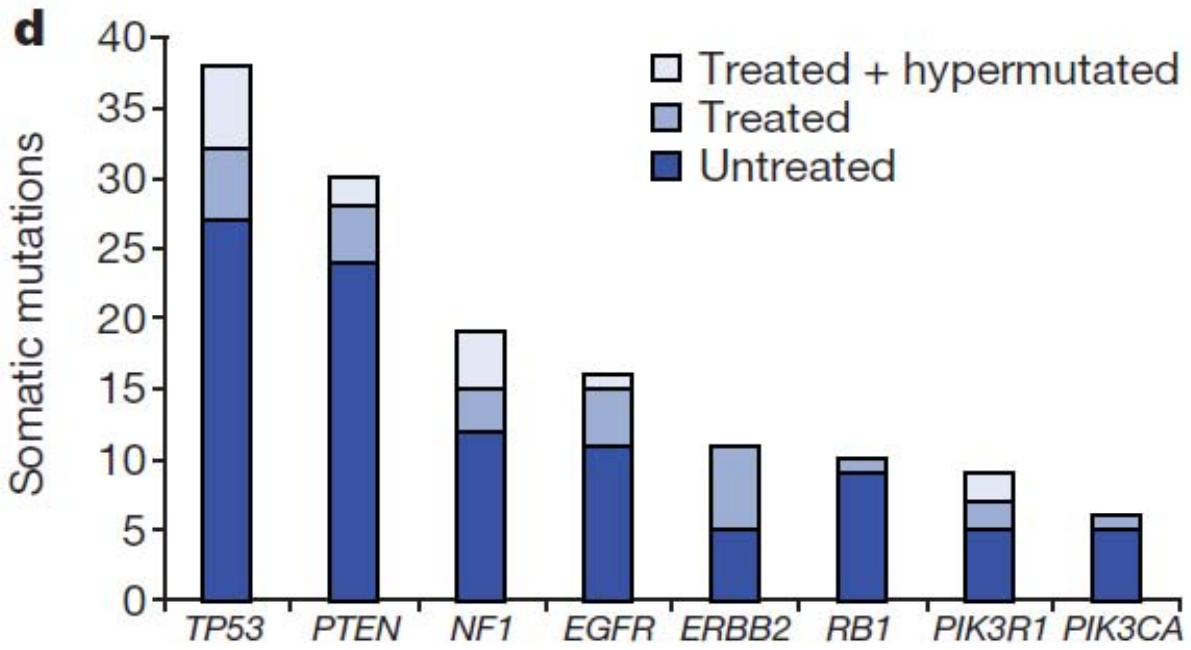
Why Study Signaling Networks?

Define better therapeutic targets, or combinations of therapeutic targets for cancer or other human diseases

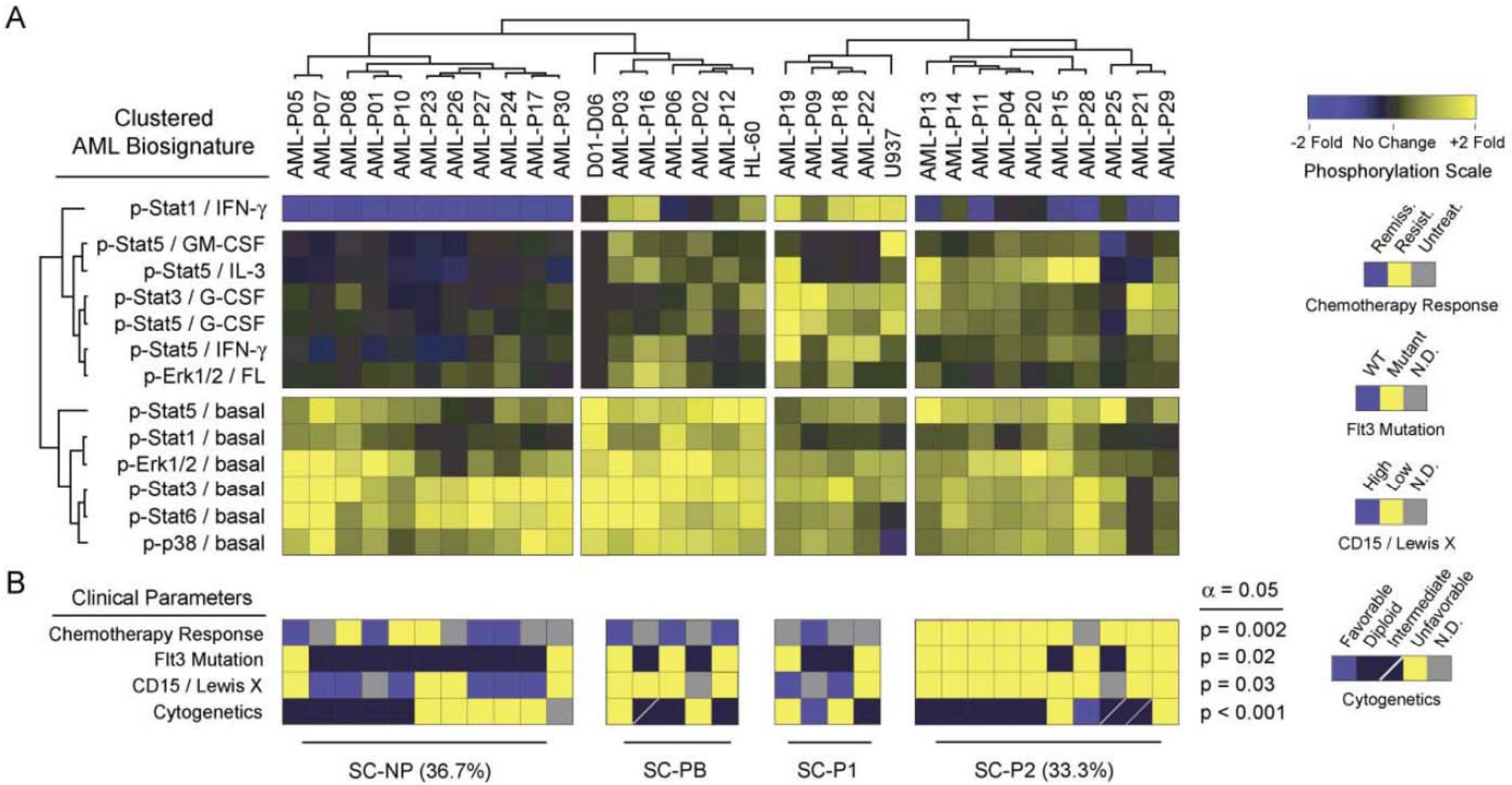
The Challenge: How do we get from genetic mutation to therapeutic targets?



Mutations in human glioblastoma tumor tissue, TCGA, Nature, 2008

Why Study Signaling Networks?

Disease Stratification and Personalized Medicine: Analyzing signaling networks can identify activated pathways and thereby highlight therapeutic options



Why Study Signaling Networks?

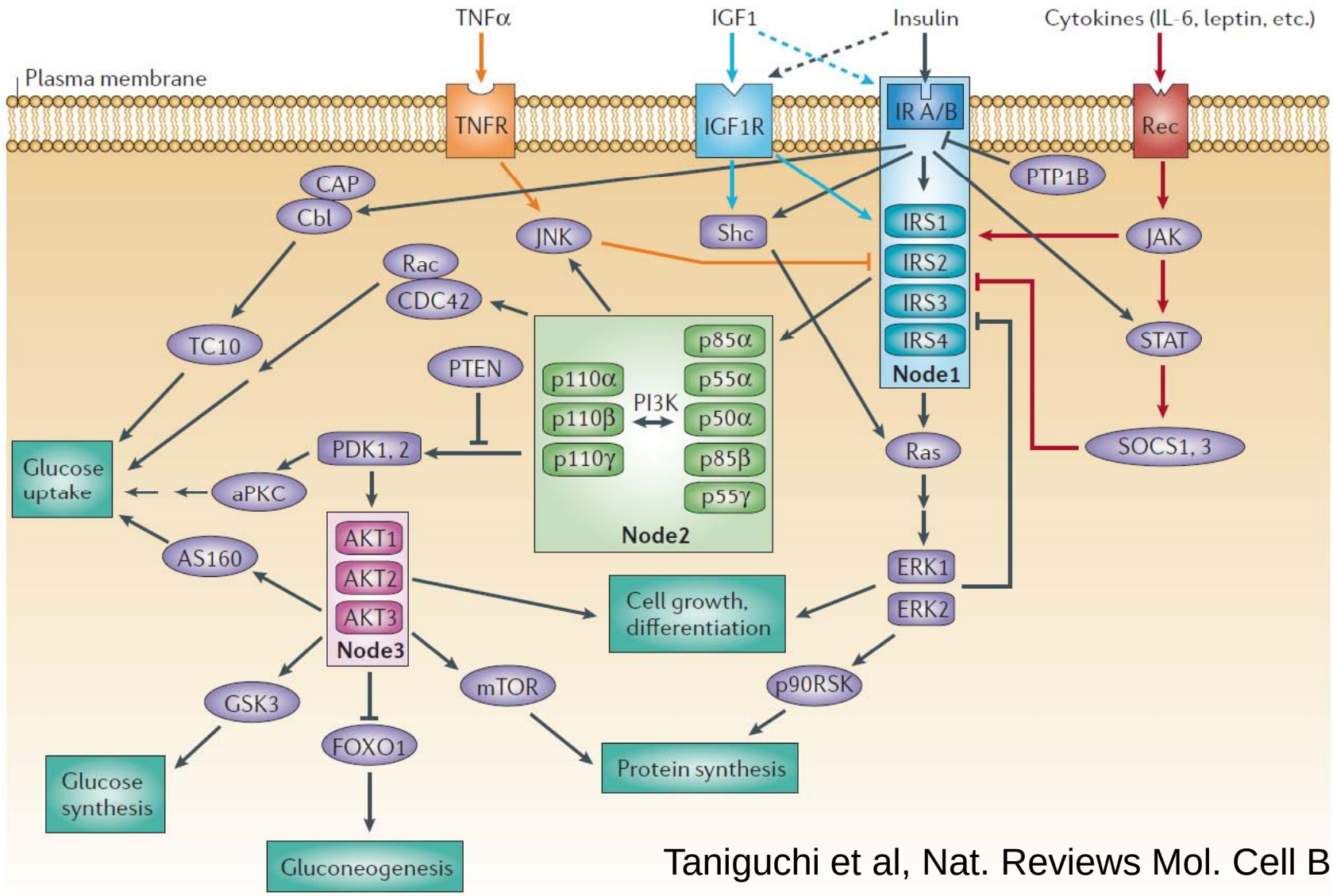
Therapeutic Targeting and Efficacy – is the kinase inhibitor actually affecting the targeted kinase? How are cells in a tumor responding to therapy?

