Cell Signaling Technology

PTMScan Direct: Multipathway v2.0

Proteomics Service Group

January 14, 2013



PhosphoScan Deliverables

- Project Overview
- Methods
- PTMScan Direct: Multipathway V2.0 (Tables 1,2)
 - Qualitative Results (Details tab)
 - Quantitative Results
 - Summary tab
 - Fold Change by Protein Type
- Informatics Table (Table 3)
- Overview of Quantitative Results
- Study Consultations





Method Summary

Phosphorylated peptide enrichment using the following antibodies:

PTMScan Direct: Multipathway Reagent V2.0 (1006 sites to 409 proteins, 807 sites to 313 proteins excluding isoforms)

LC-MS/MS Analysis using LTQ-Orbitrap-VELOS, ESI-CID

Sorcerer search results Label-free quantitation of phosphorylated peptide intensities (**Tables 1,2**).

Informatics table (Table 3).









LC Methods

Peptides were loaded directly onto a 10 cm x 75 μ m PicoFrit capillary column packed with Magic C18 AQ reversed-phase resin.

The column was developed with a 72-minute linear gradient of acetonitrile in 0.125% formic acid delivered at 280 nL/min.

MS Parameter Settings: MS Run Time 96 min, MS1 Scan Range (300.0 – 1500.00), Top 20 MS/MS (Min Signal 500, Isolation Width 2.0, Normalized Coll. Energy 35.0, Activation-Q 0.250, Activation Time 20.0, Lock Mass 371.101237, Charge State Rejection Enabled, Charge State 1+ Rejected, Dynamic Exclusion Enabled, Repeat Count 1, Repeat Duration 35.0, Exclusion List Size 500, Exclusion Duration 40.0, Exclusion Mass Width Relative to Mass, Exclusion Mass Width 10ppm)





Informatics

MS/MS spectra were evaluated using SEQUEST 3G and the SORCERER 2 platform from Sage-N Research (v4.0, Milpitas CA) (Lundgren et al., *Curr Protoc Bioinformatics*, 2009).

Searches were performed against the most recent update of the NCBI *homo sapiens* database with mass accuracy of +/-50 ppm for precursor ions and 1 Da for product ions.

Results were filtered with mass accuracy of +/- 5 ppm on precursor ions and for validated Multipathway Reagent peptide ions.







CST Products for Subsequent Validation

A (a) DNLA Due durat Links

			Gene Name (SIRNA Product Link)
			✓ pathway ▼ more info ▼ application references ■ datasheet PDF ■ MSDS PDF ■ protocols
Gene Name	Protein Name	Site	SignalSilence [®] PTEN siRNA I #6251
AKT1	Akt1	305	PhosphoSitePlus [®] protein, site, and accession data: PTEN
AKT1	Akt1	§308	
AKT1	Akt1	§308, §315	
AKT1	Akt1	312	Protein Name (Total Antibody Product Link)
AKT1	Akt1	§315	d pathway
AKT1	Akt1	§473	
AKT1	Akt1	§473, 479	Akt (pan) (11E7) Rabbit mAb #4685
AKT1	Akt1	§474	PhosphoSitePlus [®] protein, site, and accession data: Akt1, Akt2, Akt3
AKT1	Akt 🕽	§474, 479	
AKT2	Akt2	§309	
AKT2	Akt2	§309, 313	Site (Site Specific Antibody Product Link)
AKT2	Akt2	313	■ nathway ▼ more info ▼ application references ■ datasheet PDE ■ MSDS PDE ▼ protocols
AKT2	Akt2	§4 74	Photocols
AKT2	Akt2	§474, §478	> Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb #4060
AKT2	Akt2	475	PhosphoSitePlus [®] protein, site, and accession data: Akt1, Akt2, Akt3
AKT2	Akt2	475, §478	
AKT2	Akt2	§476, §478	

Blue text = hyperlink to CST product pages for siRNA products (Gene Name column), total antibodies (Protein Name column), and site-specific antibodies (Site column).



Experimental Flow Diagram: Multipathway



3 samples, duplicate injections = 6 LC-MS/MS experiments



Experimental Flow Diagram: Multipathway



3 samples, duplicate injections = 6 LC-MS/MS experiments





						Validated Pepti	de Assignments
Experiment	Cell Type	Treatment	Antibody	cs	Protease	Redundant	Non-Redundant
1	CST Cell Line	Pervanadate	Multipathway V2.0	15316	trypsin	1382	839
2	CST Cell Line	Pervanadate	Multipathway V2.0	15317	trypsin	1393	844
3	HeLa	Control	Multipathway V2.0	15318	trypsin	621	350
4	HeLa	Control	Multipathway V2.0	15319	trypsin	642	354
5	HeLa	UV	Multipathway V2.0	15320	trypsin	912	428
6	HeLa	UV	Multipathway V2.0	15321	trypsin	872	416

A total of **2,903** redundant validated peptide assignments (**Table 1**, Details tab) to **952** nonredundant validated peptides (**Table 1**, Summary tab) for the PTMScan Direct: Multipathway Reagent V2.0.

A 5% default false positive rate was used to filter the SORCERER results.





						Validated Pepti	de Assignments
Experiment	Cell Type	Treatment	Antibody	cs	Protease	Redundant	Non-Redundant
1	CST Cell Line	Pervanadate	Multipathway V2.0	15316	trypsin	1382	839
2	CST Cell Line	Pervanadate	Multipathway V2.0	15317	trypsin	1393	844
7	MCF7	Control	Multipathway V2.0	15322	trypsin	718	381
8	MCF7	Control	Multipathway V2.0	15323	trypsin	713	379
9	MCF7	EGF	Multipathway V2.0	15324	trypsin	769	400
10	MCF7	EGF	Multipathway V2.0	15325	trypsin	783	413

A total of **2,903** redundant validated peptide assignments (**Table 2**, Details tab) to **945** nonredundant validated peptides (**Table 2**, Summary tab) for the PTMScan Direct: Multipathway Reagent V2.0.

A 5% default false positive rate was used to filter the SORCERER results.



Table Overview

Each PTMScan Direct table consists of 4 sheets:

Column Definitions tab Details (qualitative) tab Summary (quantitative) tab Fold Change by Protein Type

Tables will open to the Fold Change by Protein Type tab.

Other tabs are accessed by clicking on sheet names at bottom of window (blue boxes in following slides).





Table Overview: Column Definitions

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Column Definitions tab contains description of column headers in data tables.



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Details Tab contains a redundant list of all validated peptides identified in the study with their accompanying SORCERER metrics.



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Summary Tab (quantitative results) is a non-redundant list of sites quantified in the study (**BLUE BOX**). The table header (**BLACK BOX**) contains information about the study and legend text.





