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Yi Wei, Ph.D, Jia Zhang, M.D., Ph.D., Xingmei Wu, M.D., Ph.D, Wei Sun, M.D., Ph.D, Fanqin Wei, M.D., Ph.D, Wendong Liu, M.D., Tong Lu, M.D., Weidong Ji, M.D., Ph.D, Huabin Li, M.D., Ph.D, Weiping Wen, M.D., Ph.D.

PII: S0091-6749(20)30072-5

DOI: <https://doi.org/10.1016/j.jaci.2020.01.009>

Reference: YMAI 14375

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

Received Date: 19 June 2019

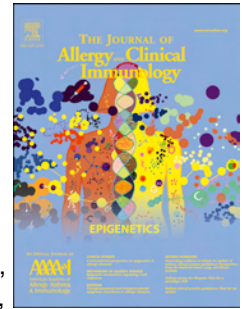
Revised Date: 7 January 2020

Accepted Date: 9 January 2020

Please cite this article as: Wei Y, Zhang J, Wu X, Sun W, Wei F, Liu W, Lu T, Ji W, Li H, Wen W, Activated pyrin domain containing 3 (NLRP3) inflammasome in neutrophilic chronic rhinosinusitis with nasal polyps (CRSwNP), *Journal of Allergy and Clinical Immunology* (2020), doi: <https://doi.org/10.1016/j.jaci.2020.01.009>.

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**Activated pyrin domain containing 3 (NLRP3) inflammasome in neutrophilic
chronic rhinosinusitis with nasal polyps (CRSwNP)**

Yi Wei, Ph.D.^{a, b, c*}, Jia Zhang, M.D., Ph.D.^{f*}, Xingmei Wu, M.D., Ph.D.^{a, b, c*}, Wei
Sun, M.D., Ph.D.^{a, b, c}, Fanqin Wei, M.D., Ph.D.^{a, b, c}, Wendong Liu, M.D.^{a, b, c}, Tong
Lu, M.D.^{a, b, c}, Weidong Ji, M.D., Ph.D.^{e§}, Huabin Li, M.D., Ph.D.^{f§}, Weiping Wen,
M.D., Ph.D.^{a, b, c, d§}

From the Departments of ^aOtorhinolaryngology Hospital, The First Affiliated Hospital
of Sun Yat-sen University, Guangzhou; ^bOtorhinolaryngology
Institute, Sun Yat-sen University; ^cGuangzhou Key Laboratory of
Otorhinolaryngology, Guangzhou; ^dDepartment of Otolaryngology, the Sixth
Affiliated Hospital of Sun Yat-sen University, Guangzhou; ^eTranslational medicine
center, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou;
^fDepartment of Otolaryngology, Head and Neck Surgery, Affiliated Eye, Ear, Nose
and Throat Hospital, Fudan University, Shanghai.

E-mail: wdji2008@126.com; allergyli@163.com; wenwp@mail.sysu.edu.cn.

*These authors contributed equally to this work.

§ Address correspondence to:

Weidong Ji, Translational medicine center, The First Affiliated Hospital of Sun
Yat-sen University, No. 58, Zhongshan 2nd Road, Guangzhou, 510080, China. Phone
and fax: 8602087338926. Email: wdji2008@126.com.

Huabin Li, Department of Otolaryngology, Head and Neck Surgery, Affiliated Eye,

Ear, Nose and Throat Hospital, Fudan University, No. 83, Fenyang Road, Shanghai, 200031, China. Phone and fax: 8602087338624. Email: allergyli@163.com.

Weiping Wen, The First Affiliated Hospital of Sun Yat-sen University, No. 58, Zhongshan 2nd Road, Guangzhou, 510080, China. Phone and fax: 8602087333108. Email: wenwp@mail.sysu.edu.cn.

Funding: This study was supported by the National Natural Science Foundation of China (NSFC) grants 81400451 (Y.W.), 81670902, 81870696 (W.P.W.), 81725004 (L.H.B) and 81772699 (W.D.J.), Guangdong Natural Science Foundation of China grants 2017A030313667 (Y.W.) and 2018B030312008 (W.P.W.), Guangzhou Science and Technology Project of China grant 201704020098 and 201605030003 (W.P.W.), Guangzhou People's Livelihood Science and Technology Project of China grant 201803010052 (W.D.J.) and Shanghai Science and Technology Committee grant 16410723600 (L.H.B.).

Disclosure of conflict of interest: The authors declare no conflict of interest.

Capsule Summary:

NLRP3 inflammasome and IL-1 β were significantly correlated and upregulated in high neutrophil infiltrated CRSwNP. The antigen and proinflammatory cytokines chemotaxis neutrophil infiltration by the NLRP3–IL-1 β axis in polyp tissues, although glucocorticoid treatment alleviates it.

Key Words:

NLR family; pyrin domain containing 3; interleukin (IL)-1 β ; chronic rhinosinusitis;

51 nasal polyps; neutrophil; eosinophil

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54 To Editor:

55 Chronic rhinosinusitis with nasal polyps (CRSwNPs) is a common chronic nasal
56 inflammatory disease with high heterogeneity worldwide. The endotypes of CRSwNP
57 are divided into eosinophilic infiltration (EOS), Th2 response and neutrophilic (NEU)
58 infiltration, mixed Th2/Th17 responses.¹ Moreover, in the eosinophilic subjects, 35%
59 showed a mixed eosinophil and neutrophil infiltration.² Detailed mechanisms
60 regarding regulation of different types of inflammatory cells await further
61 investigation.

62 NLR family, pyrin domain containing 3 (NLRP3) inflammasomes characterized
63 as multiprotein signaling complexes, plays important roles in both the innate and
64 adaptive immune response. The NLRP3 and its downstream interleukin (IL)-1 β were
65 identified to be potent inducers of neutrophilic inflammation in various inflammatory
66 diseases.³ At the same time, a positive regulation between the NLRP3 and Th2
67 program was indicated in several independent research studies.^{4,5} Our study, together
68 with Bruchard et al's report, showed that NLRP3 was highly expressed in nasal
69 polyps (NPs) from CRSwNP subjects.⁶ However, the role of the NLRP3
70 inflammasome in CRSwNP remains unclear. We therefore evaluated the expression
71 profile of the NLRP3 inflammasome in NPs and examined its relationship with NP
72 endotypes.

73 We enrolled 82 patients with CRSwNP, 32 patients with chronic rhinosinusitis
74 without nasal polyps (CRSSNP), and 35 healthy control subjects to address this issue.
75 The clinical characteristics are listed in Table E1 in this article's Online Repository at
76 www.jacionline.org. The study protocols were approved by ethics committee boards,
77 and all subjects gave written informed consent. Expression of NLRP3 inflammasomes,
78 and the proinflammatory cytokines IL-1 β and IL-4 were examined in nasal tissues. In
79 addition, expression of NLRP3 and IL-1 β in response to specific inflammatory
80 stimulation was measured in cultured single-cell suspension of NPs. Details of the
81 materials and methods used in this study are shown in the Methods section in this

article's Online Repository at www.jacionline.org.

Our results showed mRNA levels of NLRP3, NALP1 NLRC4, and IL-1 β were significantly higher in NPs than in uncinat tissues (UTs) from control subjects, patients with CRSsNP, or patients with CRSwNP ($P < .05$; Fig E1 A-D). No significant differences in ASC or caspase-1 mRNA expression were observed in NPs and UTs between patients with CRSsNP and CRSwNP (Fig E1 E, F).

To evaluate whether the NLRP3 inflammasome and IL-1 β are expressed in different polyp tissue subsets, we determined the presence of eosinophils and neutrophils in nasal tissues by H&E and immunohistochemical staining for neutrophilic (Fig 1 A). On the basis of defined cutoff values (Fig E2 and Table E2), we divided the polyp tissues into 4 subtypes: eosinophilic and neutrophilic (EOS⁺NEU⁺), noneosinophilic and neutrophilic (EOS⁻NEU⁺), eosinophilic and nonneutrophilic (EOS⁺NEU⁻), noneosinophilic and nonneutrophilic (EOS⁻NEU⁻).

We observed that the staining intensity and mean number of the NLRP3 inflammasome and IL-1 β were significantly increased in polyp tissues from patients with EOS⁺NEU⁺ and EOS⁻NEU⁺ than EOS⁺NEU⁻ and EOS⁻NEU⁻ CRSwNP ($P < .01$; Fig 1 A-C). Confirming the histologic findings, we found that polyp tissues from the EOS⁺NEU⁺ and EOS⁺NEU⁻ CRSwNP subsets exhibited significantly higher NLRP3 mRNA and protein levels than those from the EOS⁺NEU⁻ and EOS⁻NEU⁻ subsets ($P < .001$, Fig 1 D, E). Although the mRNA level and precursor protein (pro-IL-1 β , p37) of IL-1 β were not different between the 4 CRSwNP subsets (Fig 1 F), the activation protein (p17) and the secretion of IL-1 β were significantly increased in the EOS⁺NEU⁺ and EOS⁻NEU⁺ than EOS⁺NEU⁻ and EOS⁻NEU⁻ CRSwNP ($P < .001$, Fig 1 E, G). Those results indicated that the higher expression of NLRP3 in the EOS⁺NEU⁺ and EOS⁻NEU⁺ subsets lead to an increase in IL-1 β protein expression, whereas this situation was not apparent in the EOS⁺NEU⁻ and EOS⁺NEU⁻ CRSwNP.

Antigen stimulation and inflammation, what are important pathologic environments for different CRSwNP subsets, can activate NLRP3 inflammasome. To

establish the direct relationship between TLR agonists, proinflammatory cytokines, and neutrophil infiltration through the NLRP3–IL-1 β axis in CRSwNP, we examined the effects of TLR activation and proinflammatory cytokine stimulation on inflammasome complex expression in a cultured single-cell suspension of NPs and perform neutrophil chemotaxis in vitro. As shown in Fig 2 A-K, NLRP3, IL-1 β mRNA and protein levels were significantly increased after stimulating the cultured NPs single-cell suspension with TLR agonists and proinflammatory cytokines, especially TLR2, 3, 4 agonists LPS and poly (I:C) and IL-4 ($P < .01$). Of note, IL-4, a typical Th2 cytokine, which is beyond our expectations, induced high NLRP3 and promoted subsequent IL-1 β secretion in single-cell suspension of NPs as well ($P < .01$)

To clarify which type of cell expresses NLRP3, we determined the cellular localization of NLRP3 by immunofluorescence. Then we found that NLRP3 was co-localized with CD68 and MUC5AC in the human NPs tissue (Fig. E6). This finding indicated that the macrophages and epithelial cells were the main source of NLRP3 in the polyp tissues. Moreover, we examined the inflammasome complex mRNA or protein levels, in the macrophage or epithelial cells from CRSwNP patients, responded to TLR agonists and proinflammatory cytokines. Then we found that the TLR agonists (LPS and Poly I: C) and proinflammatory cytokines (IL-4) increased the mRNA and protein levels of NLRP3/ IL-1 β (Fig.E7), the results were consistent with the mixed cell experiments.

Next, we found the mRNA levels of TLR2, 3, 4 had the highest expression in polyp tissues from EOS⁻NEU⁺, followed by EOS⁺NEU⁺, and the lowest expression was in EOS⁺NEU⁻ and EOS⁻NEU⁻ CRSwNP, as our previous studies⁷ ($P < .001$, Fig E3 A-C). Accordingly, mRNA levels of TH2 cytokines IL-4, 5, 13 were significantly higher in EOS⁺NEU⁺ and EOS⁺NEU⁻ polyp tissues than in EOS⁻NEU⁺ and EOS⁻NEU⁻ polyp tissues ($P < .001$, Fig E3 D-F). However, mRNA expressions of the IFN- γ , and IL-17a were not significantly different between the 4 CRSwNP subsets (Fig E3 G, H). The results suggested that antigen stimulation was the major factor leading to elevated

NLRP3 and IL-1 β expressions in EOS⁺NEU⁺ and EOS⁻NEU⁺ CRSwNP subsets, moreover IL-4 enhanced this effect in the EOS⁺NEU⁺ subset.

