Quantitative Profiling of Signaling Pathways Using PTMScan® Direct

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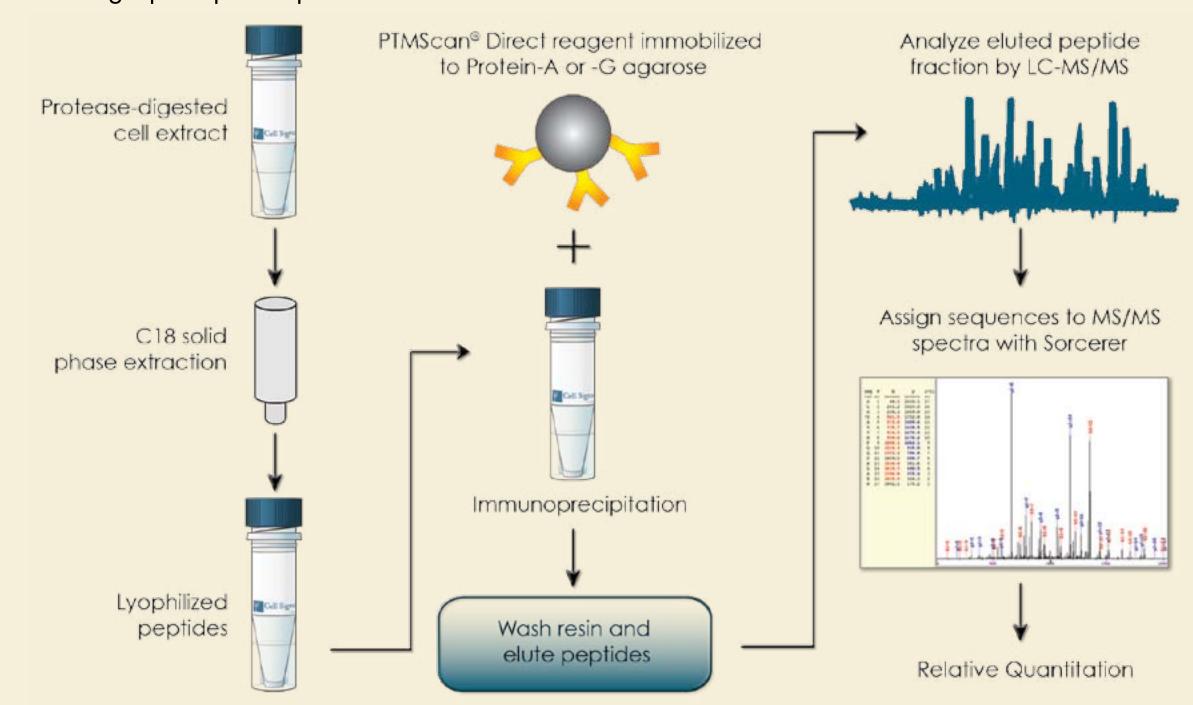
Introduction

A challenge to proteomic studies of post-translational modifications (PTMs) is the ability to focus on proteins of interest rather than randomly identified proteins typified by data dependent analysis. Methods that target specific proteins and pathways known to be critical to cellular signaling are therefore desirable. Cell Signaling Technology (CST) has developed an immunoaffinity-based LC-MS/MS method, called PTMScan® Direct, for identification and quantification of hundreds of peptides from particular signaling pathways or protein types. Cell lines, tissues, or xenografts can be used as starting material. Quantification may be performed using isotopic labeling methods such as SILAC or label-free quantification of chromatographic peak areas. PTMScan Direct is a powerful new method that combines the specificity of antibody-based methods with the sensitivity and high duty cycle of LC-MS/MS analysis allowing thousands of measurements.

