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IL-37 regulates allergic inflammation by counterbalancing pro-inflammatory IL-1 and IL-33

Short running title: IL-37 inhibits pro-inflammatory IL-1 β and IL-33

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Abstract

Background: Children with asthma have impaired production of interleukin (IL) 37; in mice IL-37 reduces hallmarks of experimental allergic asthma (EAA). However, it remains unclear how IL-37 exerts its inhibitory properties in asthma. This study aimed to identify the mechanism(s) by which IL-37 controls allergic inflammation.

Methods: IL-37 target cells were identified by single-cell RNA-seq of IL-1R5 and IL-1R8. Airway tissues were isolated by laser capture microdissection and examined by microarray-based gene expression analysis. Mononuclear cells (MNC) and airway epithelial cells (AECs) were isolated and stimulated with allergen, IL-1 β or IL-33 together with recombinant human (rh) IL-37. Wild-type, IL-1R1- and IL-33-deficient mice with EAA were treated with rhIL-37. IL-1 β , IL-33, and IL-37 levels were determined in sputum and nasal secretions from adult asthma patients without glucocorticoid-therapy.

Results: IL-37 target cells included AECs, T cells, and dendritic cells. In mice with EAA, rhIL-37 led to differential expression of >90 genes induced by IL-1 β and IL-33. rhIL-37 reduced production of Th2 cytokines in allergen-activated MNCs from wild-type but not from IL-1R1-deficient mice, and inhibited IL-33-induced Th2 cytokine release. Furthermore, rhIL-37 attenuated IL-1 β - and IL-33-induced pro-inflammatory mediator expression in murine AEC cultures. In contrast to wild-type mice, rhIL-37 had no effect on EAA in IL-1R1- or IL-33-deficient mice. We also observed that expression/production ratios of both IL-1 β and IL-33 to IL-37 were dramatically increased in asthma patients compared to healthy controls.

Conclusion: IL-37 down-regulates allergic airway inflammation by counterbalancing the disease-amplifying effects of IL-1 β and IL-33.

Key words: asthma, inflammation, interleukin, asthma treatment

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Introduction

Asthma, one of the most common chronic diseases worldwide,¹ is highly heterogeneous, with a range of endotypes.^{2,3} However, all endotypes are associated with chronic airways inflammation, with most patients experiencing symptoms.^{2,4,5} Mostly, this inflammatory response is orchestrated by T helper (Th) 2 cells and their characteristic array of cytokines that overwhelm the counterbalancing properties of cells like Th1 and/or regulatory T cells (Tregs).⁶⁻⁹ Nevertheless, the mechanisms leading to development of allergic inflammation (and thus of asthma) remain elusive. In an attempt to understand these mechanisms, the role of innate immune functions and the breakdown of the local immuno-homeostasis are being studied. Airway epithelial cells (AECs) and antigen-presenting cells (APCs) produce factors that amplify or dampen acute inflammatory reactions: On the one hand, they augment allergic airway inflammation and thus formation of pathophysiological hallmarks of asthma by releasing pro-inflammatory cytokines such as interleukin (IL) 1 β and IL-6, while epithelium-derived factors such as IL-33 promote Th2 and innate lymphoid tissue cell 2 (ILC2) differentiation that direct allergic immune responses via release of IL-4, IL-5, and IL-13.¹⁰⁻¹³ On the other hand, they maintain local immuno-homeostasis and so prevent tissue damage by the actions of anti-inflammatory factors including IL-10 and IL-37, and an impairment of this immuno-regulatory function could be a central factor in the pathogenesis of chronic inflammatory diseases such as asthma.^{14,15}

Originally described as a fundamental inhibitor of innate immune functions,^{16,17} the member of the IL-1 β cytokine family, IL-37, has been shown to impact adaptive immune responses, with production reduced in children with asthma.¹⁸⁻²⁰ Moreover, local administration of IL-37 not only reduces allergic airway inflammation but ameliorates hallmarks of experimental allergic asthma (EAA) in mice. On a cellular basis, administration of IL-37 reduces the release of Th2-type cytokines as well as of several pro-inflammatory mediators, indicating that IL-37 impacts different cell types involved in asthma pathogenesis.^{18,21-23} The anti-inflammatory properties of IL-37 are mediated by the IL-37 receptor complex composed of IL-1R5 and IL-1R8: Initial binding of monomeric IL-37 to IL-1R5 is followed by recruitment of IL-1R8, which acts as a co-receptor and transduces anti-inflammatory signals by suppressing NF- κ B (nuclear factor κ -light-chain-enhancer of activated B cells) and MAPK (mitogen-activated protein kinase) and by activating the signaling molecules Mer, PTEN (Phosphatase and Tensin homolog), STAT (signal transducer and activator of transcription proteins) 3 and dok (downstream of kinase).^{24,16,17,25} Since impaired production of

IL-37 in patients with asthma could lead to dysregulation of allergic inflammation, clarification of the underlying mechanism potentially identifies new targets for therapeutic intervention. The aim of this study was therefore to elucidate the mechanism(s) by which IL-37 controls allergic inflammation. Our hypothesis is that IL-37 directly inhibits the pro-inflammatory effects of other members of the IL-1 family namely IL-1 β and IL-33.

Material & methods

Analysis of publicly available single-cell RNA-seq (scRNA-seq) data

Overview analysis of publicly available scRNA-seq data from healthy Human individuals^{26,27} and control Mice^{28,29} was performed using Scanpy (v.1.6.0.)³⁰ and Seurat (v.4.0.1)³¹, respectively.

Animals and experimental protocol

Female wild-type (WT) C57BL/6 mice (Charles River Laboratories, Sulzfeld, Germany), IL-1R1,³² and IL-33 deficient,³³ aged 6–8 weeks, were housed under specific pathogen-free conditions, receiving an ovalbumin (OVA)-free diet and water *ad libitum*. All experimental procedures were approved by the animal ethics committee at the MELUND, Kiel, Germany (V312-72241.123-3(83-7/11), V244-28793/2016(44-4/16), V244-6919/2018(19-3/18)). Induction of EAA and administration of IL-37 was performed as described previously.¹⁸ For details, see the online supplement.

Bronchoalveolar lavage (BAL) and differential cell count was performed as previously described.³⁴ Lung specimens were sampled and prepared for immunohistochemistry and quantitative morphology as previously described,³⁵ as were immunohistochemistry and quantitative morphology,³⁶ isolation and stimulation of mononuclear cells (MNCs) and CD4⁺ T cells in cell culture,³⁶ and assessment of cytokine levels.³⁶ Furthermore, laser-capture microdissection,³⁷ whole genome array analysis of RNA samples,³⁸ air-liquid interface (ALI) culture of murine tracheal epithelial cells,³⁶ and reverse transcription and quantitative real-time polymerase chain reaction on murine and human samples were all performed as previously described.^{37,39} For details, see online supplement.

Cross-sectional human study cohort and protocol

Patient characteristics can be found in Supplementary Table 1. Patients with asthma discontinued corticosteroid medication (inhaled and oral) for two weeks before analysis. For this analysis, we examined twelve patients with grass-pollen allergic asthma with hay fever, and 23 age-matched healthy controls during pollen season. All patients reported clinical symptoms to grass pollen, however none of the control subjects. Clinical outcomes were assessed using the GINA (Global

Initiative for Asthma) score,⁴⁰ striving for an optimal individual therapy setting. The study was approved by the ethics commission of the Technical University of Munich (5534/12). After written and informed consent, and in accordance with the Helsinki Declaration, induced sputum and nasal secretions were obtained from patients during the grass pollen season from May to July 2014 and 2015 and analyzed as previously described.^{41–43}

Statistics

If not stated otherwise, results are presented as mean $\pm$ SEM. One-way analysis of variance with subsequent Tukey's test was used to determine significance of differences. Human samples from each group were summarized taking the mean $\pm$ S.D.. Mann-Whitney tests were used to determine significant differences of expression ratios. Statistically significant differences were defined as *P* values **P*<0.05, ***P*<0.01, ****P*<0.001, and *****P*<0.0001.

Results

IL-1R5 and IL-1R8 are expressed by various cells associated with asthma pathogenesis

We have previously shown that topic treatment with rhIL-37 reduces the pathophysiologic features of EAA, including mucus hyperproduction, allergic airway inflammation and release of Th2 type cytokines.²⁵ In order to determine which cells are influenced by IL-37, and to subsequently reveal its mechanism of action on a cellular level, we first analyzed the cellular expression of the two chains forming the IL-37 receptor signaling complex, namely IL-1R5 and IL-1R8. In human cells, we found mRNA expression of both receptor chains in a variety of cells associated with asthma pathogenesis, including CD4⁺ T cells, dendritic cells (DCs), ILCs (Suppl. Fig. 1A, B), and different AECs (Fig. 1A). Despite differences of the expression level, we further confirmed mRNA-levels of both chains for T cells, DCs, ILCs, and AECs (Fig. 1B) in mice and on protein level for AECs and inflammatory cells infiltrating airway tissue under the pathologic conditions of EAA (Fig. 1C), suggesting that these cells potentially respond to IL-37.

IL-37 attenuates Th2 type cytokine production in allergen-restimulated MNCs but not in anti-CD3/CD28 restimulated Th2 cells: role of IL-10

In mouse models of inflammatory diseases, such as contact hypersensitivity and atherosclerosis, the inhibitory effects of IL-37 are associated with recruitment of Tregs and tolerogenic DCs that downregulate inflammation by release of anti-inflammatory IL-10.^{44–46} Therefore, we determined the levels of IL-10 in BALF of mice with EAA. Remarkably, IL-37 treatment did not enhance IL-10 levels in BALF. Accordingly, addition of IL-37 did not increase IL-10 release in OVA-restimulated MNCs from of these animals (Fig. 2A,B) indicating that IL-37 does not control allergic inflammation through induction of IL-10 release in Tregs or DCs. Thus, we investigated the effect of IL-37 on Th2 cell stimulation, since these cells are pivotal for orchestrating allergic inflammation. CD4⁺ T cells isolated from OVA-sensitized animals were restimulated in vitro by anti-CD3/CD28, which resulted in markedly increased production of the Th2 type cytokines IL-4, IL-5, and IL-13. The addition of IL-37 had no significant effect on these cytokine levels (Fig. 2C) indicating that IL-37 does not affect T cell activation via TCR/CD80/CD86. We therefore studied the effect of IL-37 on an allergen-specific restimulation of Th2 cells by stimulating MNCs from

OVA-sensitized animals that include DCs, ILCs, and OVA-specific Th2 cells with OVA.⁴⁷ This led to enhanced production of IL-4, IL-5, and IL-13 (Fig. 2D); IL-37 significantly diminished the release of these cytokines mirroring the effects of IL-37 treatment in animals with EAA. IL-37 induced no effects on either restimulated CD4⁺ T cells or MNCs.

Expression of IL-1 β - and IL-33-pathway members is upregulated in EAA and differentially regulated by IL-37 treatment

To get a further insight into the mechanisms through which IL-37 downregulates allergen-triggered airway inflammation, we compiled gene expression profiles of airway tissues from healthy mice, mice with EAA and mice with EAA treated with IL-37. As expected, in animals with EAA numerous genes associated with inflammatory pathways were upregulated compared to healthy controls, including those triggered by IL-1 β and IL-33 (Suppl. Fig. 3A,C, Suppl. Tables S2, S4), two pro-inflammatory cytokines associated with the pathogenesis of asthma.^{48–51} Importantly, these two pathways were differentially expressed after IL-37 treatment, with several target genes downregulated (Suppl. Fig. 3B,D, Suppl. Tables S3, S5).

IL-37 attenuates Th2 type cytokine production in allergen-restimulated MNCs dependent on IL-1 β and IL-33 signaling

Based on the finding that increased production of IL-1 β in OVA-restimulated MNCs (Suppl. Fig. 4) is not observed in anti-CD3/CD28 restimulated CD4⁺ cells, we hypothesized that IL-37 may exert its regulatory functions on MNCs by interfering with pro-inflammatory IL-1 β - and/or IL-33 signals that augment cytokine release of both, Th2 cells and ILC2s.^{11–13} We therefore repeated OVA-restimulation of MNCs from OVA-sensitized IL-1R1 deficient mice that cannot react to IL-1 β . Again, OVA-restimulation resulted in increased production of IL-4, IL-5, and IL-13, although to a lesser extent than MNCs from WT animals. However, in contrast to WT cells, co-administration of IL-37 did not result in any reduction in Th2 type cytokines (Fig. 3A). The same effect was observed in IL-37-treated WT cells, when IL-1-signaling was inhibited by IL-1Ra: As observed in MNCs from IL-1R1 deficient mice, IL-1Ra administration lowered Th2 type cytokine release. Consistent with the experiments with IL-1R1 deficient cells, co-administration of IL-37 to

IL-1Ra-treated and OVA-stimulated MNCs did not show any further reduction in Th2 type cytokine release (Fig. 3B).

In contrast to IL-1 β , which is produced by APCs, IL-33 is not produced in MNC cultures. However, since IL-33 exerts considerable effects on Th2 cells and ILCs,^{11–13,52,53} we further examined whether IL-37 could also impact this cytokine. Therefore, we added IL-33 to allergen-restimulated MNCs from OVA-sensitized WT animals. We observed enhanced production of IL-5 and IL-13, but not IL-4; not unexpectedly, production of IL-5 and IL-13 was largely prevented by co-administration of IL-37 (Fig. 3C).

IL-37 attenuates IL-1 β - and IL-33-induced expression of pro-inflammatory mediators in AECs

Similar to Th2 cells, AECs not only play a significant role in the pathogenesis of asthma, but are putative target cells for IL-37 (Fig. 1). Thus, we investigated the in vitro effect of IL-37 on IL-1 β or IL-33 stimulation by culturing murine AECs under ALI-conditions, before stimulation with either IL-1 β or IL-33 in the presence or absence of IL-37. First, we examined the effect of IL-1 β stimulation. We observed enhanced expression of pro-inflammatory molecules including cytokines such as *Il6* and *Tnf*, chemokines such as *Kc* and *Mcp1*, *Gmcsf*, as well as the adhesion molecule *Icam1*; each of these have been implicated in the development and perpetuation of allergic asthma.⁵⁴ Co-administration of recombinant IL-37 markedly reduced the level of mRNA coding for these genes on AECs (Fig. 4A).

Stimulation of AECs with IL-33 also resulted in upregulation of *Gmcsf*, *Icam1*, and *Kc*, and importantly, upregulation of these pro-inflammatory molecules was also counterbalanced by co-administration of IL-37 (Figure 4B). IL-37 alone did not affect the expression of the respective genes in AECs.

IL-37 downregulates allergic airway inflammation only in animals with a functional IL-1 β and IL-33 signaling

Next, we investigated the effect of IL-37 on IL-1 β and IL-33 under physiological conditions. WT, IL-1R1 and IL-33 deficient mice with EAA were treated with IL-37. In WT animals, this resulted

in reduced features of the disease, including eosinophil counts in BAL (Fig. 5A), goblet cell (GC) hyperplasia (Fig. 5B), and BAL levels of IL-4 (Fig. 5C) and IL-6 (Fig. 5D). IL-37 treatment did not reduce these features in IL-1R1 deficient animals (Fig. 5A-D). In contrast, in IL-33 deficient animals, IL-37 treatment reduced BAL levels of IL-4 and IL-6, although not reaching statistical significance (Fig. 5C,D). Eosinophil counts in BAL and GC hyperplasia remained largely unaffected (Fig. 5A,B).