Table Overview: Fold Change by Protein Type Tab

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19	8	418	6.0	2,112,601	A	11.1	BLM	BLM	74		
20	9	421	-4.7	431,876	н	20.2	H3F3A; hCG1639761; H	I H3F3A; hCG1639761; H	3		
21	10	425	-3.0	174,960	A	20.8	NFKBIA	IkB-alpha	§36		
22	11	437	4.8	21,618,781	A	10.2	SBN01	SBN01	§815		
23	12	442	31.7	924,829	A	25.4	SBN01	SBN01	§815, 823		
24	13	449	4.5	18,547,430	A	11.0	SBN01	SBN01	§817		
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Fold Change by Protein Type tab provides the overall quantitative results sorted by protein type containing only those sites that increase above 2.5-fold (GREEN) and those sites that decrease below -2.5-fold (RED).



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5 5 7 7 10 11 12 13 14 15 16 17 18 19 20 21 22	Index Index 1 2 3 4 5 6 7 8 9 10 11	#1: CST Cell les: CST Cell Index in Detail Adaptor/scaffold 2 5 7 11 14 16 17 18 19 20	Line Control, F Line Control = horylation, # - Normalized Fold Change UV : Control	HeLa Cells; Tr CS 15316, 1 oxidized me Max Intensity 303,305 - 52,004 469,595 - - - - - - - - - - - - -	rypsin 5317; thionin Height H H H H H H H H H H H A	Digest; M HeLa Cor he, § - pu Max % CV 13.5 - 21.1 13.2 - - - - - - - - - - - - - - - - -	ABI2; ABI2 ABI2; ABI2 ABI2; ABI2 BCAR1 BCAR1 CAV1 CAV1 CAV1 CAV1 CAV1 CAV1 CAV1 CAV	Agent V2.0 8, 15319; HeLa + UE Text - CST sik Protein Name Abi-2; Abi-2 iso2 Abi-2; Abi-2 iso2 P130Cas P130Cas Caveolin-1 caveolin-1 caveolin-1 CD2AP CD2AP	UV = CS 1532 RNA/antibody a Site \$213; \$207 \$213, \$216; \$207, 115 \$385 \$6, \$14 \$6, \$14, \$25 \$6, \$15 \$6, \$15 \$6, \$15 \$6, \$15 \$539, \$46, \$55 \$541, \$551
5 5 7 7 10 111 12 13 14 15 16 17 18 19 20 21 22 23	Index 1 2 3 4 5 6 7 8 9 10 11 12	#1: CST Cell les: CST Cell d: * - phosph Adaptor/scaffold 2 5 7 11 14 16 17 18 19 20 23	Line Control, F Line Control = horylation, # - Normalized Fold Change UV : Control 1.1 - 1.5 1.4 - - - - - - - - -	HeLa Cells; Ti CS 15316, 1 oxidized me Max Intensity 303,305 	rypsin 5317; thionin Area or Height H H H H H H H H H H A A	Digest; M HeLa Cor he, § - pu Max % CV 13.5 - 21.1 13.2 - - - - - - - - - - - - - - - - - - -	ABI2; ABI2 ABI2; ABI2 ABI2; ABI2 ABI2; ABI2 BCAR1 BCAR1 CAV1	Agent V2.0 8, 15319; HeLa + UE Text - CST sik Protein Name Abi-2; Abi-2 iso2 Abi-2; Abi-2 iso2 P130Cas P130Cas P130Cas caveolin-1 caveolin-1 caveolin-1 CD2AP CD2AP CD2AP	UV = CS 1532 RNA/antibody a Site \$213; §207 §213, §216; §207, 115 §385 §6, §14 §6, §14, §25 §6, §15, §25 §6, §15, §25 \$39, \$46, §55 \$41, §551 §542, §548
5 5 7 7 10 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24	Index 1 2 3 4 5 6 7 8 9 10 11 12 13	#1: CST Cell les: CST Cell d: * - phosph Adaptor/scaffold 2 5 7 111 14 16 17 18 19 20 23 25	Line Control, F Line Control = horylation, # - Normalized Fold Change UV : Control	HeLa Cells; Ti CS 15316, 1 oxidized me Max Intensity 303,305 - 52,004 469,595 - - - - - - - - - - - - -	rypsin 5317; thionin Area or Height H H H H H H H H H H H H H H H H H H H	Digest; M HeLa Cor e, § - pu Max % CV 13.5 - - 21.1 13.2 - - - - - - - - - - - - - - - - - - -	ABI2; ABI2 ABI2; ABI2 ABI2; ABI2 ABI2; ABI2 ACAV1 CAV1 CAV1 CAV1 CAV1 CAV1 CAV1 CD2AP CD2AP CD2AP CD2AP	Agent V2.0 8, 15319; HeLa + ue Text - CST siR Protein Name Abi-2; Abi-2 iso2 Abi-2; Abi-2 iso2 P130Cas Caveolin-1 caveolin-1 caveolin-1 caveolin-1 CD2AP CD2AP CD2AP CD2AP	UV = CS 1532 RNA/antibody a Site \$213; §207 §213, §216; §207, 115 §385 §6, §14 §6, §14, §25 §6, §15, §25 \$39, 546, §55: \$41, §551 §542, §548 §542, §548, §55

Summary Tab (quantitative results) is a non-redundant list of sites quantified in the study (**BLUE BOX**). The table header (**BLACK BOX**) contains information about the study and legend text.





		Normalized Fold Change						
Index	Index in Detail	UV : Control	Max Intensity	Area or Height	Max % CV	Gene Name	Protein Name	Site
470	1077	-1.2	523,753	Н	16.5	MAPK1	ERK2	
471	1084	1.7	12,803,987	A	2.9	MAPK1	ERK2	§185
472	1092	1.5	20,993,600	Н	37.9	MAPK1	ERK2	§185, §187
473	1104	1.1	90,295	Н	39.8	MAPK1	ERK2	§187
474	1109	28.4	459,188	Н	23.4	MAPK10; MAPK10; MAPK	JNK3; JNK3 iso2; JNK1;	§221, §223; §183, §185
475	1127	15.2	715,846	Α	34.7	MAPK10; MAPK10; MAPK	JNK3; JNK3 iso2; JNK1;	§223; §223; §185; §185
476	1129	1.4	124,965	Н	14.4	MAPK12	p38-gamma	§183
477	1133	2.1	1,227,660	Α	12.4	MAPK12	p38-gamma	§183, §185
478	1134	1.6	350,227	H	14.6	MAPK12	p38-gamma	§185
479	1142	-	-	Н	-	MAPK13	p38-delta	§180, §182
480	1144	4.1	1,902,560	Н	5.6	MAPK14; MAPK14	p38-alpha; p38-alpha iso	§180, §182; §180, §182
481	1159	3.2	1,642,382	Α	1.7	MAPK14; MAPK14	p38-alpha; p38-alpha iso	§182; §182
482	1163	1.3	155,500	Н	7.7	MAPK3	ERK1	§202
483	1167	1.3	3,310,535	Н	19.2	MAPK3	ERK1	§202, §204
484	1175	-1.1	780,041	Н	2.9	MAPK3	ERK1	§204
485	1179	-9.3	347,096	Α	13.0	MAPK7	ERK5	§219, §221
486	1183	-9.2	7,367,057	Α	13.9	MAPK7	ERK5	§221
487	1189	55.4	36,814,462	A	12.9	MAPK9; MAPK9; MAPK9	JNK2; JNK2 iso2; JNK2 is	§185; §183, §185; §183,
488	1218	38.4	25,265,503	Α	41.3	MAPK9; MAPK9; MAPK9	JNK2; JNK2 iso2; JNK2 is	§188; §183, §188; §183,
489	1219	10.1	517,181	Α	19.4	MAPK9; MAPK9; MAPK9	JNK2; JNK2 iso2; JNK2 is	§185; §185; §185

