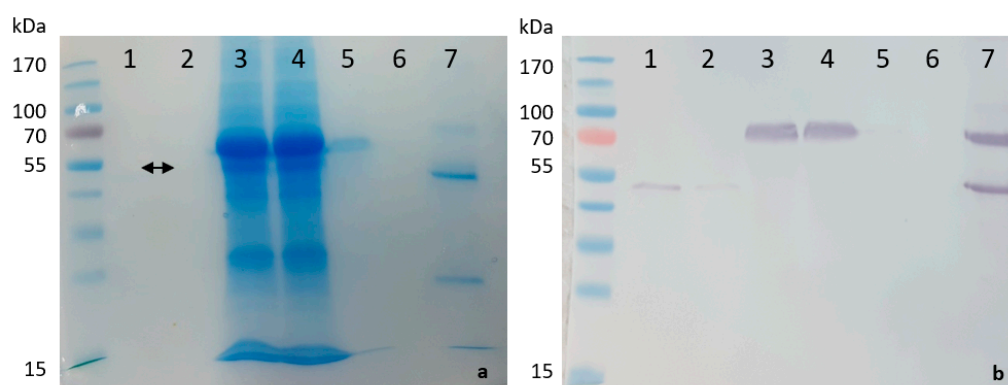
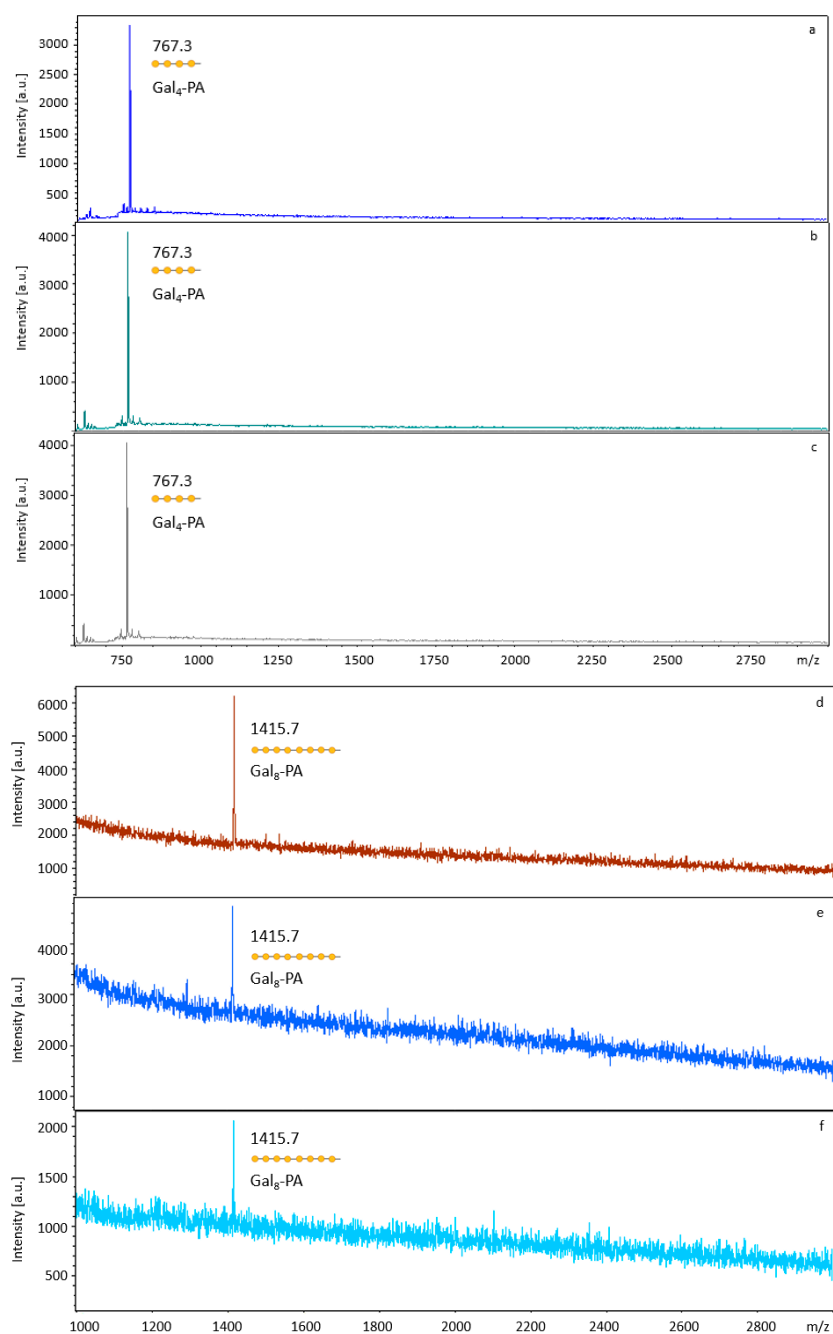


