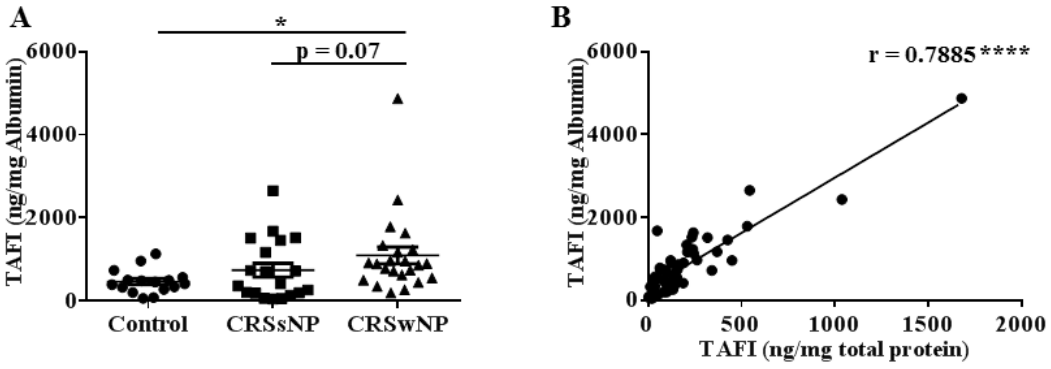
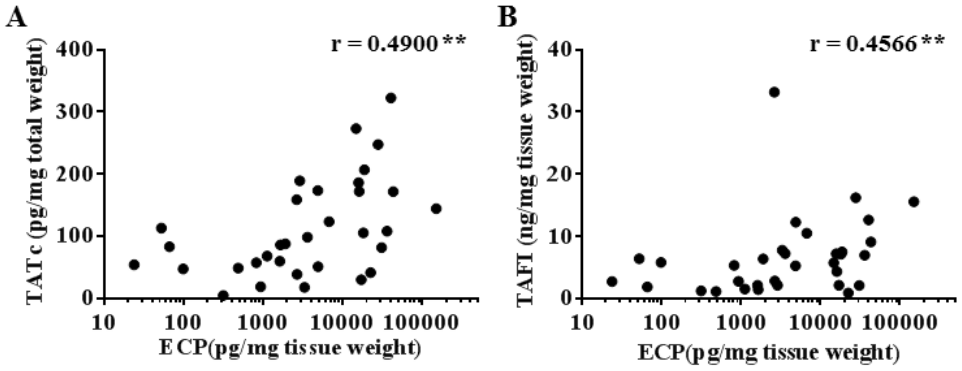
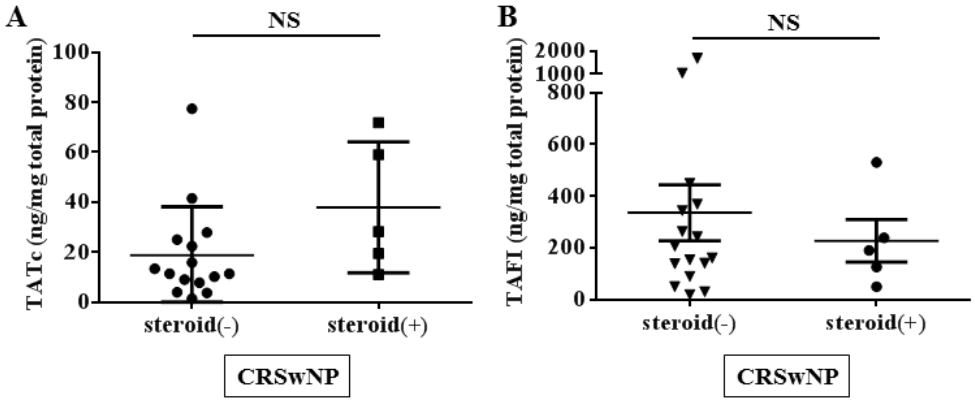
Supplementary Figure 1



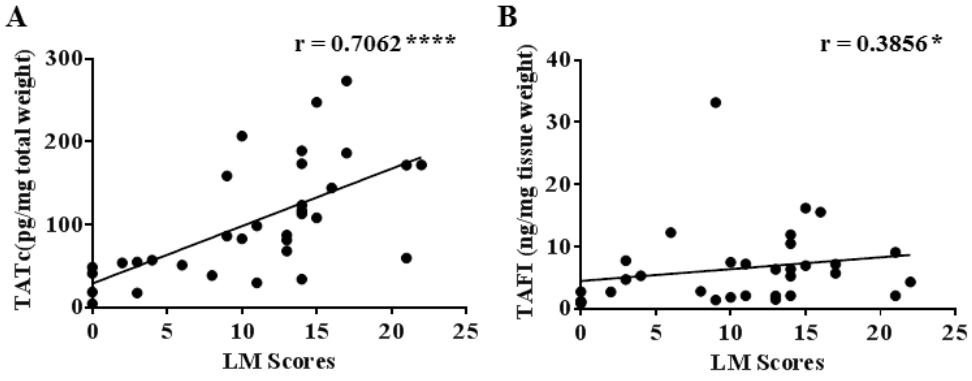
Supplementary Figure 2



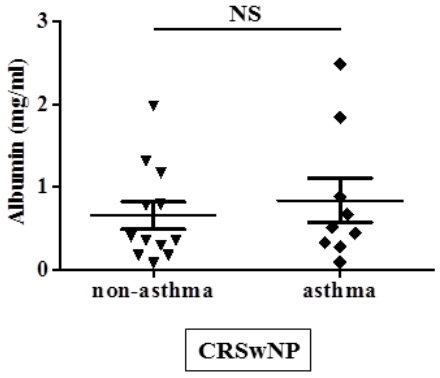
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Online Data Supplement

Title:

Increased thrombin activatable fibrinolysis inhibitor (TAFI) in chronic rhinosinusitis with nasal polyps

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Patients and biopsy specimens.

