

Supplemental Information

Clinical importance of high-mannose, fucosylated, and complex N-glycans in breast cancer metastasis

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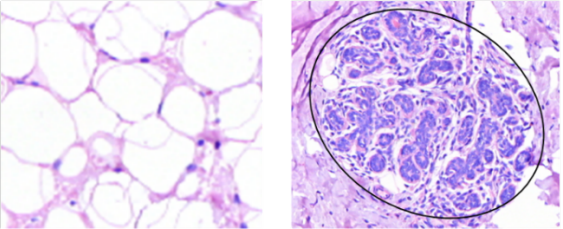
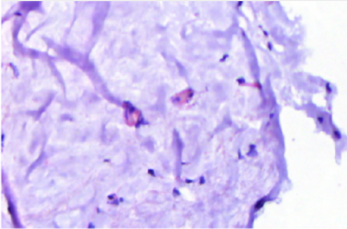
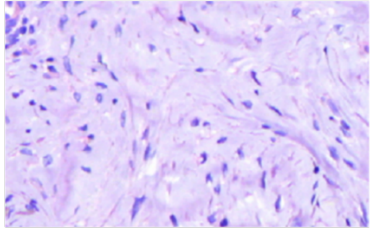
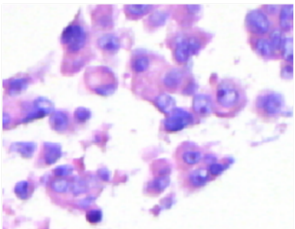
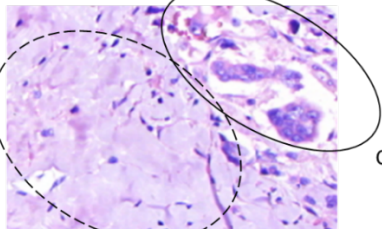
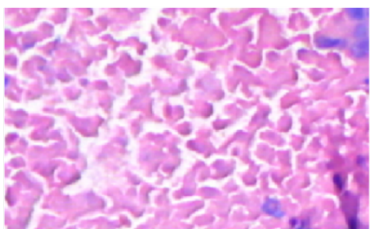
Conflict of Interest: The authors have declared that no conflict of interest exists.

Supplemental Methods

1. Pathology characterization of annotation categories

Annotation	Description
1. Normal	Normal histological variant; non-neoplastic and native to the anatomic site
2. Stroma	Hematopoietic cells + fibroblasts + blood vessels; Non-neoplastic and native to the anatomic site
3. Cancer-associated stroma	Extracellular matrix + fibroblasts + blood vessels; Type of Stroma: desmoplastic, fibrous, or hyalinized
4. Cancer	Glandular pattern carcinoma; Neoplastic
5. Cancer mixed with cancer-associated stroma	Glandular pattern carcinoma; Neoplasm + Extracellular matrix + fibroblasts + blood vessels Type of Stroma: desmoplastic, fibrous, or hyalinized
6. Necrosis	Irreversible cell injury

Note: H&E images below show examples of each category for a breast tissue. Other tissues within this multi-organ cohort were annotated similarly.

1. Breast – Normal  Adipose Tissue Terminal ductal lobular unit	2. Breast - myxoid benign stroma 	3. Breast – Cancer associated stroma 
4. Breast - Cancer 	5. Breast - Cancer + Cancer associated stroma  Cancer Cancer associated stroma	6. Breast - Necrosis 

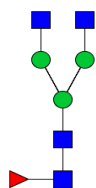
2. N-glycan nomenclature and symbolism

Throughout our publication, we used the standardized nomenclature and symbolism for glycans (1, 2). In the following, we present a brief summary of the terminology and symbols.

Supplemental scheme: Overview of generic and specific monosaccharide names and symbols.

General sugar structure	Specific sugar structure				
	Glucose	Mannose	Galactose	Fucose	Sialic acid
Hexose ○	Glc ●	Man ●	Gal ●		
HexNAc (N-Acetylhexosamine) □	GlcNAc ■	ManNAc ■	GalNAc ■		
Deoxyhexose △				Fuc ▲	
Di-deoxynonulosonate ◇					Neu5Ac ◆

An example of N-glycan nomenclature:



Hex3dHex1HexNAc4 – this naming reflects the general monosaccharide content, i.e., 3 hexoses, 1 deoxyhexose and 4 N-acetyl hexosamines.

3. BC molecular subtypes

Breast cancers (BC) are classified in several ways. The most common subtyping of clinical BC is based on a few key genes that the cancer does or does not express (3). The main subtypes are:

Luminal (Lum)

Luminal tumors are estrogen receptor–positive (ER-positive), and often progesterone receptor–positive (PR-positive), but HER2 receptor–negative (HER2-negative). Since we are studying primary tumor as well as distant metastases, we further distinguish the following 2 notations: Lum and Lum/loss. Lum means that the primary as well as the distant metastatic tumors are ER-/PR-positive, whereas Lum/loss signifies that the primary tumor had ER-/PR-positive status, but the distant metastases lost ER/PR positivity.

HER2-positive (HER2+)

HER2-positive breast cancer is hormone-receptor negative (ER- and PR-negative) and HER2-positive. Similarly, we also have Her2+ and Her2+/loss types, where Her2+ means ER- and PR-negative and Her2+ in primary as well as metastatic tumors, while Her2+/loss indicates the loss of Her2+ in distant metastases.

Basal-like (BLC)

The last molecular subtype is basal-like or so-called triple-negative breast tumors. These tumors are negative for hormone-receptors (ER and PR) and HER2.

4. BC stage and tumor type

Stage at diagnosis determined by TNM scoring

Clinical assessment of the tumor size and spread is reported using the TNM (Tumor, lymph Node, Metastasis) scoring system. We used this classification in Supplemental Table 1 detailing the clinicopathological information for each case (main text).

<i>The number following the letter T stands for the size of the tumor.</i>	
T1	≤2 cm across
T2	2–5 cm across
T3	>5 cm across
T4	Tumor of any size with direct extension to the chest wall and/or to the skin
TX	Size could not be assessed
<i>The number following N describes the degree of cancer spreading to the lymph nodes.</i>	
N0	No lymph nodes affected
N	Cancer cells are in the lymph nodes in the armpit but the nodes are not attached to surrounding tissues
N2	Cancer cells in the lymph nodes in the armpit, which are attached to each other and to other structures
N3	Cancer cells in the lymph nodes below/above the collarbone and/or armpit lymph node and/or behind the breastbone
NX	Lymph nodes could not be assessed for cancer cells
<i>The number following the letter M indicates the spread of cancer cells to distant organs.</i>	
M0	No distant organs involved
M1	Cancer has spread to another part of the body
MX	The spread of cancer cells to distant organs could not be assessed

Tumor type (4)

In the studied patient cohort, two types of primary breast cancer (Supplemental Table 1) occurred: infiltrating lobular carcinoma (ILC) and infiltrating ductal carcinoma (IDC). ILC starts in the lobules (milk glands) of the breast and often metastasizes to other organs. ILC accounts for 10% to 15% of all breast cancers. IDC begins in the milk ducts of the breast, then penetrates the ductal wall, invades the surrounding fatty breast tissue, and eventually may spread to other parts of the body. IDC is the most common type of breast cancer, accounting for 80% of breast cancer diagnoses.

5. Sample preparation for N-glycan MALDI-MSI analysis

For analysis, the glass slide was removed from the -80 °C freezer and dried in a vacuum desiccator for 30 min. The slide was subsequently placed on a heating plate set to 60 °C for 60 min. Next, the slide was deparaffinized by dipping it twice into 100% xylene (Biosolve Chimie SARL) for 10 min each, followed by a rehydration series of two 100% ethanol (Biosolve Chimie SARL,) washes for 2 min each and two milliQ water washes for 5 min each. The following step was antigen retrieval to reverse the protein cross-linking. For that purpose, the slide was submerged into 10 mM citric acid buffer at pH 6.0 and heated under pressure for 20 min using the Antigen Retriever 2100 (Aptum Biologics). After antigen retrieval, the slide was rinsed twice in milliQ water for 1 min each and dried using nitrogen gas. Next, 15 layers of

200 ng/ μ L water-dissolved PNGaseF (N-Zyme Scientifics) were applied to the slide using a SunCollect pneumatic sprayer (SunChrom GmbH) with a constant 10 μ L/min flowrate, track spacing of 1 mm, nozzle height of 25 mm, and a nozzle speed of 900 mm/min. After enzyme application, the slide was incubated for 3 h at 37 °C in a humid chamber. Then, water-based Tipp-Ex (BIC S.A.) fiducial markers were placed on the glass slide and the slide was scanned at 2000 dpi resolution with desktop slide scanner Super CoolScan 5000 (Nikon). Afterwards, 8 layers of 5 mg/mL alpha-cyano-4-hydroxycinnamic acid (CHCA, Sigma-Aldrich) in 50% acetonitrile (Biosolve Chimie SARL) and 0.2% trifluoroacetic acid (Sigma-Aldrich, The Netherlands) were applied using HTX TM Sprayer (HTX Technologies) with 0.05 ml/min flowrate, 1100 mm/min velocity, 30 °C temperature, and 2 mm track spacing.

6. Acquisition and analysis of high mass resolution MALDI imaging data

We acquired high mass resolution MALDI imaging data on a Velos Pro linear ion trap (Thermo Scientific, San Jose, USA) equipped with a National High Magnetic Field Laboratory designed external linear quadrupole ion trap, quadrupole ion transfer optics, and a novel dynamically harmonized ion cyclotron resonance (ICR) cell, which is operated at 7.5 V trapping potential (5). The commercial ion source and stacked ring ion guide were replaced with an elevated-pressure MALDI ion source incorporating a dual-ion funnel interface (Spectrograph LLC, Kennewick, USA) as previously described (6). The mass spectrometer was operated with an ion injection time of 250 ms and automatic gain control (AGC) was turned off. A transient duration of 3.1 s was used for ultra-high mass resolving power analyses, resulting in a 4 s acquisition time per-pixel. Tandem MS data-dependent acquisition (DDA) of the samples were acquired on sequential pixels, with both operated at 3.1 s transients. MS¹ mass spectra were captured between 500-2000 m/z in positive ion mode, with MS² spectra acquired from 200-2000 m/z . Samples were post-acquisition recalibrated in the MS¹ domain, using the known CHCA peak clusters available within this spectral window, while MS² spectra were not recalibrated. To enable ease of use during data interpretation, MS¹ m/z windows were shortened to 850-2000 after recalibration to significantly decrease data size. Data were peak picked in mMass (mmass.org) and loaded into GlycoWorkbench for further analysis. The GlycoWorkbench (v2.1) software was used to search the GlycomeDB database with default settings and assign m/z values to their corresponding N-glycan identifications. Default settings were as follows: derivatization - underivatized (und), reducing end - freeEnd, fragment types – B, C, Y, Z, maximum number of cleavages – 2, maximum number of cross rings – 1, derive options from parent ions – yes, maximum number of charges – 1, accuracy – 1.0 Da. The structures for each m/z of interest were then fragmented, and the MS² spectra were annotated with these fragments using GlycoWorkbench at a tolerance of 1.0 Da. The consolidated annotation reports are available in Supplemental Tables 10-21. Annotated spectra were generated on GlycoWorkbench using the reporting tool and are available in Supplemental Figures 6-17.

7. H&E staining

Following MALDI-MSI measurement, the slides were submerged for 3 min each in a series of ethanol (Biosolve Chimie SARL) in water solutions in the order listed: 100% twice, 96% twice, and 70% twice. Next, the slides were washed for 3 min in water and submerged for 3 min in hematoxylin (Biosolve Chimie SARL). Next, the hematoxylin solution was washed off under running water and the slides were submersed in eosin (Merck KGaA) for 30 sec. The eosin was washed off under running water and the slides were submerged in 100% ethanol for 1 min and 100% xylene (Biosolve Chimie SARL) for 5 min. The final step was mounting of a glass cover slip using Entellan mounting medium (Merck KGaA) followed by drying for at least 12 hours.

8. Co-registration of MALDI-MSI and annotations

Prior to each MSI experiment, an optical image of the specimen was taken and used to define the measurement area by loading it into the FlexImaging software (v 5.0, Bruker Daltonik) where a rough co-registration was performed between the optical image and the sample stage coordinate system of the mass spectrometer. Three manually selected control points provided by the fiducial markers visible both in a camera within the mass spectrometer accessed via the FlexControl software (v 5.0, Bruker Daltonik) and the optical image in FlexImaging, were employed for co-registration.

The optical image taken prior to acquisition thus defines the coordinate space for every MALDI-MSI dataset. Next, to facilitate the co-registration of the MSI data with the H&E image, both the optical image as well as the corresponding H&E image were imported into MATLAB R2018b (MathWorks). There, the high-resolution H&E image was co-registered to the optical image by using at least three manually selected control points based on prominent morphological features of the tissue that were visible in both, H&E and optical scanner image (MATLAB command `cpselect`), resulting in the affine geometric transformation $\Delta_{\text{HE-optical}}$ (MATLAB command `fitgeotransform`). Then, the annotations from CaseViewer were imported from the previously exported and corresponding XML file. The coordinates of each annotation were then transformed by applying the geometric transformation $\Delta_{\text{HE-optical}}$ to the coordinate space of the MALDI-MSI dataset. These were finally written into the .mis file (Bruker data file) as “Region of interest”.

9. Details of GE analyses

Gene expression of N-glycan biosynthesis genes

To determine whether expression profiles among genes associated with N-glycosylation were consistent with the glycosylation profiles observed by MALDI-MSI, two publicly available datasets (GSE26338 (7), GSE110590 (8)) were analyzed, comparing gene expression among normal, primary, and metastatic tissues. In GSE26338 (7), expression profiling was done by Agilent-012097 Human 1A Microarray (V2) G4110B of unmatched breast tissue, primary tumor, and metastatic tissue. In GSE110590 (8), expression profiling was done by high-throughput sequencing (Illumina HiSeq 2000) of matched primary breast tumor and metastatic tissue. Expression of aforementioned genes were analyzed in lymph, adrenal, brain, bone, liver, lung metastases, and primary tumor from 15 patients. The following samples were analyzed from GSE26338, including 5 normal breast, 18 primary tumor, and 25 metastases: GSM34427, GSM34440, GSM34441, GSM34465, GSM34490, GSM34476, GSM34477, GSM34491, GSM34492, GSM34504, GSM34505, GSM34506, GSM34508, GSM34512, GSM34514, GSM34523, GSM34527, GSM34528, GSM50154, GSM53475, GSM53476, GSM53477, GSM80245, GSM80246, GSM34442, GSM34464, GSM50147, GSM50148, GSM52910, GSM52911, GSM52912, GSM52914, GSM52915, GSM52917, GSM52919, GSM52920, GSM52921, GSM52922, GSM80213, GSM81165, GSM635382, GSM635389, GSM635391, GSM635398, GSM635403, GSM635406, GSM635407, GSM635409.

Cell specific gene expression of FUT8

To assess the native expression of FUT8 in the bone marrow microenvironment, the Human Cell Atlas (9) bone marrow tissue project, which comprises >100,000 hematopoietic cells sourced from eight healthy donors, was analyzed to identify the distribution of FUT8 expression across cell-types. In addition, a second single-cell RNA sequencing dataset (GSE144568) wherein $10 \times$ scRNA sequencing had been performed on 135,929 CD34+ lineage hematopoietic stem and progenitor cells was analyzed. As described by Psaila *et al.* (10), after identification of highly variable genes by fitting a gamma-generalized linear model and correction of batch effects via the `sva` package in R (v4.0.0), principal component analysis and uniform manifold approximation and projection (UMAP) were performed. The expression of FUT8 in each cell type was identified and superimposed on a plot of the UMAP embedding. Annotations of cell

type were determined by labels established by Psaila *et al.* (10). Analyses of these datasets were performed using R, with the Seurat package used for analysis of single cell data.

Functional characterization of the N-glycosylated proteome

To analyze enrichment of cancer-related proteins among the N-glycosylated proteome, the dbPTM database was used to extract ~1300 unique human proteins that display N-glycosylation. Using the aforementioned datasets from the GEO, as well as the TCGA Breast Cancer dataset (11), genes differentially expressed (FDR < 0.15) between normal breast and primary tumor (TCGA) and between primary tumor and metastasis (GSE26338) were identified. A list of N-glycosylated proteins encoded by those differentially expressed genes was subsequently compiled. Moreover, functional annotation of the list of N-glycosylated proteins was performed using the Enrichr package in R, with the WikiPathways (WP) database used as a library of pathway annotations and an enrichment FDR > 0.25 considered significant. For analysis of the N-glycosylated proteome, dbPTM was used to obtain a curated list of proteins with available evidence of N-glycosylation. A second database, GlycoProtDB, was used to obtain information pertaining to the lectins to which each protein binds. Analysis of TCGA samples was performed by comparing expression of genes encoding N-glycosylated proteins between normal tissue and primary tumor samples from the TCGA Breast Cancer dataset (n=1211, including 114 normal breast and 1097 primary tumor samples). The differentially expressed genes that overlapped with the following WP datasets and gene ontology (GO) terms was selected for further analysis: Amplification and Expansion of Oncogenic Pathways as Metastatic Traits (WP), Matrix Metalloproteinases (WP), TGF- β Signaling in Thyroid Cells for Epithelial-Mesenchymal Transition (WP), Integrin-mediated Cell Adhesion (WP), Focal Adhesion (WP), Focal Adhesion-PI3K-Akt-mTOR-signaling pathway (WP), Extracellular Matrix Organization (GO), Positive Regulation of Cell Proliferation (GO), Regulation of Cell Migration (GO), Regulation of Cell Proliferation (GO), Extracellular Matrix Disassembly (GO), Positive Regulation of Cell Migration (GO), Cell-Cell Adhesion via Plasma-Membrane Adhesion Molecules (GO), Homophilic Cell Adhesion via Plasma Membrane Adhesion Molecules (GO), Heterotypic Cell-Cell Adhesion (GO), Regulation of Focal Adhesion Assembly (GO), Negative Regulation of Cell Motility (GO), Angiogenesis Involved in Wound Healing (GO), Positive Regulation of Cell-Matrix Adhesion (GO), Positive Regulation of Adherens Junction Organization (GO), Positive Regulation of Epithelial Cell Migration (GO), Regulation of Extracellular Matrix Organization (GO), yielding 224 genes. Genes among this list represented among the differentially expressed genes in GSE26338 were considered for subsequent analysis. Kaplan-Meier curves were drawn using data from TCGA samples hosted via the open-source functional genomics explorer, Xena (12). Among the N-glycosylated proteins encoded by genes upregulated in metastasis, EPCAM was selected for further analysis. Boxplots were drawn to display differences in expression of EPCAM among normal tissue, primary tumor, and metastasis (GSE26338), and specifically among primary tumor and lymph, bone, brain, liver, and lung metastases (GSE110590).

