

Quantitative proteomics for elucidating protein-protein interactions and pathways

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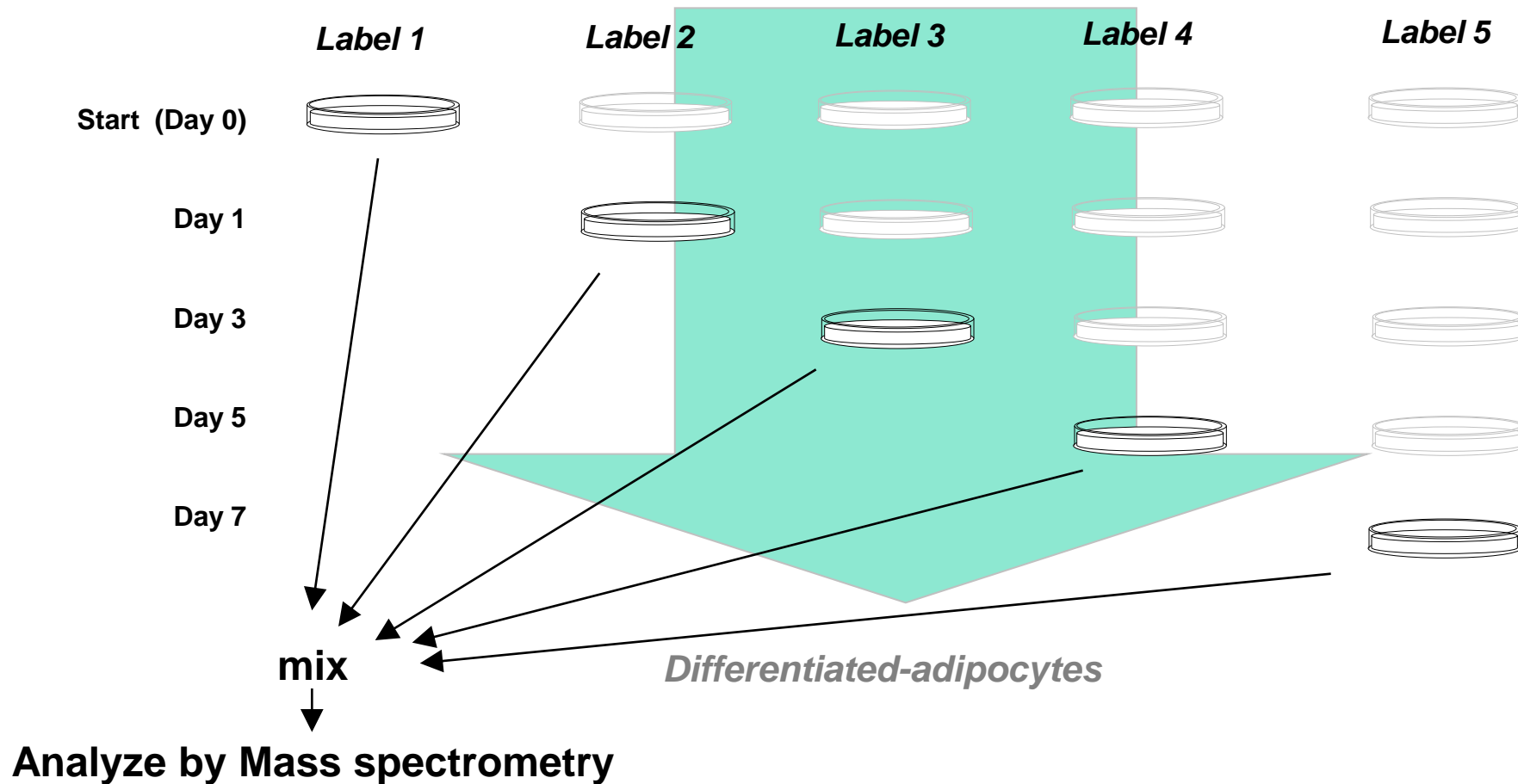
Outline

- SILAC for quantitative proteomics
- Characterizing protein complex association with an adapter protein, Odin, in EGFR signaling
- Signaling pathways activated by TSLPR, a receptor involved in the pathogenesis of asthma and acute lymphoblastic leukemias

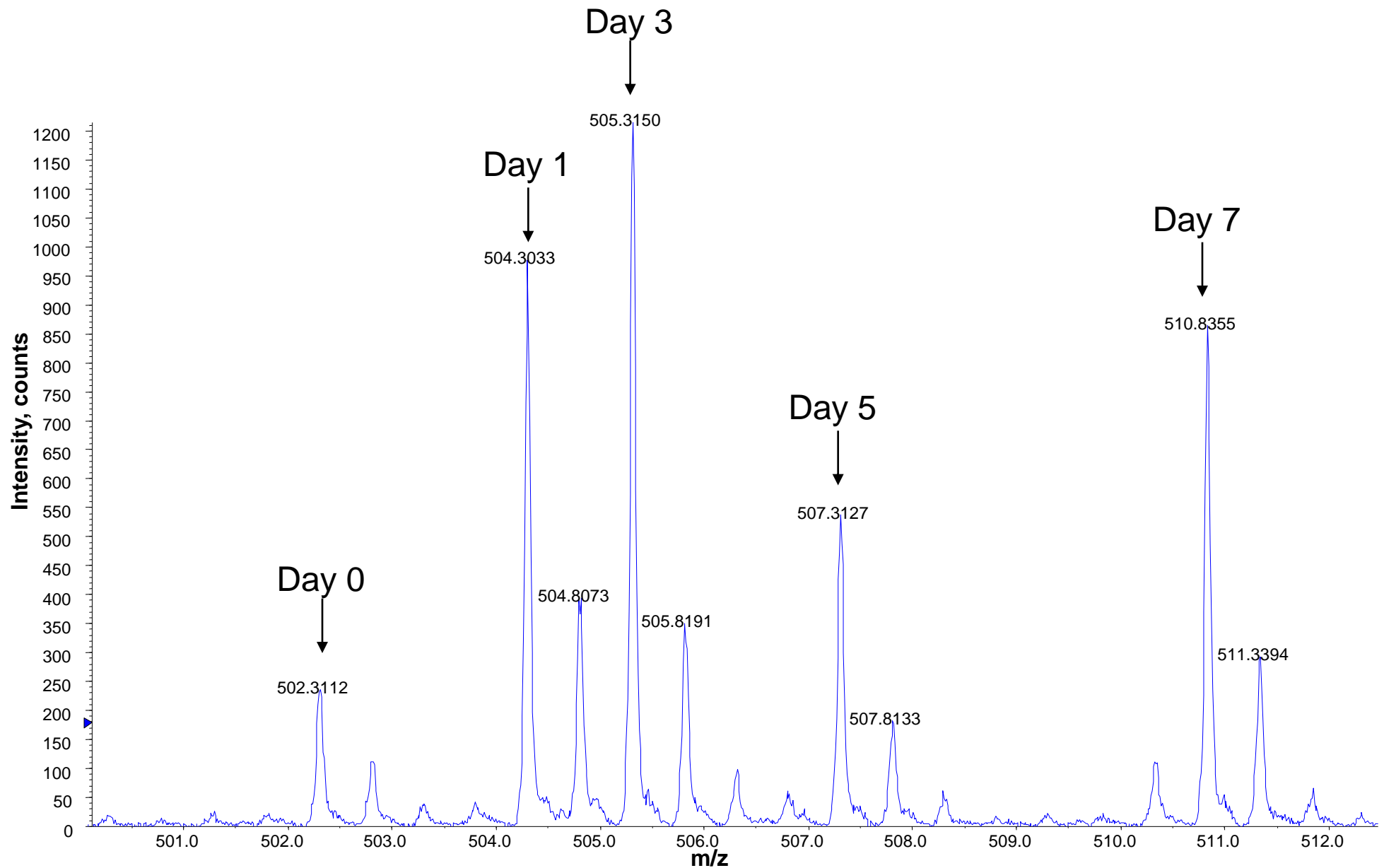
SILAC for *in vivo* labeling

- Simple
- Does not require any extra processing steps
- All proteins are uniformly labeled
- Complete and predictable incorporation
- Choice of labeled amino acids
- De novo sequencing can be performed
- www.silac.org

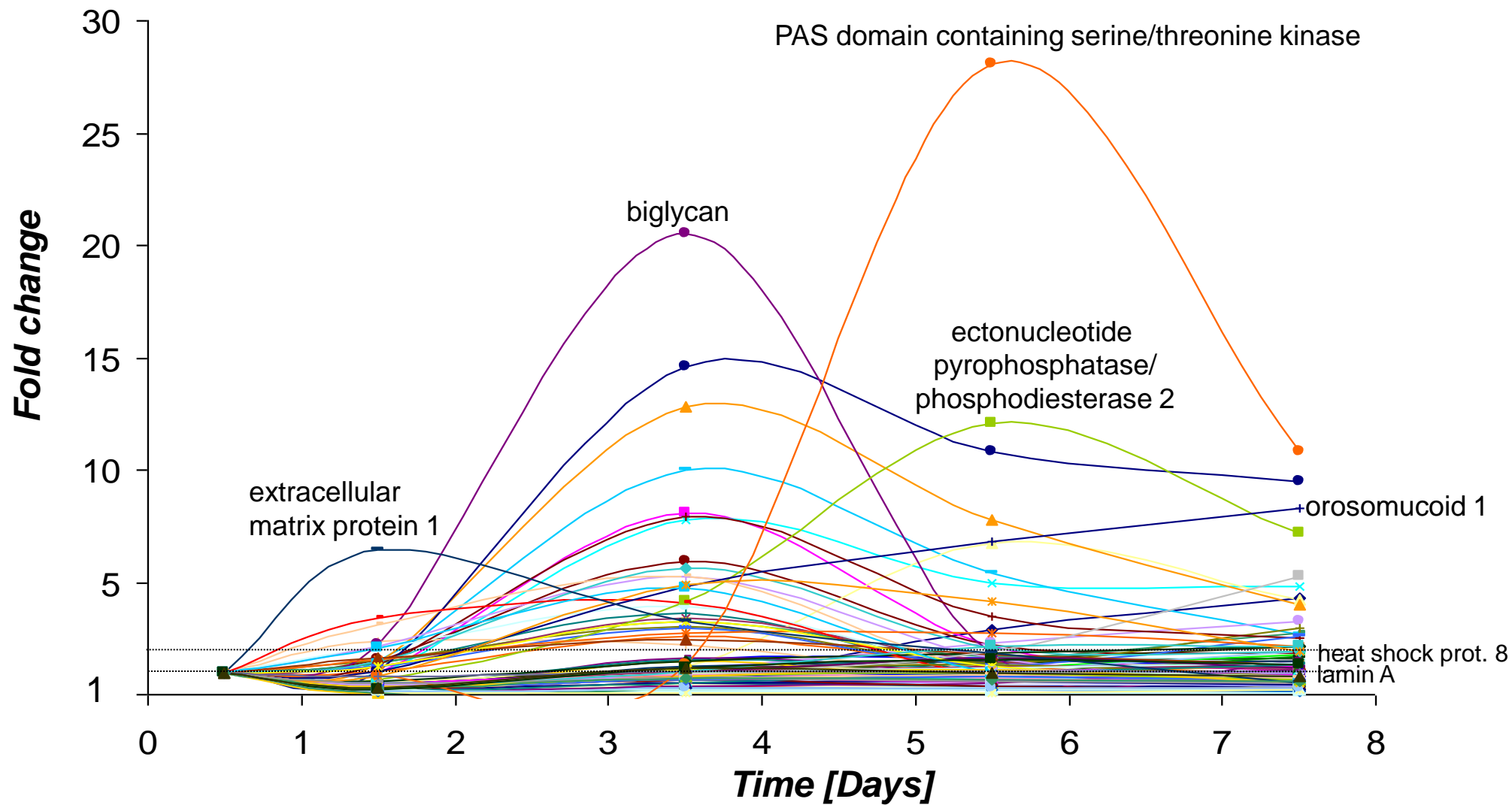
5-state SILAC experiment to study proteome dynamics



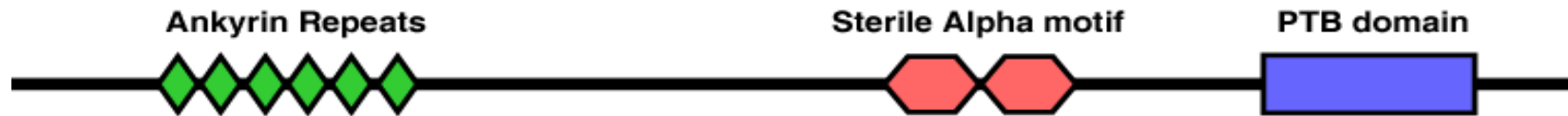
A Mass Spectrum From a 5-Plex Experiment



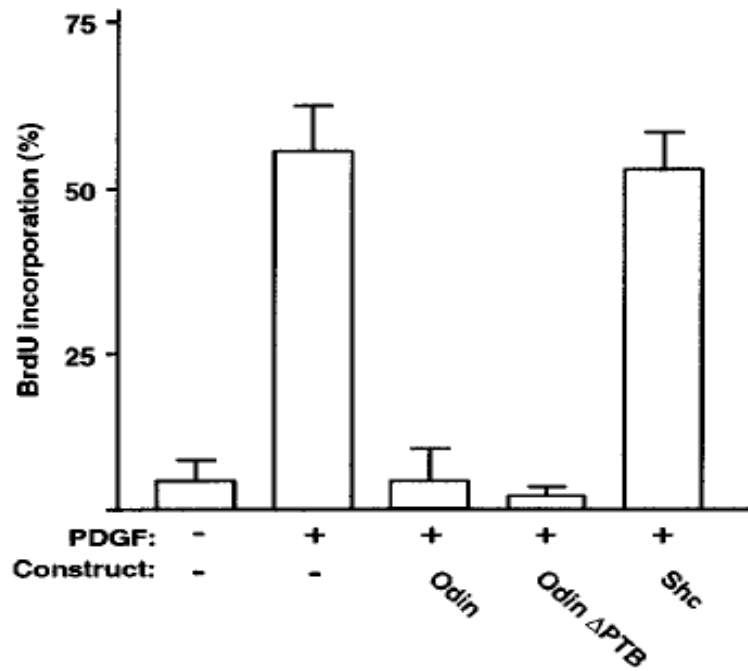
Protein Dynamics of >100 Proteins Measured by SILAC



