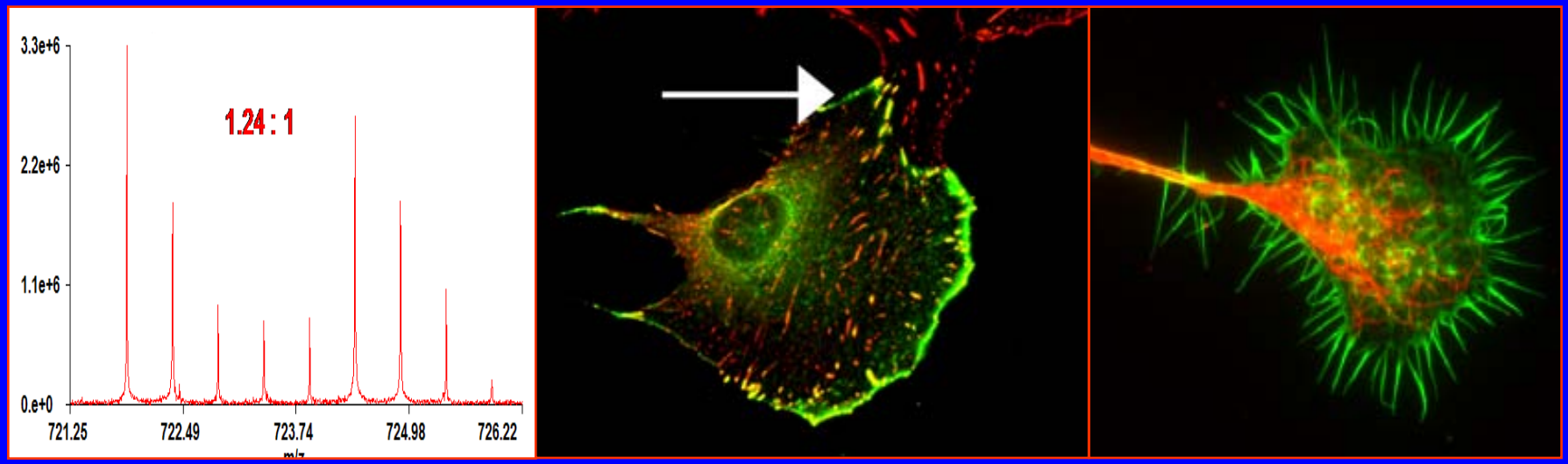
Deciphering the Signaling Network in the Leading Edge of the Migrating Cells



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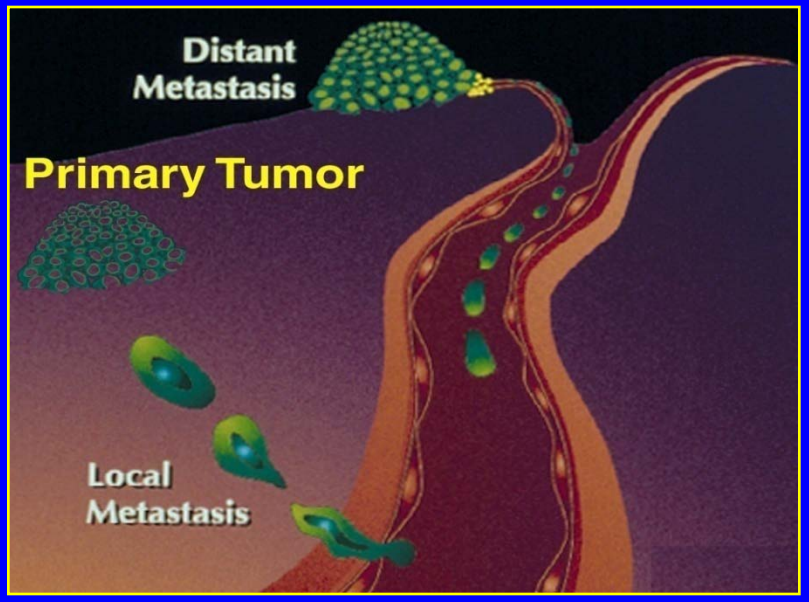
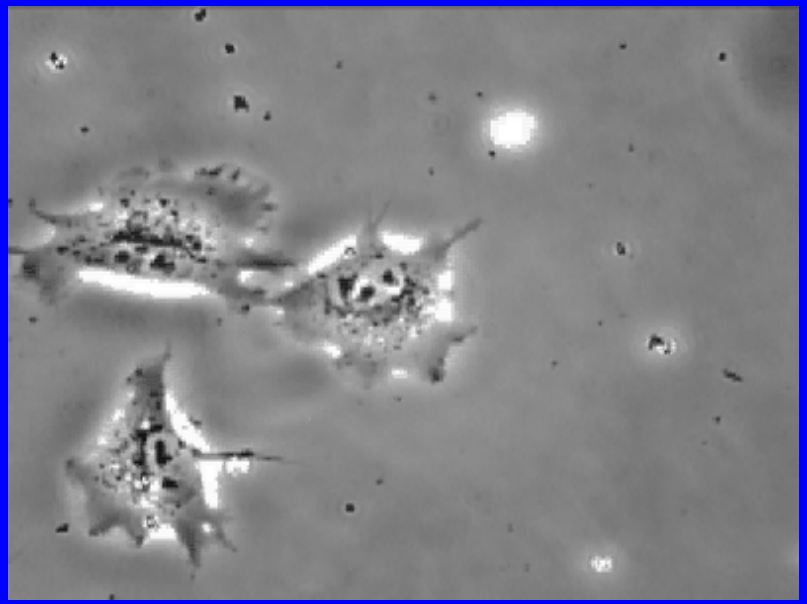
Chemotaxis Requires Directional Pseudopodium Formation

Chemotaxis plays an integral role in many biological processes:

1. Immune function
2. Embryo development
3. Wound repair
4. Tumor formation and metastasis



Cell polarization to form dominant pseudopodium/invadopodium is necessary for sustained migration and metastasis



Important Questions/Goals

How are molecular signals temporally and spatially organized in polarized cells to regulate and maintain persistent directional cell migration?

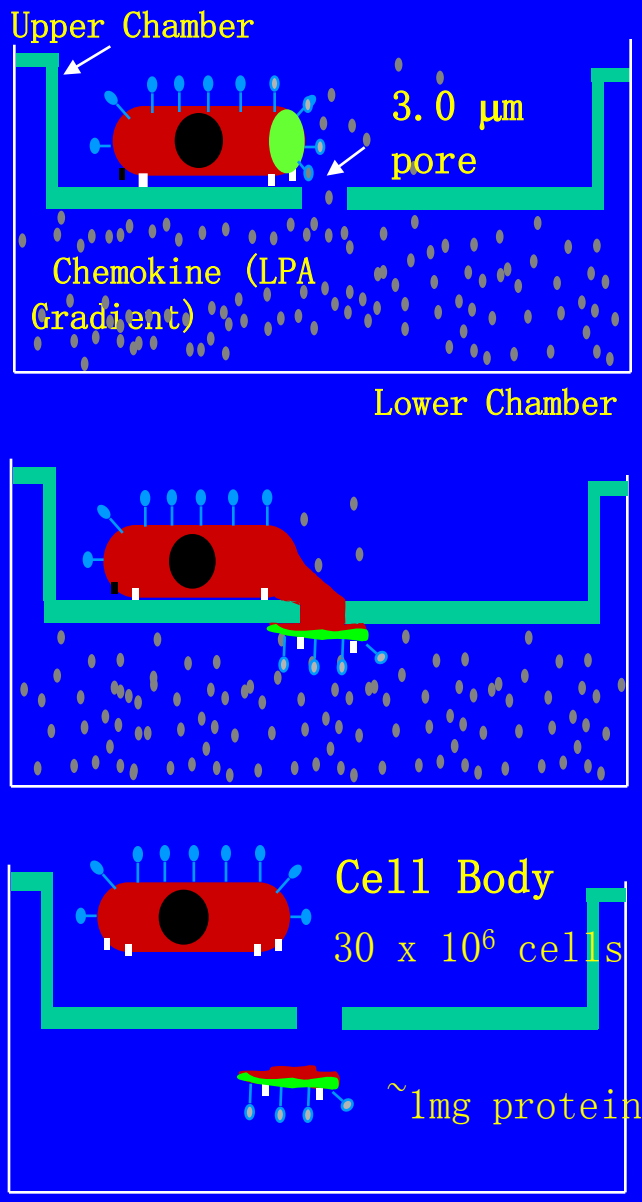
- **Targeting of specific proteins to different poles of migrating cells.**
- **Phosphorylation/activation changes.**

Goals:

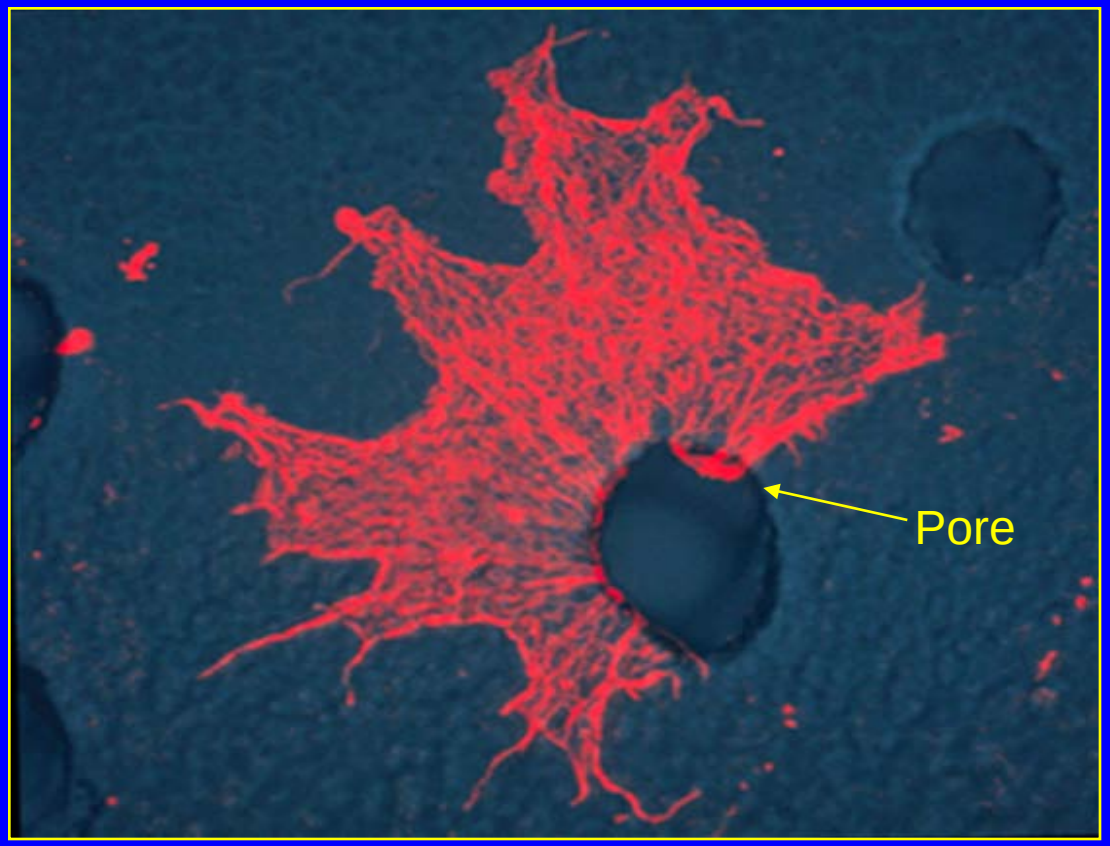
- **Understand the mechanism of cell migration and caner metastasis.**
- **Identification of pseudopodium-specific proteins and phosphoproteins will reveal markers of metastatic cells for drugable targets**

Isolate the cell body and pseudopodium compartments for large scale proteomics analysis.

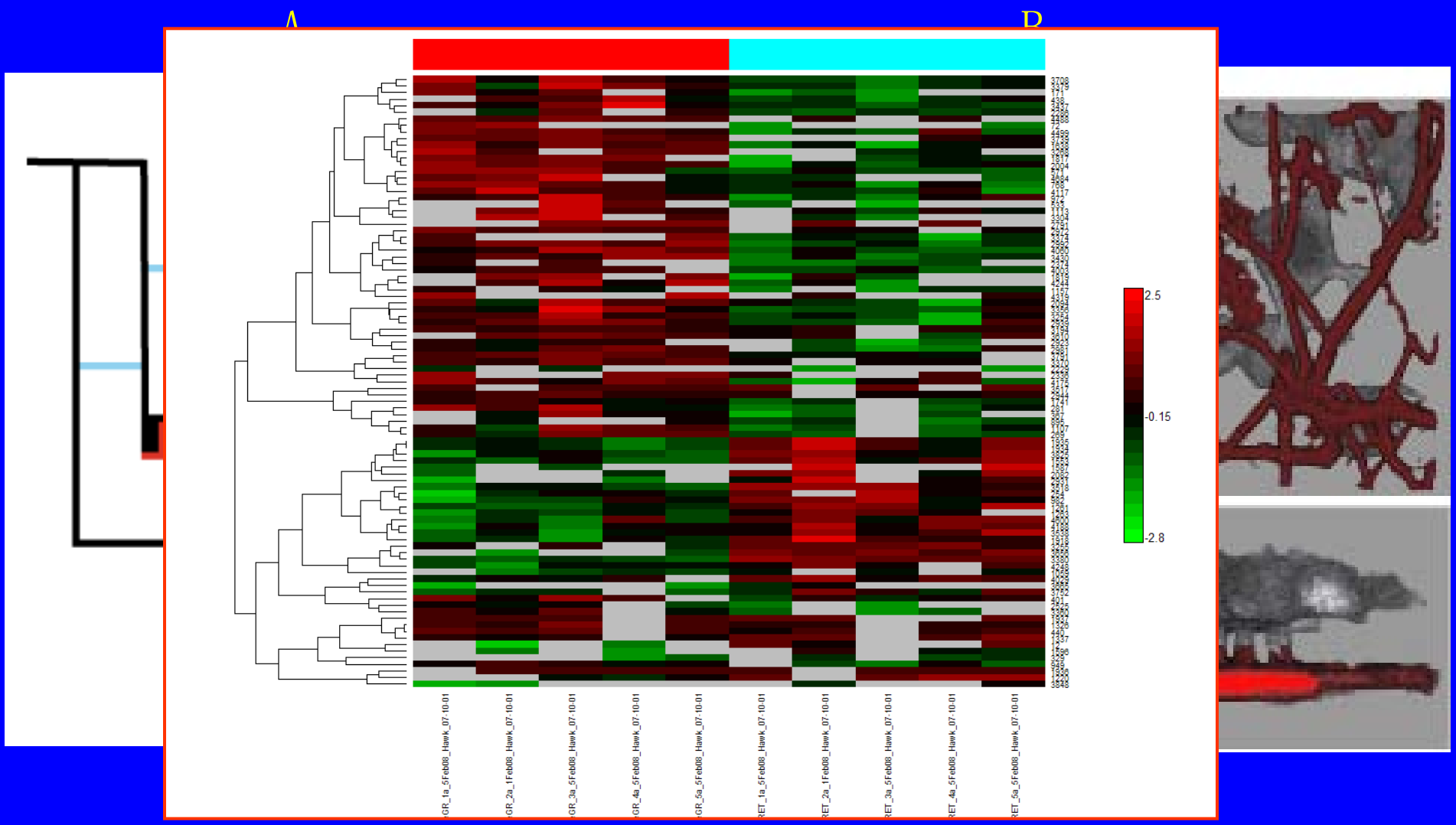
Model for Cell Polarity and Pseudopodia Purification