Lower and upper airways in asthma display increased expression/production ratios of IL-1 β /IL-37 and IL-33/IL-37

Since our in vitro and in vivo experiments demonstrated that IL-37 limits the pro-inflammatory effects of IL-1 β and IL-33 of cells in the pathogenesis of asthma (e.g., MNCs and AECs), we hypothesized that patients with asthma exhibit an imbalance between anti-inflammatory IL-37 versus pro-inflammatory IL-1 β and IL-33. Since glucocorticoids directly and indirectly inhibit the expression and production of IL-1 β ,^{55–57} IL-33,^{58–60} and IL-37,⁶¹ we determined the mRNA-expression and secreted protein levels of these cytokines in sputum cells of the lower airways and nasal secretions from healthy individuals and patients with allergic asthma (after glucocorticoid therapy had been discontinued) (Fig. 6 and Suppl. Table S1). Asthma patients showed significantly increased expression levels for *Il1beta*, *Il33*, but decreased *Il37* mRNA expression in sputum cells (Fig. 6A-C). Accordingly, patients with asthma displayed significantly higher expression ratios of *Il1beta* and *Il33* to *Il37* (Fig. 6D,E). These differences were also confirmed at the protein level in sputum supernatants (Fig. 6F-J) as well as nasal lining fluids (Fig. 6K-O), suggesting an impaired counterbalance of these pro-inflammatory cytokines by IL-37.

Discussion

This study aimed to elucidate the mechanisms through which IL-37 controls allergic airway inflammation underlying the development of asthma. We identified various cells involved in the pathogenesis of asthma (including Th2 cells, DCs, ILC2s, and AECs) as potential responders to this cytokine and demonstrated that IL-37 limits the pro-inflammatory signals of IL-1 β and IL-33 on a cellular level as well as in an EAA mouse model. Our additional finding that adult patients with asthma had enhanced expression/production ratios of the pro-inflammatory IL-1 β and IL-33 to its anti-inflammatory counterpart IL-37 further supports the concept that impaired IL-37 production and, thus an impaired capacity to counterbalance pro-inflammatory signals in order to maintain the local immuno-homeostasis, is of central importance in asthma pathogenesis.

We have previously shown that the therapeutic effects of local IL-37 administration require IL-1R5 and IL-1R8,²⁵ acting as the receptor for this cytokine.⁶² Expression of these receptor chains was confirmed in a variety of cells involved in asthma pathogenesis in both, humans and mice. The differences in the expression level of the respective receptor chains between both species could be explained by the lack of pro-inflammatory stimuli that impact the expression of IL-1R5 and IL-1R8 in mice housed under specific pathogen-free conditions.⁶³ We hypothesized that co-expression of both receptors indicates putative responders to IL-37 and selected MNCs (including Th2 cells, ILC2s, and DCs) and AECs for further experiments, since they play central roles in asthma pathogenesis: Th2 cells and ILC2s orchestrate allergic inflammatory reactions and enhance mucus production by releasing the Th2 type cytokines IL-4, IL-5, and IL-13.^{64–66} In turn, AECs provide part of the physical barrier of the airways that is repeatedly exposed to allergens and pathogens, which trigger the release of several pro-inflammatory mediators (e.g., TNF- α , IL-6, IL-8/KC) thereby initializing and supporting acute inflammatory reactions.⁶⁷

Since successful treatment of EAA in mice is associated with a reduced inflammatory response in the airways, we first checked for an enhanced IL-10 release (e.g. by Tregs or DCs) as described in other mouse models of inflammatory disease.^{44,45} As we found no increased release of IL-10 in vivo or in vitro, such an effect can be excluded. This rather surprising finding suggests that the short-term effects of IL-37 as described in this study are different from its long-term effects caused by ten weeks of treatment or transgenic overexpression.¹⁸ We therefore further tested whether IL-37 impacts Th2 cell restimulation with anti-CD3/CD28. Unexpectedly, this was not the case, indicating that IL-37 does not interfere directly with the activation of these cells via the

TCR and/or co-stimulation via CD80/86. Subsequently, we mimicked allergen-specific restimulation of MNCs isolated from OVA-sensitized animals with its specific antigen. Co-culture with IL-37 resulted in significant reduction in Th2 type cytokine production, consistent with our previous animal experiments. In contrast to our first experiment, MNC cultures comprised Th2 cells, ILC2s, and APCs, in which IL-37 could affect activation of APCs and ILC2s or interfere with the signals provided by these cells.¹⁶ Thus, we returned to the animal model and created differential gene expression profiles from airway tissues from mice with EAA undergoing IL-37 or sham treatment. Among the several pathways that revealed differential expression upon IL-37 treatment, we observed those induced by IL-1 β and IL-33. Thus, we hypothesized that IL-37 could exert its anti-inflammatory effects by interfering with their signals. We started by delineating the interrelationship of IL-1 β and IL-37, since IL-1 β release was elevated in mice with EAA as well as in allergen-restimulated MNCs. IL-1 β is central for the activation, development and proliferation of Th2 cells and ILC2s.^{11–13,68–74} Accordingly, increased release of IL-1 β is observed in patients with asthma.^{20,75,76} Furthermore, administration of IL-1 β worsens allergic airway inflammation and hallmarks of EAA in mice,⁷⁷ while blocking of IL-1 β signaling leads to the opposite.^{78,79} Our in vitro findings are consistent since allergen-restimulation of MNCs from IL-1R1 deficient mice that cannot respond to IL-1 β displayed markedly reduced release of IL-4, IL-5, and IL-13. This is also the case for OVA-restimulated MNCs from WT animals receiving IL-1Ra. Importantly, in both settings co-application of IL-37 had no further impact on the production of Th2 type cytokines. As the regulatory effect of IL-37 is not observed if the pro-inflammatory effect of IL-1 β is absent, we suggest that IL-37 counterbalances the pro-inflammatory effect of IL-1 β on MNCs. This is supported by both, the in vivo experiment demonstrating that IL-37 has no significant reduction on Th2 type cytokine production, airway eosinophilia, or mucus hyperproduction in IL-1R1 deficient mice, and also by the AEC ALI-cultures. Here, upregulation of pro-inflammatory molecules, which have been implicated in asthma pathogenesis and mouse models of EAA^{80,81}, by IL-1 β could be directly reduced by addition of IL-37. Hence, IL-37 limits the effects of IL-1 β , in terms of both its promotion of Th2 type cytokine production by MNCs and the expression of pro-inflammatory molecules by AECs, ultimately suppressing its amplifying effects on allergic airway inflammation.

Since we also found IL-33 pathway members to be differentially expressed by IL-37 treatment of mice with EAA, we speculated on a counterbalancing effect of IL-37 on this pathway. Alike IL-1,

IL-33 considerably impacts allergic inflammation: it promotes Th2 cell differentiation^{48,82}, activates ILCs and enhances their production of IL-5 and IL-13,^{11–13,49,50} and activates AECs and amplifies their release of pro-inflammatory mediators such as IL-8/KC.⁸³ Accordingly, local application of IL-33 alone to mice induces all the pathophysiologic features of EAA.⁸⁴ We therefore cultured allergen-restimulated MNCs with IL-33, which indeed resulted in markedly enhanced release of IL-5 and IL-13. Similarly, in AEC cultures IL-33 stimulation resulted in upregulation of pro-inflammatory molecules including *gmcsf*, *icam1*, and *kc*. Strikingly, both effects were dampened by presence of IL-37. Furthermore, the effects of IL-37 on Th2 type cytokine levels in BAL, airway eosinophilia, and mucus production were considerably diminished in IL-33 deficient animals indicating an inhibiting effect of IL-37 on proinflammatory IL-33.

Finally, we sought to translate our experimental findings to the clinical situation. We determined the levels of IL-1 β and IL-33 and their counterpart IL-37 in patients with mild allergic asthma who discontinued glucocorticoid treatment. Without the inhibiting effect of glucocorticoids on the release of all these cytokines,^{55–61} patients with asthma had a dramatically enhanced ratio of both IL-1 β to IL-37 and IL-33 to IL-37 as compared to healthy individuals. This predominant release of pro-inflammatory cytokines that is not counterbalanced by comparable IL-37 could be the basis for the persistence of allergic airway inflammation in adult patients. The reasons for this imbalance and for an impaired release of IL-37 in patients with asthma patients remain unclear. However, this finding is in line with a previous study that described loss of anti-inflammatory functions of four different genetic variants of IL-37 in patients with gout, characterized by severe IL-1-mediated joint inflammation.⁸⁵ Together with this our findings highlight the therapeutic potential of recombinant IL-37 for the treatment of inflammatory diseases.

Taken together, we demonstrate that IL-37 exerts its anti-inflammatory action on allergic airway inflammation by counterbalancing the disease-amplifying effects of two pro-inflammatory cytokines that are central to asthma pathogenesis, namely IL-1 β and IL-33. We demonstrated this mechanism in MNCs and AECs, which play pivotal roles in asthma pathogenesis. However, we suggest that the same mechanism also affects other cells that are influenced by IL-1 β and/or IL-33 and IL-37. As we did not find any signs for the induction of IL-10 we suggest that our findings describe a new mechanism by which IL-37 directly controls allergic inflammation. We suggest that over prolonged time this mechanism could contribute to the development of tolerogenic DCs and/or Tregs by persistent abrogation of pro-inflammatory signals, which is in line with the recent

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presentation of IL-37 as an active inhibitor of trained immunity effects.⁸⁶ Together with its previously described balancing effects on the immunometabolism this regulatory effect of IL-37 on allergic inflammation establishes IL-37 as a central factor for the maintenance of the local immunohomeostasis.^{87,88} Consequently, these findings suggest specifically targeting these pro-inflammatory cytokine effects (e.g., by compensating for the impaired IL-37 response or by inhibiting IL-1R3)⁵¹ will be superior to glucocorticoid treatment that also decreases the inherent regulatory effects of IL-37.

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Figure legends

Fig. 1: IL-1R5 and IL-1R8 are expressed by various cells associated with asthma pathogenesis

(A) Gene expression of IL-1R5 and IL-1R8 in different cell types in the human lung assessed by single cell RNA-seq. (B) Gene expression of IL-1R5 and IL-1R8 in different cell types in the murine lung assessed by single cell RNA-seq. (C) Immunohistochemically-stained lung cross-sections of EAA mice with α -IL-1R5 and α -IL-1R8 antibodies. An isotype matched IgG was used as negative control instead of the primary antibody. Scale bar, 20 μ m. IL-1R5: interleukin-1 receptor 5, IL-1R8: interleukin-1 receptor 8, AT1: alveolar epithelial type 1 cells, AT2: alveolar epithelial type 2 cells, AEC: airway epithelial cells, ILC: innate lymphoid tissue cells.

Fig. 2: IL-37 attenuates Th2 type cytokine production in allergen-restimulated MNCs but not in anti-CD3/CD28 restimulated Th2 cells: role of IL-10

(A) Concentration of IL-10 in BALF. (B) MNCs were isolated from wild-type mice sensitized to OVA and restimulated with 50 μ g/mL OVA for 48 h. (C) CD4⁺ T lymphocytes were isolated from mice sensitized to OVA and restimulated with α -CD3/ α -CD28 coated beads in the presence or absence of 100 ng/mL rhIL-37 for 48 h. (D) MNCs were isolated from mice sensitized to OVA and restimulated with OVA in the presence or absence of 100 ng/mL rhIL-37 for 48 h. Cytokine concentrations in BALF and cell culture supernatants were assessed by cytometric bead array. Cytokine concentrations are shown as mean $\pm$ SEM. N = 8 (A, B) or 11 (C, D) biological repeats. Statistical significance was assessed using one-way analysis of variance and Tukey's multiple comparison post-hoc analyses, **P<0.01; ***P<0.001. BALF: bronchoalveolar lavage fluid, MNC: mononuclear cells, OVA: ovalbumin, PBS, phosphate buffer solution.

Fig. 3: IL-37 attenuates Th2 cytokines production in allergen-restimulated MNCs dependent on IL-1 β and IL-33 signaling

MNC were isolated from IL-1R1 deficient mice (A) or wild-type mice (B,C) sensitized to OVA and restimulated with 50 μ g/mL OVA in the presence or absence of 100 ng/mL rhIL-37 and/or 1 μ g/mL IL-1Ra (B) or 10 ng/mL IL-33 (C) for 48 h. Cytokine concentrations in the supernatant

were assessed by Cytometric Bead Array. Cytokine concentrations are shown as mean $\pm$ SEM. N=7 (A), N=16 (B), N=6 (C) biological repeats. Statistical significance was assessed using one-way analysis of variance and Tukey's multiple comparison post-hoc analyses, *P<0.05; **P<0.01. MNC: mononuclear cells, OVA: ovalbumin, IL-1Ra: interleukin-1 receptor antagonist.

Fig. 4: IL-37 attenuates IL-1 β - and IL-33-induced expression of pro-inflammatory mediators in AECs

After differentiation at ALI for 8–11 days, cells were incubated with or without 100 ng/mL rhIL-37 and two hours later stimulated with 10 ng IL-1 β (A) or IL-33 (B), respectively. Four hours later mRNA was isolated and the expression of different genes was analyzed via qRT-PCR. *Rpl32* was used as housekeeping gene. Expression values were normalized to IL-1 β alone group (A) or IL-33 alone group (B). The fold changes were calculated and are graphed as mean $\pm$ SEM of N=3 biological repeats. Statistical significance was assessed using repeated measure one-way analysis of variance and Tukey's multiple comparison post hoc analyses, *P<0.05; **P<0.01, ***P<0.001; ****P<0.0001. ALI: air-liquid interface, AEC: airway epithelial cells, qRT-PCR: quantitative real-time polymerase chain reaction, *Mcp1*: monocyte chemotactic protein-1 gene, *KC*: keratinocytes-derived chemokine gene, *Gmcsf*: granulocyte-macrophage colony-stimulating factor gene, *Il6*: interleukine-6 gene, *Tnfa*: tumor necrosis factor α gene, *Icam1*: intercellular adhesion molecule 1 gene.

Fig. 5: IL-37 downregulates allergic airway inflammation only in animals with a functional IL-1 β and IL-33 signaling

(A) Total numbers of eosinophils in BALF, (B) area of EBM covered by goblet cells per epithelial basal membrane, and (C, D) cytokine level in BALF of healthy (PBS), asthmatic (OVA) and IL-37-treated asthmatic (OVA+IL-37) wild-type, IL-1R1 deficient, and IL-33 deficient mice. Data are shown as mean $\pm$ SEM. N=6–9. Statistical significance was assessed using ordinary one-way analysis of variance and Tukey's multiple comparison post hoc analyses, *P<0.05; **P<0.01; ***P<0.001. BALF: bronchoalveolar lavage fluid, EBM, epithelial basal membrane, OVA: ovalbumin, PBS, phosphate buffer solution.

Fig. 6: Lower and upper airways in asthma display increased expression/production ratios of IL-1 β /IL-37 and IL-33/IL-37

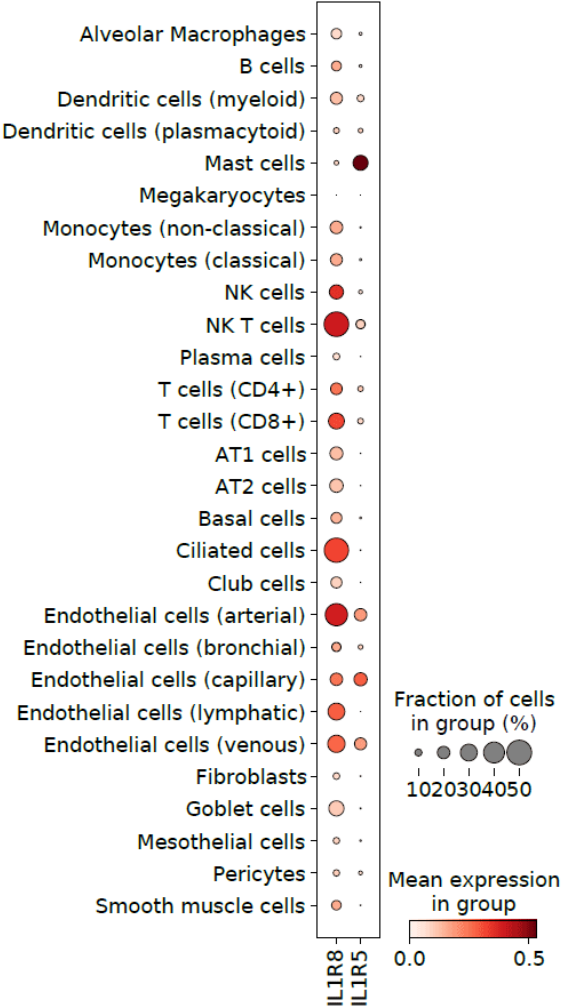
Expression levels of (A) *Il1beta*, (B) *Il33*, and (C) *Il37* as well as ratio of expression levels of (D) *Il1beta* to *Il37* or (E) *Il33* to *Il37* in induced sputum cells from healthy controls and patients with allergic asthma. Secreted protein levels were measured in sputum supernatants of healthy controls and patients suffering from allergic asthma for (F) IL-1 β , (G) IL-33, (H) IL-37 and the ratio of secreted levels of (I) IL-1 β to IL-37 and (J) IL-33 to IL-37. Levels of secreted cytokines were also measured in upper airway lining fluids in healthy controls (n = 23) and patients suffering from allergic asthma (n = 12) for (K) IL-1 β , (L) IL-33, (M) IL-37 and the ratio of secreted levels of (N) IL-1 β to IL-37 and (O) IL-33 to IL-37. Data are presented as mean $\pm$ SD. Statistical significance was assessed using Mann-Whitney tests, *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.

