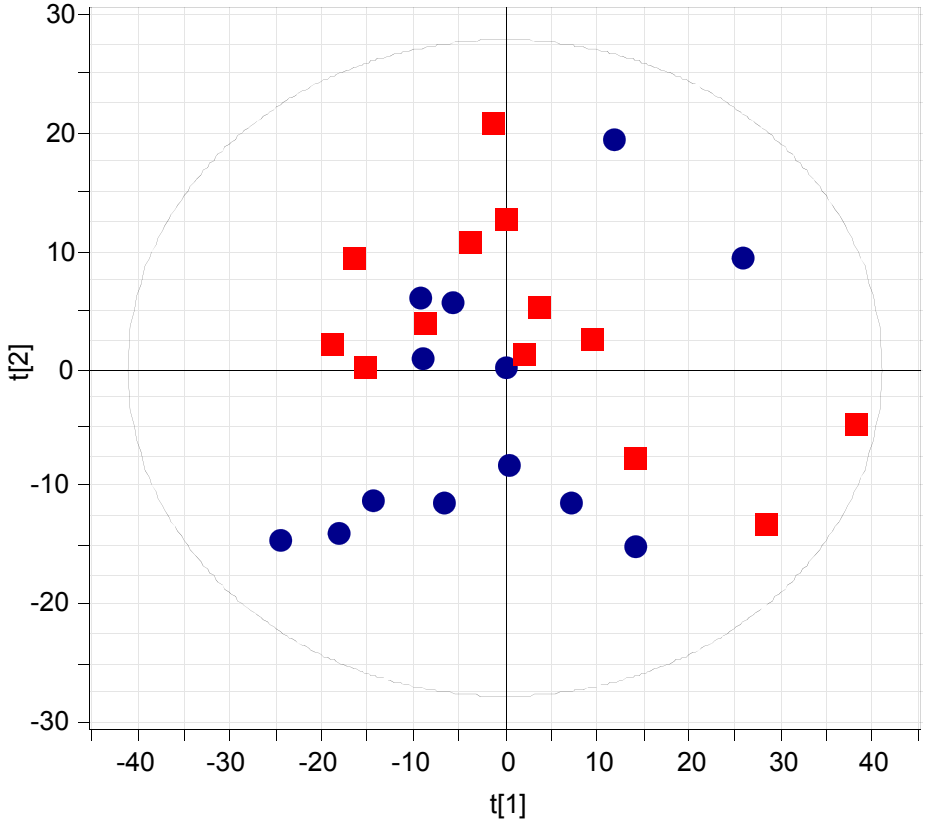
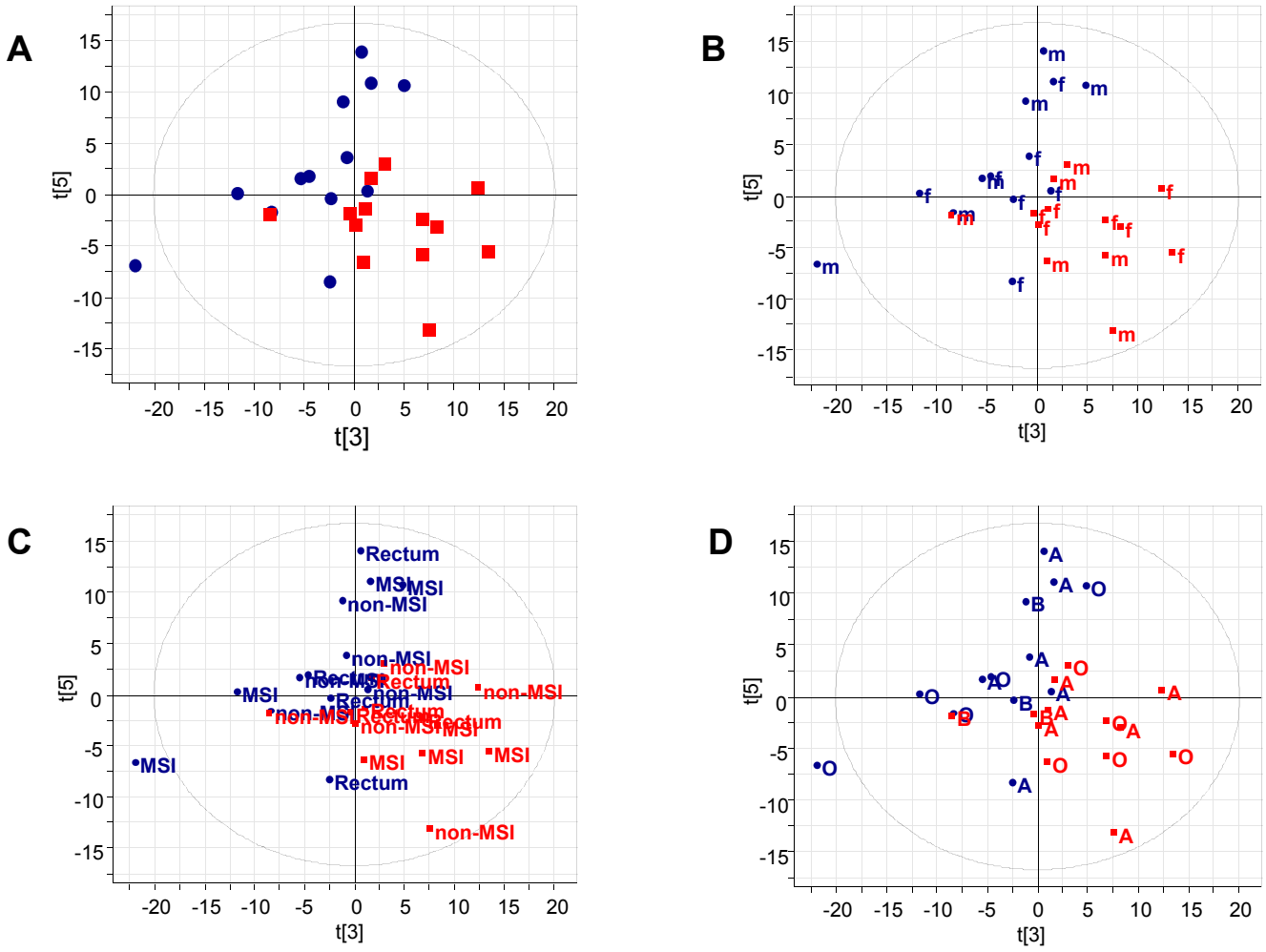
