



PhosphoScan/PTMScan Proteomics Services & Products

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Cell Signaling Technology, Inc.

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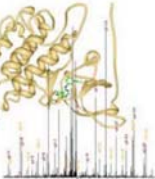



PTMScan Services & Products

PTMScan: Proteomic profiling and quantification of
100's – 1,000's of post-translational modifications (PTMs)

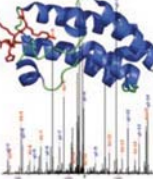
PhosphoScan
p-S, p-T, p-Y

PhosphoScan[®] Service
Provides a powerful strategy for kinome-wide phosphoproteomics employing Phospho-Motif antibodies to direct analysis to the relevant regions of the kinome.



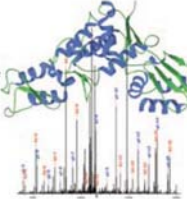
AcetylScan
Ac-K


AcetylScan[™] Service
Provides equally comprehensive analysis of protein acetylation using proprietary acetylated-lysine antibodies optimized for AcetylScan[™].



UbiScan
K-e-GG

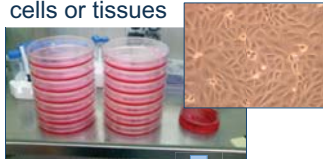
UbiScan[™] Service
Similarly powerful method utilizing a unique ubiquitin-branch antibody for ubiquitinated sequence identification and the most comprehensive analysis of cellular ubiquitin pathways.





PTMScan Services & Products

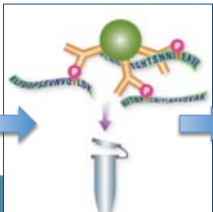
Customer-provided cells or tissues



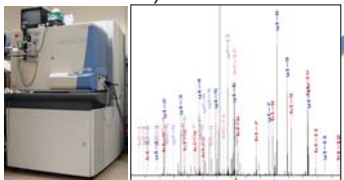
Lysis
Extraction
Digest


Total Peptide Fraction

p-S/T,Y; Ac-K; K-GG
Immunoaffinity Purification (IAP)




Liquid Chromatography – Tandem Mass Spectrometry (LC – MS/MS)





PTMScan Report



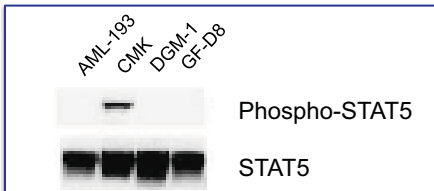
Tyrosine Kinase PhosphoScan

Activating alleles of *JAK3* in acute megakaryoblastic leukemia

Denise K. Walters,^{1,2,*} Thomas Mercher,^{3,*} Ting-Lei Gu,^{4,*} Thomas O'Hare,^{1,2} Jeffrey W. Tyner,² Marc Loriaux,⁵ Valerie L. Goss,⁶ Kimberly A. Lee,⁴ Christopher A. Eide,² Matthew J. Wang,² Eric P. Stoffregen,² Laura McGreevey,³ Julie Nardone,⁴ Sandra A. Moore,³ John Crispino,⁷ Titus J. Boggon,⁸ Michael C. Heinrich,^{2,8} Michael W. Deininger,² Roberto D. Polakiewicz,⁴ D. Gary Gilliland,³ and Brian J. Druker^{1,2,*}

CANCER CELL 10, 65-75, JULY 2006 ©2006 ELSEVIER INC. DOI 10.1016/j.ccr.2006.06.002


Phosphoproteomic Strategy: Constitutive phosphorylation of STAT5 may identify AML disease mechanism based on tyrosine kinase activation

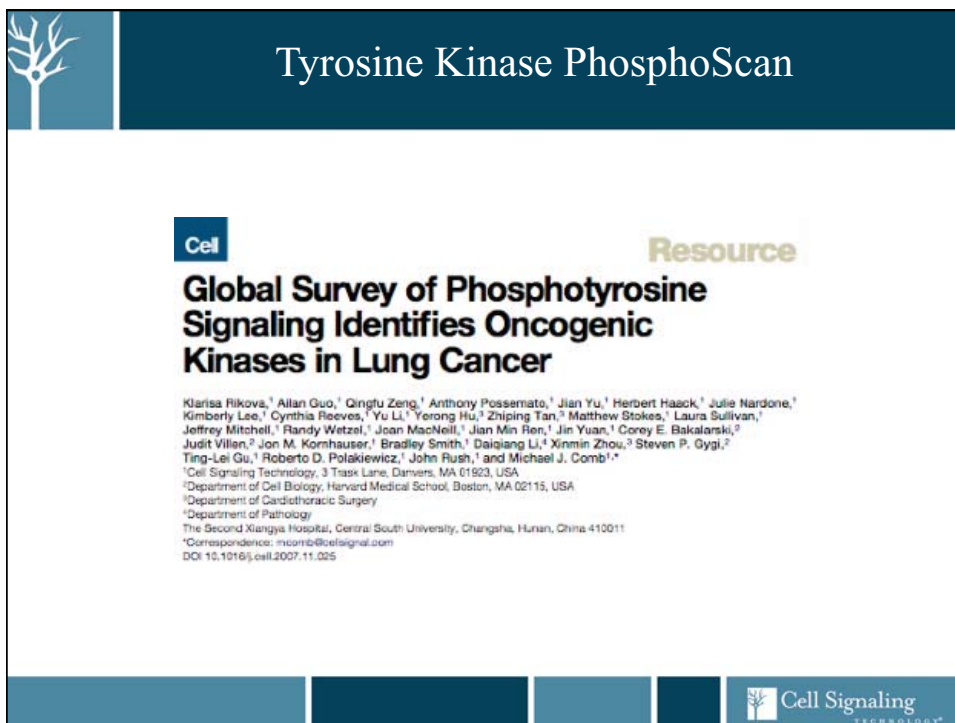
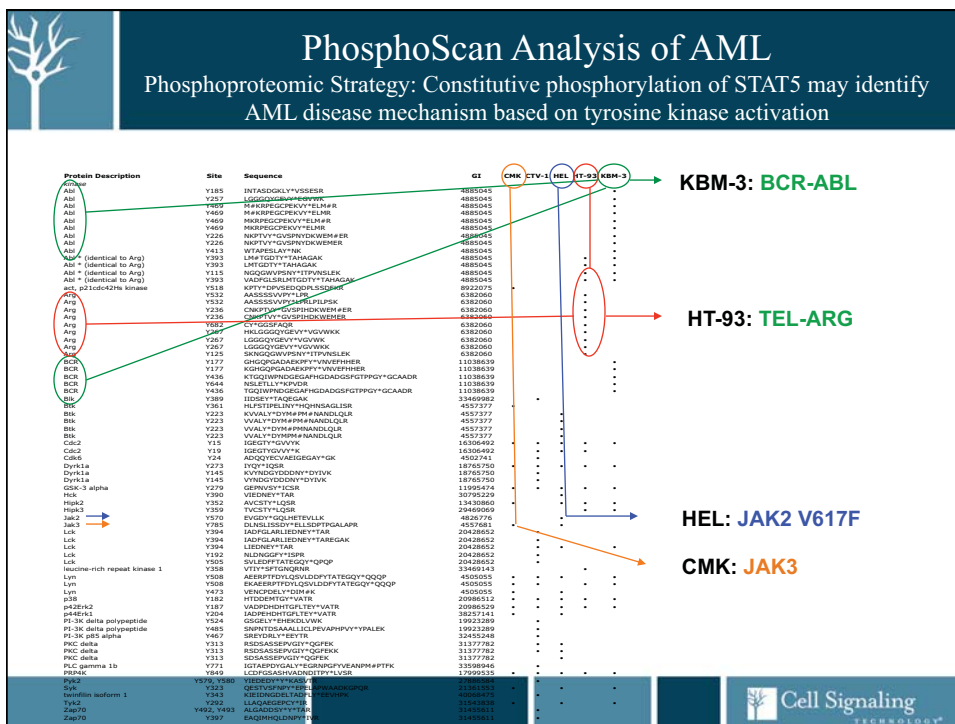


