



# Cell Signaling Technology

PTMScan Direct: Multipathway v2.0

Proteomics Service Group

January 14, 2013

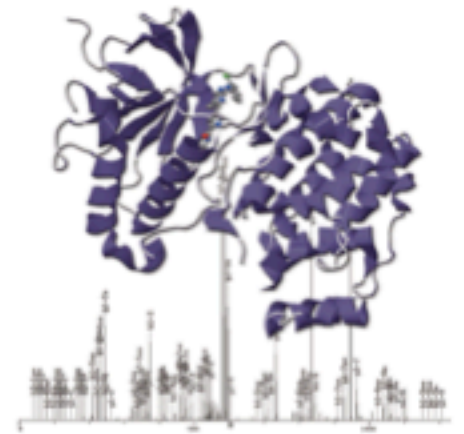


Cell Signaling  
TECHNOLOGY®



# 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

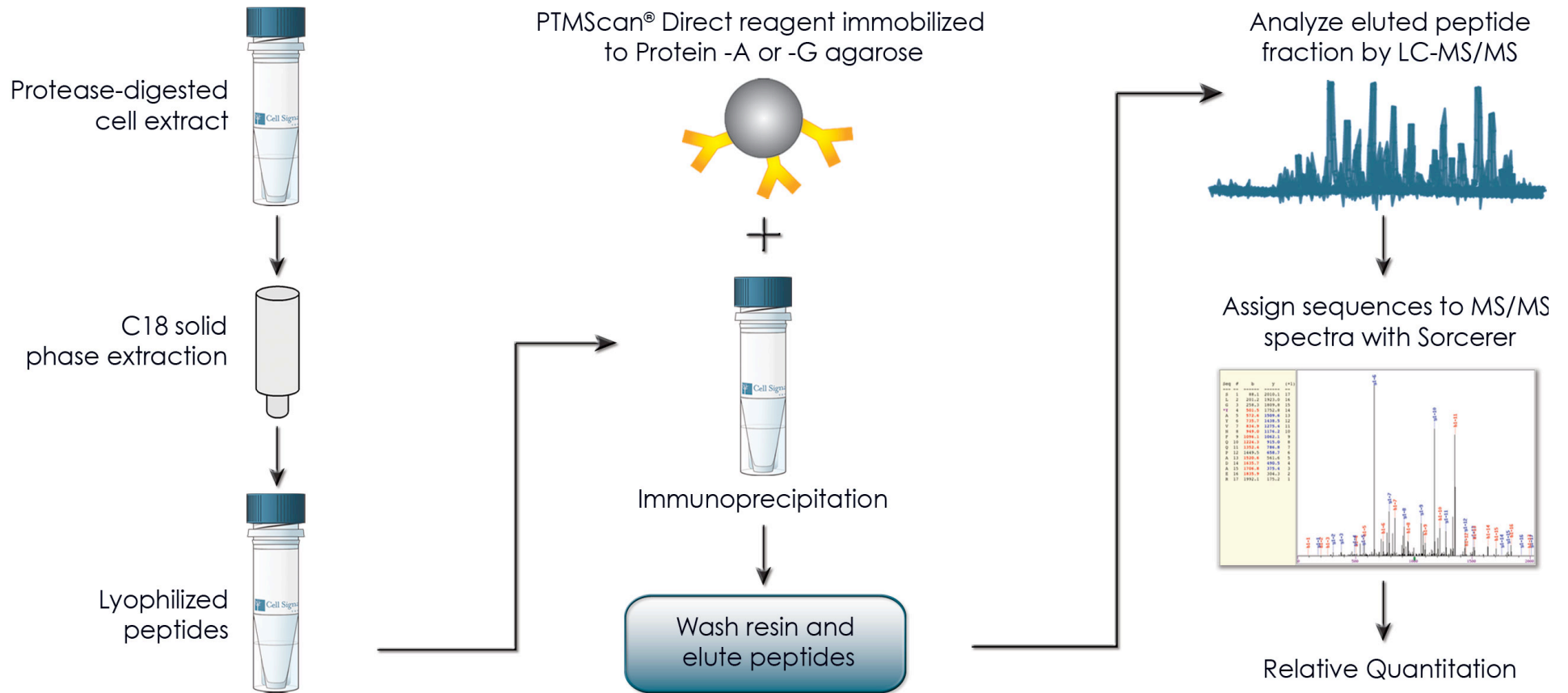
Label-free quantitation of phosphorylated peptide intensities (**Tables 1,2**).

Informatics table (**Table 3**).





# PTMScan Direct Method





# LC Methods

Peptides were loaded directly onto a 10 cm x 75  $\mu\text{m}$  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)



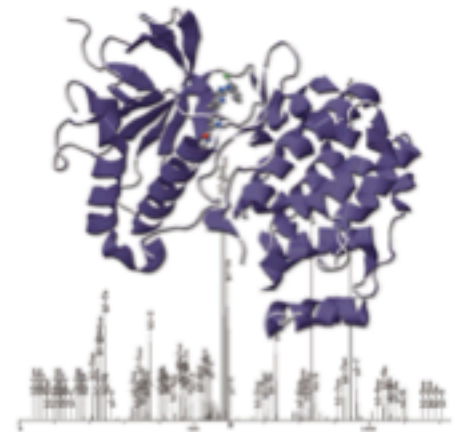


# Informatics

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

Gene Name	Protein Name	Site
AKT1	Akt1	305
AKT1	Akt1	§308
AKT1	Akt1	§308, §315
AKT1	Akt1	312
AKT1	Akt1	§315
AKT1	Akt1	§473
AKT1	Akt1	§473, 479
AKT1	Akt1	§474
AKT1	Akt1	§474, 479
AKT2	Akt2	§309
AKT2	Akt2	§309, 313
AKT2	Akt2	313
AKT2	Akt2	§474
AKT2	Akt2	§474, §478
AKT2	Akt2	475
AKT2	Akt2	475, §478
AKT2	Akt2	§476, §478

## Gene Name (**siRNA Product Link**)

◀ pathway ▼ more info ▼ application references ■ datasheet PDF ■ MSDS PDF ■ protocols

**SignalSilence® PTEN siRNA I #6251**

PhosphoSitePlus® protein, site, and accession data: [PTEN](#)

## Protein Name (**Total Antibody Product Link**)

◀ pathway ▼ more info ▼ application references ■ datasheet PDF ■ MSDS PDF ▼ protocols

**Akt (pan) (11E7) Rabbit mAb #4685**

PhosphoSitePlus® protein, site, and accession data: [Akt1](#), [Akt2](#), [Akt3](#)

## Site (**Site Specific Antibody Product Link**)

◀ pathway ▼ more info ▼ application references ■ datasheet PDF ■ MSDS PDF ▼ protocols