Fig. 1: IL1R5 and IL1R8 are expressed by various cells associated with asthma pathogenesis



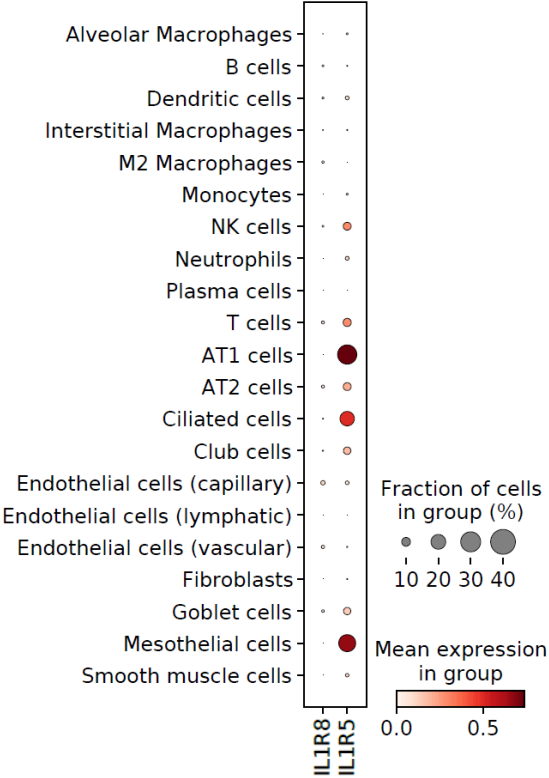
(A)

human, healthy



(B)

mouse, healthy



(C)

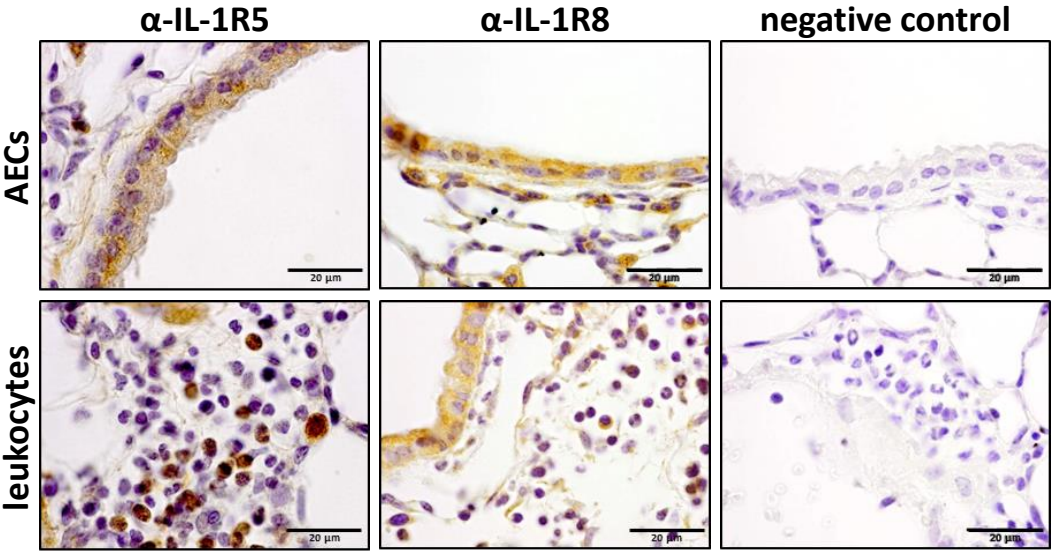


Fig. 2: : IL-37 attenuates Th2 type cytokine production in allergen-restimulated MNCs but not in anti-CD3/CD28 restimulated Th2 cells: role of IL-10

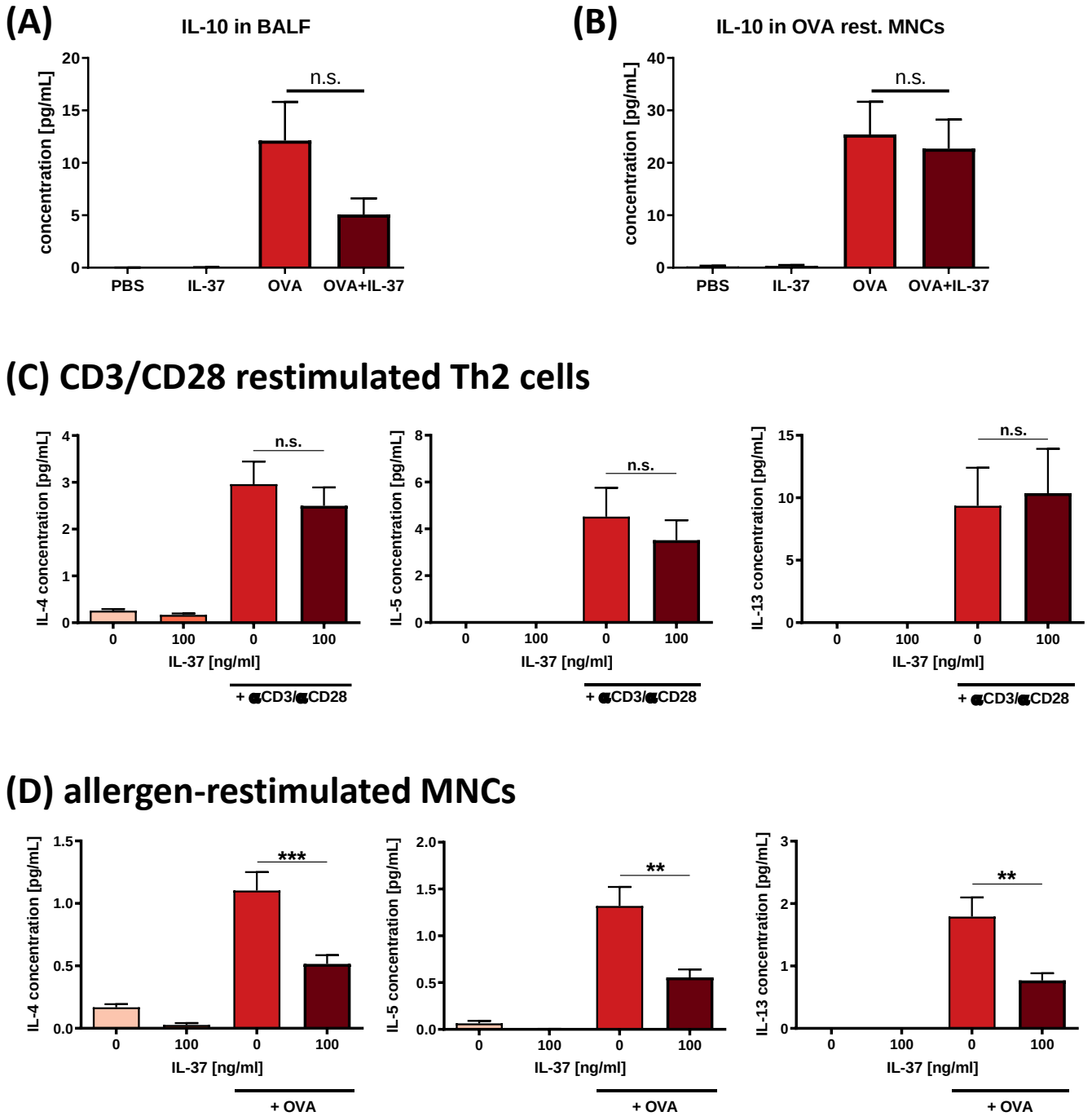
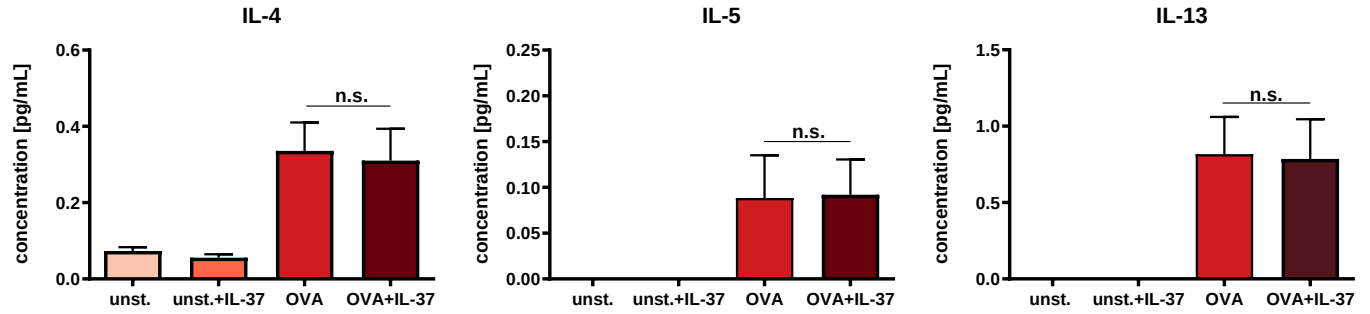


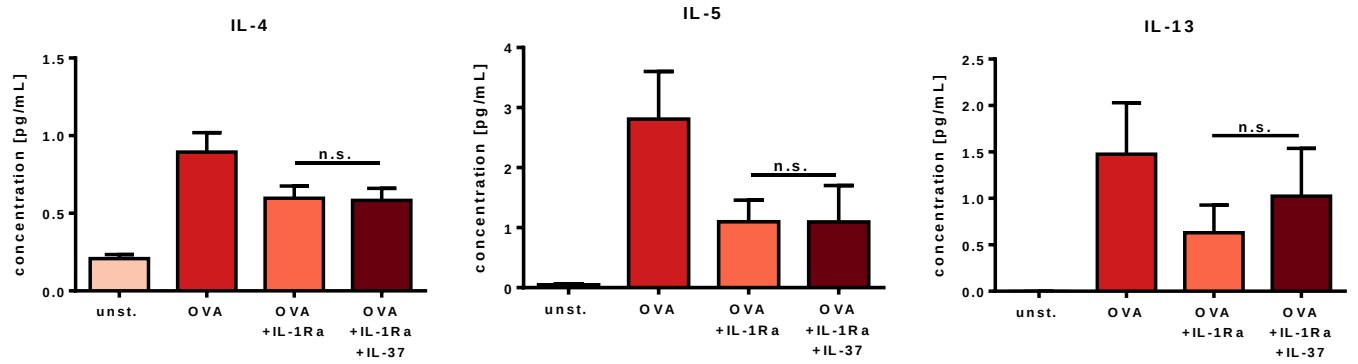
Fig. 3: IL-37 attenuates Th2 cytokines production in allergen-restimulated murine MNCs dependent on IL-1 β and IL-33 signaling



(A) IL-1R1 deficient



(B) wildtype + rIL-1Ra



(C) wildtype + IL-33

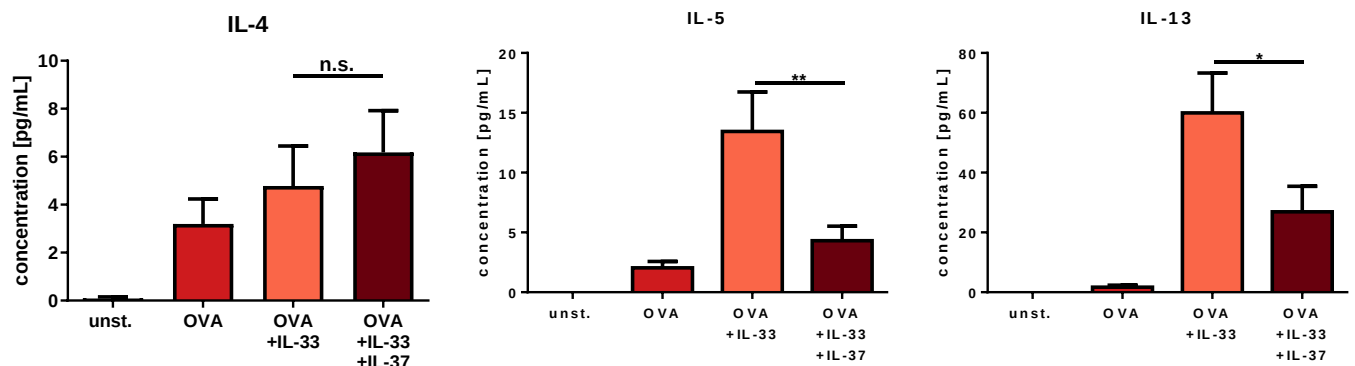
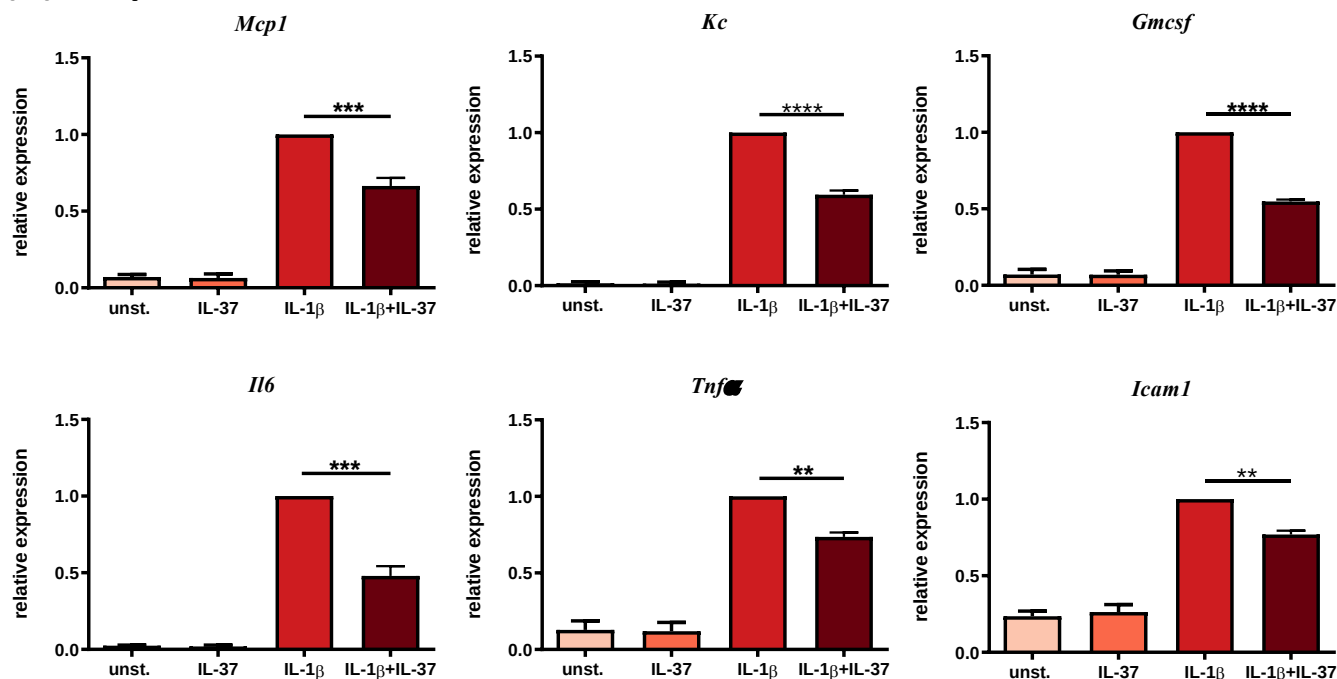


