

Supplementary data 1

For biological replicate, same procedure was used to prepare samples as described in the manuscript except that, channels 114 and 115 were used to label peptides from PC3 cell lines and channel 116 was used to label peptides from LNCap cell lines.

In the biological replicate, the proteins identified, glycosites identified, glycoproteins identified using SPEG and final glycopeptides identified in global analysis are in table 8-11, respectively.

Protein changes involved in glycan biosynthesis identified in LNCap and PC3 cells via proteomics are summarized in the following table for original experiment (Run1) and the biologically repeated experiment (Run2):

Accession	Description	Run1	Run2
		PC3/LNCap	PC3/LNCap
20302153	hyaluronan synthase 3 isoform a	6.27	3.00
34447231	bifunctional 3'-phosphoadenosine 5'-phosphosulfate synthase 2 isoform a	3.46	3.09
21361621	phosphoglucomutase-1 isoform 1	3.45	2.18
30410726	alpha-(1,6)-fucosyltransferase	3.41	3.07
4502343	UDP-GalNAc:beta-1,3-N-acetylgalactosaminyltransferase 1	2.76	2.67
190014632	bifunctional UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase isoform 1	2.71	3.17
11321585	guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	2.29	1.94
194097330	hexokinase-3	2.27	1.77
42516563	UDP-glucuronic acid decarboxylase 1 isoform 2	2.13	1.90
240255483	extracellular sulfatase Sulf-2 isoform a	2.11	5.90
13027378	glucosamine-6-phosphate isomerase 1	2.02	0.34
189011548	acid ceramidase isoform a	0.49	0.27
109148550	N-acylethanolamine-hydrolyzing acid amidase isoform 1	0.46	0.17

4505235	mannose-6-phosphate isomerase	0.45	0.24
189011550	acid ceramidase isoform c	0.45	0.27
4504373	beta-hexosaminidase subunit beta	0.43	0.23
119360348	tissue alpha-L-fucosidase	0.42	0.36
285002251	N(4)-(beta-N-acetylglucosaminy)-L-asparaginase isoform 1 preproprotein	0.42	0.62
38026892	dolichyl pyrophosphate Man9GlcNAc2 alpha-1,3-glucosyltransferase	0.38	0.44
4507303	sulfotransferase 1A2	0.37	0.17
4758092	di-N-acetylchitobiase	0.36	0.16
189181666	beta-hexosaminidase subunit alpha	0.34	0.10
66346698	alpha-N-acetylglucosaminidase	0.32	0.27
29550921	sulfotransferase 1A3/1A4	0.24	0.22

The data generated in the biological replicate verified our initial analysis, ratio of alpha-(1,6)-fucosyltransferase (FUT8) expression between PC3/LNCap was 3.41 in the original excrement and 3.07 in the biological replicate in Run2.

The intact glycopeptides, when identified and quantified with sufficient MS/MS spectra, showed consistent trend in the table below. The glycosite from adipocyte plasma membrane protein showed increased fucosylation and decreased oligomannose structures in PC3 cells comparing to LNCap cells.

This observation is consistent between replicate analyses.

Peptide Sequence	HexNAc	Hexose	Fucose	PC3/LNCap
AGPNGTLFVADAYK	3	3	1	2.68
AGPNGTLFVADAYK	2	3	1	1.73
AGPNGTLFVADAYK	2	3	1	2.19
AGPNGTLFVADAYK	2	7	0	0.76
AGPNGTLFVADAYK	2	6	0	0.19
AGPNGTLFVADAYK	2	5	0	0.31
AGPNGTLFVADAYK	2	5	0	0.43
AGPNGTLFVADAYK	2	6	0	0.28
AGPNGTLFVADAYK	2	5	0	0.20

NLEKNSTKQEILAALEK	2	5	1	0.86
NLEKNSTKQEILAALEK	2	4	0	0.09
NLEKNSTKQEILAALEK	2	3	0	0.12
HNNDTQHIWESDSNEFSVIADPR	5	3	1	1.27
HNNDTQHIWESDSNEFSVIADPR	3	3	1	2.02
HNNDTQHIWESDSNEFSVIADPR	2	3	1	1.46
HNNDTQHIWESDSNEFSVIADPR	4	3	1	1.84
HNNDTQHIWESDSNEFSVIADPR	2	3	1	2.12
HNNDTQHIWESDSNEFSVIADPR	2	5	0	1.69
HNNDTQHIWESDSNEFSVIADPR	2	5	0	1.44
HNNDTQHIWESDSNEFSVIADPR	2	5	0	0.81
HNNDTQHIWESDSNEFSVIADPR	2	6	0	0.48
HNNDTQHIWESDSNEFSVIADPR	2	6	0	0.48
HNNDTQHIWESDSNEFSVIADPR	2	6	0	0.32
HNNDTQHIWESDSNEFSVIADPR	2	6	0	0.42
HNNDTQHIWESDSNEFSVIADPR	2	6	0	0.64
HNNDTQHIWESDSNEFSVIADPR	2	6	0	0.51
HNNDTQHIWESDSNEFSVIADPR	2	6	0	0.49
HNNDTQHIWESDSNEFSVIADPR	2	6	0	0.39
HNNDTQHIWESDSNEFSVIADPR	2	6	0	0.42
HNNDTQHIWESDSNEFSVIADPR	2	7	0	0.73
HNNDTQHIWESDSNEFSVIADPR	2	7	0	0.82
HNNDTQHIWESDSNEFSVIADPR	2	7	0	0.73
HNNDTQHIWESDSNEFSVIADPR	2	8	0	0.56
HNNDTQHIWESDSNEFSVIADPR	2	8	0	0.56
HNNDTQHIWESDSNEFSVIADPR	2	8	0	0.48
HNNDTQHIWESDSNEFSVIADPR	2	8	0	0.66
HNNDTQHIWESDSNEFSVIADPR	2	8	0	0.62
HNNDTQHIWESDSNEFSVIADPR	2	8	0	0.71
HNNDTQHIWESDSNEFSVIADPR	2	8	0	0.86
HNNDTQHIWESDSNEFSVIADPR	2	9	0	0.35