

Supporting Information associated with manuscript:

Protein-specific differential Glycosylation of Immunoglobulins in serum of Ovarian Cancer Patients

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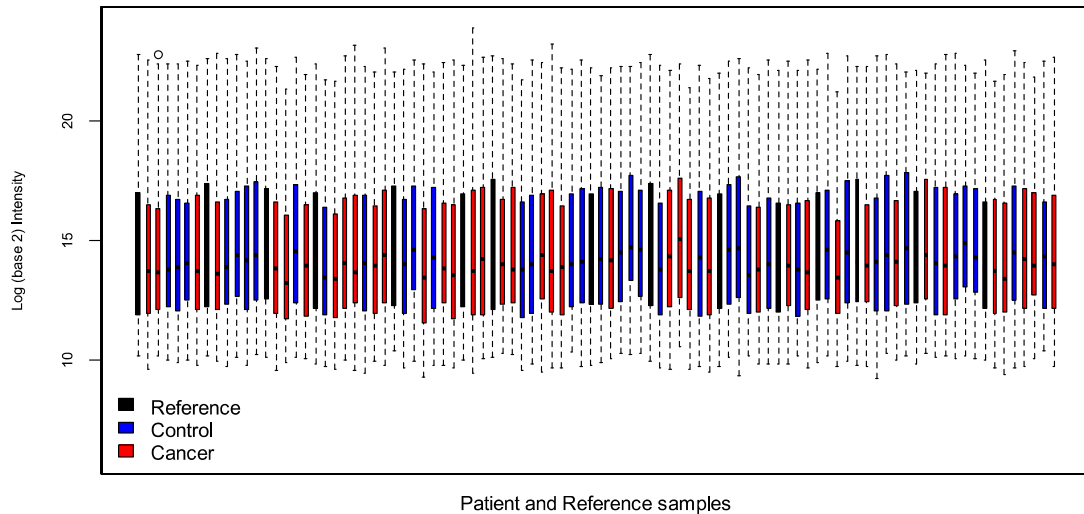
SUPPLEMENTARY INFORMATION

Supplementary Table ST1. Characteristics of the transitions monitored in this study.

No.	Peptide	Glycopeptide	Protein	Peptide	Site	Glycan Moiety	Precursor Ion	Product Ion	Ret Time (min)	Collision Energy (eV)
1	x		IgA1	TPLTATLSK	-	-	466.3	415.8	9.0	15
2	x		IgA1/2	YLTWASR	-	-	448.7	620.3	10.8	12
3	x		IgA2	DASGATFTWTPSSGK	-	-	756.9	475.3	11.9	25
4	x		IgG1	FNWYVDGVEVHNAK	-	-	839.4	968.5	5.4	13.0
5	x		IgG1/2/3/4	DTLMISR	-	-	418.2	506.3	4.5	9.0
6	x		IgG2	CCVECPAPPPVAGPSVFLFPPKPK	-	-	970.1	1100.6	6.1	28.0
7	x		IgG3	WYVDGVEVHNAK	-	-	472.9	534.3	4.7	6.0
8	x		IgG4	TTPPVLDSDGSFFLYSR	-	-	634.7	425.2	6.2	9.0
9	x		IgM	FTCTVTHTDLPSPLK	-	-	573.0	734.9	12.1	15
10		x	IgA1/2	LSLHRPALEDLLLSEANLTCTLTGLR	144/131	H3N5F0S0	1117.1	366.1	16.0	25.0
11		x	IgA1/2		144/131	H4N4F0S1	943.9	366.1	16.0	20.0
12		x	IgA1/2		144/131	H4N5F0S0	1157.6	366.1	16.0	30.0
13		x	IgA1/2		144/131	H4N5F0S1	1230.4	366.1	16.0	25.0
14		x	IgA1/2		144/131	H5N4F0S0	1147.3	366.1	16.0	25.0
15		x	IgA1/2		144/131	H5N4F0S1	976.3	366.1	16.0	25.0
16		x	IgA1/2		144/131	H5N4F0S2	1292.9	366.1	16.2	30.0
17		x	IgA1/2		144/131	H5N5F0S0	1198.1	366.1	16.0	25.0
18		x	IgA1/2		144/131	H5N5F0S1	1016.9	366.1	16.0	25.0
19		x	IgA1/2		144/131	H5N5F0S2	1075.1	366.1	16.4	25.0
20		x	IgA2	TPLTANITK	205	H4N5F1S0	923.5	366.1	6.5	18.0
21		x	IgA2		205	H5N4F1S0	909.8	366.1	6.2	19.0
22		x	IgA2		205	H5N4F1S1	1006.8	366.1	6.9	25.0
23		x	IgA2		205	H5N4F1S2	1103.8	366.1	7.5	25.0

