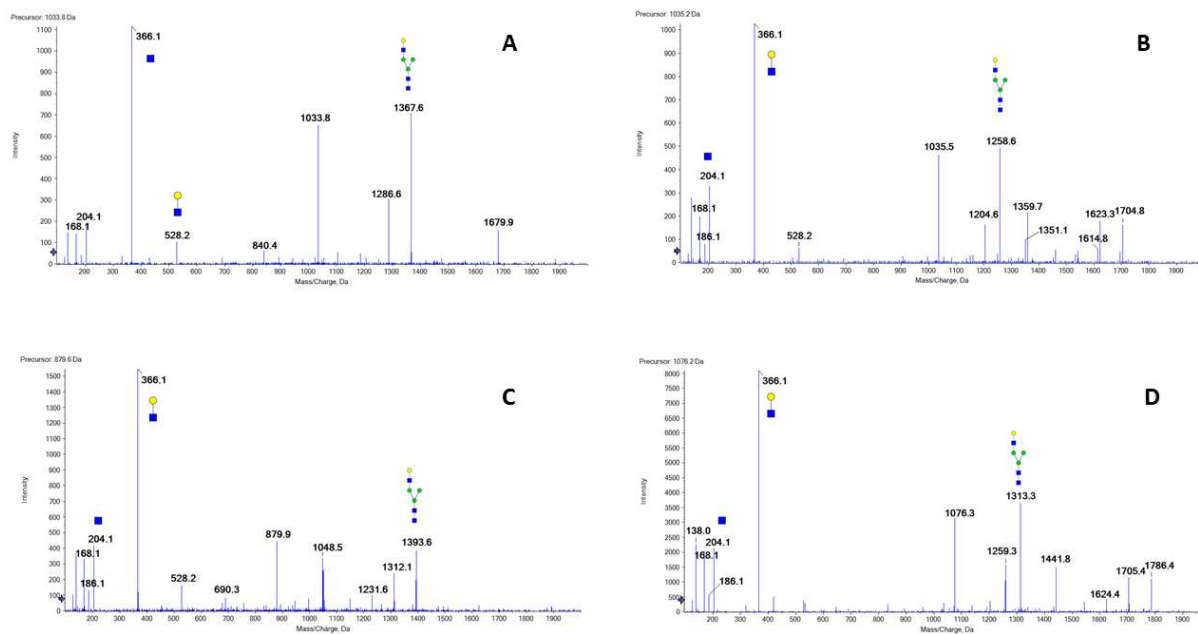


**Analytical and Bioanalytical Chemistry**

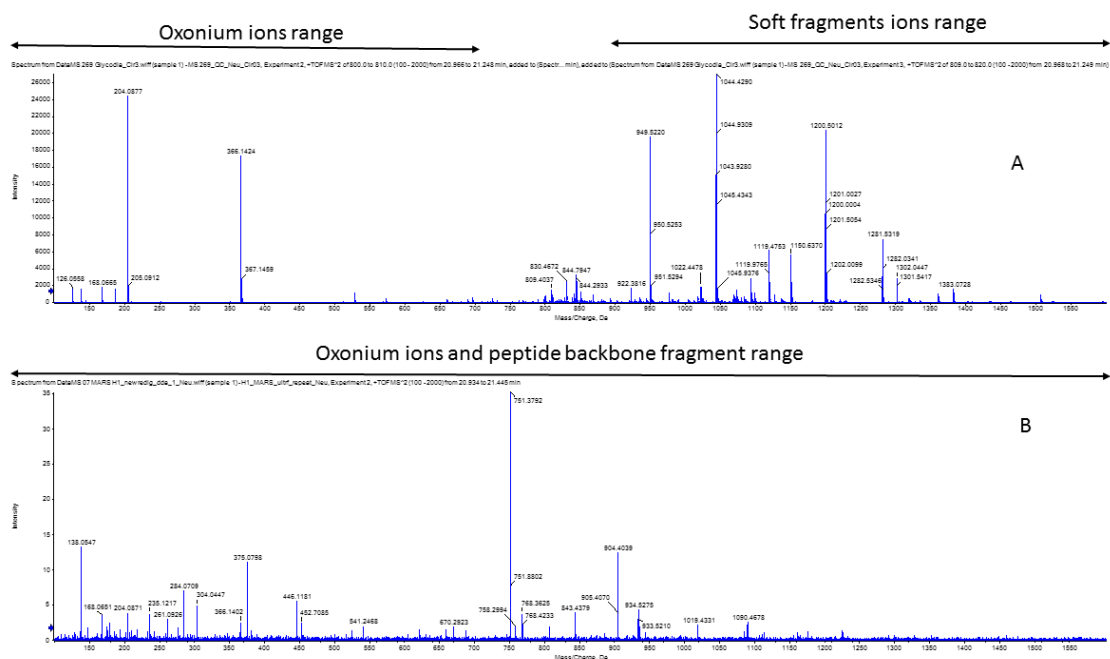
**Electronic Supplementary Material**

**Site-specific analysis of changes in the glycosylation of proteins in liver cirrhosis using data-independent workflow with soft fragmentation**

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**Fig S1** Soft fragmentation of four glycopeptides (bi-antennary structures) derived from neuraminidase treated tryptic digest of proteins retained on the MARS14 column. The soft fragments used for quantification of each glycopeptide are highlighted by the structural schematics. A. CGLVPVLAENYNK peptide of serotransferrin; B. GTAGNALMDGASQLMGENR peptide of fibrinogen; C. QQHLFGSNVTDCSGNFCLFRSETK peptide of serotransferrin; and D. MVSHHNLTTGATLINEQWLLTTAK peptide of haptoglobin



**Fig S2** CID spectra of all precursors 700-1250 m/z in the retention time window of IgG2 N-glycoforms obtained by tryptic digest of the proteins retained on the MARS 14 column. A. Low CID setting selected for fragmentation of the N-glycopeptides; and B. CID settings optimized for fragmentation of the peptides

**Table