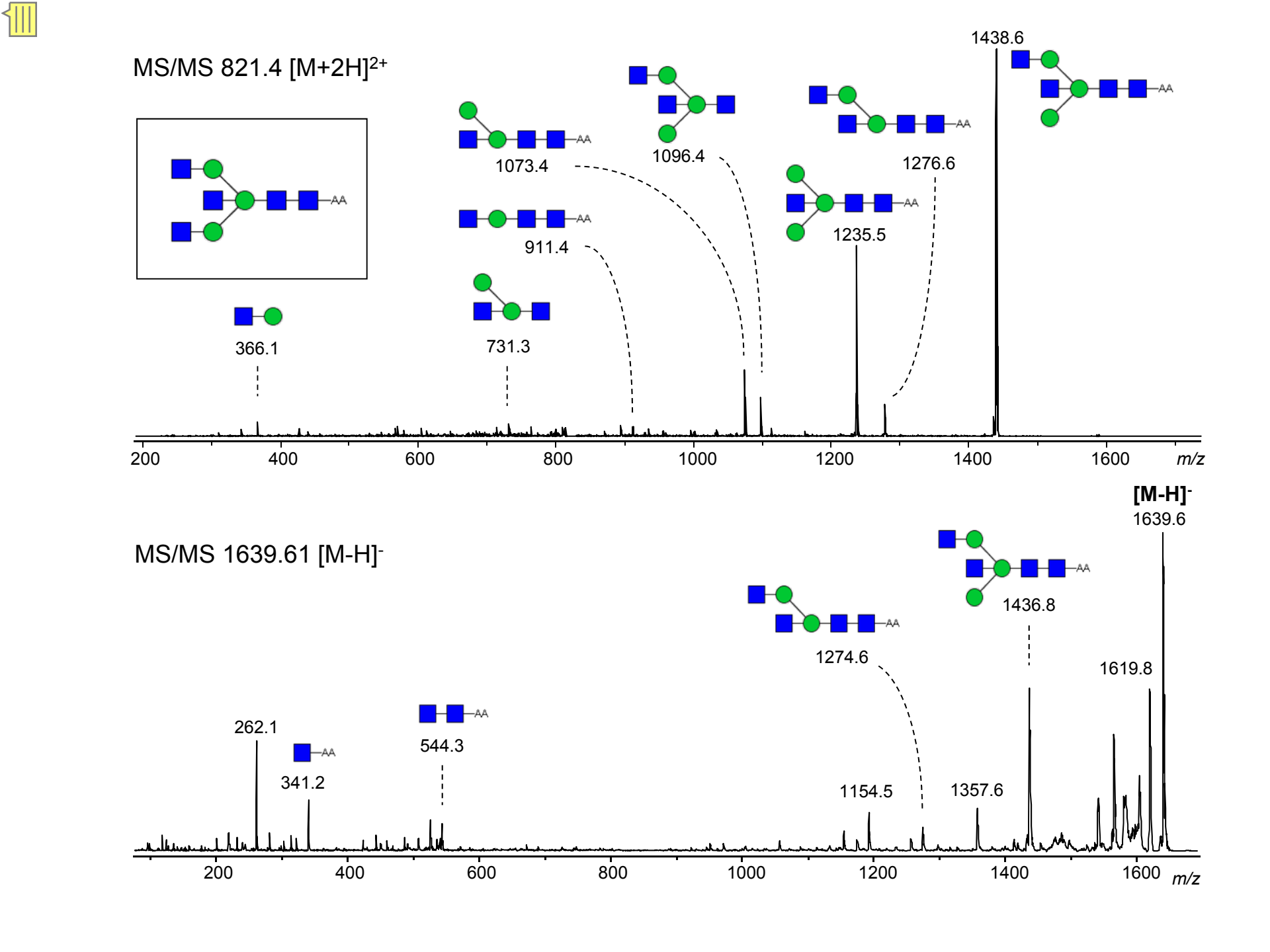


