

PRM-MS quantitative analysis of isomeric *N*-glycopeptides derived from human serum haptoglobin of patients with cirrhosis and hepatocellular carcinoma

Cristian D. Gutierrez Reyes^{1‡}, Yifan Huang^{1‡}, Mojgan Atashi¹, Jie Zhang², Jianhui Zhu², Suyu Liu³, Neehar D. Parikh⁴, Amit G. Singal⁵, J. Dai³, David M. Lubman² and Yehia Mechref^{1*}

1. Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX
2. Department of Surgery, The University of Michigan, Ann Arbor, MI 48109
3. Department of Biostatistics, University of Texas MD Anderson Cancer Center, Houston, TX 77030
4. Division of Gastroenterology and Hepatology, The University of Michigan, Ann Arbor, MI 48109
5. Division of Digestive and Liver Diseases, University of Texas Southwestern Medical Center, Dallas, TX 75390

[‡] These authors contribute equally to this work

*Corresponding author

Department of Chemistry and Biochemistry

Texas Tech University

Lubbock, TX 79409-1061

Email: yehia.mechref@ttu.edu

Tel: 806-742-3059

Fax: 806-742-1289

Table of Contents:

Supplementary Figures:

Supplementary Figure S1. Representative mass spectra of the three glycosylation sites. **a)** MVSHHN₁₈₄LTTGATLINE = Asn184, **b)** NLFLN₂₀₇HSE = Asn207, and **c)** VVLHPN₂₄₁YSQVDIGLIK = Asn241. * Common fragment ion with different charge.

Supplementary Figure S2. Interpretation of fucosylated glycopeptides; **a)** *N*-glycopeptide NLFLN₂₀₇HSE + 4-5-1-2 isomers 1 and 2, **b)** *N*-glycopeptide NLFLN₂₀₇HSE + 6-7-1-1. NLFLN₂₀₇HSE = Asn207.

Supplementary Figure S3. Sialic acid linkage of important and abundant haptoglobin *N*-glycopeptides (NLFLN₂₀₇HSE = Asn207).

Supplementary Figure S4. EICs showing the achieved isomeric separation of important haptoglobin *N*-glycopeptides. MVSHHN₁₈₄LTTGATLINE = Asn184, NLFLN₂₀₇HSE = Asn207, and VVLHPN₂₄₁YSQVDIGLIK = Asn241.

Supplementary Figure S5. PCA plot of *N*-glycopeptides derived from human serum haptoglobin from patients with cirrhosis and HCC, glycosylation site NLFLN₂₀₇HSE. Gender evaluation, **a)** cirrhosis (female) “blue” vs. HCC (female) “red”, **b)** cirrhosis (male) “blue” vs. HCC (male) “red”.

Supplementary Figure S6. Receiver operating characteristic (ROC) curve for the glycopeptide structures with statistically significance between cirrhosis and HCC patients. MVSHHN₁₈₄LTTGATLINE = Asn184, NLFLN₂₀₇HSE = Asn207, and VVLHPN₂₄₁YSQVDIGLIK = Asn241. Glycan nomenclature: HexNAc, Hex, Fuc, NeuAc (*N*-acetylhexosamine, Hexose, Fucose, *N*-acetylneuraminic acid).

Supplementary Tables:

Supplementary Table S1. PRM information for each of the target haptoglobin *N*-glycopeptides.

Supplementary Table S2. Peak area and normalized abundance of all identified haptoglobin *N*-glycopeptides from patients with cirrhosis and hepatocellular carcinoma (HCC).

Supplementary Table S3. Complete clinical information.

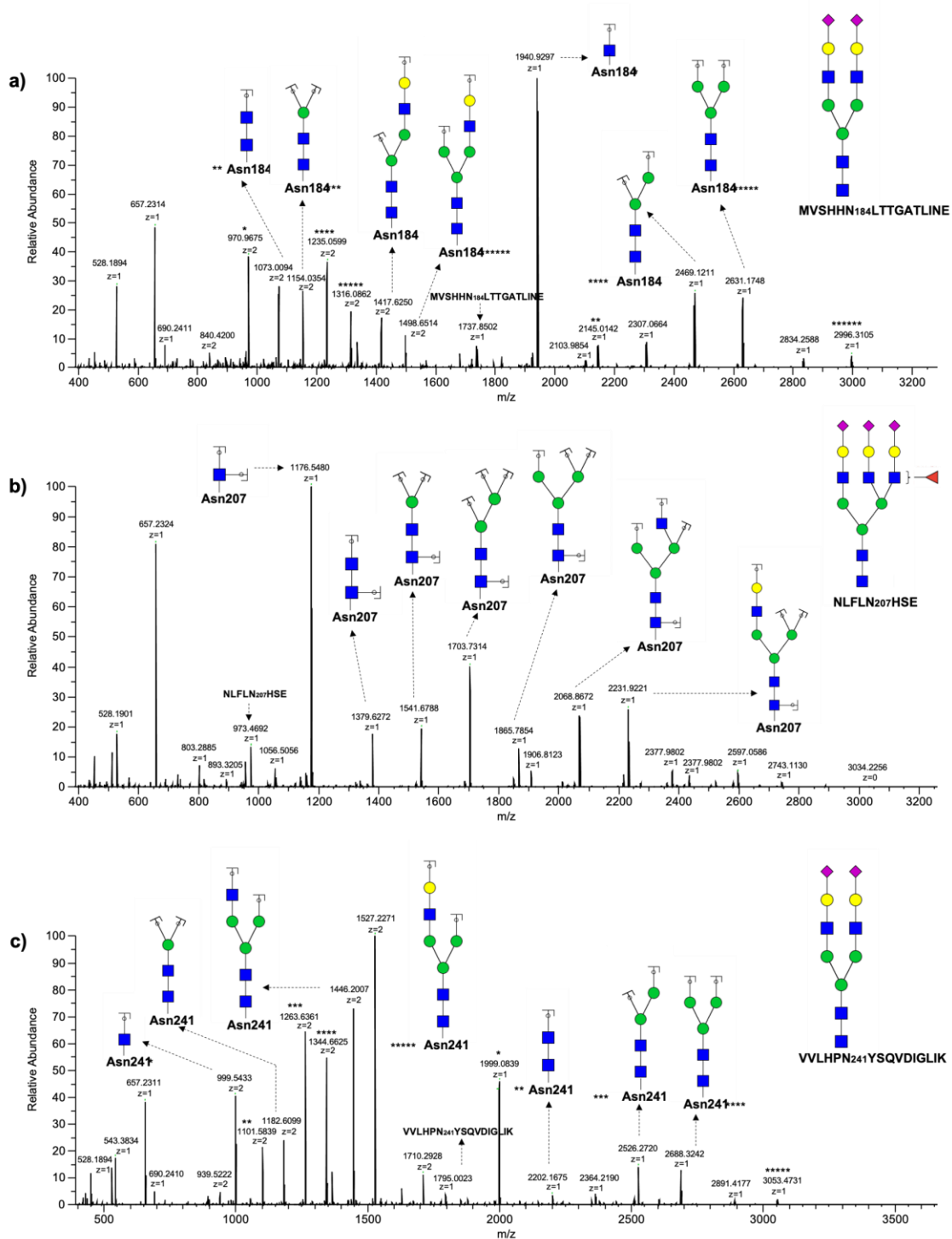
Supplementary Table S4. Descriptive statistics of haptoglobin *N*-glycopeptides with important changes between cirrhosis and HCC samples. MVSHHN₁₈₄LTTGATLINE = Asn184, NLFLN₂₀₇HSE = Asn207, VVLHPN₂₄₁YSQVDIGLIK = Asn241, and AFP = alpha-fetoprotein. Glycan nomenclature: HexNAc, Hex, Fuc, NeuAc (*N*-acetylhexosamine, Hexose, Fucose, *N*-acetylneuraminic acid).

Supplementary Table S5. Determination of haptoglobin *N*-glycopeptides with significant changes in abundance between cirrhosis and HCC using same ratio female : male in both sample cohorts (*p* value <0.05). MVSHHN₁₈₄LTTGATLINE = Asn184, NLFLN₂₀₇HSE = Asn207, VVLHPN₂₄₁YSQVDIGLIK = Asn241, and AFP = alpha-fetoprotein. Glycan nomenclature: HexNAc, Hex, Fuc, NeuAc (*N*-acetylhexosamine, Hexose, Fucose, *N*-acetylneuraminic acid).

Supplementary Table S6. Statistical comparison between gender groups. **a)** Comparison of the statistically significant *N*-glycopeptides between gender groups. **b)** Multiple-variable model adding gender. Single and group glycopeptide models, differentiation of cirrhosis and HCC. **c)** The corresponding logistic regression model. NLFLN₂₀₇HSE = Asn207, VVLHPN₂₄₁YSQVDIGLIK = Asn241, and AFP = alpha-fetoprotein. Glycan nomenclature: HexNAc, Hex, Fuc, NeuAc (*N*-acetylhexosamine, Hexose, Fucose, *N*-acetylneuraminic acid).

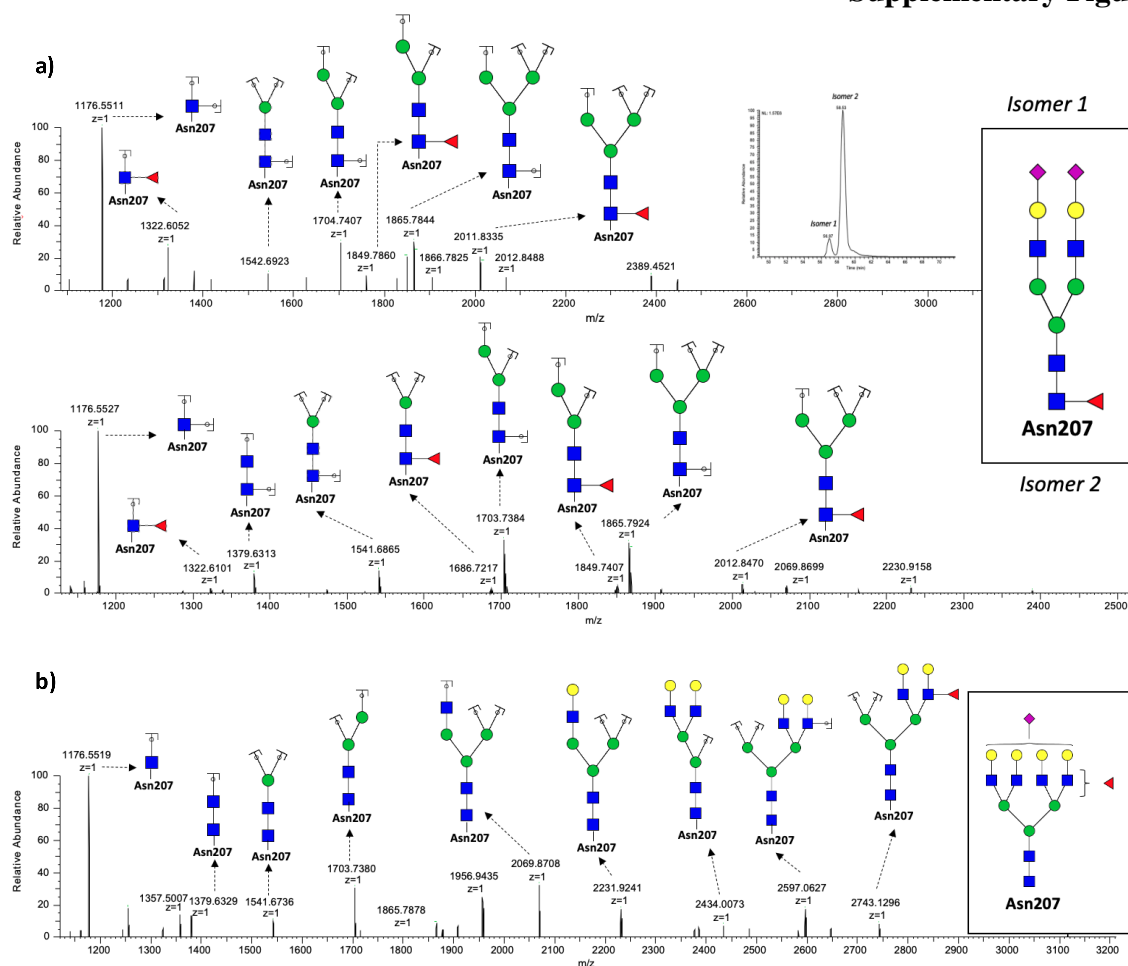
Supplementary Table S7. Determination of haptoglobin *N*-glycopeptides with significant changes in abundance between cirrhosis and **early HCC** using same gender ratio female : male in both sample cohorts. MVSHHN₁₈₄LTTGATLINE = Asn184, NLFLN₂₀₇HSE = Asn207, VVLHPN₂₄₁YSQVDIGLIK = Asn241, and AFP = alpha-fetoprotein. Glycan nomenclature: HexNAc, Hex, Fuc, NeuAc (*N*-acetylhexosamine, Hexose, Fucose, *N*-acetylneuraminic acid).