S1** Identified and quantified glycopeptide structures at each site of N-glycosylation with precursor m/z information, dominant fragment selected for DIA quantification, and ratio of average fucosylation in cirrhosis vs control samples

Protein/peptide	GLYCAN FORMULA	PRECURSOR m/z	QUANTIFIED or DETECTED	PRECURSOR CHARGE STATE	SOFT FRAGMENT m/z	Mean CIRR/ Mean CTRL
<b>ALPHA2-MACROGLOBULIN</b>						
VSNQTLSLF FTVLQDVPVR	A2G2	947.2	Q	4	1140.88	--
	A2G2F1	983.71	Q	4	1189.57	0.74
	A3G3	1038.48	Q	4	1262.59	--
	A3G3F1	1074.99	Q	4	1311.28	1.12
	A3G3F2	111.51	Q	4	1311.28	1.45
IITILEEEMNVSVCGLYTYGK	A2G2	1014.45	Q	4	1230.56	--
	A2G2F1	1050.98	Q	4	1279.24	1.11
SINTTNVMGTSLTVR	A2G2	1072.81	Q	3	1426.14	--
	A2G2F1	1121.51	Q	3	1499.17	0.73
	A3G3	1194.51	D	3	1608.71	--
GCVLLSYLNETVTVSASLESVR	A2G2	1005.57	Q	4	1218.9	--
	A2G2F1	1042.1	Q	4	1267.58	0.79
	A3G3	1096.85	D	4	1340.61	--
IYVLDYLNQQLTPEIK	A2G2	951.44	Q	4	1146.54	--
	A2G2F1	987.96	D	4	1146.54	--
	A3G3	1042.71	Q	4	1268.25	--
	A3G3F1	1079.24	Q	4	1268.25	1.23
<b>HAPTOGLOBIN</b>						
MVSHHNLTTGATLINEQWLLTTAK	A2G2	1076.25	Q	4	1312.95	--
	A2G2F1	1112.77	Q	4	1361.64	1.26
	A3G3	1167.52	Q	4	1434.67	--
	A3G3F1	1204.05	Q	4	1483.35	2.53