Pseudopodia on lower Membrane Surface
Stained with Rodamine Phalloidin

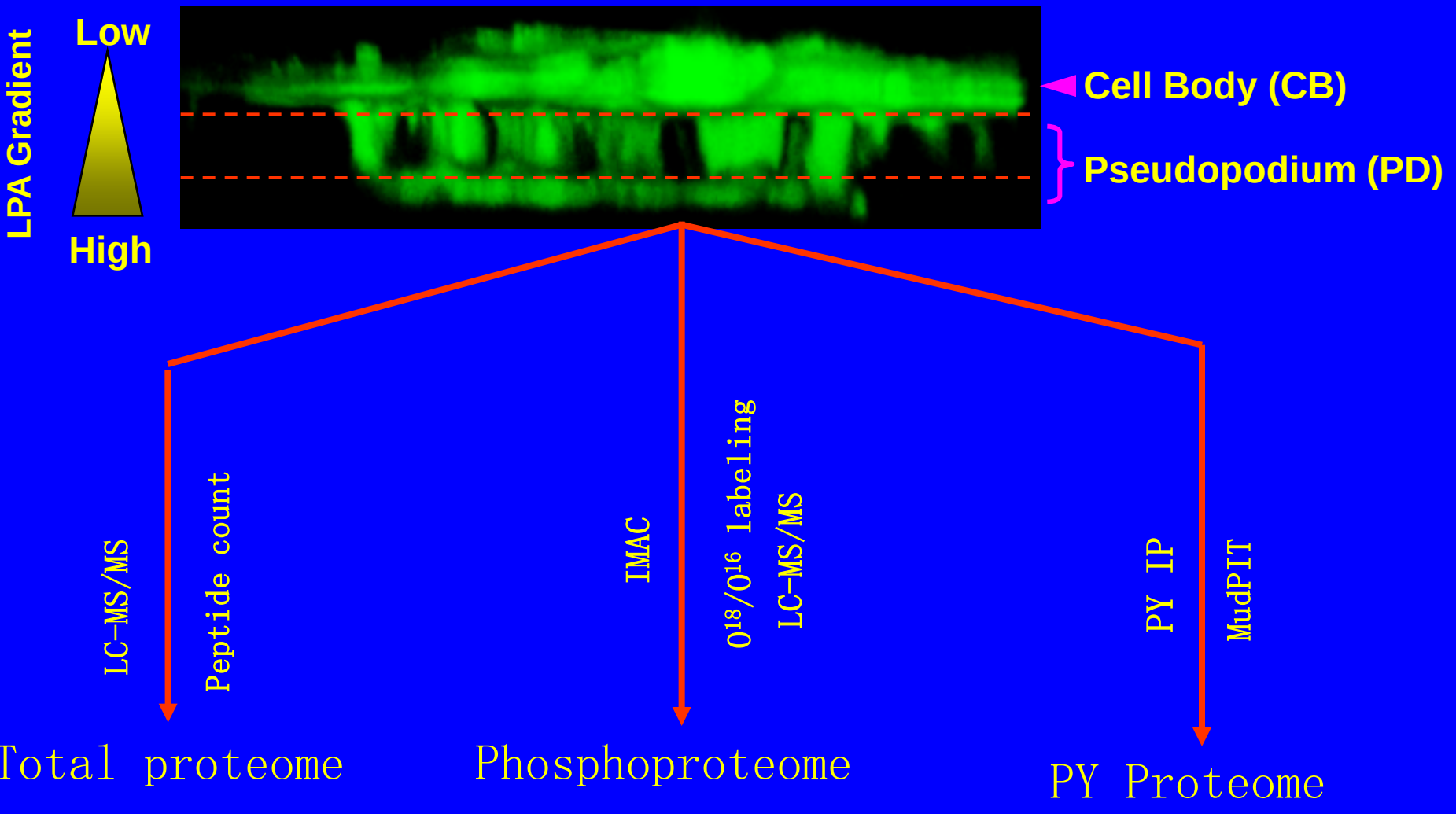


Application of the Model in Neurite Purificaion for Proteomics Study



PNAS, 2008, 105:1931-6

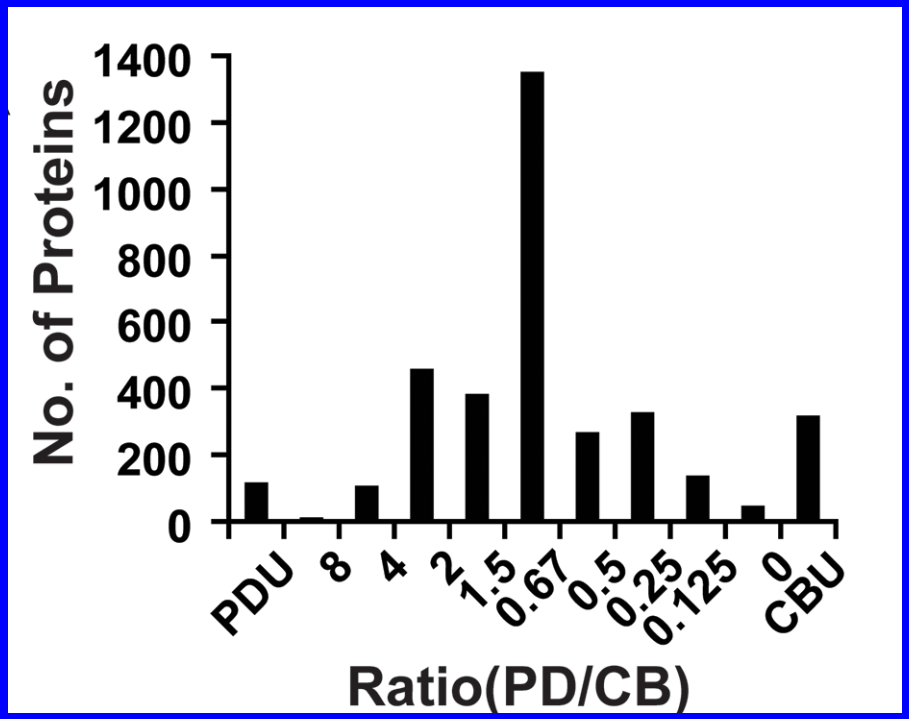
Strategies for Proteomics Study of PD/CB Specific Protein

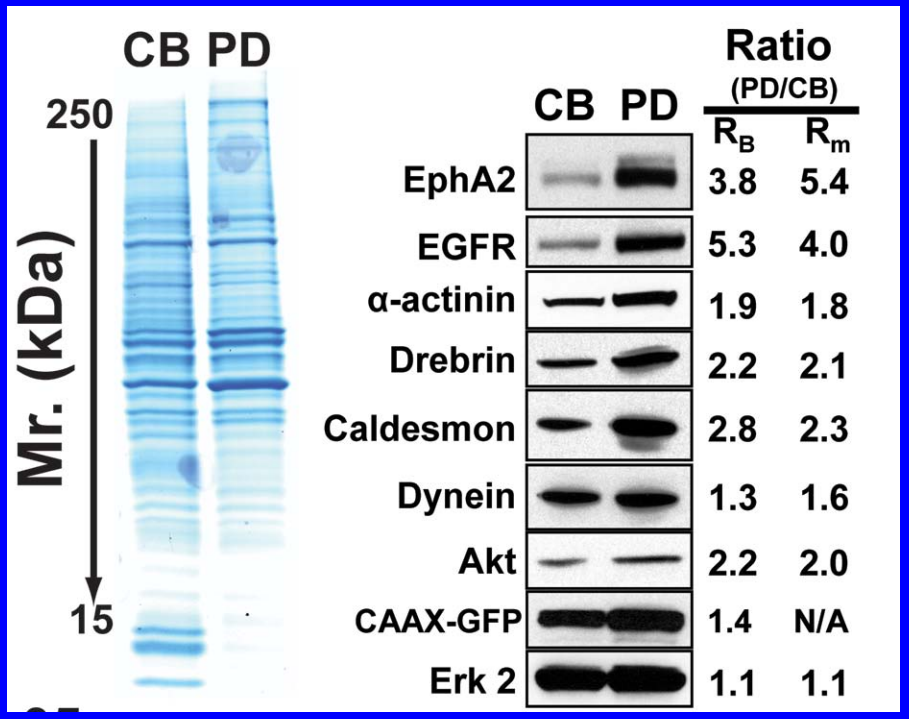


PNAS, 104: 8328-33
Sci. STKE, 2007, 400(p14)
Methods Mol Biol, 379:55-66

PNAS, 2010,107:10920-5

Quantitative Identification CB and PD Proteome

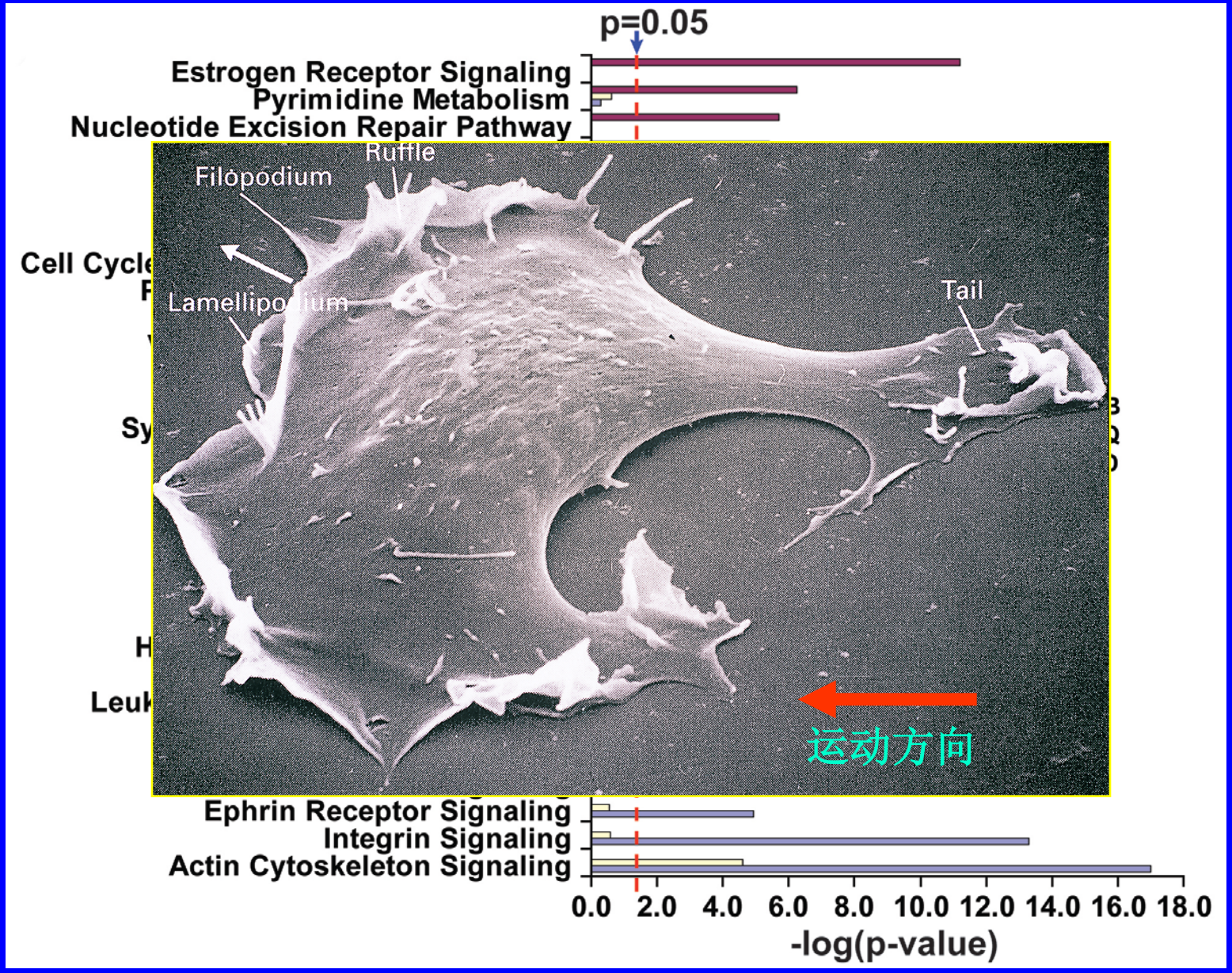




From > 5000 identified proteins, the relative abundances of 3509 proteins that have at least two peptides identified were determined by peptide spectrum counting.

T-test: P<0.001

Pathways Enriched in PD or CB



Database: KEGG Pathways

Quantitative Identification of Phosphoproteome in CB and PD

Proteins

↓

Enzyme Digest, $^{18}O/^{16}O$ labeling, and Methyl Ester Conversion

↓

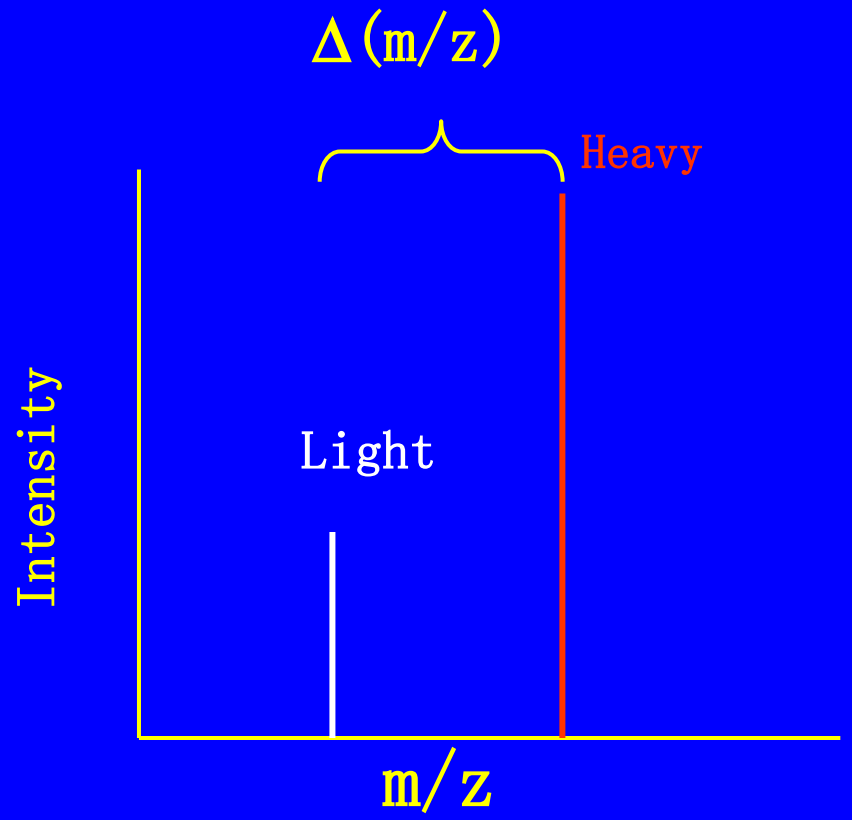
Immobilized Metal Affinity Chromatography (IMAC)

↓

RP-HPLC-MS/MS (MS^3)

