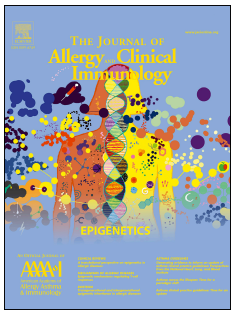
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IL-37-Mex3B-TLR3 axis in epithelial cells in eosinophilic chronic rhinosinusitis with nasal polyps

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PII: S0091-6749(19)30945-5

DOI: https://doi.org/10.1016/j.jaci.2019.07.009

Reference: YMAI 14093

To appear in: *Journal of Allergy and Clinical Immunology*

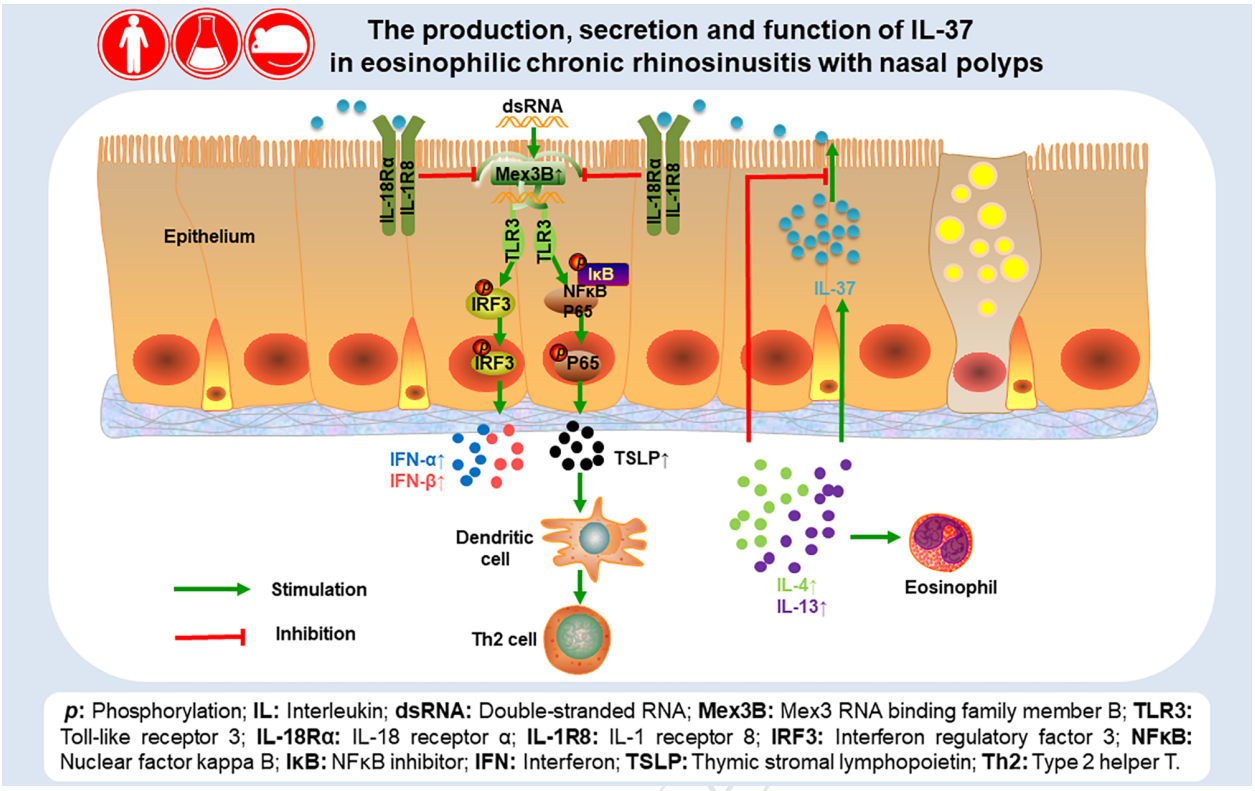
Received Date: 17 December 2018

Revised Date: 3 June 2019

Accepted Date: 9 July 2019

Please cite this article as: Liu J-X, Liao B, Yu Q-H, Wang H, Liu Y-B, Guo C-L, Wang Z-C, Li Z-Y, Wang Z-Z, Ruan J-W, Pan L, Yao Y, Chen C-L, Wang H, Liang Y-X, Zhen G-H, Liu Z, IL-37-Mex3B-TLR3 axis in epithelial cells in eosinophilic chronic rhinosinusitis with nasal polyps, *Journal of Allergy and Clinical Immunology* (2019), doi: https://doi.org/10.1016/j.jaci.2019.07.009.

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***p*:** Phosphorylation; **IL:** Interleukin; **dsRNA:** Double-stranded RNA; **Mex3B:** Mex3 RNA binding family member B; **TLR3:** Toll-like receptor 3; **IL-18Rα:** IL-18 receptor α; **IL-1R8:** IL-1 receptor 8; **IRF3:** Interferon regulatory factor 3; **NFκB:** Nuclear factor kappa B; **IκB:** NFκB inhibitor; **IFN:** Interferon; **TSLP:** Thymic stromal lymphopoietin; **Th2:** Type 2 helper T.

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IL-37-Mex3B-TLR3 axis in epithelial cells in eosinophilic chronic rhinosinusitis with nasal polyps

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Running Head: IL-37-Mex3B-TLR3 axis in eosinophilic CRSwNP

Grant Support: This study was supported by the National Natural Science Foundation of China (NSFC) grants 81630024, 81570899 and 81325006 (Z.L.), and

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81873694 (H.W.) and 81700893 (B.L.), Hubei Province Natural Science Foundation grants 2017CFA016 (Z.L.) and 2017CFB477 (H.W.), and "Ten thousand plan"-National high-level talents special support plan (Z.L.).

Disclosure of conflict of interest: None

Body word count: 4,283

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AUTHORS' CONTRIBUTIONS

Jin-Xin Liu and Bo Liao performed cell culture, immunohistochemistry, immunofluorescence, western blotting, ELISA and PCR assay, analyzed data, and prepared manuscript. Qi-Hong Yu and Hai Wang performed mouse and cell culture

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experiments. Yi-Bo Liu, Cui-Lian Guo and Zhi-Chao Wang performed cell culture and PCR assay. Zhi-Yong Li and Zhe-Zheng Wang performed flow cytometry. Jian-Wen Ruan and Li Pan performed immunohistochemistry. Yin Yao, Cai-ling Chen and Heng Wang participated in tissue sample collection and data discussion. Yu-Xia Liang and Guo-Hua Zhen performed the study of induced sputum and bronchial brushing epithelial cells. Zheng Liu designed the study and prepared the manuscript.

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ABSTRACT

Background: The role of IL-37, an immune suppressive cytokine, in inflammatory diseases is unclear.

Objective: To explore the expression and pathogenic function of IL-37 in chronic rhinosinusitis (CRS).

Methods: The expression of IL-37, IL-18Rα, IL-1R8, Mex3 RNA binding family member B (Mex3B), and TSLP in nasal samples were studied by quantitative RT-PCR, immunohistochemistry, western blotting, and ELISA. Human nasal epithelial cells (HNECs) and BEAS-2B cell line were stimulated with various cytokines and TLR agonists. In some experiments, BEAS-2B cells were transfected with Mex3B siRNA or overexpressing lentiviruses. Genes regulated by IL-37b in HNECs were studied by RNA sequencing analysis. IL-37b function was confirmed in mice *in vivo*.

Results: Compared with controls, although IL-37 mRNA and protein expression were upregulated in diseased tissues, especially in nasal epithelial cells, in CRS patients without or with nasal polyps (CRSwNP), IL-37 levels in nasal secretions were reduced in patients with eosinophilic CRSwNP. Type-2 cytokines inhibited IL-37 secretion from HNECs. HNECs expressed IL-37 receptors, IL-18Rα and IL-1R8. IL-37b downregulated the expression of Mex3B, a TLR3 co-receptor, in HNECs. IL-37b suppressed poly(I:C)-induced TSLP production in HNECs *in vitro* and in murine nasal epithelial cells *in vivo*. Knocking down or overexpressing Mex3B in BEAS-2B cells abolished the inhibitory effect of IL-37b. Secreted IL-37 levels negatively correlated with Mex3B and TSLP levels, and eosinophil numbers in

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eosinophilic CRSwNP patients.

Conclusions: The suppressed IL-37 secretion caused by type-2 milieu may enhance Mex3B-mediated TLR3 activation and subsequent TSLP production in nasal epithelial cells, and therefore promotes eosinophilic inflammation in CRSwNP.

Key words: Eosinophil, epithelial cell, chronic rhinosinusitis, IL-37, Mex3 RNA binding family member B, nasal polyp, toll-like receptor 3

Clinical implications: Reduced IL-37 secretion may contribute to the development of eosinophilic inflammation in CRSwNP. IL-37 might be a potential therapeutic target for patients with eosinophilic CRSwNP.

Capsule summary: Reduced IL-37 secretion caused by type-2 milieu may promote Mex3B expression and activation of TLR3 signaling pathway in nasal epithelial cells, which in turn exaggerates type-2 response biased eosinophilic inflammation in CRSwNP.

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ABBREVATIONS

AR: allergic rhinitis

CRS: chronic rhinosinusitis

CRSsNP: chronic rhinosinusitis without nasal polyps

CRSwNP: chronic rhinosinusitis with nasal polyps

ELISA: enzyme-linked immunosorbent assay

GAPDH: glyceraldehyde 3-phosphate dehydrogenase

HNECs: human nasal epithelial cells

IFN：interferon

IL-18Rα: IL-18 receptor α

IL-37: interleukin-37

IL-1R8: IL-1 receptor 8

LP: lamina propria

LPS: lipopolysaccharide

Ltv-Mex3B: lentiviruses carrying human Mex3B gene

Mex3B: Mex3 RNA binding family member B

Poly(I:C): polyinosinic: polycytidylic acid

RT-PCR: reverse transcription-polymerase chain reaction

TLR3: toll-like receptor 3

TNF: tumor necrosis factor

TSLP: thymic stromal lymphopoietin

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INTRODUCTION

Chronic rhinosinusitis (CRS) is a multifactorial and heterogeneous disorder of sinonasal mucosa.[1-3] Clinically, CRS can be divided into CRS with nasal polyp (CRSwNP) and CRS without nasal polyp (CRSsNP) based on the presence or absence of nasal polyp. Both CRSsNP and CRSwNP are characterized by an imbalance between the effector and regulatory activities of nasal mucosal immunity, with a preponderance of pro-inflammatory response.[4] Generally, CRSsNP is associated with a skewed type-1/type-17 response, whereas CRSwNP demonstrates a type-2 response dominated eosinophilic inflammation.[5] Nevertheless, immune responses in CRS may vary across different geographic areas and populations with distinct racial backgrounds. Over half of CRSwNP patients in East Asia, including those in China, demonstrate non-eosinophilic inflammation.[5] Despite the difference in prevalence of eosinophilic inflammation between Caucasian and Asian patients with CRSwNP, eosinophilia consistently associates with high disease severity and poor surgical outcome in both populations.[6,7] Therefore, elucidating the factors contributing to eosinophilia in CRSwNP holds important translational relevance, in that they may guide the development of novel therapeutic agents.

A number of cytokines, chemokines, and immunoglobulins have been implicated in the migration, survival, and activation of eosinophils in CRSwNP.[8,9] Recently, epithelial cell-derived thymic stromal lymphopoietin (TSLP), IL-25 and IL-33 have been indicated to initiate and promote type-2 immune responses and eosinophilic inflammation in CRSwNP.[9,10] Despite these advances, the regulation of eosinophilic

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inflammation in CRSwNP are far from being clarified.

Human interleukin (IL)-37 is a recently characterized member of the IL-1 family, which is unique in that it limits excessive inflammatory responses and prevents runaway inflammation, by suppressing innate as well as acquired immunity.[11-14] IL-37 gene consists of five splice variants (IL-37a-e), and IL-37b is the largest variant including five of the six exons.[14] Similar to IL-1α and IL-33, IL-37 emerges as a dual-function cytokine exerting potent anti-inflammatory effects via two distinct mechanisms, either extracellular (receptor-mediated) or intracellular (nuclear function). The precursor form of IL-37 can be processed by caspase-1 into mature form, and mature form interacts with Smad3 and translocates into the nucleus to exert its function.[14,15] Recently, more attention has been paid to the extracellular function of IL-37, which is mediated by hijacking the IL-18Rα and recruiting the orphan decoy IL-1R8.[15,16] Although the altered IL-37 levels have been reported in several human inflammatory and immune diseases, the pathological role of IL-37 in human diseases and some aspects of IL-37 biology remain unclarified.

In this study, we found that nasal epithelial cells were the major source of IL-37 in sinonasal cavity, and the synthesis of IL-37 was upregulated in eosinophilic and non-eosinophilic CRSwNP, and CRSsNP. However, strikingly, the secretion of IL-37 was significantly inhibited in eosinophilic CRSwNP, which might be caused by the type-2 cytokine milieu. Interestingly, for the first time, we demonstrated that IL-37 downregulated the expression of Mex3 RNA binding family member B (Mex3B), an RNA-binding protein and co-receptor of toll-like receptor 3 (TLR3), and therefore

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177 inhibited dsRNA-induced TSLP production in nasal epithelial cells. The reduced
178 IL-37 secretion associated with eosinophilic inflammation in CRSwNP. Moreover, we
179 revealed similar changes of IL-37 and Mex3B expression in other type-2 diseases,
180 including allergic rhinitis (AR) and eosinophilic asthma.
181

182 **METHODS**

183 **Subjects**

184 This study was approved by the Ethics Committee of Tongji Hospital of
185 Huazhong University of Science and Technology and every patient provided written
186 informed consent. For CRS study, 113 control subjects, 121 patients with eosinophilic
187 CRSwNP, 118 patients with non-eosinophilic CRSwNP, and107 patients with
188 CRSsNP were recruited. The diagnosis of CRSsNP and CRSwNP was made
189 according to European Position Paper on Rhinosinusitis and Nasal Polyps 2012.[1]
190 CRSwNP was classified as eosinophilic when the percentage of tissue eosinophils
191 exceeded 10% of the total number of infiltrating cells, as defined by our previous
192 study.[5] This cutoff was calculated as twice of the standard deviation of the mean
193 eosinophil percentage in controls.[5] Control subjects were those undergoing
194 septoplasty because of anatomic variations and did not have other sinonasal diseases.
195 Diseased ethmoid sinus mucosal samples from CRSsNP patients, polyp tissues from
196 CRSwNP patients, and inferior turbinate mucosal tissues from controls were collected
197 during surgery. Human nasal epithelial cells (HNECs) were scraped from polyps of
198 CRSwNP patients and from middle meatus of CRSsNP patients and control

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subjects.[10] Nasal secretions were collected using sinus packs and processed as previously described.[10,17,18] In addition, 16 allergic rhinitis (AR) subjects with concomitant nasal septum deviation along with 23 control subjects with nasal septum deviation alone were recruited. Ten subjects with eosinophilic asthma, 9 subjects with non-eosinophilic asthma, and 6 control subjects were also enrolled. Nasal epithelial cells and nasal secretions, and bronchial brushing epithelial cells and induced sputum samples were collected from AR and asthmatic patients, respectively. More information is provided in the Online Repository including Table E1.

Histology and immunohistochemistry

Hematoxylin-eosin staining, and immunohistochemical and immunofluorescence staining with specific primary antibodies (Tables E2-E4), and cell counting were conducted as previously reported.[5] More information is provided in the Online Repository.

Quantitative RT-PCR

Quantitative RT-PCR was performed with specific primers (Tables E5 and E6) as previously described.[19,20] More information is provided in the Online Repository.

Enzyme-linked immunosorbent assay (ELISA)

IL-37 and TSLP levels in the nasal secretions, tissue homogenates, induced sputum and cell culture supernatants were detected by using commercial ELISA

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kits.[19,21,22] More information is provided in the Online Repository.

Western blotting

Western blotting analysis was performed as previously reported.[23,24] More information is provided in the Online Repository including Table E7.

Cell culture and treatment

HNECs were cultured with an air-liquid interface method and treated with various cytokines and TLR agonists as mentioned elsewhere.[25-28] The BEAS-2B cell line (American Type Culture Collection, Manassas, VA), derived from human bronchial epithelium, was cultured submerged as previously described.[25] BEAS-2B cells were transfected with small interfering RNA targeting Mex3B (siMex3B; Invitrogen Thermo Fisher Scientific Company, Waltham, MA, USA) or recombinant lentiviral vectors carrying human Mex3B gene (Ltv-Mex3B; GeneChem, Shanghai, China) as previously described.[29,30] After transfection, BEAS-2B cells were further stimulated with poly(I:C) (Tocris Bioscience, Bristol, UK) and rhIL-37b (Val46-Asp218; R&D systems, Minneapolis, MN, USA). More information is provided in the Online Repository.

Flow cytometry

Cell staining and flow cytometric analysis were conducted as mentioned elsewhere.[10] More information is provided in the Online Repository including Table

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E8.

RNA sequencing analysis

Total RNA was extracted from HNECs stimulated with or without rhIL-37b (R&D systems) using TRIzol (Invitrogen, Carlsbad, Calif, USA). Library construction and sequencing were performed by Annoroad Gene Technology (Beijing, China). The libraries were sequenced on an Illumina HiSeq^{TM} 2500 platform (Illumina, San Deego, Calif, USA) according to the manufacturer's protocols with a 50-bp single-read reads length.[31,32] All primary data have been uploaded to the Sequence Read Archive (NCBI) under the accession number SRP140737. More information is provided in the Online Repository.