PTMScan® Direct Reagents

1. Multipathway Reagent
2. Serine/Threonine Kinases Reagent
3. Tyrosine Kinases Reagent
4. Akt / PI3K Pathway Reagent
5. DNA Damage / Cell Cycle Reagent
6. Apoptosis / Autophagy Reagent
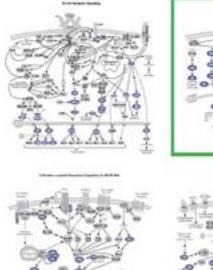
PTMScan® Direct Method

Figure 1: PTMScan Direct work flow diagram. PTMScan Direct is a published method (Stokes et al, 2012) adapted from the original PhosphoScan® method developed at Cell Signaling Technology (Rush et al, 2005, Patent # 20030044848). Cell lines, tissues, xenografts, or other biological starting materials are lysed under denaturing conditions, digested with trypsin, and desalted over C18 columns. Target peptides are immunoprecipitated using the appropriate PTMScan Direct reagent. Immunoprecipitated peptides are separated on a reversed-phase Magic C18 AQ column and data-dependent MS methods are performed with an LTQ-Orbitrap Velos or Elite mass spectrometer. MS/MS spectra are evaluated using SEQUEST 3G and the SORCERER 2 v4.0 platform from Sage-N Research (Lundgren et al., 2009). Quantification is performed using chromatographic peak apex areas or intensities.

Multipathway Reagent Selected Targets

Figure 2: Coverage of selected pathways using the PTMScan Direct Multipathway Reagent. Peptides from purple proteins are identified using the Multipathway Reagent.