↓

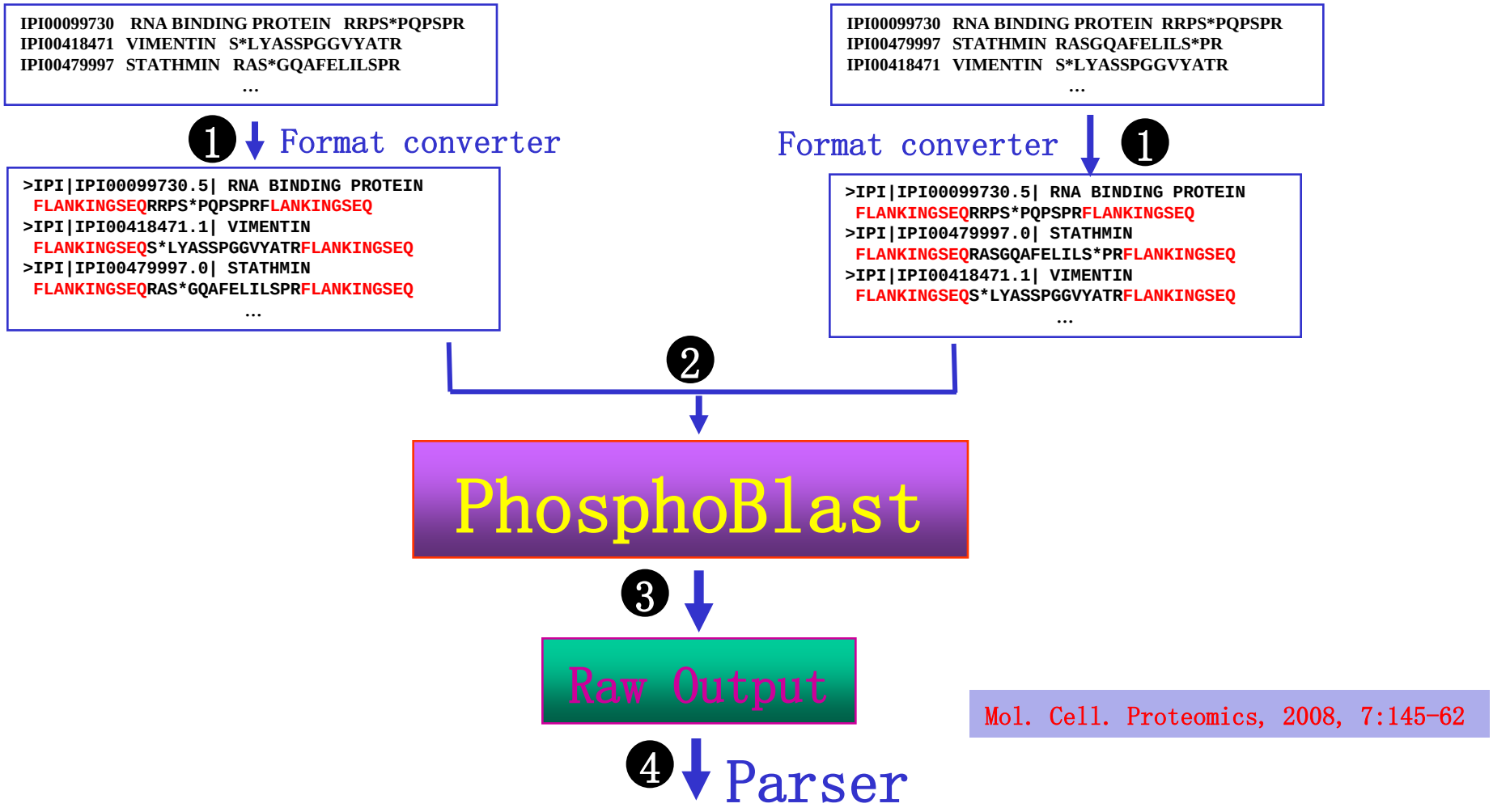
Data search & manual interpretation



Summary of PhosphoProteomics

- A total of 228 phosphopeptides were identified.
- 77 showed a 1.5 fold or more enrichment in PD.
- 96 showed a 1.5 fold or more enrichment in CB.

Conservative Analysis of Phosphosites

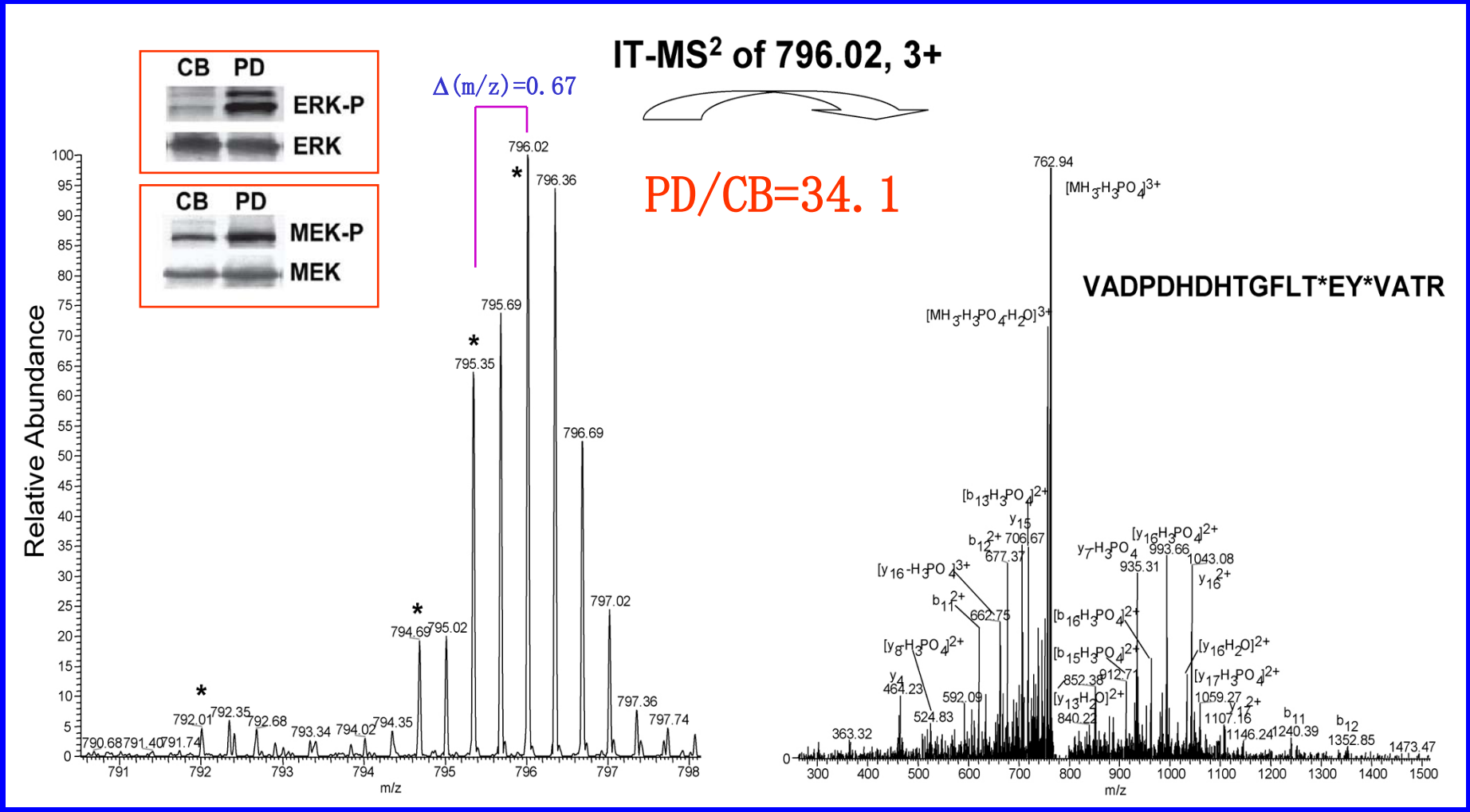


Mol. Cell. Proteomics, 2008, 7:145-62

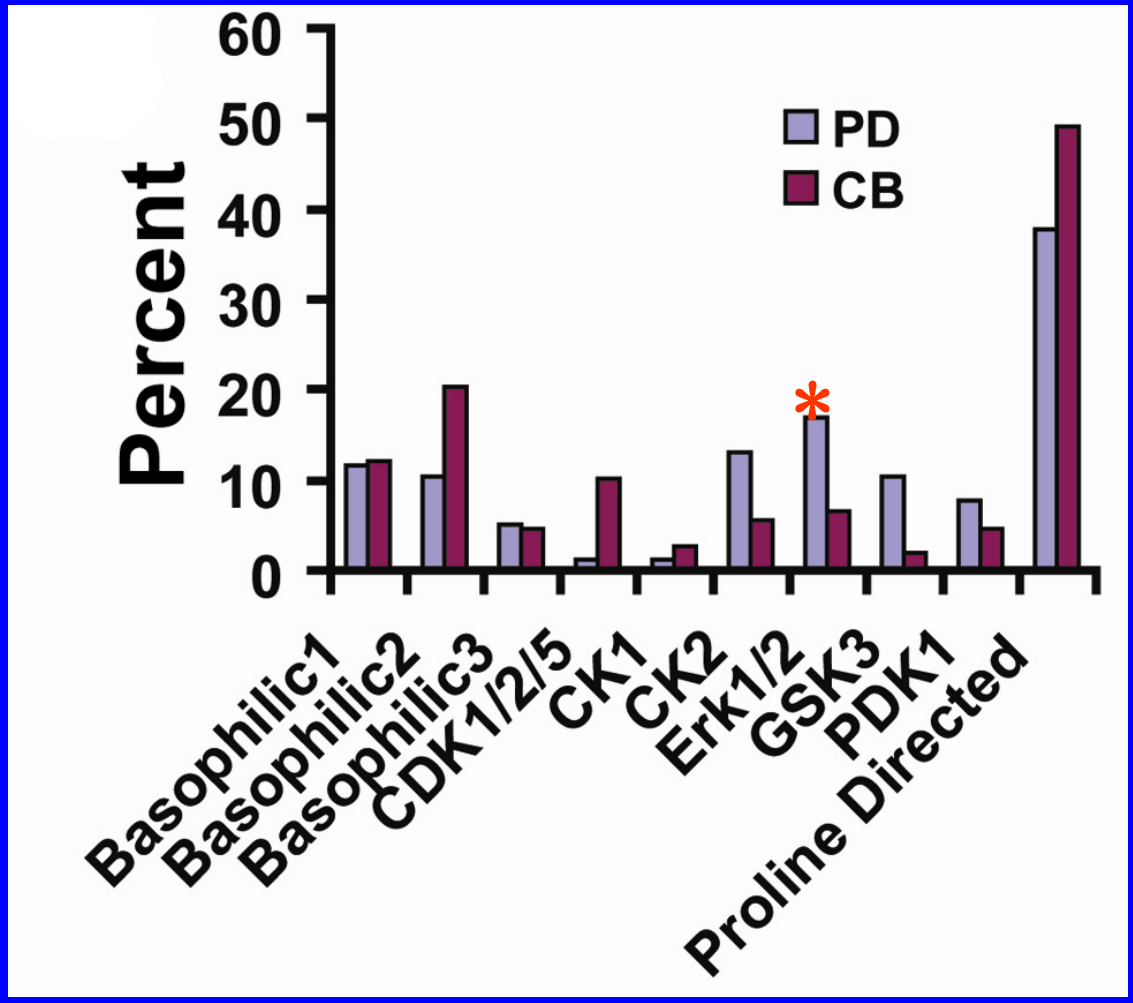
Accession	Query Name	Subject Name	Score (bits)	E-Value	Similarity (%)	Alignment
IPI00099730.5	RNA BINDING PROTEIN	RNA BINDING PROTEIN	36	7.00E-09	100	Query: 1 FLANKINGSEQRRPS*PQPSPRFLANKINGSEQ 11 Sbjct: 1 FLANKINGSEQRRPS*PQPSPRFLANKINGSEQ 11
IPI00418471.1	VIMENTIN	VIMENTIN	46	1.00E-11	100	Query: 1 FLANKINGSEQS*LYASSPGGVYATRFLANKINGSEQ 15 Sbjct: 1 FLANKINGSEQS*LYASSPGGVYATRFLANKINGSEQ 15
IPI00479997.0	STATHMIN	STATHMIN	46	6.00E-12	100	Query: 1 FLANKINGSEQRAS*GQAFELILSPRFLANKINGSEQ 15 Sbjct: 1 FLANKINGSEQRAS*GQAFELILSPRFLANKINGSEQ 15

- 89%, 80%, and 44% of the phosphosites are conserved in mouse, rat, and zebrafish, respectively.

Erk is Highly Phosphorylated (activated) in PD

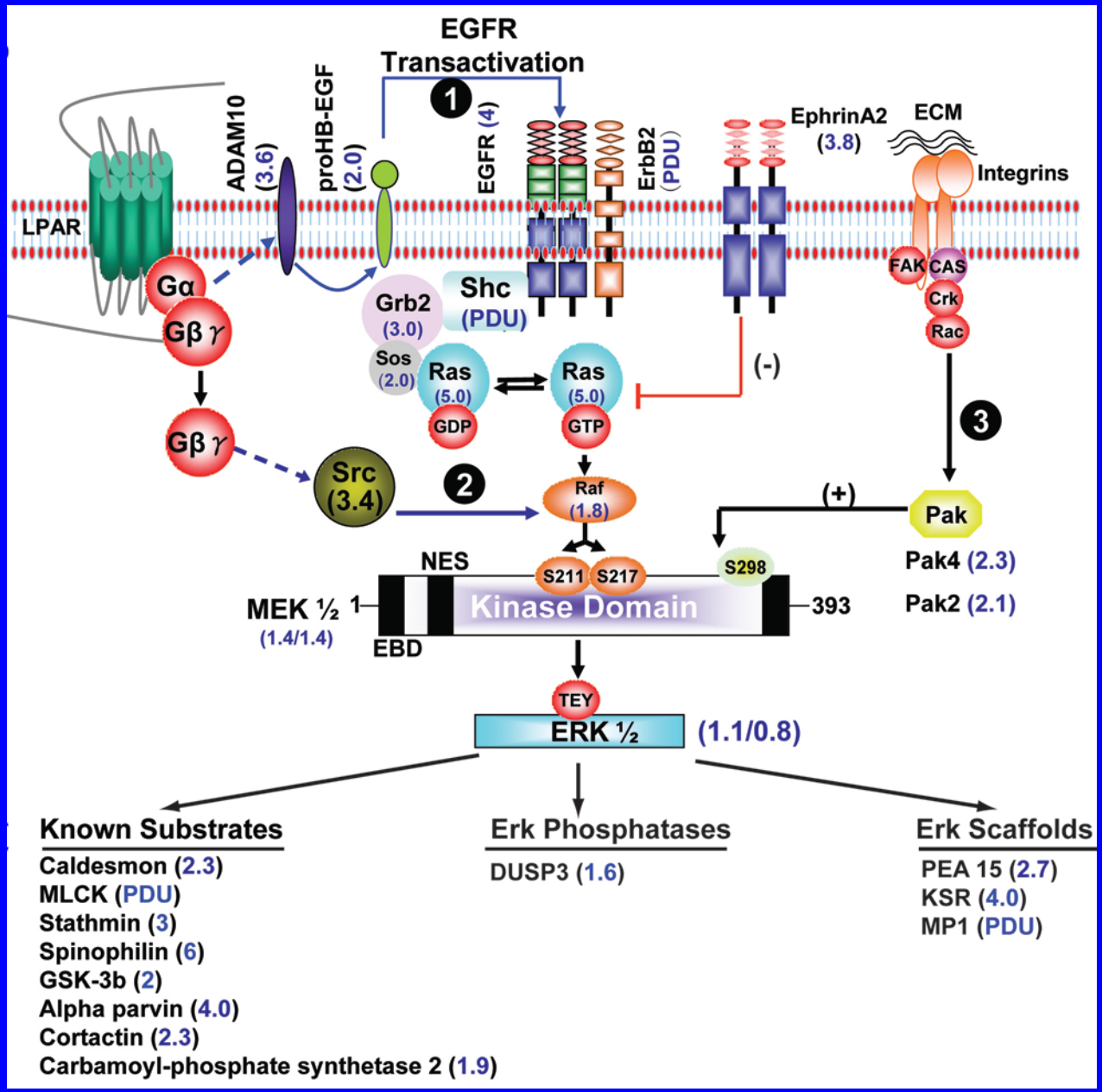


Comparison of CB-/PD- Enriched Phosphorylation Motifs



The distribution pattern of kinase phosphorylation motifs and associated kinase classes of identified phosphopeptides.