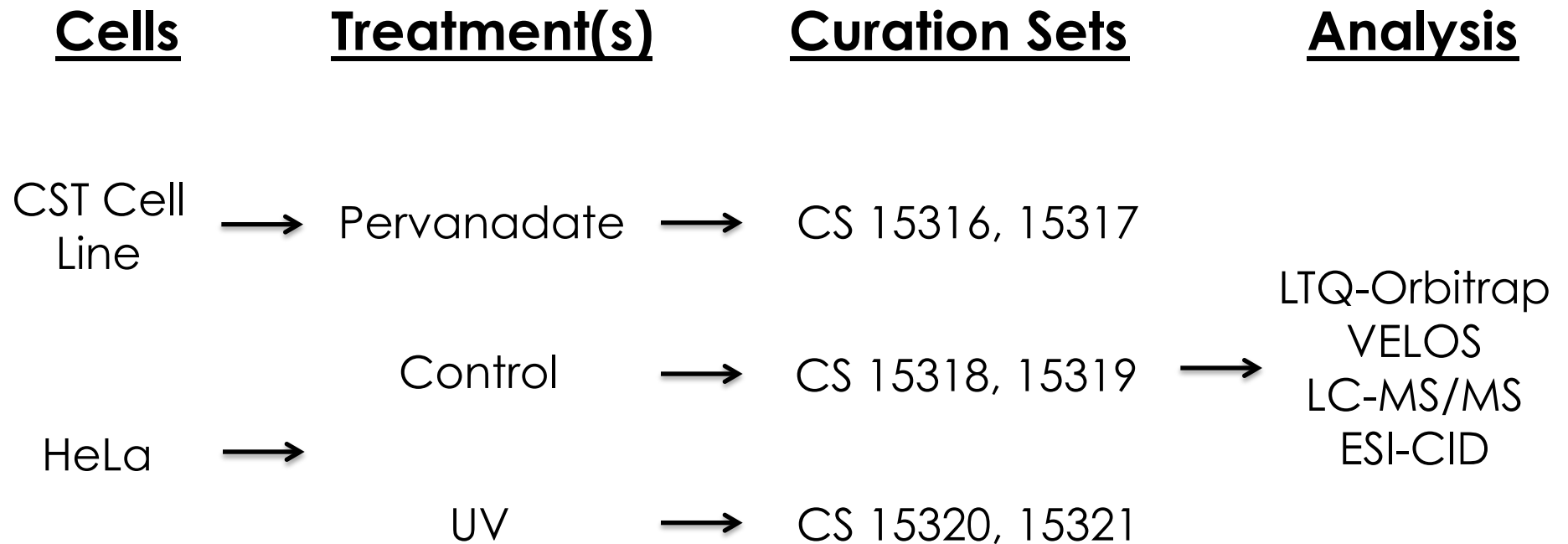
**Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb #4060**

PhosphoSitePlus® protein, site, and accession data: [Akt1](#), [Akt2](#), [Akt3](#)

**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

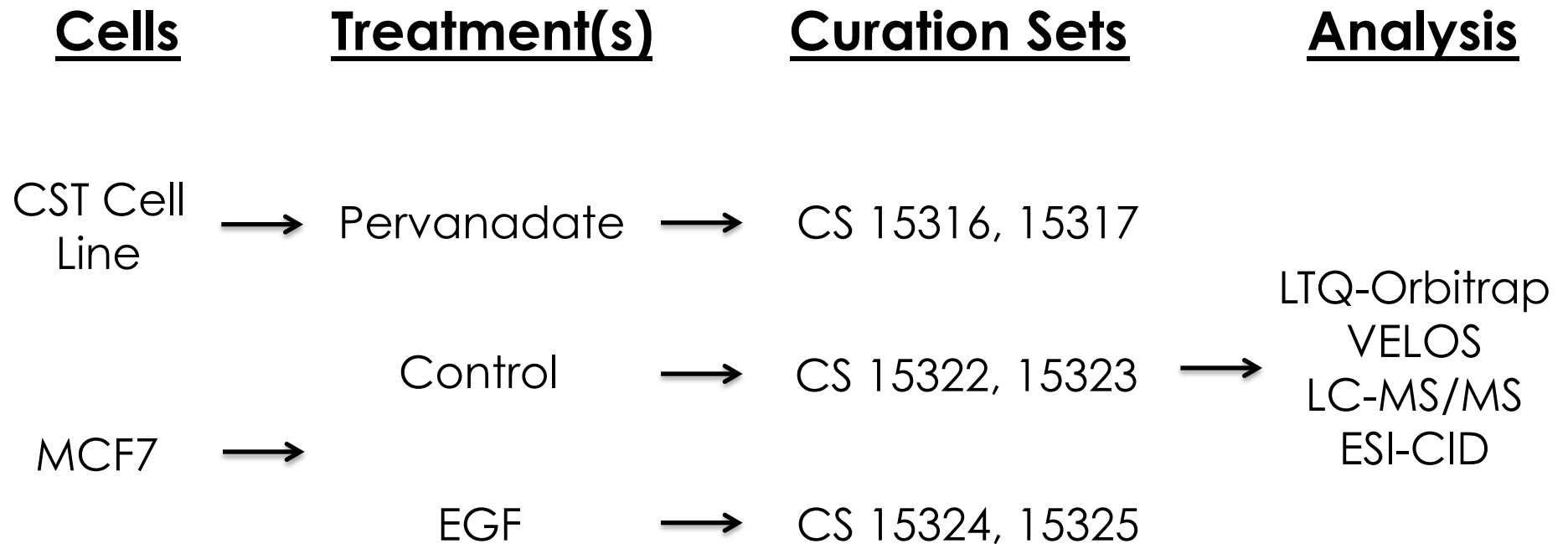


3 samples, duplicate injections = 6 LC-MS/MS experiments





# Experimental Flow Diagram: Multipathway



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



# Qualitative Results: HeLa -/+ UV

Experiment	Cell Type	Treatment	Antibody	CS	Protease	Validated Peptide Assignments	
						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** non-redundant 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.



# Qualitative Results: MCF7 -/+ EGF

Experiment	Cell Type	Treatment	Antibody	CS	Protease	Validated Peptide Assignments	
						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** non-redundant 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

The screenshot shows an Excel spreadsheet with the following table structure:

Column Name	Definition	In Details	In Summary
Accession	is the UniProt accession number for the assigned protein	Y	Y
Calculated m/z	is the calculated, accurate mass-to-charge ratio of the identified peptide from the primary amino acid sequence and associated modifications	Y	Y
Charge	is the observed charge state for the identified peptide	Y	Y
Count in Details	is the number of times the designated protein - modification site is represented from all peptide assignments in the Details tab. These redundant assignments can be as a result of multiple charge states, presence of oxidized methionine and overlapping sequence.		Y
Delta Cn	is the delta Xcorr value. In general, a Delta Cn of 0.1 or greater is good	Y	
Description	is the FASTA database description of the protein entry	Y	Y
Fold Change	is the relative fold-change between the integrated peak area of the control (denominator) and experimental (numerator) conditions. A negative value indicates the peptide is more abundant in the control condition		Y
Gene Name	is the gene name for the assigned protein. In some cases, the Gene Name is hyperlinked (when in blue text) when there are protein-specific antibodies available at CST	Y	Y
kD	is the translated molecular weight of the assigned protein	Y	
Mass Accuracy (ppm)	is the mass measurement error obtained between the observed and theoretical mass of the assigned peptide	Y	
MS2 Spectrum Number	is the spectrum number of the MS2 channel of the raw LC-MS/MS data file	Y	

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



# Table Overview: Details Tab

Table1\_Multipathway\_V2\_HeLa\_UV\_FINAL.xlsx

Search in Sheet

Calibri (Body) 11 B I U

Home Layout Tables Charts SmartArt Formulas Data Review

Table #1: 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, # - oxidized methionine, § - published site, Blue Text - CST siRNA/antibody a

