SUPPORTING INFORMATION

Data independent analysis of IgG glycoforms in samples of unfractionated human plasma.

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Study Population

Applicability of the method was documented on plasma samples of healthy controls (n=10) and patients with cirrhosis of the liver (n=10). All participants were recruited under protocols approved by the Georgetown University's Institutional Review Board in collaboration with the Department of Hepatology and Liver Transplantation, Georgetown University Hospital, Washington D.C. The healthy controls and cirrhotic patients were matched on age, race (60% Caucasian, 40% African-American), and gender (80% males). The samples of each study group were divided into two subsets (n=5 each) and were pooled for analysis by equal volume as described in detail previously 27.

DIA analysis of the glycoforms of IgG1 glycopeptides

Plasma samples were diluted and prepared as described above. Samples were measured under optimized conditions using rolling CE (CE_{3+}=0.03*M-3) and 5 Da SWATH window step. Tryptic digest of unfractionated plasma was separated using 90 mins gradient elution as follows: A 1 min trapping step using 2% ACN, 0.1% formic acid at 15 µl/min was followed by chromatographic separation at 0.4 µl/min as follows: starting conditions 1% ACN, 0.1% formic acid; 0-1 min 1-5% ACN, 1-60 min, 5–40% ACN, 0.1% formic acid; 60-65 min, 40–98% ACN, 0.1% formic acid; 65-70 min 98% ACN, 0.1% formic acid followed by equilibration to starting conditions for additional 20 minutes. Y-ion isotope cluster with isolation of a window of 1.2 Da, extracted from the SWATH MS/MS with a 5 Da step window, was used for analysis of the glycopeptide intensities **(Supplemental Table 2)**.

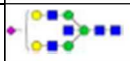
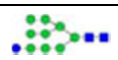
Formula	Structure	IgG1 P01857 EEQYN$_{297}$STYR		IgG2/IgG3 P01859 EEQFN$_{297}$STFR		IgG4 P01861 EEQFN$_{297}$STYR	
		Product $[M+2H]^{2+}$	delta ppm	Product $[M+2H]^{2+}$	delta ppm	Product $[M+2H]^{2+}$	delta
G0		1142.958	1.40	1126.963	3.55	1134.960	13.39
G0N		1244.497	4.82	1228.503	0.98	1236.500	12.13
G0FN		1317.526	2.35	1301.532	0.31	1309.529	1.68
G0F		1215.987	1.15	1199.992	0.50	1207.989	2.40
G1		1142.958	0.61	1126.963	4.97	1134.960	0.88
G1N		1244.497	0.96	1228.503	2.93	ND	NA
G1FN		1317.526	0.68	1301.532	4.07	1309.529	5.12
G1FNS		1317.526	3.49	1301.532	2.46	1309.529	0.61
G1NS		1244.497	4.82	ND	NA	ND	NA
G1F		1215.987	0.16	1199.992	0.75	1207.989	1.08
G1FS		1215.987	1.56	1199.992	0.75	1207.989	5.30
G2		1223.984	0.16	1207.989	1.90	1215.987	0.74
G2N		1325.524	12.30	1309.529	4.81	1317.526	0.08
G2FN		1398.553	1.64	1382.558	0.65	1390.555	0.36
G2NS		1325.524	2.04	ND	NA	ND	NA
G2F		1297.013	0.15	1281.018	2.73	1289.016	1.16
G2FS		1297.013	0.15	1281.018	0.86	1289.016	0.23
G2S		1223.984	4.74	1207.989	6.95	1215.987	1.81
G2S2		1369.532	6.94	ND	NA	ND	NA
Man6		1392.591*	11.78	1360.602*	11.91	ND	NA
Man8		1392.591*	1.36	1360.602*	8.01	1376.597*	14.60
Man9		1392.591*	0.22	1360.602*	14.63	1376.597*	5.09
Man9Glc		1392.591*	0.07	ND	NA	ND	NA
G0F-N		1114.447	2.78	1098.452	5.01	1106.449	10.76
G1F-N		1114.447	4.04	1098.452	6.83	1106.450	9.58
G1FS-N		1114.447	13.82	1098.452	3.10	ND	NA

Table T-1 Mass accuracy of Y-ion fragments of IgG glycoforms measured using independent analysis (DIA) and reported in Table 1.

