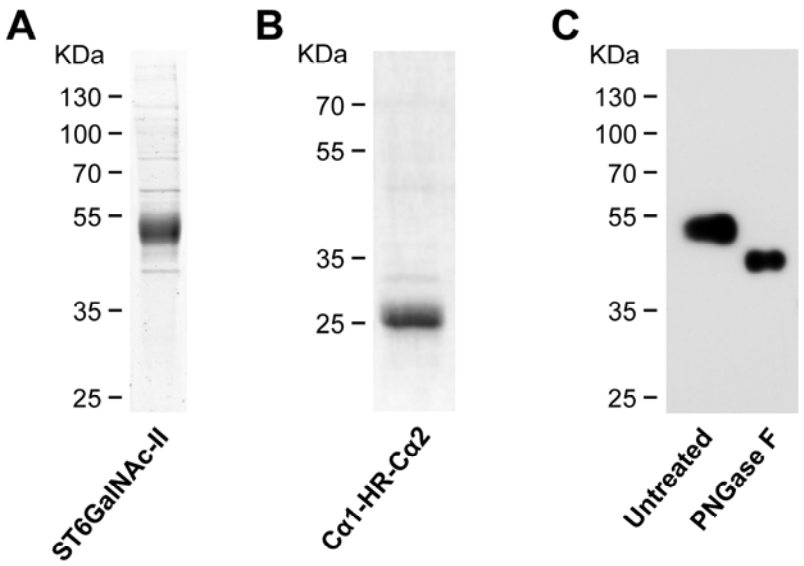
Supplementary Fig. 1 SDS-PAGE characterization and purity analysis of secreted ST6GalNAc-II and IgA1 Cα1-HR-Cα2 fragment



(**A**) Recombinant ST6GalNAc-II expressed in 293F cells was purified by Ni-NTA affinity chromatography under native conditions concentrated to reach ~0.5 mg/ml. Theoretical prediction of molecular weight of ST6GalNAc-II based on amino acid sequence is 51 KDa. Purity of the final preparation was assessed by SDS-PAGE analysis, followed by silver staining (**B**) The recombinant Cα1-HR-Cα2 expressed in *E. coli* was purified by Co-NTA affinity chromatography under denaturation conditions and concentrated to reach ~1 mg/ml. Theoretical prediction of the molecular weight of Cα1-HR-Cα2 based on amino acid sequence is 27 KDa. Purity of the final preparation was assessed by SDS-PAGE analysis, followed by Coomassie Brilliant Blue R-250 staining. (**C**) Two-µg aliquots of recombinant ST6GalNAc-II were divided into two tubes, denaturation reaction buffer was added, reaction was heated to 100°C for 5 min,

detergent solution was added, and 1 μl of PNGase F was added to one tube at volumes recommended by manufacturer (Prozyme, Hayward, CA). Both tubes were incubated overnight at 37°C. Samples were separated by SDS-PAGE, semi-dry blotted to PVDF membrane, developed with anti V5 antibody diluted 1:7,000 in SuperBlock, and signal was detected by a cooled CCD camera. In the figure, the left band corresponds to untreated and the right band corresponds to PNGase F-treated ST6GalNAc-II. The mobility shift was from approximately 54 KDa for the untreated protein to 51 KDa for the PNGase F-treated protein.