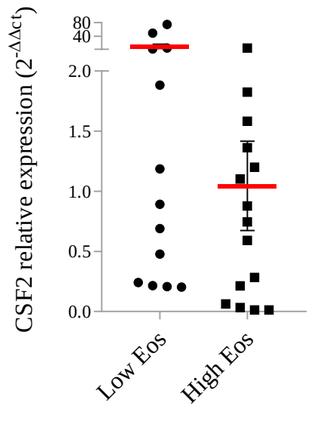
1.5
1.0
0.5
0.0
Low Eos
High Eos

b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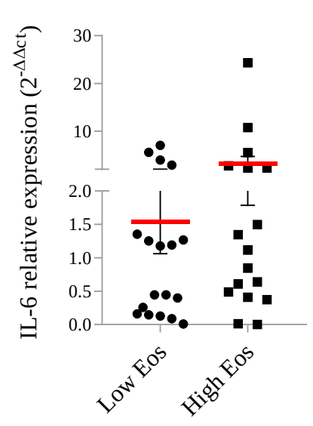
Low Eos
High Eos

c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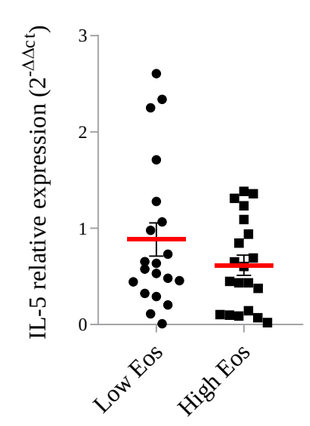
0.0
Low Eos
High Eos

d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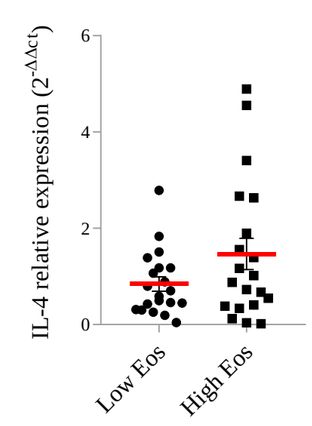
0
Low Eos
High Eos

e


IL-4 relative expression (2-ΔΔct)
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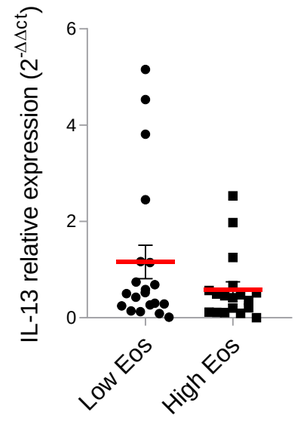
4
2
0
Low Eos
High Eos

f


IL-13 relative expression (2-ΔΔct)
6
4
2
0
Low Eos
High Eos