Supplementary Materials

Serum *N*-glycosylation RPLC-FD-MS Assay to Assess Colorectal Cancer Surgical Interventions

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Supplementary Materials – Figures

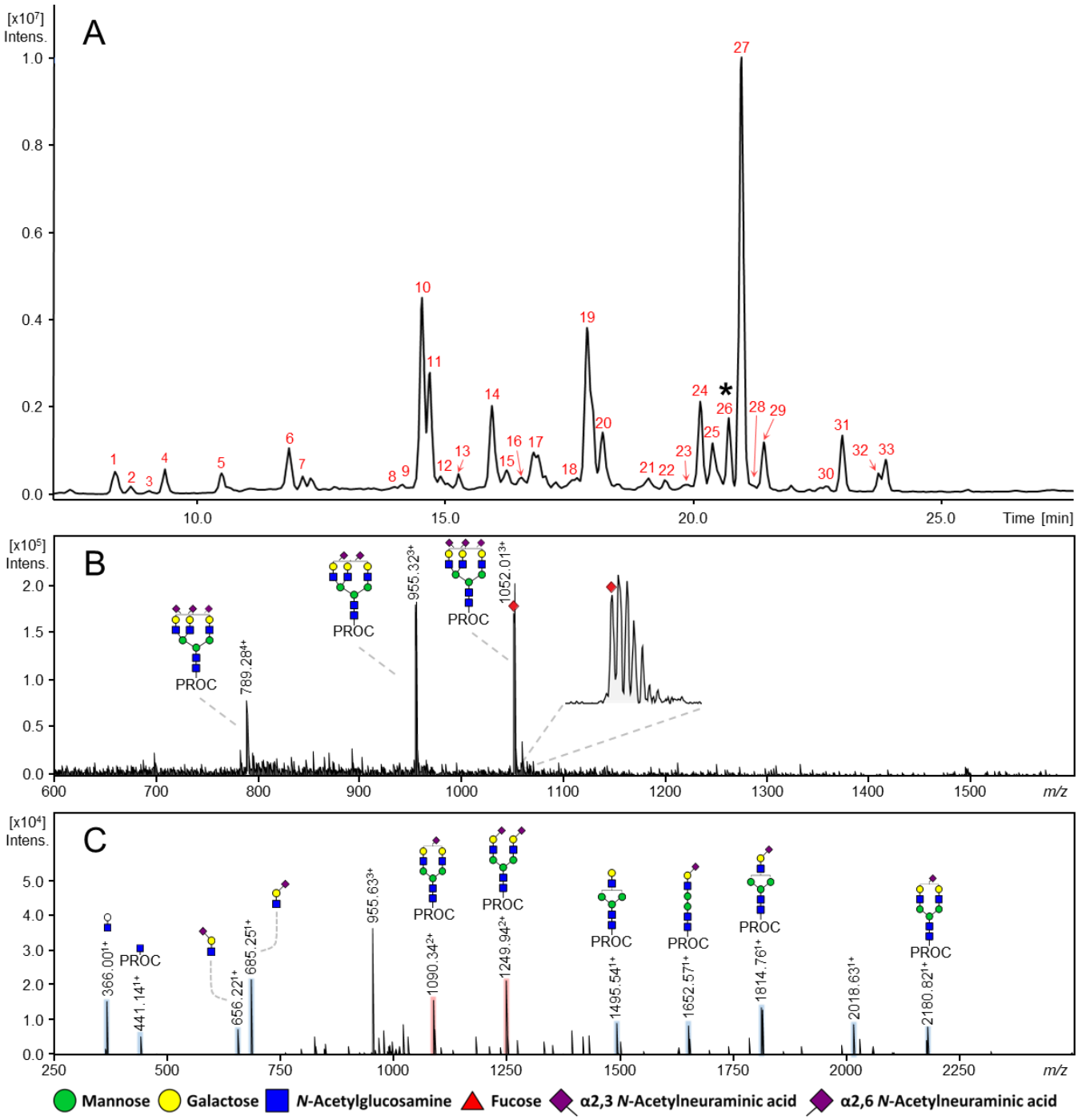


Figure S1. Representative *N*-glycan profile measured by RPLC-FD-MS. (A) Fluorescent chromatogram with peaks numbered from 1 – 33. Asterisk denotes that peak 26 is shown is panel B. **(B)** MS spectrum of peak 26 whereby the assignments of H6N5S$_{2,3}$1S$_{2,6}$2 (isomer 2) (*m/z* 789.28^{4+}, 1052.01^{3+}) as well as H6N5S$_{2,6}$2 (isomer 1) (*m/z* 955.32^{3+}) are shown. An inset with a close-up of the isotopic profile *m/z* 1052.01^{3+} is provided. The red diamond indicates the precursor that was selected for MS/MS. **(C)** MS/MS spectrum of *m/z* 1052.01^{3+} with annotated fragment ions [M+H]$^{1+}$ (blue) and [M+2H]$^{2+}$ (red).

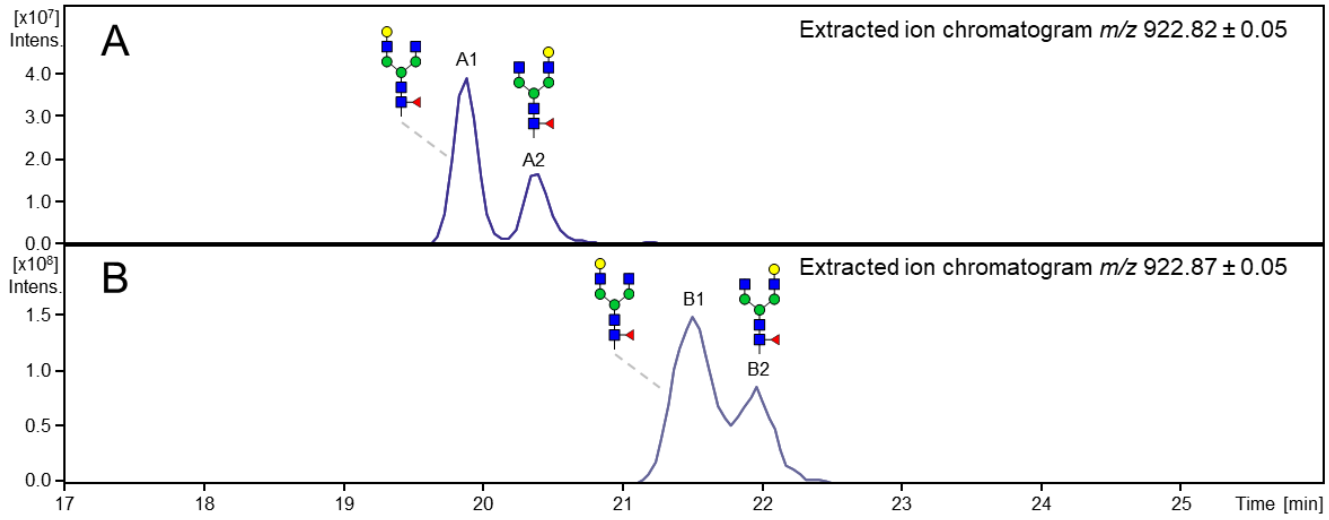


Figure S2. Extracted ion chromatograms of H4N4F1 from IgG. The extracted *m/z* of $[M+2H]^{2+}$ shown. **(A)** HILIC separation of these glycoforms following procainamide labeling. The assignment of the isomers in peaks A1 and peak A2 has been reported in the literature [1]. The gradient consisted of: 0 to 53.5 min, 76 to 55% B (0.4 mL/min); 53.5 to 54.5 min, 55 to 0% B (0.4 to 0.2 mL/min); 54.5 to 57.5 min, 0% B (0.2 mL/min); 57.5 to 59.5 min, 0 to 76% B (0.2 mL/min); 59.5 to 70 min, 76% B (0.2 to 0.4 mL/min). **(B)** RPLC separation following procainamide labeling and sialic acid ethyl esterification and amidation. Peaks B1 and B2 are tentatively assigned based on the precursor *m/z* and relative peak ratio, which is similar to (A). The following gradient was used: 0 to 53.5 min, 40 to 80% B; 53.5 to 57.5 min, 80% B; 57.5 to 59.5 min, 80 to 40% B; 59.5 to 70.1 min, 40% B; 0.2 mL/min.