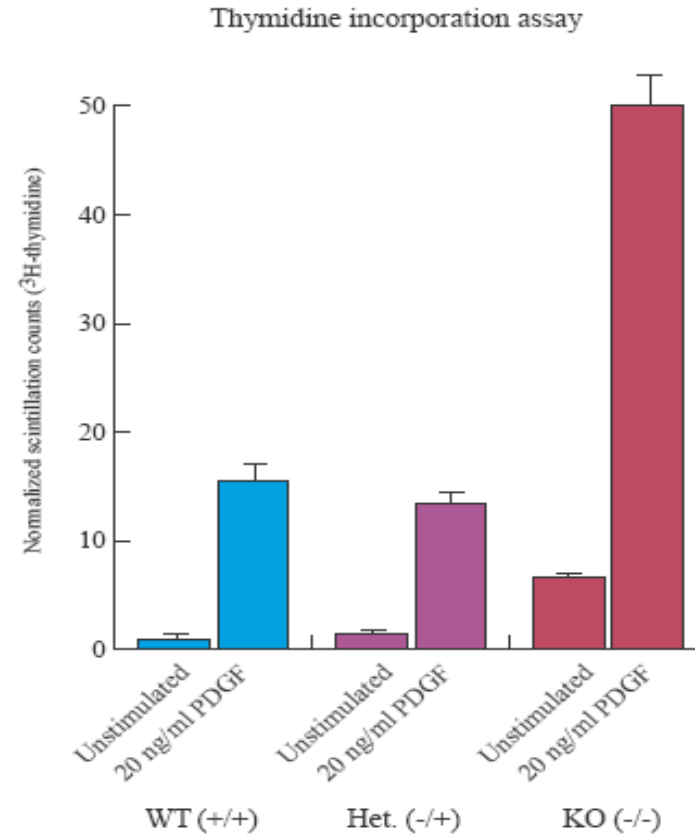
Function of Odin in Growth Factor Signaling



Odin is a negative regulator of growth factor signaling

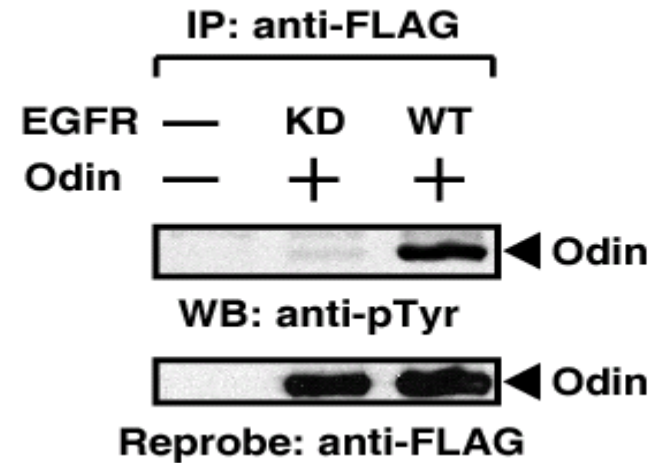
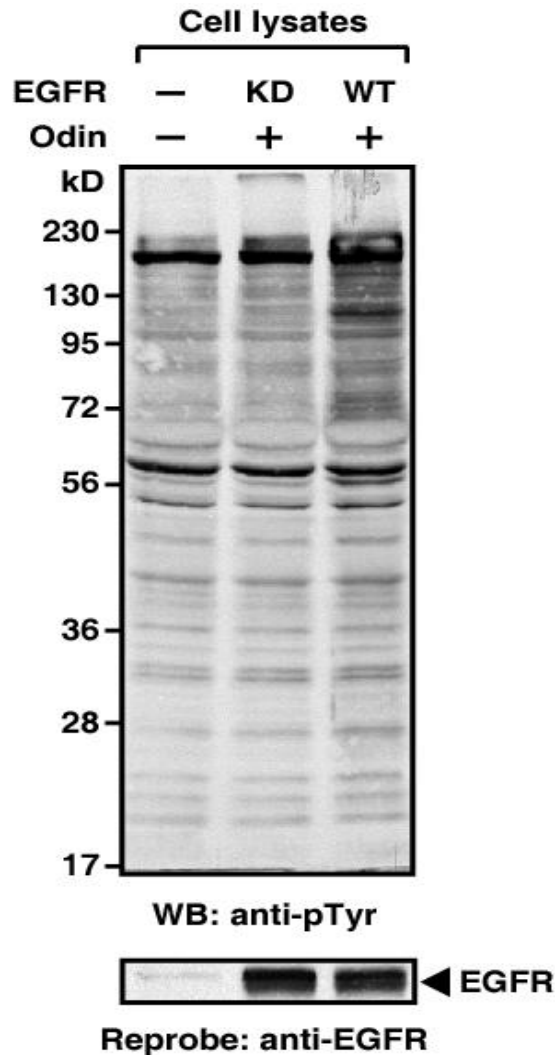


