Comprehensive analysis of subclass-specific IgG glycosylation in colorectal cancer progression by nanoLC-MS/MS

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Supporting Figures

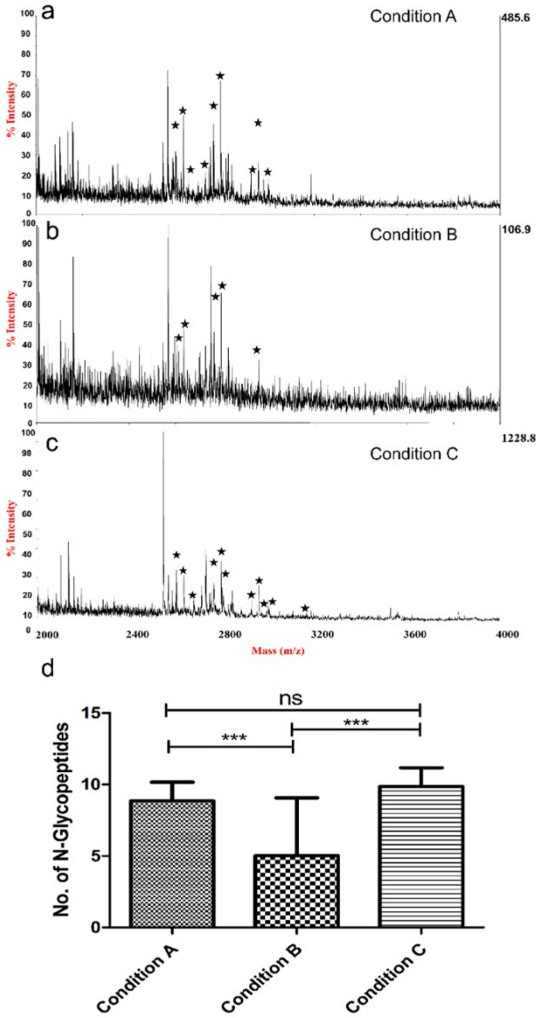


Figure S1. Comparison of the efficiency of ice-cold acetone for the enrichment of IgG glycopeptides. (a-c) MALDI-MS spectra for IgG glycopeptides purified by acetone using different conditions; (d) Statistical analysis of the number of glycopeptides identified under different conditions. Glycopeptides are marked with asterisk. 1-way ANOVA is performed to assess the difference in number of observed glycopeptides between different groups with post hoc Bonferroni test. *$P<0.05$, **$P<0.01$, ***$P<0.001$. ns, nonsignificant.

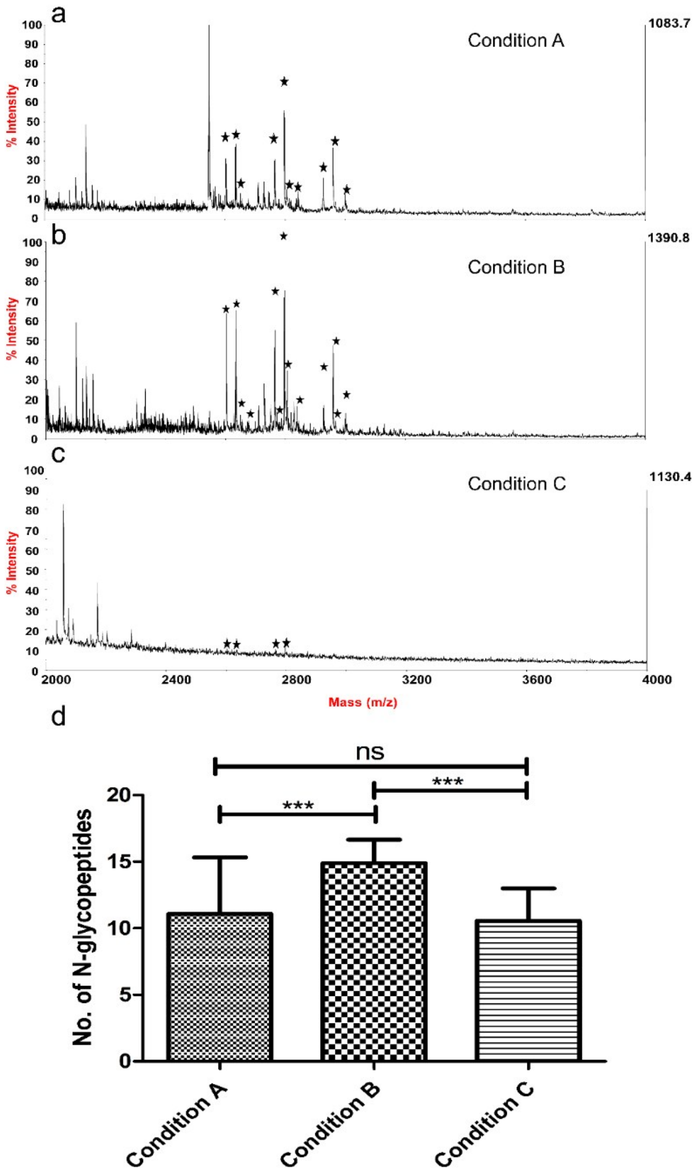


Figure S2. Comparison of the efficiency of DPA-6S for the enrichment of IgG glycopeptides. (a-c) MALDI-MS spectra for IgG glycopeptides purified by acetone using different conditions; (d) Statistical analysis of the number of glycopeptides identified under different conditions. Glycopeptides are marked with asterisk. 1-way ANOVA is performed to assess the difference in number of observed glycopeptides between different groups with post hoc Bonferroni test. **P*<0.05, ***P*<0.01, ****P*<0.001. ns, nonsignificant.