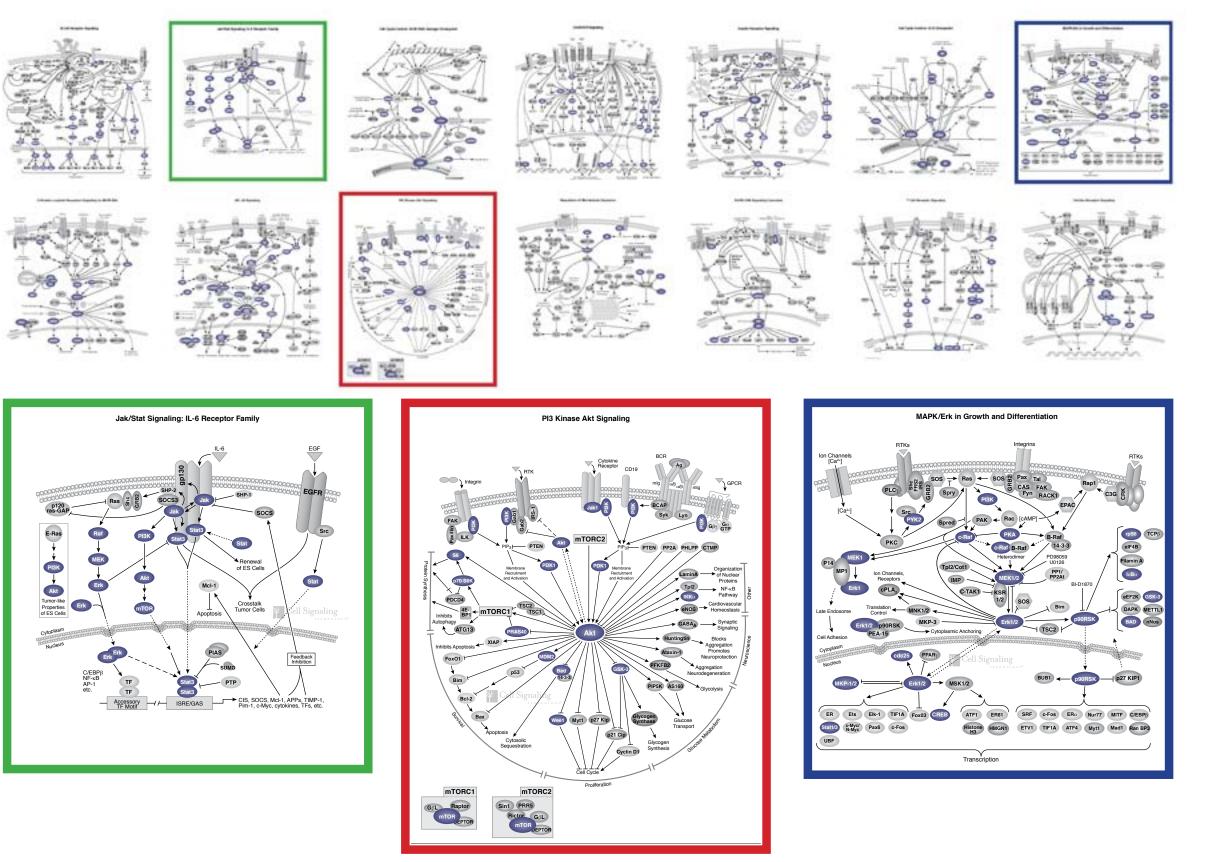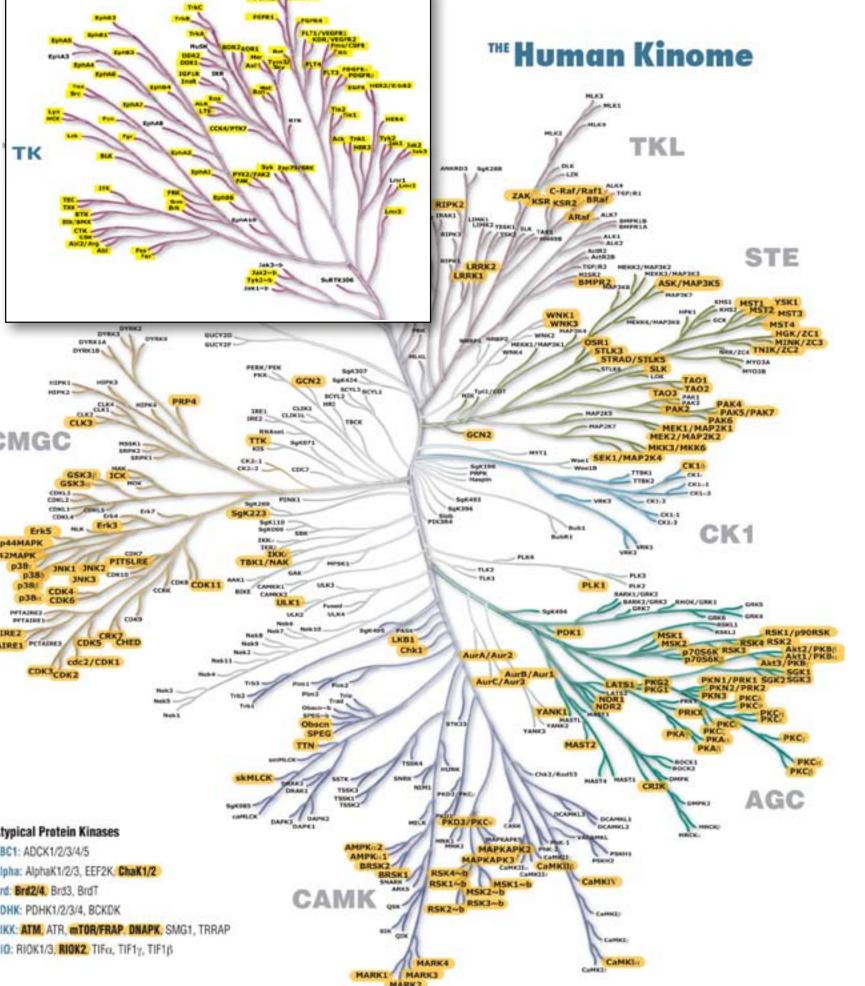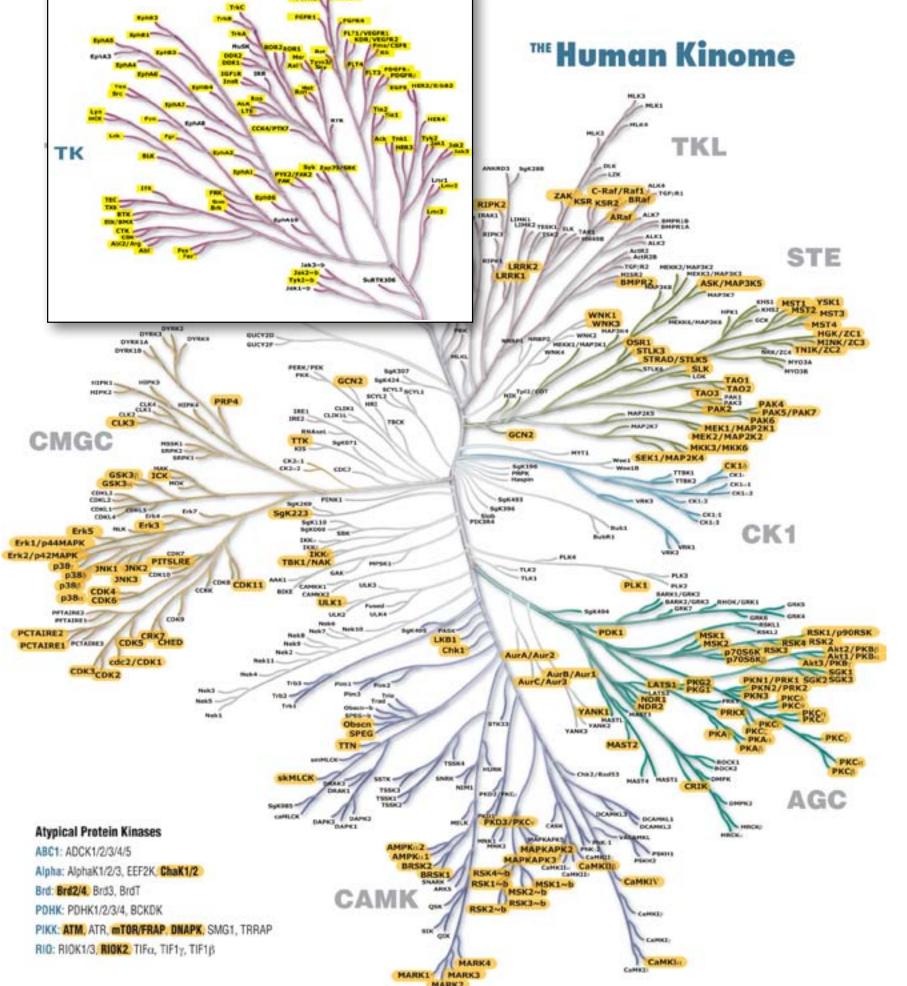