# Interleukin-1/inhibitory kappa B kinase epsilon-mediated increases in glycolysis augment epithelial effector function and promote house dust mite-induced allergic airways disease

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**List of abbreviations:**

NECs: Nasal epithelial cells

MTE cells: Mouse tracheal epithelial cells

WT: Wild-type

Rn: Newtonian resistance

G: Tissue resistance

H: Elastance

BAL: Bronchoalveolar lavage

ELISA: Enzyme-linked immunosorbent assay

qRT-PCR: Quantitative reverse transcription-polymerase chain reaction

ECAR: Extracellular acidification rate

OCR: Oxygen consumption rate

BSA: Bovine serum albumin

MAC: Macrophages

NEU: Neutrophils

EOS: Eosinophils

LYM: Lymphocytes

KC: Chemokine (C-X-C motif) ligand 1

CCL20: Chemokine (C-C motif) ligand 20;

TSLP: Thymic stromal lymphopoietin

GM-CSF: Granulocyte-macrophage colony-stimulating factor

Mct4: Monocarboxylate transporter 4

Pfkl: Phosphofructokinase, live type

Hk2: Hexokinase 2

Glut: Glucose transporter

Pkm2: Pyruvate kinase isoenzyme type M2

Ldha: Lactate dehydrogenase A

Pgm1: Phosphoglucomutase 1

Pdk1: Pyruvate dehydrogenase kinase 1

Tpi1: Triosephosphate isomerase 1

Eno1: Enolase 1

Pgk1: Phosphoglycerate kinase 1

Gpi: Glucose-6-phosphate isomerase

Pfkfb3: 6-Phosphofructo-2-Kinase/Fructose-2,6-Biphosphatase 3

Mct1: Monocarboxylate transporter 1

## Supplemental Materials and Methods

### Sputum induction

Sputum was induced and processed, as described previously (1-3). Prior to sputum induction, subjects inhaled 400 µg salbutamol using a metered-dose inhaler (+spacer). Sputum was induced using an ultrasonic nebulizer (ultra-Neb 2000, Devilbiss; output set at 0.9 ml/min). Subjects inhaled hypertonic saline (NaCl 5%) when FEV1 post salbutamol was ≥ 65% predicted and isotonic saline (NaCl 0.9%) when FEV1 was <65% predicted. The aerosol was inhaled for three consecutive periods of 5 min. FEV1 was monitored every 5 minutes for safety reasons, and when FEV1 dropped to 80% of the post-bronchodilator values, the induction procedure was stopped. The whole sputum was weighted and three volumes of PBS were added. After homogenizing by manual agitation for 30 sec and centrifugation (800 g) for 10 min at 4℃, the cell pellet and supernatant were separated. Cells were treated with Sputolysin® 0.1% (Calbiochem, Germany), washed with PBS and resuspended in 1 ml. Total cell counts, % squamous cells and cell viability (trypan blue staining) were determined with a manual hemocytometer. Sputum cell differentials were determined by counting 500 cells non squamous cells on Cytospin samples that were stained with RAPI-DIFF II stain (Atom Scientific, Manchester, United Kingdom).

### Cell culture and treatments

Primary human nasal epithelial cells (NECs) were isolated from 6 healthy volunteers and 6 patients with allergic rhinitis and asthma by gentle stroking of the inferior turbinate surface with a Rhino-Probe curette and cultured as recently described (4) in bronchial epithelial cell growth medium (Lonza). Atopy was confirmed by positive skin tests and elevated serum IgE (>100 IU/ml), and asthma was diagnosed by physicians, confirmed by positive response to bronchodilator (≥200 cc and 12% improvement in FEV1 and/or FVC) or a positive methacholine challenge test (PC20 < 8 mg/ml), and had rhinitis with a sinonasal questionnaire (SNQ) score (5) of greater than 1. Healthy volunteers had no history of rhinitis

or asthma, negative skin tests, negative methacholine challenge tests, and a SNQ score of less than 1. For experiments, NECs were plated on collagen-coated 12-well plates at a density of $2 \times 10^5$ cells/well and cultured in a 1:1 mixture of bronchial epithelial cell basic medium and DMEM-H with SingleQuot supplements (Cambrex), bovine pituitary extract (13 mg/ml), bovine serum albumin (1.5 µg/ml), and nystatin (20 units). Following 2 h starvation in basal medium, NECs were treated with 50ug/ml of HDM D. pteronyssinus; GREER, Lenoir, NC; 144.9 endotoxin units/mg protein, lot 290903) for 24 h. Primary mouse tracheal epithelial (MTE) cells were isolated from wild-type (WT) C57BL/6 mice or C57BL/6 mice lacking the Inhibitor of κB kinase ε gene (referred to herein as *Ikbke*$^{-/-}$) and cultured as previously described (6, 7). After reaching confluence, MTE cells were incubated for 16 h in serum-free medium. Cells were stimulated with IL-1α (10 ng/mL), IL-1β (10 ng/mL), IL-6 (10 ng/mL), IL-13 (5 ng/mL), IL-33 (5 ng/mL), TGF-β1 (5 ng/mL), TNFα (5 ng/mL), IL-17 (20 ng/mL), lipopolysaccharide (LPS, 1 µg/mL), or HDM (10 µg/mL or 50 µg/mL as indicated in the Figure Legend) for 2 or 24 hr.

