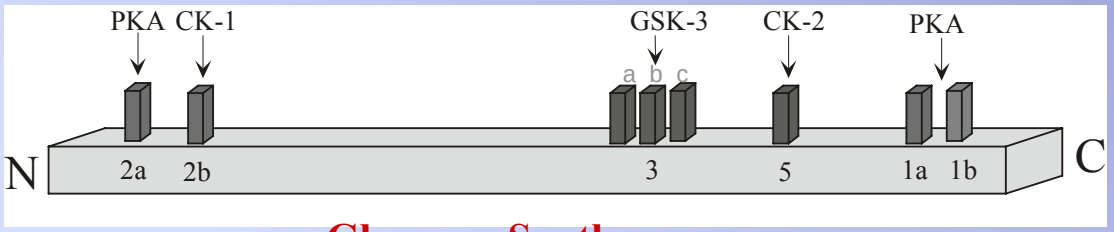
GSK-3- A Novel Drug Discovery Target For Diabetes

Prof. Hagit Eldar-Finkelman
Department of Human Molecular Genetics and Biochemistry
Sackler School of Medicine

October 2007

GSK-3 inhibits glycogen synthase



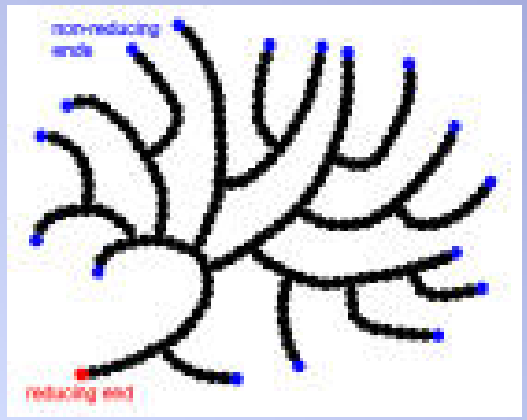
Glycogen Synthase

GSK-3

CK-2

RPASVPPSPSLSRHSSPHQ S(p)EDEE

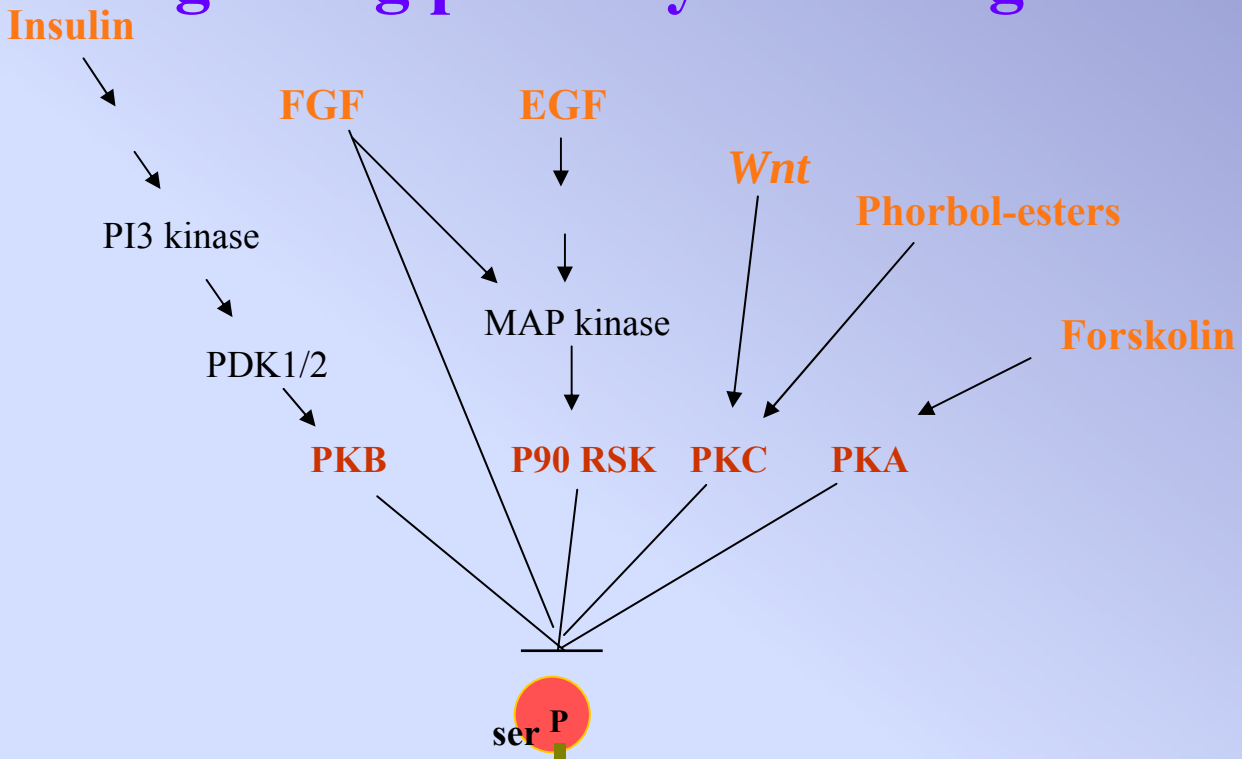
Inhibition of Glycogen Synthase



Block glycogen synthesis

GSK-3 was first discovered as a protein kinase that inhibits glycogen synthase and glycogen metabolism

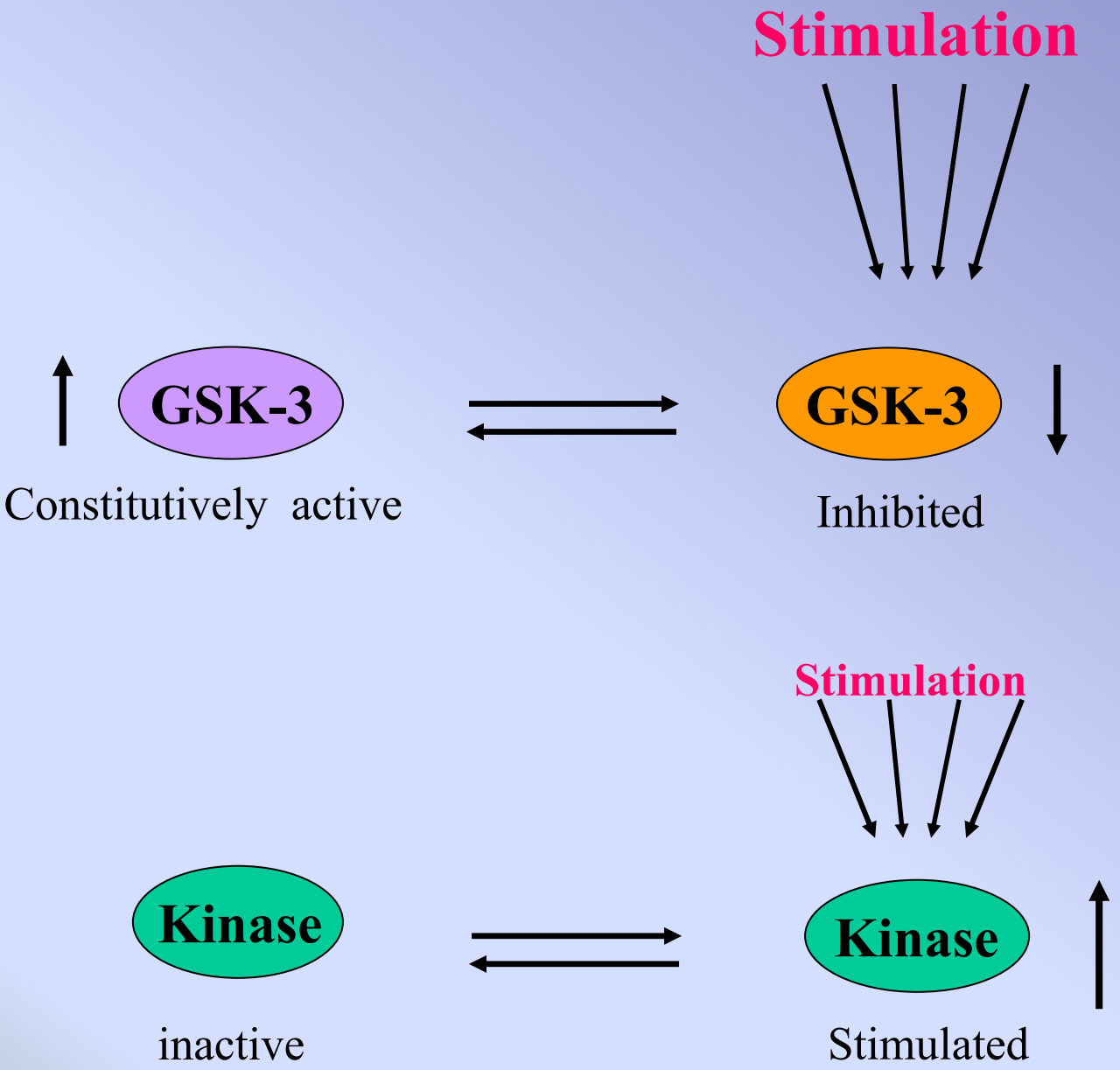
Signaling pathways Converge at GSK-3



GSK-3 (inhibited)

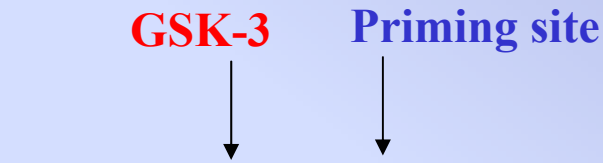
***glycogen synthesis**
***transcription factors**
***substrates dephosphorylation**

GSK-3 is inhibited by multiple pathways via serine phosphorylation



Unlike many protein kinases GSK-3 is constitutively active and undergoes inhibition by external stimulation. This suggests that GSK-3 is a negative regulator of signaling pathways