24		x	IgA2		205	H5N5F1S0	977.5	366.1	6.4	25.0
25		x	IgA2		205	H5N5F1S1	1074.5	366.1	6.9	25.0
26		x	IgA2		205	H5N5F1S2	878.9	366.1	7.7	17.0
27		x	IgG1	EEQYNSTYR	180	H3N4F1S0	878.8	204.1	2.3	13.0
28		x	IgG1		180	H3N5F1S0	946.5	204.1	2.2	15.0
29		x	IgG1		180	H4N4F0S0	884.1	204.1	2.2	14.0
30		x	IgG1		180	H4N4F1S0	932.8	204.1	2.2	15.0
31		x	IgG1		180	H4N4F1S1	1029.8	204.1	2.6	16.0
32		x	IgG1		180	H4N5F0S0	951.7	204.1	2.3	15.0
33		x	IgG1		180	H4N5F1S0	1000.5	204.1	2.3	16.0
34		x	IgG1		180	H5N4F0S0	938.1	366.1	2.3	14.0
35		x	IgG1		180	H5N4F1S0	986.8	366.1	2.3	15.0
36		x	IgG1		180	H5N4F1S1	1083.8	366.1	2.6	17.0
37		x	IgG1		180	H5N5F1S0	1054.5	366.1	2.3	17.0
38		x	IgG2	EEQFNSTFR	176	H3N4F1S0	868.1	204.1	4.2	13.0
39		x	IgG2		176	H3N5F1S0	935.8	204.1	4.1	14.0
40		x	IgG2		176	H4N4F0S0	873.4	204.1	4.2	13.0
41		x	IgG2		176	H4N4F1S0	922.1	204.1	4.1	25.0
42		x	IgG2		176	H4N4F1S1	1019.1	204.1	4.3	25.0
43		x	IgG2		176	H4N5F0S0	941.1	204.1	4.2	15.0
44		x	IgG2		176	H4N5F1S0	989.9	204.1	4.1	15.0
45		x	IgG2		176	H5N4F1S0	976.1	366.1	4.1	15.0
46		x	IgG2		176	H5N4F1S1	1073.1	366.1	4.2	25.0
47		x	IgG2		176	H5N5F1S0	1043.8	366.1	4.2	17.0
48		x	IgG3/4	EEQYNSTFR/EEQFNSTRY	227/177	H4N4F1S1	1024.5	204.1	3.8	17.0
49		x	IgG3/4		227/177	H4N5F1S0	995.1	204.1	3.7	15.0
50		x	IgG3/4		227/177	H5N4F1S1	1078.4	366.1	3.8	17.0