To address the importance of glycolysis or IKKε/TBK1 in IL-1β induced pro-inflammatory responses in MTE cells, cells were pre-treated with the hexokinase inhibitor, 2-Deoxyglucose (2-DG, 10 mM) for 1 h prior to exposure to IL-1β for 24 h. Alternatively, cells were incubated with the lactate dehydrogenase A inhibitor, oxamate (10 mM), or the IKKε/TBK1 inhibitor, Amlexanox (10-100 µM, Tocris) overnight, followed by the stimulation IL-1β (10 ng/mL) for 24 h. To determine whether IL-1β-induced glycolysis or IKKε/TBK1 augmented the subsequent response to HDM, in select experiments, cells were washed post inhibitor/IL-1β treatment, incubated with DMEM/F12 medium for 2 h before stimulation with HDM for an additional 2 hours according the schematic illustrations shown in the relevant figures. To address the role of glycolysis in IL-1α-dependent pro-inflammatory responses, MTE cells were pre-incubated with 10 mM 2-DG for 1 h or 10 mM oxamate for 16 h prior to exposure to IL-1α for 24 h and subsequent assessment of pro-inflammatory mediators in

supernatants. Note that absolute values of KC vary between studies due to freezing of some supernatants.

**Mouse studies**

Age-matched, 8- to 12-week-old mice were used (The Jackson Laboratory, Bar Harbor, ME) for all experiments. Wild-type (WT, C57BL6/NJ), *Rag*$^{-/-}$ (C57BL6/J), or *Ikbke*$^{-/-}$ (C57BL6/J) mice along with their strain-matched controls were sensitized (Days 1 and 8), challenged (Days 15-19), and rechallenged (Days 29, 32, 36, and 39) with HDM extract containing 10 µg protein (GREER, Lenoir, NC; 144.9 endotoxin units/mg protein, lot 290903) as shown in Figure 1A. Mice were euthanized and analyzed at different end points at Days 1 (2 h post HDM sensitization), 2 (24 h post HDM sensitization), 20 (24 h post the last challenge of 5 consecutive HDM challenge), 29 (2 h post the first HDM re-challenge), 30 (24 h post the first HDM rechallenge), and/or 40 (24 h post the last HDM rechallenge). The control group was subjected to saline as a vehicle control. In the *Ldha* siRNA knockdown studies, WT C57BL6/NJ mice were anesthetized with isofluorane and subjected to 10 mg/kg of siRNA targeting *Ldha* or scrambled small interfering siRNA oropharyngeally on days 26, 30, 33, and 37 post-initiation of the HDM exposure regimen, and mice were harvested in day 40 (24h post the last HDM re-challenge). In the IL-1 neutralization experiments, WT C57BL6/J mice were challenged with HDM on days 15, 16, 17, and 18, and analyzed on day 20. Mice received 5 mg/kg of IL-1 Trap (Regeneron Pharmaceuticals, Tarrytown, NY) on Day 14 and Day 17 by i.p. injections, based upon a previous study demonstrating that this dosing regimen of IL-1 Trap attenuated cardiac remodeling after experimental acute myocardial infarction in mice (8). In select experiments, 1 µg of IL-1β (R&D Systems, resuspended in 0.1% BSA in PBS) was directly administered intranasally.

**Assessment of airway hyperresponsiveness**

Following completion of the HDM protocol, mice were anesthetized with intraperitoneal pentobarbital sodium (90 mg/kg), tracheotomized, and mechanically ventilated at 200 breaths/min. Mice were subjected to increasing doses of methacholine (0, 12.5, 25, 50, and 100 mg/mL) administered via ultrasonic nebulization, and respiratory

mechanics were assessed using a forced oscillation technique on a computer-controlled small animal ventilator (SCIREQ, QC, Canada), as previously described (9, 10). Parameters of Newtonian resistance (Rn), tissue resistance (G) and elastance (H) were calculated and quantified by averaging the three highest measurements obtained at each incremental methacholine dose for each mouse (9, 10).

**Assessment of mucus metaplasia:** Airway mucus was stained via Periodic acid Schiff (PAS) and the staining intensity was evaluated by scoring of slides by two independent blinded investigators(11). Levels of MUC5AC were evaluated in lung tissue or BAL via ELISA (My Biosource).

**Bronchoalveolar lavage fluid processing**

After mice were euthanized, bronchoalveolar lavage (BAL) was performed using 1 ml PBS. BAL was collected and total cell counts were determined using an Advia 120 Automated Hematology Analyzer. BAL was spun down at 1200xg for 5 min. Cells were transferred to slides using a cytospin, fixed in methanol and stained using the Hema3 kit (Fisher Scientific, Kalamazoo, MI) and analyzed by counting a minimum of 300 cells per mouse, as described elsewhere (11). Supernatants were flash frozen in liquid nitrogen and stored at -80 ℃ until analysis.

**Enzyme-Linked Immunosorbent Assay (ELISA)**

IL-1α, IL-1β, IL-6, TNFα, IL-17, GM-CSF, and CCL20 were detected by ELISA in lung homogenates (normalized for protein) or supernatants from cell culture, according to the manufacturer's instructions (R&D Systems, Minneapolis, MN).

**Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)**

RNA was extracted using miRNeasy columns (Qiagen, Valencia, CA) as directed by the manufacturer. One µg of RNA was reverse transcribed to cDNA for gene analysis using SYBR Green (Bio-Rad; Hercules, CA, USA) to assess expression of *Mct4*, monocarboxylate transporter 4; *Pfkl*, phosphofructokinase, live type; *Hk2*, Hexokinase 2; *Glut1*, glucose transporter 1; *Glut3*, glucose transporter 3; *Pkm2*, pyruvate kinase isoenzyme type M2; *Ldha*, lactate dehydrogenase A; *Pgm1*, phosphoglucomutase 1; *Pdk1*, pyruvate

dehydrogenase kinase 1; *Tpi1*, triosephosphate isomerase 1; *Eno1*, enolase 1; *Pgk1*, phosphoglycerate kinase 1; *Gpi*, glucose-6-phosphate isomerase; *Pfkfb3*, 6-Phosphofructo-2-Kinase/Fructose-2,6-Biphosphatase 3; *Mct1*, monocarboxylate transporter 1; *Glut2*, glucose transporter 2; and *Ikbke*, inhibitory kappa B kinase ε. Expression values were normalized to the house keeping gene cyclophilin. Detailed primer sequences are provided in the online supplement, Table E3.