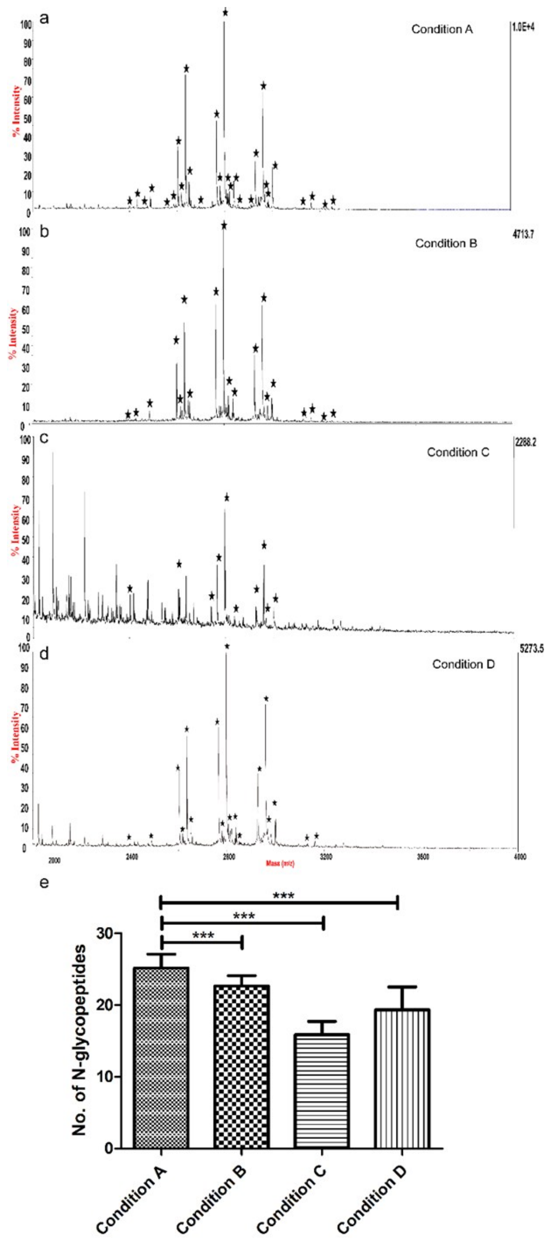


Figure S3. Comparison of the efficiency of MCC for the enrichment of IgG glycopeptides. (a-d) MALDI-MS spectra for IgG glycopeptides purified by acetone using different conditions; (e) Statistical analysis of the number of glycopeptides identified under different conditions. Glycopeptides are marked with asterisk. 1-way ANOVA is performed to assess the difference in number of observed glycopeptides between different groups with post hoc Bonferroni test. **P*<0.05, ***P*<0.01, ****P*<0.001.

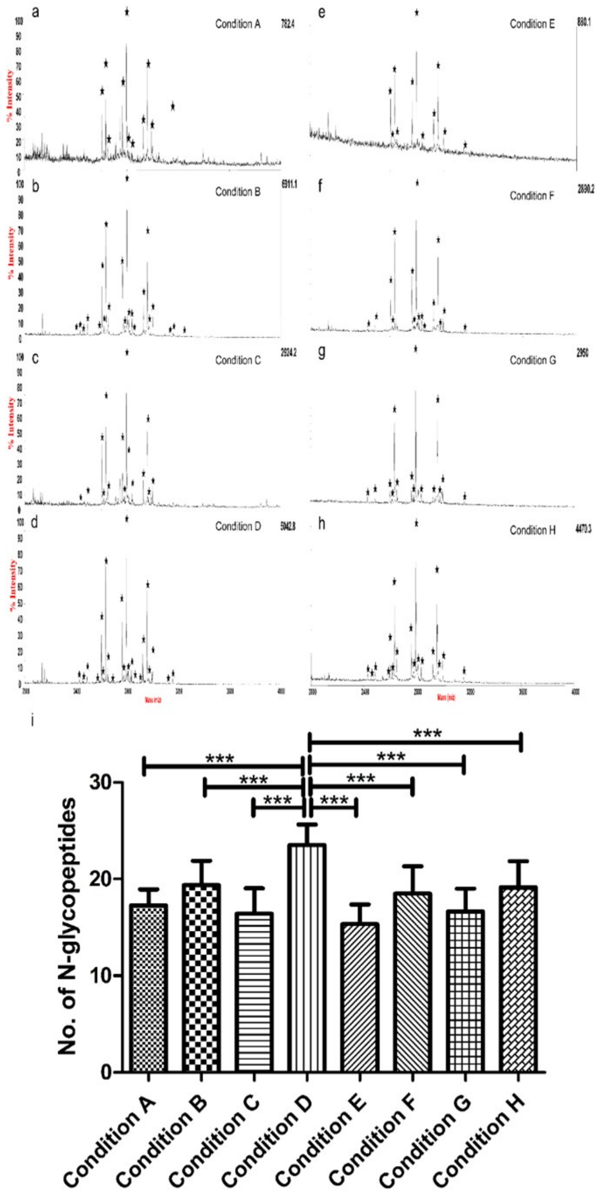


Figure S4. Comparison of the efficiency of ZIC-HILIC for the enrichment of IgG glycopeptides. (a-h) MALDI-MS spectra for IgG glycopeptides purified by acetone using different conditions; (i) Statistical analysis of the number of glycopeptides identified under different conditions. Glycopeptides are marked with asterisk. 1-way ANOVA is performed to assess the difference in number of observed glycopeptides between different groups with post hoc Bonferroni test. *P<0.05, **P<0.01, ***P<0.001.

