



7. Sandrini A, Rolland JM, O'Hehir RE. Current developments for improving efficacy of allergy vaccines. *Expert Rev Vaccines*. 2015;14:1073-1087.
8. Wambre E, DeLong JH, James EA, et al. Specific immunotherapy modifies allergen-specific CD4⁺ T-cell responses in an epitope-dependent manner. *J Allergy Clin Immunol*. 2014;133:872-879.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

DOI: 10.1111/all.13919

Subsetting reveals CD16^{high} CD62L^{dim} neutrophils in chronic rhinosinusitis with nasal polyps

To the Editor:

Neutrophils are continuously present in the nasal mucosa, and frequently overlooked when listing potential factors involved in the pathology of chronic rhinosinusitis with nasal polyps (CRSwNP). The recent discovery of different neutrophil subsets, with diverse roles during inflammatory conditions, motivates a re-examination of their role in this disease. In the present investigation, the manifestation of neutrophil subsets was determined in CRSwNP patients, and the ability of CD16^{high} CD62L^{dim} neutrophils to phagocytose and form reactive oxygen species (ROS) was examined in vitro (Methods in the online Supporting Information).

An increase in eosinophil fractions in blood and nasal tissue from patients with CRSwNP was confirmed (Figure S1 A,B). This was not seen for neutrophil fractions (Figure S1 C,D). The remaining CD45⁺ cells constituted mostly of monocytes, lymphocytes, macrophages, and basophils (data not shown). When the neutrophil population was subdivided according to the individual cellular expression of FcγRIII (CD16) and L-selectin (CD62L), three distinct sets of neutrophils could be demonstrated as previously described: CD16^{dim} CD62L^{high} (recently released from the bone marrow, regarded immature), CD16^{high} CD62L^{high} (mature neutrophils), and CD16^{high} CD62L^{dim} (thought to be more activated, displaying a hyper-segmented nuclear morphology accordingly) (Figure 1 A,B) (see full gating procedures in Figure S2 A,B).¹ The neutrophil subset CD16^{high} CD62L^{dim} was significantly higher in polyp mucosa (Figure 1C). The mature neutrophil subset, CD16^{high} CD62L^{high}, dominated in control nasal mucosa (Figure 1C). Nasal polyps exhibited a higher ratio of CD16^{high} CD62L^{dim} / CD16^{high} CD62L^{high} neutrophils compared with control nasal mucosa (Figure 1D). The normal mature neutrophil subset, CD16^{high} CD62L^{high}, dominated in peripheral blood in both CRSwNP patients and healthy controls (Figure S2 C).

The protein integrin CD11b forms a heterodimer with CD18 and mediates inflammation by regulating leukocyte adhesion and migration through binding to the cell surface molecule ICAM-1. CD11b is therefore often used as a marker of neutrophil activation and was presently shown to be upregulated on systemic and local neutrophils from CRSwNP patients (Figure S3). The expressed levels of CD11b were markedly increased on CD16^{high} CD62L^{high} and CD16^{high}

CD62L^{dim} neutrophils in patient blood, nasal mucosa, and polyps (Figure 1 E-G). No such shift was seen in healthy controls (Figure S4 A-C). Correspondingly, ICAM-1 (full gating procedures shown in Figure S5) was upregulated on epithelial cells from CRSwNP patients, suggesting a mechanism for a neutrophil-epithelial cell interaction in nasal polyps (Figure 1 H,I). Recent use of topical steroids did not seem to affect the expression of CD16, CD62L, CD11b, or ICAM-1 (data not shown).

Neutrophils isolated from blood from CRSwNP patients were treated in vitro with LPS for 1 hour for activation (Figure 2A) resulting in neutrophils highly differentiating to the CD16^{high} CD62L^{dim} subset (Figure 2A, LPS(+), *S aureus* BP(-)). *S aureus* bioparticles (ie, biomolecules from killed bacteria) were subsequently added. LPS-treated neutrophils displayed a greater capacity to phagocytose *S aureus* bioparticles (Figure 2B). The levels were comparable with positive control assays with ionomycin (data not shown). Assays with neutrophils and DHR-123 revealed more ROS formation with LPS-treated neutrophils (constituting mostly of CD16^{high} CD62L^{dim} neutrophils, Figure 2A) (Figure 2C). These data indicate that CD16^{high} CD62L^{dim} neutrophils are prone to phagocytose bacterial particles and induce oxidative burst (Figure 2D).

In 2012, Pillay et al¹ identified the existence of different neutrophil subsets. The CD16^{high} CD62L^{dim} subset, regarded activated, exhibited T cell-suppressing capacities and decreased adherence to the endothelium during induced acute inflammatory conditions.² The same subset has been shown to enhance bradykinin-dependent contraction of smooth muscle cells in airways, suggesting a role for neutrophils in asthmatic airway hyperactivity.³

More than 10% of the Western population suffers from CRS, and recent findings have stressed the immunological complexity of the disease, making it obvious that the concept of Th1/Th2 inflammation is not sufficient to explain the pathology. *S aureus* is found intramucosally, and colonizing the mucosal surface and its products has been demonstrated within the polyp tissue-stimulating immune responses.⁴ Neutrophils generate ROS during phagocytosis and in response to soluble agonists. This functional response, termed oxidative burst, contributes to host defense and is together with phagocytosis a critical antimicrobial mechanism