**Bioenergetics**

The extracellular acidification rate (ECAR) was measured using the Seahorse Extracellular Flux (XF24) Analyzer (Agilent Technologies). MTE cells were seeded onto 24-well seahorse plate at a density of 50,000 cells per well and cultured with or without 10 ng/mL IL-1β for 24 h. Cells were then washed 3 times with Seahorse stress test glycolysis assay media (DMEM without glucose, L-glutamine, phenol red, sodium pyruvate, and sodium bicarbonate [Sigma-Aldrich] supplemented with 1.85 g/l sodium chloride, 2mM L-glutamine, and 3 mg/l phenol red [GlycoStress Assay], pH 7.35). The plate was incubated in a 37℃ non-$CO_2$ incubator for 1 h. The plate was then transferred to the Seahorse XF24 Analyzer for analysis and subjected to ECAR measurements followed by successive treatments with glucose (10 mM), oligomycin (0.25 μM), and 2-deoxyglucose (100 mM).

**Glucose measurements**

Glucose consumption and uptake in MTE cells were measured 24 h post stimulation with IL-1β, by measuring glucose concentration in the cell culture supernatants (Eton Bioscience) and cellular incorporation of fluorescent glucose analog [2-NBDG, 2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose, Life technology] using a plate reader (Biotek, Winooski, VT).

**Immunohistochemistry**

Fixed sections were prepared for immunostaining by deparaffinizing with xylene and rehydrating through a series of ethanols. For antigen retrieval, slides were heated for 20 min in 95℃ citrate buffer (pH 6.0), then rinsed in dis tilled water. Sections were then blocked for 1 h in blocking serum as per manufacturer's instructions (Vectastain Alkaline Phosphatase

Universal, Vector). Slides were then washed in TBS with 0.1% TWEEN-20 3×5 min, followed by incubation with primary antibody for lactate dehydrogenase A overnight at 4℃. Sections were washed again and incubated with a biotinylated universal secondary antibody (Vectastain Alkaline Phosphatase Universal, Vector) for 30 min at room temperature. Slides were washed and incubated with the Vectastain ABC-AP reagent (prepared as per manufacturer's instructions) for 30 min at room temperature. Sections were then incubated with Vector Red/Vector Blue Alkaline Phosphatase Substrate Kit I (Vector) for 10 min at room temperature, rinsed with tap water, and counterstained with Mayer's Hemotoxylin.

**Immunofluorescence**

Following euthanization, left lobes were fixed with 4% paraformaldehyde, stored at 4℃ overnight for fixation of the tissue, mounted in paraffin, and 5 µm sections were affixed to glass microscope slides for histopathology as previously described (12). For antigen retrieval, slides were heated for 20 min in 95℃ citrate buffer (pH 6.0) with 0.05% TWEEN-20 then rinsed in distilled water. Sections were then blocked for 1 h in 1% bovine serum albumin (BSA) in PBS, followed by incubation with primary antibody for IKBKE (Cell Signaling Technology, Danvers, MA) at 1:100, overnight at 4℃. Slides were then washed 3x5min in PBS, incubated with Alexafluor 647, and counterstained with DAPI in PBS for nuclear localization. Sections were imaged using a Zeiss 510-META confocal laser scanning microscope.

**Lactate assay**

The concentration of lactate in the medium, BAL, and lung homogenates was assessed with a Lactate Assay Kit (Eton Bioscience) according to each manufacturer's recommendations.

## Figure legend

**Figure E1:** Airway inflammation in mice exposed to house dust mite (HDM). (**A**) Total and (**B**) differential cell counts in bronchoalveolar lavage (BAL) in response to saline or HDM, (see schematic in **Figure 1A)**. Data are expressed as means (± SEM) (n = 5 mice per group). *P < 0.05 (Student's t test) compared with saline controls.

**Figure E2:** Assessment of levels of IL-1α and IL-1β in lung tissues from mice subjected to vehicle control or IL1 Trap. Mice were challenged with HDM on days 15, 16, 17, and 18, and received 5 mg/kg of IL-1 Trap or vehicle on days 14 and 17 intraperitoneally. Levels of IL-1α and IL-1β in lung tissues homogenates were evaluated on day 20 via ELISA. *P < 0.05 compared to saline control, † P< 0.05 compared to HDM/Veh group (ANOVA).

**Figure E3:** Evaluation of pro-inflammatory mediators and airway inflammation in mice exposed to interleukin (IL)-1β. **A**: Lung tissue levels of KC, TSLP, CCL20 and GM-CSF following intranasal administration of IL-1β or vehicle. Total (**B**) and differential cell counts (**C**) in BAL from the mice at multiple time points post intranasal administration of vehicle or IL-1β. Data are expressed as means (± SEM) (n = 5 mice per group). *P < 0.05 (ANOVA) compared with Vehicle controls.

**Figure E4: A:** Assessment of viability following exposure to MTE cells to oxamate or 2-deoxyglucose. Epithelial cells were exposed to IL-1β in the presence or absence of inhibitors. Cell survival was evaluated via crystal violet staining of cells. Results were expressed as % survival compared to untreated control cultures. **B:** Impact of 2-DG or oxamate on IL1α-mediated increases in lactate and the indicated pro-inflammatory mediators measured 24 hr post exposure to IL-1α. *P < 0.05 (ANOVA) compared to the sham group. †P < 0.05 compared to the IL-1β treated Vehicle group (ANOVA).

**Figure E5: A:** Quantification of Western blots shown in Figure 8A. Data reflects n=3 healthy subjects and n=3 asthmatics. Data were normalized to β-actin and are expressed as fold change from healthy sham controls *P < 0.05 compared to the sham healthy group. †P < 0.05 compared to HDM healthy group (ANOVA). **B:** Sub-analysis of sputum lactate and IL-1β in asthmatics with normal neutrophils (cut off ≤ 61%) or high neutrophils (cut off > 61%),

controlled (ACQ ≤ 1.5) or uncontrolled asthma (ACQ > 1.5). p-values (ANOVA or Wilcoxon rank sum test) are provided in each of the figures.