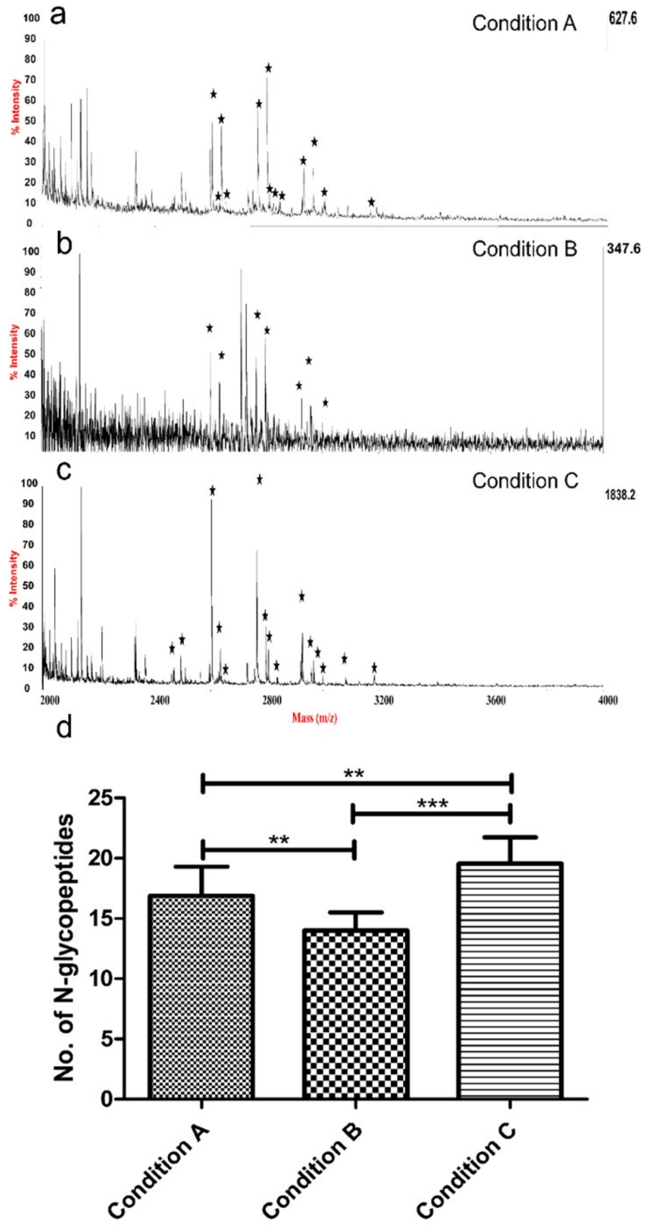


Figure S5. Comparison of the efficiency of PGC for the enrichment of IgG glycopeptides. (a-c) MALDI-MS spectra for IgG glycopeptides purified by acetone using different conditions; (d) Statistical analysis of the number of glycopeptides identified under different conditions. Glycopeptides are marked with asterisk. 1-way ANOVA is performed to assess the difference in number of observed glycopeptides between different groups with post hoc Bonferroni test. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

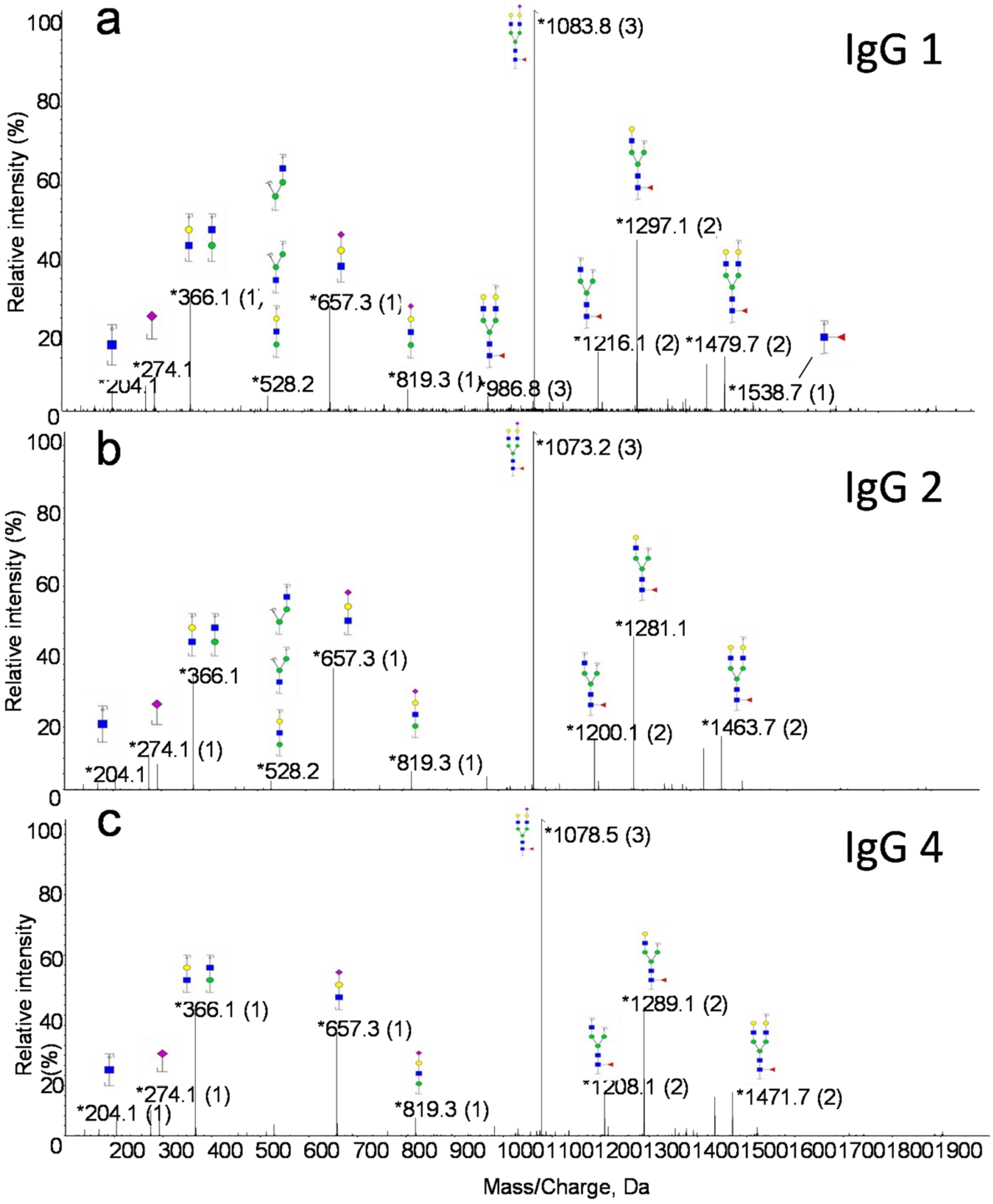


Figure S6. Representative MS/MS spectra IgG glycopeptide, the biantennary mono-sialylated glycan of fucosylation, in each subclass. MS/MS analysis was acquired by collision-induced dissociation (CID). The mass charge was labeled with bracketed numbers. Green circle: mannose; yellow circle: galactose; blue square: *N*-acetylglucosamine; red triangle: fucose; pink diamond: *N*-acetylneuraminic acid.