NIH3T3 fibroblasts

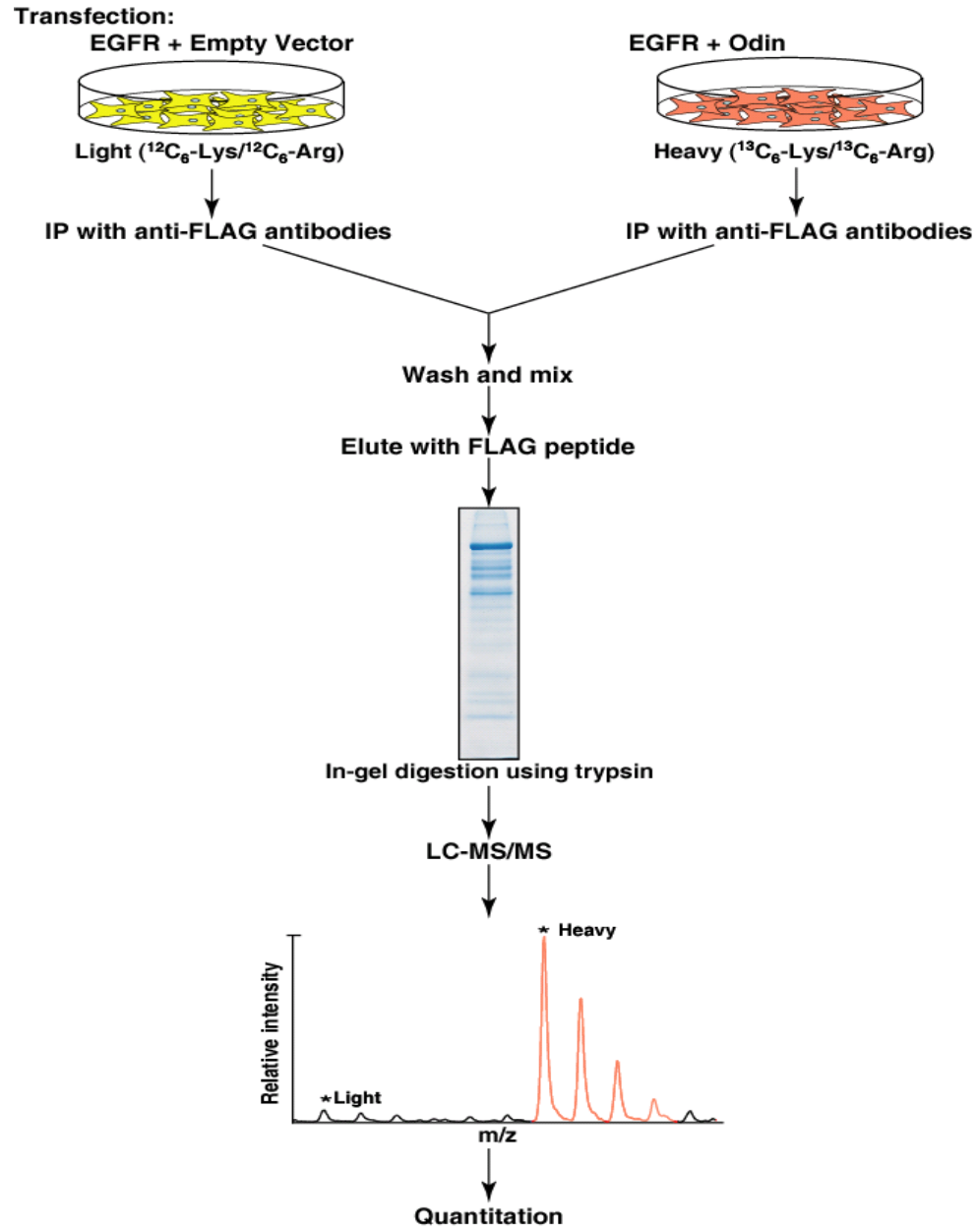


Primary mouse embryo fibroblasts treated with or without 20ng/ml PDGF-BB for 8h

Odin undergoes tyrosine phosphorylation in receptor tyrosine kinase signaling

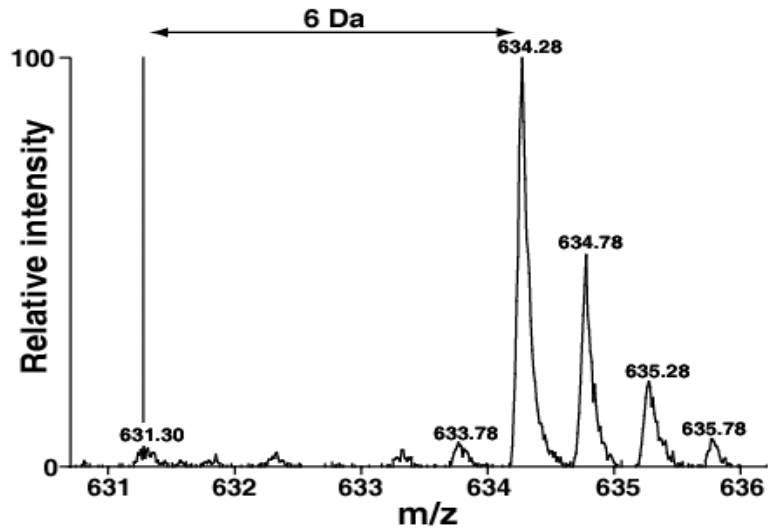


Identifying Odin Interactors Using SILAC

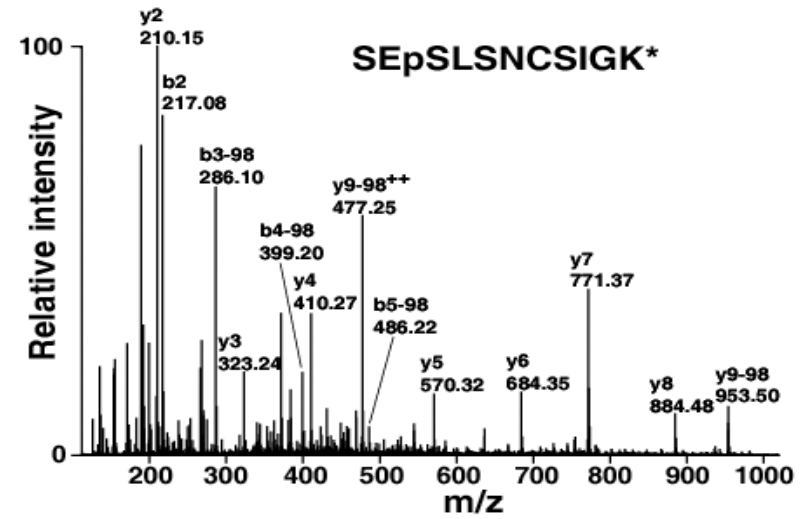


Odin is identified from Transfected Cells

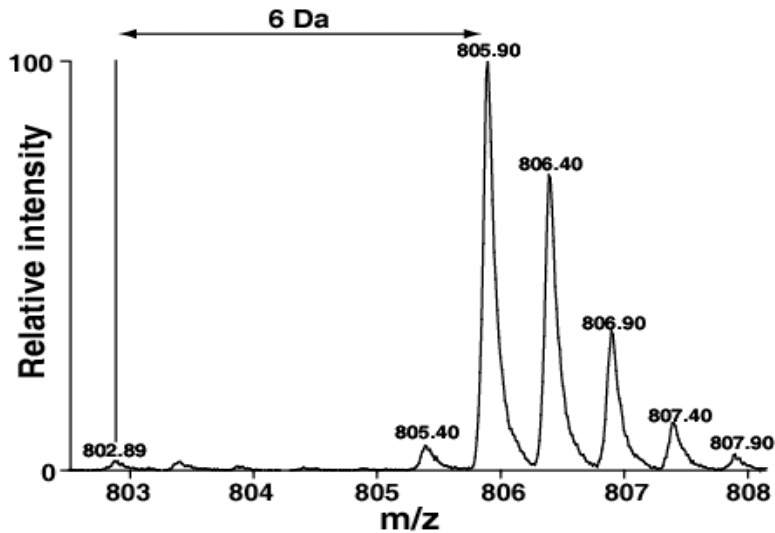
A



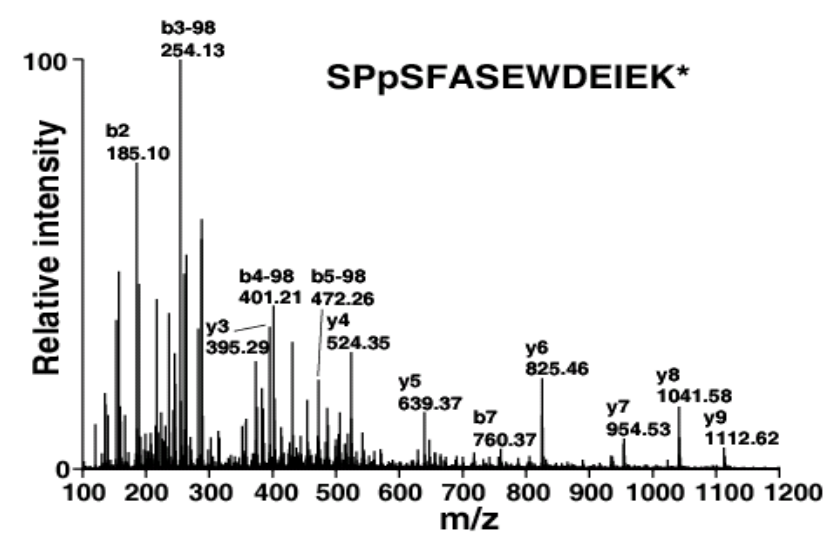
B



C

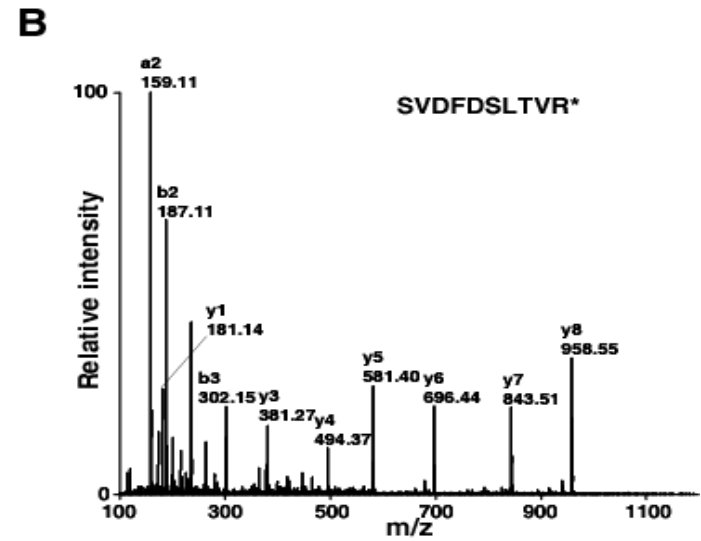
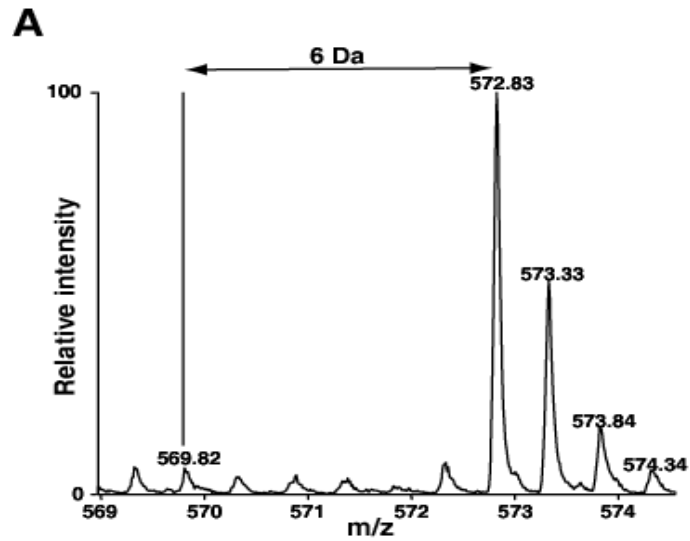


D

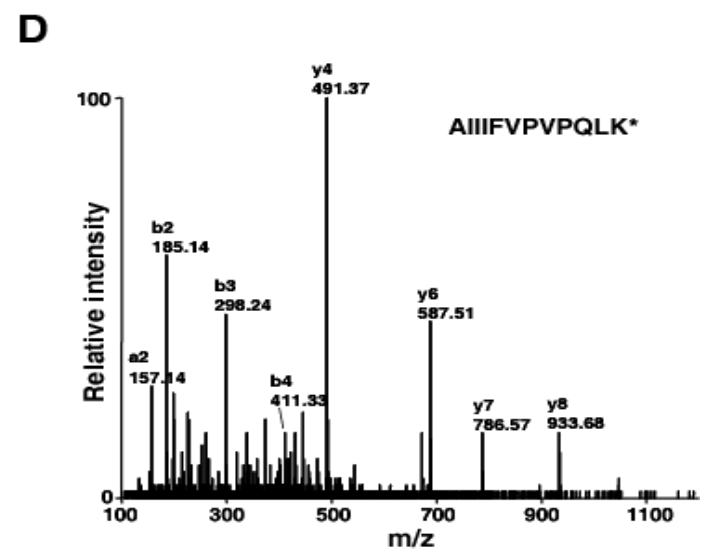
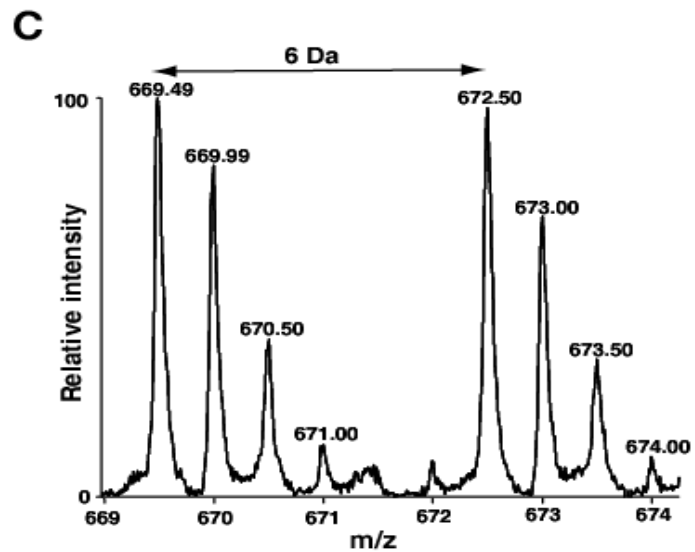


Specific and non-specific Odin Interactors

CD2-associated protein



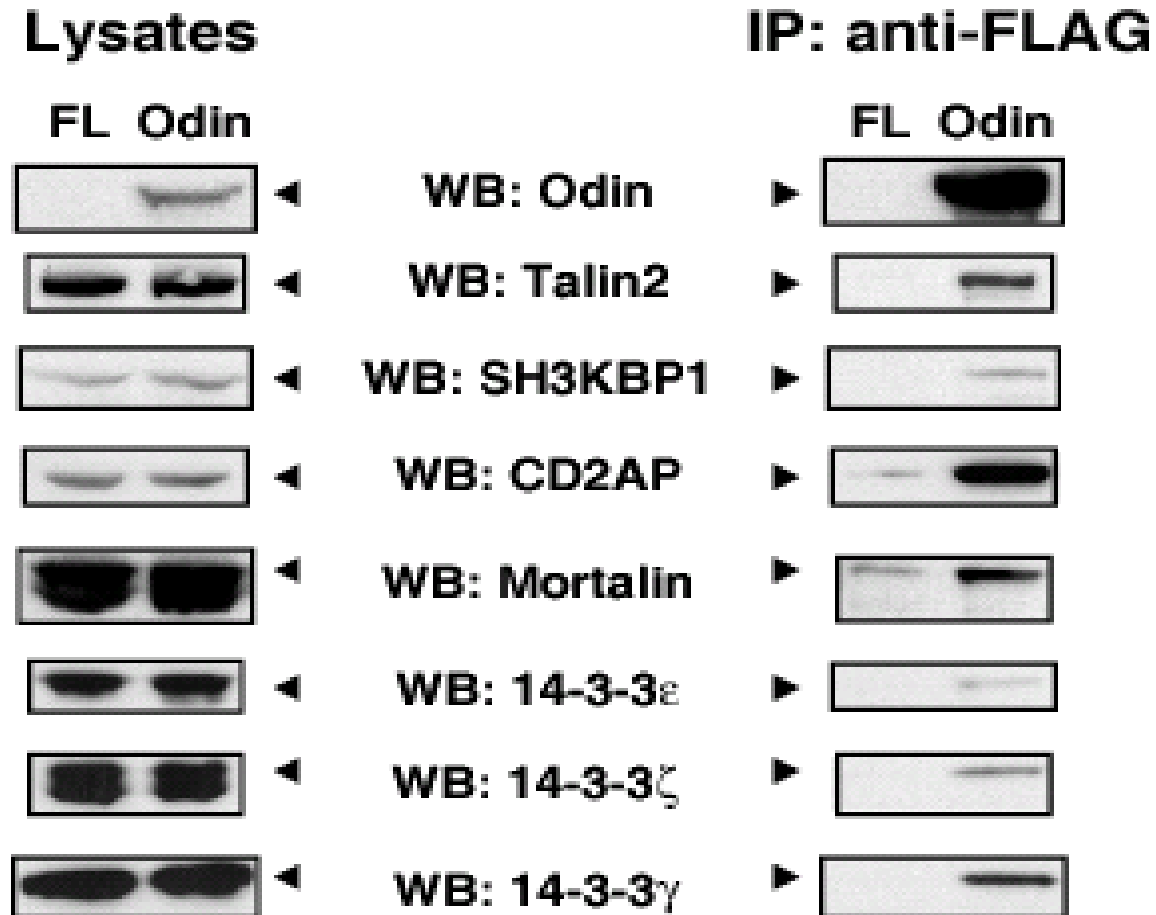
Ribosomal protein S7



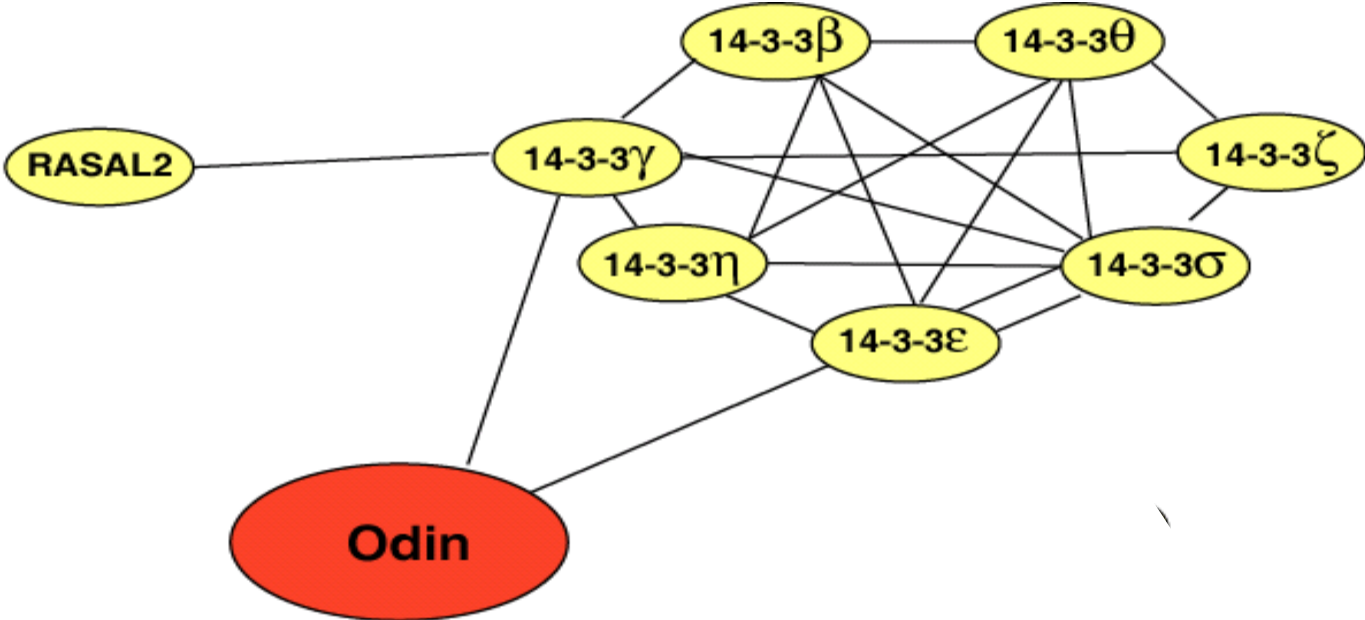
Specific Odin Interactors

	Gene Symbol	Protein
1	<i>YWHAG</i>	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, gamma polypeptide (14-3-3 gamma)
2	<i>YWHAZ</i>	Tyrosine 3/tryptophan 5 -monooxygenase activation protein, zeta polypeptide (14-3-3 theta)
3	<i>YWHAB</i>	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta polypeptide (14-3-3 beta)
4	<i>YWHAH</i>	Tyrosine 3/tryptophan 5 -monooxygenase activation protein, eta polypeptide (14-3-3 eta)
5	<i>YWHAE</i>	Tyrosine 3/tryptophan 5 -monooxygenase activation protein, epsilon polypeptide (14-3-3 epsilon)
6	<i>SFN</i>	Stratifin (14-3-3 sigma)
7	<i>SH3KBP1</i>	SH3-domain kinase binding protein 1
8	<i>CD2AP</i>	CD2-associated protein (CMS)
9	<i>RASAL2</i>	RAS protein activator like 2
10	<i>YWHAQ</i>	Tyrosine 3/tryptophan 5 -monooxygenase activation protein, theta polypeptide (14-3-3 theta)
11	<i>CAPZB</i>	F-actin capping protein beta subunit
12	<i>DAB2IP</i>	DAB2 interacting protein
13	<i>TLN2</i>	Talin 2
14	<i>GART</i>	Phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase
15	<i>VAPA</i>	Vesicle-associated membrane protein (VAMP)-associated protein A
16	<i>ARHGAP10</i>	Rho GTPase activating protein 10
17	<i>HSPA9</i>	Heat shock 70 kDa protein 9 (mortalin)
18	<i>UACA</i>	Uveal autoantigen