Possible Pathways that Activate Erk in PD



Phosphotyrosine Proteome in CB and PD

PY proteins are low abundant
PY proteins can be specifically enriched by anti-PY antibody IP.

Lysates (CB or PD)

↓

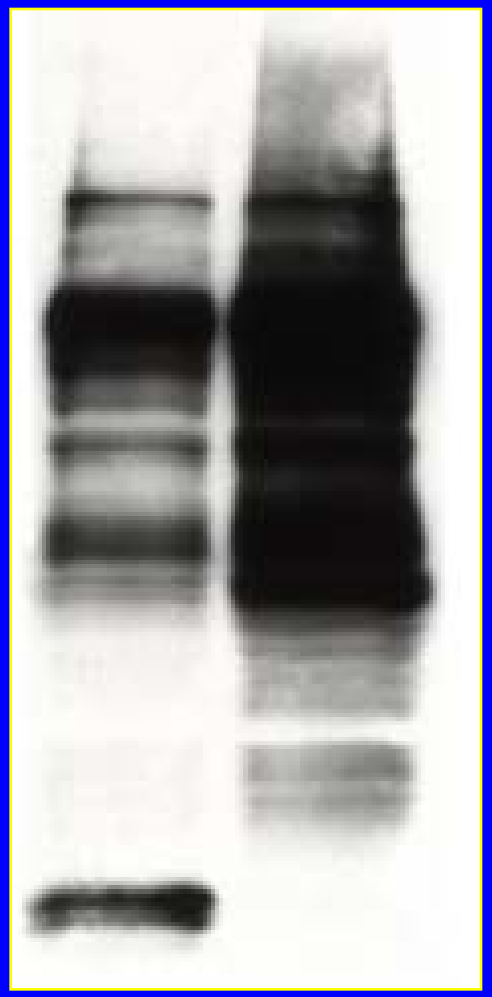
Anti-PY IP

↓

MudPIT

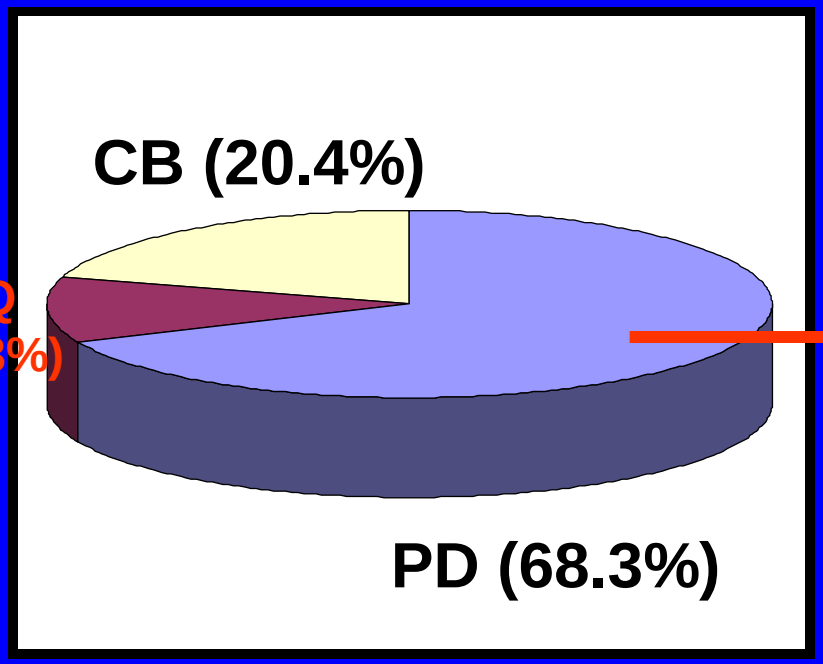
↓

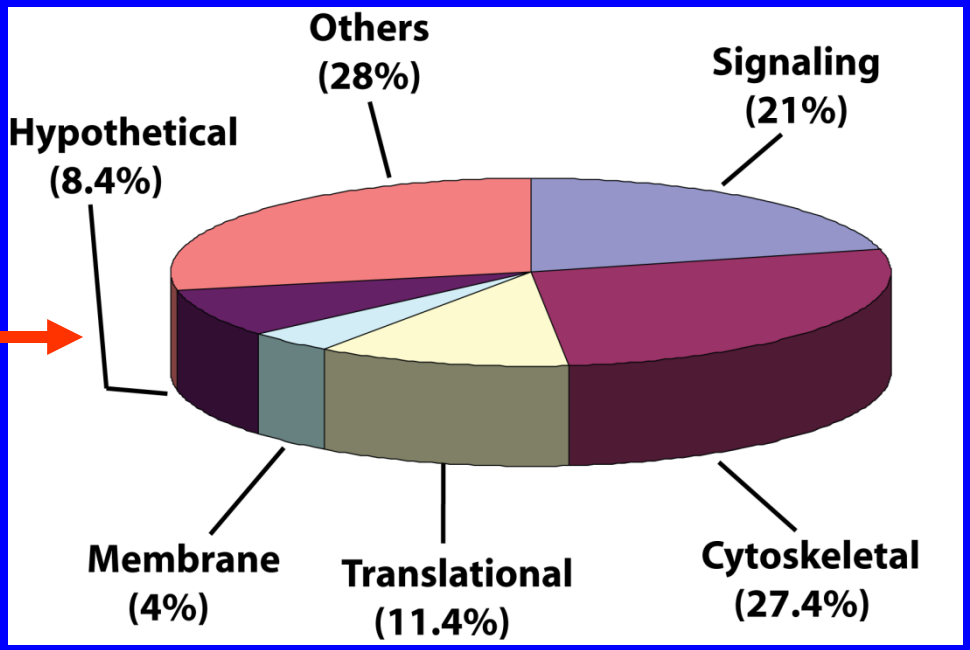
Sequest database searching for identification
Peptide spectrum count for quantitation



Blot: PY

PY Protein Distribution in Polarized Cells

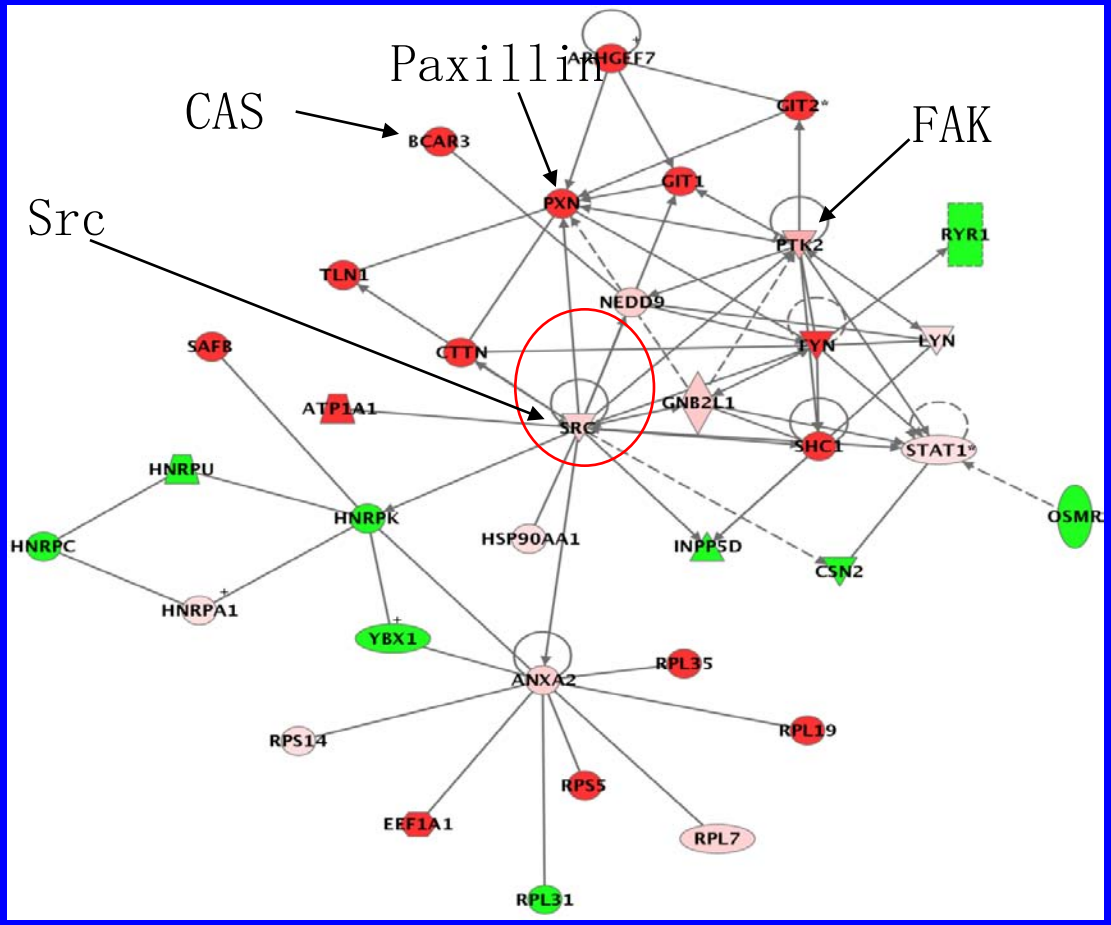




Functional group of PD enriched proteins

- 309 PY proteins were identified by a two peptide match.
- PD indicates the PY proteins are enriched in pseudopodium,
- CB indicates the PY proteins are enriched in cell body
- EQ indicates the PY proteins are equally distributed in both fractions.
- The data is from the pool of five independent experiments.

PY Interactome of Polarized Cells



PY Drives Pseudopodial Formation and Migration

Integrin Ligation

↓

FAK/Src

↓

Substrates (p130CAS)

↓

Focal Adhesions

↓

Cytoskeletal Changes

↓

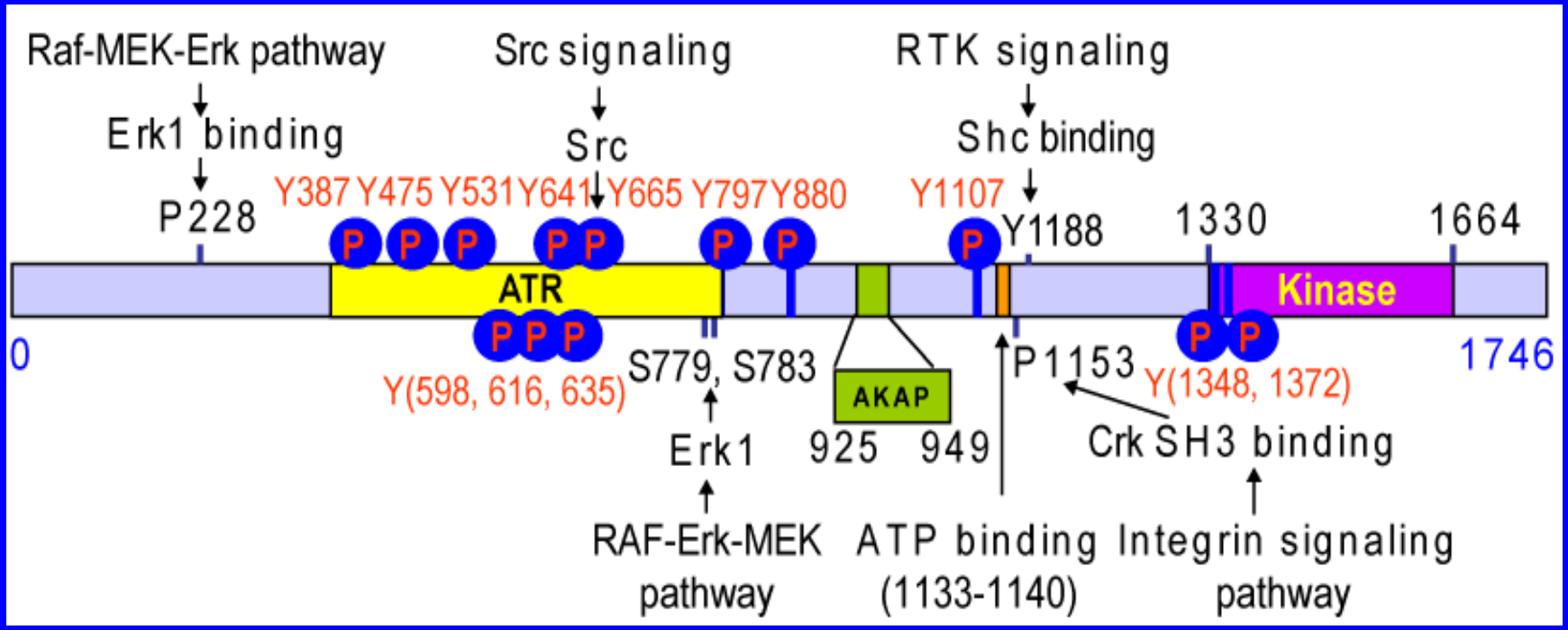
Pseudopodial Extension and Adhesion

Red indicates that the protein is enriched in pseudopodium.

Green indicates that the protein is enriched in cell body.

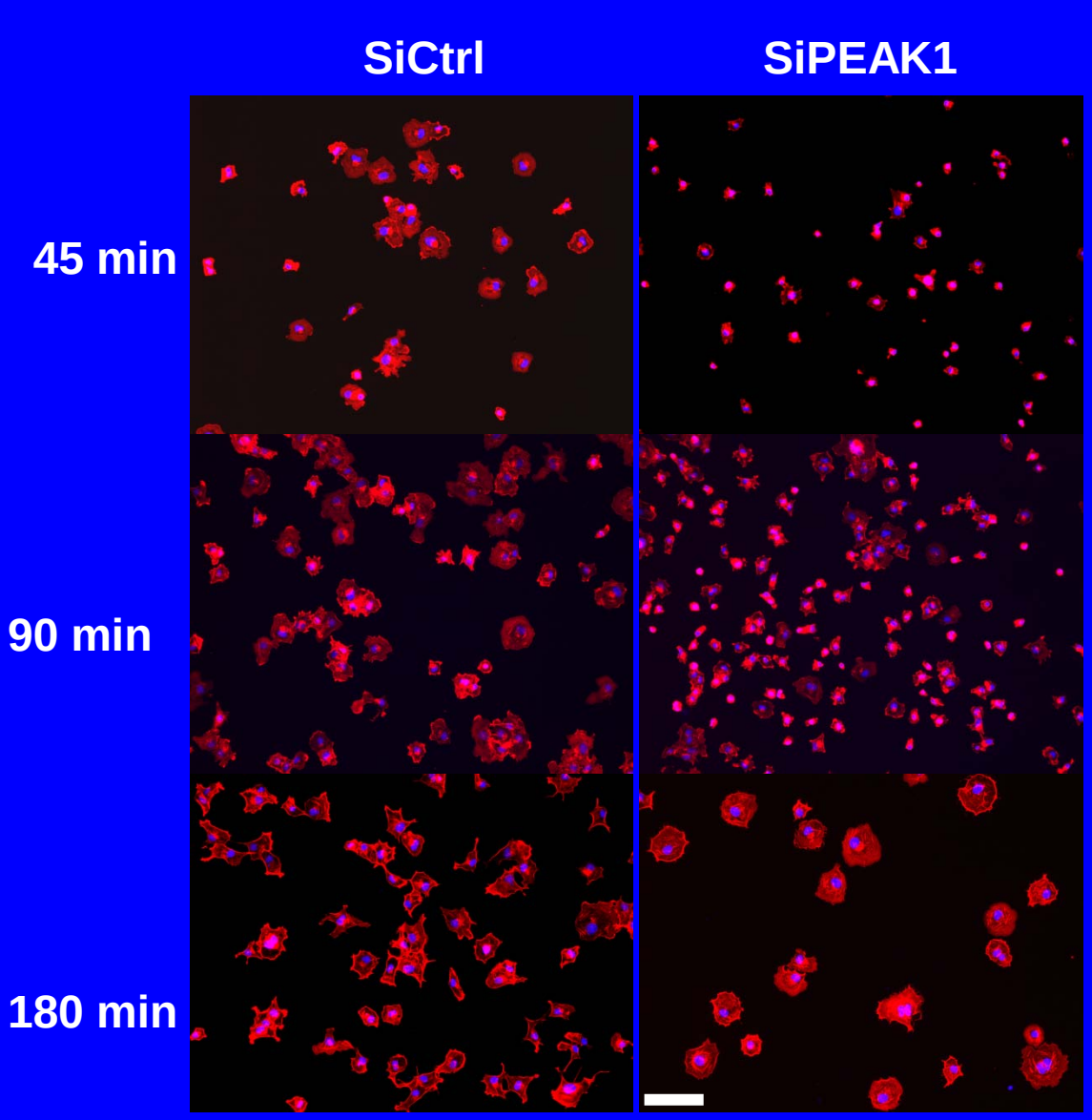
The network was generated using Ingenuity software.