GSK-3 Recognition Motif



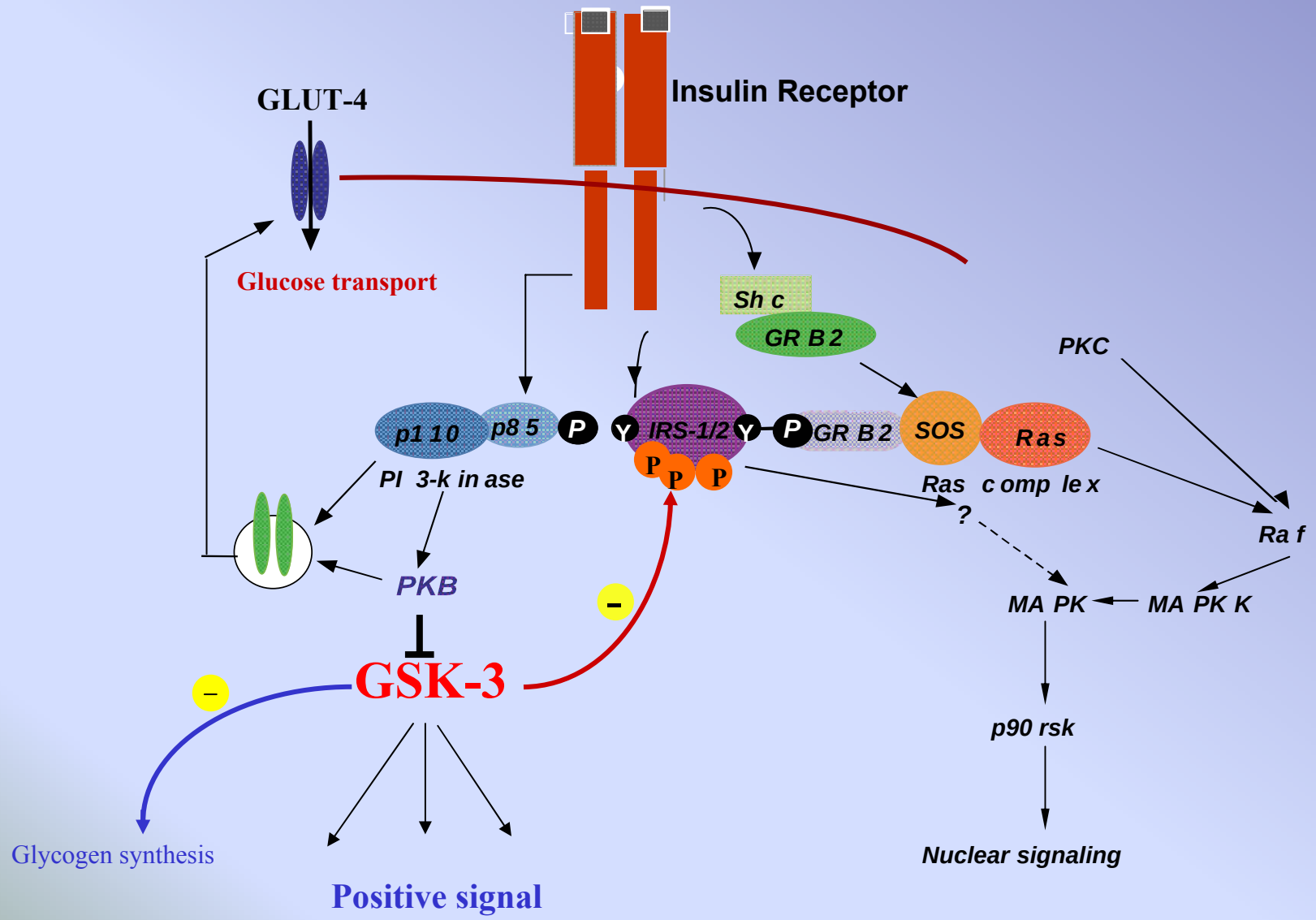
KRREILSRRPS(p)YR	CREB peptide
RPASVPPSPSLSRHSSPHQ S(p)EDEE	Glycogen Synthase peptide
SYLDSGIHSGATTTAPSLGKG	β-Catenin
KEEPPSPPQS(p)PRV	Heat shock factor-1

Motif: SXXXS(P)

GSK-3 has a unique recognition motif on its substrates. That is GSK-3 requires pre-phosphorylation of its substrates

GSK-3 in Type 2 Diabetes

GSK-3 is a negative regulator in insulin signaling

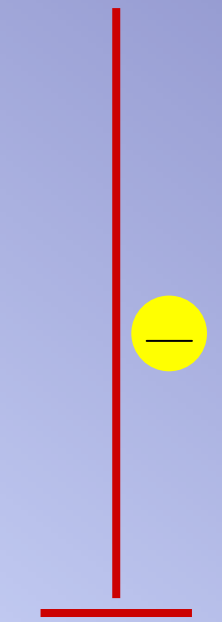


GSK-3 inhibits insulin signaling via serine phosphorylation of IRS proteins

GSK-3 in Diabetes

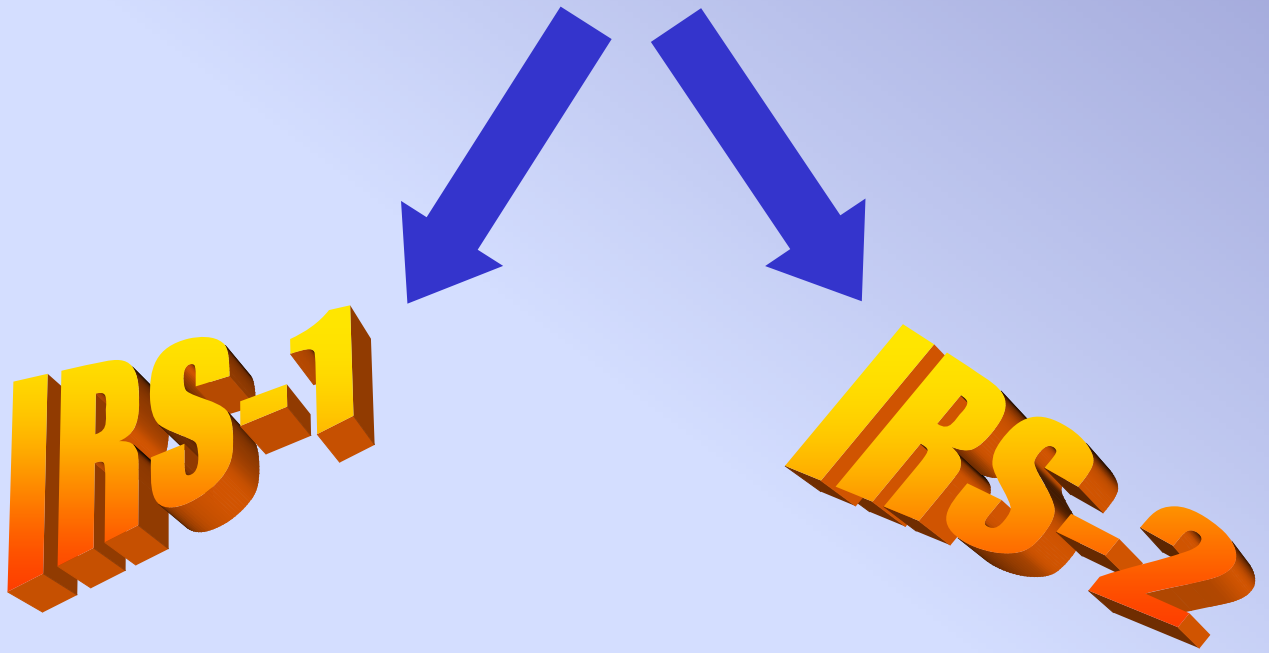
Additional evidence for GSK-3 target important in diabetes

1. Expression of GSK-3 inhibits insulin signaling
2. GSK-3 activity is elevated in diabetes
3. GSK-3 transgenic animals develops diabetes
4. GSK-3 affects key targets: IRS-1, IRS-2, glycogen synthase, gluconeogenic enzymes.
5. Treatment with GSK-3 inhibitors has anti-diabetic effects
6. Recent work also implicates GSK-3 in type 1 diabetes



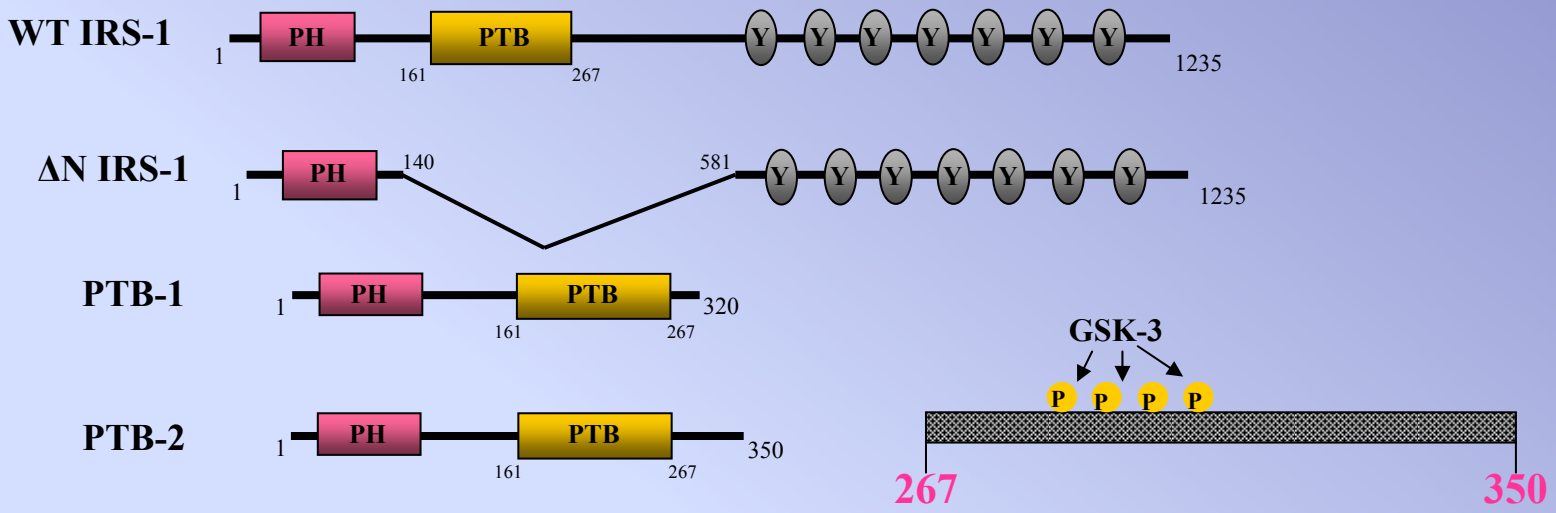
This suggests that inhibition of GSK-3 may be of therapeutic benefits in treating diabetes