Merrimack – Her3 antibody

AstraZeneca – Gefitinib (Iressa)

Why Study Signaling Networks?

Understanding mechanisms of resistance



Taniguchi et al, Nat. Reviews Mol. Cell Biol, 2006

How to analyze signaling networks

Classical Approach: Western blots

Reverse-Phase Protein Microarray

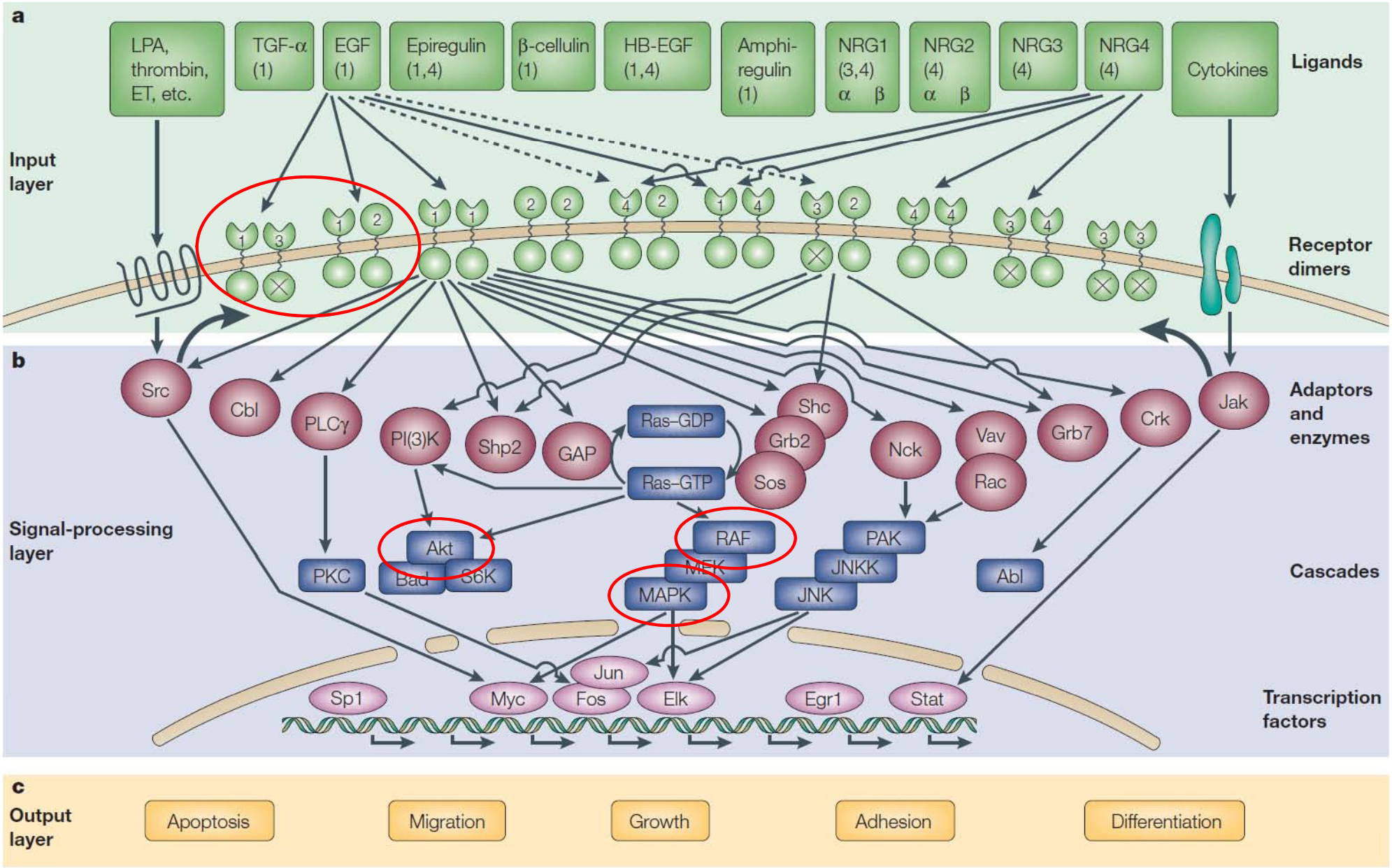
Luminex/ELISA

Mass Spectrometry

Phospho-FACS

Kinase Activity Assays

Classical Approach: Western blots – Target Selection?



Yarden and Slikowski, Nat. Reviews Mol. Cell Biol, 2001

Limitations of the Western blots for signaling analysis

Labor-intensive

Limited number of targets

Antibodies – not as specific as advertised

No discovery possibility

Quantitation sub-optimal

Micro-Western Arrays: Tackling the rate-limiting throughput of Western Blots

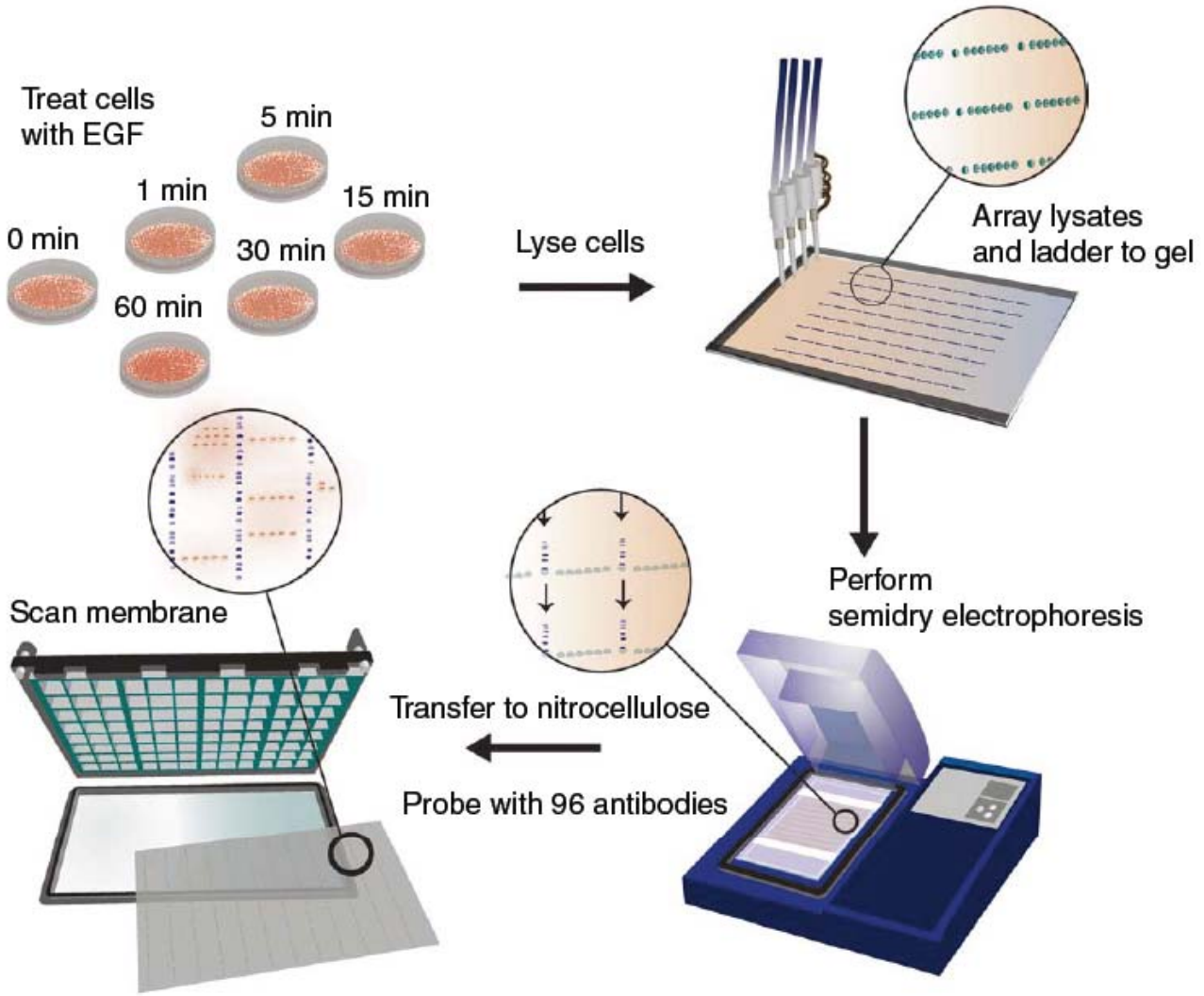
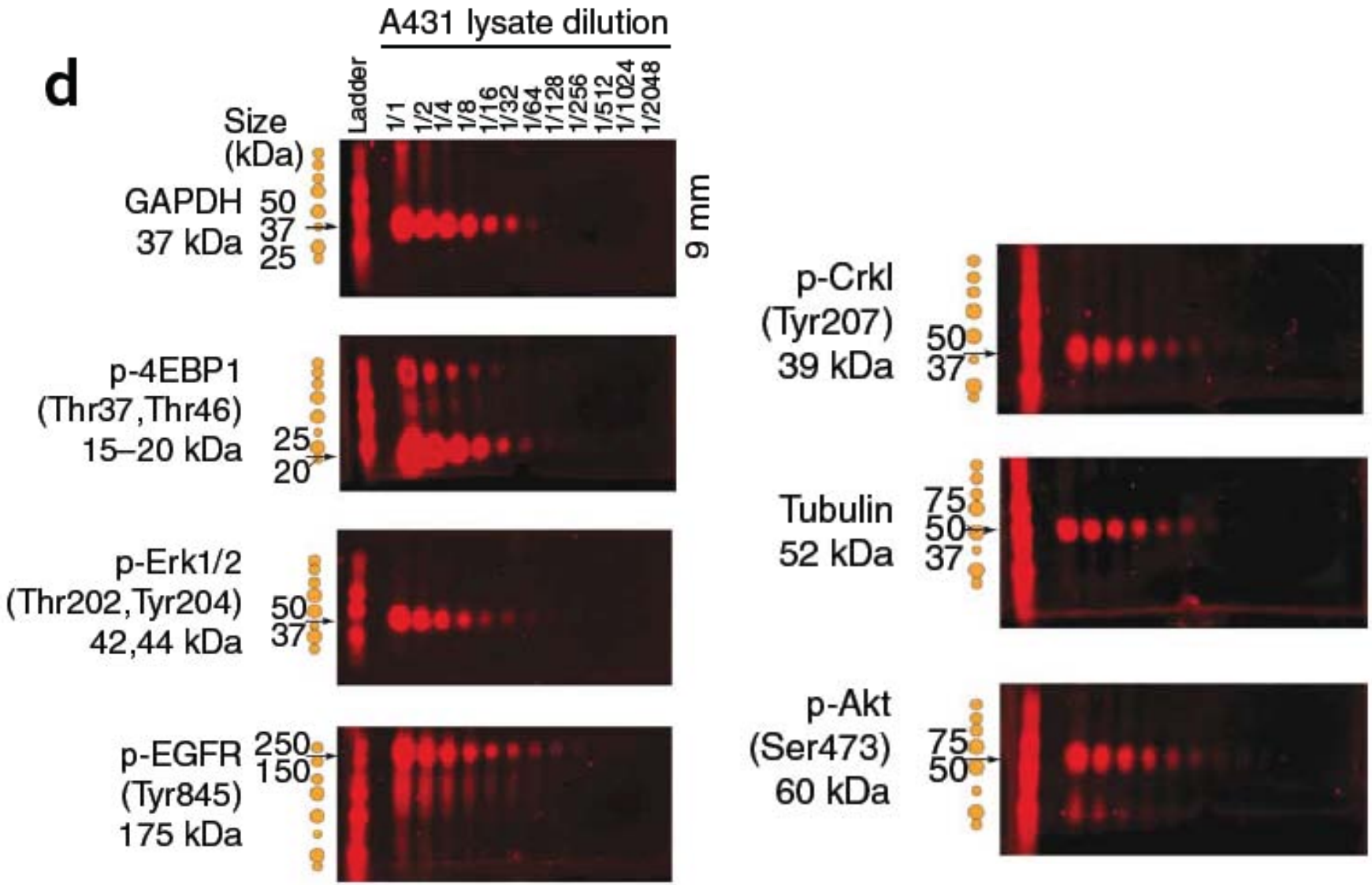


