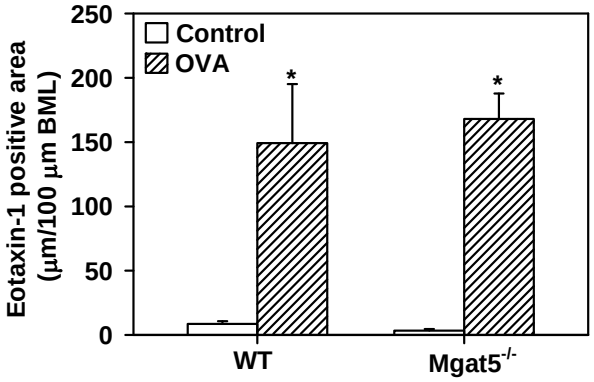
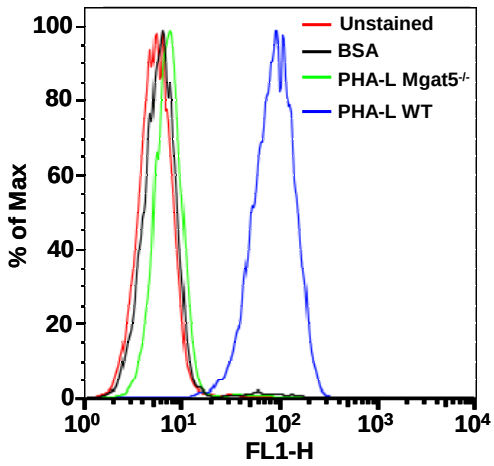
Supplemental Data

N-glycans differentially regulate eosinophil and neutrophil recruitment during allergic airway inflammation