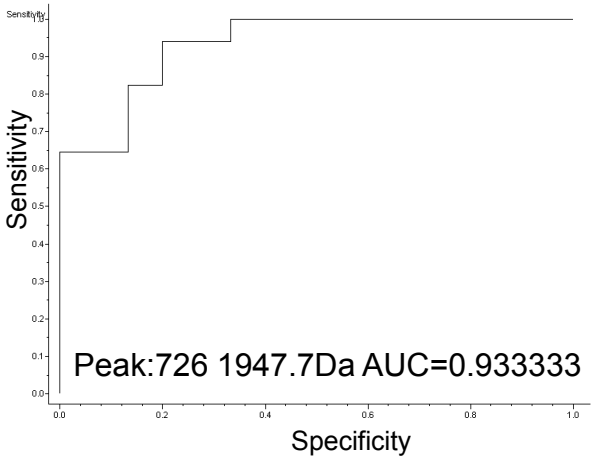
Supplementary Figure 2. PCA scores plot (PC1 *vs.* PC2) of the MALDI-ToF-MS profiles of the AA-labeled N-glycans released from 13 colon cancer and their corresponding control tissues. PCA analysis shows a separation tendency between cancer and control samples based on the MS N-glycan profiles. The samples were colored for the 13 colon control tissues (blue dots) and for the 13 colon cancer tissues (red squares) with control samples clustering to the bottem side and the cancer samples clustering to the upper side of the plot.