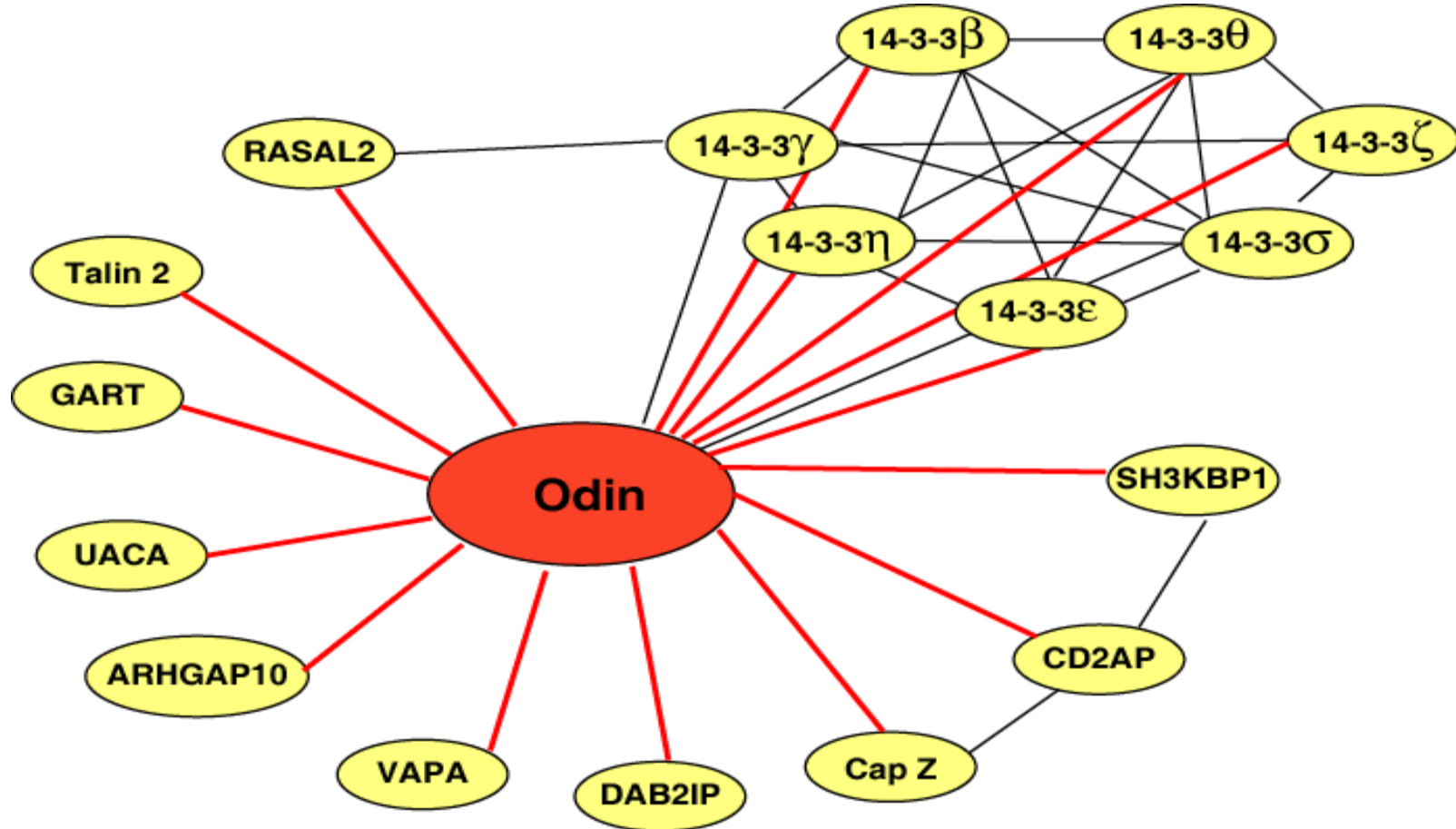
Validation of protein interactions



Known Interaction Network between Odin and its Interactors



Expanded Odin Interaction Network



Summary

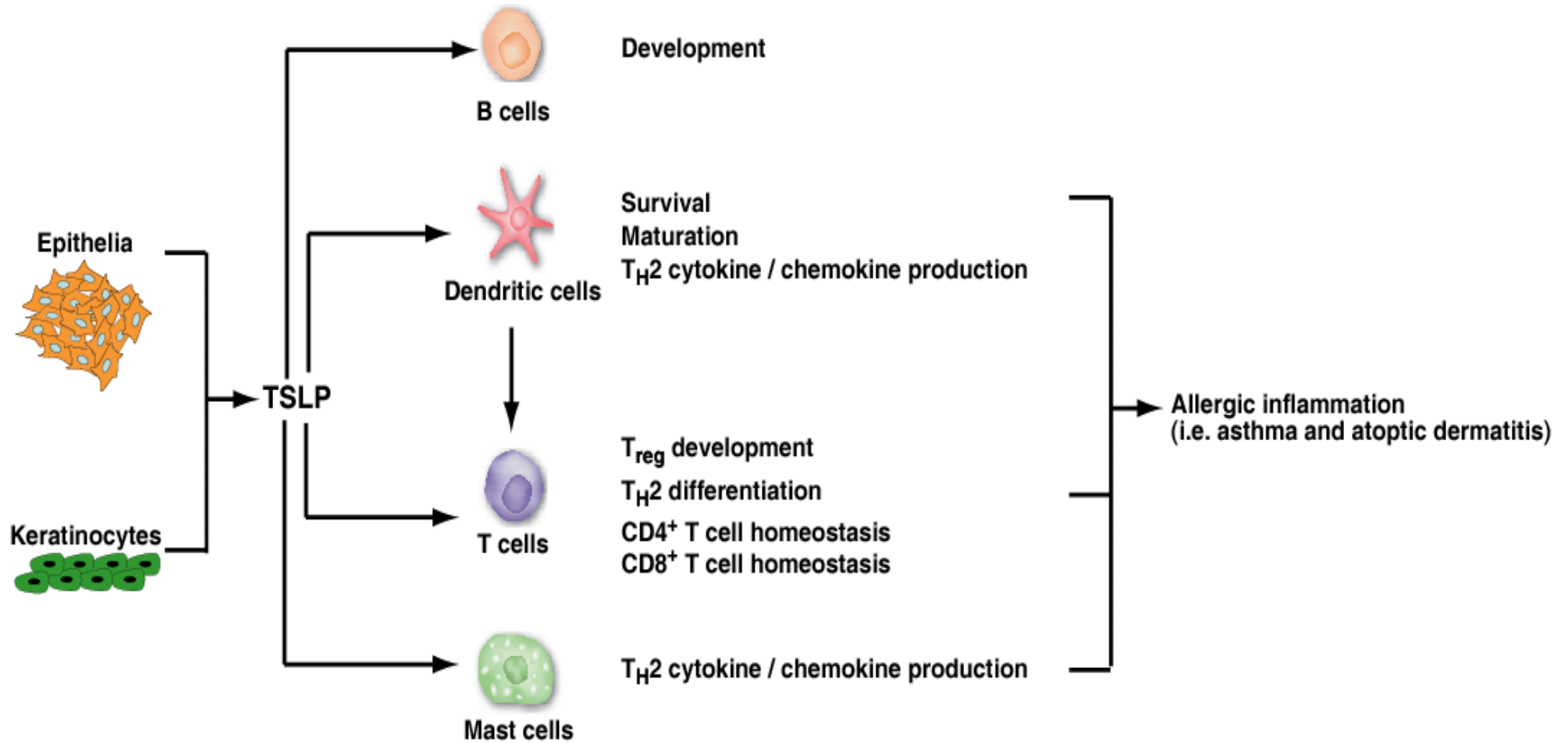
- It is possible to derive 'clues' about the function of a protein from protein complex studies
- Odin is possibly involved in endocytosis pathway (guilt by association) but additional directed experiments need to be carried out

Not all protein complexes are stable...

Thymic Stromal Lymphopoietin (TSLP)

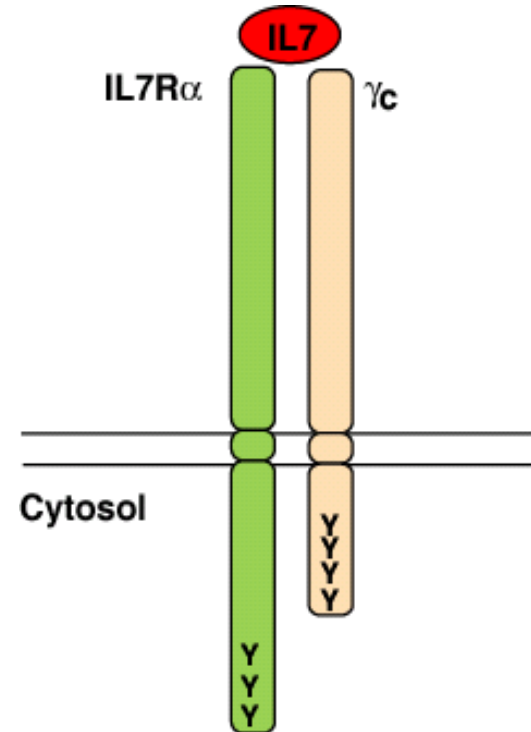
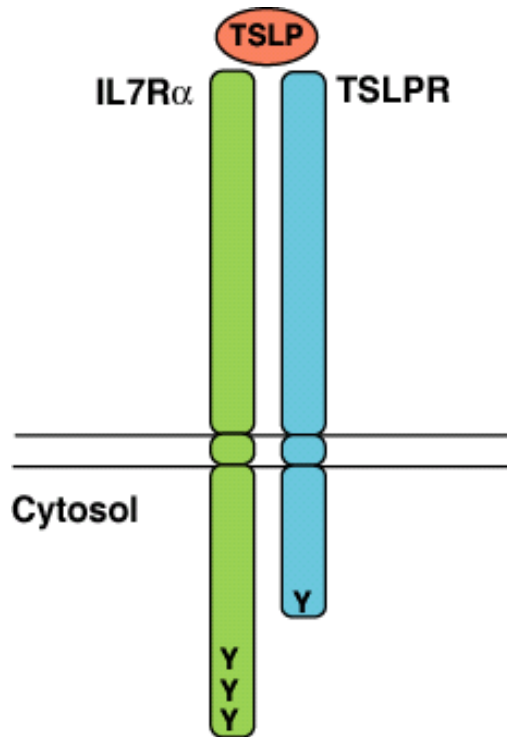
- A novel IL-7-like cytokine
- Originally identified from a murine thymic stromal cell line
- Important in the pathogenesis of asthma
- Mutations recently identified in certain types of leukemias

Biological Functions of TSLP



TSLP receptor complex

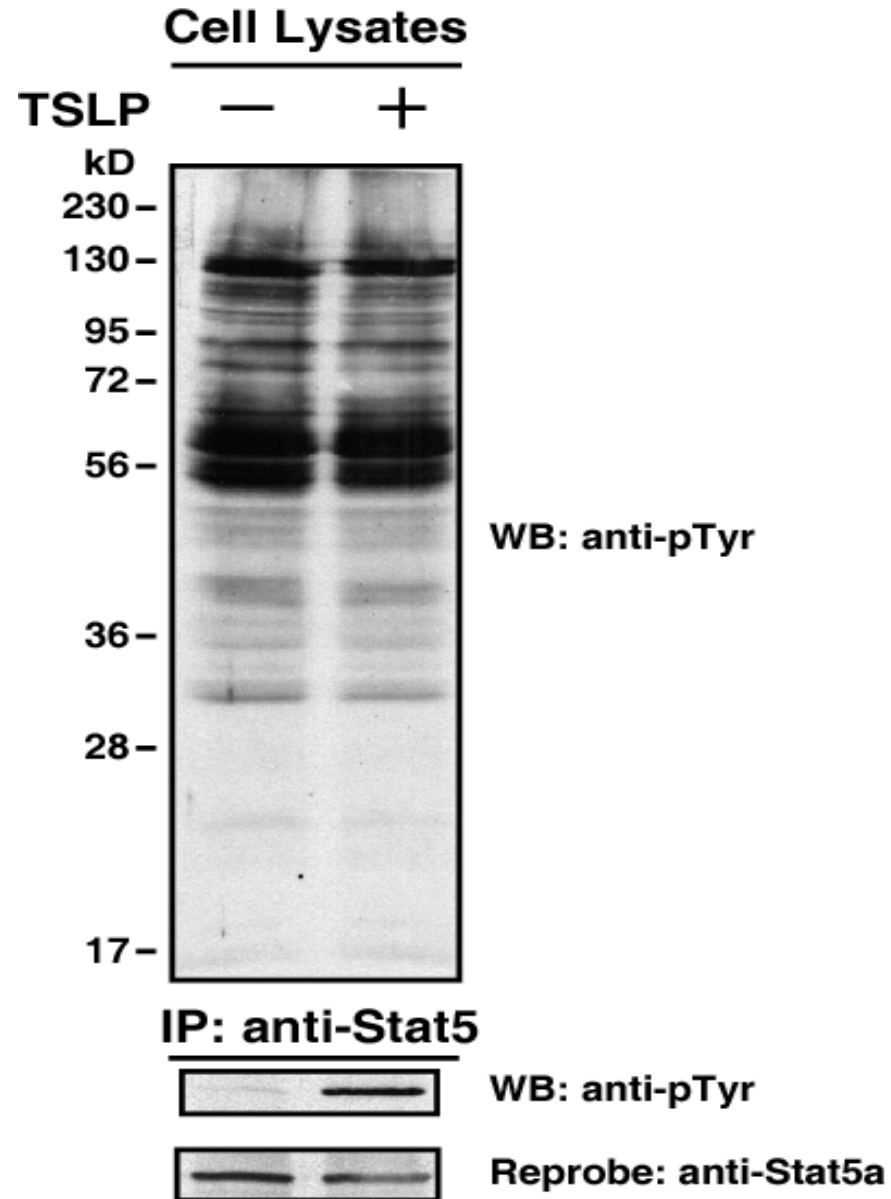
- Consists of two receptor subunits
 - Interleukin 7 receptor alpha chain (shared with another cytokine, IL-7)
 - A unique receptor designated TSLPR