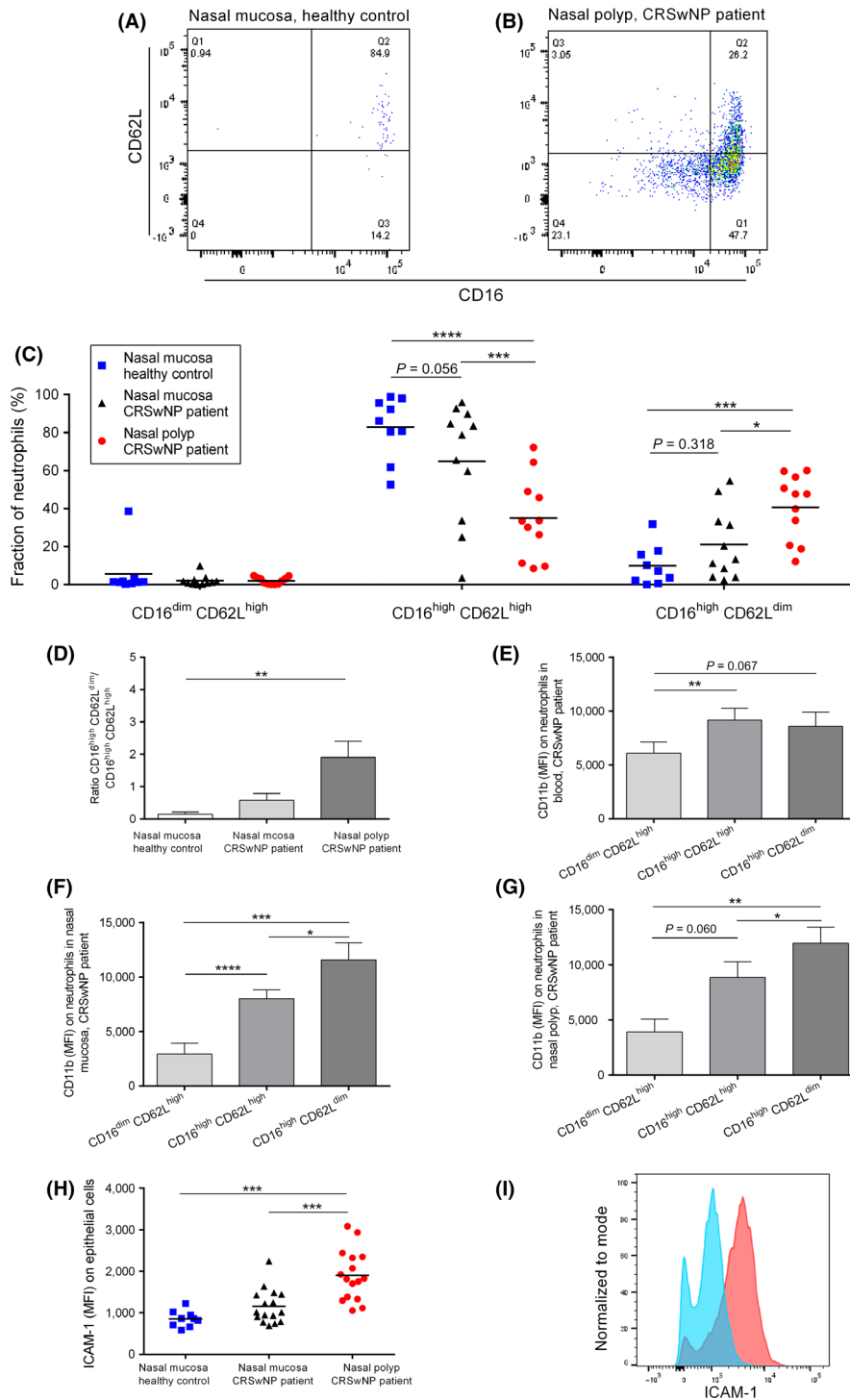
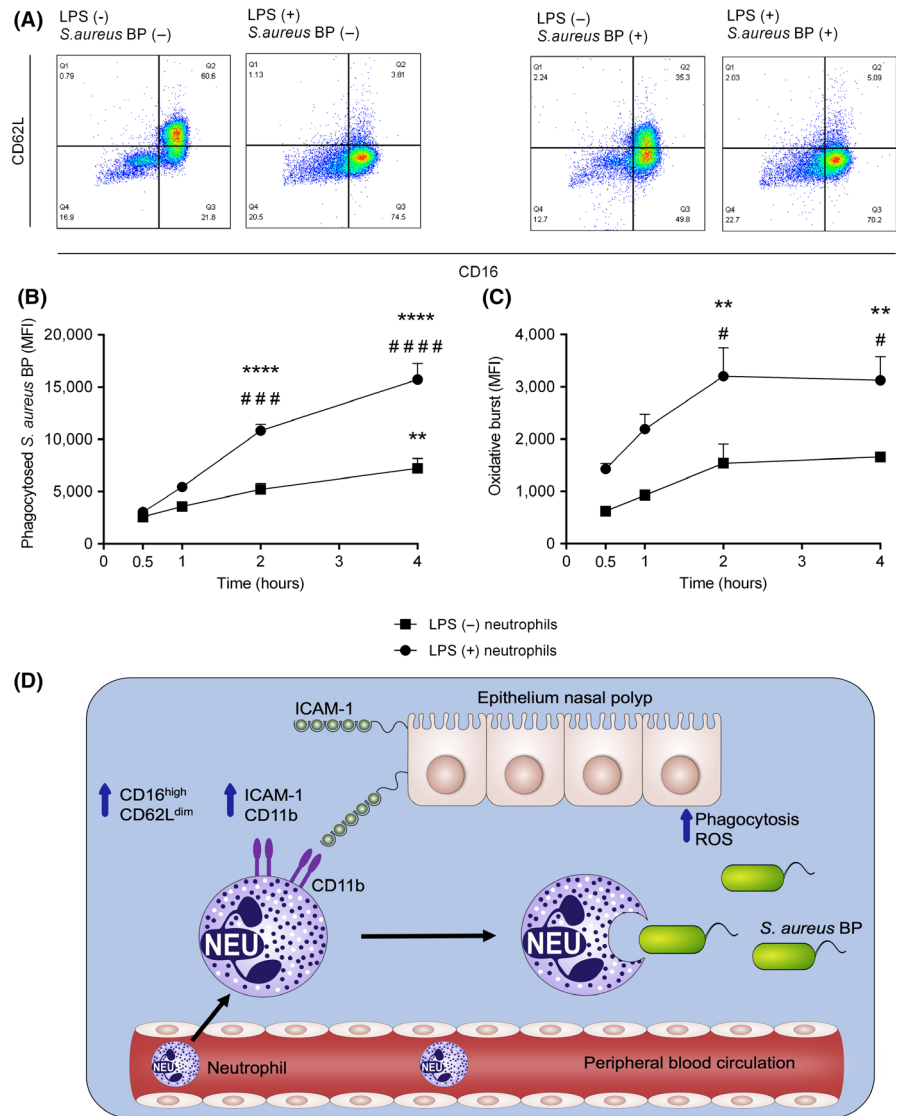


FIGURE 1 Representative dot plots showing gated neutrophil subsets in (A) control nasal mucosa and (B) nasal polyp from CRSwNP patient. (C) Neutrophil subsets in nasal mucosa and polyps from CRSwNP patients ($n = 11$) and control mucosa ($n = 9$). (D) Ratio of $CD16^{\text{high}} CD62L^{\text{dim}} / CD16^{\text{high}} CD62L^{\text{high}}$ neutrophils. Expression of CD11b (MFI) on neutrophils on subset level in (E) blood ($n = 16$), (F) nasal mucosa ($n = 11$), and (G) polyps from CRSwNP patients ($n = 11$). (H) Expression of ICAM-1 on epithelial cells from nasal mucosa and polyps from CRSwNP patients ($n = 16$) and control mucosa ($n = 9$). (I) ICAM-1 expression in polyp epithelium (CRSwNP patients) (red) compared with control nasal mucosa (blue)

of neutrophils. The clinical significance of oxidative burst in CRS is however still somewhat unclear, since ROS potentiate cellular injury and inflammation.⁵ $CD16^{\text{high}} CD62L^{\text{dim}}$ neutrophils have in the present study been shown to have increased capacity to

phagocytose *S aureus* bioparticles and form ROS. In contrast, $CD16^{\text{high}} CD62L^{\text{dim}}$ neutrophils from elderly patients had a lower phagocytic index and exhibited a lower oxidative burst compared with the mature neutrophil subset.⁶ However, these subsets were