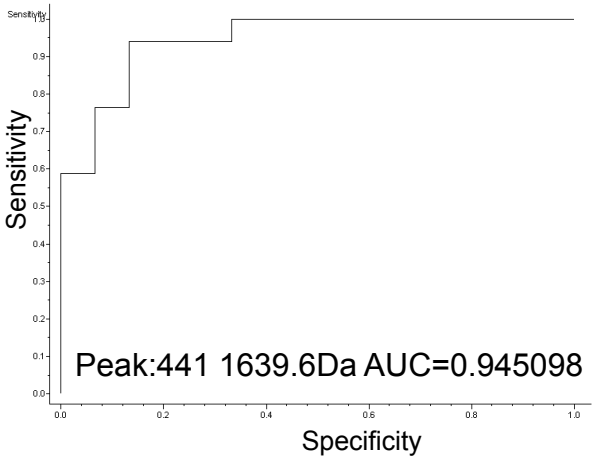


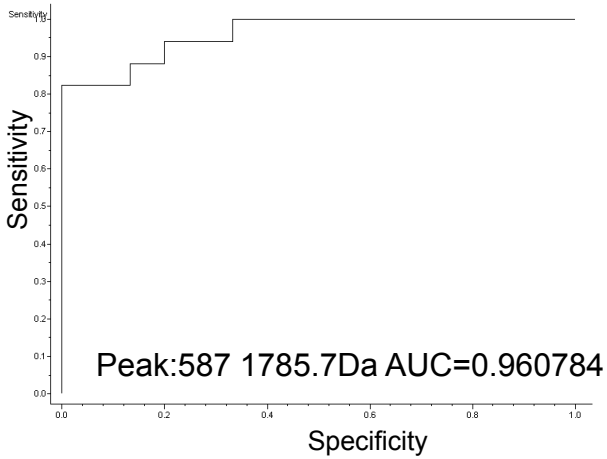
Supplementary Figure 3. PCA scores plot (PC3 *vs.* PC5) of the MALDI-ToF-MS profiles of the AA-labeled N-glycans released from 13 colon cancer and their corresponding control tissues. A) PCA analysis shows a separation tendency between cancer and control samples based on the MS N-glycan profiles. The samples were colored for the 13 colon control tissues (blue dots) and for the 13 colon cancer tissues (red squares) with control samples clustering to the right section and the cancer samples clustering to the left side of the plot. **B,C,D)** The PCA score plots of component 3 and 5 show no clustering according to sex (B) (m=male; f=female), tumor type (C) (MSI=Microsatellite instability) and blood group (D).


MS/MS 821.4 [M+2H]2+
AA
1438.6
1096.4
1073.4
1276.6
911.4
1235.5
366.1
731.3
200
400
600
800
1000
1200
1400
1600
m/z
[M-H]-
MS/MS 1639.61 [M-H]-
1639.6
1436.8
1274.6
1619.8
262.1
544.3
341.2
1154.5
1357.6
200
400
600
800
1000
1200
1400
1600
m/z

