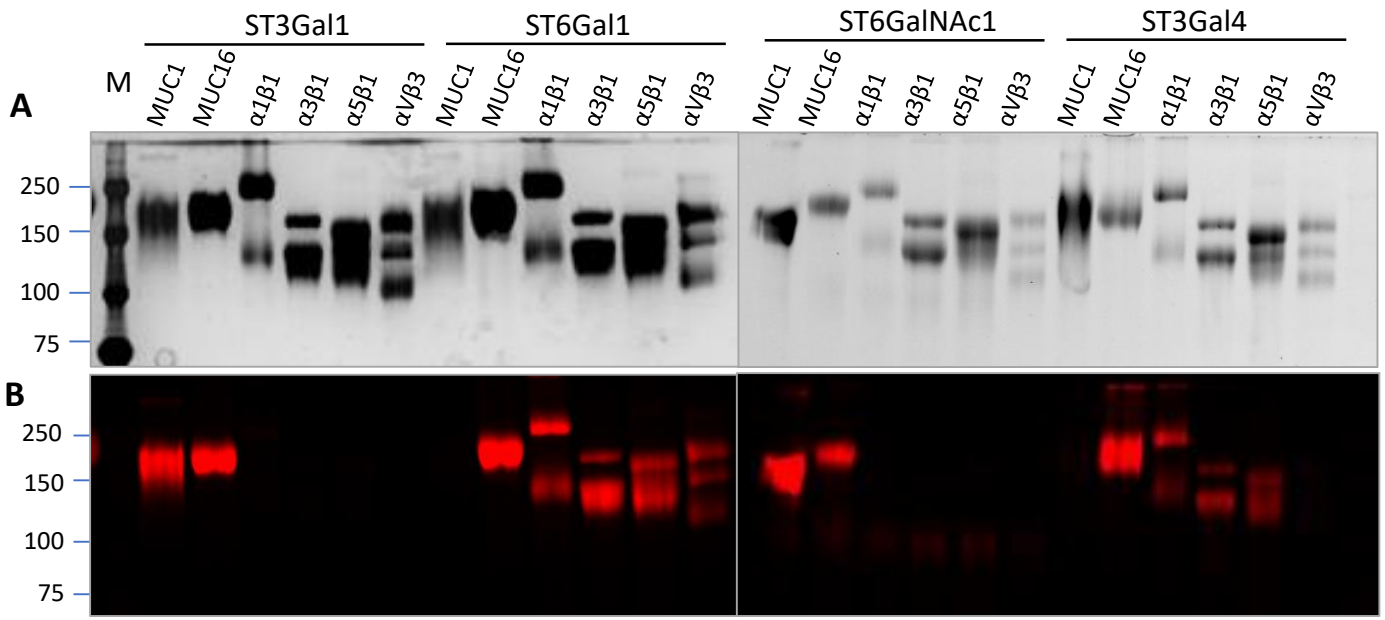
Supplemental Table 1. Relative tolerance of the three fluorophores by the sialyltransferases used in this report.

