

Supplemental Figure Legends.

Supplemental Figure 1. Gating strategy to identify eosinophils in sputum and BAL. Flow cytometry was performed on samples of peripheral blood (A, D, G, J); sputum (B, E, H, K) and BAL (C, F, I, L). Samples were first sequentially gated using SSC-A vs. FSC-A (A-C); SSC-A vs. CD45+ cells with high SSC (D-F); and then with isotype controls (G-I) or monoclonal antibodies against CCR3 and CD16 (J-L). Representative examples from NS subjects.

Supplemental Figure 2. Expression of various eosinophil receptors (as percentage positive) by sample type in all subjects. A, CD11b; B, CD34; C, CD49d; D, CD69; E, CD125. Data are median \pm interquartile (IQR) range, 95th & 5th percentiles, with outliers shown as symbols. Note that the median for BAL cells expressing CD34 is 99%. Samples with fewer than 100 events in the eosinophil positive gate were excluded. *, p<0.05; **, p<0.01; ***, p<0.000; ****, p<0.00001; ANOVA with Holm-Sidak post-hoc testing. We interpret the reductions in sputum eosinophil expression of CD49d and CD125 to be artefactual and due to the effects of dithiothreitol, as previously shown⁴.

Supplemental Figure 3. Eosinophil expression of adhesion and activation receptors (as MFI) by BAL eosinophils, divided by subject group. A, Representative histograms from never-smokers are shown for CD11b, CD34, CD49d, CD69, and CD125 for blood, sputum, and BAL. Red histogram, isotype control; Blue histogram, mAb-specific staining. B, Cumulative expression by BAL eosinophils of CD11b, CD49d. NS, never-smokers; FS-NAO, former smokers, no airflow obstruction; CS-NAO, current smokers, no airflow obstruction; FS-COPD, former smokers with COPD; CS-COPD, current smokers with COPD. Data are median, IQR, 95th & 5th percentiles, with outliers shown as symbols. Samples with fewer than 100 events in

the eosinophil positive gate were excluded. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.000$; ANOVA with Holm-Sidak post-hoc testing.





