Protein Oxidation of Fucose Environments (POFE) Reveals Fucose-protein Interactions

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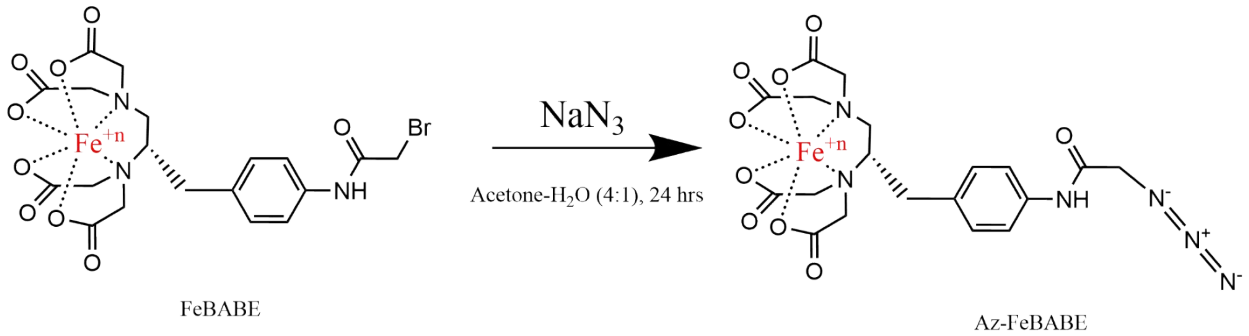
Methods

Cell culture. Human immortalized prostate epithelium PNT2 and human colon adenocarcinoma Caco-2 cells were obtained from American Type Culture Collection (ATCC, Manassas, VA). The PNT2 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium, and Caco-2 cells were grown in Eagle's Minimum Essential Medium (EMEM). The medium were supplemented with 10% (v/v) fetal bovine serum (FBS), 0.1% (v/v) penicillin and streptomycin, and 1% (v/v) GlutaMAX. Cells were maintained in a humidified incubator at 37°C with 5% CO_2 and subcultured at 80% confluency. For the fucose reporter treatment, cells were treated with 100 μM of 6AlkFuc, 6AzFuc, or 7AlkFuc for 72 hours.

Confocal image analysis of fucose-labeled glycoproteins. The cells were seeded onto FluoroDish cell culture dishes (WPI, FL) coated with Poly-D-Lysine with appropriate density. At 40% confluency, cells were treated with 100 μM of fucose reporters supplemented growing media for 72 hours. Afterward, the treated cells were rinsed with phosphate-buffered saline (PBS) and treated with 50 μM of coumarin azide (for labeling alkynyl groups) or DBCO-Cy3 (for labeling azido groups) in PBS at room temperature for 1 hour, followed by the fixation with 4% paraformaldehyde at room temperature for 1 hour. The plasma membrane and the nucleus were stained with CellMask Deep Red plasma membrane stain and Hoechst 33342, respectively, at 37°C for 10 minutes. Fluorescent images were captured by Leica TCS SP8 STED 3X Super-Resolution Confocal Microscope (Wetzlar, Germany).



FeBABE

Az-FeBABE

Schematic 1. Synthesis of the Az-FeBABE probe.

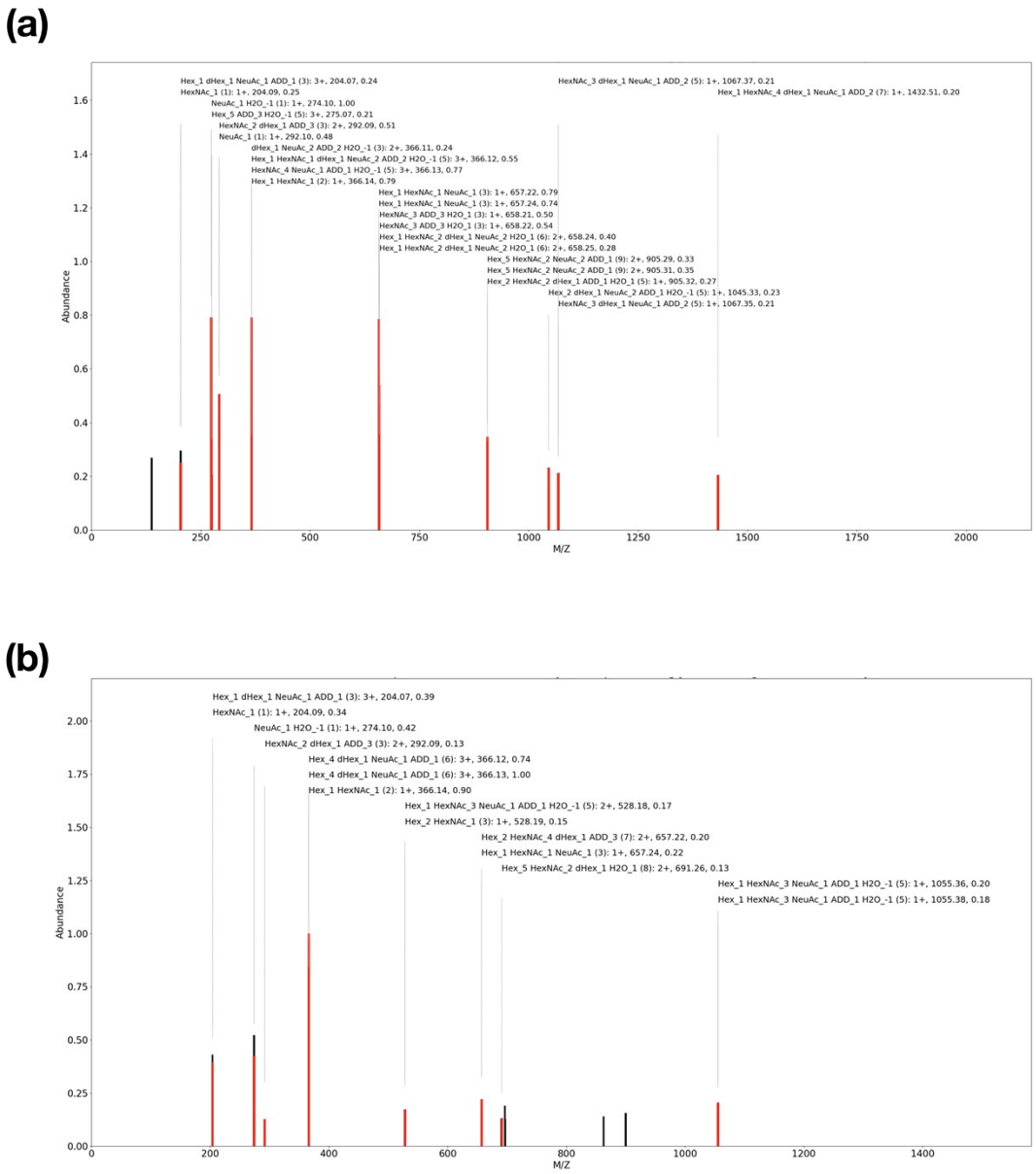


Figure S1. Tandem mass spectra of selected compounds (a) $Hex_{(5)}HexNAc_{(4)}7AlkFuc_{(1)}Sia_{(2)}$ and (b) $Hex_{(5)}HexNAc_{(4)}7AlkFuc_{(1)}Sia_{(1)}$. The glycan structures and fragments were annotated using GlycoNote.