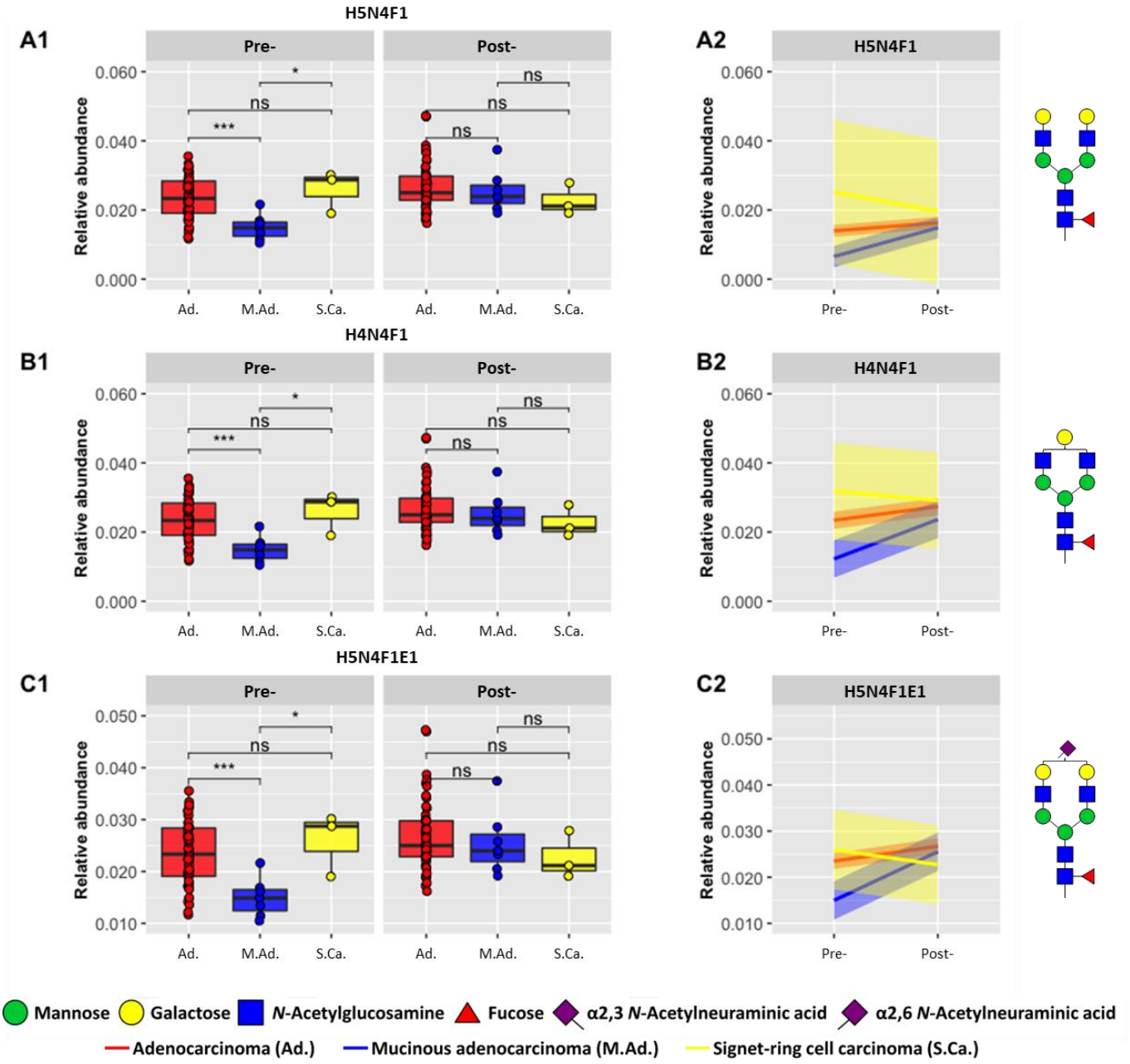


Figure S3. Differences in histological type between pre- and post-operative samples measured by MALDI-MS. The eight significant *N*-glycans found by RPLC-FD-MS were assessed in the pre- and post-operative MALDI-MS data set. [2] Three *N*-glycans differentiated significantly based upon histological type, adenocarcinoma (*n* = 57), mucinous adenocarcinoma (*n* = 7) and signet-ring cell carcinoma (*n* = 3), as denoted by each row: Row A (H5N4F1), row B (H4N4F1) and row C ($H5N4F1S_{2,6}1$). **(1)** Boxplots of relative abundances observed in pre- and post-operative samples. **(2)** Trend observed following surgery, from pre- to post-operative samples from the same patients. The 95% CI is shown as colored bands around each line. Asterisks denote significance with *p*-value < 0.05 (*), 0.01 (**), 0.001 (***), 0.0001 (****). Abbreviations: Not significant (ns), hexose (H), *N*-acetylhexosamine (N), fucose (F), amidated α2,3-linked *N*-acetylneuraminic acid (Am) and ethyl esterified α2,6-linked *N*-acetylneuraminic acid (E).