Index	Row Index	Protein Type	Gene Name	Protein Name	Site
1	<b>Adaptor/scaffold</b>				
2	1	Adaptor/scaffold	ABI2; ABI2	Abi-2; Abi-2 iso2	%213; %207
3	2	Adaptor/scaffold	ABI2; ABI2	Abi-2; Abi-2 iso2	%213; %207
4	3	Adaptor/scaffold	ABI2; ABI2	Abi-2; Abi-2 iso2	%213; %207
5	4	Adaptor/scaffold	ABI2; ABI2	Abi-2; Abi-2 iso2	%213, %216; %207, %21
6	5	Adaptor/scaffold	ABI2; ABI2	Abi-2; Abi-2 iso2	%213, %216; %207, %21
7	6	Adaptor/scaffold	ABI2; ABI2	Abi-2; Abi-2 iso2	%213, %216; %207, %21
8	7	Adaptor/scaffold	BCAR1	P130Cas	115
9	8	Adaptor/scaffold	BCAR1	P130Cas	115
10	9	Adaptor/scaffold	BCAR1	P130Cas	115
11	10	Adaptor/scaffold	BCAR1	P130Cas	%128
12	11	Adaptor/scaffold	BCAR1	P130Cas	%385
13	12	Adaptor/scaffold	CAV1	caveolin-1	%14

Column Definitions Details Summary Fold Change by Protein Type

Count Numbers=2903

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

# Table Overview: Summary Tab

**PTMSCAN DIRECT: MULTIPATHWAY V2.0 RESULTS**

**Table #1: 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, # - oxidized methionine, § - published site, Blue Text - CST siRNA/antibody a**

Index	Index in Detail	Normalized Fold Change	UV : Control	Max Intensity	Area or Height	Max % CV	Gene Name	Protein Name	Site
1	<b>Adaptor/scaffold</b>								
2	2	1.1		303,305	H	13.5	ABI2; ABI2	Abi-2; Abi-2 iso2	§213; §207
3	5	-		-	H	-	ABI2; ABI2	Abi-2; Abi-2 iso2	§213, §216; §207,
4	7	1.5		52,004	H	21.1	BCAR1	P130Cas	115
5	11	1.4		<b>469,595</b>	A	13.2	BCAR1	P130Cas	§385
6	14	-		-	H	-	CAV1	caveolin-1	§6, §14
7	16	-		-	H	-	CAV1	caveolin-1	§6, §14, §25
8	17	-		-	H	-	CAV1	caveolin-1	§6, §15
9	18	-		-	H	-	CAV1	caveolin-1	§6, §15, §25
10	19	-		-	H	-	CD2AP	CD2AP	539, 546, §551
11	20	-		-	A	-	CD2AP	CD2AP	541, §551
12	23	-		-	A	-	CD2AP	CD2AP	§542, §548
13	25	-		-	H	-	CD2AP	CD2AP	§542, §548, §551
14	27	-		-	H	-	CD2AP	CD2AP	§542, §550, §551

**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

**Table #1: 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, # - oxidized methionine, § - published site, **Blue Text** - CST siRNA/antibody a

Index	Index in Detail	UV : Control	Max Intensity	Area or Height	Max % CV	Gene Name	Protein Name	Site
1	<b>Adaptor/scaffold</b>							
2	41	7.6	184,192	A	39.7	CD2AP	CD2AP	550
3	60	5.9	15,605,340	A	2.5	HGS	Hrs	239
4	66	5.9	15,605,340	A	2.5	HGS	Hrs	§240
5	<b>Adhesion or extracellular matrix protein</b>							
6	249	-16.8	963,002	A	53.6	CTNNB1	CTNNB1	§675, §681
7	<b>Chromatin, DNA-binding, DNA repair or DNA replication protein</b>							
8	418	6.0	2,112,601	A	11.1	BLM	BLM	74
9	421	-4.7	431,876	H	20.2	H3F3A; hCG1639761; HI	H3F3A; hCG1639761; HI	
10	425	-3.0	174,960	A	20.8	NFKBIA	IkB-alpha	§36
11	437	4.8	21,618,781	A	10.2	SBNO1	SBNO1	§815
12	442	31.7	924,829	A	25.4	SBNO1	SBNO1	§815, 823
13	449	4.5	18,547,430	A	11.0	SBNO1	SBNO1	§817
14	452	31.7	817,822	A	25.4	SBNO1	SBNO1	§817, 822

**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**).



# Table Overview: Summary Tab

**PTMSCAN DIRECT: MULTIPATHWAY V2.0 RESULTS**

**Table #1: 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, # - oxidized methionine, § - published site, **Blue Text** - CST siRNA/antibody a

Index	Index in Detail	Normalized Fold Change	UV : Control	Max Intensity	Area or Height	Max % CV	Gene Name	Protein Name	Site
1	<b>Adaptor/scaffold</b>								
2	2	1.1		303,305	H	13.5	ABI2; ABI2	Abi-2; Abi-2 iso2	§213; §207
3	5	-		-	H	-	ABI2; ABI2	Abi-2; Abi-2 iso2	§213, §216; §207,
4	7	1.5		52,004	H	21.1	BCAR1	P130Cas	115
5	11	1.4		<b>469,595</b>	A	13.2	BCAR1	P130Cas	§385
6	14	-		-	H	-	CAV1	caveolin-1	§6, §14
7	16	-		-	H	-	CAV1	caveolin-1	§6, §14, §25
8	17	-		-	H	-	CAV1	caveolin-1	§6, §15
9	18	-		-	H	-	CAV1	caveolin-1	§6, §15, §25
10	19	-		-	H	-	CD2AP	CD2AP	539, 546, §551
11	20	-		-	A	-	CD2AP	CD2AP	541, §551
12	23	-		-	A	-	CD2AP	CD2AP	§542, §548
13	25	-		-	H	-	CD2AP	CD2AP	§542, §548, §551
14	27	-		-	H	-	CD2AP	CD2AP	§542, §550, §551

**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.



# Quantitative Table: Summary Tab

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



# Quantitative Table: Summary Tab

Index	Index in Detail	Normalized Fold Change		Area or Height	Max % CV			
		UV : Control	Max Intensity					
470	1077	-1.2	523,753	H	16.5			
471	1084	1.7	<b>12,803,987</b>	A	2.9			
472	1092	1.5	20,993,600	H	37.9			
473	1104	1.1	90,295	H	39.8			
474	1109	28.4	<b>459,188</b>	H	23.4			
475	1127	15.2	<b>715,846</b>	A	34.7			
476	1129	1.4	124,965	H	14.4			
477	1133	2.1	<b>1,227,660</b>	A	12.4			
478	1134	1.6	350,227	H	14.6			
479	1142	-	-	H	-			
480	1144	4.1	<b>1,902,560</b>	H	5.6			
481	1159	3.2	<b>1,642,382</b>	A	1.7			
482	1163	1.3	<b>155,500</b>	H	7.7	MAPK3	ERK1	§202
483	1167	1.3	3,310,535	H	19.2	MAPK3	ERK1	§202, §204
484	1175	-1.1	<b>780,041</b>	H	2.9	MAPK3	ERK1	§204
485	1179	-9.3	<b>347,096</b>	A	13.0	MAPK7	ERK5	§219, §221
486	1183	-9.2	<b>7,367,057</b>	A	13.9	MAPK7	ERK5	§221
487	1189	55.4	<b>36,814,462</b>	A	12.9	MAPK9; MAPK9; MAPK9	JNK2; JNK2 iso2; JNK2 iso3	§185; §183, §185; §183
488	1218	38.4	<b>25,265,503</b>	A	41.3	MAPK9; MAPK9; MAPK9	JNK2; JNK2 iso2; JNK2 iso3	§188; §183, §188; §183
489	1219	10.1	<b>517,181</b>	A	19.4	MAPK9; MAPK9; MAPK9	JNK2; JNK2 iso2; JNK2 iso3	§185; §185; §185

Fold changes should be considered in the context of intensity – the higher the intensity, the more accurate the fold change.

