Electronic Supplementary Material (ESI) for Analyst.

Supporting information

Boronic acid-functionalized mesoporous magnetic particles with a hydrophilic surface for multimodal enrichment of glycopeptides for glycoproteomics

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Contents:

Mass spectrometry analysis

MALDI–TOF MS experiments were operated in positive ion mode on a 5800 Proteomics Analyzer (Applied Biosystems, Framingham, MA, USA) with the Nd-YAG laser at 355 nm, a repetition rate of 400 Hz and an acceleration voltage of 20 kV.

For experiment of nano-LC-MS/MS anaylsis, the sample was loaded on the trap column (Thermo Scientific Acclaim PepMap C18, 100 μm × 2 cm) in 3 min at a flow rate of 10 μL/min. The sample was subsequently separated with an analytical column (Thermo Scientific Acclaim PepMap C18, 75 μm × 25 cm). For gradient separation, H_2O/FA (99.9: 0.1) was used as the mobile phase A, and ACN/FA (99.9: 0.1) was the mobile phase B. The gradient elution was performed as follows: 2% B was held for 1 min. Then, from 2% to 30%, B was held for 104 min, and from 30% to 90%, B was held for 5 min. Next, the mobile phase B was held at 90% for 5 min, and then from 90% to 1% for 0.1 min. Finally, at 1%, B was held for 4.9 min. The column flow rate was maintained at 300 nL/min and column temperature was maintained at 40°C. The electrospray voltage of 2.0 kV versus the inlet of the mass spectrometer was used. The Nano Aquity UPLC system (Waters Corp.) was coupled to an Orbitrap Fusion mass spectrometer equipped with a nanospray source. Survey full-scan MS spectra (m/z 350−1600) were acquired in Orbitrap with a mass resolution of 120000 at m/z. The AGC target was set to 1000000, and the maximum injection time was 50ms. MS/MS acquisition was performed in Orbitrap in 3 s cycle time. The intensity threshold was 50000, and the isolation window was 2 m/z. Ions with charge states 2+, 3+, and 4+ were orderly fragmented by HCD with NCE of 35% and fixed first mass was set at 110.

Supporting Figures

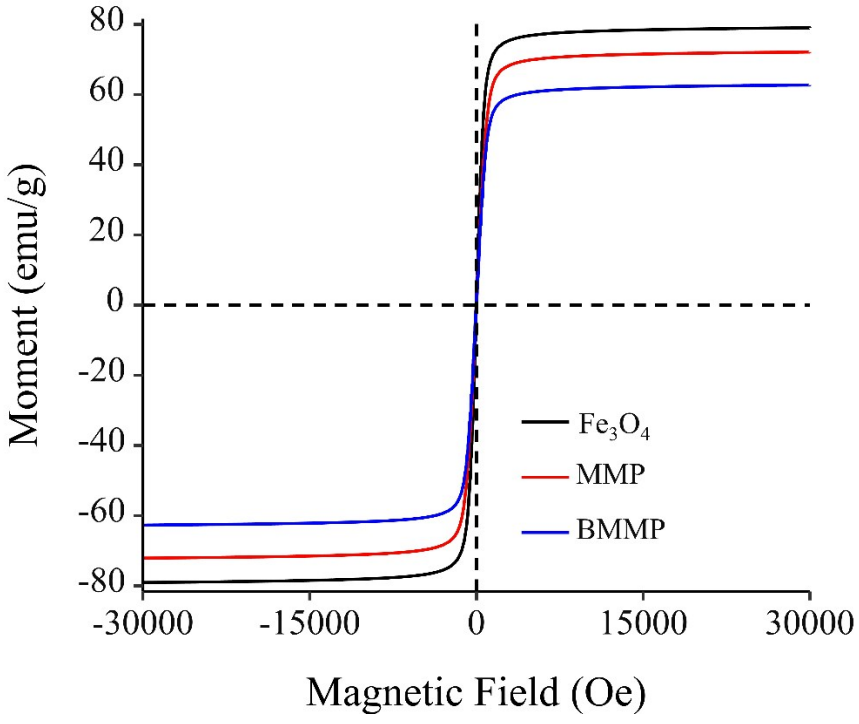

Figure S1. Magnetic hysteresis curves of Fe_3O_4, MMP and BMMP.

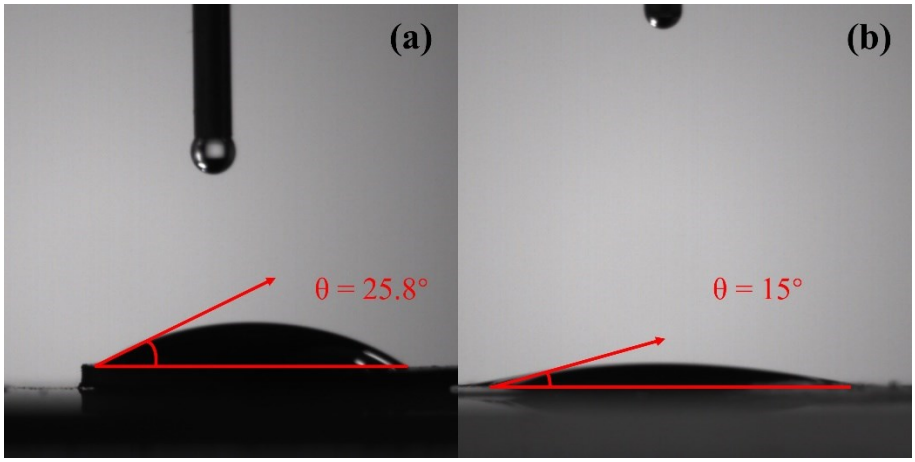

Figure S2. Water contact angles of (a) BMMP and (b) MMP.

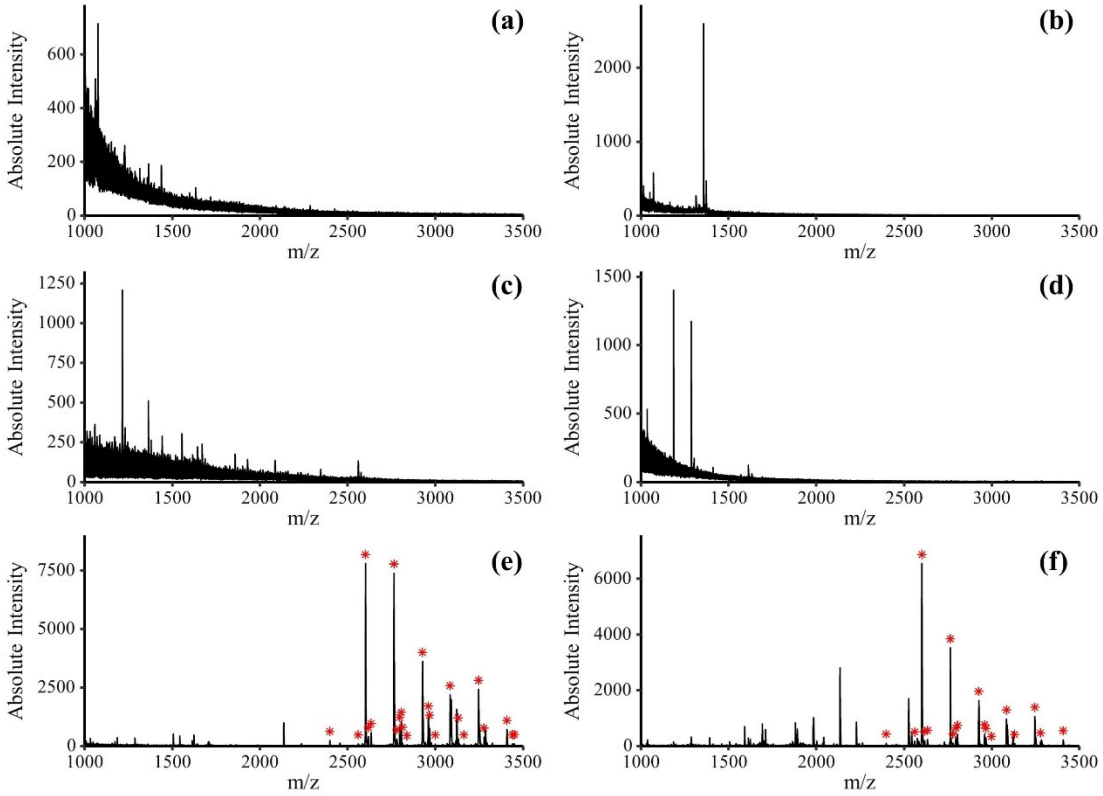

Figure S3. Mass spectra of IgG tryptic digestion enrichment with BMMP under 50% (a), 60% (b), 70% (c), 80% (d), 90% (e), 95% (f) ACN concentrations loading buffer with 0.1% TFA.