Redundancy is eliminated from the Details Table to provide a summary of the quantitative results (**black box**). The table is indexed for easy re-sorting to original order (**blue box**), and index number from the Details tab is included for easy lookup of peptides between tabs ("Index in Detail", **red box**)



		Normalized Fold Change							
Index	Index in Detail	UV : Control	Max Intensity	Area or Height	Max % CV	Fold cha	anges shou	ld be	
470	1077	-1.2	523,753		16.5			I I	
471	1084	1.7	12,803,987	A	2.9	consider	ea in the co	ontext	
472	1092	1.5	20,993,600	н	37.9				37
473	1104	1.1	90,295	н	39.8	ofinton	sitv <u> tho</u> h	ighor	
474	1109	28.4	459,188	н	23.4		Sity then	igner	33, §185
475	1127	15.2	715,846	Α	34.7				5; §18
476	1129	1.4	124,965	н	14.4	the inte	ensity, the r	nore	
477	1133	2.1	1,227,660	Α	12.4		1,		35
478	1134	1.6	350,227	н	14.6	accurate	the fold ch	αρησο	
479	1142	-	-	н	-	accurate		lange.	32
480	1144	4.1	1,902,560	н	5.6				30, §18
481	1159	3.2	1,642,382	Α	1.7				32
482	1163	1.3	155,500	н	7.7	MAPK3	ERK1	§202	
483	1167	1.3	3,310,535	н	19.2	MAPK3	ERK1	§202, §2	.04
484	1175	-1.1	780,041	н	2.9	MAPK3	ERK1	§204	
485	1179	-9.3	347,096	Α	13.0	MAPK7	ERK5	§219, §2	21
486	1183	-9.2	7,367,057	Α	13.9	MAPK7	ERK5	§221	
487	1189	55.4	36,814,462	Α	12.9	MAPK9; MAPK9; MAPK9	JNK2; JNK2 iso2; JNK2 i	s§185; §183, §1	85; §18
488	1218	38.4	25,265,503	Α	41.3	MAPK9; MAPK9; MAPK9	JNK2; JNK2 iso2; JNK2 i	s§188; §183, §1	88; §18
489	1219	10.1	517,181	A	19.4	MAPK9: MAPK9: MAPK9	JNK2: JNK2 iso2: JNK2 i	\$185: \$185:	6185

Redundancy is eliminated from the Details Table to provide a summary of the quantitative results (**black box**). The table is indexed for easy re-sorting to original order (**blue box**), and index number from the Details tab is included for easy lookup of peptides between tabs ("Index in Detail", **red box**)





		Normalized Fold Change				Peak n	neasureme	nts can		
Index	Index in Detail	UV : Control	Max Intensity	Area or Height	Max % CV	ha	he area or height			
470	1077	-1.2	523,753	н		be	area or nei	gni.		
471	1084	1.7	12,803,987	Α	2.9	MAPI		•		
472	1092	1.5	20,993,600	н	37.9	MAPK1	ERK2	§185, §187		
473	1104	1.1	90,295	н	39.8	MAPK1	ERK2	§187		
474	1109	28.4	459,188	н	23.4	MAPK10; MAPK10; MAPH	JNK3; JNK3 iso2; JNK1;	§221, §223; §183, §185		
475	1127	15.2	715,846	Α	34.7	MAPK10; MAPK10; MAPH	JNK3; JNK3 iso2; JNK1;	§223; §223; §185; §185		
476	1129	1.4	124,965	н	14.4	MAPK12	p38-gamma	§183		
477	1133	2.1	1,227,660	Α	12.4	MAPK12	p38-gamma	§183, §185		
478	1134	1.6	350,227	н	14.6	MAPK12	p38-gamma	§185		
479	1142	-	-	н	-	MAPK13	p38-delta	§180, §182		
480	1144	4.1	1,902,560	н	5.6	MAPK14; MAPK14	p38-alpha; p38-alpha iso	§180, §182; §180, §182		
481	1159	3.2	1,642,382	Α	1.7	MAPK14; MAPK14	p38-alpha; p38-alpha iso	§182; §182		
482	1163	1.3	155,500	н	7.7	MAPK3	ERK1	§202		
483	1167	1.3	3,310,535	н	19.2	MAPK3	ERK1	§202, §204		
484	1175	-1.1	780,041	н	2.9	MAPK3	ERK1	§204		
485	1179	-9.3	347,096	Α	13.0	MAPK7	ERK5	§219, §221		
486	1183	-9.2	7,367,057	Α	13.9	MAPK7	ERK5	§221		
487	1189	55.4	36,814,462	Α	12.9	MAPK9; MAPK9; MAPK9	JNK2; JNK2 iso2; JNK2 is	§185; §183, §185; §183		
488	1218	38.4	25,265,503	Α	41.3	MAPK9; MAPK9; MAPK9	JNK2; JNK2 iso2; JNK2 is	§188; §183, §188; §183		
489	1219	10.1	517,181	A	19.4	MAPK9; MAPK9; MAPK9	JNK2; JNK2 iso2; JNK2 is	§185; §185; §185		

Redundancy is eliminated from the Details Table to provide a summary of the quantitative results (**black box**). The table is indexed for easy re-sorting to original order (**blue box**), and index number from the Details tab is included for easy lookup of peptides between tabs ("Index in Detail", **red box**)



					Normalized Fold		
The maxi					Change		
			Area or				
cooffici	Gene Name	Max % CV	Height	Max Intensity	UV : Control	Index in Detail	Index
CUEIIICI	MADI/1	16.5	Н	523,753	-1.2	1077	470
• .•	MAPK1	2.9	A	12,803,987	1.7	1084	471
variation	MAPK1	37.9	Н	20,993,600	1.5	1092	472
	MAPK1	39.8	н	90,295	1.1	1104	473
given for r	MAPK10; MAPK10; N	23.4	н	459,188	28.4	1109	474
giveniori	MAPK10; MAPK10; N	34.7	Α	715,846	15.2	1127	475
	MAPK12	14.4	н	124,965	1.4	1129	476
injection	MAPK12	12.4	Α	1,227,660	2.1	1133	477
ingeociona	MAPK12	14.6	Н	350,227	1.6	1134	478
	MAPK13	-	Н	-	-	1142	479
same sam	MAPK14; MAPK14	5.6	Н	1,902,560	4.1	1144	480
	MAPK14; MAPK14	1.7	Α	1,642,382	3.2	1159	481
lower the v	МАРКЗ	7.7	Н	155,500	1.3	1163	482
	МАРКЗ	19.2	Н	3,310,535	1.3	1167	483
alacar th	МАРКЗ	2.9	Н	780,041	-1.1	1175	484
cioser tr	MAPK7	13.0	Α	347,096	-9.3	1179	485
	MAPK7	13.9	Α	7,367,057	-9.2	1183	486
intensi	МАРК9; МАРК9; МА	12.9	Α	36,814,462	55.4	1189	487
interis	МАРК9; МАРК9; МА	41.3	Α	25,265,503	38.4	1218	488
PK9 JNK2; JNK2 ISO2; JNK2	МАРК9; МАРК9; МА	19.4	Α	517,181	10.1	1219	489

The maximum % coefficient of variation (CV) is given for replicate injections of the same sample. The lower the value the closer the two intensities.