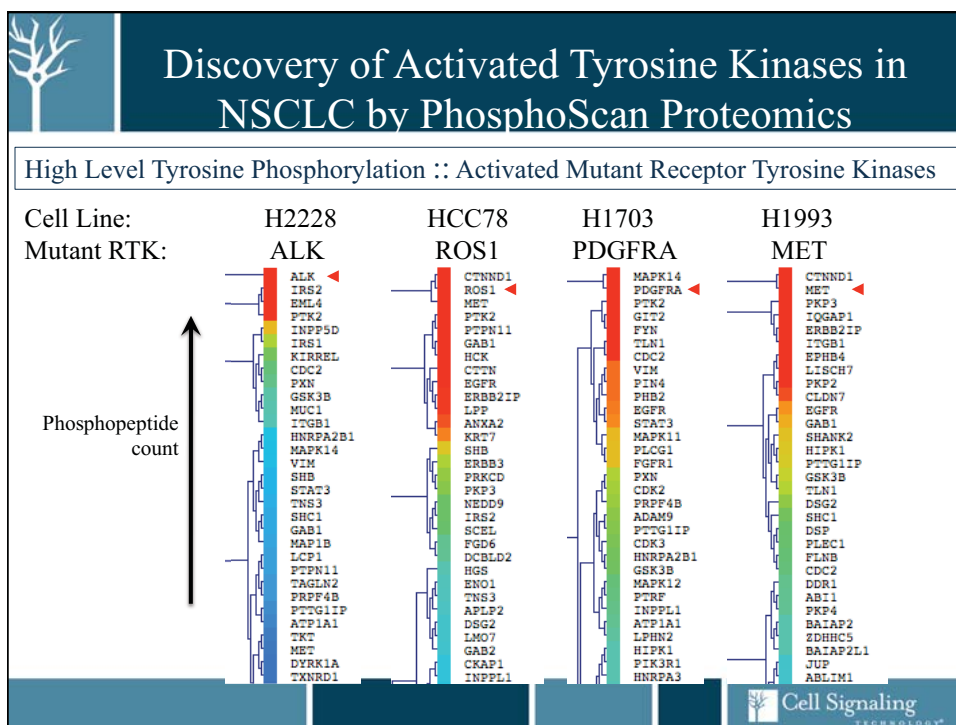
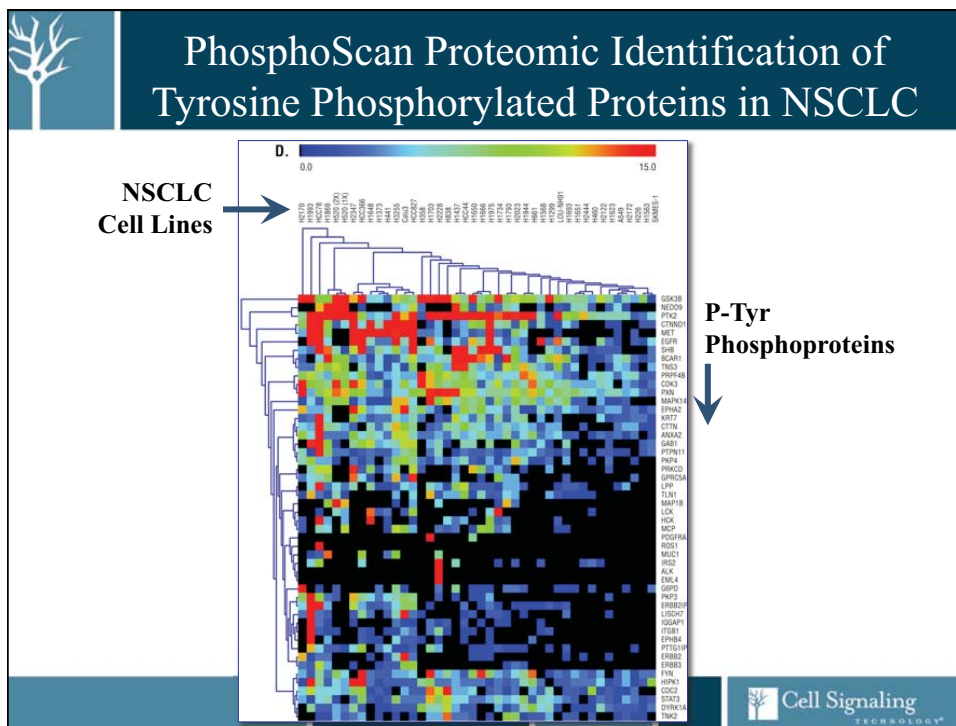
AML-193
CMK
DGM-1
GF-D8

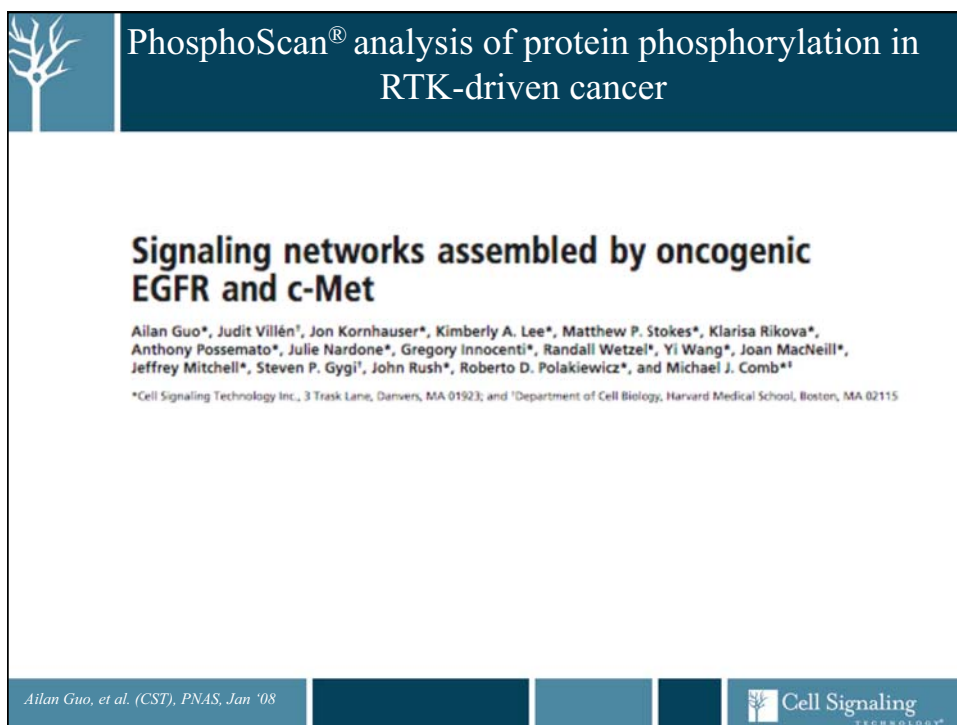
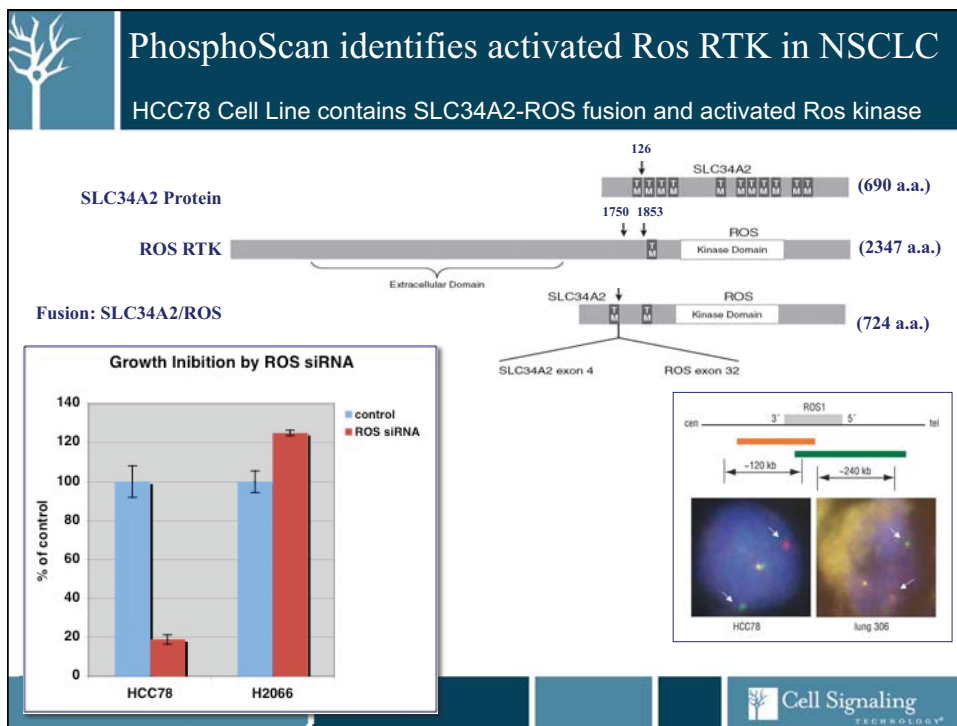
Phospho-STAT5

STAT5









PhosphoScan® analysis of protein phosphorylation in RTK-driven cancer

Table 2. Summary of the PhosphoScan-SILAC results for H3255 treated with gefitinib and for MKN45 treated with Su11274

| | H3255 (gefitinib) | | | MKN45 (Su11274) | | |
|--------------------------|-----------------------|-----|------|-----------------------|-----|------|
| | 3-h and 24-h combined | 3-h | 24-h | 3-h and 24-h combined | 3-h | 24-h |
| p-Peptides | 393 | 308 | 337 | 1181 | 976 | 903 |
| p-Proteins | 185 | 160 | 167 | 535 | 469 | 447 |
| p-Sites | 301 | 245 | 267 | 945 | 793 | 756 |
| Sites affected >2.5-fold | | 110 | 161 | | 586 | 603 |
| Sites affected >5-fold | | 84 | 133 | | 423 | 462 |