Furthermore, The supernatant of the NP single-cell treatment with LPS, poly I: C, IL-4, and ATP significantly chemotaxis more neutrophil compared with control, and those trends could be suppressed by small-molecule siRNA inhibiting NLRP3 or neutralizing antibody against IL-1 β (Fig 2 L and Fig E4 A-C, $P < .001$). Moreover, we did the neutrophils chemotaxis by culture supernatant from different treatments with macrophage or epithelial cells from CRSwNP patients. Then we found that LPS, Poly I: C, IL-4 also could chemotaxis neutrophils in the macrophage or epithelial cells (Fig. E8), the results were consistent with the mixed cell experiments. Inhibition of NLRP3 expression in single-cell suspension of NPs abrogated the effect of either LPS, poly (I:C), or IL-4 exposure on IL-1 β secretion and neutrophil recruitment, indicating that NLRP3 is a central component in both tissue eosinophilia and neutrophilia. Meanwhile, the inhibitor of NLRP3 or abolished downstream IL-1 β maturation played a potential role in the control of chronic nasal mucosal inflammation.

Because glucocorticoid treatment is recommended as the primary treatment for patients with CRSwNP, we found the treatment with glucocorticoids significantly downregulated NLRP3 mRNA and IL-1 β protein levels and alleviated the chemotactic neutrophils by IL-4 but not for LPS, poly I: C and ATP (Fig 2 L, Fig E4 D and Fig E5, $P < .001$). This finding indicates that glucocorticoid treatment might partially exert its anti-inflammatory effect by inhibiting the NLRP3 inflammasome and IL-1 β expressions and reducing local mucosal neutrophilic infiltration.

In summary, these studies suggest that the NLRP3 inflammasome and its associated downstream IL-1 β were significantly correlated and upregulated in high-neutrophil-infiltrated NPs, including both mixed eosinophil and neutrophil infiltration subjects and singular neutrophil infiltration subjects. The antigen stimulated high expression of NLRP3 and secretory IL-1 β may be the key factor in neutrophilic inflammation of EOS⁻NEU⁺ and EOS⁺NEU⁺ CRSwNP subsets.

Meanwhile, IL-4 induced NLRP3 expression promotes mixed eosinophilic and neutrophilic infiltration in the EOS⁺NEU⁺ CRSwNP. Gurung et al reported that NLRP3 was the trigger of an adaptive Th2-biased immune response upon parasite infection.⁸ In contrast, Niebuhr et al's published data from subjects with atopic dermatitis indicated that the function of the NLRP3 inflammasome was impaired in keratinocytes under a dominant Th2 milieu.⁹ It is possible that NLRP3 inflammasome signaling exerts different roles in different types of cells, under certain disease settings. IL-4–NLRP3 signaling interactions may amplify IL-1 β production in single-cell suspension of NPs and modulate neutrophil chemotaxis, thus contributing to NP pathogenesis in vivo. These studies indicate that the NLRP3 pathway may be used as a therapeutic approach to target a certain CRSwNP subset.

Yi Wei, Ph.D. ^{a, b, c*}

Jia Zhang, M.D., Ph.D. ^{f*}

Xingmei Wu, M.D., Ph.D. ^{a, b, c*}

Wei Sun, M.D., Ph.D. ^{a, b, c}

Fanqin Wei, M.D., Ph.D. ^{a, b, c}

Wendong Liu, M.D. ^{a, b, c}

Tong Lu, M.D. ^{a, b, c}

Weidong Ji, M.D., Ph.D. ^{e§}

Huabin Li, M.D., Ph.D. ^{f§}

Weiping Wen, M.D., Ph.D. ^{a, b, c, d§}

From the Departments of ^aOtorhinolaryngology Hospital, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou; ^bOtorhinolaryngology

Institute, Sun Yat-sen University; ^cGuangzhou Key Laboratory of
 Otorhinolaryngology, Guangzhou; ^dDepartment of Otolaryngology, the Sixth
 Affiliated Hospital of Sun Yat-sen University, Guangzhou; ^eTranslational medicine
 center, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou;
^fDepartment of Otolaryngology, Head and Neck Surgery, Affiliated Eye, Ear, Nose
 and Throat Hospital, Fudan University, Shanghai.

REFERENCES

1. Zhang Y, Gevaert E, Lou H, Wang X, Bachert C, Zhang N, et al. Chronic rhinosinusitis in Asia. *J Allergy Clin Immunol* 2017; 140:1230-1239.
2. Wen W, Liu W, Zhang L, Bai J, Li H, Xu G, et al. Increased neutrophilia in nasal polyps reduces the response to oral corticosteroid therapy. *J Allergy Clin Immunol* 2012; 129:1522-1528.
3. Haneklaus M, O'Neill LA. NLRP3 at the interface of metabolism and inflammation. *Immunology Rev* 2015; 265:53-62.
4. Ting JP, Harton JA. NLRP3 moonlights in TH2 polarization. *Nat Immunol*. 2015; 16:794-796.
5. Bruchard M, Rebe C, Derangere V, Togbe D, Vegran F, Ghiringhelli F, et al. The receptor NLRP3 is a transcriptional regulator of TH2 differentiation. *Nat Immunol*. 2015; 16:859-70.
6. Lin H, Li Z, Lin D, Zheng C, Zhang W. Role of NLRP3 Inflammasome in Eosinophilic and Non-eosinophilic Chronic Rhinosinusitis with Nasal Polyps. *Inflammation* 2016; 39:2045-2052.
7. Wei Y, Xia W, Ye X, Fan Y, Yang P, Li H, et al. The antimicrobial protein short palate, lung, and nasal epithelium clone 1 (SPLUNC1) is differentially modulated in eosinophilic and noneosinophilic chronic rhinosinusitis with nasal

- 217 polyps. J Allergy Clin Immunol. 201; 133:420-8.
- 218 8. Gurung P, Karki R, Vogel P, Watanabe M, Bix M, Lamkanfi M, Kanneganti TD.
219 An NLRP3 inflammasome-triggered Th2-biased adaptive immune response
220 promotes leishmaniasis. J Clin Invest. 2015; 125:1329-1338.
- 221 9. Niebuhr M, Baumert K, Heratizadeh A, Satzger I, Werfel T. Impaired NLRP3
222 inflammasome expression and function in atopic dermatitis due to Th2 milieu.
223 Allergy. 2014; 69:1058-1067.

1 **Figure legends**

2

3 **Figure 1. NLRP3, IL-1 β mRNA and protein expression in 4 CRSwNP subsets.**

4 (A) Representative NEU, H&E, NLRP3 and IL-1 β staining ($\times 400$). (B, C)
 5 Quantitative analysis of NLRP3 and IL-1 β staining numbers. (D, F) mRNA
 6 expression of NLRP3 and IL-1 β . (E) Representative Western blot results of NLRP3
 7 and IL-1 β , pro-IL-1 β (p37), cleaved IL-1 β (p17). (G) ELISA analysis of IL-1 β .
 8 Results are expressed as the medians ((IQRs). No statistical significance is ns, *P
 9 < .05, **P < .01, and ***P < .001; as determined by nonparametric Mann-Whitney
 10 U-test.

11

12 **Figure 2. Inflammasome complex expression level and neutrophils chemotaxis** 13 **from different treatments' single cells of polyp tissues.**

14 (A, C) NLRP3, (B, D) IL-1 β mRNA expression after a 24 hours stimulation. (E, F)
 15 Representative NLRP3, pro-IL-1 β (p37) and cleaved IL-1 β (p17) western blot results
 16 of cell lysates, and (G, I) densitometric analysis of NLRP3 after a 48 hours
 17 stimulation. (H, K) ELISA analysis of IL-1 β after a 72 hours stimulation. (L) Analysis
 18 of neutrophils numbers by different treatments. Results represent mean values (SEMs)
 19 from 3 independent experiments. No statistical significance is ns, *P < .05, **P < .01,
 20 and ***P < .001, as determined by unpaired Student's, t-test. Comparison among
 21 groups, ###P < .001, as determined by 1-way Analysis of Variance (ANOVA).

Activated pyrin domain containing 3 (NLRP3) inflammasome in neutrophilic chronic rhinosinusitis with nasal polyps (CRSwNP)

METHODS

Patients and tissue samples

This study was approved by the local ethics committee boards, and written informed consent was obtained from each subject. Adult patients with CRS (CRSwNP, n = 82; chronic rhinosinusitis without nasal polyps [CRSsNP], n = 32) and 35 control subjects were randomly recruited from the First Affiliated Hospital of Sun Yat-sen University. CRSwNP and CRSsNP diagnoses were made according to the current European position paper on rhinosinusitis and NPs.¹⁻² Skin-prick tests were performed to evaluate the atopic status of the patients. Asthma was diagnosed by a pneumologist based on disease history and evaluation of airway responsiveness. The polyp tissues were sampled from CRSwNP patients, and the uncinated tissues were sampled of CRS patients and control subjects. The demographic data of all subjects enrolled in the study are listed in Table E1.

Tissue samples were divided into 3 pieces: the first was frozen in liquid nitrogen and stored at -80 °C for subsequent RNA extraction, the second was used for protein extraction, and the third was fixed overnight in a freshly prepared fixative containing 4% paraformaldehyde in phosphate-buffered saline (PBS; pH 7.4) and embedded in paraffin wax for histologic assessment. In addition, single-cell suspension of human NPs was isolated and cultured from fresh polyp tissues.

District CRSwNP subsets

Different CRSwNP subsets were identified by eosinophilic and/or neutrophilic cells counted in hematoxylin and eosin (H&E) and neutrophil elastase (NEU)

immunohistochemically staining. After counting the number of positive cells, the cutoff value for the eosinophilic phenotype was set at 16 eosinophilic cells/high-power field (hpf; 200 $\times$), which is equivalent to the mean value in the noneosinophilic subjects (5.86) plus 2 times the standard deviation (SD; $4.60 \times 2 = 9.2$; cutoff value, $5.86 + 9.2 = 15.06$). The cutoff value for the neutrophilic phenotype was set at 17 neutrophilic cells/hpf (200 $\times$), which is equivalent to the mean value in the nonneutrophilic subjects (6.37) plus 2 times the SD ($4.87 \times 2 = 9.74$; cutoff value, $6.37 + 9.74 = 16.44$). On the basis of defined cutoff values, we divided the polyp tissues into 4 subtypes: eosinophilic and neutrophilic (EOS⁺NEU⁺), noneosinophilic and neutrophilic (EOS⁻NEU⁺), eosinophilic and nonneutrophilic (EOS⁺NEU⁻), noneosinophilic and nonneutrophilic (EOS⁻NEU⁻). As shown in Table E2 and Fig E2, the median number of eosinophils per hpf in the polyp tissues was EOS⁺NEU⁺ 34.4 (IQR, 21.2-49.8), EOS⁻NEU⁺ 4.3 (IQR, 0.6-15.2), EOS⁺NEU⁻ 28.6 (IQR, 18.2-43.2), and EOS⁻NEU⁻ 3.4 (IQR, 0.6-15.8), respectively. The median number of neutrophils per hpf in the polyp was EOS⁺NEU⁺ 34 (IQR, 21.8-53.6), EOS⁻NEU⁺ 40.5 (IQR, 25.4-57.8), EOS⁺NEU⁻ 4.4 (IQR, 0.5-16.9), and EOS⁻NEU⁻ 4.7 (IQR, 0.6-15.8), respectively. The demographic data of these subjects are listed in Table E2.

Quantitative real-time polymerase chain reaction (qRT-PCR)

The mRNA expression levels were evaluated by using qRT-PCR analysis. Total RNA was extracted with TRIzol reagent (Invitrogen) according to the manufacturer's instructions. The total RNA was then used to synthesize the first-strand cDNA using the 5X Prime Script Kit (TaKaRa). qPCR was performed on the ABI7500 system (Invitrogen). For the relative quantification of mRNA expression, the expression of β -actin served as an internal control. Perfecta SYBR Green (Invitrogen) was used as a DNA intercalator dye to monitor amplified DNA quantification. The amplification conditions were 40 cycles for 2-stage PCR (95 $^{\circ}$ C for 12 s and 60 $^{\circ}$ C for 1 min). The following primers are shown in Table E3.