## References


1. Manise M, Holtappels G, Van Crombruggen K, Schleich F, Bachert C, Louis R. Sputum IgE and cytokines in asthma: relationship with sputum cellular profile. PloS one. 2013;8(3):e58388.
2. Delvaux M, Henket M, Lau L, Kange P, Bartsch P, Djukanovic R, et al. Nebulised salbutamol administered during sputum induction improves bronchoprotection in patients with asthma. Thorax. 2004;59(2):111-5.
3. Maes T, Cobos FA, Schleich F, Sorbello V, Henket M, De Preter K, et al. Asthma inflammatory phenotypes show differential microRNA expression in sputum. Journal of Allergy and Clinical Immunology. 2016;137(5):1433-46.
4. Müller L, Brighton LE, Carson JL, Fischer WA. Culturing of human nasal epithelial cells at the air liquid interface. Journal of visualized experiments: JoVE. 2013(80).
5. Dixon AE, Sugar EA, Zinreich SJ, Slavin RG, Corren J, Naclerio RM, et al. Criteria to screen for chronic sinonasal disease. CHEST Journal. 2009;136(5):1324-32.
6. Wu R, Smith D. Continuous multiplication of rabbit tracheal epithelial cells in a defined, hormone-supplemented medium. In vitro. 1982;18(9):800-12.
7. Alcorn JF, Guala AS, van der Velden J, McElhinney B, Irvin CG, Davis RJ, et al. Jun N-terminal kinase 1 regulates epithelial-to-mesenchymal transition induced by TGF-β1. Journal of cell science. 2008;121(7):1036-45.
8. Van Tassell BW, Varma A, Salloum FN, Das A, Seropian IM, Toldo S, et al. Interleukin-1 trap attenuates cardiac remodeling after experimental acute myocardial infarction in mice. Journal of cardiovascular pharmacology. 2010;55(2):117-22.
9. Tomioka S, Bates JH, Irvin CG. Airway and tissue mechanics in a murine model of asthma: alveolar capsule vs. forced oscillations. Journal of Applied Physiology. 2002;93(1):263-70.
10. Riesenfeld E, Allen GB, Bates JH, Poynter ME, Wu M, Aimiand S, et al. The temporal evolution of airways hyperresponsiveness and inflammation. Journal of allergy & therapy. 2012;1(5):1.
11. Tully JE, Hoffman SM, Lahue KG, Nolin JD, Anathy V, Lundblad LK, et al. Epithelial NF-κB orchestrates house dust mite–induced airway inflammation, hyperresponsiveness, and fibrotic remodeling. The Journal of Immunology. 2013;191(12):5811-21.
12. Anathy V, Aesif SW, Hoffman SM, Bement JL, Guala AS, Lahue KG, et al. Glutaredoxin-1 attenuates S-glutathionylation of the death receptor fas and decreases resolution of Pseudomonas aeruginosa pneumonia. American journal of respiratory and critical care medicine. 2014;189(4):463-74.

**Table E1.** Demographic, functional, and inflammatory characteristics of the study cohort

| | Healthy subjects | Asthmatic patients | *P* value |
|---|---|---|---|
| n | 20 | 94 | |
| Age (yr) | 53 ± 3 | 52 ± 2 | 0.86 |
| BMI (kg/m2) | 24.30 ± 1.05 | 27.28 ± 0.48 | 0.01 |
| Age asthma onset (range) | - | 41 (12-54) | - |
| Gender (M/F) | 10/10 | 43/51 | 0.3 |
| Atopy*, no. (%) | 4 (20) | 42 (45) | 0.03 |
| Positive Dpt*, no. (% atopy) | 3 (75) | 25 (60) | |
| FEV1 % predicted | 101 ± 4.62 | 81.56 ± 2.07 | 0.0001 |
| FEV1/FVC ratio | 77.68 ± 2.51 | 72.41 ± 1.13 | 0.0291 |
| FENO (ppb), median (IQR) | 20 (17.3-26.8) | 17.5 (10-36.25) | 0.13 |
| Eosinophils (%), median (IQR) | 0.2 (0-0.9) | 0.9 (0.2-5.45) | 0.03 |
| **Medication use** | | | |
| Not treated | - | 5 (5) | - |
| ICS/LABA, no. (%) | - | 58 (62) | - |
| eq Beclomethasone µg/ml | - | 1000 (400-2000) | |
| ICS alone, no. (%) | - | 2 (2) | - |
| OCS, no. (%) | - | 3 (3) | - |
| SABA, no. (%) | - | 64 (68) | - |
| SABA only, no. (%) | - | 19 (20) | - |
| LTRA, no. (%) | - | 24 (26) | - |

Data are expressed as means ± SDs, or medians with interquartile range (IQR). P values are based on the student's t test (mean ± SD), the $\chi 2$ test for proportions (sex), the Wilcoxon rank sum test (median [range]), or Poisson regression (atopy). Atopy is defined as positive test results for at least 1 specific IgE to common aeroallergens. FENO: Fraction of exhaled nitric oxide. * Dpt.: Dermatophagoid pteronyssinus. ICS: inhaled corticosteroid. LABA: long acting beta agonist. OCS: oral corticosteroids. SABA: Short acting beta agonist. LTRA: leukotriene receptor agonists. Ethnicity: All subjects are Caucasian except for 2 African subjects.