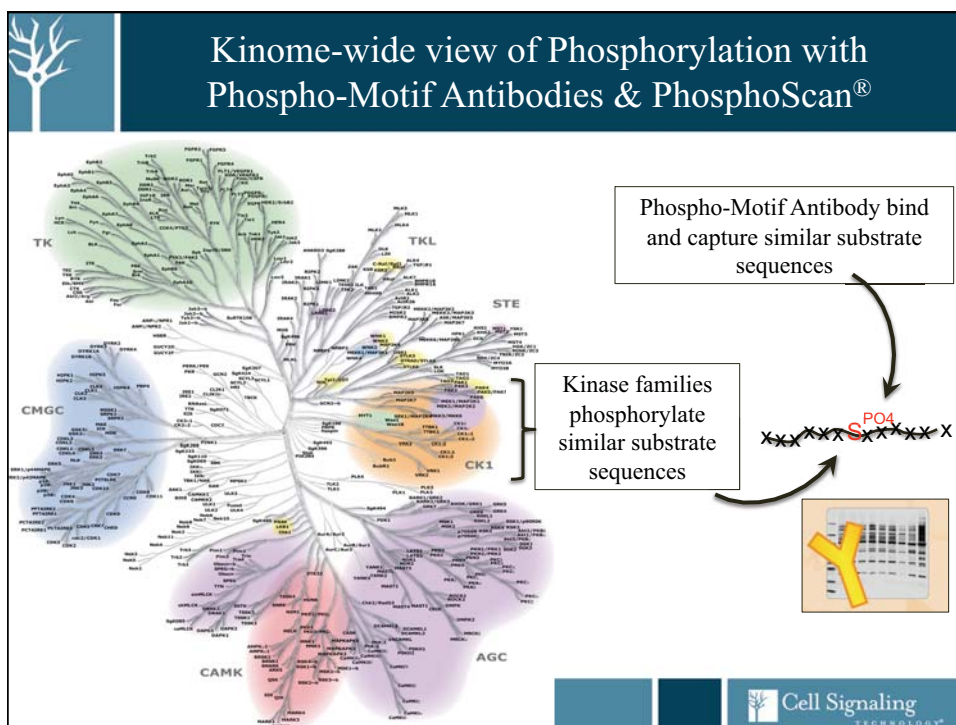
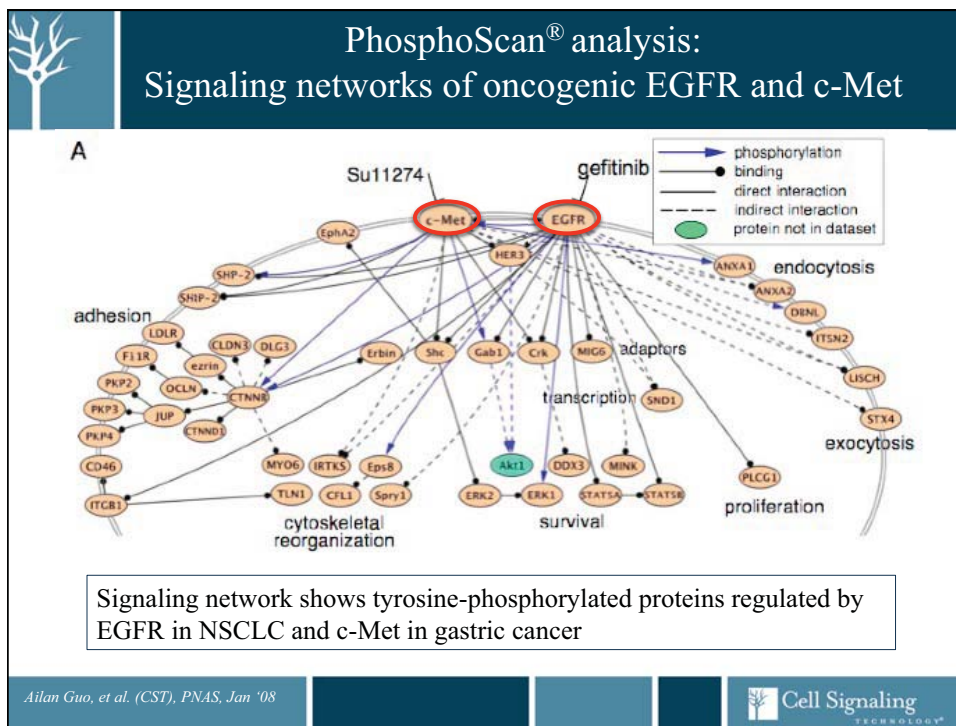
Ailan Guo, et al. (CST), PNAS, Jan '08 Cell Signaling TECHNOLOGY®

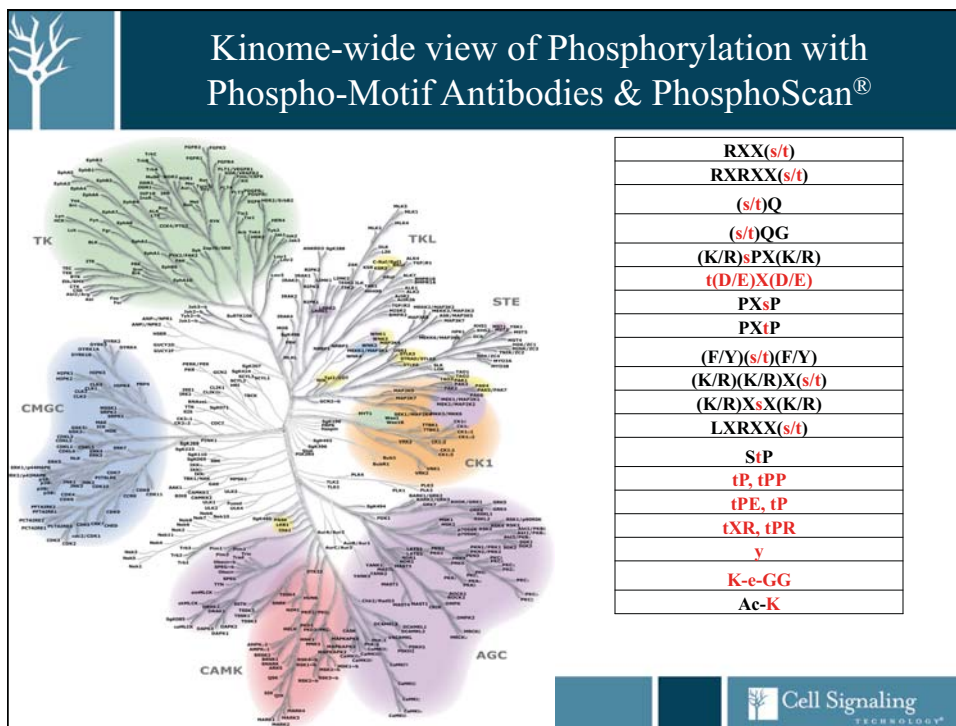
PhosphoScan Report: MKN-45: Control vs. SU11274 Tyrosine Kinase Inhibitor P-Tyr site list & fold-change quantification

PHOSPHOSCAN® RESULTS
Table #1: MKN-45 Cells; Trypsin Digest; Phosphotyrosine (CST# 9411)
Samples: Control = CS 3476 & 3477; SU11274 Treated = CS 3474 & 3475
Legend: * - phosphorylation, # - oxidized methionine, § - published site, Blue Text - CST antibody

| 11 | Index | Index in Detail | Normalized Fold Change | Gene Name | Protein Name | Site | Descrip |
|-----|-------|-----------------|------------------------|-------------------|------------------------|------------------------|-----------------|
| 547 | 536 | 902 | 2.3 | EPH4 | Eph4 | §987 | EPH receptor |
| 548 | 537 | 903 | -12.1 | ERBB2 | HER2 | §1005 | erbB-2 isoforr |
| 549 | 538 | 905 | 5.9 | ERBB2 | HER2 | §877 | erbB-2 isoforr |
| 550 | 539 | 906 | -6.3 | ERBB3 | HER3 | §1307 | erbB-3 isoforr |
| 551 | 540 | 908 | -6.0 | ERBB3 | HER3 | §1328 | erbB-3 isoforr |
| 552 | 541 | 909 | 1.2 | IGF1R; INSR; INSR | IGF1R; InSR; InSR iso2 | §1161; §1185; §1173 | insulin-like gr |
| 553 | 542 | 910 | -1.6 | IGF1R; INSR; INSR | IGF1R; InSR; InSR iso2 | §1161; §1165; §1185; § | insulin-like gr |
| 554 | 543 | 913 | 1.1 | IGF1R; INSR; INSR | IGF1R; InSR; InSR iso2 | §1165; §1189; §1177 | insulin-like gr |
| 555 | 544 | 915 | -4.0 | MERTK; TYRO3 | Mer; Tyro3 | §753; §685 | MER receptor |
| 556 | 545 | 916 | 5.8 | MERTK; TYRO3 | Mer; Tyro3 | §754; §686 | MER receptor |
| 557 | 546 | 917 | -2.0 | MET | Met | §1003 | met proto-onc |
| 558 | 547 | 920 | 3.6 | MET | Met | §1093 | met proto-onc |
| 559 | 548 | 921 | -56.3 | MET | Met | §1230; §1234 | met proto-onc |
| 560 | 549 | 926 | -34.1 | MET | Met | §1230; §1234; §1235 | met proto-onc |
| 561 | 550 | 928 | -1.3 | MET | Met | §1234 | met proto-onc |
| 562 | 551 | 938 | -60.9 | MET | Met | §1234; §1235 | met proto-onc |
| 563 | 552 | 947 | -2.7 | MET | Met | §1235 | met proto-onc |
| 564 | 553 | 950 | -11.5 | MET | Met | §1349; §1356 | met proto-onc |
| 565 | 554 | 952 | -5.8 | MET | Met | §1356 | met proto-onc |
| 566 | 555 | 953 | -9.0 | MST1R | Ron | §1238 | macrophage |