Supplementary Figure S1



Supplementary Figure S1. Representative mass spectra of the three glycosylation sites. **a)** MVSHHN₁₈₄LTTGATLINE = Asn184, **b)** NLFLN₂₀₇HSE = Asn207, and **c)** VVLHPN₂₄₁YSQVDIGLIK = Asn241. * Common fragment ion with different charge.

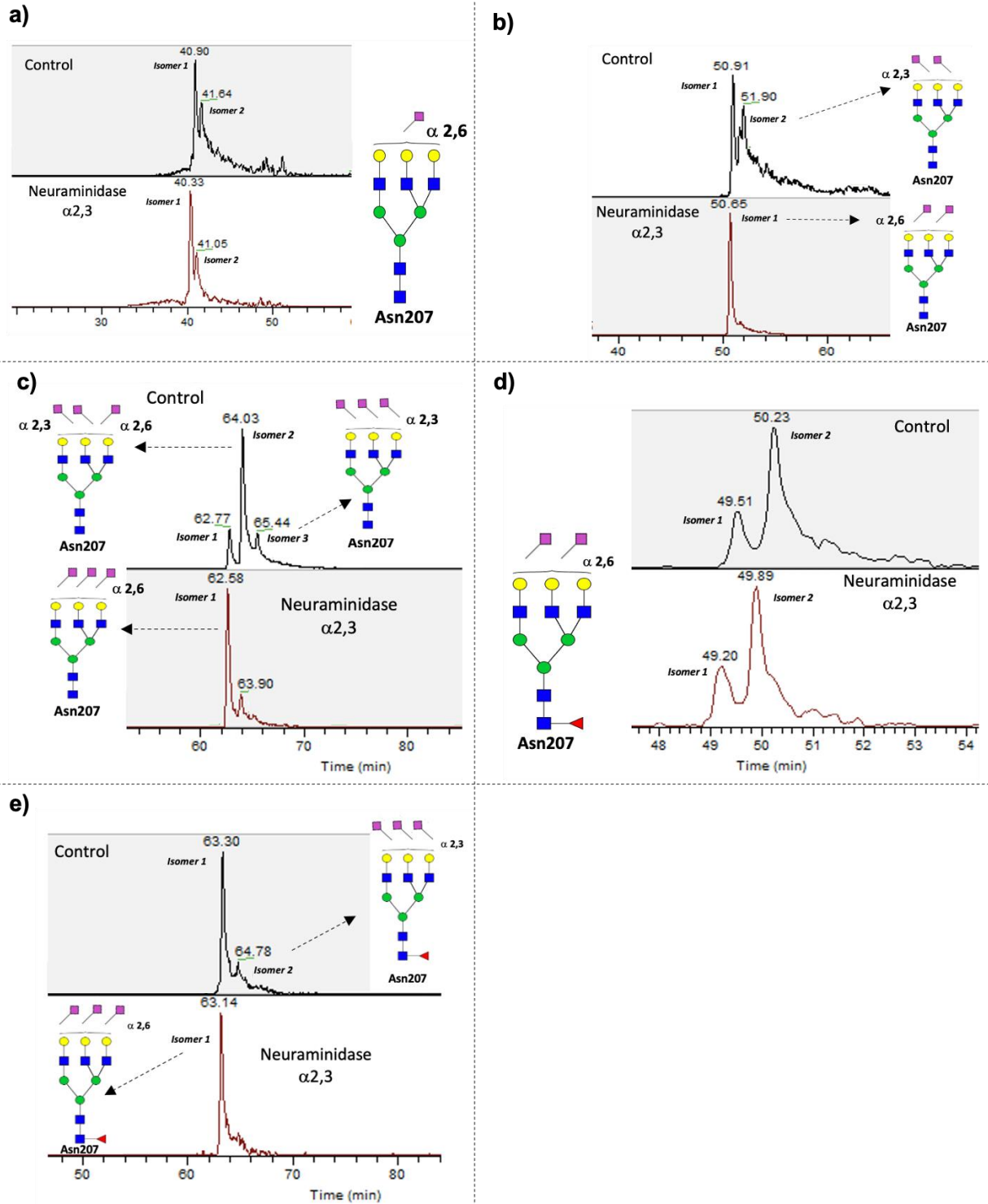
Supplementary Figure S2



Supplementary Figure S2. Interpretation of fucosylated glycopeptides; **a)** *N*-glycopeptide NLFLN₂₀₇HSE + 4-5-1-2 isomers 1 and 2, **b)** *N*-glycopeptide NLFLN₂₀₇HSE + 6-7-1-1. NLFLN₂₀₇HSE = Asn207.

The core or branch fucosylation of most abundant *N*-glycopeptides was performed by the evaluation of their fragmentation. The high abundance of core fragments allowed us to identify this type of structures, **Supplementary Figure S1a** shows the NLFLN₂₀₇HSE + 4-5-1-2 **core** fucosylated glycopeptide where the core-fucose fragments were observed in both isomers. Otherwise, the low abundance of branched fragments made difficult the identification of this type of glycopeptides, **Supplementary Figure S1b** shows the NLFLN₂₀₇HSE + 6-7-1-1 **branch** fucosylated glycopeptide, a branch-fucose fragment with a *m/z* of 2743.1296 was observed in the EIC, additionally the absence of core-fucose fragments gave us a clear conclusion of the branch fucosylation.

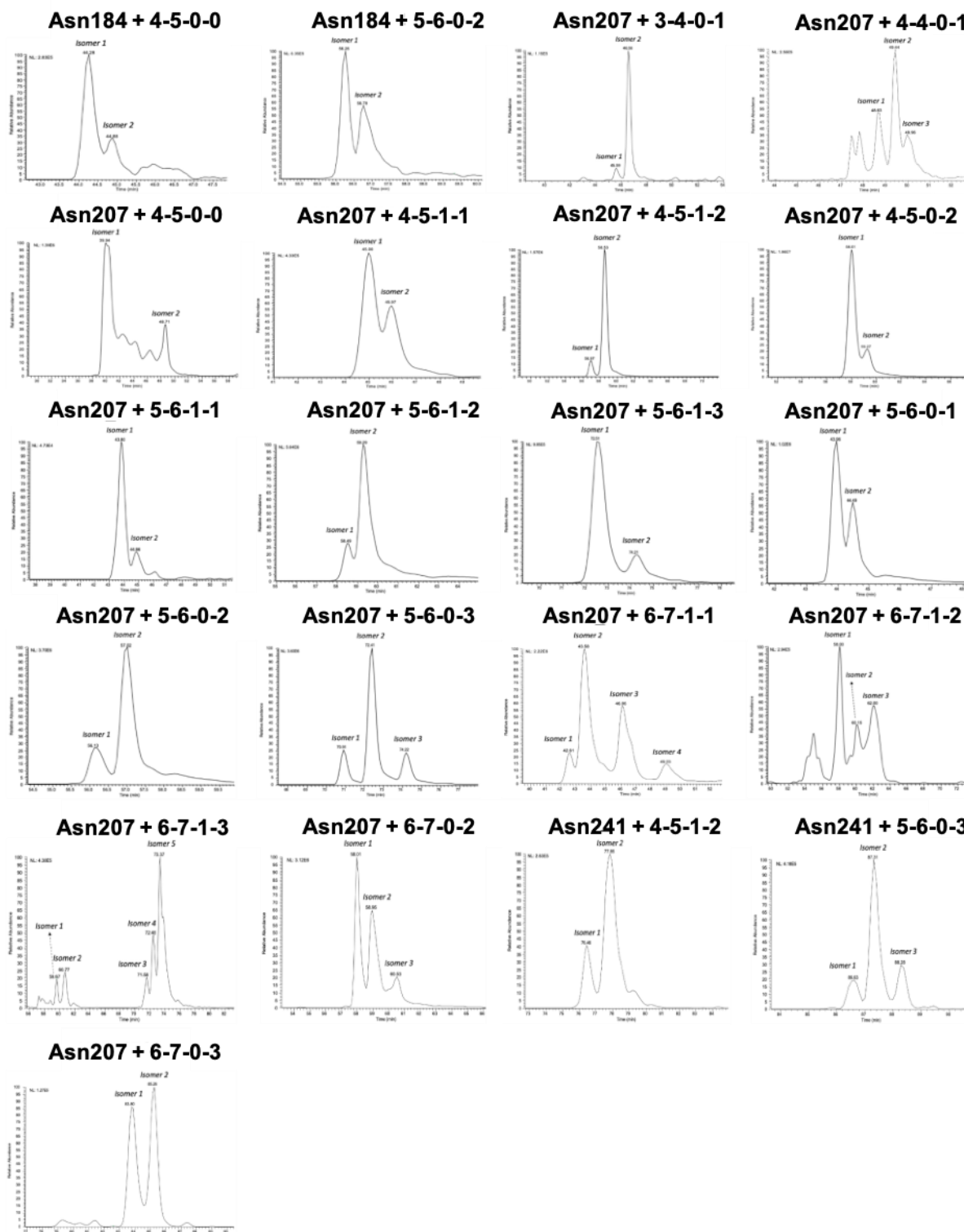
Supplementary Figure S3



Supplementary Figure S3. Sialic acid linkage of important and abundant haptoglobin *N*-glycopeptides (NLFLN₂₀₇HSE = Asn207).