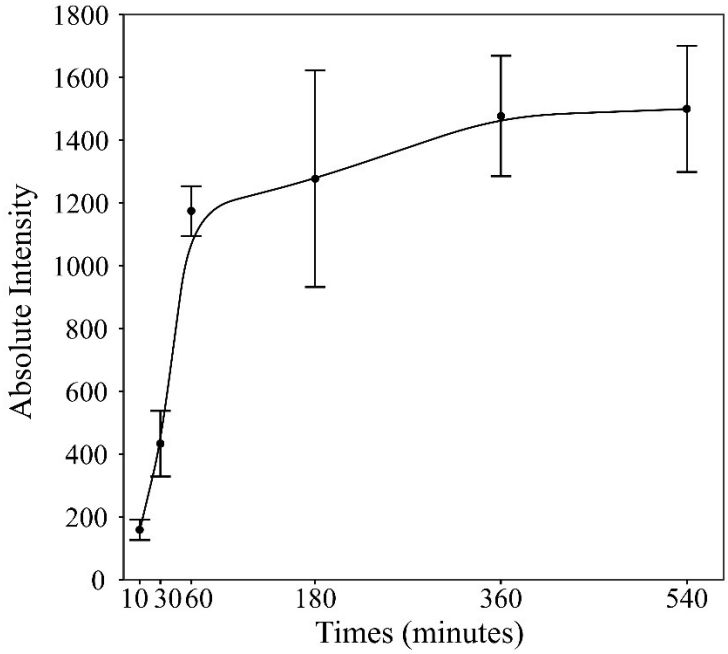

Figure S4. The x axis indicated the loading time, from 10 min to 9 hours. The y axis represented the absolute intensity of the glycopeptide peak (m/z = 2602.4) enriched by BMMP.

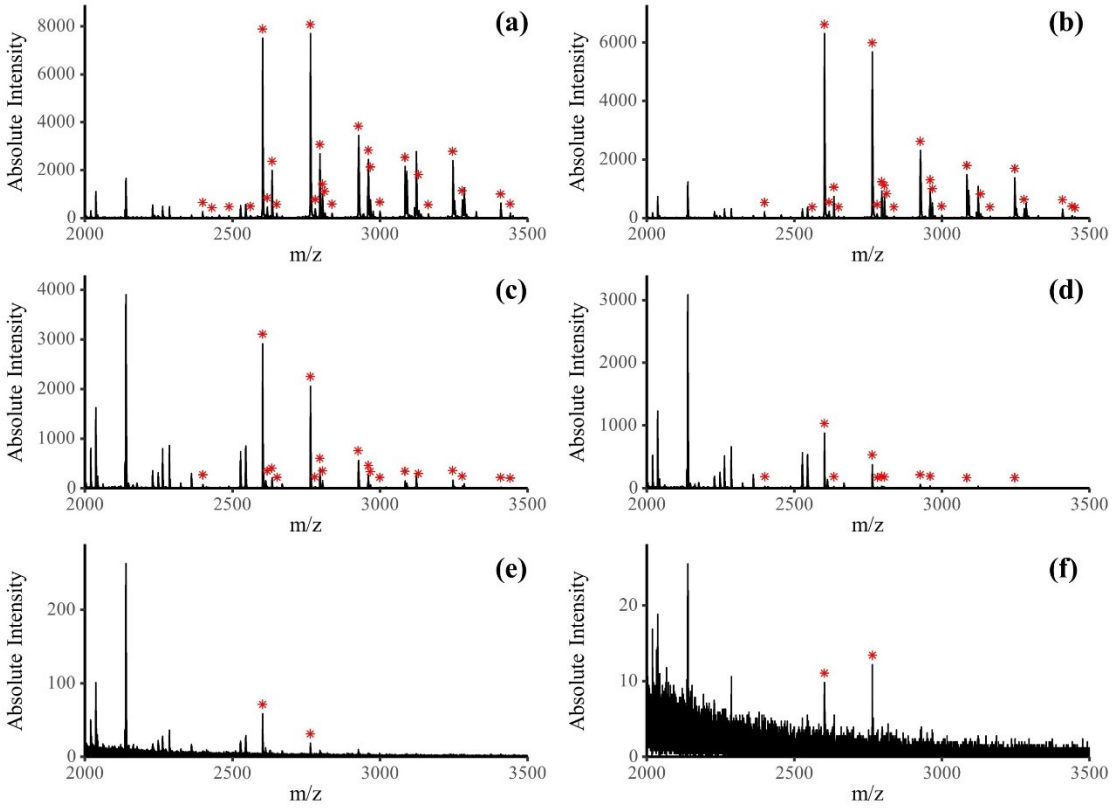

Figure S5. Mass spectra of 6.66 pmol/μL(a), 3.33 pmol/μL(b), 0.333 pmol/μL(c), 33.3 fmol/μL(d), 3.33 fmol/ μL(e), 0.33 fmol/ μL(f) IgG tryptic digestion after enrichment with BMMP.

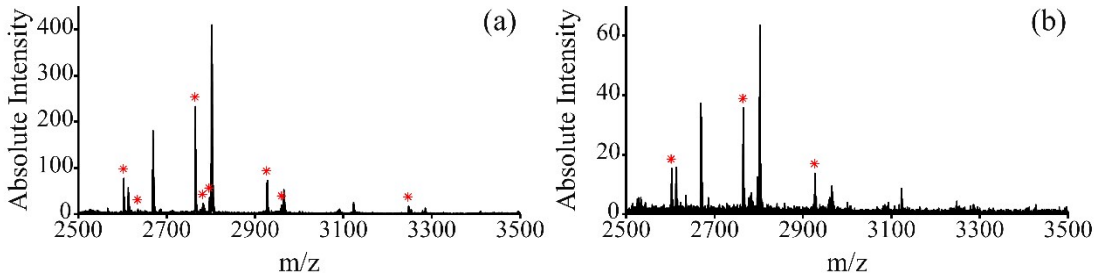

Figure S6. Mass spectra of enrichment from IgG and BSA tryptic digestion mixture at molecular ratio of 1:50 (a) and 1:100 (b).