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**)



# Quantitative Table: Summary Tab

Index	Index in Detail	Normalized Fold Change		Area or Height	Max % CV		
		UV : Control	Max Intensity				
470	1077	-1.2	523,753	H			
471	1084	1.7	<b>12,803,987</b>	A	2.9	MAPK1	
472	1092	1.5	20,993,600	H	37.9	MAPK1	ERK2 §185, §187
473	1104	1.1	90,295	H	39.8	MAPK1	ERK2 §187
474	1109	28.4	<b>459,188</b>	H	23.4	MAPK10; MAPK10; MAPK10	JNK3; JNK3 iso2; JNK1; JNK2 §221, §223; §183, §185
475	1127	15.2	<b>715,846</b>	A	34.7	MAPK10; MAPK10; MAPK10	JNK3; JNK3 iso2; JNK1; JNK2 §223; §223; §185; §185
476	1129	1.4	124,965	H	14.4	MAPK12	p38-gamma §183
477	1133	2.1	<b>1,227,660</b>	A	12.4	MAPK12	p38-gamma §183, §185
478	1134	1.6	350,227	H	14.6	MAPK12	p38-gamma §185
479	1142	-	-	H	-	MAPK13	p38-delta §180, §182
480	1144	4.1	<b>1,902,560</b>	H	5.6	MAPK14; MAPK14	p38-alpha; p38-alpha iso2 §180, §182; §180, §182
481	1159	3.2	<b>1,642,382</b>	A	1.7	MAPK14; MAPK14	p38-alpha; p38-alpha iso2 §182; §182
482	1163	1.3	<b>155,500</b>	H	7.7	MAPK3	ERK1 §202
483	1167	1.3	3,310,535	H	19.2	MAPK3	ERK1 §202, §204
484	1175	-1.1	<b>780,041</b>	H	2.9	MAPK3	ERK1 §204
485	1179	-9.3	<b>347,096</b>	A	13.0	MAPK7	ERK5 §219, §221
486	1183	-9.2	<b>7,367,057</b>	A	13.9	MAPK7	ERK5 §221
487	1189	55.4	<b>36,814,462</b>	A	12.9	MAPK9; MAPK9; MAPK9	JNK2; JNK2 iso2; JNK2 iso3 §185; §183, §185; §183
488	1218	38.4	<b>25,265,503</b>	A	41.3	MAPK9; MAPK9; MAPK9	JNK2; JNK2 iso2; JNK2 iso3 §188; §183, §188; §183
489	1219	10.1	<b>517,181</b>	A	19.4	MAPK9; MAPK9; MAPK9	JNK2; JNK2 iso2; JNK2 iso3 §185; §185; §185

Peak measurements can be area or height.

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**)



# Quantitative Table: Summary Tab

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

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**)



# Quantitative Table: Summary Tab

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



# Quantitative Table: Summary Tab

Protein Name	Site	Description	Accession	URL
ERK2		mitogen-activated protein kinase 1	P28482	<a href="http://www.phosphosite.org">http://www.phosphosite.org</a>
ERK2	§185	mitogen-activated protein kinase 1	P28482	<a href="http://www.phosphosite.org">http://www.phosphosite.org</a>
ERK2	§185, §187	mitogen-activated protein kinase 1	P28482	<a href="http://www.phosphosite.org">http://www.phosphosite.org</a>
ERK2	§187	mitogen-activated protein kinase 1	P28482	<a href="http://www.phosphosite.org">http://www.phosphosite.org</a>
JNK3; JNK3 iso2; JNK1; JNK1 iso2	§221, §223; §183, §185	mitogen-activated protein kinase 10 isoform 1	P53779; P53779-2; P45984	<a href="http://www.phosphosite.org">http://www.phosphosite.org</a>
JNK3; JNK3 iso2; JNK1; JNK1 iso2	§223; §223; §185; §185	mitogen-activated protein kinase 10 isoform 1	P53779; P53779-2; P45984	<a href="http://www.phosphosite.org">http://www.phosphosite.org</a>
p38-gamma	§183	mitogen-activated protein kinase 12	P53778	<a href="http://www.phosphosite.org">http://www.phosphosite.org</a>
p38-gamma	§183, §185	mitogen-activated protein kinase 12	P53778	<a href="http://www.phosphosite.org">http://www.phosphosite.org</a>
p38-gamma	§185	mitogen-activated protein kinase 12	P53778	<a href="http://www.phosphosite.org">http://www.phosphosite.org</a>
p38-delta	§180, §182	mitogen-activated protein kinase 13	O15264	<a href="http://www.phosphosite.org">http://www.phosphosite.org</a>
p38-alpha; p38-alpha iso2	§180, §182; §180, §182	mitogen-activated protein kinase 14 isoform 1	Q16539; Q16539-2	<a href="http://www.phosphosite.org">http://www.phosphosite.org</a>
p38-alpha; p38-alpha iso2	§182; §182	mitogen-activated protein kinase 14 isoform 1	Q16539; Q16539-2	<a href="http://www.phosphosite.org">http://www.phosphosite.org</a>
ERK1	§202	mitogen-activated protein kinase 3 isoform 2	P27361	<a href="http://www.phosphosite.org">http://www.phosphosite.org</a>
ERK1	§202, §204	mitogen-activated protein kinase 3 isoform 2	P27361	<a href="http://www.phosphosite.org">http://www.phosphosite.org</a>
ERK1	§204	mitogen-activated protein kinase 3 isoform 2	P27361	<a href="http://www.phosphosite.org">http://www.phosphosite.org</a>
ERK5	§219, §221	mitogen-activated protein kinase 7 isoform 1	Q13164	<a href="http://www.phosphosite.org">http://www.phosphosite.org</a>
ERK5	§221	mitogen-activated protein kinase 7 isoform 1	Q13164	<a href="http://www.phosphosite.org">http://www.phosphosite.org</a>
JNK2; JNK2 iso2; JNK2 iso3	§185; §183, §185; §183	mitogen-activated protein kinase 9 isoform JNK2 gamma	P45984; P45984-2; P45984	<a href="http://www.phosphosite.org">http://www.phosphosite.org</a>
JNK2; JNK2 iso2; JNK2 iso3	§188; §183, §188; §183	mitogen-activated protein kinase 9 isoform JNK2 gamma	P45984; P45984-2; P45984	<a href="http://www.phosphosite.org">http://www.phosphosite.org</a>
JNK2; JNK2 iso2; JNK2 iso3	§185; §185; §185	mitogen-activated protein kinase 9 isoform JNK2 gamma	P45984; P45984-2; P45984	<a href="http://www.phosphosite.org">http://www.phosphosite.org</a>



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**).