Mouse experiment

Female C57BL/6 mice (5-6 weeks old; Hubei Research Center of Laboratory Animal, Wuhan, China) were housed under specific pathogen-free conditions. All mice were used following protocols approved by the Animal Care and Use Committee of Tongji Hospital of Huazhong University of Science and Technology. Mice were treated with PBS or rhIL-37b (R&D systems) intranasally followed by poly(I:C) (Tocris Bioscience) treatment.[33] Histology study of murine sinonasal cavity was performed as previously reported.[25] In some experiments, respiratory sinonasal mucosa was dissected under a microscope and total RNA was extracted for quantitative RT-PCR assay.[25,29] More information is provided in the Online

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Repository.

Statistical analysis

Data distribution was tested for normality using a Kolmogorov-Smirnov test or a Shapiro-Wilk test. For continuous variables in the human tissue study, the results are expressed as median and interquartile ranges or in box and whisker plots displaying median and interquartile ranges. A Kruskal-Wallis H test was used to assess significant intergroup variability and a Mann-Whitney U 2-tailed test was used for between-group comparison. For dichotomous variables, a chi-square test or Fisher's exact test was performed to determine the difference between groups. Cell culture and animal study data are expressed as mean ± SEM and analyzed by paired or unpaired Student *t* test. Significance was accepted at $P < 0.05$. Data were analyzed by SPSS 22.0 software (SPSS Inc, Chicago, IL, USA).

For RNA sequencing analysis, the gene expression was compared between cells with and without exposure using DESeq R package (v1.18.0) and genes with corrected *P* value < 0.05 and $|\log2 \text{ (fold change)}| \geq 1$ between two groups were considered to have significantly differential expressions.[32]

RESULTS

IL-37 mRNA and protein expression are upregulated in nasal tissues in CRS

We first found that IL-37b mRNA expression was upregulated in diseased sinus mucosa from CRSsNP patients and polyp tissues from both eosinophilic and

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non-eosinophilic CRSwNP patients in comparison to inferior turbinate tissues from controls (Fig 1, A). Consistently, we found that IL-37 protein levels in tissues were elevated in CRS patients compared with control subjects as measured by ELISA (Fig 1, B). Immunohistochemistry and immunofluorescence study demonstrated the immunoreactivity of IL-37 in nasal epithelial cells and infiltrating cells in lamina propria (LP) in sinonasal tissues (Fig 1, C and Fig E1, A). The staining intensity of IL-37 in epithelial cells and the number of IL-37$^+$ cells in LP in sinonasal tissues were increased in CRS patients compared to control subjects (Fig 1, D and E). In LP, we found that IL-37 was expressed by CD68$^+$ macrophages, tryptase$^+$ mast cells, CD3$^+$ T cells and CD19$^+$ B cells (Fig E2). However, we did not find significant expression of IL-37 in submucosal glands or mucin 5AC positive goblet cells in epithelium (Fig E1).

IL-37 levels in nasal secretions are downregulated in eosinophilic CRSwNP

Given the significant expression of IL-37 in nasal epithelial cells in CRS as revealed by immunohistochemistry, we further measured IL-37 expression in scraped HNECs (Fig 2, A and B). Enhanced mRNA and protein expression of IL-37 were confirmed in nasal epithelial cells from CRS patients by quantitative RT-PCR and western blotting analysis, respectively (Fig 2, A and B). IL-37 can translocate into the nucleus to exert its anti-inflammatory effect.[14,15] However, we did not find significant presence of IL-37 in nucleus in nasal epithelial cells in different subject groups (Fig E3), disfavoring an intracellular mechanism for IL-37 in CRS. We therefore turned to

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detect the secreted IL-37 and were surprised to find a significant reduction of IL-37 levels in nasal secretions in eosinophilic CRSwNP patients compared with patients with other types of CRS and control subjects (Fig 2, C). We further confirmed this finding by western blotting analysis (Fig 2, D). In western blotting analysis, two forms of IL-37 with different molecular weights (25 and 45 KDa) were identified (Fig 2, B and D), indicating the presence of both monomeric and dimeric IL-37.[14]

Type-2 cytokines inhibit the secretion of IL-37 from nasal epithelial cells

The reduced IL-37 levels in nasal secretions in eosinophilic CRSwNP drive us to explore the regulation of IL-37 production and secretion by the cytokines and TLR agonists which are implicated in the pathogenesis of CRS.[6,10] We found that IL-37b mRNA levels in HNECs obtained from control subjects were upregulated by TLR agonists (LPS, CpG, and poly(I:C)), proinflammatory cytokines (TNF-α), type-1 cytokine (IFN-γ), type-2 cytokines (IL-4 and IL-13), and type-17 cytokine (IL-17A) (Fig 3, A). Consistently, western blotting analysis showed the up-regulation of IL-37 production, particularly the dimeric form at 45KDa, by these cytokines and TLR agonists in HNECs (Fig 3, B). In accordance with the IL-37 production in HNECs, we found that IL-37 levels in culture supernatants were enhanced by LPS, CpG, IFN-γ, TNF-α and poly(I:C) (Fig 3, C). However, in contrast to IL-37 production in HNECs, IL-37 levels in culture supernatants were significantly downregulated by IL-4 and IL-13 after 24-hour treatment (Fig 3, C). Furthermore, we found that LPS and CpG promoted, whereas IL-4 and IL-13 suppressed, ATP-induced IL-37 secretion from

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HNECs to culture supernatants after 3-hour pretreatment (Fig 3, D). We found that HNECs from CRS patients and BEAS-2B cells had similar response pattern in IL-37 expression to the treatment with these stimulators in comparison to HNECs from control subjects (data not shown).

IL-37 downregulates Mex3B in nasal epithelial cells

Given to the reduced extracellular IL-37 levels in eosinophilic CRSwNP, we next specifically explored extracellular mechanisms for IL-37 in CRS. Extracellular IL-37 acts through binding to IL-18Rα and IL-1R8. By immunofluorescence staining, we found obvious co-localization of IL-18Rα and IL-1R8 mainly in epithelial cells in nasal tissues (Fig 4, A). Flow cytometric analysis of dispersed nasal tissue cells confirmed that IL-18Rα and IL-1R8 were co-expressed on nasal epithelial cells, and the co-expression of IL-18Rα and IL-1R8 was elevated in nasal epithelial cells in CRS patients (Fig E4).

To dissect the mechanisms underlying IL-37 function, we treated HNECs obtained from eosinophilic CRSwNP patients with or without rhIL-37b. By RNA sequencing analysis, we identified 3 upregulated genes and 17 downregulated genes in rhIL-37b treated HNECs compared with those without rhIL-37b treatment (control) (Fig 4, B). Some of these genes are involved in innate immune response, whereas others are involved in cell proliferation, negative regulation of transcription, various metabolic pathways and other functions. Among these genes, Mex3B showed consistent expression across different samples in both groups with and without

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rhIL-37 treatment, and its mRNA expression was downregulated by IL-37 (IL-37 treatment vs. control: 3.03-fold decrease). Recently, Mex3B has been identified as a co-receptor of TLR3 that is important for innate antiviral response.[34] To validate the RNA sequencing results, we treated HNECs obtained from additional eosinophilic CRSwNP patients with or without rhIL-37b. Again, we found that Mex3B was downregulated by rhIL-37b in HNECs at both mRNA and protein level by quantitative RT-PCR and Western blotting analysis, respectively (Fig 4, C and D).

As well as in HNECs, we found the co-expression of IL-18Rα and IL-1R8 in BEAS-2B cells by immunofluorescence staining (Fig E5, A). We also demonstrated dose-dependent inhibition of Mex3B expression in BEAS-2B cells by rhIL-37b (Fig E5, B and C).

Mex3B expression is upregulated in epithelial cells in eosinophilic CRSwNP

Exploring Mex3B expression in CRS, we found that Mex3B mRNA expression was upregulated in scrapped HNECs from CRS patients compared with those from control subjects and importantly eosinophilic CRSwNP showed the highest level of expression (Fig 5, A). Immunohistochemistry study revealed Mex3B expression in epithelial cells and infiltrating cells in sinonasal tissues, and the staining intensity of Mex3B in epithelium was increased in all types of CRS compared with controls (Fig 5, B and C). Again, eosinophilic CRSwNP demonstrated the highest level of Mex3B protein expression in epithelial cells (Fig 5, C). By western blotting analysis, we confirmed enhanced protein expression of Mex3B in epithelial cells in CRS,

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375 especially in eosinophilic CRSwNP (Fig 5, D). Interestingly, we found a negative
376 correlation between IL-37 level in nasal secretions and Mex3B mRNA level in nasal
377 epithelial cells in patients with eosinophilic CRSwNP (Fig 5, E).

378

379 **IL-37b suppresses TLR3 signaling activation by downregulating Mex3B**
380 **expression in nasal epithelial cells**

381 Recently, Mex3B has been identified to be a co-receptor of TLR3.[34] By
382 immunohistochemical staining, we first found that TLR3 was expressed by epithelial
383 cells and infiltrating cells in LP in sinonasal mucosal tissues and the staining intensity
384 of TLR3 was significantly upregulated in eosinophilic CRSwNP, but not other types
385 of CRS, compared with controls (Fig E6, A and B). Consistently, we observed an
386 upregulation of TLR3 mRNA expression in scrapped nasal epithelial cells from
387 eosinophilic CRSwNP patients compared with those from control subjects and
388 patients with other types of CRS (Fig E6, C).

389 We next discovered the co-localization of Mex3B and TLR3 in epithelial cells
390 and infiltrating cells in LP in sinonasal tissues, especially in eosinophilic nasal polyps
391 (Fig 6, A). We then studied the effect of IL-37 on TLR3 pathway activation in HNECs
392 from eosinophilic CRSwNP. We found that poly(I:C) enhanced Mex3B mRNA
393 expression, which was abolished by rhIL-37b pretreatment (Fig 6, B). Moreover, we
394 found that the poly(I:C)-induced production of TSLP, interferon (IFN)-α, and IFN-β
395 mRNA in HNECs was markedly suppressed by rhIL-37b pretreatment (Fig 6, C-E).
396 Western blotting analysis demonstrated the similar changes of Mex3B and TSLP

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protein expression after poly(I:C) and rhIL-37b treatment as their mRNA expression (Fig 6, F). The anti-IL-18Rα pre-incubation was able to diminish the effects of rhIL-37b on poly(I:C)-induced Mex3B and TSLP mRNA production in HNECs (Fig 6, G and H), confirming the utilization of IL-18Rα by extracellular IL-37 to exert its function.

Furthermore, we found that knocking down Mex3B expression with specific small interfering RNA abolished the induction of Mex3B and TSLP in BEAS-2B cells by poly(I:C) at both mRNA and protein level (Fig E7, A-C). Importantly, we found that rhIL-37b was unable to further downregulate Mex3B and TSLP mRNA and protein expression after siMex3B treatment in BEAS-2B cells (Fig 7, A-C). In addition, rhIL-37b lost its inhibitory effect on poly(I:C)-induced TSLP expression if Mex3B was overexpressed by transfecting with Ltv-Mex3B (Fig 7, D-F). Transfection efficiency were confirmed at both mRNA and protein level (Fig E7, D-E). In contrast, we did not find significant effect of rhIL-37b on TLR3 expression in BEAS-2B cells and HNECs from patients with eosinophilic CRSwNP (Fig E8). These findings suggest that IL-37 may inhibit TLR3 activation by modulating the expression of Mex3B.

Finally, we explore the IL-37 effect *in vivo*. Although the IL-37 homologue is absent in the mouse, human IL-37 has been found functional in several murine models.[13,33,35,36] We demonstrated co-expression of IL-18Rα and IL-1R8 in nasal epithelial cells in mice (Fig E9), and found that poly(I:C) induced the mRNA expression of Mex3B, TSLP, IFN-α, and IFN-β in murine nasal tissues, which was

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significantly suppressed by rhIL-37b treatment (Fig E10, A). Immunohistochemistry study consistently showed the changes of those molecules in nasal epithelial cells at protein level after treatment with poly(I:C) and rhIL-37b (Fig E10, B and C).

Correlations of IL-37 with TSLP and eosinophilic inflammation in patients with eosinophilic CRSwNP

Our previous study demonstrated significantly increased expression of TSLP in nasal epithelial cells, which correlated with mucosal eosinophil infiltration, in patients with eosinophilic CRSwNP.[10] In this study, we further discovered enhanced levels of TSLP in nasal secretions from patients with eosinophilic CRSwNP compared to those from controls and patients with other types of CRS (Fig 8, A), which also correlated with eosinophil infiltration in eosinophilic polyps (Fig 8, B). Moreover, we found negative correlations of IL-37 levels in nasal secretions with TSLP levels in nasal secretions and mucosal eosinophilia in patients with eosinophilic CRSwNP (Fig 8, C and D). In addition, a positive correlation between Mex3B mRNA levels in epithelial cells and TSLP levels in nasal secretions was revealed in patients with eosinophilic CRSwNP (Fig 8, E).

Expression of IL-37 and Mex3B in AR and eosinophilic asthma

In order to validate the importance of IL-37 in type-2 diseases, we further explored the expression of IL-37 and Mex3B in patients suffering from AR and asthma. We found that the mRNA expression of IL-37 was upregulated in airway

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441 epithelial cells, whereas the IL-37 protein levels in airway fluids were downregulated,
442 in subjects with AR and eosinophilic asthma (Fig E11, A and B). In addition, we
443 found that Mex3B mRNA expression levels were elevated in nasal and bronchial
444 epithelial cells in AR and eosinophilic asthmatic patients, respectively (Fig E11, C
445 and D). There were negative correlations between Mex3B mRNA expression in nasal
446 epithelial cells and IL-37 level in nasal secretions, and between Mex3B mRNA
447 expression in bronchial epithelial cells and IL-37 level in induced sputum (Fig E11, C
448 and D).
449

450 **DISCUSSION**

451 The biology of IL-37 and its pathological role in human diseases remain to be
452 defined. In this study, we provided evidence for a novel Mex3B-mediated mechanism
453 for IL-37 to finely tune the activation of TLR3 in nasal epithelial cells and its
454 involvement in the development of eosinophilic CRSwNP.

455 IL-37 is expressed by various immune and non-immune cells, including
456 monocytes, macrophages, dendritic cells, and epithelial cells of skin and gut.[14,37,38] Its
457 expression was found either upregulated or downregulated in different inflammatory
458 and immune conditions.[33,38] Lunding L et al found that the IL-37 production from
459 peripheral blood mononuclear cells was significantly lower in allergic asthmatics
460 compared to healthy children, whereas Saenz et al reported that IL-37 expression was
461 significantly upregulated in intestinal epithelial cells in patients with inflammatory
462 bowel diseases.[33,38] In the present study, we found that IL-37 production was

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up-regulated in sinonasal tissues, especially in epithelial cells, in all types of CRS in comparison with that in controls. However, we were surprised to find that the secretion of IL-37 to nasal cavity was significantly downregulated in eosinophilic CRSwNP characterized by a predominate type-2 response. We further found that type-2 cytokines, but not type-1 or type-17 cytokines, significantly inhibited IL-37 release from nasal epithelial cells although they promoted its production. Previous studies reported that IL-4 inhibited NLRP3-dependent caspase-1 activation and subsequent secretion of IL-1β, another IL-1 cytokine family member, from epidermal cells, monocytes and macrophages.[39,40] Human IL-37 also has a caspase-1 cleavage site and the precursor form of IL-37 can be processed by caspase-1 into mature form, which can be translocated to nuclear or secreted to extracellular fluid.[14,15] Our preliminary data showed that IL-4 and IL-13, but not IFN-γ or IL-17A，inhibited caspase-1 activity in nasal epithelial cells (data not shown), which may contribute to the reduced secretion of IL-37 induced by type-2 cytokines. However, the precise mechanisms underlying the inhibition of IL-37 secretion from nasal epithelial cells by type-2 cytokines certainly require further investigations. Compared to controls, there was no upregulation of IL-37 in nasal secretions in patients with CRSsNP or non-eosinophilic CRSwNP, suggesting the presence of other undefined factors in suppressing IL-37 secretion from nasal epithelial cells in CRS. In addition to epithelial cells, IL-37 was also expressed by inflammatory cells in LP. The regulation of IL-37 production in these cells require further investigations as well.

IL-37 functions either extracellularly (receptor-mediated) or intracellularly

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(nuclear function).[14-16,33,41] In this study, we only detected weak IL-37 expression in nuclei of nasal epithelial cells and no significant difference was found among different study groups, disfavoring an intracellular mechanism for IL-37 in CRS. In contrast, the reduced abundance of IL-37 in nasal secretions in eosinophilic CRSwNP likely points to an extracellular mechanism for IL-37 in eosinophilic CRSwNP. Extracellular IL-37 acts through binding to its receptors IL-18Rα and IL-1R8.[16] IL-18Rα has been found expressed on intestinal epithelial cells, GC B cells, peripheral T lymphocytes, and murine tracheobronchial epithelial cells.[42-45,] While IL-1R8 has been reported to be expressed on murine tracheobronchial epithelial cells, monocytes and macrophages.[41,43] In the present study, for the first time, we demonstrated co-expression of IL-18Rα and IL-1R8 predominantly in nasal epithelial cells in both human and murine nasal mucosa, suggesting that nasal epithelial cells are an important and previously unrecognized target of extracellular IL-37.