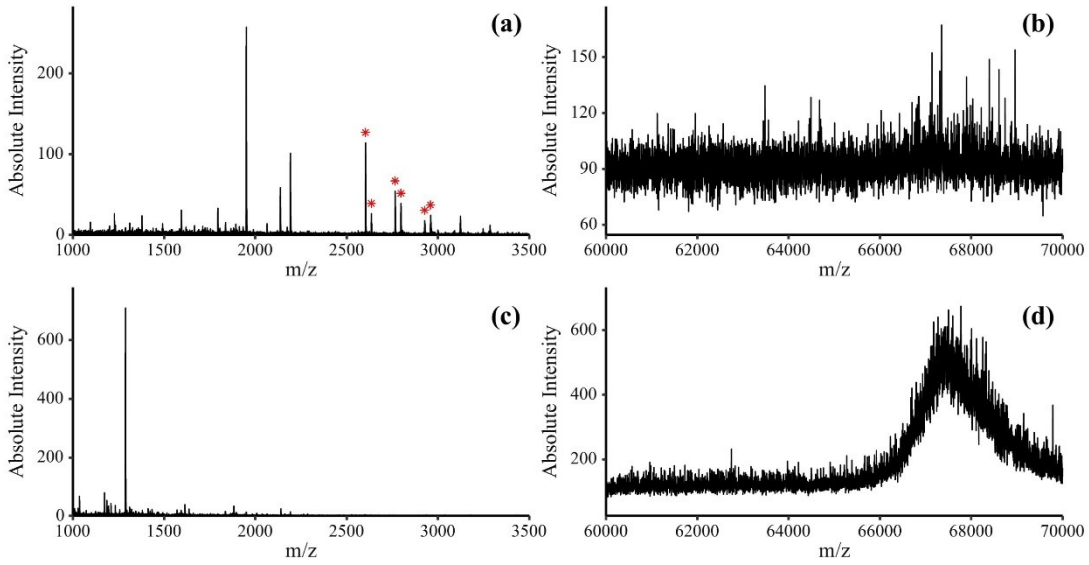

Figure S7. The reflection model (a) and liner model (b) mass spectra of elution buffer of IgG tryptic digestion mixed with 100 times mole number BSA protein after enrichment. The reflection model (c) and the liner model (d) of the supernatant of the mixture after enrichment.

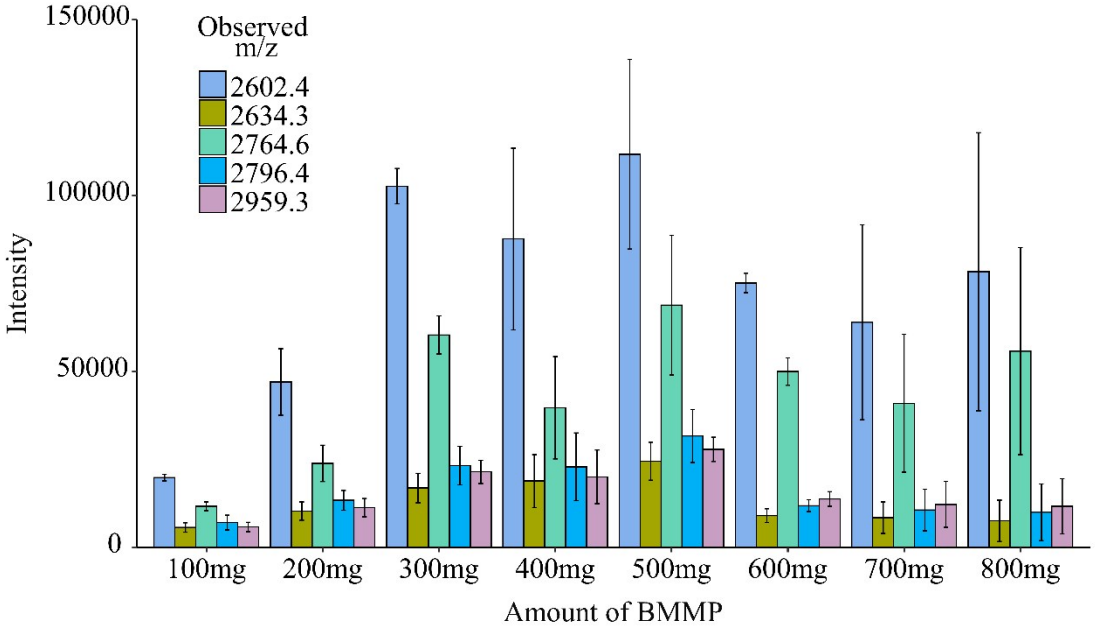

Figure S8. The MS intensities of five selected glycopeptides from tryptic digests of human IgG after enrichment with different amounts of BMMP.

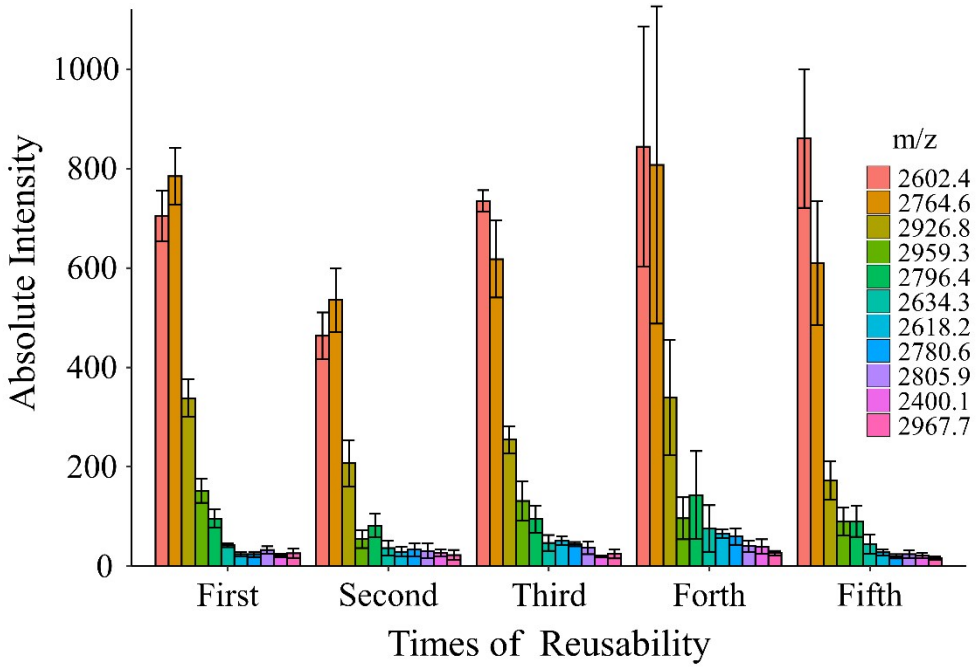

Figure. S9 Mass spectrum intensity of the glycopeptides from Human IgG tryptic digest after enrichment by BMMP for five times continuously.

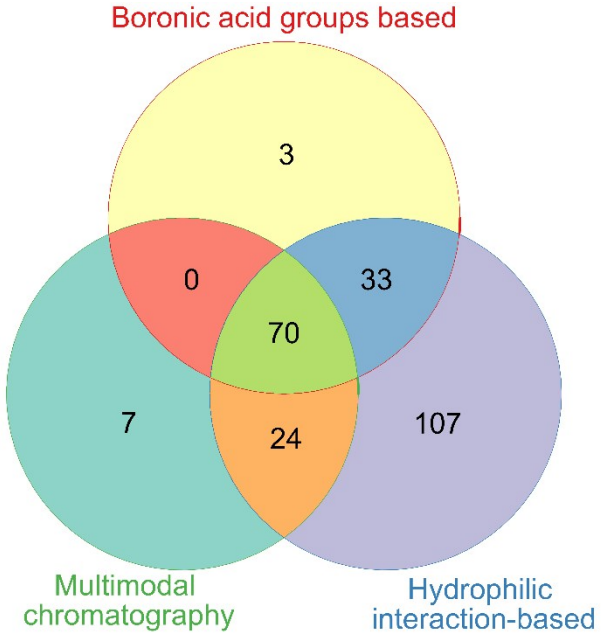

Figure S10. Venn plot of glycoproteins identified by hydrophilicity interaction, boronic acid groups based and multimodal enrichment.