55

56 **Histologic staining**

57 H&E and immunohistochemical staining for NEU, NLRP3, and IL-1 β were
58 performed according to the manufacturer's instructions. Rabbit anti-NEU antibody,
59 Abcam, ab68672, 1:200; Mouse anti-NLRP3 antibody, Enzo, ALX-804-818, 1:100;
60 Rabbit anti-IL-1 β antibody, Abcam, ab2105, 1:100; Mouse, IgG1 isotype, Abcam,
61 ab81216, 1:100; Rabbit, IgG isotype, Abcam, ab37415, 1:100, were using as the
62 primary antibodies according to the manufacturer's instructions (Fig E9). Each
63 section was incubated with secondary antibody and then with horseradish peroxidase
64 (HRP)-labeled streptavidin complex (DAKO). Distribution of peroxidase was
65 revealed by incubating the sections in a solution containing 3% 3, 3-diaminobenzidine
66 tetrahydrochloride before being counterstained with hematoxylin and cover slipped.
67 Negative control studies were performed using isotype control IgG and IgG1 to
68 replace the primary antibody in appropriate concentration. The histological analyses
69 were performed in a double-blind manner. The immunoreactivity of five randomly
70 selected epithelial or subepithelial areas (200 $\times$) on each section of the subjects was
71 quantitatively scored using Image-Pro Plus 6.0 software, and the average optical
72 density value was calculated.¹⁻⁴

73

74 **Immunofluorescence**

75 Nasal polyp tissues embedded in paraffin were cut into 4 μ m sections and placed
76 onto glass slides. Immunofluorescence (IF) staining was performed as previously
77 described.⁵ The tissues were incubated with a primary antibody overnight at 4°C,
78 including mouse anti-CD68 (1:100, Abcam, ab955), mouse anti-MUC5AC (1:50,
79 SANTA CRUZ, sc-20118), goat anti- NLRP3 (1:100, Abcam, ab4207), rabbit anti-
80 IL-1 β (1:100, Abcam, ab2105), and mouse IgG1 isotype control (Abcam, ab81216),
81 goat IgG polyclonal isotype control (Abcam, ab37373), rabbit IgG polyclonal isotype

control (Abcam, ab37415). This was followed by the incubation with fluorescent□
conjugated secondary antibodies for 50 minutes at room temperature, including
donkey anti-mouse IgG H&L (Alexa Fluor® 555, Abcam, ab150106) (1:500), donkey
anti-goat IgG H&L (Alexa Fluor® 647, Abcam, ab150131) (1:500), and donkey
anti-rabbit IgG H&L (Alexa Fluor® 488, Abcam, ab150073) (1:500). All the
secondary antibodies were purchased from Life Technologies. Nuclear DNA was
detected by incubation of DAPI (1:500, Roche) for 10 minutes at room temperature.
Coverslips were mounted in ProLong Antifade Reagent. Images were captured with a
confocal microscopy (Leica, 780).

Western blot analysis

The protein levels were evaluated by means of Western blot analysis as
previously described.¹⁻² The protein extracts from nasal tissues underwent sodium
dodecyl sulfate–polyacrylamide gel electrophoresis and were transferred to a
polyvinylidene fluoride membrane. The membranes were blocked and incubated with
primary antibody (Mouse anti-NLRP3 antibody, Enzo, ALX-804-818, 1:1000; Rabbit
anti-IL-1 β antibody, Abcam, ab2105, 1:1000) and then incubated with the
anti-mouse/rabbit IgG HRP secondary antibody and detected using enhanced
chemiluminescence detection reagent (Millipore). Western blot images were scanned
and analyzed using GeneTools from stngene software (SYNGENE).

Enzyme-linked immunosorbent assay (ELISA)

Polyp tissues were homogenized with 2 times their weight of PBS (mL/mg), and
the final concentration was calculated from the weight of the tissue. The levels of IL- β
in the homogenized supernatants of polyp tissues was measured by ELISA using a
series of commercially available ELISA kits (IL- β , R&D Systems, SLB50) according
to the manufacturer's instructions.

109

110 NPs Cell culture and stimulation in vitro

111 Polyp tissues were collected from randomly selected patients with CRSwNP, and
112 single-cell suspension of polyp tissues was isolated by collagenase V digesting for 1
113 hour at 37°C. Then, we added RPMI 1640 medium containing 20 % FBS to terminate
114 digestion, filtered cell suspensions and centrifuged at 400g for 5 min. We washed the
115 cells with PBS (37 °C, pH 7.4) and cultured them into RPMI 1640 medium (Gibco)
116 containing 10 % FBS at 37°C with 5% CO₂ as previously described.¹⁻⁴

117 After preparing a single cell suspension, we added either 20μl PBS (blank
118 control), 20μl Toll-like receptor agonists (Catalog no. and working concentration
119 shown in Table E4), or 20μl recombinant proinflammatory cytokines (Catalog no. and
120 working concentration shown in Table E5) to the medium immediately. The cells
121 were harvested for assaying mRNA by PCR after 24 hours, assaying protein by
122 western blotting after 48 hours, and the supernatants were collected for assaying
123 IL-1β protein levels by ELISA after 72 hours.

124 In some experiments, the single-cell suspension of polyp tissues were incubated
125 with either 20μl PBS (blank control), 20μl LPS, 10μl Poly I: C LMW +10μl Poly I: C
126 HMW, 20μl IL-4, or 5 mM adenosine triphosphate (ATP, Invitrogen™, 18330-019;
127 positive control) for 24 hours. Then we added either 60μl PBS (blank control), 60μl
128 small-molecule small interfering RNA (siRNA) inhibiting NLRP3 (20 nM,
129 GenePharma), 60μ neutralizing antibodies against IL-β (1 μg/mL, R&D Systems,
130 MAB201), or 60μ glucocorticoids (including 100 μM dexamethasone, 100 μM
131 budesonide, and 100 μM fluticasone) continue to culture for 48 hours. After 72 hours,
132 the supernatant of treated single cells from polyp tissues was collected for the
133 neutrophil chemotaxis experiment.

134

135 Macrophages culture

PBMCs were isolated from buffy coats of blood from CRSwNP patients by Ficoll density gradient centrifugation, as previously described⁶. Monocytes were selected from PBMCs by anti-CD14 magnetic beads in a MACS column purification system (Miltenyi Biotec). To generate normal or activated Macrophages, the purified monocytes were cultured for 6–7 days in complete RPMI medium supplemented with 40 ng/ml GM-CSF, half of the culture medium was replaced on day 3 and day 5.

HNECs culture

Polyp tissues were collected from 4 randomly selected patients with CRSwNP, and human nasal epithelial cells (HNECs) were isolated, as we previously described⁷. The NP tissue was cut into small pieces (3-5mm), incubated with Dispase 2 (Sigma, D4693-1G) overnight and EDTA-Trypsin (0.5%, GIBCO, 25300-054) at 37°C with 5% CO₂, 15 minutes, neutralized with DMEM (10% FBS). The tissue pieces thoroughly dispersed into a single cell suspension, cultured in the 6-well plate (1x10⁵ cells), with DMEM (10% FBS), at 37°C with 5% CO₂, overnight. Drew the suspended cells, used for the non-HNECs. The adherent cells were HNECs. The HNECs were culture at air-liquid interface (ALI) using BEGM™ Bronchial Epithelial Cell Growth Medium (LONZA, USA) in the polyester membrane transwell-clear inserts (Corning, Lowell, MA, USA), at 37°C with 5% CO₂.

Macrophages and HNECs stimulation

After macrophages or HNECs achieving 70–80% confluence, 20μl PBS (blank control), 20μl LPS, 10μl Poly I: C LMW +10μl Poly I: C HMW, 20μl IL-4 were adding to bottom chamber. The cells were harvested for assaying mRNA by PCR after 24 hours, assaying protein by western blotting after 48 hours, and the supernatants were collected for neutrophil chemotaxis after 72 hours.

Neutrophil chemotaxis in vitro

Neutrophils were isolated from buffy coats of blood from healthy donors by Ficoll density gradient centrifugation and red cell lysis. The polymorphonuclear neutrophils (PMNs) were selected by anti-CD16 magnetic beads in a MACS column purification system (Miltenyi Biotech) and checked by Trypan Blue exclusion staining (>97% viable). They were resuspended in RPMI 1640 medium (Gibco) containing 10% FBS (Sigma-Aldrich). Neutrophil chemotaxis was assessed in a 48-well chemotaxis chamber (Neuro Probe), with a 5- μ m polycarbonate filter between the upper and lower chamber and a 2- μ m filter lining the bottom of the lower chamber. The lower chamber contained 50 μ L of the supernatant of the polyp tissue single cells treated as described above or serum-free RPMI 1640 medium (control for random migration). The upper chamber contained 50 μ L PMN suspension (3×10^6 cells/mL) with serum-free RPMI 1640 medium. The chambers were incubated for 1 hour at 37°C with 5% CO₂. The upper chamber contents were discarded, and nonmigrated PMNs were wiped off the upper side of the 5- μ m filter per the manufacturer's instructions. Cells were counted on the lower side of the 5- μ m filters under a light microscope (Olympas). Neutrophil migration can be expressed as PMNs/hpf.

Statistical analysis

Continuous variables are expressed as means and the standard errors of the medians and interquartile ranges (IQRs). Data were analyzed using the nonparametric Mann-Whitney *U* test. The Spearman rank correlation test was used to analyze the correlations among different parameters. For in vitro assays, the data were expressed as the standard error of the mean of 3 independent experiments. One-way analysis of variance and paired or unpaired Student's *t*-test were used for the statistical analysis. A *P* value less than .05 was considered statistically significant.

REFERENCES

1. Wen W, Liu W, Zhang L, Bai J, Li H, Xu G, et al. Increased neutrophilia in nasal polyps reduces the response to oral corticosteroid therapy. *J Allergy Clin Immunol.* 2012; 129:1522-1528.
2. Wei Y, Xia W, Ye X, Fan Y, Yang P, Li H, et al. The antimicrobial protein short palate, lung, and nasal epithelium clone 1 (SPLUNC1) is differentially modulated in eosinophilic and noneosinophilic chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol.* 2014; 133:420-8.
3. Lin H, Li Z, Lin D, Zheng C, Zhang W. Role of NLRP3 Inflammasome in Eosinophilic and Non-eosinophilic Chronic Rhinosinusitis with Nasal Polyps. *Inflammation.* 2016; 39:2045-2052.
4. Barac A, Pekmezovic M, Spiric VT, Trivic A, Marinkovic J, Arsenijevic VA8, et al. Chronic rhinosinusitis: association of recalcitrant nasal polyposis and fungal finding in polyp's single-cell suspension. *Eur Arch Otorhinolaryngol.* 2015; 272: 3727-34.
5. Hong HY, Chen FH, Sun YQ, Xu R, Li HB, Shi JB, et al. Local IL-25 contributes to Th2-biased inflammatory profiles in nasal polyps. *Allergy.* 2018; 73: 459-69.
6. Zhao Q, Xiao X, Wu Y, Wei Y, Zhu LY, Kuang DM, et al. Interleukin-17-educated monocytes suppress cytotoxic T-cell function through B7-H1 in hepatocellular carcinoma patients. *Eur J Immunol.* 2011; 41: 2314-22.
7. Lai Y, Chen B, Shi J, Palmer JN, Kennedy DW, Cohen NA. Inflammation-mediated upregulation of centrosomal protein 110, a negative modulator of ciliogenesis, in patients with chronic rhinosinusitis. *J Allergy Clin Immun.* 2011; 128: 1207-1215.

Repository E Figure legends**Figure E1. Inflammasome complex mRNA expression in nasal tissues.**

(A) NLRP3, (B) NALP1, (C) NLRC4, (D) IL-1 β , (E) ASC, and (F) Caspase-1 mRNA levels were significantly increased in polyp tissues from patients with CRSwNP compared with values seen in the other subgroups. Results are expressed as the medians ((IQRs). *P < .05, **P < .01, and ***P < .001; as determined by nonparametric Mann-Whitney U-test.

Figure E2. Number of tissue eosinophils and neutrophils in EOS⁺NEU⁺, EOS⁺NEU⁻, EOS⁻NEU⁺ and EOS⁻NEU⁻ polyp tissues.

(A) Eosinophils staining with H&E (B) neutrophils anti-NEU Ab, positive cells per high-power field [HPF], X200 magnification. Results are expressed as the medians ((IQRs). *P < .05 and ***P < .001; as determined by nonparametric Mann-Whitney U-test.

Figure E3. mRNA expression of the TLR2,TLR3, TLR4, IL-4, IL-5, IL-13, IFN- γ , and IL-17a in 4 CRSwNP subsets.

mRNA expression of the (A) TLR2, (B) TLR3, (C) TLR4, (D) IL-4, (E) IL-5, (F) IL-13, (G) IFN- γ , and (H) IL-17a, in 4 CRSwNP subsets. Results are expressed as the medians ((IQRs). No statistical significance is ns, **P < .01, and ***P < .001; as determined by nonparametric Mann-Whitney U-test.