FIGURE 2 (A) Subset dot plots of neutrophils from blood (CRSwNP patients) without LPS (LPS(-)), LPS-treated (LPS(+)), cocultured without *S aureus* bioparticles (*S aureus* BP(-)), and cocultured with *S aureus* bioparticles (*S aureus* BP(+)). In vitro assays determining (B) phagocytosis and (C) ROS formation of both untreated (LPS(-)) and LPS-treated (LPS(+)) neutrophils. (D) Summarizing figure of CD16^{high} CD62L^{dim}-induced modulation of phagocytosis and ROS formation in nasal polyp. ***P* < 0.01; *****P* < 0.0001 (compared with control value at 30 min). #*P* < 0.05; ###*P* < 0.001; #####*P* < 0.0001 (LPS(-) compared with LPS(+))



pre-activated with TLR agonists and fMLP, a process we have suspended since it routinely leads to over-activation, turning the cells to CD16^{dim} CD62L^{dim} neutrophils (considered the end-stage neutrophil before apoptosis).

Three distinct neutrophil subsets have for the first time been demonstrated in CRSwNP patients. A disease-related shift in the composition of these subsets, favoring the CD16^{high} CD62L^{dim} subset, was shown. The local activation of neutrophils likely takes place in an IL-8 and IL-17 cytokine-rich environment.⁷ The connection between local expressed ICAM-1 on epithelial cells and neutrophils has previously been suggested in the intestine⁸ but not heretofore in nasal polyps. Accordingly, we suggest that neutrophils from CRSwNP patients have an increased ability to adhere and subsequently migrate to the local site of nasal inflammation. Since Pillay et al identified CD16^{high} CD62L^{dim} neutrophils, this subset has mostly been studied in cancer. CD16^{high} CD62L^{dim} neutrophils were prone to migrate into the tumor in patients with head and neck squamous cell carcinoma (HNSCC), due to increased CD11b and CD18 expression, and inhibited migration and proliferation and

induced apoptosis of FaDu cancer cells.⁹ Interestingly, an increased fraction of activated CD16^{high} CD62L^{dim} neutrophils among HNSCC patients correlated with increased survival rate, altogether establishing CD16^{high} CD62L^{dim} neutrophils as a protective marker in cancer.⁹ This article is the first attempt to study the role of this neutrophil subset in CRSwNP. It is tempting to speculate that CD16^{high} CD62L^{dim} neutrophils might in some way modulate CRSwNP disease by their capacity to phagocytose *S aureus* bioparticles and form ROS.

ACKNOWLEDGMENTS

We thank the surgeons at the ENT department along with the nurses Eva Fransson, Carina Hedenström, and Anna Drevland for assisting with the specimen sampling.

CONFLICT OF INTEREST

The authors have no conflicts to declare.

FUNDING INFORMATION

This work was supported by grants from the Swedish Research Council, Karolinska Institutet, Karolinska University Hospital, the Hesselman's Research Foundation, the Acta Oto-Laryngologica Foundation, the Nachmanssöns Foundation, the Swedish Association for Otorhinolaryngology Head and Neck Surgery (SFOHH), and the European Rhinology Society (ERS).

Julia Arebro^{1,2} 

Cecilia Drakskog¹

Ola Winqvist³

Claus Bachert^{1,2,4} 

Susanna Kumlien Georen¹

Lars-Olaf Cardell^{1,2} 

¹Division of ENT Diseases, Department of Clinical Science, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden

²Department of ENT Diseases, Karolinska University Hospital, Stockholm, Sweden

³Department of Medicine, Immunology and Allergy Unit, Karolinska University Hospital, Stockholm, Sweden

⁴Upper Airways Research Laboratory, Ghent University, Ghent, Belgium

Correspondence

Lars-Olaf Cardell, Professor and Head of Department of ENT-Diseases, Karolinska University Hospital, 141 86 Stockholm, Sweden.

Email: lars-olaf.cardell@ki.se

ORCID

Julia Arebro  <https://orcid.org/0000-0002-2584-0941>

Claus Bachert  <https://orcid.org/0000-0003-4742-1665>

Lars-Olaf Cardell  <https://orcid.org/0000-0003-0538-9580>

REFERENCES

1. Pillay J, Kamp VM, van Hoffen E, et al. A subset of neutrophils in human systemic inflammation inhibits T cell responses through Mac-1. *J Clin Invest*. 2012;122(1):327-336.
2. Kamp VM, Pillay J, Lammers JW, Pickkers P, Ulfman LH, Koenderman L. Human suppressive neutrophils CD16bright/CD62Ldim exhibit decreased adhesion. *J Leukoc Biol*. 2012;92(5):1011-1020.
3. Ekstedt S, Safholm J, Georen SK, Cardell LO. Dividing neutrophils in subsets reveals a significant role for activated neutrophils in the development of airway hyperreactivity. *Clin Exp Allergy*. 2019;49(3):285-291.
4. Chalermwatanachai T, Vilchez-Vargas R, Holtappels G, et al. Chronic rhinosinusitis with nasal polyps is characterized by dysbacteriosis of the nasal microbiota. *Sci Rep*. 2018;8(1):7926.
5. Fordham MT, Mulligan JK, Casey SE, et al. Reactive oxygen species in chronic rhinosinusitis and secondhand smoke exposure. *Otolaryngol Head Neck Surg*. 2013;149(4):633-638.
6. Sauce D, Dong Y, Campillo-Gimenez L, et al. Reduced Oxidative Burst by Primed Neutrophils in the Elderly Individuals Is Associated With Increased Levels of the CD16bright/CD62Ldim Immunosuppressive Subset. *J Gerontol A Biol Sci Med Sci*. 2017;72(2):163-172.
7. Mantovani A, Cassatella MA, Costantini C, Jaillon S. Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat Rev Immunol*. 2011;11(8):519-531.
8. Sumagin R, Robin AZ, Nusrat A, Parkos CA. Transmigrated neutrophils in the intestinal lumen engage ICAM-1 to regulate the epithelial barrier and neutrophil recruitment. *Mucosal Immunol*. 2014;7(4):905-915.
9. Millrud CR, Kagedal A, Kumlien Georen S, et al. NET-producing CD16(high) CD62L(dim) neutrophils migrate to tumor sites and predict improved survival in patients with HNSCC. *Int J Cancer*. 2017;140(11):2557-2567.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

