Supplementary information associated with

HILIC-MRM-MS for linkage-specific separation of sialylated glycopeptides to quantify Prostate-Specific Antigen Proteoforms

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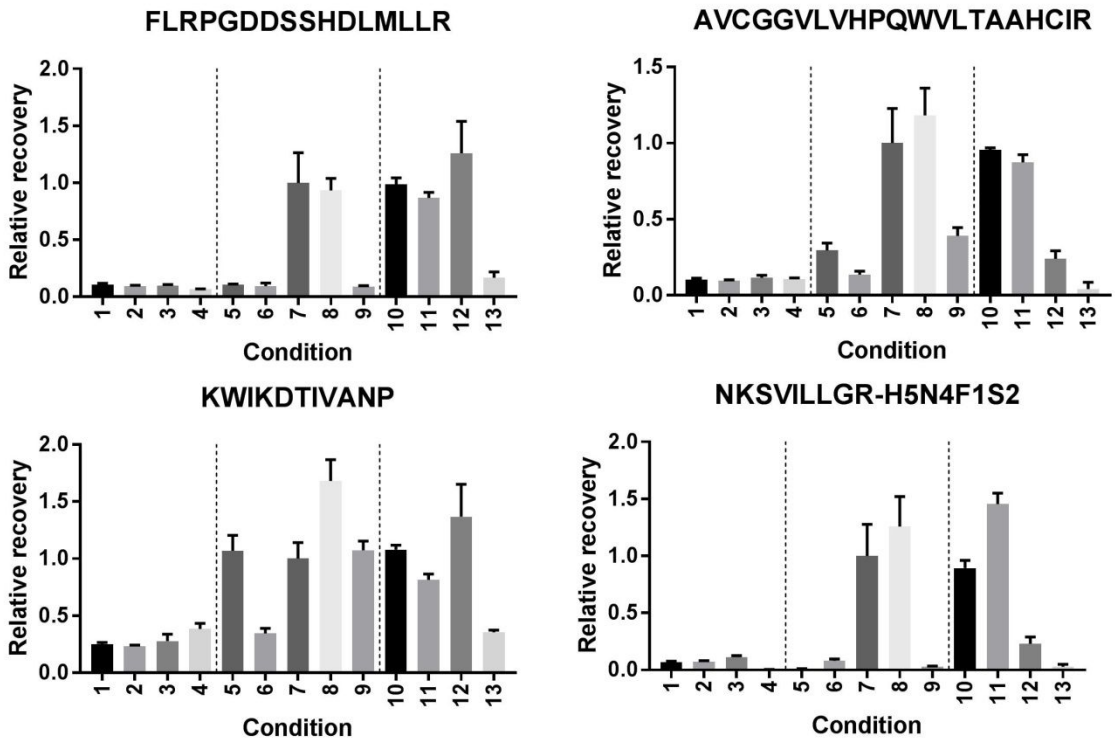
Supplementary Table S1. Transitions developed for tryptic peptides and glycopeptides from PSA.

		RT	Precursor	Quantifier		Qualifier 1		Qualifier 2	
AA	peptide	min	m/z	m/z	CE	m/z	CE	m/z	CE
25-33	IVGGWECEK	3.4	539.2 (2+)	865.4 (y7, 1+)	15	964.4 (y8, 1+)	18	213.2 (b2, 1+)	15
110-125	FLRPGDDSSHDLMLLR	3.2	468.7 (4+)	532.3 (y4, 1+)	15	760.4 (y6, 1+)	18	645.4 (y5, 1+)	18
126-137	LSEPAELTDAVK	3.6	636.8 (2+)	943.5 (y9, 1+)	18	1072.6 (y10, 1+)	20	201.2 (b2, 1+)	22
-	SIL-FLRPGDDSSHDLMLLR	3.2	471.2 (4+)	542.3 (y4, 1+)	15	770.4 (y6, 1+)	18	655.4 (y5, 1+)	18
-	SIL-LSEPAELTDAVK	3.6	640.8 (2+)	951.5(y9, 1+)	18	1080.6 (y10, 1+)	20	201.2 (b2, 1+)	22
69-70	NK-H5N4F1S2	6.2, 6.3, 6.4	871.3 (3+)	204.0 (1+)	30	274.0 (1+)	30	-	-
69-70	NK-H5N4F0S2		822.6 (3+)	204.0 (1+)	30	274.0 (1+)	30	-	-
69-70	NK-H4N5F1S2		885.0 (3+)	204.0 (1+)	30	274.0 (1+)	30	-	-
69-70	NK-H4N5F0S2		836.3 (3+)	204.0 (1+)	30	274.0 (1+)	30	-	-
69-70	NK-H3N6F1S2		898.7 (3+)	204.0 (1+)	30	274.0 (1+)	30	-	-
69-70	NK-H5N4F1S1		774.3 (2+)	204.0 (1+)	30	274.0 (1+)	30	-	-
69-70	NK-H4N3F1S1		978.4 (2+)	204.0 (1+)	30	274.0 (1+)	30	-	-
69-70	NK-H4N3F0S1		905.4 (2+)	204.0 (1+)	30	274.0 (1+)	30	-	-
69-70	NK-H3N4F1S1		998.9 (2+)	204.0 (1+)	30	274.0 (1+)	30	-	-
69-70	NK-H5N4F1S0		1015.4 (2+)	204.0 (1+)	30	366.0 (1+)	30	-	-
69-70	NK-H4N3F1S0		832.8 (2+)	204.0 (1+)	30	366.0 (1+)	30	-	-
69-70	NK-H4N4F1S0		934.4 (2+)	204.0 (1+)	30	366.0 (1+)	30	-	-
69-70	NK-H4N5F1S0		1035.9 (2+)	204.0 (1+)	30	366.0 (1+)	30	-	-