51		x	IgG3/4		227/177	H3N5F1S0	941.1	204.1	3.7	15.0
52		x	IgG3/4		227/177	H4N4F1S0	927.4	204.1	3.7	14.0
53		x	IgM	YKNNSDISSTR	46	H4N3F1S1	993.8	366.1	2.2	25.0
54		x	IgM		46	H4N4F0S1	1012.8	366.1	2.2	25.0
55		x	IgM		46	H5N4F1S1	1115.5	366.1	2.2	25.0
56		x	IgM		46	H5N4F1S2	909.6	366.1	2.3	14.0
57		x	IgM		46	H5N5F0S1	851.1	366.1	2.2	14.0
58		x	IgM		46	H5N5F0S2	923.9	366.1	2.3	19.0
59		x	IgM		46	H5N5F1S1	1183.2	366.1	2.3	25.0
60		x	IgM		46	H5N6F0S1	901.9	366.1	2.3	14.0
61		x	IgM	GLTFQQNASSMCVPDQDTAIR	209	H4N5F1S1	1110.8	366.1	12.4	24.0
62		x	IgM		209	H5N4F1S1	1100.5	366.1	12.4	19.0
63		x	IgM		209	H5N4F1S2	1173.3	366.1	12.5	23.0
64		x	IgM		209	H5N5F1S1	1151.3	366.1	12.4	25.0
65		x	IgM		209	H5N5F1S2	1224.1	366.1	12.5	25.0
66		x	IgM	STGKPTLYNVSLVMSDTAGTCY	439	H5N2F0S0	1194.5	1284.7	13.6	24.0
67		x	IgM		439	H6N2F0S0	1248.5	1284.7	13.6	24.0
68		x	IgM		439	H7N2F0S0	1302.6	1284.7	13.6	27.0
69		x	IgM		439	H8N2F0S0	1356.6	1284.7	13.5	27.0
70		x	IgM		439	H9N2F0S0	1058.3	1284.7	13.5	27.0
71		x	IgM		439	-	1183.1	342.1	14.3	38.0



Supplementary Figure SF1. Stability of the method used. The distribution of log₂ transformed intensities is plotted for each sample, including the reference samples. These results indicate good stability and repeatability of the method used.

Protein	Glycopeptide	AUC	Accuracy	Sensitivity	Specificity
IgG 1	N¹⁸⁰ - H5N5F1	0.941	0.886	0.846	0.925
IgG 1	N¹⁸⁰ - H5N4F1	0.924	0.848	0.897	0.800
IgG 1	N¹⁸⁰ - H5N4F1S1	0.908	0.824	0.872	0.775
IgG 1	N¹⁸⁰ - H3N4F1	0.842	0.810	0.718	0.900
IgG 1	N¹⁸⁰ - H4N5F1	0.904	0.797	0.821	0.775
IgG 1	N¹⁸⁰ - H5N4	0.852	0.785	0.821	0.750
IgG 1	N¹⁸⁰ - H4N5	0.851	0.785	0.821	0.750
IgG 1	N¹⁸⁰ - H4N4F1	0.865	0.759	0.769	0.750
IgG 2	N¹⁷⁶ - H4N5F1	0.733	0.696	0.641	0.750
IgG 1	N ¹⁸⁰ - H4N4F1S1	0.717	0.696	0.718	0.675
IgG 2	N ¹⁷⁶ - H3N4F1	0.714	0.696	0.615	0.775
IgG 2	N ¹⁷⁶ - H4N4F1S1	0.666	0.671	0.462	0.875
IgG 1	N ¹⁸⁰ - H4N4	0.843	0.671	0.718	0.625
IgG 2	N ¹⁷⁶ - H5N4F1	0.729	0.658	0.615	0.700
IgG	Total protein	0.630	0.633	0.359	0.900
IgG 2	N ¹⁷⁶ - H4N4F1	0.688	0.607	0.564	0.650
IgG 1	Total subclass 1	0.669	0.595	0.590	0.600
IgG 3/4	N ²²⁷ /N ¹⁷⁷ - H3N4F1	0.671	0.595	0.615	0.575
IgG 3/4	N ²²⁷ /N ¹⁷⁷ - H4N5F1	0.648	0.519	0.538	0.500

Supplementary Table ST2. Classification metrics for each peptide/glycopeptide from IgG of which levels were significantly different for EOC in the discovery set. Accuracy was estimated using LOOCV. AUC, Fold change, Threshold, sensitivity and specificity were calculated using the whole discovery set. Bold font indicates that these glycopeptides were included in the best performing multiplexed classifier.

