SUPPORTING INFORMATION

Quantification of fucosylated hemopexin and complement factor H in plasma of patients with liver disease.

Julius Benicky[#a], Miloslav Sanda[#a], Petr Pompach[b,c], Jing Wu[a], Radoslav Goldman[a,d,*]

[a] *Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University. Washington, D.C. 20057;*

[b] *Institute of Microbiology v.v.i., Czech Academy of Sciences, Prague, Czech Republic;*

[c] *Department of Biochemistry, Faculty of Sciences, Charles University, Prague, Czech Republic*

[#] Authors contributed equally

* Corresponding author. Tel: 202-687-9868, Fax: 202-687-1988, E-mail: rg26@georgetown.edu

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Table S-1

Protein *UNIPROT #*	Glycosite position	Glycopeptide
Hemopexin	N64	CSDGWSFDATTLDD***N***^{64}GTMLFFK
P02790	N187	SWPAVG***N***^{187}CSSALR
	N240/N246	***N***^{240}GTGHG***N***^{246}STHHGPEYMR
	N453	ALPQPQ***N***^{453}VTSLLGCTH
CFH	N217	SPDVI***N***^{217}GSPISQK
P08603	N529	L***N***^{529}DTLDYECHDGYESNTGSTTGSIVCGYNGWSDLPICYER
	N718	IQCVDGEWTTLPVCIVEESTCGDIPELEHGWAQLSSPPYYYGDSVEF***N***^{718}CSESFTMIGHR
	N802 /N822	WDPEV***N***^{802}CSMAQIQLCPPPPQIPNSH***N***^{822}MTTTLNYR
	N882	IPCSQPPQIEHGTI***N***^{882}SSR
	N911	ISEE***N***^{911}ETTCYMGK
	N1029	MDGAS***N***^{1029}VTCINSR
	N1095	SPYEMFGDEEVMCLNG***N***^{1095}WTEPPQCK

Table S-1. Glycopeptides obtained by tryptic digest of hemopexin and CFH

Table S-2

Protein *UNIPROT ID*	Peptide	UNIPROT position	Site occupancy (%) H	CIR	HCC
Hemopexin	CSDGWSFDATTLDDNGTMLFFK	N64	96	96	97
P02790	SWPAVGNCSSALR	N187	100	100	100
	ALPQPQNVTSLLGCTH	N453	99	99	98
CFH	SPDVINGSPISQK	N217	87	92	91
P08603	IPCSQPPQIEHGTINSSR	N882	76	78	80
	ISEENETTCYMGK	N911	93	96	93
	MDGASNVTCINSR	N1029	99	99	99

Table S-2. Site occupancy. Site occupancy, shown as percent of glycosylated peptide, was determined in pooled samples (n=2 per group, 5 patient per pool) of healthy controls (H), cirrhosis (CIR) and HCC patients for all detected singly glycosylated sites as described in Material and Methods. The site occupancy of 100% means that non-glycosylated form of peptide was not detected. The results represent average of the two pooled samples. None of the sites changes significantly ($p<0.05$) in occupancy with disease state.