# Quantitative Table: Summary Tab

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	VADPDHDHTGFLT*EY*VATR	3	768.6505	86
ERK2	§187	VADPDHDHTGFLTEY*VATR	4	556.7481	32
JNK3; JNK3 iso2; JNK1; JNK1 iso2	§221, §223; §183, §185	TAGTSFM#M#T*Y*VVTR	2	927.3662	86
JNK3; JNK3 iso2; JNK1; JNK1 iso2	§223; §223; §185; §185	TAGTSFM#MTPY*VVTR	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 iso2	§180, §182; §180, §182	HTDDEMT*GY*VATR	2	828.2941	84
p38-alpha; p38-alpha iso2	§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 iso2	§185; §183, §185; §183	TACTNFM#MT*PY*VVTR	2	984.3788	127
JNK2; JNK2 iso2; JNK2 iso2	§188; §183, §188; §183	TACTNFM#M#T*PYVVT*R	3	661.9199	3
JNK2; JNK2 iso2; JNK2 iso2	§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.





# Quantitative Table: Summary Tab

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

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.



# Quantitative Table: Summary Tab

Protein Name	Site	Average Raw Intensity		% CV		Raw Ratio
		Control	UV	Control	UV	UV : Control
ERK2		523,753	516,182	1.7	16.5	0.99
ERK2	§185	<b>6,461,650</b>	<b>12,803,987</b>	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		<b>13,733</b>	<b>459,188</b>	23.4	21.5	33.44
JNK3; JNK3 iso2; JNK1; §223; §223; §185; §185		<b>39,958</b>	<b>715,846</b>	34.7	7.3	17.91
p38-gamma	§183	75,877	124,965	4.6	14.4	1.65
p38-gamma	§183, §185	<b>496,371</b>	<b>1,227,660</b>	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 iso §180, §182; §180, §182		<b>398,392</b>	<b>1,902,560</b>	5.6	3.7	4.78
p38-alpha; p38-alpha iso §182; §182		<b>441,258</b>	<b>1,642,382</b>	0.7	1.7	3.72
ERK1	§202	<b>100,750</b>	<b>155,500</b>	1.8	7.7	1.54
ERK1	§202, §204	2,224,370	3,310,535	4.8	19.2	1.49
ERK1	§204	<b>715,813</b>	<b>780,041</b>	1.4	2.9	1.09
ERK5	§219, §221	<b>347,096</b>	<b>44,053</b>	10.3	13.0	0.13
ERK5	§221	<b>7,367,057</b>	<b>947,482</b>	13.9	2.6	0.13
JNK2; JNK2 iso2; JNK2 is §185; §183, §185; §183		<b>564,326</b>	<b>36,814,462</b>	12.9	1.6	65.24
JNK2; JNK2 iso2; JNK2 is §188; §183, §188; §183		<b>558,854</b>	<b>25,265,503</b>	41.3	11.2	45.21
JNK2; JNK2 iso2; JNK2 is §185; §185; §185		<b>43,478</b>	<b>517,181</b>		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.



# Quantitative Table: Summary Tab

Protein Name	Site	Log2 Ratio	Normalized Log2 Ratio	Raw Fold Change	Normalized Fold Change	Species
		UV : Control	UV : Control	UV : Control	UV : Control	
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 iso2; §180, §182; §180, §182		2.26	2.02	4.78	4.05	human
p38-alpha; p38-alpha iso2; §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 iso2; §185; §183, §185; §183		6.03	5.79	65.24	55.38	human
JNK2; JNK2 iso2; JNK2 iso2; §188; §183, §188; §183		5.50	5.26	45.21	38.38	human
JNK2; JNK2 iso2; JNK2 iso2; §185; §185; §185		3.57	3.34	11.90	10.10	human

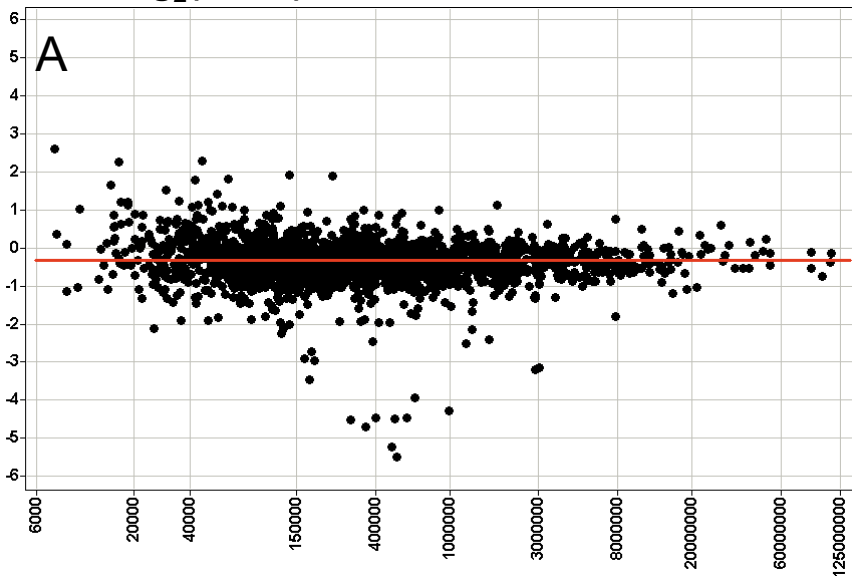


Table includes Log2 Ratio (**GREEN BOX**) Normalized Log2 Ratio (**RED BOX**), Raw Fold Change (**BLACK BOX**), and Normalized Fold Change (**BLUE BOX**). Normalized Log 2 Ratios are calculated by subtracting the median Log2 fold change for each comparison. Species of tissue profiled is shown (**green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