Protein	Glycopeptide	AUC	Accuracy	Sensitivity	Specificity
IgA 1/2	N¹⁴⁴/N¹³³ – H4N5	0.810	0.788	0.717	0.926
IgA 1/2	N¹⁴⁴/N¹³³ – H4N4S1	0.804	0.763	0.839	0.714
IgA 1/2	N¹⁴⁴/N¹³³ – H5N4S1	0.783	0.750	0.685	0.885
IgA 1/2	N¹⁴⁴/N¹³³ – H3N5	0.791	0.738	0.711	0.771
IgA 1/2	N¹⁴⁴/N¹³³ – H4N5S1	0.867	0.738	0.757	0.721
IgA 2	N²⁰⁵ – H5N5F1	0.868	0.738	0.732	0.744
IgA 2	N²⁰⁵ – H5N5F1S1	0.695	0.738	0.673	0.880
IgA 2	N ²⁰⁵ – H5N5F1S2	0.821	0.725	0.737	0.714
IgA 2	Total isoform 2	0.762	0.700	0.629	0.944
IgA 1/2	N ¹⁴⁴ /N ¹³³ - H5N4	0.746	0.700	0.711	0.691
IgA 2	N ²⁰⁵ – H4N5F1	0.744	0.688	0.667	0.714
IgA 1	Total isoform 1	0.723	0.663	0.676	0.651
IgA 1/2	N ¹⁴⁴ /N ¹³³ – H5N5S1	0.702	0.663	0.633	0.710
IgA 2	N ²⁰⁵ – H5N4F1S1	0.645	0.663	0.659	0.667
IgA 1/2	N ¹⁴⁴ /N ¹³³ – H5N4S2	0.721	0.638	0.597	0.739
IgA	Total IgA	0.657	0.575	0.594	0.563

Supplementary Table ST3. Classification metrics for each peptide/glycopeptide from IgA of which levels were significantly different for EOC in the discovery set. Accuracy was estimated using LOOCV. AUC, Fold change, Threshold, sensitivity and specificity were calculated using the whole discovery set. Bold font indicates that these glycopeptides were included in the best performing multiplexed classifier.

Protein	Glycopeptide	AUC	Accuracy	Sensitivity	Specificity
IgM	N²⁰⁹ - H4N5F1F1	0.797	0.750	0.727	0.778
IgM	N⁴⁶ - H5N4F1S1	0.739	0.713	0.698	0.730
IgM	N⁴⁶ - H4N3F1S1	0.719	0.700	0.682	0.722
IgM	N²⁰⁹ - H5N4F1S1	0.736	0.700	0.654	0.786
IgM	N²⁰⁹ - H5N5F1S2	0.707	0.688	0.660	0.727
IgM	Total protein	0.685	0.663	0.628	0.724
IgM	N ⁴³⁹ - H8N2	0.660	0.663	0.651	0.676
IgM	N ⁴³⁹ - H7N2	0.683	0.613	0.596	0.636
IgM	N ²⁰⁹ - H5N4F1S2	0.743	0.575	0.583	0.568
IgM	N ⁴⁶ - H5N5S2	0.658	0.500	0.500	0.500

Supplementary Table ST4. Classification metrics for each peptide/glycopeptide from IgM of which levels were significantly different for EOC in the discovery set. Accuracy was estimated using LOOCV. AUC, Fold change, Threshold, sensitivity and specificity were calculated using the whole discovery set. Bold font indicates that these glycopeptides were included in the best performing multiplexed classifier.