Table S-3

Protein *UNIPROT #*	Glycosite position	Glycan structure	m/z(charge)	H (SI)	*H [%]*	CIR (SI)	*CIR [%]*	HCC (SI)	*HCC [%]*
Hemopexin	N64	A2G2S1	1111.1937 (4+)	10090±1337	*8.3*	7523±870	*6.6*	9351±432	*6.6*
P02790		A2G2S2	1183.9676 (4+)	101335±9745	*83.4*	90072±9254	*79.1*	112117±11511	*79.1*
		A2G2S2F1	1220.4821 (4+)	10115±1223	*8.3*	16344±1185	*14.3*	*20354±1585	*14.4*
	N187	A2G2S1	1106.7872 (3+)	8001±990	*4.0*	8112±135	*3.9*	9075±1064	*3.7*
		A2G2S2	1203.8190 (3+)	80533±6953	*40.7*	92559±3187	*44.3*	101139±7309	*41.7*
		A2G2S2F1	939.6307 (4+)	13005±1674	*6.6*	26131±3548	*12.5*	*28105±3245	*11.6*
		A3G3	1131.4661 (3+)	7957±563	*4.0*	8503±928	*4.1*	10202±456	*4.2*
		A3G3S1	1228.4979 (3+)	11316±1164	*5.7*	12299±1071	*5.9*	11319±1345	*4.7*
		A3G3S2	994.3992 (4+)	17563±2235	*8.9*	*7834±332	*3.8*	11891±1093	*4.9*
		A3G3S2F1	1030.9137 (4+)	18037±1530	*9.1*	25218±1544	*12.1*	*30159±3621	*12.4*
		A3G3S3	1067.1731 (4+)	21269±3309	*10.8*	*8823±998	*4.2*	12707±1617	*5.2*
		A3G3S3F1	1103.6876 (4+)	8790±1616	*4.4*	8174±751	*3.9*	14899±2212	*6.1*
		A4G4	1253.1768 (3+)	11247±1201	*5.7*	11232±1056	*5.4*	13119±605	*5.4*
	N453	A2G2S1	913.1450 (4+)	12156±549	*5.5*	9513±172	*2.7*	9832±973	*2.8*
		A2G2S1F1	949.6595 (4+)	10077±894	*4.6*	16144±1866	*4.6*	20512±2518	*5.9*
		A2G2S2	985.9189 (4+)	180111±19486	*82.0*	270251±19091	*77.3*	260039±27066	*74.2*
		A2G2S2F1	1022.4333 (4+)	17368±2316	*7.9*	*53664±7335	*15.4*	*60098±6294	*17.1*
CFH	N217	A2G2S2	887.3742 (4+)	20173±1864	*93.5*	16092±1863	*83.8*	21153±1992	*82.4*
P08603		A2G2S2F1	923.8887 (4+)	1406±122	*6.5*	*3102±182	*16.2*	*4529±486	*17.6*
	N882	A2G2	911.6480 (4+)	28007±2505	*6.7*	*16110±1860	*5.3*	*16998±1631	*4.4*
		A2G2S1	984.4218 (4+)	10029±947	*2.4*	18205±2320	*6.0*	16005±1907	*4.2*
		A2G2S2	1057.1957 (4+)	350215±34546	*83.7*	*220038±16664	*72.7*	300138±23621	*78.5*
		A2G2S2F1	1093.7102 (4+)	21312±2728	*5.1*	36216±3892	*12.0*	35835±3905	*9.4*
		A3G3S2	1148.4787 (4+)	2506±190	*0.6*	3431±294	*1.1*	*4112±385	*1.1*
		A3G3S2F1	1184.9932 (4+)	1009±124	*0.2*	*3837±514	*1.3*	*3061±337	*0.8*
		A3G3S3	1221.2526 (4+)	5546±432	*1.3*	5017±293	*1.7*	5998±309	*1.6*
	N911	A2G2S1	869.5878 (4+)	14141±1915	*3.1*	7900±925	*2.0*	13006±1725	*3.0*
		A2G2S1F1	906.1023 (4+)	15034±1502	*3.3*	*30171±2637	*7.6*	*35208±3704	*8.0*
		A2G2S2	942.3617 (4+)	420406±43788	*91.9*	320524±37147	*80.3*	351044±41205	*80.0*
		A2G2S2F1	978.8762 (4+)	908±105	*0.2*	*32353±3951	*8.1*	*30213±1692	*6.9*
		A3G3S2	1033.6447 (4+)	6008±893	*1.3*	6025±760	*1.5*	8121±1107	*1.8*
		A3G3S3	1106.4186 (4+)	1081±25	*0.2*	*2107±184	*0.5*	#1415±130	*0.3*
	N1029	A2G2S1	835.3315 (4+)	18064±1954	*10.4*	20144±2774	*12.1*	16928±705	*9.3*
		A2G2S1F1	871.8460 (4+)	4549±743	*2.6*	*10108±1380	*6.1*	*10975±515	*6.0*
		A2G2S2	908.1054 (4+)	145238±22655	*83.3*	120541±15146	*72.6*	141714±18929	*77.8*
		A2G2S2F1	944.6199 (4+)	6018±383	*3.5*	*12010±1366	*7.2*	9947±1106	*5.5*
		A3G3S2	999.3884 (4+)	503±29	*0.3*	*3226±201	*1.9*	*2605±188	*1.4*

Table S-3. Relative intensities of identified site-specific glycoforms of tryptic peptides of HPX and CFH. Glycopeptide signal intensities (SI) in pooled samples of healthy controls (H), cirrhosis (CIR) and HCC patients (2 pools per group, 5 patients per pool) are presented as peak area of precursor ion XIC normalized to the intensity of a stable non-glycosylated tryptic peptide (GGYTLVSGYPK for hemopexin and SSNLIILEEHLK for CFH). Percent value indicates the relative distribution of individual glycoforms at a particular glycosylation site. Results are shown as mean ± SD, * $P<0.05$ vs H, # $P<0.05$ HCC vs CIR