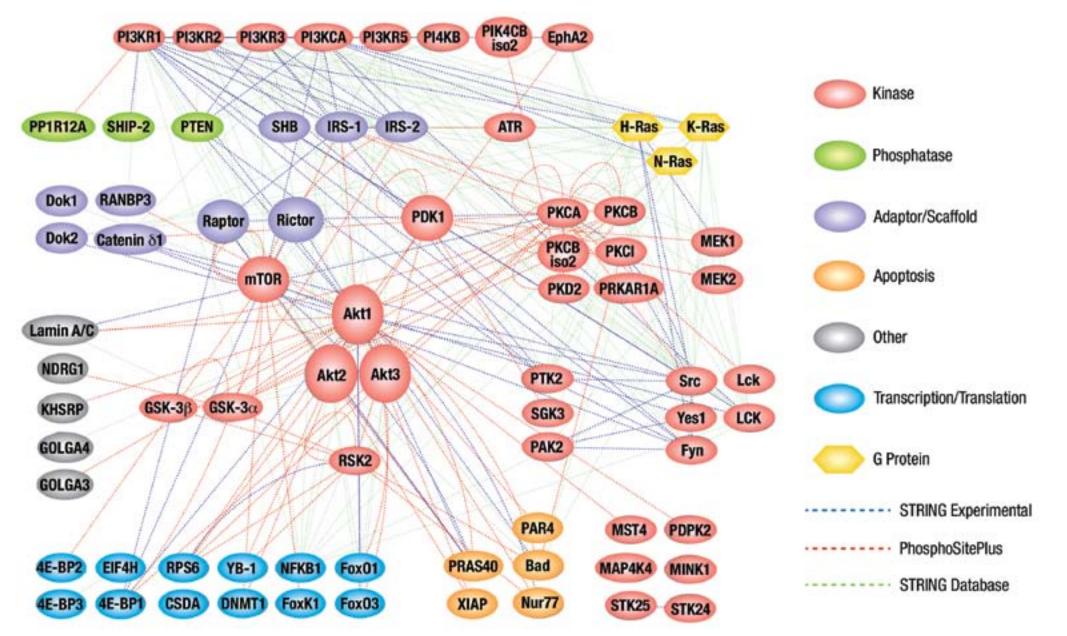## Ser/Thr Kinases and Tyr Kinases Reagent Targets