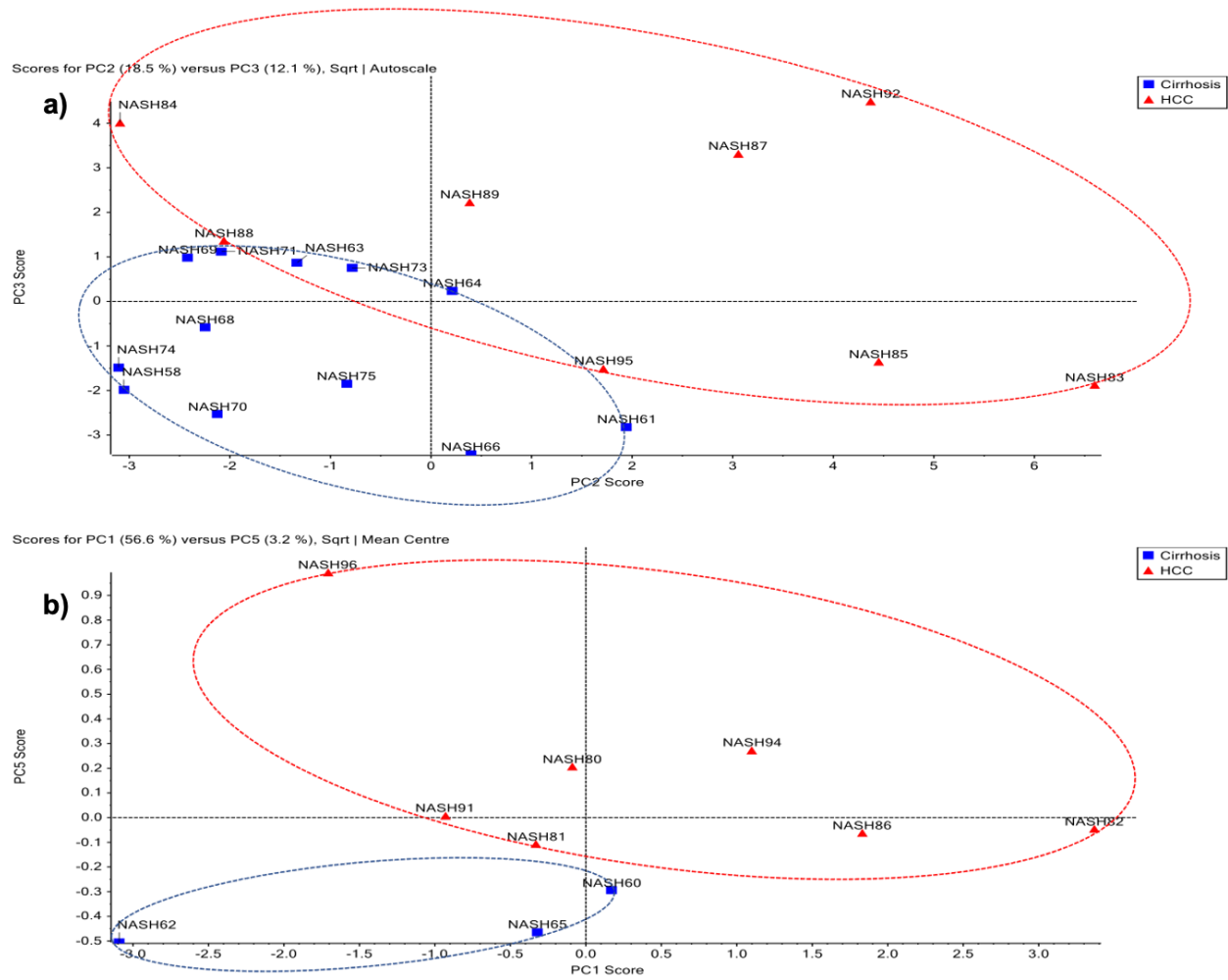
A pooled sample was used to determine the sialic acid linkage of some abundant *N*-glycopeptides by the application of α 2,3 neuraminidase enzyme digestion. The sample was fractionated using the LC method mentioned in section 2.4, the fractions were collected considering the retention time ranges \pm 2 min aforementioned for the elution of mono-, di, and tri-sialylated glycopeptides (**Supplementary Table S1**). The fractionation of the sample was performed to avoid peak overlapping of the digested sialylated glycopeptide structures and the present sialylated glycopeptides in the sample, the fractions were divided in two, control and digested samples. The samples were analyzed in an scan range of 300 – 2000 *m/z*. We were able to identified the linkage of some high abundant sialylated glycopeptides. **a)** For the *N*-glycopeptide NLFLN₂₀₇HSE + 5-6-0-1 both isomeric structures have sialic acid linkage α 2,6, no changes were observed after enzymatic digestion. **b)** For the *N*-glycopeptide NLFLN₂₀₇HSE + 5-6-0-2; the shift in mass of isomer 2 indicated an α 2,3 linkages, and therefore no changes in the isomer 1 indicated α 2,6 linkages. **c)** For the *N*-glycopeptide NLFLN₂₀₇HSE + 5-6-0-3; isomer 1 have α 2,6 linkages, the mass shift of isomer 3 after enzymatic digestion indicated a structure with α 2,3 linkages. Meanwhile for isomer 2 the incomplete digestion indicated by a remaining peak suggest the presence of sialic acid molecules with both linkages, the change in peak area was about 1/3 of the initial which suggest a ratio 1 to 3 of linkages α 2,6 and α 2,3. Huang et al. reported these three isomeric structures in their glycomic analysis of serum haptoglobin [1]. **d)** For the *N*-glycopeptide NLFLN₂₀₇HSE + 5612 both isomeric structures have sialic acid linkage α 2,6, no changes were observed after enzymatic digestion. **e)** For the *N*-glycopeptide NLFLN₂₀₇HSE + 5-6-1-3; the shift in mass of isomer 2 indicated an α 2,3 linkages, and therefore no changes in the isomer 1 indicated α 2,6 linkages.

Supplementary Figure S4



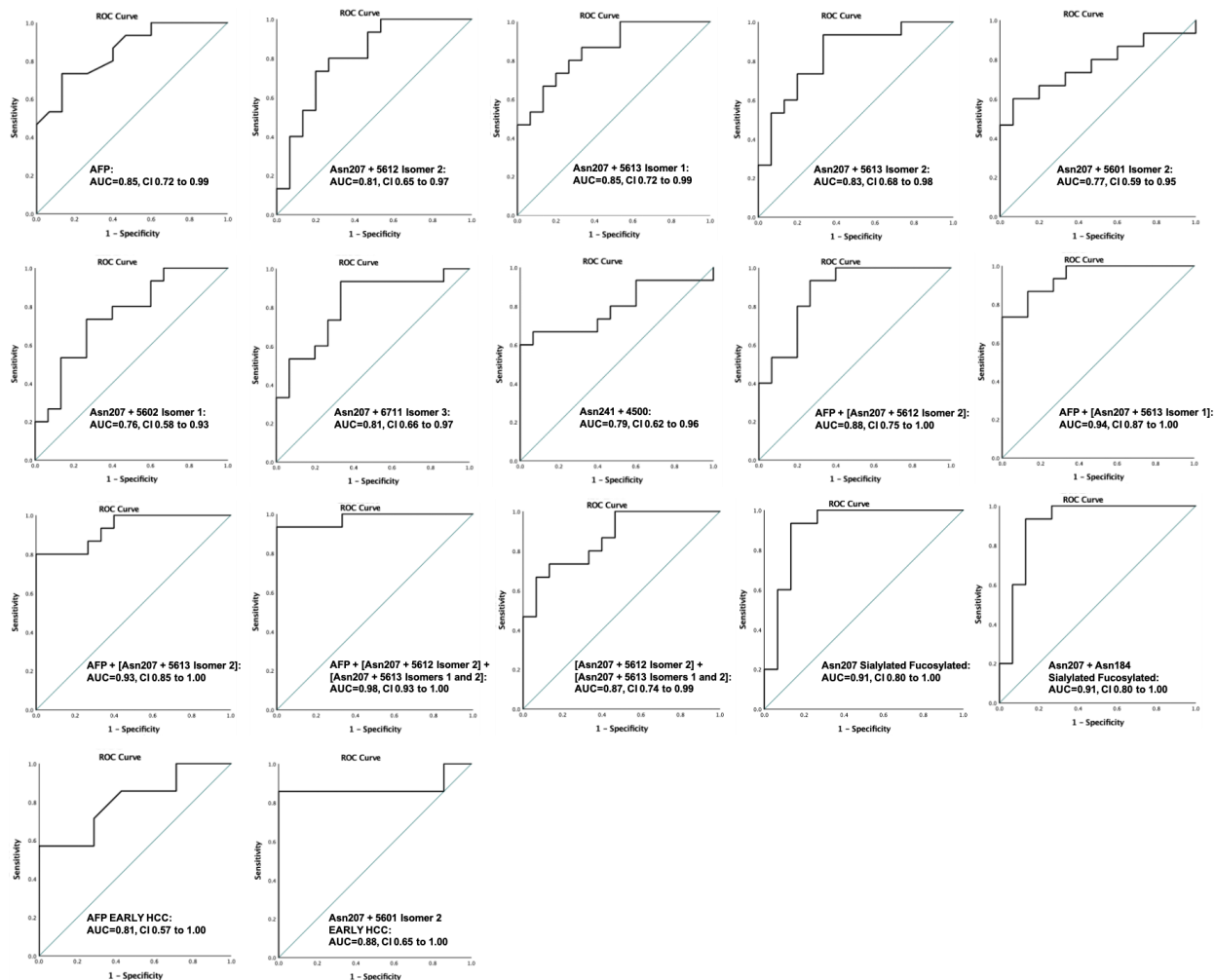
Supplementary Figure S4. EICs showing the achieved isomeric separation of important haptoglobin N-glycopeptides. MVSHHN₁₈₄LTGATLINE = Asn184, NLFLN₂₀₇HSE = Asn207, and VVLHPN₂₄₁YSQVDIGLIK = Asn241.

Supplementary Figure S5



Supplementary Figure S5. PCA plot of *N*-glycopeptides derived from human serum haptoglobin from patients with cirrhosis and HCC, glycosylation site NLFLN₂₀₇HSE. Gender evaluation, **a)** cirrhosis (female) “blue” vs. HCC (female) “red”, **b)** cirrhosis (male) “blue” vs. HCC (male) “red”.

Supplementary Figure S6



Supplementary Figure S6. Receiver operating characteristic (ROC) curve for the glycopeptide structures with statistically significance between cirrhosis and HCC patients. MVSHHN₁₈₄LTTGATLINE = Asn184, NLFLN₂₀₇HSE = Asn207, and VVLHPN₂₄₁YSQVDIGLIK = Asn241. Glycan nomenclature: HexNAc, Hex, Fuc, NeuAc (*N*-acetylhexosamine, Hexose, Fucose, *N*-acetylneuraminic acid).

Supplementary Table S1. PRM information for each of the target haptoglobin *N*-glycopeptides.