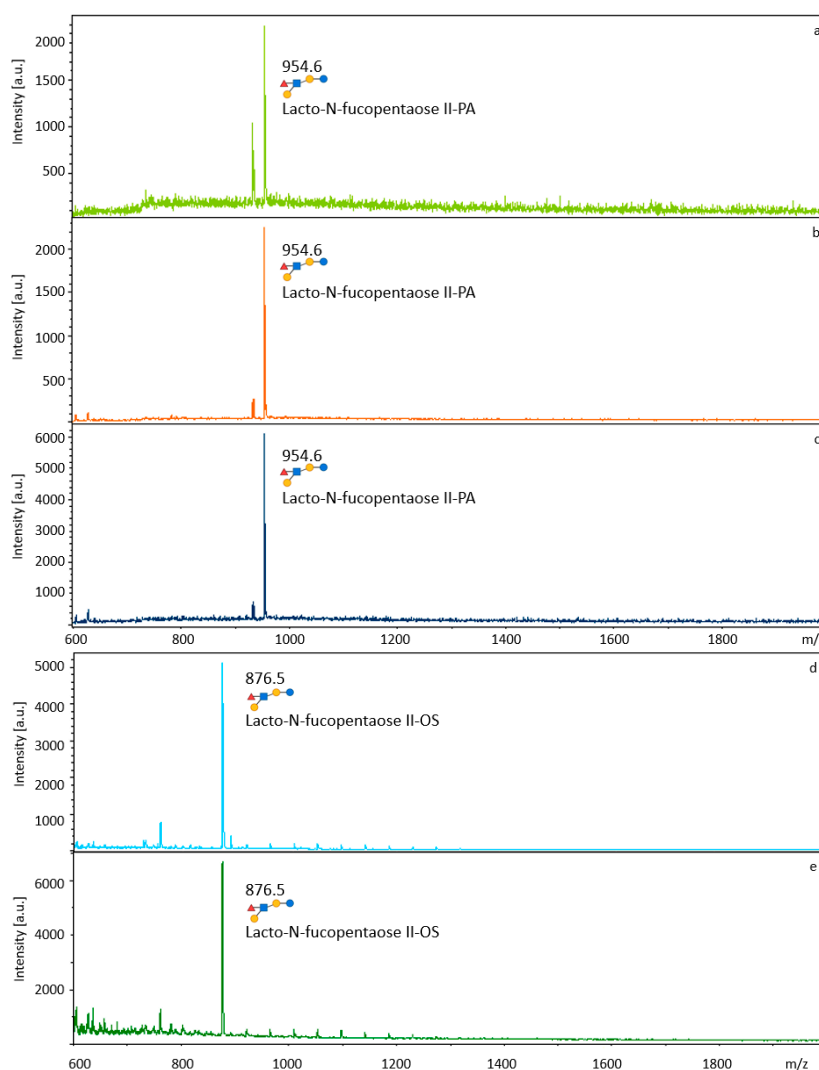
## Supplementary data



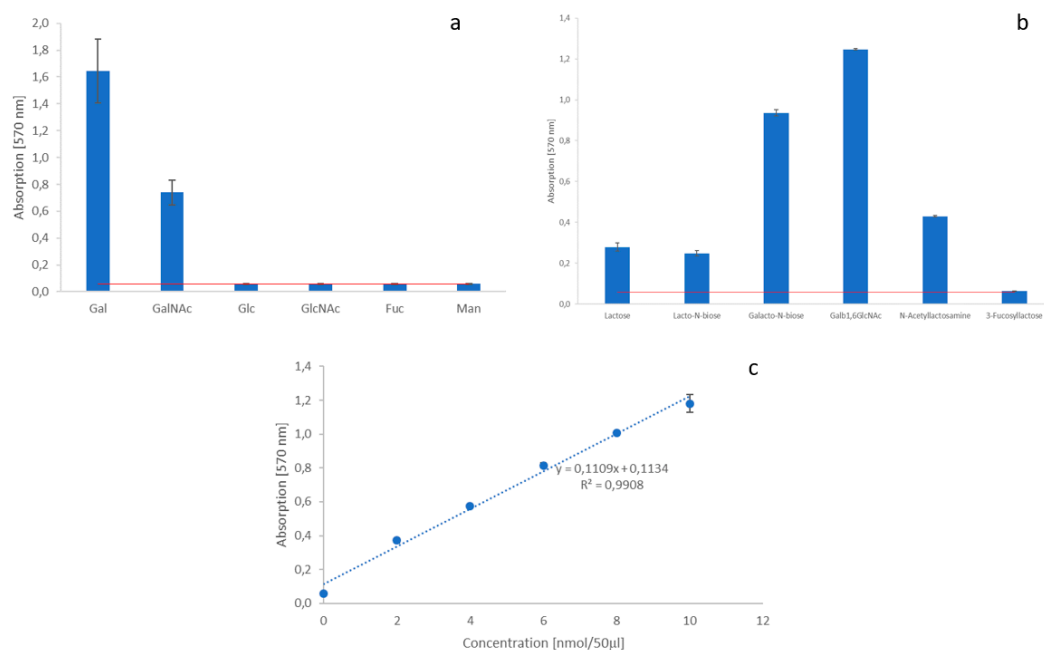
**Figure S1.** Purification procedure of  $\beta$ -galactosidase from *C. gigas* via immunoprecipitation. (a) SDS-PAGE Coomassie staining, (b) Western Blot using mouse anti-Penta-Histidine tag:HRP monoclonal antibody (1:2500, BIORAD – Vienna, Austria) followed by alkaline phosphatase conjugated anti-mouse IgG from goat (1:4000, Sigma-Aldrich, Vienna, Austria). (1) His-tag antibody prior to linkage to agarose beads, (2) remaining His-tag antibody in solution after linkage to agarose beads, (3) Sf9 lysate fraction containing recombinant  $\beta$ -galactosidase, (4) unbound proteins after incubation with agarose beads, (5) washing step 1, (6) washing step 2, (7) purified  $\beta$ -galactosidase (~78 kDa) and heavy chain of mouse anti-Penta-His-tag antibody (~55 kDa).



**Figure S2** MALDI-TOF analysis of  $\beta$ -galactosidase activity towards galactan chains. (a) Gal<sub>4</sub>-PA substrate, (b) Gal<sub>4</sub>-PA incubated with purified  $\beta$ -galactosidase from *C. gigas*, (c) Gal<sub>4</sub>-PA incubated with purified  $\beta$ -galactosidase from *A. vulgaris*, (d) Gal<sub>8</sub>-PA substrate, (e) Gal<sub>8</sub>-PA incubated with purified  $\beta$ -galactosidase from *C. gigas*, (f) Gal<sub>8</sub>-PA incubated with purified  $\beta$ -galactosidase from *A. vulgaris*. N-Glycan