Figure 1 | Microwestern array (MWA) method. Schematic of the procedure.

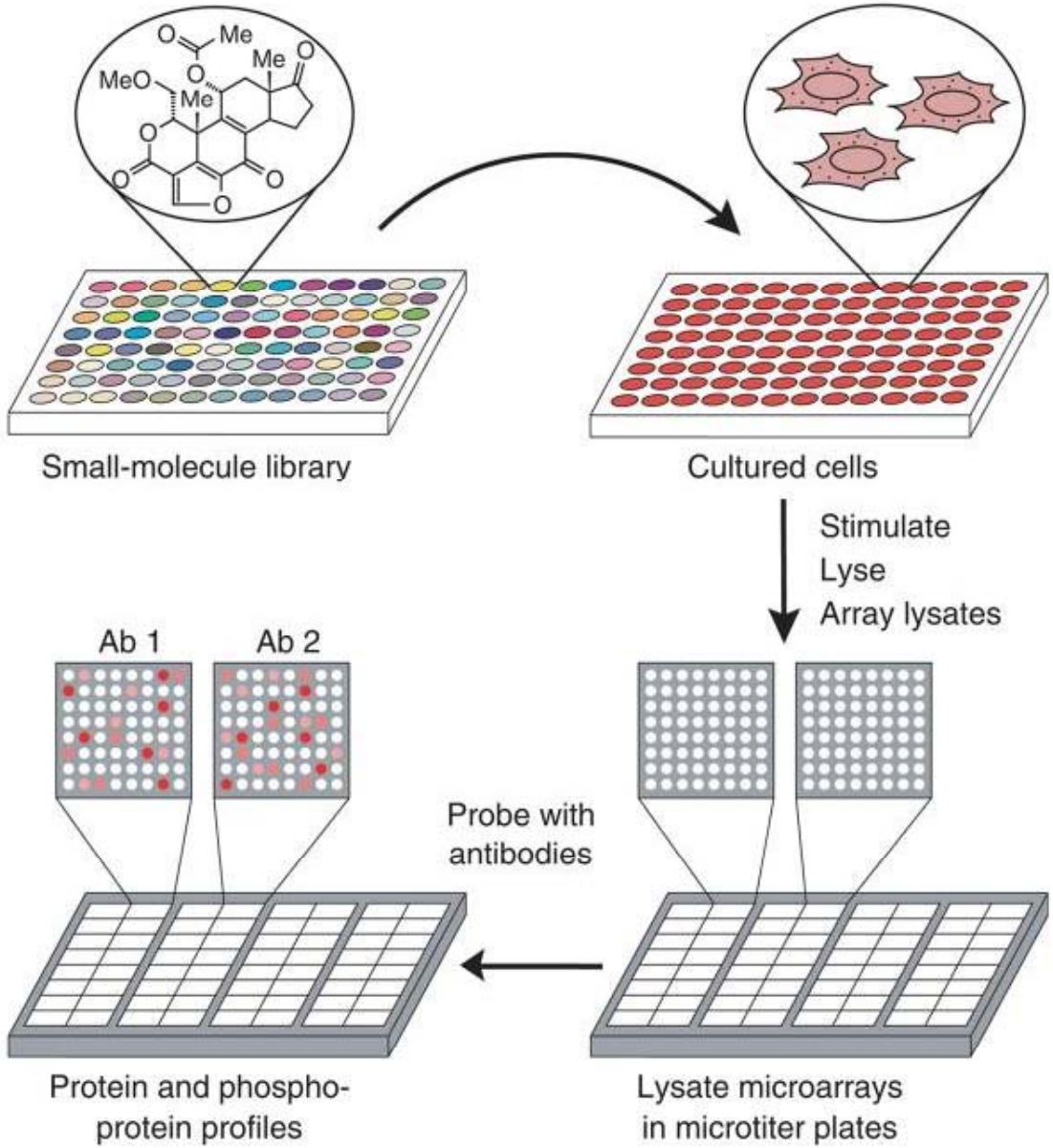
Ciaccio et al, Nat. Methods 2010

Micro-Western Arrays: Not the best looking westerns, but still functional



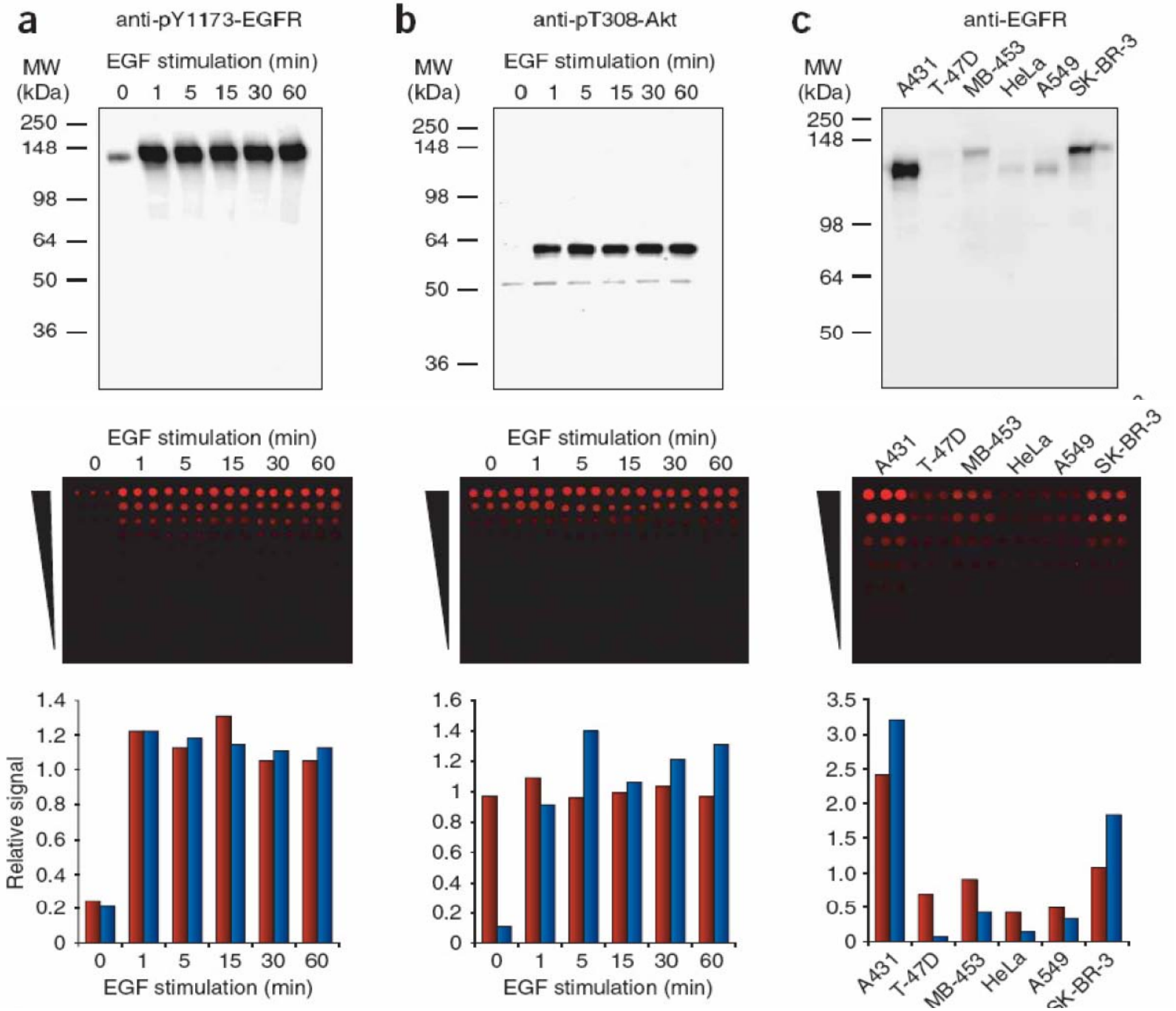
Ciaccio et al, Nat. Methods 2010

Reverse-phase microarrays: an alternate high-throughput strategy for quantifying signaling networks