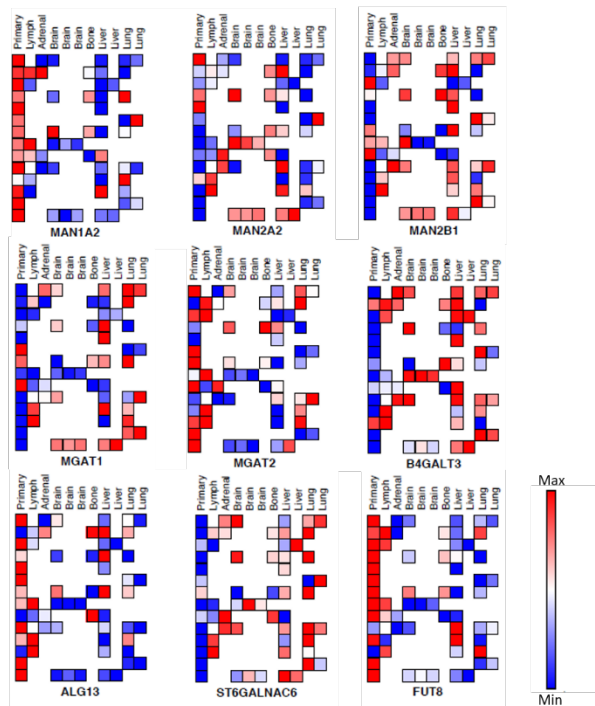
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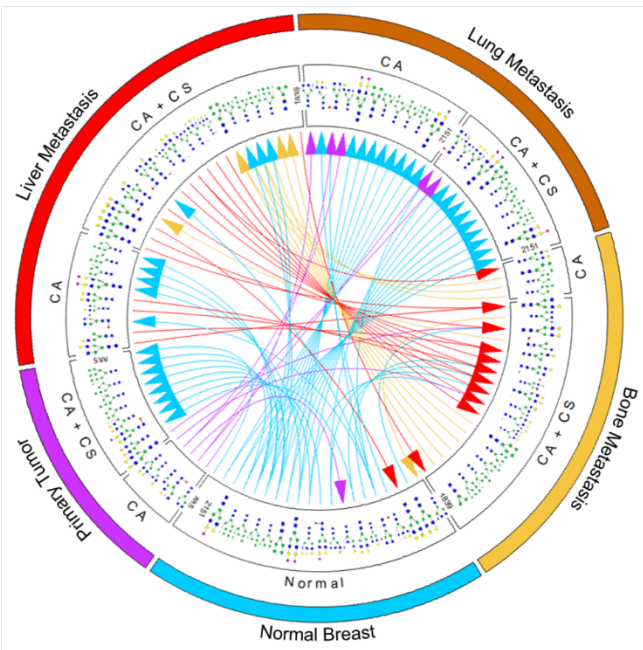
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Supplemental Figures

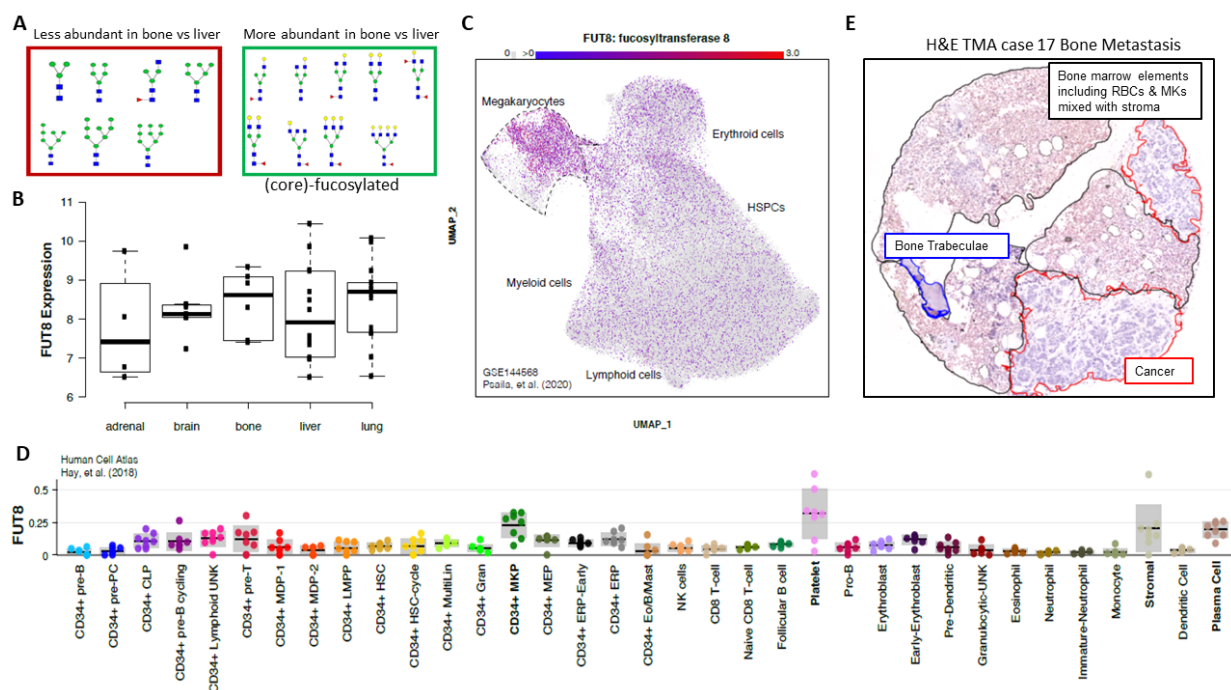
A GEO:GSE110590



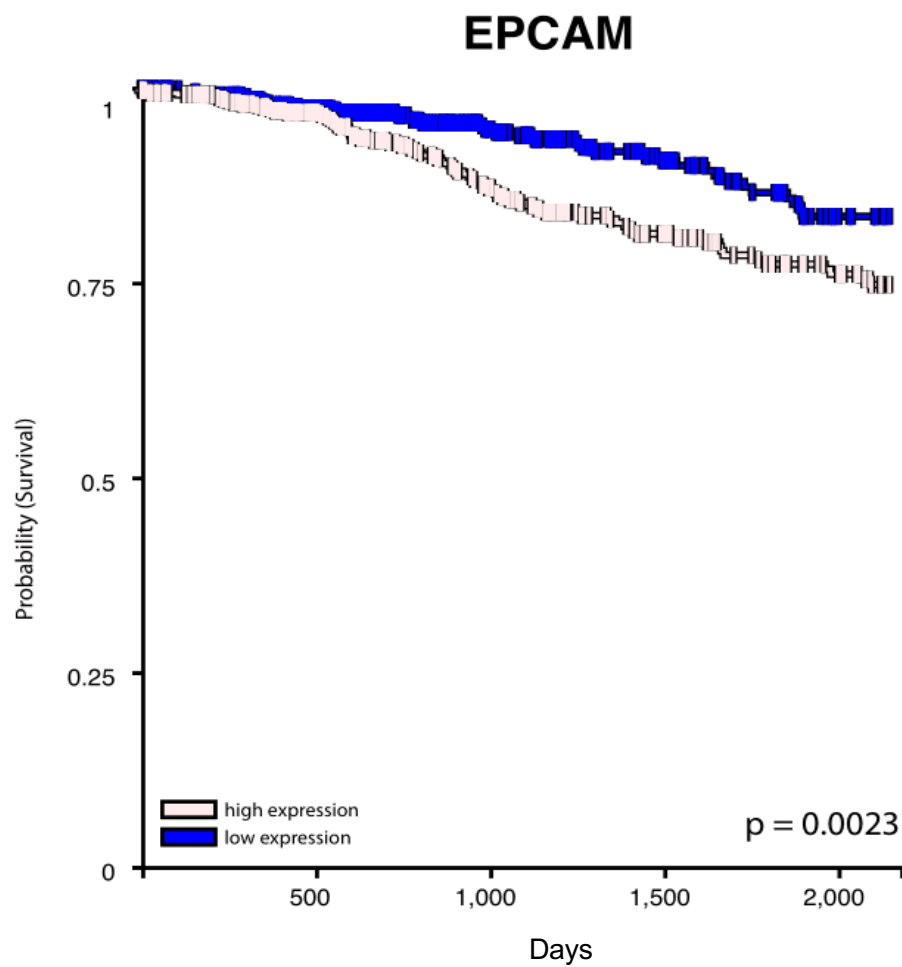
B MALDI-MSI



Supplemental Figure 1: Paired PT and organ-specific metastases mRNA sequencing data further supports gene alterations. (A) Publicly available dataset GEO:GSE110590 re-analyzed for N-glycosylation genes. Heat maps showing significantly altered genes between primary and metastatic tumors matched per patient ($p < 0.05$, $FDR < 0.15$). **(B)** MALDI-MSI comparisons of N-glycan abundances between normal breast & primary tumor, and between bone, lung, and liver metastatic sites. Bone metastasis consists of pooled bone, spine, vertebra, and rib metastases. The arrows indicate increases in abundance relative to the color-coded tissue. CA – cancer, CA+CS – cancer with cancer-associated stroma.

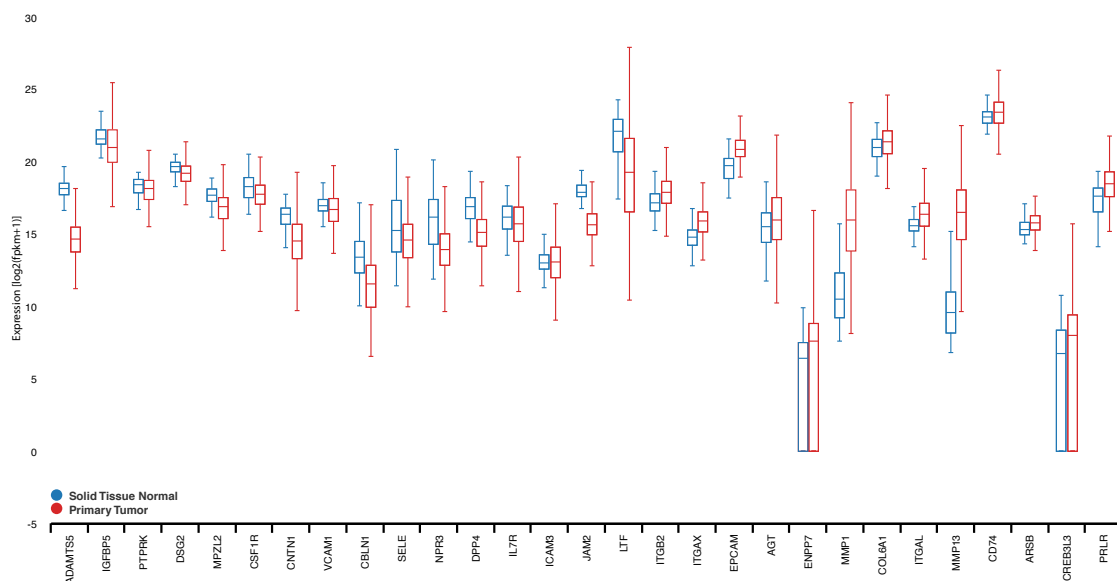


Supplemental Figure 2: Cell-type specific bone expression reveals preferential FUT8 expression by bone marrow elements. (A) N-glycans were significantly lower (red) or higher (green) in bone compared to liver metastases. Most of the abundant N-glycans in bone were fucosylated. (B) Publicly available dataset GEO:GSE110590 containing matched primary breast tumors with site-specific metastases re-analyzed for FUT8 expression per metastases. (C) Publicly available single-cell RNA sequencing dataset (GEO:GSE144568) analyzed to identify the distribution of FUT8 expression across cell types. Uniform manifold approximation and projection (UMAP) was performed and plotted to display the FUT8 cell type expression, with megakaryocytes having the highest one. (D) Human Cell Atlas and its bone marrow tissue project, containing >100,000 hematopoietic cells sourced from 8 healthy donors was analyzed to identify the distribution of FUT8 expression across cell types. (E) H&E staining of TMA #17 from our MALDI-MSI cohort, showing increased presence of megakaryocytes and red blood cells.

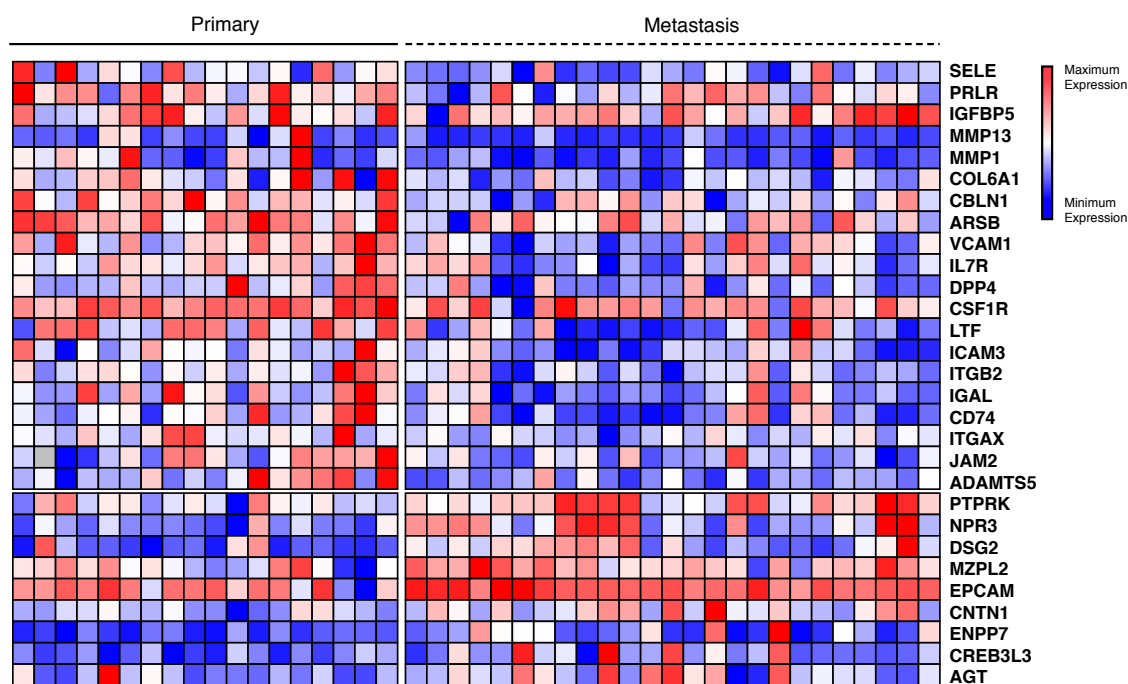


Supplemental Figure 3: Kaplan-Meier curve using the TCGA dataset of EpCAM (blue – lower expression) showing improved probability of survival with low EpCAM expression.