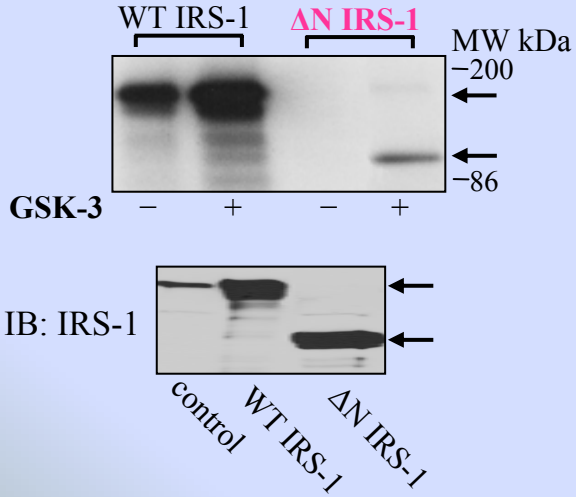
GSK-3

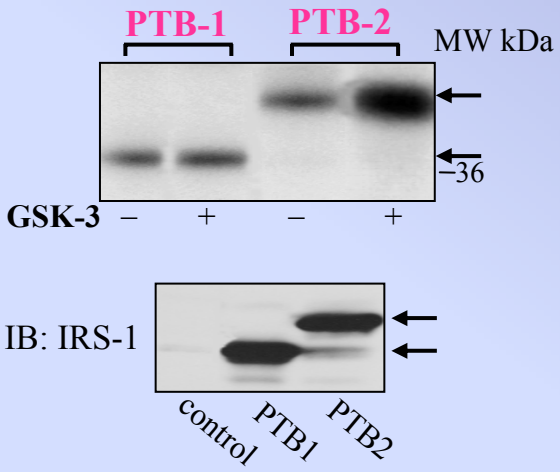


We focus on GSK-3 phosphorylation of IRS-1 and IRS-2

Mapping IRS-1 Phosphorylation sites

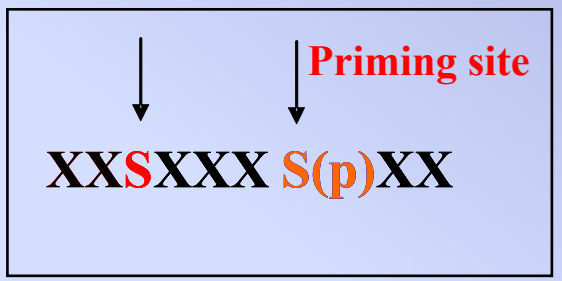


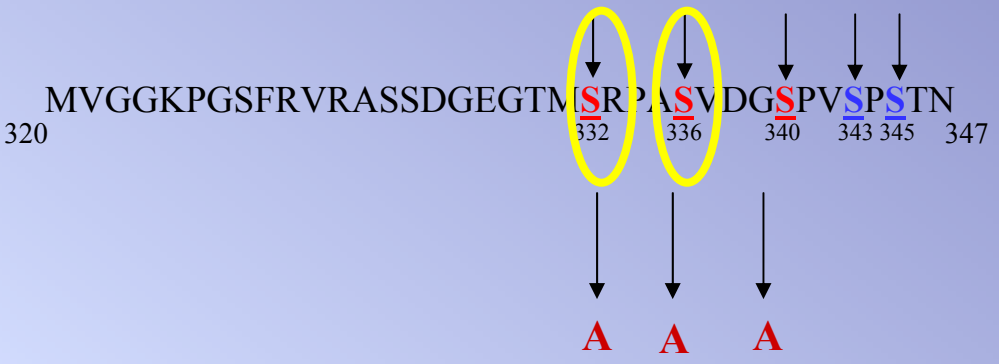


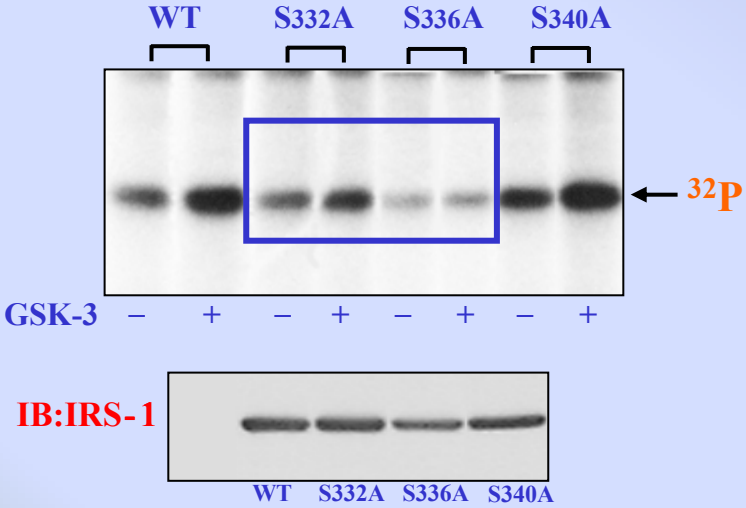


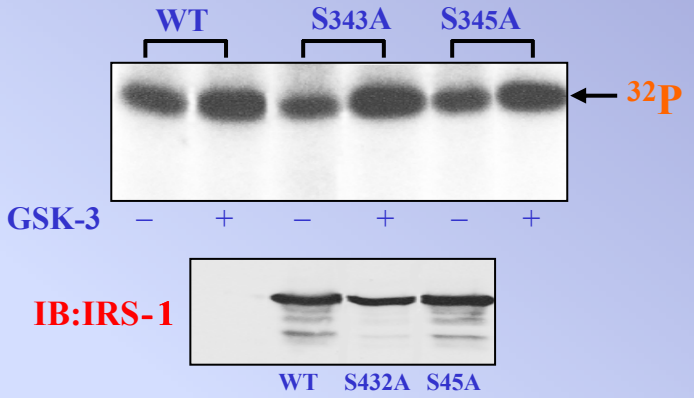
Molecular biology approach enabled us to localize GSK-3-IRS-1 phosphorylation sites in segment 267-350

Recognition motif



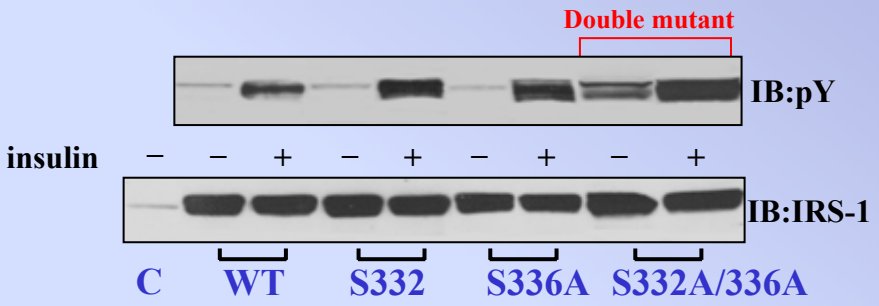


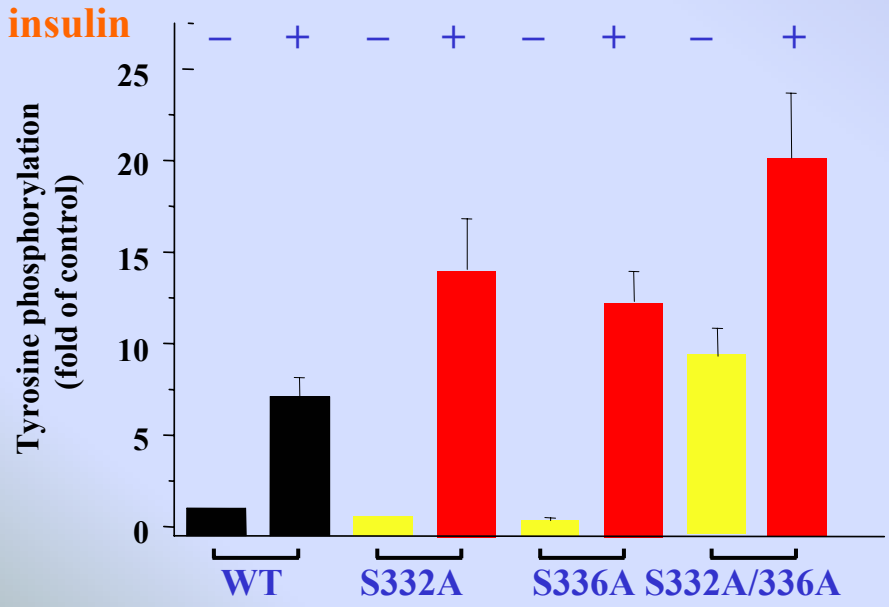


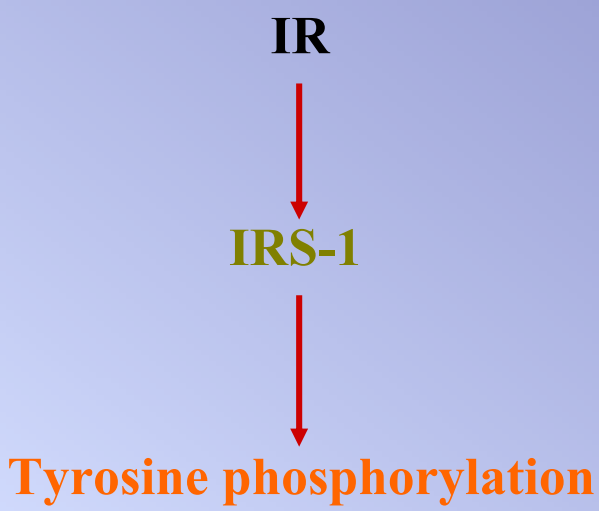


Mutation of potential sites that follows GSK-3 motif: **SXXXS** confirmed that serine 332 and 336 are the relevant GSK-3 phosphorylation sites (332 as the direct site and 336 as the priming site)