Figure E4. Neutrophils chemotaxis by cultural supernatant from different treatments' single cells of polyp tissues.

Representative neutrophils images by nasal polyps single cells supernatant treatment with (A) LPS, Poly I: C, IL-4 and ATP, (B) presence small molecule siRNA inhibiting NLRP3 (si-NLRP3), (C) neutralizing antibody against IL-1 β (anti-IL1 β), and (D) glucocorticoids dexamethasone (DXM).

Figure E5. mRNA expression of NLRP3 and IL-1 β in the single cell suspension of nasal polyps with glucocorticoid treatment.

NLRP3 and IL-1 β mRNA levels after stimulation with glucocorticoid alone (A, E) or glucocorticoid in combination with the IL-4 (B, F), LPS (C, G), and Poly I: C (D, H). Results represent mean values (SEMs) from 3 independent experiments. No statistical significance is ns, *P < .05, **P < .01, and ***P < .001, as determined by unpaired Student's, t-test. Bud, Budesonide; Dex, dexamethasone; Flu, fluticasone.

Figure E6. The cellular localization of NLRP3 in nasal polys.

Determined the cellular localization of NLRP3 by immunofluorescence, the NLRP3 and IL-1 β were co-localized with CD68 (A) and MUC5AC (B) in the human NPs tissue.

Figure E7. LPS, Poly I: C and IL-4 stimulated macrophage or epithelial cells from CRSwNP patients.

(A, B) mRNA levels of NLRP3 and IL- β in macrophage, (C, D) mRNA levels of NLRP3 and IL- β in epithelial cells, (E) protein levels of NLRP3/ IL-1 β in macrophage or epithelial cells. Results represent mean values (SEMs) from 3 independent experiments. ***P < .001, as determined by unpaired Student's, t-test.

Figure E8. Neutrophils chemotaxis by culture supernatant from LPS, Poly I: C, IL-4 with macrophage or epithelial cells from CRSwNP patients.

(A) Representative neutrophils images by nasal polyps single cells supernatant treatment with LPS, Poly I: C, IL-4, (B) summary graphs. Results represent mean values (SEMs) from 3 independent experiments. ***P < .001, as determined by unpaired Student's, t-test.

Figure E9. Immunohistochemically isotype staining for NEU, IL-1 β , and NLRP3.

(A) Neutrophils staining with isotype control, (B) Neutrophils staining with anti-NEU Ab, (C) IL-1 β staining with isotype control, (D) IL-1 β staining with anti-IL-1 β , (E) NLRP3 staining with isotype control, (F) NLRP3 staining with anti- NLRP3, red arrow indicates positive cells, X200 magnification.

Repository E Tables

Table E1. Demographics of subjects.

	Control Subjects	Patients with CRSsNP	Patients with CRSwNP
No.	35	32	82
Tissue sampled	Uncinate	Uncinate	Uncinate (34)/ Nasal polyps (82)
Age, mean (SD), (y)	27.8 (22-55)	33.8 (21-57)	30.6 (21-58)
Atopy, no. (%)	4 (11.43)	5 (15.63)	19 (23.17)*
Asthma, no. (%)	0	2 (6.25)	8 (10.98)
Aspirin sensitivity, no.	0	0	2 (2.44)
Sex (male/female)	17/ 18	15/17	39/43
CT Scores, mean (SD)	NA	5.56 (3.53)	15.79 (10.03)
Total IgE, mean (SD), (IU/mL)	11.95 (3.96)	88.46 (44.81)	101.74 (51.54)*
Blood eosinophils, mean (SD), ($10^9/L$)	0.02 (0.05)	0.18 (0.19)	0.35 (0.38)*
Methodologies used			
Tissue IHC	35	32	34/82
Tissue mRNA	35	32	34/82
Tissue WB	12	14	13/15

Definition of abbreviations: CRSsNP= chronic rhinosinusitis without nasal polyps.

CRSwNP=chronic rhinosinusitis with nasal polyps. * $P<0.05$, compared with control subjects.

Table E2. Characteristics of patients with EOS+NEU+, EOS+NEU-, EOS-NEU+ and EOS-NEU- CRSwNP subsets

	EOS+NEU+ subsets	EOS+NEU- subsets	EOS-NEU+ subsets	EOS-NEU- subsets
No.	20	20	21	21
Age, mean (SD), (y)	31.8 (22-57)	29.4 (21-56)	30.2 (21-58)	30.8 (23-55)
Atopy, no. (%)	9 (45)*	7 (35)*	2 (9.5)	1 (4.67)
Asthma, no. (%)	4 (20)*	4 (20)*	NA	NA
Aspirin sensitivity, no.	1 (5)	1 (5)	NA	NA
Sex (male/female)	11/ 9	10/10	12/9	10/11
CT Scores, mean (SD)	18.67 (11.86)	14.12 (8.97)	8.82 (5.60)	5.54 (3.52)
Total IgE, mean (SD), (IU/mL)	110.52 (55.99)	104.48 (52.93)	88.54 (44.85)	74.58 (37.78)
Blood eosinophils, mean (SD), ($10^9/L$)	0.45 (0.49)*	0.41 (0.45)*	0.22 (0.24)	0.19 (0.21)
Methodologies used				
Tissue IHC	20	20	21	21
Tissue mRNA	20	20	21	21
Tissue WB	7	7	6	7

Definition of abbreviations: CRSwNP=chronic rhinosinusitis with nasal polyps. * $P<0.05$, compared with control subjects.

Table E3. Primer sequences for quantitative PCR analysis

Primer	Forward	Reverse
NLRP3	5'-AAGGAAGTGGACTGCGAGAA-3'	5'-ACGTTTCGTCCTTCCTTCCTT -3'
IL-1 β	5'-GGGCCTCAAGGAAAAGAATC-3'	5'-TTCTGCTTGAGAGGTGCTGA-3'
NALP1	5'-GCAGTGCTAATGCCCTGGAT-3'	5'-GAGCTTGGTAGAGGAGTGAGG-3'
NLRC4	5'-ATCTTTTCGGGATAGGTTCTGT-3'	5'-ACACGATCTCTGTTATAGGAGCA-3'
ASC	5'-TGGATGCTCTGTACGGGAAG-3'	5'-CCAGGCTGGTGTGAAACTGAA-3'
caspase-1	5'-TTTCCGCAAGGTTTCGATTTTCA-3'	5'-GGCATCTGCGCTCTACCATC-3'
TLR2	5'-GGTCCTGTGCCACCGTTTC-3'	5'-GATGTTCCCTGCTGGGAGCTTT-3'
TLR3	5'-TGATGCTCCGAAGGGTGG-3'	5'-CAGGGTTTGCGTGTTTCC-3'
TLR4	5'-GGATGAGGACTGGGTAAGGAATG-3'	5'-CACACCGGGAATAAAGTCTCTGTA-3'
IL-4	5'-TTGCTGCCTCCAAGAACACAACCTG-3'	5'-TTCCTGTGAGCCGTTTCAGGAAT-3'
IL-5	5'-TCTACTCATCGAACTCTGCTGA-3'	5'-CCCTTGACACAGTTTGACTCTC-3'
IL-13	5'-TGGTCAACATCACCCAGAACCAGA-3'	5'-AGCCTGACACGTTGATCAGGGATT-3'
IFN- γ	5'-TGCAGGTCATTCAGATGTAGCGGA-3'	5'-TGTCTTCCTTGATGGTCTCCACACTC-3'
IL-17a	5'-CAACCGATCCACCTCACC-3'	5'-AGCCACGGACACCAGTA-3'
β -actin	5'-AAGTGTGACGTTGACATCCG-3'	5'-GATCCACATCTGCTGGAAGG-3'

Table E4. Toll-like receptor agonists

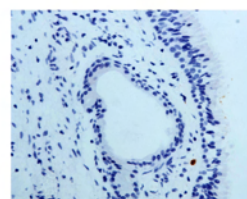
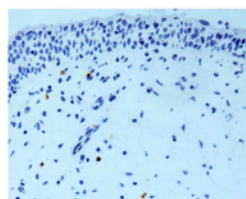
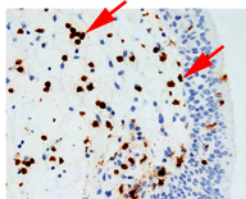
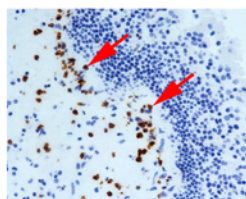
Agonist	Company	Catalog no.	Ingredient	Receptors	Working Concentration
Pam3CSK4	invivogen	tlrl-pms	Synthetic triacylated lipopeptide	TLR1/2	100 ng/ml
PGN	invivogen	tlrl-pgns2	Peptidoglycan	TLR2	10 µg/ml
PolyI:C LMW	invivogen	tlrl-picw	Polyinosinic-polycytidylic acid, from 0.2 kb to 1 kb	TLR3	100 ng/ml
PolyI:C HMW	invivogen	tlrl-pic	Polyinosinic-polycytidylic acid, from 1.5 kb to 8 kb	TLR3	100 ng/ml
LPS	invivogen	tlrl-pb5lps	Lipopolysaccharide	TLR4	100 ng/ml
FLA-ST	invivogen	tlrl-stfla	Recombinant Flagellin from <i>S.</i> <i>typhimurium</i>	TLR5	100 ng/ml
FSL-1	invivogen	tlrl-fsl	synthetic lipopeptide contained a diacylated cysteine residue	TLR2/6	100 ng/ml
Imiquimod-R 837	invivogen	tlrl-imqs	An imidazoquinoline amine analog to guanosine	TLR7/8	1 µg/ml
ssRNA	invivogen	tlrl-lrna40	Single-stranded RNA	TLR7/8	1 µg/ml
ODN2359	invivogen	tlrl-2395	Synthetic oligonucleotides	TLR9	1 µM

Table E5. Recombinant proinflammatory cytokines

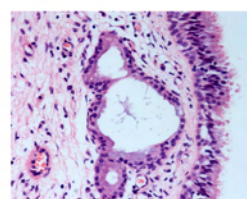
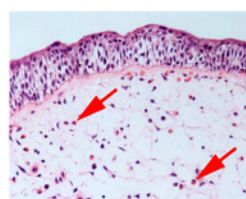
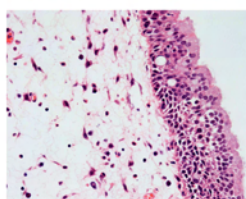
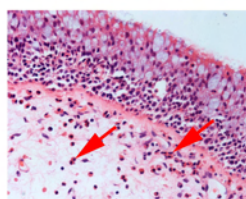
Cytokines	Company	Catalog no.	Working concentration
IL-1 β	Invivogen	rcyec-hil1b	100 ng/ml
IL-4	Invivogen	rcyec-hil4	100 ng/ml
IL-5	Sino Biological Inc	15673-HNCE	100 ng/ml
IL-13	Peprtech	200-13	100 ng/ml
IL-17a	Invivogen	rcyc-hil17a	100 ng/ml
IFN- γ	Invivogen	rcyec-hifng	100 ng/ml
TGF- β	Invivogen	rcyc-htgfb1	100 ng/ml

A

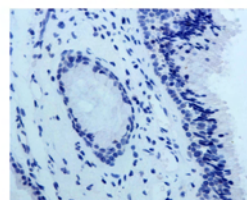
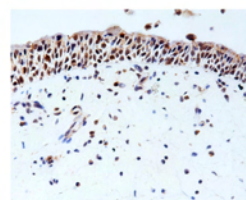
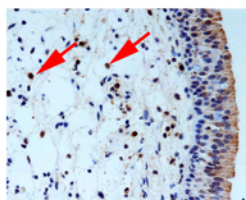
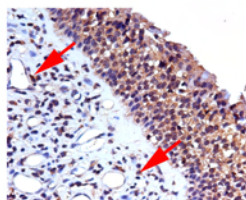
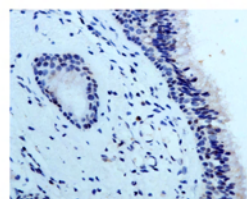
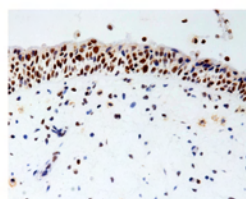
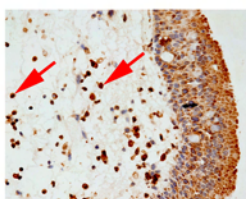
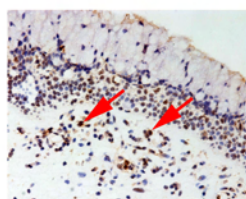
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HE