Formula	Ctrl Avg	Ctrl SD	Cirr Avg	Cirr SD	Cirr/Ctrl Ratio
G0	0.336	0.072	0.702	0.571	2.1
G0F	4.600	0.375	14.023	5.257	3.1
G0FN	6.026	0.198	17.251	0.798	2.9
G0N	0.954	0.316	1.223	0.185	1.3
G1	1.212	0.064	2.611	1.155	2.2
G1F	9.260	0.034	15.149	6.486	1.6
G1FN	3.616	0.269	6.874	0.260	1.9
G1FNS	0.173	0.019	0.385	0.051	2.2
G1FS	1.272	0.051	3.102	0.121	2.4
G1N	0.356	0.072	0.471	0.056	1.3
G2	0.872	0.185	0.832	0.198	1.0
G0F-N	0.017	0.004	0.024	0.007	1.4
G1F-N	0.088	0.009	0.190	0.074	2.2
G1FS-N	0.152	0.031	0.266	0.008	1.8
G2F	7.883	0.755	9.260	3.125	1.2
G2FN	1.675	0.294	1.582	0.698	0.9
G2FS	7.059	0.853	7.158	3.656	1.0
G2N	0.654	0.005	1.248	0.038	1.9
G2NS	0.141	0.018	0.174	0.004	1.2
G2S	0.675	0.008	0.514	0.250	0.8
Man 6	0.019	0.001	0.032	0.014	1.7
Man 8	0.025	0.003	0.052	0.005	2.1
Man 9	0.026	0.006	0.018	0.005	0.7
Man9Glc	0.075	0.014	0.093	0.008	1.2

Table T-2 Data independent analysis (DIA) of IgG1 glycoforms in two control (Ctrl) and two cirrhosis (Cirr) plasma pools (5 samples per pool) based on the intensity of Y-ion fragments reported in Table 1.

	IgG1	IgG2/3	IgG4
G0	4.82	11.11	13.64
G0F	7.80	9.40	12.63
G2F	6.61	2.81	3.15
G2FS	3.53	14.65	3.94

Table T-3 Relative standard deviations (RSDs) of parallel measurement of soft fragment intensities under optimized collision energy