DOI: 10.1111/all.13921

Differential allergen expression in three *Tyrophagus putrescentiae* strains inhabited by distinct microbiome

To the Editor,

Allergen production by house dust mites is influenced directly by antibacterial compounds and digestive enzymes¹ or indirectly via interactions with nutrients linked to associated microorganisms.¹ Importantly, heat-stable lipopolysaccharides (endotoxins) from associated Gram-negative bacteria can affect the efficacy of allergen immunotherapy sera produced from house dust mites.^{1,2} House dust mites serve as carriers of bacteria, and it is possible that these bacteria are responsible for the induction of IgE sensitization to microbial antigens.³ For this reason, the microbiomes of allergen-producing mites have recently become

the subject of intensive research.⁴ Unfortunately, the existence of variability in microbiome composition among different mite populations or strains is insufficiently considered (Erban et al,⁵ Lee et al⁶), despite the evidence that different strains of mites may harbor different and persistent microbial communities. In *Tyrophagus putrescentiae*, for example, these communities include the following bacteria: *Wolbachia*, *Cardinium*, *Solitalea*, *Blattabacterium*-like (intracellular), *Bartonella*-like, and *Bacillus* sp. (gut-associated).⁵ These observations open the question of whether mite strains harboring different bacterial communities differ in allergen production.

Figure S1

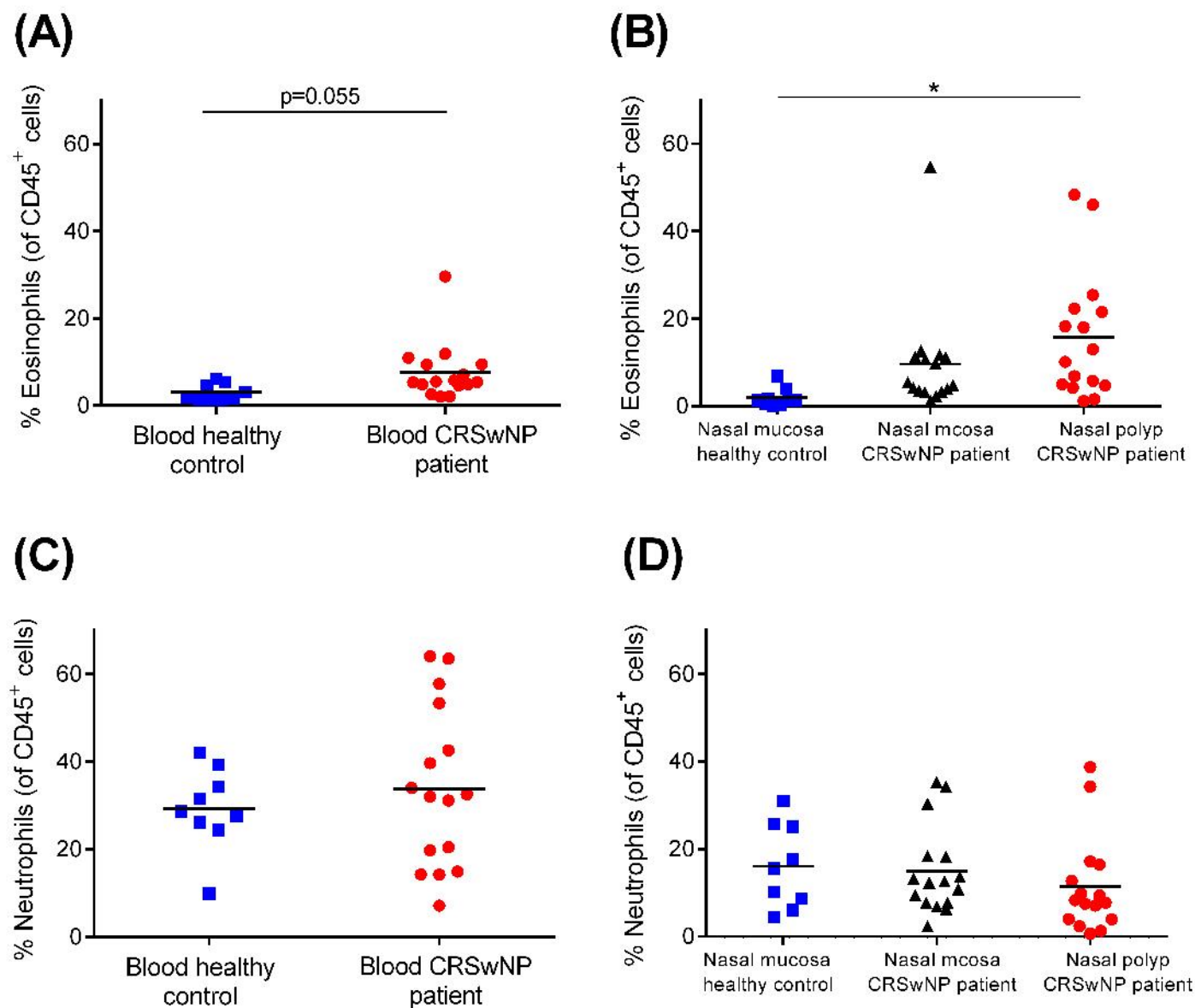
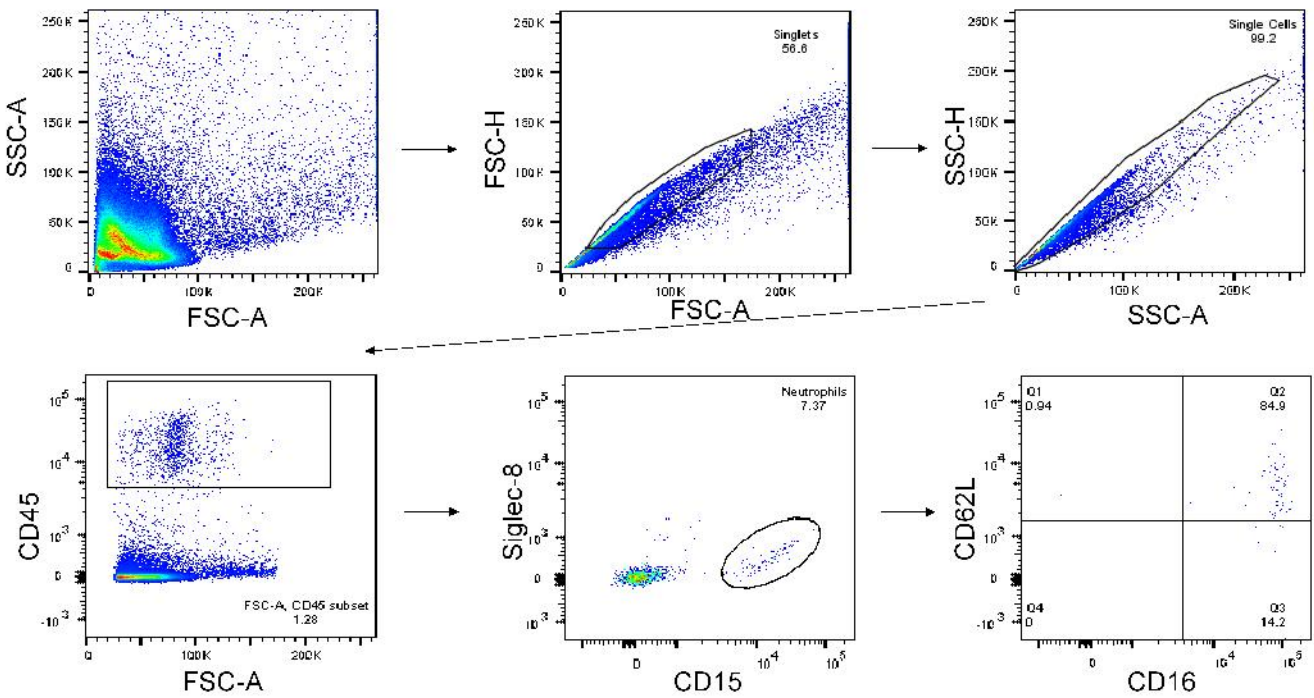
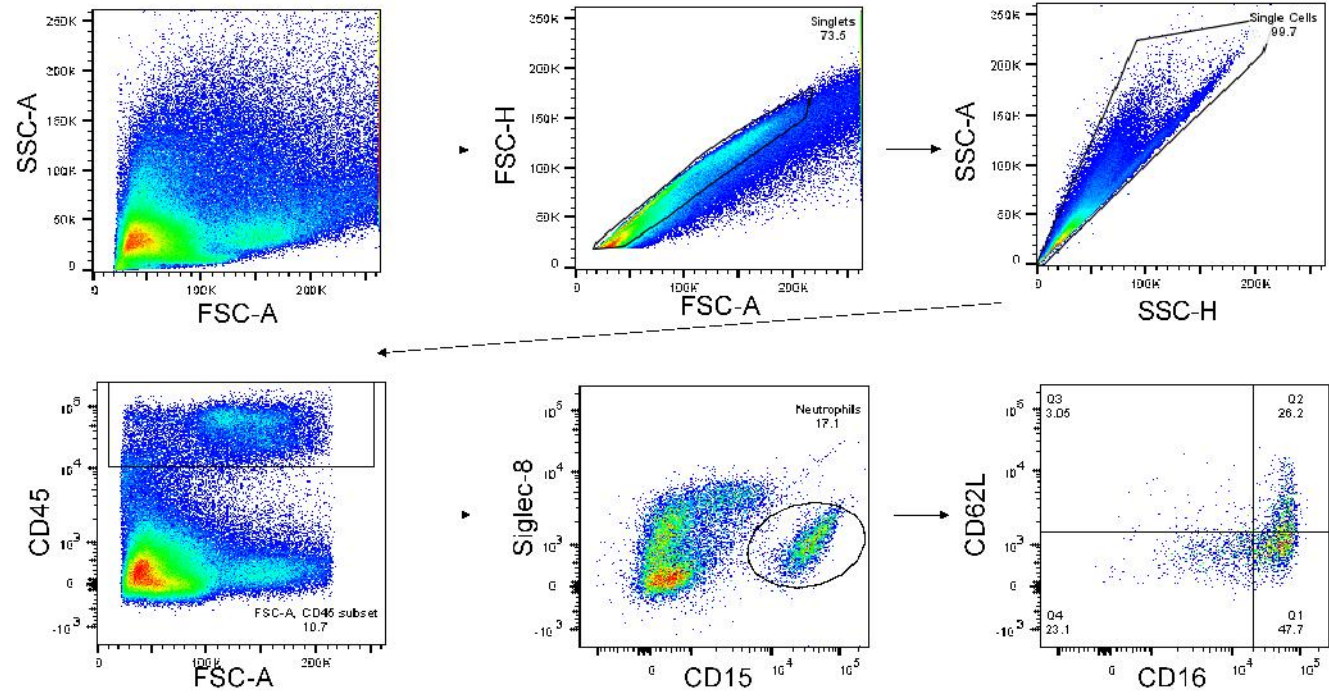