NLRP3

IL-1 β 

200X

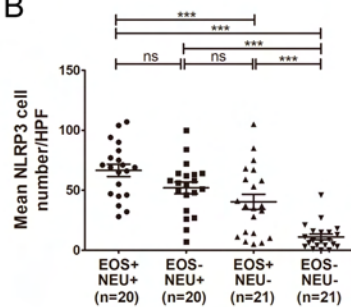
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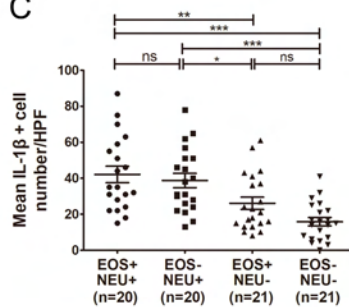
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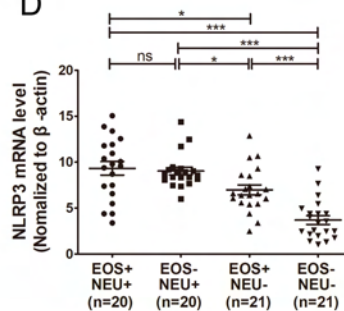
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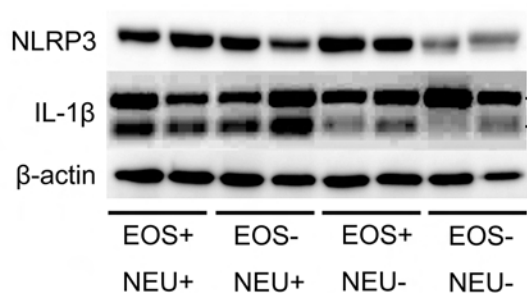
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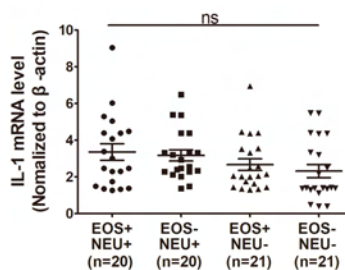
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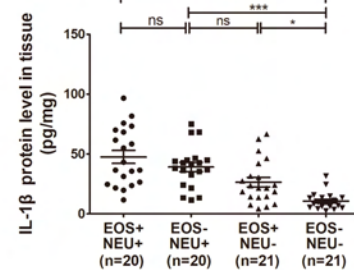
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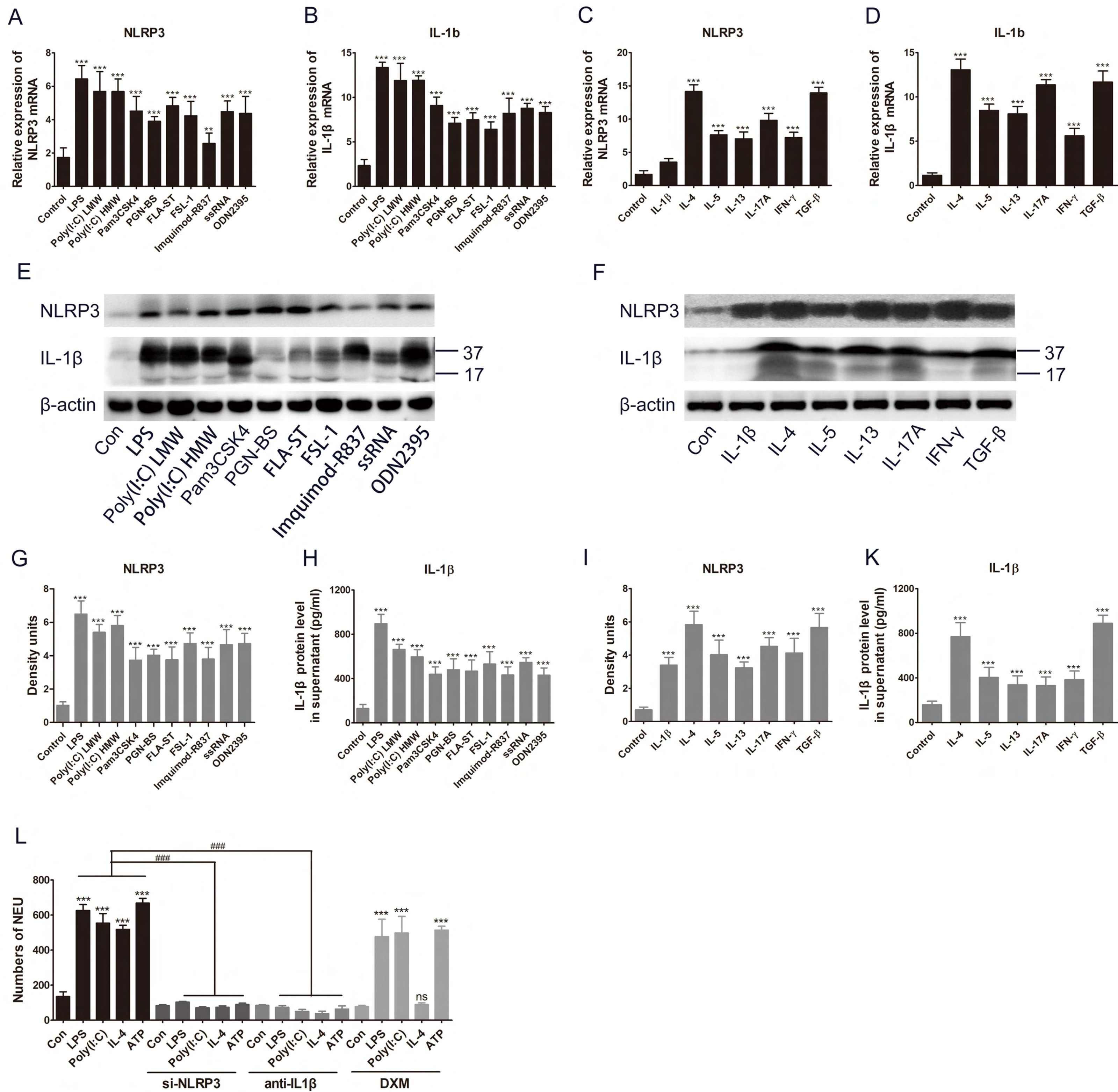


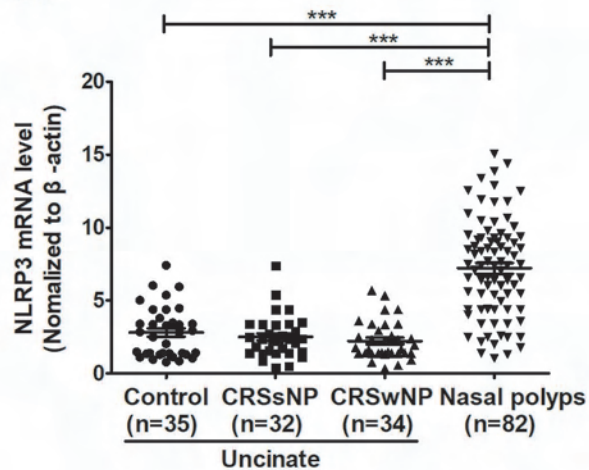
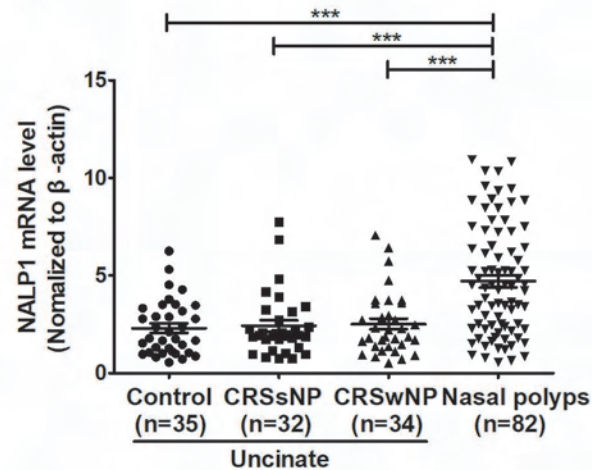
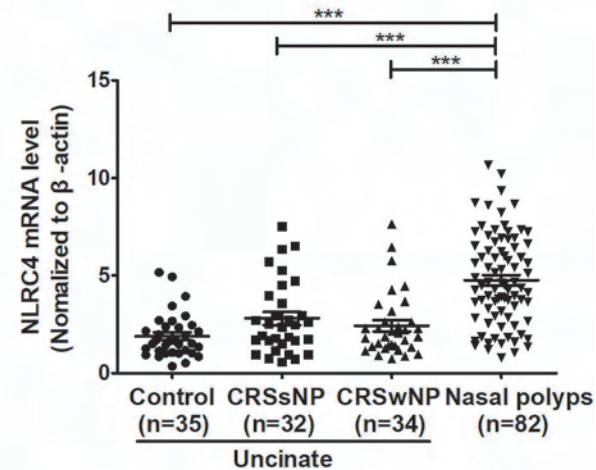
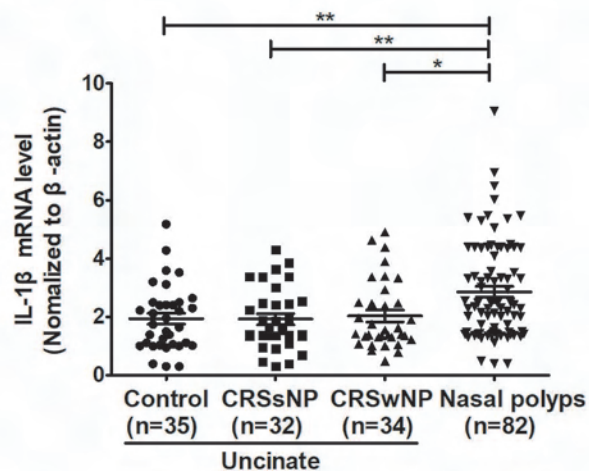
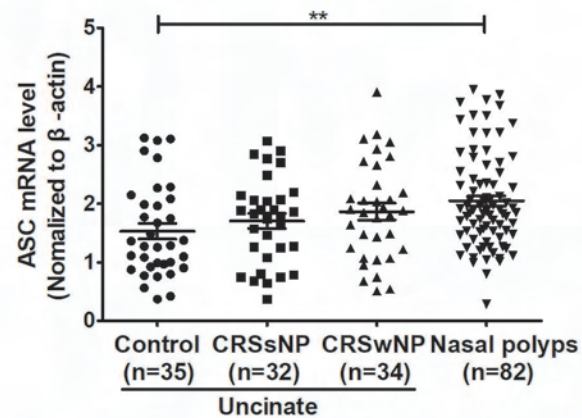
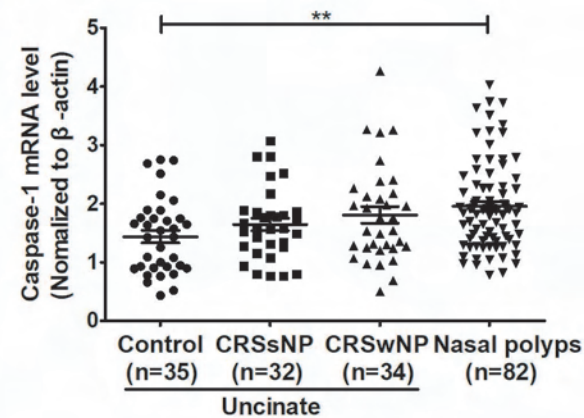
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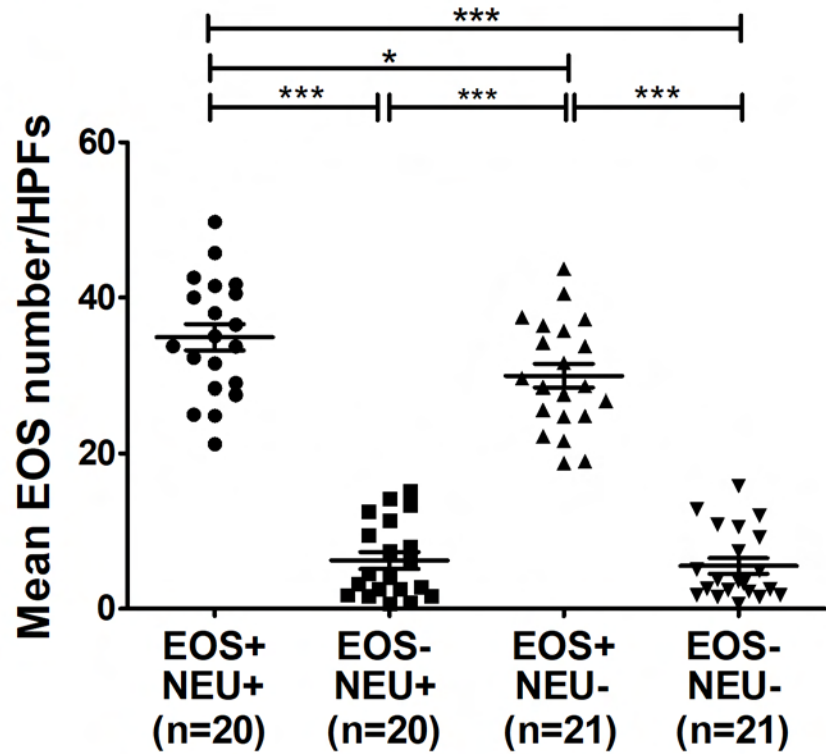
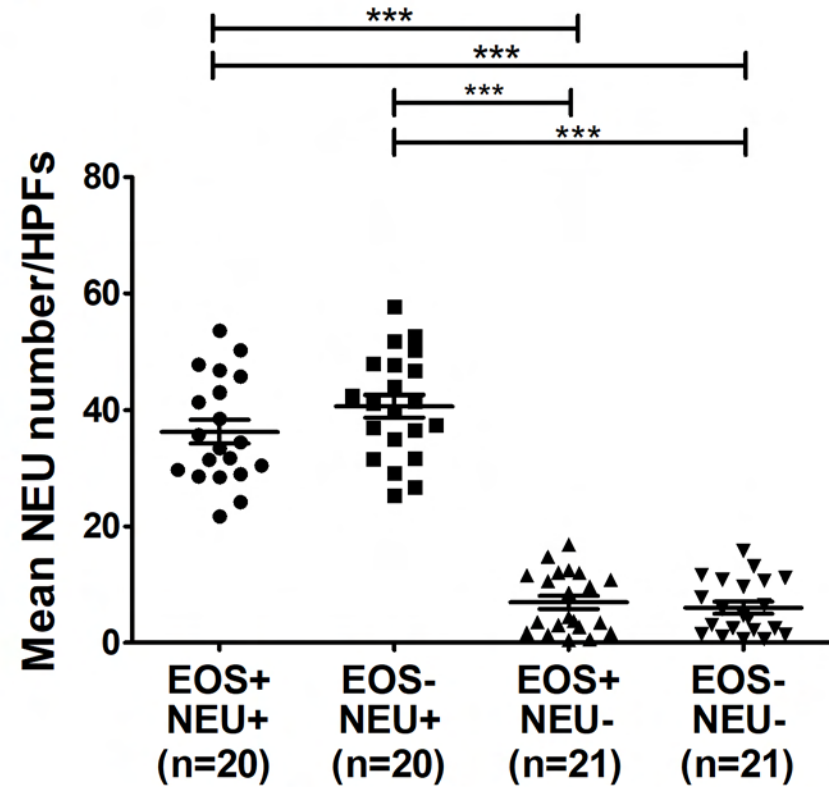