Mutation at serines 332 and 336 enhance insulin signaling

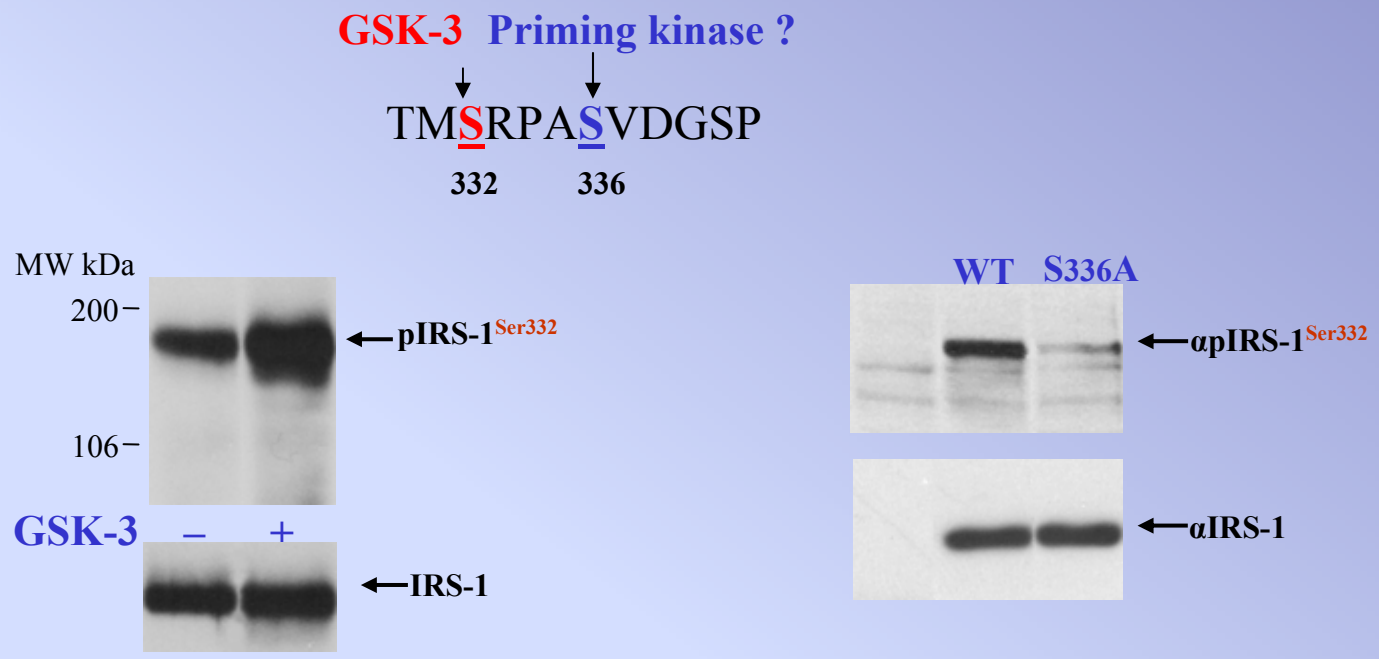






Phosphorylation of IRS-1 at GSK-3-phosphorylation sites inhibits insulin signaling
(reduced insulin-induced tyrosine phosphorylation of IRS-1)

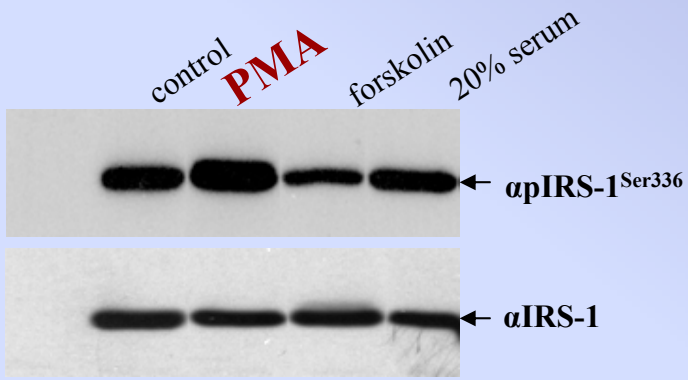
Serine 336 is the Priming Site



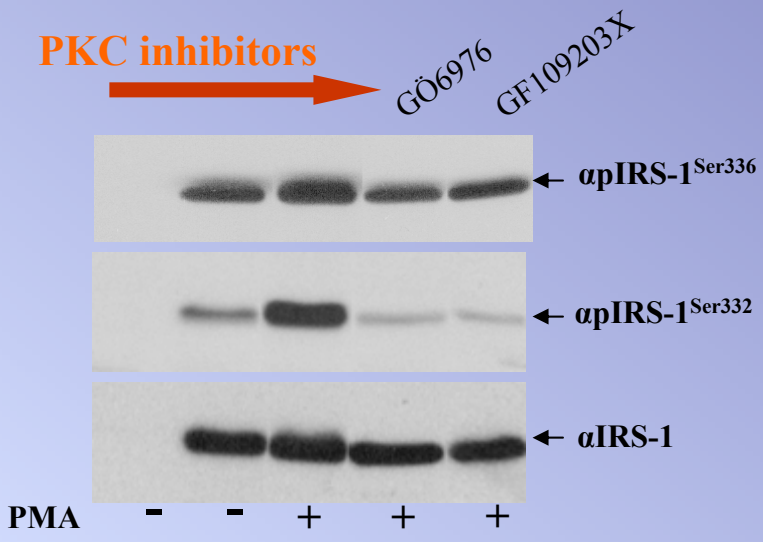
We generated specific anti-phospho IRS-1 antibody the recognize IRS-1 phosphorylation at serine 332 or 336. Left panel shows that GSK-3 phosphorylates IRS-1 at serine 332. Right panel shows that mutation at serine336 prevents IRS-1 phosphorylation at serine 332 meaning that serine 336 is the priming site.

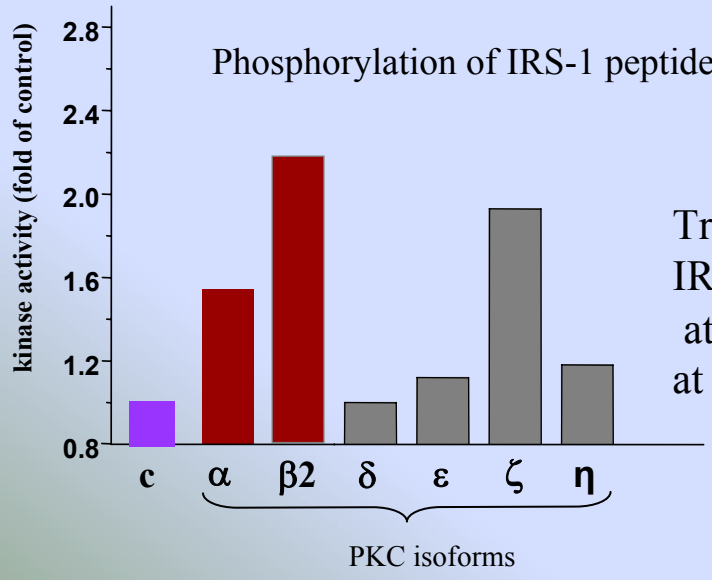
PKC is the priming kinase

TMSRPASVDGSP



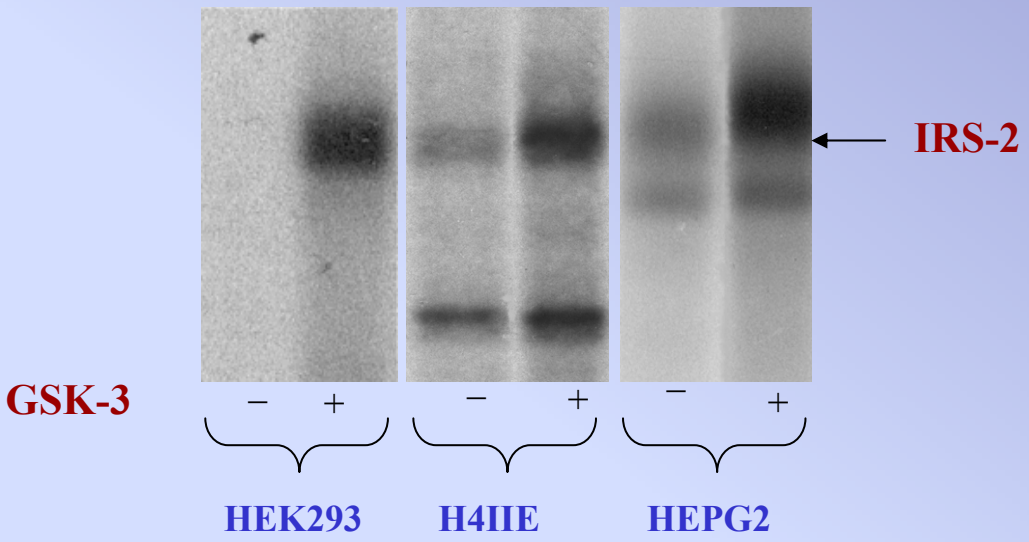
PKC → IRS-1^{336} → IRS-1^{332}





Treatment with PKC activator phorbol-estres (PMA) enhances IRS-1 phosphorylation at serine 336 and primes for phosphorylation at serine 332.I n addition PKC α and β can phosphorylate IRS-1 at serine 336. This suggest that PKC is the priming kinase.

GSK-3 phosphorylates IRS-2

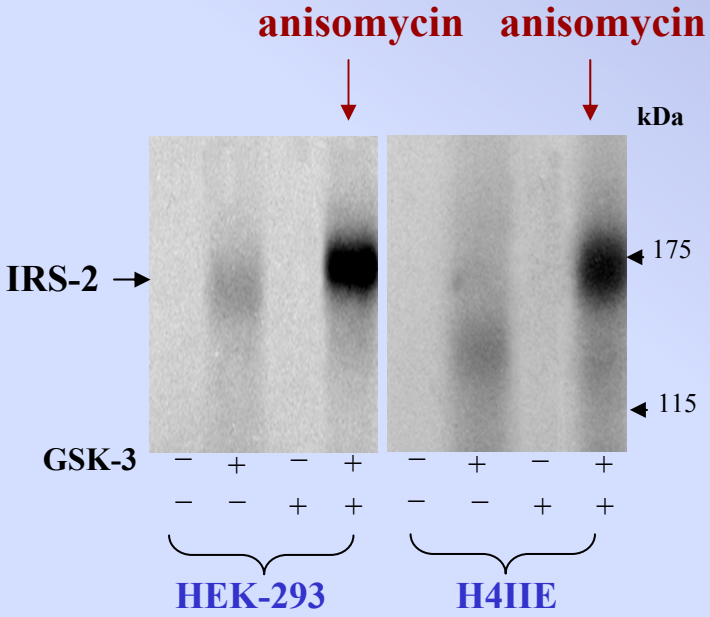


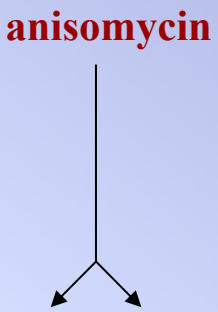
Priming kinase (?)