Table S-4

Protein *UNIPROT #*	Glycosite position	Glycan structure	m/z(charge)	H (SI)	*H [%]*	CIR (SI)	*CIR [%]*	HCC (SI)	*HCC [%]*
Hemopexin	N64	A2G2	1038.4199 (4+)	115256±8208	*91.2*	1324541±30205	*84.4*	145095±13404	*87.4*
P02790		A2G2F1	1074.9344 (4+)	7370±812	*5.8*	*18683±2315	*11.9*	14779±1859	*8.9*
		A3G3	1129.7029 (4+)	2561±559	*2.0*	848±81	*0.5*	1044±70	*0.6*
		A3G3F1	1166.2174 (4+)	1186±95	*0.9*	4951±1737	*3.2*	5077±1889	*3.1*
	N187	A2G2	1009.7554 (3+)	468677±54669	*79.9*	365518±19391	*75.4*	420926±76770	*72.4*
		A2G2F1	1058.4413 (3+)	62393±2179	*10.6*	63878±6919	*13.2*	88403±16542	*15.2*
		A3G3	1131.4661 (3+)	35557±17850	*6.1*	21607±3622	*4.5*	29680±2072	*5.1*
		A3G3F1	1180.1521 (3+)	14796±933	*2.5*	22069±1614	*4.5*	*30220±2899	*5.2*
		A3G3F2	1228.8380 (3+)	840±63	*0.1*	2406±465	*0.5*	*3071±335	*0.5*
		A4G4	940.1346 (4+)	3771±1498	*0.6*	6923±5556	*1.4*	6238±3513	*1.1*
		A4G4F1	976.6491 (4+)	376±134	*0.1*	2677±3255	*0.6*	2724±2575	*0.5*
	N453	A2G2	1120.1589 (3+)	913342±80225	*96.3*	886004±57384	*93.7*	868559±88078	*92.5*
		A2G2F1	1168.8449 (3+)	16337±3811	*1.7*	34644±11919	*3.7*	45813±7939	*4.9*
		A3G3	1241.8697 (3+)	16663±7058	*1.8*	16466±326	*1.7*	15580±1794	*1.7*
		A3G3F1[@]	1290.5556 (3+)	2288±488	*0.2*	*8233±894	*0.9*	*8745±1237	*0.9*
CFH	N217	A2G2	741.8265 (4+)	16722±560	*91.6*	12472±270	*86.1*	11888±4332	*89.4*
P08603		A2G2F1	1074.9344 (4+)	1532±321	*8.4*	2012±241	*13.9*	1416±856	*10.6*
	N882	A2G2	911.6480 (4+)	213380±87237	*85.2*	167264±37896	*71.1*	424843±7208	*70.1*
		A2G2F1	948.1625 (4+)	16348±7626	*6.5*	38123±6225	*16.2*	#*101879±13057	*16.8*
		A3G3	1002.9310 (4+)	14419±117	*5.8*	12298±4205	*5.2*	#*31377±1302	*5.2*
		A3G3F1	1039.4455 (4+)	6278±1555	*2.5*	17541±7113	*7.5*	#*48017±2769	*7.9*
	N911	A2G2	1062.0827 (3+)	116372±13814	*83.7*	102527±3449	*69.1*	124283±39793	*66.1*
		A2G2F1	1110.7687 (3+)	7297±1760	*5.2*	19919±4302	*13.4*	24900±8858	*13.2*
		A3G3	888.0970 (4+)	9895±1617	*7.1*	11834±2846	*8.0*	16746±938	*8.9*
		A3G3F1	924.6115 (4+)	5492±1478	*3.9*	14086±490	*9.5*	*22209±5271	*11.8*
	N1029	A2G2	762.5577 (4+)	33755±8178	*90.9*	31458±859	*80.0*	35627±20698	*76.3*
		A2G2F1	799.0722 (4+)	3376±513	*9.1*	7876±1058	*20.0*	11074±7923	*23.7*

Table S-4. Relative intensities of site-specific glycoforms in de-sialylated pooled samples from healthy controls (H), cirrhosis (CIR) and HCC patients. Glycopeptide signal intensities (SI) are presented as peak area of precursor ion XIC normalized to the intensity of internal control non-glycosylated peptide (GGYTLVSGYPK for hemopexin and SSNLIILEEHLK for CFH). Percent value indicates the relative distribution of individual glycoforms at a particular glycosylation site. Results are shown as mean ± SD, * P<0.05 vs H, # P<0.05 HCC vs CIR, @ Structure out of fragmentation range confirmed by retention time and exact mass