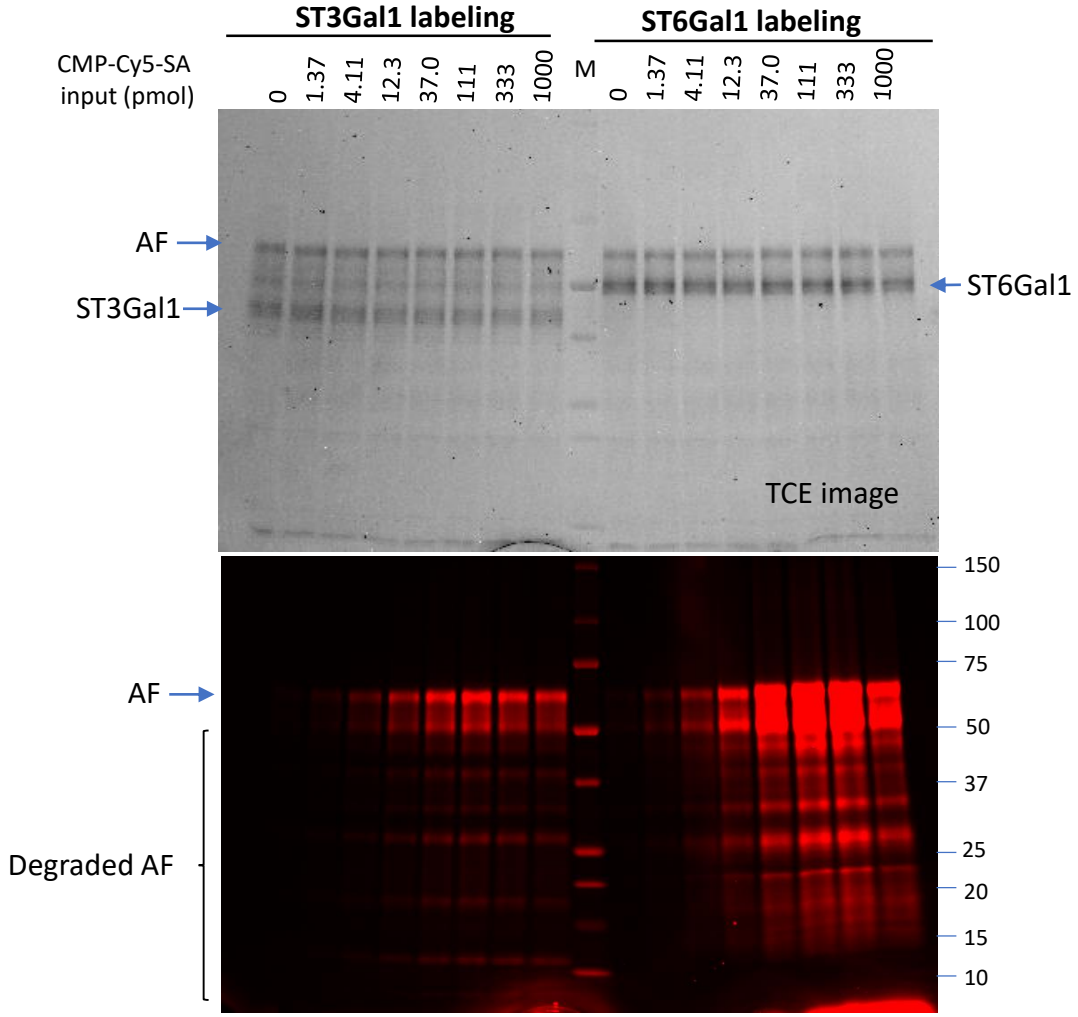
Sialyltransferases	Acceptor glycan type*	Alexa Fluor™ 555**	Alexa Fluor™ 488**	Cy5**
ST3Gal1	Gal of Core-1 O-glycan	++	+	++
ST3Gal2	Gal of Core-1 O-glycan	+++	+++	+++
ST3Gal4	Gal of N-glycan	++	+	+++
ST6Gal1	Gal of N-glycan	++	+++	+++
ST6GalNAc1	O-GalNAc, sialylation on terminal Gal is flexible	++	++	++
ST6GalNAc2	O-GalNAc, sialylation on terminal Gal is flexible	+	-	+
ST6GalNAc4	O-GalNAc, with sialylated terminal Gal	+++	+	-

Note: *Detailed acceptor glycan structures for these enzymes may need further characterization. **The tolerance of the three fluorophores is based on the corresponding fluorescent intensity of the labeled fetuin or asialofetuin in Fig. 2 and is indicated by + and – signs only. As the fluorescent intensity of each labeled band is also dependent on the abundance of the acceptor glycan for the labeling enzyme on fetuin, comparison of the tolerance of fluorophores by different labeling enzymes is not valid.



Supplemental Fig.1. Differential labeling of O-glycans and N-glycans on recombinant mucins and integrins. O-glycans and N-glycans on recombinant mucins and integrins were labeled by ST3Gal1, ST6Gal1, ST6GalNAc1 and ST3Gal4 with Cy5. All samples were pretreated with recombinant *C. perfringens* neuraminidase to remove preexisting sialic acids. The labeling reactions were separated on SDS-PAGE and imaged by silver staining (**A**) and fluorescent imager (**B**). Same amount of protein (2.5 µg) was loaded into each lane. M, BioRad Western molecular marker.

