Supplementary Data

Enterocyte glycosylation is responsive to changes in extracellular conditions: Implications for membrane functions

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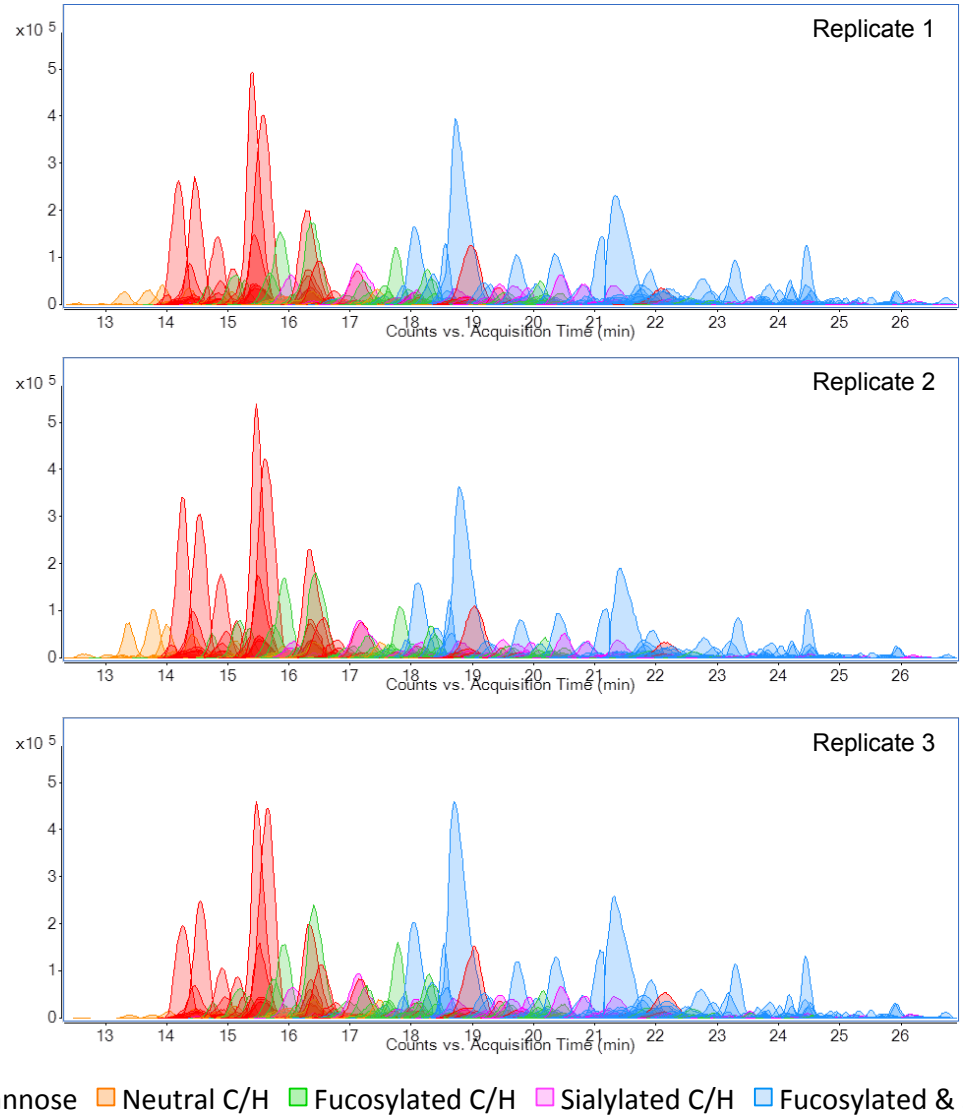


Figure S1. Stacked chromatograms of three biological replicates of identified Caco-2 cell surface N-glycans treated with high galactose (25 mM). Each peak represents a glycan compound and is colored according to type as indicated by the legend where C = Complex, H = Hybrid. Separation was performed using PGC-LC.