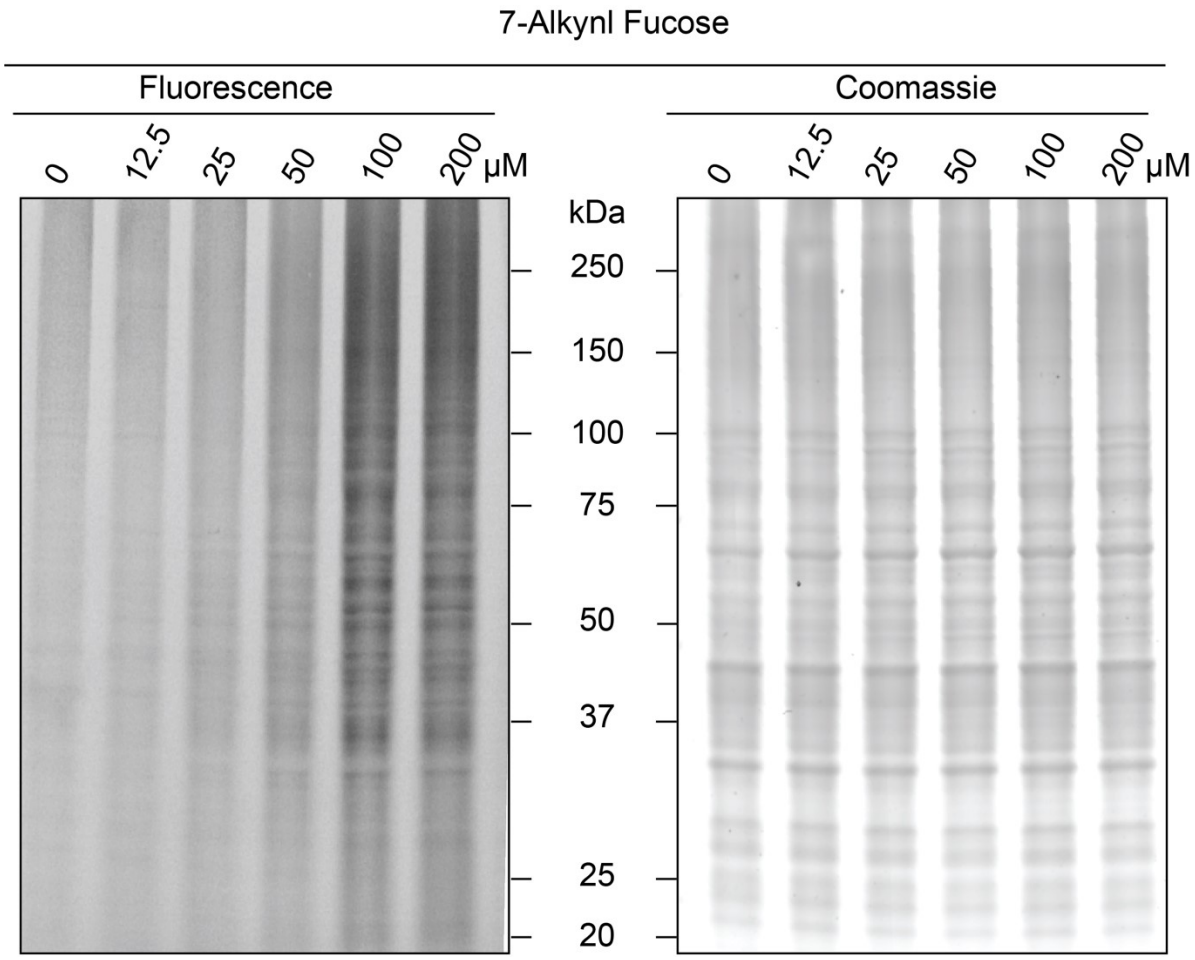


Figure S2. Dose-dependent gel-based profiles of PNT2 cells treated by 7AlkFuc. The cells were incubated for varying amounts of probe (0, 12.5, 25, 50, 100, and 200 μM). Corresponding expression profiles are shown after Coomassie staining. Concentrations and molecular weight markers are indicated.

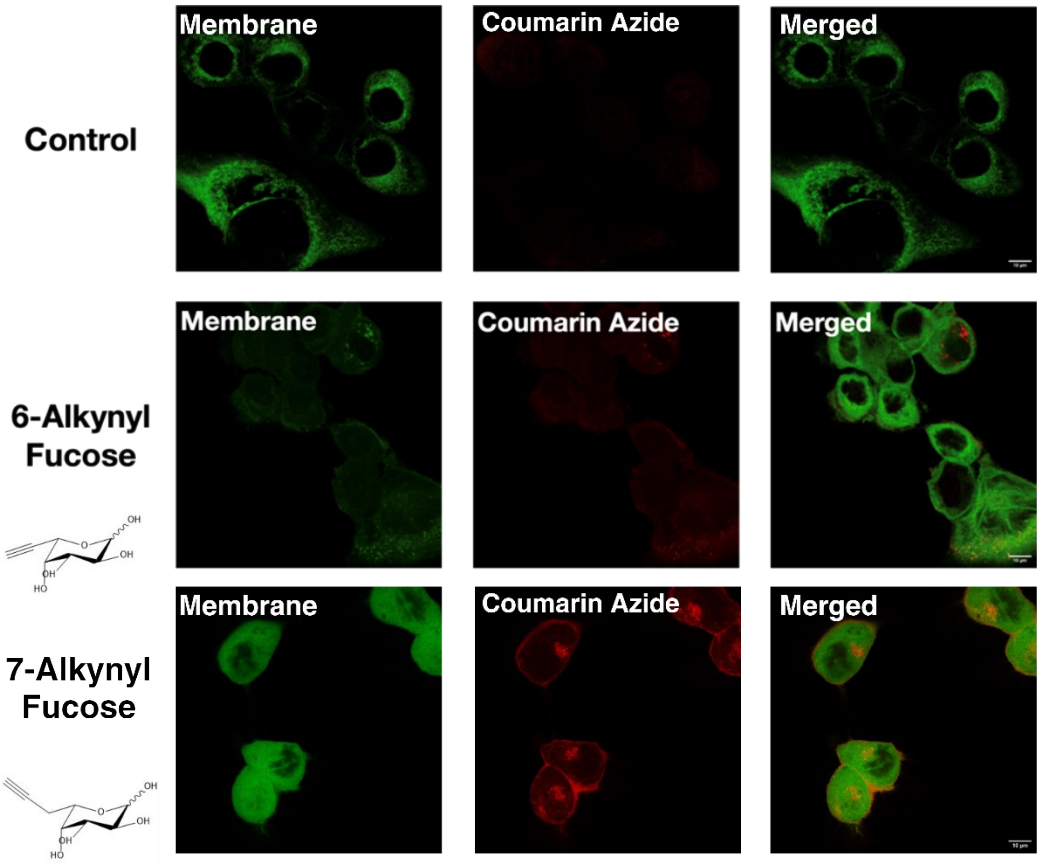


Figure S3. Validation of the labeling of the fucose probes using confocal microscopy. PNT2 Cells were treated with DMSO (**top**), 6AlkFuc (**middle**), and 7AlkFuc (**bottom**). The cell membrane was stained with CellMask™ Deep Red Plasma membrane Stain.

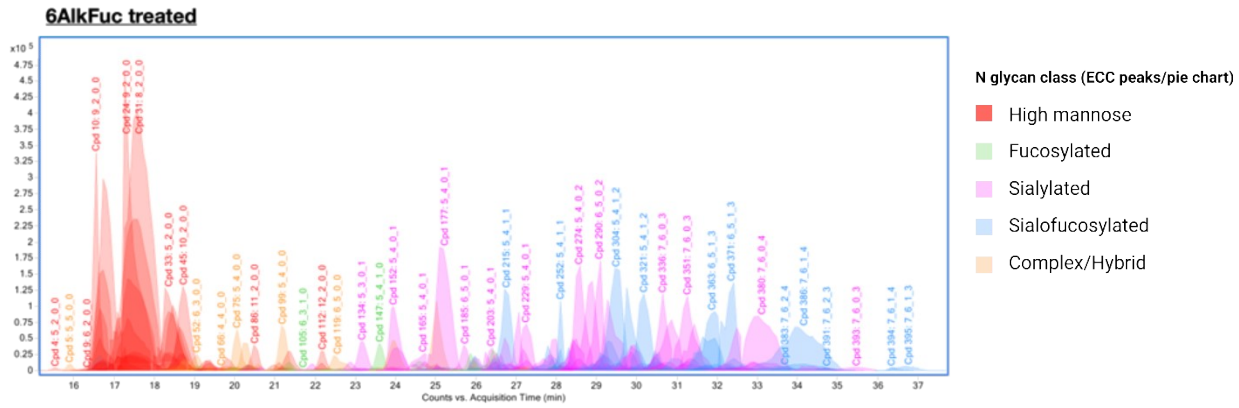


Figure S4. LC-MS profile of N-Glycans released from 6AlkFuc-treated PNT2 cells. Annotated structures are putative based on mass and composition. LC-MS peaks were color coded to assign glycan subtypes.