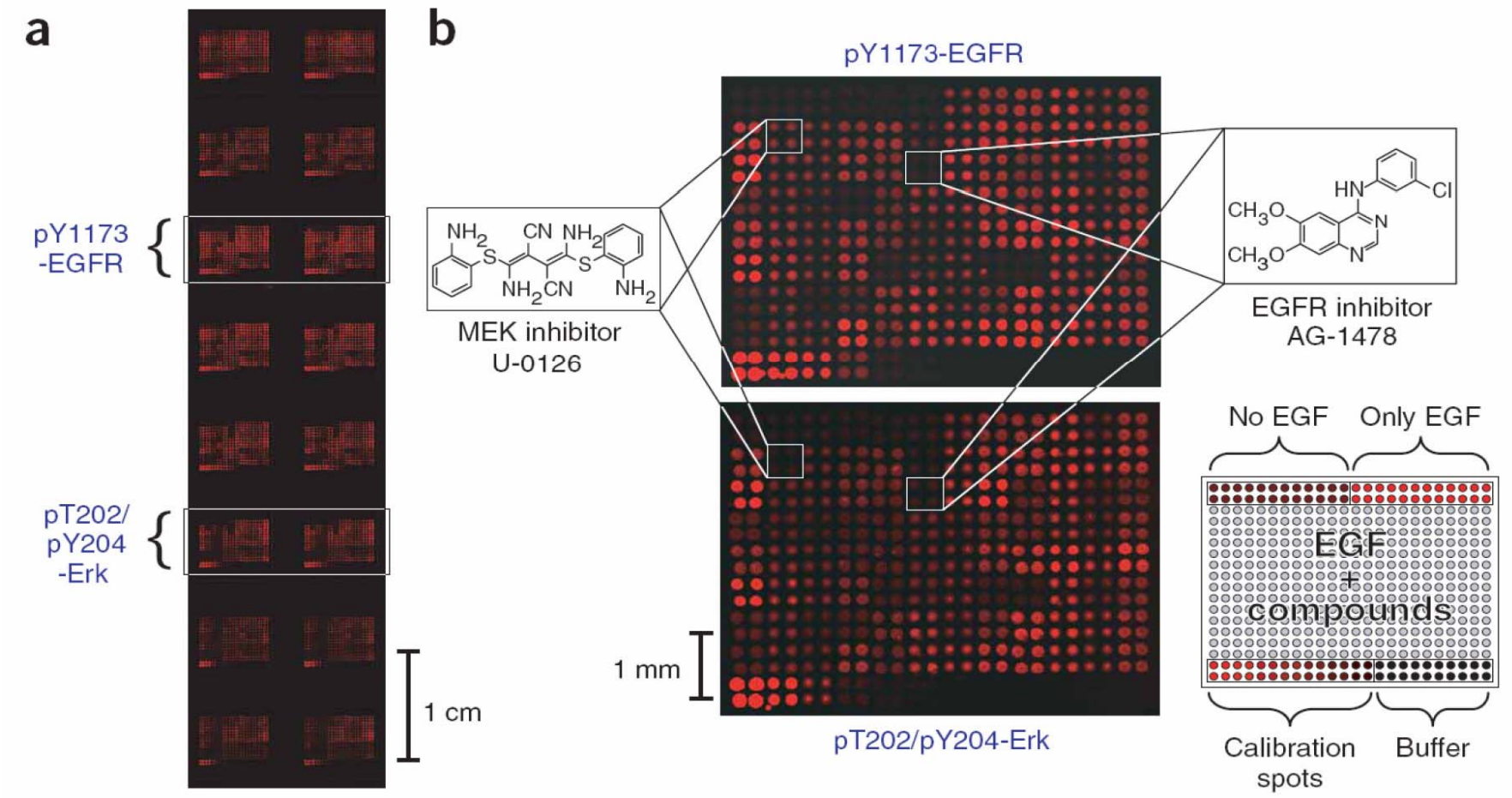


Sevecka et al, Nat. Methods 2006

How accurate are reverse-phase microarrays?



Reverse-phase arrays: lots of data on a single chip



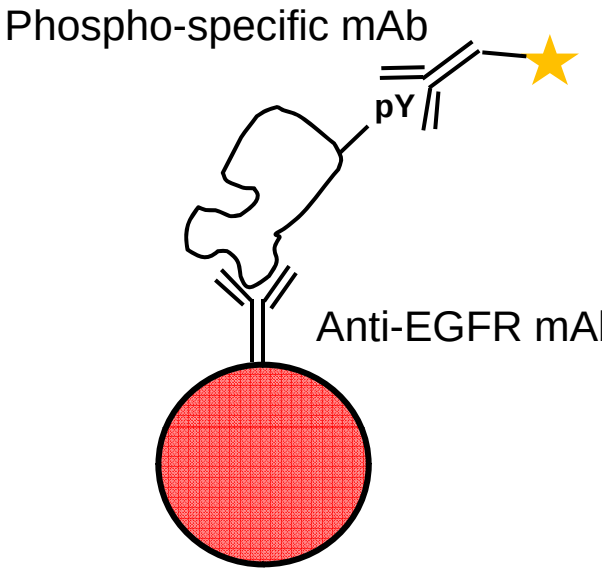
Sevecka et al, Nat. Methods 2006

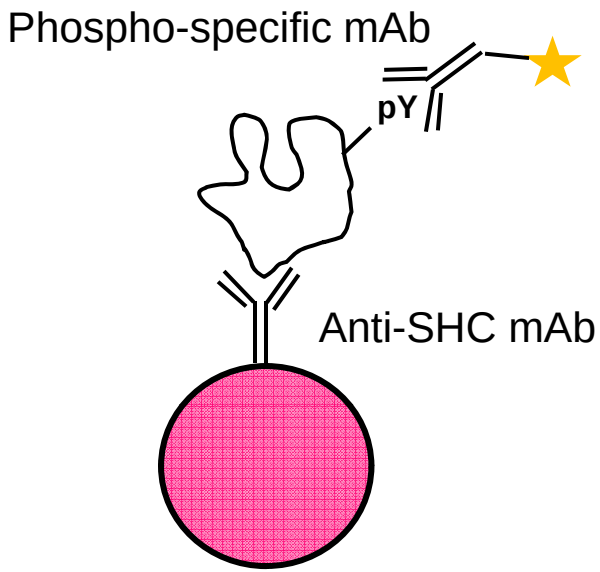
Using reverse-phase arrays to understand signaling networks

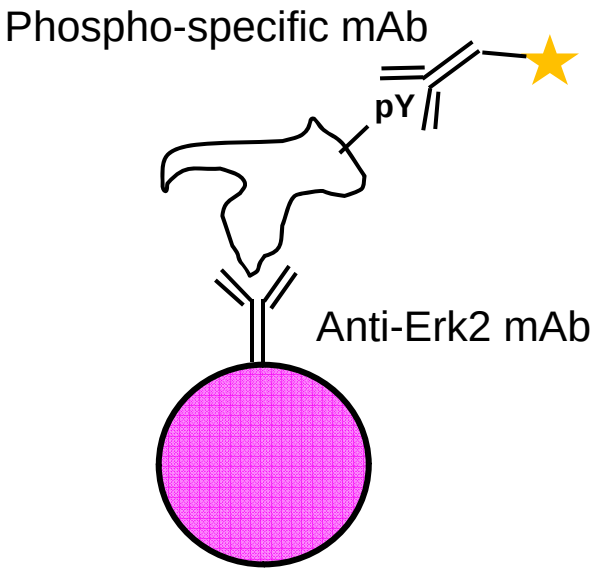
Sevecka et al, Nat. Methods 2006

Reverse-phase microarrays work, but accurate quantification is limited by non-specificity of the antibody. How can we overcome this limitation?

Luminex – improved specificity due to sandwich format, improved throughput with ~FACS-based quantification







Up to 100 different beads/targets per analysis

Sandwich-ELISA format improves specificity

http://www.luminexcorp.com/

All of these techniques are limited to well-characterized nodes in the signaling network, and limited by existing reagents. How can we discover novel pathway/network components while maintaining high coverage of the network?