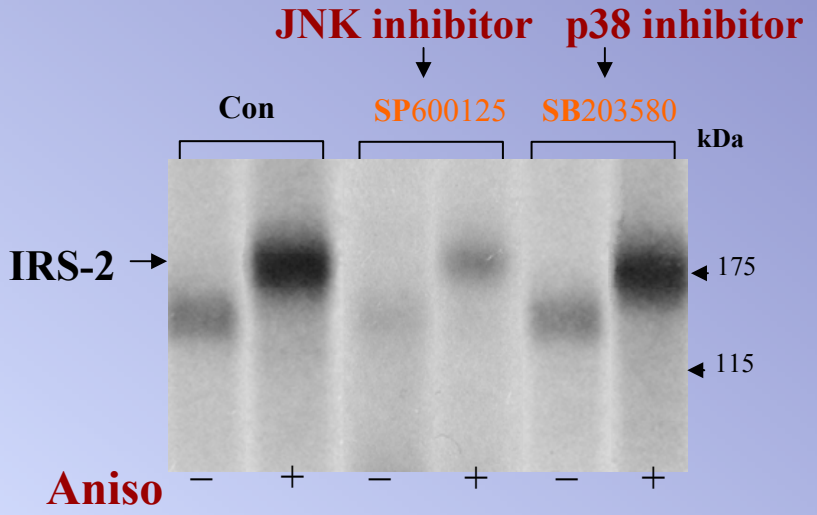
SXXXS(p)

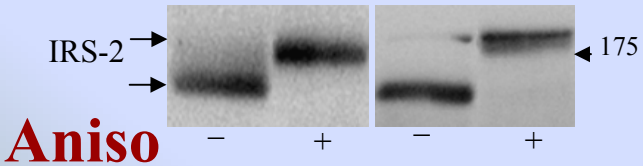
Studies in mouse rat and human IRS-2suggest that GSK-3 phosphorylates IRS-2 at conserved site

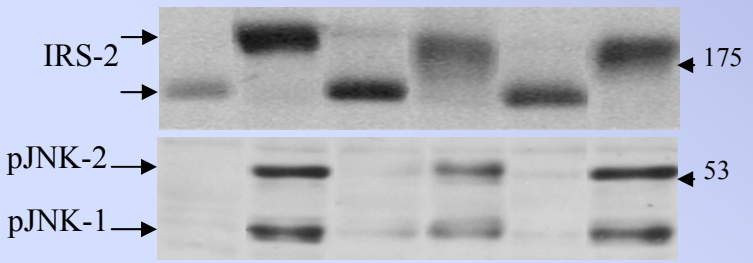
JNK Primes IRS-2 for GSK-3





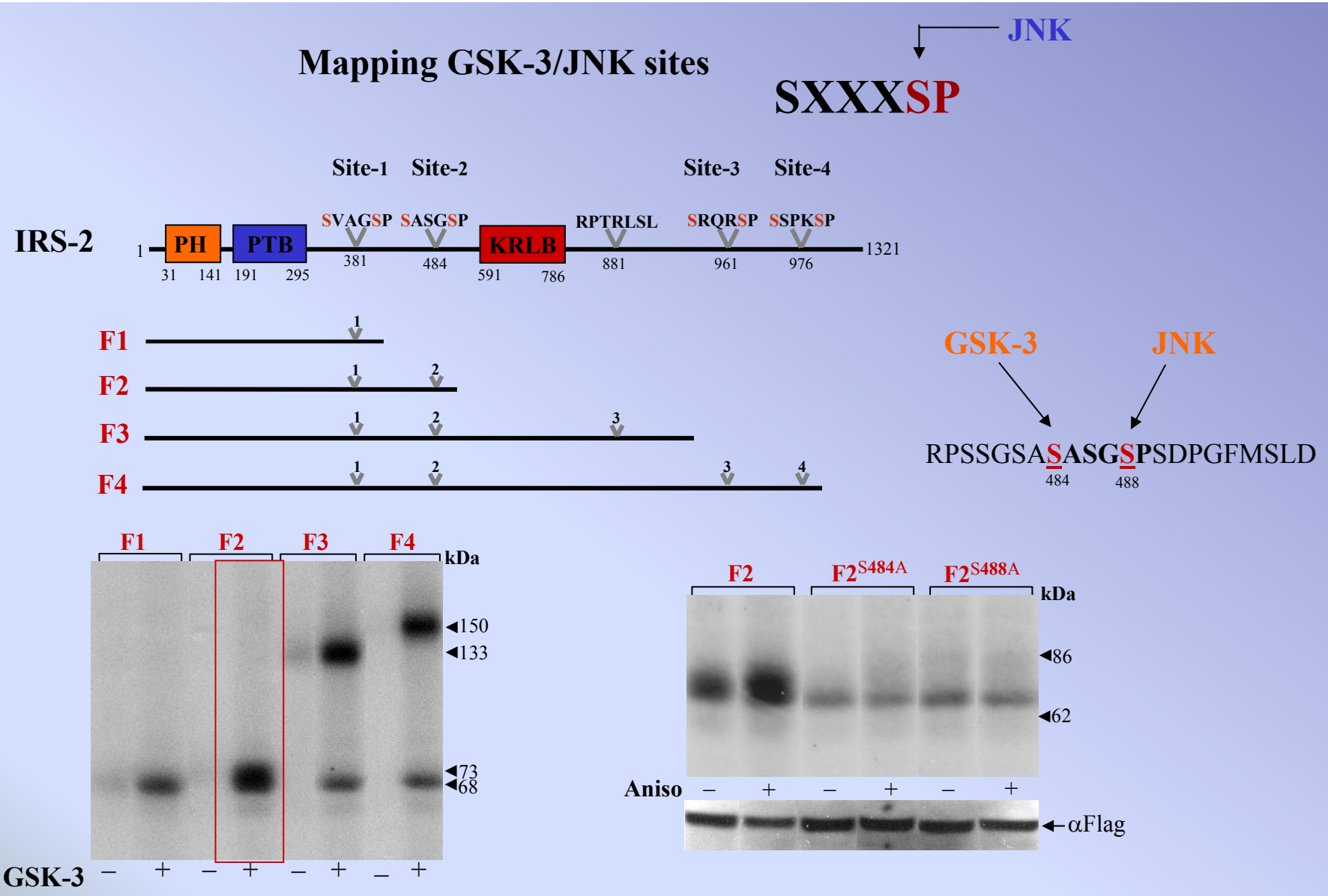






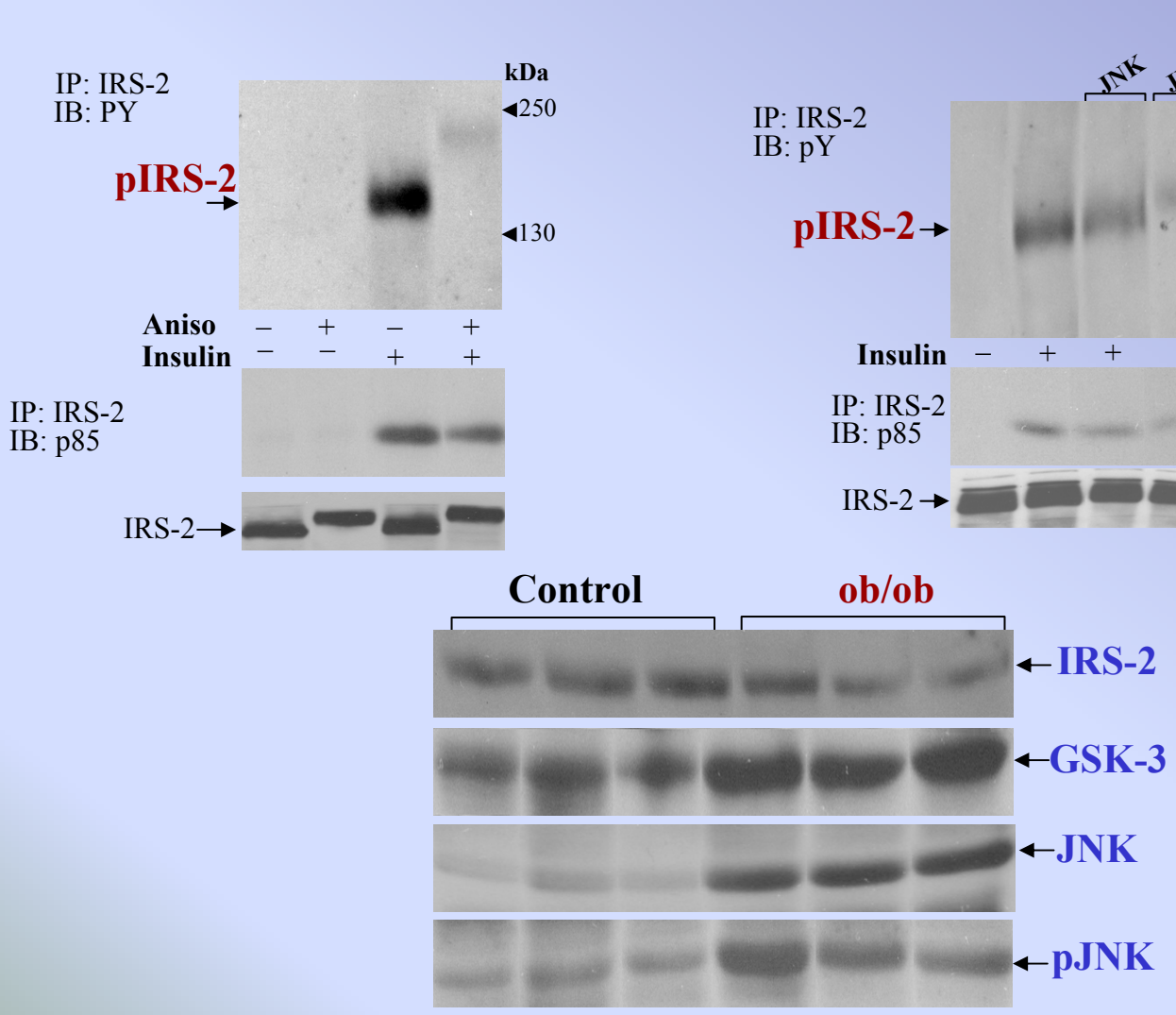
Our strategy was to identify the priming kinase first. We show that the stress activator anisomycin enhances greatly the ability of GSK-3 to phosphorylates IRS-2. Furthermore it is shown that JNK and not p38 is the priming kinase.