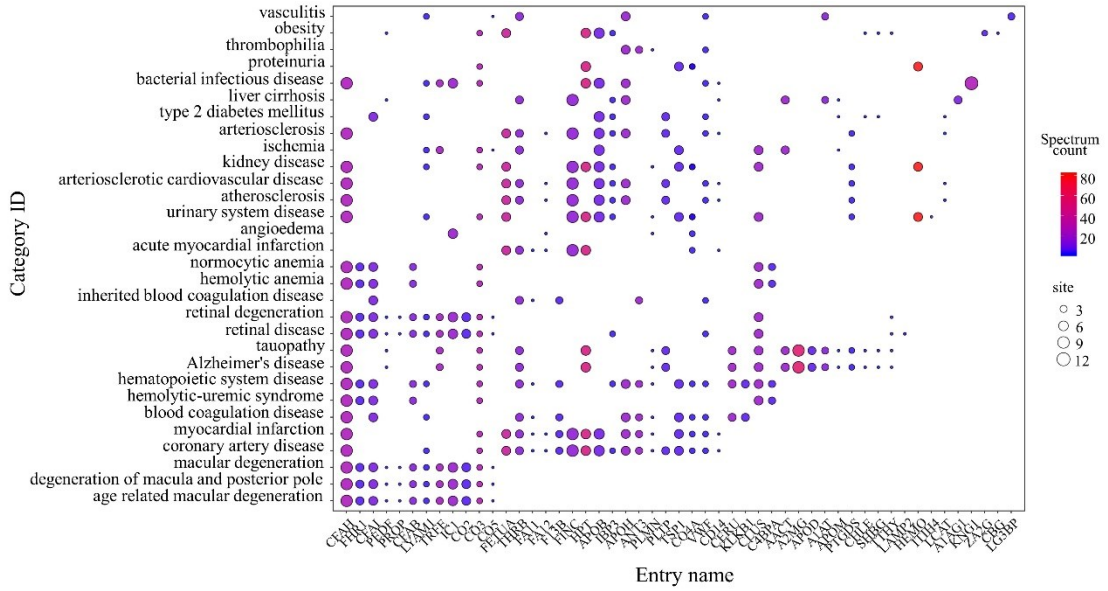

Figure S11. Disease Ontology analysis of the glycoprotein identified in human serum. the size of the points was related to the glycosylation sites amount, the color represents the spectrum count.

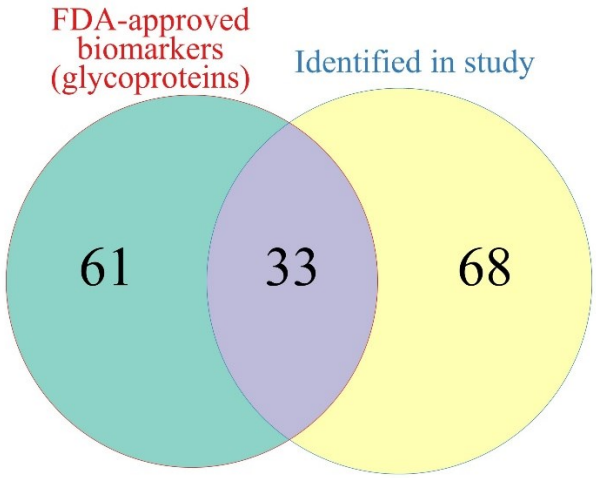

Figure S12. Venn diagram depicting the overlap of FDA-approved biomarkers and glycoproteins identified in this study.

Table S1. Detailed information of the glycopeptides enriched with BMMP from human IgG tryptic digest. N# denotes the N-linked glycosylation site.

No.	Observed m/z	Glycan	Peptide sequence
1	2235.9	[Hex]3[HexNAc]2[Fuc]1	EEQFN#STFR
2	2397.9	[Hex]3[HexNAc]3[Fuc]1	EEQFN#STFR
3	2429.9	[Hex]3[HexNAc]3[Fuc]1	EEQYN#STYR
4	2455.4	[Hex]3[HexNAc]4	EEQFN#STFR
5	2559.9	[Hex]4[HexNAc]3[Fuc]1	EEQFN#STFR
6	2600.9	[Hex]3[HexNAc]4[Fuc]1	EEQFN#STFR
7	2616.9	[Hex]4[HexNAc]4	EEQFN#STFR

8	2632.8	[Hex]3[HexNAc]4[Fuc]1	EEQYN#STYR
9	2648.9	[Hex]4[HexNAc]4	EEQYN#STYR
10	2660.7	[Hex]3[HexNAc]5	EEQFN#STYR
11	2762.9	[Hex]4[HexNAc]4[Fuc]1	EEQFN#STFR
12	2778.8	[Hex]5[HexNAc]4	EEQFN#STFR
13	2794.8	[Hex]4[HexNAc]4[Fuc]1	EEQYN#STYR
14	2803.8	[Hex]3[HexNAc]5[Fuc]1	EEQFN#STFR
15	2811.3	[Hex]5[HexNAc]4	EEQYN#STYR
16	2835.8	[Hex]3[HexNAc]5[Fuc]1	EEQYN#STYR
17	2924.8	[Hex]5[HexNAc]4[Fuc]1	EEQFN#STFR
18	2941.4	[Hex]5[HexNAc]4[Fuc]1	EEQFN#STYR
19	2956.9	[Hex]5[HexNAc]4[Fuc]1	EEQYN#STYR
20	2965.8	[Hex]4[HexNAc]5[Fuc]1	EEQFN#STFR
21	2997.8	[Hex]4[HexNAc]5[Fuc]1	EEQYN#STYR
22	3088.9	[Hex]4[HexNAc]4[Fuc]1[NeuAc]1	EEQYN#STFR
23	3129.8	[Hex]5[HexNAc]5[Fuc]1	EEQFN#STFR
24	3161.9	[Hex]5[HexNAc]5[Fuc]1	EEQYN#STYR
25	3250.9	[Hex]5[HexNAc]4[Fuc]1[NeuAc]1	EEQYN#STYR
26	3282.8	[Hex]4[HexNAc]4[Fuc]1	TKPYEEQYN#STYR
27	3407.0	[Hex]5[HexNAc]4[Fuc]1	TKPREEQFN#STFR
28	3440.4	[Hex]5[HexNAc]4[Fuc]1	TKPYEEQYN#STYR

Table S2. Detailed information of the glycopeptides enriched with BMMP from HRP tryptic digest. N# denotes the N-linked glycosylation sites.