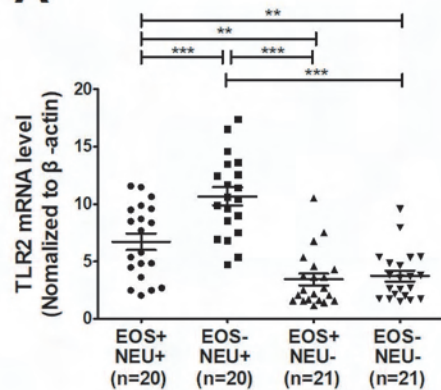
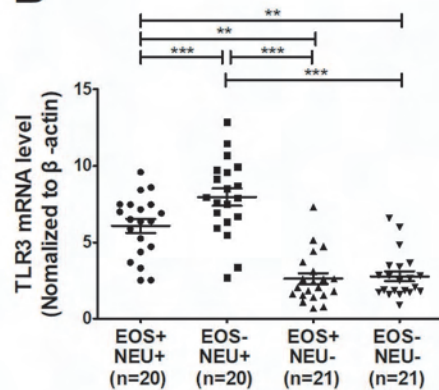
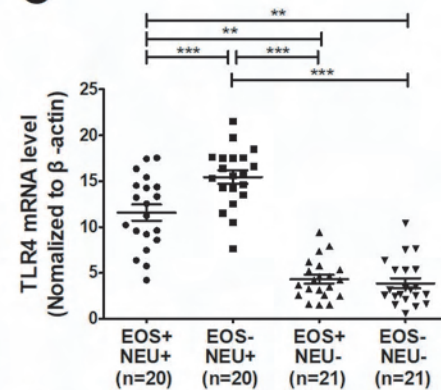
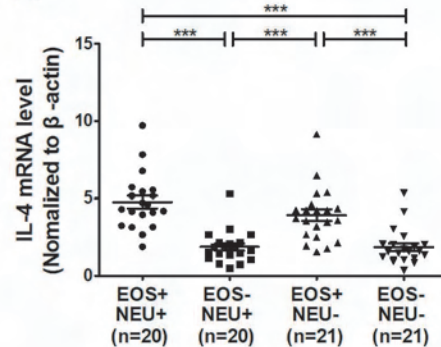
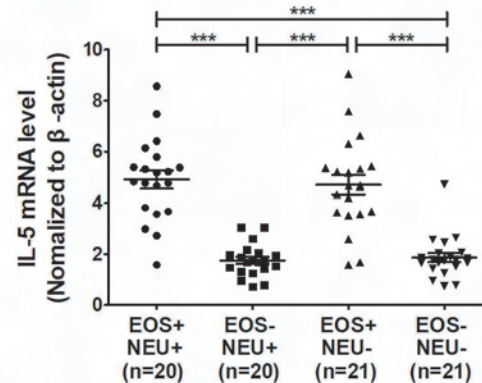
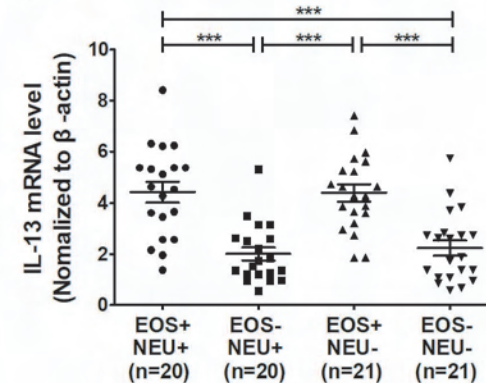
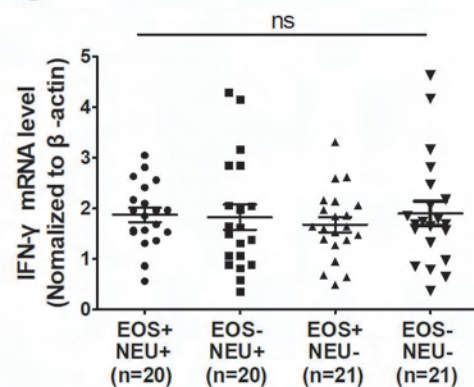
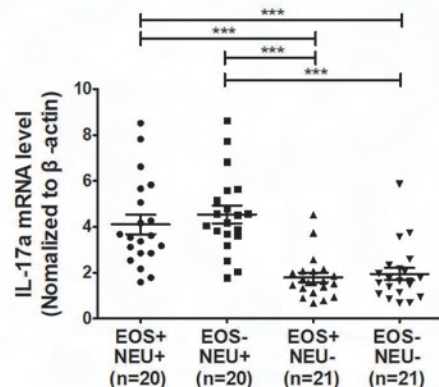
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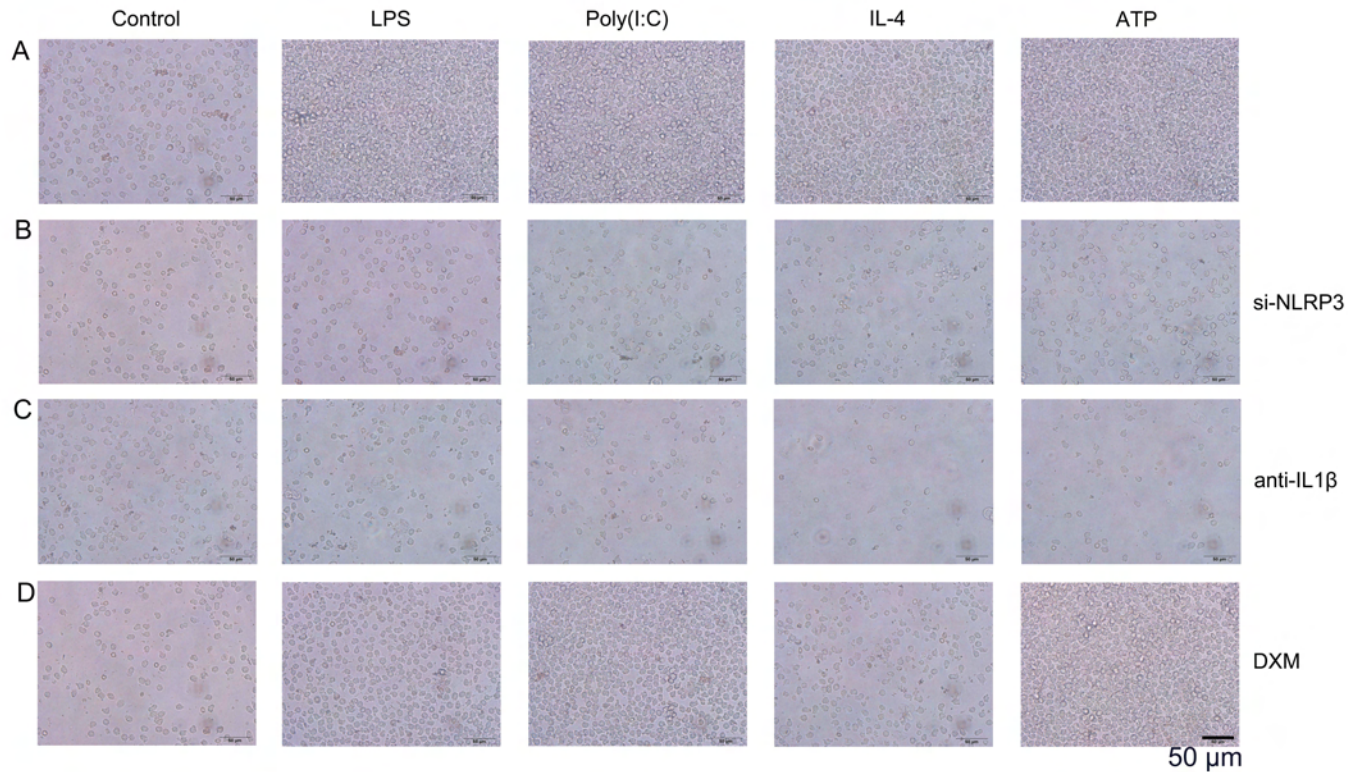