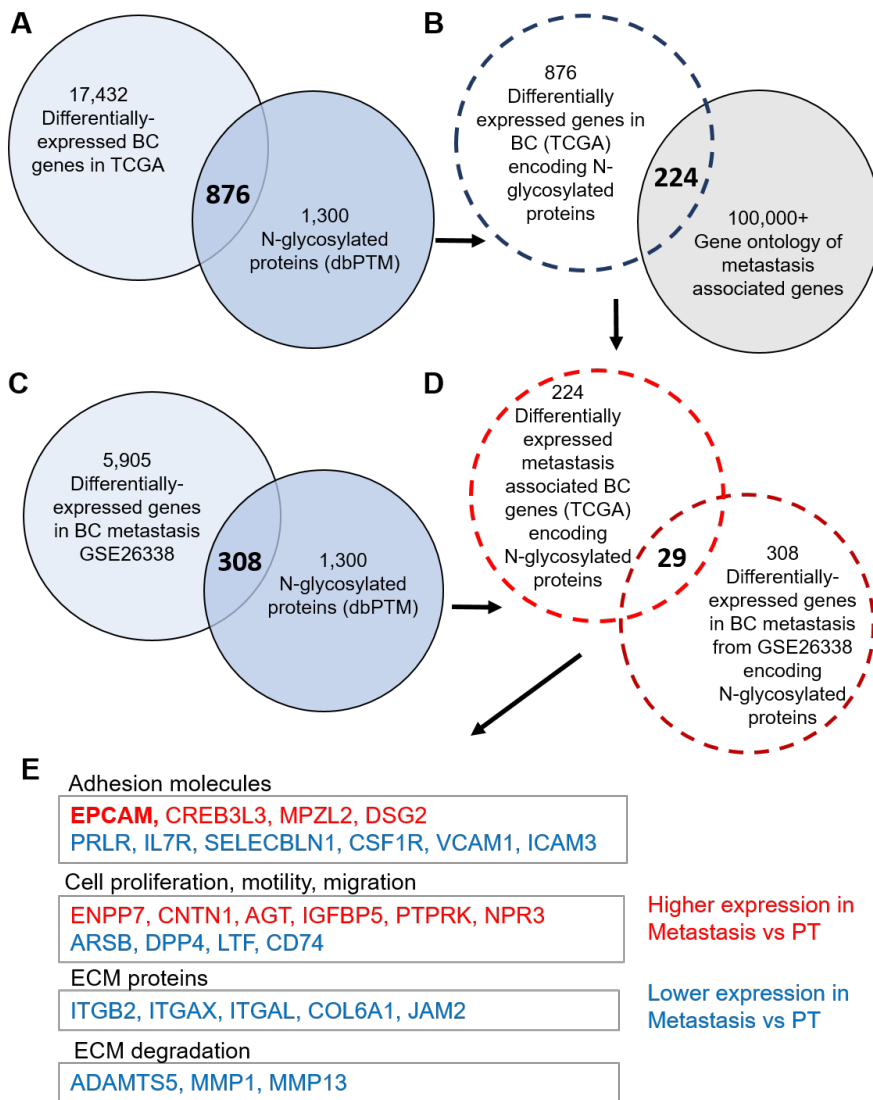
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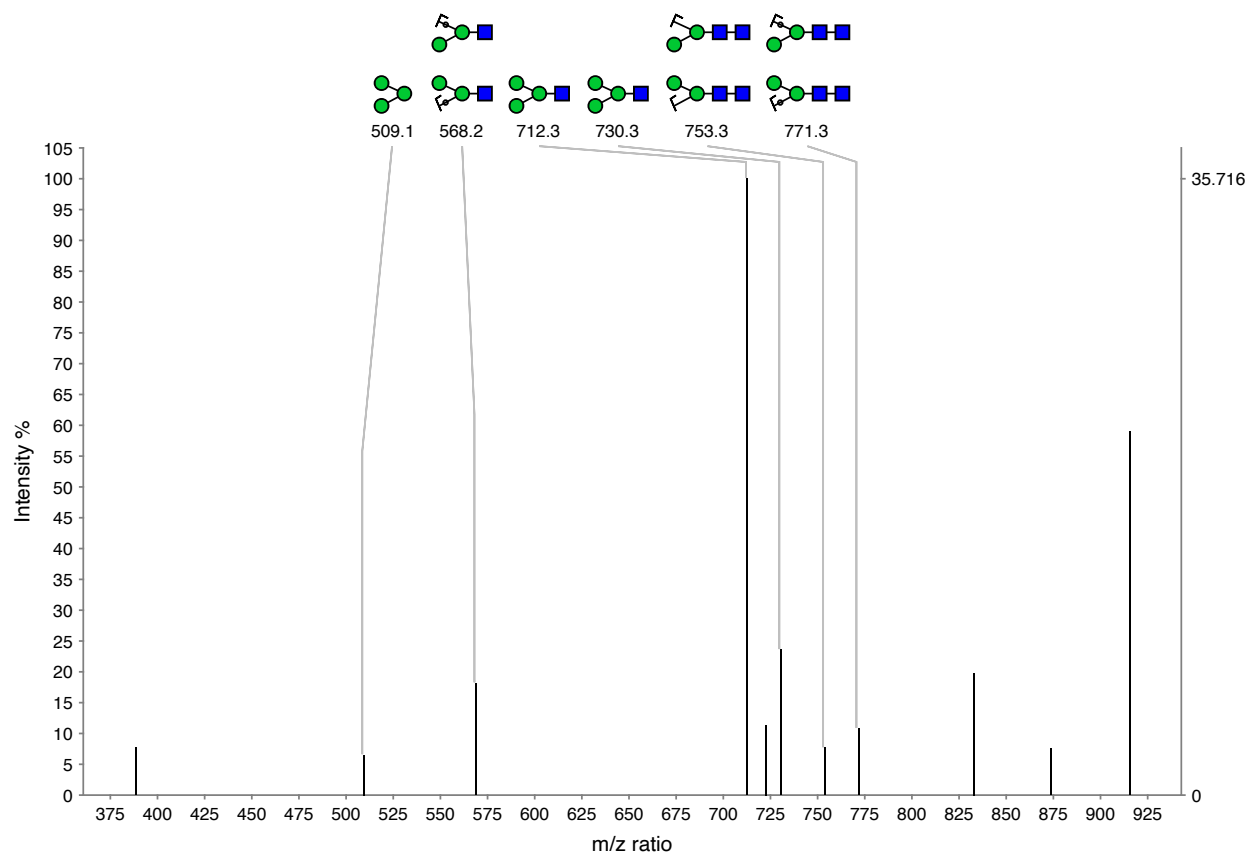
B GSE26338



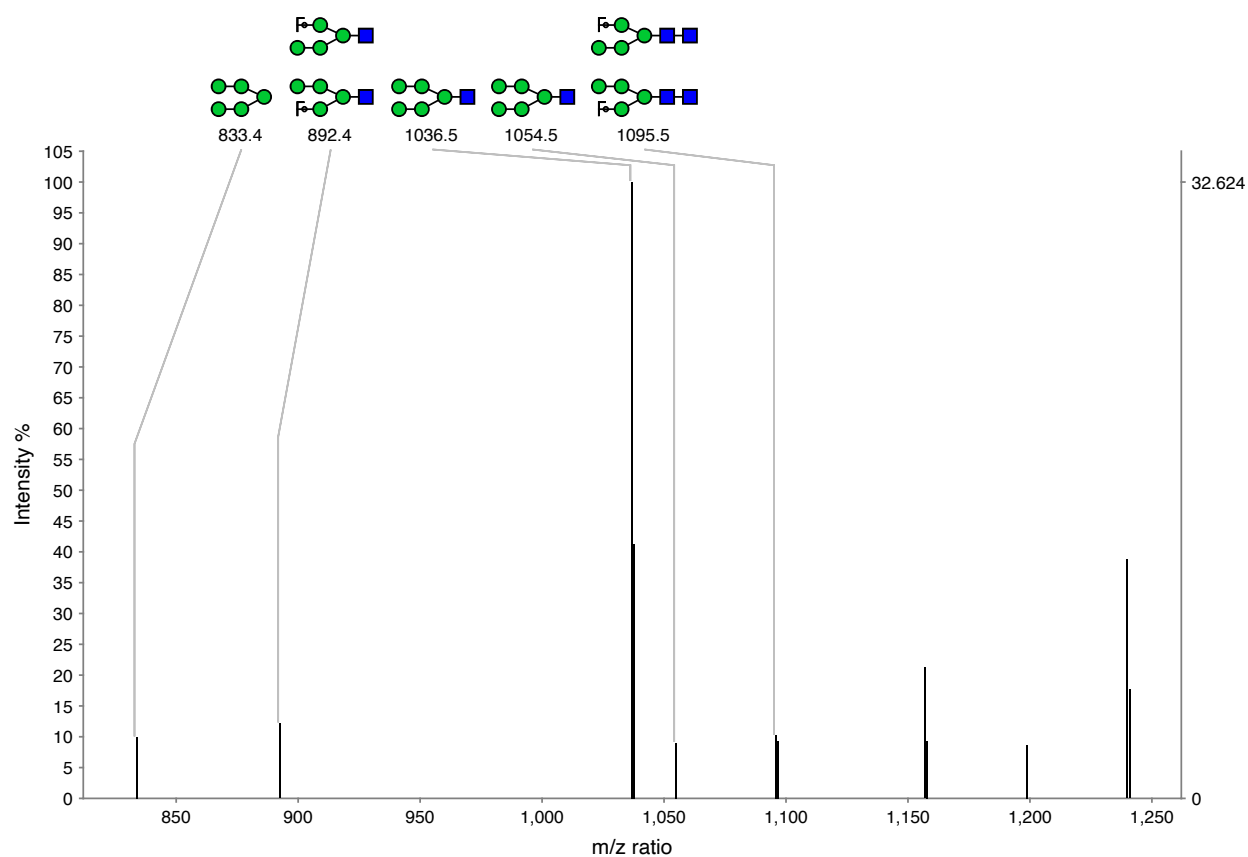
Supplemental Figure 4: Detailed GE of 29 metastasis-associated N-glycosylated and DE genes in BC. (A) GE of TCGA dataset, normal breast compared to PT and (B) GE of GSE26338 dataset, PT compared to metastasis.



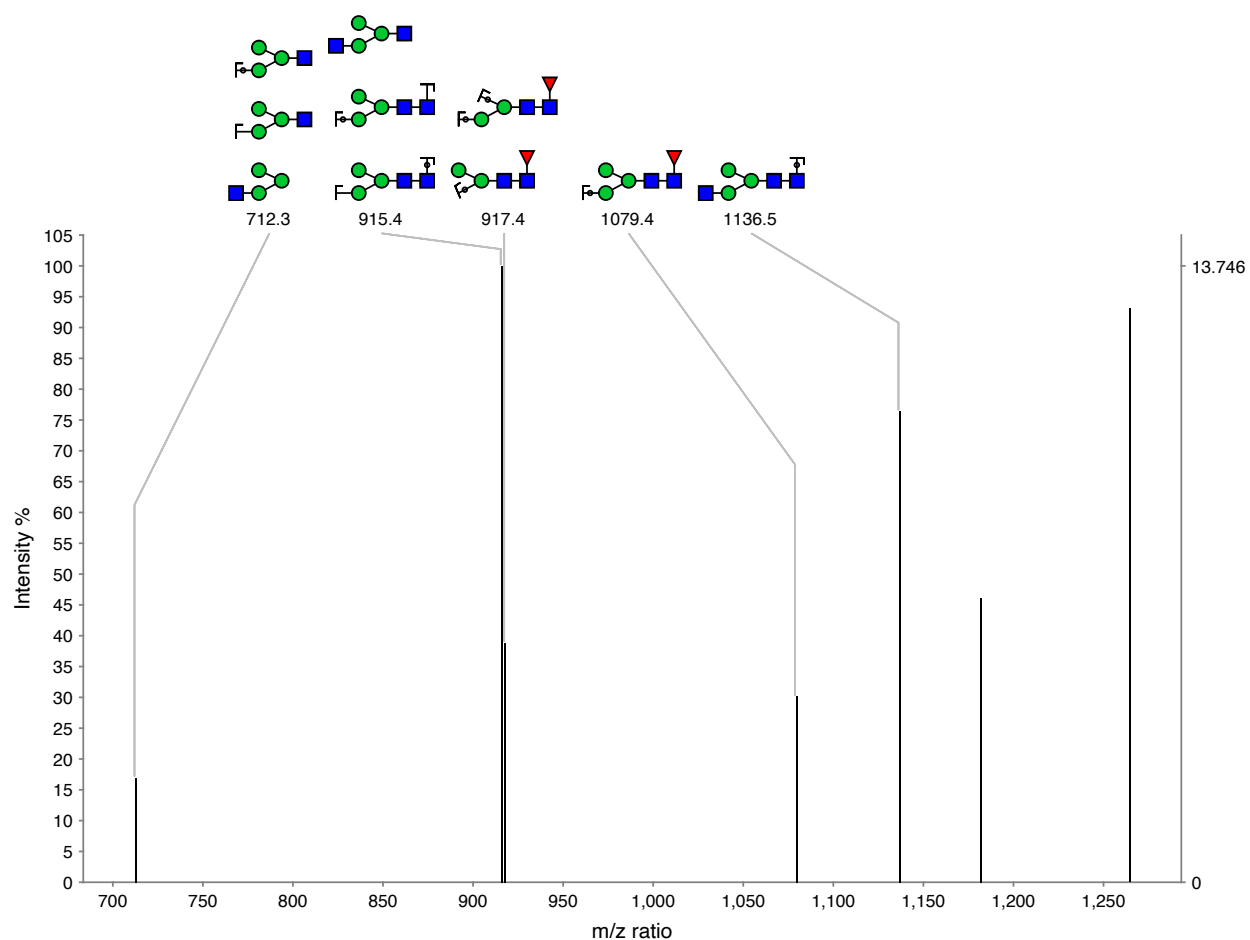
Supplemental Figure 5: Genes encoding N-glycosylated proteins are frequently differentially expressed (DE) in metastasis. (A) Overlap between DE genes in the TCGA breast cancer dataset and known N-glycosylated proteins from dbPTM. **(B)** Overlap between the DE genes encoding N-glycosylated proteins from A with the gene ontology term “metastasis”. **(C)** Overlap between DE genes in GSE26338 dataset and known N-glycosylated proteins from dbPTM. **(D)** Overlap between DE genes encoding N-glycosylated proteins in GSE26338 in C and TCGA metastasis-associated genes encoding N-glycosylated proteins in B. **(E)** List of the overlapping genes encoding N-glycosylated proteins in D according to their function and color-coded by expression levels in metastasis compared to PT: red – higher, blue – lower.



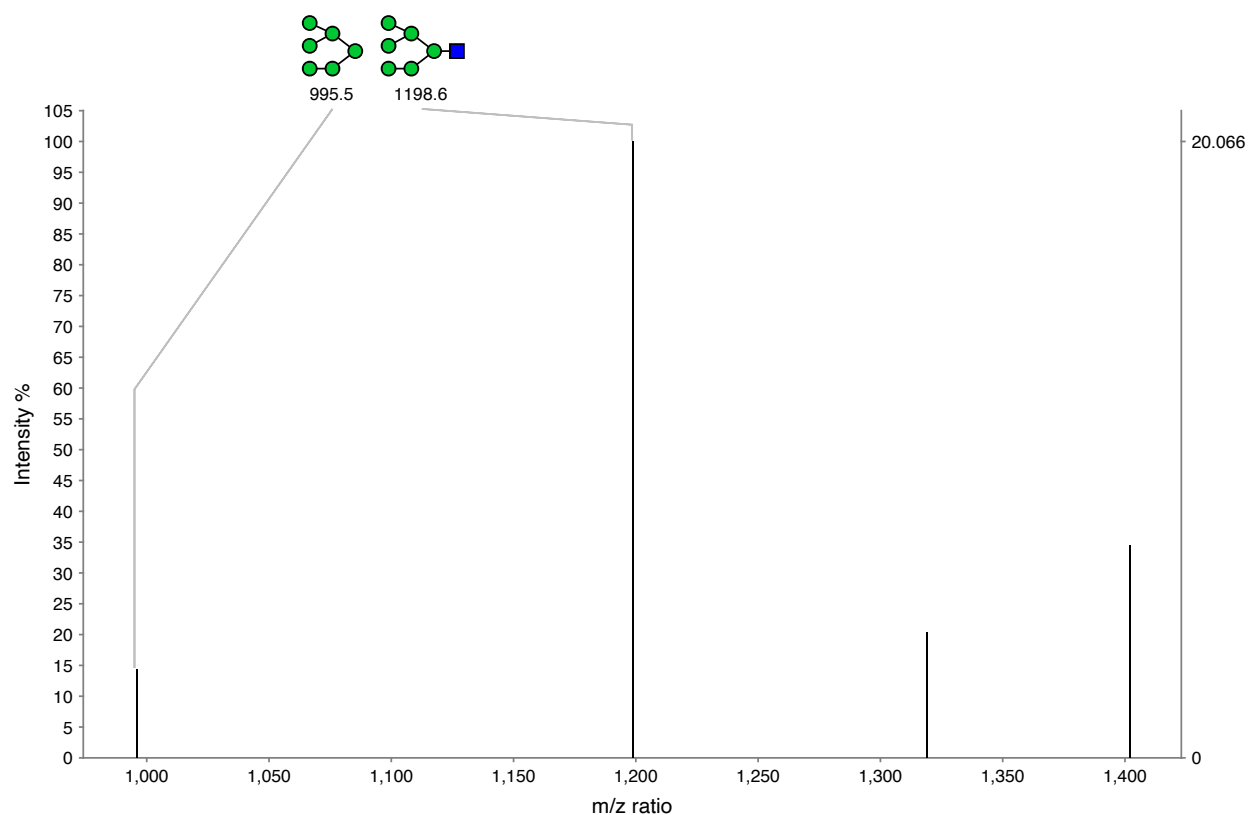
Supplemental Figure 6: Glycworkbench generated annotation of 21T MALDI FTICR DDA fragmentation spectra of molecular ion m/z 933.31688. Fragmentation spectra was annotated based on the most relevant glycoform as circled in red in Supplemental Table 10, which was identified from our data and additional literature review.