Fig. 4: IL-37 attenuates IL-1 β - and IL-33-induced expression of pro-inflammatory mediators in AECs



(A) IL-1 β



(B) IL-33

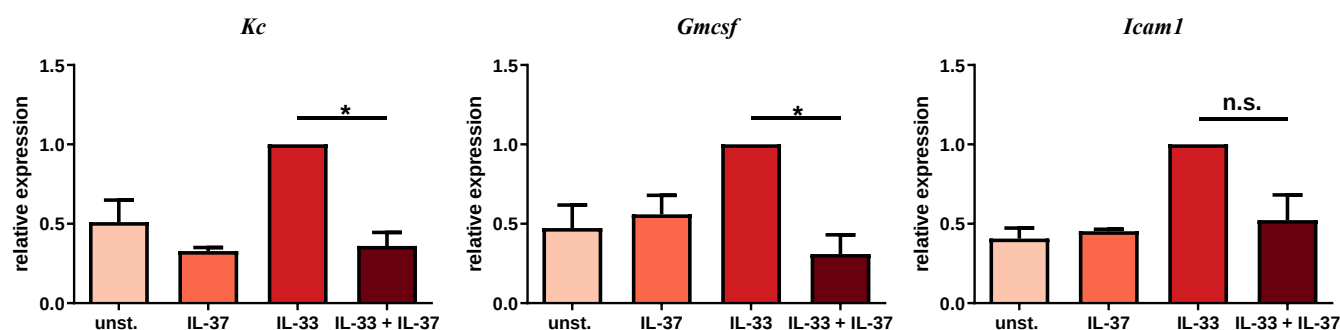


Fig. 5: IL-37 downregulates allergic airway inflammation only in animals with a functional IL-1 β and IL-33 signaling



wildtype

IL-1R1 deficient

IL-33 deficient

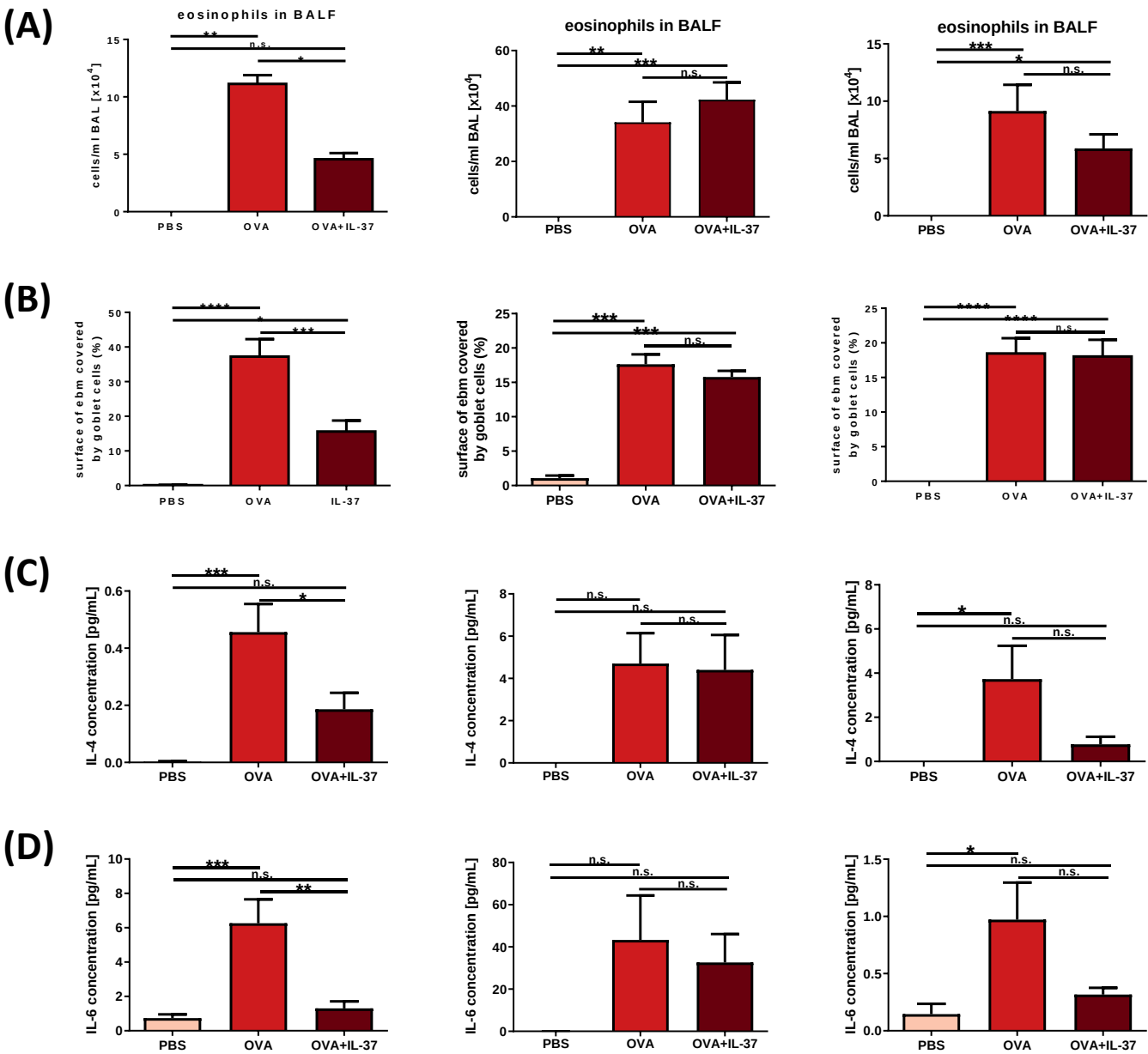
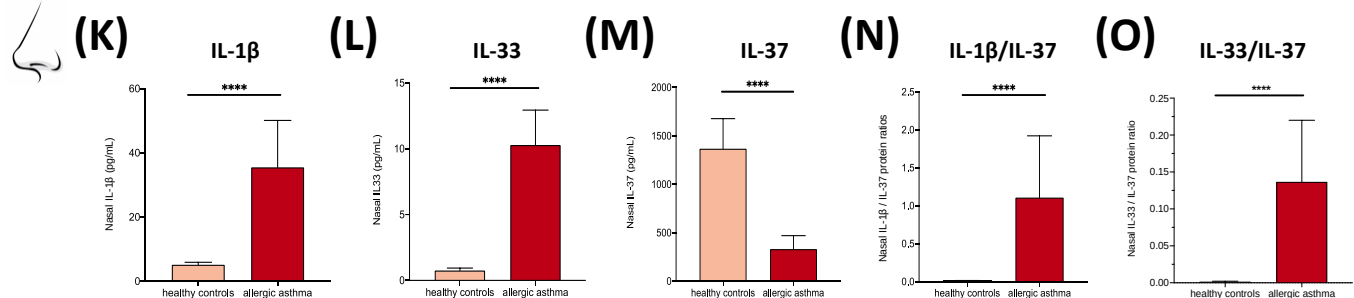
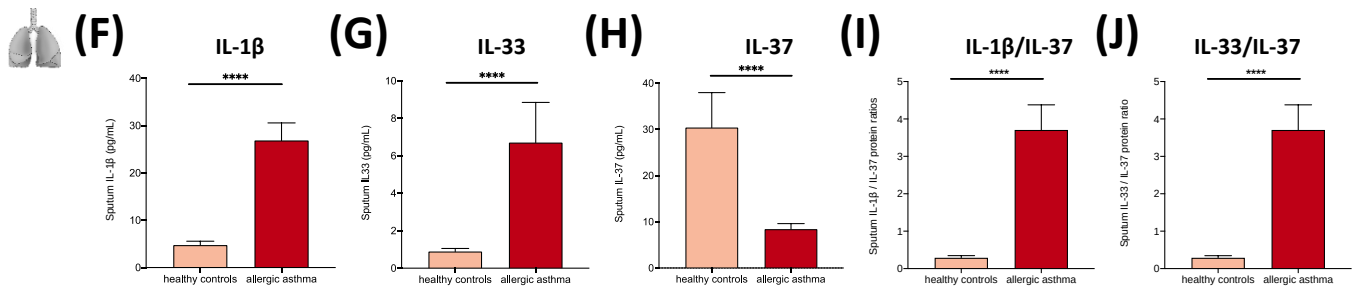
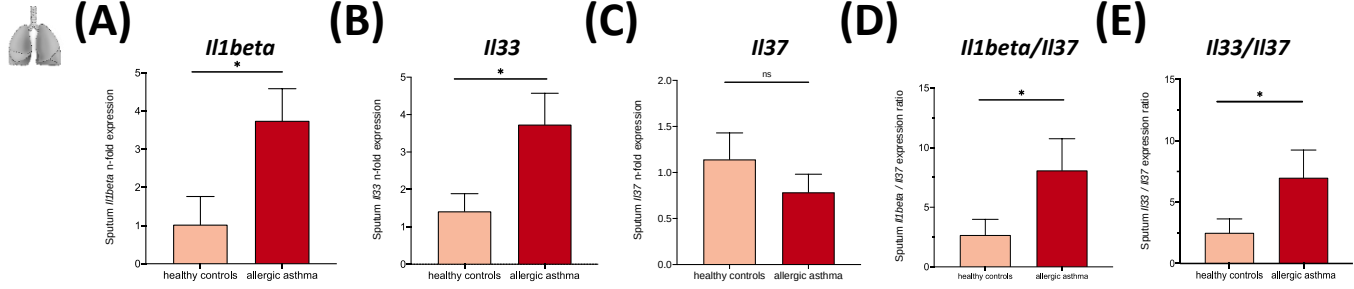
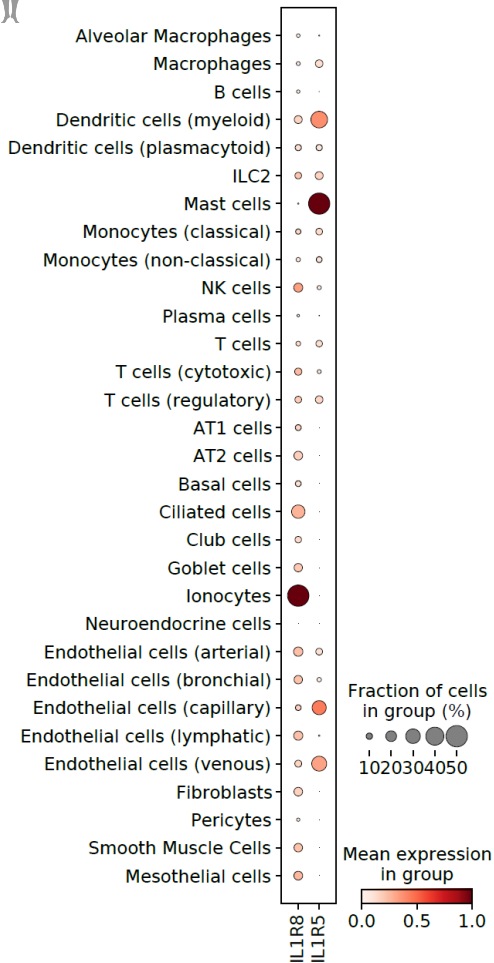


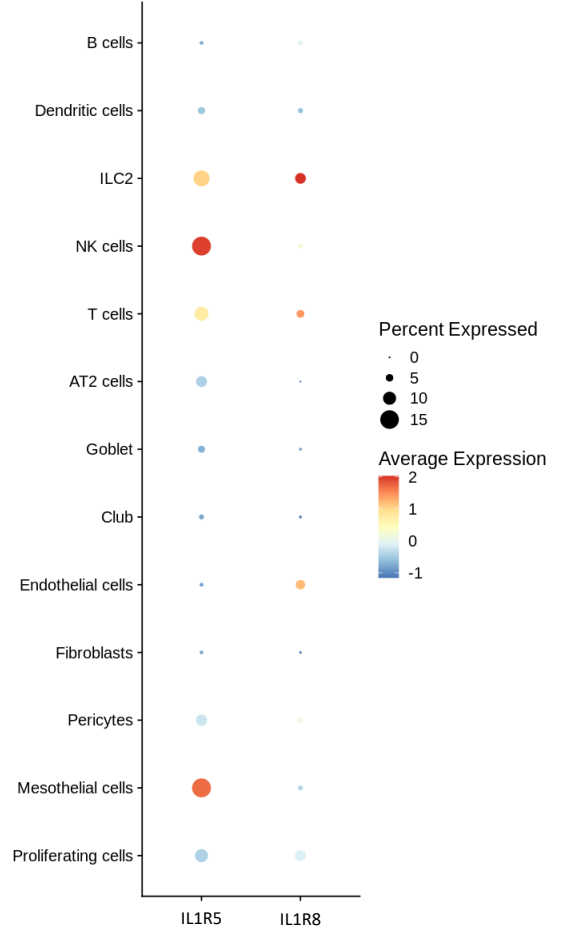
Fig. 6: Lower and upper airways in asthma display increased expression/production ratios of IL-1 β /IL-37 and IL-33/IL-37



(A)

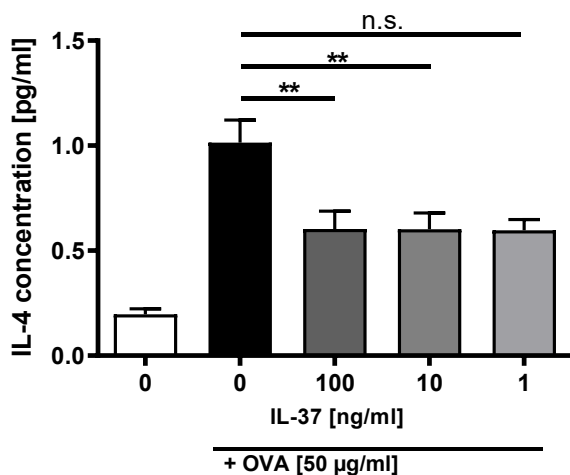


(B)

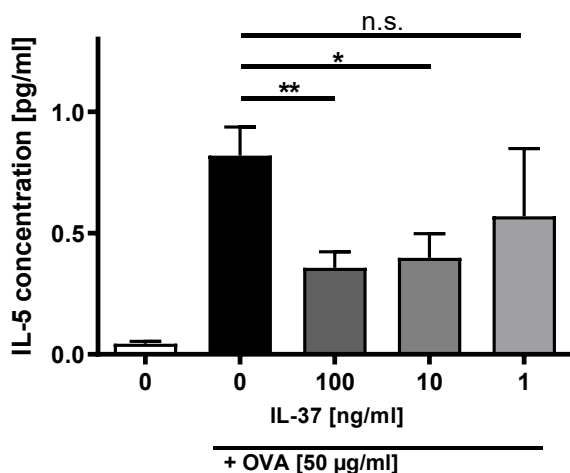


Suppl. Fig. 2: Dose-response effects of IL-37 on IL-4 IL-5, and IL-13 release in OVA restimulated MNCs

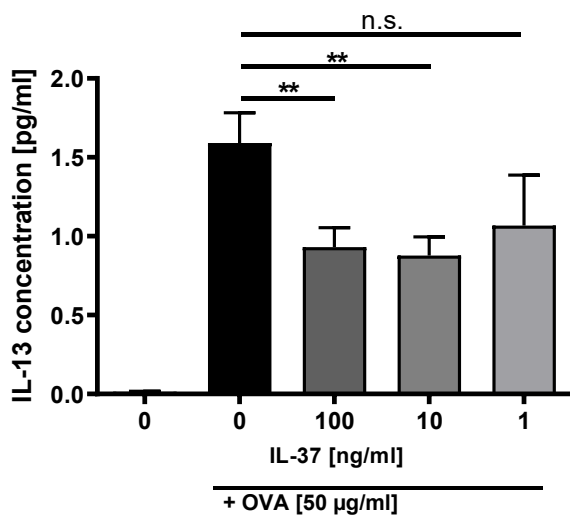
(A)