Table S-5

Protein *UNIPROT ID*	Peptide	UNIPROT position	Glycan structure	Precursor m/z (charge)
Hemopexin *P02790*	CSDGWSFDATTLDDNGTMLFFK	N64	A2G2	1038.4 (4+)
			A2G2F1	1074.9 (4+)
			A3G3	1129.7 (4+)
			A3G3F1	1166.2 (4+)
	SWPAVGNCSSALR	N187	A2G2	1009.7 (3+)
			A2G2F1	1058.4 (3+)
			A3G3	1131.4 (3+)
			A3G3F1	1180.1 (3+)
			A3G3F2	1228.8 (3+)
			A4G4	940.1 (4+)
			A4G4F1	976.7 (4+)
			A4G4F2	1013.2 (4+)
			A4G4F3	1049.7 (4+)
	ALPQPQNVTSLLGCTH	N453	A2G2	1120.1 (3+)
			A2G2F	1168.8 (3+)
CFH *P08603*	SPDVINGSPISQK	N217	A2G2	741.8 (4+)
			A2G2F	778.3 (4+)
			A3G3	833.1 (4+)
			A3G3F	869.6 (4+)
	IPCSQPPQIEHGTINSSR	N882	A2G2	911.6 (4+)
			A2G2F	948.1 (4+)
			A3G3	1002.9 (4+)
			A3G3F	1039.4 (4+)
	ISEENETTCYMGK	N911	A2G2	1062.1 (3+)
			A2G2F	1110.7 (3+)
			A3G3	888.1 (4+)
			A3G3F	924.6 (4+)
	MDGASNVTCINSR	N1029	A2G2	762.6 (4+)
			A2G2F	799.1 (4+)

Table S-5. List of precursor ions selected for MRM transitions.

Table S-6

	Hemopexin				Complement factor H						
Position	**N64**	**N187**	**N187**	**N453**	**N217**	**N217**	**N882**	**N882**	**N911**	**N911**	**N1029**
Structure	A2G2F	A2G2F	A3G3F	A2G2F	A2G2F	A3G3F	A2G2F	A3G3F	A2G2F	A3G3F	A2G2F
Healthy 1	8.5	14.1	40.9	2.5	5.9	44.8	8.3	55.9	10.3	19.2	50.2
	12.4	18.0	38.8	1.9	10.1	50.0	12.4	95.8	17.5	32.4	45.2
	14.1	10.9	30.1	1.9	4.5	18.4	10.9	83.7	32.5	49.2	35.7
	11.5	14.5	38.6	1.2	9.0	19.5	8.9	52.9	25.5	30.2	28.6
	7.7	14.8	66.5	2.9	10.7	34.6	8.7	70.9	10.6	37.1	36.5
Healthy 2	4.9	9.5	20.1	1.0	8.2	39.5	10.9	56.0	28.4	28.0	64.6
	10.6	14.9	32.7	2.4	8.2	30.2	12.3	60.3	19.1	37.4	37.6
	6.8	15.7	19.6	1.6	12.3	22.9	9.5	36.6	17.4	16.4	40.2
	8.1	12.6	18.4	1.4	9.7	18.1	8.6	27.3	12.0	8.6	27.8
	7.9	14.6	34.6	2.9	16.3	27.4	9.9	52.1	12.1	23.1	30.5
Cirrhosis 1	14.3	22.9	68.3	2.8	7.4	33.0	12.6	68.0	26.6	46.0	22.7
	14.6	8.4	59.5	5.0	13.1	92.9	31.9	168.4	35.8	121.3	54.2
	16.4	28.9	101.9	4.4	23.5	34.9	27.4	152.8	18.7	92.1	53.8
	16.3	21.3	130.5	3.5	40.7	96.9	44.4	193.5	44.4	168.4	81.0
	25.0	26.5	161.3	8.3	54.9	156.6	47.7	398.8	43.8	391.1	67.4
Cirrhosis 2	13.9	13.3	45.0	3.0	17.7	40.4	20.6	65.6	36.1	53.8	61.1
	23.1	21.1	73.3	3.3	21.2	46.4	25.8	121.5	45.2	94.2	30.9
	18.3	23.8	51.4	5.8	13.4	34.8	29.8	67.1	41.7	46.6	24.1
	61.2	35.9	107.3	12.8	47.9	177.9	72.8	222.8	84.2	307.8	88.0
	97.4	22.1	107.8	5.6	54.9	110.9	63.5	243.9	130.9	46.1	102.1
HCC 1	13.6	19.4	64.8	3.9	11.2	57.8	15.9	81.7	25.2	70.0	26.8
	17.9	22.9	152.4	8.3	14.8	102.6	25.7	169.1	27.3	132.4	35.8
	11.2	28.5	78.1	3.8	17.9	54.3	23.3	87.0	30.1	67.1	44.1
	34.1	18.2	140.6	10.1	39.8	217.6	48.8	319.2	53.5	213.8	76.5
	20.9	22.8	70.0	8.1	36.4	76.8	32.1	162.2	44.9	94.6	94.3
HCC 2	16.7	23.7	85.3	2.5	10.6	42.7	19.3	107.6	34.6	68.1	46.4
	17.8	21.5	139.5	5.7	12.9	72.1	23.4	138.2	28.8	96.0	35.2
	20.5	17.7	68.2	4.4	12.0	28.2	18.7	105.0	25.6	49.9	46.8
	59.1	27.8	130.7	10.4	62.5	199.7	65.6	384.9	85.9	289.6	78.2
	43.5	30.2	161.1	10.9	110.7	185.5	48.5	226.6	72.4	190.7	83.4