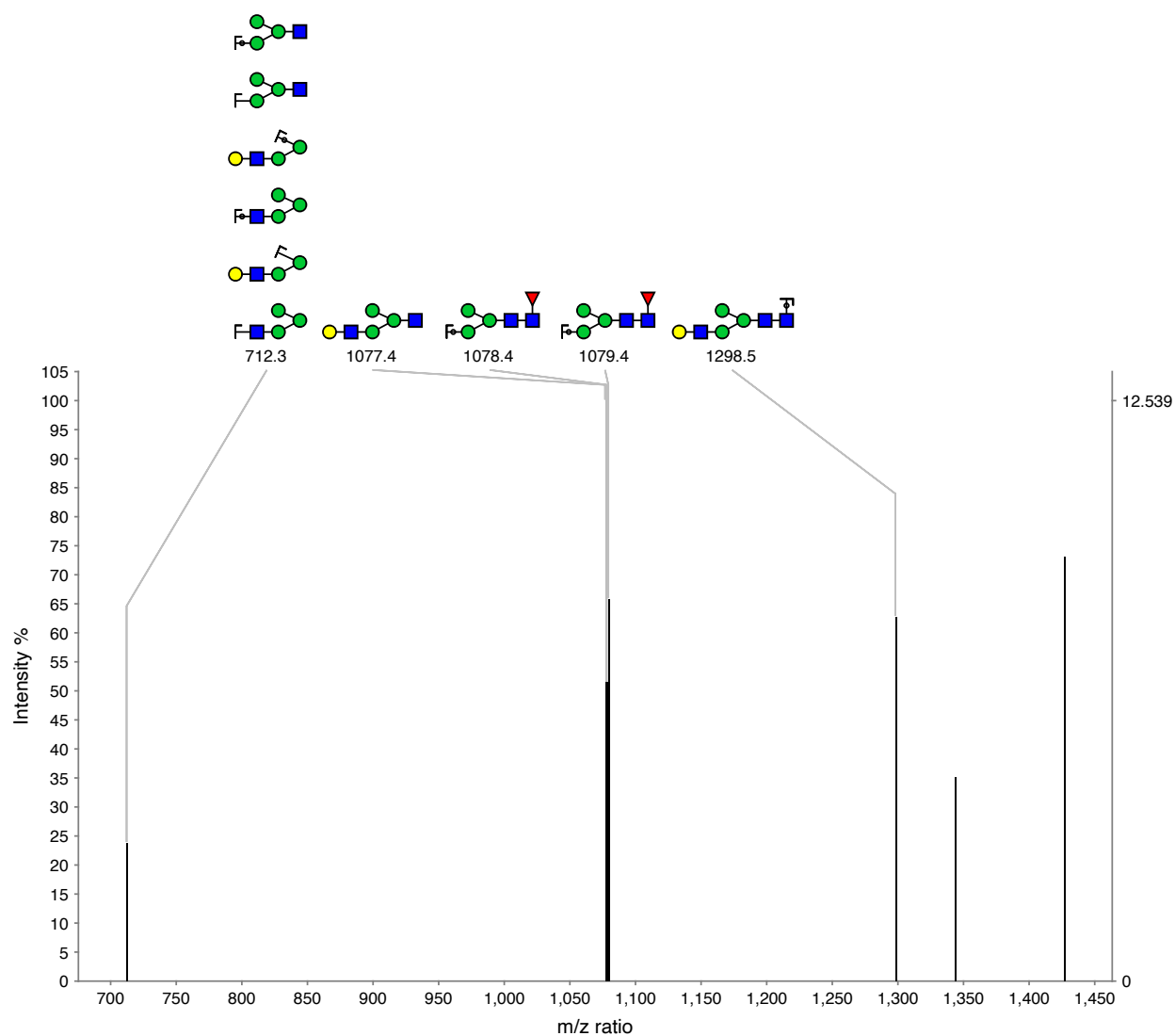
Supplemental Figure 7: Glycoworkbench generated annotation of 21T MALDI FTICR DDA fragmentation spectra of molecular ion m/z 1257.42247. Fragmentation spectra was annotated based on the most relevant glycoform as circled in red in Supplemental Table 11, which was identified from our data and additional literature review.



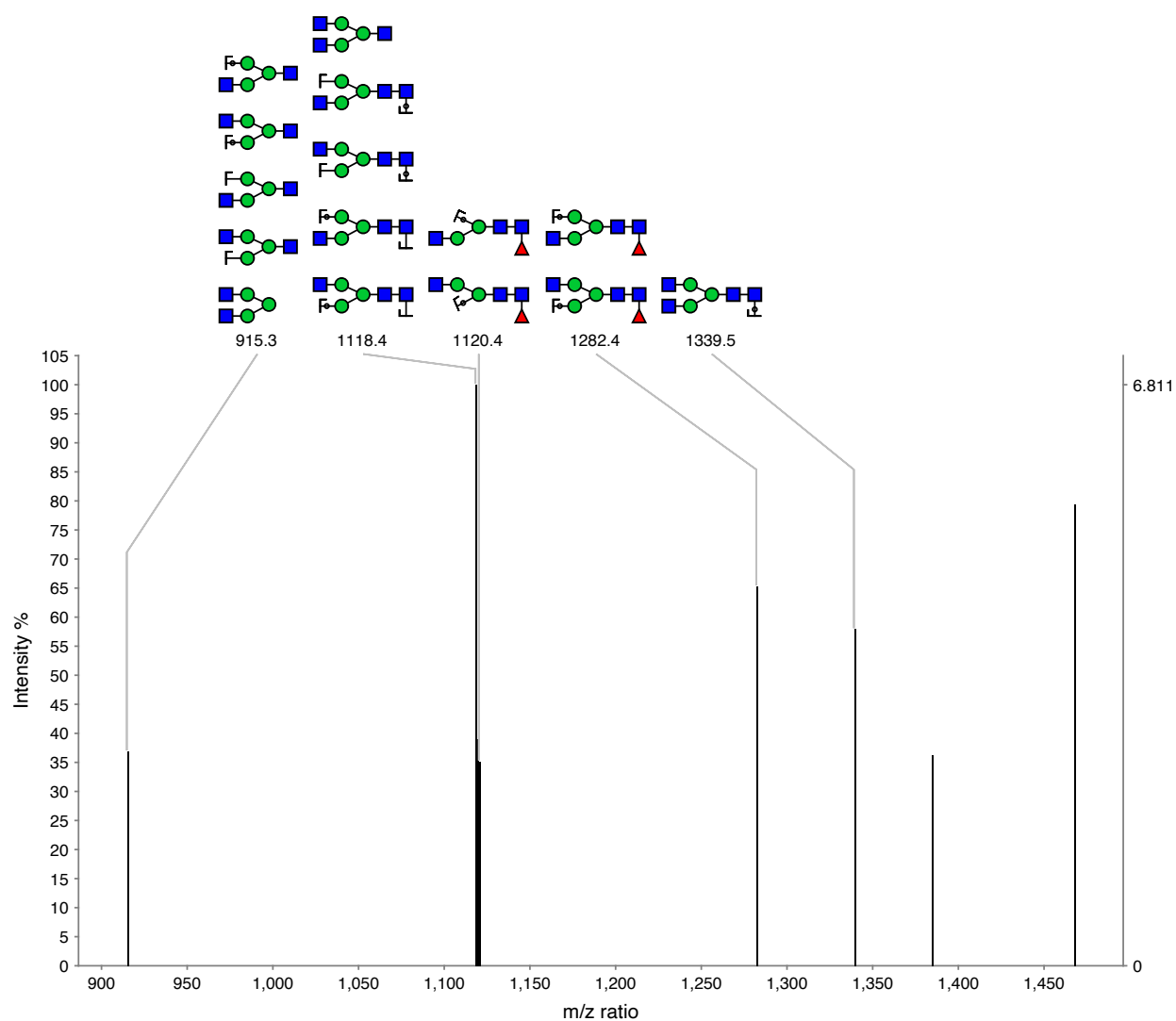
Supplemental Figure 8: Glycoworkbench generated annotation of 21T MALDI FTICR DDA fragmentation spectra of molecular ion m/z 1282.45432. Fragmentation spectra was annotated based on the most relevant glycoform as circled in red in Supplemental Table 12, which was identified from our data and additional literature review.



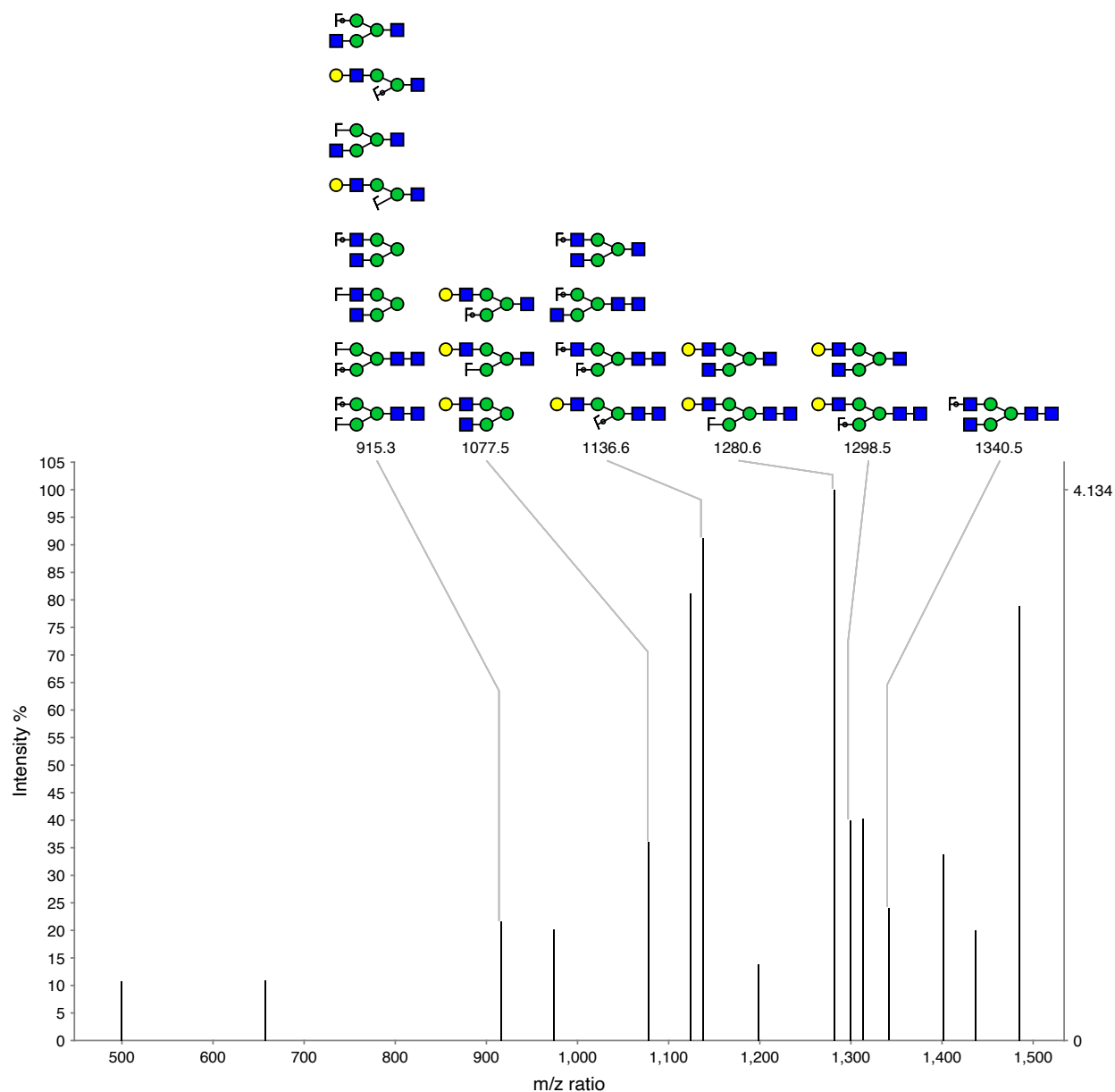
Supplemental Figure 9: Glycoworkbench generated annotation of 21T MALDI FTICR DDA fragmentation spectra of molecular ion m/z 1419.47530. Fragmentation spectra was annotated based on the most relevant glycoform as circled in red in Supplemental Table 13, which was identified from our data and additional literature review.



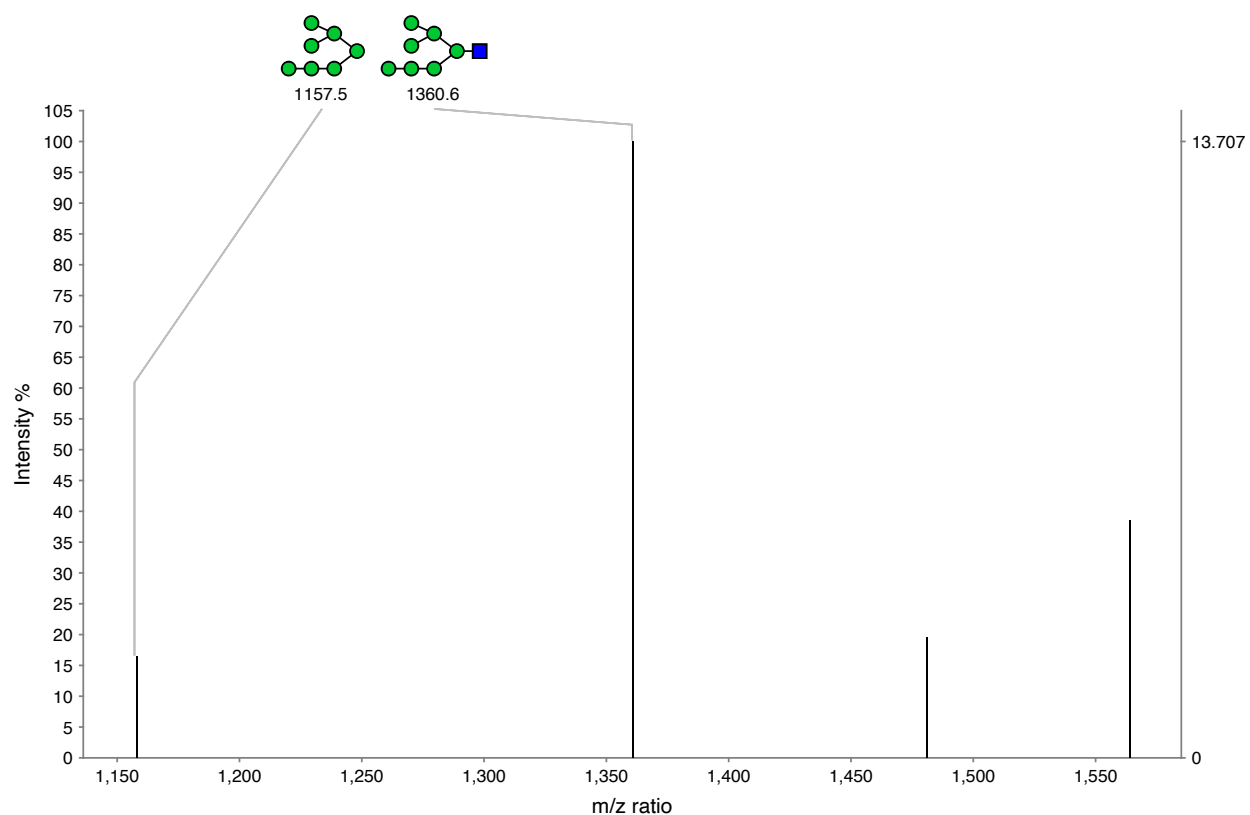
Supplemental Figure 10: Glycoworkbench generated annotation of 21T MALDI FTICR DDA fragmentation spectra of molecular ion m/z 1444.50788. Fragmentation spectra was annotated based on the most relevant glycoform as circled in red in Supplemental Table 14, which was identified from our data and additional literature review.



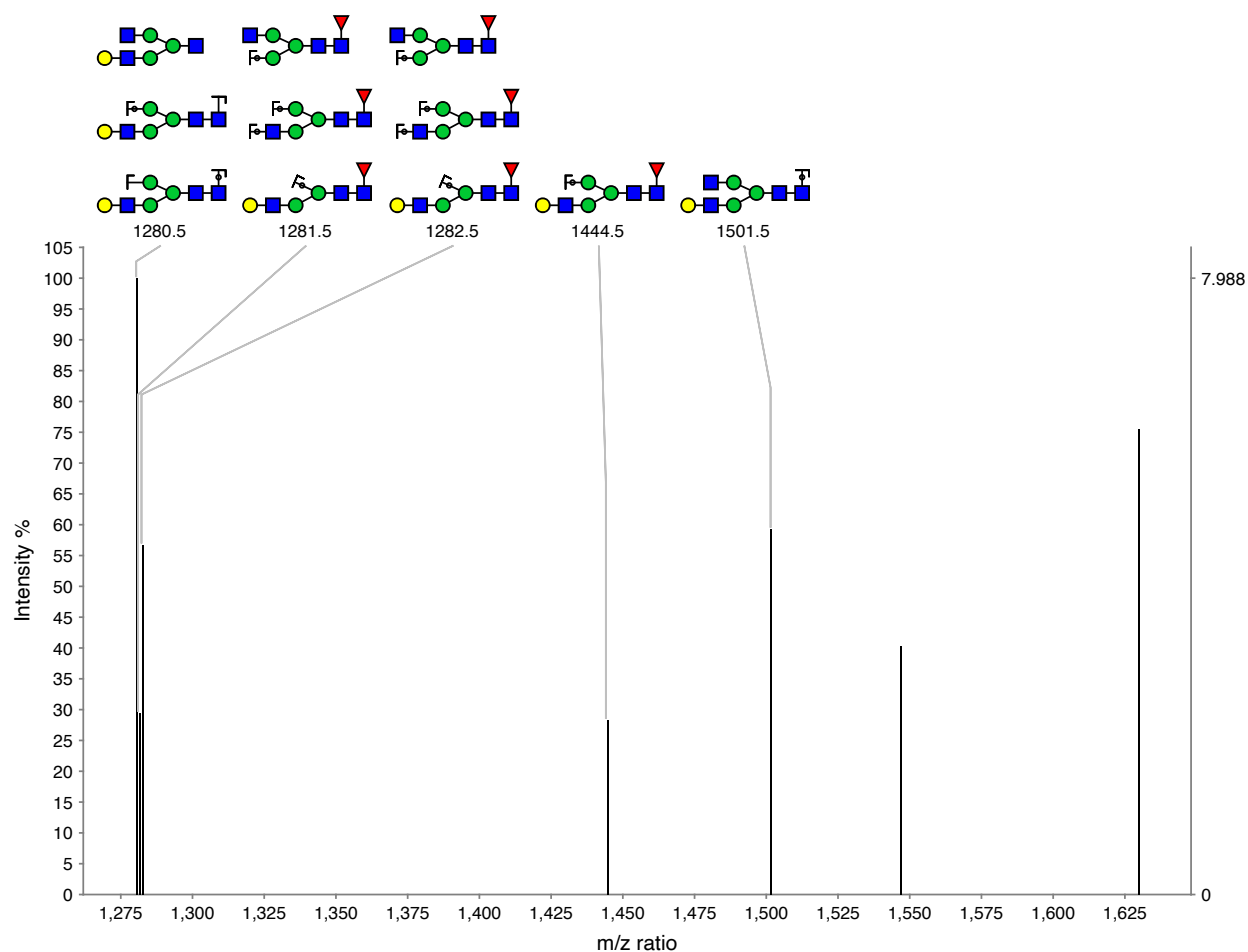
Supplemental Figure 11: Glycworkbench generated annotation of 21T MALDI FTICR DDA fragmentation spectra of molecular ion m/z 1485.53418. Fragmentation spectra was annotated based on the most relevant glycoform as circled in red in Supplemental Table 15, which was identified from our data and additional literature review.