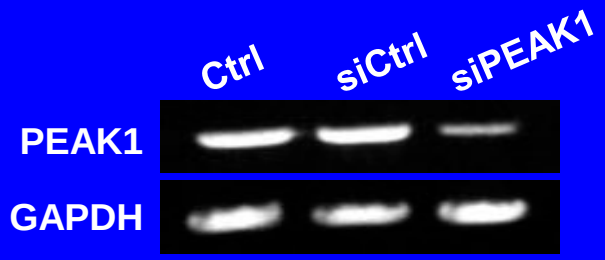
PEAK1: A Novel Pseudopodium Enriched Atypical Kinase

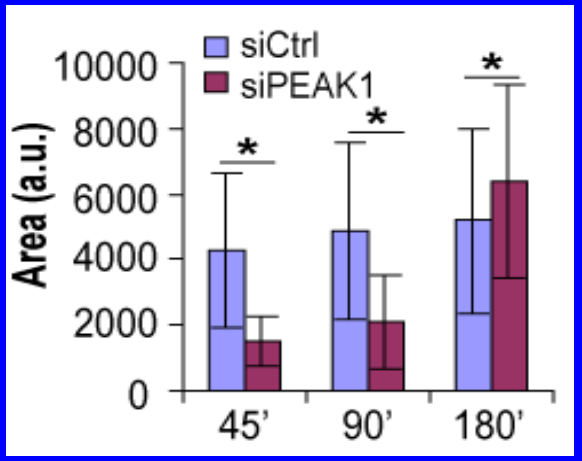


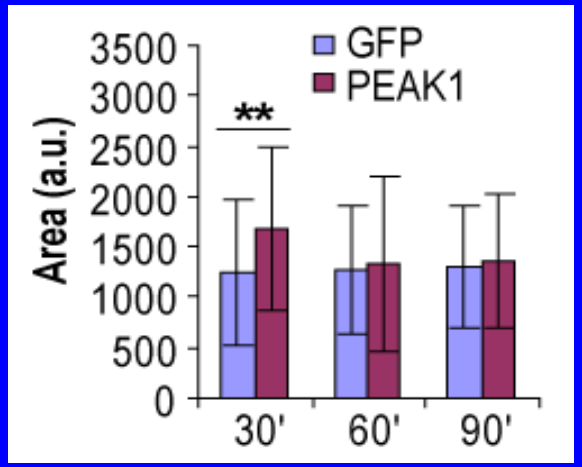
- 1746 aa, 193 KD protein.
- 10 peptides were identified by MudPIT. 5 independent experiments.
- 15 tyrosine sites are predicted to be phosphorylated by NetPhos 2.0
- No transmembrane helix was predicted by TMHMM program.
- Highly conserved from Zebrafish to Human.

PEAK1 Regulates Early Stage Cell Spreading







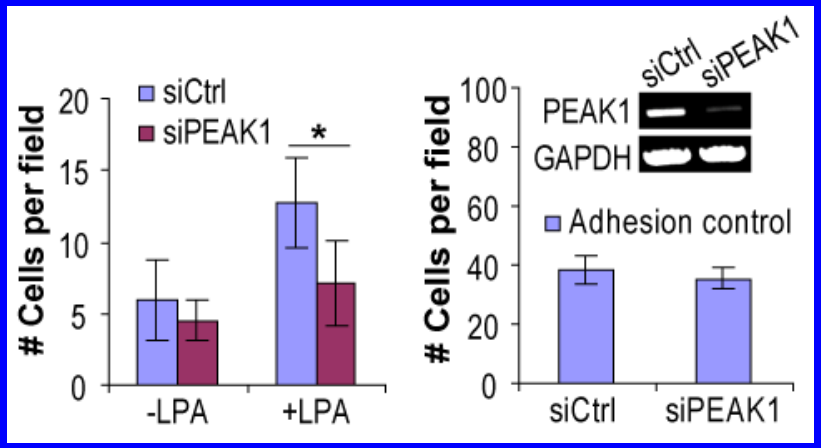


Attach to 5 ug/ml fibronectin

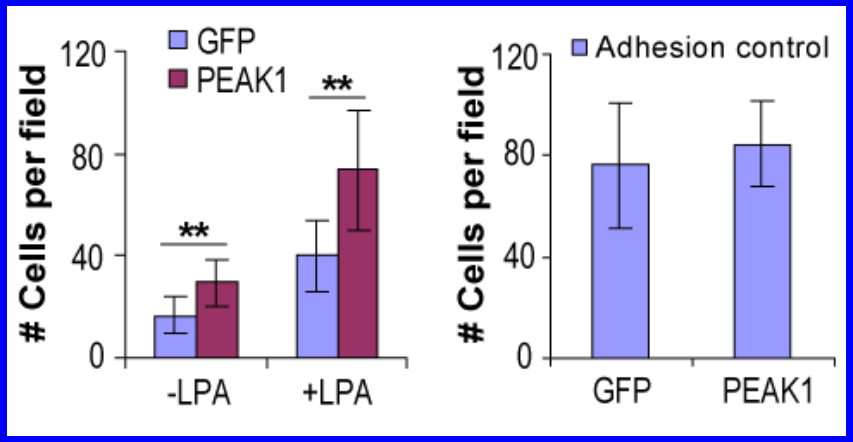
Scale bar: 100 um

(*, P<0.001; **, P<0.01)

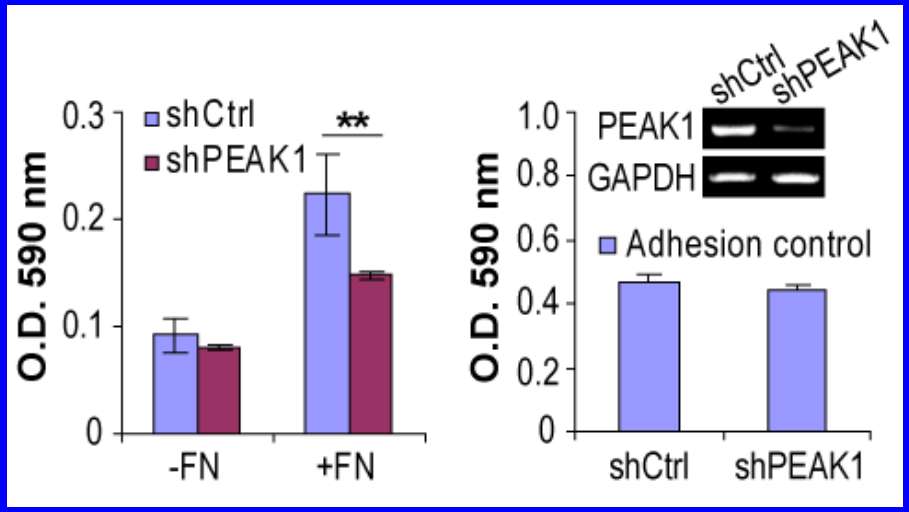
PEAK1 Is Necessary for Optimal Cell Migration



Depletion of PEAK1 Inhibits Chemotaxis

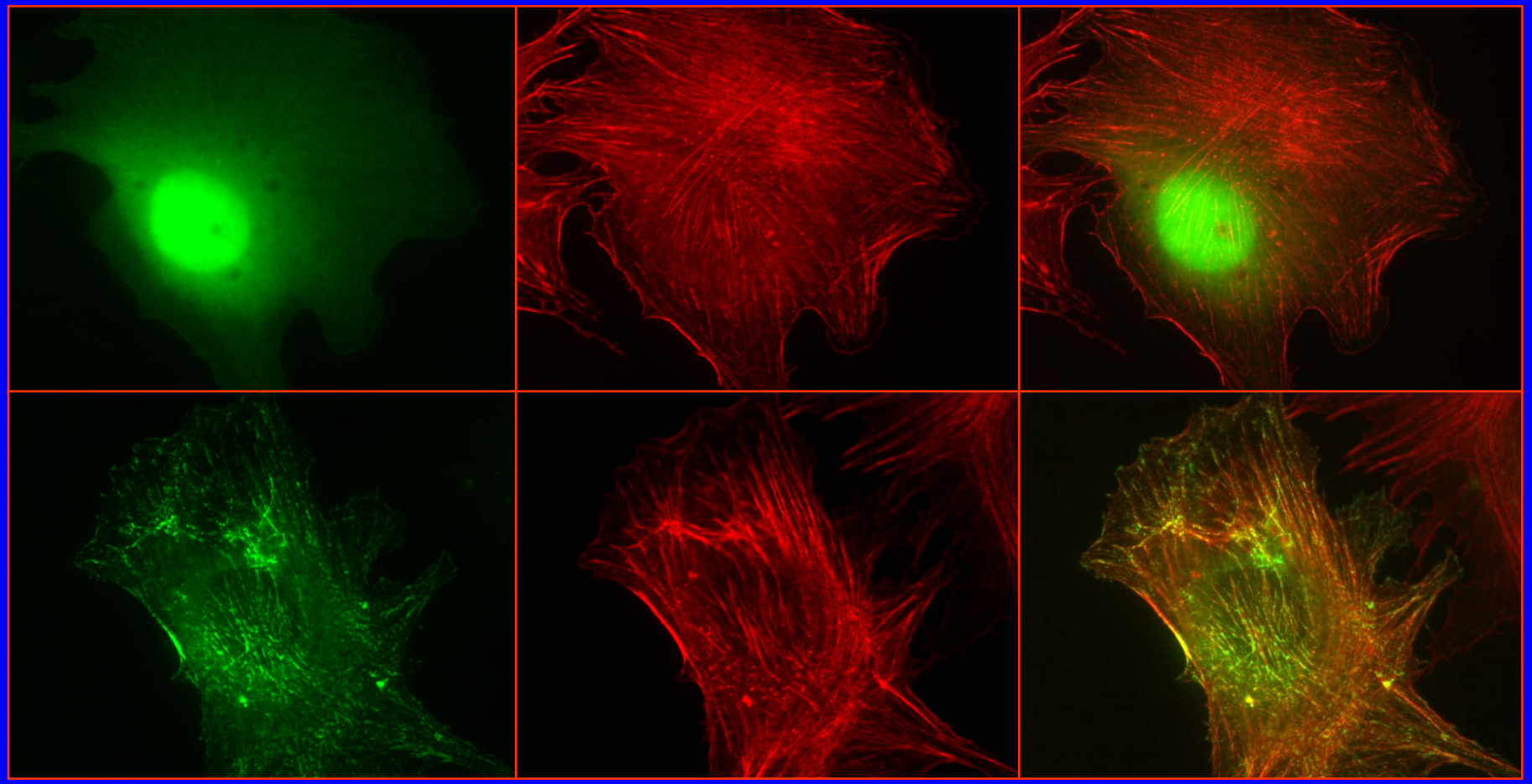


Over-expression of PEAK1 enhances chemotaxis



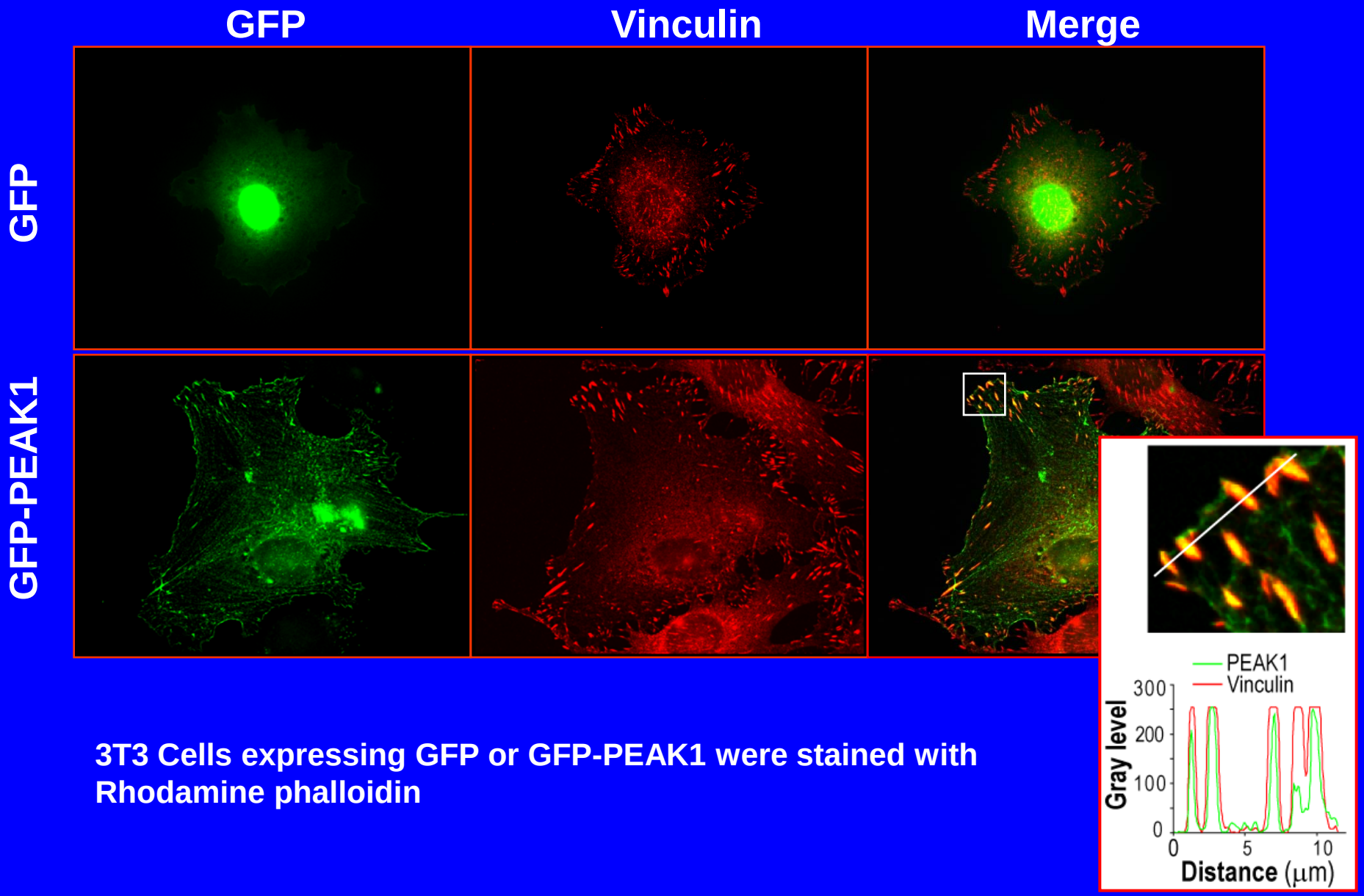
Depletion of PEAK1 also inhibits haptotaxis