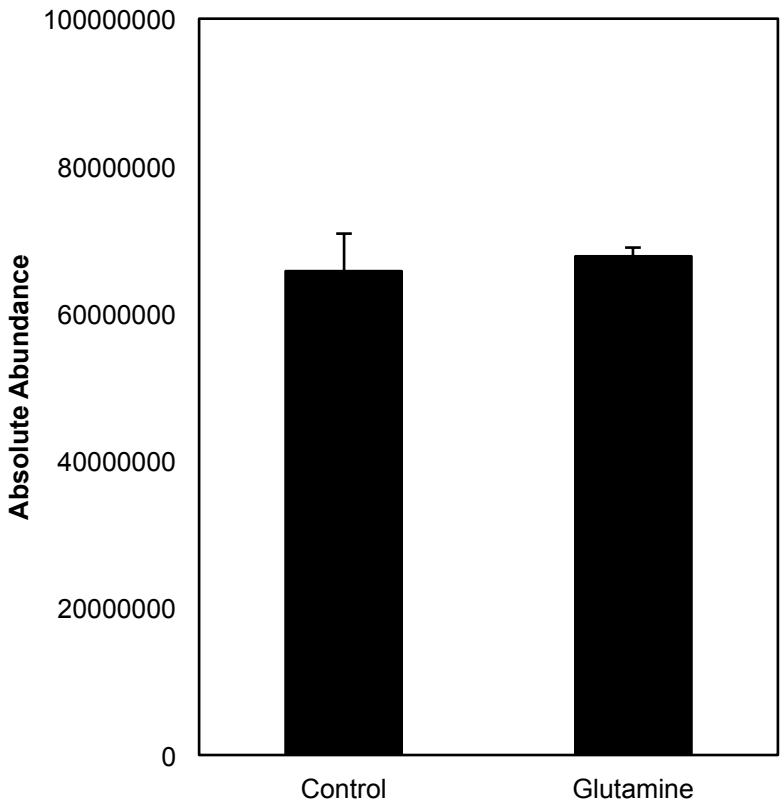


Figure S2. Summed absolute abundances of all sialylated structures profiled on Caco-2 cells without and with glutamine supplementation (n = 3).

◆ Precursor *m/z* = 960.698 (z=3)