The downstream events of IL-37-IL-18Rα/IL-1R8 signaling activation remain largely unknown. In this study, by employing high-throughput RNA sequencing analysis, we identified a novel target of IL-37-IL-18Rα/IL-1R8 signaling activation, Mex3B, in nasal epithelial cells. A recent elegant study demonstrated that Mex3B is a co-receptor of TLR3 and positively regulates TLR3 signaling activation.[34] TLR3 activation not only induces the production of type I IFN, the cytokines critical for anti-viral defense, but also the production of TSLP, which plays an important role in initiation and exaggeration of type-2 response, from airway epithelial cells.[10,46] Previous studies suggest a potential role of viral infection in CRS.[47-49] Viral infection

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may not only arouse immune response of host but also impair the nasal epithelial barrier function and lead to subsequent bacterial infection in CRS.[50] Jang et al demonstrated that rhinovirus, which was absent in control patients, could be detected in 21% of patients with CRS.[48] We previously showed that one of the four common respiratory viruses including picornavirus, influenza virus types A and B, and respiratory syncytial virus could be detected in 70% of patients with eosinophilic CRSwNP.[51] In addition, upregulation of TSLP in epithelial cells has been well documented in CRSwNP, associating with eosinophilic inflammation.[10,52] We therefore investigated whether IL-37 can regulate TLR3 activation via Mex3B in nasal epithelial cells.

Here, we provide several lines of novel evidence for the Mex3B-mediated suppressive function of IL-37 on TLR3 signaling activation. First, we demonstrated colocalization of TLR3 and Mex3B in nasal epithelial cells. Second, we found that IL-37 could suppress the activation of TLR3 as reflected by the downregulation of type I IFN and TSLP production in nasal epithelial cells both *in vitro* and *in vivo*. Third and most importantly, we demonstrated that knocking down or overexpressing Mex3B abolished the suppressive effect of IL-37. Fourth, negative correlations of IL-37 levels in nasal secretions with Mex3B mRNA expression in nasal epithelial cells and TSLP levels in nasal secretions were revealed in patients with eosinophilic CRSwNP. In this study, we found that TLR3 activation upregulated Mex3B expression, suggesting that TLR3 activation can self-amplify its signaling transduction.

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One limitation of our present study is the small sample size for the RNA sequencing study. Although we cannot exclude the possibility of missing some important genes regulated by IL-37 due to the small sample size, the regulation of Mex3B by IL-37 has been confirmed by additional studies with different techniques and larger sample size.

In summary, our study highlights a novel Mex3B-mediated mechanism for the inhibitory effect of extracellular IL-37 on TLR3 activation in airway epithelial cells. Type-2 cytokines inhibit the secretion of IL-37 from nasal epithelial cells. Such inhibition may result in exaggerated activation of TLR3 signaling pathway and excessive production of TSLP, and therefore promote the development of mucosal type-2 response and eosinophilic inflammation in CRSwNP, as supported by the negative correlation between IL-37 levels in nasal secretions and mucosal eosinophilia in patients with eosinophilic CRSwNP. The similar change of IL-37 and Mex3B expression was also noted in AR and eosinophilic asthma, indicating a broad role of IL-37 in the pathogenesis of type-2 diseases. Our findings here not only extend our understanding of the pathogenesis of CRSwNP, but also add new knowledge to the mechanisms underlying the inhibitory effect of IL-37 on innate immunity.

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FIGURE LEGENDS

Fig 1. IL-37 mRNA and protein production are upregulated in nasal tissues in CRS, especially in epithelial cells. A, IL-37b mRNA expression in sinonasal mucosal samples from different study groups as detected by quantitative RT-PCR. **B,** The protein levels of IL-37 in tissue homogenates from different study groups as measured by ELISA. **C,** The immunoreactivity of IL-37 in sinonasal mucosa as detected by immunohistochemistry. The representative photomicrographs are shown (original magnification × 400). **D,** The staining intensity in epithelium was quantified and the results were presented as average optical density value per unit area. **E,** The positive cells in the lamina propria (LP) were counted at high-power field (HPF). Eos CRSwNP, eosinophilic chronic rhinosinusitis with nasal polyps; Non-Eos CRSwNP, non-eosinophilic chronic rhinosinusitis with nasal polyps; CRSsNP, chronic

rhinosinusitis without nasal polyps.

Fig 2. Reduced secretion of IL-37 in eosinophilic CRSwNP. A, IL-37b mRNA expression in scrapped nasal epithelial cells from different study groups as detected by quantitative RT-PCR. **B,** The protein levels of IL-37 in whole cell lysates of nasal epithelial cells as detected by western blotting. Representative blots are shown and densitometric analysis of blots was performed. **C，** The protein levels of IL-37 in nasal secretions as measured by ELISA. **D,** IL-37 in nasal secretions as detected by western blotting. Reversible Ponceau staining was applied to check equal loading. Representative blots are shown. Eos CRSwNP, eosinophilic chronic rhinosinusitis with nasal polyps; Non-Eos CRSwNP, non-eosinophilic chronic rhinosinusitis with nasal polyps; CRSsNP, chronic rhinosinusitis without nasal polyps.

Fig 3. Type-2 cytokines inhibit the secretion of IL-37 from nasal epithelial cells. Human nasal epithelial cells (HNECs) from control subjects were stimulated with various cytokines and TLR agonists. **A,** After 6-hour stimulation, cells were subjected to quantitative RT-PCR analysis of IL-37 mRNA expression (n = 6). **B,** After 24-hour stimulation, cells were subjected to western blotting analysis of IL-37 protein expression. Representative blots are shown and densitometric analysis of blots was performed (n = 6). **C,** After 24-hour of stimulation, IL-37 levels in culture supernatants were measured by ELISA (n = 6). **D,** After 3-hour pre-incubation of various cytokines and TLR agonists, cells were further stimulated with 5 mM ATP for

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30 min. IL-37 levels in culture supernatants were measured by means of ELISA (n = 6). * $P < 0.05$ and ** $P < 0.01$, as compared to control.

Fig 4. IL-37b downregulates the Mex3B expression in nasal epithelial cells. A, Co-localization of IL-18Rα and IL-1R8 expression in nasal epithelial cells in nasal tissues as revealed by immunofluorescence staining. The representative photomicrographs show immunostaining of tissue section from a patient with eosinophilic CRSwNP (original magnification × 400). Insets show a higher magnification of the outline area, and arrows denote representative positive cells. **B,** The heat map shows genes significantly regulated by rhIL-37b in human nasal epithelial cells (HNECs) from 3 patients with eosinophilic CRSwNP as detected by RNA sequencing. **C-D,** Confirmation of downregulation of Mex3B gene by rhIL-37b in HNECs from additional 9 eosinophilic CRSwNP patients as detected by quantitative RT-PCR (C) and western blotting analysis (D). In Fig 4, D, representative blots are shown and densitometric analysis of blots was performed.

Fig 5. Mex3B expression is upregulated in nasal epithelial cells in eosinophilic CRSwNP. A, Mex3B mRNA expression in scrapped nasal epithelial cells from different study groups as detected by quantitative RT-PCR. **B,** The immunoreactivity of Mex3B in sinonasal tissues as detected by immunohistochemistry. The representative photomicrographs are shown (original magnification × 400). **C,** The staining intensity of Mex3B in epithelium was quantified and the results were

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794 presented as average optical density value per unit area. **D,** Mex3B protein expression
795 in scrapped nasal epithelial cells from different study groups as detected by western
796 blotting. Representative blots are shown and densitometric analysis of blots was
797 performed. **E,** The IL-37 levels in nasal secretions negatively correlated with the
798 Mex3B mRNA expression levels in nasal epithelial cells in patients with eosinophilic
799 CRSwNP.
800
801 **Fig 6. IL-37b suppresses Mex3B expression and TLR3 signaling activation in**
802 **nasal epithelial cells. A,** Co-localization of Mex3B and TLR3 expression in sinonasal
803 mucosa, particularly in epithelial cells, as revealed by immunofluorescence staining.
804 The representative photomicrographs show immunostaining of tissue section from a
805 patient with eosinophilic CRSwNP (original magnification × 400). Insets show a
806 higher magnification of the outline area, and arrows denote representative positive
807 cells. **B-E,** rhIL-37b inhibited the mRNA expression of Mex3B (B), TSLP (C), IFN-α
808 (D), and IFN-β (E) in human nasal epithelial cells (HNECs) induced by poly(I:C) as
809 detected by quantitative RT-PCR (n = 6). **F,** rhIL-37b inhibited the protein expression
810 of Mex3B and TSLP in HNECs induced by poly(I:C) as detected by western blotting.
811 Representative blots are shown and densitometric analysis of blots was performed (n
812 = 6). $^{**}P < 0.01$. **G and H,** Anti-IL-18Rα treatment abolished the inhibitory effect of
813 rhIL-37b on Mex3B and TSLP mRNA expression in HNECs induced by poly(I:C) (n
814 = 6). HNECs were obtained from patients with eosinophilic CRSwNP.
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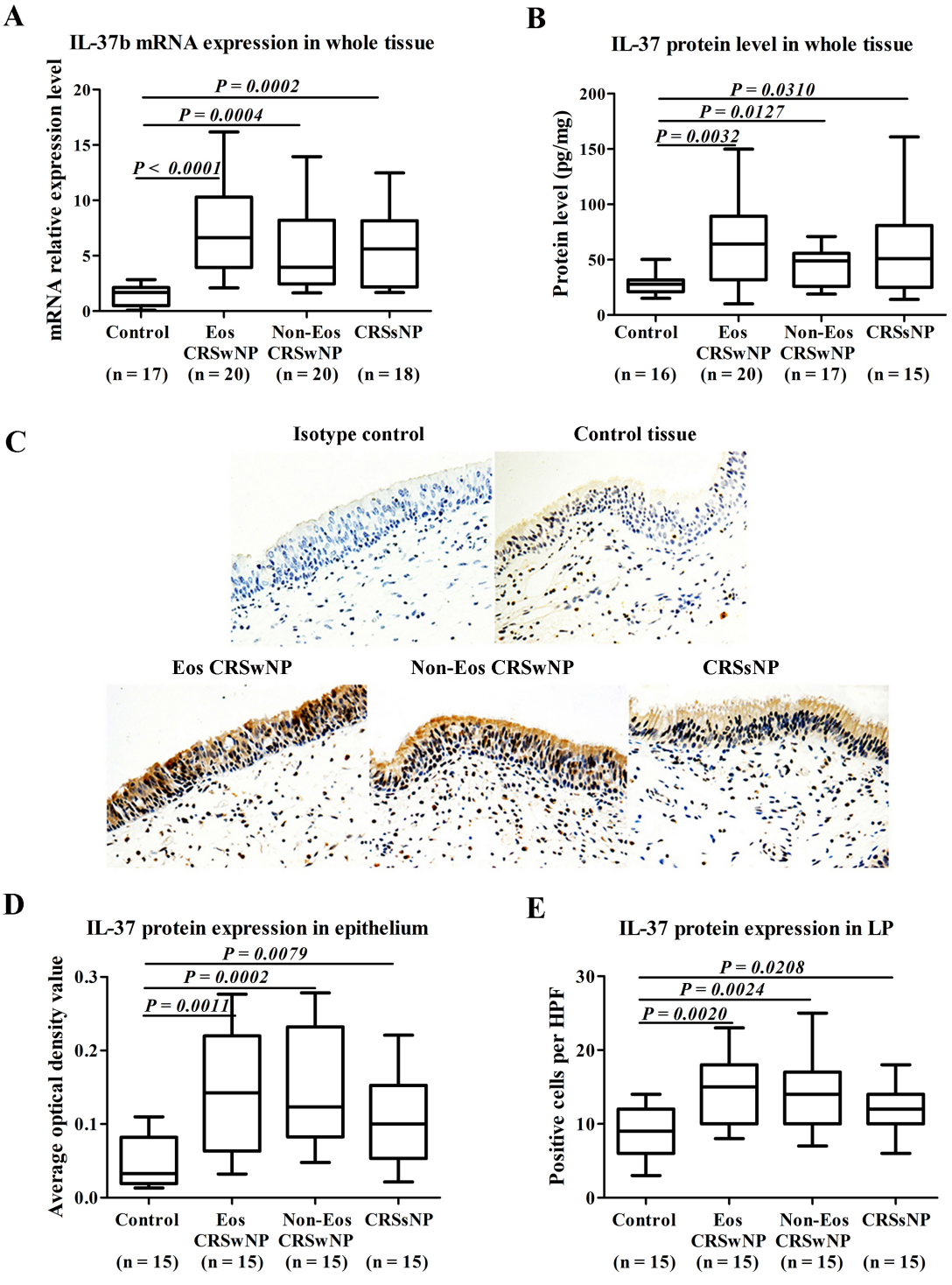
Fig 7. The inhibitory effect of IL-37b on TLR3 signaling activation is dependent on Mex3B. A-C, After siMex3B transfection, rhIL-37b was unable to further downregulate the Mex3B and TSLP mRNA and protein expression in poly(I:C)-treated BEAS-2B cells as detected by RT-PCR (A and B) and western blotting (C), respectively. In Fig 7, C, representative blots are shown and densitometric analysis was performed (n = 6). **D-F,** After Ltv-Mex3B transfection, rhIL-37b was unable to inhibit the Mex3B and TSLP mRNA and protein expression induced by poly(I:C) in BEAS-2B cells as detected by RT-PCR (D and E) and western blotting (F), respectively. In Fig 7, F, representative blots are shown and densitometric analysis was performed (n = 6). $^{**}P < 0.01$. siNC, negative control siRNA; Ltv-vector, negative control lentiviruses.

Fig 8. Correlations of IL-37 with TSLP and eosinophilic inflammation in patients with eosinophilic CRSwNP. A, The protein levels of TSLP in nasal secretions as measured by ELISA. **B,** The mucosal eosinophil counts positively correlated with TSLP levels in nasal secretions. **C,** The IL-37 and TSLP levels in nasal secretions were negatively correlated. **D,** The mucosal eosinophil counts negatively correlated with IL-37 levels in nasal secretions. **E,** Mex3B mRNA expression levels in nasal epithelial cells positively correlated with TSLP levels in nasal secretions.