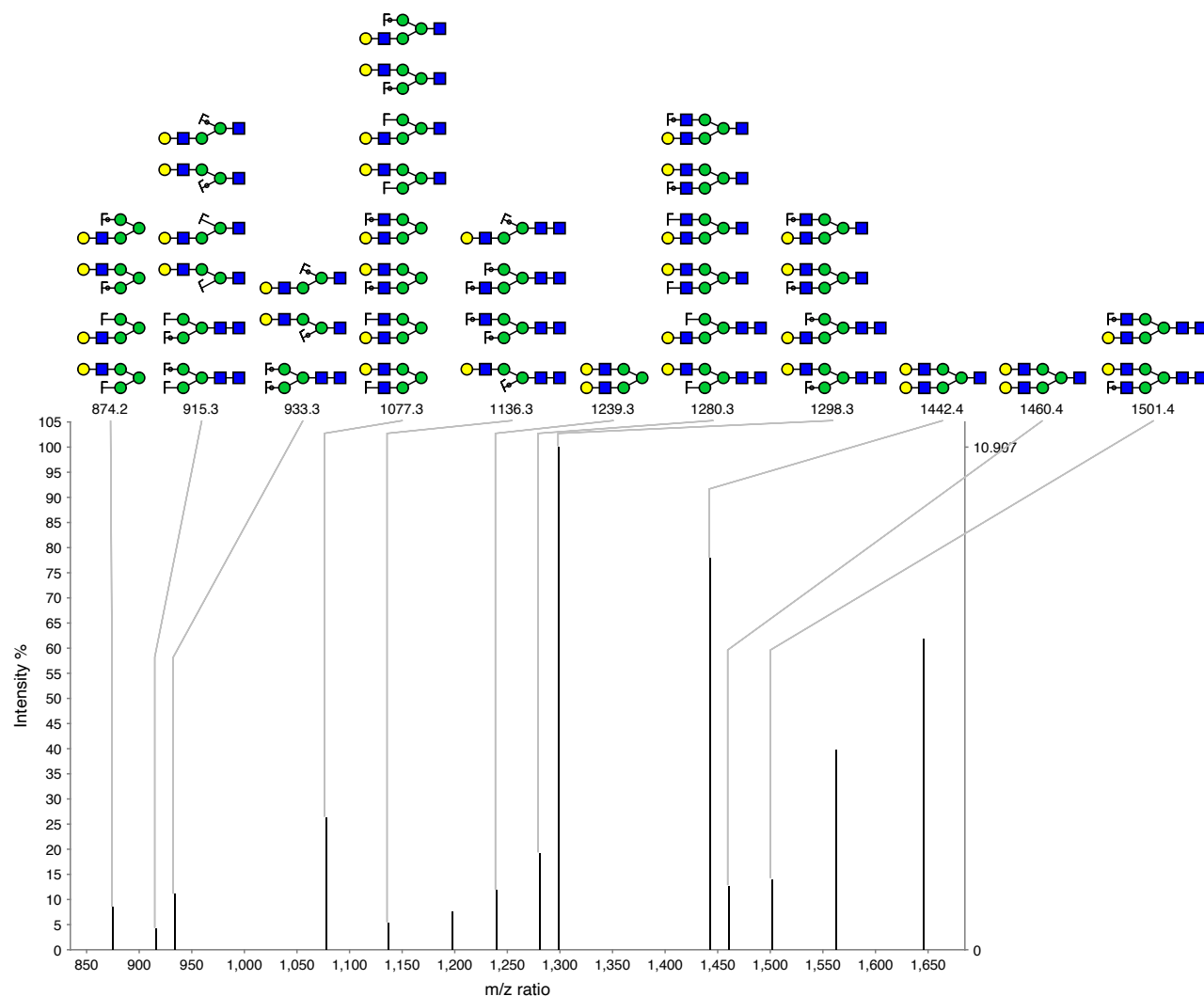
Supplemental Figure 12: Glycworkbench generated annotation of 21T MALDI FTICR DDA fragmentation spectra of molecular ion m/z 1501.52837. Fragmentation spectra was annotated based on the most relevant glycoform as circled in red in Supplemental Table 16, which was identified from our data and additional literature review.



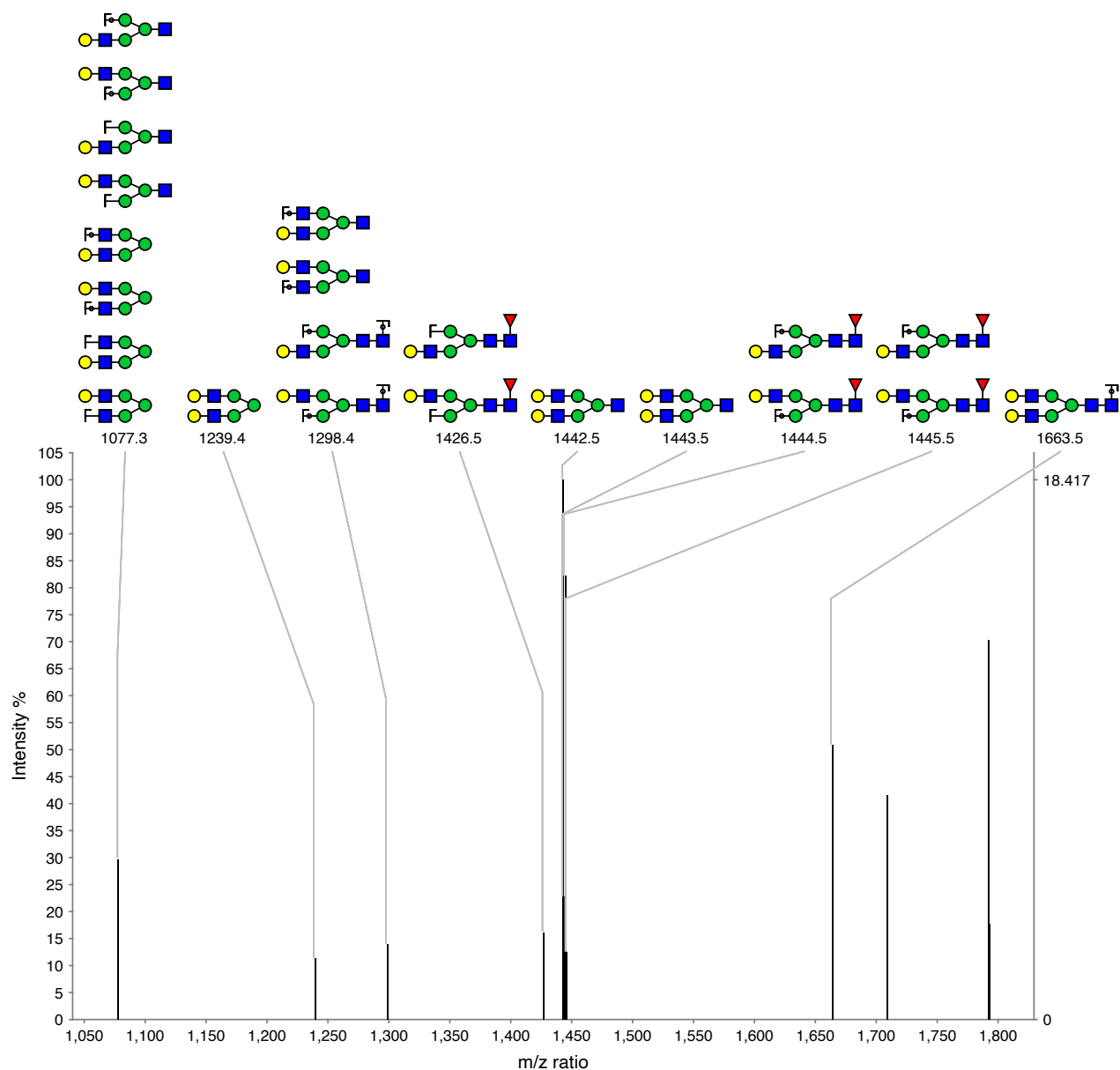
Supplemental Figure 13: Glycoworkbench generated annotation of 21T MALDI FTICR DDA fragmentation spectra of molecular ion m/z 1581.52821. Fragmentation spectra was annotated based on the most relevant glycoform as circled in red in Supplemental Table 17, which was identified from our data and additional literature review.



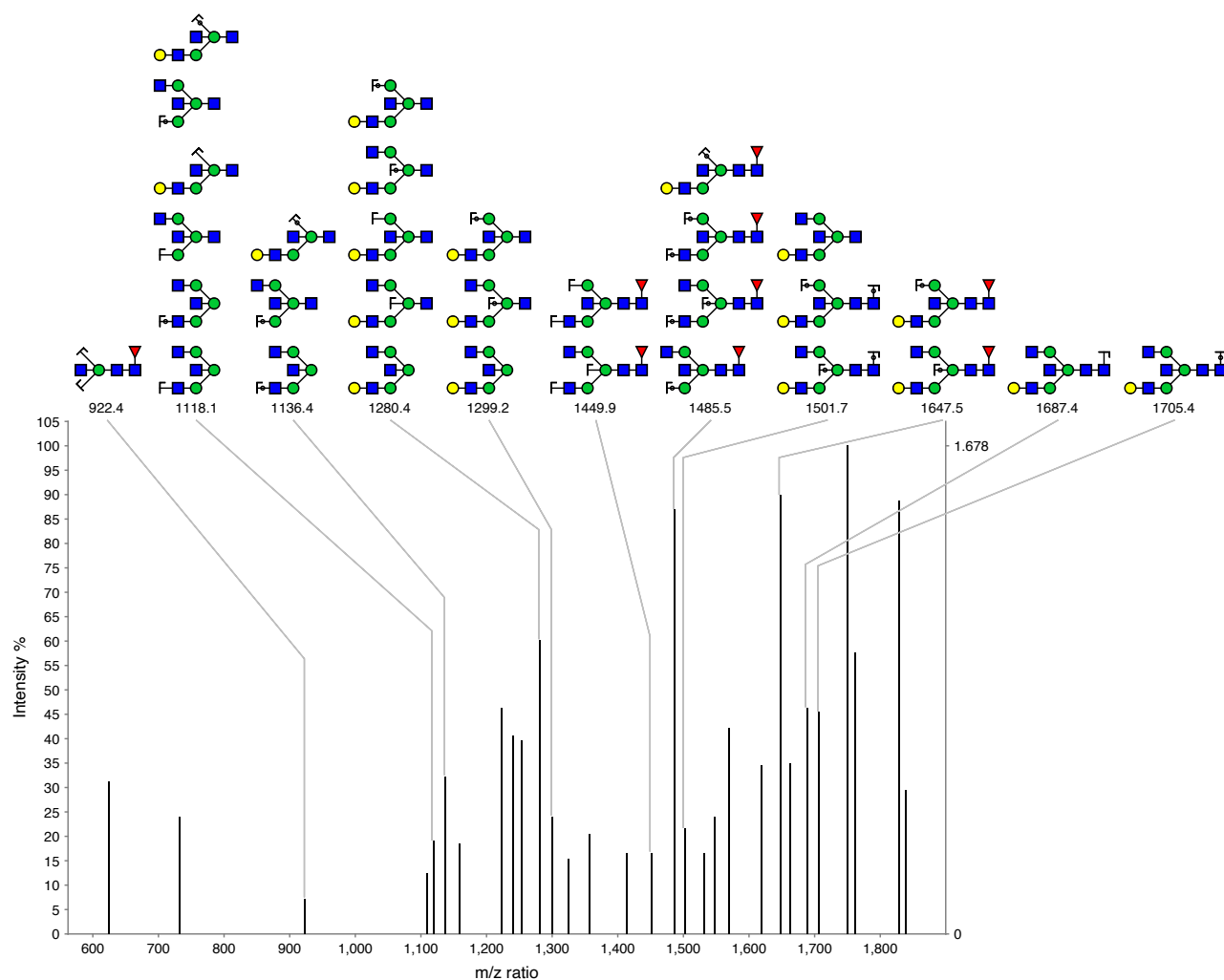
Supplemental Figure 14: Glycoworkbench generated annotation of 21T MALDI FTICR DDA fragmentation spectra of molecular ion m/z 1647.58700. Fragmentation spectra was annotated based on the most relevant glycoform as circled in red in Supplemental Table 18, which was identified from our data and additional literature review.



Supplemental Figure 15: Glycoworkbench generated annotation of 21T MALDI FTICR DDA fragmentation spectra of molecular ion m/z 1663.58113. Fragmentation spectra was annotated based on the most relevant glycoform as circled in red in Supplemental Table 19, which was identified from our data and additional literature review.



Supplemental Figure 16: Glycoworkbench generated annotation of 21T MALDI FTICR DDA fragmentation spectra of molecular ion m/z 1809.63931. Fragmentation spectra was annotated based on the most relevant glycoform as circled in red in Supplemental Table 20, which was identified from our data and additional literature review.



Supplemental Figure 17: Glycoworkbench generated annotation of 21T MALDI FTICR DDA fragmentation spectra of molecular ion m/z 1850.66465. Fragmentation spectra was annotated based on the most relevant glycoform as circled in red in Supplemental Table 21, which was identified from our data and additional literature review.

Supplemental Tables

Supplemental Table 1

Table 1: Frequency overview of tissue and annotation types present in the patient cohort																					
	Apoptotic Bodies	Cancer	Cancer mixed with Cancer-associated stroma	Cancer mixed with gliosis	Cancer mixed with lymphoplasmacytic infiltrate	Cancer mixed with meninges	Cancer mixed with stroma	DCIS mixed with lymphoplasmacytic infiltrate	Cancer-associated stroma	Necrosis	Normal	Stroma	Stroma (gliosis) adjacent to cancer	Stroma adjacent to cancer	Tumor infiltrating lymphocytes	Tumor stroma	Total TMAs for tissue type	Legend			
Abdominal Lymph Node	2	3	3	0	0	0	0	0	2	2	3	1	0	1	0	0	3	Number of annotations	14		
Adrenal Metastasis	0	5	6	0	0	0	0	0	3	2	5	1	0	1	0	0	7				
Adrenal Normal	0	1	1	0	0	0	0	0	1	0	10	1	0	0	0	0	10				
ANOTHER PATIENT Breast Primary	0	2	4	0	1	0	0	0	2	1	3	2	0	1	0	0	4				
Axillary Lymph Node	1	4	2	0	1	0	0	0	2	1	4	1	0	1	0	0	4				
Bone Metastasis	1	4	4	0	0	0	0	0	1	1	5	1	0	1	0	0	5				
Brain Metastasis	1	3	1	2	0	1	0	0	0	2	3	0	1	0	0	0	3		0		
Breast Normal	0	2	1	0	1	0	0	0	1	1	14	11	0	0	0	0	15				
Breast Primary	1	12	13	0	6	0	0	0	10	6	10	4	0	3	1	0	16				
Diaphragm Metastasis	0	4	5	0	0	0	0	0	4	3	1	1	0	0	0	1	5				
Initial Lymph Node Metastasis	1	3	2	0	2	0	1	0	0	1	3	0	0	1	0	0	3				
Kidney Normal	0	0	0	0	0	0	0	0	0	0	5	1	0	0	0	0	5				
Liver Metastasis	1	9	12	0	0	0	0	0	8	6	13	3	0	2	0	0	13				
Liver Normal	0	0	1	0	0	0	0	0	0	0	8	0	0	0	0	0	8				
Lung Metastasis	4	10	9	0	0	0	0	0	8	6	11	2	0	2	0	0	11				
Lung Normal	0	2	1	0	0	0	0	0	1	2	3	0	0	0	0	0	3				
Mediastinal Lymph Node	2	4	4	0	0	0	0	0	3	4	2	0	0	1	0	0	4				
Omentum Metastasis	1	2	3	0	0	0	0	0	1	3	2	1	0	1	0	0	3				
Ovary Normal	0	0	0	0	0	0	0	0	0	0	4	1	0	0	0	0	4				
Pericardium Metastasis	0	2	2	0	0	0	0	0	1	1	0	0	0	1	0	0	3				
Pleura Metastasis	0	2	4	0	0	0	0	0	2	1	4	0	0	0	0	0	4				
Rib Metastasis	1	3	3	0	0	0	0	0	1	1	2	0	0	0	0	0	3				
Spleen Normal	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	4				
Vertebra Metastasis	1	2	3	0	1	0	0	0	2	0	3	0	0	0	0	0	3				
Frequency table reduced to annotation types present in most tissue types																					
	Apoptotic bodies	Cancer	Cancer mixed with Cancer-associated stroma	Cancer-associated stroma	Necrosis	Normal	Stroma	Stroma adjacent to cancer	Total TMAs for tissue type												
Abdominal Lymph Node	2	3	3	2	2	3	1	1	3												
Adrenal Metastasis	0	5	6	3	2	5	1	1	7												
Adrenal Normal	0	1	1	1	0	10	1	0	10												
ANOTHER PATIENT Breast Primary	0	2	4	2	1	3	2	1	4												
Axillary Lymph Node	1	4	2	2	1	4	1	1	4												
Bone Metastasis	1	4	4	1	1	5	1	1	5												
Brain Metastasis	1	3	1	0	2	3	0	0	3												
Breast Normal	0	2	1	1	1	14	11	0	15												
Breast Primary	1	12	13	10	6	10	4	3	16												
Diaphragm Metastasis	0	4	5	4	3	1	1	0	5												
Initial Lymph Node Metastasis	1	3	2	0	1	3	0	1	3												
Kidney Normal	0	0	0	0	0	5	1	0	5												
Liver Metastasis	1	9	12	8	6	13	3	2	13												
Liver Normal	0	0	1	0	0	8	0	0	8												
Lung Metastasis	4	10	9	8	6	11	2	2	11												
Lung Normal	0	2	1	1	2	3	0	0	3												
Mediastinal Lymph Node	2	4	4	3	4	2	0	1	4												
Omentum Metastasis	1	2	3	1	3	2	1	1	3												
Ovary Normal	0	0	0	0	0	4	1	0	4												
Pericardium Metastasis	0	2	2	1	1	0	0	1	3												
Pleura Metastasis	0	2	4	2	1	4	0	0	4												
Rib Metastasis	1	3	3	1	1	2	0	0	3												
Spleen Normal	0	0	0	0	0	4	0	0	4												
Vertebra Metastasis	1	2	3	2	0	3	0	0	3												