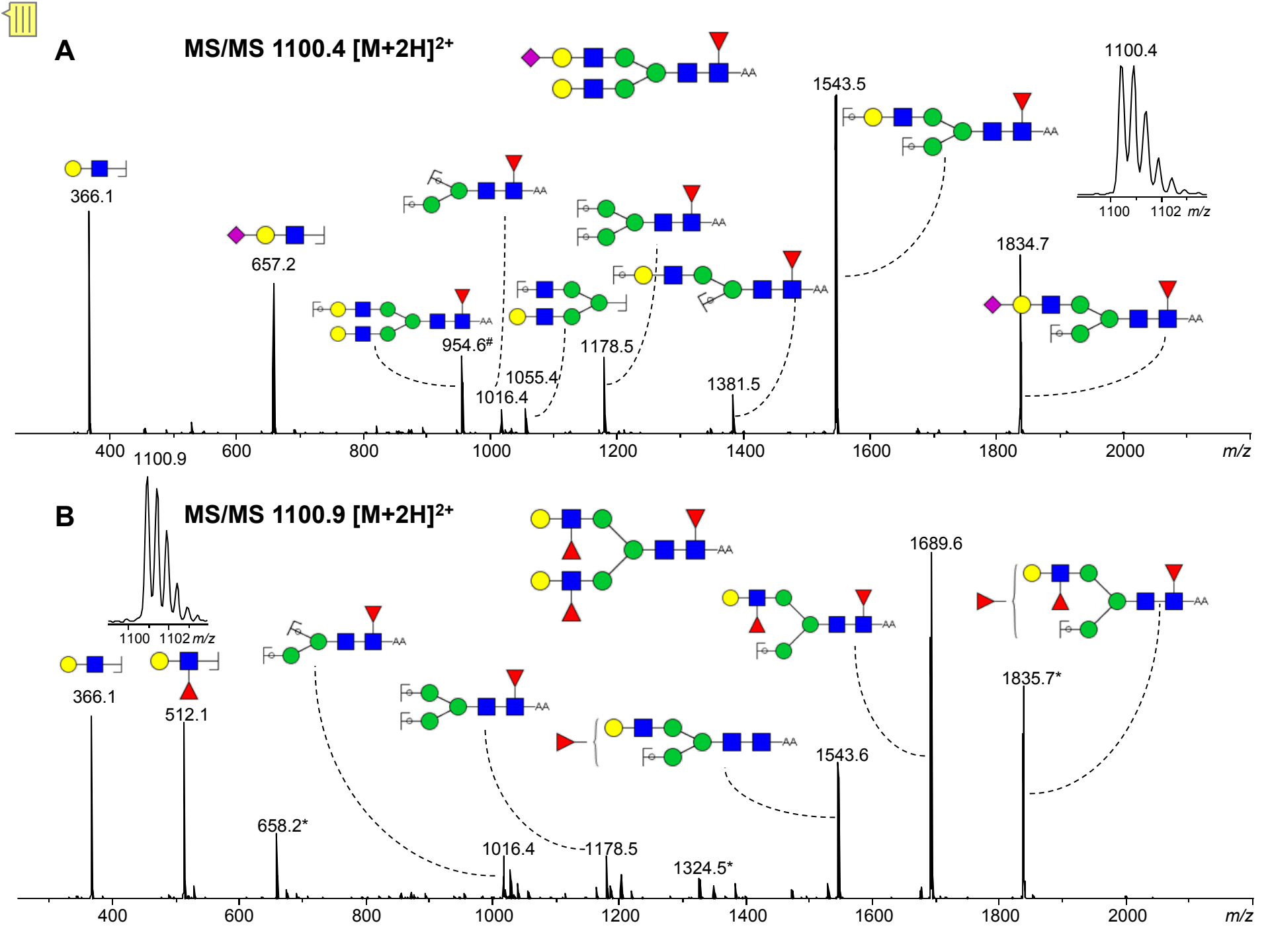
Supplementary Figure 4. MS/MS spectrum of a discriminative AA-labeled N-glycans carrying a bisecting GlcNAc. (A) The MS/MS spectrum of *m/z* 821.4 $[M+2H]^{2+}$ (H_3N_5-AA) acquired by nano-LC-MS/MS. (B) The MALDI-ToF/ToF-MS spectrum of *m/z* 1639.61 $[M-H]^-$ (H_3N_5-AA). The spectra contain fragments indicating the presence of a bisecting GlcNac. Monosaccharides are represented according to MS-Tools from EUROcarbDB (G*reen circle,* mannose; *blue square*, *N*-acetylglucosamine).





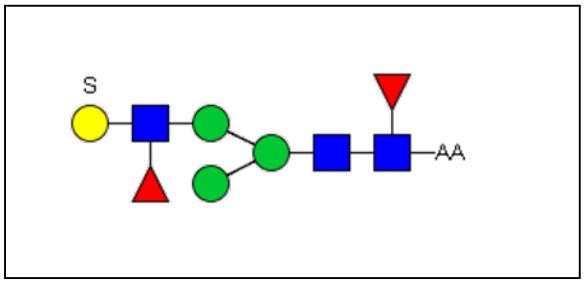


Supplementary Figure 5. ROC curves of the first isotopes of the AA-labeled N-glycans carrying a bisecting GlcNAc *m/z* 1639.61, *m/z* 1785.67 and *m/z* 1947.73. The area under the ROC curve, which serves as measure for the overall ability of a structure to discriminate control versus cancer tissues is given within the plot.