Redundancy is eliminated from the Details Table to provide a summary of the quantitative results (**black box**). The table is indexed for easy re-sorting to original order (**blue box**), and index number from the Details tab is included for easy lookup of peptides between tabs ("Index in Detail", **red box**)





		Normalized Fold Change						
Index	Index in Detail	UV : Control	Max Intensity	Area or Height	Max % CV	Gene Name	Protein Name	Site
470	1077	-1.2	523,753	Н	16.5	MAPK1	ERK2	
471	1084	1.7	12,803,987	A	2.9	MAPK1	ERK2	§185
472	1092	1.5	20,993,600	Н	37.9	MAPK1	ERK2	§185, §187
473	1104	1.1	90,295	Н	39.8	MAPK1	ERK2	§187
474	1109	28.4	459,188	Н	23.4	MAPK10; MAPK10; MAPK	JNK3; JNK3 iso2; JNK1;	§221, §223; §183, §185
475	1127	15.2	715,846	Α	34.7	MAPK10; MAPK10; MAPK	JNK3; JNK3 iso2; JNK1;	§223; §223; §185; §185
476	1129	1.4	124,965	Н	14.4	MAPK12	p38-gamma	§183
477	1133	2.1	1,227,660	Α	12.4	MAPK12	p38-gamma	§183, §185
478	1134	1.6	350,227	Н	14.6	MAPK12	p38-gamma	§185
479	1142	-	-	н	-	MAPK13	p38-delta	§180, §182
480	1144	4.1	1,902,560	н	5.6	MAPK14; MAPK14	p38-alpha; p38-alpha iso	§180, §182; §180, §182
481	1159	3.2	1,642,382	A	1.7	MAPK14; MAPK14	p38-alpha; p38-alpha iso	§182; §182
482	1163	1.3	155,500	н	7.7	MAPK3	ERK1	§202
483	1167	1.3	3,310,535	н	19.2	MAPK3	ERK1	§202, §204
484	1175	-1.1	780,041	н	2.9	MAPK3	ERK1	§204
485	1179	-9.3	347,096	Α	13.0	MAPK7	ERK5	§219, §221
486	1183	-9.2	7,367,057	Α	13.9	MAPK7	ERK5	§221
487	1189	55.4	36,814,462	A	12.9	MAPK9; MAPK9; MAPK9	JNK2; JNK2 iso2; JNK2 is	§185; §183, §185; §183,
488	1218	38.4	25,265,503	Α	41.3	MAPK9; MAPK9; MAPK9	JNK2; JNK2 iso2; JNK2 is	§188; §183, §188; §183,
489	1219	10.1	517,181	Α	19.4	MAPK9; MAPK9; MAPK9	JNK2; JNK2 iso2; JNK2 is	§185; §185; §185

The Summary table contains Gene Name, Protein Name, and modification Site for each identified peptide. siRNA products are available for Gene Names in blue text (**GREEN BOX**). Total antibodies or phosphorylation site-specific antibodies are available for Protein Names or Sites in **blue text** (**BLUE BOX**). Published (§, **RED BOX**) and novel sites (**not shown**) of phosphorylation.





Protein Name	Site	Description	Accession	URL
ERK2		mitogen-activated protein kinase 1	P28482	http://www.phosphosite.
ERK2	§185	mitogen-activated protein kinase 1	P28482	http://www.phosphosite.
ERK2	§185, §187	mitogen-activated protein kinase 1	P28482	http://www.phosphosite.
ERK2	§187	mitogen-activated protein kinase 1	P28482	http://www.phosphosite.
JNK3; JNK3 iso2; JNK1; .	§221, §223; §183, §185	mitogen-activated protein kinase 10 isoform 1	P53779; P53779-2; P459	http://www.phosphosite.
JNK3; JNK3 iso2; JNK1; .	§223; §223; §185; §185	mitogen-activated protein kinase 10 isoform 1	P53779; P53779-2; P459	http://www.phosphosite.
p38-gamma	§183	mitogen-activated protein kinase 12	P53778	http://www.phosphosite.
p38-gamma	§183, §185	mitogen-activated protein kinase 12	P53778	http://www.phosphosite.
p38-gamma	§185	mitogen-activated protein kinase 12	P53778	http://www.phosphosite.
p38-delta	§180, §182	mitogen-activated protein kinase 13	015264	http://www.phosphosite.
p38-alpha; p38-alpha iso	§180, §182; §180, §182	mitogen-activated protein kinase 14 isoform 1	Q16539; Q16539-2	http://www.phosphosite.
p38-alpha; p38-alpha iso	§182; §182	mitogen-activated protein kinase 14 isoform 1	Q16539; Q16539-2	http://www.phosphosite.
ERK1	§202	mitogen-activated protein kinase 3 isoform 2	P27361	http://www.phosphosite.
ERK1	§202, §204	mitogen-activated protein kinase 3 isoform 2	P27361	http://www.phosphosite.
ERK1	§204	mitogen-activated protein kinase 3 isoform 2	P27361	http://www.phosphosite.
ERK5	§219, §221	mitogen-activated protein kinase 7 isoform 1	Q13164	http://www.phosphosite.
ERK5	§221	mitogen-activated protein kinase 7 isoform 1	Q13164	http://www.phosphosite.
JNK2; JNK2 iso2; JNK2 is	§185; §183, §185; §183	mitogen-activated protein kinase 9 isoform JNK2 gamma	P45984; P45984-2; P459	http://www.phosphosite.
JNK2; JNK2 iso2; JNK2 is	§188; §183, §188; §183	mitogen-activated protein kinase 9 isoform JNK2 gamma	P45984; P45984-2; P459	http://www.phosphosite.
JNK2; JNK2 iso2; JNK2 is	§185; §185; §185	mitogen-activated protein kinase 9 isoform JNK2 gamma	P45984; P45984-2; P459	http://www.phosphosite.

Multiple isoforms for a single peptide are separated by semicolon (**RED BOX**). A link to the PhosphoSitePlus page for each protein is given (Accession, **BLUE BOX**, **blue text**) and (URL, **BLUE ARROW**).