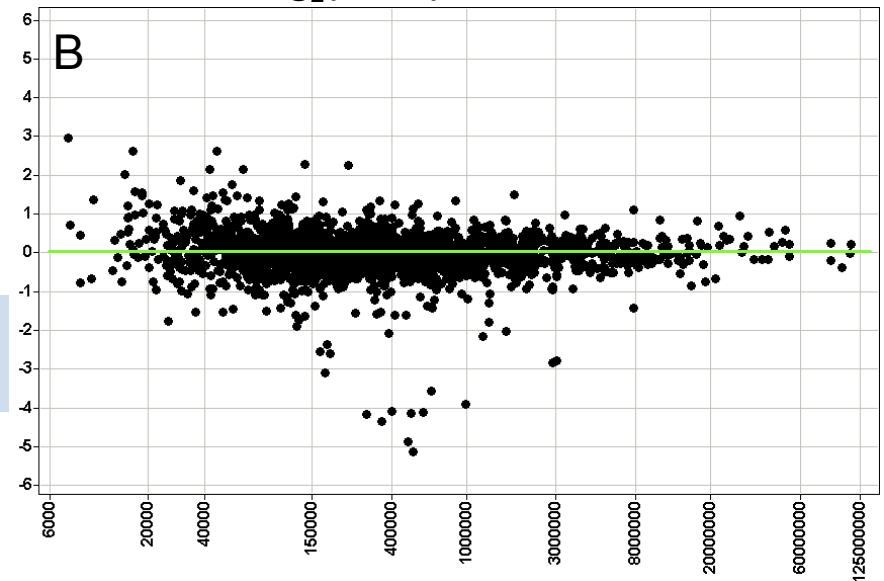


# Quantitative Table: Normalization

Raw  $\log_2(\text{Ratio})$  Values



Normalized  $\log_2(\text{Ratio})$  Values



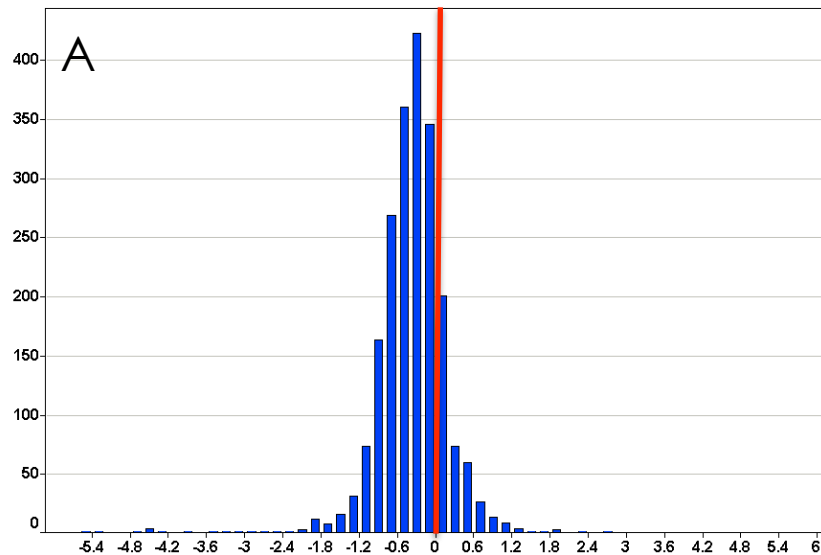
Log<sub>2</sub> ratio  
correction  
0.30

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  $\log_2(\text{ratio})$  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.

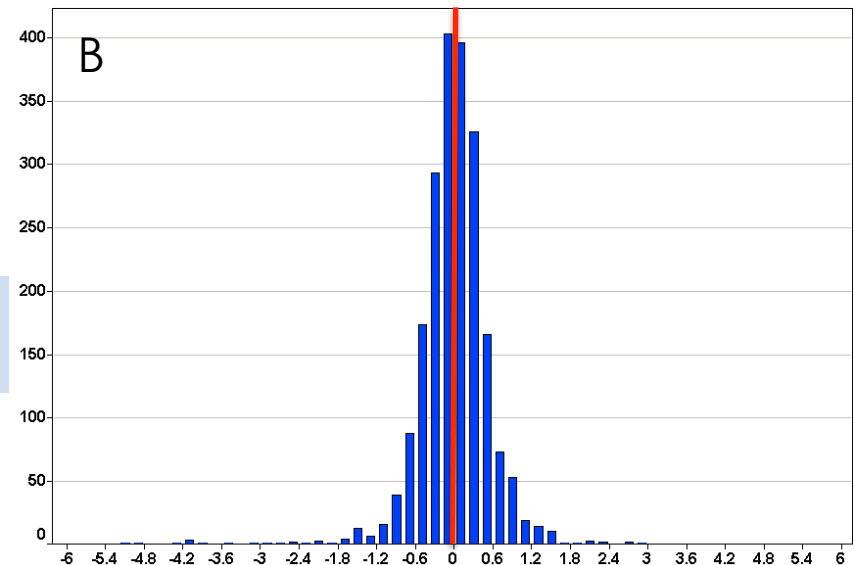


# Quantitative Table: Normalization

Raw  $\log_2(\text{Ratio})$  Values



Normalized  $\log_2(\text{Ratio})$  Values



$\log_2$  ratio  
correction  
0.30

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



# Table Overview: Details Tab

Table1\_Multipathway\_V2\_HeLa\_UV\_FINAL.xlsx

Search in Sheet

Calibri (Body) 11 B I U

Home Layout Tables Charts SmartArt Formulas Data Review

Table #1: 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, # - oxidized methionine, § - published site, Blue Text - CST siRNA/antibody a

Index	Row Index	Protein Type	Gene Name	Protein Name	Site	
1	<b>Adaptor/scaffold</b>					
2	1	Adaptor/scaffold	ABI2; ABI2	Abi-2; Abi-2 iso2	%213; %207	abl interactor 2
3	2	Adaptor/scaffold	ABI2; ABI2	Abi-2; Abi-2 iso2	%213; %207	abl interactor 2
4	3	Adaptor/scaffold	ABI2; ABI2	Abi-2; Abi-2 iso2	%213; %207	abl interactor 2
5	4	Adaptor/scaffold	ABI2; ABI2	Abi-2; Abi-2 iso2	%213, %216; %207, %21	abl interactor 2
6	5	Adaptor/scaffold	ABI2; ABI2	Abi-2; Abi-2 iso2	%213, %216; %207, %21	abl interactor 2
7	6	Adaptor/scaffold	ABI2; ABI2	Abi-2; Abi-2 iso2	%213, %216; %207, %21	abl interactor 2
8	7	Adaptor/scaffold	BCAR1	P130Cas	115	breast cancer anti-es
9	8	Adaptor/scaffold	BCAR1	P130Cas	115	breast cancer anti-es
10	9	Adaptor/scaffold	BCAR1	P130Cas	115	breast cancer anti-es
11	10	Adaptor/scaffold	BCAR1	P130Cas	%128	breast cancer anti-es
12	11	Adaptor/scaffold	BCAR1	P130Cas	%385	breast cancer anti-es
13	12	Adaptor/scaffold	CAV1	caveolin-1	%14	caveolin-1 isoform b
14	13	Adaptor/scaffold	CAV1	caveolin-1	%6, %14	caveolin-1 isoform b

Column Definitions Details Summary Fold Change by Protein Type +

Normal View Ready Count Numbers=2903

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



# Qualitative Table: Details Tab

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 iso	%182; %182	41; 41	TDDEMTGY*VATRWYR; TDDEMTGY	ASK1, HePTP, MEKK6, M	PKCZ, p38-alpha; PKCZ,
p38-alpha; p38-alpha iso	%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, M	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**).



# Qualitative Table: Details Tab

Protein Name	Site	MS2 Spectrum Number				Peak Apex MS Spectrum Number			
		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.





# Qualitative Table: Details Tab

Protein Name	Site	Retention Time				Xcorr			
		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.



# Qualitative Table: Details Tab

Protein Name	Site	Mass Accuracy (ppm)				DeltaCN			
		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	-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.