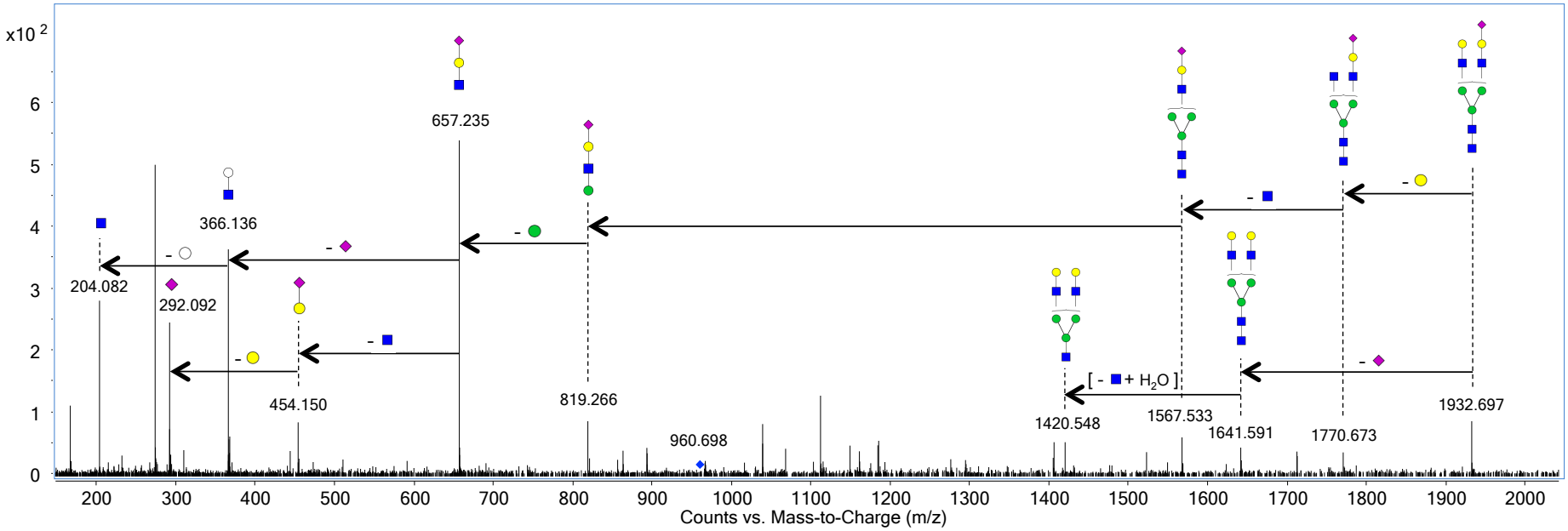
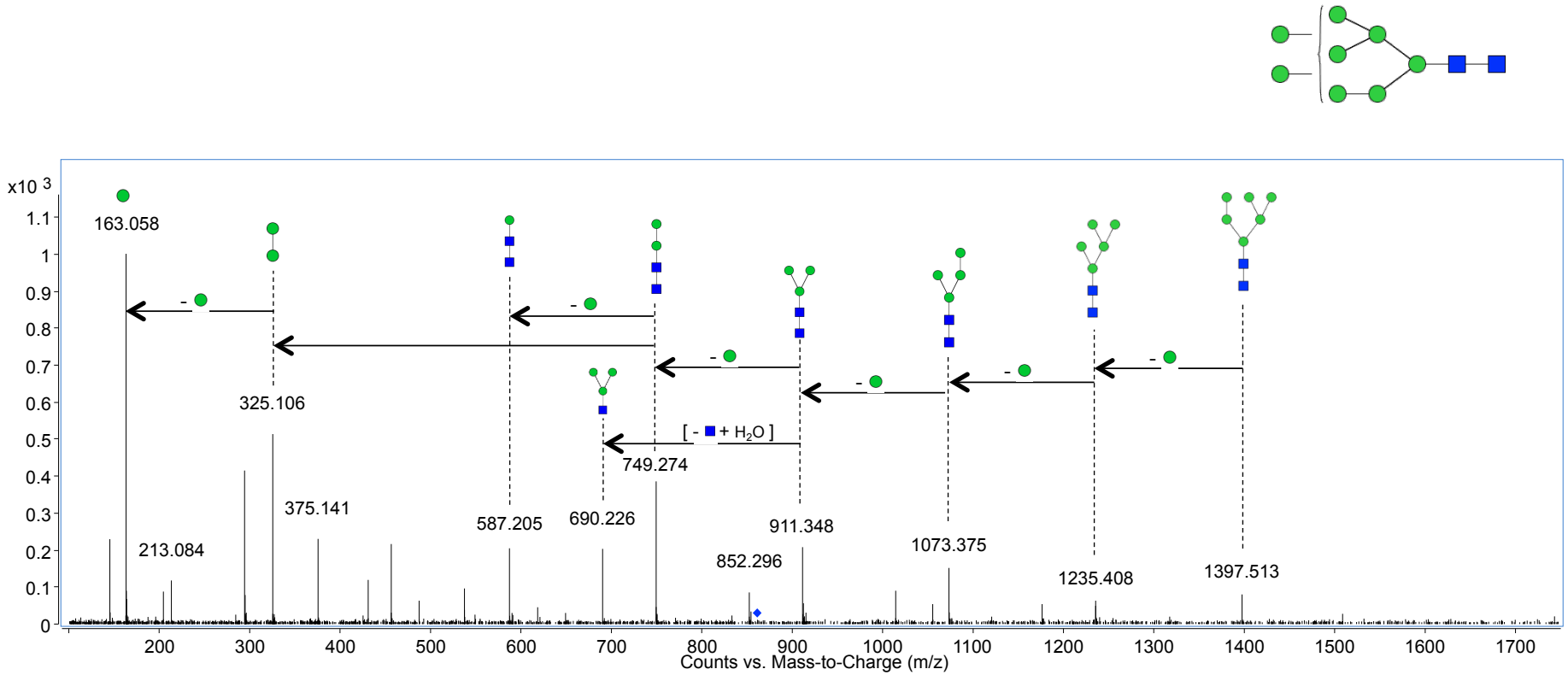


Figure S3. CID fragmentation spectra of N-glycan structures derived from Caco-2 as specified by *m/z* and symbolic representation. Sequential losses are annotated inset. Open symbols are used to indicate fragments with more than one possible position (*e.g.*, chitobiose/antennae) and/or composition (*e.g.*, Man/Gal). Monosaccharide symbols follow the SNFG (Symbol Nomenclature for Glycans) system details at NCBI (Varki, A., Cummings, R.D., et al. 2015).

