

Supplemental Figure 1 LC-  $MS^2$  spectra of sulfated core 1 O-glycans collected after fragmentation of the precursor ion at *m*/*z* 464 ([M-H]-) from recombinantly produced PSGL-1/mlgG2b protein purified from CHO cells. PSGL-1 was co-transfected with Gal3ST4 (A) or CHST1 (B). Included in each figure is EIC of *m*/*z* 464 from LC-MS run. For key of symbols, see Figure 1.



Supplemental Figure 2 Fragmentation of the disulfated isomer 910-4. Glycan sequence was interpreted from MS2, MS3 and MS4 experiments. (A) MS<sup>2</sup> spectrum of parent detected as  $[M-2H]^{2-}$  ions at m/z 454.2<sup>2-</sup> (inserted EIC). Fragment ions are annotated with proposed compositions. For some fragment ions, more than one composition may be possible. (B) MS<sup>3</sup> spectrum of the m/z 373.0<sup>2-</sup> from the MS<sup>2</sup> experiment shown in (A). (C) MS4 spectra of the ions at m/z 261.1<sup>2-</sup> from the MS<sup>3</sup> experiment shown in (B). For key of symbols, see Figure 1.



Supplemental Figure 3 Table of glycans detected after transfection of CHO-cells with PSGL-1/mIgG2b and GCNT1 contructs together with constructs for sulfotransferases. Included is also the relative abundance as measured by their ion intensity of parent ions from the LC-MS runs. For key of symbols, see Figure 1.