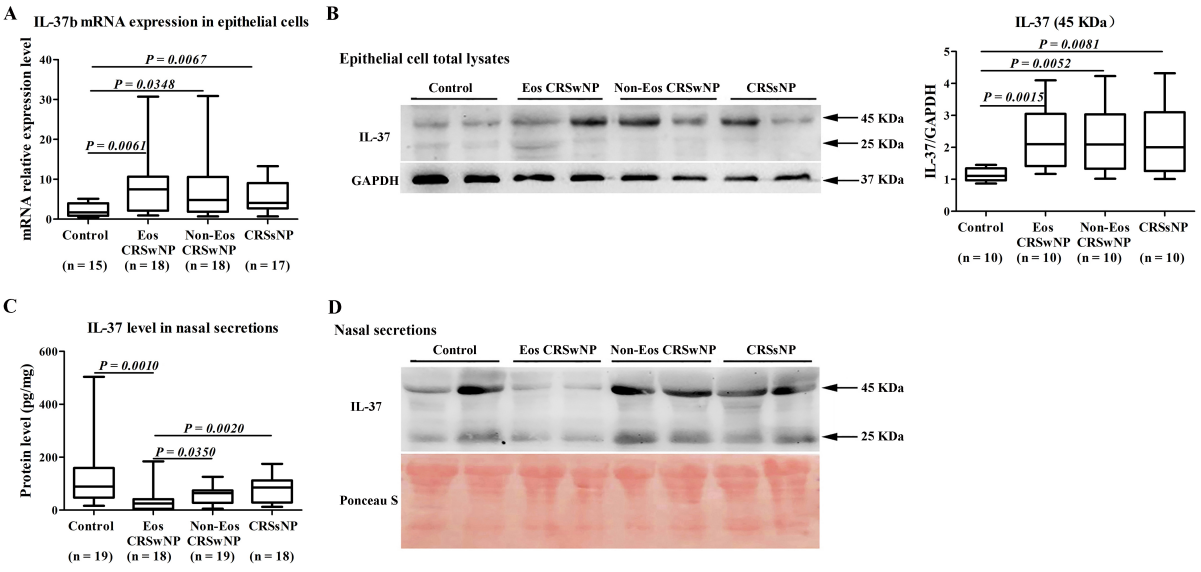
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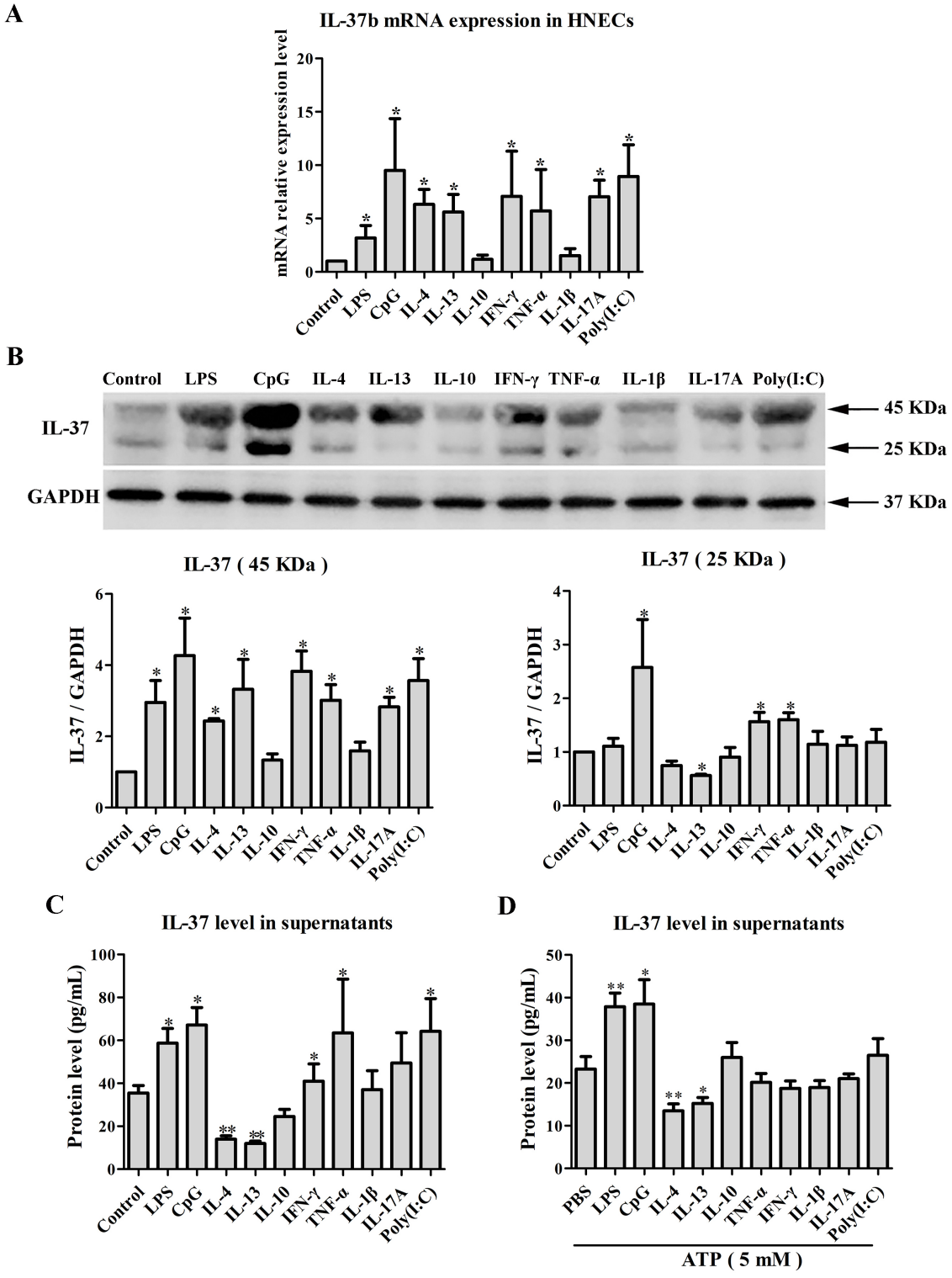
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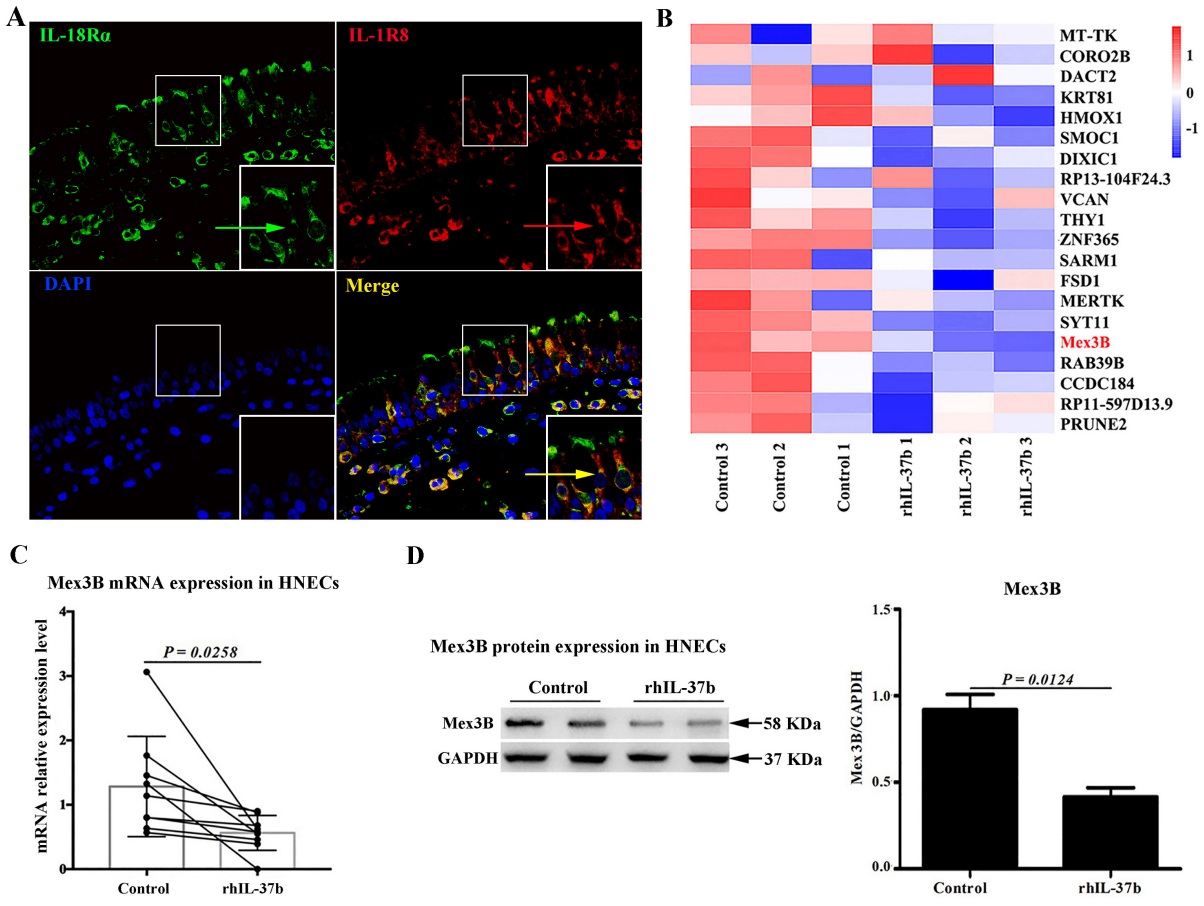

A
IL-37b mRNA expression in whole tissue
mRNA relative expression level
P = 0.0002
P = 0.0004
P < 0.0001
Control (n = 17)
Eos CRSwNP (n = 20)
Non-Eos CRSwNP (n = 20)
CRSsNP (n = 18)
B
IL-37 protein level in whole tissue
Protein level (pg/mg)
P = 0.0310
P = 0.0127
P = 0.0032
Control (n = 16)
Eos CRSwNP (n = 20)
Non-Eos CRSwNP (n = 17)
CRSsNP (n = 15)
C
Isotype control
Control tissue
Eos CRSwNP
Non-Eos CRSwNP
CRSsNP
D
IL-37 protein expression in epithelium
Average optical density value
P = 0.0079
P = 0.0002
P = 0.0011
Control (n = 15)
Eos CRSwNP (n = 15)
Non-Eos CRSwNP (n = 15)
CRSsNP (n = 15)
E
IL-37 protein expression in LP
Positive cells per HPF
P = 0.0208
P = 0.0024
P = 0.0020
Control (n = 15)
Eos CRSwNP (n = 15)
Non-Eos CRSwNP (n = 15)
CRSsNP (n = 15)

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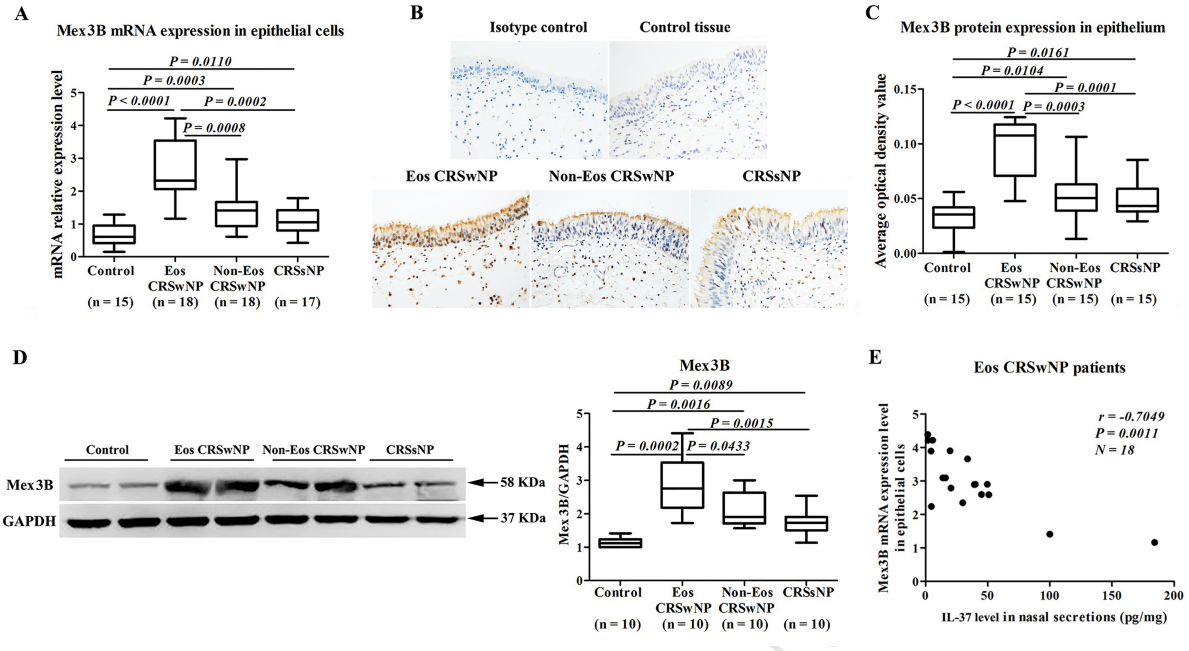

A
IL-37b mRNA expression in HNECs
mRNA relative expression level
Control
LPS
CpG
IL-4
IL-13
IL-10
IFN-γ
TNF-α
IL-1β
IL-17A
Poly(I:C)
B
IL-37
GAPDH
45 KDa
25 KDa
37 KDa
IL-37 (45 KDa)
IL-37 / GAPDH
IL-37 (25 KDa)
C
IL-37 level in supernatants
Protein level (pg/mL)
D
IL-37 level in supernatants
PBS
ATP (5 mM)

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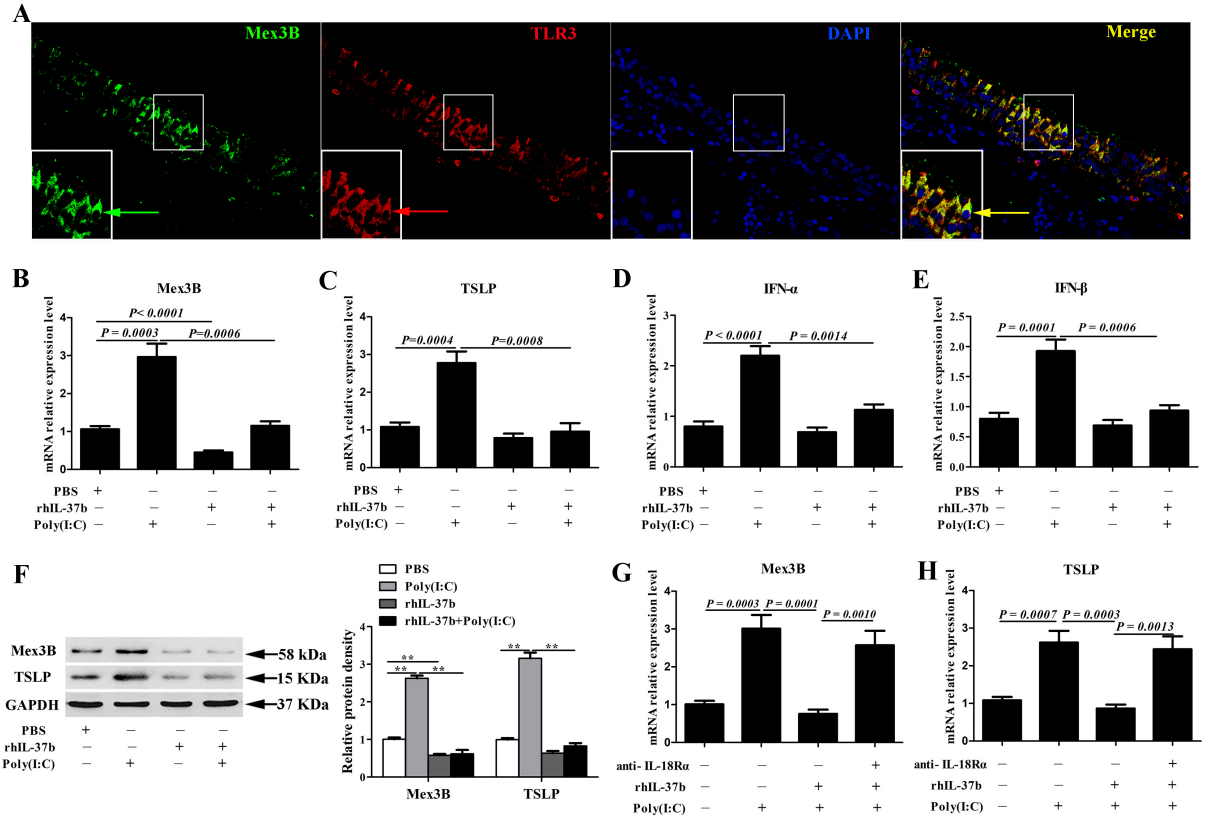