No.	Observed m/z	Glycan	Peptide sequence
1	1842.9	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	NVGLN#R
2	2067.1	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	PN#VSNIVR
3	2163.2	[Hex]3[HexNAc]2[Fuc]1	LYN#FSN#TGLP
4	2286.1	[Hex]2[HexNAc]2[Xyl]1	SILLDN#TTSFR
5	2445.3	[Hex]3[HexNAc]2[Xyl]1	PTLN#TTYLQTLR
6	2532.3	[HexNAc]1[Fuc]1	SFAN*STQTFFNAFVEAMDR
7	2541.2	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	SSPN#ATDTIPLVR
8	2591.3	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	PTLN#TTYLQTLR
9	2611.3	[Hex]3[HexNAc]2[Xyl]1	MGN#ITPLTGTQGQIR
10	2705.5	[HexNAc]1	GLIQSDQELFSSPN#ATDTIPLVR
11	2851.4	[HexNAc]1[Fuc]1	GLIQSDQELFSSPN#ATDTIPLVR
12	3047.7	[Hex]2[HexNAc]2[Xyl]1	SFAN#STQTFFNAFVEAMDR
13	3073.4	[HexNAc]1[Fuc]1	LHFHDCFVNGCDASILLDN#TTSFR

14	3087.6	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	GLCPLNGN#LSALVDFDLR
15	3191.5	[Hex]2[HexNAc]2[Fuc]1[Xyl]1	SFAN#STQTFFNAFVEAMDR
16	3207.5	[Hex]3[HexNAc]2[Xyl]1	SFAN#STQTFFNAFVEAMDR
17	3221.5	[Hex]3[HexNAc]2[Fuc]1	SFAN#STQTFFNAFVEAMDR
18	3321.5	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	QLTPTFYDNSCPN#VSNIVR
19	3353.4	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	SFAN#STQTFFNAFVEAMDR
20	3369.5	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	SFAN#STQTFFNAFVEAM*DR
21	3388.4	[Hex]6[HexNAc]4[Fuc]2[Xyl]1	DSFRNVGLN*R
22	3508.6	[Hex]2[HexNAc]2[Fuc]1[Xyl]1	GLIQSDQELFSSPN#ATDTIPLVR
23	3527.2	[Hex]3[HexNAc]2[Xyl]1	GLIQSDQELFSSPN#ATDTIPLVR
24	3539.8	[Hex]3[HexNAc]2[Fuc]1	GLIQSDQELFSSPN#ATDTIPLVR
25	3605.3	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	NQCRGLCPLNGN#LSALVDFDLR
26	3671.6	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	GLIQSDQELFSSPN#ATDTIPLVR
27	3894.2	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	LHFHDCFVNGCDASILLDN#TTSFR
28	4056.7	[Hex]3[HexNAc]2[Xyl]1	QLTPTFYDNSC(AAVESACPR)PN#VSNIVR.H2O
29	4221.9	[Hex]3[HexNAc]2[Fuc]1[Xyl]1	QLTPTFYDNSC(AAVESACPR)PN#VSNIVR
30	4720.0	[Hex]3[HexNAc]2[Fuc]1, [Hex]3[HexNAc]2[Fuc]1	LYN#FSNTGLPDPTLN#TTYLQTLR
31	4820.1	[Hex]2[HexNAc]2[Fuc]1[Xyl]1, [Hex]2[HexNAc]2[Xyl]1	LYN#FSNTGLPDPTLN#TTYLQTLR
32	4837.0	[Hex]3[HexNAc]2[Fuc]1[Xyl]1, [Hex]3[HexNAc]2[Xyl]1	LYN#FSNTGLPDPTLN#TTYLQTLR
33	4851.1	[Hex]3[HexNAc]2[Fuc]1, [Hex]3[HexNAc]2[Fuc]1[Xyl]1	LYN#FSNTGLPDPTLN#TTYLQTLR
34	4982.8	[Hex]3[HexNAc]2[Fuc]1[Xyl]1, [Hex]3[HexNAc]2[Fuc]1[Xyl]1	LYN#FSNTGLPDPTLN#TTYLQTLR
35	5067.0	[Hex]3[HexNAc]2[Xyl]1	QLTPTFYDNSC(AAVESACPR)PN#VSNIVR

Table S3. Detailed information of the recovery yield of BMMP for each glycopeptides.

Observed m/z	Glycan	Peptides sequences	Recovery of BMMP %
2839.85	[Hex]5[HexNAc]4[NeuAc]2	RFNGSVSFFR	97.28±4.61
3877.97	[Hex]5[HexNAc]4[NeuAc]2	NVNISYTVNDSFFPQRPQK	105.46±14.27

2782.8	[Hex]5[HexNAc]4[NeuAc]2	GHTLTLNFTR	102.1±15.4

Table S4. Detailed information of the observed N-glycopeptides obtained from tryptic digests of human serum of 2 μL. N# denoted the N-linked glycosylation site. Minuscule represent the post-translation sites on the peptides.

No.	Accessions	sequence	Scores
1	Q08380	AAIPSALDTN#SSK	77
2	P02765	AALAAFnAQnN#GSNFQLEEISR	44
3	P02765	AALAAFnAQNN#GSnFQLEEISR	44
4	P02765	AALAAFnAQNN#GSNFQLEEISR	126
5	P02765	AALAAFNAQnN#GSnFQLEEISR	44
6	P02765	AALAAFNAQnN#GSNFQLEEISR	119
7	P02765	AALAAFNAQNN#GSnFQLEEISR	134
8	P02765	AALAAFNAQNN#GSNFQLEEISR	126
9	P0DOY3; A0M8Q6	AAPSVTLFPPSSEELQAN#K	13
10	P05090	ADGTVnQIEGEATPVN#LTEPAKLEVK	17
11	P05090	ADGTVNQIEGEATPVN#LTEPAKLEVK	42
12	P01009	ADTHDEILEGLNFN#LTEIPEAQIHEGFQELLR	87
13	P04180	AELSN#HTRPVILVPGcLGNQLEAK	38
14	P22792	AFGSNPN#LTK	30
15	P26927	AFHYN#VSSHGcQLLPWTQHSPHTR	31
16	P35858	AGAFLGLTNVAVMN#LSGNcLR	47
17	P00450	AGLQAFFQVQEcN#K	70
18	P00450	AGLQAFFQVQEcN#KSSSK	37
19	P00450	AGLQAFFQVQEcN#KSSSKDNIR	64
20	Q9HDC9	AGPN#GTLFVADAYK	51
21	P0DOY3	AGVETTTPSKQSNN#K	15
22	P02766	ALGISPFHEHAEVVFTAN#DSGPR	27
23	P02766	ALGISPFHEHAEVVFTAN#DSGPRR	45
24	P02790	ALPQPQN#VTSLLGcTH	79
25	P00751	ALQAVYSMMSWPDDVPPEGWN#R	44
26	P01031	AN#ISHKDMQLGR	34
27	P19827	AN#LSSQALQmSLDYGFVTPLTSMSIR	12
28	P19827	AN#LSSQALQMSLDYGFVTPLTSMSIR	115
29	P01011	APDKNVIFSPLSISTALAFLSLGAHN#TTLTEILK	86
30	P08185	AQLLQGLGFN#LTER	78
31	P25311	AREDIFMETLKDIVEYYN#DSN#GSHVLQGR	60
32	P25311	AREDIFMETLKDIVEYYN#DSNGSHVLQGR	46
33	P25311	AREDIFMETLKDIVEYYNDSN#GSHVLQGR	81
34	P02748	AVN#ITSENLIDDVVSLIR	107
35	P17936	AYLLPAPPAPGN#ASESEEDRSAGSVESPSVSSTHR	27
36	P23142	cATPHGDN#ASLEATFVKR	51