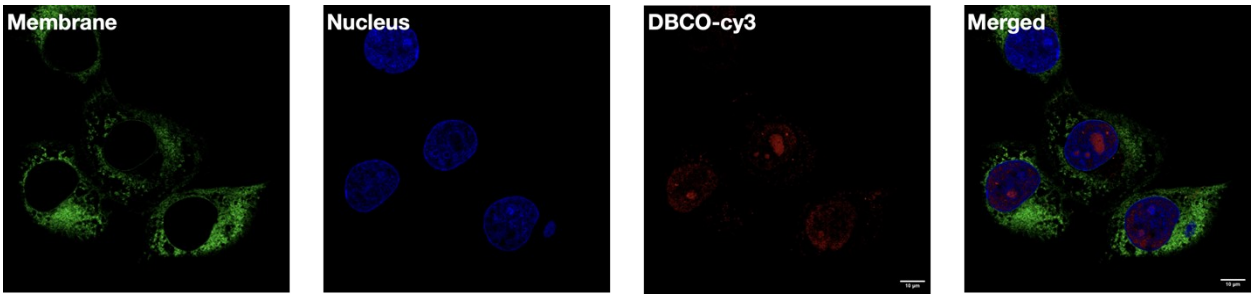


Figure S5. Validation of the labeling of the 6AzFuc probe using confocal microscopy. The incorporated azido group was labeled using DBCO-cy3. And the cell membrane and nucleus were stained with CellMask™ Deep Red Plasma membrane Stain and Hoechst 33342, respectively.

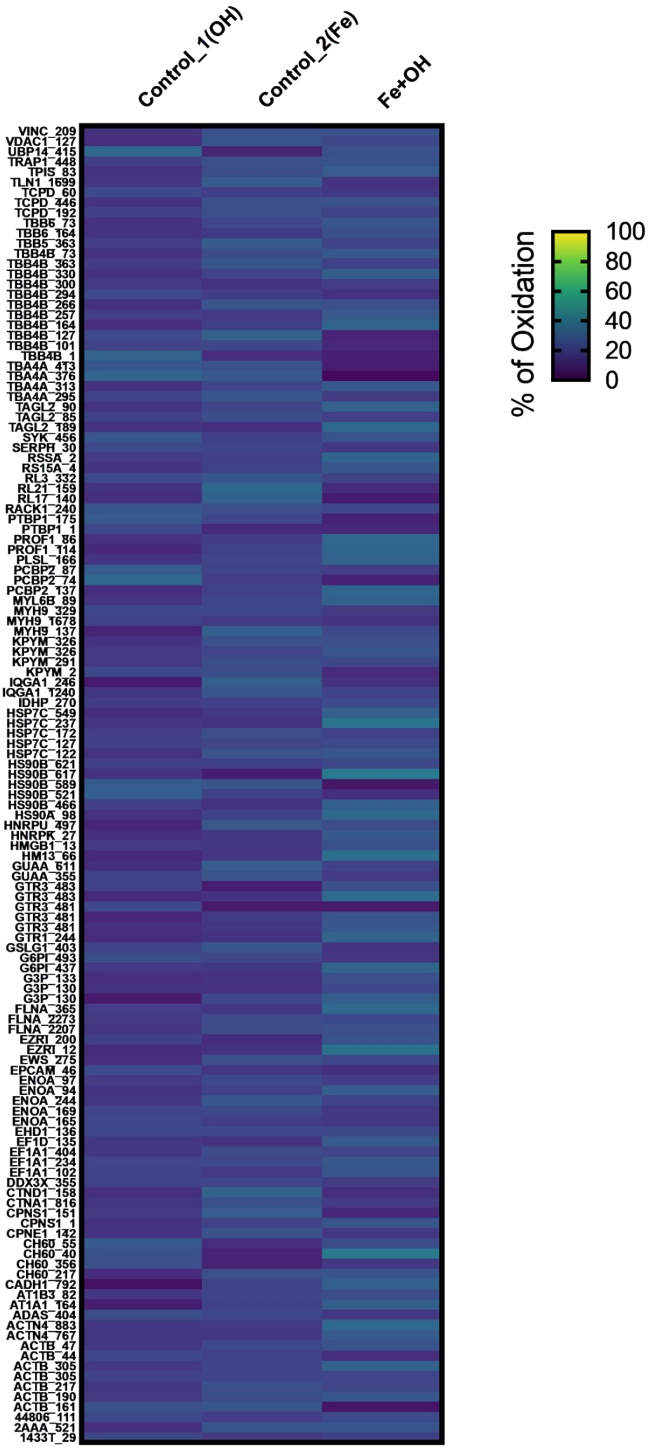


Figure S6. The extent of background oxidation quantified by Byologic. All experiments (including controls) were conducted in copper-environments to account for background oxidation by copper.

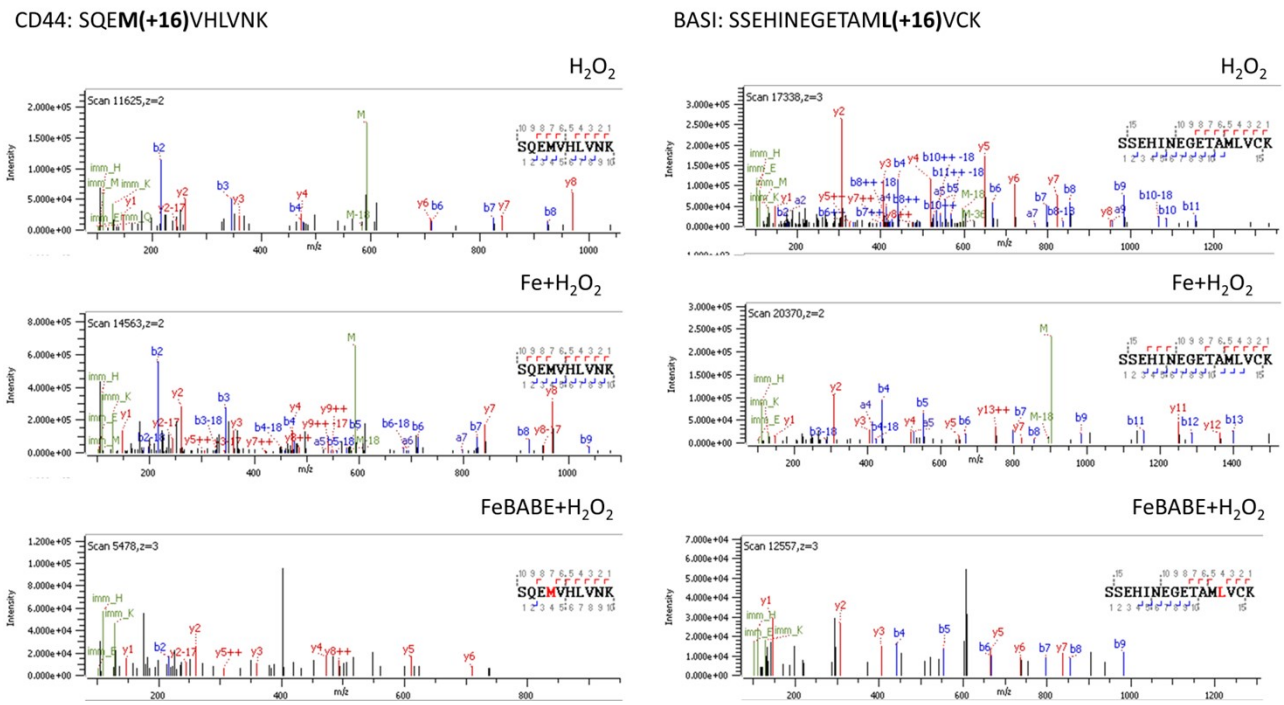


Figure S7. Annotated spectra of CD44 and BASI proteins in the control, H_2O_2, and FeBABE-H_2O_2 treatments.