Mapping GSK-3/JNK sites



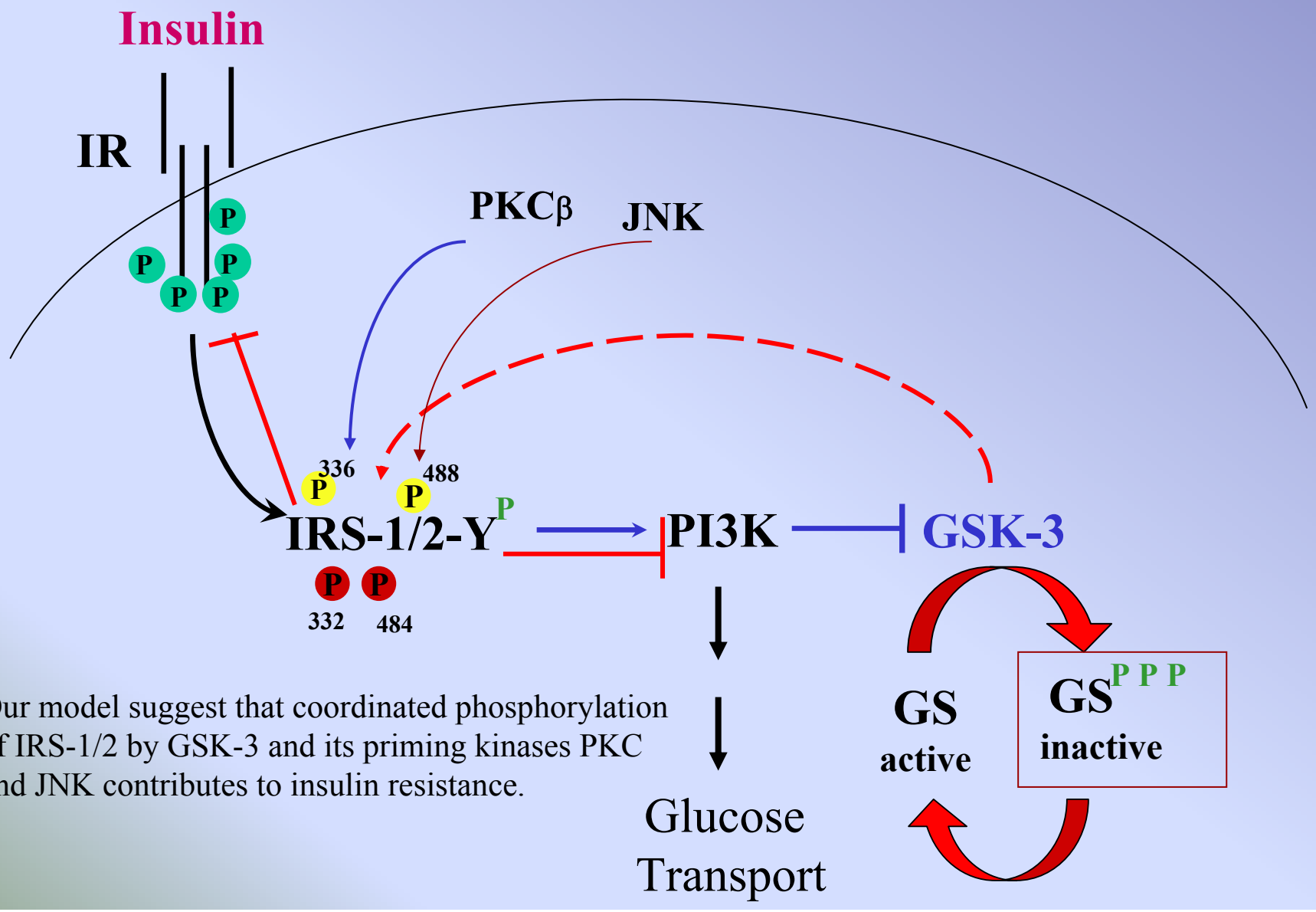
We searched for SXXXSP motifs as the phosphorylated sequence. Accordingly 4 deleted IRS-2 fragments were generated (upper panle). It was found that F2 is phosphorylated by GSK-3 (lower left panel). Mutation at serines 484 and 488 in F-2 confirmed that GSK-3 phosphorylates 484 and that JNK phosphorylates serine 488 (Lower right panel)

Phosphorylation of IRS-2 inhibits insulin signaling in liver cells



Enhanced IRS-2 serine phosphorylation inhibits insulin signaling in liver cells. In addition We found that both GSK-3 and JNK are elevated in the diabetic liver (lower panel).

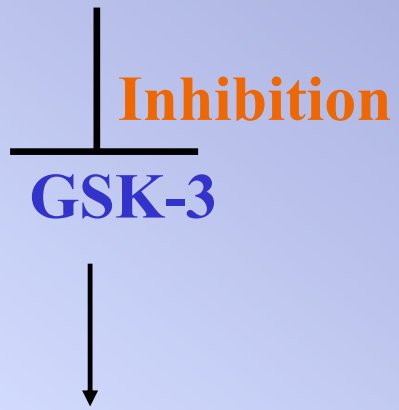
The role of GSK-3: *Negative Feedback Regulation of Insulin Signaling*



Our model suggest that coordinated phosphorylation of IRS-1/2 by GSK-3 and its priming kinases PKC and JNK contributes to insulin resistance.

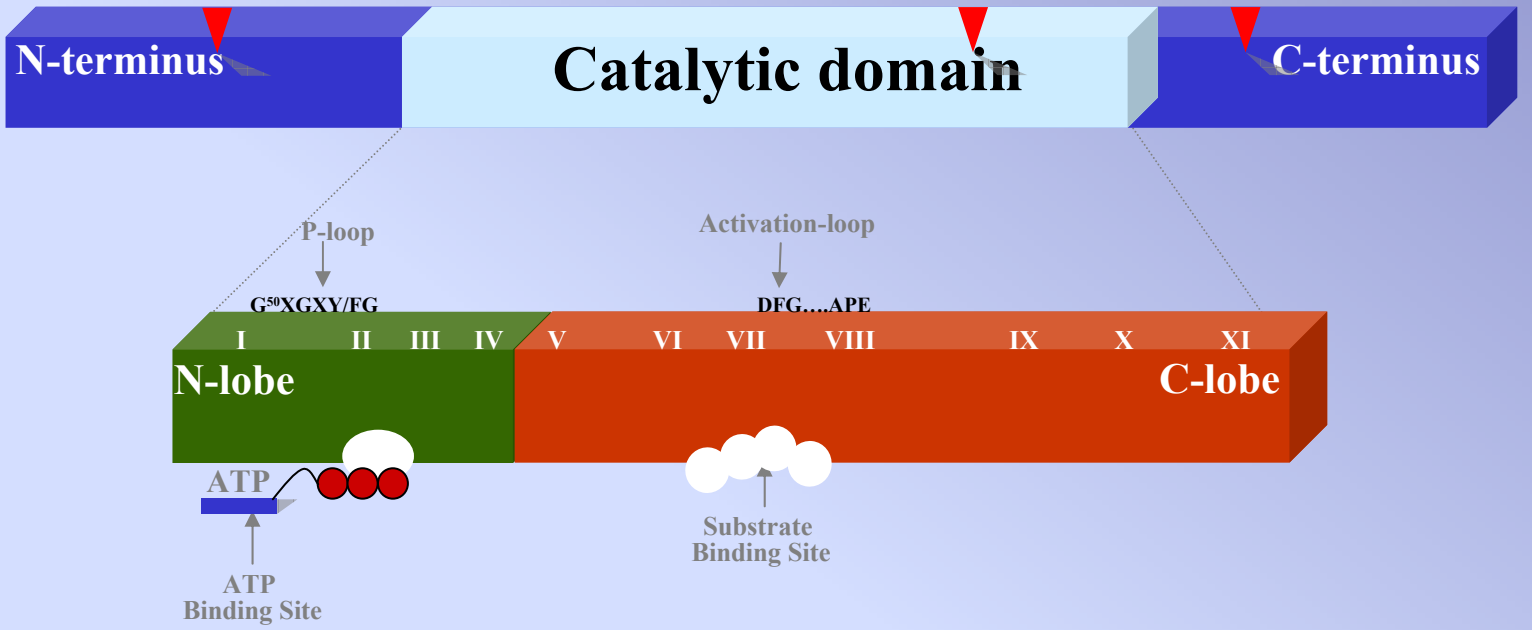
Rationale for development of GSK-3 Inhibitors

- GSK-3 impairs Insulin action
- Elevated levels of GSK-3 activity are associated with insulin resistance and type 2 diabetes



- mimic insulin signaling pathway
- useful in condition of Insulin resistance and type 2 diabetes

Protein kinase inhibitors



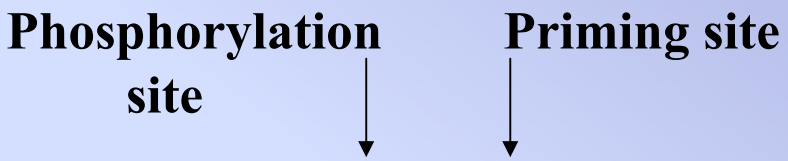
ATP competitive

substrate competitive

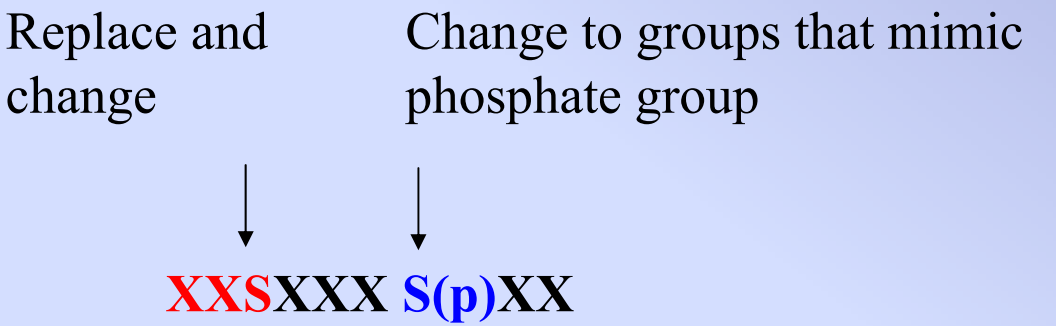
More specific, safer, less toxic effects