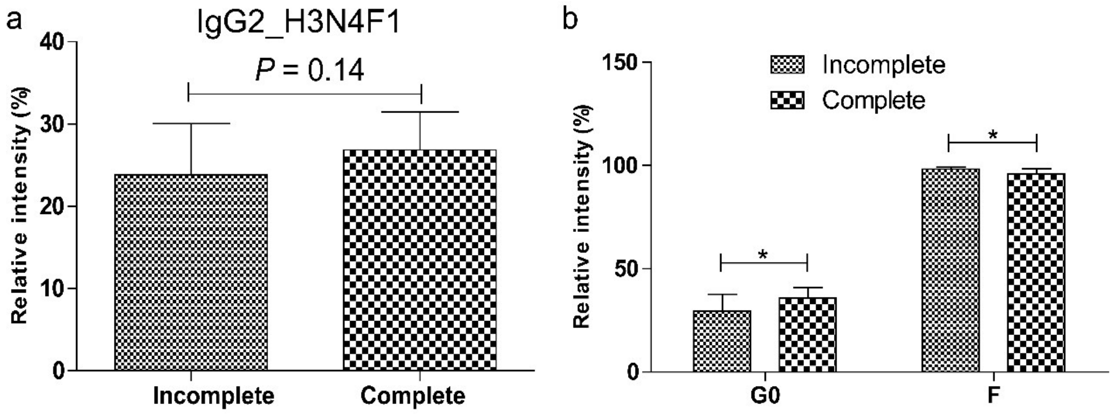


Figure S7. Bar graph describing the statistical difference between incomplete digestion and complete digestion in analysis of representative individual glycopeptide and corresponding glycan features. H, hexose; N, N-acetylglucosamine; S, N-acetylneuraminic acid. G0, agalactosylation; F, fucosylation. Mann-Whitney U test is performed to assess the difference in statistical analysis between different groups. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

Supporting Tables

Table S1. Descriptive statistics of the difference between normal group and colon or rectal cancer group in the factors including age, sex and TNM stages. P values result from one-way ANOVA analysis. P-value less than 0.05 is set as significant. [a]Comparison between normal and colon cancer; [b]Comparison between normal and rectal cancer; [c]Comparison between normal and colon cancer. IQR, interquartile range.

	Discovery set				Validation set			
Characteristic	Normal (N=24)	Colon (N=17)	Rectal (N=16)	*P*	Normal (N=17)	Colon (N=23)	Rectal (N=23)	*P*
Age,y, median (IQR)	53 (50, 55)	53 (51, 54)	52 (47, 60)	0.26 [a] 0.86 [b] 0.34 [c]	55 (37, 60)	57 (47, 67)	58 (53, 65)	0.16 [a] 0.06 [b] 0.59 [c]
Sex, male, n (%)	13 (54.2)	11 (64.7)	13 (81.2)	0.51 [a] 0.08 [b] 0.30 [c]	9 (52.9)	10 (43.5)	14 (60.9)	0.57 [a] 0.63 [b] 0.25 [c]
TNM stage, early stages, n (%)	–	8 (47.1)	10 (62.5)	0.39 [c]	–	9 (39.1)	12 (52.2)	0.39 [c]

Table S2. Number of IgG glycopeptides that could be identified using different enrichment methods under different reaction conditions. The optima condition is labeled with “*”.

Enrichment methods	Condition	Reaction conditions			No. of glycopeptide ± S.D.
		Washing buffer	loading/equilibrium buffer	Elution buffer	
PGC	A	water	water	30% ACN/0.1% TFA	16.89 ± 2.41
	B	water	80% ACN/0.1% TFA	30% ACN/0.1% TFA	14.00 ± 1.50
	C*	water	0.1% TFA	30% ACN/0.1% TFA	19.56 ± 2.19
ZIC-HILIC	A	water	80% ACN/0.5% FA	0.5% FA	17.26 ± 1.68
	B	water	80% ACN/0.1% TFA	water	19.37 ± 2.53
	C	80% ACN/0.5% FA	80% ACN/0.5% FA	1% FA	16.41 ± 2.62
	D*	0.5% FA	90% ACN/0.1% TFA	0.5% TFA	23.52 ± 2.12
	E	water	80% ACN/1% TFA	0.1% TFA	15.33 ± 2.02
	F	0.1% TFA	80% ACN/0.1% TFA	0.1% TFA	18.48 ± 2.83
	G	1% TFA	80% ACN/1% TFA	0.1% TFA	16.63 ± 2.37
	H	80% ACN/5% TFA	80% ACN/5% TFA	30% ACN/0.1% TFA	19.15 ± 2.68
MCC	A*	water	80% ACN/0.1% TFA	water	25.19 ± 1.92
	B	water	80% ACN/0.1% TFA	50% ACN/0.1% TFA	22.67 ± 1.41
	C	70% ACN	70% ACN	10% ACN	15.85 ± 1.90
	D	water	80% ACN/0.1% TFA	0.1% TFA	19.33 ± 3.20
DPA-6S	A	95% ACN	95% ACN	20% ACN	11.07 ± 4.25
	B*	95% ACN/0.1% TFA	95% ACN/0.1 % TFA	20% ACN/0.1% TFA	14.89 ± 1.76
	C	H_2O	80% ACN/ 0.1% TFA	0.1% TFA	10.56 ± 2.44
		Centrifugal speed (g)	Centrifugal Temperature (℃)	Centrifugal Time (min)	
Ice-cold acetone	A	12000	20	10	8.85 ± 1.32
	B	16000	20	15	5.04 ± 4.03
	C*	10000	20	15	9.85 ± 1.32

Table S3. Overview of the glycoforms detected by nanoLC-MS/MS. Product ion is defined to be the higher abundance in the comparison between two fragment ions at m/z 204.1 or m/z 366.1. For all glycoforms, green circle: mannose; yellow circle: galactose; blue square: N-acetylglucosamine; red triangle: fucose; pink diamond: N-acetylneuraminic acid. For glycan compositions, H: hexose; F, fucose; N: N-acetylglucosamine; S, N-acetyl neuraminic acid.