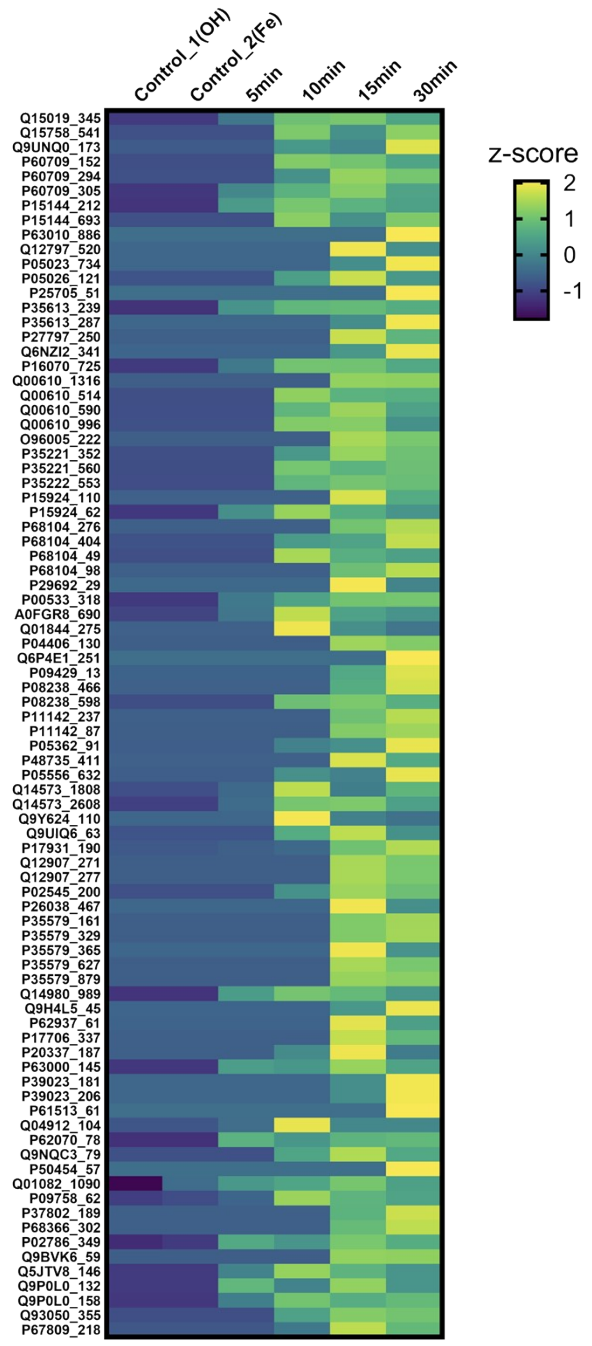
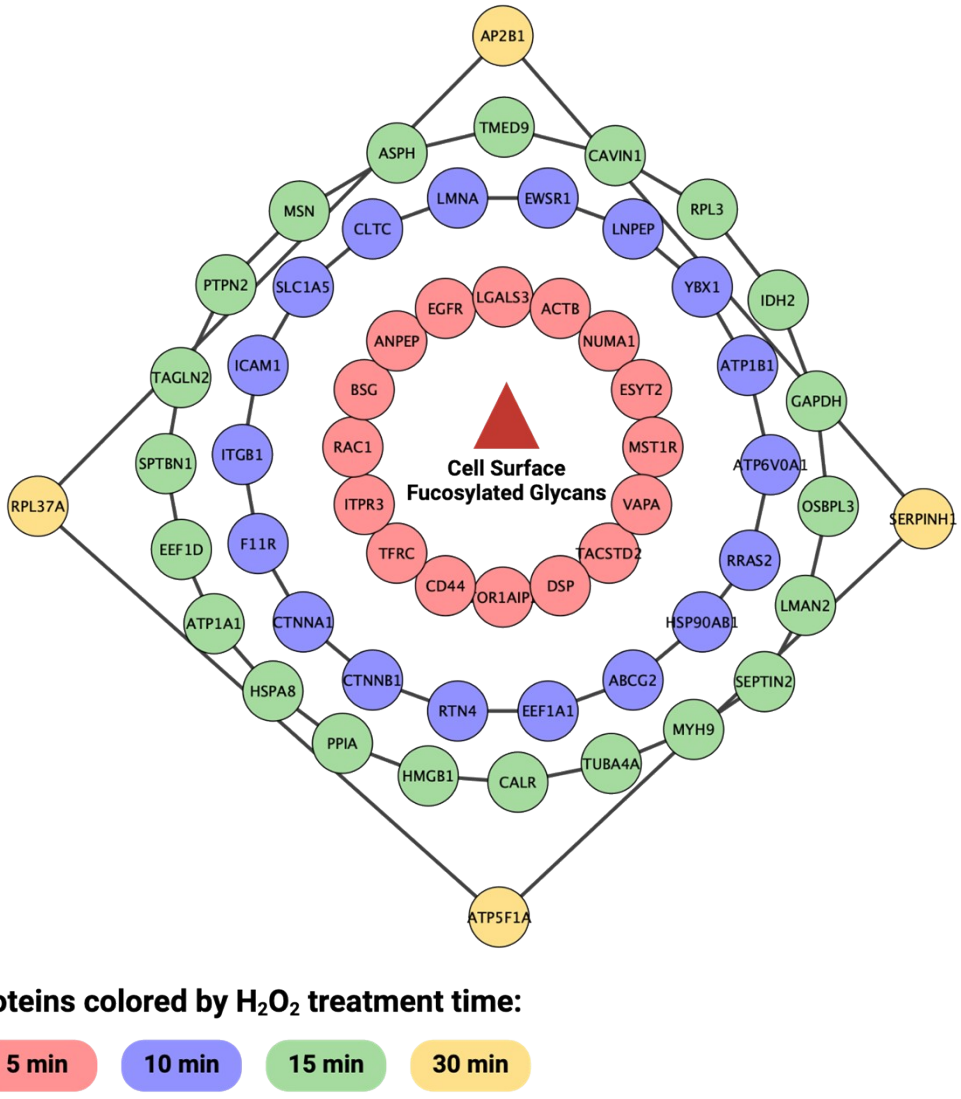


Figure S8. The extent of oxidation quantified by Byologic for selected proteins oxidized in PNT2 cells. Each column represents one treatment condition, and each row represents one oxidation site of a selected protein.



Proteins colored by H_2O_2 treatment time:

5 min | 10 min | 15 min | 30 min

Figure S9. Proteins from PNT2 within the spatial environment of cell-surface fucose identified by POFE. The spatial information was revealed by different H_2O_2 treatment time. Proteins with different spatial distances (reported as unit of time) relative to the fucose were labeled with different colors. Colors correspond to incubation time: 5 minutes (red), 10 minutes (blue), 15 minutes (green), and 30 minutes (orange).