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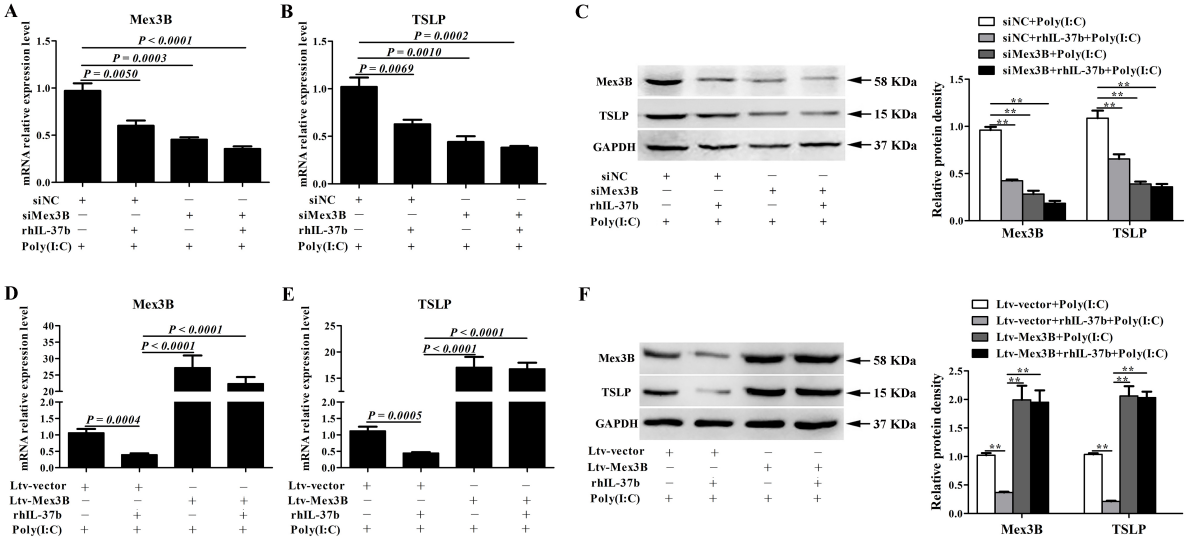
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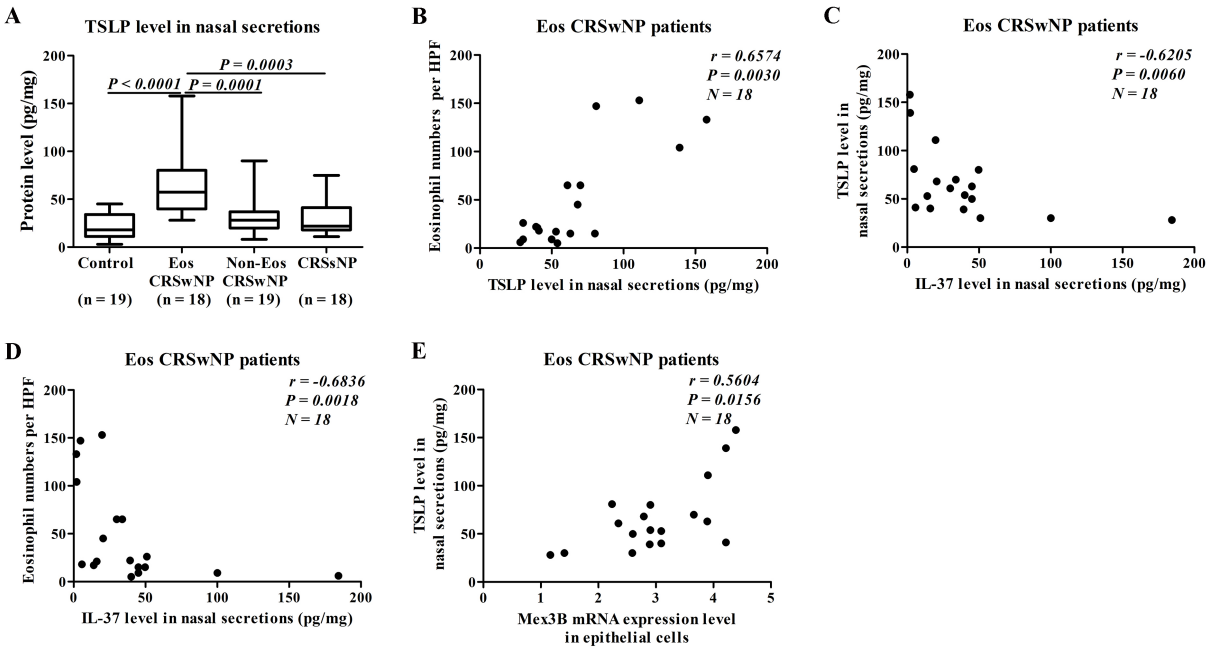
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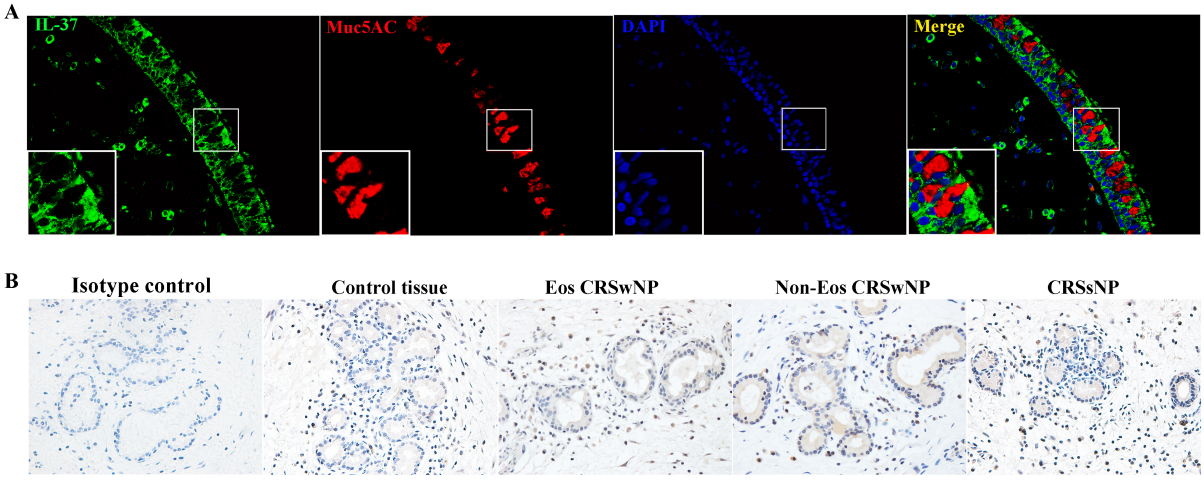
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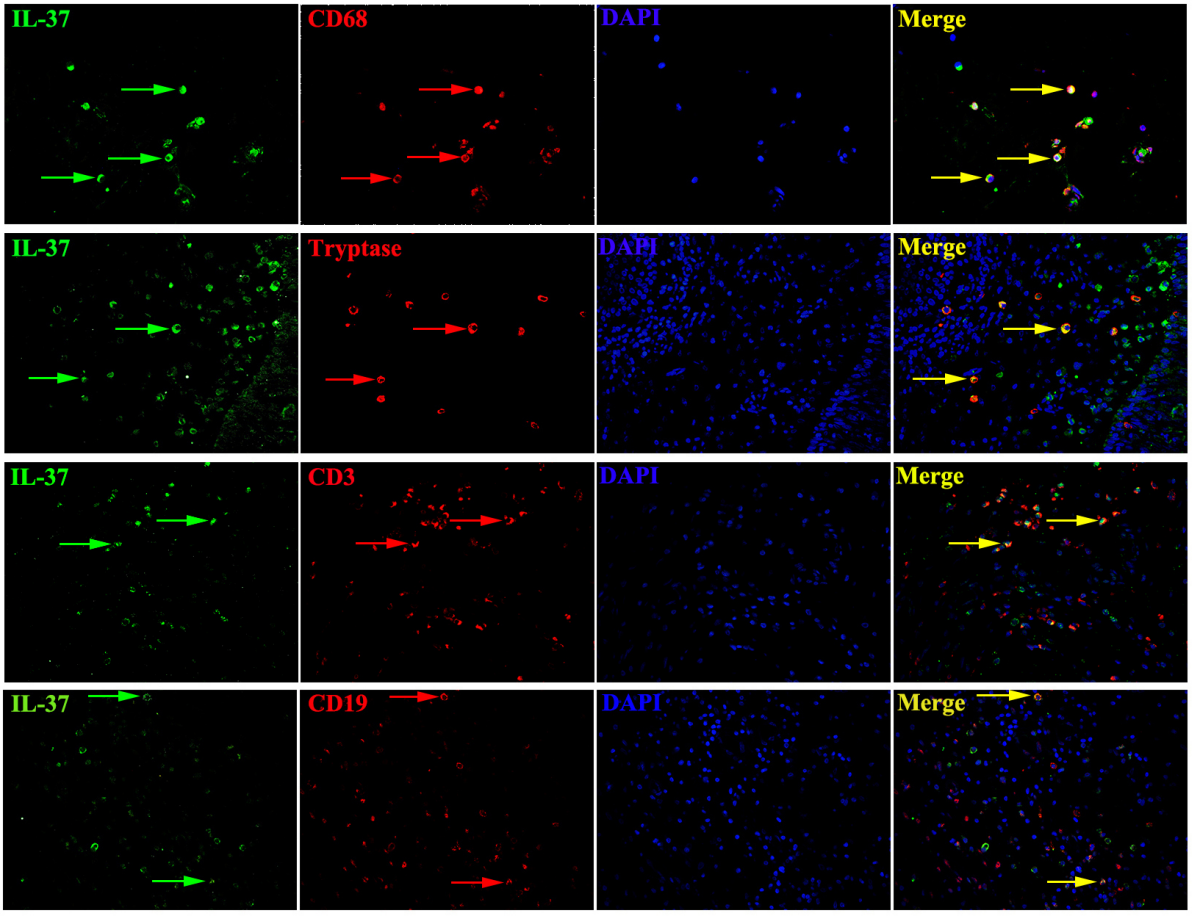
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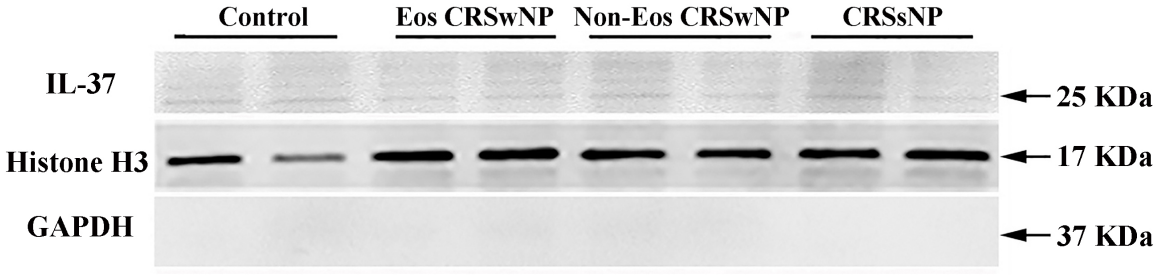
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IL-37
CD68
DAPI
Merge
IL-37
Tryptase
DAPI
Merge
IL-37
CD3
DAPI
Merge
IL-37
CD19
DAPI
Merge

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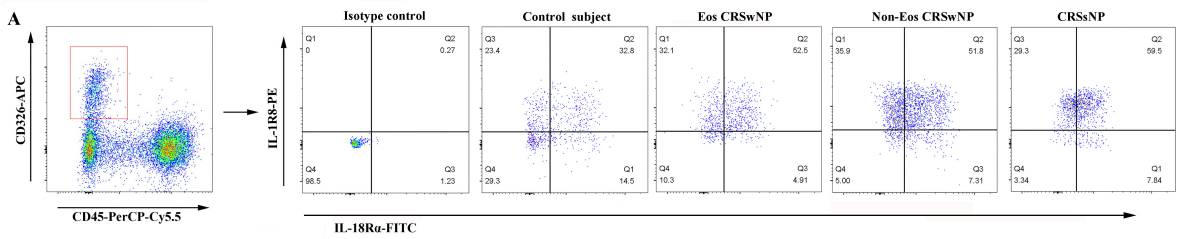
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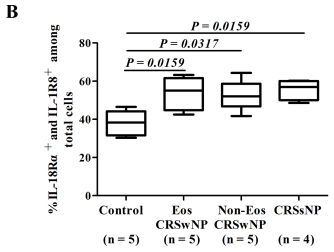
Epithelial cell nucleus lysates

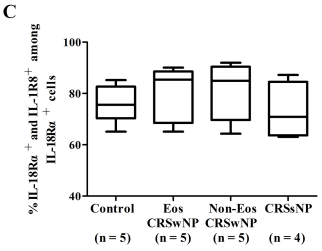


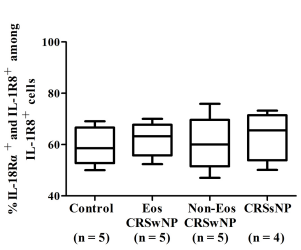
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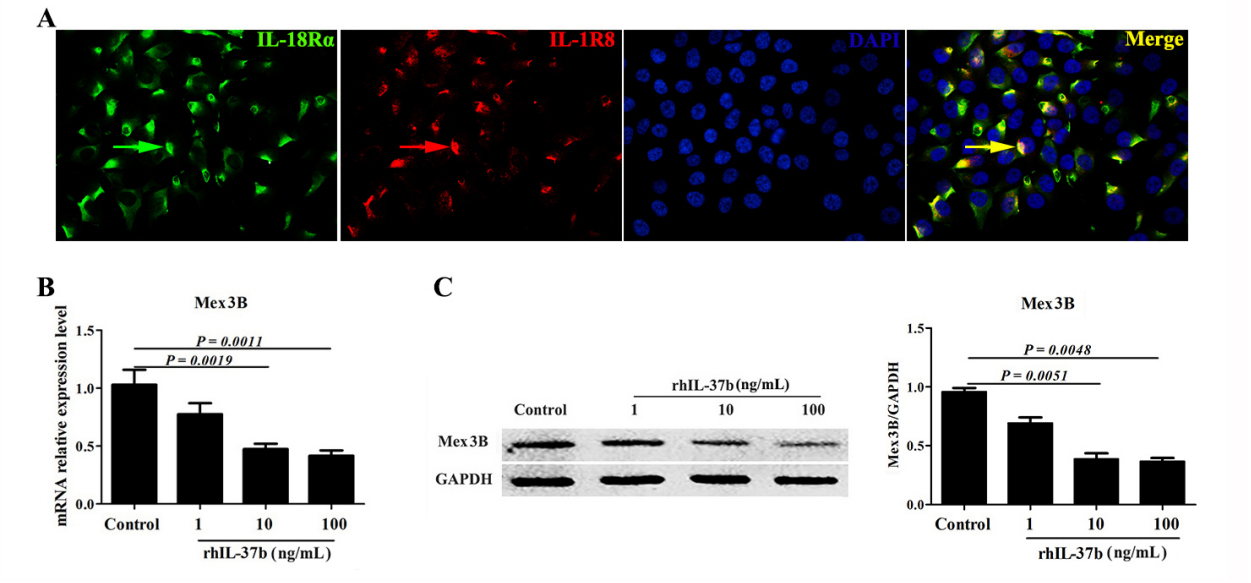
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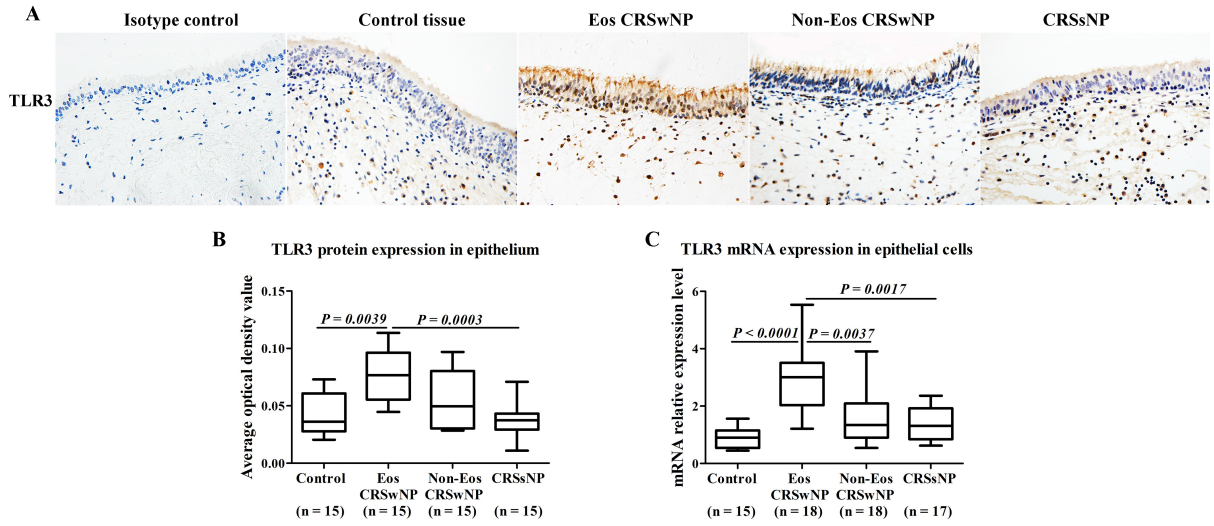