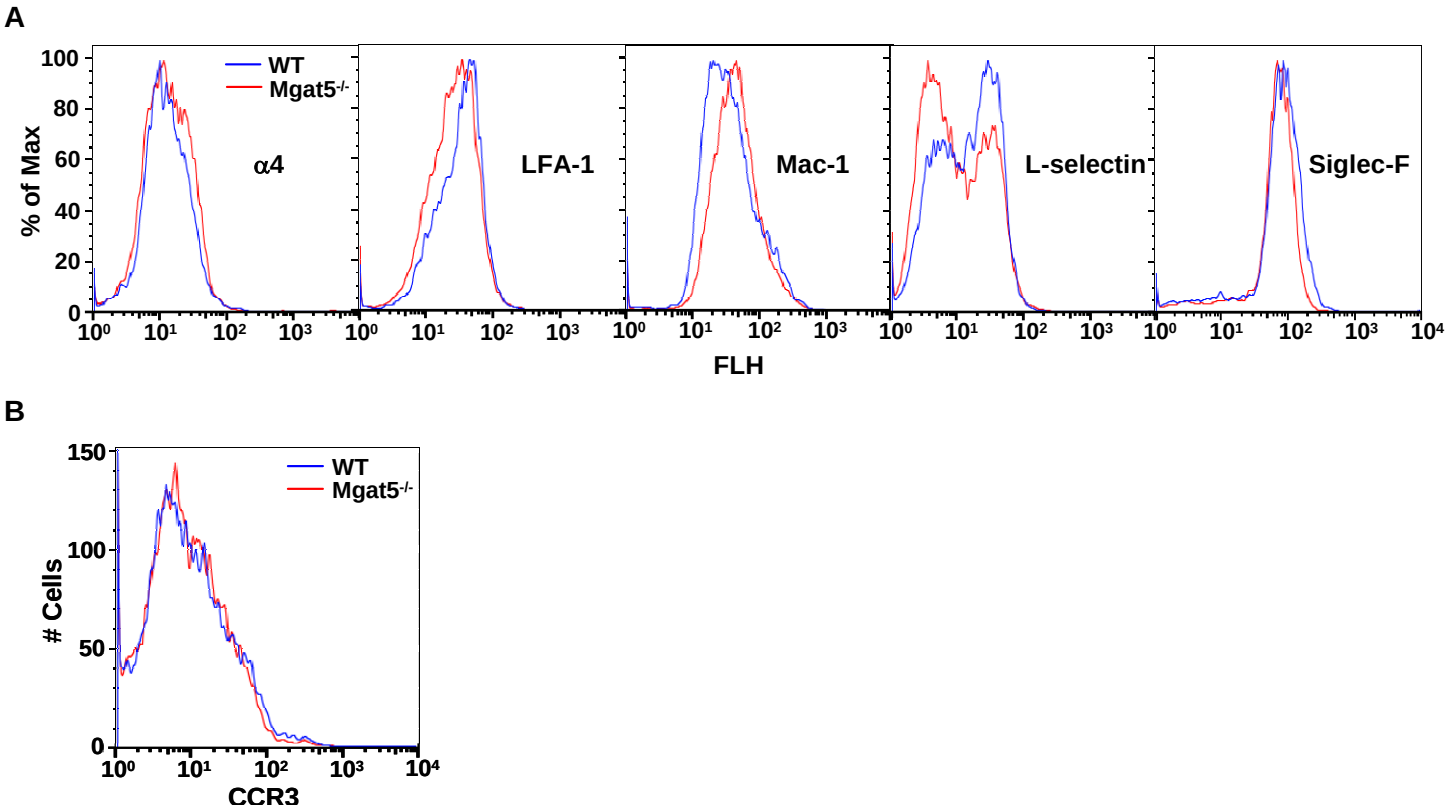
Supplemental Fig. 1

Supplemental Fig. 1. Allergen-challenged Mgat5$^{-/-}$ mice exhibit similar levels of lung eotaxin-1. Expression of eotaxin-1 in lung tissue was examined by immunostaining using rat mAb against murine eotaxin-1 at 20 μg/ml. Quantitation of eotaxin-1 expression in peribronchial areas (150 μm below the basement membrane) was accomplished with ImageJ analysis. Results are reported as eotaxin-1 positive area per 100 μm of basement membrane length (BML) and represent mean ± SEM values. n = 4-5 mice per experimental group. *p <0.05 for comparison of control versus allergen-challenged groups.