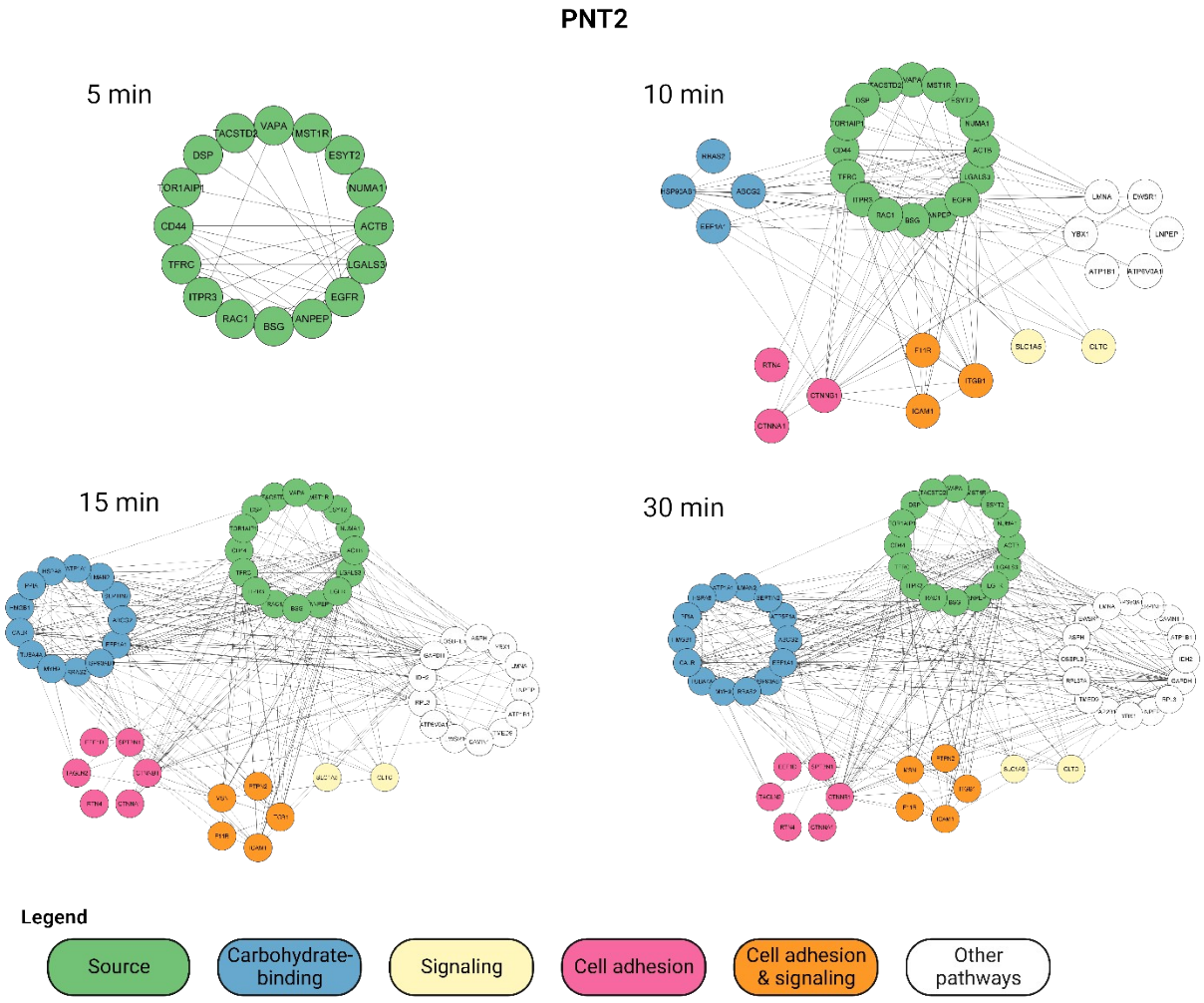


Figure S10. Interaction networks of PNT2. Proteins are categorized and color-coded into different biological pathways based on being the Source protein (green) or as Molecular Functions: Carbohydrate binding (blue), Signaling (yellow), Cell adhesion (pink), Cell adhesion and signaling (orange), and Other pathways (white).

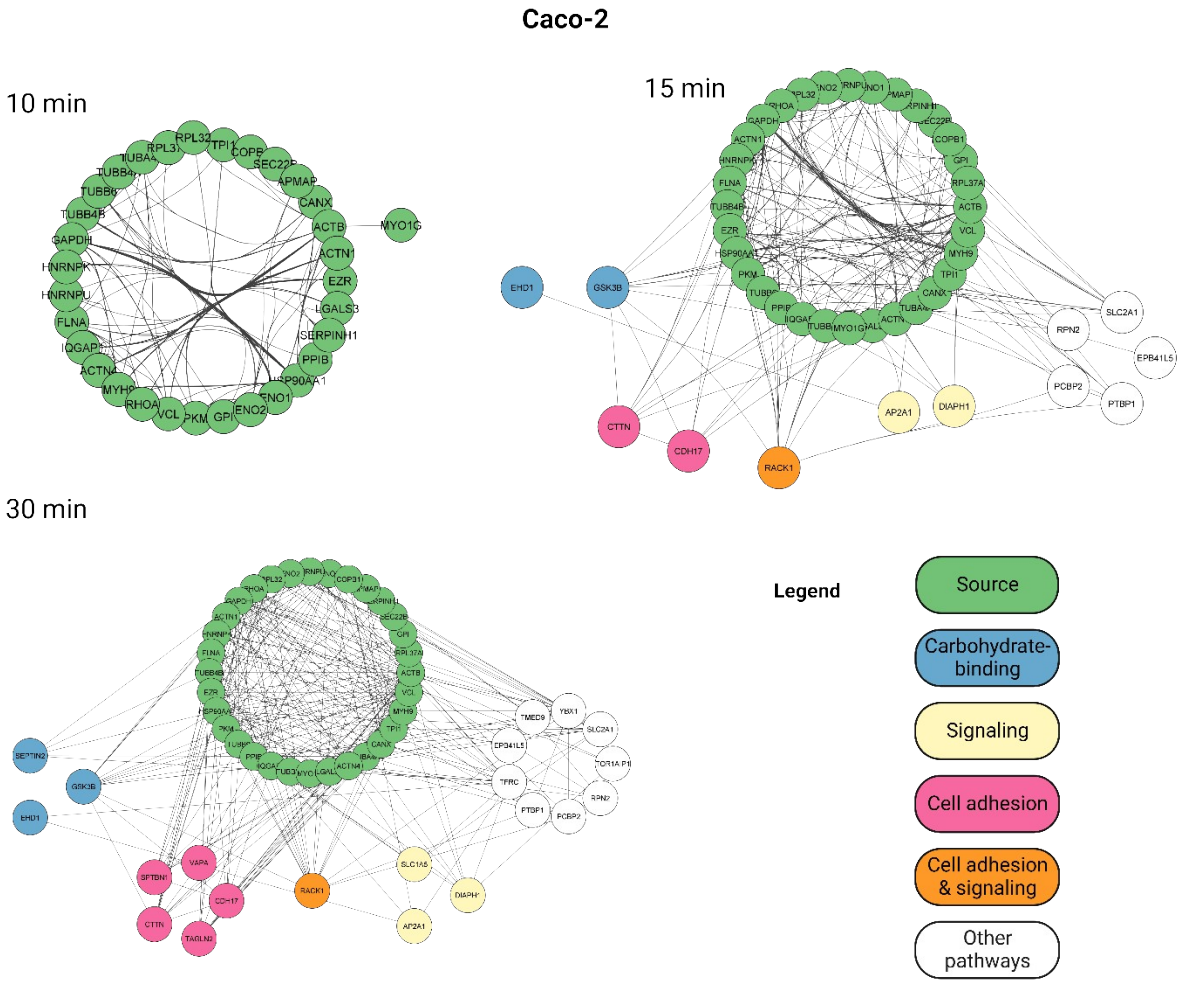


Figure S11. Interaction networks of Caco-2. Proteins are categorized and color-coded into different biological pathways based on being the Source protein (green) or as Molecular Functions: Carbohydrate binding (blue), Signaling (yellow), Cell adhesion (pink), Cell adhesion and signaling (orange), and Other pathways (white).