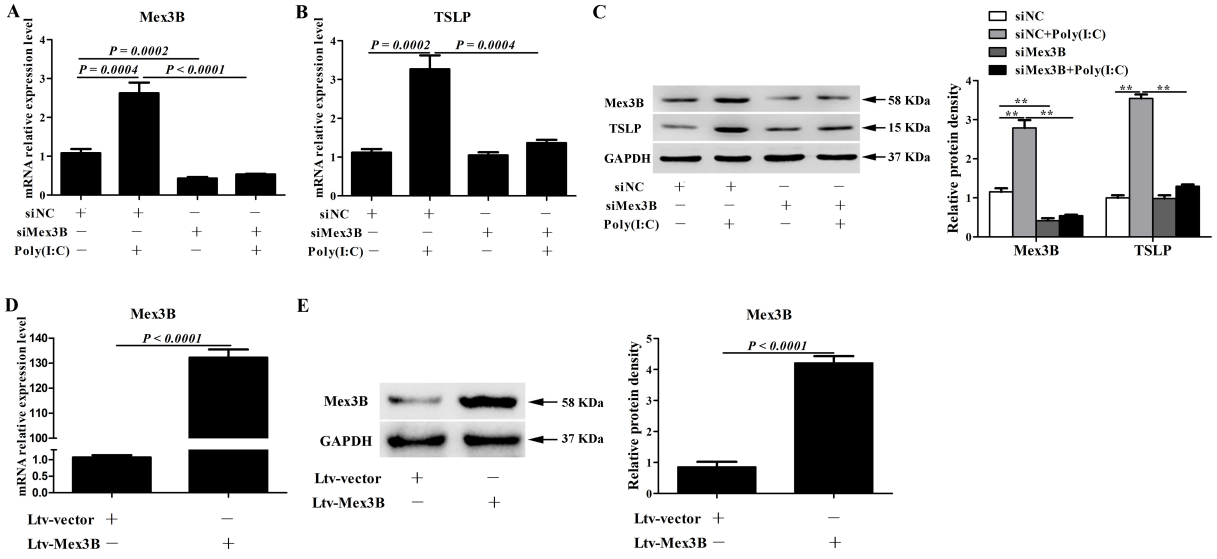


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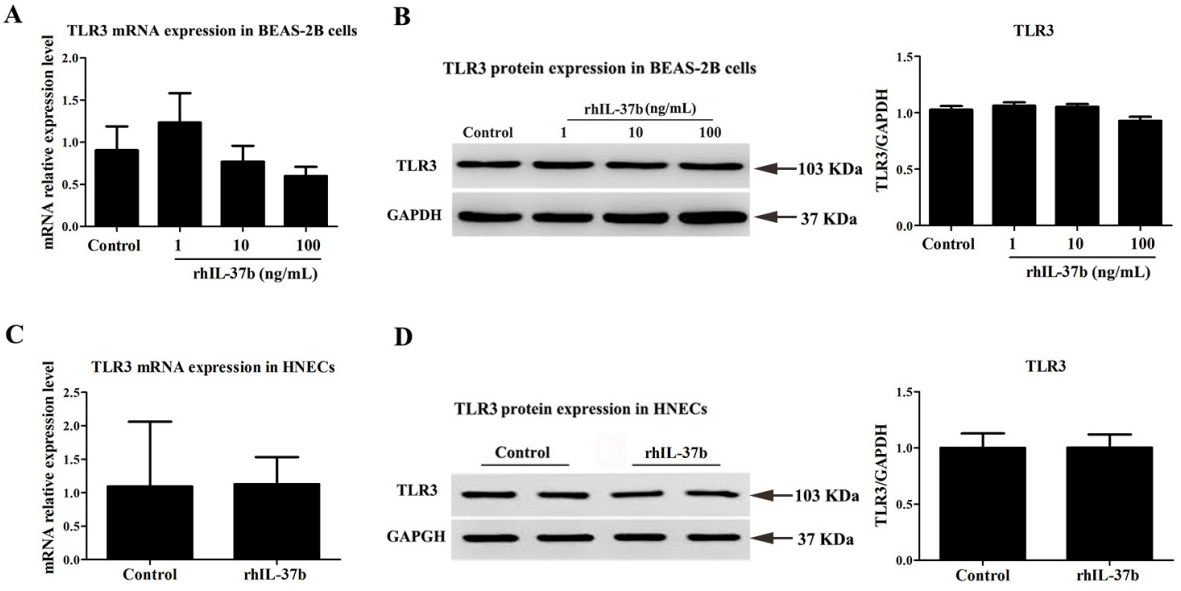
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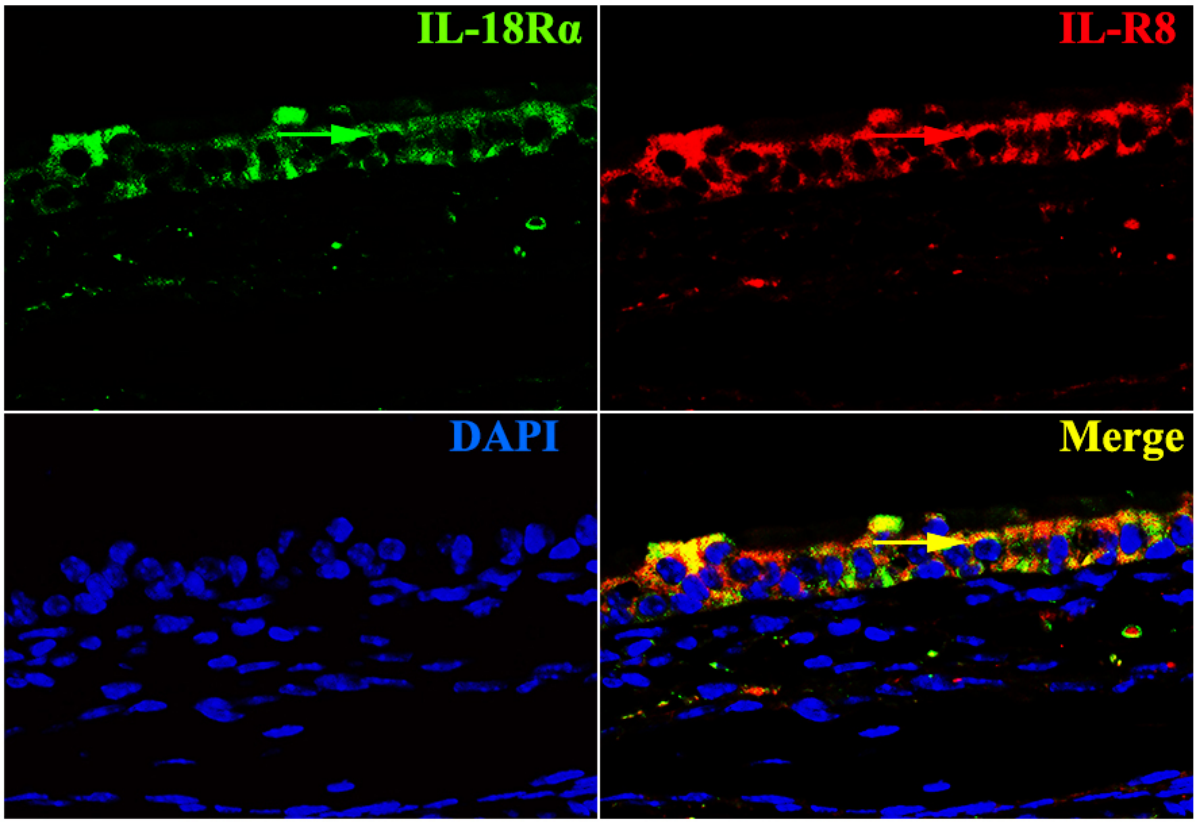


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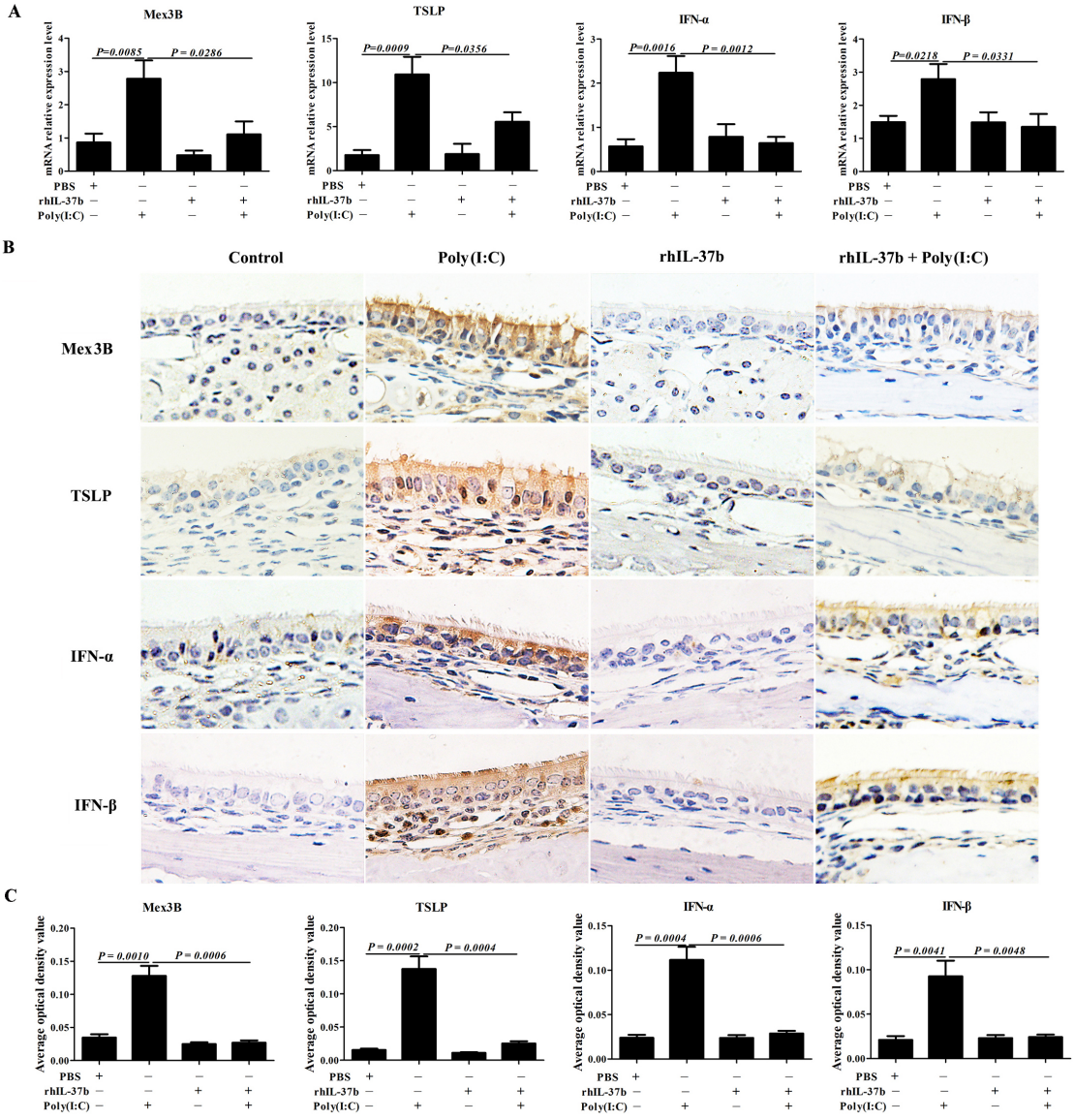
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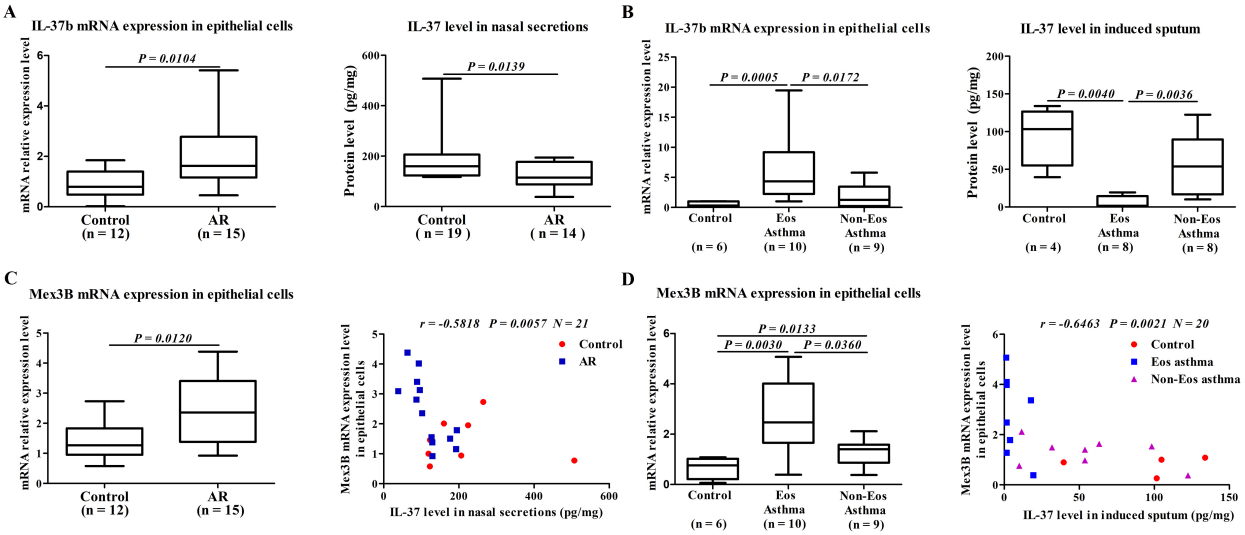


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IL-37-Mex3B-TLR3 axis in epithelial cells in eosinophilic chronic rhinosinusitis with nasal polyps

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Running Head: IL-37-Mex3B-TLR3 axis in eosinophilic CRSwNP

Grant Support: This study was supported by the National Natural Science

Foundation of China (NSFC) grants 81630024, 81570899, and 81325006 (Z.L.), 81873694 (H.W.), and 81700893 (B.L.), Hubei Province Natural Science Foundation grants 2017CFA016 (Z.L.) and 2017CFB477 (H.W.), and "Ten thousand plan"-National high-level talents special support plan (Z.L.).

Disclosure of conflict of interest: None

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METHODS

Subjects and sample collection

This study was approved by the Ethics Committee of Tongji Hospital of Huazhong University of Science and Technology and every patient signed informed consent before enrollment. For CRS study, 113 control subjects, 121 patients with eosinophilic CRSwNP, 118 patients with non-eosinophilic CRSwNP, and 107 patients with CRSsNP were recruited. The diagnosis of CRSsNP and CRSwNP was made based on the guideline of European Position Paper on Rhinosinusitis and Nasal Polyps 2012.[E1] CRSwNP was classified as eosinophilic when the percentage of tissue eosinophils exceeded 10% of the total number of infiltrating cells, as defined by our previous study.[E2] This cutoff was calculated as twice of the standard deviation of the mean eosinophil percentage in control subjects.[E2] Control subjects were those undergoing septoplasty because of anatomic variations and did not have other sinonasal diseases. For allergic rhinitis (AR) study, 16 subjects (10 male and 6 female subjects; age, 36 (23-47) years) with house dust mite induced AR and concomitant nasal septum deviation and 23 control subjects (13 male and 10 female subjects; age, 34 (26-38) years) with nasal septum deviation alone were recruited.[E3] AR and control subjects had no other sinonasal disease. For asthma study, 6 control subjects (5 male and 1 female subjects; age, 37 (31-45) years), 10 subjects (4 male and 6 female subjects; age, 38 (33-42) years) with eosinophilic asthma, and 9 subjects (4 male and 5 female subjects; age, 43 (32-48) years) with non-eosinophilic asthma were enrolled. None of the control subjects had a history of respiratory diseases or evidence of

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bronchial hyperreactivity to methacholine challenge.[E4] A cut point of 3% of eosinophils in total sputum inflammatory cells was used to distinguish eosinophilic from non-eosinophilic asthma.[E5, E6]

The atopic status was evaluated by using skin prick test with a standard panel of inhalant allergens common in our region and/or using the ImmunoCAP to detect IgE antibodies against common inhalant allergens (Phadia, Uppsala, Sweden).[E7] The diagnosis of allergic rhinitis was based on the concordance between the atopic status and typical allergic symptoms. The diagnosis of asthma was based on history and physician's diagnosis according to Global Initiative for Asthma.[E8] All asthma subjects had symptoms of episodic cough, wheeze and/or dyspnea; and had accumulated dosage of methacholine provoking a 20% fall of forced expiratory volume in the first second (FEV1) < 2.505 mg and/or ≥12% increase in FEV1 following inhalation of 200 μg salbutamol. Oral glucocorticoid and intranasal steroid sprays were discontinued at least 3 months and 1 month before surgery, respectively. Antileukotrienes were discontinued at least 1 month before inclusion. Subjects with an acute upper respiratory tract infection and acute asthma episode within 4 weeks of entering the study, and patients under immunotherapy were excluded. In addition, subjects who had an antrochoanal polyp, fungal sinusitis, cystic fibrosis, primary ciliary dyskinesia, immunodeficiency, systemic vasculitis, or gastroesophageal reflux disease were excluded from this study, because these are discrete disorders with unique pathophysiology. None of the subjects had a history of aspirin sensitivity. The details of subjects' characteristics in CRS study are summarized in Table E1.

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For CRS study, diseased ethmoid sinus mucosal samples from CRSsNP patients, polyp tissues from CRSwNP patients, and inferior turbinate mucosal tissues from control subjects were collected during surgery. Human nasal epithelial cells (HNECs) were scraped from polyps of CRSwNP patients, and from middle meatus of CRSsNP patients and control subjects using a sterile Rhino-Pro curette (Arlington Scientific Inc, Springville, UT) as previously described.[E9] Each nasal scraping specimen yielded 1-2 $\times 10^6$ cells, of which more than 95% were epithelial cells based on cytokeratin indirect immunofluorescence staining with anti-pan cytokeratin antibody (Abcam, Cambridge, UK).[E10] Nasal secretions were collected using sinus packs and processed as stated elsewhere.[E9,E11,E12] Briefly, a sinus sponge pack was inserted into each nasal cavity for 5 min. After collection, 2 mL of 0.9% sodium chloride solution was added to sponge packs. Sponges were transferred to a 10 mL syringe. The samples were squeezed out with the piston of the syringe, and centrifuged at 1,500g for 15 min at 4°C. The supernatants were stored in aliquots at -80°C for further use. Not all samples were included in every experiment protocol because of limited quantity.

For AR study, nasal epithelial cells were scraped from inferior turbinate mucosa of AR patients and control subjects, and nasal secretions were collected. For asthma study, human bronchial epithelial cells collected by bronchial brushing technique as previously described.[E4,E13] Briefly, 10 sites within the subsegmental bronchi of right middle and lower lobes were brushed. The dissociated cells were recovered by vortexing the brush in ice-cold RPMI-1640 medium. Sputum induction with hypertonic saline (4.5%) was performed as mentioned elsewhere with minor modifications.[E14,E15]

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Brifely, the sputum was isolated from saliva and dispersed using dithiothreitol and then filtered through a 40 μm Nitex filter to remove mucus. The resulting suspension was centrifuged at 1000 rpm for 10 min at 4 °C and the supernatants were collected and stored at -80°C for later analysis. The cell pellet was resuspended in PBS and total cell count and viability were assessed. For inflammatory cell counts, the cell suspension was centrifuged by a cytospin (Andreas Hettich, Tuttlingen, Germany) and stained with Wright-Giemsa solution (Leagene Biotechnology, Beijing, China). Cell differential counts were determined by counting at least 400 nonsquamous cells.[E4,E5]

Immunohistochemistry and immunofluorescence

Fresh tissue samples were fixed in formaldehyde solution and embedded in paraffin. Paraffin sections (4 μm) were prepared from tissue blocks. After deparaffinization and rehydration, sections were stained with hematoxylin and eosin to determine the general pathologic features and to count the number of eosinophils and total inflammatory cells as previously reported.[E2]

Human and murine tissue sections were subject to heat-induced antigen retrieval using Target Retrieval Solution (Dako, Carpinteria, Calif, USA). The 3% hydrogen peroxidase was used for endogenous peroxidase inhibition.[E16] To block nonspecific binding, 5% bovine serum albumin was used.[E16] Sections were stained with primary antibodies listed in Table E2 and E3. For immunohistochemistry, all antigens were detected by using the streptavidin-peroxidase complex method with a histostain-plus kit (Boster Biotechnology, Wuhan, China) according to the manufacturer's

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instructions.[E16] Color development was achieved with 3', 3'-diaminobenzidine, which rendered positive cells brown. Sections were counterstained with hematoxylin and mounted. For immunofluorescence staining, fluorescence conjugated secondary antibodies (Table E4) were used. Species- and subtype-matched antibodies were used as negative controls.

