

**Sialylation of MUC4 β N-glycans by ST6GAL1 orchestrates human airway
epithelial cell differentiation associated with Type-2 inflammation**

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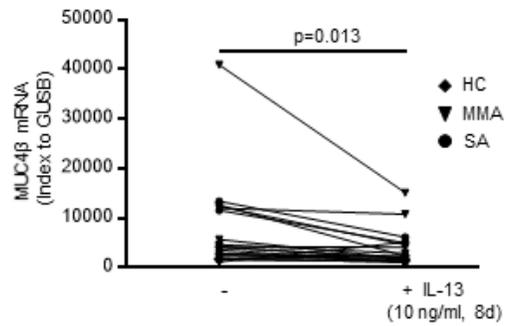
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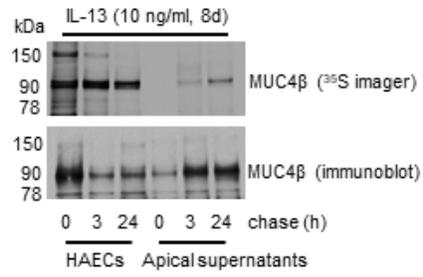
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Conflict of interest: ERB has performed clinical trials through his former employer, the Wake Forest School of Medicine and his current employer, the University of Arizona, and has served as a paid consultant for AstraZeneca/Medimmune, GSK, Novartis, Regeneron, and Sanofi Genzyme. SEW has been site and study PI on studies sponsored by AstraZeneca, GSK and Sanofi-Aventis. Other authors have declared that no conflict of interest exists.

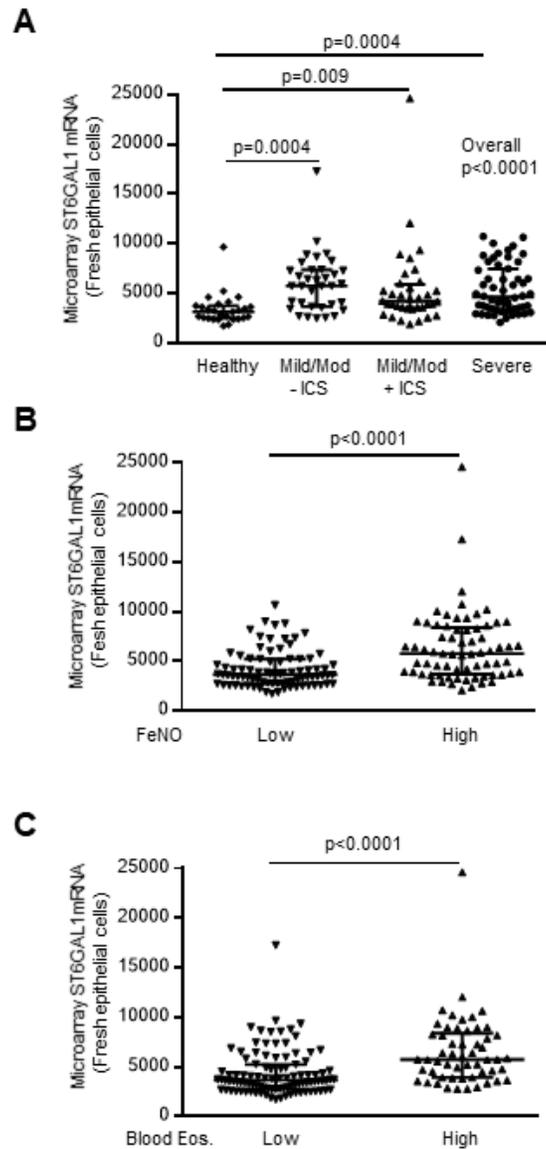
Supplemental figure legends



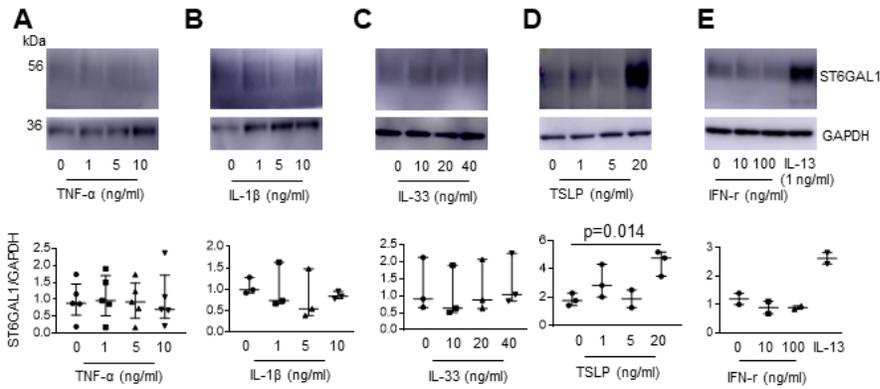
Supplemental Figure 1. Chronic IL-13 exposure for 8 d decreased MUC4β mRNA in ALI cultured HAECs. Non-parametric paired t test identified the difference.