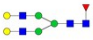
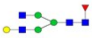
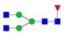
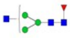
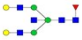
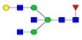
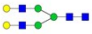
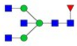
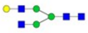
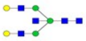
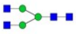
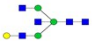
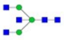
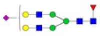
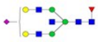
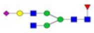
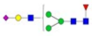
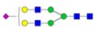
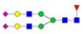
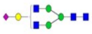
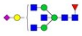
Glycan structure	Glycan species	IgG1 (EEQYNSTYR)		IgG2 (EEQFNSTFR)		IgG4 (EEQFNSTYR)	
		Precursor ion (m/z) $[M+3H]^{3+}$	Product ion (m/z) $[M+H]^{+}$	Precursor ion (m/z) $[M+3H]^{3+}$	Product ion (m/z) $[M+H]^{+}$	Precursor ion (m/z) $[M+3H]^{3+}$	Product ion (m/z) $[M+H]^{+}$
	H5N4F1	986.7407	366.1446	976.0744	366.1454	981.4106	366.1393
	H4N3F1	865.0244	366.1427	854.3621	366.1436	_	_
	H4N4F1	932.7208	204.0937	922.0578	366.1455	927.4307	366.1447
	H3N4F1	878.7008	204.0934	868.0381	204.0811	873.3666	204.0808
	H3N3F1	810.9946	204.0932	800.3302	204.0897	805.6600	204.0803
	H5N5F1	1054.4347	366.1441	1043.7715	366.1446	1049.1042	366.1450
	H4N5F1	1000.4159	204.0926	989.7521	204.0813	995.0633	204.0805
	H5N4	938.0517	366.1459	927.3982	366.1000	_	_
	H3N5F1	946.3934	204.0808	935.7326	204.0815	941.0633	204.0798
	H4N4	884.0167	204.0931	873.3547	204.0890	_	_
	H5N5	1005.7485	366.1436	_	_	_	_
	H3N4	830.0128	204.0936	819.3352	204.0897	_	_
	H4N5	951.7281	204.0811	_	_	_	_
	H3N5	897.7078	204.0809	_	_	_	_
	H5N4F1S1	1083.7752	366.1463	1073.1134	366.1459	1078.1069	366.1429
	H5N5F1S1	1151.4717	366.1459	1140.8092	366.1429	_	_
	H4N4F1S1	1029.7565	204.0818	1019.0935	366.1443	1024.4236	366.1423
	H4N3F1S1	962.0617	366.1433	951.3969	366.1464	_	_
	H5N4S1	1035.0876	366.1439	1024.4292	366.1457	_	_
	H5N4F1S2	1180.8108	366.1423	_	_	_	_
	H4N4S1	981.0467	366.1441	_	_	_	_
	H4N5F1S1	_	_	1086.7633	204.0808	_	_

Table S4. Subclass-specific IgG glycan feature calculations. The individual glycoforms with the same glycan features were grouped and the percentage was calculated by the formula. Glycan structures are annotated as described in Table S3.

IgG Subclass	Glycan features	Description	Formula
IgG 1	**G0**	Fraction of agalactosylated glycan	A2G0F+A1G0FN+A2G0FN+A2G0+A2G0N
	G1	Fraction of monogalactosylated glycans	A1G1FN+A2G1F+A2G1FN+A2G1+A2G1N+A2G1FS+A1G1FNS+A2G1S
	G2	Fraction of digalactosylated glycans	A2G2F+A2G2FN+A2G2+A2G2N+A2G2FS+A2G2FNS+A2G2S+A2G2FS2
	S	Fraction of sialylated glycans	A2G2FS+A2G2FNS+A2G1FS+A1G1FNS+A2G2S+A2G2FS2+A2G1S
	B	Fraction of bisected glycans	A1G1FN+A1G0FN+A2G2FN+A2G1FN+A2G0FN+A2G2N+A2G1N+A2G0N+A2G2FNS+A1G1FNS
	F	Fraction of fucosylated glycans	A1G1FN+A2G1F+A2G0F+A1G0FN+A2G2FN+A2G1FN+A2G0FN+A2G2FS+A2G2FNS+A2G1FS+A1G1FNS+A2G2FS2
	A2G	Galactosylation per antenna on diantennary glycans	A2G2F+A2G2FN+A2G2+A2G2N+A2G2FS+A2G2FNS+A2G2S+A2G2FS2+(A2G1F+A2G1FN+A2G1+A2G1N+A2G1FS+A2G1S)/2
	A2S	Sialylation per antenna on diantennary glycans	A2G2FS2+(A2G2FS+A2G2FNS+A2G2S+A2G1S)/2
	A2GS	Sialylation per galactose on diantennary glycans	A2S/A2G
IgG 2	**G0**	Fraction of agalactosylated glycan	A2G0F+A1G0FN+A2G0
	G1	Fraction of monogalactosylated glycans	A1G1FN+A2G1FN+A2G1F+A2G1+A2G1FNS+A2G1FS+A1G1FNS
	G2	Fraction of digalactosylated glycans	A2G2F+A2G2FN+A2G2FNS+A2G2FS+A2G2+A2G2S
	S	Fraction of sialylated glycans	A2G2FNS+A2G2FS+A2G1FNS+A2G1FS+A1G1FNS+A2G2S
	B	Fraction of bisected glycans	A2G2FN+A1G1FN+A2G1FN+A1G0FN+A2G0FN+A2G2FNS+A2G1FNS +A1G1FNS
	F	Fraction of fucosylated glycans	A2G2F+A2G2FN+A1G1FN+A2G1FN+A2G1F+A2G0F+A1G0FN+A2G0FN+A2G2FNS+A2G2FS+A2G1FNS+A2G1FS+A1G1FNS
	A2G	Galactosylation per antenna on diantennary glycans	A2G2F+A2G2FN+A2G2FNS+A2G2FS+A2G2+A2G2S+(A2G1FN+A2G1F+A2G1+A2G1FNS+A2G1FS)/2
	A2S	Sialylation per antenna on diantennary glycans	(A2G2FNS+A2G2FS+A2G1FNS+A2G1FS+A2G2S)/2
	A2GS	Sialylation per galactose on diantennary glycans	A2S/A2G
IgG 4	**G0**	Fraction of agalactosylated glycan	A2G0F+A1G0F+A2G0FN
	G1	Fraction of monogalactosylated glycans	A2G1F+A2G1FN+A2G1FS
	G2	Fraction of digalactosylated glycans	A2G2F+A2G2FN+A2G2FS
	S	Fraction of sialylated glycans	A2G2FS+A2G1FS
	A2FB	GlcNAcylation of fucosylated diantennary glycans	A2G2FN+A2G1FN+A2G0FN
	A2FG	Galactosylation per antenna on fucosylated diantennary glycans	A2G2F+A2G2FN+A2G2FS+(A2G1F+A2G1FN+A2G1FS)/2
	A2FS	Sialylation per antenna on fucosylated diantennary glycans	(A2G2FS+A2G1FS)/2
	A2FGS	Sialylation per galactose on fucosylated diantennary glycans	A2FS/A2FG

Table S5. Comparison of the result for the quantitation of individual IgG glycopeptide between normal and cancer groups by incomplete or complete digestion. P values result from Mann-Whitney U test. P-value less than 0.05 is set as significant, which are emphasized with bold. SD, standard deviation. Glycan structures are annotated as described in Table S3.