Cell Signaling TECHNOLOGY®





KinomeView Services

Western Blot Profiling – Casting a wide net

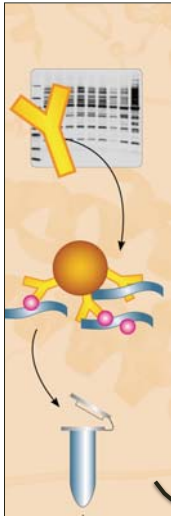
Phospho-Motif Antibody Western Blots

Strategy:

Focus PhosphoScan proteomics on phosphorylation that is regulated or de-regulated


Cell Signaling TECHNOLOGY®

From KinomeView to Focused PhosphoScan

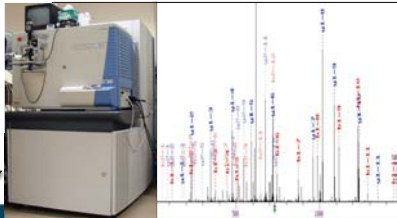



Phospho-Motif Antibody that shows changes by western...

... is the Phospho-Motif Antibody that will be used to capture phosphopeptides for ID and quantification.

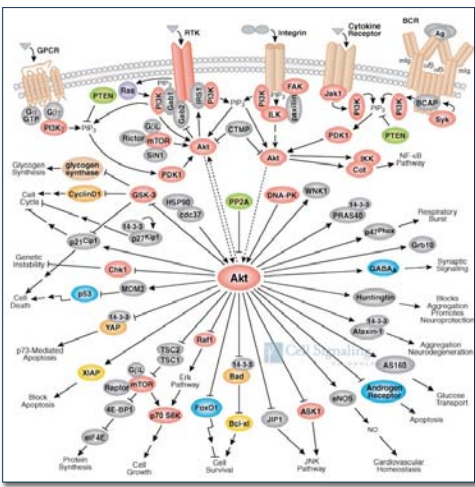


PTMScan Report




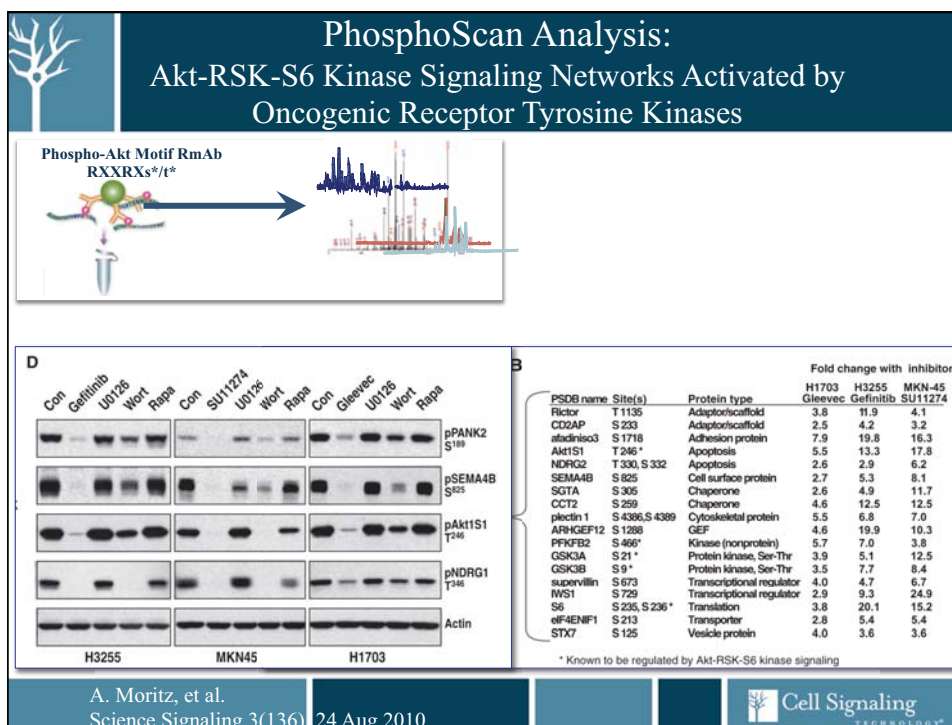
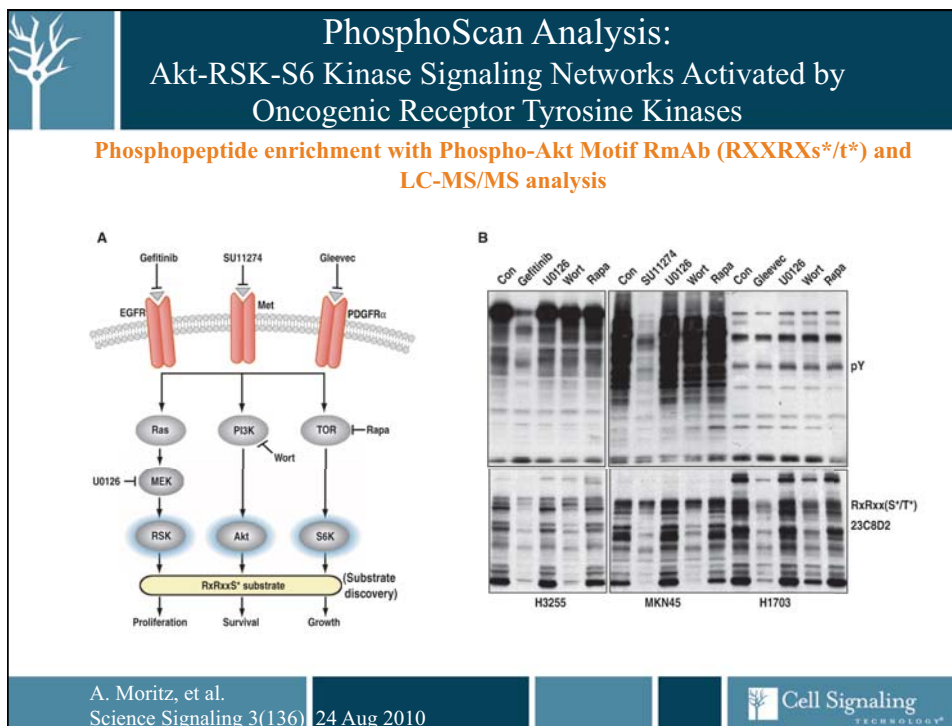


