

1 **Supplementary Information**

2 **Glycomic profiling of targeted serum haptoglobin for gastric**
3 **cancer using nano LC/MS and LC/MS/MS**

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1 Supplemental Tables and Figures

2 **Suppl Table 1.** Clinical and histopathological information of serum samples used in this
3 study

Characteristic	Number of samples (%)
<i>General Classification</i>	
Healthy Control	30
Gastric Cancer	30
<i>Age in Years (median, range)</i>	
Healthy Control	49.4, 33-77
Gastric Cancer	50.7, 31-76
<i>Patient Sex</i>	
Male	15 (50%)
Female	15 (50%)
<i>Pathological Stage</i>	
IV	30 (100%)
<i>Cancer type</i>	
Adenocarcinoma	30 (100%)

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2 **Suppl Table 2.** Protein identification for lectin-binding target spots using LC-MS/MS

3 analysis. Identified proteins showed more than 60 mowse score and 9% coverage were listed.

Spot label	Protein identified by LC-MS/MS	Accession number (NCBI BLAST)	Mowse score	Peptides matched	Coverage(%)
Normal	haptoglobin	gi 176792	115	15	15
	haptoglobin	gi 466455	106	15	15
	haptoglobin-related protein	gi 210148826	102	15	11
Gastric Cancer	haptoglobin	gi 176792	145	33	16
	haptoglobin	gi 466455	116	32	12
	haptoglobin-related protein	gi 210148826	111	32	9

The spots listed correspond to lanes in rectangular inset in Supple Fig. 1A.

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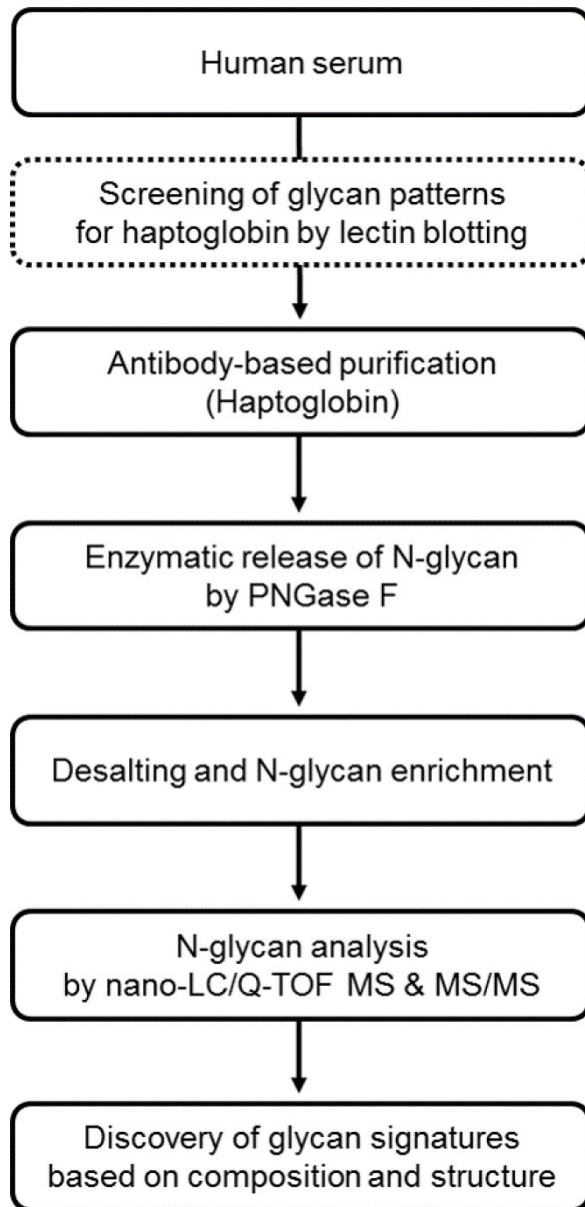
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- 2 **Suppl Table 3.** Relative abundance of 35 distinct N-glycans of haptoglobin found in healthy
3 controls, gastric cancer patients as well as commercial standard (C.standard) sera. Criteria:
4 100% frequency in C.standard and top 99% in all groups