Figure S2

(A)



(B)



(C)

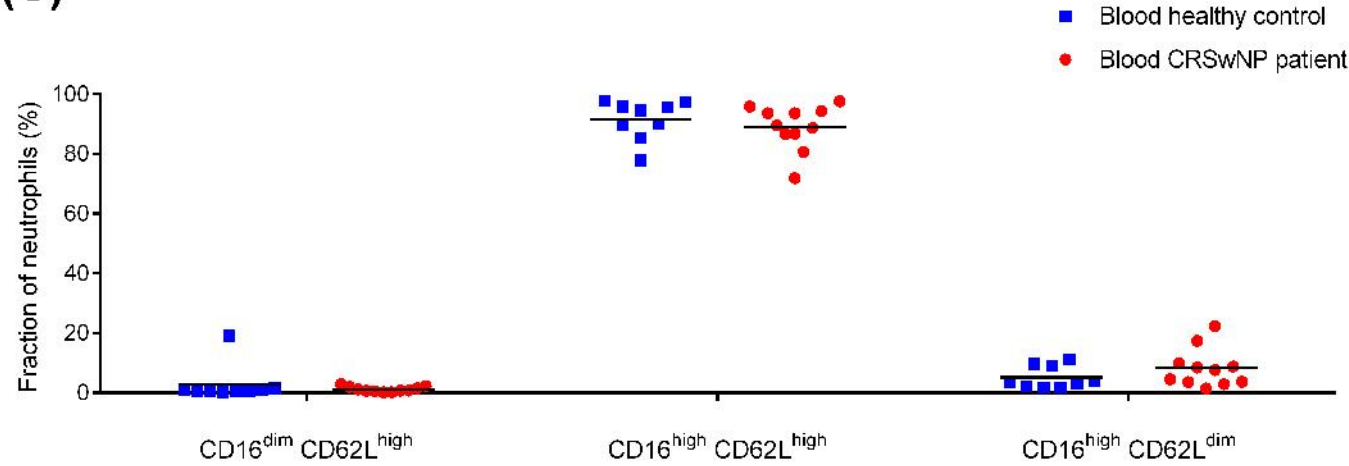
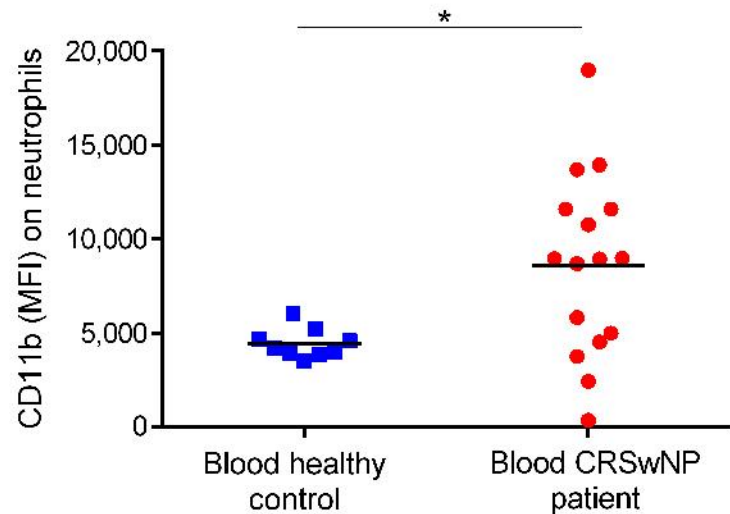


