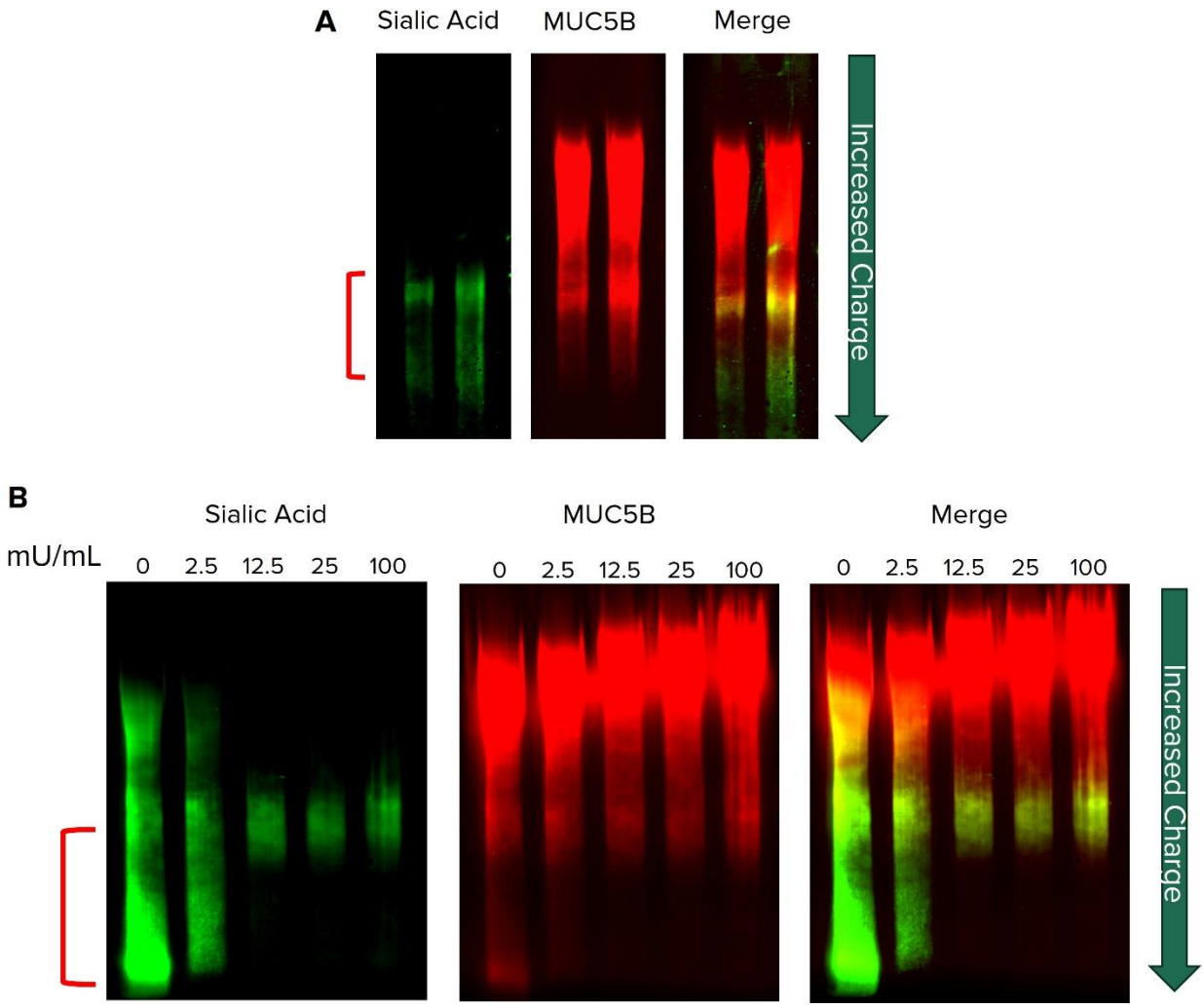
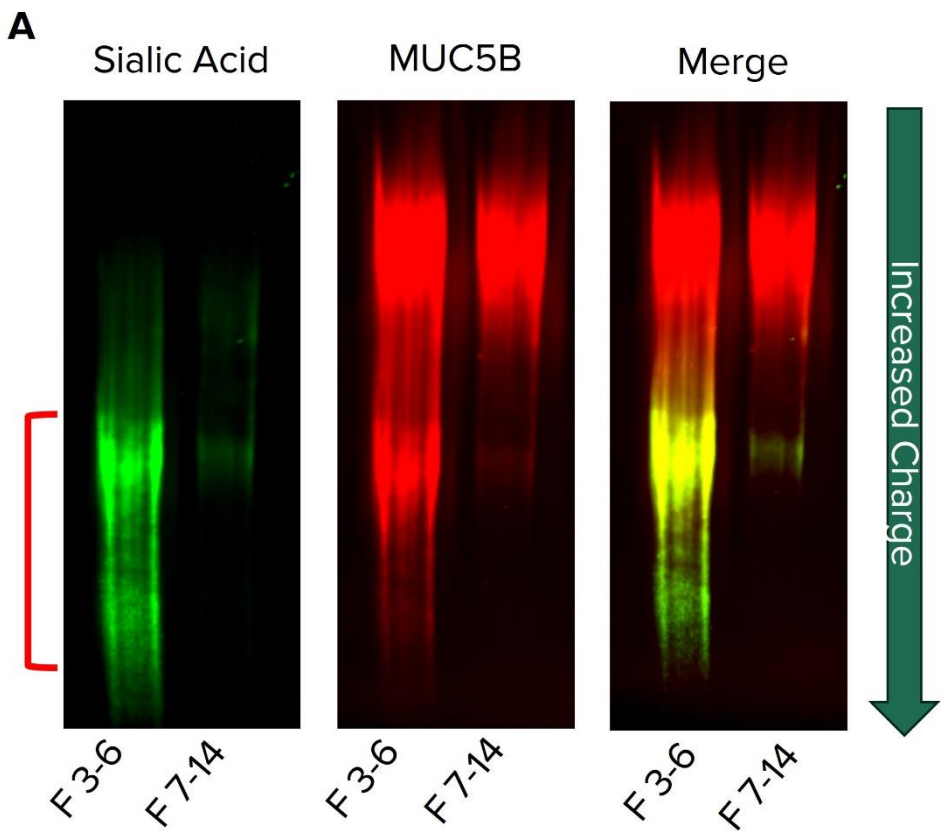