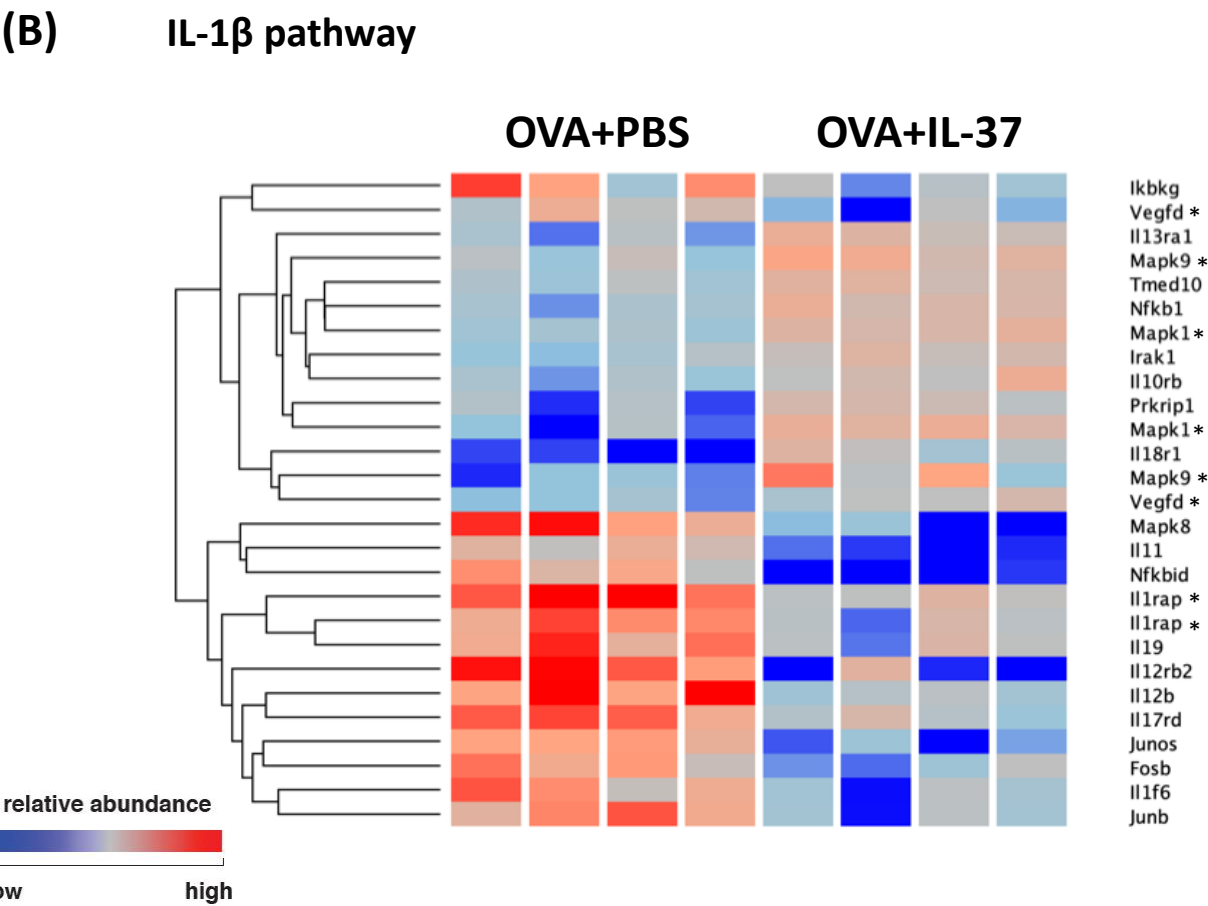
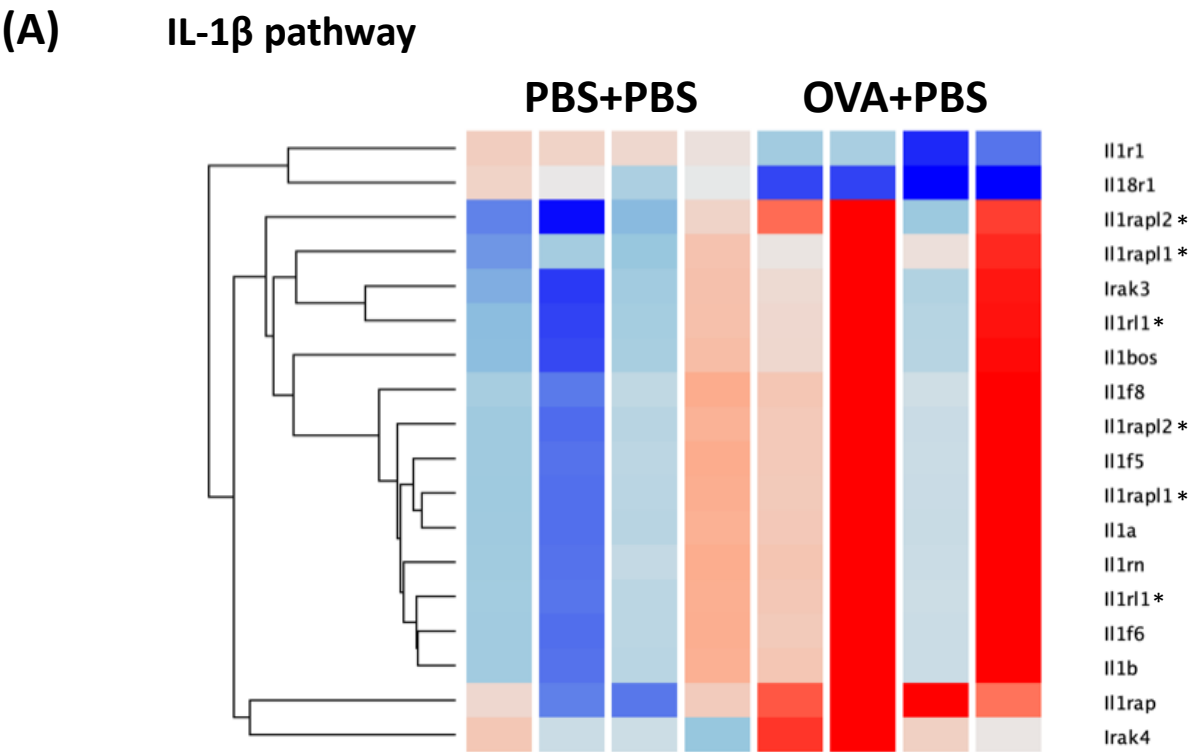
(B)



(C)

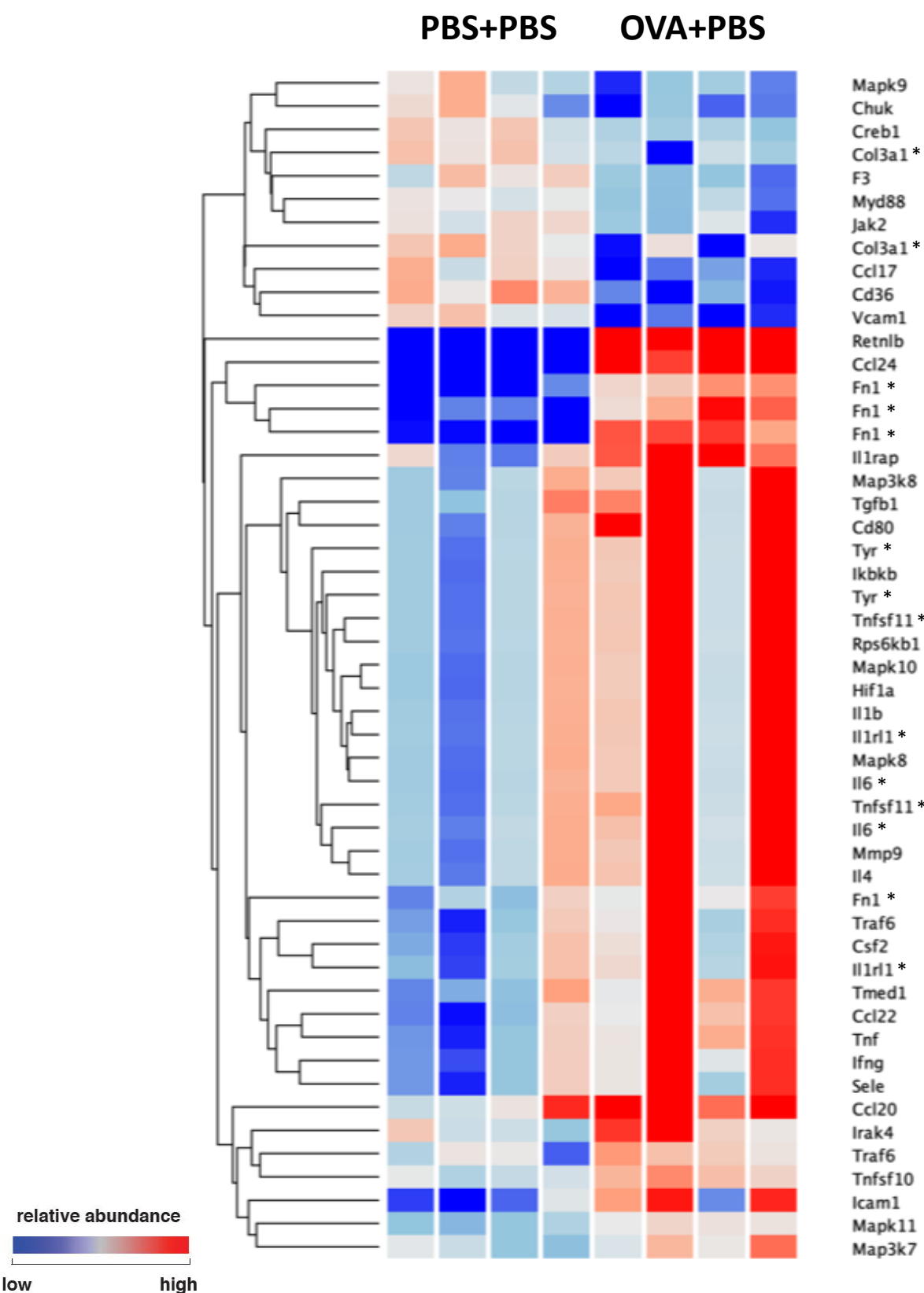


Suppl. Fig. 3: Expression of IL-1 β - & IL-33-pathway members is upregulated in EAA and differentially regulated by IL-37 treatment



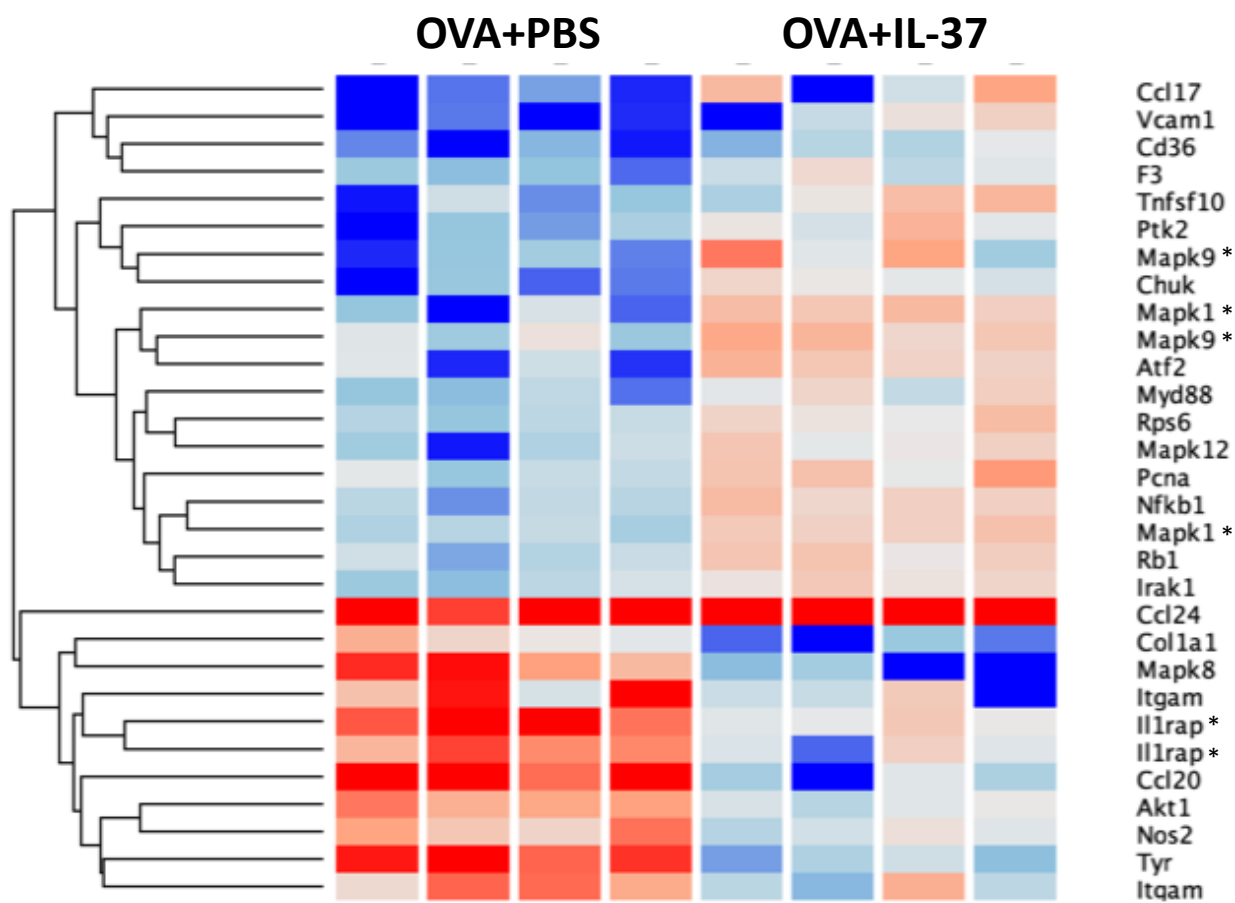
Suppl. Fig. 3: Expression of IL-1 β - & IL-33-pathway members is upregulated in EAA and differentially regulated by IL-37 treatment

(C) IL-33 pathway



Suppl. Fig. 3: Expression of IL-1 β - & IL-33-pathway members is upregulated in EAA and differentially regulated by IL-37 treatment

(D) IL-33 pathway



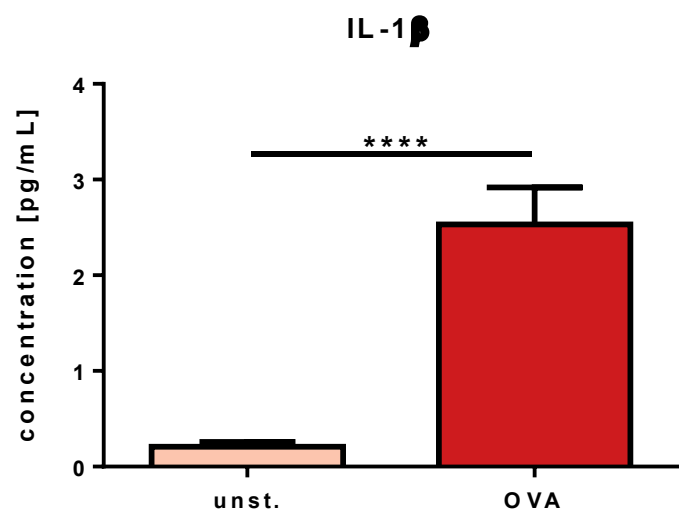
relative abundance



low

high

Suppl. Fig. 4: Allergen-restimulation of murine MNCs leads to IL-1 β release



Suppl. Tab. S1: Cohort characteristics

	Controls	Allergic asthma
	(n=23)	(n=12)
Age [years]	24.30 (±0.54)	30.50 (±3.28)
Sex (m/f)	9/14	7/5
RQLQ Score	0.47 (±0.18)	2.6 (±0.44) ****
IgE [IU/L]	37.41(±12.41)	249,70 (±78.07) ***
FEV ₁ [L]	4.12 (±0.20)	4.28 (±0.35)
FVC [L]	4.48 (±0.24)	4.45 (±0.35)
MEF25 [L]	4.04 (±0.21)	3.56 (±0.52)
Total cells x10 ⁴ /mL	31.85 (±5.23)	82.95 (±22.61)
Macrophages [%]	78.14 (±4.74)	82.15 (±8.74)
Neutrophils [%]	19.58 (±4.65)	13.32 (±8.25)
Eosinophils [%]	0.12 (±0.08)	2.58 (±0.96)
Lymphocytes [%]	2.14 (±0.56)	2.60 (±0.72)
IL1beta expression n-fold	1.03 (±0.48)	3.75 (±0.84) *
IL33 expression n-fold	1.42 (±0.47)	3.74 (±0.84) *
IL37 expression n-fold	1.14 (±0.29)	0.78 (±0.20)
sputum IL-1beta (pg/mL)	4.76 (±0.81)	26.88 (±3.71) ****
sputum IL-33 (pg/mL)	0.89 (±0.16)	6.71 (±2.15) ****
sputum IL-37 (pg/mL)	30.41 (±7.55)	8.41 (±1.20) ****
nasal IL-1beta (pg/mL)	5.12 (±0.79)	35.47 (±14.64) ****
nasal IL-33 (pg/mL)	0.76 (±0.18)	10.28 (±2.64) ****
nasal IL-37 (pg/mL)	1367.00 (±310.60)	330.60 (±139.60) ***

Data are presented as mean±SEM. * Significance in relation to healthy controls.