Glycan Mass/Da	Composition				C.standard		Healthy controls		Cancer patients	
	Hex	HexNAc	Fuc	NeuAc	<u>Avg(%)</u>	<u>Ste</u>	<u>Avg(%)</u>	<u>Ste</u>	<u>Avg(%)</u>	<u>Ste</u>
2222.78296	5	4	0	2	27.544	1.547	29.726	1.081	29.789	1.043
2879.01056	6	5	0	3	21.935	0.464	24.911	1.314	20.980	1.101
2587.91516	6	5	0	2	13.001	0.234	11.506	0.555	9.365	0.596
1931.68756	5	4	0	1	12.389	0.270	11.738	0.295	10.989	0.260
3025.06846	6	5	1	3	6.251	0.149	5.966	0.508	9.850	0.920
2296.81976	6	5	0	1	2.869	0.085	2.454	0.116	2.079	0.122
2733.97306	6	5	1	2	2.754	0.042	1.864	0.187	3.304	0.334
1640.59216	5	4	0	0	2.219	0.038	1.266	0.110	1.257	0.118
2953.04736	7	6	0	2	1.897	0.042	1.928	0.112	1.676	0.107
2368.84086	5	4	1	2	1.495	0.048	1.455	0.078	1.876	0.166
3244.14276	7	6	0	3	1.080	0.091	1.221	0.111	1.069	0.099
2077.74546	5	4	1	1	0.819	0.031	0.673	0.037	0.950	0.082
2661.95196	7	6	0	1	0.745	0.029	0.591	0.041	0.504	0.040
3390.20066	7	6	1	3	0.385	0.016	0.318	0.043	0.803	0.119
2005.72436	6	5	0	0	0.355	0.009	0.183	0.014	0.168	0.013
2442.87766	6	5	1	1	0.327	0.024	0.219	0.020	0.325	0.032
2280.82486	5	5	1	1	0.298	0.011	0.415	0.025	0.311	0.026
3099.10526	7	6	1	2	0.285	0.017	0.236	0.024	0.467	0.056
2093.74036	6	4	0	1	0.272	0.011	0.273	0.014	0.319	0.020
1566.55536	4	3	0	1	0.215	0.012	0.195	0.011	0.239	0.018
3171.12636	6	5	2	3	0.181	0.024	0.179	0.034	0.563	0.129
1769.63476	4	4	0	1	0.155	0.005	0.136	0.006	0.131	0.010
3042.08376	7	5	2	2	0.134	0.004	0.128	0.014	0.220	0.020
2134.76696	5	5	0	1	0.127	0.003	0.095	0.005	0.118	0.013
2370.85656	7	6	0	0	0.122	0.040	0.055	0.010	0.055	0.021
1989.72946	5	5	1	0	0.107	0.005	0.107	0.008	0.087	0.008
2425.86236	5	5	0	2	0.105	0.006	0.076	0.005	0.096	0.012
2571.92026	5	5	1	2	0.103	0.004	0.121	0.008	0.089	0.008
1624.59726	4	4	1	0	0.103	0.004	0.148	0.010	0.136	0.014
1275.45996	4	3	0	0	0.101	0.004	0.074	0.005	0.092	0.008
1786.65006	5	4	1	0	0.099	0.008	0.080	0.007	0.097	0.010
1462.54446	3	4	1	0	0.095	0.006	0.125	0.011	0.157	0.019
1827.67666	4	5	1	0	0.092	0.007	0.109	0.012	0.106	0.011
2808.00986	7	6	1	1	0.090	0.005	0.063	0.007	0.110	0.014
1234.43336	5	2	0	0	0.079	0.003	0.093	0.007	0.072	0.007

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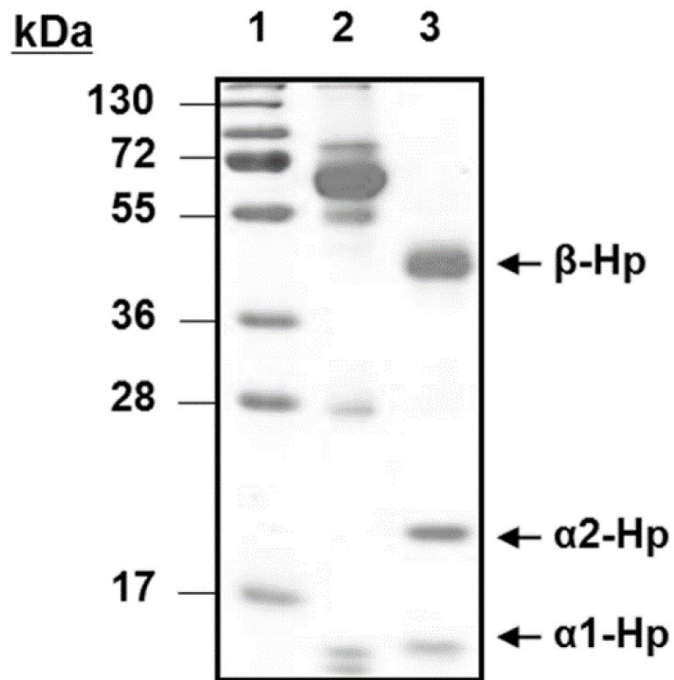
2 **Suppl Figure 1.** The experimental workflow for N-glycan analysis of targeted serum
3 haptoglobin based on immunoaffinity purification followed by chip-based nano-LC/Q-TOF
4 MS and MS/MS