Figure 3: PTMScan Direct Ser/Thr Kinases Reagent and Tyr Kinases Reagent targets mapped onto the human kinome tree. Yellow highlighting indicates kinases identified using the reagents. Tyr Kinases Reagent coverage is shown in the inset ("TK").
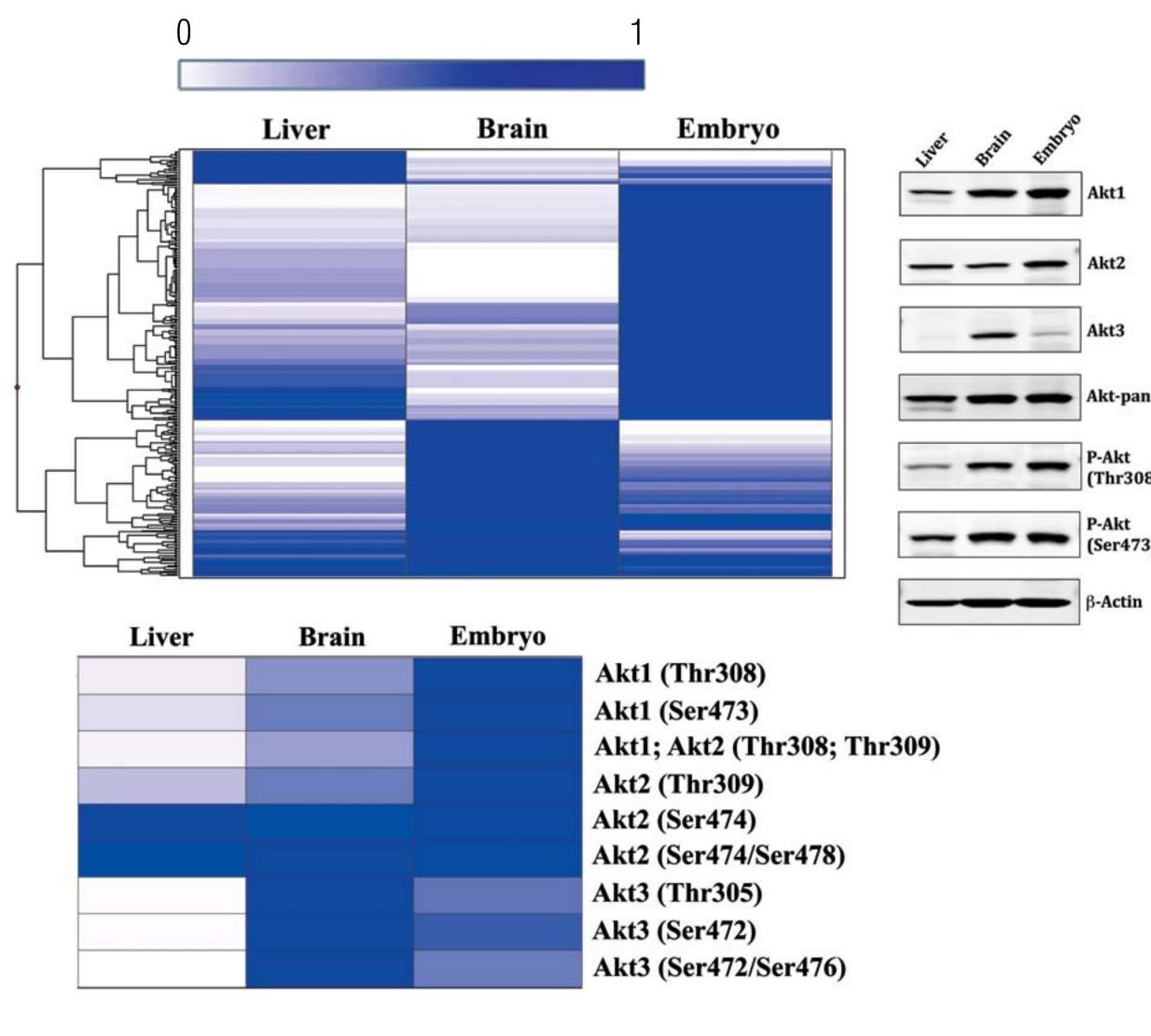

