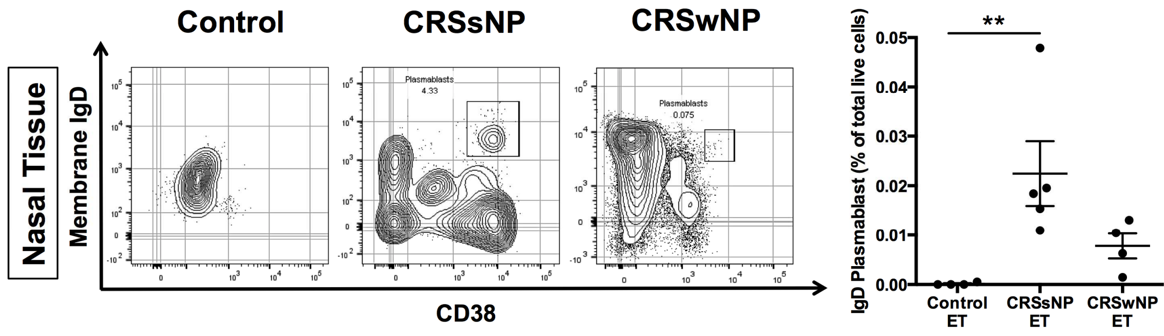
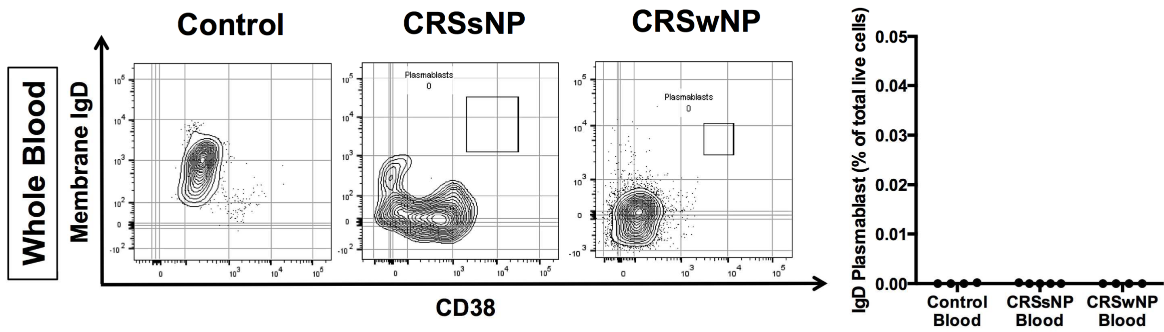
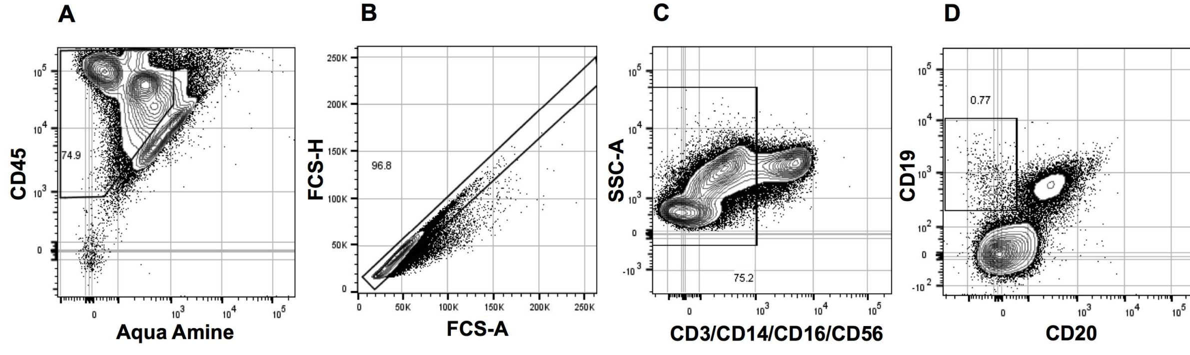
A