Sample 1

Sample 2

Sample 3

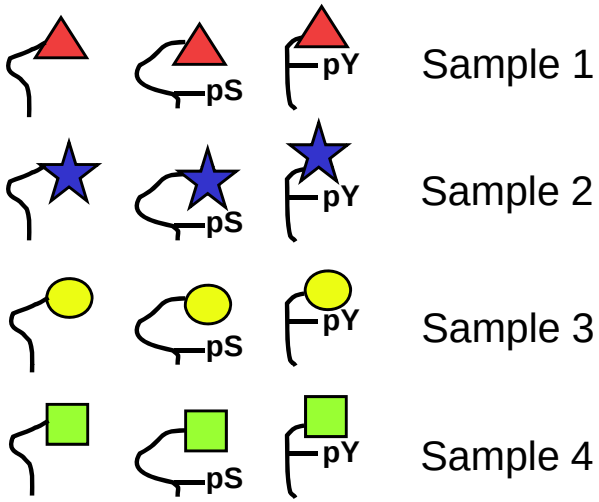
Sample 4

Biological Samples: cells, tumors, etc…

Lysis

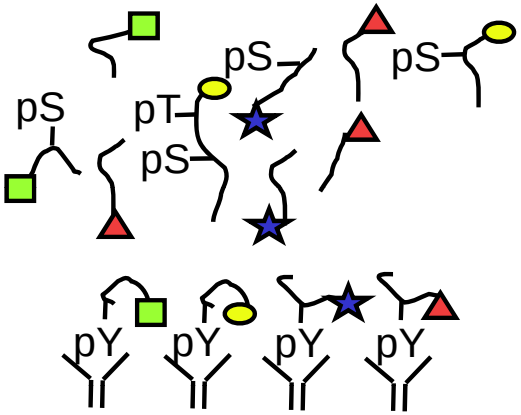
Protein Proteolysis

Desalt



Stable isotope (iTRAQ) labeling

Mix



pTyr-Peptide Immunoprecipitation

IMAC phosphopeptide enrichment

LC MS/MS analysis

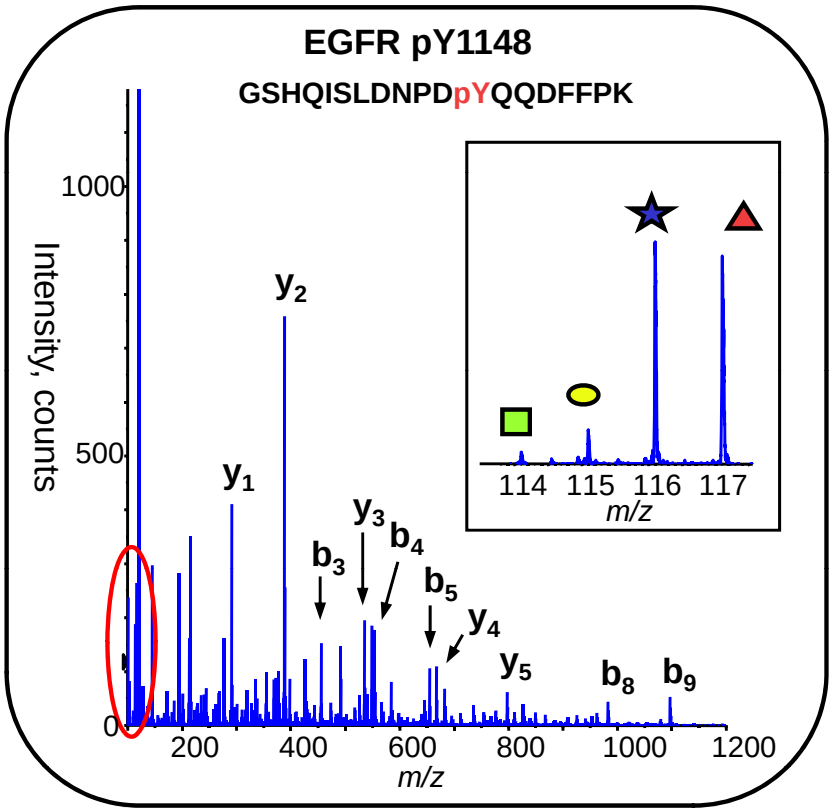


Photo of QSTAR® XL Hybrid LC/MS/MS System removed due to copyright restrictions.

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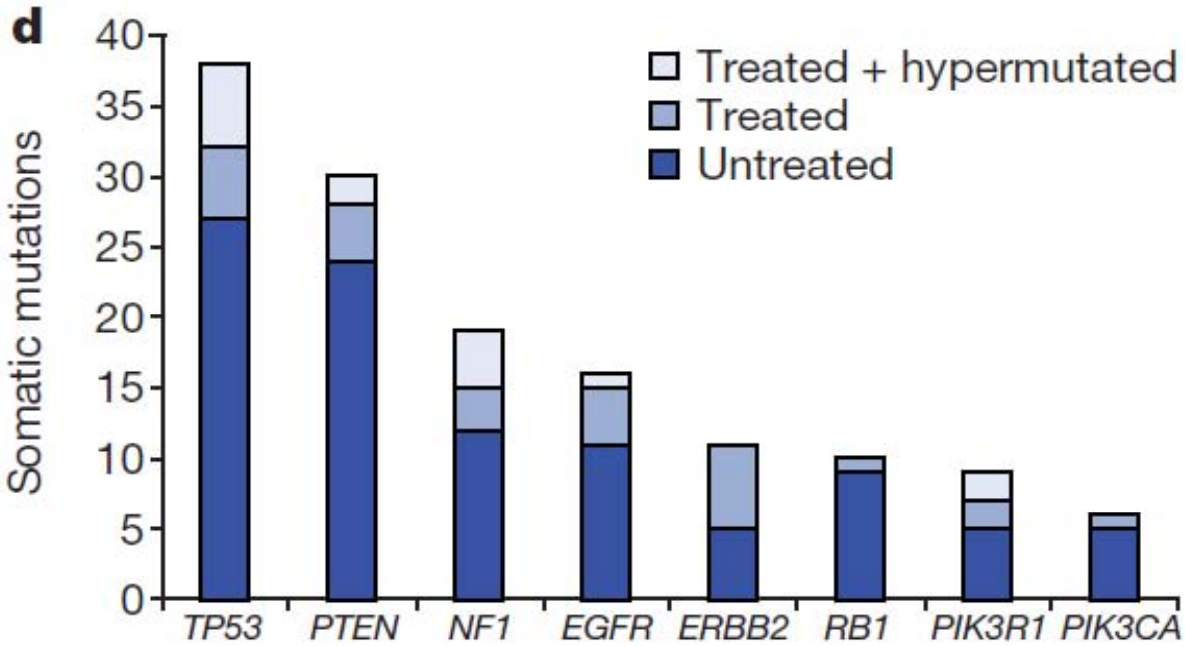
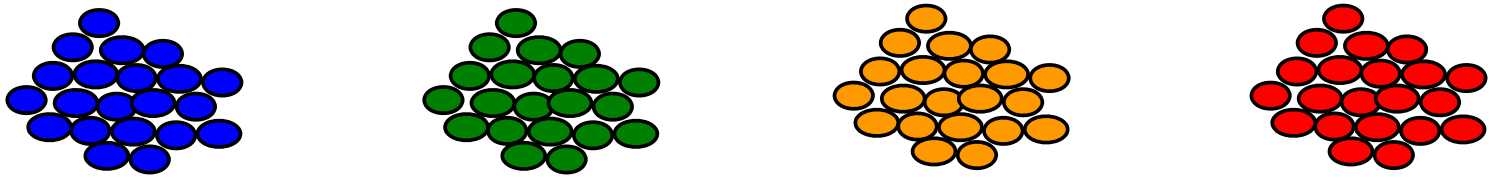


Mutations in human glioblastoma tumor tissue, TCGA, Nature, 2008

EGFRvIII Signaling Network Analysis

U87 Glioblastoma cell line



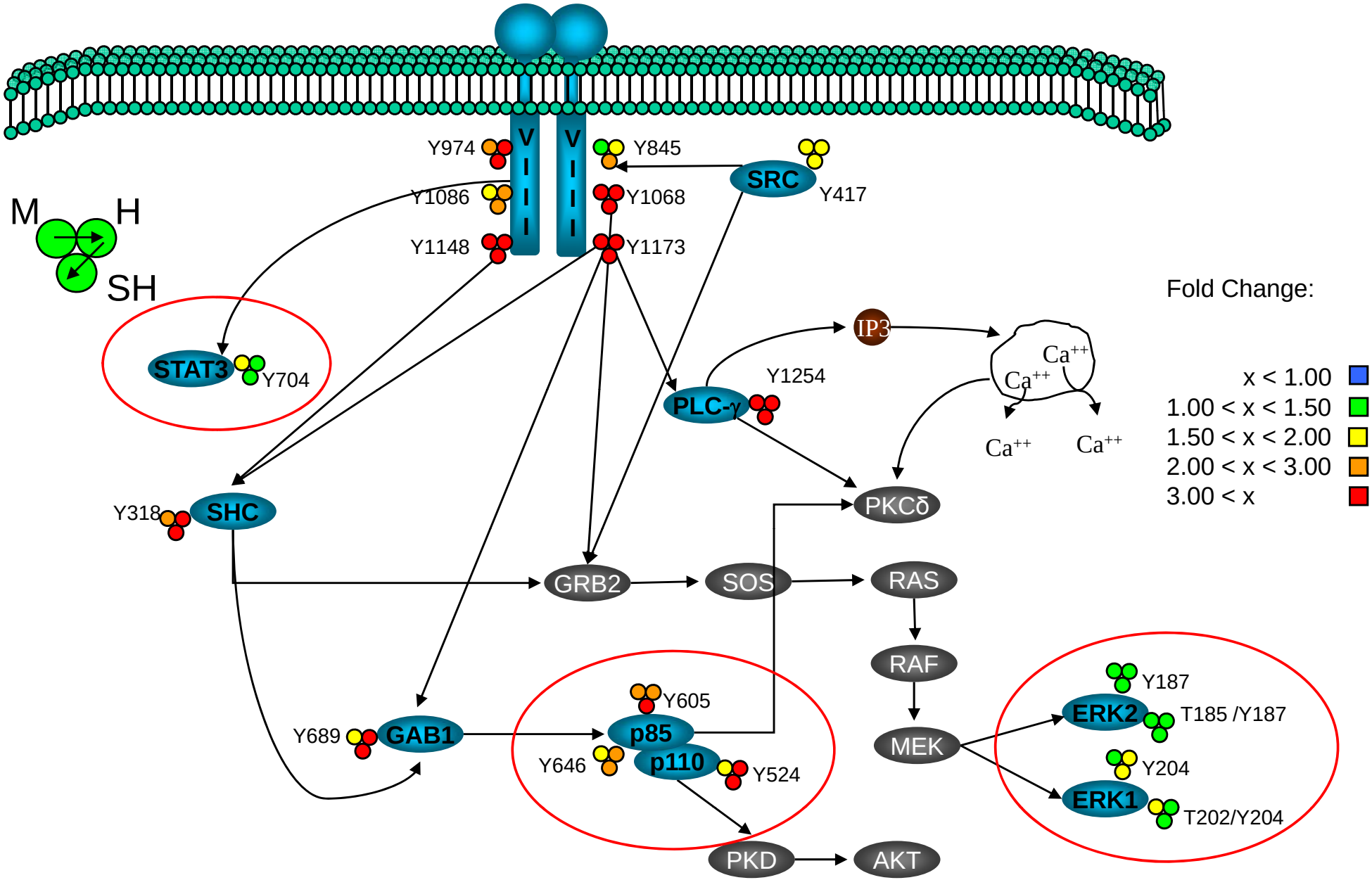
	Kinase-dead	Medium	High	Super High
EGFR:	100,000	100,000	100,000	100,000
EGFRvIII:	2,000,000	1,500,000	2,000,000	3,000,000
	Kinase dead			