Supplemental Table 2

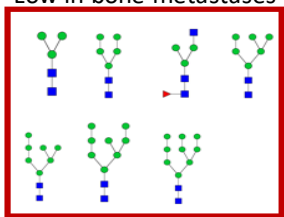
Supplementary Table 2: Pairwise t-test complete list of results						
Structure	Name	Experimental mass Rapiflex (+/- 0.2 Da)	p-value	Class 1	Class 2	Class 1 increased expression
	Not identified	885.5	0.048	Breast Normal_Normal	Breast Primary_Cancer mixed with cancer-associated stroma	FALSE
	Hex3HexNac2	933.4	0.046	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma	FALSE
	Hex5HexNac2	1257.5	0.025	Bone Metastasis_Cancer	Liver Metastasis_Cancer mixed with cancer-associated stroma	FALSE
		1257.5	0.008	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma	FALSE
		1257.5	0.019	Breast Normal_Normal	Lung Metastasis_Cancer	FALSE
		1257.5	0.012	Breast Normal_Normal	Lung Metastasis_Cancer mixed with cancer-associated stroma	FALSE
	Hex3dHex1HexNac3	1282.6	0.043	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma	FALSE
	Hex6HexNac2	1419.5	0.044	Bone Metastasis_Cancer	Liver Metastasis_Cancer mixed with cancer-associated stroma	FALSE
		1419.5	0.015	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma	FALSE
		1419.5	0.008	Breast Normal_Normal	Breast Primary_Cancer mixed with cancer-associated stroma	FALSE
		1419.5	0.021	Breast Normal_Normal	Liver Metastasis_Cancer	FALSE
		1419.5	0.036	Breast Normal_Normal	Liver Metastasis_Cancer mixed with cancer-associated stroma	FALSE
		1419.5	0.012	Breast Normal_Normal	Lung Metastasis_Cancer	FALSE
		1419.5	0.012	Breast Normal_Normal	Lung Metastasis_Cancer mixed with cancer-associated stroma	FALSE
	Hex4dHex1HexNac3	1444.6	0.012	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer	TRUE
		1444.6	0.023	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma	TRUE
	Hex3dHex1HexNac4	1485.6	0.021	Breast Normal_Normal	Liver Metastasis_Cancer	FALSE
		1485.6	0.036	Breast Normal_Normal	Liver Metastasis_Cancer mixed with cancer-associated stroma	FALSE
		1485.6	0.023	Breast Normal_Normal	Lung Metastasis_Cancer	FALSE
		1485.6	0.026	Breast Normal_Normal	Lung Metastasis_Cancer mixed with cancer-associated stroma	FALSE
		1485.6	0.042	Breast Primary_Cancer	Lung Metastasis_Cancer	FALSE
		1485.6	0.018	Breast Primary_Cancer	Lung Metastasis_Cancer mixed with cancer-associated stroma	FALSE
	Hex4HexNac4	1501.6	0.002	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer	TRUE
		1501.6	0.008	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma	TRUE
	Hex3HexNac5	1542.7	0.049	Bone Metastasis_Cancer mixed with cancer-associated stroma	Breast Normal_Normal	FALSE
		1542.7	0.042	Breast Primary_Cancer	Lung Metastasis_Cancer	FALSE
	Hex7HexNac2	1581.6	0.048	Bone Metastasis_Cancer	Liver Metastasis_Cancer mixed with cancer-associated stroma	FALSE
		1581.6	0.043	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma	FALSE
		1581.6	0.015	Breast Normal_Normal	Breast Primary_Cancer mixed with cancer-associated stroma	FALSE
		1581.6	0.021	Breast Normal_Normal	Liver Metastasis_Cancer	FALSE
		1581.6	0.036	Breast Normal_Normal	Liver Metastasis_Cancer mixed with cancer-associated stroma	FALSE
		1581.6	0.014	Breast Normal_Normal	Lung Metastasis_Cancer	FALSE
		1581.6	0.012	Breast Normal_Normal	Lung Metastasis_Cancer mixed with cancer-associated stroma	FALSE
	Hex4dHex1HexNac4	1647.7	0.049	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma	TRUE
	Hex5HexNac4	1663.7	0.040	Bone Metastasis_Cancer mixed with cancer-associated stroma	Breast Normal_Normal	TRUE
		1663.7	0.019	Bone Metastasis_Cancer mixed with cancer-associated stroma	Breast Primary_Cancer mixed with cancer-associated stroma	TRUE
		1663.7	0.019	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma	TRUE
	Hex4HexNac5	1704.8	0.043	Breast Normal_Normal	Liver Metastasis_Cancer mixed with cancer-associated stroma	TRUE
	Hex8HexNac2	1743.7	0.039	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma	FALSE
		1743.7	0.021	Breast Normal_Normal	Breast Primary_Cancer mixed with cancer-associated stroma	FALSE
		1743.7	0.038	Breast Normal_Normal	Liver Metastasis_Cancer	FALSE
		1743.7	0.049	Breast Normal_Normal	Liver Metastasis_Cancer mixed with cancer-associated stroma	FALSE
		1743.7	0.012	Breast Normal_Normal	Lung Metastasis_Cancer	FALSE
	Hex4dHex2HexNac4	1743.7	0.012	Breast Normal_Normal	Lung Metastasis_Cancer mixed with cancer-associated stroma	FALSE
		1793.7	0.049	Bone Metastasis_Cancer mixed with cancer-associated stroma	Breast Normal_Normal	TRUE
		1793.7	0.014	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer	TRUE
		1793.7	0.008	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma	TRUE
	Hex5dHex1HexNac4	1809.8	0.044	Bone Metastasis_Cancer	Liver Metastasis_Cancer mixed with cancer-associated stroma	TRUE
		1809.8	0.049	Bone Metastasis_Cancer mixed with cancer-associated stroma	Breast Normal_Normal	TRUE
		1809.8	0.001	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer	TRUE
		1809.8	0.008	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma	TRUE
		1809.8	0.008	Breast Normal_Normal	Breast Primary_Cancer mixed with cancer-associated stroma	FALSE
	Hex4dHex1HexNac5	1838.8	0.015	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma	TRUE
	Hex3dHex1HexNac6	1850.8	0.028	Breast Normal_Normal	Breast Primary_Cancer	TRUE
		1891.8	0.049	Bone Metastasis_Cancer mixed with cancer-associated stroma	Breast Normal_Normal	TRUE
		1891.8	0.046	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma	TRUE
		1891.8	0.022	Breast Normal_Normal	Lung Metastasis_Cancer mixed with cancer-associated stroma	FALSE
		1891.8	0.042	Breast Primary_Cancer	Lung Metastasis_Cancer	FALSE
	Hex9HexNac2	1891.8	0.006	Breast Primary_Cancer	Lung Metastasis_Cancer mixed with cancer-associated stroma	FALSE
		1905.8	0.046	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma	FALSE
		1905.8	0.046	Breast Normal_Normal	Breast Primary_Cancer mixed with cancer-associated stroma	FALSE
		1905.8	0.023	Breast Normal_Normal	Lung Metastasis_Cancer	FALSE
	Hex5dHex1HexNac4NeuAc1 + 2Na	1905.8	0.015	Breast Normal_Normal	Lung Metastasis_Cancer mixed with cancer-associated stroma	FALSE
		1976.8	0.023	Breast Normal_Normal	Lung Metastasis_Cancer	FALSE
		1976.8	0.012	Breast Normal_Normal	Lung Metastasis_Cancer mixed with cancer-associated stroma	FALSE
	Hex6HexNac5	2028.8	0.023	Breast Normal_Normal	Lung Metastasis_Cancer	FALSE
	Hex5dHex1HexNac4NeuAc1 + 2Na	2122.9	0.040	Breast Normal_Normal	Breast Primary_Cancer mixed with cancer-associated stroma	FALSE
	Hex6dHex1HexNac5	2150.8	0.023	Breast Normal_Normal	Lung Metastasis_Cancer	FALSE
		2150.8	0.015	Breast Normal_Normal	Lung Metastasis_Cancer mixed with cancer-associated stroma	FALSE
		2174.9	0.049	Bone Metastasis_Cancer mixed with cancer-associated stroma	Breast Normal_Normal	TRUE
		2174.9	0.014	Bone Metastasis_Cancer mixed with cancer-associated stroma	Breast Primary_Cancer	TRUE
	Hex5HexNac4NeuAc2 + 3Na	2174.9	0.029	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma	TRUE
		2174.9	0.046	Breast Normal_Normal	Breast Primary_Cancer mixed with cancer-associated stroma	FALSE
		2174.9	0.046	Breast Normal_Normal	Lung Metastasis_Cancer mixed with cancer-associated stroma	FALSE
	Hex7dHex1HexNac6	2289.9	0.048	Breast Normal_Normal	Liver Metastasis_Cancer	FALSE
		2289.9	0.014	Breast Normal_Normal	Lung Metastasis_Cancer	FALSE
		2289.9	0.012	Breast Normal_Normal	Lung Metastasis_Cancer mixed with cancer-associated stroma	FALSE
	Hex7dHex1HexNac6	2540.1	0.049	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma	TRUE

Supplemental Table 3

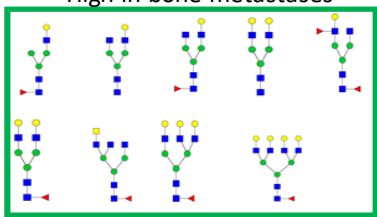
Supplementary Table 3: Significant N-glycans in pairwise comparisons between bone and liver metastases

m/z	p-value	class 1	class 2
933.4	0.046	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma
1257.5	0.008	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma
1282.5	0.043	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma
1419.6	0.015	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma
1444.6	0.023	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma
1501.6	0.008	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma
1581.6	0.043	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma
1647.7	0.049	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma
1663.7	0.019	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma
1743.6	0.039	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma
1793.7	0.008	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma
1809.7	0.008	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma
1838.7	0.015	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma
1891.8	0.046	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma
1905.7	0.046	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma
2174.9	0.029	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma
2540.1	0.049	Bone Metastasis_Cancer mixed with cancer-associated stroma	Liver Metastasis_Cancer mixed with cancer-associated stroma

Low in bone metastases



High in bone metastases



Supplemental Table 4

Supplementary Table 4: Surgical and therapeutic interventions of patients in cohort

[illegible]

Supplemental Table 5

Supplementary Table 5: Correlation analysis between levels of N-glycans and EpCAM in PT versus metastases

m/z value	Corrected p-value
1485.6	0.003
1257.5	0.013
1976.8	0.013
1647.7	0.04
1891.8	0.04
2028.8	0.04
2150.8	0.04

This list displays the overlap between differentially-expressed genes in the TCGA breast cancer dataset and known N-glycosylated proteins from dbPTM.

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Supplemental Table 7

Supplementary Table 7: Functional annotation of the 876 DE genes encoding N-glycosylated proteins from TCGA dataset

	Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score
Gene Ontology (GO)	extracellular matrix organization (GO:0030198)	72/229	6.77E-42	3.46E-38	0	0	7.178321469	680.4754505
	positive regulation of cell proliferation (GO:0008284)	66/424	1.77E-19	1.13E-16	0	0	3.553889894	157.4600434
	regulation of cell migration (GO:0030334)	54/316	5.72E-18	2.24E-15	0	0	3.901508583	154.902106
	cell-matrix adhesion (GO:0007160)	24/90	5.12E-13	7.46E-11	0	0	6.088280061	172.3025223
	regulation of cell proliferation (GO:0042127)	78/740	5.25E-13	7.45E-11	0	0	2.406516105	68.04403989
	extracellular matrix disassembly (GO:0022617)	22/78	1.38E-12	1.76E-10	0	0	6.439526987	175.8703527
	positive regulation of cell migration (GO:0030335)	37/221	1.94E-12	2.41E-10	0	0	3.822393025	103.0912297
	cell-cell adhesion via plasma-membrane adhesion molecules (GO:0098742)	28/142	1.78E-11	1.93E-09	0	0	4.501897228	111.438742
	homophilic cell adhesion via plasma membrane adhesion molecules (GO:0007156)	15/64	7.95E-08	4.22E-06	0	0	5.351027397	87.47724248
	heterotypic cell-cell adhesion (GO:0034113)	9/25	6.17E-07	2.58E-05	0	0	8.219178082	117.5170183
	negative regulation of cell adhesion (GO:0007162)	14/65	6.55E-07	2.61E-05	0	0	4.917456972	70.01967743
	regulation of focal adhesion assembly (GO:0051893)	11/44	2.17E-06	7.54E-05	0	0	5.707762557	74.42487642
	positive regulation of focal adhesion assembly (GO:0051894)	7/17	3.99E-06	1.27E-04	0	0	9.401020682	116.8649546
	negative regulation of cell motility (GO:2000146)	16/97	4.55E-06	1.43E-04	0	0	3.765946429	46.32102334
	angiogenesis involved in wound healing (GO:0060055)	5/8	7.99E-06	2.38E-04	0	0	14.26940639	167.4810028
	positive regulation of cell-matrix adhesion (GO:0001954)	9/33	8.50E-06	2.49E-04	0	0	6.226650062	72.70190731
	positive regulation of adherens junction organization (GO:1903393)	7/19	9.58E-06	2.75E-04	0	0	8.411439558	97.20360476
	positive regulation of epithelial cell migration (GO:0010634)	12/74	8.30E-05	0.001586046	0	0	3.702332469	34.79024848
	regulation of extracellular matrix organization (GO:1903053)	4/7	1.15E-04	0.002017599	0	0	13.04631442	118.331476
WikiPathways (WP)	Focal Adhesion-PI3K-Akt-mTOR-signaling pathway WP3932	60/303	3.29E-23	3.89E-21	0	0	4.521000045	234.0412101
	Focal Adhesion WP306	31/198	6.79E-10	2.46E-08	0	0	3.574558369	75.46255924
	Integrin-mediated Cell Adhesion WP185	14/101	1.28E-04	0.001311146	0	0	3.164700032	28.37214194
	TGF- β Signaling in Thyroid Cells for Epithelial-Mesenchymal Transition WP3859	5/18	8.47E-04	0.007406884	0	0	6.341958397	44.85883056
	Matrix Metalloproteinases WP129	5/30	0.009128085	0.051909109	0	0	3.805175038	17.87062168
	Amplification and Expansion of Oncogenic Pathways as Metastatic Traits WP3678	3/17	0.03598745	0.164913364	0	0	4.029008864	13.39478247

Supplemental Table 8

Supplementary Table 8: Resulting 308 DE genes encoding N-glycosylated proteins in GSE26338 dataset

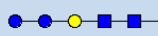
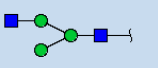
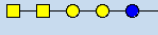
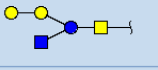
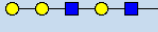
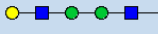
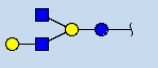
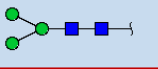
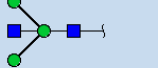
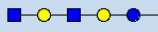
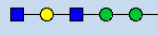
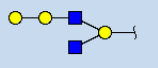
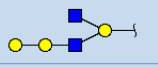
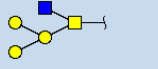
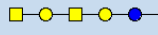
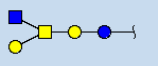
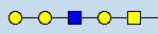
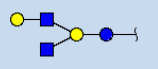
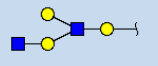
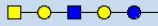
This list displays the overlap between differentially-expressed genes in the GSE26338 dataset and known N-glycosylated proteins from dbPTM.