◆ Precursor *m/z* = 861.306 (z=2)



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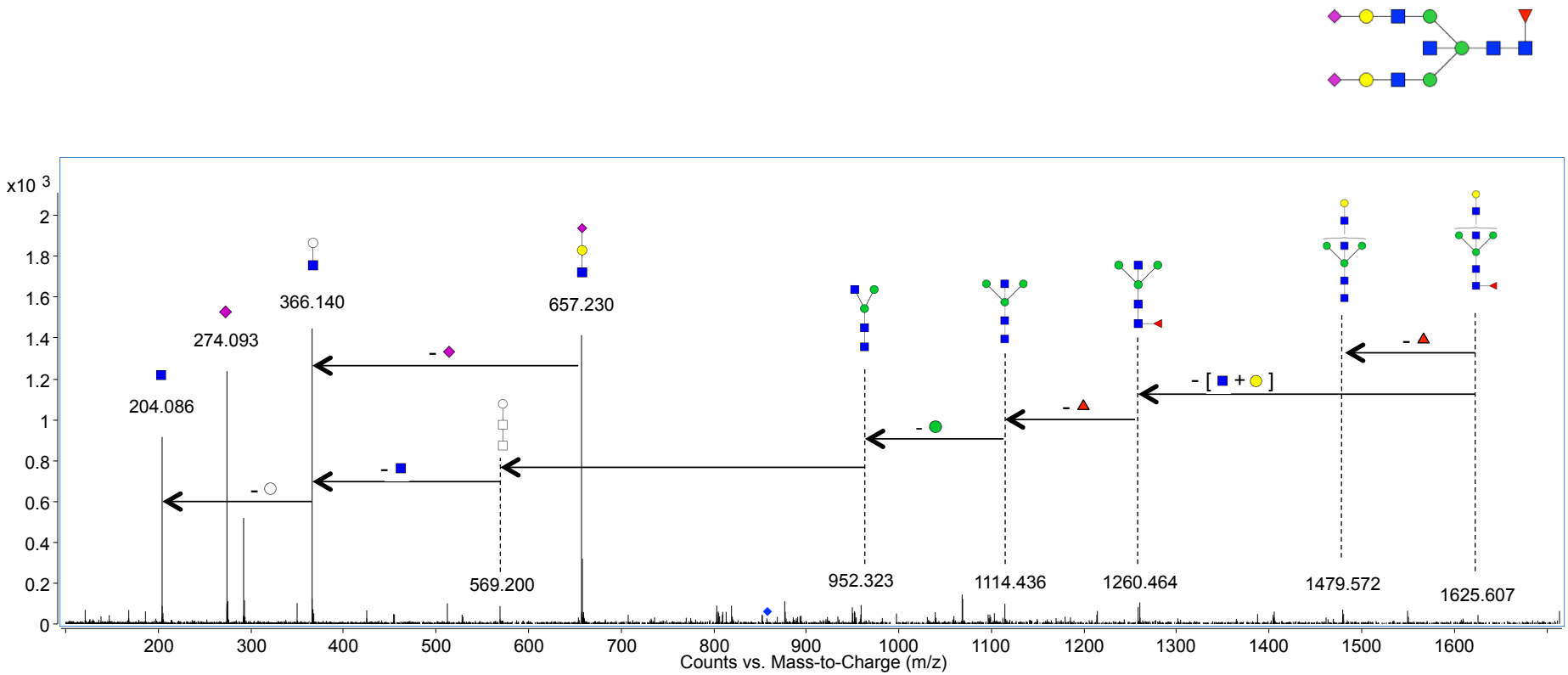
◆ Precursor *m/z* = 858.315 (z=3)