*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Suppl.Table S2 (Suppl.Figure 1A)

ProbeName	p ([OVA+PBS] Vs [PBS+PBS])	Regulation ([OVA+PBS] Vs [PBS+PBS])	FC ([OVA+PBS] Vs [PBS+PBS])	[PBS+PBS](normalized)	GeneSymbol	Description
A_55_P1962400	0,048523765	up	2,3919942	-0,33069986	Il1rn	Mus musculus interleukin 1 receptor antagonist (Il1rn), transcript variant 2, mRNA [NM_001039701]
A_52_P386627	0,0459856	up	2,4259505	-0,5707132	Irak3	Mus musculus interleukin-1 receptor-associated kinase 3 (Irak3), mRNA [NM_028679]
A_55_P2800007	0,02681492	up	2,338204	-0,44330442	Il1rapl1	interleukin 1 receptor accessory protein-like 1 [Source:MGI Symbol;Acc:MGI:2687319] [ENSMUST00000113966]
A_55_P2071221	0,048846956	up	2,3859086	-0,34778512	Il1f5	interleukin 1 family, member 5 (delta) [Source:MGI Symbol;Acc:MGI:1859325] [ENSMUST0000028360]
A_51_P497833	0,047626723	up	2,3975992	-0,36333215	Il1rapl1	Mus musculus interleukin 1 receptor accessory protein-like 1 (Il1rapl1), mRNA [NM_001160403]
A_51_P271503	0,003910286	down	-2,139289	0,21124893	Il1r1	Mus musculus interleukin 1 receptor, type I (Il1r1), transcript variant 1, mRNA [NM_008362]
A_66_P109708	0,047928534	up	2,390186	-0,358423	Il1f6	Mus musculus interleukin 1 family, member 6 (Il1f6), mRNA [NM_019450]
A_55_P2027461	0,046630792	up	2,3982089	-0,31667328	Il1f8	Mus musculus interleukin 1 family, member 8 (Il1f8), mRNA [NM_027163]
A_52_P207374	0,002128227	up	3,6447833	-0,38343757	Il1rap	Mus musculus interleukin 1 receptor accessory protein (Il1rap), transcript variant 4, mRNA [NM_001159318]
A_52_P494020	0,04897617	up	2,8407683	-0,5146991	Il1bos	Mus musculus interleukin 1 beta, opposite strand (Il1bos), long non-coding RNA [NR_015474]
A_66_P125238	0,018456839	up	3,1709623	-0,75144243	Il1rapl2	MMU277831 IL1RAPL-2 related protein {Mus musculus} (exp=-1; wgp=0; cg=0), complete [TC1592067]
A_52_P100926	0,04523412	up	2,4226882	-0,37284017	Il1a	Mus musculus interleukin 1 alpha (Il1a), mRNA [NM_010554]
A_55_P2027731	0,045396753	up	2,4068255	-0,5354777	Il1rl1	Mus musculus interleukin 1 receptor-like 1 (Il1rl1), transcript variant 1, mRNA [NM_001025602]
A_55_P2720183	0,0439351	up	2,4331443	-0,36239618	Il1b	interleukin 1 beta [Source:MGI Symbol;Acc:MGI:96543] [ENSMUST00000155994]
A_55_P1954246	0,044531077	up	2,460427	-0,37971282	Il1rapl2	Mus musculus interleukin 1 receptor accessory protein-like 2 (Il1rapl2), mRNA [NM_030688]
A_55_P2852406	0,042071126	up	2,1774585	-0,20663881	Irak4	Mus musculus interleukin-1 receptor-associated kinase 4 (Irak4), mRNA [NM_029926]
A_55_P2092492	0,001058167	down	-2,499063	-0,06985736	Il18r1	Mus musculus interleukin 18 receptor 1 (Il18r1), transcript variant 2, mRNA [NM_001161842]
A_51_P339793	0,045645945	up	2,3952558	-0,34681582	Il1rl1	Mus musculus interleukin 1 receptor-like 1 (Il1rl1), transcript variant 2, mRNA [NM_010743]

Suppl.Table S3 (Suppl.Figure 1B)

ProbeName	p [(OVA+IL37] Vs [OVA+PBS]	Regulation [(OVA+IL37] Vs [OVA+PBS]	FC [(OVA+IL37] Vs [OVA+PBS]	GeneSymbol	Description
A_55_P2741233	0,002209273	down	-3,2340298	Il12b	Mus musculus interleukin 12b (Il12b), mRNA [NM_001303244]
A_51_P267783	2,69E-04	down	-2,9693198	Il11	Mus musculus interleukin 11 (Il11), transcript variant 1, mRNA [NM_008350]
A_55_P2745489	0,017736582	up	1,7871048	Nfkb1	GB
A_52_P27244	0,016483808	up	2,2451634	Mapk9	Mus musculus mitogen-activated protein kinase 9 (Mapk9), transcript variant alpha2, mRNA [NM_207692]
A_55_P2720030	0,009217014	down	-2,386879	Il1f6	Mus musculus interleukin 1 family, member 6 (Il1f6), mRNA [NM_019450]
A_55_P1973289	0,032886755	up	1,7244323	Mapk9	Mus musculus mitogen-activated protein kinase 9 (Mapk9), transcript variant alpha1, mRNA [NM_001163672]
A_52_P207374	0,0027066	down	-2,6545482	Il1rap	Mus musculus interleukin 1 receptor accessory protein (Il1rap), transcript variant 4, mRNA [NM_001159318]
A_55_P2011106	0,003905897	down	-2,5834336	Junb	Mus musculus jun B proto-oncogene (Junb), mRNA [NM_008416]
A_55_P2541276	3,20E-04	down	-3,460643	Junos	PREDICTED: Mus musculus jun proto-oncogene, opposite strand (Junos), transcript variant X19, misc_RNA [XR_867924]
A_51_P380201	0,042397264	down	-1,9843551	Ikbg	Mus musculus inhibitor of kappaB kinase gamma (Ikbg), transcript variant 3, mRNA [NM_001136067]
A_55_P1970489	7,30E-04	down	-4,9568667	Il12rb2	Mus musculus interleukin 12 receptor, beta 2 (Il12rb2), transcript variant 1, mRNA [NM_008354]
A_51_P117739	0,0435739	up	1,6161219	Vegfd	Mus musculus vascular endothelial growth factor D (Vegfd), transcript variant 1, mRNA [NM_010216]
A_66_P107225	9,03E-05	down	-5,0253186	Nfkbid	nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, delta [Source:MGI Symbol;Acc:MGI:3041243] [ENSMUST00000108176]
A_51_P407004	0,037496828	up	1,8366007	Prkrip1	Mus musculus Prkr interacting protein 1 (Il11 inducible) (Prkrip1), mRNA [NM_025774]
A_55_P2041432	0,010864749	down	-2,1999094	Il1rap	interleukin 1 receptor accessory protein [Source:MGI Symbol;Acc:MGI:104975] [ENSMUST00000096129]
A_55_P2806002	0,022539267	up	1,8449837	Il13ra1	Mus musculus interleukin 13 receptor, alpha 1 (Il13ra1), mRNA [NM_133990]
A_51_P481788	0,01831616	up	1,7114094	Mapk1	Mus musculus mitogen-activated protein kinase 1 (Mapk1), transcript variant 1, mRNA [NM_011949]
A_55_P2099080	0,044393465	up	1,5977938	Irak1	Mus musculus interleukin-1 receptor-associated kinase 1 (Irak1), transcript variant 4, mRNA [NM_008363]
A_65_P02960	0,003934927	up	2,4662304	Mapk1	Mus musculus mitogen-activated protein kinase 1 (Mapk1), transcript variant 2, mRNA [NM_001038663]
A_55_P2092492	0,001213878	up	2,5598114	Il18r1	Mus musculus interleukin 18 receptor 1 (Il18r1), transcript variant 2, mRNA [NM_001161842]
A_51_P406454	0,042260654	up	1,6463801	Il10rb	Mus musculus interleukin 10 receptor, beta (Il10rb), mRNA [NM_008349]
A_55_P2001489	0,020508934	down	-2,0676305	Il19	Mus musculus interleukin 19 (Il19), mRNA [NM_001009940]
A_51_P371993	0,04848195	up	1,5673592	Tmed10	Mus musculus transmembrane p24 trafficking protein 10 (Tmed10), mRNA [NM_026775]
A_66_P112629	0,038323242	down	-1,8781896	Vegfd	Mus musculus vascular endothelial growth factor D (Vegfd), transcript variant 2, mRNA [NM_001308489]
A_55_P2113051	0,005254265	down	-2,4066079	Fosb	Mus musculus FBI osteosarcoma oncogene B (Fosb), transcript variant 1, mRNA [NM_008036]
A_52_P12243	0,004604997	down	-2,2275047	Il17rd	Il17RD_MOUSE (Q8JZL1) Interleukin-17 receptor D precursor (IL-17 receptor D) (IL-17RD) (Interleukin-17D receptor) (IL-17D receptor) (Interleukin-17 receptor-like protein) (Sef homolog) (mSef), partial (25%) [TC1625893]
A_55_P2808167	3,00E-04	down	-5,021745	Mapk8	Mus musculus mitogen-activated protein kinase 8 (Mapk8), transcript variant 1, mRNA [NM_016700]

Suppl. Table S4 (Suppl.Fig.1C)

ProbeName	p ([OVA+PBS] Vs [PBS+PBS])	Regulation ([OVA+PBS] Vs [PBS+PBS])	FC ([OVA+PBS] Vs [PBS+PBS])	GeneSymbol	Description
A_51_P375146	4,16E-04	down	-3,5288804	Cd36	Mus musculus CD36 antigen (Cd36), transcript variant 2, mRNA [NM_007643]
A_51_P114462	0,003163945	down	-3,1436243	Ccl17	Mus musculus chemokine (C-C motif) ligand 17 (Ccl17), mRNA [NM_011332]
A_51_P210956	6,20E-04	down	-3,163463	Vcam1	Mus musculus vascular cell adhesion molecule 1 (Vcam1), mRNA [NM_011693]
A_55_P2717454	0,04553689	down	-2,2503874	Col3a1	collagen, type III, alpha 1 [Source:MGI Symbol;Acc:MGI:88453] [ENSMUST00000129611]
A_52_P27244	0,026606103	down	-1,8619629	Mapk9	Mus musculus mitogen-activated protein kinase 9 (Mapk9), transcript variant alpha2, mRNA [NM_207692]
A_55_P2829726	0,023127524	down	-2,0732043	Chuk	Mus musculus conserved helix-loop-helix ubiquitous kinase (Chuk), transcript variant 2, mRNA [NM_001162410]
A_51_P515605	0,034462616	down	-1,9272999	Col3a1	Mus musculus collagen, type III, alpha 1 (Col3a1), mRNA [NM_009930]
A_55_P1971889	0,010112971	down	-1,9293777	F3	Mus musculus coagulation factor III (F3), mRNA [NM_010171]
A_55_P2804962	0,0265187	down	-1,6551781	Creb1	Mus musculus cAMP responsive element binding protein 1 (Creb1), transcript variant B, mRNA [NM_009952]
A_52_P282762	0,030341782	down	-1,6231253	Myd88	Mus musculus myeloid differentiation primary response gene 88 (Myd88), mRNA [NM_010851]
A_66_P106863	0,03320577	down	-1,7521583	Jak2	Mus musculus Janus kinase 2 (Jak2), transcript variant 1, mRNA [NM_008413]
A_51_P428360	2,78E-07	up	138,29422	Retnlb	Mus musculus resistin like beta (Retnlb), mRNA [NM_023881]
A_52_P237357	0,03893899	up	2,8696234	Fn1	fibronectin 1 [Source:MGI Symbol;Acc:MGI:95566] [ENSMUST00000189160]
A_55_P2718745	0,049256984	up	2,3889549	Map3k8	mitogen-activated protein kinase kinase kinase 8 [Source:MGI Symbol;Acc:MGI:1346878] [ENSMUST00000172805]
A_55_P2852406	0,042071126	up	2,1774585	Irak4	Mus musculus interleukin-1 receptor-associated kinase 4 (Irak4), mRNA [NM_029926]
A_55_P2737229	0,024755515	up	2,4701858	Tmed1	Mus musculus transmembrane p24 trafficking protein 1 (Tmed1), transcript variant 1, mRNA [NM_010744]
A_51_P102311	0,045007642	up	2,419303	Tyr	Mus musculus tyrosinase (Tyr), transcript variant 1, mRNA [NM_011661]
A_66_P135688	0,044642292	up	2,5845628	Tgfb1	Mus musculus transforming growth factor, beta 1 (Tgfb1), mRNA [NM_011577]
A_66_P130432	0,016809005	up	2,9185102	Cd80	Mus musculus CD80 antigen (Cd80), mRNA [NM_009855]
A_55_P2733612	0,042119198	up	2,4417667	Il6	Mus musculus interleukin 6 (Il6), transcript variant 1, mRNA [NM_031168]
A_55_P2039359	0,032493066	up	2,5451705	Tnfsf11	Mus musculus tumor necrosis factor (ligand) superfamily, member 11 (Tnfsf11), mRNA [NM_011613]
A_55_P2744624	0,039153803	up	2,6723292	Tyr	Mus musculus tyrosinase (Tyr), transcript variant 1, mRNA [NM_011661]
A_55_P2731881	0,045861088	up	2,6282754	Ikbkb	inhibitor of kappaB kinase beta [Source:MGI Symbol;Acc:MGI:1338071] [ENSMUST00000149093]
A_55_P1997756	0,045137044	up	2,4062266	Il6	Mus musculus interleukin 6 (Il6), transcript variant 1, mRNA [NM_031168]
A_52_P537438	0,045792546	up	2,4276714	Mapk10	mitogen-activated protein kinase 10 [Source:MGI Symbol;Acc:MGI:1346863] [ENSMUST00000133069]
A_55_P2149011	0,045312084	up	2,4362984	Hif1a	hypoxia inducible factor 1, alpha subunit [Source:MGI Symbol;Acc:MGI:106918] [ENSMUST00000110464]
A_51_P237865	0,045732953	up	2,4088767	Il4	Mus musculus interleukin 4 (Il4), transcript variant 1, mRNA [NM_021283]
A_52_P507214	0,046190787	up	2,4063735	Mmp9	Mus musculus matrix metalloproteinase 9 (Mmp9), mRNA [NM_013599]
A_51_P339793	0,045645945	up	2,3952558	Il1rl1	Mus musculus interleukin 1 receptor-like 1 (Il1rl1), transcript variant 2, mRNA [NM_010743]
A_52_P559044	0,04801827	up	2,3911805	Mapk8	HSU35004 JNK1 beta1 protein kinase {Homo sapiens} (exp=-1; wgp=0; cg=0), partial (22%) [TC1616561]
A_55_P2720183	0,0439351	up	2,4331443	Il1b	interleukin 1 beta [Source:MGI Symbol;Acc:MGI:96543] [ENSMUST00000155994]
A_52_P488947	0,044808418	up	2,4296768	Tnfsf11	Mus musculus tumor necrosis factor (ligand) superfamily, member 11 (Tnfsf11), mRNA [NM_011613]
A_52_P193994	0,04343828	up	2,421713	Rps6kb1	
A_52_P207374	0,002128227	up	3,6447833	Il1rap	Mus musculus interleukin 1 receptor accessory protein (Il1rap), transcript variant 4, mRNA [NM_001159318]
A_51_P171075	0,0486226	up	2,3925388	Csf2	Mus musculus colony stimulating factor 2 (granulocyte-macrophage) (Csf2), mRNA [NM_009969]
A_55_P2027731	0,045396753	up	2,4068255	Il1rl1	Mus musculus interleukin 1 receptor-like 1 (Il1rl1), transcript variant 1, mRNA [NM_001025602]
A_66_P109553	0,017626211	up	1,734652	Tnfsf10	tumor necrosis factor (ligand) superfamily, member 10 [Source:MGI Symbol;Acc:MGI:107414] [ENSMUST00000174840]
A_51_P385099	0,00669283	up	2,996957	Tnf	Mus musculus tumor necrosis factor (Tnf), transcript variant 1, mRNA [NM_013693]
A_51_P455326	0,045431893	up	2,4106586	Sele	Mus musculus selectin, endothelial cell (Sele), mRNA [NM_011345]
A_51_P327996	0,008823973	up	2,93664	Ccl22	Mus musculus chemokine (C-C motif) ligand 22 (Ccl22), mRNA [NM_009137]
A_52_P68893	0,023702849	up	2,8132553	Ifng	Mus musculus interferon gamma (Ifng), mRNA [NM_008337]
A_51_P408595	0,006805345	up	2,9016323	Ccl20	Mus musculus chemokine (C-C motif) ligand 20 (Ccl20), transcript variant 1, mRNA [NM_016960]
A_55_P1998380	0,04756942	up	1,7371361	Traf6	
A_55_P2640840	0,040640235	up	1,7677218	Map3k7	Mus musculus mitogen-activated protein kinase kinase kinase 7 (Map3k7), transcript variant A, mRNA [NM_172688]
A_52_P168449	0,011737688	up	1,7281101	Mapk11	Mus musculus mitogen-activated protein kinase 11 (Mapk11), mRNA [NM_011161]
A_51_P495259	0,047744483	up	2,3884795	Traf6	AV244412 RIKEN full-length enriched, 0 day neonate head Mus musculus cDNA clone 4831430116 3'. [AV244412]
A_52_P613241	0,015125608	up	3,2328665	Icam1	Mus musculus intercellular adhesion molecule 1 (Icam1), mRNA [NM_010493]
A_52_P18116	6,06E-07	up	16,546972	Ccl24	Mus musculus chemokine (C-C motif) ligand 24 (Ccl24), mRNA [NM_019577]
A_51_P100856	7,27E-05	up	4,5166965	Fn1	Mus musculus fibronectin 1 (Fn1), transcript variant 1, mRNA [NM_010233]
A_55_P2743309	1,65E-04	up	4,474534	Fn1	Mus musculus fibronectin 1 (Fn1), transcript variant 7, mRNA [NM_001276413]
A_55_P2130178	2,59E-06	up	6,0092697	Fn1	Mus musculus fibronectin 1 (Fn1), transcript variant 1, mRNA [NM_010233]