Figure S3

(A)



(B)

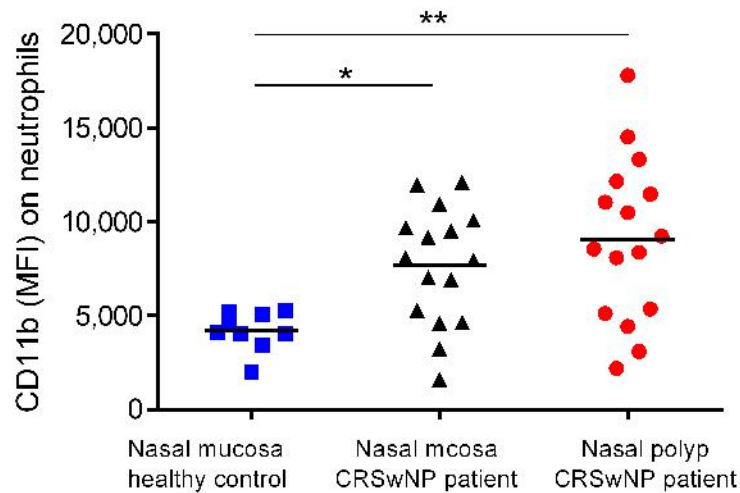


Figure S4

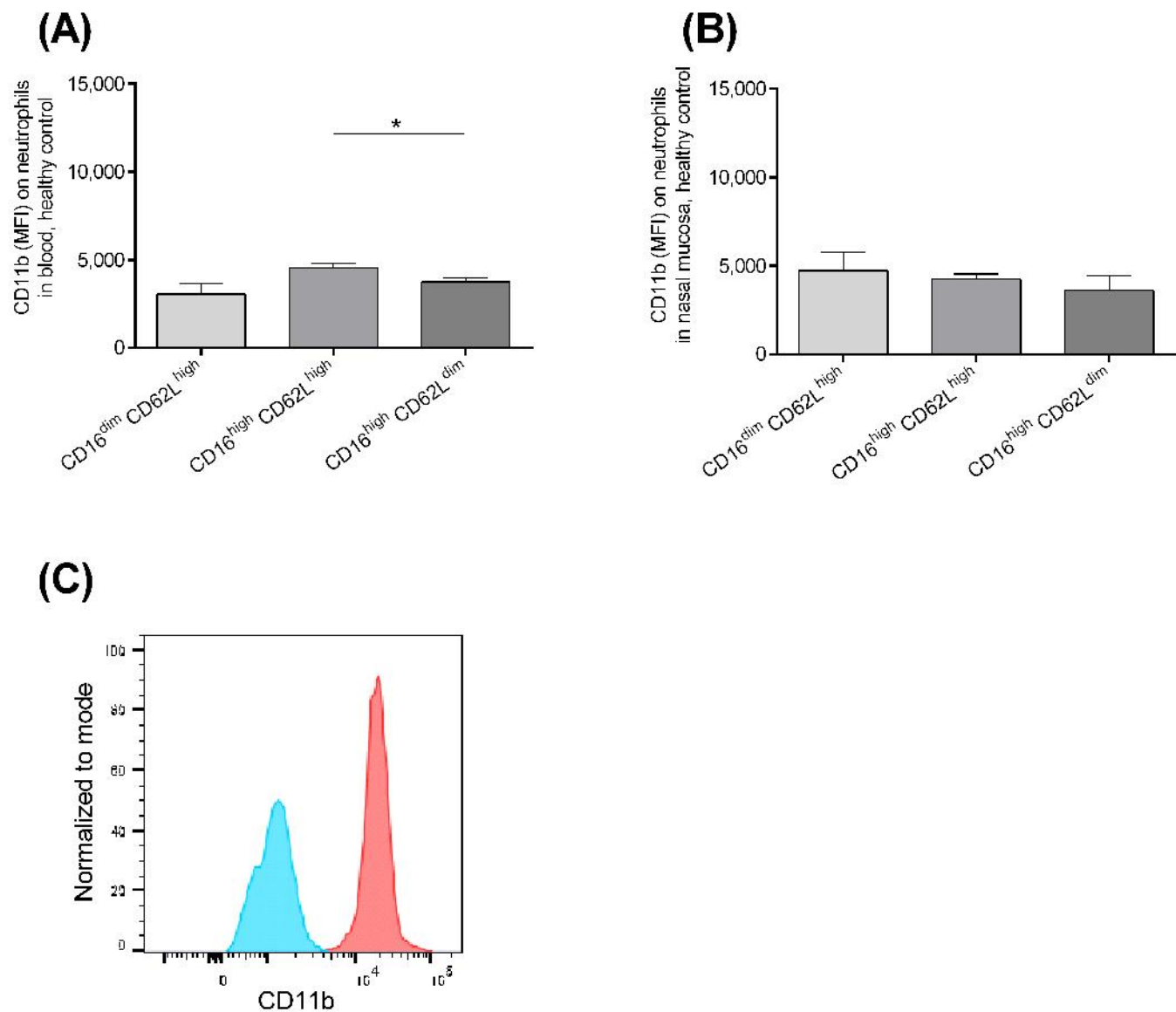
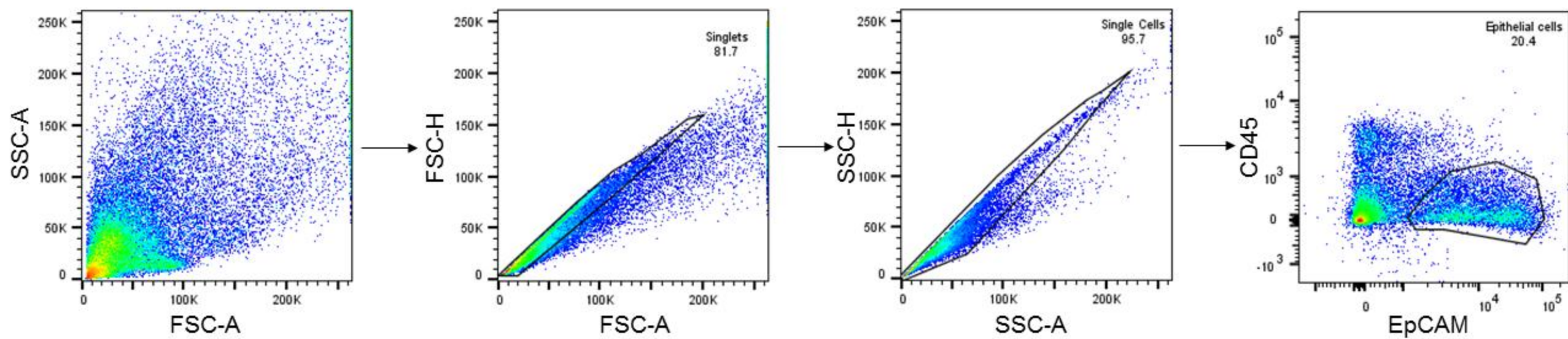