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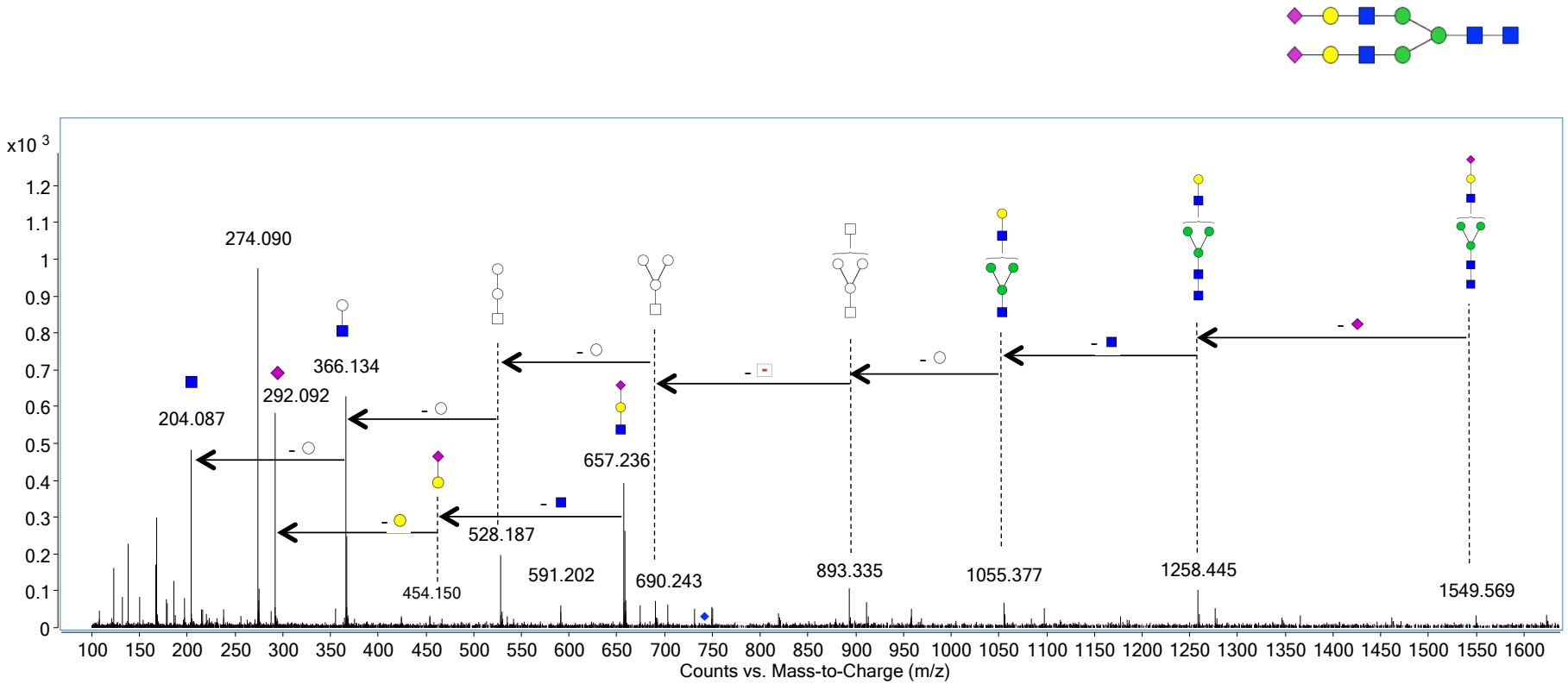
◆ Precursor *m/z* = 741.935 (z=3)



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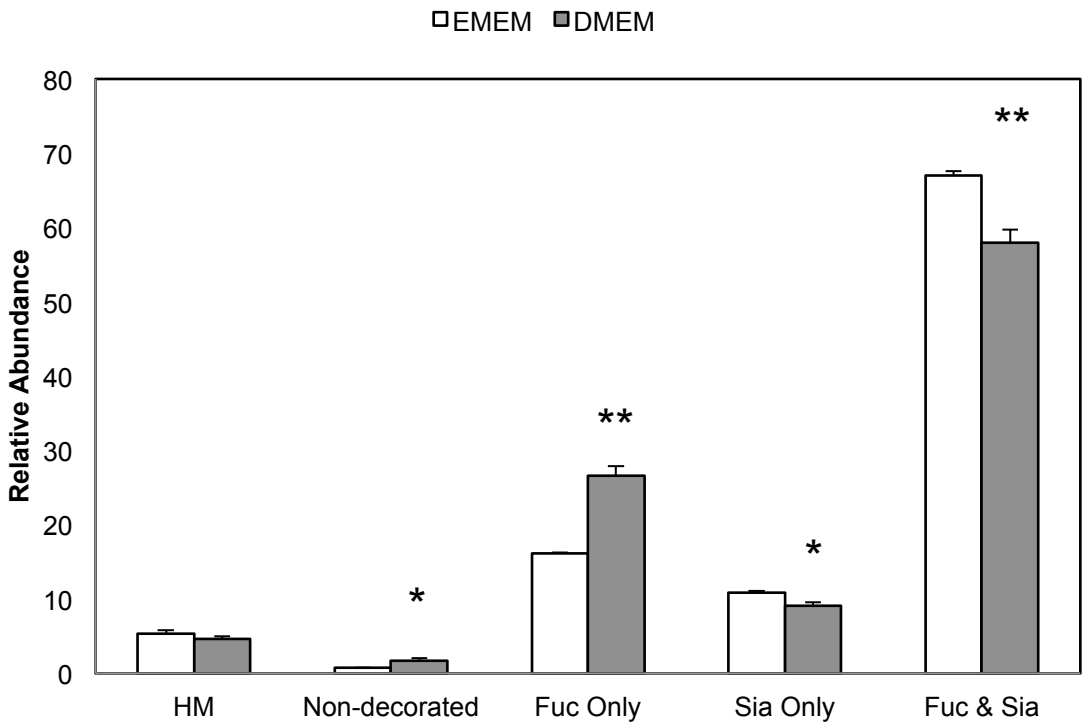


Figure S4. Summed relative abundances of cell surface N-glycans identified in Caco-2 cells grown in EMEM versus DMEM basal media. Data are compared by glycan types, where HM = High Mannose, Fuc = Fucosylated, and Sia = Sialylated. Error bars represent standard deviation. Astericks indicate statistical significant changes (EMEM to DMEM), where *p<0.05 and **p<0.01.