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2 **Suppl Fig 2.** Purification of haptoglobin from commercial serum. Haptoglobin was purified
3 from 450 μ L serum by anti-haptoglobin affinity chromatography. The purified haptoglobin
4 was subjected to 12.5% SDS-PAGE, and bands were stained by Coomassie Brilliant Blue.
5 Lane 1: size marker. Lane 2: 1:100 diluted serum. Lane 3: purified haptoglobin.

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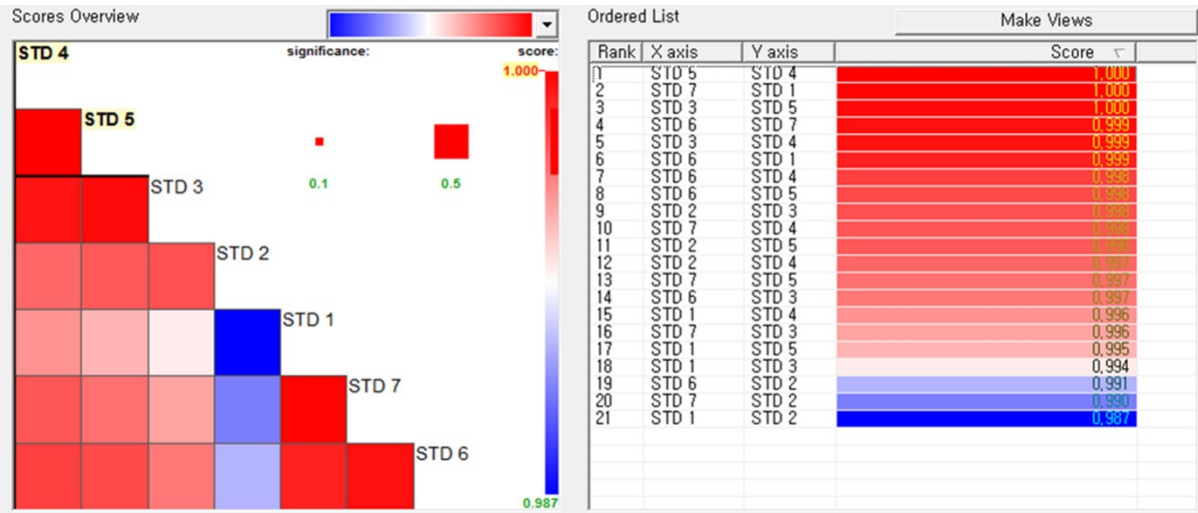
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 2 **Suppl Fig 3.** Pearson correlation coefficient (R) based on haptoglobin glycan composition
 3 profiles derived from 7 commercial sera samples.