Protein Name	Site	Peptide	Charge	Calc. m/z	Count in Details
ERK2		TQHLSNDHICYFLYQILR	4	581.0428	13
ERK2	§185	VADPDHDHTGFLT*EYVATR	3	741.9951	9
ERK2	§185, §187	VADPDHDHTGFL [*] EY*VATR	3	768.6505	86
ERK2	§187	VADPDHD HTGFL EY*VATR	4	556.7481	32
JNK3; JNK3 iso2; JNK1;	§221, §223; §183, §185	TAGTSFM#M#TY*VVTR	2	927.3662	86
JNK3; JNK3 iso2; JNK1;	§223; §223; §185; §185	TAGTSF	2	879.3855	2
p38-gamma	§183	QADSEM#T*GYVVTR	2	776.8211	2
p38-gamma	§183, §185	QADSEM#T*GY*VVTR	2	816.8043	12
p38-gamma	§185	QADSEMTGY*VVTR	2	768.8236	6
p38-delta	§180, §182	HADAEM#T*GY*VVTR	2	813.3070	22
p38-alpha; p38-alpha isc	§180, §182; §180, §182	HTDDEMT*GY*VATR	2	828.2941	84
p38-alpha; p38-alpha isc	§182; §182	HTDDEMTGY*VATR	2	788.3109	9
ERK1	§202	IADPEHDHTGFLT*EYVATR	3	751.3388	10
ERK1	§202, §204	IADPEHDHTGFLT*EY*VATR	3	777.9943	63
ERK1	§204	IADPEHDHTGFLTEY*VATR	3	751.3388	29
ERK5	§219, §221	GLCTSPAEHQYFM#T*EY*VATR	3	846.3353	4
ERK5	§221	GLCTSPAEHQYFM#TEY*VATR	3	819.6799	22
JNK2; JNK2 iso2; JNK2 is	§185; §183, §185; §183	TACTNFM#MT*PY*VVTR	2	984.3788	127
JNK2; JNK2 iso2; JNK2 is	§188; §183, §188; §183	TACTNFM#M#T*PYVVT*R	3	661.9199	3
JNK2; JNK2 iso2; JNK2 is	§185; §185; §185	TACTNFM#M#TPY*VVTR	2	952.3931	2

Table includes protein/peptide/site information (Peptide, Charge, Calc. m/z, Count in Details).

phosphorylation (*, **RED ARROW**), oxidized methionine (#, **BLUE ARROW**).

Count in Details (**RED BOX**): number of MS/MS identifications in the Details Tab for each site.





						Raw In	tensity		
Protein Name	Site	Area or Height	Average RT	CST Cell Line (CS15316)	CST Cell Line (CS15317)	HeLa Control (CS15318)	HeLa Control (CS15319)	HeLa + UV (CS15320)	HeLa + UV (CS15321)
ERK2		н	72.82	326,061	288,421	517,373	530,132	456,080	576,283
ERK2	§185	Α	56.39	19,223,947	19,967,309	6,330,180	6,593,120	13,000,501	12,607,473
ERK2	§185, §187	н	51.46	111,001,000	112,356,000	11,438,400	11,722,100	26,613,100	15,374,100
ERK2	§187	н	47.73	39,023	55,831	48,877	87,174	86,268	94,321
JNK3; JNK3 iso2; JNK1;	§221, §223; §183, §185	н	47.57	462,565	493,611	11,461	16,005	389,247	529,128
JNK3; JNK3 iso2; JNK1; 3	§223; §223; §185; §185	A	56.22			30,160	49,756	752,732	678,959
p38-gamma	§183	н	30.35	2,895	9,945	73,407	78,346	112,219	137,710
p38-gamma	§183, §185	A	30.86	986,131	906,043	490,049	502,692	1,119,777	1,335,543
p38-gamma	§185	н	39.80			195,490	187,168	386,401	314,052
p38-delta	§180, §182	н	28.03	469,323	672,504				
p38-alpha; p38-alpha iso	§180, §182; §180, §182	н	33.29	3,526,840	3,779,960	414,115	382,669	1,853,120	1,952,000
p38-alpha; p38-alpha iso	§182; §182	Α	31.62	76,981	123,555	438,975	443,540	1,622,795	1,661,968
ERK1	§202	н	57.70	571,000	528,000	102,000	99,500	147,000	164,000
ERK1	§202, §204	н	53.21	34,299,900	45,964,900	2,149,010	2,299,730	3,760,760	2,860,310
ERK1	§204	н	49.61	723,745	831,121	708,795	722,831	796,074	764,007
ERK5	§219, §221	Α	51.65	1,151,502	961,312	321,913	372,279	40,000	48,105
ERK5	§221	A	49.89	428,311	382,844	6,642,775	8,091,339	929,937	965,027
JNK2; JNK2 iso2; JNK2 is	§185; §183, §185; §183	A	57.66	17,697,900	18,945,063	512,900	615,751	37,229,505	36,399,418
JNK2; JNK2 iso2; JNK2 is	§188; §183, §188; §183	A	47.20	19,156,849	16,517,409	395,795	721,912	23,271,767	27,259,239
JNK2; JNK2 iso2; JNK2 is	§185; §185; §185	A	46.36				43,478	446,174	588,187

Table includes Average Retention Time of each peptide (**BLACK BOX**), whether measurement is height (H) or area (A) (**RED BOX**), and intensities (**BLUE BOX**). **Bold values** = intensities manually reviewed; **Red values** (not shown) = multiple identifications for 1 m/z measurement due to ambiguous phosphorylation site localization.





		Average Raw Intensity		%	cv	Raw Ratio
		, it charge it a				
Protein Name	Site	Control	UV	Control	UV	UV : Control
ERK2		523,753	516,182	1.7	16.5	0.99
ERK2	§185	6,461,650	12,803,987	2.9	2.2	1.98
ERK2	§185, §187	11,580,250	20,993,600	1.7	37.9	1.81
ERK2	§187	68,026	90,295	39.8	6.3	1.33
JNK3; JNK3 iso2; JNK1;	§221, §223; §183, §185	13,733	459,188	23.4	21.5	33.44
JNK3; JNK3 iso2; JNK1;	§223; §223; §185; §185	39,958	715,846	34.7	7.3	17.91
p38-gamma	§183	75,877	124,965	4.6	14.4	1.65
p38-gamma	§183, §185	496,371	1,227,660	1.8	12.4	2.47
p38-gamma	§185	191,329	350,227	3.1	14.6	1.83
p38-delta	§180, §182					
p38-alpha; p38-alpha isc	§180, §182; §180, §182	398,392	1,902,560	5.6	3.7	4.78
p38-alpha; p38-alpha iso	§182; §182	441,258	1,642,382	0.7	1.7	3.72
ERK1	§202	100,750	155,500	1.8	7.7	1.54
ERK1	§202, §204	2,224,370	3,310,535	4.8	19.2	1.49
ERK1	§204	715,813	780,041	1.4	2.9	1.09
ERK5	§219, §221	347,096	44,053	10.3	13.0	0.13
ERK5	§221	7,367,057	947,482	13.9	2.6	0.13
JNK2; JNK2 iso2; JNK2 is	§185; §183, §185; §183	564,326	36,814,462	12.9	1.6	65.24
JNK2; JNK2 iso2; JNK2 is	§188; §183, §188; §183	558,854	25,265,503	41.3	11.2	45.21
JNK2; JNK2 iso2; JNK2 is	§185; §185; §185	43,478	517,181		19.4	11.90

Table includes Average Intensity (**RED BOX**), % CV(**BLUE BOX**), and Raw Ratio (**GREEN BOX**). % CV is a measure of variation in intensity between the replicate injections.