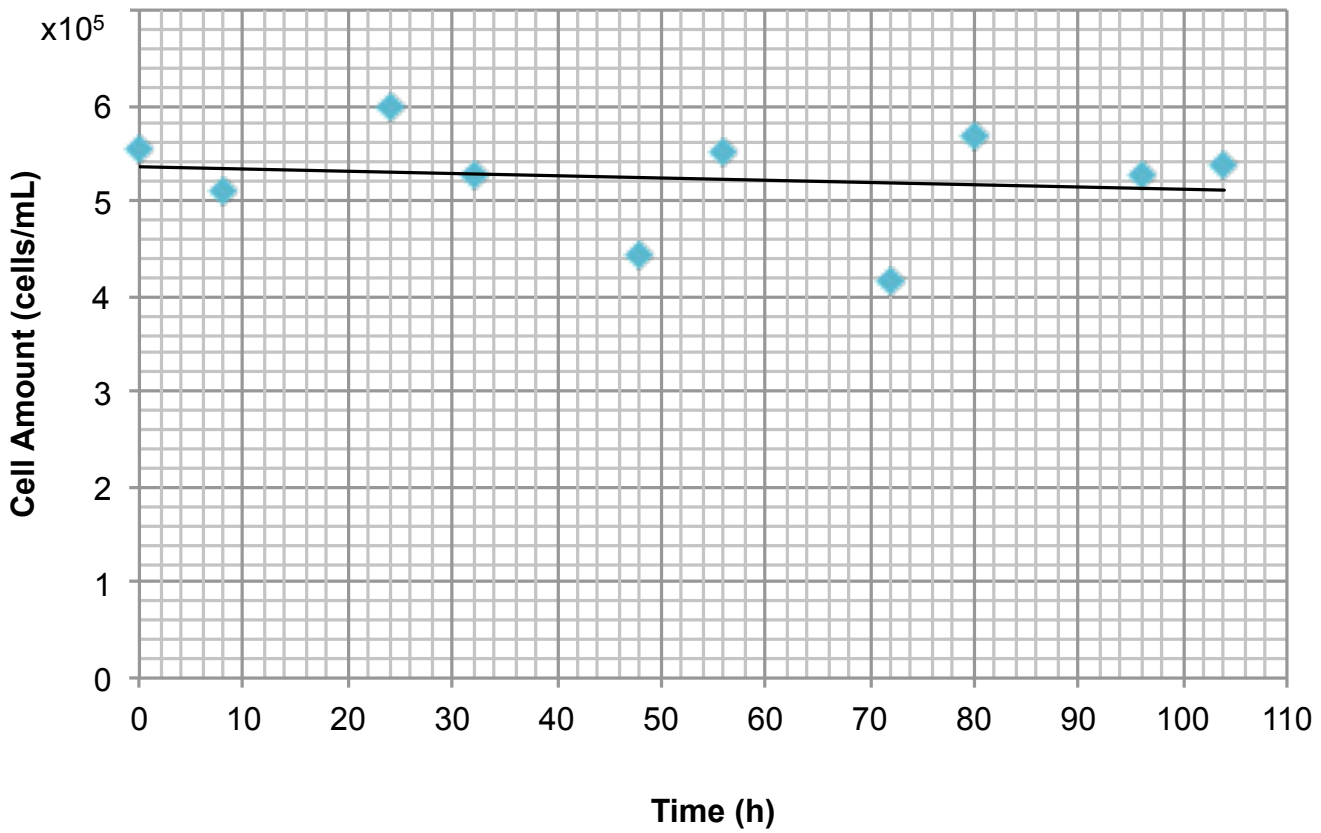


Figure S5. Number of viable Caco-2 cells over time from the initial introduction of kifunensine to 104 h post-treatment. The regression line is plotted inset.