Akt / PI3K Pathway Reagent

INTERACTION MAP

Figure 4: PTMScan Direct: Akt/PI3K Pathway Reagent interaction map. Interactions were derived from STRING using high confidence scores (>0.700) from experimental, database, and textmining lines of evidence and the substrate search function in PhosphoSitePlus®. Node colors/ shapes denote different protein classes. Edge color denotes interaction type.

MOUSE TISSUE STUDY

Figure 5: Akt/PI3K Pathway Reagent mouse tissue study. Heat map of relative intensities for mouse liver, brain, and embryo. Each row represents a different peptide and each column represents a different tissue. The max intensity across the three tissues was set to 1 and the other two intensities normalized to the max. Blue indicates higher intensity.
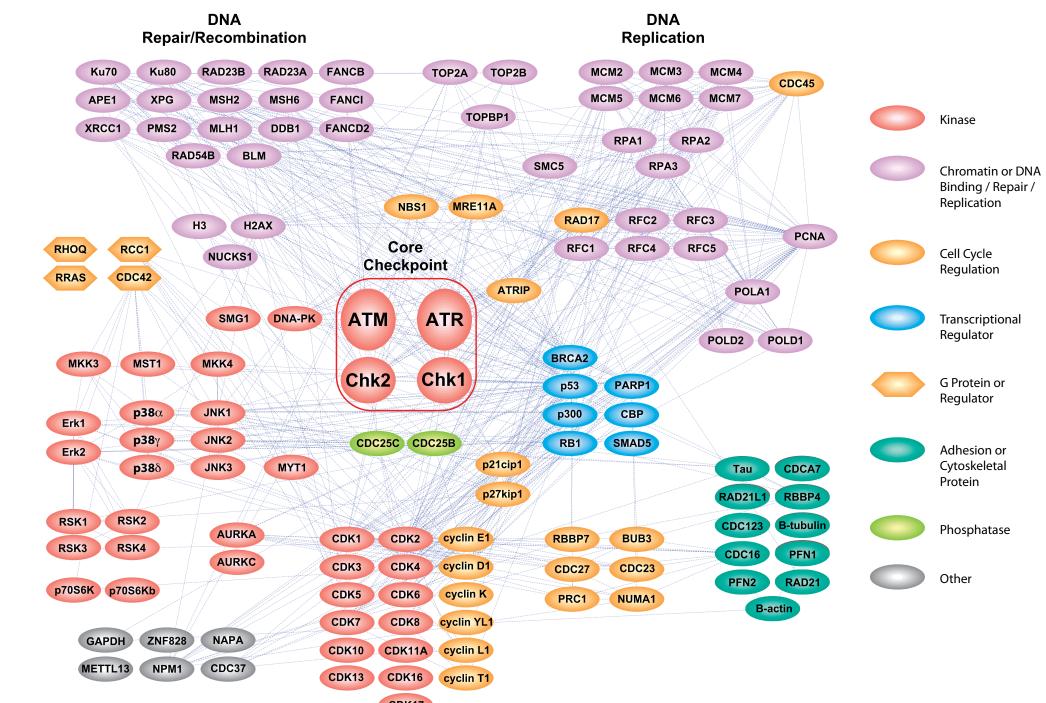

DNA Damage/Cell Cycle Reagent

INTERACTION MAP

Figure 6: PTMScan Direct: DNA Damage / Cell Cycle Reagent interaction map. Interactions were derived from STRING using using h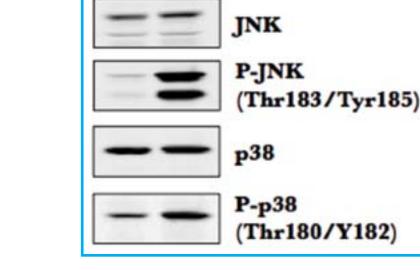igh confidence scores (>0.700) from experimental, database, and textmining lines of evidence. Node colors/shapes denote different protein classes.

HeLa -/+ UV DAMAGE

Figure 7: DNA Damage/Cell Cycle Reagent: HeLa cell UV damage study. HeLa cells were untreated or treated with 500 mJ/cm² UV light and harvested at 2 hr post treatment. Fold changes were calculated from chromatographic peak heights/areas. Green cells indicate peptides more abundant with UV damage, Red cells indicate peptides less abundant. Selected DNA damage response proteins are shown in detail with accompanying western blots.

