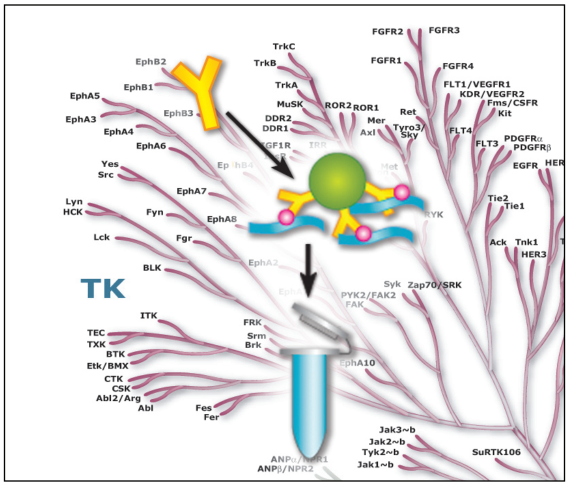
Tyrosine - PhosphoScan® Proteomics

Step 1: PhosphoScan® Analysis

1. Experimental objectives and design consultation with CST scientists
2. Determine samples and experimental parameters for study.
3. Quality assurance of samples by western blot with P-Tyr-100 mAb.
4. Phospho-tyrosine peptide immuno-affinity purification (IAP) with P-Tyr-100.
5. Tandem mass spectrometry (LC-MS/MS) analysis of enriched phosphopeptides for qualitative sequence and phospho-site identification.
6. Quantitative analysis of phosphopeptide fold-change between study samples.



Step 2: PhosphoScan® Report and Consultation

1. PhosphoScan report with qualitative and quantitative results.
2. Report contains sequence assignments in table format and detailed explanation of table contents and guidelines for data review.
3. Detailed discussion and review of report with CST scientists.
4. PhosphoScan timeline: approximately 5 weeks; preliminary results delivery in 2–3 weeks; timeline will vary with project size.

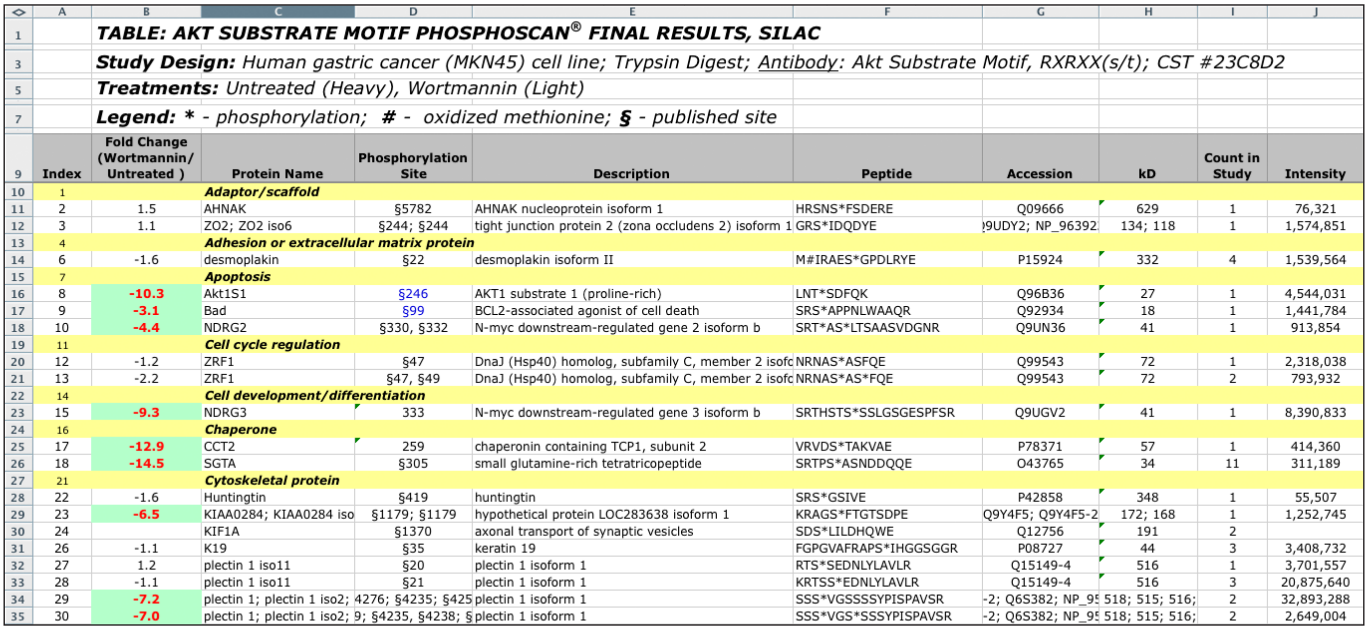


TABLE: AKT SUBSTRATE MOTIF PHOSPHOSCAN® FINAL RESULTS, SILAC

Study Design: *Human gastric cancer (MKN45) cell line; Trypsin Digest; Antibody: Akt Substrate Motif, RXRXX(s/t); CST #23C8D2*