Entry Order	Protein	Glycopeptide	Accuracy
1	IgG 1	N ¹⁸⁰ - H5N5F1	88.6
2	IgG 1	N ¹⁸⁰ - H5N4F1	86.7
3	IgA 2	N ²⁰⁵ - H5N5F1S2	91.1
4	IgM	N ⁴⁶ - H5N4F1S1	89.2
5	IgA 1	Total isoform 1	91.1
6	IgG 1	N ¹⁸⁰ - H3N4F1	90.5
7	IgM	N ²⁰⁹ - H5N5F1S2	92.4
8	IgA 1/2	N ¹⁴⁴ /N ¹³³ - H5N4	91.1
9	IgM	N ⁴⁶ - H5N5S2	92.4
10	IgG 2	N ¹⁷⁶ - H3N4F1	92.4
11	IgG 1	N ¹⁸⁰ - H4N5F1	96.2
12	IgA 1/2	N ¹⁴⁴ /N ¹³³ - H4N4S1	94.3
13	IgG 1	N ¹⁸⁰ - H4N4F1S1	96.2
14	IgG	Total protein	94.3
15	IgG 1	N ¹⁸⁰ - H4N4	96.2
16	IgM	N ⁴³⁹ - H8N2	94.9
17	IgA 2	N ²⁰⁵ - H4N5F1	96.2
18	IgA 2	N ²⁰⁵ - H5N4F1S1	94.3
19	IgA	Total IgA	94.9
20	IgG 1	N ¹⁸⁰ - H5N4	94.3
21	IgG 2	N ¹⁷⁶ - H4N4F1S1	94.9
22	IgG 2	N ¹⁷⁶ - H4N5F1	93.7
23	IgA 1/2	N ¹⁴⁴ /N ¹³³ - H4N5	94.9
24	IgG 1	N ¹⁸⁰ - H5N4F1S1	94.3
25	IgM	Total protein	94.9
26	IgG 1	N ¹⁸⁰ - H4N5	93.7
27	IgA 1/2	N ¹⁴⁴ /N ¹³³ - H3N5	94.9
28	IgA 1/2	N ¹⁴⁴ /N ¹³³ - H4N5S1	94.3
29	IgG 3/4	N ²²⁷ /N ¹⁷⁷ - H4N5F1	94.9
30	IgG 3/4	N ²²⁷ /N ¹⁷⁷ - H3N4F1	94.3
31	IgG 2	N ¹⁷⁶ - H4N4F1	94.9
32	IgG 1	N ¹⁸⁰ - H4N4F1	93.7
33	IgG 1	Total subclass 1	93.7
34	IgM	N ⁴⁶ - H4N3F1S1	92.4
35	IgA 1/2	N ¹⁴⁴ /N ¹³³ - H5N5S1	92.4
36	IgG 2	N ¹⁷⁶ - H5N4F1	92.4
37	IgM	N ²⁰⁹ - H5N4F1S1	92.4
38	IgA 1/2	N ¹⁴⁴ /N ¹³³ - H5N4S2	92.4
39	IgA 2	Total isoform 2	92.4
40	IgA 2	N ²⁰⁵ - H5N5F1	90.5
41	IgA 1/2	N ¹⁴⁴ /N ¹³³ - H5N4S1	89.9
42	IgM	N ²⁰⁹ - H5N4F1S2	88.0
43	IgA 2	N ²⁰⁵ - H5N5F1S1	86.1
44	IgM	N ²⁰⁹ - H4N5F1F1	85.4
45	IgM	N ⁴³⁹ - H7N2	83.5

Supplementary Table ST5. Entry order and classification metrics for each peptide/glycopeptide from all three proteins of which levels were significantly different for EOC in the discovery set. Accuracy represents the accuracy of a multiplexed classifier including the glycopeptide indicated and all of the

glycopeptides above. Accuracies were estimated using LOOCV using set OC1. **Bold font indicates that these glycopeptides were included in the best performing multiplexed classifier.**