Figure S5



ONLINE SUPPORTING INFORMATION

ADDITIONAL REFERENCES, LETTER TO THE EDITOR

1. Kolaczowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol*. 2013;13(3):159-175.
2. Manley HR, Keightley MC, Lieschke GJ. The Neutrophil Nucleus: An Important Influence on Neutrophil Migration and Function. *Front Immunol*. 2018;9:2867.
3. Mesnil C, Raulier S, Paulissen G, et al. Lung-resident eosinophils represent a distinct regulatory eosinophil subset. *J Clin Invest*. 2016;126(9):3279-3295.
4. Tomassen P, Vandeplas G, Van Zele T, et al. Inflammatory endotypes of chronic rhinosinusitis based on cluster analysis of biomarkers. *J Allergy Clin Immunol*. 2016;137(5):1449-1456.e1444.6
5. Derycke L, Eyerich S, Van Crombruggen K, et al. Mixed T helper cell signatures in chronic rhinosinusitis with and without polyps. *PloS One*. 2014;9(6):e97581.
6. Zhang N, Van Zele T, Perez-Novó C, et al. Different types of T-effector cells orchestrate mucosal inflammation in chronic sinus disease. *J Allergy Clin Immunol*. 2008;122(5):961-968.
7. Bachert C, Hamilos DL. Are Antibiotics Useful for Chronic Rhinosinusitis? *J Allergy Clin Immunol Pract*. 2016;4(4):629-638.
8. Park IH, Park SJ, Cho JS, et al. Role of reactive oxygen species in transforming growth factor beta1-induced alpha smooth-muscle actin and collagen production in nasal polyp-derived fibroblasts. *Int Arch Allergy Immunol*. 2012;159(3):278-286.
9. Uneri C, Ozturk O, Polat S, Yuksel M, Haklar G. Determination of reactive oxygen species in nasal polyps. *Rhinology*. 2005;43(3):185-189.
10. Olcott CM, Han JK, Cunningham TD, Franzese CB. Interleukin-9 and interleukin-17C in chronic rhinosinusitis. *Int Forum Allergy Rhinol*. 2016;6(8):841-847.

11. Eyerich S, Eyerich K, Cavani A, Schmidt-Weber C. IL-17 and IL-22: siblings, not twins. *Trends Immunol.* 2010;31(9):354-361.
12. Xia W, Bai J, Wu X, et al. Interleukin-17A promotes MUC5AC expression and goblet cell hyperplasia in nasal polyps via the Act1-mediated pathway. *PloS One.* 2014;9(6):e98915.
13. Laan M, Lotvall J, Chung KF, Linden A. IL-17-induced cytokine release in human bronchial epithelial cells in vitro: role of mitogen-activated protein (MAP) kinases. *Br J Pharmacol.* 2001;133(1):200-206.
14. Hao S, Andersen M, Yu H. Detection of immune suppressive neutrophils in peripheral blood samples of cancer patients. *Am J Blood Res.* 2013;3(3):239-245.
15. Millrud CR, Mansson Kvarnhammar A, Uddman R, Bjornsson S, Riesbeck K, Cardell LO. The activation pattern of blood leukocytes in head and neck squamous cell carcinoma is correlated to survival. *PloS One.* 2012;7(12):e51120.
16. Sauce D, Dong Y, Campillo-Gimenez L, et al. Reduced Oxidative Burst by Primed Neutrophils in the Elderly Individuals Is Associated With Increased Levels of the CD16bright/CD62Ldim Immunosuppressive Subset. *J Gerontol A Biol Sci Med Sci.* 2017;72(2):163-172.

METHODS

Patients and controls

Sixteen patients (mean age 57 years; M:F ratio 12:4) with CRSwNP (defined by EPOS criteria [European Position Paper on Rhinosinusitis and Nasal Polyps] based on symptoms, results of nasal endoscopy and computed tomography of the sinuses) were included in the study.¹ In eight patients, steroids were withheld during at least four weeks (nasal) and two months (oral) prior to functional endoscopic sinus surgery (FESS). None of these eight

patients used daily-inhaled steroid medication and had no history of asthma. The remaining eight patients used steroids nasally and four used daily-inhaled steroids due to asthma. Two out of 16 patients were smokers and one had history of allergy (birch, cat and horse). Thirteen patients underwent surgery for CRSwNP for the first time, two for the second time and one for the fourth time. Excluded were patients younger than 18 years and patients with immunodeficiencies or cystic fibrosis. In addition, nine non-pregnant healthy controls (mean age 30 years; M:F ratio 6:3) with no history of sinus disease, asthma or allergy were included. None had a history of steroid use or were smokers. The patients and healthy controls reported no acute infections in the airways or in the rest of the body three weeks prior to participating. The study was approved by the local ethical committee, all patients and healthy controls included in the study gave their written informed consent and all methods were performed in accordance with the relevant guidelines and regulations.