Patients with chronic rhinosinusitis (CRS) were recruited from the Allergy-Immunology and Otolaryngology Clinics at Northwestern University and the Northwestern Sinus Center. All subjects signed informed consent, and the protocol governing procedures for study have been approved by the Institutional Review Board of Northwestern University Feinberg School of Medicine. All subjects met the criteria for CRS as defined by the American Academy of Otolaryngology-Head and Neck Surgery Chronic Rhinosinusitis Task Force (1,2). The presence of sinusitis or bilateral nasal polyps was confirmed by office endoscopy and computed tomography imaging. Patients with an established immunodeficiency, pregnancy, coagulation disorder, diagnosis of classic allergic fungal sinusitis, Churg-Strauss syndrome, or cystic fibrosis were excluded from the study. All patients scheduled for surgery had previously failed to respond to adequate trials of conservative medical therapy (e.g. prolonged antibiotic regimens, nasal steroid sprays, oral steroids, saline irrigations, and decongestants) for control of symptoms. Sinonasal and nasal polyp tissues were obtained from routine functional endoscopic sinus surgery in patients with CRS. Specimens from patients without CRS were obtained during the performance of skull base tumor excision, facial fracture repair, lacrimal duct surgery, and orbital decompression surgery. Details of subjects' characteristics are described in Table 1.

Nasal Lavage Fluid Sampling

At the time of surgery, the nasopharynx was cleared of secretions, then 8 mL of PBS was sprayed via a syringe towards the middle meatus, and fluids were subsequently collected with a Lukens trap (Covidien, Mansfield, MA). The lavage fluids were then centrifuged for 10 minutes at 3000 rpm, and the resulting supernatant was subsequently concentrated 2-fold by

centrifugation in an Amicon Ultra-4 10K Centrifugal Filter Unit (EMD Millipore, Billerica, MA) at 3,000 rpm for 10 minutes at 4°C. The retentate was stored at -80°C until use.

Extraction and Measurement of Total Protein from Nasal Tissue

Weighed uncinate tissue (UT) and NP samples were placed in PBS-TWEEN containing a 1% protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO) and frozen at -80°C until processing. For total protein extraction, samples were minced with a surgical scalpel and then homogenized using a Next Advance bullet blender per the manufacturer's instructions (Next Advance Inc, Averill Park, NY). The samples were then centrifuged at 4,000 rpm for 20 minutes at 4°C and the supernatants were retained. For normalization purposes, total protein concentration was measured with a Bicinchoninic acid Protein Assay Kit per the manufacturer's instructions (Thermo Fisher, Watham, MA).

Supplementary Figure Legend

Supplementary Figure 1. Levels of TAFI normalized to albumin in nasal lavage fluids. (A) Increased TAFI in nasal lavage fluids in CRSwNP (n = 15-21) (●; control, ■; CRSsNP, ▲; CRSwNP). (B) The correlation of TAFI levels normalized to total protein and albumin in nasal lavage fluids. The levels of TAFI protein in nasal lavage fluids were measured by ELISA. The correlation in nasal lavage fluid was assessed using a Spearman rank correlation test. $*P < .05$ and $****P < .0001$.

Supplementary Figure 2. The correlation of levels of ECP with TATc (A) and TAFI (B) in nasal tissue. The correlation was assessed using a Spearman rank correlation test. $**P < .01$.

Supplementary Figure 3. The levels of TATc and TAFI in nasal lavage fluids of CRSwNP subjects according to the intake of oral glucocorticoids. No significant differences were seen (n = 5-11). NS, not significant.

Supplementary Figure 4. (A) The correlation of Lund-MacKay score and TATc levels in nasal tissue. (B) The correlation of Lund-MacKay score and TAFI levels in nasal tissue. The correlations were assessed using a Spearman rank correlation test. $*P < .05$, and $**** P < .0001$.

Supplementary Figure 5. The levels of albumin in nasal lavage fluids among CRSwNP subjects according to comorbidity with asthma. No significant differences were seen (n = 9-12). NS, not significant.

1. Meltzer EO, Hamilos DL, Hadley JA, Lanza DC, Marple BF, Nicklas RA et al. Rhinosinusitis: establishing definitions for clinical research and patient care. *J Allergy Clin Immunol* 2004;**114**:155–212.

2. Pearlman AN, Conley DB. Review of current guidelines related to the diagnosis and treatment of rhinosinusitis. *Curr Opin Otolaryngol Head Neck Surg* 2008;**16**:226–230.