Constitutive signaling

24 hours serum starvation

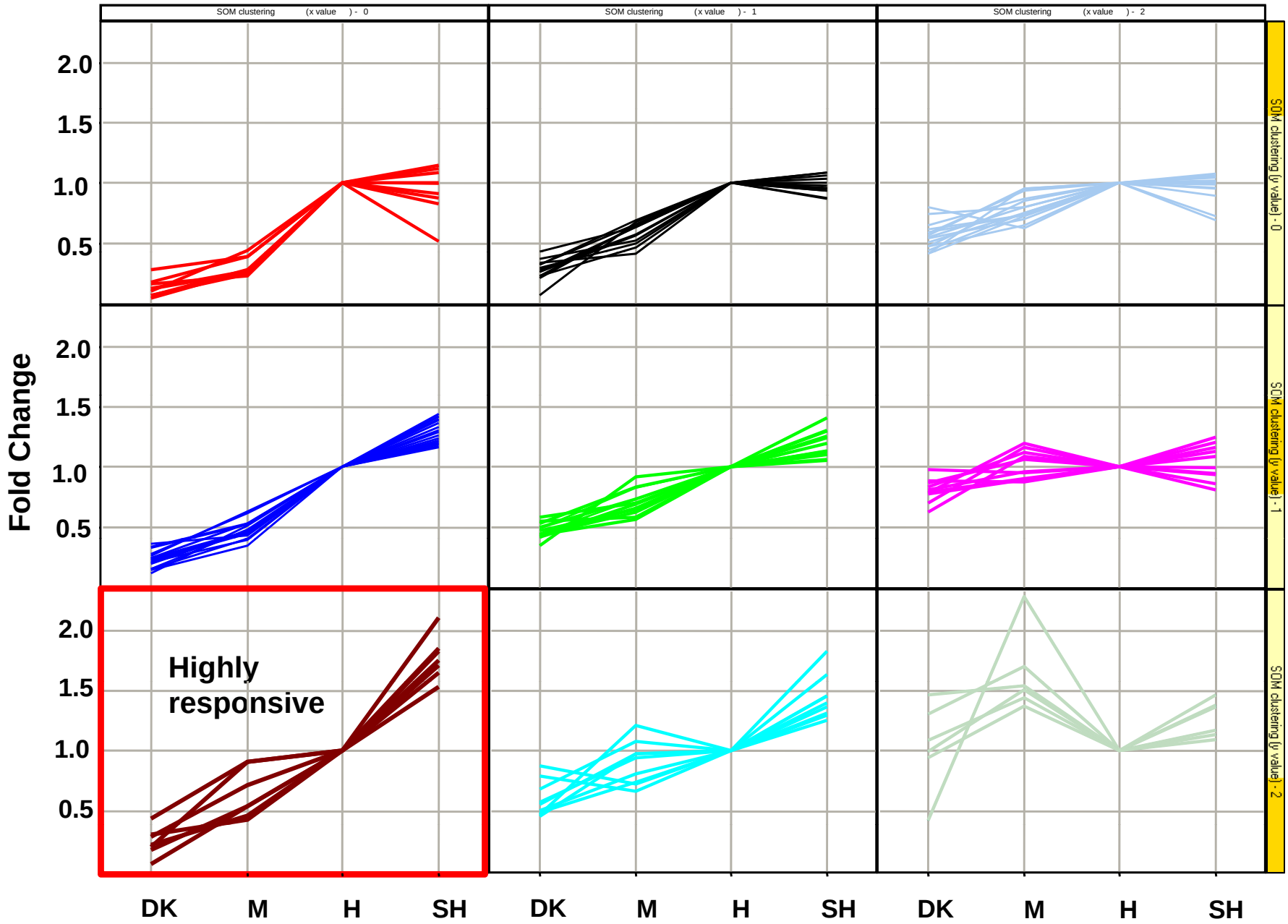
w/ Frank Furnari, Web Cavenee

EGFRvIII signaling preferentially activates PI3K/Akt



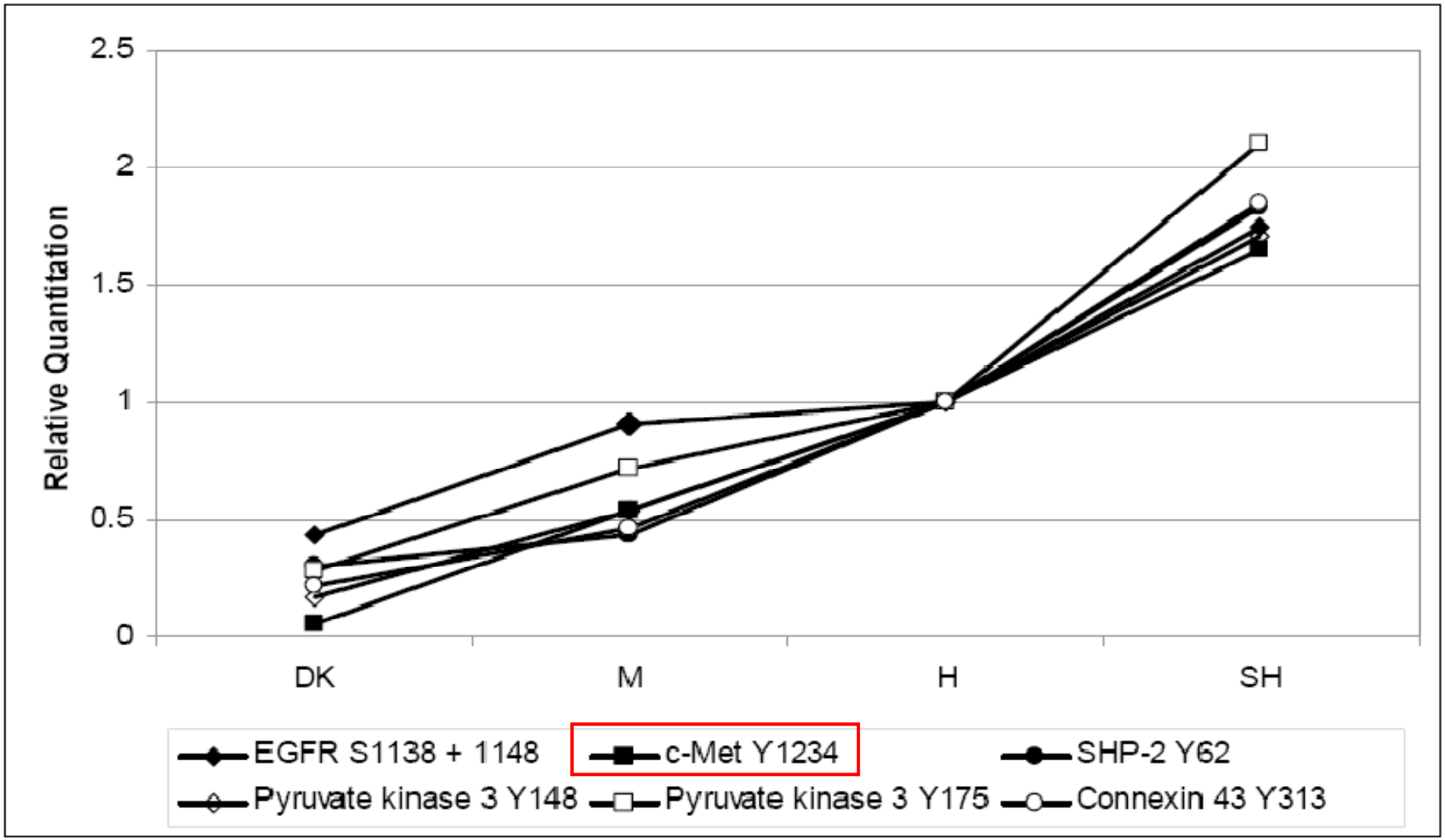
Paul Huang

Self-similar phosphorylation profiles revealed by self-organizing map



Paul Huang

The highly responsive cluster contains tyrosine phosphorylation of the c-Met activation site