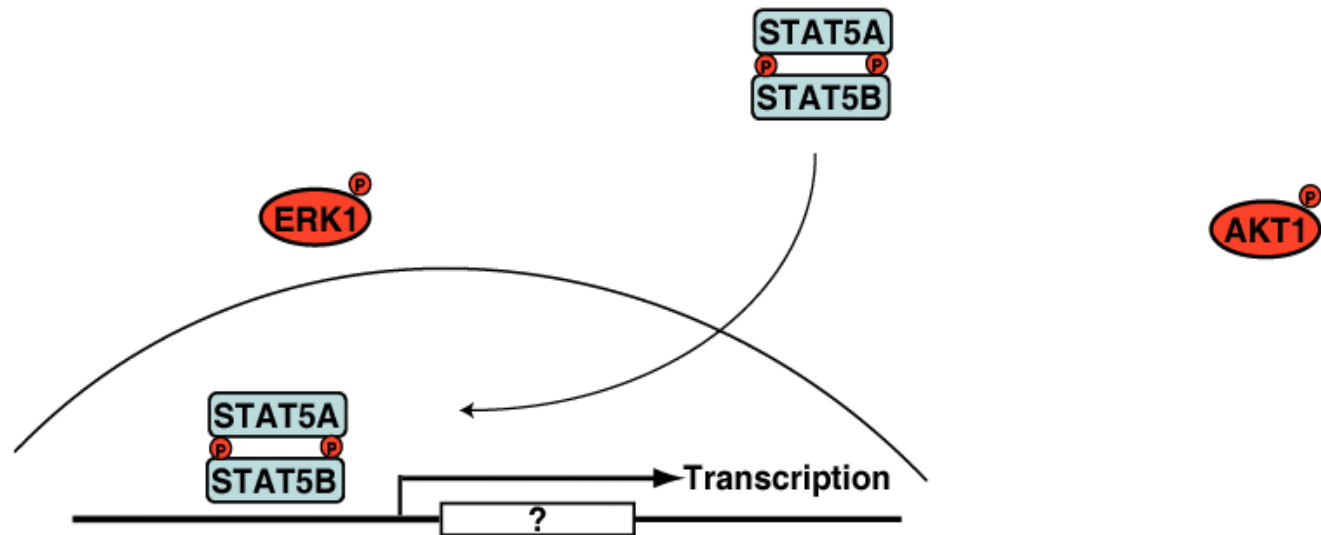
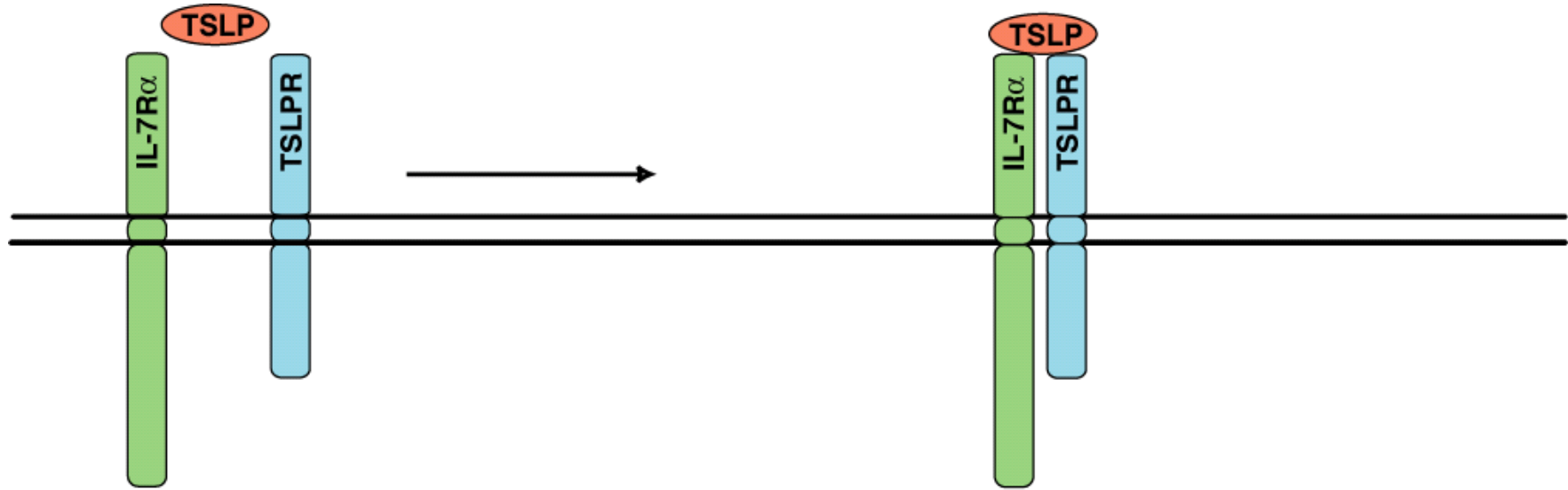
TSLP Signaling

- JAK family kinases shown not to be activated based on Western blotting data (lack of increased tyrosine phosphorylation in phosphotyrosine Western blots)
- Scientists in the field trying to identify the 'elusive' kinase(s)

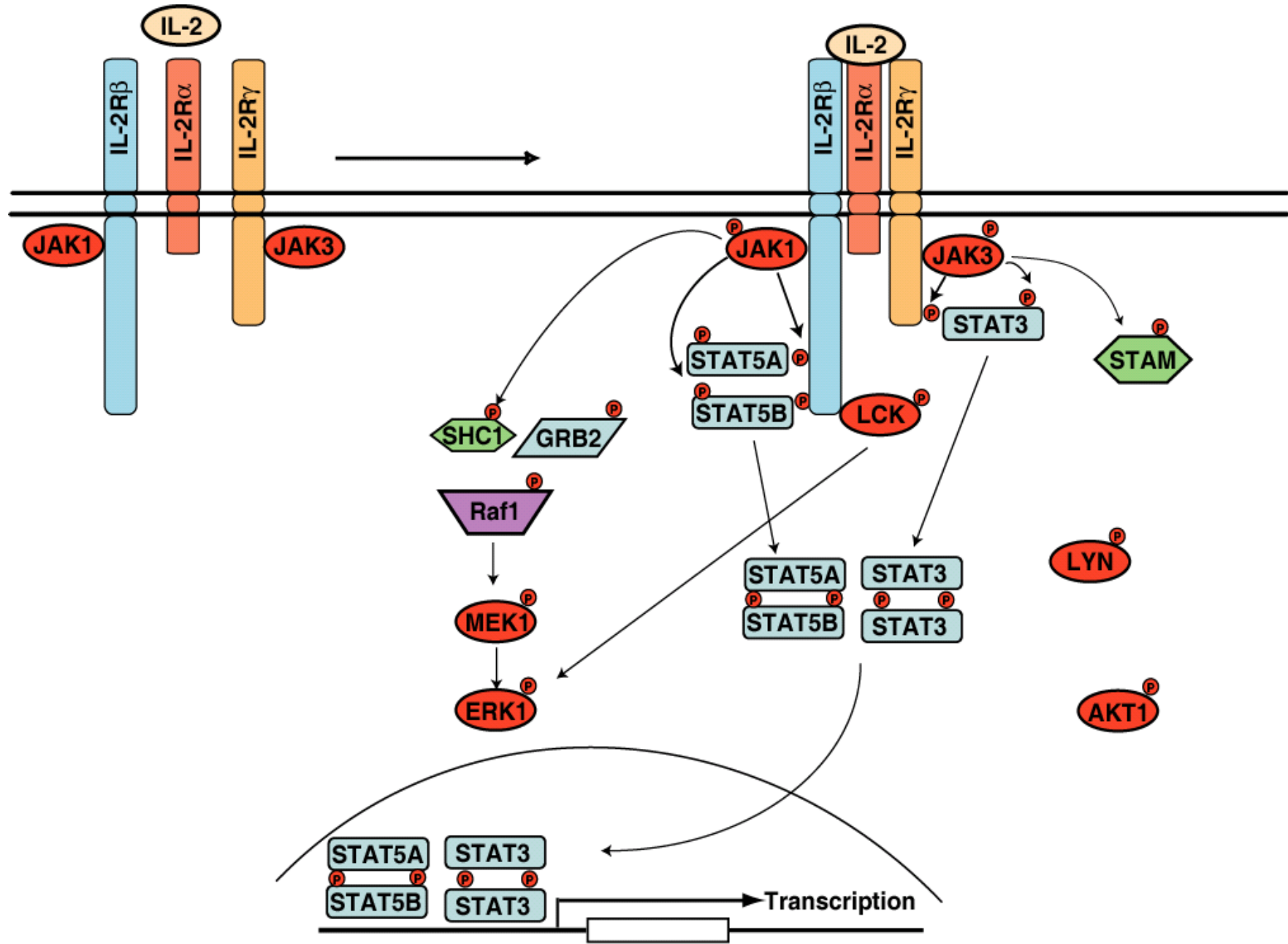
TSLP signaling – a Western blot perspective



TSLP Signaling



IL-2 Signaling: Several Decades of Studies



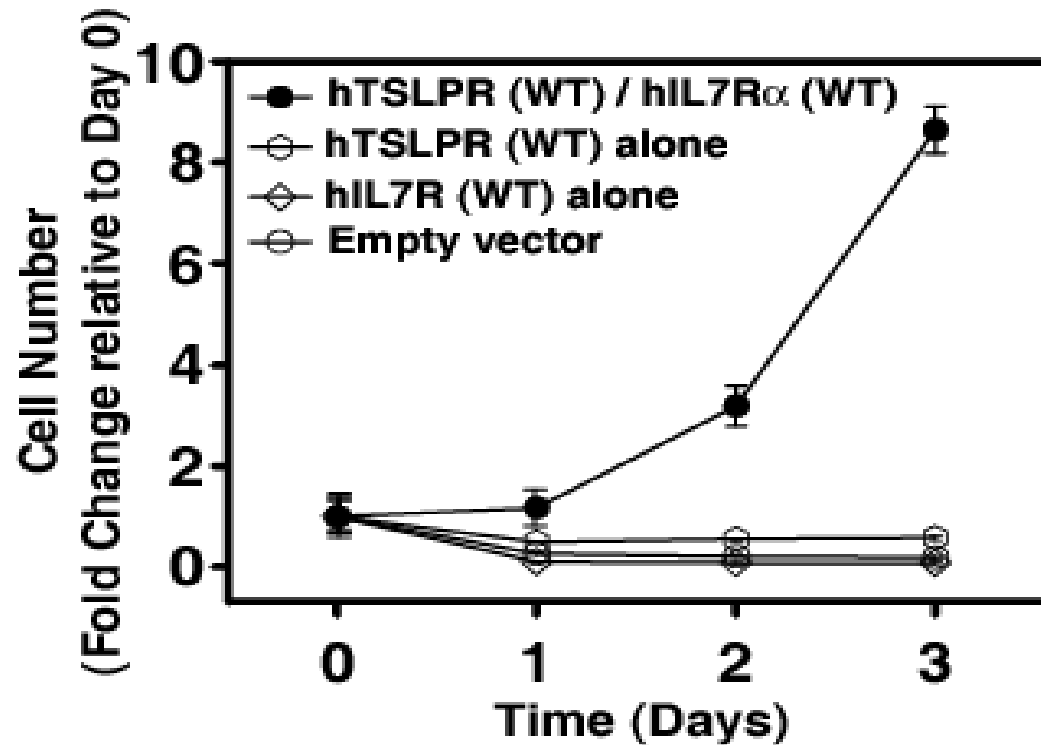
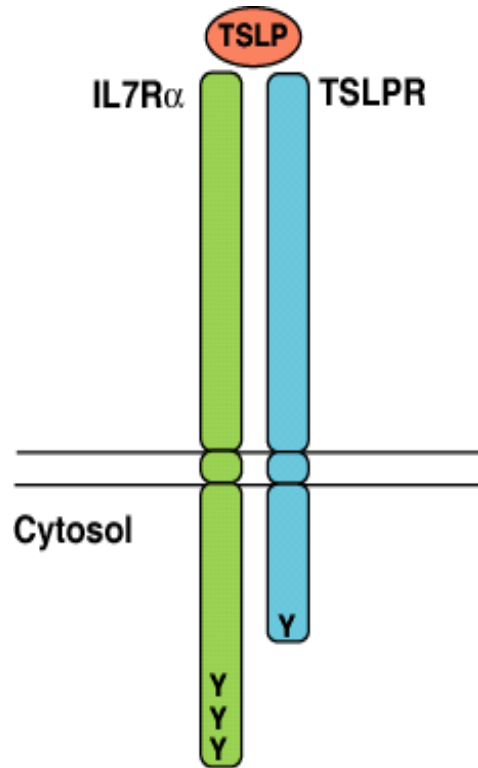
How do we start to map the detailed circuitry in signaling pathways?