Gene.symbol	adj.P.Val	Gene.symbol	adj.P.Val	Gene.symbol	adj.P.Val
C7	0.00021473	NUP214	0.07291294	OGN	0.13599787
COL17A1	0.00032831	IGSF11	0.07379834	MFAP4	0.13768498
OXTR	0.00045779	LGR4	0.07391314	EMILIN2	0.13774519
RELN	0.00062775	IKBIP	0.07482864	FASLG	0.13774519
FAM3D	0.000799	CD28	0.07574103	NCEH1	0.14086556
IGFBP6	0.00084894	LALBA	0.07580181	NPR3	0.14127643
PIGR	0.00109503	OLFM1	0.0764059	IL1RN	0.14120773
FCER1A	0.00161609	NPC2	0.0765377	NAGA	0.14149459
SLC6A1	0.00271445	PTPRC	0.07667442	FGI2	0.14187972
FCGR1A	0.00312369	NPR3	0.0772577	PKD2	0.14258903
JAM2	0.00351703	HTR2C	0.07744703	CD69	0.14258903
NEFH	0.0039843	HTR7	0.08028724	CD276	0.14352513
FCN3	0.00517982	ENPP4	0.08037265	LPL	0.14394055
CD1B	0.00566241	GGH	0.08075994	MAN2A2	0.14429294
CYBB	0.00738655	TNFRSF21	0.08105535	CFP	0.14435221
IL4I1	0.00785827	CD1B	0.08143671	TINAGL1	0.14435326
MME	0.00795772	SLC2A1	0.08205011	FOLR1	0.14605548
DLK1	0.00824349	ITGA4	0.08220142	PTPRK	0.14660686
ADAMTS5	0.00834761	OPTC	0.08267688	PTPRC	0.14647697
CLU	0.00837689	CTBS	0.08523224	CIQA	0.14738122
LTF	0.00901516	PROS1	0.08581285	TRPM8	0.14761953
JCHAIN	0.00952398	ITGAL	0.08581285	SLC20A2	0.14883849
KIT	0.00999397	CD3G	0.08602945	HEXA	0.14919919
APOD	0.01122914	MAN2B1	0.08749123	IFNA14	0.15018937
SMPD1	0.01241789	SELE	0.08776969	DRD5	0.15044521
CHST11	0.01405047	HLA-DPA1	0.08863785	SLC4A4	0.15044521
CD1A	0.01581637	SULF2	0.08900559	PTPRC	0.15046371
NTRK1	0.01593238	CD48	0.08925463	LGR5	0.15102788
TNFSF13B	0.01613476	DMBT1	0.08933714	PRRC2C	0.15110715
FN1	0.0170812	GCGR	0.09087724	CD52	0.15173987
ACPS	0.01738137	CAOM4	0.09107363	CLN5	0.15184923
SERPINA5	0.01956864	TIMP1	0.09162626	PAMR1	0.15226899
SFRP1	0.02115689	KLK6	0.0946198	AZGP1	0.15251309
MYC	0.02410919	ITGB2	0.09517767	ADGRE5	0.15400052
LY96	0.02637397	ADAMTS14	0.0952361	AGA	0.15415969
HCFC1	0.02646028	CD8D	0.09524821	LIFR	0.15518347
PTPRC	0.02707138	SULF1	0.09524821	GNPTG	0.15650897
TCN1	0.02714003	HP	0.09528021	PRB4	0.15674411
VCAM1	0.02987589	CD83	0.09617571	CP	0.15785171
PIGR	0.03053991	CD244	0.0970441	TEK	0.15941683
AOAH	0.03139933	DSG2	0.09764836	GC	0.16005128
GLA	0.03151257	SAA4	0.09818756	IMPAD1	0.16036034
HBB	0.03467566	HGSNAT	0.09842361	CD69	0.16070966
LAMP3	0.03555362	IL7R	0.09857093	FCGR3A	0.16086515
PTPRC	0.0365266	DSC2	0.09882618	PLXNB1	0.16117859
CTSF	0.03657617	HLA-DRA	0.10051936	MAFK	0.16200912
ADAMDEC1	0.03662282	PPT1	0.10097342	VNN2	0.16266619
PLXNB2	0.03662282	F10	0.10160893	CD69	0.16374546
EFNB2	0.03729664	IGFBP4	0.10209807	ITGA7	0.16536035
IL2RA	0.03855741	SIAE	0.10262728	FSTL1	0.16546558
PAHA1	0.03872473	PLXNC1	0.10399168	PTPRK	0.16584515
CLEC2D	0.03908168	LGALS3BP	0.10576008	LAMP1	0.16612852
IL18BP	0.03964618	HLA-A	0.1060094	APOM	0.16613051
CD33	0.03964618	CNTN1	0.10616418	LAMB2	0.16757858
GZMA	0.04111542	HEG1	0.10616418	HSPG2	0.16953671
EPCAM	0.04139243	LRP1	0.10616418	SAA4	0.17181065
CBLN1	0.0415397	COL6A1	0.1084542	CALU	0.17313706
PLXND1	0.0415397	ATP1B1	0.10813222	SPP1	0.1736391
MAL2	0.04179288	HIVEP1	0.10849819	LAMA2	0.17429482
CLEC3B	0.04194968	SIT1	0.10877759	ATF6	0.17490383
CPVL	0.0421194	TG	0.10909493	TNFRSF14	0.1751816
ACKR1	0.04298696	SSR1	0.10909493	CDH6	0.17654757
C2	0.04352683	ACAN	0.10965237	IGFBP7	0.17656222
EPHA2	0.04588013	DPP4	0.10993933	JAM3	0.17778858
HLA-DMB	0.04787539	HSP90B1	0.11133507	MST1R	0.17799033
CD33	0.04817963	ENPP7	0.11191161	POFUT1	0.17824051
GAL3ST1	0.05085268	USP9X	0.11193591	PRNP	0.17848802
YTHDF3	0.05136267	LRPAP1	0.11306918	SIRPG	0.18035182
LTPB1	0.0518538	FAM209B	0.11306918	SPP1	0.18037749
CERS2	0.05240546	BP1FA2	0.11316527	CLEC12A	0.18140224
CD163	0.05354541	CTSA	0.11354125	LAIR1	0.18184225
PIOD2	0.05446937	POGLUT1	0.11354125	TXNDC15	0.18227661
IGF2	0.05456526	LBP	0.11370696	PNLIPRP2	0.18269568
F3	0.05458351	C3	0.11419148	G6PC2	0.18282461
EMC10	0.05827802	CREB3L3	0.11484129	AGT	0.18282461
C15	0.05980578	ICOSLG	0.11482638	SEMA4B	0.18307833
OLFML3	0.06134661	TRPV6	0.11491244	ENPEP	0.18324374
GRN	0.06148578	PTK7	0.1151565	SPP1	0.18546298
TSPAN9	0.06218356	FIP1L1	0.11598467	CD69	0.18565236
CP	0.06385236	CHST7	0.116163	P2RX7	0.1860947
KCNE1	0.06486243	LRIG1	0.11715651	F7	0.18674083
NPTX1	0.0651879	SIRPA	0.11796554	ICAM3	0.18731227
MMP1	0.06618106	B2M	0.1202161	RHO	0.1872057
EDEM3	0.06711955	STT3B	0.12056288	COLGALT1	0.18768322
SEL1L	0.06749721	PTGIR	0.120585	FKBP9	0.18860475
B3GNT2	0.06790647	ITGAX	0.12195725	S100BPB	0.18922597
SIGLEC7	0.06828863	STIM1	0.12177285	SPP1	0.18948869
FSTL3	0.06828863	SLC14A2	0.12254411	CD69	0.18988816
CD59	0.06852069	ANPEP	0.1246027	SGCE	0.19011233
CD74	0.06951933	CD14	0.12623751	SPP1	0.19023851
CD300LF	0.0700461	CES1	0.12661855	TNFRSF11B	0.19023851
HLA-C	0.07030561	BPTF	0.1266272	SPP1	0.19053247
DCN	0.07037481	FCGR2A	0.12664867	WNT3	0.19058873
ATP1B1	0.07063892	MFSO2A	0.12695713	ERMP1	0.19069836
HLA-DRB5	0.07118308	ST8SIA4	0.12861265	ARSD	0.19069836
CSF2RA	0.07146285	SLC4A7	0.12964471	ICAM1	0.19096054
HLA-G	0.07181205	KCNH6	0.13034256	FLRT2	0.1911438
TMEM26	0.07203801	KITLG	0.13101943	GUSB	0.19307662
HLA-DRB1	0.07203801	CSF1R	0.13128311	HLA-B	0.19384656
CTSB	0.072515	UMOD	0.13469094	CD69	0.19422823
HLA-DRB3	0.07290741	PLXDC2	0.13560113	AOC3	0.19504846
				HLA-DRB5	0.19520794
				F12	0.19560954
				HLA-A	0.19560954

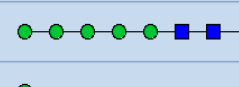
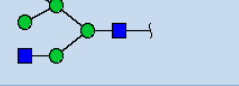
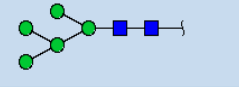
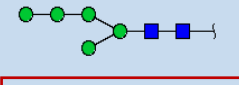
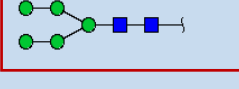
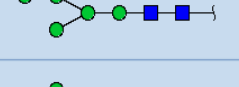
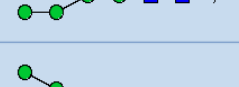
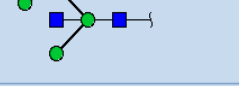
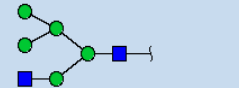
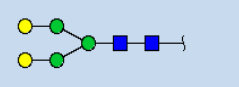
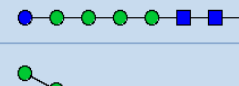
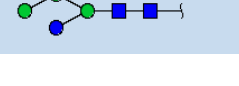
Supplemental Table 9: Accurate masses from 21T MALDI-FTICR DDA data

Name	21T experimental mass	Theoretical mass	ppm error
Hex3HxNAc2	933.31688	933.317001	-0.13
Hex5HexNAc2	1257.42247	1257.422647	-0.14
Hex3dHex1HexNAc3	1282.45432	1282.454283	0.03
Hex6HexNAc2	1419.47530	1419.475470	-0.12
Hex4dHex1HexNAc3	1444.50788	1444.507106	0.54
Hex3dHex1HexNAc4	1485.53418	1485.533656	0.35
Hex4HexNAc4	1501.52837	1501.528570	-0.13
Hex7HexNAc2	1581.52821	1581.528293	-0.05
Hex4dHex1HexNAc4	1647.58700	1647.586479	0.32
Hex5HexNAc4	1663.58113	1663.581393	-0.16
Hex5dHex1HexNAc4	1809.63931	1809.639302	0.00
Hex4dHex1HexNAc5	1850.66465	1850.665852	-0.65

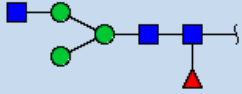
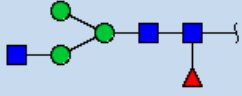
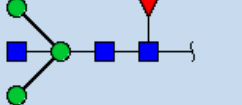
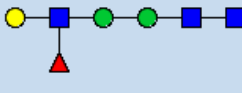
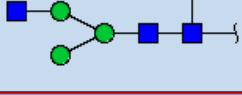
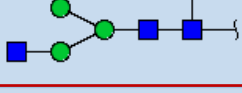
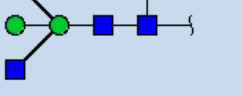
Supplemental Table 10: Glycoworkbench table for m/z 933.31688 (21T MALDI-FTICR DDA data). The N-glycan circled in red was selected as the most relevant glycoform detected based on additional literature review and shown as annotated spectra in Supplemental Figure 6.

Structure	Coverage	RMSD	RMSD PPM	≧ Assigned	> 10% assigned	> 5% assigned
	64.0979	0.0389	54.5255	7/11 (63.64%)	4/7 (57.14%)	7/11 (63.64%)
	64.0979	0.0389	54.5255	7/11 (63.64%)	4/7 (57.14%)	7/11 (63.64%)
	64.0979	0.0389	54.5255	7/11 (63.64%)	4/7 (57.14%)	7/11 (63.64%)
	64.0979	0.0389	54.5255	7/11 (63.64%)	4/7 (57.14%)	7/11 (63.64%)
	64.0979	0.0389	54.5255	7/11 (63.64%)	4/7 (57.14%)	7/11 (63.64%)
	64.0979	0.0389	54.5255	7/11 (63.64%)	4/7 (57.14%)	7/11 (63.64%)
	64.0979	0.0389	54.5255	7/11 (63.64%)	4/7 (57.14%)	7/11 (63.64%)
	61.7049	0.0416	57.7674	6/11 (54.55%)	4/7 (57.14%)	6/11 (54.55%)
	61.7049	0.0416	57.7674	6/11 (54.55%)	4/7 (57.14%)	6/11 (54.55%)
	61.7049	0.0416	57.7674	6/11 (54.55%)	4/7 (57.14%)	6/11 (54.55%)
	61.7049	0.0416	57.7674	6/11 (54.55%)	4/7 (57.14%)	6/11 (54.55%)
	61.7049	0.0416	57.7674	6/11 (54.55%)	4/7 (57.14%)	6/11 (54.55%)
	61.2430	0.0419	58.3262	6/11 (54.55%)	4/7 (57.14%)	6/11 (54.55%)
	61.2430	0.0419	58.3262	6/11 (54.55%)	4/7 (57.14%)	6/11 (54.55%)
	61.7049	0.0416	57.7674	6/11 (54.55%)	4/7 (57.14%)	6/11 (54.55%)
	61.7049	0.0416	57.7674	6/11 (54.55%)	4/7 (57.14%)	6/11 (54.55%)
	61.7049	0.0416	57.7674	6/11 (54.55%)	4/7 (57.14%)	6/11 (54.55%)
	61.7049	0.0416	57.7674	6/11 (54.55%)	4/7 (57.14%)	6/11 (54.55%)
	61.2430	0.0419	58.3262	6/11 (54.55%)	4/7 (57.14%)	6/11 (54.55%)
	61.7049	0.0416	57.7674	6/11 (54.55%)	4/7 (57.14%)	6/11 (54.55%)
	61.7049	0.0416	57.7674	6/11 (54.55%)	4/7 (57.14%)	6/11 (54.55%)
	61.7049	0.0416	57.7674	6/11 (54.55%)	4/7 (57.14%)	6/11 (54.55%)
	61.7049	0.0416	57.7674	6/11 (54.55%)	4/7 (57.14%)	6/11 (54.55%)
	61.7049	0.0416	57.7674	6/11 (54.55%)	4/7 (57.14%)	6/11 (54.55%)
	61.7049	0.0416	57.7674	6/11 (54.55%)	4/7 (57.14%)	6/11 (54.55%)

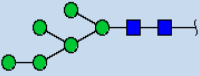
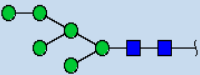
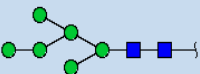
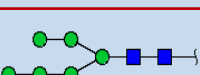
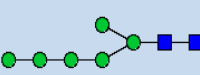
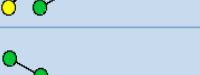
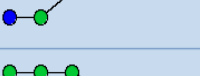
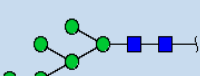
Supplemental Table 11: Glycworkbench table for m/z 1257.42247 (21T MALDI-FTICR DDA data). The N-glycan circled in red was selected as the most relevant glycoform detected based on additional literature review and shown as annotated spectra in Supplemental Figure 7.

Structure	Coverage	RMSD	RMSD PPM	Assigned	>10% assigned	>5% assigned
	49.1446	0.1358	136.2377	5/12 (41.67%)	3/7 (42.86%)	5/12 (41.67%)
	49.1446	0.1358	136.2377	5/12 (41.67%)	3/7 (42.86%)	5/12 (41.67%)
	49.1446	0.1358	136.2377	5/12 (41.67%)	3/7 (42.86%)	5/12 (41.67%)
	49.1446	0.1358	136.2377	5/12 (41.67%)	3/7 (42.86%)	5/12 (41.67%)
	49.1446	0.1358	136.2377	5/12 (41.67%)	3/7 (42.86%)	5/12 (41.67%)
	49.1446	0.1358	136.2377	5/12 (41.67%)	3/7 (42.86%)	5/12 (41.67%)
	49.1446	0.1358	136.2377	5/12 (41.67%)	3/7 (42.86%)	5/12 (41.67%)
	49.1446	0.1358	136.2377	5/12 (41.67%)	3/7 (42.86%)	5/12 (41.67%)
	49.1446	0.1358	136.2377	5/12 (41.67%)	3/7 (42.86%)	5/12 (41.67%)
	49.1446	0.1358	136.2377	5/12 (41.67%)	3/7 (42.86%)	5/12 (41.67%)
	49.1446	0.1358	136.2377	5/12 (41.67%)	3/7 (42.86%)	5/12 (41.67%)
	49.1446	0.1358	136.2377	5/12 (41.67%)	3/7 (42.86%)	5/12 (41.67%)
	49.1446	0.1358	136.2377	5/12 (41.67%)	3/7 (42.86%)	5/12 (41.67%)
	49.1446	0.1358	136.2377	5/12 (41.67%)	3/7 (42.86%)	5/12 (41.67%)
	49.1446	0.1358	136.2377	5/12 (41.67%)	3/7 (42.86%)	5/12 (41.67%)
	49.1446	0.1358	136.2377	5/12 (41.67%)	3/7 (42.86%)	5/12 (41.67%)
	49.1446	0.1358	136.2377	5/12 (41.67%)	3/7 (42.86%)	5/12 (41.67%)

Supplemental Table 12: Glycworkbench table for m/z 1282.45432 (21T MALDI-FTICR DDA data). The N-glycan circled in red was selected as the most relevant glycoform detected based on additional literature review and shown as annotated spectra in Supplemental Figure 8.