37		cFLGN#GTGYR	18
38	P02787	cGLVPVLAENYN#K	54
39	P05090	cIQAN#YSLMEnGK	17
40	P05090	cIQAN#YSLMENGK	26
41	P02790	cSDGWSFDATTLDDN#GTmLFFK	45
42	P02790	cSDGWSFDATTLDDN#GTMLFFK	82
43	P43652	DIENFN#STQK	28
44	P06276	DN#YTKAEEILSR	65
45	P02751	DQcIVDDITYnVN#DTFHKR	37
46	P02751	DQcIVDDITYNVN#DTFHKR	64
47	P02749	DTAVFEcLPQHAMFGN#DTITcTTHGN#WTK	17
48	P02749	DTAVFEcLPQHAmFGN#DTITcTTHGN#WTKLPEcR	22
49	P05155	DTFVN#ASR	42
50	P43251	DVQIIVFPEDGIHGFN#FTR	49
51	P01859	EEQFN#STFR	56
52	P01861	EEQFN#STYR	33
53	P01860	EEQYN#STFR	58
54	P0DOX5	EEQYN#STYR	56
55	P04217	EGDHEFLEVPEAQEDVEATFPVHQPGN#YScSYR	30
56	P55058	EGHFYYN#ISEVK	31
57	P49908	EGYSN#ISYIVVNHQGISSR	39
58	P00450	EHEGAIYPDN#TTDFQR	90
59	P10909	EIRHN#STGcLR	31
60	P00450	ELHHLQEQN#VSNAFLDK	42
61	P00450	ELHHLQEQN#VSNAFLDKGEFYIGSK	103
62	P19652	EN#GTVSRYEGGR	24
63	P01591	EN#ISDPTSPLR	63
64	P00450	EN#LTAPGSDSAVFFEQGTTR	85
65	P02790	ERSWPAVGN#cSSALR	48
66	P20851	EWDN#TTTEcR	46
67	P00450	EWEKELHHLQEQN#VSNAFLDKGEFYIGSK	58
68	P07358	EYESYSDFERN#VTEK	69
69	P14151	FcRDN#YTDLVAIQNK	24
70	P04004	FEDGVLDPDYPRN#ISDGFDGIPDNVDAALALPAHSY SGR	52
71	P04114	FEVDSPVYN#ATWSASLK	74
72	P48740	FGYILHTDN#R	35
73	P05156	FLnN#GTcTAEGK	61
74	P05156	FLNN#GTcTAEGK	40
75	P43251	FN#DTEVLQR	51
76	P01011	FN#LTETSEAEIHQSFQHLLR	95
77	P04114	FN#SSYLQGTNQITGR	28
78	P04003	FSLLGHASIScTVEN#ETIGVWRPSPPTcEK	66

79	P02748	FSYSKN#ETYQLFLSYSSK	34
80	P0DOX5	FTISRN#DSK	26
81	P55058	GAFFPLTERN#WSLPNR	30
82	P19823	GAFISN#FSmTVDGK	45
83	P19823	GAFISN#FSMTVDGK	67
84	O75882	GcScFSDWQGPGcSVPVPAN#QSFWTR	12
85	P01023	GcVLLSYLN#ETVTVSASLESVR	145
86	P01023	GcVLLSYLN#ETVTVSASLESVRGN#R	51
87	A0A0B4J1Y8	GDGIPDRFSVLGSGLN#R	33
88	Q96PD5	GFGVAIVGN#YTAALPTEAALR	78
89	Q9NZP8	GFLALYQTVAVN#YSQPISEASR	43
90	P02751	GGnSnGALcHFPFLYNNHN#YTDcTSEGR	19
91	P02751	GGnSNGALcHFPFLYnNHN#YTDcTSEGR	19
92	P02751	GGnSNGALcHFPFLYNnHN#YTDcTSEGR	19
93	P02751	GGnSNGALcHFPFLYnNHN#YTDcTSEGRR	27
94	P02751	GGnSNGALcHFPFLYNnHN#YTDcTSEGRR	27
95	P02751	GGnSNGALcHFPFLYNNHN#YTDcTSEGRR	21
96	P02751	GGNSnGALcHFPFLYNNHN#YTDcTSEGRR	21
97	P07357	GGSSGWSGGLAQN#R	67
98	P02790	GHGHRN#GTGHGN#STHHGPEYMR	55
99	P00734	GHVN#ITR	38
100	P17936	GLcVN#ASAVSR	61
101	P01011	GLKFN#LTETSEAEIHQSFQHLLR	57
102	P0C0L4; P0C0L5	GLN#VTLSSTGR	54
103	P0C0L4; P0C0L5	GLN#VTLSSTGRnGFK	24
104	P01871; P0DOX6	GLTFQQN#ASSmcVPDQDTAIR	70
105	P01871; P0DOX6	GLTFQQN#ASSMcVPDQDTAIR	145
106	P01023	GNEAnYYSN#ATTDEHGLVQFSIN#TTNVMGTSLTVR	35
107	P01023	GNEANYYSN#ATTDEHGLVQFSIN#TTNVMGTSLTVR	57
108		GNRN#NSQLEKTTPSK	16
109	P00747	GNVAVTVSGHTcQHWSAQTPHTHN#R	51
110	Q14624	GPDVLTATVSGKLPTQN#ITFQTESSVAEQEAEFQSPK	23
111	P04275	GQVYLQcGTPcN#LTcR	25
112	P26927	GTAN#TTTAGVPcQR	59
113	P03952	GVNFN#VSK	40
114	P05155	GVTSVSQIFHSPDLAIRDTFVN#ASR	53
115	P02751	HEEGHMLN#cTcFGQGR	54
116	P01042	HGIQYFNN#NTQHSSLFmLNEVK	15

117	P01042	HGIQYFNN#NTQHSSLFMLNEVK	42
118	P01042	HGIQYFnN#NTQHSSLFMLNEVKR	15
119	P01042	HGIQYFNN#nTQHSSLFMLNEVKR	15
120	P01042	HGIQYFNN#NTQHSSLFmLNEVKR	27
121	P01042	HGIQYFNN#NTQHSSLFMLnEVKR	17
122	P01042	HGIQYFNN#NTQHSSLFMLNEVKR	59
123	P05160	HGVIISSTVDTYEN#GSSVEYR	86
124		HIPGLIHN#MTAR	12
125	P10909	HN#STGcLR	41
126	P00450	HYYIAAEEIIWNYAPSGIDIFTKEN#LTAPGSDSAVFFEQGTTR	57
127	P04196	IADAHLDRVEN#TTVYYLVLDVQESDcSVLSR	81
128	P19827	IcDLLVAnNHFAHFFAPQN#LTNMNK	15
129	P19827	IcDLLVANnHFAHFFAPQN#LTNMNK	15
130	P19827	IcDLLVANNHFAHFFAPQN#LTNMNK	13
131	P14151	IGGIWTWVGTN#K	47
132	P01023	IITILEEEMN#VSVcGLYTYGKPVPGHVTVSIcR	61
133	P01591	IIVPLnNREN#ISDPTSPLR	34
134	P01591	IIVPLNnREN#ISDPTSPLR	35
135	P01591	IIVPLNNREN#ISDPTSPLR	39
136	P08603	IPcSQPPQIEHGTIN#SSR	60
137	Q7Z478	ISAVSLAN#R	41
138	P08603	ISEEN#ETTcYMGK	70
139	P01042	ITYSIVQTN#cSK	61
140	P01042	ITYSIVQTN#cSKENFLFLTPDcK	16
141	P03952	IVGGTN#SSWGEWPWQVSLQVK	35
142	P00751	IVLDPSGSMNIYLVLDGSDSIGASN#FTGAK	53
143	P03952	IYPGVDFGGEELN#VTFVK	18
144	P55058	IYSN#HSALESLALIPLQAPLK	37
145	P00751	KALQAVYSMMSWPDDVPPEGWN#R	39
146	Q9Y5Y7	KANQQLN#FTEAK	32
147	P05160	KEHETcLAPELYnGN#YSTTQK	28
148	P00751	KIVLDPSGSMNIYLVLDGSDSIGASN#FTGAK	30
149	P10909	KKEDALN#ETR	41
150	P51884	KLHInHNN#LTESVGPLPK	19
151	P51884	KLHINHnN#LTESVGPLPK	19
152	P51884	KLHINHNN#LTESVGPLPK	23
153	P02750	KLPPGLLAN#FTLLR	71
154	P20851	KTLFcN#ASKEWDN#TTTEcR	44
155	P04275	KTTcNPcPLGYKEEN#NTGEccGR	15
156	P02765	KVcQDcPLLAPLN#DTR	106
157	P01876	LAGKPTHVN#VSVVmAEVDGTcY	23
158	P01876	LAGKPTHVN#VSVVMAEVDGTcY	57