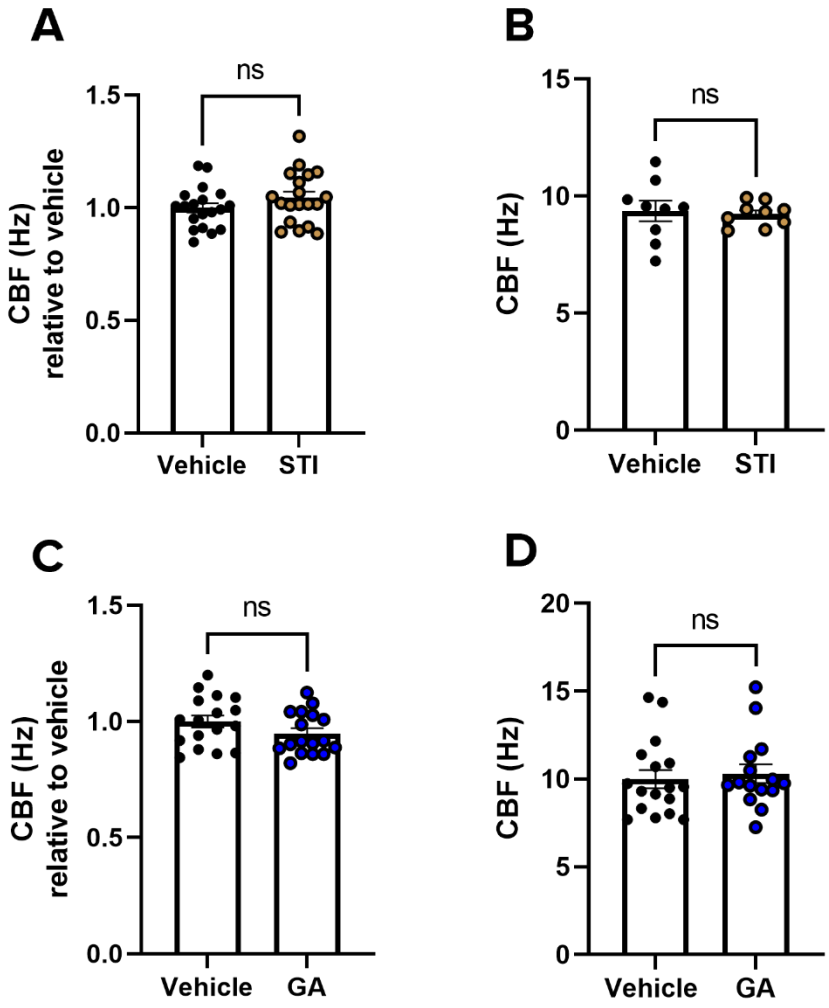
SI Figure 1. Sialylation correlates to high charged salivary MUC5B, and reduction of sialylation contributes to a lower charged MUC5B form.