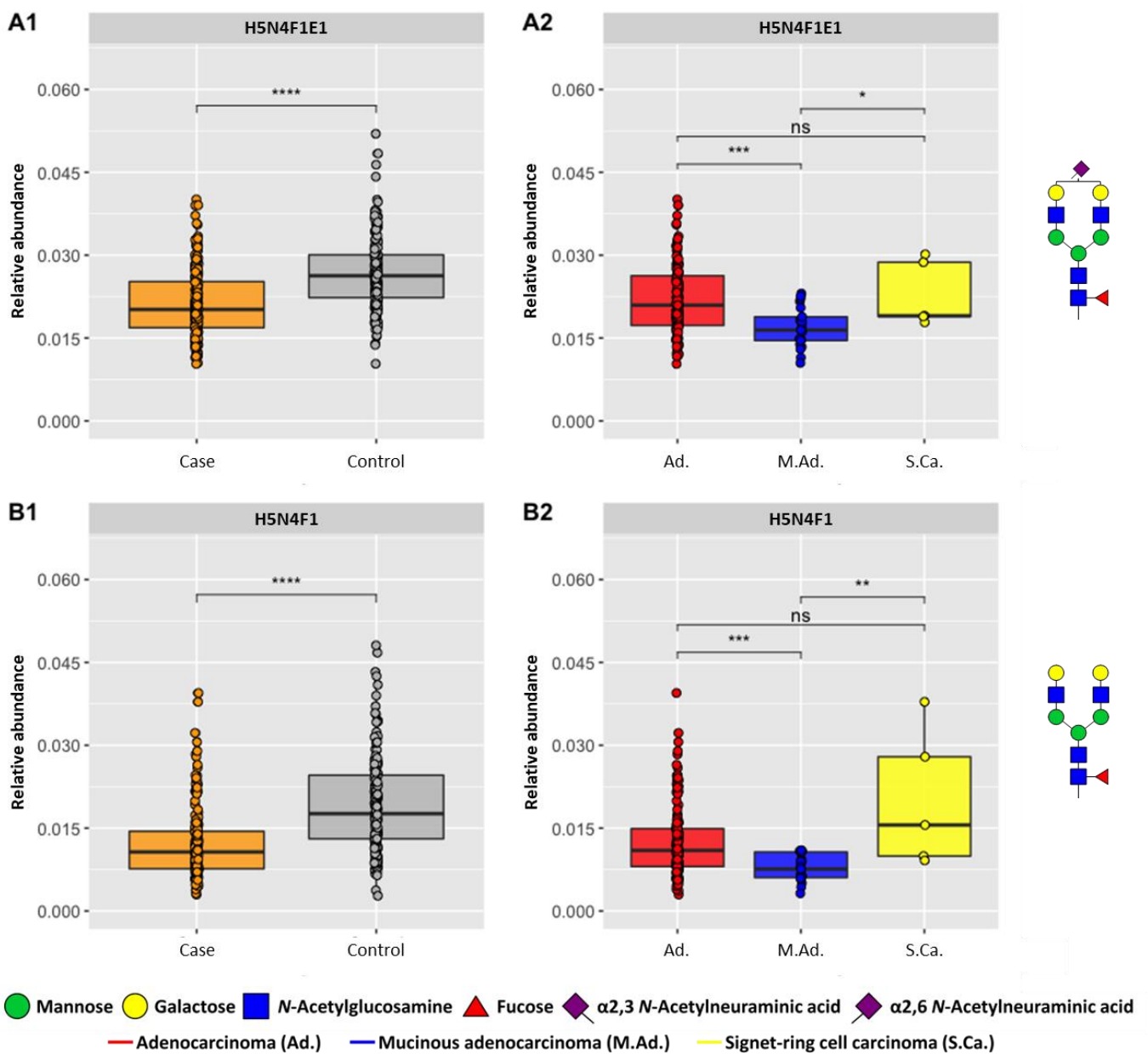
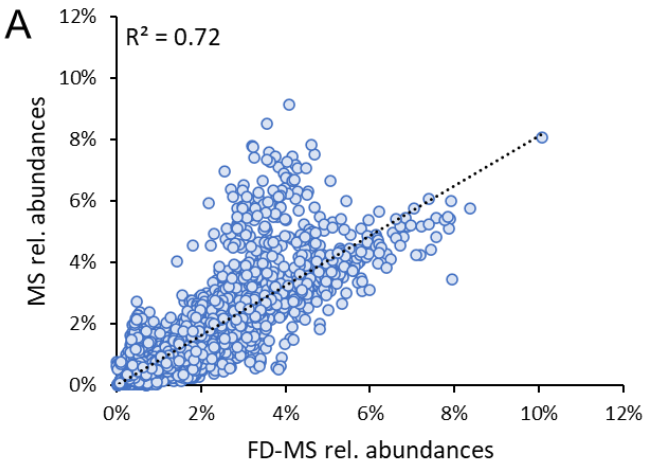