	A3G3F2	1240.57	Q	4	1483.35	3.11
	A4G4	1007.24	Q	5	1167.53	--
	A4G4F1	1065.48	D	5	1204.05	--
	A4G4F2	1094.9	D	5	1204.05	--
NLFLNHSENATAK	A2G2	1176.73	Q	4	1446.93	--
	A2G2F1	1213.26	Q	4	1495.61	2.22
	A2G2F2	1249.78	D	4	1544.3	--
VVLHPNYSQVDIGLIK	A2G2	855.15	Q	4	1018.16	--
	A2G2F1	891.68	Q	4	1066.84	0.95
	A3G3	946.43	Q	4	1139.87	--
	A3G3F1	982.95	Q	4	1188.55	2.41
	A3G3F2	1019.48	D	4	1188.55	--
	AG44	1074.23	Q	4	1261.58	--
	A4G4F1	1110.75	Q	4	1310.27	8.8
	A4G4F2	1147.28	D	4	1310.27	--
SEROTRANSFERRIN						--
QQQHILFGSNVTDCSGNFCLFRSETK	A2G2	1035.18	Q	4	1258.2	--
	A2G2F1	1071.71	Q	4	1306.88	0.97
	A3G3	1126.46	Q	4	1379.91	--
	A3G3F1	1162.98	Q	4	1428.59	2.33
	A3G3F2	1199.51	Q	4	1428.59	4.14
	A3G3F3	1236.03	D	4	1477.28	--
	A4G4	974.39	D	5	1126.46	--
CGLVPVLAENYNK	A2G2	1033.78	Q	3	1367.6	--
	A2G2F1	1082.48	Q	3	1440.63	2.26
	A3G3	1155.48	Q	3	1550.17	--
	A3G3F1	1204.18	Q	3	1623.2	8.44
	A3G3F2	939.91	D	4	1131.15	--
	AG44	958.14	D	4	1155.49	--
	A4G4F1	1030.94	D	4	1204.18	--

<b>ANTITRYPSIN</b>						
QLAHQSNSTNIFFSPVSIATAFAMLSLGTK	A2G2	961.65	Q	5	1110.53	--
	A2G2F1	1020.09	D	5	1147.04	--
	A3G3	1034.67	Q	5	1201.81	--
ADTHDEILEGLNFNLTEIPEAQIHEGFQELLR	A2G2	1063.69	Q	5	1238.07	--
	A2G2F1	1092.91	Q	5	1274.59	1.43
	A3G3	1136.71	Q	5	1329.36	--
	A3G3F1	1165.93	Q	5	1365.87	1.72
	A3G3F2	1195.15	Q	5	1365.87	2.84
	A3G3F3	1224.37	D	5	1402.39	--
	A4G4	1238.95	Q	5	1420.64	--
	A4G4F1	1056.81	Q	6	1065.92	1.11
	A4G4F2	1081.32	D	6	1165.92	--
	A4G4F3	1105.67	D	6	1195.14	--
YLGNATAIFFLPDEGK	A2G2	1126.83	Q	3	1507.18	--
	A2G2F1	1175.53	Q	3	1580.2	1.94
	A3G3	1248.53	Q	3	1689.74	--
	A3G3F1	973.17	D	4	1175.56	--
	A3G3F2	1009.05	D	4	1224.23	
<b>FIBRINOGEN</b>						
DLQSLEDILHQVENK	A2G2	851.63	Q	4	1013.46	--
	A2G2F1	888.15	Q	4	1062.14	2.81
	A3G3	942.9	D	4	1135.17	--
GTAGNALMDGASQLMGENR	A2G2	879.62	Q	4	1050.77	--
	A2G2F1	916.14	Q	4	1099.46	7.28
	A3G3	970.89	D	4	1172.49	--
<b>Ig A</b>						
PALEDLLLSEANLTCTLTGLR	A2G2	995.71	Q	4	1205.57	--
	A2G2F1	1032.24	Q	4	1254.25	0.82
	A3G3	1086.99	Q	4	1327.28	--

	A3G3F1	1123.51	Q	4	1375.96	--
	A2G1	955.19	Q	4	1151.55	--
	A2G1F1	991.72	Q	4	1200.24	0.64
	AB1G1	1005.97	Q	4	1219.24	--
	AB1G1F1	1042.49	Q	4	1267.93	0.87
	A2G2B	1046.49	Q	4	1273.26	--
	A2G2BF1	1083.01	Q	4	1321.95	1.16
	A2	914.67	D	4	1151.55	--
	A2F	951.2	Q	4	1151.55	--
TPLTANITK	A2G2	861.53	Q	3	1108.51	--
	A2G2F1	910.23	Q	3	1181.54	1.27
	A2G1	807.51	Q	3	1291.07	--
	A2G1F1	856.21	Q	3	1364.1	1.33
	AB1G1	875.21	Q	3	1129.02	--
	AB1G1F1	923.91	Q	3	1202.05	--
	A2G2B	929.23	Q	3	1210.05	--
	A2G2BF1	977.93	Q	3	1283.08	1.2
<b>ALPHA1-ACID GYCOPROTEIN</b>						
QDQCIYNTTYLNVQR	A2G2	885.36	D	4	1058.45	--
	A3G3	976.64	Q	4	1180.16	--
	A3G3F1	1013.16	Q	4	1228.85	1.53
	A3G3F2	1049.69	D	4	1228.85	--
	AG44	1104.45	Q	4	1301.87	--
	A4G4F1	1140.97	D	4	1350.56	--
	A4G4F2	1177.48	D	4	1399.25	
	A4G4F3	1214	D	4	1447.93	
SVQEIQATFFYFTPDK	A3G3	977.67	Q	4	1181.52	--
	AG44	1068.96	Q	4	1303.23	--
	A4G4F1	1105.47	Q	4	1351.91	2.28
	A4G4F2	1141.98	D	4	1351.91	--