			Normalized Log2		Normalized Fold	
		Log2 Ratio	Ratio	Raw Fold Change	Change	
Protein Name	Site	UV : Control	UV : Control	UV : Control	UV : Control	Species
ERK2		-0.02	-0.26	-1.01	-1.20	human
ERK2	§185	0.99	0.75	1.98	1.68	human
ERK2	§185, §187	0.86	0.62	1.81	1.54	human
ERK2	§187	0.41	0.17	1.33	1.13	human
JNK3; JNK3 iso2; JNK1; .	§221, §223; §183, §185	5.06	4.83	33.44	28.38	human
JNK3; JNK3 iso2; JNK1;	§223; §223; §185; §185	4.16	3.93	17.91	15.21	human
p38-gamma	§183	0.72	0.48	1.65	1.40	human
p38-gamma	§183, §185	1.31	1.07	2.47	2.10	human
p38-gamma	§185	0.87	0.64	1.83	1.55	human
p38-delta	§180, §182					human
p38-alpha; p38-alpha iso	§180, §182; §180, §182	2.26	2.02	4.78	4.05	human
p38-alpha; p38-alpha iso	§182; §182	1.90	1.66	3.72	3.16	human
ERK1	§202	0.63	0.39	1.54	1.31	human
ERK1	§202, §204	0.57	0.34	1.49	1.26	human
ERK1	§204	0.12	-0.11	1.09	-1.08	human
ERK5	§219, §221	-2.98	-3.21	-7.88	-9.28	human
ERK5	§221	-2.96	-3.20	-7.78	-9.16	human
JNK2; JNK2 iso2; JNK2 is	§185; §183, §185; §183	6.03	5.79	65.24	55.38	human
JNK2; JNK2 iso2; JNK2 is	§188; §183, §188; §183	5.50	5.26	45.21	38.38	human
JNK2; JNK2 iso2; JNK2 is	§185; §185; §185	3.57	3.34	11.90	10.10	human

Table includes Log2 Ratio (**GREEN BOX**) Normalized Log2 Ratio (**RED BOX**), Raw Fold Change (**BLACK BOX**).and Normalized Fold Change (**BLUE BOX**). Normalized Log 2 Ratios are calculated by subtracting the median Log2 fold change for each comparison. Species of tissue profiled is shown (**green arrow**).



Quantitative Table: Normalization



The scatter plots show relative changes in peptide abundance between treated and control conditions. Each black circle represents the relative abundance for a peptide between the two conditions (treated versus control). The relative abundance values are plotted as $log_2(ratio)$ on the Y-axis and the corresponding intensity of the peptide in the control condition on the X-axis. Panel **A** shows the relative abundance based on raw peptide intensity values. Panel **B** shows the relative abundance of all peptides following a median offset correction (normalization) based on all the relative abundance values in the data set. Plots were generated using sample data not from this project.



Quantitative Table: Normalization







The histogram plots show a distribution of the log_2 (ratio) values of all quantified modified peptides before and after the median offset correction normalization. Before normalization the median log_2 (ratio) is 0.30 and after normalization the median log_2 (ratio) is ZERO. The median normalization is based on all the relative abundance values in the data set. The log_2 (ratio) correction is 0.30 or 1.28-fold offset correction. Plots were generated using sample data not from this project.



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Details Tab contains a redundant list of all validated peptides identified in the study with their accompanying SORCERER metrics.





Protein Name	Site	kD	-7/+7 peptide	Upstream Kinase	Downstream Target
p38-alpha; p38-alpha iso	%182; %182	41; 41	TDDEMTGY*VATRWYR; TDDEMTGY	ASK1, HePTP, MEKK6, M	PKCZ, p38-alpha; PKCZ,
p38-alpha; p38-alpha isc	%182; %182	41; 41	TDDEMTGY*VATRWYR; TDDEMTGY	ASK1, HePTP, MEKK6, M	PKCZ, p38-alpha; PKCZ,
p38-alpha; p38-alpha isc	%182; %182	41; 41	TDDEMTGY*VATRWYR; TDDEMTGY	ASK1, HePTP, MEKK6, M	PKCZ, p38-alpha; PKCZ,
ERK1		43			
ERK1	%202	43	HDHTGFLT*EYVATRW	DUPD1, MEK1, MEK2, MK	KSR, TACE
ERK1	%202, %204	43	HDHTGFLT*EYVATRW, HTGFLTEY*	DUPD1, ERK1, JAK2, Lck	ERK2, KSR, TACE
ERK1	%202, %204	43	HDHTGFLT*EYVATRW, HTGFLTEY*	DUPD1, ERK1, JAK2, Lck	ERK2, KSR, TACE
ERK1	%202, %204	43	HDHTGFLT*EYVATRW, HTGFLTEY*	DUPD1, ERK1, JAK2, Lck	ERK2, KSR, TACE
ERK1	%202, %204	43	HDHTGFLT*EYVATRW, HTGFLTEY*	DUPD1, ERK1, JAK2, Lck	ERK2, KSR, TACE
ERK1	%202, %204	43	HDHTGFLT*EYVATRW, HTGFLTEY*	DUPD1, ERK1, JAK2, Lck	ERK2, KSR, TACE
ERK1	%202, %204	43	HDHTGFLT*EYVATRW, HTGFLTEY*	DUPD1, ERK1, JAK2, Lck	ERK2, KSR, TACE
ERK1	%202, %204	43	HDHTGFLT*EYVATRW, HTGFLTEY*	DUPD1, ERK1, JAK2, Lck	ERK2, KSR, TACE
ERK1	%202, %204	43	HDHTGFLT*EYVATRW, HTGFLTEY*	DUPD1, ERK1, JAK2, Lck	ERK2, KSR, TACE
ERK1	%202, %204	43	HDHTGFLT*EYVATRW, HTGFLTEY*	DUPD1, ERK1, JAK2, Lck	ERK2, KSR, TACE
ERK1	%202, %204	43	HDHTGFLT*EYVATRW, HTGFLTEY*	DUPD1, ERK1, JAK2, Lck	ERK2, KSR, TACE
ERK1	%204	43	HTGFLTEY*VATRWYR	DUPD1, ERK1, JAK2, Lck	ERK2, KSR, TACE
ERK1	%204	43	HTGFLTEY*VATRWYR	DUPD1, ERK1, JAK2, Lck	ERK2, KSR, TACE
ERK1	%204	43	HTGFLTEY*VATRWYR	DUPD1, ERK1, JAK2, Lck	ERK2, KSR, TACE
ERK1	%204	43	HTGFLTEY*VATRWYR	DUPD1, ERK1, JAK2, Lck	ERK2, KSR, TACE
ERK1	%204	43	HTGFLTEY*VATRWYR	DUPD1, ERK1, JAK2, Lck	ERK2, KSR, TACE

 The Details Tab includes the peptide sequence 7 amino acids to each side of the modified residue (BLUE BOX), the molecular weight of the protein from which the peptide is derived (BLACK BOX), and upstream kinases and downstream targets of the protein/site (RED BOX).