Glycopeptide ^{a, b}	Charge	rt (min)	Precursor ion (<i>m/z</i>)	Fragment ions (<i>m/z</i>)
Asn184 + 4-5-0-0	+ 3	42.24	1120.8183	1940.9333, 1072.5091, 1153.5359, 1234.5626, 1315.5888, 1417.1236
Asn184 + 4-5-1-1	+ 3	48.01	1266.5361	1940.9345, 1072.5099, 1153.5363, 1234.5613, 1315.5918, 1417.1268
Asn184 + 4-5-1-2	+ 3	57.07	1363.5679	1940.9336, 2086.9927, 2306.0684, 2468.1247, 2614.1797, 2630.1775
Asn184 + 4-5-0-1	+ 3	48.16	1217.8501	1940.9348, 1072.5088, 1153.5328, 1234.5603, 1315.5876, 1417.1272
Asn184 + 4-5-0-2	+ 3	54.73	1314.8819	1940.9337, 1072.5088, 1153.5358, 1234.5612, 1315.5887, 1498.1566
Asn184 + 4-6-0-1	+ 3	47.70	1271.8677	1940.9342, 2468.1247, 2630.1767, 2792.2461, 1336.5882, 1417.1265
Asn184 + 5-6-1-1	+ 3	47.18	1388.2468	1940.9331, 1072.5094, 1153.5355, 1234.5614, 1336.1014, 1417.1266
Asn184 + 5-6-1-2	+ 4	56.36	1114.2108	1940.9355, 2086.9979, 2306.0781, 2468.1318, 2630.1824, 2995.3198
Asn184 + 5-6-1-3	+ 3	57.30	1582.3104	1940.9361, 1459.6648, 1585.6160, 2101.8861, 2468.1117, 2995.3202
Asn184 + 5-6-0-1	+ 3	47.48	1339.5609	1940.9336, 1072.5086, 1153.5362, 1234.5614, 1315.5884, 1498.1562
Asn184 + 5-6-0-2	+ 3	54.08	1436.5927	1940.9337, 1072.5088, 1153.5349, 1234.5614, 1315.5827, 1417.1324
Asn207 + 3-4-0-1	+ 3	49.81	1533.6345	1176.5484, 1056.5070, 1379.6285, 1541.6803, 1703.7228, 1865.7870
Asn207 + 4-4-0-1	+ 2	49.44	1261.513	1176.5498, 1379.6307, 1541.6826, 1703.7349, 1865.7875, 1906.8068
Asn207 + 4-5-0-0	+ 2	40.39	1363.0527	1176.5490, 1379.6296, 1703.7336, 1865.7869, 2068.8884, 2230.9209
Asn207 + 4-5-1-1	+ 2	45.27	1298.5314	1176.5490, 1379.6298, 1541.6806, 1703.7309, 1865.7865, 2011.8485
Asn207 + 4-5-1-2	+ 3	57.98	1011.7411	1176.5505, 1703.7195, 1865.7773, 2068.8518, 2011.8485, 2230.9328
Asn207 + 4-5-0-1	+ 3	46.13	1108.7729	1176.5489, 1379.6279, 1541.6831, 1703.7328, 1865.7862, 2068.8710
Asn207 + 4-5-0-2	+ 2	57.49	1444.0791	1176.5495, 1541.6829, 1703.7334, 1865.7890, 2068.8653, 2230.9326
Asn207 + 5-6-1-1	+ 3	47.31	1060.0869	1176.5492, 1379.6293, 1541.6816, 1703.7332, 1865.7881, 2068.8665
Asn207 + 5-6-1-2	+ 3	56.34	1133.4518	1176.5496, 1379.6278, 1541.6826, 1703.7333, 1865.7822, 2068.8705
Asn207 + 5-6-1-3	+ 3	70.56	1230.4836	1176.5496, 1379.6244, 1541.6758, 1703.7313, 2068.8542, 2230.9204
Asn207 + 5-6-0-1	+ 3	45.18	1327.5154	1176.5497, 1379.6282, 1541.6810, 1703.7330, 1865.7879, 2230.9212
Asn207 + 5-6-0-2	+ 3	56.25	1084.7659	1176.5498, 1379.6283, 1541.6814, 1703.7339, 1865.7873, 2230.9204
Asn207 + 5-6-0-3	+ 3	71.39	1181.7977	1176.5502, 1379.6268, 1541.6714, 1703.7332, 2068.8577, 2230.9304
Asn207 + 6-7-1-1	+ 3	46.06	1278.8295	1176.5494, 1703.7345, 1932.9272, 2068.8702, 2230.9241, 1541.6814
Asn207 + 6-7-1-2	+ 3	58.07	1255.1626	1176.5503, 1129.5302, 1210.5564, 2068.8720, 2230.9256, 2596.0621
Asn207 + 6-7-1-3	+ 3	60.00	1352.1944	1176.5499, 1703.7356, 1875.9048, 1892.9331, 2068.8711, 2230.9238
Asn207 + 6-7-0-1	+ 3	43.81	1449.2262	1176.5494, 1379.6287, 1541.6718, 1703.7314, 2068.8716, 2596.0640
Asn207 + 6-7-0-2	+ 3	57.79	1206.4766	1176.5493, 1379.6289, 1703.7333, 2068.8687, 2230.9216, 2596.0574
Asn207 + 6-7-0-4	+ 3	60.29	1303.5084	1176.5509, 1201.5585, 1642.6395, 1516.6875, 1220.5885, 2528.0915
Asn241 + 4-4-0-1	+ 4	77.35	1123.4308	1998.0903, 2231.9111, 2069.8580, 1866.7745, 1177.5254, 1542.6679
Asn241 + 4-5-0-0	+ 3	61.74	1182.8833	1998.0914, 1101.0855, 1182.1120, 1263.1389, 1344.1667, 1445.7048
Asn241 + 4-5-1-2	+ 3	78.62	1139.8691	1998.0943, 2013.9092, 1290.7283, 1445.7031, 1486.7358, 1526.7318
Asn241 + 4-5-0-1	+ 3	67.98	1382.6187	1998.0868, 1101.0865, 1182.1102, 1344.1648, 1445.7029, 1526.7317
Asn241 + 4-5-0-2	+ 3	75.35	1236.9009	1999.0859, 1263.1281, 1344.1588, 1445.6924, 1526.7240, 1285.5670
Asn241 + 5-6-1-2	+ 3	78.15	1333.9327	1998.0907, 1128.6021, 1347.7465, 1445.6995, 1526.7316, 1709.3043
Asn241 + 5-6-0-1	+ 4	69.80	1128.4989	1998.0891, 1174.5344, 1417.6190, 1472.2158, 1580.6812, 1624.7572
Asn241 + 5-6-0-2	+ 4	77.58	1019.2106	1998.0902, 1263.1382, 1709.2971, 1344.1647, 1182.1115, 1628.2694
Asn241 + 5-6-0-3	+ 4	87.31	1091.9844	1998.0890, 1101.0842, 1263.1386, 1345.7034, 1526.7317, 1709.2975
Asn241 + 6-7-0-1	+ 4	69.23	1164.7583	1998.0898, 1209.6461, 1445.7010, 1827.7471, 1451.6415, 1288.5770
Asn241 + 6-7-0-2	+ 4	77.30	1110.4936	1998.0909, 1177.5336, 1445.7034, 1704.7222, 2231.9072, 2069.8579
Asn241 + 6-7-0-3	+ 4	87.23	1183.2675	1998.0868, 1645.7706, 1445.7043, 1255.5942, 1150.5383, 1037.4541

^a PEPTIDE_HexNAc, Hex, Fuc, NeuAc (*N*-acetylhexosamine, Hexose, Fucose, *N*-acetylneuraminic acid)^b MVSHHN₁₈₄LTTGATLINE = Asn184, NLFLN₂₀₇HSE = Asn207, and VVLHPN₂₄₁YSQVDIGLIK=Asn241

Supplementary Table S2. Peak area (A) and normalized intensity (B) of all identified haptoglobin *N*-glycopeptides from patients with cirrhosis and hepatocellular carcinoma (HCC).

(A)

Glycosylation site MVSHHN ₁₆ LTGATLINE		N- Glycopeptide		NASH58	NASH60	NASH61	NASH62	NASH63	NASH64	NASH65	NASH66	NASH69	NASH70	NASH71	NASH72	NASH74	NASH75	NASH80	NASH81	NASH82	NASH83	NASH84	NASH85	NASH87	NASH88	NASH89	NASH91	NASH92	NASH93	NASH95	NASH96
Alan 1841 + HexNAc4,Hex5 isomer 1	7440	154119	4621175	1522050	3973189	2603015	524102	8292467	15502089	14828814	784811	2500303	3434500	33679	879778	1800806	3959444	511973	5303890	27176	1686248	349657	6901076	490656	48479	137898	8049183	4616472	1108892	2932329	
Alan 1841 + HexNAc4,Hex5 isomer 2	0	28672	1602476	377697	180735	547631	549839	1580735	549839	1580735	549839	1580735	549839	1580735	549839	1580735	549839	1580735	549839	1580735	549839	1580735	549839	1580735	549839	1580735	549839	1580735	549839	1580735	
Alan 1841 + HexNAc4,Hex5,Neu5Ac2 isomer 1	1105699	740100	19053466	360440	1379515	265840	864734	7242502	7317703	1361290	477485	2463004	3436307	89412	13819952	5922200	13341262	3436307	6626819	47485	1336484	1293190	8882707	39448	438556	775803	1383645	15707994	29262419	739278	
Alan 1841 + HexNAc4,Hex5,Neu5Ac2 isomer 2	186780	14602609	34135313	2244560	11077811	11089716	3902769	8000030	21365443	37755734	5229704	2817973	31426000	445100	28946436	45664919	38257658	69040535	23668114	858400	2131793	866757	26495158	2800667	2507282	12159931	24801518	88040636	15079988	6603121	
Alan 1841 + HexNAc4,Hex5,Neu5Ac2 isomer 3	12778974	27690120	30959052	157152053	397133458	78191564	8718055	43236453	86298329	69436456	13056471	59169578	61472744	44671304	46571364	28902377	25774008	550217484	68045925	51923487	55172827	54470647	42704627	451133994	40812651	156041454	390915952	15079988	6603121		
Alan 1841 + HexNAc4,Hex5,Neu5Ac2 isomer 4	7444807	73507798	83659254	34140217	35648351	30964023	901743819	1946321369	211079145	300148434	155229694	220044173	151388134	191097465	323004763	107762318	130630339	10701445	80485157	20661202	179451593	60432458	223436861	307120884	113511718	9753393	110554055	110554055	110554055		
Alan 1841 + HexNAc4,Hex5,Neu5Ac2 isomer 5	19179	551314	3074203	360232	1175134	963023	236576	3891636	6993899	10312403	286446	2807864	3384138	67910	625877	321252	3814552	11505162	1977381	33426	2908596	63008	3464046	60334	34097	180194	1535982	4685556	105801	1260389	
Alan 1841 + HexNAc4,Hex5,Neu5Ac2 isomer 6	79257	145668	1346874	746751	229138	120449	257509	867222	2487346	2976756	262489	1196897	466526	26326	30470225	1222452	1592353	2167433	943065	52413	3211047	133749	2628778	55150	99513	29239	5189600	743191	2413909	145211	
Alan 1841 + HexNAc4,Hex5,Neu5Ac2 isomer 7	4728311	1338674	5948499	105133	385949	1875560	946489	1025446	3177214	3014438	271663	370010	667698	19883	4144014	3764970	6862359	9917609	13151839	34711	9450855	536342	5934786	31755	159943	287124	4646523	9212442	2510355	251249	
Alan 1841 + HexNAc4,Hex5,Neu5Ac2 isomer 8	801747	12321159	4473341	3871663	2455140	5221159	1618173	1537910	2068950	2798446	952181	213003	4462180	5004128	2631750	2277654	2557176	285136	2140878	6432428	26656072	755435	1552747	3021117	2424022	3249373	6239214	3121631	7327678		
Alan 1841 + HexNAc4,Hex5,Neu5Ac2 isomer 9	87046	1095570	16172029	3189334	5452995	3940488	1307800	27071359	21393172	23880571	1488750	7730888	11331014	120150	3044241	6995991	12244326	3253405	28777699	102225	8389901	8455774	791975	790846	445743	13145626	1756449	3202342	3727322		
Alan 1841 + HexNAc4,Hex5,Neu5Ac2 isomer 10	756532	984362	20037891	8273556	10581282	3847899	4650210	23651588	3378962	76454991	7939856	16883167	2385411	840574	5358231	2279502	45283200	2780024	36382871	91391	13424354	5587545	45066907	6872041	4139932	13506517	42164278	12578442	3423490	9050512	
Alan 1841 + HexNAc4,Hex5,Neu5Ac2 isomer 11	79887	321947	12515748	6132831	9297283	7817283	1342506	12033485	23315074	20776183	2604468	22811721	1988904	250818	17630556	11992317	8817974	2907121	22269688	814891	9631615	503805	4320049	2207318	173597	188571	11361168	12520768	262265	728592	
TOTAL	95120215	80296500	125000891	518279654	1435236768	71638653	412725543	1464241729	2953217362	3704502309	641424618	1929656471	2951149284	83523095	2343196561	2211686213	2802214342	1306751758	2008039336	143147297	100465233	786472902	2492677924	429973363	284863373	493013858	808939557	1734305381	797474807	1471739596	
Glycosylation site NLFN ₂₀ RHSE		N- Glycopeptide		NASH58	NASH60	NASH61	NASH62	NASH63	NASH64	NASH65	NASH66	NASH69	NASH70	NASH71	NASH72	NASH74	NASH75	NASH80	NASH81	NASH82	NASH83	NASH84	NASH85	NASH87	NASH88	NASH89	NASH91	NASH92	NASH93	NASH95	NASH96
Alan 2071 + HexNAc4,Hex5,Neu5Ac2 isomer 1	16070	236663	3154005	700370	350715	1559870	413120	2489044	4980722	3818347	625098	1867355	1897557	14861	5749497	1778732	972118	1217835	338675137	191373	716335	1760757	4630519	453826	1179324	137824	1760757	4630519	453826	1179324	
Alan 2071 + HexNAc4,Hex5,Neu5Ac2 isomer 2	490491	2342112	30531243	9481389	3963399	5665450	2638226	27552100	17857500	33254684	14026119	11057289	15075554	588434	58627628	117341319	25111050	2796644	3602576	3195704	30284152	8013131	57947356	3047940	1681812	5574782	103154473	18725542	17270156	8599848	
Alan 2071 + HexNAc4,Hex5,Neu5Ac2 isomer 3	41756	738179	1887480	2364200	445317	1330541	1010639	4398062	6610480	3730682	15700499	3849157	4971789	34428	8807897	224167	2119101	480345	494156	65940	427787	411169	33241	133141	688426	93547	309746	1151885	4684426	93547	
Alan 2071 + HexNAc4,Hex5,Neu5Ac2 isomer 4	12523	505814	379347	570130	219651	815548	342687	581672	139966	1802429	371148	759338	994316	7083	2458487	1175545	577241	483992	166725	154675	389928	5254438	203966	188872	497912	1242529	2111608	235304	703821		
Alan 2071 + HexNAc4,Hex5,Neu5Ac2 isomer 5	186780	511370	7380875	26074995	16308120	2624352	60714573	10068893	72911815	3618879	4865206	6093304	205326	14125962	3122544	1887697	649474	3022294	331709	3748993	8841327	51172000	4435969	850483	518868	3436767	5798521	1361768	44312720		
Alan 2071 + HexNAc4,Hex5,Neu5Ac2 isomer 6	10872	145634	1929072	8391516	480740	638214	2525709	1668187	3140044	1662705	3076762	796381	1168647	21250	4273332	922983	5867807	699952	914770	102748	1397842	1749212	845341	1387284	44893	174843	68740	4150548	246112	1180713	
Alan 2071 + HexNAc4,Hex5,Neu5Ac2 isomer 7	26033761	17028380	16897409	5520596	13011221	45461145	34846312	21192035	5332704	21916688	1596267	17040106	3688527	601554	105799901	73844460	81535880	26781055	11342115	21637272	24240276	71732918	388181	966678	1193580	34551100	342099757	168022144	32071403		
Alan 2071 + HexNAc4,Hex5,Neu5Ac2 isomer 8	4287348	9018014	32377373	2972870	12717910	16290056	11640733	11293060	46871813	17610340	2455144	8775957	1371772	1358572	31445520	12759048	2487835	29718863	14283204	40251947	14452423	368845	6864671	5374848	1351858	3898951	18954627	14095427	14291365		
Alan 2071 + HexNAc4,Hex5,Neu5Ac2 isomer 9	67046	29800	1602476	377697	177949	340535	1537395	1056450	2498466	2607213	1686457	6027213	1889775	2080967	688373	5719295	2680620	2340826	2351736	2603656	2340826	2351736	2603656	2340826	2351736	2603656	2340826	2351736	2603656	2340826	
Alan 2071 + HexNAc4,Hex5,Neu5Ac2 isomer 10	4145052	4638185	98877009	4725372	28818359	10572114	39578253	14575522	83212795	26885456	18849279	42654491	2624407	76052415	9685554	62682627	132020570	97919352	38457038	14387356	62588000	82840125	6269878	23396633	93595907	204252921	4272188	90520378	9272188		
Alan 2071 + HexNAc4,Hex5,Neu5Ac2 isomer 11	2315108	24149165	125085342	796317071	120580342	67274228	168412021	360145589	3165884	145462100	141667014	31122664	30008240	111808008	795724050	260293175	127834793	219442207	38873627	156478457	26401805	15136380	14363401	74631400	14023104	42686423	105964972	105964972	105964972		
Alan 2071 + HexNAc4,Hex5,Neu5Ac2 isomer 12	114541096	822334871	3457426957	89625918	913208978	270938251	493415861	400895228	533348878	734849137	186319578	1542891970	9881209	157162338	159562661	362118821	101029886	196085358	40914709	86331409	618180232	186512272	42344005	288915066	514113871	352607878	67811841	17780831	17780831		
Alan 2071 + HexNAc4,Hex5,Neu5Ac2 isomer 13	656736	17912696	38128608	17543373	2868159	64724268	17195062	46274676	16328070	16328070	16328070	16328070	16328070	16328070	16328070	16328070	16328070	16328070	16328070	16328070	16328070	16328070	16328070	16328070	16328070	16328070	16328070	16328070	16328070	16328070	
Alan 2071 + HexNAc4,Hex5,Neu5Ac2 isomer 14	474047	975753	159586902	6323453	6821990	2889025	1768714	9911075	11756851	19512500	1587876	3208493	1438473	1840274	5276181	74028010	32890213	13134472	2507357	3897635	1480738	577485	3268213	1338733	577485	3268213	1338733	577485	3268213	1338733	
Alan 2071 + HexNAc4,Hex5,Neu5Ac2 isomer 15	341815	2700073	2218677	904997	1477993	3181068	3809675	5311206	691180	5983452	1483034	2087779	4572086	167866	10524049	1873204	5103412	5445682	3621701	1026571	2904475	3170088	7311570	687252	923776	748815	3705459	7908800	687463	3473573	
Alan 2071 + HexNAc4,Hex5,Neu5Ac2 isomer 16	8890352	1204295	32152292	3564863	726781	16328070	12816164	2052385	16298844	12816164	2052385	16298844	12816164	2052385	16298844	12816164	2052385														