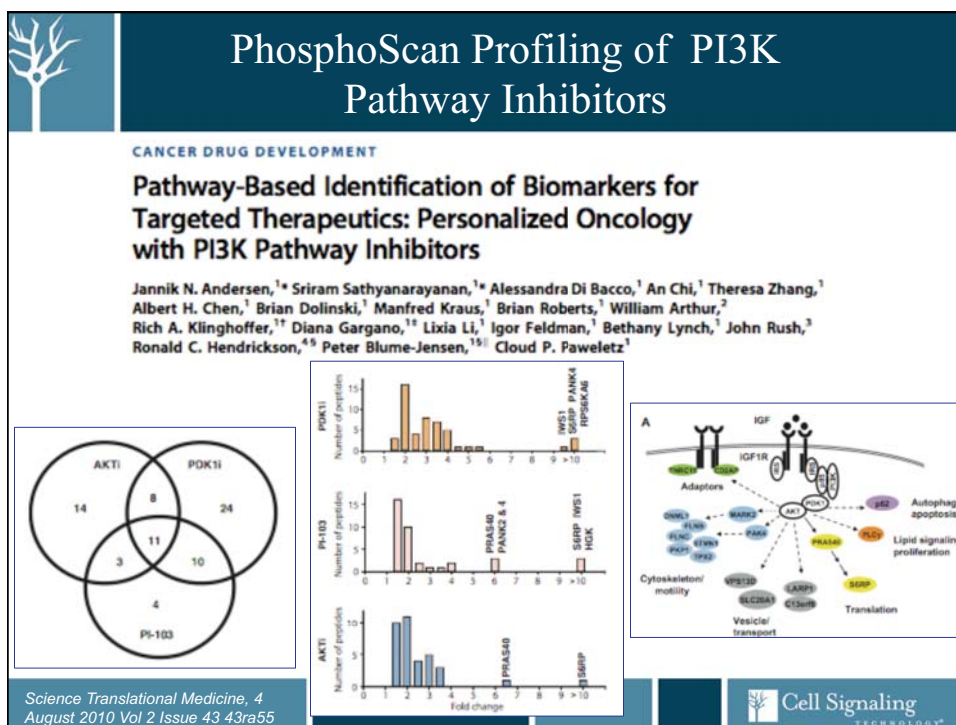
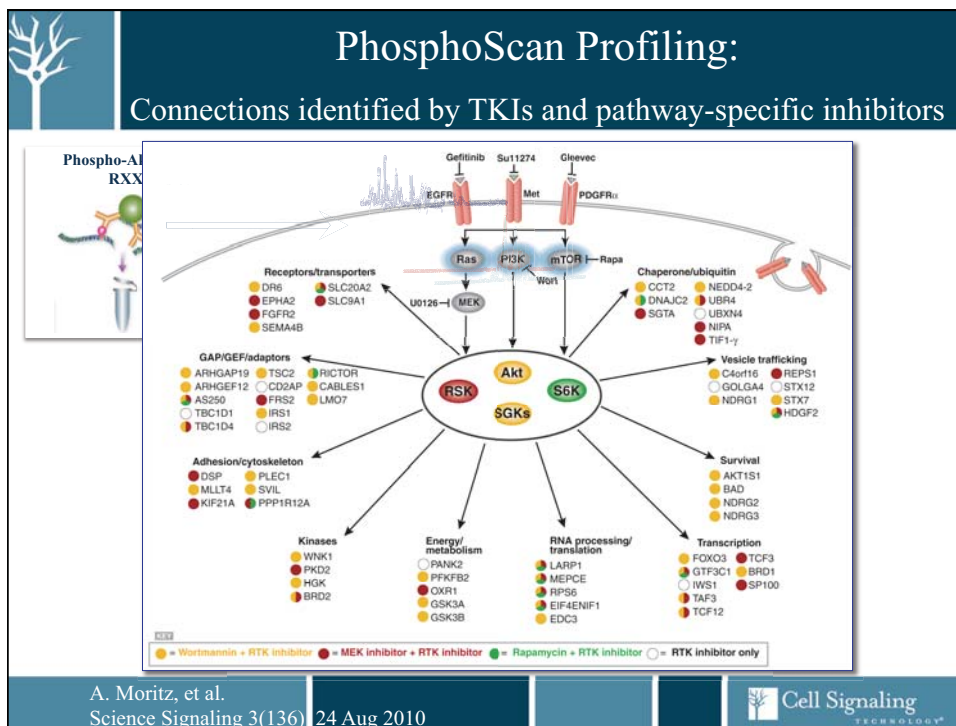
PhosphoScan Profiling of Akt Substrate Phosphorylation



PhosphoScan-based discovery of drug-response Akt substrates

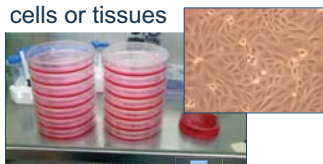






PTMScan Services & Products

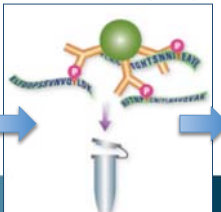
Customer-provided cells or tissues



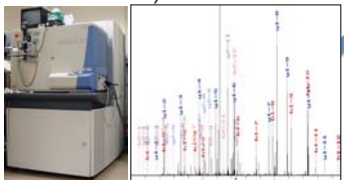
Lysis
Extraction
Digest


Total Peptide Fraction

p-S/T,Y; Ac-K; K-GG
Immunoaffinity Purification (IAP)




Liquid Chromatography – Tandem Mass Spectrometry (LC – MS/MS)



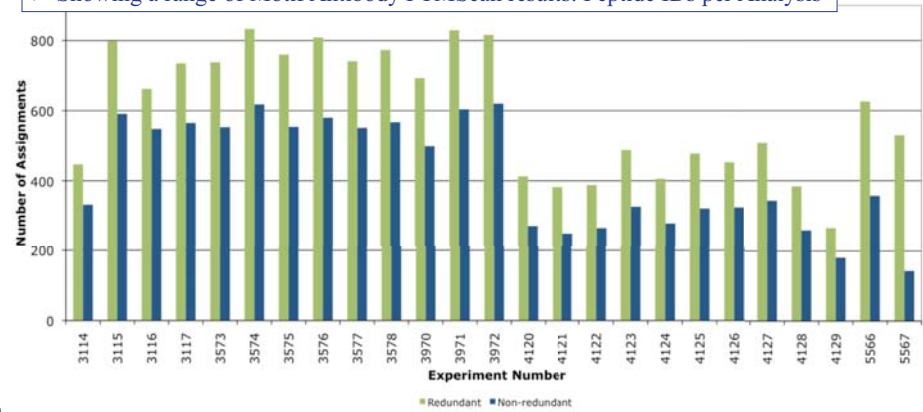


PTMScan Report




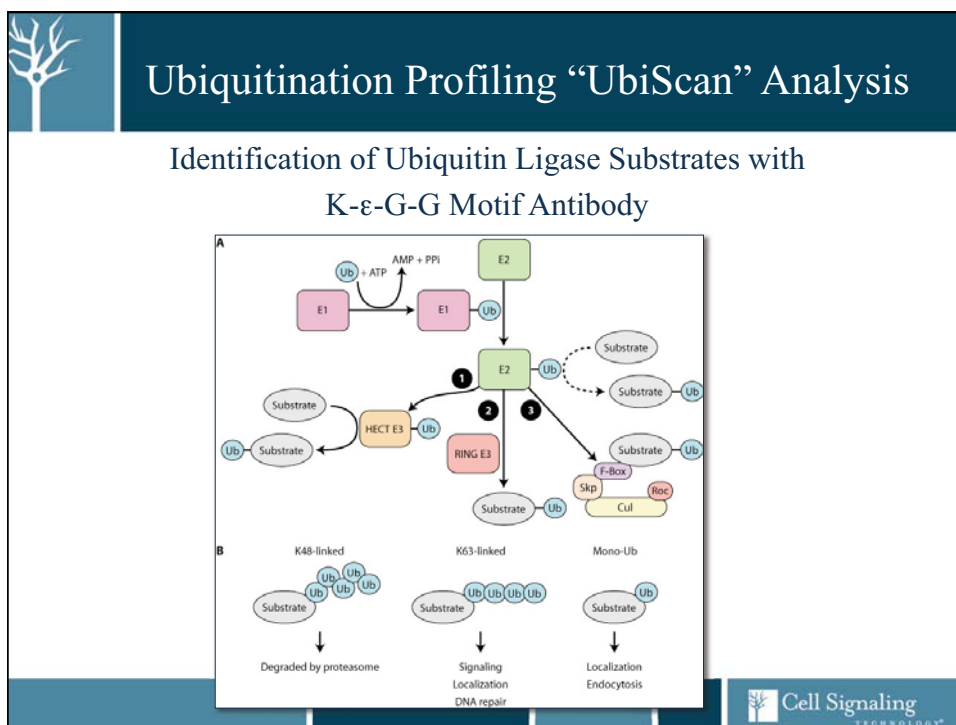
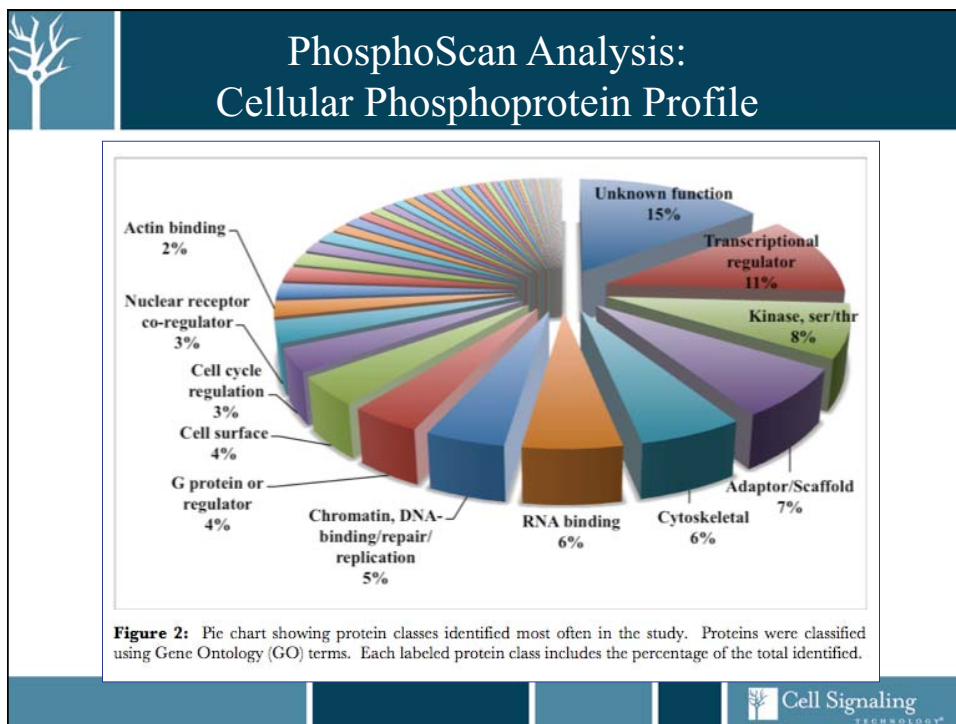
PTMScan/PhosphoScan Profiling Results: Site Identifications: 100 – 1,000