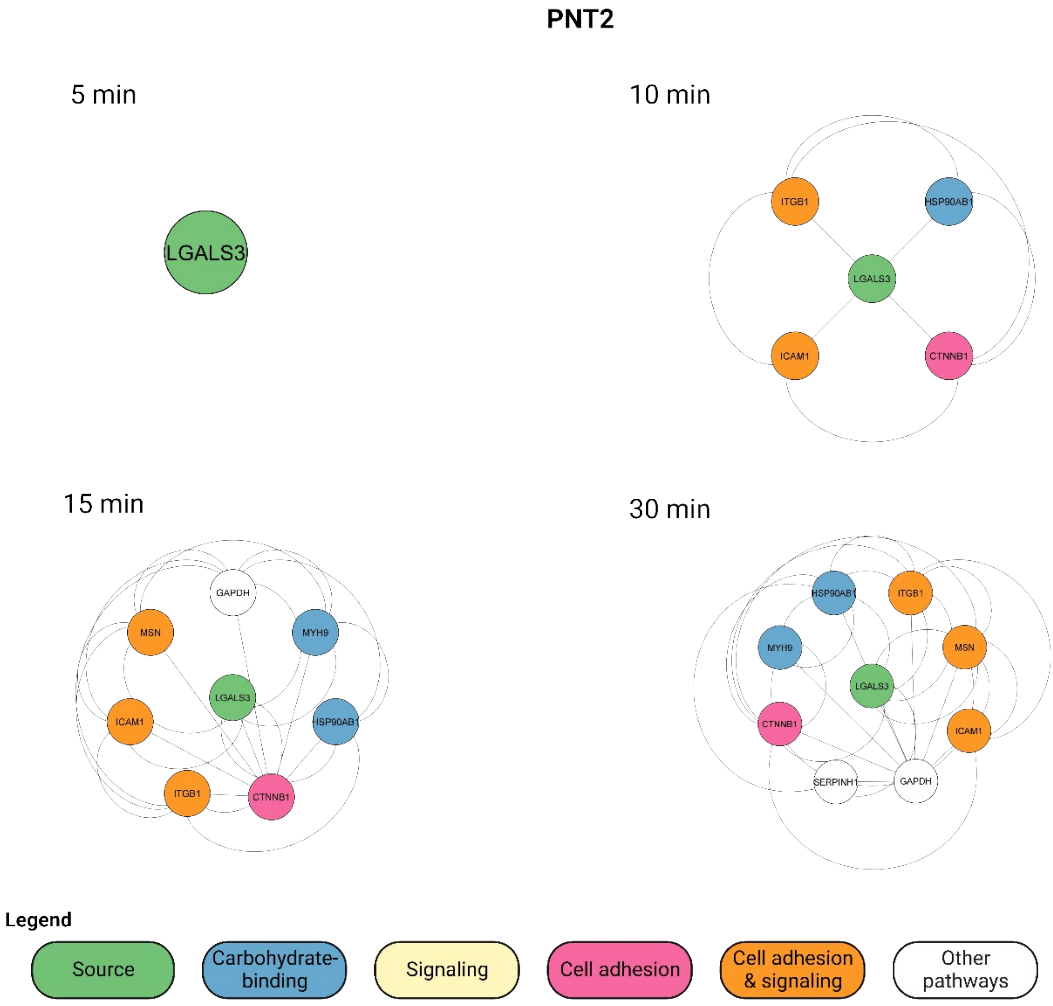


Figure S12. Interaction networks of LGALS3 in PNT2. Proteins are categorized and color-coded into different biological pathways based on being the Source protein (green) or as Molecular Functions: Carbohydrate binding (blue), Signaling (yellow), Cell adhesion (pink), Cell adhesion and signaling (orange), and Other pathways (white).

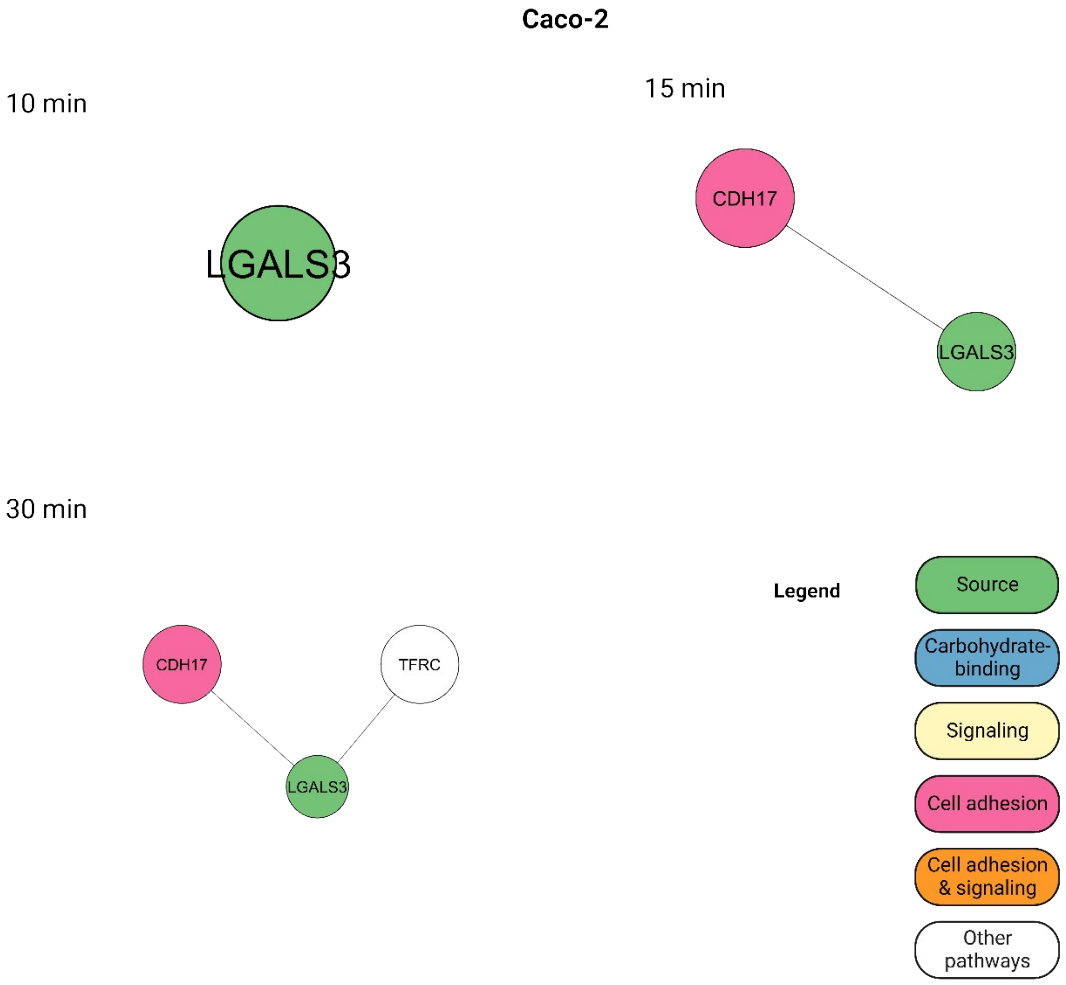


Figure S13. Interaction networks of LGALS3 in Caco-2. Proteins are categorized and color-coded into different biological pathways based on being the Source protein (green) or as Molecular Functions: Carbohydrate binding (blue), Signaling (yellow), Cell adhesion (pink), Cell adhesion and signaling (orange), and Other pathways (white).

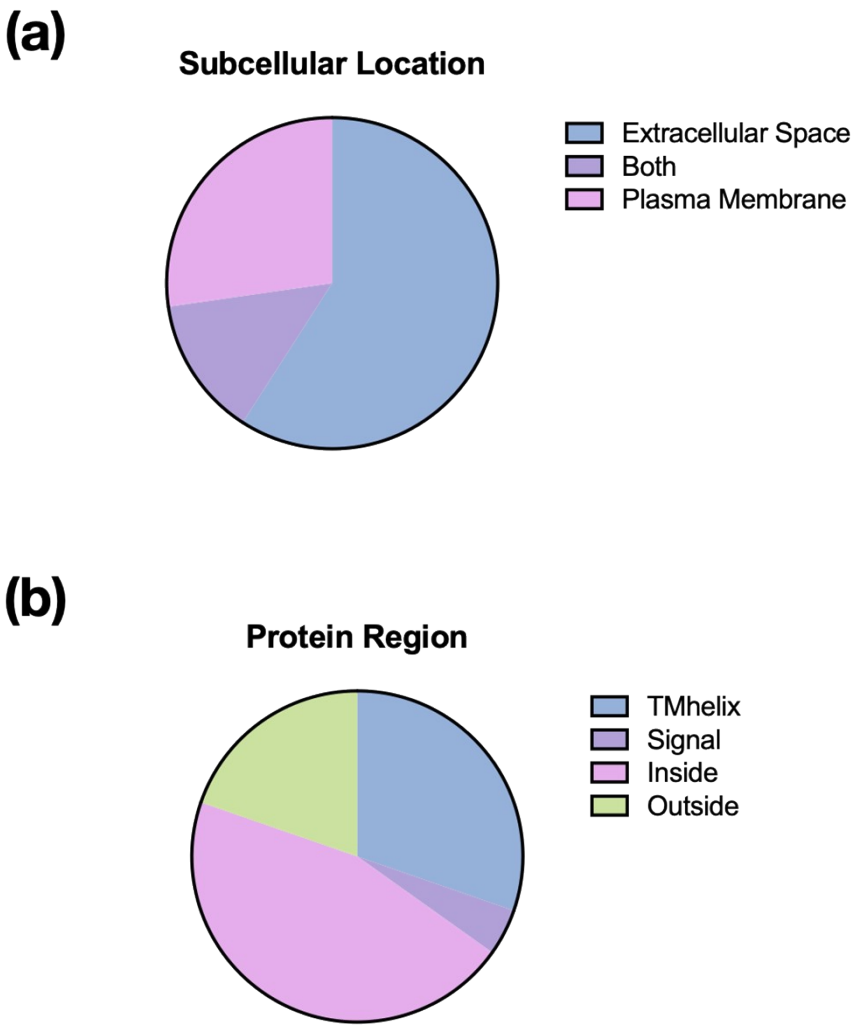


Figure S14. Annotated Location of identified proteins from PNT2. (a) The annotated subcellular location of oxidized proteins (level 3) by Gene Ontology (GO). **(b)** The predicted protein region distribution of oxidized proteins (level 3) by DeepTMHMM.