**Table E2.** Demographic, functional, and inflammatory characteristics of the study cohort enrolled at the University of Vermont Medical Center

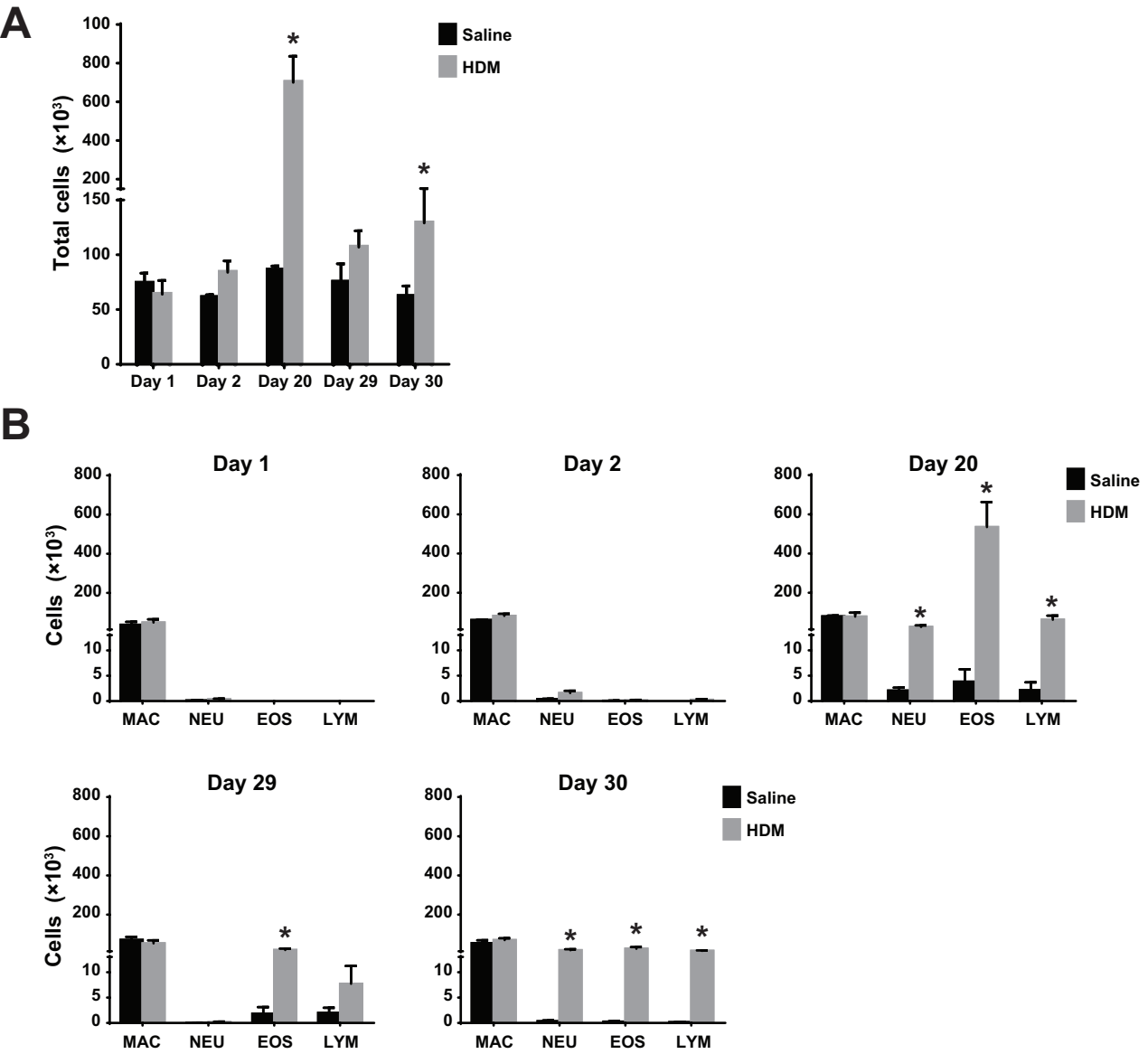
| | Healthy subjects | Asthmatic patients |
|---|---|---|
| n | 7 | 6 |
| Age (range) | 23 (19-27) | 23 (19-45) |
| Gender (M/F) | 0/6 | 3/4 |
| Age asthma onset (range) | - | 4 (1-18) |
| BMI | 23.2 (19.8-26.3) | 28.6 (22.0-35.0) |
| Atopy*, no. (%) | - | 7 (100) |
| **Medication use** | | |
| ICS_LABA | - | 3 |
| ICS | - | 3 |
| SABA only | - | 1 |
| SNQ | 0.4 (0-0.8) | 1.4 (1.2-1.6) |

Data are expressed as median (range). ICS_LABA: inhaled corticosteroid_long acting beta agonist. ICS: inhaled corticosteroid. SABA: short acting beta agonist. SNQ: sinonasal questionnaire. *All participants were allergic to house dust mite as determined either by positive skin prick test to D. pteronyssinus or positive serum IgE to D. pteronyssinus. All subject are Caucasian.