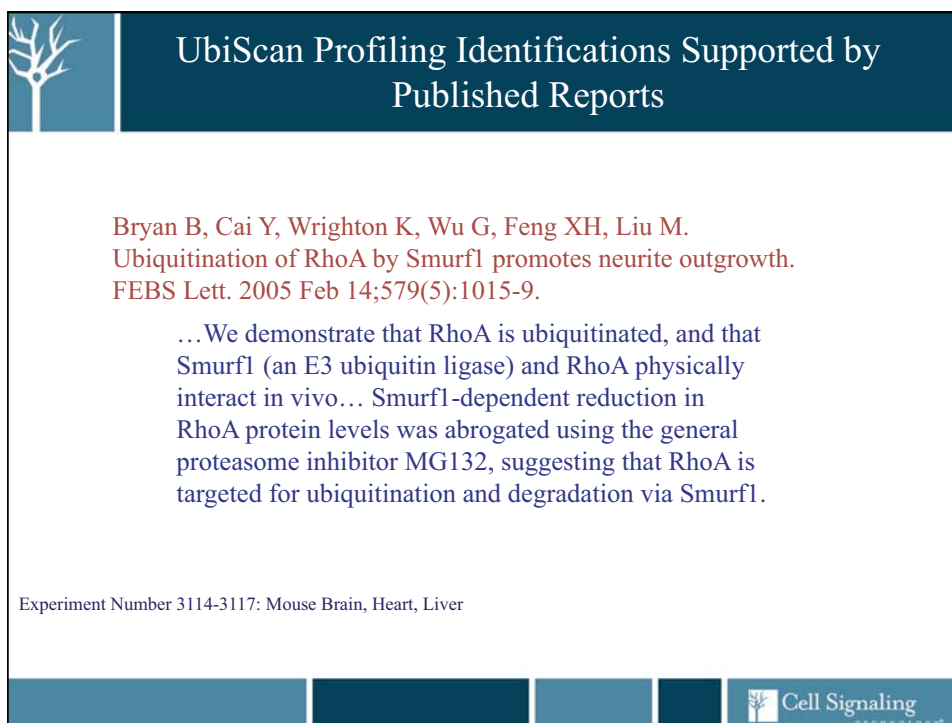
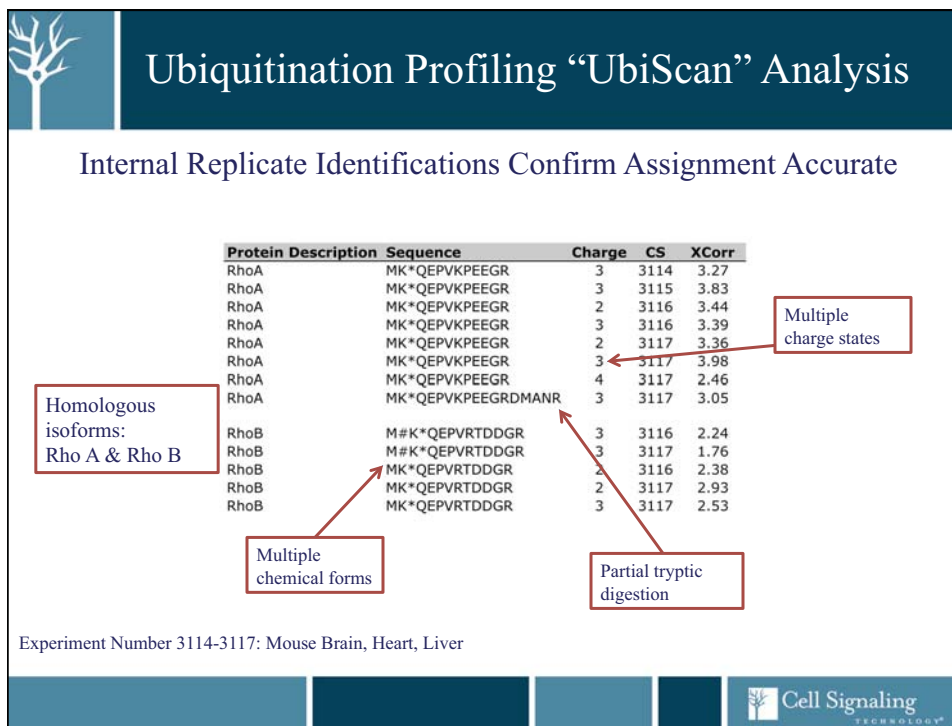
- 5 Example Studies – Mouse tissues and cell lines
- 25 PTMScan MS Analyses
- Showing a range of Motif Antibody PTMScan results: Peptide IDs per Analysis



| Experiment Number | Redundant | Non-redundant |
|-------------------|-----------|---------------|
| 3114 | 450 | 350 |
| 3115 | 800 | 600 |
| 3116 | 680 | 550 |
| 3117 | 750 | 580 |
| 3573 | 750 | 550 |
| 3574 | 850 | 620 |
| 3575 | 780 | 580 |
| 3576 | 800 | 600 |
| 3577 | 750 | 580 |
| 3578 | 780 | 600 |
| 3970 | 700 | 500 |
| 3971 | 850 | 620 |
| 3972 | 820 | 600 |
| 4120 | 420 | 280 |
| 4121 | 380 | 250 |
| 4122 | 400 | 280 |
| 4123 | 480 | 350 |
| 4124 | 420 | 300 |
| 4125 | 480 | 350 |
| 4126 | 450 | 350 |
| 4127 | 500 | 380 |
| 4128 | 380 | 280 |
| 4129 | 280 | 200 |
| 5566 | 650 | 400 |
| 5567 | 550 | 150 |