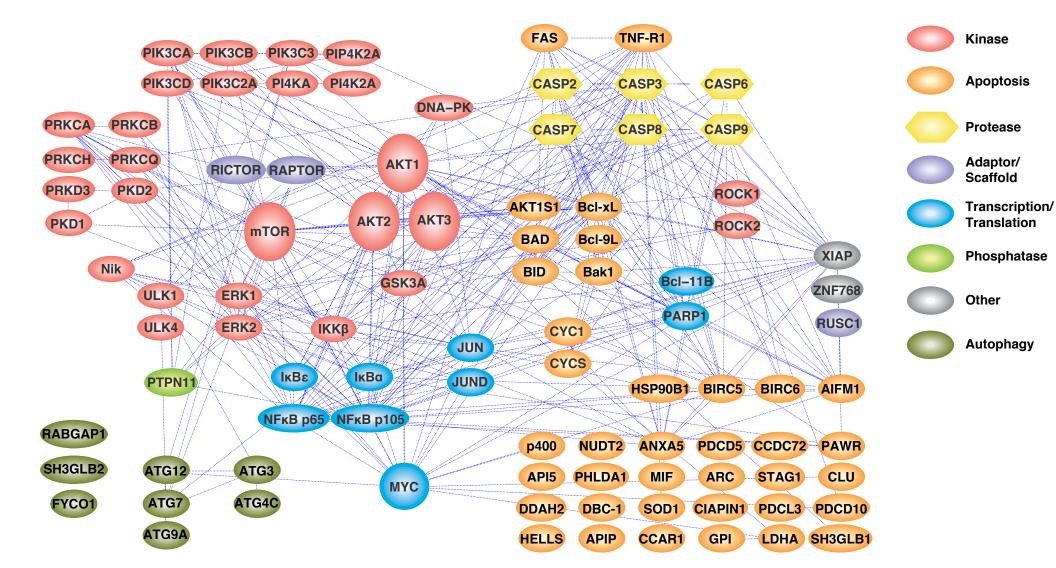| Fold Change UV / Control	Protein Name	Site	Peptide	Max Intensity
1.1	ATM		QAGIIQALGNLGCHILSVYLK	299,739
5.5	ATM	§1981	SLAFEEGSQSTTISSLSEK	20,664,088
-1.6	ATR	–	YIVLHFGR	46,407
-1.2	ATR	§428	M#ESM#EIIEEIQCQTQQENLSSNSD	43,724
-1.3	Chk1		FFHQLM#AGVVYLHGIGITHR	48,715
3.2	Chk1	§317	YSSS*QPEPR	698,572
4.1	Chk1	§345	LVQGISFS*QPTCPDHM#ALLNSQLLG	1,840,956
-1.1	Chk2		FTTEEALRHPWLQDEDM#K	7,865,595
9.1	Chk2	§379	ILGETS*LM#R	1,010,005
260.2	JNK1; JNK3	§183, §185; §221, §223	TAGTSFM#MT*PY*VVTR	8,543,090
98.0	JNK2	§183, §185	TACTNFMM#T*PY*VVTR	15,427,858
4.7	p38-alpha	§180, §182	HTDDEMT*GY*VATR	18,211,944
4.1	p38-gamma	§183, §185	QADSEM#T*GY*VVTR	1,421,705