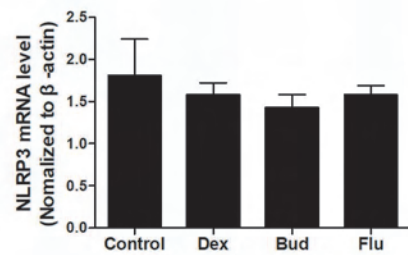
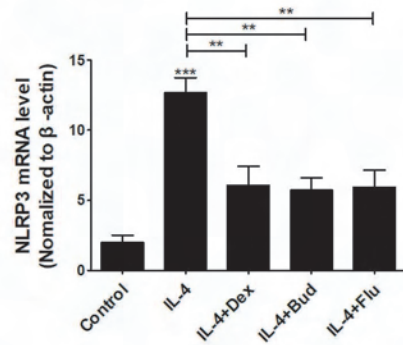
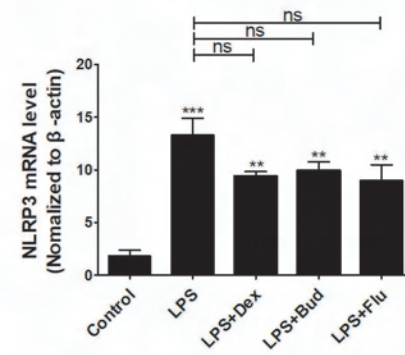
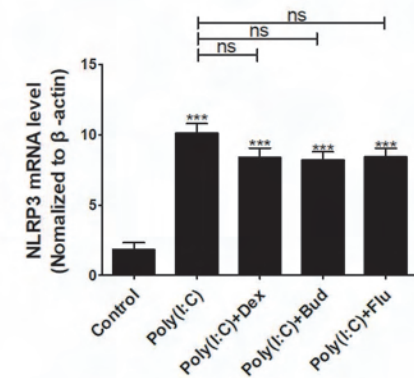
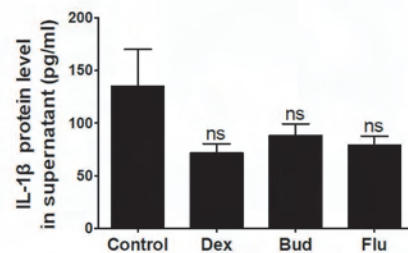
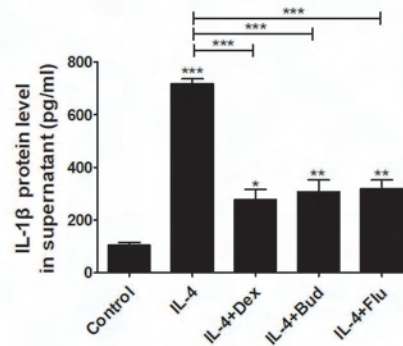
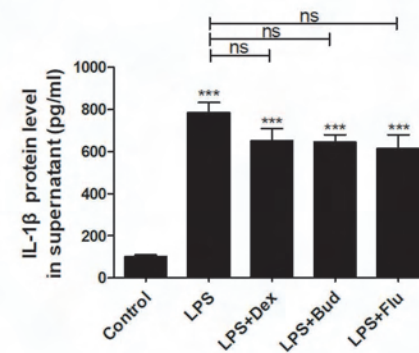
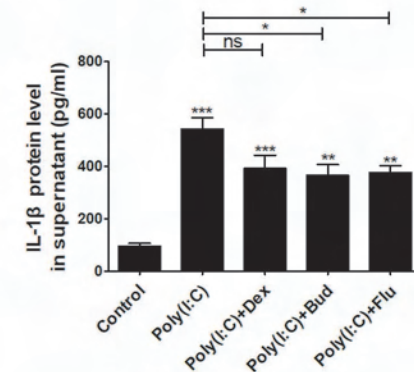


A**B****C****D****E****F**

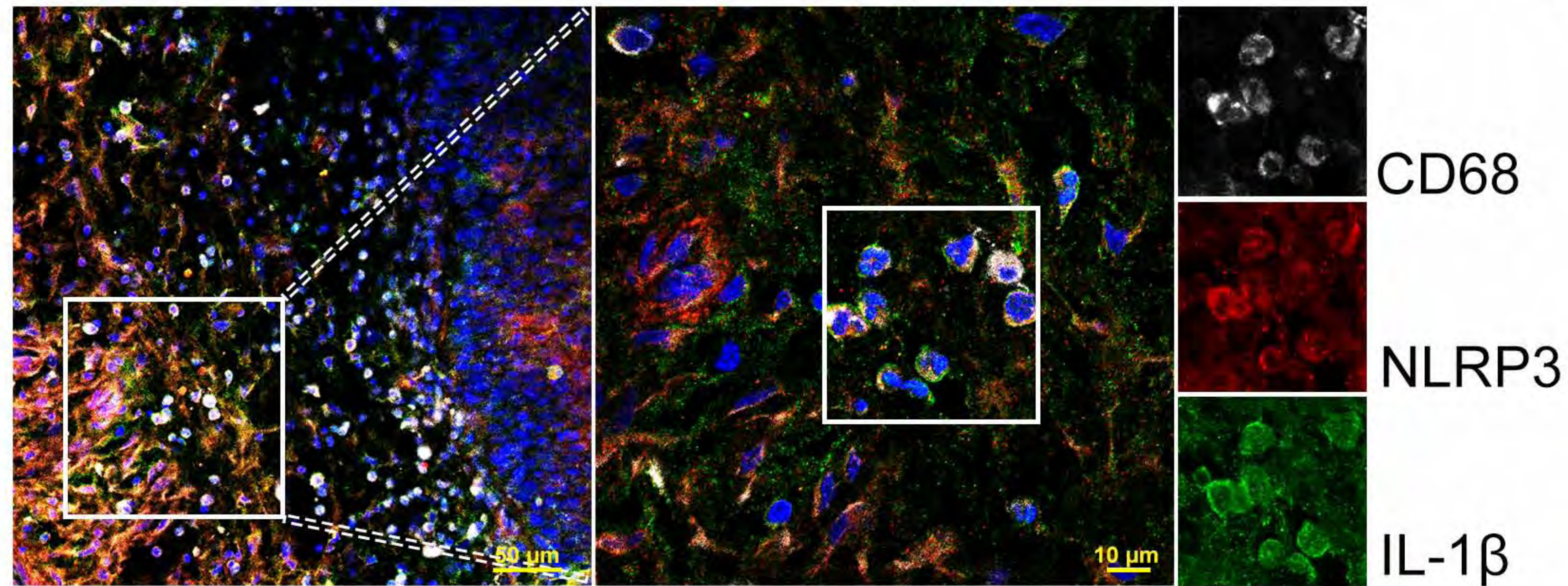
A**B**

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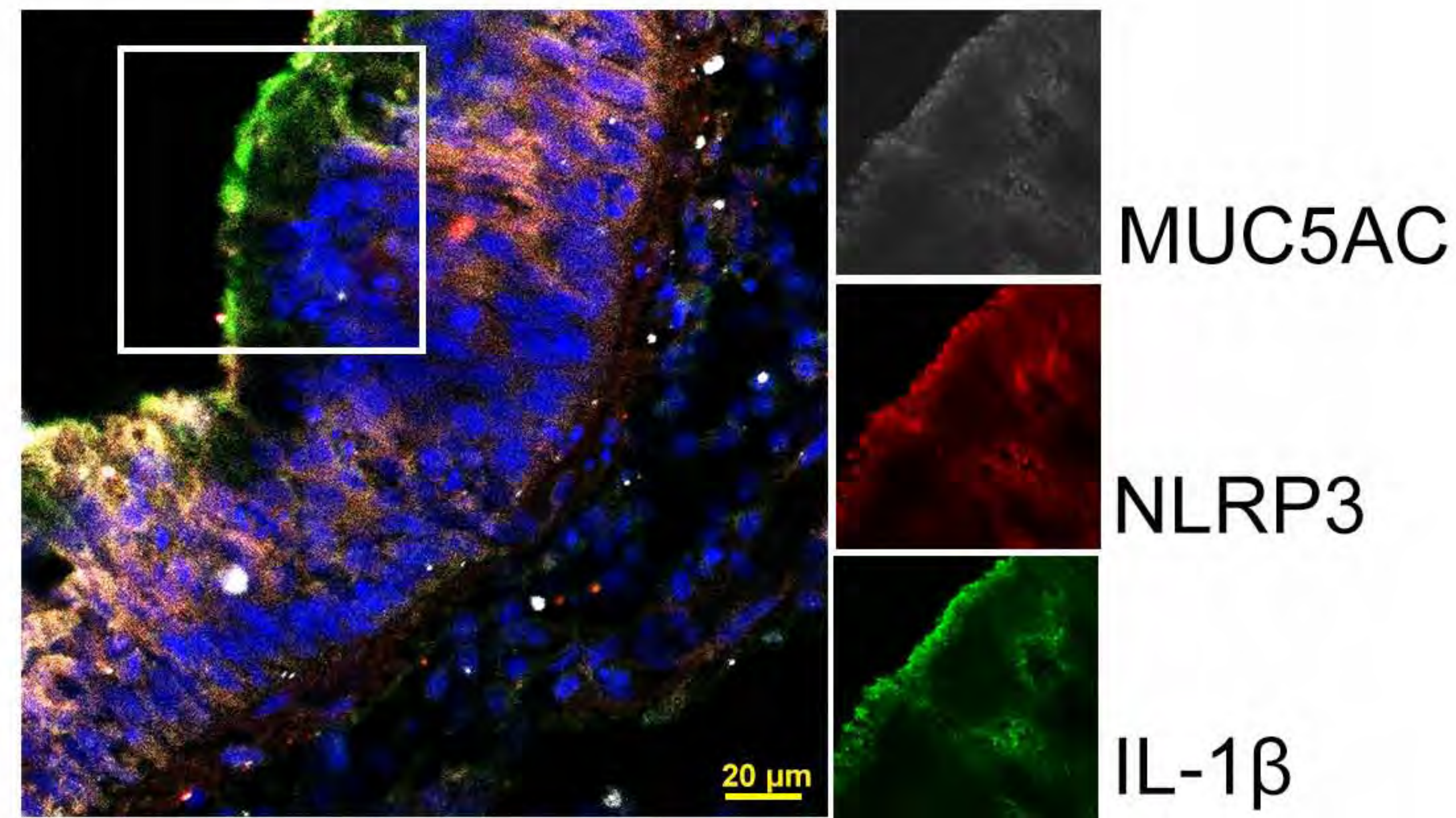


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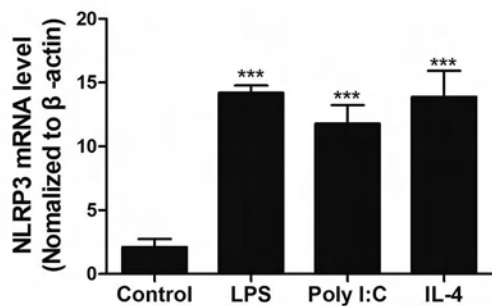
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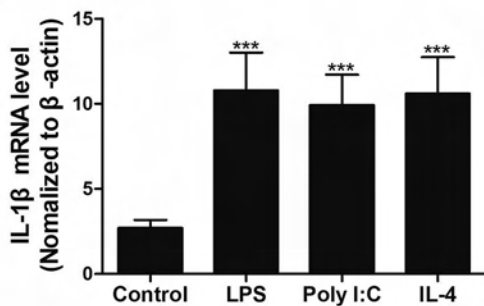
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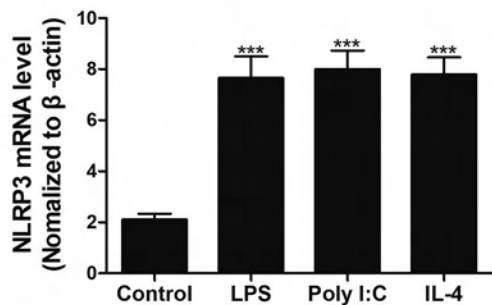
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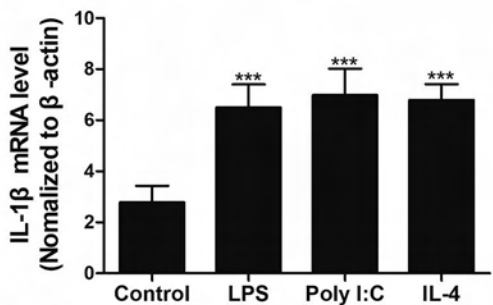
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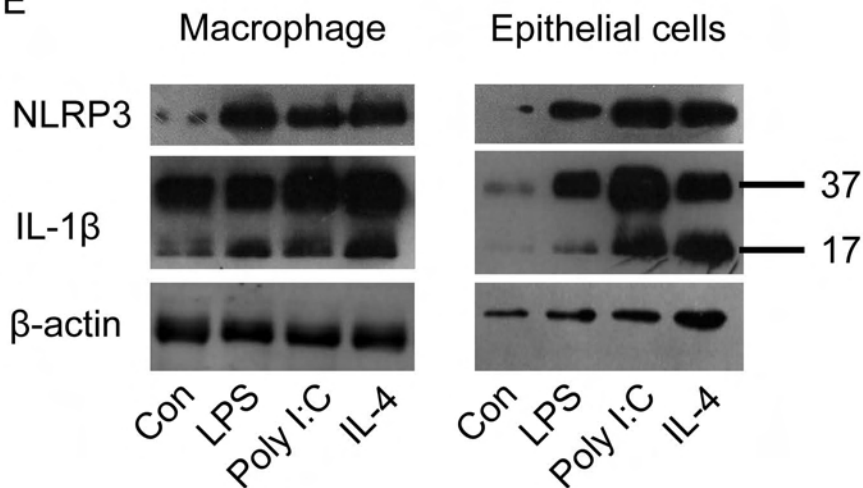
C