As previously stated,[E2, E17] the positive cells in the lamina propria (LP) were counted at high-power field (HPF) and 10 HPFs were randomly selected and analyzed.[E2,E16] The positive staining intensity in epithelium was analyzed by using image pro-plus 6.0 analysis software (Media Cybernetics, Inc. Silver Spring, MD, USA) and the results were presented as average optical density value per unit area.[E18]

Quantitative RT-PCR

Total RNA was extracted from human and murine tissue samples by using TRIzol reagent (Invitrogen, Carlsbad, Calif, USA). A total of 1 μg of RNA was reverse-transcribed to cDNA using a PrimeScript RT reagent kit (TaKaRa Biotechnology, Dalian, China). The PCR was performed by using the SYBR Premix Ex Taq kit (TaKaRa Biotechnology, Dalian, China) with appropriate primers (Table E5 and E6). cDNA equivalent to 50 ng of total RNA was used and quantitative PCR was performed on StepOnePlus (Applied Biosystems, Foster City, Calif, USA). Amplification was as follows: 95 °C for 2 min, followed by 40 cycles of 95 °C for 10 sec, specific annealing temperature for 10 sec, and 72 °C for 15 sec. After PCR, a melting curve was constructed by increasing the temperature from 65 to 95°C with a

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temperature transition rate of 0.1°C/s. Relative gene expression was calculated by using the 2(-Delta Delta CT) method.[E19] Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene for normalization of gene expression.

ELISA

Tissue samples were weighted and 1 mL of 0.9% sodium chloride solution supplemented with 10 μL of 100 mM phenylmethylsulfonyl fluoride was added per every 0.1 g tissue.[E16] Tissue was homogenized on ice and centrifuged at 3,000 rpm for 10 min at 4 °C.[E16] Then the supernatants were harvested and stored at -80 °C for further use. The nasal secretions, induced sputum, and cell culture supernatants were also collected for ELISA. The IL-37 protein levels in nasal secretions, induced sputum, tissue homogenates, and cell culture supernatants were measured by using a commercial ELISA kit (Adipogen, Liestal, Switzerland) according to the manufacturer's instructions. The TSLP levels in nasal secretions were also detected by a commercial ELISA kit (R&D systems, Minneapolis, MN, USA). The lower detection limit was 0.016 ng/mL for IL-37 and 31.25 pg/mL for TSLP, respectively. The total protein levels were measured by using a bicinchoninic acid (BCA) protein detection kit (Guge Biotechnology, Wuhan, China) and the cytokine levels in nasal secretions and tissue homogenates were expressed as protein levels per mg of total protein.

Western blotting

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Total cellular protein was extracted from HNECs or BEAS-2B cells in Radio-Immunoprecipitation Assay lysis buffer containing 2% cocktail of protease inhibitor (Guge Biotechnology). The processed cells were centrifuged at 12,000 rpm for 20 min at 4 °C and the supernatants were harvested.

The nuclear protein of HNECs was extracted as previously described with minor modifications.[E20] The HNECs were suspended in cytoplasmic extraction buffer and lysed on ice for 30 min. Nuclear pellets were separated from cytoplasm by centrifugation at 12,000 rpm for 10 min. The supernatants (cytoplasmic extract) were removed and nuclear pellets were washed with PBS twice. The pellets were resuspended in the nuclear extraction buffer (containing 2% protease inhibitor cocktail), sonicated, and kept on ice for 30 min for further lysis. Then the lysates were centrifuged at 12,000 rpm for 10 min at 4°C and the supernatants were collected as nuclear protein extracts.

Nasal secretions were subjected to a membrane-based concentration step (10 kDa of Amicon Ultracel-10 Membrane; Merck Millipore, Billerica, MA, USA) under the centrifugation at 3,000 rpm for 10 min at 4°C. The supernatants were collected and then added with appropriate buffer (containing 2% protease inhibitor cocktail).

The protein concentrations of the extracts were measured with a BCA protein assay kit (Guge Biotechnology). Samples containing 40 μg of proteins were separated on sodium dodecyl sulfate-polyacrylamide gels (SDS/PAGE) under reducing conditions and transferred onto polyvinylidene difluoride (PVDF) membranes (Guge Biotechnology). The membranes were incubated with 5% fat-free skin milk in

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Tris-buffered saline with Tween-20 (TBS-T, Guge Biotechnology) for 1 hour at room temperature and then incubated with primary antibodies as listed in Table E7. After washing 3 times in TBS-T buffer, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody (goat anti-rabbit or goat anti-mouse antibody, Boster Biotechnology) for 1 hour at room temperature. After that, the members were processed by using an ECL chemiluminescence reaction kit (Guge Biotechnology) and followed by exposure on chemiluminescent film to visualize the proteins. GAPDH and histone H3 were used as internal standard to correct for variations in total cellular and nuclear protein loading, respectively. As to the analysis of nasal secretions, we applied reversible Ponceau staining to check equal loading of gels.[E21,E22] Densitometric analysis of the blots was performed by using the software AlphaEase FC (Alpha Innotech, Silicon Valley, Calif, USA). IL-37 was identified as two bands around 25-45 KDa, representing monomeric and dimeric form, respectively.[E23] Mex3B and TSLP were identified as a single band around 58 KDa and 15 KDa, respectively.

Cell culture and treatment

HNECs were cultured with an air-liquid interface (ALI) method as mentioned elsewhere[E24-E27]. Briefly, HNECs obtained by nasal scrapping were washed twice in Dulbecco modified Eagle medium (Gibco-BRL, Invitrogen, Carlsbad, Calif, USA) with penicillin/streptomycin and then grown submerged in bronchial epithelial cell basal medium (BEBM, Lonza, Walkersville, MD, USA) supplemented with

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SingleQuot Kit Suppl (Lonza) in 6-well plates coated with rat tail collagen type I (Sigma-Aldrich, St. Louis, MO, USA) in a 5% CO_2-humidified atmosphere at 37 °C. Upon confluence, the cells were passaged to collagen-coated 0.4 μm pore membrane transwell culture plates (Becton Dickson, Franklin Lake, NJ, USA) and were cultured with BEBM (Lonza). When they were confluent, they were shifted to air-liquid interface (ALI) culture by removing all of the apical medium and medium in the basal chamber was replaced with an ALI medium consisting of BEBM/Dulbecco's modified Eagle's medium H (50: 50; Invitrogen). HNECs were maintained at ALI culture for 21 days for differentiation.[E24,E27] After HNECs were differentiated, they were stimulated with CpG, IL-4, IL-13, IL-10, IFN-γ, TNF-α, IL-1β and IL-17A at 10 ng/mL (R&D systems), LPS at 1 μg/mL (Invivogen, San Diego, Calif, USA), poly(I:C) at 25 μg/mL (Tocris Bioscience, Bristol, UK), and rhIL-37b (Val46-Asp218, R&D systems) at 10 ng/mL.[E28] Cells were harvested for quantitative RT-PCR assay and RNA sequencing analysis 6 hours after stimulation. Culture supernatants and cells were collected for ELISA and western blotting analysis 24 hours after stimulation, respectively.

In some experiments, HNECs cells were pretreated with rhIL-37b at 10 ng/mL (R&D systems) for 24 hours. Then, HNECs were stimulated with poly(I:C) at 25 μg/mL (Tocris Bioscience). After 6-hour and 24-hour poly(I:C) stimulation, cells were harvested and subject to quantitative RT-PCR and Western blotting, respectively. To study the secretion of IL-37, HNECs were pre-incubated with various cytokines and TLR agonists for 3 hours, and further stimulated with 5 mM ATP for 30 min. IL-37 levels in culture supernatants were measured by means of ELISA.

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In order to investigate the involvement of IL-18Rα in IL-37 signaling, HNECs were pre-incubated with anti-IL-18Rα (100 ng/mL, R&D systems) for 1 hour and then stimulated with rhIL-37b (R&D systems) and poly(I:C) (Tocris Bioscience) as mentioned above. Cells were harvested 6 hours after stimulation and subject to quantitative RT-PCR analysis.

The BEAS-2B cells (American Type Culture Collection, Manassas, VA), derived from human bronchial epithelium, were cultured submerged as mentioned elsewhere.[E24] The BEAS-2B cells were culture with Dulbecco's modified Eagle's medium/F-12 supplemented with 10% fetal calf serum (Invitrogen) at 37 □ with 5% CO_2 in humidified air. When the cells reach 80 to 90% confluence, medium was changed to a serum-free one, and the cells were treated with rhIL-37b (1, 10, 100 ng/mL, R&D systems) directly. After treatment for 6 or 24 hours, the cells were harvested to detect the mRNA and protein level of Mex3B and TLR3, respectively.

For the transfection experiments, when BEAS-2B cells reached 30 to 50% confluence, the cells were transfected with Mex3B siRNA or Mex3B overexpressing lentiviruses. SiRNA transfection was performed as previously described.[E29] Transfection reagents and siRNA were purchased from Invitrogen Thermo Fisher Scientific Company (Waltham, MA, USA). The sense sequence of Mex3B siRNA was 5'-CCCGGAAUAAGAACACGGCACUCAA-3' (ID: HSS130731), and scrambled siRNA was used as negative control (siNC). BEAS-2B cells were transfected with 100 nM of siNS or siMex3B using RNAimax (Invitrogen Thermo Fisher Scientific Company) for 8 hours. After a recovery period of 24 hours, medium was changed and

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cells were further stimulated with rhIL-37b and/or poly(I:C) as mentioned above. After culture, cells were harvested and subject to quantitative RT-PCR and Western blotting, respectively.

Recombinant lentiviruses carrying human Mex3B gene (GenBank accession no. XM_032246) (Ltv-Mex3B; GeneChem, Shanghai, China) or a nonspecific control sequence (Ltv-vector; GeneChem) were used to infect BEAS-2B cells according to the manufacturer's protocol at a multiplicity of infection of 100 in medium containing polybrene (5 μg/mL, GeneChem). The 5′-3′ sequence for Ltv-Mex3B is: sense, AGGTCGACTCTAGAGGATCCCGCCACCATGCCCAGCTCGCTGTTCGCAGAC, and anti-sense, TCCTTGTAGTCCATACCAGAAAAGATGCGGATGGCCTGAGTG. Transfection efficiency was identified by observing fluorescence under fluorescence microscope three days after transfection. Total RNA and protein were extracted 5 days and 7 days post-transfection to further detect transfection efficiency, respectively. After transfection, BEAS-2B cells were treated with rhIL-37b and poly(I:C) as mentioned above. After culture, cells were harvested and subjected to quantitative RT-PCR and Western blotting, respectively.

Flow cytometry

Dispersed sinonasal tissue cells were prepared from sinonasal tissues by means of mechanical dissociation as previously reported.[E30] The resulting cells were depleted of mononuclear cells by density gradient centrifugation over Ficoll (Tianjin Haoyang Biological Manufacture, Tianjin, China).[E30] After that, cells were stained with mouse

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monoclonal anti-human IL-1R8 antibody on ice for 30 min. After wash, the cells were further stained with donkey anti-mouse IgG-PE for 30 min in the dark. After that, the cells were stained with FITC-conjugated mouse monoclonal antibody against human IL-18Rα, APC-conjugated mouse monoclonal antibody against human CD326, PerCP-Cy5.5-conjugated mouse monoclonal antibody against human CD45 for 30 min at 4°C in the dark.[E31,E32] Species and subtype-matched antibodies were used as negative controls. The detailed information of primary antibodies used are listed in Table E8. The stained cells were analyzed by using LSRII flow cytometer (Becton Dickinson Biosciences, San Jose, Calif, USA). Fluorescence was determined for all cells in each sample after debris, dead cells, and aggregates were excluded by forward angle and side scatter gating.[E10] HNECs were gated as $CD326^{+}CD45^{-}$ cells.[E33] The expression of IL-18Rα and IL-1R8 on HNECs was analyzed. Data were analyzed using FlowJo software (TreeStar, Ashland, OR, USA).

RNA sequencing analysis

HNECs obtained from 3 patients with eosinophilic CRSwNP were cultured and treated with or without rhIL-37b (Val46-Asp218, R&D systems) at 10 ng/mL for 6 hours. After that, total RNA was extracted using TRIzol (Invitrogen) from cells. A total amount of 5 μg total RNA per sample was used as input material for the RNA sample preparation. RNA integrity and concentration were assessed with an Agilent 2100 Bioanalyzer and an RNA Nano 6000 Assay Kit (Agilent Technologies, Santa Clara, Calif, USA).

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Sequencing libraries were generated according to the manufacturer's protocol index codes were added to attribute sequences to each sample. Briefly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. Fragmentation was carried out using divalent cations under elevated temperature by using NEBNext RNA Library Prep Kit (#E7530L, NEB, USA). First strand cDNA was synthesized using random hexamer primer and RNase H, and second strand cDNA was subsequently synthesized. The library fragments were purified with QiaQuick PCR kits (QIAGEN, Duesseldorf, Germany) and eluted with EB buffer. Following these, the terminal repair, A-tailing, and adapter adding were implemented and the aimed products were retrieved. Then PCR amplification was carried out and the library quality was evaluated by an Agilent 2100 Bioanalyzer system (Agilent Technologies). The final library products were deep sequenced on an Illumina Hiseq 2500 platform with 50 bp single-read reads length.

In order to analyze differential expression pattern, the reads count of unambiguous clean tags for each gene was calculated by using RPKM (Reads Per Kilobase Millon Mapped Reads) method.[E34] The RPKM method could eliminate the effect of sequencing depth and gene length on gene expression levels. Therefore, the data could be directly used for comparing the differential gene expression among samples.

The DESeq R package (v1.18.0) software was used to analyze the differential expression patterns of two groups.[E35] In comparison, genes with *P*-value <0.05 and $|\log2 \text{ (fold change)}| \geq 1$ between two groups were considered to have significantly

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differential expression.

Mouse experiment

Female C57BL/6 mice (5-6 weeks old) were purchased from Hubei Research Center of Laboratory Animal, Wuhan, China. The mice were raised in specific pathogen-free condition under a controlled environment with a 12-hour light/dark cycle.

Mice were treated with 50 μL sterile PBS (25 μL per nostril) or 1 μg rhIL-37b (Val46-Asp218, R&D systems) in 50 μL sterile PBS (25 μL per nostril) intranasally from Days 1-5.[E36] Some mice were given 50 μg of poly(I:C) (Tocris Bioscience) in 50 μL sterile PBS (25 μL per nostril) 30 min after rhIL-37b treatment from Days 2-5.[E24] Mice were sacrificed on Day 6 and sinonasal cavity structures were dissected, decalcified, embedded in paraffin as previously reported.[E24] The tissue was cut in coronal sections at 4 μm of thickness. The protein expression of IL-18Rα, IL-1R8, Mex3B, TSLP, IFN-α, and IFN-β were detected by immunofluorescence or immunohistochemical staining as described previously with proper primary antibodies (Table E4). In some experiments, respiratory sinonasal mucosa was dissected under a microscope and total RNA was extracted for quantitative RT-PCR assay.[E37] The mRNA expression levels of Mex3B, TSLP, IFN-α, and IFN-β were detected by RT-PCR with specific primers (Table E6). All mice were used following protocols approved by the Animal Care and Use Committee of Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology.