Table S-6. Fucosylation of desialylated glycopetides in individual samples of healthy controls (H), cirrhosis (CIR) and HCC patients. Tryptic glycopeptides of hemopexin and CFH from heme-bound fraction of individual patient samples were quantified by LC-MS MRM. Data are expressed as a relative ratio of signal intensities of fucosylated glycopeptide to its non-fucosylated counterpart monitored as the 366 transition (Hex-HexNAc) and shown as percent of the non-fucosylated form for each individual patient sample.

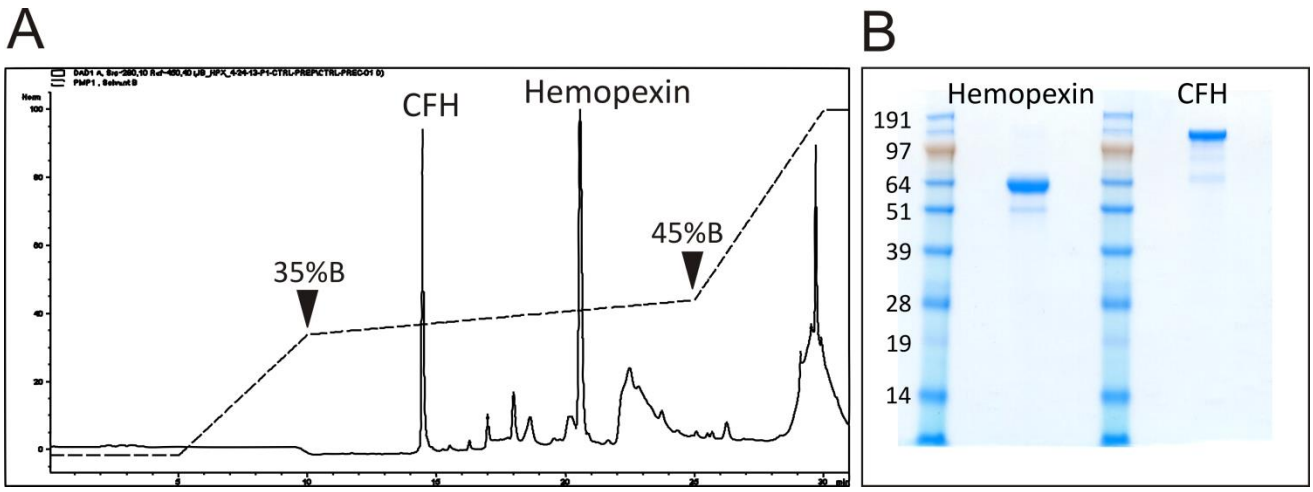

Figure S-1. Purification of HPX and CFH A. Representative chromatogram of hemin-bound fraction of plasma proteins separated on a C18 column. Two major peaks corresponding to CFH and hemopexin are labeled and the elution gradient is indicated by a dashed line. **B.** Peaks corresponding to CFH and hemopexin separated by SDS-PAGE and stained with Coomassie Blue. The two fractions were free of major contaminants.