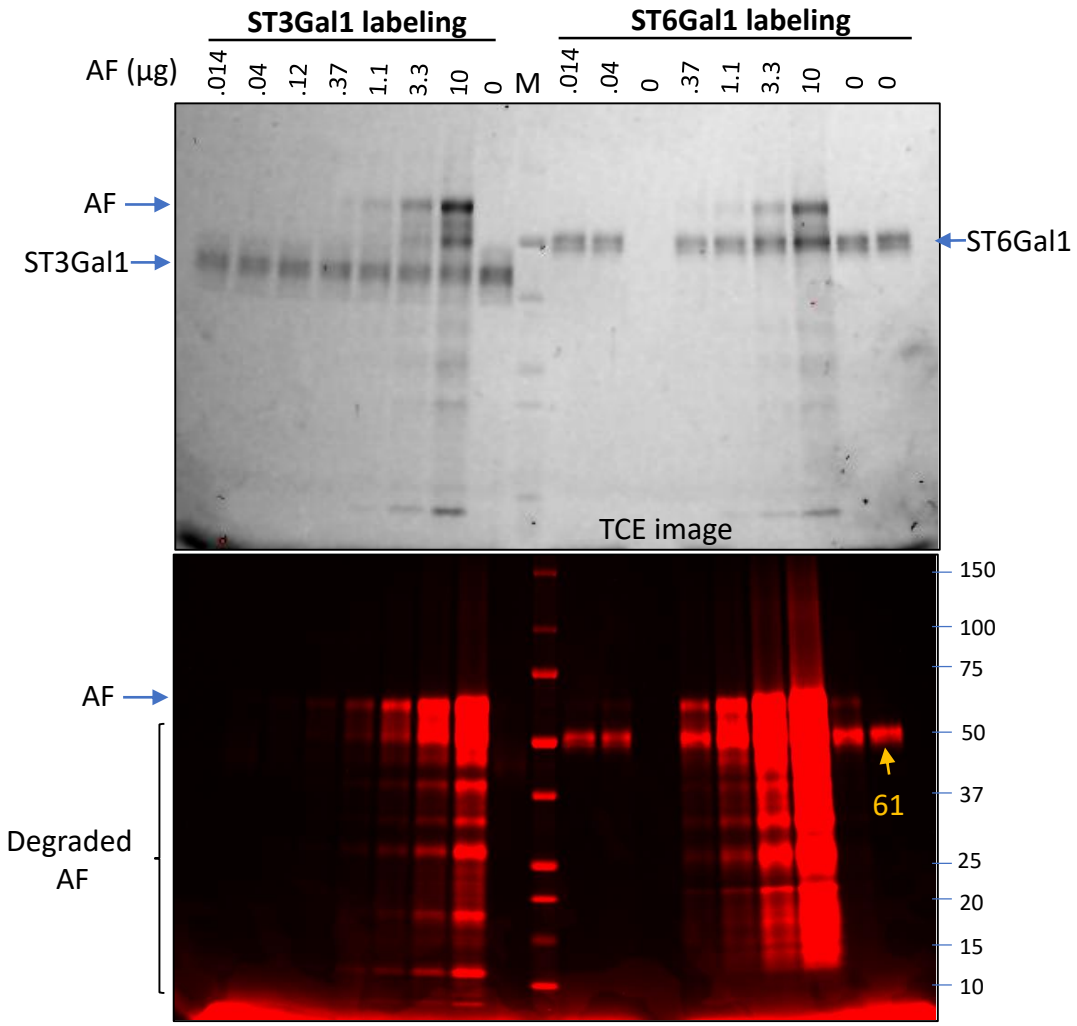
- MUC1 exclusively contains O-glycans, as it was only labeled by ST3Gal1 and ST6GalNAc1;
- MUC16 contains both N- and O-glycans, as it was labeled strongly by all four enzymes.
- All integrins exclusively contain N-glycans, as they were only labeled by ST6Gal1 and ST3Gal4 but not ST3Gal1 and ST6GalNAc1



Supplemental Fig. 2. Maximal labeling of asialofetuin (AF) is achieved with 1.2 µM of CMP-Cy5-SA.