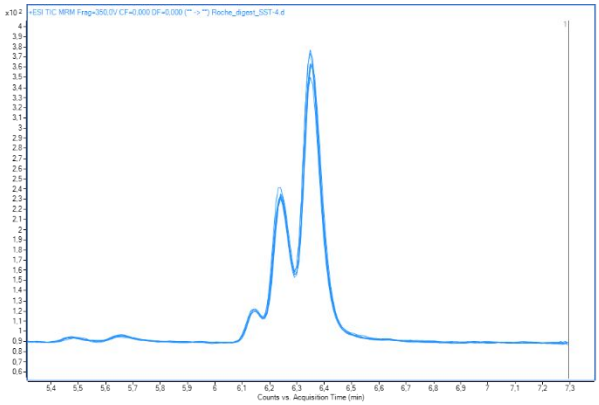
Supplementary Table S2. Transitions developed for ArgC peptides and glycopeptides from PSA

AA	peptide	Precursor	Quantifier		Qualifier 1		Qualifier 2	
		m/z	m/z	CE	m/z	CE	m/z	CE
48-68	AVCGGVLVHPQWVLTAAHCIR	586.8 (4+)	544.3 (b6, 1+)	12	941.5 (y8, 1+)	25	726.4 (y6, 1+)	15
110-125	FLRPGDDSSHDLMLLR	468.7 (4+)	532.3 (y4, 1+)	15	760.4 (y6, 1+)	18	645.4 (y5, 1+)	18
251-261	KWIKDTIVANP	428.9 (3+)	116.1 (y1, 1+)	10	1055.6 (b9, 1+)	12	885.5 (b7, 1+)	15
-	SIL-FLRPGDDSSHDLMLLR	471.2 (4+)	542.3 (y4, 1+)	15	770.4 (y6, 1+)	18	655.4 (y5, 1+)	18
-	SIL-KWIKDTIVANP	430.9 (3+)	116.1 (y1, 1+)	10	1061.6 (b9, 1+)	12	885.5 (b7, 1+)	15
69-77	NKSVILLGR-H5N4F1S2	1117.5	204.0 (1+)	30	274.0 (1+)	30	-	-
69-77	NKSVILLGR-H5N4F0S2	1068.8	204.0 (1+)	30	274.0 (1+)	30	-	-
69-77	NKSVILLGR-H4N5F1S2	1131.2	204.0 (1+)	30	274.0 (1+)	30	-	-
69-77	NKSVILLGR-H4N5F0S2	1082.5	204.0 (1+)	30	274.0 (1+)	30	-	-
69-77	NKSVILLGR-H3N6F1S2	1144.8	204.0 (1+)	30	274.0 (1+)	30	-	-
69-77	NKSVILLGR-H5N4F1S1	1020.5	204.0 (1+)	30	274.0 (1+)	30	-	-
69-77	NKSVILLGR-H4N3F1S1	898.7	204.0 (1+)	30	274.0 (1+)	30	-	-
69-77	NKSVILLGR-H4N3F0S1	850.1	204.0 (1+)	30	274.0 (1+)	30	-	-
69-77	NKSVILLGR-H3N4F1S1	912.4	204.0 (1+)	30	274.0 (1+)	30	-	-
69-77	NKSVILLGR-H5N4F1S0	923.4	204.0 (1+)	30	366.0 (1+)	30	-	-
69-77	NKSVILLGR-H4N3F1S0	801.7	204.0 (1+)	30	366.0 (1+)	30	-	-
69-77	NKSVILLGR-H4N4F1S0	869.4	204.0 (1+)	30	366.0 (1+)	30	-	-
69-77	NKSVILLGR-H4N5F1S0	937.1	204.0 (1+)	30	366.0 (1+)	30	-	-

Condition	Buffer	CaCl2	EDTA	ArgC
1	50 mM TRIS	50 mM	50 mM	1:35
2	50 mM TRIS	50 mM	50 mM	1:20
3	50 mM TRIS	50 mM	50 mM	1:10
4	50 mM TRIS	50 mM	50 mM	1:5
5	50 mM TRIS	50 mM	0 mM	1:35
6	50 mM TRIS	0 mM	50 mM	1:35
7	50 mM TRIS	0 mM	0 mM	1:35
8	50 mM TRIS	5 mM	0 mM	1:35
9	50 mM TRIS	20 mM	0 mM	1:35
10	10 mM TRIS	0 mM	0 mM	1:35
11	100 mM TRIS	0 mM	0 mM	1:35
12	50 mM ABC	5 mM	0 mM	1:35
13	50 mM ABC	50 mM	0 mM	1:35



Supplementary Figure S1. Optimization of digestion conditions.



Supplementary Figure S2. Overlay of seven analyses of a PSA standard digest (for experimental details, see the main publication, experimental section). These digests were measured over a 48 h. time interval. The injection volume was reduced to 1 µL to allow for a larger number of injections from the same vial. These results indicate the repeatability of the LC-MRM-MS measurement both in terms of quantitation as well as retention time.