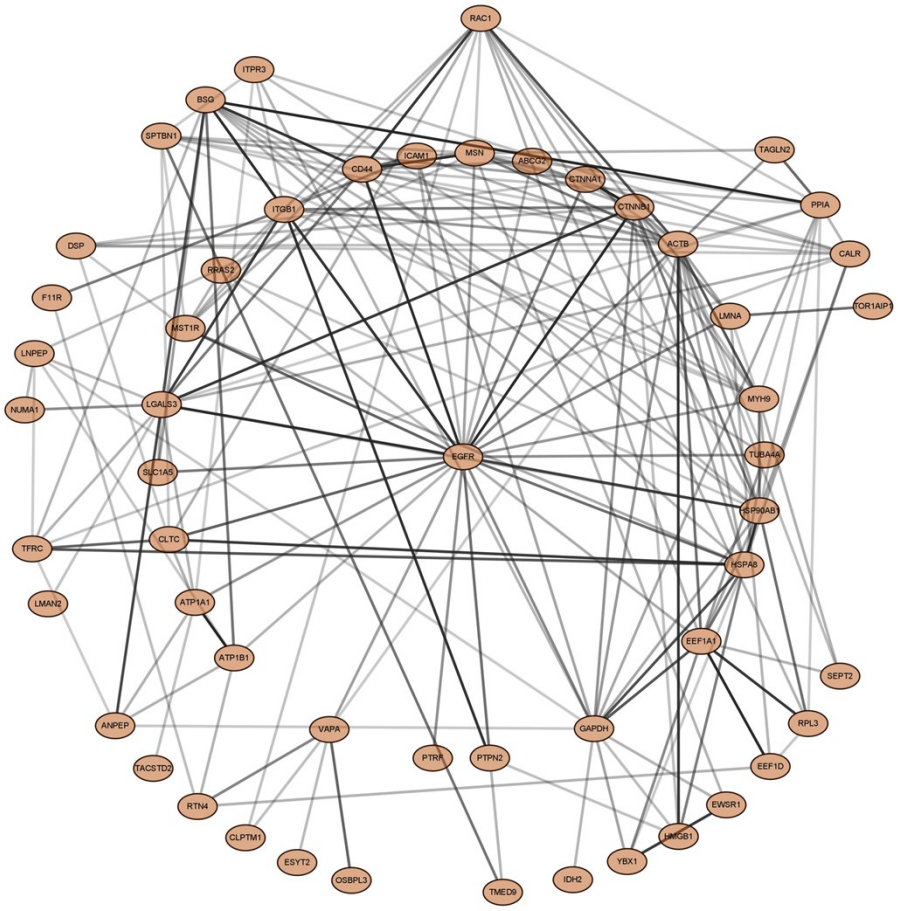


Figure S15. Interaction network of the identified proteins in proximity to the cell-surface fucose. All the interactions were identified by STRING and visualized by Cytoscape.

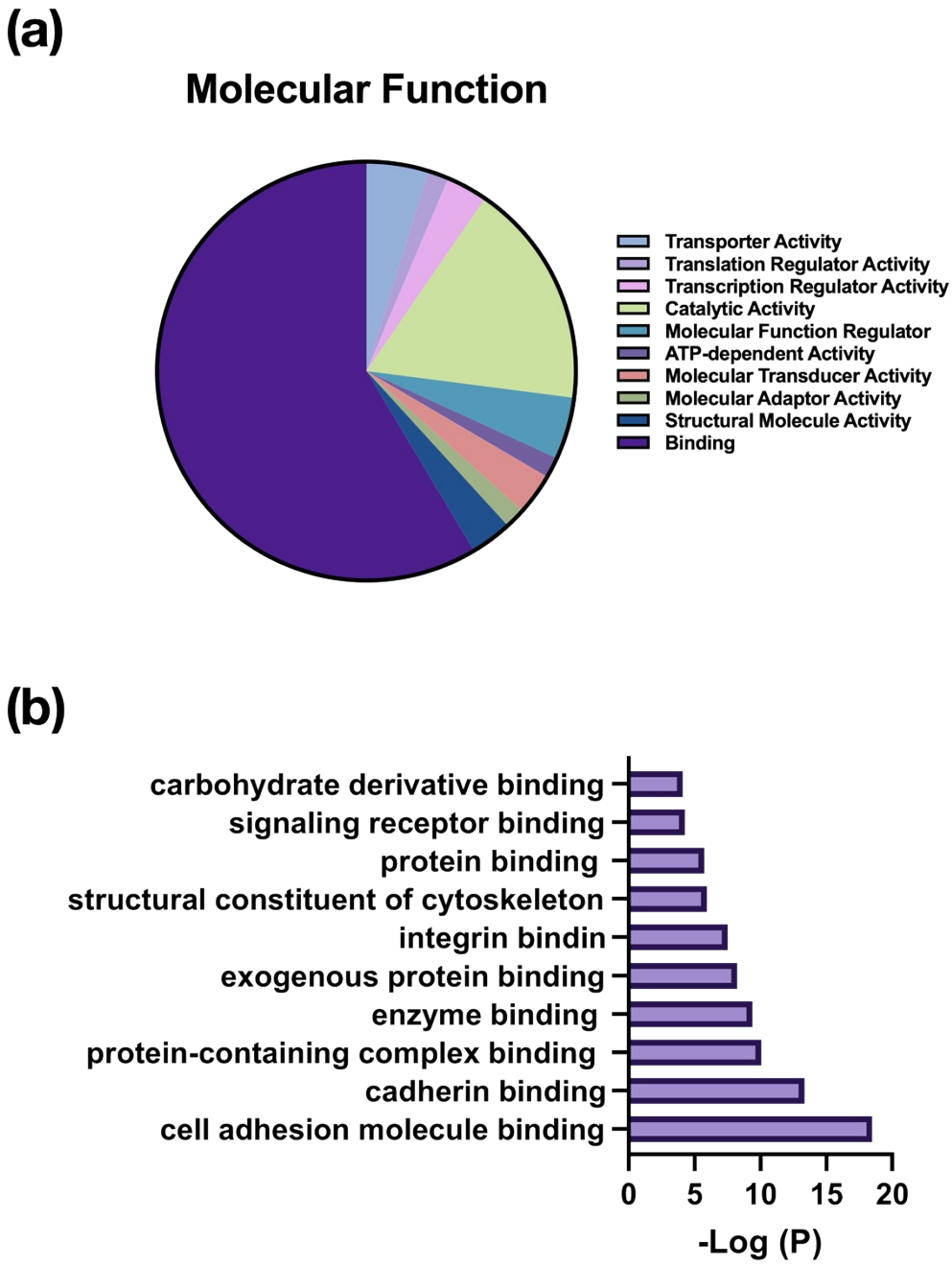
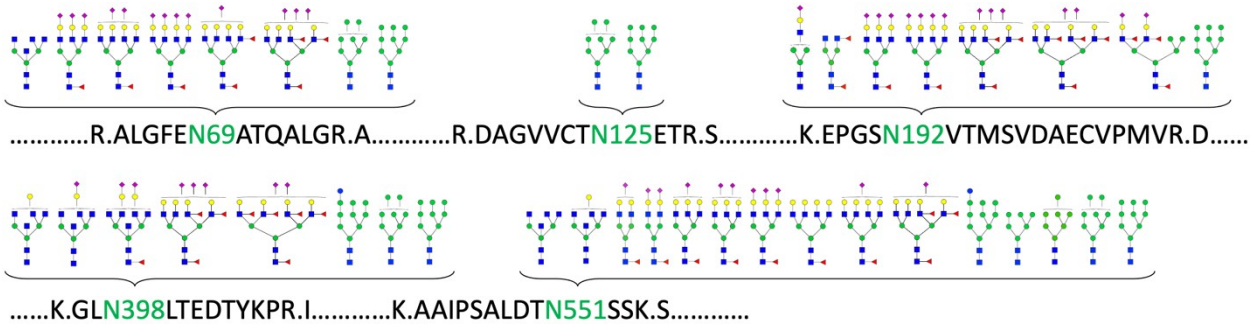


Figure S16. Functional annotation of identified proteins from PNT2 by POFE. (a) Molecular functions of the identified proteins. **(b)** Binding function of the identified proteins.