Ubiquitination Profiling “UbiScan” Analysis

Identification of All Ubiquitination Sites on Ubiquitin

| Sequence | Charge | CS | XCorr | Sequence | Charge | CS | XCorr | Sequence | Charge | CS | XCorr |
|------------------|--------|------|-------|---------------------------------|--------|------|-------|---------------------------------|--------|------|-------|
| AK*IQDK*EGIPDQQR | 3 | 3115 | 2.61 | IQDK*EGIPDQQR | 2 | 3117 | 1.99 | LIFAGK*QLEDGRTLSDYNIQKESTLHLVLR | 5 | 3115 | 3.27 |
| AK*IQDK*EGIPDQQR | 3 | 3116 | 2.09 | IQDK*EGIPDQQR | 3 | 3117 | 1.76 | MQIFVK*TLTGK | 2 | 3116 | 3.69 |
| AK*IQDK*EGIPDQQR | 3 | 3117 | 2.49 | LIFAGK*QLEDGR | 2 | 3114 | 3.38 | MQIFVK*TLTGK | 2 | 3117 | 3.19 |
| AK*IQDK*EGIPDQQR | 3 | 3114 | 2.90 | LIFAGK*QLEDGR | 2 | 3115 | 3.55 | MQIFVK*TLTGK | 2 | 3114 | 2.21 |
| AK*IQDK*EGIPDQQR | 2 | 3115 | 4.32 | LIFAGK*QLEDGR | 2 | 3115 | 3.27 | MQIFVK*TLTGK | 2 | 3116 | 3.34 |
| AK*IQDK*EGIPDQQR | 2 | 3115 | 3.90 | LIFAGK*QLEDGR | 2 | 3116 | 3.47 | MQIFVK*TLTGK | 2 | 3117 | 3.61 |
| AK*IQDK*EGIPDQQR | 2 | 3115 | 3.79 | LIFAGK*QLEDGR | 2 | 3117 | 3.31 | MQIFVK*TLTGK | 3 | 3115 | 2.64 |
| AK*IQDK*EGIPDQQR | 2 | 3115 | 3.74 | LIFAGK*QLEDGR | 2 | 3117 | 2.56 | MQIFVK*TLTGK | 3 | 3116 | 3.55 |
| AK*IQDK*EGIPDQQR | 2 | 3115 | 2.88 | LIFAGK*QLEDGR | 3 | 3114 | 2.65 | MQIFVK*TLTGK | 3 | 3117 | 3.34 |
| AK*IQDK*EGIPDQQR | 2 | 3116 | 2.41 | LIFAGK*QLEDGR | 3 | 3115 | 2.66 | TITLEVEPSDTIENVK*AKIQDKEGIPDQQR | 4 | 3117 | 2.61 |
| AK*IQDK*EGIPDQQR | 2 | 3117 | 3.41 | LIFAGK*QLEDGR | 3 | 3116 | 2.47 | TLSDYNIQK*ESTLHLVLR | 2 | 3115 | 4.61 |
| AK*IQDK*EGIPDQQR | 3 | 3115 | 2.80 | LIFAGK*QLEDGR | 3 | 3117 | 2.81 | TLSDYNIQK*ESTLHLVLR | 2 | 3116 | 4.72 |
| AK*IQDK*EGIPDQQR | 3 | 3115 | 2.77 | LIFAGK*QLEDGR | 3 | 3117 | 2.58 | TLSDYNIQK*ESTLHLVLR | 2 | 3117 | 4.63 |
| AK*IQDK*EGIPDQQR | 3 | 3116 | 2.47 | LIFAGK*QLEDGR | 3 | 3117 | 1.53 | TLSDYNIQK*ESTLHLVLR | 3 | 3114 | 3.78 |
| AK*IQDK*EGIPDQQR | 3 | 3116 | 2.22 | LIFAGK*QLEDGRTLSDYNIQK | 2 | 3115 | 4.87 | TLSDYNIQK*ESTLHLVLR | 3 | 3114 | 2.77 |
| AK*IQDK*EGIPDQQR | 3 | 3117 | 2.62 | LIFAGK*QLEDGRTLSDYNIQK | 2 | 3116 | 5.04 | TLSDYNIQK*ESTLHLVLR | 3 | 3115 | 2.87 |
| AK*IQDK*EGIPDQQR | 3 | 3117 | 2.47 | LIFAGK*QLEDGRTLSDYNIQK | 2 | 3116 | 3.26 | TLSDYNIQK*ESTLHLVLR | 3 | 3116 | 3.63 |
| AK*IQDK*EGIPDQQR | 3 | 3117 | 1.66 | LIFAGK*QLEDGRTLSDYNIQK | 3 | 3114 | 3.56 | TLSDYNIQK*ESTLHLVLR | 3 | 3117 | 4.00 |
| AK*IQDK*EGIPDQQR | 4 | 3115 | 2.77 | LIFAGK*QLEDGRTLSDYNIQK | 3 | 3115 | 2.75 | TLSDYNIQK*ESTLHLVLR | 3 | 3117 | 3.72 |
| AK*IQDK*EGIPDQQR | 4 | 3115 | 2.52 | LIFAGK*QLEDGRTLSDYNIQK | 3 | 3116 | 2.40 | TLSDYNIQK*ESTLHLVLR | 3 | 3117 | 2.03 |
| IQDK*EGIPDQQR | 2 | 3114 | 2.30 | LIFAGK*QLEDGRTLSDYNIQK | 3 | 3117 | 3.54 | TLSDYNIQK*ESTLHLVLR | 3 | 3117 | 2.00 |
| IQDK*EGIPDQQR | 2 | 3115 | 3.23 | LIFAGK*QLEDGRTLSDYNIQK | 4 | 3115 | 2.52 | TLSDYNIQK*ESTLHLVLR | 4 | 3117 | 2.45 |
| IQDK*EGIPDQQR | 2 | 3115 | 1.96 | LIFAGK*QLEDGRTLSDYNIQK | 4 | 3116 | 2.10 | TLTGK*TITLEVEPSDTIENVK | 3 | 3114 | 4.25 |
| IQDK*EGIPDQQR | 2 | 3116 | 3.37 | LIFAGK*QLEDGRTLSDYNIQK | 4 | 3117 | 2.23 | TLTGK*TITLEVEPSDTIENVK | 3 | 3115 | 3.88 |
| IQDK*EGIPDQQR | 2 | 3117 | 2.70 | LIFAGK*QLEDGRTLSDYNIQKESTLHLVLR | 3 | 3115 | 6.65 | TLTGK*TITLEVEPSDTIENVK | 3 | 3116 | 4.36 |
| IQDK*EGIPDQQR | 2 | 3117 | 2.48 | LIFAGK*QLEDGRTLSDYNIQKESTLHLVLR | 4 | 3115 | 3.99 | TLTGK*TITLEVEPSDTIENVKAK | 3 | 3117 | 3.85 |
| IQDK*EGIPDQQR | 2 | 3117 | 2.26 | LIFAGK*QLEDGRTLSDYNIQKESTLHLVLR | 4 | 3117 | 4.48 | | | | |

MQIFVK₆TLTGK₁₁TITLEVEPSDTIENVK₂₇AK₂₉IQDK₃₃EGIPDQQRLIFAGK₄₈QL
EDGRTLSDYNIQK₆₃ESTLHLVLRLRGG

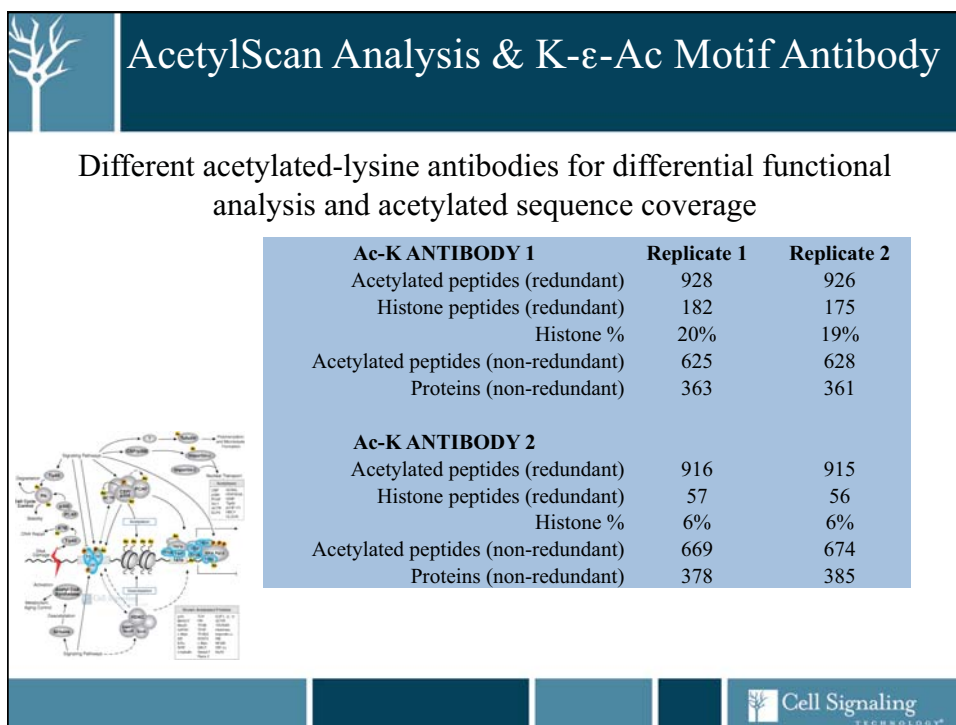
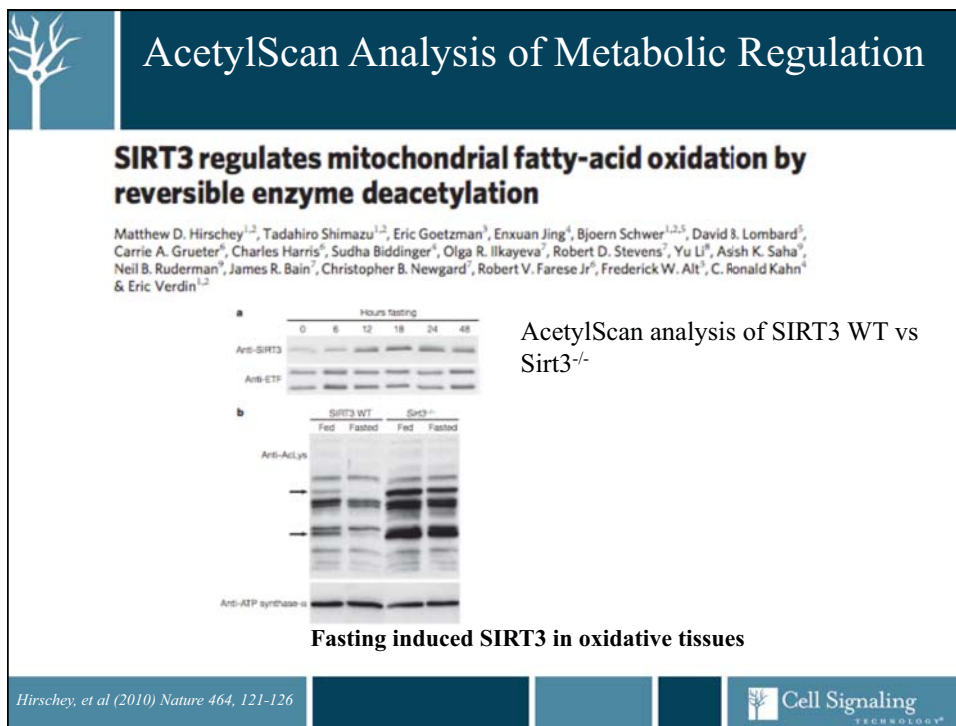
Lysine 48-Linkage Specific Polyubiquitin Antibody #4289