Our approach is to develop substrate competitive inhibitors for GSK-3

GSK-3 Recognition Motif



KRREILSRRPS(p)YR	CREB peptide
RPASVPPSPSLSRHSSPHQ S(p)EDEE	Glycogen Synthase peptide
SYLDSGIHSGATTTAPSLGKG	β-Catenin
KEEPPSPPQS(p)PRV	Heat shock factor-1



The strategy is based on the unique recognition motif of GSK-3

L803-mts A Novel GSK-3 Peptide Inhibitor

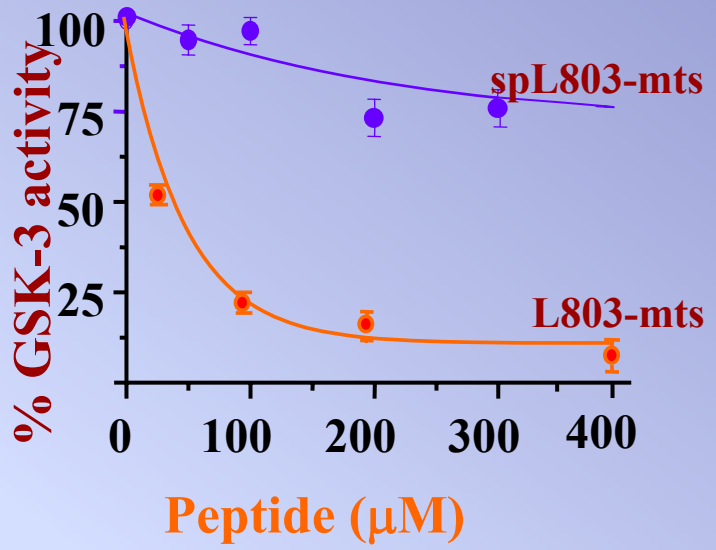
Length: 11 amino acids: **KEAPPAPPQS(p)P**

Solubility: **H2O, DMSO**

IC50: **40μM**

Substrate competitive

Specificity: tested toward CdC2, PKC, ERK, JNK, PKA, PKB, PDK1at 300μM (no inhibition)

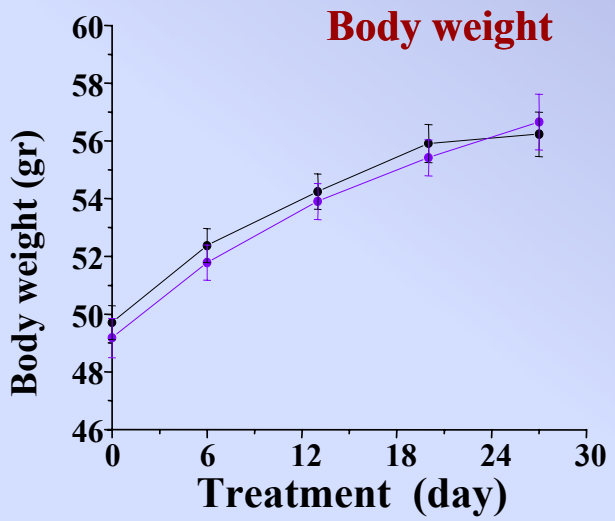


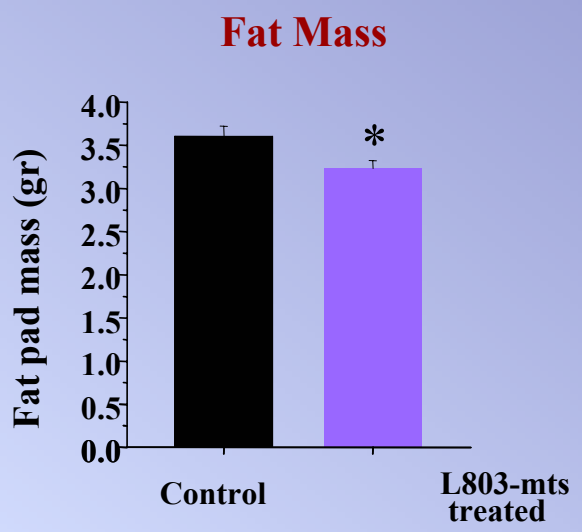
Stability: at least 8 hr in serum

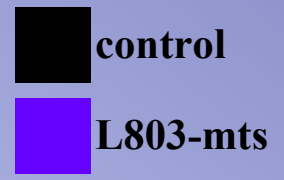
Safety: maximal Tolerated Dose 120mg/kg

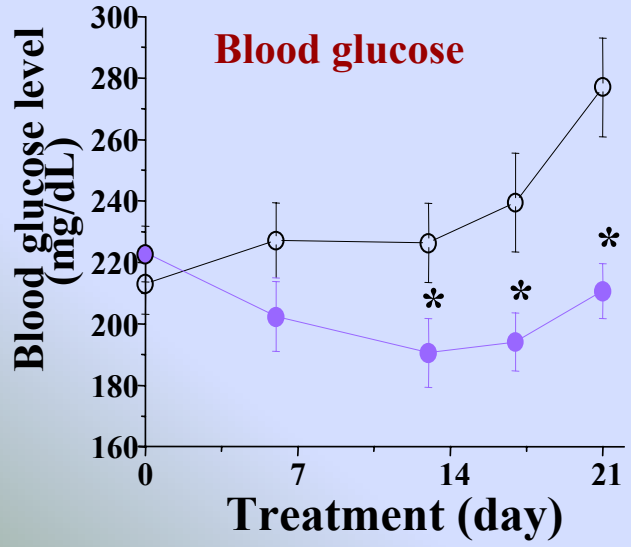
L803-mts is our GSK-3 inhibitor leading compound

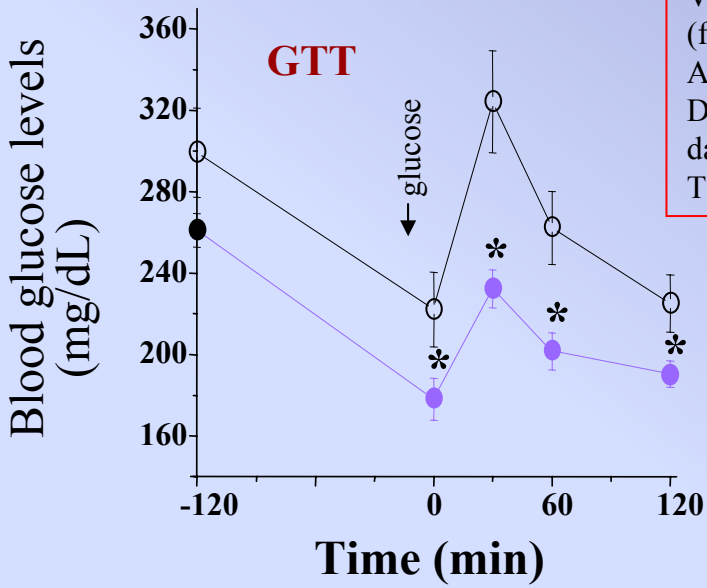
Long-Term Effect of L803-mts in Diabetic ob/ob Mice







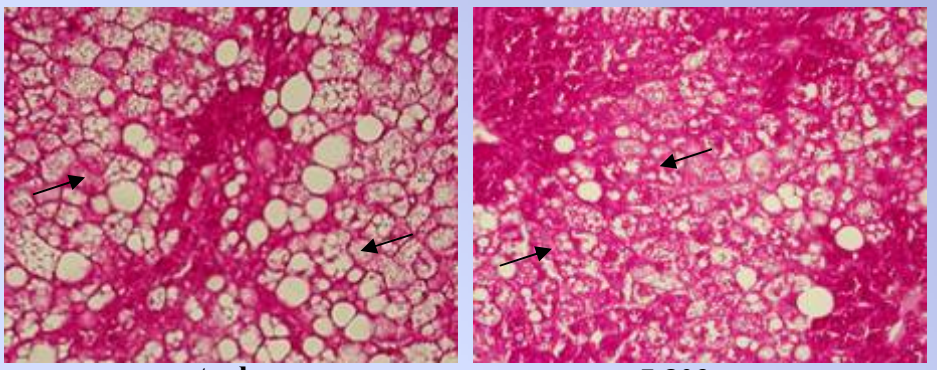




n=12-14
Vehicle: 2.6% DMSO (final 0.65%)
Administration: ip
Dose: 11.6 mg/Kg daily
Time: 3 weeks

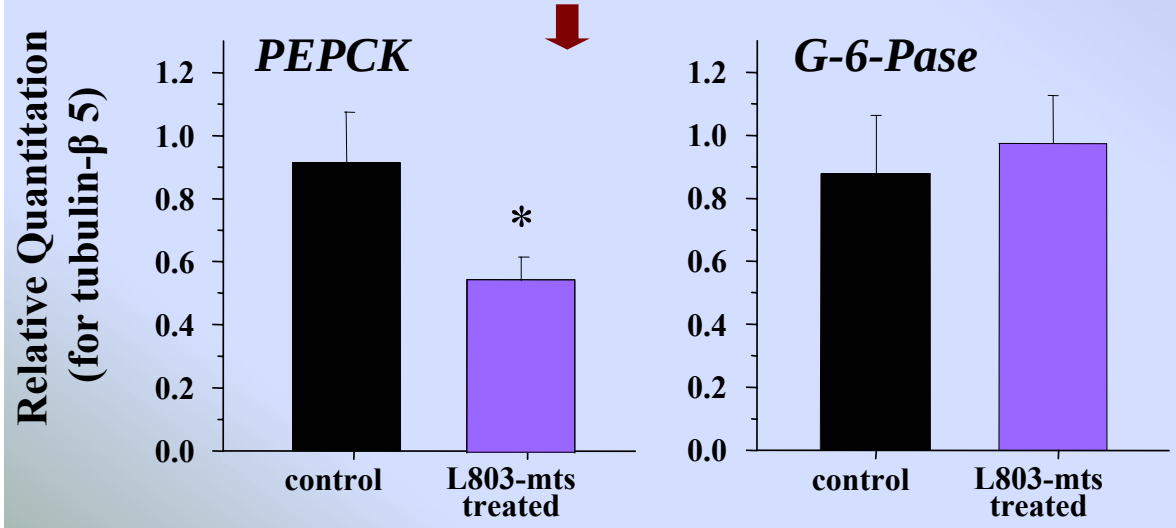
Animals (ob/ob mice) were treated daily with L803-mts for 3 weeks. L803-mts reduced blood glucose and improved glucose tolerance