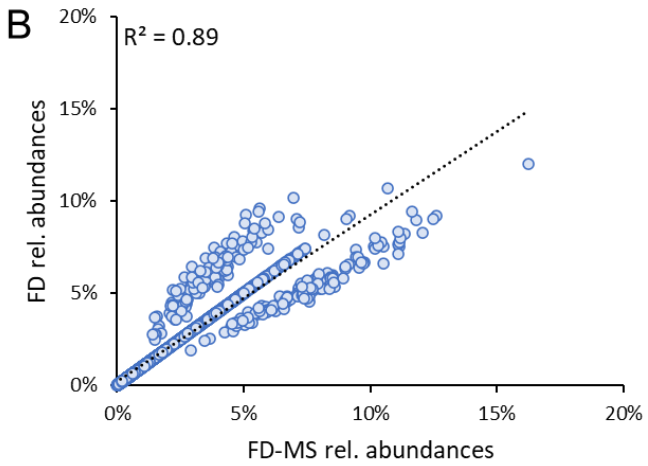
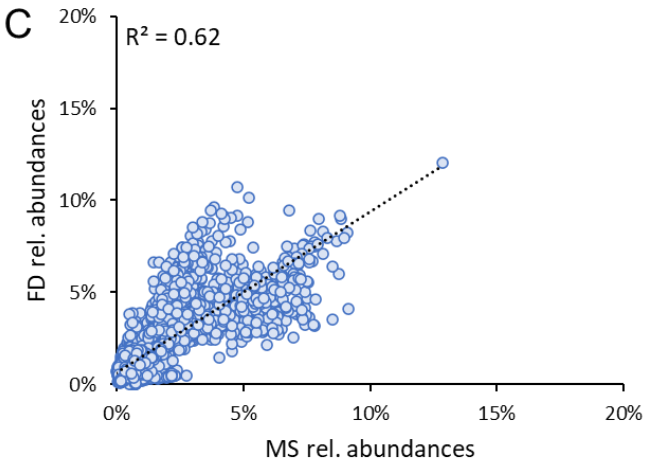