D

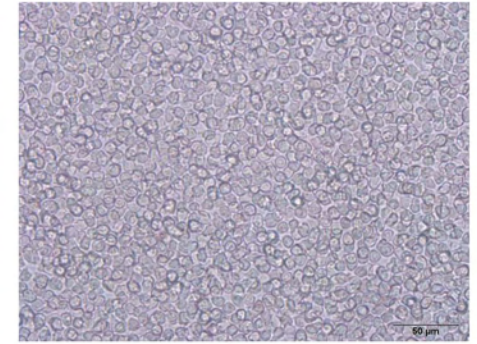
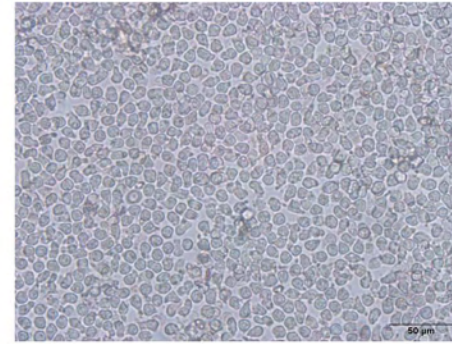
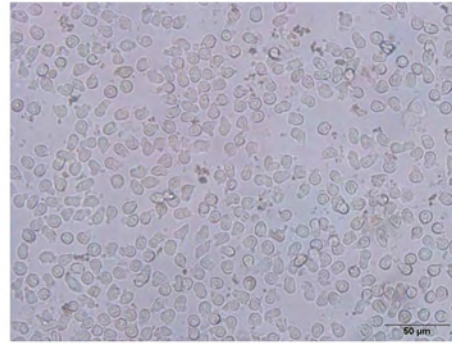
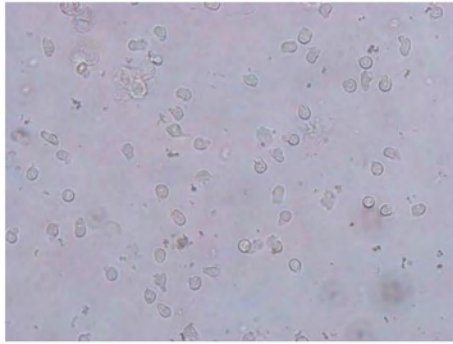


E

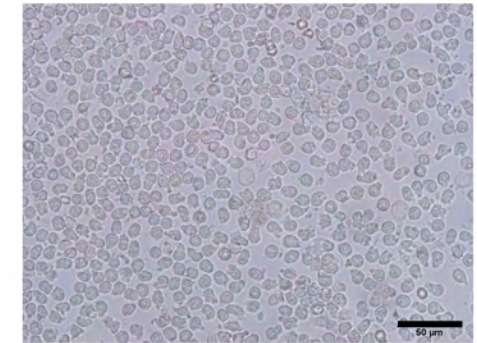
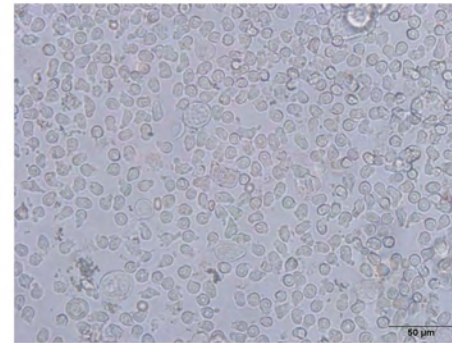
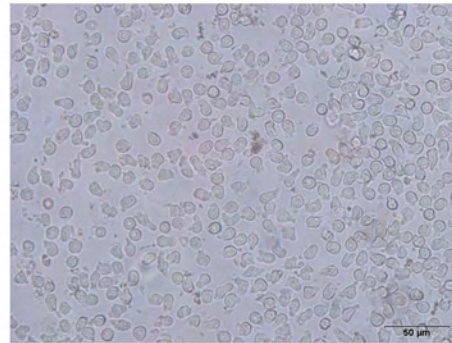
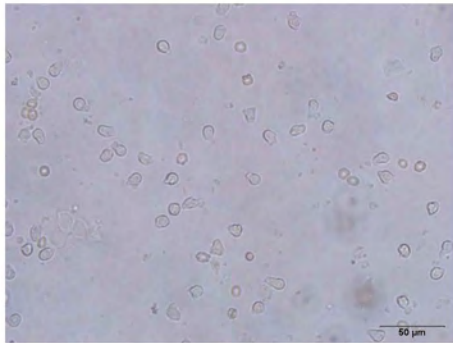


A

Macrophage



Epithelial cells



50 µm

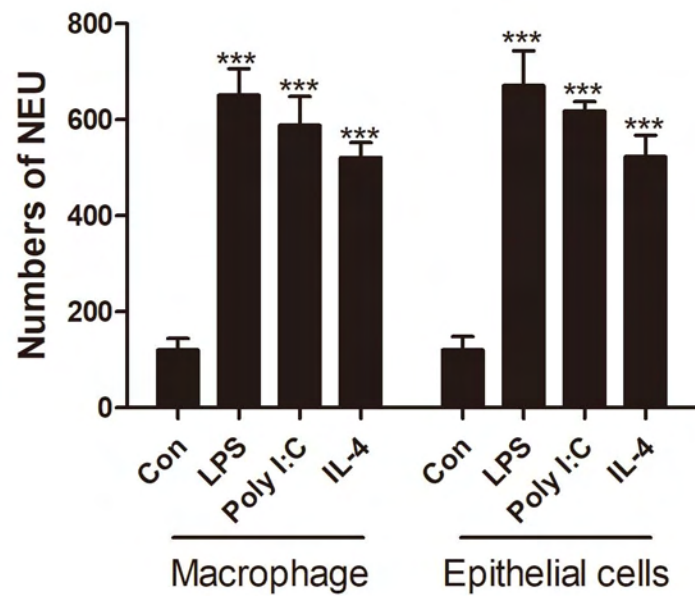
Control

LPS

Poly I:C

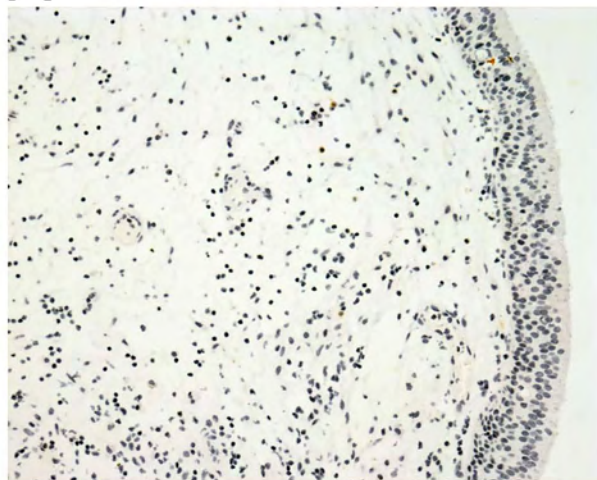
IL-4

B



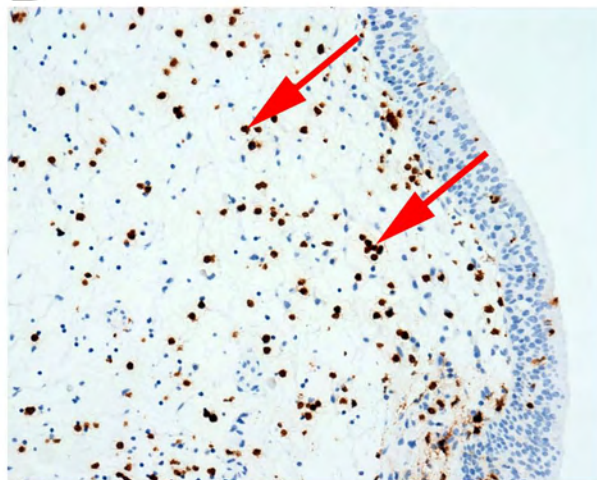
Isotype control

A

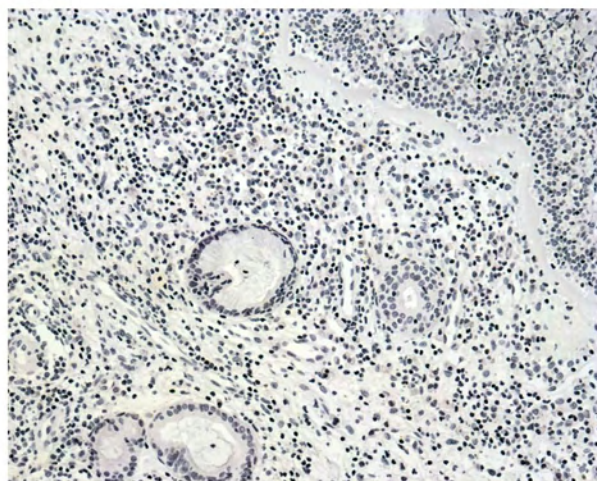


NEU

B

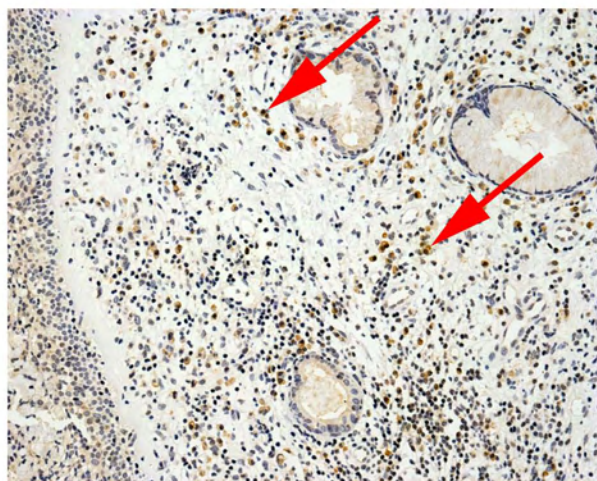


C

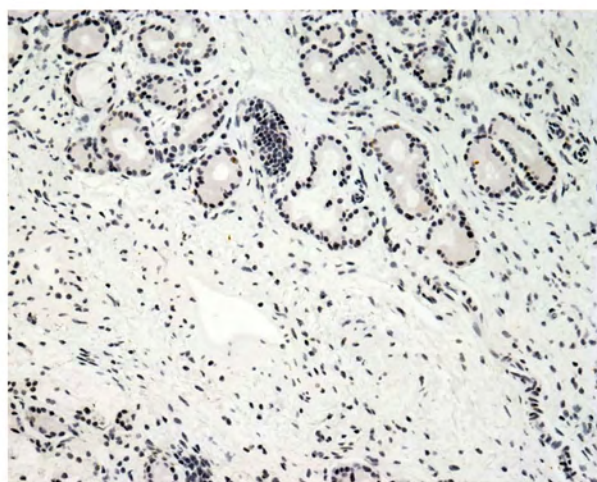


IL-1 β

D

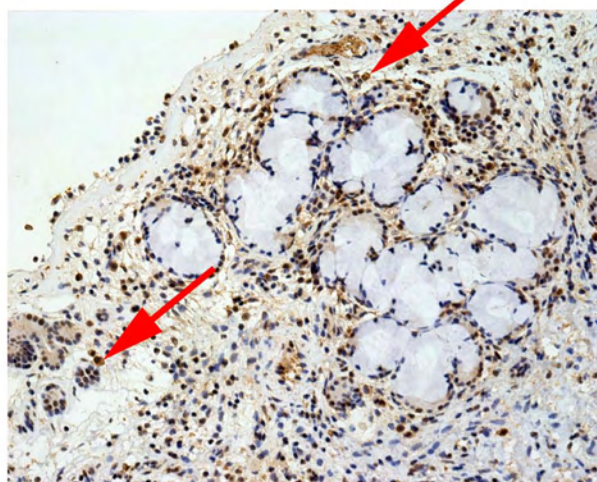


E



NLRP3

F



200X