B





A
B
C
D
74.9
CD45
Aqua Amine
96.8
FCS-H
FCS-A
75.2
SSC-A
CD3/CD14/CD16/CD56
0.77
CD19
CD20

1 **Supplementary Methods**

2 *Subjects*

3 All CRS subjects met clinical criteria for CRS as defined by the American
4 Academy of Otolaryngology-Head and Neck Surgery Chronic Rhinosinusitis Task Force
5 and underwent routine functional endoscopic sinus surgery.[1, 2] Patients with established
6 immunodeficiency, pregnancy, coagulation disorder, classic allergic fungal sinusitis,
7 isolated antrochoanal polyps, or cystic fibrosis were excluded from the study. The
8 presence of sinusitis or NP was confirmed by office endoscopy and CT imaging. Control
9 subjects without history of CRS undergoing nasal surgery for a variety of non-CRS
10 indications (e.g. septoplasty, approaches to the orbit or anterior skull base) were
11 recruited from the otolaryngology clinic at Northwestern University.
12
13 *Sample collection and preparation*

14 Standardized nasal tissue samples consisting of UT and NP were obtained
15 during routine functional endoscopic sinus surgery. Specimens from control subjects
16 were obtained during endoscopic skullbase tumor excisions, intranasal procedures for
17 obstructive sleep apnea, and facial fracture repairs for patients without a history of
18 sinonasal inflammation. After removal, weighed tissue samples were placed in PBS-
19 Tween, supplement with a cocktail of protease inhibitors (Sigma-Aldrich) added at a
20 1:100 dilution. For total protein extraction, samples were homogenized with a Bullet
21 Blender Blue (Next Advance, Averill Park, NY) per manufacturer's instructions. The
22 samples were then centrifuged at 4,000 rpm for 20 minutes at 4°C and the supernatants
23 were frozen at -20°C until analysis.

24 Nasal lavage fluid was also obtained prior to initiating surgery from control subjects
25 and patients with CRS. After suctioning the nasopharynx, 8 ml of PBS was sprayed via a
26 syringe towards the middle meatus, and resultant fluid was collected with a Lukens trap

27 (Covidien, Mansfield, MA). Collected nasal lavage fluid was spun down for 10 minutes at
28 3000 rpm, with the resulting supernatant concentrated by centrifugation in a Amicon
29 Ultra-4 10K Centrifugal Filter Unit (EMD Millipore) at 3000 rpm for 10 minutes at 4°C.
30 The supernatant was stored at -20°C until use.
31 Blood samples were also collected prior to initiating surgery from control subjects
32 and patients with CRS for IgD measurements in the serum.
33
34 *Immunofluorescence*
35 Paraffin-embedded tissue sections were rehydrated, treated with antigen retrieval
36 unmasking reagent (Vector Laboratories, Burlingame, CA), rinsed, and blocked with 5%
37 goat serum (Vector Laboratories) for at least 1 hr. Then, the sections were incubated
38 with PE- conjugated goat anti-human IgD antibody (1:400; Southern Biotech) overnight
39 at 4°C. After washing, slides were mounted with SlowFade Gold Antifade Reagent with
40 4,6-diamidino-2-phenylindole (DAPI) counterstain (Life Technologies) and the slides
41 were stored in the dark at 4°C. Representative images from immunofluorescence slides
42 were obtained with an Olympus IX71 inverted research microscope (Olympus, Center
43 Valley, PA) by using a X200 or X400 objective lens and a MicroFire AR digital
44 microscope camera (Optronics, Goleta, CA).
45 The number of positive cells in 5 random fields was counted in 5 sections at a
46 magnification X400 from each tissue specimen. Each section was randomly selected
47 and diagnosis was blinded to the observers.
48
49 *Cell isolation and flow cytometry*
50 ET collected into cold RPMI-1640 medium (Life Technologies) supplemented
51 with 3% FCS (Atlanta Biologicals) during sinus surgery were processed within 2 hr. To
52 increase the number of isolated cells, we obtained ET instead of UT. Bone and cartilage

53 was removed and ET was minced into small pieces followed by digestion with 0.25
54 mg/mL collagenase IV (Sigma-Aldrich) for 1 hr at 37°C. The softened tissue was then
55 placed between two frosted ends of microscope slides, gently ground, and filtered
56 through a 70 µm Falcon™ cell strainer (Thermo Fisher Scientific). The filtrate was
57 layered atop FCS and centrifuged at low speed (300 x g) to remove additional debris
58 and yield a pellet of monodispersed cells. In addition, blood from the same subjects was
59 collected into heparin-containing vacutainers and lysed with standard ACK RBC lysis
60 buffer to obtain erythrocyte-free leukocytes.