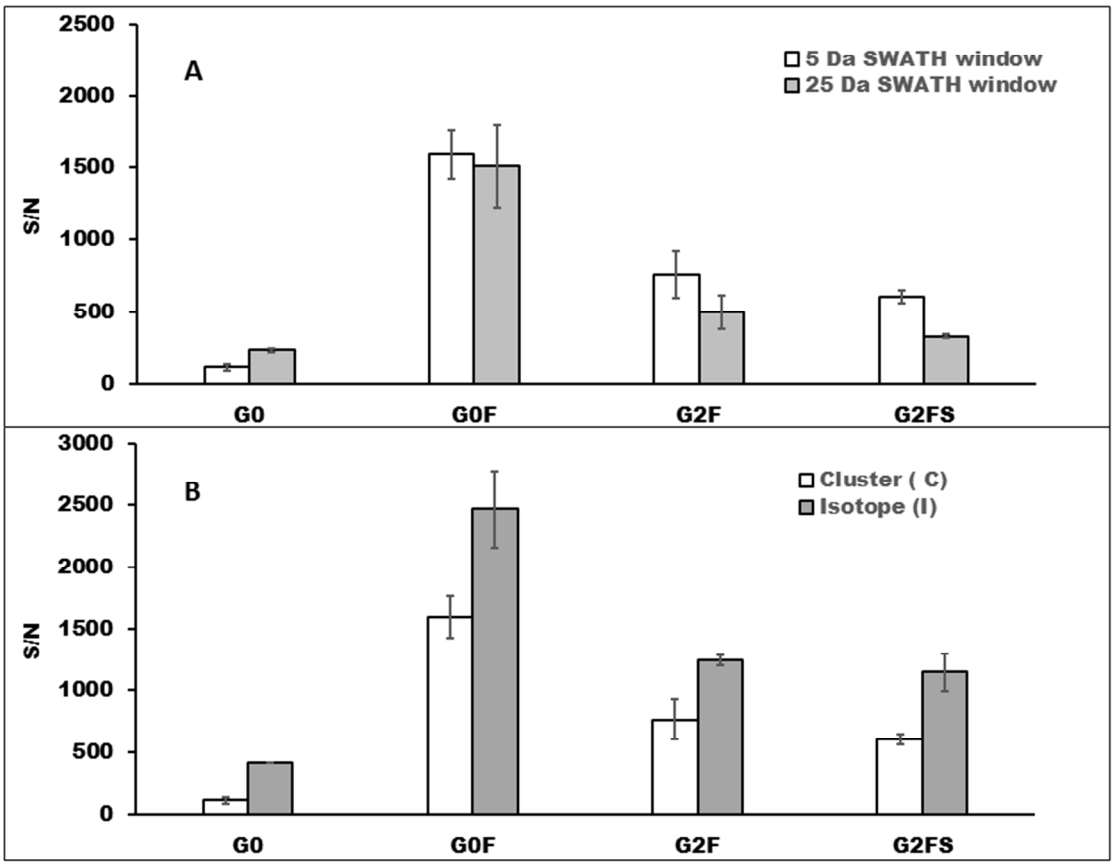


Figure S-1. Optimization of the signal to noise (S/N) on G0, G0F, G2F, and G2FS glycoforms of IgG1: **A.** comparison of acquisition using 5 Da and 25 Da isolation windows; **B.** comparison of 'soft' fragment XIC of entire isotope cluster with width of window 1.2Da (C) and sum of XICs of three major isotopes with window width 0.1 Da (I)

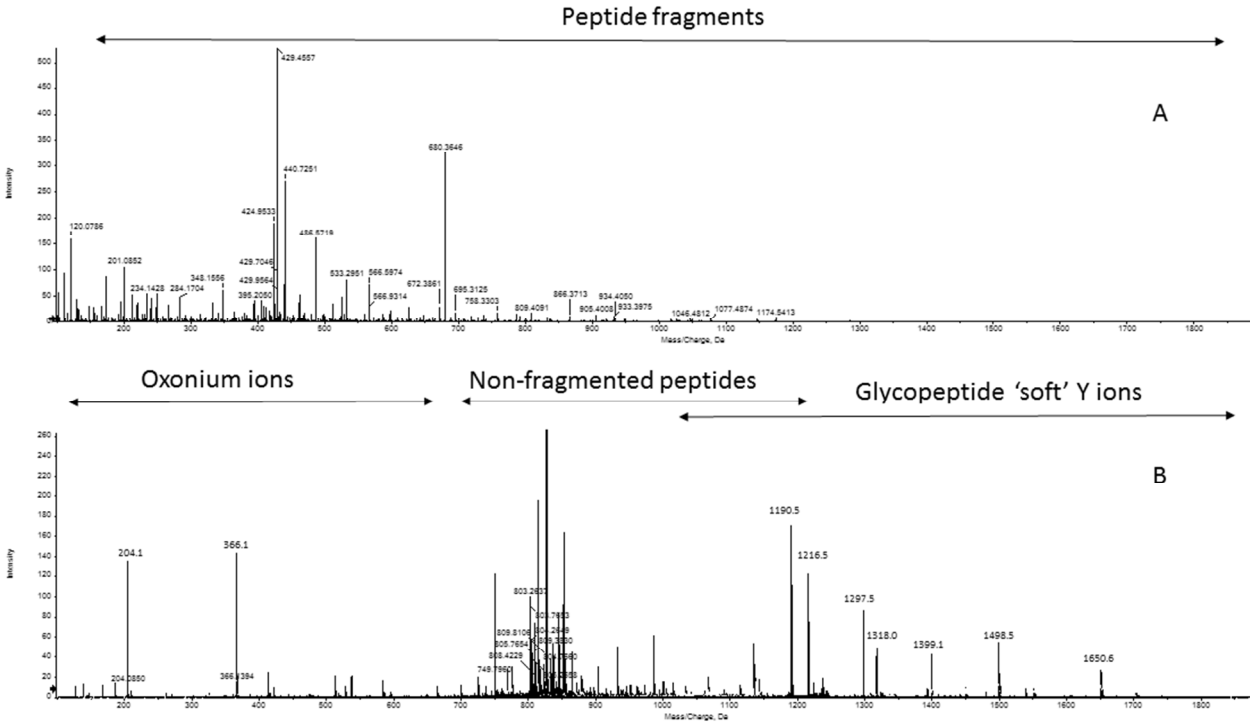


Figure S-2. Fragmentation spectra acquired under the following conditions: **A.** CE typical for peptide fragmentation; and **B.** CE optimized for 'soft' glycopeptide fragmentation; the signal represents overlaid fragmentation spectra in a 1 minute chromatographic elution time of the IgG1 glycopeptides.