Suppl.Table S5 (Suppl.Figure 1D)

ProbeName	p ([OVA+IL37] Vs [OVA+PBS])	Regulation ([OVA+IL37] Vs [OVA+PBS])	FC ([OVA+IL37] Vs [OVA+PBS])	GeneSymbol	Description
A_51_P375146	0,037770852	up	1,7871212	Cd36	Mus musculus CD36 antigen (Cd36), transcript variant 2, mRNA [NM_007643]
A_51_P114462	0,041321505	up	2,5110793	Ccl17	Mus musculus chemokine (C-C motif) ligand 17 (Ccl17), mRNA [NM_011332]
A_51_P210956	0,030723536	up	2,225912	Vcam1	Mus musculus vascular cell adhesion molecule 1 (Vcam1), mRNA [NM_011693]
A_55_P1971889	0,04487728	up	1,5997661	F3	Mus musculus coagulation factor III (F3), mRNA [NM_010171]
A_55_P1963817	0,045801397	up	1,5850313	Rps6	Mus musculus ribosomal protein S6 (Rps6), mRNA [NM_009096]
A_52_P252258	0,03070413	up	1,8063349	Mapk12	Mus musculus mitogen-activated protein kinase 12 (Mapk12), mRNA [NM_013871]
A_52_P282762	0,03449267	up	1,6959126	Myd88	Mus musculus myeloid differentiation primary response gene 88 (Myd88), mRNA [NM_010851]
A_52_P27244	0,016483808	up	2,2451634	Mapk9	Mus musculus mitogen-activated protein kinase 9 (Mapk9), transcript variant alpha2, mRNA [NM_207692]
A_55_P2742078	0,014485709	up	2,1673589	Ptk2	Mus musculus PTK2 protein tyrosine kinase 2 (Ptk2), transcript variant 2, mRNA [NM_001130409]
A_55_P2829726	0,004550871	up	2,1636393	Chuk	Mus musculus conserved helix-loop-helix ubiquitous kinase (Chuk), transcript variant 2, mRNA [NM_001162410]
A_55_P1973289	0,032886755	up	1,7244323	Mapk9	Mus musculus mitogen-activated protein kinase 9 (Mapk9), transcript variant alpha1, mRNA [NM_001163672]
A_51_P396351	0,04032645	up	1,6768153	Pcna	Mus musculus proliferating cell nuclear antigen (Pcna), mRNA [NM_011045]
A_55_P2099080	0,044393465	up	1,5977938	Irak1	Mus musculus interleukin-1 receptor-associated kinase 1 (Irak1), transcript variant 4, mRNA [NM_008363]
A_51_P136888	0,034114465	up	1,6639918	Rb1	Mus musculus RB transcriptional corepressor 1 (Rb1), mRNA [NM_009029]
A_55_P2745489	0,017736582	up	1,7871048	Nfkb1	GB
A_51_P481788	0,01831616	up	1,7114094	Mapk1	Mus musculus mitogen-activated protein kinase 1 (Mapk1), transcript variant 1, mRNA [NM_011949]
A_55_P2018017	0,028817436	up	1,9546722	Tnfsf10	Mus musculus tumor necrosis factor (ligand) superfamily, member 10 (Tnfsf10), mRNA [NM_009425]
A_55_P2062891	0,014742407	up	2,1619256	Atf2	Mus musculus activating transcription factor 2 (Atf2), transcript variant 1, mRNA [NM_001025093]
A_65_P02960	0,003934927	up	2,4662304	Mapk1	Mus musculus mitogen-activated protein kinase 1 (Mapk1), transcript variant 2, mRNA [NM_001038663]
A_52_P18116	0,003100902	up	2,5447254	Ccl24	Mus musculus chemokine (C-C motif) ligand 24 (Ccl24), mRNA [NM_019577]
A_55_P2717469	0,002827636	down	-2,7093008	Col1a1	collagen, type I, alpha 1 [Source:MGI Symbol;Acc:MGI:88467] [ENSMUST00000148593]
A_55_P2808167	3,00E-04	down	-5,021745	Mapk8	Mus musculus mitogen-activated protein kinase 8 (Mapk8), transcript variant 1, mRNA [NM_016700]
A_52_P207374	0,0027066	down	-2,6545482	Il1rap	Mus musculus interleukin 1 receptor accessory protein (Il1rap), transcript variant 4, mRNA [NM_001159318]
A_55_P1977934	0,032439925	down	-2,3886049	Itgam	Mus musculus integrin alpha M (Itgam), transcript variant 1, mRNA [NM_001082960]
A_55_P2041432	0,010864749	down	-2,1999094	Il1rap	interleukin 1 receptor accessory protein [Source:MGI Symbol;Acc:MGI:104975] [ENSMUST00000096129]
A_55_P2158741	0,03895413	down	-1,6723412	Nos2	Mus musculus nitric oxide synthase 2, inducible (Nos2), transcript variant 1, mRNA [NM_010927]
A_66_P107415	0,008726046	down	-1,8991823	Akt1	thymoma viral proto-oncogene 1 [Source:MGI Symbol;Acc:MGI:87986] [ENSMUST00000159815]
A_55_P1977926	0,032278433	down	-1,9460342	Itgam	Mus musculus integrin alpha M (Itgam), transcript variant 2, mRNA [NM_008401]
A_66_P135323	7,94E-05	down	-3,7695727	Tyr	Mus musculus tyrosinase (Tyr), transcript variant 2, mRNA [NM_001317397]
A_51_P408595	1,48E-04	down	-5,4047885	Ccl20	Mus musculus chemokine (C-C motif) ligand 20 (Ccl20), transcript variant 1, mRNA [NM_016960]

1IL-37 regulates allergic inflammation by counterbalancing proinflammatory IL-1 and 2IL-33

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30Supplementary Material and Methods

31Analysis of publicly available single-cell RNA-seq (scRNA-seq) data

32Overview analysis of publicly available scRNA-seq data from healthy Human individuals^{1,2}
33and control Mice^{3,4} was performed using Scanpy (v.1.6.0.)⁵ and Seurat (v.4.0.1). Scanpy's
34function *scanpy.pl.dotplot* was used to compute and visualize the average scaled expression
35per cell type and the percentage of cells expressing *IL1R5/ilr5* and *IL1R8/il1r8*. For detailed
36analysis of *IL1R5/il1r5* and *IL1R8/il1r8* expression in ILC2s we relied on publicly available
37scRNA-seq data from healthy Human individuals and one ROR α lineage tracer mouse
38(labelling ILC2s) each containing at least one cluster of ILC2s. Average scaled expression per
39cell type and the percentage of cells expressing IL1R5 and IL1R8 were computed and
40visualized with Scanpy³ and Seurat (v.4.0.1.)⁶, respectively.

41

42Animal treatment protocol

43All mechanistic studies on the effects of IL-37 have been carried out in mice with
44experimental allergic asthma (EAA) and in cells isolated from these animals. Though mouse
45IL-37 has not been identified yet, recombinant human (rh) IL-37 has been shown to deploy
46murine IL-1R5 and IL-1R8 to provide its anti-inflammatory effects in-vitro and in-vivo.
47Induction of EAA and administration of rhIL-37 (*E. coli*-derived human IL-37/IL-1F7 protein
48Lys27-Asp192, R&D Systems, Minneapolis, MN, USA) was performed as described
49previously.⁷ In brief, mice (N=8) were sensitized to ovalbumin (OVA) by three intraperitoneal
50(i.p.) injections of 10 μ g OVA (OVA grade VI, Sigma-Aldrich, St. Louis, MO, USA) diluted
51in 100 μ L phosphate-buffered saline (PBS) together with 100 μ L ImjectTM Alum Adjuvant
52(Thermo Fischer Scientific, Waltham, MA, USA) on Days 1, 14, and 21, followed by
53exposure to an OVA (OVA grade V, Sigma-Aldrich) aerosol (1% w/v in PBS) on Days 26,

5427, and 28. Then, 1 µg recombinant human rhIL-37 was administered intranasally (i.n.) 24 h
55before each OVA aerosol challenge. All analysis were performed on Day 29.

56

57Bronchoalveolar lavage and differential cell count

58Lungs were rinsed with 1 mL of ice-cold PBS containing protease inhibitor (Complete, Roche
59Applied Science, Penzberg, Germany) via a tracheal cannula and obtained cells were counted
60using a Neubauer counting chamber. Next, 50 µL aliquots of lavage fluids were cytopinned
61(Cytospin™, Thermo Fischer Scientific, Waltham, MA, USA), and stained with Diff-Quick
62(Medion Diagnostic, Düringen, Switzerland), and cells were microscopically differentiated
63according to morphologic criteria.

64

65Isolation and co-stimulation of mononuclear cells (MNCs) and CD4⁺ T lymphocytes

66EAA was induced in C57BL/6 mice as described above. Subsequently, a single-cell
67suspension from spleen was generated by gentle passage through a nylon cell strainer (pore
68size: 70 µm). Red blood cells were depleted by using a red blood cell lysis buffer (BioLegend
69San Diego, CA, USA). CD4⁺ T Lymphocytes were isolated using a CD4⁺ T cell Isolation Kit
70(Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's guidelines.
71Purity and viability was assed via flow cytometry (97% CD4⁺, 98% viable cells). As
72described previously,⁸ cells were resuspended in culture media (TexMACS™ (Miltenyi
73Biotec), 10% FBS, 1% P/S) and activation of cells occurred via anti-CD3 and anti-CD28
74coated particles (T Cell activation/Expansion Kit, Miltenyi Biotec) in the presence or absence
75of 100 ng rhIL-37 for 48 h, a concentration that turned out to be effective in dose-response
76experiments using 1, 10, and 100 ng rhIL-37 (Suppl. Fig. 2).

77

78Isolation and stimulation of mononuclear cells

79The spleen was isolated from OVA sensitized and challenged C57BL/6 or IL-1R1^{-/-} mice.
80Subsequently, tissue was mashed by gentle passage through a nylon cell strainer (pore size: 70
81µm) to generate a single-cell suspension. MNCs were separated by density-gradient
82centrifugation with Lympholyte M (Cedarlane, Hornby, Ontario, Canada) and cultured in
83Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal calf serum (FCS, PAA
84Laboratories, Paschingen, Austria) and 1% penicillin/streptomycin (P/S, Capricorn Scientific
85GmbH, Ebsdorfergrund, Germany). The cells were then antigen-specifically activated with 50
86µg/mL OVA (OVA grade V, Sigma-Aldrich) in the presence or absence of 100 ng rhIL-37
87and/or 1 µg/mL IL-1 receptor antagonist (IL-1Ra) (Amgen, Thousand Oaks, CA, USA)
88and/or 10 ng/mL IL-33 (PeproTech, Rocky Hill, NJ, USA) for 48 h.

89

90Assessment of cytokines

91Levels of IL-4, IL-5, IL-6, IL-10, and IL-13 in bronchoalveolar lavage (BAL) fluid or cell
92culture supernatants were assessed using enhanced cytometric bead array (Flex Set Kits, BD
93Bioscience, Franklin Lakes, NJ, USA) and BD AccuriTM C6 flow cytometer (BD Bioscience)
94according to the manufacturer's guidelines. The analysis was performed using FCAP ArrayTM
95v3.0 Software (BD Bioscience).

96

97Lung histology

98The lungs were inflated and fixed ex-situ by instillation of 4% (w/v) phosphate-buffered
99paraformaldehyde under constant pressure for 20 min. Subsequently, the lung was kept at 4°C
100in paraformaldehyde overnight and embedded in paraffin. The orientation of the lungs was
101randomized according to the orientator technique.⁹ For analysis of lung inflammation, 2 µm
102sections were stained with periodic acid-Schiff (PAS) or with hematoxylin and eosin (HE),
103respectively. Photomicrographs were recorded by a digital camera (DP-25, Olympus, Tokyo,

104Japan) attached to a microscope (BX-51, Olympus) with a 20-fold magnification objective
105using Olympus cell^A software.

106

107**Immunohistochemistry**

108Immunohistochemical staining was performed against IL-1R5 (rabbit; polyclonal; 1:50;
109Cloud-Clone Corp, Katy, TX, USA) and IL-1R8 (rabbit; polyclonal; 1:50; Abcam,
110Cambridge, UK). Prior to staining, sections were exposed to 1 mg/mL protease from
111*Streptomyces griseus* (Pronase, Sigma-Aldrich) for 2 min. Antibody–antigen complexes were
112detected using biotinylated secondary antibody (goat anti-rabbit IgG antibody (H+L)
113biotinylated, Vector Laboratories, Burlingame, CA, USA) followed by avidin-biotin
114peroxidase (Vectastain Elite kit, Vector Laboratories) and 3,3'-diaminobenzidine (DAB)
115substrate (Sigma-Aldrich). Sections were counterstained with Mayer's hematoxylin (Merck
116Millipore, Darmstadt, Germany). Polyclonal rabbit IgG isotype were used as control detection
117antibody (IS067-Rb01; 1:50; Cloud-Clone Corp).