Glycan species	Incomplete Digestion					Complete Digestion				
	Normal		Cancer			Normal		Cancer		
	Mean	SD	Mean	SD	*P*	Mean	SD	Mean	SD	*P*
IgG1										
H5N4F1	14.38	4.41	13.41	4.48	4.47E-01	14.38	4.01	11.04	2.95	**1.00E-03**
H4N3F1	0.53	0.14	0.50	0.17	5.70E-01	0.80	0.38	0.63	0.18	1.05E-01
H4N4F1	29.75	4.26	31.13	4.75	2.97E-01	26.05	3.77	25.98	4.22	9.54E-01
H3N4F1	24.41	7.11	26.08	8.53	4.75E-01	17.54	5.14	23.24	6.25	**1.00E-03**
H3N3F1	0.45	0.28	0.42	0.26	6.30E-01	0.46	0.12	0.82	2.41	5.43E-01
H5N5F1	0.70	0.25	0.69	0.27	9.37E-01	1.40	0.78	0.82	0.35	**9.00E-03**
H4N5F1	4.90	1.26	4.77	1.47	7.52E-01	6.69	1.82	6.26	1.82	4.01E-01
H5N4	2.57	1.16	2.33	1.07	4.35E-01	2.32	1.33	2.23	1.02	7.58E-01
H3N5F1	3.83	1.60	3.95	1.54	7.95E-01	4.86	1.40	5.20	1.47	4.21E-01
H4N4	3.57	2.08	3.09	1.96	4.00E-01	2.91	1.42	3.45	1.53	2.17E-01
H5N5F1	0.14	0.14	0.14	0.14	8.99E-01	1.24	1.25	0.89	0.99	3.05E-01
H3N4	2.49	1.50	2.45	1.48	9.23E-01	1.76	1.09	2.80	1.39	**7.00E-03**
H4N5	0.65	0.41	0.61	0.34	7.05E-01	2.32	1.83	2.25	1.89	8.97E-01
H3N5	0.47	0.35	0.47	0.33	9.96E-01	1.25	1.09	1.94	2.18	2.13E-01
H5N4F1S1	7.41	2.88	6.64	2.69	3.27E-01	10.46	3.23	7.58	1.94	**2.00E-03**
H5N5F1S1	0.13	0.11	0.11	0.08	5.15E-01	0.34	0.27	0.27	0.25	3.49E-01
H4N4F1S1	2.00	0.99	1.81	0.80	4.40E-01	2.41	1.21	2.30	0.79	6.83E-01
H4N3F1S1	0.12	0.04	0.11	0.05	3.37E-01	0.35	0.44	0.47	1.16	6.94E-01
H5N4S1	1.14	0.56	0.94	0.47	1.64E-01	1.54	0.73	1.23	0.52	1.20E-01
H5N4F1S2	0.12	0.13	0.11	0.10	8.64E-01	0.40	0.58	0.21	0.30	2.05E-01
H4N4S1	0.27	0.12	0.26	0.17	8.19E-01	0.53	0.47	0.42	0.46	3.76E-01
IgG2										
H5N4F1	15.99	5.01	12.18	3.95	**2.00E-03**	12.46	2.99	9.67	3.20	**3.00E-03**
H5N5F1	1.04	0.23	0.83	0.34	**2.50E-02**	0.99	0.22	0.76	0.28	**4.00E-03**
H4N3F1	0.45	0.08	0.44	0.11	7.14E-01	0.66	0.37	0.57	0.15	3.14E-01
H4N5F1	4.40	1.02	4.23	0.93	5.44E-01	4.67	0.80	4.27	0.88	1.02E-01
H4N4F1	33.36	1.80	31.15	4.97	8.00E-02	32.11	3.20	30.88	3.82	2.42E-01
H3N4F1	23.83	6.21	31.27	8.13	**1.00E-03**	26.84	4.59	33.12	6.39	**4.55E-04**
H3N3F1	0.35	0.22	0.50	0.27	**4.50E-02**	0.65	0.27	0.60	0.15	4.60E-01
H3N5F1	4.97	1.88	6.15	1.57	**1.50E-02**	5.74	1.50	6.64	1.65	5.20E-02
H4N4	0.79	0.77	1.05	1.53	5.03E-01	0.66	0.27	0.69	0.33	7.26E-01
H3N4	0.46	0.32	0.57	0.26	1.40E-01	2.66	2.78	1.67	1.36	1.79E-01
H5N5F1S1	0.11	0.04	0.09	0.04	**4.60E-02**	0.15	0.10	0.12	0.13	4.52E-01
H5N4F1S1	8.02	2.95	6.01	2.92	**1.80E-02**	6.48	1.77	5.18	1.62	**8.00E-03**
H4N5F1S1	0.16	0.07	0.13	0.05	**4.70E-02**	0.28	0.15	0.23	0.14	2.59E-01
H4N4F1S1	5.34	1.11	4.68	1.58	1.23E-01	4.59	1.46	4.51	1.06	8.09E-01
H4N3F1S1	0.27	0.08	0.26	0.09	8.48E-01	0.38	0.16	0.43	0.59	6.96E-01
H5N4	0.36	0.28	0.35	0.28	9.20E-01	0.50	0.35	0.44	0.33	5.20E-01
H5N4S1	0.11	0.04	0.10	0.07	7.00E-01	0.19	0.14	0.20	0.32	8.34E-01
IgG4										
H5N4F1	20.18	8.56	14.43	6.41	**5.00E-03**	15.44	5.37	12.47	6.21	8.60E-02
H4N4F1	21.52	14.19	17.48	12.76	2.83E-01	36.91	23.51	32.84	20.60	5.05E-01
H5N5F1	0.55	0.45	0.82	1.23	3.70E-01	1.23	0.50	1.19	0.86	8.43E-01
H3N4F1	34.82	12.69	42.60	17.15	9.40E-02	28.58	14.18	32.33	13.82	3.46E-01
H3N3F1	4.68	6.00	5.82	7.63	5.80E-01	0.63	0.34	1.51	2.39	**1.90E-02**
H4N5F1	4.20	6.46	3.54	2.63	5.69E-01	4.18	2.40	4.20	2.65	9.80E-01
H3N5F1	7.98	3.91	8.89	2.99	3.26E-01	6.61	3.84	8.57	5.27	1.67E-01
H5N4F1S1	2.02	1.62	2.11	1.39	8.26E-01	3.47	3.34	3.44	3.51	9.76E-01
H4N4F1S1	4.06	2.44	4.31	1.98	6.78E-01	2.94	1.44	3.45	3.83	5.98E-01

Table S6. Comparison of the result for the quantitation of IgG glycan features between normal and cancer groups by partial and complete digestion. *P* values result from Mann-Whitney U test. *P*-value less than 0.05 is set as significant, which are emphasized with bold. SD, standard deviation.