- Experimentally identify components of signaling pathways
- Determine the ordering of the components by biochemical experiments
- Determine the interactions among the components
- Mapping of enzymes and their substrates in signaling is a key step

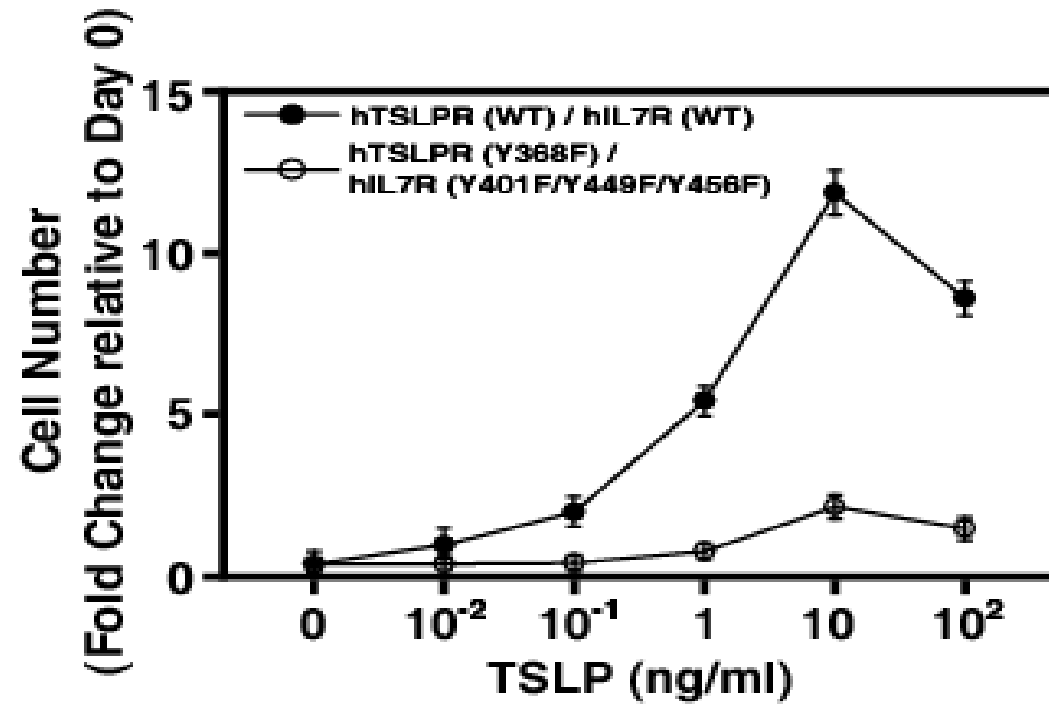
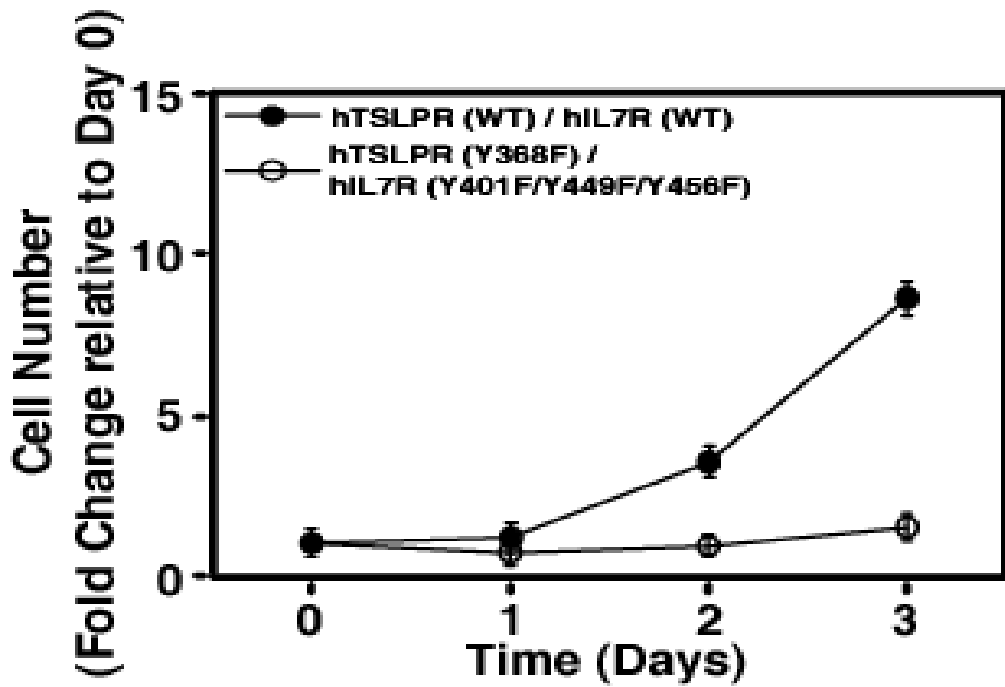
Profiling of activated kinases: Identifying direct kinase substrates is difficult

- Kinase-substrate interactions are transient
- Stoichiometry of phosphorylation is low
- Analytical methods to identify phosphorylation sites are difficult and not comprehensive
- Establishing a protein as a direct substrate of a kinase is not trivial

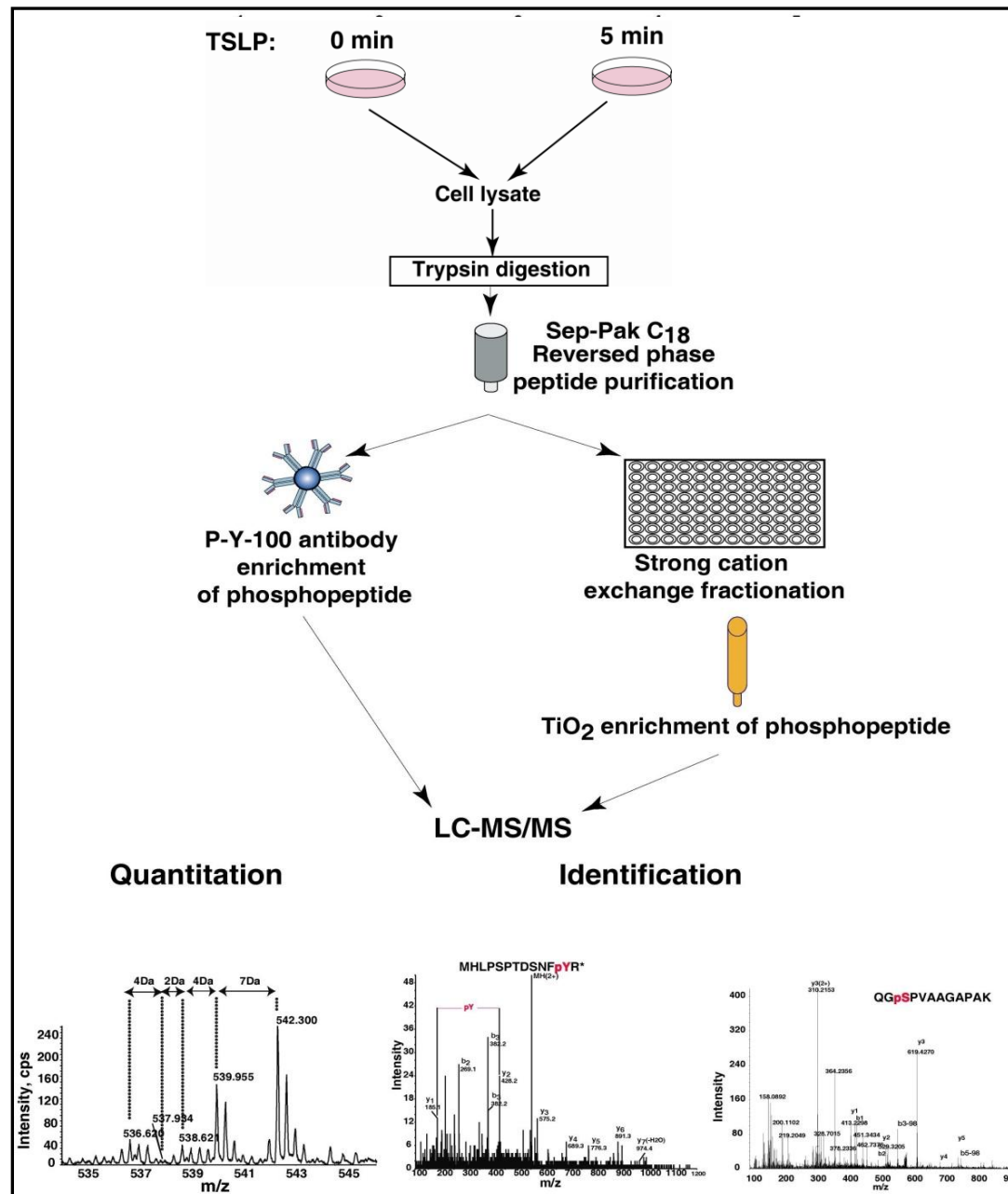
TSLP requires IL-7R and TSLPR to transmit signals



TSLP requires at least one cytoplasmic tyrosine residue to transmit proliferative signals



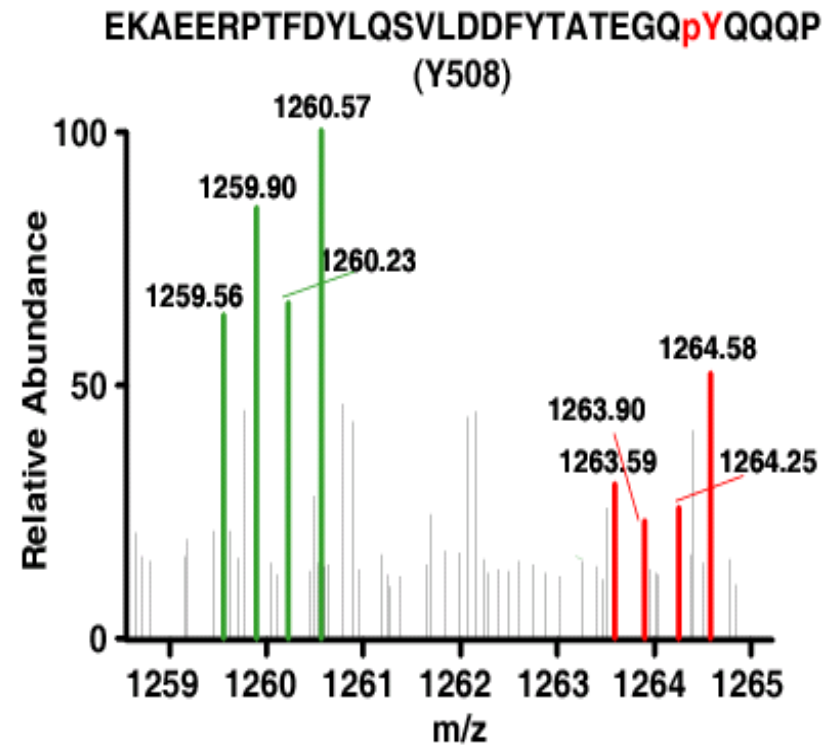
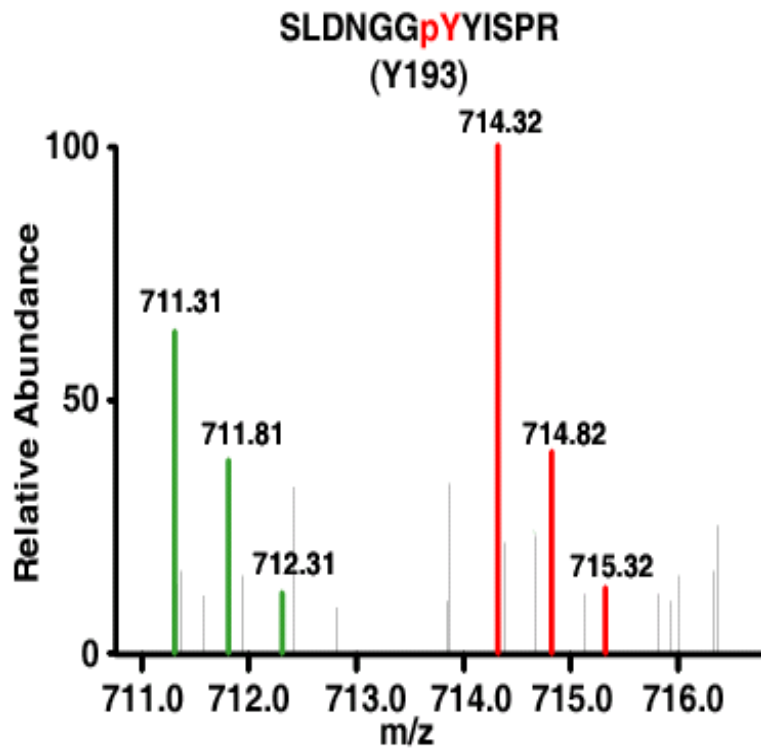
SILAC-based pTyr and pSer/pThr proteome profiling