# Qualitative Table: Details Tab

Protein Name	Site	Rsp				PP Probability			
		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	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

Protein Name	Site	Peptide	Charge	MS2 Spectrum Number			
				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

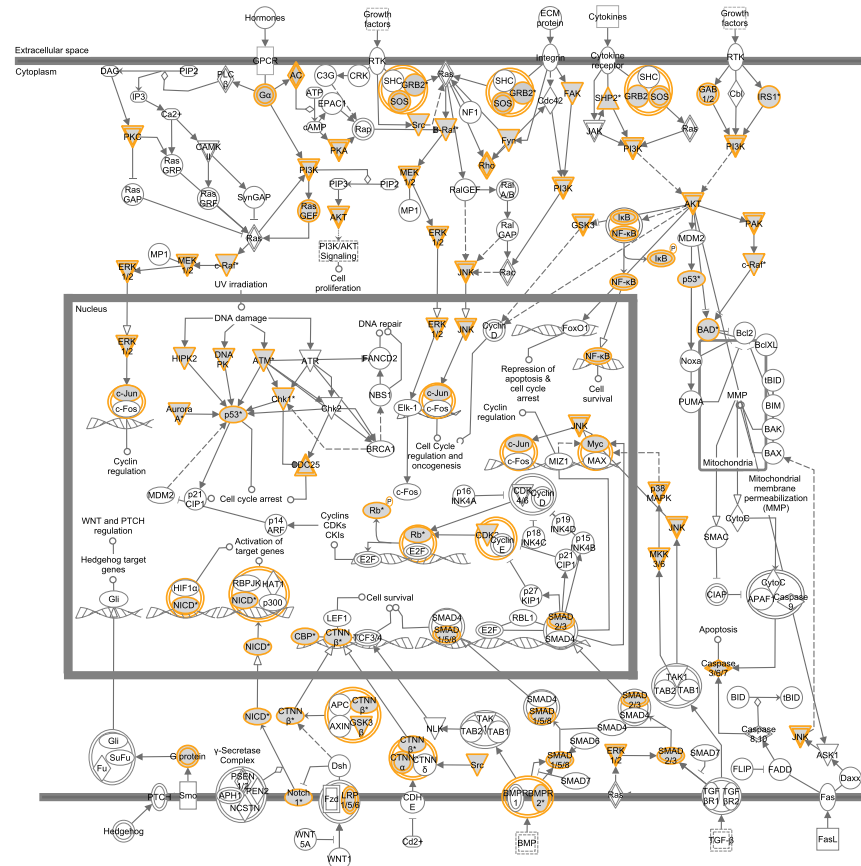
**Legend:** \* - phosphorylation, ^ - multiple protein IDs for one peptide

gene_name	species	protein_type	protein_function_description	entrez_geneid	NCBI_acc	NCBI_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
SBNO1	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	T818	
SBNO1	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	S817	
SBNO1	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	S816	
SBNO1	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	S816	
SBNO1	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	S816	
SBNO1	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	T834	
SBNO1	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	S822	
SBNO1	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	S814	
SBNO1	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	S814	
SBNO1	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	S814	
SBNO1	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	S814	
SBNO1	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	S814	
SBNO1	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	S836	
SBNO1	human	Chromatin, DNA-binding, DNA rep	Belongs to the SBNO family. 4 isoforms of	55206	NP_060653	T834	
SBNO1	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.



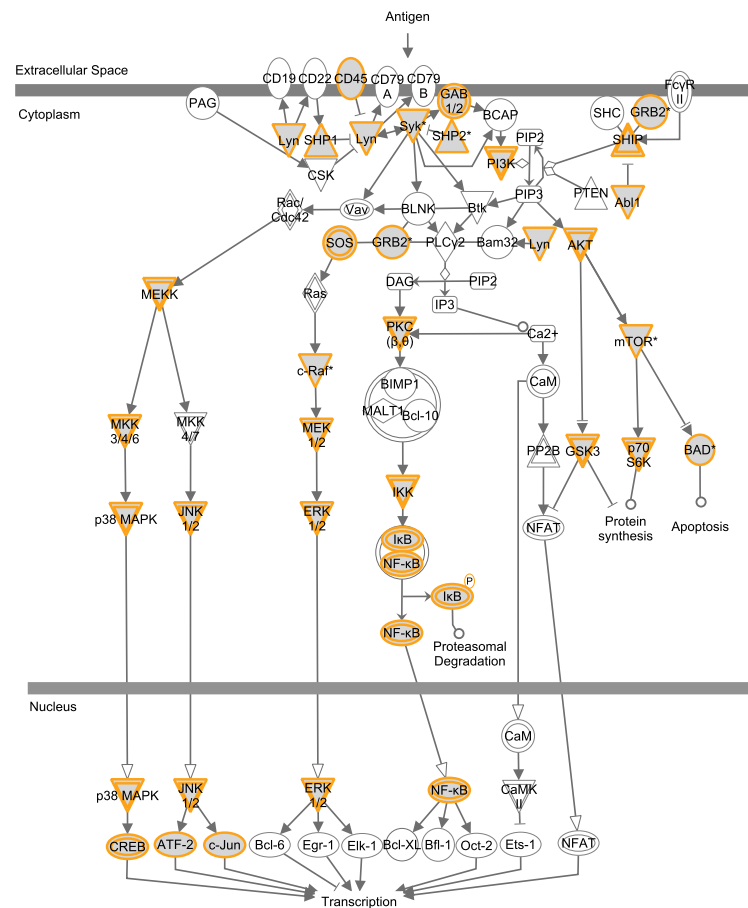
# Pathway Analysis using Ingenuity



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.