Agarose-PAGE western blots of MUC5B isolated from saliva. **(A)** Salivary MUC5B was separated by gel electrophoresis and evaluated for sialic acid (SIAFIND™ Pan Lectenz) (green) and MUC5B (red) in duplicate. The fastest migrating/ most charged MUC5B (red bracket) strongly colocalized with sialic acid (yellow). **(B)** Salivary MUC5B was treated with increasing concentrations of neuraminidase, ranging from 0 to 100mu/mL, to remove sialic acid and separated by gel electrophoresis before being probed for sialic acid (green) and MUC5B (red). The faster migrating/mostly charged species (red bracket) shifted with neuraminidase treatment. The gel mobility of MUC5B was decreased as sialic acid was removed indicating a decrease in charge. Blots were cropped to improve clarity and conciseness; the original, uncropped blots are presented in SI Figure 4B-C.



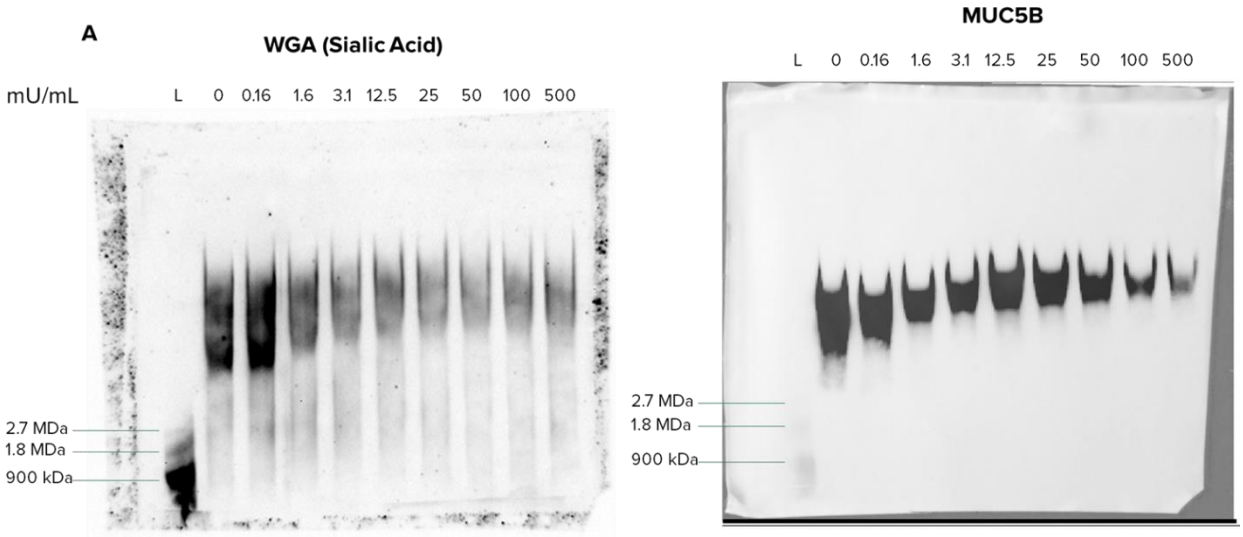
SI Figure 2. Slower sedimenting MUC5B exhibits increased sialylation and the high charged form of MUC5B.