PEAK1 Colocalizes with Actin Cytoskeleton

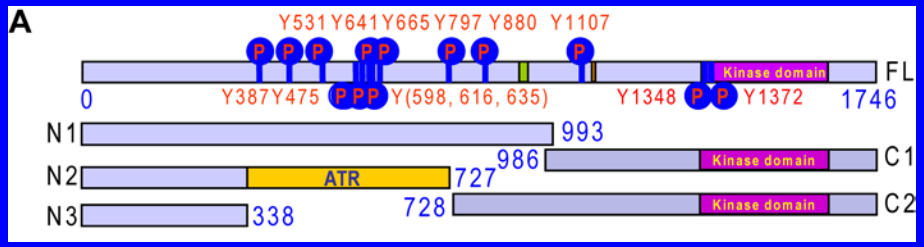


3T3 Cells transfected with GFP or GFP-PEAK1 were stained with Rhodamine phalloidin

PEAK1 Colocalizes with Focal Adhesions

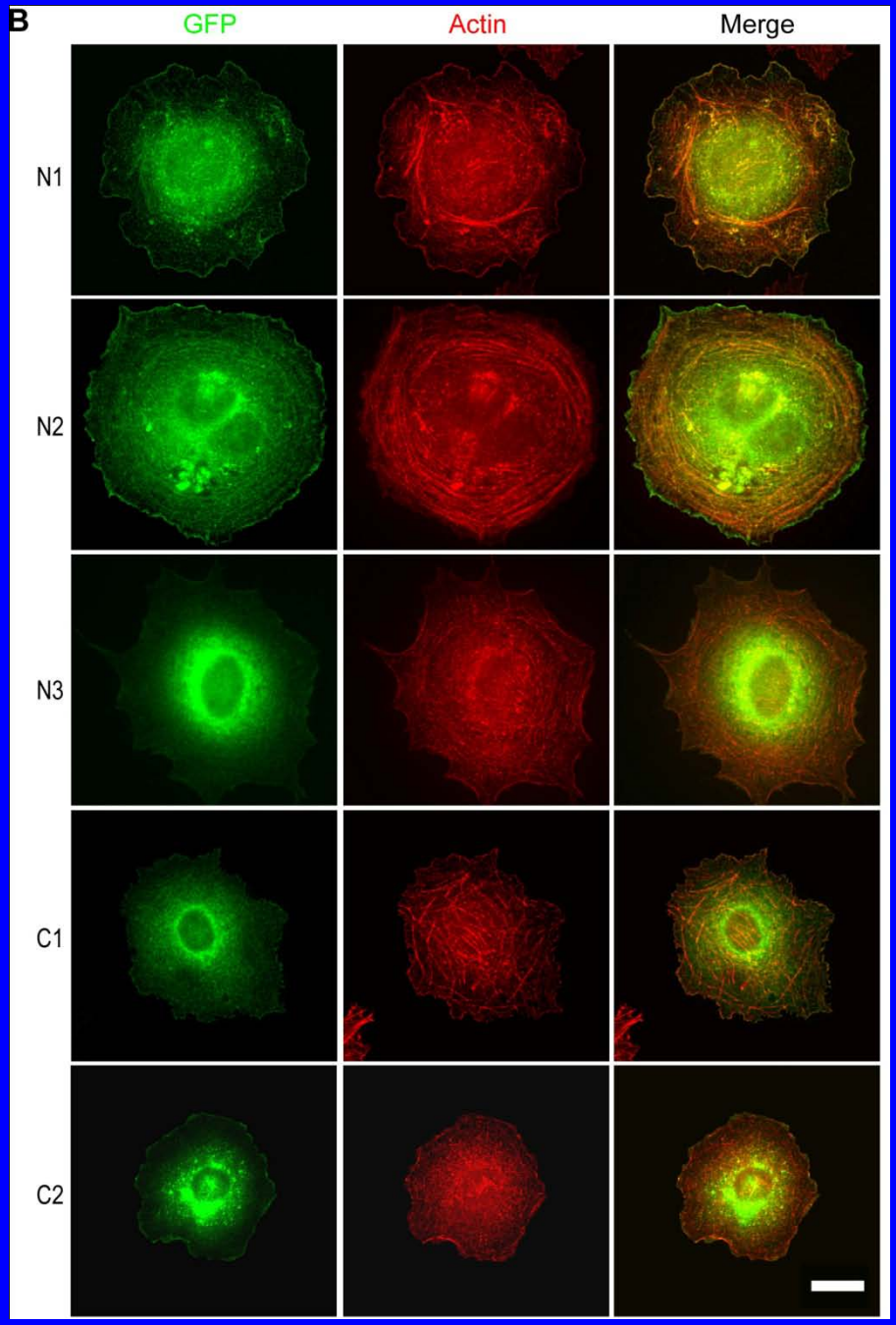


3T3 Cells expressing GFP or GFP-PEAK1 were stained with Rhodamine phalloidin



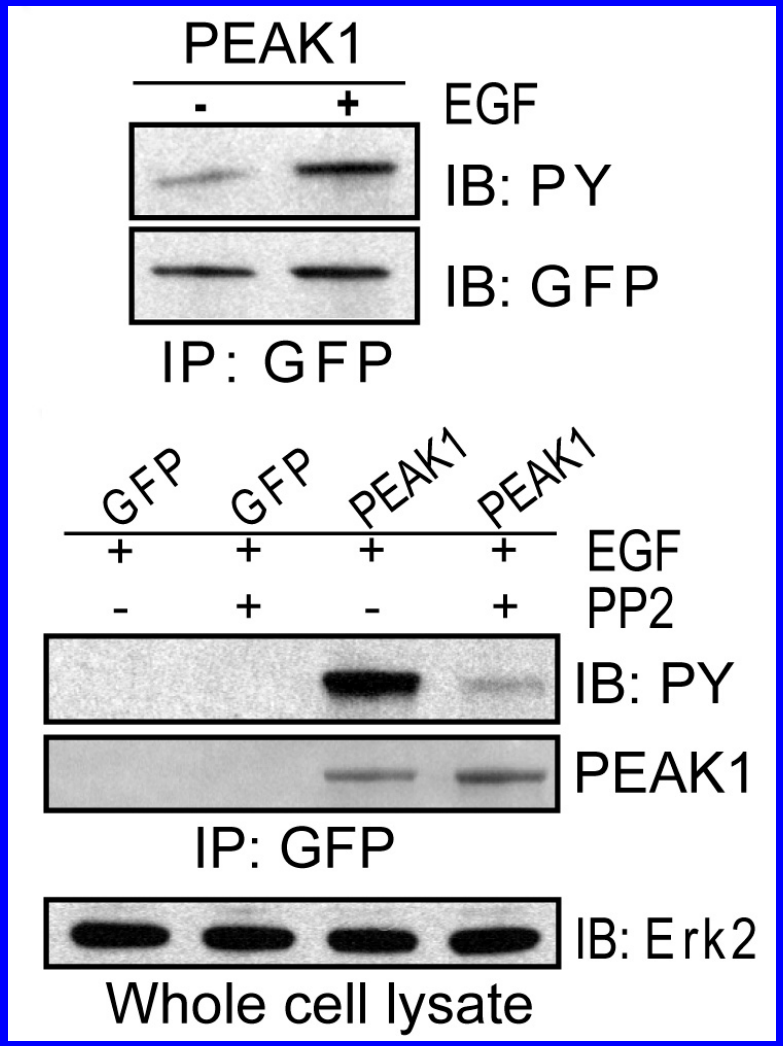
C

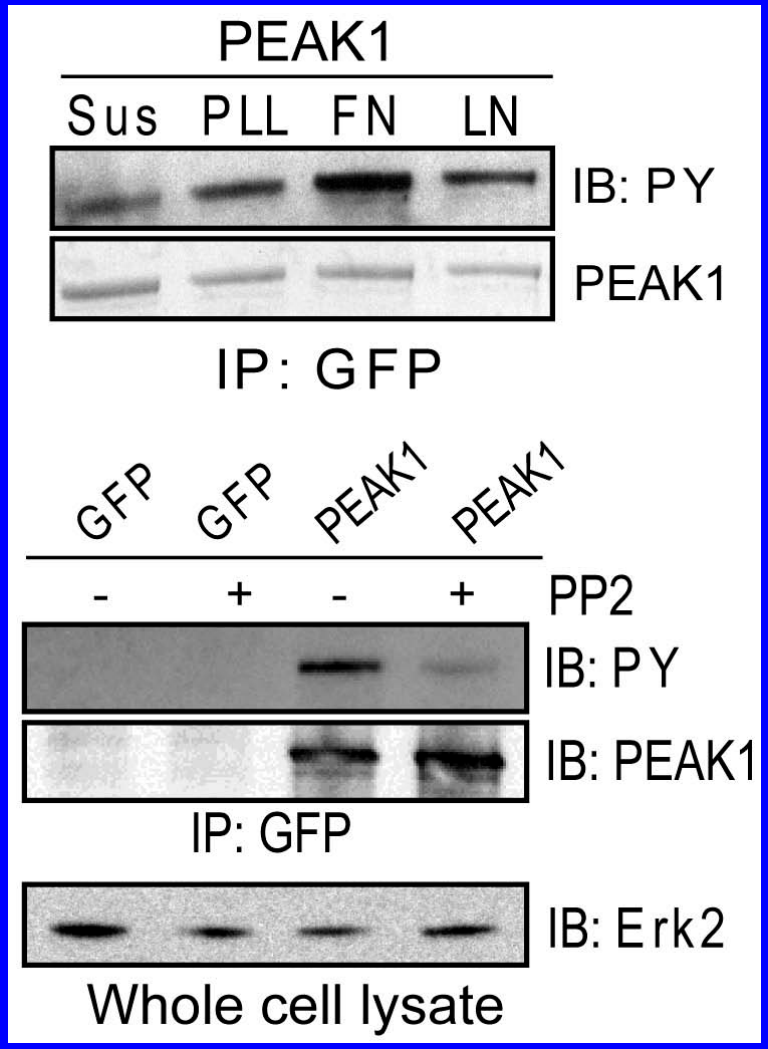
	GFP	N1	N2	N3	C1	C2	FL
Starve	-	+	+++	-	-	-	+
Serum	-	++	+++	-	-	-	+++
PDGF	-	++	+++	-	-	-	+++



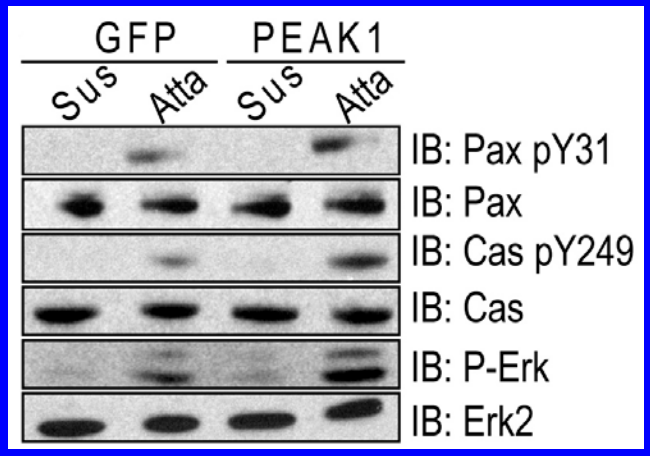
PEAK1 Actin-Cytoskeleton localization Depends on Growth Factor Signaling

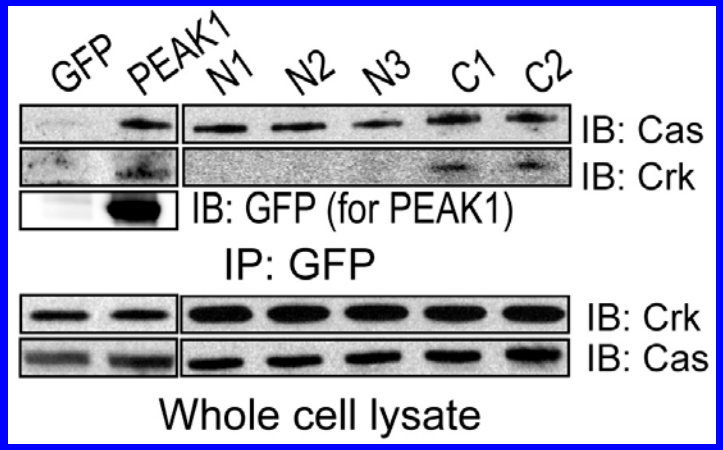
PEAK1 Undergoes Src-dependent Tyrosine Phosphorylation in Response to Cell Adhesion or EGF Stimulation

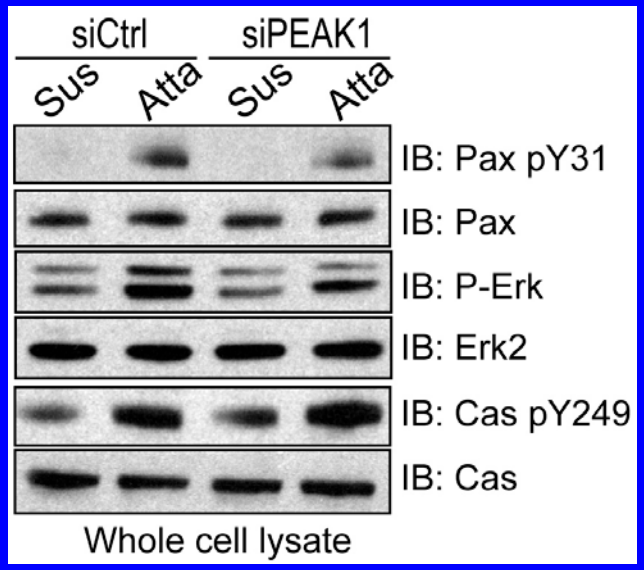




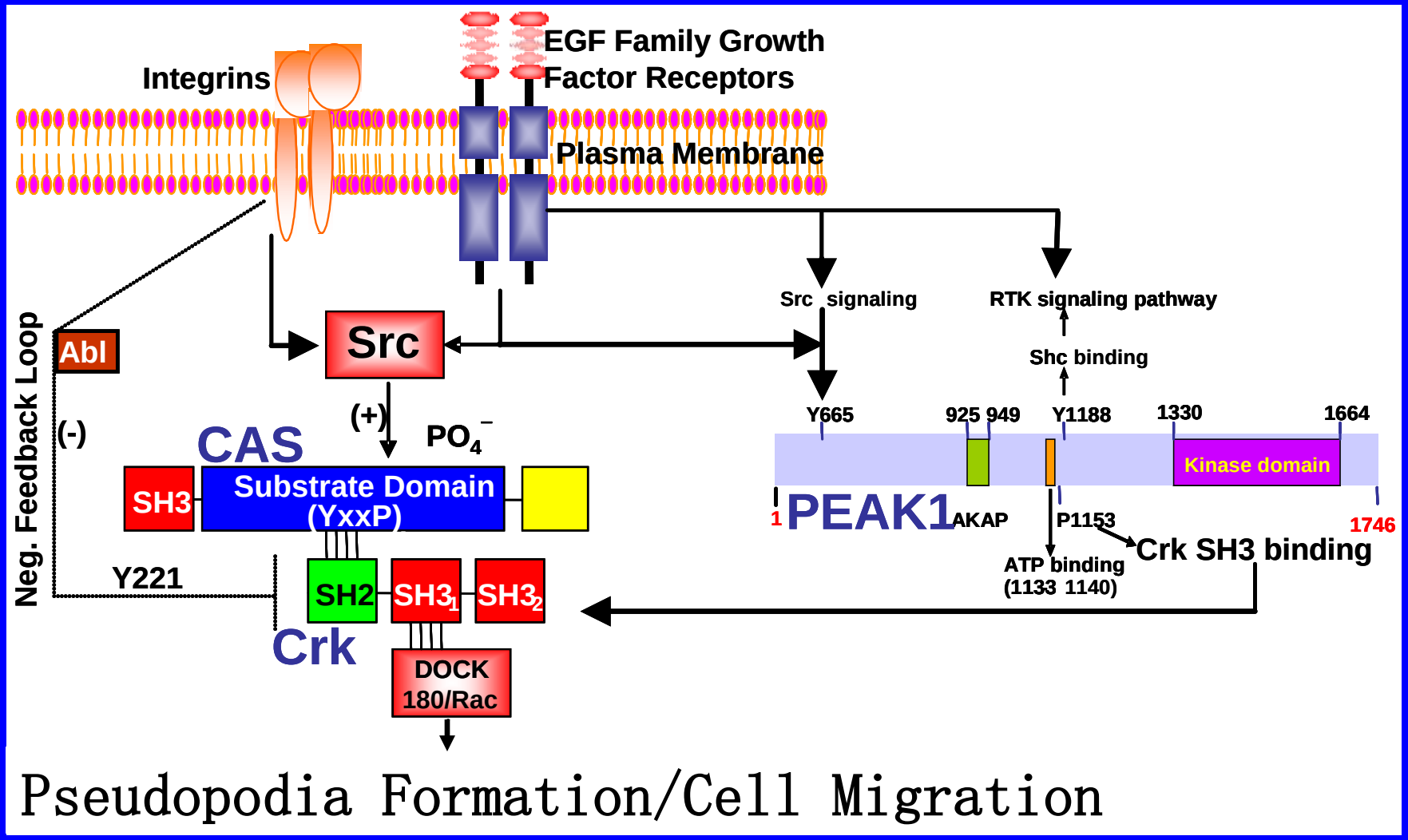
PEAK1 Regulates Cytoskeletal and Focal Adhesion Proteins