<b>IgM</b>						
GLTFQQNASSMCVPDQDTAIR	A2G1F	987.17	Q	4	1194.18	--
	A2G2F	1027.68	Q	4	1248.2	--
	A2G1BF	1037.94	D	4	1261.87	--
	A2G2B1F	1078.45	Q	4	1315.89	--
<b>NNSDISSTR</b>	A2	764.63	D	3	1044.93	--
	A2F	813.33	D	3	1117.96	--
	A2G1	818.66	Q	3	1044.93	--
	A2G1F	867.36	Q	3	1117.96	1.98
	A2G2	872.69	Q	3	1125.96	--
	A2G2F	921.39	Q	3	1198.99	1.01
	A2G1B	886.36	D	3	1146.47	--
	A2G1BF	935.06	Q	3	1219.5	--
	A2G2B	940.39	D	3	1227.5	--
	A2G2B1F	989.09	Q	3	1300.53	--
	A3G3	994.39	Q	3	1308.52	--
	A3G3F	1043.0877	Q	3	1381.55	1.11
<b>COMPLEMENT C3</b>						
TVLTPATNHMGNVFTTIPANR	M5	1157.87	Q	3	1229.62	--
	M6	1211.88	Q	3	1229.62	--
<b>IgG1</b>						
EEQYNSTYR	A2	830.001	Q	3	1142.958	--
	A1F	865.0113	Q	3	1114.447	--
	A2F	878.687	Q	3	1215.987	1.06
	A2G1	884.018	Q	3	1142.958	--
	A2B	897.694	Q	3	1244.497	--
	A2G1F	932.704	Q	3	1215.987	1
	A2G2	938.036	Q	3	1223.984	--
	A2FB	946.38	Q	3	1317.526	--
	A2G1B	951.712	Q	3	1244.497	--



	A2G2F	986.722	Q	3	1297.013	1.01
	A2G1FB	1000.398	Q	3	1317.526	--
	A2G2B	1005.729	Q	3	1325.524	--
	A2G2BF1	1054.415	Q	3	1398.553	1
<b>IgG2/3</b>						
EEQFNSTFR	A1F	800.331	Q	3	1098.452	--
	A2	819.338	Q	3	1126.963	--
	A1G1F	854.348	Q	3	1098.452	--
	A2F	868.024	Q	3	1199.992	1.01
	A2G1	873.355	Q	3	1126.963	--
	A2B	887.031	Q	3	1228.503	--
	A2G1F	922.041	Q	3	1199.992	1
	A2G2	927.373	Q	3	1207.989	--
	A2BF	935.717	Q	3	1301.532	--
	A2G2F	976.059	Q	3	1281.018	0.98
	A2G1B	989.734	Q	3	1301.532	--
	A2G1BF	1043.752	Q	3	1382.558	--
<b>IgG4</b>						
EEQF(Y)NSTY(F)R	A2	824.669	Q	3	1134.96	--
	A1G1F	859.68	D	3	1106.45	--
	A2F	873.355	Q	3	1207.989	1.04
	A2G1	878.687	Q	3	1134.96	--
	A2B	892.362	D	3	1236.5	--
	A2G1F	927.373	Q	3	1207.989	0.8
	A2G2	932.704	Q	3	1215.987	--
	A2FB1	941.048	Q	3	1309.529	--
	A2G2F	981.39	Q	3	1289.016	0.91
	A2G1BF	995.066	Q	3	1309.529	--
	A2G2B	1000.398	Q	3	1317.526	--
	A2G2BF1	1049.084	D	3	1390.555	--