structures were created using bioRENDER



**Figure S3** MALDI-TOF analysis of  $\beta$ -galactosidase activity towards lacto-N-fucopentaose II. (a) Lacto-N-fucopentaose II-PA substrate, (b) lacto-N-fucopentaose II-PA incubated with purified  $\beta$ -galactosidase from *C. gigas*, (c) lacto-N-fucopentaose II-PA incubated with purified  $\beta$ -galactosidase from *A. vulgaris*, (d) lacto-N-fucopentaose II-OS substrate, (e) lacto-N-fucopentaose II-OS incubated with purified  $\beta$ -galactosidase from *C. gigas*. N-Glycan structures were created using bioRENDER



**Figure S4.** Background of the Galactose Assay Kit (ab83382; ABCAM – Cambridge, UK) with (a) different monosaccharides and (b) di- and trisaccharides without addition of  $\beta$ -galactosidase. Red line depicts a H<sub>2</sub>O control without any addition of sugars. (c) Represents the galactose standard calculation curve according to supplier's instructions.



**Figure S5.** Schematic structure of recombinant full-length  $\beta$ -galactosidase from *C. gigas* (NCBI Ref. Nr.: XP\_034310761.1). The recombinant protein consists of gene sequence (grey), N-terminal gp64 leader sequence (purple) for secretion out of Sf9 insect cells and a 6xHis-tag (green) for purification at the C-terminus. Short 3 amino-acid linkers were added to the gene at the N- and C-terminus to connect gp64 leader sequence and 6xHis-tag (not shown).