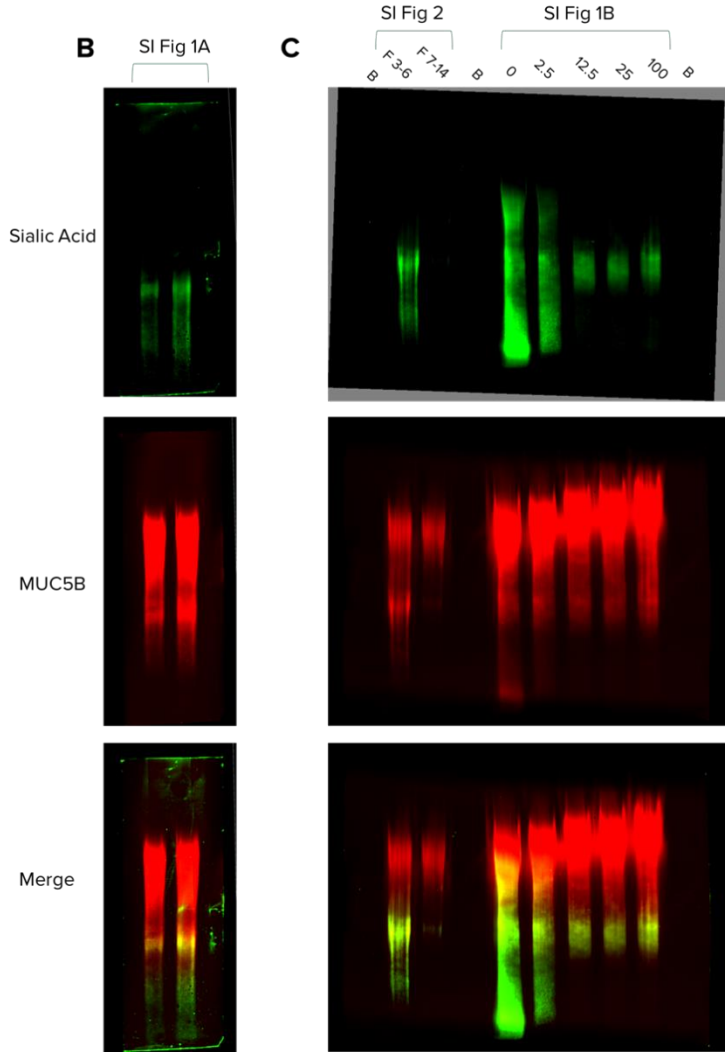
Agarose-PAGE western blots of MUC5B following rate zonal centrifugation. Untreated MUC5B was separated on 10-35% Sucrose gradient by rate zonal centrifugation to separate MUC5B by shape (Fig 2C-D); more compact mucins sediment faster. **(A)** Fractions containing MUC5B were combined into two pools: 1) Fractions 3-6 representing the slower (more linear) MUC5B, and 2) fractions 7-14 representing the faster sedimenting (more compact) MUC5B. These pools were separated by gel electrophoresis and evaluated for sialic acid (SIAFIND™ Pan Lectenz) (green) and MUC5B (red). More sialic acid was present in pool 1, and sialic acid strongly colocalized with the fastest migrating/more charged MUC5B (red bracket). This high charged form was nearly absent in pool 2, indicating that increased MUC5B charge conferred by sialic acid correlates to slower sedimenting (more linear) MUC5B and promotes mucin linearization. Blots were cropped to improve clarity and conciseness; the original, uncropped blots are presented in SI Figure 4C.