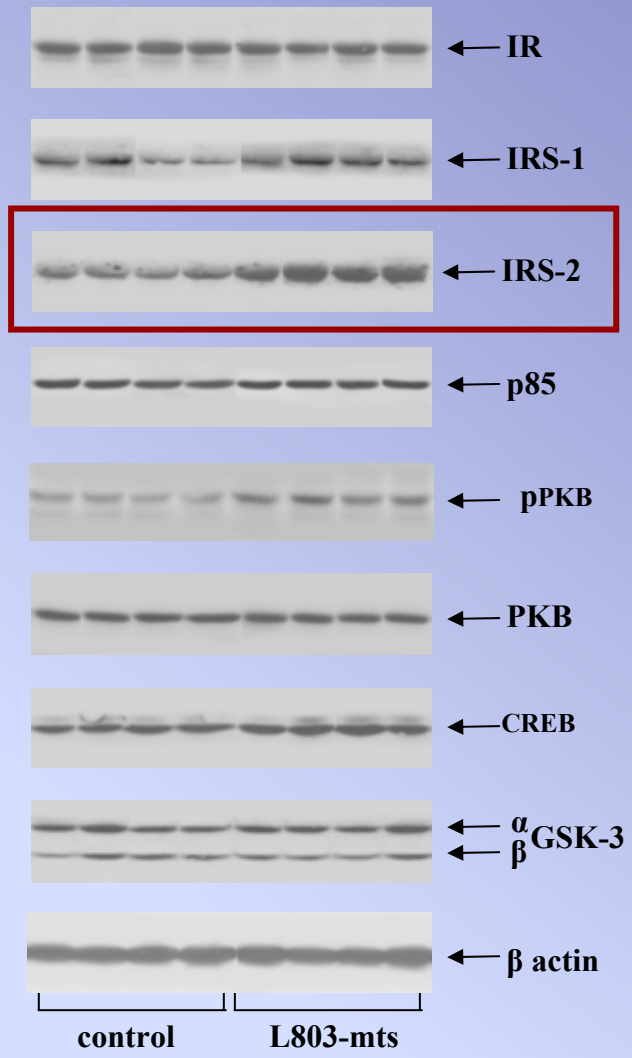
L803-mts affects hepatic glucose metabolsim



Lipid accumulation

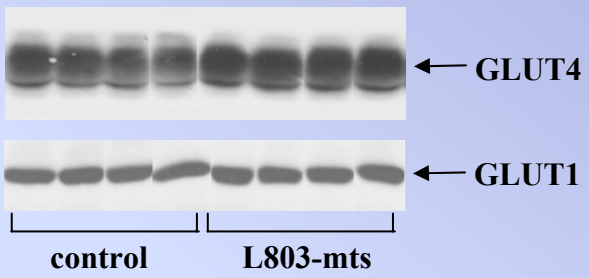
Gluconeogenesi s enzymess

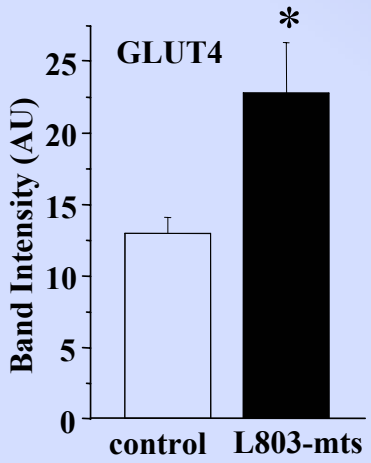


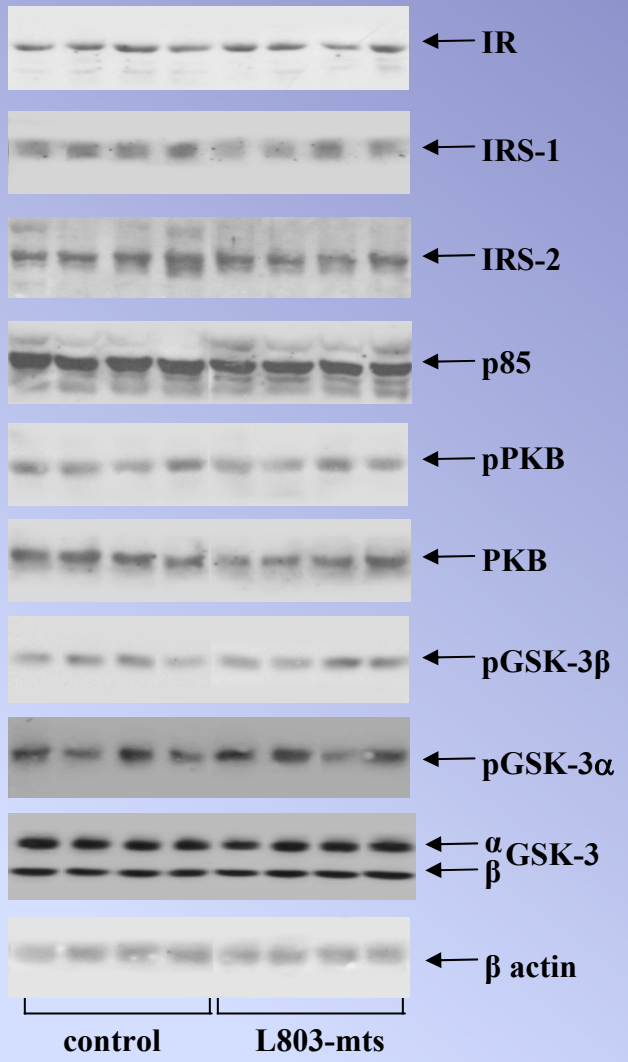


Treatment with L803-mts upregulates hepatic glycogen content, inhibits gluconeogenesis and increases IRS-2 expression levels in the liver.

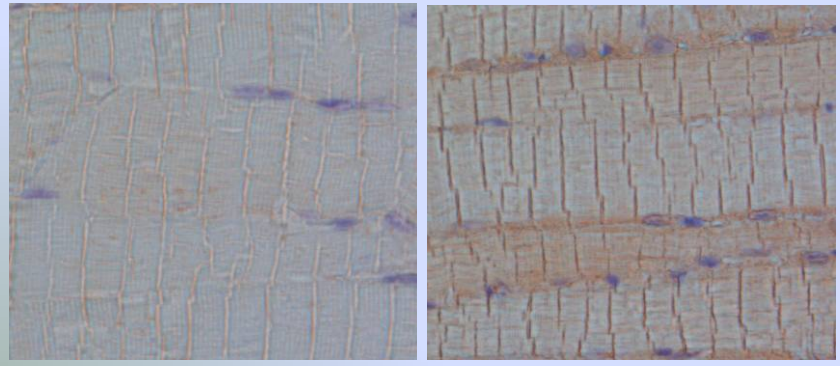
L80-3-mts enhaces Muscle GLUT4 expression







GLUT4

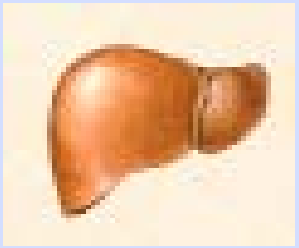


In muscle L803-mts upregulates GLUT4

L803-mts Anti-diabetic activity

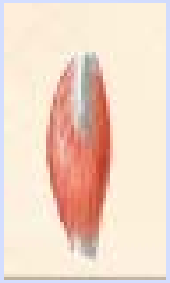
Suppression of gluconeogenesis

Hepatic glucose output ↓



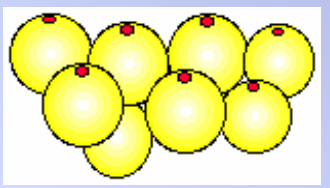
Liver

Glucose transport ↑



Muscle

Inflammation ↓

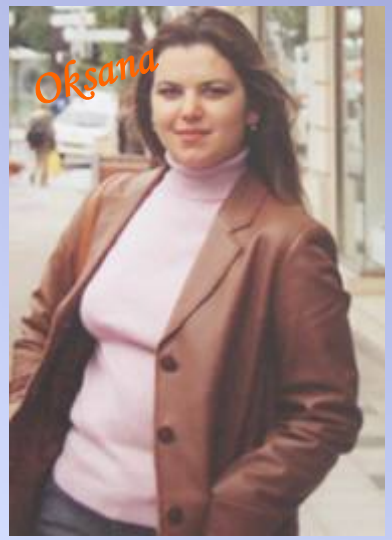


Fat

Anti-Diabetes Effects

Summary

- We suggest a working model in which GSK-3 phosphorylate IRS proteins which in turn inhibits insulin signaling
- Phosphorylation of IRS-1/2 by GSK-3 is strictly dependent on pre-phosphorylation at a priming site catalyzed by the priming kinases PKC and JNK. The phosphorylation sites were mapped as serines 332/336 in IRS-1 and serines 384/488 in IRS-2
- We suggest that inhibition of GSK-3 is a potential and promising strategy for treatment of insulin resistance and type 2 diabetes
- We developed a peptide inhibitor termed L803-mts. L803mts is a bio-available substrate competitive inhibitor of GSK-3
- Proof of concept work showed that L803mts improves glucose homeostasis and insulin sensitivity in diabetic animals



Acknowledgments

Zival Liberman- IRS-1 phosphorylation

Hadar Sharfi- IRS-2 phosphorylation

Oksana Kaidanovich-Beilin- in vivo studies