Structure	Coverage	RMSD	RMSD PPM	Assigned	>10% assigned	>5% assigned
	65.3292	0.0766	85.5373	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)
	65.3292	0.0766	85.5373	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)
	65.3292	0.0766	85.5373	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)
	65.3292	0.0766	85.5373	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)
	65.3292	0.0766	85.5373	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)
	65.3292	0.0766	85.5373	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)
	65.3292	0.0766	85.5373	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)

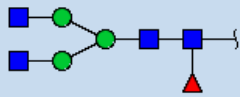
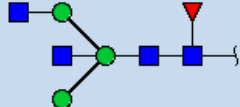
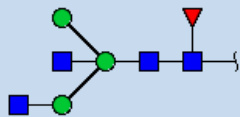
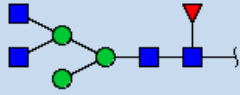
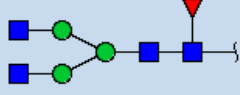
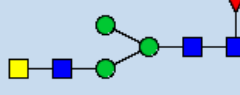
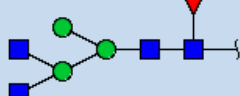
Supplemental Table 13: Glycoworkbench table for m/z 1419.47530 (21T MALDI-FTICR DDA data). The N-glycan circled in red was selected as the most relevant glycoform detected based on additional literature review and shown as annotated spectra in Supplemental Figure 9.

Structure	Coverage	RMSD	RMSD PPM	Assigned	>10% assigned	>5% assigned
	67.6042	0.1709	155.8453	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	67.6042	0.1709	155.8453	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	67.6042	0.1709	155.8453	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	67.6042	0.1709	155.8453	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	67.6042	0.1709	155.8453	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	67.6042	0.1709	155.8453	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	67.6042	0.1709	155.8453	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	67.6042	0.1709	155.8453	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	67.6042	0.1709	155.8453	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	67.6042	0.1709	155.8453	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	67.6042	0.1709	155.8453	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	67.6042	0.1709	155.8453	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	67.6042	0.1709	155.8453	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	67.6042	0.1709	155.8453	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	67.6042	0.1709	155.8453	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)

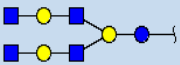
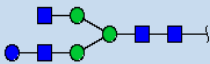
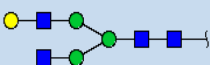
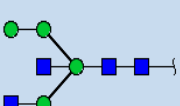
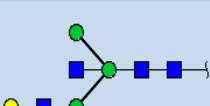
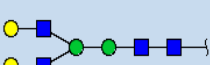
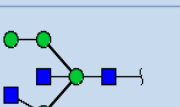
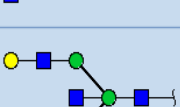
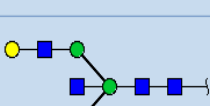
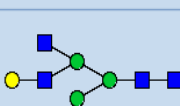
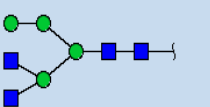
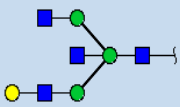
Supplemental Table 14: Glycoworkbench table for m/z 1444.50788 (21T MALDI-FTICR DDA data). The N-glycan circled in red was selected as the most relevant glycoform detected based on additional literature review and shown as annotated spectra in Supplemental Figure 10.

Structure	Coverage	RMSE	RMSE PPM	Assigned	>10% assigned	>5% assigned
	73.7158	0.4409	408.9515	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)
	73.7158	0.4409	408.9515	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)
	73.7158	0.4409	408.9515	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)
	73.7158	0.4409	408.9515	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)
	73.7158	0.4409	408.9515	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)
	73.7158	0.4409	408.9515	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)
	73.7158	0.4409	408.9515	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)
	73.7158	0.4409	408.9515	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)
	73.7158	0.4409	408.9515	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)
	73.7158	0.4409	408.9515	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)
	73.7158	0.4409	408.9515	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)
	73.7158	0.4409	408.9515	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)

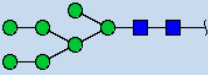
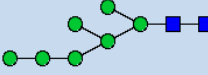
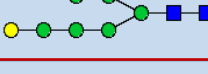
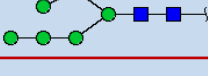
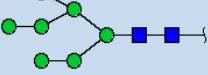
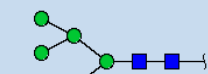
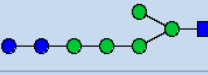
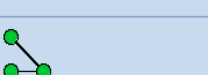
Supplemental Table 15: Glycoworkbench table for m/z 1485.53418 (21T MALDI-FTICR DDA data). The N-glycan circled in red was selected as the most relevant glycoform detected based on additional literature review and shown as annotated spectra in Supplemental Figure 11.

Structure	Coverage	RMSD	RMSD PPM	Assigned	>10% assigned	>5% assigned
	65.6390	0.0175	13.6311	5/8 (62.50%)	5/8 (62.50%)	5/8 (62.50%)
	65.6390	0.0175	13.6311	5/8 (62.50%)	5/8 (62.50%)	5/8 (62.50%)
	65.6390	0.0175	13.6311	5/8 (62.50%)	5/8 (62.50%)	5/8 (62.50%)
	65.6390	0.0175	13.6311	5/8 (62.50%)	5/8 (62.50%)	5/8 (62.50%)
	65.6390	0.0175	13.6311	5/8 (62.50%)	5/8 (62.50%)	5/8 (62.50%)
	65.6390	0.0175	13.6311	5/8 (62.50%)	5/8 (62.50%)	5/8 (62.50%)
	65.6390	0.0175	13.6311	5/8 (62.50%)	5/8 (62.50%)	5/8 (62.50%)
	65.6390	0.0175	13.6311	5/8 (62.50%)	5/8 (62.50%)	5/8 (62.50%)

Supplemental Table 16: Glycworkbench table for m/z 1501.52837 (21T MALDI-FTICR DDA data). The N-glycan circled in red was selected as the most relevant glycoform detected based on additional literature review and shown as annotated spectra in Supplemental Figure 12.

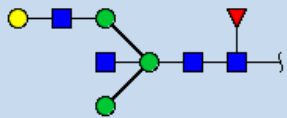
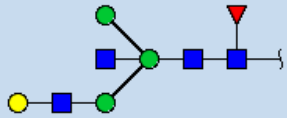
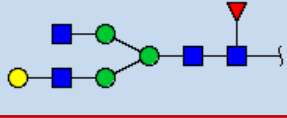
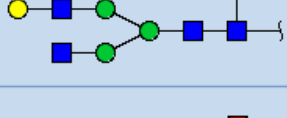
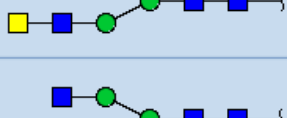
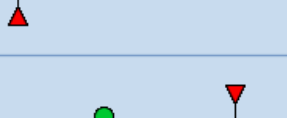
Structure	Coverage	RMSD	RMSD PPM	Assigned	>10% assigned	>5% assigned
	50.2272	0.4171	314.0458	6/15 (40.00%)	6/15 (40.00%)	6/15 (40.00%)
	50.2272	0.4171	314.0458	6/15 (40.00%)	6/15 (40.00%)	6/15 (40.00%)
	50.2272	0.4171	314.0458	6/15 (40.00%)	6/15 (40.00%)	6/15 (40.00%)
	50.2272	0.4171	314.0458	6/15 (40.00%)	6/15 (40.00%)	6/15 (40.00%)
	50.2272	0.4171	314.0458	6/15 (40.00%)	6/15 (40.00%)	6/15 (40.00%)
	50.2272	0.4171	314.0458	6/15 (40.00%)	6/15 (40.00%)	6/15 (40.00%)
	50.2272	0.4171	314.0458	6/15 (40.00%)	6/15 (40.00%)	6/15 (40.00%)
	50.2272	0.4171	314.0458	6/15 (40.00%)	6/15 (40.00%)	6/15 (40.00%)
	50.2272	0.4171	314.0458	6/15 (40.00%)	6/15 (40.00%)	6/15 (40.00%)
	50.2272	0.4171	314.0458	6/15 (40.00%)	6/15 (40.00%)	6/15 (40.00%)
	50.2272	0.4171	314.0458	6/15 (40.00%)	6/15 (40.00%)	6/15 (40.00%)
	50.2272	0.4171	314.0458	6/15 (40.00%)	6/15 (40.00%)	6/15 (40.00%)
	50.2272	0.4171	314.0458	6/15 (40.00%)	6/15 (40.00%)	6/15 (40.00%)
	50.2272	0.4171	314.0458	6/15 (40.00%)	6/15 (40.00%)	6/15 (40.00%)

Supplemental Table 17: Glycworkbench table for m/z 1581.52821 (21T MALDI-FTICR DDA data). The N-glycan circled in red was selected as the most relevant glycoform detected based on additional literature review and shown as annotated spectra in Supplemental Figure 13.

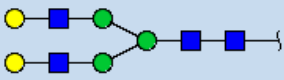
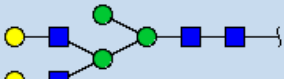
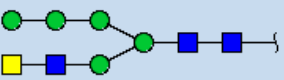
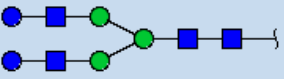
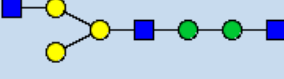
Structure	Coverage	RMSD	RMSD PPM	Assigned	>10% assigned	>5% assigned
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)

Structure	Coverage	RMSD	RMSD PPM	Assigned	>10% assigned	>5% assigned
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)
	66.7536	0.1553	128.4401	2/4 (50.00%)	2/4 (50.00%)	2/4 (50.00%)

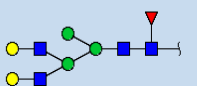
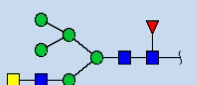
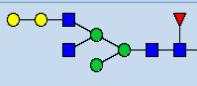
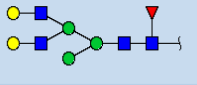
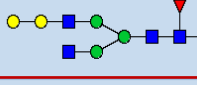
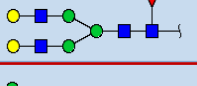
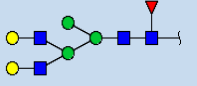
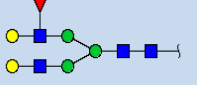
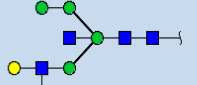
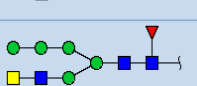
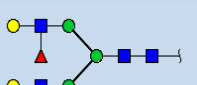
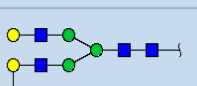
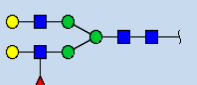
Supplemental Table 18: Glycworkbench table for m/z 1647.58700 (21T MALDI-FTICR DDA data). The N-glycan circled in red was selected as the most relevant glycoform detected based on additional literature review and shown as annotated spectra in Supplemental Figure 14.

Structure	Coverage	RMSD	RMSD PPM	Assigned	>10% assigned	>5% assigned
	70.2555	0.4387	342.2293	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)
	70.2555	0.4387	342.2293	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)
	70.2555	0.4387	342.2293	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)
	70.2555	0.4387	342.2293	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)
	70.2555	0.4387	342.2293	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)
	70.2555	0.4387	342.2293	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)
	70.2555	0.4387	342.2293	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)
	70.2555	0.4387	342.2293	5/7 (71.43%)	5/7 (71.43%)	5/7 (71.43%)

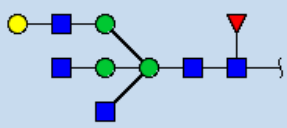
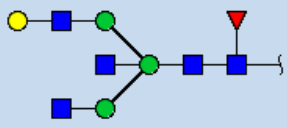
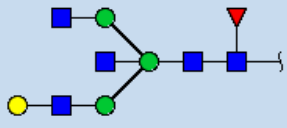
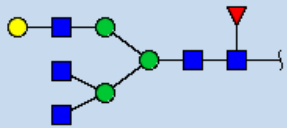
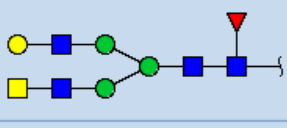
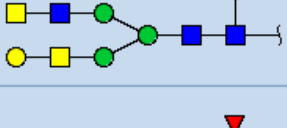
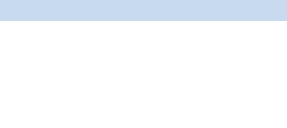
Supplemental Table 19: Glycoworkbench table for m/z 1663.58113 (21T MALDI-FTICR DDA data). The N-glycan circled in red was selected as the most relevant glycoform detected based on additional literature review and shown as annotated spectra in Supplemental Figure 15.

Structure	Coverage	RMSD	RMSD PPM	Assigned	>10% assigned	>5% assigned
	72.7077	0.1107	86.1643	11/14 (78.57%)	8/10 (80.00%)	10/13 (76.92%)
	72.7077	0.1107	86.1643	11/14 (78.57%)	8/10 (80.00%)	10/13 (76.92%)
	72.7077	0.1107	86.1643	11/14 (78.57%)	8/10 (80.00%)	10/13 (76.92%)
	72.7077	0.1107	86.1643	11/14 (78.57%)	8/10 (80.00%)	10/13 (76.92%)
	72.7077	0.1107	86.1643	11/14 (78.57%)	8/10 (80.00%)	10/13 (76.92%)
	72.7077	0.1107	86.1643	11/14 (78.57%)	8/10 (80.00%)	10/13 (76.92%)
	72.7077	0.1107	86.1643	11/14 (78.57%)	8/10 (80.00%)	10/13 (76.92%)

Supplemental Table 20: Glycoworkbench table for m/z 1809.63931 (21T MALDI-FTICR DDA data). The N-glycan circled in red was selected as the most relevant glycoform detected based on additional literature review and shown as annotated spectra in Supplemental Figure 16.

Structure	Coverage	RMSD	RMSD PPM	Assigned	>10% assigned	>5% assigned
	72.3711	0.4630	320.5543	9/12 (75.00%)	9/12 (75.00%)	9/12 (75.00%)
	72.3711	0.4630	320.5543	9/12 (75.00%)	9/12 (75.00%)	9/12 (75.00%)
	72.3711	0.4630	320.5543	9/12 (75.00%)	9/12 (75.00%)	9/12 (75.00%)
	72.3711	0.4630	320.5543	9/12 (75.00%)	9/12 (75.00%)	9/12 (75.00%)
	72.3711	0.4630	320.5543	9/12 (75.00%)	9/12 (75.00%)	9/12 (75.00%)
	72.3711	0.4630	320.5543	9/12 (75.00%)	9/12 (75.00%)	9/12 (75.00%)
	72.3711	0.4630	320.5543	9/12 (75.00%)	9/12 (75.00%)	9/12 (75.00%)
	72.3711	0.4630	320.5543	9/12 (75.00%)	9/12 (75.00%)	9/12 (75.00%)
	72.3711	0.4630	320.5543	9/12 (75.00%)	9/12 (75.00%)	9/12 (75.00%)
	72.3711	0.4630	320.5543	9/12 (75.00%)	9/12 (75.00%)	9/12 (75.00%)
	72.3711	0.4630	320.5543	9/12 (75.00%)	9/12 (75.00%)	9/12 (75.00%)
	72.3711	0.4630	320.5543	9/12 (75.00%)	9/12 (75.00%)	9/12 (75.00%)
	72.3711	0.4630	320.5543	9/12 (75.00%)	9/12 (75.00%)	9/12 (75.00%)
	72.3711	0.4630	320.5543	9/12 (75.00%)	9/12 (75.00%)	9/12 (75.00%)
	72.3711	0.4630	320.5543	9/12 (75.00%)	9/12 (75.00%)	9/12 (75.00%)
	72.3711	0.4630	320.5543	9/12 (75.00%)	9/12 (75.00%)	9/12 (75.00%)

Supplemental Table 21: Glycworkbench table for m/z 1850.66465 (21T MALDI-FTICR DDA data). The N-glycan circled in red was selected as the most relevant glycoform detected based on additional literature review and shown as annotated spectra in Supplemental Figure 17.

Structure	Coverage	RMSD	RMSD PPM	Assigned	> 10% assigned	> 5% assigned
	39.3507	0.4594	303.6927	11/30 (36.67%)	10/29 (34.48%)	11/30 (36.67%)
	39.3507	0.4594	303.6927	11/30 (36.67%)	10/29 (34.48%)	11/30 (36.67%)
	39.3507	0.4594	303.6927	11/30 (36.67%)	10/29 (34.48%)	11/30 (36.67%)
	40.8122	0.5290	470.1836	11/30 (36.67%)	11/29 (37.93%)	11/30 (36.67%)
	40.8122	0.5290	470.1836	11/30 (36.67%)	11/29 (37.93%)	11/30 (36.67%)
	40.8122	0.5290	470.1836	11/30 (36.67%)	11/29 (37.93%)	11/30 (36.67%)
	40.8122	0.5290	470.1836	11/30 (36.67%)	11/29 (37.93%)	11/30 (36.67%)
	40.8122	0.5290	470.1836	11/30 (36.67%)	11/29 (37.93%)	11/30 (36.67%)