159	P10909	LAN#LTQGEDQYYLR	89
160	P04114	LATALSLSNKFVEGSHN#STVSLTTK	18
161	P02751	LDAPTNLQFVN#ETDSTVLVR	60
162	P04278	LDVDQALN#R	68
163	Q96PD5	LEPVHLQLQcMSQEQLAQVAAN#ATKEFTEAFLGcPAIHPR	80
164	P03951	LETTVN#YTDSQRPIcLPSKGDR	41
165	P01008	LGAcN#DTLQQLmEVFK	34
166	P01008	LGAcN#DTLQQLMEVFK	57
167	P01008	LGAcN#DTLQQLmEVFKFDTISEK	28
168	P01008	LGAcN#DTLQQLMEVFKFDTISEK	71
169	P20851	LGHcPDPVLVnGEFSSSGPVN#VSDK	22
170	P02749	LGN#WSAmPScK	68
171	P02749	LGN#WSAMPScK	55
172	P51884	LGSFEGLVN#LTFIHLQHNR	89
173	P06681	LGSYPVGGN#VSFEcEDGFILR	18
174	P80108	LGTSLSSGHVLMN#GTLK	68
175	P51884	LHINHNN#LTESVGPLPK	38
176	P05156	LISN#cSK	47
177	P10909	LKELPGVcN#ETMMALWEEcKPcLK	37
178	A0A0A0MRZ8	LLIYDASN#R	50
179	P08603	LN#DTLDYEcHDGYESNTGSTTGSIVcGYnGWSDLPIcYER	59
180	P01042	LnAENN#ATFYFK	14
181	P01042	LNAEnN#ATFYFK	52
182	P01042	LNAENN#ATFYFK	60
183	P80108	LNVEAAN#WTVR	75
184	P02750	LPPGLLAN#FTLLR	46
185	Q14624	LPTQN#ITFQTESSVAEQEAEFQSPK	68
186	P03952	LQAPLN#YTEFQKPIcLPSK	39
187	Q03591	LQNNEnN#IScVER	31
188	Q03591	LQNNENN#IScVER	23
189	P08571	LRN#VSWATGR	64
190	P05156	LSDLSIN#STEcLHVHcR	28
191	P01876; P0DOX2	LSLHRPALEDLLLGSEAN#LTcTLTGLR	106
192	P04003	LSVDKDQYVEPEN#VTIQcDSGYGVVGPQSITcSGN#R	27
193	P06681	LTDTIcGVGN#MSAN#ASDQER	66
194	Q96PD5	LYHFLLGAWSLN#ATELDPcPLSPELLGLTK	64
195	P08603	mDGASN#VTcINSR	100
196	P08603	MDGASN#VTcInSR	52
197	P08603	MDGASN#VTcINSR	68

198	P02750	MFSQN#DTR	36
199	P10909	mLN#TSSLLEQLNEQFNWVSR	124
200	P10909	MLN#TSSLLEQLNEQFNWVSR	123
201	P00738	mVSHHN#LTTGATLInEQWLLTTAK	52
202	P00738	mVSHHN#LTTGATLINEQWLLTTAK	77
203	P00738	MVSHHN#LTTGATLInEQWLLTTAK	59
204	P00738	MVSHHN#LTTGATLINEQWLLTTAK	73
205	P04004	N#GSLFAFR	58
206	P02790	N#GTGHGN#STHHGPEYmR	57
207	P02790	N#GTGHGN#STHHGPEYMR	82
208	O75882	N#HScSEGQISIFR	56
209	P04004	N#ISDGFDGIPDNVDAALALPAHSYSGR	65
210	P80108	N#LTTSLTESVDR	76
211	P80108	N#LTTSLTESVDRNIN#YTER	18
212	P80108	N#LTTSLTESVDRnINYTER	18
213	P0DP01	N#TSISTAYMELSSLR	51
214	P27918	N#VTFWGRPLPR	16
215	P09871	NcGVN#cSGDVFTALIGEIASPnYPKPYPENSR	20
216	P09871	NcGVN#cSGDVFTALIGEIASPNYPKPYPENSR	11
217	P02763; P19652	NEEYN#KSVQEIQATFFYFTPN#KTEDTIFLR	68
218	P02763; P19652	nEEYNKSVQEIQATFFYFTPN#KTEDTIFLR	23
219	P07996	nKGcSSSTSVLLTLDNNVVN#GSSPAIR	43
220	P07996	NKGcSSSTSVLLTLDnNVVN#GSSPAIR	43
221	P07996	NKGcSSSTSVLLTLDNnVVN#GSSPAIR	43
222	P00738	nLFLN#HSEN#ATAK	25
223	P00738	NLFLN#HSEN#ATAK	70
224	P00738	NLFLN#HSEN#ATAKDIAPTLTLYVGK	38
225	P00738	NLFLN#HSEN#ATAKDIAPTLTLYVGKK	43
226	P04004	NN#ATVHEQVGGPSLTSDLQAQSK	83
227	P43251	NPVGLIGAEN#ATGETDPSHSK	32
228	P01011	NVIFSPLSISTALAFLSLGAHN#TTLTEILK	22
229	P02763	QDQcIYN#TTYLNVQR	83
230	P01009	QLAHQSN#STNIFFSPVSIATAFAmLSLGTK	83
231	P01009	QLAHQSN#STNIFFSPVSIATAFAMLSLGTK	88
232	P00738	QLVEIEKVVLHPN#YSQVDIGLIK	15
233	P01871	QNGEAVKTHTN#ISESHPN#ATFSAVGEASIcEDDWNSGER	28
234	P01871	QnGEAVKTHTN#ISESHPNATFSAVGEASIcEDDWNSGER	28
235	P01871	QnGEAVKTHTNISESHPN#ATFSAVGEASIcEDDWNSGER	28

236	P19652	QNQcFYN#SSYLNVQR	74
237	P02787	QQQHLFGSN#VTDcSGnFcLFR	72
238	P02787	QQQHLFGSN#VTDcSGNFcLFR	84
239	P06681	QSVPAHFVALN#GSK	44
240	P04114	QVFPGLnYcTSGAYSN#ASSTDSASYYPLTGDTR	49
241	P02751	RHEEGHMLN#cTcFGQGR	59
242	P00748	RN#HScEPcQTLAVR	51
243	P05156	SIPAcVPWSPYLFQPN#DTcIVSGWGR	52
244	P01023	SLGnVN#FTVSAEALESQELcGTEVPSVPEHGR	31
245	P01023	SLGNVN#FTVSAEALESQELcGTEVPSVPEHGR	86
246	P01023	SLGNVN#FTVSAEALESQELcGTEVPSVPEHGRK	55
247	P01008	SLTFN#ETYQDISELVYGAK	66
248	P08603	SPDVIN#GSPISQK	45
249	P08603; Q03591	SPYEMFGDEEVMcLnGN#WTEPPQcK	10
250	P08603; Q03591	SPYEMFGDEEVMcLNGN#WTEPPQcK	14
251	P00751	SPYYN#VSDEISFHcYDGYTLR	62
252	P29622	SQILEGLGFN#LTELSESDVHR	106
253	P00734	SRYPHKPEIN#STTHPGADLQENFcR	66
254	A0A075B6I0	SSGVPDRFSGSILGN#K	31
255	P01871; P0DOX6	STGKPTLYN#VSLVMSDTAGTcY	30
256	P02763; P19652	SVQEIQATFFYFTPN#KTEDTIFLR	95
257	P41222	SVVAPATDGGLN#LTSTFLR	26
258	P02790	SWPAVGN#cSSALR	77
259	O95445	TELFSSScPGGIMLN#ETGQGYQR	57
260	P01023	TEVSSNHVLIYLDKVSN#QTLSLFFTVLQDVPVR	70
261	P0DOX6	THTN#ISESHPN#ATFSAVGEASIcEDDDWSGER	11
262	P01871	THTN#ISESHPN#ATFSAVGEASIcEDDWnSGER	43
263	P01871	THTN#ISESHPN#ATFSAVGEASIcEDDWNSGER	89
264	P0DOX6	THTNISESHPN#ATFSAVGEASIcEDDDWSGER	20
265	P01871	THTNISESHPN#ATFSAVGEASIcEDDWnSGER	20
266	P01859	TKPREEQFN#STFR	35
267	P01861	TKPREEQFN#STYR	20
268	P01860	TKPREEQYN#STFR	23
269	P0DOX5	TKPREEQYN#STYR	51
270	P20851	TLFcN#ASKEWDN#TTTEcR	38
271	P0DOX3	TLLN#ASR	47
272	P01011	TLN#QSSDELQLSMGNAMFVK	70
273	P06681	TmFPN#LTDVR	30
274	P0DOX2	TPLTAN#ITK	52