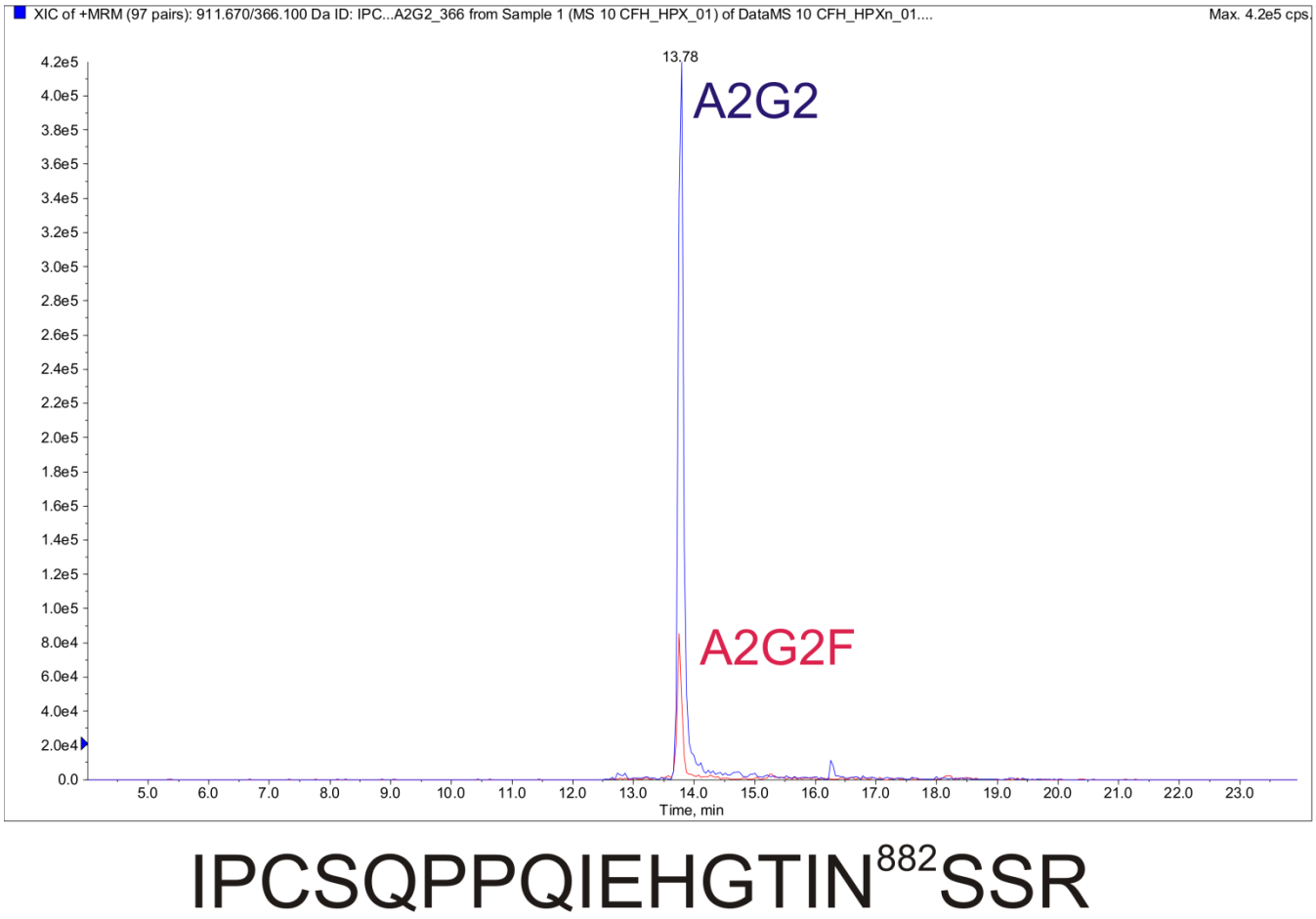

Figure S-2. Extracted ion chromatogram (XIC) of fucosylated (red) and non-fucosylated (blue) forms of bi-antennary glycans at N882 of tryptic glycopeptide IPCSQPPQIEHGTIN*SSR of CFH shows that retention times of both glycoforms are nearly identical.