Treatments: *Untreated (Heavy), Wortmannin (Light)*

Legend: ** - phosphorylation; # - oxidized methionine; § - published site*

9	Index	Fold Change (Wortmannin/ Untreated)	Protein Name	Phosphorylation Site	Description	Peptide	Accession	kD	Count in Study	Intensity
10	1		***Adaptor/scaffold***							
11	2	1.5	AHNAK	§5782	AHNAK nucleoprotein isoform 1	HRSNS*FSDERE	Q09666	629	1	76,321
12	3	1.1	ZO2; ZO2 iso6	§244; §244	tight junction protein 2 (zona occludens 2) isoform 1	GRS*IDQDYE	9UDY2; NP_96392	134; 118	1	1,574,851
13	4		***Adhesion or extracellular matrix protein***							
14	6	-1.6	desmoplakin	§22	desmoplakin isoform II	M#IRAES*GPDLRYE	P15924	332	4	1,539,564
15	7		***Apoptosis***							
16	8	**-10.3**	Akt1S1	§246	AKT1 substrate 1 (proline-rich)	LNT*SDFQK	Q96B36	27	1	4,544,031
17	9	**-3.1**	Bad	§99	BCL2-associated agonist of cell death	SRS*APPNLWAAQR	Q92934	18	1	1,441,784
18	10	**-4.4**	NDRG2	§330, §332	N-myc downstream-regulated gene 2 isoform b	SRT*AS*LTSAASVDGNR	Q9UN36	41	1	913,854
19	11		***Cell cycle regulation***							
20	12	-1.2	ZRF1	§47	DnaJ (Hsp40) homolog, subfamily C, member 2 isofc	NRNAS*ASFQE	Q99543	72	1	2,318,038
21	13	-2.2	ZRF1	§47, §49	DnaJ (Hsp40) homolog, subfamily C, member 2 isofc	NRNAS*AS*FQE	Q99543	72	2	793,932
22	14		***Cell development/differentiation***							
23	15	**-9.3**	NDRG3	333	N-myc downstream-regulated gene 3 isoform b	SRTHSTS*SSLGSGESPFSR	Q9UGV2	41	1	8,390,833
24	16		***Chaperone***							
25	17	**-12.9**	CCT2	259	chaperonin containing TCP1, subunit 2	VRVDS*TAKVAE	P78371	57	1	414,360
26	18	**-14.5**	SGTA	§305	small glutamine-rich tetratricopeptide	SRTPS*ASNDDQQE	O43765	34	11	311,189
27	21		***Cytoskeletal protein***							
28	22	-1.6	Huntingtin	§419	huntingtin	SRS*GSIVE	P42858	348	1	55,507
29	23	**-6.5**	KIAA0284; KIAA0284 iso	§1179; §1179	hypothetical protein LOC283638 isoform 1	KRAGS*FTGTSDPE	Q9Y4F5; Q9Y4F5-2	172; 168	1	1,252,745
30	24		KIF1A	§1370	axonal transport of synaptic vesicles	SDS*LILDHQWE	Q12756	191	2	
31	26	-1.1	K19	§35	keratin 19	FGPGVAFRAPS*IHGGSGGR	P08727	44	3	3,408,732
32	27	1.2	plectin 1 iso11	§20	plectin 1 isoform 1	RTS*SEDNLYLAVLR	Q15149-4	516	1	3,701,557
33	28	-1.1	plectin 1 iso11	§21	plectin 1 isoform 1	KRTSS*EDNLYLAVLR	Q15149-4	516	3	20,875,640
34	29	**-7.2**	plectin 1; plectin 1 iso2;	4276; §4235; §425	plectin 1 isoform 1	SSS*VGSSSSYPISPAVSR	-2; Q6S382; NP_95	518; 515; 516;	2	32,893,288
35	30	**-7.0**	plectin 1; plectin 1 iso2;	9; §4235, §4238; §	plectin 1 isoform 1	SSS*VGS*SSSYPISPAVSR	-2; Q6S382; NP_95	518; 515; 516;	2	2,649,004

Cell Signaling
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