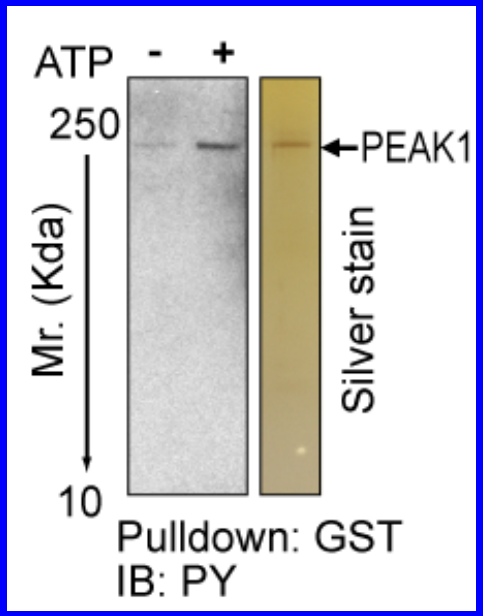




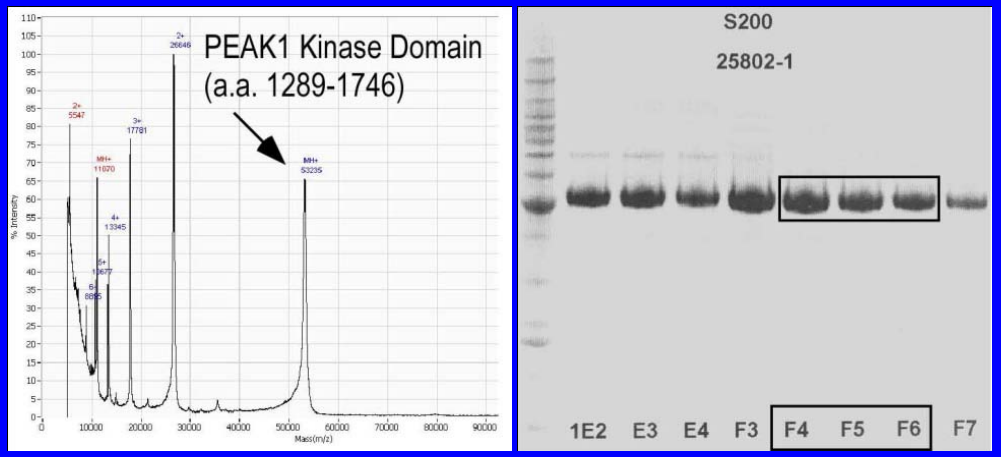
Proposed Signaling Pathway of PEAK1-mediated Cell Migration



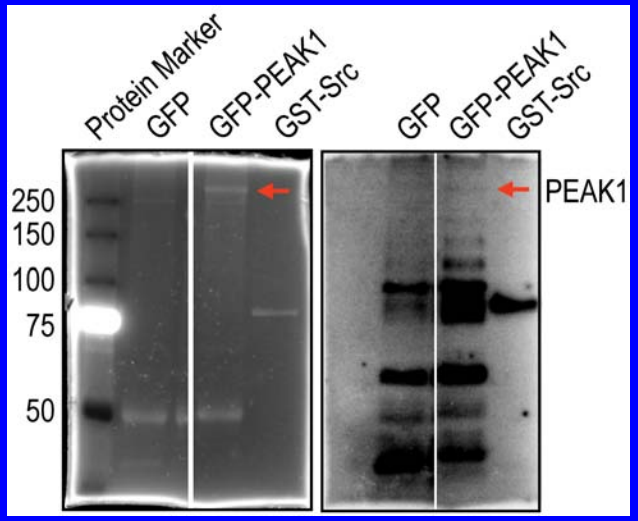
PEAK1 Is an Active Kinase



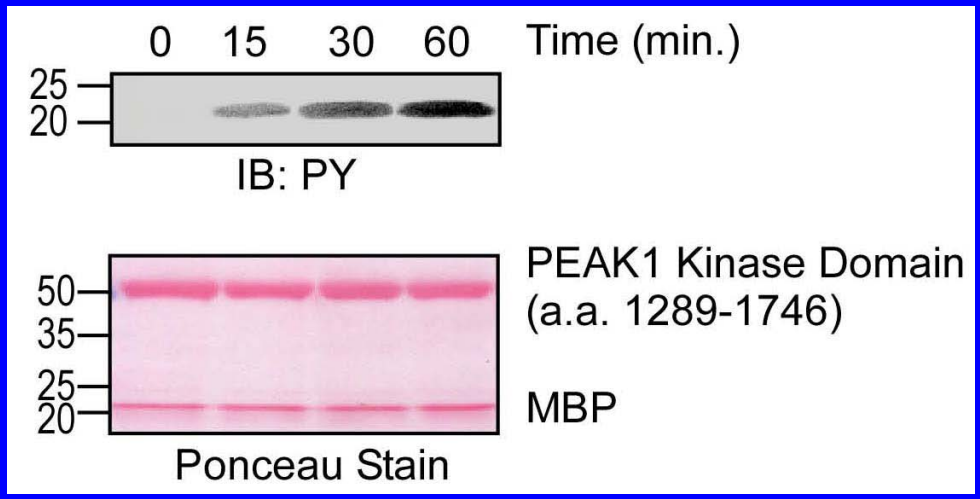
PEAK1 autophosphorylation



Size exclusion chromatography
purification of PEAK1 kinase domain

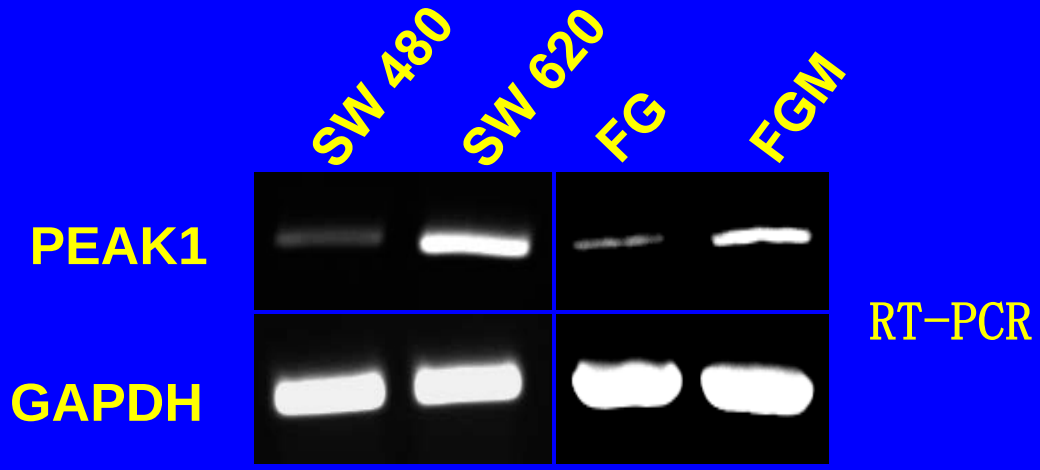


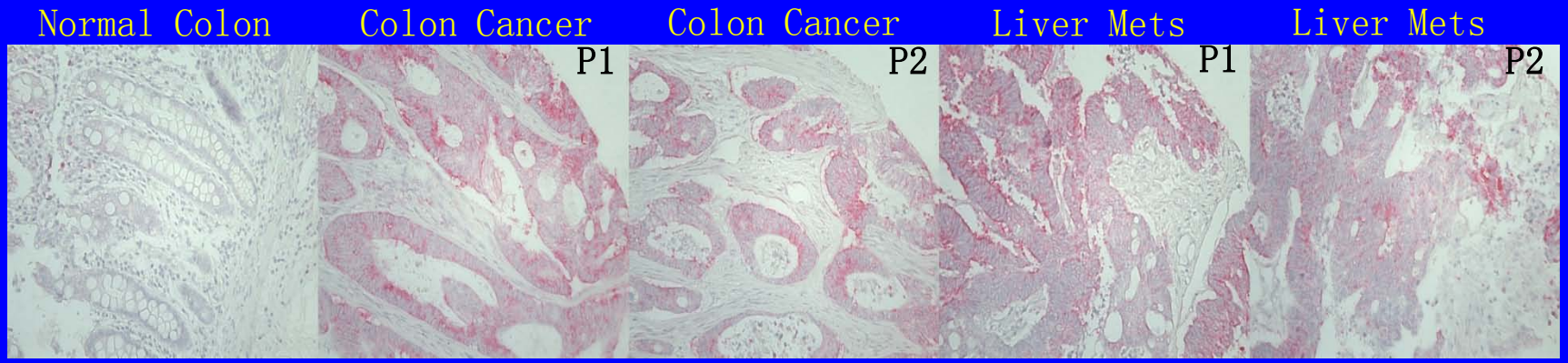
In-gel kinase assay



In-vitro kinase assay

Amplification of PEAK1 in Metastatic Cancer Cell Lines and Cancer Patients

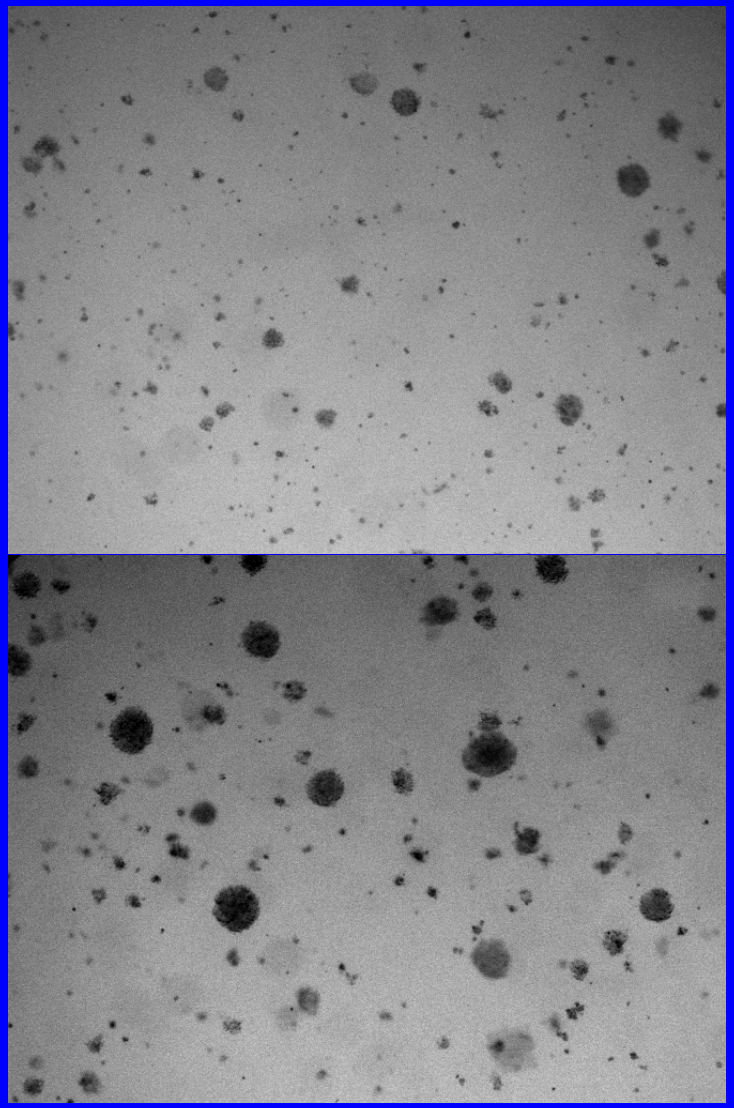


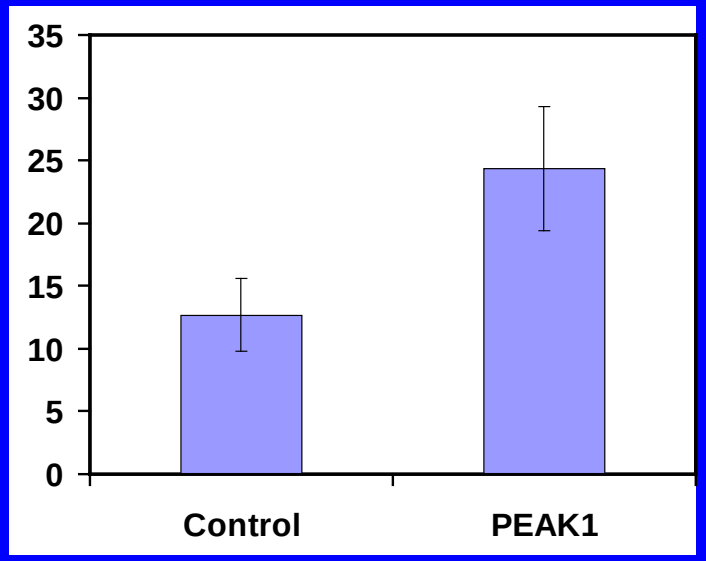


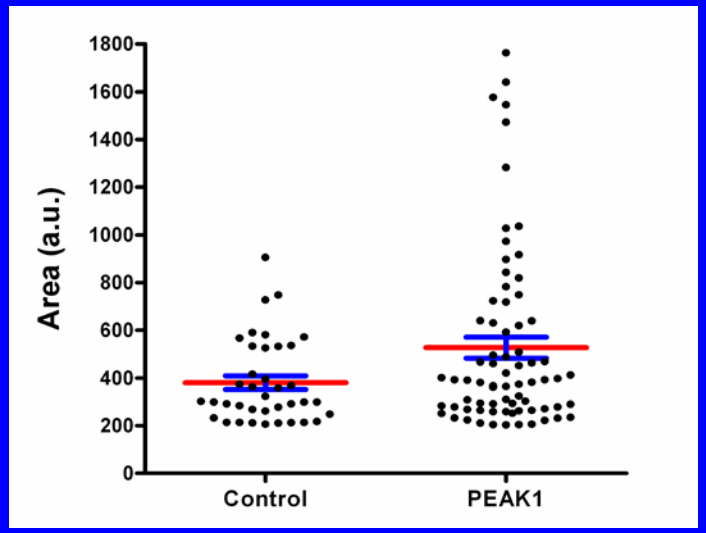
P1=patient 1
P2=patient 2

Human cancer tissue array indicates that PEAK1 is amplified in ~82% of colon cancers and corresponding liver metastases 22 patient array