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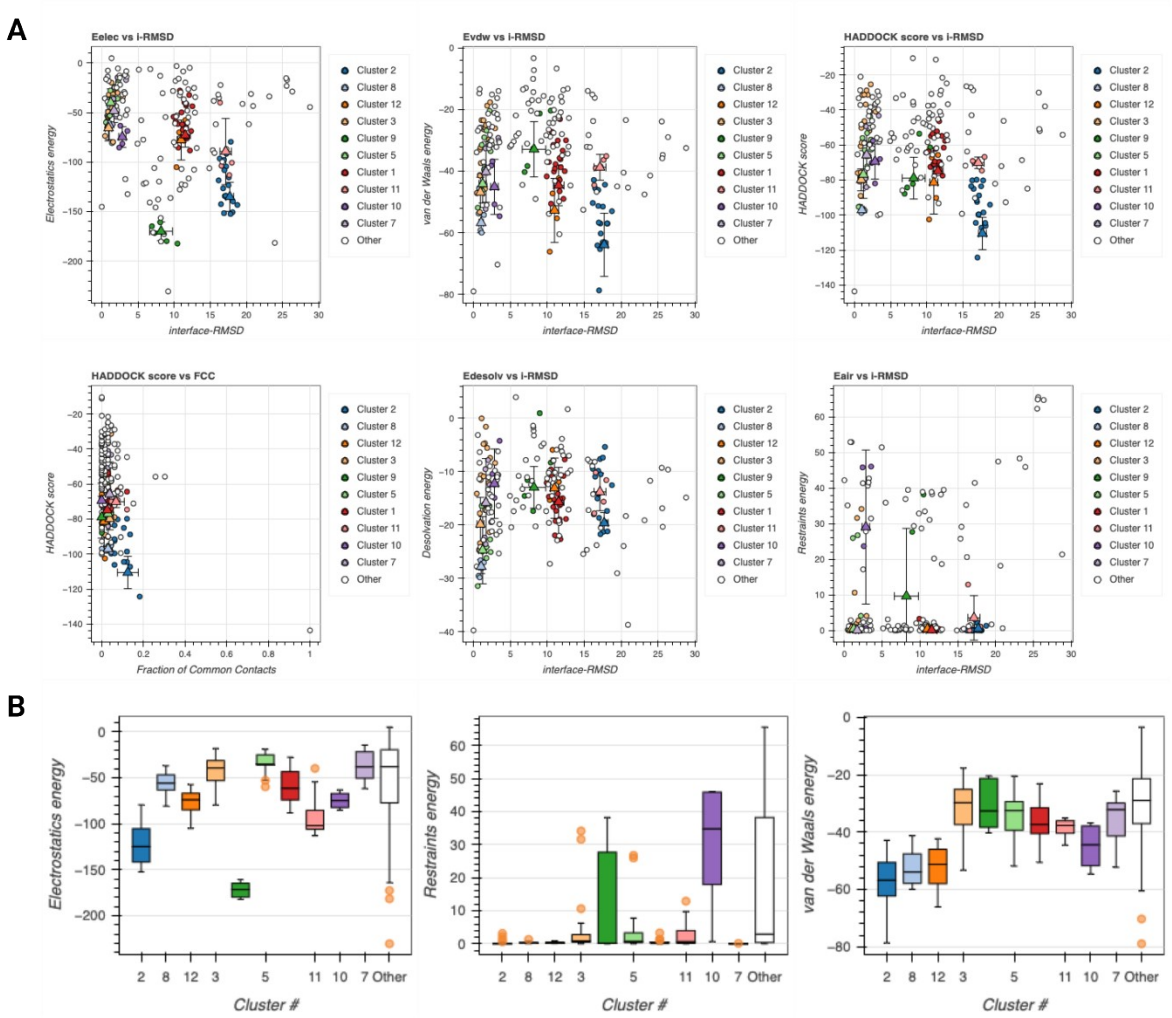

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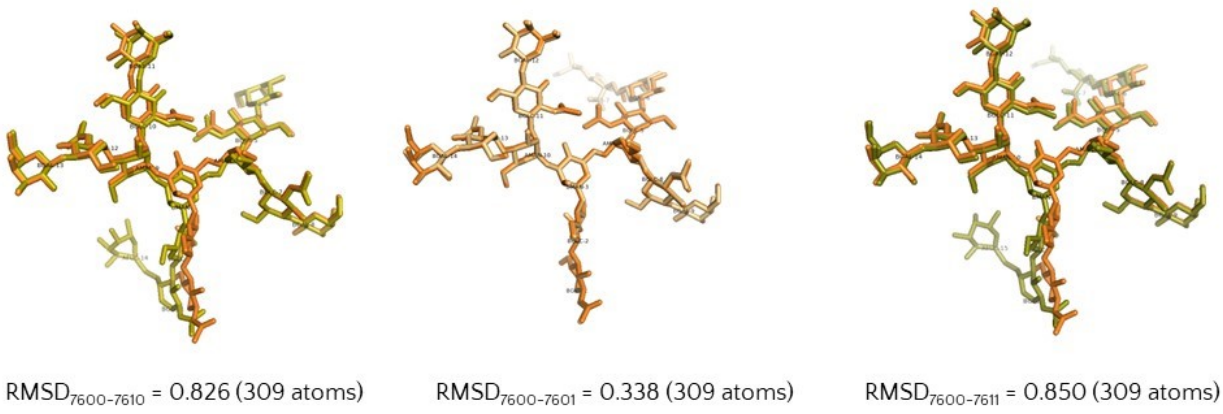

Figure S19. Modeling of glycan structures with or without the addition fucose. The conformation of fucosylated N-glycan structures significantly changed compared to the undecorated N-glycan.

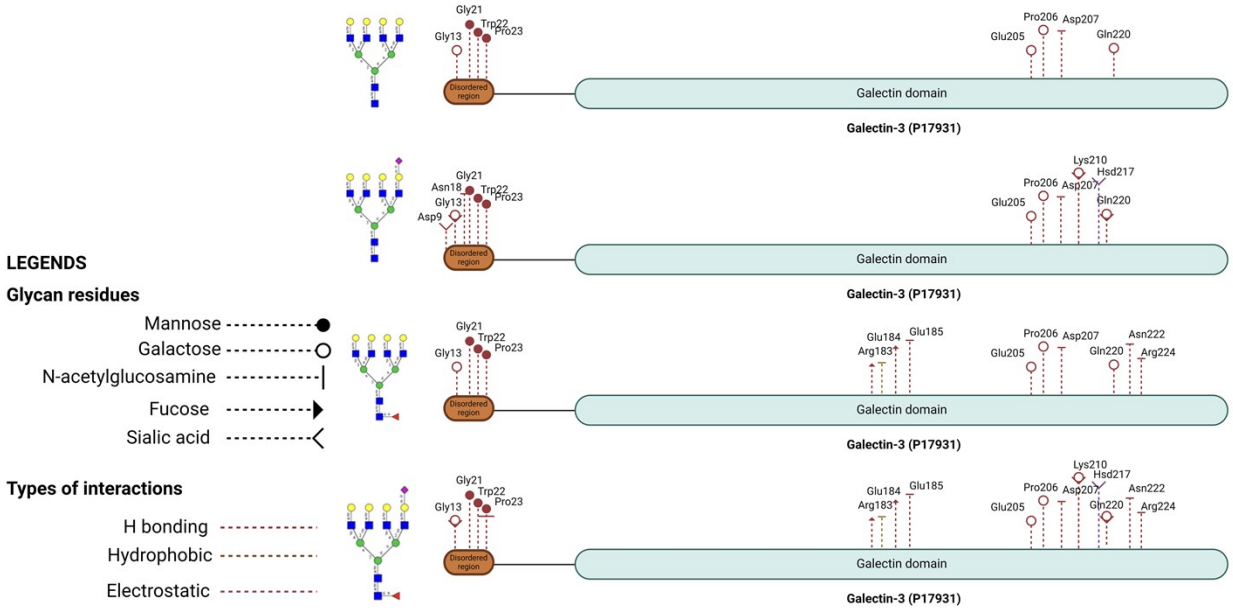

Figure S20. Summary of the glycan-protein interaction maps between LEG3 and LG3BP-Asn511 N-glycans. The domain information for LEG3 was obtained from Uniprot. The N-glycans were found to interact primarily with the Galectin domain of LEG3.