**Table E3. The primers used in this study**

| Genes | Forward | Reverse |
|---|---|---|
| ***Mct4*** | 5′-ATCGTGGGCACTCAGAAGTT-3′ | 5′-CGCCAGGATGAACACATACTT-3′ |
| ***Pfkl*** | 5′-CATATATGTGGGGGCCAAAG-3′ | 5′-GACACACAGGTTGGTGATGC-3′ |
| ***Hk2*** | 5′-GGGACGACGGTACACTCAAT-3′ | 5′-GCCAGTGGTAAGGAGCTCTG-3′ |
| ***Glut1*** | 5′-TCTCTGTCGGCCTCTTTGTT-3′ | 5′-CCAGTTTGGAGAAGCCCATA-3′ |
| ***Glut2*** | 5′-GCCTGTGTATGCAACCATTG-3′ | 5′-GAAGATGGCAGTCATGCTCA-3′ |
| ***Glut3*** | 5′-TGTCACAGGAGAAGCAGGTG-3′ | 5′-GCTCCAATCGTGGCATAGAT-3′ |
| ***Pkm2*** | 5′-CTGCAGGTGAAGGAGAAAGG-3′ | AGATGCAAACACCATGTCCA-3′ |
| ***Ldha*** | 5′-GGAAGGAGGTTCACAAGCAG-3′ | 5′-ACCCGCCTAAGGTTCTTCAT-3′ |
| ***Pgm1*** | 5′-TCAGGCCATTGAGGAAAATC-3′ | 5′-CGAACTTCACCTTGCTCTCC-3′ |
| ***Pdk1*** | 5′-GGCGGCTTTGTGATTTGTAT-3′ | 5′-ACCTGAATCGGGGGATAAAC-3′ |
| ***Tpi1*** | 5′-CCTGGCCTATGAACCTGTGT-3′ | 5′-CAGGTTGCTCCAGTCACAGA-3′ |
| ***Eno1*** | 5′-CTGCCTCCGAGTTCTACAGG-3′ | 5′-CGCTTAGGGTTGGTCACTGT-3′ |
| ***Pgk1*** | 5′-CAAGGCTTTGGAGAGTCCAG-3′ | 5′-TGTGCCAATCTCCATGTTGT-3′ |
| ***Gpi*** | 5′-GTGGTCAGCCATTGGACTTT-3′ | 5′-CTGGAAATAGGCAGCAAAGC-3′ |
| ***Pfkfb3*** | 5′-CAGCTACCAGCCTCTTGACC-3′ | 5′-AACTTCTTGCCTCTGCTGGA-3′ |
| ***Mct1*** | 5′-TCCAGTAATGATCGCTGGTG-3′ | 5′-AGTTGAAAGCAAGCCCAAGA-3′ |
| ***Ikbke*** | 5′-CTGGATGTCCCAAAGTTCGT-3′ | 5′-AGGCTGCTGCTGAGGTAGAG-3′ |