275	P01871; P0DOX6	TVDKSTGKPTLYN#VSLVMSDTAGTcY	35
276	Q08380	TVIRPFYLTN#SSGVD	45
277	P01024	TVLTPATnHMGN#VTFTIPANR	56
278	P01024	TVLTPATNHmGN#VTFTIPANR	75
279	P01024	TVLTPATNHMGN#VTFTIPANR	97
280	P02765	VcQDcPLLAPLN#DTR	83
281	P02765	VcQDcPLLAPLN#DTRVVHAAK	16
282	O75636	VELEDFNGN#R	30
283	O75882	VFHIHN#ESWVLLTPK	14
284	P05155	VGQLQLSHN#LSLVILVPQNLK	58
285	P04196	VIDFN#cTTSSVSSALANTK	77
286	Q9Y6R7	VITVQVAN#FTLR	29
287	P13671	VLN#FTTK	46
288	P05155	VLSN#nSDANLELINTWVAK	52
289	P05155	VLSN#NSDAnLELINTWVAK	50
290	P05155	VLSN#NSDANLELInTWVAK	17
291	P05155	VLSN#NSDANLELINTWVAK	89
292	Q9UGM5	VLYLAAYN#cTLRPVSK	60
293	P04114	VNQNLVYESGSLN#FSK	18
294	P13473	VQPFN#VTQGK	55
295	P07357	VRGGSSGWSGGLAQN#R	30
296	P49908	VSEHIPVYQQEEN#QTDVWTLLN#GSKDDFLIYDR	30
297	P01023	VSN#QTLSLFFTVLQDVPVR	149
298	P01023	VSN#QTLSLFFTVLQDVPVRDLKPAIVK	29
299	P55058	VSN#VScQASVSR	109
300		VSTVYANN#GSVLQGTSVASVYHGK	18
301	P36955	VTQN#LTLIEESLTSEFIHDIDRELK	48
302	P00738	VVLHPN#YSQVDIGLIK	46
303	P07996	VVN#STTGPGEHLR	63
304	P02749	VYKPSAGN#nSLYR	60
305	P02749	VYKPSAGN#NSLYR	66
306	P41222	WFSAGLASN#SSWLR	34
307	P43251	WNPcLEPHRFN#DTEVLQR	30
308	P08603	WQSIPLcVEKIPcSQPPQIEHGTIN#SSR	26
309	P00734	WVLTAAHcLLYPPWDKN#FTEnDLLVR	59
310	P00734	WVLTAAHcLLYPPWDKN#FTENDLLVR	61
311	P01008	WVSN#KTEGR	56
312	P43652	YAEDKFN#ETTEK	57
313	P04114	YDFN#SSmLYSTAK	47
314	Q08380	YKGLN#LTEDTYKPR	27
315	P01871	YKN#nSDISSTR	56
316	P01871	YKN#NSDISSTR	74

317	P01009	YLGN#ATAIFFLPDEGK	44
318	P01009	YLGN#ATAIFFLPDEGKLQHLENELTHDIITK	26
319	Q9Y6R7	YLPVN#SSLLTSDcSER	21
320	P01042	YnSQN#QSNNQFVLYR	94
321	P01042	YNSQN#QSnNQFVLYR	101
322	P01042	YNSQN#QSNnQFVLYR	99
323	P01042	YNSQN#QSNNQFVLYR	34
324	P00734	YPHKPEIN#STTHPGADLQENFcR	34
325	P27918	YPPTVSMVEGQGEKN#VTFWGRPLPR	41
326	P01011	YTGN#ASALFILPDQDKmEEVEAMLLPETLKR	24
327	P01011	YTGN#ASALFILPDQDKMEEVEAmLLPETLKR	24
328	P01011	YTGN#ASALFILPDQDKMEEVEAMLLPETLKR	94

Table S5. The amount of glycopeptides identified by previous researches.

material	sample	mechanism	glycoprotein counts	glycopeptide counts	ref
magG@PF@APB	1 μl human serum	boronic acid	11	17	1
Fe_3O_4@SiO_2@PSV	human serum	boronic acid	46	101	2
Fe_3O_4–MPBA	1 μl human serum	boronic acid	93	230	3
BA@fibrous cellulose	2 μl human serum	boronic acid	54	84	4
MMP	0.35 μl human serum	hydrophilicity interaction	119	356	5
Fe_3O_4@mSiO_2-IDA	2 μl human serum	hydrophilicity interaction	140	424	6
GO-PEI-Carr	2 μl human serum	hydrophilicity interaction	56	76	7
Ce-MOF@PA	2 μl human serum	hydrophilicity interaction	155	422	8
BMMP	2 μl human serum	Multimodal enrichment	101	328	this study

Table S6. FDA-cleared biomarkers in blood identified in this study

No	ProteinName	UniprotKB
1	Alpha-1-Acid Glycoprotein (Orosomucoid)	P02763
2	Alpha-1-Acid Glycoprotein (Orosomucoid)	P19652
3	Alpha-1-Antitrypsin	P01009
4	Alpha-2-HS-Glycoprotein	P02765
5	Alpha-2-Macroglobulin	P01023
6	Antithrombin III (ATIII)	P01008

7	Apolipoprotein B	P04114
8	Biotinidase	P43251
9	Ceruloplasmin	P00450
10	Cholinesterase	P06276
11	Complement C1	P09871
12	Complement C1 Inhibitor	P05155
13	Complement C3	P01024
14	Complement C4	P0C0L4
15	Complement C5	P01031
16	Factor XIII	P05160
17	Fibronectin	P02751
18	Haptoglobin	P00738
19	Hemopexin	P02790
20	Immunoglobulins IgA	P01876
21	Immunoglobulins IgD	P0DOX3
22	Immunoglobulins IgG	P0DOX5
23	Immunoglobulins IgG	P01859
24	Immunoglobulins IgG	P01860
25	Immunoglobulins IgM	P01871
26	Immunoglobulins IgM	P0DOX6
27	Insulin-like Grwth Factor Bind. Protein3 (IGFBP-3)	P17936
28	Plasminogen	P00747
29	Prealbumin	P02766
30	Properdin factor B	P00751
31	Pseudocholinesterase	P06276
32	Sex Hormone Binding Globulin	P04278
33	Transferrin	P02787
34	Von Willebrand Factor	P04275

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