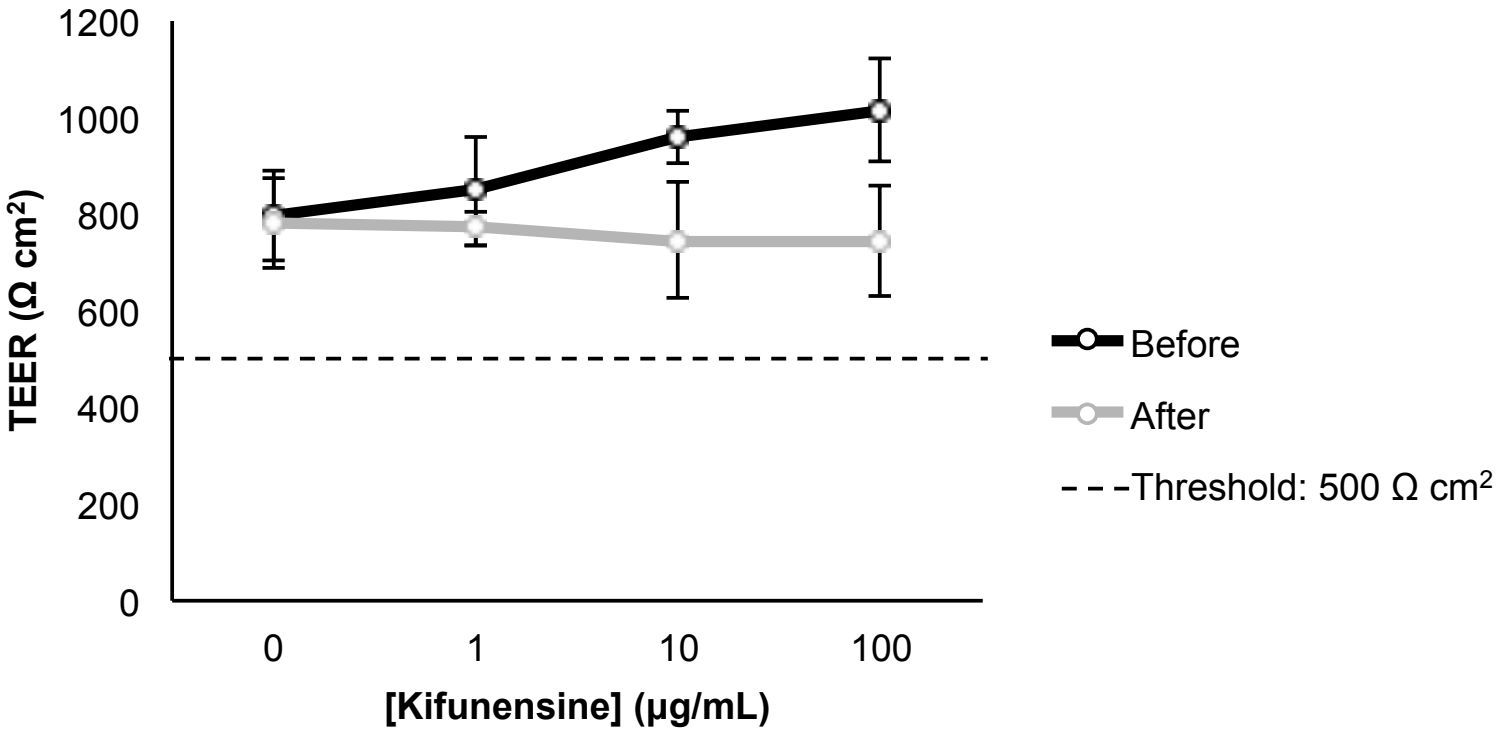


Figure S6. Transepithelial electrical resistance (TEER) measurements in Caco-2 monolayers grown on permeable supports as a function of the amount of kifunensine added. A threshold of 500 Ω cm^2 was used to ensure monolayer integrity.