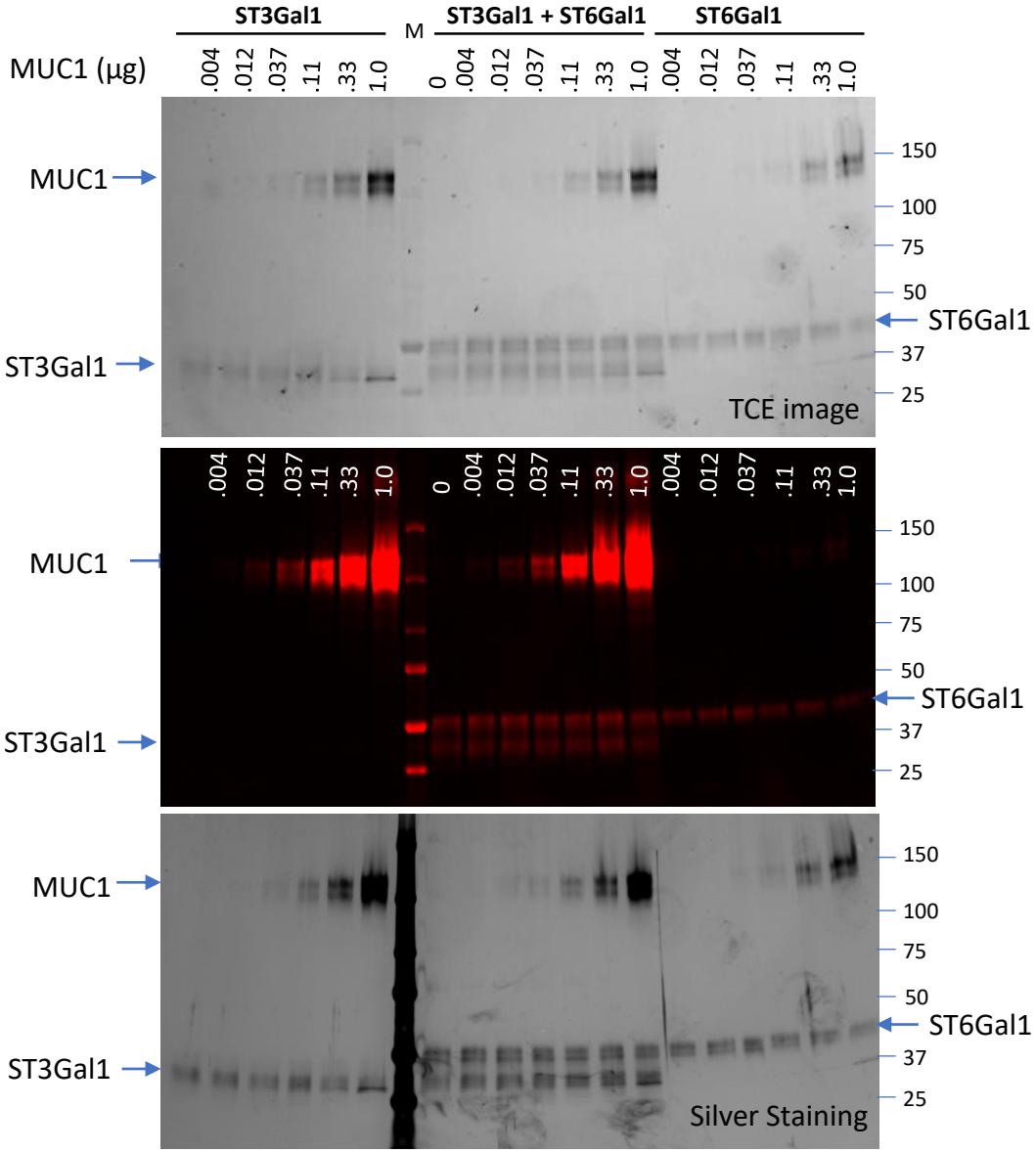
Labeling of 5 µg asialofetuin (Sigma Aldrich) by 0.2 µg of ST3Gal1 or ST6Gal1 with variable amounts of CMP-Cy5-SA in 30 µL of reaction volume. The labeling reactions were incubated at 37° C for 30 minutes and terminated by 6x SDS gel loading buffer. Labeled samples were separated on SDS-PAGE and visualized by trichloroethanol (TCE) imaging (top panel) and fluorescent imaging (lower panel). Visible labeling on asialofetuin by ST3Gal1 was observed at 4.11 pmol of CMP-Cy5-SA and maximal labeling by ST3Gal1 was achieved at 37.0 pmol of CMP-Cy5-SA (1.2 µM). Visible labeling on asialofetuin by ST6Gal1 was observed at 1.37 pmol of CMP-Cy5-SA level and maximal labeling by ST6Gal1 was achieved at 37.0 pmol of CMP-Cy5-SA (1.2 µM). Asialofetuin was partially degraded and the degradation products are visible in the fluorescent image. M, Western molecular marker from BioRad.