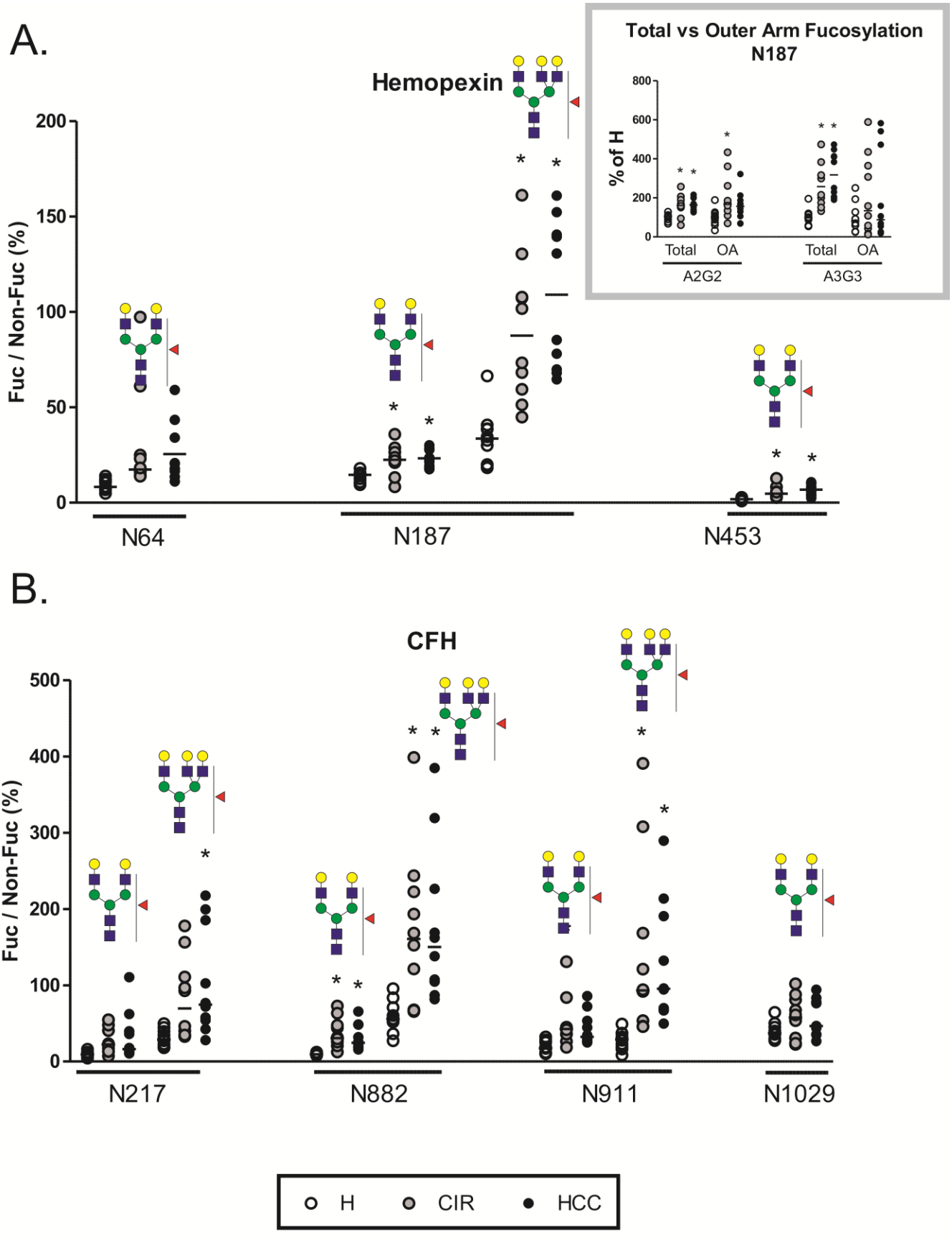
A.
Hemopexin
Fuc / Non-Fuc (%)
200
150
100
50
0
N64
N187
N453
Total vs Outer Arm Fucosylation
N187
% of H
800
600
400
200
0
Total
OA
Total
OA
A2G2
A3G3
B.
CFH
Fuc / Non-Fuc (%)
500
400
300
200
100
0
N217
N882
N911
N1029
H
CIR
HCC

Figure S-3. Fucosylation of desialylated glycopetides in individual samples of healthy controls (H), cirrhosis (CIR) and HCC patients. Tryptic glycopeptides of **A.** Hemopexin, **B.** CFH from heme-bound fraction of individual patient samples were quantified by LC-MS MRM. Data are expressed as a relative ratio of signal intensities of fucosylated glycopeptide to its non-fucosylated counterpart monitored as the 366 transition (Hex-HexNAc) and shown as percent of the non-fucosylated form. Glycan structures representing specific glycoforms are indicated above each group of corresponding bars; position of the glycosylation site in protein sequence is shown below. **Insert**. Comparison of total and outer arm fucosylation at N187 site of hemopexin. Outer arm fucosylation was quantified as 512 transition (Fuc-GlcNAc-Gal) of fucosylated precursor normalized to the 366 transition of its non-fucosylated counterpart; total fucosylation was quantified as above. The relative change in fucosylation in liver disease groups is shown as percent of H. OA; outer arm fucosylation. Distribution of individual values is shown as aligned dot plot, median is indicated by horizontal line., * $P < 0.05$ vs H.