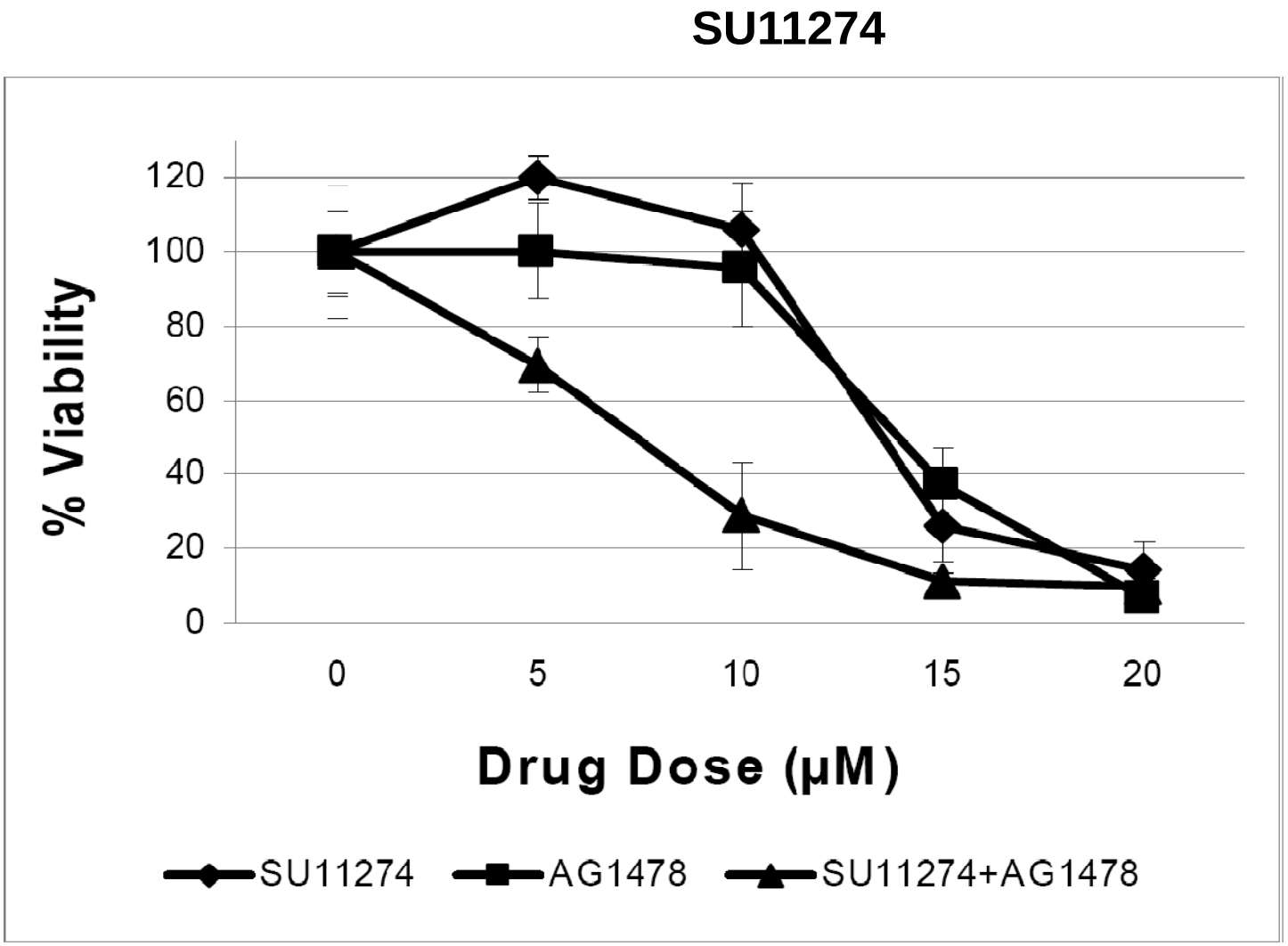


Source: Huang, Paul H., et al. "Quantitative Analysis of EGFRvIII Cellular Signaling Networks Reveals a Combinatorial Therapeutic Strategy for Glioblastoma." *Proceedings of the National Academy of Sciences* 104, no. 31 (2007).

Paul Huang

Combinatorial (c-Met and EGFR) inhibition decreases cell viability

Figure removed due to copyright restrictions. See Figure 1 (top) from Sattler, Martin, et al. "A Novel Small Molecule Met Inhibitor Induces Apoptosis in Cells Transformed by the Oncogenic TPR-MET Tyrosine Kinase." *Cancer Research* 63 (2003).

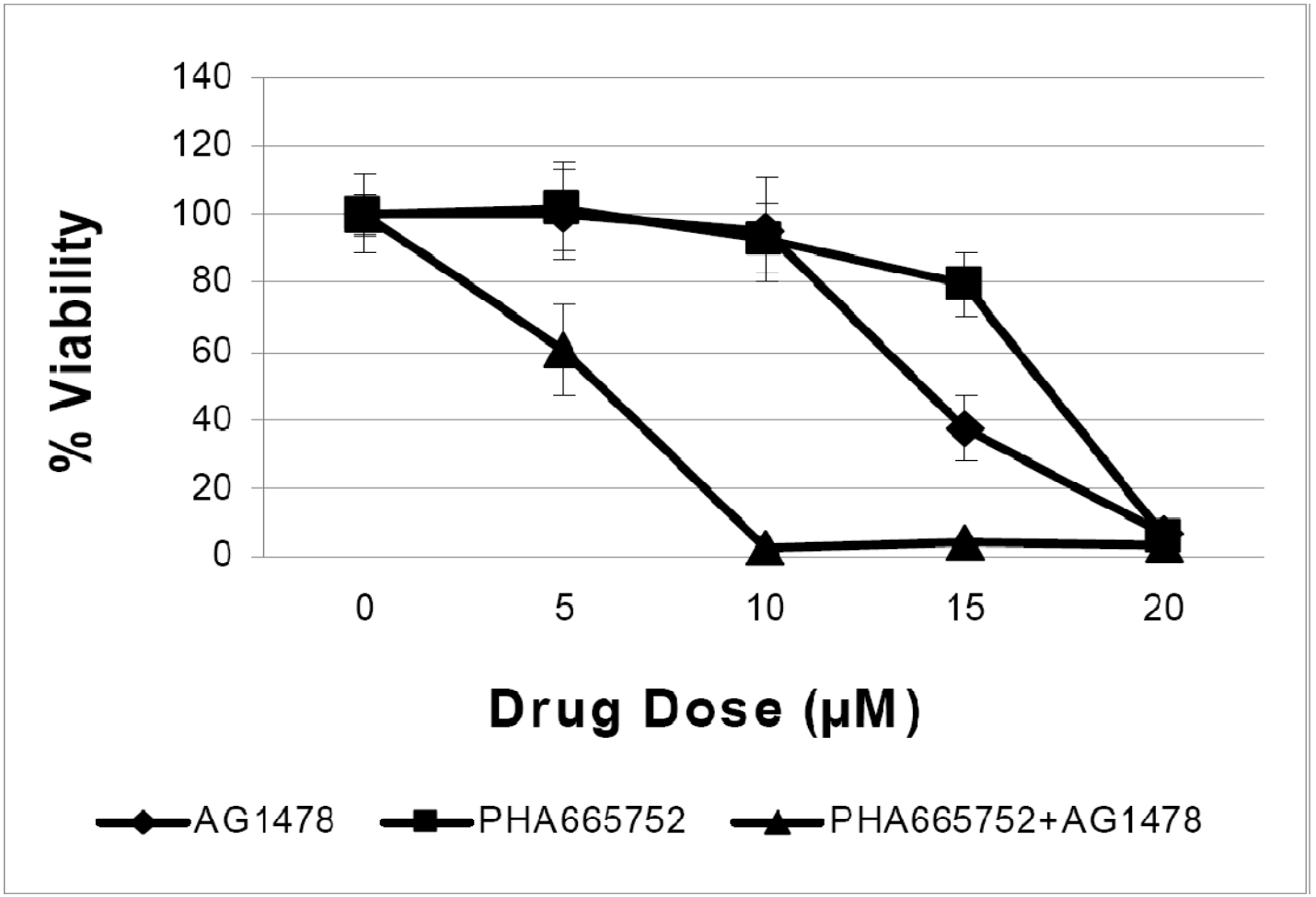


Source: Huang, Paul H., et al. "Quantitative Analysis of EGFRvIII Cellular Signaling Networks Reveals a Combinatorial Therapeutic Strategy for Glioblastoma." *Proceedings of the National Academy of Sciences* 104, no. 31 (2007). © 2007 National Academy of Sciences, USA.

Paul Huang

A second, more potent c-Met inhibitor displays similar behavior

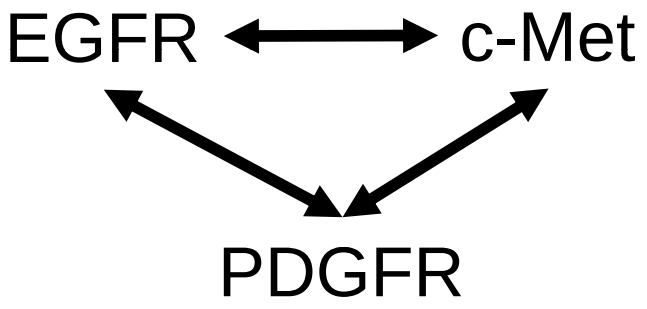
Figure removed due to copyright restrictions. See Figure 1 from Christensen, James G., et al. "A Selective Small Molecule Inhibitor of c-Met Kinase Inhibits c-Met-Dependent Phenotypes in Vitro and Exhibits Cytoreductive Antitumor Activity *in Vivo*." *Cancer Research* 63 (2003).



Source: Huang, Paul H., et al. "Quantitative Analysis of EGFRvIII Cellular Signaling Networks Reveals a Combinatorial Therapeutic Strategy for Glioblastoma." *Proceedings of the National Academy of Sciences* 104, no. 31 (2007). © 2007 National Academy of Sciences, USA.

Huang et al., *Proc Natl Acad Sci U S A.* (2007) 104:12867.

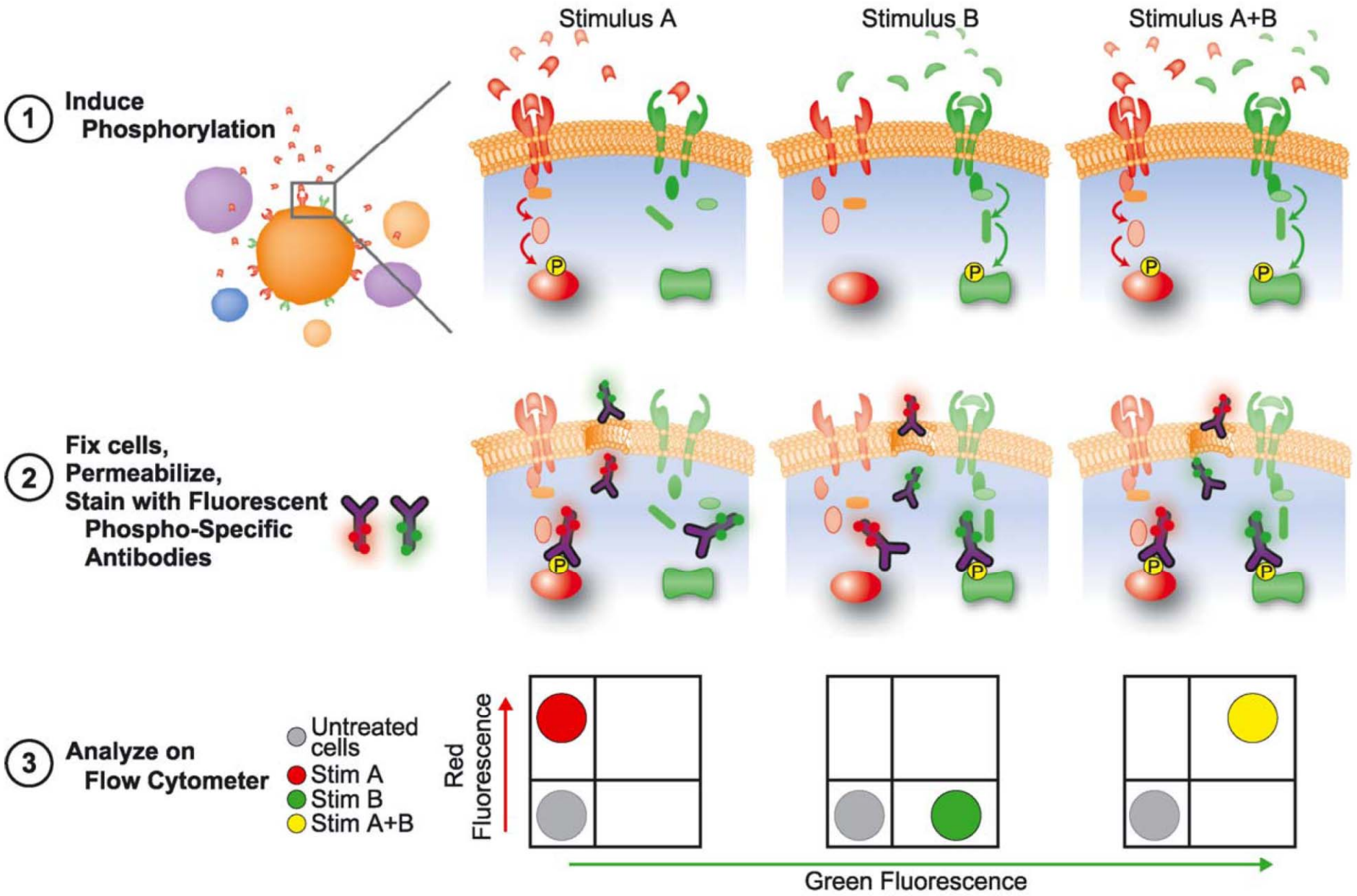
Independent validation of combinatorial targeting of EGFR and c-Met in GBM