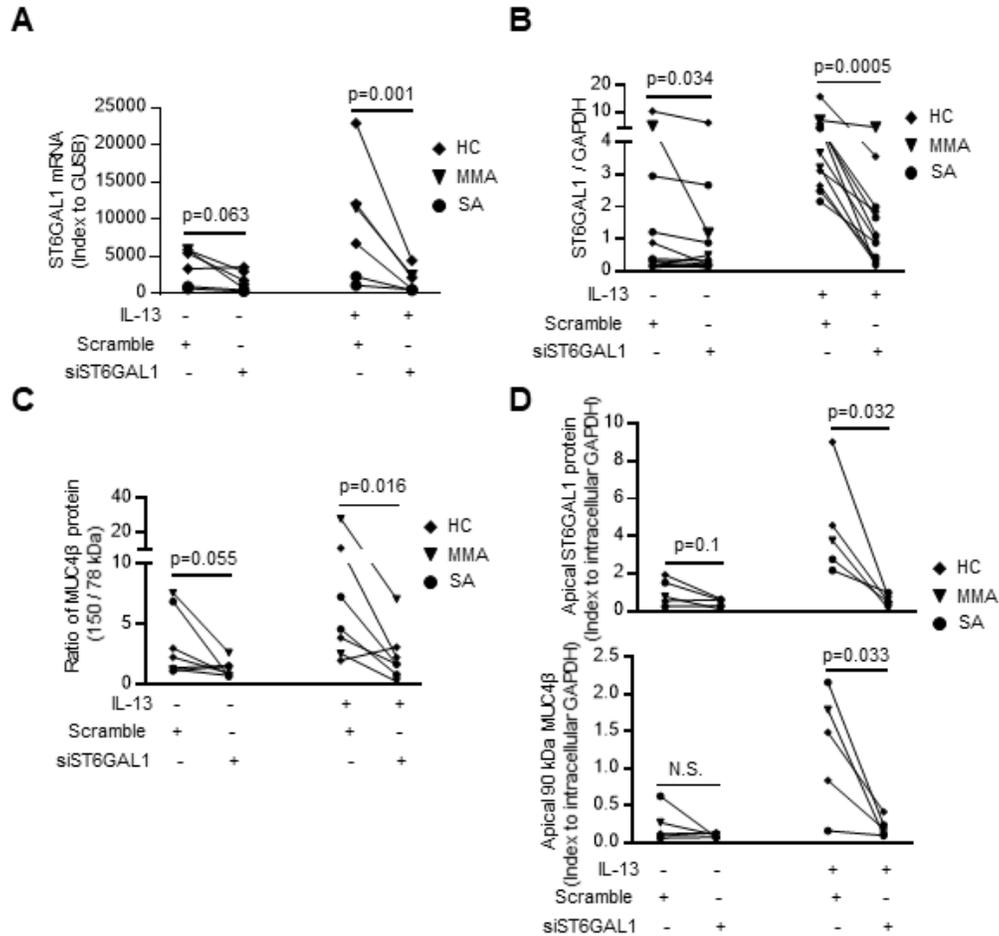
Supplemental Figure 2. Pulse-chase experiments in 8 d IL-13 treated ALI cultured HAECs. Representative imaging for [³⁵S] Met/Cys incorporated MUC4β and Western blot (n=3).



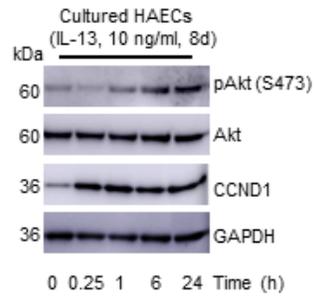
Supplemental Figure 3. The levels of ST6GAL1 mRNA (microarray in freshly isolated epithelial cells) and protein (sputum supernatant) association with T2 inflammation. (A) Comparison of ST6GAL1 mRNA level among 4 groups (26 HC, 37 MMA (-ICS), 41 MMA (+ICS), 51 SA). (B and C) Microarray ST6GAL1 mRNA was associated to T2 marker FeNO and blood eosinophils. Wilcoxon test identified the overall differences among the 4 groups. When overall $p \leq 0.05$, intergroup comparisons were further assessed. Non-parametric paired t test identified the difference between 2 independent groups.



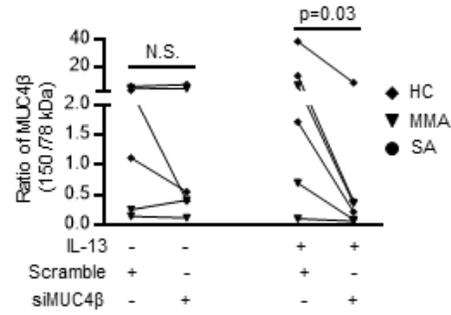
Supplemental Figure 4. Representative Western blots and densitometry of ST6GAL1 protein expression in ALI cultured HAECs (n=2-5). (A-D) Different doses of TNF- α , IL-1 β , IL-33 or TSLP treated HAECs for 8 d. **(E)** Pretreated with IL-13 (1 ng/ml) for 6 d, then further treated either with IL-13 or different doses of IFN- γ for 2 d.