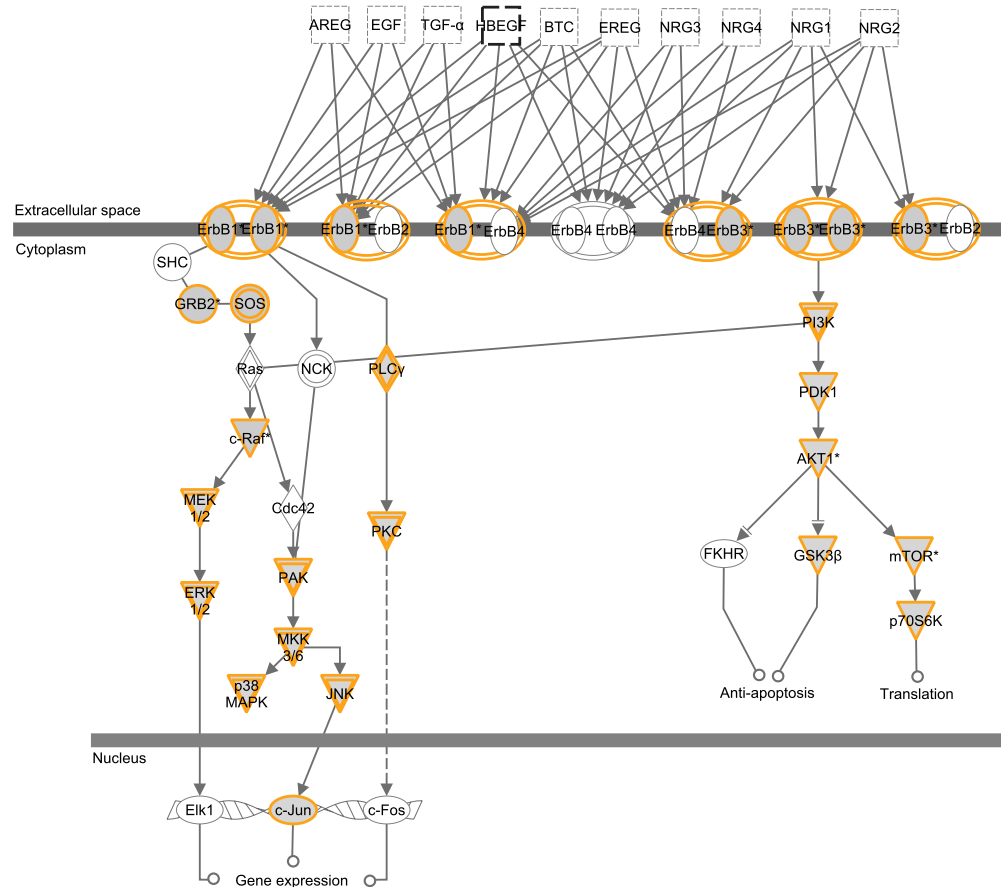
# Pathway Analysis using Ingenuity



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



# Pathway Analysis using Ingenuity

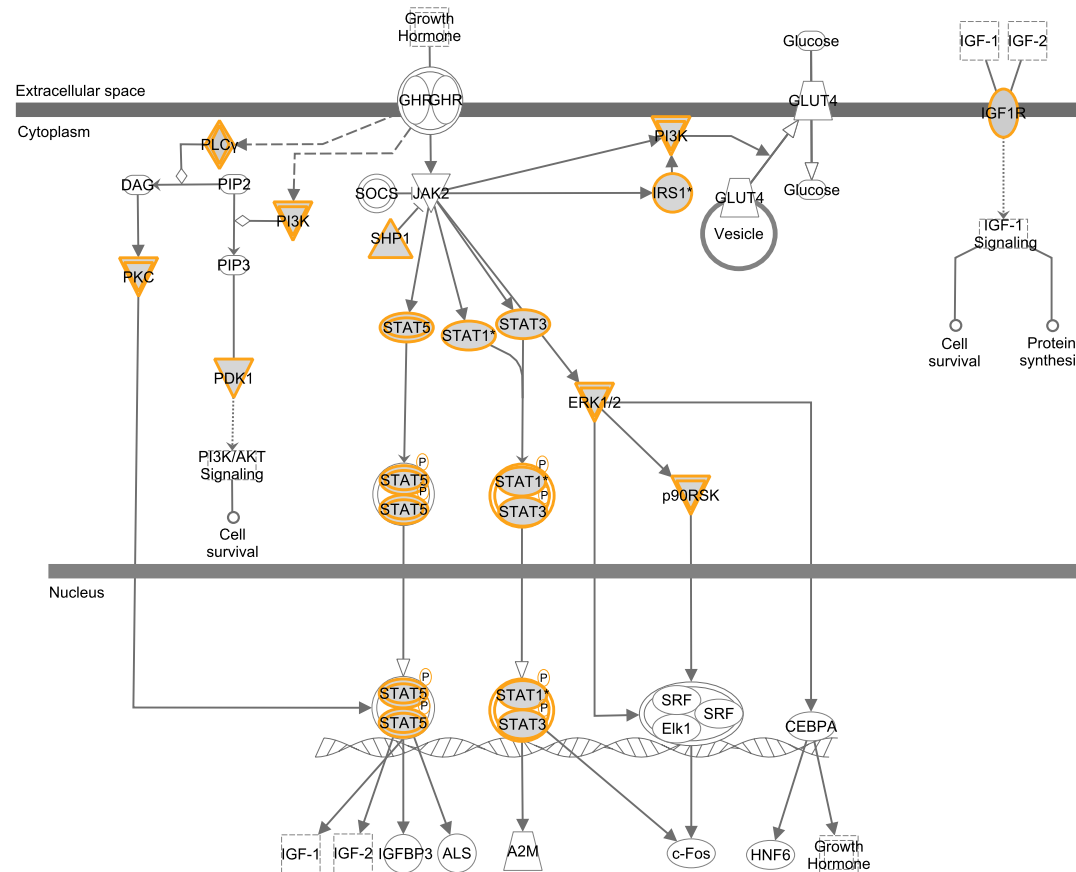


Sampling of pathways monitored by PTMScan Multipathway using Ingenuity, Erb Signaling. Proteins that are monitored by PTMScan Direct Multipathway are highlighted in ORANGE.



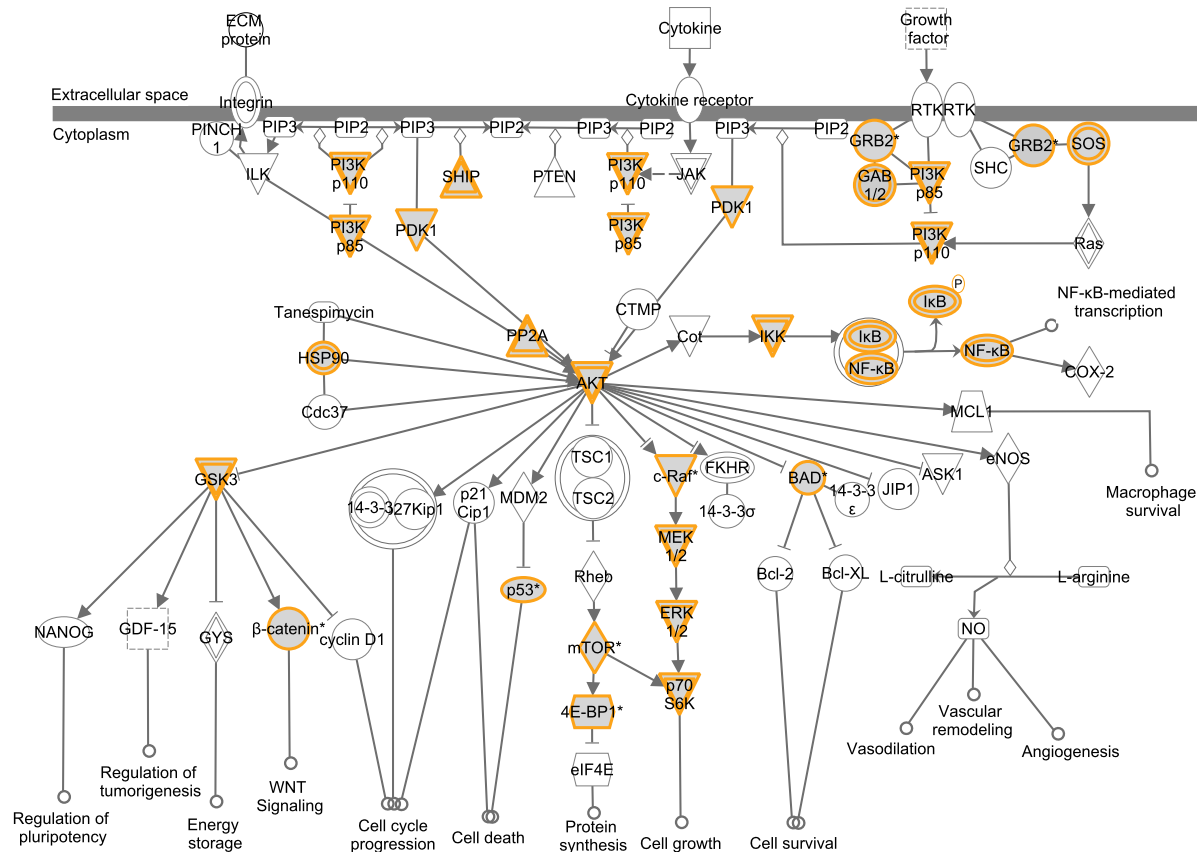


# Pathway Analysis using Ingenuity



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

# Pathway Analysis using Ingenuity



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.