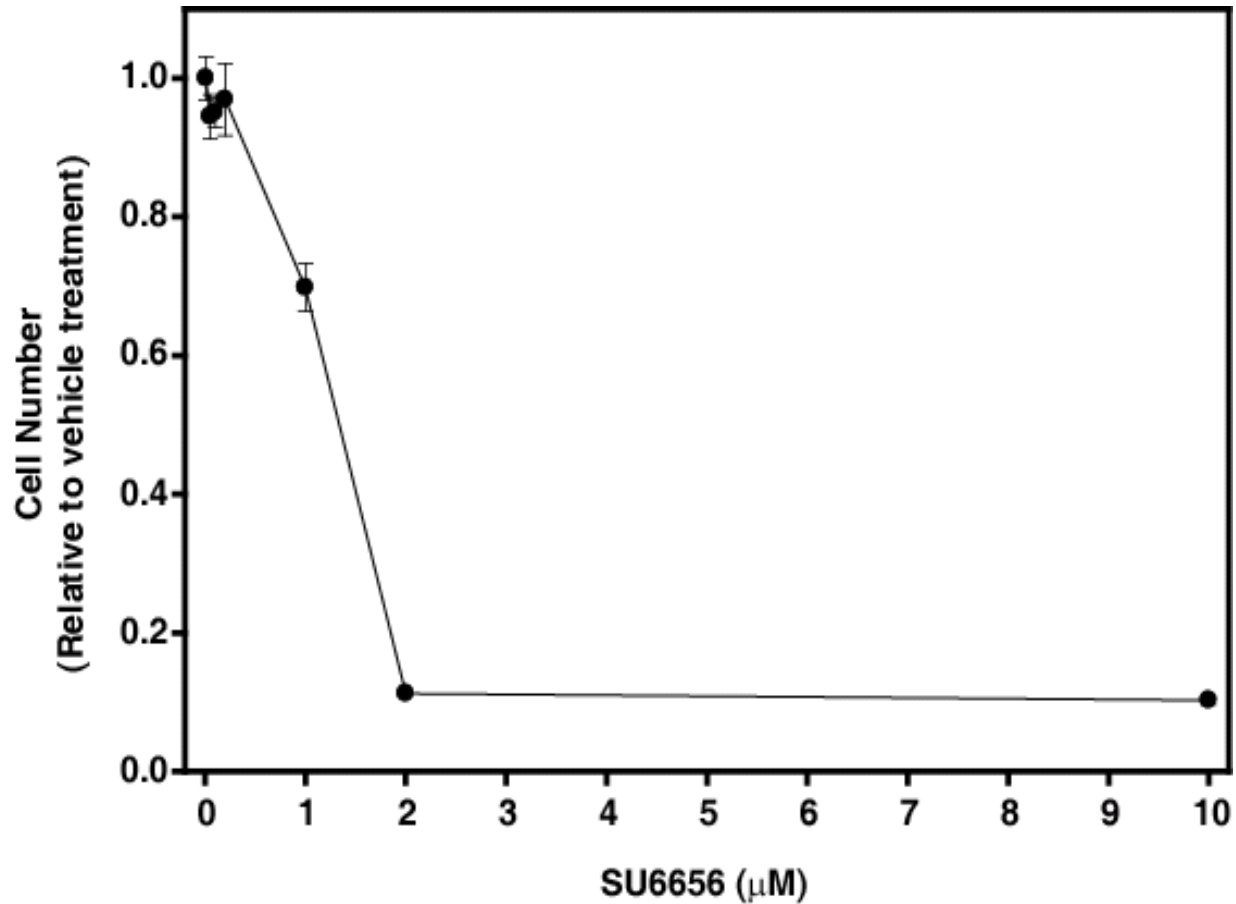
TSLP induces tyrosine phosphorylation of signaling molecules

Protein	Phosphopeptides with sites	Fold upregulation
STAT5A	AKAVDGpYVKPQIK	3.2
STAT5B	AKAADGpYVKPQIK	10.0
TEC	YVLDDQpYTSSSGA	1.4
BTK	HYVVCSTPQSQpYYLAEK	1.4
SHIP1	LpYDFVKTERDESSGMK	2.0
JAK2	EVGDpYGQLHKTEVLLK	1.4
	REVGDPYGQLHK	1.8
FcR γ	SQETpYETLK	1.8
FYB	TTAVEIDpYDSLK	2.0
SHP2	IQNTGDpYYDLYGGEK	1.8
LYN	EKAEERPTFDYLQSVLDDFYTATEGQpYQQQP	0.5
	SLDNGGpYYISPR	1.5
ERK1	IADPEHDHTGFLTEpYVATR	1.3
CDK3	VEKIGEGTpYGVVpYK	1.0
GSK3A	GEPNVSpYICSR	1.0

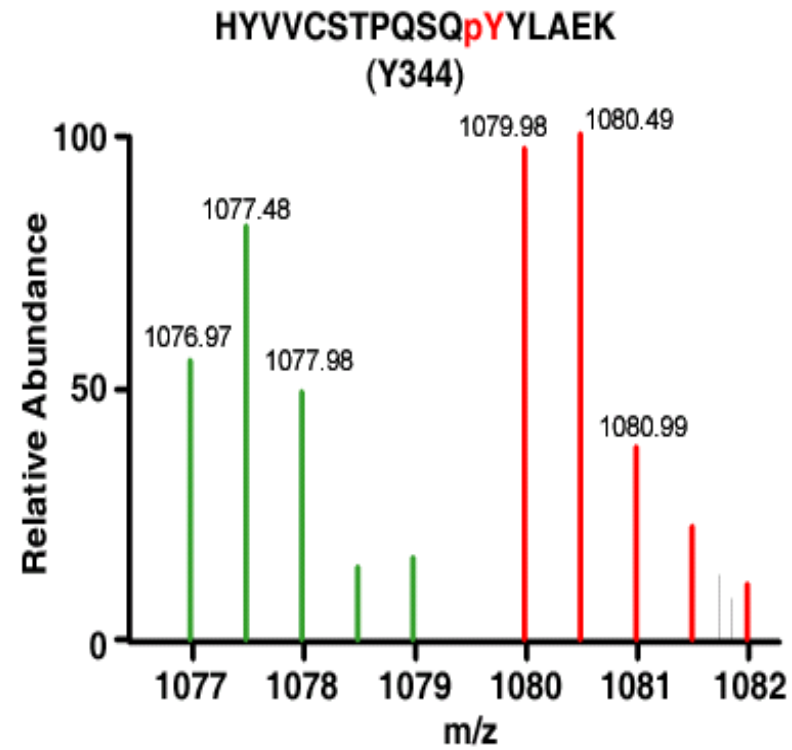
Phosphorylation changes in Lyn reflect activation



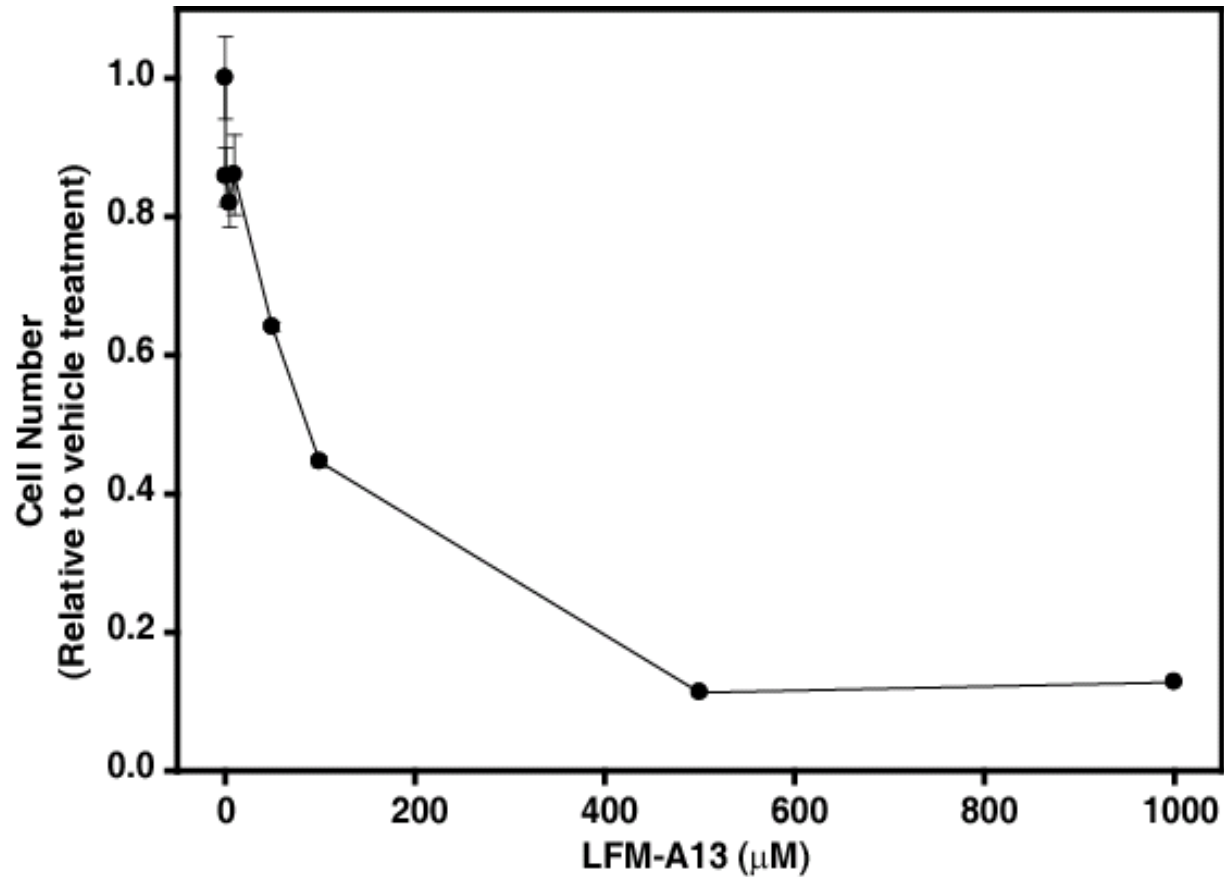
Src family kinase inhibitor, SU6656, inhibits TSLP-induced cell proliferation



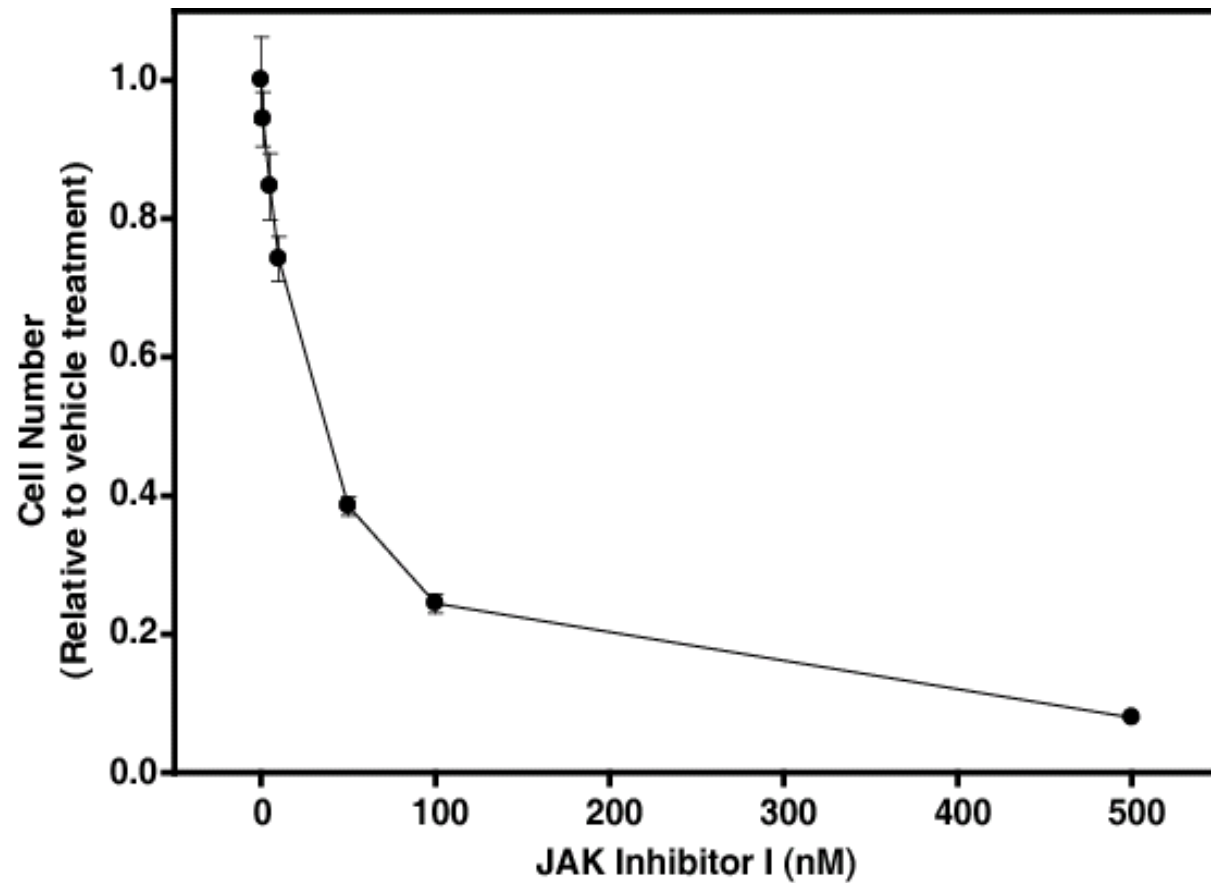
Bruton's tyrosine kinase



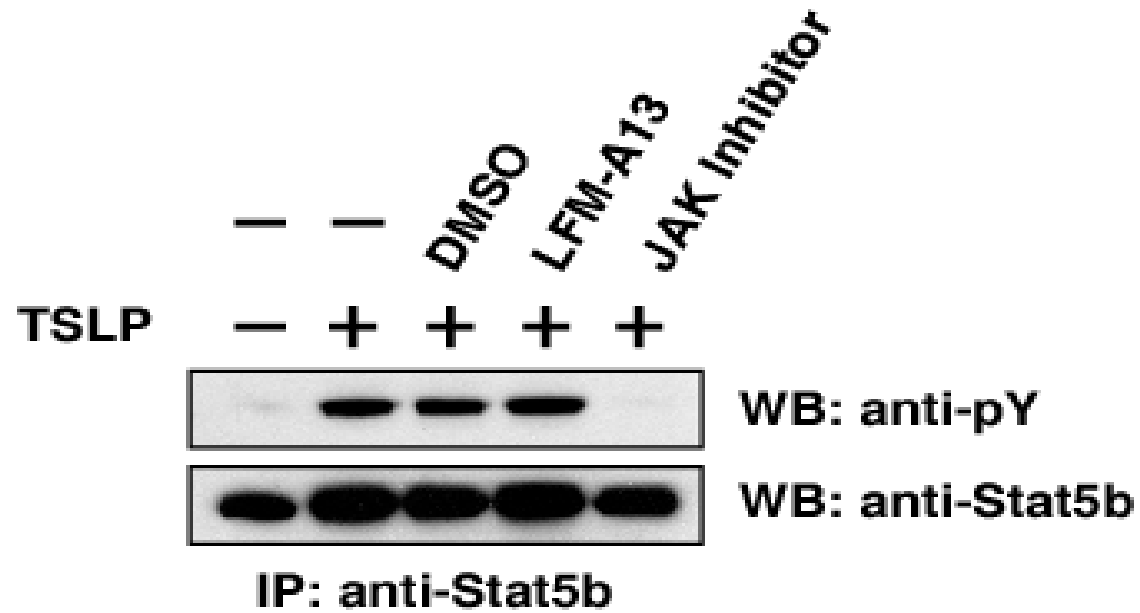
BTK kinase inhibitor, LFM-A13, inhibits TSLP-mediated proliferation