Figures from *Science* removed due to copyright restrictions.
See Figures 2d and 3b from Stommel, Jayne M., et al. "Coactivation of Receptor Tyrosine Kinases Affects the Response of Tumor Cells to Targeted Therapies." *Science* 318 (2007).

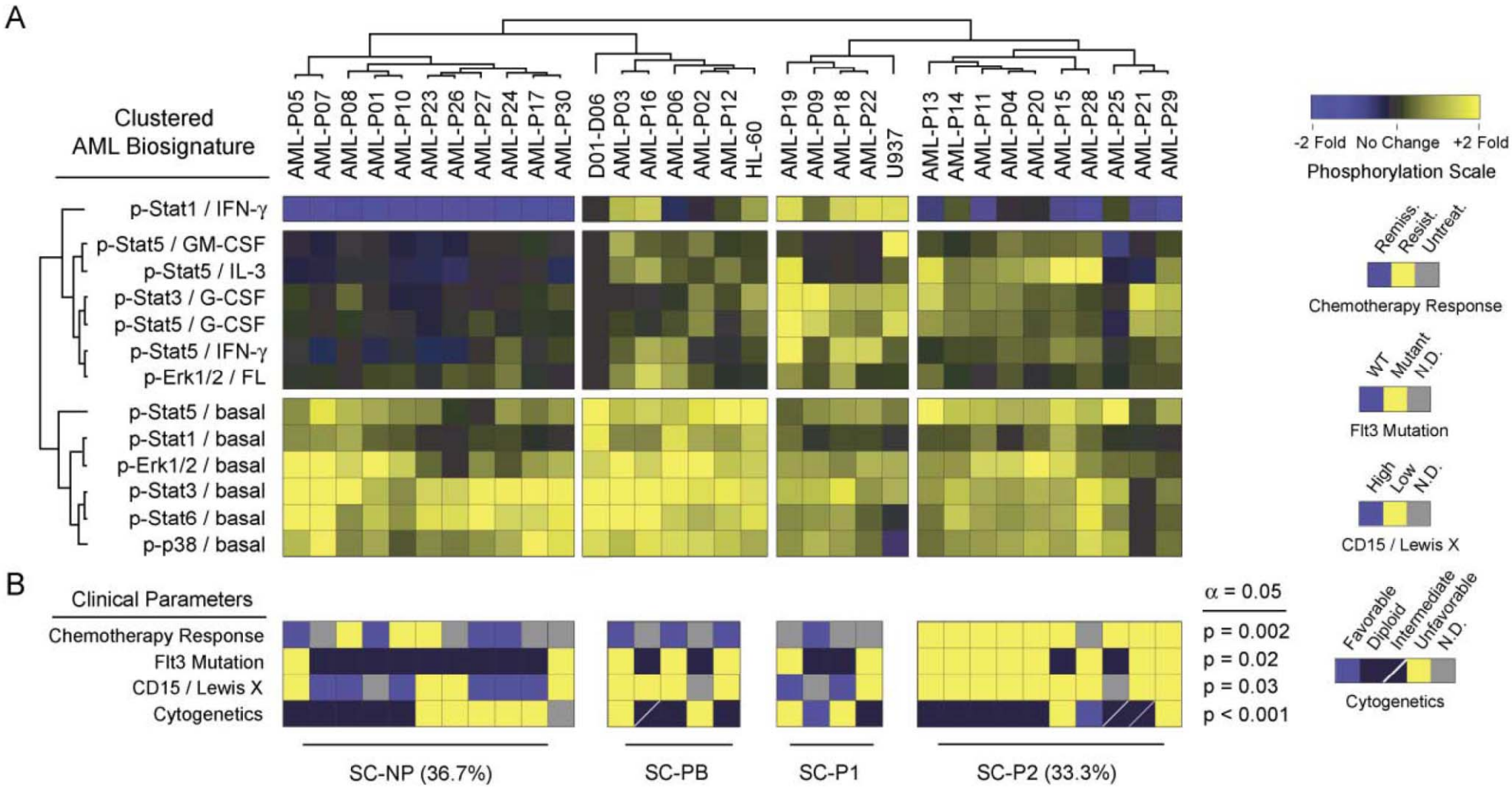
Is there a technique that can determine which of these signals are occurring in the same cell?

Phospho-FACS: Single cell signaling analysis



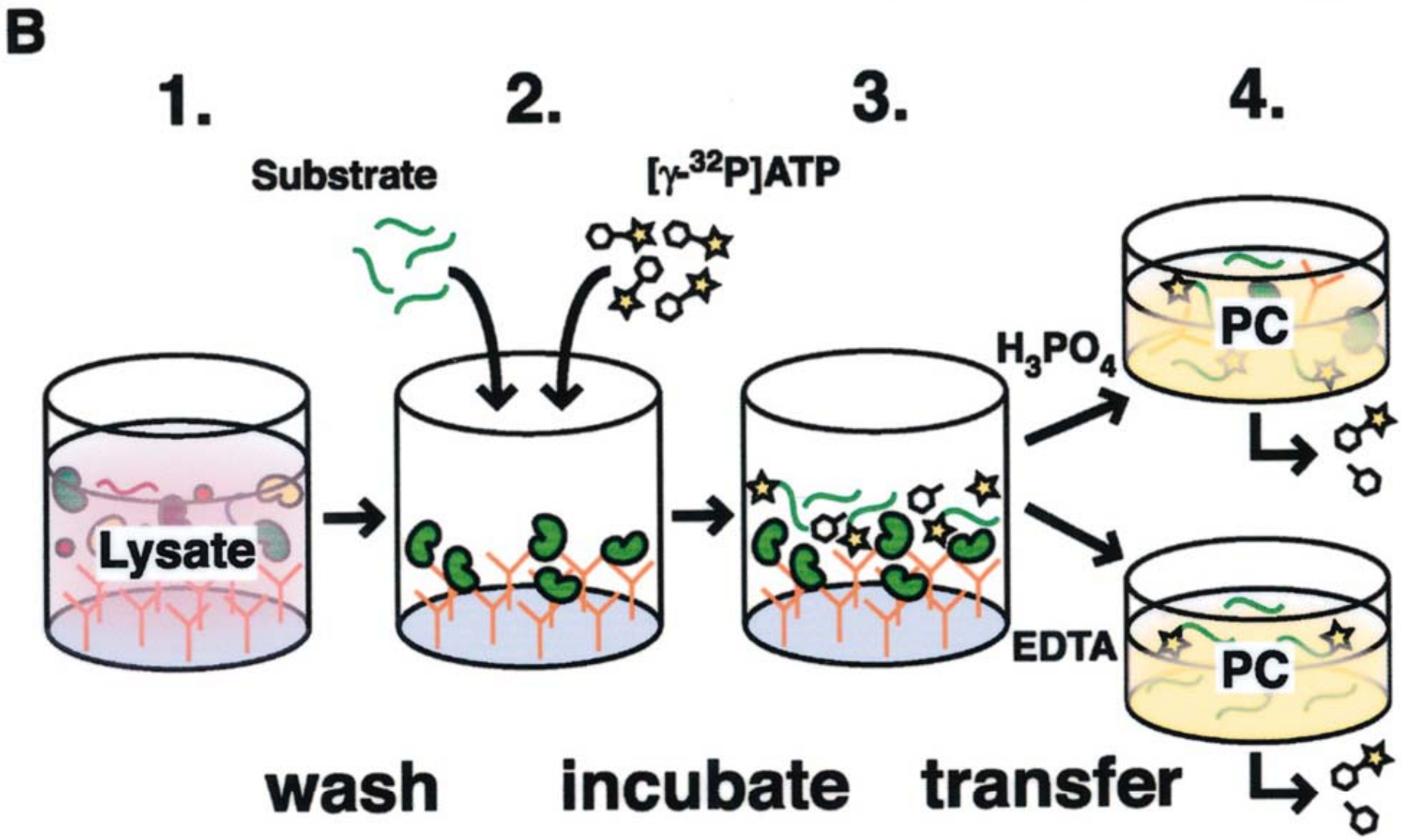
Krutzik *et al.,* Clinical Immunology , 2004.

Personalized medicine by Phospho-FACS



Irish *et al., Cell* , 2004.

All of these techniques measure phosphorylation, but this does not directly measure activity…



Janes, K. A. (2003) Mol. Cell. Proteomics 2: 463-473

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Therapeutic Targeting and Efficacy – is the kinase inhibitor actually affecting the targeted kinase? How are cells in a tumor responding to therapy?

Understanding mechanisms of resistance

To clear up my mistake last Thursday:

Modafinil ≠ Monoxidil

Provigil ≠ Rogaine

20.380J / 5.22J Biological Engineering Design

Spring 2010