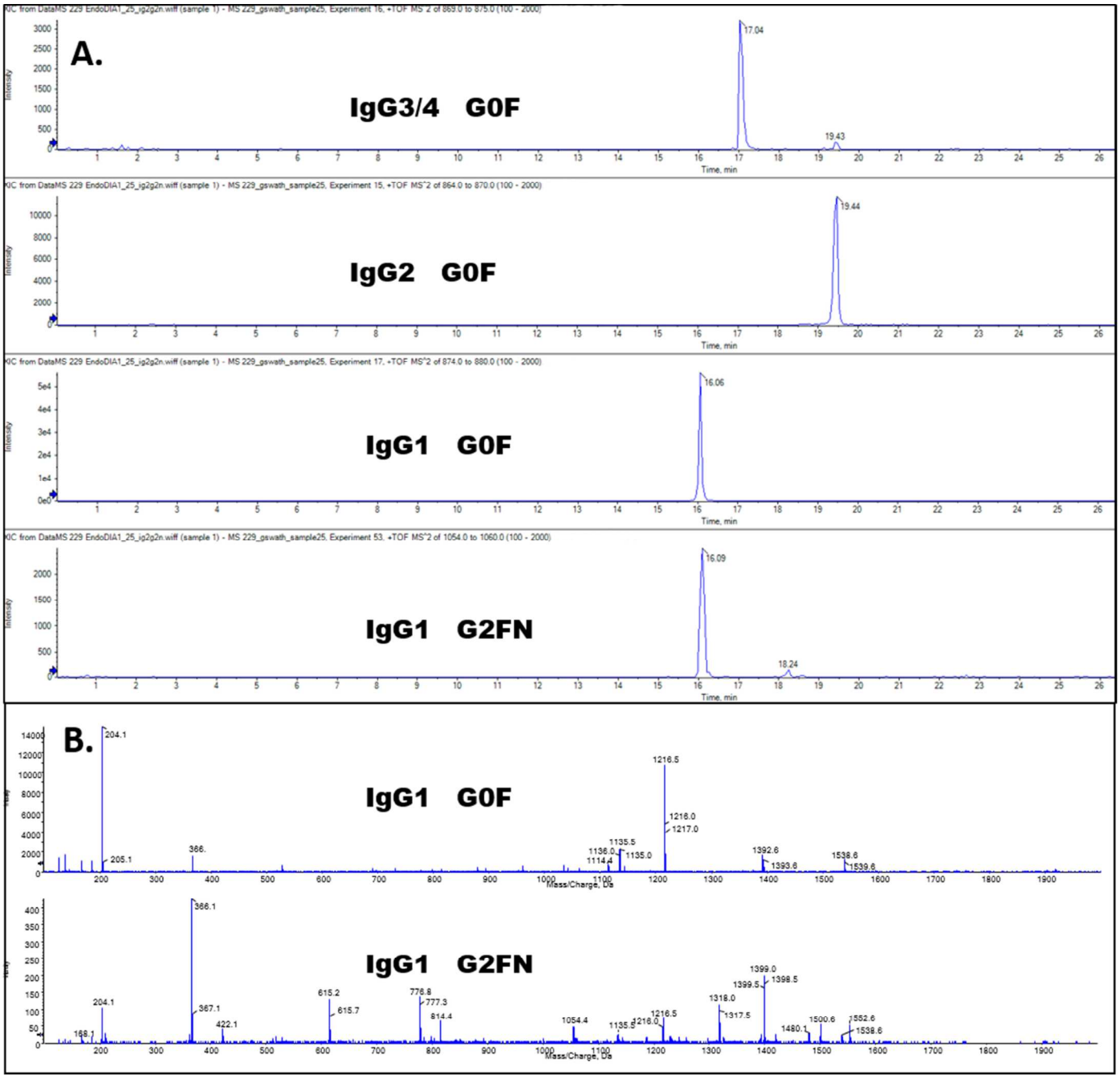


Figure S-3. Detection of the glycoforms of IgG by SWATH DIA of a tryptic digest of unfractionated human plasma: **A.** XIC of G0F, a major glycoforms of the IgG1, IgG2/3, and IgG4 subclasses, and G2NB, a minor glycoform of IgG1; **B.** Soft fragmentation spectra of the G0F and G2N glycoforms of IgG1 documenting the Y-ions used for XIC.