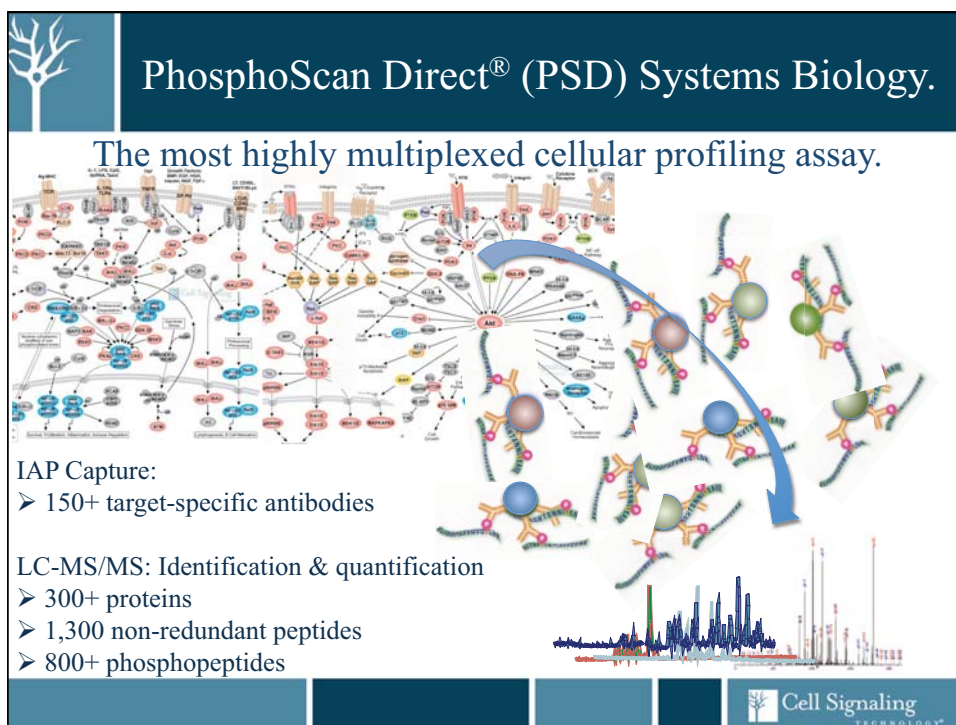
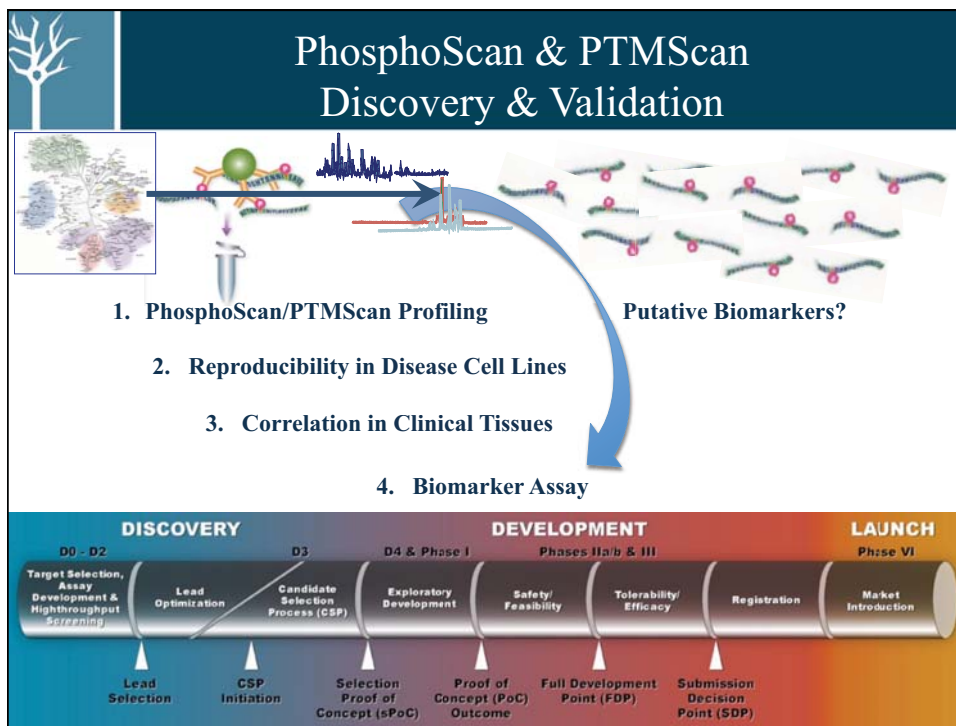
Experiment Number 3114-3117: Mouse Brain, Heart, Liver

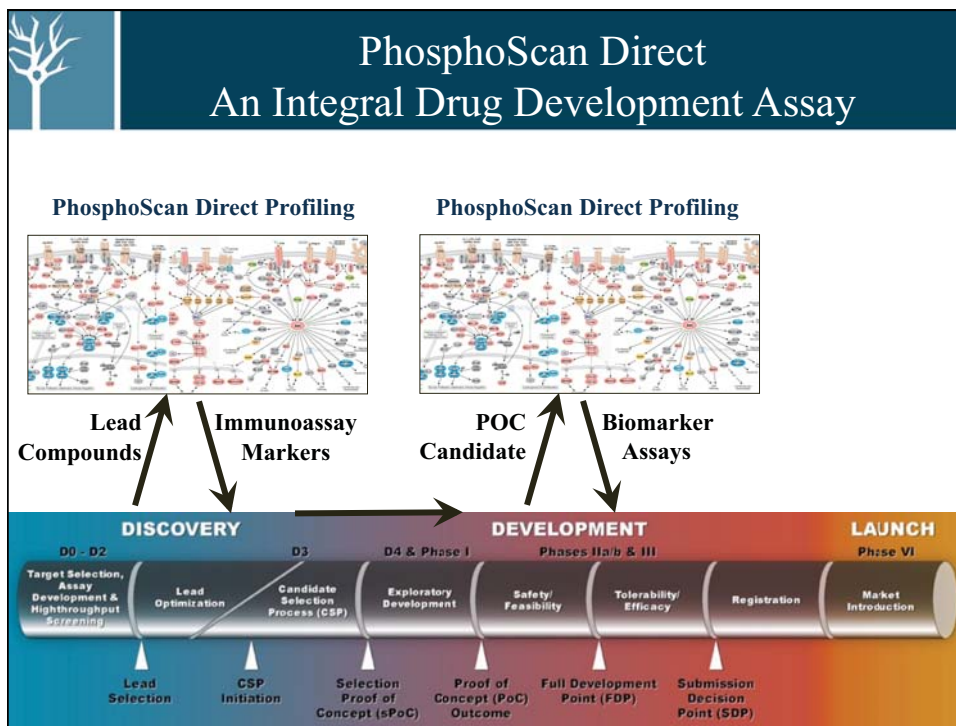
AcetylScan Analysis & K-ε-Ac Motif Antibody

AcetylScan & MethylScan data
 at www.phosphosite.org

| Known Acetylated Proteins | | |
|---------------------------|--------|------------|
| p53 | TCF | E2F1, 2, 3 |
| MtG17 | PI1 | ACTR |
| MycD | TFIIID | TRIM33 |
| GATA1 | TFIIIF | Histones |
| α-Myb | TFIIIB | Importin-α |
| AR | STAT3 | RB |
| ERα | α-Myc | NP-KB |
| SRP | ERK1 | HIF-1α |
| α-tubulin | Smad7 | Ko70 |
| | | Flax 2 |







PTMScan™ Services

Proteomics of Regulatory Post-Translational Modifications

John Rush, Ph.D.
Sr. Director of Protein Chemistry

Jeffrey Silva, Ph.D.
PTMScan Project Manager

Chuck Farnsworth, Ph.D.
PTMScan Scientist

Matt Stokes, Ph.D.
PTMScan Scientist



Xiaoying Xia
PTMScan Associate

Cell Signaling
TECHNOLOGY®



Acknowledgements

Cell Signaling Technology's Leadership, Scientists, Managers, Sales & Marketing
&
CST's Worldwide Distribution Partners



Cell Signaling Technology

Cell Signaling Technology, Inc.

- Mission: To develop and provide innovative tools that help to define mechanisms underlying cell function and disease
- Founded by scientists from New England BioLabs (NEB)
- 380+ employees and growing
- Located in Danvers, MA USA; 100,000 sq R&D facility
- Subsidiaries: CST-China, CST-Japan, CST-Europe