- 1.2 µM of CMP-Cy5-SA is needed for maximal labeling on AF by both ST3Gal1 and ST6Gal1.



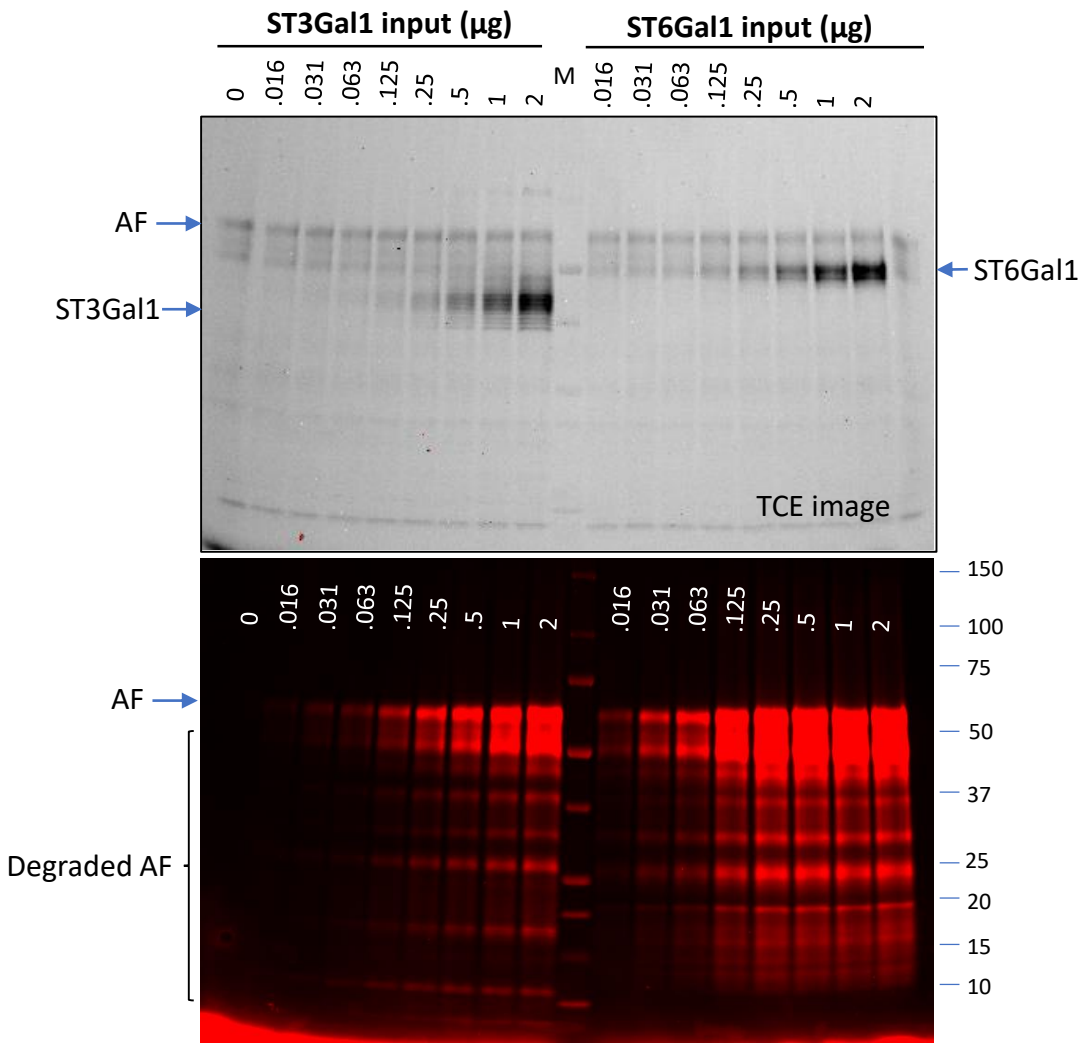
Supplemental Fig. 3. Lower limit of detection for asialofetuin labeled by ST3Gal1 and ST6Gal1 is at sub-microgram level. Variable amounts of **a**sialofetuin (Sigma Aldrich) as indicated were labeled by 0.2 µg of ST3Gal1 or ST6Gal1 with 0.2 nmol of Cy5-conjugated CMP-Sialic acid in 30 µL of reaction volume. The labeling reactions were incubated at 37° C for 30 minutes and terminated by 6x SDS gel loading buffer. The samples were separated on SDS-PAGE and visualized by trichloroethanol (TCE) imaging (top panel) and fluorescent imaging (lower panel). In both cases, 0.37 µg of asialofetuin is the limit that can be detected. However, due to the significant degradation of asialofetuin, the actual limit for detection could be much lower. ST6Gal1 (61) showed significant self labeling. M, Western molecular marker from BioRad.