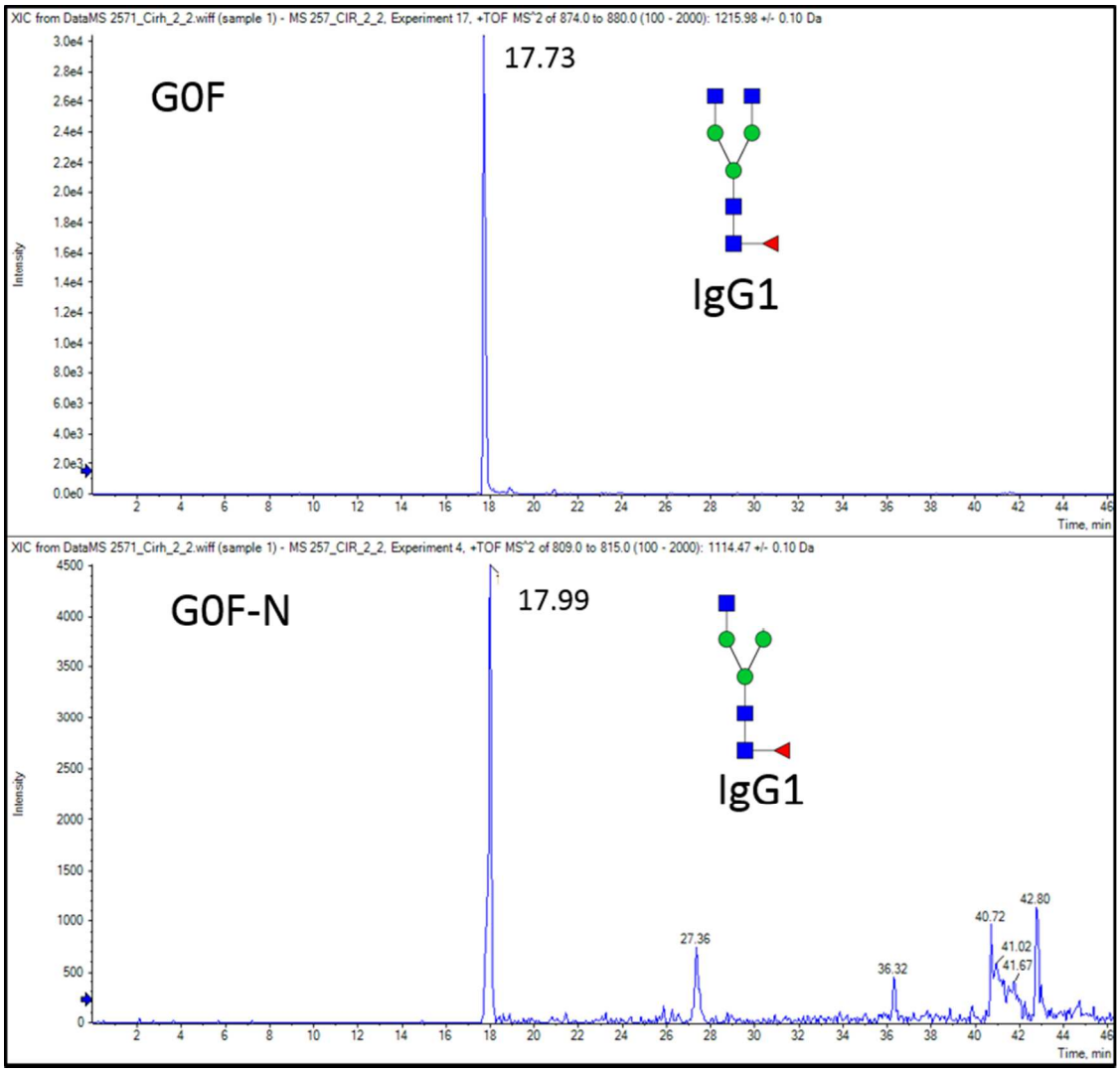


Figure S-4. XIC chromatograms of the G0F and G0F-N glycoforms of IgG1 detected at different retention times which excludes in source fragmentation as origin of the G0F-N glycoform.