Nasal biopsies and peripheral blood

Biopsies from polyp tissue and inferior or middle turbinates (referred to as ‘nasal mucosa’) as well as peripheral blood were obtained from patients with CRSwNP during FESS at Karolinska University Hospital, Stockholm. Peripheral blood and nasal mucosal biopsies from the inferior turbinate from healthy controls were taken following a 20 min of topical application of local anesthesia (lidocainhydrochloride/nafazoline (34 mg/mL/0.17 mg/mL)) at the outpatients clinic. Biopsy tissue obtained for flow cytometry was put through a 100 µm cell strainer (BD Falcon, Franklin Lakes, NJ, USA), into Dulbecco’s modified Eagle’s medium and Nutrient Mixture Ham’s F12 Medium (DMEM/F-12) (Gibco®, Paisley, UK) containing 10% FBS (Gibco®, Paisley, UK) and incubated at RT for 5 min. Cells were washed and centrifuged once and the pellet was dissolved in PBS. Samples were blocked non-specific binding with Human Fc Block Pure (BD Biosciences, San Jose, CA, USA) and stained with relevant antibodies before flow cytometric analysis. The peripheral blood was

collected in heparin tubes (BD Biosciences). Erythrocytes were lysed with a hypotonic solution, and finally stained with different antibodies for flow cytometry. Cell definitions: neutrophils CD45⁺/CD15⁺/Siglec8⁻, eosinophils CD45⁺/CD15⁺/Siglec8⁺ and epithelial cells CD45⁻/EpCAM⁺.

Phagocytosis and oxidative burst

Additional four patients with CRSwNP were included for *in vitro* experiments. Peripheral blood was collected in heparin tubes. Neutrophils were isolated using the MACSxpress Neutrophil Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany, 130-104-434) and MACSxpress Erythrocyte Depletion Kit (Miltenyi Biotec 130-098-196) according to the manufacturer's instructions. Briefly, neutrophils from blood was negatively selected by binding of non-target cells to magnetic beads, followed by addition of magnetic bead binding of erythrocytes resulting in pure neutrophils. The neutrophils were counted, pelleted and re-suspended to approximately 5×10^6 cells/ml in RPMI with 10% FBS (both Gibco®). Half of the sample were then activated with 1 µg/ml LPS (product no: L2654, Sigma-Aldrich, St. Louis, MO, USA) for 1 hour and then washed twice in RPMI with 10% FBS. Un-activated cells were left for 1 hour in RPMI with 10% FBS. pHrodo™ Red *S. aureus* Bioparticles™ Conjugate for Phagocytosis (Invitrogen, Carlsbad, California, USA) (5µg/mL) and Dihydrorhodamine-123 (DHR-123) (Sigma-Aldrich®, Saint Louis, MO, USA) (40 ng/mL) were then added to both activated and un-activated cells. Samples for flow cytometry analysis were collected at 0, 0.5, 1, 2 and 4 hours. The cells were stained for CD16 and CD62L before flow cytometry analysis. The amount of fluorescence in neutrophils were measured using MFI (as recommended by the manufacturer) which directly correlated to the number of phagocytosed conjugated *S. aureus* bioparticles or amount of ROS using DHR-123 (converted to Rhodamine-123).

Antibodies and flow cytometry

The following monoclonal antibodies were purchased from BD Biosciences: anti-human EPCAM (APC, clone EBA-1), HLA-DR (PE-Cy7, clone G46-6), CD11b (AF700, clone ICRF44), CD15 (BV711, clone W6D3), CD16 (V450, clone 3G8), CD45 (APC Cy7, clone 2D1), CD45 (PE-CF594, clone HI30), CD62L (BV510, clone DREG-56), CD66b (PERCP 5.5, clone G10F5), CD69 (APC CY7, clone FN50), CD80 (PE-Cy7, clone L307,4), CD80 (FITC, clone BB1), CD86 (PE, clone 2331). Anti-human HLA-DR (PERCP 5.5, clone L243) and SIGLEC-8 (APC, clone 7C9) were purchased from BioLegend® (San Diego, CA, USA). Anti-human ICAM-1 (FITC, clone BBIG-I1) was purchased from R&D (Minneapolis, MN, USA). All antibodies were titrated for optimum concentration before use, with dilutions ranging from 1:10 to 1:50. Cells were identified based on expression of specific antigens. Cells were analysed on an LRSFortessa analyser (BD Biosciences) and data were processed using FlowJo software (©Tree Star, Inc., Ashland, USA). Mean fluorescence intensity (MFI) was calculated as geometric mean.

Statistics

Statistical differences between patients with CRSwNP and healthy controls were performed using unpaired t-tests (Mann-Whitney). For non-repeated measurements, a one-way ANOVA with a Tukey post-test was performed. For more than two sets of matched data, a two-way ANOVA with a Tukey post-test was made. Calculations of ratio were made using a non-parametric (Kruskal-Wallis) test together with a Dunn's post-test. Calculations of *in vitro* assays were made with two-way ANOVA with a Sidak (phagocytosis) or Tukey (ROS) post-test. Samples with <100 neutrophil counted events in the flow cytometer were excluded in the analysis (both figure and statistical analysis) since the sample then was regarded unreliable. A p-value of 0.05 or less was considered statistically significant (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; #p < 0.05; ####p < 0.001; #####p < 0.0001). Statistical analyses were performed using GraphPad Prism software (version 6.0, GraphPad Software, La Jolla, CA,

USA). Flow cytometry data are shown as individual data points with mean or as bars with mean $\pm$ S.E.M. n equals the number of patients or controls.

REFERENCE

1. Fokkens WJ, Lund VJ, Mullol J, et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2012. *Rhinol Suppl.* 2012(23):3 p preceding table of contents, 1-298.

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Total eosinophil fractions of CD45⁺ cells in (A) peripheral blood and in (B) nasal mucosa and nasal polyps from CRSwNP patients ($n = 16$) and in control nasal mucosa ($n = 9$). Total neutrophil fractions of CD45⁺ cells in (C) peripheral blood and in (D) nasal mucosa and nasal polyps from CRSwNP patients ($n = 16$) and in control nasal mucosa ($n = 9$).

Figure S2. Gating procedures of neutrophil subsets in nasal mucosa from healthy controls (A) and of neutrophil subsets in nasal polyps from CRSwNP patients (B). Neutrophil subsets in peripheral blood from CRSwNP patients ($n = 11$) and from healthy controls ($n = 9$), respectively. No statistically significant differences.

Figure S3. Expression of CD11b (MFI) on neutrophils. Detected in (A) peripheral blood and in (B) nasal mucosa and nasal polyps from CRSwNP patients ($n = 16$) and control nasal mucosa ($n = 9$).

Figure S4. Expression of CD11b (MFI) on neutrophils on subset level in (A) peripheral blood from healthy controls ($n = 9$) and (B) control nasal mucosa ($n = 9$). (C) Histogram of CD11b on CD16^{high} CD62L^{dim} neutrophils from nasal polyps from CRSwNP patients (red) compared to from nasal mucosa from healthy controls (blue).

Figure S5. Gating procedures of epithelial cells from nasal polyp mucosa.