Figure S4. Significant *N*-glycans for histological type in MALDI-MS discovery and validation study. The eight significant *N*-glycans found by RPLC-FD-MS were assessed between cases (*n* = 185) and controls (*n* = 185) in the discovery and validation MALDI-MS data set. [2] Two *N*-glycans differentiated significantly based upon histological type, adenocarcinoma (*n* = 154), mucinous adenocarcinoma (*n* = 21) and signet-ring cell carcinoma (*n* = 7), as denoted by rows A (H5N4F1$S_{2,6}$1) and B (H5N4F1). **(1)** Boxplots of relative abundances observed in cases *versus* healthy controls (*n* = 185). **(2)** Boxplots of relative abundances (cases) observed across the three histological types. Metadata was not collected in five patients, therefore the number of plotted cases is *n* = 180. Asterisks denote significance with *p*-value < 0.05 (*), 0.01 (**), 0.001 (***), 0.0001 (****). Abbreviations: Not significant (ns), hexose (H), *N*-acetylhexosamine (N), fucose (F), amidated α2,3-linked *N*-acetylneuraminic acid (Am) and ethyl esterified α2,6-linked *N*-acetylneuraminic acid (E).







D

Comparison	Analytes[1,2]	Measurements	R^2 A	R^2 B
FD-MS vs. MS	48	128	0.94	0.72
FD-MS vs. FD	32	128	0.98	0.89
MS vs. FD	32	128	0.91	0.62

[1] 48 *N*-glycans determined by MS were compared
[2] 32 analytes were compared whereby MS signals for *N*-glycans eluting under the same FD peak were summed to compare with FD. FD peaks 2 and 3 were summed
A All analytes included
B Excluding the two most abundant analytes

Figure S5. Linear regression comparison. (A) Comparison of FD-MS and MS relative abundances. **(B)** Comparison of FD-MS and FD relative abundances. A discrepancy may have been introduced due to batch correction which was performed after FD-MS calculation **(C)** Comparison of MS and FD relative abundances. **(D)** Summary table of all comparisons. *N*-glycans signals eluting under the same fluorescent peak were summed in order to perform the comparison with FD quantification. Similarly, FD peaks 2 and 3 were summed as H7N2 isomers were quantified as one signal by MS quantification approaches. Correlation between quantification approaches is given via the R^2 values, colored from 0 (red) to 0.5 (yellow) to 1 (green).

References

1. Keser, T.; Pavić, T.; Lauc, G.; Gornik, O. Comparison of 2-Aminobenzamide, Procainamide and RapiFluor-MS as Derivatizing Agents for High-Throughput HILIC-UPLC-FLR-MS N-Glycan Analysis. *Front Chem* **2018**, *6*, doi:10.3389/fchem.2018.00324.
2. de Vroome, S.W.; Holst, S.; Girondo, M.R.; van der Burgt, Y.E.M.; Mesker, W.E.; Tollenaar, R.A.E.M.; Wuhrer, M. Serum N-Glycome Alterations in Colorectal Cancer Associate with Survival. *Oncotarget* **2018**, *9*, 30610–30623, doi:10.18632/oncotarget.25753.