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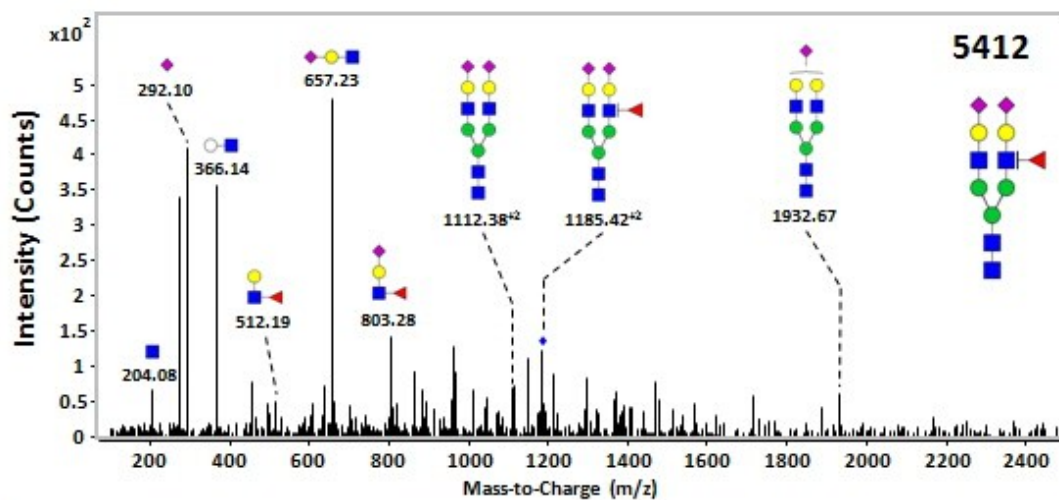
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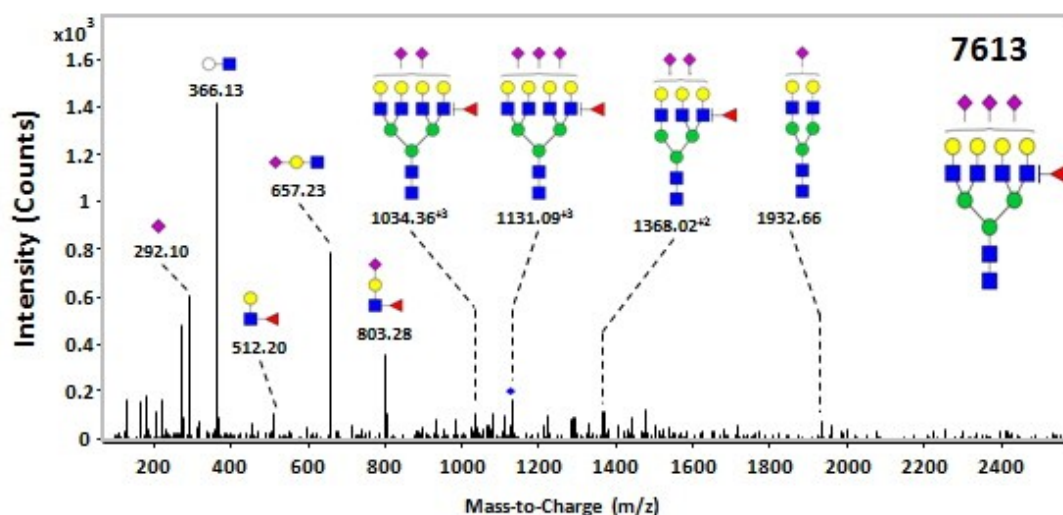
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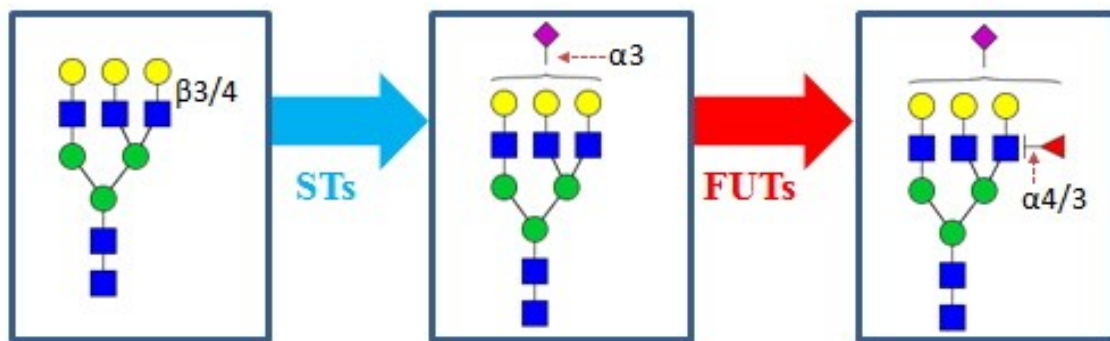


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2 **Suppl Fig 4.** Identification of fucose location of serum haptoglobin. Targeted CID MS/MS
3 spectrums of bi-sialylated, mono-fucosylated, bi-antennary N-glycans (A) and
4 mono-fucosylated, tetra-antennary (B) glycans taken from the major isomer during LC
5 elution of the composition Hex₅HexNAc₄Fuc₁NeuAc₂ and Hex₇HexNAc₆Fuc₁NeuAc₃,
6 respectively.

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2 **Suppl Fig 5.** A representative processes related to *in vivo* synthesis pathway of SLe^a or SLe^x.

3 Red and blue arrows indicate fucosylation and sialylation, respectively.

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