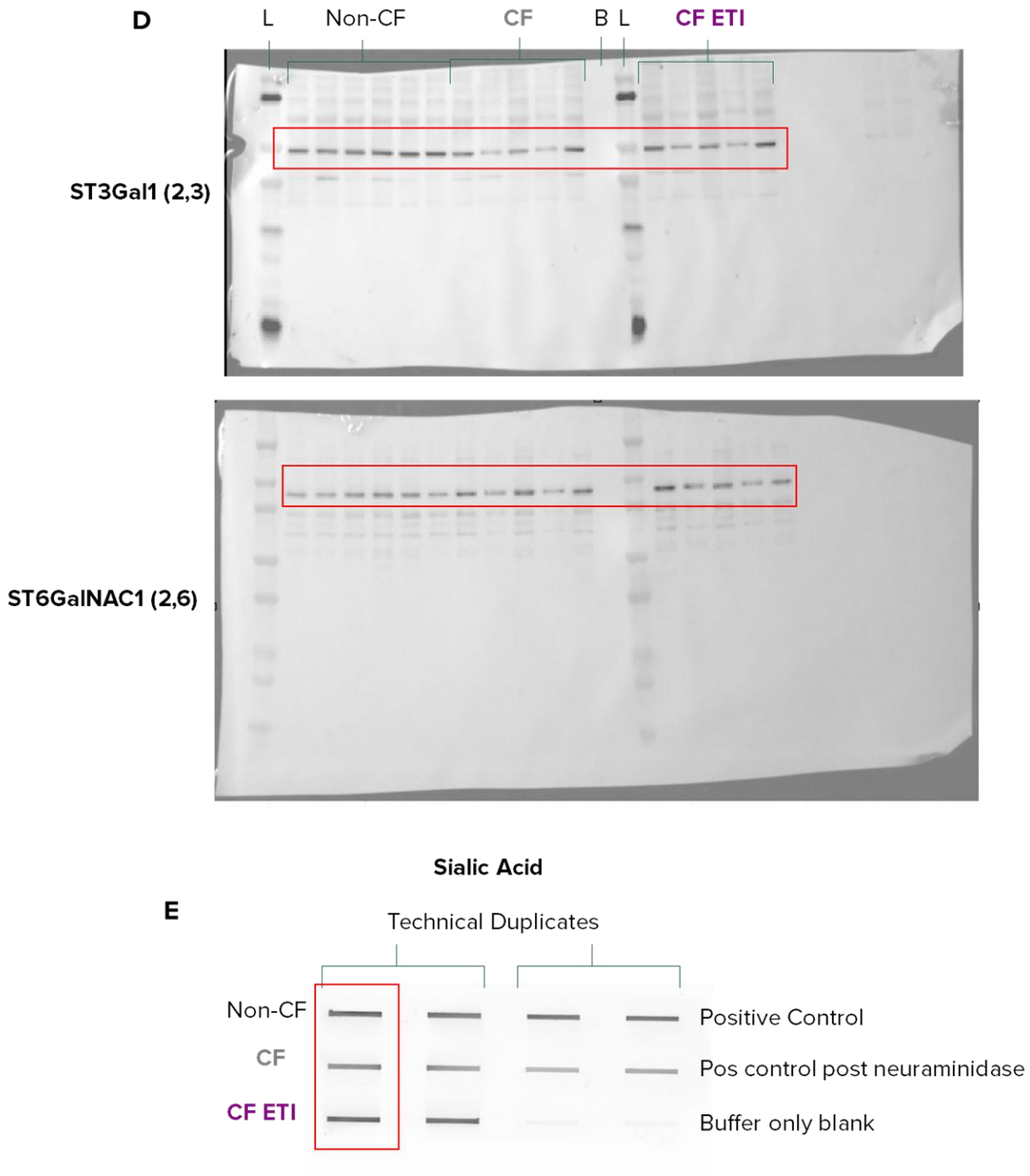


SI Figure 3. CBF of HBECs and trachea after sialyltransferase inhibition

Quantification of CBF from (A) HBECs treated with either vehicle or 200uM STI, (B) excised WT tracheae from rats treated with either PBS vehicle or 500 μM STI, (C) HBECs treated with either vehicle or 120 μM GA, and (D) excised WT tracheae from rats treated with either PBS vehicle or 300 μM GA. N=9-18/condition. nsP>0.05 by unpaired T-test.


A
WGA (Sialic Acid)
mU/mL
L 0 0.16 1.6 3.1 12.5 25 50 100 500
2.7 MDa
1.8 MDa
900 kDa
MUC5B
L 0 0.16 1.6 3.1 12.5 25 50 100 500
2.7 MDa
1.8 MDa
900 kDa


B
SI Fig 1A
C
SI Fig 2
SI Fig 1B
B F 3-6 F 7-14 B 0 2.5 12.5 25 100 B
Sialic Acid
MUC5B
Merge



SI Figure 4. Original and uncropped blots from figures.

(A) Original blots from Figure 1 A-B, blotted for sialic acid (WGA) and MUC5B following. Blots were cropped to the 25mU/mL neuraminidase treatment, because that is when the MUC5B migration plateaued. "L" indicates well with molecular weight ladder. (B) Original blot from SI Figure 1A, blotted for sialic acid (green) and MUC5B (red) in duplicate. (C). Original blot from SI Figure 1B and SI Figure 2A. The lanes are notated with green brackets to indicate the figure

they correspond to. “B” indicated wells loaded with buffer only. (D) Original blots from Figure 7I, J. “B” indicates wells loaded with buffer only, and “L” indicates wells with molecular weight ladder. The cropped regions presented in the manuscript are outlined with a red box. (E) Original blot from Figure 7O. Technical duplicates are