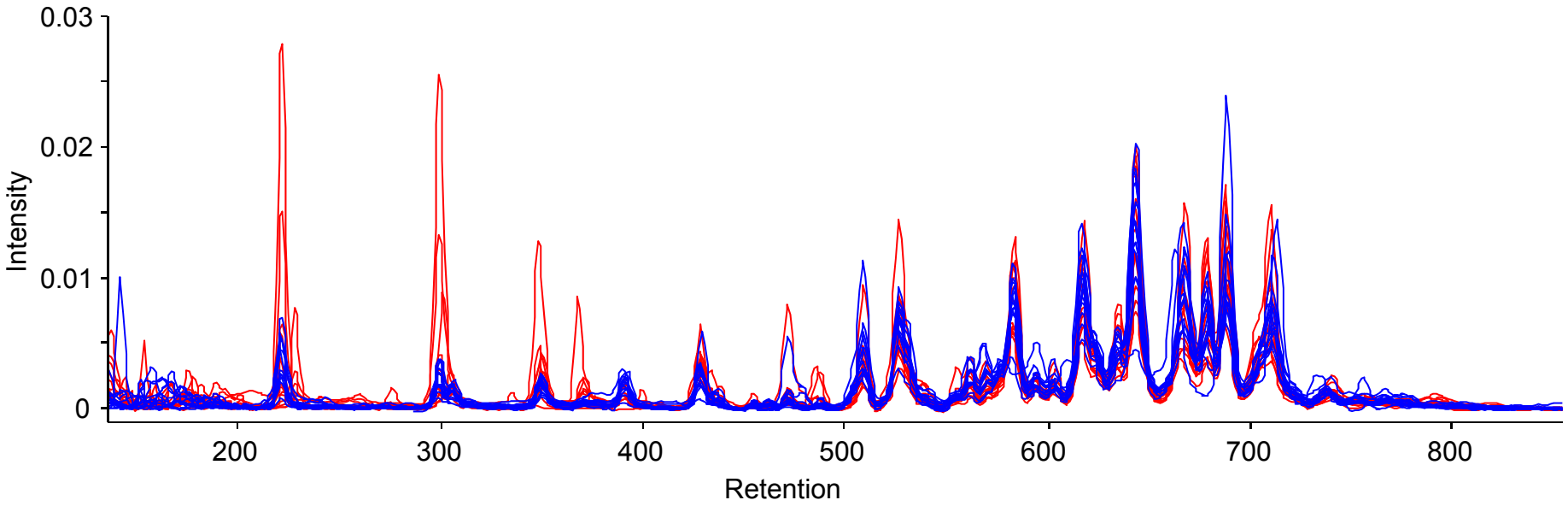

A
MS/MS 1100.4 [M+2H]2+
1100.4
1100
1102 m/z
1543.5
366.1
657.2
954.6#
1055.4
1016.4
1178.5
1381.5
1834.7
400
600
800
1000
1200
1400
1600
1800
2000
m/z
B
MS/MS 1100.9 [M+2H]2+
1100.9
1100
1102 m/z
366.1
512.1
658.2*
1016.4
1178.5
1324.5*
1543.6
1689.6
1835.7*
400
600
800
1000
1200
1400
1600
1800
2000
m/z

Supplementary Figure 6. Ion trap-MS/MS spectra of two AA-labeled N-glycans coeluting in the HILIC-HPLC separation. The MS/MS of *m/z* 1100.4 [M+2H]2+ clearly indicates the presence of a sialic acid (fragment ion at *m/z* 657.2) whereas the MS/MS of *m/z* 1100.9 [M+2H]2+ clearly indicates the presence of a core fucose (fragment ion at *m/z* 1016.4 and *m/z* 1178.5) and the presence of fucose on the antennae (fragment ion at *m/z* 512.1). The sialylated biantennary N-glycan was shown to be decreased in colon cancer tissues while the N-glycan contain Lewis type elements was shown to be slightly increased in colon cancer tissues (Table 2). Monosaccharides are represented according to MS-Tools from EUROcarbDB (Y*ellow circle*, galactose; *green circle,* mannose; *blue square*, *N*-acetylglucosamine; *purple diamond*, sialic acid).