Supplemental Figure 5. Knockdown of ST6GAL1 decreased the mRNA / protein of ST6GAL1, and the ratio of intracellular 150 kDa/78 kDa MUC4 β , as well as the apical secreted ST6GAL1 and MUC4 β in ALI cultured HAECs. (A) ST6GAL1 mRNA was decreased by ST6GAL1 knockdown. (B) Densitometry of ST6GAL1 protein was decreased by ST6GAL1 knockdown. (C) The ratio of 150 kDa to 78 kDa MUC4 β was decreased by ST6GAL1 knockdown in the presence of IL-13. (D) Densitometry of ST6GAL1 and 90 kDa MUC4 β in apical supernatant (index to cell lysate GAPDH) was decreased by siST6GAL1 in the presence of IL-13. Non-parametric paired t test identified the difference between siST6GAL1 and scramble.



Supplemental Figure 6. Acute IL-13 stimulation on pAkt and CCND1 protein in ALI cultured HAECs. Representative Western blot of pAkt and CCND1 (n=3).



Supplemental Figure 7. Knockdown of MUC4β decreased the ratio of 150 kDa to 78 kDa MUC4β in the presence of IL-13. The densitometry ratio of 150 kDa to 78 kDa MUC4β was compared between MUC4β knockdown and scramble. Non-parametric paired t test identified the difference between siMUC4β and scramble.

Supplemental Table 1 Demographics of ex vivo fresh epithelial cells for qRT-PCR

	Healthy Control (HC, n=7)	Mild/Moderate (MMA, n=13)	Severe (SA, n=9)	Overall p value
Gender (M/F)	3/4	5/8	3/6	0.702
Race (C/AA/Others)	6/0/1	6/5/2	6/2/1	0.234
Age (yrs)	21.8 (19.1-56.8)	40.2 (21.4-51.0)	41.7 (33.7-56.3)	0.276
BMI (kg/m ²)	22.4 (21.7-23.7)	26.3 (23.1-38.6)	30.9 (26.9-34.8)	0.098
Baseline FEV1 (% predicted)	96 (95-103)	75 (63-89)	70.5 (35-88)	0.0054
FeNO (ppb)	14.1 (9.3-77.3)	21.0 (16.7-29.4)	20.3 (14.4-33.2)	0.375
Blood eosinophils (/μL)	100 (0-200)	200 (100-300)	200 (100-400)	0.105
Serum IgE (kU/L)	13 (6.0-42.0)	137.5 (20.5-337.8)	118 (33.8-1603.5)	0.092
ICS (yes/no)	None	12/1	1/8	0.0001
OCS (yes/no)	None	13/0	6/3	0.022

Categorical variables were analyzed using Pearson ChiSquare tests. Continuous variables were not normally distributed and analyzed using Wilcoxon/Kruskal-Wallis tests and presented as medians and interquartile range (25th-75th percentile). C: Caucasian; AA: African American; BMI: Body mass index; FeNO: Fractional exhaled nitric oxide; ICS: Inhaled corticosteroid; OCS: Oral corticosteroid

Supplemental Table 2 Demographics of ex vivo fresh epithelial cells for Western blot

	Healthy Control (HC, n=9)	Mild/Moderate (MMA, n=13)	Severe (SA, n=11)	Overall p value
Gender (M/F)	2/7	6/7	3/8	0.44
Race (C/AA/Others)	9/0/0	8/2/0	7/2/1	0.38
Age (yrs)	35.0 (30.5-42.0)	28.0 (20.0-43.0)	52.0 (43.0-57.0)	0.005
BMI (kg/m ²)	27.5 (23.0-33.2)	25.2 (21.8-29.3)	29.5 (25.0-35.9)	0.49
Baseline FEV1 (% predicted)	93 (88-110)	89 (83-98)	51 (29-71)	0.0003
FeNO (ppb)	15.0 (11.0-20.0)	36.0 (19.0-44.0)	66.0 (31.0-119.0)	0.003
Blood eosinophils (/μL)	166 (25-318)	1150 (400-2900)	723 (0-3050)	0.048
Serum IgE (kU/L)	134.5 (20.0-135.6)	129.3 (81.9-435.3)	118.8 (28.0-343.5)	0.057
ICS (yes/no)	None	4/9	11/0	<0.0001
OCS (yes/no)	None	2/11	11/0	<0.0001

Categorical variables were analyzed using Pearson ChiSquare tests. Continuous variables were not normally distributed and analyzed using Wilcoxon/Kruskal-Wallis tests and presented as medians and interquartile range (25th-75th percentile). C: Caucasian; AA: African American; BMI: Body mass index; FeNO: Fractional exhaled nitric oxide; ICS: Inhaled corticosteroid; OCS: Oral corticosteroid