Glycan features	Incomplete Digestion					Complete Digestion				
	Normal		Cancer			Normal		Cancer		
	Mean	SD	Mean	SD	*P*	Mean	SD	Mean	SD	*P*
IgG1										
G0	25.83	6.69	33.36	9.41	**4.00E-03**	25.86	6.06	34.00	7.17	**1.03E-04**
G1	42.72	3.55	42.27	5.28	7.50E-01	42.06	2.47	41.75	3.13	7.13E-01
G2	31.45	7.59	24.37	7.08	**1.00E-03**	32.08	6.81	24.26	5.54	**1.71E-05**
S	12.65	4.64	9.98	3.40	**1.50E-02**	16.03	4.84	12.47	2.90	**1.00E-02**
B	11.55	3.73	11.76	2.91	8.17E-01	19.70	4.29	19.53	6.75	9.27E-01
F	90.10	5.65	89.72	4.17	7.69E-01	86.12	4.74	84.80	5.57	3.89E-01
A2G	52.81	6.93	45.51	7.90	**1.00E-03**	53.11	6.33	44.58	6.27	**1.13E-05**
A2S	6.39	2.32	5.05	1.71	**1.50E-02**	8.22	2.56	6.11	1.32	**4.00E-03**
A2GS	0.12	0.03	0.11	0.03	3.20E-01	0.15	0.04	0.14	0.02	1.23E-01
IgG2										
G0	29.61	8.02	38.50	9.24	**1.00E-03**	35.89	4.87	42.03	7.51	**3.00E-03**
G1	44.76	2.45	41.95	4.78	**2.40E-02**	43.35	2.51	41.59	3.88	8.70E-02
G2	25.52	7.59	19.55	6.69	**3.00E-03**	20.76	4.77	16.38	4.54	**1.00E-03**
S	14.00	3.86	11.27	4.06	**1.90E-02**	12.06	2.96	10.68	2.81	9.30E-02
B	11.74	2.72	12.63	1.93	1.53E-01	13.51	1.99	13.63	2.26	5.40E-01
F	98.29	0.92	97.92	1.58	3.73E-01	96.00	3.26	96.99	1.95	2.50E-01
A2G	48.00	7.71	40.53	7.71	**1.00E-03**	41.92	4.59	36.67	5.86	**1.00E-03**
A2S	7.00	1.93	5.63	2.03	**1.90E-02**	5.84	1.45	5.12	1.31	6.50E-02
A2GS	0.14	0.02	0.14	0.03	3.63E-01	0.14	0.03	0.14	0.03	8.32E-01
IgG4										
G0	47.48	12.59	57.31	13.87	**1.30E-02**	35.82	17.88	42.41	16.18	1.68E-01
G1	29.78	13.74	25.33	12.56	2.29E-01	44.04	20.36	40.49	17.62	4.99E-01
G2	22.74	8.38	17.36	6.96	**1.20E-02**	20.14	5.20	17.10	6.72	6.50E-02
S	6.07	3.08	6.42	2.29	6.34E-01	6.41	2.66	6.89	5.01	7.10E-01
A2FB	12.72	9.51	13.26	4.23	7.54E-01	12.03	6.11	13.96	7.59	6.50E-01
A2FG	37.63	8.20	30.02	9.00	**3.00E-03**	42.16	8.35	37.34	8.71	5.30E-02
A2FS	3.04	1.54	3.21	1.15	6.35E-01	3.21	1.33	3.45	2.50	7.10E-01
A2FGS	0.09	0.04	0.11	0.05	**4.80E-02**	0.08	0.03	0.10	0.07	2.68E-01

Table S7. Diagnostic performance of the candidate glyco-biomarkers for diagnosis of different stages of CRC. *P*-values less than 0.05 and AUC scores over 0.70 are emphasized with bold. Glycan structures are annotated as described in Table S3.

Glycan species	Normal vs Benign		Normal vs Cancer		Normal vs Early Stages		Normal vs Late Stages	
	P	AUC	*P*	AUC	*P*	AUC	*P*	AUC
IgG1_H3N4F1	**3.30E-03**	**0.78**	**1.00E-03**	**0.77**	**6.90E-03**	**0.76**	**2.10E-03**	**0.78**
IgG1_H5N5F1	**1.37E-02**	**0.74**	**4.00E-04**	**0.80**	**1.50E-03**	**0.80**	**1.80E-03**	**0.79**
IgG1_H3N4	1.00E-01	0.66	**1.10E-03**	**0.77**	**9.00E-03**	**0.75**	**1.90E-03**	**0.79**
IgG1_H5N4F1S1	5.27E-02	0.69	**1.80E-03**	**0.76**	**5.30E-03**	**0.77**	**6.60E-03**	**0.75**
IgG2_H5N4F1	**1.26E-02**	**0.74**	**3.20E-03**	**0.74**	**8.20E-03**	**0.75**	**1.04E-02**	**0.74**

Table S8. Diagnostic performance of the glycan features for diagnosis of different stages of CRC. *P*-values less than 0.05 and AUC scores over 0.70 are emphasized with bold.