			MS2 Spectro	um Number		Peak Apex MS Spectrum Number			
Protein Name	Site	CST Cell Line (CS15316)	CST Cell Line (CS15317)	HeLa Control (CS15318)	HeLa Control (CS15319)	CST Cell Line (CS15316)	CST Cell Line (CS15317)	HeLa Control (CS15318)	HeLa Control (CS15319)
p38-alpha; p38-alp	%182; %182			5065	5048		5199	5067	5034
p38-alpha; p38-alp	%182; %182								3072
p38-alpha; p38-alp	%182; %182								3072
ERK1									20606
ERK1	%202	12843	12928	12373					
ERK1	%202, %204	11228	11338			11228	11372		
ERK1	%202, %204	11410	11520	11023	11143	11595	11561	11036	11156
ERK1	%202, %204	11595				11595	11561	11036	11156
ERK1	%202, %204	11389	11730	10985	11116	11471	11500	11036	11156
ERK1	%202, %204	11900	11500	11058		11471	11500	11036	11156
ERK1	%202, %204	11620	12008			11471	11500	11036	11156
ERK1	%202, %204		24461			11471	11500	11036	11156
ERK1	%202, %204					11471	11561	11036	11156
ERK1	%202, %204	10219	10298			10232	10298		9917
ERK1	%202, %204	10193	10272	9827	9912	10232	10272	9839	9917
ERK1	%204		10447			10358	10430	9944	10022
ERK1	%204	10300	10394	9905	10007	10337	10430	9944	10043
ERK1	%204				12494	10337	10430	9944	10043
ERK1	%204					10358	10430	9944	10043
ERK1	%204					8930	9023	8579	8636

The Details Tab includes the MS/MS (or MS2, **BLUE BOX**) and MS (**RED BOX**) scan numbers for each peptide assignment. Experimental details that are mainly used as a reference to the raw data.





			Potonti	on Time			Ye	orr	
Protein Name	Site	CST Cell Line (CS15316)	CST Cell Line (CS15317)	HeLa Control (CS15318)	HeLa Control (CS15319)	CST Cell Line (CS15316)	CST Cell Line (CS15317)	HeLa Control (CS15318)	HeLa Control (CS15319)
p38-alpha; p38-alp	%182; %182		31.53	31.77	31.33			4.368	4.273
p38-alpha; p38-alp	%182; %182				24.24				
p38-alpha; p38-alp	%182; %182				24.24				
ERK1					81.89				
ERK1	%202					3.027	3.378	3.344	
ERK1	%202, %204	52.62	52.43			3.213	3.649		
ERK1	%202, %204	53.28	53.02	53.24	53.04	3.634	3.594	3.405	3.846
ERK1	%202, %204	53.28	53.02	53.24	53.04	3.162			
ERK1	%202, %204	53.28	53.02	53.24	53.04	3.875	3.797	4.036	3.741
ERK1	%202, %204	53.28	53.02	53.24	53.04	3.749	3.533	3.107	
ERK1	%202, %204	53.28	53.02	53.24	53.04	3.630	2.881		
ERK1	%202, %204	53.28	53.02	53.24	53.04		2.447		
ERK1	%202, %204	53.28	53.02	53.24	53.04				
ERK1	%202, %204	49.25	49.00		48.95	3.674	4.565		
ERK1	%202, %204	49.25	49.00	49.22	48.95	3.112	4.211	3.083	3.397
ERK1	%204	49.71	49.44	49.60	49.31		3.379		
ERK1	%204	49.63	49.44	49.60	49.38	4.398	4.825	4.870	5.324
ERK1	%204	49.63	49.44	49.60	49.38				3.373
ERK1	%204	49.71	49.44	49.60	49.38				
ERK1	%204	44.76	44.64	44.78	44.60				

The Details Tab includes the Retention Time (**black box**), and Xcorr values (or Sorcerer cross-correlation value, **red box**) for each peptide assignment. Experimental details are used as a reference and measure of confidence for the peptide assignment.





			Mass Accu	racy (ppm)			Delt	aCN	
Protein Name	Site	CST Cell Line (CS15316)	CST Cell Line (CS15317)	HeLa Control (CS15318)	HeLa Control (CS15319)	CST Cell Line (CS15316)	CST Cell Line (CS15317)	HeLa Control (CS15318)	HeLa Control (CS15319)
p38-alpha; p38-alp	%182; %182			-0.2663	-0.0461			0.5290	0.4860
p38-alpha; p38-alp	%182; %182								
p38-alpha; p38-alp	%182; %182								
ERK1									
ERK1	%202	-0.4172	-0.4975	-1.0086		0.2900	0.3630	0.3240	
ERK1	%202, %204	-0.1462	-0.1462			0.1430	0.3570		
ERK1	%202, %204	-0.2881	-0.1595	0.4147	-0.2757	0.4100	0.3680	0.3540	0.4020
ERK1	%202, %204	-0.2890				0.2700			
ERK1	%202, %204	2.0803	1.8483	-0.4588	-0.5578	0.3140	0.3370	0.4260	0.3170
ERK1	%202, %204	-0.5621	1.8123	0.7278		0.3190	0.2460	0.2350	
ERK1	%202, %204	2.1347	-0.5827			0.2810	0.0990		
ERK1	%202, %204		-0.3181				0.0560		
ERK1	%202, %204								
ERK1	%202, %204	-0.2957	-0.0620			0.3170	0.5120		
ERK1	%202, %204	-0.3085	-0.2076	-0.1904	-0.4683	0.2700	0.4100	0.3240	0.3700
ERK1	%204		-0.0282				0.3480		
ERK1	%204	-0.3110	-0.4376	-0.5473	-0.6641	0.4120	0.3930	0.3400	0.4160
ERK1	%204				-1.4709				0.4010
ERK1	%204								
ERK1	%204								

The Details Tab includes the measured peptide mass accuracy (**BLACK BOX**), and DeltaCN value (**BLUE BOX**) for each peptide assignment. Experimental details that are used as a reference and measure of confidence to the peptide assignment.