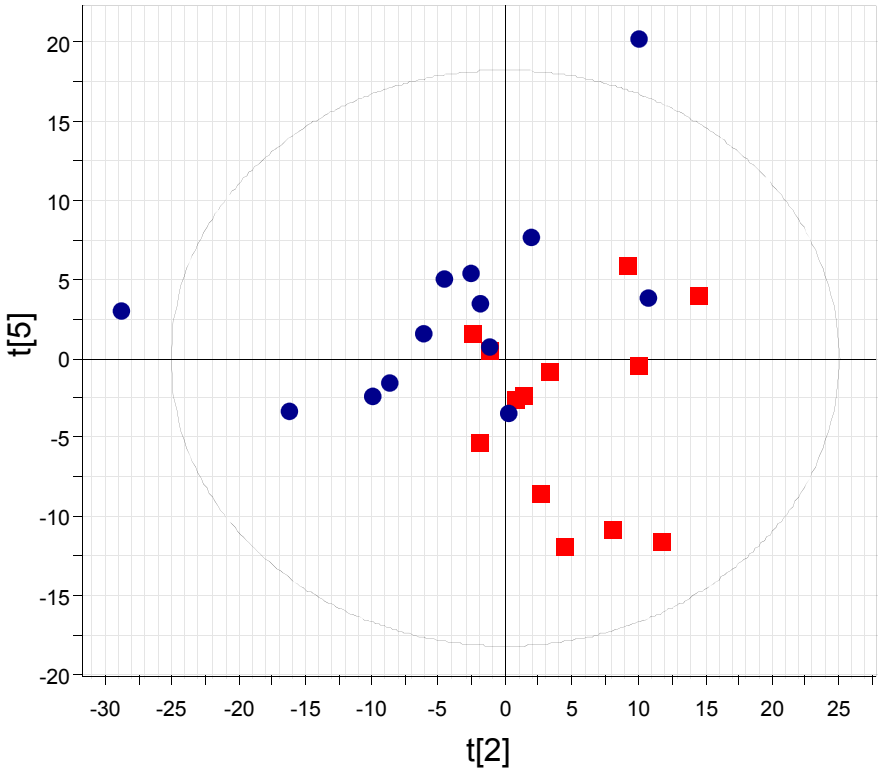
MS/MS 1767.57 [M-H]-



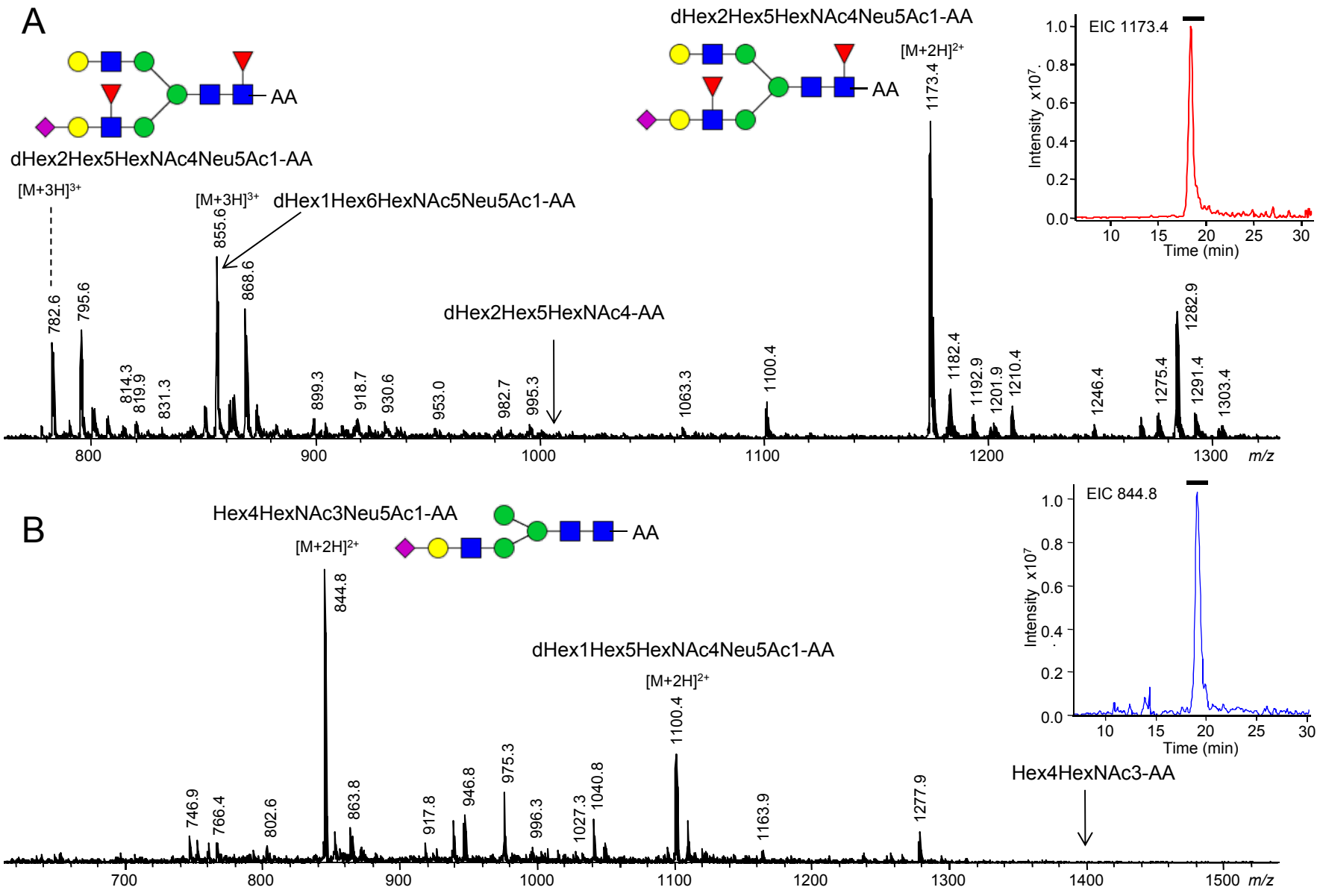
Supplementary Figure 7. MALDI-ToF/ToF-MS spectrum of *m/z* 1767.7 [M-H]$^-$ ($H_4N_3F_2$(S)-AA). The AA-labeled N-glycan carrying a sulfate group was showing to be increased in colon cancer tissues. The spectrum contains fragments indicating the presence of a sulfated Lewis-type epitope. Monosaccharides are represented according to MS-Tools from EUROcarbDB (Y*ellow circle*, galactose; *green circle,* mannose; *blue square*, *N*-acetylglucosamine; (S), sulfate group).