(B)

Glycosylation site MVSHHN14dLTGTAGLINE

N - Glycopeptide		NASH58	NASH60	NASH61	NASH62	NASH63	NASH64	NASH65	NASH66	NASH68	NASH69	NASH70	NASH71	NASH73	NASH74	NASH75	NASH80	NASH81	NASH82	NASH83	NASH84	NASH85	NASH86	NASH87	NASH88	NASH89	NASH91	NASH92	NASH94	NASH95	NASH96
[Asn 184] + HexNAc,Hex5,Isomer 1	0.007918	0.019219	0.368221	0.239674	0.276834	0.363456	0.126986	0.581347	0.509632	0.400292	0.122356	0.150548	0.117174	0.039121	0.380200	0.076778	0.136428	0.039179	0.258841	0.018985	0.157844	0.044459	0.276854	0.099570	0.017018	0.027970	0.444808	0.266286	0.136582	0.195936	
[Asn 184] + HexNAc,Hex5,Isomer 2	0.000000	0.003571	0.246412	0.072856	0.132200	0.054095	0.040000	0.223151	0.184023	0.157365	0.040403	0.082636	0.048030	0.007659	0.189769	0.022292	0.029037	0.004223	0.073114	0.003366	0.008002	0.007592	0.039570	0.048914	0.003633	0.005874	0.281220	0.205903	0.067575	0.070289	
[Asn 184] + HexNAc,Hex5,Fuc,Neu5Ac	1.161812	0.002021	1.534140	0.059119	0.059847	0.398339	0.209518	0.507739	0.247795	0.036747	0.066677	0.027640	0.179563	0.004059	0.511500	0.267772	0.390781	1.257815	0.313783	0.031822	1.229775	0.164269	0.396469	0.008012	0.146932	0.055871	0.766122	0.329127	0.665852	0.050247	
[Asn 184] + HexNAc,Hex5,Fuc,Neu5Ac2	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	0.196289	
[Asn 184] + HexNAc,Hex5,Neu5Ac	13.427493	1.564304	24.379860	29.279565	27.266370	10.374153	21.123044	30.311150	29.122684	18.743804	18.714727	30.537466	20.972233	19.840474	27.163072	11.518841	17.924847	6.952215	29.978960	25.518750	11.636440	6.600069	22.133958	15.101246	17.372233	8.661953	24.860860	23.532466	15.449415	22.366583	
[Asn 184] + HexNAc,Hex5,Neu5Ac2,Isomer 1	78.327980	91.545699	66.854025	65.872197	69.380120	83.337399	75.023293	63.217956	65.907165	76.882748	78.100526	85.204814	75.340556	77.893565	68.410384	81.882090	76.831838	82.481419	74.758279	80.113019	90.235303	72.196074	82.017382	79.138627	86.577634	66.712546	65.453939	72.098423	75.142125	72.128525	
[Asn 184] + HexNAc,Hex5,Neu5Ac2,Isomer 2	0.013848	0.068660	0.244956	0.067576	0.051876	0.137259	0.057320	0.272824	0.236829	0.280040	0.044689	0.145502	0.115454	0.007633	0.271835	0.145388	0.131436	0.088017	0.006257	0.023351	0.289513	0.077562	0.138969	0.012739	0.011970	0.036549	0.106708	0.280323	0.132310	0.086666	
[Asn 184] + HexNAc,Hex5,Fuc,Neu5Ac	0.083929	0.018588	0.266671	0.144083	0.019585	0.016818	0.067329	0.060482	0.085466	0.090493	0.060493	0.060216	0.051009	0.031384	0.116531	0.056316	0.054873	0.165864	0.003363	0.038615	0.131968	0.017006	0.040560	0.003073	0.034934	0.039391	0.038975	0.047602	0.302403	0.070078	
[Asn 184] + HexNAc,Hex5,Fuc,Neu5Ac2	4.968268	0.156716	0.474063	0.020285	0.028891	0.261184	0.220079	0.071891	0.107588	0.081372	0.043055	0.019175	0.022779	0.029663	0.179057	0.170231	0.208887	0.738974	0.649595	0.022466	0.026276	0.066924	0.238089	0.006441	0.056137	0.058267	0.256883	0.513189	0.315189	0.210727	
[Asn 184] + HexNAc,Hex5,Neu5Ac	0.842435	0.375845	0.754847	0.362499	0.171063	0.729010	0.206780	0.107816	0.067180	0.074759	0.090870	0.104321	0.082379	0.006965	0.226199	1.773195	0.784592	2.246933	0.315243	0.199470	0.213006	0.187883	0.170980	0.152224	0.051075	0.050620	1.340860	1.354648	0.786268	0.312777	
[Asn 184] + HexNAc,Hex5,Neu5Ac	0.091943	0.139480	1.289521	0.615311	0.379949	0.595027	0.316695	1.456313	0.737908	0.617642	0.263125	0.400625	0.386972	0.114817	1.306673	0.275789	0.421893	0.248788	1.363527	0.074908	0.851506	0.090275	0.339224	0.160652	0.274112	0.098525	0.745444	1.017482	0.401422	0.296397	
[Asn 184] + HexNAc,Hex5,Neu5Ac2,Isomer 1	0.749025	0.077180	1.668365	1.598306	0.737255	0.537276	1.126683	1.638807	1.143496	2.049411	0.512730	1.237820	0.873757	0.006389	2.315100	1.008954	1.560298	0.212743	1.883836	0.658897	1.358653	1.038117	1.397370	1.453326	1.861287	2.330011	0.940001	0.753773	0.429075	0.614507	
[Asn 184] + HexNAc,Hex5,Neu5Ac2,Isomer 2	0.083941	0.000497	0.997270	1.183267	0.647792	1.906891	0.325278	0.843613	0.789505	0.602015	0.400044	0.171800	0.535276	0.300265	2.761703	0.540416	1.200467	0.222056	0.057166	0.956710	0.064059	0.173317	0.427469	0.060765	0.199938	0.628938	0.721947	0.329096	0.465052	0.615447	
TOTAL	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	