indicated by green brackets. The positive control was MUC5B isolated from saliva. The negative control was an equal volume of isolated MUC5B treated with neuraminidase. The cropped region used in the manuscript is outlined with a red box.

Headings for Supplemental Videos:

S1 Video. Representative µOCT video of non-CF HBECs treated with DMSO vehicle for 24 hours (STI control).

S2 Video. Representative µOCT video of non-CF HBECs treated with 200 µM STI for 24 hours.

S3 Video. Representative µOCT video of excised trachea from a WT rat after intratracheal instillation of DMSO vehicle diluted in PBS daily for 7 days (STI littermate control).

S4 Video. Representative µOCT video of excised trachea from a WT rat after intratracheal instillation of 500 µM STI daily for 7 days.

S5 Video. Representative µOCT video of non-CF HBECs treated with DMSO vehicle for 24 hours (GA control).

S6 Video. Representative μOCT video of non-CF HBECs treated with 120μM GA for 24 hours.

S7 Video. Representative μOCT video of excised trachea from a WT rat after intratracheal instillation of DMSO vehicle diluted in PBS daily for 7 days (GA littermate control).

S8 Video. Representative μOCT video of excised trachea from a WT rat after intratracheal instillation of 300 μM GA daily for 7 days.

S9 Video. Representative μOCT video of non-CF HBECs treated with DMSO vehicle for 72 hours.

S10 Video. Representative μOCT video of CF HBECs treated with DMSO vehicle for 72 hours.

S11 Video. Representative μOCT video of CF HBECs treated with Elexacaftor/Tezacaftor/Ivacaftor for 72 hours.