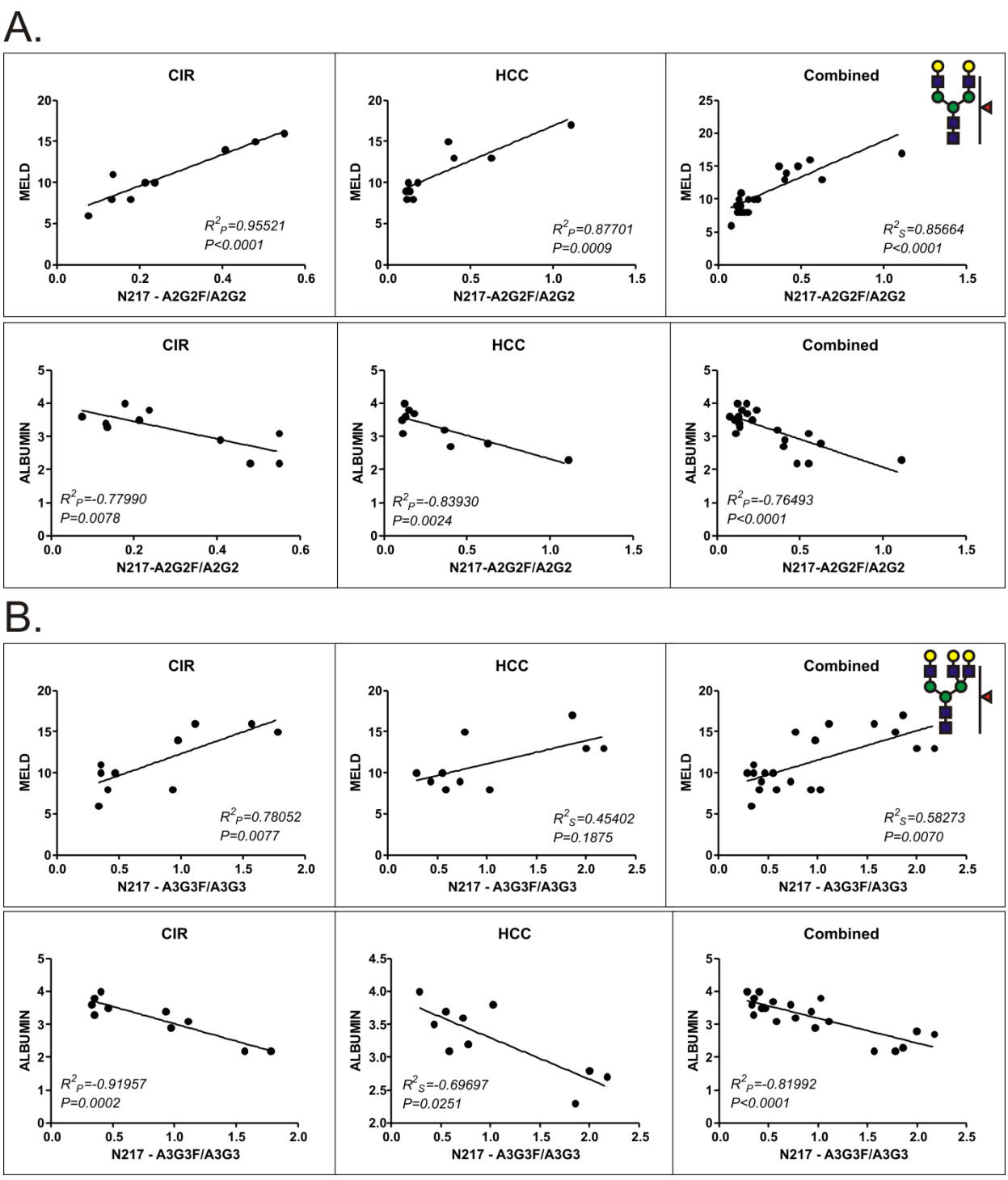

Figure S-4. Correlation between the level of fucosylation of bi- (A.) and tri- (B) antennary glycans with MELD score and serum albumin concentration, parameters of liver damage, at N217 site of CFH. The level of fucosylation is expressed as ratio of fucosylated to non-fucosylated forms quantified on individual samples by MRM protocol as described in Methods. Correlation was assessed by Pearson (P) or Spearman (S) correlation analysis depending on the sample distribution. R^2 and P values are indicated in the figure.