Glycosylation site NFLN207HSE

N - Glycopeptide		NASH58	NASH60	NASH61	NASH62	NASH63	NASH64	NASH65	NASH66	NASH68	NASH69	NASH70	NASH71	NASH73	NASH74	NASH75	NASH80	NASH81	NASH82	NASH83	NASH84	NASH85	NASH86	NASH87	NASH88	NASH89	NASH91	NASH92	NASH94	NASH95	NASH96
Asn 207 + HexNAc3,Hex4,Neu5Ac,Isomer 1	0.006232	0.011295	0.029950	0.020689	0.011911	0.015102	0.026039	0.034982	0.048514	0.025340	0.026828	0.024444	0.027496	0.006804	0.031981	0.028618	0.017964	0.042034	0.137769	0.016231	0.067952	0.010097	0.028216	0.024247	0.007159	0.015969	0.034982	0.047491	0.021655	0.020788	
Asn 207 + HexNAc3,Hex4,Neu5Ac,Isomer 2	0.195270	0.111780	0.290125	0.280436	0.134676	0.080727	0.159302	0.358531	0.159569	0.231428	0.077438	0.163304	0.207679	0.272163	0.323880	0.220668	0.464037	0.130974	0.750238	0.162564	0.280888	0.105624	0.207833	0.247867	0.176351	0.633276	0.270488	0.192032	0.606987	0.515194	
Asn 207 + HexNAc3,Hex4,Neu5Ac,Isomer 3	0.016192	0.035240	0.017163	0.069727	0.015022	0.018533	0.040260	0.061064	0.059001	0.026158	0.079122	0.059621	0.084345	0.015658	0.043288	0.030568	0.031650	0.025622	0.125866	0.041155	0.073914	0.023102	0.080583	0.020966	0.035434	0.016293	0.056423	0.048044	0.040469	0.053054	
Asn 207 + HexNAc3,Hex4,Neu5Ac,Isomer 4	0.027510	0.040478	0.053875	0.049523	0.025886	0.021016	0.051683	0.026862	0.027695	0.018161	0.063832	0.025019	0.025831	0.018065	0.030877	0.020206	0.007386	0.015619	0.015341	0.033820	0.040189	0.005111	0.047421	0.047041	0.031105	0.021392	0.039534	0.021428	0.020205	0.022465	
Asn 207 + HexNAc3,Hex4,Neu5Ac,Isomer 5	0.005915	0.021410	0.036400	0.016842	0.007460	0.011621	0.026182	0.009146	0.008401	0.013250	0.001740	0.011218	0.015986	0.000367	0.013371	0.018977	0.010667	0.016705	0.020435	0.011319	0.036042	0.004581	0.017351	0.014835	0.002041	0.012994	0.033098	0.016128	0.011228	0.024462	
Asn 207 + HexNAc3,Hex5,Isomer 1	0.007240	0.023868	0.019434	0.170259	0.565571	0.402312	0.381164	0.710191	0.954765	0.103522	0.169028	0.839679	0.003291	0.696028	0.003869	0.003291	0.778308	0.010484	0.438836	0.224617	0.431746	0.118307	0.350131	0.309929	0.737339	0.306865	0.902907	0.348300	0.014294	0.555313	0.781540
Asn 207 + HexNAc3,Hex5,Isomer 2	0.040280	0.078954	0.183180	0.247889	0.168711	0.119446	0.125514	0.232639	0.280537	0.109411	0.115747	0.112987	0.193236	0.109000	0.235961	0.112291	0.108434	0.058643	0.112771	0.086744	0.129645	0.061132	0.117853	0.093630	0.037506	0.090931	0.183331	0.145312	0.121495	0.209894	
Asn 207 + HexNAc3,Hex5,Fuc,Neu5Ac,Isomer 1	10.056690	0.859597	1.600596	0.163236	0.441900	0.744074	2.205187	0.298275	0.476609	0.674307	0.800232	0.251133	0.504890	0.738822	0.604972	1.188073	0.758455	0.848333	0.353607	0.961972	0.250458	0.849733	1.000015	0.293979	1.043906	0.798435	0.920351	0.508641	0.817695	0.532548	
Asn 207 + HexNAc3,Hex5,Fuc,Neu5Ac,Isomer 2	1.662578	0.430002	0.315960	0.088540	0.413840	0.274379	0.702894	0.167106	0.412790	0.123296	0.372150	0.145879	0.189287	0.616639	0.173088	0.205221	0.459741	0.938774	0.182746	1.023016	0.373340	0.505677	0.452160	0.268088	0.739016	0.355492	0.359973	0.104871	0.904468	0.251970	
Asn 207 + HexNAc3,Hex5,Fuc,Neu5Ac,Isomer 3	0.260220	0.388199	0.155958	0.136091	0.295192	0.167862	0.102163	0.231543	0.163161	0.247348	0.301860	0.250275	0.386779	0.317614	0.141989	0.476333	0.322657	0.558526	0.287550	0.445350	0.119041	0.221642	0.331775	0.227738	0.195138	0.349877	0.364351	0.320381	0.342358	0.129165	
Asn 207 + HexNAc3,Hex5,Fuc,Neu5Ac,Isomer 4	1.719781	1.827417	0.557181	0.139529	0.910832	1.086584	1.203287	0.204167	0.734227	0.412573	0.395271	0.293160	0.988676	1.201744	0.181772	0.909912	1.139859	0.456584	0.837300	0.309096	1.311377	2.193857	1.189780	0.627624	2.506511	1.281109	1.438308	2.048627	2.182229	0.688229	
Asn 207 + HexNAc3,Hex5,Neu5Ac	9.577777	11.525424	20.471523	23.523206	17.889457	9.585885	16.286281	20.787229	23.303692	18.692841	16.022717	21.483699	23.319475	14.154319	19.839913	17.988602	14.704519	9.840699	15.759479	18.620298	11.186538	19.202011	21.814354	19.203121	16.295059	15.264865	19.809255	15.202890	20.524044	26.609598	
Asn 207 + HexNAc3,Hex5,Neu5Ac,Isomer 1	0.4431769	38.203748	32.814717	26.475509	31.018991	38.109894	38.269295	29.793579	29.787351	35.765939	37.343847	37.001912	24.526965	28.742687	34.898987	34.194519	34.948442	34.948442	34.948442	34.948442	34.948442	34.948442	34.948442	34.948442	34.948442	34.948442	34.948442	34.948442	34.948442	34.948442	
Asn 207 + HexNAc3,Hex5,Neu5Ac,Isomer 2	1.536390	0.501296	3.620710	5.182317	0.755221	2.228991	1.138357	6.535549	9.450117	11.433031	8.586334	9.398917	8.221775	1.968565	6.888055	4.951332	8.789853	4.844401	5.173134	12.631302	4.367146	7.577074	8.238718	1.648460	8.753132	1.701629	8.804718	1.475312	4.284161	6.626784	
Asn 207 + HexNAc3,Hex5,Neu5Ac,Isomer 3	1.734951	0.655003	1.892022	0.184110	0.755221	0.230592	0.413063	1.058477	0.407682	0.158678	0.719549	0.349946	0.179955	0.882421	0.290701	1.398262	0.086088	0.264122	0.160356	0.212691	0.760236	0.316849	0.411524	0.302165	0.362165	0.121656	0.897436	0.368170	0.320727	0.203035	
Asn 207 + HexNAc3,Hex5,Fuc,Neu5Ac,Isomer 1	0.132550	0.128894	0.210983	0.026754	0.059197	0.184800	0.197281	0.074376	0.080862	0.041682	0.074330	0.030834	0.026933	0.076339	0.082776	0.140138	0.094308	0.181959	0.047642	0.74583	0.111126	0.101935	0.049987	0.099449	0.049527	0.080676	0.081114	0.236753	0.036120		
Asn 207 + HexNAc3,Hex5,Fuc,Neu5Ac,Isomer 2	0.207468	0.026203	0.620349	0.493331	0.330727	0.565959	0.348956	0.340547	0.350565	0.268279	0.342668	0.352369	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	
Asn 207 + HexNAc3,Hex5,Fuc,Neu5Ac,Isomer 3	5.587051	4.287176	2.058000	0.094040	0.264963	0.154467	0.480362	0.964213	1.397297	0.61138	1.246517	0.200371	1.726610	1.436078	0.737289	0.948138	0.948138	0.948138	0.948138	0.948138	0.948138	0.948138	0.948138	0.948138	0.948138	0.948138	0.948138	0.948138	0.948138	0.948138	
Asn 207 + HexNAc3,Hex5,Fuc,Neu5Ac,Isomer 4	5.298888	7.298640	3.909751	1.319867	0.377726	0.481242	6.533267	1.342834	2.760766	1.538825	1.354298	2.480227	0.886664	4.027393	2.181139	9.516469	4.788600	10.410533	3.903931	6.902539	6.721355	4.847175	2.942124	4.350621	5.297907	11.004796	7.560279	4.956540	4.956540	4.956540	
Asn 207 + HexNAc3,Hex5,Fuc,Neu5Ac,Isomer 5	0.424927	0.094501	0.451021	0.151280	0.654001	0.182432	0.729599	0.223143	0.517789	0.338655	0.177264	0.040032	0.396637	0.300034	0.676697	0.984994	0.658977	0.612790	0.418980	0.170194	0.103477	1.188708	0.570428	1.521716	1.101870	1.818796	1.128297	0.373762	0.557112		
Asn 207 + HexNAc3,Hex5,Neu5Ac,Isomer 1	0.794249	1.505967	2.041742	0.912778	1.044743	2.847465	3.147582	2.717584	0.725233	0.965719	1.404879	0.868896	1.362386	0.804507	0.565471	1.930201	1.378082	0.439060	0.859160	0.355249	2.383324	0.809111	2.393987	0.352642	2.286846	1.458231	0.693546	2.691267	2.691267	2.691267	
Asn 207 + HexNAc3,Hex5,Neu5Ac,Isomer 2	1.110392	0.908482	5.242332	3.430779	1.481918	1.611943	3.554493	0.945655	3.246227	0.326628	5.620947	1.862527	1.302365	0.263477	4.156152	1.546652	3.102372	0.420780	1.988094	0.355249	1.721122	0.449540	0.566488	1.382824	1.448023	0.942313	1.029626	1.510388	1.929565	1.632387	
Asn 207 + HexNAc3,Hex5,Neu5Ac,Isomer 3	1.507468	0.926033	3.620349	0.493331	0.330727	0.565959	0.348956	0.340547	0.350565	0.268279	0.342668	0.352369	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	0.270692	
Asn 207 + HexNAc3,Hex5,Neu5Ac,Isomer 4	1.432501	0.410643	6.203727	0.496781	0.114878	0.703212	7.443994	2.746529	5.571725	1.777558	1.715581	0.291488	2.769842	5.112825	0.845404	5.112825	0.845404	5.112825	0.845404	5.112825	0.845404	5.112825	0.845404	5.112825	0.845404	5.112825	0.845404	5.112825	0.845404	5.112825	0.845404
Asn 207 + HexNAc3,Hex5,Neu5Ac,Isomer 5	1.927679	1.429638	2.131436	2.564878	1.254162	1.266887	1.483758	2.808102	1.720176	0.722217	1.655317	0.249148	1.769822	1.501941	1.749237	3.967208	3.076188	1.439772	2.758083	1.987162	3.661288	3.966351	1.968609	2.948921	1.430025	1.745500	3.210289	1.925837	1.240641	1.469449	
Asn 207 + HexNAc3,Hex5,Neu5Ac,Isomer 6	0.448985	0.484968	0.484968	0.932211	0.010516	0.789344	0.496883	0.629977	0.581191	0.211313	0.654010	1.336636	9.293304	2.213557	0.103571	0.741810	0.251738	0.164707	0.265205	0.316980	0.278428	2.998827	0.4254196	0.2312764	5.192192	7.637855	0.864158	1.881740	0.522448	0.737769	
Asn 207 + HexNAc3,Hex5,Neu5Ac,Isomer 7	0.956724	0.602536	0.617276	1.657803	0.199712	0.264014	0.930321	1.232307	1.128148	0.319829	0.943107	0.769665	2.225607	0.285788	1.861842	0.628135	1.215330	0.092076	0.826013	0.059711	0.115820	0.468551	1.334688	0.222196	1.738204	2.136692	0.874171	0.370646	0.440137	1.520882	
Asn 207 + HexNAc3,Hex7,Fuc,Neu5Ac,Isomer 1	0.058994	0.045325	0.059058	1.218242	0.613646	0.183831	0.197700	0.548739	0.726325	0.234708	0.148489	0.610194	0.508990	0.294407	0.096023	0.238630	0.138456	0.140437	1.189947	0.342750	0.292427	0.103646	0.460326	0.245554	0.390547	0.089381	0.530403	0.904991	1.967978	0.884121	
Asn 207 + HexNAc3,Hex7,Fuc,Neu5Ac,Isomer 2	0.085031	0.210213	0.095017	0.341208	0.535778	0.214790	0.287620	0.369596	0.231402	0.059028	0.088848	0.120954	0.188988	0.289440																	

Supplementary Table S3. Complete clinical information.