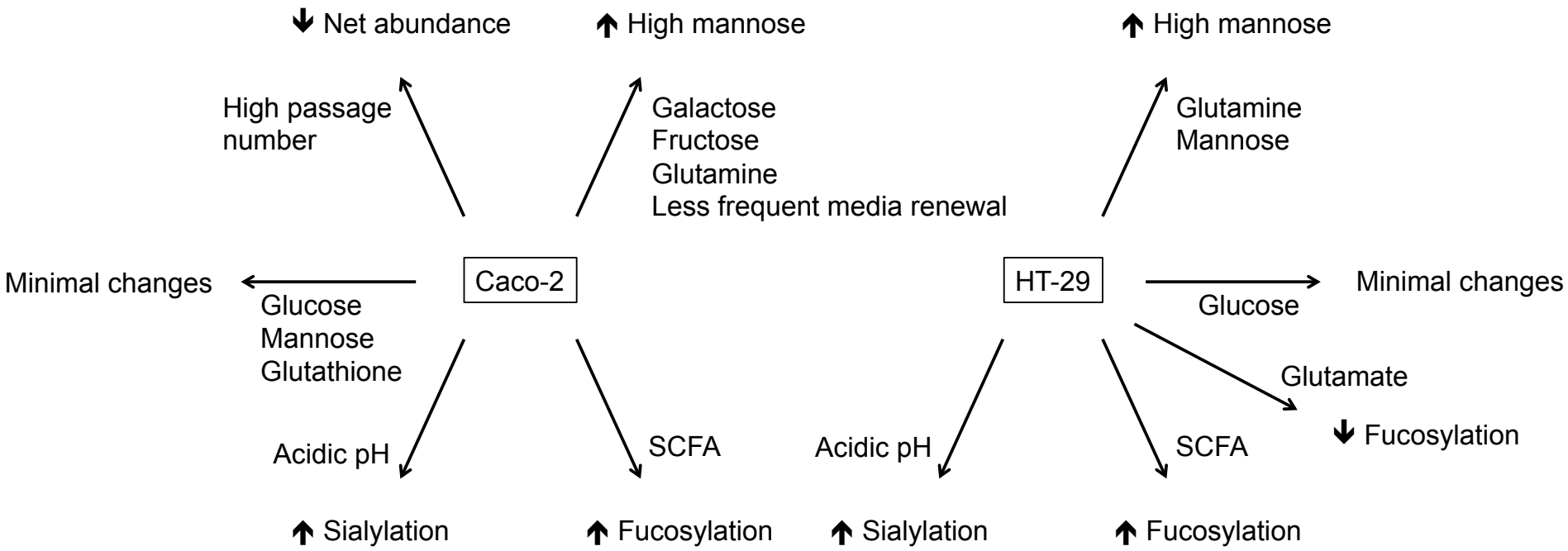


Figure S7. Summary of major changes observed in the cell surface N-glycan profiles of Caco-2 and HT-29 with metabolite and growth manipulations.

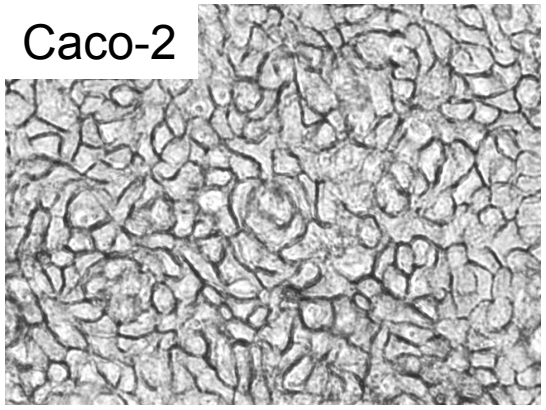
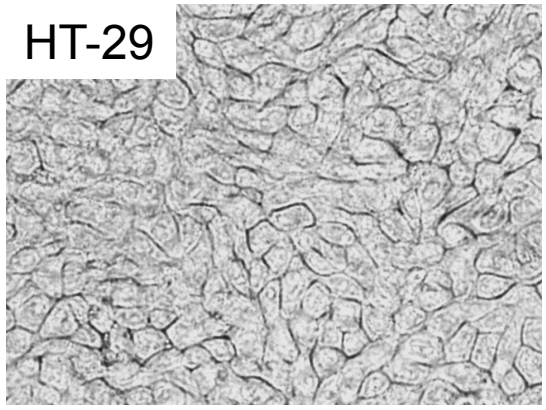
	Caco-2	HT-29
		
Species	Homo sapiens	Homo sapiens
Origin	Caucasian Male 72 years	Caucasian Female 44 years
Tissue	Colon	Colon
Cell Type	Epithelial	Epithelial
Differentiation	Spontaneous Enterocyte-like	Not spontaneous Enterocyte-like or goblet-like
Growth	Adherent	Adherent
Base Medium	Eagle's Minimum Essential Medium	McCoy's 5A Medium
Passage Number (used in this study)	P16 – P31	P15 – P29

Figure S8. Characteristics and representative microscope images of fully differentiated Caco-2 and partially differentiated HT-29 cells in culture. Images were collected at 40X magnification on a Leica DMI3000B. To follow the differentiation process, focus position was adjusted to visualize the formation of distinct outlines of cell junctions.