# Figure E1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Day 29
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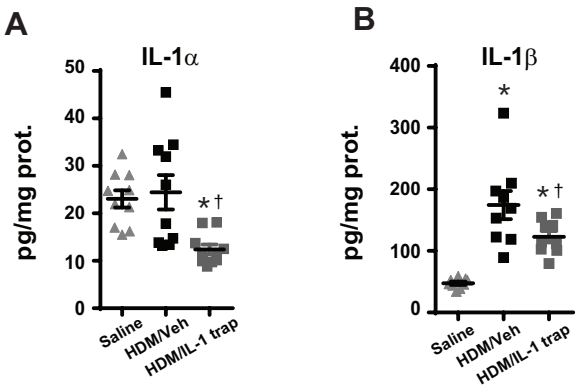
## Figure E2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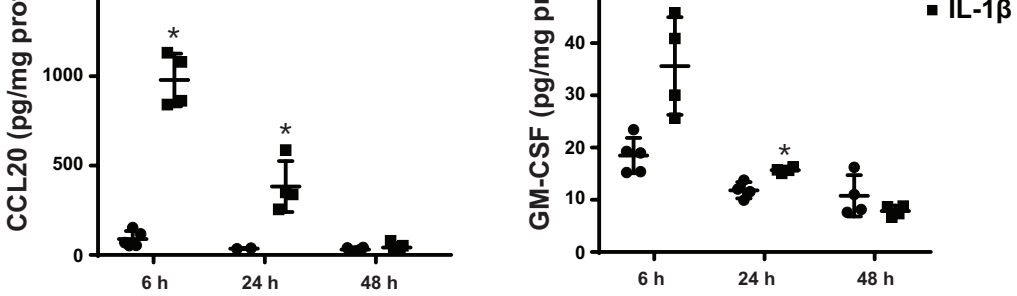
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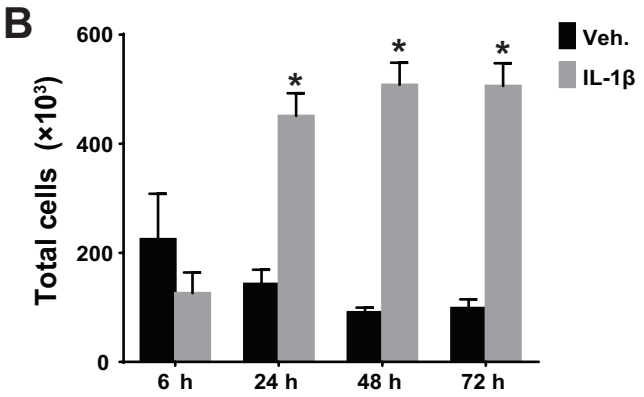
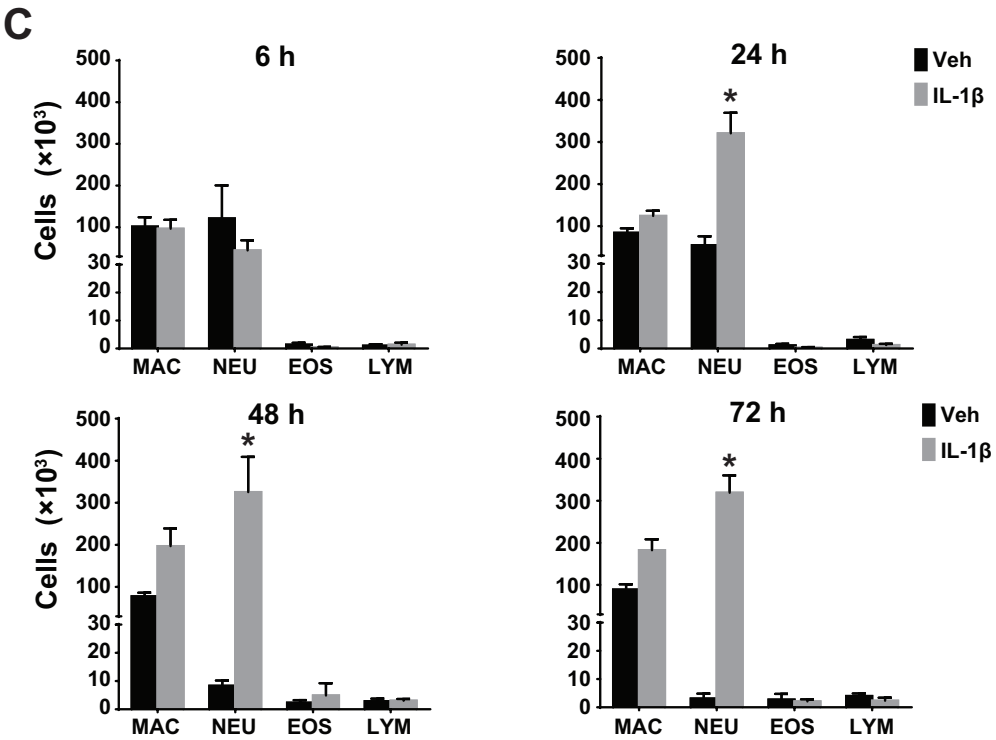
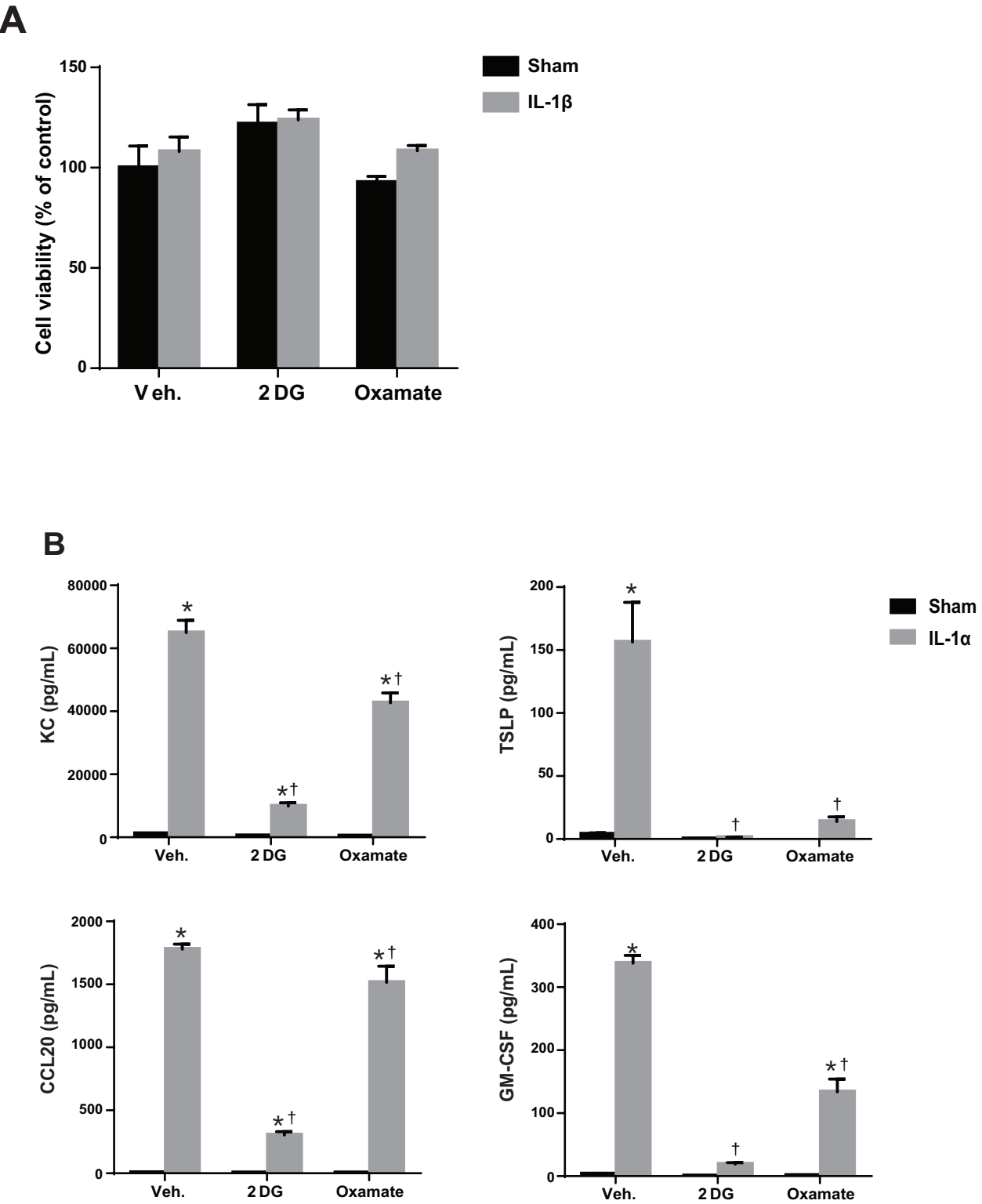
0
IL-1β