61 For flow cytometric analysis, cell viability and absolute counts were assessed
62 with ethidium bromide/acridine-orange vital staining under a fluorescent microscope.
63 Cells were resuspended in staining medium at ~10^8 cells/ml and first incubated with an
64 Fc Block reagent (Miltenyi Biotec, Auburn, CA) for 10 minutes at room temperature
65 followed by staining with the amine reactive dye Zombie Aqua™ (BioLegend) to mark
66 dead cells. All antibodies were obtained from BioLegend, except for IgM (BD Bioscience)
67 and BLIMP-1 (Novus Biologicals,Littleton, CO). The following fluorophore-conjugated
68 monoclonal antibodies and dilutions were used to stain the surface of cells: CD3 (clone
69 UCHT1), CD14 (M5E2), CD16 (3G8), CD19 (HIB19), CD20 (2H7), CD27 (O323), CD38
70 (HIT2), CD45 (HI30), CD56 (HCD56), CD138 (MI15), IgD (IA-62), and IgM (G20-127).
71 Following the procedures for surface marker staining, cells were fixed/permeabilized by
72 using Cytofix/Cytoperm™ Kit (BD Biosciences) according to manufacturer instructions,
73 and stained with fluorescent antibody reagents specific for intracellular IgD (clone IA-62)
74 and BLIMP-1 (clone 3H2-E8).

75 Using FlowJo software (FlowJo-LLC), all samples were subjected to a sequential
76 gating strategy to remove first dead cells (Zombie Aqua^{TM+}) and all non-leukocytes
77 events ($CD45^-$). Subgates excluded doublets and triplet events with a FSC-H vs FSC
78 diagonal gate, where events within the gate are considered singlets. Finally, all T cells

79 ($CD3^+$), monocytes ($CD14^+$), granulocytes ($CD16^+$) and natural killer (NK) cells ($CD56^+$)
80 were excluded by using a dump channel. Following this filtration, B cell populations
81 were gated using $CD19^+CD20^-$ staining, along with relevant plasmablast markers.
82
83 *Real-time PCR*
84 Total RNAs were isolated from whole tissue extracts and cDNA was prepared as
85 previously described.[3, 4] Briefly, total RNAs were isolated from whole cell extracts and
86 treated with DNase I by using NucleoSpin RNA II (MACHEREY-NAGEL, Bethlehem, PA)
87 according to the manufacturer's instructions. The quality of total RNA was assessed with
88 a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) by using a RNA 6000 Nano
89 LabChip (Agilent Technologies). Single-strand cDNA was synthesized from 0.5 μg of
90 total RNAs with SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA) and
91 random primers. Semi-quantitative real-time PCR was performed with a TaqMan method
92 by using an Applied Biosystems 7500 Sequence Detection System (Applied Biosystems,
93 Foster City, CA) in 20 μL reactions (10 μL of 2x TaqMan Master mix [Applied
94 Biosystems], 400 nmol/L of each primer, and 200 nmol/L of TaqMan probe plus 10 ng of
95 cDNA). Primer and probe sets for β-glucuronidase (Hs00939627_m1), IL-2
96 (Hs00171146_m1), IL-21 (Hs00222327_m1), IL-15 (Hs01003716_m1), CD40L
97 (Hs00163934_m1), and BAFF (Hs00198106_m1) were purchased from Applied
98 Biosystems. All expression values were normalized to housekeeping gene β-
99 glucuronidase (GUSB), and expressed as $2^{-\Delta Ct}$.
100
101 *Nasal swab culture*
102 To obtain the specimens, the swab was left in the middle meatus or sinus cavity
103 of patients for few seconds until moistened and was rolled back and forth gently and

104 then was carefully removed. The samples were collected using two swabs per patient.
105 For aerobic microbiological examination, quantitative cultures were processed according
106 to the standard laboratory protocol and Gram staining was performed. The swabs
107 specimens were inoculated directly onto the aerobic media including Sheep Blood Agar,
108 Chocolate Agar, and a Mac Conkey Agar. All plates were incubated at 35 °C for 24 hr. If
109 there was no bacterial growth, the medium was further incubated for 24 hr more before it
110 was reported as negative. In addition, Gram staining was performed in smear and the
111 presence of any microorganisms and white blood cells were identified.