NASH label	Cirrhosis/HCC	AFP	MELD score	TNM	Gender	Race	Age	Etiology	Lab Date	Sodium	Creatinine	Albumin	Total bilirubin	INR	Acetate: 1-None 2-Mild 3-Severe	Encephalopathy: 1-none 2-Cont 3-Uncont	Child Pugh (CTP) Score	No. Lesions	Max diameter (cm)	Portal vein invasion	Lymph node involvement	Evidence of metastases	Presence of cirrhosis	ALT	AST	ALP	PHOS	NA	WBC	PLT	ALBI score	Infiltrative	Hepatic vein thrombosis	Location of mets	Smoking	Alcohol use	Diabetes	Functional status (ECOG)	Cancer history	Family history of HCC		
58	Cirrhosis	5.7	8	No data	Female	Caucasian	67.0	NASH	1/8/2018	142	1.21	4.1	0.6	1.0	1	1	5	No data	No data	No data	No data	No data	No data	31	46	155	No data	No data	147	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	
59	Cirrhosis	No data	9	No data	Male	Caucasian	64.0	NASH	1/1/2018	136	1.25	2.9	1.0	1.0	1	1	6	No data	No data	No data	No data	No data	No data	33	38	129	No data	No data	189	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	
61	Cirrhosis	3.0	8	No data	Female	Hispanic	62.0	NASH	1/18/2018	134	0.92	3.9	0.3	1.2	1	1	5	No data	No data	No data	No data	No data	No data	55	54	236	No data	No data	85	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data
62	Cirrhosis	2.8	11	No data	Male	Caucasian	63.0	NASH	1/29/2018	140	0.53	4.1	1.9	1.2	1	1	5	No data	No data	No data	No data	No data	No data	67	60	64	No data	No data	104	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data
63	Cirrhosis	4.1	6	No data	Female	Caucasian	61.0	NASH	1/29/2018	147	0.59	4.1	0.8	1.0	1	1	5	No data	No data	No data	No data	No data	No data	20	24	54	No data	No data	218	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data
64	Cirrhosis	1.7	10	No data	Female	Caucasian	76.0	NASH	2/5/2018	143	1.52	3.7	0.2	0.9	1	1	5	No data	No data	No data	No data	No data	No data	19	29	156	No data	No data	335	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data
65	Cirrhosis	4.3	7	No data	Male	Caucasian	63.0	NASH	2/6/2018	138	0.71	3.3	0.9	1.1	1	1	6	No data	No data	No data	No data	No data	No data	20	32	102	No data	No data	244	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data
66	Cirrhosis	4.0	9	No data	Female	Hispanic	62.0	NASH	2/8/2018	139	0.81	3.5	1.2	1.2	1	1	6	No data	No data	No data	No data	No data	No data	30	43	148	No data	No data	97	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data
68	Cirrhosis	2.2	10	No data	Female	Caucasian	58.0	NASH	2/15/2018	138	1.07	4.2	0.3	1.3	1	2	6	No data	No data	No data	No data	No data	No data	24	22	113	No data	No data	194	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data
69	Cirrhosis	2.0	7	No data	Female	Caucasian	52.0	NASH	2/16/2018	139	0.62	4.1	0.6	1.1	1	1	5	No data	No data	No data	No data	No data	No data	19	28	57	No data	No data	92	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data
70	Cirrhosis	2.6	6	No data	Female	Caucasian	66.0	NASH	2/26/2018	143	0.81	4.2	0.5	1.0	1	1	5	No data	No data	No data	No data	No data	No data	46	48	146	No data	No data	115	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data
71	Cirrhosis	4.0	6	No data	Female	Hispanic	49.0	NASH	3/6/2018	142	0.88	4.6	0.3	1.0	1	1	5	No data	No data	No data	No data	No data	No data	33	26	63	No data	No data	182	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data
73	Cirrhosis	2.3	8	No data	Female	Caucasian	60.0	NASH	3/8/2018	142	0.81	3.9	0.4	1.2	1	1	5	No data	No data	No data	No data	No data	No data	34	31	92	No data	No data	92	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data
74	Cirrhosis	6.0	9	No data	Female	Caucasian	66.0	NASH	3/15/2018	137	1.13	4.0	1.3	1.1	1	1	5	No data	No data	No data	No data	No data	No data	69	97	84	No data	No data	96	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data
75	Cirrhosis	1.9	6	No data	Female	Asian	61.0	NASH	3/15/2018	140	0.78	4.5	0.4	1.0	1	1	5	No data	No data	No data	No data	No data	No data	82	84	69	No data	No data	215	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data
80	HCC	2.5	14	1	Male	White	65.0	NASH	10/30/2017	No data	1.18	2.8	2.2	1.2	2	1	8	1	3.1	No	No	No	Yes	21	35	161	136	475	159	1.098	No	No	No	Quit	Quit (heavy hx)	Yes, on insulin	0	Yes	None	None		
81	HCC	68.0	7	3	Male	White	65.1	NASH	10/13/2017	No data	0.88	3.8	1.0	1.1	1	1	5	2	10	Yes	No	No	No	Yes	121	85	184	142	657	168	0.272	No	No	No	Quit	Quit (heavy hx)	Yes	0	No	One 1st degree relative	None	
82	HCC	2221.0	30	3	Male	Black	79.2	NASH	10/5/2017	No data	7.47	2.5	2.3	1.9	3	1	11	98	NA	Yes	No	No	No	No	36	169	147	138	932	118	1.383	Yes	No	No	Quit	Quit (heavy hx)	Yes, on insulin	3	No	None	None	
83	HCC	4.6	7	3	Female	White	58.9	NASH	10/6/2017	No data	0.90	3.8	0.4	1.1	1	1	5	>5	15	Yes	No	No	No	Yes	50	71	308	144	11.80	493	-0.877	Yes	No	No	None	None	Yes, on insulin	0	No	None	None	
84	HCC	82173.0	11	4	Female	White	59.8	NASH	11/16/2017	No data	0.65	2.8	1.9	1.1	1	1	6	98	NA	Yes	No	Yes	Yes	Yes	78	166	698	133	7.80	282	1.002	Yes	No	No	None	None	No data	2	No	None	None	
85	HCC	6.0	11	3	Female	Hispanic	43.4	NASH	12/8/2018	No data	0.49	1.9	1.9	1.2	2	1	6	2	9.6	No	No	No	Yes	66	103	130	152	9.20	167	1.767	No	No	No	No	None	None	No data	0	No	None	None	
86	HCC	60500.0	14	4	Male	Hispanic	47.2	NASH	1/5/2018	No data	0.72	3.6	0.7	1.6	1	1	5	2	17.2	Yes	Yes	Yes	Yes	29	46	146	135	7.69	200	-0.337	Yes	Yes	Lung/pancreas	Quit	Quit (heavy hx)	No data	0	No	None	None		
87	HCC	3.2	10	1	Female	Other	61.8	NASH	1/21/2017	No data	1.00	3.7	2.1	1.1	1	2	7	1	2.5	No	No	No	Yes	49	48	225	150	3.95	199	0.303	No	No	No	None	None	Yes, on insulin	0	Yes	None	None		
88	HCC	7.0	21	1	Female	White	73.8	NASH	1/18/2018	No data	1.53	3.6	0.3	2.5	1	1	7	1	2.7	No	No	No	Yes	23	31	63	139	10.48	192	-0.897	No	No	No	None	None	Yes, on insulin	0	No	None	None		
89	HCC	310.4	9	1	Female	White	75.3	NASH	3/6/2018	No data	0.59	3.3	0.6	1.3	1	1	6	1	3	No	No	No	Yes	30	55	87	137	5.52	70	-0.184	No	No	No	None	None	Yes, on insulin	0	No	None	None		
91	HCC	5.3	8	3	Male	White	60.0	NASH	3/25/2018	No data	0.85	3.3	1.4	1.0	2	1	7	98	NA	No	No	No	Yes	60	56	120	138	10.25	465	0.375	No	No	No	Quit	Quit (heavy hx)	No data	No data	No	None	None		
92	HCC	4.0	8	3	Female	Hispanic	64.0	NASH	4/13/2018	No data	0.63	3.2	0.6	1.2	1	1	6	1	8.6	Yes	No	No	Yes	24	48	274	137	7.36	152	-0.099	No	No	No	Quit	None	No data	No data	No	None	None		
94	HCC	4.6	9	1	Male	White	69.2	NASH	6/5/2018	No data	0.78	3.9	0.8	1.3	2	1	6	1	4.9	No	No	No	Yes	48	59	168	134	4.72	67	-0.504	No	No	No	Quit	Quit (heavy hx)	Yes, on insulin	0	No	None	None		
95	HCC	12.0	21	1	Female	Hispanic	68.4	NASH	6/6/2018	No data	2.53	2.6	2.3	1.3	2	1	10	2	3.6	No	No	No	Yes	20	35	79	138	3.86	91	1.298	No	No	No	None	None	Yes, on insulin	No data	No	None	None	None	
96	HCC	3.0	6	1	Male	Hispanic	63.4	NASH	7/13/2018	No data	0.79	4.5	0.4	1.0	1	1	5	1	2.5	No	No	No	No	42	27	59	142	7.54	213	-1.472	No	No	No	None	None	Yes, on metformin	No data	No	None	None	None	

Supplementary Table S4. Descriptive statistics of haptoglobin *N*-glycopeptides with important changes between cirrhosis and HCC samples. MVSHHN₁₈₄LTTGATLINE = Asn184, NLFLN₂₀₇HSE = Asn207, VVLHPN₂₄₁YSQVDIGLIK = Asn241, and AFP = alpha-fetoprotein. Glycan nomenclature: HexNAc, Hex, Fuc, NeuAc (*N*-acetylhexosamine, Hexose, Fucose, *N*-acetylneuraminic acid).

Variable	Diagnosis	Sample number	Relative response (%)		<i>p</i> value
			Mean	Standard deviation	
AFP	Cirrhosis	15	3.11	1.59	0.001
	HCC	15	7688.31	19821.80	
Asn184 + 4-5-1-2	Cirrhosis	15	0.94	0.42	0.131
	HCC	15	1.64	1.21	
Asn184 + 5-6-1-3	Cirrhosis	15	0.38	0.39	0.077
	HCC	15	0.83	0.63	
Asn184 + 5-6-0-2 Isomer 2	Cirrhosis	15	0.67	0.34	0.128
	HCC	15	0.41	0.33	
Asn207 + 4-4-0-1 Isomer 3	Cirrhosis	15	0.01	0.01	0.132
	HCC	15	0.02	0.02	
Asn207 + 4-5-0-0 Isomer 2	Cirrhosis	15	0.16	0.07	0.104
	HCC	15	0.11	0.05	
Asn207 + 5-6-1-2 Isomer 2	Cirrhosis	15	2.43	1.55	0.012
	HCC	15	4.23	1.59	
Asn207 + 5-6-1-3 Isomer 1	Cirrhosis	15	3.51	1.94	0.001
	HCC	15	6.85	2.48	
Asn207 + 5-6-1-3 Isomer 2	Cirrhosis	15	0.50	0.29	0.005
	HCC	15	0.96	0.42	
Asn207 + 5-6-0-1 Isomer 2	Cirrhosis	15	2.75	1.64	0.010
	HCC	15	1.31	0.59	
Asn207 + 5-6-0-2 Isomer 1	Cirrhosis	15	3.67	1.09	0.038
	HCC	15	2.70	0.89	
Asn207 + 5-6-0-2 Isomer 2	Cirrhosis	15	7.11	1.78	0.033
	HCC	15	5.16	2.12	
Asn207 + 6-7-1-1 Isomer 3	Cirrhosis	15	0.08	0.11	0.085
	HCC	15	0.02	0.02	
Asn207 + 6-7-1-2 Isomer 1	Cirrhosis	15	0.19	0.15	0.096
	HCC	15	0.47	0.46	
Asn207 + 6-7-1-2 Isomer 3	Cirrhosis	15	0.05	0.02	0.014
	HCC	15	0.08	0.02	
Asn207 + 6-7-1-3 Isomer 5	Cirrhosis	15	0.07	0.07	0.094
	HCC	15	0.13	0.08	
Asn241 + 4-5-0-0	Cirrhosis	15	0.03	0.03	0.050
	HCC	15	0.01	0.01	
Asn241 + 4-5-0-1	Cirrhosis	15	5.73	0.95	0.065
	HCC	15	4.95	0.78	
AFP (early HCC)*	Cirrhosis	7	3.10	0.9	0.354
	HCC	7	48.96	115.33	
Asn207 + 5-6-0-1 Isomer 2 (early HCC)*	Cirrhosis	7	3.88	1.61	0.006
	HCC	7	1.43	0.42	

* Cirrhosis samples; NASH60, 61, 62, 65, 66, 70, and 75. Early HCC samples; NASH80, 87, 88, 89, 94, 95, and 96.
p – value obtained from T – Test and corrected using Bonferroni Correction.