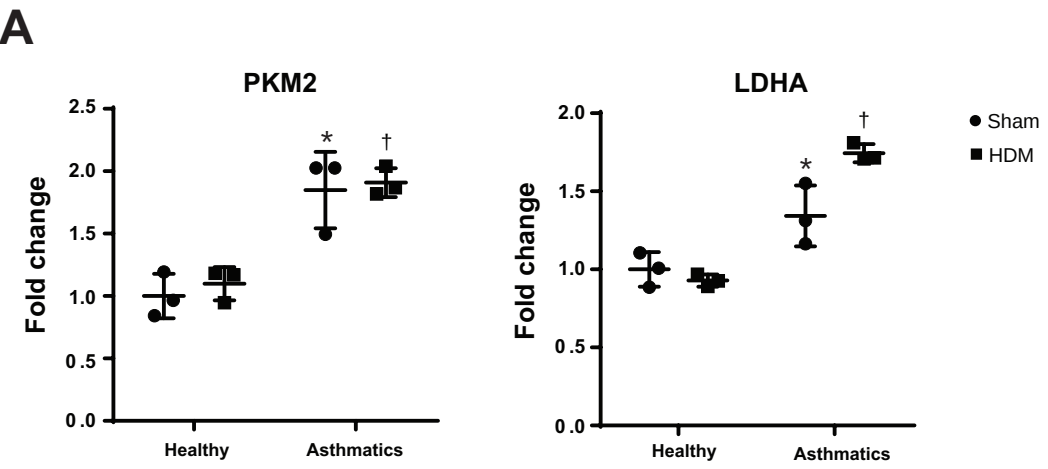

B
Total cells (×10³)
600
400
200
0
6 h
24 h
48 h
72 h
Veh.
IL-1β

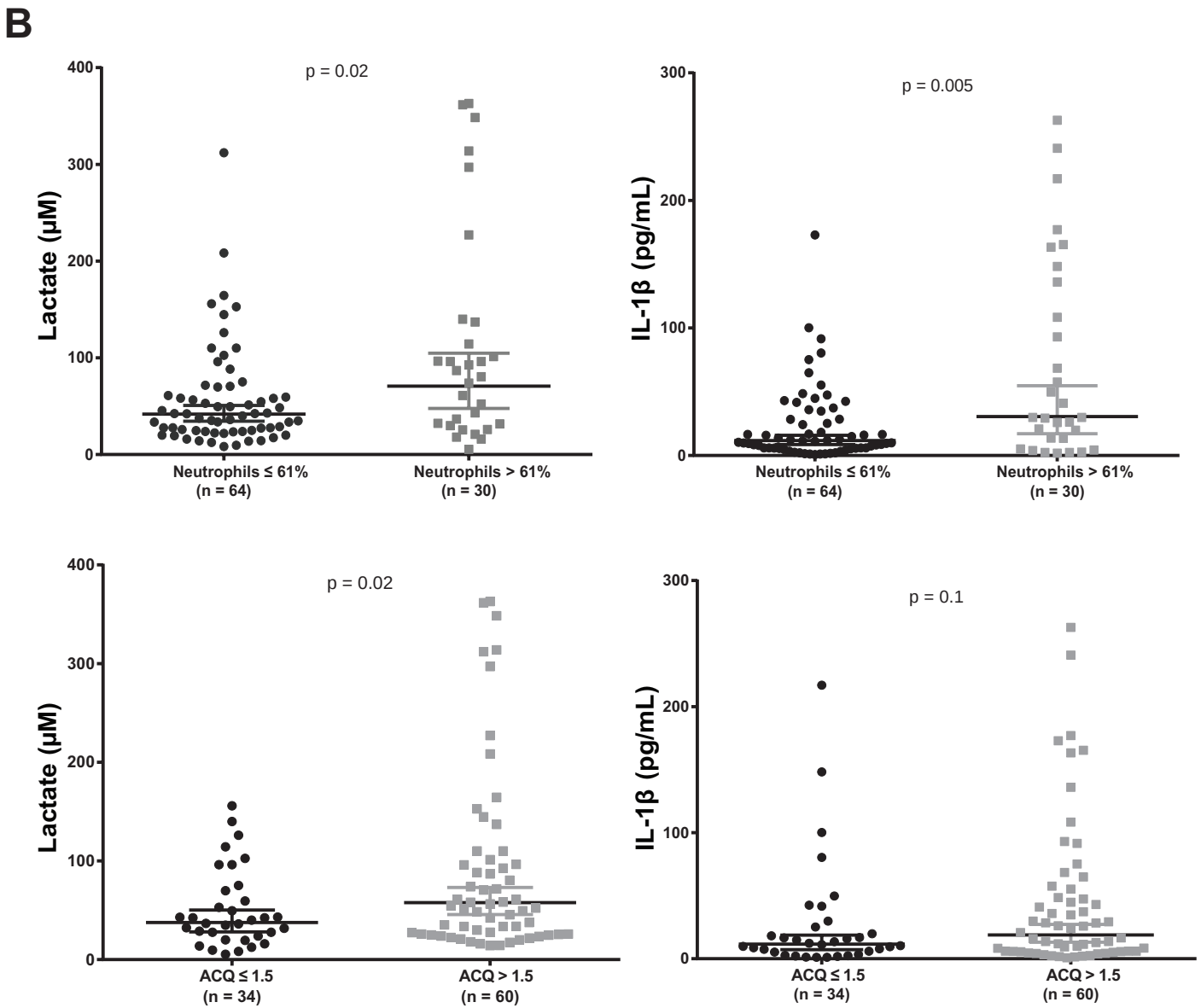

C
6 h
24 h
48 h
72 h
Cells (×10³)
500
400
300
200
100
30
20
10
0
MAC
NEU
EOS
LYM
Veh
IL-1β

## Figure E4


A
Cell viability (% of control)
150
100
50
0
Veh.
2 DG
Oxamate
Sham
IL-1β
B
KC (pg/mL)
80000
60000
40000
20000
0
TSLP (pg/mL)
200
150
100
50
CCL20 (pg/mL)
2000
1500
1000
500
GM-CSF (pg/mL)
400
300
Veh.
2 DG
Oxamate
Sham
IL-1α

## Figure E5


A
PKM2
LDHA
Fold change
2.5
2.0
1.5
1.0
0.5
0.0
*
†
Healthy
Asthmatics
Sham
HDM


B
Lactate (μM)
IL-1β (pg/mL)
p = 0.02
p = 0.005
p = 0.02
p = 0.1
Neutrophils ≤ 61%
(n = 64)
Neutrophils > 61%
(n = 30)
ACQ ≤ 1.5
(n = 34)
ACQ > 1.5
(n = 60)