- The lower detection limit for AF labeled by ST3Gal1 and ST6Gal1 could be much lower than 0.37 µg



Supplemental Fig. 4. Lower limit of detection for recombinant MUC1 labeled by ST3Gal1 is at nanogram level. Variable amounts of MUC1 (R&D Systems) as indicated were labeled by 0.2 µg of ST3Gal1 or ST6Gal1 with 0.2 nmol of Cy5-conjugated CMP-Sialic acid (CMP-Cy5-SA) in 30 µL of reaction volume. The labeling reactions were incubated at 37° C for 30 minutes and terminated by 6x SDS gel loading buffer. The samples were separated on SDS-PAGE and visualized by trichloroethanol (TCE) imaging (top panel), fluorescent imaging (middle panel), and silver staining (lower panel). ST3Gal1 strongly labeled MUC1 with the lower limit of detection around 0.012 µg. ST6Gal1 didn't label MUC1 but labeled itself. ST3Gal1 didn't label itself but was labeled by ST6Gal1. M, Western molecular marker from BioRad.

- The lower limit of detection for MUC1 labeled by ST3Gal1 is around 0.012 µg



Supplemental Fig. 5. Optimal amount ST3Gal1 and ST6Gal1 for asialofetuin (AF) labeling is below 1 µg.

Five µg of asialofetuin (Sigma Aldrich) was labeled by variable amounts of ST3Gal1 or ST6Gal1 with 0.2 nmol of Cy5-conjugated CMP-sialic acid in 30 µL of reaction volume. The labeling reactions were incubated at 37° C for 30 minutes and terminated by 6x SDS gel loading buffer. Labeled samples were separated on SDS-PAGE and visualized by trichloroethanol (TCE) imaging (top panel) and fluorescent imaging (lower panel). ST3Gal1 at 0.063 µg level showed clear visible labeling on asialofetuin and at 0.5 µg level achieved maximal labeling. ST6Gal1 at 0.016 µg level showed clear visible labeling on asialofetuin and at 0.25 µg level achieved maximal labeling. Asialofetuin was partially degraded and the degradation products are visible by the labeling. M, Western molecular marker from BioRad.

- 0.5 µg of ST3Gal1 is needed for maximal labeling of AF.
- 0.25 µg of ST6Gal1 is needed for maximal labeling of AF.