JAK2 inhibitor inhibits TSLP-mediated proliferation



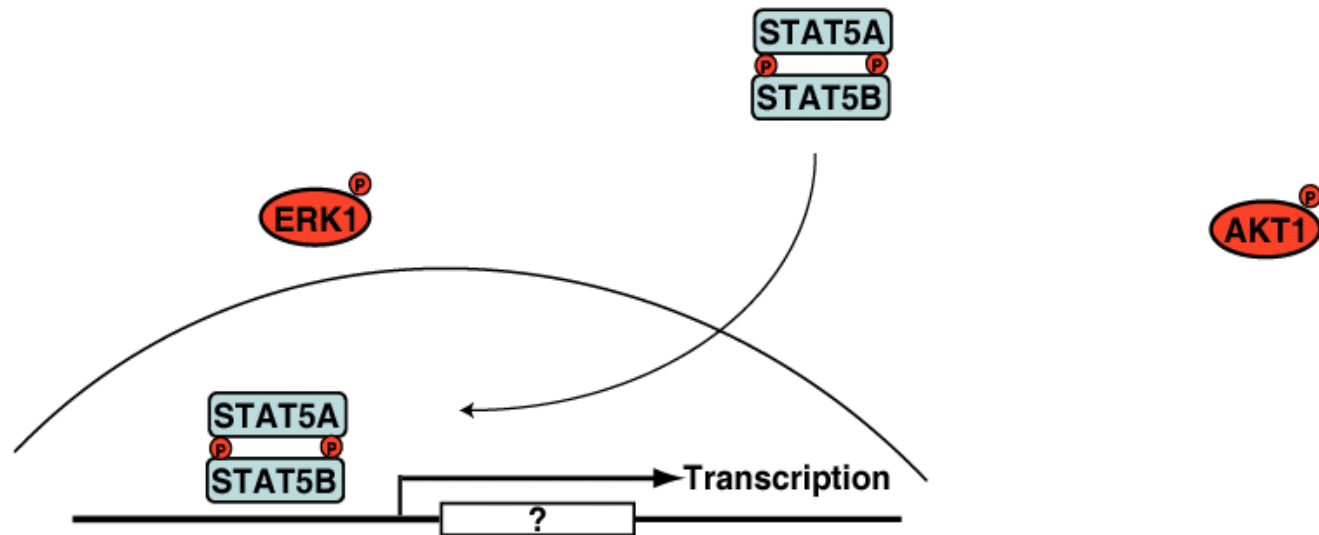
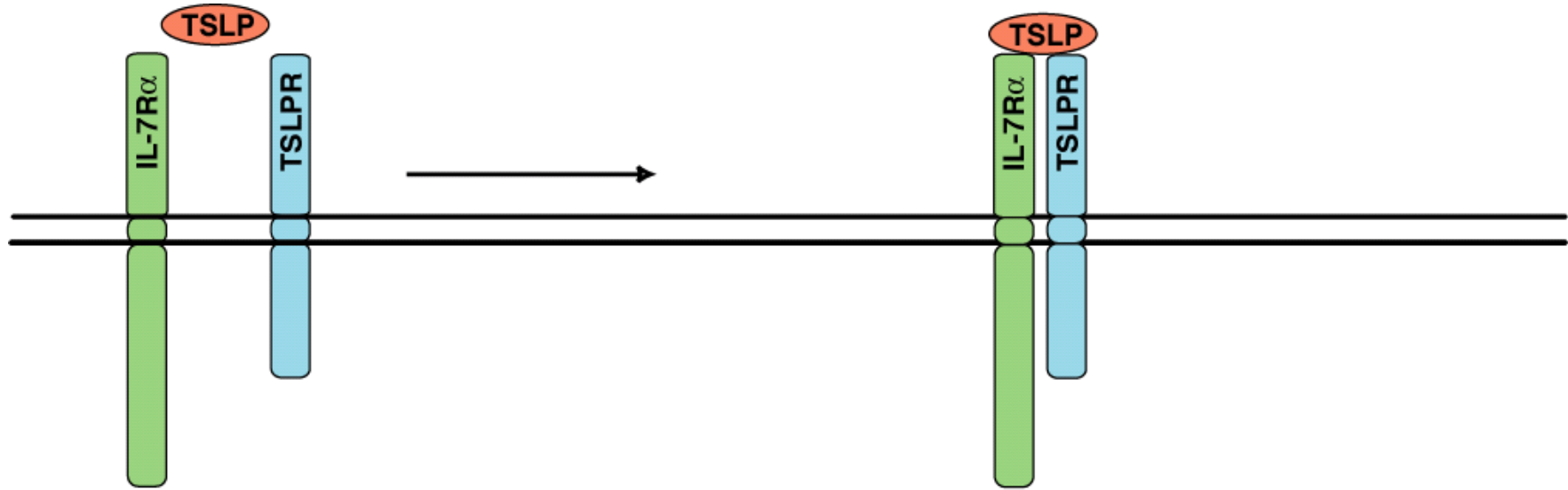
JAK2 inhibitor inhibits TSLP-induced Stat5 phosphorylation



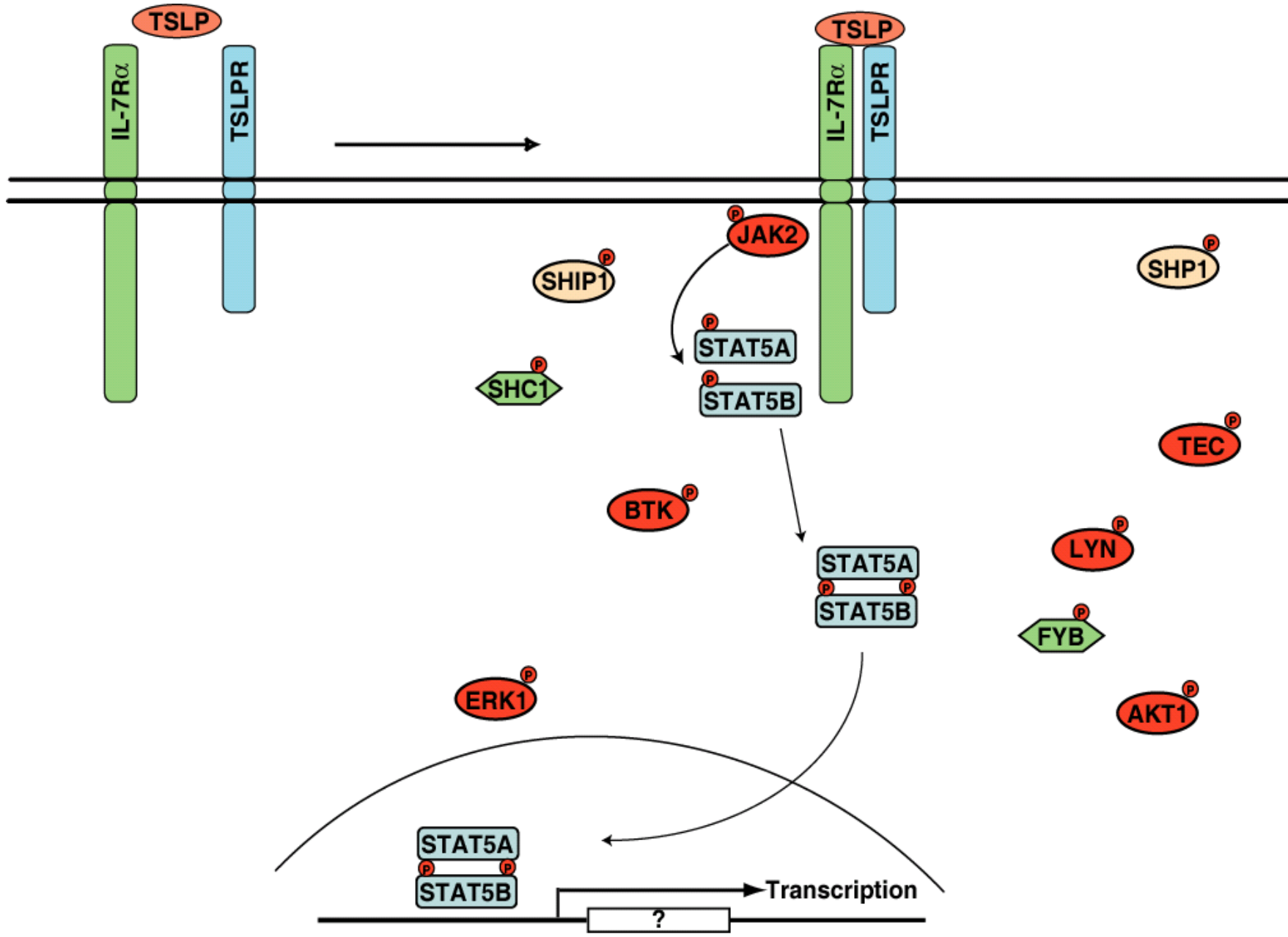
A partial list of serine/threonine phosphorylated peptides identified from SCX fractionation experiments

Protein Name	Phosphopeptides	Fold upregulation with TSLP treatment
Pumilio homolog 2	pTPGSRQA <p>S</p> PTEVVER	4.3
Programmed cell death protein 5	KVMD <p>S</p> DEDDADY	10.0
Protein transport protein Sec61 subunit beta	PGPTPSGTNVG <p>S</p> SGR <p>S</p> PSK	6.6
60S ribosomal protein L8	GAG <p>S</p> VFR	4.5
Plectin1	SS <p>S</p> VGSSSSYPISSAGPR	5.5
Heterogeneous nuclear ribonucleoprotein A3	SSGSPYGGGYG <p>S</p> GGGSGGYGSR	5.2
Programmed cell death protein 4	FV <p>S</p> EGDGGR	3.8
Rho guanine nucleotide exchange factor 1	<p>S</p> ESLRV <p>S</p> DR	6.7
FcR γ	SQE <p>T</p> YETLK	2.5
Nucleolar RNA helicase 2	SNSSDAPGEE <p>S</p> Sp <p>S</p> ETEKEIPVEQK	1.0
Eukaryotic translation initiation factor 4E transporter	SS <p>S</p> PVGLAK	1.0

TSLP Signaling: BEFORE



TSLP Signaling: AFTER



Lessons Learnt

- Exercise caution in interpreting negative data – ‘not detectable’ is not the same as ‘not occurring in cells’
- It is possible to take unbiased discovery approaches to dissect signaling pathways in a global fashion using quantitative proteomics
- Activated kinases identified using this proteomic approach are candidate therapeutic targets in asthma and leukemias

NetPath

NetPath

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- [Alpha6 Beta4 Integrin](#)
- [ID](#)
- [Hedgehog](#)
- [Notch](#)
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- [AR](#)
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News

[PhosphoMotif Finder, published in *Nature Biotechnology*](#)
[Comparison of Protein-Protein Interaction Databases, published in *BMC Bioinformatics*](#)



Highlights

[PhosphoMotif Finder](#)

Allows you to check if your protein contains any phosphorylation motif described in the literature

[Pathways](#)

A set of 20 curated signaling pathways are available as part of a new pathway resource that we have developed called 'NetPath.'

[HPRD Release 7](#) ^{New}

The latest Release 7 is available for download. [Click here ...](#)

Statistics

Protein Entries	25,661
Protein-Protein Interactions	38,167
Domains	455
PTMs	16,972
PubMed Links	270,466

Vimentin – tissue expression from *Human Proteinpedia*

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Vimentin

Molecular Class	Cytoskeletal protein
Molecular Function	Structural constituent of cytoskeleton
Biological Process	Cell growth and/or maintenance



ALTERNATE NAMES DISEASES PTMs & SUBSTRATES

SUMMARY SEQUENCE INTERACTIONS EXTERNAL LINKS

General

Gene Symbol:	VIM	Molecular Weight (Da) :	53652	Gene Map Locus:	10p13
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Localization

Primary	Cytoplasm GO	Alternate	Extracellular GO Nucleolus GO
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Domains and Motifs

Domains	Motifs
CC 94 - 257	
CC 294 - 394	

Expression

Site of Expression

[Muscle](#)

[Ovary](#)

[T Cell](#)

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Human Proteinpedia

Site of Expression

Normal Tissue	Cancer Tissue	Cell Line
Adrenal gland	Breast cancer	HeLa
Appendix	Cervical cancer	
B Cell	Colo-rectal cancer	
Blood plasma	Endometrial cancer	
Bone marrow	Head & neck cancer	
Breast	Liver cancer	
Cerebral cortex	Lung cancer	

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