Supplementary Table S5. Determination of haptoglobin *N*-glycopeptides with significant changes in abundance between cirrhosis and HCC using same gender ratio female : male in both sample cohorts (p value <0.05). MVSHHN₁₈₄LTTGATLINE = Asn184, NLFLN₂₀₇HSE = Asn207, VVLHPN₂₄₁YSQVDIGLIK = Asn241, and AFP = alpha-fetoprotein. Glycan nomenclature: HexNAc, Hex, Fuc, NeuAc (*N*-acetylhexosamine, Hexose, Fucose, *N*-acetylneuraminic acid).

<i>N</i>-Glycopeptides	Samples: 15 Cirrhosis 15 HCC	Samples: 7 Cirrhosis; M/F* (43/57 %) 15 HCC ; M/F (43/57 %)	
		<i>*For each test different female cirrhosis samples were used</i>	
COMPARISON	1	3	4
MVSHHN₁₈₄LTTGATLINE	<i>p</i> value^{A, C}	<i>p</i> value^{B, C}	<i>p</i> value^{B, C}
Asn184 + 4-5-1-2	0.131	0.560	0.656
Asn184 + 5-6-1-3	0.077	0.553	0.379
Asn184 + 5602 Isomer 2	0.128	0.048	0.368
NLFLN₂₀₇HSE			
Asn207 + 4-4-0-1 Isomer 3	0.132	1.000	0.513
Asn207 + 4-5-0-0 Isomer 2	0.104	0.029	0.242
Asn207 + 5-6-1-2 Isomer 2	0.012	0.059	0.123
Asn207 + 5-6-1-3 Isomer 1	0.001	0.027	0.048
Asn207 + 5-6-1-3 Isomer 2	0.005	0.048	0.050
Asn207 + 5-6-0-1 Isomer 2	0.010	0.001	0.006
Asn207 + 5-6-0-2 Isomer 1	0.038	0.016	0.018
Asn207 + 5-6-0-2 Isomer 2	0.033	0.113	0.051
Asn207 + 6-7-1-1 Isomer 3	0.085	0.063	0.010
Asn207 + 6-7-1-2 Isomer 1	0.096	0.516	0.404
Asn207 + 6-7-1-2 Isomer 3	0.014	0.504	1.014
Asn207 + 6-7-1-3 Isomer 5	0.094	0.825	0.934
VVLHPN₂₄₁YSQVDIGLIK			
Asn241 + 4-5-0-0	0.050	0.142	0.121
Asn241 + 4-5-0-1	0.065	0.620	0.698

Comparison 1. Cirrhosis samples; NASH58, 60, 61, 62, 63, 64, 65, 66, 68, 69, 70, 71, 73, 74 and 69.

HCC samples; NASH80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 91, 92, 94, 95 and 96.

Comparison 2. Cirrhosis samples; NASH60, 61, 62, 65, 66, 68, and 71.

Comparison 3. Cirrhosis samples; NASH60, 62, 65, 71, 73, 74, and 75.

^A p – value obtained from T – Test.

^B p – value obtained from Wilcoxon test.

^C p – values were corrected according to Bonferroni Correction ($n = 3$, $\alpha = 0.05$).

Initially, we observe seventeen haptoglobin *N*-glycopeptides with significant changes in abundance between cirrhosis and HCC samples (Supplementary Table S4). However, the cirrhosis cohort have a large number of female samples. Therefore, to avoid gender bias, the performance of the significant *N*-glycopeptides was evaluated by using a constant female : male (57:43 %) ratio for both cohorts. To complete the evaluation three cirrhosis sample groups were tested, different female cirrhosis samples were selected for each evaluation (Supplementary Table S5). The large number of parameter used in this evaluation can increase the probability that the observed results are accidental. Therefore, the “Bonferroni Correction” was applied to the observed p values. The corrected p values can be observed in supplementary table S5. Despite the applied correction the

N-glycopeptides Asn207 + 5-6-1-3 Isomer 1, Asn207 + 5-6-1-3 Isomer 2, Asn207 + 5-6-0-1 Isomer 2, and the Asn207 + 5-6-0-2 Isomer 1 have *p* values lower than 0.05 in all tested sample groups. By using this evaluation we intended to increase the accuracy of the presented results in the main manuscript.

Supplementary Table S6. Statistical comparison between gender groups. NLFLN₂₀₇HSE = Asn207, VVLHPN₂₄₁YSQVDIGLIK = Asn241, and AFP = alpha-fetoprotein. Glycan nomenclature: HexNAc, Hex, Fuc, NeuAc (*N*-acetylhexosamine, Hexose, Fucose, *N*-acetylneuraminic acid).

To prove that the *N*-glycopeptides selected to compare the cirrhosis and HCC were not biased by the gender groups the following statistical analysis was completed (a, b, and c).

a) Comparison of the statistically significant *N*-glycopeptides between gender groups.

Variable	Gender ^{1,2}	N	Min	Median	Max	Mean	SD	<i>p</i> value ³
Asn207 + 5-6-1-3 Isomer 1	Female	20	1.34	3.97	11.00	4.46	2.62	0.035
	Male	10	1.32	7.01	10.41	6.62	2.61	
Asn241 + 4-5-0-0	Female	20	0.00	0.02	0.12	0.02	0.03	0.091
	Male	10	0.00	0.01	0.02	0.01	0.01	
Asn207 + 5-6-1-2 Isomer 2	Female	20	0.84	2.56	5.59	2.92	1.68	0.169
	Male	10	0.96	4.14	7.37	4.14	1.84	
Asn207 + 5-6-1-3 Isomer 2	Female	20	0.17	0.53	1.82	0.70	0.48	0.287
	Male	10	0.15	0.78	1.13	0.78	0.30	
Asn207 + 6-7-1-1 Isomer 3	Female	20	0.01	0.02	0.42	0.06	0.10	0.328
	Male	10	0.00	0.02	0.12	0.03	0.04	
Asn207 + 5-6-0-1 Isomer 2	Female	20	0.33	1.80	5.69	2.21	1.54	0.422
	Male	10	0.42	1.52	3.52	1.67	1.11	
Asn207 + 5-6-0-2 Isomer 1	Female	20	1.50	3.32	6.30	3.28	1.08	0.746
	Male	10	1.24	3.24	5.00	3.00	1.15	

¹ Female samples; NASH58, 61, 63, 64, 66, 68, 69, 70, 71, 73, 74, 75, 83, 84, 85, 87, 88, 89, 92, and 95.

² Male samples; NASH60, 62, 65, 80, 81, 82, 86, 91, 94, and 96.

³ *p* – value obtained from Wilcoxon test.

Based on the Wilcoxon test, only Asn207 + 5-6-1-3 Isomer 1 had statistically significant difference between gender groups, and Asn241 + 4-5-0-0 showed marginally significant difference between gender groups.

We have added gender to the single and group glycopeptide panels, the estimated AUC and its 95% CI were summarized in b). We also completed the corresponding logistic regression model for the evaluated glycopeptides, see evaluation in c).

b) Multiple-variable model adding gender. Single and group glycopeptide models, differentiation of cirrhosis and HCC.

Glycopeptide	AUC	CI ¹ (low)	CI ¹ (high)
[Asn207 + 5-6-1-3 Isomer 1]+gender	0.86	0.69	0.97
[Asn241 + 4-5-0-0]+gender	0.79	0.62	0.93
AFP + [Asn207 + 5-6-1-3 Isomer 1] +gender	0.95	0.86	1.00

¹ Confidence interval (95% CI).

c) The corresponding logistic regression model.

Model	Variable	Coefficient	p value
1	Asn207 + 5-6-1-3 Isomer 1	0.66	0.012
	Gender	0.16	0.887
2	Asn241 + 4-5-0-0	-98.72	0.030
	Gender	0.48	0.611
	AFP	0.77	0.044
3	Asn207 + 5-6-1-3 Isomer 1	0.55	0.039
	Gender	1.36	0.337

The similarity of the AUC results when gender is added to the evaluation and the initial values (Table 2 in the manuscript). It was obvious that adding gender to the panels did not change the performance evidence by similar estimated AUC. The effect of gender in all the three logistic models was also not statistically significant. Therefore, no significant bias was introduced by gender.

Supplementary Table S7. Determination of haptoglobin *N*-glycopeptides with significant changes in abundance between cirrhosis and **early HCC** using same gender ratio female : male in both sample cohorts. MVSHHN₁₈₄LTTGATLINE = Asn184, NLFLN₂₀₇HSE = Asn207, VVLHPN₂₄₁YSQVDIGLIK = Asn241, and AFP = alpha-fetoprotein. Glycan nomenclature: HexNAc, Hex, Fuc, NeuAc (*N*-acetylhexosamine, Hexose, Fucose, *N*-acetylneuraminic acid).

<i>N</i>-Glycopeptides	Samples: 7 Cirrhosis; M/F* (42.9%/57.1%) 7 EARLY HCC; M/F (42.9%/57.1%) <i>*For each test different female cirrhosis samples were used</i>		
	1	2	3
COMPARISON	1	2	3
MVSHHN₁₈₄LTTGATLINE	<i>p</i> value^{A, B}	<i>p</i> value^{A, B}	<i>p</i> value^{A, B}
Asn184 + 4-5-1-2	1.000	1.000	0.746
Asn184 + 5-6-1-3	0.667	0.965	0.415
Asn184 + 5602 Isomer 2	0.191	0.119	0.440
NLFLN₂₀₇HSE			
Asn207 + 4-4-0-1 Isomer 3	1.000	1.000	0.679
Asn207 + 4-5-0-0 Isomer 2	0.173	0.554	0.350
Asn207 + 5-6-1-2 Isomer 2	0.241	0.376	0.375
Asn207 + 5-6-1-3 Isomer 1	0.159	0.234	0.234
Asn207 + 5-6-1-3 Isomer 2	0.192	0.630	0.334
Asn207 + 5-6-0-1 Isomer 2	0.006	0.034	0.040
Asn207 + 5-6-0-2 Isomer 1	0.032	0.050	0.175
Asn207 + 5-6-0-2 Isomer 2	0.322	0.670	0.600
Asn207 + 6-7-1-1 Isomer 3	0.318	1.000	0.147
Asn207 + 6-7-1-2 Isomer 1	0.702	1.000	0.394
Asn207 + 6-7-1-2 Isomer 3	0.750	0.199	0.560
Asn207 + 6-7-1-3 Isomer 5	1.000	1.000	1.000
VVLHPN₂₄₁YSQVDIGLIK			
Asn241 + 4-5-0-0	0.527	0.338	0.751
Asn241 + 4-5-0-1	1.000	0.567	1.000

Comparison 1. Cirrhosis samples; NASH60, 61, 62, 65, 66, 70, and 75.

Comparison 2. Cirrhosis samples; NASH60, 61, 62, 64, 65, 68, and 70.

Comparison 3. Cirrhosis samples; NASH60, 62, 65, 66, 68, 73, and 74.

^A*p* – value obtained from T – Test.

^B*p* – values were corrected according to Bonferroni Correction ($n = 3$, $\alpha = 0.05$).

The efficacy of the initial seventeen haptoglobin *N*-glycopeptides (Supplementary Table S4) was tested for the HCC samples in TNM 1 stage (early HCC). To avoid gender bias, the performance of the *N*-glycopeptides was evaluated by using a constant female : male (57:43 %) ratio for both cohorts. To complete the evaluation three cirrhosis sample groups were tested, different female cirrhosis samples were selected for each evaluation (Supplementary Table S7). The large number of parameter used in this evaluation can increase the probability that the observed results are accidental. Therefore, the “Bonferroni Correction” was applied to the observed *p* values. The corrected *p* values can be observed in supplementary table S7. Despite the applied correction the *N*-glycopeptide Asn207 + 5-6-0-1 Isomer 2 has *p* values lower than 0.05 in

all tested sample groups. By using this evaluation we intended to increase the accuracy of the presented results in the main manuscript.