Apoptosis / Autophagy Reagent

INTERACTION MAP

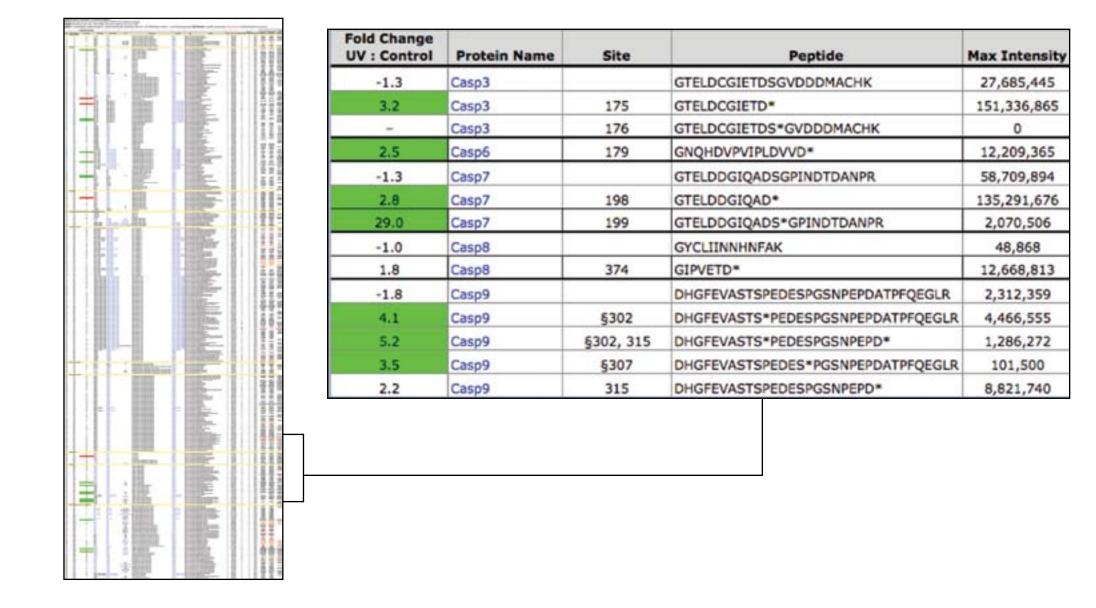**Figure 8: PTMScan Direct: Apoptosis / Autophagy Reagent interaction map.** Interactions were derived from STRING using using high confidence scores (>0.700) from experimental, database, and textmining lines of evidence. Node colors/shapes denote different protein classes.

HeLa -/+ UV DAMAGE

Figure 9: Apoptosis / Autophagy Reagent: HeLa cell UV damage study. Quantitative data for caspase peptides identified with the Apoptosis/Autophagy Reagent used to probe UV damaged HeLa cells (500 mJ/cm², harvest 2 hr post treatment). Green indicates increased abundance relative to control.

Fold Change UV / Control	Protein Name	Site	Peptide	Max Intensity
-1.3	Casp3		GTELDCCGGETDSGVDDDMACHK	27,685,445
6.2	Casp3	175	GTELDCGGETD*	151,336,865
-1.6	Casp3	176	GTELDCGETDS*DVDDDMACHK	0
-1.2	Casp3	179	GNQHDVPVDPLIVG*	12,209,363
-1.3	Casp7		GTELDCGGQEDSGPENDTDANPR	58,709,894
2.8	Casp7	198	GTELDCGQAD*	135,291,676
29.0	Casp7	199	GTELDCGQADS*GPENDTDANPR	2,070,506
-1.0	Casp8		GYCLIINNHNFAK	48,868
1.8	Casp8	374	GIFVETD*	12,668,813
3.2	Casp8		DHGFEVASTSPEDESPGSNPEPDATPFQEGLR	2,312,308
5.5	Casp8	§302	DHGFEVASTS*PEDESPGSNPEPDATPFQEGLR	4,466,555
2.3	Casp8	§303, 315	DHGFEVASTS*PEDESPGSNPEPD*	1,286,272
3.5	Casp8	§303	DHGFEVASTSPEDESPGSNPEPD*	101,500
2.2	Casp9	315	DHGFEVASTSPEDESPGSNPEPD*	8,821,740

Summary

PTMScan Direct is a novel method that allows identification and quantification of hundreds of peptides from selected protein types or signaling pathways. This allows focus on proteins of interest, bypassing the random sampling of peptides that occurs in traditional data-dependent proteomic analysis. PTMScan Direct is widely applicable in drug development and discovery, as well as in any application where monitoring of known signaling pathways is desired.

References

1. Rush J. et. al. (2005) *Nat. Biotechnol.* 23: 94-101
2. Lundgren, et. al. (2009) *Curr. Protoc. Bioinformatics.* 13: Unit 13-3
3. Stokes M.P. et. al. (2012) *Mol. Cell Proteomics.* Epub Feb 9th

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