Glycan features	Normal vs Benign		Normal vs Cancer		Normal vs Early Stages		Normal vs Late Stages	
	P	AUC	*P*	AUC	*P*	AUC	*P*	AUC
IgG1_G0	**2.80E-03**	**0.79**	**2.00E-04**	**0.81**	**5.00E-03**	**0.83**	**1.40E-03**	**0.80**
IgG1_G2	**5.30E-03**	**0.77**	**1.00E-04**	**0.82**	**8.00E-03**	**0.82**	**6.00E-04**	**0.82**
IgG1_S	6.87E-02	0.68	**1.14E-02**	**0.71**	8.33E-02	0.67	**7.70E-03**	**0.75**
IgG1_A2G	**1.70E-03**	**0.80**	**< 0.0001**	**0.83**	**2.00E-03**	**0.85**	**5.00E-04**	**0.82**
IgG1_A2S	**4.92E-02**	0.69	**4.50E-03**	**0.74**	**2.77E-02**	**0.71**	**5.70E-03**	**0.76**
IgG2_G0	**8.20E-03**	**0.75**	**2.20E-03**	**0.75**	**3.70E-03**	**0.78**	**1.20E-02**	**0.73**
IgG2_G1	**4.59E-02**	0.69	1.43E-01	0.62	1.13E-01	0.65	3.05E-01	0.60
IgG2_G2	**3.71E-02**	**0.70**	**3.00E-03**	**0.75**	**7.60E-03**	**0.76**	**1.04E-02**	**0.74**
IgG2_A2G	**3.45E-02**	**0.70**	**9.50E-03**	**0.71**	**1.37E-02**	**0.74**	**3.34E-02**	**0.70**

Table S9. Statistical analysis of the glycosylation changes between normal and different tumor location of CRC patients in the discovery set. Mean Diff, mean difference; 95% CI, 95% confidence interval. Glycan features were annotated as in Table S5.

Glycan features	Normal vs Colon			Normal vs Rectal			Colon vs Rectal		
	Mean Diff.	95% CI	*P*	Mean Diff.	95% CI	*P*	Mean Diff.	95% CI	*P*
IgG1									
G0	-6.68	(-11.23, 2.12)	**2.00E-03**	-2.90	(-7.54, 1.73)	3.83E-01	3.77	(-1.23, 8.78)	2.04E-01
G1	0.91	(-2.56, 4.37)	1.00E+00	-0.12	(-3.65, 3.40)	1.00E+00	-1.03	(-4.84, 2.78)	1.00E+00
G2	5.77	(0.88, 10.66)	**1.60E-02**	3.03	(-1.95, 8.01)	4.20E-01	-2.74	(-8.12, 2.63)	6.38E-01
S	7.26	(2.53, 11.99)	**1.00E-03**	4.78	(-0.04, 9.60)	5.20E-02	-2.48	(-7.68, 2.72)	7.30E-01
B	1.73	(-3.77, 7.23)	1.00E+00	0.96	(-4.64, 6.56)	1.00E+00	-0.78	(-6.82, 5.27)	1.00E+00
F	2.52	(-0.32, 5.36)	9.90E-02	3.86	(0.97, 6.75)	**5.00E-03**	1.34	(-1.78, 4.47)	8.76E-01
A2G	4.65	(-0.54, 9.83)	9.30E-02	1.77	(-3.51, 7.05)	1.00E+00	-2.88	(-8.58, 2.82)	6.50E-01
A2S	1.98	(0.46, 3.48)	**6.00E-03**	0.90	(-0.64, 2.43)	4.68E-01	-1.08	(-2.73, 0.58)	3.45E-01
A2GS	0.03	(0.003, 0.06)	**2.40E-02**	0.02	(-0.01, 0.04)	3.82E-01	-0.01	(-0.04, 0.02)	8.58E-01
IgG2									
G0	-5.57	(-9.52, -1.63)	**3.00E-03**	-2.01	(-6.23, 2.01)	6.66E-01	3.56	(-0.77, 7.90)	1.42E-01
G1	1.93	(-0.41, 4.27)	1.40E-01	1.50	(-0.88, 3.88)	3.78E-01	-0.43	(-2.99, 2.14)	1.00E+00
G2	3.65	(0.42, 6.88)	**2.20E-02**	0.51	(-2.78, 3.80)	1.00E+00	-3.14	(-6.69, 0.41)	1.00E-01
S	1.00	(-1.64, 3.64)	1.00E+00	-1.72	(-4.40, 0.97)	3.58E-01	-2.72	(-5.62, 0.18)	7.20E-02
B	0.28	(-1.73, 2.28)	1.00E+00	-0.46	(-2.50, 1.58)	1.00E+00	-0.74	(-2.94, 1.47)	1.00E+00
F	1.88	(0.39, 3.38)	**9.00E-03**	2.27	(0.74, 3.79)	**2.00E-03**	0.38	(-1.26, 2.02)	1.00E+00
A2G	4.45	(1.06, 7.85)	**6.00E-03**	1.12	(-2.34, 4.58)	1.00E+00	-3.33	(-7.06, 0.40)	9.50E-02
A2S	0.13	(-1.20, 1.46)	1.00E+00	-1.38	(-2.73, -0.03)	**4.40E-02**	-1.51	(-2.97, -0.05)	**4.00E-02**
A2GS	-0.02	(-0.04, 0.01)	2.42E-01	-0.04	(-0.06, -0.01)	**1.00E-03**	-0.02	(-0.05, 0.01)	1.68E-01
IgG4									
G0	7.44	(-1.21, 16.10)	1.14E-01	8.78	(-0.02, 17.59)	5.10E-02	1.34	(-8.16, 10.84)	1.00E+00
G1	-1.25	(-10.71, 8.21)	1.00E+00	-4.57	(-14.20, 5.07)	7.40E-01	-3.32	(-13.71, 7.08)	1.00E+00
G2	-6.19	(-13.65, 1.26)	1.35E-01	-4.22	(-11.81, 3.37)	5.26E-01	1.98	(-6.21, 10.17)	1.00E+00
S	-10.65	(-18.32, -2.98)	**3.00E-03**	-8.90	(-16.70, -1.09)	**2.00E-02**	1.76	(-6.67, 10.18)	1.00E+00
A2FB	2.96	(-2.64, 8.55)	5.91E-01	0.04	(-5.66, 5.74)	1.00E+00	-2.92	(-8.55, 2.64)	5.91E-01
A2FG	-6.82	(-13.36, -0.28)	**3.80E-02**	-6.50	(-13.16, 0.16)	5.80E-02	0.32	(-6.87, 7.51)	1.00E+00
A2FS	-5.33	(-9.16, -1.49)	**3.00E-03**	-4.45	(-8.35, -0.54)	**2.00E-02**	0.88	(-3.33, 5.09)	1.00E+00
A2FGS	-0.09	(-0.15, -0.02)	**3.00E-03**	-0.07	(-0.13, -0.004)	**3.00E-02**	0.02	(-0.05, 0.09)	1.00E+00

Supporting File

AreaProportion_simple.txt file. The MATLAB code for the integration and quantitation of subclass-specific IgG glycosylation.