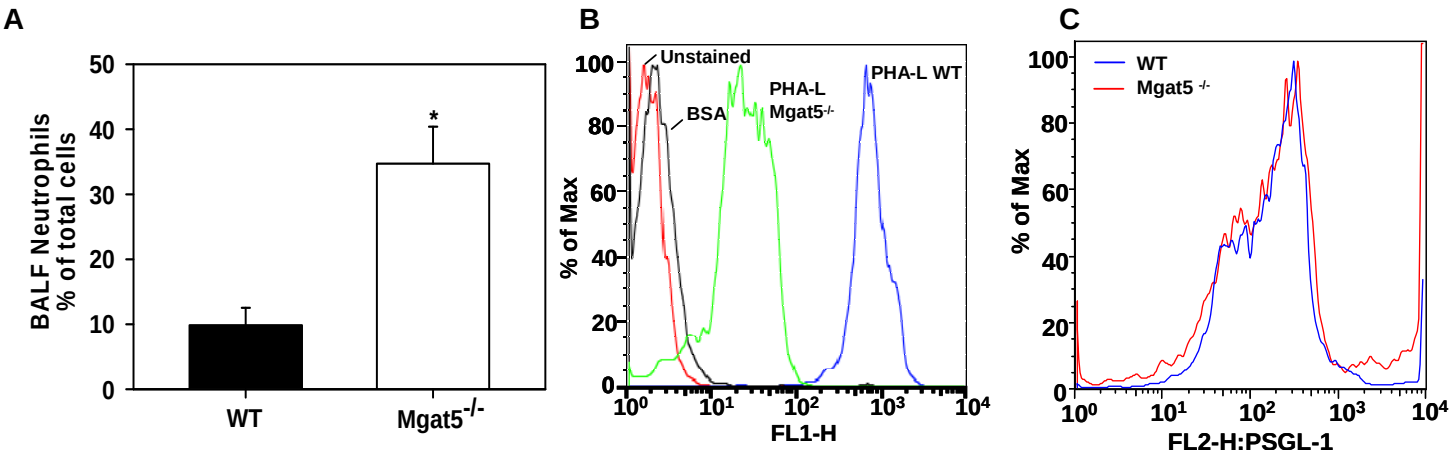
Supplemental Fig. 2

<u>Supplemental Fig. 2.</u> Eosinophils from Mgat5$^{-/-}$ mice do not express branched N-glycans. Expression of branched N-glycans by eosinophils cultured from BM of WT and Mgat5$^{-/-}$ mice was evaluated based on PHA-L-binding by flow cytometry using FITC-PHA-L (1 μg/ml) with FITC-BSA as a control. FlowJo flow cytometry software was used for data analysis. Representative histogram of PHA-L binding by eosinophils cultured from BM of WT and Mgat5$^{-/-}$ mice is shown. WT and Mgat5$^{-/-}$ eosinophils exhibited similar binding to BSA, therefore only WT BALF eosinophil binding to BSA is shown. n = 3 mice per group.



Supplemental Fig. 3

<u>Supplemental Fig. 3.</u> Expression of cell surface adhesion molecules and CCR3 by WT and Mgat5$^{-/-}$ eosinophils (A) Expression of α4, LFA-1, Mac-1, L-selectin and siglec-F by WT and Mgat5$^{-/-}$ eosinophils was assessed by flow cytometry using rat mAbs against murine α4 (PS/2), CD11a, CD11b, siglec-F and CD62L. Depending on the mAb, rat IgG2a or 2b was used as the isotype matched control. (B) Expression of CCR3, the receptor for eotaxin-1, by eosinophils from WT and Mgat5$^{-/-}$ mice by flow cytometry using FITC-conjugated anti-mouse CCR3 (5 μg/ml) with FITC-conjugated rat IgG2a as the isotype matched control antibody. FlowJo flow cytometry software was used for data analysis in all cases and histograms shown are representative of three experiments in duplicate with eosinophils from three different mice for each group.



Supplemental Fig. 4

<u>Supplemental Fig. 4.</u> Allergen-challenged Mgat5$^{-/-}$ mice develop airway neutrophilia despite lack of branched N-glycan expression (A) Differential cell counts in BALF of control and allergen (OVA) challenged WT and Mgat5$^{-/-}$ mice collected 18-24 h after that last allergen challenge were determined from Hema 3-stained cytocentrifuged slides. The number of neutrophils present is shown as a percentage of the total number of cells recovered from the BALF. No neutrophils were detected in BALF of control mice. Combined data from experiments repeated at least three times is shown. Data represent mean ± SEM for OVA-challenged groups. n = 12 mice per group. *p <0.01 compared to WT mice. (B) Expression of branched N-glycans by neutrophils was evaluated based on PHA-L-binding by flow cytometry using FITC-PHA-L (1 µg/ml) with FITC-BSA as a control. FlowJo flow cytometry software was used for data analysis Representative histogram of PHA-L binding by mature neutrophils from BM of WT and Mgat5$^{-/-}$ mice is shown. WT and Mgat5$^{-/-}$ neutrophils exhibited similar binding to BSA, therefore only WT BALF neutrophil binding to BSA is shown. n = 3 mice per group. (C) Expression of PSGL-1 by WT and Mgat5$^{-/-}$ neutrophils was assessed by flow cytometry using PE-conjugated anti mouse PSGL-1 (5 µg/ml) with PE-conjugated rat IgG1 as the isotype matched control. n = 4 mice per group.