Supplementary Figure 8. HILIC-HPLC profiles of AA-labeled N-glycans from tumor and control tissues. The purified and AA-labeled N-glycans, released from 13 tumor and corresponding control tissues were profiled by hydrophilic interaction liquid chromatography with fluorescence detection. The chromatograms of the cancer samples (red) and control samples (blue) were baseline corrected and aligned from 135 to 890 data points.



Supplementary Figure 9. PCA scores plot (PC2 *vs.* PC5) of the HILIC-HPLC profiles of the AA-labeled N-glycans released from 13 colon cancer and their corresponding control tissues. The samples were colored for the 13 colon control tissues (blue dots) and for the 13 colon cancer tissues (red squares) with control samples clustering to the left section and the cancer samples spreading mainly to the right side of the plot.


A
dHex2Hex5HexNAc4Neu5Ac1-AA
AA
dHex2Hex5HexNAc4Neu5Ac1-AA
[M+2H]2+
1173.4
EIC 1173.4
Intensity x10^7
Time (min)
[M+3H]3+
[M+3H]3+
dHex1Hex6HexNAc5Neu5Ac1-AA
855.6
868.6
782.6
795.6
814.3
819.9
831.3
899.3
918.7
930.6
953.0
982.7
995.3
dHex2Hex5HexNAc4-AA
1063.3
1100.4
1182.4
1192.9
1201.9
1210.4
1246.4
1275.4
1282.9
1291.4
1303.4
m/z
B
Hex4HexNAc3Neu5Ac1-AA
AA
[M+2H]2+
844.8
EIC 844.8
Intensity x10^7
Time (min)
dHex1Hex5HexNAc4Neu5Ac1-AA
[M+2H]2+
1100.4
746.9
766.4
802.6
863.8
917.8
946.8
975.3
996.3
1027.3
1040.8
1163.9
1277.9
Hex4HexNAc3-AA
m/z

Supplementary Figure 10. No desialylation of glycans observed in glycan analysis by LC-MS/MS. The extracted ion chromatograms (EIC) of two sialylated, AA-labeled glycans observed in different LC-MS/MS runs are shown in the insets. Sum mass spectra are displayed for the elution time window indicated by horizontal bars. The sialylated AA-labeled N-glycan dHex2Hex5HexNAc4Neu5Ac1-AA was observed as double and triple charged species (A), and the corresponding desialylated species (expected *m/z* value of the double protonated species indicated by a vertical arrow) was not observed. The sialylated AA-labeled N-glycan Hex4HexNAc3Neu5Ac1-AA was observed as double charged species (B), and the corresponding desialylated species (expected *m/z* value of the single protonated species indicated by a vertical arrow) was not observed. The fragmentation spectra of these glycans are depicted in Spectrum 112 (dHex2Hex5HexNAc4Neu5Ac1-AA) and 42 (Hex4HexNAc3Neu5Ac1-AA) in Supplementary Figure 1.