118

119**Quantitative morphology**

120For mucus assessment, systematic uniform random samples of lung tissue were taken
121according to standard methods.¹⁰ The surface area of mucin-containing goblet cells (S_{gc}) per
122total surface area of airway epithelial basal membrane (S_{ep}) and the volume of PAS-stained
123stored epithelial mucus (V_{mucus}) per S_{ep} were determined using a computer-assisted stereology
124tool box (newCAST, Visiopharm, Hoersholm, Denmark)^{11,12} according to the following
125formulae: $S_{gc}/S_{ep} = \sum I_{gc} / \sum I_{ep}$ and $V_{mucus}/S_{ep} = LP \times \sum P_{mucus} / 2 \times \sum I_{ep}$ where $\sum I_{gc}$ is the
126sum of intersections of test lines with goblet cells; $\sum I_{ep}$ is the sum of all intersections of test
127lines with epithelial basal membrane; $\sum P_{mucus}$ is the sum of all points hitting mucus and LP
128is the test line length at final magnification.⁸

130Laser-capture microdissection from snap-frozen lung tissue

131Laser-capture microdissection (LCM) was carried as described previously¹³ in order to
132subsequently perform differential gene expression profiling of airway tissues and to avoid
133contamination by non-inflamed lung parenchyma. In brief, the trachea of the mice was
134cannulated on Day 29 of the treatment protocol, and the lungs were inflated with cryo-
135protective Tissue-Tek OCT (Hartenstein, Würzburg, Germany) diluted 1:1 in PBS.
136Subsequently, the lung was removed, snap-frozen in liquid nitrogen and stored at
137–80°C. Afterwards cryosections (7 µm) were prepared on a cryotom, fixed on PALM
138membrane slides (Carl Zeiss AG, Jena, Germany) with 70% ethanol and stained with cresyl
139violet solution (0.5% w/v in ethanol) (Sigma-Aldrich). The airways were marked, isolated
140using the PALM Microbeam microdissection system (Carl Zeiss AG) and collected in RLT
141buffer (Qiagen, Hilden, Germany).

142

143Air-liquid interface (ALI) culture

144Dissected mouse trachea were incubated in HAMs F12 Medium (Gibco, Fisher Scientific
145GmbH, Schwerte, Germany) containing 0.2% Pronase (Roche Diagnostics, Mannheim,
146Germany), 1% P/S and 0.1% amphotericin B (Gibco) for 12 h. Digestion was stopped by
147addition of 20% FCS (Capricorn Scientific GmbH, Ebsdorfergrund, Germany) and followed
148by incubation with 1% DNase I (Sigma-Aldrich, Munich, Germany) solution (HAMs F12
149medium, 1% P/S, 0.1% amphotericin B, and 1% BSA) on ice for 5 min. To remove
150fibroblasts, cells were incubated in murine tracheal epithelial cells (mTEC) basic medium
151(DMEM/F12; Sigma-Aldrich) with 1.5 M HEPES (Sigma-Aldrich), 4mM glutamine
152(Glutamax, Gibco), 1% P/S, 0.1 amphotericin B, 3% NaHCO₃ (Merck Millipore, Darmstadt,
153Germany). Subsequently, mTEC were washed and 1x10⁶ cells were plated on collagen-
154coated, polycarbonate transwell plates (0.4 µm pore diameter, Corning™, Fisher Scientific) in

155mTEC proliferation medium (consisting of mTEC basic medium, 0.5% FCS [Sigma-Aldrich],
156100 nM retinoic acid [Sigma-Aldrich], 10 µg/mL insulin, 25 ng/mL EGF [Sigma-Aldrich], 60
157µg/mL bovine pituitary gland extract [Gibco], 5 µg/mL transferrin [Sigma-Aldrich], 100 ng/mL
158cholera toxin [Sigma-Aldrich]). Apical medium was removed after cells reached confluence,
159which was monitored by measuring transepithelial cell resistance using an electrode (EVOM
160Ohmvoltometer, World Precision Instruments, Sarasota, FL, USA). After differentiation at
161air-liquid interface (ALI) for 8–11 days, cells were treated with 100 ng/mL rhIL-37 (*E. coli*-
162derived human IL-37/IL-1F7 protein Lys27-Asp192, R&D Systems, Minneapolis, MN, USA)
163for 2 h, and the cells were then stimulated with 10 ng/mL IL-1β (PeproTech, Rocky Hill, NJ,
164USA) or 10 ng/mL IL-33 (PeproTech) in mTEC basic medium containing 2% Corning™
165nuSerum (Fisher Scientific). After 4 h, mRNA was isolated and the expression of different
166genes was analyzed via qPCR.

167

168Reverse transcription and quantitative real-time PCR for murine samples

169RNA was isolated using RNeasy® Micro Kit (Qiagen, Hilden, Germany) and reversely
170transcribed into cDNA using Maxima First Strand cDNA Synthesis Kit (Thermo Fisher
171Scientific, Waltham, MA, USA) according to the manufacturer's guidelines. Real-time PCR
172was performed on a LightCycler 480 II instrument (Roche Applied Science) according to the
173manufacturer's instruction with the Light Cycler 480 Sybr Green II Master (Roche Applied
174Science, Penzberg, Germany) in a total volume of 10 µL. Cycling conditions were as follows:
175one cycle at 95°C for 10 min followed by 45 cycles touch-down 63–58°C for 8 sec with
1760.5°C/s and 72°C for 10 s. For each primer pair, a standard curve was established by serial
177cDNA dilutions. *Rpl-32* was used as housekeeping gene. The following primers were used:
178*Mcp1* forward 5'- GGCTCAGCCAGATGCAGTTA-3', reverse 5'-
179GCTGCTGGTGATCCTCTTGT-3'; *Kc* forward 5'- CAGACCATGGCTGGGATTC-3',
180reverse 5'- GAACCAAGGGAGCTTCAG-3'; *Gmcsf* forward 5'-

181TGCCTGTCACGTTGAATGAAG-3', reverse 5'- AGGCTGTCTATGAAATCCGCA-3'; *Il6*
182forward 5'- CTCCCAACAGACCTGTCTATAC-3', reverse 5'-
183GTGCATCATCGTTGTTTCATAC-3'; *Tnfa* forward 5'- TCGTAGCAAACCACCAAGTG-3',
184reverse 5'- AGATAGCAAATCGGCTGACG-3'; *Icam1* forward 5'-
185ACCGTTGTGATCCCTGGGCCT-3', reverse 5'- AAGCGGACGACTGCACGGTG-3'. Data
186were analyzed employing the 'advanced relative quantification' and 'standard curve method'.
187A calibrator cDNA was included in each run to correct for run-to-run differences.
188Amplification specificity was checked using melting curve. PCR products were visualized by
189agarose gel electrophoresis.

190

191Lung function testing of patients

192Using pneumotachography device (Jaeger SpiroPro; Würzburg, Germany), three
193measurements of baseline lung function were performed according to ERS guidelines (VC,
194FEV1, FEV1/VC, and maximum expiratory flow 25% (MEF 25%) were recorded. Lung
195function testing was repeated three times after 15 minutes to test the bronchodilator
196reversibility after 400µg of salbutamol. Between increase of the hypertonic saline
197concentrations, one lung function test was performed to monitor changes.

198

199Sputum Collection and Processing

200Participants first inhaled 400 µg salbutamol and then nebulized hypertonic saline at increasing
201concentrations of 3%, 4%, and 5% NaCl every 7 min according to previous publications.¹⁴
202During this procedure, participants cleaned their noses and rinsed their mouths to reduce
203squamous epithelial cells in the samples. Sputum was processed within 1 h of collection. The
204selected sputum plugs, which contained as little saliva as possible were placed in a weighed
205Eppendorf tube and processed with 4x w/v of sputolysin[®] reagent working solution
206(Calbiochem Merck, Darmstadt, Germany). Afterwards, 2x w/v of PBS was added. Samples

207were filtered through a 70 μ m mesh and centrifuged for 10 min at 790xg to remove the cells.
208Resulting cell-free supernatants were stored at -80°C for additional analysis using a protein
209assay. Appropriate sputum samples were obtained from 23 healthy controls and 12 patients
210with untreated asthma. In addition, nasal lining fluids were obtained, which are natively
211absorbed through a membrane from the main nasal cavity and is eluted in the following step
212by centrifugation in a portafilter cryotube with 200 μ L PBS as described before.^{15,16}

213

214**Identification of sputum cell populations by differential cell count**

215Cell suspension was centrifuged on a slide in a Labofuge 400 cytopsin centrifuge (Heraeus,
216Hanau, Germany) at 44xg for 6 min. The slides were air-dried, fixed with methanol for 5 min
217and stained using the Pappenheim method.¹⁴ Specimens with a content of less than 15% of
218squamous epithelial cells were considered adequate. Three investigators blinded to the subject
219classification read at least 400 non-squamous cells on each sputum slide. Neutrophils,
220lymphocytes, eosinophils, basophils, and macrophages were calculated as proportions of the
221total cell counts.

222

223**Human and murine RNA isolation**

224RNA was extracted using RNeasy Micro Kit (Qiagen, Hilden, Germany) with on-column
225DNase digestion (Qiagen) to avoid DNA contamination. RNA was quantified by ultraviolet–
226visible spectrophotometry (Nanodrop Technologies, Wilmington, DE, USA), with RNA
227integrity assessed by the RNA 6000 Nano Chip Kit with the Agilent 2100 Bioanalyzer
228(Agilent Technologies, Waldbronn, Germany).

229

230

231**Reverse transcription and quantitative real-time PCR for human samples**

232Isolated human total RNA was subjected to reverse transcription using a high-capacity cDNA
233kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions.

Real-time PCR profiles were visualized using the commercially available FastStart Universal SYBR Green Mastermix (Roche, Basel, Swiss) and quantified by the ViiA 7 Real-Time PCR System (Applied Biosystems). QuantiTect primer assays were used in the real-time PCR (Qiagen, Hilden, Germany). The amount of *IL1B*, *IL33* and *IL37* mRNA expression was normalized with endogenous control *TFRC*, *HPRT*, and β -*ACTIN* (housekeeping gene index, Δ Ct values) and the relative quantification and calculation of range of confidence was performed using the comparative threshold cycle ($2^{-\Delta\Delta C_t}$) method (relative gene expression) as previously described.¹⁷ All amplifications were carried out at least in duplicates.

Murine whole genome microarray

Total RNA was extracted from LCM-isolated airway tissue of PBS treated (n = 4) and IL-37 treated mice with EAA (n = 4) as well as of PBS treated healthy mice (n = 4) and amplified and Cy3-labeled by using the one-color Low Input Quick Amp Labeling Kit (Agilent Technologies) according to the manufacturer's protocol. Hybridization to SurePrint G3 Mouse Gene Expression 8x60K Microarrays (Agilent Technologies) was performed with the Gene Expression Hybridization Kit (Agilent Technologies).

Murine microarray data analysis strategy

Upon data import a standard baseline transformation to the median of all values was performed, including log transformation and computation of fold changes. Subsequently, a principle component analysis (PCA) was conducted and revealed a homogenous component distribution. Compromised array signals (array spot is non-uniform if the pixel noise of the feature exceeds the threshold or is above saturation threshold) were excluded from further analysis. Genes with an absolute log₂ fold change larger than 1.5 and a p-value smaller than the testing level of 0.05 by using the Moderated T-Test were defined as significantly differentially expressed hits. The significantly regulated genes were summarized in entity lists

260(Supplementary Tab. 2, 3, 4, 5). Manhattan cityblock on entities (Ward's linkage) was used to
261cluster changes in gene expression.

262

**263Protein measurement in human nasal secretions and sputum supernatants by multi-
264array technology.**

265Sputum supernatants as well as nasal secretions were stored at -80 °C for analysis of protein
266levels. Total amount of proteins in sputum supernatants and nasal secretions were determined
267using the Pierce™ Coomassie Plus (Bradford) Assay Kit (Thermo Fisher Scientific, USA)
268according to manufacturers instructions. Protein levels of IL-1 β , IL-33, and IL-37 in human
269nasal secretions and sputum supernatants in nasal secretions were detected in duplicates by
270the highly-sensitive MesoScaleDiscovery's (MSD) Multi-Array technology (MSD Mesoscale,
271USA) as described before. The lower limits of detection of the cytokines/chemokines are: IL-
2721 β : 0.067 pg/mL; IL-33: 0.216 pg/mL; IL-37: 2.185 pg/mL.

273

274

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324Supplementary Figures

325Fig. 1: IL-1R5 and IL-1R8 are expressed by various cells associated with asthma 326pathogenesis

327(A) Gene expression of IL-1R5 and IL-1R8 in different cell types in the human lung assessed
328by single cell RNA-seq. (B) Gene expression of IL-1R5 and IL-1R8 in different cell types in
329the murine lung assessed by single cell RNA-seq. IL-1R5: interleukin-1 receptor 5, IL-1R8:
330interleukin-1 receptor 8, AT1: alveolar epithelial type 1 cells, AT2: alveolar epithelial type 2
331cells, AEC: airway epithelial cells.

332

333Suppl. Fig. 2: Dose-response effects of IL-37 on IL-4 IL-5, and IL-13 release in OVA 334restimulated MNCs

335MNC were isolated from wildtype mice sensitized to OVA and restimulated with 50 μ g/mL
336OVA in the presence or absence of 1, 10, or 100 ng/mL IL-37, respectively for 48 h. IL-4 (A),
337IL-5 (B), and IL-13 (C) concentrations in the supernatant were assessed by Cytometric Bead
338Array. Cytokine concentrations are shown as mean $\pm$ SEM. Statistical significance was
339assessed using one-way analysis of variance and Tukey's multiple comparison post-hoc
340analyses, *P<0.05; **P<0.01. MNC: mononuclear cells, OVA: ovalbumin.

341

342Suppl. Fig. 3: Expression of IL-1 β - & IL-33-pathway members is upregulated in EAA,
343and differentially regulated by IL-37 treatment

344RNA from laser microdissected murine airways was isolated and a one-color microarray-
345based gene expression analysis was performed. **(A)** Comparison of IL-1 pathway members
346between healthy (PBS+PBS; n = 4) and OVA treated asthmatic mice (OVA+PBS; n = 4). **(B)**
347Comparison of IL-1-related entities, extracted from a Venn diagram cut set, between
348asthmatic mice (OVA+PBS; n = 4) and IL-37-treated asthmatic mice (OVA+IL-37; n = 4).
349**(C)** Comparison of IL-33 pathway members between healthy (PBS+PBS; n = 4) and
350asthmatic mice (OVA+PBS; n = 4). **(D)** Comparison of IL-33-related entities, extracted from
351a Venn diagram cut set, between asthmatic mice (OVA+PBS; n = 4) and IL-37-treated
352asthmatic mice (OVA+IL-37; n = 4). The color code indicates the abundance of transcripts
353(average means of 4 animals per group) ranging from low (blue) to high (red). Asterisks
354indicate two isoforms that were present in the analysis. RNA: ribonucleic acid, PBS:
355phosphate buffered saline, OVA: ovalbumin, IL-37: interleukin 37.

356

357Suppl. Fig. 4: Antigen-restimulation of murine MNCs leads to IL-1 β release

358MNCs were isolated from wildtype mice sensitized to OVA and restimulated with 50 μ g/mL
359OVA for 48 h. IL-1 β concentrations in the supernatant was assessed by cytometric bead array.
360Cytokine concentration is graphed as mean $\pm$ SEM. N=8 biological repeats. Statistical
361significance was assessed using t-test, ***P<0.0001. MNC: mononuclear cell, OVA:
362ovalbumin.

363

364Suppl. Tab. S1: Cohort characteristics

365Mini rhinoconjunctivitis quality of life questionnaire (mRQLQ) score, Immunoglobulin E
366(IgE), forced expiratory volume in 1 second (FEV₁), forced vital capacity (FVC), maximal

367expiratory flow at 25% of FVC (MEF25). Data are mean $\pm$ SD.