PEAK1 Promotes Oncogeneic Growth in Cancer Cells





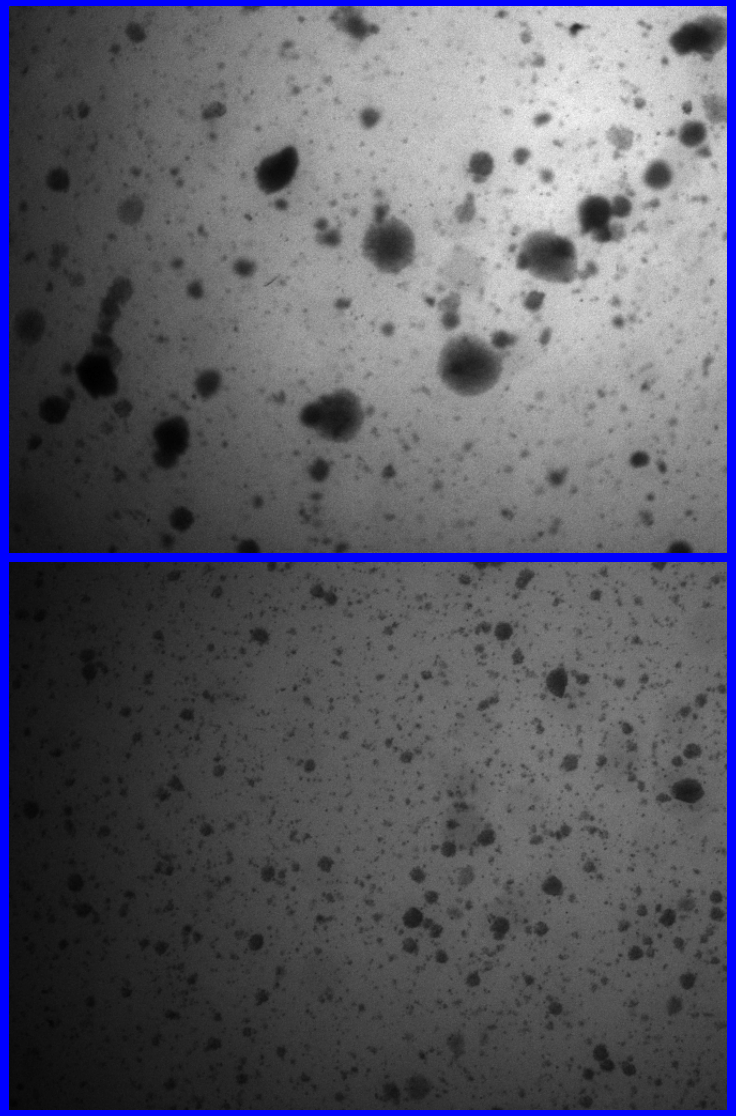


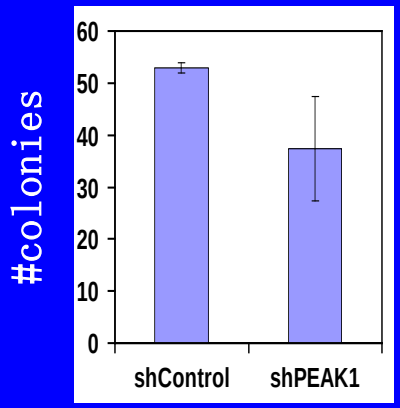
MDA435 cells stably expressing GFP (control) or GFP-PEAK1 fusion protein (PEAK1) were allowed to form colonies in soft agar supplemented with 10% FBS for 14 days. The number and the sizes of the colonies were measured by the software MetaMorph.

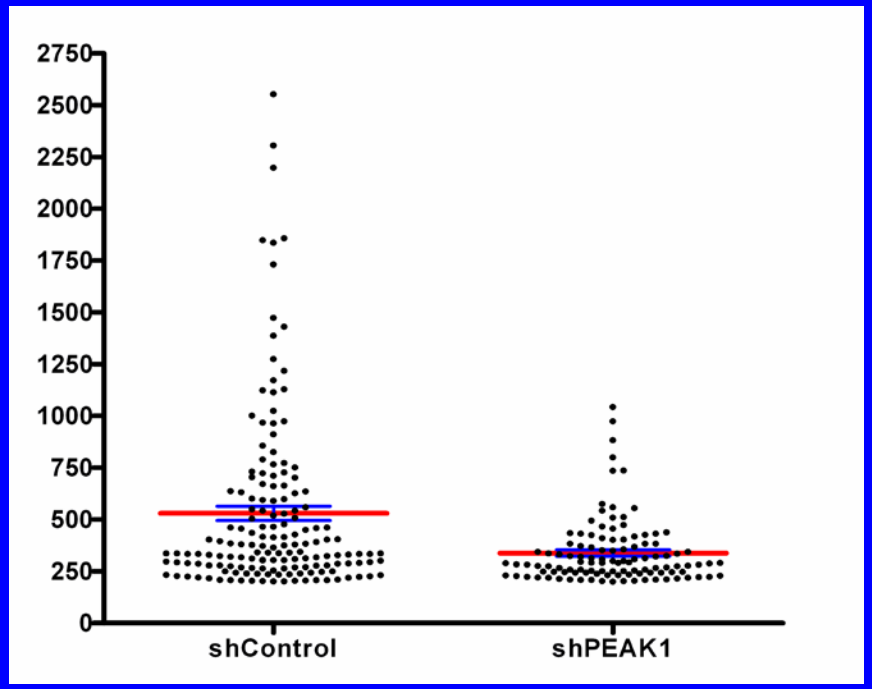
Reducing PEAK1 Inhibits Oncogenic Growth

shControl

shPEAK1



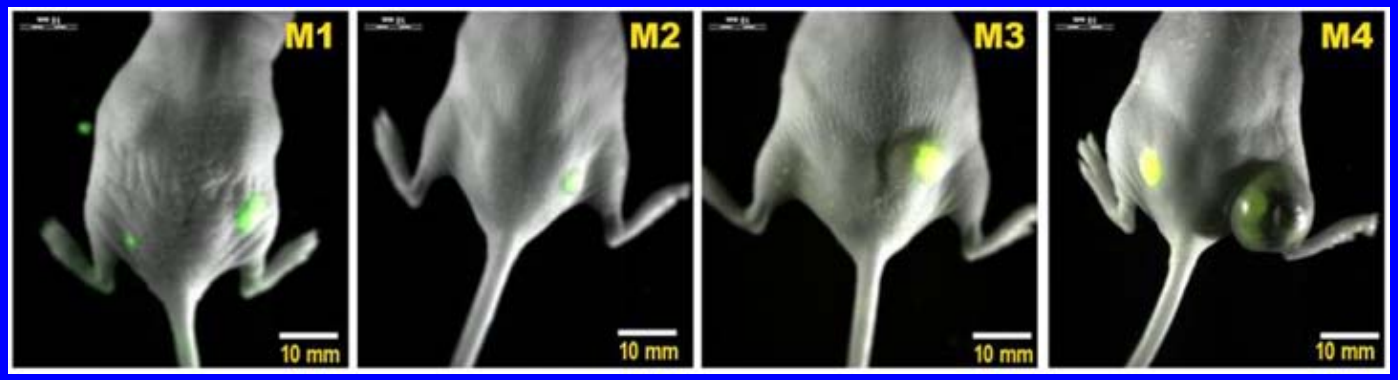


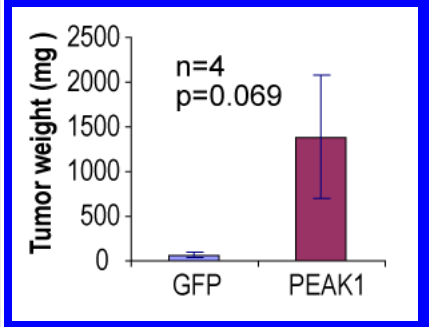


MDA-435 cells stably expressing PEAK1 were infected with shRNA lentivirus. The cells were then cultured in softagar to form colonies

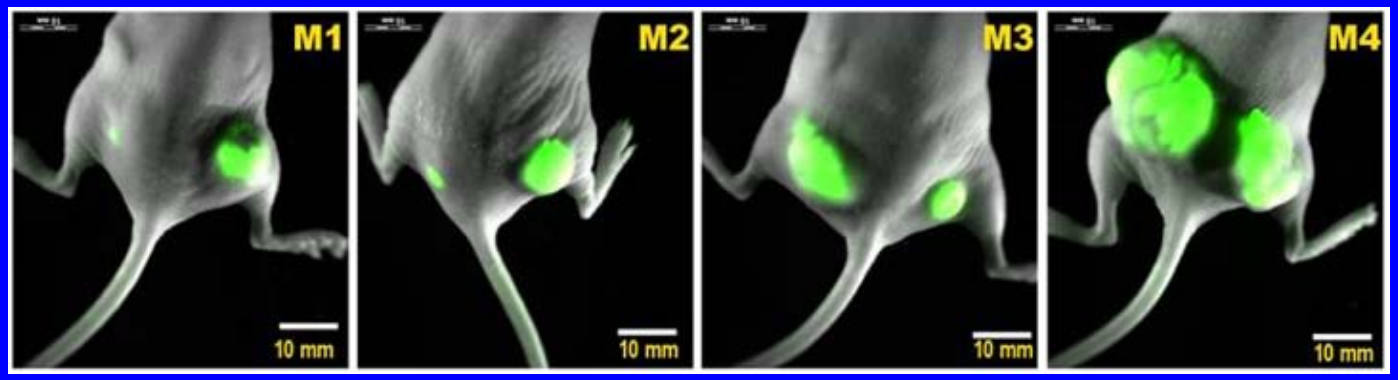
PEAK1 Promotes Tumor Progression in vivo

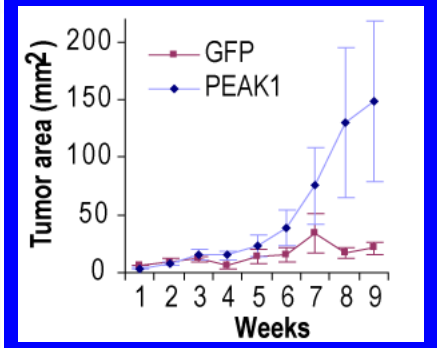
GFP



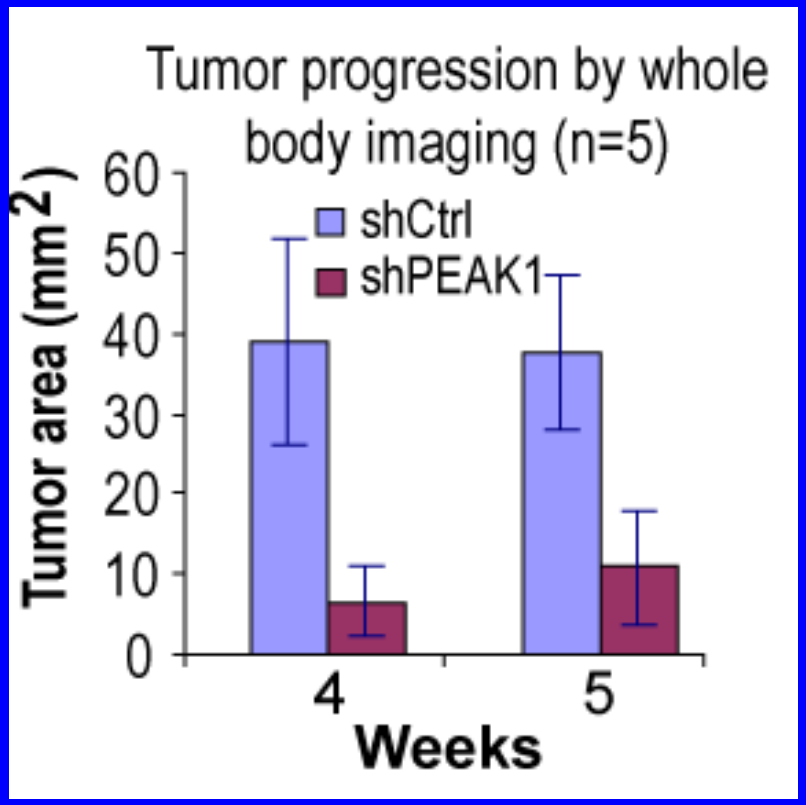


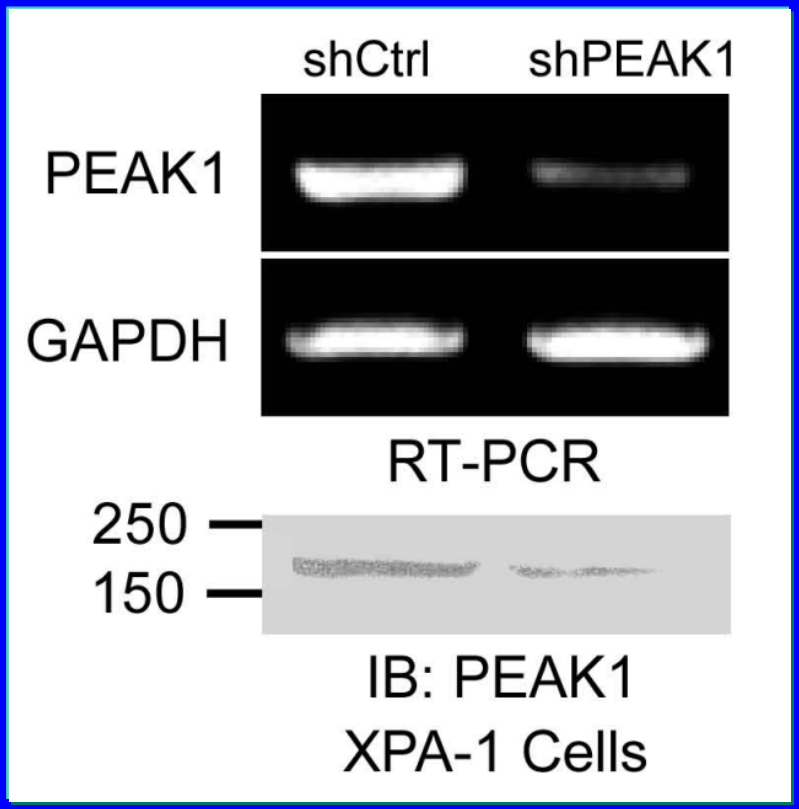
GFP-PEAK1





Depletion of PEAK1 Inhibits Tumor Growth





Summary

1. Systematical identification of proteome and phosphoproteome in PD and CB.

2. Cytoskeletal and signaling proteins are highly enriched in PD while cell cycle and metabolism proteins are enriched in CB.

3. Discovered a novel PD-enriched kinase PEAK1 that is colocalized with cytoskeleton and is necessary for cell migration and tumor progression.

Future Work

- Study signaling mechanism underlying cell migration and cancer metastasis.
- Develop bioinformatics tool to facilitate analyzing proteomics data.
- Membrane proteomics.

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