Supplementary Fig. 1

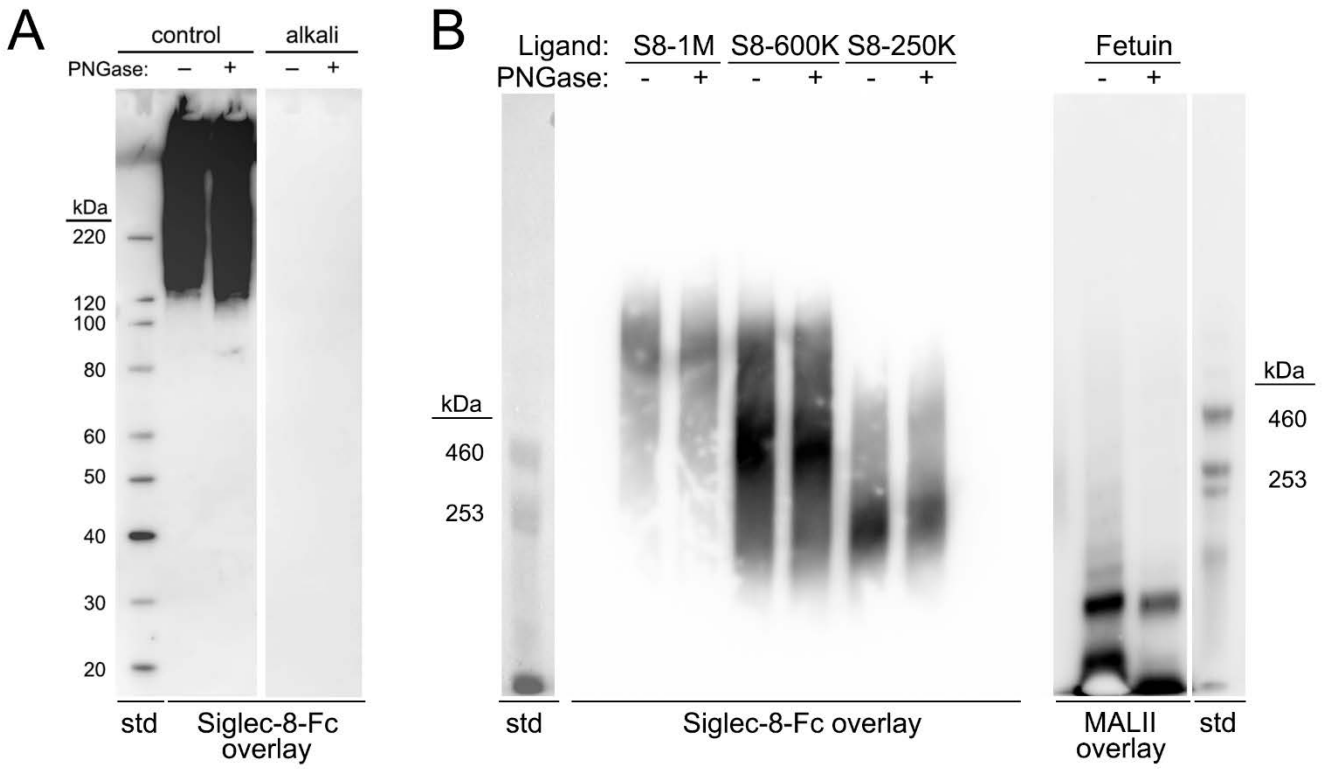

Fig. S1. Siglec-8 ligands extracted from human trachea are PNGase insensitive and alkali labile. (**A**) Siglec-8 ligands were extracted from postmortem human trachea in 6 M GuHCl as described previously (Yu et al. 2017), salt exchanged into 1 M urea, 20 mM Tris-HCl pH 8, and subjected to size exclusion chromatography on a 1 cm x 20 cm column of Sepharose CL-4B in the same buffer. Siglec-8 ligands (250-1,000 kDa) were pooled. Replicate aliquots of ligand were incubated for 10 min at 100°C in 0.5% SDS, 40 mM DTT to denature. After cooling, the reactions were brought to 50 mM sodium phosphate pH 7.5, 1% NP-40. Reactions were incubated with (+) or without (–) 2500 manufacturer units of PNGase F (New England Biolabs P7050S) for 16 h. Replicate (–) and (+) enzyme aliquots were resolved on a 4-12% NuPAGE Novex Bis-Tris gel (Invitrogen) and transferred to PVDF. The blot was washed with water, cut lengthwise and half was incubated in 0.1 M NaOH for 16 h at 45°C (alkali). Control and alkali-treated blots were probed with Siglec-8-Fc as described in Methods. (**B**) Purified Siglec-8 ligands of each size class (as indicated) were treated in 20 µl of buffer with or without 1000 manufacturer units of PNGase F for 25 h at 37°C. As a positive control, 16 µg of fetuin (Sigma) was equally treated. Treated and untreated samples as indicated were resolved by 1.5% acrylamide, 2% agarose gel electrophoresis, blotted to PVDF, and ligand lanes probed with Siglec-8-Fc as described in the text. Blots of lanes with fetuin were overlaid with biotinylated MALII (Vector) precomplexed with HRP-Streptavidin. Bound lectin was detected as described in the text. Siglec-8 ligands were not significantly shifted or diminished, while fetuin bands shifted downward and MALII staining was diminished. Molecular weight standards (std) were MagicMark XP (panel A) or HiMark (panel B), both from Thermo Fisher.