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476 **Table E1. Demographic characteristics of enrolled subjects for CRS study**

	Control	Eos CRSwNP	Non-Eos CRSwNP	CRSsNP	*P* value
Total subjects enrolled	113	121	118	107	
Methodology used					
Immunohistochemistry and immunofluorescence					
Subject number	35	38	39	30	
Gender, male	26 (74%)	28 (74%)	25 (64%)	17 (57%)	*0.360*
Age (years)	31 (25-38)	39 (32-47)	42 (22-52)	33 (22-47)	*0.055*
Patients with atopy	7 (20%)	11 (29%)	10 (26%)	7 (23%)	*0.841*
Patients with AR	0 (0)	5 (13%)	3 (8%)	4 (13%)	*0.153*
Patients with asthma	0 (0)	4 (11%)	0 (0)	1 (3%)	***0.042***
RT-PCR					
Subject number	30	38	38	30	
Gender, male	26 (87%)	28 (74%)	22 (58%)	19 (63%)	*0.058*
Age (years)	35 (30-44)	43 (31-53)	43 (35-50)	38 (25-43)	*0.080*
Patients with atopy	6 (20%)	15 (39%)	7 (18%)	9 (30%)	*0.153*
Patients with AR	0 (0)	8 (21%)	2 (5%)	4 (13%)	***0.023***
Patients with asthma	0 (0)	9 (24%)	1 (3%)	1 (3%)	***0.001***
ELISA					
Subject number	31	34	35	28	
Gender, male	26 (84%)	24 (71%)	20 (57%)	18 (64%)	*0.157*
Age (years)	29 (21-37)	41 (26-52)	30 (21-43)	36 (21-45)	*0.146*

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Patients with atopy	6 (19%)	11 (32%)	11 (31%)	10 (36%)	*0.524*
Patients with AR	0 (0)	8 (24%)	5 (14%)	2 (7%)	***0.023***
Patients with asthma	0 (0)	6 (18%)	3 (9%)	1 (4%)	***0.048***
Western blotting					
Subject number	20	20	20	20	
Gender, male	17 (85%)	15 (75%)	13 (65%)	13 (65%)	*0.430*
Age (years)	26 (21-36)	40 (28-50)	38 (18-47)	34 (24-51)	*0.255*
Patients with atopy	6 (30%)	10 (50%)	7 (35%)	7 (35%)	*0.589*
Patients with AR	0 (0)	6 (30%)	3 (15%)	5 (25%)	*0.064*
Patients with asthma	0 (0)	3 (15%)	0 (0)	0 (0)	***0.025***
RNA sequencing analysis					
Subject number	-	3	-	-	-
Gender, male	-	3 (100%)	-	-	-
Age (years)	-	42 (41-42)	-	-	-
Patients with atopy	-	2 (67%)	-	-	-
Patients with AR	-	1 (33%)	-	-	-
Patients with asthma	-	0 (0)	-	-	-
Validation for RNA sequencing analysis					
Subject number	-	9	-	-	-
Gender, male	-	6 (66.7%)	-	-	-
Age (years)	-	47 (28, 52)	-	-	-
Patients with atopy	-	6 (66.7%)	-	-	-
Patients with AR	-	2 (22.2%)	-	-	-
Patients with asthma	-	2 (22.2%)	-	-	-
Cell culture study					
Subject number	6	12	-	-	-
Gender, male	4 (67%)	8 (67%)	-	-	-
Age (years)	28 (22-32)	31 (26-34)	-	-	-

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Patients with atopy	1 (17%)	3 (25%)	-	-	-
Patients with AR	0 (0)	2 (17%)	-	-	-
Patients with asthma	0 (0)	0 (0)	-	-	-
Flow cytometry					
Subject number	5	5	5	4	
Gender, male	3 (60%)	3 (60%)	4 (80%)	2 (50%)	*0.921*
Age (years)	32 (30-35)	34 (24-35)	31 (27-40)	37 (28-48)	*0.893*
Patients with atopy	0 (0)	2 (40%)	1 (20%)	1 (20%)	*0.871*
Patients with AR	0 (0)	1 (20%)	1 (20%)	0 (0)	*1.000*
Patients with asthma	0 (0)	1 (20%)	0 (0)	0 (0)	*1.000*

For continuous variables, results are expressed as medians and interquartile ranges. Categorical variables are summarized using percentage. Eos CRSwNP, eosinophilic chronic rhinosinusitis with nasal polyps; Non-Eos CRSwNP, non-eosinophilic chronic rhinosinusitis with nasal polyps; CRSsNP, chronic rhinosinusitis without nasal polyps.

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Table E2. Primary antibodies used in immunohistochemistry and immunofluorescence for human tissues

Antibody	Application	Species	Concentration	Clone ID	Reference	Source
IL-37	IHC, IF	rabbit	1:100	polyclonal	K110419	Adipogen (Liestal, Switzerland)
IL-18Rα	IF	rabbit	1:100	polyclonal	ab117432	Abcam (Cambridge, UK)
IL-1R8	IF	mouse	1:100	2H3C12	NBP2-52491	Novus Biologicals (Littleton, CO, USA)
Mex3B	IHC, IF	rabbit	1:50	polyclonal	sc-135304	Santa Cruz Biotechnology (Santa Cruz, Calif, USA)
TLR3	IHC, IF	mouse	1:100	TLR3.7	ab12085	Abcam
Muc5AC	IF	mouse	1:100	45M1	ab2649	Abcam
CD68	IF	mouse	1:100	PG-M1	ZM-0464	Zhongshan Golden Bridge Biotechnology (Beijing, China)
Tryptase	IF	mouse	1:200	AA1	ab2378	Abcam
CD3	IF	mouse	1:100	LN10	ZM-0417	Zhongshan Golden Bridge Biotechnology
CD19	IF	mouse	1:100	UMAB103	ZM-0038	Zhongshan Golden Bridge Biotechnology

IL-37, interleukin-37; IL-18Rα, IL-18 receptor α; IL-1R8，IL-1 receptor 8; Mex3B, Mex3 RNA binding family member B; TLR3, Toll-like receptor 3; IHC, immunohistochemistry; IF, immunofluorescence.

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Table E3. Secondary antibodies used in immunofluorescence

Antibody	Concentration	Clone ID	Reference	Source
IFKine™ Green donkey anti-rabbit IgG	1:100	polyclonal	A24221	Abbkine Scientific Company (Wuhan, China)
IFKine™ Red donkey anti-mouse IgG	1:100	polyclonal	A24411	Abbkine

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Table E4. Primary antibodies used in immunohistochemistry and immunofluorescence for mouse experiment

Antibody	Application	Species	Concentration	Clone ID	Reference	Source
IL-18Rα	IF	rabbit	1:100	polyclonal	ab117432	Abcam (Cambridge, UK)
IL-1R8	IF	mouse	1:100	2H3C12	NBP2-52491	Novus Biologicals (Littleton, CO, USA)
Mex3B	IHC	rabbit	1:50	polyclonal	sc-135304	Santa Cruz Biotechnology (Santa Cruz, CA, USA)
TSLP	IHC	rabbit	1:100	polyclonal	bs-0449R	Biosynthesis Biotechnology (Beijing, China)
IFN-α	IHC	rabbit	1:100	polyclonal	bs-8995R	Biosynthesis Biotechnology
IFN-β	IHC	rabbit	1:100	polyclonal	bs-0784R	Biosynthesis Biotechnology

Mex3B, Mex3 RNA binding family member B; TSLP**,** thymic stromal lymphopoietin; IFN-α, interferon-α; IFN-β, interferon-β.

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Table E5. Primers used in quantitative PCR analysis for human tissues

Primer	Primer sequences	Expected product size (bp)	Annealing temperature (°C)
IL-37b	(F)5'-CCGGCCCTTCATCTTTTATAG -3'	210	60
	(R)5'-GTTTCCTAATCGCTGACCTCAC -3'		
Mex3B	(F)5'-AAGAGCGTGAACATGACCGAG -3'	90	60
	(R)5'-CGCTTTGATTTTACAACCTTGCC-3'		
TLR3	(F)5'-TTGCCTTGTATCTACTTTTGGGG-3'	157	60
	(R)5'-TCAACACTGTTATGTTTGTGGGT-3'		
TSLP	(F)5'-CCCAGGCTATTCGGA AACTCA-3'	118	60
	(R)5'-ACG CCACAATCCTTGTAATTGTG-3		
IFN-α	(F)5'-GCCTCGCCCTTTGCTTTACT-3'	89	59
	(R)5'-CTGTGGGTCTCAGGGAGATCA-3'		
IFN-β	(F)5'-ATGACCAACAAGTGTCTCCTCC-3'	88	60
	(R)5'-GGAATCCAAGCAAGTTGTAGCTC-3'		
GAPDH	(F)5'-ACCCAGAAGACTGTGGATGG-3'	201	60
	(R)5'-TTCTAGACGGCAGGTCAGGT-3'		

IL-37, interleukin-37; Mex3B, Mex3 RNA binding family member B; TLR3, Toll-like receptor 3; TSLP, thymic stromal lymphopoietin; IFN-α, interferon-α; IFN-β, interferon-β; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

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Table E6. Primers used in quantitative PCR analysis for mouse experiment

Primer	Primer sequences	Expected product size (bp)	Annealing temperature (°C)
Mex3B	(F)5'-GGGCTTTGATCTGCATCACG-3'	122	60
	(R)5'-AGCTGTCGTTGCGATAGCTG-3'		
TSLP	(F)5'-ACGGATGGGGCTAACTTACAA-3'	128	60
	(R)5'-AGTCCTCGATTTGCTCGAACT-3'		
IFN-α	(F)5'-CTGCTACTTGGAATGCAACTC-3'	251	60
	(R)5'-CAGTCTTGCCAGCAAGTTGG-3'		
IFN-β	(F)5'-AGCTCCAAGAAAGGACGAACA-3'	82	60
	(R)5'-GCCCTGTAGGTGAGGTTGAT-3'		
GAPDH	(F)5'-AGGTCGGTGTGAACGGATTTG-3'	95	62
	(R)5'-GGGGTCGTTGATGGCAACA-3'		

Mex3B, Mex3 RNA binding family member B; TSLP, thymic stromal lymphopoietin; IFN-α, interferon-α; IFN-β, interferon-β; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

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Table E7. Primary antibodies used in western blotting

Antibody	Species	Concentration	Clone ID	Reference	Source
IL-37	rabbit	1:500	polyclonal	K110419	Adipogen (Liestal, Switzerland)
Mex3B	rabbit	1:500	polyclonal	sc-135304	Santa Cruz Biotechnology (Santa Cruz, CA, USA)
TSLP	rabbit	1:500	polyclonal	bs-0449R	Biosynthesis Biotechnology (Beijing, China)
GAPDH	mouse	1:1000	monoclonal	GB12002	Guge Biotechnology, (Wuhan, China)
Histone H3	rabbit	1:1000	polyclonal	GB11026	Guge Biotechnology

IL-37, interleukin-37; Mex3B, Mex3 RNA binding family member B; TSLP, thymic stromal lymphopoietin

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Table E8. Antibodies used in flow cytometry

Antigen-Fluorophore	Manufacturer	Clone ID	Source	Isotype	Dilution
IL-18Rα-FITC	R&D systems, (Minneapolis, MN, USA)	70625	mouse	IgG1	1:20
IL-1R8	Novus Biologicals (Littleton, CO, USA)	2H3C12	mouse	IgG	1:50
CD326-APC	Biolegend (San Diego, CA, USA)	9C4	mouse	IgG2b, κ	1:20
CD45-PerCP-Cy5.5	BD Biosciences (Franklin Lakes, NJ, USA)	HI30	mouse	IgG1, κ	1:20
Anti-mouse IgG-PE	Biolegend	-	donkey	IgG	1:20

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FIGURE LEGENDS

Fig E1. The IL-37 was not expressed by mucin 5AC positive goblet cells in epithelium (A) and submucosal glands in lamina propria (B). In Fig E1, A, representative photomicrographs show immunostaining of tissue section from a patient with eosinophilic CRSwNP. Insets show a higher magnification of the outline area. Original magnification × 400.

Fig E2. The IL-37 positive cells in lamina propria in sinonasal mucosa. Double immunofluorescence staining demonstrated that IL-37 was expressed by CD68 positive macrophages, tryptase positive mast cells, CD3 positive T cells, and CD19 positive B cells. Representative photomicrographs show immunostaining of tissue sections from patients with eosinophilic CRSwNP (original magnification × 400). Arrows indicate the representative double positive cells.

Fig E3. The nuclear IL-37 levels in nasal epithelial cells as examined by western blotting. No significant nuclear IL-37 expression was detected. Image is representative blots of target protein.

Fig E4. The co-expression of IL-18Rα and IL-1R8 on nasal epithelial cells from

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different study groups as detected by flow cytometry. A, Dispersed sinonasal tissue cells were depleted of mononuclear cells and subject to flowcytometric analysis of cell surface expression of IL-18Rα and IL-1R8. $CD326^{+}CD45^{-}$ epithelial cells were gated and analyzed for the IL-18Rα and IL-1R8 expression. Gating strategy and representative flow plots are shown. **B,** The frequencies of IL-18Rα and IL-1R8 double positive cells in total nasal epithelial cells are increased in CRS patients. **C,** IL-18Rα and IL-1R8 double positive cells account for about 70% of $IL\text{-}18R\alpha^{+}$ epithelial cells. **D,** IL-18Rα and IL-1R8 double positive cells account for about 60% of $IL\text{-}1R8^{+}$epithelial cells. Eos CRSwNP, eosinophilic chronic rhinosinusitis with nasal polyps; Non-Eos CRSwNP, non-eosinophilic chronic rhinosinusitis with nasal polyps; CRSsNP, chronic rhinosinusitis without nasal polyps.

Fig E5. IL-37 inhibits Mex3B expression in BEAS-2B cells. A, Co-localization of IL-18Rα and IL-1R8 expression in BEAS-2B cells as revealed by immunofluorescence staining. Representative photomicrographs are shown (original magnification × 400). Arrows indicate the representative double positive cells. **B and C,** The dose-dependent effect of rhIL-37b on Mex3B mRNA (B) and protein expression (C) in BEAS-2B cells. BEAS-2B cells were stimulated with rhIL-37b for 6 hours or 24 hours and then subject to quantitative RT-PCR or western blotting analysis, respectively. In Fig E5, C, images are representative blots of target proteins. Densitometric analysis was performed and results are presented as the relative ratio of Mex3B to GAPDH (n = 6).

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Fig E6. TLR3 expression is upregulated in nasal epithelial cells in eosinophilic CRSwNP. A, The protein expression of TLR3 in sinonasal mucosal samples from different study groups as detected by immunohistochemistry. The representative photomicrographs are shown (original magnification × 400). **B,** The quantification of TLR3 staining intensity in epithelium. The results are presented as average optical density value per unit area. **C,** The mRNA expression of TLR3 in scrapped nasal epithelial cells from different study groups as detected by quantitative RT-PCR. Eos CRSwNP, eosinophilic chronic rhinosinusitis with nasal polyps; Non-Eos CRSwNP, non-eosinophilic chronic rhinosinusitis with nasal polyps; CRSsNP, chronic rhinosinusitis without nasal polyps.

Fig E7. The transfection efficiency of siMex3B and Ltv-Mex3B in BEAS-2B cells. A-C, After siMex3B transfection, poly(I:C) was unable to induce Mex3B and TSLP mRNA and protein expression in BEAS-2B cells as detected by RT-PCR (A and B), and western blotting (C), respectively (n = 6). In Fig E7, C, representative blots are shown and densitometric analysis was performed. **D-E**, Ltv-Mex3B transfection efficiency in BEAS-2B cells was identified at mRNA and protein level by RT-PCR (D) and western blotting (E), respectively (n = 6). In Fig E7, E, representative blots are shown and densitometric analysis of blots was performed.

Fig E8. IL-37b has no effect on TLR3 expression in airway epithelial cells. A and

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B, The mRNA (A) and protein expression (B) of TLR3 in BEAS-2B cells after treatment with various dose of rhIL-37b (n = 6). **C and D,** The mRNA (C) and protein expression (D) of TLR3 in human nasal epithelial cells (HNECs) after treatment with rhIL37b at 10 ng/mL. HNECs were obtained from eosinophilic CRSwNP patients (n = 9). Cultured cells were stimulated with rhIL-37b for 6 hours or 24 hours and then subject to quantitative RT-PCR or western blotting analysis, respectively. In Fig E8, B and D, images are representative blots of target proteins. Densitometric analysis was performed and results are presented as the relative ratio of TLR3 to GAPDH.

Fig E9. The co-localization of IL-18Rα and IL-1R8 in nasal epithelial cells of murine nasal cavity. Co-localization of IL-18Rα and IL-1R8 immunoreactivity in nasal epithelial cells of murine nasal cavity as revealed by immunofluorescence staining. The representative photomicrographs are shown (original magnification × 400). Arrow indicates the representative positive cell.

Fig E10. **Exogenous IL-37b decreases the production of Mex3B, TSLP, IFN-α, and IFN-β in nose in mice.** Mice were treated with rhIL-37b intranasally from Days 1-5 and poly(I:C) intranasally from Days 2-5. **A,** After treatment, the mRNA expression of Mex3B, TSLP, IFN-α and IFN-β in nasal mucosal samples were examined by quantitative RT-PCR (n = 7). **B,** Immunohistochemical staining demonstrated the protein expression of Mex3B, TSLP, IFN-α and IFN-β in nasal

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tissues, especially in epithelium. The representative photomicrographs are shown (original magnification × 400). **C,** The quantification of Mex3B, TSLP, IFN-α and IFN-β staining intensity in epithelium. The results are presented as average optical density value per unit area (n = 5).

Fig E11. The expression of IL-37 and Mex3B in allergic rhinitis and asthma. A, the mRNA expression of IL-37b was upregulated in scraped nasal epithelial cells, whereas the IL-37 protein levels were downregulated in nasal secretions, in allergic rhinitis (AR) patients, in comparison with control subjects. **B**, IL-37b mRNA levels were increased in bronchial brushing epithelial cells, whereas IL-37 protein levels were decreased in induced sputum in patients with eosinophilic asthma, but not in patients with non-eosinophilic asthma, in comparison with control subjects. **C,** Mex3B mRNA expression levels in scraped nasal epithelial cells were elevated in AR patients. There was a negative correlation between Mex3B mRNA expression levels in scraped nasal epithelial cells and IL-37 protein levels in nasal secretions. **D,** Mex3B mRNA levels in bronchial brushing epithelial cells were increased in patients with asthma, especially in patients with eosinophilic asthma. There was a negative correlation between Mex3B mRNA expression levels in scraped nasal epithelial cells and IL-37 protein levels in induced sputum.