			R	sp		PP Probability				
Protein Name	Site	CST Cell Line (CS15316)	CST Cell Line (CS15317)	HeLa Control (CS15318)	HeLa Control (CS15319)	CST Cell Line (CS15316)	CST Cell Line (CS15317)	HeLa Control (CS15318)	HeLa Control (CS15319)	
p38-alpha; p38-alp	%182; %182			1	1			1.000	1.000	
p38-alpha; p38-alp	%182; %182									
p38-alpha; p38-alp	%182; %182									
ERK1										
ERK1	%202	1	1	1		1.000	1.000	1.000		
ERK1	%202, %204	2	1			0.862	0.999			
ERK1	%202, %204	1	1	1	1	1.000	1.000	1.000	1.000	
ERK1	%202, %204	1				1.000				
ERK1	%202, %204	1	1	1	1	1.000	1.000	1.000	1.000	
ERK1	%202, %204	1	1	1		1.000	1.000	1.000		
ERK1	%202, %204	1	23			1.000	0.995			
ERK1	%202, %204		1				0.992			
ERK1	%202, %204									
ERK1	%202, %204	1	1			0.992	1.000			
ERK1	%202, %204	9	1	12	1	0.975	1.000	0.990	0.999	
ERK1	%204		1				1.000			
ERK1	%204	1	1	1	1	1.000	1.000	1.000	1.000	
ERK1	%204				14				1.000	
ERK1	%204									
ERK1	%204									

The Details Tab includes the Rsp value (**BLUE BOX**), the Peptide Prophet Probability (**RED BOX**) for each peptide assignment. Experimental details that are used as a reference and measure of confidence to the peptide assignment.





Qualitative Table: MS/MS Assignments

				MS2 Spectrum Number					
Protein Name	Site	Peptide	Charge	CST Cell Line (CS15316)	CST Cell Line (CS15317)	HeLa Control (CS15318)	HeLa Control (CS15319)		
ERK2	%185, %187	DPDHDHTGFLT*EY*VATR	2	11236	11358				
ERK2	%185, %187	DPDHDHTGFLT*EY*VATR	2	11250					
ERK2	%185, %187	DPDHDHTGFLT*EY*VATR	3		11331				
ERK2	%185, %187	PDHDHTGFLT*EY*VATR	2	10444	10523				
ERK2	%185, %187	PDHDHTGFLT*EY*VATR	3	10443	10516	10095	10150		
ERK2	%185, %187	VADPDHDHTGFLT*EY*VATR	2	11081	11170	10481	10591		
ERK2	%185, %187	VADPDHDHTGFLT*EY*VATR	2	10843	10935				
ERK2	%185, %187	VADPDHDHTGFLT*EY*VATR	3	11052	11268	10662	10717		
ERK2	%185, %187	VADPDHDHTGFLT*EY*VATR	3	11521	11504	10447	10551		
ERK2	%185, %187	VADPDHDHTGFLT*EY*VATR	3	11753	11037	10449	11028		
ERK2	%185, %187	VADPDHDHTGFLT*EY*VATR	3	11284	11736	10903	10898		
ERK2	%185, %187	VADPDHDHTGFLT*EY*VATR	3	10822	11976	10798	10891		
ERK2	%185, %187	VADPDHDHTGFLT*EY*VATR	3	24437	10815				
ERK2	%185, %187	VADPDHDHTGFLT*EY*VATR	3	12011	12229				
ERK2	%185, %187	VADPDHDHTGFLT*EY*VATR	3		12483				

The sites, T185/Y187, are assigned 41 times in the first four curation sets (**RED BOX**, count number of MS2 entries).

The site is represented in 3 overlapping sequences (incomplete digestion, red arrow).

The phosphorylated peptide assignments are seen in multiple charge states (blue arrow).





Informatics Table

PTMSCAN DIRECT: MULTIPATHWAY V2.0 INFORMATICS TABLE

 Table #3: CST Cell Line Control, HeLa Cells; Trypsin Digest; Multipathway Reagent V2.0

 Samples: CST Cell Line Control = CS 15316, 15317; HeLa Control = CS 15318, 15319; HeLa + UV = CS 15320, 15321

 Legend: * - phosphorylation, ^ - multiple protein IDs for one peptide

6000 00m0	cracios	protoin typo	protoin function description	ontron conoid	NCBT acc		
gene_name	species	protein_type	protein_runction_description	entrez_geneiu	NCDI_acc	NCDI_site	SWISSPROT_acc
CRIP2	human	Adhesion or extracellular matrix p	Interacts with TGFB1I1. Note: This descrip	1397	NP_001303	Y75	P52943
CRIP2	human	Adhesion or extracellular matrix p	Interacts with TGFB1I1. Note: This descrip	1397	NP_001303	Y77	P52943
CRIP2	human	Adhesion or extracellular matrix p	Interacts with TGFB1I1. Note: This descrip	1397	NP_001303	S74	P52943
CRIP2	human	Adhesion or extracellular matrix p	Interacts with TGFB1I1. Note: This descrip	1397	NP_001303	Y77	P52943
SBN01	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	T818	
SBN01	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	S817	
SBN01	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	S816	
SBN01	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	S816	
SBN01	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	S816	
SBN01	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	T834	
SBN01	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	S822	
SBN01	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	S814	
SBN01	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	S814	
SBN01	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	S814	
SBN01	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	S814	
SBN01	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	S836	
SBN01	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	T834	
SBN01	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	S822	
PLEKHG4	human	G protein or regulator	Possible role in intracellular signaling and	25894	NP_056247	S677	Q58EX7

The informatics table contains specific information for each identified peptide: Gene ontology protein type, accession numbers, site designation, PubMed references, ScanSite, Upstream and downstream genes. The table also includes statistical data within the context of this study, peptide count in each curation set and in each cell type.





Sampling of pathways monitored by PTMScan Multipathway using Ingenuity, Molecular Mechanisms of Cancer. Proteins that are monitored by PTMScan Direct Multipathway are highlighted in ORANGE.





Sampling of pathways monitored by PTMScan Multipathway using Ingenuity, Bcr Signaling. Proteins that are monitored by PTMScan Direct Multipathway are highlighted in ORANGE.





Sampling of pathways monitored by PTMScan Multipathway using Ingenuity, Erb Signaling. Proteins that are monitored by PTMScan Direct Multipathway are highlighted in ORANGE.





Sampling of pathways monitored by PTMScan Multipathway using Ingenuity, Growth Hormone Signaling. Proteins that are monitored by PTMScan Direct Multipathway are highlighted in ORANGE.





Sampling of pathways monitored by PTMScan Multipathway using Ingenuity, PI3K/Akt Signaling. Proteins that are monitored by PTMScan Direct Multipathway are highlighted in ORANGE.



Follow-up for PTMScan Data

- 1. Filter candidates based on MS and MS/MS metrics.
 - Intensity, % CV, MS/MS scoring, etc.
 - See "Prioritizing Follow-up Candidates" document.
- 2. Perform initial biochemical follow-up.
 - Western blots, IP-western, siRNA studies, etc.
- 3. Consider LCMS-based follow-up
 - Synthetic peptides (for MS/MS identification)
 - Different cell lines/tissues, doses, times, treatments, etc for PTMScan analysis



Cell Signaling Technology Consultation

Study Design

Discuss details of study, samples, sample preparation.

Final Results

Provide final results, report, highlight sites of interest.

Follow-up

Review final results, discuss additional experiments.