112 **References**

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126

127 **Supplementary Figure Legends**

128 **Figure E1.** Representative gating strategy used for flow cytometric analysis. Collected
129 cells were gated for **A,** live leukocytes (Aqua$^-$CD45$^+$), and then gated based on **B,**
130 forward scatter A (FCS-A) versus FCS-H for mono-dispersed cells or singlets. **C,** Next,
131 cells were gated out based on a dump channel indicating positive staining for T-cells
132 (CD3$^+$), monocytes (CD14$^+$), granulocytes (CD16$^+$), and NK cells (CD56$^+$). **D,** The
133 remaining cells (CD3$^-$CD14$^-$CD16$^-$CD56$^-$) were segmented into CD19$^+$/CD20$^-$,
134 CD19$^+$/CD20$^+$ and CD19$^-$/CD20$^-$ populations. Further characterization for IgD$^+$ B cells
135 was mined in the CD19$^+$CD20$^-$ population.
136

137 **Figure E2.** Levels of inflammatory mediators in nasal tissue. **A,** mRNA expression of IL-
138 2 was quantitated by real-time PCR and protein levels of **B**, IL-2, **C**, IL-10, and **D**, BAFF
139 were measured by using multiplex immunoassay. Dot plots illustrate individual data
140 points, and solid lines represent means ± SEMs. *P < .05.

141 Table E1. Clinical characteristics of subjects
142

	Control	CRSsNP	CRSwNP	
Total no. of subjects (M/F)	64 (31/33)	86 (39/47)	96 (53/43)	
Age (y), median (range)	52 (18-78)	39 (19-70)	46 (19-74)	
Atopy (Y/N/U)	12/49/2	40/40/6	55/28/13	
Asthma (Y/N/U)	3/59/1	24/62/0	52/43/1	
Prior nasal surgery (Y/N)	0/63	5/81	23/73	
Methodology used:				
Tissue extract				
Tissue type, n (M/F)	UT, 30 (12/18)	UT, 30 (11/19)	UT, 30 (19/11)	NP, 30 (21/9)
Age (y), median (range)	52 (19-78)	43 (19-70)	42 (24-62)	45 (27-74)
Nasal lavage fluid				
n (M/F)	30 (15/15)	30 (17/13)	30 (17/13)	
Age (y), median (range)	45 (21-78)	43 (19-70)	45 (27-72)	
Serum				
n (M/F)	30 (15/15)	30 (15,15)	30 (19/11)	
Age (y), median (range)	53 (19-78)	39 (19-71)	46 (26-72)	
Tissue RNA				
Origin, n (M/F)	UT, 19 (7/12)	UT, 11(4/7)	UT, 7 (4/3)	NP, 15 (11/4)
Age (y), median (range)	52 (27-72)	47 (19-55)	45 (37-61)	44 (32-74)
Immunofluorescence				
Origin, n (M/F)	UT, 15(6/9)	UT, 15 (8/7)	UT, 15 (11/4)	NP, 15 (11/4)
Age (y), median (range)	53 (27-72)	47 (19-70)	42 (27-72)	47 (27-74)
Flow Cytometry				
Origin, n (M/F)	ET and blood, 4 (3/1)	ET and blood, 5 (2/3)	ET and blood, 4 (2/2)	
Age (y), median (range)	52 (20-63)	45 (21-55)	59 (32-67)	
Supernatants from Ex vivo tissue explants culture				
Origin, n (M/F)	UT, 4 (2/2)	UT, 10 (5/5)	UT, 8 (4/4)	NP, 19 (14/5)
Age (y), median (range)	54 (38-72)	40 (23-65)	47 (28-60)	52 (23-66)
Nasal swab culture				
n (M/F)	4 (3/1)	29 (11/18)	18 (8/10)	
Age (y), median (range)	45 (30-61)	38 (23-69)	42 (19-55)	

143
144 CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis

145 with nasal polyps; M, male; F, female; Y, yes; N, no; U, unknown; UT, uncinate tissue;

146 NP, nasal polp; IT, inferior turbinate; ET, ethmoid tissue